BioSensors and Bio-Inspired Sciences

5 September 2008

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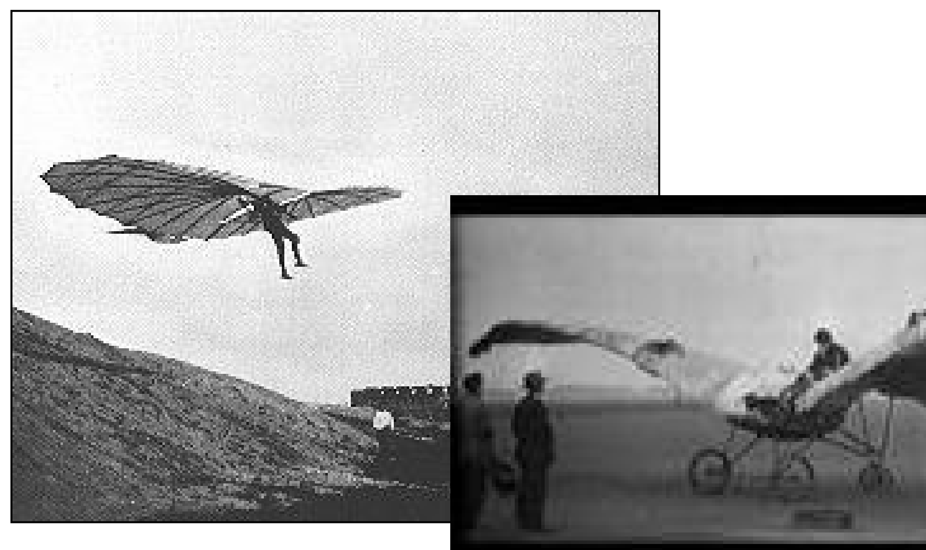
AFRL
THE AIR FORCE RESEARCH LABORATORY
LEAD | DISCOVER | DEVELOP | DELIVER

Biomimetics and Bio-Inspiration

• Bio-inspiration has always been a part of humanity's technological pursuits
• When do we copy nature versus just get inspiration from nature?

vs



NanoBio History:
So What's Different Now?

Info Timeline

Intel 4004
1971

Bio Timeline

- 1973: "Modern" Era Begins
- 1975: Alsilomar Conference
- 1976: First Biotech Company
- 1978: Smith & Nathans win Nobel Prize
- 1985: Mullis publishes PCR paper
- 1993: Mullis wins Nobel Prize

Nano Timeline

- 1981: Binning and Rohrer invent STM
- 1985: Binning, Quate, and Gerber invent AFM
- 1986: Binning and Rohrer win Nobel Prize
- 1989: First commercial AFM

NanoBio History: So What's Different Now?

1985

Democratization Happened…

What Henry Ford did for automobiles, PCR and AFM did for biotechnology and nanotechnology, respectively and independently.

Economics α scope of adoption = N (collective of researchers)

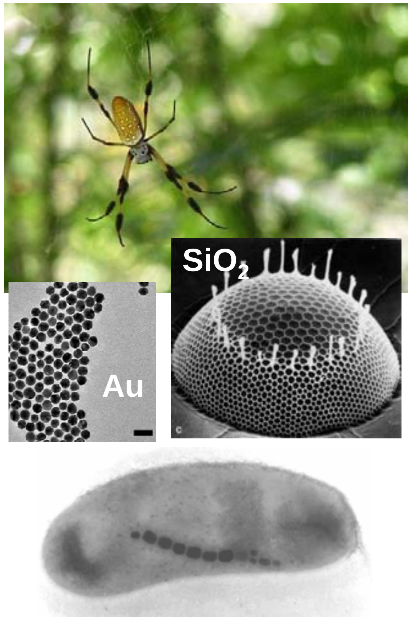
A "Wisdom of Crowds" effect for the entire research area

Bionanotechnology Future

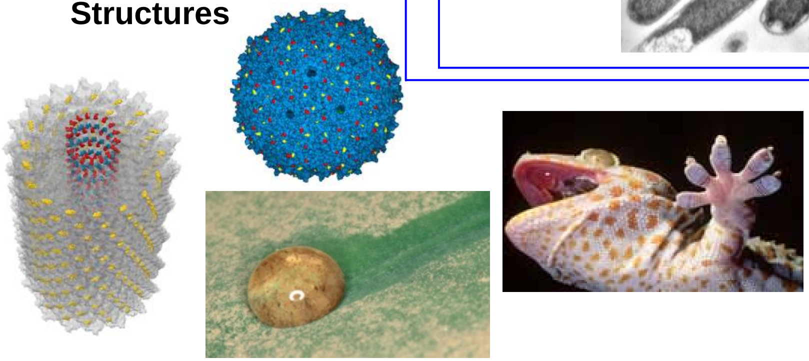
Sensors

Optics

Materials

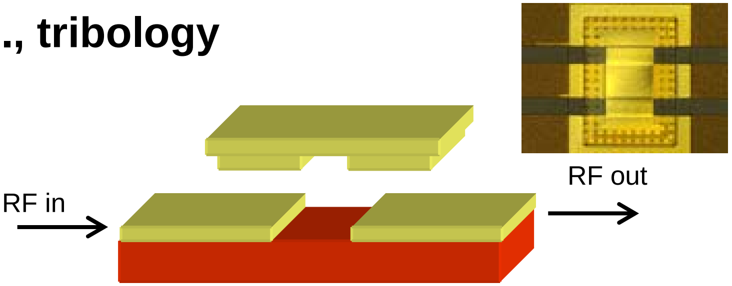


(Self-Assembled)
Structures

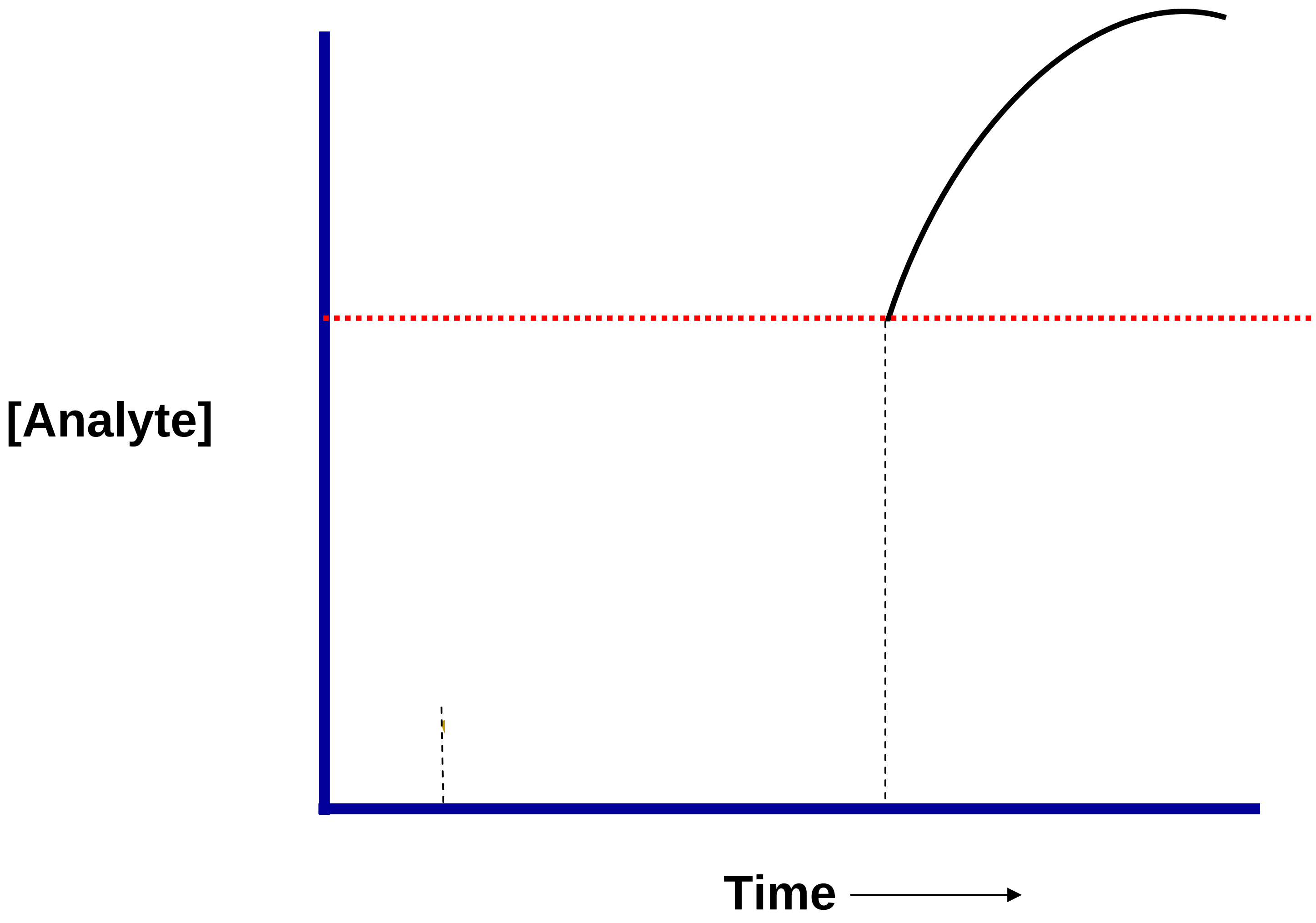


- **Diagnostics – medical and human performance**
- **Nano-Manufacturing – self-assembly and directed assembly, but, defect/error tolerance, design tools, thermodynamics are plotting against you**
- **“Non-obvious” and “non-sexy” areas, i.e., tribology**

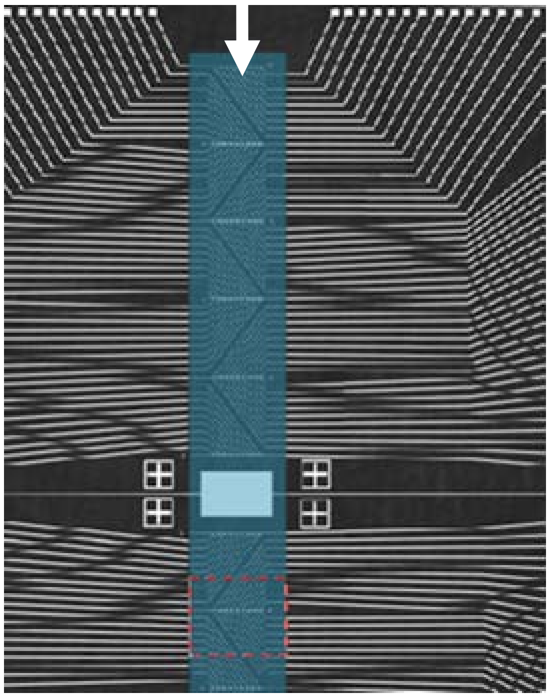
Au/Pd NPs

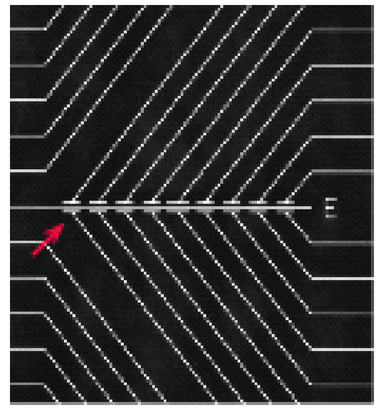


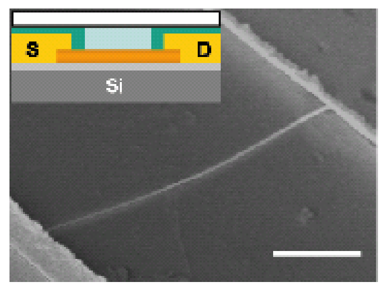
NanoDiagnostics Impact

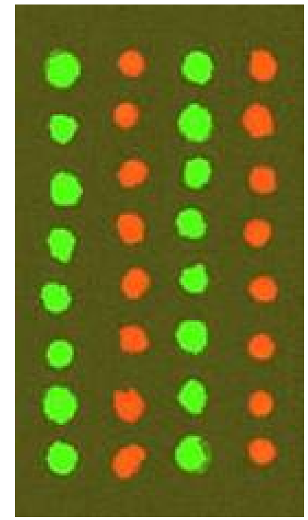


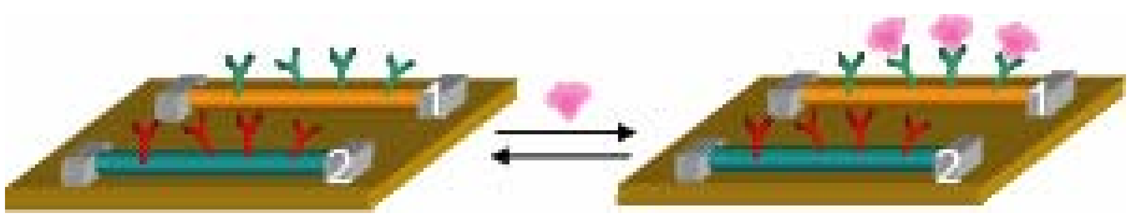
Sensor Chip for Flexible Detection









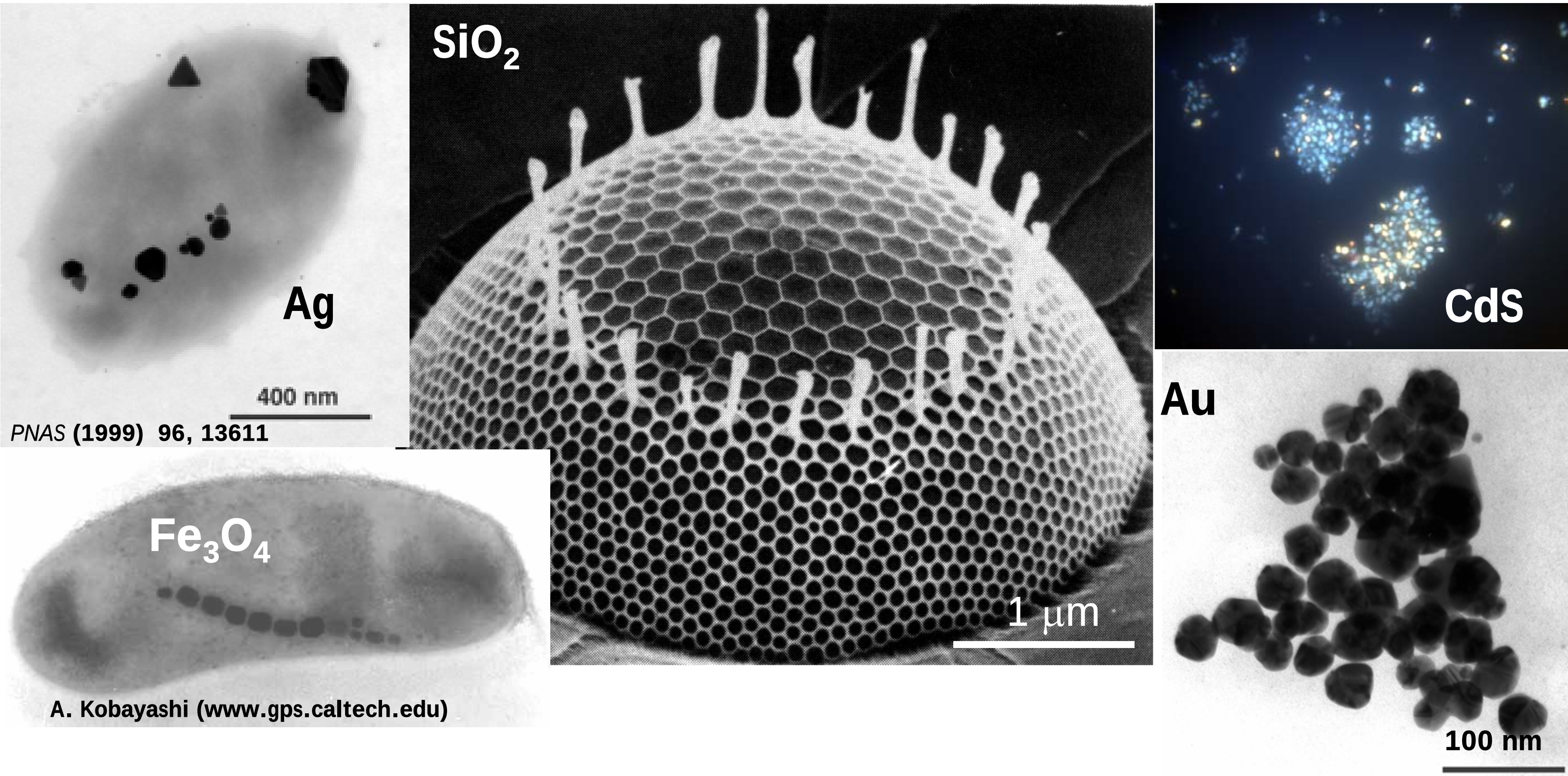


< pg/ml sensitivity

Independently incorporate many distinct receptors and nanowires with excellent reproducibility!

Patolsky, Zheng, Hayden, Lakadamyali, Zhuang & Lieber, *PNAS* **101**, 14017 (2004)

Inorganic Structures in Biology



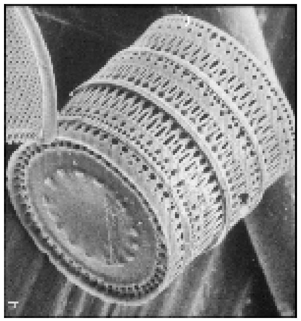
PNAS **(1999) 96, 13611**

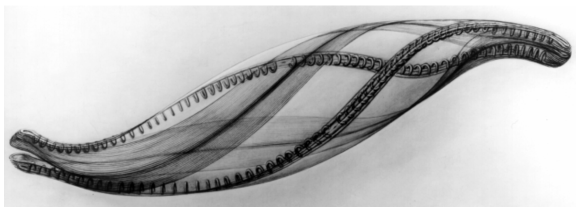
A. Kobayashi (www.gps.caltech.edu)

- Biology has evolved the ability to synthesize inorganic materials
- The master of “ambient conditions” materials science
- Proteins control the nucleation and growth of inorganic structures from nano- to macroscopic scales

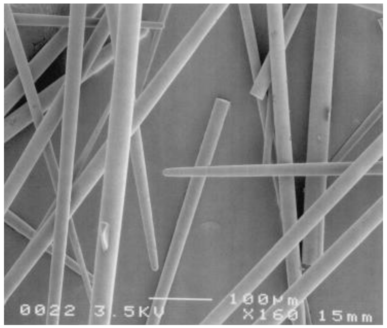
Silica in Biology

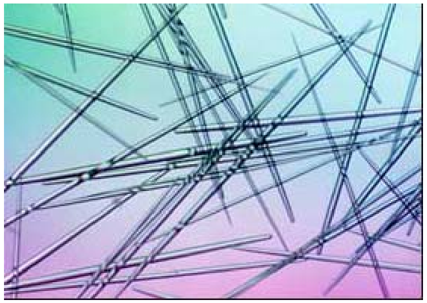
Biosilicification – formation of amorphous silica (diatoms, sponges mollusks)





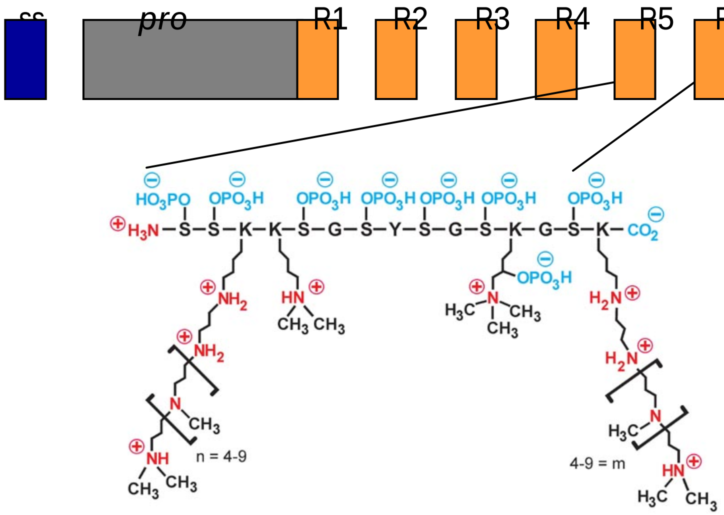
Diatoms





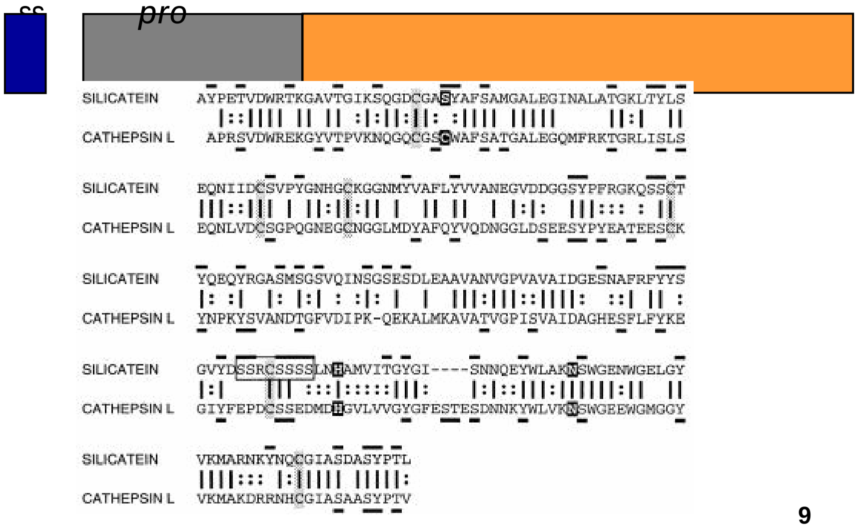
Marine Glass Sponge (*T. aurantia*) Spicules

Silaffin Protein



Kroger et al., *Science* (1999) **286**: 1129

Silicatein α Protein



Morse et al., PNAS. (1998) **95**:6234

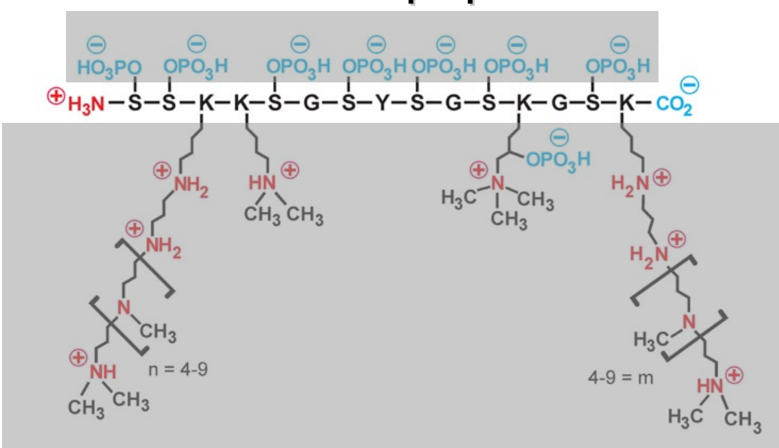
Formation of Biosilica

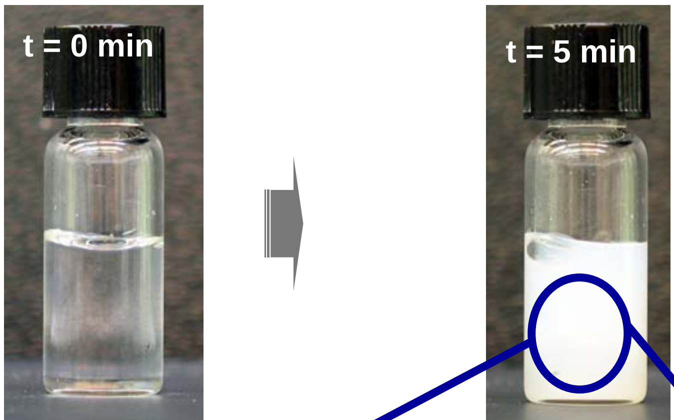
+ Silicic acid (TMOS)

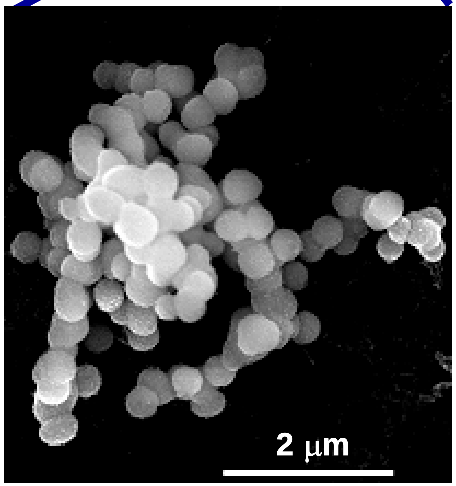
H_2N-SSKKSGSYSGSKGSKRRIL-COOH

R5 peptide

Silaffin peptide







Silica formation under ambient conditions: room temperature, neutral pH

Peptide Binder Library

COBALT OXIDE
Co-12 HYPTLPLGSSTY
Co-14 QYKHHPQKAAHI
Co-17 QLLPLTPSLLQA
Co-18 CFSQLNALPLIL
Co-9 KLHSSPHTPLVQ

CARBON NANOTUBES
CN-1 HSSYWYAFNNKT
CN-2 HTSYWYAFNTKT
CN-3 YTTHVLPFAPSS
CN-4 HAWVDWIRPIHS

GADOLINIUM
Gd-1 TTFSHYANQVHR
Gd-2 AETVESCLAKSH
Gd-3 LPYGTSNRHAPV
Gd-6 SLASYLQSWLGS
Gd-7 TKNMLSLPVGPG
Gd-8 EDNLAVRSQRIM

GERMANIA
Ge-8 SLKMPHWPHLLP
Ge-34 TGHQSPGAYAAH
Ge-10 SFLYSYTGPRPL
Ge-18 HATGTHGLSLSH

RUBY
Ru-1 AHRPLSANPFTA
Ru-2 HHKPWHPGKLLI
Ru-10 HSNWRVPSPWQL

IRON OXIDE
Fe-1 LPDSHHYKSDDH
Fe-2 QHMQQPQTQGIQ
Fe-4 SLYSNPTVPYSY
Fe-7 LPGSHQYQQQLL
Fe-8 QHITQSIWPGVR
Fe-10 QQLPKNGCLPAV

TITANIA
Ti-1 QPYLFATDSLIK
Ti-2 DLNYFTLSSKRE
Ti-7 SSWSSPITTAAV
Ti-5 GHTHYHAVRTQT

TIN OXIDE
Sn2-1 KNAGQYPPSALM
Sn4-1 SPSHSADHTPPT
Sn4-2 TPTLRSMSSLLF
Sn4-3 STLTQSTSSLVA

SILVER
Ag4 NPSSLFRYLPSD
Ag27 PWATAVSGCFAP
Ag28 SPLLYATTSNQS
Ag35 WSWRSPTPHVVT

SILICA
Si-3 KPHHHHTHHMYT
Si-8 KPSHHHHHTGAN
Si-7 APPGHHHWHIHH
Si-1 MSPHPHPRHHHT
Si-4 MSASSYASFSWS

ZINC OXIDE
ZnO1 GLHIPTGSYSHR
ZnO2 NLLTSNSHWPPR
ZnO3 TPSATMQTRPGL

COBALT PLATINUM
CoPt KYHNLHSHPLHK
CoPt KTHSLHSPLSHK
CoPt HLKHLPHTLPHK
CoPt KLHSSPHTPLVQ

PALLADIUM
Pd2 NFMSLPRLGHMH
Pd4 TSNAVHPTLRHL
Pd5 TTTKSITLTLSV

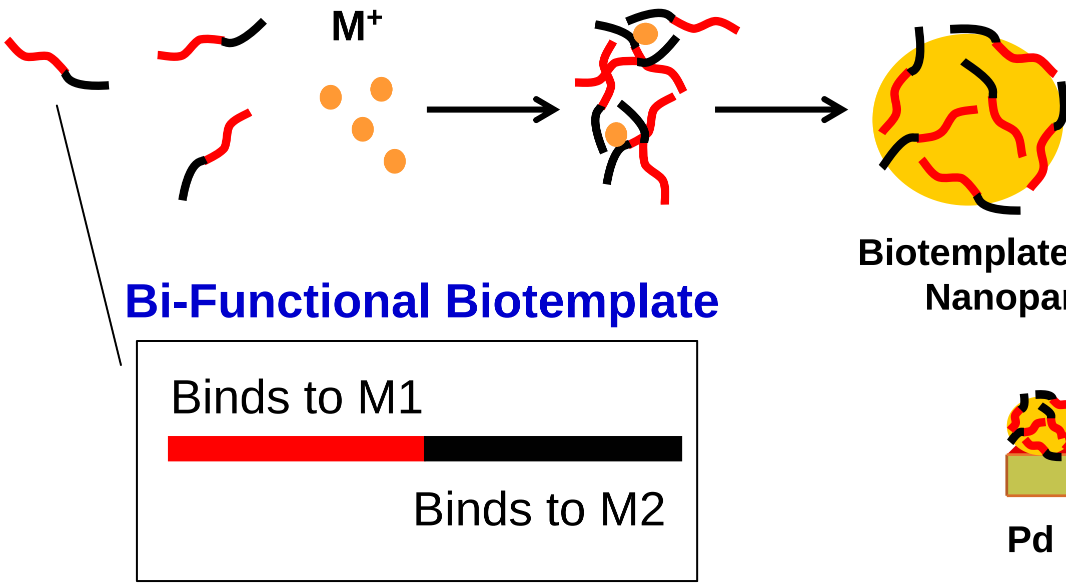
GOLD
Au3 AYSSGAFPPMPPF
Flg1 DYKDDDDK

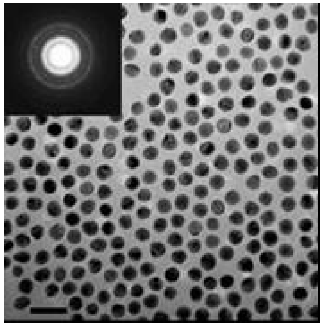
Na^{+}-MONTMORILLONITE
Mt1 WPSSYLSPIPYS
Mt4 AVTTLTLVPAGT

Positively charged
Aromatic, hydrophobic
Hydroxyl
Sulfhydryl
Negatively charged

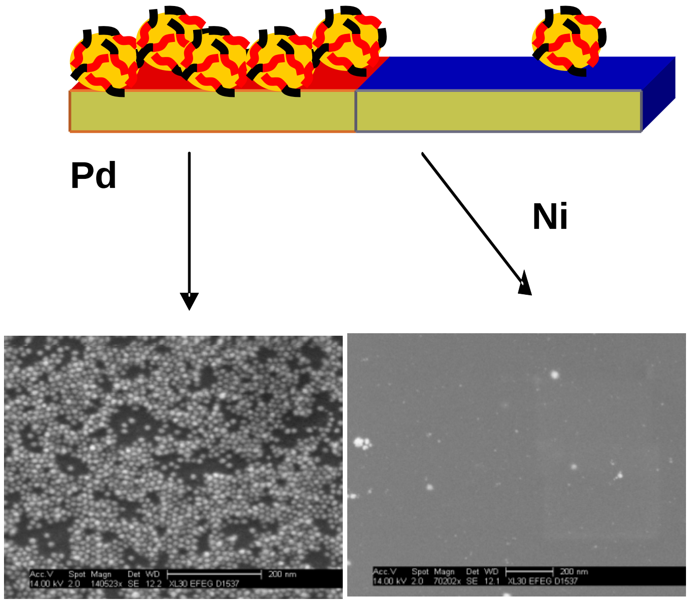
J. Nanosci & Nanotech (2002) **2**, 95-100
Nature Materials (2002) **1**, 169-172
Adv. Func. Maters. (2004) **14**, 25-30
Chem. Commun. (2004) 1, 1776-1777

Addressable Biomolecular Domains on Nanoparticle Surfaces





Monodispersed NPs



4200 particles /μm² | 70 particles /μm²

Site-Specific Deposition of Nanoparticles

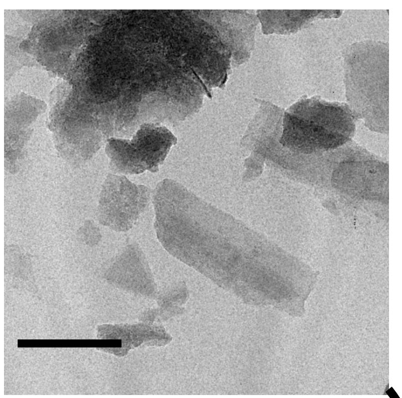
Advanced Materials 2006, 18, 1988

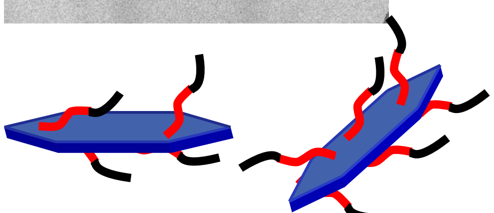
Controlling Nanomaterial Properties

Clay Binding Domain **Metal Binding/Bioactive Peptide**

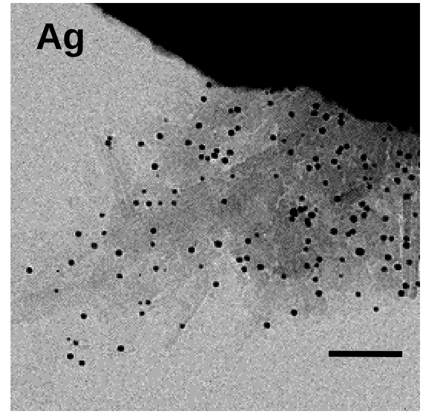
Bifunctional Biotemplate

Peptide-functionalized Clays

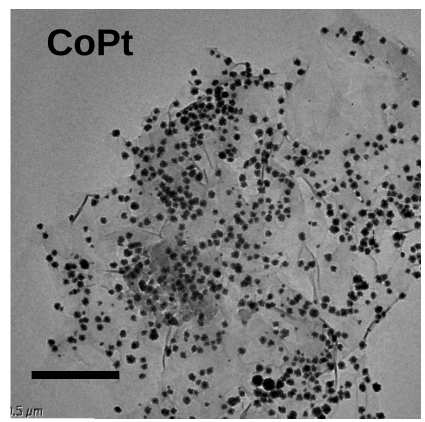




S2 Peptide



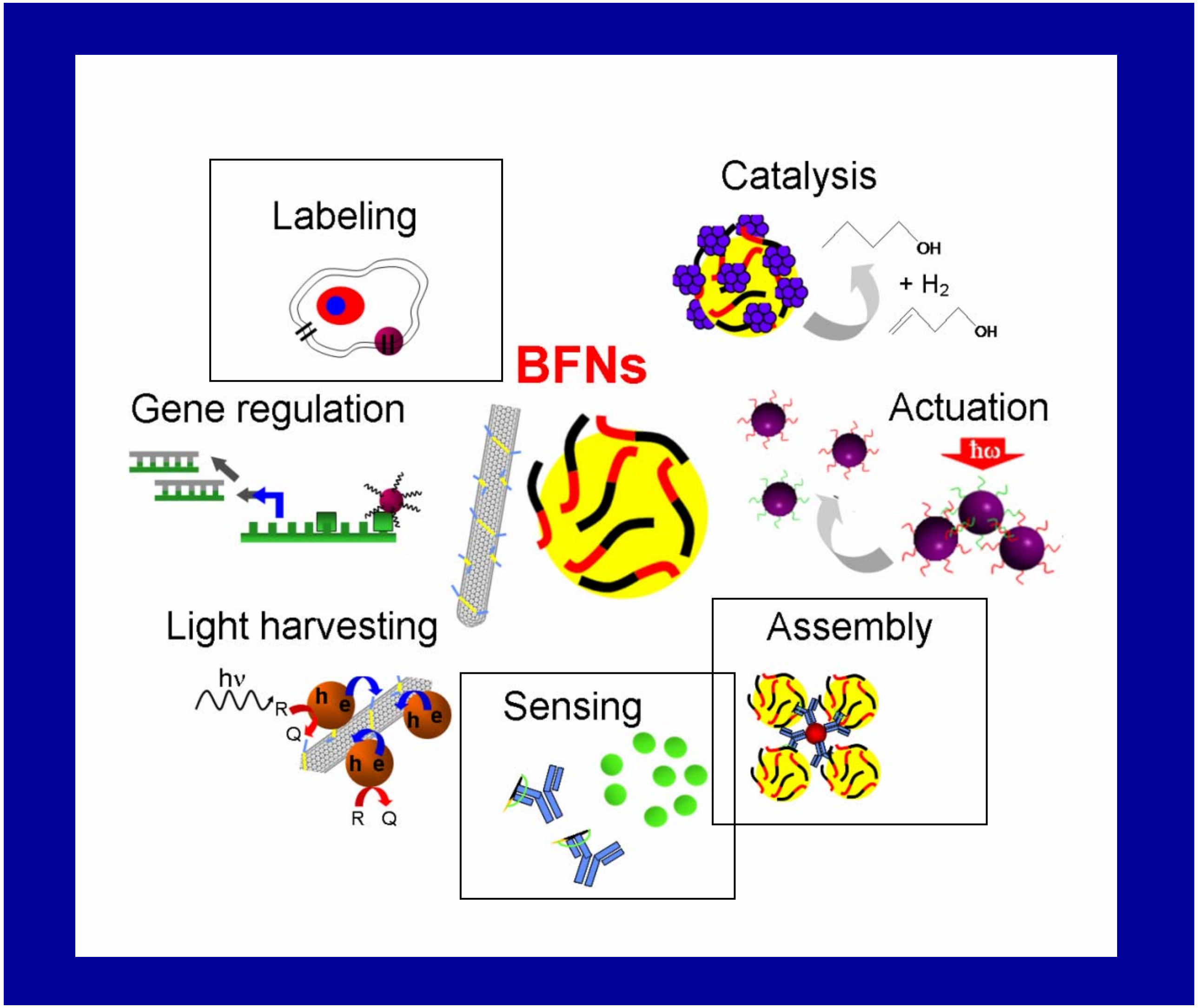
→ Antimicrobial



→ Magnetic

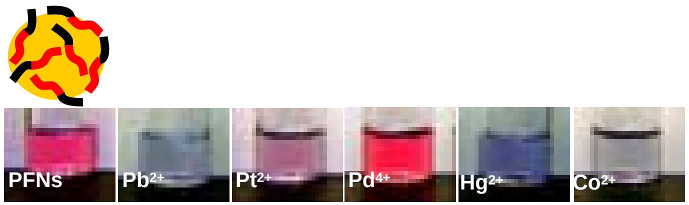
(Drummy, Vaia & Naik Submitted)

Bio-Functionalized Nanomaterials

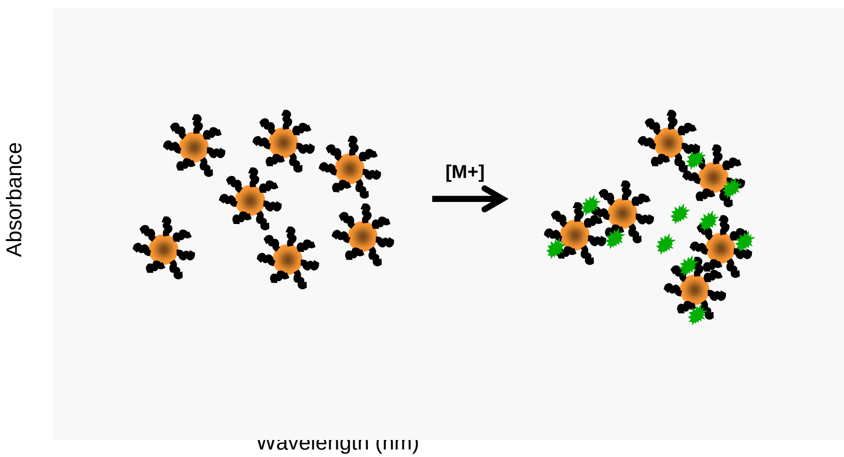


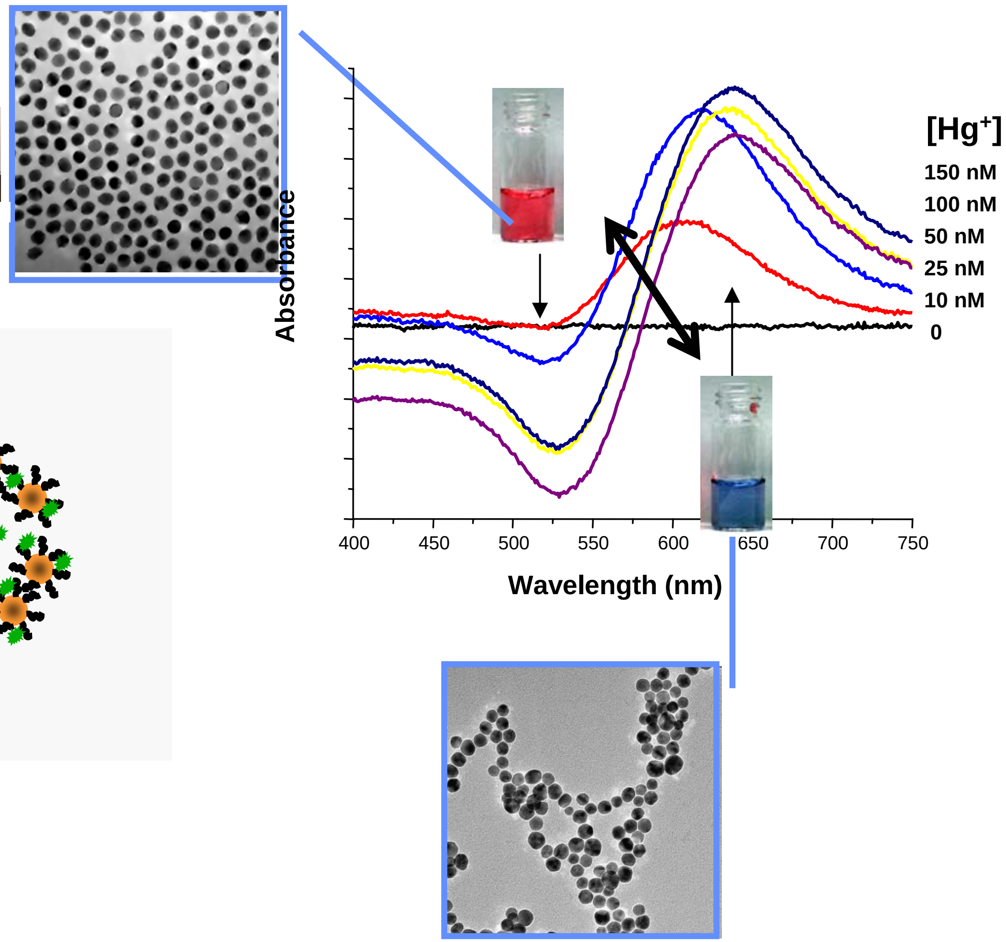
Colorimetric Response of Biofunctionalized Nanoparticles

A



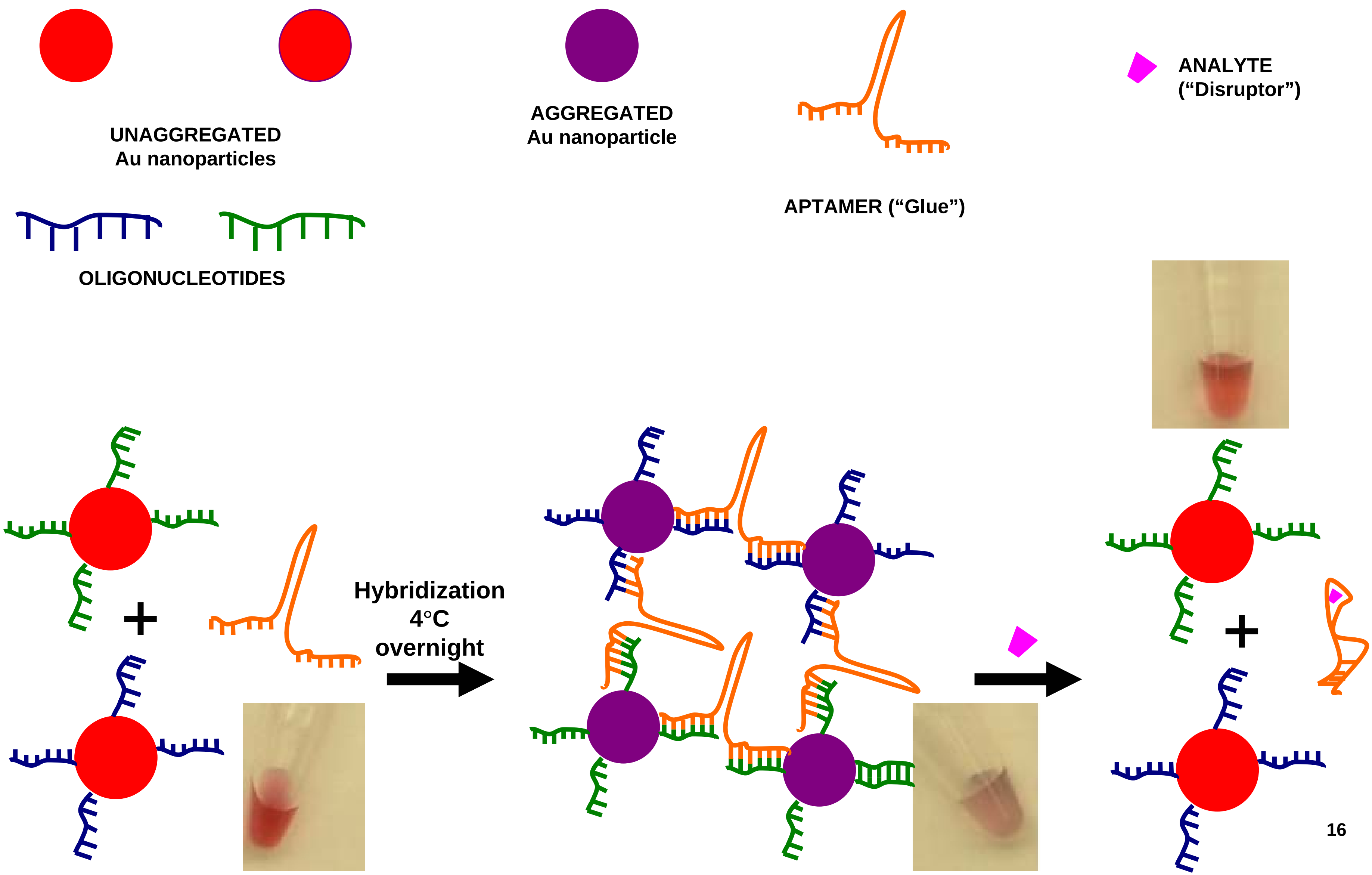
B





Small (2007) In Press

Molecular Target Assays
3 Part Design



Air Force Research Lab

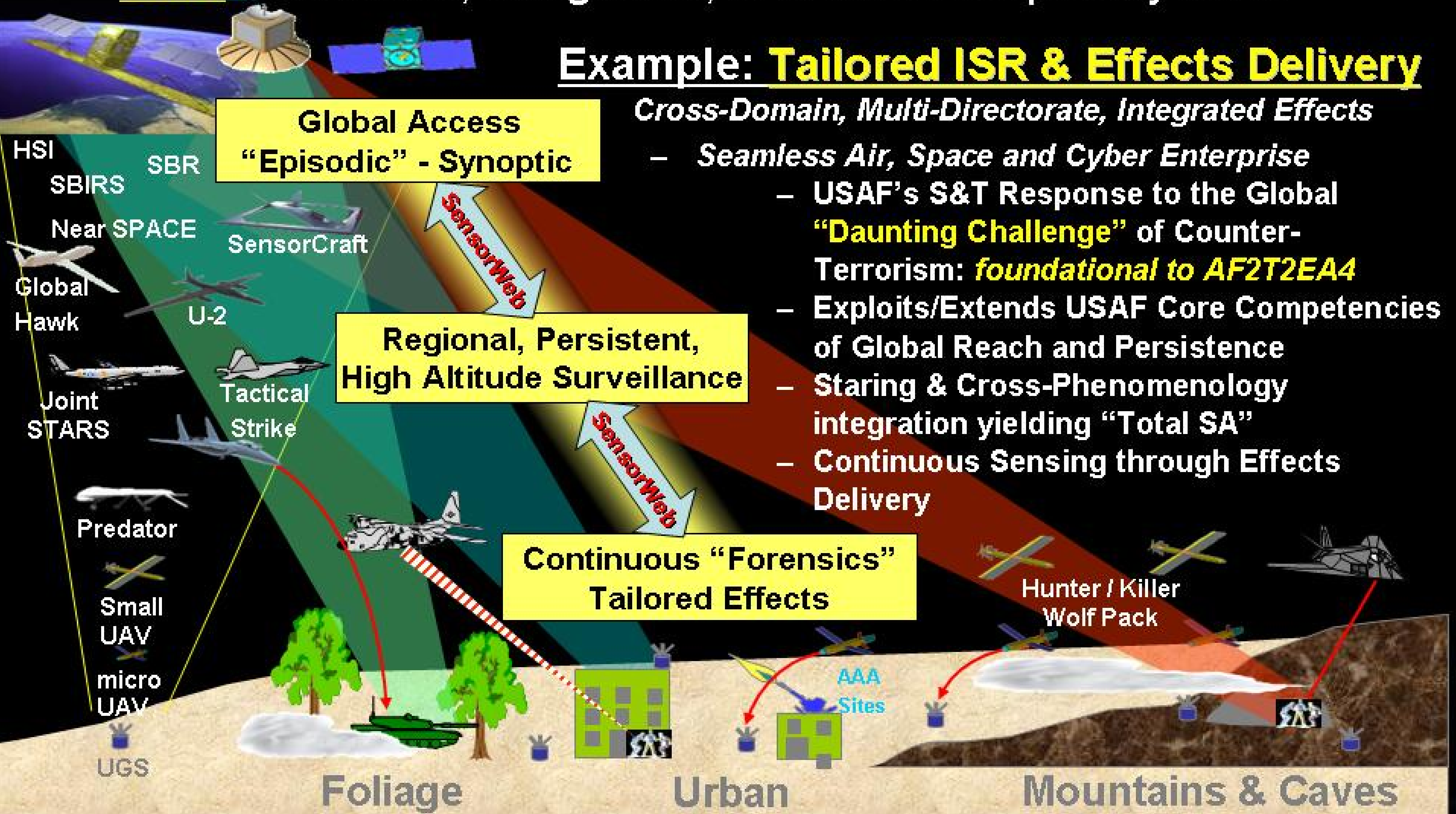
We must continue to:

- <u>Lead</u> innovative, integrated, and multidisciplinary R&D

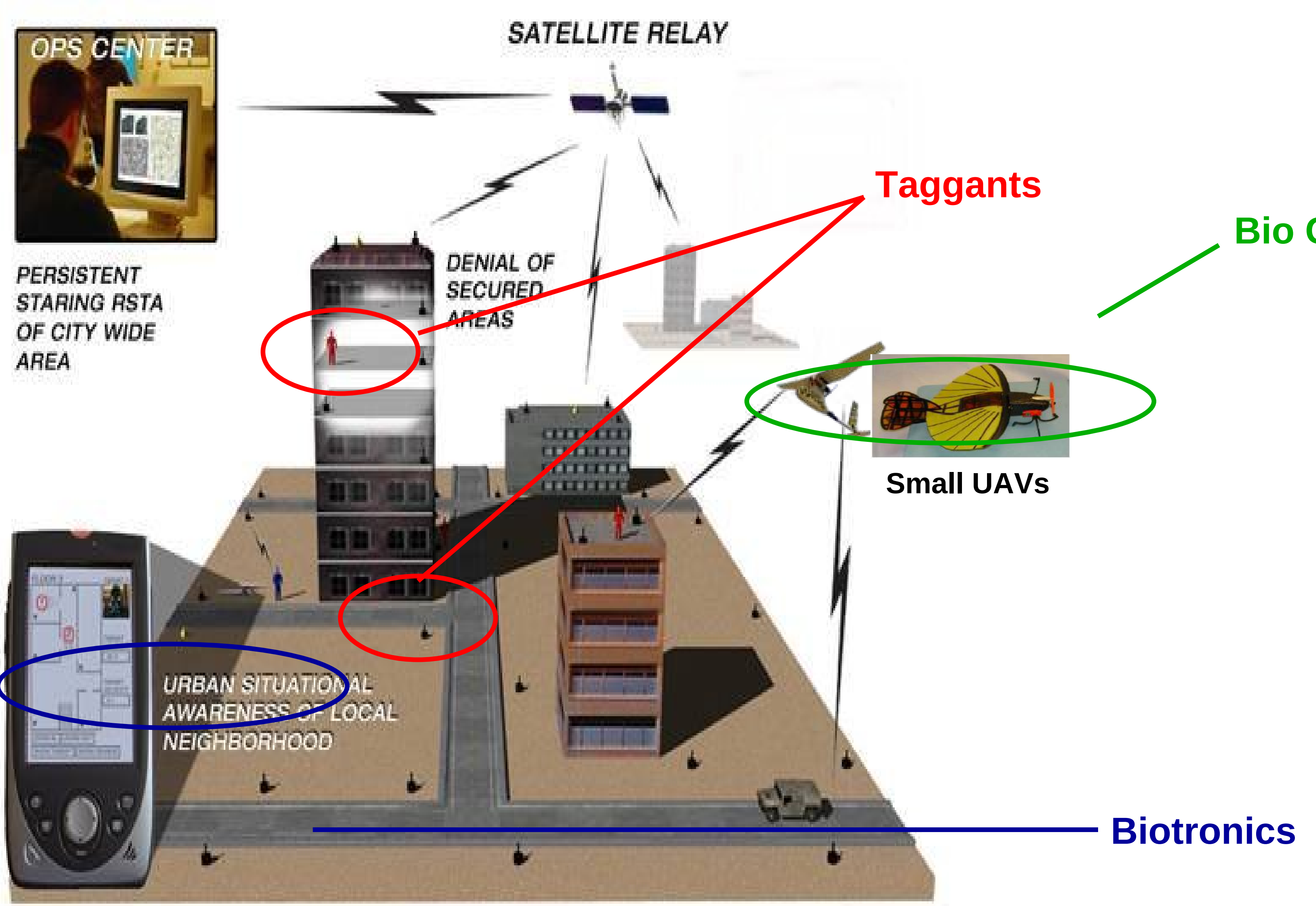
Example: Tailored ISR & Effects Delivery

Cross-Domain, Multi-Directorate, Integrated Effects

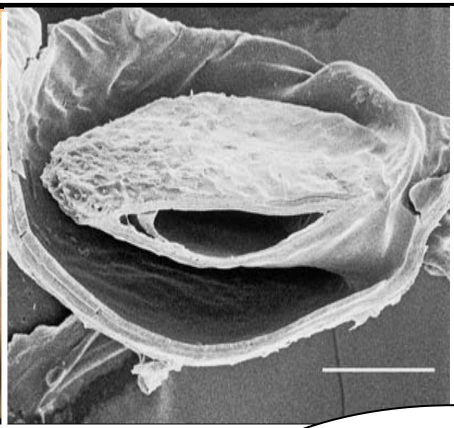
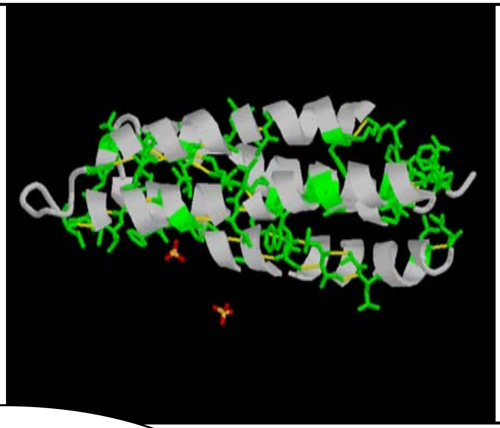
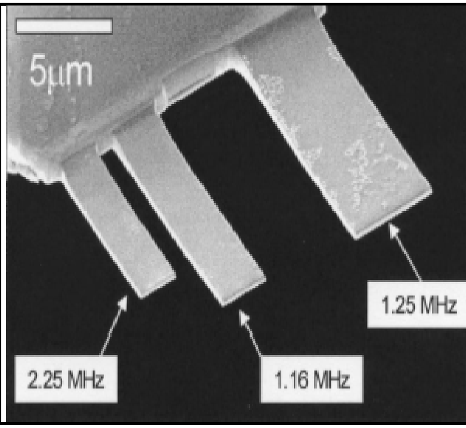
- *Seamless Air, Space and Cyber Enterprise*
  - USAF's S&T Response to the Global "Daunting Challenge" of Counter-Terrorism: *foundational to AF2T2EA4*
  - Exploits/Extends USAF Core Competencies of Global Reach and Persistence
  - Staring & Cross-Phenomenology integration yielding "Total SA"
  - Continuous Sensing through Effects Delivery

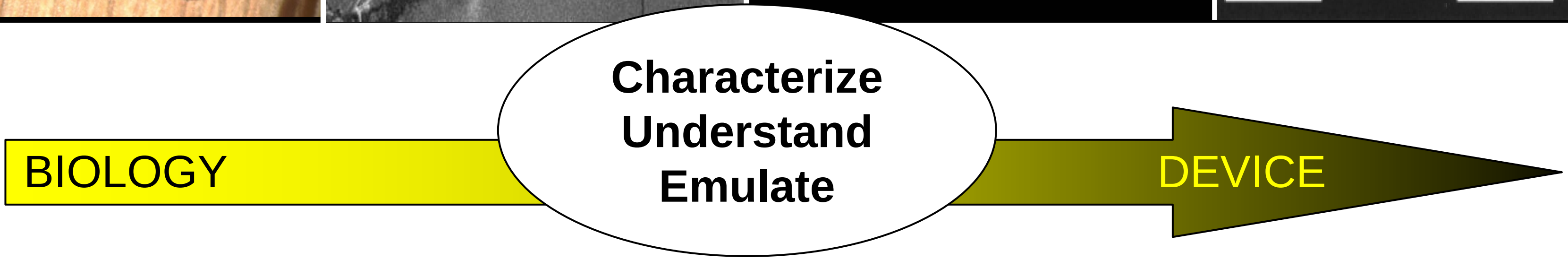


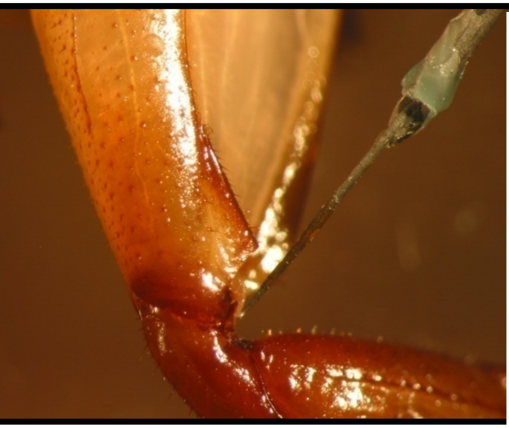
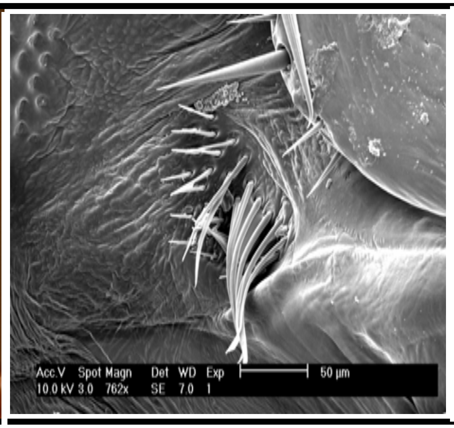
Bio-X STT
Urban Environment Game Changers

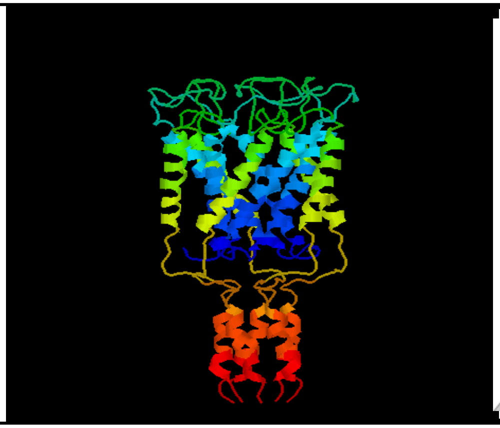
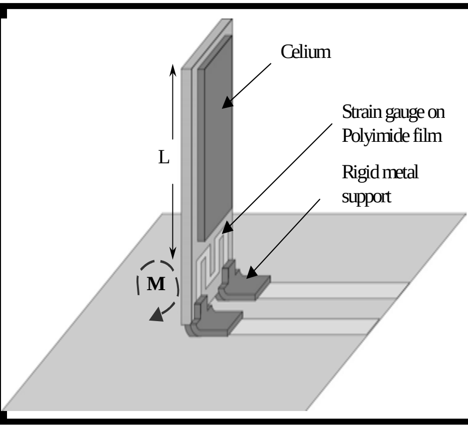


Program Vision



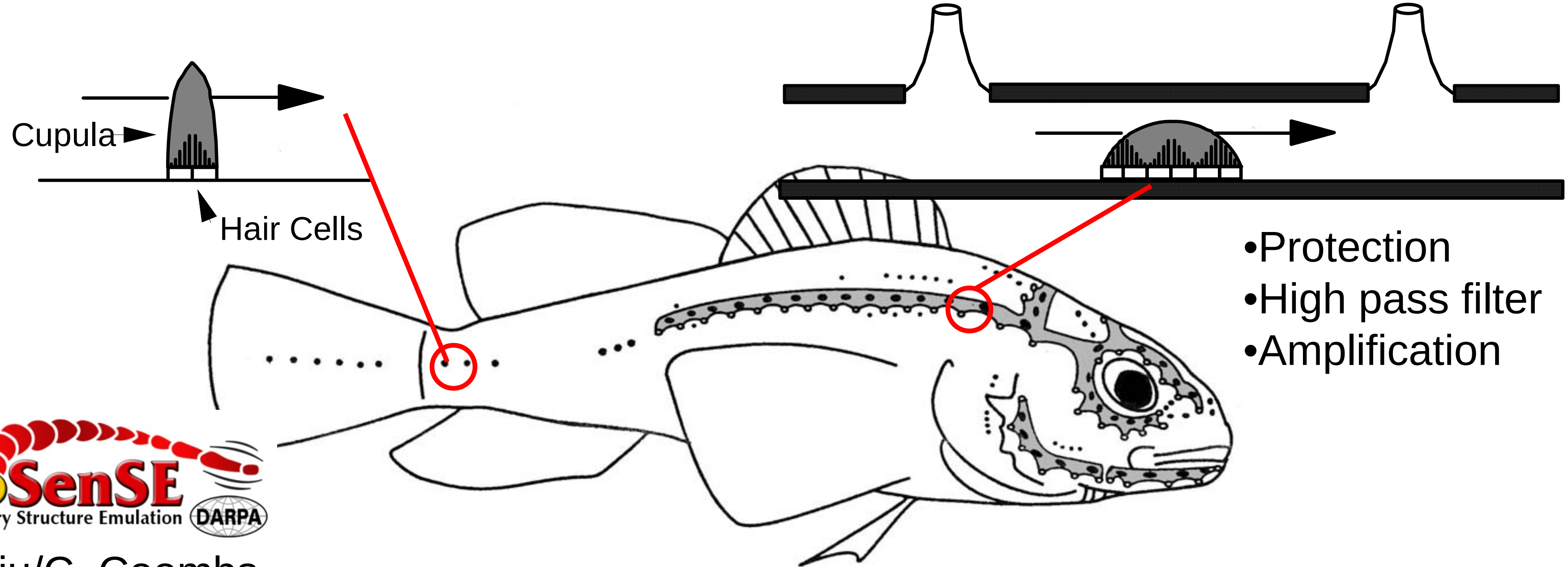



Lateral Line System

Superficial Neuromast

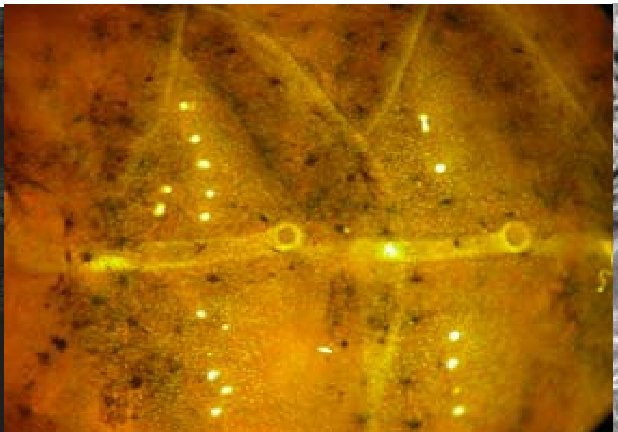
Canal Neuromast

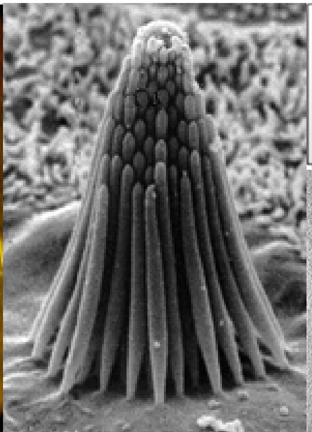


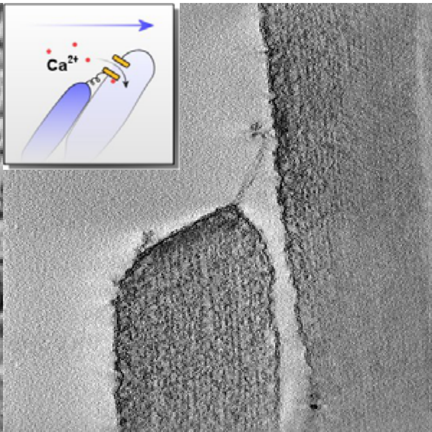
- Protection
- High pass filter
- Amplification

BioSenSE
Bio-Sensory Structure Emulation DARPA

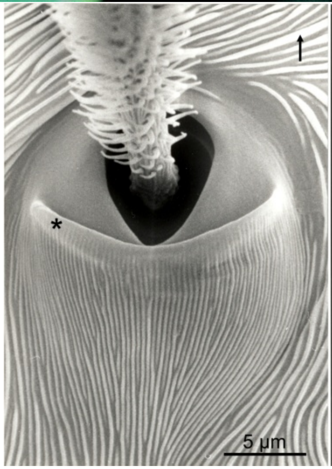
C. Liu/C. Coombs







Spider flow and vibration tracking



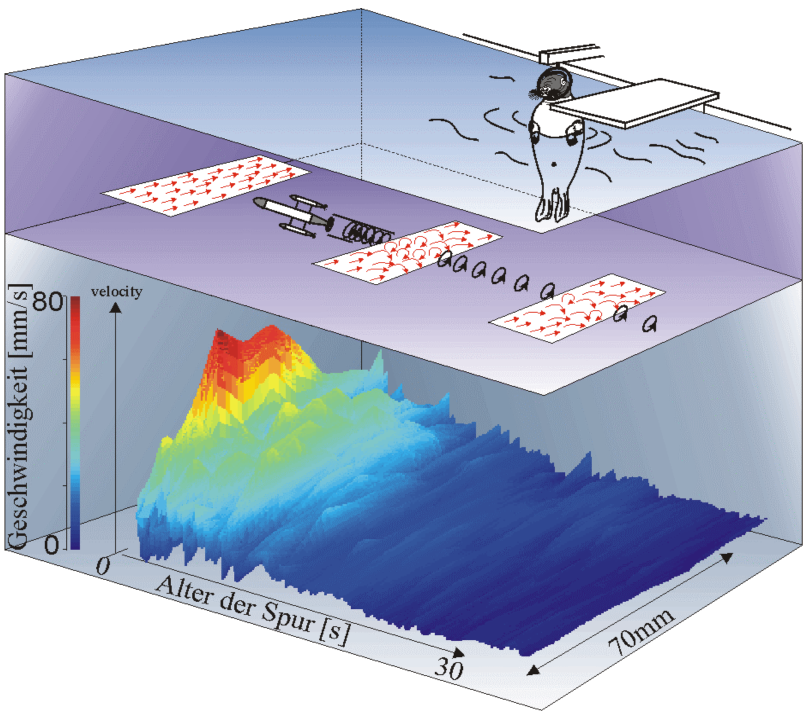
BioSenSE
Bio-Sensory Structure Emulation DARPA

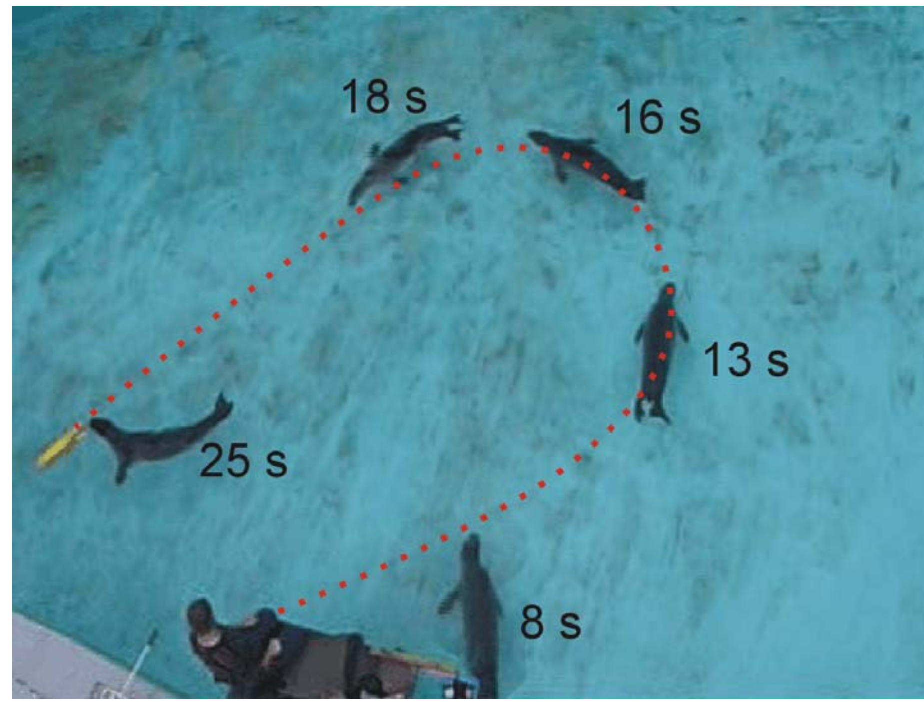
C. Liu/F. Barth

Wake tracking in seals

BioSenSE
Bio-Sensory Structure Emulation
DARPA

C. Liu/H. Bleckmann



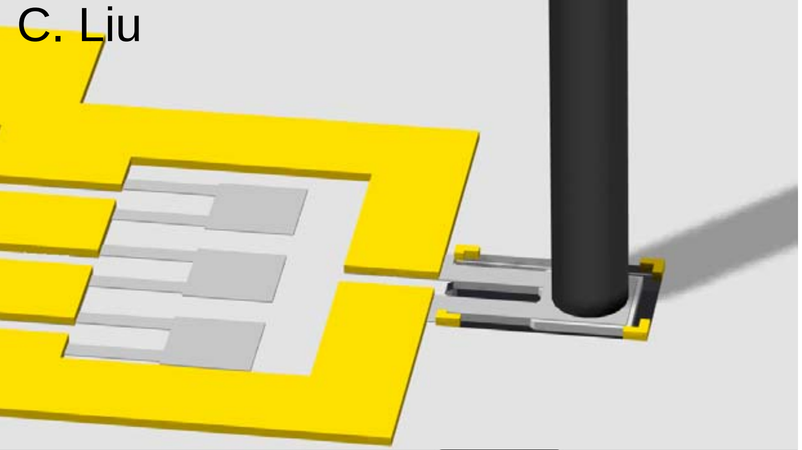


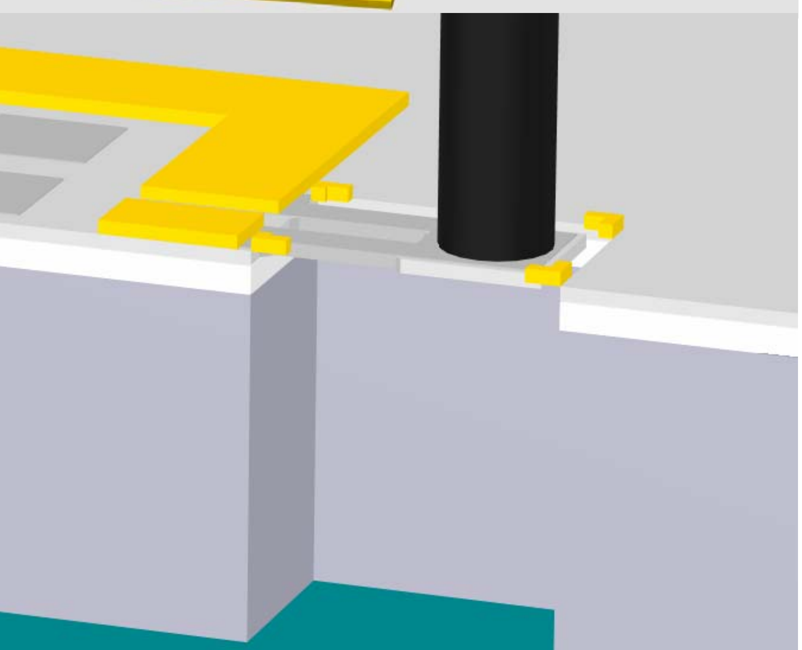
Dehnhardt, Mauck, Hanke & Bleckmann, Science 292 (2001)

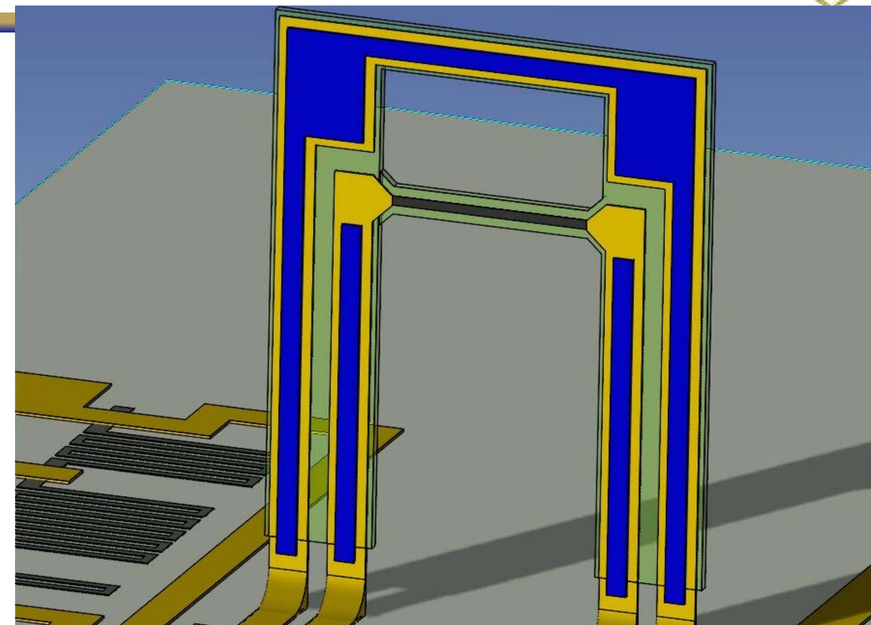
Artificial Hair Sensor

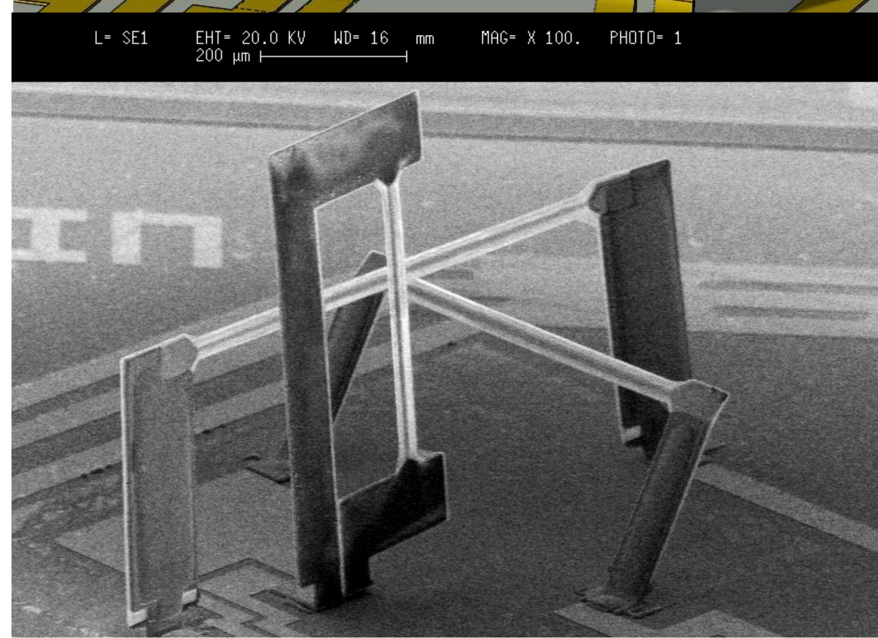
BioSenSE
Bio-Sensory Structure Emulation DARPA

C. Liu

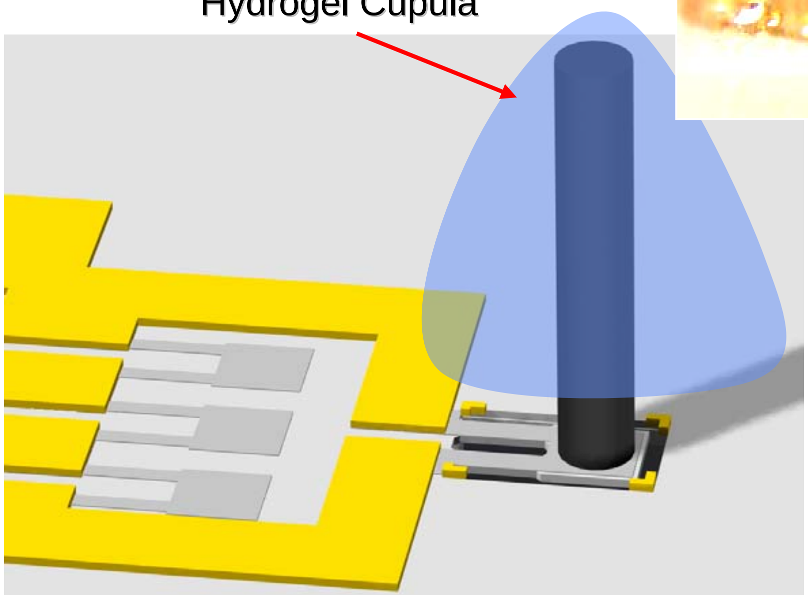


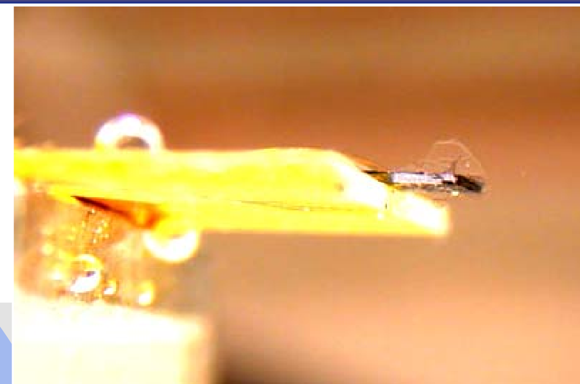


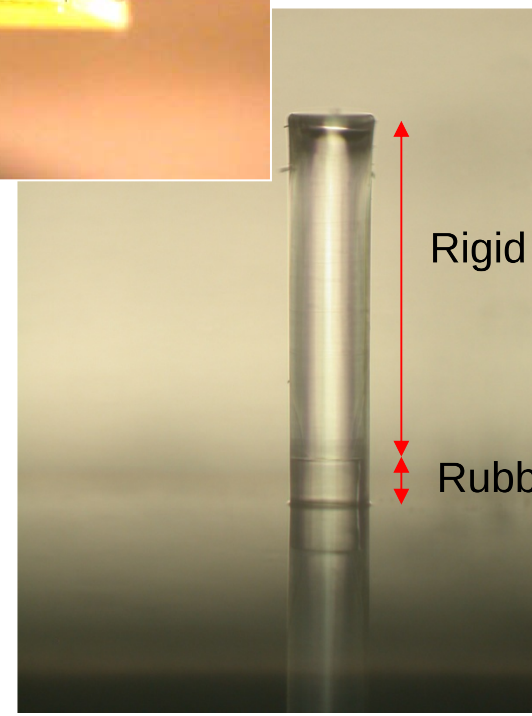




Device Improvement via Cupula





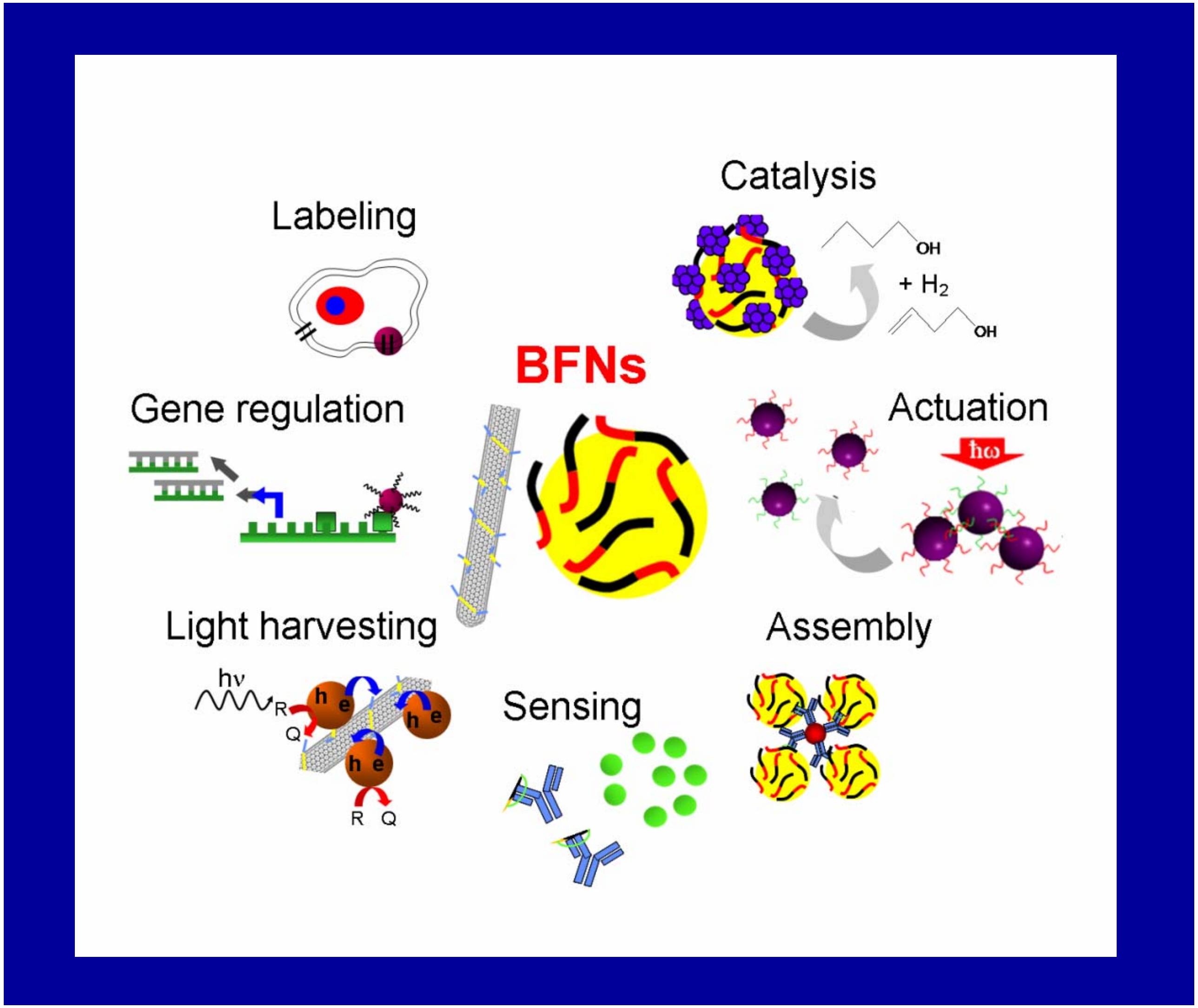


Hydrogel cupula is assembled to the hair to increase sensor sensitivity.

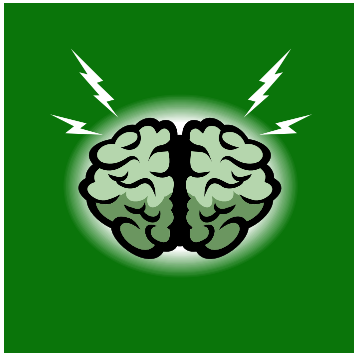
Bi-sectional hair with rubbery base is designed to improve device robustness.

Bionanotechnology: Technology Impact

Bio-Functionalized Nanomaterials (BFN)



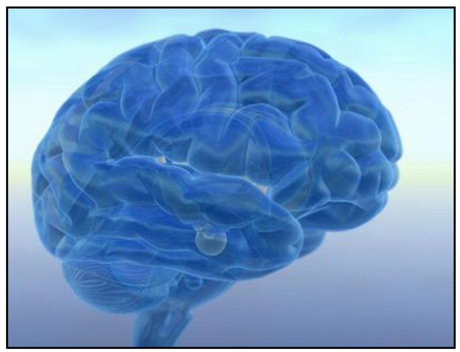
Cognition Impact



External Physiological Effects (TMS & tDCS)

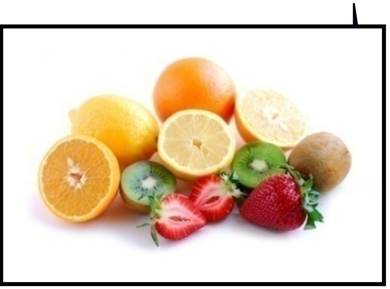
AF Training

"COGNITION"



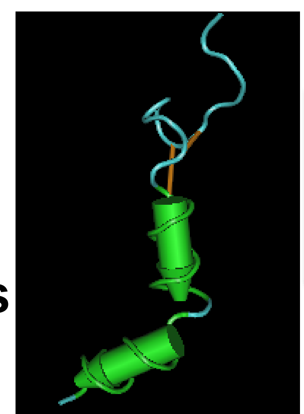
EFFECT : Cognition Enhancement, e.g., Accelerated Learning

Diet



Cognitive/ Physiological Loop

Molecular Aspects



mTOR: molecular role in moving memory from short-term to long-term

Molecular Targets of Opportunity: Cognition

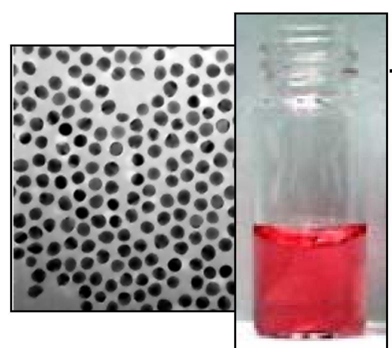
Stress,
Sleep Deprivation
& Cognitive function

Molecular Signatures
Orexin ↓
S6K+/- P
Stress hormones ↑

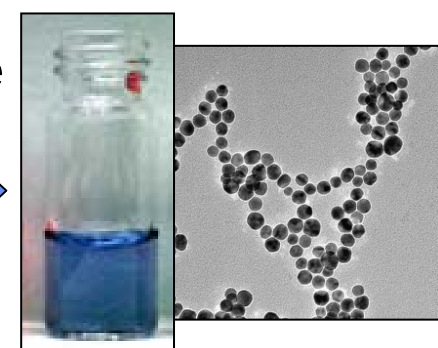
Rodent Models:
Molecular and Neuro-Behavioral

+ leucine

Enhanced Molecular Signatures
↑ Orexin
S6K-P
↓ Stress hormones



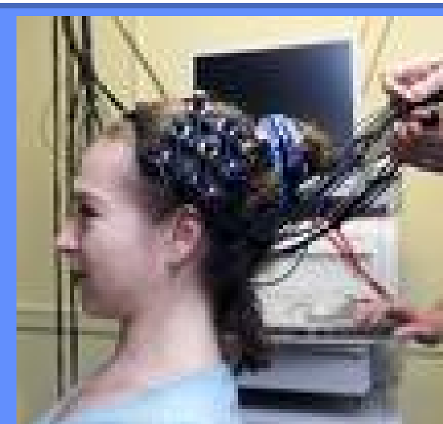
+ cognitive marker



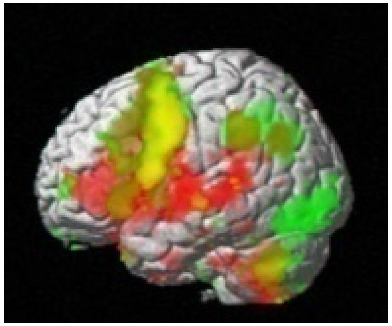
Nano in Biology:
Nanoshells / Nanoparticles

Nanoshells exhibit a unique electromagnetic resonance response

VS.



Revolutionized Airman Selection, Training, and Effectiveness via Innovation in Cognition



Academic partners are telling us which areas of the brain are important in a given task – i.e., visual acuity

We can *safely* stimulate the those regions of the brain via TMS or TDCS in RHP

We can directly measure warfighter improvement via Live, Virtual, Constructed (LVC) environment of RHA

Revolutionize pilot training and effectiveness via recent advances in neuroscience and cognition!

Study Methodology

TMS Treatment

- **Subjects**
  - **20 healthy volunteers (ag**
  - **Medical/Neurological Eva**
  - **Subjects with metal in the head, neurological disord seizure risk, history of seizures will be excluded**
- **Task**
  - **Difficult Surveillance and Reconnaissance (SAR) Task**
    - **Target vehicles among similar distracter non-target vehicles**
    - **Three levels of search difficulty – vary # of targets and distracters**
    - **Accuracy and reaction time data will be recorded**

Valid Targets

Invalid Targets

Parting Thoughts
(truth in advertising)

“The not so good…”

- **Bio-based approaches can be *very* expensive (good plug for bio-inspiration)**
- **Bio-based approaches can be very slow (but that is not always a bad thing)**
- **Response & signal transduction: What’s your ROC curve look like? (know your engineering brethren)**

“And now the good…”

- **Great lessons in the non-obvious**
- **Mastery of the ambient**
- **Truly unique surfactants for materials control**

ACKNOWLEDGMENTS

Materials and Manufacturing Directorate
(R. Naik, Bio RL)

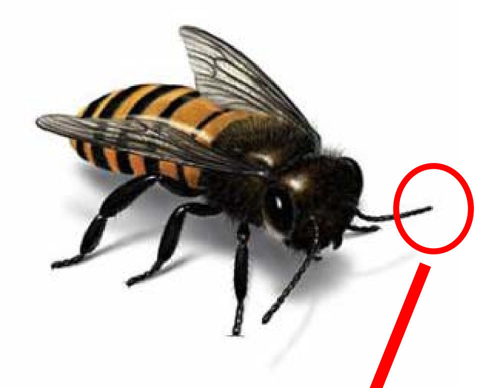
Human Effectiveness Directorate

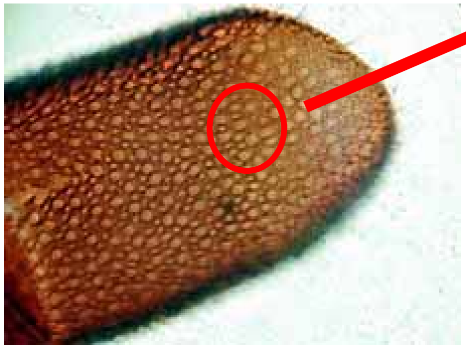
Bio-X STT

Air Force Office of Scientific Research

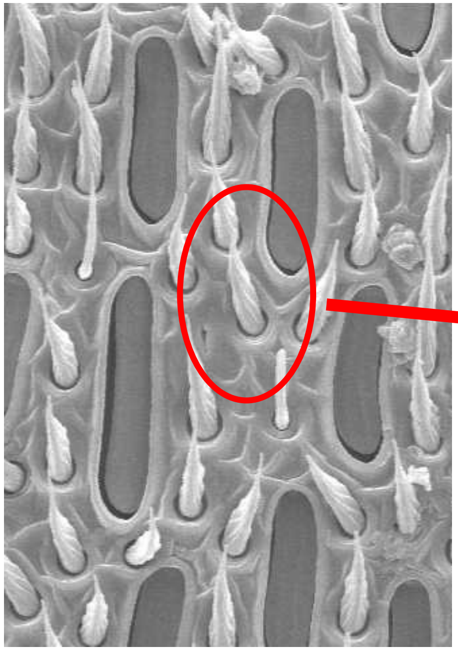
DARPA

Insects Sensing Using OBPs

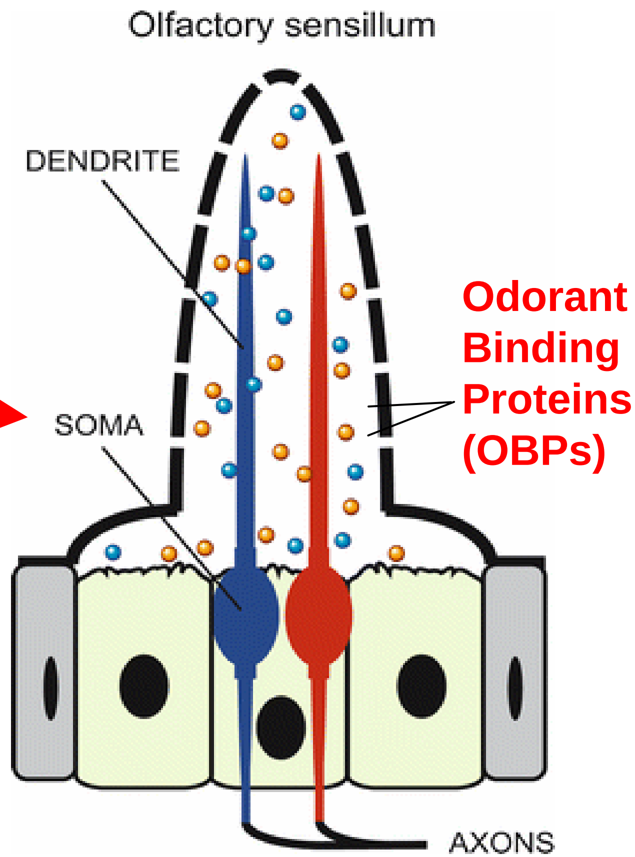




bee antennae, 400X

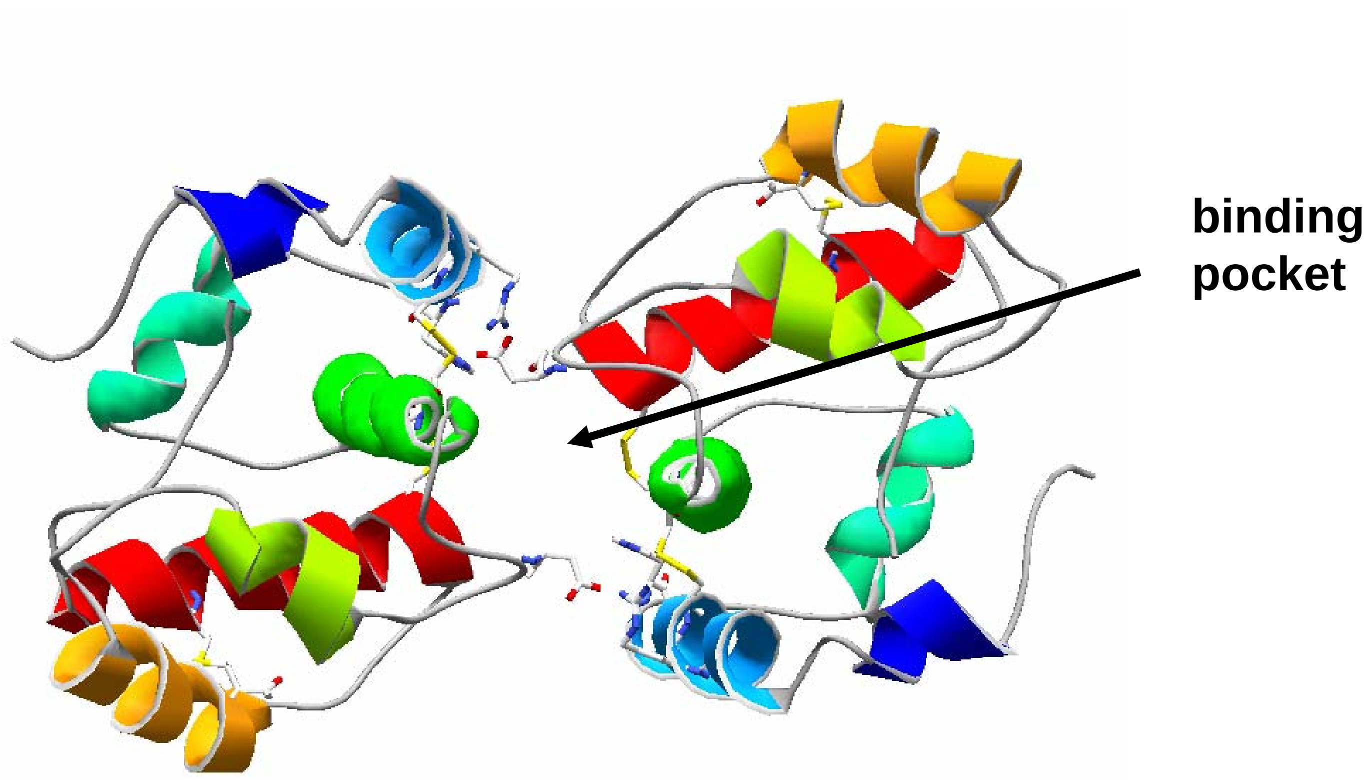


surface of bee antennae



M Rutzler and LJ Zwiebel, J Comp Physiol A (2005) 191: 777-790

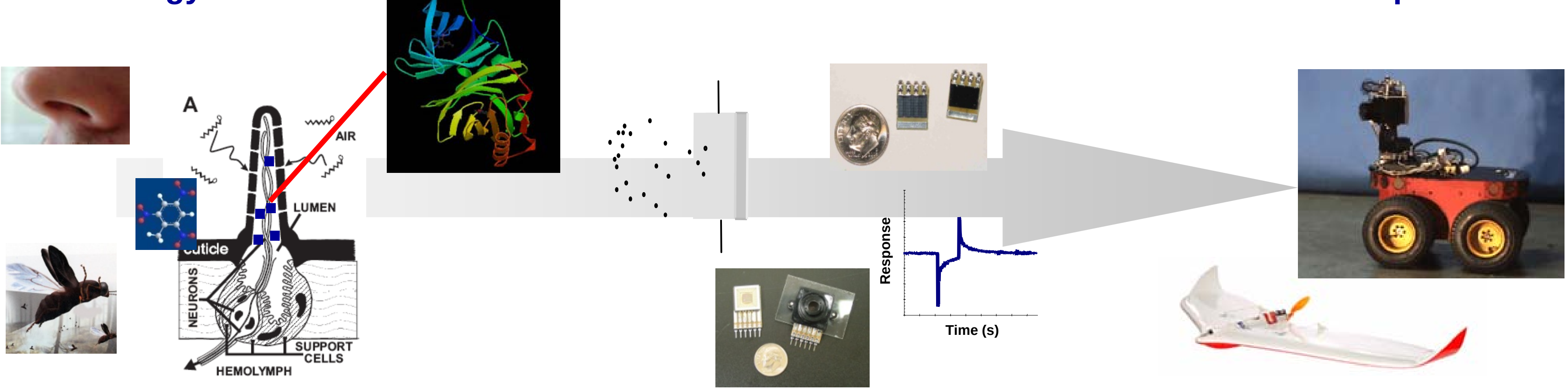
Crystal Structure of ASP1 Homodimer



ASP1 has large hydrophobic binding pocket similar to pheromone binding proteins from other insects.

Biomimetic Sniffer

From Biology… **….to sensor platforms.**



Sensitivity in nM-μM

Goal: Genetic engineering of Odor Binding Protein (OBP) for chemical agent detection.

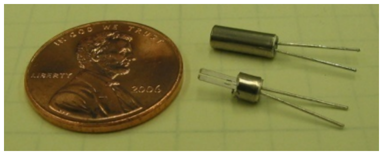
Specific Objectives: Clone OBPs from insects into systems that would allow genetic manipulation, screen against target compounds and incorporate into solid state device.

Approach:
Develop a genetic screen for identifying OBP variants that bind to a specific chemical ligand using a molecular biology and computational approach. Develop a biomimetic sensor that can detect chemical signatures.

Benefits:

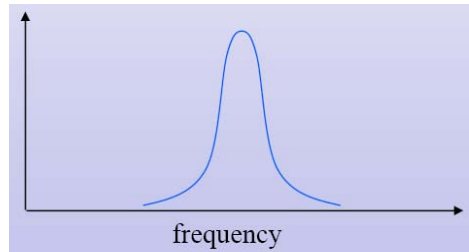
- Sense, and ID chemical threats/signatures.
- Hybrid, multifunctional, sensors: Low-cost, distributed, omni-present lightweight sensors.

Quartz Tuning Fork (QTF) Sensor



Quartz tuning fork

- Inexpensive
- Used in watches
- High quality factor of 90,000
- Low power consumption

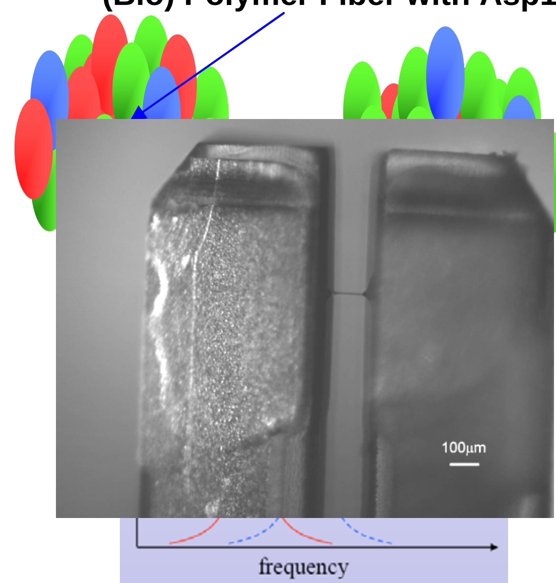


Tuning fork vibrates at a highly defined frequency

Benefits:

- Inexpensive.
- Highly selective.
- Tunable.
- Robust.

(Bio) Polymer Fiber with Asp1



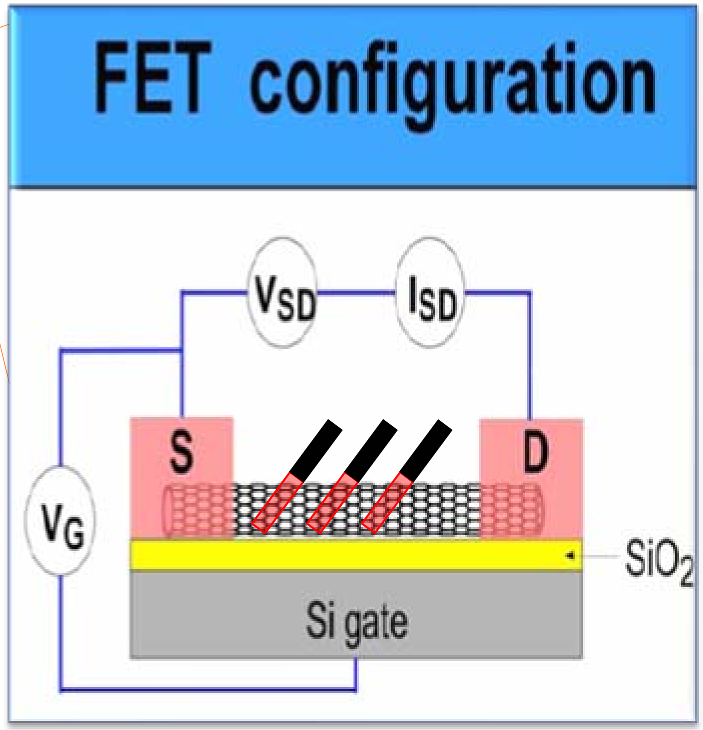
Sensor fabrication

- Using photolithography, bridge the forks with a polymer
- Polymer has a protein-binding functional group
- Immerse the tuning fork in a solution of OBPs
- OBPs are now attached to the polymer wire
- Expose the QTF to air
- As material bind to the OBPs, the tension of the polymer wire changes, which changes the frequency of the QTF

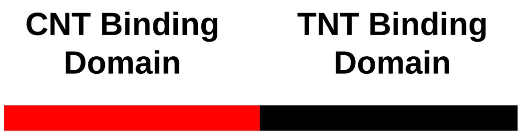
CNT Chemosensor

nanomaterials (sensitivity) + biomolecules (selectivity) = chemosensor

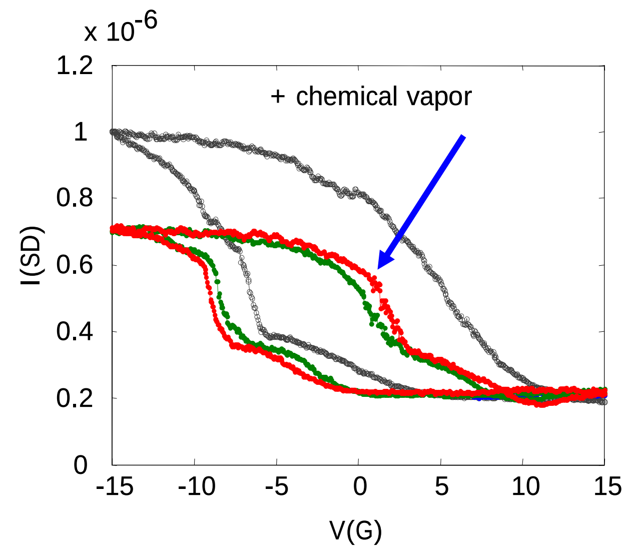
Insect Odor Sensing Mechanism



Functionalize CNTs with engineered biomolecules

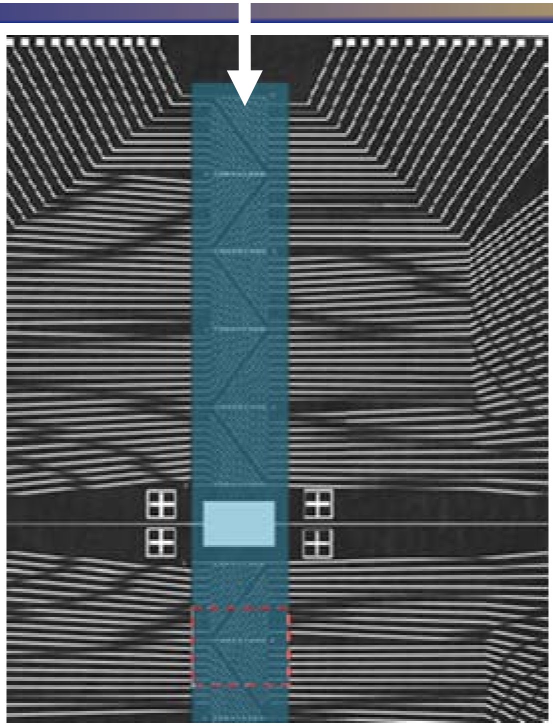


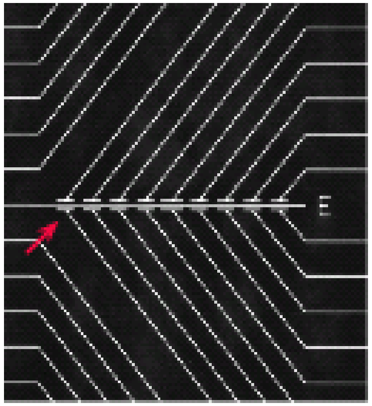
Bifunctional Biotemplate

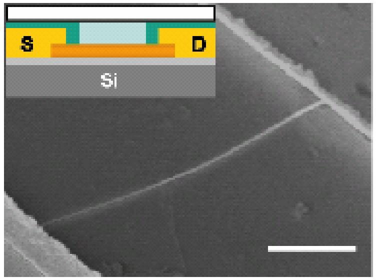


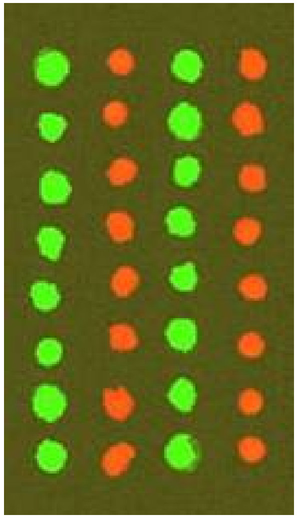
Biomimetic Chemosensor

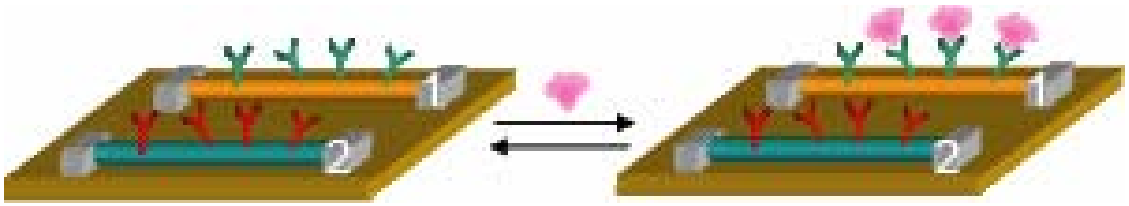
Sensor Chip for Flexible Detection











< pg/ml sensitivity

Independently incorporate many distinct receptors and nanowires with excellent reproducibility!

Patolsky, Zheng, Hayden, Lakadamyali, Zhuang & Lieber, *PNAS* **101**, 14017 (2004)

NanoBio History:
So What's Different Now?

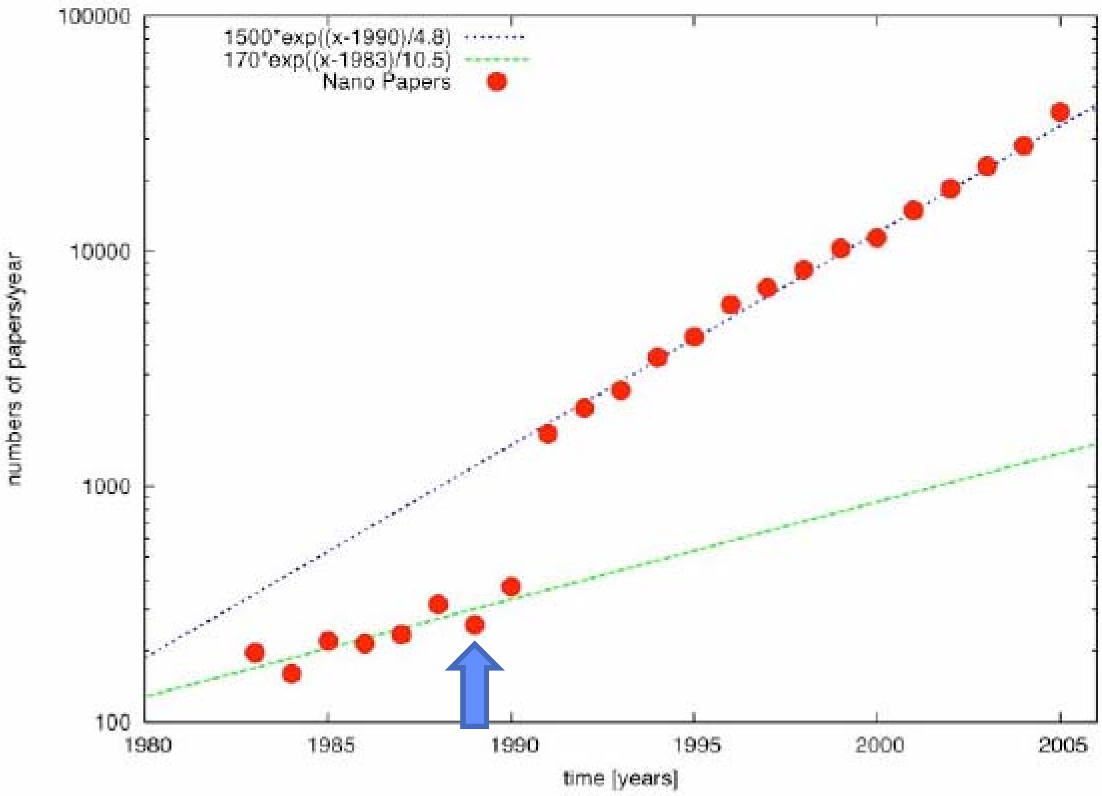


Fig. 1. Number of articles on nano science and engineering, based on the presence of the string "nano*" in article titles, abstracts, and keywords in the ISI Web of Science database. Data provided by David Wojick; figure prepared by Luis Bettencourt.

D. Kaiser, "Notes toward a Nanotech Timeline," OSTI Working Paper 6-06-001 (5 June 2006)

4 “Groups” in Synthetic Biology

1. Biologists – test current understanding of natural biological systems
2. Chemists – an extension of synthetic chemistry
3. ‘Re-writers’ – Genomes encoding natural biological systems can be ‘re-written’, producing engineered surrogates that might usefully ~~supplant~~ some natural biological systems
4. Engineers – biology is a technology with an emphasis on development of foundational technologies to make the design and construction easier

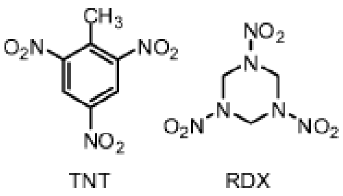
~~augment~~

CONTROL

D. Endy (2005) “Foundations for engineering biology,” *Nature* **438** (24): 449.

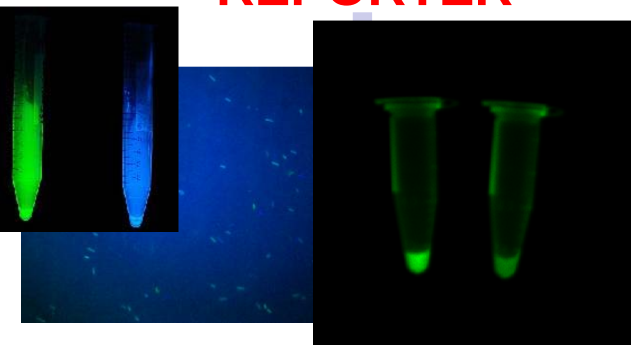
In vivo circuits: Riboswitch Strategy

SWITCH/SENSOR



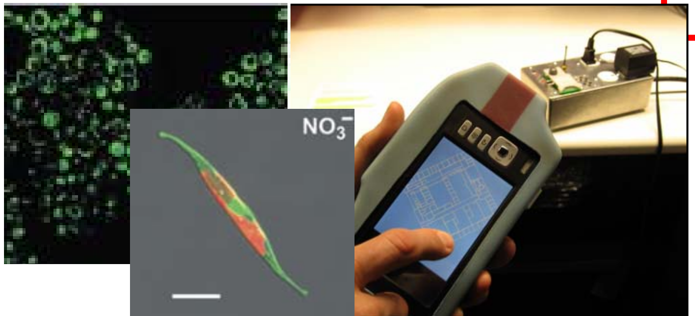
Identification/recognition elements that are coupled to genetic control

REPORTER



Development of pathway/reporter system (optical and redox-based assays)

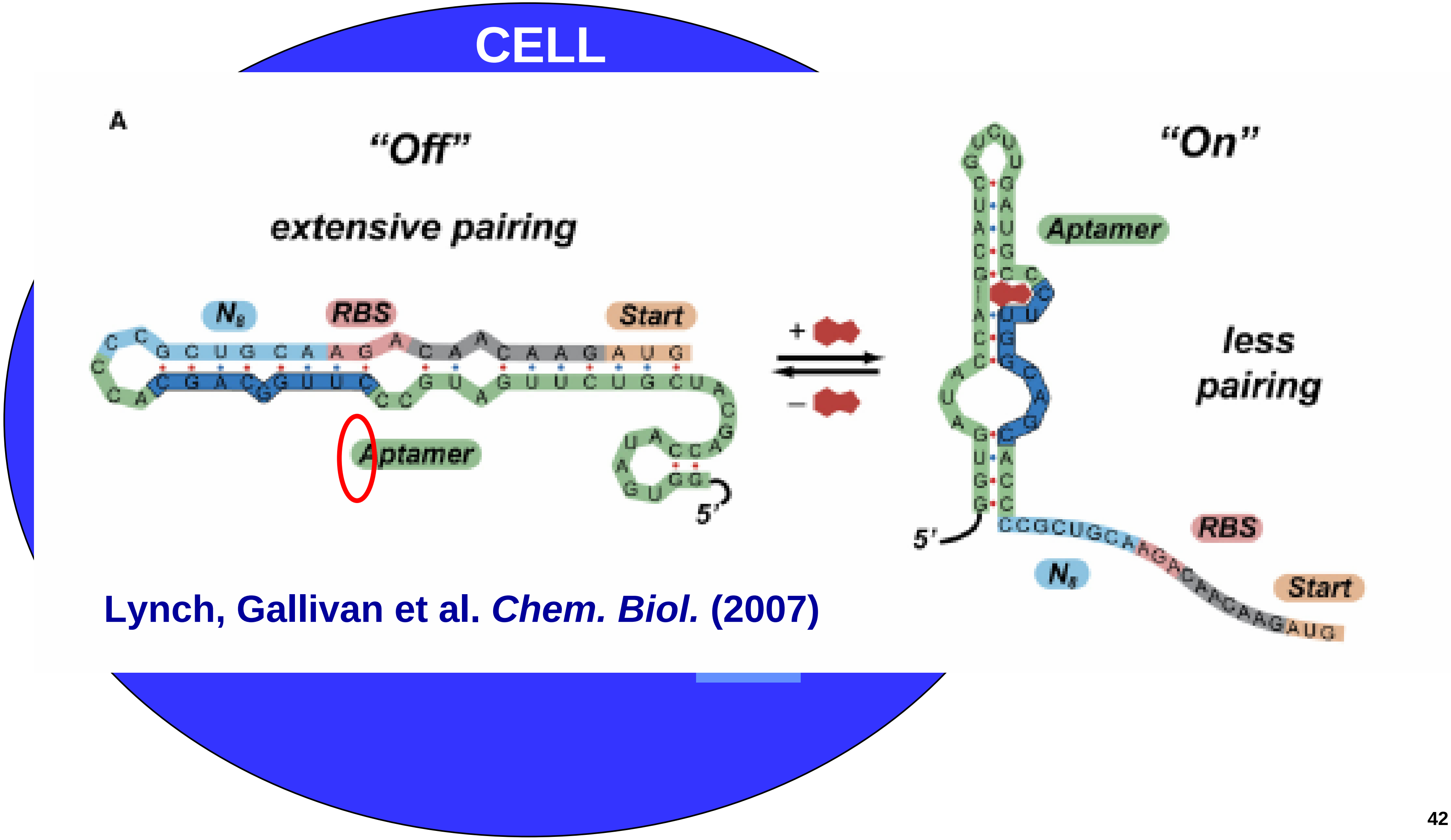
INTEGRATION



Packaging of genetic switches and reporter assay for device integration

Riboswitch Mode of Action: *Modularity*

Engineer Synthetic Riboswitch triggered by small molecule



Lynch, Gallivan et al. *Chem. Biol.* (2007)

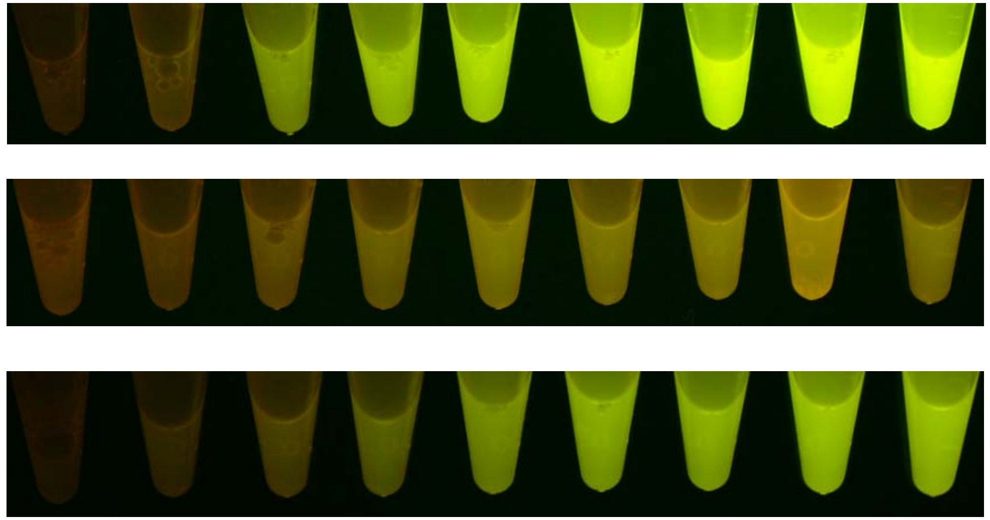
Cell-Based Sentinel

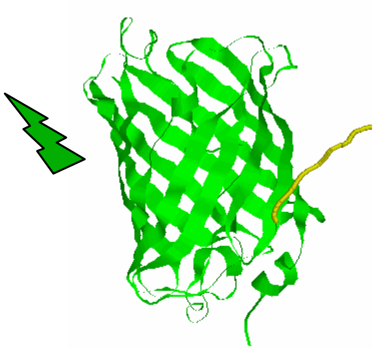
CELL LYSATES

Positive Control 0-8hrs

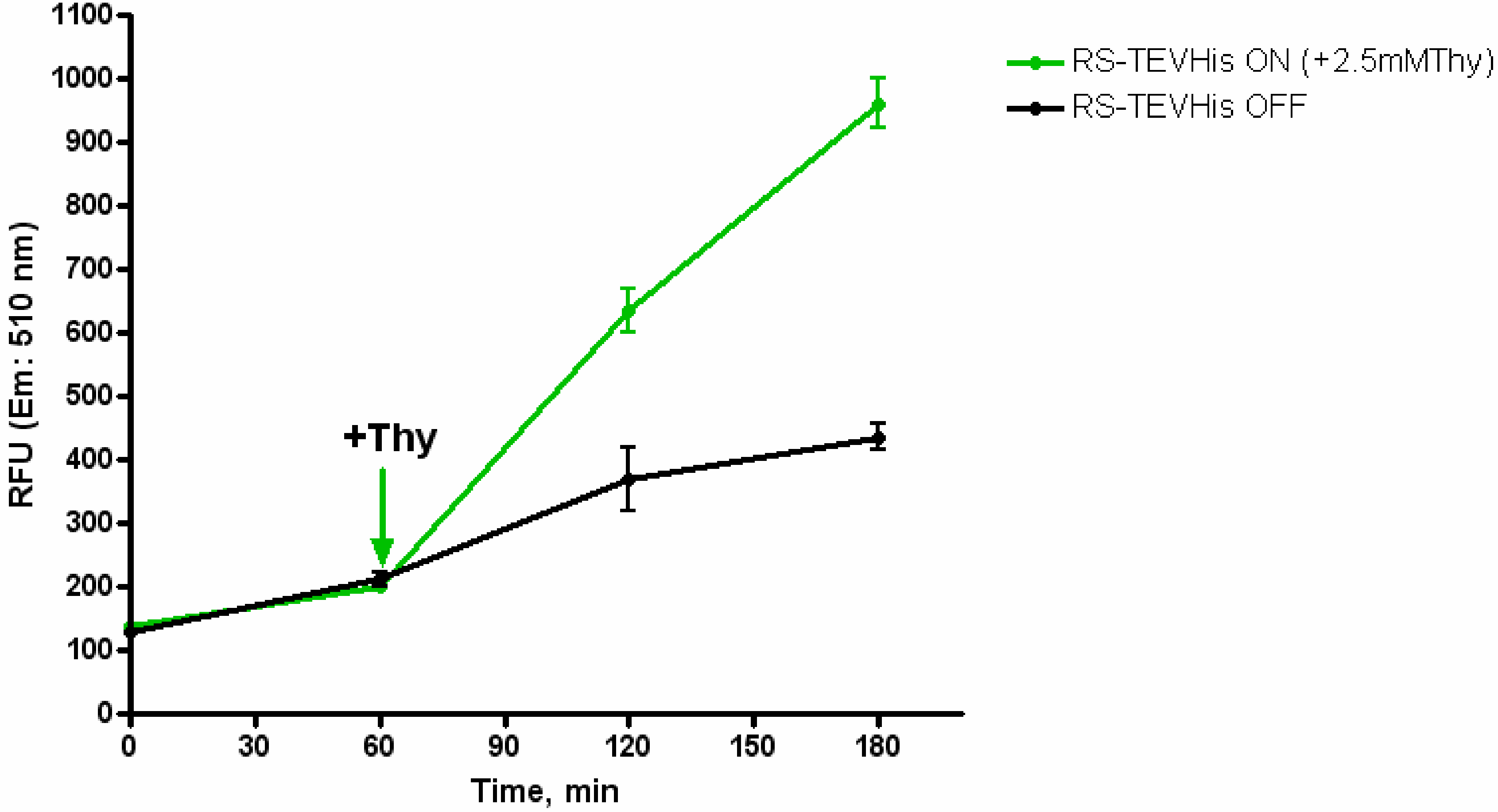
Riboswitch Off 0-8hrs

Riboswitch On 0-8hrs

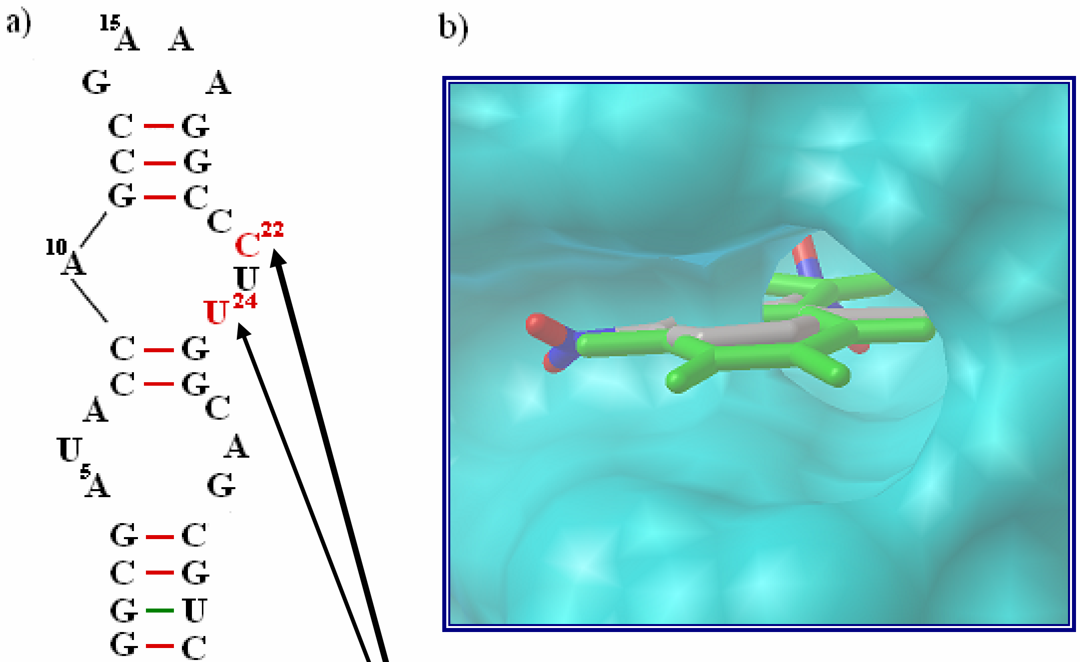




Cell-Based Sentinel Data



Autodock4* Results: Riboswitch Binding Pocket



Superimposition of 2,4-DNT and theophylline within aptamer binding pocket

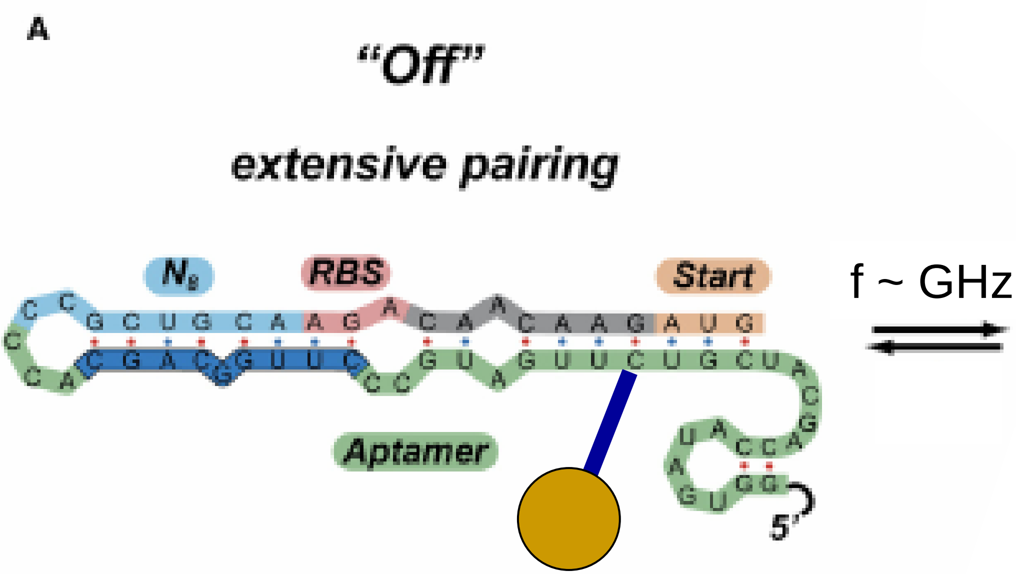
Critical role of cytosine and uracil at positions 22 and 24

Zimmermann et al., *Nature Struct. Biology*, 1997

Software - Autodock4 (Scripps Research Institute)
Huey, Morris, Olson and Goodsell, *J. Comput. Chem*. **2007**, *28*, 1145.

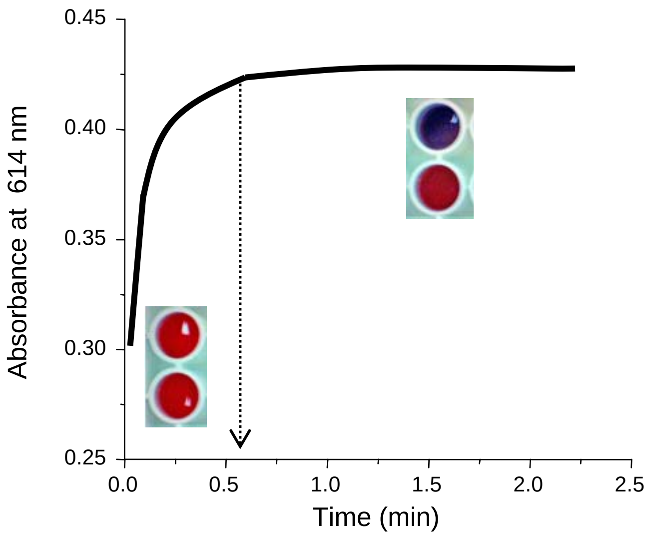
Riboswitch Mode of Action: *Remote Activation*

Engineer Synthetic Riboswitch triggered by ~~small molecule~~ external field

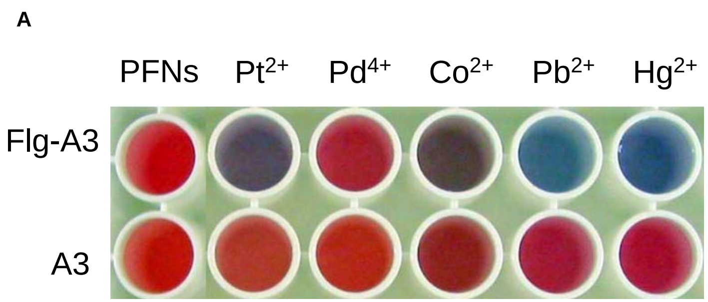


Response of PFN Sensor

Response Time



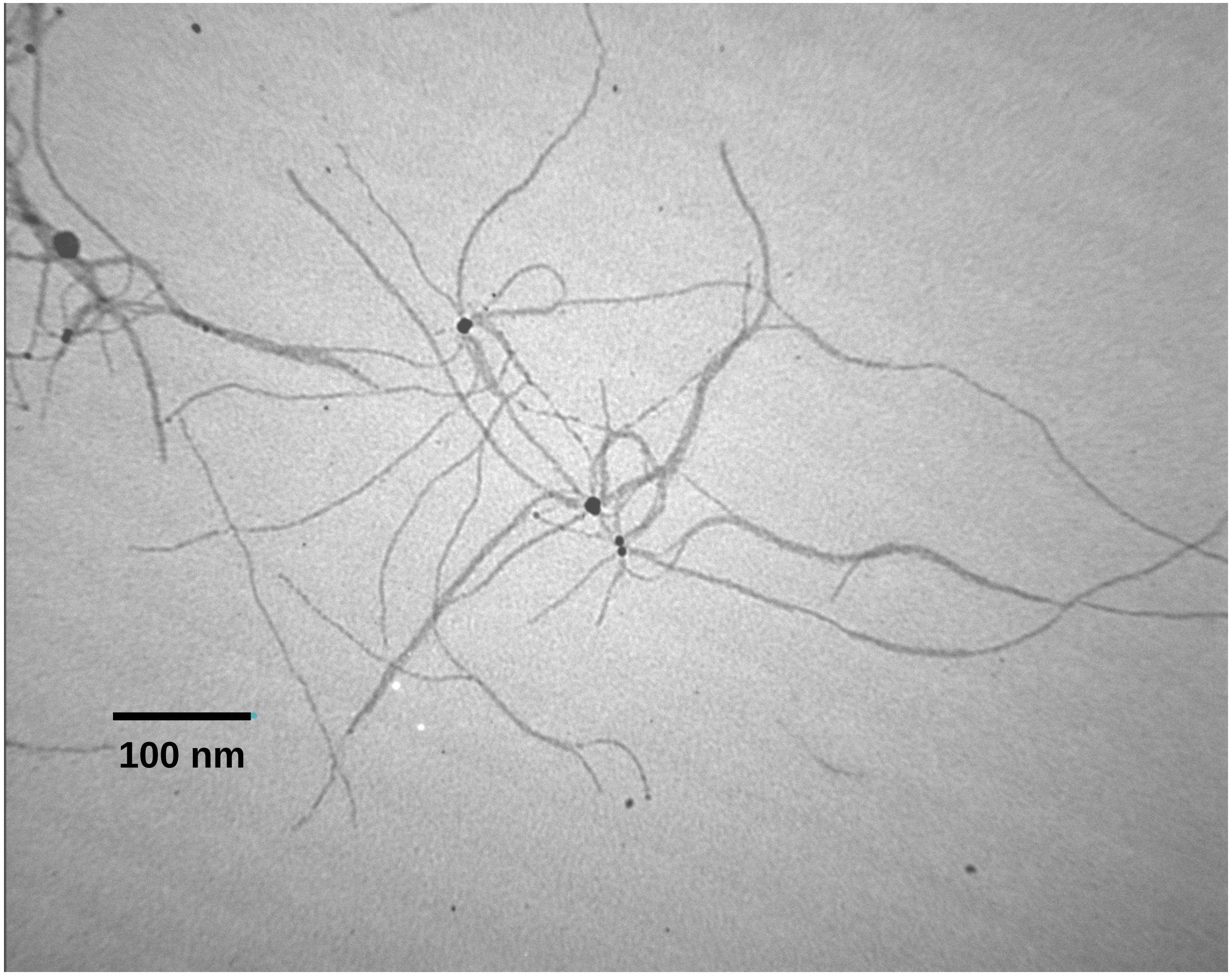
Flg Domain Effect



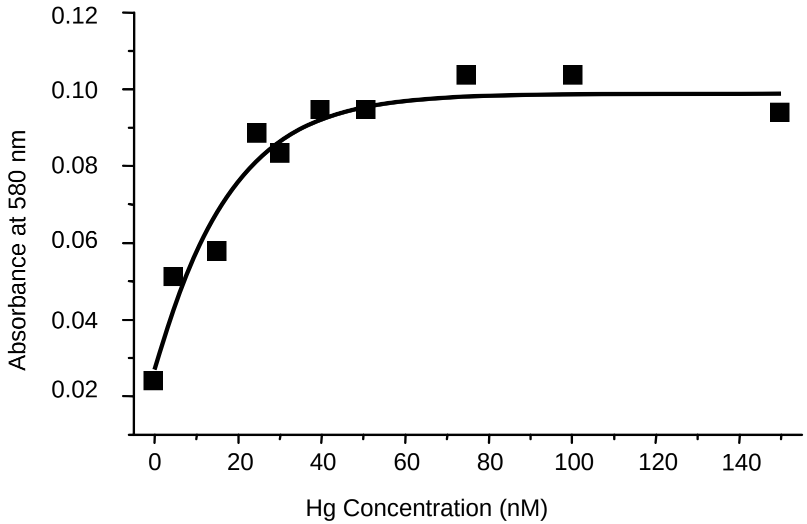
Small **(2007) In Press**

Phage Peptide Display (PD)

Low voltage TEM image of M13 phage selected for binding to Co/Pt nanoparticles



Peptide Functionalized NPs Detection Limit



Peptide Functionalized NPs

PFN + [M+]	Absorbance (nm)	Metal ion Conc.(nM)	Metal ion Conc. (ppb)
PFNs	539	NA	NA
Pt^{2+}	542	22.6 ± 0.1	4.5 ± 0.1
Pd^{4+}	617	31.0 ± 11.0	3.3 ± 1.2
Co^{2+}	593	191.4 ± 52.7	11.3 ± 3.1
Pb^{2+}	614	242.0 ± 8.6	50.2 ± 1.8
Hg^{2+}	580	26.4 ± 11.3	5.3 ± 2.2

	Recognition element	Metal ion	Conc.
Mirkin	DNA	Hg^{2+}	100 nM
Chang	DNAzyme	Pb^{2+}	400 nM
Lu	mercapto	Hg^{2+}	3 µM

1. Lu, *Chem. Mater.* **2004**, 3231.
2. Chang, *Chem. Commun.* **2007, 1215.**
3. Mirkin, *Angew. Chem. Int. Ed.* **2007**, 4093