REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Service, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188) Washington, DC 20503.
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1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
02-07-2013	Final Technical Report	April 2012 - January 2013

4. TITLE AND SUBTITLE
Workshop on Gas Channels

5a. CONTRACT NUMBER

5b. GRANT NUMBER
N00014-12-1-0646

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Walter F. Boron

5d. PROJECT NUMBER
12PR07821-00

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Case Western Reserve University, Department of Physiology & Biophysics
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Office of Naval Research
ONR Code 342
875 North Randolph Street
Arlington, VA 22203

10. SPONSOR/MONITOR'S ACRONYM(S)
ONR

11. SPONSORING/MONITORING AGENCY REPORT NUMBER

12. DISTRIBUTION AVAILABILITY STATEMENT
Distribution Statement A/ Approved for public release

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The aforementioned grant supported a workshop on gas channels highlighting the work related to Gas Channels in Walter Boron's Laboratory. In addition several faculty from other institutions were invited speakers at the workshop.

15. SUBJECT TERMS
Gas channels, aquaporins, Rh proteins, red blood cells, mass spectroscopy, modular dynamics, carbon dioxide (C02), ammonia (NH3), surface-pH measurements. Xenopus oocytes.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE	UU		Morley Schwebel
U	U	U			19b. TELEPONE NUMBER *(Include area code)* 216-368-3401

I. Heading

A. **PI Name: Walter F. Boron M.D, Ph.D.**

B. **Organization: Case Western Reserve University (CWRU)**

C. **ONR Award Number: N00014-12-1-0646**

D. **Award Title: Gas Channel Workshop**

II. Scientific and Technical Objectives

The grant has two main objectives:

AIM 1: ***CONVENE A GAS-CHANNEL WORKSHOP.*** Topics to be discussed on Day 1 include: (A) Factors limiting gas permeable through the membrane's lipid phase. (B) Molecular mechanism of gas permeation through AQPs and Rh proteins. (C) Physiological significance of gas channels. (D) O_2 channels. (E) Relevant technologies, including electrophysiology, stopped-flow techniques, mass spectrometry, x-ray crystallography, molecular dynamics and other modeling approaches. Day 2 will be devoted to assessing options for future work.

AIM 2: ***GENERATE A REPORT OF THE MAJOR CONCLUSIONS OF THE WORKSHOP.*** We will record the presentations (audio, video, as well as content of projector electronic whiteboard) and generate a written summary of the entire Workshop. We will also generate parallel multimedia presentations on our Website as well as in an iPad Textbook format.

III. Approach (N/A)

IV. Concise Accomplishments

We arranged a 2-day meeting—Thursday September 6 through Friday September 7, 2012—held in the Department of Physiology and Biophysics at Case Western Reserve University in Cleveland, Ohio. We hosted 7 outside speakers plus the PI, 3 postdoctoral participants from CWRU, and two additional attendees (see "Gas Channels Workshop Participants" in Appendix). On the evening preceding the Workshop, we held a welcoming reception at the PI's home (funded privately). In addition, we held a dinner on Thursday evening (funded privately). Finally, several participants remained for an additional day for scientific discussions.

Aim 1 (Convene a Gas-Channel Workshop). On the first day of the Workshop (see "Gas Channels Workshop Agenda" in Appendix), the faculty participants made presentations on a range of carefully selected topics. These included a keynote lecture by Robert Stroud at 4PM that was attended by about 160. The other lectures were attended by 50-100. On the second day, three postdoctoral fellows made shorter presentations. In addition, we held a wide-ranging discussion in which we addressed specific questions on the future of gas-channel research.

Aim 2 (Generate a Report). We video-taped the lectures on Day 1 (see videos posted on the Website, described below), and recorded the more informal proceedings of Day 2. The videos are being edited and will be posted on the Website in the near future. In addition, we took notes of all Workshop activities, including the individual presentations (see "Workshop Notes on Presentations--Rossana" and "Workshop Notes on Presentations--Walter" in Appendix) and the General Discussion during the latter part of Day 2 (see "Workshop Notes--General Discussion" in Appendix). After the Workshop, we collected the presentations of all but one of the presenters. Finally, we created a site on the PI's departmental Website (see http://physiology.case.edu/events/symposia/gas-channels-workshop-2012/), where we present the agenda, participants, notes, and links to the presentations. This site is available to all interested parties and will remain live indefinitely.

The consensus among the invited attendees as well as Clevelanders was that Workshop was a great success—in terms of the quality of the participants, the presentations, and the discussions that surrounded the formal presentations. Moreover, the participants strongly indicated that it would be most helpful for the group to get together regularly, perhaps next in 2014.

V. Expanded Accomplishments (N/A)

VI. Work Plan (N/A)

VII. Major Problems/Issues

The only negative aspect regarding the Workshop is that final permission go forward came relatively late. Although we had tentative commitments from the participants, a firm commitment obviously depended upon the dates chosen for the Workshop and the calendars of the participants remaining open. Immediately upon getting approval for the Workshop, the ONR and the organizers at CWRU did an outstanding job to assemble a first-rate meeting. On the other hand, we could have attracted a few more outstanding speakers as well as representatives of other funding agencies if we had been able to give them more notice. Thus, I recommend that—if we do go ahead with a follow-up meeting, say in 2014—we announce the meeting 8-12 months in advance.

VIII. Technology Transfer

None.

IX. Foreign Collaborations and Supported Foreign Nationals

Two of the meeting participants, Gerolf Gros and Volker Endeward, are from Hannover in Germany.

X. Productivity (N/A)

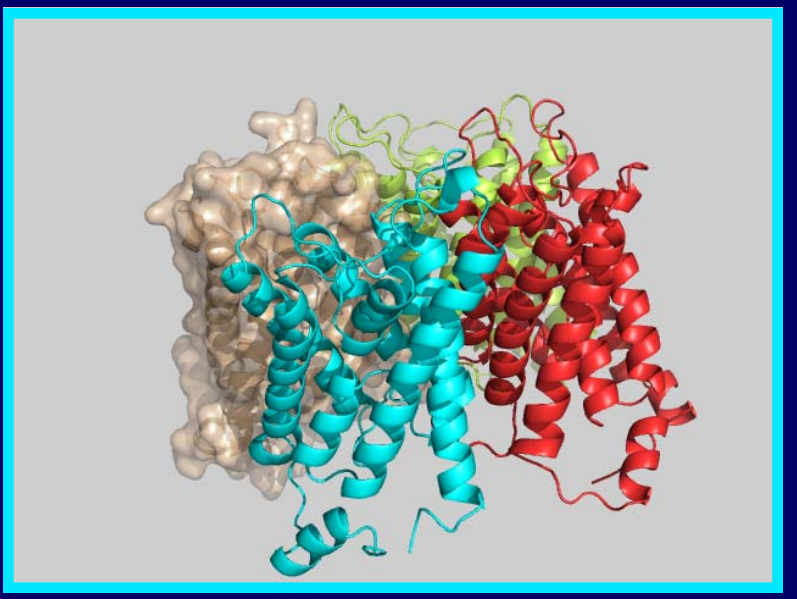
XI. Award Participants

Military personnel: None

Walter F. Boron, M.D., Ph.D—PI

For other participants, see Gas Channels Workshop Participants in the Appendix.

Gas Channels Workshop



September 6 - 7, 2012

Case Western Reserve University
School of Medicine
Cleveland, Ohio

Sponsored by
The Office of Naval Research

Gas Channels Workshop

Thursday, September 6 &
Friday, September 7, 2012

Department of Physiology and Biophysics
Case Western Reserve University
School of Medicine

Sponsored by
The Office of Naval Research

Welcome to the Workshop on Gas Channels, sponsored by the Office of Naval Research and the Department of Physiology and Biophysics in the School of Medicine at Case Western Reserve University.

The ONR handles the science and technology programs of the US Navy and Marine Corps. Divers, submariners, and individuals ascending to altitude may face a range of medical issues related to dissolved (or undissolved) gases. These include decompression illness, N_2 narcosis, O_2 toxicity, CO_2 narcosis, and hypoxia. Thus, the ONR has a strong and longstanding interest in gas transport.

The Department of Physiology and Biophysics at Case Western Reserve University is one of the few in the world that studies physiological problems from the level of the atom—through molecules, cellular organelles, whole cells, tissues, and organs—to the whole organism. We focus on the nervous, cardiovascular, and renal systems. In the past few years, we have recruited eight outstanding new faculty members. In addition, we completed a major renovation of about 40,000 gross square feet of space. We have also established three major core facilities to support our work: A Protein Expression, Purification, and Crystallization Core (PEPCC, 5th floor), a Molecular Biophysics Core (6th floor), and a Mouse Physiological Phenotyping Core (MPPC, basement).

We hope that you enjoy the Workshop. If during your visit you would like to see our facilities, we would be happy to arrange a tour.





WORKSHOP AGENDA
Thursday, September 6, 2012

7:45 - 8:15am
Registration & Continental Breakfast

8:15am - 8:20am
Welcome/ Introduction
-Walter F. Boron, M.D., Ph.D.

8:20am - 9:00am
Walter Boron, M.D., Ph.D.
Title: *"Gas Channels"*

9:00am - 9:10am: ***Question/Answer Session***

9:10am - 9:50am
Emad Tajkhorshid, Ph.D.
Title: *"Visualizing gas permeation pathways through proteins at sub-Angstrom resolution"*

9:50am - 10:00am: ***Question/Answer Session***

10:00am - 10:25am: **Morning Break**

10:25am - 11:05am
Gerolf Gros, Ph.D.
Title: *"Measuring cellular CO_2 permeability by ^{18}O ex change—methodology and results on red blood cells"*

11:05am - 11:15am: ***Question/Answer Session***

11:15am - 11:55am
Volker Endeward, Ph.D.
Title: *"Intrinsic CO_2 permeability of cell membranes and effect of cholesterol and aquaporin"*

11:55am - 12:05pm: ***Question/Answer Session***

12:05pm - 1:05pm
Lunch –On your own
(Lunch provided for invited speakers in E-504)

1:05pm - 1:45pm
Bhanu Jena, Ph.D.
Title: *"Involvement of elevated membrane cholesterol on G-protein regulated water and gas transport in biological membranes"*

1:45pm- 1:55pm: ***Question/Answer Session***

1:55pm - 2:35pm
Jeffrey Garvin, Ph.D.
Title: *"NO transport by aquaporin 1"*

2:35pm - 2:45pm: ***Question/Answer Session***

2:45pm - 3:10pm: **Afternoon Break**

3:10pm - 3:50pm
David Weiner, M.D.
Title: *"Role of Rh glycoproteins in gas transport — lessons from in vitro model systems"*

3:50pm - 4:00pm: ***Question/Answer Session***

4:00pm - 5:00pm
Robert Stroud, Ph.D.
Title: *"What do structures of the Aquaporins, and Ammonia transporters tell us about conduction of gases?"*

WORKSHOP AGENDA
Friday, September 7, 2012

8:00am - 8:30am
Continental Breakfast

8:30am – 8:35am
Introduction/Welcome
-Dr. Walter Boron, M.D., Ph.D.

8:40am – 9:00am
Speaker: Ryan Geyer, Ph.D.
Title: *"Role of membrane proteins in oxygen transport in red blood cells"*

9:05am – 9:25am
Speaker: Rossana Occhipinti, Ph.D.
Title: *"Mathematical modeling of gas movements in an oocyte "*

9:30am - 9:50am
Speaker: Xue Qin, Ph.D.
Title: *"Structure determinants for CO_2 transport of human aquaporin 5"*

10:00am – 10:30am
Morning Break
(Refreshments served)

10:30am - 1:00pm
Gas Workshop Meeting

1:00pm - 2:00pm
Lunch

2:00pm - 3:30pm
Gas Workshop Meeting

3:30pm –End of Meeting

Walter F. Boron, M.D., Ph.D.
–Case Western Reserve University
Cleveland, Ohio

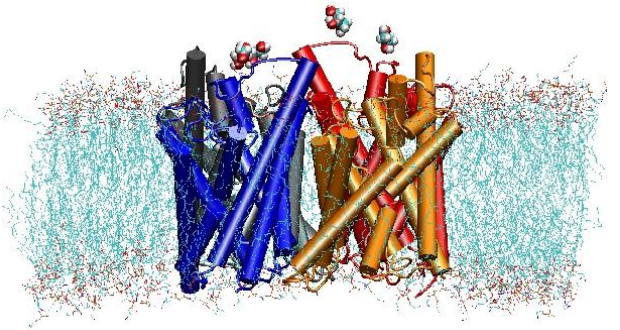


Dr. Boron is the David N. and Inez Myers/ Antonio Scarpa Professor & Chairman of the Department of Physiology and Biophysics at Case Western Reserve University. He earned his AB in chemistry at Saint Louis University, and his M.D. and Ph.D. (Physiology & Biophysics) at Washington University in St. Louis. He joined Yale University as a post-doctoral fellow with Emile Boulpaep in 1978, and remained there for the next 29 years, serving as Chairman of the Department of Cellular & Molecular Physiology for three 3-year terms (1989-1998). In 2007 he returned to his hometown of Cleveland. He is the former President of the American Physiological Society (APS) and is currently Secretary-General of the International Union of Physiological Sciences (IUPS). He is the former editor-in-chief of Physiological Reviews and is the former editor-in-chief of Physiology. He and Emile Boulpaep co-edit the textbook Medical Physiology. He developed his life-long interest in acid-base transport and intracellular-pH regulation with his Ph.D. mentor Albert Roos as well as Paul De Weer, and his complementary interest in renal HCO_3^- transport with Boulpaep. His group currently focuses on three related areas: the molecular physiology of the Na^+-coupled HCO_3^- transporters, molecular CO_2/HCO_3^- sensors, and gas channels. Among his previous honors are a Young Investigator Award (American Society of Nephrology/American Heart Association, 1986), the Robert F. Pitts Award (IUPS, 1993), the Gottschalk Award (APS, 1998), an NIH MERIT Award (2002), the Homer Smith Award (ASN, 2005), the Sharpey-Schafer Award (The Physiological Society, 2008), and the Palade Gold Medal (shared with William Catterall and Richard Tsien, Wayne State University, 2010).

Emad Tajkhorshid, Ph.D.
-University of Illinois at Urbana-Champaign
Urbana, Illinois



Emad Tajkhorshid received his initial training as a pharmacist from Tehran University. After attending two Ph.D. programs, one in medicinal chemistry and pharmacology at Tehran University of Medical Sciences and another one in molecular biophysics at the University of Heidelberg, he started his postdoctoral training in Computational Biophysics in the Theoretical and Computational Biophysics Group at the University of Illinois at Urbana-Champaign in 2001. In 2003 he became the assistant director of research of the NIH Center for Macromolecular Modeling and Bioinformatics at the Beckman Institute for Advanced Science and Technology. He started his independent career as an assistant professor of biophysics, biochemistry, and pharmacology in 2007 and was promoted to associate professor in 2010. The primary focus of his research is on understanding the structural and dynamical properties of membranes and membrane proteins as a basis for their biological function. Employing computational methodologies, his group investigates a wide range of membrane proteins and membrane-associated phenomena in biological systems, in particular the mechanisms of passive and active transport across the membrane.



Gerolf Gros, M.D., Ph.D.
–Hannover Medical School
Hannover, Germany



Dr. Gros is Professor of Physiology at the Department of Physiology at the Medizinische Hochschule Hannover/Germany. He was Professor and Chairman of this Department from 1986 to 2008. He earned his MD degree in 1969 at the University of Tübingen/Germany, followed by one year of practical clinical work. In 1970 he joined Hannover Medical School as a postdoc with Waldemar Moll, and joined his mentor when he moved to the Department of Physiology at the University of Regensburg in 1972. Intermittently, he worked at the Department of Physiology with Robert E. Forster in 1973-1974. He obtained his "Habilitation" in Physiology after returning to Regensburg in 1976. From 1978-1986 he was Associate Professor of Physiology at the University of Essen, and thereafter moved to Hannover to become Full Professor and Department Chairman. He was President of the German Physiological Society in 2007, and President of the Annual Congress of Physiology held in Hannover in 2007. He developed a lifelong interest in CO_2 and O_2 transport in the body, in carbonic anhydrases and in acid-base physiology, initially stimulated by Waldemar Moll and Robert E. Forster. After his move to Hannover, he developed a second field of interest in studying the molecular mechanisms of skeletal muscle plasticity. His work was continuously supported by the Deutsche Forschungsgemeinschaft. His most recent interest is in the field of CO_2 channels in biological membranes, in combination with developing a novel method to determine the CO_2 permeability of cell membranes, and in the molecular mechanism of HCO_3^- transfer across the red cell membrane.

Volker Endeward, Ph.D.
–Hannover Medical School
Hannover, Germany



Dr. Endeward is presently Asst. Professor of Physiology in the Department of Physiology of the Medizinische Hochschule Hannover/Germany. From 1983-1995 he studied Physics at the University of Hannover and obtained his "Diplom" in 1995. Partly simultaneously, he studied Medicine at the Medizinische Hochschule Hannover from 1986-1995. From 1996 to 1997 he practiced Surgery at the Agnes-Karll hospital in Laatzen/Hannover. In 1998 he joined the Department of Physiology of Hannover Medical School and developed his research interests in CO_2 and O_2 transport and acid-base physiology in Gerolf Gros' laboratory. He has worked and published on several topics in these areas, but his main interest over the last years has been CO_2 channels in biological membranes. He has essentially contributed to the development of the mass spectrometric ^{18}O exchange technique to measure CO_2 permeabilities of cell and vesicle membranes, including the complex mathematical description of this process and a numerical procedure to derive CO_2 and bicarbonate permeabilities from mass spectrometric measurements. He has further developed this technique by an analysis of the size and role of unstirred layers and by modelling the intracellular processes influencing the process of ^{18}O exchange. He has presented a comprehensive experimental analysis of the role of aquaporin 1 as a CO2 channel in the human red cell membrane, as well as the first report that the red cell Rhesus protein RhAG also acts as a CO_2 channel. Most recently he has shown that the intrinsic permeability of many biological membranes is low and identified the molecular cause of this property. In addition, he has presented a comprehensive reinvestigation of the so-called metabolon hypothesis, which proposes the existence of a functionally relevant complex of the anion exchanger 1 and carbonic anhydrase 2 in the red cell membrane. His scientific success was recognized by a special personal grant awarded to him by the Deutsche Forschungsgemeinschaft in 2009.

Bhanu Jena, Ph.D.
–Wayne State University School of Medicine
Detroit, Michigan

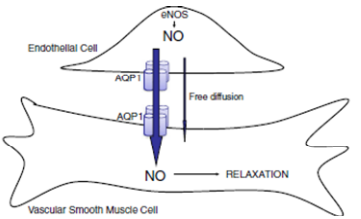


Dr. Bhanu Jena was born in a small town in Orissa, India, [redacted], to Manju Prova and Prafulla K. Jena, a chemist. He spent his early childhood in several remote villages in India, where his grandfather practiced medicine. The dedication of his father and grandfather to science and medicine and their service to humanity greatly influenced his choice for a career in science. Dr. Jena majored in Chemistry, Zoology and Botany from BJB College in India (B.Sc., 1975) and studied Reproductive Endocrinology at Utkal University, (M.Sc., 1978). He graduated top of his class in the Masters program receiving the Prasant Ku. Memorial Prize and the Utkal University Gold Medal. In December 1988, Dr. Jena received his Ph.D. degree in Reproductive Endocrinology, and the Research Excellence Award from Iowa State University. Following postdoctoral training at Yale University, he-joined Yale University as an Assistant Professor. In 2000, Dr. Jena moved to the Department of Physiology, at Wayne State University School of Medicine, as a tenured Professor, and Founder-Director of the Institute of NanoBioScience. His foray into science began 40 years ago, when he published his first scientific paper. His enquiry on how cells secrete, led to the serendipitous discovery of the ***"porosome"*** *- a new cellular structure universally present in all secretory cells at the cell plasma membrane, and involved in secretion. In early 2012, the neuronal porosome proteome was determined. The current focus of the laboratory is to further determine the structure and conformation of the neuronal porosome using cryo electron crystallography.*

Jeffrey Garvin, Ph.D.
-Henry Ford Hospital
Detroit, Michigan



Jeffrey Garvin, Ph.D. is currently Professor of Physiology at Wayne State University and Division Head of the Hypertension and Vascular Research Division of Henry Ford Hospital. He received his B.S. from the University of Miami in Biology and Chemistry in 1979 and his Ph.D. from Duke University in 1984. Dr. Garvin did his postdoctoral training in the Laboratory of Kidney and Electrolyte Metabolism at the National Institutes of Health under Maurice Burg, Mark Knepper and Kenneth Spring where he was supported by a National Kidney Foundation fellowship and two National Research Service Awards. In 1988 he joined the Hypertension and Vascular Research Division of Henry Ford Hospital and became Division Head in 2009. His research deals with the regulation of transport processes in the kidney and how disregulation of these systems can contribute to hypertension. Currently he has more than 125 original publications on renal physiology. Dr. Garvin is a fellow of the Council for High Blood Pressure Research of the American Heart Association and has served on several NIH study sections. He also is an Associate Editor of The American Journal of Physiology: Renal Physiology. His research is now supported by three NIH grants, including a Program Project Grant entitled "Blood Pressure Regulation: Novel Roles for the Kidney.



David Weiner, M.D.
-University of Florida
Gainesville, Florida



Dr. Weiner's primary research interests involve examining the mechanisms and regulation of renal ammonia metabolism and transport. Ammonia plays a central role in acid-base homeostasis, as it is the primary component of basal net acid excretion and changes in ammonia excretion comprise almost 90% of the renal response to acid-base alterations. Renal ammonia transport has traditionally been believed to involve "ammonium (NH_4^+) trapping" and diffusive NH_3 movement.

Dr. Weiner's laboratory examines the specific mechanisms of renal NH_3 movement, and has shown that, in contrast to previously thought models, that NH_3 transport involves specific proteins, namely, Rh glycoproteins. These proteins are widely expressed in ammonia transporting tissues, and Dr. Weiner's studies, using a variety of in vivo and in vitro models, including transgenic animal models utilizing cell-specific gene deletion, have shown the central role of these proteins in renal ammonia, and thereby acid-base, homeostasis.



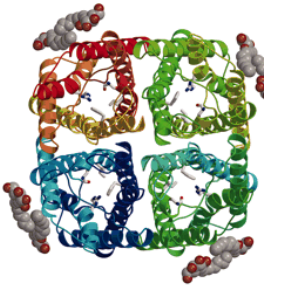
Robert Stroud, Ph.D.
-University of California San Francisco
San Francisco, California



Dr. Stroud was the first to discover fundamental mechanisms of transmembrane proteins by 'Aquaporins' at atomic resolution. These included GlpF, AqpZ, the eye lens AQP0, the H_2S channel, and the essential glycerol channel of the malaria parasite P.falciparum. He defined the structure and regulatory mechanisms of the ammonia channel AmtB and the 'Rh factors'. He revealed the atomic basis for 'signal sequence' dependant membrane protein synthesis, signaling by EPO (erythropoietin) via its receptors. Stroud also determined the mechanisms of enzyme drug targets thymidylate synthase, HIV protease, HIV integrase, and KSHV protease and used these to facilitate drug discovery for human health.

He was elected to the National Academy of Sciences (of the USA) in 2003, President of the Biophysical Society (of the United States) from 1986-1987, and Founding Fellow of the Society in 2000. Dr. Stroud is a member of the Committee for the International Union of Pure and Applied Biophysics. In 1984 he was elected the DeWitt Stetten Lecturer of the National Institutes of Health (NIH). Dr. Stroud was elected as a Fellow of the Royal Society of Medicine (United Kingdom) in 1992.

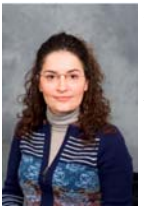




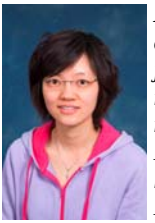
Case Western Reserve University
Boron Lab Post-doctorates



R. Ryan Geyer, Ph.D. *is currently a postdoctoral research fellow in the Department of Physiology and Biophysics at Case Western Reserve University. He received his B.A. in biology from Earlham College in 1998 and his Ph.D. in biochemistry and molecular biology from Wright State University in 2007. In 2008, Dr. Geyer joined Dr. Boron's laboratory and has focused his attention towards elucidating the role of membrane proteins in red blood cell oxygen transport. Dr. Geyer is currently supported by a postdoctoral fellowship from the Office of Naval Research.*



Rossana Occhipinti, Ph.D. *joined Dr. Boron's laboratory as a postdoctoral fellow in October of 2009 shortly after obtaining her Ph.D. in Applied Mathematics from Case. During her Ph.D. studies she developed mathematical models of cellular brain metabolism and numerical methods combining optimization algorithms with Bayesian statistics. She is currently developing mathematical models to investigate the movement of acid-base equivalents across the plasma membrane. In 2009, she received the Melvin H. Knisely International Award and in 2012 the Cell & Molecular Physiology Section Research Recognition Award. Her work is currently supported by an AHA Postdoctoral Fellowship.*



Xue Qin, Ph.D. *earned her Ph.D. in Pathophysiology at Peking University in China. In 2008 she joined Case Western Reserve University in Dr. Boron's lab. Dr. Qin's Ph.D. work was about the signaling pathway of Nitric Oxide, cGMP and Protein Kinase G in coronary arteries. In Dr Boron's Lab, her research has mainly focused on gas channels. Dr. Qin uses surface pH method to study the structural functional relationships of human aquaporin 5. Her work has been supported by American Heart Association Postdoctoral Fellowship. In 2010 she won the Cell & Molecular Physiology Section Research Recognition Award.*


SCHOOL OF MEDICINE
1843
CASE WESTERN RESERVE UNIVERSITY

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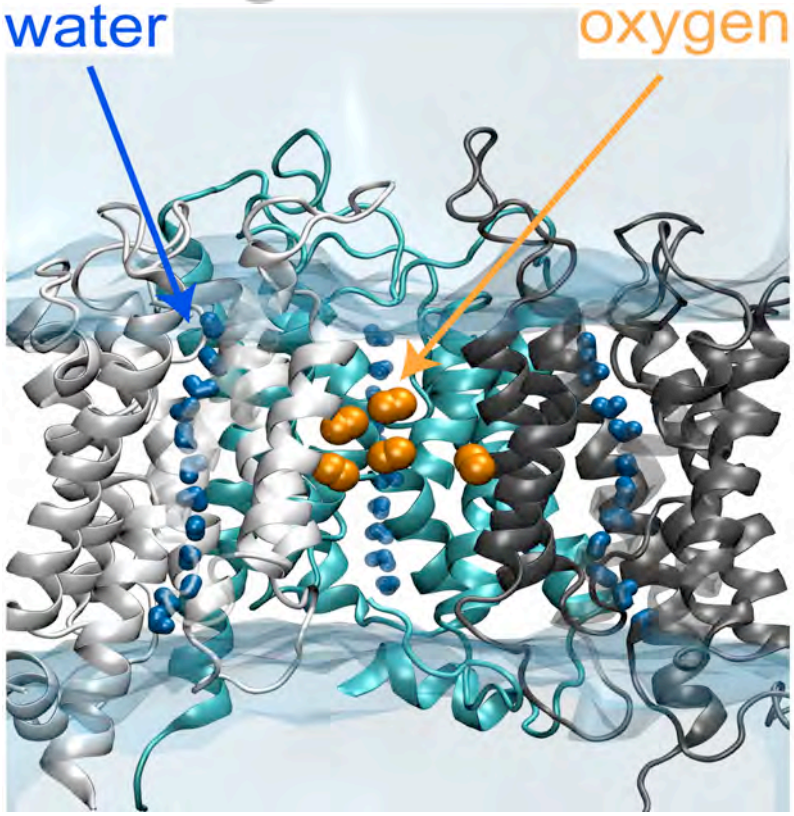
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Email: malmstad@usc.edu

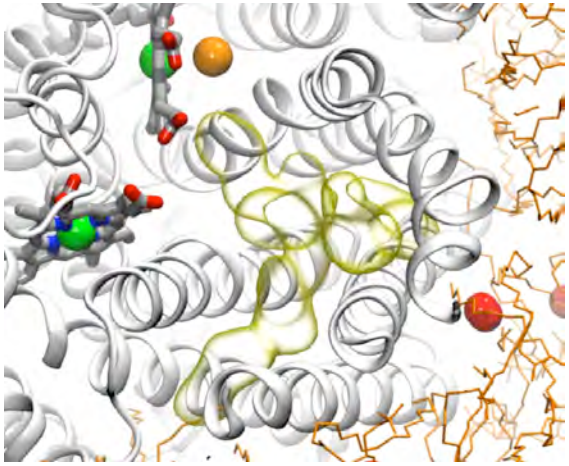
Visualizing Gas Permeation Pathways Through Proteins at Sub-Angstrom Resolution

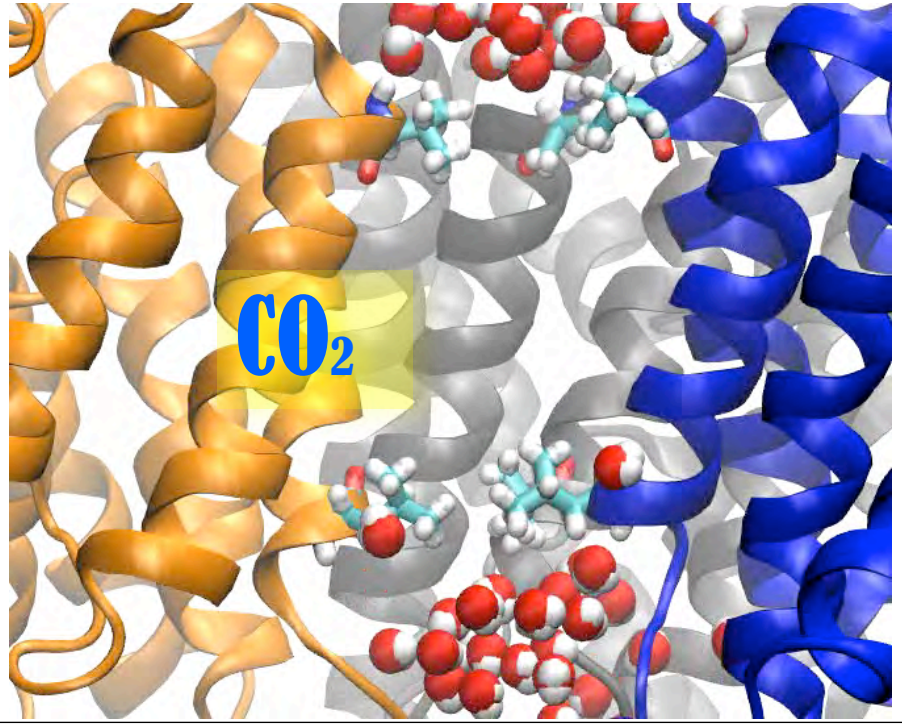


Emad Tajkhorshid

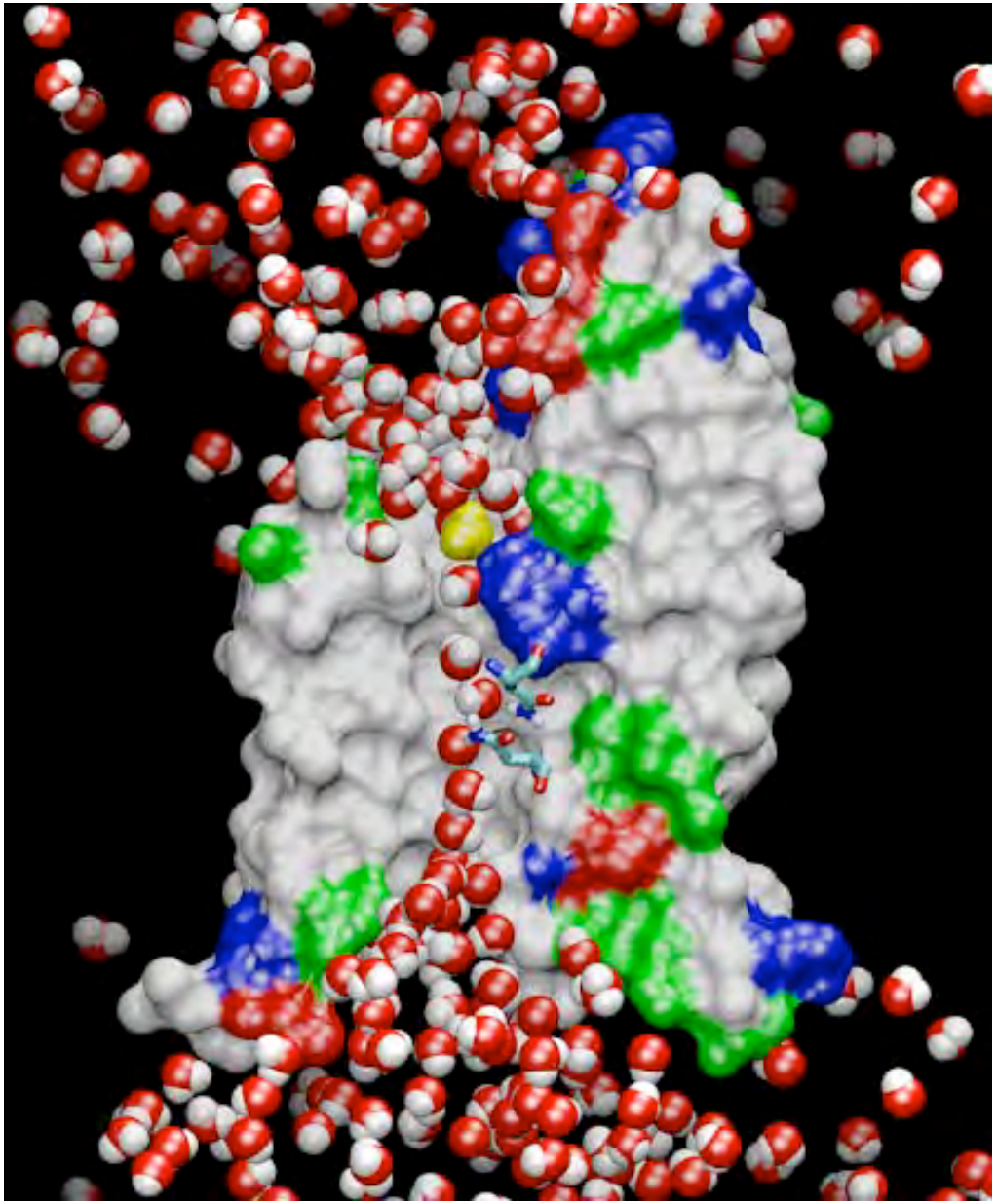
Computational Structural Biology and Molecular Biophysics
www.csbmb.beckman.illinois.edu

Department of Biochemistry
Center for Biophysics and Computational Biology
Beckman Institute for Advanced Science and Technology
University of Illinois at Urbana-Champaign





Molecular Dynamics Simulations



nobelprize.org/nobel_prizes/chemistry/laureates/2003/animations.html

Solving the Newtonian equations of motion for all particles at every time step

SPEED LIMIT

1 fs

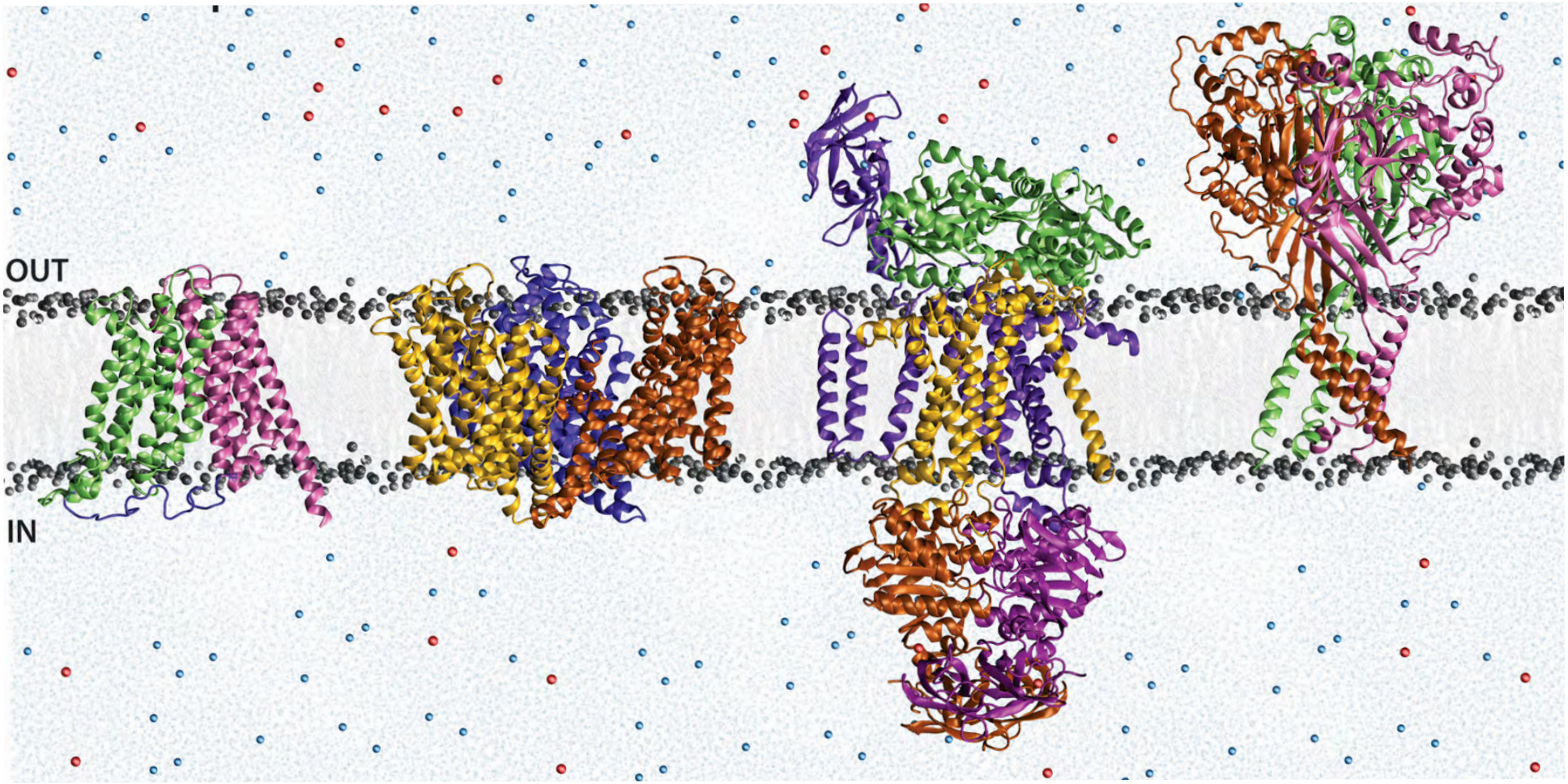
Major limitations:

- Time scale / sampling
- Force field approximations

Major advantages:

- Providing a dynamical description
- Unparalleled spatial and temporal resolutions, **simultaneously**

In situ Molecular Dynamics Simulations



Atom count: 100-500k
~10 ns/day on 128-1024 processors
100-500 ns for each system

Fast Growth of Computational Power

HP 735 cluster
12 processors
(1993)





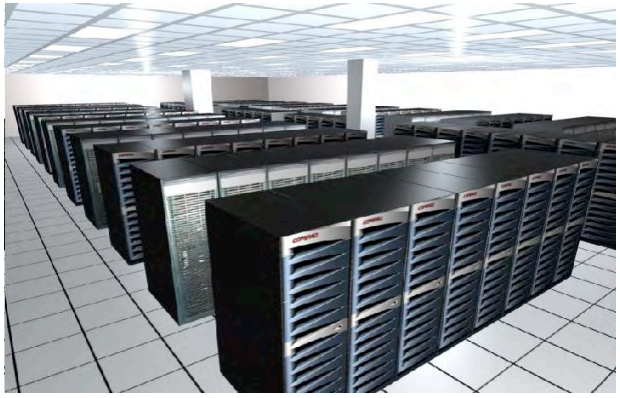
SGI Origin 2000
128 processors (1997)



Blue Waters (UIUC)
200,000+ processors (2013)



Anton/DESHAW/PSC
512 processors (2010)

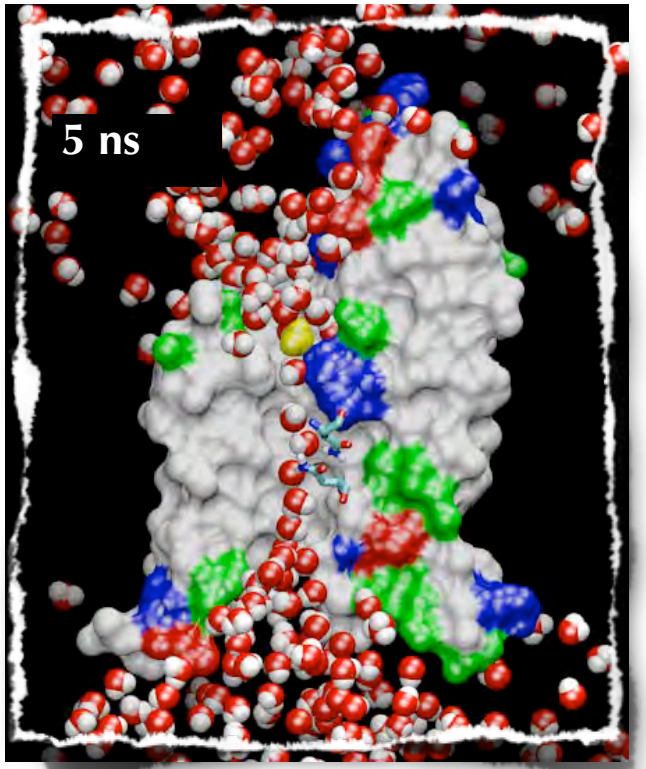


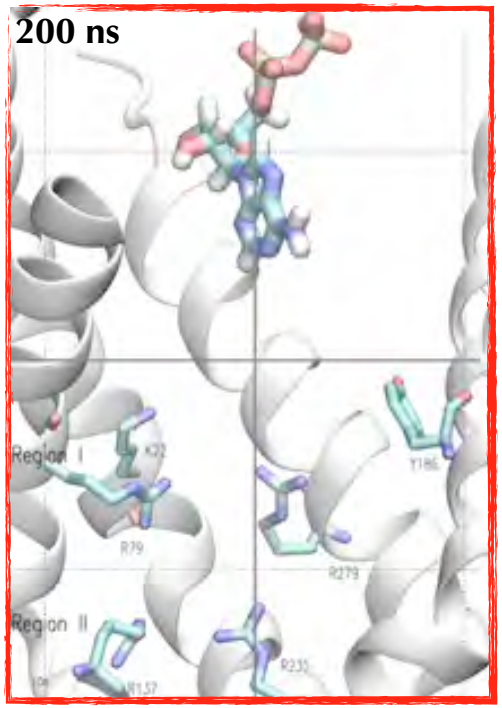
PSC LeMieux AlphaServer SC
3000 processors (2002)

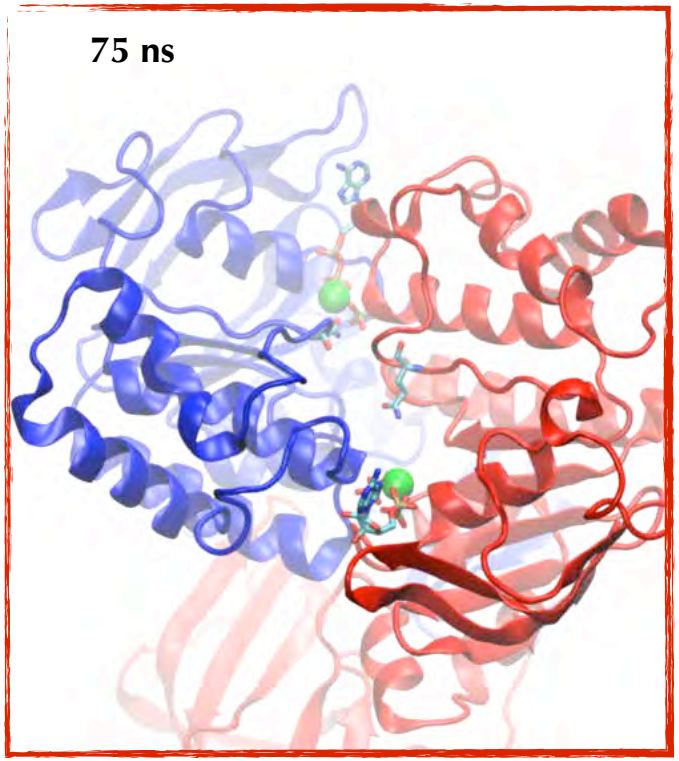


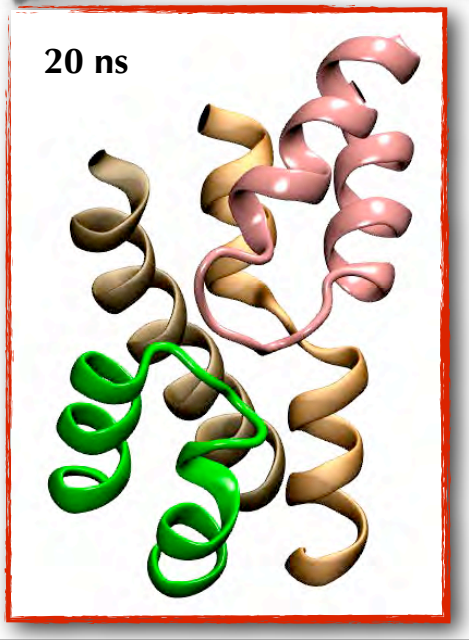
Ranger/Kraken
~60,000 processors (2007)

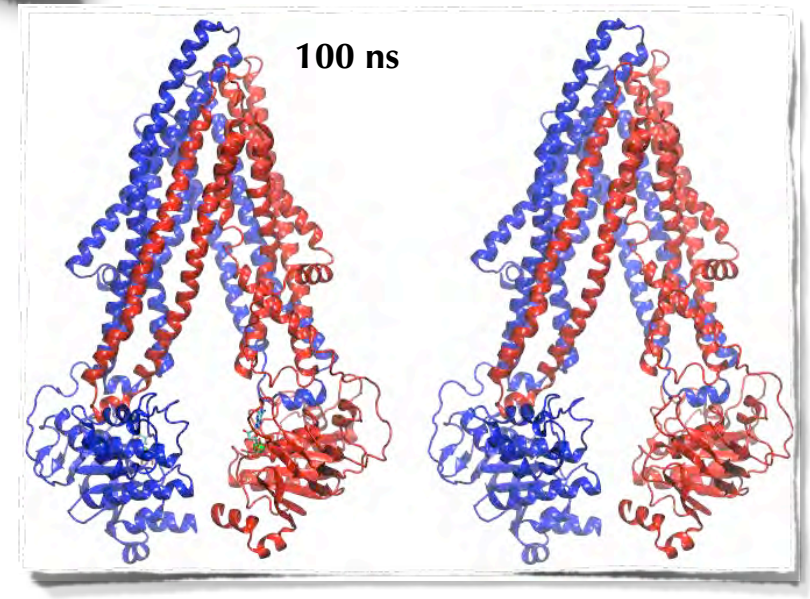
Capturing Biology at sub-Å Resolution

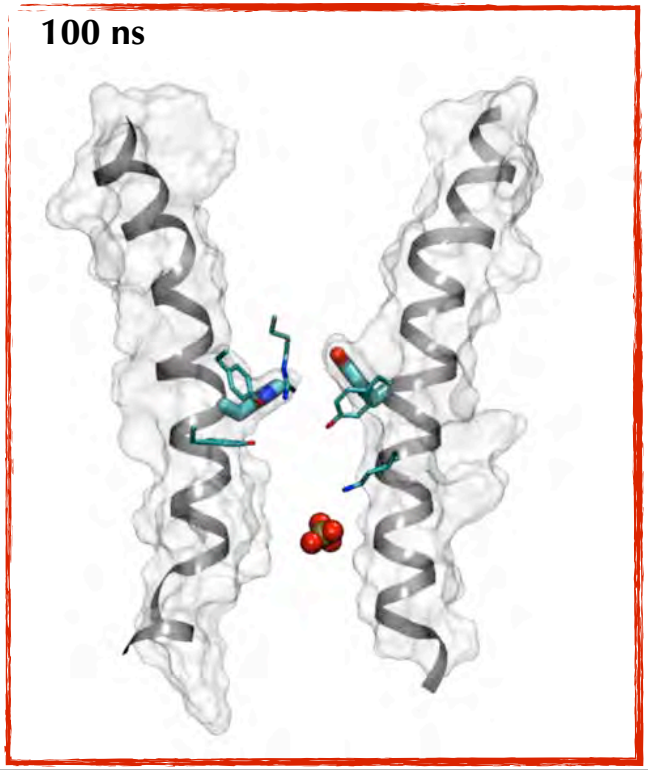




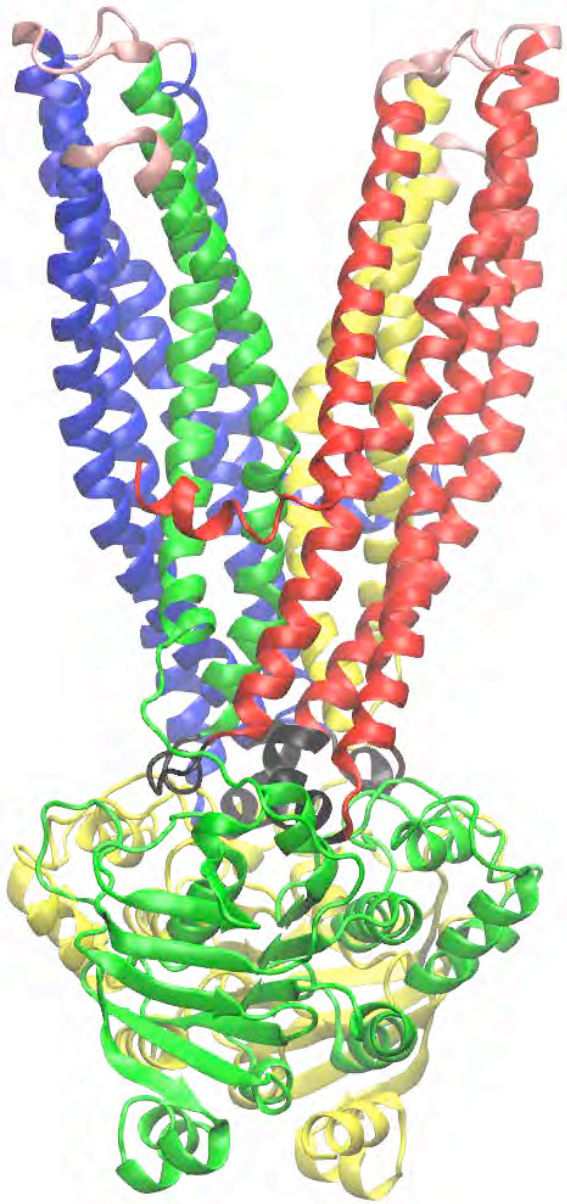


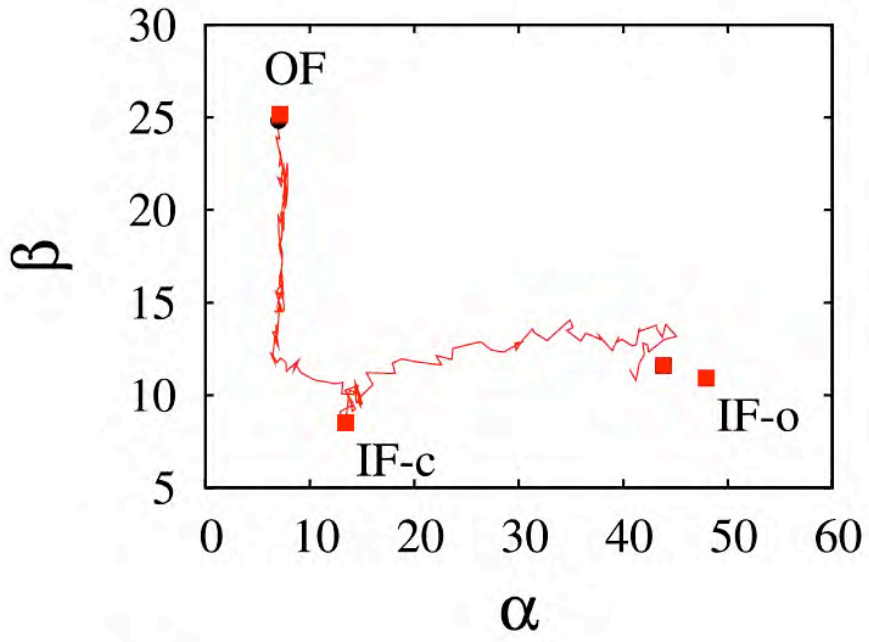


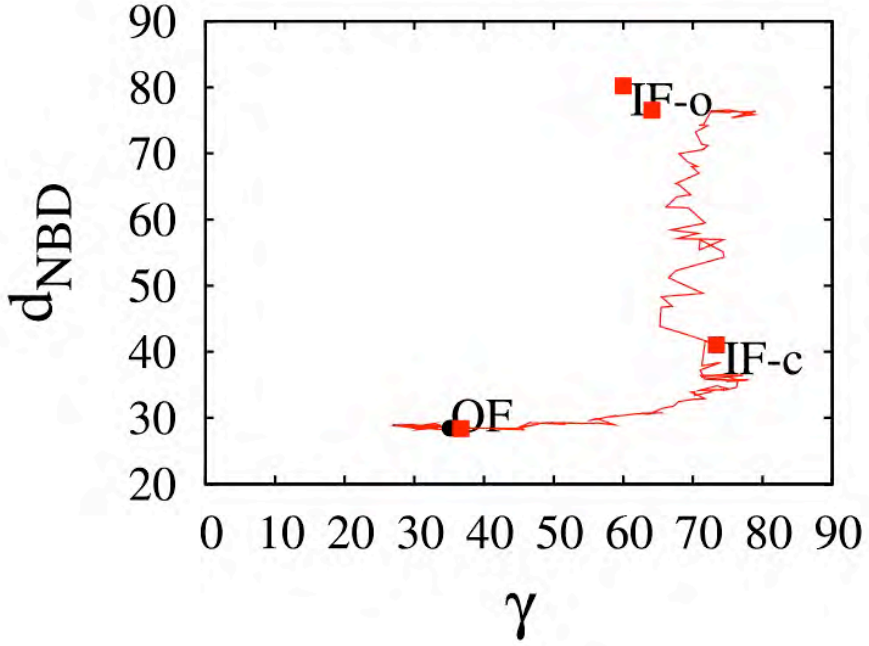




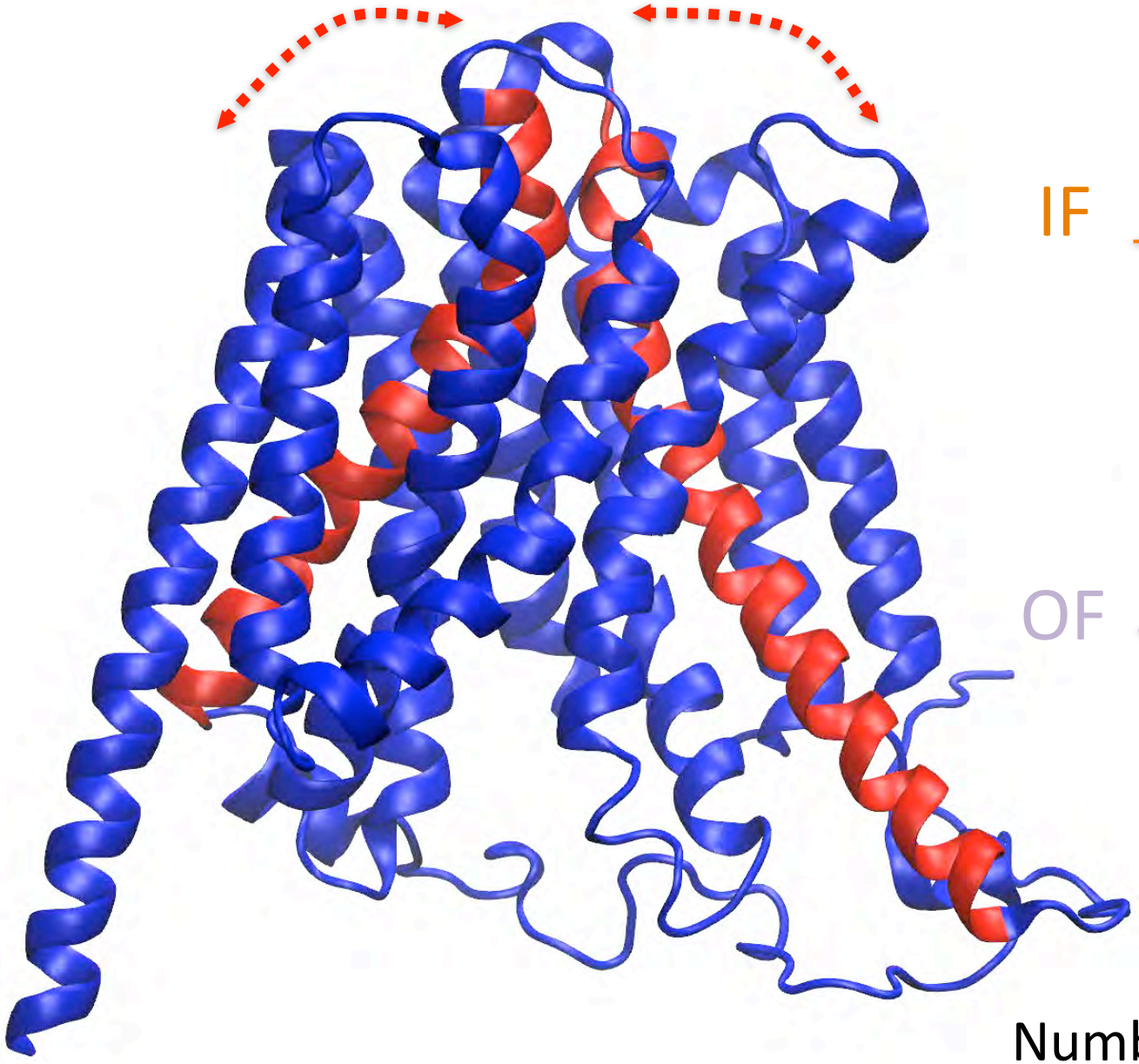
Large-Scale Transition of an ABC Transporter in the Membrane

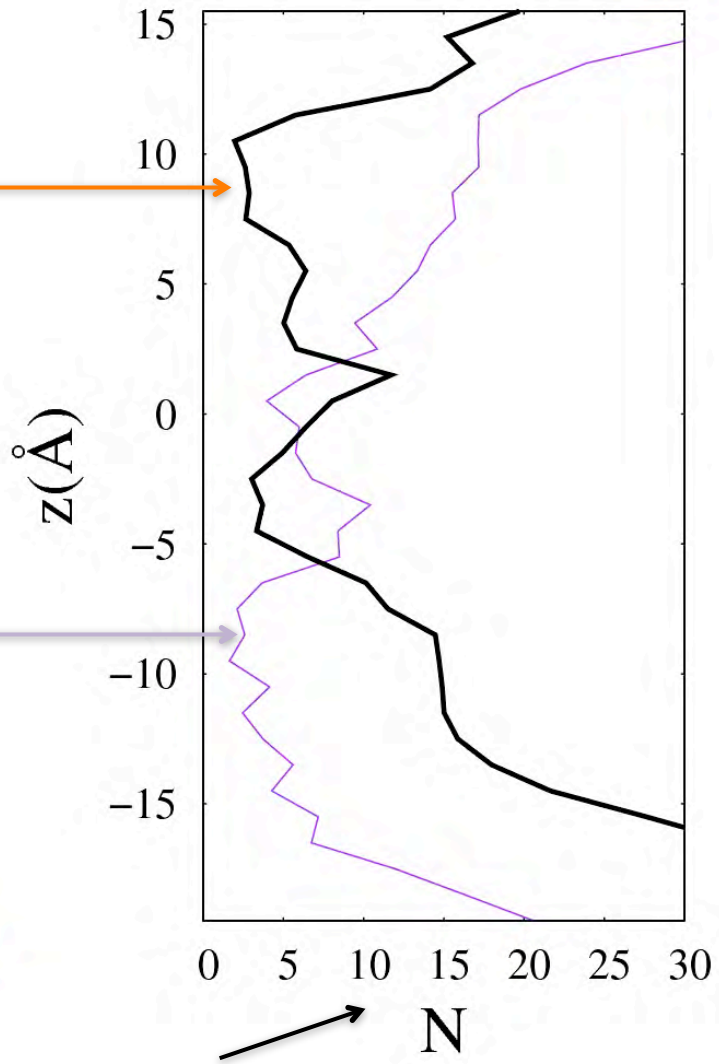






IF↔OF transition in an MFS Transporter in Membrane

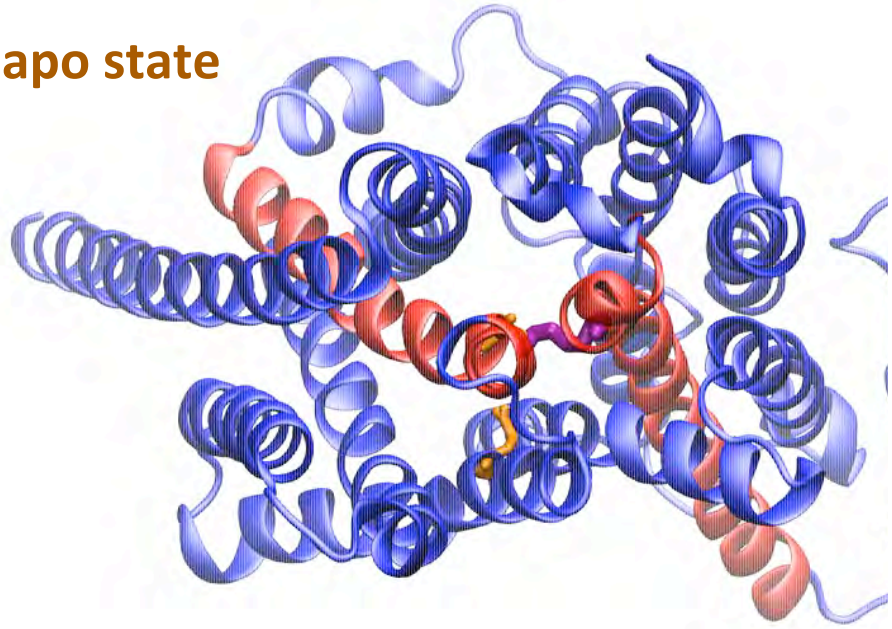




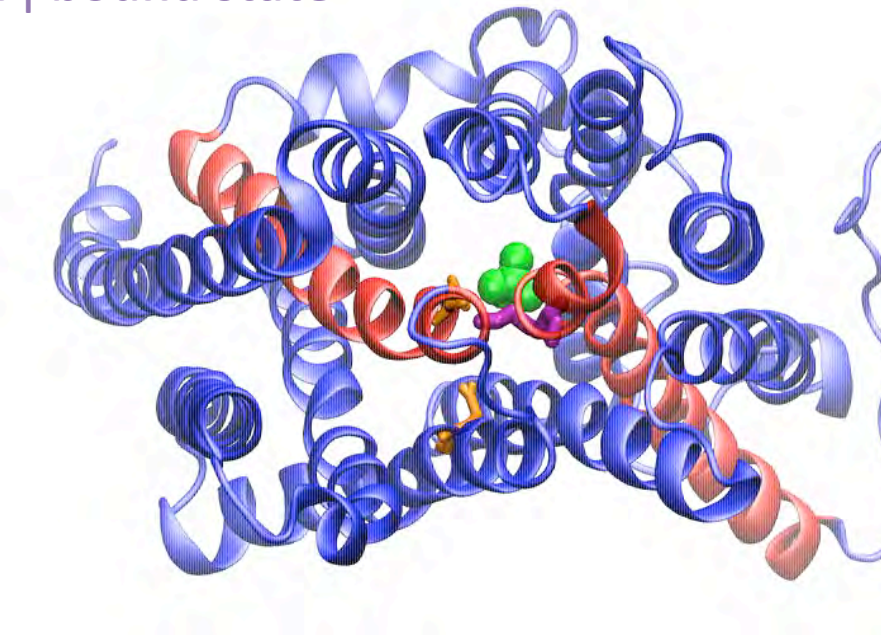
Number of water molecules
(averaged over a 1 ns window)

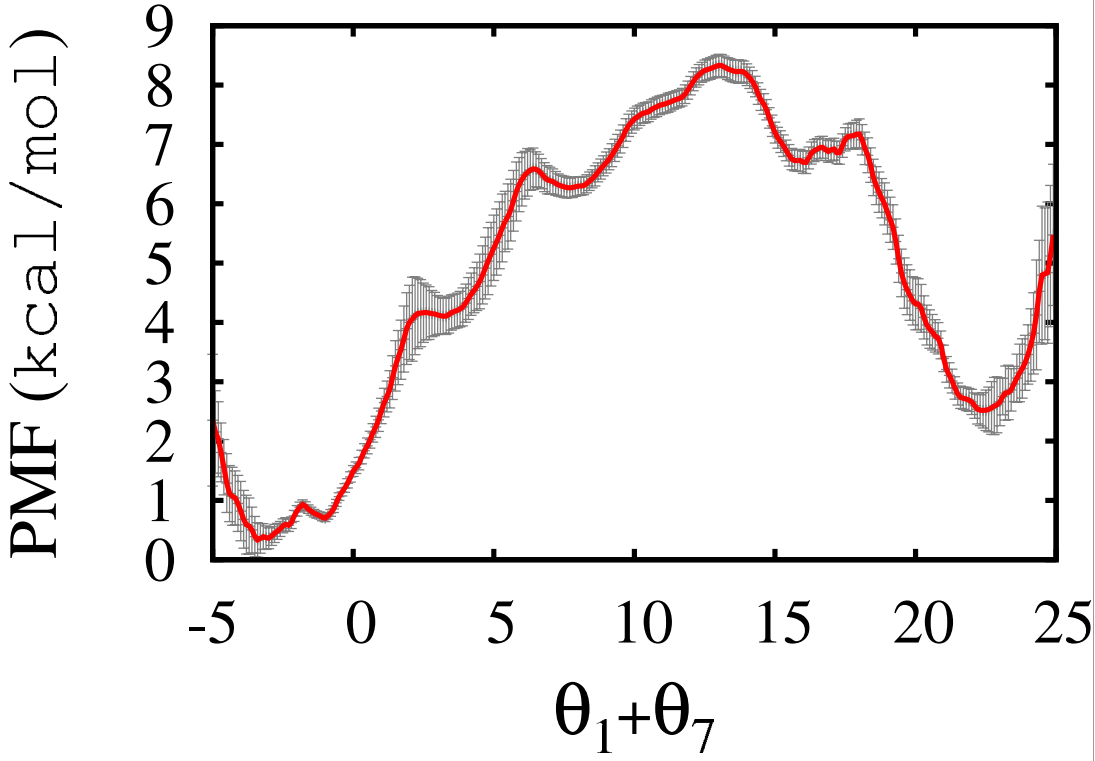
Chemomechanical Coupling in GlpT

apo state

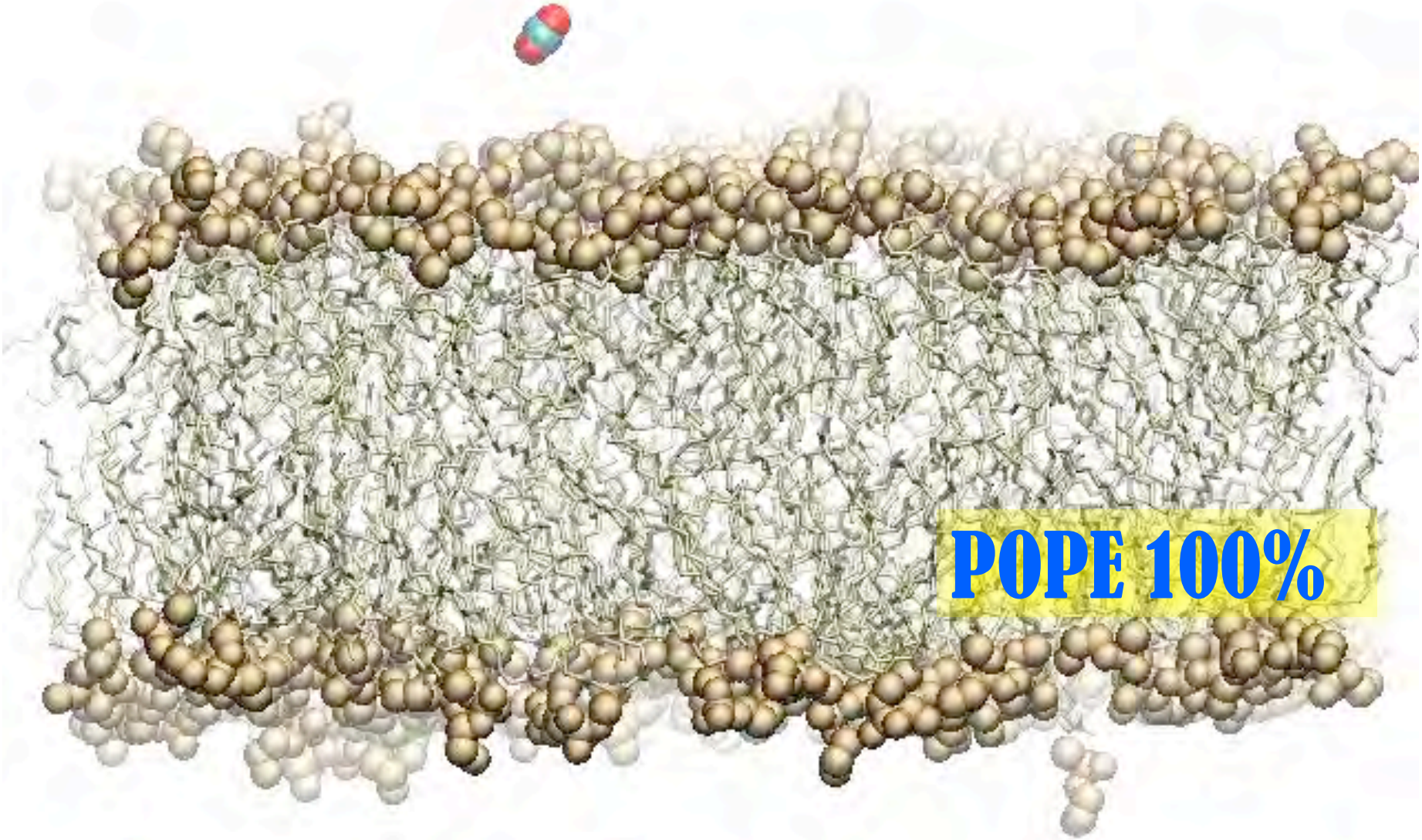


P_i-bound state



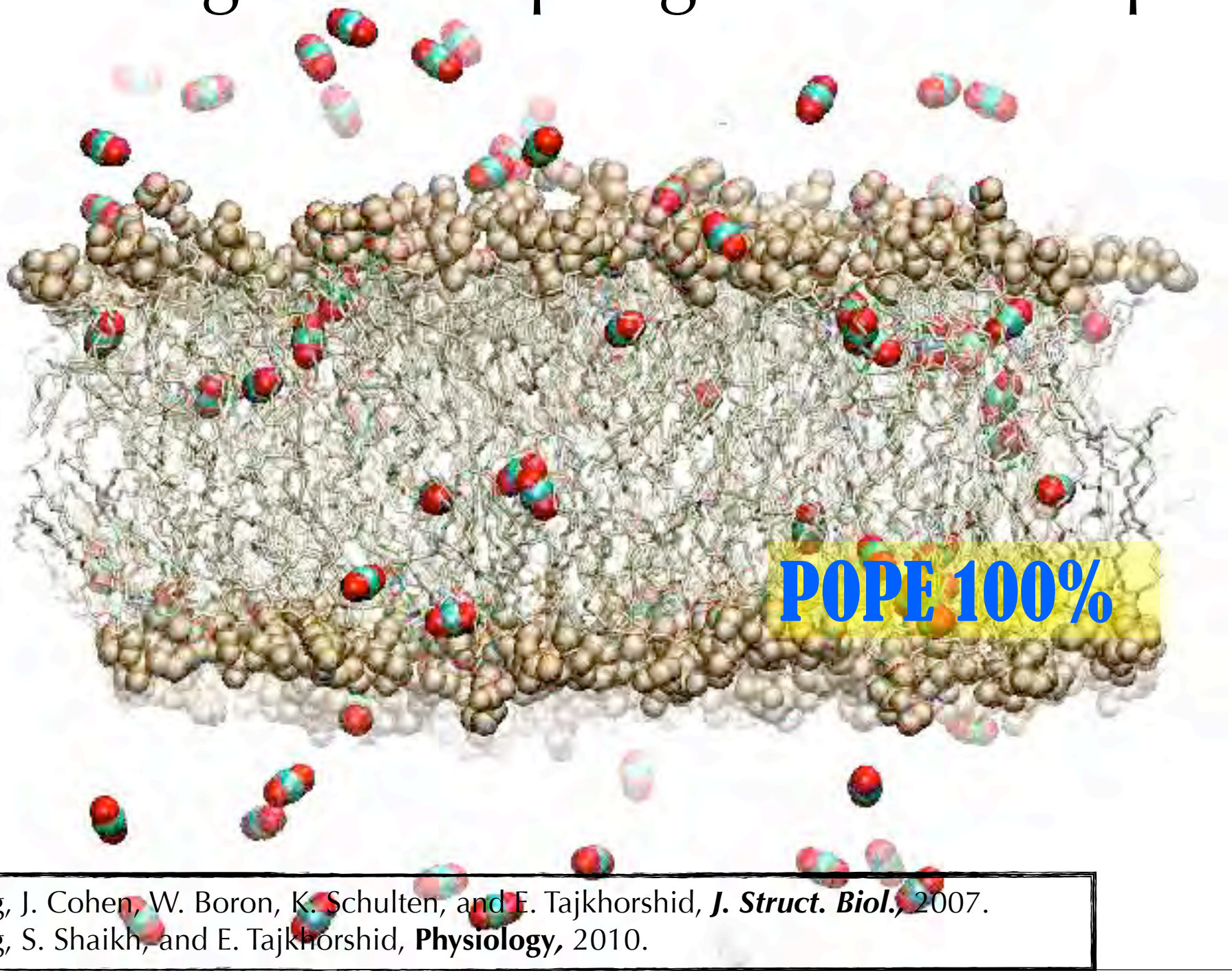


Explicit Ligand Sampling of Gas Transport



Y. Wang, J. Cohen, W. Boron, K. Schulten, and E. Tajkhorshid, ***J. Struct. Biol.,*** 2007.
Y. Wang, S. Shaikh, and E. Tajkhorshid, **Physiology,** 2010.

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Y. Wang, S. Shaikh, and E. Tajkhorshid, **Physiology,** 2010.

Lipid/Water Partition Coefficients

Simulation	
CO_2 in POPE	3.50
CO_2 in POPC	2.74
$O_{2(P)}$ in POPC	4.04
$O_{2(N)}$ in POPC	3.46
$O_{2(P)}$ in POPE	4.73
$O_{2(N)}$ in POPE	5.79

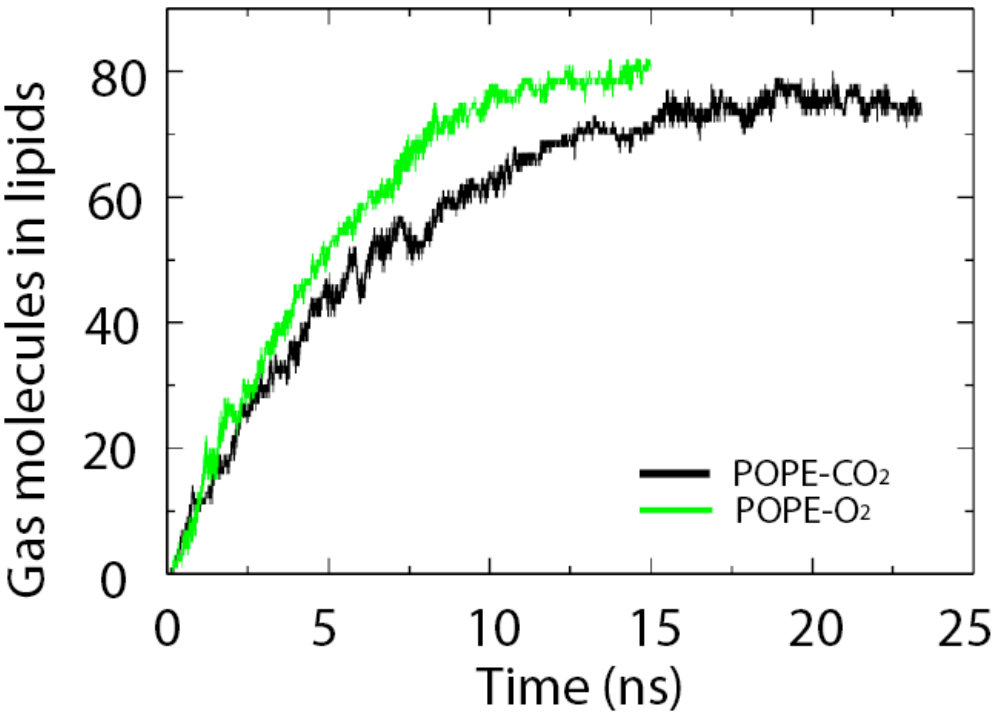
Experiment

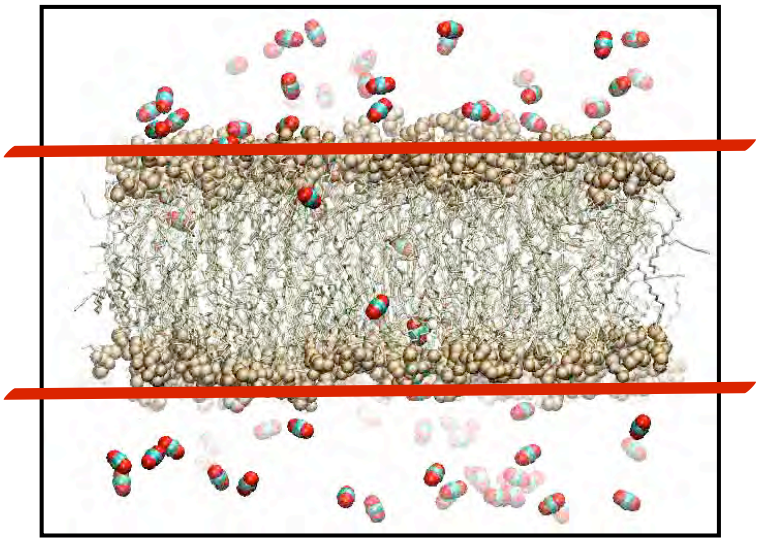
CO2

- Octanol: 1.3
- Hexadecane: 1.5
- Olive oil: 1.7

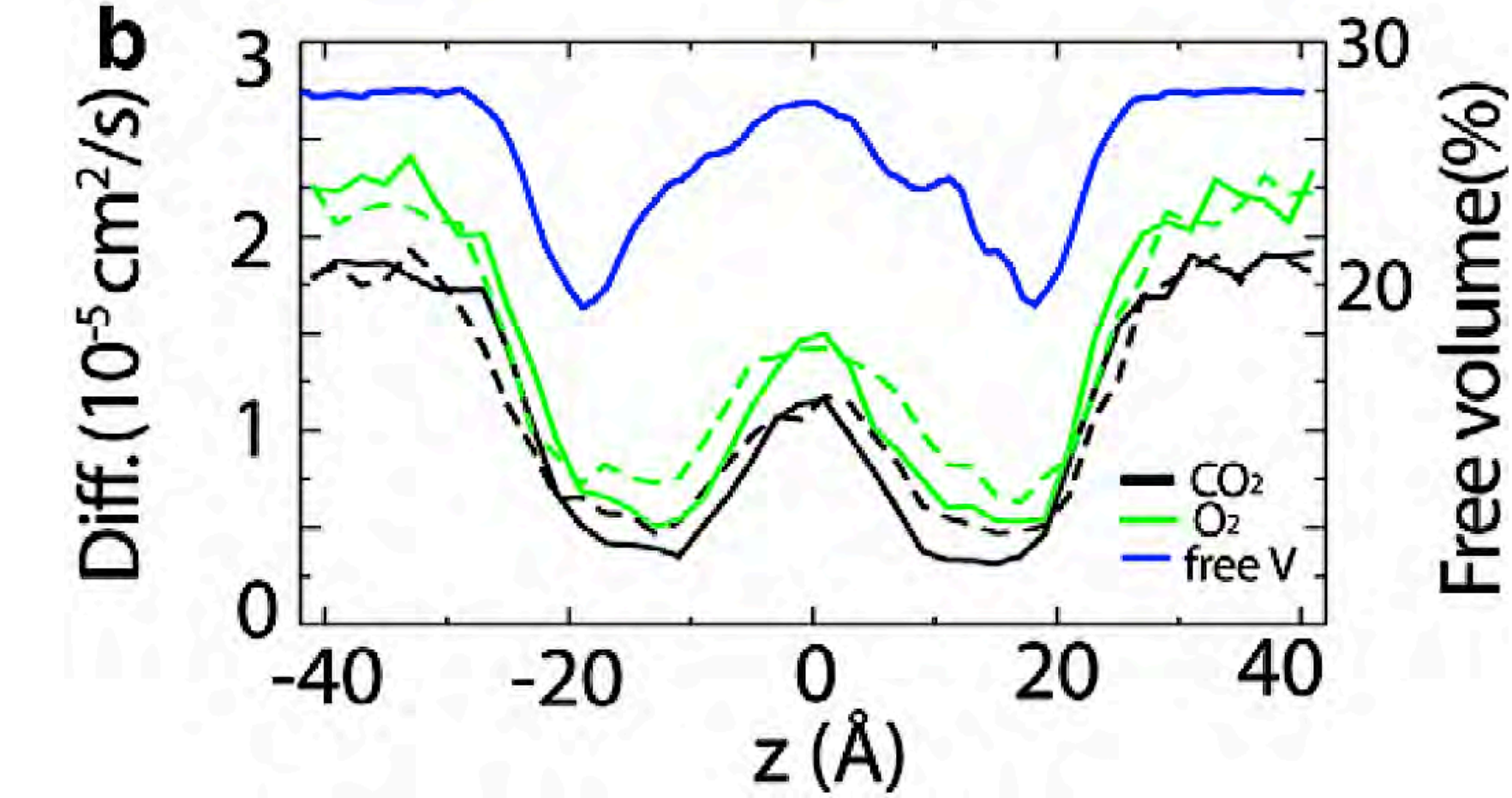
O2

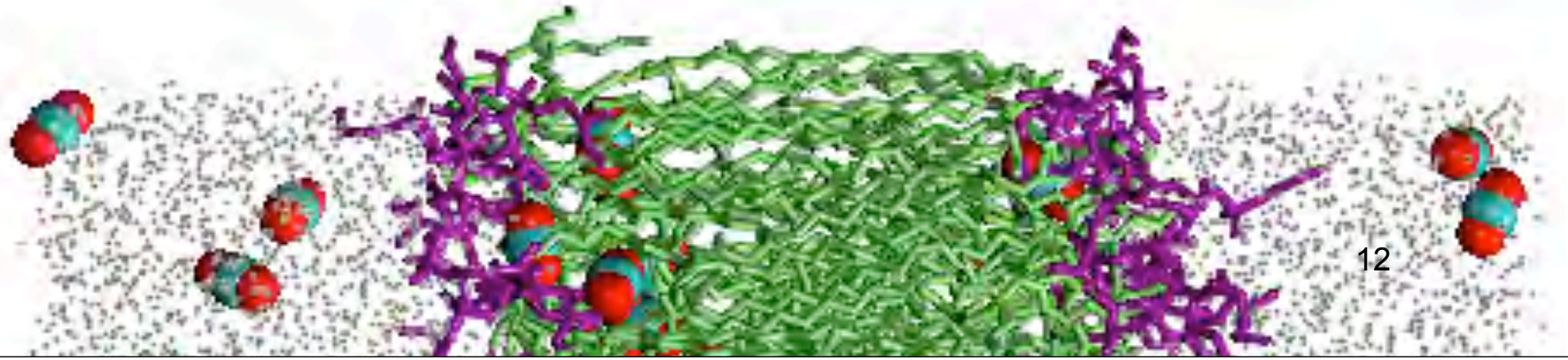
- Liposome: 3.9



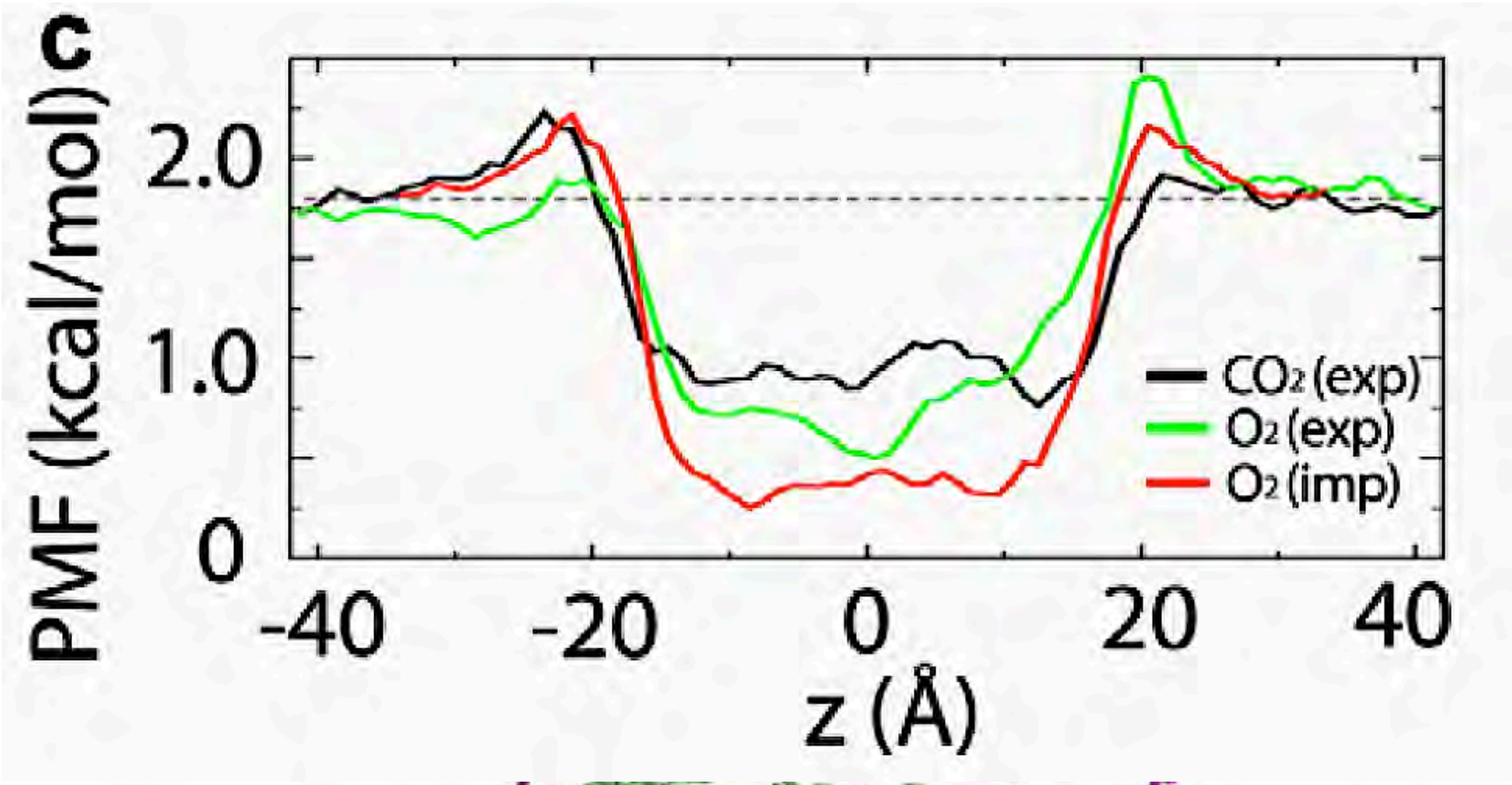


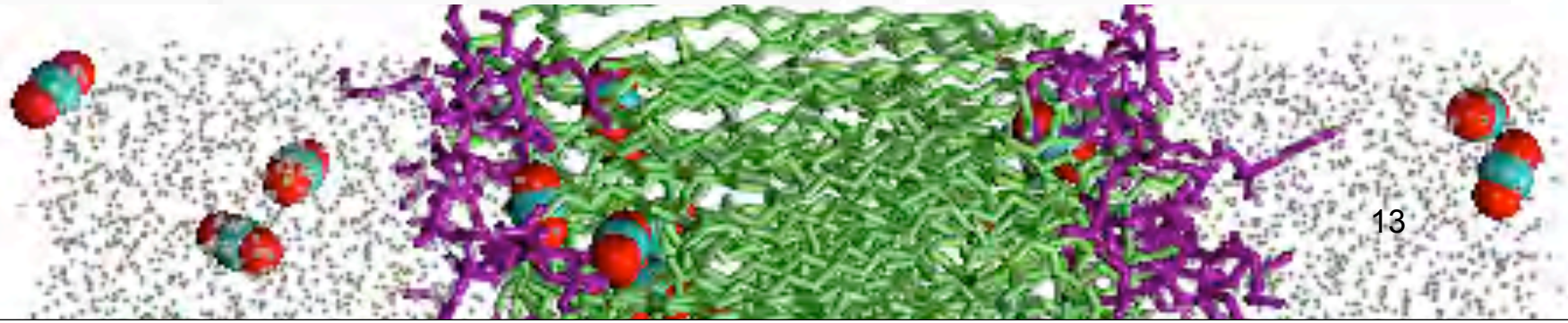
Gas Diffusion Inside the Lipid Bilayer



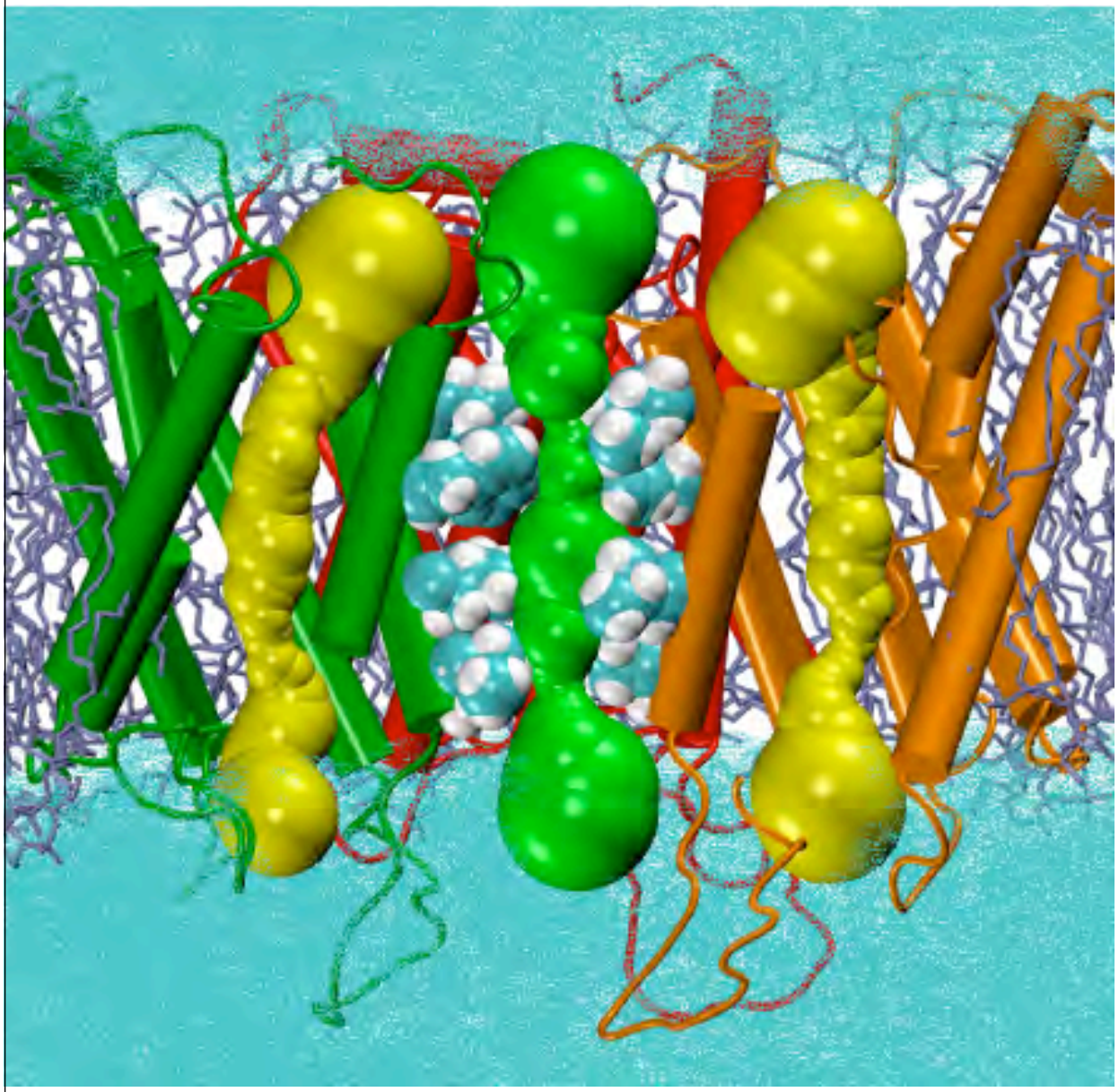


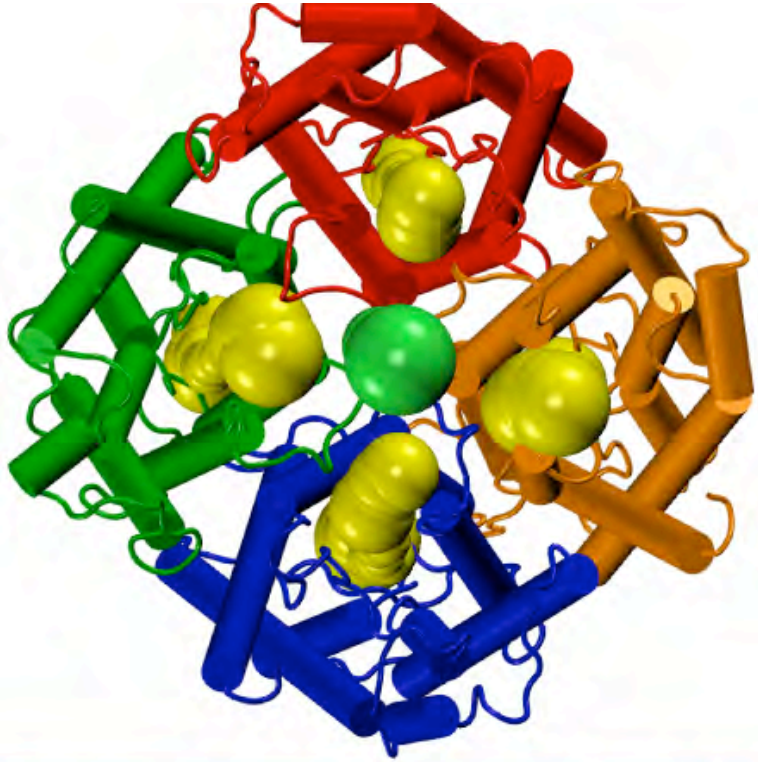
Gas Diffusion Inside the Lipid Bilayer





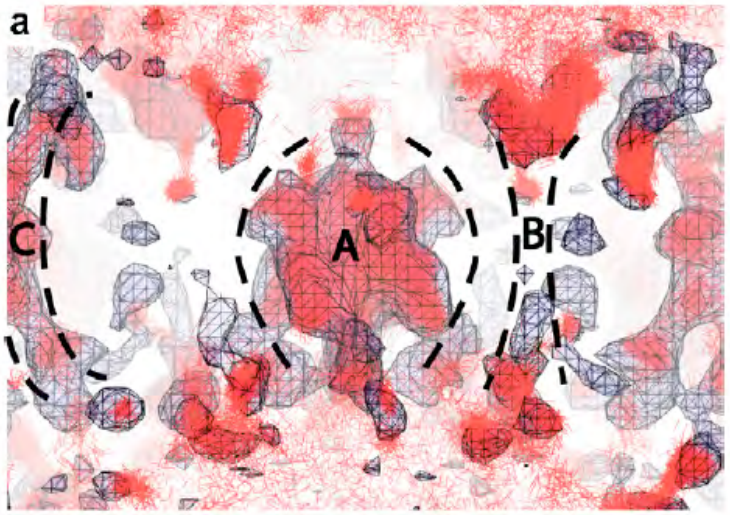
Aquporin **Water**/**Gas** Channels



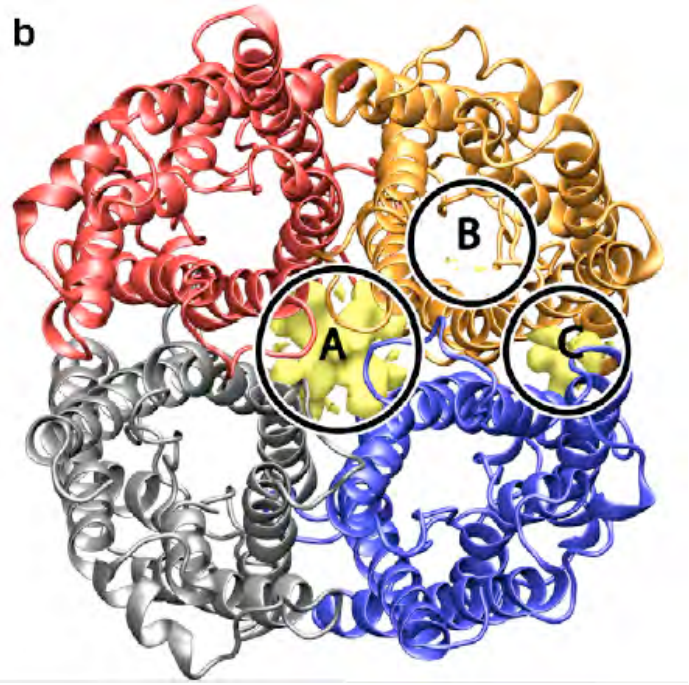


Why Tetramers?

Implicit Ligand Sampling



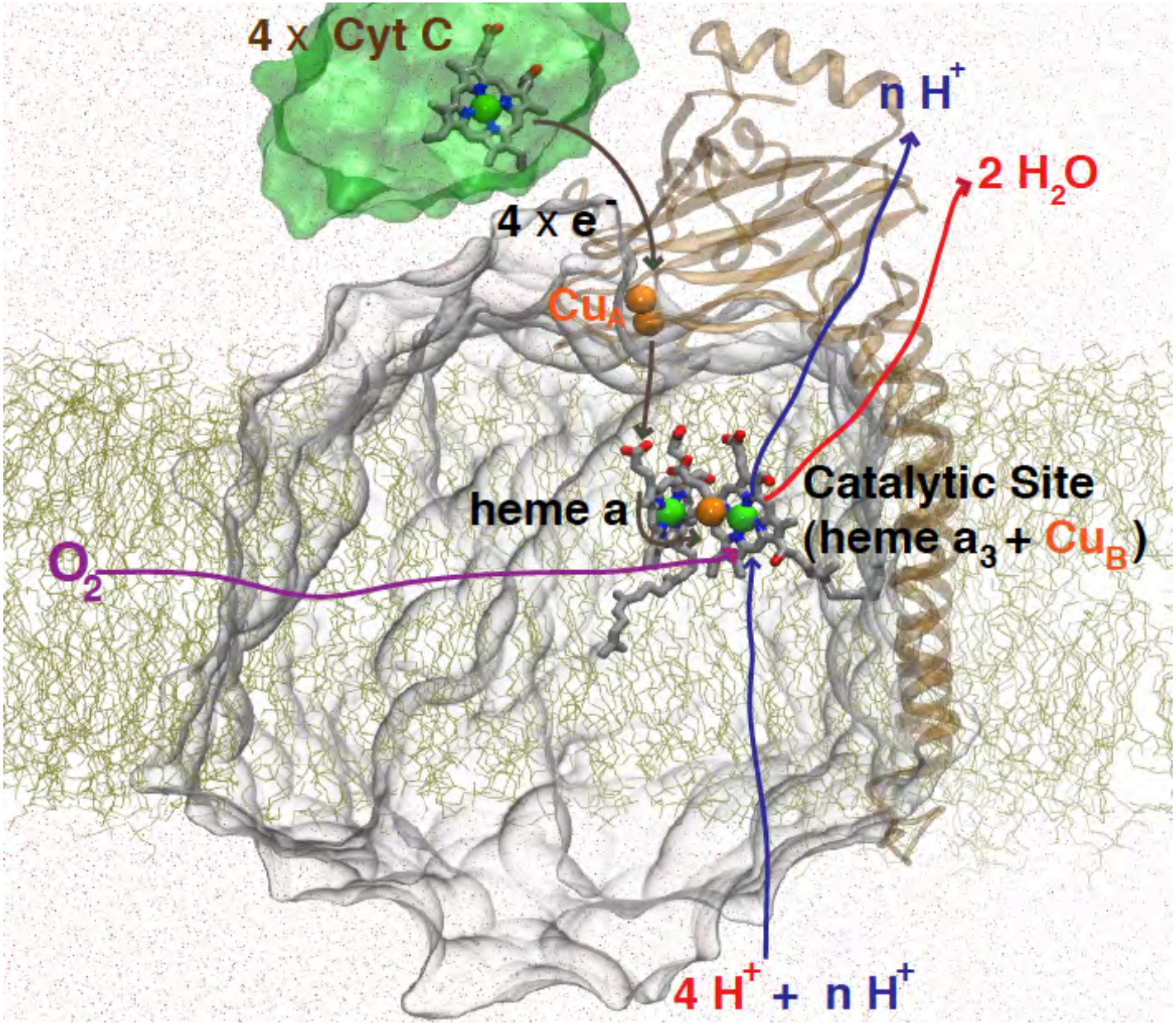
$$\mathcal{W}(\mathbf{r}) = -k_{\mathrm{B}}T\ln\left[\frac{\rho(\mathbf{r})}{\rho_{\mathrm{o}}}\right]$$



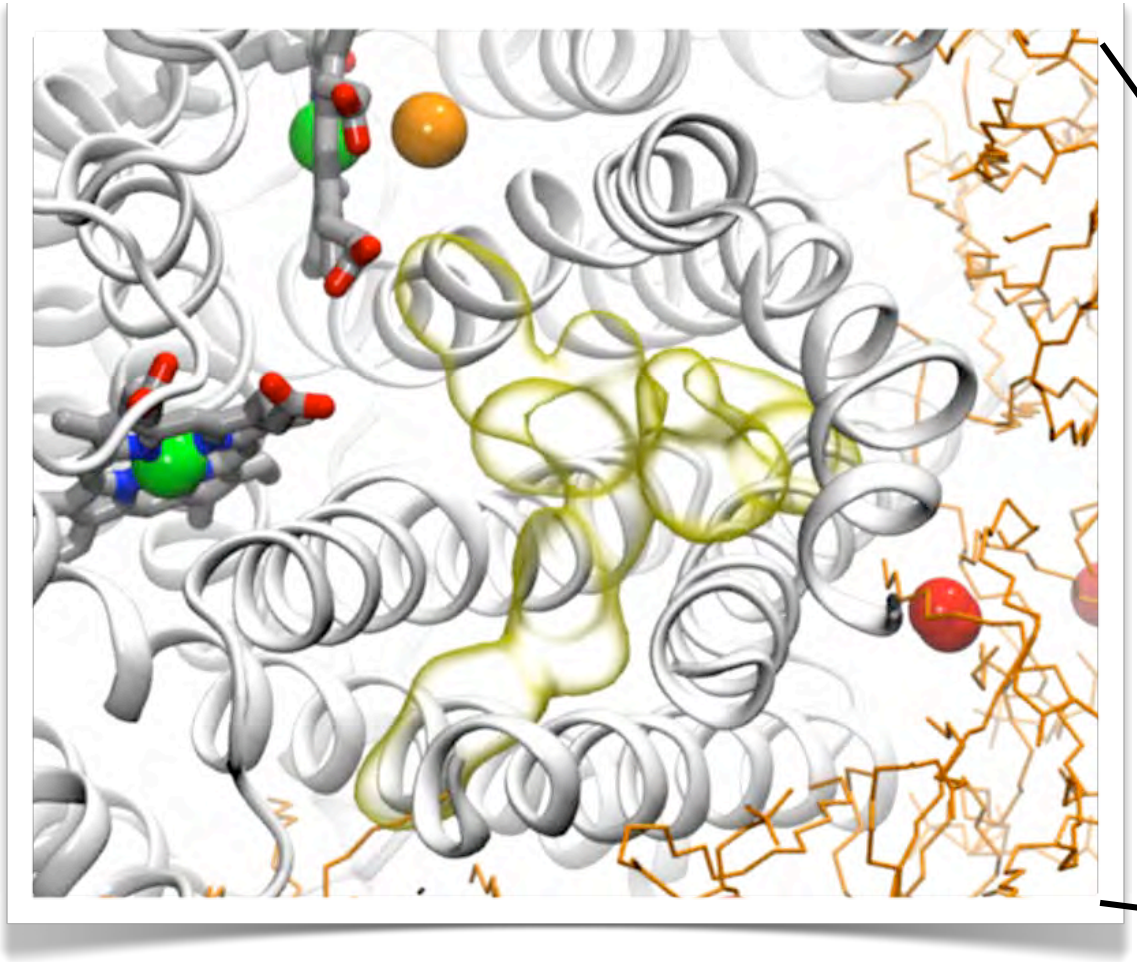
$$F(z) = -RT\ln\sum_{x,y=0}^{L_x,L_y}\frac{\mathrm{e}^{-F(x,y,z)/RT}}{L_xL_y}$$

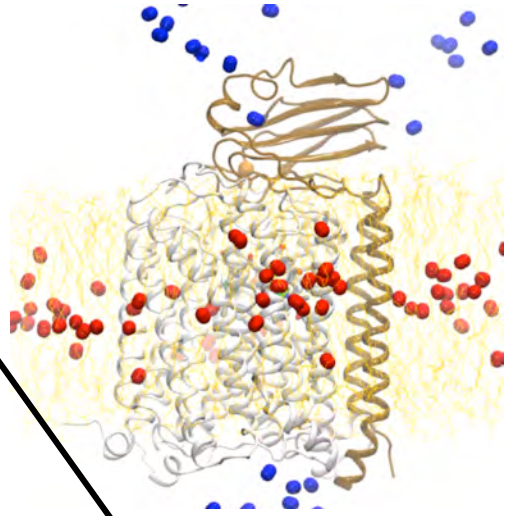
Cohen, et al., 2006; Wang, et al., 2007

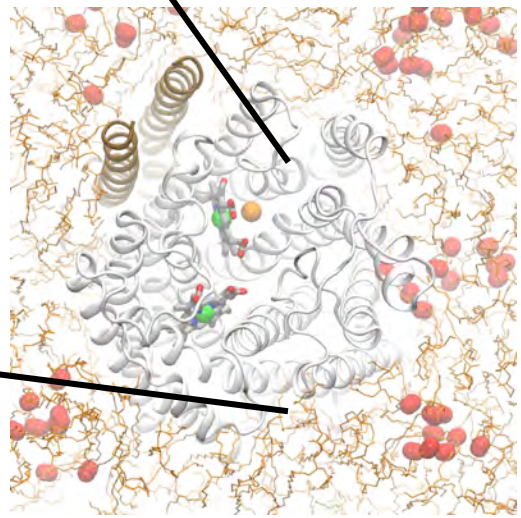
Oxido-reductase and Proton Pump



Rapid O_2 Permeation via the Hydrophobic Channel in Cytochrome C Oxidase

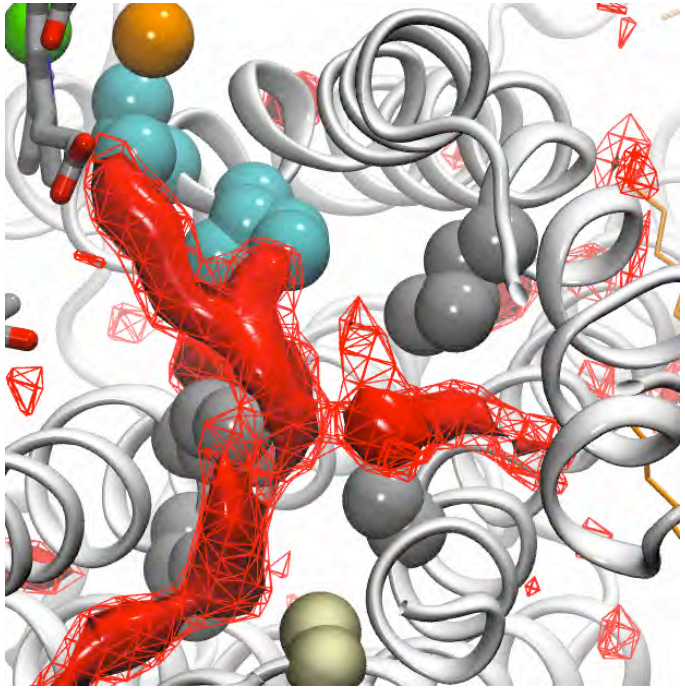




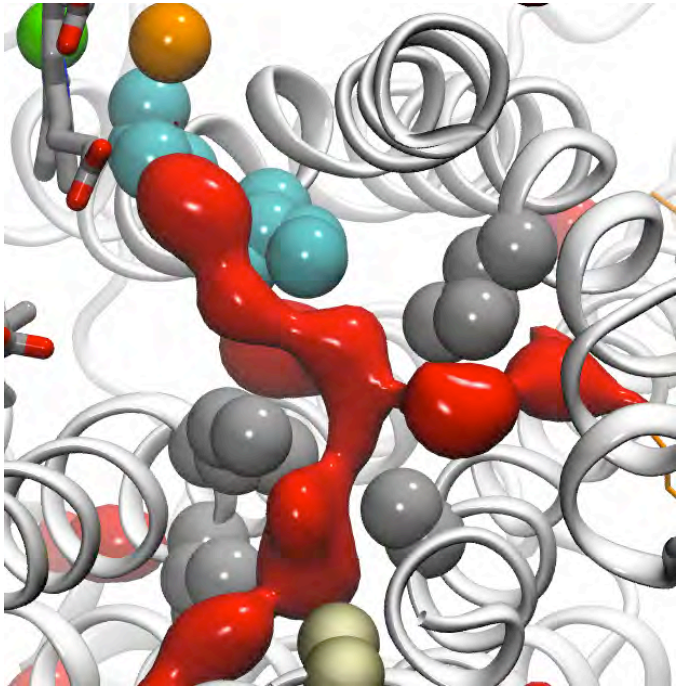


O_2 Pathway in Cytochrome C Oxidase

Implicit ligand sampling

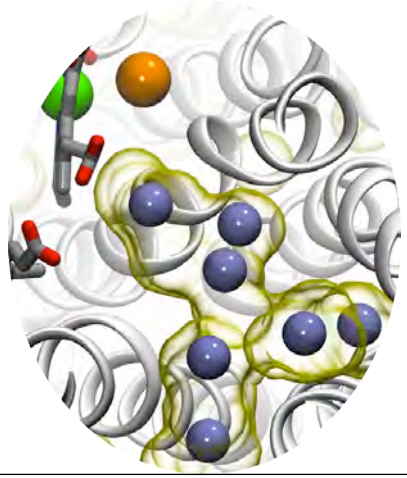


Explicit O_2 simulation



Reddish solid: ΔΔG map of ~ -3.5 kcal/mol ; Reddish wireframe: ΔΔG map of ~ -3.0 kcal/mol

Observed Xenon binding in CcO ba_3 crystal structures

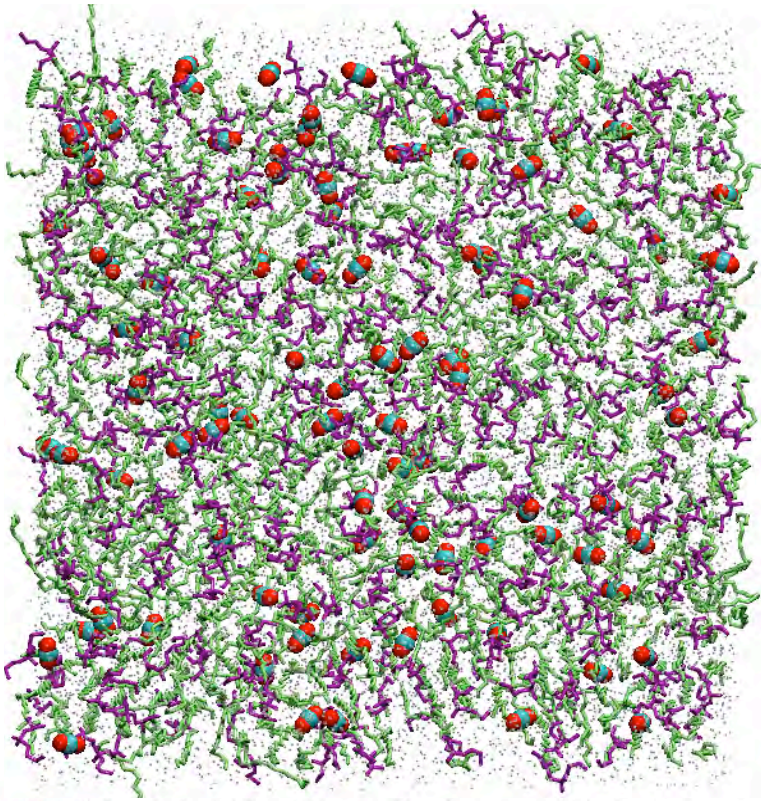


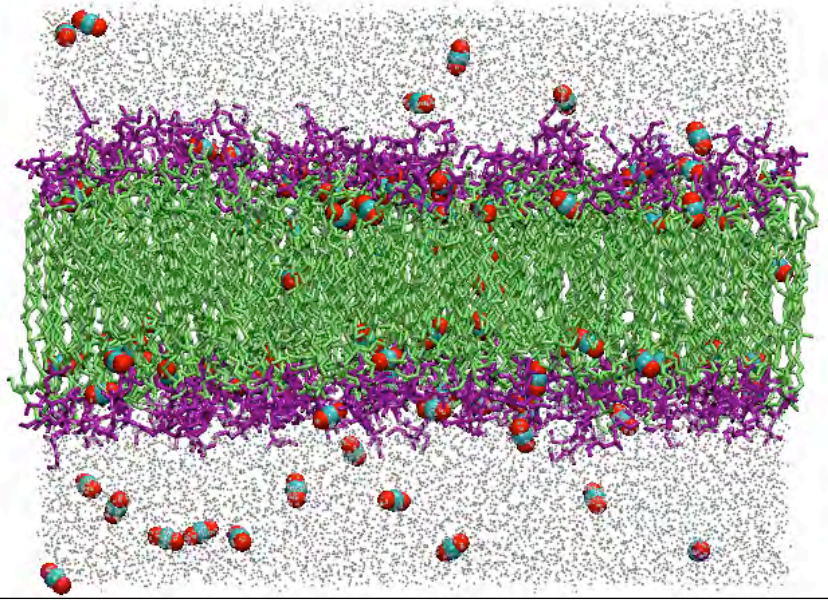
All located along the hydrophobic channel

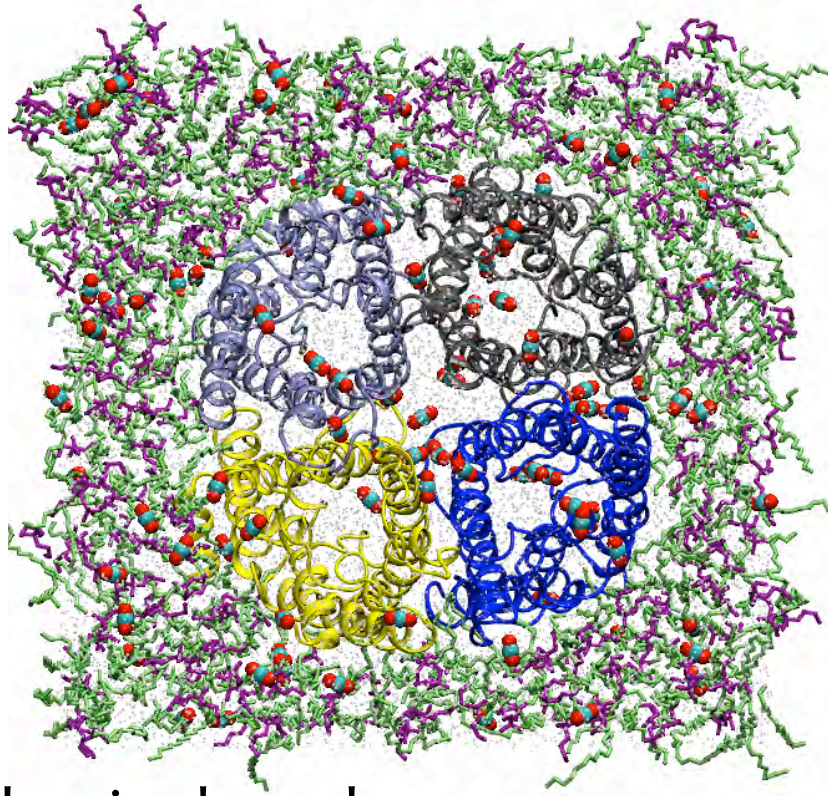
Luna VM, Chen Y., Fee JA and Stout CD (2008)
Biochemistry, 47, 4657-4665 (PDB entry 3BVD)

Luna VM, Fee JA, Deniz AA and Stout CD (2012)
Biochemistry, 51, 4669-4676

Simulating Membrane Gas Transport







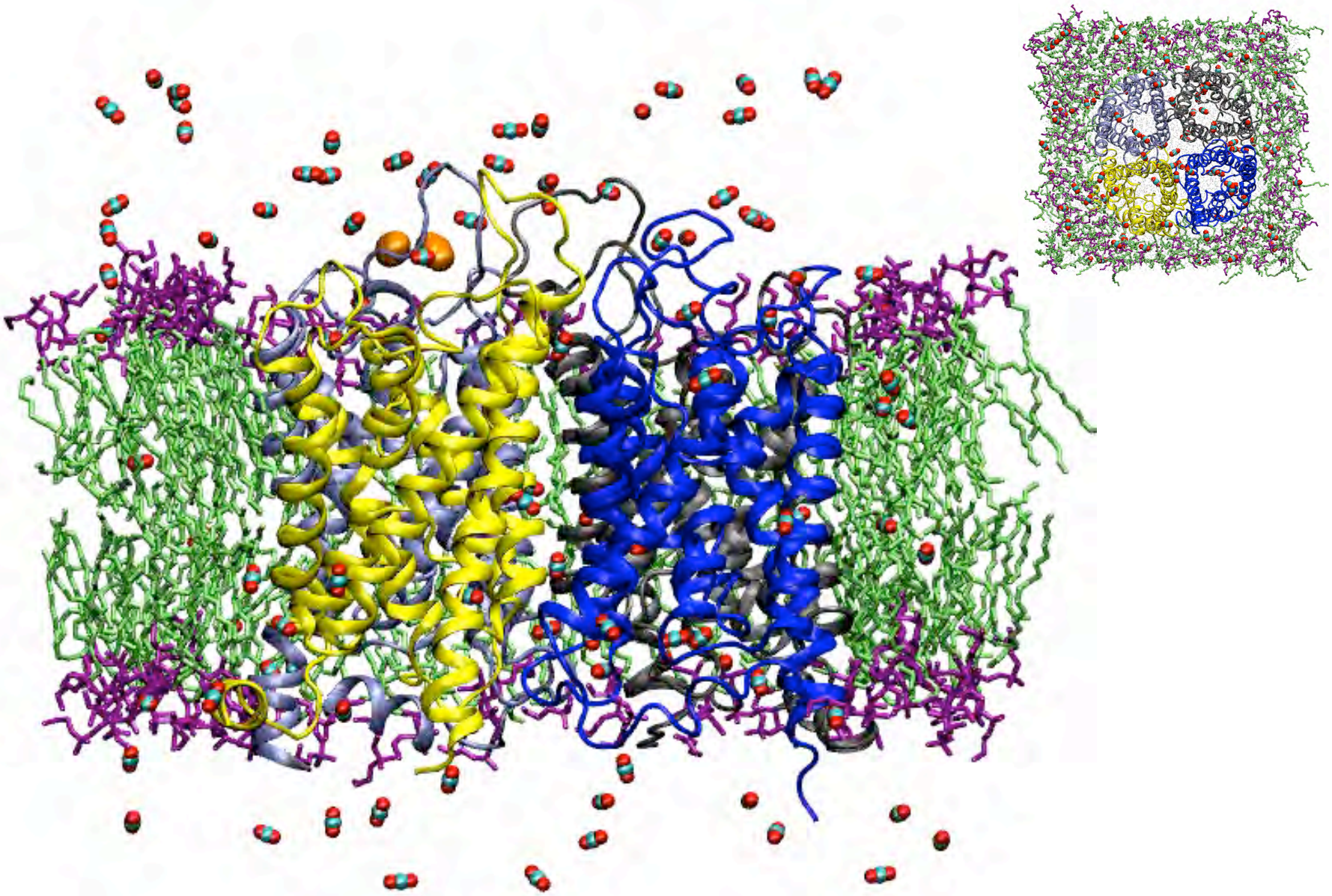
Identical total areas

Calculating permeation rate in MD simulations

AQP1 AQP4

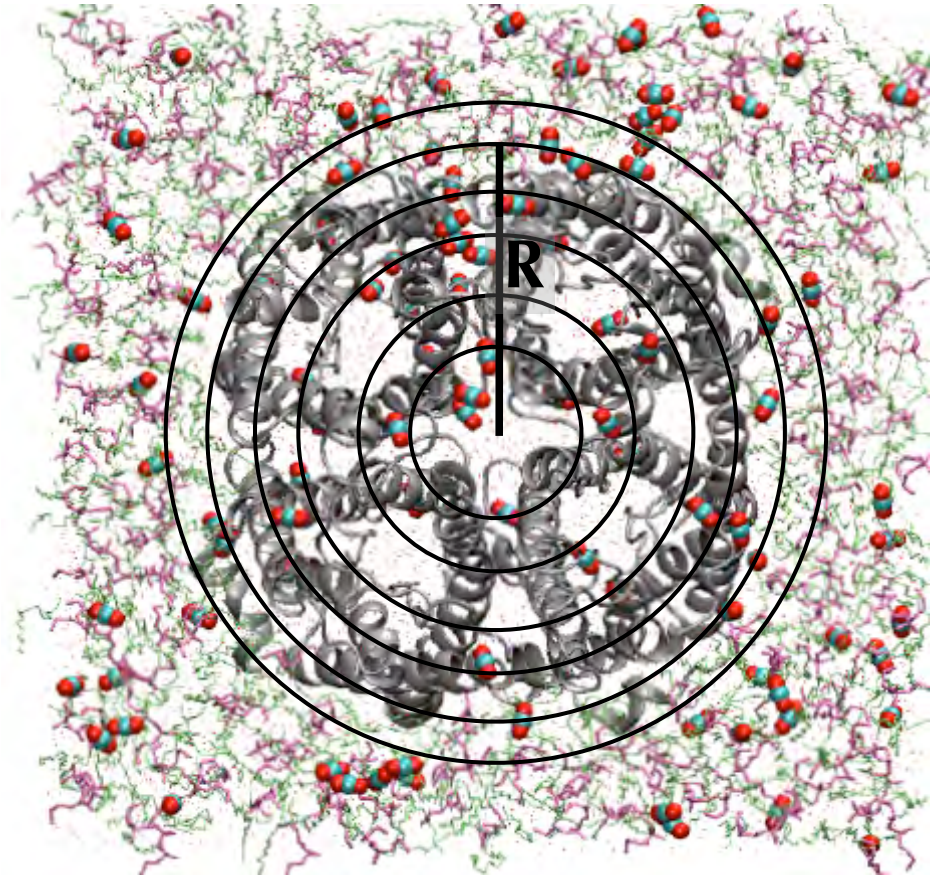
CO_2 O_2 NO

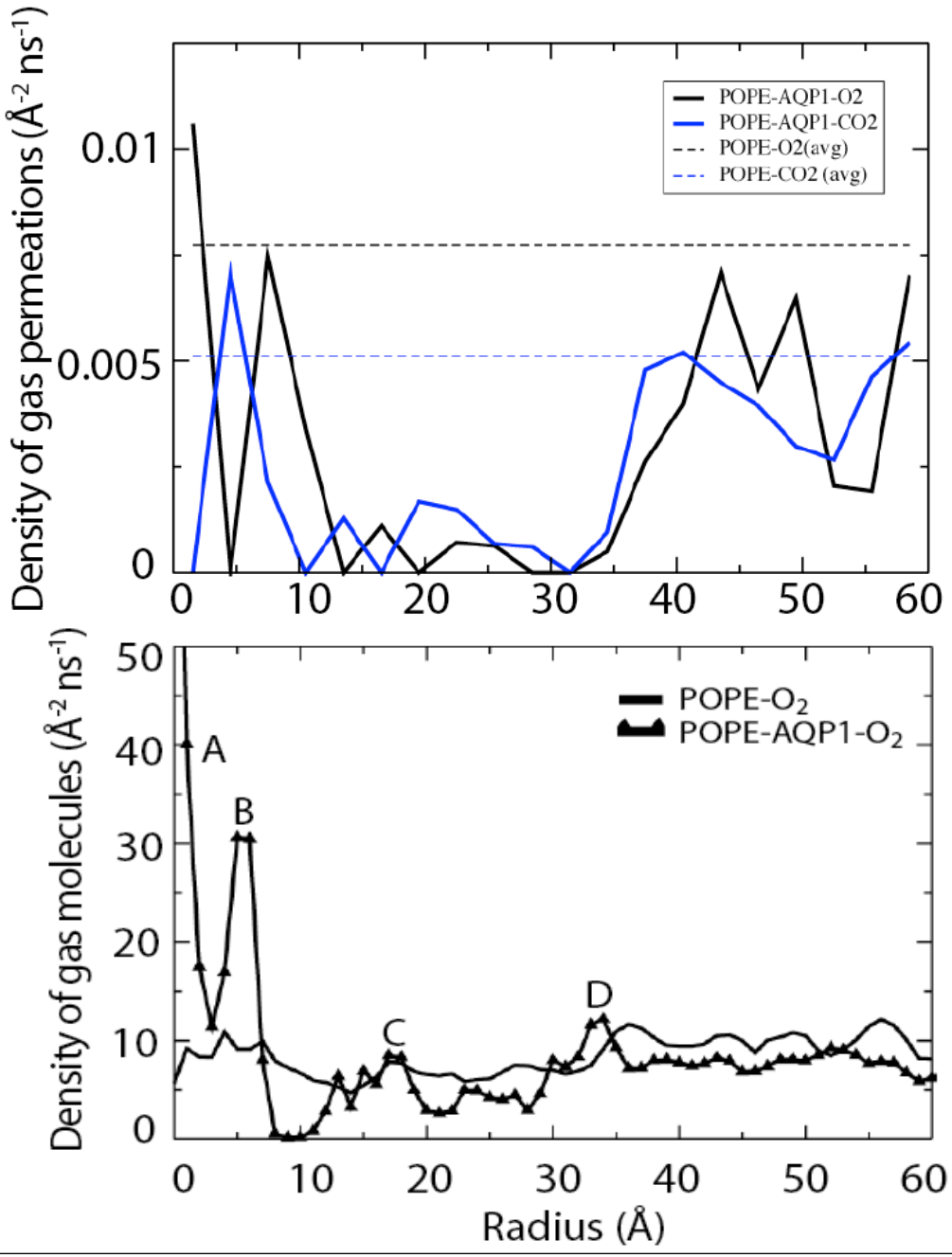
Gas Transport through Aquaporins



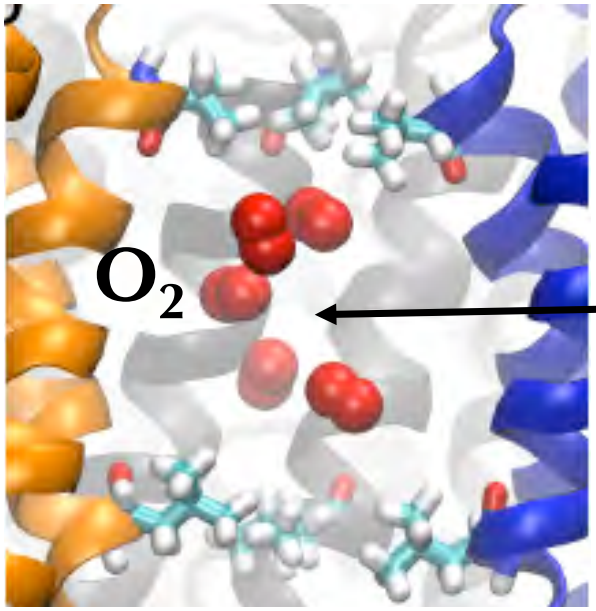
Typical permeation events (300-400 ps)

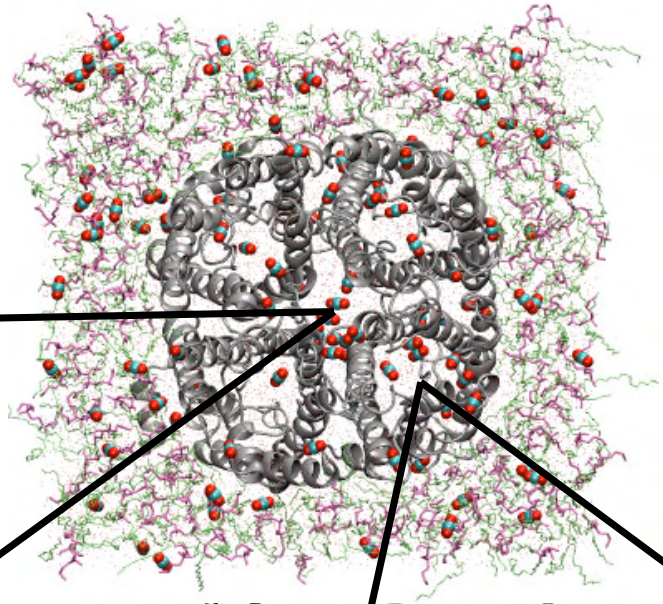
Gas Occupancy/Permeation Radial Distribution



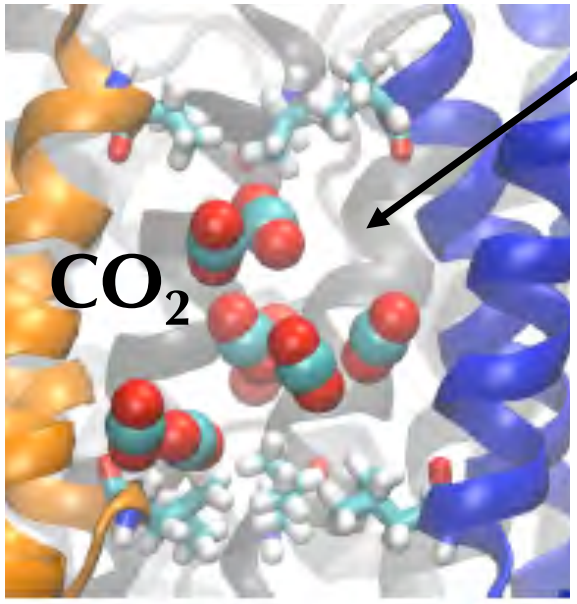


A Role for the Central Pore!

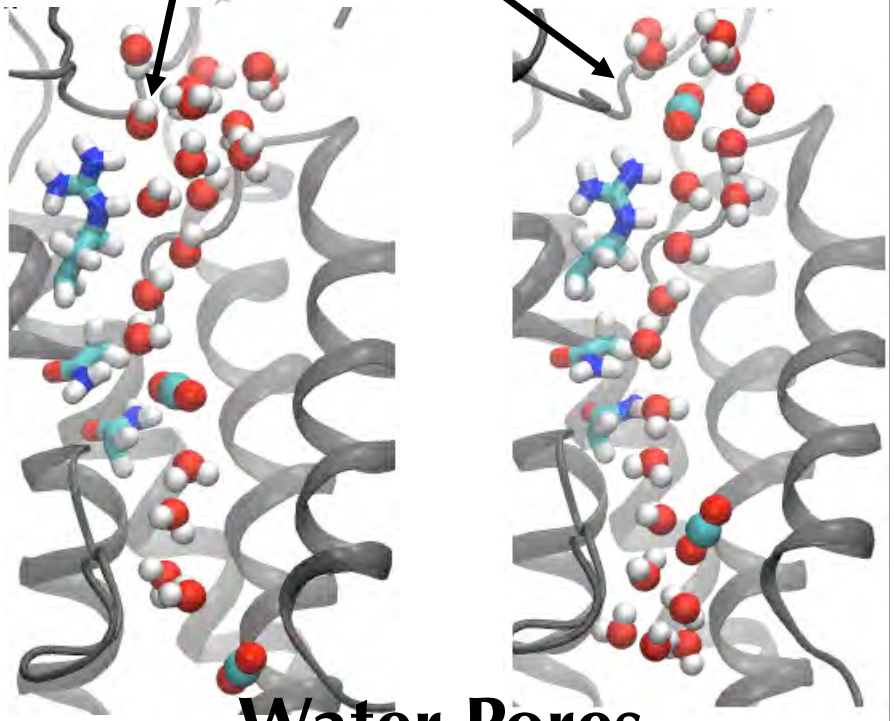




Central Pore



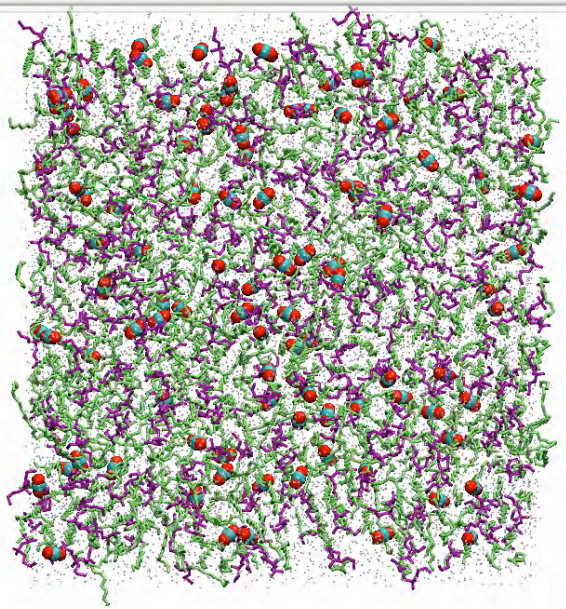
CO_2

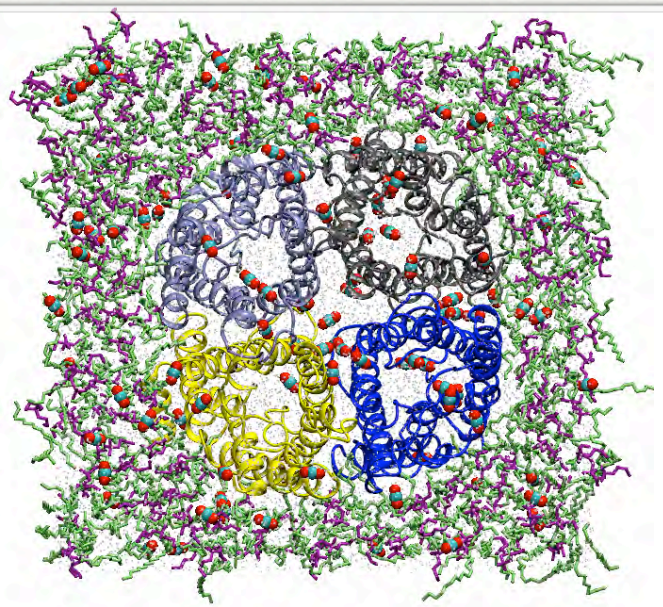


Water Pores

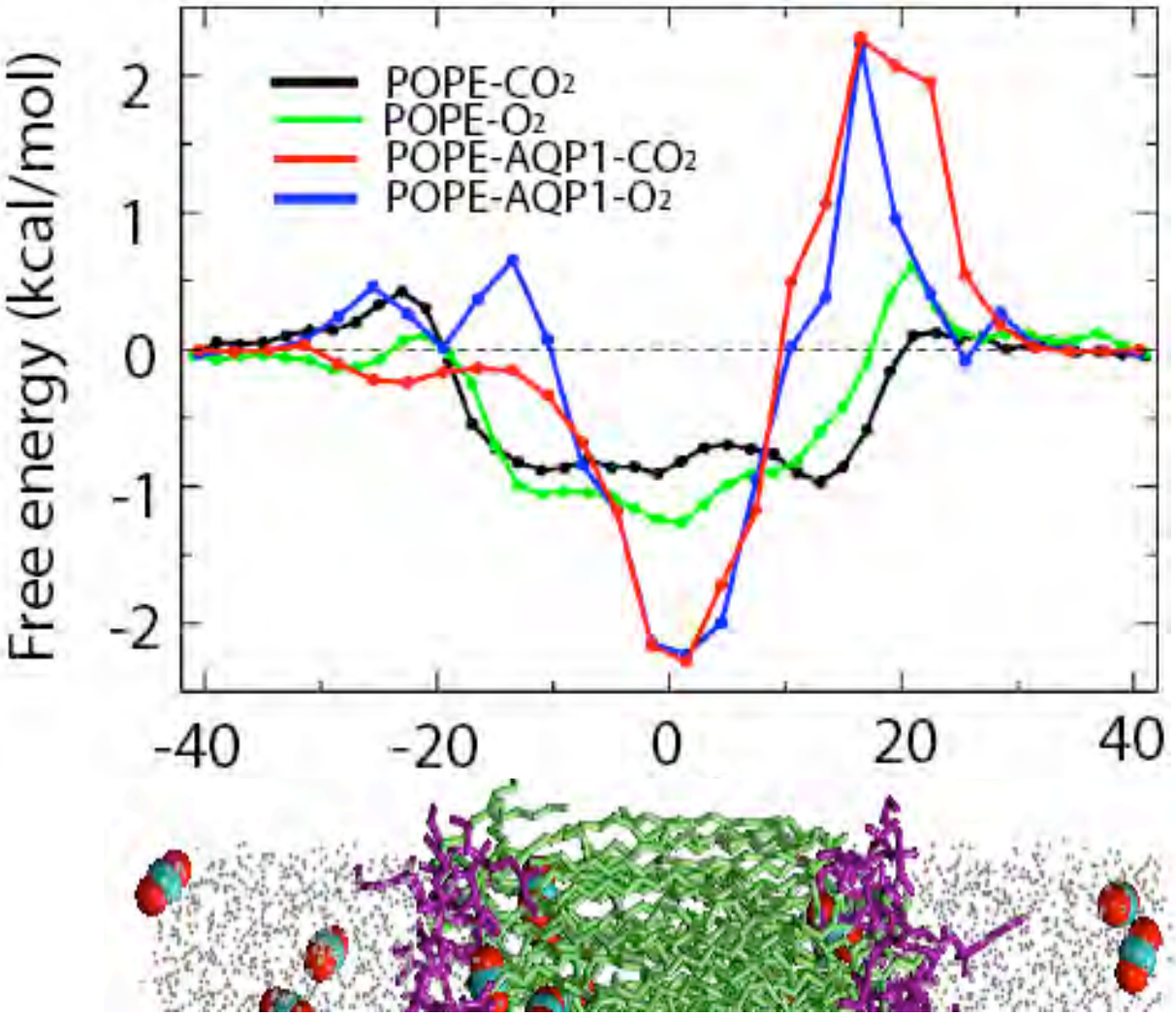
Gas Transport through Aquaporins

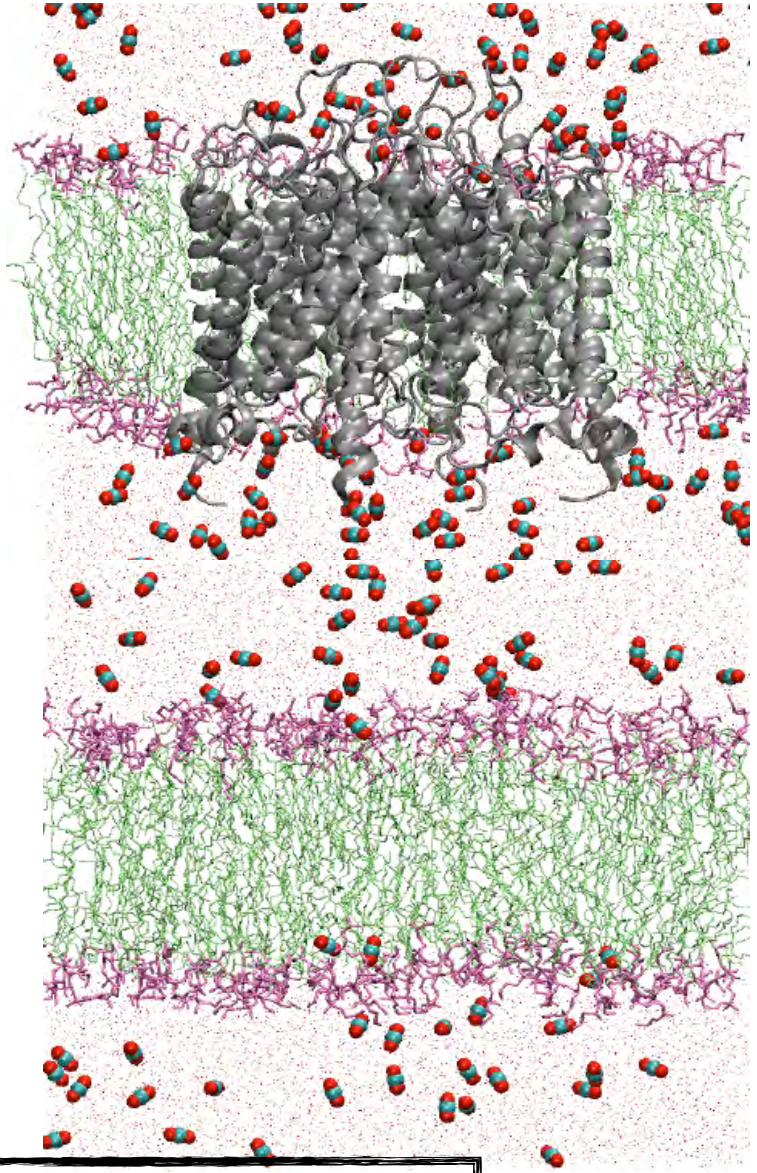
SYSTEM	TOTAL (100x100 A^2)	WATER PORES (4)	CENTRAL PORE (1)
Equi POPE-CO_2	*3*	*N/A*	*N/A*
Equi POPC-CO_2	*5*	*N/A*	*N/A*
Equi POPC-$O_{2(P)}$	*16*	*N/A*	*N/A*
Equi POPE-$O_{2(P)}$	*11*	*N/A*	*N/A*
Press POPE-CO_2	168	N/A	N/A
Press POPC-CO_2	160	N/A	N/A
Press POPE-$O_{2(P)}$	310	N/A	N/A
Press POPC-$O_{2(P)}$	208	N/A	N/A
Press POPE-AQP1-CO_2	**76**	**6**	**4**
Press POPE-AQP1-$O_{2(P)}$	**79**	**1**	**6**





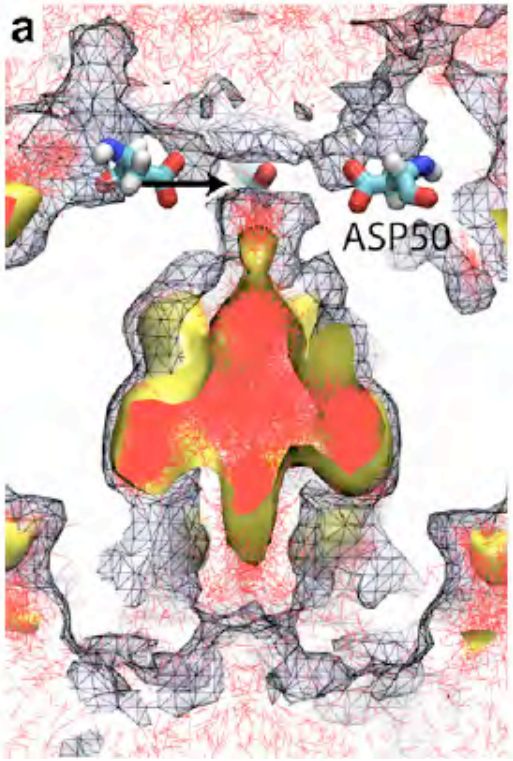
Free Energy Profiles for O_2 and CO_2

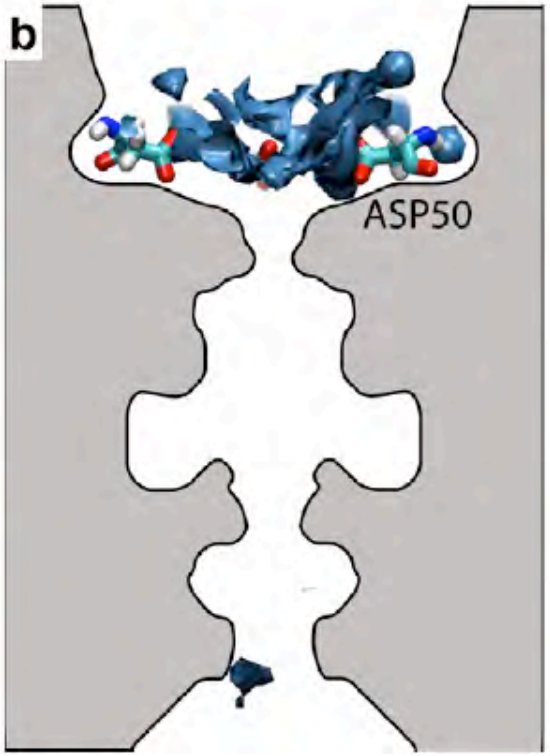


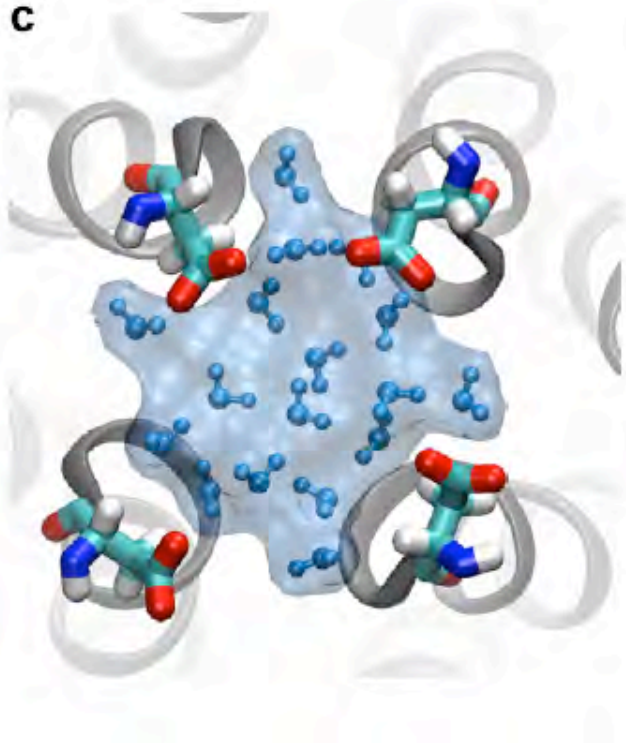


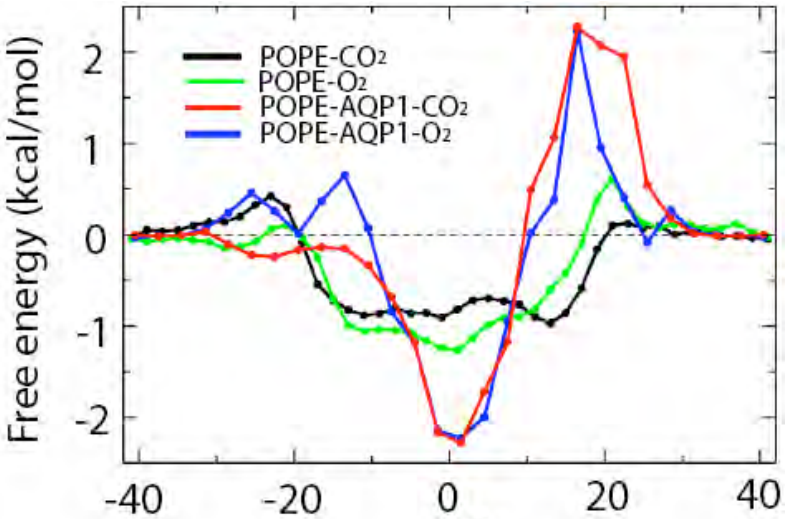
Y. Wang, J. Cohen, W. Boron, K. Schulten, and E. Tajkhorshid, ***J. Struct. Biol.,*** 2007.
Y. Wang, S. Shaikh, and E. Tajkhorshid, **Physiology,** 2010.

Major Barrier Generated by Structured Water





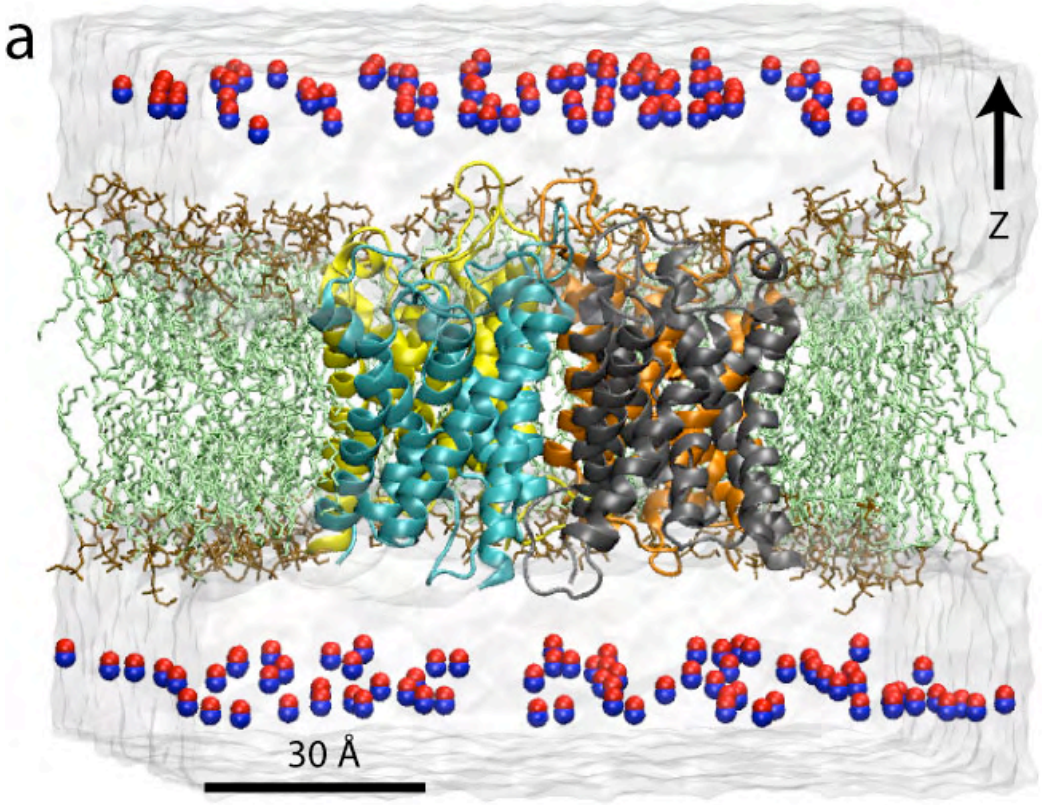


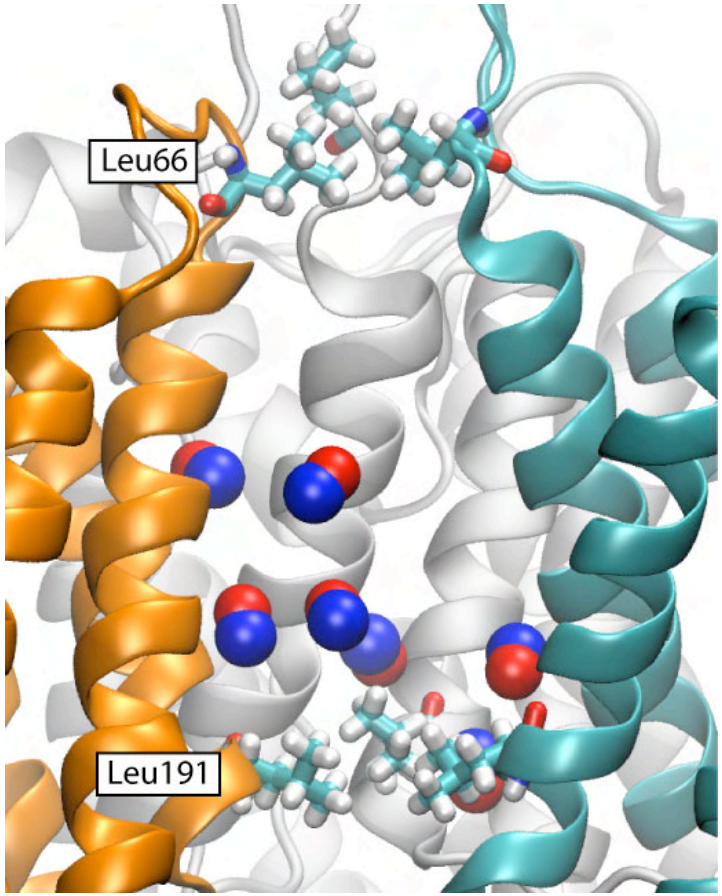


Barrier identified and characterized through combining the implicit and explicit approaches

Y. Wang, J. Cohen, W. Boron, K. Schulten, and E. Tajkhorshid, ***J. Struct. Biol.,*** 2007.
Y. Wang, S. Shaikh, and E. Tajkhorshid, **Physiology,** 2010.

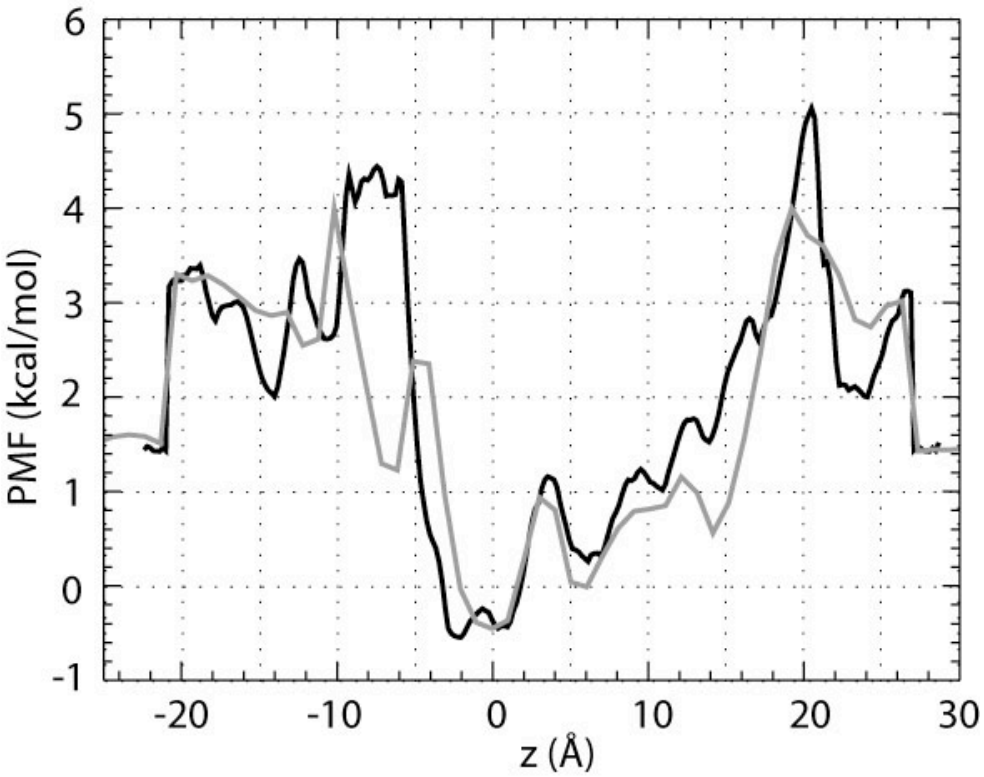
$NO^{\bullet}$ Permeation Through AQP4





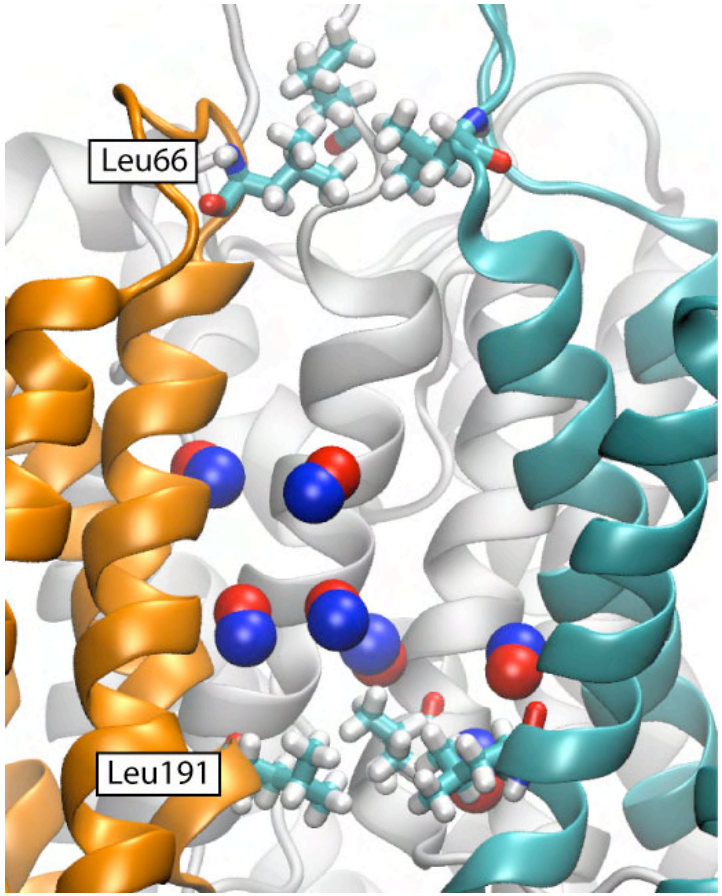
Y. Wang, and E. Tajkhorshid, ***Proteins,*** 2010.

$NO^{\bullet}$ Permeation Through AQP4



--- Umbrella sampling

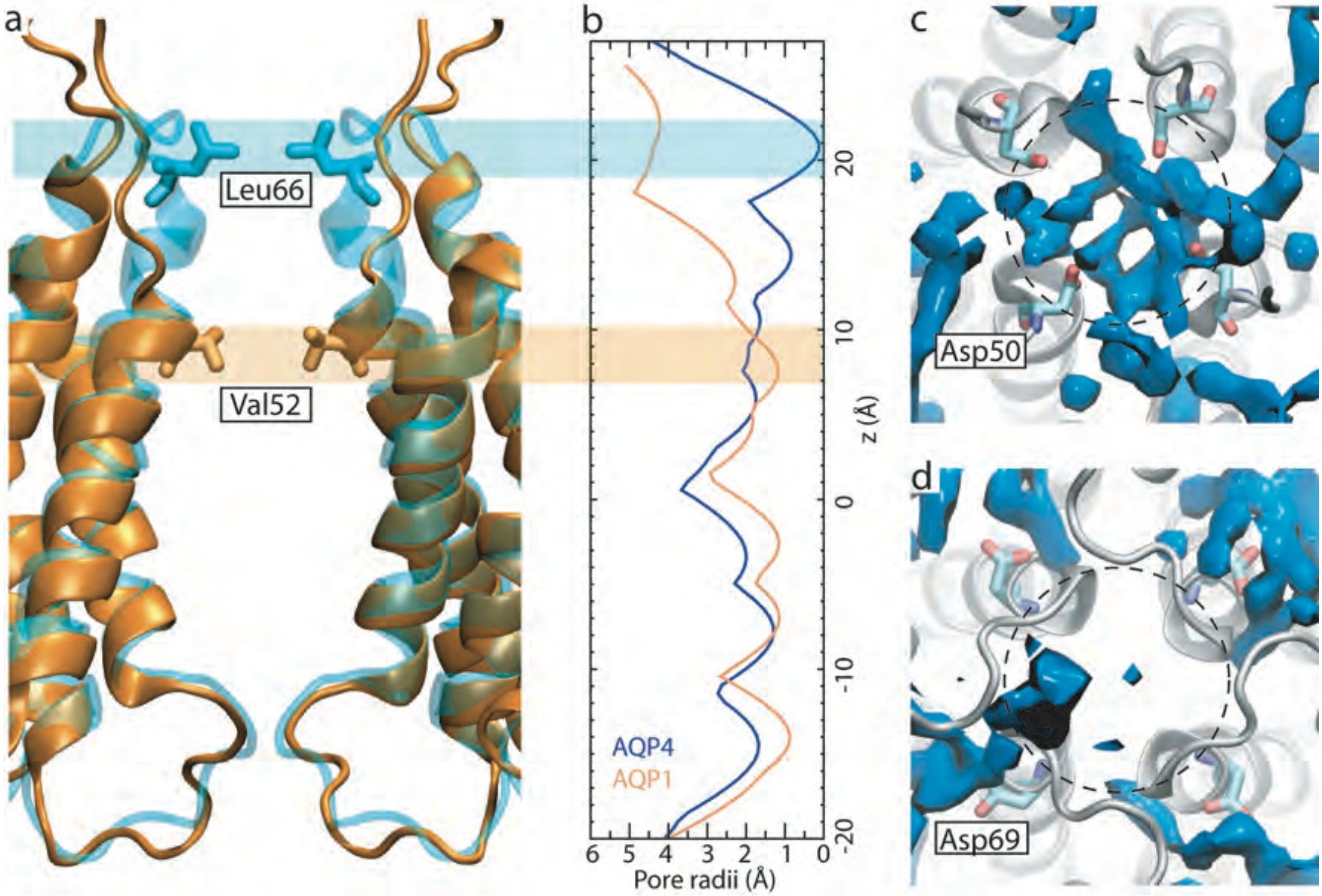
--- Implicit sampling



50 ns equilibrium simulation

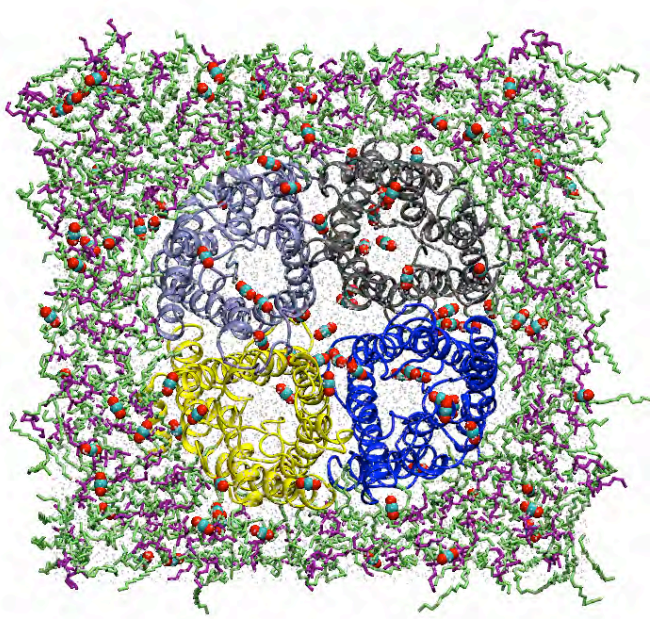
Y. Wang, and E. Tajkhorshid, ***Proteins,*** 2010.

Comparison of the Central Pore in AQP1 and AQP4



Gas Transport through Aquaporins

✦ Computational evidence for gas transport through a membrane channel



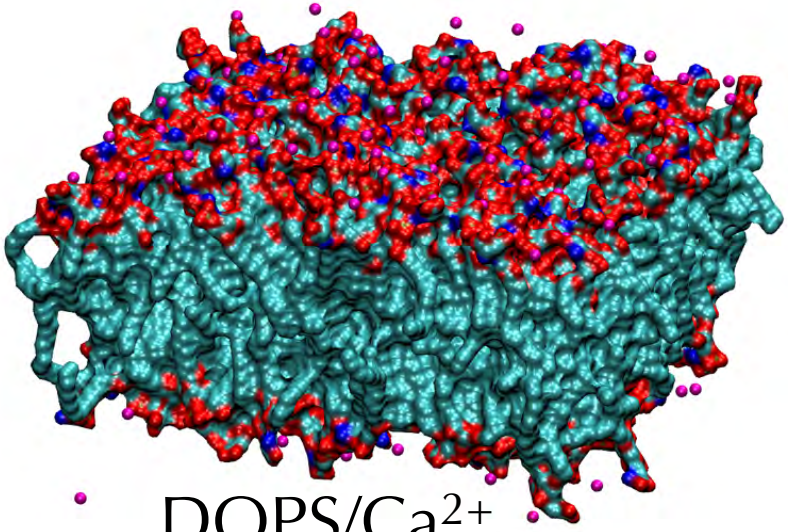
✦ Central Pore in AQPs is an optimal pathway for gas diffusion

Shared by other oligomeric membrane proteins?

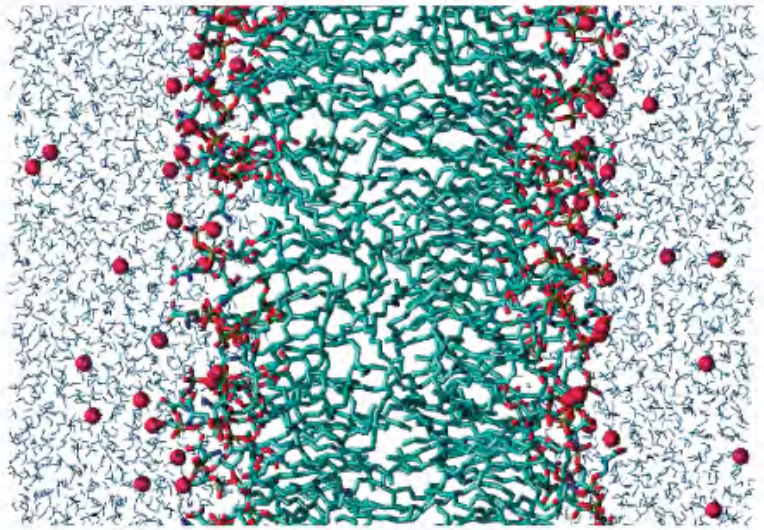
✦ AQPs can be physiologically relevant gas channels in lipid bilayer with low gas permeability

✦ We can simulate very efficiently the process of gas diffusion, but we rely heavily on reliable initial configurations of lipids/ protein

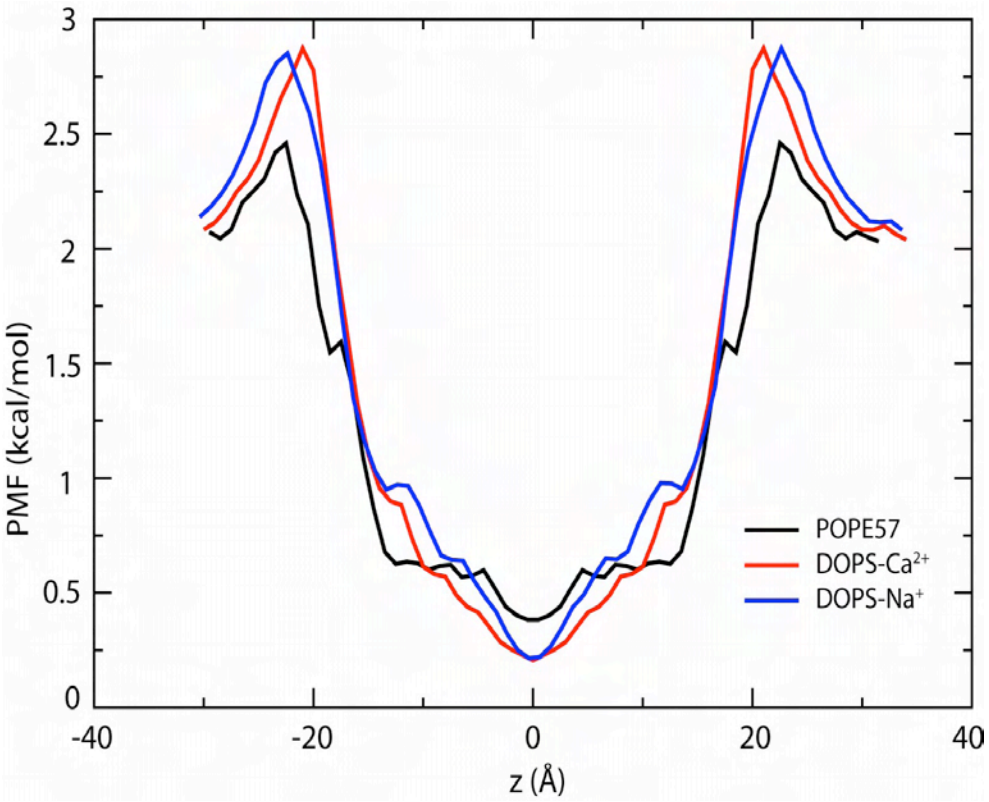
Free Energy of O_2 Permeation Across Charged Lipid Bilayers



DOPS/Ca^{2+}



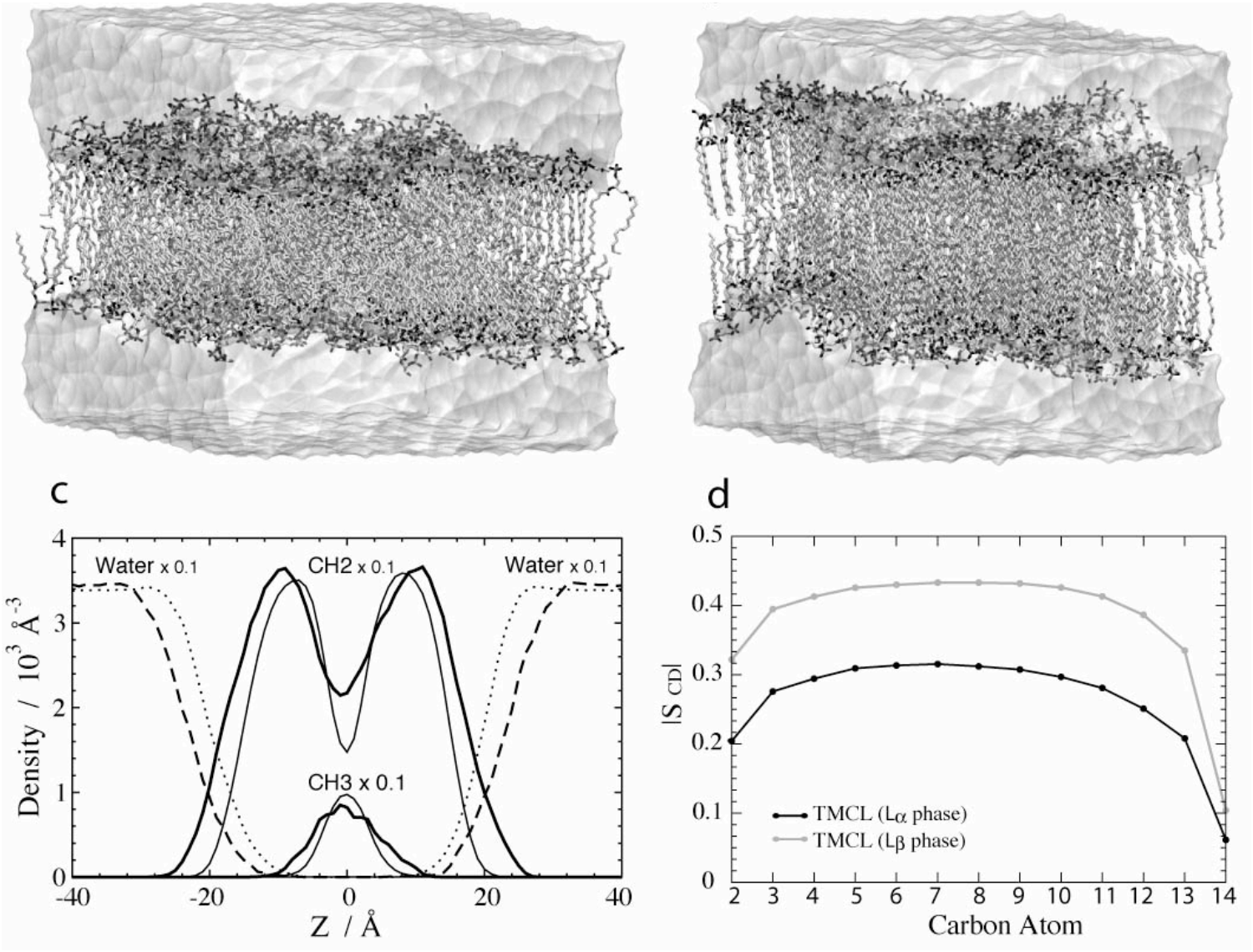
DOPS / Na^+



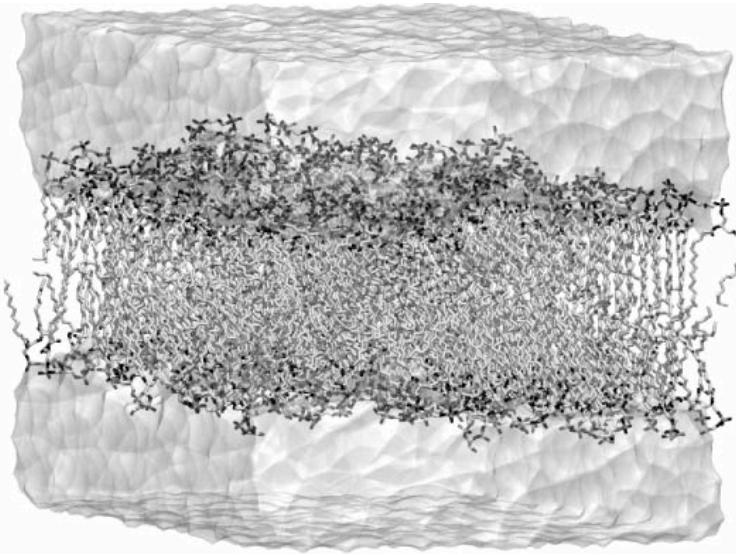
Lipid Phase and Gas Permeation

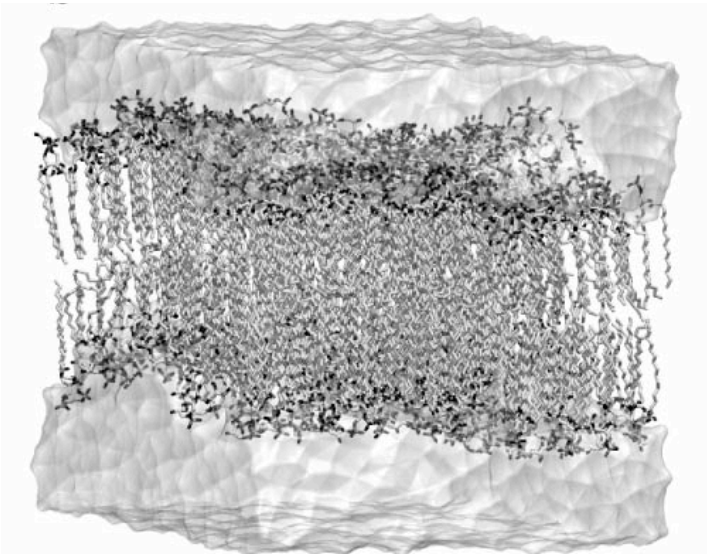
Liquid phase (30 ns)

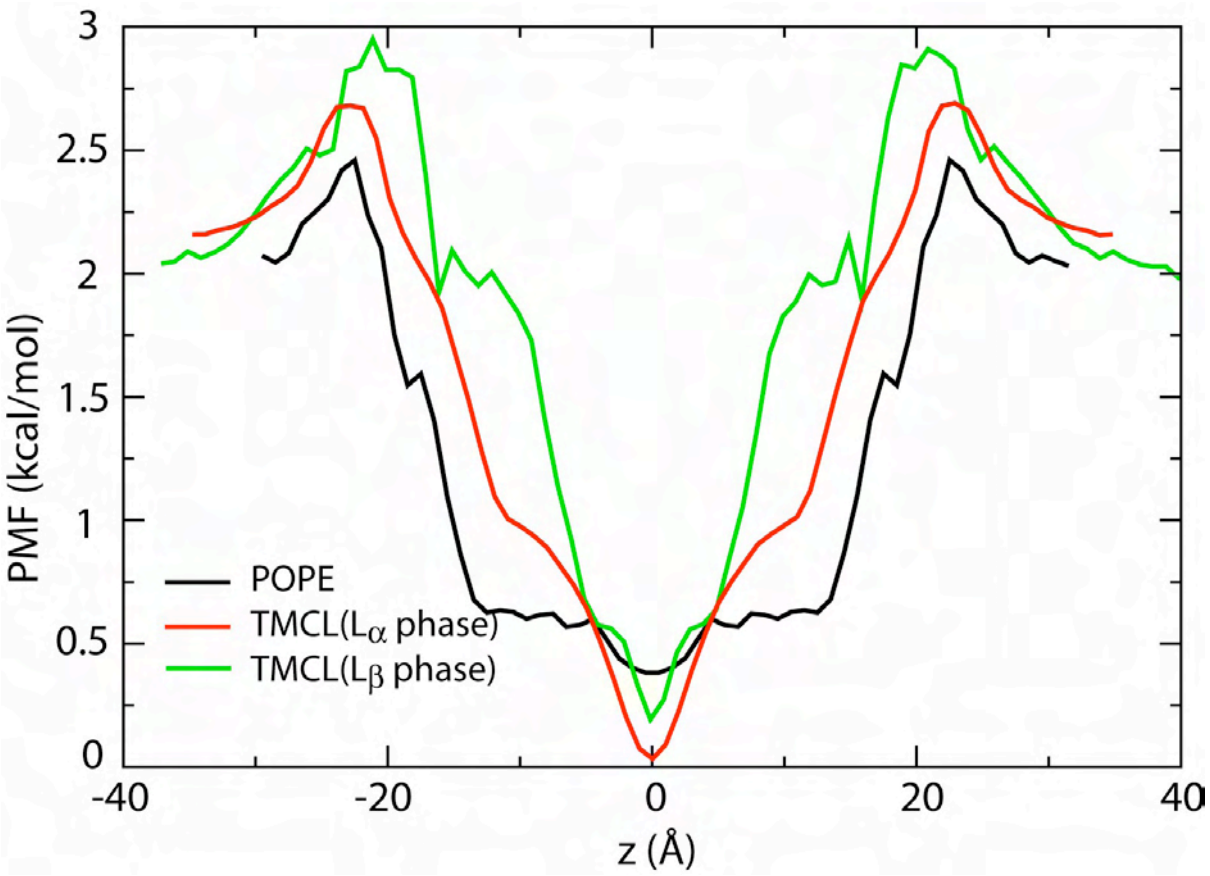
Gel phase (30 ns)



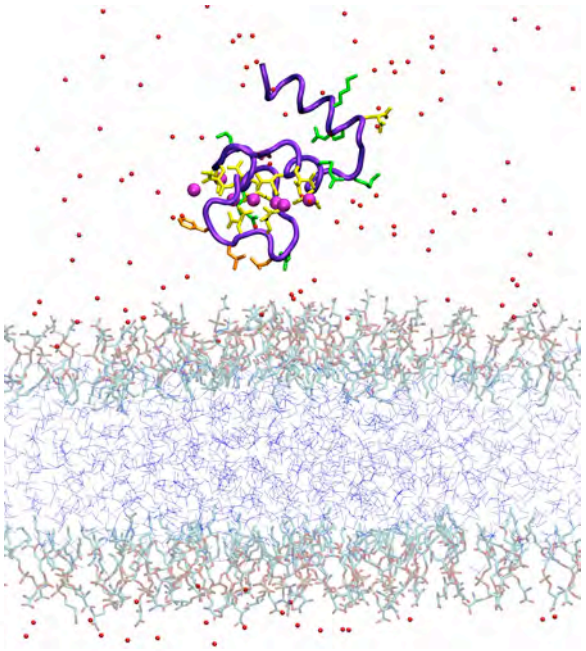
Lipid Phase and Gas Permeation

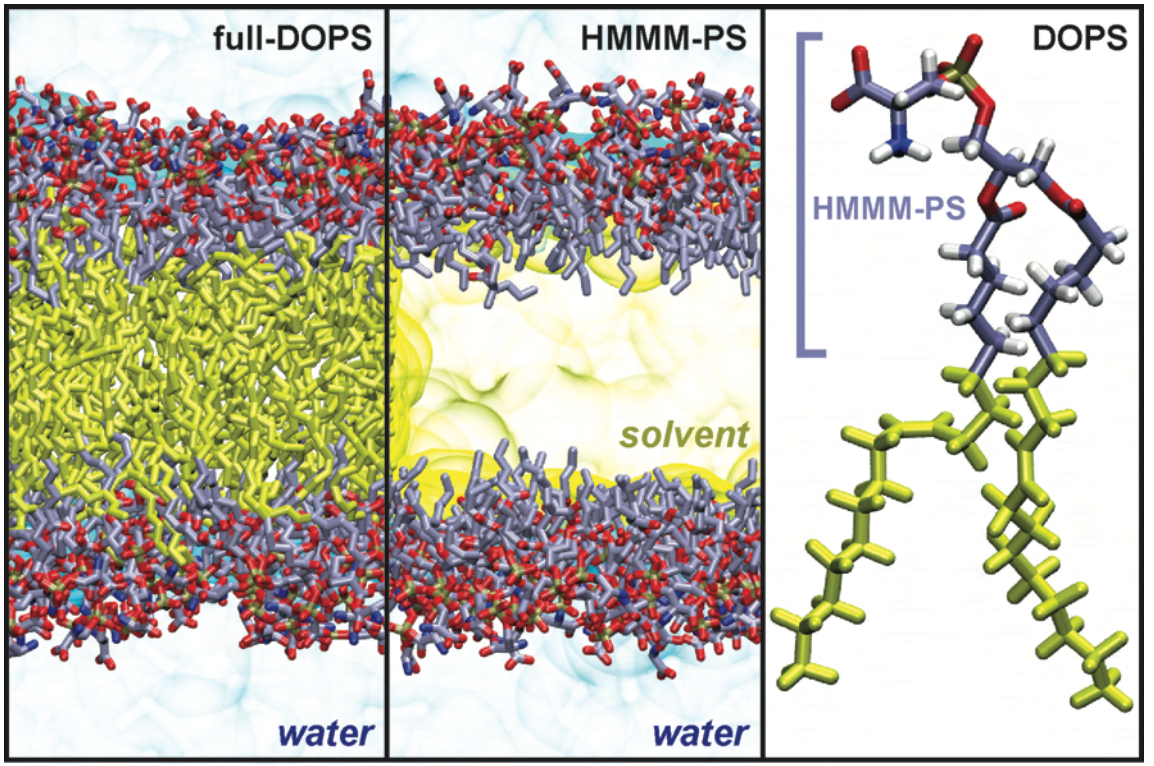


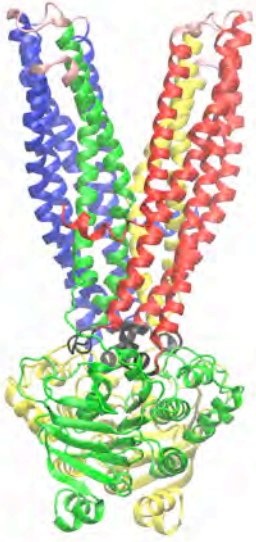


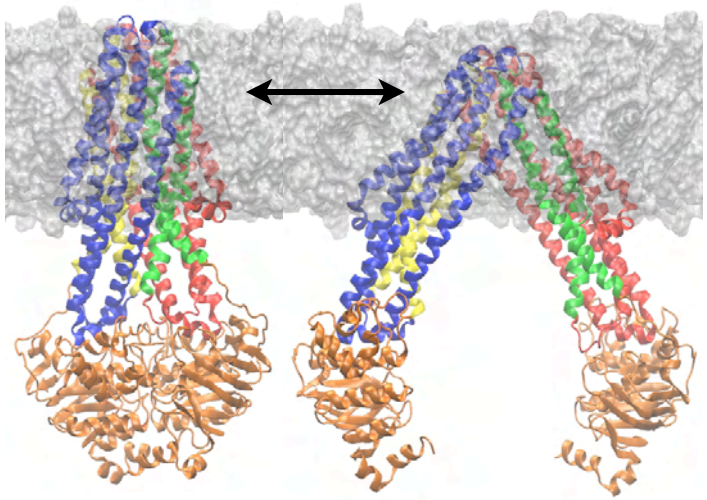


Highly **M**obile **M**embrane **M**imetic (**HMMM**) Model for Membrane Proteins and Phenomena





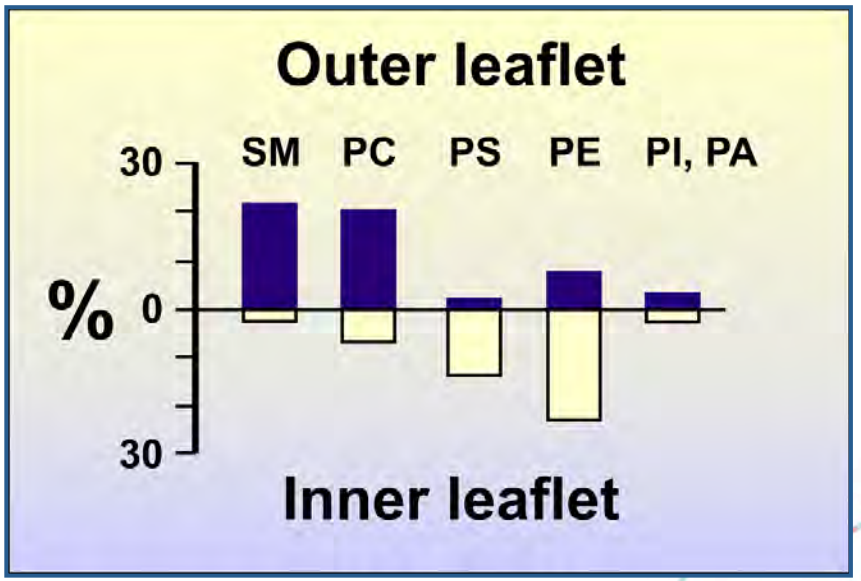


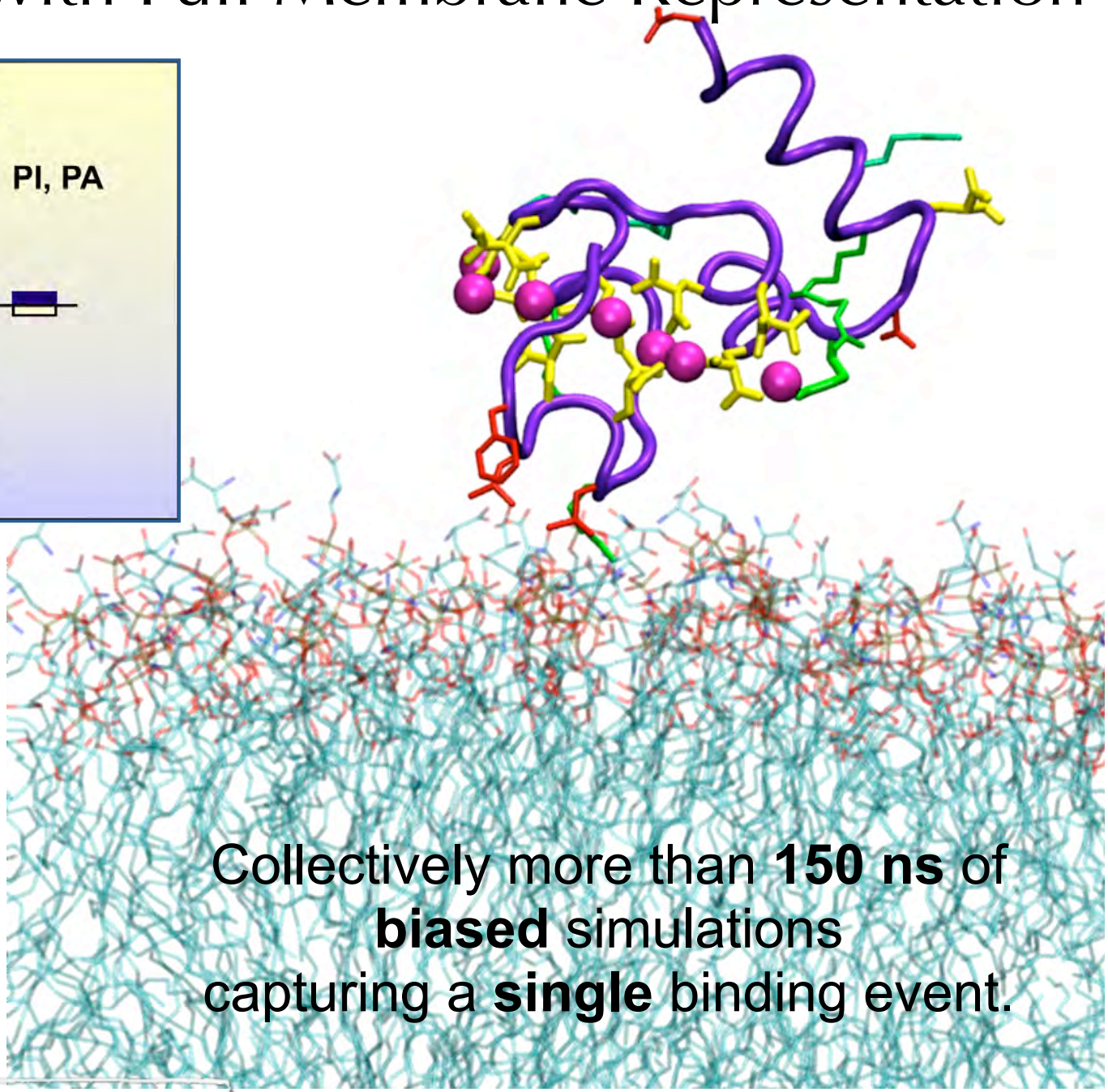




Ohkubo, Pogorelov, Arcario, Christensen, Tajkhorshid, *Biophysical J. May 2012.*

MD Simulation with Full Membrane Representation



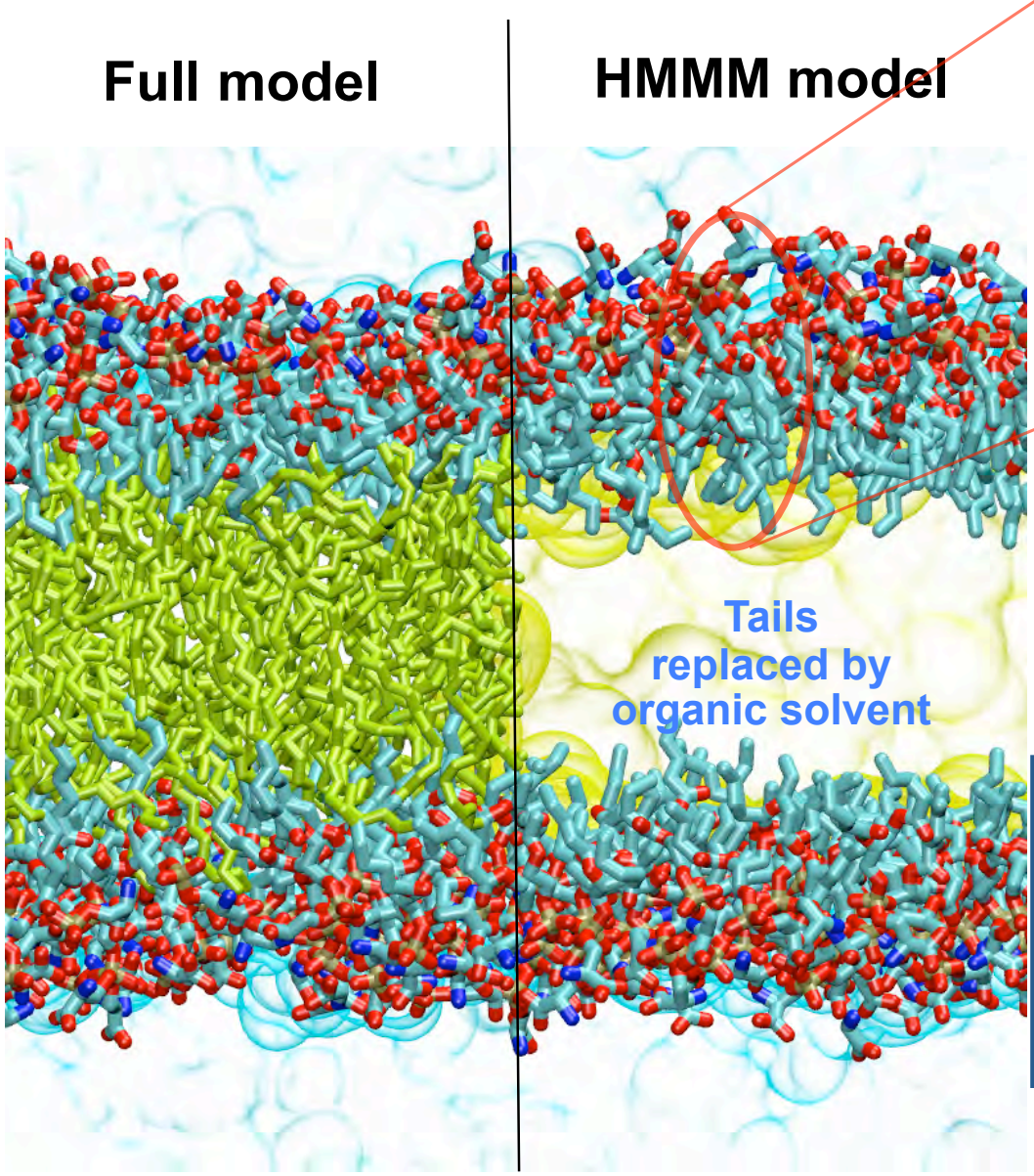


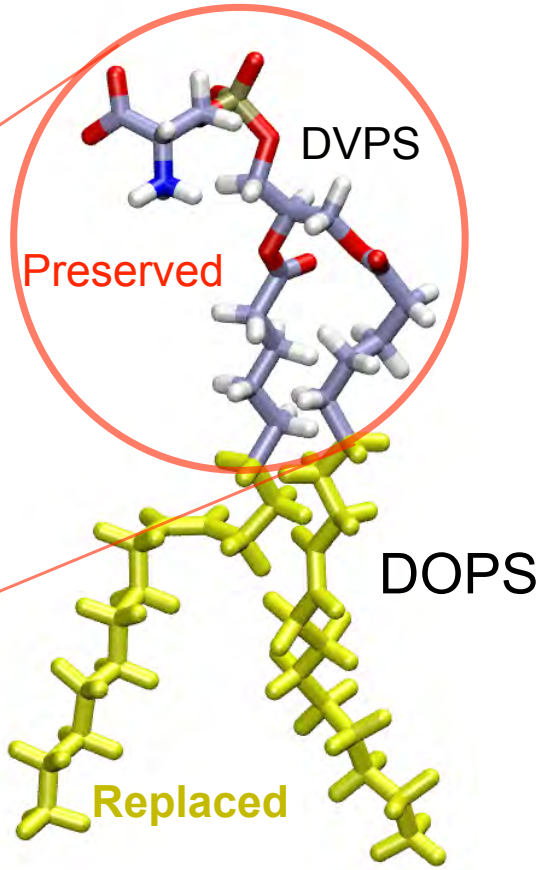
Collectively more than **150 ns** of **biased** simulations capturing a **single** binding event.

Z. Ohkubo and E. Tajkhorshid, *Structure* **2008**

HMMM model

Highly Mobile Membrane Mimetic model





Advantages

Increased mobility of lipids

Retain explicit headgroups allowing for atomic details


Zenmei Ohkubo

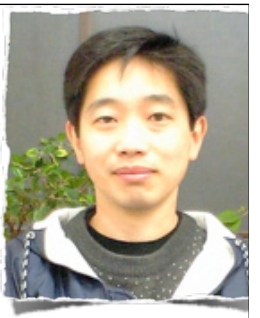

Mark Arcario


Taras Pogorelov

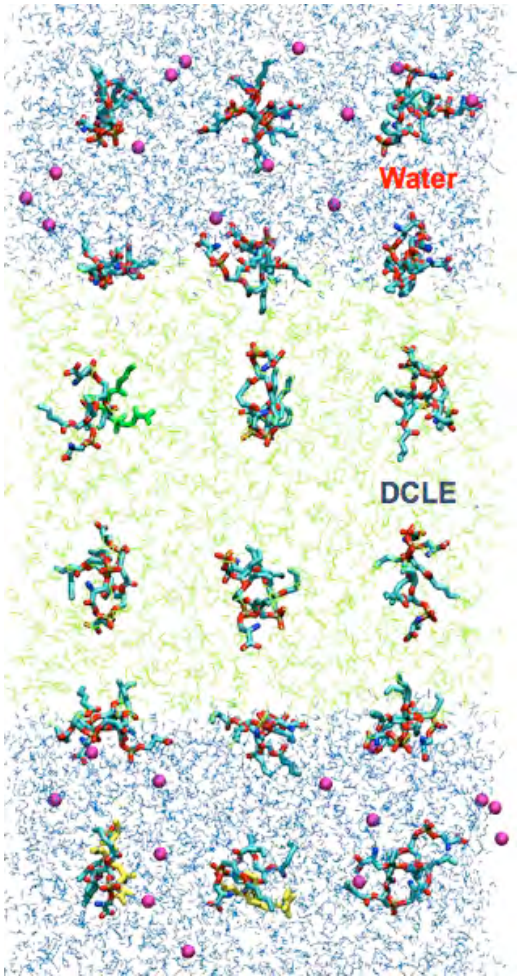

Josh Vermaas


Javier Baylon

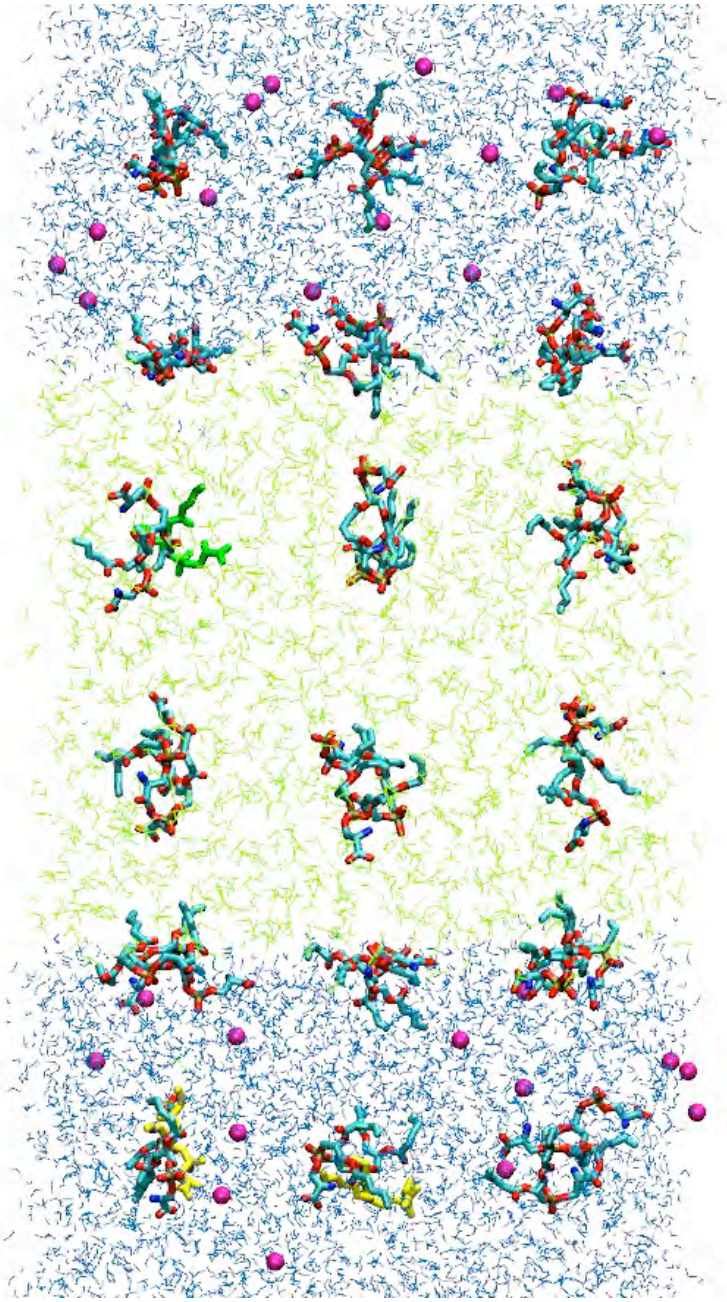
Spontaneous and Rapid Formation of a Bilayer



Zenmei Ohkubo





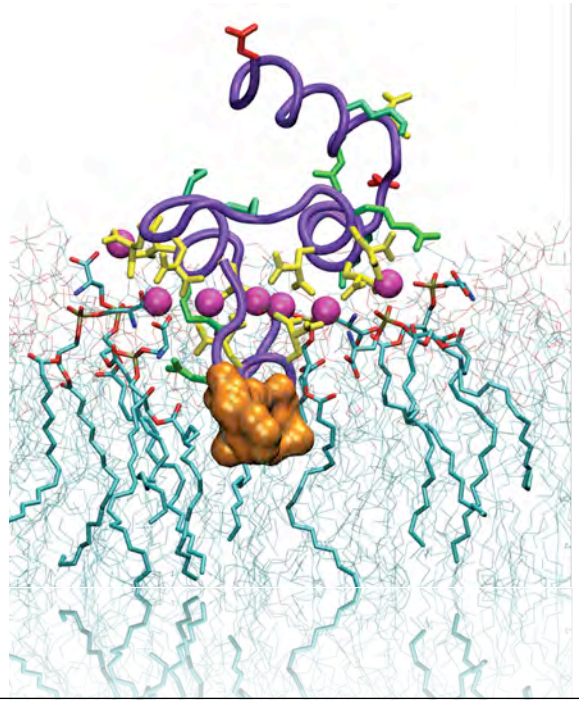


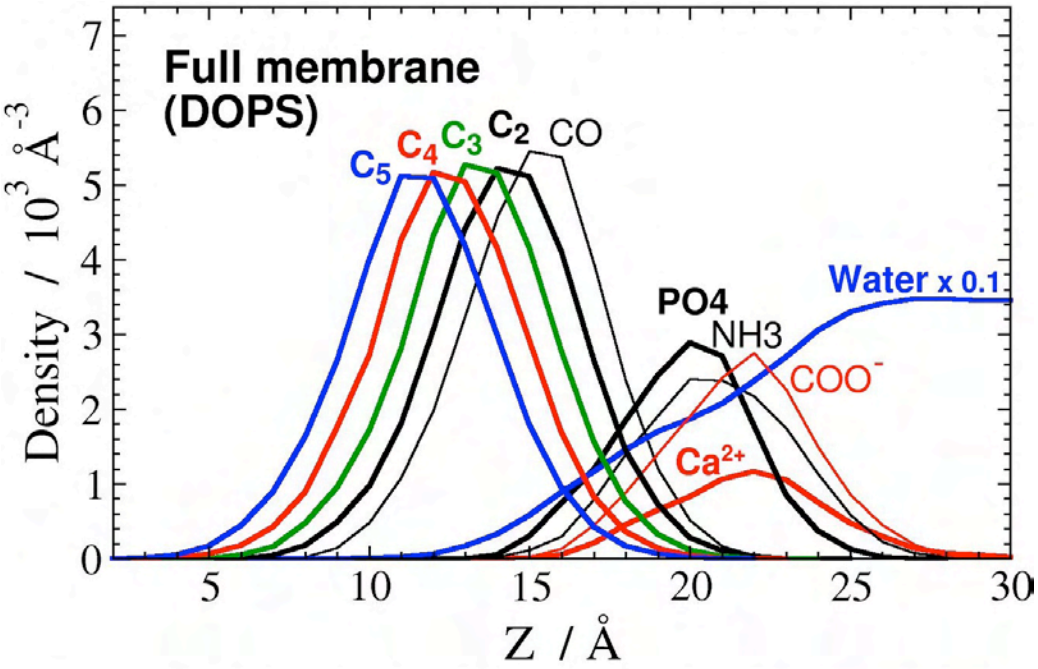
60 x 60 x 120 Å
DVPSs at 3 x 3 x 6 grid points
(22 ns)

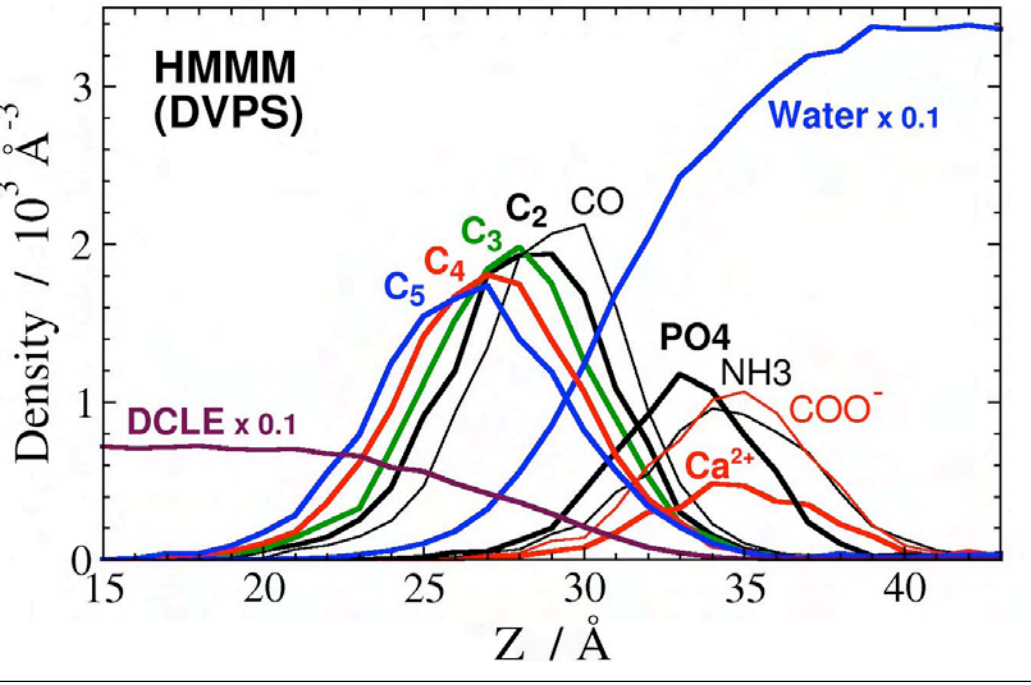
HMMM- Preserving the "Face" of the Lipid Bilayer

Perfect match in the membrane profile particularly in the head group region

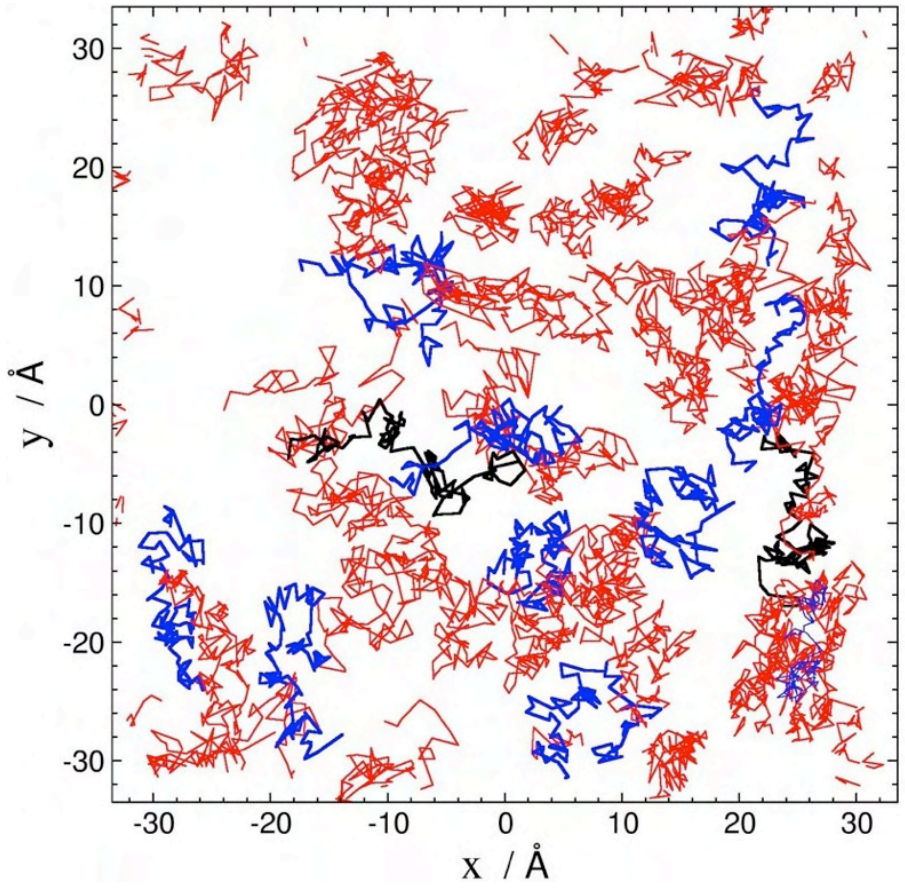
Critical for proper description of lipid protein interactions

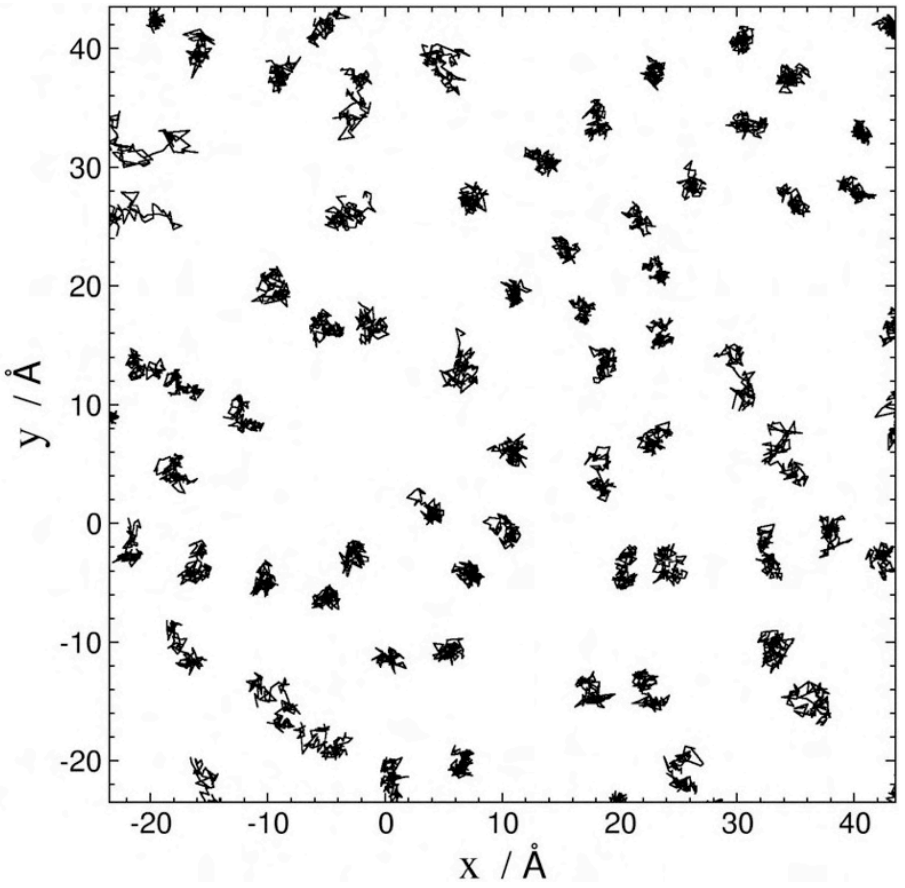




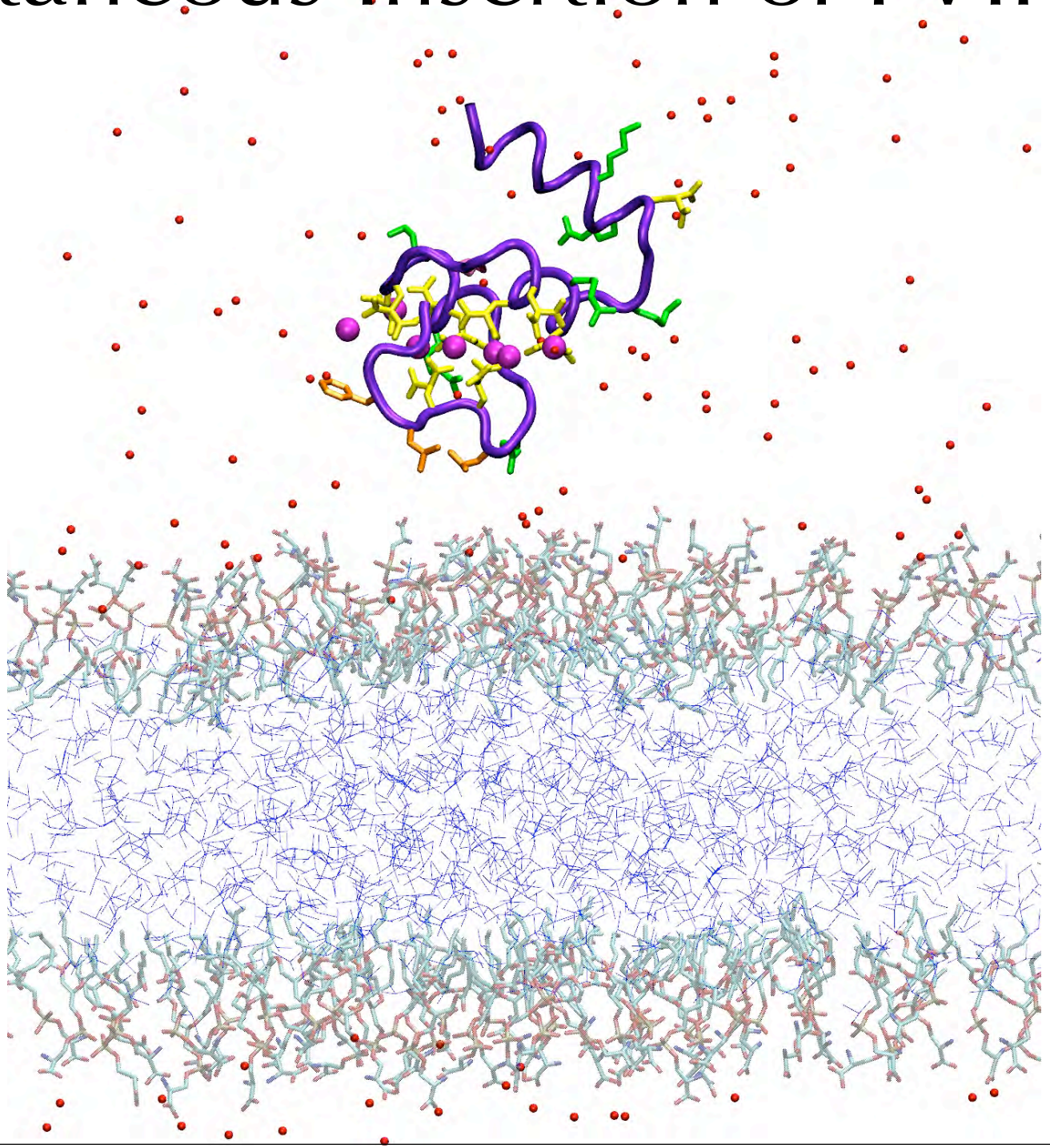


HMMM – lipids are more mobile than full-lipids

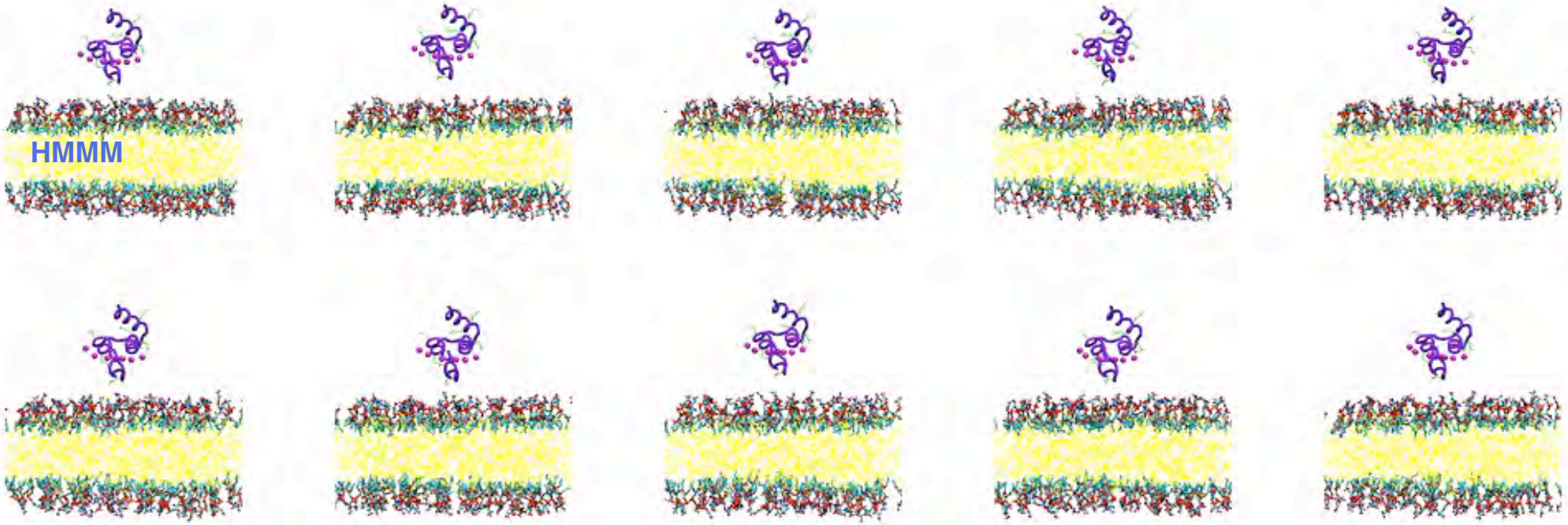


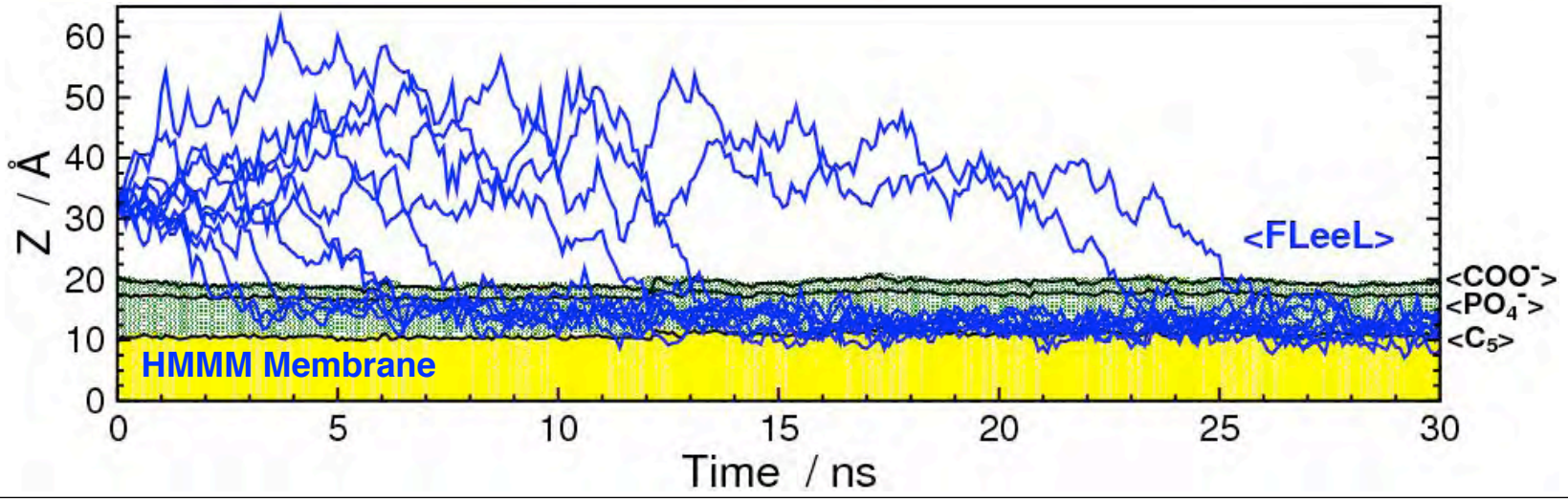


Spontaneous Insertion of FVII-GLA



Spontaneous Membrane Binding (*n = 10*)



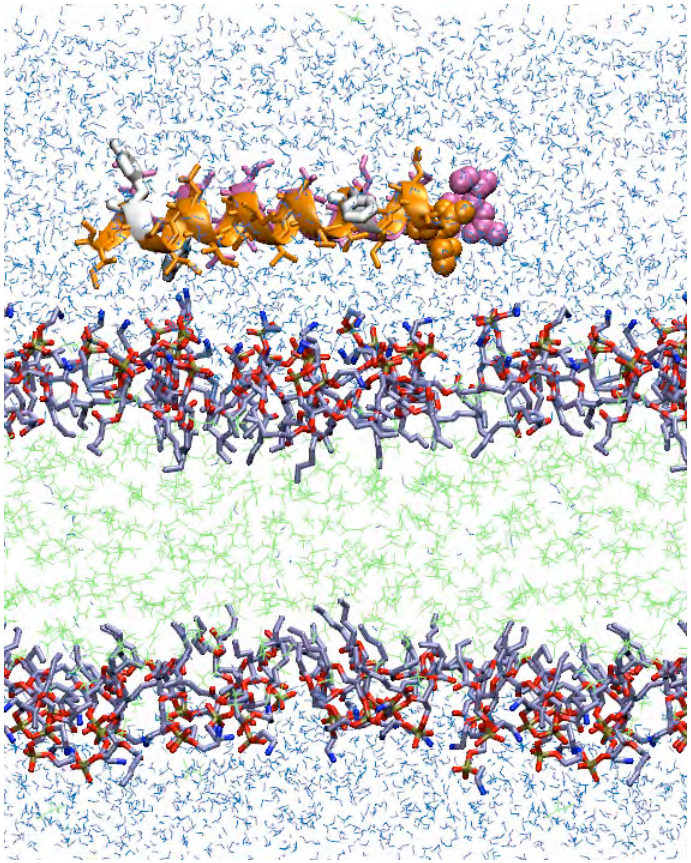


Spontaneous Insertion of Transmembrane Helices



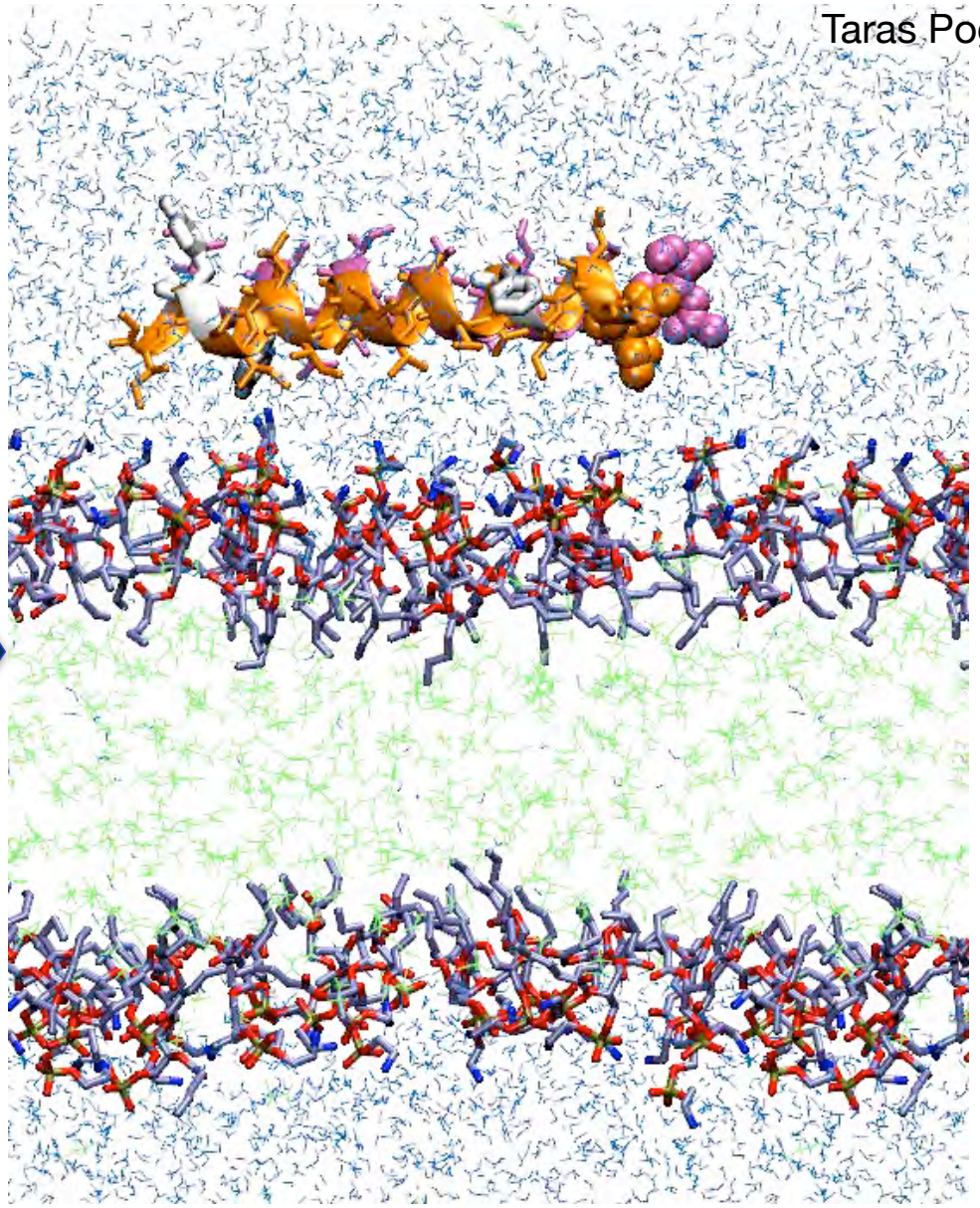
Taras Pogorelov

t = 0

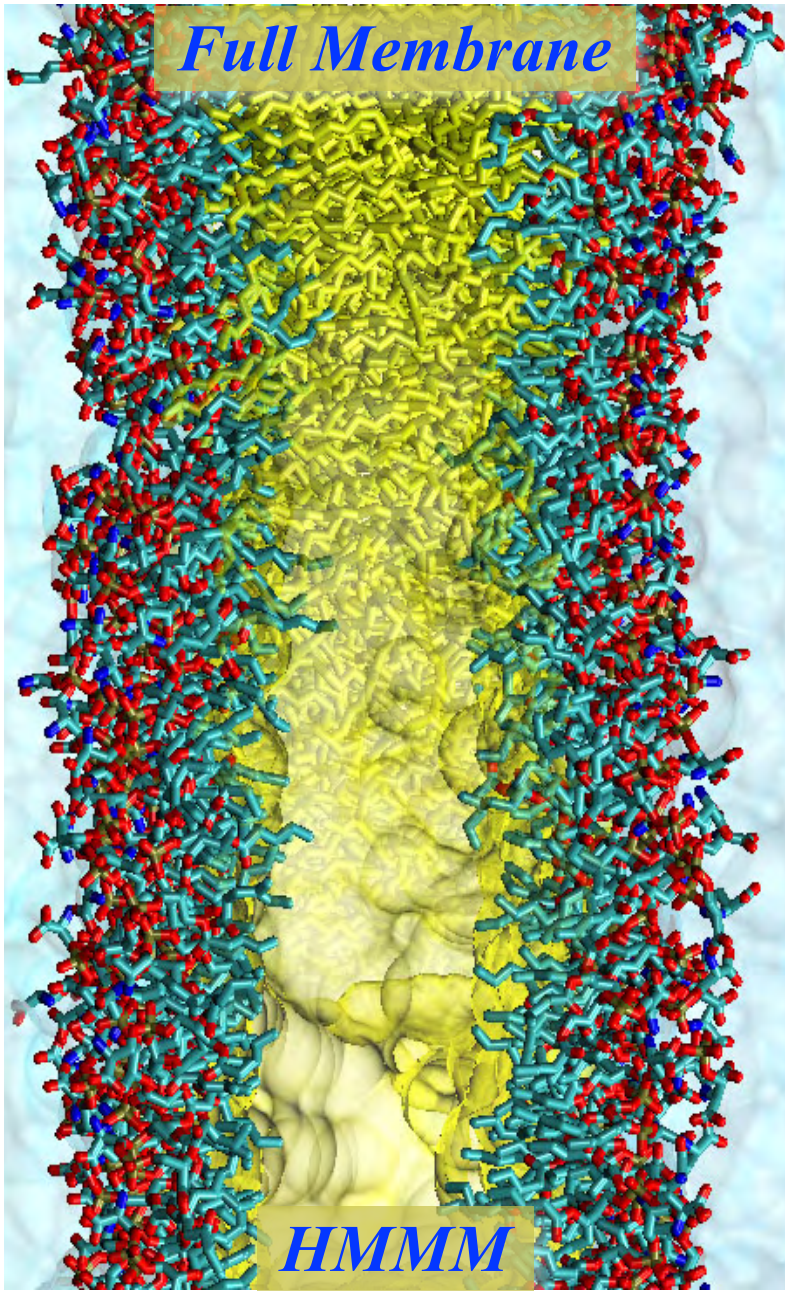


50 x 50 x 75 Å
Glycophorin A monomers: 2
z-constraint on 2 carbonyl carbons

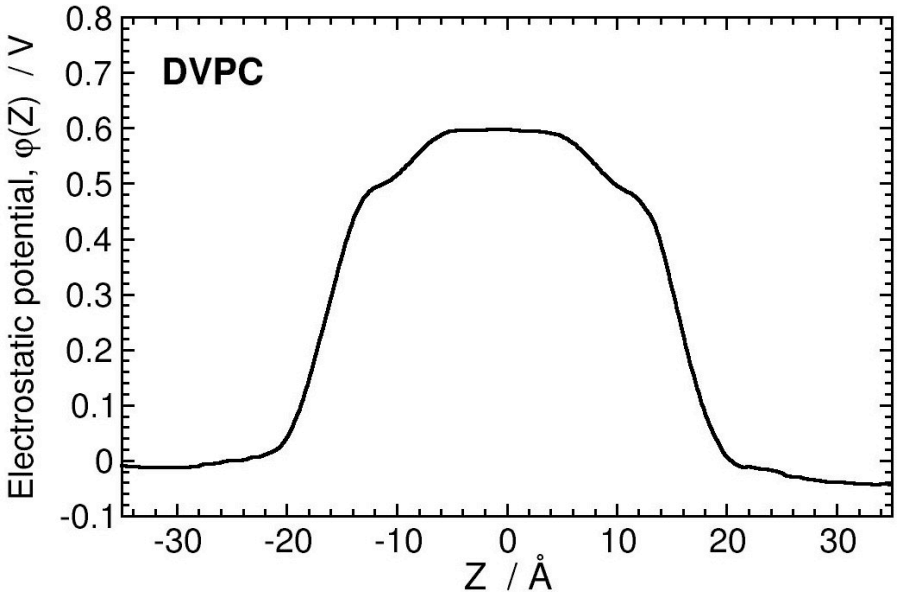
12 ns

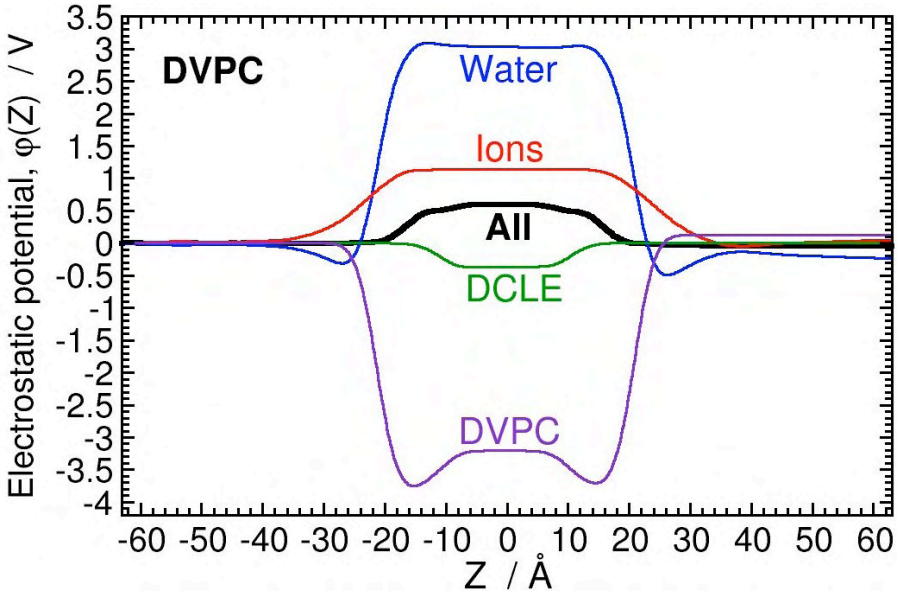


Quantitative Characterization and Optimization of HMMM



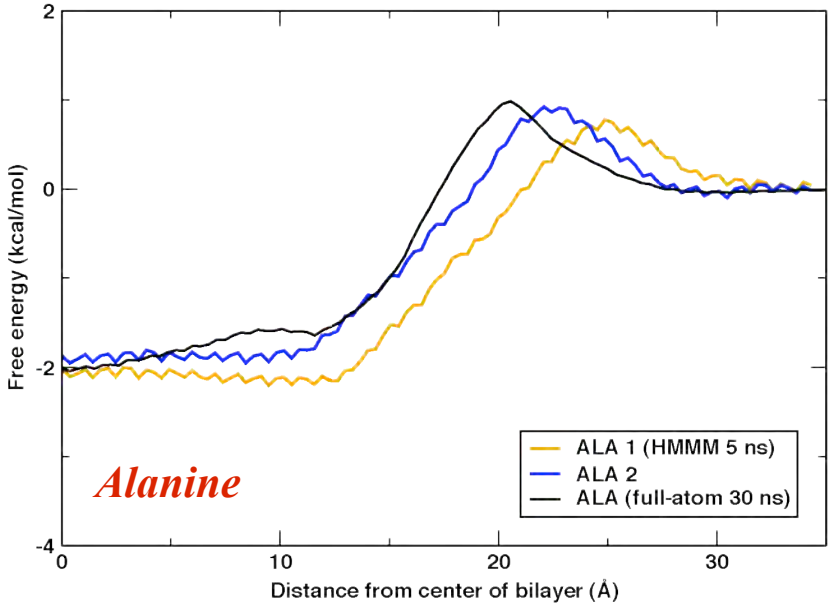
Membrane Dipolar Potential

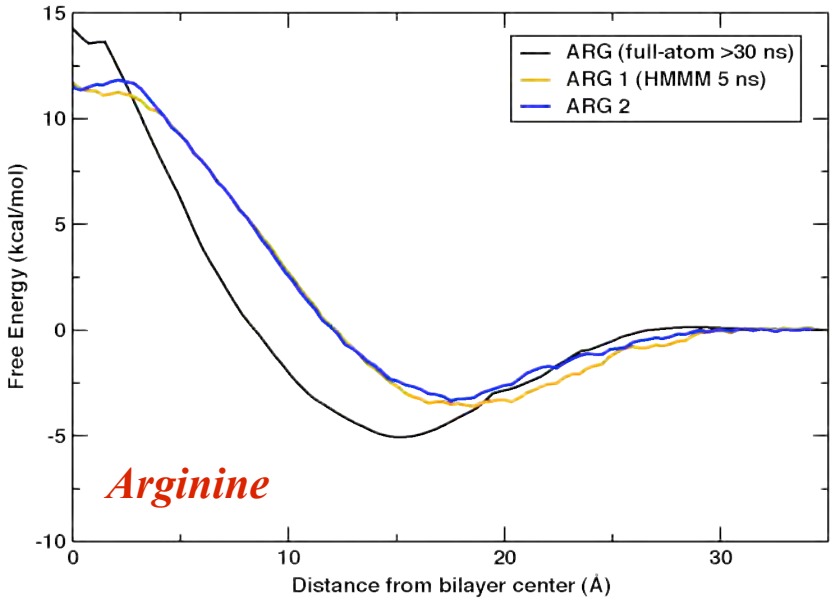


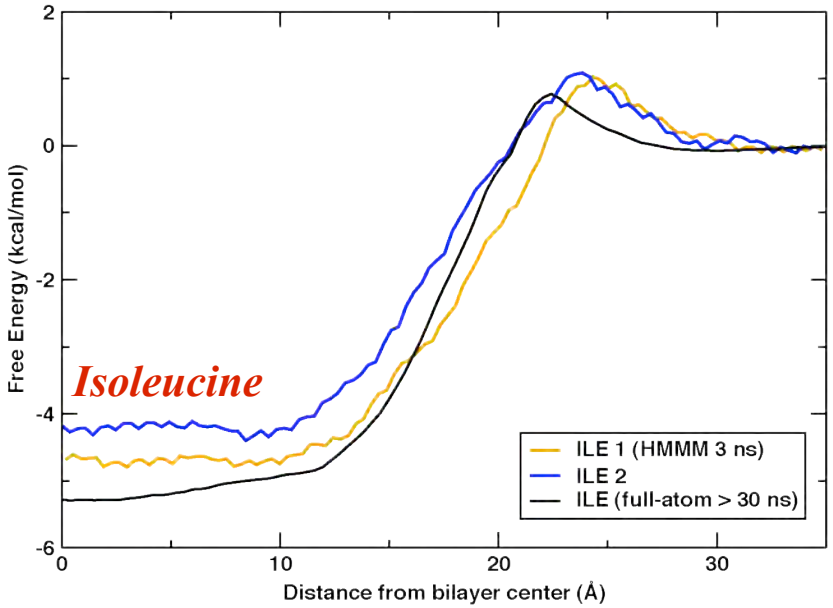


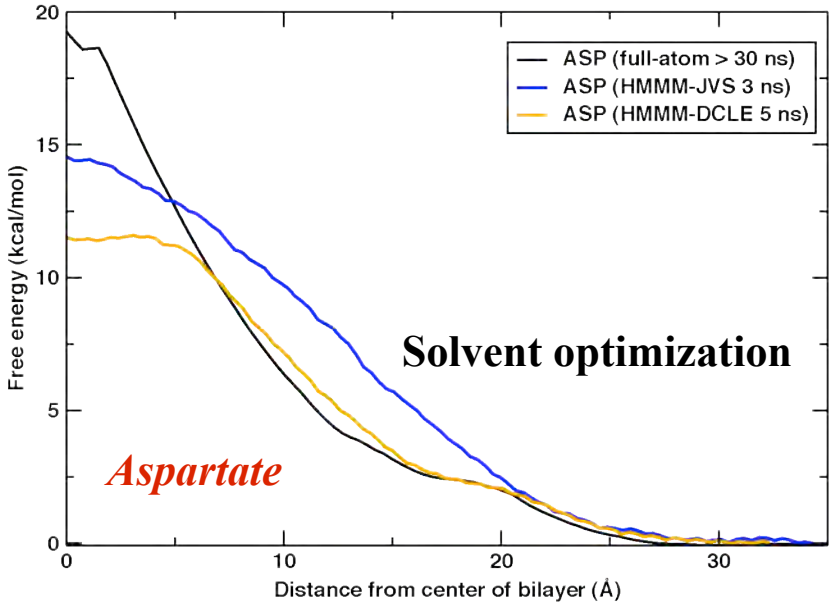
Quantitative Characterization and Optimization of HMMM

PMF of Amino Acid Insertion







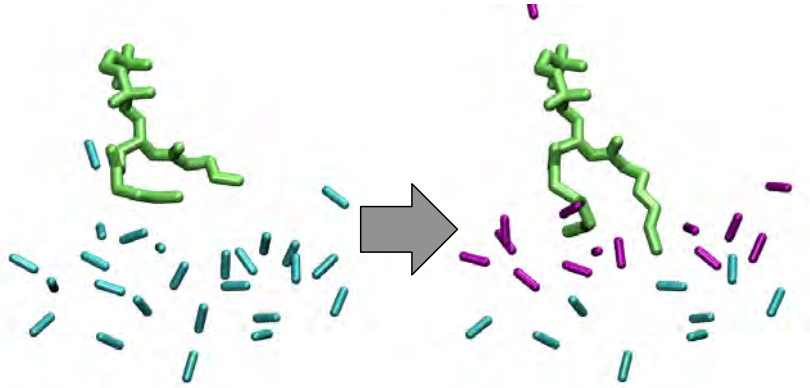


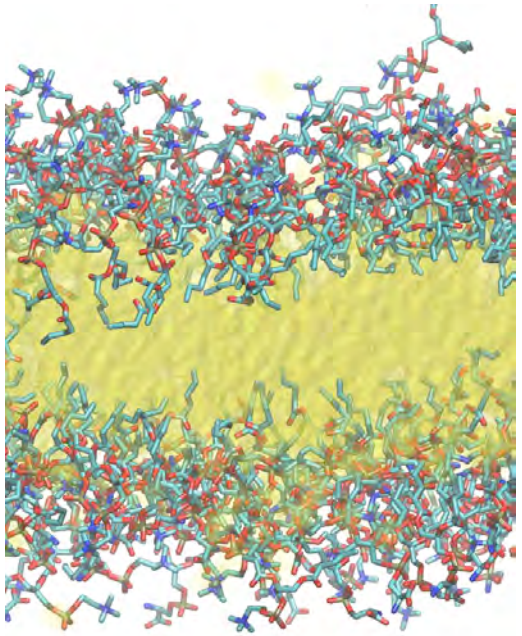
* Black lines: Full membrane data from Biophys. J. 94, 3393, 2008.

Tail-Gro - Stepwise transformation of HMMM to full membrane representation

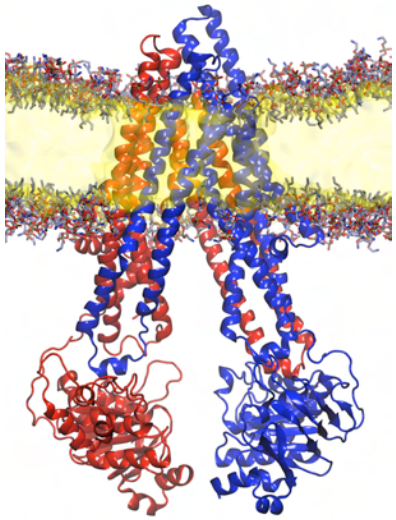


Josh Vermaas



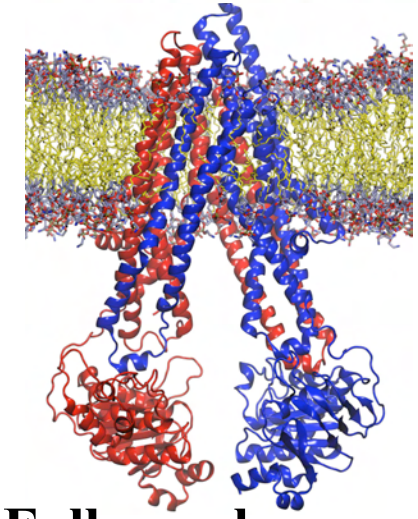


Step-wise insertion of P-glycoprotein



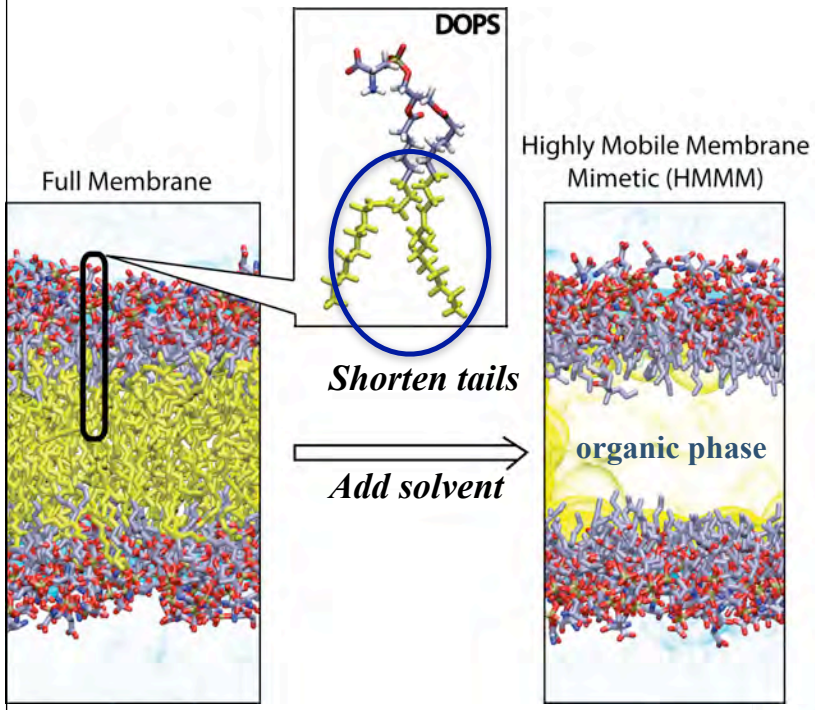
HMMM

Grow tails

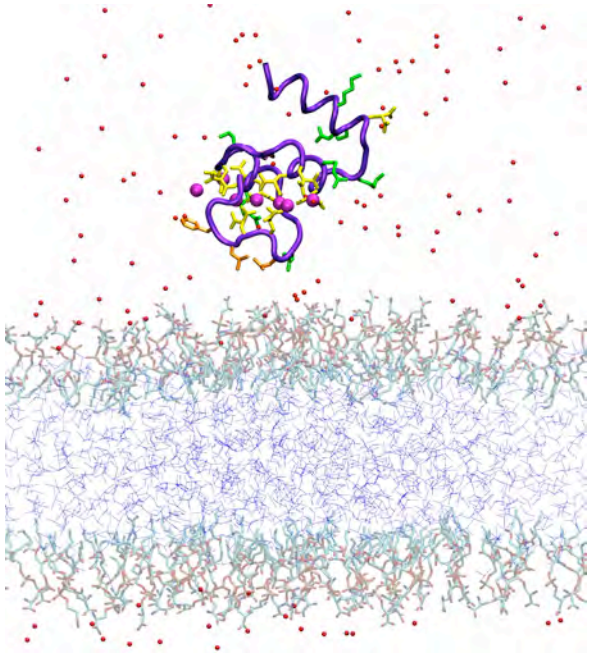


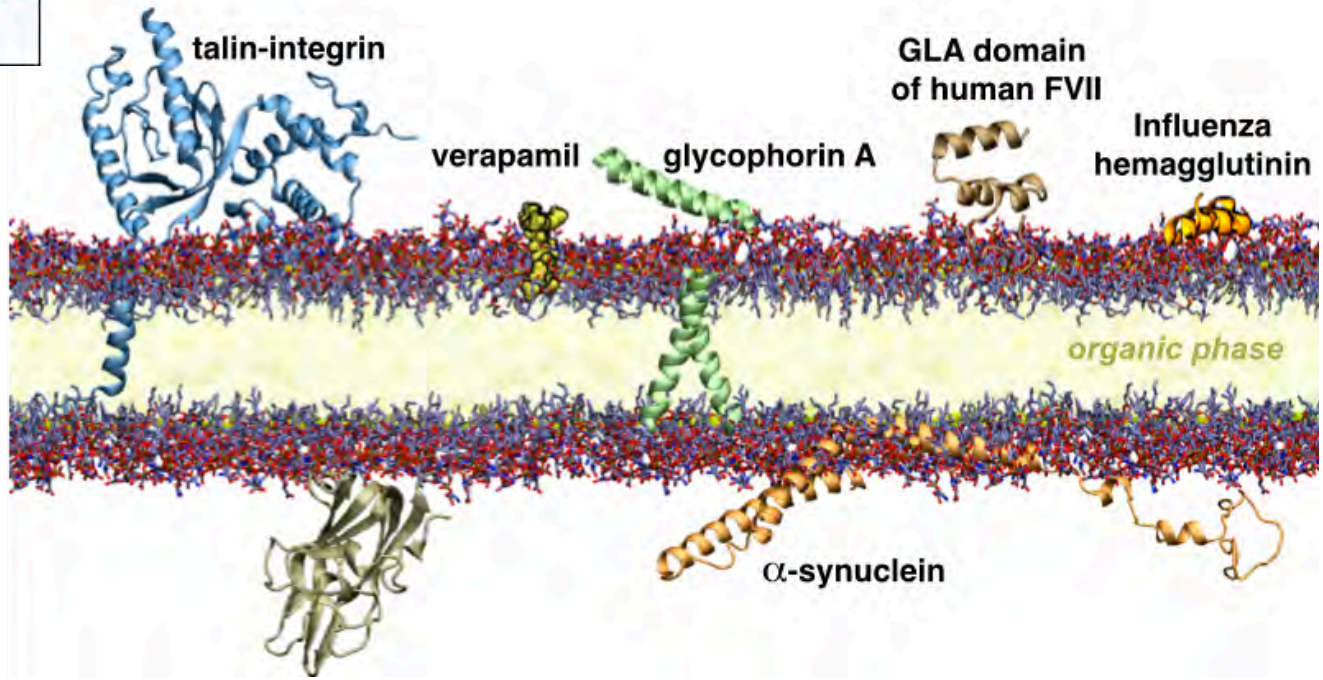
Full membrane

Highly Mobile Membrane Mimetic Model (HMMM)

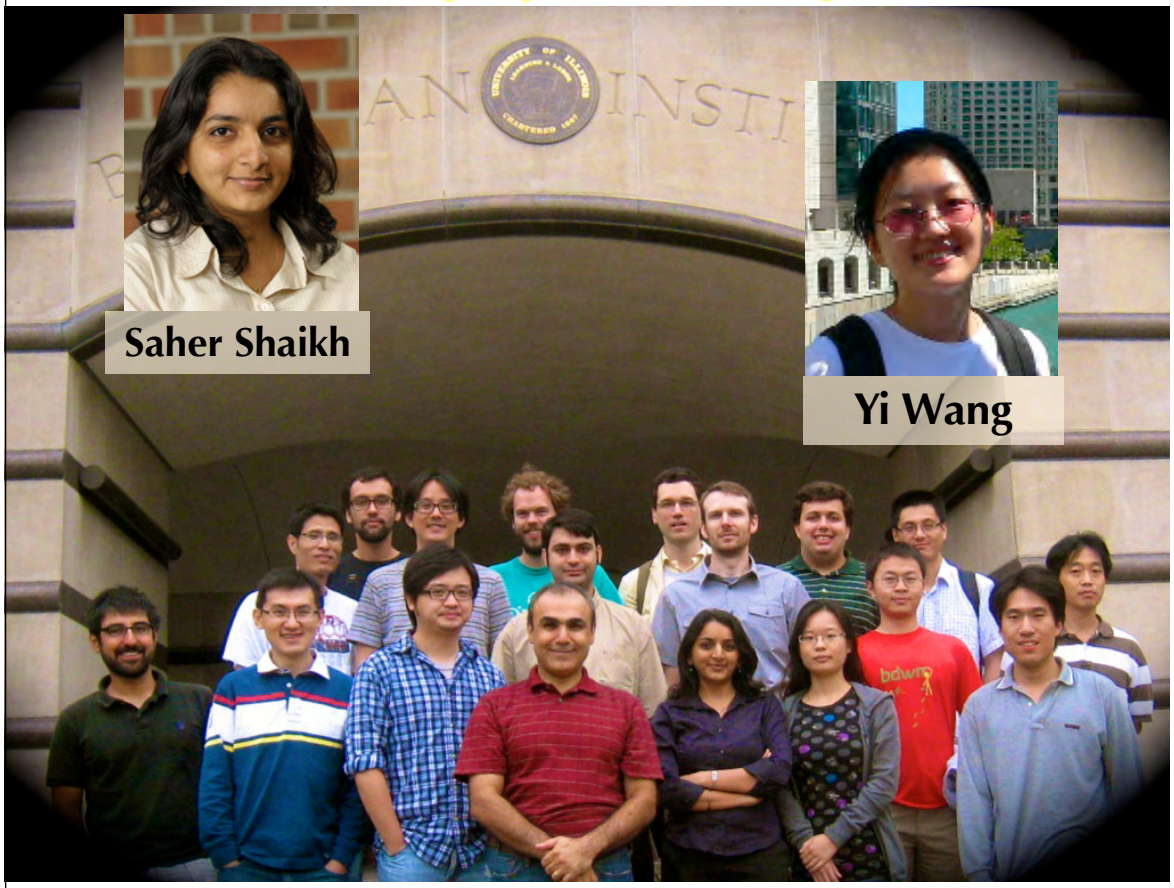


Facilitating dynamical studies of membrane-associated phenomena





Computational Structural Biology and Molecular Biophysics Group (CSBMB)



csbmb.beckman.illinois.edu

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R01-GM086749 U54-GM087519
R01-GM101048 P41-GM104601

Measuring Cellular CO_2 Permeability by ^{18}O Exchange – Methodology and Results on Red Blood Cells

Gerolf Gros and Volker Endeward

Zentrum Physiologie
Medizinische Hochschule Hannover
Germany

Methods Available to Measure Membrane CO_2 Permeability

- Surface pH transients in Xenopus oocytes
- Kinetics of cellular CO_2 uptake recorded by intracellular pH measurement
- pH gradients in the surface region of epithelial cell layers
- Stopped flow rapid reaction spectrophotometry
- ^{18}O exchange between CO_2, HCO_3^- and H_2O

Earlier Measurements of CO_2 permeability of membranes

P_{CO2} of planar phospholipid bilayers from CO_2 flux measurements

0.35 cm/s (Gutknecht et al., 1977)

3.2 cm/s (Missner et al., 2008)

P_{CO2} of phospholipid vesicles by stopped flow spectrophotometry

~ 10^{-3} cm/s (Prasad et al., 1998)

~ 10^{-3} cm/s (Yang et al., 2000)

Can the kinetics of CO_2 and O_2 uptake by red cells be reliably measured by stopped flow techniques?

$t_{1/2}$ of CO_2 uptake by human red cells: 13 ms

(Holland and Forster, 1975)

continuous-flow rapid reaction apparatus

$t_{1/2}$ of CO_2 uptake by red cells by theory: ~ 12 ms

(Endeward et al., 2008)

$t_{1/2}$ of O_2 uptake by human red cells: ~ 80 ms

(Vandegriff and Olson, 1984)

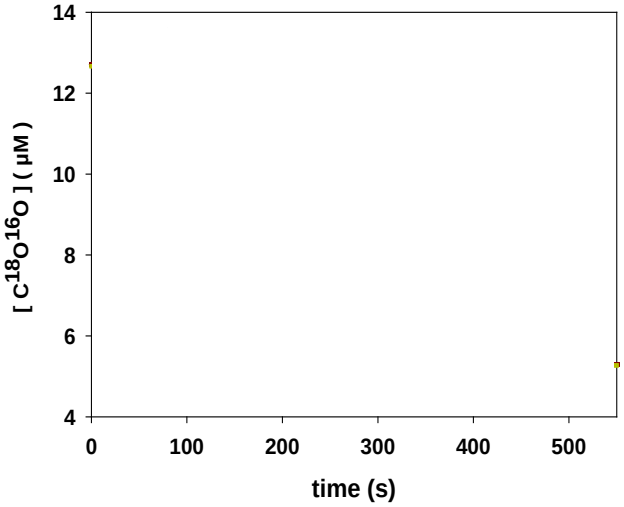
Determining Membrane Permeabilities of CO_2 and HCO_3^- by the ^{18}O Exchange Technique

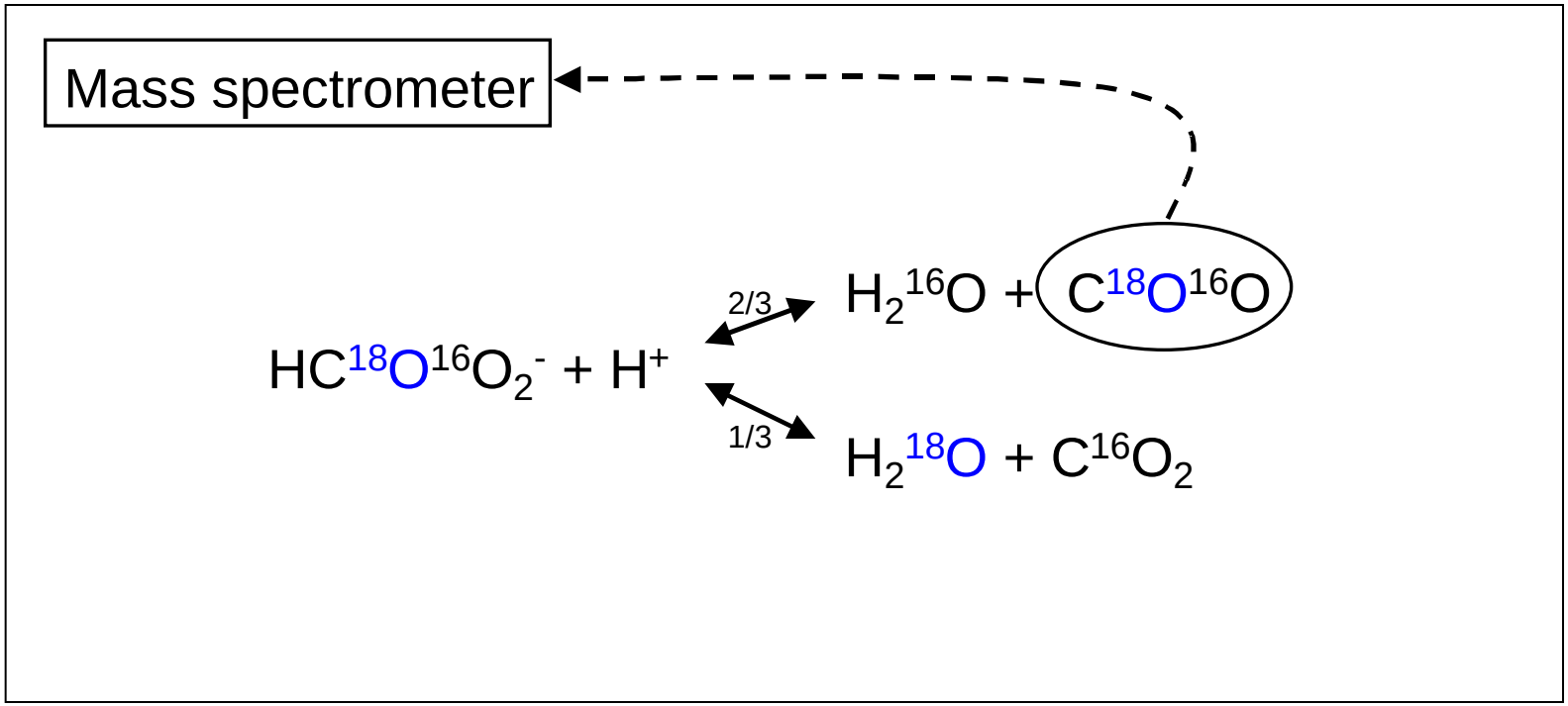
Has been applied to

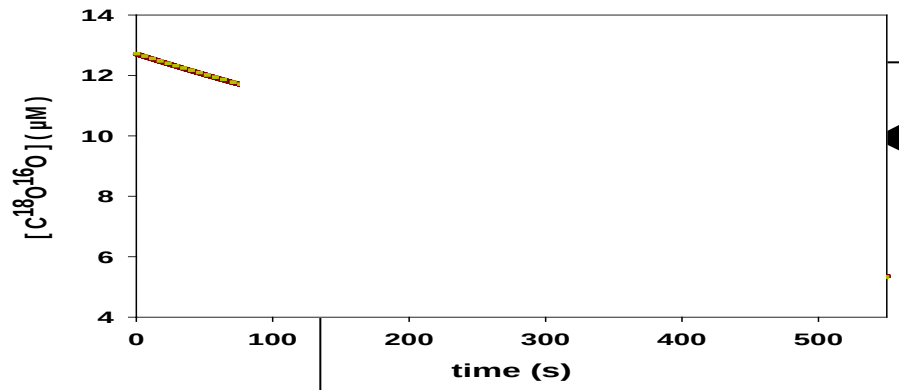
Isolated cells in suspension: red blood cells, MDCK and tsA201 cells

Phospholipid vesicles in suspension

Intact colon epithelium


14
12
10
8
6
4
0
100
200
300
400
500
[C18O16O] (µM)
time (s)


Mass spectrometer
HC18O16O2- + H+
2/3
1/3
H216O + C18O16O
H218O + C16O2



$H_2{}^{16}O + C^{18}O^{16}O$

2/3

$HC^{18}O^{16}O_2^- + H^+$

1/3

$H_2{}^{18}O + C^{16}O_2$

P_{HCO3-}

P_{H2O}

P_{CO2}

1/3

$H_2{}^{18}O + C^{16}O_2$

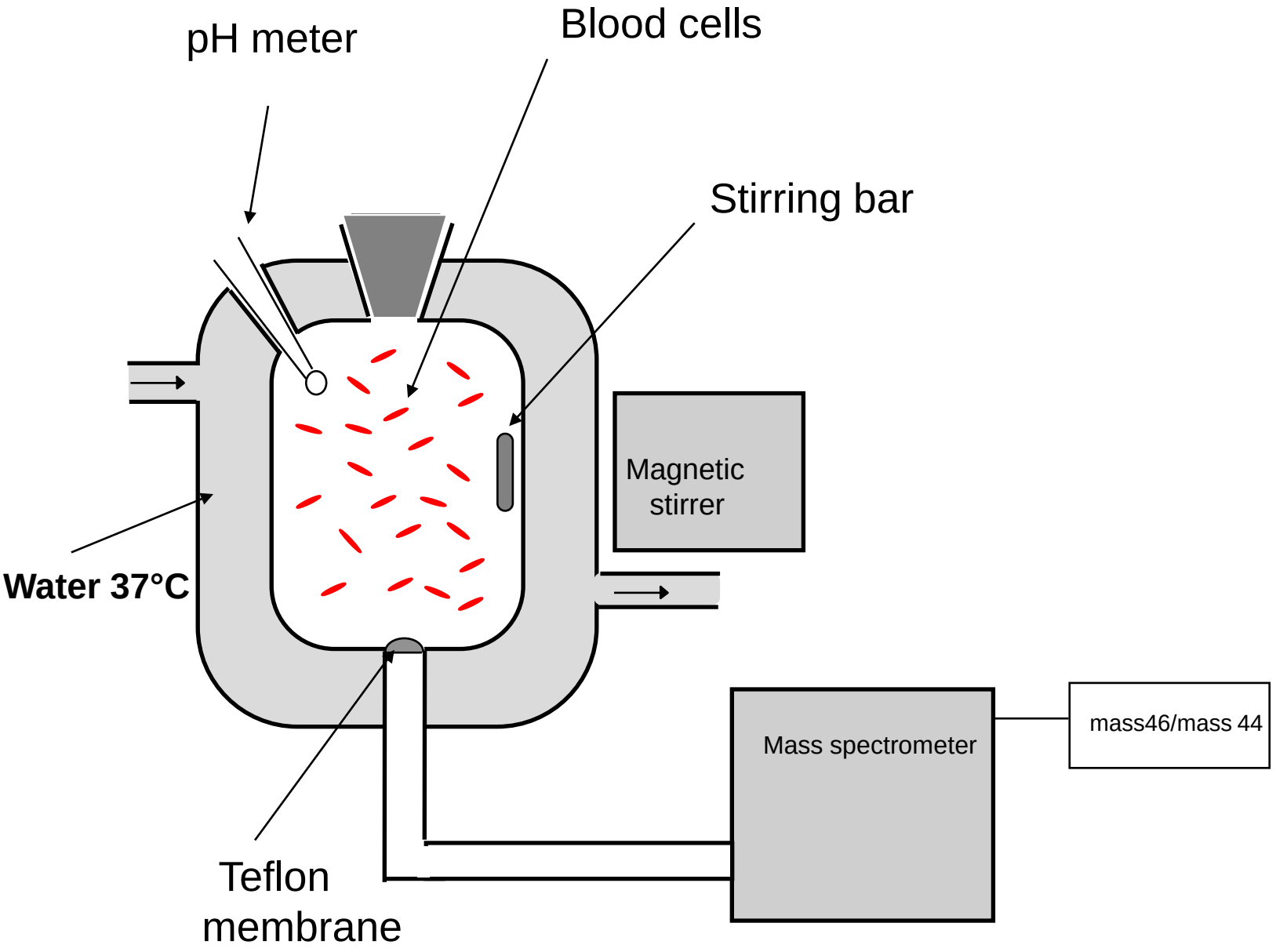
$H\,C^{18}O^{16}O_2^- + H^+$

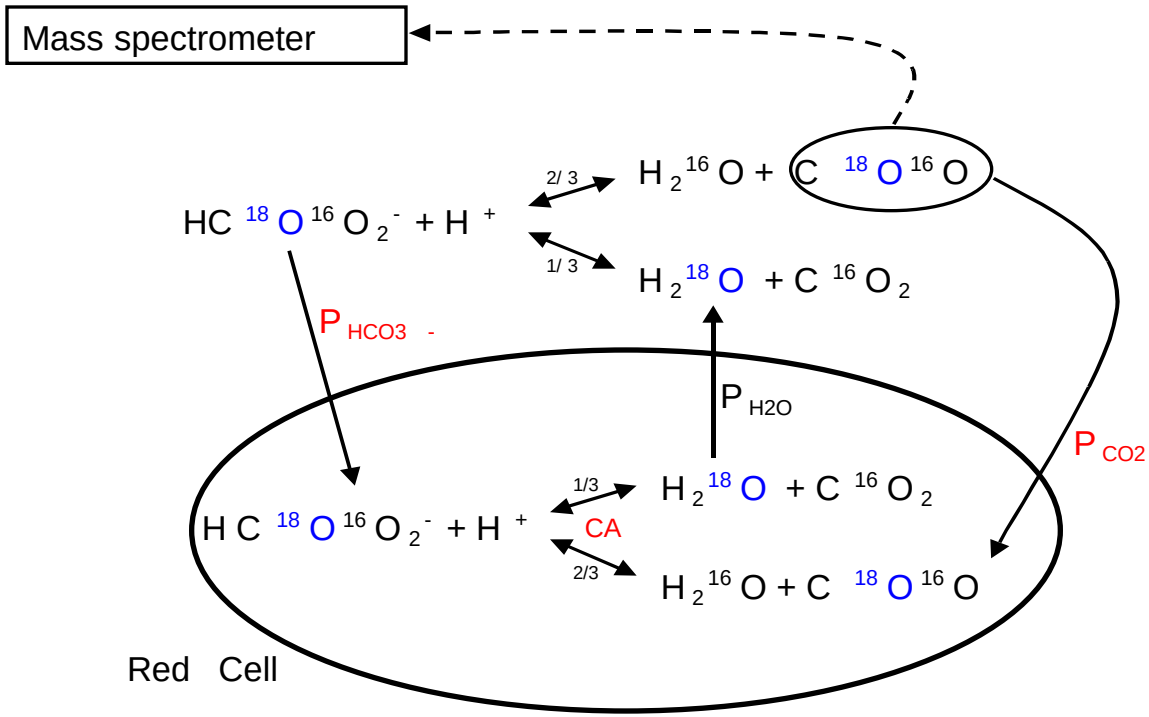
CA

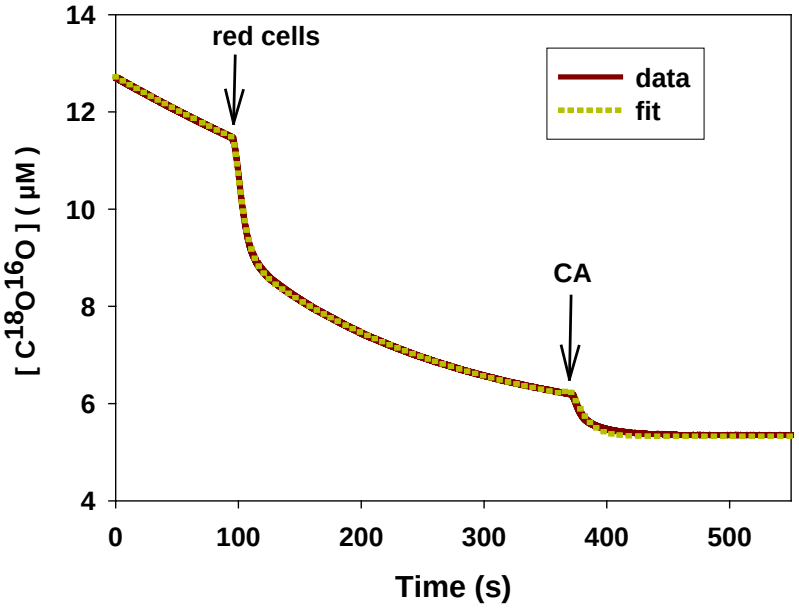
2/3

$H_2{}^{16}O + C^{18}O^{16}O$

Cell


pH meter
Blood cells
Stirring bar
Magnetic stirrer
Water 37°C
Mass spectrometer
mass46/mass 44
Teflon membrane


Mass spectrometer
HC 18O 16O2- + H+
2/ 3
1/ 3
H2 16O + C 18O 16O
H2 18O + C 16O2
P HCO3 -
P H2O
P CO2
H C 18O 16O2- + H+
1/3
CA
2/3
H2 18O + C 16O2
H2 16O + C 18O 16O
Red Cell


red cells
CA
data
fit
[C18O16O] (µM)
Time (s)
14
12
10
8
6
4
0
100
200
300
400
500

$$\frac{d\left[C^{18}O^{16}O\right]_{ex}(t)}{dt} = -k_u A_{ex}\left[C^{18}O^{16}O\right]_{ex}(t) + \frac{2k_u}{3K_1'} A_{ex}\left[H^+\right]_{ex}\left[HC^{18}O^{16}O_2^-\right]_{ex}(t) + P_{CO_2}\, a\frac{v}{1-v}\left\{\left[C^{18}O^{16}O\right]_{in}(t) - \left[C^{18}O^{16}O\right]_{ex}(t)\right\}$$

$$\frac{d\left[C^{18}O^{16}O\right]_{in}(t)}{dt} = -k_u A_{in}\left[C^{18}O^{16}O\right]_{in}(t) + \frac{2k_u}{3K_1'} A_{in}\left[H^+\right]_{in}\left[HC^{18}O^{16}O_2^-\right]_{in}(t) - P_{CO_2}\, a\left\{\left[C^{18}O^{16}O\right]_{in}(t) - \left[C^{18}O^{16}O\right]_{ex}(t)\right\}$$

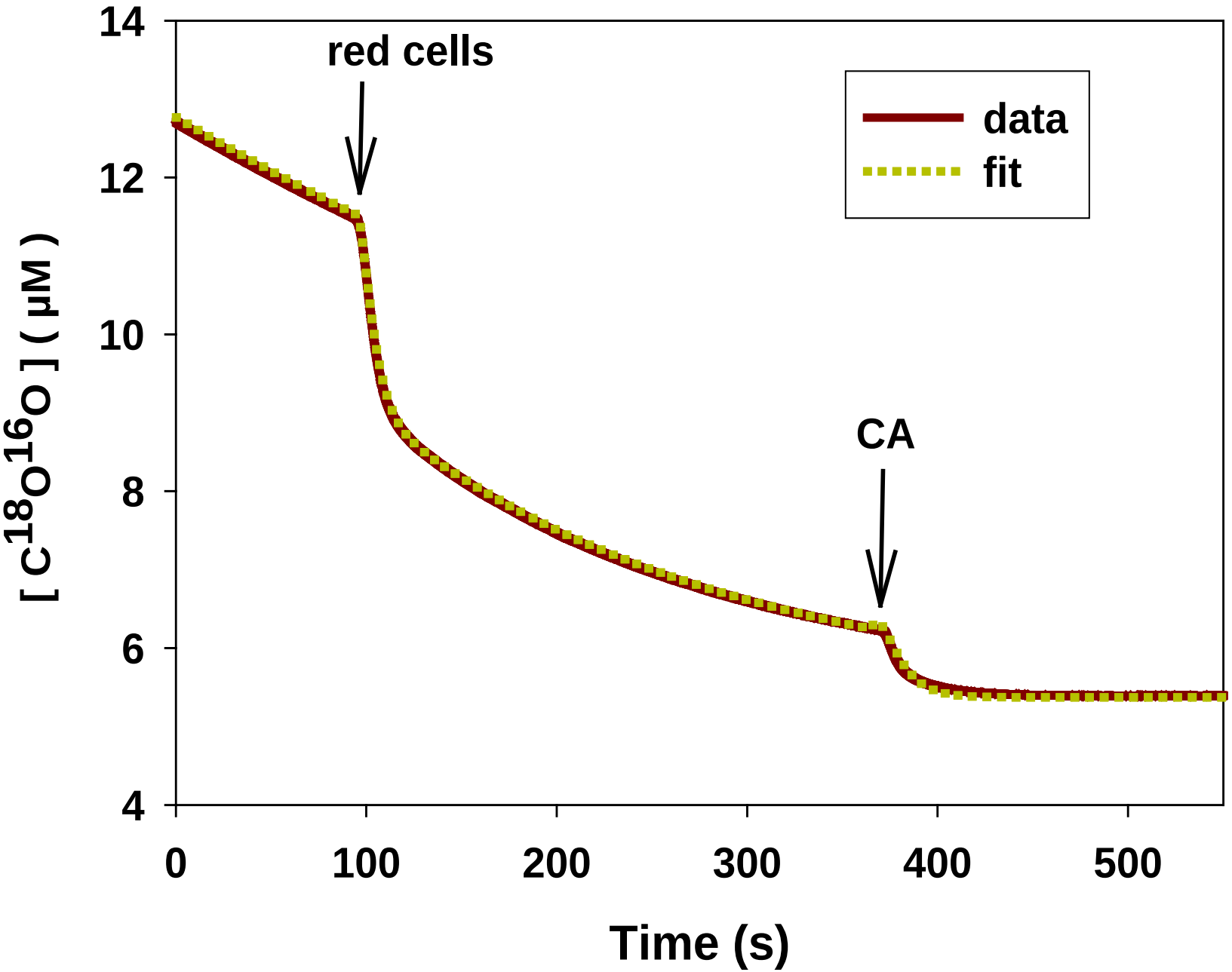
$$\frac{d\left[HC^{18}O^{16}O_2^-\right]_{ex}(t)}{dt} = k_u A_{ex}\left(\left[C^{18}O^{16}O\right]_{ex}(t) + [CO_2]\frac{\left[H_2{}^{18}O\right]_{ex}(t)}{[H_2O]}\right) - \frac{k_u}{K_1'} A_{ex}\left[H^+\right]_{ex}\left[HC^{18}O^{16}O_2^-\right]_{ex}(t) - P_{HCO_3^-}\, a\frac{v}{1-v}\left\{\frac{\left[H^+\right]_{ex}}{\left[H^+\right]_{in}}\left[HC^{18}O^{16}O_2^-\right]_{ex}(t) - \left[HC^{18}O^{16}O_2^-\right]_{in}(t)\right\}$$

$$\frac{d\left[HC^{18}O^{16}O_2^-\right]_{in}(t)}{dt} = k_u A_{in}\left(\left[C^{18}O^{16}O\right]_{in}(t) + [CO_2]\frac{\left[H_2{}^{18}O\right]_{in}(t)}{[H_2O]}\right) - \frac{k_u}{K_1'} A_{in}\left[H^+\right]_{in}\left[HC^{18}O^{16}O_2^-\right]_{in}(t) - P_{HCO_3^-}\, a\left\{\frac{\left[H^+\right]_{ex}}{\left[H^+\right]_{in}}\left[HC^{18}O^{16}O_2^-\right]_{ex}(t) - \left[HC^{18}O^{16}O_2^-\right]_{in}(t)\right\}$$

$$\frac{d\left[H_2{}^{18}O\right]_{ex}(t)}{dt} = \frac{1}{3}\frac{k_u}{K_1'} A_{ex}\left[H^+\right]_{ex}\left[HC^{18}O^{16}O_2^-\right]_{ex}(t) - k_u A_{ex}\frac{[CO_2]}{[H_2O]}\left[H_2{}^{18}O\right]_{ex}(t) + P_{H_2O}\, a\frac{v}{1-v}\left\{\left[H_2{}^{18}O\right]_{in}(t) - \left[H_2{}^{18}O\right]_{ex}(t)\right\}$$

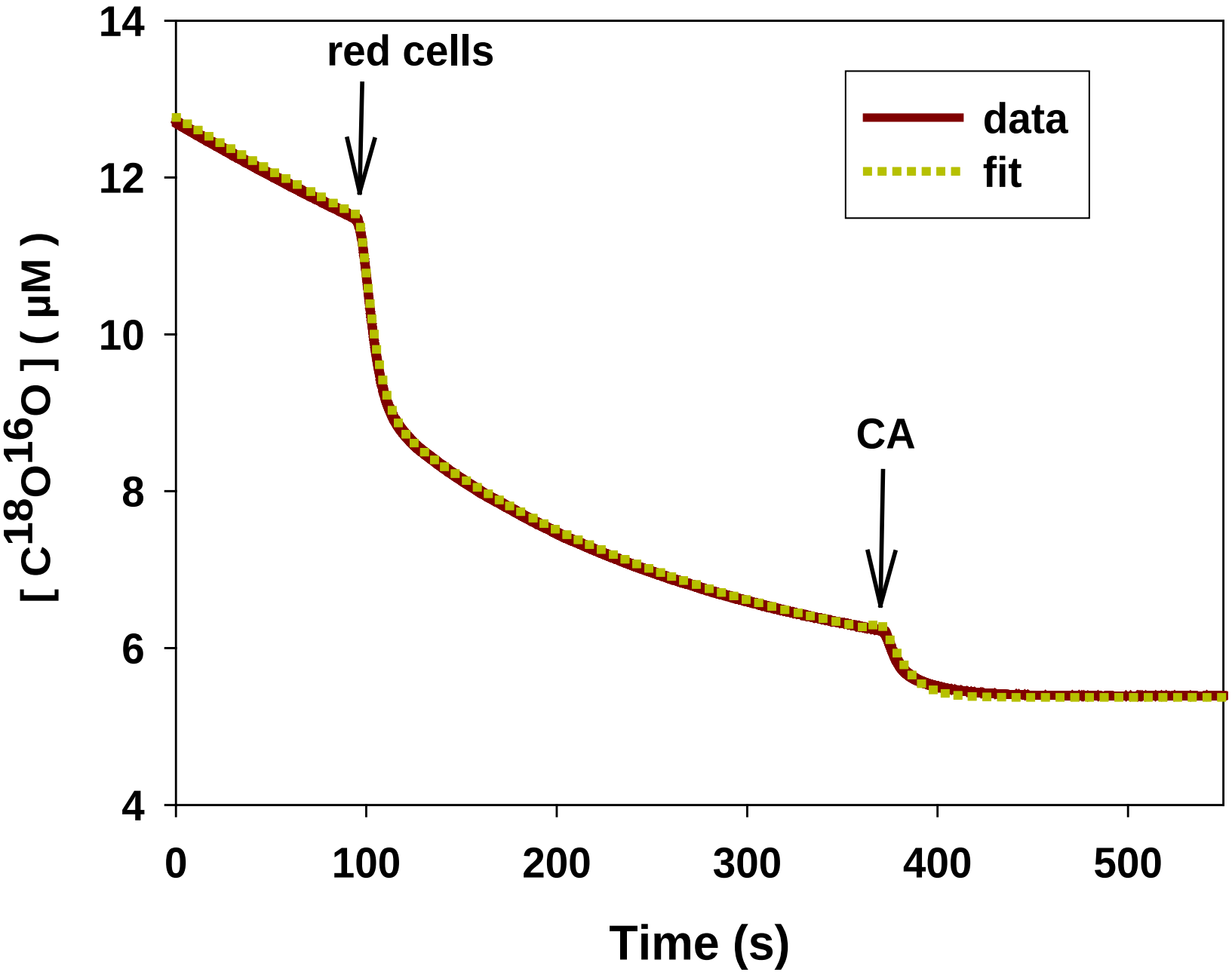
$$\frac{d\left[H_2{}^{18}O\right]_{in}(t)}{dt} = \frac{1}{3}\frac{k_u}{K_1'} A_{in}\left[H^+\right]_{in}\left[HC^{18}O^{16}O_2^-\right]_{in}(t) - k_u A_{in}\frac{[CO_2]}{[H_2O]}\left[H_2{}^{18}O\right]_{in}(t) - P_{H_2O}\, a\left\{\left[H_2{}^{18}O\right]_{in}(t) - \left[H_2{}^{18}O\right]_{ex}(t)\right\}$$

Fig.3


14
12
10
8
6
4
0
100
200
300
400
500
Time (s)
[C18O16O] (µM)
red cells
CA
data
fit

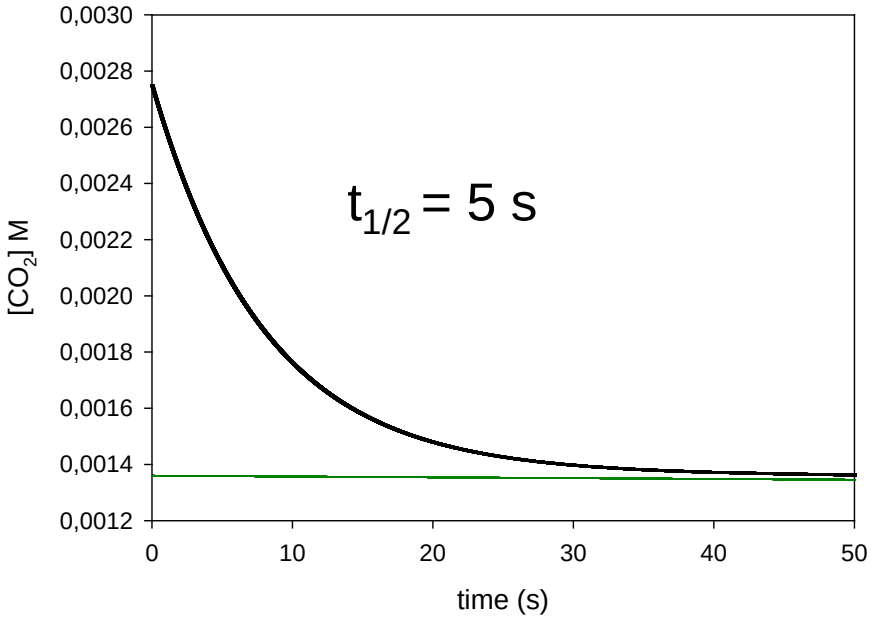
Why can we observe fast processes on such a slow time scale,

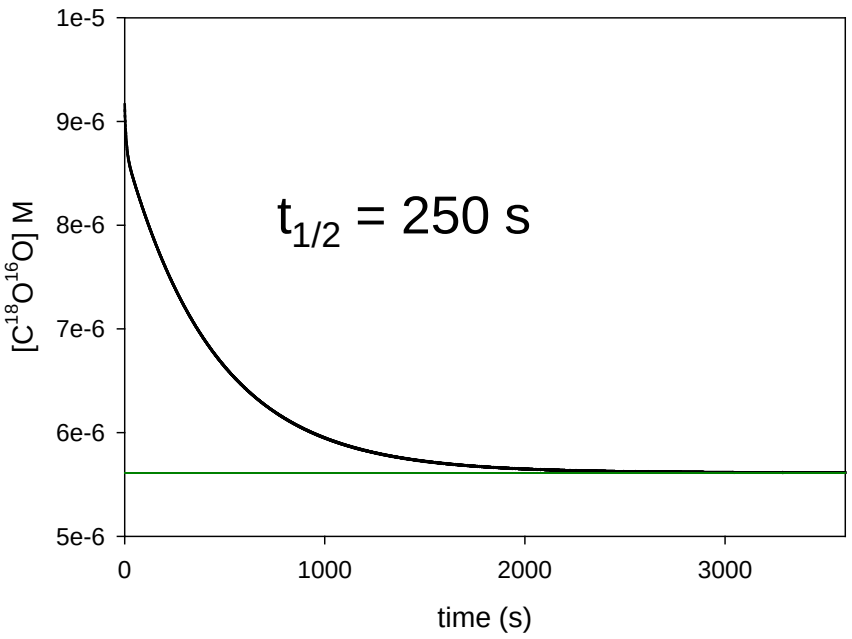
allowing us to follow these processes by mass spectrometry?


14
12
10
8
6
4
0
100
200
300
400
500
Time (s)
[C18O16O] (µM)
red cells
CA
data
fit

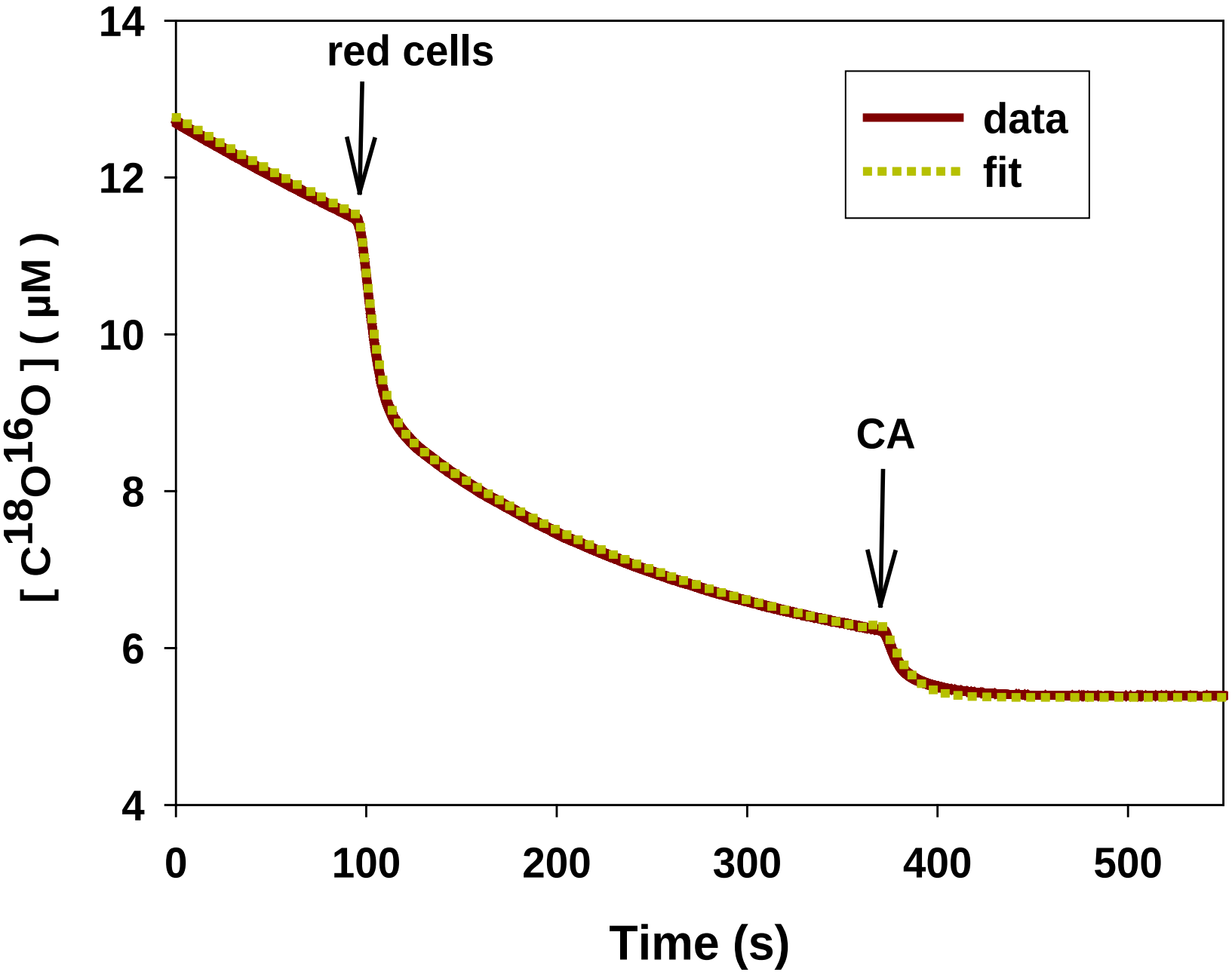
$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$$

$$HC^{18}O^{16}O_2^- + H^+ \overset{2/3}{\underset{1/3}{\leftrightarrows}} H_2{}^{16}O + C^{18}O^{16}O \quad / \quad H_2{}^{18}O + C^{16}O2$$





Kinetics of CO_2 hydration reaction vs. that of ^{18}O exchange


14
12
10
8
6
4
0
100
200
300
400
500
Time (s)
[C18O16O] (µM)
red cells
CA
data
fit

It was shown here that a time course of the decay of [$C^{18}O^{16}O$] that is measurable by mass spectrometry, is observed when the volume fraction of human red cells is extremely small, i.e. 2×10^{-4}. Raising this volume fraction by a factor of 10, to 0.002, renders the signal already too fast compared to the time resolution of the mass spectrometer in combination with the inlet system.

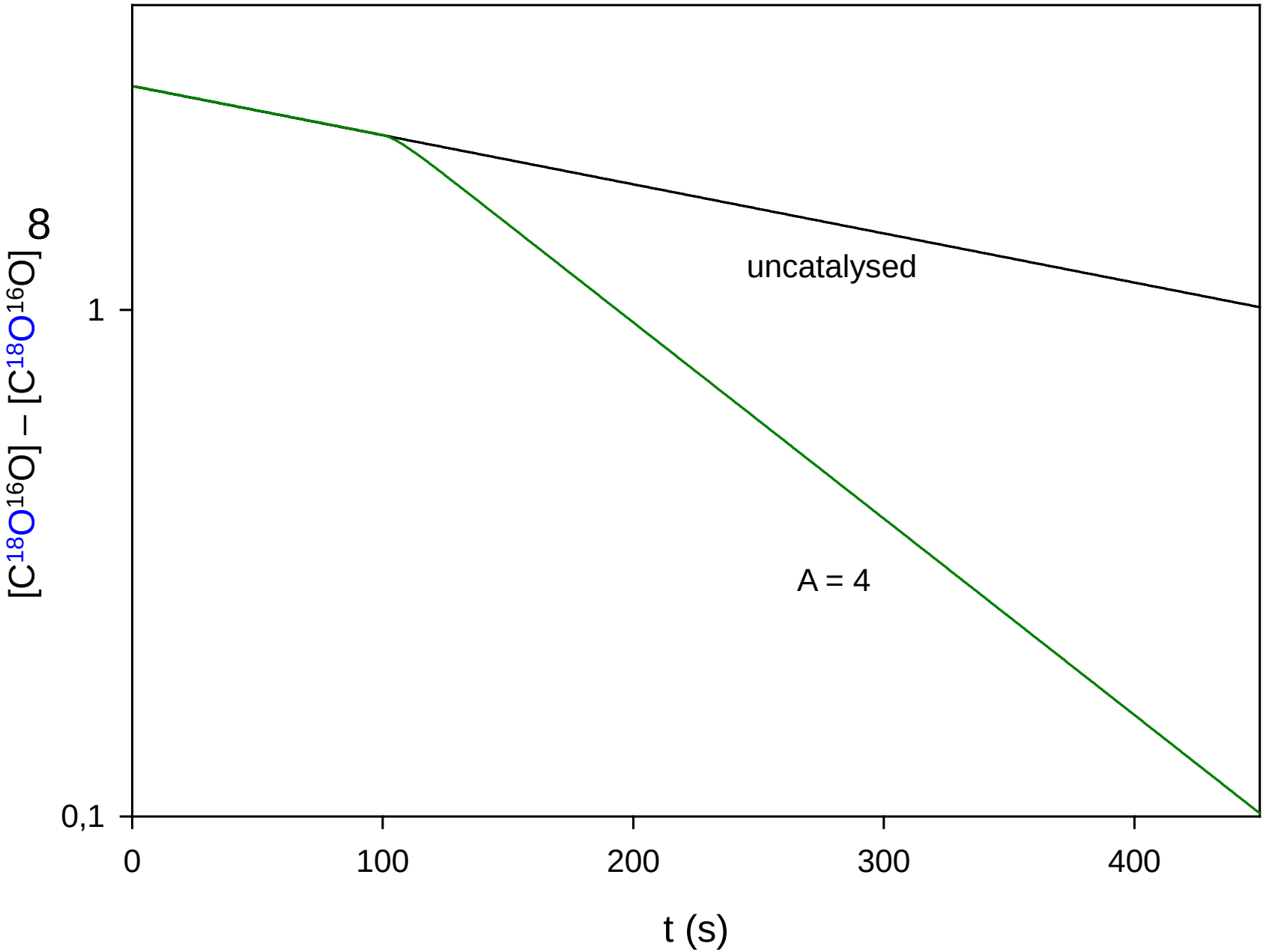
It is concluded that the process of ^{18}O exchange can be slowed down by orders of magnitude, because it is possible to use extremely small amounts of red cells and still obtain a well-defined and clear signal.

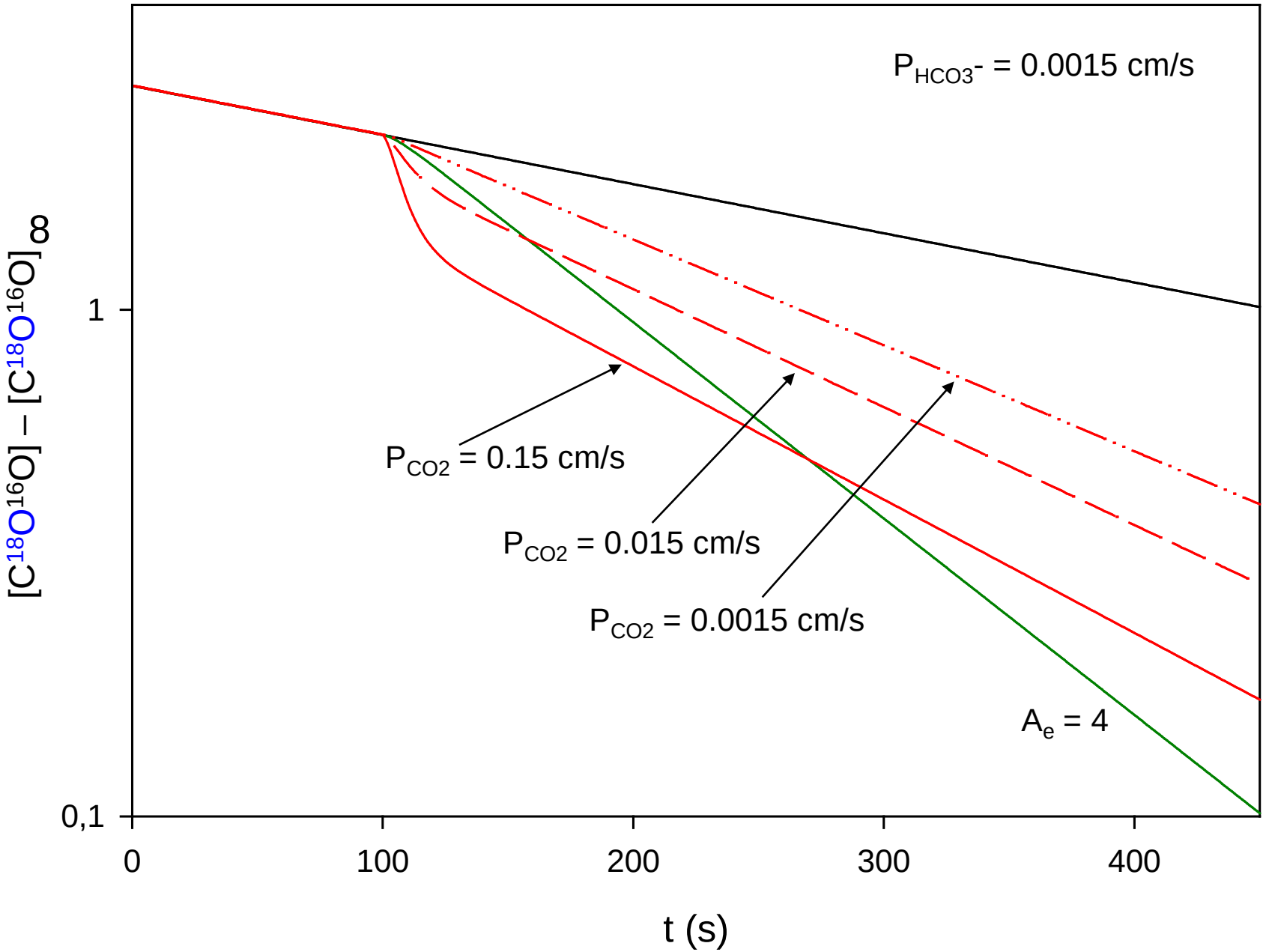
Also for this reason, the ^{18}O exchange technique allows us to observe fast processes such as the uptake of CO_2 by red cells on a very slow time scale.

How well are P_{CO2} and P_{HCO3-} defined by the experimental curves of decay of [$C^{18}O^{16}O$] ?

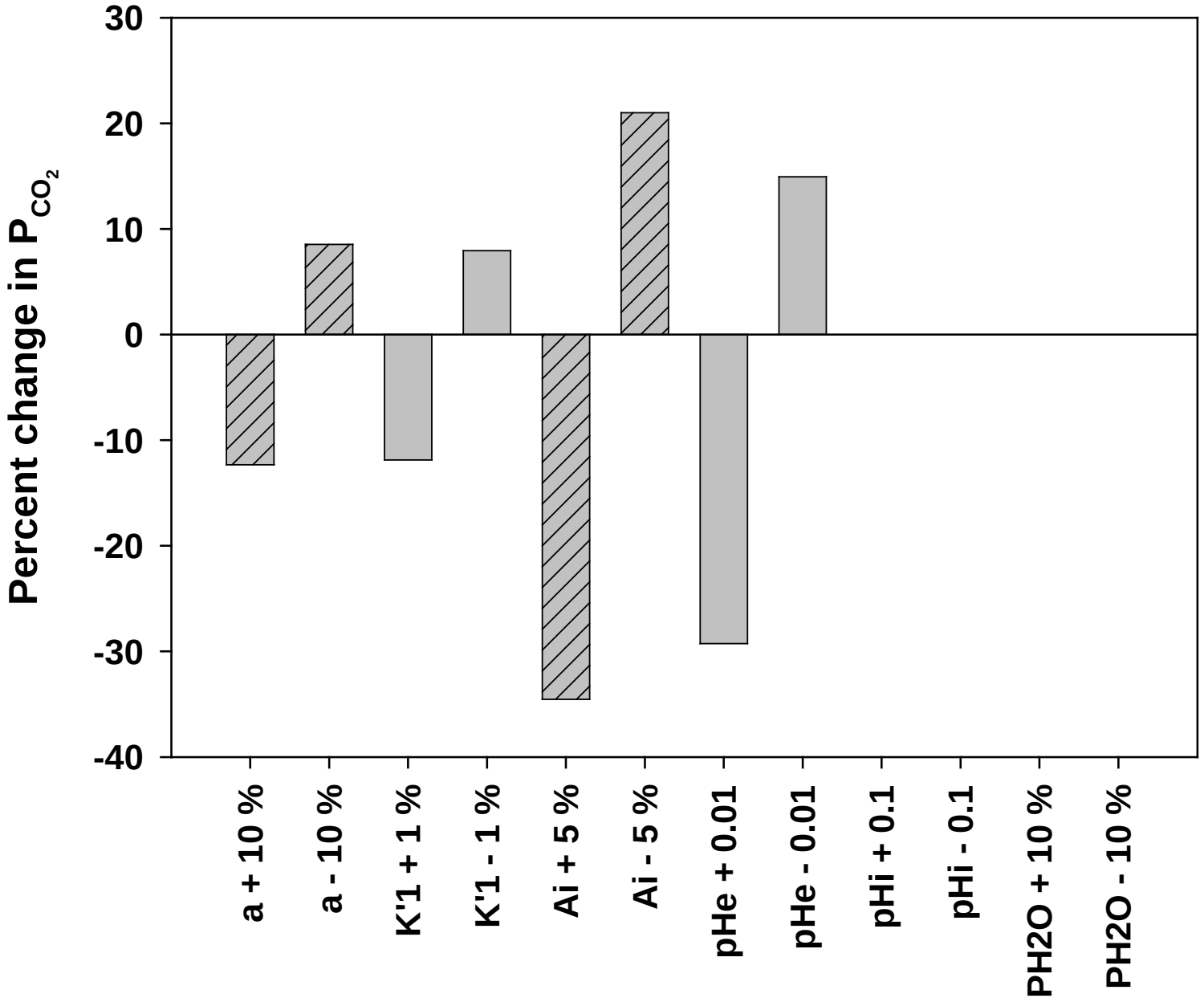
It was shown here that a well-defined minimum exists for both P_{HCO3-} and P_{CO2} in the sum of squares of deviations between the experimental data of [$C^{18}O^{16}O$] and those obtained from the best-fit calculation.

When P_{HCO3-} and P_{CO2} are varied over a wide range of values, clearly only one well-defined minimum is apparent and no local minima whatsoever are visible.


[C18O16O] – [C18O16O] 8
1
0,1
uncatalysed
A = 4
0
100
200
300
400
t (s)


P_{HCO3}- = 0.0015 cm/s
P_{CO2} = 0.15 cm/s
P_{CO2} = 0.015 cm/s
P_{CO2} = 0.0015 cm/s
A_e = 4
$[C^{18}O^{16}O] - [C^{18}O^{16}O]_B$
1
0,1
0
100
200
300
400
t (s)

Sensitivity of calculated P_{CO2} to parameter values


Percent change in P_{CO_2}
30
20
10
0
-10
-20
-30
-40
a + 10 %
a - 10 %
K'1 + 1 %
K'1 - 1 %
Ai + 5 %
Ai - 5 %
pHe + 0.01
pHe - 0.01
pHi + 0.1
pHi - 0.1
PH2O + 10 %
PH2O - 10 %

To what extent do unstirred layers around cells

affect the permeability determinations?

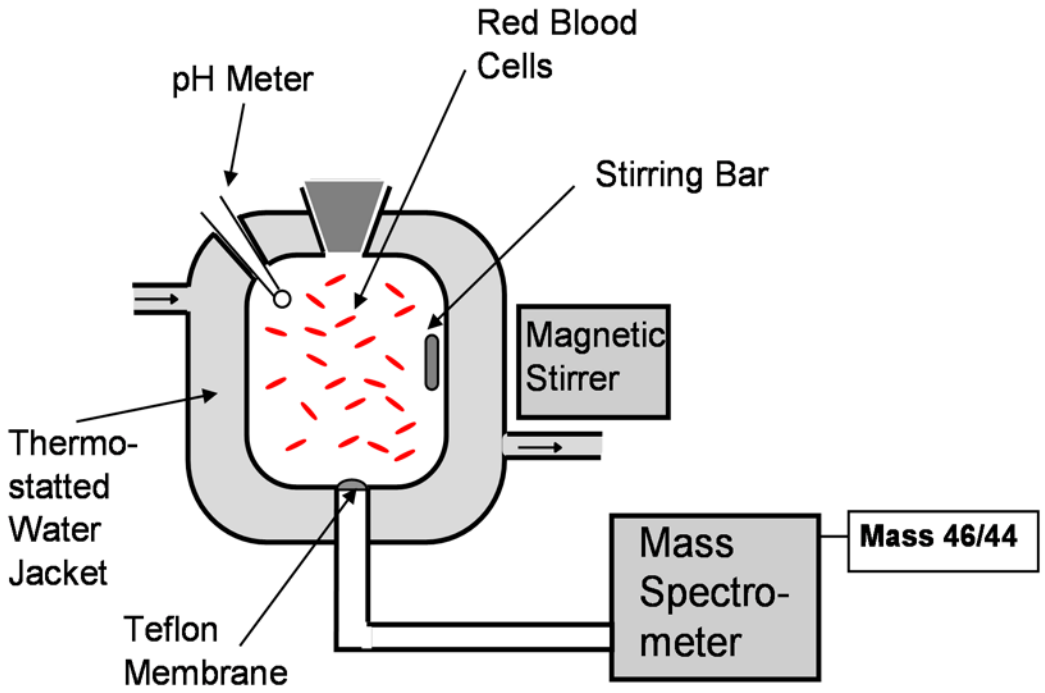
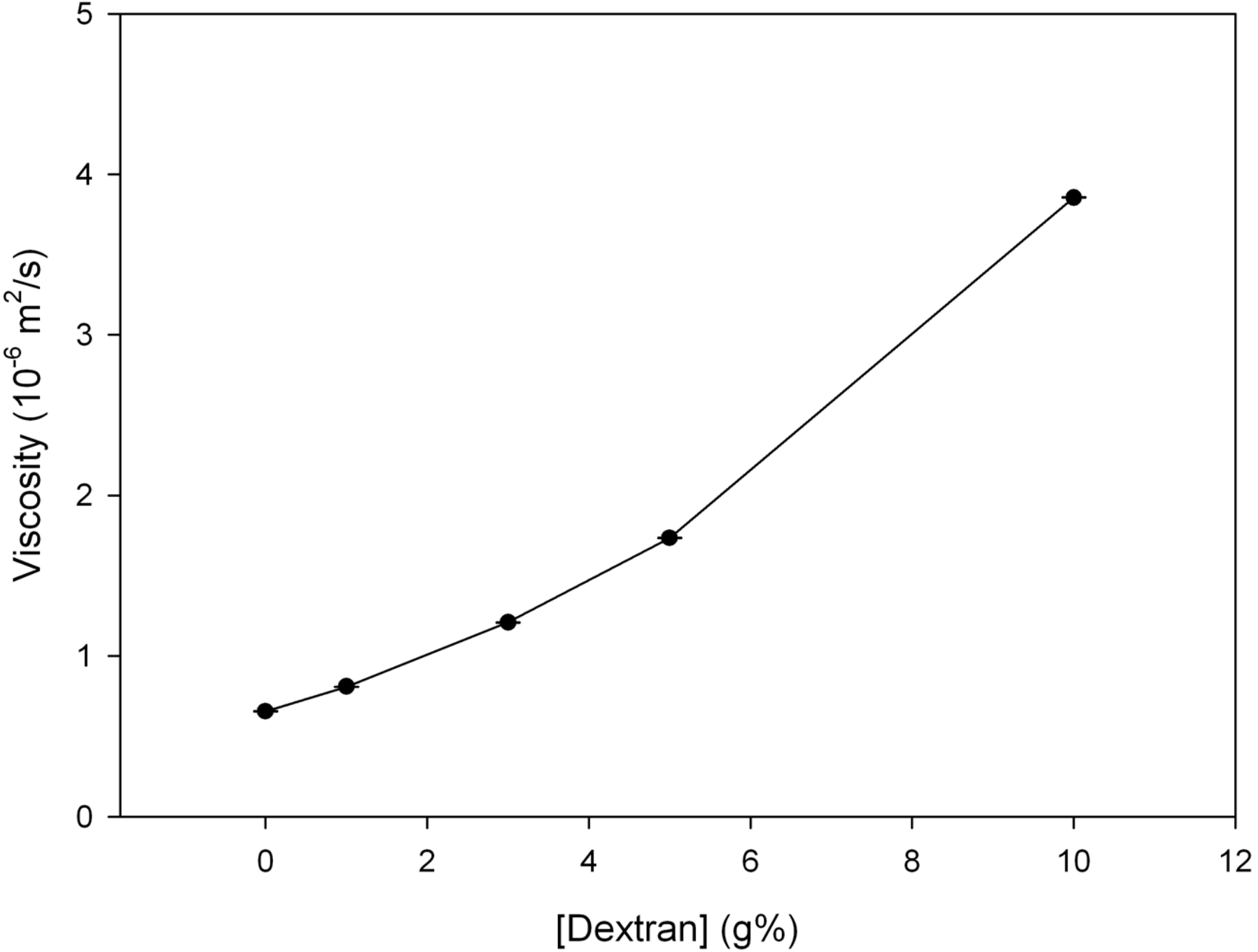

Red Blood
Cells
pH Meter
Stirring Bar
Magnetic
Stirrer
Thermo-
statted
Water
Jacket
Mass
Spectro-
meter
Mass 46/44
Teflon
Membrane

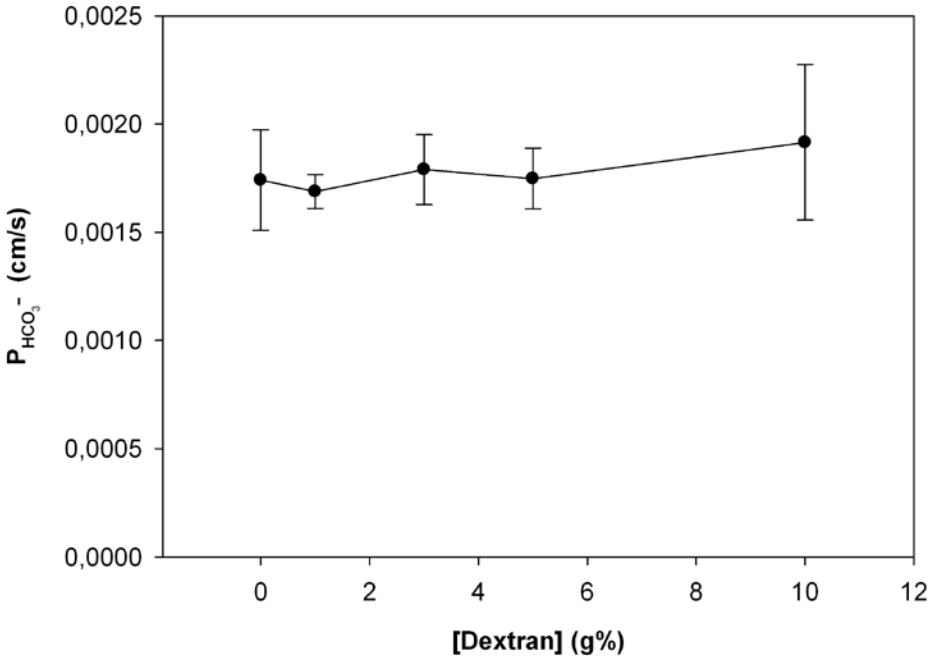
Fig. 1

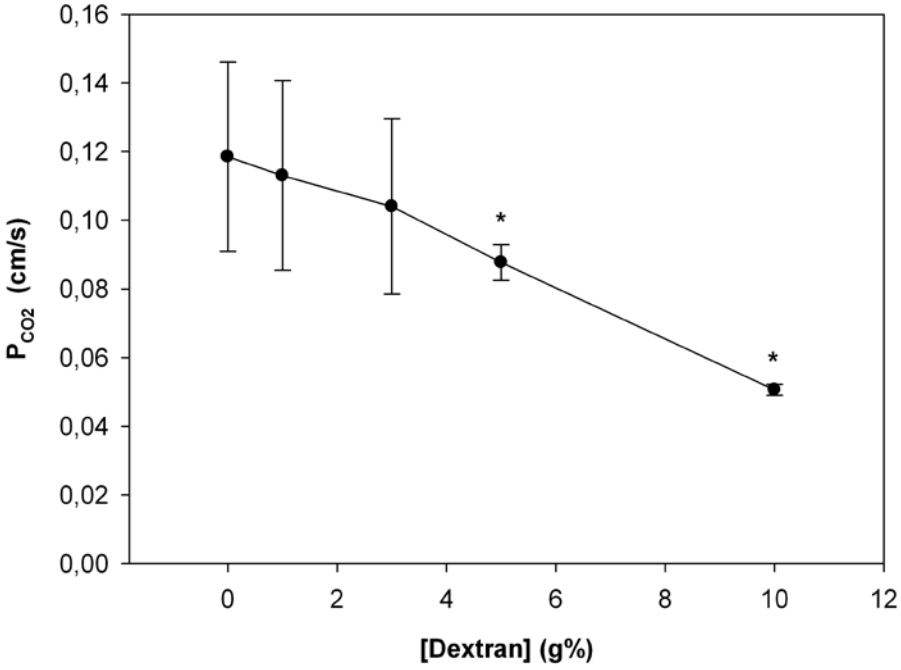
thickness of unstirred layer δ $\sim$

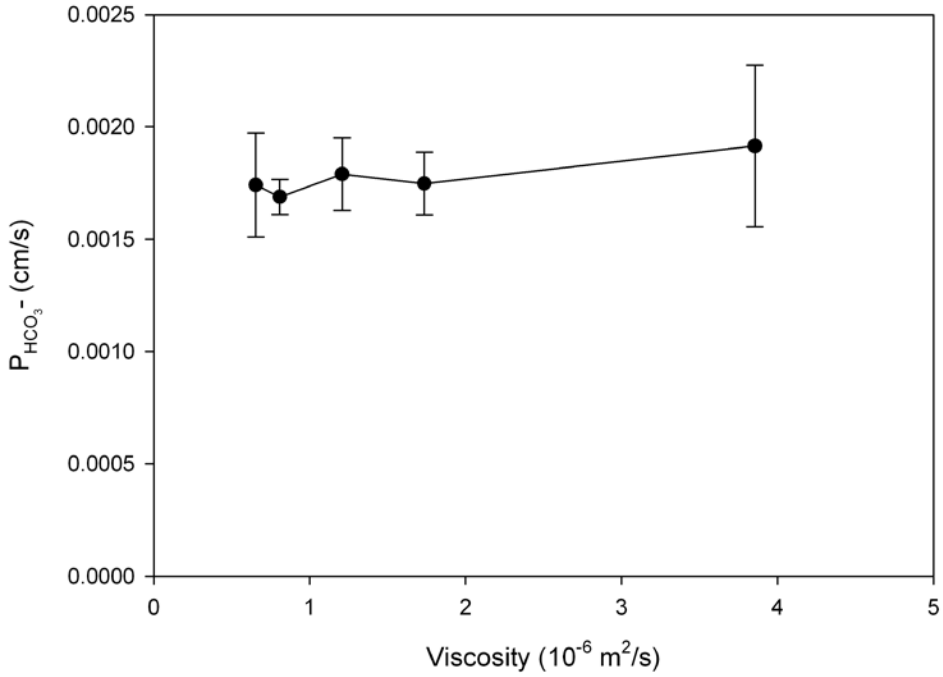
kinematic viscosity ν x $\sqrt{\text{cell diameter } \ell}$

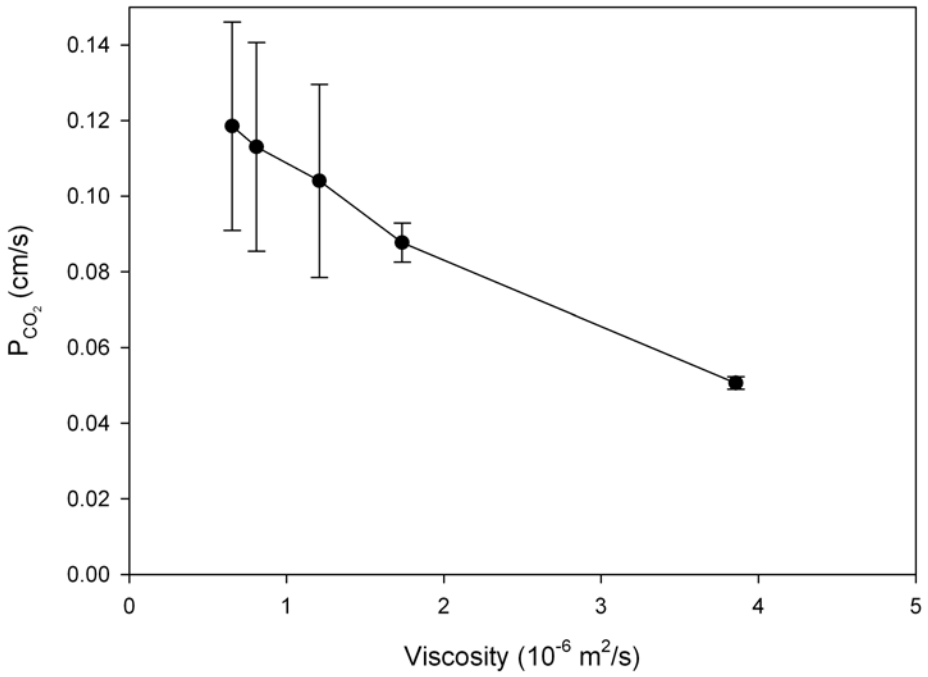
Landau LD and Lifschitz EM (1991): Hydrodynamics


5
4
3
2
1
0
Viscosity (10^{-6} m^2/s)
0
2
4
6
8
10
12
[Dextran] (g%)

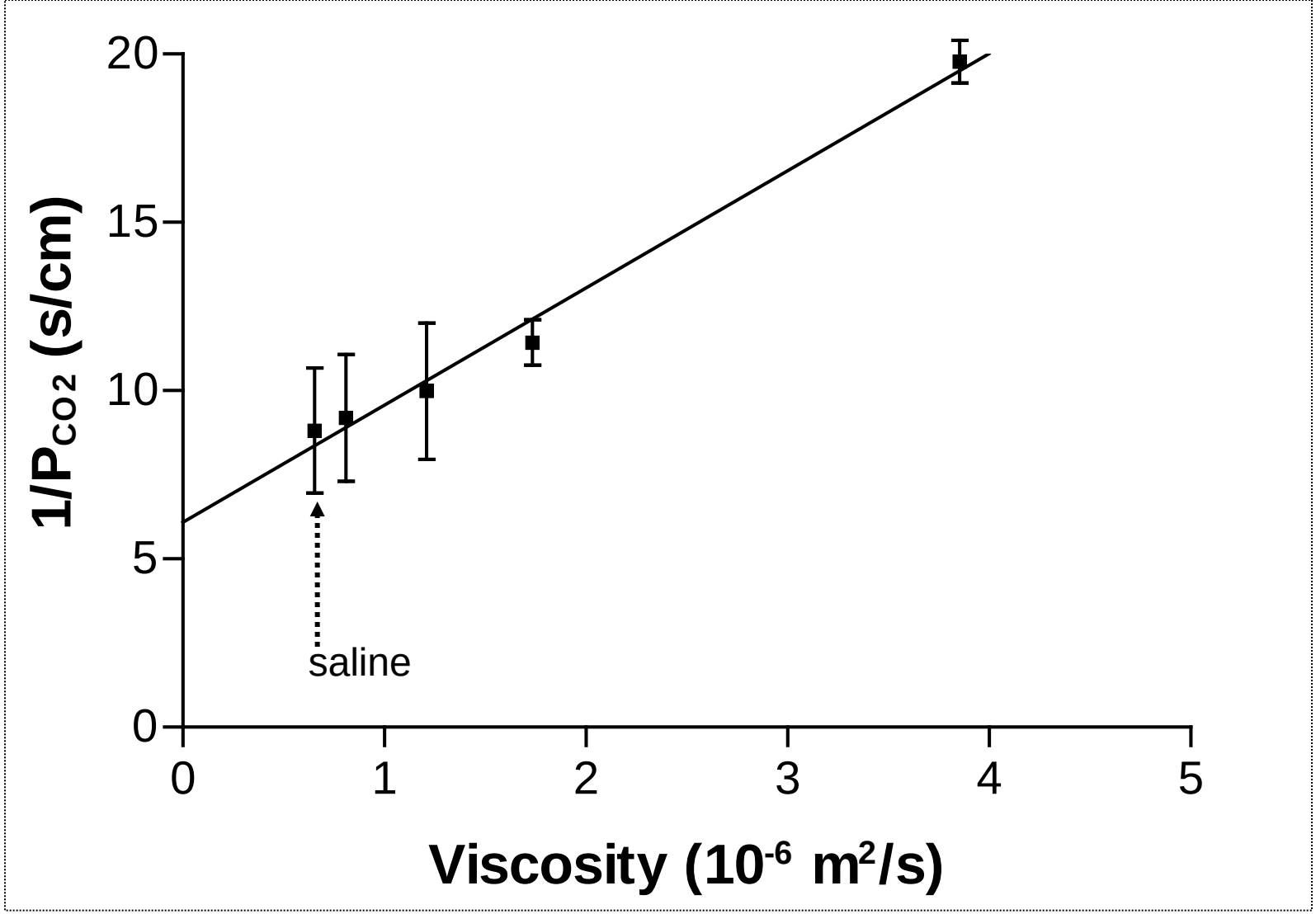

0,0025
0,0020
0,0015
0,0010
0,0005
0,0000
$P_{HCO_3^-}$ (cm/s)
0
2
4
6
8
10
12
[Dextran] (g%)


0,16
0,14
0,12
0,10
0,08
0,06
0,04
0,02
0,00
P_{CO2} (cm/s)
*
*
0
2
4
6
8
10
12
[Dextran] (g%)

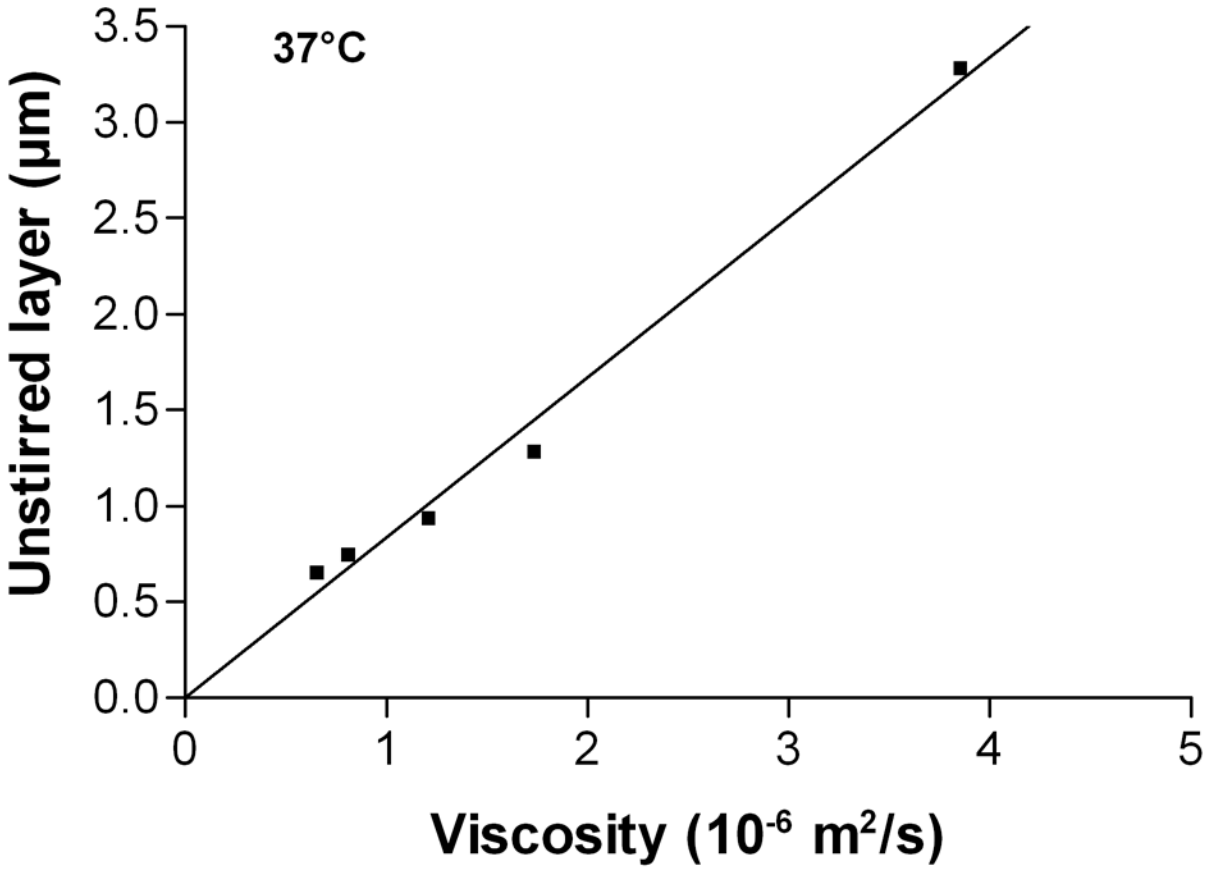




$$\frac{1}{P_{CO_2,app}} = \frac{1}{P_{CO_2,mem}} + \frac{\delta_{UL_e}}{D_{CO_2,solution}}$$



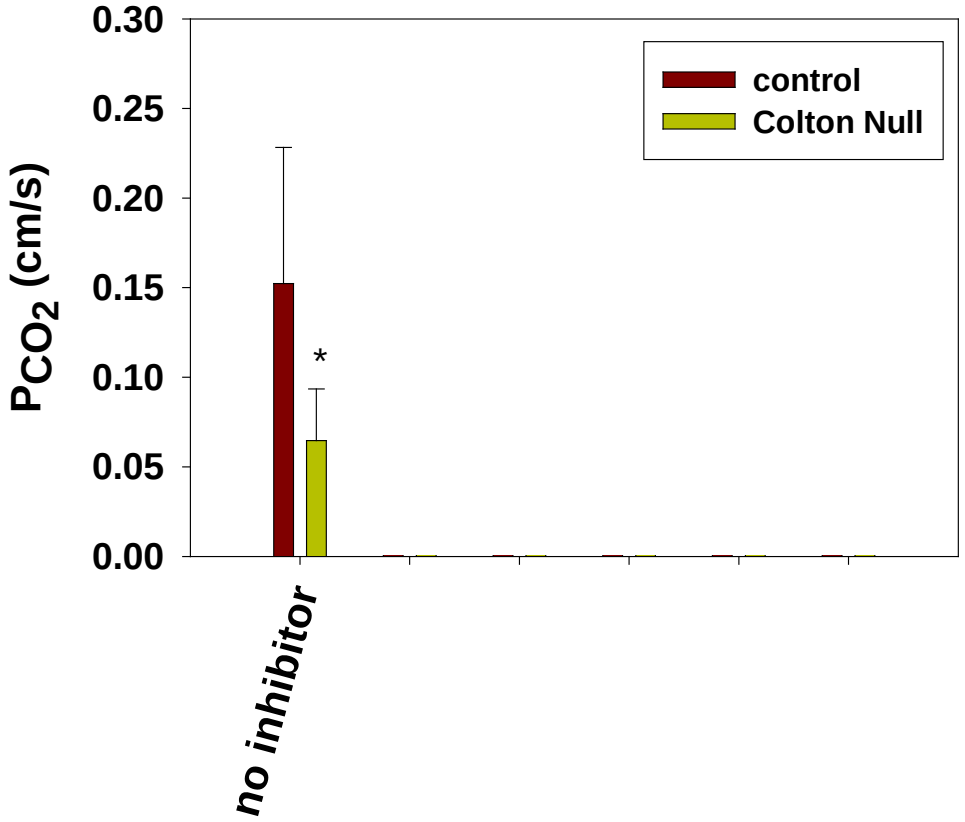
	P_{app} in saline (cm/s)	P_M (cm/s)	δ in saline (μm)
37 °C	0.12	0.16	0.5


37°C
Unstirred layer (µm)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
Viscosity (10-6 m2/s)

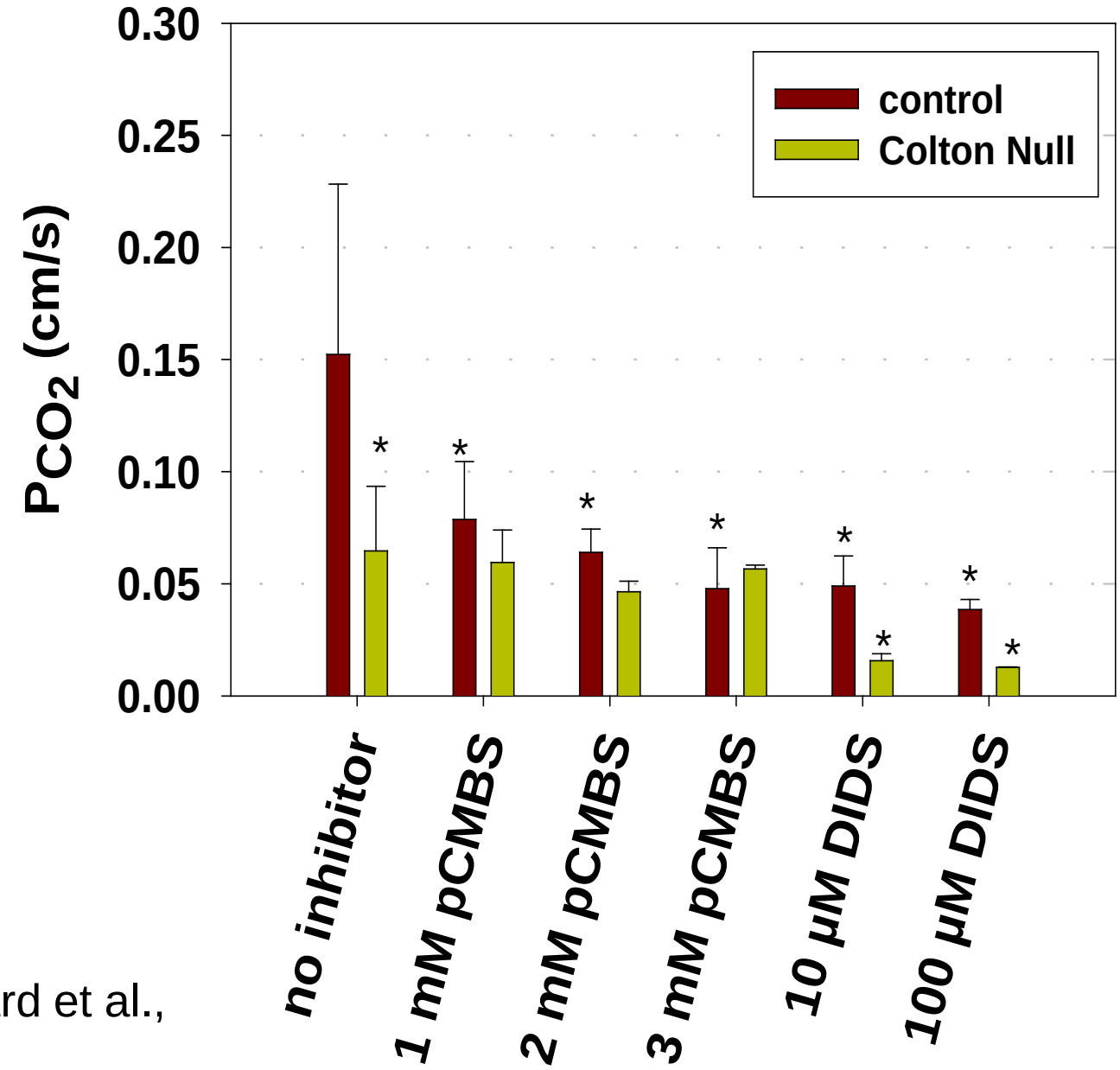
$$\delta \sim \nu$$

CO_2 Permeability of Normal and Deficient Human Red Blood Cells

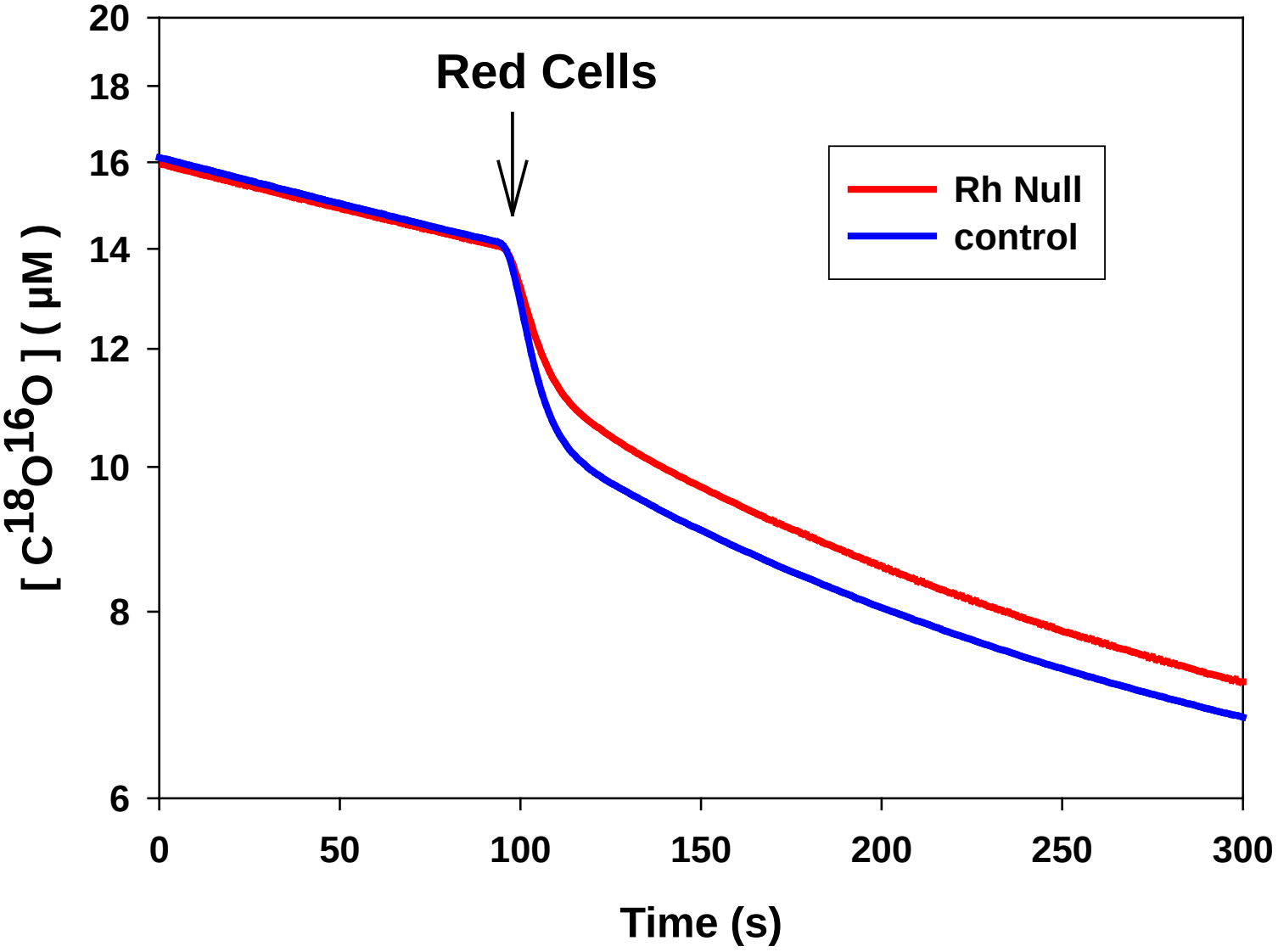
P_{CO2} of control and AQP1 deficient (Colton null) human red blood cells



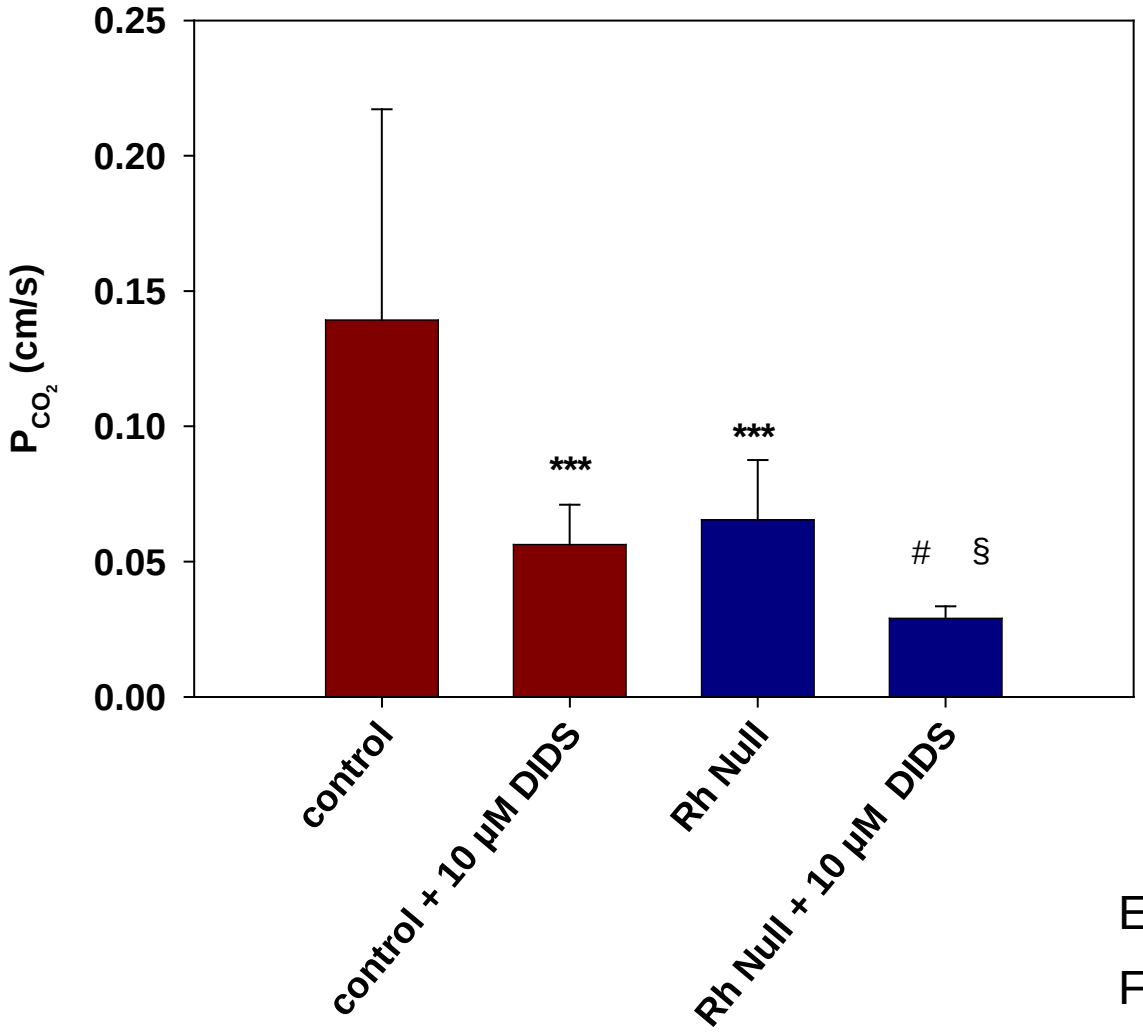
Endeward et al., FASEB J, 2006



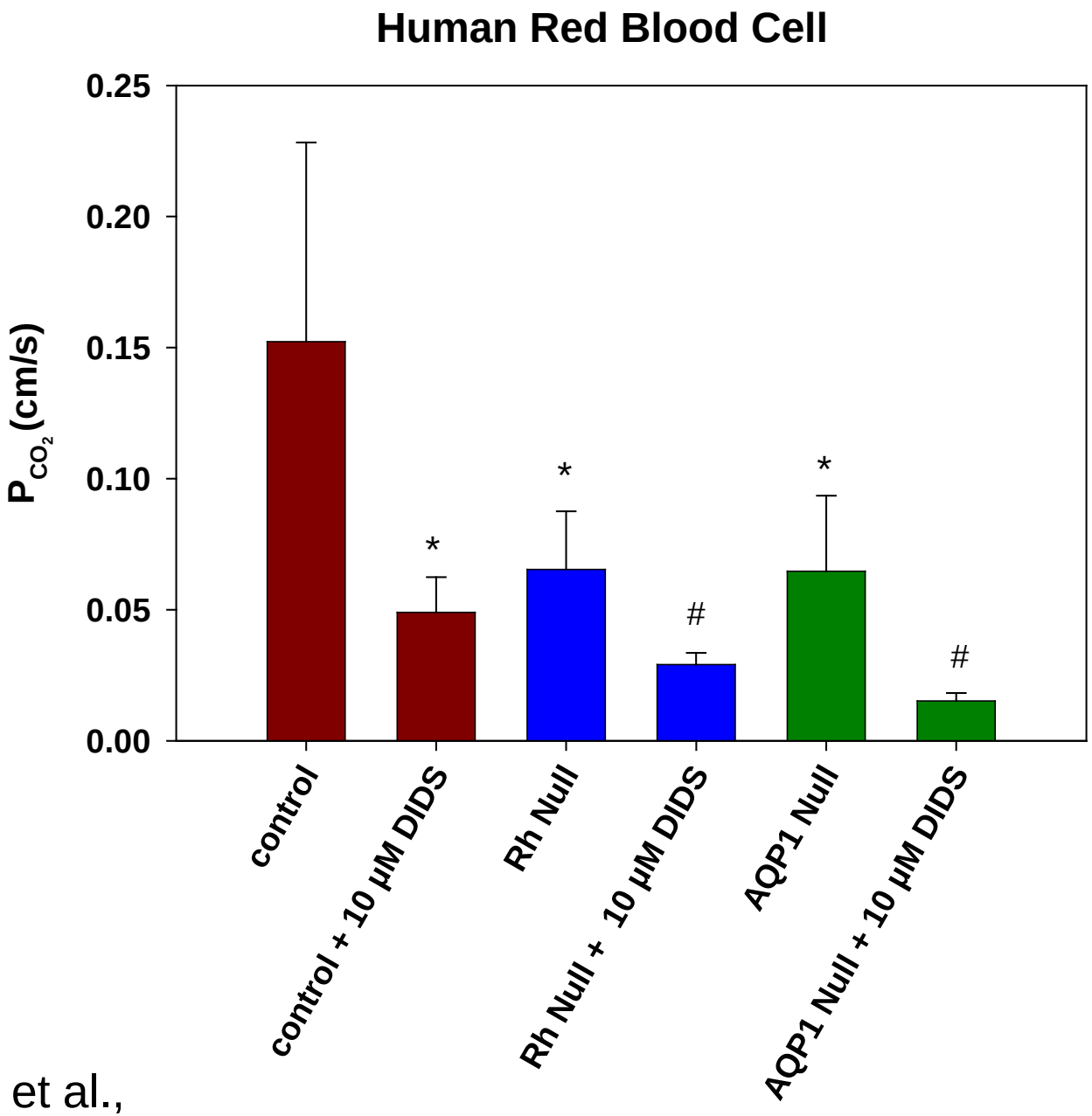
Endeward et al., 2006


Red Cells
Rh Null
control
[C18O16O] (µM)
Time (s)
20
18
16
14
12
10
8
6
0
50
100
150
200
250
300

P_{CO2} of control and Rhesus null human red blood cells



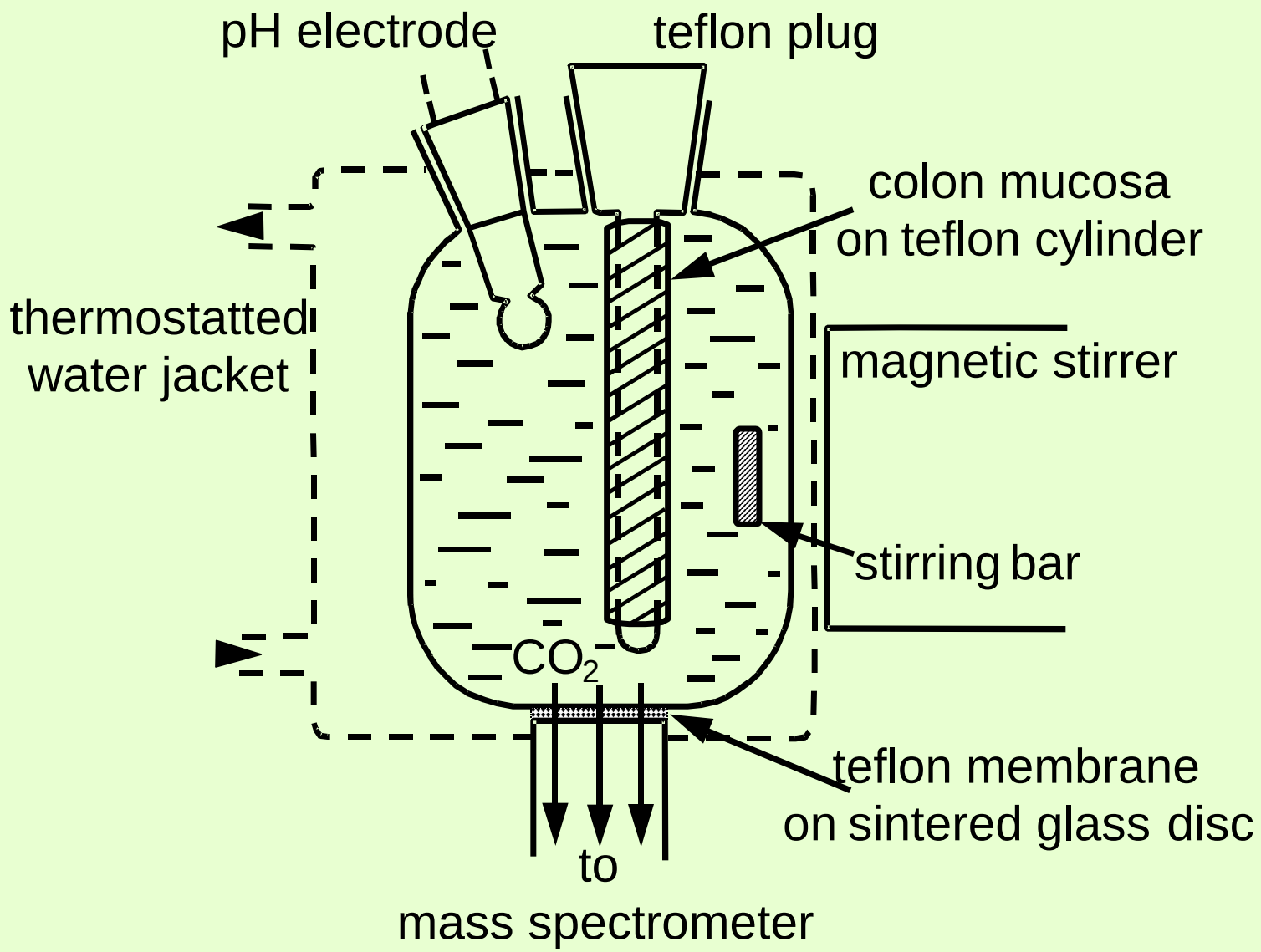
Endeward et al.,
FASEB J, 2008

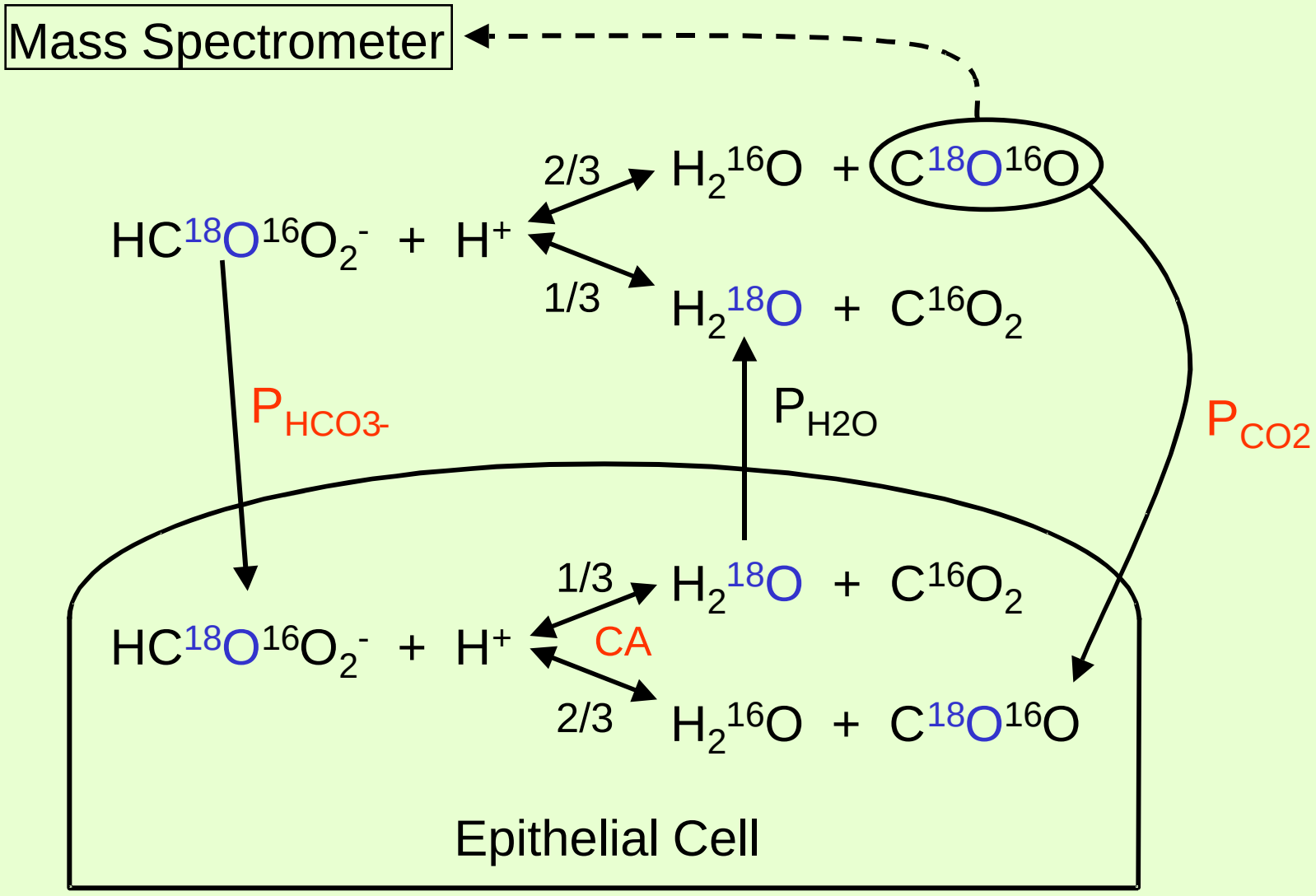


Endeward et al.,
2006, 2008

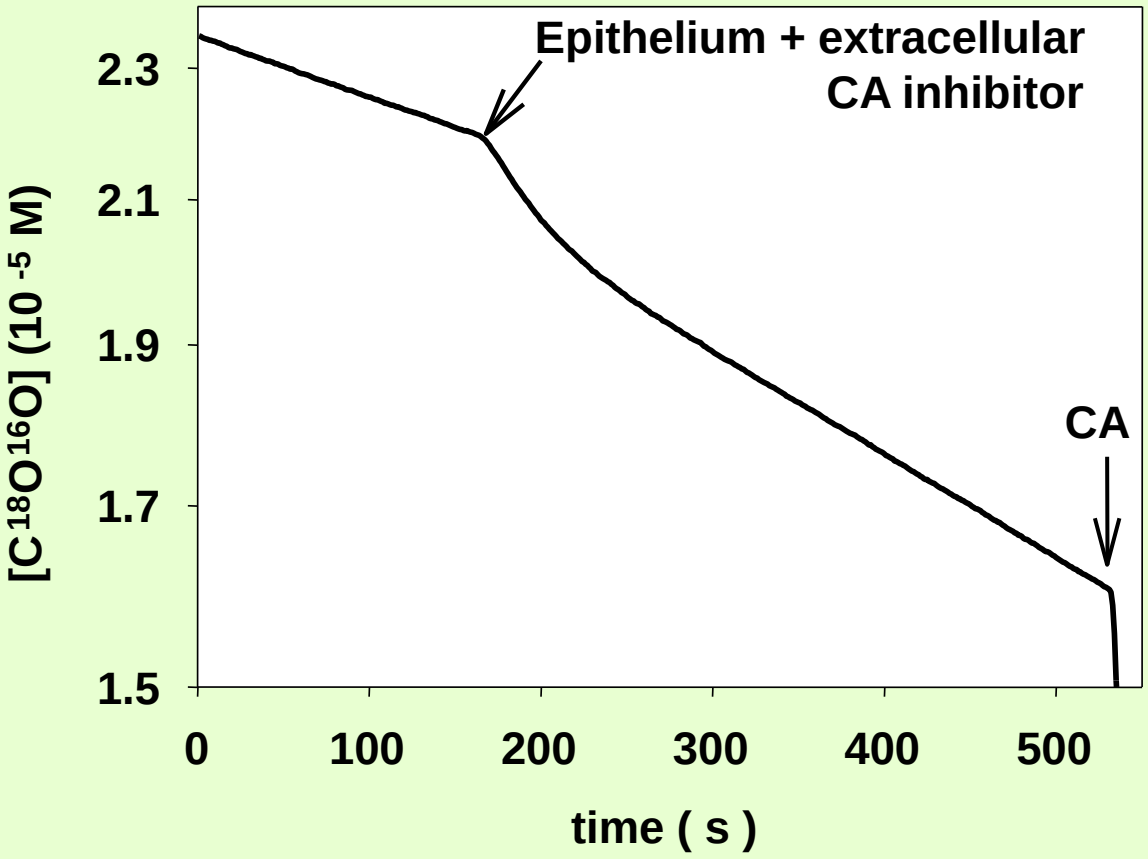
Applying the ^{18}O technique to measure the CO_2 permeability of the apical membrane of intact colon epithelium


100 µm


pH electrode
teflon plug
colon mucosa
on teflon cylinder
thermostatted
water jacket
magnetic stirrer
stirring bar
CO_2
teflon membrane
on sintered glass disc
to
mass spectrometer


Mass Spectrometer
2/3
$H_2{}^{16}O$ + $C^{18}O^{16}O$
$HC^{18}O^{16}O_2^-$ + H^+
1/3
$H_2{}^{18}O$ + $C^{16}O_2$
P_{HCO3-}
P_{H2O}
P_{CO2}
1/3
$H_2{}^{18}O$ + $C^{16}O_2$
$HC^{18}O^{16}O_2^-$ + H^+
CA
2/3
$H_2{}^{16}O$ + $C^{18}O^{16}O$
Epithelial Cell

Intact Proximal Epithelium
Apical Side



CO_2 and HCO_3^- Permeability of the Apical Membrane of Intact Guinea Pig Colon

	P_{CO2} ± SD (cm/s)	P_{HCO3^-} (cm/s)	A_{in}	n
Intact Proximal Colon	**0.0015** ± 0.0007	**$6.3 \cdot 10^{-4}$** ± $4.0 \cdot 10^{-4}$	**41 000**	**40**
Intact Distal Colon	**0.00077** ± 0.00021	**$0.87 \cdot 10^{-4}$** ± $0.56 \cdot 10^{-4}$	**900**	**23**

Endeward & Gros, 2005

Conclusions

The ^{18}O exchange technique follows the decay of ^{18}O-labelled CO_2 in the extracellular fluid by mass spectrometry

This is possible because this decay is 1.000-10.000 times slower than net CO_2 uptake by cells or vesicles

The system of differential equations describing this process yields values of P_{CO2} and P_{HCO3-} from well defined minima of a fitting procedure

P_{CO2} values can be determined over a range of 3-4 orders of magnitude

Parameters critical für calculation of P_{CO2} and P_{HCO3-} are intracellular CA activity and extracellular pH, both of which are carefully controlled

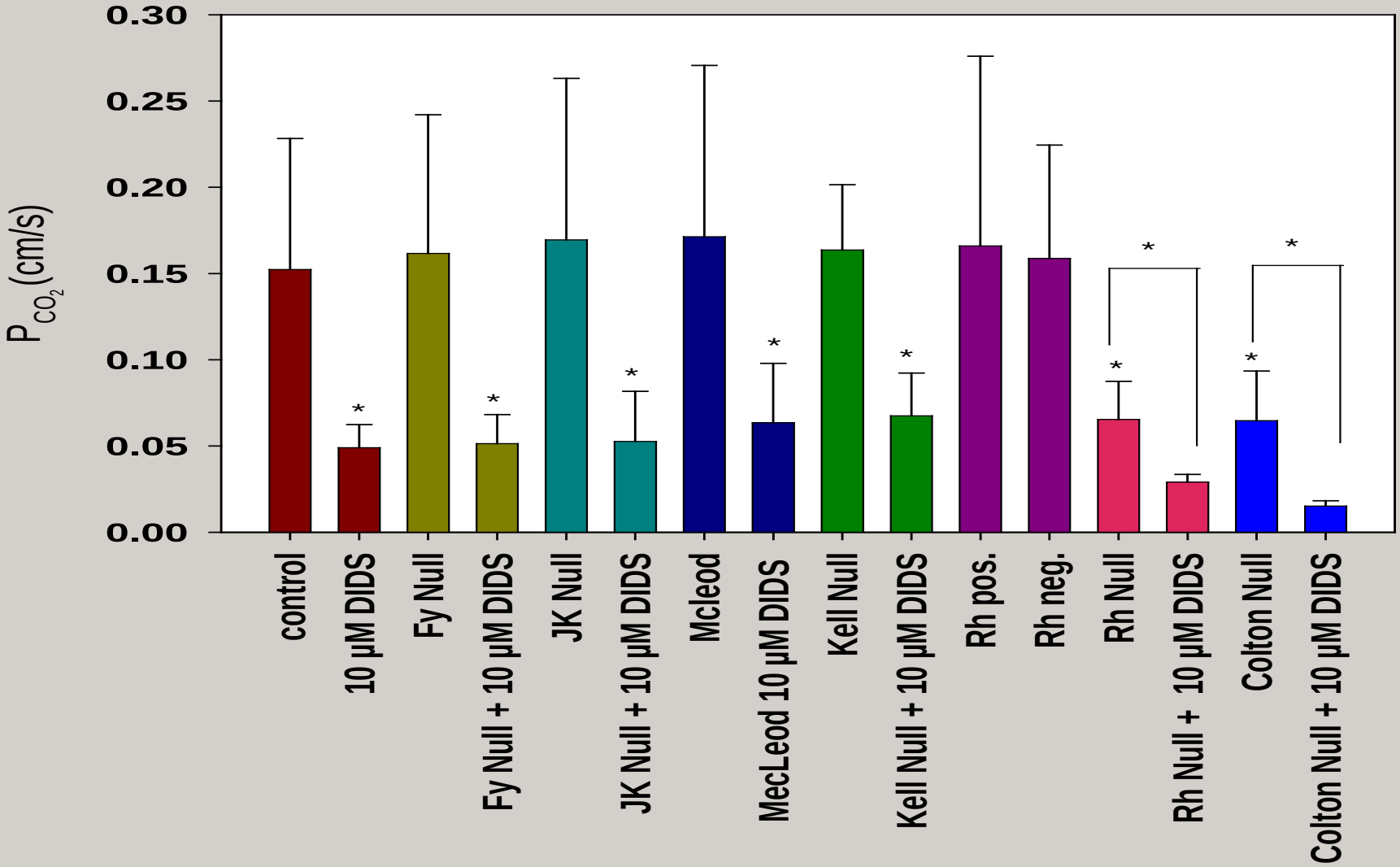
Unstirred layers affect the results by no more than ~ 20%

The method is applicable to suspensions of isolated cells or vesicles and to intact epithelia

Intrinsic CO_2 permeability of cell membranes and role of CO_2 channels

Volker Endeward, Fabian Itel, Samer Al-Samir, Mohamed Chami, Fredrik Öberg, Kristina Hedfalk, Gerolf Gros

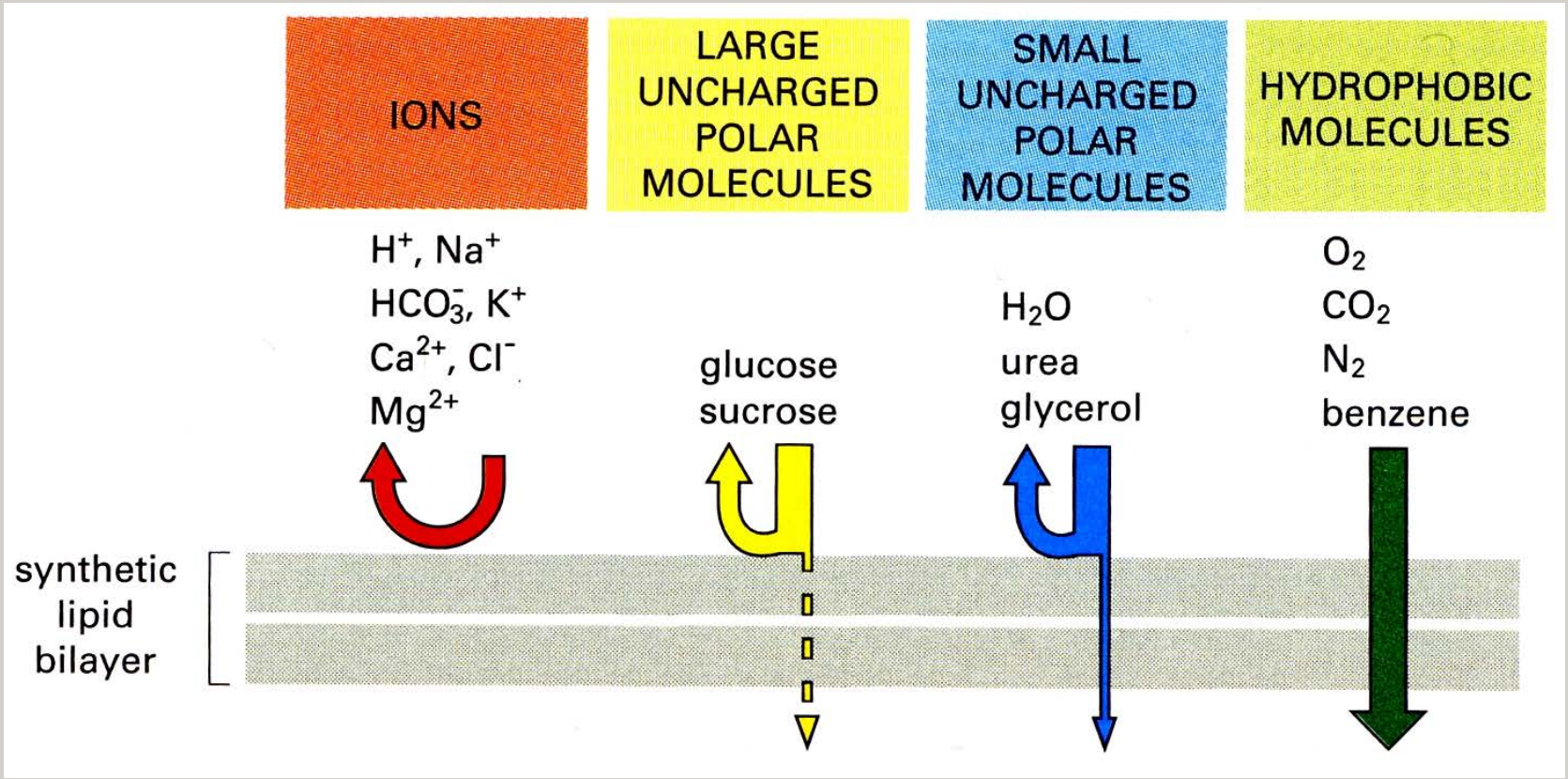
Medizinische Hochschule Hannover


0.30
0.25
0.20
0.15
0.10
0.05
0.00
P_{CO_2} (cm/s)
control
10 µM DIDS
Fy Null
Fy Null + 10 µM DIDS
JK Null
JK Null + 10 µM DIDS
Mcleod
MecLeod 10 µM DIDS
Kell Null
Kell Null + 10 µM DIDS
Rh pos.
Rh neg.
Rh Null
Rh Null + 10 µM DIDS
Colton Null
Colton Null + 10 µM DIDS
*

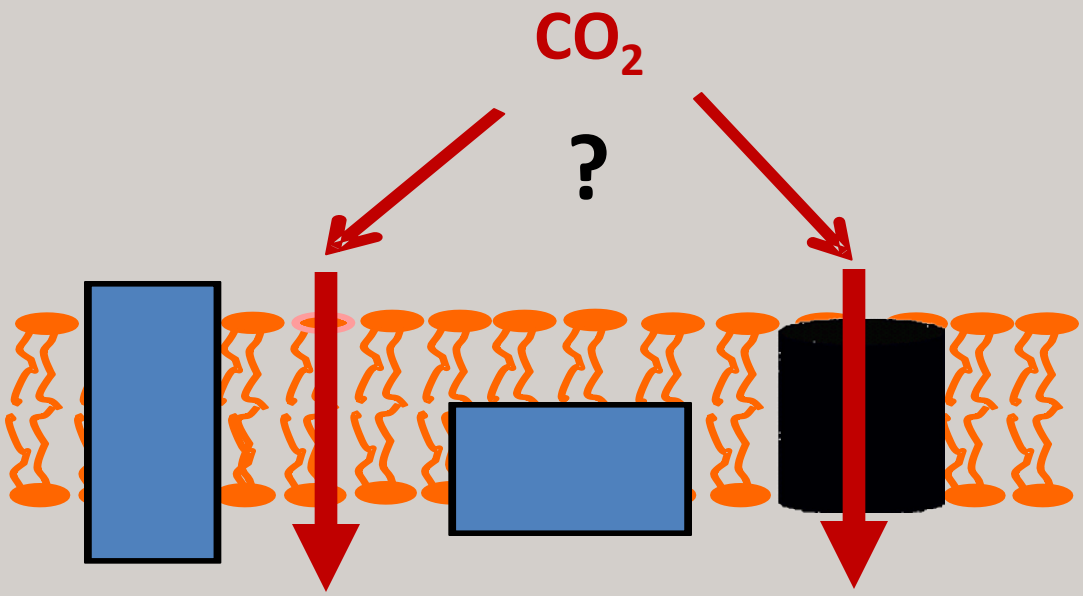
Intrinsic CO_2 permeability of a red cell membrane

	P_{CO2} (cm/s)
Red cell	**0.15** ± 0.08
Red cell ∅ AQP1, ∅ functional Rh	**0.015** ± 0.003

Gas permeability of synthetic phospholipid bilayers



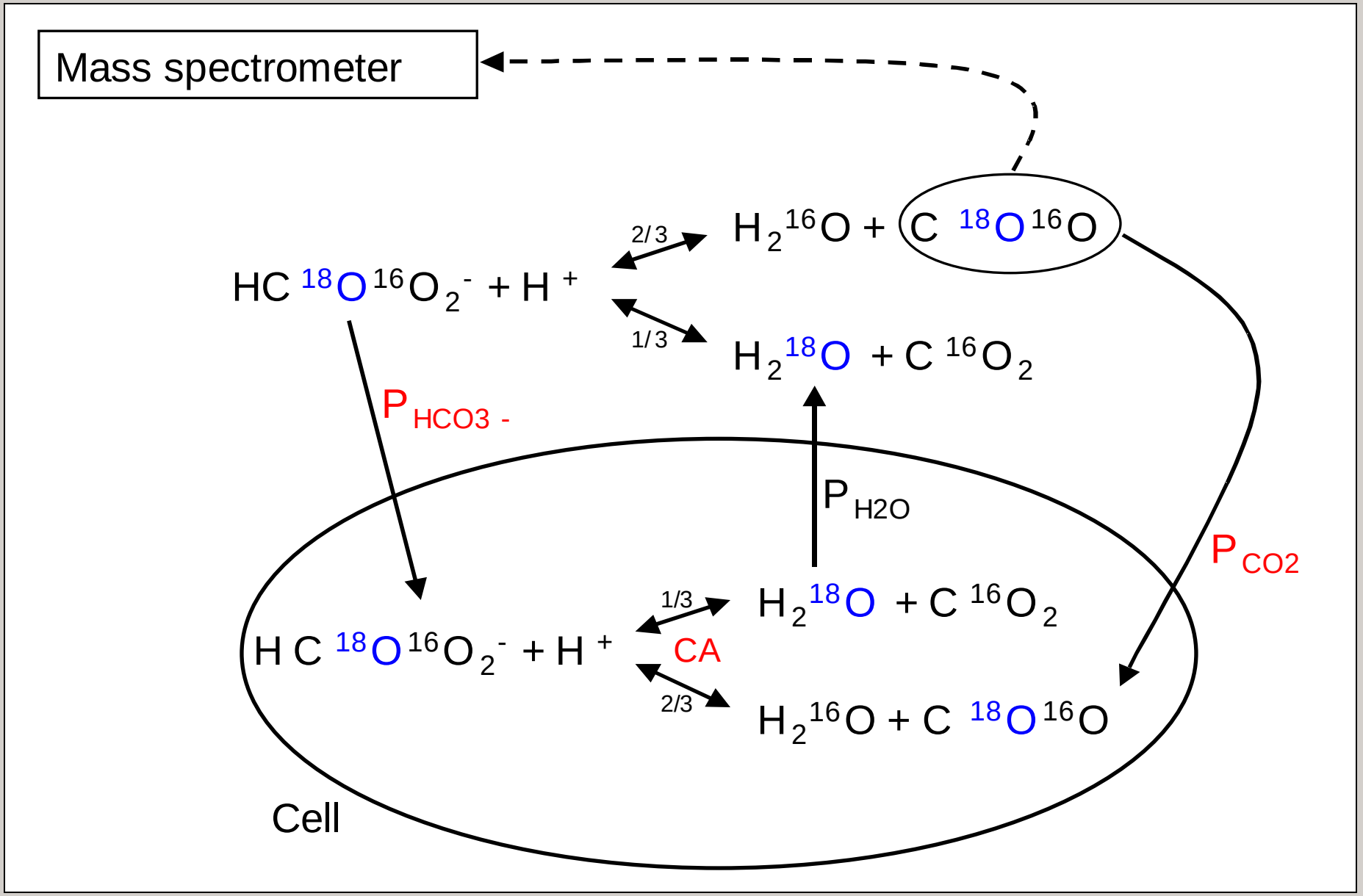
Alberts et al.
Molecular Biology Of The Cell, 4th Edition



1. What are the intrinsic CO_2 permeabilities of cell membranes?

2. Which mechanisms are responsible for the given intrinsic permeabilities of cell membranes?

1. What are the intrinsic CO_2 permeabilities of cell membranes?


Mass spectrometer
2/3
1/3
$H_2{}^{16}O + C\,{}^{18}O\,{}^{16}O$
$HC\,{}^{18}O\,{}^{16}O_2^- + H^+$
$H_2{}^{18}O + C\,{}^{16}O_2$
P_{HCO3-}
P_{H2O}
P_{CO2}
1/3
2/3
$H_2{}^{18}O + C\,{}^{16}O_2$
$H\,C\,{}^{18}O\,{}^{16}O_2^- + H^+$
CA
$H_2{}^{16}O + C\,{}^{18}O\,{}^{16}O$
Cell

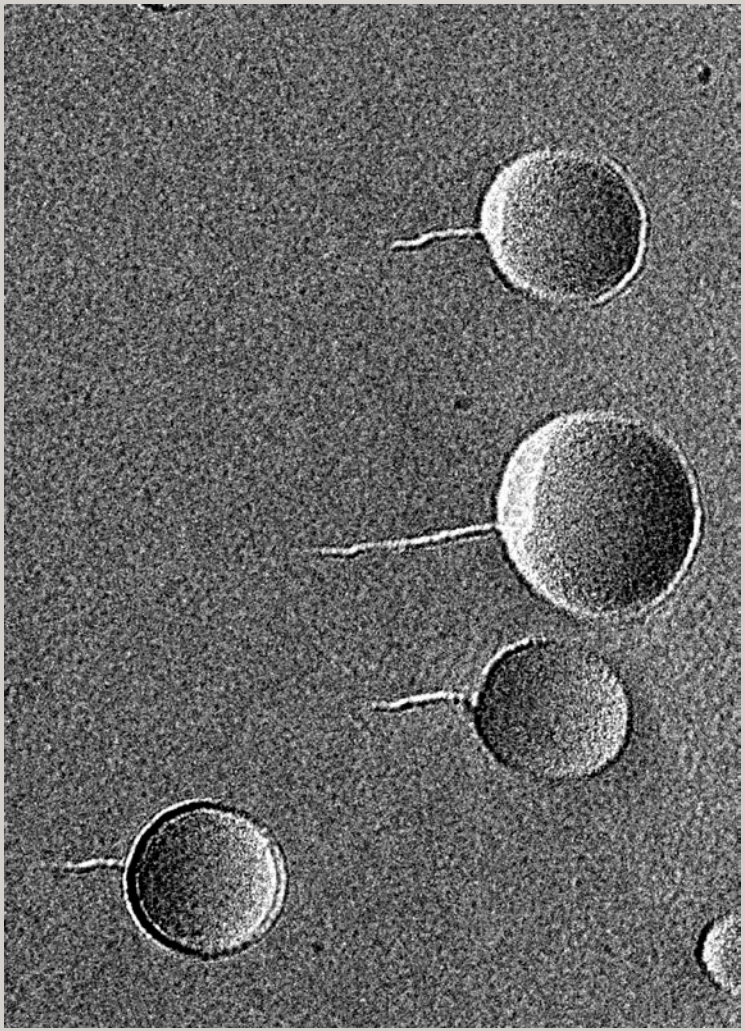
Cell membranes show CO_2 permeabilities lower then synthetic lipid bilayer

	P_{CO2} (cm/s) ± S.D.
Synthetic lipid bilayer	0.35 - 3.2
Red cell, ∅ functional gas channel	0.015 ± 0.003
MDCK	0.017 ± 0.004
tsA201	0.007 ± 0.003
Basolateral membrane of proximal colon epithelium	~ 0.022
Apical membrane of proximal colon epithelium	0.0015 ± 0.0006

2. Which mechanisms are responsible for the given intrinsic permeabilities of cell membranes?

Parameter studied	Cholesterol fraction of total bilayer lipids (mol %)	Ratio of parameter w over w/o cholesterol	
P_{NH3}	30 %	0.31	Antonenko et al. 1997
P_{NH3}	52 %	0.012	Hill & Zeidel 2000
$P_{H2O\ (f)}$	40 %	0.18	Lande et al. 1995
$P_{H2O\ (f)}$	52 %	0.026	Hill & Zeidel 2000
$P_{H2O\ (d)}$	66 % (L+Chol)	0.26	Finkelstein 1976
"	66 % (SM + Chol)	0.037	"

PC:PS:Chol – vesicles with different cholesterol content

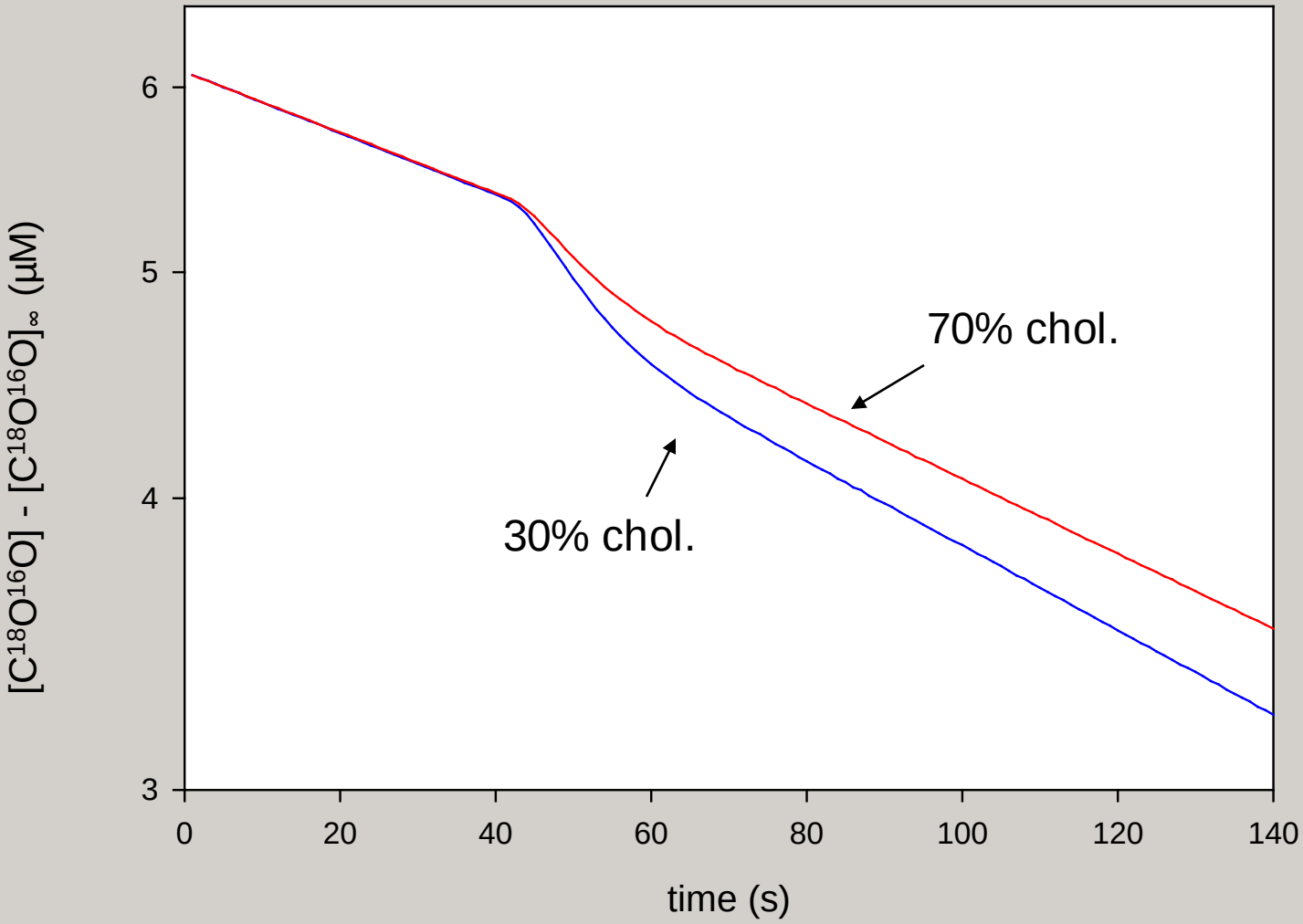


PC = Phosphatidylcholine

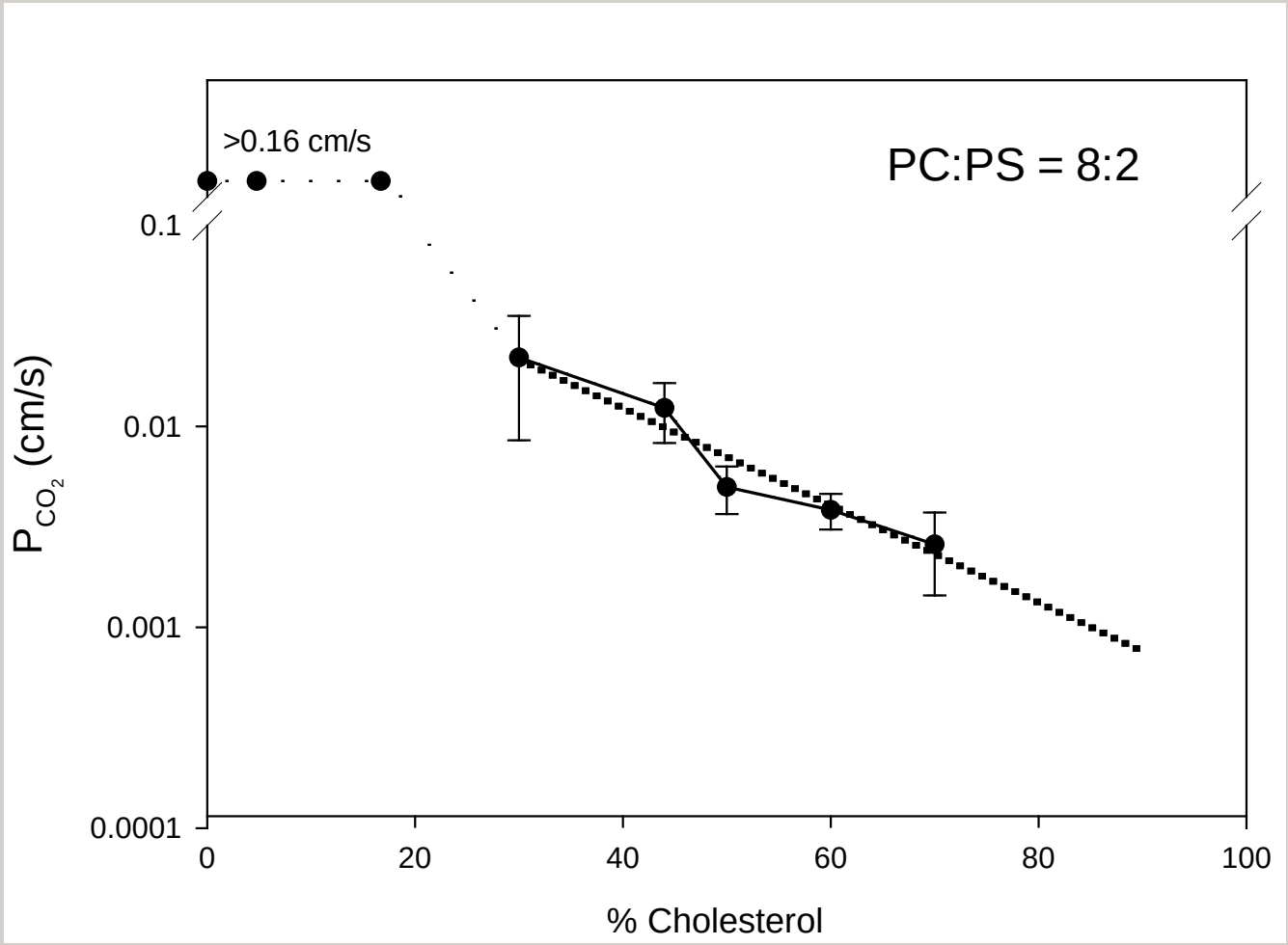
PS = Phosphatidylserine

Chol = Cholesterol (0 – 70%)

Ø = ~ 150 nm


6
5
4
3
0
20
40
60
80
100
120
140
time (s)
[C18O16O] - [C18O16O]∞ (µM)
70% chol.
30% chol.

Effect of cholesterol on lipid vesicle CO_2 permeability

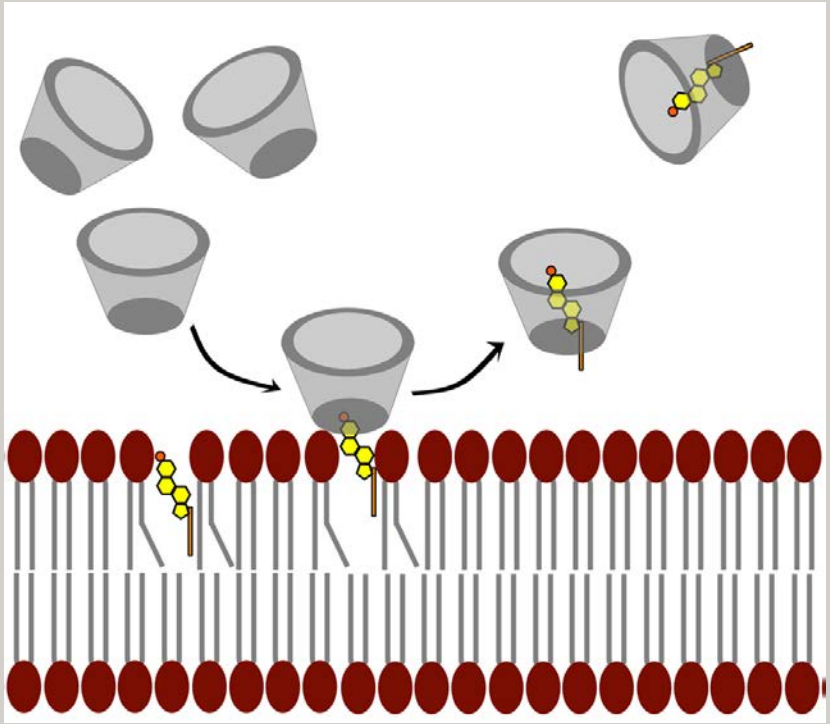


Comparison of cell membranes and cholesterol-containing vesicles

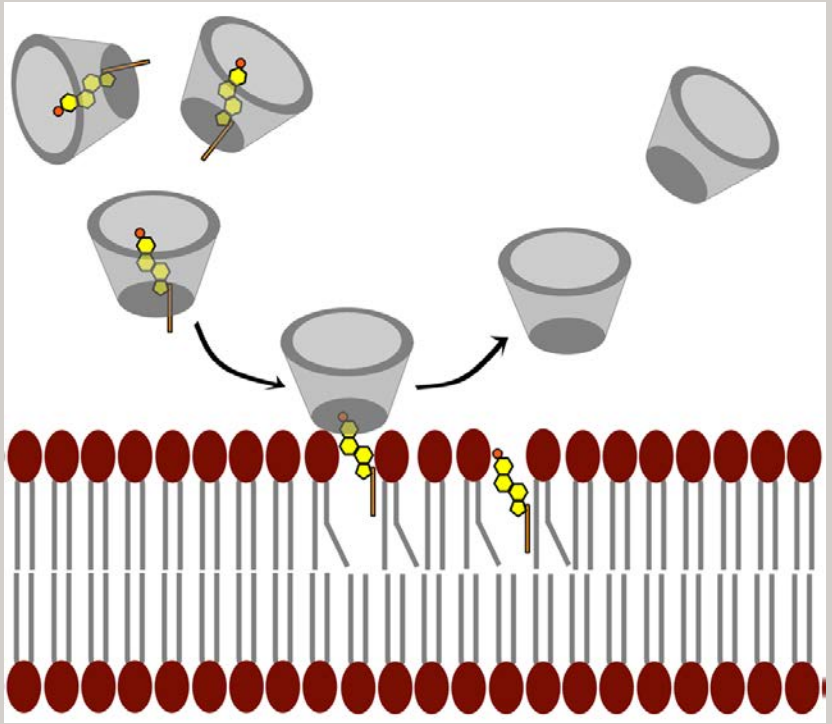
	P_{CO2} (cm/s) ± S.D	Cholesterol content (Mol%)	P_{CO2} predicted from cholesterol effect in vesicles (cm/s)
Lipid bilayer	**0.35 / 3.2**		**-**
Red cell: ∅ AQP1, ∅ functional Rh	**0.015** ± 0.003	45	**0.010**
MDCK	**0.017** ± 0.004	37	**0.015**
tsA201	**0.007** ± 0.003	-	**-**
Basolateral membrane prox colon epithelium	**~ 0.022**	42	**0.011**
Apical membrane of prox colon epithelium	**0.0015** ± **0.0006**	77	**0.0016**

CO_2 permeabilities of cell membranes appear to be essentially determined by their cholesterol content

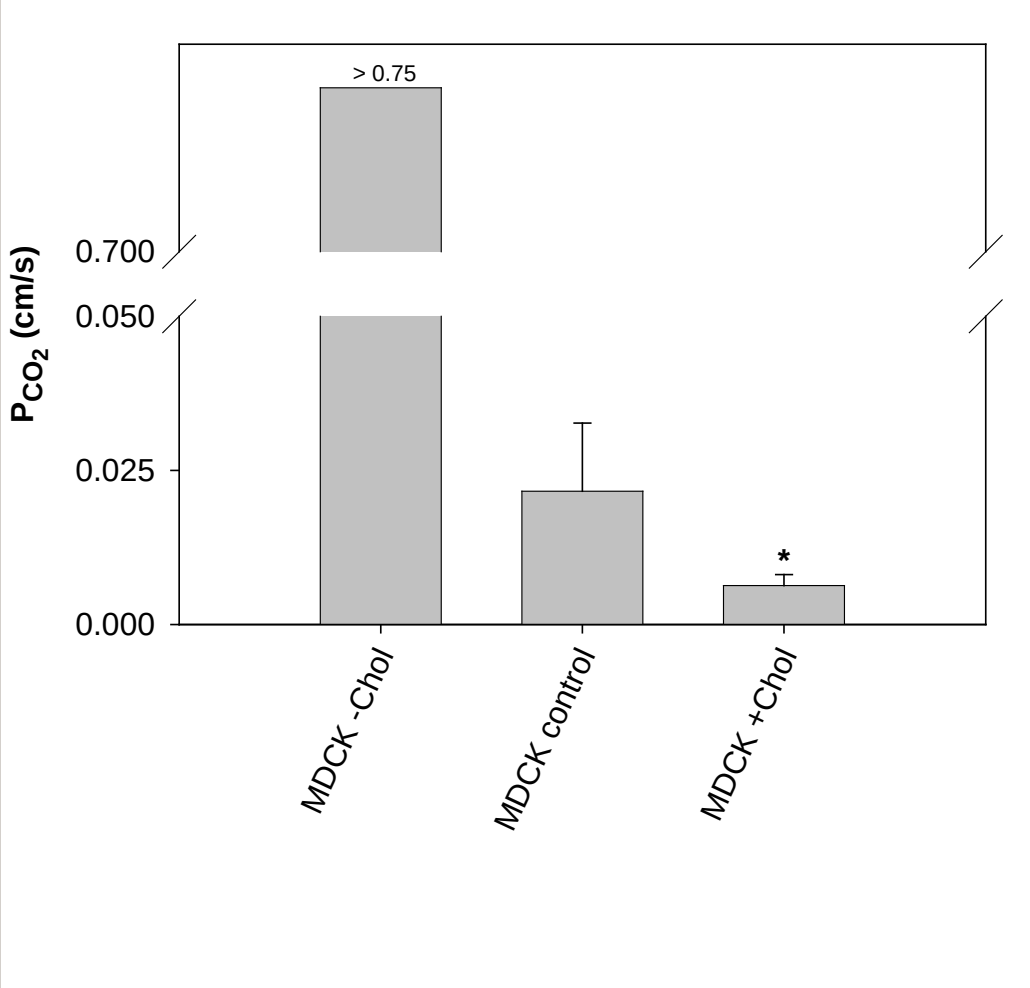
cholesterol depletion with β-cyclodextrin



cholesterol enrichment with β-cyclodextrin



Is cholesterol the cause of the low CO_2 permeability of MDCK cells?



Reduction of cholesterol with cyclodextrin raises $P_{CO2.}$

Enrichment with cholesterol lowers P_{CO2} compared to normal cells.

- We show that cell membranes possess a low intrinsic CO_2 permeability, often in the range of 0.01 cm/s.

- This permeability is 2, and in one case 3, orders of magnitude lower than the CO_2 permeability of pure artificial phospholipid bilayers.

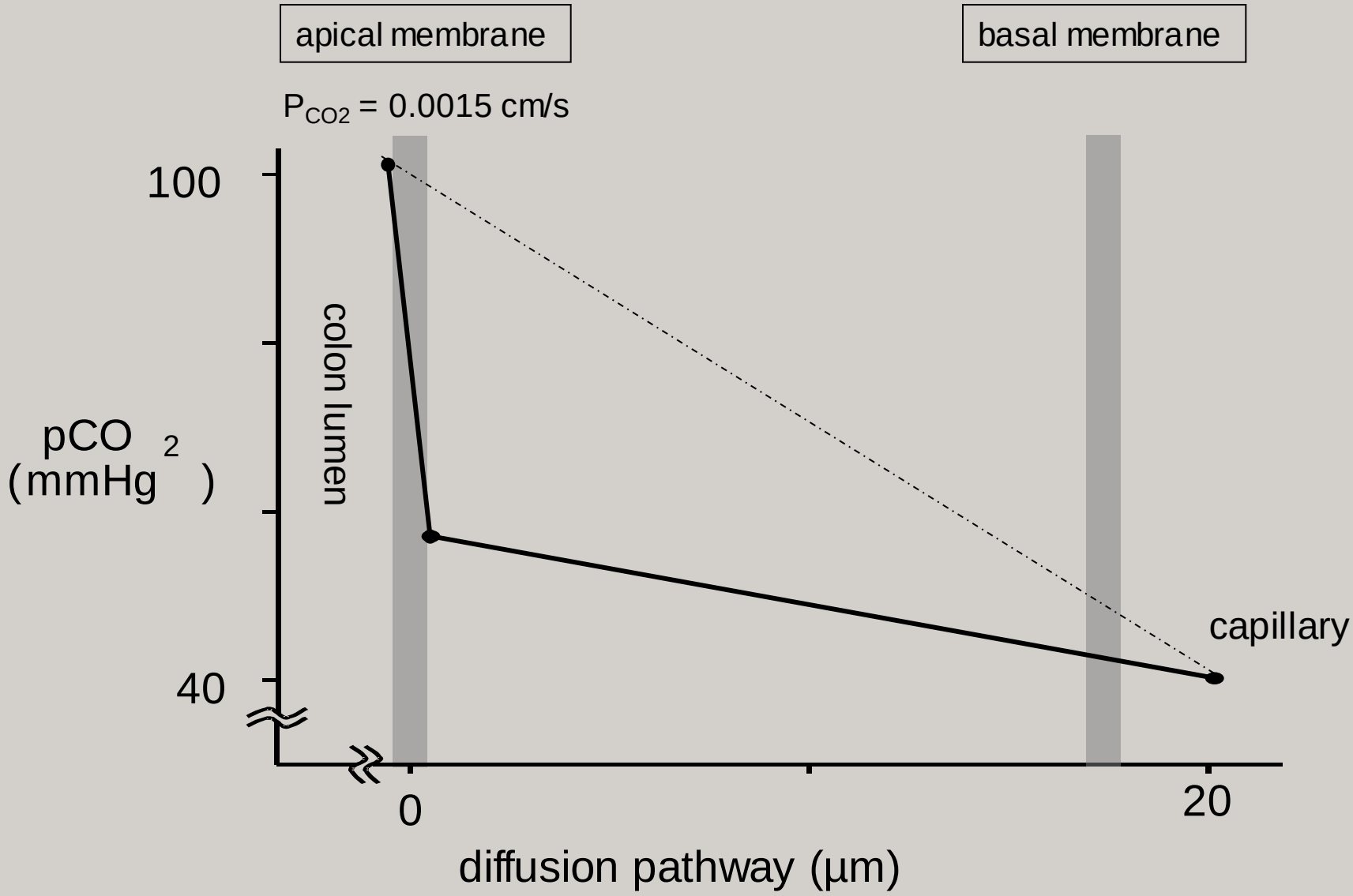
- The main cause of this low CO_2 permeability is the cholesterol content of the cell membrane. With increasing cholesterol content P_{CO2} decreases in artificial vesicles as well as in intact cells.

Physiological consequences of low CO_2 membrane permeabilities

1. Consequences of the extremely low CO_2 permeability of the apical membrane of colon epithelium

2. Effect of low CO_2 membrane permeability on red blood cell gas exchange

Consequences of low apical CO_2 permeability in colonocytes

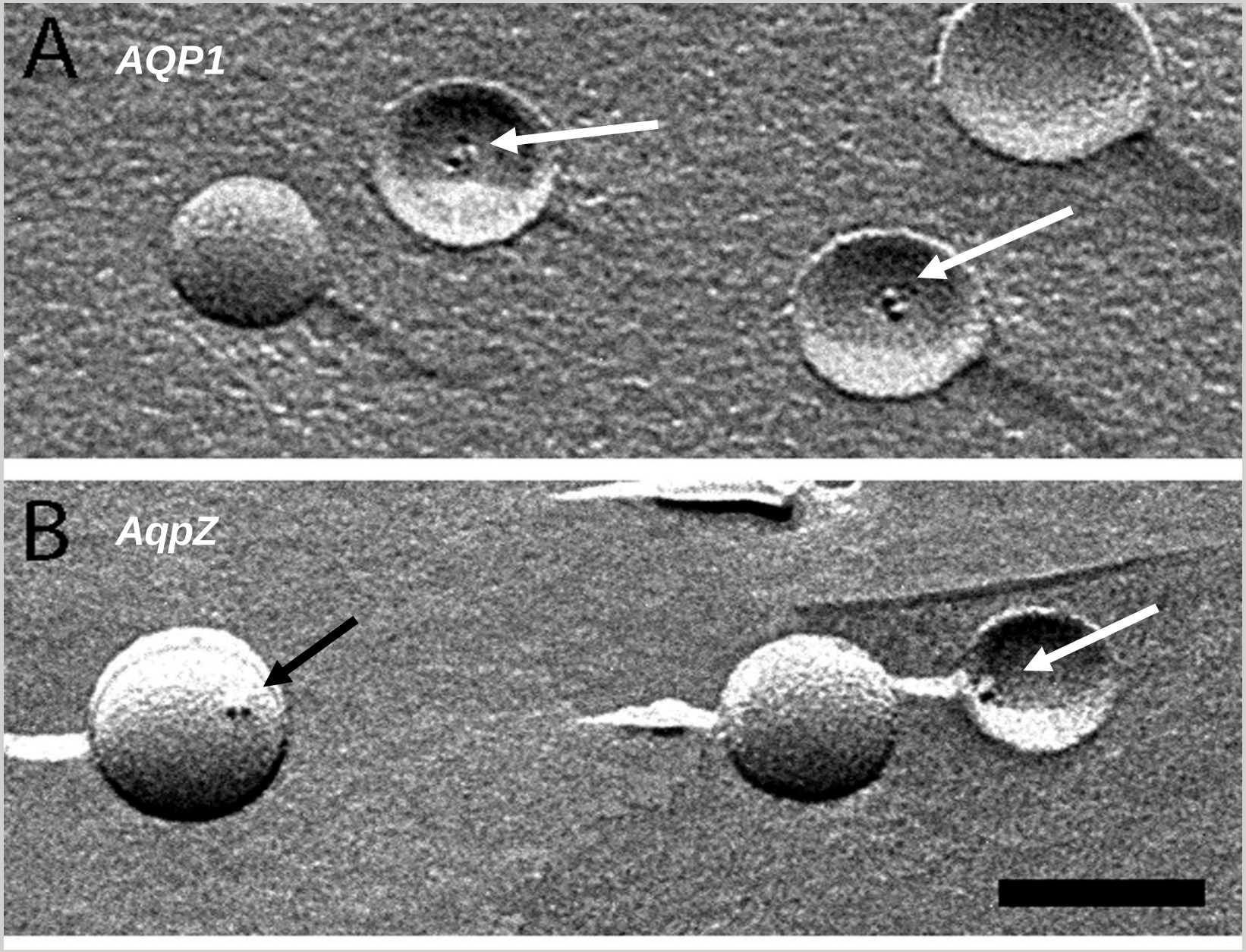


Example of a cell with a high gas exchange: red blood cell

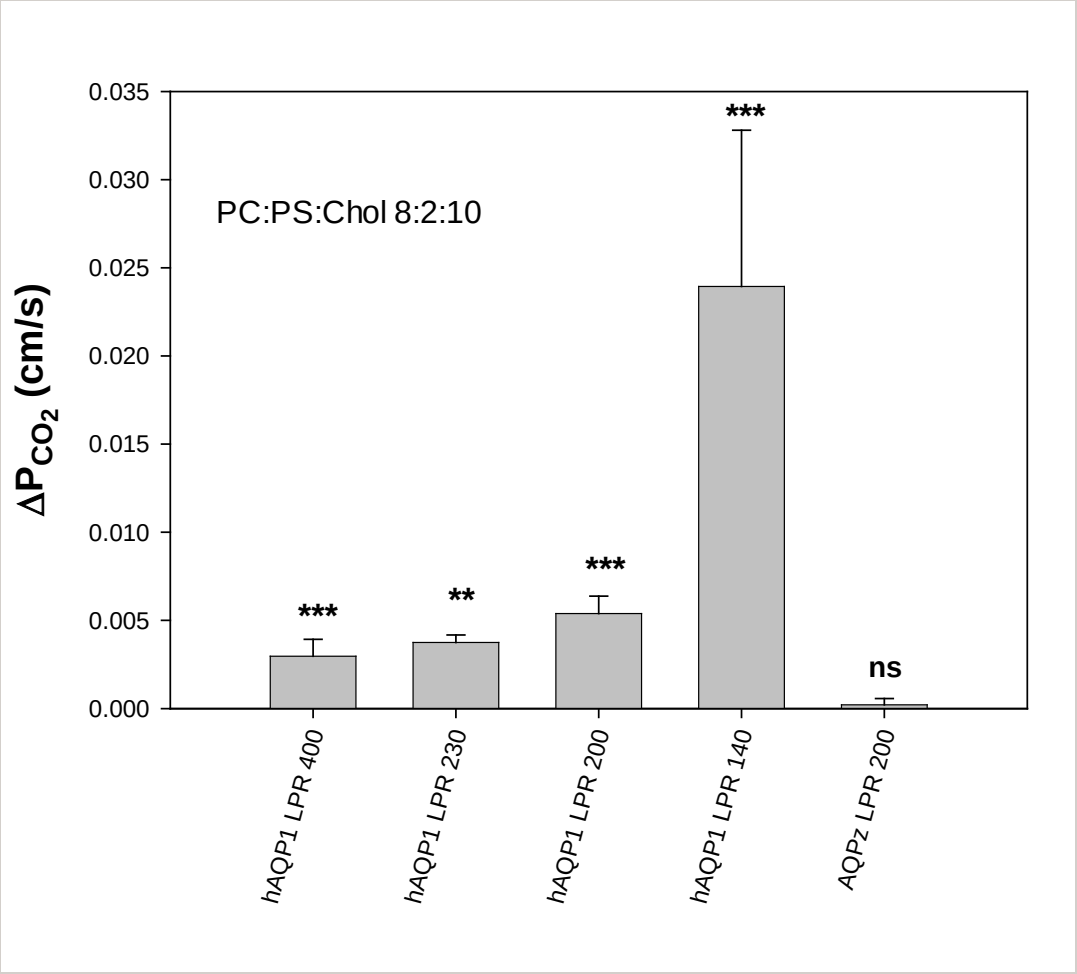
	P_{CO2} (cm/s)	t_{95} (ms)	transit time lung capillary (ms)	transit time heavy exercise (ms)
normal membrane resistance	**0.15**	**110**	**700**	**350**
permeability ∅ functional gas channel	**0.01**	**1000**	**700**	**350**

- From these considerations we can see that gas exchange of cells with a low CO_2 permeability is limited.

- Hypothesis: cell membranes with normal cholesterol and low intrinsic P_{CO2} adapt their CO_2 permeabilities to their needs by incorporating gas chanels in the membrane.


A
AQP1
B
AqpZ

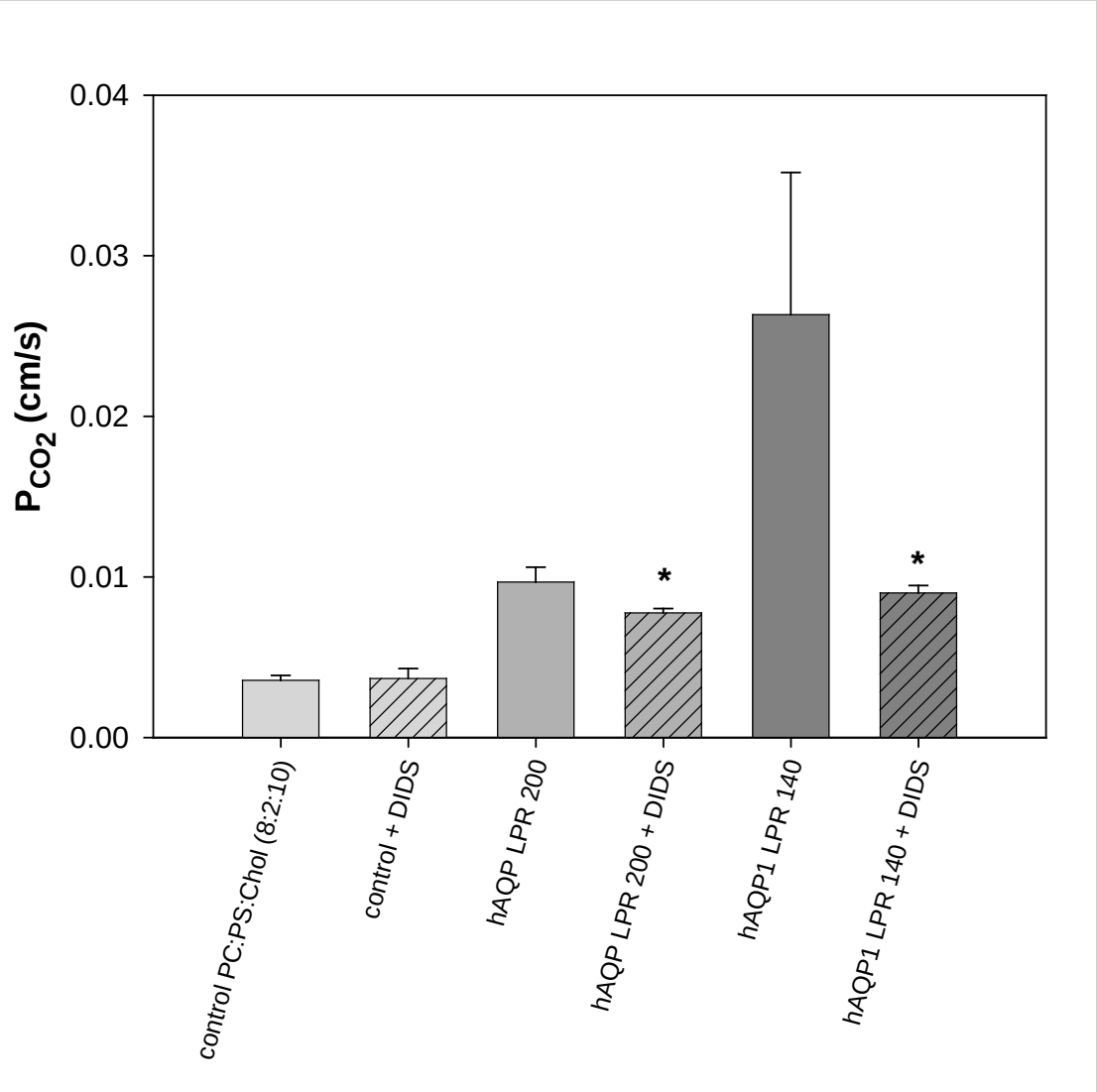
Aquaporin 1 as a CO_2 channel in cholesterol-containing lipid vesicles



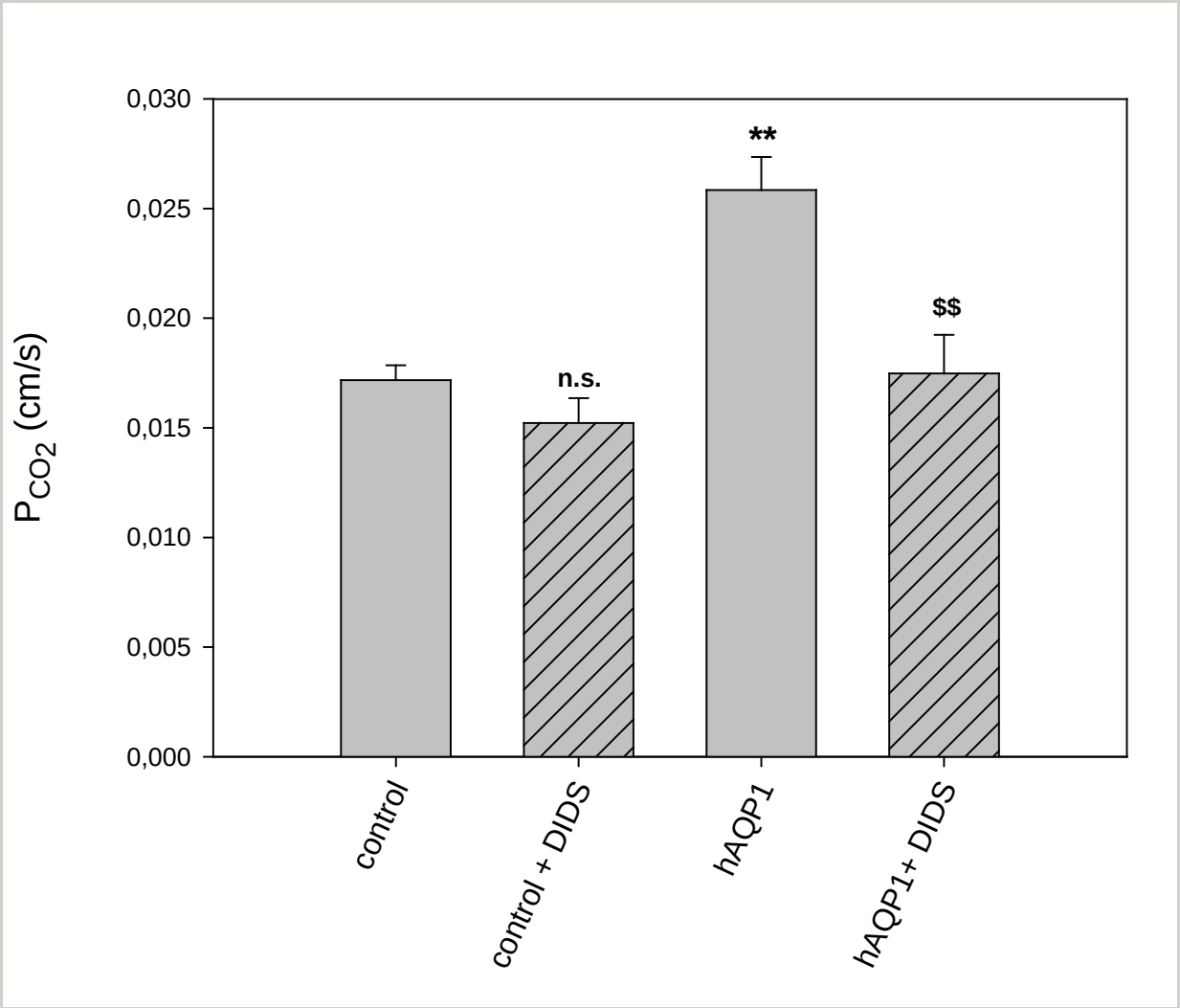
Change of P_{CO2} in vesicles with decreasing Lipid-Protein-Ratios (LPR)

Incorporation of AQP1 into vesicles causes a rise in P_{CO2}

DIDS reduces the CO_2 permeability of AQP1 containing vesicles



Aquaporin 1 as a CO_2 channel in MDCK cells



Expression of AQP1 in MDCK cells raises P_{CO2}

- We conclude that in a membrane of normal cholesterol content and low CO_2 permeability, incorporation of AQP1 into the membrane significantly increases the CO_2 permeability in a concentration dependent manner.

- AQP1 acts as a DIDS-sensitive CO_2 channel.

Gas	CO_2	O_2	NO	N_2
Lipid-water partition coefficient	0.95	2.9	3.8	4.1

	CO_2	O_2
Lipid-water partition coefficient	0.95	2.9
Reduction of membrane permeability by cholesterol	1/100	(1/100) ?
Membrane permeability	0.01 cm/s	(0.03 cm/s) ?
Heart muscle under heavy exercise: partial pressure difference across the membrane ΔP	5 mmHg	(40 mmHg) ?

Summary

With rising cholesterol content the CO_2 permeability (P_{CO2}) of lipid vesicles decreases drastically.

The intrinsic P_{CO2} of cell membranes is low due to their cholesterol content:

1) cell membranes and lipid vesicles with identical cholesterol content exhibit identical CO_2 permeability
2) cholesterol-depleted cell membranes have an increased CO_2 permeability, cholesterol-enriched cell membranes a reduced permeability

Cell membranes with normal cholesterol raise their CO_2 permeability, when functionally required, by incorporation of CO_2 channels:

1) AQP1 incorporated in lipid vesicles raises CO_2 permeability in a concentration-dependent manner
2) AQP1 expression in MDCK cells increases membrane P_{CO2}.

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Vegetative Physiologie

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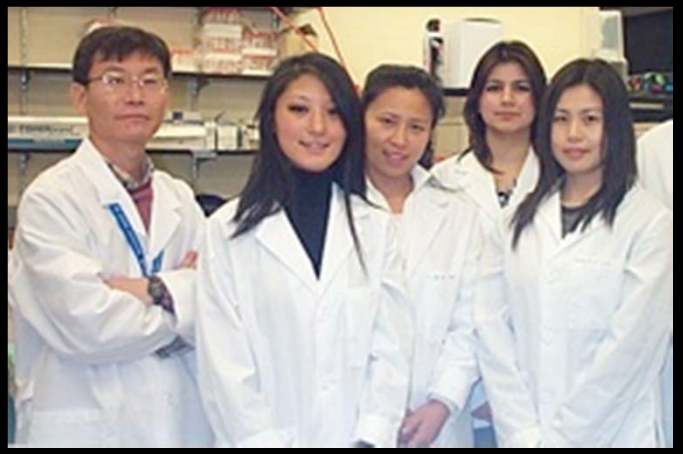
Involvement of elevated membrane cholesterol on G-protein regulated water and gas transport in biological membranes

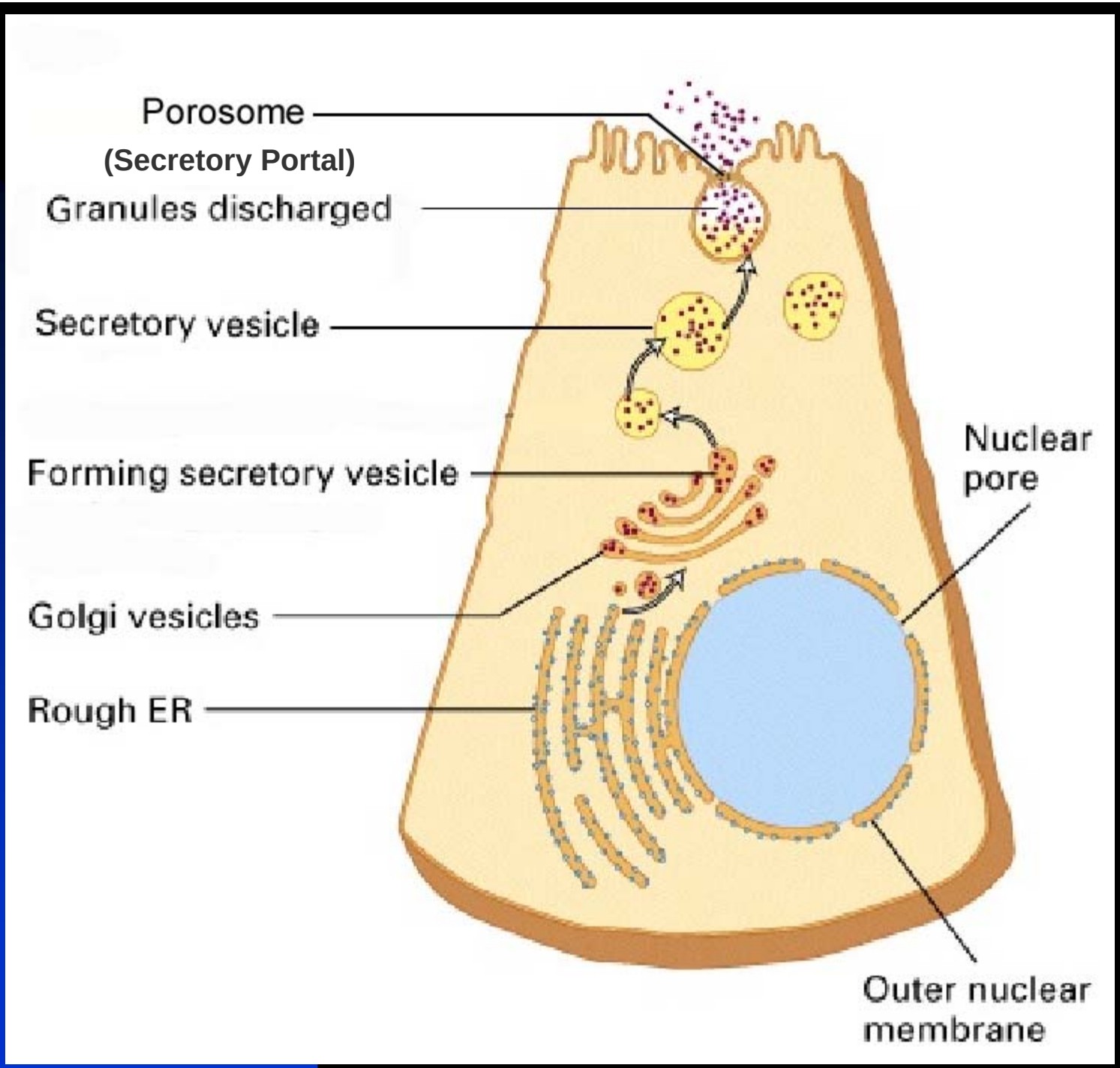
Gas Channels Workshop, Case Western Reserve University, OH; Sept. 5-7, 2012

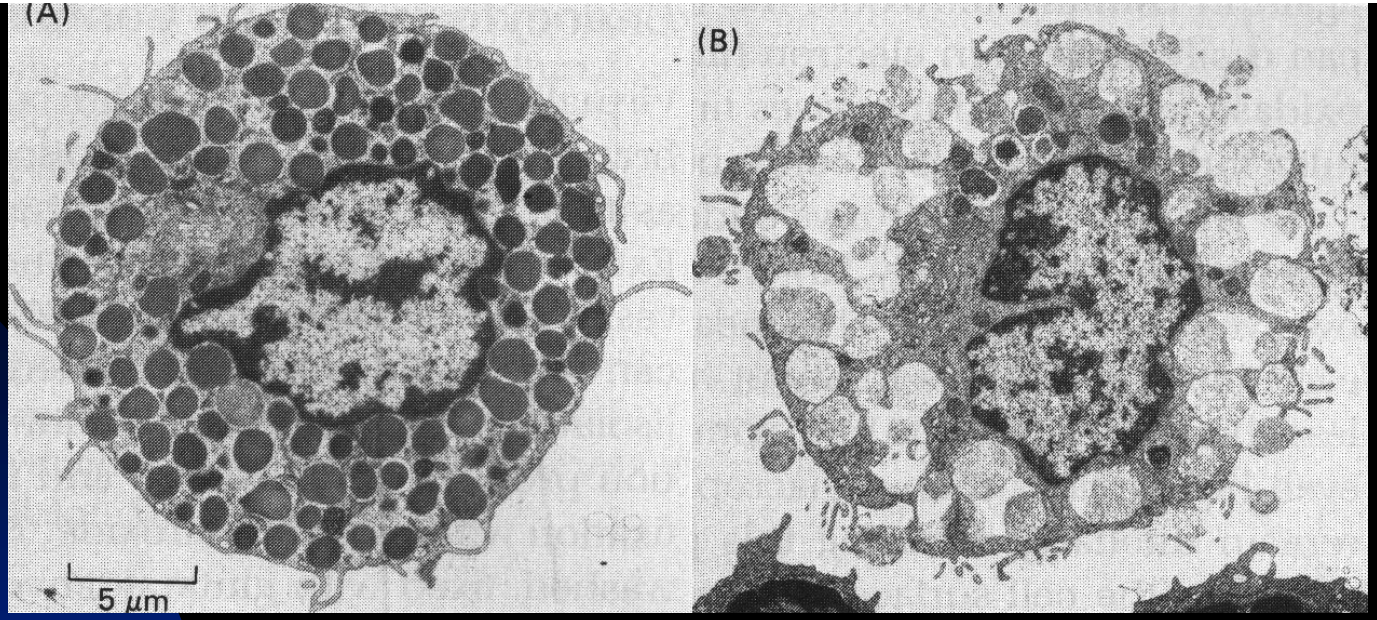
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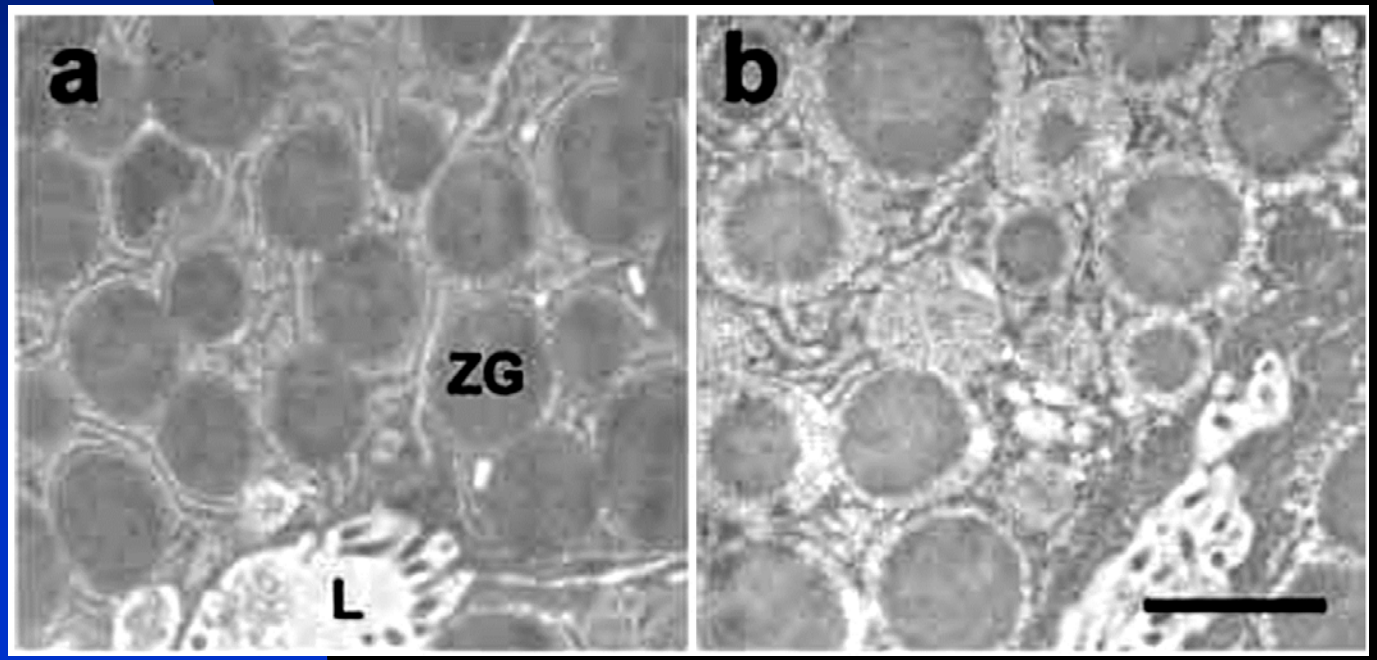
Jena Research Group




Porosome
(Secretory Portal)
Granules discharged
Secretory vesicle
Forming secretory vesicle
Golgi vesicles
Rough ER
Nuclear pore
Outer nuclear membrane

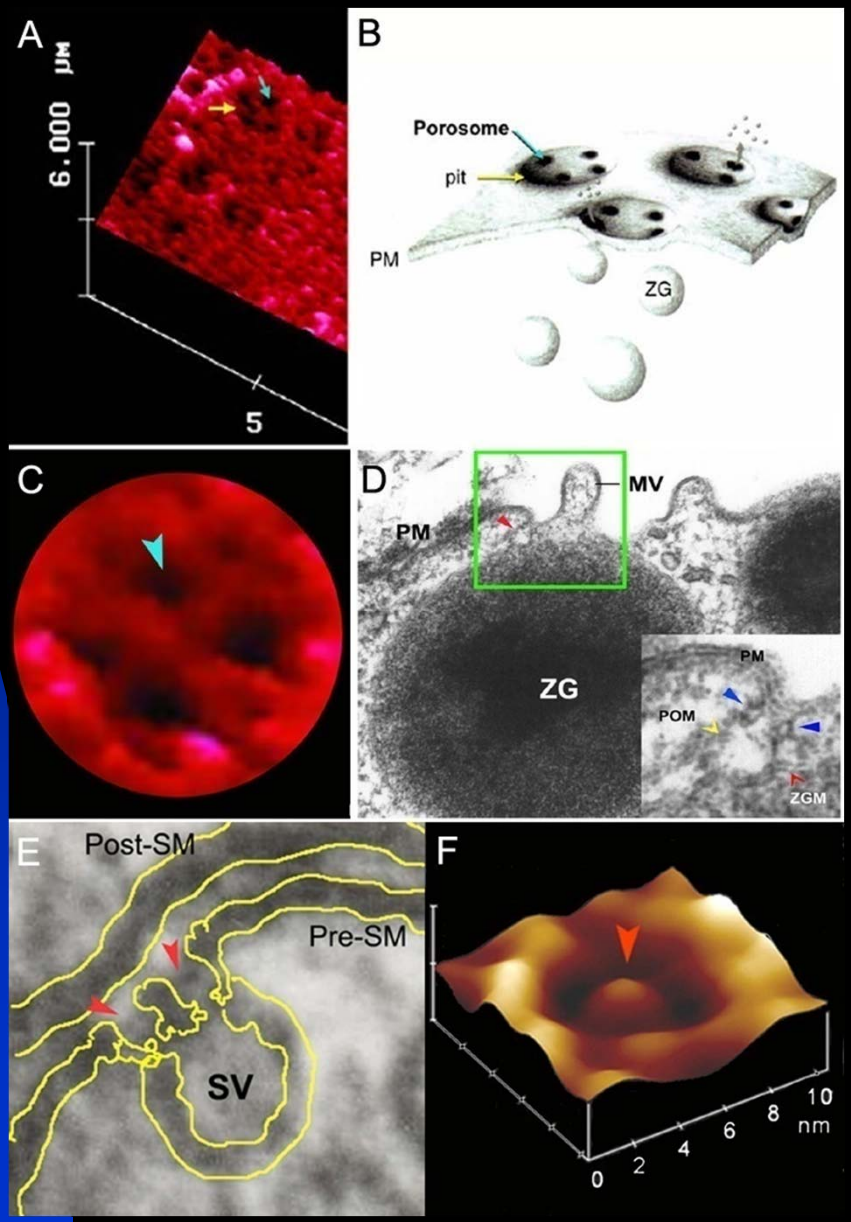


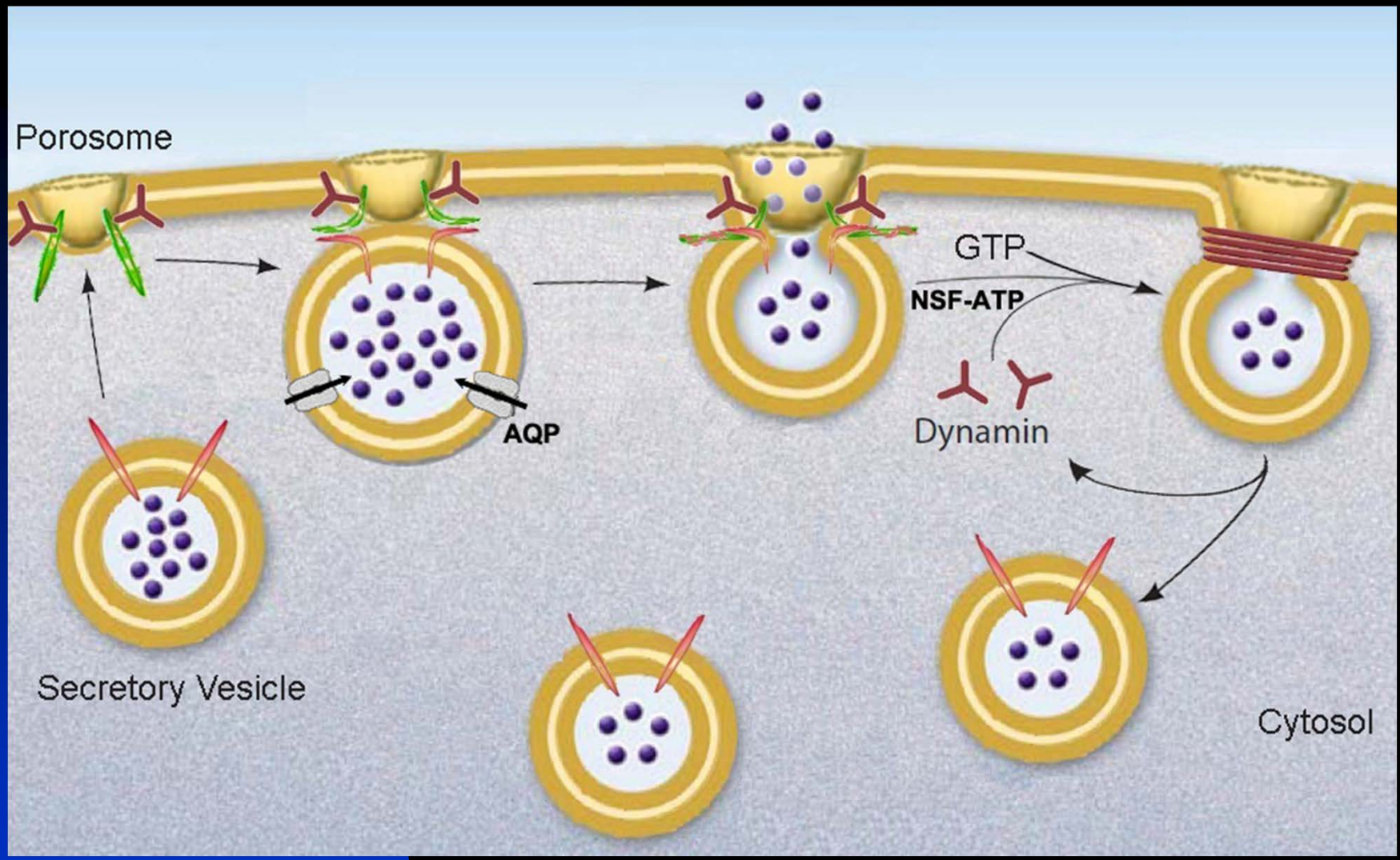
Rat Peritoneal Mast Cell- Lawson D. et al. 1975 *J. Exp. Med.* 142:391-402



Rat Pancreatic Acinar Cells- Cho SJ et al. 2002 *Cell Biol. Int.* 26:29-33

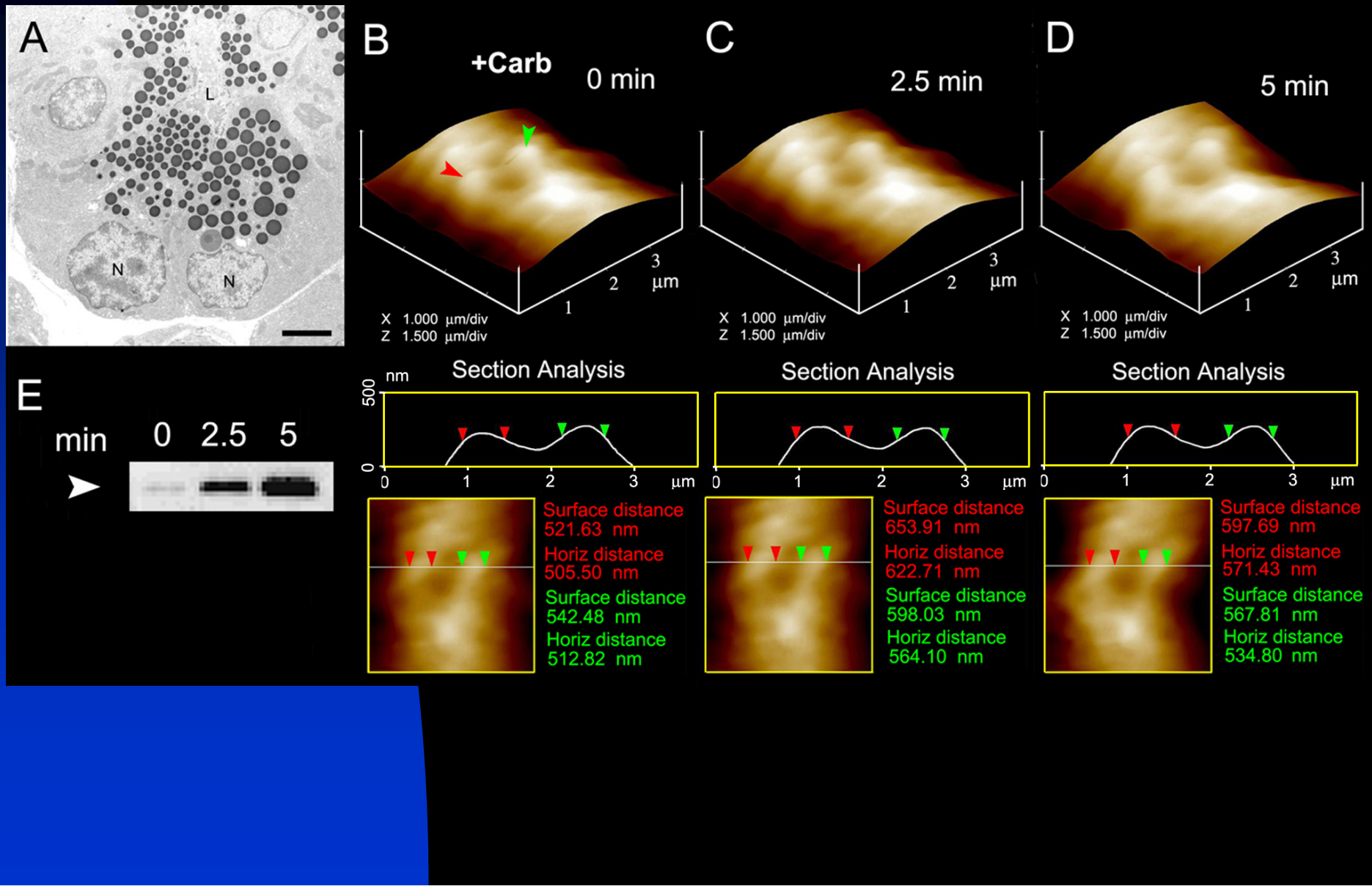
Porosome: The Universal Secretory Portal in Cells



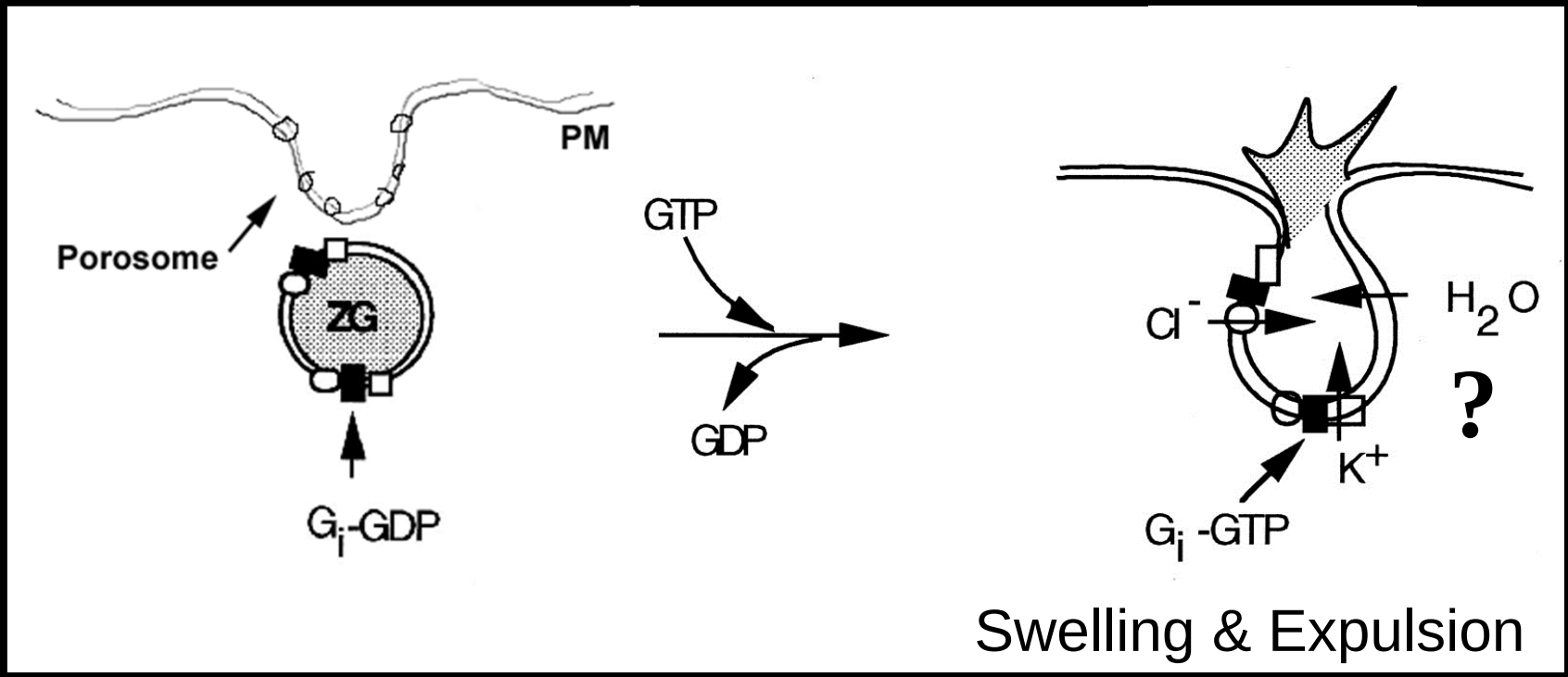




Lee et. al. 2012 J. Proteomics 75:3952-62

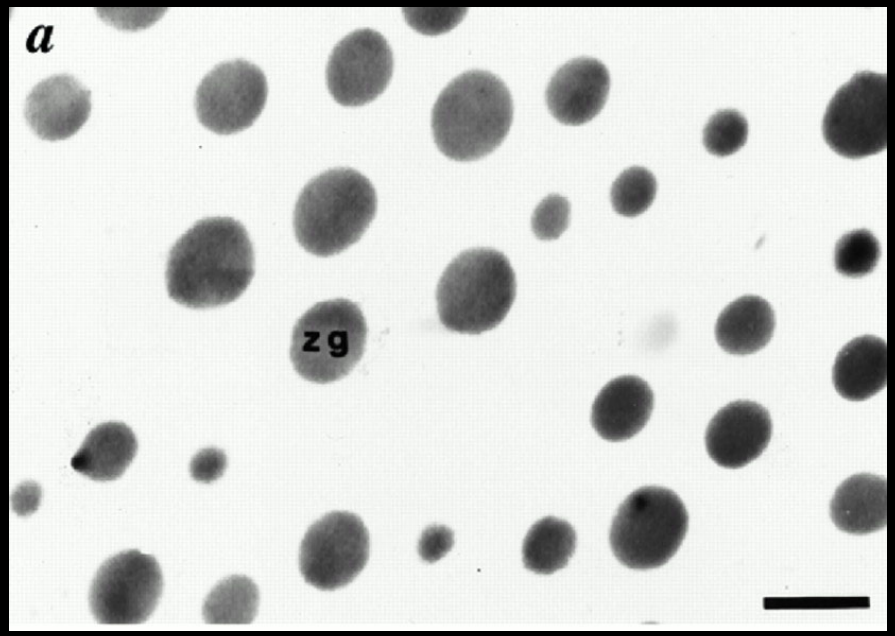

A
L
N
N
B
+Carb
0 min
C
2.5 min
D
5 min
3
2
1
µm
X 1.000 µm/div
Z 1.500 µm/div
E
min
0
2.5
5
nm
500
0
Section Analysis
0
1
2
3
µm
Surface distance
521.63 nm
Horiz distance
505.50 nm
Surface distance
542.48 nm
Horiz distance
512.82 nm
Surface distance
653.91 nm
Horiz distance
622.71 nm
Surface distance
598.03 nm
Horiz distance
564.10 nm
Surface distance
597.69 nm
Horiz distance
571.43 nm
Surface distance
567.81 nm
Horiz distance
534.80 nm

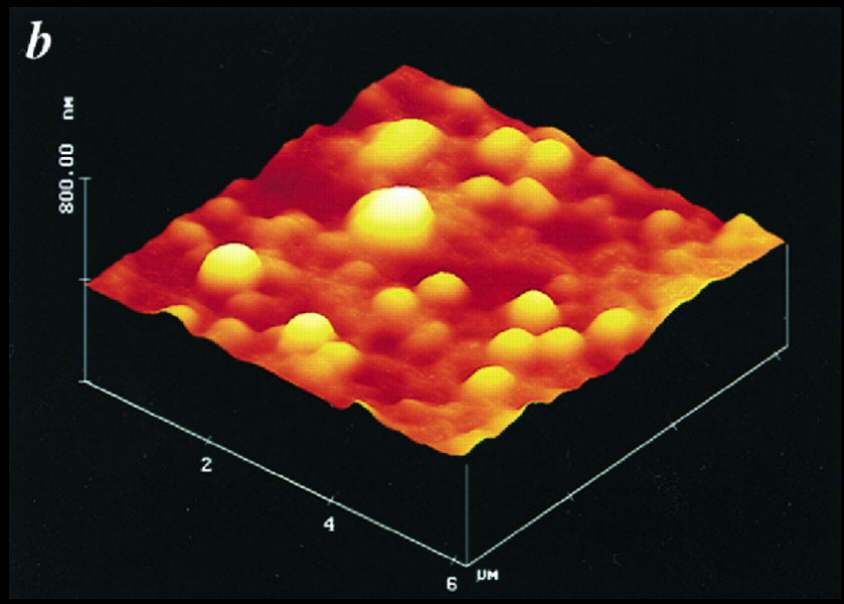
Hypothetical model



Jena et. al. 1997 Proc. Natl. Acad. Sci. USA 94:13317-22

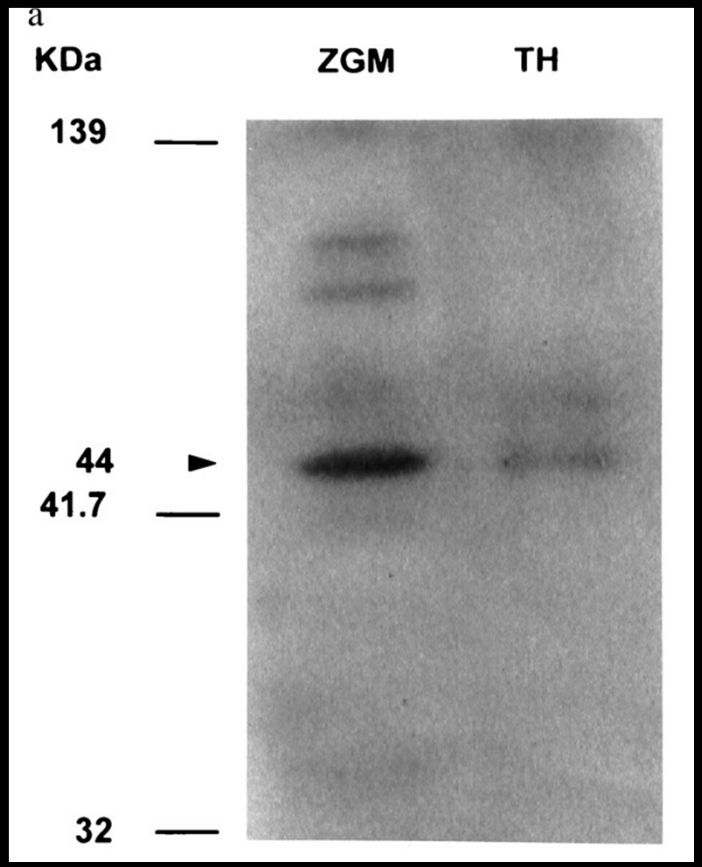
EM & AFM micrographs of Zymogen Granules

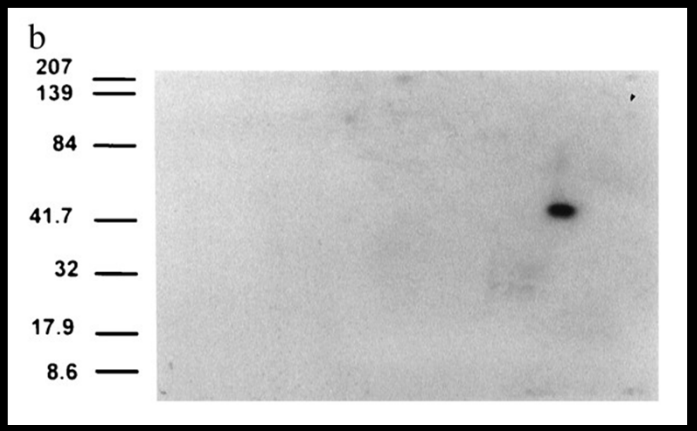


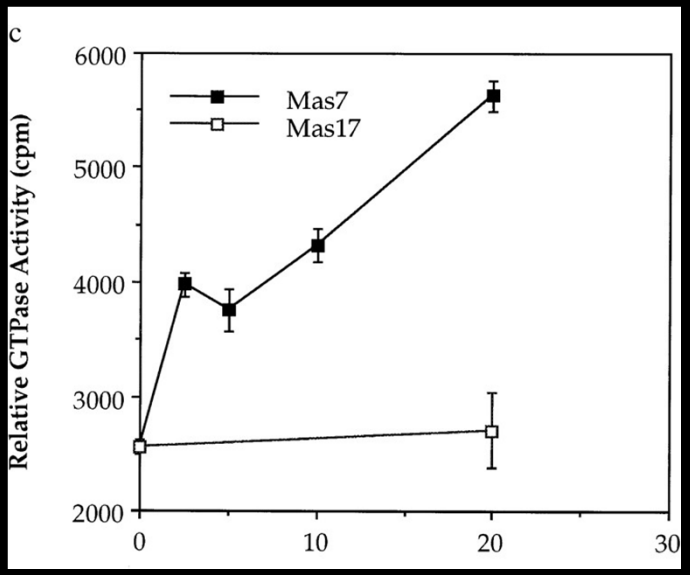


Jena et. al. 1997 Proc. Natl. Acad. Sci. USA 94:13317-22

Association of $G_{\alpha i3}$ with ZGM

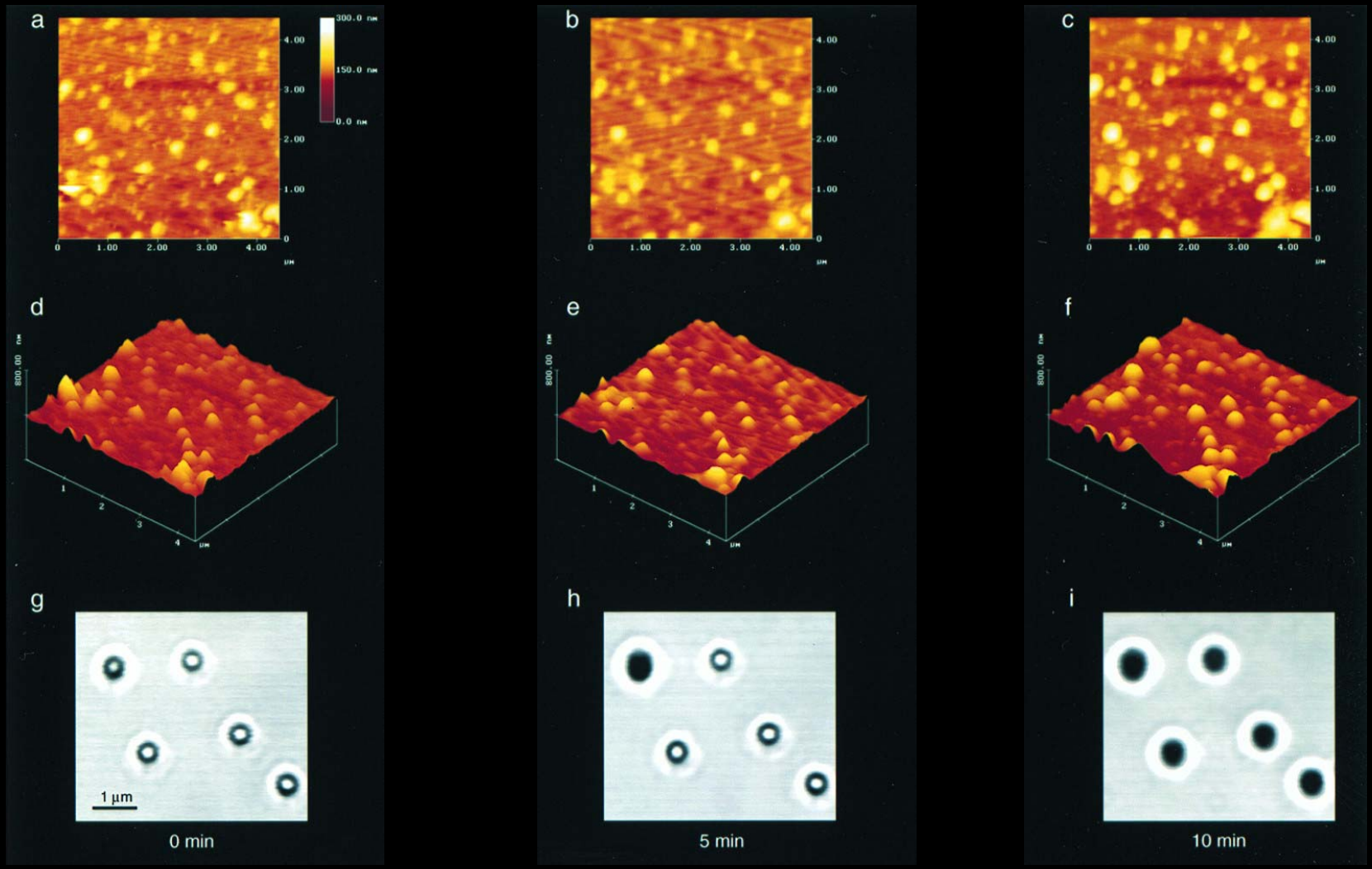




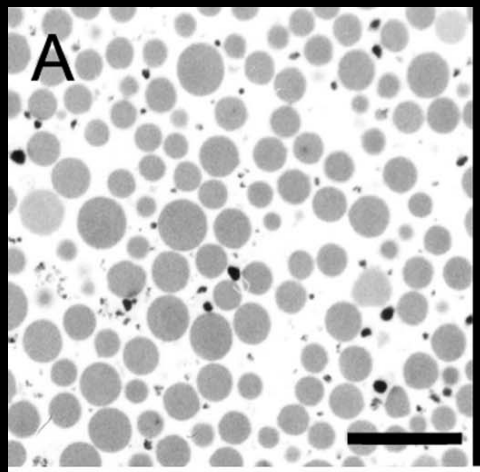


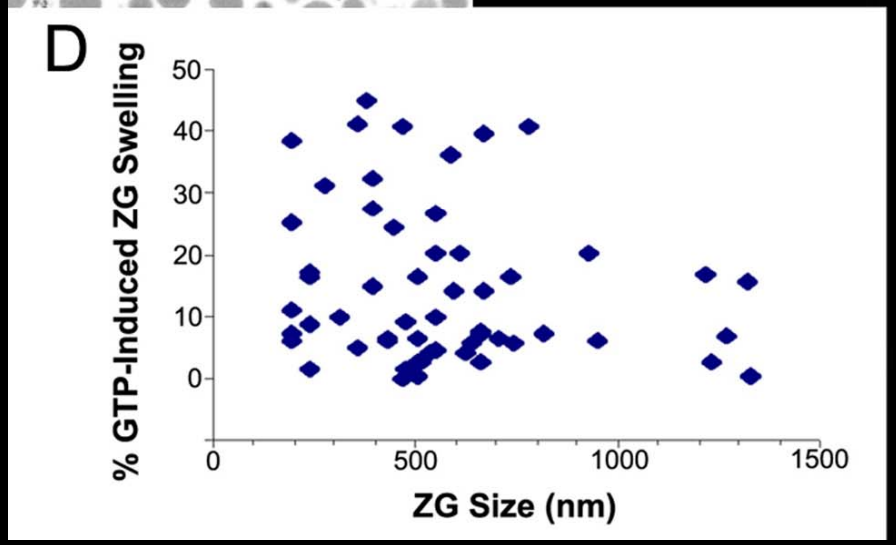
Jena et. al. 1997 Proc. Natl. Acad. Sci. USA 94:13317-22

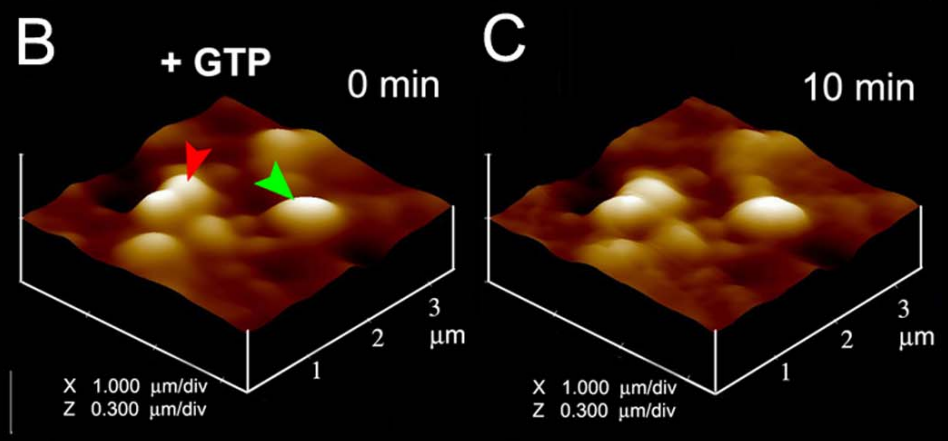
Vesicle Size Increase After Exposure to GTP

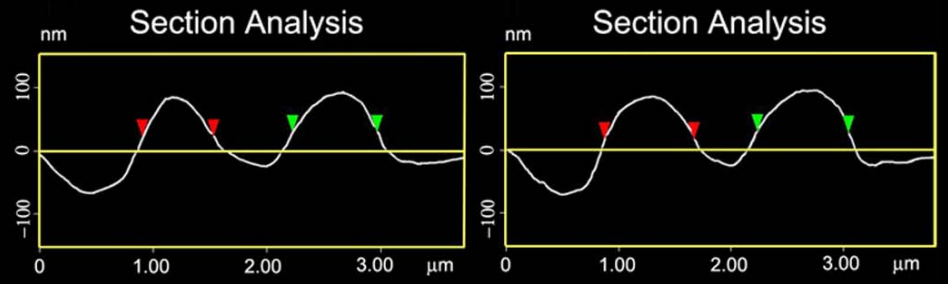


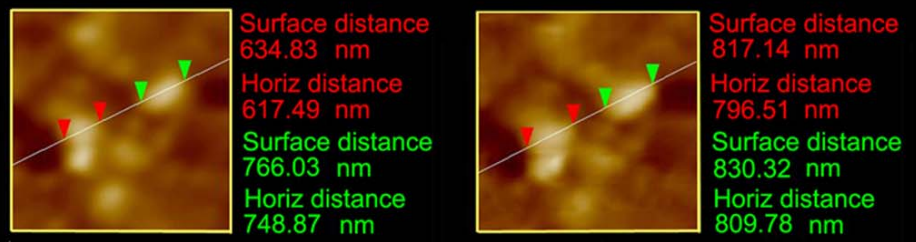
Jena et. al. 1997 Proc. Natl. Acad. Sci. USA 94:13317-22





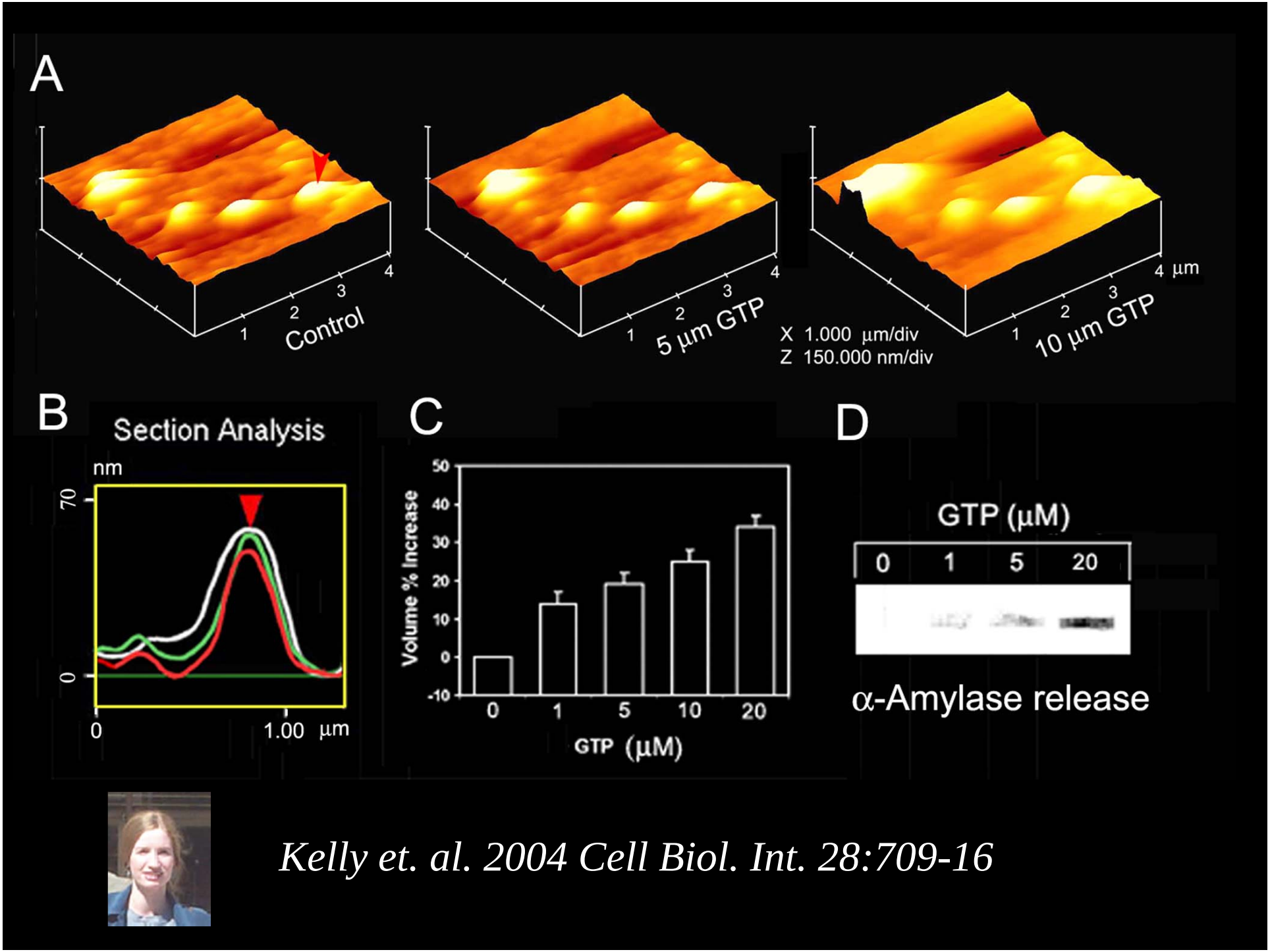




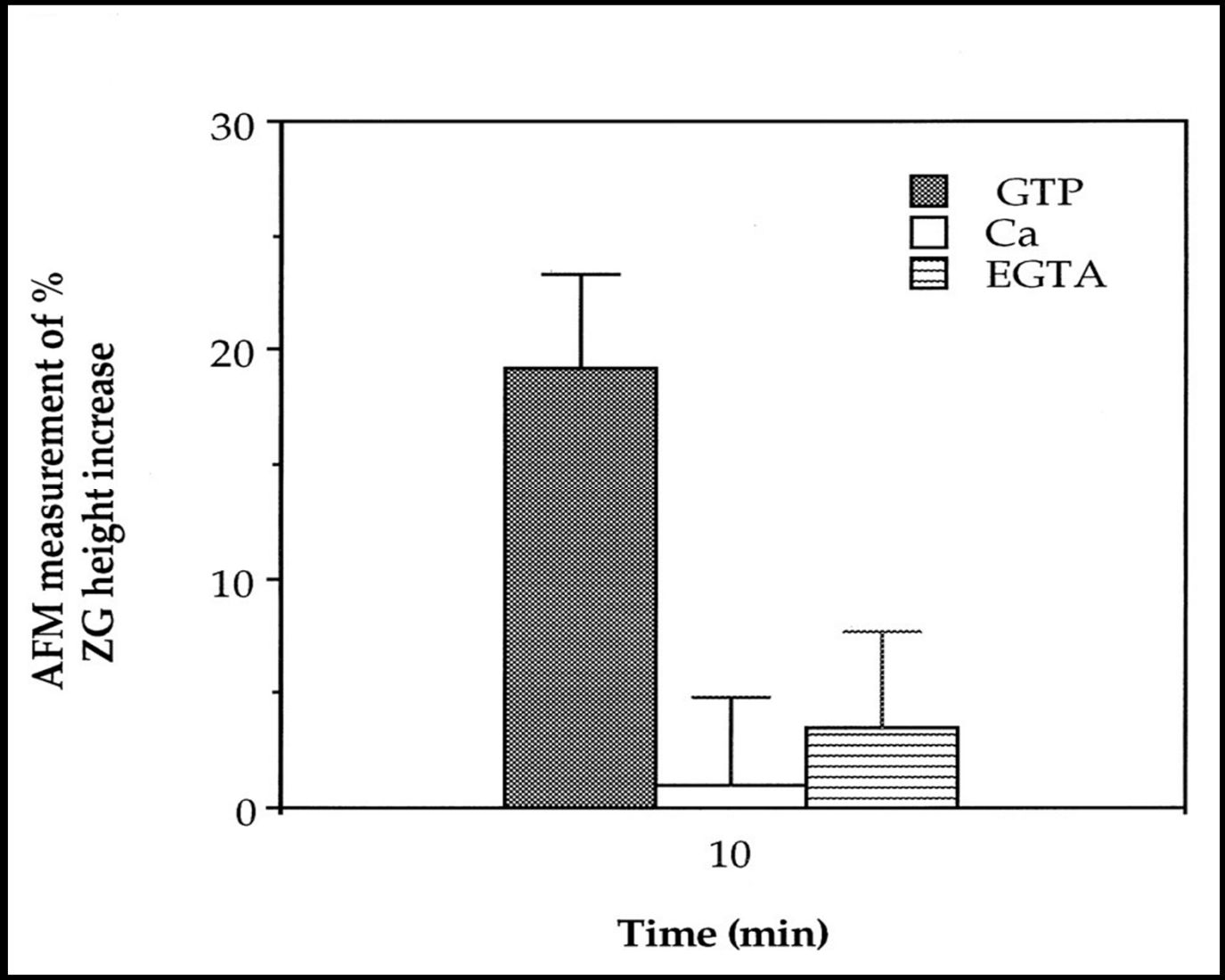




Kelly et. al. 2004 Cell Biol. Int. 28:709-16

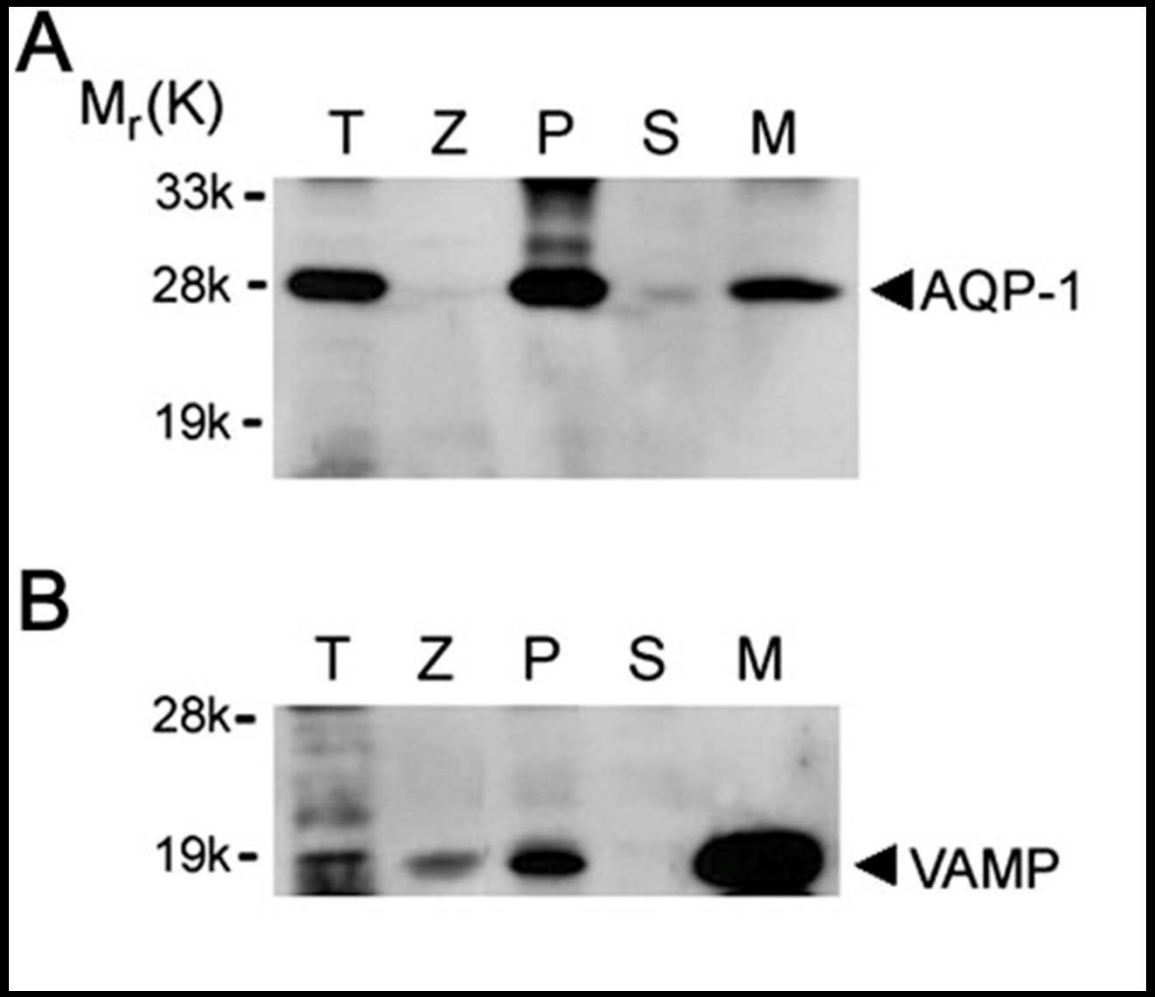


Kelly et. al. 2004 Cell Biol. Int. 28:709-16



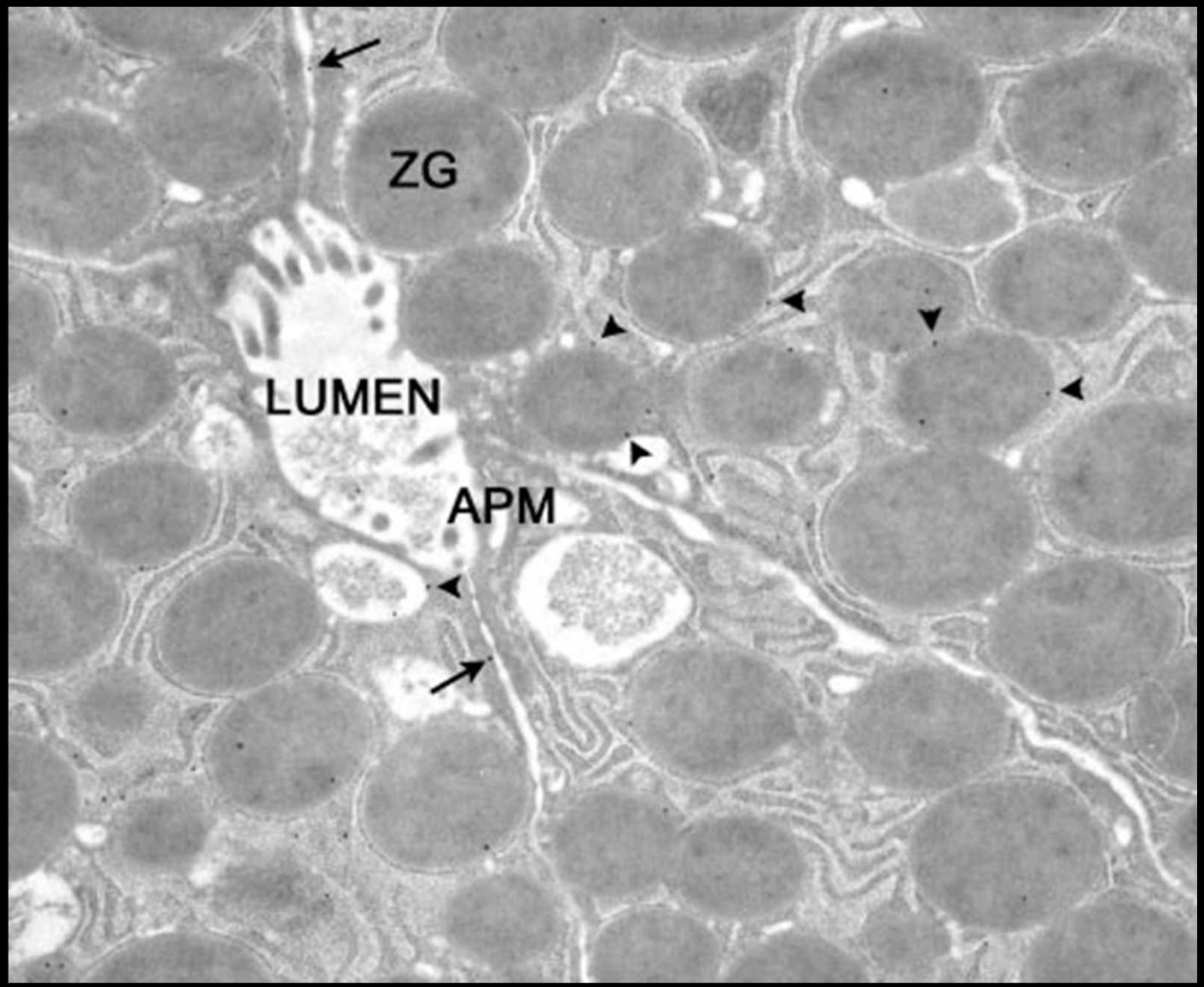
Jena et. al. 1997 Proc. Natl. Acad. Sci. USA 94:13317-22

Water-Channel AQP1 at ZG Membrane



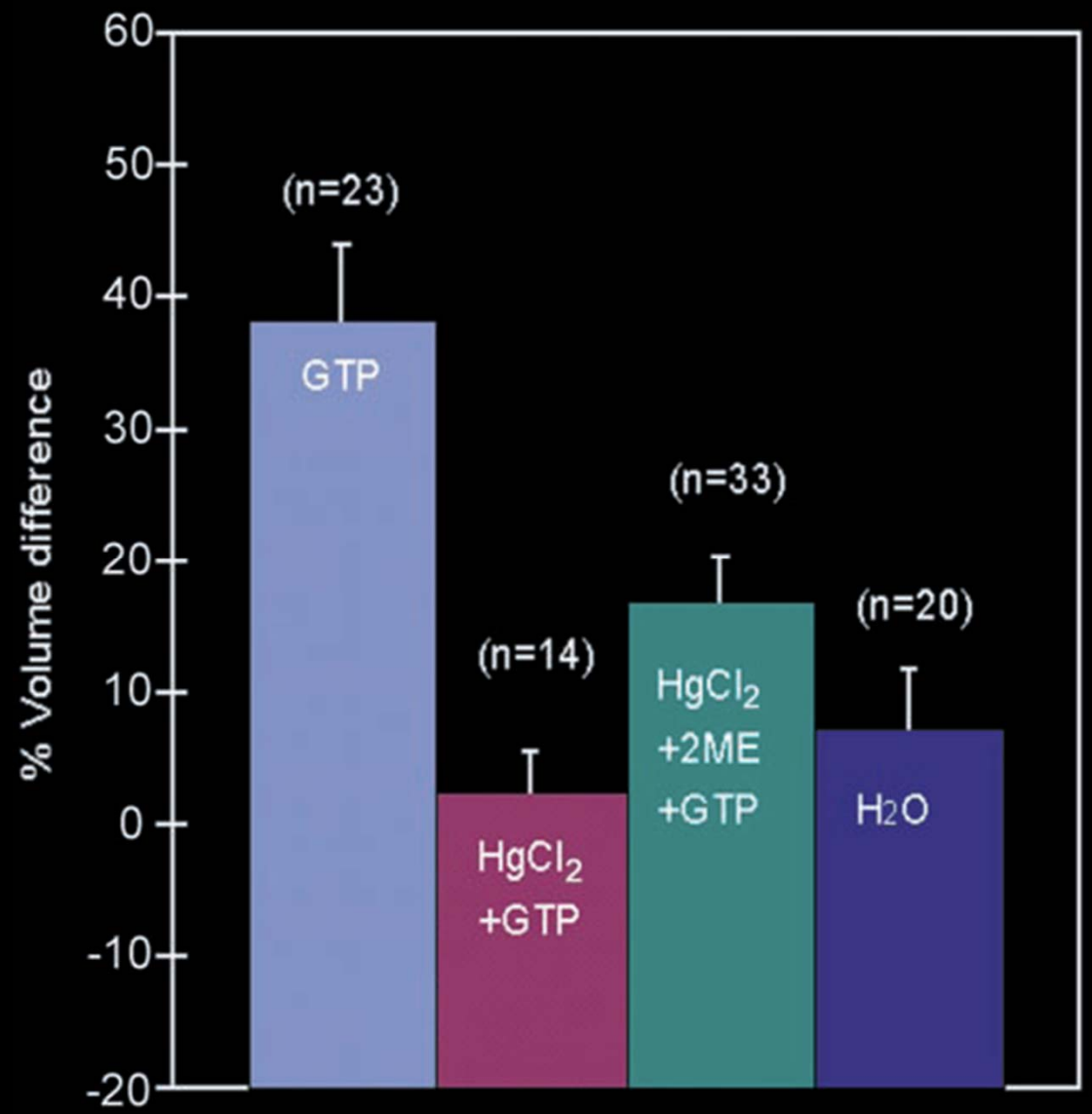


Cho et. al. 2002 Proc. Natl. Acad. Sci. USA 99:4720-24



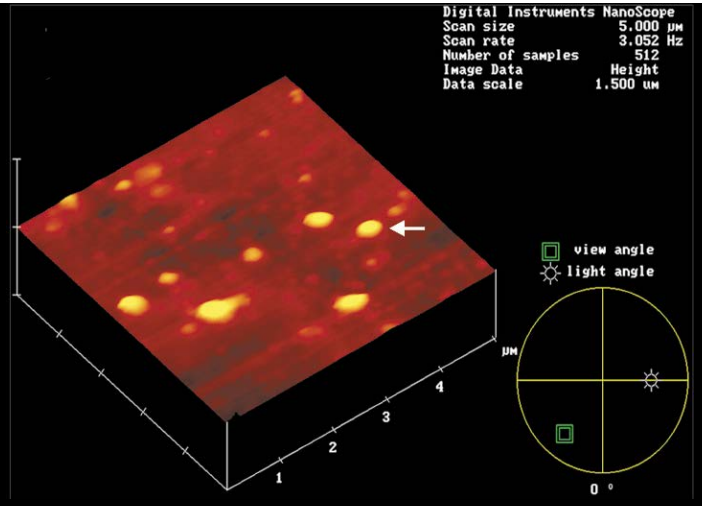


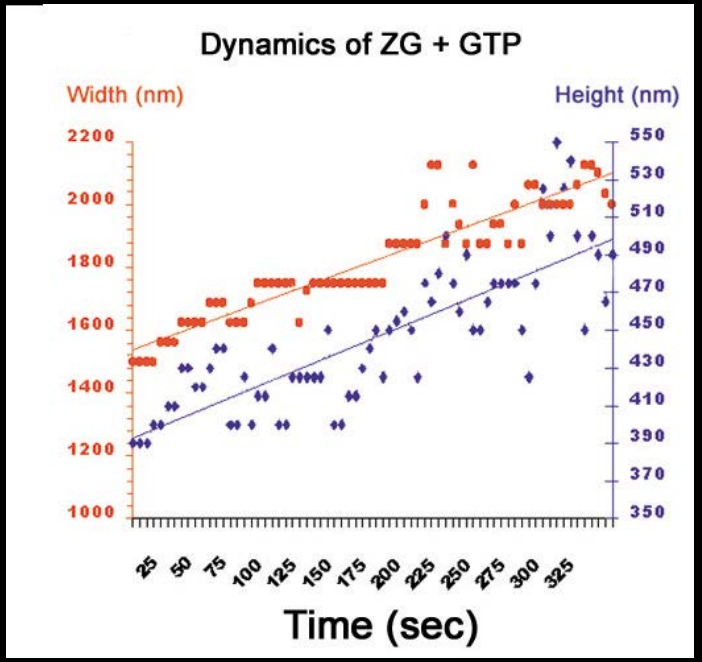
Cho et. al. 2002 Proc. Natl. Acad. Sci. USA 99:4720-24

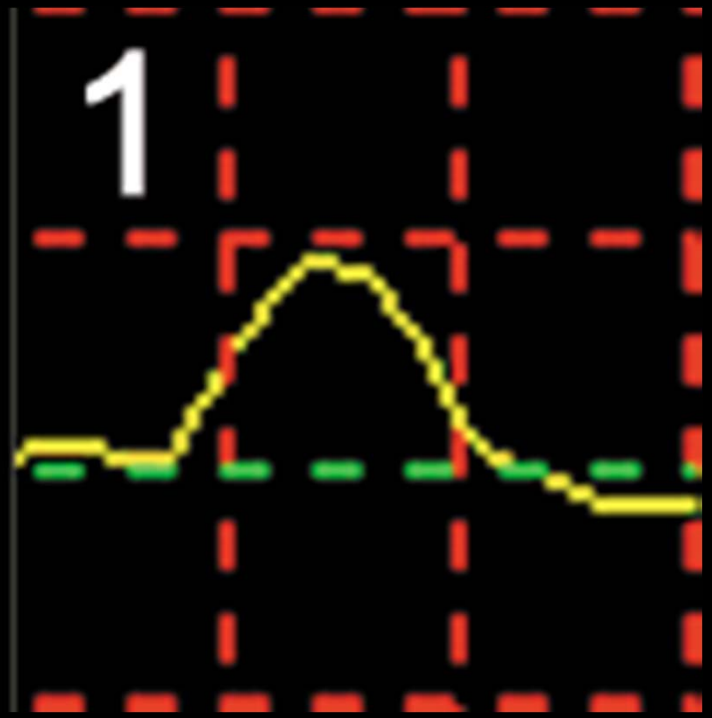




Cho et. al. 2002 Proc. Natl. Acad. Sci. USA 99:4720-24



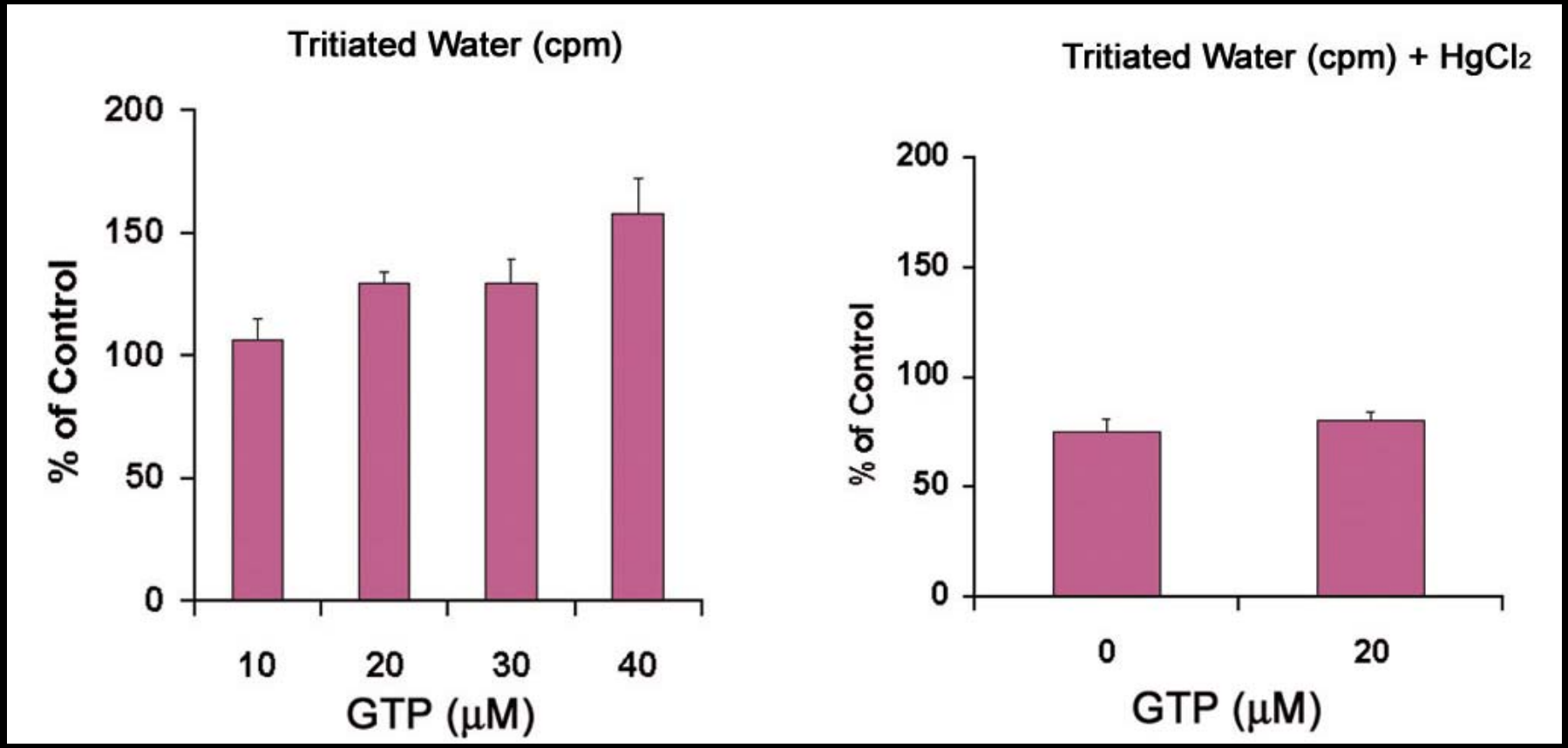






Cho et. al. 2002 Proc. Natl. Acad. Sci. USA 99:4720-24

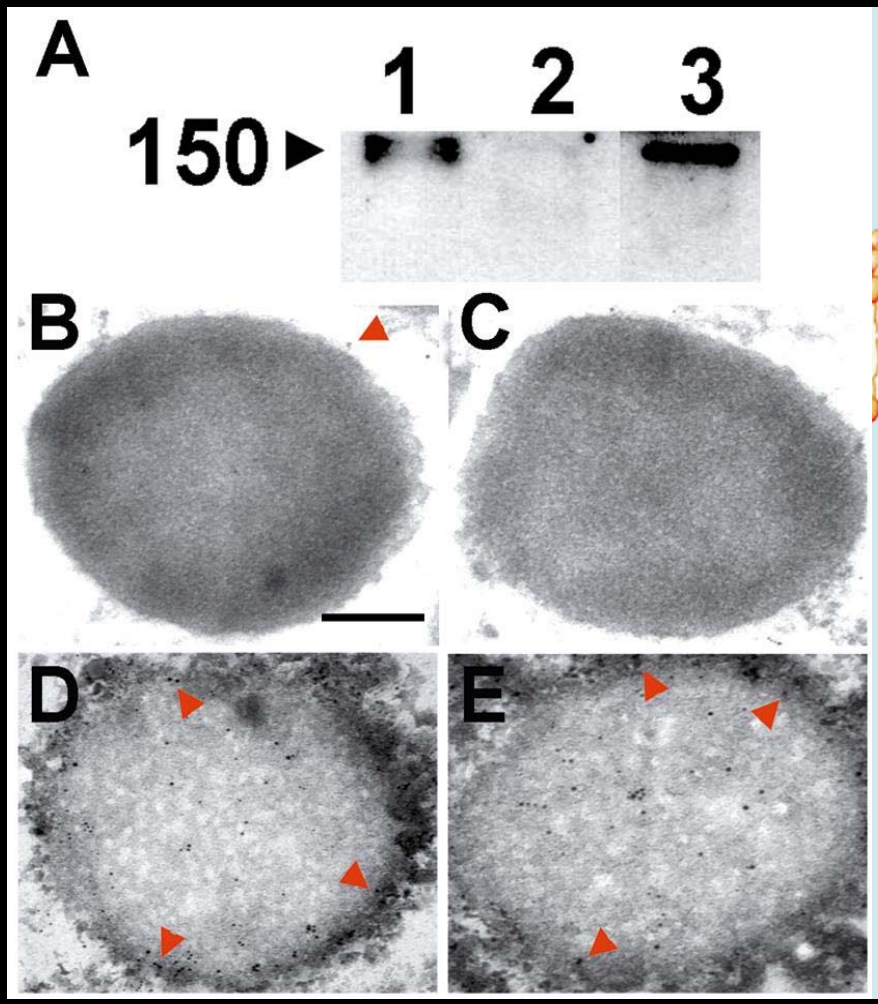
Tritiated Water Entry into ZG

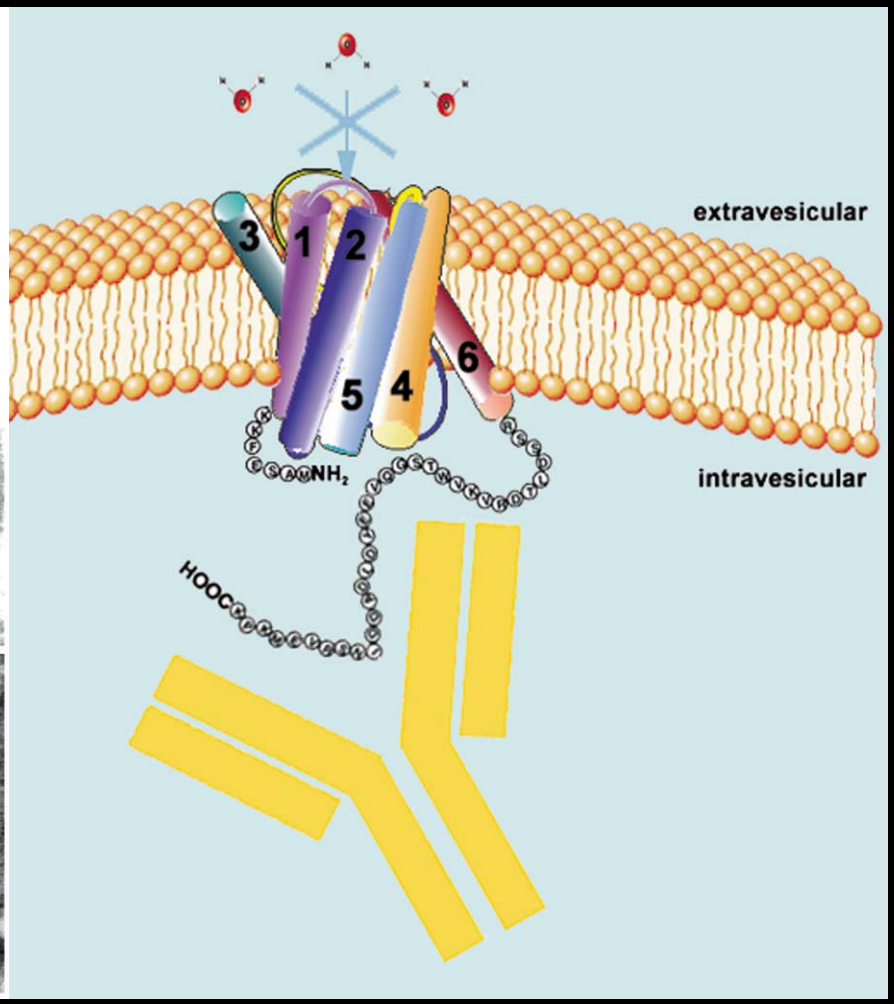




Cho et. al. 2002 Proc. Natl. Acad. Sci. USA 99:4720-24

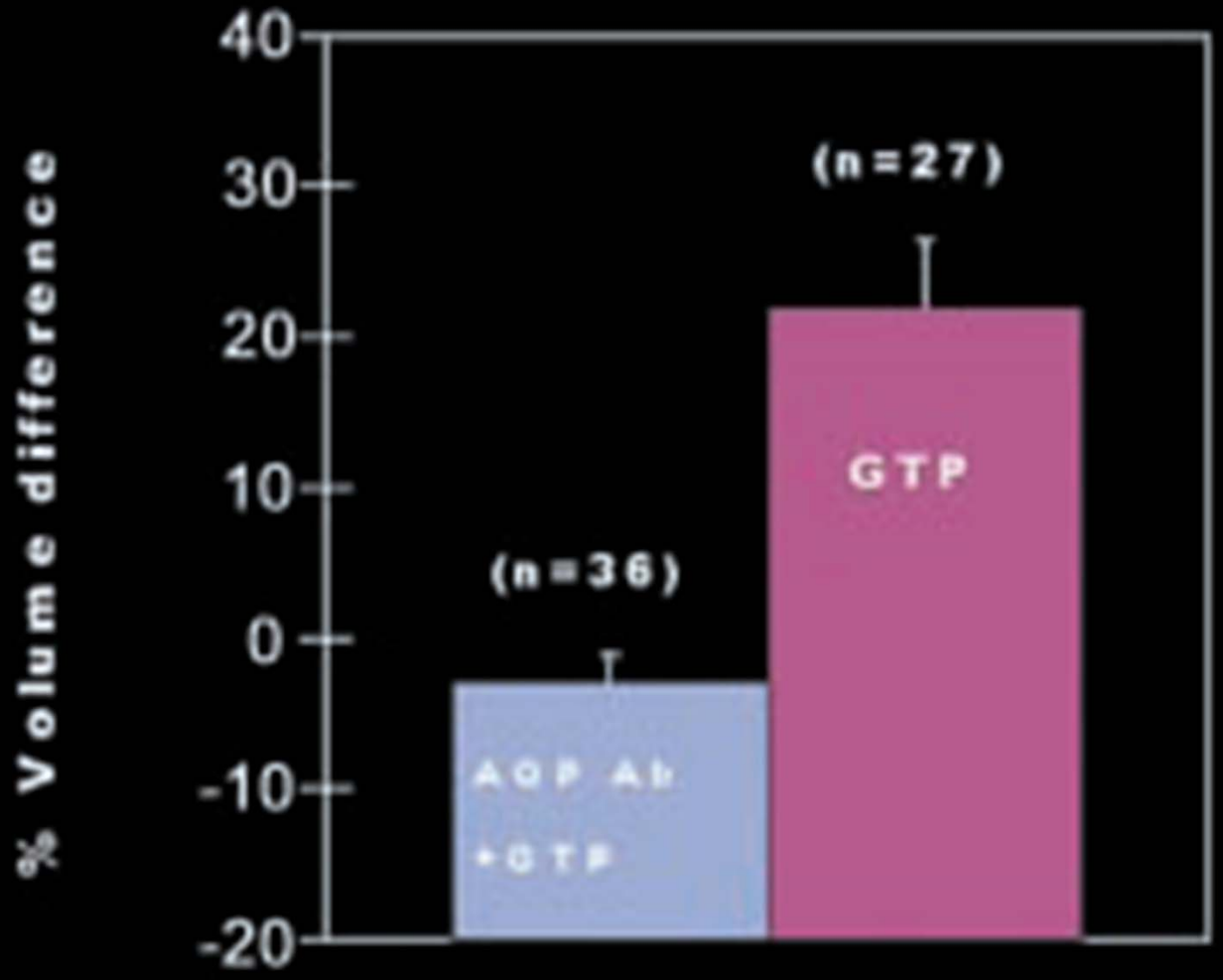
Introduction of AQP1 antibody into ZG







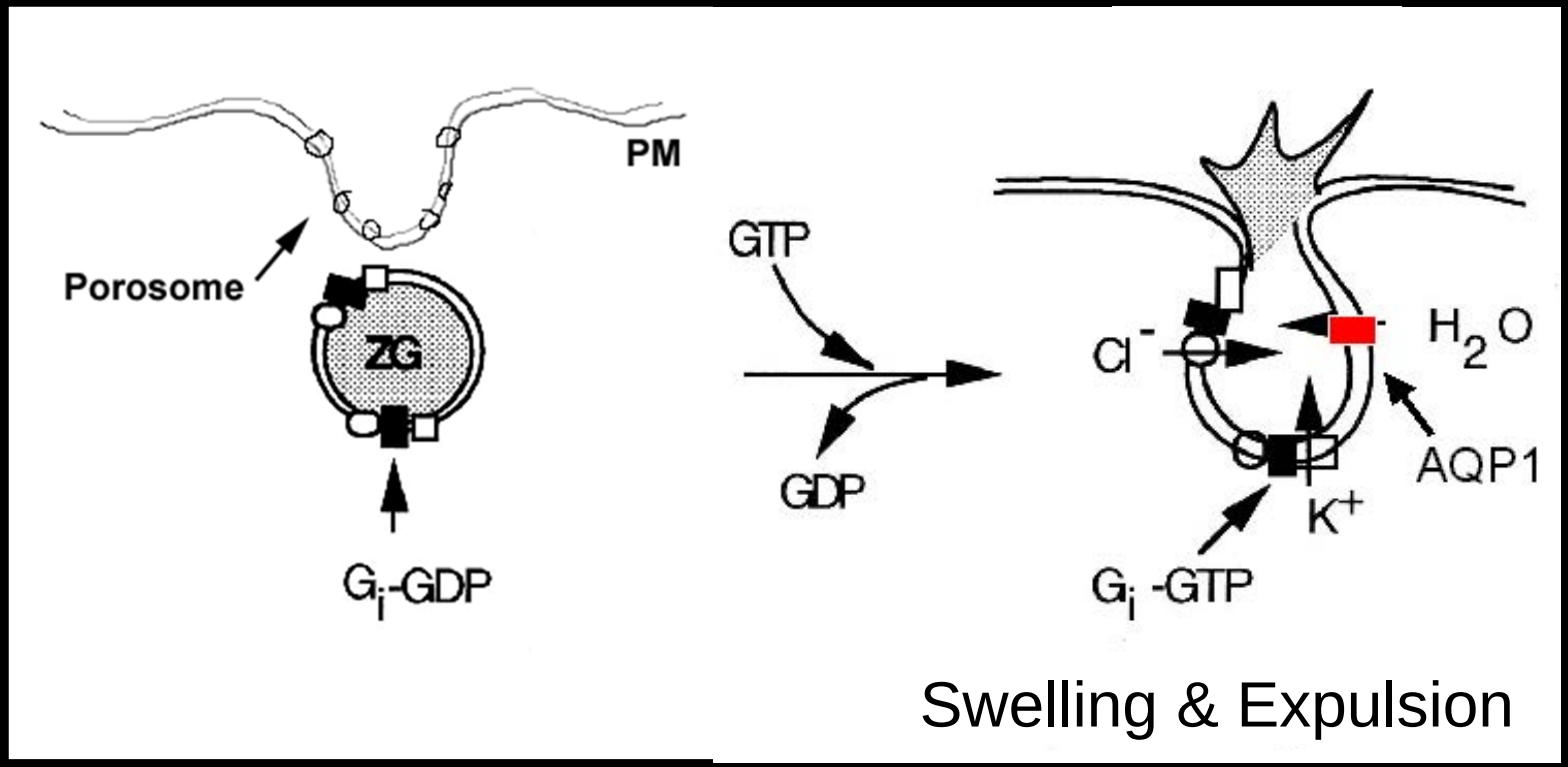
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Cho et. al. 2002 Proc. Natl. Acad. Sci. USA 99:4720-24

Regulation of water entry into ZG

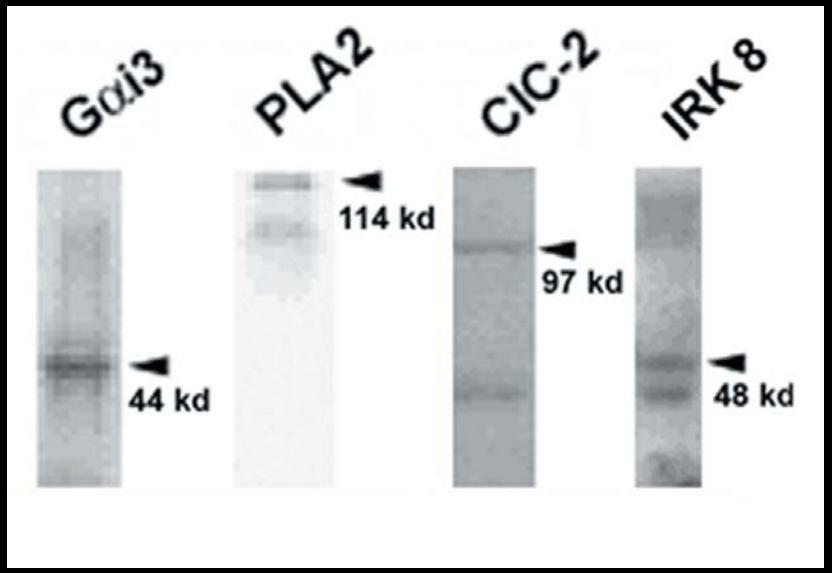


Jena et. al. 1997 PNAS Vol 94. 13317-22
Cho et. al. 2002 PNAS Vol 99. 4720-24

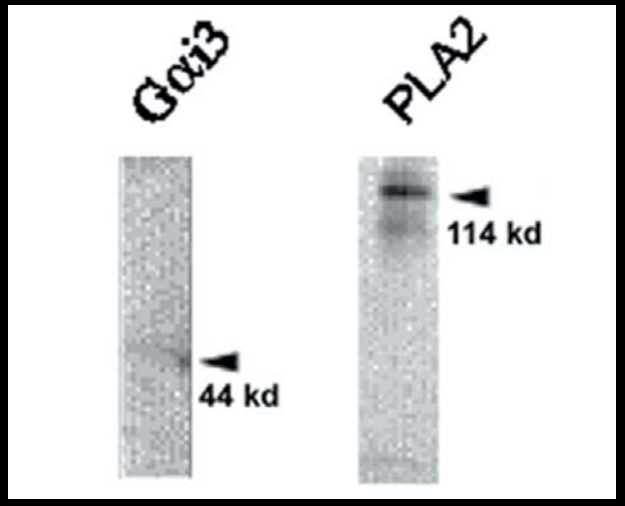
AQP1 Immunoisolated Complex

Zymogene granules





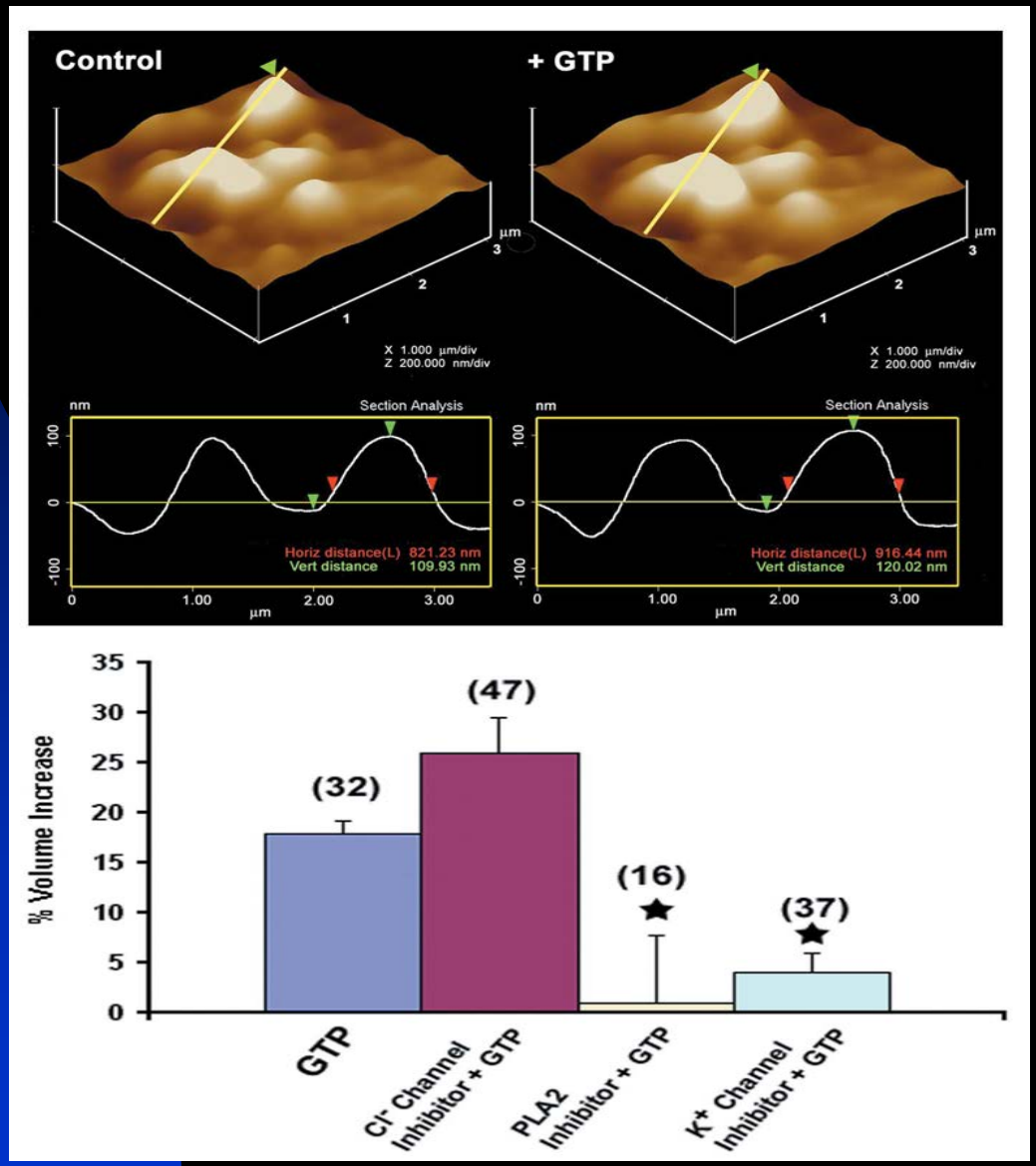
Red blood cells





Abu-Hamdah et. al. 2004 Cell Biol. Int. 28:7-17

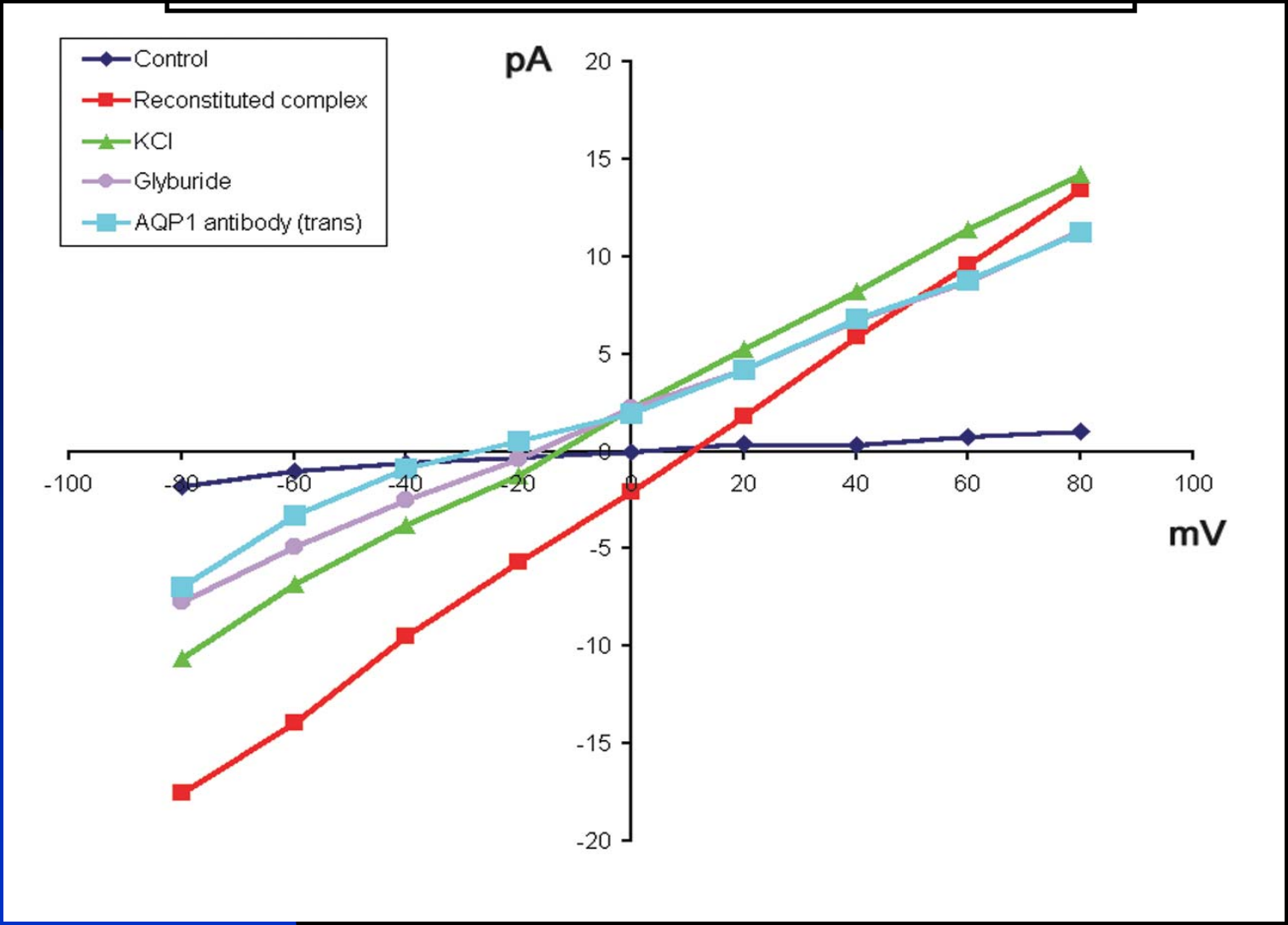
ZG volume changes measured by AFM





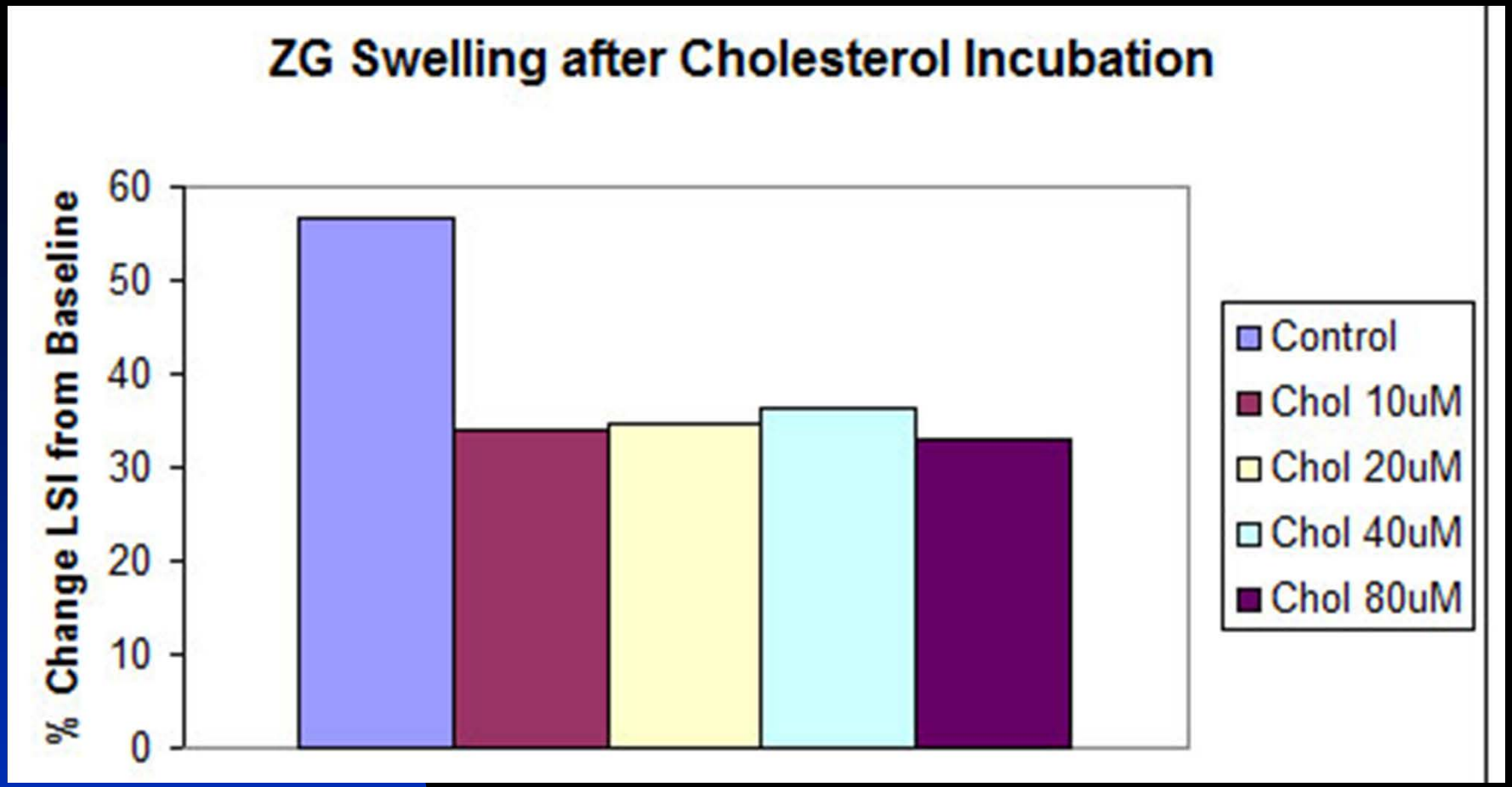
Abu-Hamdah et. al. 2004 Cell Biol. Int. 28:7-17

Electrophysiological properties of AQP1-immunocomplex reconstituted in PC:PS bilayer

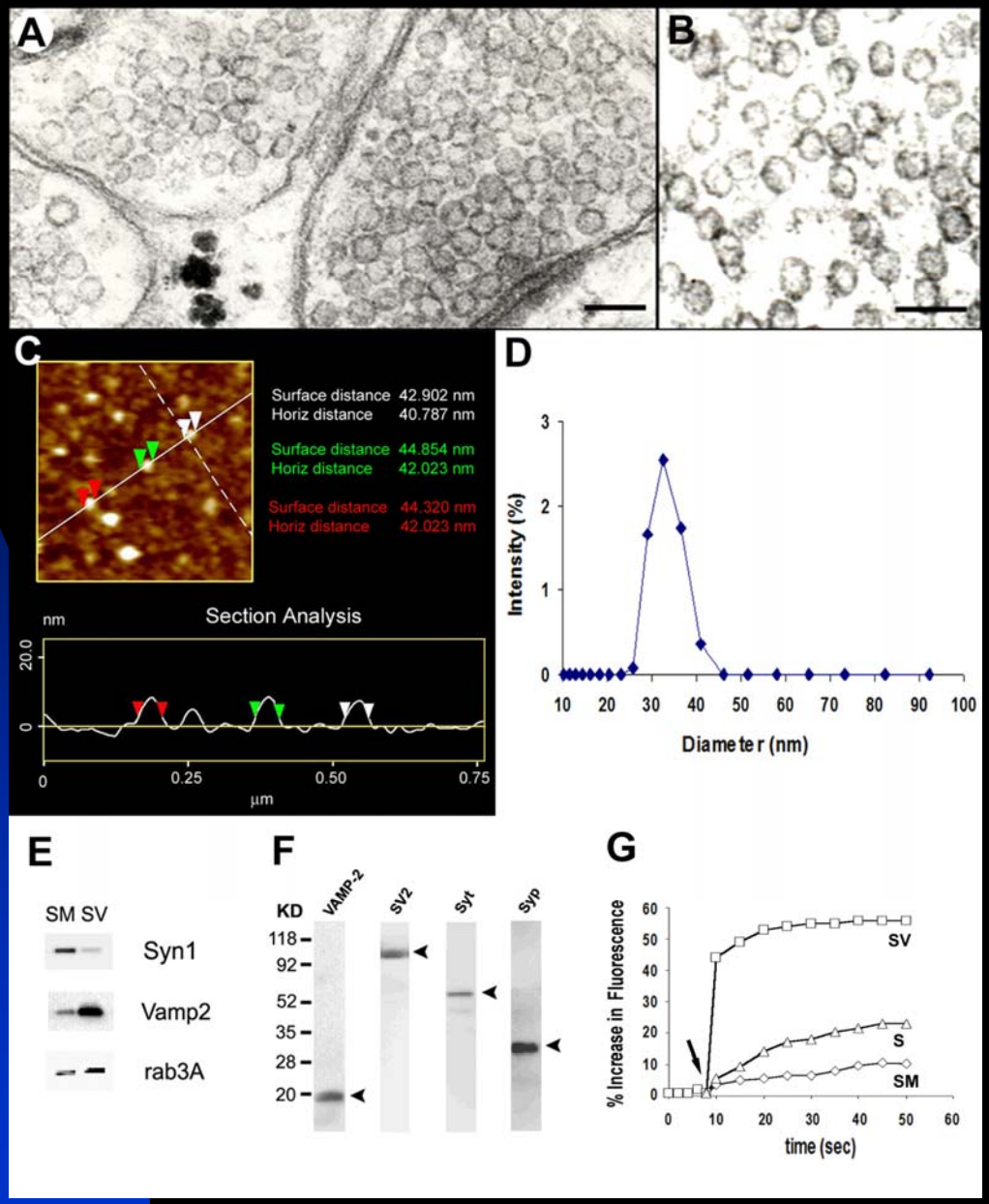




Abu-Hamdah et. al. 2004 Cell Biol. Int. 28:7-17

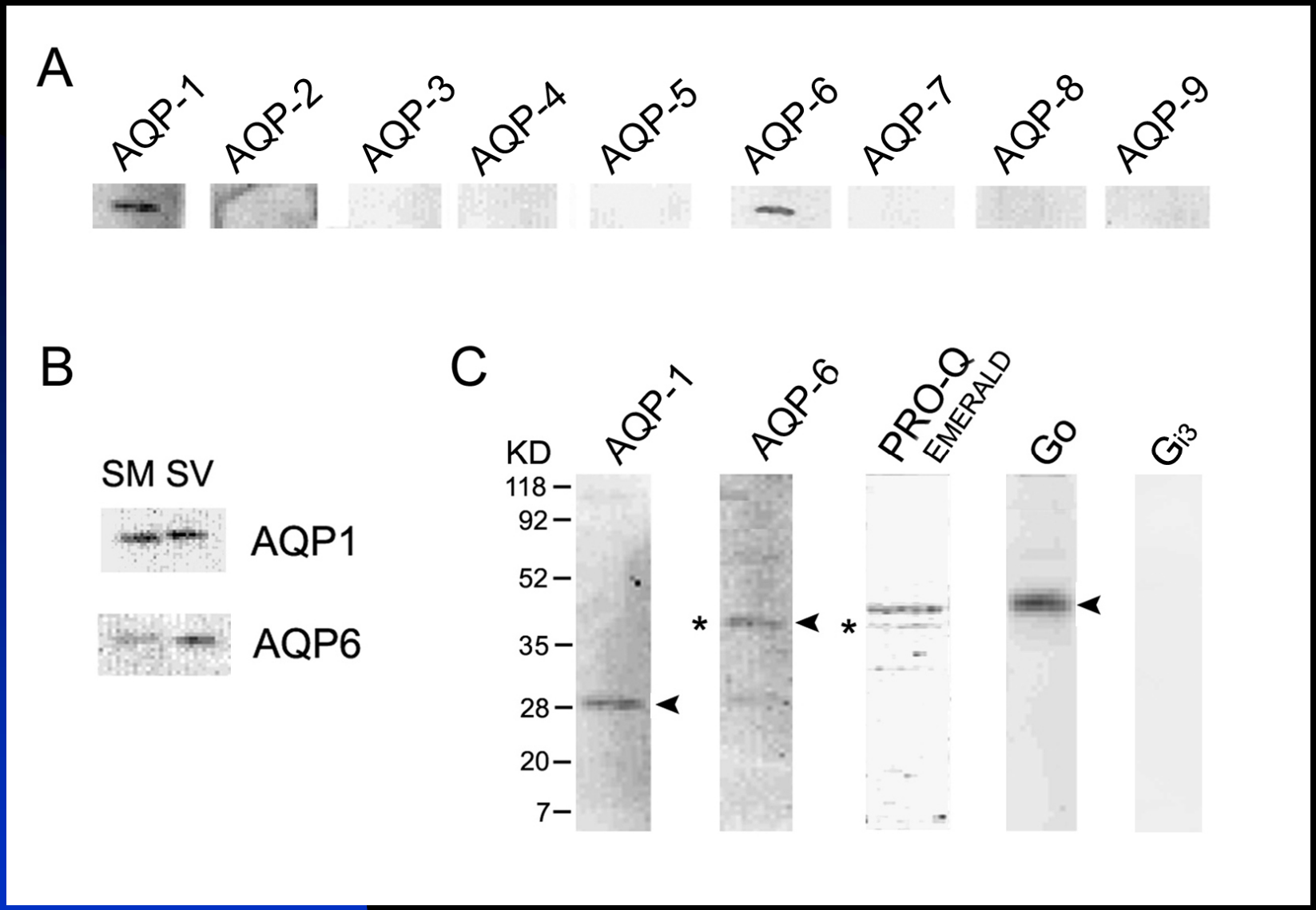


Amanda F., Jena B.P. (unpublished observation)



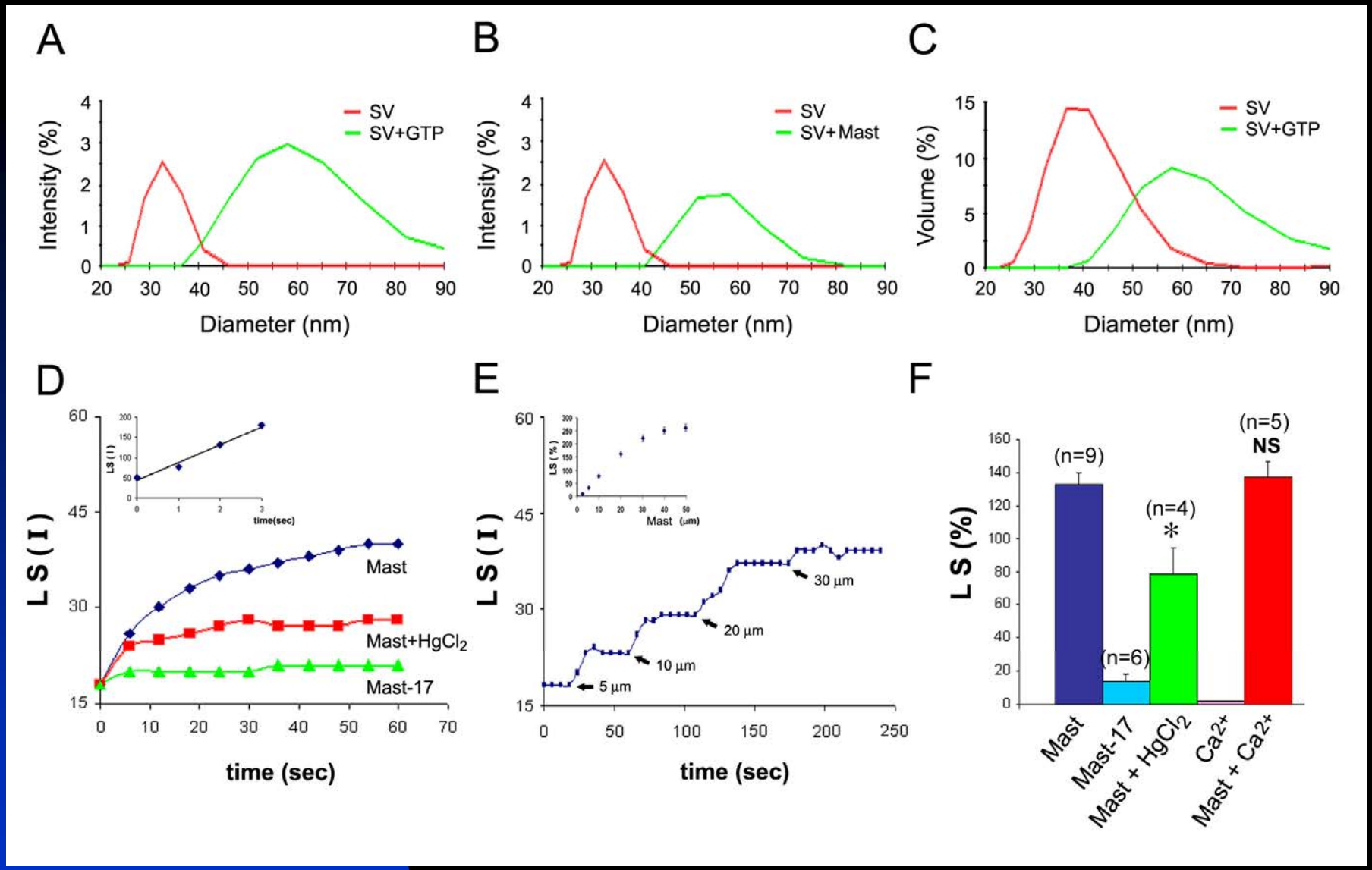


Jeremic et. al. 2005 Exp. Biol. Med. 230:674-80



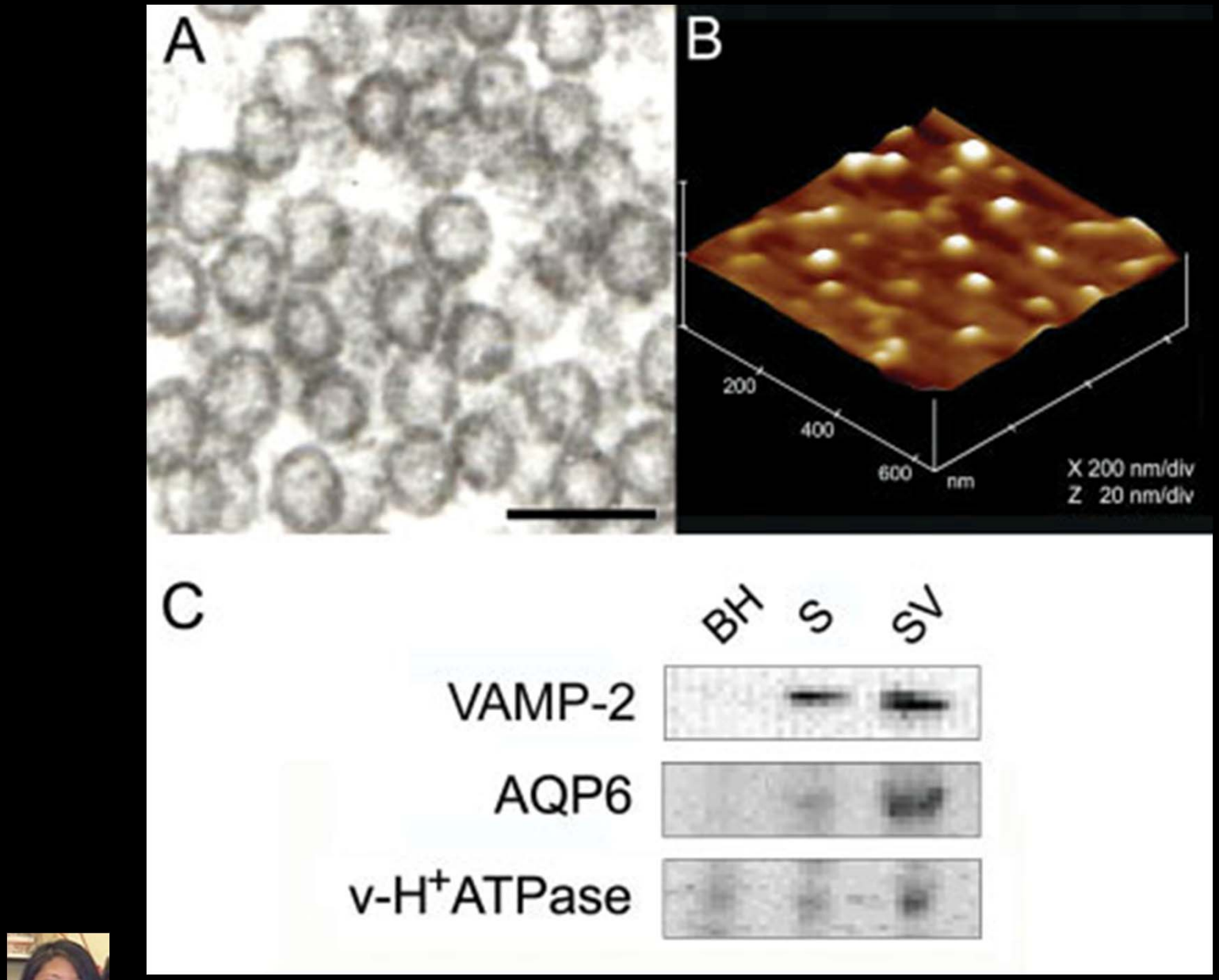


Jeremic et. al. 2005 Exp. Biol. Med. 230:674-80



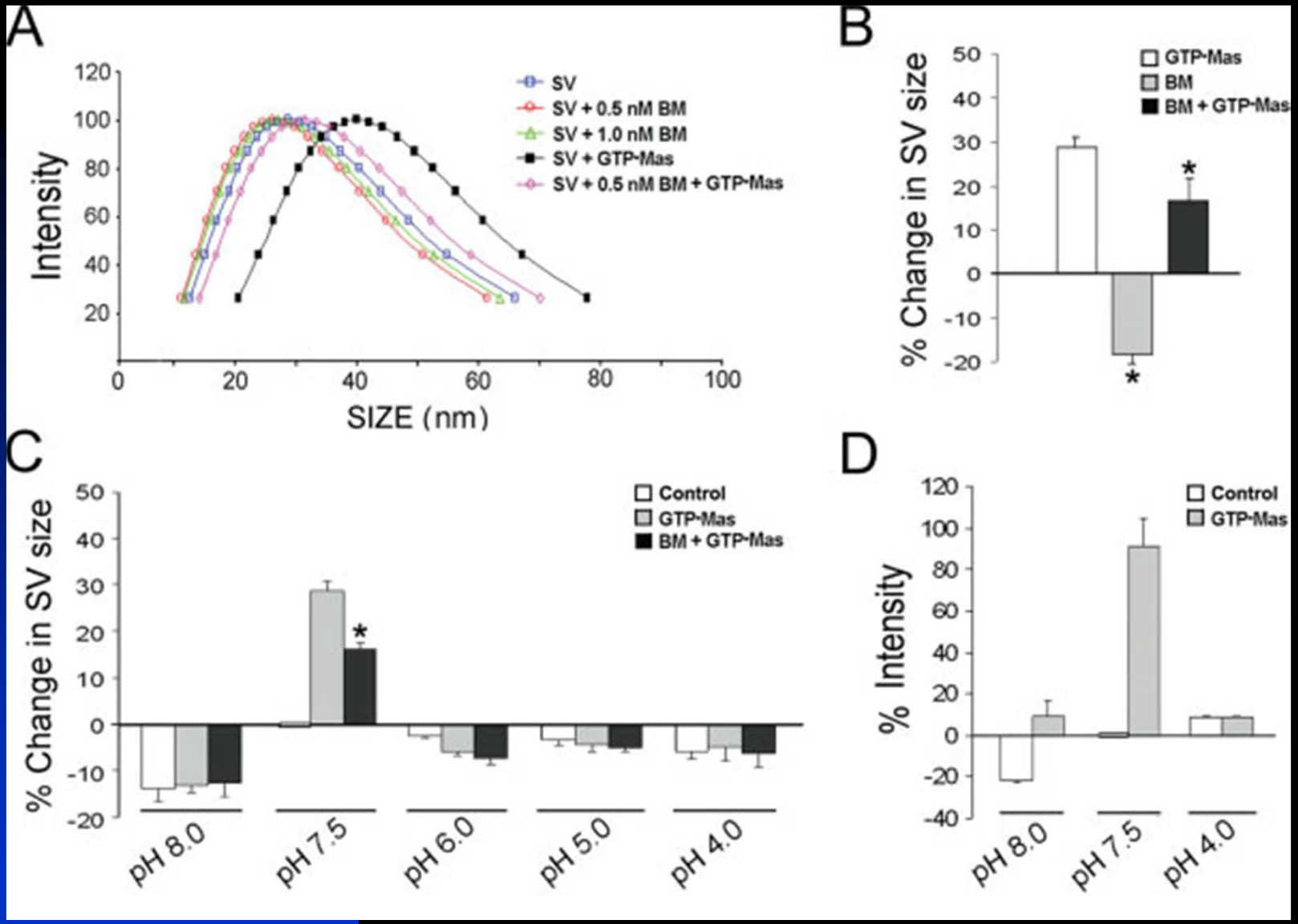


Jeremic et. al. 2005 Exp. Biol. Med. 230:674-80



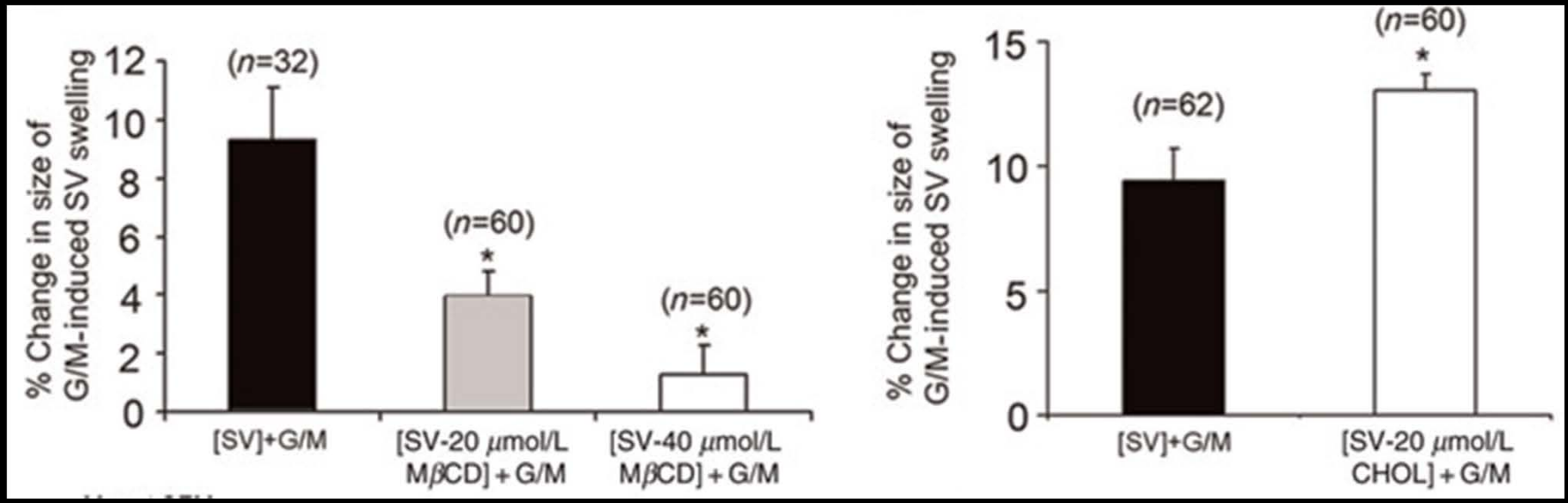


Shin et. al. 2010 J. Neurosci. Res. 88:95-101



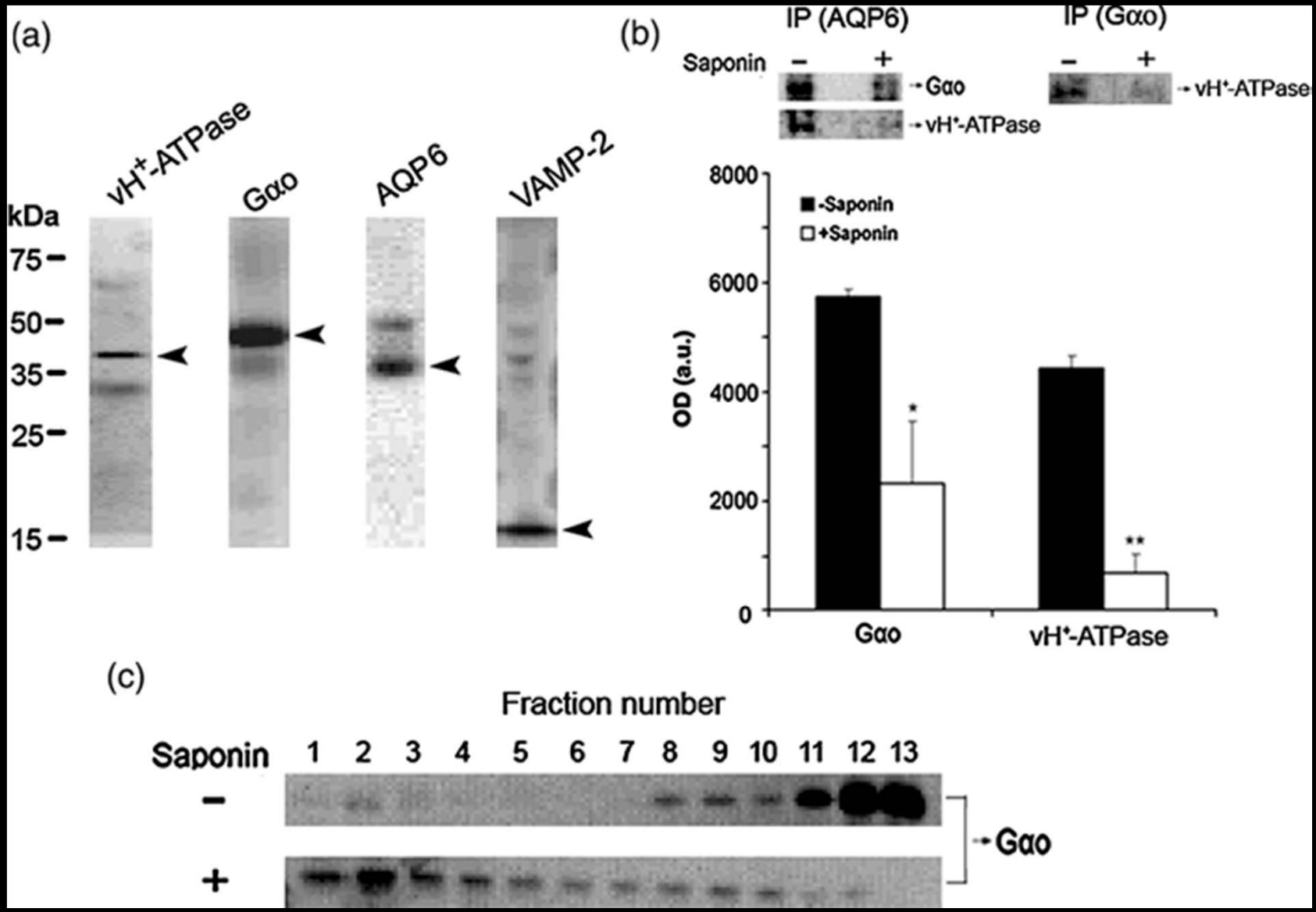


Shin et. al. 2010 J. Neurosci. Res. 88:95-101



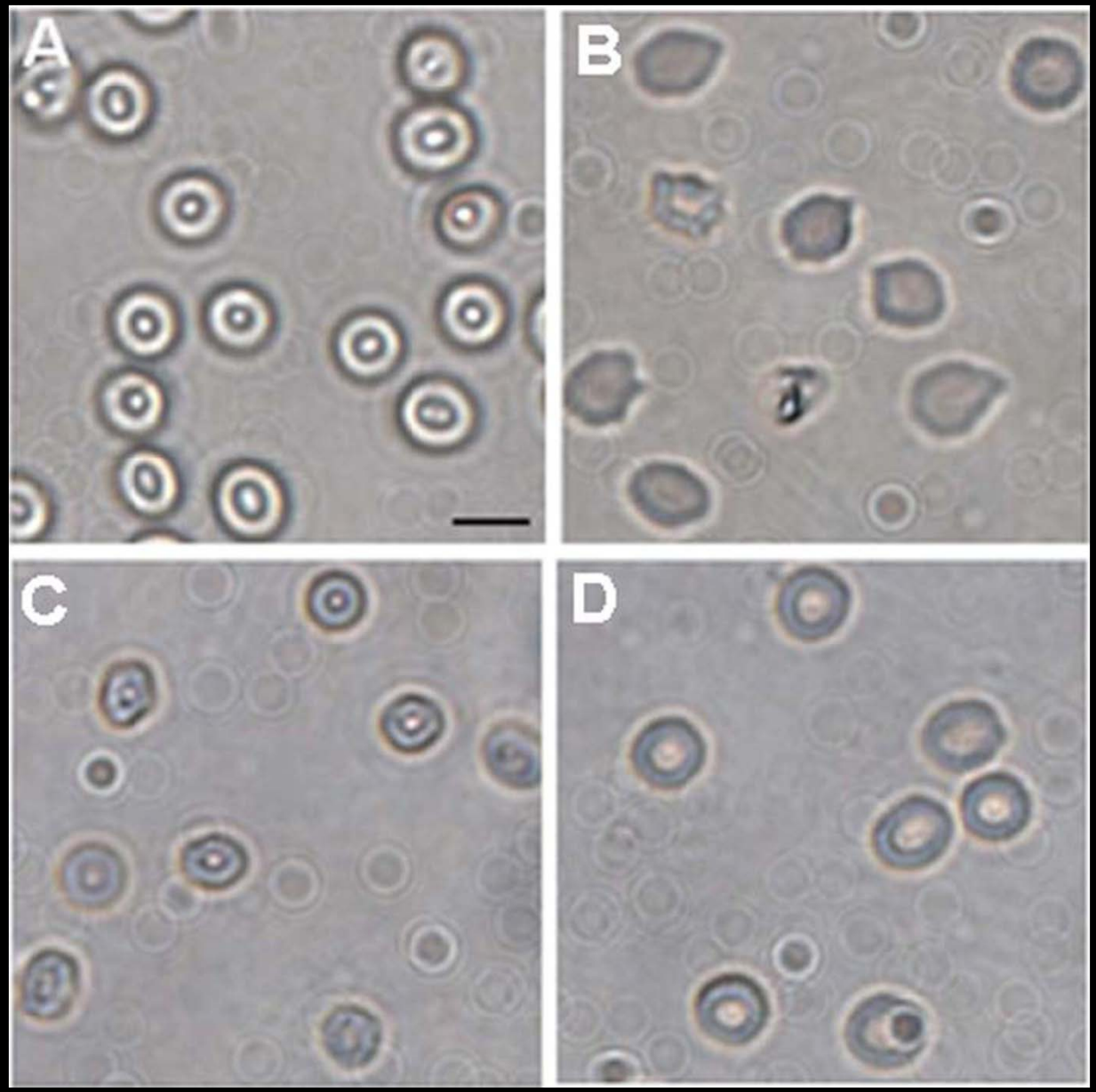


Lee et. al. 2010 Exp. Biol. Med. 235:470-7

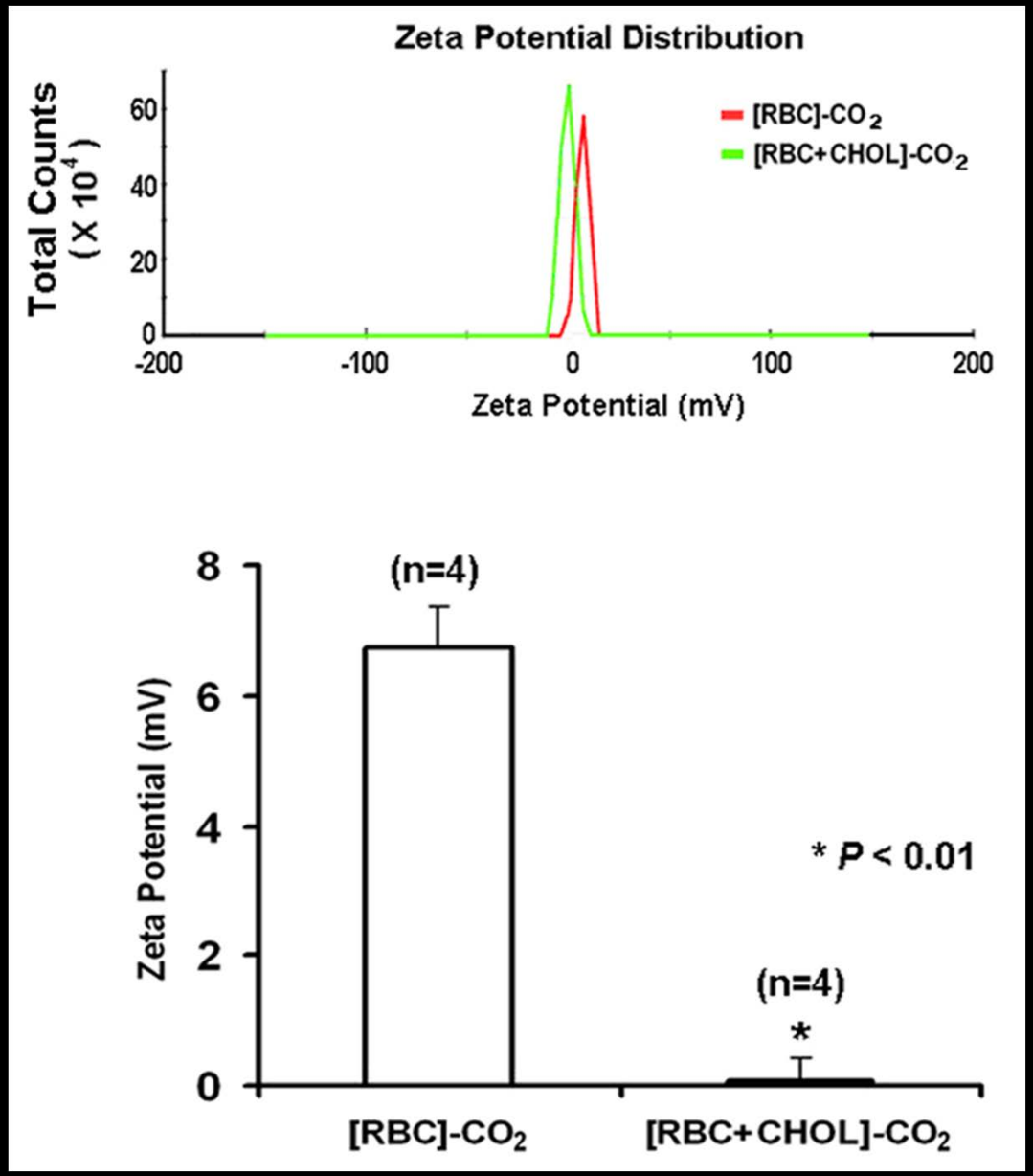




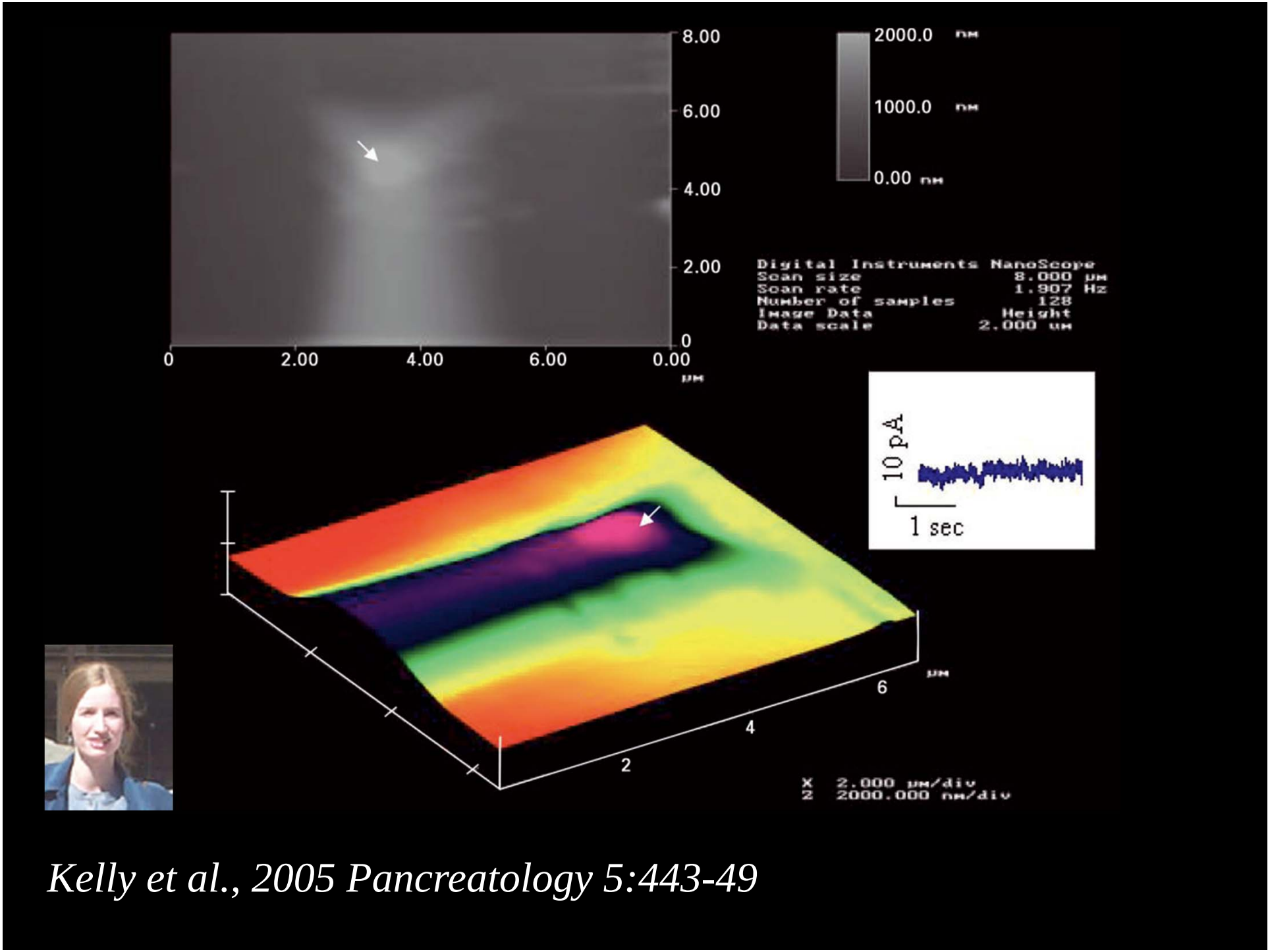
Lee et. al. 2010 Exp. Biol. Med. 235:470-7



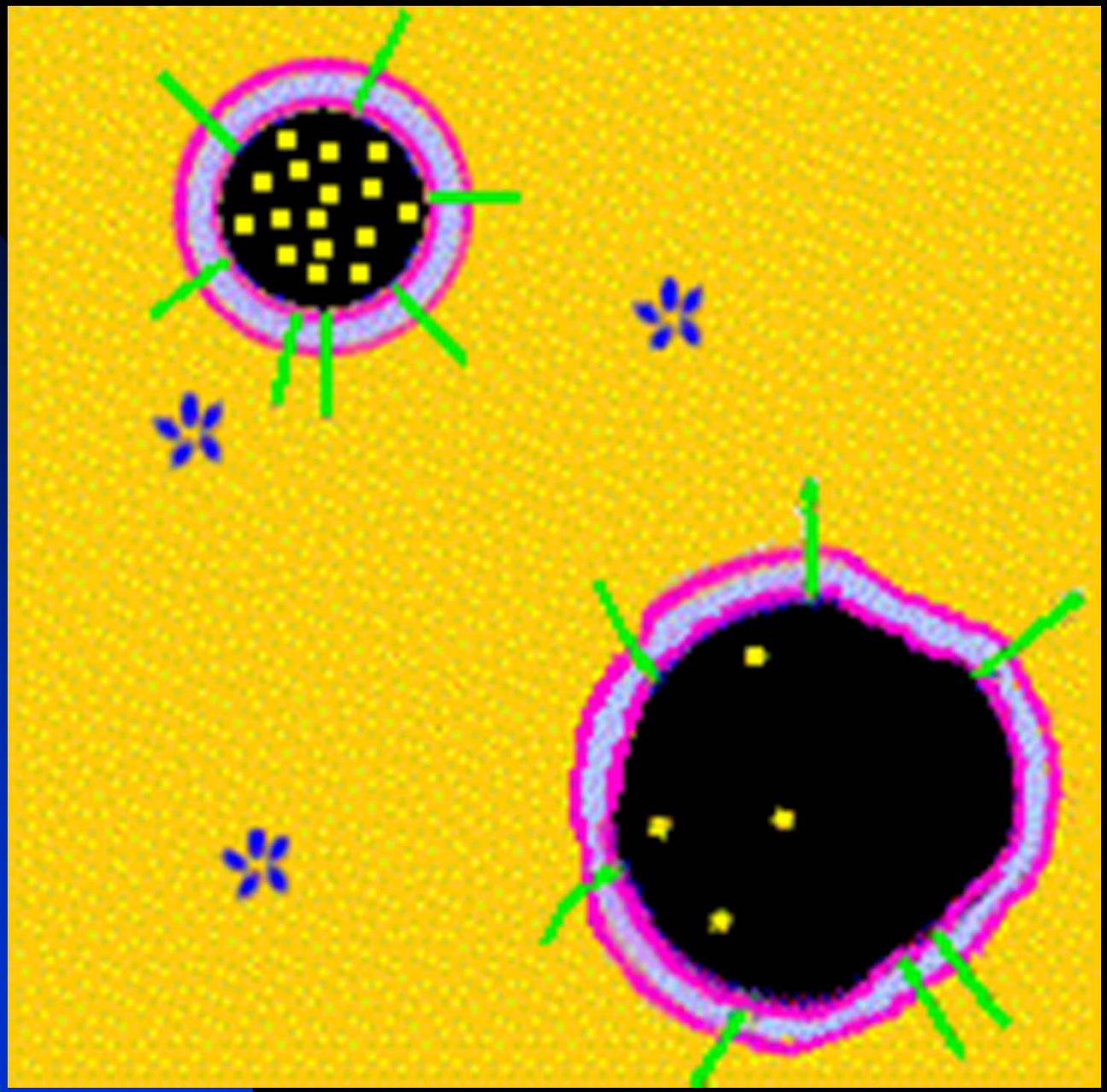
Jena S.G., Lee J.-S. 2010 J. Biol. Phys. Chem. 10:127-34



Jena S.G., Lee J.-S. 2010 J. Biol. Phys. Chem. 10:127-34



Kelly et al., 2005 Pancreatology 5:443-49

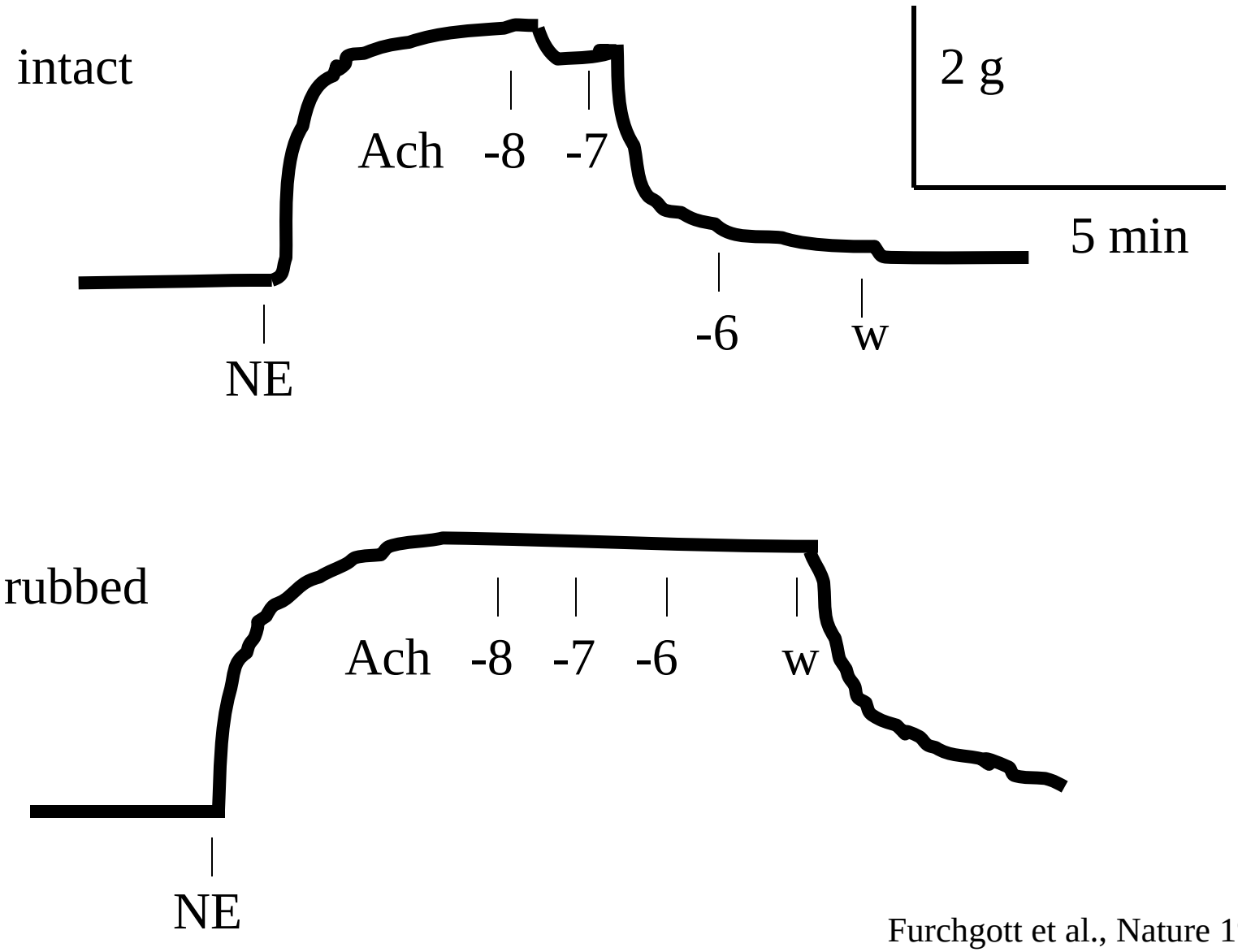


The truth about the movement of NO across cell membranes

Jeffrey Garvin

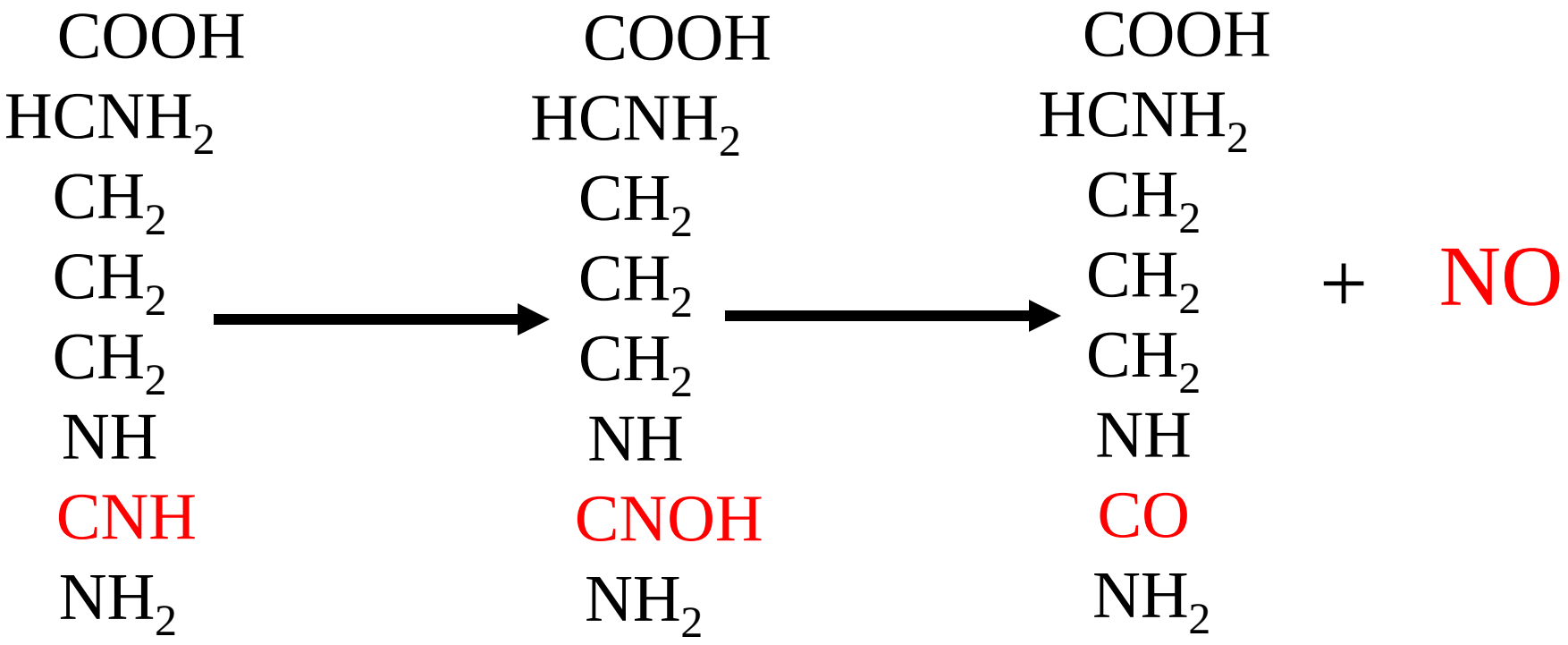
Hypertension and Vascular Research Division
Department of Internal Medicine
Henry Ford Hospital

Acetylcholine-induced EDRF release



Furchgott et al., Nature 1980

NO synthesis



L-arginine

L-citrulline

Why do we care about NO?

NO is involved in:

1. CNS function and cognition
2. Cardiac contractility
3. Peripheral vascular resistance
4. Respiration
5. Gut motility and ion absorption
6. Renal perfusion and transport
7. Reproduction

Properties of NO

1. It is small.

2. It is non-polar.

3. It is RELATIVELY lipophilic with a partition coefficient of about 5.

4. It is a gas.

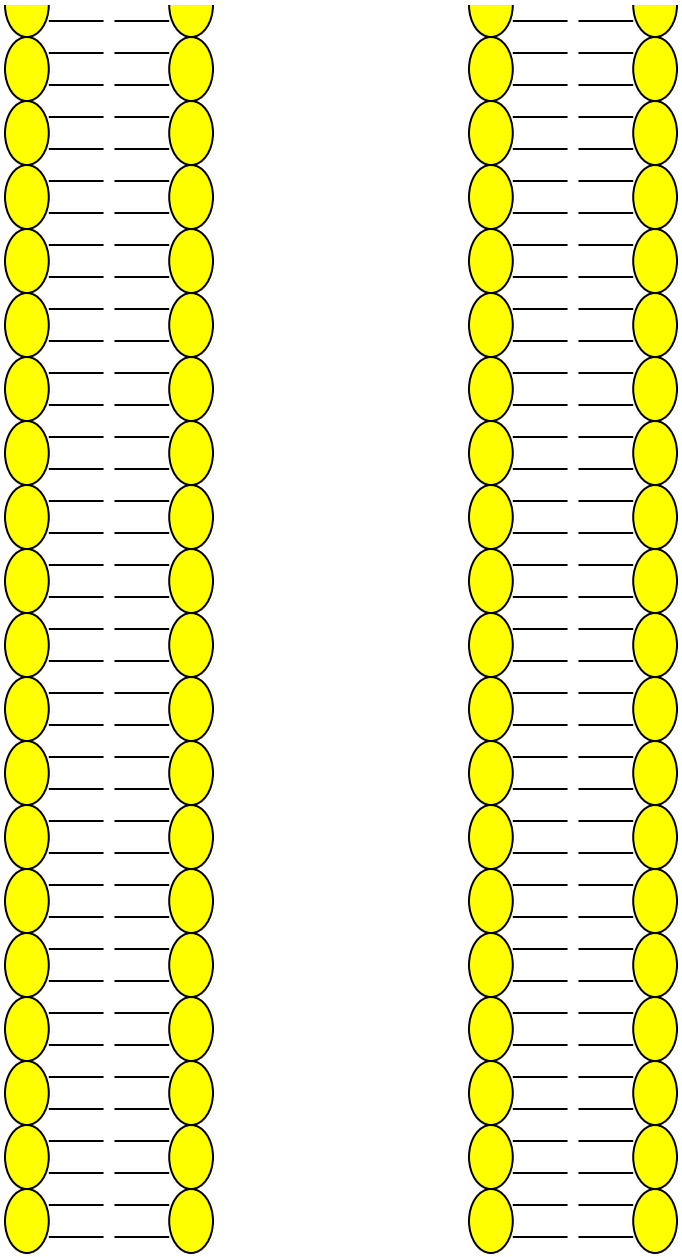
5. Its reactive (different from O_2 and CO_2).

How many think NO diffuses through two bilayers

endothelial cell

NOS 3

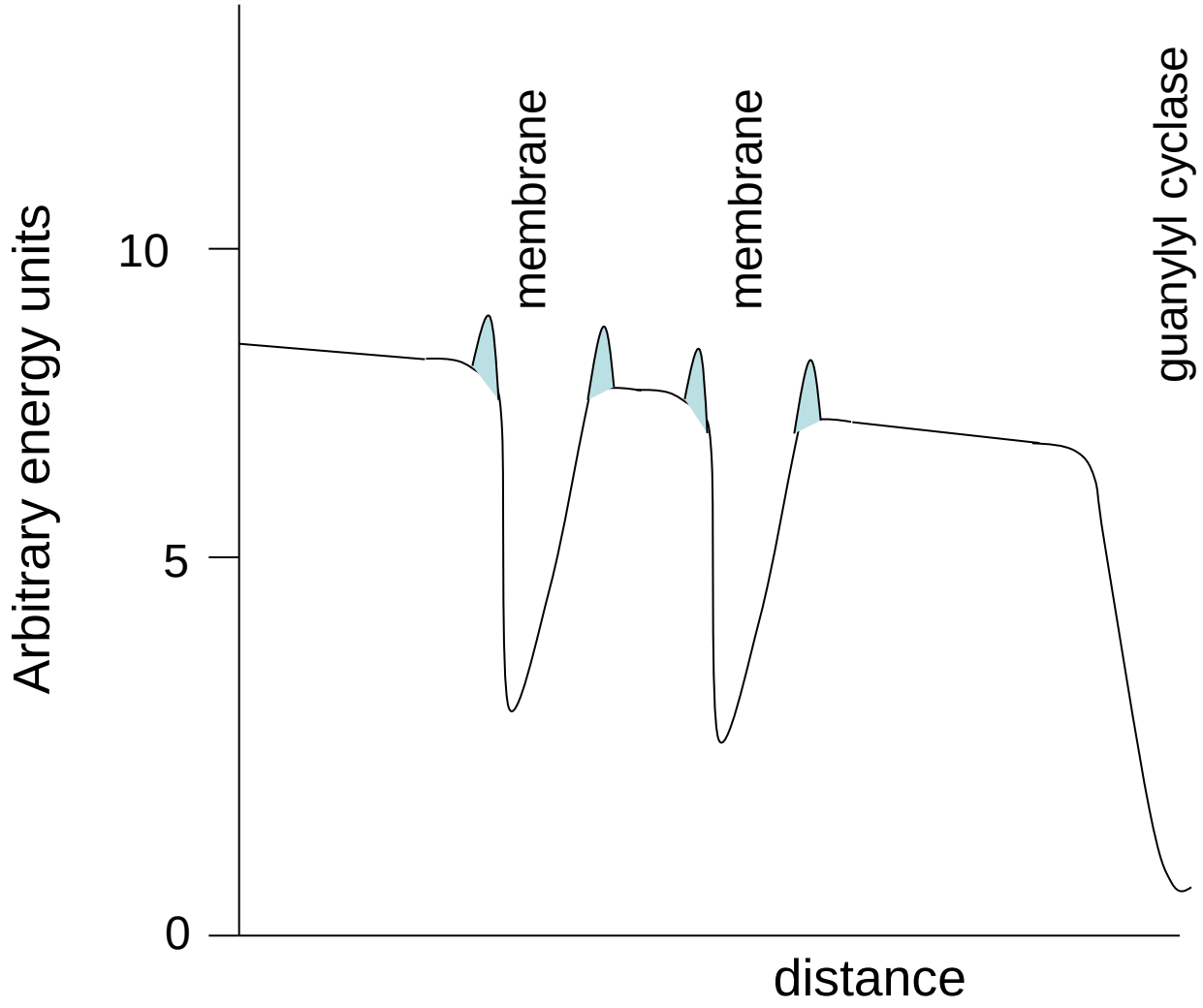
NO



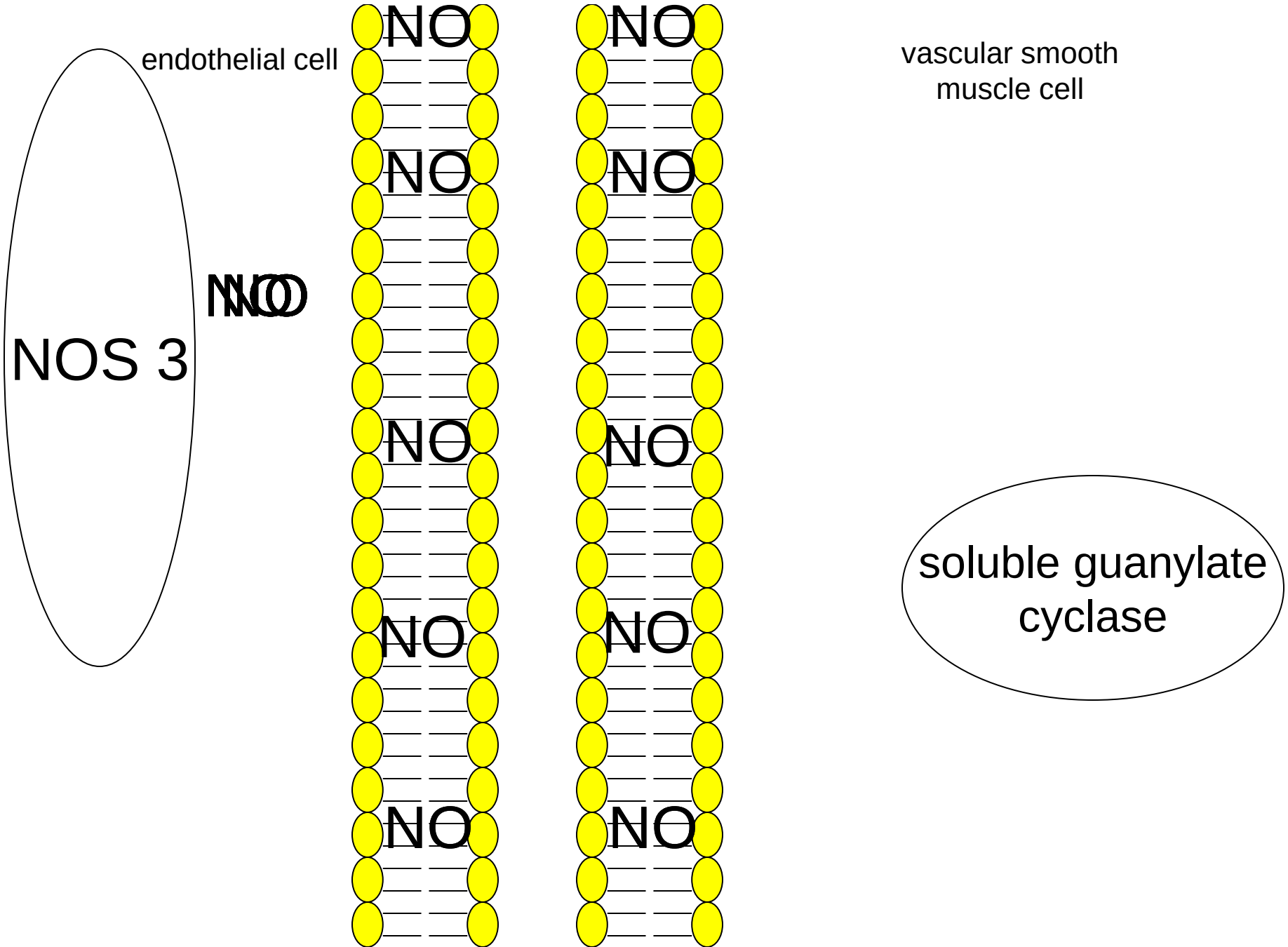
vascular smooth muscle cell

soluble guanylate cyclase

Energy profile of NO with distance based on partition coefficient



A slightly more “realistic” model of NO diffusion through bilayers



There have been no direct measurements of the NO permeability of any cell membrane!!!

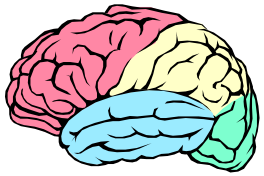
There has been one calculation which is widely cited. This value of 76 cm/s was calculated based on steady-state measurements of NO within an artificial membrane using 2 mM NO.

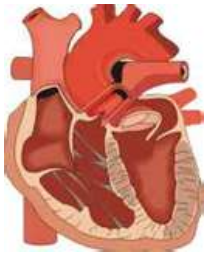
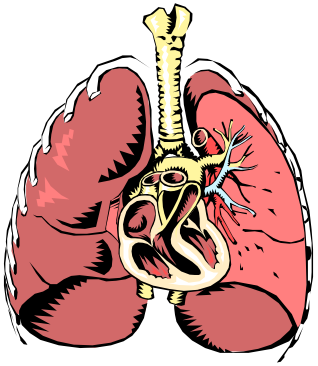
Free diffusion creates several problems:

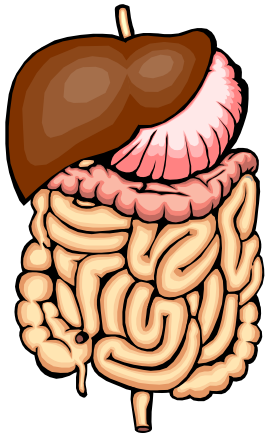
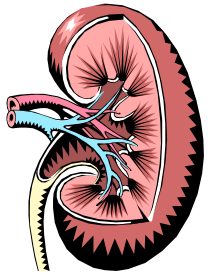
1. Free diffusion is relatively slow;
2. The amount of NO trapped in the membrane is relatively large;
3. If NO is only around transiently, the membrane could act as a trap;
4. There is no control over where NO goes;
5. There is no way to regulate NO release;
6. There is little control over NO entry.

As you have heard today “gas channels” including aquaporin-1(AQP-1) has been shown to transport CO_2 and other gases.

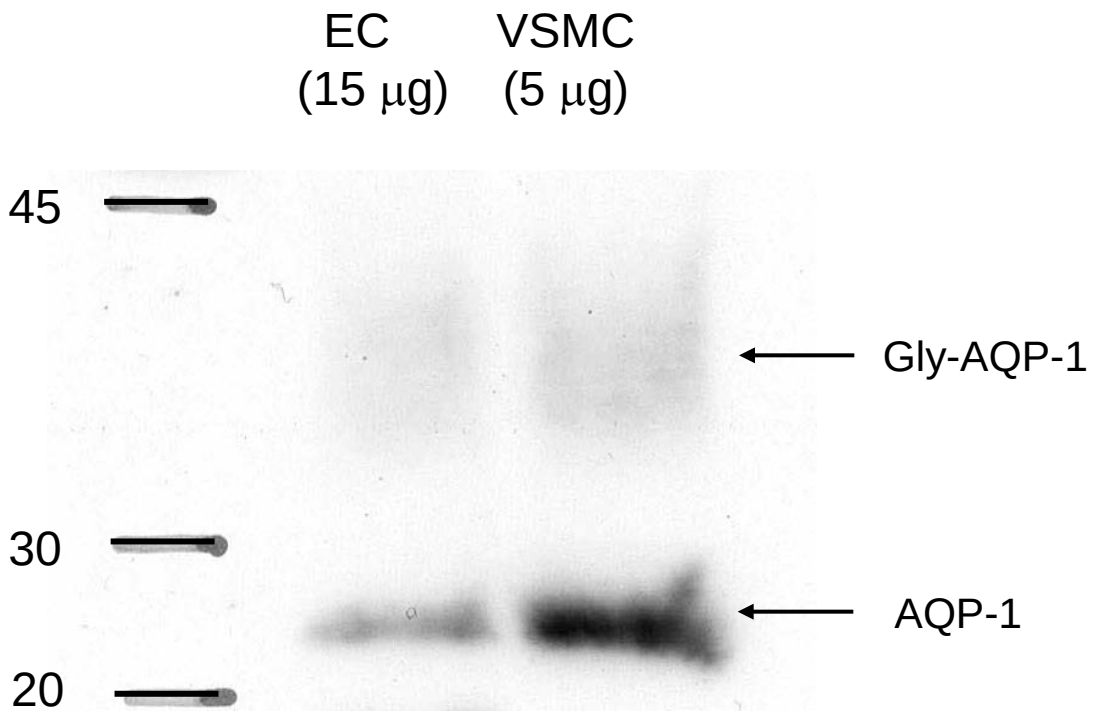
Organs where AQP-1 and NO synthase are expressed



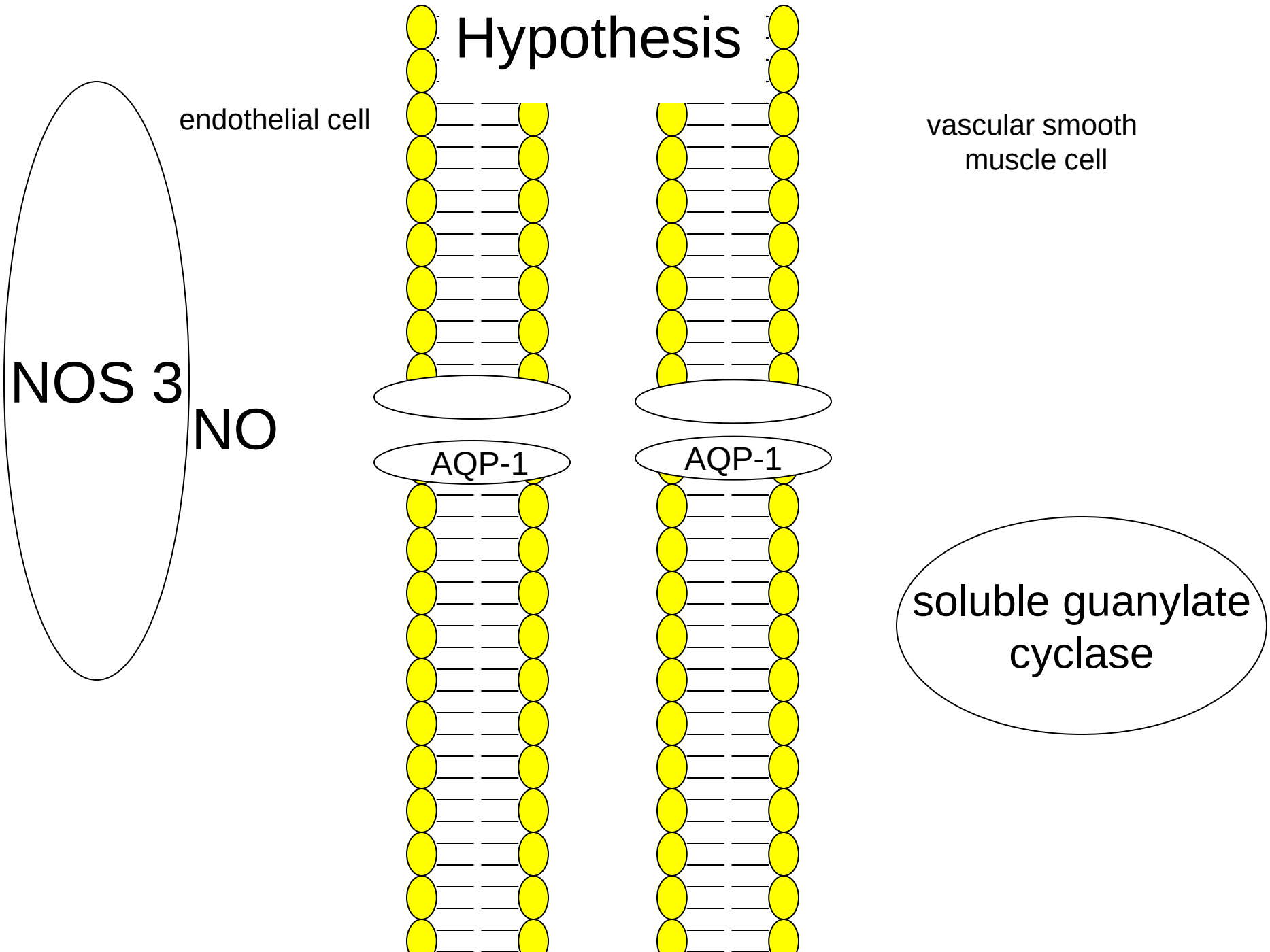




AQP-1 expression by aortic EC and VSMC isolated from CD1 mice



Hypothesis


endothelial cell
NOS 3
NO
AQP-1
AQP-1
vascular smooth muscle cell
soluble guanylate cyclase

If our hypothesis is correct:

1. NO permeability (P_{NO}) should correlate with water permeability (P_f).

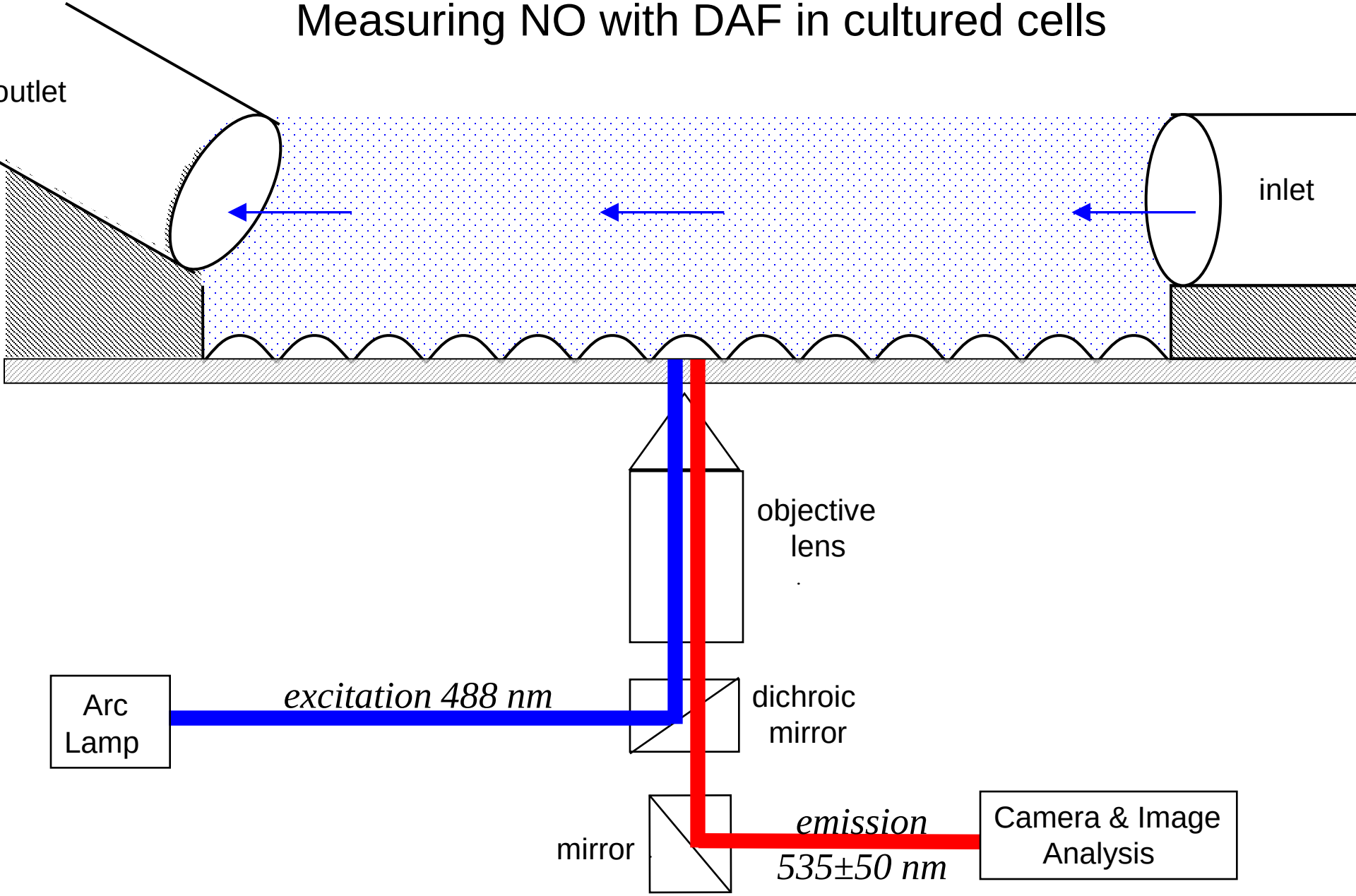
2. Increasing AQP-1expression should increase NO flux.

3. Inhibitors of AQP-1 should reduce NO flux.

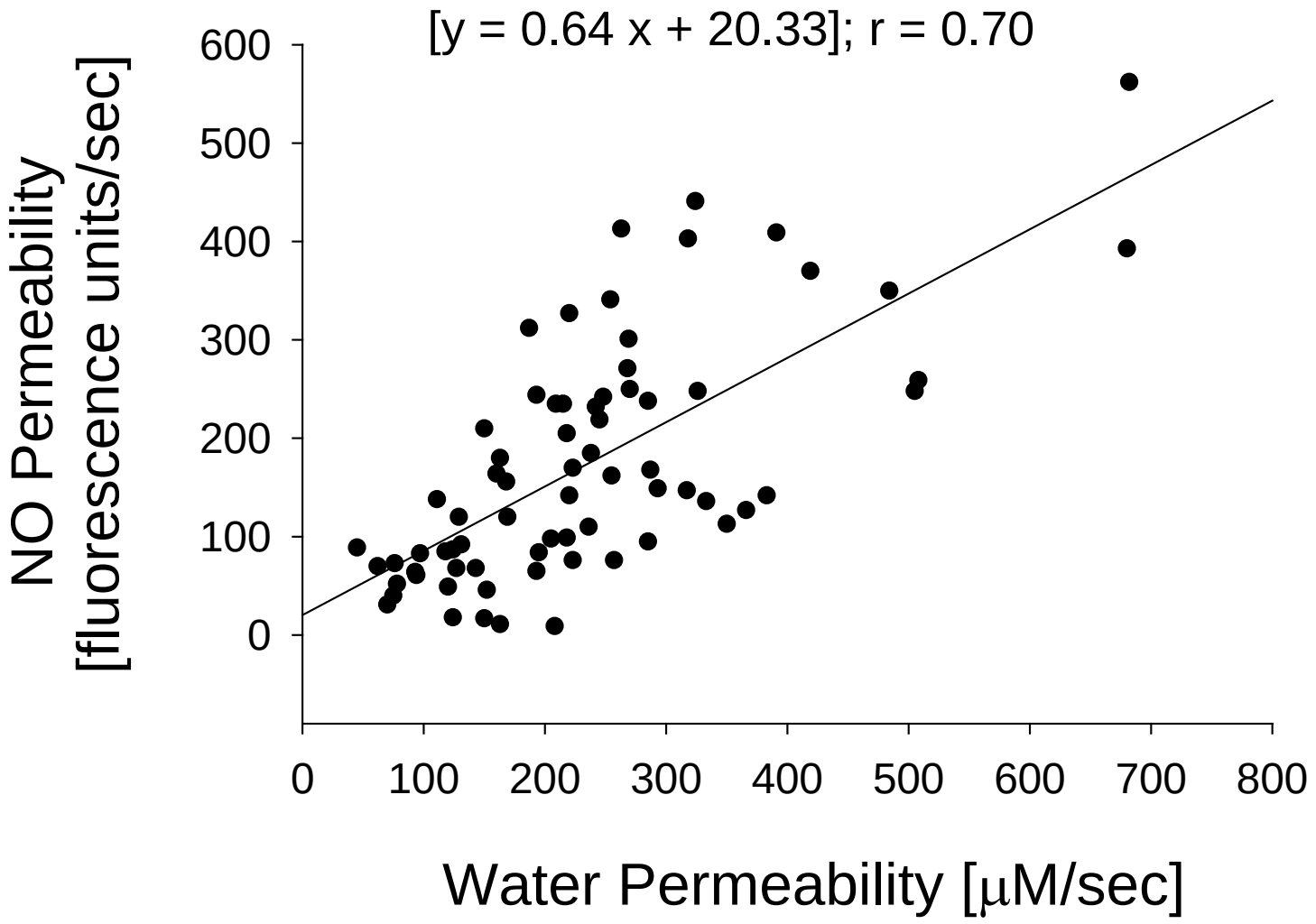
4. NO flux should be saturable.

5. Purified AQP-1 should transport NO.

Measuring NO with DAF in cultured cells



Correlation of P_{NO} and P_f in stably transfected CHO cells



1. NO permeability (P_{NO}) correlates with water permeability (P_f).

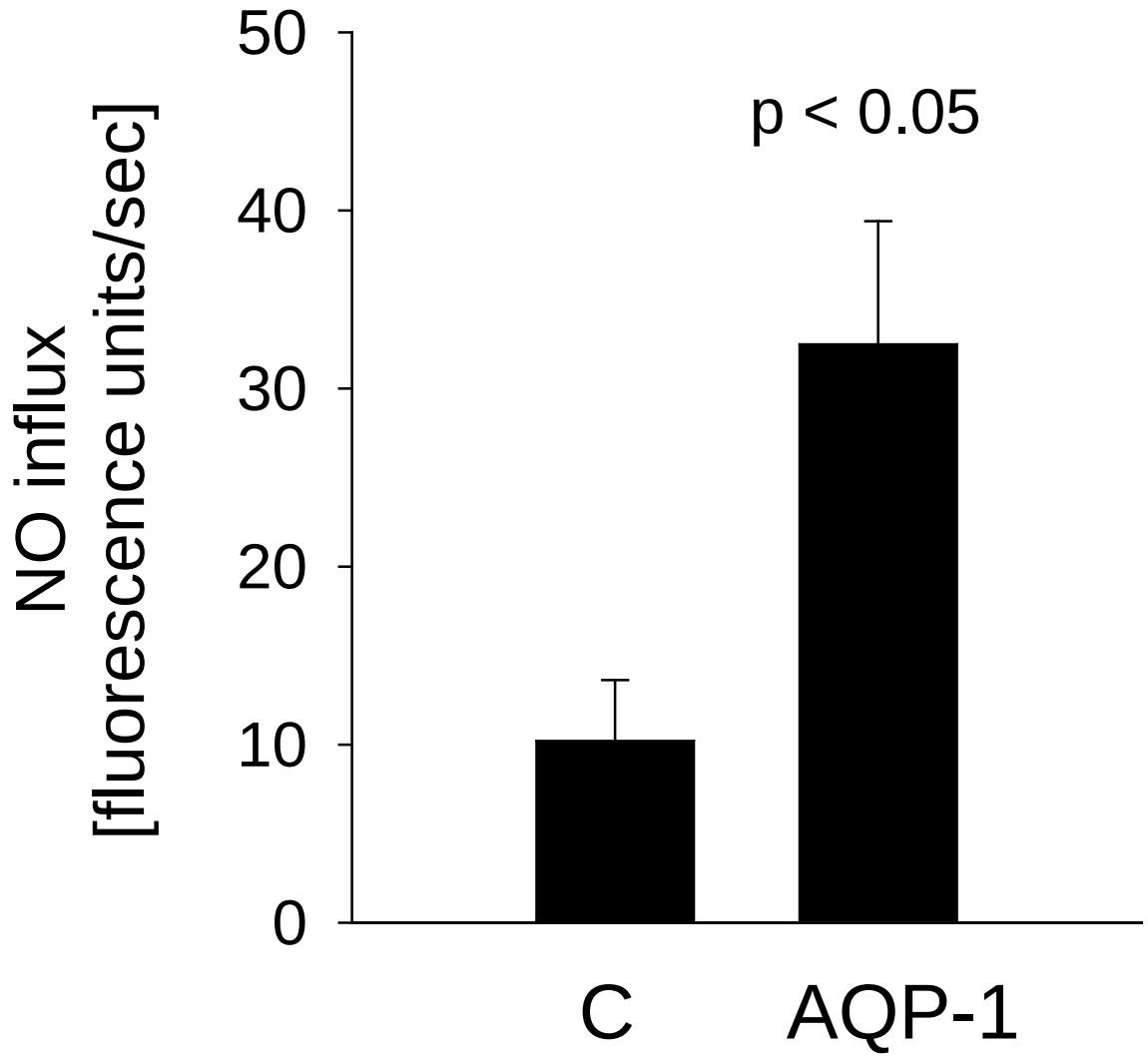
2. Increasing AQP-1 expression should increase NO flux.

3. Inhibitors of AQP-1 should reduce NO flux.

4. NO flux should be saturable.

5. Purified AQP-1 should transport NO.

Effect of transiently transfecting CHO cells with aquaporin-1 (AQP-1) on NO influx



NO gradient by SPM (5 μM NO)

1. NO permeability (P_{NO}) correlates with water permeability (P_f).

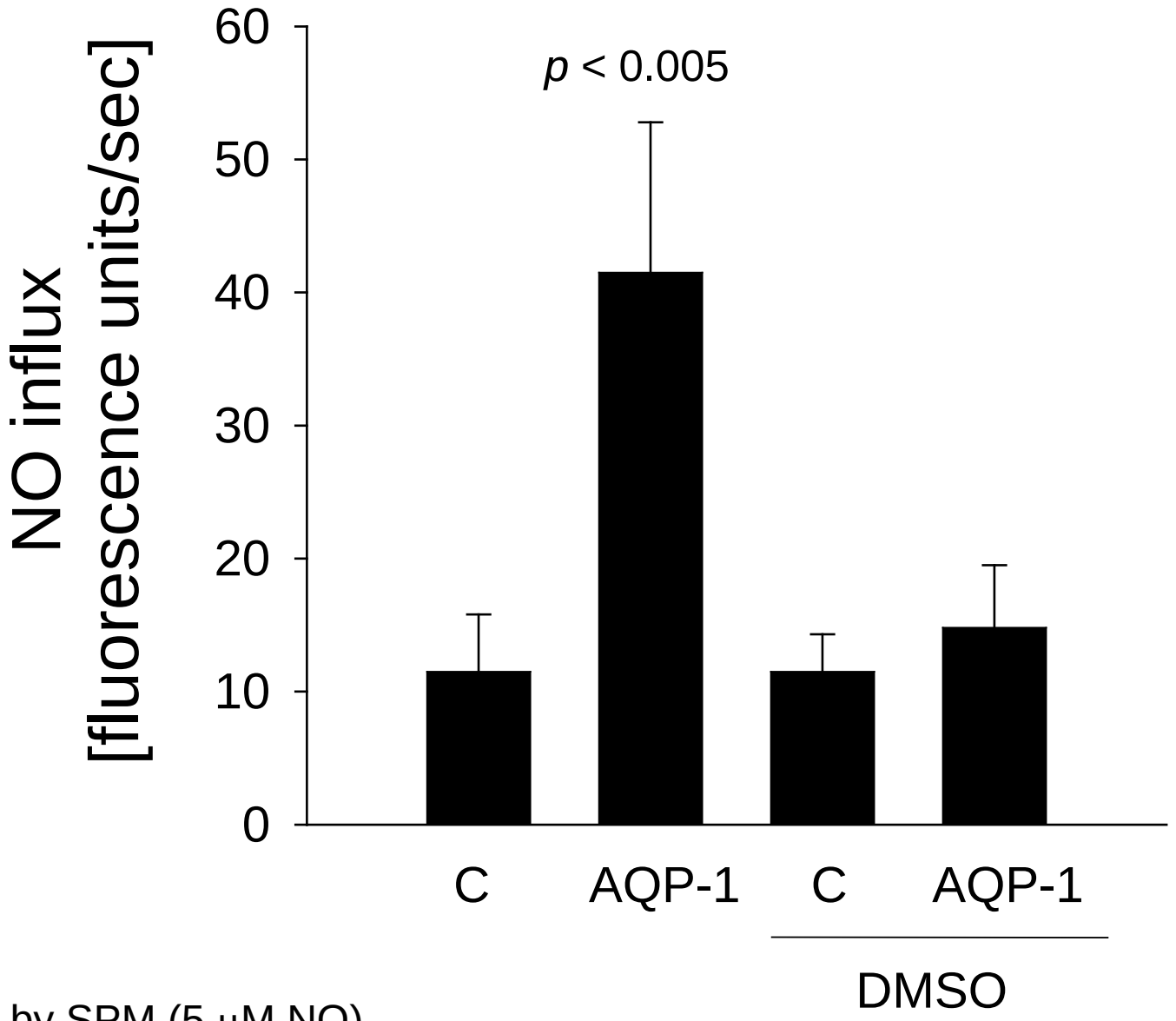
2. Increasing AQP-1 expression increases NO flux.

3. Inhibitors of AQP-1 should reduce NO flux.

4. NO flux should be saturable.

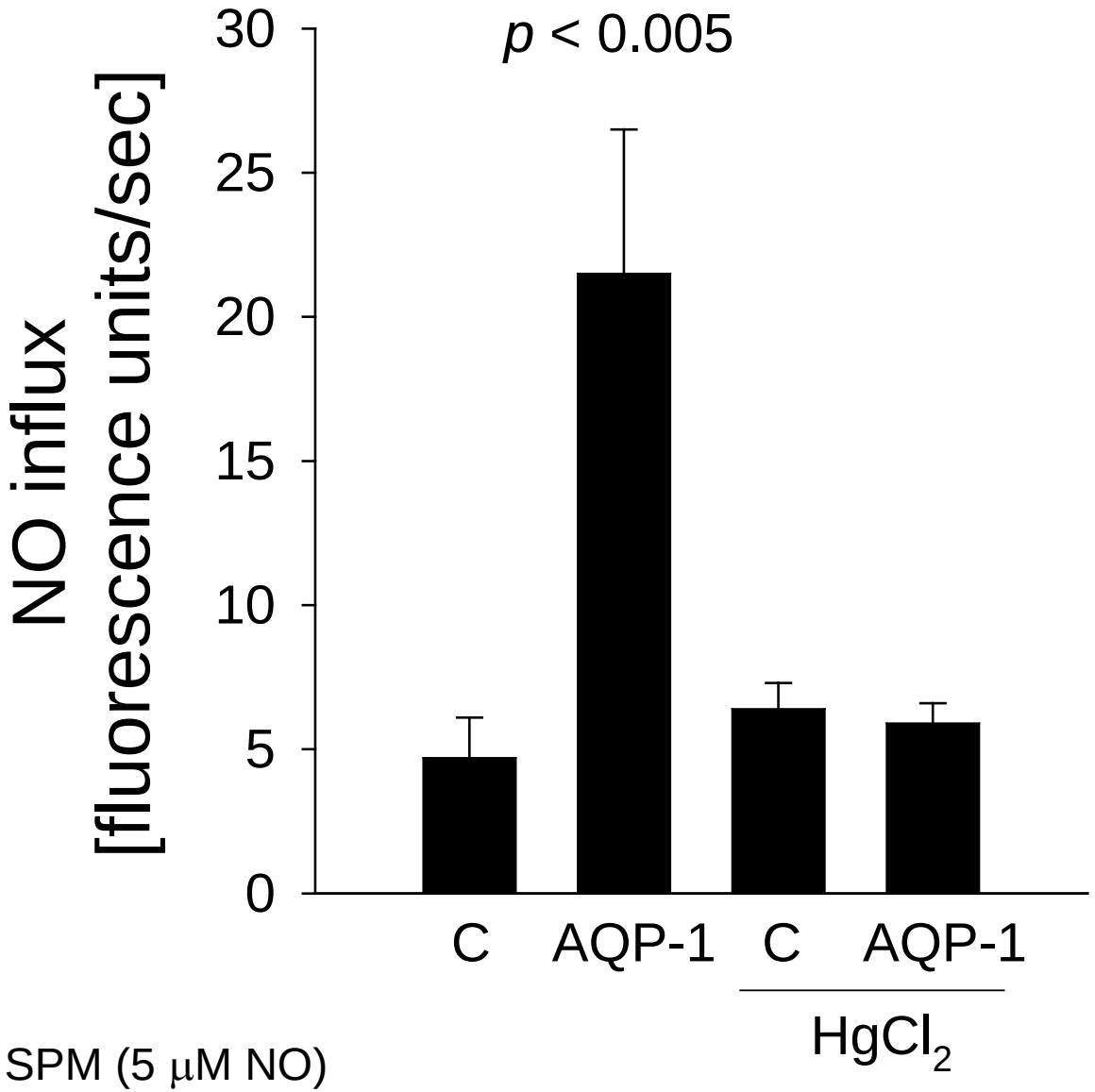
5. Purified AQP-1 should transport NO.

Effect of DMSO, an AQP-1 inhibitor, on NO influx into transiently transfected CHO cells



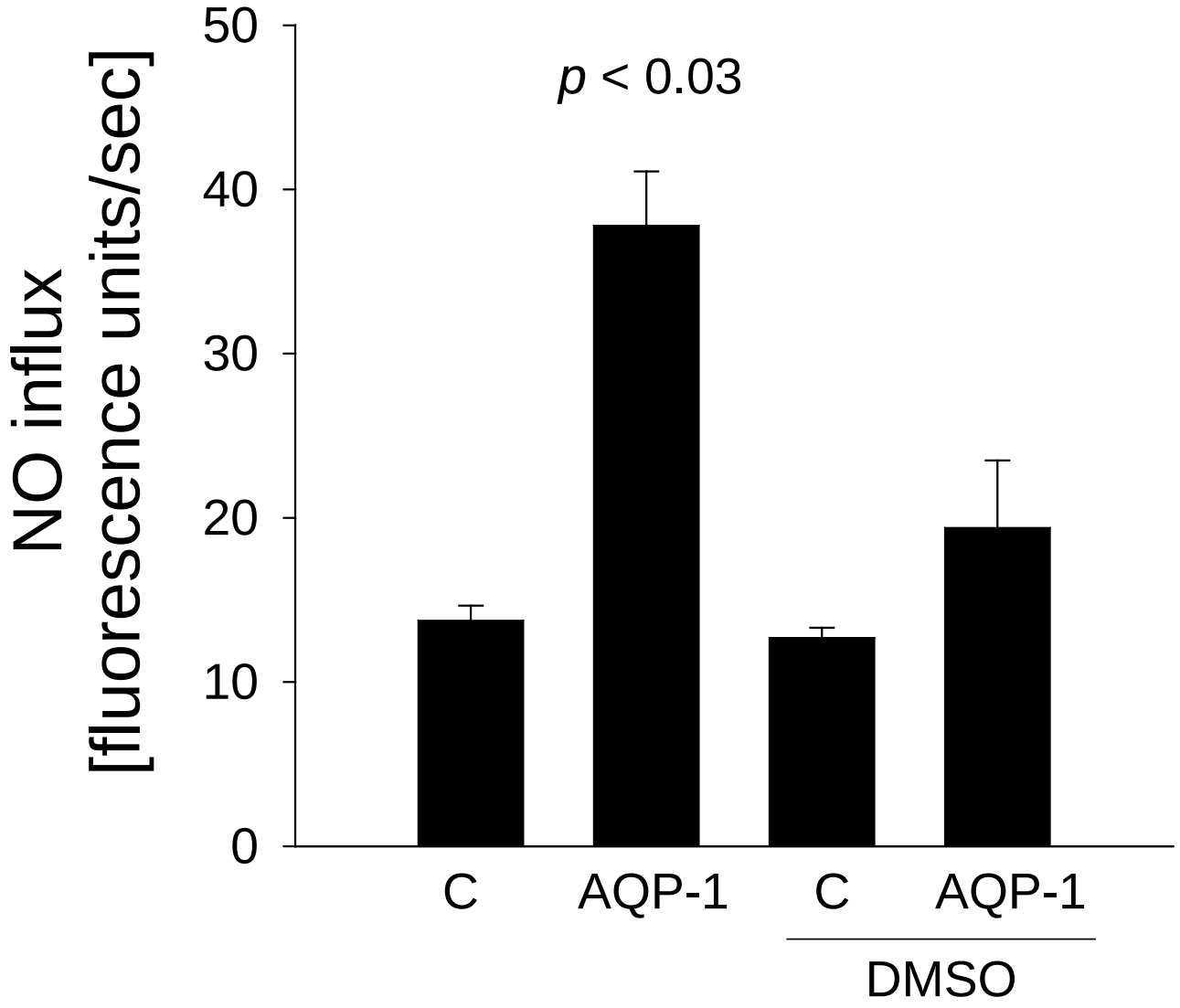
NO gradient by SPM (5 μM NO)

Effect of 20 μM $HgCl_2$, an AQP-1 inhibitor, on NO influx into transfected CHO cells



NO gradient by SPM (5 μM NO)

Effect of DMSO on NO influx into transiently transfected CHO cells



NO gradient by gas (5 μM NO)

1. NO permeability (P_{NO}) correlates with water permeability (P_f).

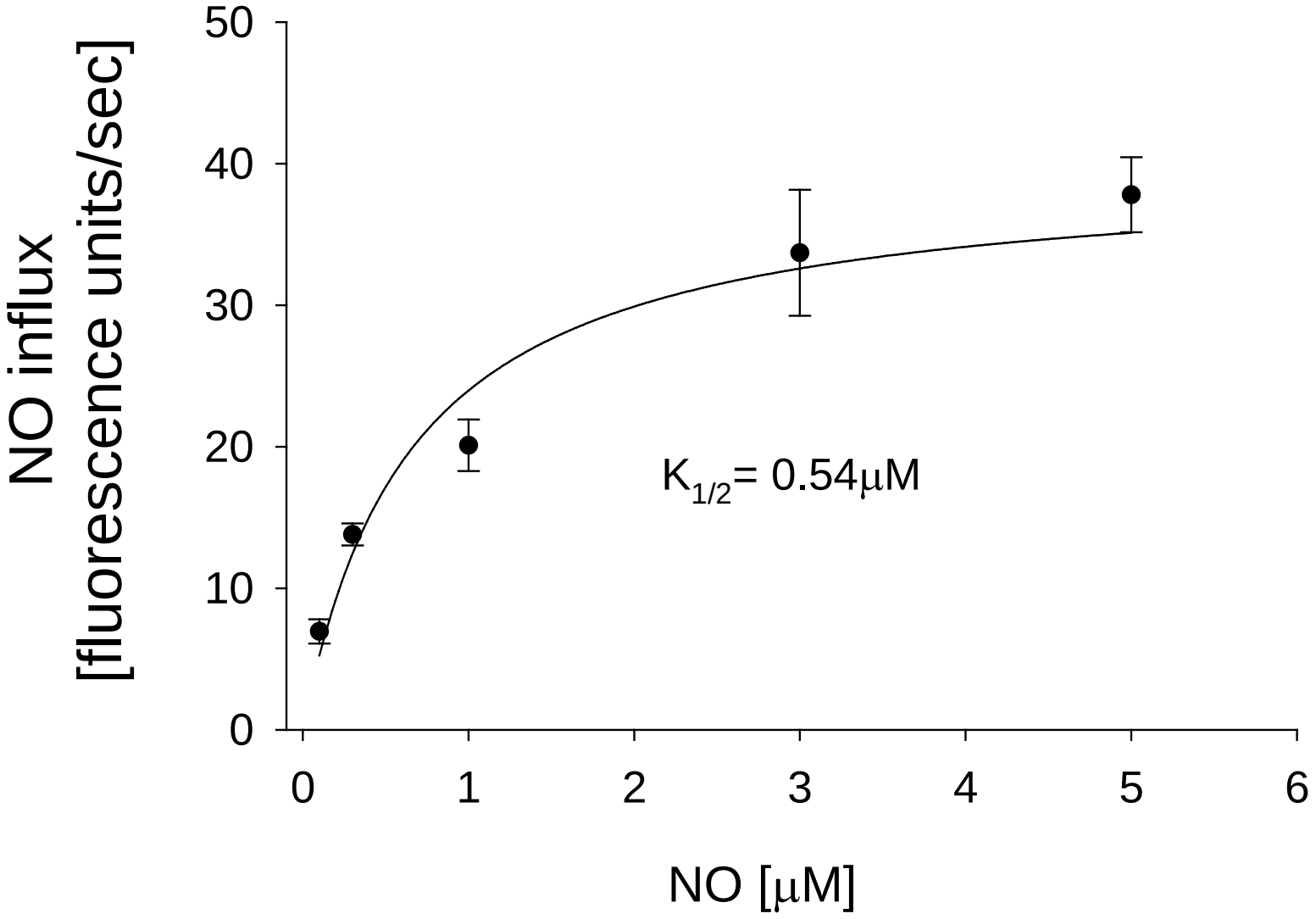
2. Increasing AQP-1 expression increases NO flux.

3. Inhibitors of AQP-1 reduce NO flux.

4. NO flux should be saturable.

5. Purified AQP-1 should transport NO.

Concentration-dependent NO flux using NO gas



1. NO permeability (P_{NO}) correlates with water permeability (P_f).

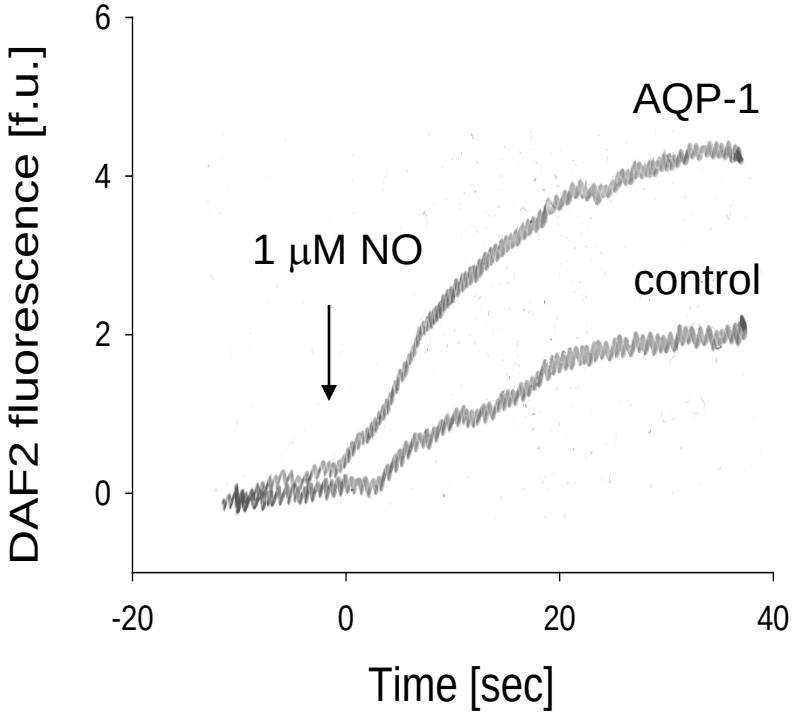
2. Increasing AQP-1 expression increases NO flux.

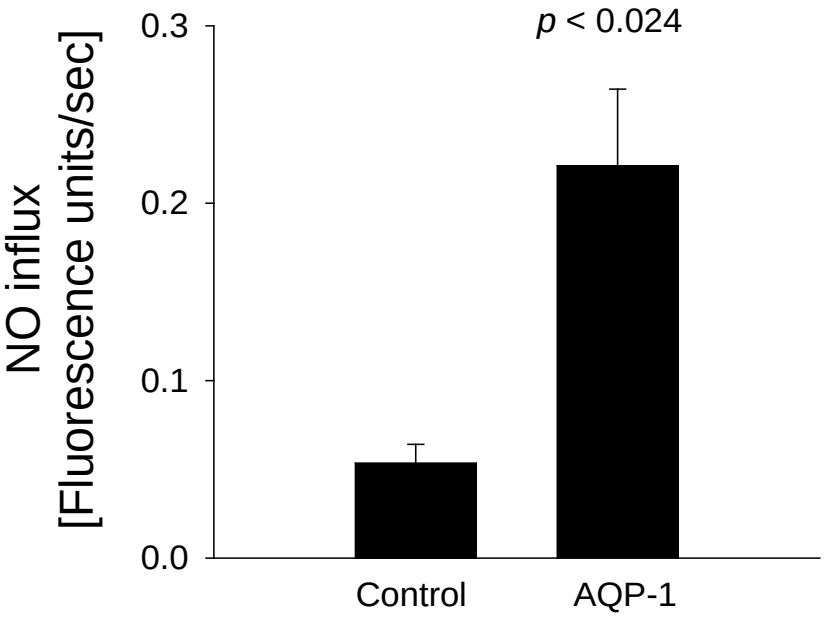
3. Inhibitors of AQP-1 reduce NO flux.

4. NO flux is saturable.

5. Purified AQP-1 should transport NO.

NO flux into proteolipisomes made with purified AQP-1





1. NO permeability (P_{NO}) correlates with water permeability (P_f).

2. Increasing AQP-1 expression increases NO flux.

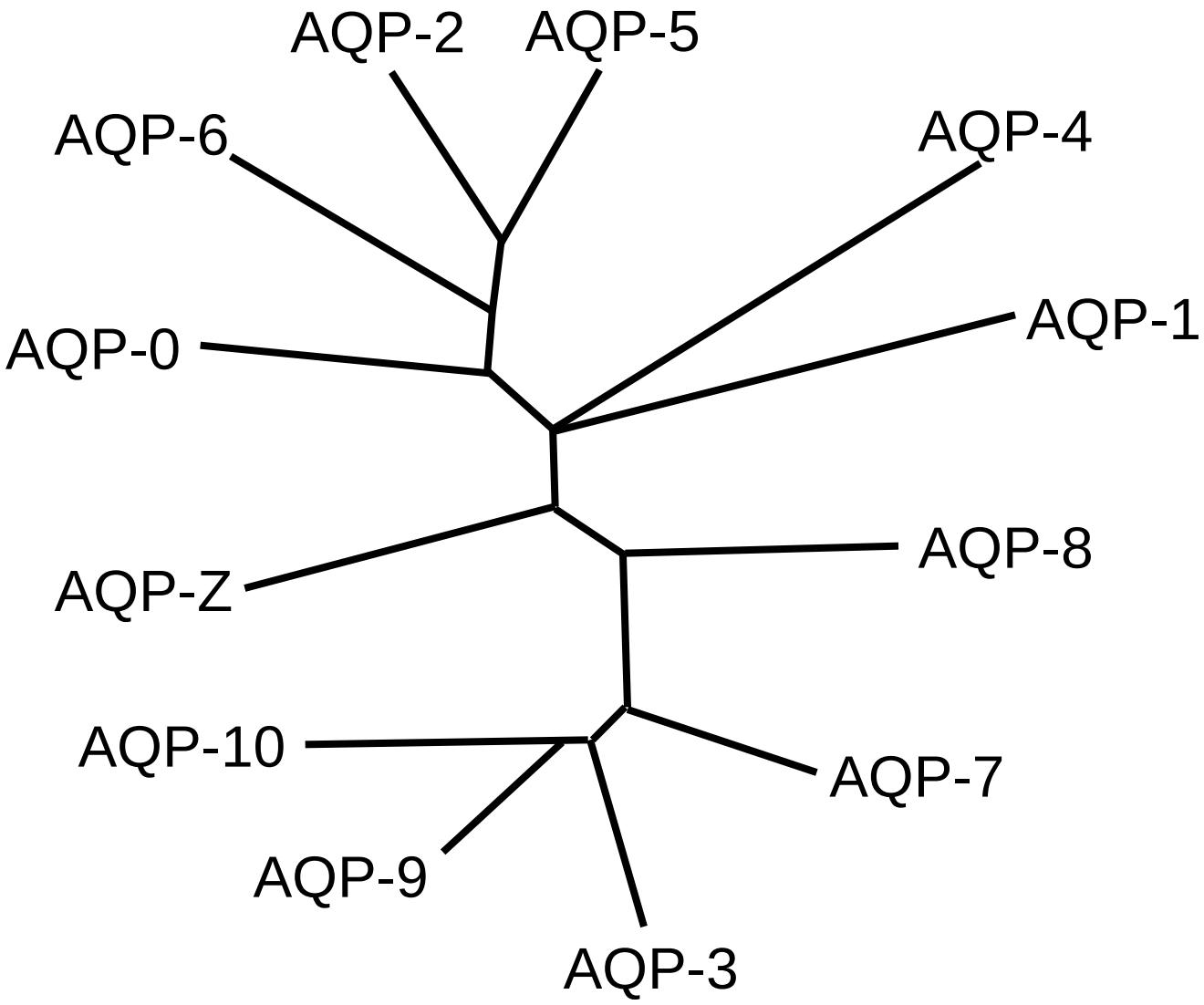
3. Inhibitors of AQP-1 reduce NO flux.

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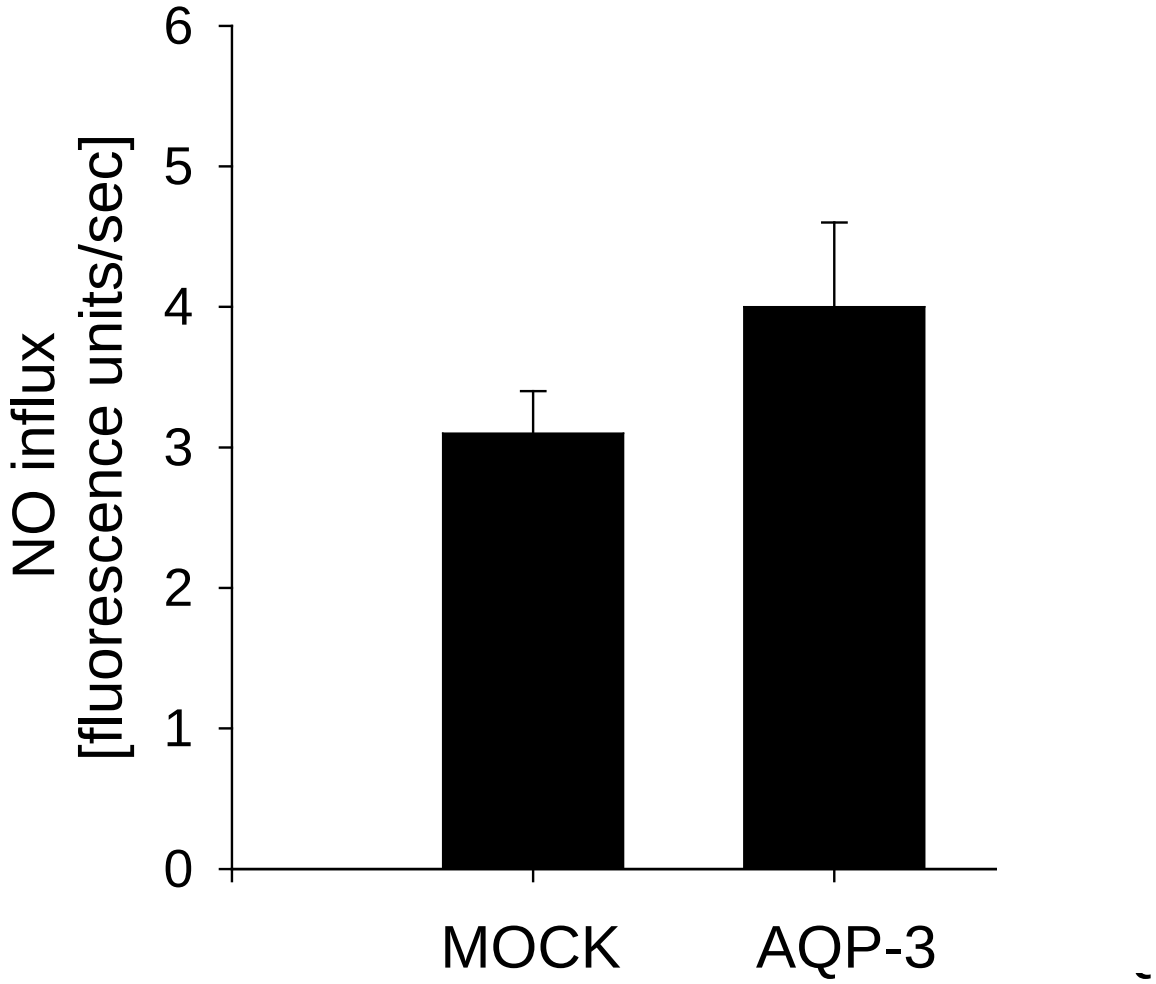
Do other aquaporins transport NO?

Partial aquaporin family tree

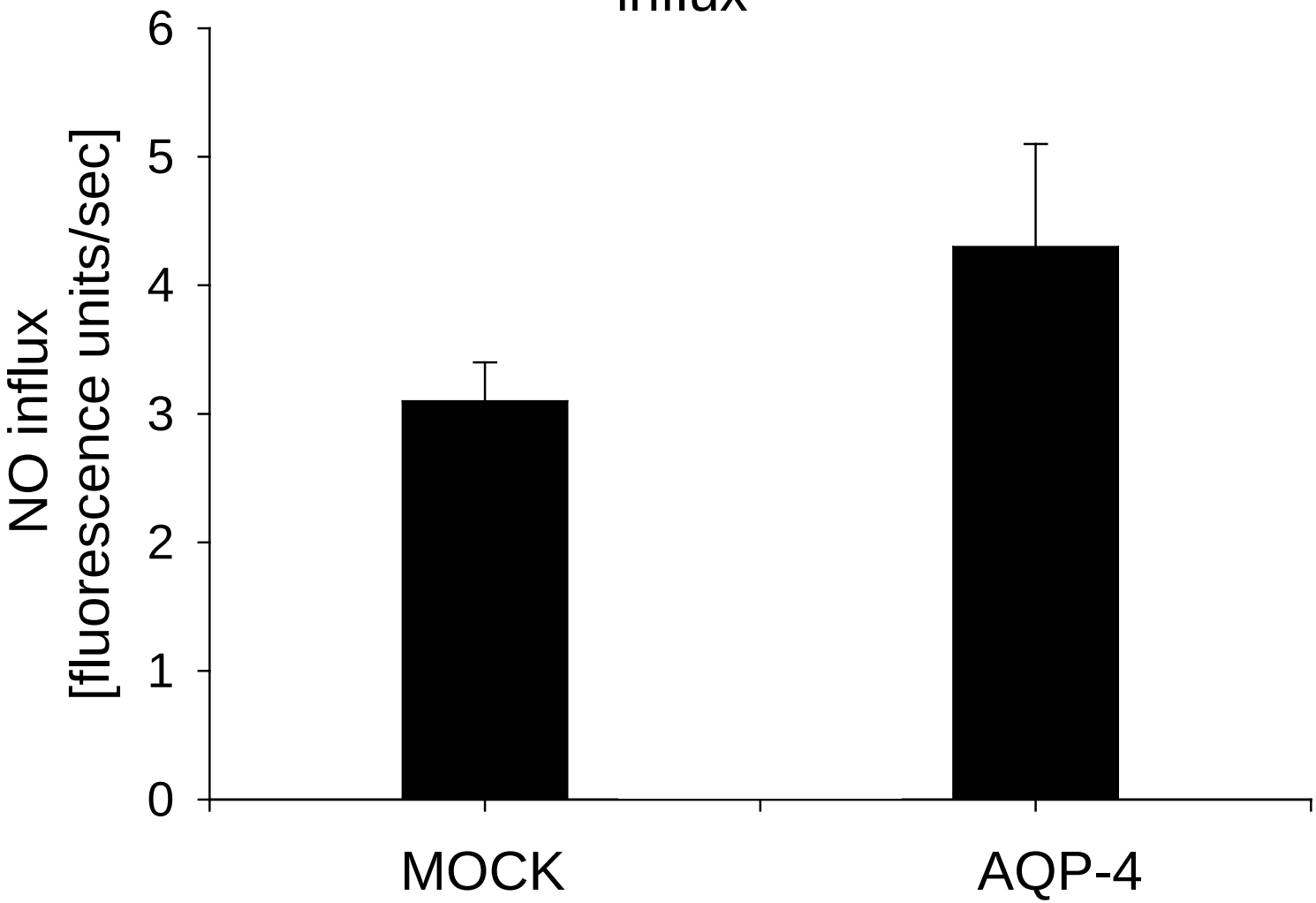


Adapted from Agre et al. J Physiol 542:3-16, 2002

Effect of transiently transfecting CHO cells with AQP-3 on NO influx



Effect of transiently transfecting CHO cells with AQP-4 on NO influx

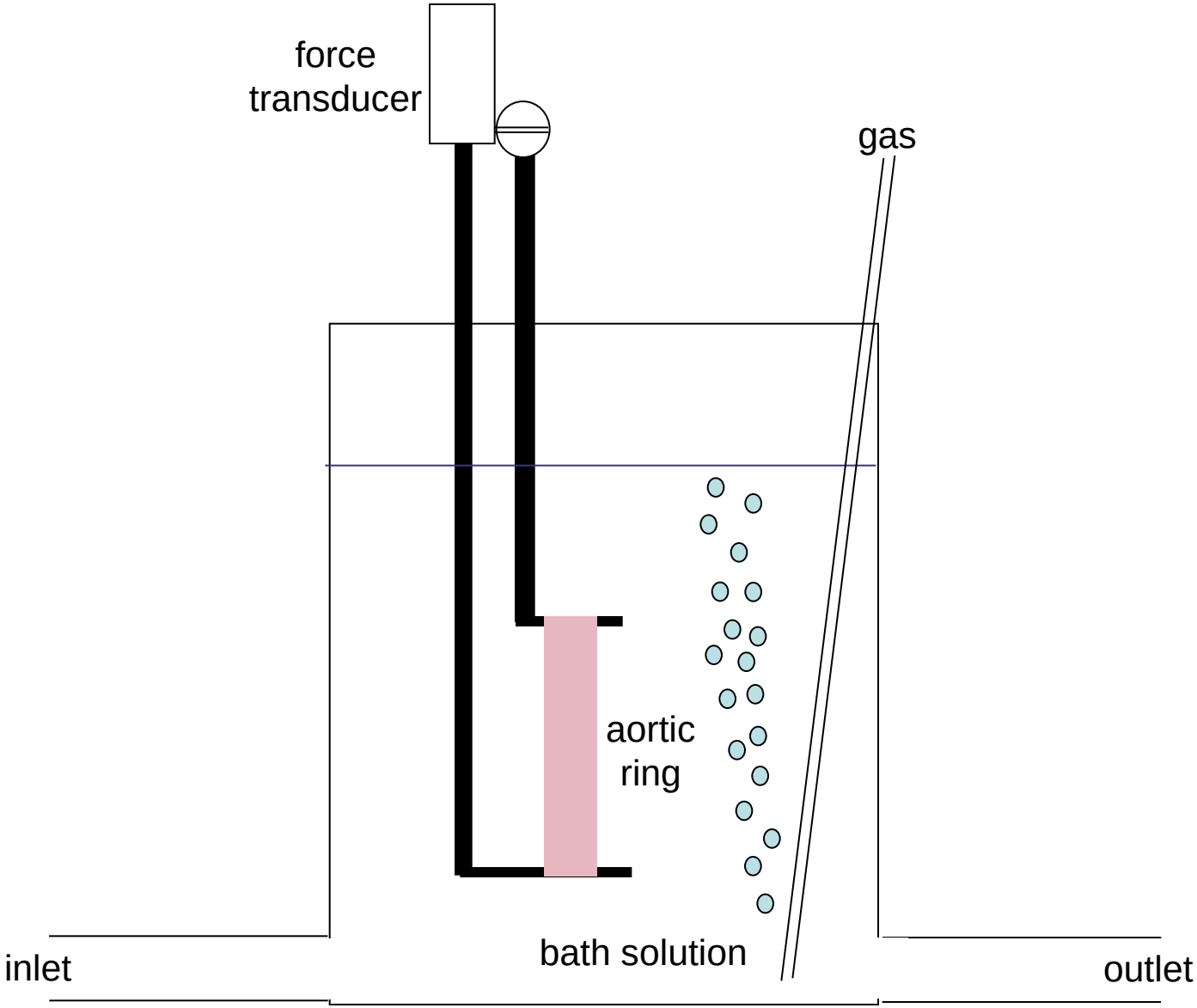


AQP-3 and AQP-4 may transport NO.
More data are required.

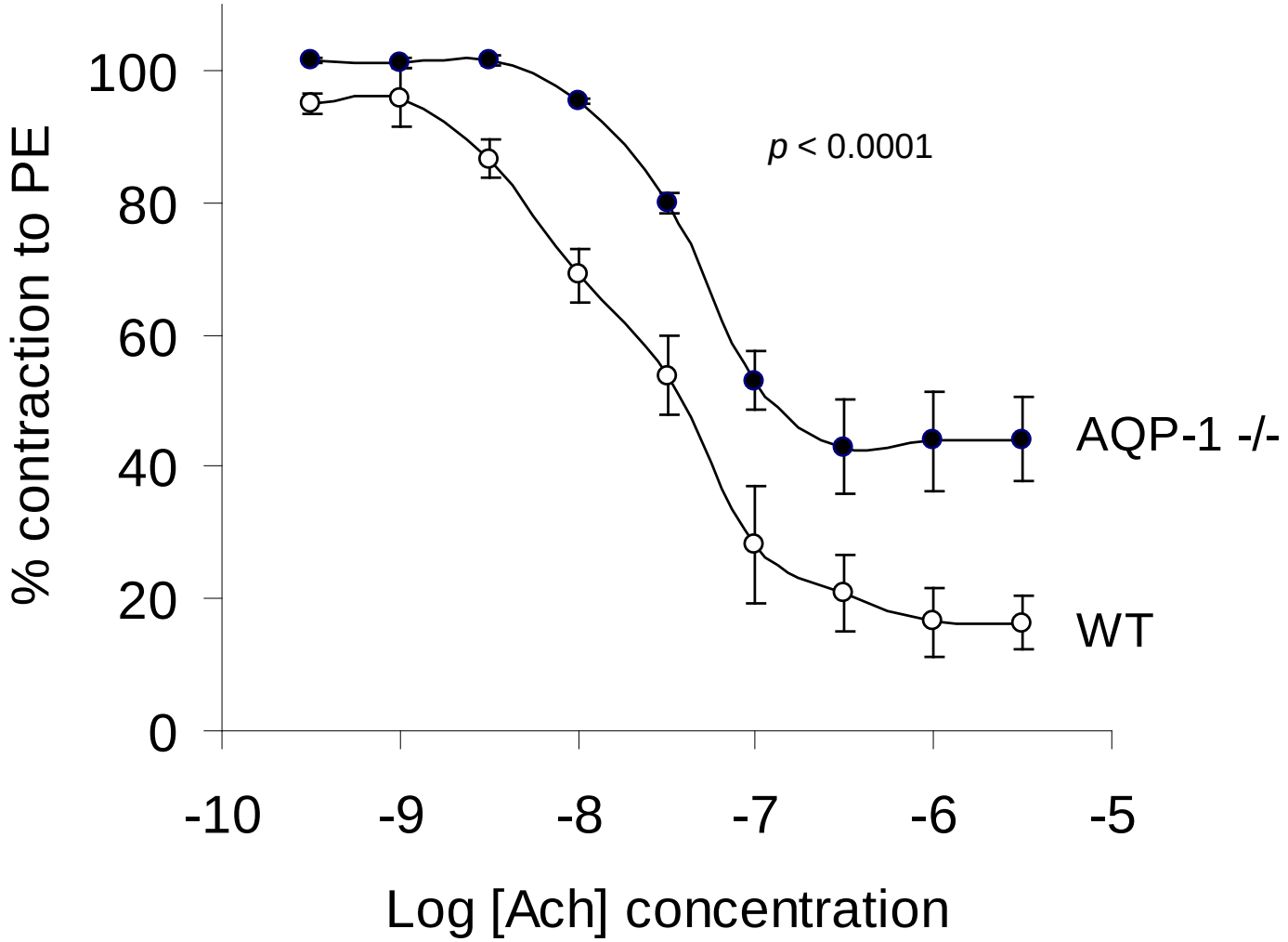
How does NO transport by AQP-1 compare to diffusion through the bilayer in “real” cells?

Is it physiologically significant?

Aortic ring preparation



Acetylcholine-dependent relaxation of aortic rings from wild type and AQP-1 -/- mice



The reduction in Ach-induced relaxation in AQP-1 -/- mice is NOT due to:

1. Less NOS 3. There is more in AQP-1 -/- mice than WT.

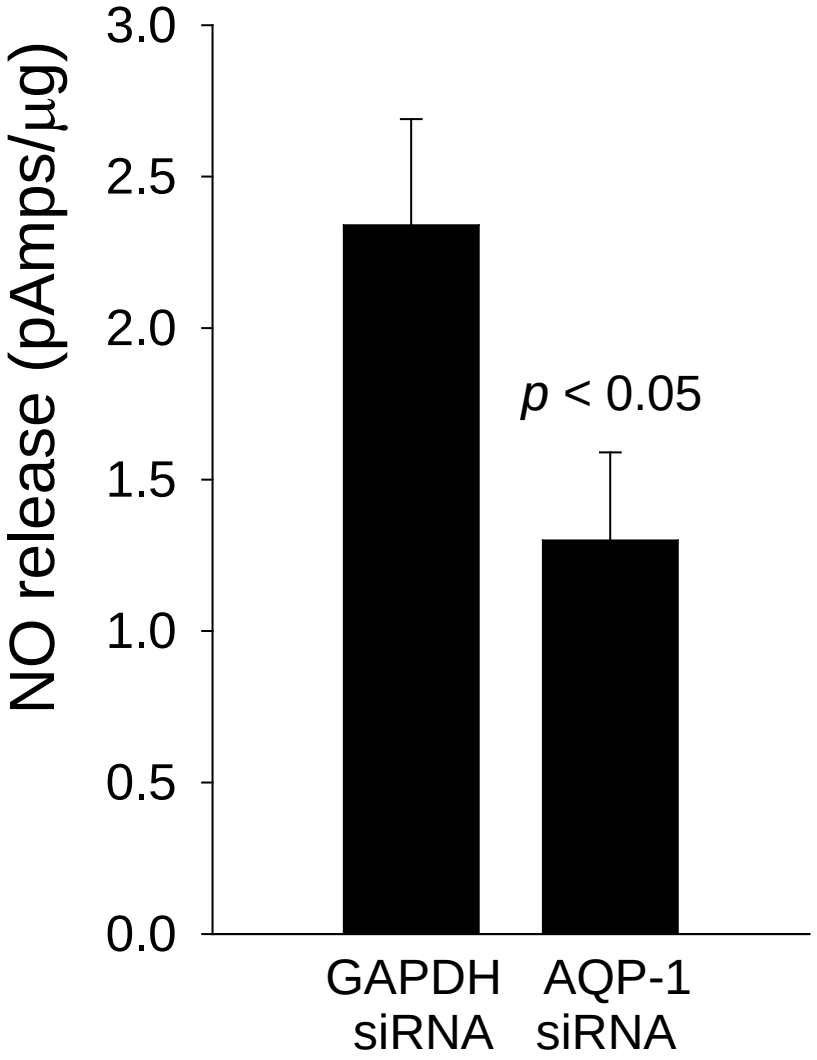
2. Defective signaling down-stream of NO. Donors that release NO inside VSMCs and cGMP relax rings from AQP-1 -/- mice the same as WT.

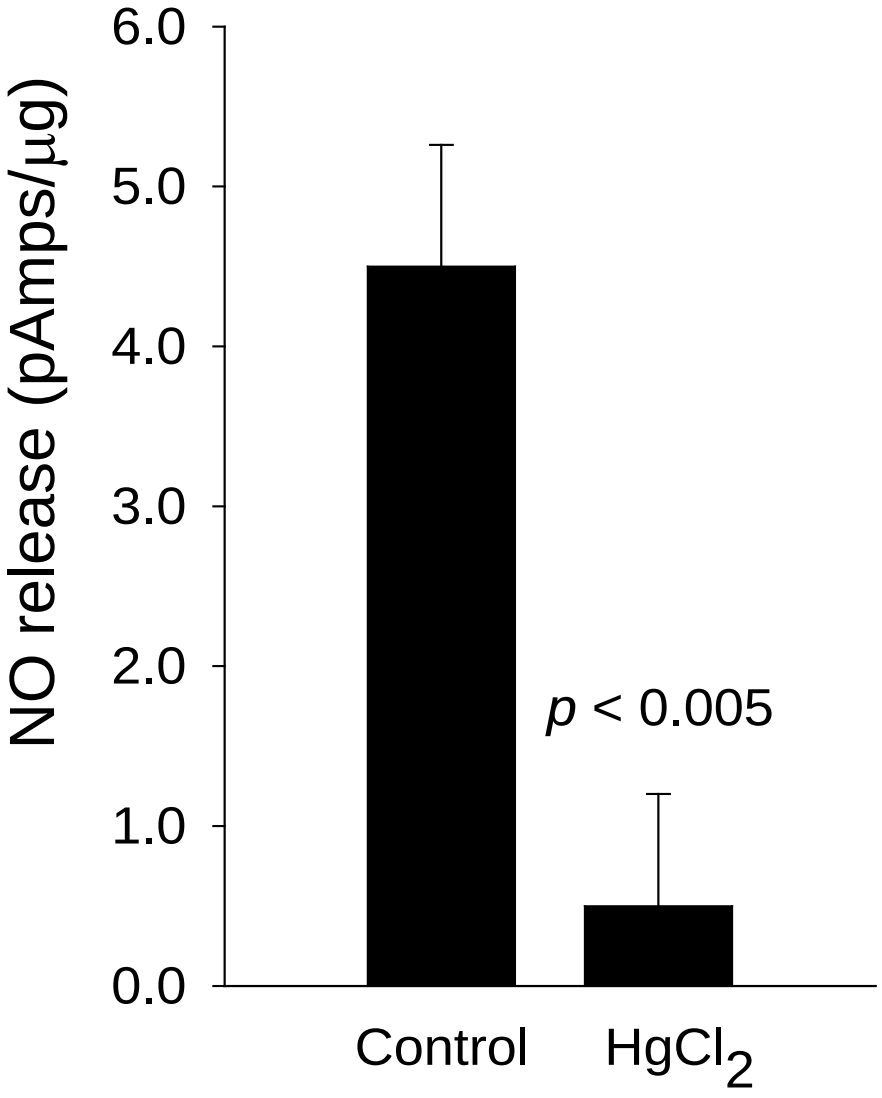
The reduction in Ach-induced relaxation in AQP-1 -/- mice could be due to:

1. Reduced NO efflux out of endothelial cells; and/or

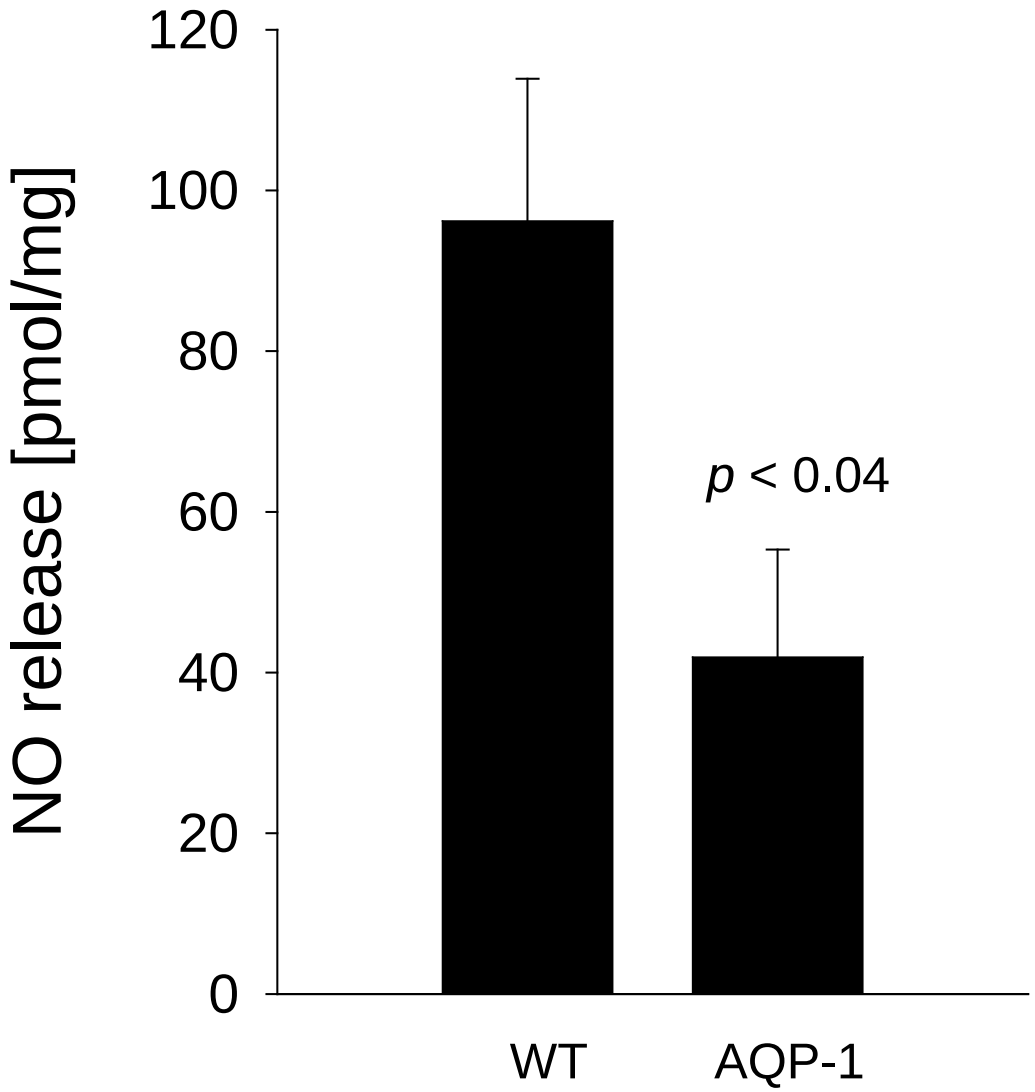
2. Reduced NO influx into vascular smooth muscle cells.

Effect of inhibiting AQP-1 on NO release by pancreatic endothelial cells

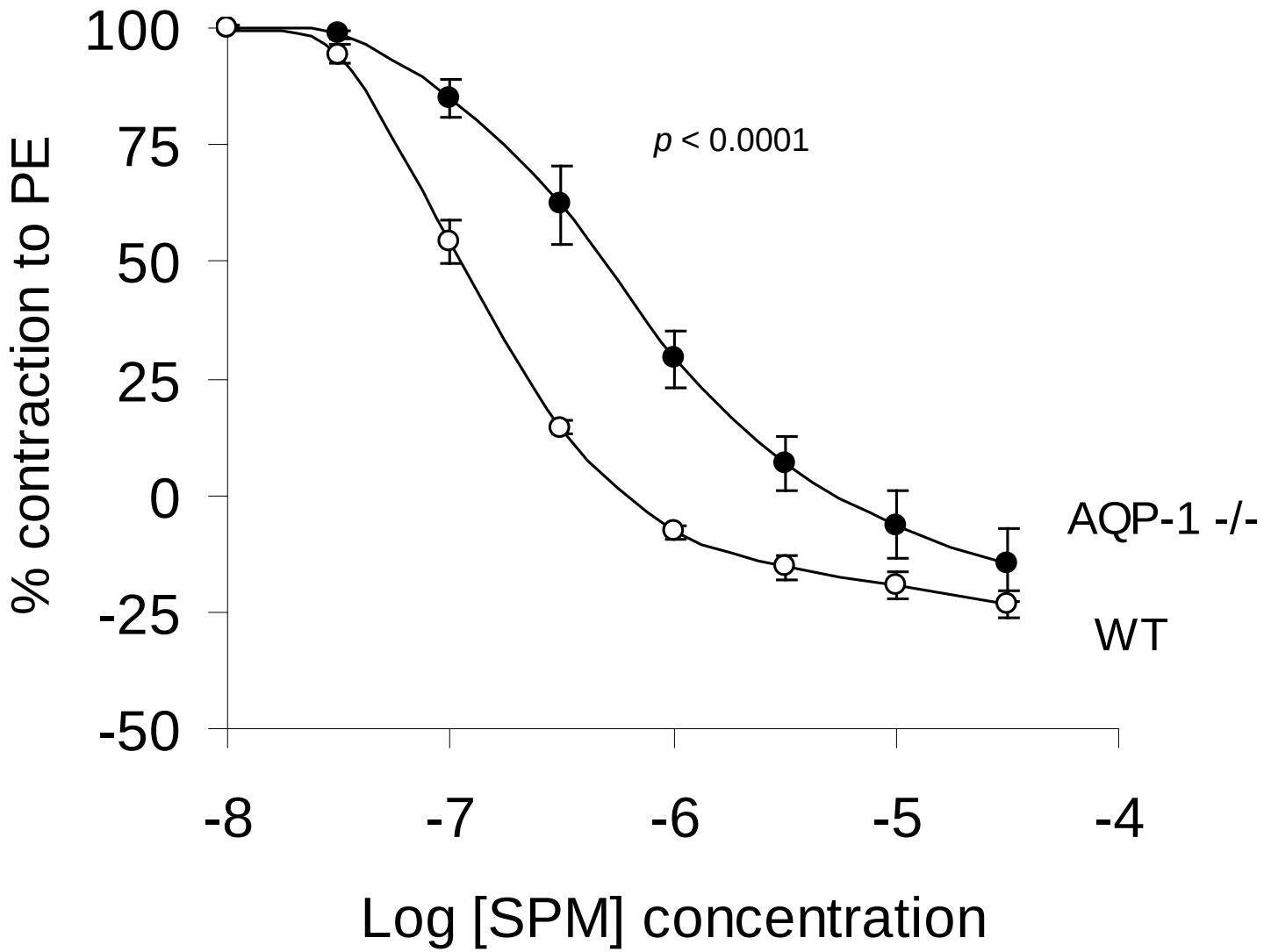




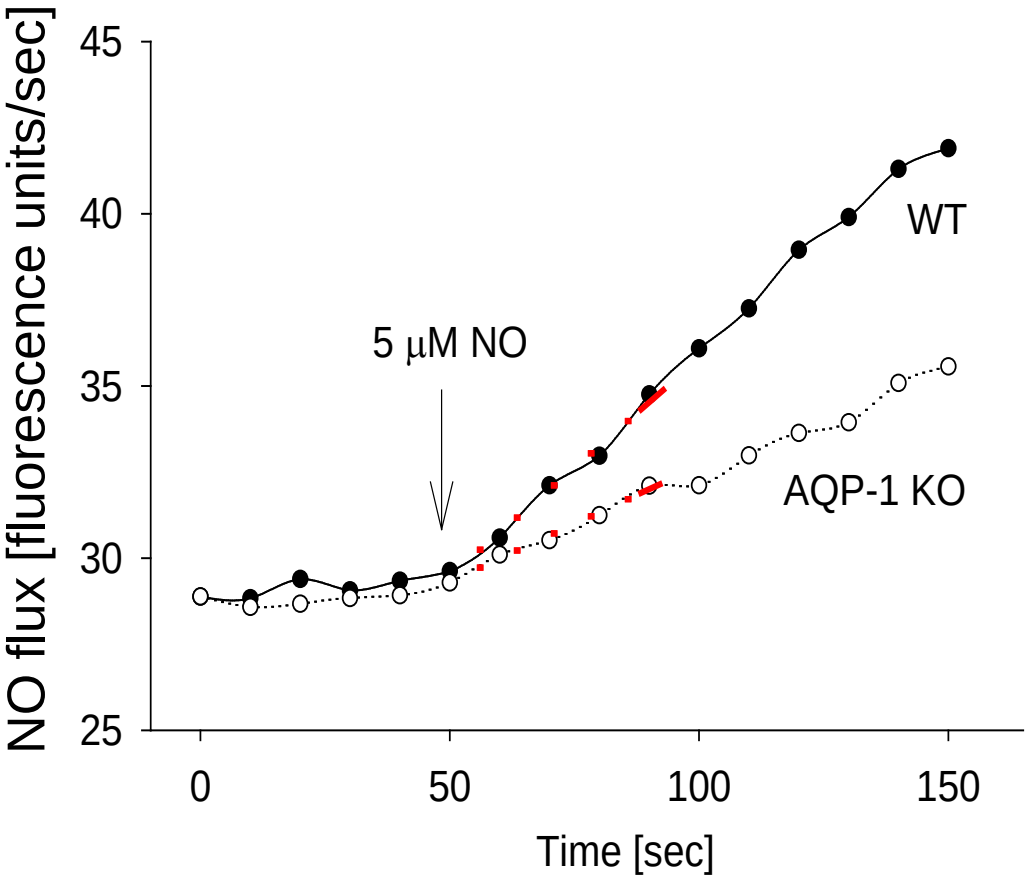
NO release by cultured aortic endothelial cells from wild type and AQP-1 -/- mice

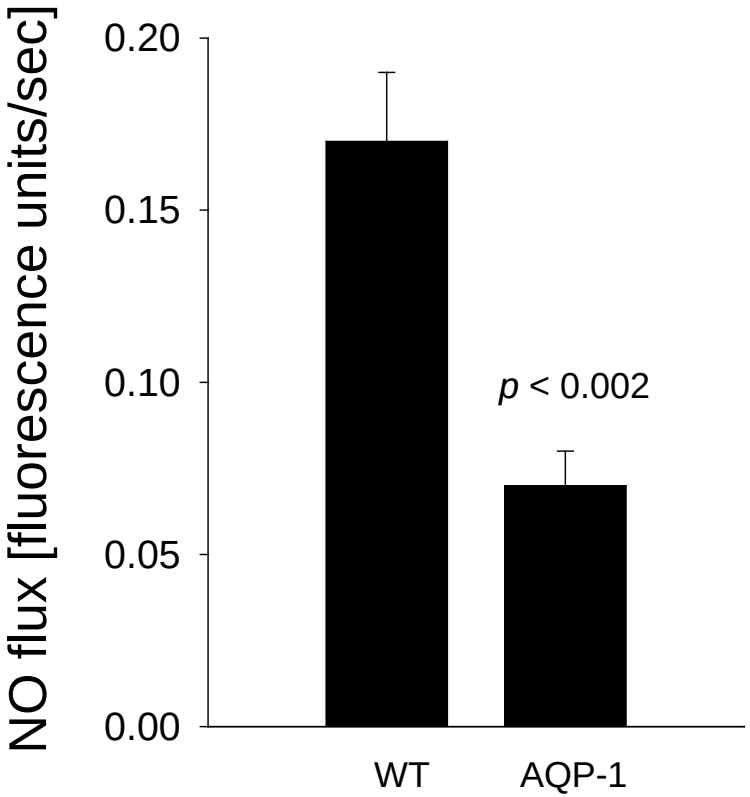


Relaxation of denuded aortic rings to spermine NONOate, an NO donor that releases NO into the bathing media



NO influx into isolated aortic vascular smooth muscle cells from wild type and AQP-1 -/- mice





We are trying to show that the reduction in NO transport by AQP-1 is physiologically relevant in vivo by showing that total peripheral resistance does not decrease in response to acetylcholine in these mice as much as wild type mice

BUT

it seems that these mice have compensation mechanism including increased prostaglandin production and NOS expression that has frustrated our attempts thus far.

Conclusion

1. AQP-1 transports NO.

2. Transport of NO by AQP-1 occurs faster than by diffusion through the bilayer by about a factor of 2.

3. Transport of NO by AQP-1 appears to be physiologically significant.

4. Reduced Ach-dependent relaxation of aortic rings from AQP-1 -/- mice is due to both reduced efflux out of endothelial cells and reduced influx into vascular smooth muscle cells.

UF UNIVERSITY of FLORIDA
Health Science Center

Role of Rh glycoproteins in NH_3 gas transport – lessons from in vivo model systems

David Weiner, M.D.

University of Florida College of Medicine and NF/SGVHS

Thanks …

Jill W. Verlander, D.V.M.
Mary E. Handlogten, M.Sc.
Hyun-Wook Lee, Ph.D.

Jesse Bishop, B.Sc.
Melanie N. Cash, B.Sc.
Amy E. Frank, B.Sc.
Hui Guo, Ph.D.
Seong-Pyo Hong, M.D., Ph.D.
George Kasnic, B.S.
Hye-Young Kim, M.D., Ph.D.
Sharon W. Matthews, Ph.D.
Kavya Mekala, M.D.
Manisa Sahni, M.D.
Sriram Seshadri, M.D.
Marshall E. Steinbaum, B.Sc.
Arthur J. Weiner
Florence Whitehill, B.A.
Li Zhang, M.D.

Cre-LoxP studies
- Donald Kohan, M.D., Ph.D.
- Peter Igarashi, M.D.
- Raoul Nelson, M.D., Ph.D.
- Lance Miller, Ph.D.

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- Bill Clapp, M.D.
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- John Leader, Ph.D.

Ewha Womans University, Seoul, Korea
- Ki-Hwan Han, M.D., Ph.D.

University of Pennsylvania
- Connie M. Westhoff, Ph.D.

Emory University
- Young-Hee Kim, M.D.
- Janet Klein, Ph.D.
- Shelley Kozlowski
- Jeff Sands, M.D.
- Tekla Smith



UF UNIVERSITY of FLORIDA College of Medicine
The Foundation for The Gator Nation

Funding support: NIH (DK045788, NS047624), Department of Veterans Affairs, American Heart Association, International Society of Nephrology, Korea Science and Engineering Foundation, Gatorade Research Foundation and NF/SGVHS Research Service

Our studies assessing the role of Rh glycoproteins in NH_3 gas transport

Is renal collecting duct NH_3 transport diffusive or transporter-mediated?

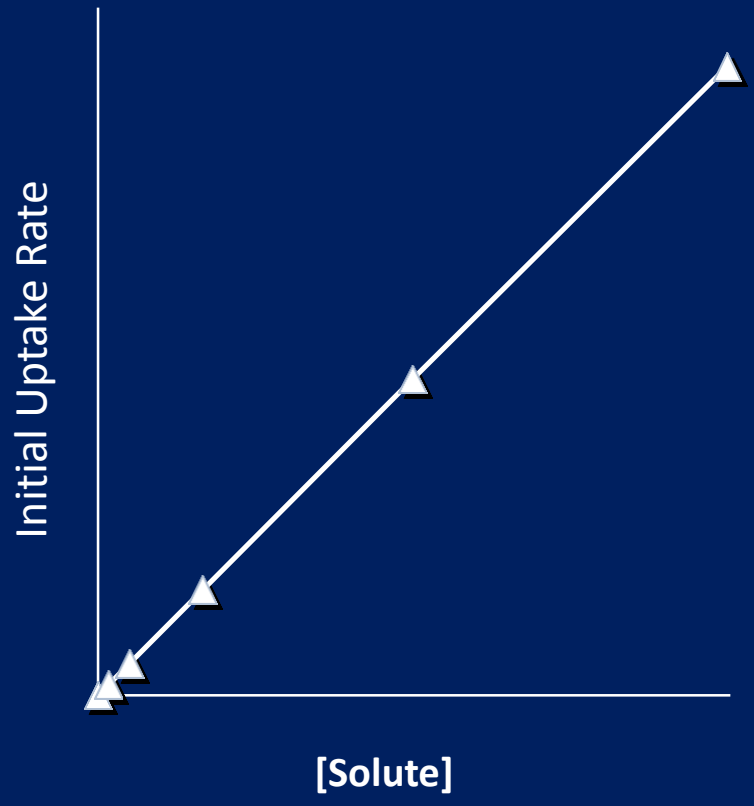
Are Rh glycoproteins present in cells that transport NH_3 gas?

Does expression parallel changes in NH_3 gas transport?

Does Rh glycoprotein inhibition alter NH_3 gas transport?

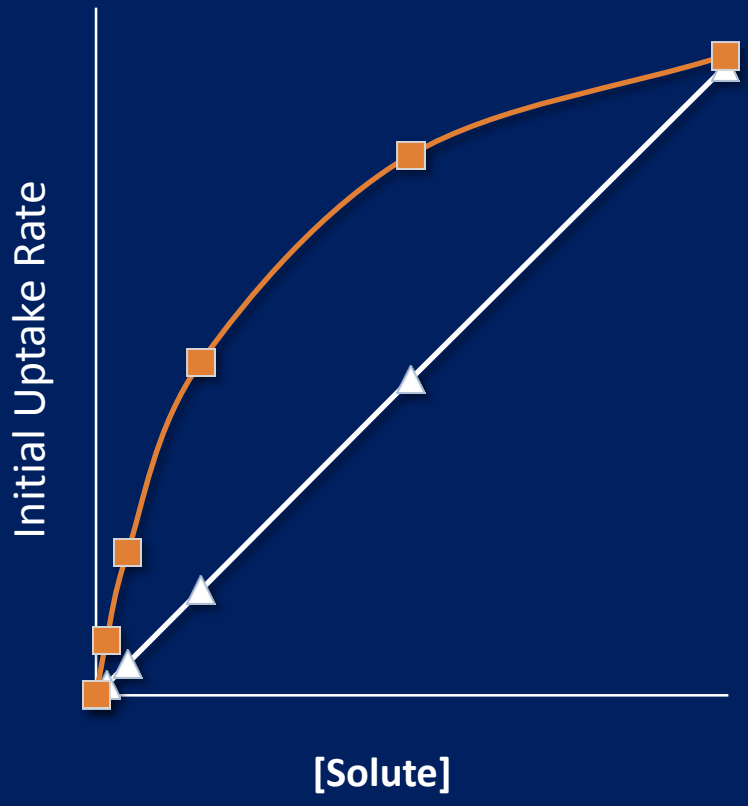
How can we determine whether collecting duct NH_3 transport is diffusive or transporter-mediated?

- Inhibitors
- Gene knock-down
- Functional tests
  - Diffusive
    - Transport proportional to concentration gradient

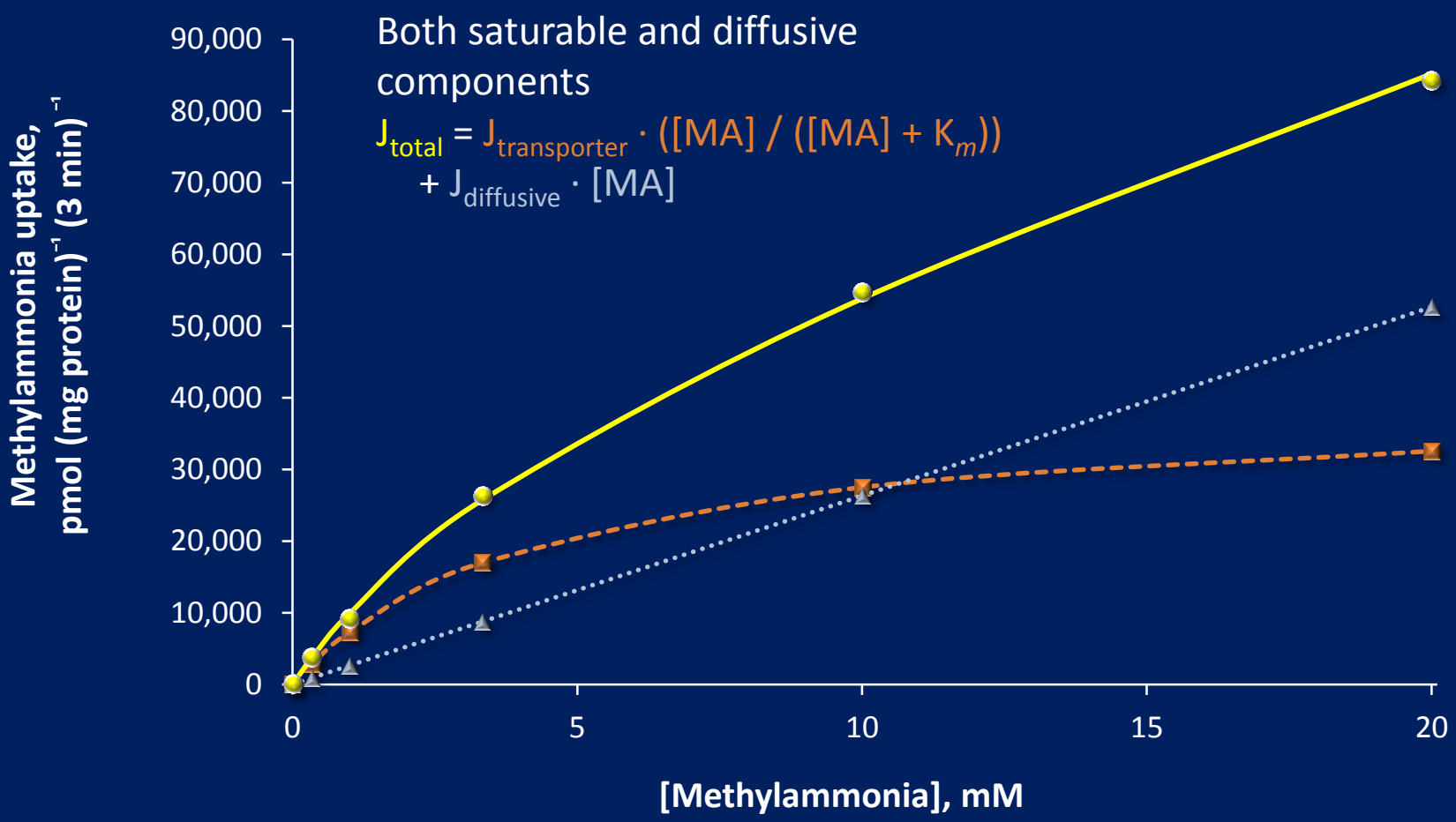


How can we determine whether collecting duct NH_3 transport is diffusive or transporter-mediated?

- Inhibitors
- Gene knock-down
- Functional tests
  - Diffusive
    - Transport proportional to concentration gradient
  - Transporter-mediated
    - Saturable

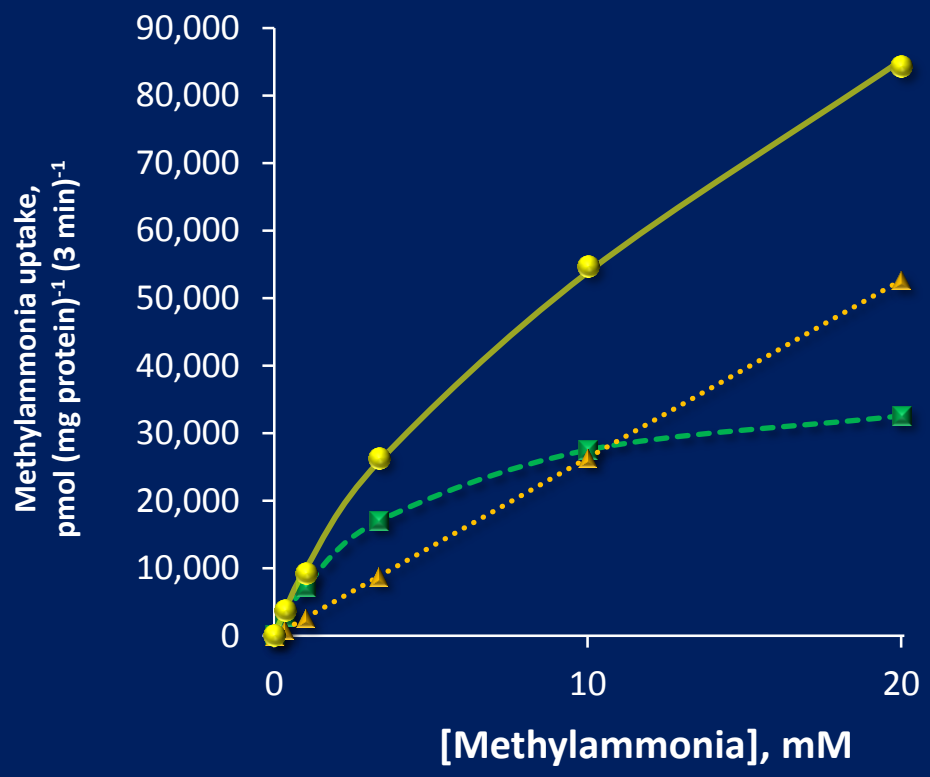


Measurement of collecting duct cell (mIMCD-3) basolateral total ammonia transport



Handlogten, et al, *AJP Renal* 287:F628-38, 2004.

Characteristics of collecting duct cell (mIMCD-3) basolateral membrane total ammonia transport



- Functional characteristics
  - Electroneutral
  - Na^+ and K^+-independent
  - Not inhibited by K^+ transporter or NHE inhibitors
  - Extracellular and intracellular pH dependent
  - NH_3 transport
- Similar findings when studying apical transport
- Similar findings in gastric, hepatic, small intestinal and colonic epithelial cells

Handlogten, et al, *AJP Renal* 287:F628-38, 2004.

Our studies assessing the role of Rh glycoproteins in NH_3 gas transport

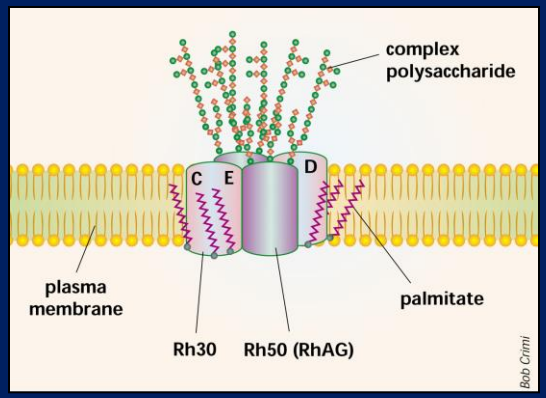
Renal collecting duct NH_3 transport is both diffusive and saturable

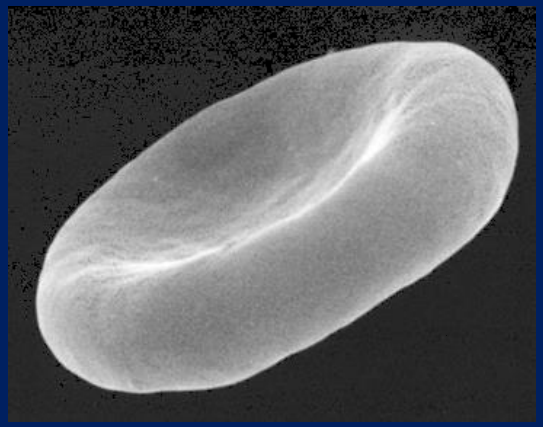
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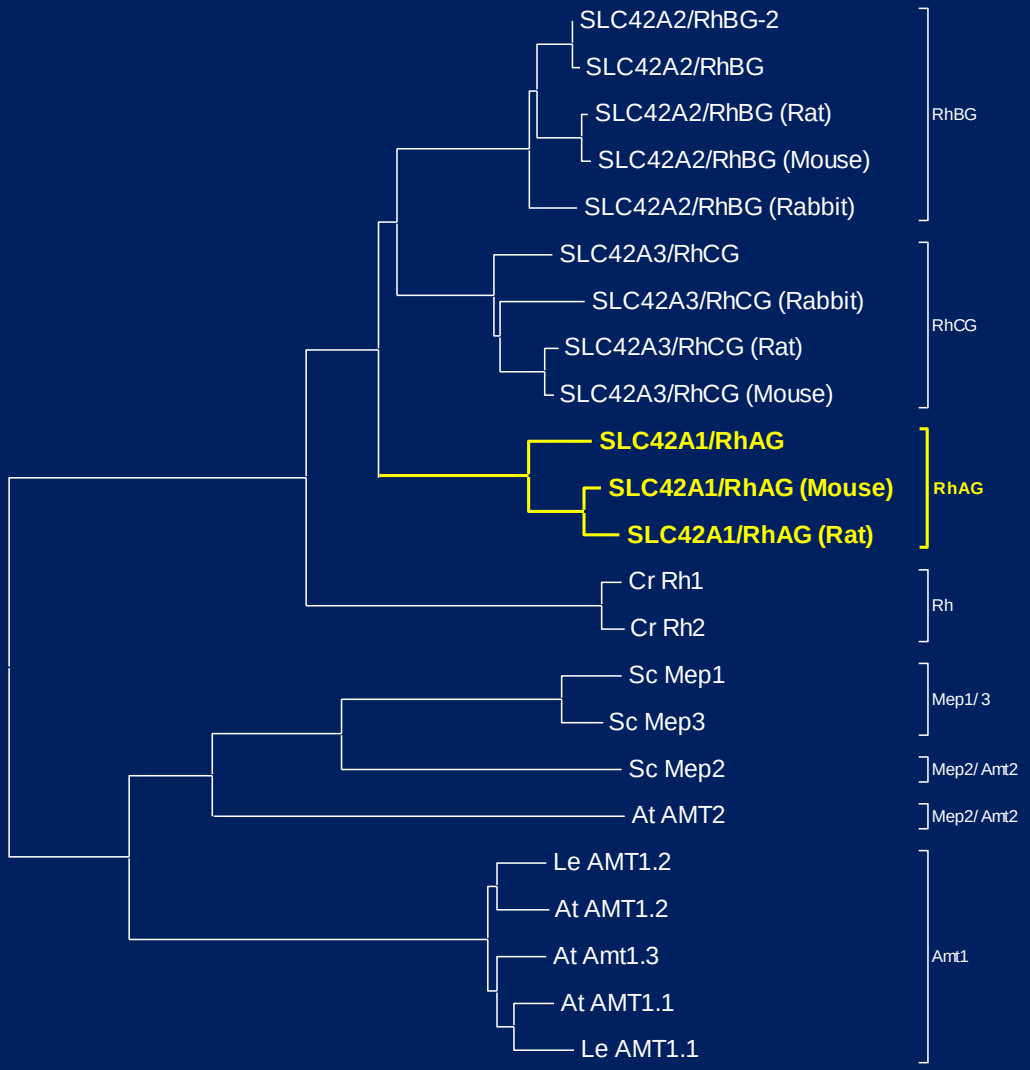
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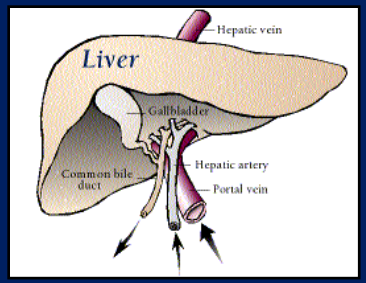
Where is RhAG/Rhag expressed?

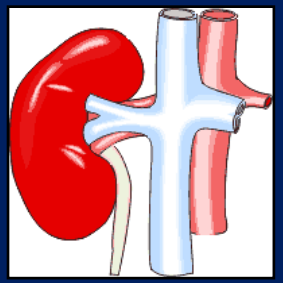


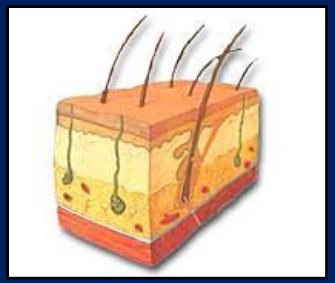




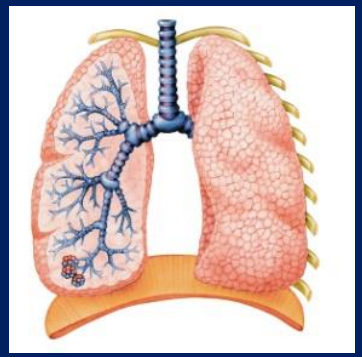
Where is Rhbg expressed?

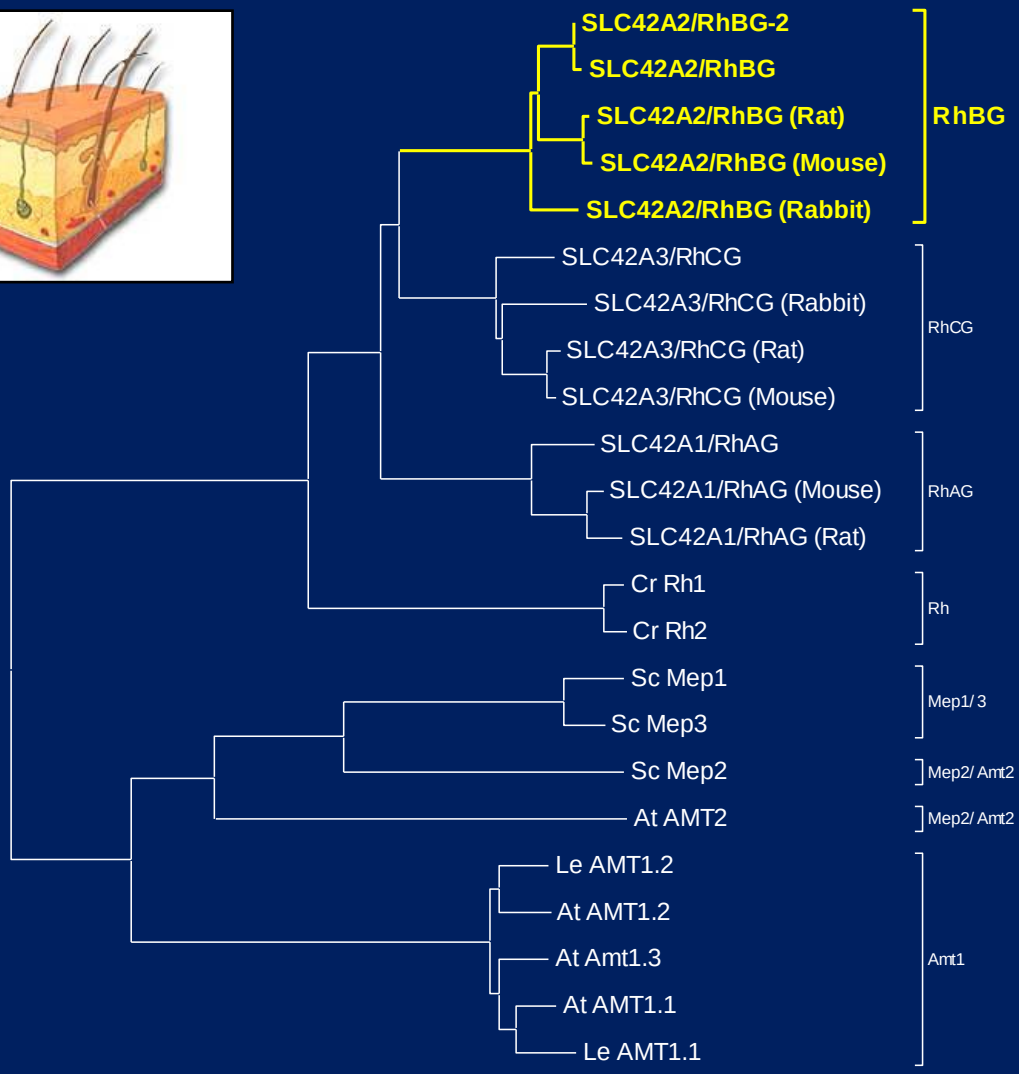




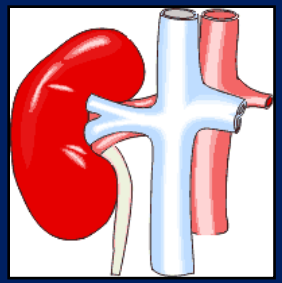








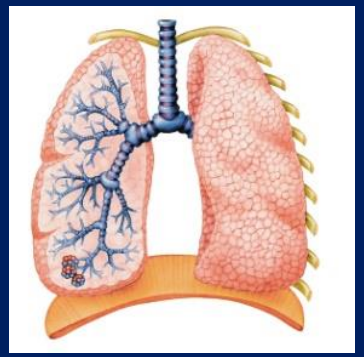
Where is Rhcg expressed?

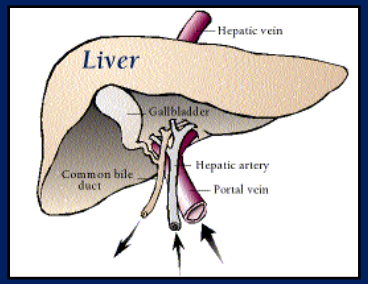




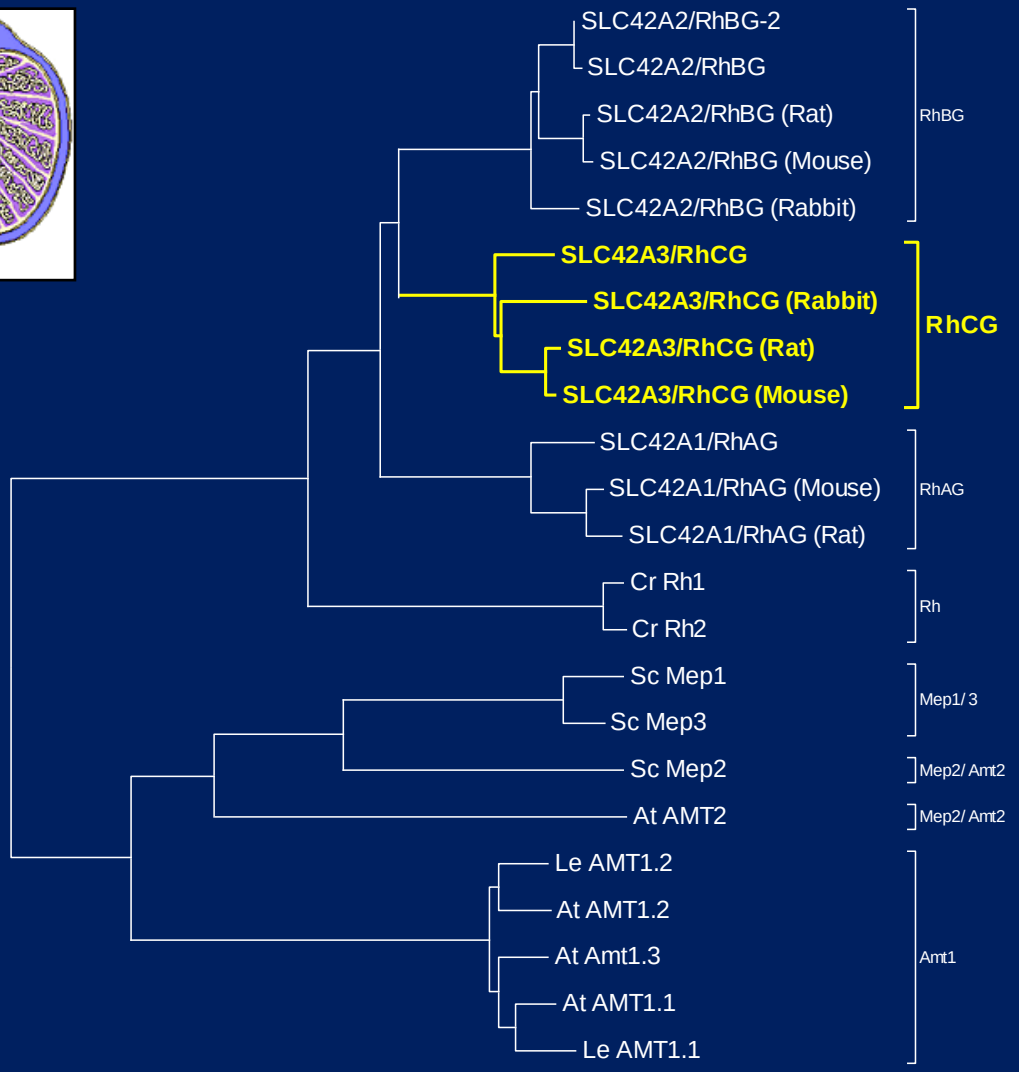






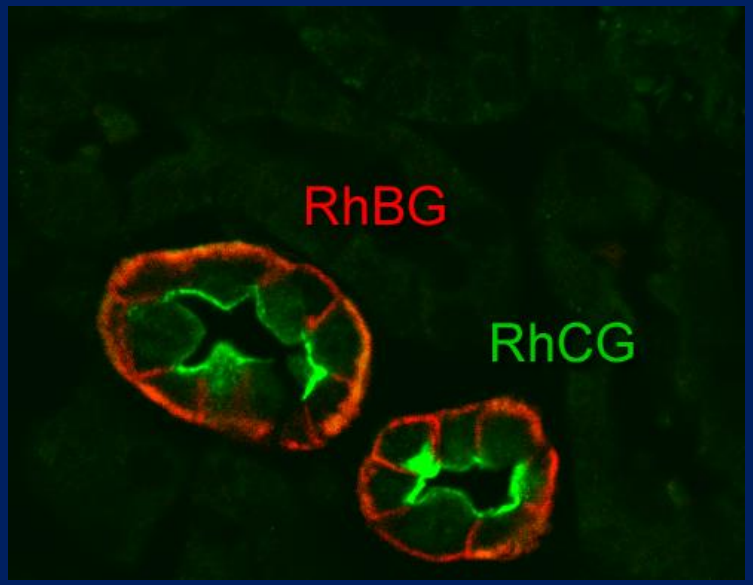


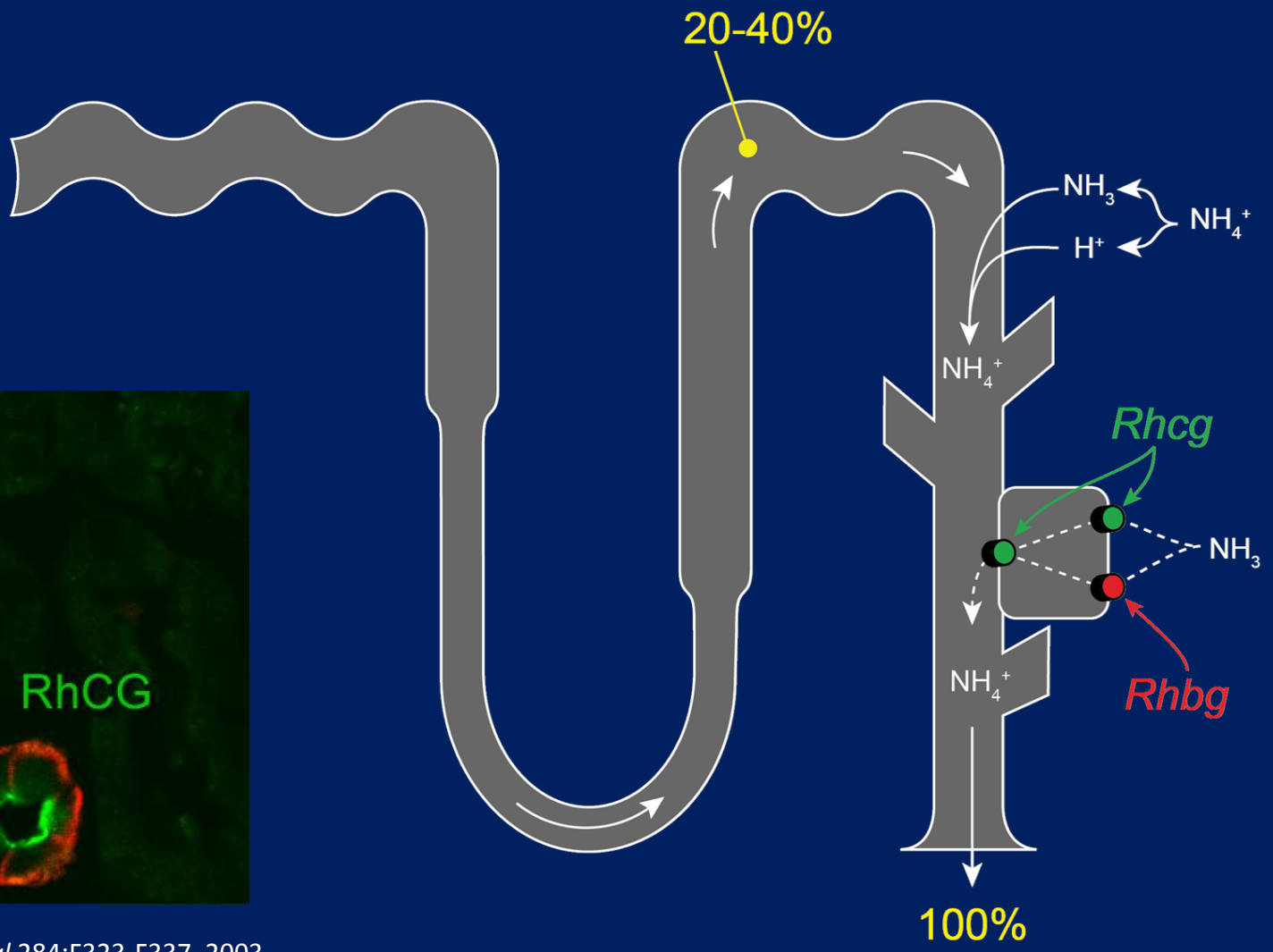




Renal Rh glycoprotein expression

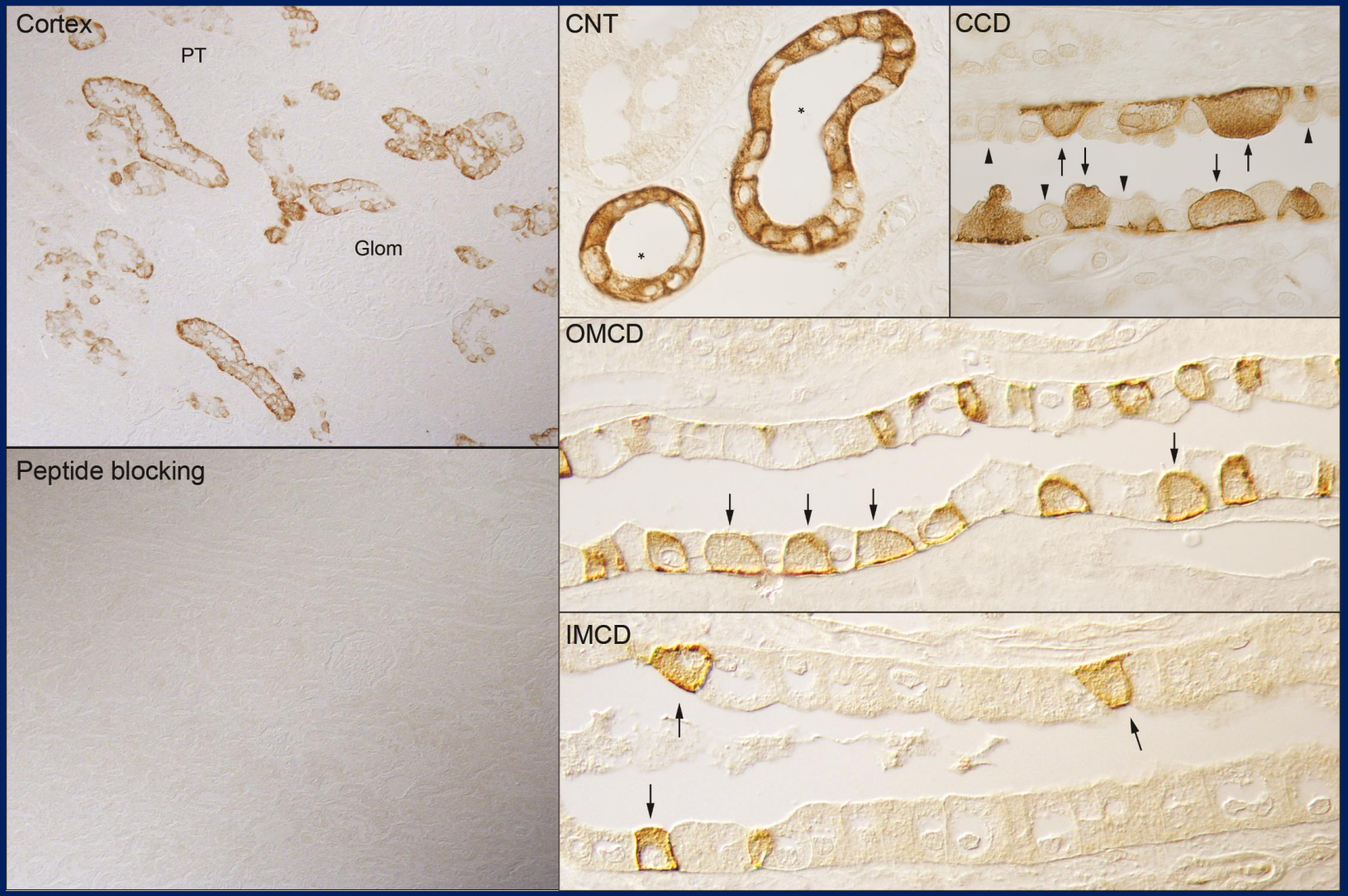






JW Verlander, et al, *AJP Renal* 284:F323-F337, 2003.

RhCG expression in human kidney



KH Han, et al. *JASN* 17:2670-9, 2006.

Our studies assessing the role of Rh glycoproteins in NH_3 gas transport

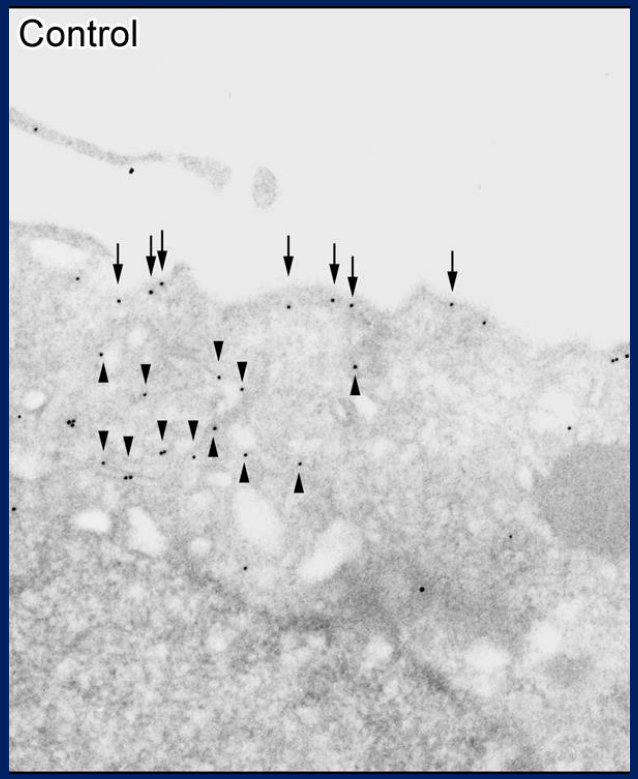
Renal collecting duct NH_3 transport is both diffusive and saturable

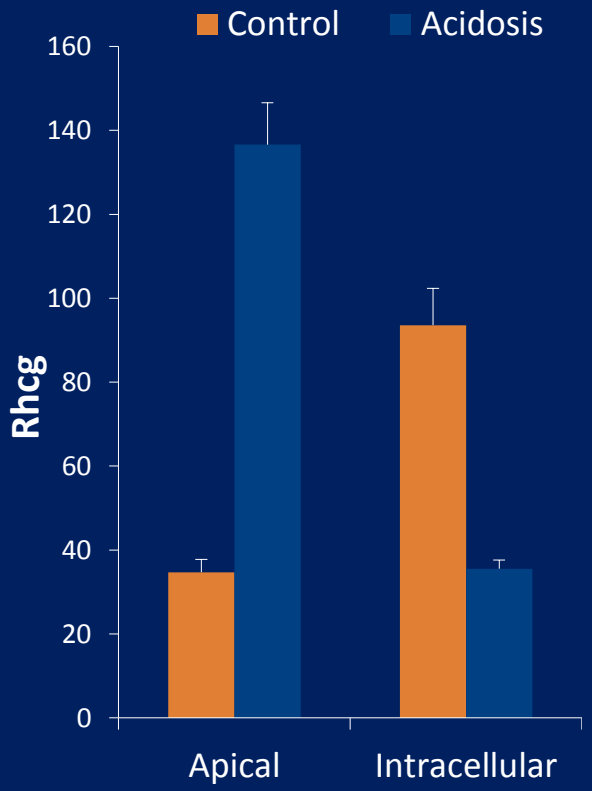
Rh glycoproteins are present specifically in cells that transport NH_3

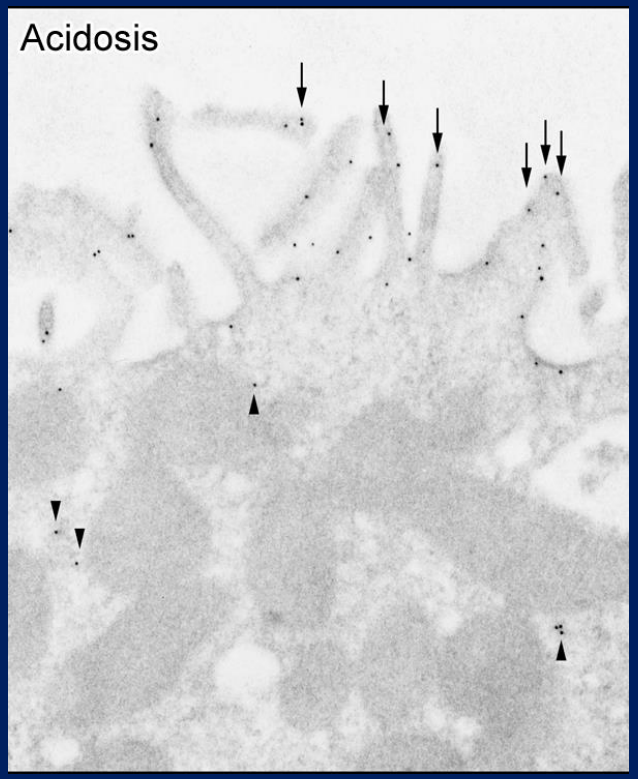
Does expression parallel changes in NH_3 gas transport?

Does Rh glycoprotein inhibition alter NH_3 gas transport?

Metabolic acidosis increases apical plasma membrane Rhcg expression







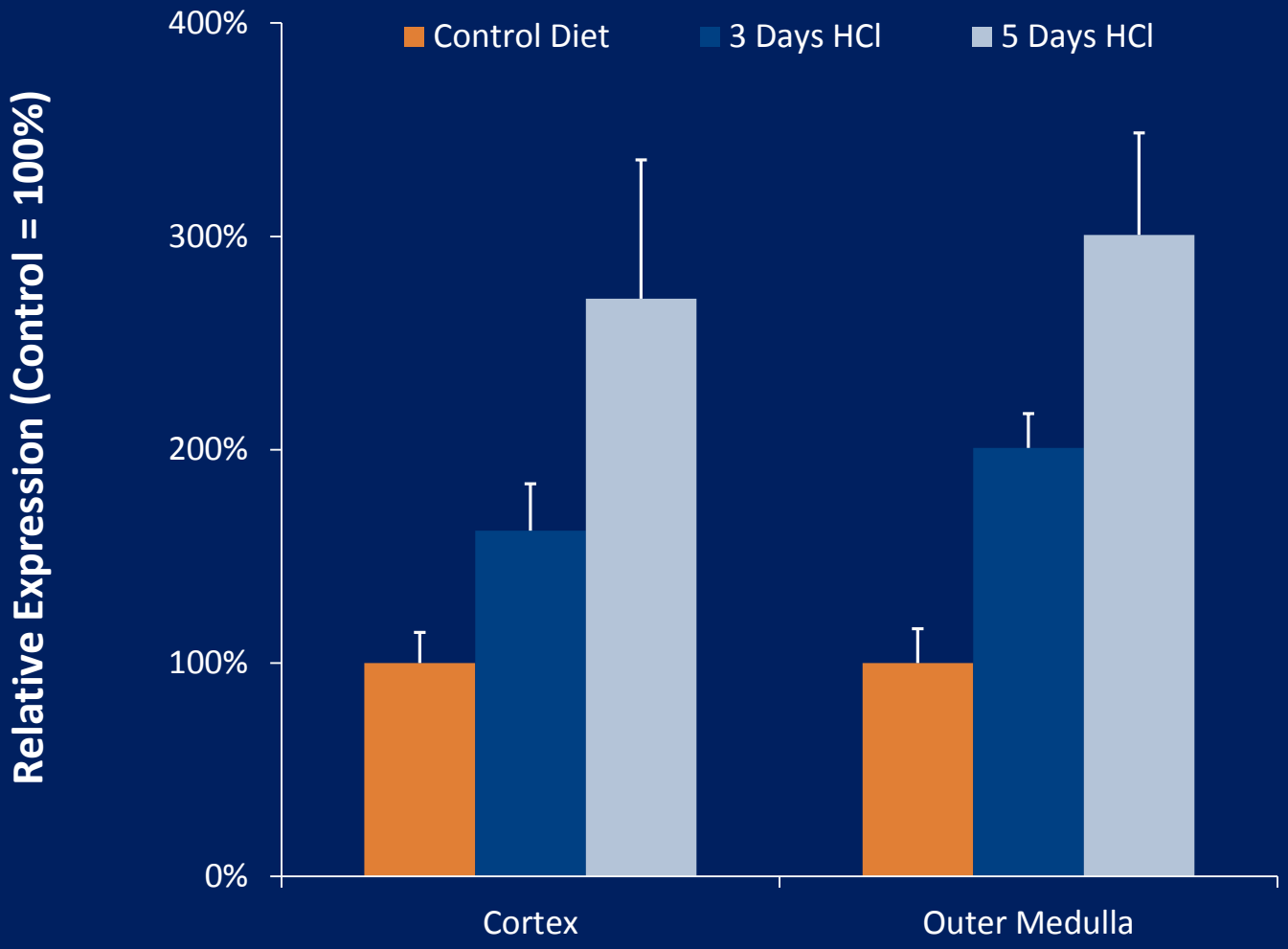
At least two modes of Rhcg regulation:

- Changes in steady-state protein expression
  - Post-translational regulation
- Trafficking to and from plasma membrane

Relative roles of each are cell-specific

Seshadri RM, et al. *AJP Renal* 290: F1443-F1452, 2006.

Metabolic acidosis increases Rhbg expression



JM Bishop, et al. *AJP Renal* 299:F1067-77, 2010.

Conditions where Rhbg and/or Rhcg expression parallels ammonia transport

- Metabolic acidosis
  - Seshadri RM, et al, *AJP Renal* 290: F397-408, 2006.
  - Seshadri RM, et al, *AJP Renal* 290: F1443-52, 2006.
  - JM Bishop, et al. *AJP Renal* 299:F1067-77, 2010.
- Reduced renal mass
  - HY Kim, et al, *AJP Renal* 293:F1238-F1247, 2007.
- Ischemia-reperfusion injury
  - KH Han, et al, *AJP Renal* 293:F1342-F1354, 2007.
- Cyclosporine A-induced renal tubular acidosis
  - SW Lim, et al, *Nephron Exp Nephrology* 110:e49-58, 2008.
- Hypokalemia
  - KH Han, et al, *AJP Renal* 301:F823-F832, 2011
- Adaptive response to deletion of other acid-base transporters
  - Pendrin
    - Kim YH, et al, *AJP Renal* 289:F1262-F1272, 2005.
  - Collecting duct Rhcg
    - HW Lee, et al, *AJP Renal* 296:F1364-F1375, 2009.
  - Intercalated cell-specific Rhcg
    - HW Lee, et al, *AJP Renal* 299:F369-F379, 2010.
  - Intercalated cell-specific Rhbg
    - JM Bishop, et al, *AJP Renal* 299:F1065-F1077, 2010.

Our studies assessing the role of Rh glycoproteins in NH_3 gas transport

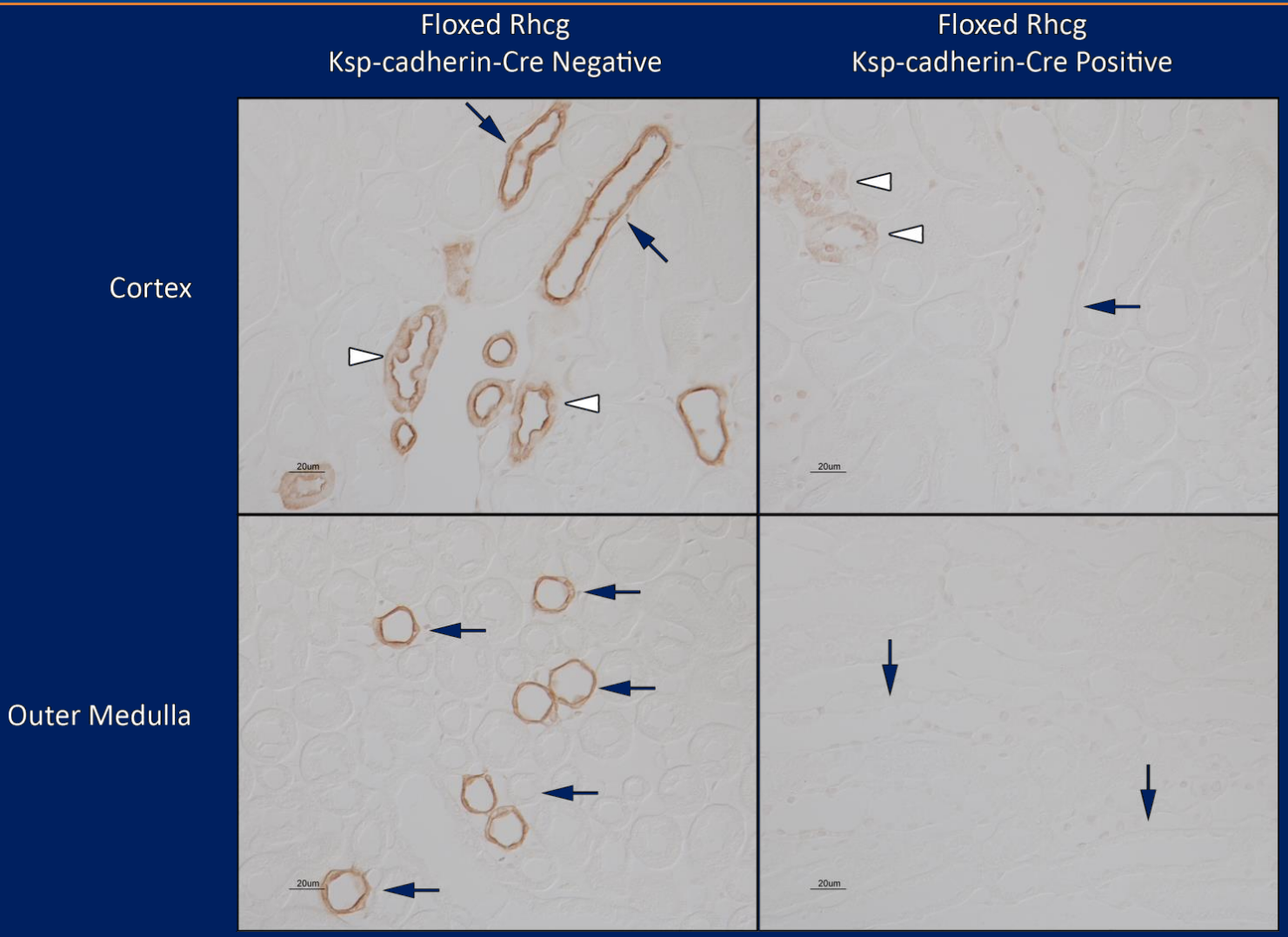
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Rh glycoproteins are present specifically in cells that transport NH_3

Rh glycoprotein expression parallels NH_3 gas transport

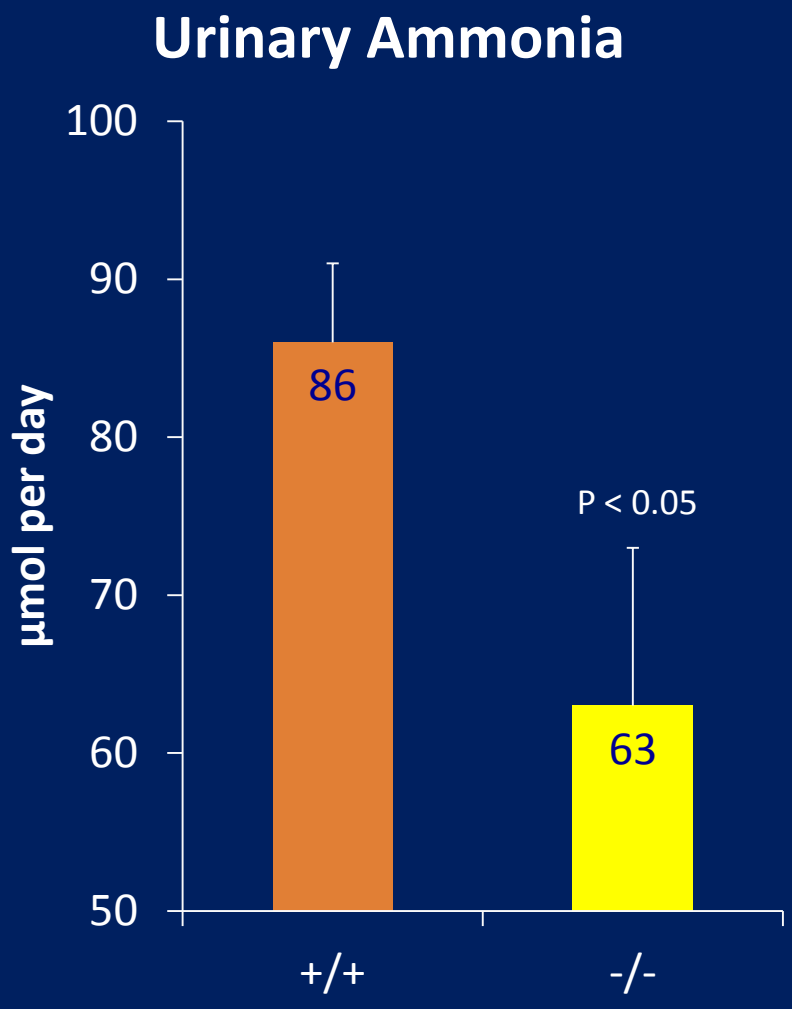
Does Rh glycoprotein inhibition alter NH_3 gas transport?

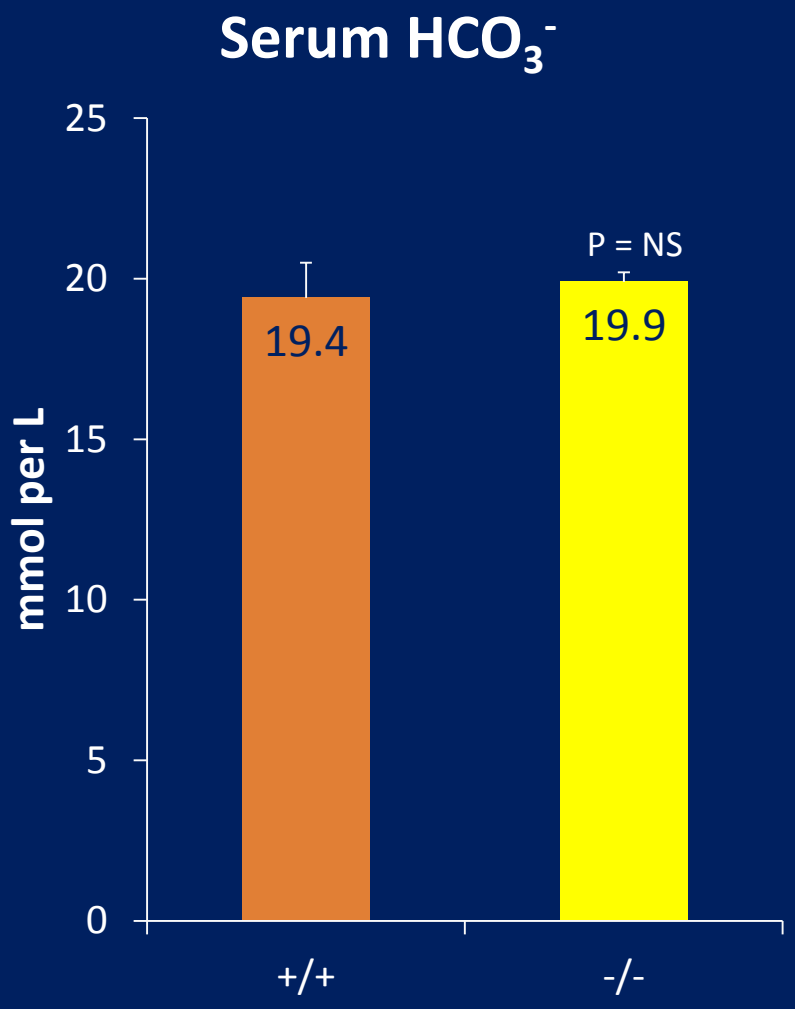
Collecting duct-specific Rhcg deletion



HW Lee, et al, *AJP Renal* 296:F1364-75, 2009.

Collecting duct-specific Rhcg deletion - basal effects

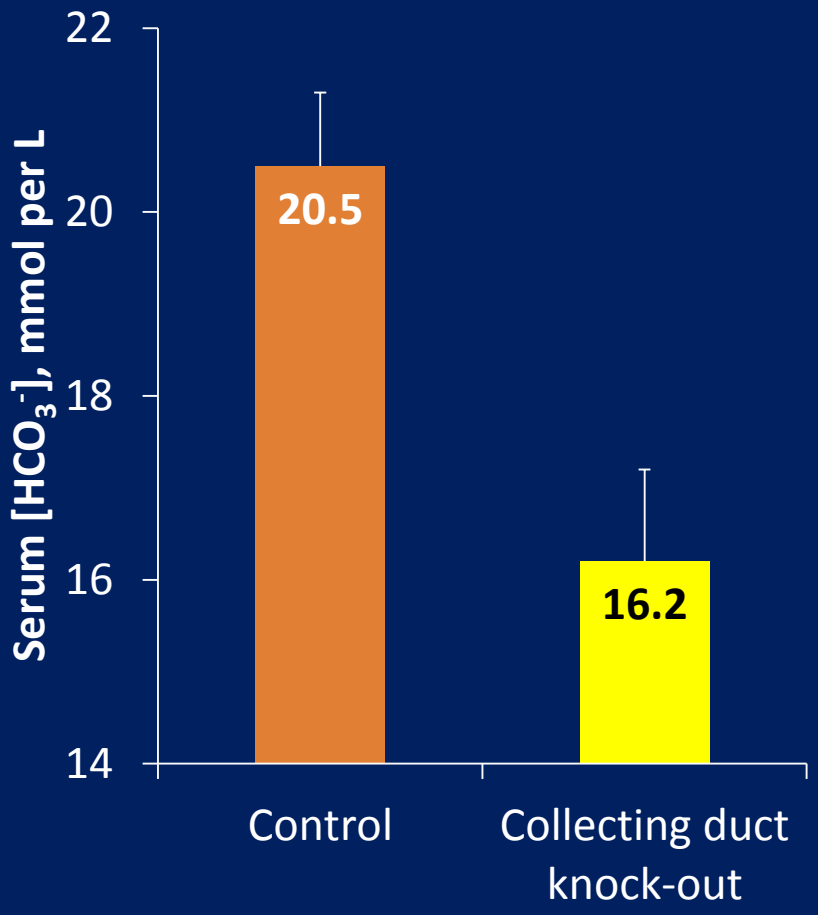




HW Lee, et al, *AJP Renal* 296:F1364-75, 2009.

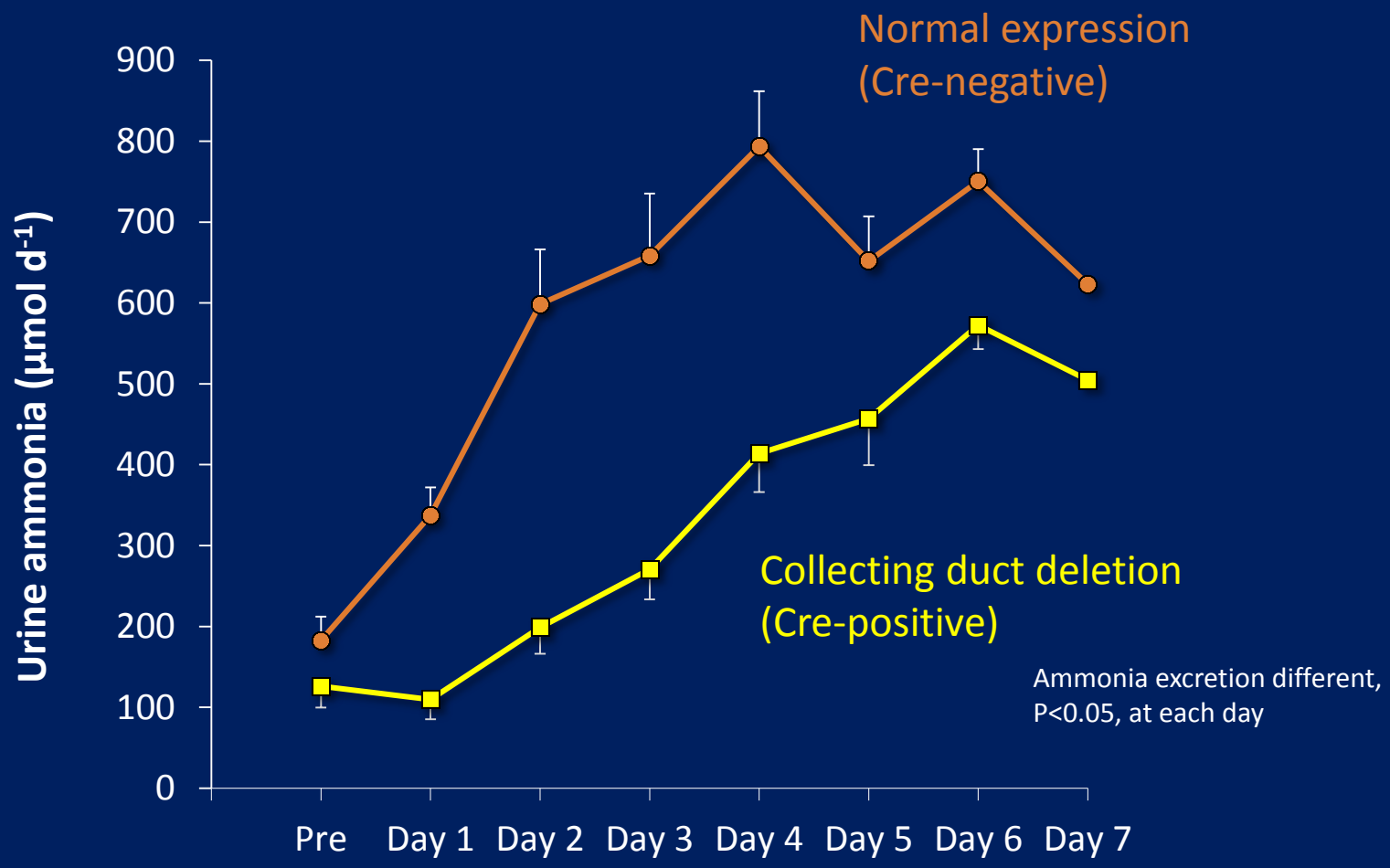
Collecting duct-specific Rhcg deletion - acid loading

- Traditional
  - Add NH_4Cl to drinking water
  - Not well tolerated
    - Add glucose
  - Hard to quantify
- "New"
  - Add HCl directly to food
  - Use powdered food
  - Easily quantified



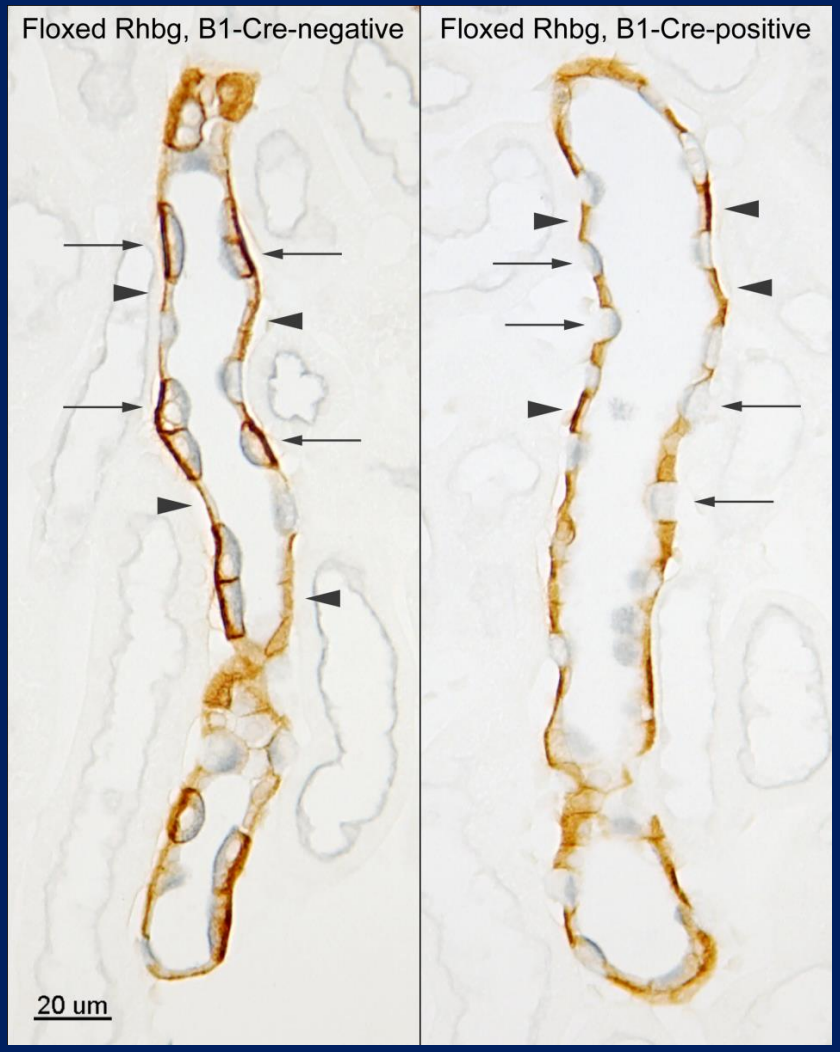
HW Lee, et al, *AJP Renal* 296:F1364-75, 2009.

Collecting duct-specific Rhcg deletion - acid loading



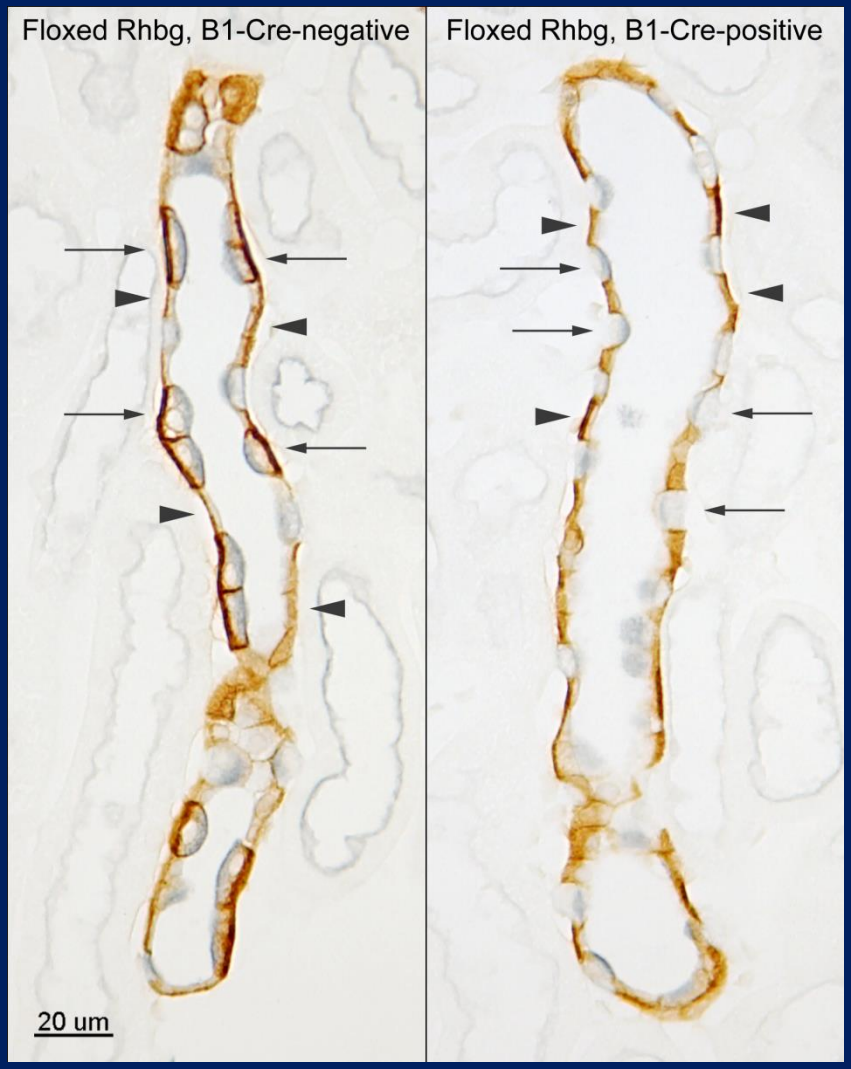
HW Lee, et al, *AJP Renal* 296:F1364-75, 2009.

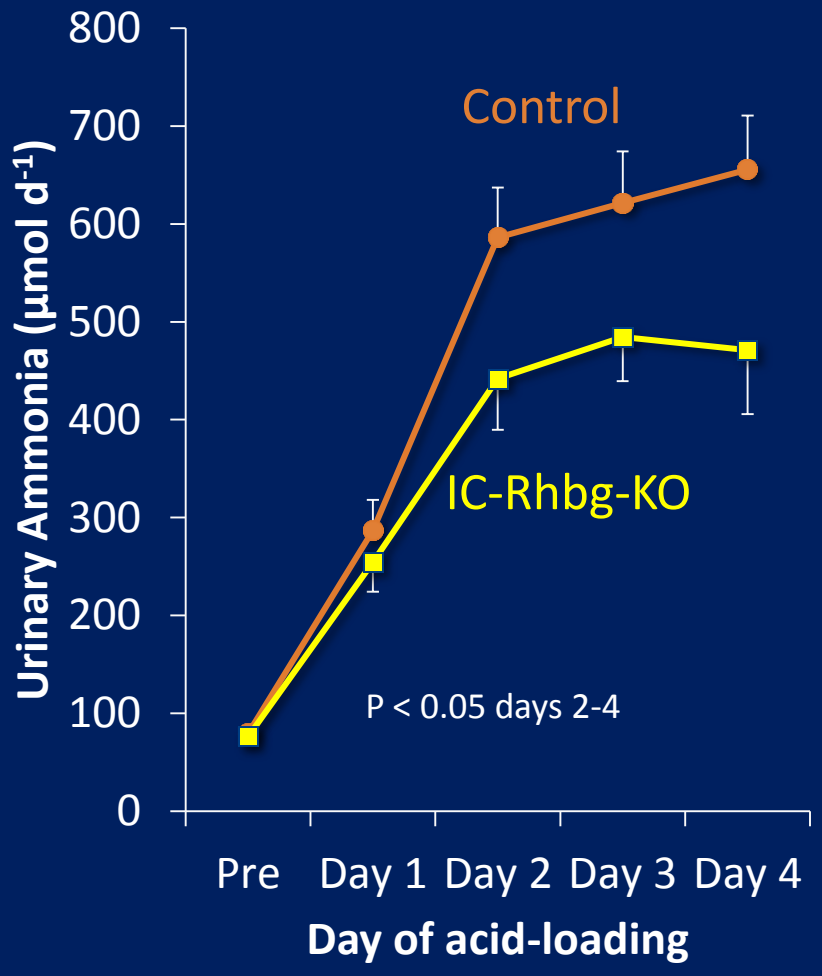
Development of intercalated cell-specific Rhbg knock-out mouse



JM Bishop, et al. *AJP Renal* 299: F1065-77, 2010.

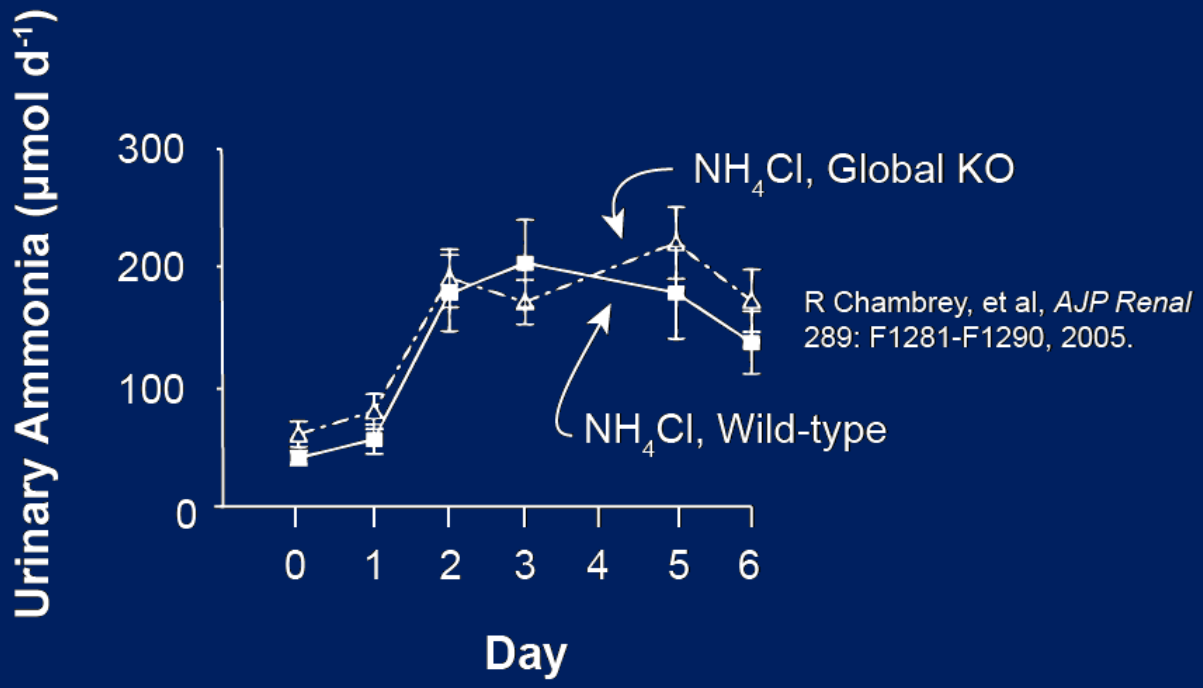
Effect of intercalated cell-specific Rhbg deletion on response to metabolic acidosis



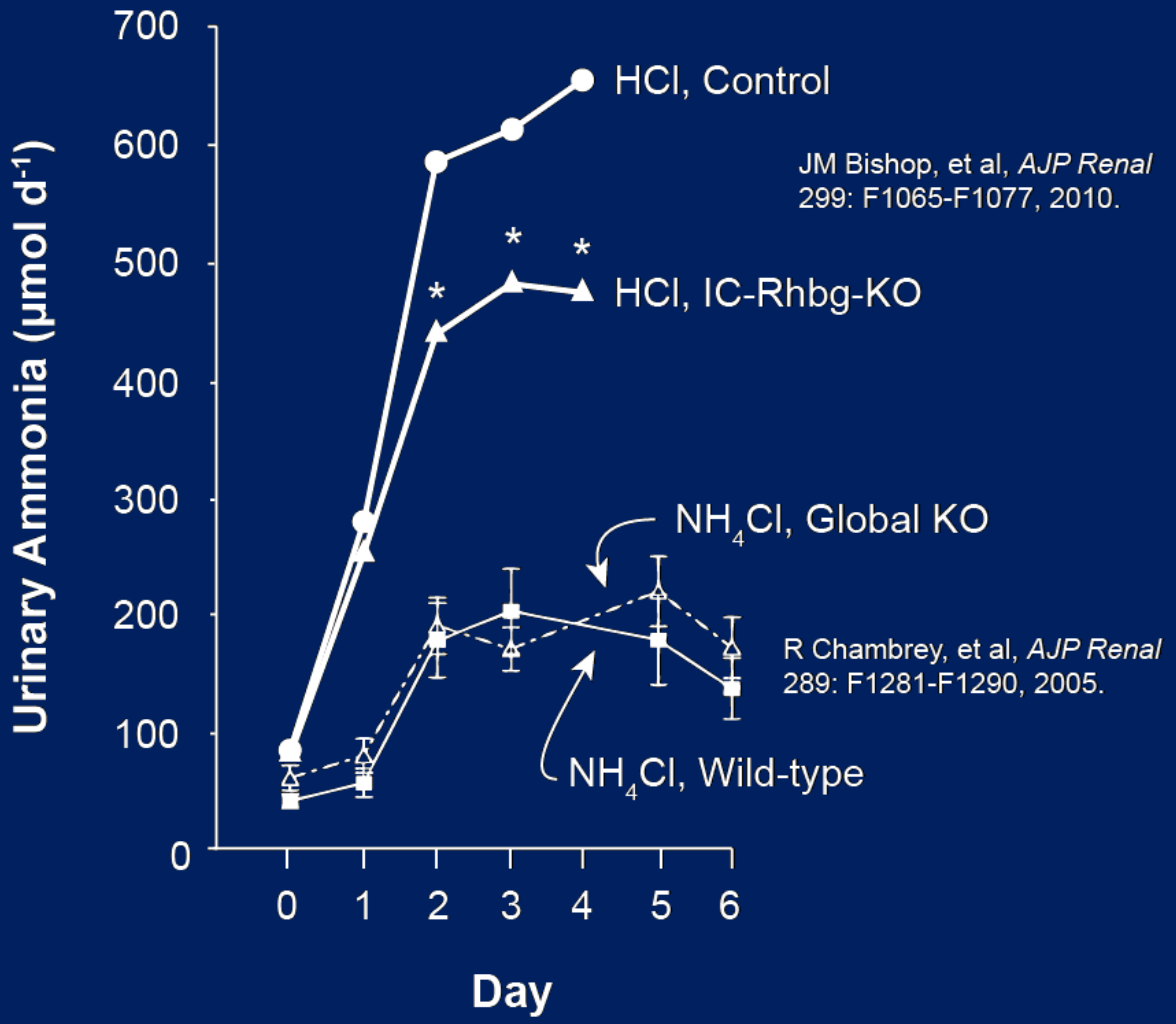


JM Bishop, et al. *AJP Renal* 299: F1065-77, 2010.

Another Rhbg gene deletion study



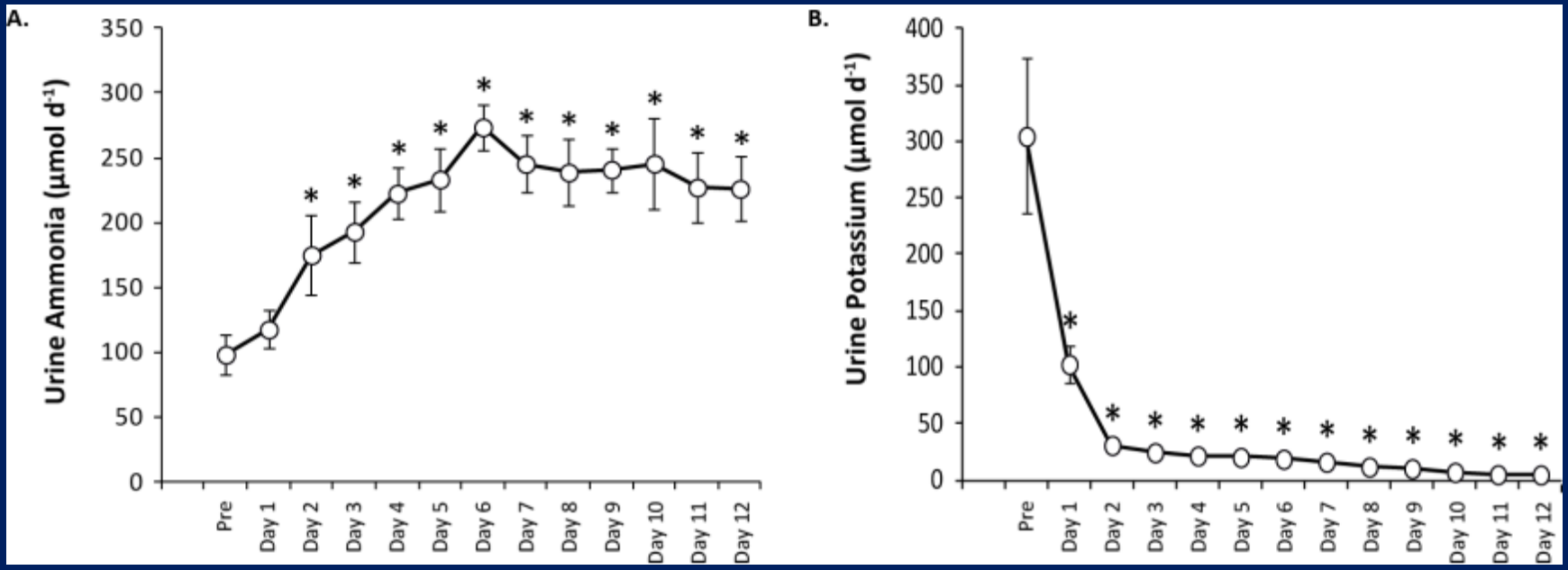
Comparison of the two Rhbg gene deletion studies



Does the role of Rhbg and Rhcg differ in different conditions?

- Hypokalemia
  - Increased urinary ammonia excretion
  - Urine alkalinization
    - Increased urine acidification cannot be the primary driving force
  - Development of metabolic alkalosis

Effect of K^+-free diet on mouse urinary electrolytes



HW Lee, et al, unpublished data.

Our studies assessing the role of Rh glycoproteins in NH_3 gas transport

Renal collecting duct NH_3 transport is both diffusive and saturable

↓

Rh glycoproteins are present specifically in cells that transport NH_3

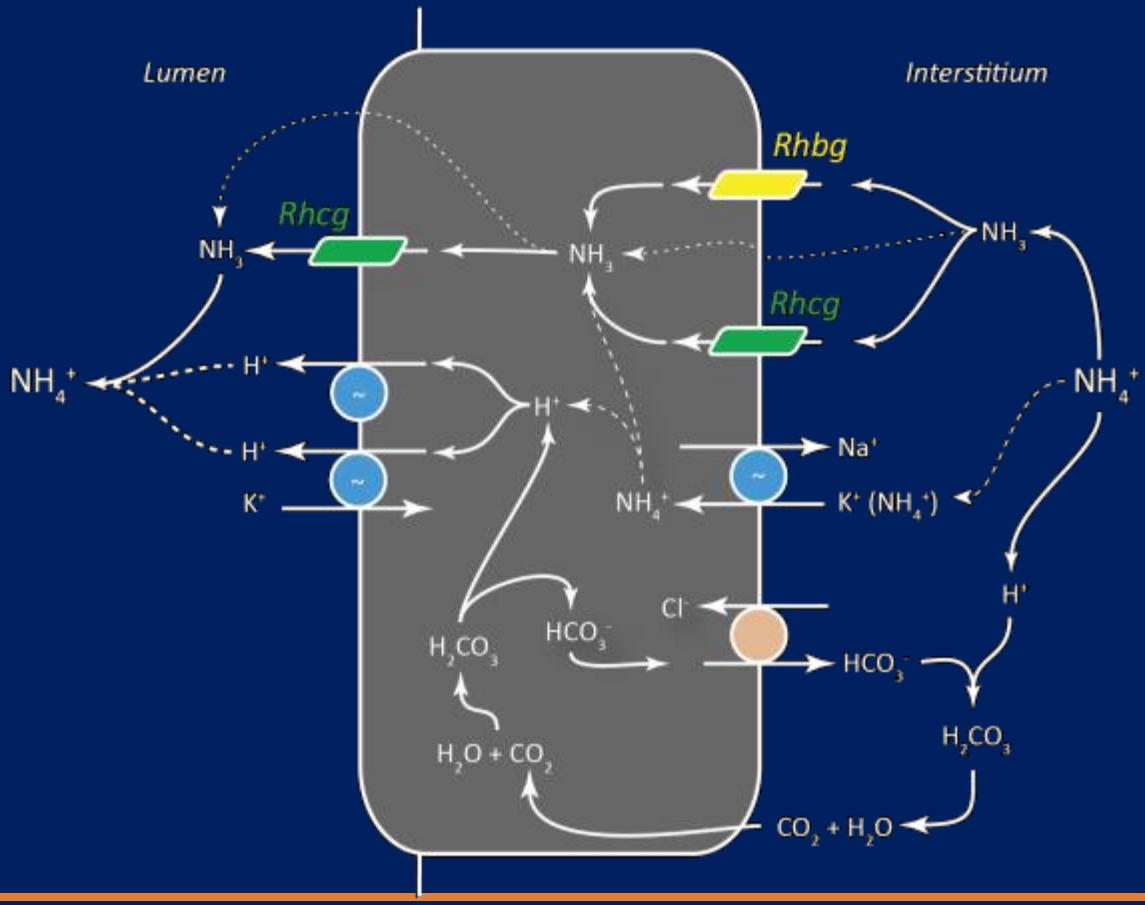
↓

Rh glycoprotein expression parallels NH_3 gas transport

↓

Rh glycoprotein gene deletion alter NH_3 gas transport

Rh glycoprotein-mediated NH_3 transport is central to renal ammonia metabolism and transport



‘Role of Membrane Proteins in Oxygen Transport in Red Blood Cells ’

R. Ryan Geyer, Ph.D.

PI: Walter F. Boron M.D., Ph.D.

Dept of Physiology & Biophysics
Case Western Reserve University School of Medicine
Cleveland, Ohio, USA

Gas Channel Workshop

September 7th, 2012 Cleveland, OH

Evidence for Gas Channels

The Boron Lab identified the first gas channel—the water channel AQP1— which exhibits permeability to CO_2.

Nakhoul et al, *AJP Cell* 274, 1998

Cooper & Boron, *AJP Cell* 275, 1998

DIDS—the anion transport inhibitor—not only reduces HCO_3^- permeability, but the CO_2 permeability in human RBCs.

Forster et al, *PNAS* 95, 1998

Later it was shown that NH_3 also passes through AQP1.

Nakhoul et al, *AJP Renal* 281, 2001

RhAG (a component of the Rh complex in human RBC) conducts NH_3.

Ripoche et al. *PNAS* 101, 2004

Lipid vesicles containing AQP1 increased Nitric Oxide influx by about 300%.

Herrera et al. *Hypertension* 48, 2006

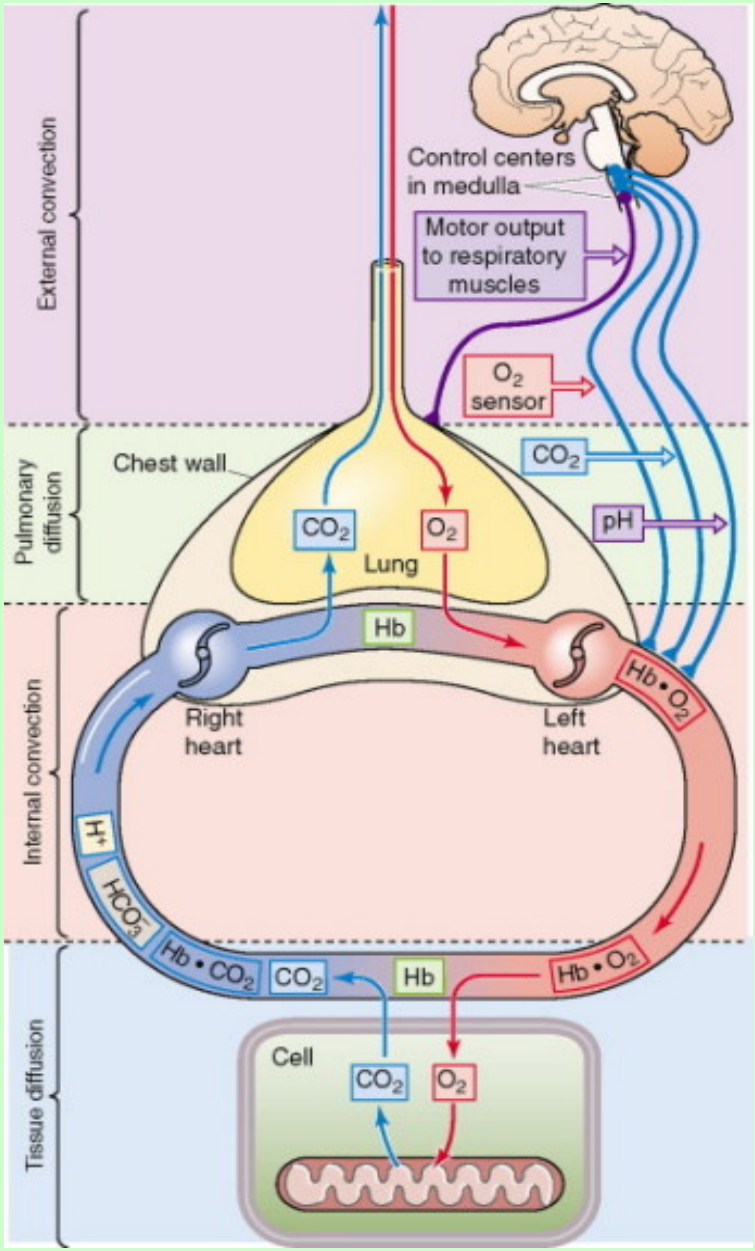
Endeward *et al.* found that the lipid of the RBC membrane has an extremely low permeability to CO_2.

Endeward et al, *FASEB J* 20, 2006

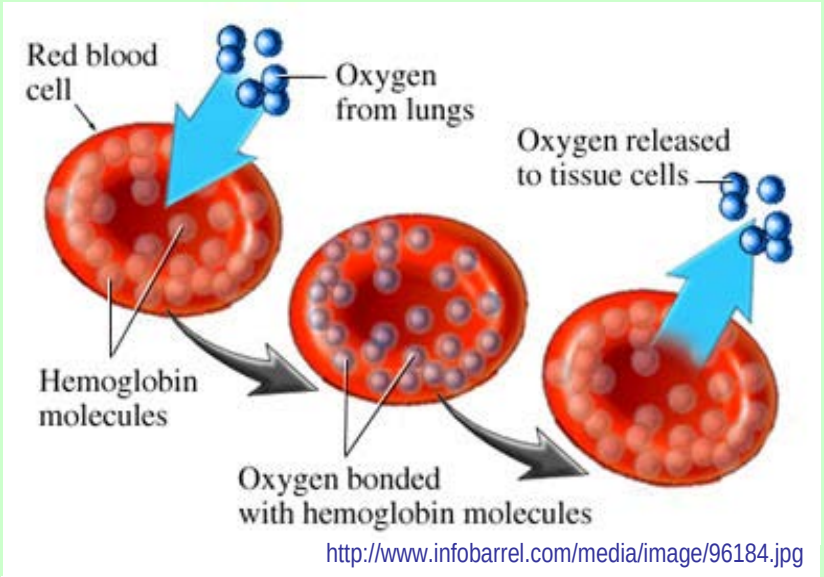
Musa-Aziz & Boron showed for the first time that gas channels—like ion channels—can exhibit selectivity for one gas over another (CO_2 *vs.* NH_3).

Musa-Aziz et al, *PNAS* 106, 2009

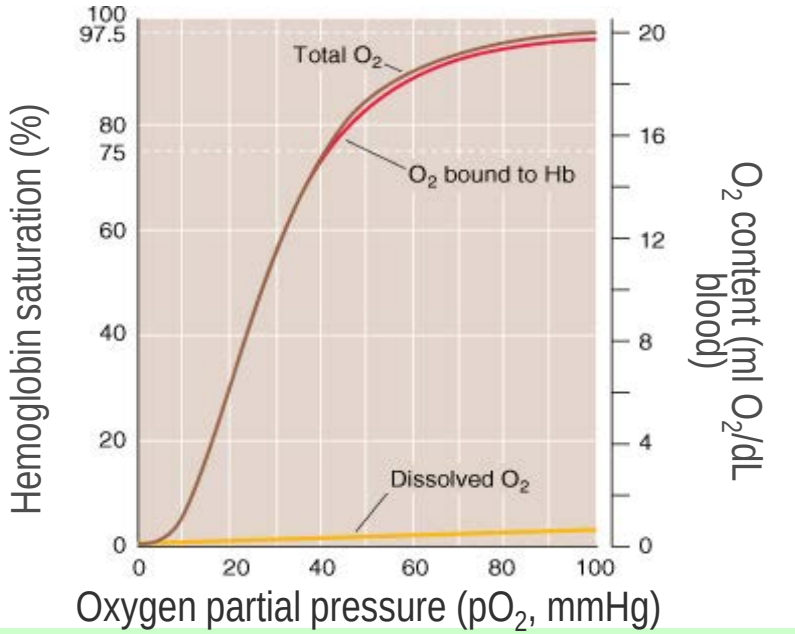
Gas Exchange



Boron, W.F. & Boulpaep, E.L. Medical Physiology



http://www.infobarrel.com/media/image/96184.jpg



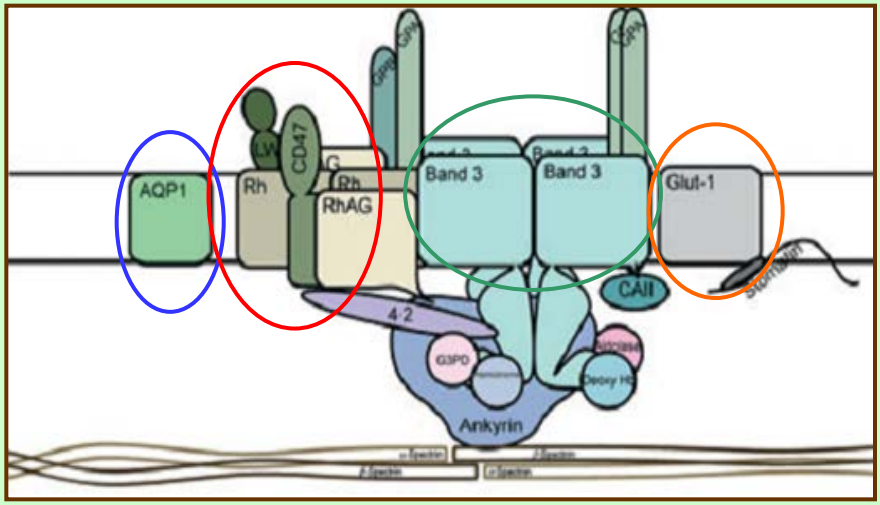
Significance

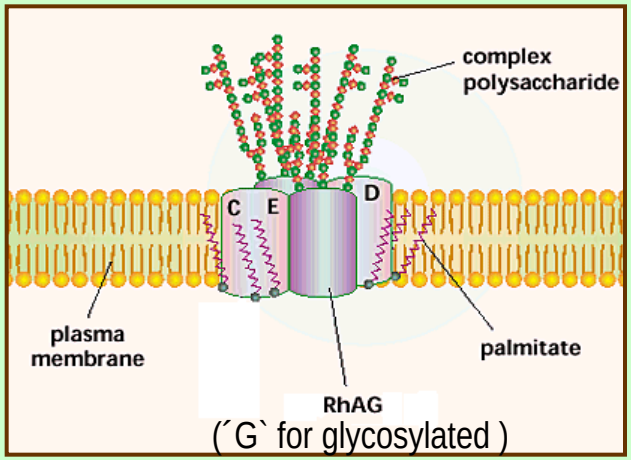
Due to the lipid and protein composition it is likely that these red blood cells (RBCs) have a low intrinsic permeability to gases.

Therefore, it would make physiological sense to have gas channel(s) to increase the O_2 flux, and that such a protein would be highly expressed in the RBC membrane.

Key Membrane Proteins Present at High Levels in the RBC

- AE1 (1 million copies per cell)
  - Cl^-/HCO_3^- co-transporter, Band 3
  - 25% of the cell membrane surface
  - Inhibited by DIDS
- GLUT1 (humans), GLUT4 (mouse) (600,000 copies per cell)
  - Glucose transporter
  - In mice there is a switch at about day 5
    Montel-Hagan et al. *Cell* 132, 2008
  - Inhibited by pCMBS and phloretin
- AQP1 (Aquaporin 1) (200,000 copies per cell)
  - H_2O, NH_3, and some CO_2 transport inhibited by pCMBS
  - Major CO_2 pathway inhibited by DIDS
- Rh-complex (Rhesus) (100,000 copies)
  - Transports NH_3
  - CO_2 transport blocked by DIDS
- MCT-1 (Monocarboxylate Transporter 1) (80,000 copies per cell)
  - Inhibited by DIDS and pCMBS
- UT-B (Urea Transporter) (15,000 copies per cell)
  - Urea transport inhibited by pCMBS and phloretin
  - We have shown that UT-B can transport H_2O & NH_3, but not CO_2



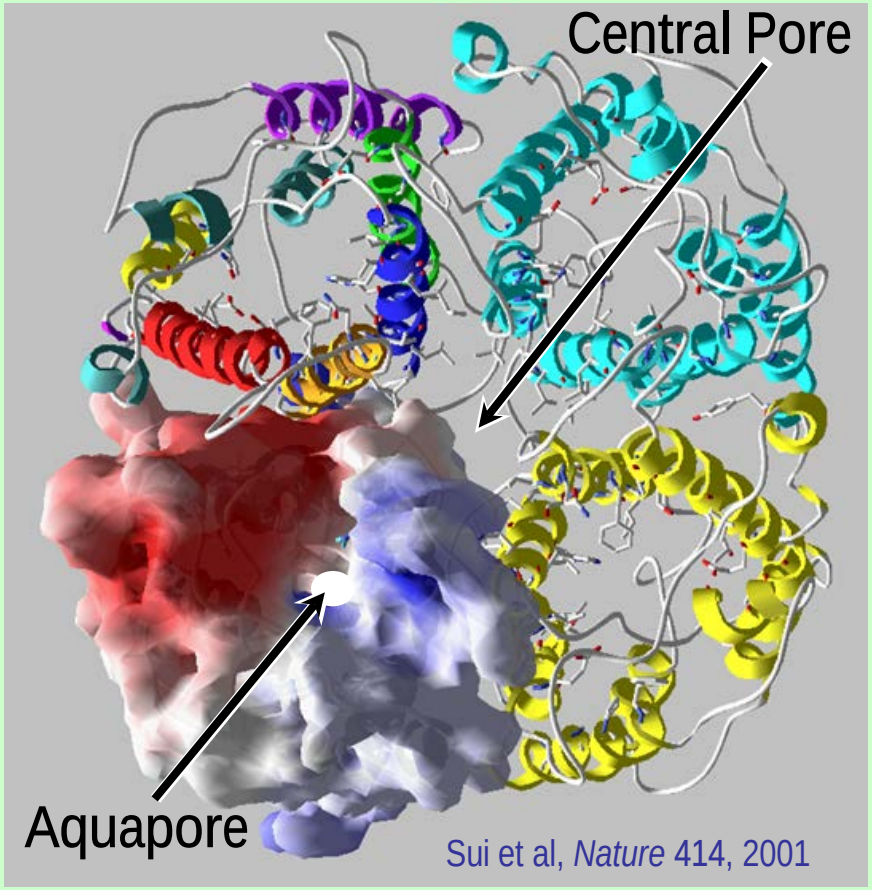


All of these proteins form homo-oligomers of dimers, trimers, or tetramers, which could form additional pores.

Bruce et al. *Blood* 101, 2003

Anstee. *VoxSanguinis* 100, 2011

AQP1 Crystal Structure

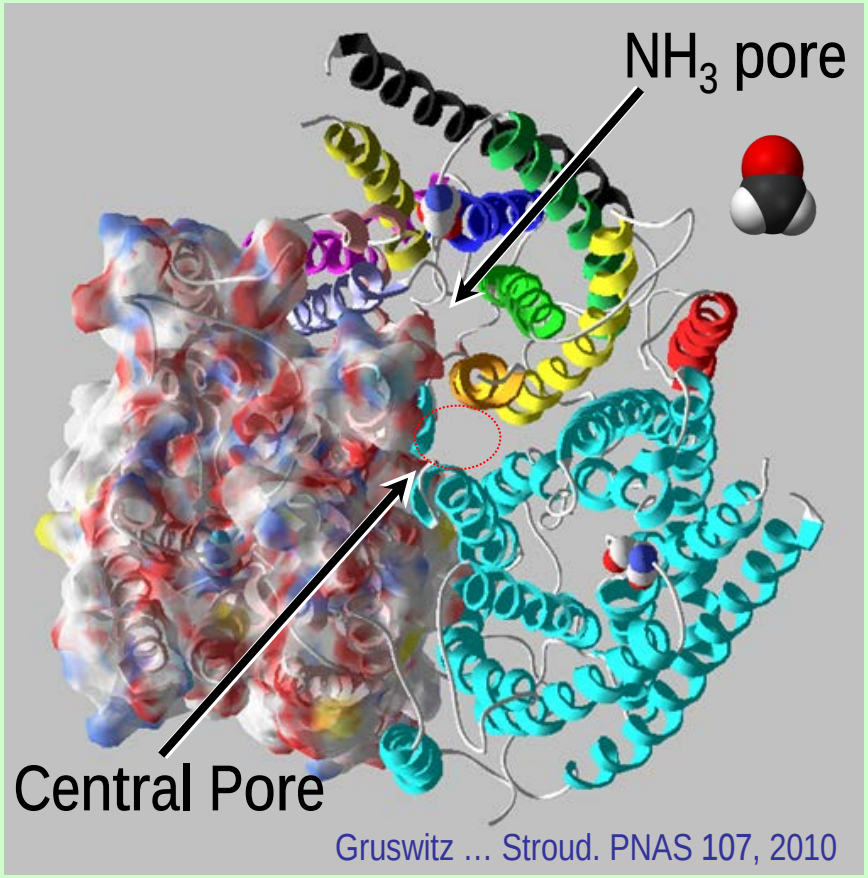


AQP1 is a **homotetrameric** protein with 4 water channels … '**aquapore**', which is lined by hydrophilic & hydrophobic residues.

There is an additional pore …the '**central pore**', which is lined by hydrophobic residues.

Our Laboratory showed that AQP1 can transport both CO_2 and NH_3.

RhCG Crystal Structure

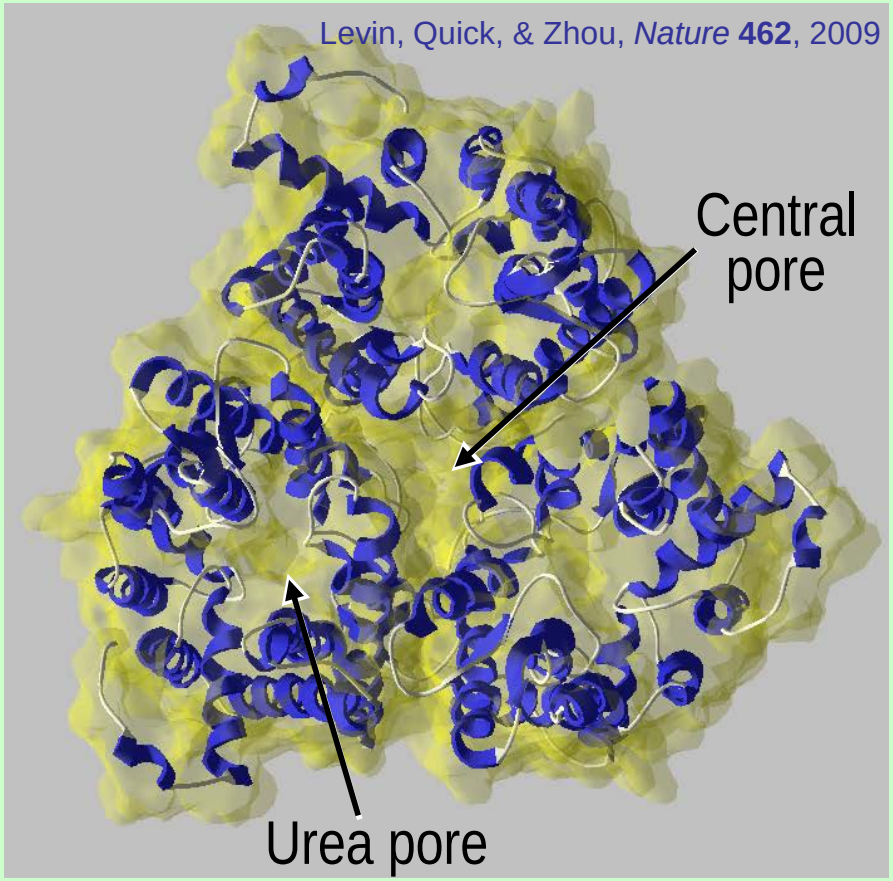


RhCG is a **homotrimeric** protein – each monomer contains 12 TMs and works as a functional ammonia pore …

… playing an essential role in the secretion of NH_3 in the kidney, which is critical to systemic acid-base homeostasis.

Hydrophobic '**central pore**' is formed at the threefold axis of symmetry.

Urea Transporter Crystal Structure



The bacterial homolog Urea Transporter (UT-B) was crystallized as a homotrimer.

Each bundle of helices forms a monomeric urea channel.

Urea transport can be inhibited by phloretin, $HgCl^{2+}$, and pCMBS.

Our Questions …

Can we quantitate the O_2 efflux and/or influx rate of intact wild-type RBCs?

If so, is it possible to determine the contribution of the CO_2 channels (AQP1 and Rh-complex) to the O_2 efflux?

Are there other RBC membrane proteins that could also contribute to the O_2 efflux?

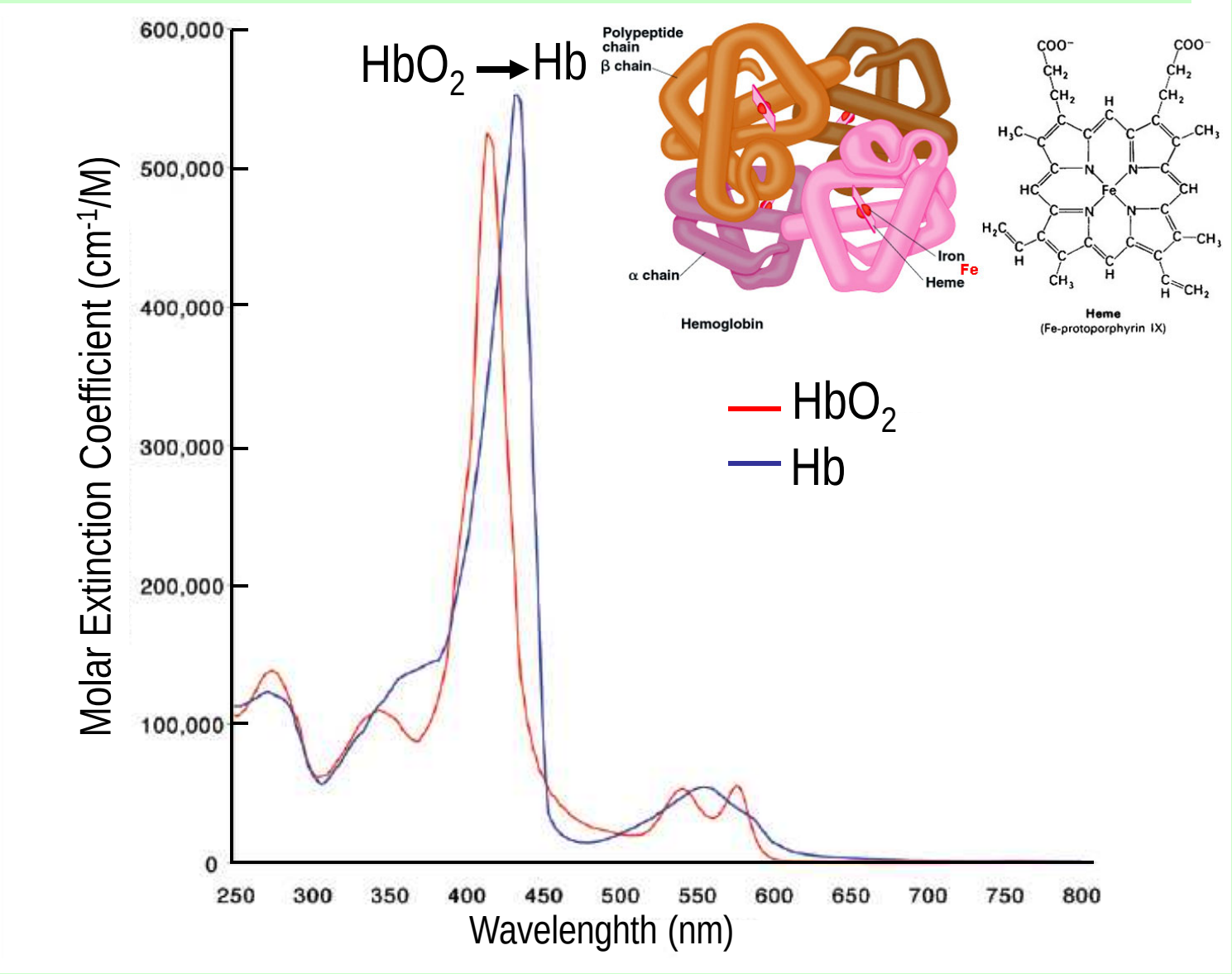
Can known inhibitors of transporters block O_2 efflux from the RBC?

pCMBS – mercurial agent that covalently reacts with cysteine thiol groups. Blocks water transport via AQPs and a variety of other transport processes.

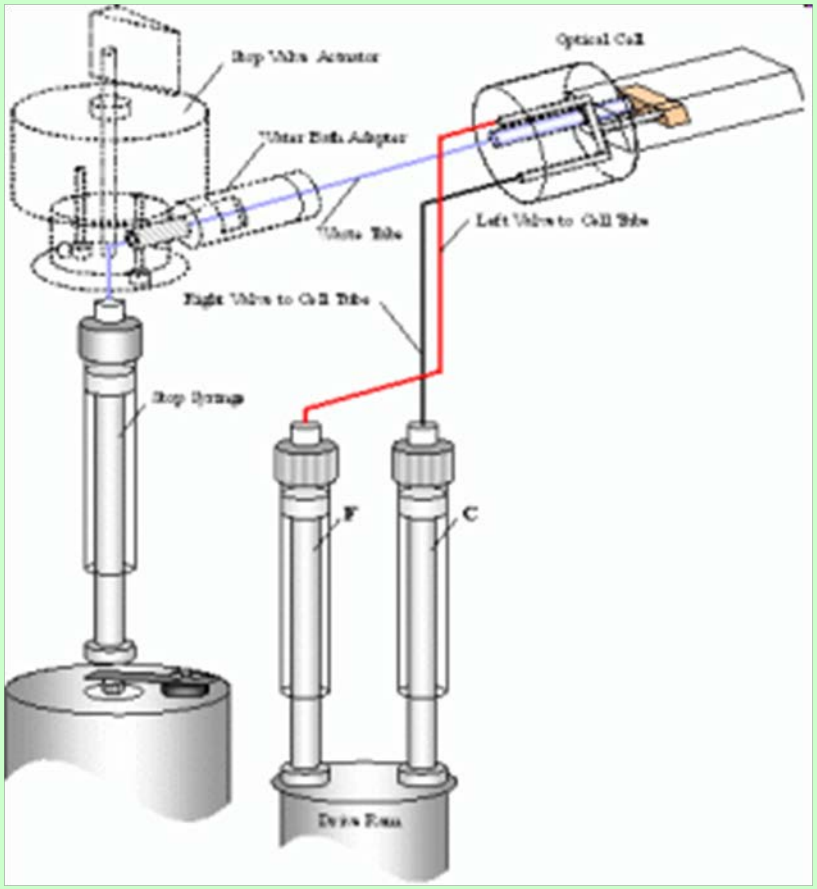
DIDS – amino-reactive agent known to inhibit the anion transporter (AE1 or Band 3) activity. The interaction can be reversible and irreversible (covalent). Also shown to reduce the CO_2 permeability of AQP1 and RhAG.

Phloretin – known to inhibit the glucose an urea transporters.

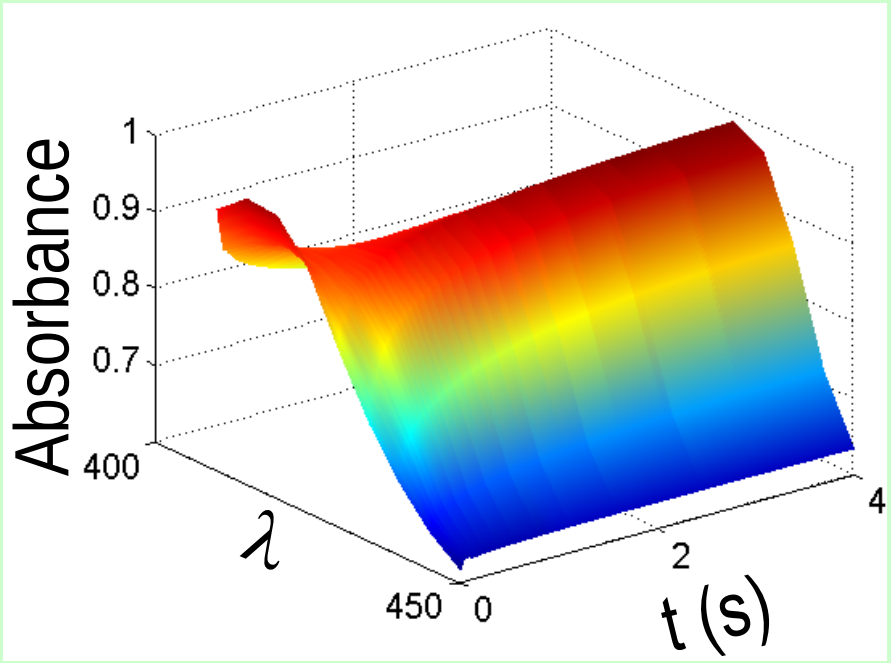
Hemoglobin Absorbance Changes



Stopped Flow Technique



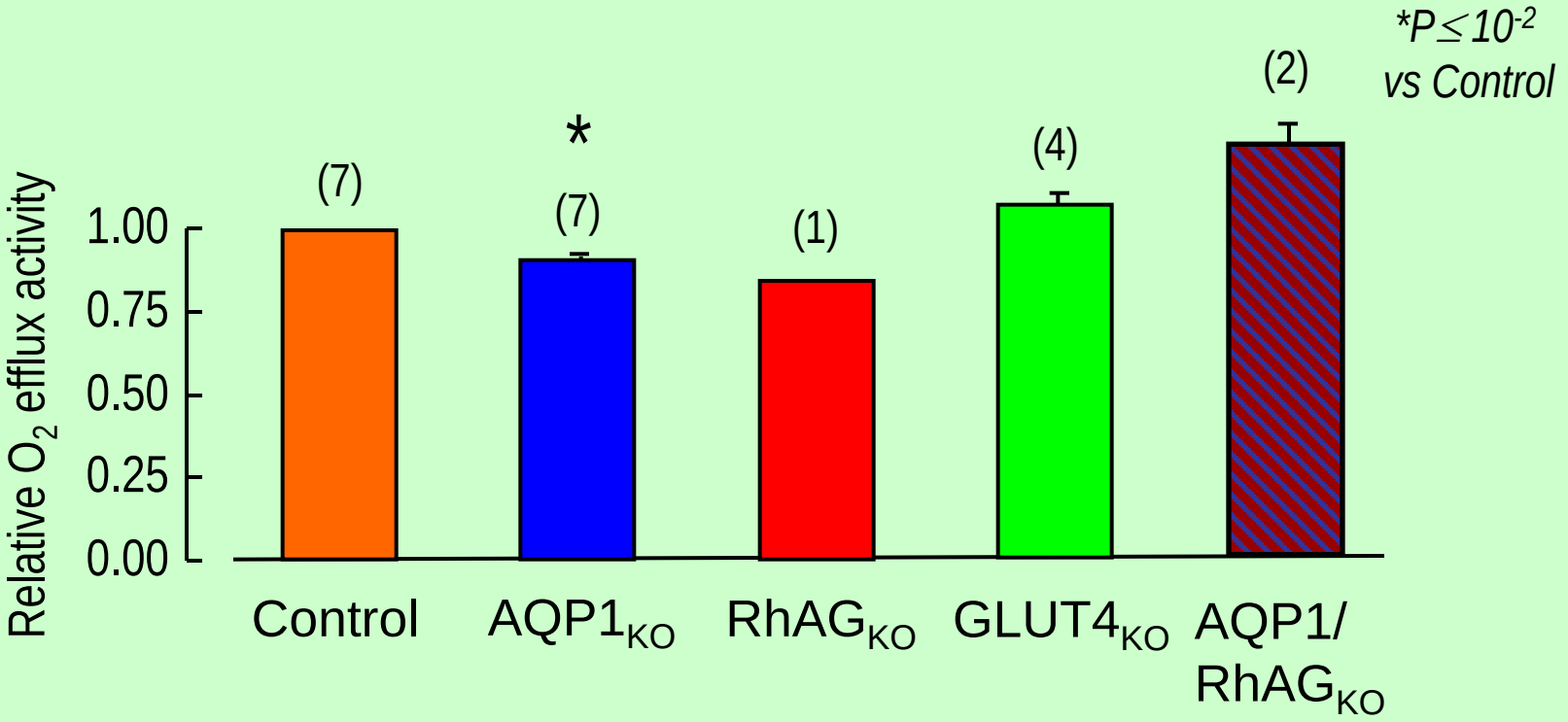
Rapidly mixes two solutions
(on a millisecond timescale)



Data from multiple wavelengths (λ) can be collected and complied into a 3-D graph (Abs. vs. Time vs. λ).

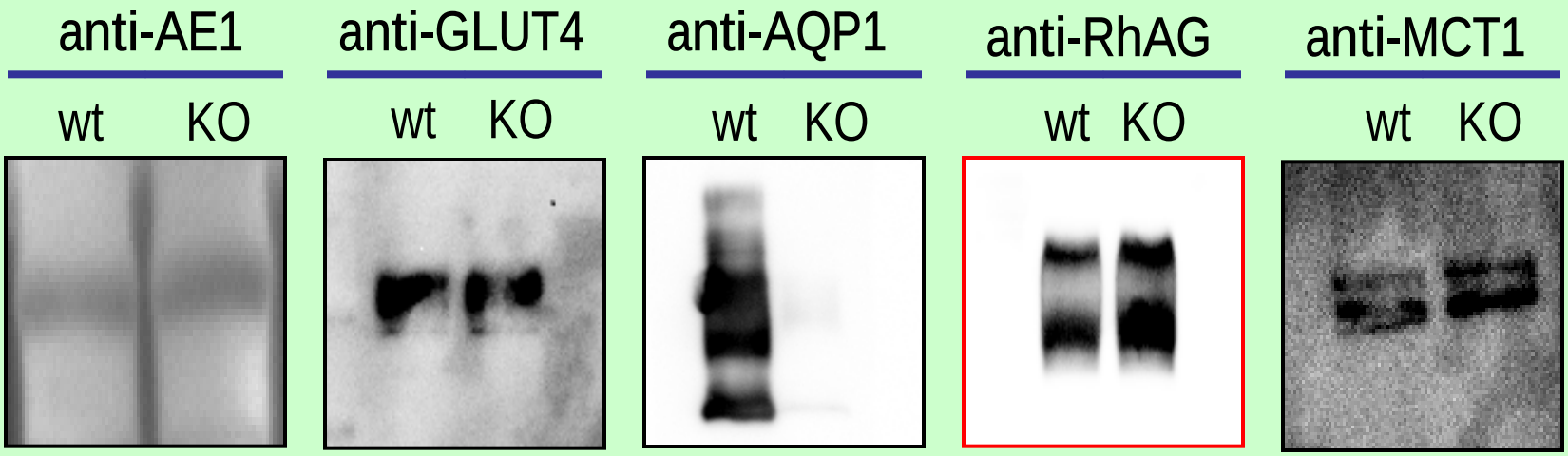
From this data we can calculate the rate of the chemical reaction.

Effect of knocking out AQP1, RhAG, GLUT4, and AQP1/RhAG on O_2 efflux in mouse RBCs

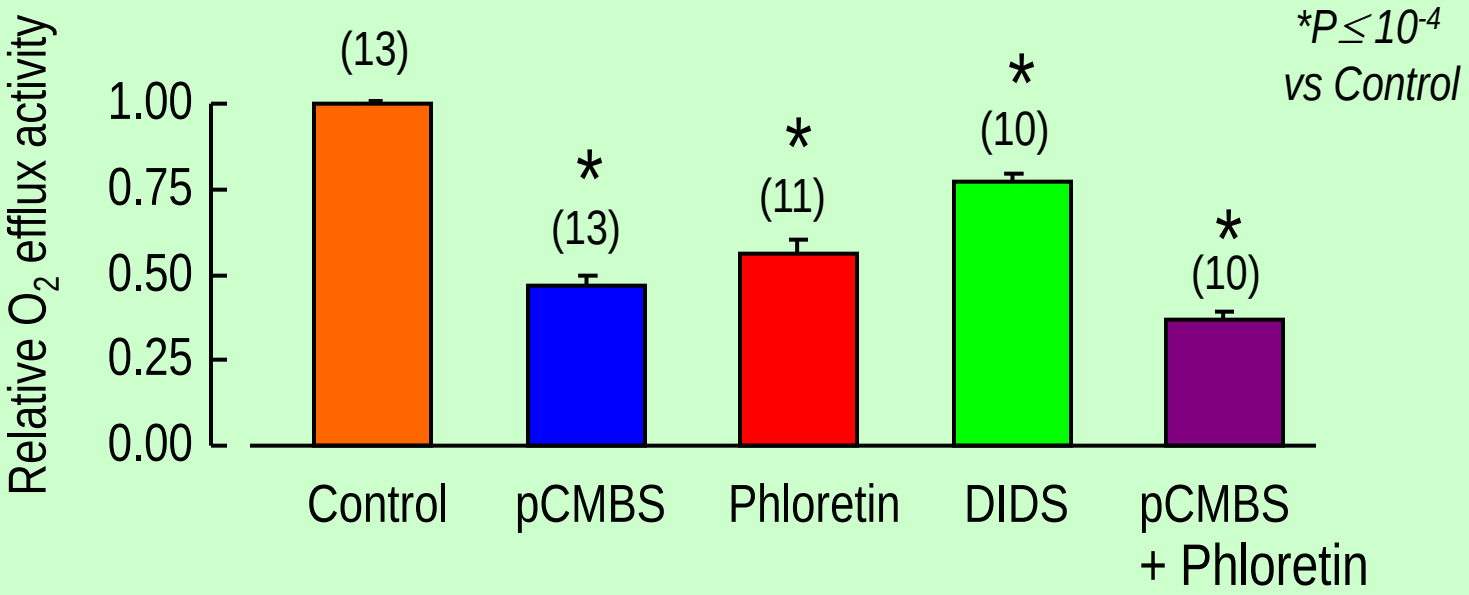


The major CO_2 channels of the RBC—AQP1 and RhAG—also function as modest O_2 channels.

Western Blot Analysis of Membrane Proteins from wild-type and AQP1-KO



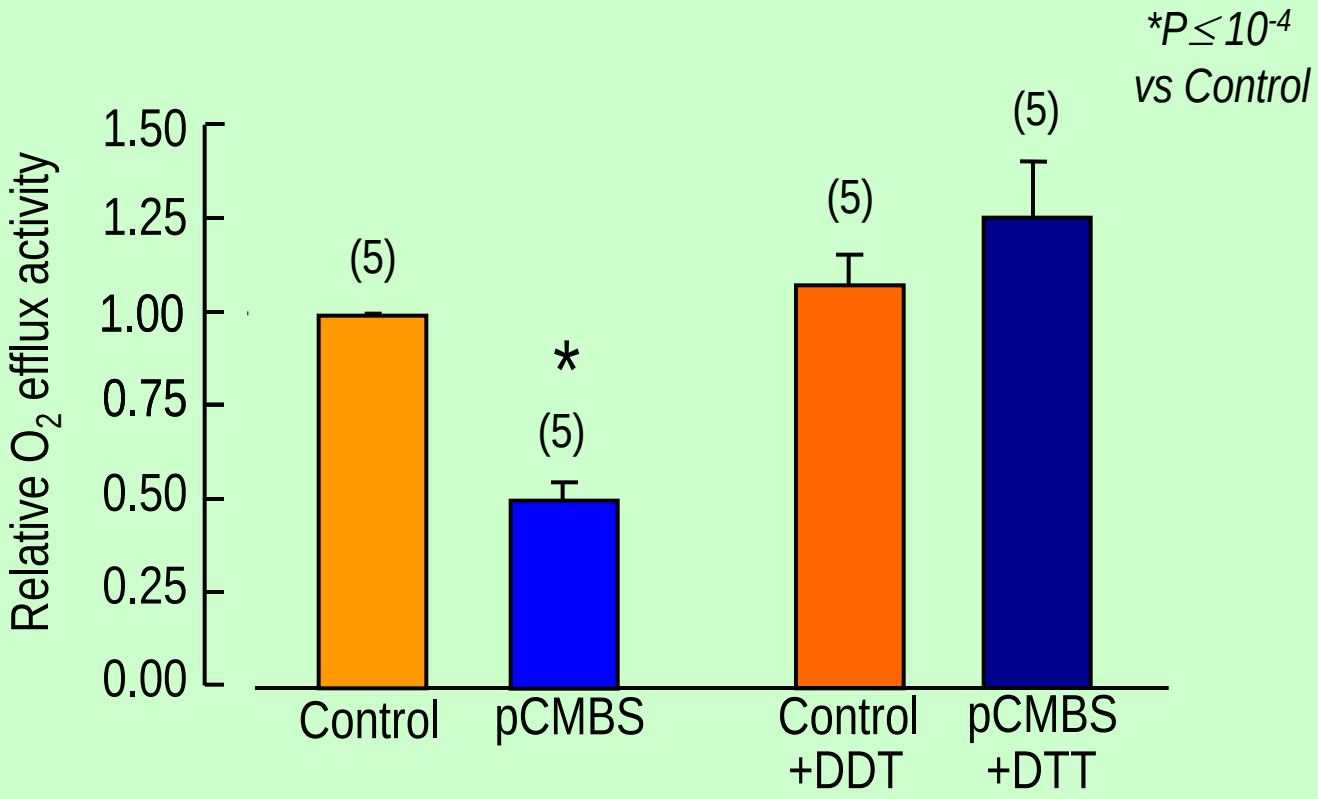
Effect of pCMBS, Phloretin, or DIDS on Oxygen Efflux from Mouse Red Blood Cells



- pCMBS reduces the O_2 efflux by 50%.
- Phloretin reduces the O_2 efflux by 40%.
- DIDS reduces the O_2 efflux by 25%.
- pCMBS + Phloretin reduce the O_2 efflux by 70%.

Inhibitor efficacy: pCMBS + phloretin > pCMBS > phloretin > DIDS

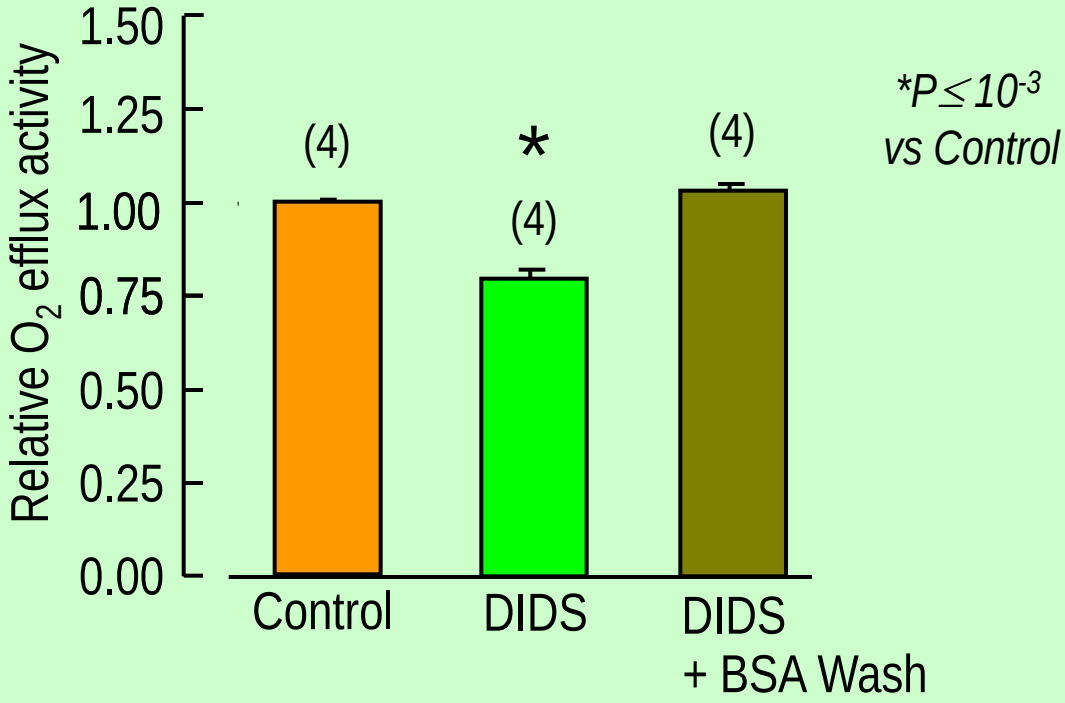
pCMBS Reversibility



As shown previously, pCMBS reduces the O_2 efflux rate by ~50%.

The inhibitory effect of pCMBS can be reversed with the addition of the reducing agent DTT.

DIDS Reversibility



The inhibition of O_2 efflux observed with DIDS appears to be non-covalent and reversible, the inhibition can be reversed when the RBCs are washed with 0.2% Bovine Serum Albumin (BSA).

Conclusions

- The data suggest that the DIDS-sensitive CO_2 channels (AQP1 and RhAG) of the RBC appear to modestly transport O_2 out of the cell.

  … because this transport process is not greatly effected by DIDS the O_2 must utilize an alternative pathway(s).

- Knockouts of GLUT4 and AQP1/RhAG have O_2 efflux rates greater (10-25%) than the wild-type controls.
- Inhibitor efficacy:

  pCMBS + phloretin > pCMBS > phloretin > DIDS

  … because the pCMBS/Phloretin inhibition was not totally additive, O_2 transport likely occurs by <u>two</u> or <u>more</u> <u>channels</u>.

- There must be a pathway (channel) that is sensitive to both pCMBS and phloretin and another pathway that is insensitive to both inhibitors.

Future Directions

- Compile inhibitor profiles for the knockout mice.
- Perform western blots on RBCs from knockout mice.
- Develop assays for monitoring nitric oxide (NO) and hydrogen sulfide (H_2S) transport in red blood cells.
- Investigate the effect of hypoxia on protein expression (XQ) and O_2 transport activity (RRG).
- Investigate the effect of adding and removing cholesterol from the RBC membranes on O_2 transport activity.

Acknowledgments

Principal Investigator

Walter F. Boron, M.D., Ph.D.

Collaborator

Raif Musa-Aziz, Ph.D. (Univ. of Sao Paulo)

Animal Technician

Thomas Radford (CWRU)



Talks & Posters

Geyer RR, MD Parker, N Burton, WF Boron, AM Toye, & R Musa-Aziz. Relative CO_2/NH_3 permeabilities of human RhAG, RhBG, and RhCG. Experimental Biology, Washington, DC, *FASEB J* 25:1040.4, 2011.

Geyer RR, R Musa-Aziz, & WF Boron. Evidence that DIDS crosslinks Aquaporin 1 monomers. Experimental Biology, Washington, DC, *FASEB J* 25:1039.26, 2011.

Musa-Aziz R, RR Geyer & WF Boron. Relative CO_2/NH_3 permeabilities of several members of the mammalian Aquaporin family: bAQP0, hAQP1, hAQP2, rAQP3, rAQP4-M1, rAPQ4-M23, and hAQP8. Experimental Biology, Washington, DC, *FASEB J* 25:1040.5, 2011.

Geyer RR & WF Boron. Gas transport through channels. Undersea & Hyperbaric Medicine Society Scientific Meeting in conjunction with The Office of Naval Research, Fort Worth, TX, June 15-18, 2011.

Geyer RR, R Musa-Aziz, & WF Boron. Movement of NH_3 through Human Urea Transporter B (UT-B)—a new member of gas channels. ASN Kidney Week, 2011.

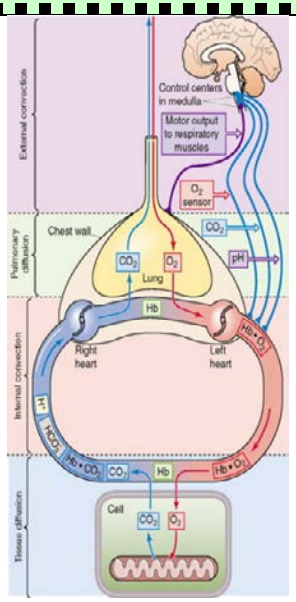
Musa-Aziz R, RR Geyer, X Qin, & WF Boron. The CO_2/NH_3 selectivities and inhibitor sensitivities of mammalian Aquaporins. ASN Kidney Week, 2011.

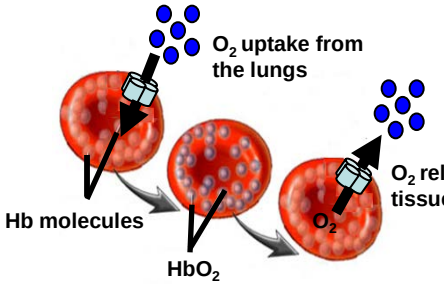
Geyer RR & WF Boron. Role of Membrane Proteins in Oxygen Transport in Red Blood Cells. International Workshop on Membrane Transport of Small Solutes, Strobl, Austria, June, 2012.

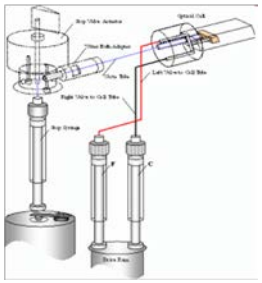
Postdoctoral Fellowship for 'Gas Transport through Channels'

Background: Red blood cells (RBCs) function to carry oxygen (O_2) to tissues and transport carbon dioxide (CO_2) away from tissues. The traditional view had been that these gases dissolve in the membrane and diffuse into or out of the cell. Our laboratory and others have shown that RBC membrane proteins can function as gas channels for the transport of CO_2 and/or NH_3. It is our hypothesis that membrane proteins can also facilitate the transport of O_2 across the RBC membrane. This research could represent a major paradigm shift, and totally reorganize our thinking of how O_2 crosses cell membranes.

Naval and Scientific Benefits: If we understand the molecular mechanism of gas transport—we could design pharmacological agents that—by inhibiting or activating gas channels—can prevent or treat **decompression illness** and **O_2 toxicity.**







Applied Photophysics Stopped Flow Mixing Chamber

Objectives: (1.) To quantitate O_2 efflux of intact RBCs using stopped-flow absorbance spectroscopy. **(2.)** Determine the contribution of the CO_2 channels (AQP1 and RhAG) to the O_2 efflux, as well as other highly expressed RBC membrane proteins (AE1, GLUT4, MCT1, and UT-B). **(3.)** Assess the effect on O_2 efflux rate when wild-type RBCs have been treated with compounds known to inhibit: H_2O permeability (pCMBS), glucose and urea transport (phloretin), and CO_2 transport (DIDS).

FY12 Accomplishments, Discoveries, & Inventions

- Determined O_2 efflux rate of intact, wild-type RBCs.
- Completed inhibitor profile of O_2 efflux from intact RBCs.
- Investigated O_2 efflux rate of intact RBCs from AQP1-null, RhAG null, and GLUT-4 null mice.

FY13 Goals

- Quantitate O_2 efflux rate of intact RBCs from AE1 null, GLUT-4 null, and UT-B null mice.
- Investigate the transport of nitric oxide (NO) and hydrogen sulfide (H_2S) in intact RBCs.

Principle Investigator: Dr. Walter F. Boron, 216-368-3400
walter.boron@case.edu

Gas Channel Workshop

Structure determinants for CO_2 transport of human aquaporin5

Xue Qin

PI: Walter F Boron

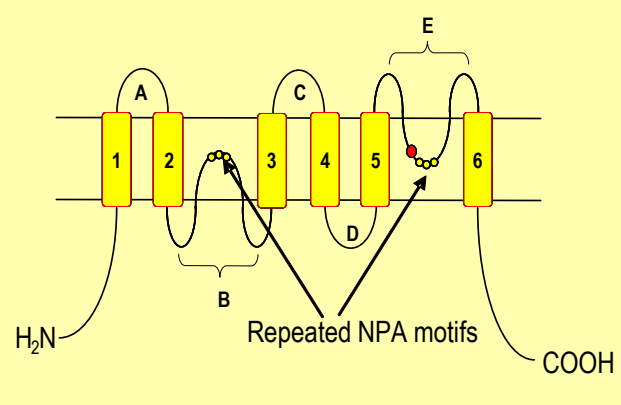
Department of Physiology and Biophysics

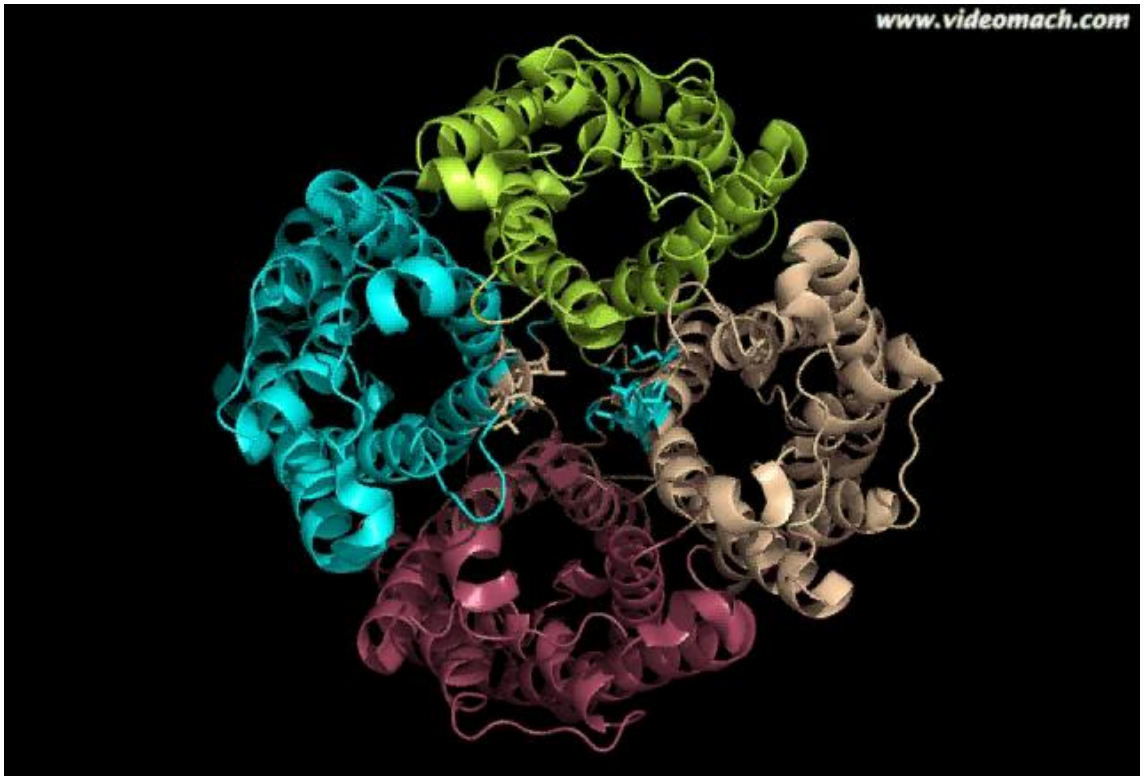
Case Western Reserve University

Background

Aquaporin 5 is a water channel highly expressed in salivary glands, eye, lung and trachea.

Aquaporins are composed of 6 transmembrane domains, with N- and C- terminus on the cytoplasmic side of the membrane.





Background

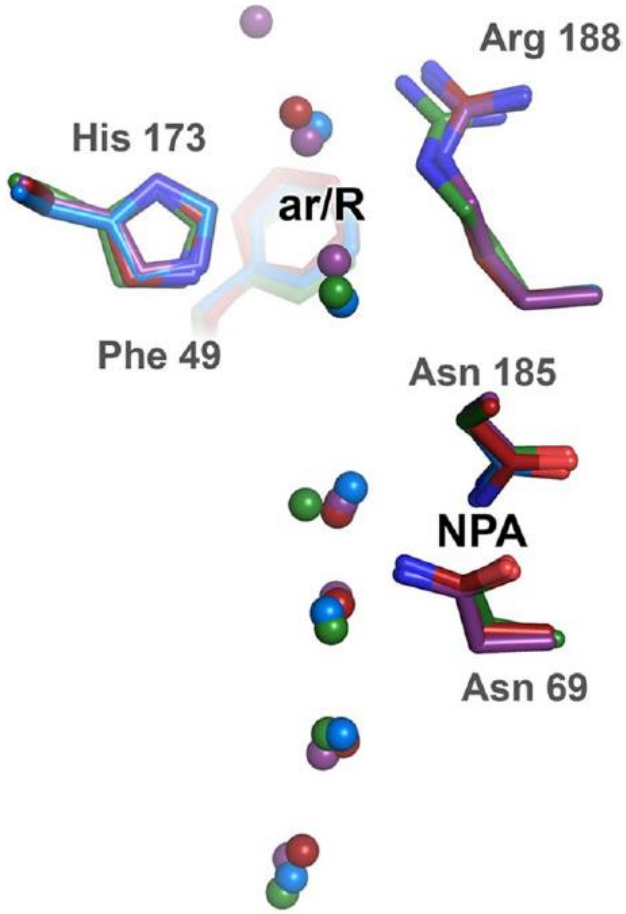
H_2O permeability

2 filter regions is important for H_2O transport

- Selectivity filter: ar/R region
- NPA region

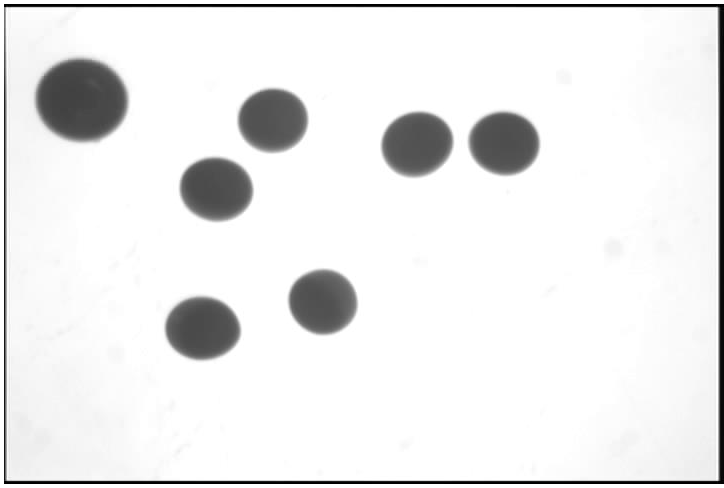
CO_2 permeability

Not well defined



Horsefield et al, 2008 PNAS

Methods

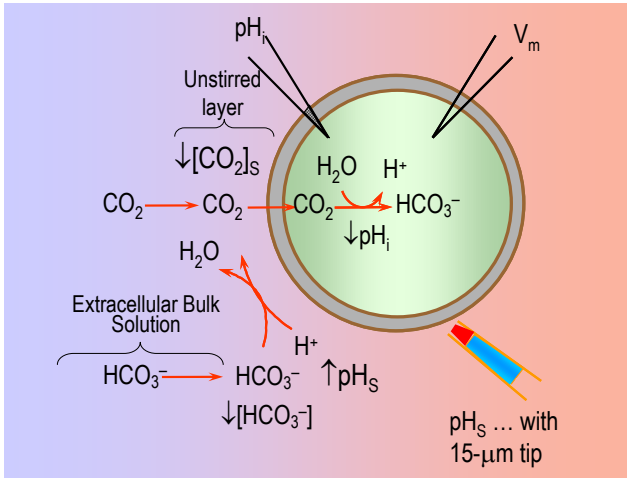


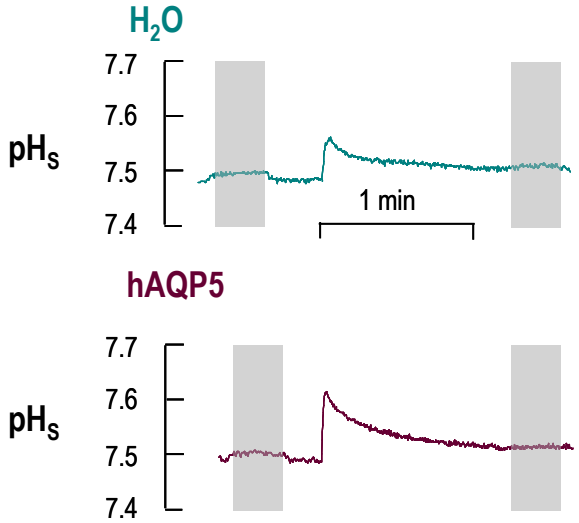
H_2O permeability--P_f

Volumetric assay to measure osmotic water permeability
How fast the volume of oocytes change with time

CO_2 permeability--ΔpH_S

Microelectrode to measure pH on the surface of the oocytes





Outline

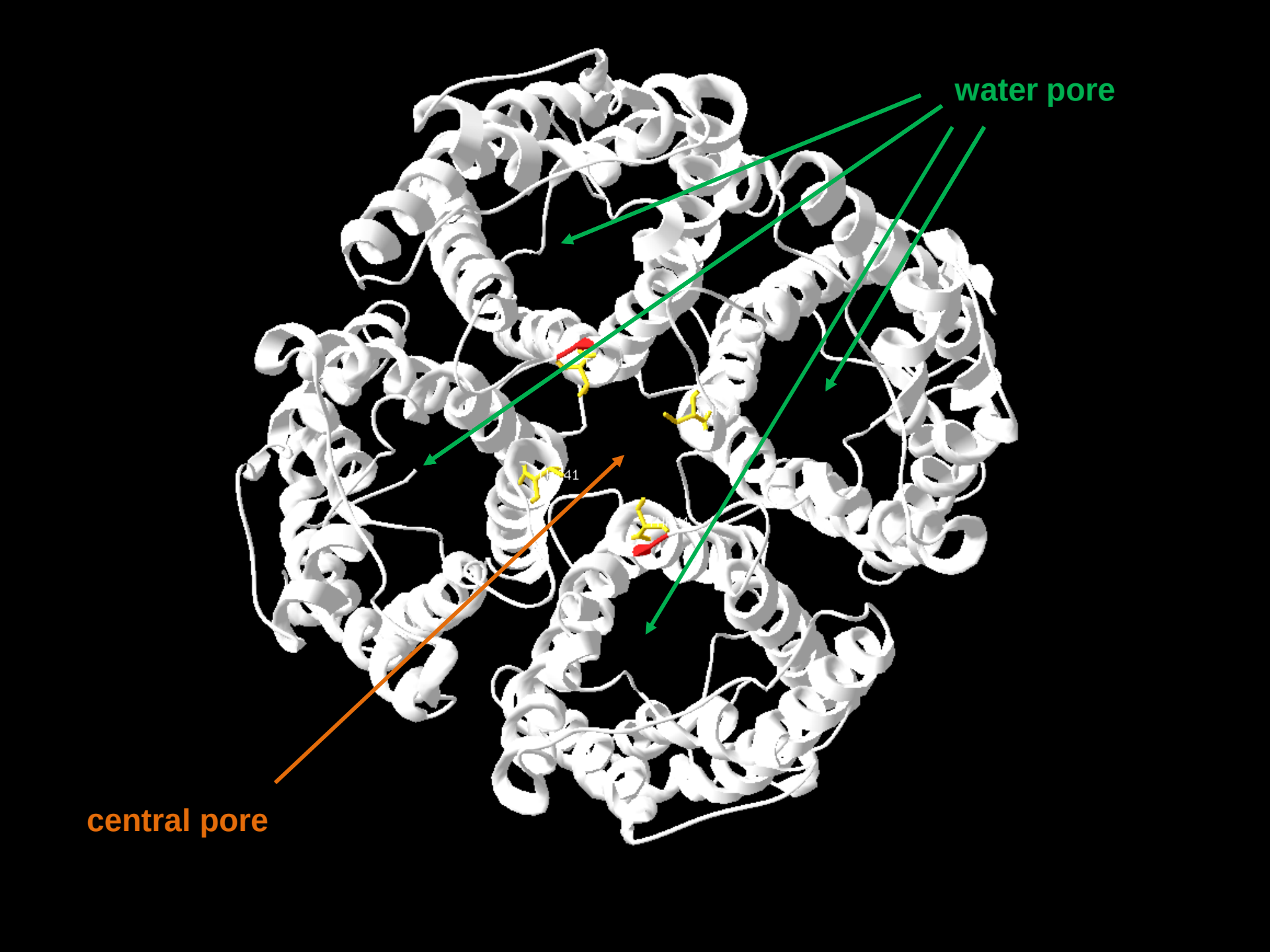
I Amino acids at the mouth of the central pore

II Amino acids lining the central pore

Outline

I Amino acids at the mouth of the central pore

II Amino acids lining the central pore


water pore
central pore

Exploring gas permeability of cellular membranes and membrane channels with molecular dynamics

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[b] *Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT 06520, USA*
[c] *Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA*
[d] *Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA*

Received 12 August 2006; received in revised form 28 October 2006; accepted 3 November 2006
Available online 4 January 2007

Abstract

Aquaporins are a family of membrane proteins specialized in rapid water conduction across biological membranes. Whether these channels also conduct gas molecules and the physiological significance of this potential function have not been well understood. Here we report 140 ns of molecular dynamics simulations of membrane-embedded AQP1 and of a pure POPE bilayer addressing these questions. The permeability of AQP1 to two types of gas molecules, O_2 and CO_2, was investigated using two complementary methods, namely, explicit gas diffusion simulation and implicit ligand sampling. The simulations show that the central (tetrameric) pore of AQP1 can be readily used by either gas molecule to permeate the channel. The two approaches produced similar free energy profiles associated with gas permeation through the central pore: a −0.4 to −1.7 kcal/mol energy well in the middle, and a 3.6–4.6 kcal/mol energy barrier in the periplasmic vestibule. The barrier appears to be mainly due to a dense cluster of water molecules anchored in the periplasmic mouth of the central pore by four aspartate residues. Water pores show a very low permeability to O_2, but may contribute to the overall permeation of CO_2 due to its more hydrophilic nature. Although the central pore of AQP1 is found to be gas permeable, the pure POPE bilayer provides a much larger cross-sectional area, thus exhibiting a much lower free energy barrier for CO_2 and O_2 permeation. As such, gas conduction through AQP1 may only be physiologically relevant either in membranes of low gas permeability, or in cells where a major fraction of the cellular membrane is occupied by AQPs.
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Keywords: Aquaporin; AQP1; Gas permeability; O_2; CO_2; Free energy profile; Gas channels

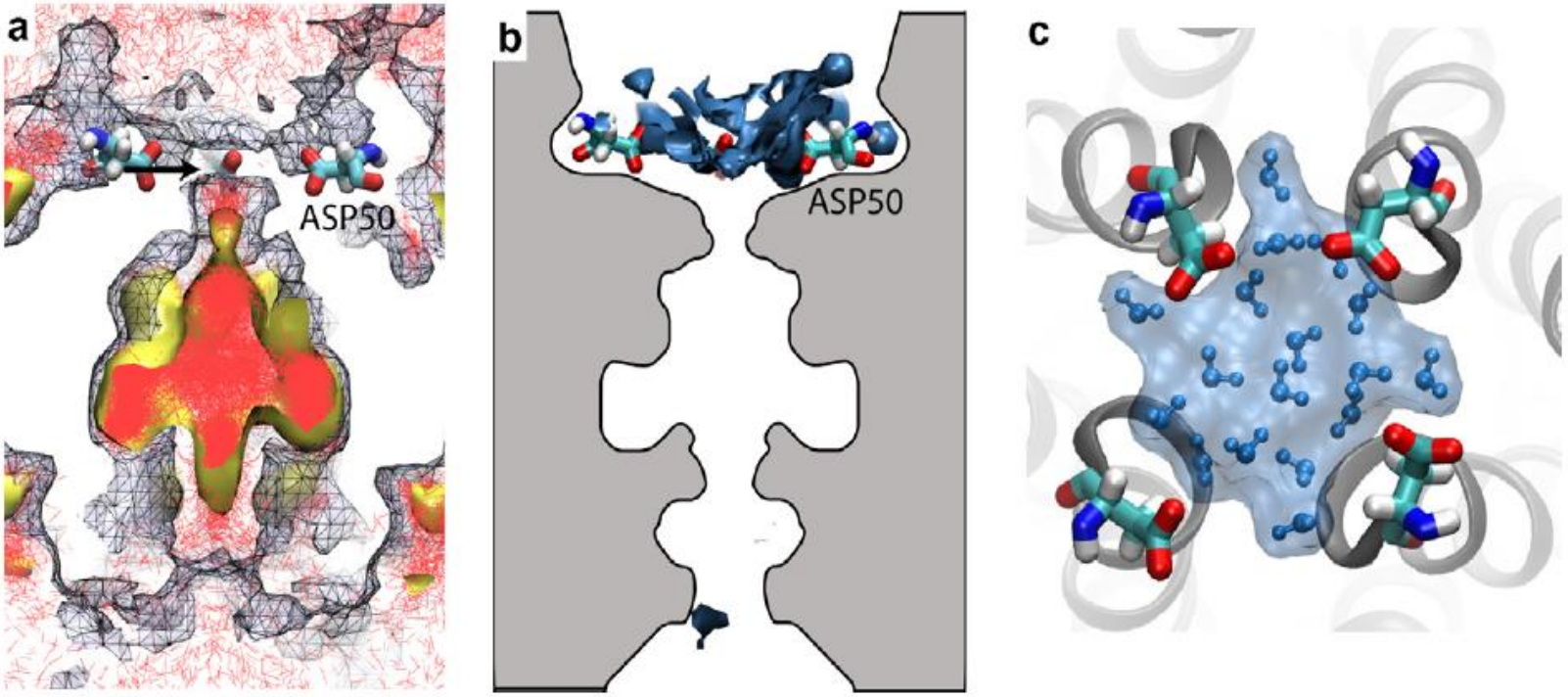
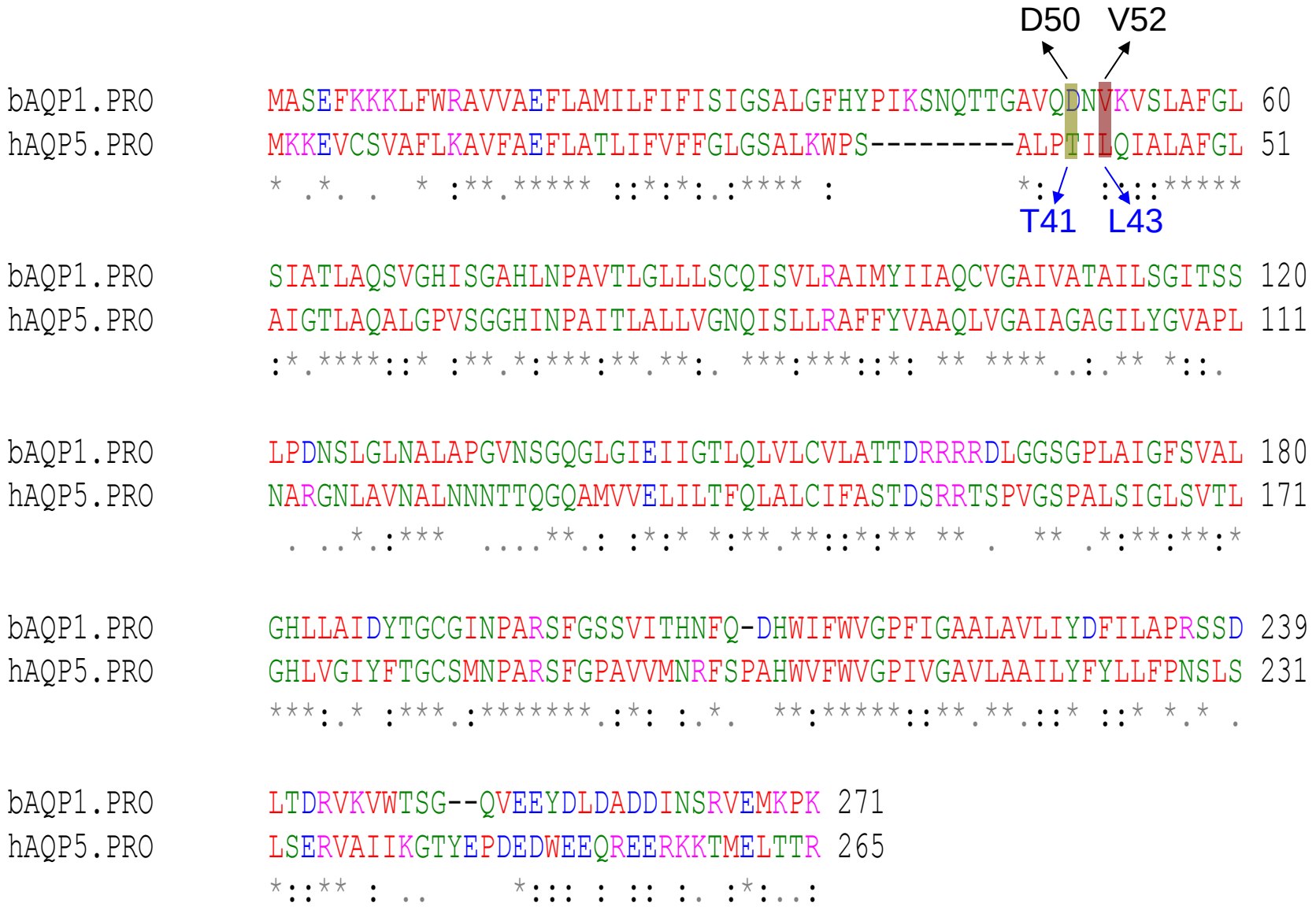
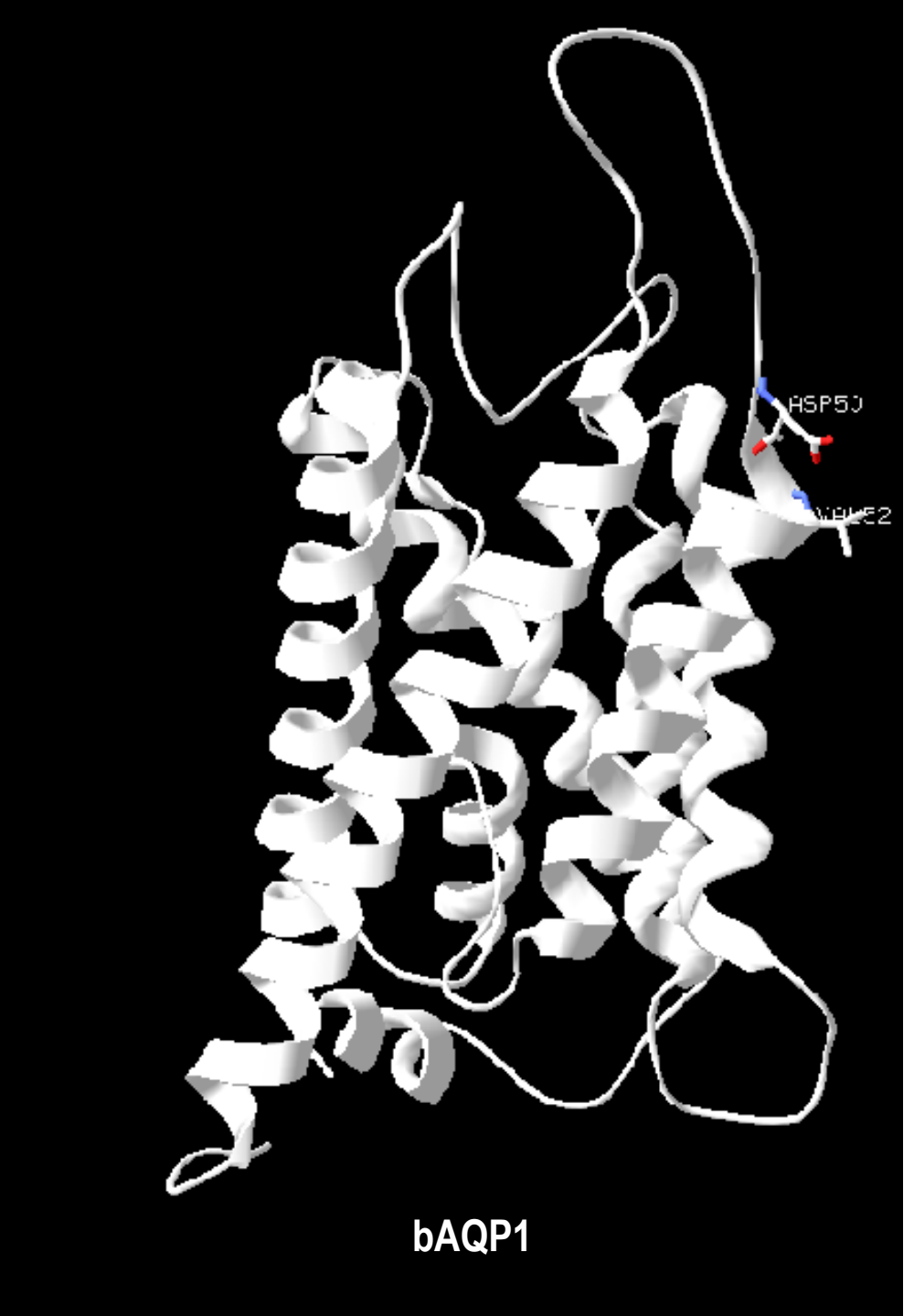


Fig. 6. (a) Close-up of the energy barrier (indicated by the black arrow) at the periplasmic mouth of the AQP1 central pore. The 0 kcal/mol (yellow) and 3 kcal/mol (black) energy isosurfaces calculated from implicit ligand sampling are shown, along with O_2 positions obtained from explicit gas diffusion simulations (red). (b) Water occupancy per $Å^3$, showing in blue the volumes occupied by water during at least 75% of the time during the 26-ns AQP1-O_2 simulation. A layer of water, denser than the bulk, is found around the Asp50 residues, corresponding to the location of the energy barrier shown in (a). Water occupancy was calculated using the volmap plugin of VMD (Humphrey et al., 1996). The profile of the central pore is shown in black lines. (c) Snapshot of water molecules around Asp50 during equilibrium simulations of AQP1-O_2. The water molecules are shown in both surface and CPK representations.

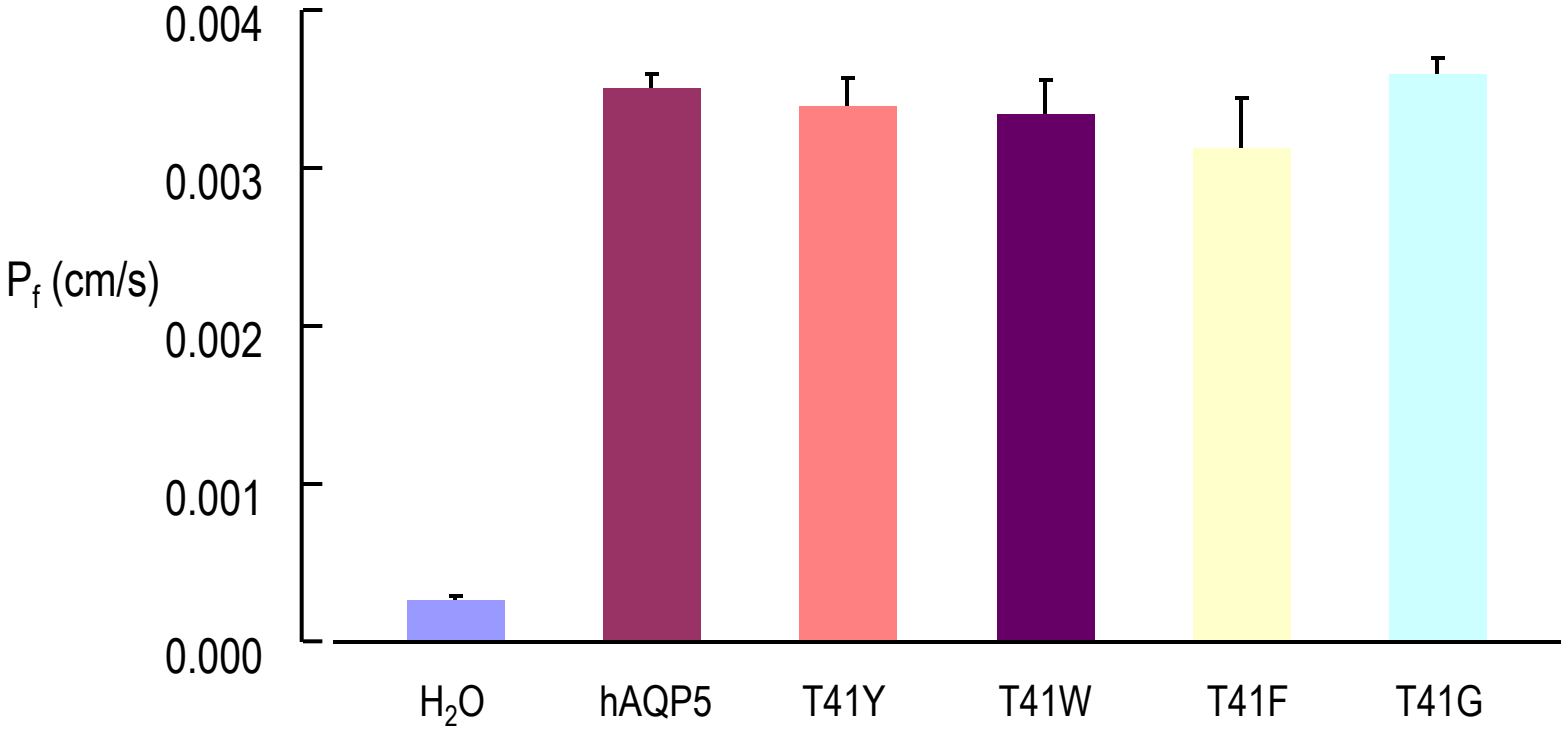
According to our PMFs, the major barrier of the central pore to gas permeation is about 3.6–4.6 kcal/mol, located at the periplasmic side of the central pore ($13 Å \leqslant z \leqslant 19 Å$). This barrier, consistently found by both our approaches, as well as by another study (Hub and de Groot, 2006), surprisingly, does not correspond to a region that is sterically blocked directly by the protein. As shown in Fig. 6a, this barrier is located above the region of maximum protein contraction where the four hydrophobic residues Val52 reside; rather, it corresponds to a region that is populated solely by water. We have created a volumetric map of the local occupancy of water, as shown in Fig. 6b. It is clear that the barrier corresponds to a dense layer of water molecules surrounded by four aspartate (Asp50) residues (Fig. 6c). With a higher density than the bulk water, this water layer reduces the chance of gas molecules to access the central pore. If these aspartate residues will be mutated to neutral residues, e.g., alanines or asparagines, the strong electrostatic effects of the quadruplets may be eliminated and a less dense water structure could be expected, which might result in a better gas-conductive central pore of AQP1. Simulations of these mutants are currently underway.

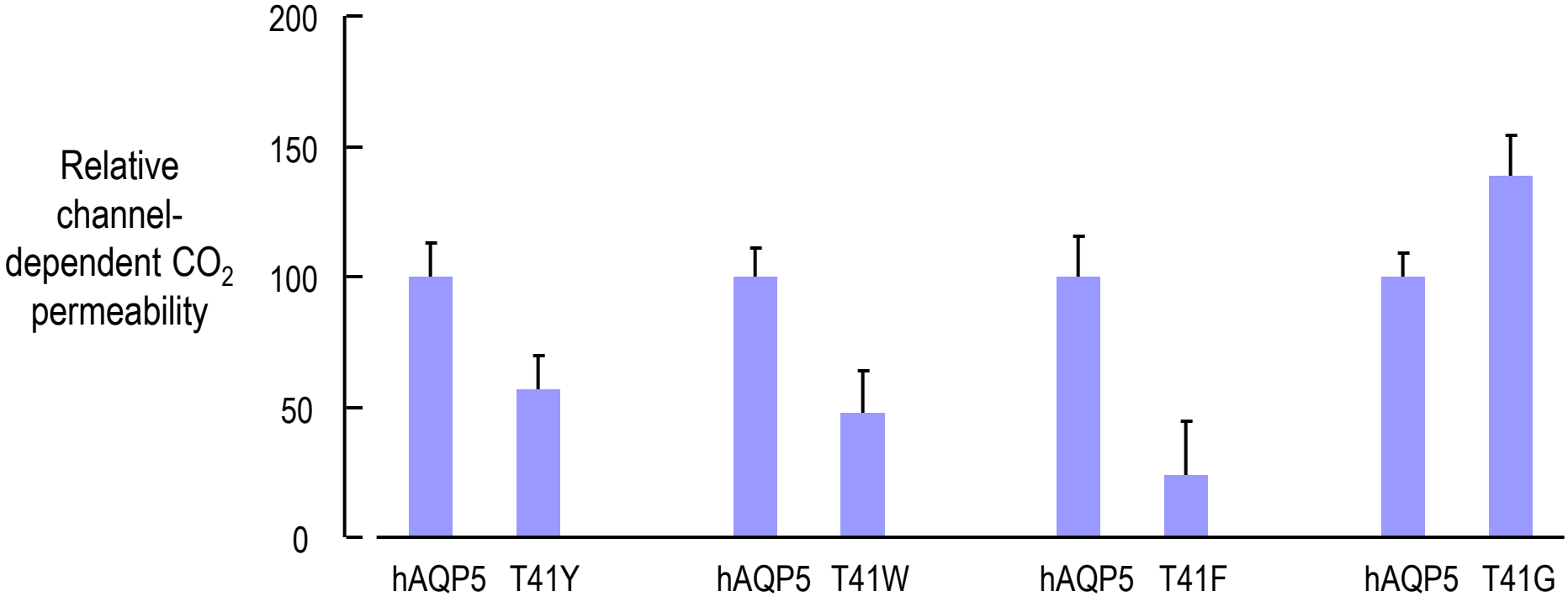
Protein sequence alignment

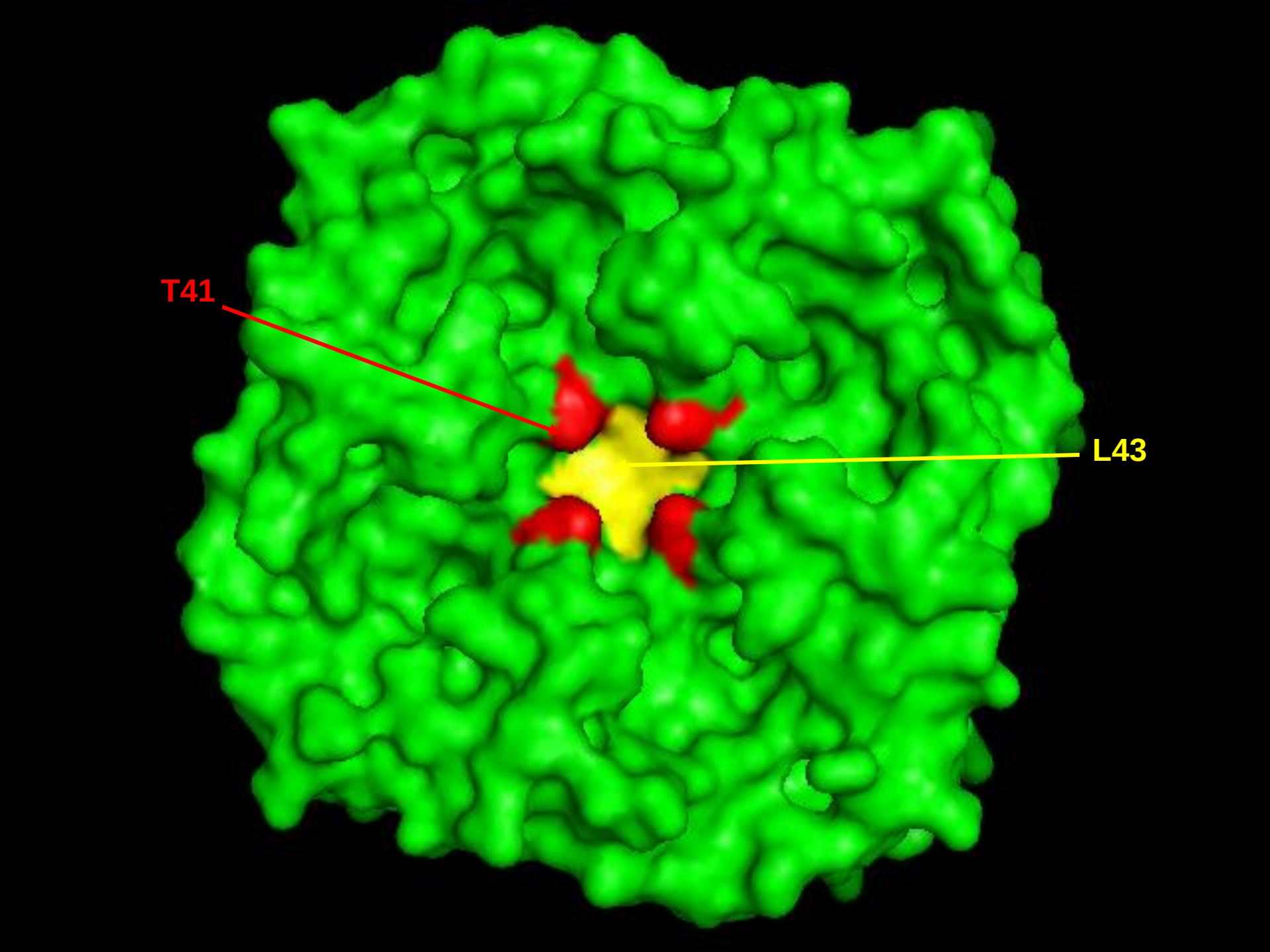


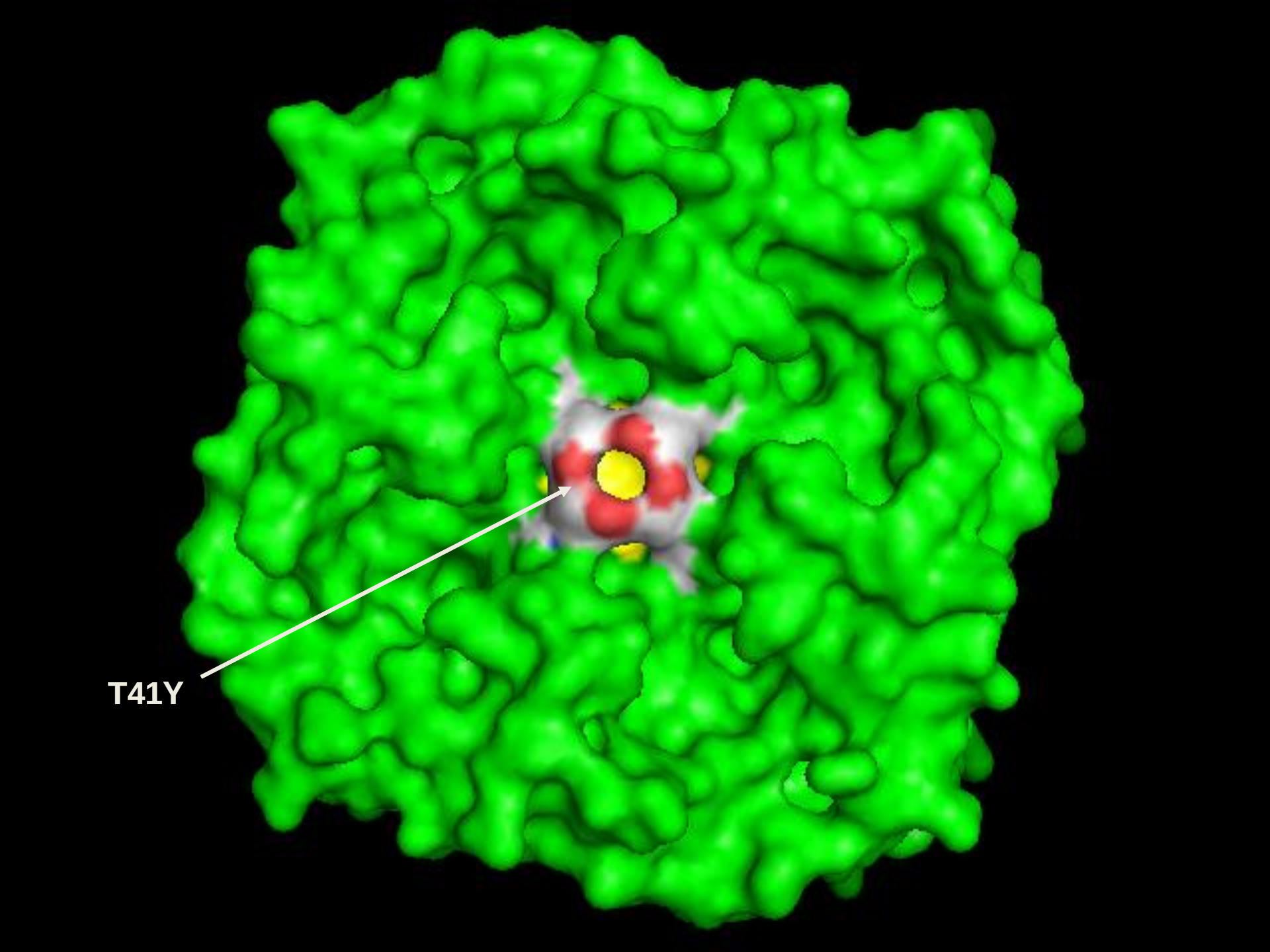

bAQP1

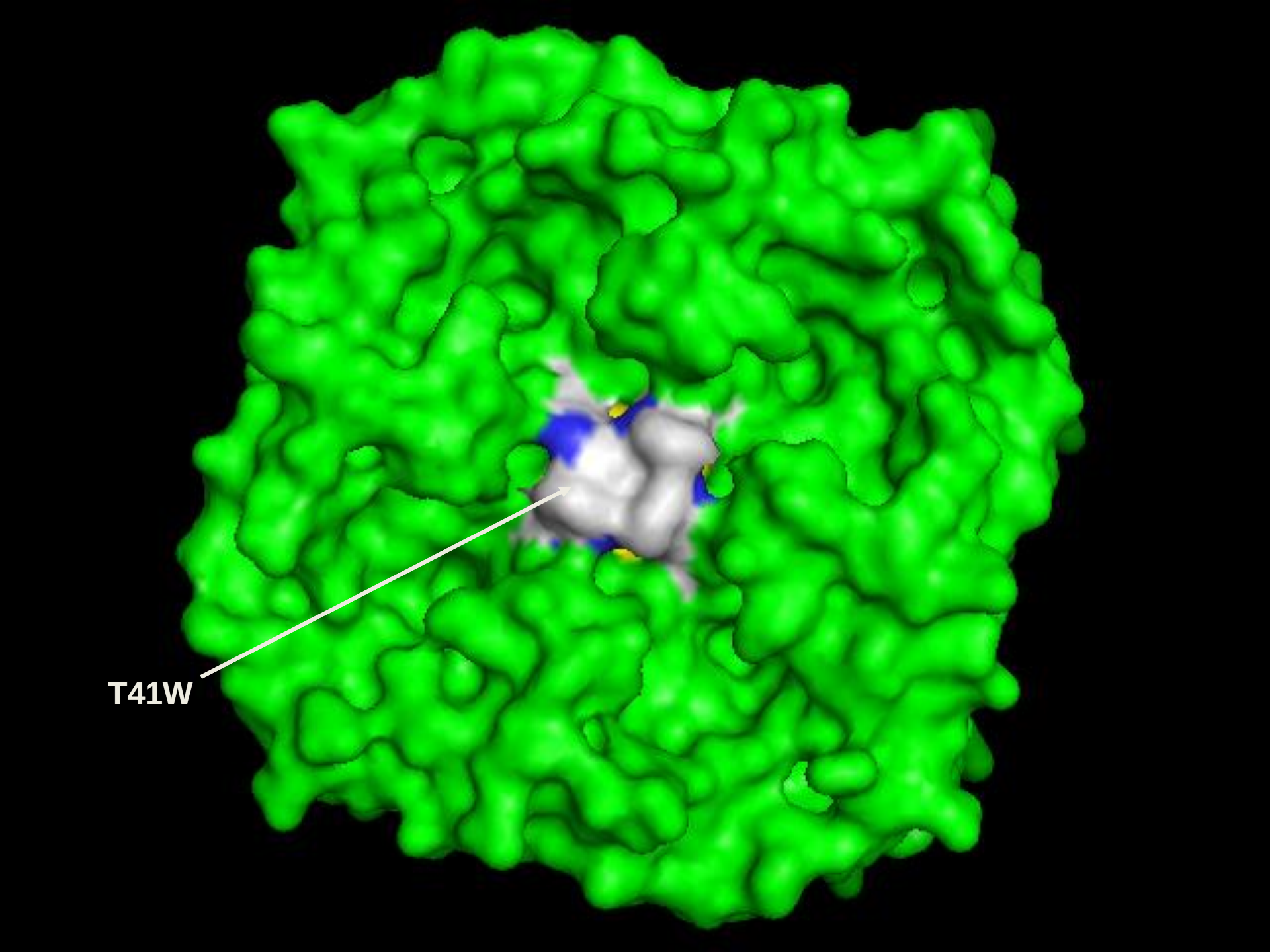

hAQP5

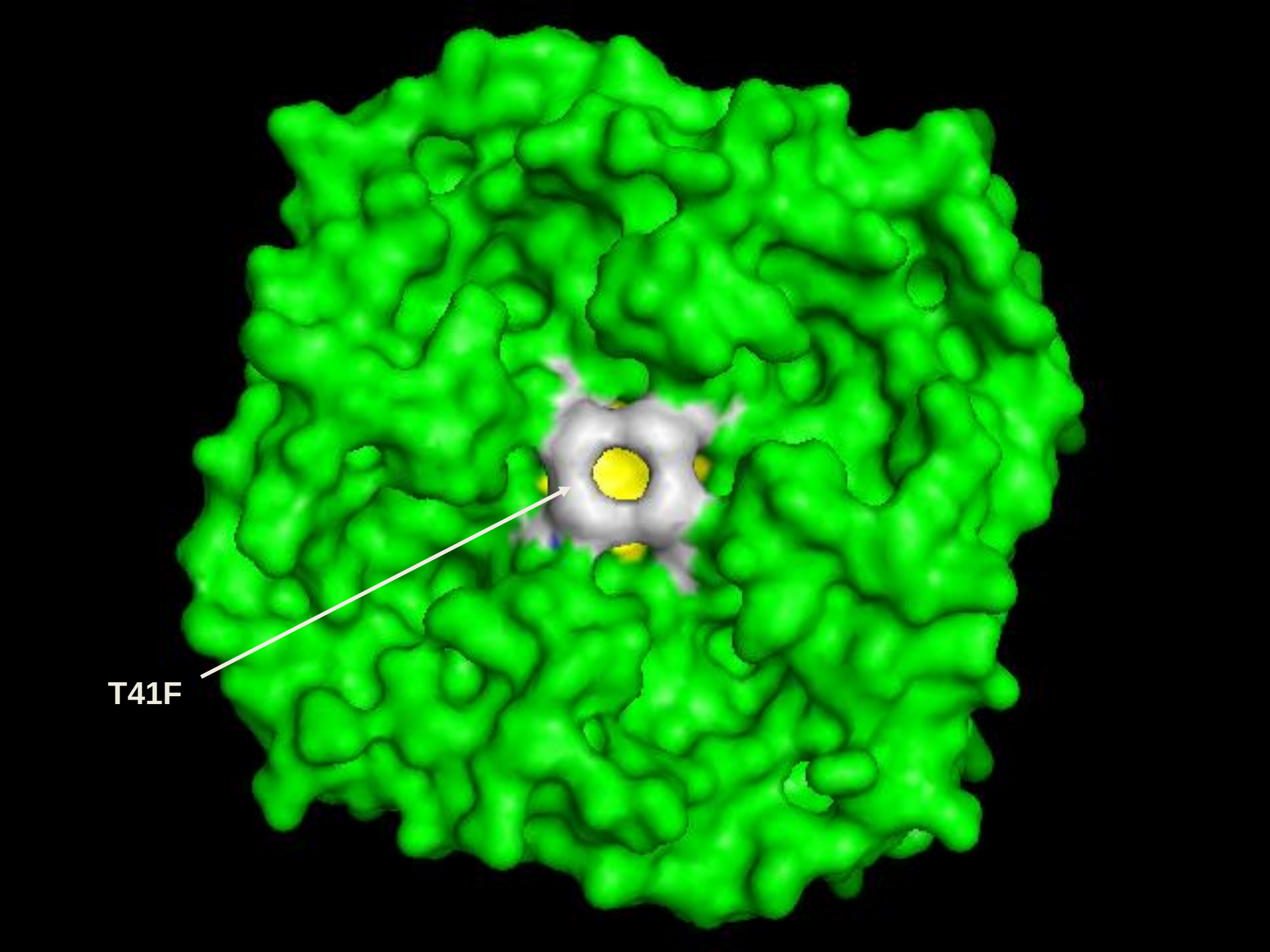

0.004
0.003
0.002
0.001
0.000
P_f (cm/s)
H_2O
hAQP5
T41Y
T41W
T41F
T41G

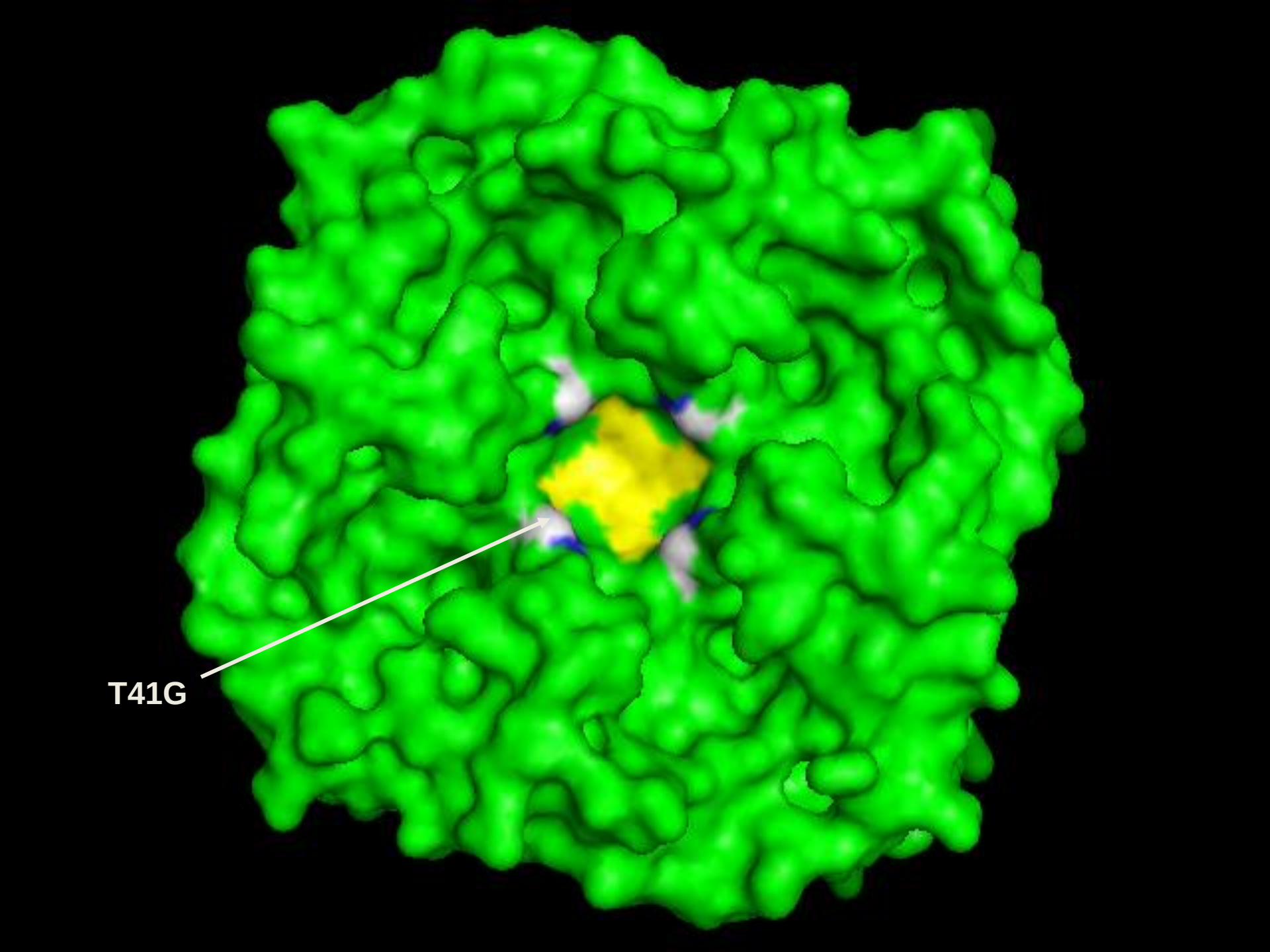

Relative channel-dependent CO_2 permeability
200
150
100
50
0
hAQP5 T41Y
hAQP5 T41W
hAQP5 T41F
hAQP5 T41G


T41
L43


T41Y


T41W


T41F


T41G

Conclusion I

I Amino acids at the mouth of the central pore

Changes of ΔpH_S (CO_2 permeability) is more sensitive than P_f (H_2O permeability)

T41 is more important than L43.

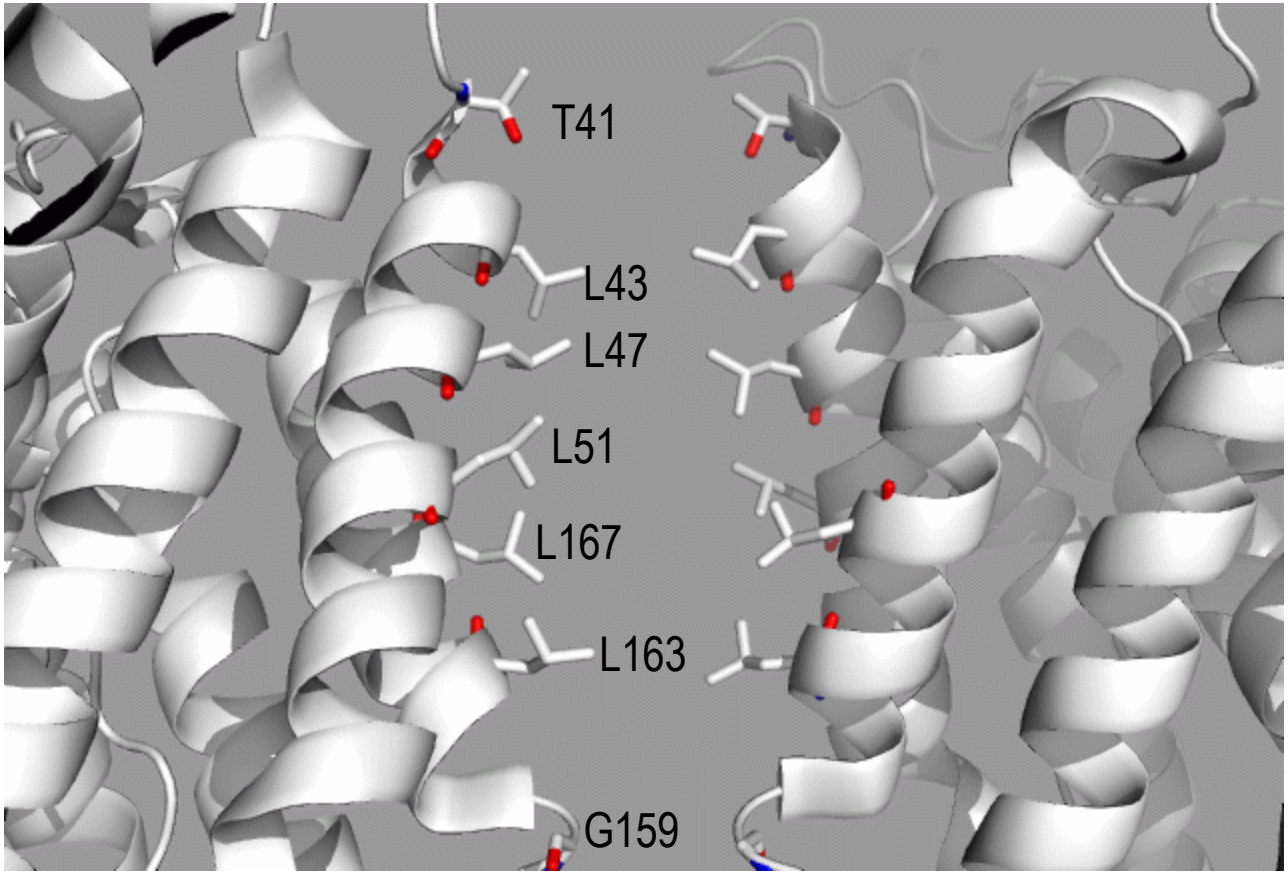
II Amino acids lining the central pore

Outline

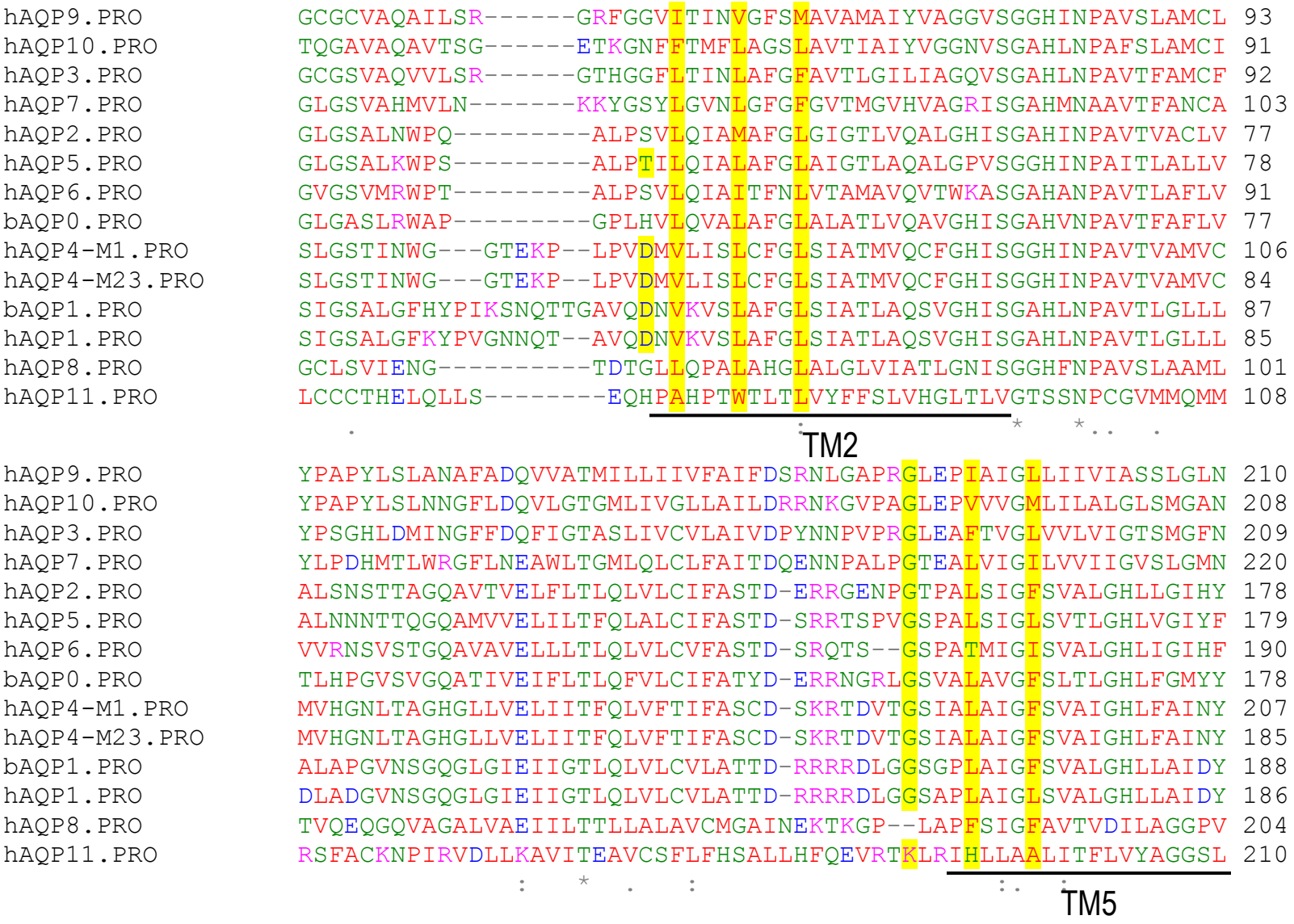
I Amino acids at the mouth of the central pore

II Amino acids lining the central pore

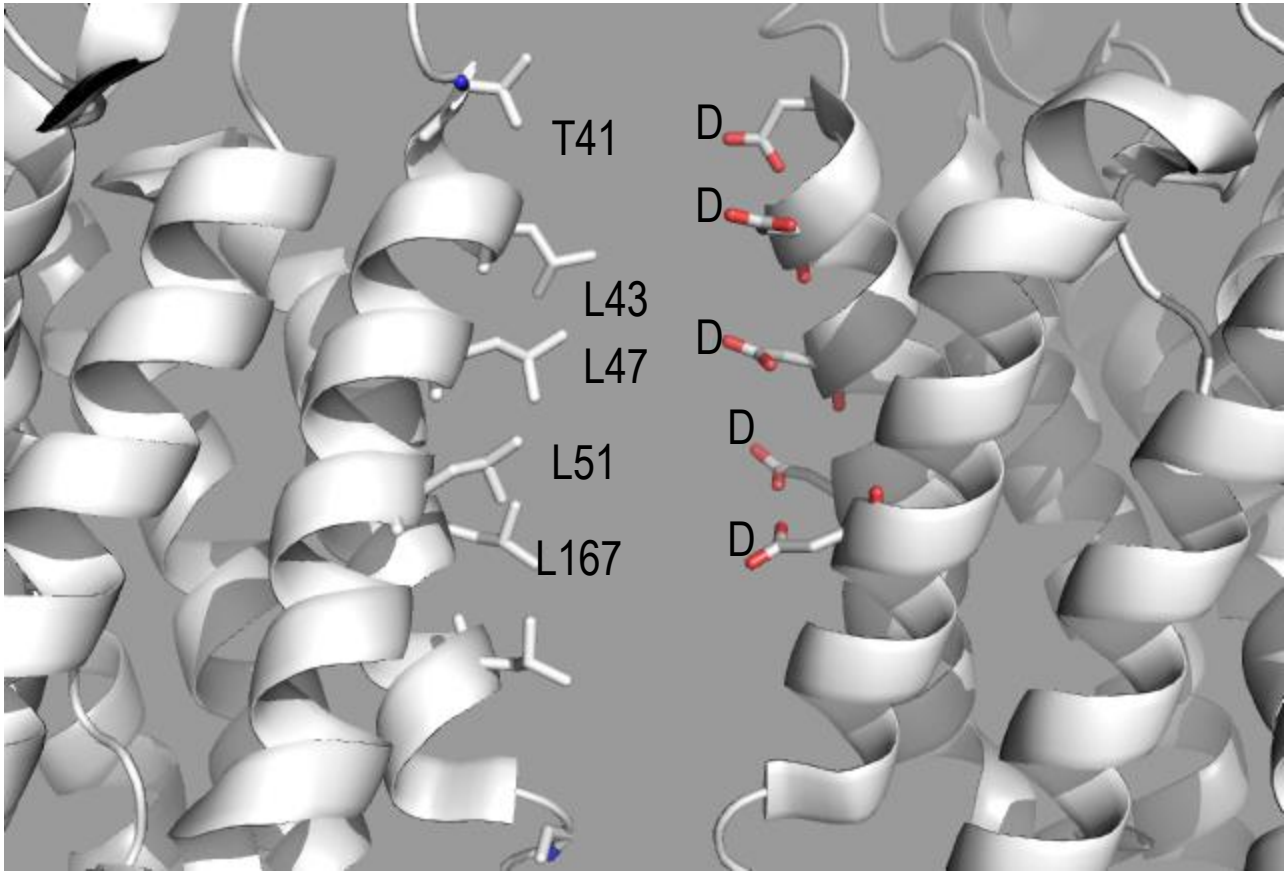
Amino acids lining the central pore



Sequence alignment of AQPs

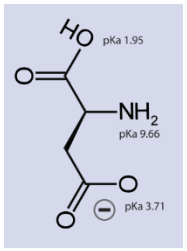


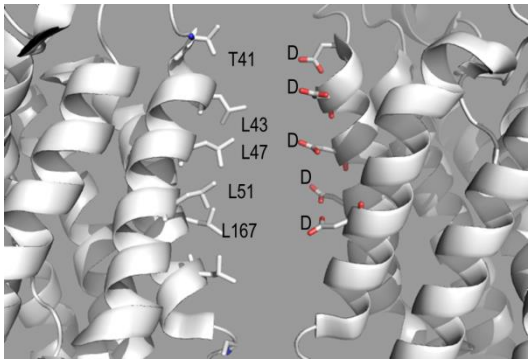
If mutate to Asp (D)



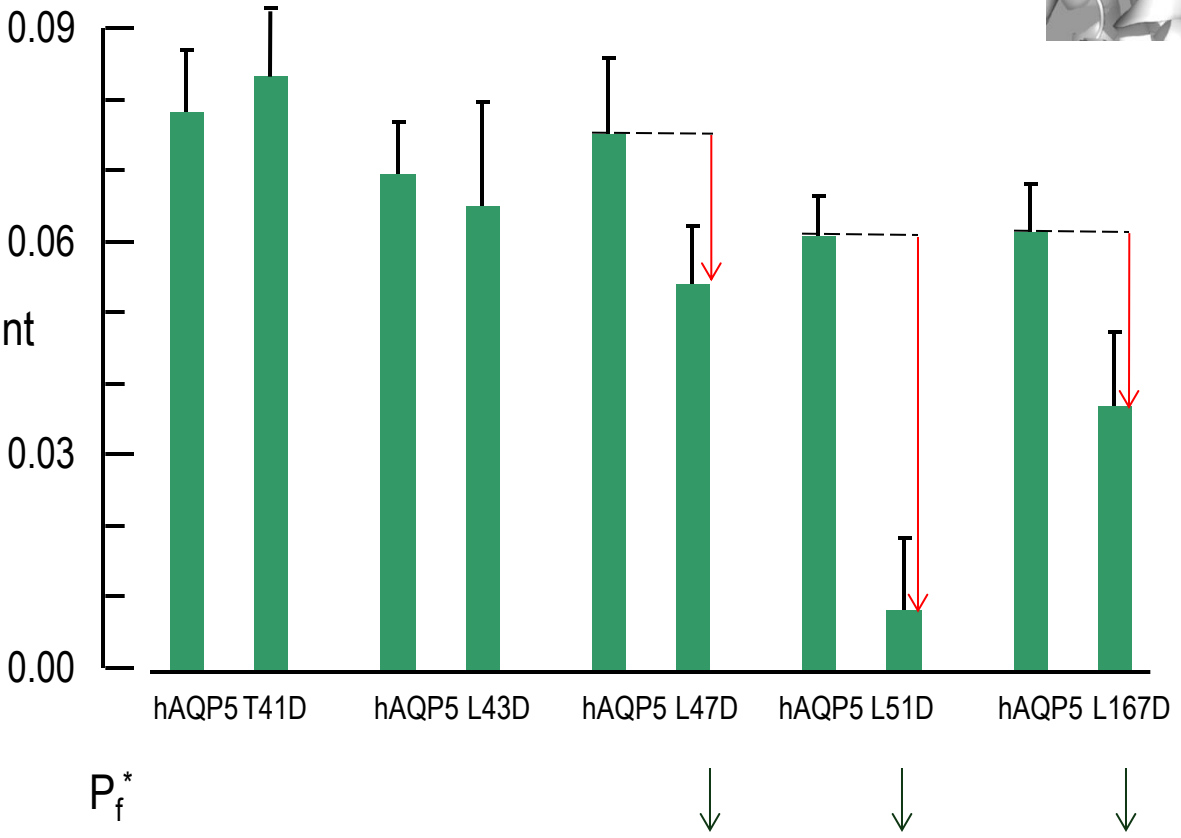
LXXD – ΔpH_S*

Aspartic Acid (Asp)



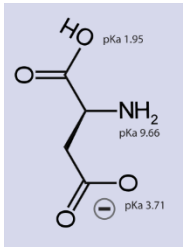


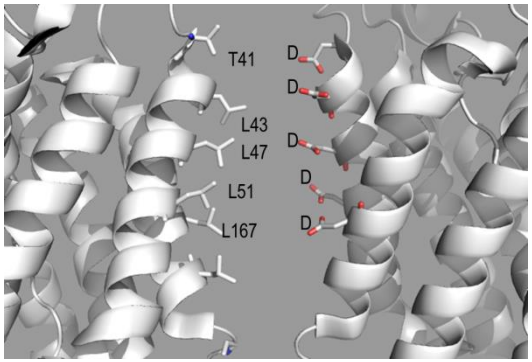
Channel-dependent ΔpH_S for CO_2

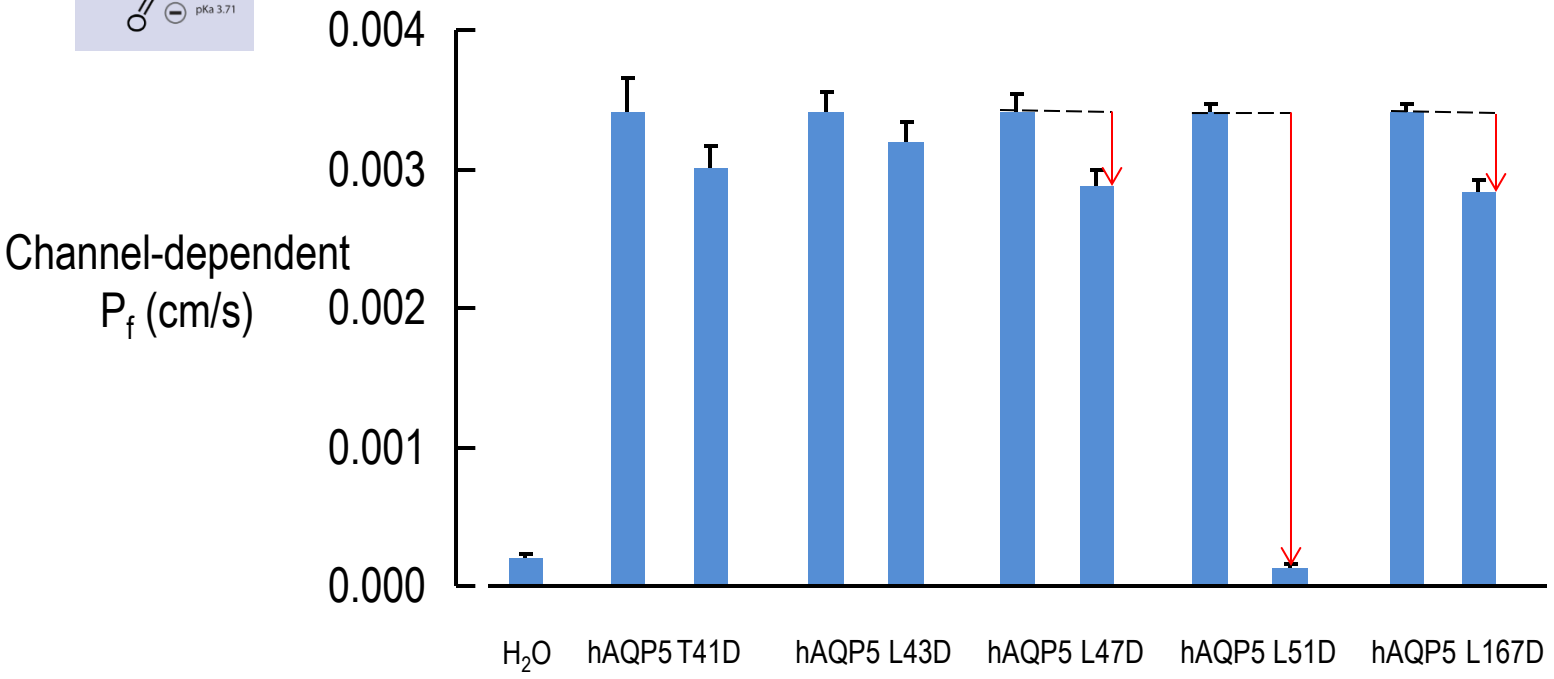


LXXD – P_f^*

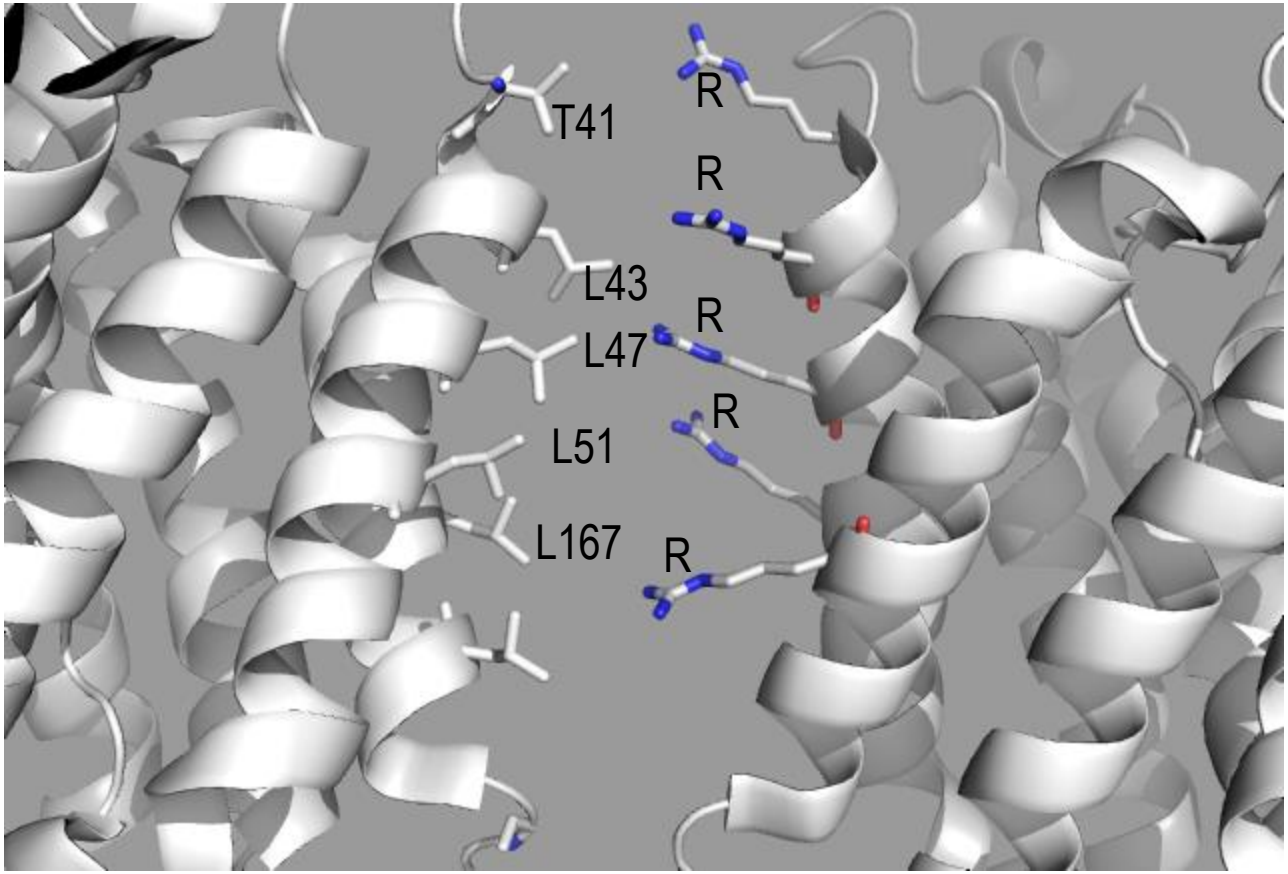
Aspartic Acid (Asp)





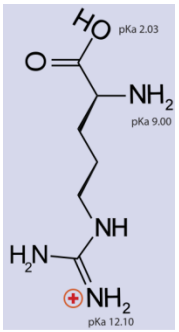


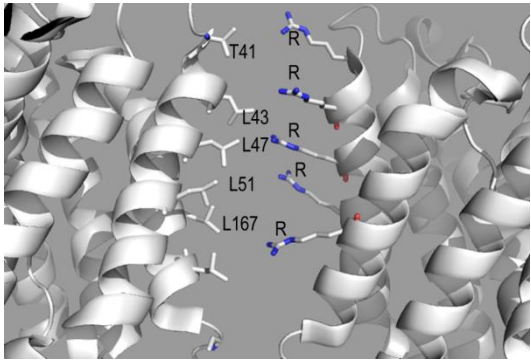
If mutate to Arg (R)



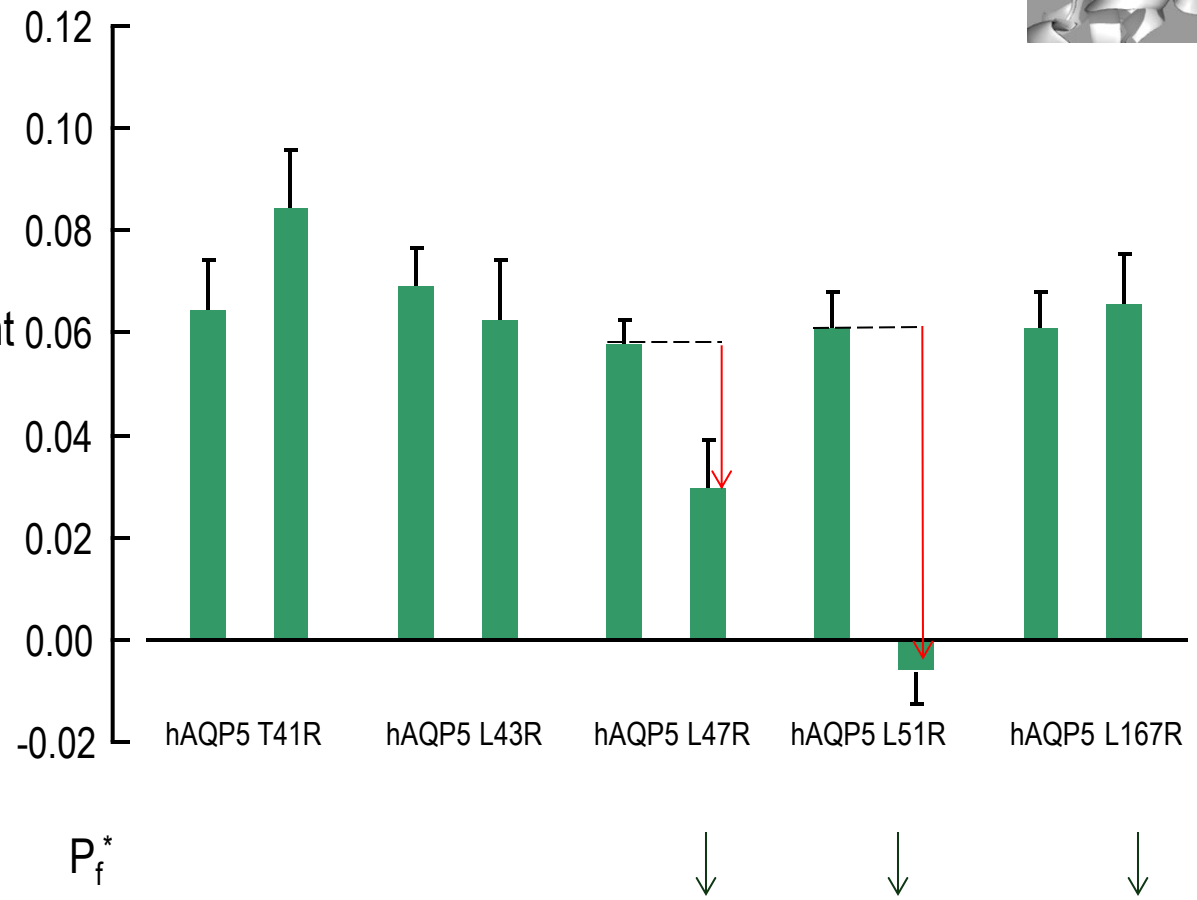
LXXR – ΔpH_S^*

Arginine (Arg)



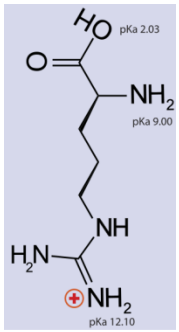


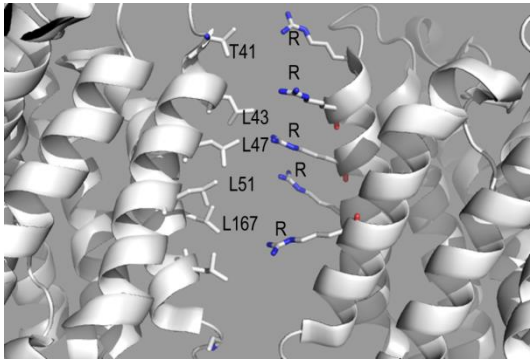
Channel-dependent ΔpH_S for CO_2

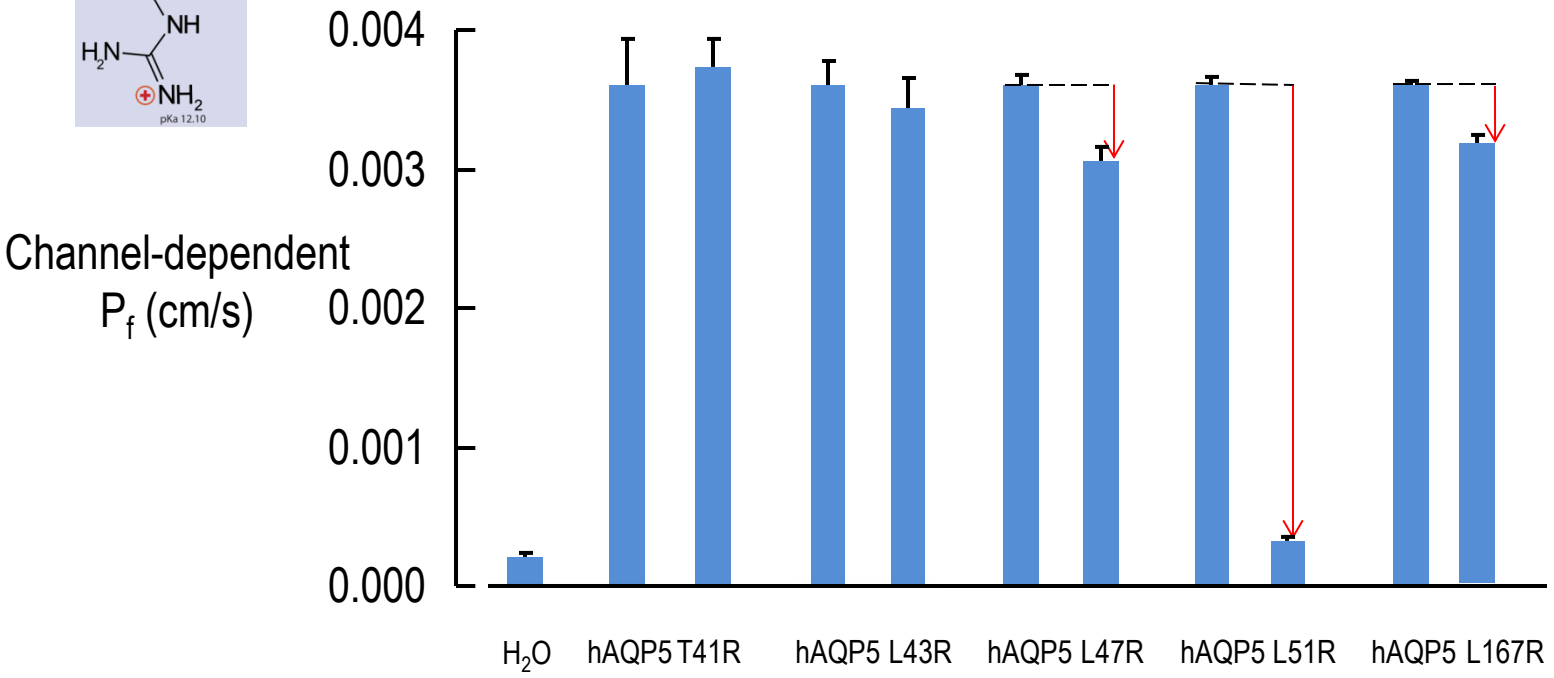


LXXR – P_f^*

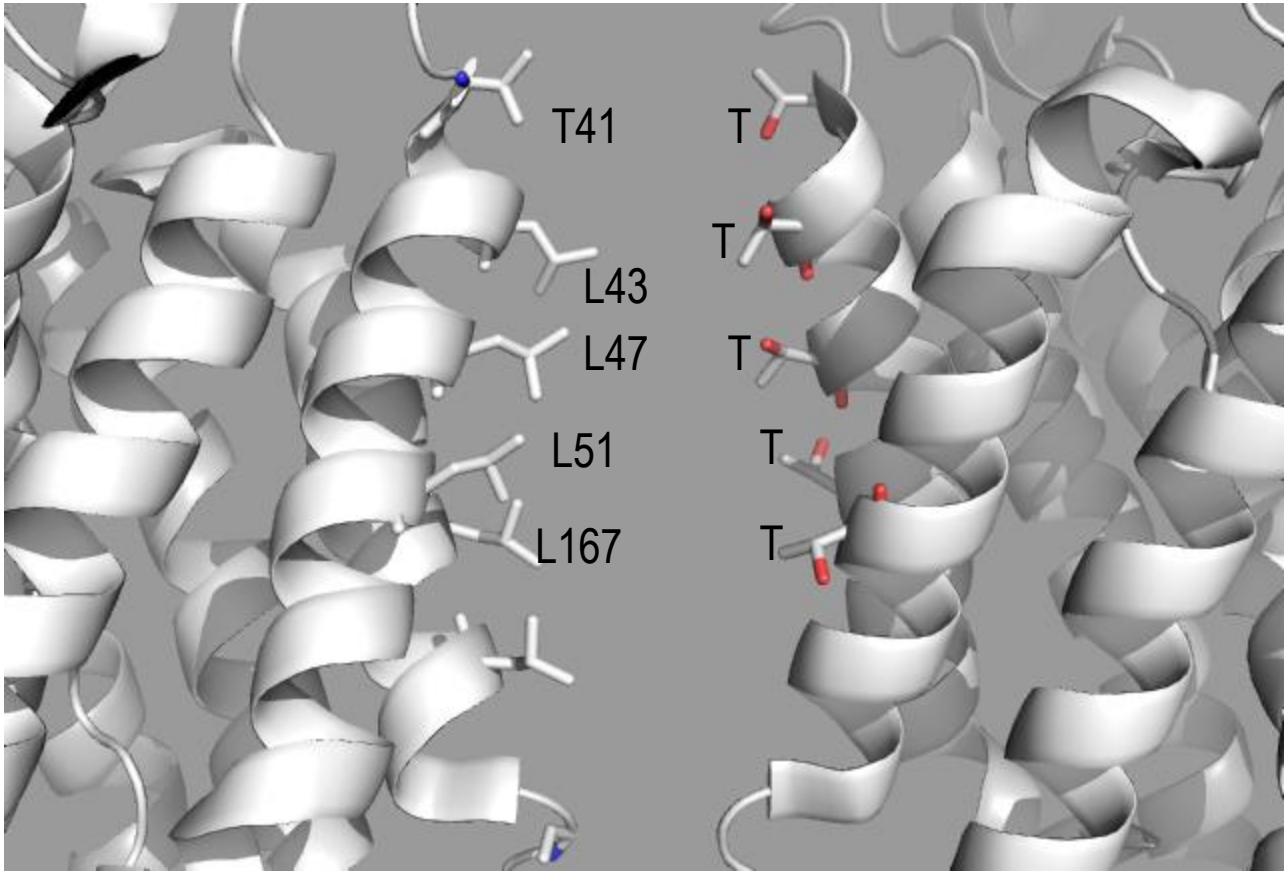
Arginine (Arg)





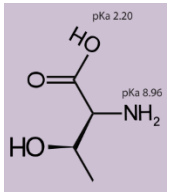


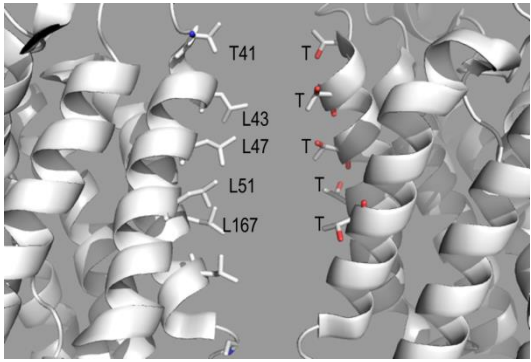
If mutate to Thr (T)



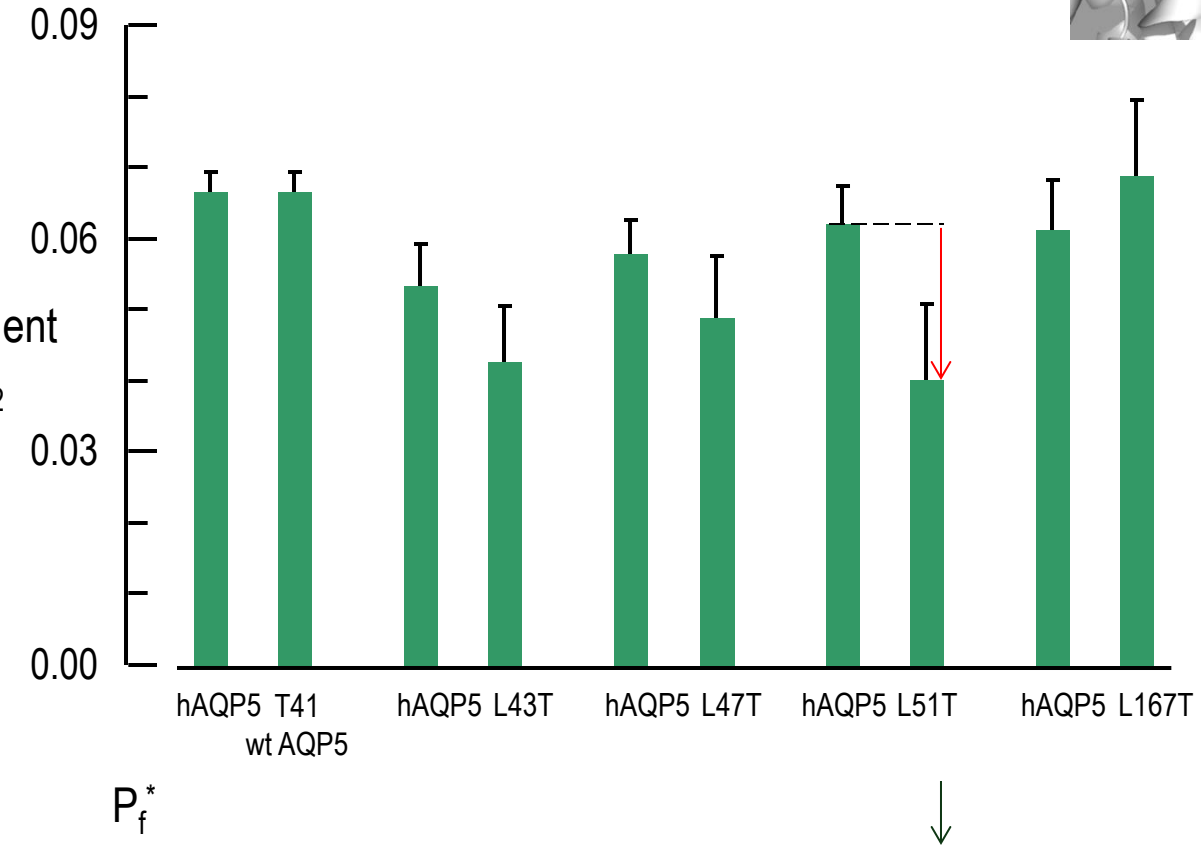
LXXT – ΔpH_S^*

Threonine (Thr)



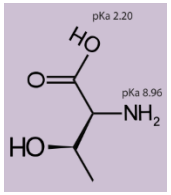


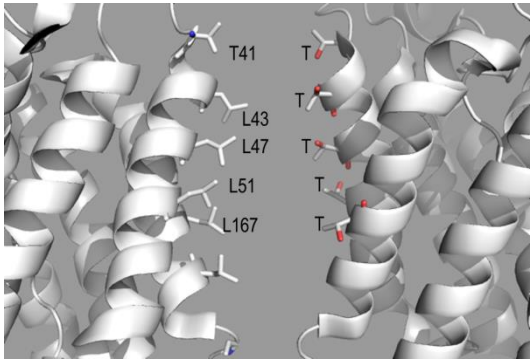
Channel-dependent ΔpH_S for CO_2

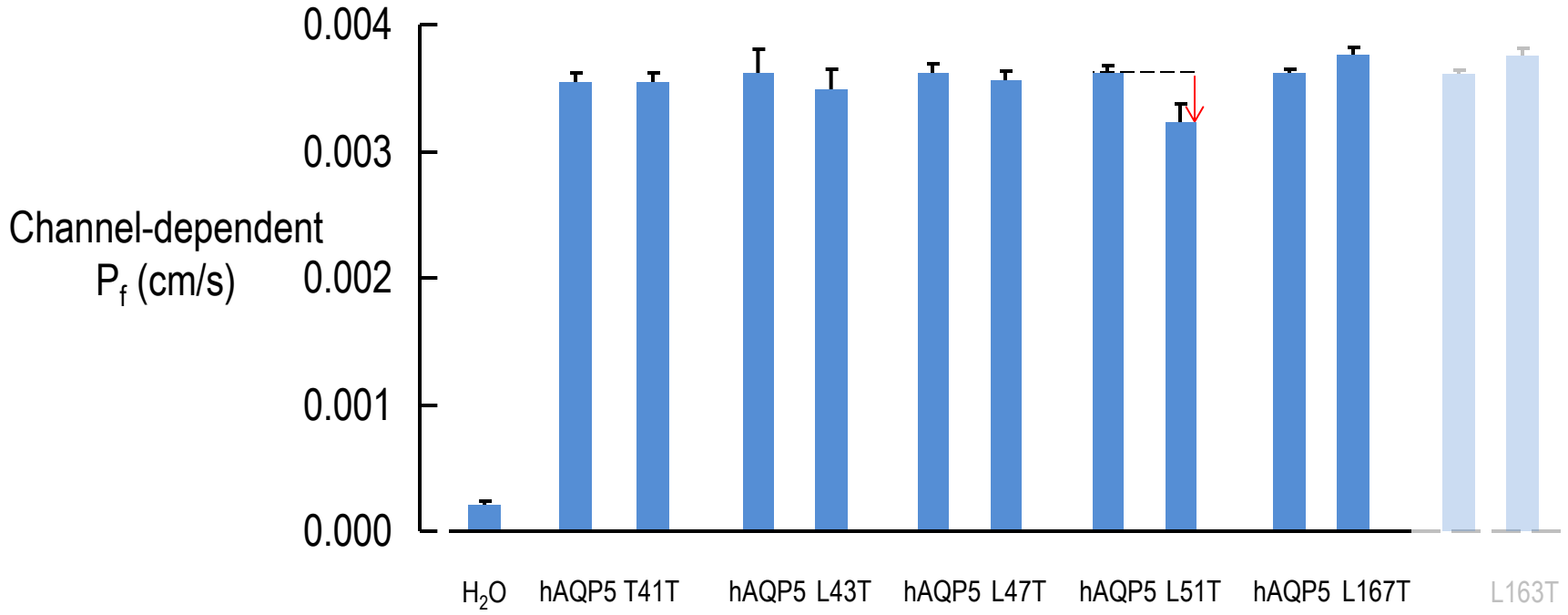


LXXT – P_f^*

Threonine (Thr)







Conclusion II

I Amino acids at the mouth of the central pore

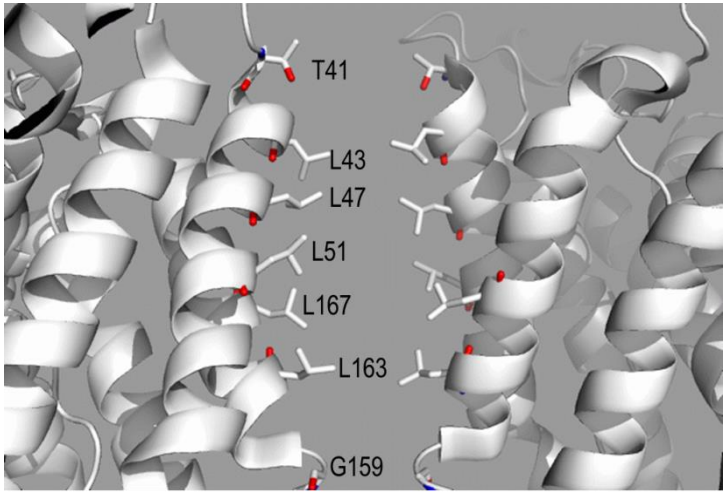
Changes of ΔpH_S (CO_2 permeability) is more sensitive than P_f (H_2O permeability)

T41 is more important than L43.

II Amino acids lining the central pore

Changes of P_f (H_2O permeability) is more sensitive than ΔpH_S (CO_2 permeability)

Of all the amino acids lining the central pore, L51 is most sensitive to determine P_f and ΔpH_S.



Acknowledgement

Collaborator
Emad Tajkhorshid

Lab
Walter F Boron
Raif Musa-Aziz
Mark D Parker





American Heart Association®
Learn and Live

Gas Channels Workshop

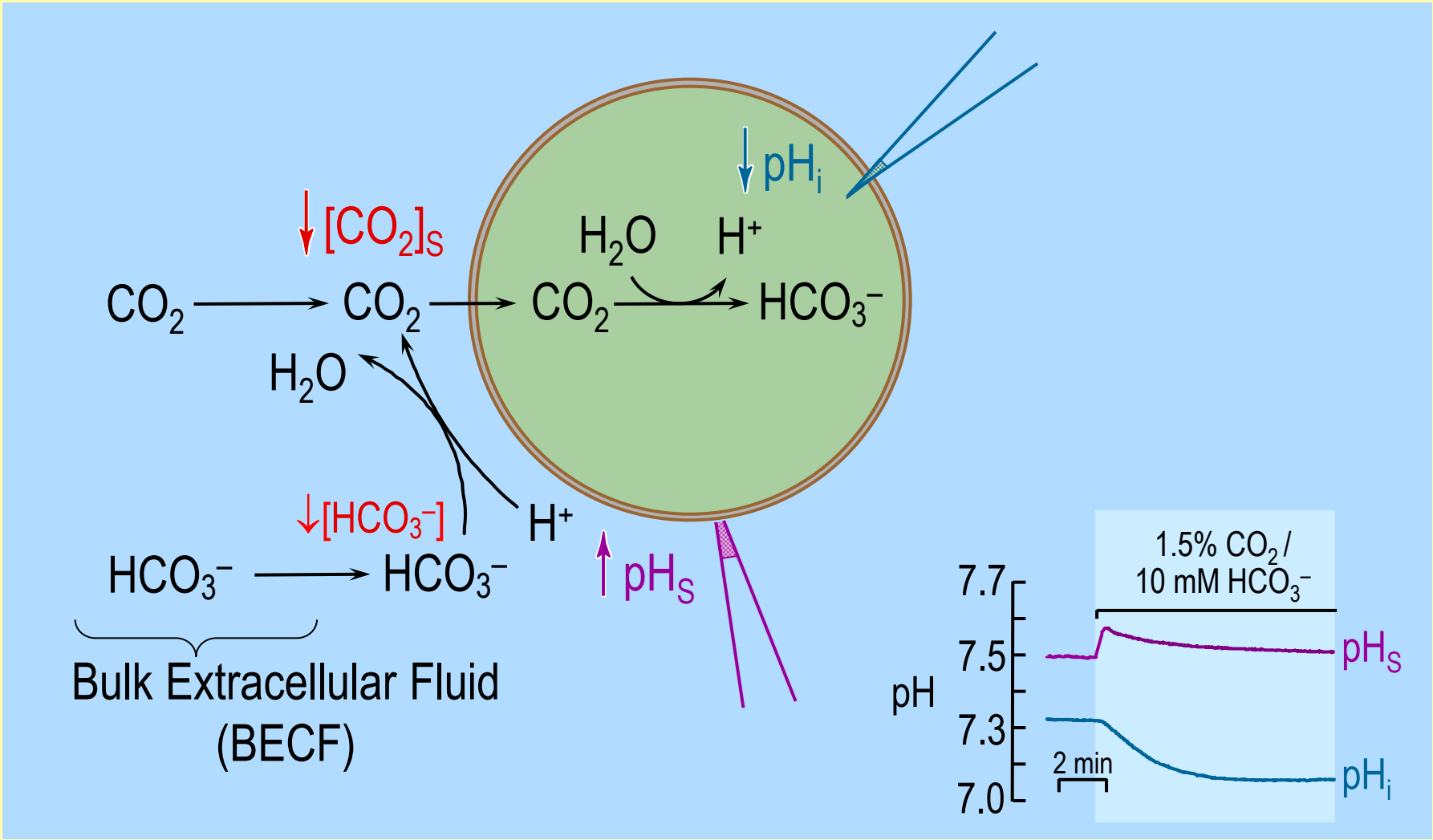
September 7, 2012
Cleveland, Ohio

Mathematical Modeling of Gas Movements in an Oocyte

Rossana Occhipinti, Ph.D.

Department of Physiology & Biophysics
Case Western Reserve University School of Medicine
10900 Euclid Avenue
Cleveland, OH 44106-4906

Xenopus oocyte: pH Changes Caused by CO_2 Influx

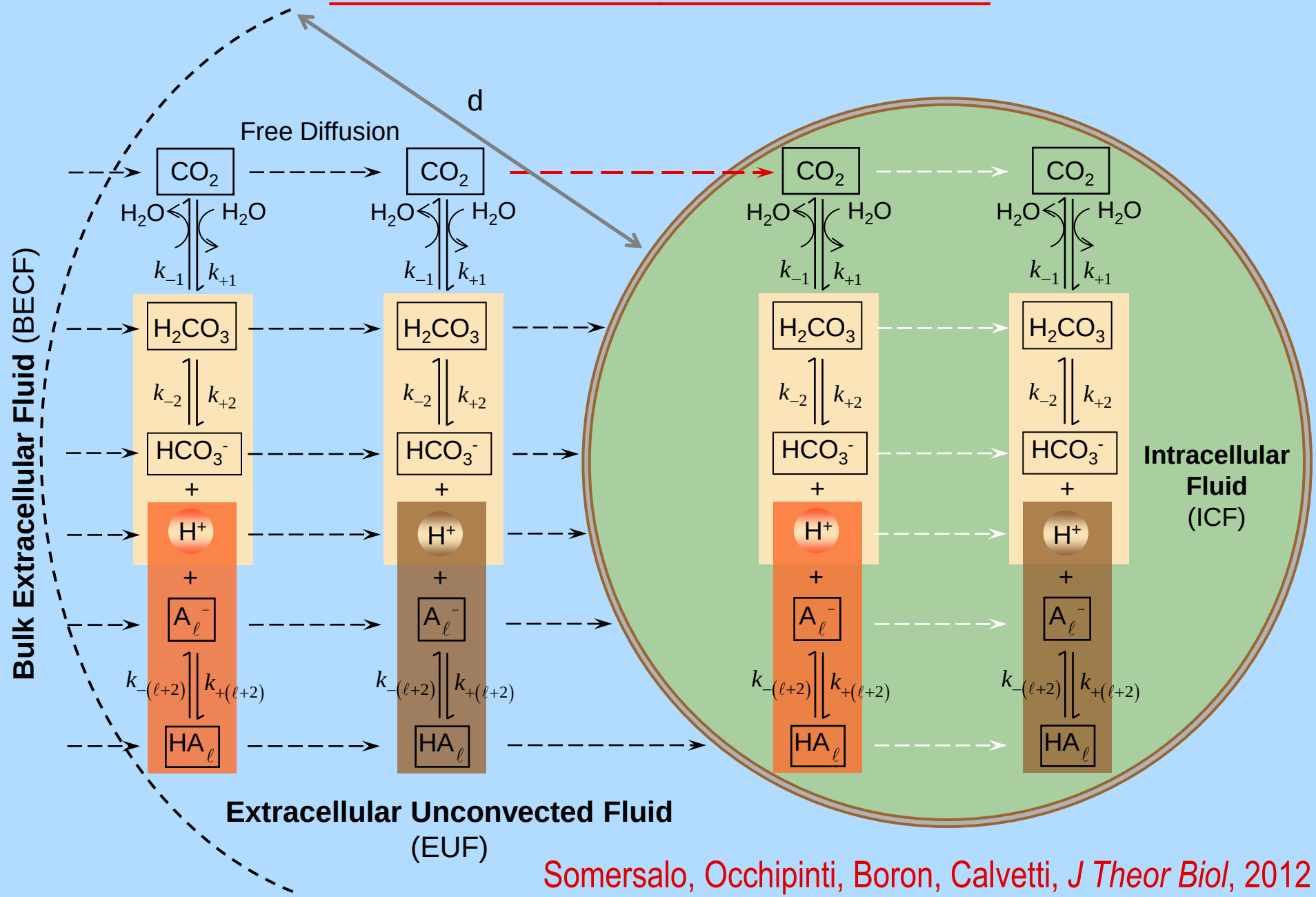


(data kindly provided by Dr. Musa-Aziz)

An appropriate mathematical model should include…

- A spherical cell
- Transport of CO_2 across the plasma membrane
- Reactions of a multitude of extra- and intracellular buffers
- Diffusion of solutes through the extra- and intracellular spaces
- Temporal and spatial variations of solute concentrations
- Carbonic anhydrase (CA) activity at specific loci

The Mathematical Model



Somersalo, Occhipinti, Boron, Calvetti, *J Theor Biol*, 2012

The Key Components of the Model

Bulk extracellular fluid (BECF)

Infinite reservoir where convection could occur but not reaction or diffusion

Extracellular unconvected fluid (EUF)

Thin layer adjacent to the surface of the oocyte where no convection occurs, but reactions and diffusion do occur

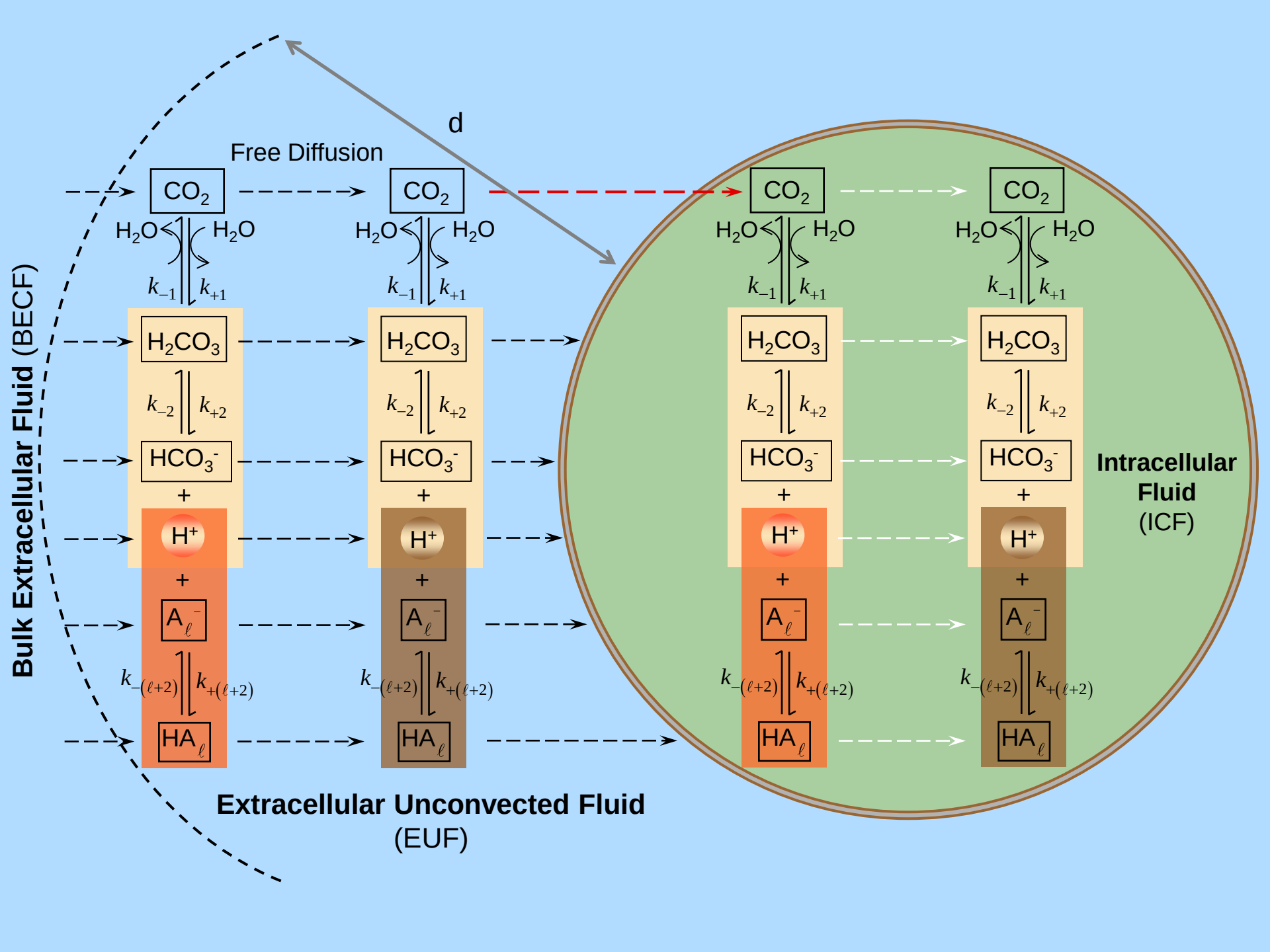
Plasma membrane

Infinitely thin and permeable only to CO_2

In both EUF and intracellular fluid (ICF)

Slow equilibration of the CO_2 hydration/dehydration reactions

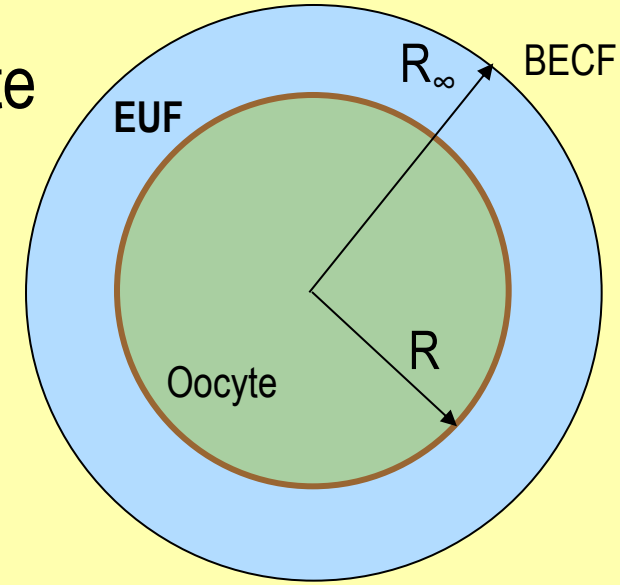
Competing equilibria among the CO_2/HCO_3^- and a multitude of non-CO_2/HCO_3^- buffers


d
Free Diffusion
CO2
H2O
H2O
k−1
k+1
H2CO3
k−2
k+2
HCO3-
+
H+
+
A−ℓ
k−(ℓ+2)
k+(ℓ+2)
HAℓ
Bulk Extracellular Fluid (BECF)
Extracellular Unconvected Fluid
(EUF)
Intracellular
Fluid
(ICF)

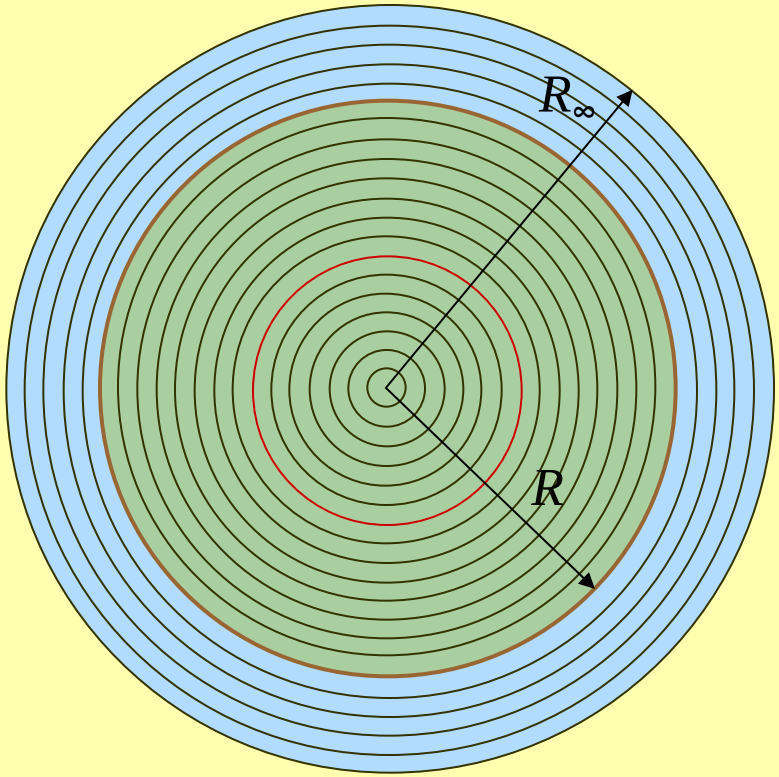
Assuming *spherical symmetry*, we write a reaction-diffusion equation for each species *j*,

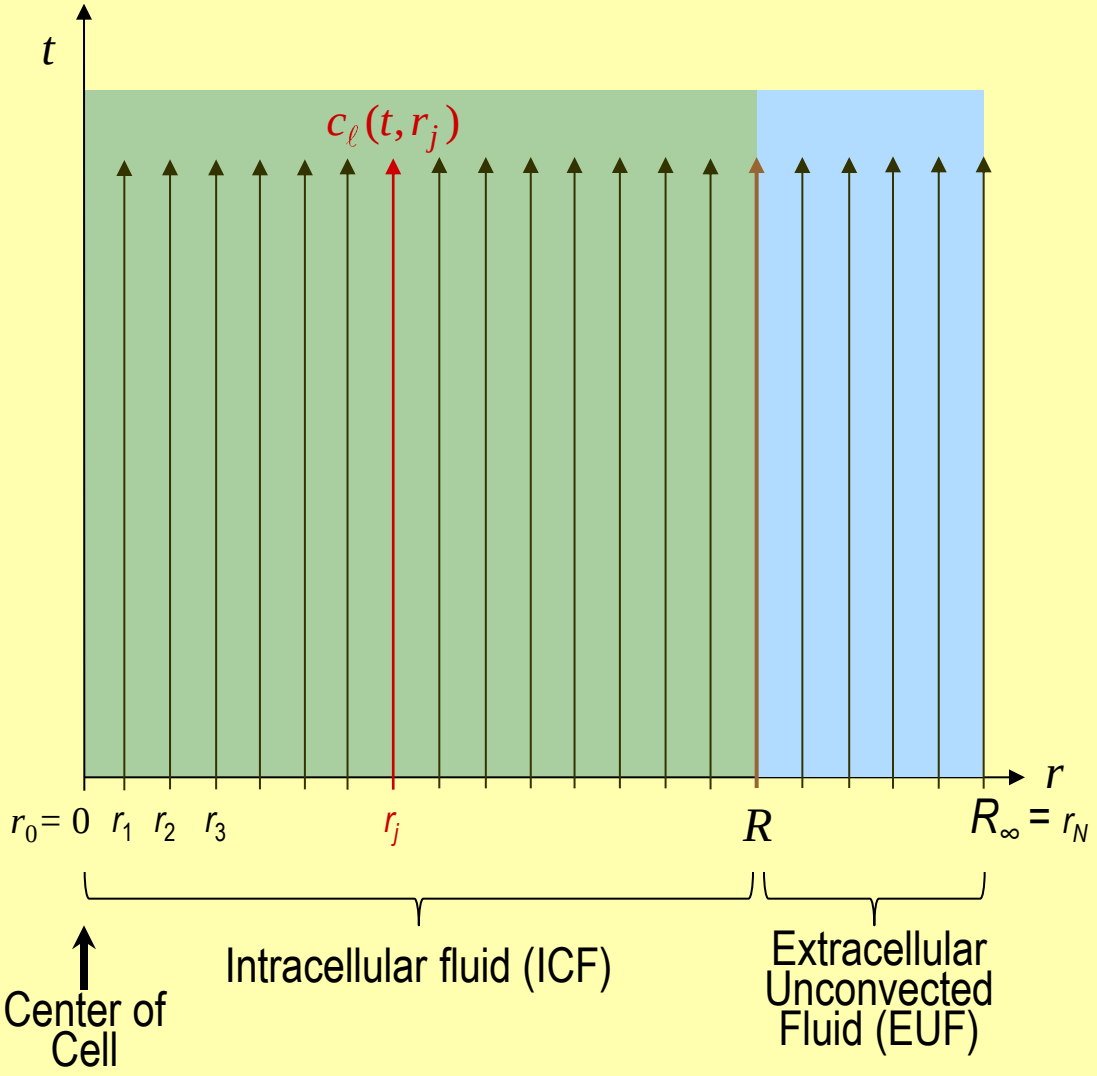
$$\frac{\partial}{\partial t}C_j(r,t) = \underbrace{\frac{1}{r^2}\frac{\partial}{\partial r}\left(D_j r^2 \frac{\partial}{\partial r}C_j(r,t)\right)}_{\text{Diffusion term (Fick's second law)}} + \underbrace{\sum_{\ell=-L-1}^{L+1} S_{j,\ell}\Phi_\ell(r,t)}_{\text{Reaction term (law of mass action)}}, \qquad 0 \le r \le R \le R_\infty,$$

with *r* distance from the center of the oocyte



Method of Lines





Somersalo, Occhipinti, Boron, Calvetti, *J Theor Biol*, 2012

Numerical Experiments

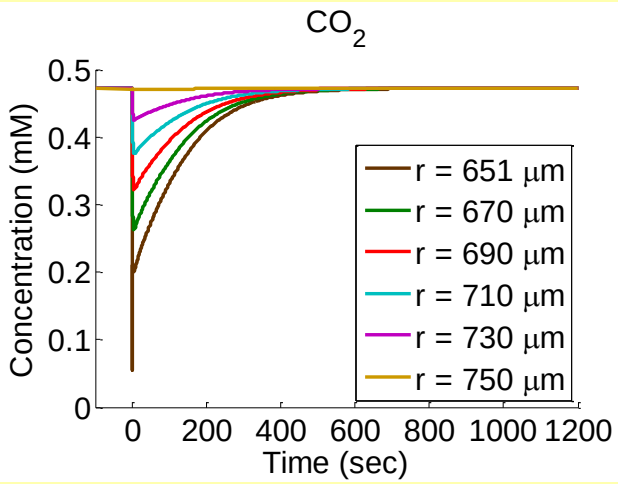
Assumptions

- The BECF, EUF, ICF and plasma membrane have same properties as water
- The EUF has thickness d = 100 μm
- Small CA-like activity uniformly distributed inside the oocyte and on the surface of the plasma membrane
- The BECF and EUF
  - contain 1.5% CO_2/9.9 mM HCO_3^- / pH 7.50
  - have a single *mobile* non-CO_2/HCO_3^- buffer with pK = 7.5 (e.g., HEPES) and [TA] = 5mM
- The ICF
  - has initial pH_i = 7.20
  - $[CO_2] = [H_2CO_3] = [HCO_3^-] = 0$ mM
  - has a single *mobile* non-CO_2/HCO_3^- buffer with pK = 7.10 and [TA] ≈ 27.31mM

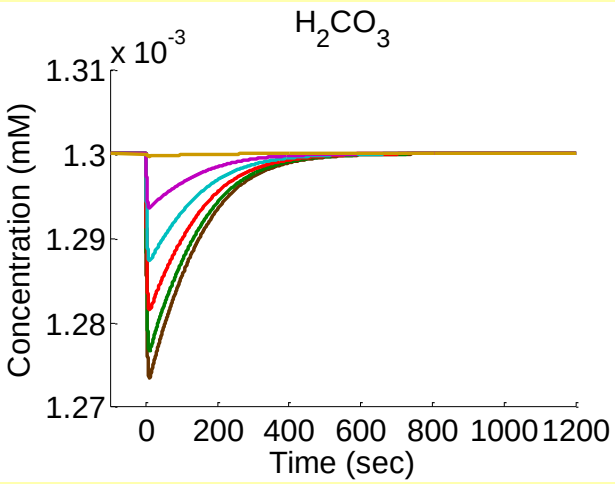
Results

Extracellular concentration-time profiles for solutes $CO_2 + H_2O \; f \;\; H_2CO_3 f \;\; HCO_3^- + H^+$ $HA_1 \; f \;\; A_1^- + H^+$

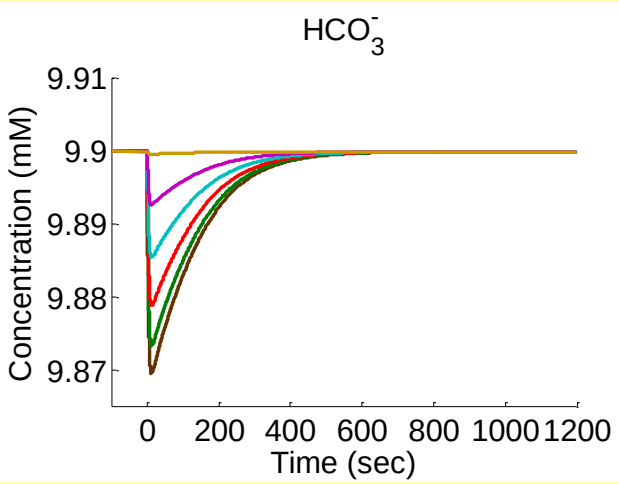
(A)



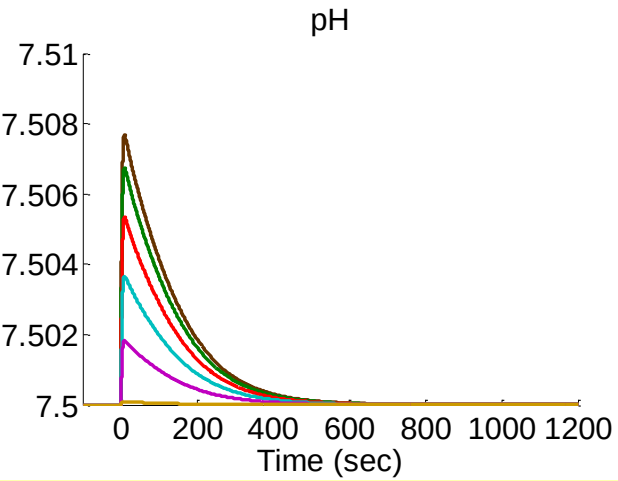
(B)



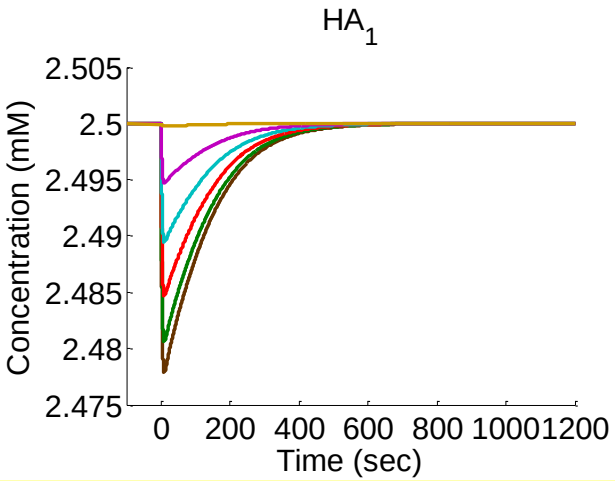
(C)



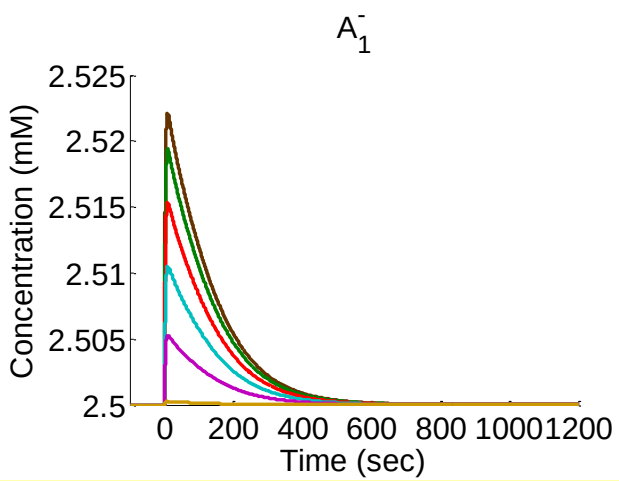
(D)



(E)



(F)

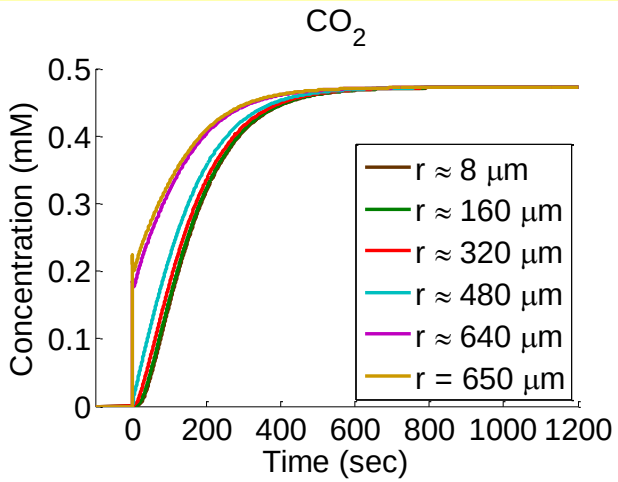


Intracellular concentration-time profiles for solutes

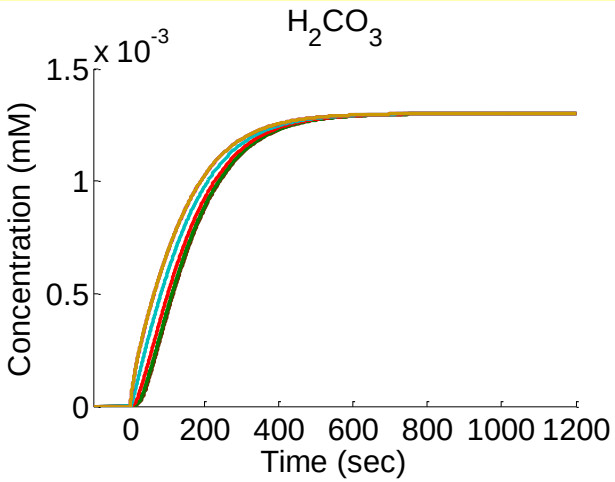
$CO_2 + H_2O \; f \;\; H_2CO_3 f \;\; HCO_3^- + H^+$

$HA_1 \; f \;\; A_1^- + H^+$

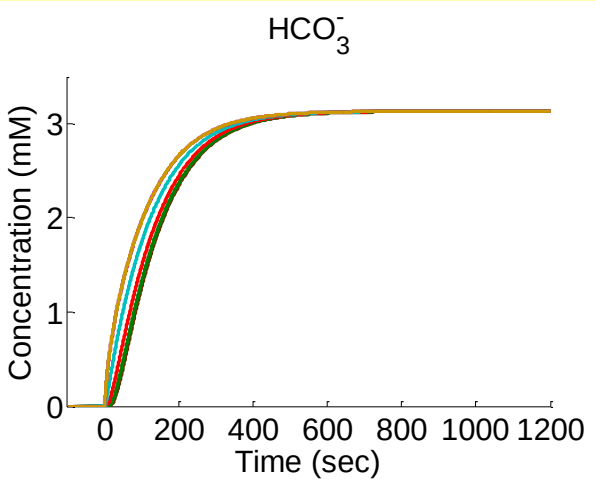
(A)



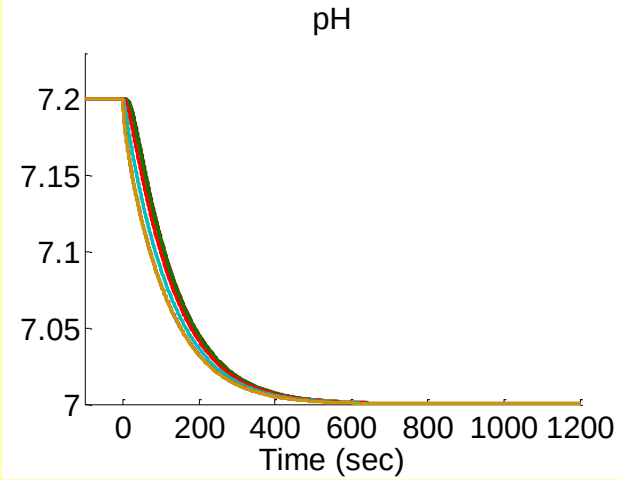
(B)



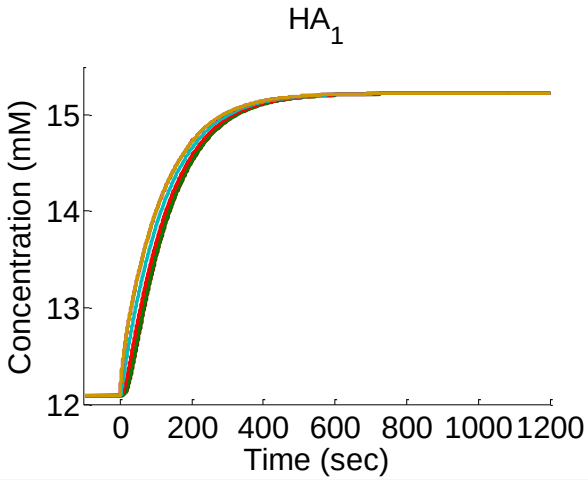
(C)



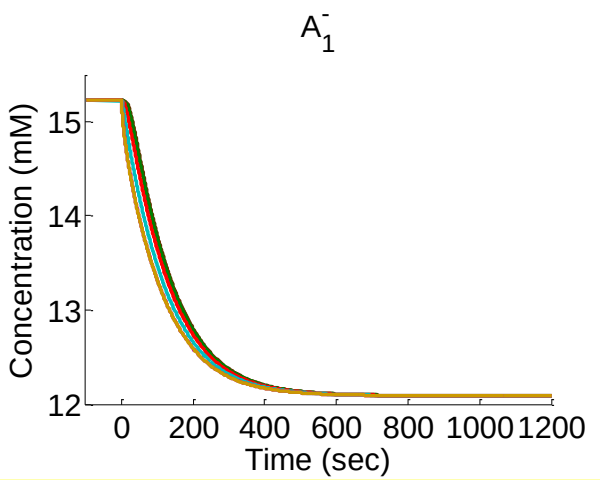
(D)



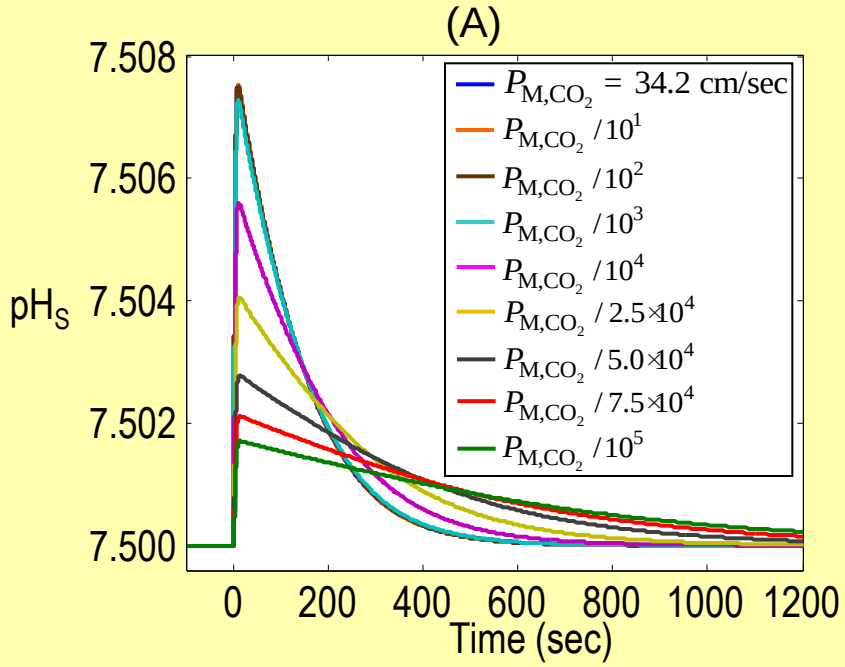
(E)

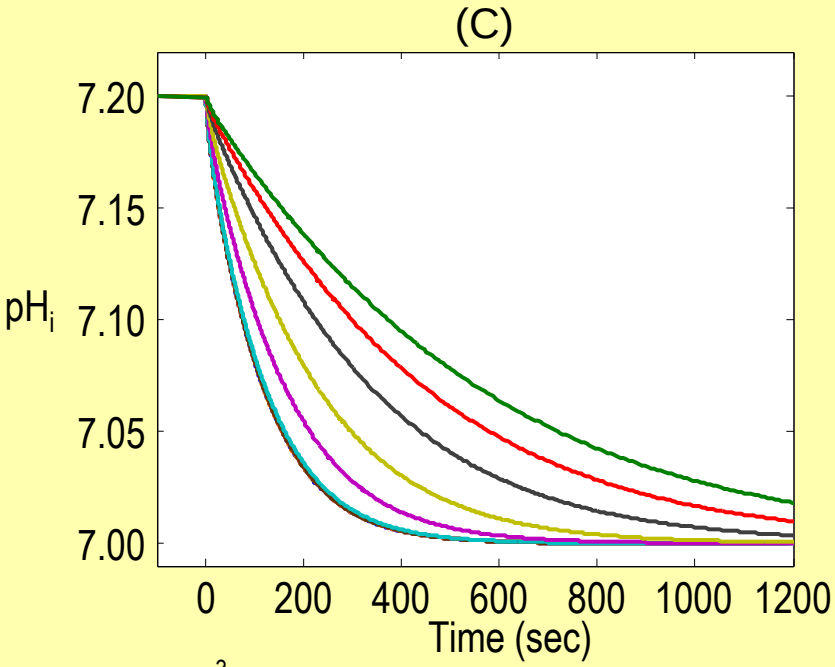


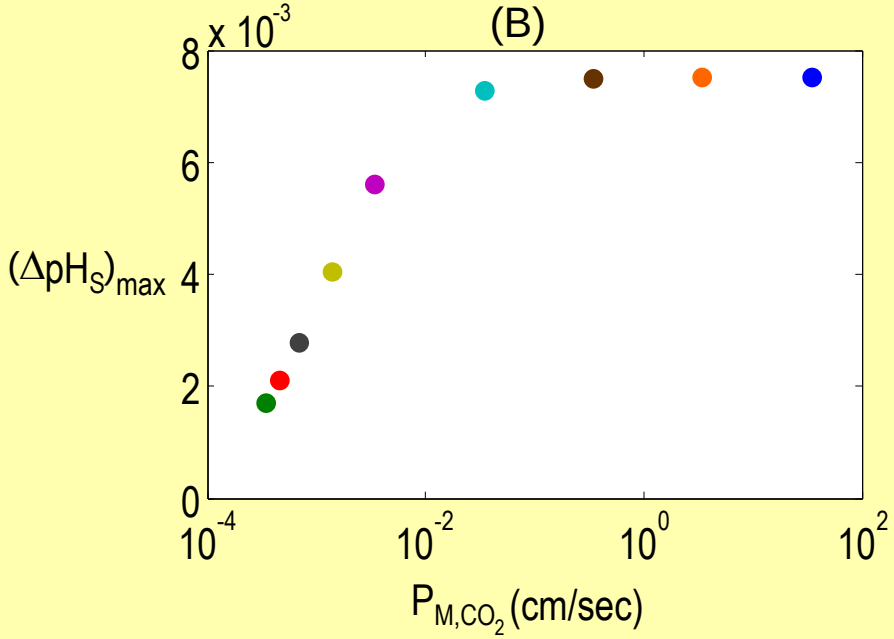
(F)

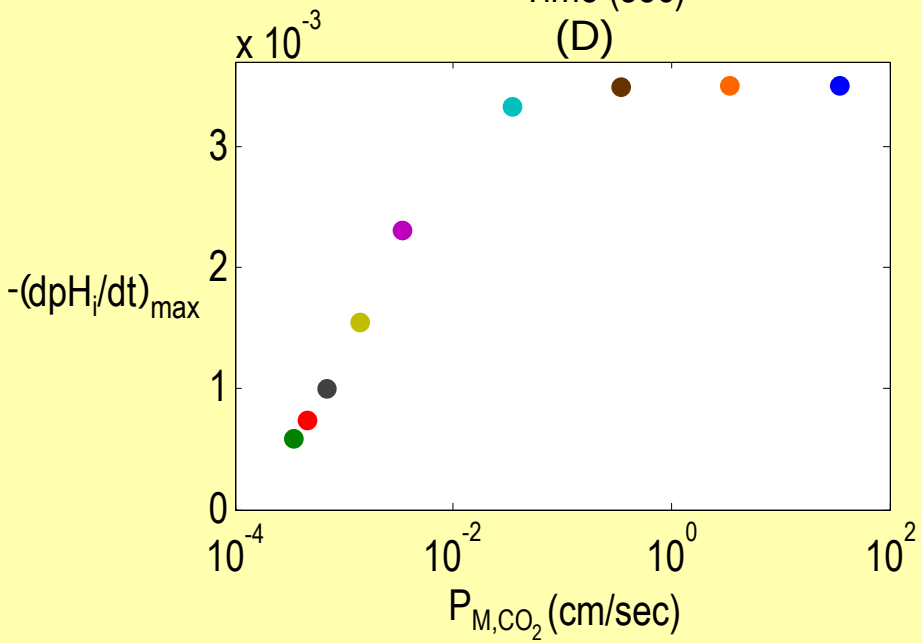


Effects of Decreasing CO_2 Membrane Permeability









Implications

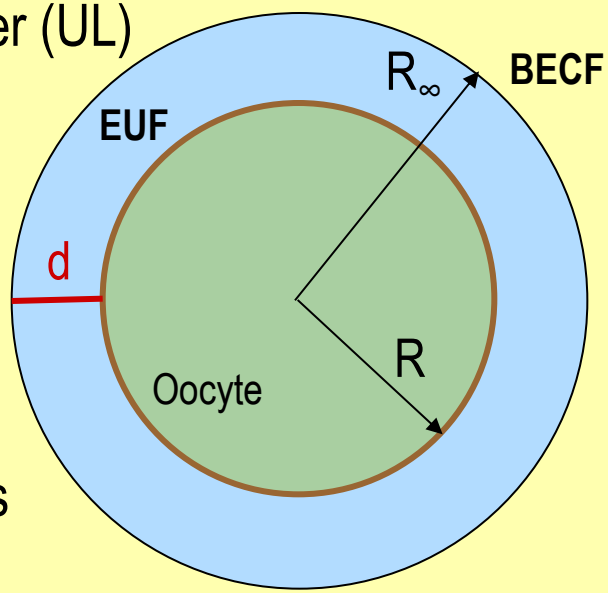
The background permeability of the membrane (i.e., in the absence of gas channels) must be very low

Given a sufficiently small $P_{M,CO2}$, gas channels could contribute to CO_2 permeability even in the presence of a large d (in our numerical experiments d = 100μm)

With additional refinements to the model, we ought to be able to estimate absolute permeabilities

Effects of Changing the Width of the EUF

The EUF is a generalization of the concept of unstirred layer (UL)



ULs are thin, diffuse layers of fluid, always present near the surface of solid bodies immersed in a fluid, where molecules move predominantly via diffusion (Dainty and House, *J Physiol*, 1966; Korjamo et al, *J Pharm Sci*, 2009)

For a particular solute, the width of the UL (δ) is defined as

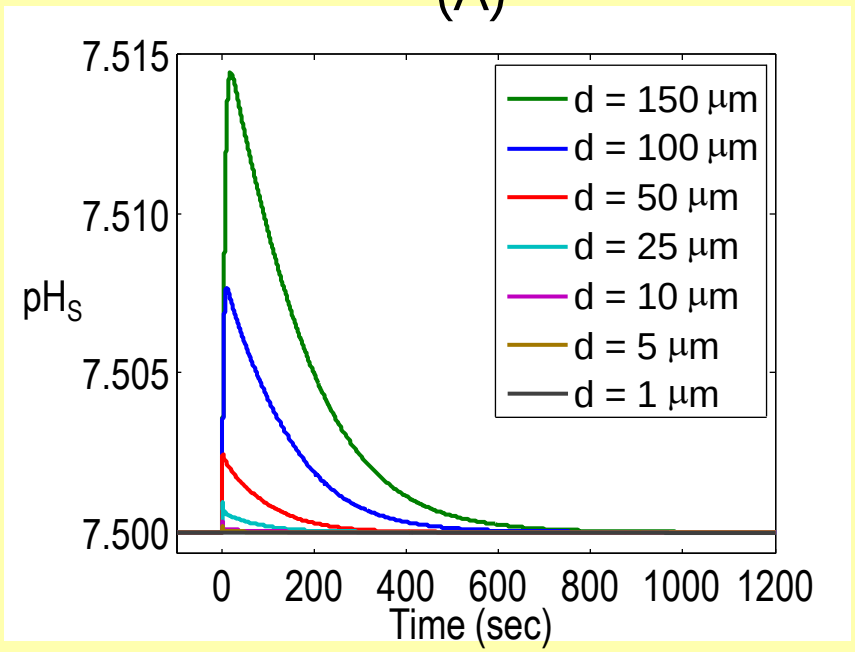
$$\delta = \frac{D}{P}$$

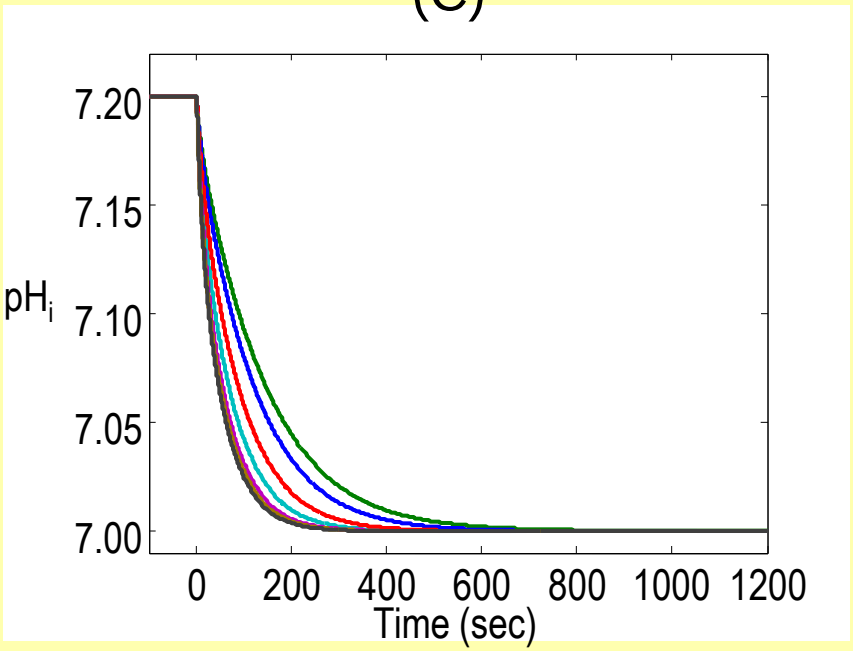
where *D* is the diffusion constant and *P* is the empirically measured permeability

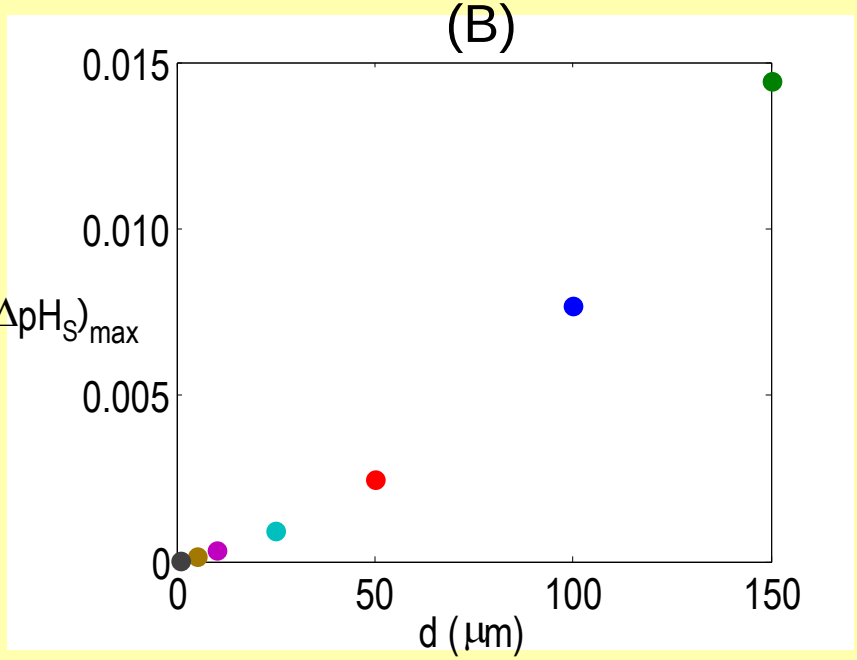
The width of the UL:

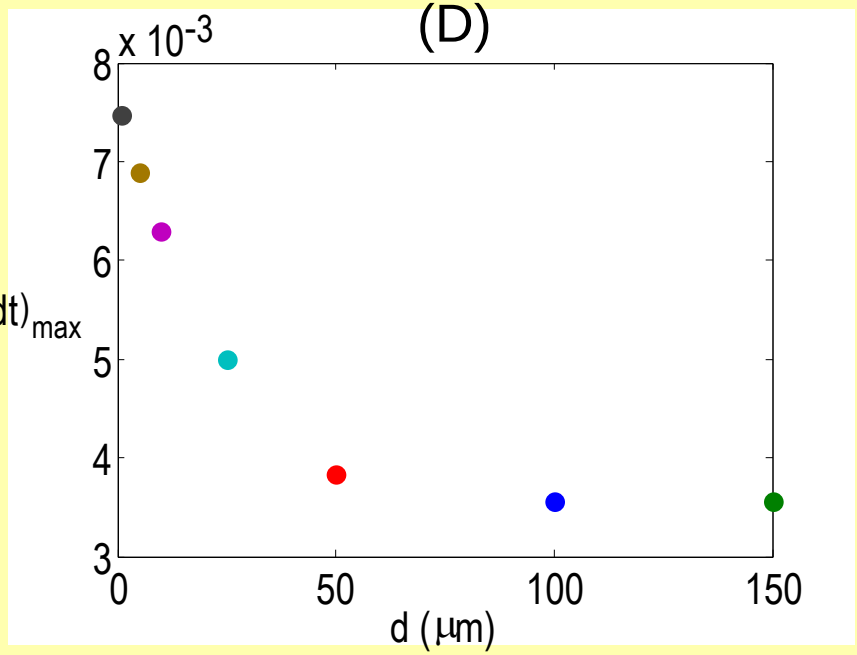
1. A steady-state concept
2. Solute-dependent
3. Ignores the effects of chemical reactions

It is because our system is dynamic, involves multiples solutes, and solutes can react in the “UL”, that we decided to define the EUF


(A)
7.515
7.510
7.505
7.500
pH_S
d = 150 μm
d = 100 μm
d = 50 μm
d = 25 μm
d = 10 μm
d = 5 μm
d = 1 μm
0
200
400
600
800
1000
1200
Time (sec)


(C)
7.20
7.15
7.10
7.05
7.00
pH_i
0
200
400
600
800
1000
1200
Time (sec)

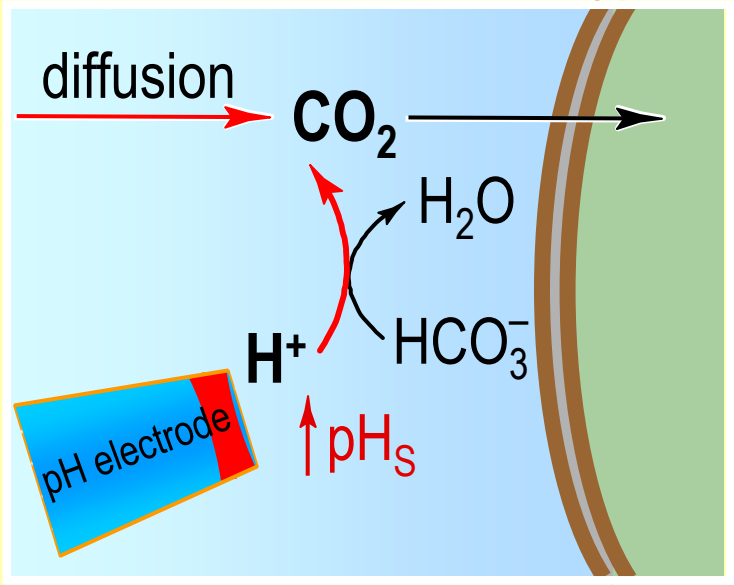

(B)
0.015
0.010
0.005
0
(ΔpH_S)_max
0
50
100
150
d (μm)


(D)
x 10^-3
8
7
6
5
4
3
-(dpH_i/dt)_max
0
50
100
150
d (μm)

Implications

There is competition between diffusion and reaction in replenishing the lost CO_2 near the outer surface of the oocyte

We quantify this competition by introducing the diffusion reaction ratio (DRR)



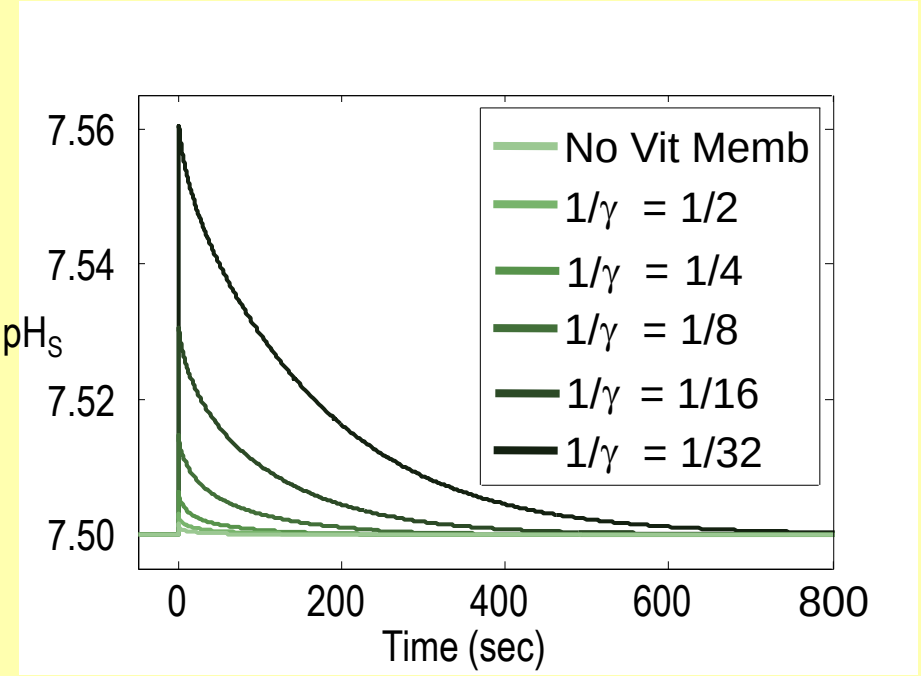
$$DRR = \frac{\text{rate of } CO_2 \text{ replenisced by diffusion}}{\text{rate of } CO_2 \text{ produced by reaction}}$$

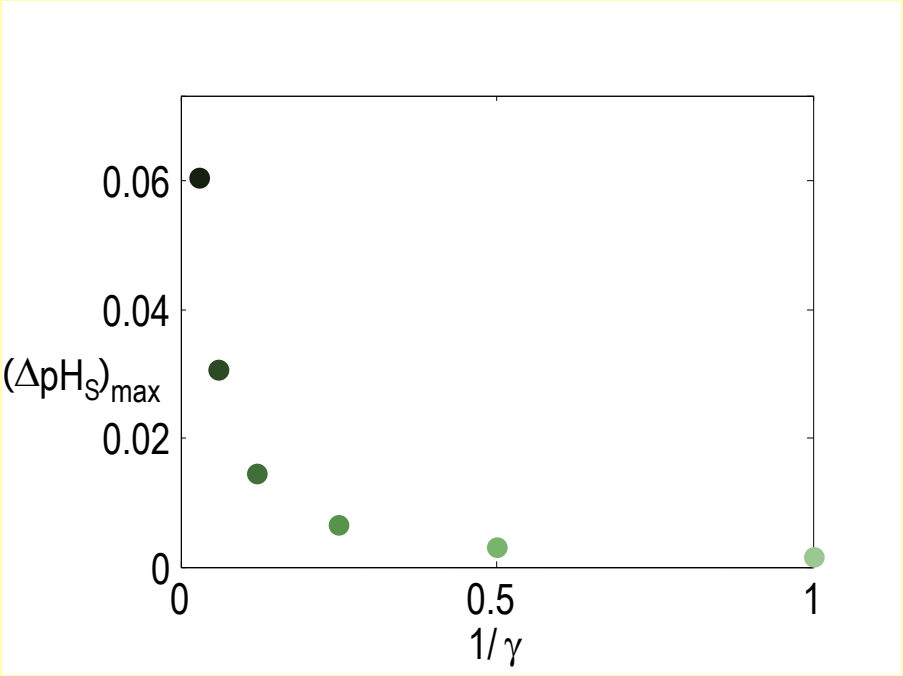
DRR rises as the width d of the EUF decreases

The Vitelline Membrane: pH_S Spike

Additional diffusion barrier to the movement of solutes

Implemented by reducing the mobility D of each solute near the outer surface of the oocyte by the same factor γ, i.e., $D_* = D/\gamma$





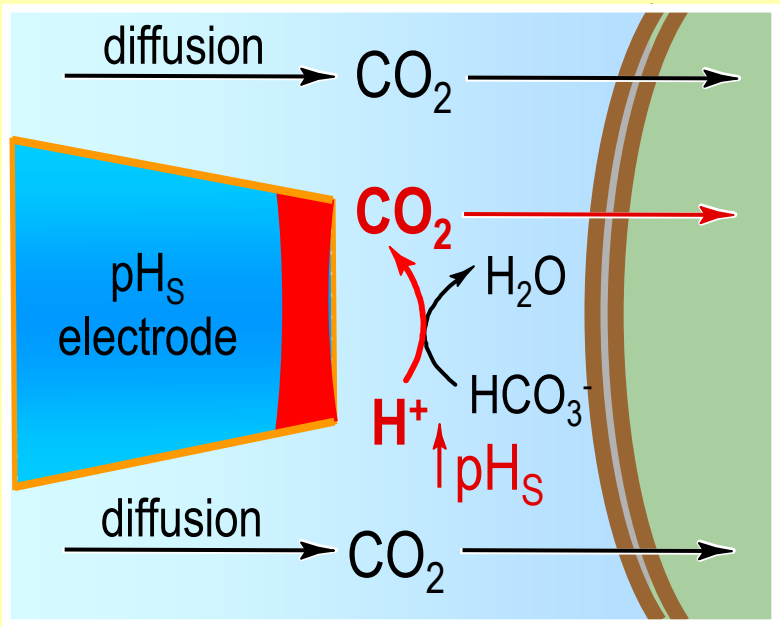
As we increase γ, the maximal height of the pH$_S$ spike, $(\Delta pH_S)_{max}$, increases

Implementation of the vitelline membrane reduces the contribution of diffusion and enhances the contribution of reaction at the surface

Implications

Implementation of the vitelline membrane – which reduces the contribution of diffusion and enhances the contribution of the reaction – can explain the height of the pH_S spike

Because the pH_S electrode creates a special environment with restricted diffusion, our implementation of the vitelline membrane somehow mimics this environment



Conclusions

The model can reproduce the pH transients observed experimentally

The simulations predict that:

1. The background permeability of the oocyte membrane must be very low
2. Given a sufficiently small $P_{M,CO2}$, gas channels could contribute to CO_2 permeability even with a large EUF

The model provides new insights into the competition between diffusion and reaction processes near the outer surface of the plasma membrane

Future Directions

Apply the model to investigate the movements of ammonia and ammonium across the plasma membrane

Model the pH_S electrode's touching on the oocyte surface to explore the special environment underneath the pH_S electrode

Acknowledgments

Principal Investigator

Walter F. Boron, M.D., Ph.D.

Collaborators

Erkki Somersalo, Ph. D. (CWRU)

Daniela Calvetti, Ph. D. (CWRU)

Raif Musa-Aziz, Ph.D. (University of Sao Paulo)

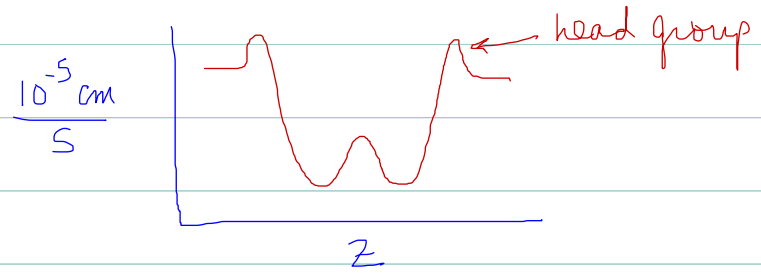




9/6/12 @ 9:10 AM
Gas Channels Worksop
Emad
MD ... Sub-angstrom resolution

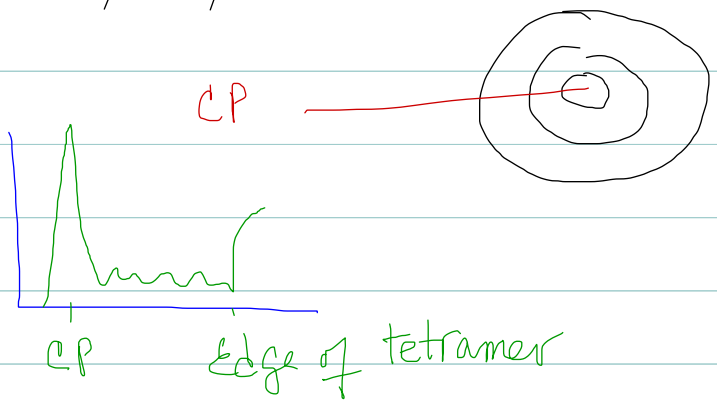
2016: Blue Waters → 200K processors
≠

POPE 100%.
Start off c 100 CO_2 near membrane
Modeling 50% Chol. is not trivial... where to place them, equilibration... have a partial solution.
His partition coeff is ~ exp. det. values
Gas reaches equil in lipid in 10-15 ns for O_2 & CO_2.



Implicit ligand sampling: Works c neutral molecules (not ions, wh. perturb protein) ... results are about same as explicit.

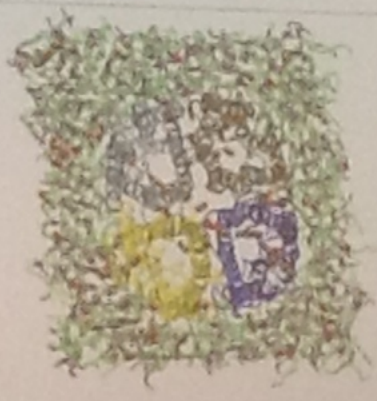
{ AQP1, 4, 5
{ CO_2, O_2, NO



through Aquaporins

SYSTEM	TOTAL (100x100 Å^2)	WATER PORES (4)	CENTRAL PORE (1)
Equi POPE-CO_2	3	N/A	N/A
Equi POPC-CO_2	5	N/A	N/A
Equi POPC-O_{2P}	16	N/A	N/A
Equi POPE-O_{2P}	11	N/A	N/A
Press POPE-CO_2	168	N/A	N/A
Press POPC-CO_2	160	N/A	N/A
Press POPE-O_{2P}	310	N/A	N/A
Press POPC-O_{2P}	208	N/A	N/A
Press POPE-AQP1-CO_2	**76**	**6**	**4**
Press POPE-AQP1-O_{2P}	**79**	**1**	**6**





AQP1: DSO → H_2O coord → major barrier

NO through AQP4

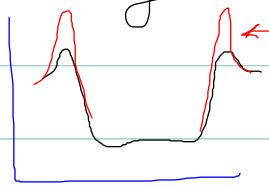
O_2 " "

(Wang, Proteins, 2010)

wB: Is $[O_2]$ in CP the same as in bulk gas phase?

AQP4 (vs. 1): diff ΔG profile

Rely heavily on init. config. of lipid



Problem: lipid molec. move v. slowly!

10^3 slower than H_2O

HMMM: highly mobile membrane mimetic

(liquid center of membrane)

Water-Oil attracts lipids to interface

Lipids are far more mobile

Even can see insertion of a peptide helix.

Chol might partition into core of bilayer, parallel to plane of membrane.

9/6/12 @ 10:25 AM
Gas Channels Worksop
Gerolf Gros, Hanniver
18O ... CO2 permeability

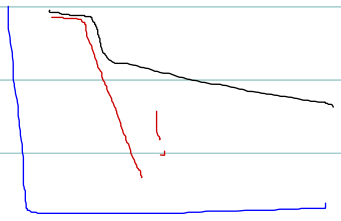
Problem ⊂ stopped-flow applied to vesicles

$t_{1/2}$ of CO_2 uptake by human RBC : 13 ms
O_2 80

This could be too fast, esp. if there is incomplete mixing. But KO of AQP1 + Rh could ↑ $t_{1/2}$ by 10x ... make a Δ measurable? WB

Measure ^{18}O-labelled CO_2
46 vs 44 Mol. Mass

"HCT" : 2×10^{-4}
2×10^{-3} ^{18}O

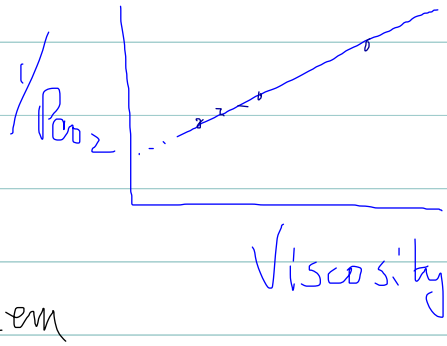


3-D curve fitting → optimal P_{CO_2} & $P_{HCO_3^-}$ ... no local minima

Critical params: A_i & pH_e ...
errors → big ΔP_{CO_2}
pH: ±0.01 → 20-30%
pH_i & P_{H_2O} are not important

Viscosity: ↑[Dextran] → ↓ P_{H_2O} (wh is so small)
= → ↓ P_{CO_2}

unstirred layer



$\delta = 0.5\,\mu m$

$P_M = 0.16\ cm/s$

∴ UL is only a minor problem if $\delta \propto$

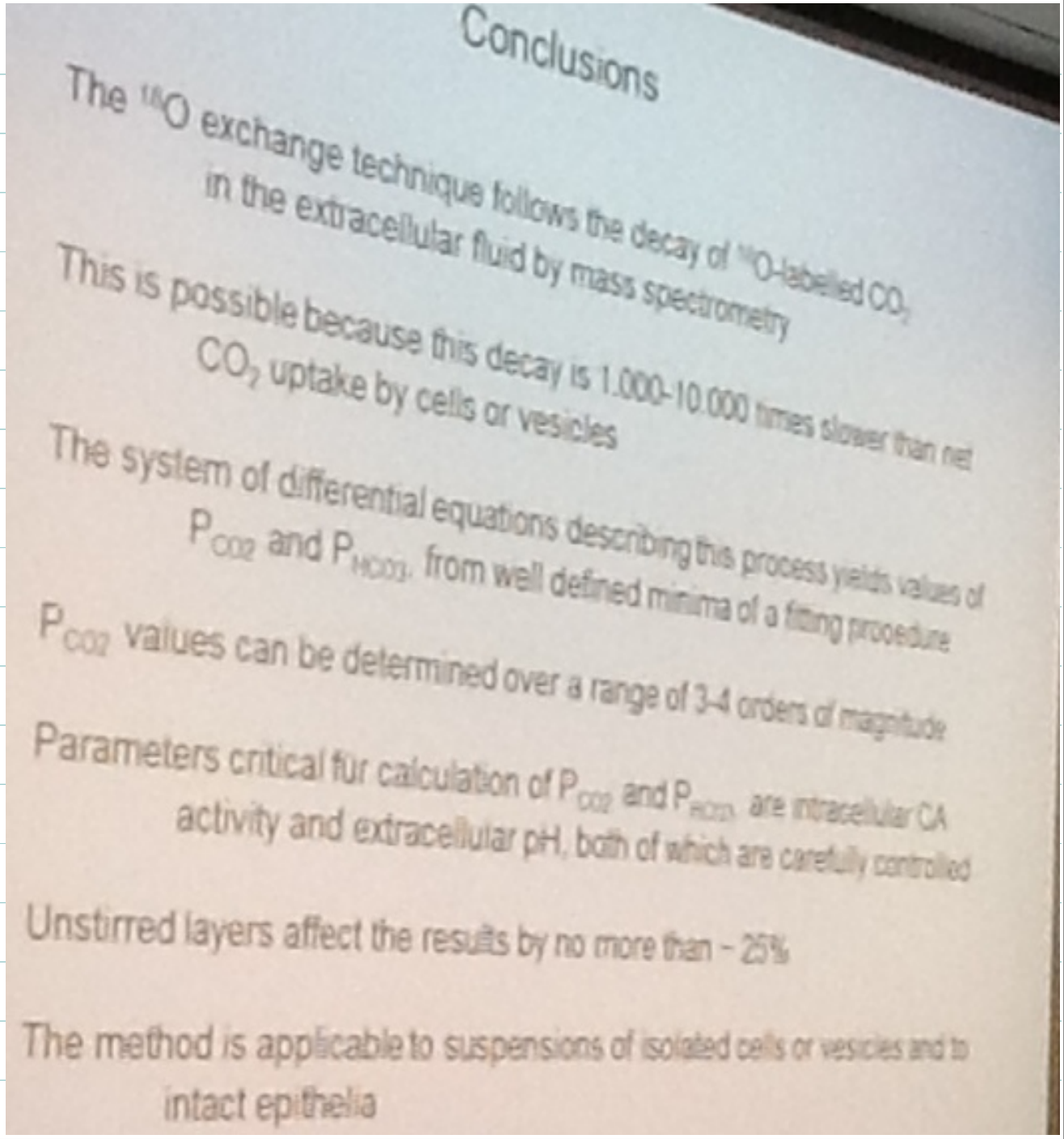
P_{O_2} in human RBC's

IDEAS FOR DISC.

AQP1 ~ 50%
Rh ~ 50%
} ~ 100% ≠

Colon:

Competition of H_2O vs. NH_3 or CO_2 through aquapore

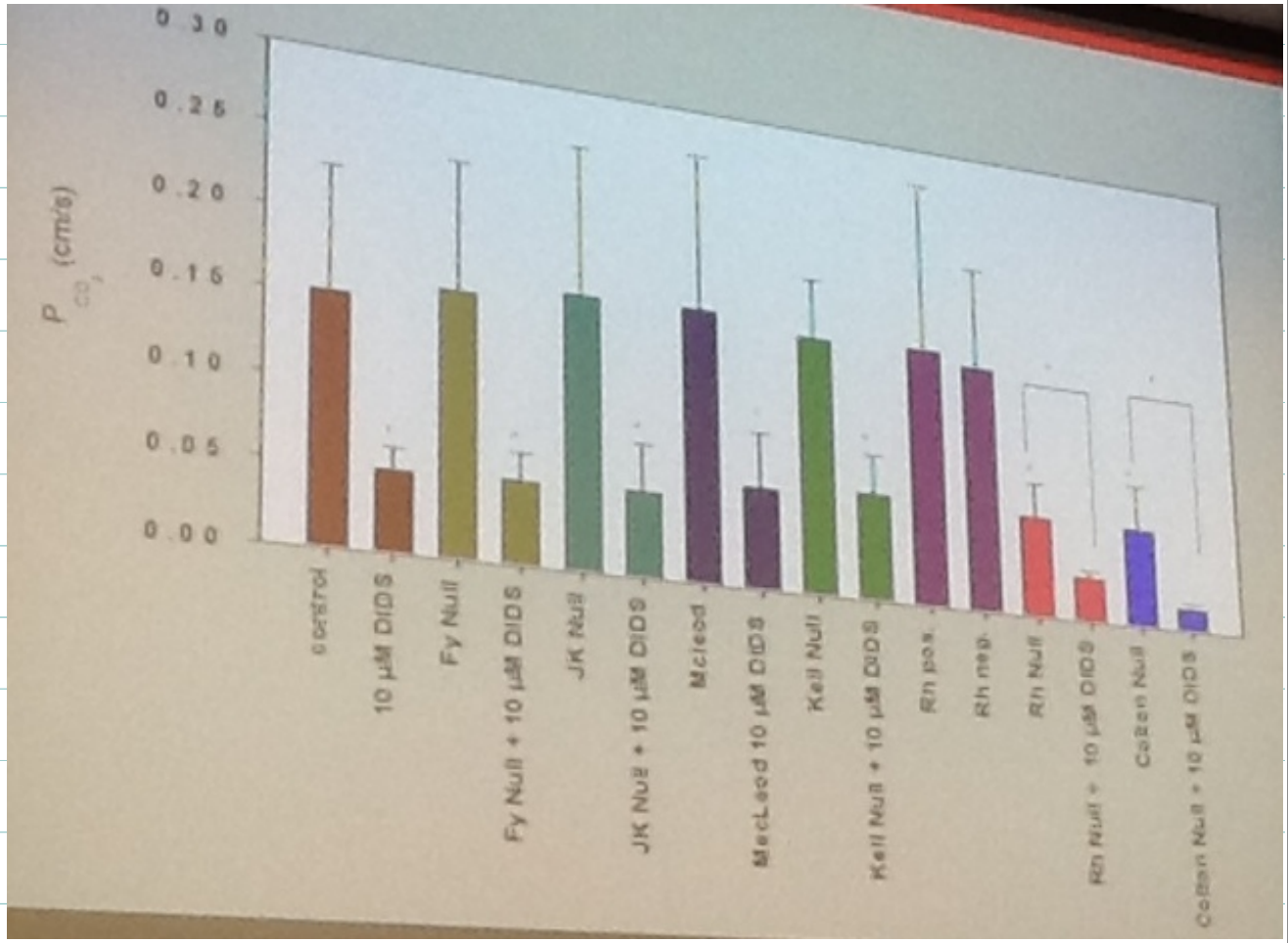

Conclusions
The ^{18}O exchange technique follows the decay of ^{18}O-labelled CO_2 in the extracellular fluid by mass spectrometry
This is possible because this decay is 1.000-10.000 times slower than net CO_2 uptake by cells or vesicles
The system of differential equations describing this process yields values of P_{CO2} and P_{HCO3} from well defined minima of a fitting procedure
P_{CO2} values can be determined over a range of 3-4 orders of magnitude
Parameters critical fur calculation of P_{CO2} and P_{HCO3} are intracellular CA activity and extracellular pH, both of which are carefully controlled
Unstirred layers affect the results by no more than ~ 25%
The method is applicable to suspensions of isolated cells or vesicles and to intact epithelia

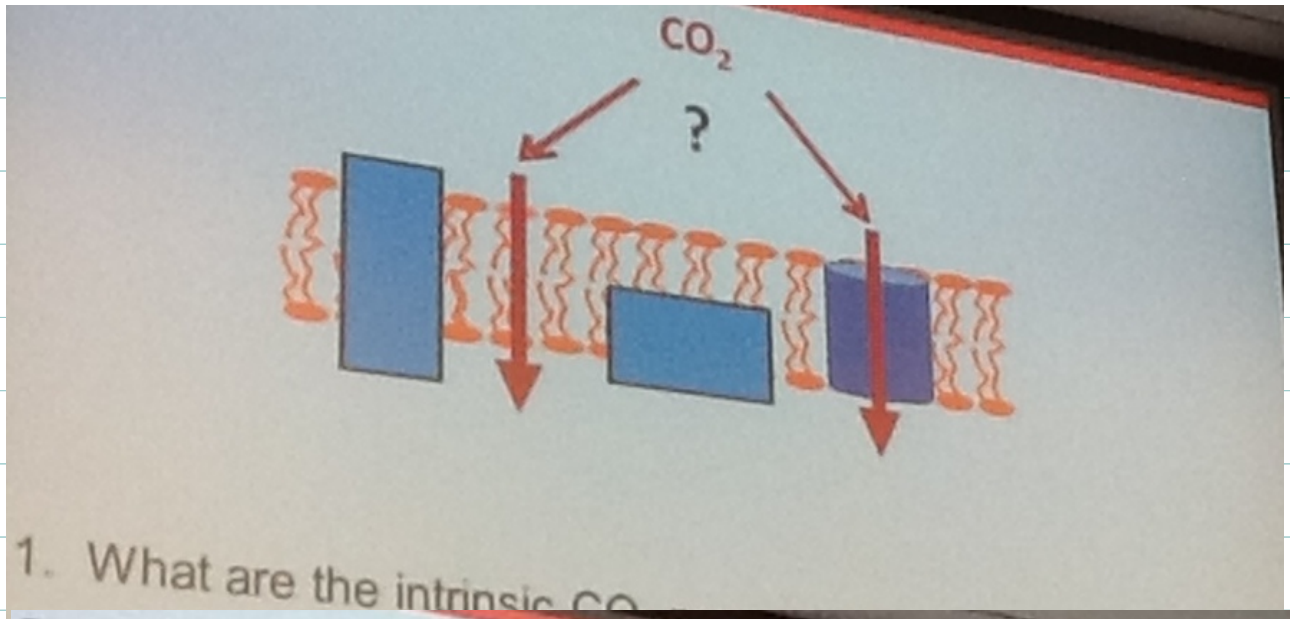
$$[x]_w = S_w P_x$$

$$[x]_L = \frac{S_L}{S_w} [x]_w = \frac{S_L}{S_w} \cdot S_w \cdot P_x$$

$$= S_L \cdot P_x$$

9/6/12 @ 11:15 AM
Gas Channels Worksop
Volker Endeward, Hannover
Background CO2 permeability





1. What are the intrinsic CO_2

Cell membranes show CO_2 permeabilities lower then synthetic lipid bilayer

	P_{CO2} (cm/s) ± S.D
Synthetic lipid bilayer	0.35 / 3.2
Red cell,	~1
Ø functional gas channel	0.015 ± 0.003
MDCK	0.017 ± 0.004
tsA201	0.007 ± 0.003
Basolateral membrane of proximal colon epithelium	~ 0.022
Apical membrane of proximal colon epithelium	0.0015 ± 0.0006

P_{CO_2} (art. lipid bilayer) >> naked mammalian membrane

Cholesterol: 98% ↓ in P_f.

$\neq$

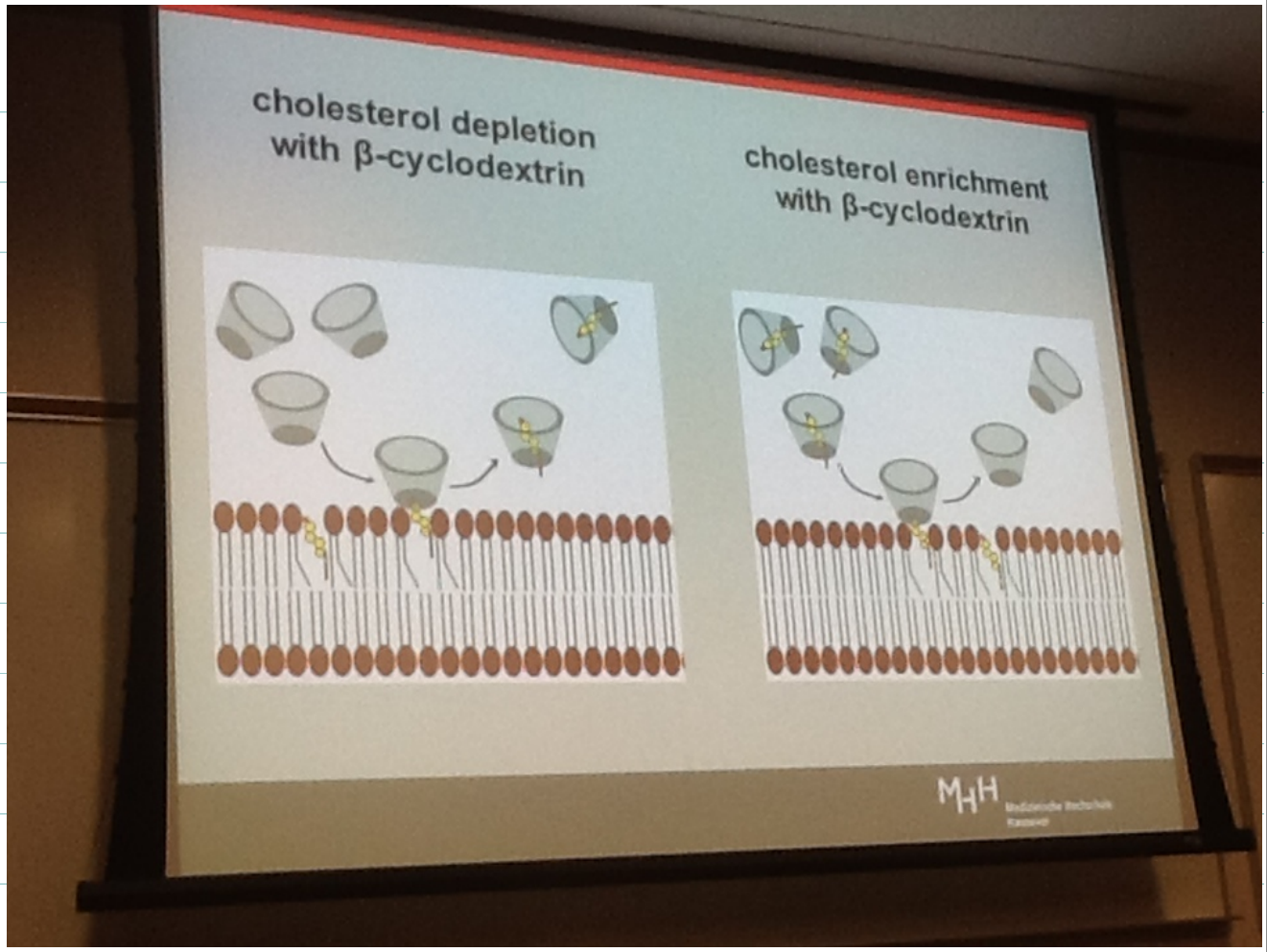
150 nm: mean vesicle diam... contain CA II

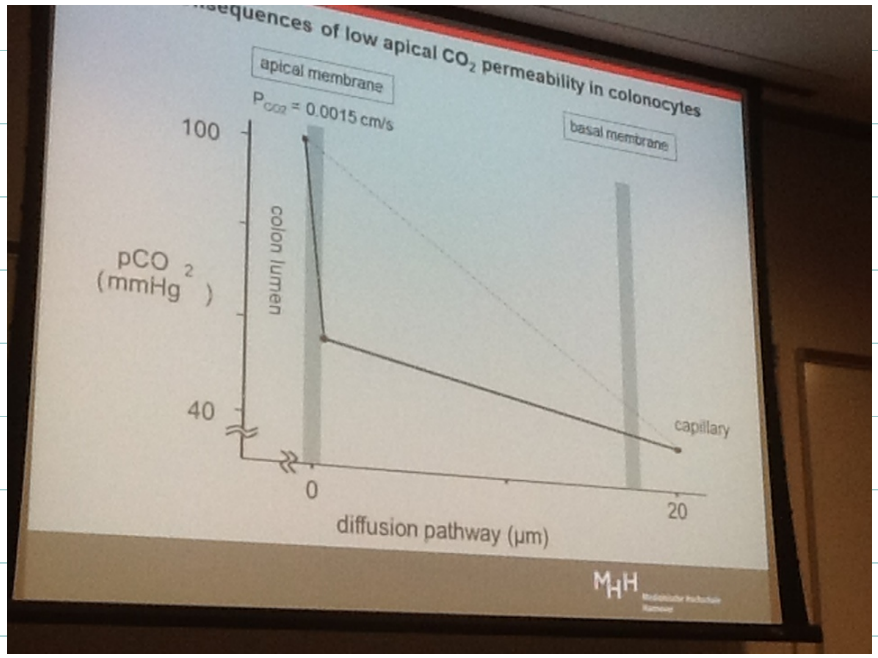
Chol: 0-20% → ⚠ measurable

30-70% → log-linear ↓ P_{CO_2} by ~ 10^2

Apical colonic membrane: 70% cholesterol

β-cyclodextrin





Could we ↓ P_{CO_2} ↑ X_o by ↑ Chol & Vice versa.

- From these considerations we can see that gas exchange of cells with a low CO_2 permeability is limited.
- Hypothesis: cell membranes with normal cholesterol and low intrinsic P_{CO2} adapt their CO_2 permeabilities to their needs by incorporating gas chanels in the membrane.

AQP1 vs. Agpz in liposomes

↑↑ P_{CO_2} ↑ ≠ P_{CO_2}

He sees a much bigger effect (? ~80%) than we do.

Gas	CO_2	O_2	NO	N_2
Lipid-water partition coefficient	0.95	2.9	3.8	4.1
Permeability (phospholipid membrane)	~1 cm/s	~3 cm/s	~4 cm/s	~4 cm/s

O_2: PL membrane 3 cm/s

Unst: 1/100

PL + Chol: 0.03?

Heart m: $\Delta P_{CO_2} = 40$ mm Hg

↑ way too high to be physiol. possible.

Summary

With rising cholesterol content the CO_2 permeability (P_{CO_2}) of lipid vesicles decreases drastically.

The intrinsic P_{CO_2} of cell membranes is low due to their cholesterol content:

1) cell membranes and lipid vesicles with identical cholesterol content exhibit identical CO_2 permeability

2) cholesterol-depleted cell membranes have an increased CO_2 permeability, cholesterol-enriched cell membranes a reduced permeability

Cell membranes with normal cholesterol and low P_{CO_2} raise their CO_2 permeability, when functionally required, by incorporation of CO_2 channels:

1) AQP1 incorporated in lipid vesicles raises CO_2 permeability in a concentration-dependent manner

2) AQP1 expression in MDCK cells increases membrane P_{CO_2}

$P_{CO_2} \propto [AQP1]^n$?

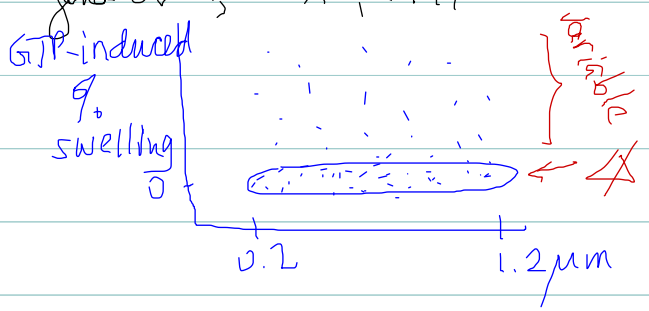
9/6/12 @ 1:05 PM
Gas Channels Worksop
Bhanu Jena, Wayne State
Cholesterol

Got interested in AQPs because of their involvement in vesicle fusion.
H_2O must enter the vesicle

$G\alpha_{i3}$: assoc. ~~c~~ ZGM

GTP → ↑ water by volume (AFM) & ^3H_2O.

Jena et al, PNAS, 1997

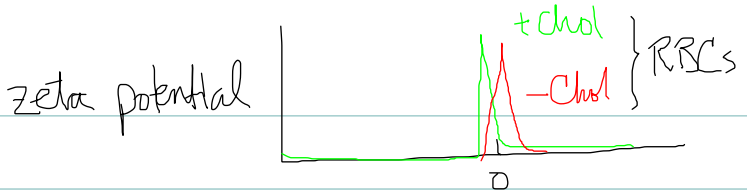


AQP1: Swelling complex } not 2,3,4,5,
" 6: " } 7,8,9

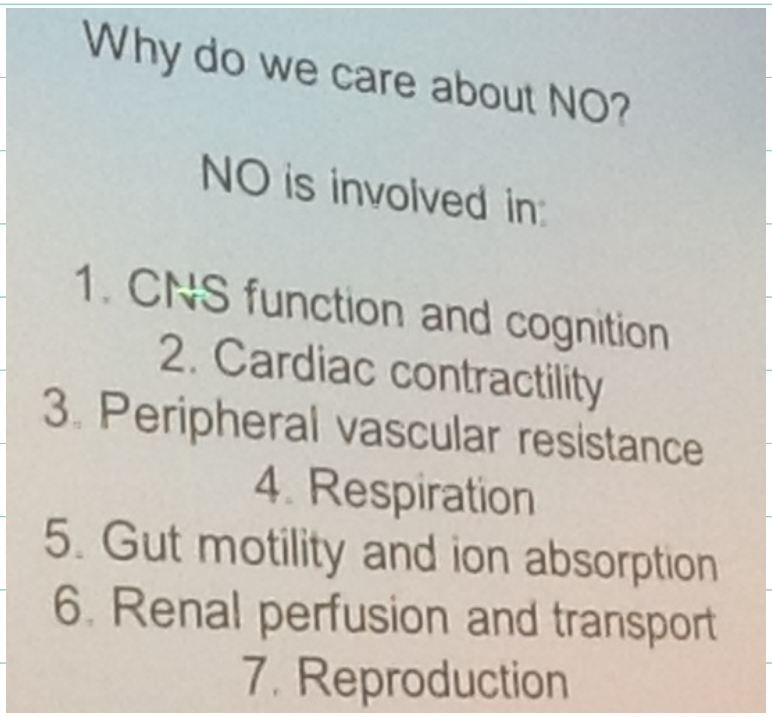
Mast (mastoparan)

% ΔVolume of granule (swelling): GTP + Mast
+ 20–40 μM MβCD (cyclodextrin)

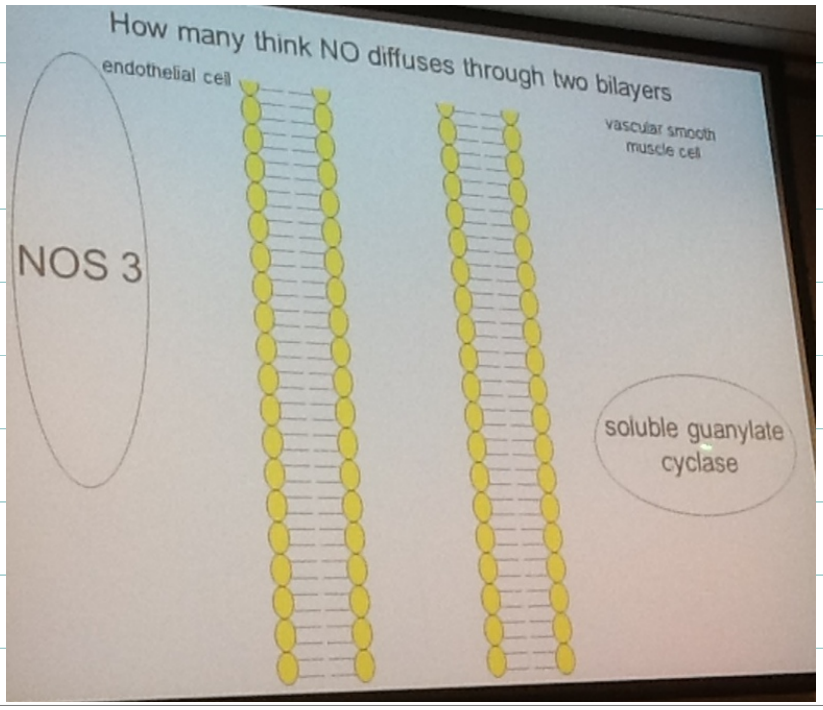
AQP6: $G\alpha_o$, V-H^+ ATPase complex (stability) req. Chol.
Remove Chol → complex falls apart.

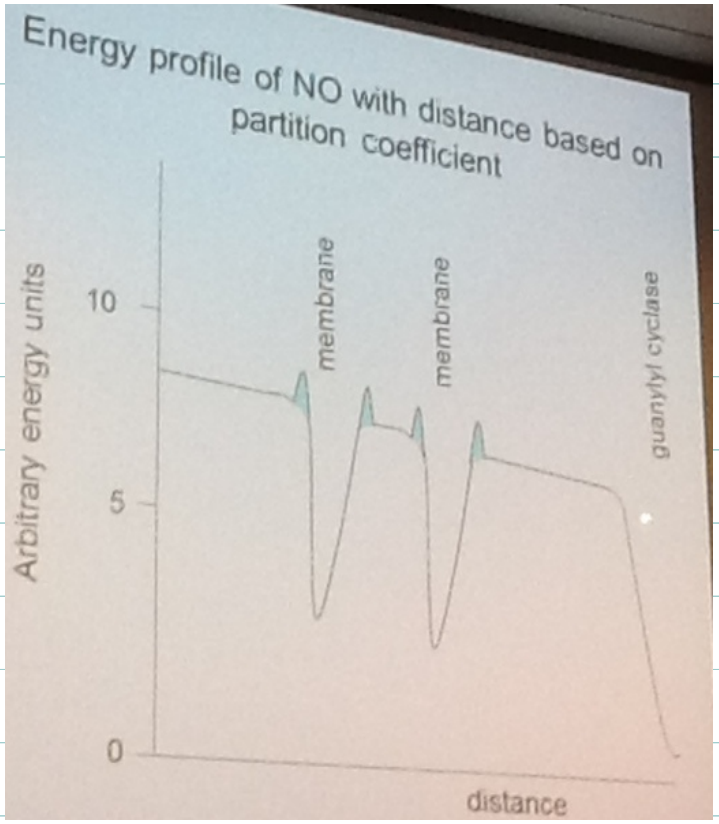

Zeta potential
+ Chol
– Chol
RRCs
0

9/6/12 @ 1:55 PM
Gas Channels Worksop
Jeff Garvin, HFH
The truth about the movement of NO across cell membranes



Partition coef: 3-5
t½ ~ 30 s



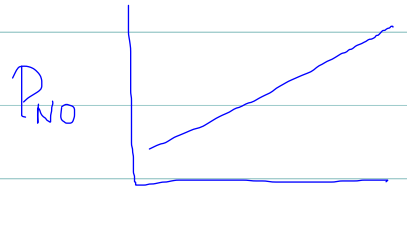


"Partition coeff say nothing about rates" ... his PhD mentor beat into him that S/S is an equilibrium parameter.

What is the chemistry of NO in lipid? More or less stable than in H_2O?

If our hypothesis is correct:

1. NO permeability (P_{NO}) should correlate with water permeability (P_f).
2. Increasing AQP-1 expression should increase NO flux.
3. Inhibitors of AQP-1 should reduce NO flux.
4. NO flux should be saturable.
5. Purified AQP-1 should transport NO.



P_{NO}: fluorescent probe DAF2

NO influx → CHO cells, transiently transfected.

NO: NO donor or gas.

Hg inhibits ≈ NO gas?

K½: 0.54 μM Physiol [NO] ≅ 0.2 μM

AQP1 reconst. into vesicles → ↑ J_{NO}

CHO cells:

AQP3: 25% ↑ over mock << AQP1. Did not ✓ expression.

" 4: 30% ↑

≠

Aortic ring: isometric force

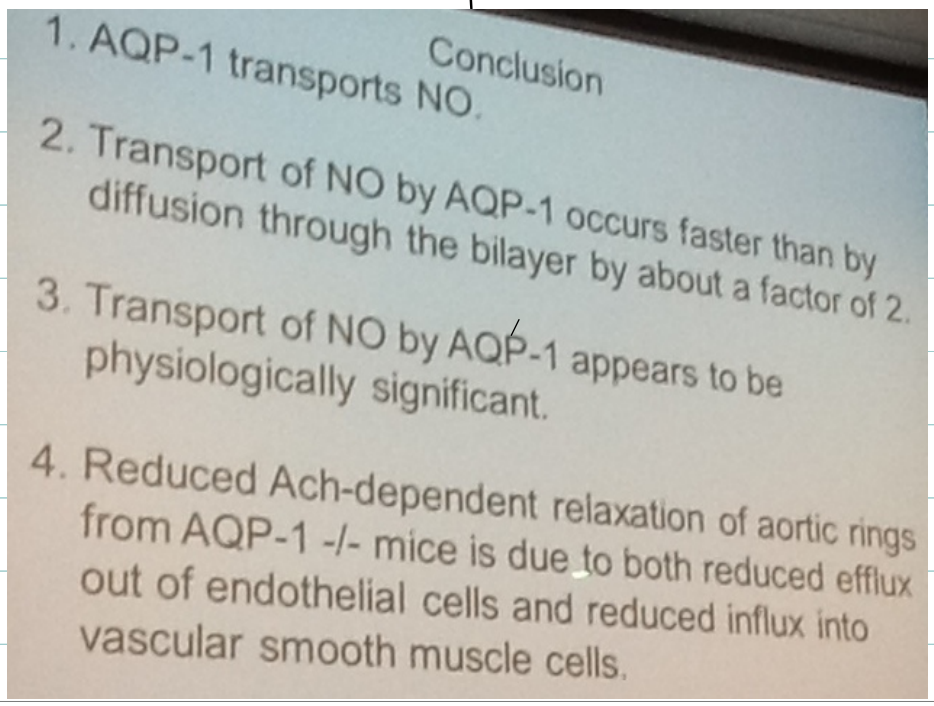
PE = Phenylephrine → contr.

Vary [ACh] to relax. AQP1 KO: ↓ ACh response

? ↓ NO efflux from EC or ↓ influx into VSMC

KO: ↓ NO release from EC

↓ " uptake into VSMC



32 in Audience

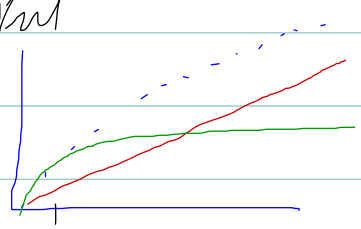
9/6/12 @ 3:10 PM
Gas Channels Worksop
David Weiner (wee)
Assessing roles of Rh glycoproteins in NH_3 gas transport?

Is NH_3 transport "diffusive" or protein mediated?

Inhibitors: none

si RNA: unsuccessful

Saturation?



Saturable component + diffusive comp.

Me-NH_2 Basolat. uptake

~3 mM $K_{1/2}$

Diffusion may dominate in inner medulla, where $[NH_3]$ is high & Rh levels are v. low.

RhAG + Rh30 $\lessgtr^{CE}_{D}$

Cannot find RhAG by Ab anywhere but RBC.

1st cloned: RhAG

#2 " BG: Perivenous cells in hepatocytes Hair follicles. $[NH_3]$ is 100× > plasma. Goes ↑ ē exercise: 2 → 10 mM.

Urine: 200-300 mM (highest NH_3 conc)

GI → 250 mmole NH_3/day. SI > Colon

Lungs: RhBG → not in alveolar cells but in Bronchial epith cells.

RhCG: ? Glu neurotransmission.

Liver: $[NH_3]$ is ↑ in bile

Muscle: Exercise → 4-5x NH_3 ... produced by sk.m. At rest, Sk.m. is a NH_3 sink. excreted

Kidney: 1-2% of NH_3 from GFR.

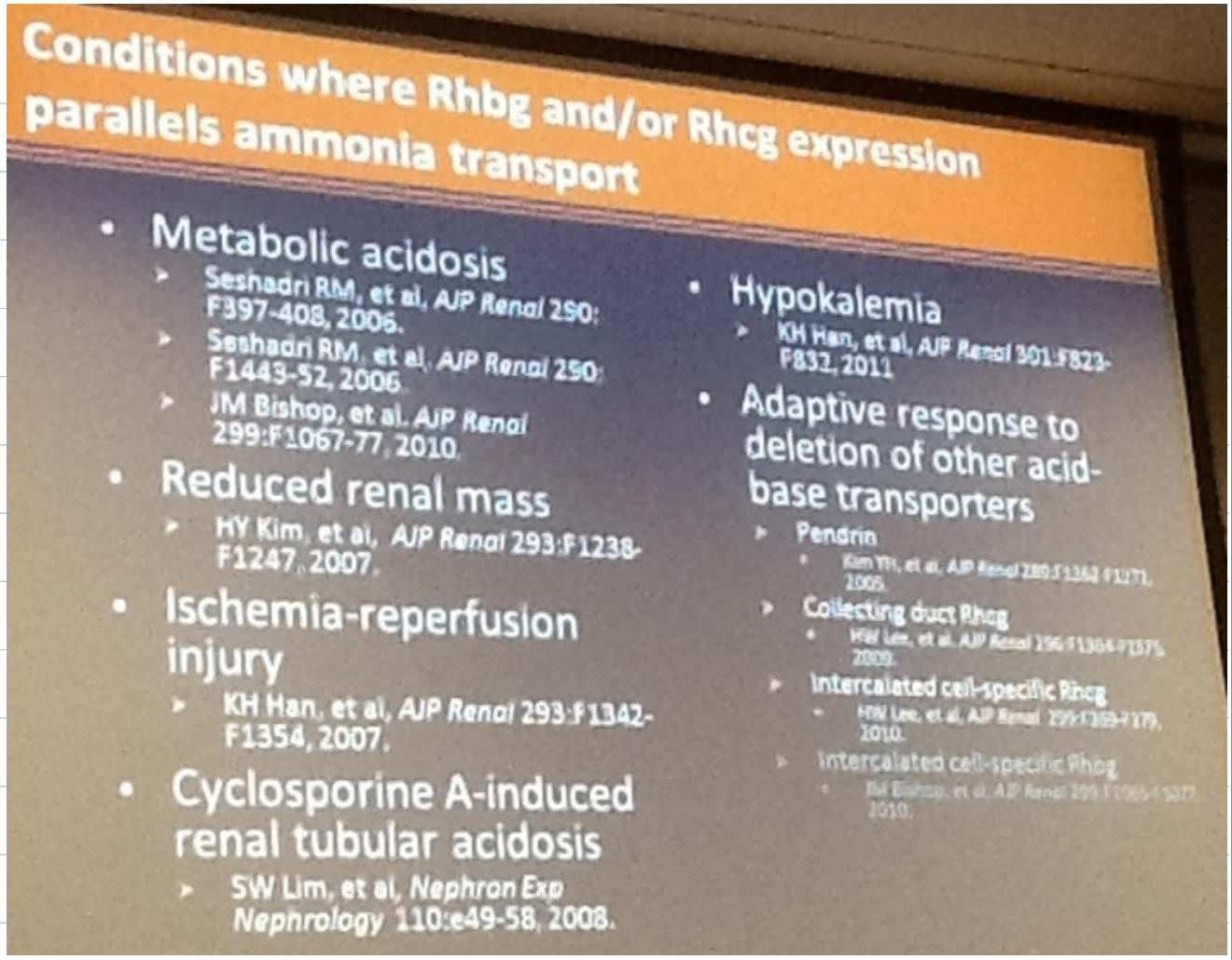
CD: RhBG: BLM ← BalbC

CG: AM + some BLM ← C57 BL6: much higher

MAc → ↑ RhBG expression

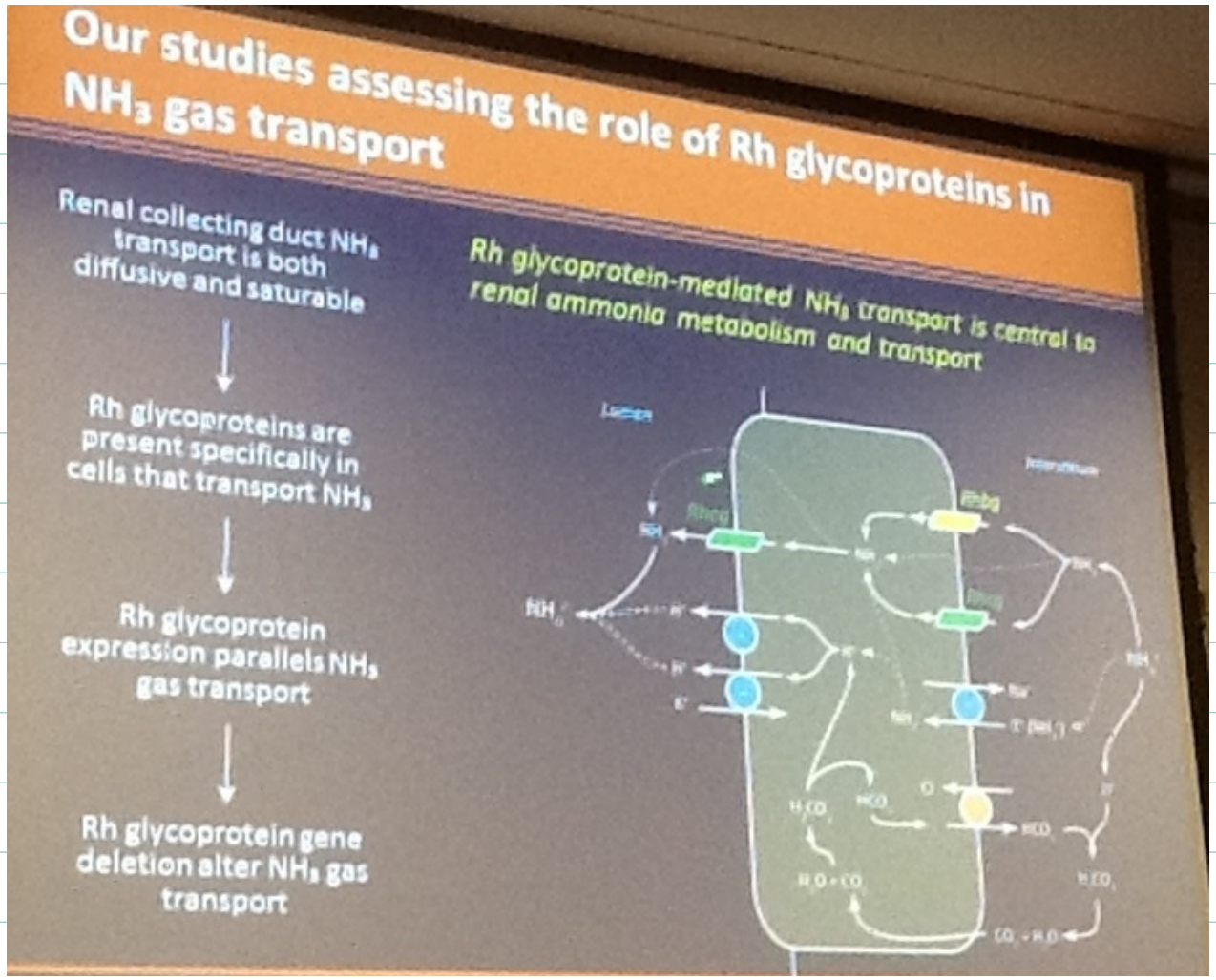
Does Teniap ↓ the "CO_2 permeability" attributable to NBC? Does it speed up pHi ↓?

Grant: Mutant NBCe1
- Cond. KO?
- Mutations



Conditional KO of Rhcg : ↓ Urinary excretion but ↔ pH_a

Slowest renal response : NH_3 transport ... req. 4-5 days


Our studies assessing the role of Rh glycoproteins in NH_3 gas transport
Renal collecting duct NH_3 transport is both diffusive and saturable
Rh glycoproteins are present specifically in cells that transport NH_3
Rh glycoprotein expression parallels NH_3 gas transport
Rh glycoprotein gene deletion alter NH_3 gas transport
Rh glycoprotein-mediated NH_3 transport is central to renal ammonia metabolism and transport

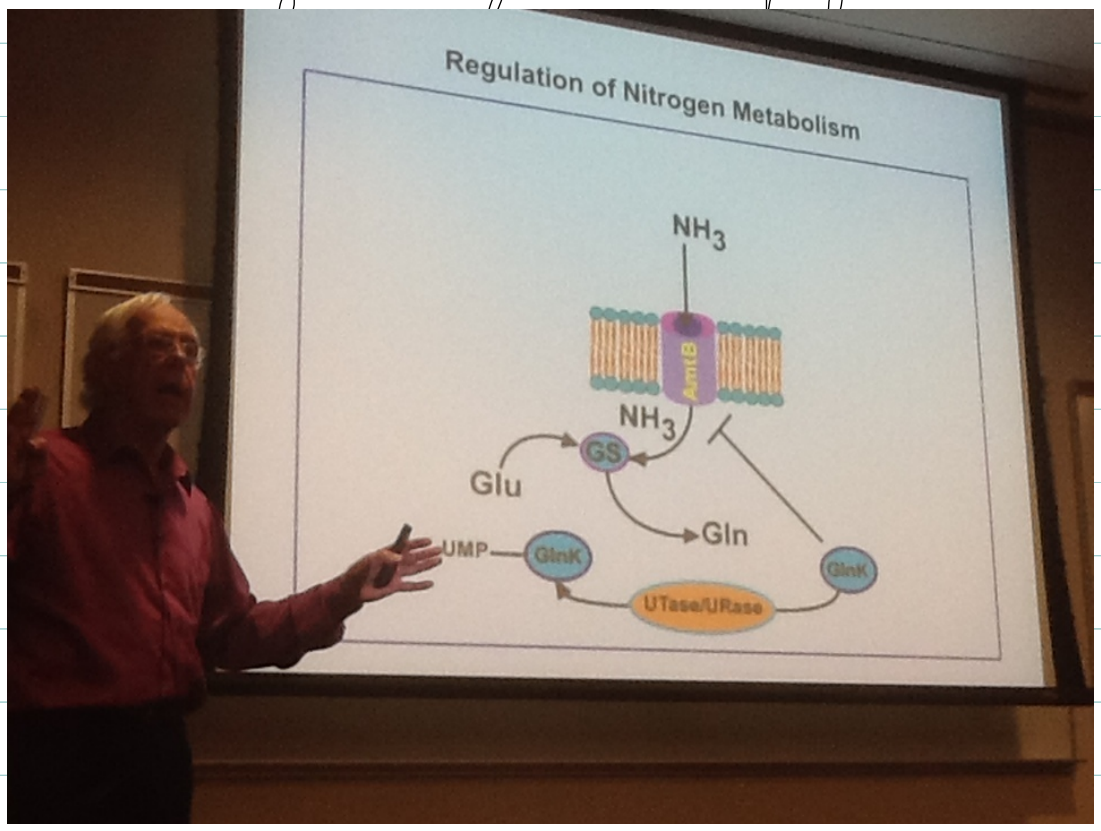
9/6/12 @ 4:00 PM
Gas Channels Worksop
Robert Stroud, UCSF
What do structures tell us about Gas Channels? QED!

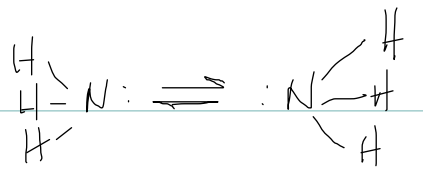
Much harder to discover channels for neutral substances (H_2O, 1990 .. gases only now).

QED: Quantum Electrodynamics

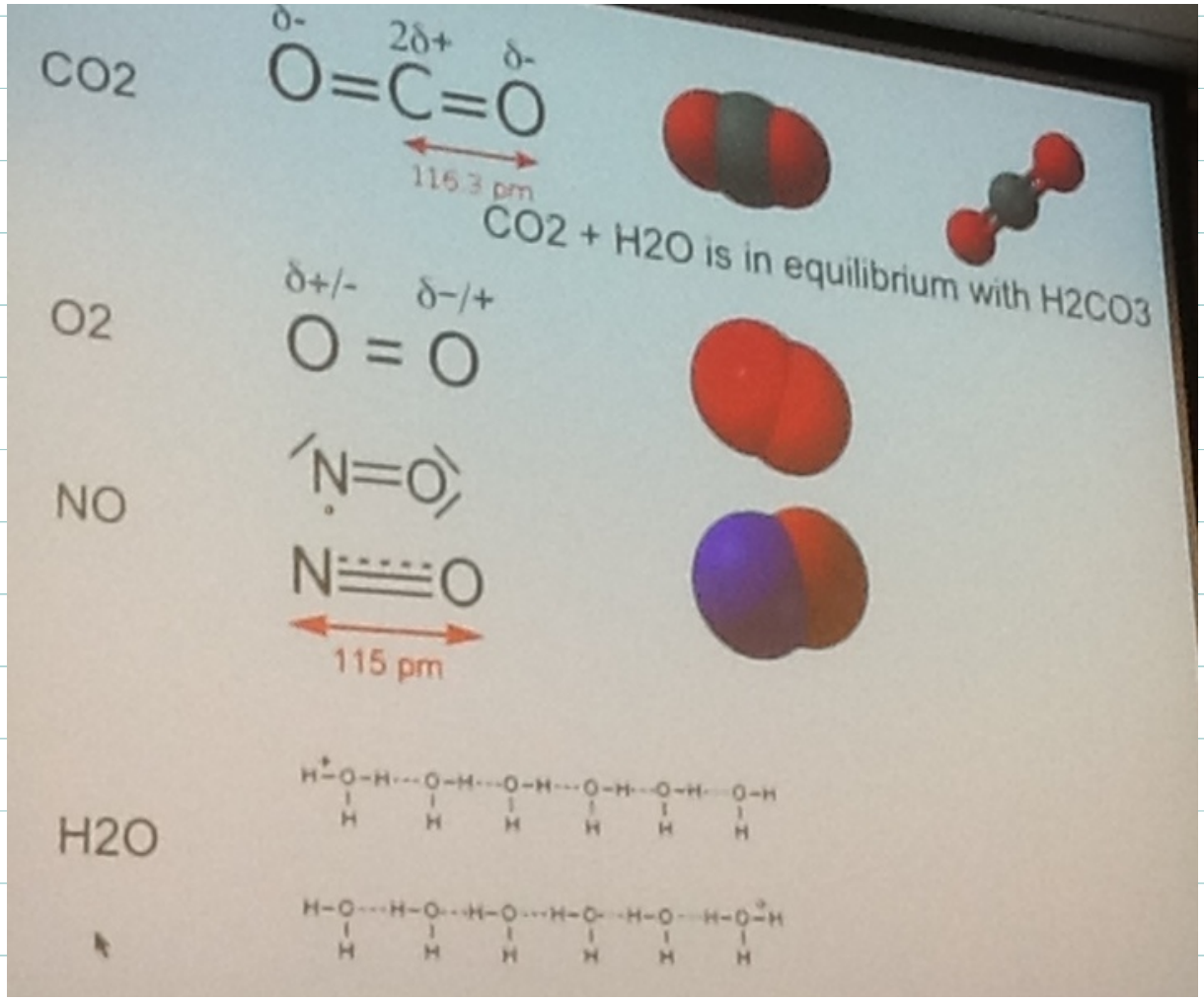
I. Rh Family (Amt/MEP)
Bact. Yeast

Bact: reg. N as food source, pref. as NH_3





Rapid inversion
Dipole moment ~ H_2O



Why is H_2O not a gas ... H-bonding

But H_2O can be a gas! WB

Some still think that Rh prot. are NH_4^+ channels!

AmtB : 1.35 Å

11 TMs

inverted repeat

NH_3: Black hole of crystallography

$10e^-$ → also Na^+, Mg^{++}

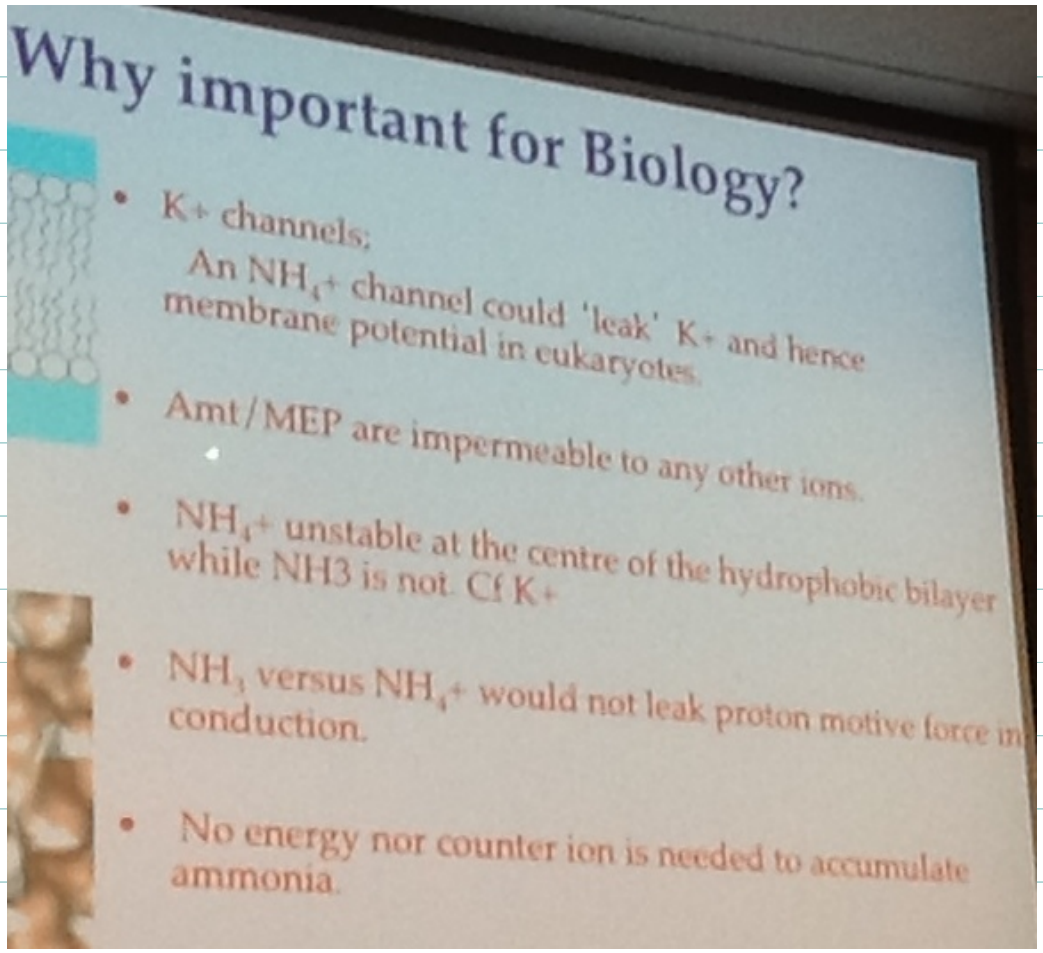
Can also use $MeNH_3$

Channel (pore): No H_2O. 1/7 occupancy by NH_3 @ 3 sites.

pKa 9.6 → < 7

binding of NH_4^+

No waters, no ions



AmtB

Completely turned off when enough NH_3 is around!

GlnK: Finger points to AmtB & blocks it.

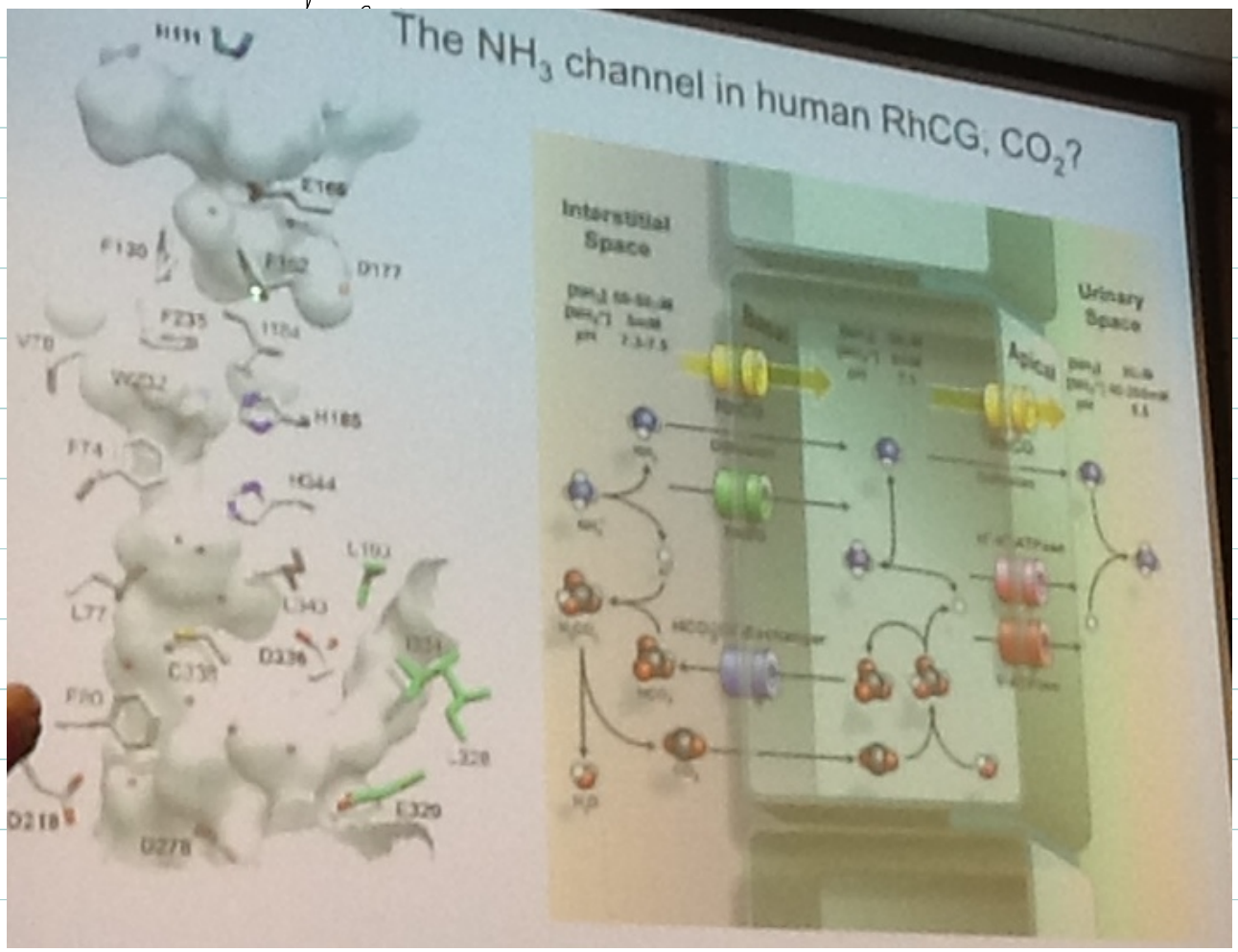
ADP bound

Prevents reprotonation of entering NH_3.

Nitrosomas europea ... more similar than Amt to mammalian Rh. Has a 'stalk' extending into cytosol. (They do not pay much attn. to it)

NeRh ≠

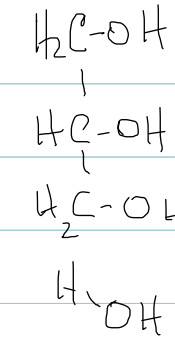
RhCG: Expressed in HEK293s



"NIH Common Fund" 4th NIH Roadmap meeting
Nov. 28-30
SFO: Westin Hotel

II. Aquaporins

Glycerol is Water like

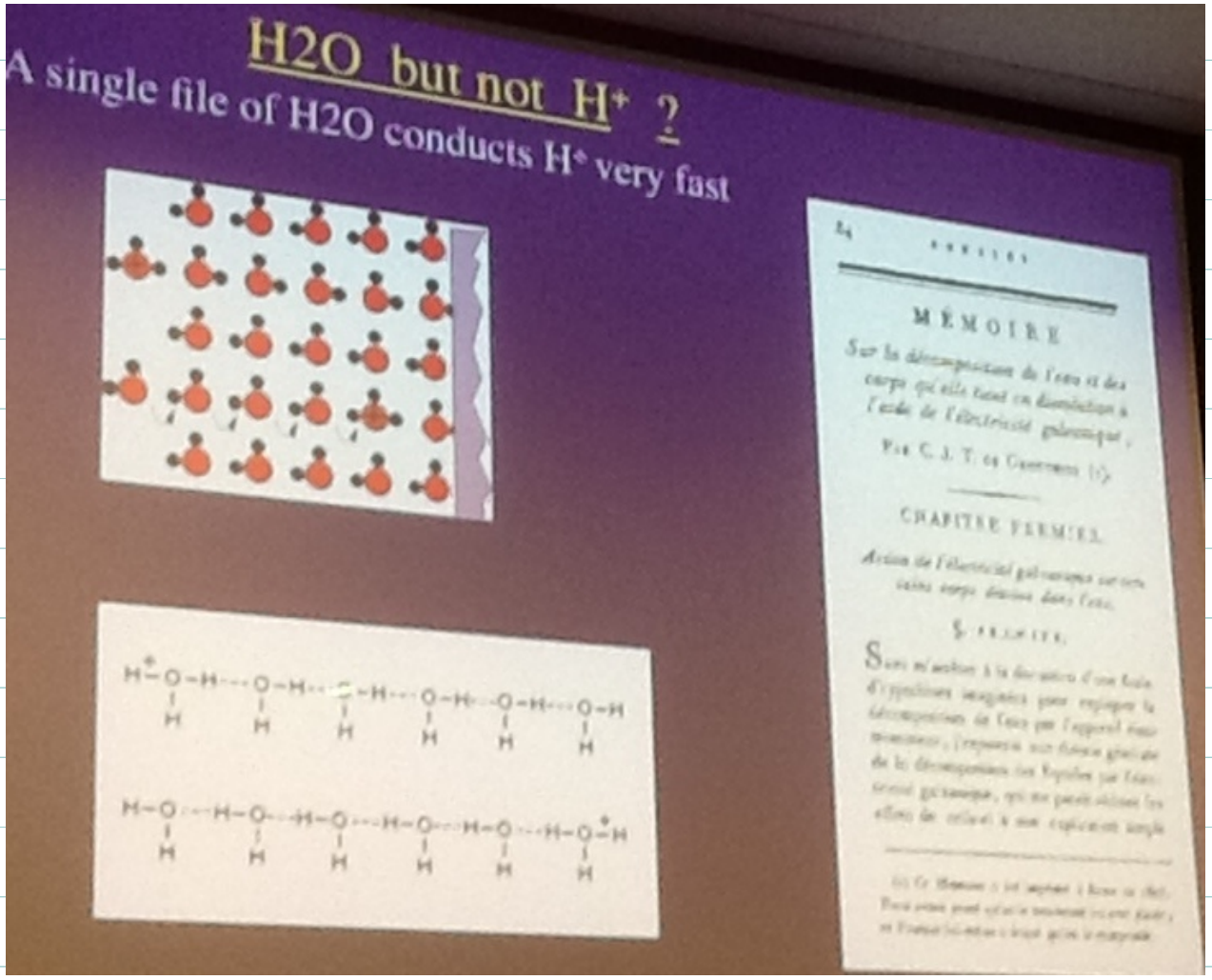


PfAQP: Plasmodium → Glycerol

1.8 Å - Rotamers can be unambiguously determined

9 H_2O molec. in a chain

- H_2O density clear
- Solutes can be identified



Beitz et al PNAS, 2004

An AQP that transport H_2O & Glycerol both v. well.

↖ Malaria

One mutation: Glu → Ser (way up above aquapore) → H_2O permeability.

↓

Removes 1 H bond.

Channel holds on to H_2O.

9/7/12 @ 8:30 AM
Gas Channels Worksop
Ryan Geyer
O2 Transport in RBCs

Ryan: What was sampling rate?
Dead time?
9 wavelengths: PMT, array?
Fumbling ∈ details cost you control of presentation.
Showing non-case KOs ∃ their controls was shooting yourself in the foot.

- Q_{10}: ?
- What was Verkman's Q_{10} for P_f? 4?
- Ck MCV: Is there a s/v Δ. Ck Hb.
- Ck P_{50} in WT vs KO vs blockers.
- 2,3 DPG, pH, etc ⇒ are addressed by P_{50}.

[WB interpretation: we'll be OK, but we have to dot i's & cross t's to be sure that your Δs are not due to something other than the cell membrane.]

9/7/12 @ 8:30 AM
Gas Channels Worksop
Rossana Occhipinti
Mathematical modeling

WB: Would be nice to have a dye to monitor plts.

DRR: spelling error.

Jeff: Animal vs. Vegital poles

Bhanu: Optical tweezers -> viscosity across the entire diameter.

9/7/12 @ 9:30 AM
Gas Channels Worksop
Xue Qin
CO2 permeability of AQP5

Emad : Rotamer search ... what is stable?

Gas channels Workshop

September 6-7 2012

Lecture 1 : Walter Boron – Gas Channels

- Solubity theory
  $P \propto S_L / S_W$

  Note: Henry's law is true at steady-state
- Solubility-Diffusion theory
- Access-Solubility-Diffusion-Egress theory

Lecture 2 : Emad

Newtonian equations

Major limitation → time scale (speed limit : 1fs)

Force field approximations

atomistic resolution

Implicit Ligand sampling $W(r) = -k_B T \ln \left[\frac{p(r)}{p_0} \right]$

$$F(z) = -RT \ln \frac{\sum e^{-F(x,y,z)/RT}}{\dots}$$

Lecture 3: Gerolf Gros – Measuring CO_2 permeability by ^{18}O Exchange

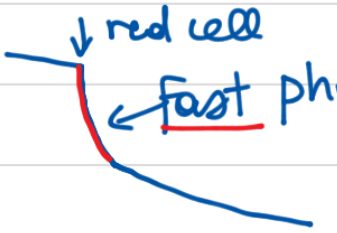
Techniques:

pH gradients in the surface of lipid bilayer

$t_{1/2}$ of CO_2 uptake ~ 12 ms (Endeward et al 2008)
In the case of CO_2 kinetics, stopped flow is not good

we have chemical eq but not isotopic equilibrium ⇒ take advantage of this in ^{18}O technique

$P_{HCO_3^-}$
P_{CO_2}
CA activity
} are the 3 main parameters



phase where P_{CO_2} dominates

monitor pHs continuously

how do extract P_{CO_2} ?

6 ODEs

Estimate P_{CO_2}, P_{HCO_3}, A_{in}, A_{out}

A_{in}, A_{out} — estimate first

fitting procedure

excellent fit

Phase 1



$t_{1/2} = 5s$ for $CO_2 \leftrightarrow HCO_3^- + H^+$

$t_{1/2} = 250s$ (isotopic exchange)

Phase 2

volume fraction of RBC is very critical (↑v => 6 time faster)

trick = use small v to reduce the time resolution for mass spectrom

P_{CO_2} = 0.15 cm/sec by RBC

Sensitivity

Keq is important

Ai is very critical parameter

ptle is also " " "

$\Rightarrow$ Ai and ptle need to be controlled

ptli is not critical

P_{H_2O} " "

How about ULs?

theoretical hydrodynamics

$\delta \sim$ viscosity $\upsilon \times \sqrt{\text{cell diameter } l} \Rightarrow$

$\upsilon = 0 \Rightarrow \delta = 0$

↑ dextran $\Rightarrow$ ↑ δ for CO_2

Extrapolate to $\upsilon = 0$

P_{m,CO_2} = 0.16 cm/sec

$P_{CO_2}^{app}$ in saline = 0.12 cm/sec

$\Rightarrow \delta$ = 0.5 μm in saline

$P_{CO_2} = 0.15$ cm/sec → 50% due to AQP1

↘ 50% due to Rh proteins

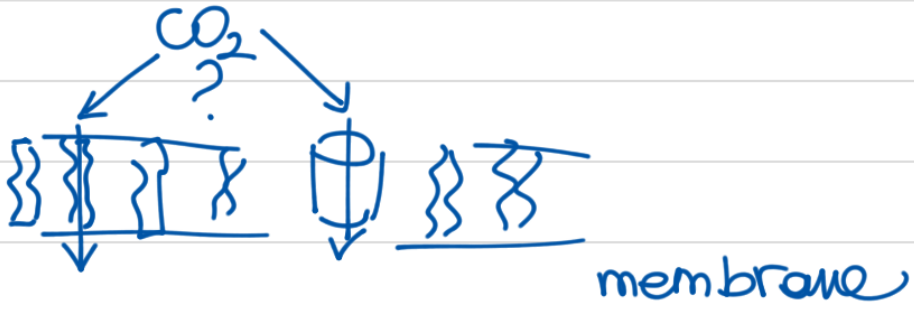
Endeward et al, 2008

2 channels

$P_{CO_2} \approx 100\, P_{HCO_3^-}$

Lecture 4: Endeward – Intrinsic CO_2 permeability of cell membrane

P_{CO_2} = 0.015 cm/sec in RBC AQP1 & Rh null



Vesicles with ≠ cholesterol content

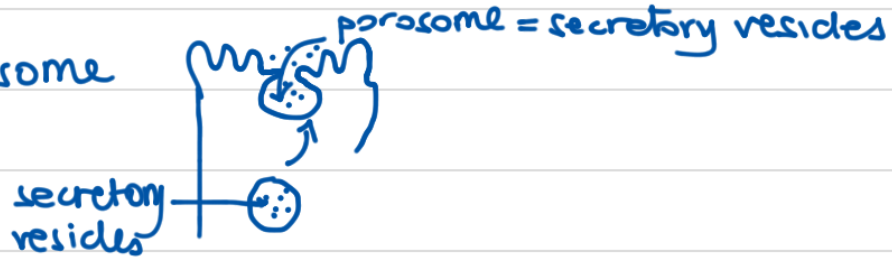
↑

contains CA II

Afternoon Sessions

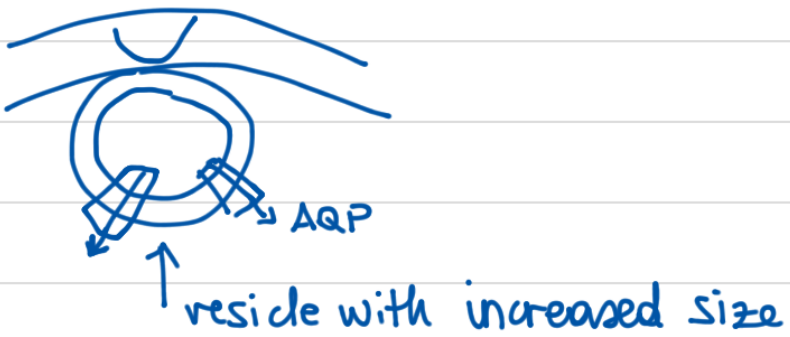
Lecture 5: Bhanu P. Jena - Involvement of elevated membrane cholesterol on G-protein regulated H_2O and gas transport in biological membranes

Porosome



We will focus on the porosome plasma membrane

in synaptic vesicles



Jena et al 1997, PNAS

Lecture 6 : Jeff Garvin – Movement of NO across cell membr.

First described by Furchgott in 1980
L-arginine → L-citrulline + NO

Why do we care about NO? – involved in brain CNS
– mitochondrial respiration
– . . . .

NO
↑ small, non-polar
reactive
is a gas

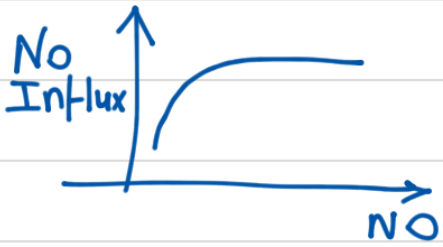
partition coefficients are measured @ equilibrium
" " say nothing about rates

Why does the heart have AQP1? It doesn't need H_2O so why?
Hp: AQP1 transports NO

Measurements: cultured cells & fluorescence
① P_{NO} correlates with P_F

② ↑ AQP1 ⇒ ↑ NO expression

③ Inhibitors of AQP1 reduce NO fluxes

④ 

⇒ NO Influx is saturable

⑤ Purified AQP-1 increases NO transport

Conclusion:
⇒ AQP1 transport NO

How about other AQPs ?
AQP3 transports NO but not as rapidly as AQP1 - Same for AQP4

Is it physiologically relevant ?
Use Aortic ring preparation

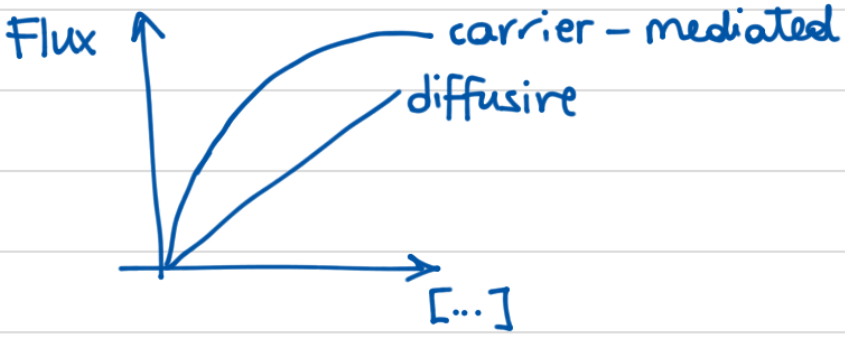
Ach

Not been able to calculate P_{NO}

Q/A:
NO electrode probably measures changes in blood flow

Lecture 7: David Weiner - Role of Rh proteins in NH_3 gas transport

Is collecting duct NH_3 diffusive or transporter-mediated ?



Data show both saturable & diffusive

$$J_{Tot} = \underbrace{J_{trans}\left([MA]/([MA]+Km)\right)}_{\text{saturable component}} + \underbrace{J_{diff}\,[MA]}_{\text{linear component}}$$

Handlogten et al, AJP Renal, 2004

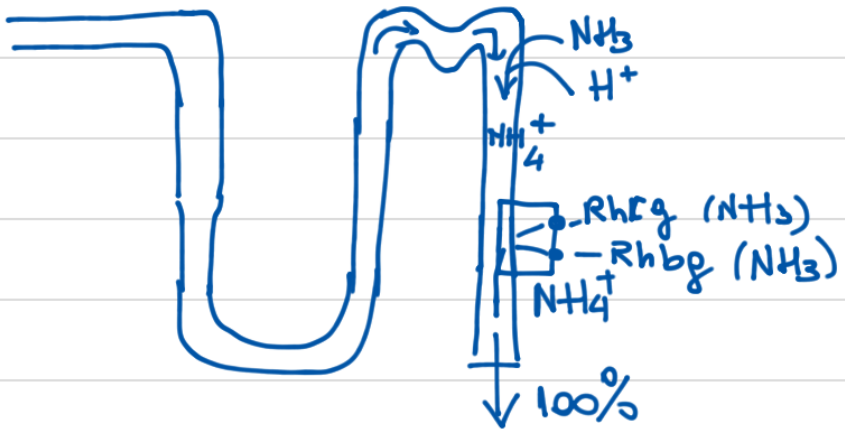
Are Rh proteins present in cells with NH_3 transport ?

RhAG  in RBC ....

RhbG in liver, kidney, sweat glands, intestine, lungs

↑ when you sweat, NH_3 ↑

RhcG in kidney, brain, testis, intestine, liver, skeletal Muscle



Rh are present in cells that transport NH_3

MAc increases Rhcg expression

Rhcg & Rhbc expression increase in :

1) MAc
2) Ischemia-reperfusion injury
3) pt acidosis
4) etc ...

Keynote speaker: Robert Stroud

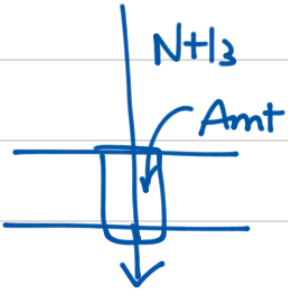
What do structures tell us about gas channels? QED!

(2) families of membrane proteins that can move gases
gases are uncharged

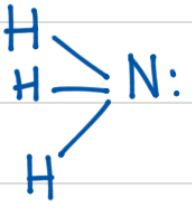
→ Rh family
AQP family

Ammonia Transport : Amt/MEP/Rh Family
↑
in bacteria

Nitrogen Metabolism in bacteria



low pH $NH_4^+ \longleftrightarrow NH_3^+ + H^+$ high pH
pK=9.25



δ^- $2\delta^+$ δ^-
O=C=O

Dipole moment

$\delta+/-$ $\delta+/-$
O=O

$NH_4^+ \longleftrightarrow NH_3 + H^+$

NH_3 channel

AmtB Crystallography

↑ trimer

lyphosome
AmtB conducts NH_3 but not H_2O

Wed 28th November : http://rmi2012.org/ San Francisco

Day 2 09/07/12

Xue Qin : AQP5

$\Delta pHs^* = (\Delta pHs)_{AQP} - (\Delta pHs)_{H_2O\ control\ /\ daily\ matched}$

T41 in AQP5
L43

No significant change in L43 mutations
Interesting changes for T41

movement of ions in the central pore
In order to see what happens we need the crystal structure

The central pore ← what is the best molecule to see what goes through the central pore

AQP6 carries very little H_2O or none
Do something to the CO_2 permeability without affecting the H_2O permeability

crystal structure ~ difficult

O_2 diffusion through cavities

nice packing between the helices
partition coefficient of water to octanol → hydrophobic channel

DIDS has no significant effect on the water permeability in AQP5 (and probably to all AQPs)

AQP4 in astrocytic endfeet
↑ P_f is insensitive to DIDS
↑
non specific
you get specificity by making mutations (in NBCs)
but for AQPs we do not know where the binding site is.

glycosylation

reaction that is covalent

Wisdom : cystines within the central pore. To do : add mercury
L43C mutant : CO_2 permeability is normal
ND96 solution
reacts those cystines with other things

AQP5 has the biggest spike
DDT doesn't do anything ...

expose to a solution to be oxidized
T41C is probably misfolded

Workshop meeting - Look at future directions

① Which other families of gas channels might be there?

So far we have looked at

CO_2

NH_3 general medicine

O_2 : EPR, Optical /Hb; we want to measure fluxes of O_2 and we want to do it faster

NO : Hb

CO : Hb

CH_4 : swamp bacteria

H_2S : purple bacteria

N_2 : nitrogenase - Raman Spectroscopy (fast but not sensitive

Ethylene : plants

H_2

How do we measure N_2 fluxes?

^{13}N - NMR (not very sensitive, slow)

$H_2S \rightleftharpoons H^+ + HS^-$

pH measurements

Signaling gas

Optical /Hb

② What other families of gas channels might be there?

- AE1; GLUT1/4; AQP1, Rh, MCT-1

RBCs proteins

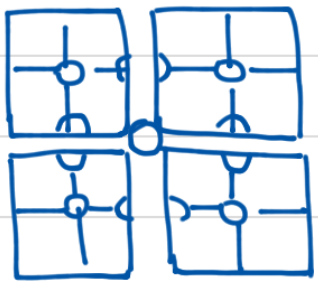
- Endothelial cells in capillaries
- BBB, BRetinaB
- BTB, BOB
- Lungs: AQP5
- Striated Muscles ... myoglobin
- Mitochondrion: CO_2 is formed into the matrix - AQP8, AQP9 cytocrome oxidase

③ Physiological implications ?

- Exercise
- Size scaling; Allometry: might expect to see a lot of gas channels in mice, but not in elephants
- Fish gills
- Zebrafish (swim bladder)

Effects of pressure on gas permeability

Pharmacological Interrention



TETRAMER

DOE experiments (Jing Lu)
NBC as a CO_2 channel

ONR global Funding Opportunities

Director's initiative : point of contact

Notes on the Discussion following Xue Qin's talk:

Clustering of AQPs: Does the gas permeability increase with clustering, suggesting an inter-tetramer contribution to permeability?

How to attack the central-pore hypothesis? The outermost residue is T41 in TM2. Reducing agents or Cu^{2+}? In a T41C mutant, Cu^{2+} should bind to Cys residues and block, reversibly. Zn^{2+} or Ni^{2+} could coordinate with His in a T41H mutant. Bob Stroud thinks that we should use $HgCl_2$ rather than pCMBS. Worries about DIDS being non-specific. Dose-response for DIDS? Others.

General Discussion

I. What gases should we be interested in?

- CO_2
- NH_3: general medicine. Agriculture. Microbiology (Army: Infectious diseases).
- O_2: Optical/Hb. Phosphorescence (if fast). EPR.
- NO: Optical/Hb. Electrodes
- CO: Optical/Hb.
- N_2: nitrogenase to turn it into NH_3? Raman Spectroscopy (fast but not very sensitive). Surface enhancement with gold particles? Agriculture. Microbiology. ^{13}N-NMR (not very sensitive … talk to NMR guys)
- H_2S: Purple bacteria. H_2S electrode. $H_2S \rightleftarrows H^+ + HS^-$ … could one use pH? Hibernation gas (LaManna). Signaling gas. Optical/Hb (650-ish nm).
- CH_4: Swamp bacteria
- Ethylene: signaling in plants
- H_2: would it need a channel

II. What other families of gas channels might there be?

Any multimeric membrane protein whose monomers are functionally active (excludes ion channels)

- RBC proteins/O_2: AE1, GLUT1/4, AQP1, Rh, MCT-1 (all ≥ 100k/cell) … dozens of proteins 10k-25k copies/cell.
- Endothelial cells in capillaries, etc:
- BBB, BRetinaB (Pigment epithelial)
- Blood-testis barrier, blood-ovary barrier
- Lungs: AQP5 (no alveolar Rh proteins).
- Striated Muscle … myoglobin
- Mitochondrion: MIM. CO_2 is formed in the matrix. Perhaps O_2 as well? AQP8, AQP9 (MIM). AQP5. H_2O???
- Associations …. Proteomics.
- Connexins, pannexins, and similar proteins
- Strategies for finding new kinds of channels: (1) subjecting mice to chronic hypoxia and harvest RBC … proteomic analysis. Check mRNA levels in retics. Normalize to 18S RNA, etc … proteomics, lipidomics (↓ cholesterol), MCV (surface-volume ratio), P50 (pH_i, 2-3-DPG). Splice variants change?

III. What are the physiological implications of gas channels?

The gas-channel hypothesis, if true, would be a major paradigm shift … changing the way we think about all processes involving gases. Game changer. Definitive health and performance issues. Gas channels provide:

- High flux
- Selectivity
- Control by signal transduction
- Pharmacological intervention: block or stimulate (signal-transduction: trafficking, post-translational modification) a specific pathways for specific gases, in specific places.
- Performance→Exercise, athletics, Warfighter performance, altitude: AQP1-null mouse has a 50% voluntary exercise deficit (performance defect). Worse at altitude. Could be due to CO_2 retention, reduced NO flux (less exercise-induced vasodilation)? Treadmill. RhAG-null. If we ever find the O_2 channel(s) … those KOs? NO channel
- Performance→mental: AQP1-null mouse has a 50% voluntary exercise deficit. Do AQP4-null mice have ↑ cerebral capillary density to compensate for low O_2 permeability? … but downside is susceptibility.
- Cerebral edema. Stroke, TBI, AMS … Aeromics has a drug that blocks the aquapores of AQP4 (and AQP2) … we hope not the gas. After the first 3 days of stroke, when edema is resolving … stimulate AQP4
- AQP5: gas permeability of the lungs … but downside is susceptibility. pulmonary edema. Selective drug to block P_f and stay away from gas (if it is important).
- Effect of pressure on gas permeability. In Fish … Different channels or splice variants at different depth.
- HRE (hypoxia-response elements): which proteins unexpectedly have HREs. HIF-1α.
- Shear stress: ↑ expression of NOS
- Are different splice variants used under different conditions?
- Size scaling, Allometry: Might expect to see a lot of gas channels in mice, but not in elephants. Also a lower O_2 consumption/gram.
- Fish gills. Compare tuna to a flounder.
- Horse has a wide range of performance.
- Joe LaManna: membranes with low intrinsic permeability—lots of proteins or cholesterol—and a high O_2 requirement, would be most likely to have gas channels. Optimizes human performance.
- Exclude gas:
- Transport gas directionally.
- Wound healing, bone-fracture healing.
- COPD: CO_2 retention,
- Stroke, MI … low gas permeability could contribute to the development of the problem??? Increasing gas permeability could help in recovery.
- Decompression illnesses (DCS +AGE, arterial gas embolism): ↓ N_2 permeability on the way down (would also solve N_2 narcosis) … increase it on the way up.
- O_2 toxicity:
- CO_2 narcosis:
- N_2 narcosis:
- Submarine escape … DCI.
- Acute mountain sickness … hypoxia
- Increase O_2 transport into tumors just before radiation
- Bacteria that need to transport gas/antibiotic. Helicobacter …
- Parasites … inhibit gas transport … if the organism has a sufficiently phunky gas channel

IV. How do gases pass through the gas channel?

- Monomeric pores: AQP1 aquapores, Rh ammoniapores, UT urea pores
- Central pores (3- or 4-fold axes of symmetry)
- Side pockets (e.g., between the edges of 2 AQP tetramers)
- Corner pockets (e.g., at the corners of 4 AQP tetramers)
- Packing?: Emad tried to pack AQP4 monomers based on Fujioshi's/Engel's EM data. He thinks that the monomers did not get close enough together. The only reference they had. Could one do Atomic Force Microscopy (Jeff)?
- WFB/Jeff: Might it be possible to push the sides of tetramers together to see if the sides like to be together?
- Arrays: AQP4/M1 forms very small arrays, AQP4/M23 (BBB) form extended arrays of tetramers. Verkman found that it is aa17-22 in M1 that obstruct array formation. AV took MM23 Nt and transplanted it to AQP1 and got AQP1 to form arrays.
- Nanotubes, peptides that form channel-like structures … NSF … conduct CO_2, O_2, etc. Could be used as sensors. Cannot emphasize medical side. They fund the basic science. Plants.

V. How can we better model the movement of gases across cell membranes?

- More crystal structures
- More molecular dynamics

VI. What funding mechanisms are possible?

Early on, we have to hit at least one home run.

- ONR-BRC (Basic Research Challenge): Navy.
- ONR-MURI (Multi-Univ Research Initiative): OSD (Office of the Secretary of Defense) oversight.
- ONR-Undersea Medicine/Stress Physiology:
- ONR Young Investigator: Tenure track.
- Chief of Naval Research (CNR/2*)
- DHP (Defense Health Program): Army is the agent. Warfighter protection/performance … AMS, …
- PPG NIH-HL/Hypertension:
- PPG NIH-HL/Blood:
- PPG NIH-HL/Lung:
- PPG NIH-DK/: NH_3 via Rh and AQPs. Acid-base balance.
- Director's Initiative ???: Sept 25 … no preliminary data necessary … high risk/high impact. Up to $5M/year. Could we get: Point of Contact. List of past recipients. Also train future scientists to carry torch. Most of winners are Associate Professor.
- ONR-G (ONR Global)/Foreign Only: VSP (Visiting Scientist Program), meetings, NICOP
- NSF: nanotubes, etc.
- Dept of Agriculture: N_2 fixation (must be done in the absence of O_2)

Ignore the following:

ACh-induced ↓ in resistance … how affected by AQP1 KO?

WFB: We need to get together in-silico and stay in touch … plan grant applications.
Jeff: we need to be focused … in each grant … stay out of KMBD (kiss of death) … aim for Kidney Pathobiology and Urologic Diseases, Hypertension and microcirculation.

Rose:

- Please collect notes from volunteers
- PPTs
- Set up a teleconference in 1 month
- Send out these notes for annotation
-