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6. AUTHORS
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14. ABSTRACT
This report describes the UCSB contributions to the Coagulopathy effort to Aim 5: Develop, Validate and Analyze a Comprehensive Mathematical Model of the Human Clotting Subsystem. Our accomplishments are in two areas: mathematical modeling, and data analytics.We made substantial progress in our Aim 4b work. We made considerable headway in the development of a new real-time sensor platform that can continuously measure protein analytes with sub-minute temporal resolution in undiluted whole blood. We have demonstrated the performance of this device in vitro on a model target and are working on increasing device stability for long-term in vivo operation

15. SUBJECT TERMS
Mathematical modeling, blood coagulation, disease states of coagulopathy in trauma, survival analysis

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a. REPORT	b. ABSTRACT	c. THIS PAGE			Francis Doyle
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Report Title

Final Report: Systems Biology of Coagulation and Trauma-Induced Coagulopathy

ABSTRACT

This report describes the UCSB contributions to the Coagulopathy effort to Aim 5: Develop, Validate and Analyze a Comprehensive Mathematical Model of the Human Clotting Subsystem. Our accomplishments are in two areas: mathematical modeling, and data analytics. We made substantial progress in our Aim 4b work. We made considerable headway in the development of a new real-time sensor platform that can continuously measure protein analytes with sub-minute temporal resolution in undiluted whole blood. We have demonstrated the performance of this device in vitro on a model target and are working on increasing device stability for long-term in vivo operation. In addition, we have made significant progress in the development of two new ultrasensitive protein detection techniques that can quantitatively discriminate small-fold changes in extremely low-abundance protein levels in complex media such as blood serum. We have demonstrated that our techniques offer performance far superior to that of gold-standard commercial assays.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
03/20/2017	26	Yuanyang Zhang, Tie Bo Wu, Bernie J. Daigle, Mitchell Cohen, Linda Petzold. Identification of disease states associated with coagulopathy in trauma, BMC Medical Informatics and Decision Making, ( ): . doi: 1,035,741.00
03/22/2017	32	Jinpeng Wang, Jingwen Yu, Qin Yang, John McDermott, Alexander Scott, Matthew Vukovich, Remy Lagrois, Qiang Gong, William Greenleaf, Michael Eisenstein, B. Scott Ferguson, H. Tom Soh. Multiparameter Particle Display (MPPD): A Quantitative Screening Method for the Discovery of Highly Specific Aptamers, Angewandte Chemie International Edition, ( ): 744. doi: 1,035,792.00
03/22/2017	28	Faye Yi Fong, Seung Soo Oh, Craig J. Hawker, H. Tom Soh. In Vitro Selection of pH-Activated DNA Nanostructures, Angewandte Chemie International Edition, ( ): 15258. doi: 1,035,743.00
03/22/2017	27	Yuanyang Zhang, Tie Bo Wu, Bernie J. Daigle, Mitchell Cohen, Linda Petzold. Identification of disease states associated with coagulopathy in trauma, BMC Medical Informatics and Decision Making, ( ): . doi: 1,035,742.00
03/22/2017	30	Andrew T. Csordas, Anna Jørgensen, Jinpeng Wang, Emily Gruber, Qiang Gong, Elizabeth R. Bagley, Margaret A. Nakamoto, Michael Eisenstein, H. Tom Soh. High-Throughput Discovery of Aptamers for Sandwich Assays, Analytical Chemistry, ( ): 10842. doi: 1,035,790.00
08/20/2014	6	Hong Li, Linda R. Petzold, Jin Fu, Sheng Wu. The time dependent propensity function for acceleration of spatial stochastic simulation of reaction–diffusion systems, Journal of Computational Physics, (10 2014): 0. doi: 10.1016/j.jcp.2014.06.025 331,664.00
08/23/2016	18	Adithya Sagar, Wei Dai, Mason Minot, Jeffrey D Varner. Reduced order modeling and analysis of the human complement system, Scientific Reports, ( ): . doi: 1,014,815.00
08/23/2016	25	Minseon Cho, Seung Soo Oh, Jeff Nie, Ron Stewart, Monte Radeke, Michael Eisenstein, Peter Coffey, James Thomson, H. Tom Soh. Array-based Discovery of Aptamer Pairs, Analytical Chemistry, ( ): 821. doi: 1,014,912.00
08/23/2016	24	Ola Jakobsson, Seung Soo Oh, Maria Antfolk, Michael Eisenstein, Thomas Laurell, H. Tom Soh. Thousand-fold volumetric concentration of live cells with a recirculating acoustofluidic device, Analytical Chemistry, ( ): . doi: 1,014,910.00
08/23/2016	22	Hao Qu, Andrew Csordas, Jinpeng Wang, Seung Soo Oh, Michael Eisenstein, Hyongsok Tom Soh. Rapid and Label-Free Strategy to Isolate Aptamers for Metal Ions, ACS Nano, ( ): A. doi: 1,014,831.00
08/23/2016	20	Adithya Sagar, Jeffrey Varner. Dynamic Modeling of the Human Coagulation Cascade Using Reduced Order Effective Kinetic Models, Processes, ( ): 178. doi: 1,014,822.00

08/23/2016	19	Adithya Sagar, Christine Shoemaker, Jeffrey Varner. Dynamic Optimization with Particle Swarms (DOPS): A metaheuristic for parameter estimation in biochemical models, Biotechnology Journal, ( ): . doi:	1,014,816.00
08/26/2015	12	Adithya Sagar, Jeffrey Varner. Dynamic Modeling of the Human Coagulation Cascade Using Reduced Order Effective Kinetic Models, Processes, (03 2015): 0. doi: 10.3390/pr3010178	364,313.00
08/26/2015	16	Faye M. Walker, Kareem M. Ahmad, Michael Eisenstein, H. Tom Soh. Transformation of Personal Computers and Mobile Phones into Genetic Diagnostic Systems, Analytical Chemistry, (09 2014): 0. doi: 10.1021/ac5022419	364,320.00
08/26/2015	15	Seung Soo Oh, Jeff Nie, Ron Stewart, Monte J. Radeke, Michael Eisenstein, Peter J. Coffey, James A. Thomson, H. Tom Soh, Minseon Cho. Array-based Discovery of Aptamer Pairs, Analytical Chemistry, (01 2015): 0. doi: 10.1021/ac504076k	364,319.00
08/26/2015	14	Ola Jakobsson, Seung Soo Oh, Maria Antfolk, Michael Eisenstein, Thomas Laurell, H. Tom Soh. Thousand-Fold Volumetric Concentration of Live Cells with a Recirculating Acoustofluidic Device, Analytical Chemistry, (08 2015): 0. doi: 10.1021/acs.analchem.5b01944	364,318.00
08/26/2015	13	Adithya Sagar, Jeffrey Varner, Joseph Wayman. Dynamic Modeling of Cell-Free Biochemical Networks Using Effective Kinetic Models, Processes, (03 2015): 0. doi: 10.3390/pr3010138	364,314.00
08/27/2014	8	Jinpeng Wang, Nupur Maheshwari, Michael Eisenstein, Mary Luz Arcila, Kenneth S. Kosik, Qiang Gong, H. Tom Soh. Particle Display: A Quantitative Screening Method for Generating High-Affinity Aptamers, Angewandte Chemie, (05 2014): 0. doi: 10.1002/ange.201309334	332,620.00
08/27/2014	9	Kevin W. Plaxco, H. Tom Soh, Allen H. J. Yang, Kuangwen Hsieh, Adriana S. Patterson, B. Scott Ferguson, Michael Eisenstein. Accurate Zygote-Specific Discrimination of Single-Nucleotide Polymorphisms Using Microfluidic Electrochemical DNA Melting Curves, Angewandte Chemie, (03 2014): 0. doi: 10.1002/ange.201310059	332,621.00
08/27/2014	10	B. S. Ferguson, D. Maliniak, K. Ploense, R. J. White, N. Woodward, K. Hsieh, A. J. Bonham, D. A. Hoggarth, M. Eisenstein, T. E. Kippin, K. W. Plaxco, H. T. Soh. Real-Time, Aptamer-Based Tracking of Circulating Therapeutic Agents in Living Animals, Science Translational Medicine, (11 2013): 0. doi: 10.1126/scitranslmed.3007095	332,622.00
08/27/2014	11	Kory Plakos, Yi Xiao, Michael Eisenstein, H. Tom Soh, Seung Soo Oh. In Vitro Selection of Shape-Changing DNA Nanostructures Capable of Binding-Induced Cargo Release, ACS Nano, (11 2013): 0. doi: 10.1021/nn404079v	332,623.00
08/30/2011	1	J. Wang, Y. Liu, T. Teesalu, K. N. Sugahara, V. R. Kotamrajua, J. D. Adams, B. S. Ferguson, Q. Gong, S. S. Oh, A. T. Csordas, M. Cho, E. Ruoslahti, Y. Xiao, H. T. Soh. Selection of phage-displayed peptides on live adherent cells in microfluidic channels, Proceedings of the National Academy of Sciences, (04 2011): 0. doi: 10.1073/pnas.1014753108	259,720.00
08/30/2011	2	Steven F. Buchsbaum, Ting-Ting Wu, B. Scott Ferguson, Kuangwen Hsieh, Yi Xiao, Ren Sun, H. Tom Soh. Genetic Analysis of H1N1 Influenza Virus from Throat Swab Samples in a Microfluidic System for Point-of-Care Diagnostics, Journal of the American Chemical Society, (06 2011): 0. doi: 10.1021/ja203981w	259,721.00
08/30/2011	3	C. Anders Olson, Jonathan D. Adams, Terry T. Takahashi, Hangfei Qi, Shannon M. Howell, Ting-Ting Wu, Richard W. Roberts, Ren Sun, H. Tom Soh. Rapid mRNA-Display Selection of an IL-6 Inhibitor Using Continuous-Flow Magnetic Separation, Angewandte Chemie International Edition, (07 2011): 0. doi: 10.1002/anie.201101149	259,722.00
08/31/2012	5	Sheng Wu, Jin Fu, Hong Li, Linda Petzold. Automatic Identification of Model Reductions for Discrete Stochastic Simulation, J Chem Phys, (07 2012): 0. doi:	284,131.00
TOTAL:	**25**		

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received	Paper

TOTAL: **1**

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Linda Petzold

University of Washington, Seattle, November 2016

University of California Santa Barbara, Center for Adaptive Network Dynamics workshop, December 2016

Jeff Varner

Sagar A, and J. Varner "Dynamic Modeling of Human Coagulation Cascade Using Reduced Order Effective Kinetic Models", ACS National Meeting, San Diego, CA, March 2016 (Winner: ACS Peterson Travel Award)

LeCover, R, Sagar A, Orfeo T, Brummel-Ziedins K, and J. Varner "Dynamic Modeling of Fibrinolysis using Reduced Order Effective Kinetic Models", Military Health System Research Symposium, Kissimmee, FL, August 2016

Number of Presentations: 4.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received		Paper
08/23/2016	21	Abolfazl Doostparast, Linda Petzold, Mitchell Cohen. Direct Higher Order Fuzzy Rule-based Classification System: Application in Mortality Prediction, 2015 IEEE International Conference on Bioinformatics and Biomedicine (BTBM). 10-NOV-15, Washington, DC. : ,

TOTAL: **1**

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received	Paper	
08/31/2012	4.00	Jin Fu, Sheng Wu, Linda R. Petzold. Time dependent Solution for Acceleration of Tau-leaping, Computational Physics (03 2012)

TOTAL: **1**

Number of Manuscripts:

Books

Received	Book

TOTAL:

Received	Book Chapter

TOTAL:

Patents Submitted

None

Patents Awarded

None

Awards

Linda Petzold
SIAM Prize for Distinguished Service to the Profession, 2016

Honorary Doctorate, Uppsala University, Sweden, 2015

Distinguished Alumni Achievement Award, Computer Science Department, University of Illinois UIUC, 2014

SIAM/ACM Prize in Computational Science and Engineering, 2013

Fellow, ACM, 2011

UCSB Faculty Research Lecturer (highest honor awarded by UCSB faculty to one of its members), 2011

Tom Soh

Chan-Zuckerberg Investigator (2017)

Jeff Varner

ACS Peterson Travel Award, Adi Sagar, ACS National Meeting, San Diego, CA March 2016

Top three poster award, Rachel LeCover, Military Health System Research Symposium (MHSRS), Kissimmee, FL, August 2016

Graduate Students

NAME	PERCENT_SUPPORTED	DISCIPLINE
Hamed Ghaffari	49	Computer and Computational Sciences
Arya Pourzanjani	49	Computer and Computational Sciences
Adithya Sagar	100	Computer and Computational Sciences
Nathan Ogden	17	
FTE Equivalent:	**2.15**	
Total Number:	**4**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Faculty Supported

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:..... 0.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME
Adithya Sagar

Total Number: 1

Names of other research staff

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Sub Contractors (DD882)

1 a. Cornell University

1 b. Office of Sponsored Programs
373 Pine Tree Road
Ithaca NY 148502820

Sub Contractor Numbers (c): KK1217
Patent Clause Number (d-1):
Patent Date (d-2):
Work Description (e): The Varnerlab team has developed a multiscale physiologically based pharmacokinetic (P
Sub Contract Award Date (f-1): 9/30/11 12:00AM
Sub Contract Est Completion Date(f-2): 12/14/16 12:00AM

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1 a. Cornell University

1 b. 373 Pine Tree Road
Ithaca NY 148502820

Sub Contractor Numbers (c): KK1217
Patent Clause Number (d-1):
Patent Date (d-2):
Work Description (e): The Varnerlab team has developed a multiscale physiologically based pharmacokinetic (P
Sub Contract Award Date (f-1): 9/30/11 12:00AM
Sub Contract Est Completion Date(f-2): 12/14/16 12:00AM

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1 a. Massachusetts Institute of Technology

1 b. 77 Massachusetts Avenue
Cambridge MA 021394307

Sub Contractor Numbers (c):
Patent Clause Number (d-1):
Patent Date (d-2):
Work Description (e): We propose to study the cytokine and cellular networks mediating the APR to inflammati
Sub Contract Award Date (f-1): 3/30/11 12:00AM
Sub Contract Est Completion Date(f-2): 12/14/15 12:00AM

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1 a. Massachusetts Institute of Technology

1 b. 77 Massachusetts Avenue
Cambridge MA 021394307

Sub Contractor Numbers (c):
Patent Clause Number (d-1):
Patent Date (d-2):
Work Description (e): We propose to study the cytokine and cellular networks mediating the APR to inflammati
Sub Contract Award Date (f-1): 3/30/11 12:00AM
Sub Contract Est Completion Date(f-2): 12/14/15 12:00AM

Inventions (DD882)

Scientific Progress

See Attached.

Technology Transfer

None

Technical Report: Scientific Progress and Accomplishments

Professor Linda Petzold, University of California Santa Barbara

Abstract

This report describes the UCSB contributions to the Coagulopathy effort to Aim 5: Develop, Validate and Analyze a Comprehensive Mathematical Model of the Human Clotting Subsystem. Our accomplishments are in two areas: mathematical modeling, and data analytics.

Key Words

Mathematical modeling, blood coagulation, disease states of coagulopathy in trauma, survival analysis

Accomplishments

Mathematical modeling

We began by implementing and validating a basic ordinary differential equation (ODE) model of the coagulation cascade developed at the University of Vermont. There were computational challenges due to extreme stiffness and rapid change as intermediate species depleted. We developed new techniques to deal with these issues. Then, we developed a partial differential equation (PDE) model, which also required development of a PDE solver that could deal with the extreme stiffness, and validated the simulations on the Aim4b microfluidic experimental results. Also, we developed new algorithms for stochastic simulation of chemical reacting systems, that were motivated by the extreme challenges of modeling the coagulation cascade.

We used our solver to study the role of thrombumodulin and flow in localized coagulopathy. Coagulation has been extensively studied in a well-mixed setting, where the activation of the anticoagulant protein C by the thrombin-thrombomodulin complex eventually leads to an inability of the blood to coagulate. By studying coagulation in a flow model, where some species are present in the endothelium and others are in the flow, we found that in contract, the act of thrombomodulin binding to thrombin at the injury site is the dominant mechanism impairing thrombin generation, leading to an inability of blood to coagulate near the

injury site. Lowering the flow rate exacerbates this process by lowering the supply of pro-coagulants.

Finally, we developed a computational model for hyperfibrinolytic onset of acute traumatic coagulopathy. We proposed that the fibrinolytic response, specifically the release of tissue-plasminogen activator, within vessels of different sizes leads to a variable susceptibility to local coagulopathy through hyperfibrinolysis. We used a partial differential equation model to examine the consequences of vessel geometry differences on fibrinolysis profiles. In addition, we simulated the efficacy of tranexamic acid treatment on coagulopathy initiated through endothelial tPA release, and were able to reproduce the time-sensitive nature of the efficacy of this treatment as observed in clinical studies.
Experiments are currently underway at the University of Vermont to measure endothelial tPA release following injury.

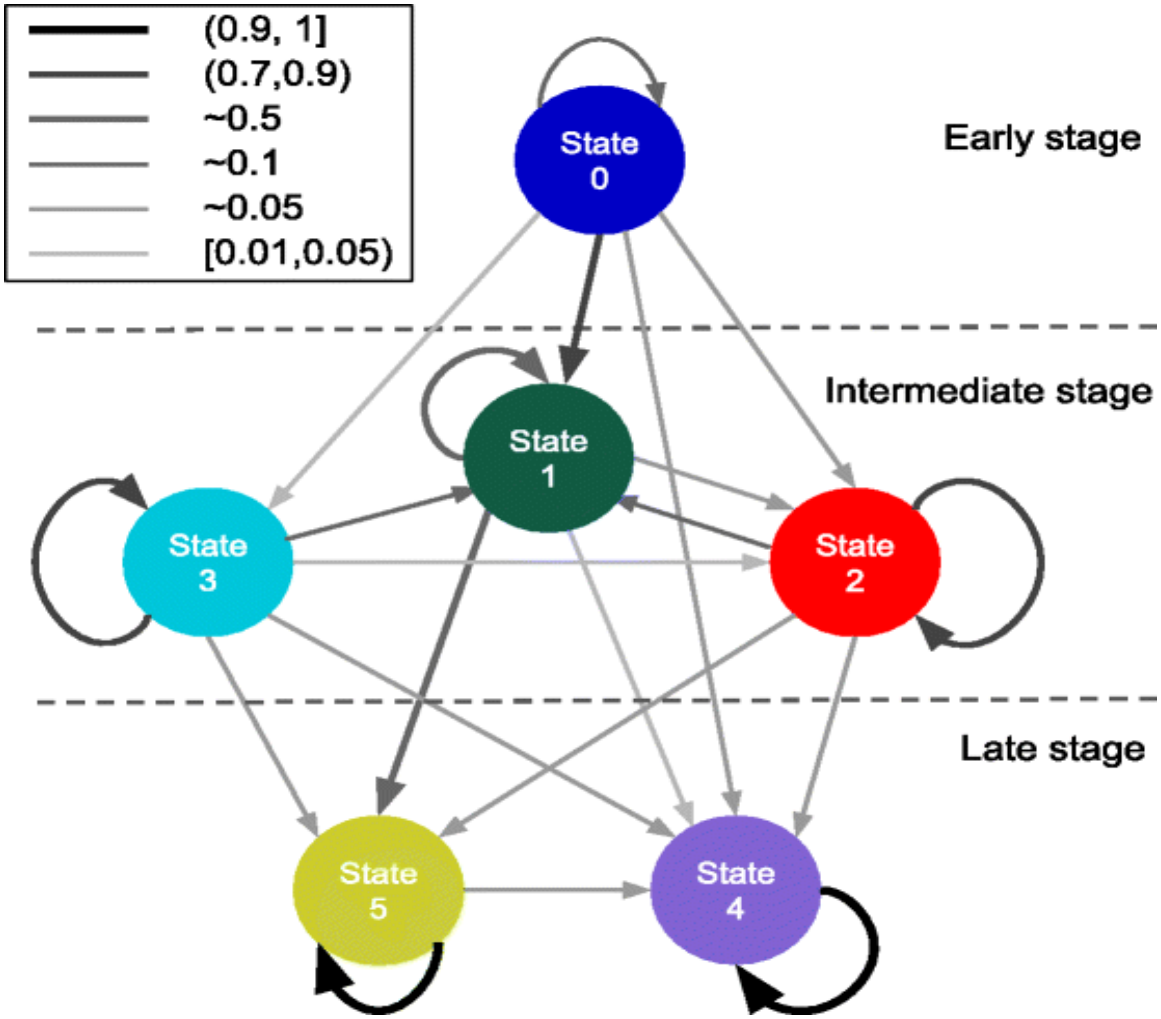
Data analytics

We developed a data-driven method to uncover the progression of patient states associated with coagulopathy in trauma, and to identify critical states that might be targeted for interventions.

In this study, we used time series clinical data for 1413 trauma patients from University of California, San Francisco (UCSF)/San Francisco General Hospital and Trauma Center to identify the disease states. The activity levels of factors II, V, VII, VIII, IX, X, ATIII and protein C, and prothrombin time and partial thromboplastin time were measured at hours 0, 2, 3, 4, 6, 12, 24, 48, 72, 96 and 120 during the course of patients' hospitalization. We applied a hidden Markov model to the temporal data and distinguished 6 disease states and 3 stages through which patients transition after injury. Specifically, we assumed that the blood factor measurements at each time are generated based on a latent disease state, and that the disease states satisfy the Markov property. With these assumptions we applied the hidden Markov model to infer the hidden states from the patients' blood data.

We found that the states fall into 3 stages: early stage (State 0), intermediate stage (State 1, 2 and 3) and late stage (State 4 and 5). We note that the temporal characterization of the stages describes the temporal relationship between the stages, but not the absolute time of occurrence of any of the states. For example, if a state in the intermediate stage is going to change to a different state, it will move into another state in the intermediate stage or a state in the late stage, but it will not move into a state in the early stage. However, that is not to say that a patient cannot arrive already in a state from the intermediate stage or the late stage as that does occur in our data. The state diagram and stage separation are shown below where a

wider and more intense color arrow indicate



Apart from temporal relationships, each state can be characterized by its blood profiles (Fig. 4), its relationships with the other states (Table 3) and its mortality rate (Table 5). We let $P_{m\to n}$ denotes $P(z_t=n|z_{t-1}=m)$. State 0, the only state in the early stage, is characterized by having the fastest clot times and average blood factor levels. We refer to the state in which patients are more likely to remain for one time interval as a *stable* state; otherwise as an *unstable* state. State 0 is the most unstable state, in that $P_{0\to0}$=0.16. Patients in this state are most likely to transition to states in the intermediate stage, with the highest probability of going to State 1 ($P_{0\to1}$=0.77). Since this state is the most unstable, it is the most critical state to influence the patient's trajectory and probably the most promising for intervention.

There are 3 states in the intermediate stage (States 1, 2 and 3). The states in this stage are more stable than the early stage state, but less stable than the late stage states. The states in this stage have the largest variation of mortality rates and therefore are the best indicators in terms of patient outcome. State 0 transitions to State 1 at the highest probability. It is the state with the lowest rate of mortality ($P_{1\to death}$ =0.316) in the intermediate stage. State 1 is characterized by average clot

times and slightly below-average factor levels. This is probably due to the initial coagulation process using up some of the blood factors, which slows down the clot times. It is a relatively unstable state, with a relatively low probability of remaining in the same state ($P_{1\to1}$=0.57). If the patient moves to a different state, he/she is most likely to move to State 5 ($P_{1\to5}$=0.36) or to one of the other intermediate stages at a much lower probability ($P_{1\to2}$=0.05, $P_{1\to3}$=0.004). The next lowest mortality rate among the intermediate stage states is State 2 ($P_{2\to death}$=0.375). It is characterized by high clot times and low blood factors, with the exception of factor VIII which is high. State 2 is a relatively stable state ($P_{2\to2}$=0.76) but is most likely to transition to State 1 ($P_{2\to1}$=0.12) and less likely to transition to the late stage states. State 3 is the state with the highest mortality rate ($P_{3\to death}$=0.6) and is characterized by having the longest clot times and the lowest blood factor levels. It is also a relatively stable state that is most likely to transition to State 1 but can also transition to the states in the late stage.

Lastly, there are two states in the late stage (States 4, 5). Both of these states are very stable and have low mortality. Both of these states have fast clot times and high factor levels. The differences between these states primarily arise from the blood factor levels, in which State 4 has higher levels of prothrombin, Factors VII, VIII, X and protein C, while State 5 has higher levels of factors V and IX and ATIII. Patients in State 4 are probably healthier as they have lower rate of mortality, shorter clot times and are more stable (State 5 can transition to State 4, but not vice versa).

It is important to note that the probabilities of the transition matrix do not include the transition to death or discharge, due to the requirement of equally spaced time points in the hidden Markov model. Furthermore, patients that recover quickly are less likely to get the blood sample showing the healthy results. It is most likely for this reason that the probability to transition to the healthiest late state (State 4) is higher for the sicker intermediate states (2 and 3) than the healthier one (State 1). It should also be mentioned that the mortality rates include causes not associated with coagulation impairment. This, combined with the fact that healthier patients are less likely to have blood factor data, are reasons why the “healthier” late stage states can have a higher mortality probability than State 0.

We have also developed models for predicting outcomes for trauma patients, based on extensions of survival analysis. While data mining techniques have been proposed to predict mortality for ICU patients using their demographic data, measurements and notes from doctors and nurses. Most of these techniques suffer from two main drawbacks. First, they model the mortality prediction problem as a binary classification problem, while ignoring the time of death as continuous values. Second, they use topic models to analyze caregiver notes, while ignoring the relationship between measurements, notes and mortality/discharge outcomes. We developed a novel model, called the survival topic model (SVTM), which models patients’ measurements, notes and mortality/discharge jointly, and predicts the probability of mortality/discharge as functions of time. The idea is that each patient has a latent distribution of disease conditions, which we call topics. These

conditions generate the measurements and notes, and determine the patients' mortality. We have derived a mean-field variational inference algorithm for this model. We fit the SVTM with two outcomes on the Medical Information Mart for Intensive Care III (MIMIC III) dataset, and obtained some important topics, as well as demonstrated the relationships between these topics.

Professor Tom Soh, Stanford University

Abstract

We made substantial progress in our Aim 4b work. We made considerable headway in the development of a new real-time sensor platform that can *continuously measure protein analytes with sub-minute temporal resolution in undiluted whole blood.* We have demonstrated the performance of this device *in vitro* on a model target and are working on increasing device stability for long-term *in vivo* operation. In addition, we have made significant progress in the development of two new ultrasensitive protein detection techniques that can *quantitatively discriminate small-fold changes in extremely low-abundance protein levels in complex media such as blood serum*. We have demonstrated that our techniques offer performance far superior to that of gold-standard commercial assays.

Key Words

Real-time sensor platform, protein detection

Accomplishments

Aim 4b: Microfluidics platform and experiment

Sub-aim I. Development of a real-time fluorescent protein sensor

One of the major goals of our group is to develop biosensor technology capable of continuous, real-time measurement of coagulopathy biomarkers directly in animals, and ultimately in human patients. Our first-generation electrochemical device was published in *Science Translational Medicine* in 2013 [1]. Meanwhile, we have developed a second-generation device which is able to utilize a closed-loop feedback system to control *in vivo* drug concentrations (P. Mage *et al*., in final review, 2017). While we have been able to leverage this platform in order to measure *in vivo* concentrations of small molecule drugs directly in live animals, difficulties with electrode biofouling have hitherto prevented us from directly measuring concentrations of proteins involved in the coagulation cascade. As a result, during the previous funding cycle we have begun development of a new sensor platform.

The new sensor is, at its core, a highly automated sandwich immunoassay, which has been adapted to give a continuous, near-real-time readout (**Fig. 1**). Our device utilizes magnetic microbeads as a solid support for a microfluidic fluorescence-

based sandwich assay with incubation times on the order of one minute. The beads are passively sorted using microfabricated ferromagnetic strips (MFS) into an optically favorable imaging buffer where they are then imaged with a CCD camera. In this way we are able to avoid the problems with biofouling which have prevented the use of previous electrochemical sensor platforms directly in whole blood to enable monitoring of coagulation proteins. Furthermore, the entire process is inherently automated as a result of the microfluidic design. Once the footage has been taken, it is sent to a real-time analysis program we have developed in parallel, allowing us to quantify the fluorescent signal. The analysis program is capable of performing approximately two measurements per second.

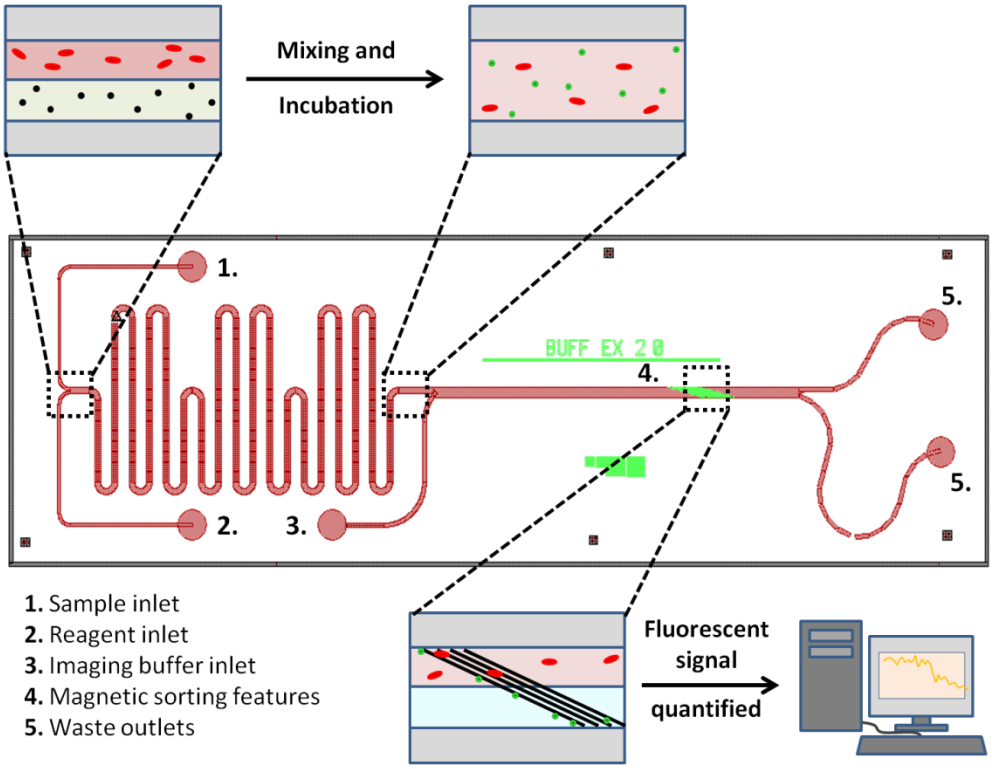


Fig. 1: Schematic showing real-time biosensor design. The blood sample is mixed on-chip with a combination of antibody-coated magnetic beads and fluorophore-labeled detection reagents. After a short incubation, the magnetic beads are passively sorted out of the blood sample stream and into a clear buffer stream. Here they are imaged with a CCD camera and their fluorescence quantified in real time.

For all of the characterization tests performed thus far, we have chosen to use the human cytokine tumor necrosis factor α (TNFα) as our model target. This is for two reasons: first, there exist a set of high-performing affinity reagents capable of forming a sandwich complex, one reagent being a monoclonal antibody and the other being a recently generated DNA aptamer selected for its specificity in serum (J. P. Wang *et al*., in review, 2016). Second, while TNFα is naturally present in the blood

stream, it typically exists at sub-pM concentrations [2]. Since this is quite low compared to the concentration of proteins we will eventually be attempting to measure (high pM to nM coagulation proteins such as thrombin), we have been able to use TNFα as a convenient stand-in protein without worrying about our assay running up against the physiological background concentration.

In order to test the accuracy and sensitivity of the device, we doped varying concentrations of TNFα ranging from 100 pM to 100 nM into undiluted whole blood. We then pumped the doped blood through our device using a syringe pump and measured the fluorescence of the resulting bead/fluorophore complexes (**Fig. 2a**). In order to verify the accuracy of our algorithm's measurements we then took the mean value of each of the traces and compared that against the median fluorescence of beads prepared on the bench top and measured with a flow cytometer (BD FACSVerse). The results are shown in **Fig. 2b**.

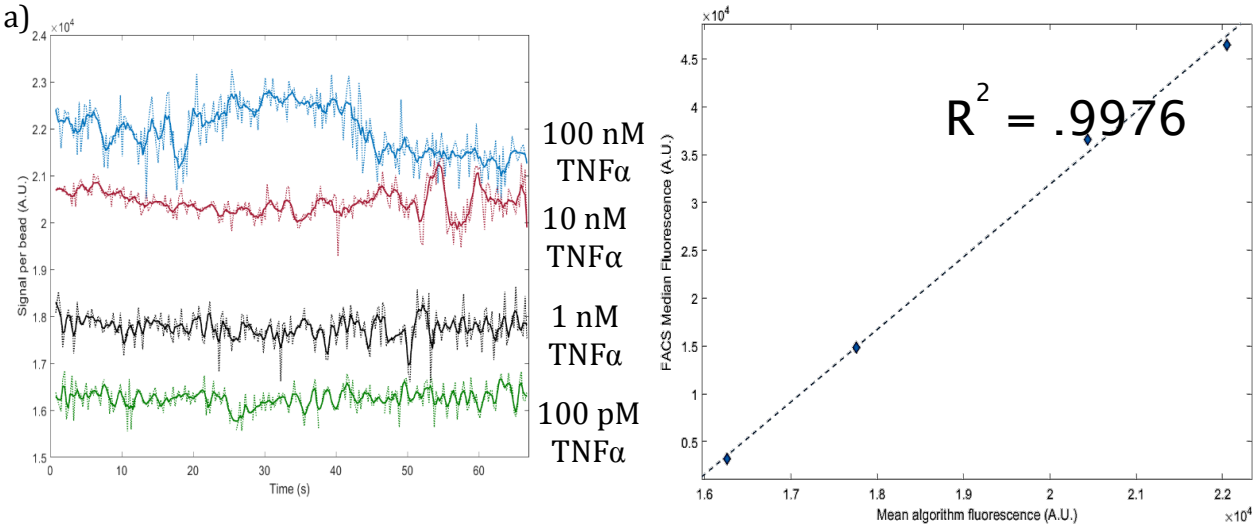


Fig. 2. (a) After doping undiluted whole blood with varying concentrations of TNFα and passing it through our device, we measured the fluorescence of the resulting beads using our detection algorithm. (b) We then compared the mean fluorescence of each trace against the median fluorescence of beads prepared with the same concentrations of TNFα on the bench top and measured with a commercial flow cytometer (BD FACSVerse).

Confident in our device's ability to quantify bead fluorescence with a high degree of accuracy and with sub-second temporal resolution, we then sought to perform long-term measurements. This objective proved to be non-trivial due to the tendency of beads to adhere to the MFS strips and subsequently photobleach during imaging. Eventually, through a combination of surface treatments, optimization of the flow rates inside the device, and modifications to the image detection algorithm, we were successful in gathering over an hour of uninterrupted data using our device. Since this platform is not designed to measure low-abundance proteins such as TNFα, we are currently focused on evaluating affinity reagents for other protein targets in the coagulation cascade, including thrombin and thrombin-antithrombin (TAT)

complex. Considering that the usage of dual affinity reagent recognition is so ubiquitous, we estimate that it should not be too difficult to adapt our platform to a new, more physiologically relevant target. Once we have successfully chosen a target and characterized the device's performance with the new affinity reagents, we will begin testing the device on live animals.

Sub-aim II. Ultrasensitive and high-resolution protein quantification in complex media

A chief goal of this work is to develop improved techniques for the quantification of protein biomarkers in complex biological media such as blood serum. We seek to utilize the extraordinary sensitivity and multiplexing capabilities of nucleic acid analysis techniques for the sensitive and quantitative detection of proteins. Our first-generation technology was published in *Angewandte Chemie International Edition* in 2010 [3]. In the past funding cycle, we have developed two second-generation assay technologies, the digital aptamer PCR assay (daPCR) and the digital immuno-PCR assay (diPCR), that improve upon the sensitivity, precision, and multiplexing capability of our first-generation technology.

Our new assay technologies use digital PCR (dPCR) to quantify proteins that are labeled by DNA aptamers or antibody/oligonucleotide conjugates. The use of dPCR readout lends the assay high sensitivity and precision, enabling the detection of small-fold changes of protein levels even at very low abundance [4].

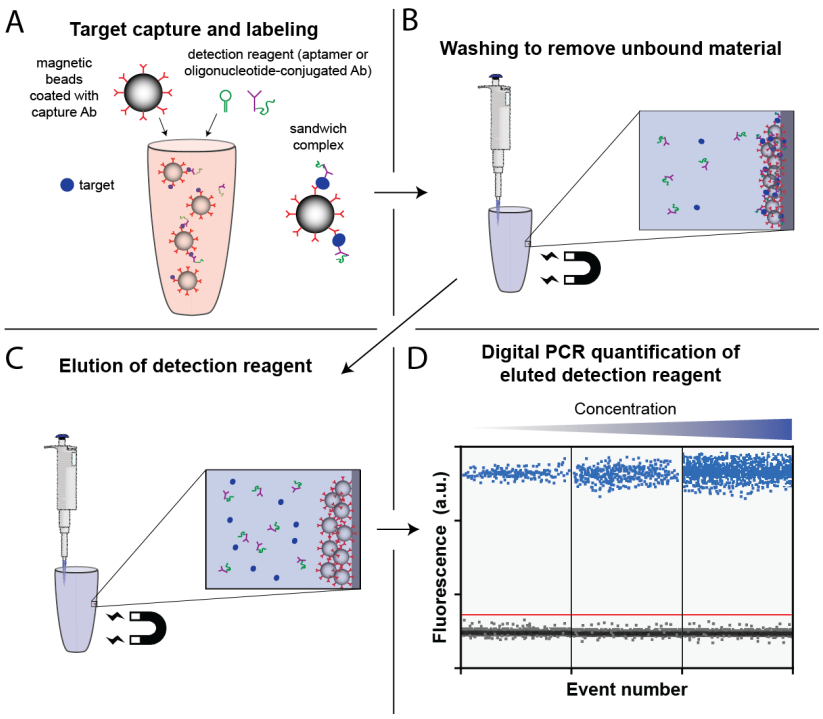


Figure 4. Scheme of daPCR/diPCR assay. (A) Target proteins in a serum sample are captured by antibodies coated on magnetic microbeads and labeled with DNA aptamers (daPCR) or antibody/oligonucleotide conjugates (diPCR) that bind to

different epitopes on the target than the capture antibody. (B) Magnetic separation allows thorough washing of the bead surfaces to remove unbound material and PCR contaminants/inhibitors. (C) The affinity reagent is eluted from the bead surface by a denaturing buffer that disrupts the affinity reagent/target sandwich complexes. (D) The eluent is analyzed via dPCR, with the measured detection reagent concentration correlating quantitatively with the amount of target protein present in the original sample.

The daPCR and diPCR assays are sandwich immunoassays that use matched antibody/aptamer or antibody/antibody pairs to specifically identify target molecules in complex samples such as blood serum (**Fig. 4**). The requirement of dual target recognition gives the assay the specificity necessary to detect low-abundance proteins even in the presence of a multitude of other protein species present at many orders of magnitude higher abundance. The use of a digital PCR readout gives the assay a linear signal response to increasing target protein concentrations, enabling the discrimination of sub-2-fold changes in low-fM protein levels in diluted serum. As a proof of concept, we developed a model daPCR assay for the quantification of the cytokine TNFα in human blood serum (**Fig. 5**). We chose TNFα as a model target because its physiological serum levels are very low (sub-picomolar, below the assay range of many commercial ELISAs) but are known to increase in response to traumatic or immune insult, and as a signaling cytokine, even small-fold changes in TNFα expression levels can have significant downstream effects [5]. Using a commercially available monoclonal antibody and a DNA aptamer we recently generated, we developed a daPCR assay protocol that enables us to discriminate 1.5-fold differences in low-fM TNFα levels in 10% serum (**Fig. 5**). The calculated limit of detection of the assay is 1.1 fM in 10% serum (or 11 fM in the original serum sample), which is more than 10x more sensitive than standard commercial ELISAs for TNFα.

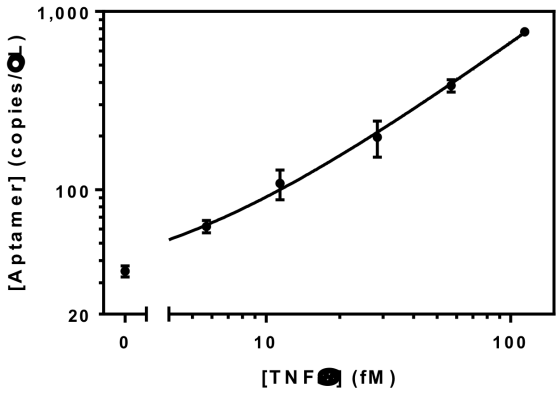


Fig. 5. Representative standard curve of daPCR assay for the quantitation of human TNFα in 10% human blood serum. A 1.5-fold change in TNFα levels can be resolved with 95% certainty even at levels as low as 5 fM.

To generalize our platform, we have begun developing a model diPCR assay (in which the aptamer is replaced with a DNA-labeled antibody) for the quantification of murine TNFα in serum. Thus far, we have tested and identified high-quality

matched antibody pairs against the target, have successfully produced and characterized highly purified and functional antibody/oligonucleotide conjugates, and have begun running and optimizing the proof-of-concept diPCR assay. Once assay performance is satisfactory (in terms of sensitivity, resolution, reproducibility, etc.), we plan to utilize the developed assay to perform serial measurements of circulating TNFα levels in mice following bacterial infection, to demonstrate the applicability of our assay platform for detecting small-fold changes in low-abundance protein levels in complex biological media such as serum or plasma. Following this, we aim to extend the platform to enable detection of another murine cytokine (such as IFNβ or IL-1β) whose endogenous circulating levels are much lower than TNFα and thus undetectable with current commercial assays. We then aim to again perform serial measurements of the circulating levels of this cytokine in infected mice, shedding new light on biology that until now has been hidden below the detection limit of commercially available assays.

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Prof Jeff Varner, Cornell University

Abstract

The Varnerlab team has developed a multiscale physiologically based pharmacokinetic (PBPK) model to simulate blood biochemistry and whole-body physiology following trauma. Our hypothesis is that therapeutic targets estimated using mechanism enhanced PBPK models, which describe both the biochemistry and physics of injury, will give higher fidelity predictions of patient response to therapeutic intervention when compared with static biochemical models. However, the challenge is scale; current biochemical models of coagulation or complement, when combined with detailed whole-body biophysical models, generate model systems that are untractable. Toward this challenge, we have developed reduced-order kinetic modeling tools, and used these to model blood coagulation, fibrinolysis and complement along with tools to estimate model parameters. The reduced order models were identified and tested using literature data, and data generated by the clinical sites on this program. The collection of reduced order models gave similar performance on typical data, compared with their full-scale counterparts, albeit with lumped or phenomenological descriptions of the underlying biochemistry in some cases. Taken together, we developed reduced-order biochemical submodels required for the multiscale PBPK trauma model. Combining these with descriptions of the biophysics of trauma, for example, heart-rate changes and vessel dilation, is the critical next step for this approach.

Key Words

Reduced order trauma modeling, multiscale modeling

Accomplishments

Aim 5: Modeling, Simulation and Data Analysis

Developed reduced order modeling and analysis framework for signal transduction networks important to hemorrhage.

Associated publications:

1. Sagar A# and J. Varner (2015) Dynamic Modeling of the Human Coagulation Cascade using Reduced Order Effective Kinetic Models. Processes, 3:178 - 203 (invited; *Special Issue Modeling and Analysis of Signal Transduction Networks*)

2. Wayman J[#], Sagar A[#] and J. Varner (2015) Dynamic Modeling of Cell Free Biochemical Networks using Effective Kinetic Models. Processes, 3:138-160 (invited; *Special Issue Dynamic Approaches to Metabolic Modeling and Metabolic Engineering*)
3. Sagar A[#], Wei D[#], Minot M[#,] and J. Varner (2016) Reduced order modeling and analysis of the human complement system. PLoS ONE, *submitted* (available at bioRxiv doi**:** http://dx.doi.org/10.1101/059386
4. Sagar A[#], LeCover R, Orfeo, T, Brummel-Ziedins K and J. Varner (2017) Effective modeling of the human coagulation and fibrinolytic pathways. J. Mil Med (2016 MHSRS Supplement), *in preparation*.

Developed model parameter estimation techniques for models with many parameters and potentially conflicting training data.

Associated publications**:**

1. Sagar A[#], Shoemaker C, and J. Varner (2016) Dynamic Optimization with Particle Swarms (DOPS): A meta-heuristic for parameter estimation in biochemical models. Biotechnol J., *in revision* (available at https://github.com/varnerlab/DOPS_Manuscript_Repository)
2. Bassen DM[#], Vikhovoy M[#], Minot M[#], Butcher J and J. Varner (2017) JuPOETs: a constrained multiobjective optimization approach to estimate biochemical model ensembles in the Julia programming language. *BMC Sys. Biol*., 11:10 DOI 10.1186/s12918-016-0380-2

Developed automatic model code generation tools to facilitate rapid model development and iteration.

Associated publications:

1. J. Varner (2015) Kwatee: A Code Generation System for Biochemical Models in the Julia Programming Language http://dx.doi.org/10.5281/zenodo.32628