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4. TITLE AND SUBTITLE Final Report: Peptide nanotube reinforced polymers: A system for tunable, composite materials			5a. CONTRACT NUMBER W911NF-12-1-0229		
			5b. GRANT NUMBER		
			5c. PROGRAM ELEMENT NUMBER 611102		
6. AUTHORS Neel Joshi, Daniel Rubin, Rajiv Desai			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
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13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.					
14. ABSTRACT D,L-cyclic peptides (DLCPs) are peptides composed of alternating D and L amino acids that self-assemble into nanotubular stacks and microcrystalline aggregates. The goal of this proposal was to determine the suitability of these self-assembled structures for the mechanical reinforcement of polymeric materials used in the fabrication of implantable medical devices. Our results show that the high aspect ratio microcrystalline aggregates (a.k.a. "microneedles") that come from the assembly process are capable of increasing the average stiffness of biodegradable polymers like poly D,L-lactic acid (PDLLA), a common polymer used in resorbable load bearing					
15. SUBJECT TERMS nanotube, peptide self-assembly, polymer reinforcement					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON Neel Joshi
a. REPORT UU	b. ABSTRACT UU	c. THIS PAGE UU			19b. TELEPHONE NUMBER 617-432-7730

Report Title

Final Report: Peptide nanotube reinforced polymers: A system for tunable, composite materials

ABSTRACT

D,L-cyclic peptides (DLCPs) are peptides composed of alternating D and L amino acids that self-assemble into nanotubular stacks and microcrystalline aggregates. The goal of this proposal was to determine the suitability of these self-assembled structures for the mechanical reinforcement of polymeric materials used in the fabrication of implantable medical devices. Our results show that the high aspect ratio microcrystalline aggregates (a.k.a. "microneedles") that come from the assembly process are capable of increasing the average stiffness of biodegradable polymers like poly-D,L-lactic acid (PDLLA), a common polymer used in resorbable load bearing implants. Preliminary experiments also demonstrated that, for a particular sequence of DLCP (cyclo-[Gln-Leu]4) the assembled microneedles do not exhibit any cytotoxicity toward sheep fibroblasts. Finally, nanomechanical characterization of the microneedles revealed that they were among the stiffest known proteinaceous substances in existence, suggesting their utility as mechanical reinforcers. However, significant challenges remain in rationally controlling the size and surface chemistry features of DLCP-derived microneedles, limiting the ease of their incorporation into biomedical implant devices.

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)

<u>Received</u>	<u>Paper</u>
11/30/2015	2.00 Shahrouz Amini, Daniel J. Rubin, Feng Zhou, Haibin Su, Ali Miserez, Neel S. Joshi. Structural, Nanomechanical, and Computational Characterization of, ACS Nano, (03 2015): 3360. doi: 10.1021/acsnano.5b00672
11/30/2015	1.00 Daniel J. Rubin, Hadi T. Nia, Thierry Desire, Peter Q. Nguyen, Michael Gevelber, Christine Ortiz, Neel S. Joshi. Mechanical Reinforcement of Polymeric Fibers through Peptide Nanotube Incorporation, Biomacromolecules, (10 2013): 3370. doi: 10.1021/bm4008293
TOTAL:	2

Number of Papers published in peer-reviewed journals:

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

<u>Received</u>	<u>Paper</u>
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TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

ACS National Meeting New Orleans (2013)
MIT Synthetic Biology Group
MRS National Meeting (Boston 2013)

Number of Presentations: 0.00

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

<u>Received</u>	<u>Paper</u>
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TOTAL:

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

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

<u>Received</u>	<u>Paper</u>
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TOTAL:

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

(d) Manuscripts

<u>Received</u>	<u>Paper</u>
-----------------	--------------

TOTAL:

Number of Manuscripts:

Books

Received Book

TOTAL:

Received Book Chapter

TOTAL:

Patents Submitted

D, L-cyclic Peptide Nanotubes as Reinforcing Agents

Patents Awarded

D, L-cyclic Peptide Nanotubes as Reinforcing Agents

Awards

Graduate Students

NAME	PERCENT SUPPORTED	Discipline
Daniel Rubin	1.00	
FTE Equivalent:	1.00	
Total Number:	1	

Names of Post Doctorates

NAME	PERCENT SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	National Academy Member
Neel Joshi	0.08	
FTE Equivalent:	0.08	
Total Number:	1	

Names of Under Graduate students supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	Discipline
Brian Borsiquot	0.00	Chemistry
FTE Equivalent:	0.00	
Total Number:	1	

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:..... 1.00

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:..... 1.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 1.00

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:..... 1.00

Names of Personnel receiving masters degrees

<u>NAME</u>
Total Number:

Names of personnel receiving PHDs

<u>NAME</u>
Daniel Rubin
Total Number:
1

Names of other research staff

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
FTE Equivalent:	
Total Number:	

Sub Contractors (DD882)

Inventions (DD882)

5 D, L-cyclic peptide nanotube reinforcing agents

Patent Filed in US? (5d-1) Y

Patent Filed in Foreign Countries? (5d-2) N

Was the assignment forwarded to the contracting officer? (5e) N

Foreign Countries of application (5g-2):

5a: Daniel Rubin

5f-1a: Harvard University

5f-c: 29 Oxford St.

Cambridge

MA 02143

5a: Neel Joshi

5f-1a: Harvard University

5f-c: 29 Oxford St.

Cambridge

MA 02138

Scientific Progress

Final Report

1) Foreword

This is the final report for an STIR grant awarded in September 2012 and ended in May 2013. The award provided important seed funding for a project that formed the basis for one PhD thesis in my lab and helped to forge two important collaborations. Below is provided a summary of the project during and after the award period. More technical details can be found in the two publications referenced in this report.

2) Statement of the problem to be studied

The purpose of this project was to investigate the use of a unique class of self-assembling crystalline nanostructures as reinforcing agents when incorporated as a minor component into polymeric composites. In particular, we were interested in self-assembling units comprised of cyclic peptides (8-mers) with alternating D- and L-amino acids. This scaffold had previously been shown to form nanotubular structures after solution-phase assembly, mediated by beta-sheet-like hydrogen bonding interactions that promoted stacking of the individual units. The rationale was that the chemical synthesis of these cyclic peptides could potentially yield a huge diversity in nanostructures by drawing upon the availability of Fmoc-protected amino acids with diverse side chains. These diverse structures could then be tailored to specific applications. At the time, a similar class of self-assembling nanostructured materials, based on the diphenylalanine scaffold, were mechanically characterized and declared to be the stiffest known organic material.¹ This, in part, inspired our investigation into the mechanical properties of D-, L-cyclic peptides (DLCPs) and their potential application in reinforcing polymeric materials. The proposal was framed in the context of stabilizing load-bearing resorbable biomedical implants, like spinal fusion cages and bone screws, which are currently made from poly lactic acid and similar synthetic polymers, and sometimes fail prematurely and lead to poor healing outcomes for patients. However, if the surface chemistry of the DLCP nanotubes could be customized, there might be many applications where customizing polymer-filler interactions would be of utmost importance.

3) Summary of most important results

The actual term of the award lasted almost 12 months, during which time we were able to complete a preliminary investigation into the primary proposal goal. First, we synthesized a particular DLCP composed of alternating glutamine and leucine amino acids, QL4. This particular peptide was known to assemble from previous literature reports, but its micro-scale structure was ill-defined. We determined that the peptides formed polydispersed crystalline needle-like aggregates, with dimensions of ~1 micron (length) and ~100 nm (diameter), composed of longitudinally aligned nanotubes. These “microcrystals” could be isolated and co-dissolved with poly(D,L-lactic acid) (PDLLA) in organic solvents and then spun into microfibers using electrospinning. The resulting fibrous meshes contained the peptide microcrystals embedded in a polymer matrix. We tested the fibers by nanoindentation and found that the microcrystals increased the average stiffness of the fibers 5-fold. This work was published in *Biomacromolecules*.²

During the course of this investigation, we were intrigued by the mechanical properties of the microcrystals, given their ability to serve as filler materials in polymer composites. Therefore, after the award term concluded, we continued to build on our previous work to perform a more detailed study on the mechanical properties of the microcrystals themselves. This led to nanoindentation experiments and three-point bending experiments on the microcrystals, done with specialized instrumentation found in our collaborators’ lab in Singapore. The results of these experiments demonstrated that the DLCP microcrystals were comparable to the most mechanically robust proteinaceous materials known. This work was published in *ACS Nano*, and was made possible by the initial seed funding from ARO.³

Overall, the DLCP self-assembling system remains intriguing for their mechanical properties. However, we found that their assembly properties were much more adversely influenced by the peptide sequence than we originally hypothesized. For example, the DLCP composed of alternating leucine and glutamic acid forms interesting nano-scale tubular structures, but they were not robust enough to be harvested and incorporated into other fabrication protocols. Other DLCP sequences either did not assemble at all, despite exploring a wide variety of assembly conditions, or formed large aggregates that could not be resuspended in any solvents. Future work may explore conjugation of polymers to the amino acid side chains to increase processability of the nanotubes and further investigations of their biocompatibility.

4) Bibliography

Please refer to our publications for relevant references

1. Even, N., Adler-Abramovich, L., Buzhansky, L., Dodiuk, H. & Gazit, E. Improvement of the Mechanical Properties of Epoxy by Peptide Nanotube Fillers. *Small* 7, 1007–1011 (2011).
2. Rubin, D. J. et al. Mechanical Reinforcement of Polymeric Fibers through Peptide Nanotube Incorporation. *Biomacromolecules* 14, 3370–3375 (2013).

3. Rubin, D. J. et al. Structural, Nanomechanical, and Computational Characterization of d, l-Cyclic Peptide Assemblies. ACS nano 9, 3360–3368 (2015).

Technology Transfer

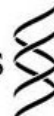
The PI traveled to give invited talks at The ARL lab in Aberdeen, MD and the AFOSR lab in Dayton, OH. Slides from the talks are included as an attachment.

Building functional materials from proteins: Assembly and dynamism

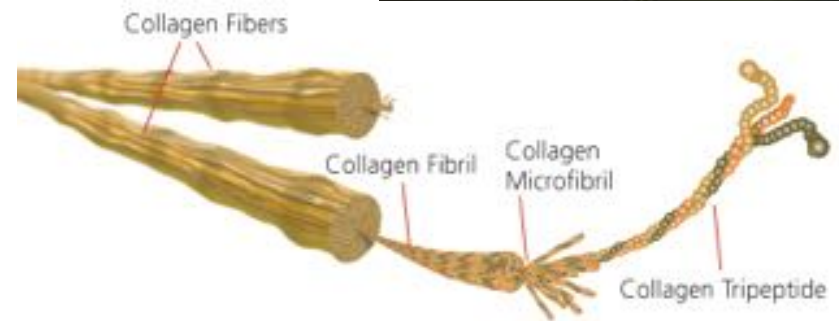
Neel Joshi
6.18.2014



HARVARD
School of Engineering
and Applied Sciences

WYSS  INSTITUTE

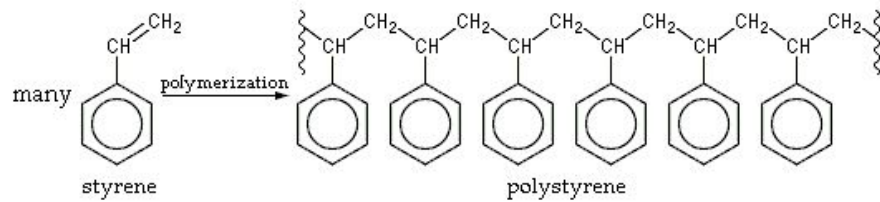
Living systems use proteins to build



SILKS

VERTEBRATE TISSUES

Synthetic Polymers



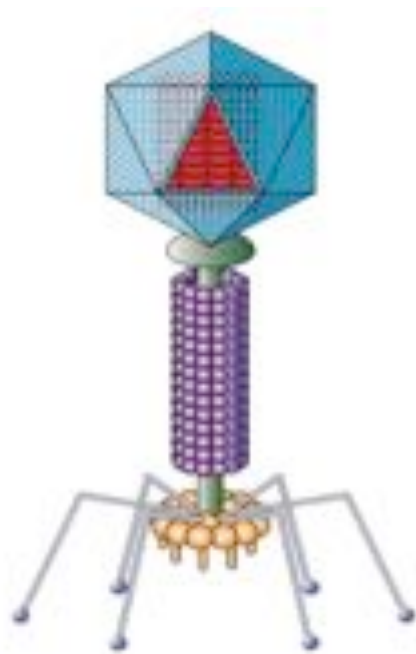
Limited sequence control

Proteins

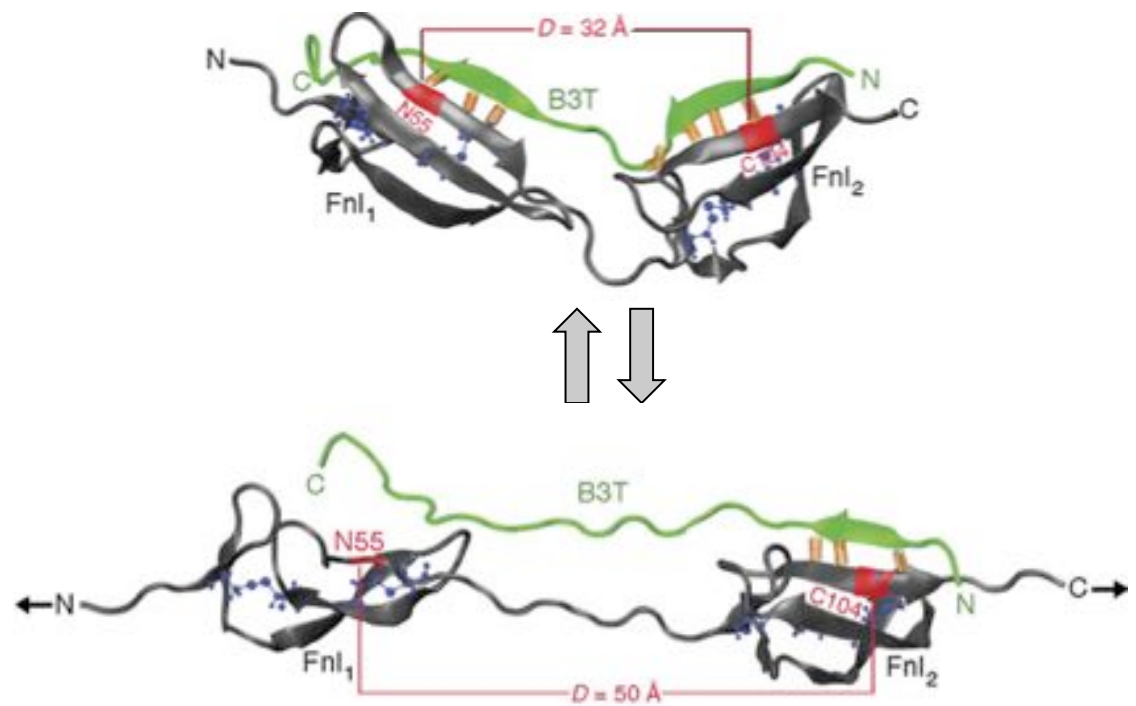


Complete sequence control

Self-assembly

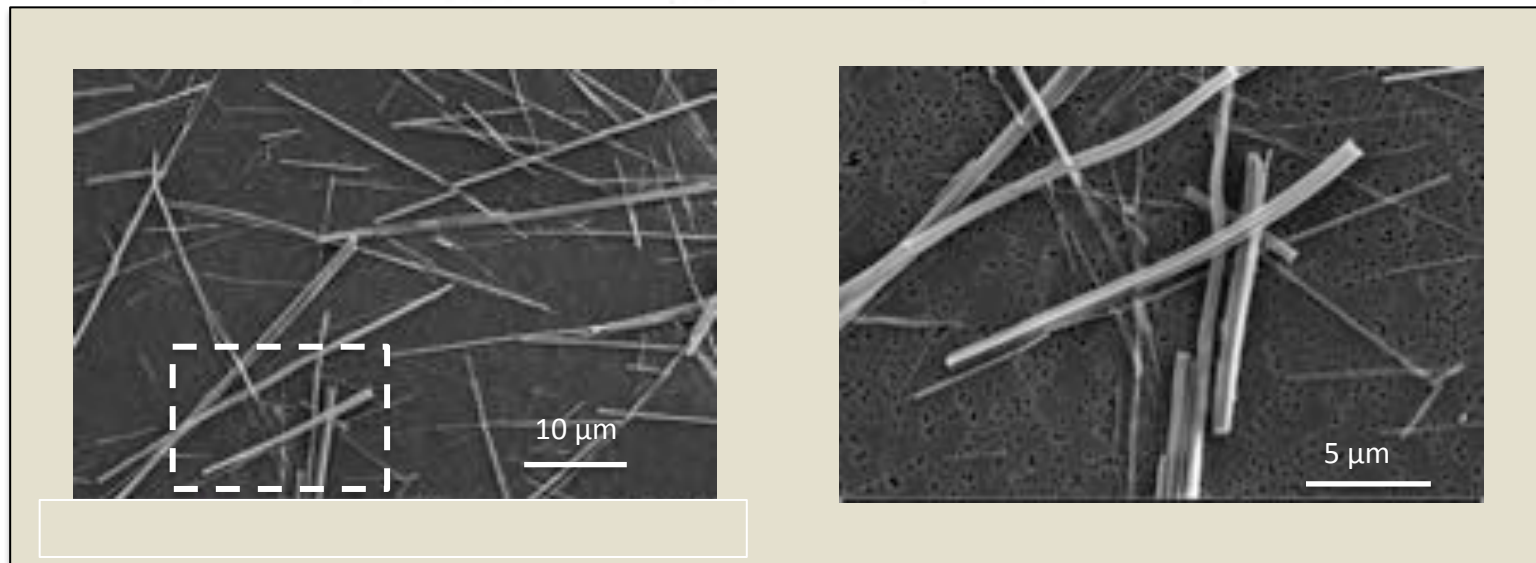
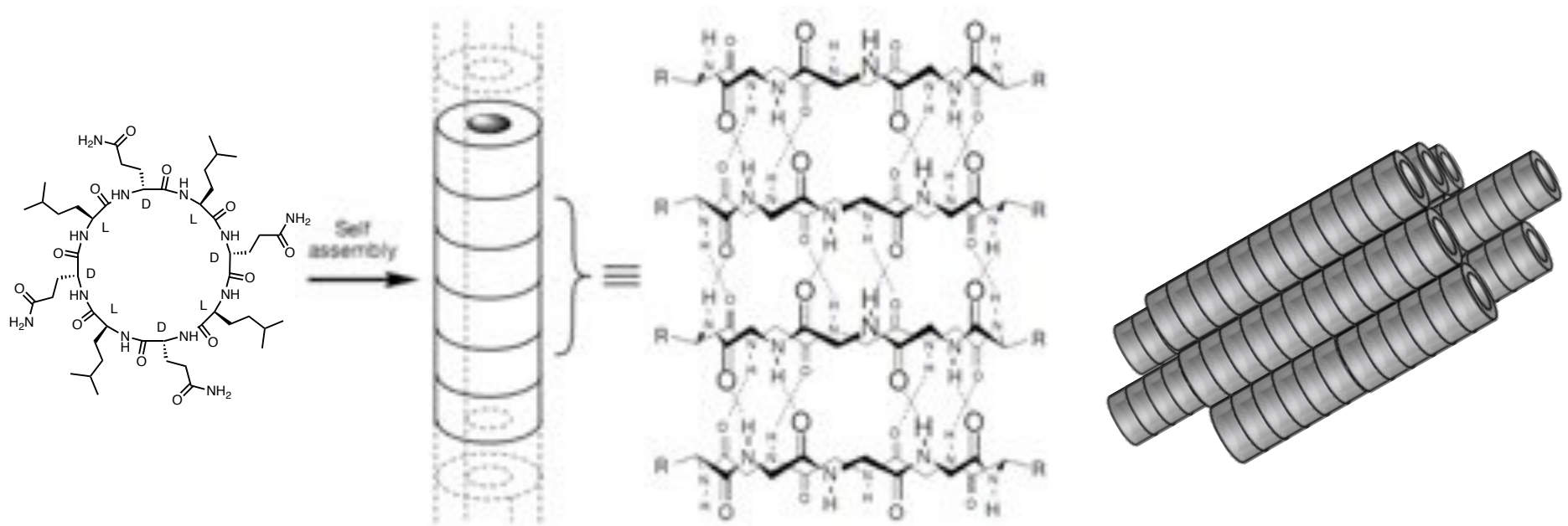


Structural Dynamism

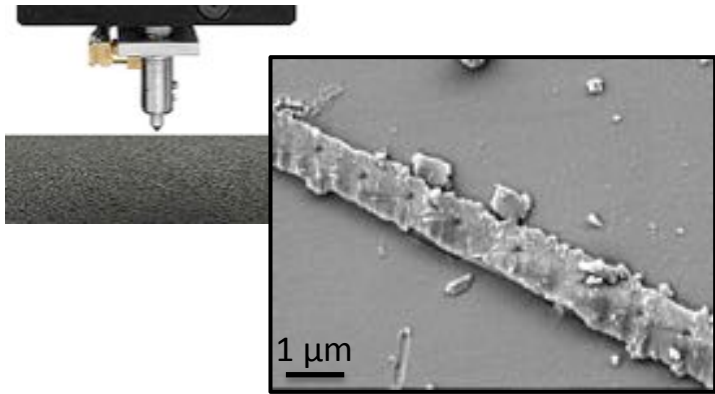


peptides -----> **proteins** -----> **networks of proteins**

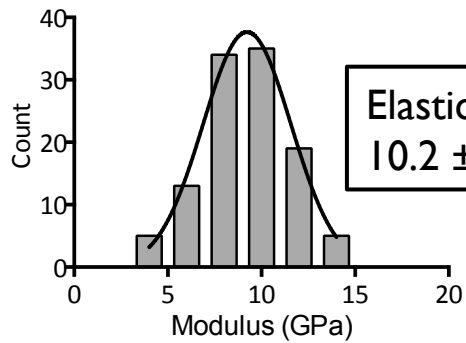
D,L-cyclic peptides (DLCPPs)



DLCPs are remarkably stiff

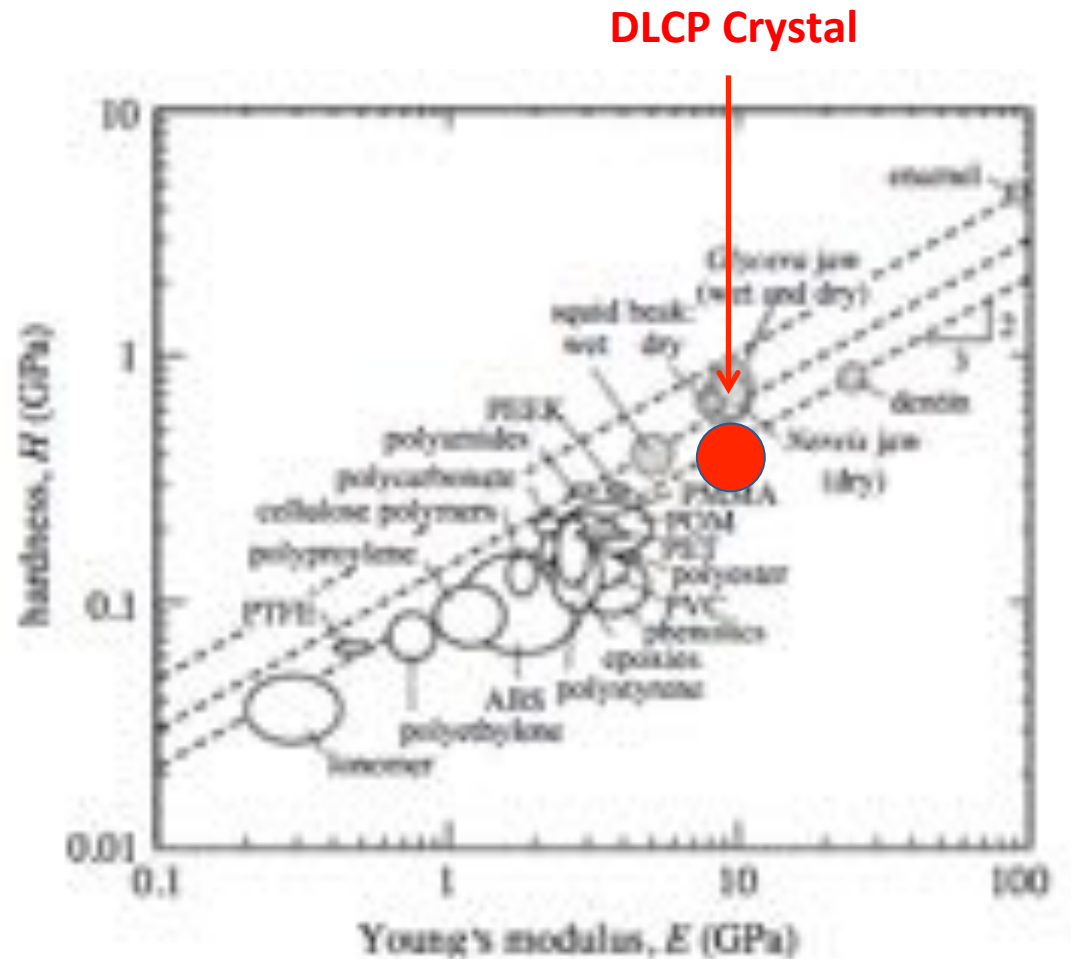
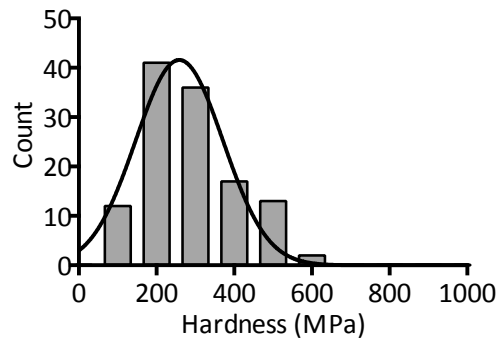


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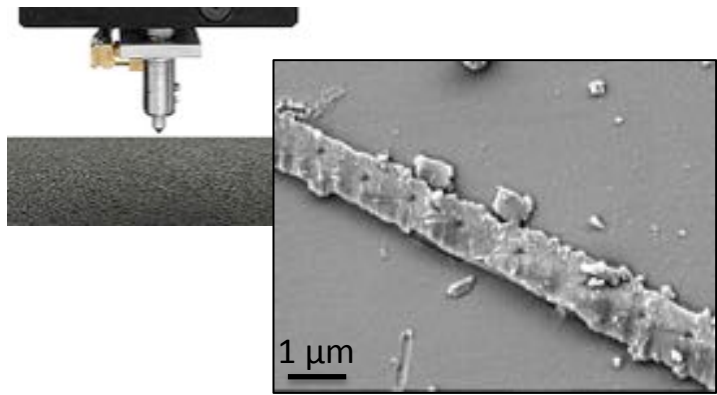


Elastic Modulus =
 10.2 ± 0.5 GPa

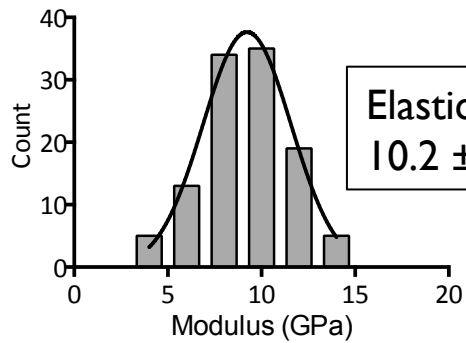
Hardness =
 343 ± 12 MPa



DLCPs are remarkably stiff

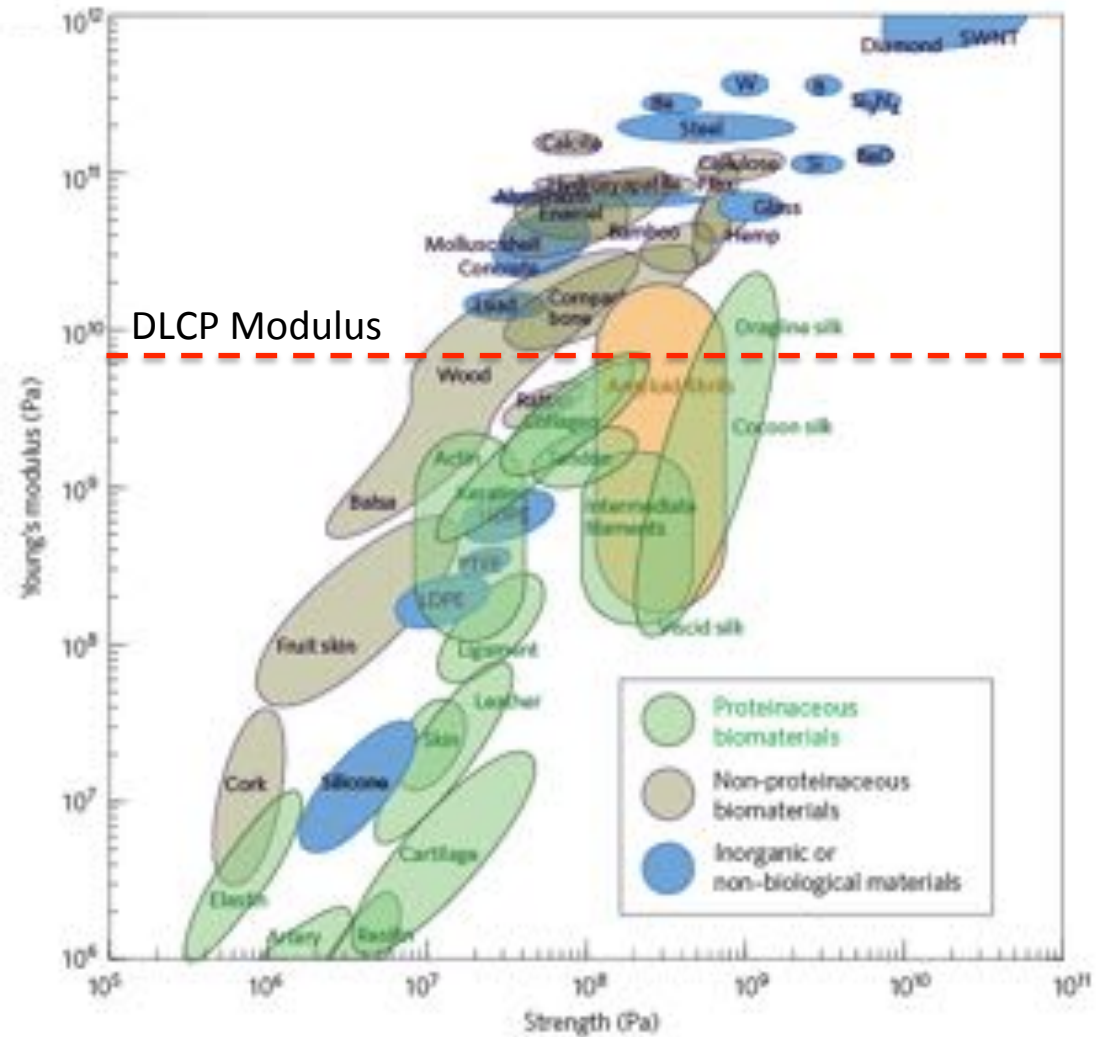
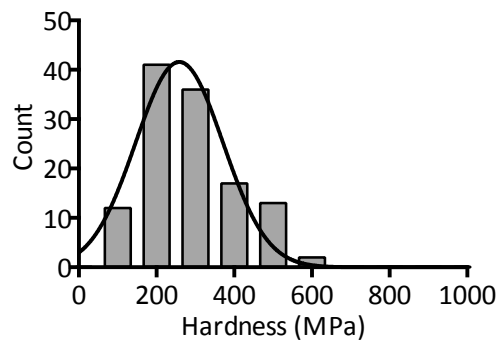


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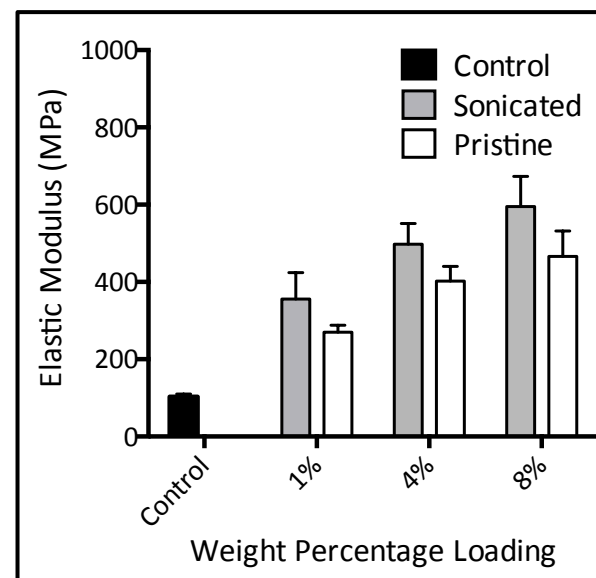
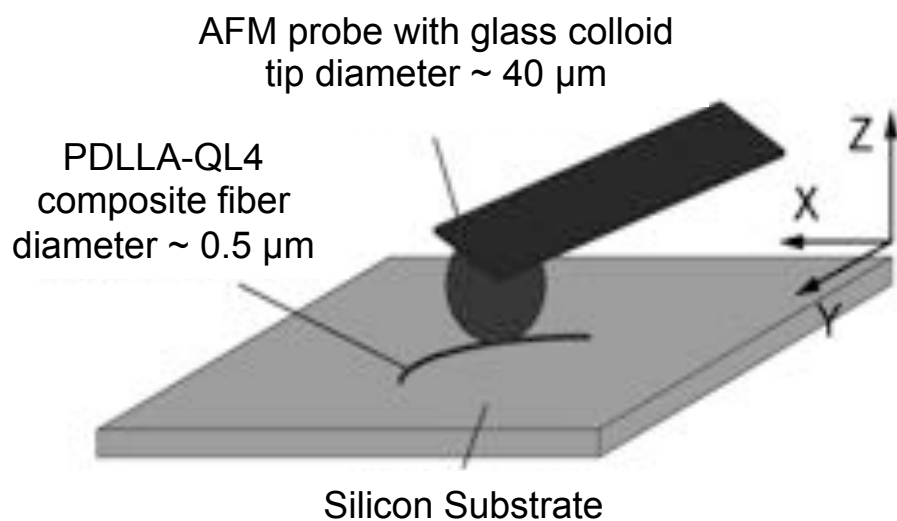
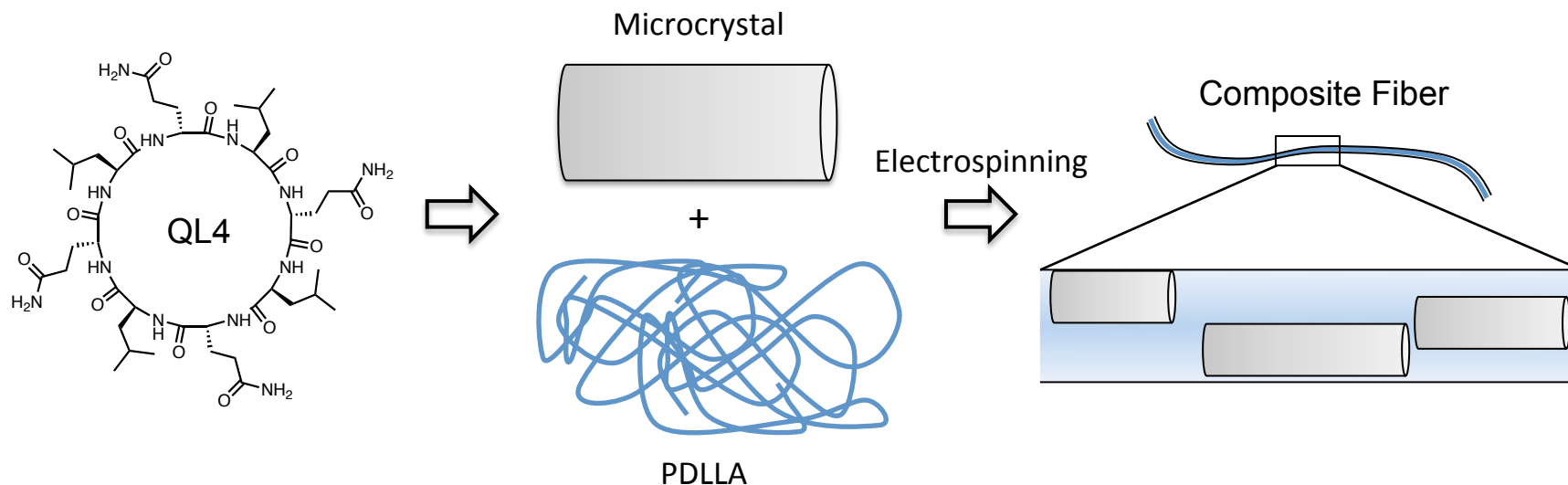


Elastic Modulus =
 10.2 ± 0.5 GPa

Hardness =
 343 ± 12 MPa

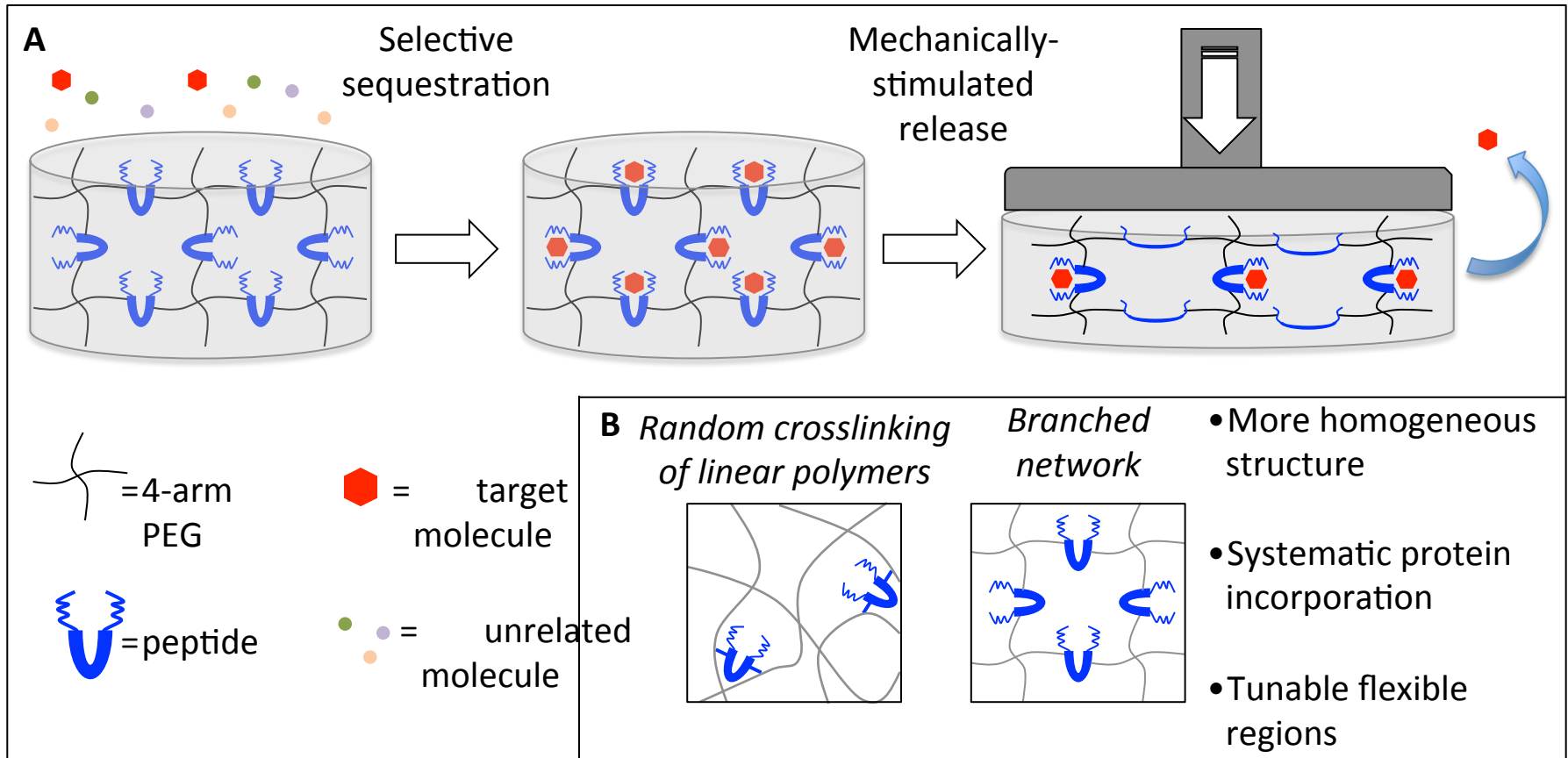


DLCPs can reinforce polymeric fibers



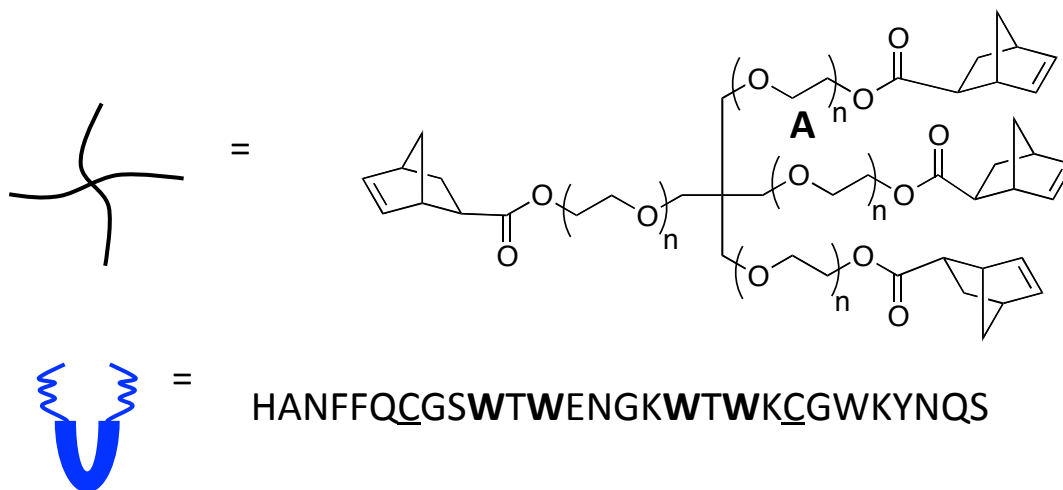
peptides -----> proteins -----> networks of proteins

Mechano-sensitive protein-polymer hybrids

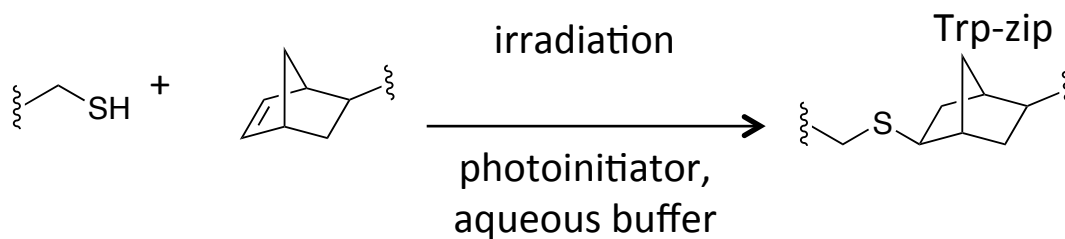


Mechano-sensitive protein-polymer hybrids

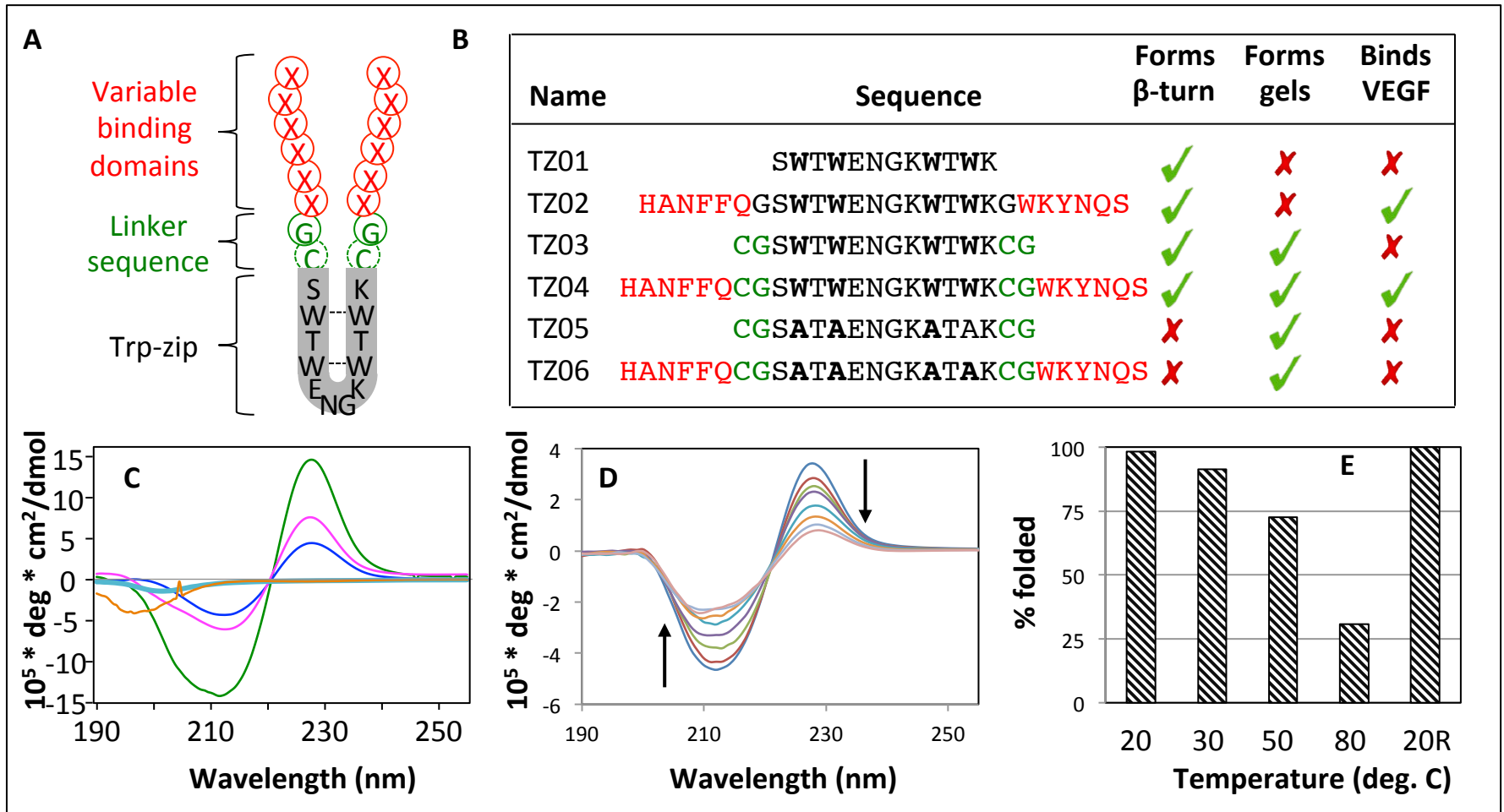
Components for gelation reaction



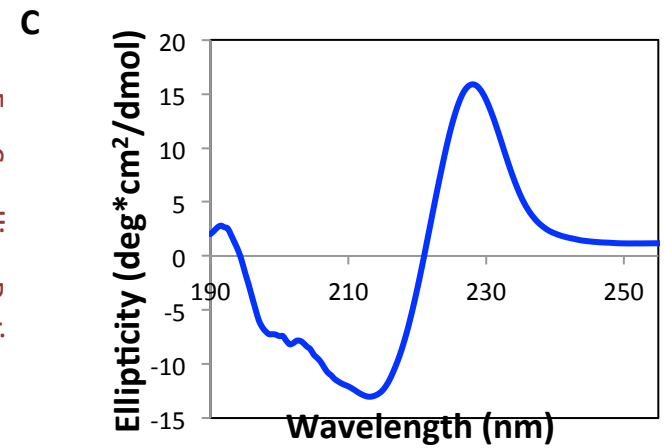
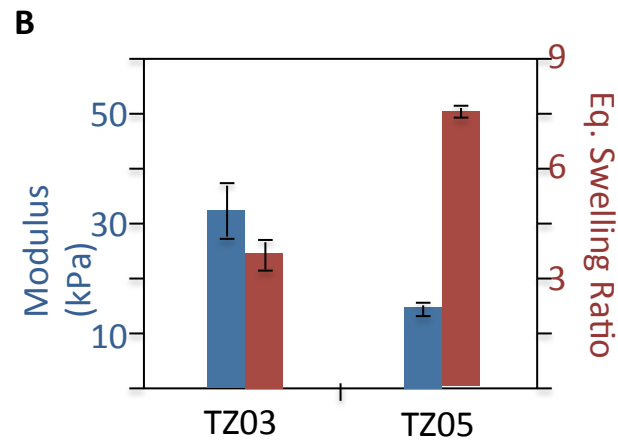
Thiol-ene reaction



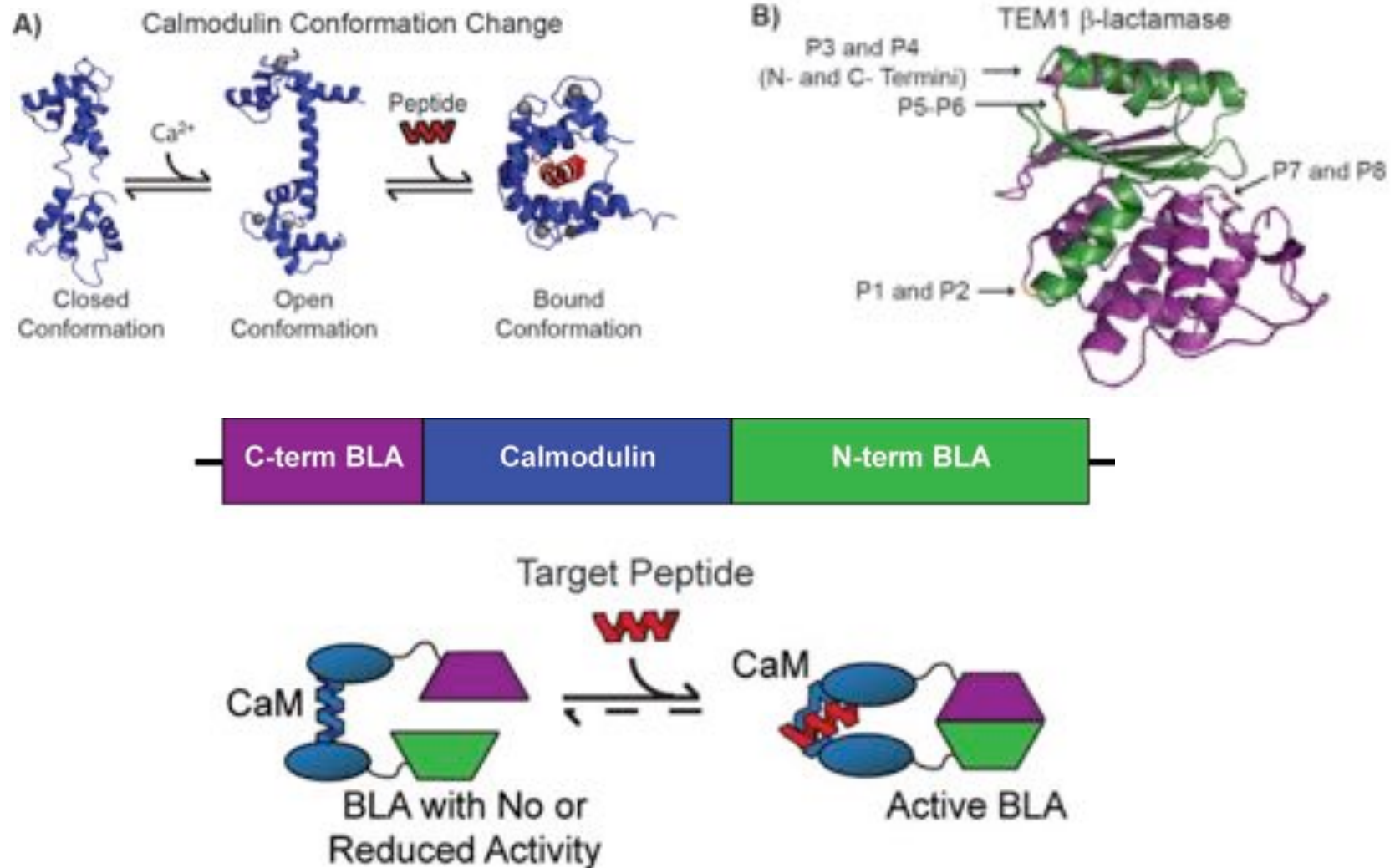
Mechano-sensitive protein-polymer hybrids



Mechano-sensitive protein-polymer hybrids



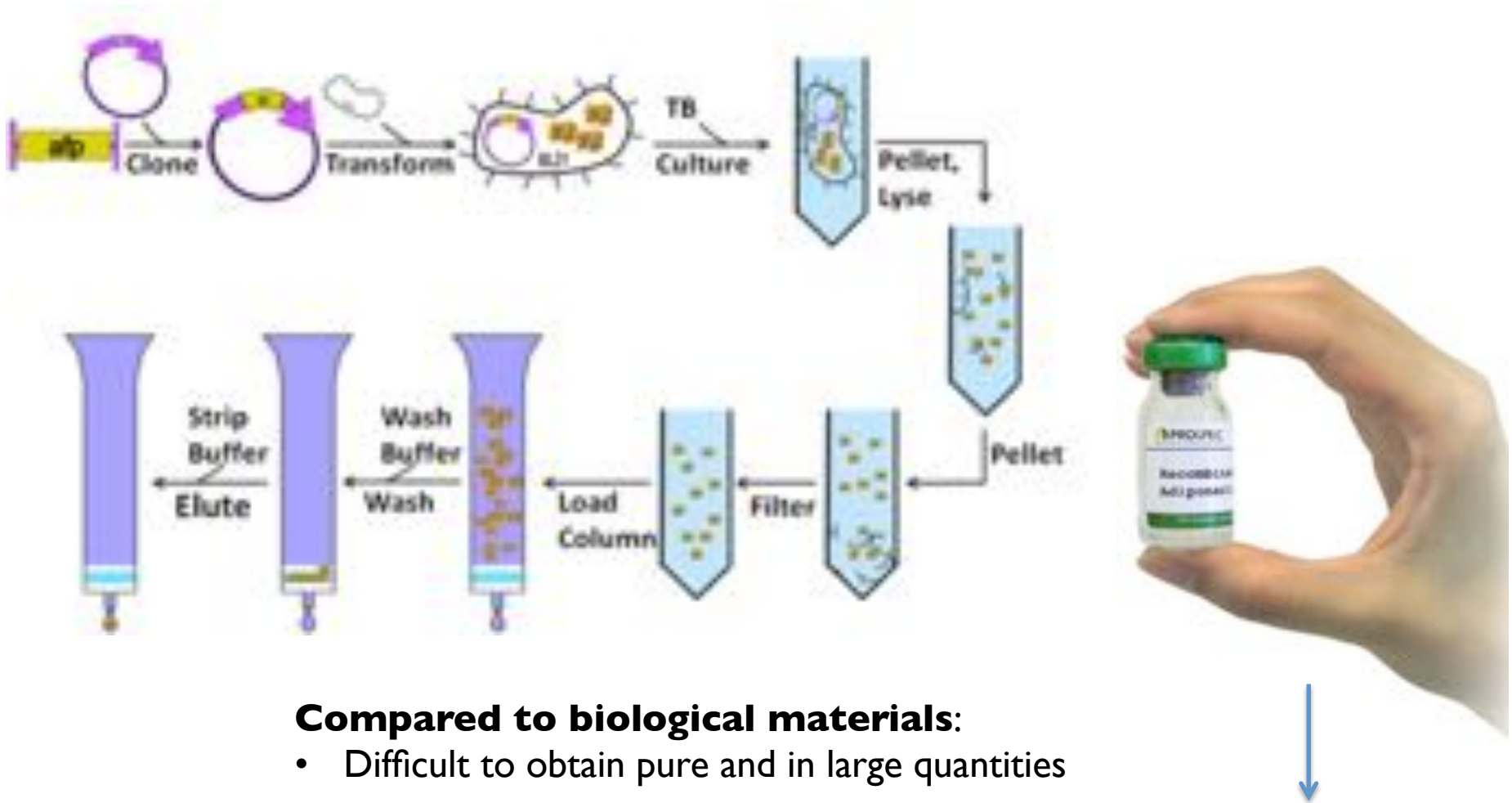
Biosensing Platforms Based on Protein Conformational Dynamics



Meister, et al. *ChemBioChem* **2013** 14(12), 1460-1467

peptides -----> proteins -----> networks of proteins

Engineered protein manufacturing



Compared to biological materials:

- Difficult to obtain pure and in large quantities
- Time consuming, less cost effective
- Not appropriate for large-scale materials

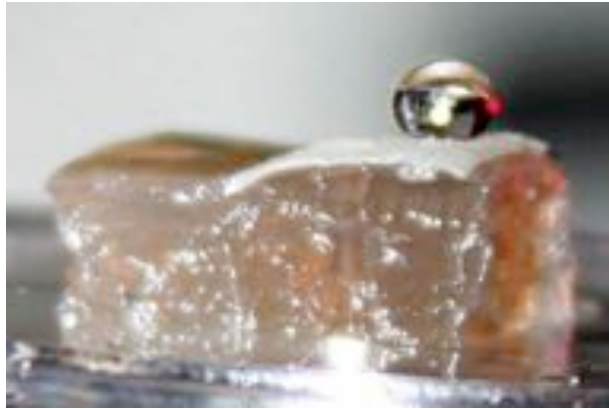
Materials
fabrication

Material design parameters

- 1) Must be able to control sequence using conventional genetic engineering
- 2) Must be able to produce material on large scales by harnessing biosynthetic potential of a living organism
- 3) No protein purification

Biofilms

Self-standing, macroscopic, biosynthetic materials



Epstein, et al. *PNAS* **2010**



<http://www.biofilm.montana.edu/node/2390>

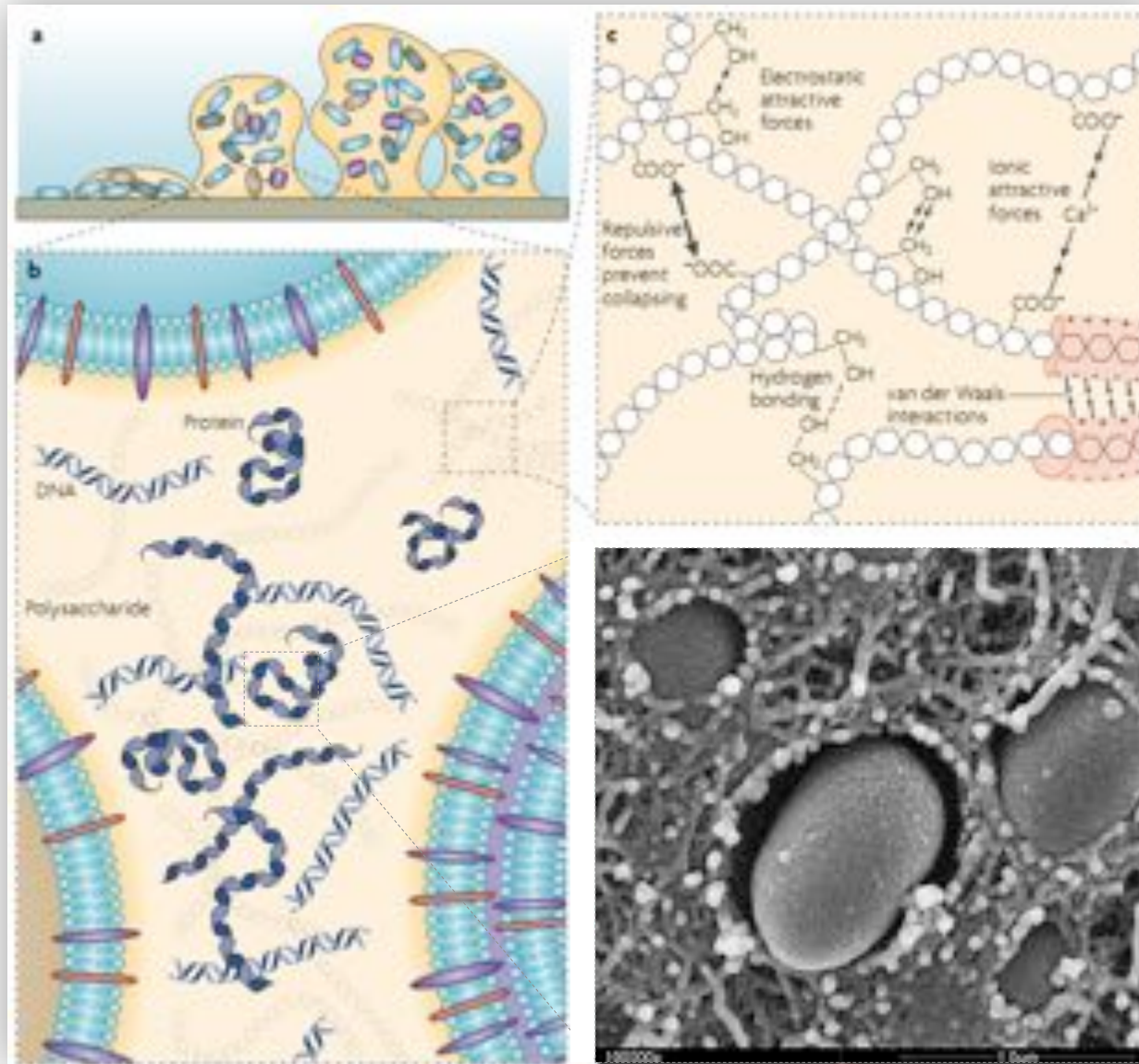


weitzlab.seas.harvard.edu



Vlamakis, et al. *Nat. Rev. Microbiol.* **2013**

Biofilm Nano-architecture



Nature Rev. Microb. 2010, 8:623.

mBio. 2013, 4(2):e00103-13

Domesticating the Microbe



Bacteria are dangerous!!!
(Germ Theory of Disease)
Pasteur and Koch, early 19th century



We understand how they work...
(Antibiotics, Microbiology, Molecular Biology)
Fleming, Watson, Crick, Lederberg, Brenner...et al.

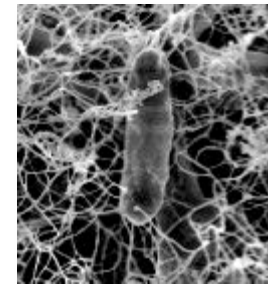
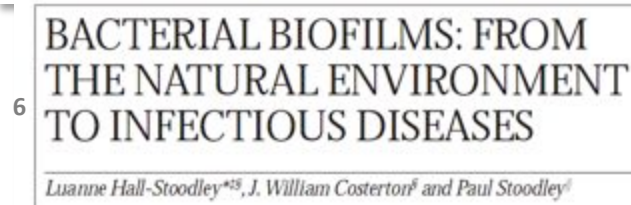
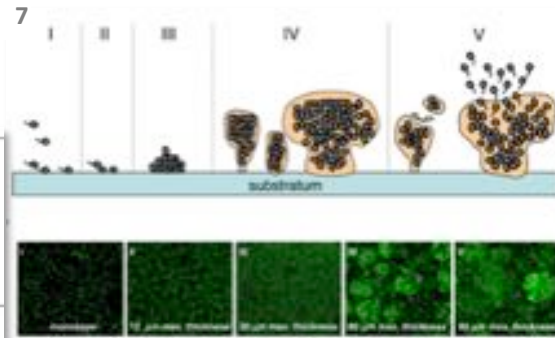
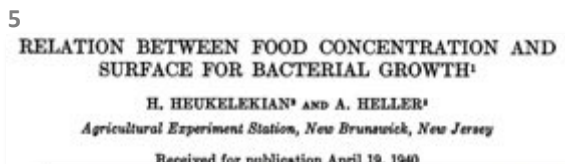


We can exploit them.
(Recombinant DNA Technology)
Cohen, Boyer, and Lloban

Biofilms are bad!!!

**We are starting to understand
how they work...**

Can we exploit them?



1,2. Wikimedia Commons, 3. Time Magazine, Inc., 4. Genentech, 5. *J. Bacteriol.*, 1940. 40(4):547, 6. *Nat. Rev. Microb.* 2004.2, 95-108, 7. *Clin Rev Allergy Immunol*, 2008. 35(3): 124, 8. Lower Lab, OSU.

Industrial Biofilm Usage

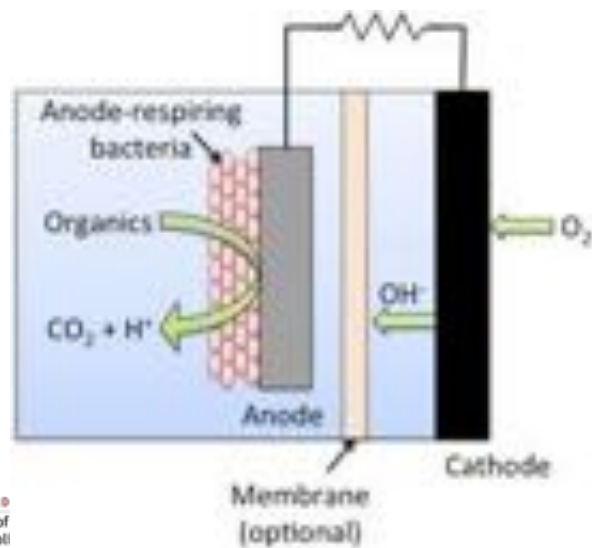
Wastewater treatment



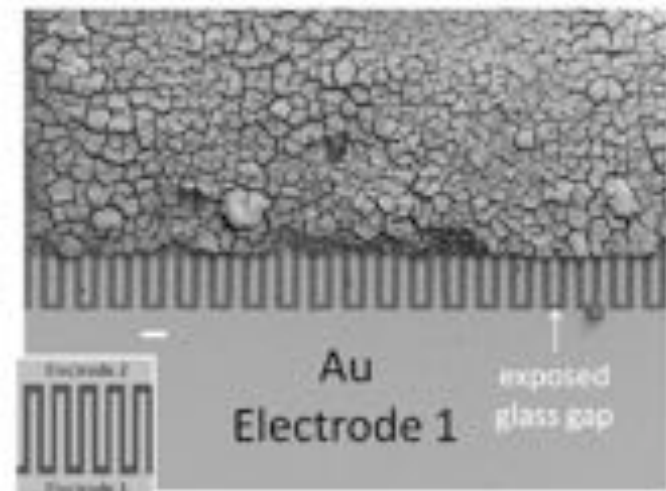
Chemical processing



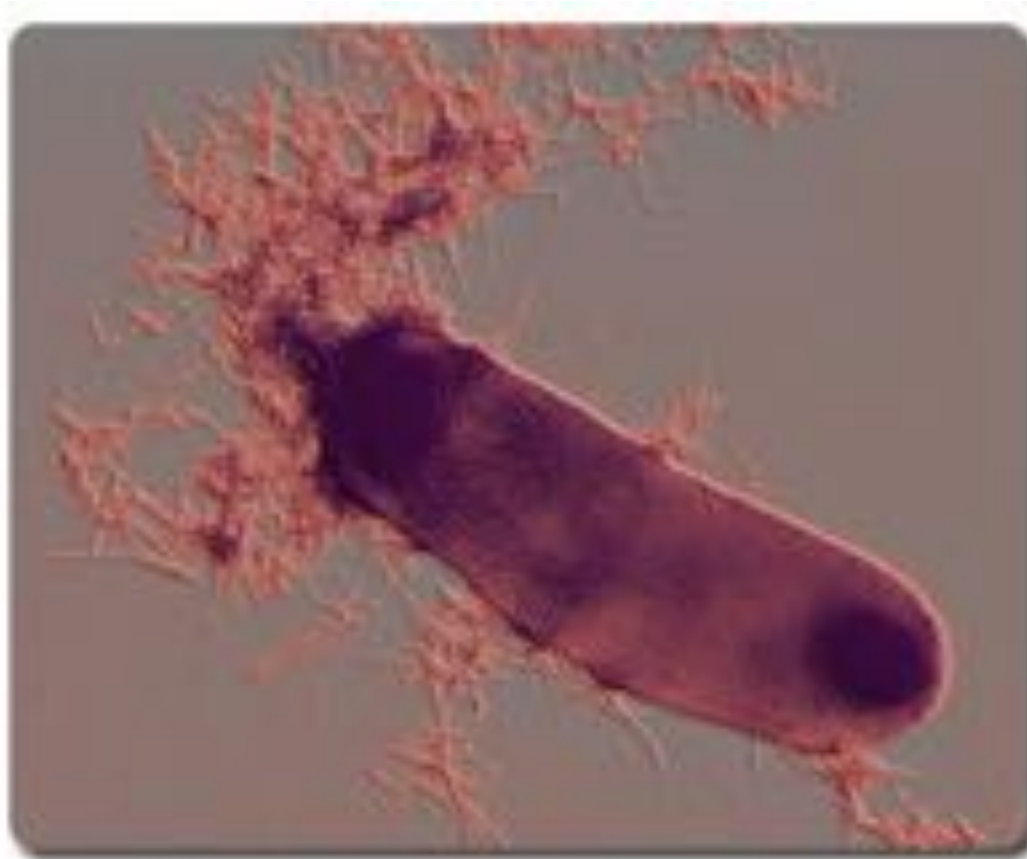
Microbial Fuel Cells



Microbial circuits



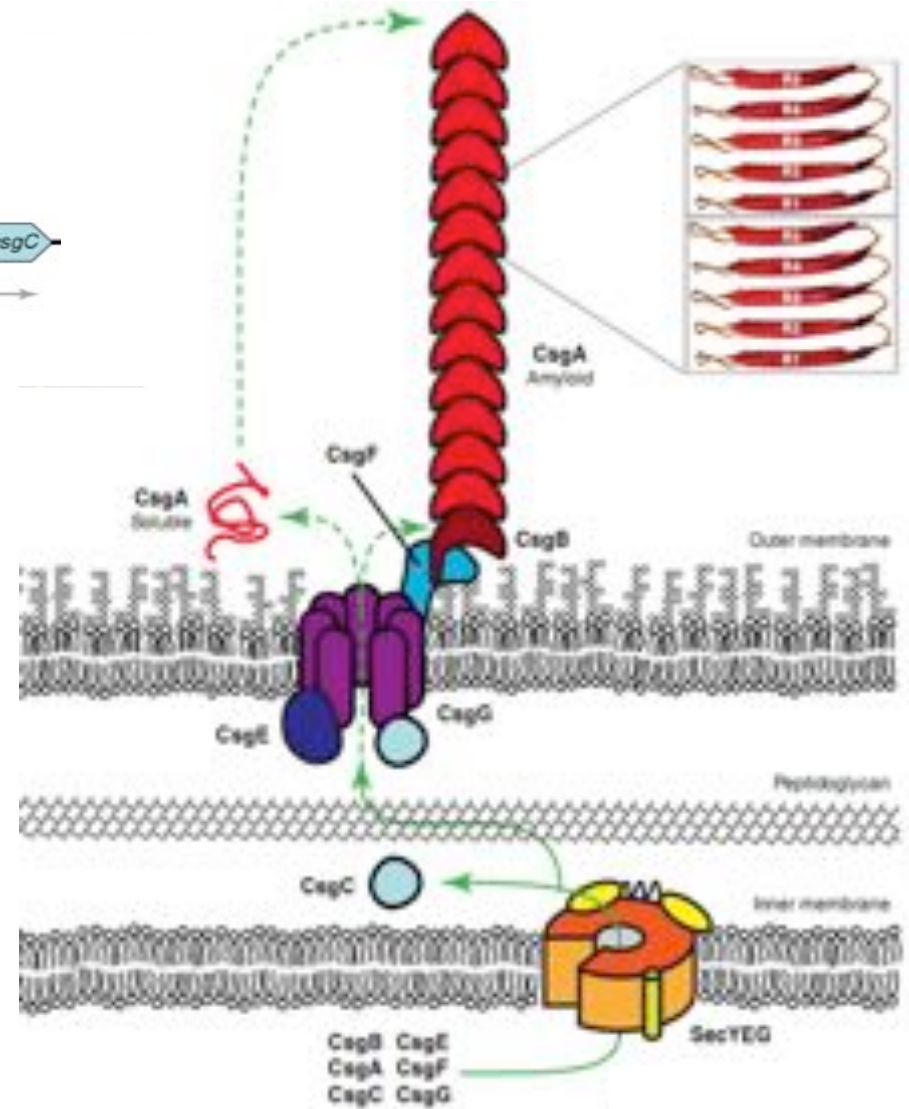
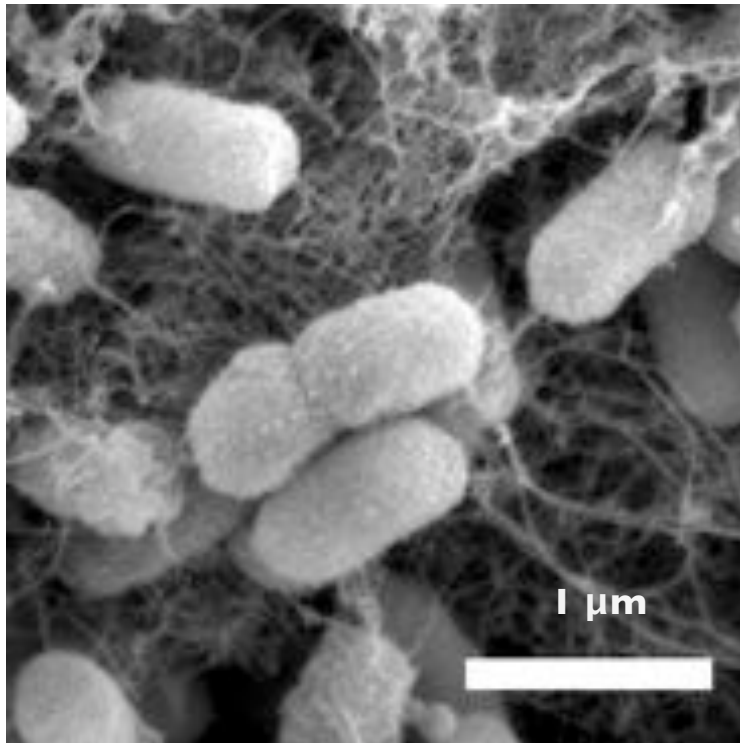
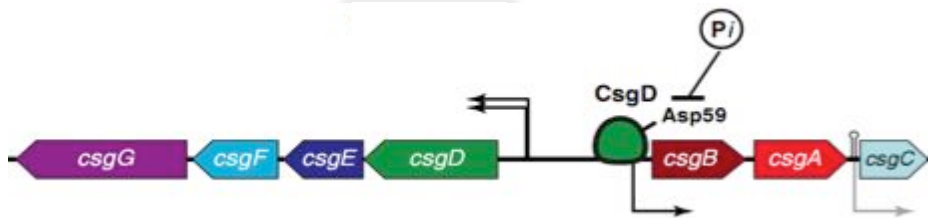
Bacterial ECM Proteins: Functional Amyloids



<http://labs.mcdb.lsa.umich.edu/labs/chapman/>

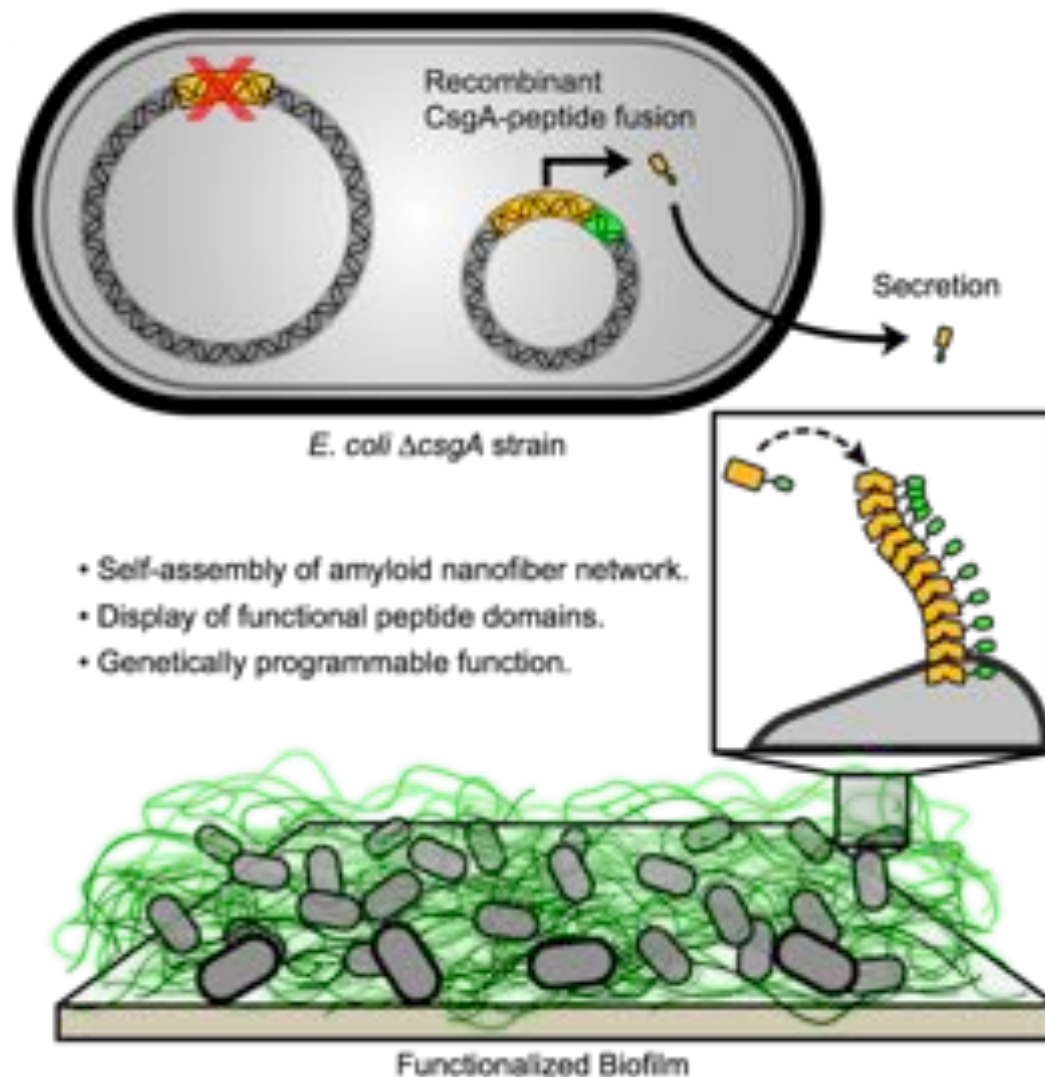
- Mediate adhesion to surfaces
- Can be up to 60% of biofilm biomass

E. Coli: Curli Biosynthesis



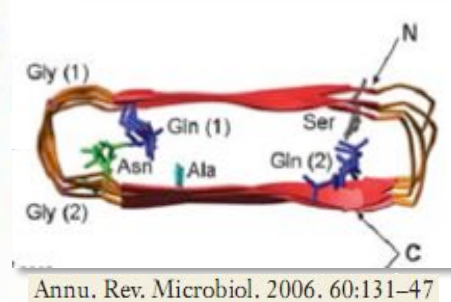
The BIND Concept

Biofilm-Integrated Nanofiber Display

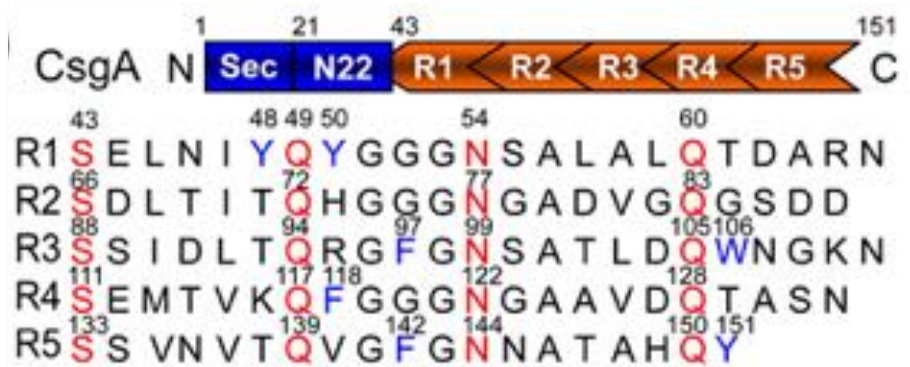


- Nanofibers have diameters from 4-7nm and are tens of microns in length.
- Nanofiber network is robust
- Amyloids: strength comparable to steel and stiffnesses comparable to silk

CsgA Protein Structure

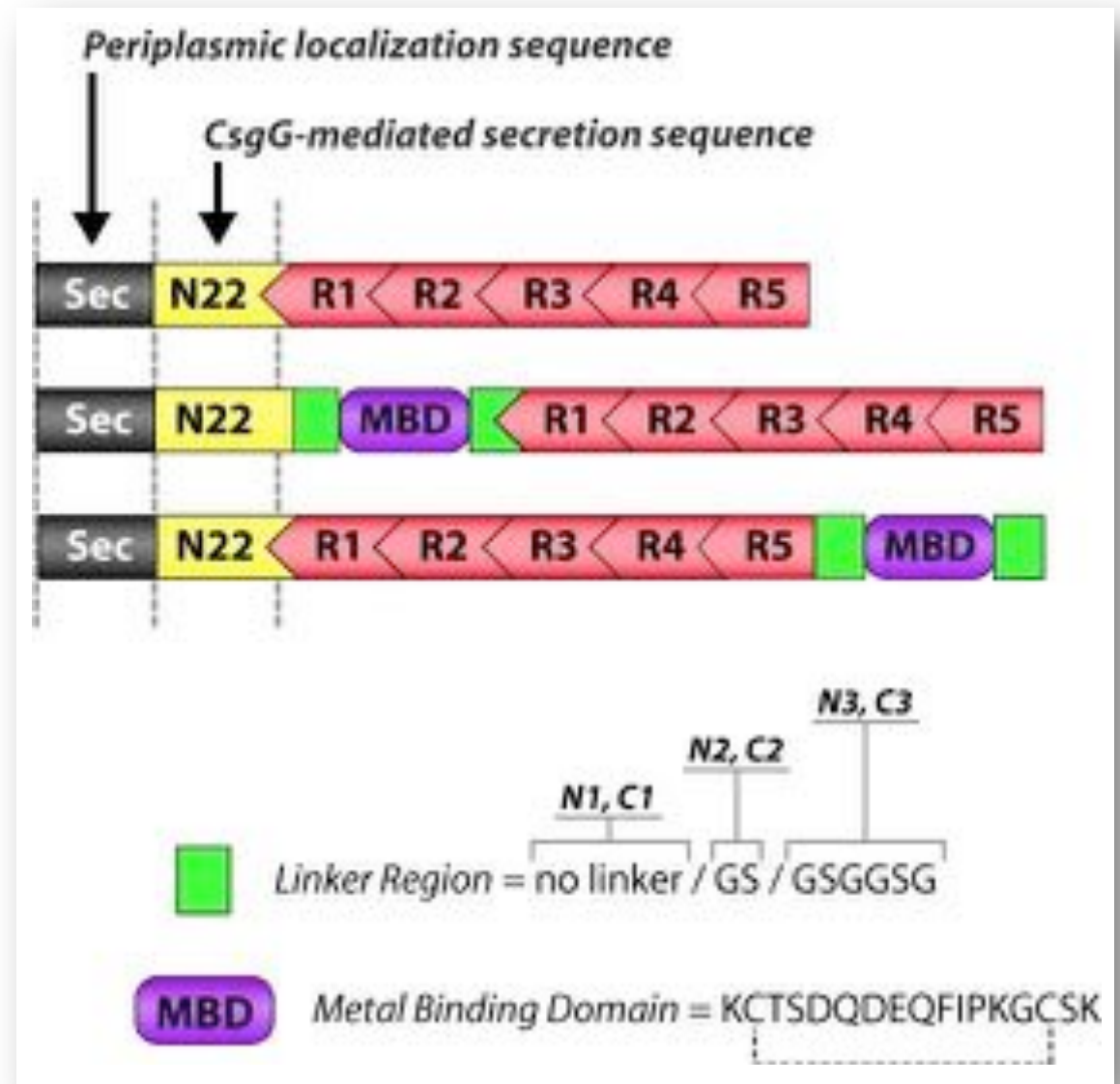
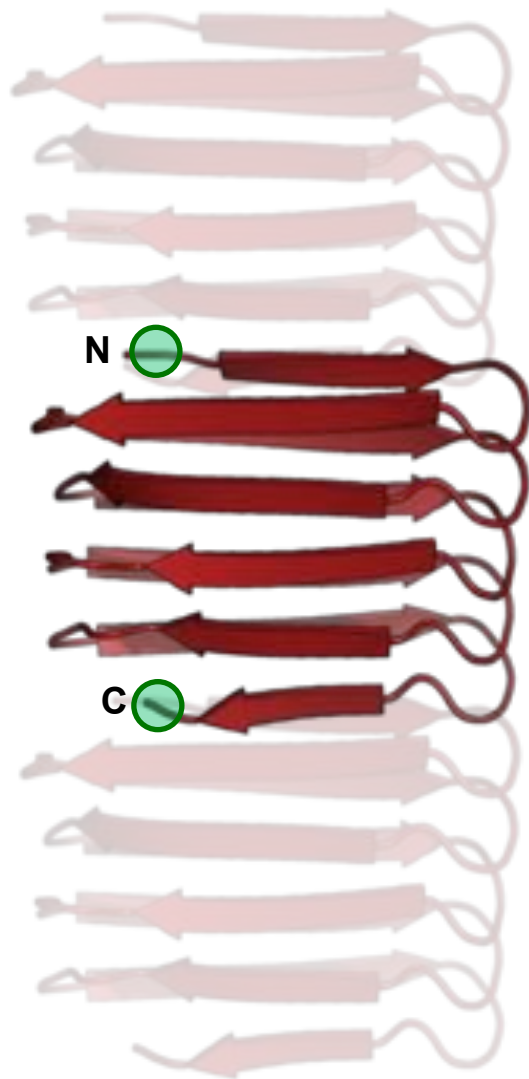


- Assembled from the secreted CsgB protein (17.5kDa) which is membrane-anchored.
- Easily detected using Congo Red, which stains amyloid fibers.
- Assembly kinetics can be monitored *in vitro* by Thioflavin T.

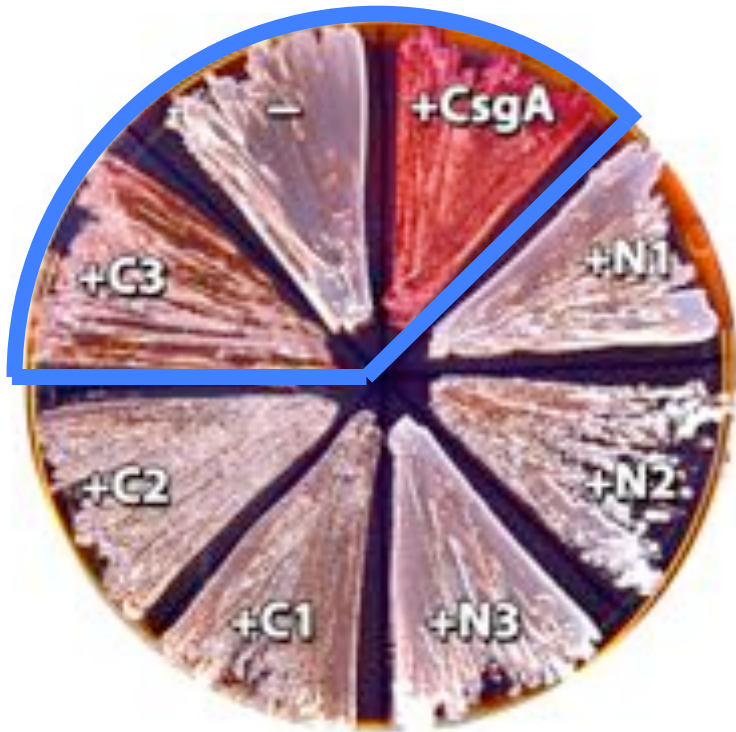


J. Mol. Biol. (2008) 380, 570-580

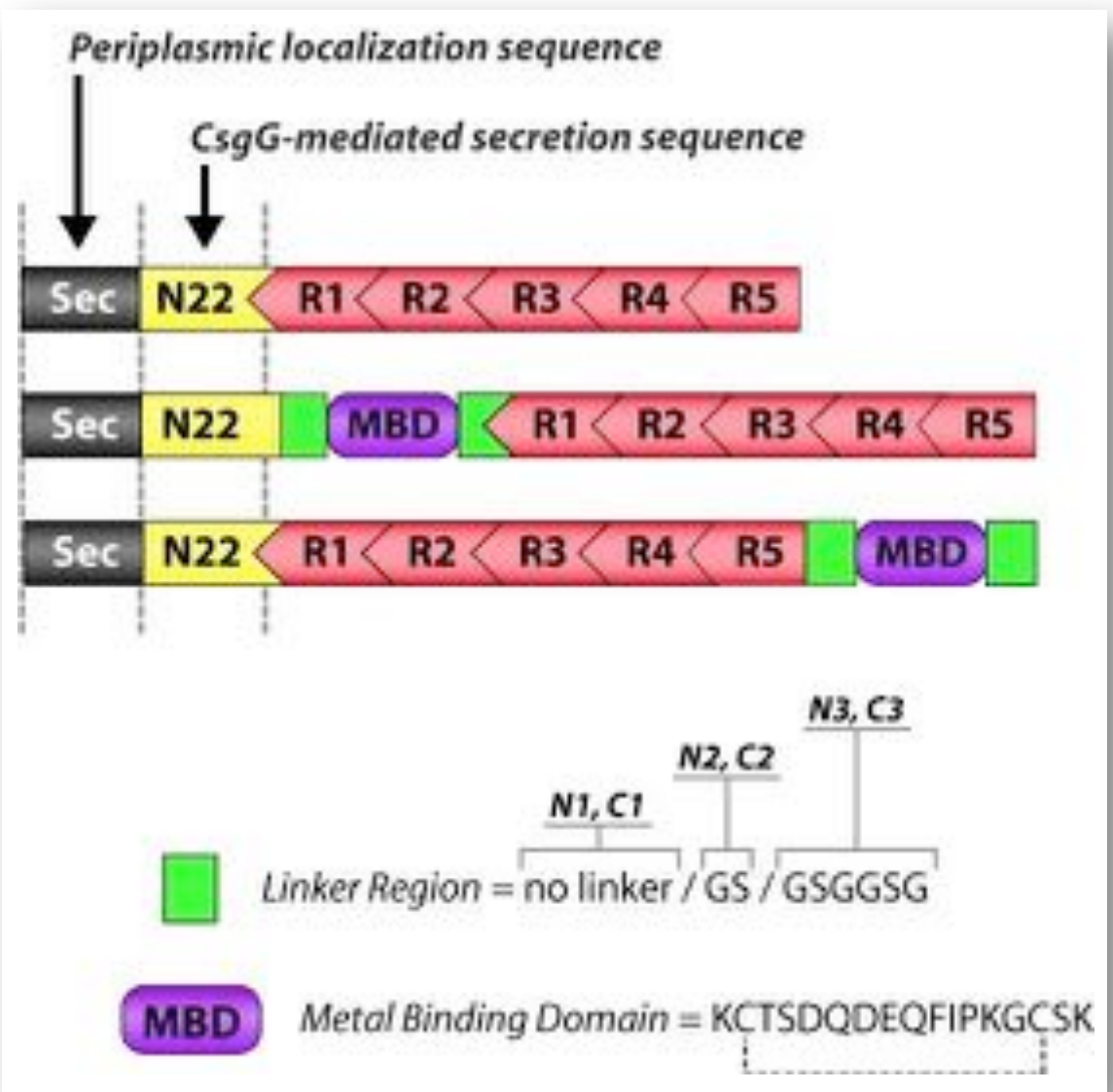
CsgA Insertion Library



CsgA Insertion Library

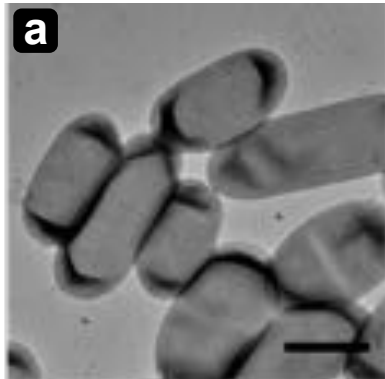


Congo Red assay
Red = amyloid formation

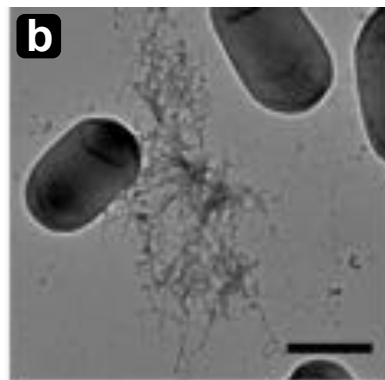


CsgA Insertion Library

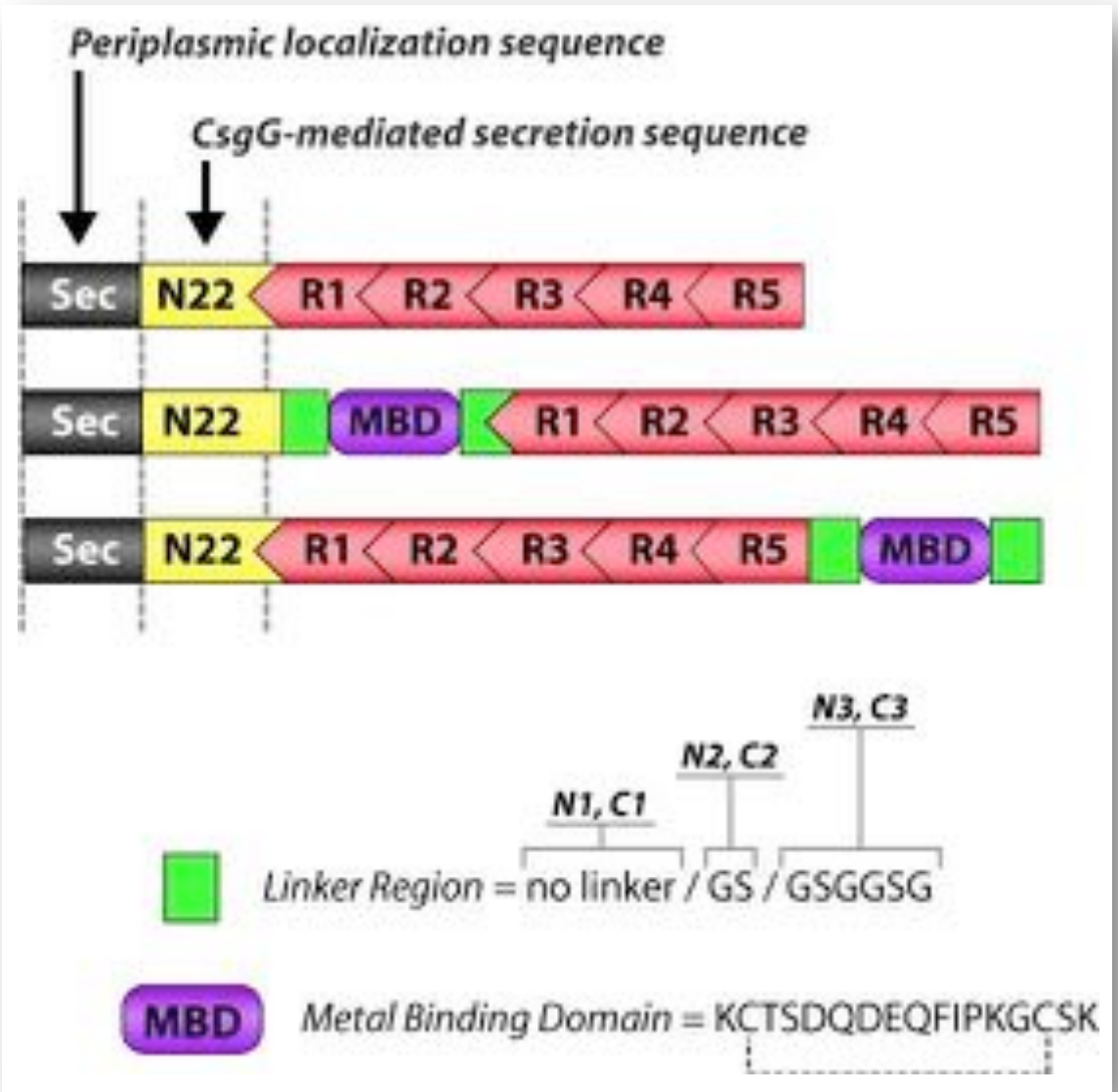
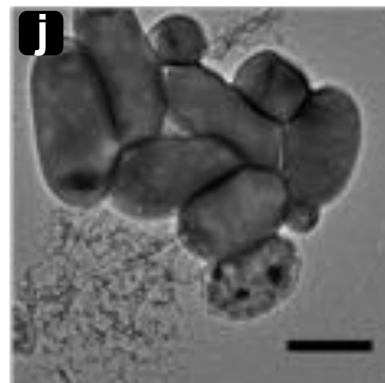
no CsgA
(-)



wt-CsgA
(+CsgA)



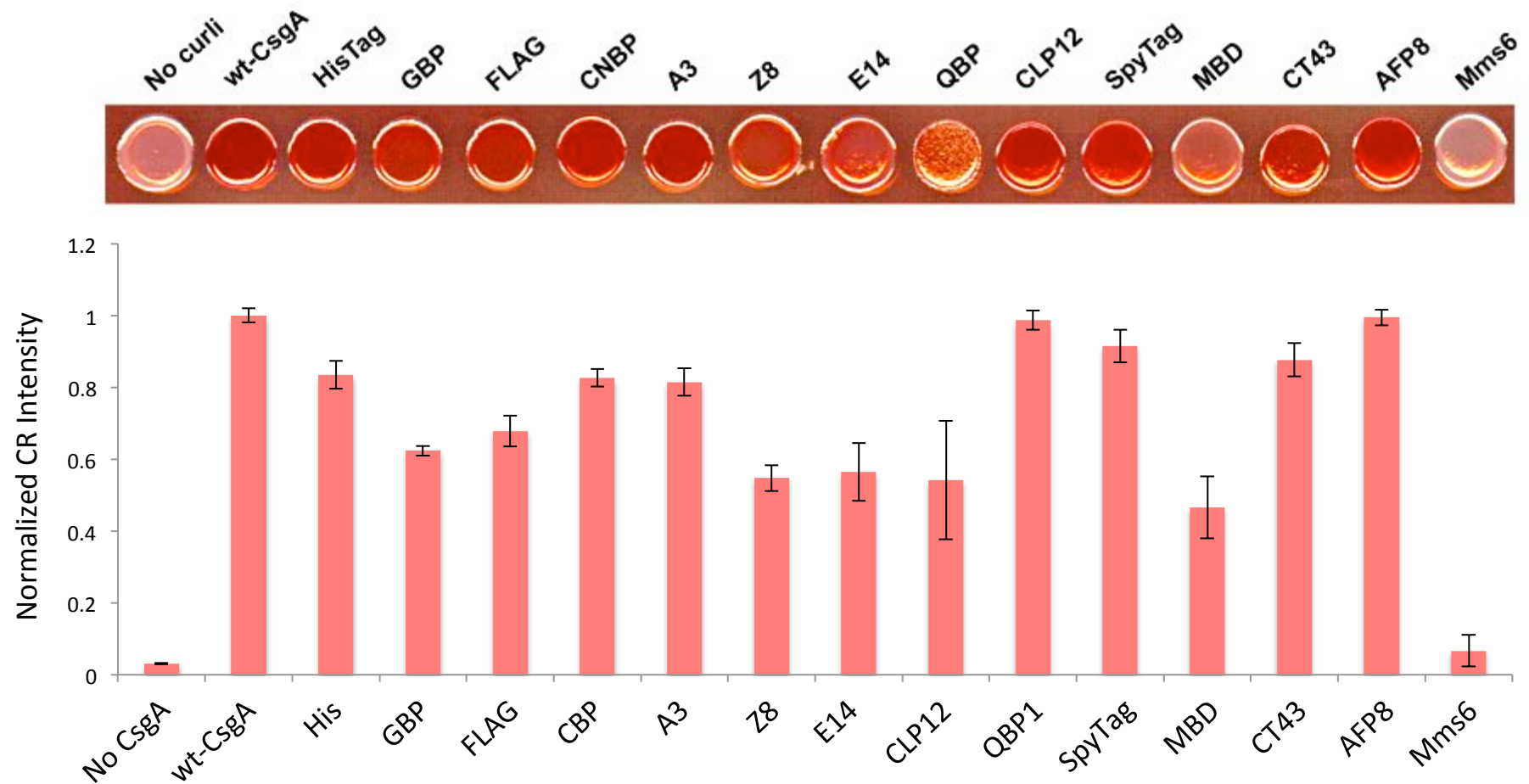
CsgA-MBD
(C3)



CsgA-peptide Insertion Library

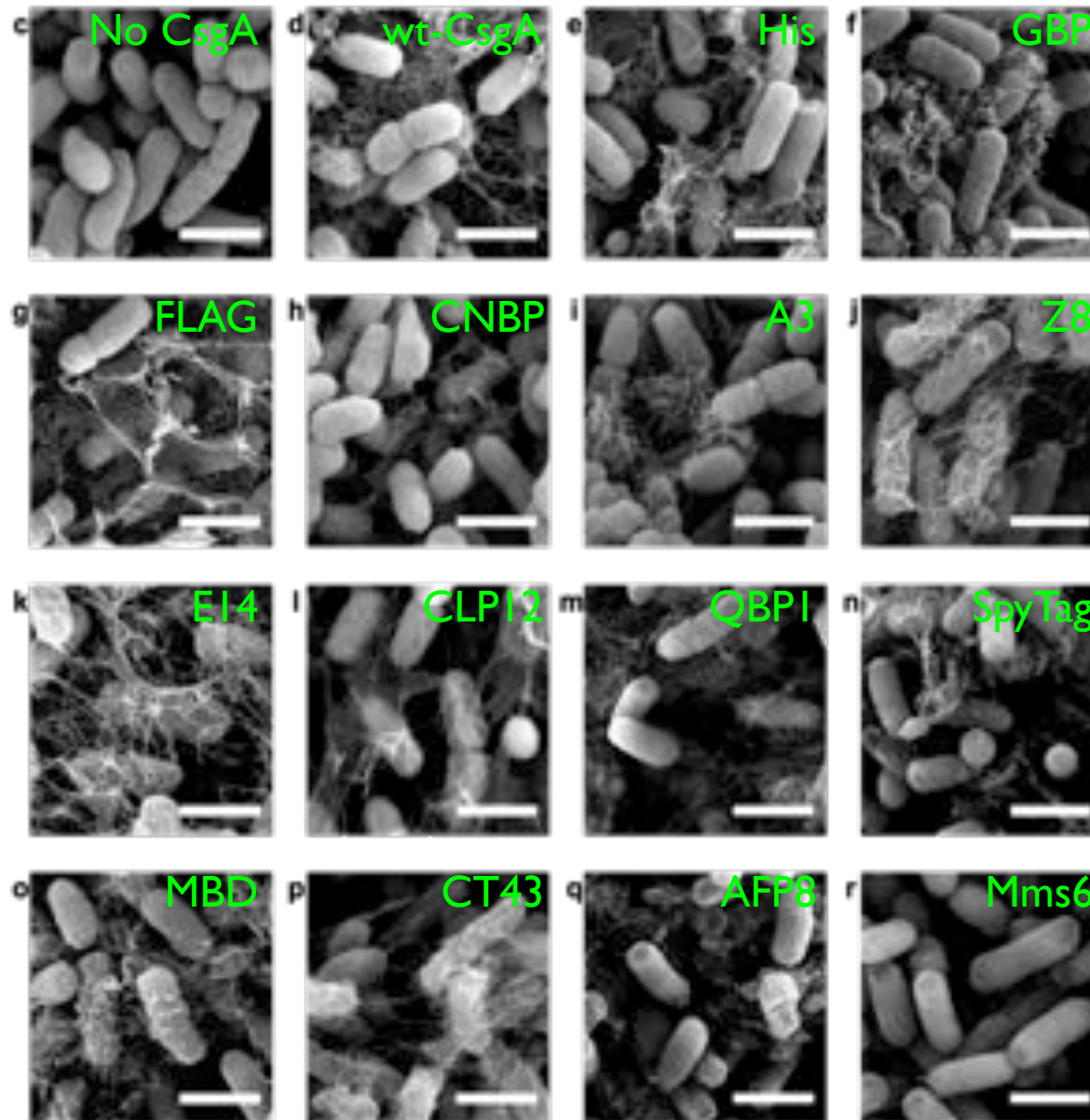
Peptide	Sequence	Length (aa)	Type	Function	Reference
HIS	HHHHHH	6	Affinity Tag	Affinity Tag	Bio/Technology 1988, 6(11): 1321.
GBP	EPLQLKM	7	Substrate Binding	Graphene edge binding	JACS 2011, 133: 14480.
FLAG	DYKDDDDK	8	Affinity Tag	Affinity Tag	Nature Biotech. 1988, 6: 1204.
CNBP	HSSYWYAFNNKT	12	Substrate Binding	Carbon nanotube binding	Nano. Lett. 2006, 6: 40.
A3	AYSSGAPMPPF	12	Substrate Binding	Gold surface binding	Small 2005, 1(11): 1048.
Z8	LRRSSEAHNSIV	12	NP templating	ZnS quantum dot templating	J. Mater. Chem. 2003, 13: 2414.
E14	PWIPTPRPTFTG	12	NP templating	CdS quantum dot templating	J. Mater. Chem. 2003, 13: 2414.
CLP12	NPYHPTIPQSVH	12	Mineral templating	Hydroxyapatite nucleation	Langmuir 2011, 27: 7620.
QBP1	PPPWLPMPPWS	12	Substrate Binding	Quartz/Glass binding	Bioinformatics 2007, 23: 2816.
SpyTag	AHIVMVDAYKPTK	13	Protein Display	General covalent capture/display of proteins	PNAS 2012, 109(12): E690.
BCCP	GLNDIFEAQKIEWH	14	Protein Display	Biotinylation tag	Prot. Sci. 1999, 8: 921.
MBD	KCTSDQDEQFIPKGCSK	17	Substrate Binding	Binding to stainless steel surfaces	Mol. Microb. 2006, 59(4): 1083.
CT43	CGPAGDSSGVDSRSVGPC	18	NP templating	ZnS quantum dot templating	JACS 2010, 132: 4731.
AFP8	DTASDAAAAAALTAANAKAAAE LTAANAAAAAATAR	37	Substrate Binding	Ice crystal binding	JBC 1998, 273(19): 11714.
Mms6	GGTIWTGKGLGLGLGLGLGAV/ GPIILGVVGAGAVYAYMKSRDI ESAQSDEEVELRDALA	59	NP templating	Magnetite NP templating	JBC 2003, 278(10): 8745.

Quantifying CsgA-peptide production



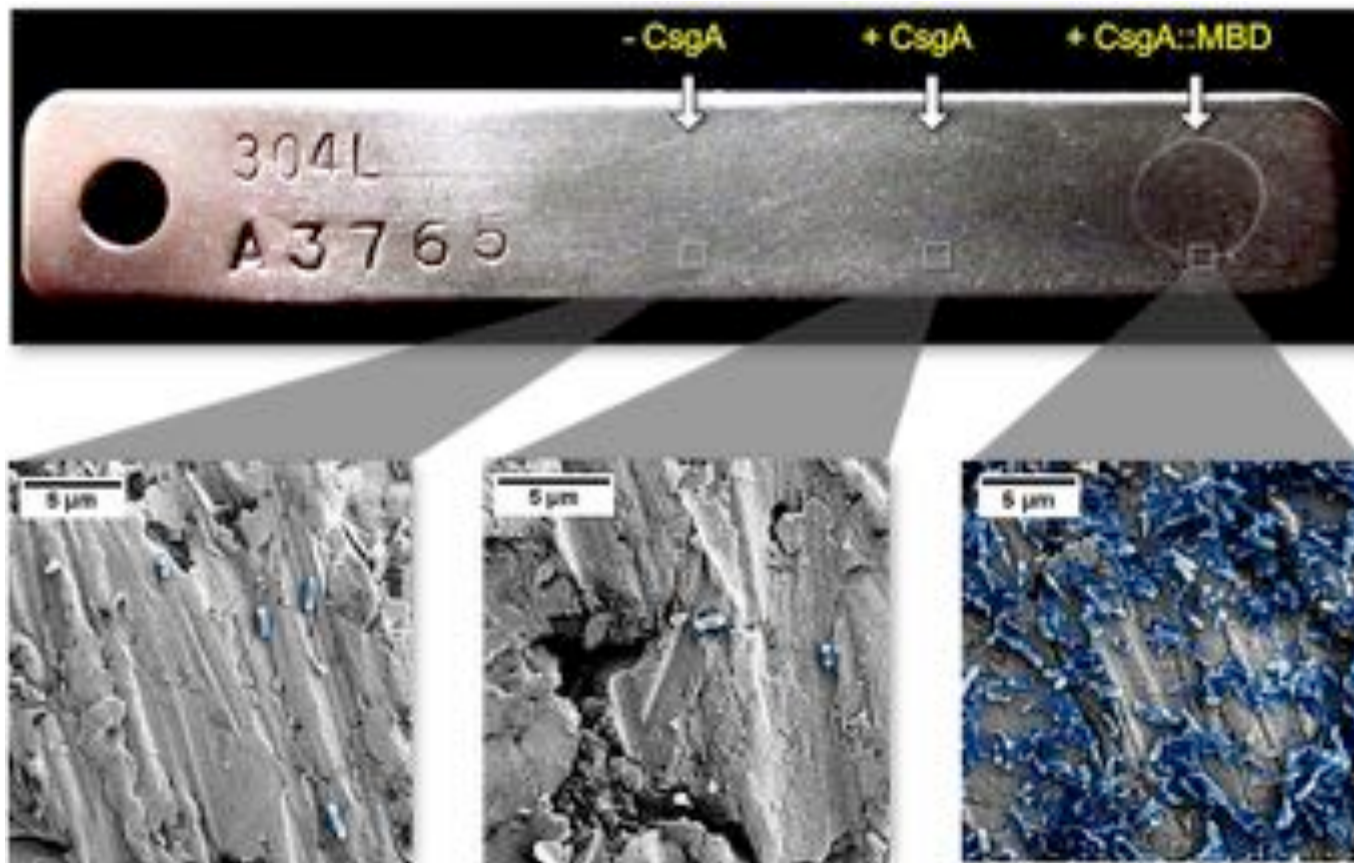
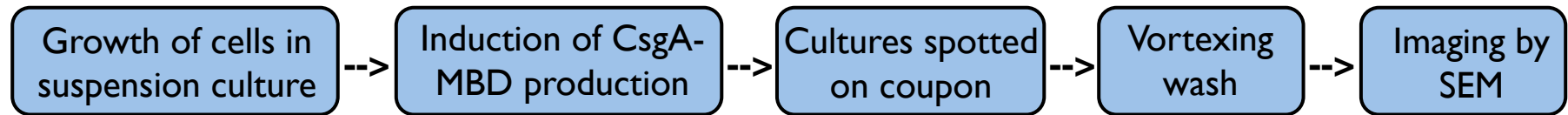
→ Most peptides <50 amino acids do not hinder protein secretion or assembly

Most CsgA-peptide mutants form amyloids



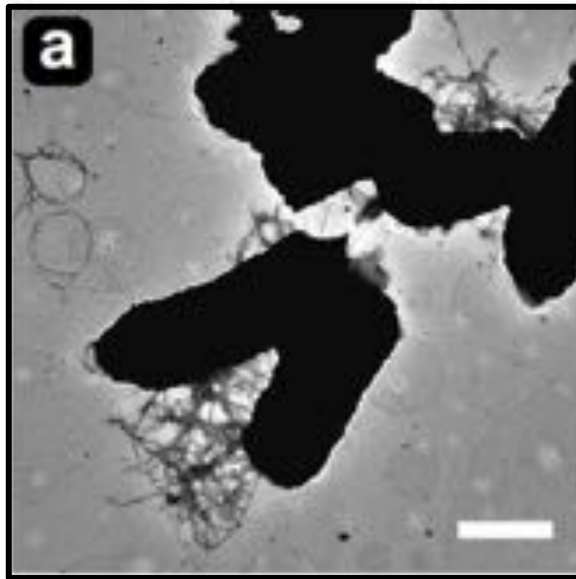
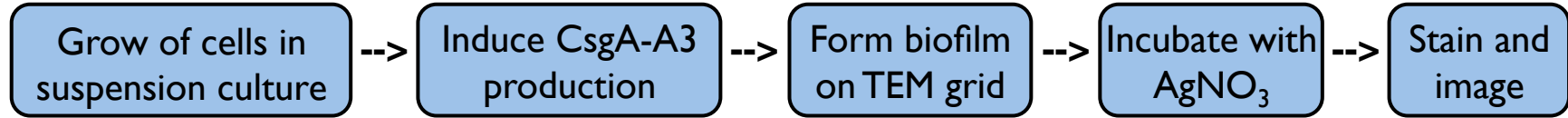
Scale bar = 1 μ m

CsgA-MBD enhances adhesion to steel

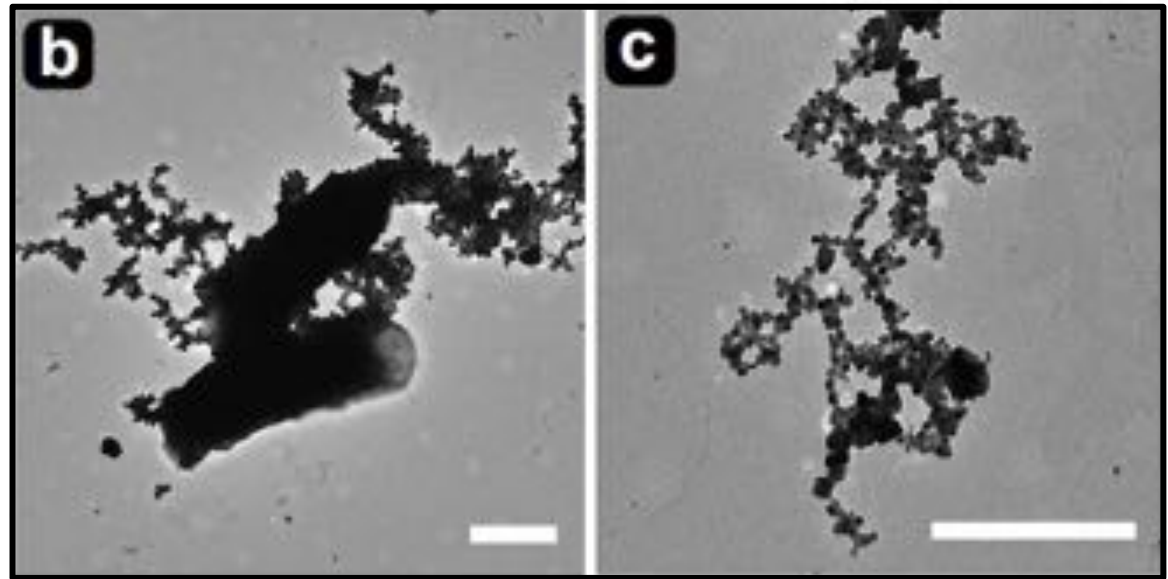


MBD	KCTSDQDEQFIPKGCSK	17	Substrate Binding	Binding to stainless steel surfaces	Mol. Microb. 2006, 59(4): 1083.
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CsgA-A3 templates AgNP growth



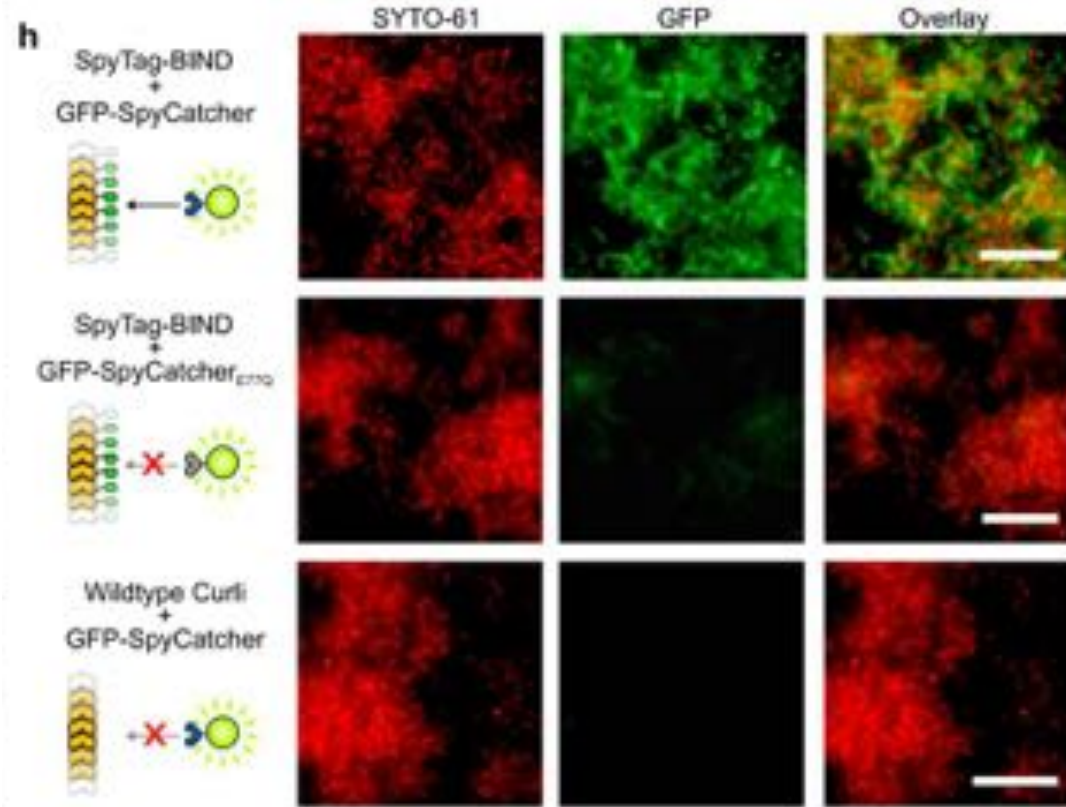
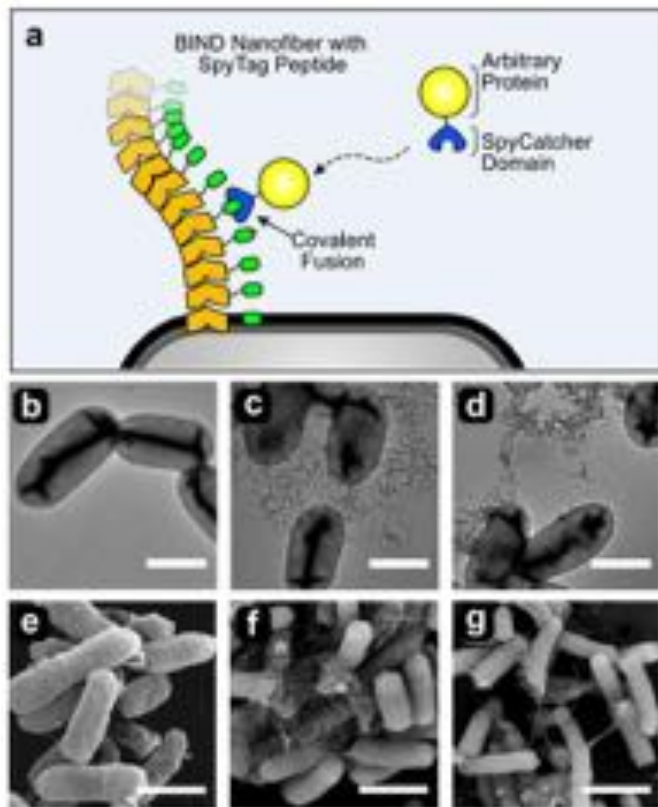
wt-CsgA



CsgA-A3

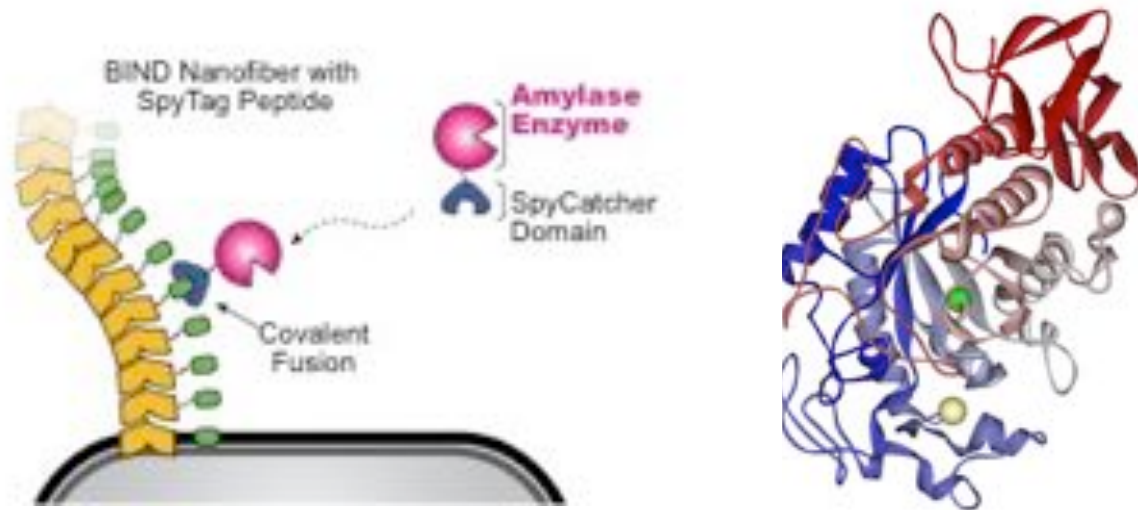
CNDP	POSTWTAFINNNI	12	Substrate binding	Carbon nanotube binding	IBRID. Lett. 2000, 0, 40.
A3	AYSSGAPPMPPF	12	Substrate Binding	Gold surface binding	Small 2005, 1(11): 1048.
79	LDSSCALNNIV	42	NP formation	ZnS quantum dot formation	J Mater Chem. 2002, 12, 2444

CsgA-SpyT enable protein immobilization



Seq 1	PPPWLPLYMPWWS	12	Substrate binding	General covalent capture/display of proteins	PNAS 2012, 109(12): E690.
SpyTag	AHIVMVDAYKPTK	13	Protein Display		

BIND as a catalytic substrate



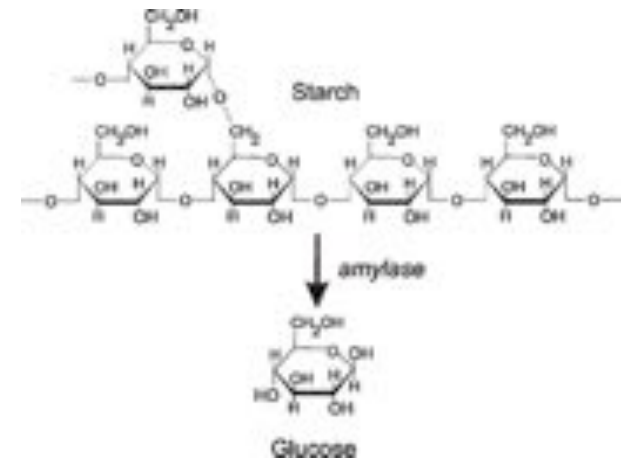
- Hydrolyzes alpha glycosidic bonds of sugars.

-

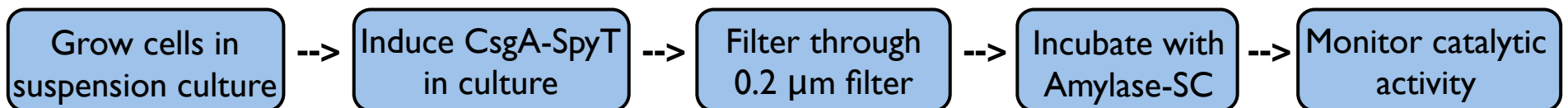
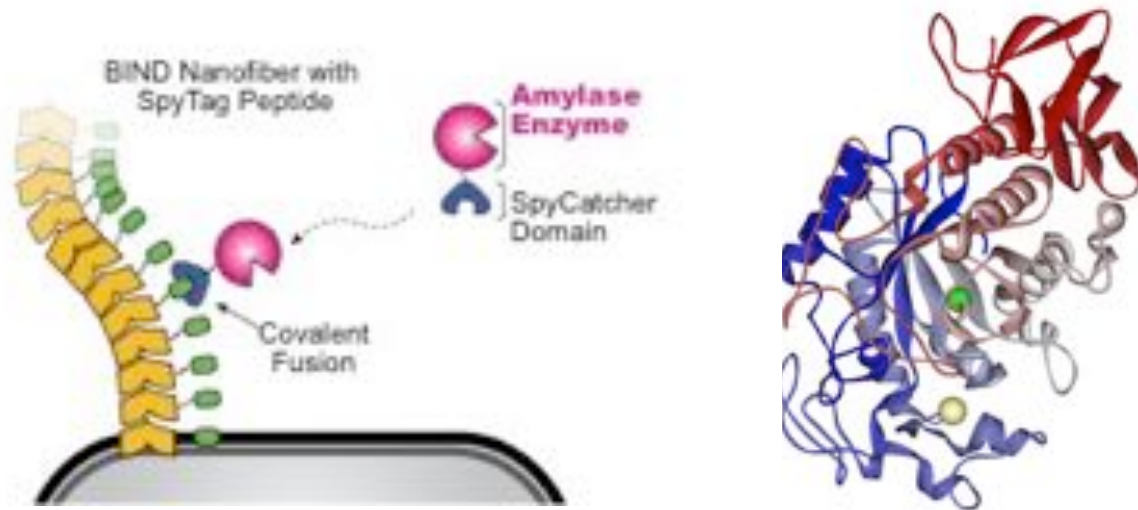
- Used for:

- Ethanol production
- HFCS production
- Laundry detergents

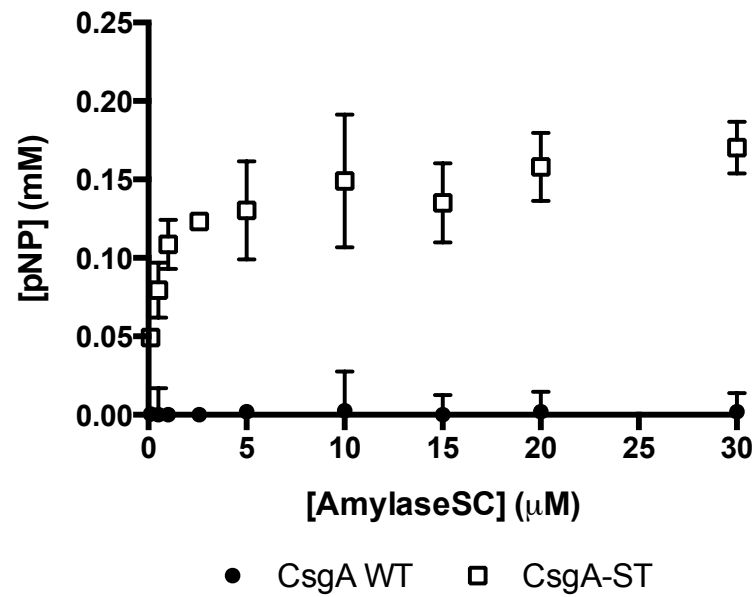
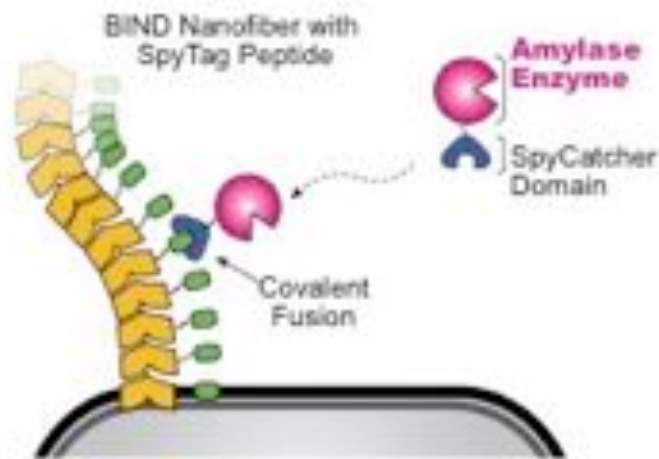
- Accounts for ~**30%** of world-wide industrial enzyme production



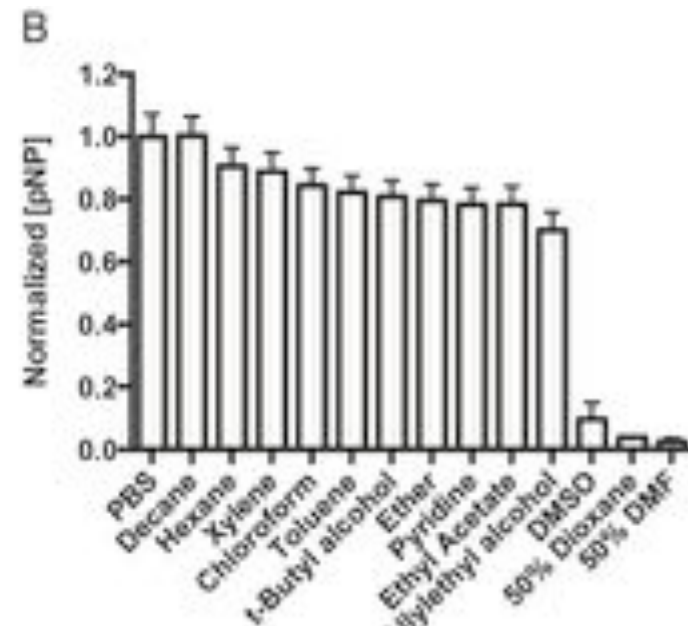
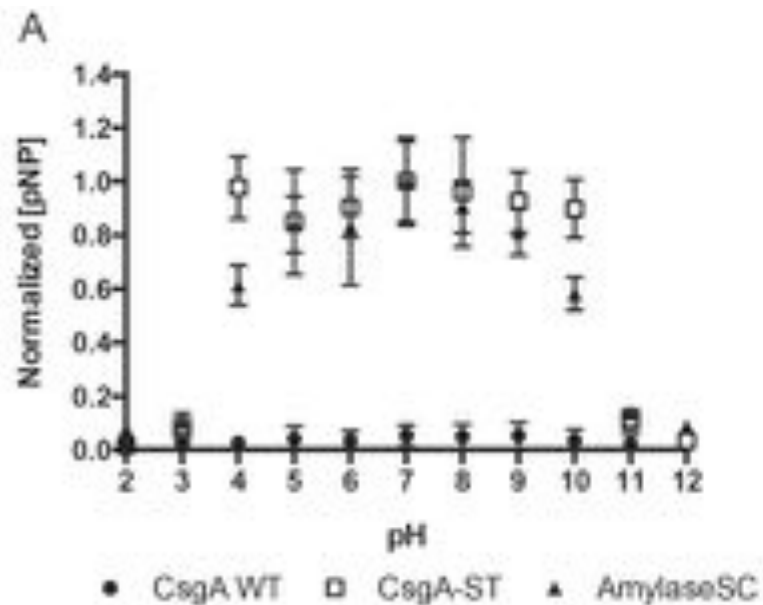
BIND as a catalytic substrate



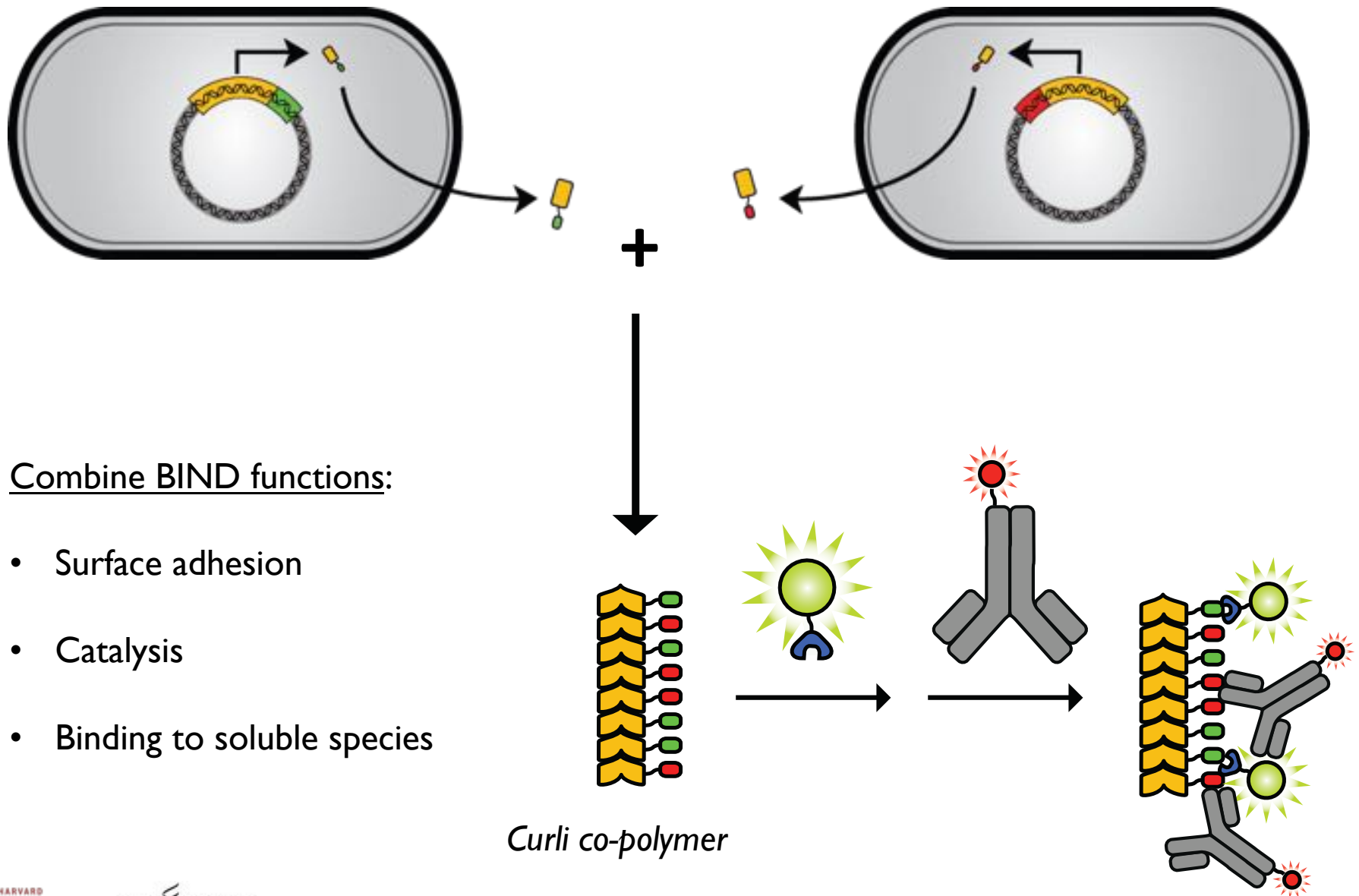
BIND as a catalytic substrate



BIND as a catalytic substrate

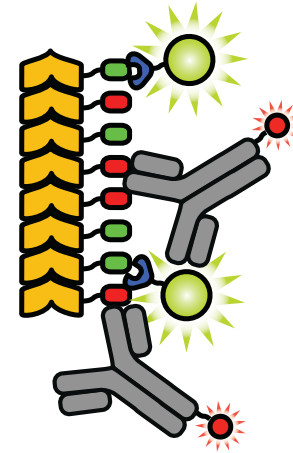


Co-assembly of Two CsgA Variants

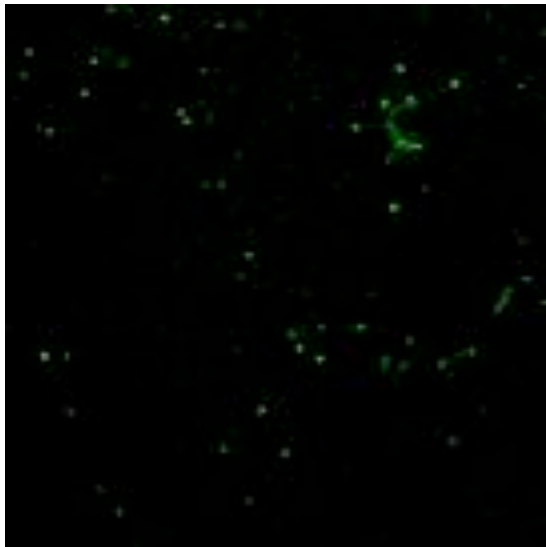


Bifunctional Curli Networks

Two CsgA::peptide variants can be displayed simultaneously to create multifunctional materials

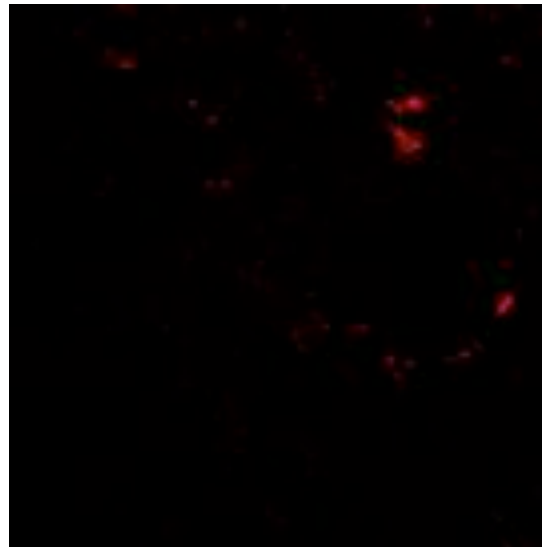


Function 1: SpyTag
(Venus::SC-immobilization)



GFP detection

Function 2: FLAG tag
(anti-FLAG 1°, 633nm Dylight 2°)

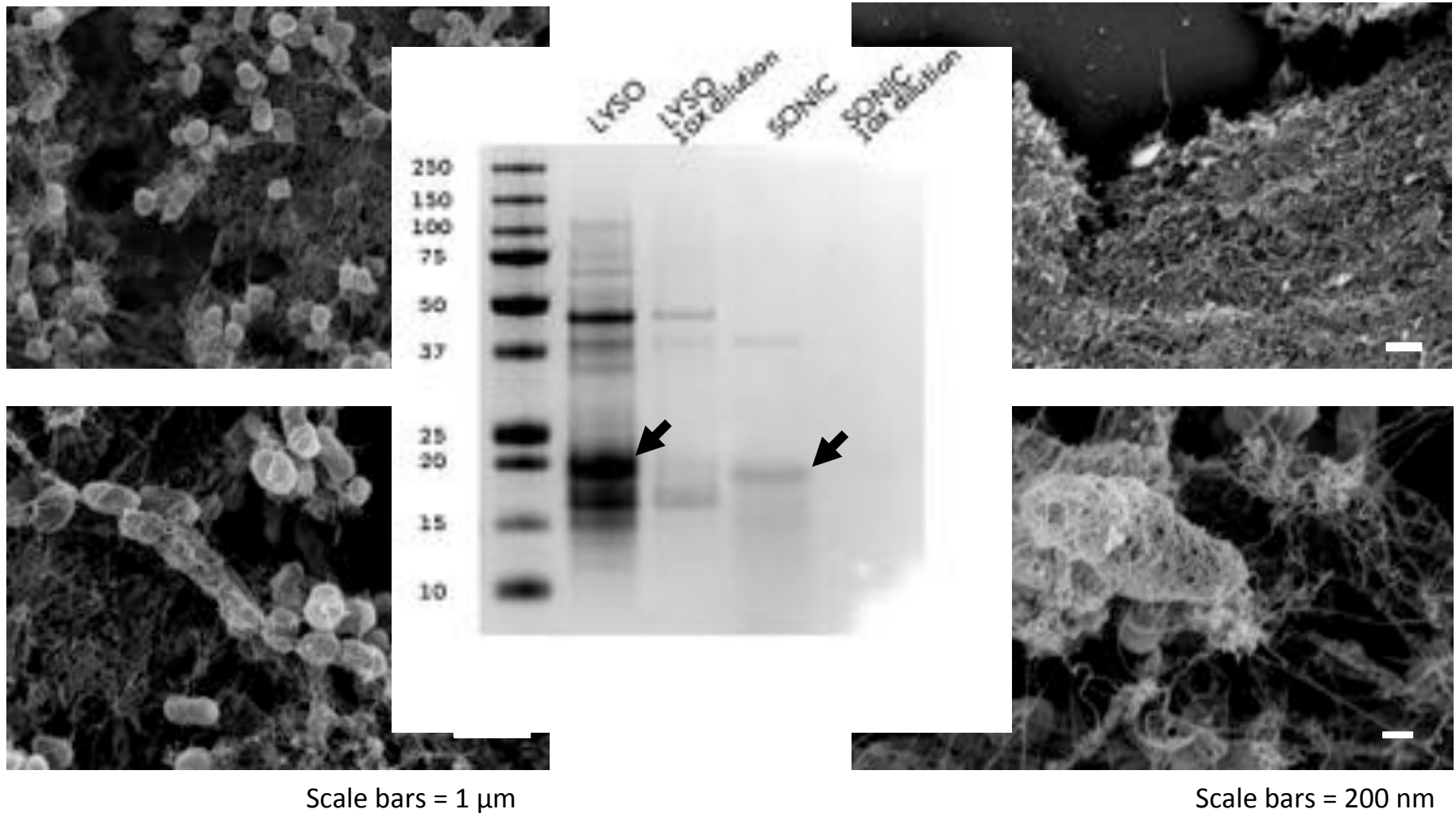


DyLight 633 detection

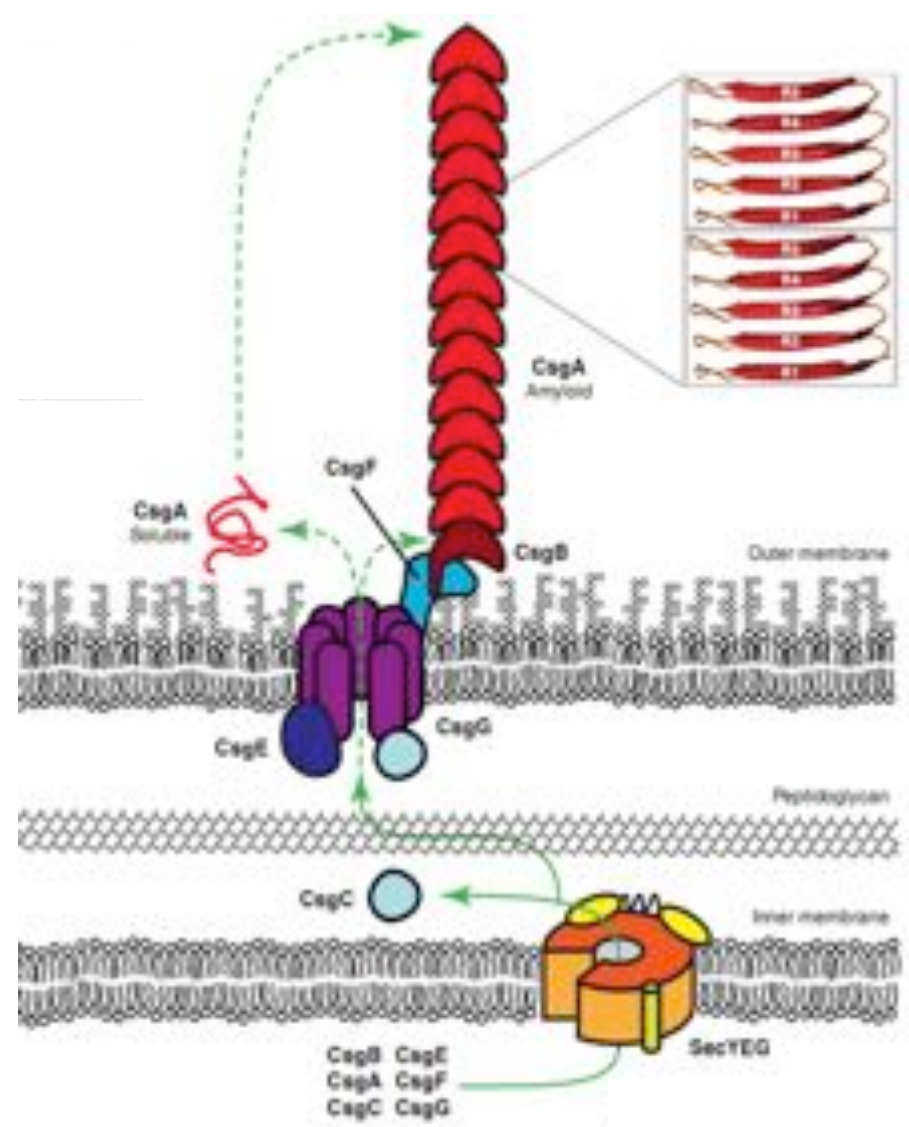


DIC

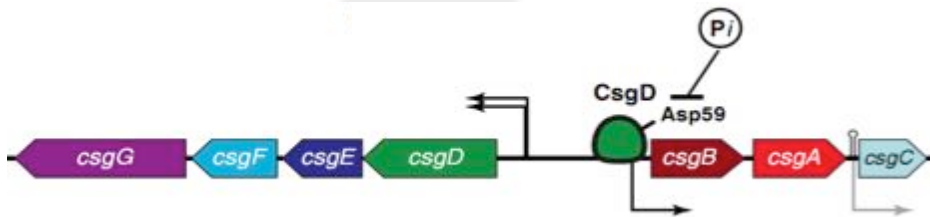
Amyloid network survives decellularization



Amyloid material remains intact after harsh treatments (solvents, pH)



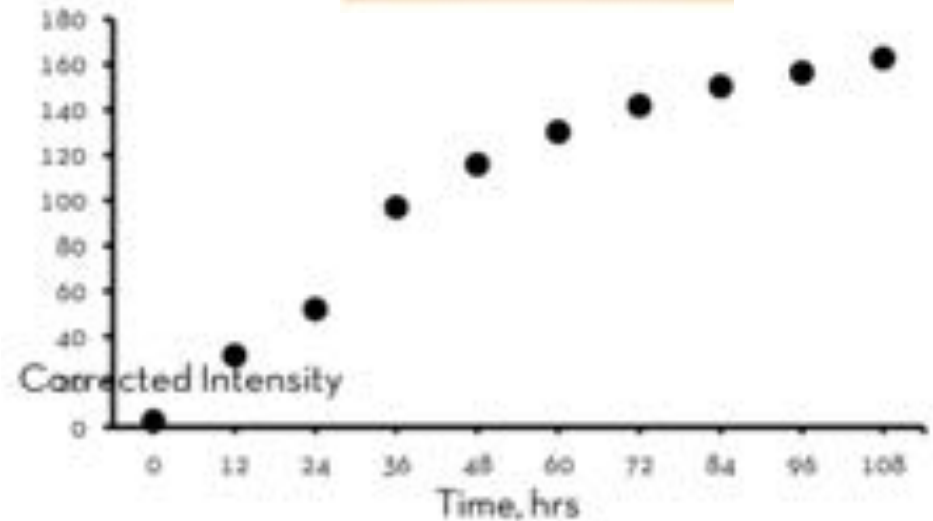
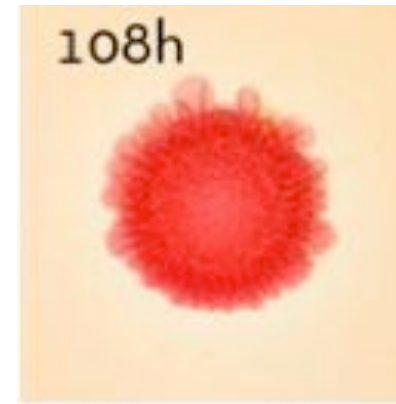
Curli operon optimization



Library of mutants with varying production levels for each music

Screen library for amyloid formation

Identify mutants with optimal curli production

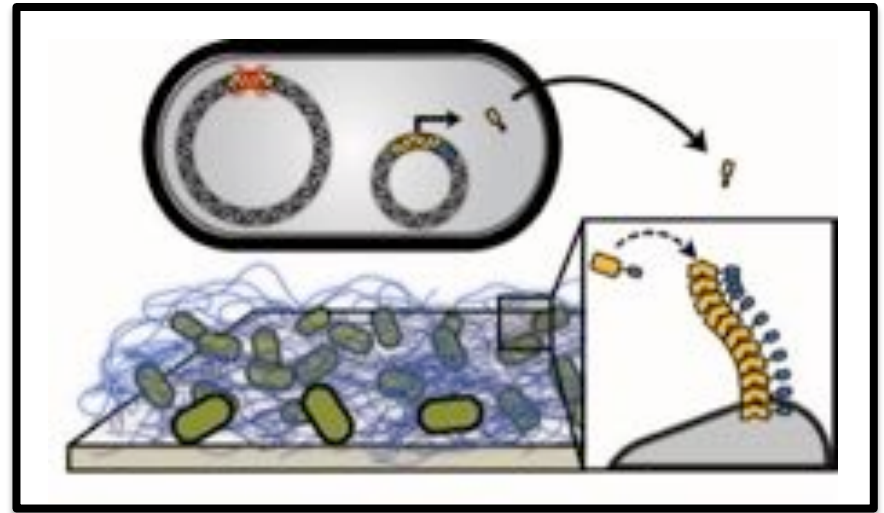


BIND: what is it good for?

Combining the versatile functions of recombinant proteins with scalable materials fabrication

CAPABILITIES

- Specific surface adhesion
- Nanoparticle templating
- Enzymatic catalysis
- Specific binding and capture of soluble entities
(**metals**, small molecules, proteins, viruses, cells)
- Large scale material production
(**biofilm paint**, spray-on coatings, self-standing 3D materials)
- Environmental responsiveness
(sensing, programmed formation/breakdown, dynamic properties)
- Programmed biological interactions
(antimicrobial coatings, **live biotherapeutic**)



BIND for biocatalysis

Solution-phase catalyst

- Poor catalyst recycling/recovery
- Limited catalyst stability

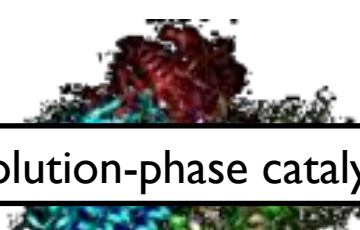
Existing biocatalysis strategies

- Limited substrate diversity
- Contaminants complicate product purification

- Activity affected by immobilization
- Cost of substrate and processing

Whole-cell catalyst

Surface-immobilized catalyst



Solution-phase catalyst

BIND for biocatalysis

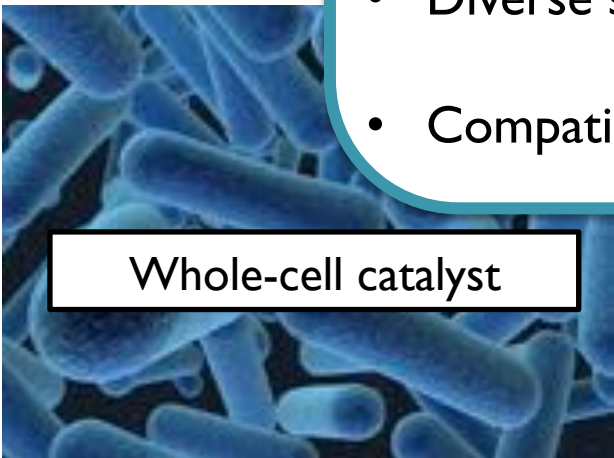
- Modular immobilization strategy
- No enzyme purification or processing
- Enhanced enzyme stability
- Diverse substrate tolerance
- Compatible w/ continuous flow processes

- Limited substrate scope
- Contaminants in feed stream require product purification

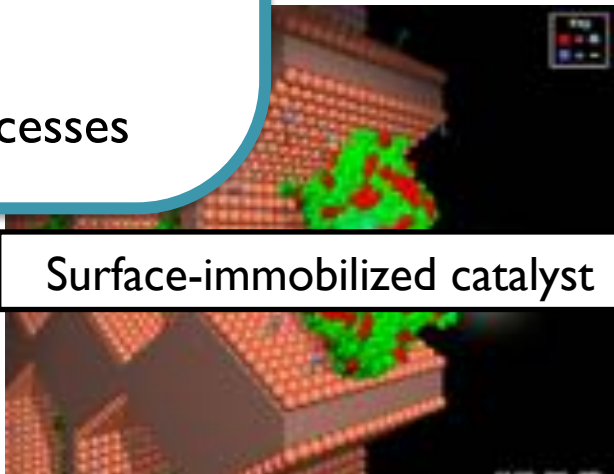
enzyme recycling/

enzyme stability

activity affected by immobilization
loss of substrate and
leakage

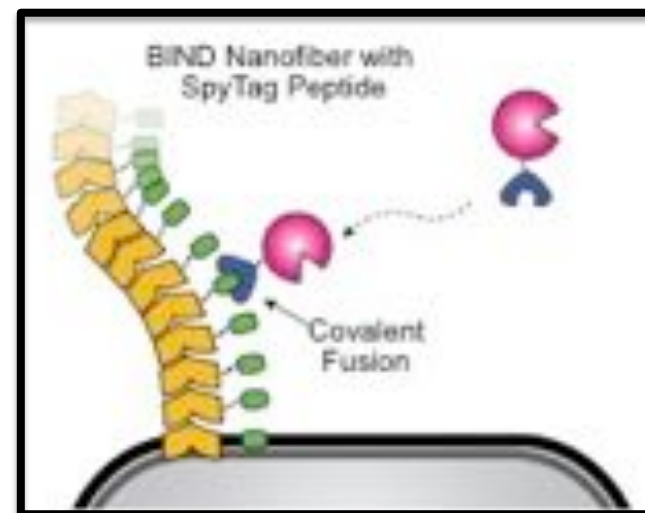
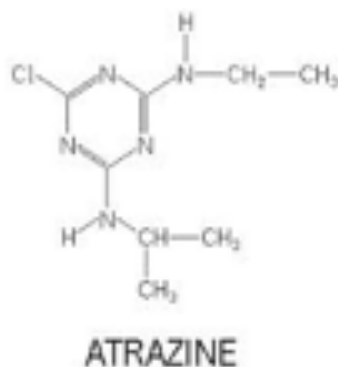


Whole-cell catalyst

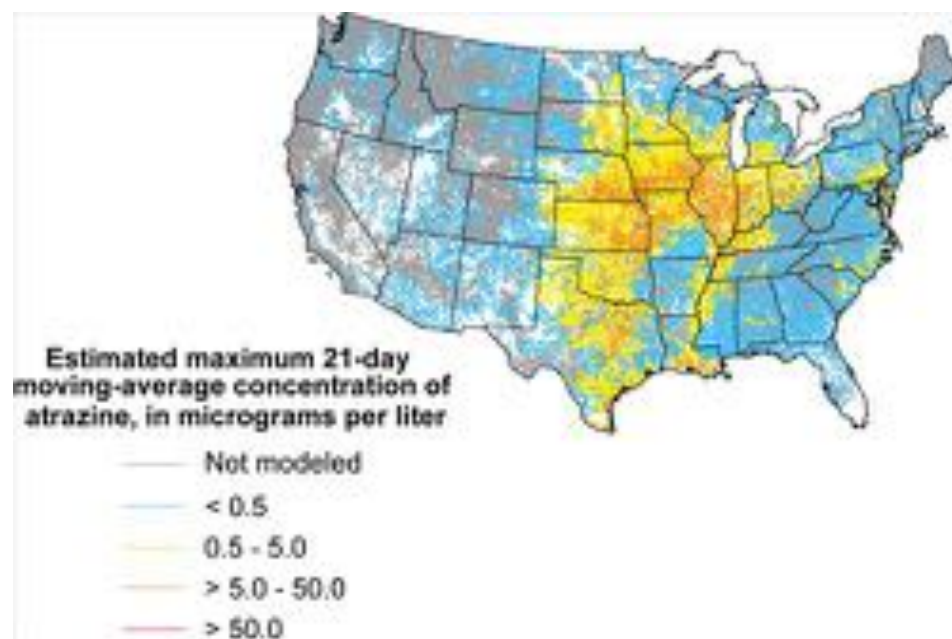


Surface-immobilized catalyst

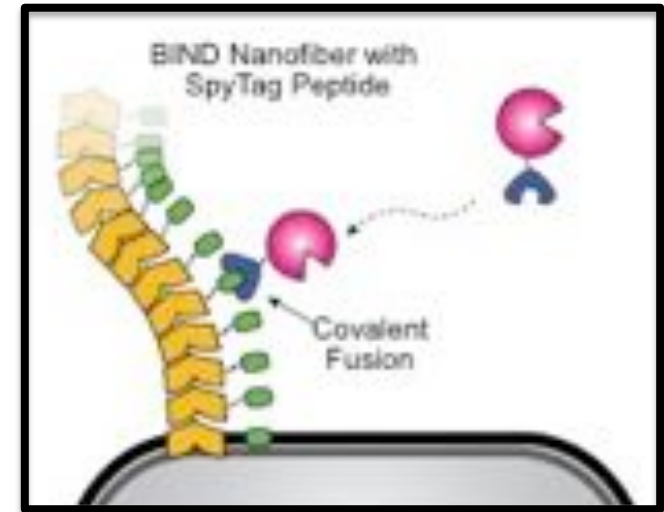
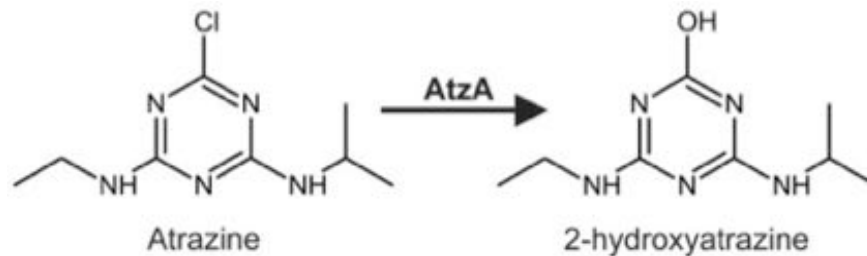
Enzymatic BIND for water decontamination



- EPA Maximum Contaminant Level (MCL) = 3ppb
- Frequently found to be above 5ppb in the mid-east and mid-west
- 0.1ppb = endocrine disruption



Enzymatic BIND for water decontamination



Enzymatic water decontamination is inefficient and expensive:

- Naturally occurring strains do not eliminate atrazine to acceptable levels
- Cost of enzyme purification is too high
- Limited substrate diffusion across cell membrane inhibits breakdown with whole cells

→ Can BIND facilitate an efficient continuous flow atrazine decontamination system by displaying enzymes?

BIND for specific metal removal/recovery

The need for rare earth metals

Rare earth minerals

Group of 17 elements used in a wide range of consumer products

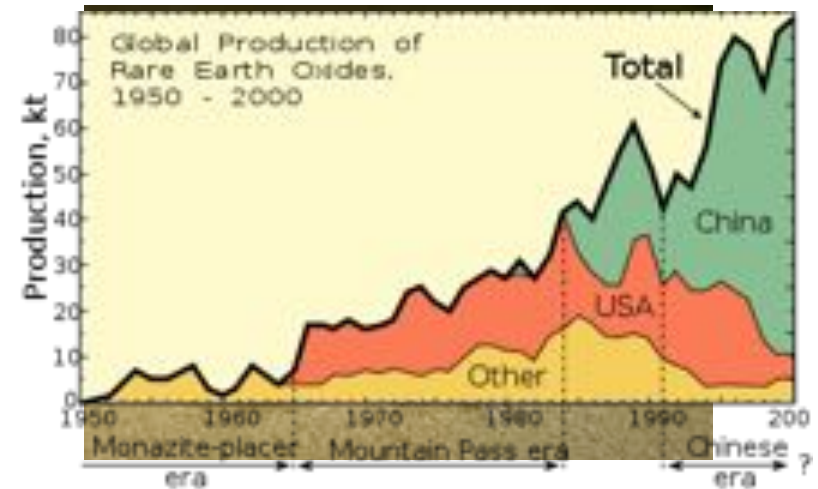
Features:

- ▶ Gray to silvery metals
- ▶ Soft, malleable and ductile
- China supplies at least 95 percent of world's rare earths

Some products that contain rare earth elements:

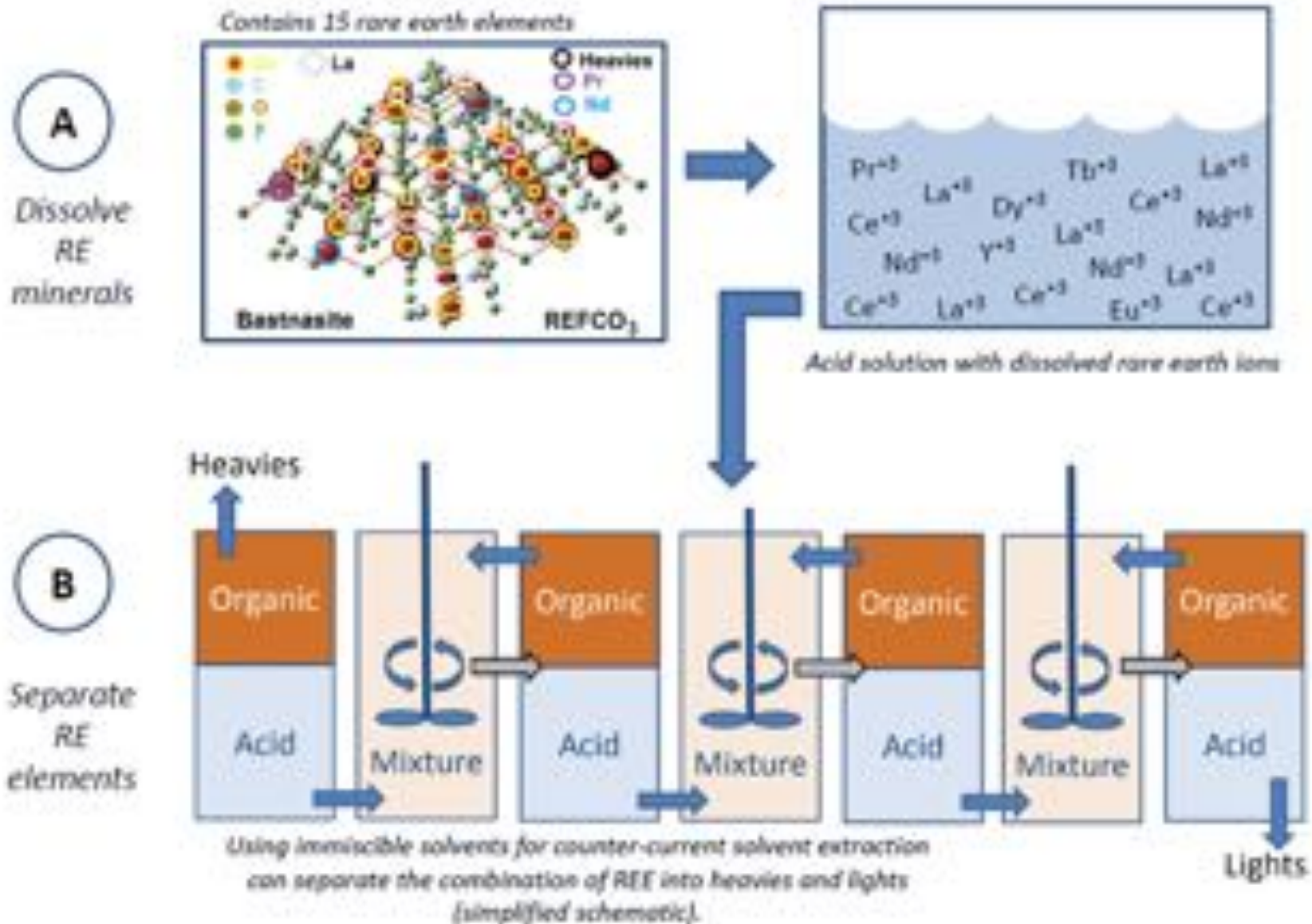
- iPods**
 dysprosium, neodymium, praseodymium, samarium, terbium
- Wind turbines**
 dysprosium, neodymium, praseodymium, terbium
- Hybrid vehicles**
 dysprosium, lanthanum, neodymium, praseodymium
- Fibre optics**
 erbium, europium, terbium, yttrium
- Energy-efficient fluorescent light bulbs**
 europium, terbium, yttrium

Source: USGS

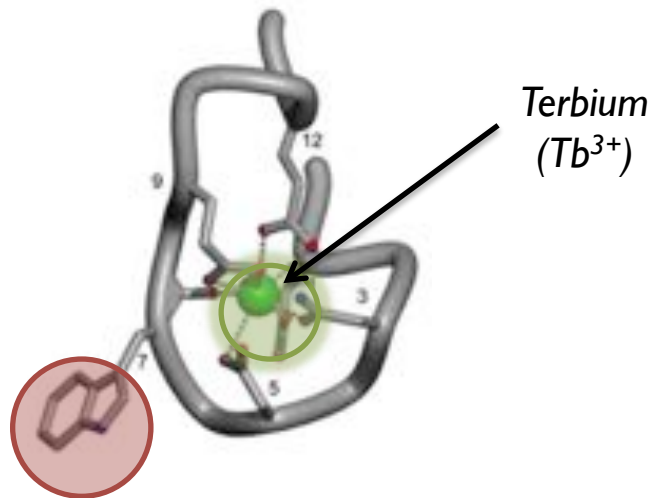


															3 IIIB 21 Sc 44.956	
															39 Y 88.906	
57	58	59	60	61	62	63	64	65	66	67	68	69	70	71		
La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu		
138.91	140.12	140.91	144.24	(145)	150.36	151.96	157.25	158.93	162.50	164.93	167.26	168.93	173.04	174.97		
LREE								HREE								

SEPARATING RARE EARTHS at MOUNTAIN PASS

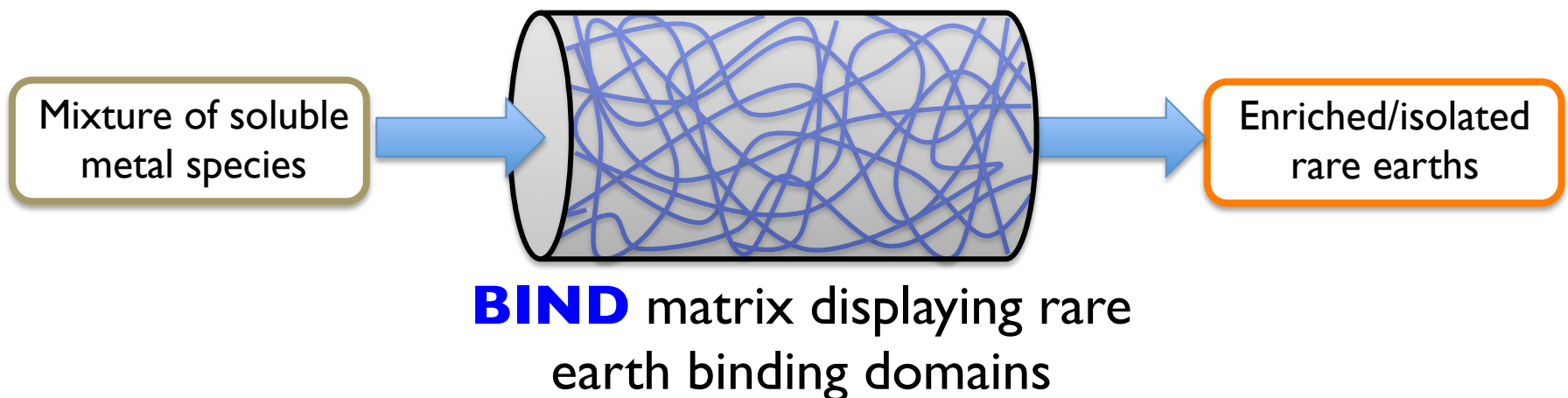
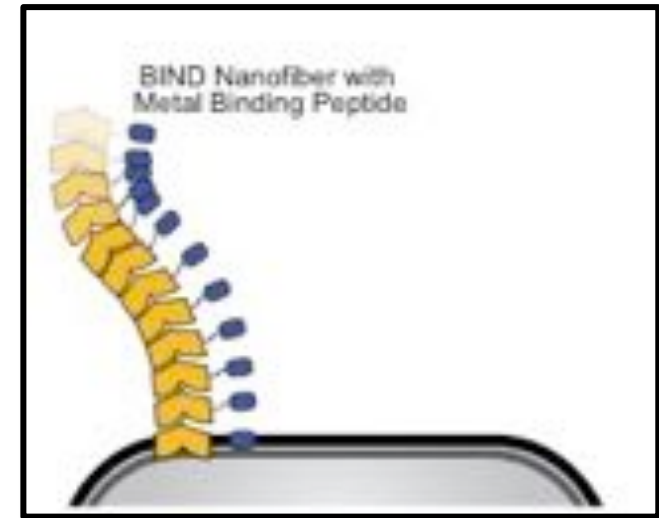


Rare earth isolation and separation



Lanthanide binding peptides

Nitz, et al. *Angewandte* **2004** 43(28) 3682.

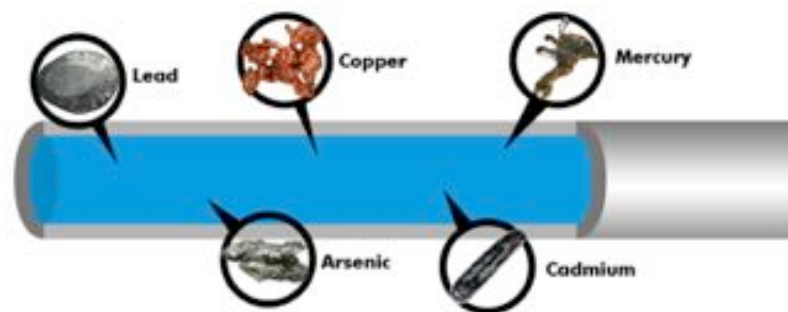


Other possible BIND applications

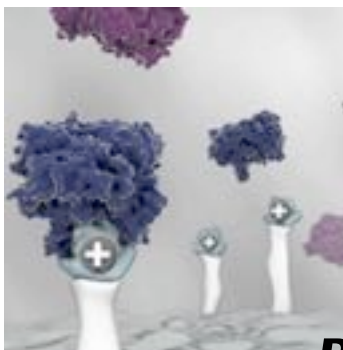
BIOFUEL PRODUCTION



WATER PURIFICATION



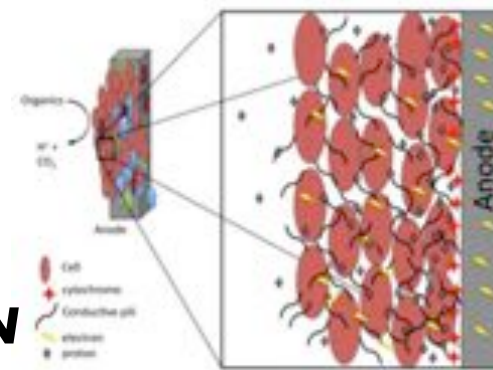
CELL CAPTURE/ AFFINITY SEPARATIONS



PROTECTIVE COATINGS



BIO-ELECTRODE INTERFACE

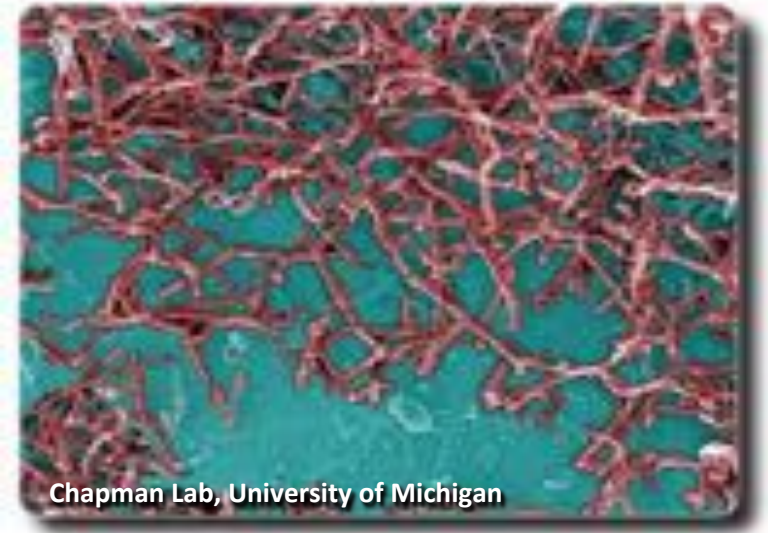


BIOSENSING DECONTAMINATION



Benefits of Biofilm Technology

- Self-generated and self-renewing scaffold; the bacterium as a nanomaterial factory.
- Vast surface area for immobilization.
- Robust – stable under conditions normally considered harsh for biology



- Easily scalable – could lead to cost effective large scale solutions.
- Living material – may allow for dynamic temporal control over material properties
- A green technology for nanomaterials.

Acknowledgements

Joshi Group

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Richie Tay

Pichet Praveschotinunt

Anna Duraj-Thatte



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Michael Gevelber (BU)

Ali Miserez (NTU, Singapore)



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