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Natural Materials, Systems & Extremophiles

06 03 2012

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2012 AFOSR SPRING REVIEW



NAME: Dr Hugh C. DeLong

BRIEF DESCRIPTION OF PORTFOLIO:

The goals of this program are to: 1) study, use, mimic, or alter how biological systems accomplish a desired (from our point of view) task, and 2) enable them to task-specifically produce natural materials and systems. Both goals are to advance or create future USAF technologies.

LIST SUB-AREAS IN PORTFOLIO:

Biomimetics

Biomaterials

BioInterfacial Sciences

Extremophiles



Program Vision



- This program not only wants to mimic existing natural systems, but also to create new capabilities in or with these organisms for more precise control over system production.
 - Protect Human Assets - Finding and Defending against militarily significant threats to humans
 - Enhance Materials Performance - Use natural systems to enhance or create new materials
 - Enhance System Operation - Mimic nature's ability to find, track, and survive the enemy



Program Trends – Program Constant with Additions Coming From Outside Program



- ➔ **Chromophores/Bioluminescence** – Bio-X STT phase 1 focus. One of its discoveries are now used by AFRL TDs, Navy & several Univ PI's
- ➔ **Bio-camouflage** – FY09 PBD 709 program: iridiphores, leucophores, chromatophores, papillae, control system. Linked: FY11 AFRL/RX pgm
- ➔ **Structural Coloration** – new area, several PIs moving in and out; MURI (Harvard)
- ➔ **Biopolymers** – Mainly silk but looking at other biopolymers. The silk work is well integrated with AFRL; many exchanges of personnel & material. Some PIs moving out with biocomposites increasing.
- ➔ **Biomolecular assembly** – New MURI (Northwestern), existing MURI (Georgia Tech), rest has remained constant.
- ➔ **Peptide Mediated Materials Synthesis** – The efforts are focused on discovering the nature of the mechanism behind this.
- ➔ **Extremophile survival** – Looking at mechanisms of protein activity under extreme conditions with the goal to transfer good ideas into weaker systems. Fewer PIs left that perform this type of work.
- ➔ **Biocombinatorics** – New BRI looking at Bio based combinatorics from a bio-nano-info basis



Other Organizations That Fund Related Work



- **Chromophores** – I currently have two grants plus work in AFRL. The work of other organizations is almost exclusively on reporter technology. The interest of the AFOSR program is on wavelength, intensity, and lifetime as it pertains to marking items.
- **Silk** – DARPA has contributed to my existing program. ARO has a single grantee. ONR funds a single investigator. NSF has several single PI grants.
- **Structural Coloration/Bio-Camouflage** – ONR has a MURI focused on vision aspect. ARO has a single grant with ICB PI. NSF has just single PI grants.
- **Biomolecular assembly** – A number of funding organizations are interested in this area, so the AFOSR program is focused on soft lithography, peptide binding, and self or directed assembly for materials. AFRL program works closely with this group for both relevance and guidance.
- **Extremophiles** – NASA has funded this area and focused on the origins of life. The focus of the AFOSR program is on radiation protection mechanisms, bio-templating, and biopolymers that can exist in extreme environments. ARO is focused on spore formers.



Sensory Mimics (Biomimetics)



- Study principles, processes, and designs as well as manipulate sensors/processing systems
- Mimicking of sensor denial systems
- The Future of Sensory Mimics:
 - Mimicking sensor motifs used by animal for flight operations
 - Complex autonomous materials (**skin-like; sensing, regulating, healing**) (w/ L. Lee)
 - Understand the complex nature of predator-prey avoidance to **hide in plain sight**
- **AF Relevance: Sensitivity, Self-healing, Stealthy**



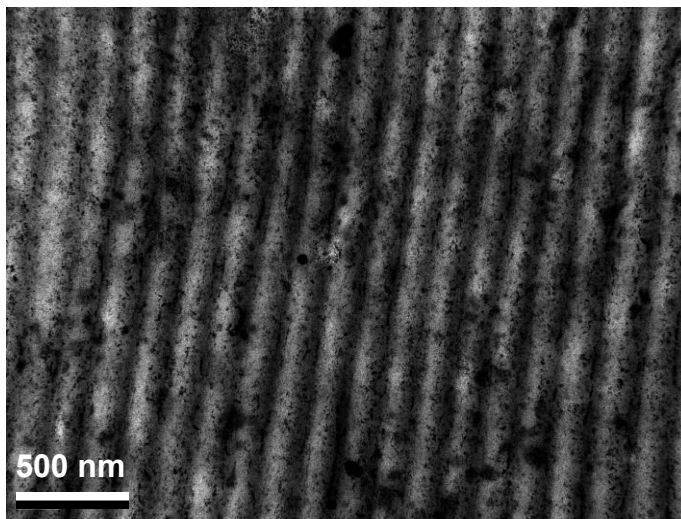
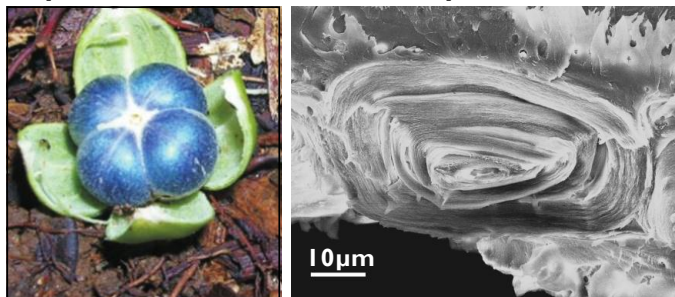
Study and Characterization of Bio-photonic Systems, P. Vukusic, U. Exeter



Interested in studying the relationship between the interplay of hierarchical structures on different length scales

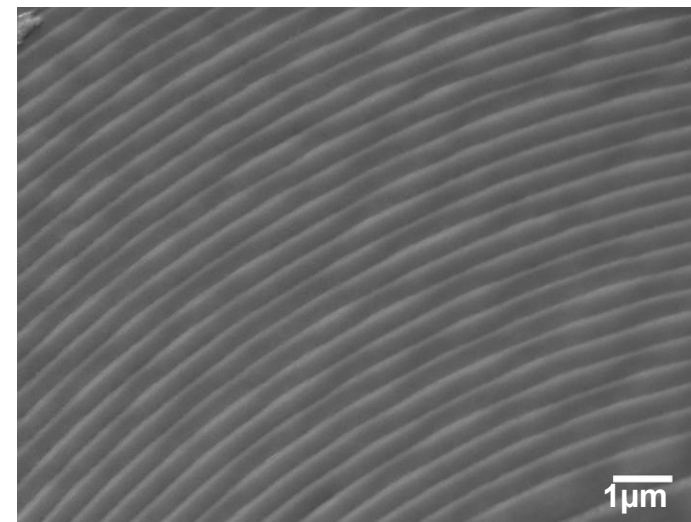
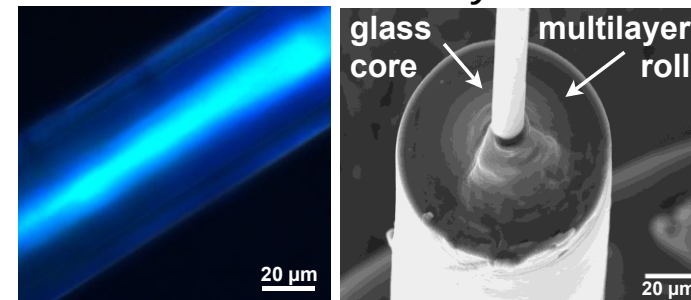
The natural system

Concentric multilayers in the epidermal cells of a plant seed



The artificial counterpart

Artificial multilayer photonic fiber with 60 - 200 layers

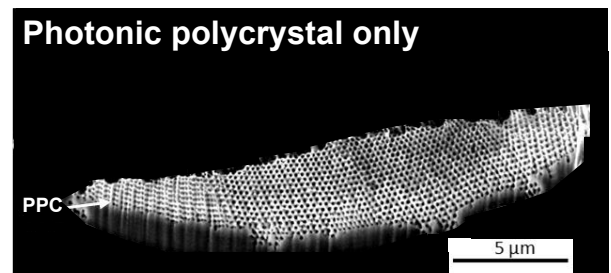
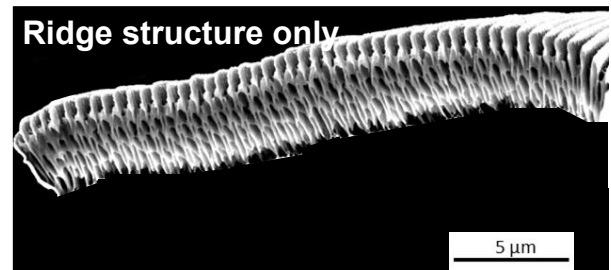
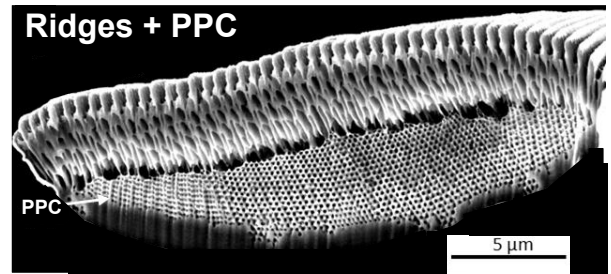
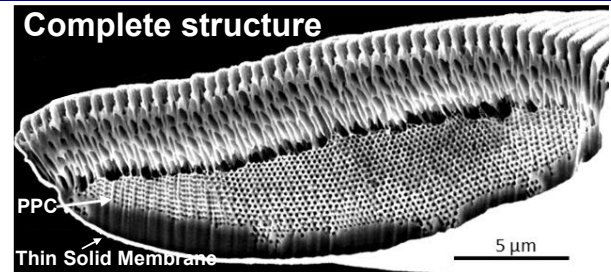
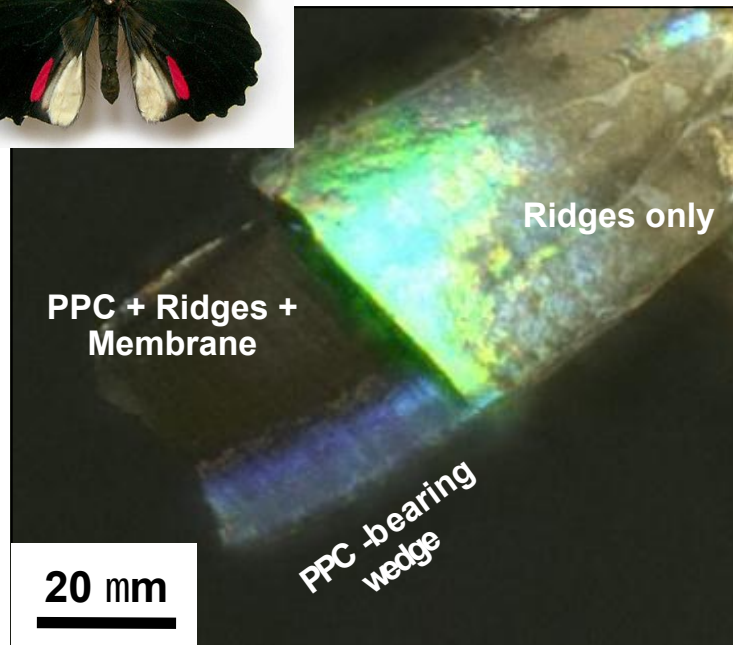




Precise deconstruction of hierarchical biological structures



Deconstruction of a wing scale of the tropical butterfly *Papilio palinurus* to analyze the optical performance of the individual elements - ridges, a photonic polycrystal and the supporting membrane - and to understand their optical interplay





3D Model Physical Fabrication

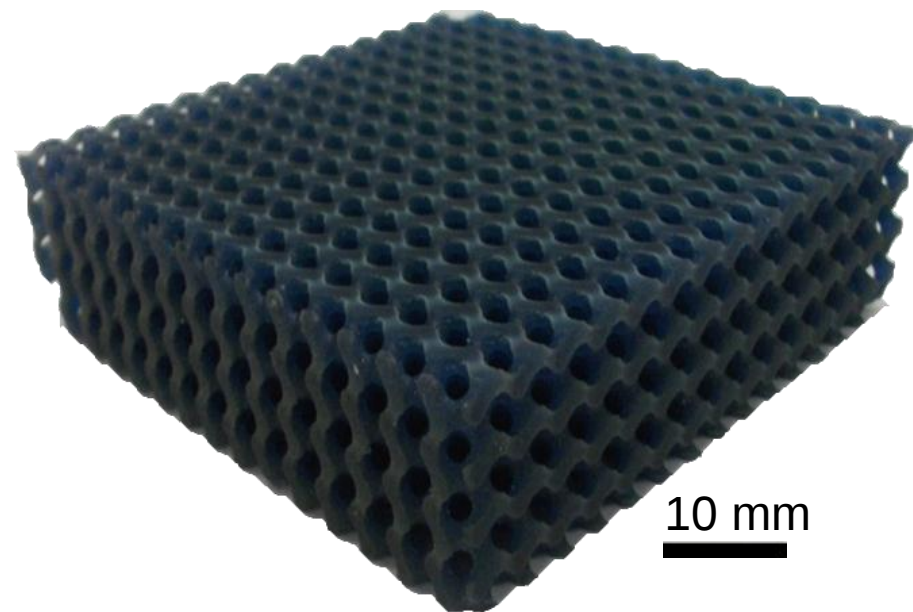
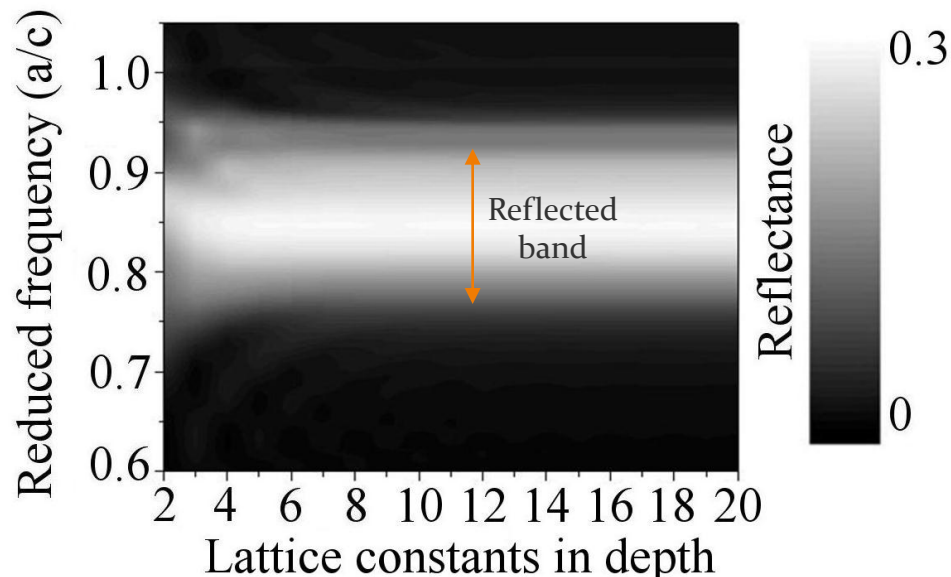


Maxwell's equations are scalable:

- Direct laser writing: resolution issues for **complex** structures therefore characterise in the microwave regime.

Stereolithography allows 3D printing of complex structures:

- Resolution $\sim 50\mu\text{m}$
- Uses dielectrics
- Potential for metals and stretchable materials





Natural Materials (Biomaterials)

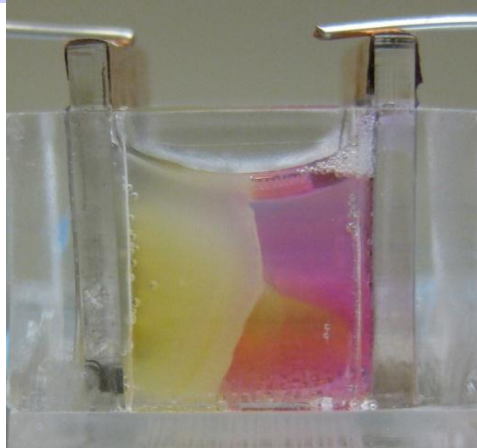


- **Mimicking** of natural materials or systems
- Using organisms as **natural material factories** for new materials
- Using **existing natural materials/organisms** as novel materials
- The Future of Natural Materials:
 - Natural Materials that can **withstand extreme environments**
 - New **optical and electronic materials** based on biology's capability to self-assemble
 - New materials **grown to order** by a biological organism (w/ J. Fuller)
 - Used as **structural materials** for advanced UAV systems
- AF Relevancy: Improved performance, Shape, Composites



Dynamic Silk Materials – electrogelation and silk processing for new functional materials

David Kaplan - Tufts University



Electrogelation – mechanisms & modes of materials formation and functions



Objective: To understand and exploit the novel dynamic properties of silks, including under applied electric fields and in aqueous environments, as a route to new functionalized materials.

Approach:

- Mechanisms – characterization of silk proteins under electric fields – structure, morphology, behavior
- *Protein assembly* – device designs to study silk assembly under electric fields, adhesion
- *Materials characterization* – use novel analytical tools to characterize assembly

Progress:

- New insight into mechanisms – pH, morphology, improved model
- New high performance materials and properties generated from silk through the process
- New silk-electronic interfaces
- New dynamic silk-based materials

Impact:

- High performance silk-based materials and processes – fibers, films, coatings
- New dynamic silk-based material systems
- New reversible adhesives
- New nano- and micro-composite materials

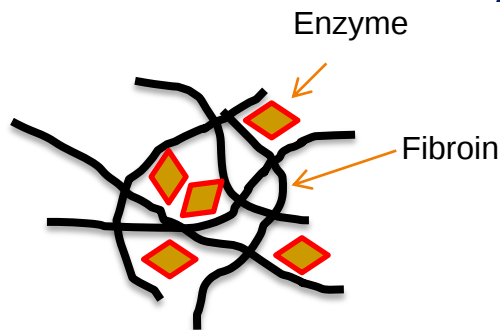


Enzyme Entrapment in Silk

Rajesh Naik, AFRL-RX

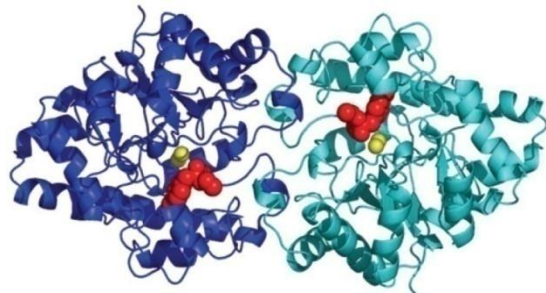


Advantages of Enzyme Stabilization in Silk fibroin Films



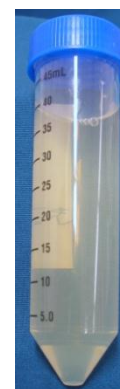
- Large hydrophobic domains and small hydrophilic spacers
- Crystalline domains (β -sheets) and less organized more flexible domains (more hydrated)
- Microenvironments sufficient hydration
- Controlled released based on silk processing conditions
- Enzymes with varied molecular weights can be entrapped

Silk Fibroin

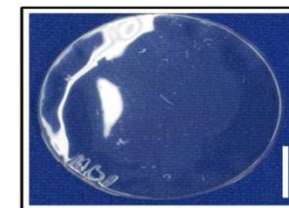


Organophosphate Hydrolase
(OPH)

~ 45kDa mol. wt +

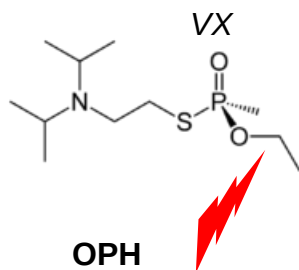


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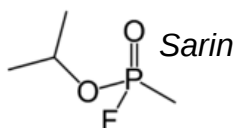


Silk Fibroin
(OPH)

(In collaboration with David Kaplan)

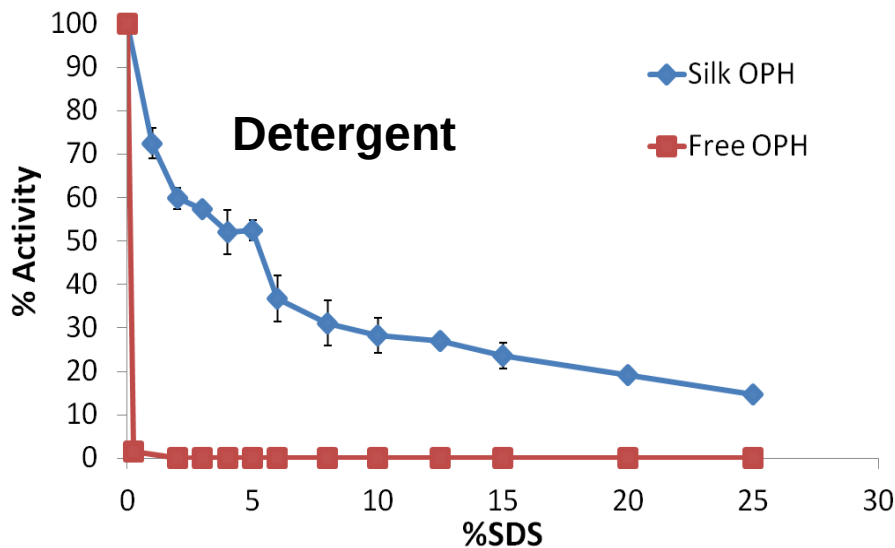
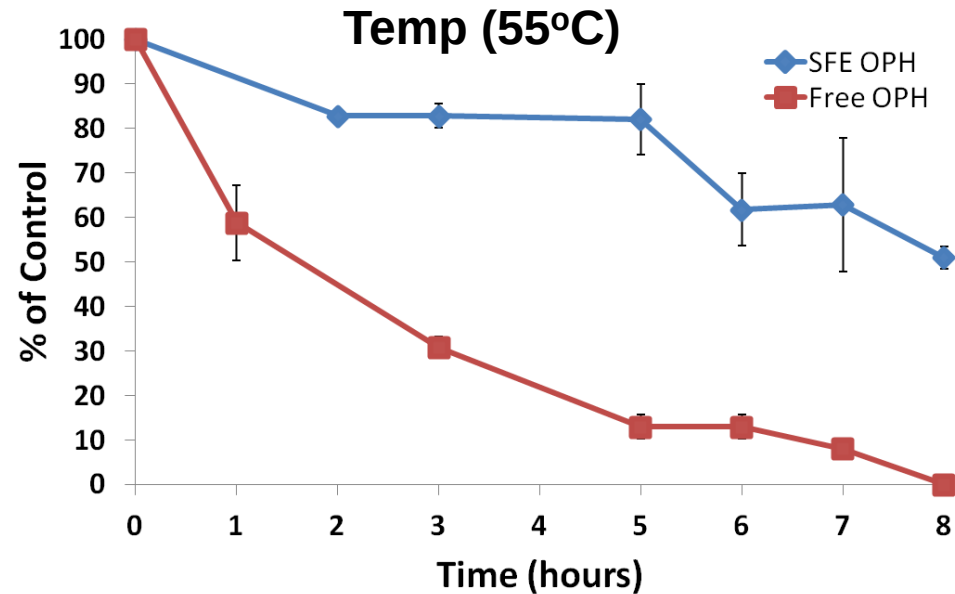
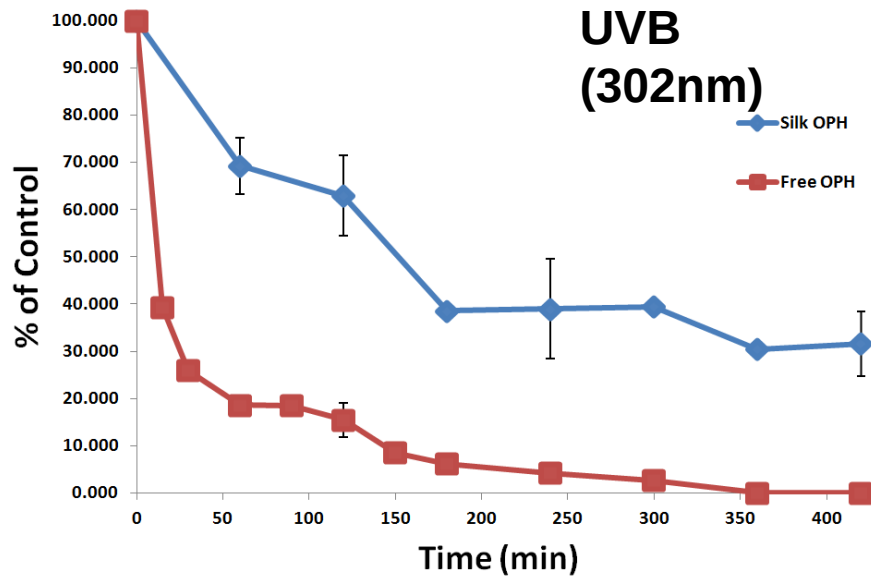


Nerve Agents





Stability of OPH Entrapped in Silk



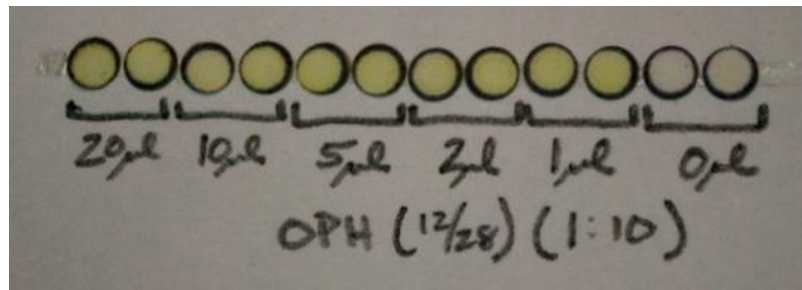
Increased protection against UV, heat and detergent of OPH-Silk films



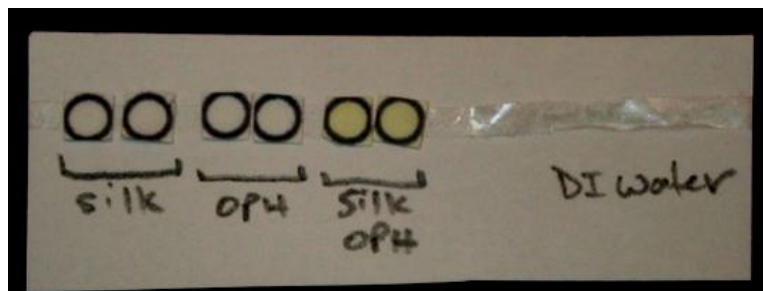
Paper Based Microfluidics for Organophosphates



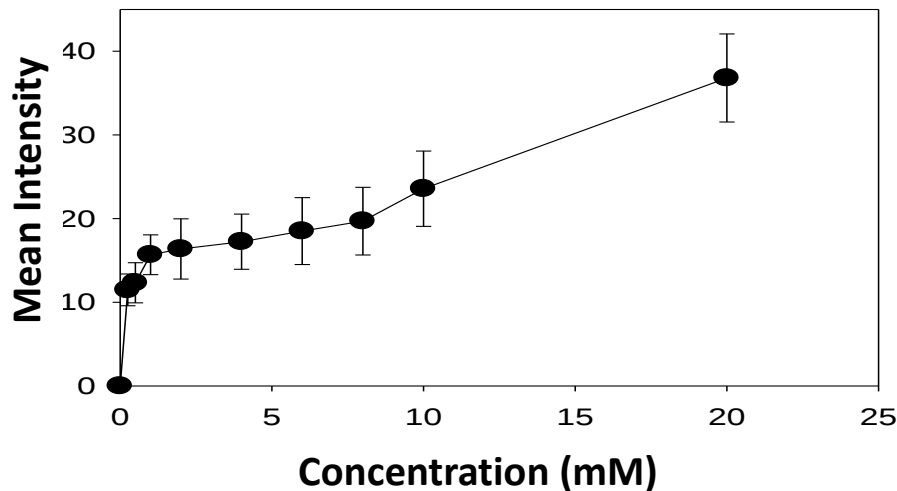
Activity of OPH-Silk
spotted onto paper



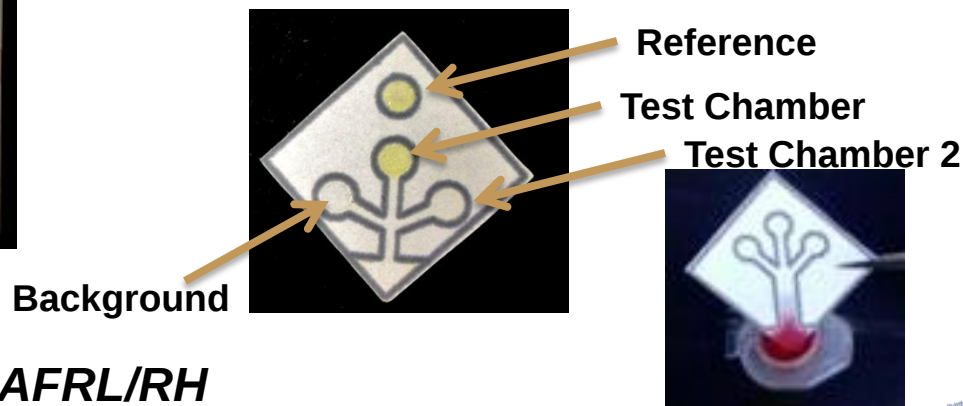
OPH is unstable on
paper



LOD = 25 μ M



Microfluidic Paper Assay
for Chem agents (μ PAC)



In Collaboration with Josh Hagen, AFRL/RH



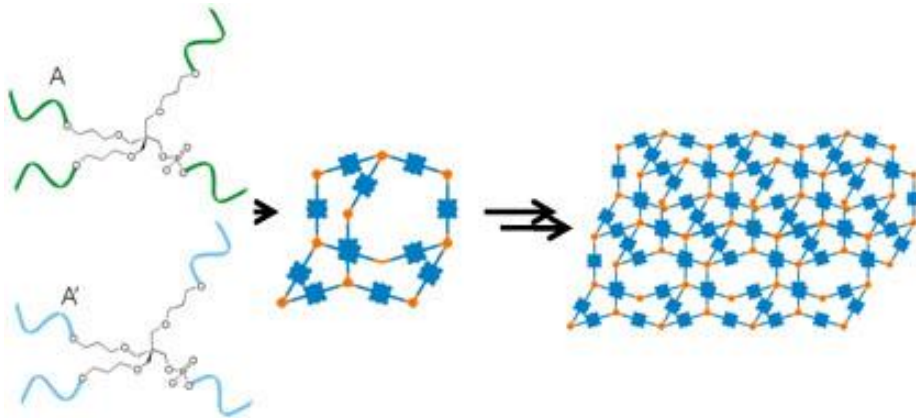
Natural/Synthetic Interfaces (Biointerfacial Sciences)



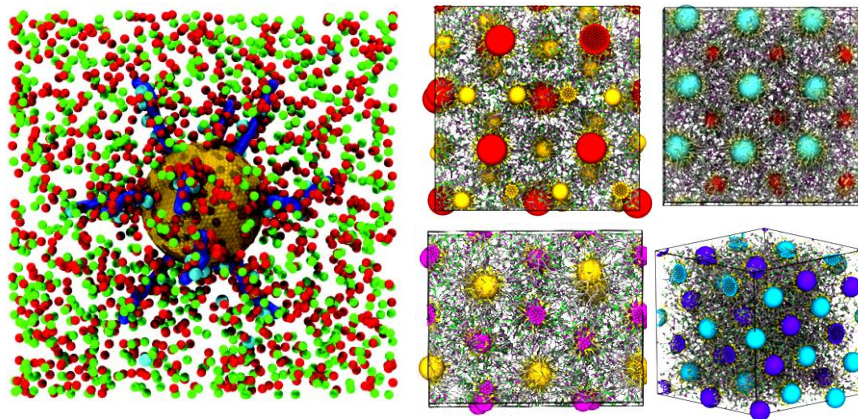
- Biotic-biotic or the biotic-abiotic interface.
- Bionanotechnology and biomesotechnology.
- Self-assembly, directed assembly
- The Future of Natural/Synthetic Interfaces:
 - **biocatalysts** for electrical power systems (providing low signature, long life ISR capability)
 - sensor applications in **extreme environments**
 - **bio-optics and bio-electronics** (w/ G. Pomrenke & H. Weinstock)
- **AF Relevancy: Nanofabrication – constraints on design & production**



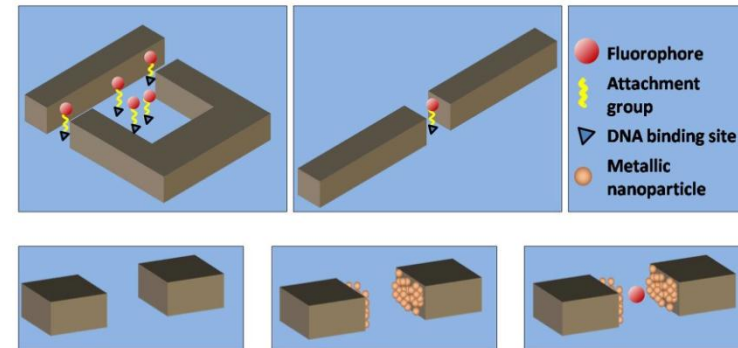
Bio-Programmable 1-, 2-, & 3-D Materials, Chad A. Mirkin, Northwestern University



**Small-molecule DNA/Peptide
Hybrid Structures**
(Nguyen, Rosi, Mirkin)



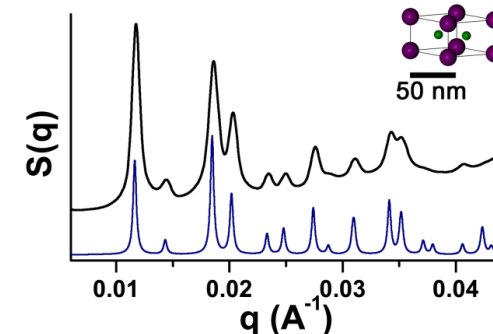
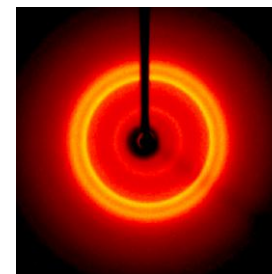
**Theoretical Examination of Nanoparticle
Assembly and Properties**
(Schatz, Olvera, Rosi, Mirkin)



Tailor properties of resonant structure
with metallic nanoparticles

Use tailored resonator to probe
emission properties of fluorophore

Large-Scale Patterned Metamaterial Arrays
(Atwater, Schatz, Mirkin)



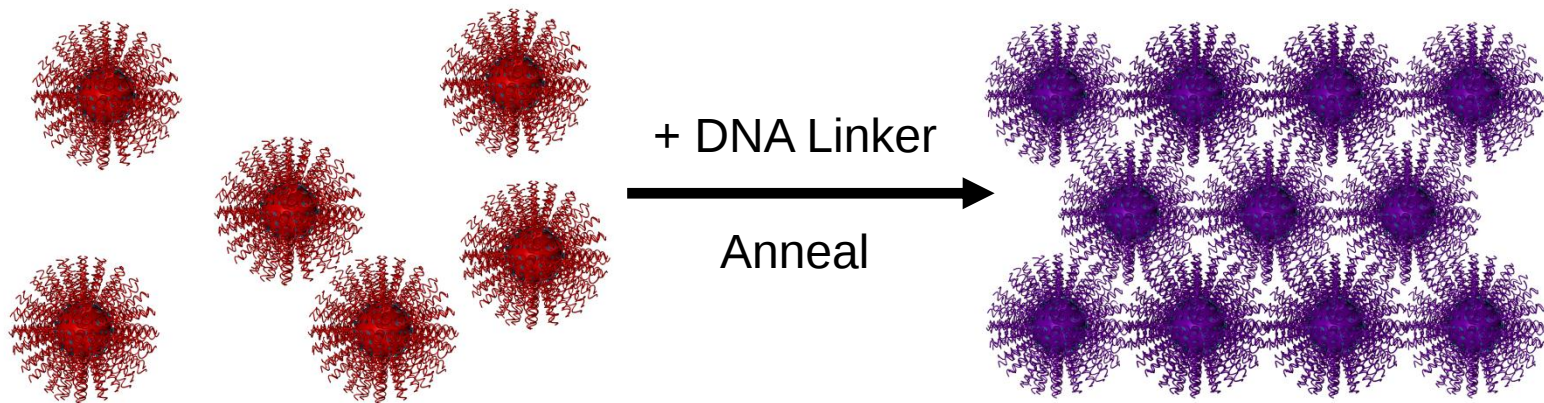
X-Ray Characterization of Materials
(Bedzyk, Rosi, Mirkin)



Key Hypothesis of DNA-Programmed Assembly:



In the context of DNA-programmable nanoparticle assembly, the structures that represent thermodynamic minima rather than kinetics will maximize the number of nearest neighbors that can form DNA connections.



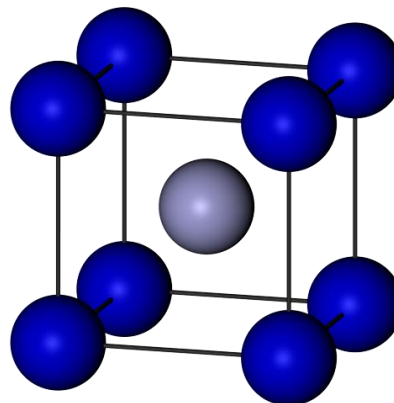
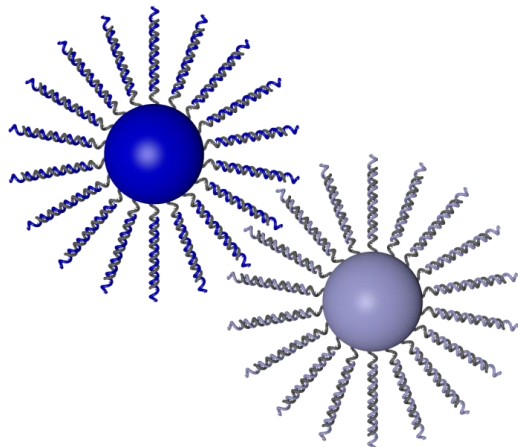
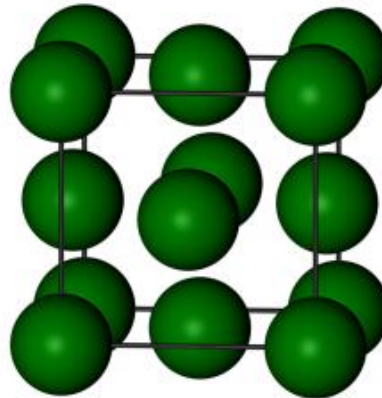
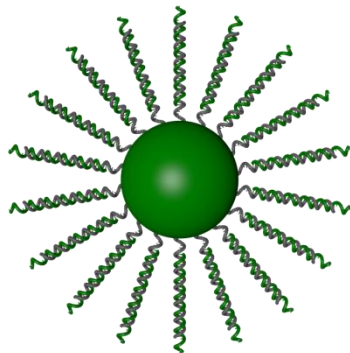
Developed Five Rules of DNA-Programmed Assembly



Rule #1: Particles of Equal Hydrodynamic Radius will Maximize Complementary Nearest Neighbors



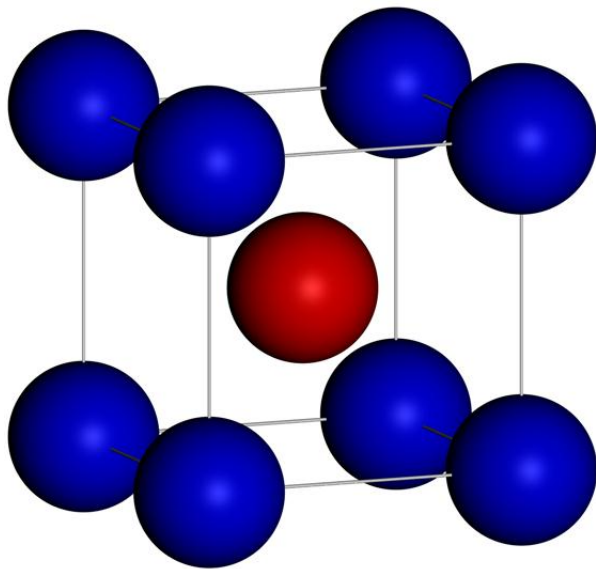
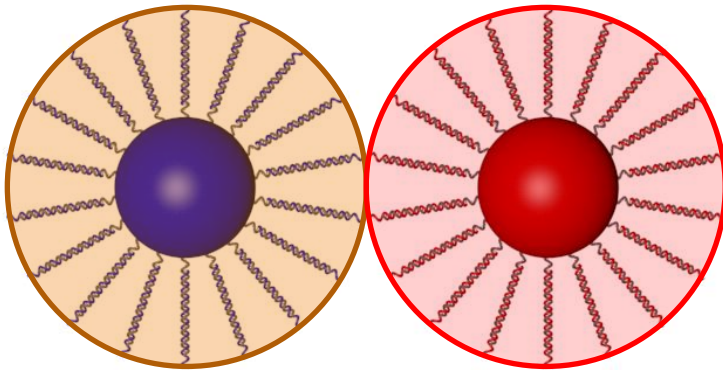
Face-Centered Cubic Lattice



Body-Centered Cubic Lattice



Rule #2: The overall hydrodynamic radius of a DNA-NP dictates its assembly and packing behavior

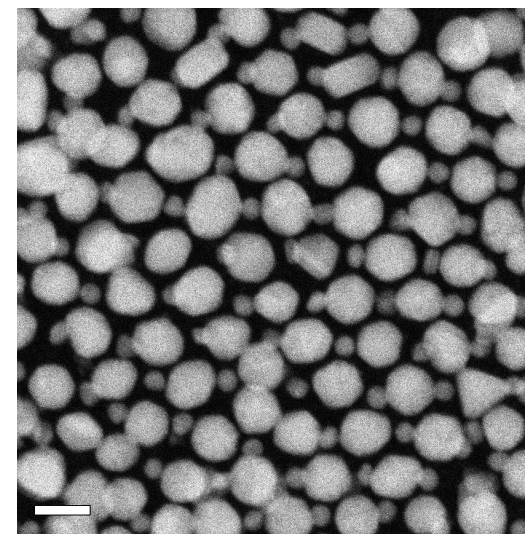
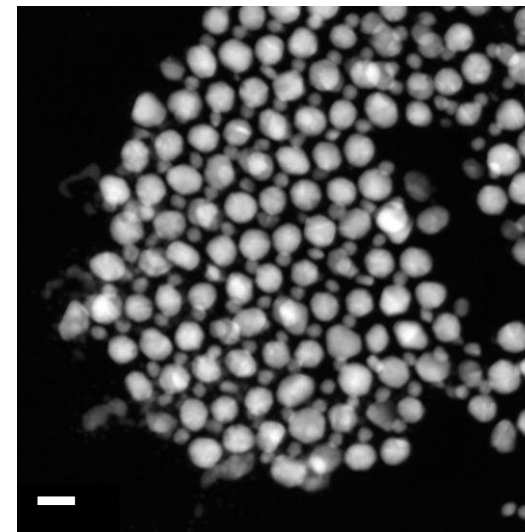
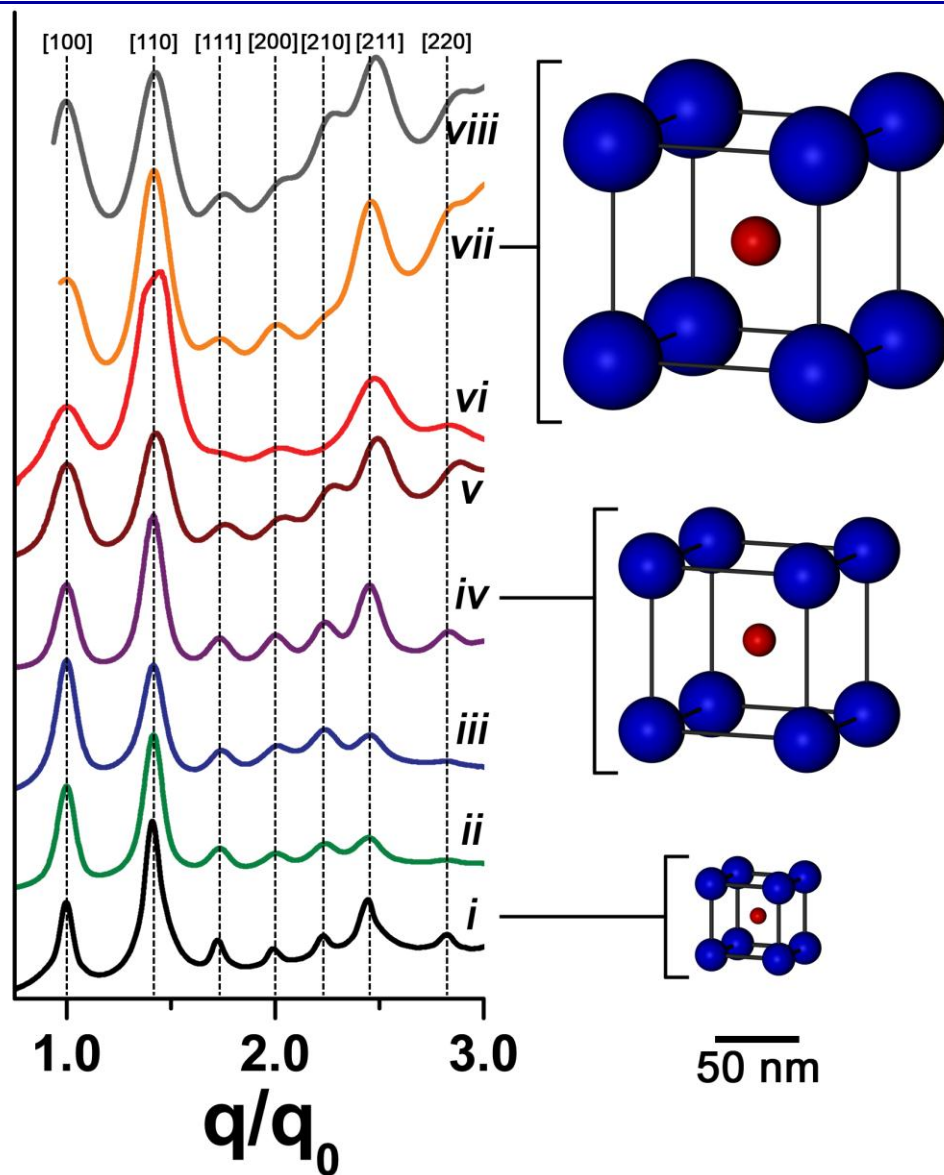


Body-Centered Cubic Lattice





Rule #2: The overall hydrodynamic radius of a DNA-NP dictates its assembly and packing behavior

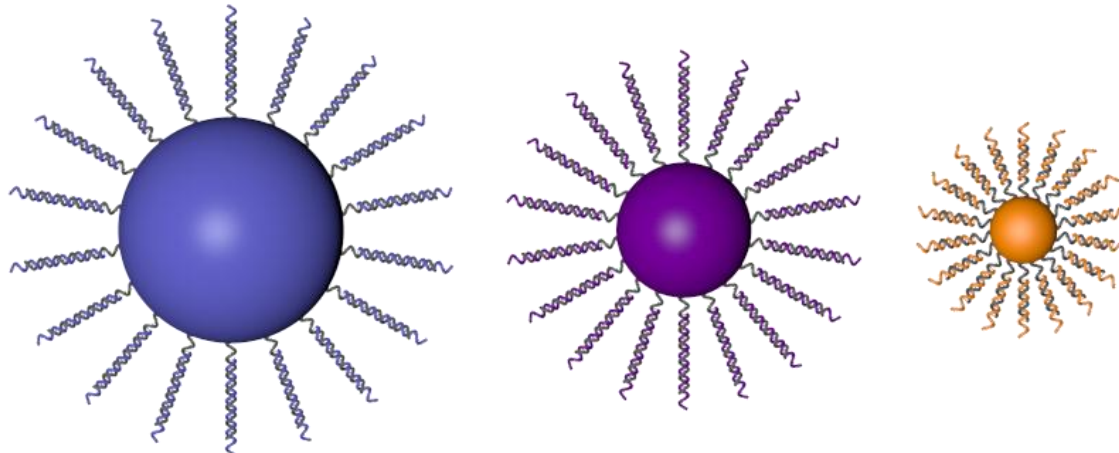




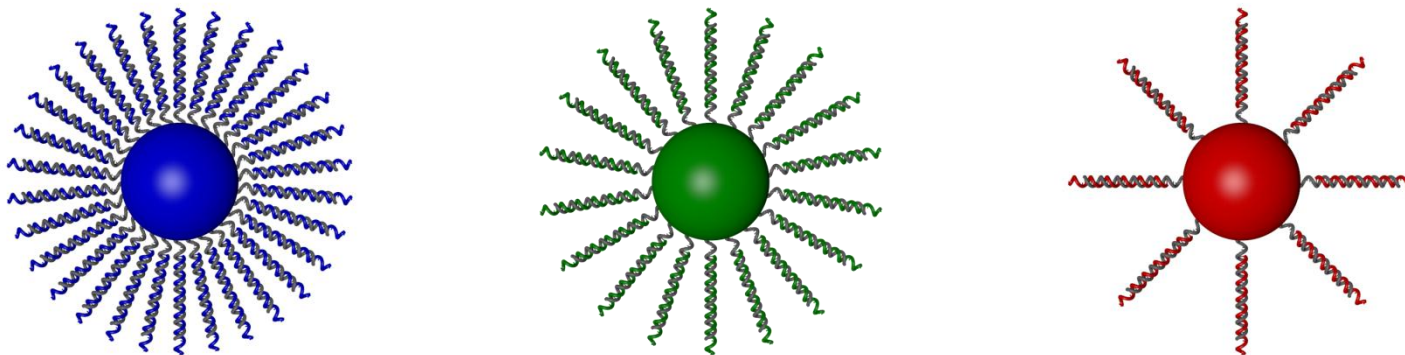
Rule #3: Particle Hydrodynamic Size ratio and DNA Linker Ratio Dictate the Thermodynamically Favored Crystal Structure



Hydrodynamic Radius



DNA Linkers per NP

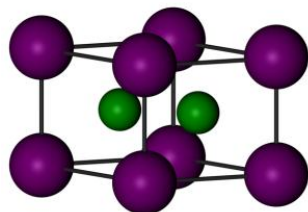




Rule #3: Particle Hydrodynamic Size ratio and DNA Linker Ratio Dictate the Thermodynamically Favored Crystal Structure

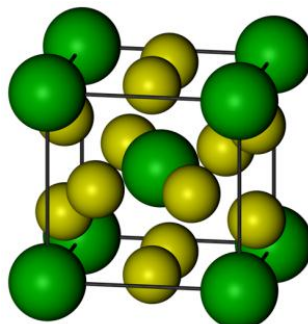


H_d Size Ratio: **0.64**
Linker Ratio: **2.4**



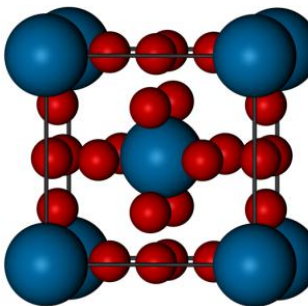
AIB₂

H_d Size Ratio: **0.37**
Linker Ratio: **2.0**



Cr₃Si

H_d Size Ratio: **0.35**
Linker Ratio: **3.0**



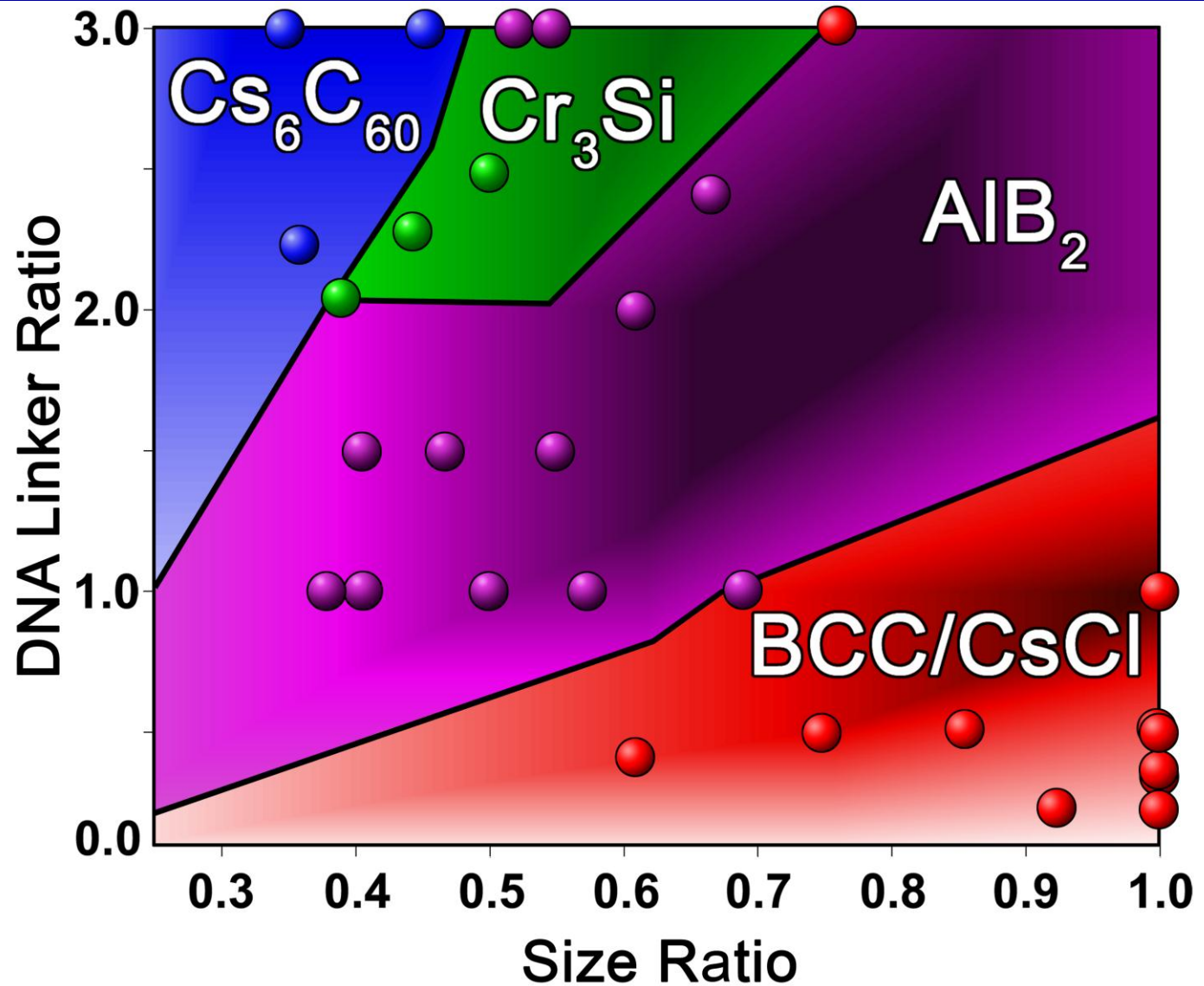
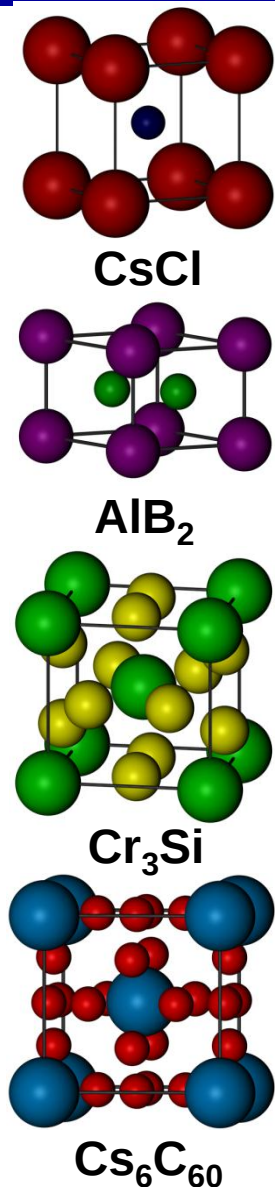
Cs₆C₆₀

DISTRIBUTION A: Approved for



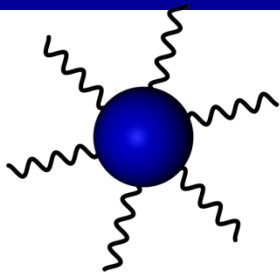


Rule #4: Two Systems With the Same Hydrodynamic Size Ratio and DNA Linker Ratio Exhibit the Same Thermodynamic Product



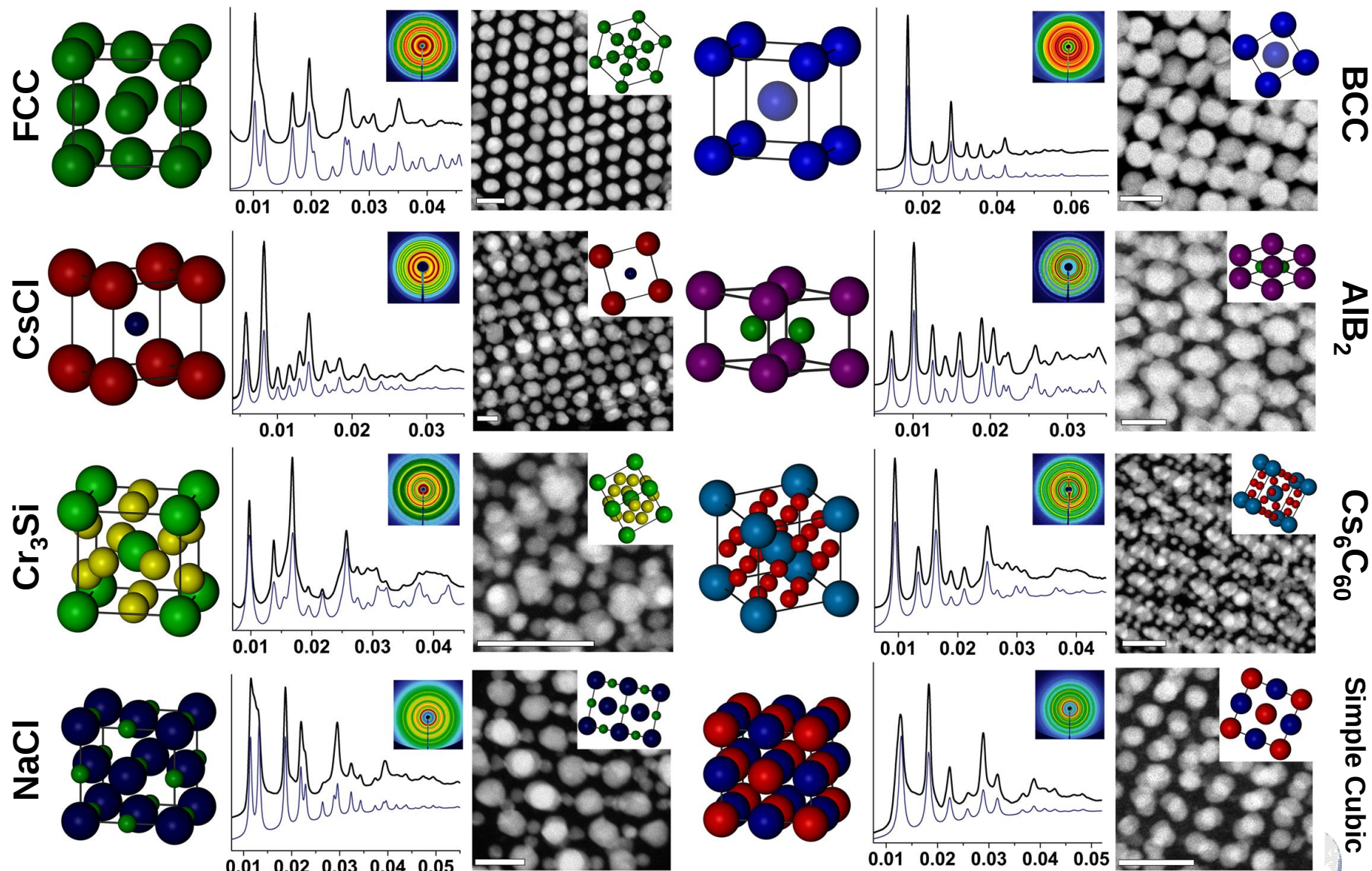


Rule #5: The Most Stable Crystal Will Maximize All Possible Types of DNA Sequence-Specific Hybridization Interactions



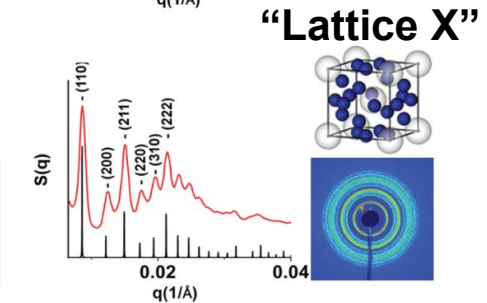
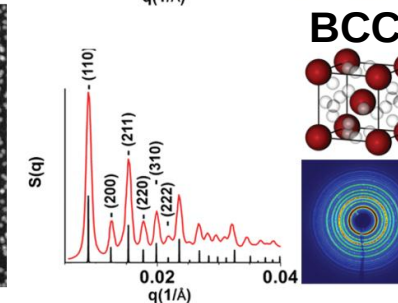
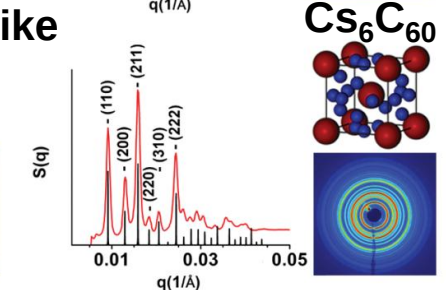
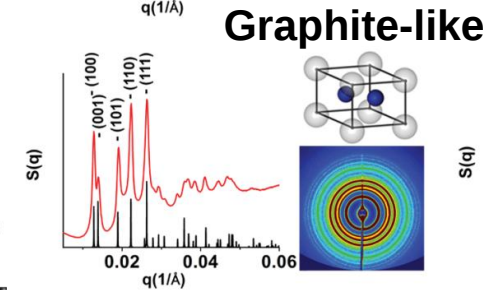
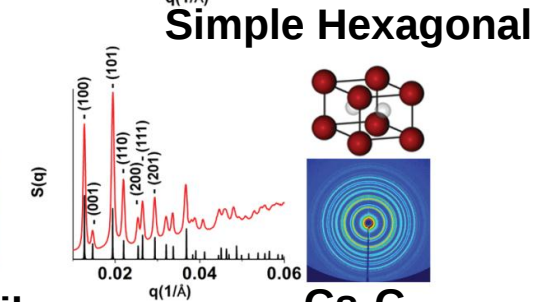
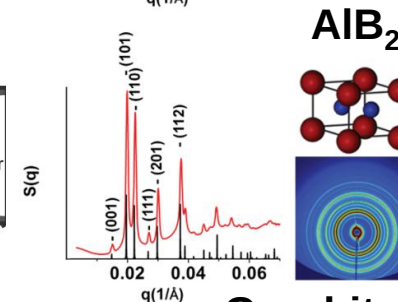
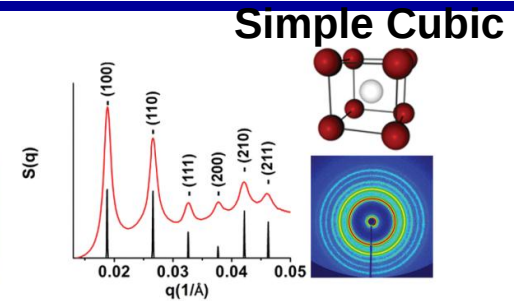
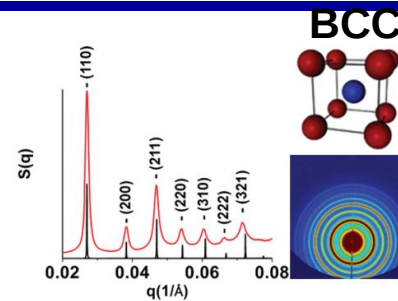
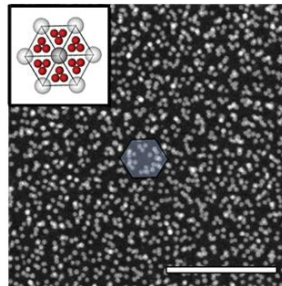
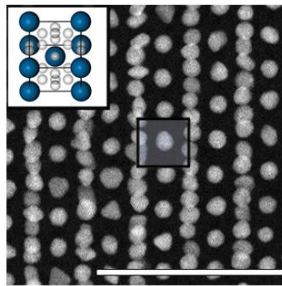
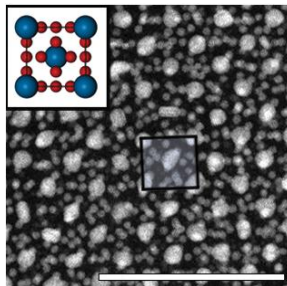
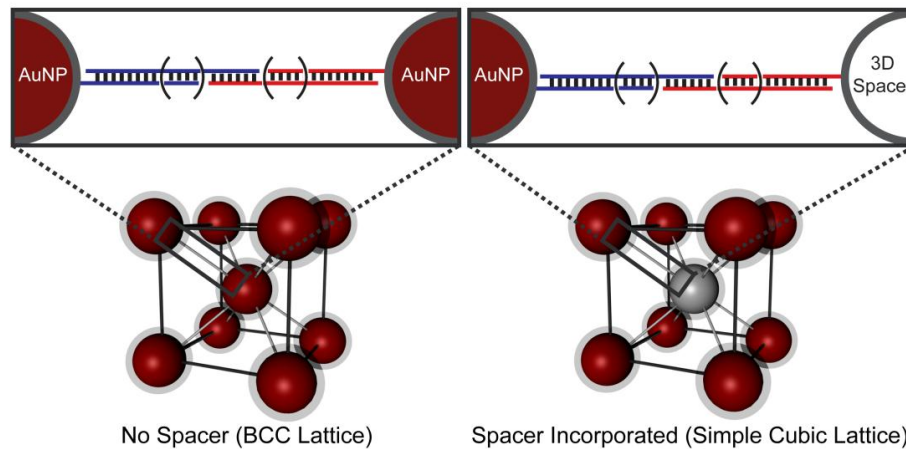
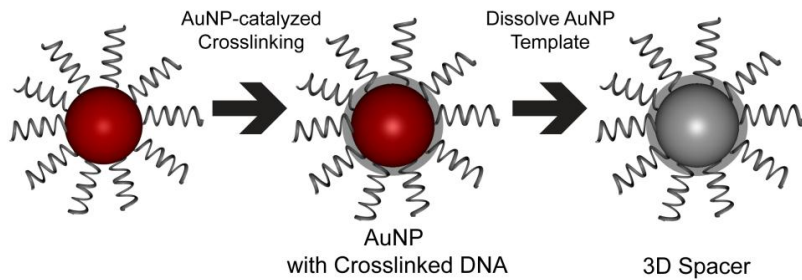


DNA-Programmable Nanoparticle Materials by Design



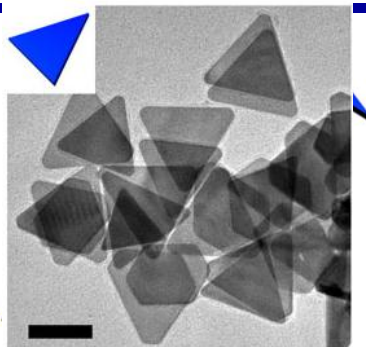


Expanding Lattice Versatility with a 3-D “Hollow Spacer”

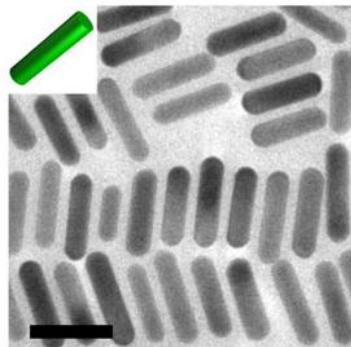
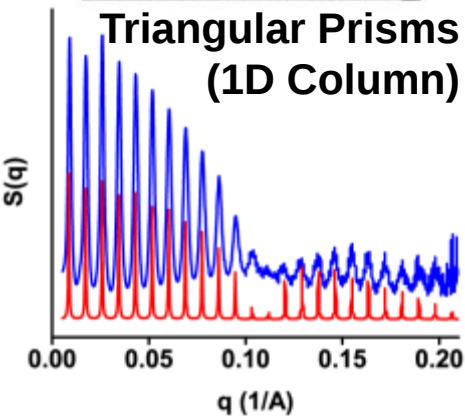




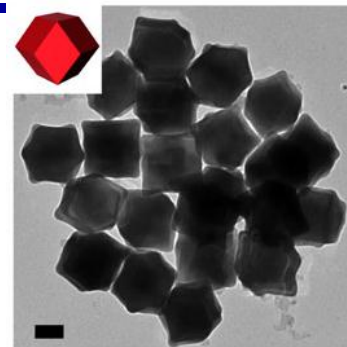
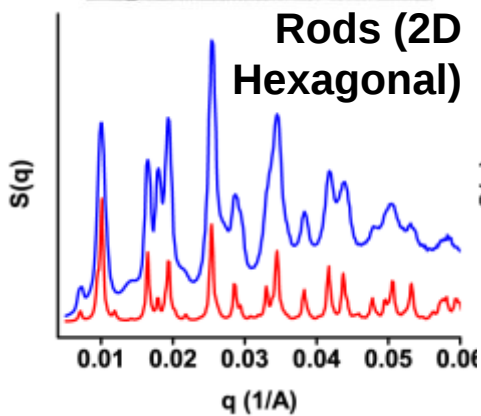
Nanoparticle Valency Imposed by Flat Surfaces Yields Ordered Superlattices



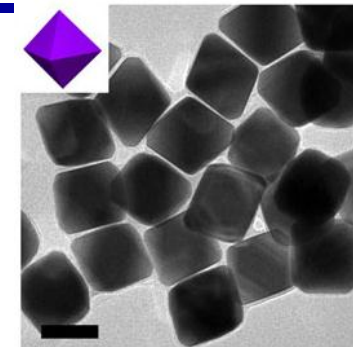
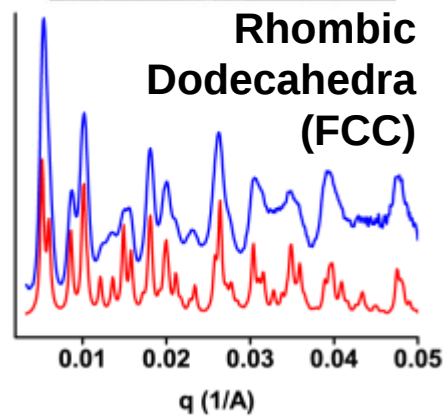
**Triangular Prisms
(1D Column)**



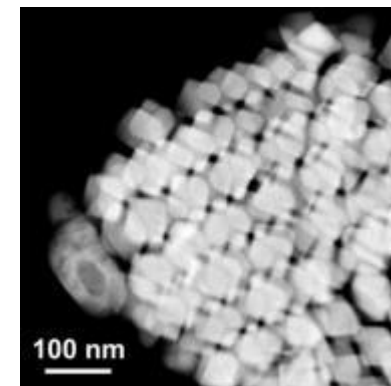
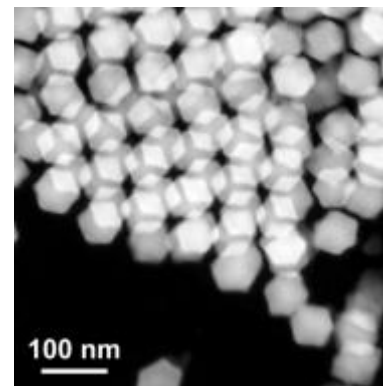
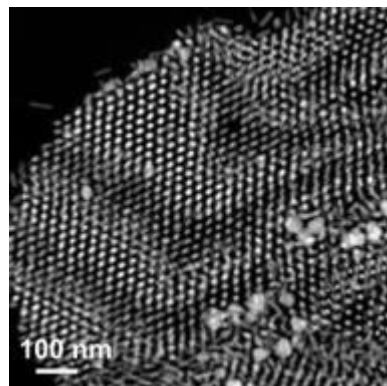
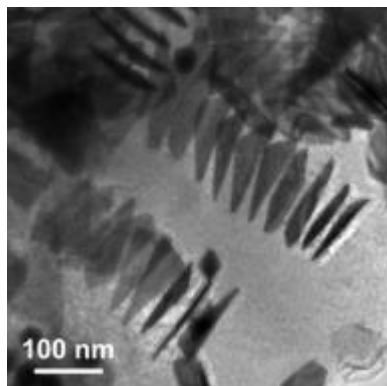
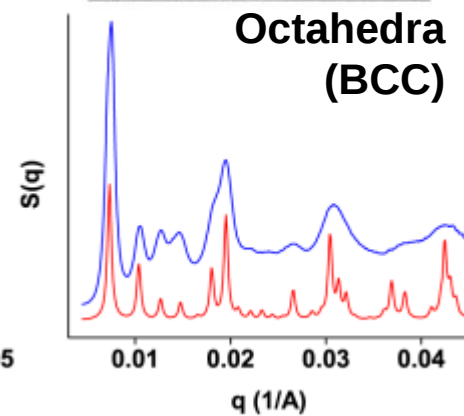
**Rods (2D
Hexagonal)**



**Rhombic
Dodecahedra
(FCC)**



**Octahedra
(BCC)**





Physical Mechanisms of Natural Systems Under Environmental Distress (Extremophilies)



- Focused on discovering and understanding basic natural mechanisms
- Increasingly used as catalysts, sensors, and as materials, so necessary to understand how can use in extreme environments, while incorporating change.
- The Future of Physical Mechanisms of Natural Systems Under Environmental Distress:
 - the mechanisms for survival and protein stability in **extremophilic archaea & their viruses**, and enzymatic engineering for faster **catalysis** in material degradation designs.
- **AF Relevancy: New catalysts, sensors, and as materials**



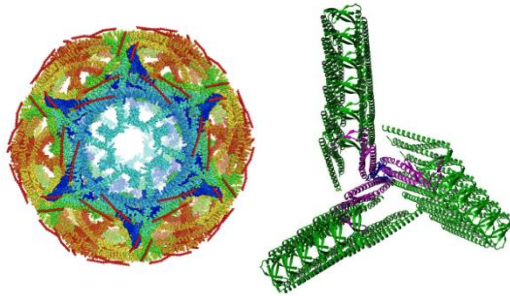
Engineering Ultrastable Protein Filaments into 2D & 3D Templates for Materials Design— Douglas Clark, UC Berkeley



From Hyperstable Filaments to Self-Assembling Ovaloids: Expanding the Dimensions of Protein Design

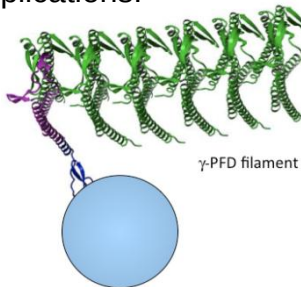
STATUS QUO

Current protein templates and architectures for nanoscale device fabrication are limited to natural molecules owing to difficulties associated with generating new full-domain protein shapes.



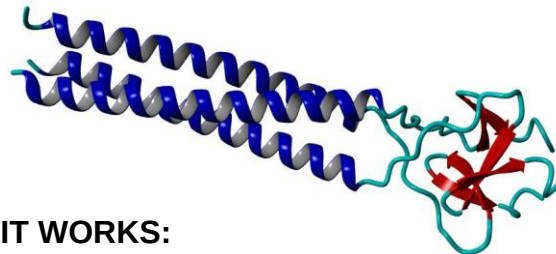
NEW INSIGHTS

We have demonstrated that the γ -PFD is exceptionally stable and can be engineered for numerous possible applications.



MAIN ACHIEVEMENTS:

- Characterized γ -PFD assembly as function of T; discovered new filament morphology.
- Designed and expressed 2D and 3D connector proteins and demonstrated binding with γ -PFD.
- Developed γ -PFD variant with greater thermostability.
- Engineered system for secretion of γ -PFD.



HOW IT WORKS:

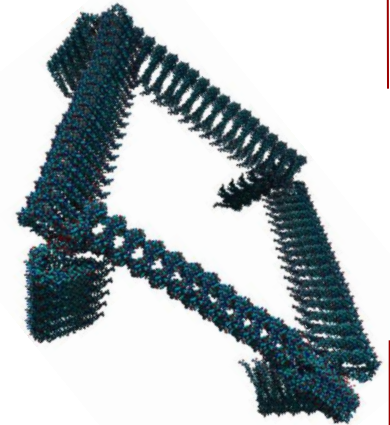
- 3D Protein connectors are based on hub region of the cage-like protein clathrin and the foldon from viral protein fibrin.
- Ultrastable γ -PFD mutant rationally engineered using structure-stability relationships but filament formation of mutant must still be engineered
- γ -PFD secreted from *B. subtilis* for assembly of filaments *ex vivo*.

ASSUMPTIONS AND LIMITATIONS:

2D structure of “pinwheel” construct requires further confirmation; assembly of more complex 3D structures not yet accomplished.

DISTRIBUTION A: Approved for public release; distribution is unlimited.

Generating highly stable proteins that assemble into 2D and 3D shapes of controllable size and symmetry will increase the dimensional space for template-based construction of advanced biomaterials.



Rational design and construction of modular 2D and 3D protein architectures that will serve as lattices and scaffolds in protein-based and hybrid biomaterials.

QUANTITATIVE IMPACT

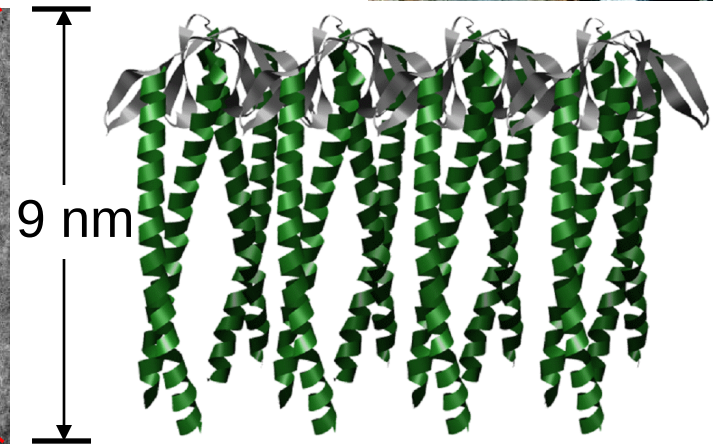
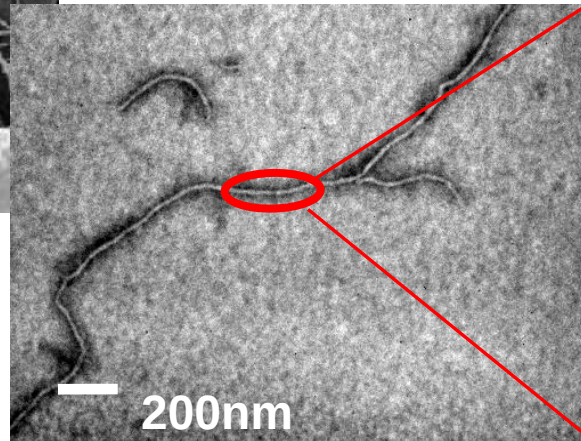
END-OF-PHASE GOAL



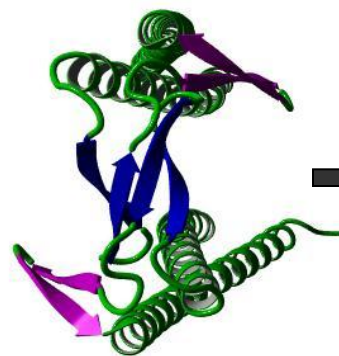
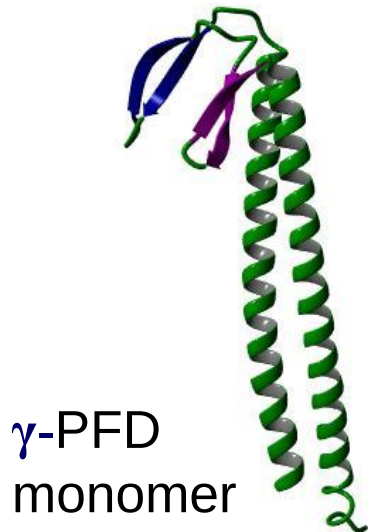
Filamentous γ -Prefoldin (γ -PFD)



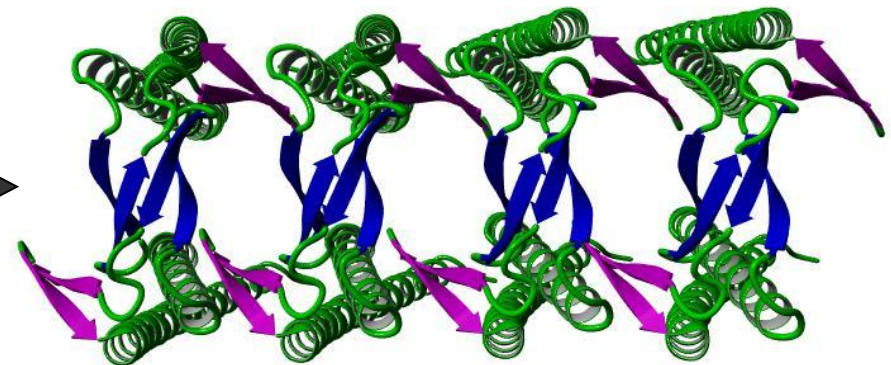
Methanocaldococcus jannaschii



γ -prefoldin (γ -PFD)



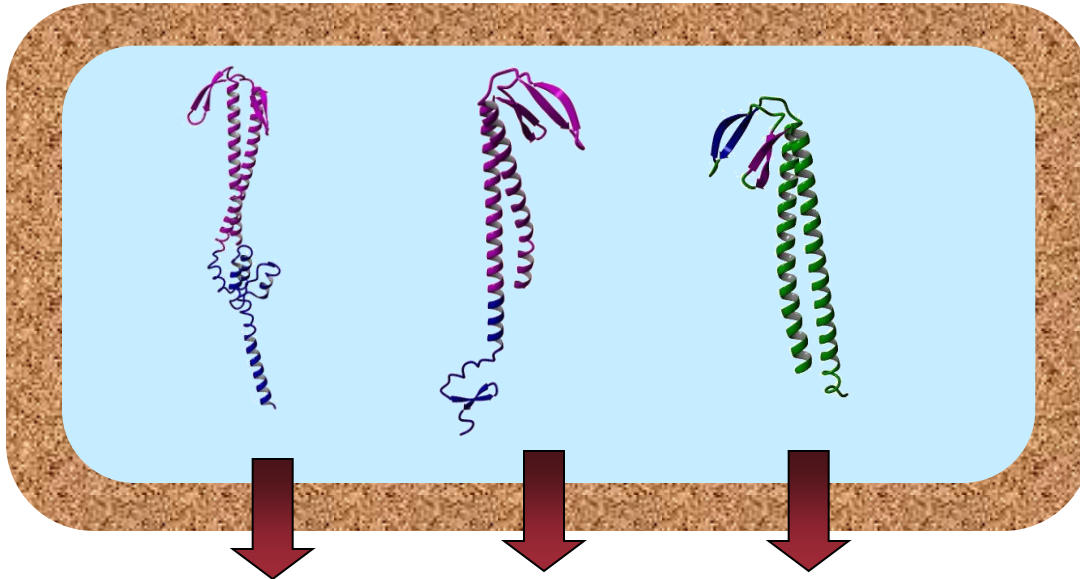
γ -PFD dimer



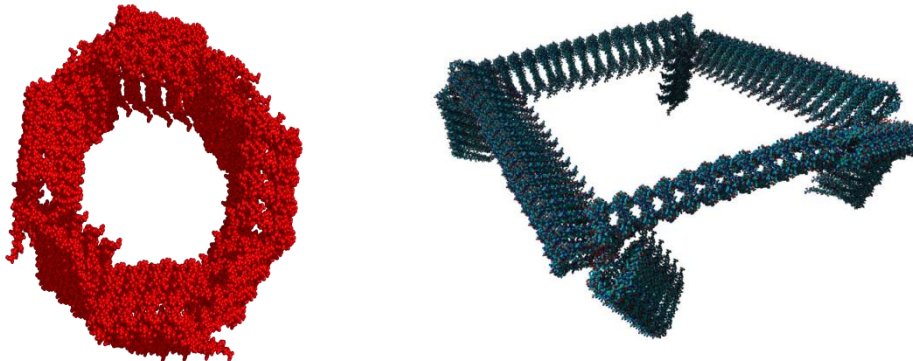
γ -PFD filament

Microbial factories for controlling protein assembly

B. subtilis engineered to express γ -PFD protein parts



Rods and connectors expressed and secreted from *B. subtilis* in a controlled manner



Controlled assembly of higher-order structures *ex vivo*

2-way and 3-way/filament assemblies

Figure 10.1. Assembly of protein filaments; distribution is unlimited.



Transitions



- **AFRL/RX – Collaboration with GE on bio-inspired photonic sensors (CRADA)**
- **AFRL/RX – DTRA funding for biofunctionalized textiles for Chem-Bio (jointly with AFRL/RH)**
- **AFRL/RX – Invention disclosure filed on Halamine functionalized biomaterials for decon application**
- **Northwestern – Patent application “Tunable compliant optical metamaterial structures (US 13/200,273)**
- **Connecticut College – Luciferase product development of patent (US Patent # 7,807,429 B2; UK, Germany EP 2 002 007 B1) license holder Targeting Systems, El Cajon, CA.**
- **UCSD – Invention disclosure (Dec., 2010). Cvario: A new pliable biophotonic material with low degradation in seawater. UCSD docket# (in process). (Deheyn DD)**
- **UC Berkeley – γ -PFD filaments to template organic semi-conductors (Monash University, Australia)**
- **UC Berkeley – γ -PFD filaments for magnetically driven protein assembly (Rice University)**
- **Northwestern – Invention disclosure filed on Functionalization of Anisotropic Nanostructures - NU 2010-094**
- **Northwestern – Invention disclosure filed on Short-Duplex Probes for Enhanced Target Nucleic Acid Hybridization - NU 29147**
- **Northwestern – Nanoflare technology licensed to Aurasense.**