MURI-Funded Scientific and Technological Blockbusters from Northwestern University

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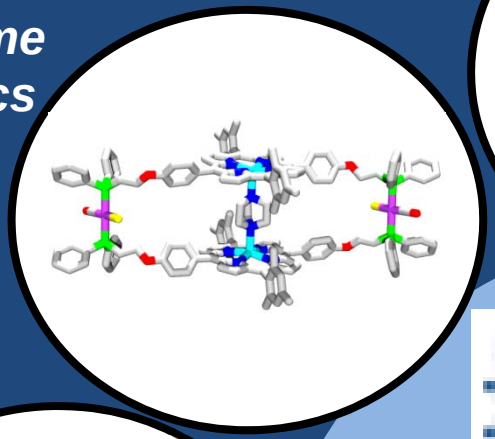
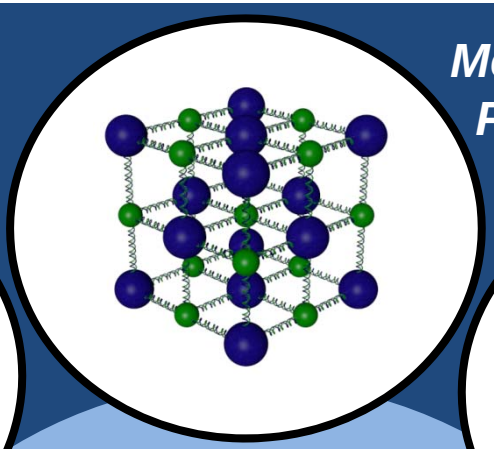
MURI Support at Northwestern University

- **MURI-00**: Surface Templated Bio-Inspired Synthesis and Fabrication of Functional Materials (F49620-00-1-0283/P01, 2000-2006)
- **DURINT-01**: Ultrasensitive and Selective Chip Based Detection of DNA (F49620-01-1-0401, 2001-2007)
- **MURI-04**: Biomechanical Interfaces for Cell-based Microsystems (W911NF-04-1-0171, 2004-2009)
- **MURI-07**: Bio-inspired Supramolecular Enzymatic Systems (FA9550-07-1-0534, 2007-2012)
- **MURI-11**: Bioprogrammable One-, Two-, and Three-Dimensional Materials (FA9550-11-1-0275, 2011-2014)
- **MURI-11**: Conductive DNA Systems and Molecular Devices (N00014-11-1-0729, 2011-2014)

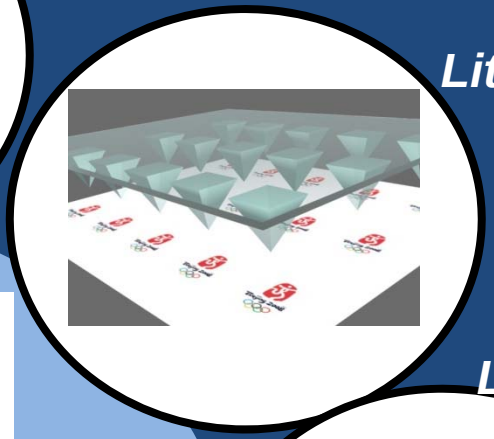
Supramolecular Enzyme Mimics



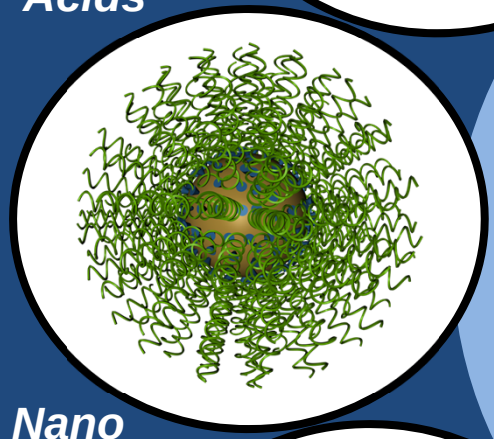
Metal-Organic Frameworks



Polymer Pen Lithography



Spherical Nucleic Acids



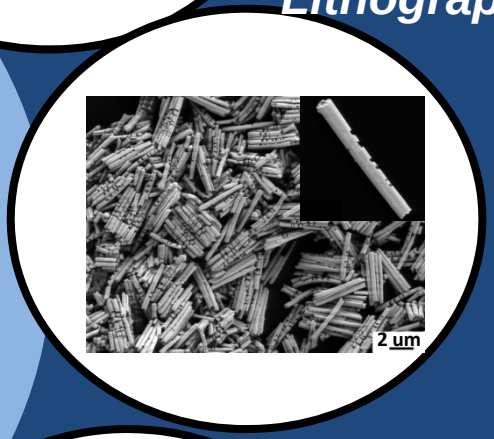
DDRE

AIR FORCE OFFICE OF SCIENTIFIC RESEARCH

MURI

AIR FORCE RESEARCH LABORATORY

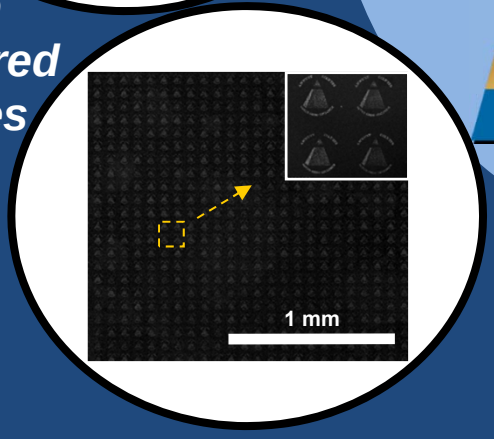
On-Wire Lithography



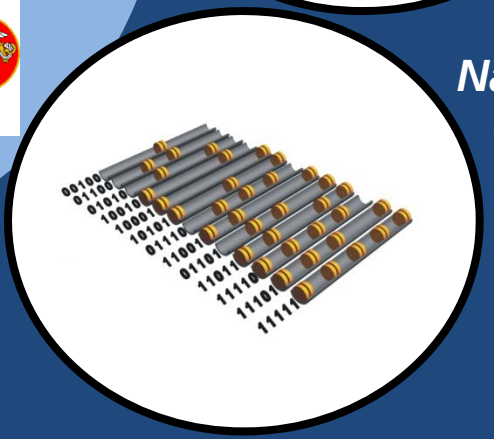
ARL

OFFICE OF NAVAL RESEARCH ONR

Nano Structured Surfaces



NanoDisk Codes



MURI-00: Surface-Templated, Bio-Inspired Synthesis and Fabrication of Functional Materials

Team

- Program Manager: H. DeLong
- NU
  - C. Mirkin, V. Chandrasekhar, V. Dravid, R. Letsinger, G. Schatz, S. Stupp, D. Ginger
- Harold Washington
  - T. Higgins
- Tufts
  - D. Kaplan
- Scripps
  - M. Ghadiri
- Perkin Elmer Applied Biosystems
  - E. Mayrand
- Lucent Technologies
  - P. Wiltzius
- DoD Labs
  - Valdes, Stone, Naik

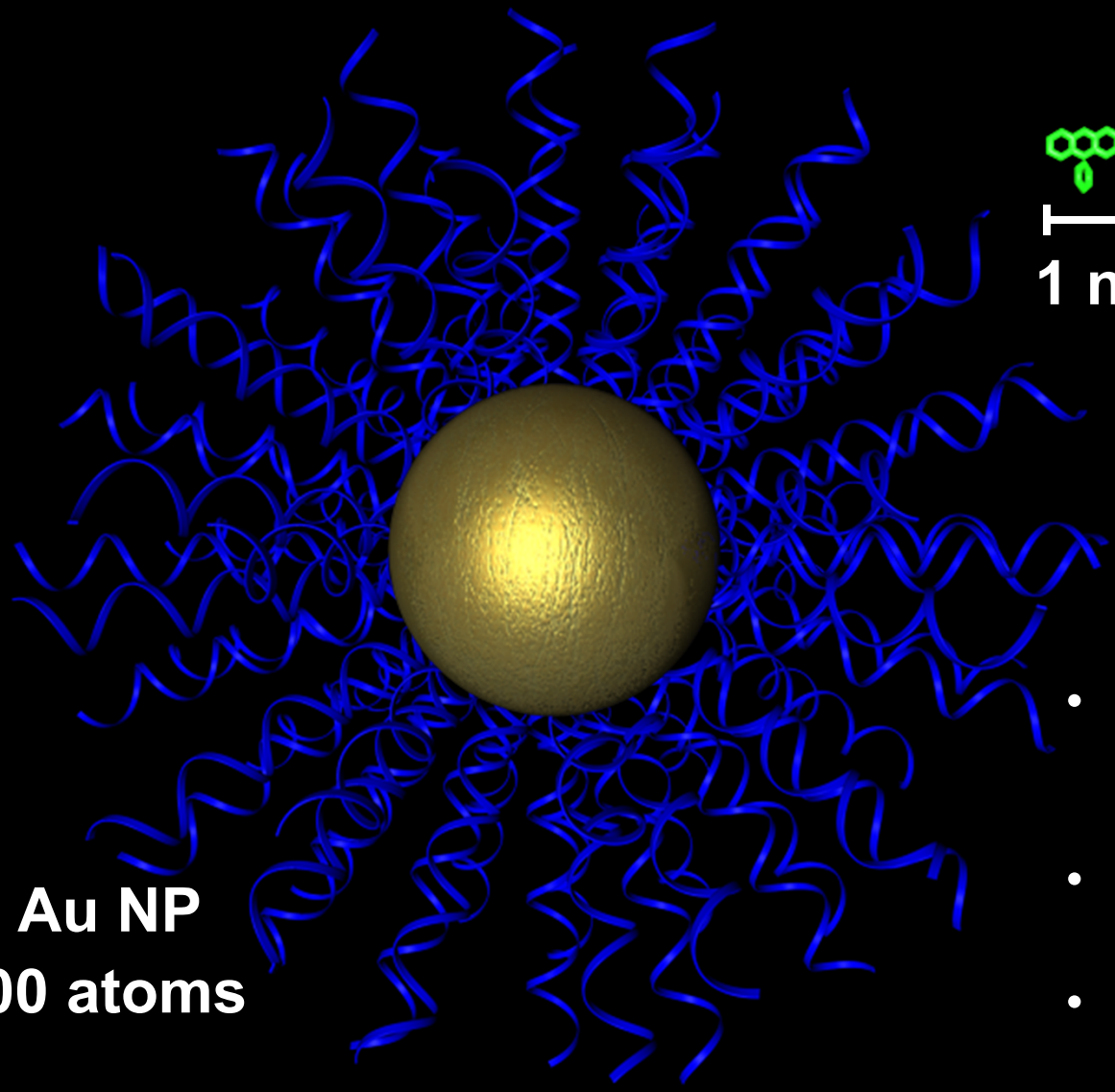
Goals

- Establish rules that can be used in 2D and 3D assembly of biomolecules
- Merge solution phase assembly with DPN
- Develop computational tools to predict the properties of assembled nanostructures

Outcome

- *Design rules for assembling particles into colloidal crystals with pre-conceived structures*
- *An understanding of the fundamental factors that control molecular transport from tip-based scanning probes*

Spherical Nucleic Acid Nanostructures



Fluorescein

37 atoms

1 nm

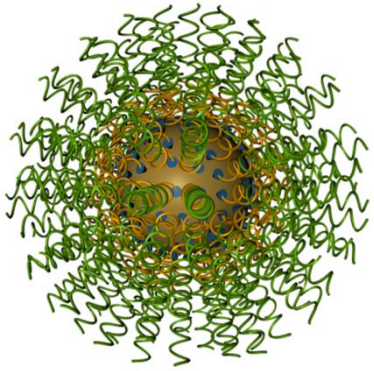
40-mer
Oligonucleotide
1400 atoms

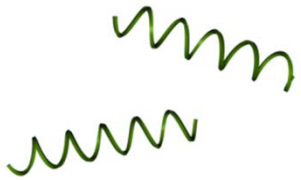
- Synthetically Programmable Recognition
- Multivalency and Multi-functionality
- New Properties: Cooperative binding, Catalysis

13 nm Au NP
~67,500 atoms

Mirkin *et al, Nature* 1996 (382) 607-609

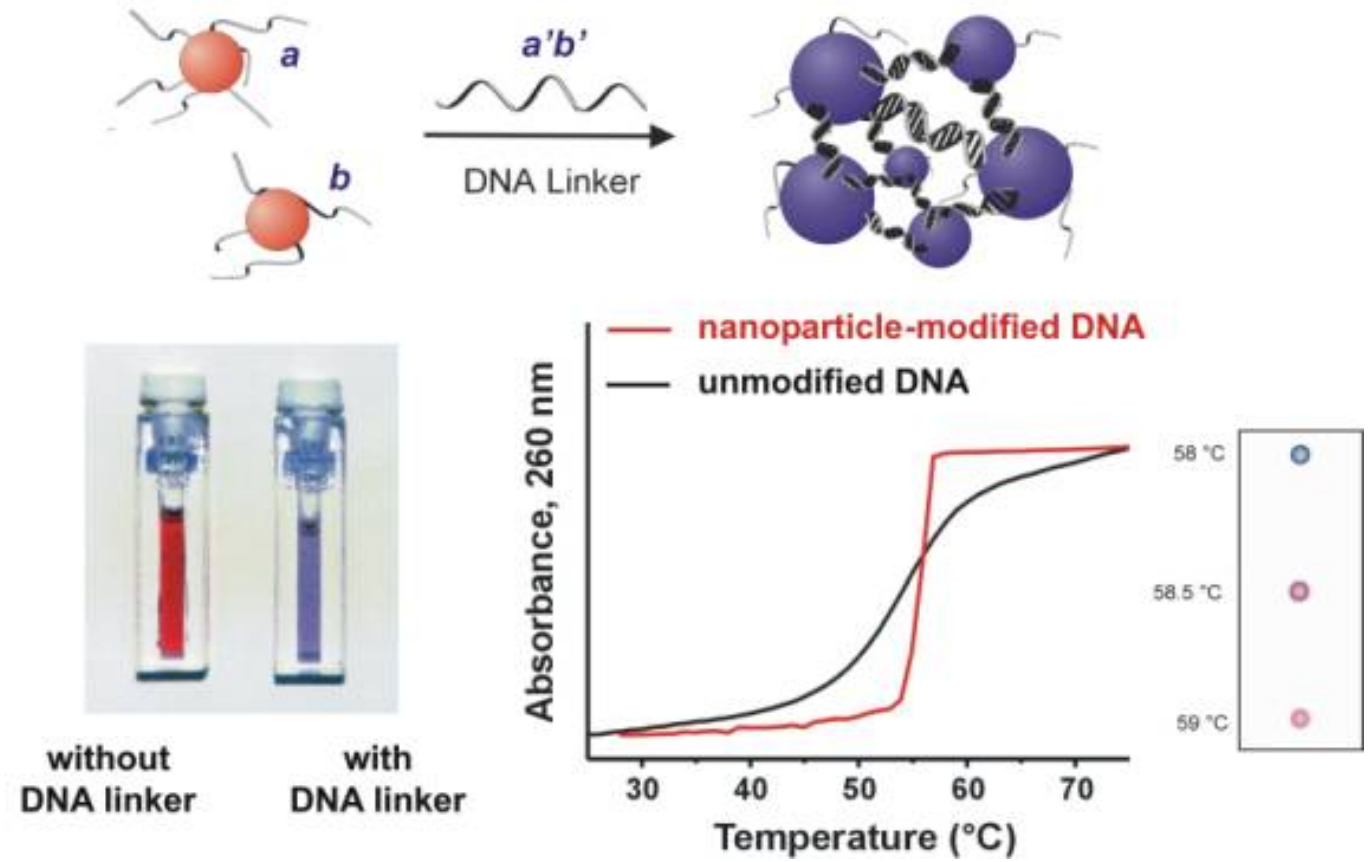
SNAs Have Unique Properties Distinct From Their Linear Counterparts





Property	Spherical Nucleic Acids	Linear Nucleic Acids
Melting Transition	Cooperative and Narrow (~2-8ºC)	Broad (~20ºC)
Cellular Uptake	Transfection agents NOT required	Lipofectamine™, Dharmafect™, etc
Immune Response	Minimal	Elevated Interferon-β
Stability	Resistance to Nucleases	Rapid Degradation
Inorganic Core's	Plasmonic, Catalytic, Magnetic, Luminescent	N/A
Binding Strength	$K_{eq} = 1.8 \times 10^{14}$	$K_{eq} = 1.8 \times 10^{12}$

Properties of Hybridized Nanoparticle Probes



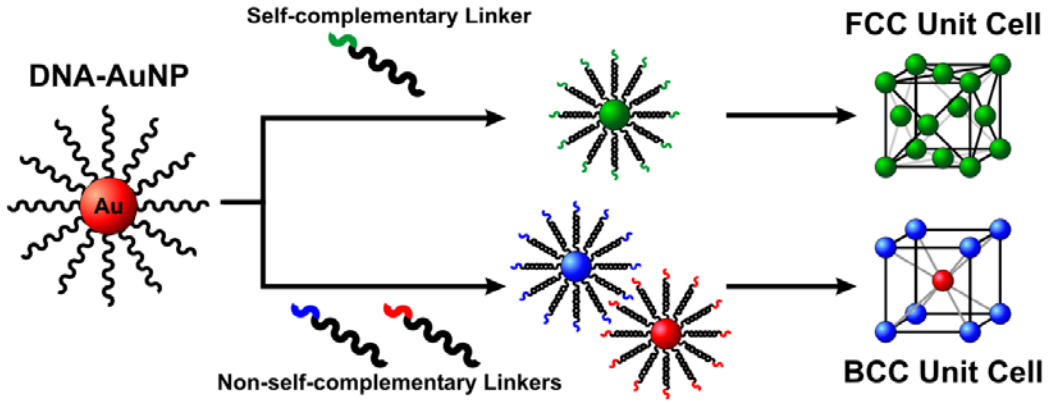
Color: Hybridized aggregates of DNA functionalized Au nanoparticles show distinct color changes in their hybridized (purple) and unhybridized (red) forms.

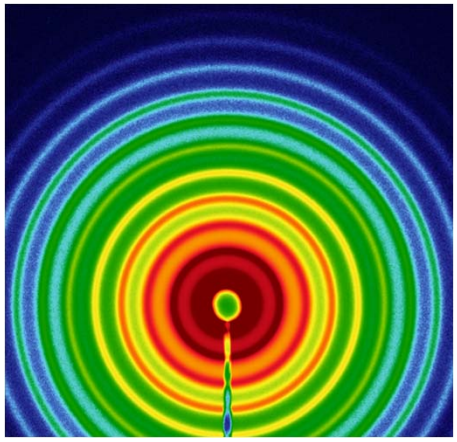
Cooperativity: Hybridized aggregates of DNA functionalized Au nanoparticles show sharper melting transitions than the same DNA duplex free in solution.

Mirkin *et al, Nature* 1996, Elghanian, R. *et al, Science,* 1997

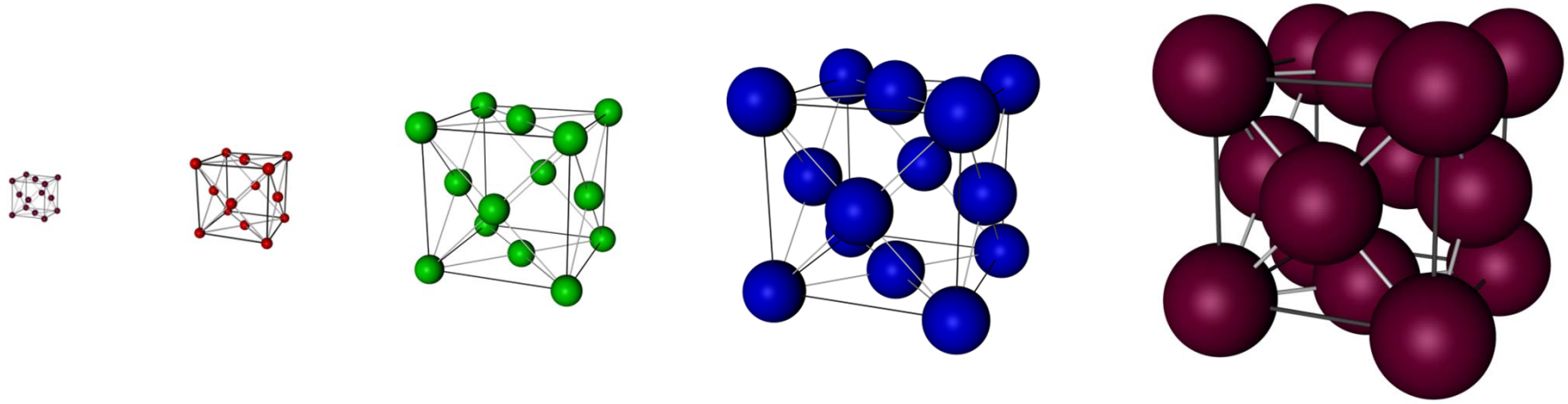
DNA-Programmable Nanoparticle Crystallization

- DNA guides the assembly of the same inorganic particle into different crystalline states
- Solution based
- Crystallization driven by maximizing hybridization interactions
- Independently tailorable design parameters (NP size, interparticle distance, crystallographic symmetry)

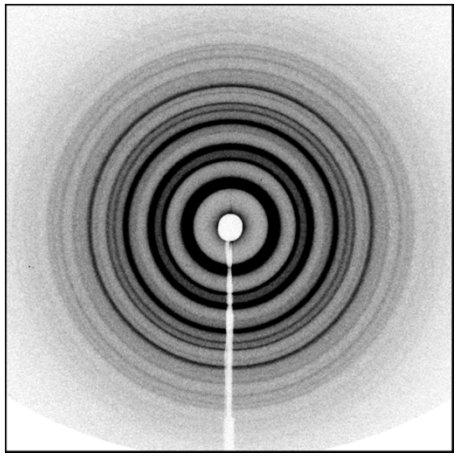




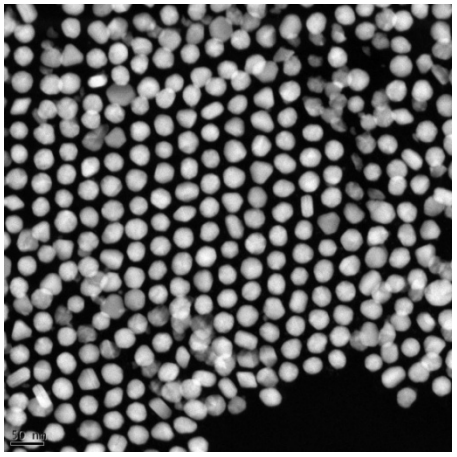
Crystallization Over an Order of Magnitude of Sizes



5 nm → Diameter of NPs Crystallized → 80 nm



5 nm AuNPs, 38 nm DNA

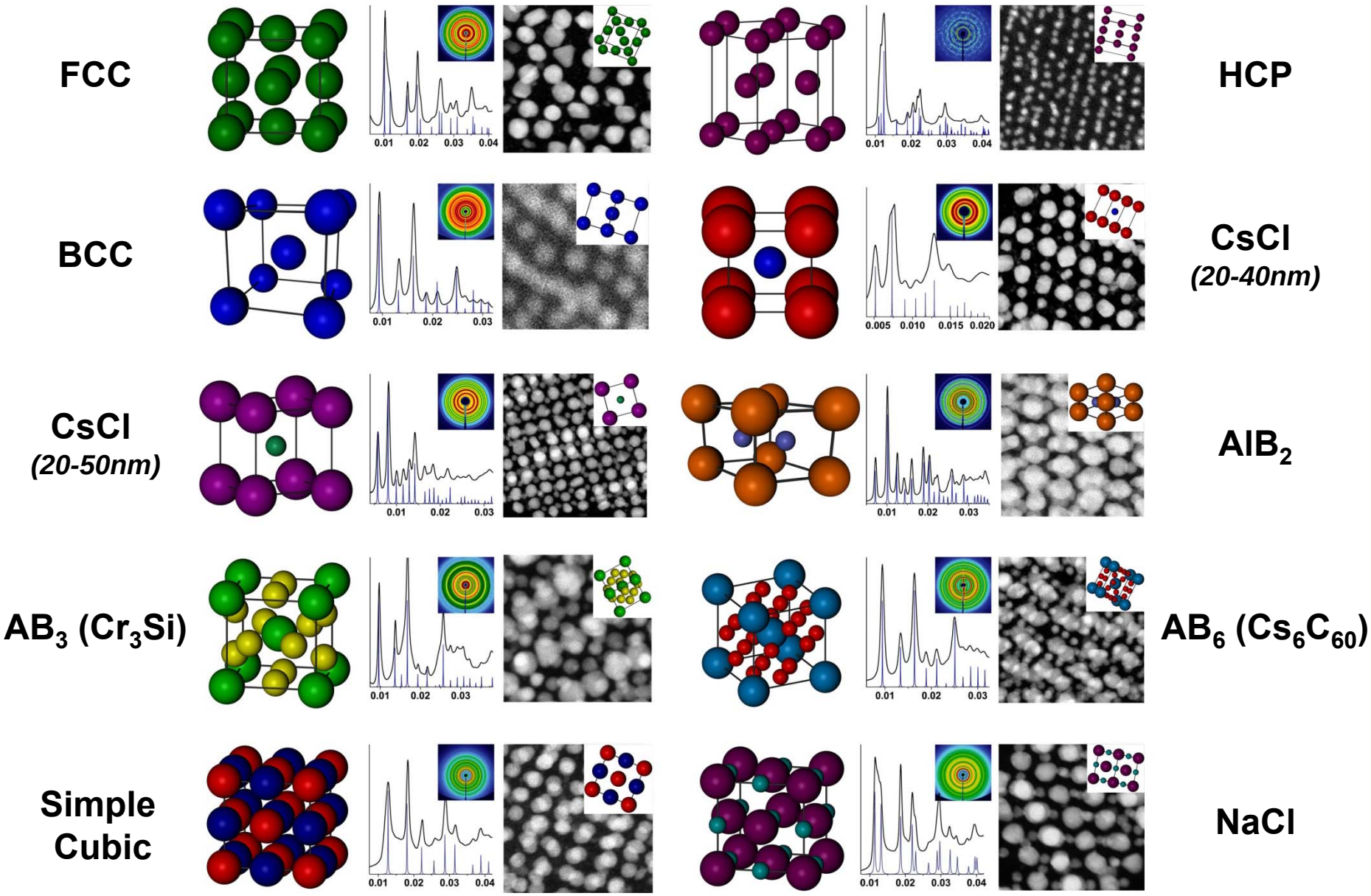


30nm NPs, [111] axis
Unit Cell Edge Length
~100 nm
(Before embedding)

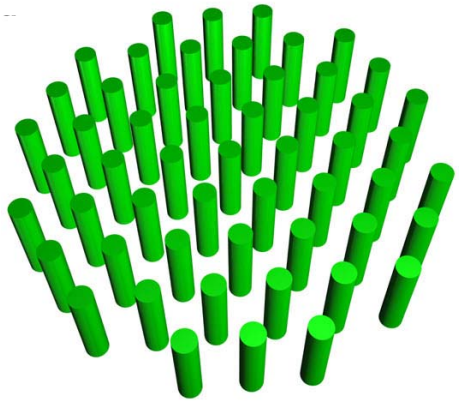
Diameters of NPs:
5 nm – 80 nm
Crystal Lattice Parameters:
25 nm – 225 nm
Average Crystal Size: 1.5 μm

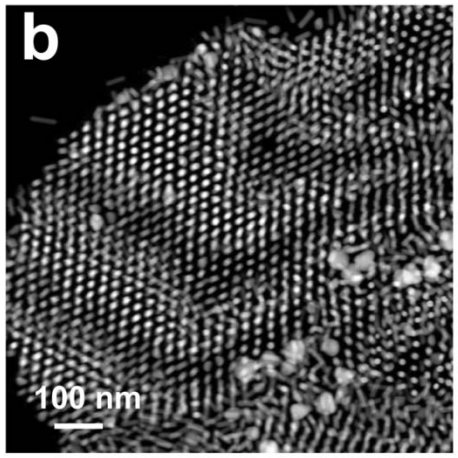
Macfarlane et. al., *Ange. Chemie Int. Ed.* 2010, *49*, 4589

Different Crystallographic Symmetries

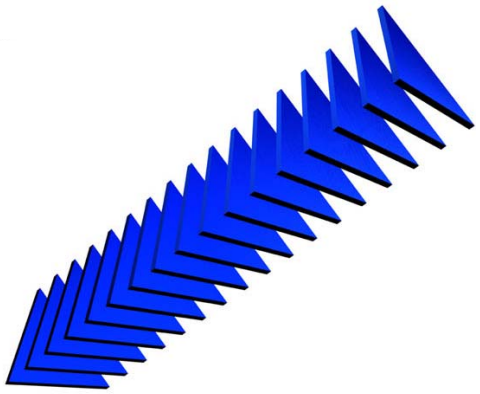


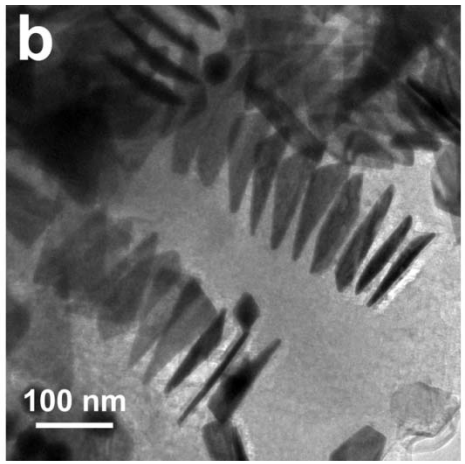
Anisotropic Particle Assembly: Introducing Valency Into the Process



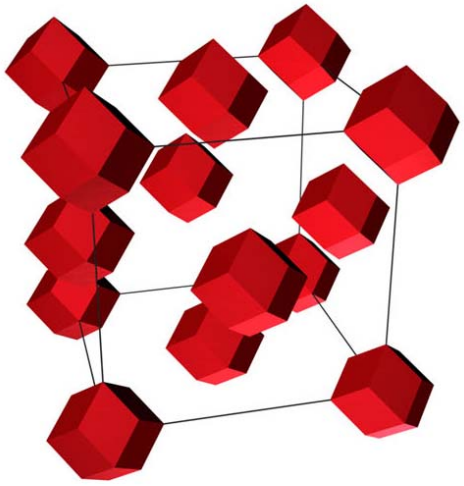


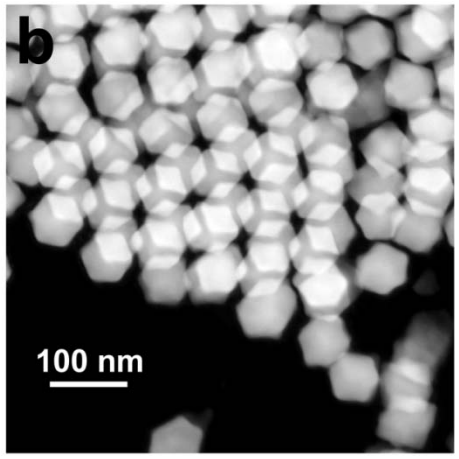
Nanorods ("1D" Structures) form 2D Hexagonal Arrays



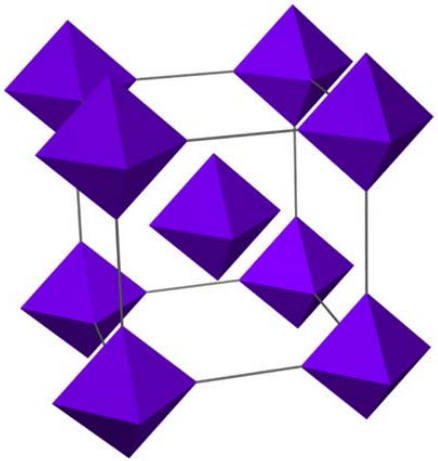


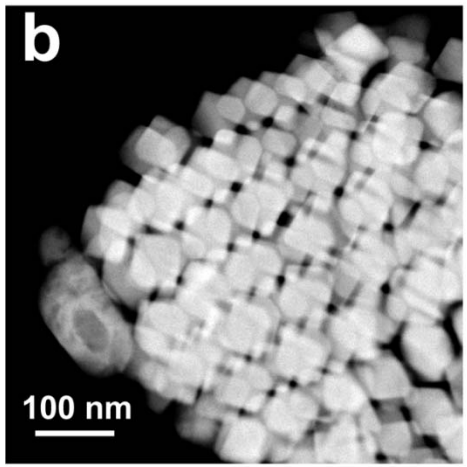
Nanoprisms ("2D" Structures) form Linear 1D Arrays





Rhombic Dodecahedra form FCC Lattices





Octahedra can form BCC or FCC Lattices Depending on DNA Length

MURI-04: Biomechanical Interfaces for Cell-Based Microsystems

Team

- **Program Manager:**
  B. LaMattina (ARO)
- **University of Chicago**
  - M. Mrksich, A. Dinner
- **NU**
  - C. Mirkin
- **CalTech**
  - M. Roukes
- **University of Pennsylvania**
  - C. Chen
- **UCSB**
  - A. Evans, R. McMeeking

DoD Labs
L. Whitman, M. Stone

Goals

- Develop an integrated platform for installing mechanical and chemical interfaces to cells.
- Employ platform in investigating chemo-mechanical signatures and actuation of cellular behavior.
- Prototype cell-based devices with high impact for the DoD.

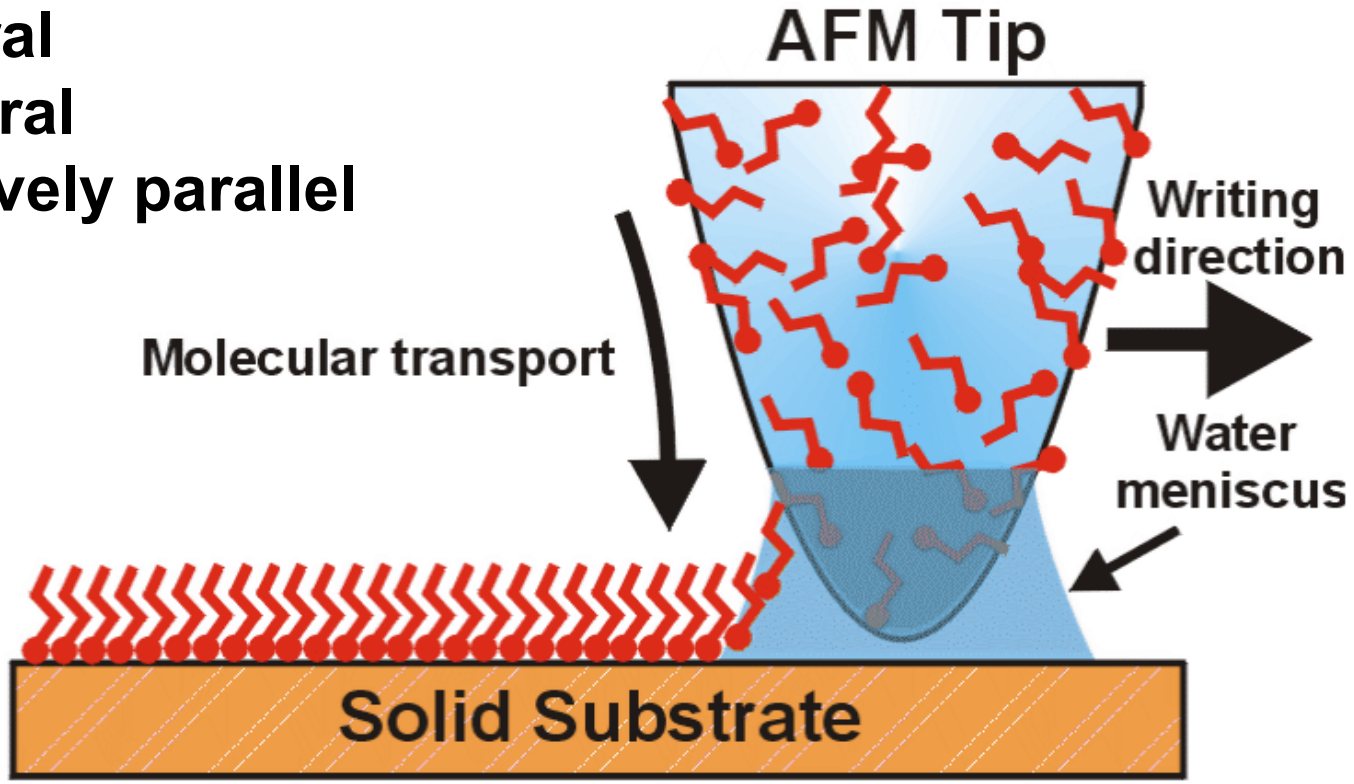
Outcome

- *An understanding of how to use scanning probe molecular printing techniques to reconstruct models of extracellular matrices.*
- *Unprecedented ability to manipulate individual biological entities for cell based technologies.*

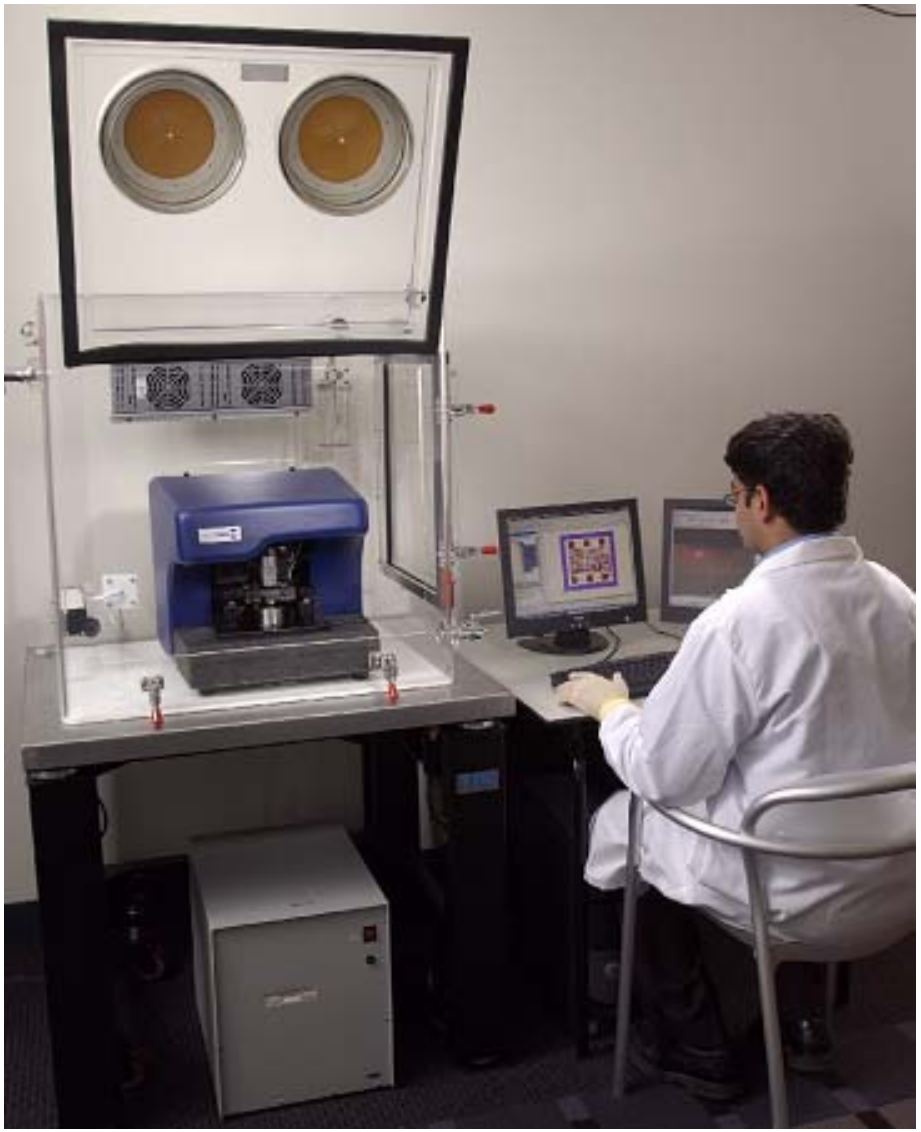
Dip Pen Nanolithography (DPN)

Attributes of DPN:

- Direct-write
- High resolution: 10 nm line width, ~5 nm spatial resolution
- Positive printing
- Writing and imaging with same tool
- Molecule general
- Substrate general
- Serial or massively parallel



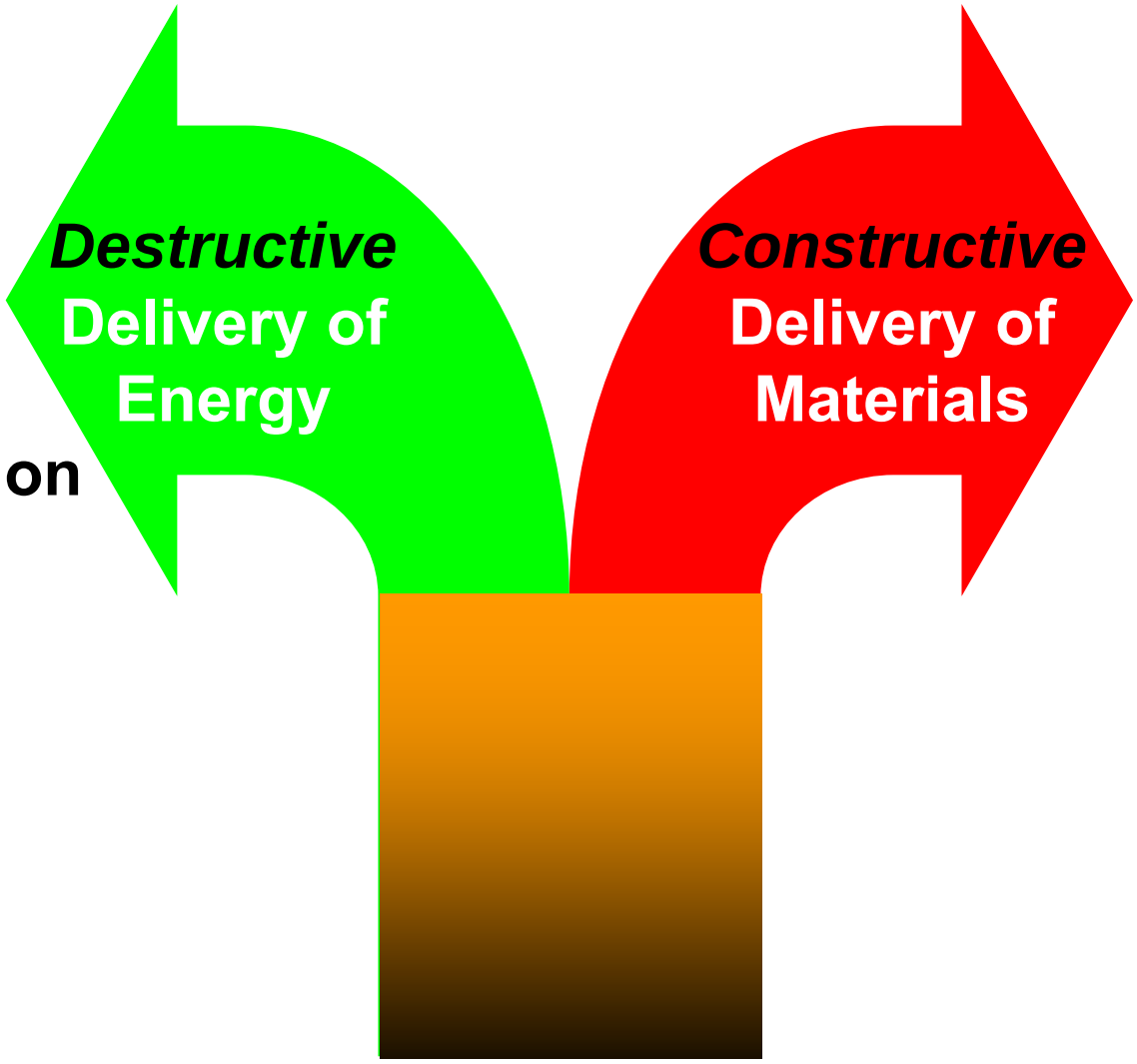
The NSCRIPTOR™
An Integrated DPN System



NanoINK INC.

Scanning Probe Lithography: A Dichotomy is Emerging

Nanografting
Nanoshaving
Anodic Oxidation
"Millipede"



DPN

Development of Writing & Printing Tools

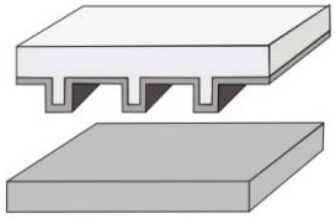
Parallel Printing

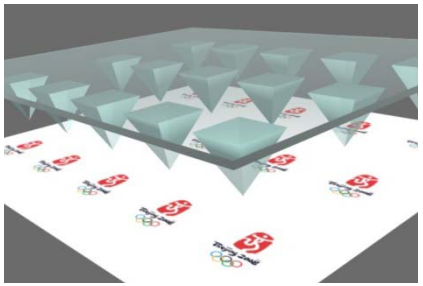
Woodblock Printing
(China ~200)

Printing Press
(Gutenberg, 1439)

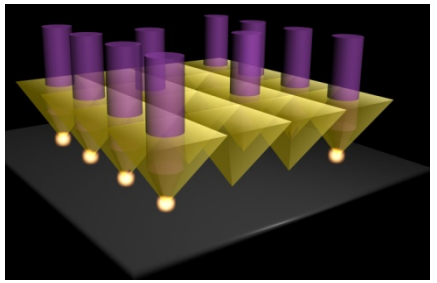
Movable Type
(Bi Sheng, ~1041-1048)

μ-Contact Printing
(Whitesides, 1993)





Polymer Pen Lithography (PPL)
(2008)



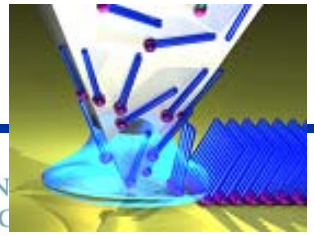
Beam Pen Lithography (BPL)
(2010)

Serial Writing

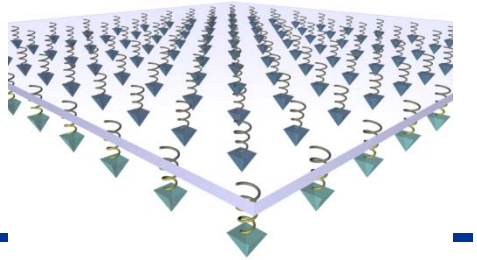
Quill Pen
(~2000 BC)

Ball-Point
(Loud, 1888)

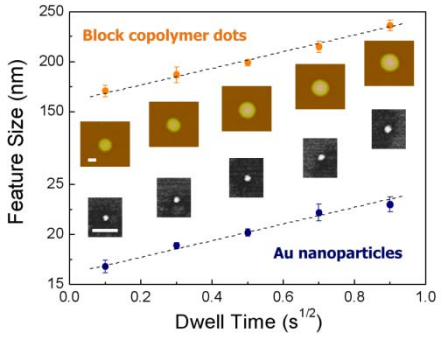
Dip-Pen Nanolithography (DPN)
(Mirkin, 1999)



Hard Tip, Soft Spring Lithography
(2010)



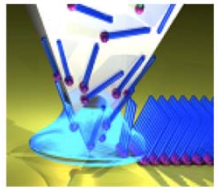
Scanning Probe Block Copolymer Lithography
(2010)



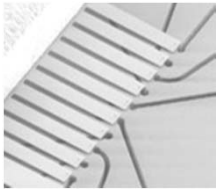
NTERNATION
OR NANOTEC
orthwestern University

Scanning Probe Molecular Printing

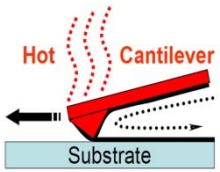
Cantilever-Based



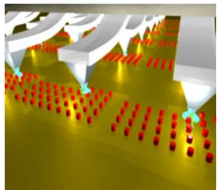
DPN (1999)



1-D Multipen Cantilever Array (2000)

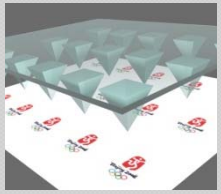


Thermal DPN (2004)

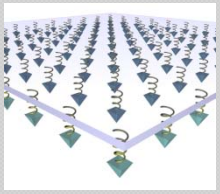


2-D 55,000 Pen Cantilever Array (2006)

Cantilever-Free



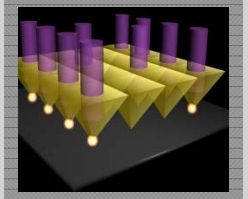
Polymer Pen Lithography (2008)



Hard Tip, Soft Spring Lithography (2011)

Key Advance 1: Deposition of materials (through a meniscus) rather than energy

Key Advance 2: Move the "spring" in a cantilever to an elastomeric pyramid on a solid backing for cantilever-free printing

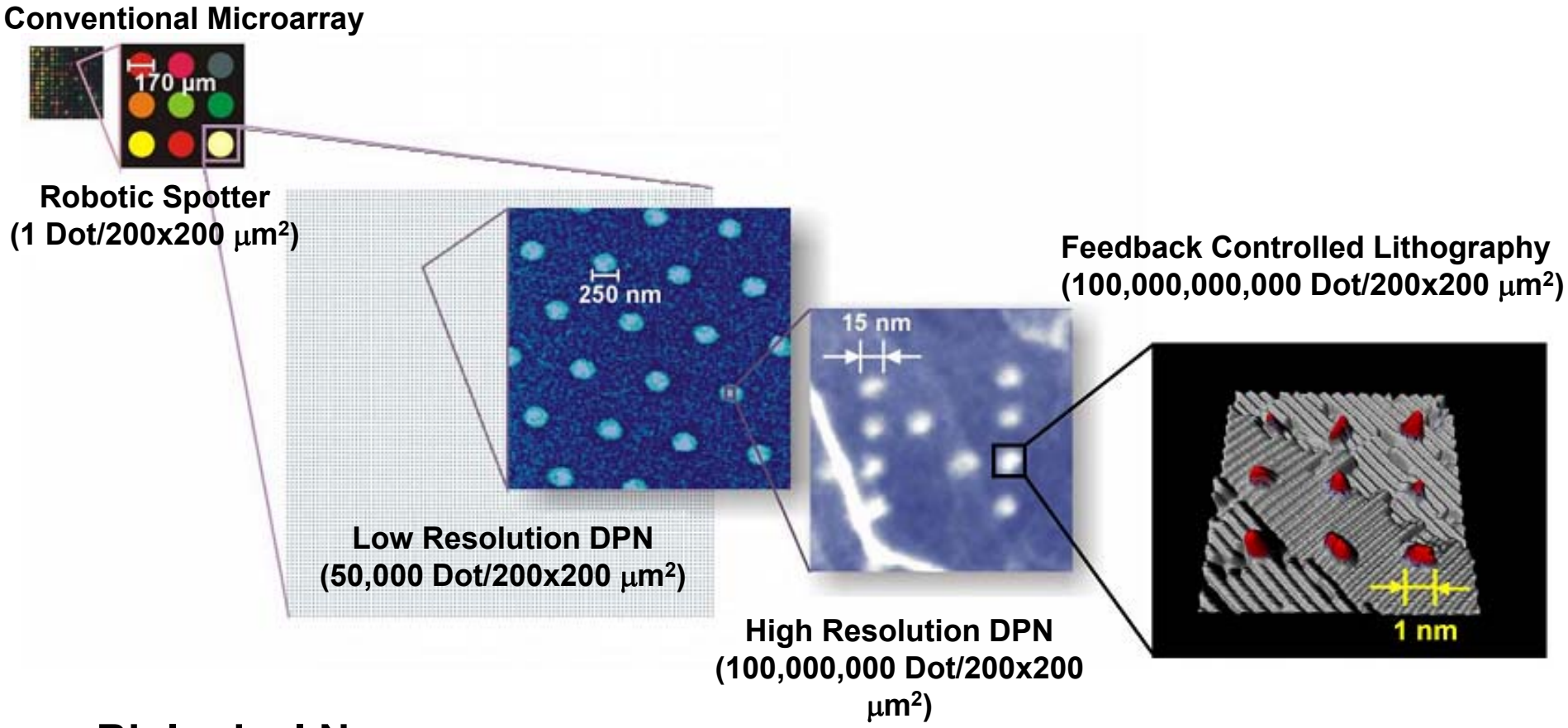


Beam Pen Lithography (2010)

Key Advance 3: Move the "spring" from the tip (in PPL) to a polymer backing layer

Giam, LR and Mirkin, CA. *Angew. Chem*. 2011. *50*, 7482.

The Ultimate in High Density Arrays

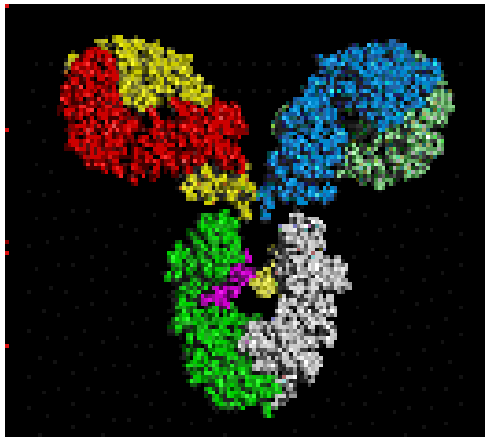


Biological Nanoarrays:

- More than just miniaturization with higher density
- New opportunities for biodetection and studying biorecognition
- Templates for guiding the assembly of larger building blocks
- Open up the opportunity to study multivalency and surface cooperativity

Can DPN be Used To Generate Multicomponent Templates that are Used to Recognize and Larger Biological structures and Organisms?

8.5 nm

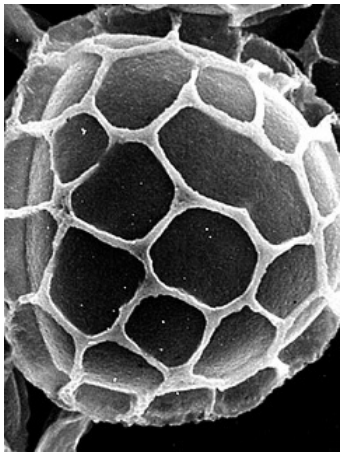


Protein
(Human IgG)

120 nm

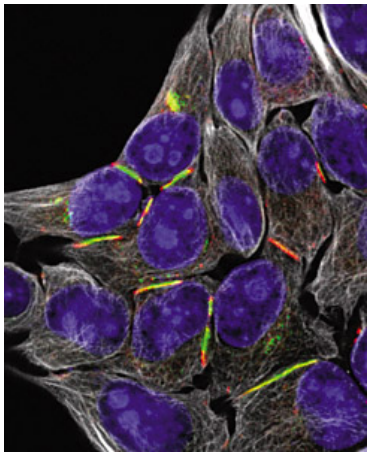
Virus
(HIV)

~20 µm



Spores
(Anthrax)

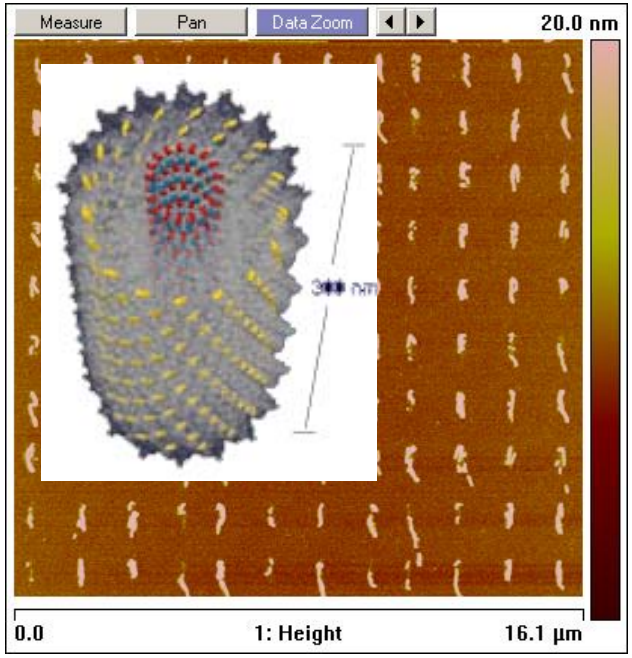
~15 µm

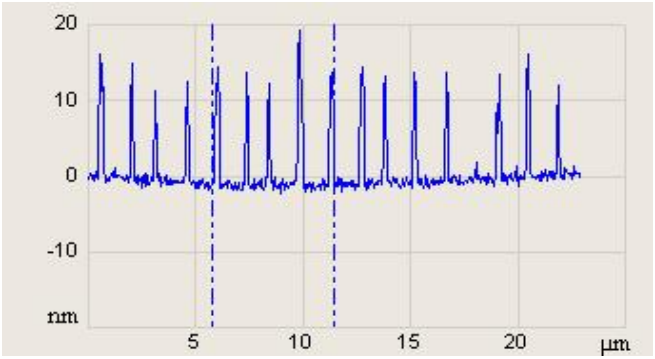


Living Cells

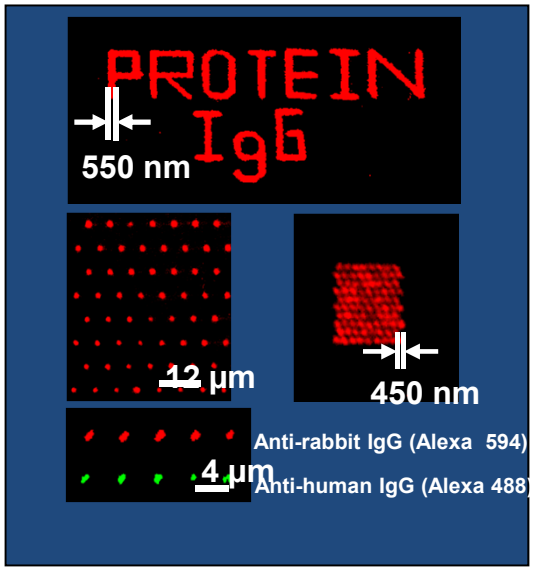
Patterning of Biological Structures

Viruses (TMV)

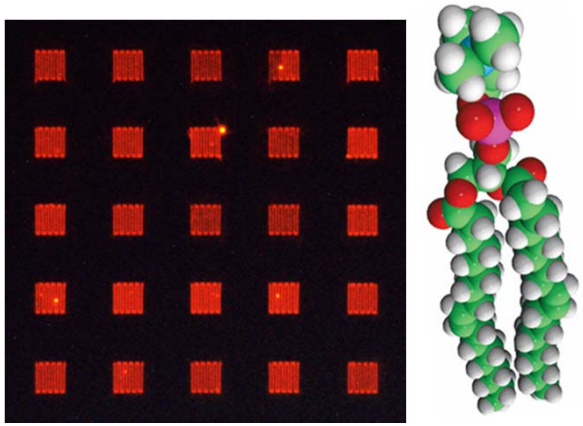




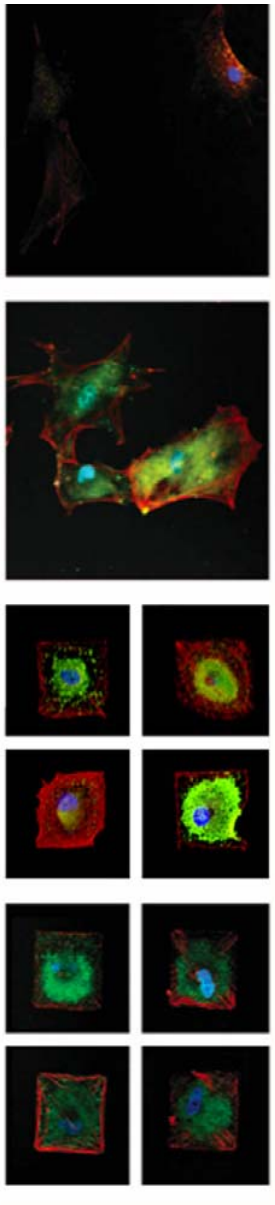
Proteins



Lipids



Cells



DURINT-01: Ultrasensitive and Selective Chip Based Detection of DNA

Team

- Program Manager: H. DeLong
- NU
  - C. Mirkin, M. Ratner, A. Baron, C. Liu, G. Schatz
- DoD Labs: J. Valdes, M. Goode, M. Stone

Outcomes

- *Design and creation of novel chip-based detection platforms for the detection of DNA, proteins and peptides that are currently being commercialized by Nanosphere, Inc. and AuraSense, LLC.*

Goals

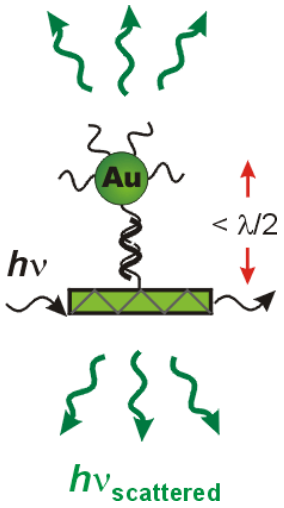
- Develop understanding of nanoparticle-based sensors for DNA
- Engineer chip-based detection platforms
- Design and interface target isolation and purification to integrate DNA analysis systems
- Create chip based detection strategies for rapid identification of biological warfare agents

The Properties of Spherical Nucleic Acid (SNA) Nanoparticle Conjugates

Optical

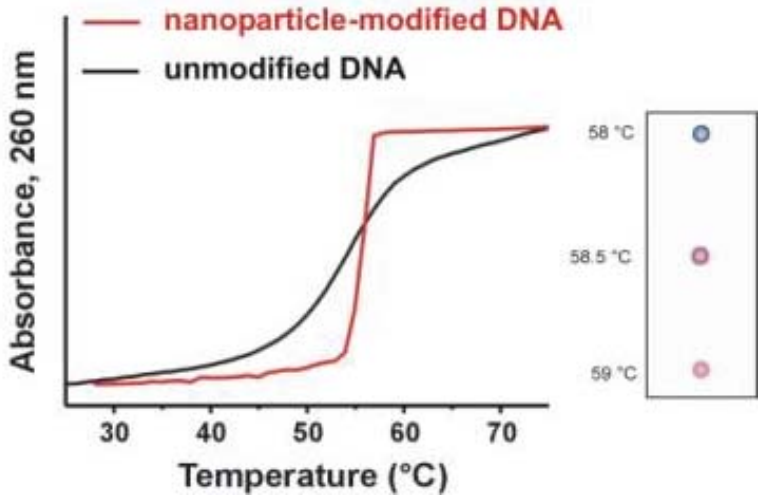
Nature, 1996, Science, 1997

Plasmonic



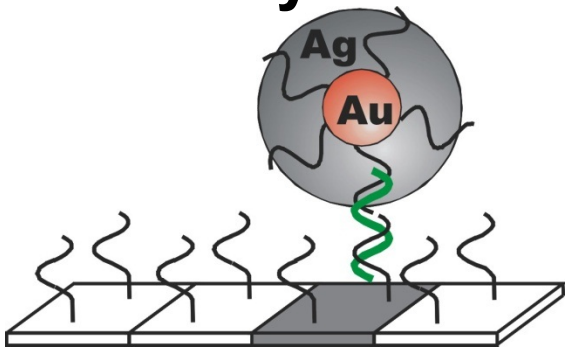
JACS, 2000

Cooperative Binding



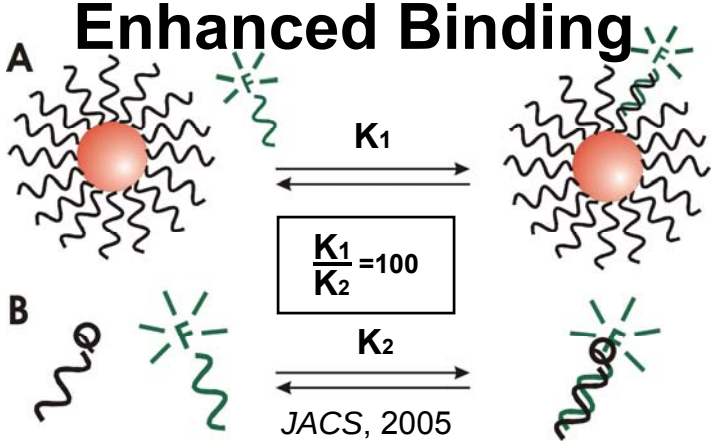
Nature, 1996, Science, 1997

Catalytic



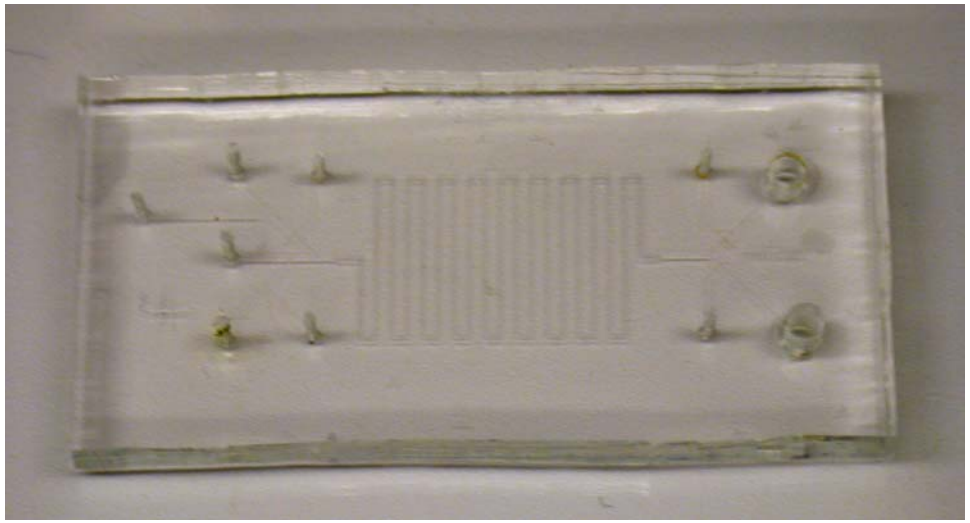
Science, 2000

Enhanced Binding



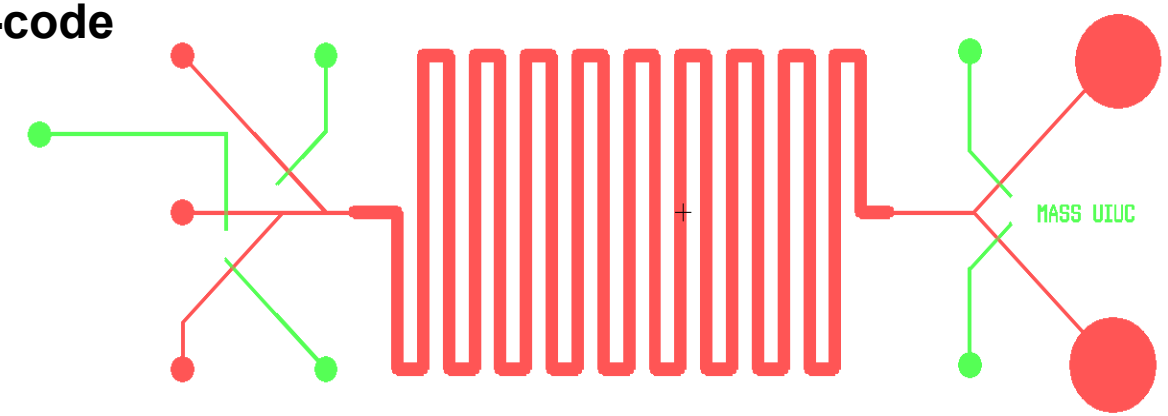
JACS, 2005

Chip-Based Bio-bar-code Assay



Several iterations of protocol development were performed to adapt the standard bio-bar-code assay to micro-channels

- PDMS microfluidic chip on a glass substrate
- A magnet is placed under the chip to immobilize magnetic micro-particles

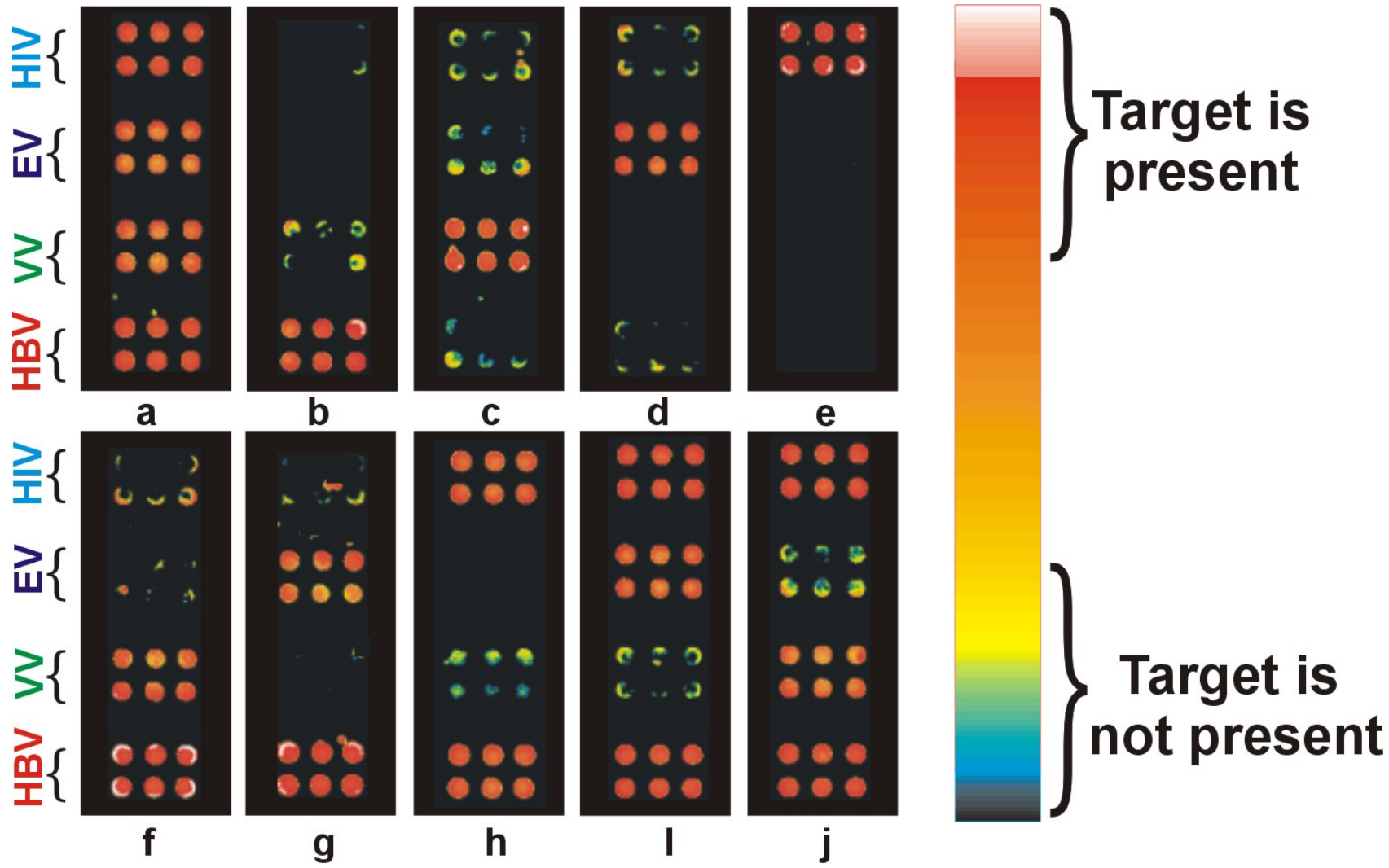


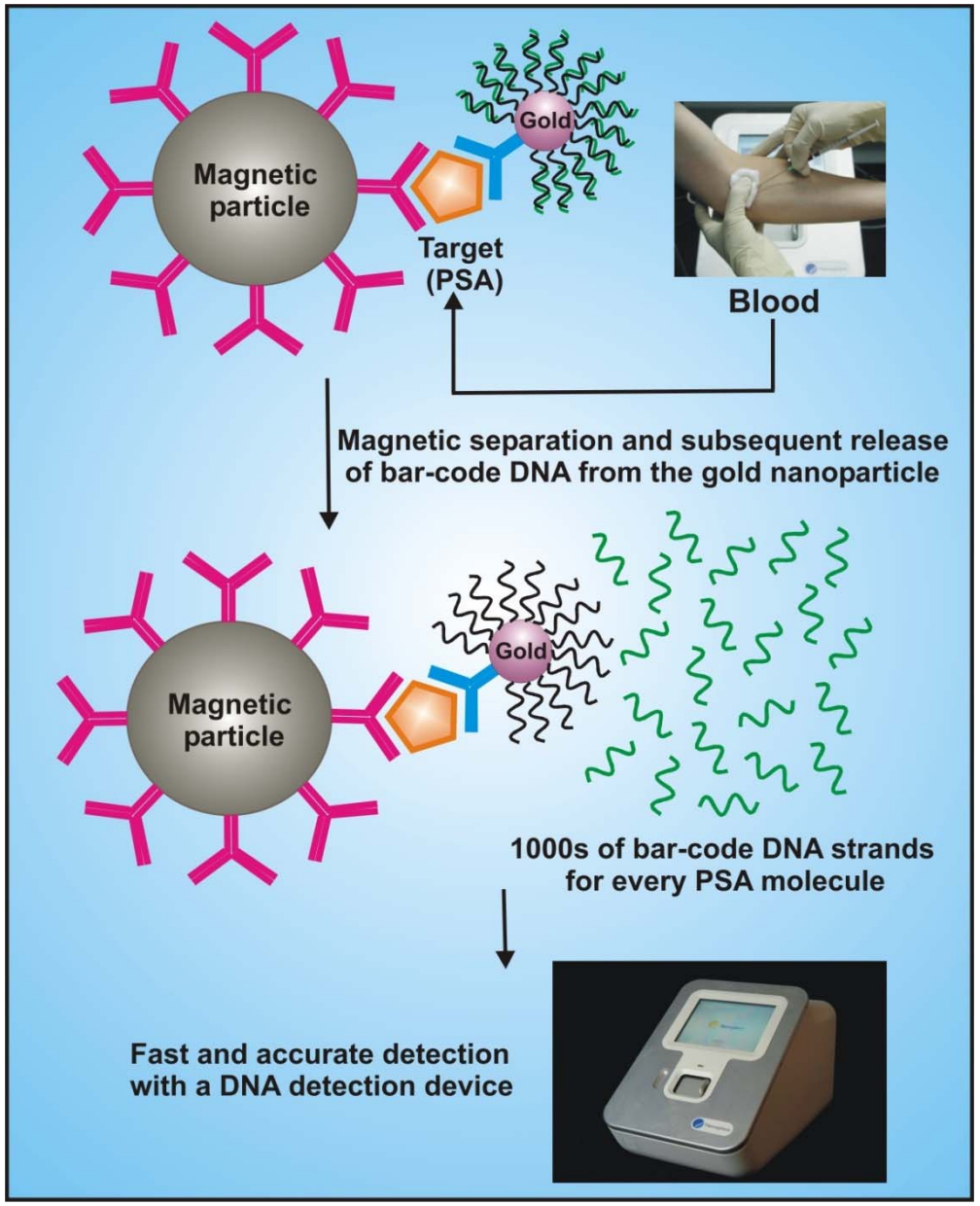
Verigene™ System

FDA-Cleared Hypercoagulation, Warfrin Metabolism, Cystic Fibrosis, and Influenza Assays

- Direct genomic detection
- ~100 aM (10^{-18}) LOD
- Multiplexed targets
- Automated assay process
- Ease of use
  - Minimal training required
  - Automated data tracking
  - No interpretation required

Multiplexed DNA Detection (HIV, Ebola Virus, Small Pox, Hepatitis B): Nucleic Acid Markers





Advantages of the Nanoparticle-based Bio-Barcode Assay

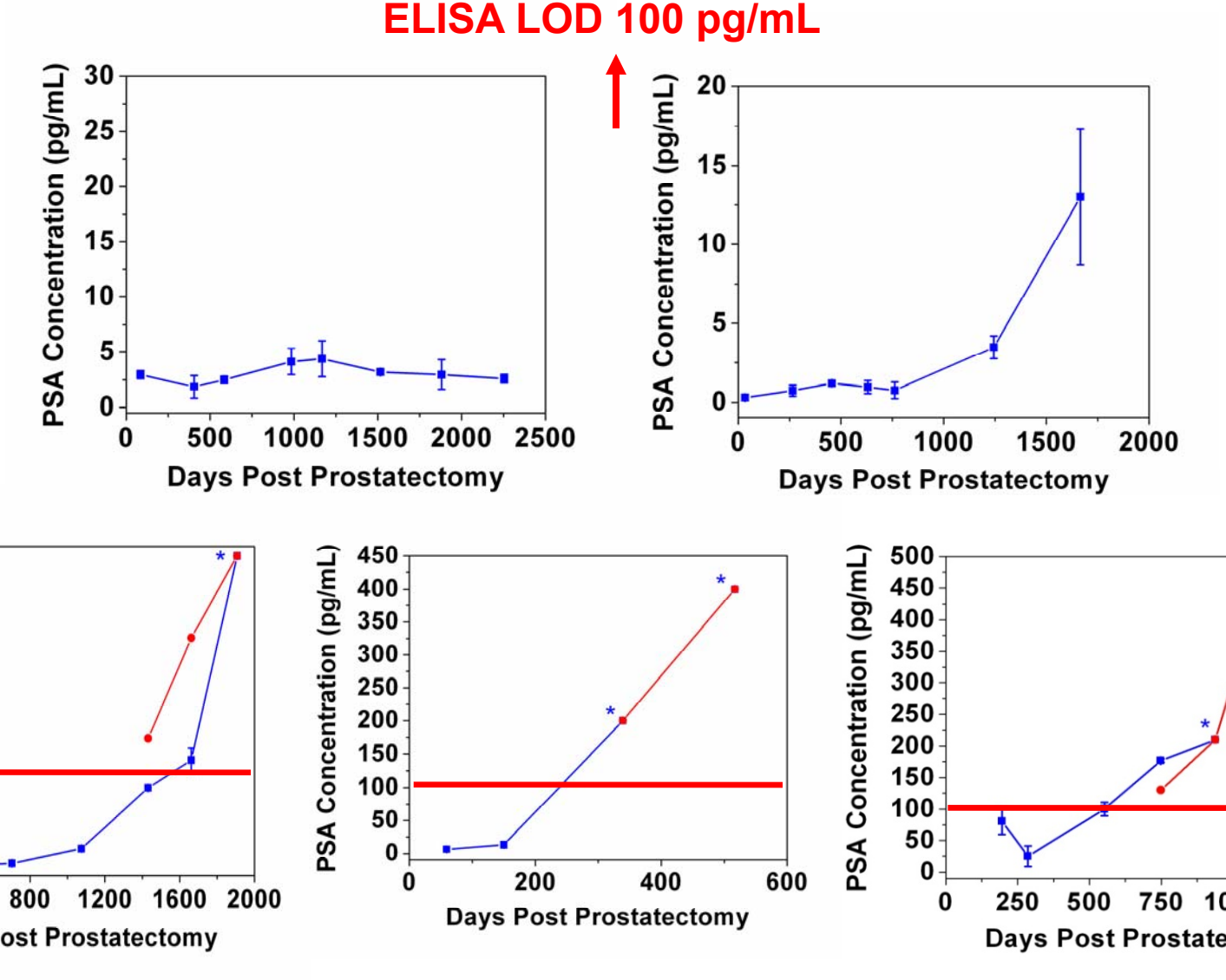
1. Up to 10^6 times more sensitive than conventional ELISAs.
2. Evaluate new biomarkers for diagnosing and following human diseases (e.g. HIV, Cancer, and Alzheimer's Disease).
3. Single-cell protein expression experiments.

Nanosphere

Field Defining Technologies

Concentration	Molecule/Drop	Detection/ Targets/Disease
10^{-3}- Millimolar	Quadrillions	Colorimetric/ Enzymatic Chemistry
10^{-6}- Micromolar	Trillions	Blood Sugar (Diabetes)
10^{-9}- Nanomolar	Billions	ELISA & Chemiluminesence
10^{-12}- Picomolar	Millions	Troponin, CK-MB, BNP, βHCG
10^{-15}- Femtomolar	Thousands	Bio-barcode Technology
10^{-18}- Attomolar	Tens	Alzheimer's Disease, Mad Cow, Ovarian, Breast, and many other cancers, Pulmonary Disease, Cardiovascular Disease
10^{-21}- Zeptomolar	<1	

Bio-barcode Assay Detects PSA Levels Undetectable by ELISA (450 patient study)



Acknowledgements

ASD (RE), AFOSR, ARO
DARPA, and ONR