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-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)
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

13 nm Au NP
~67,500 atoms

Fluorescein
37 atoms
1 nm

40-mer Oligonucleotide
1400 atoms

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

SNAs Have Unique Properties Distinct From Their Linear Counterparts

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

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

Diameter of NPs Crystallized

5 nm – 80 nm

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

- FCC
- BCC
- CsCl (20-50nm)
- AB$_3$ (Cr$_3$Si)
- Simple Cubic
- NaCl
- HCP
- CsCl (20-40nm)
- AlB$_2$
- AB$_6$ (Cs$_6$C$_{60}$)
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
Scanning Probe Lithography: A Dichotomy is Emerging

- Destructive Delivery of Energy
- Constructive Delivery of Materials

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)
- Dip-Pen Nanolithography (DPN) (Mirkin, 1999)
- Scanning Probe Block Copolymer Lithography (2010)
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

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

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?

- **Protein (Human IgG)**: 8.5 nm
- **Virus (HIV)**: 120 nm
- **Spores (Anthrax)**: ~20 µm
- **Living Cells**: ~15 µm
Patterning of Biological Structures

Viruses (TMV)

Proteins

Cells

Lipids
**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**


**Plasmonic**

JACS, 2000

**Cooperative Binding**


**Catalytic**

Science, 2000

**Enhanced Binding**

JACS, 2005

\[
\frac{K_1}{K_2} = 100
\]
Chip-Based Bio-bar-code Assay

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

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

- 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

FDA-Cleared Hypercoagulation, Warfrin Metabolism, Cystic Fibrosis, and Influenza Assays
Multiplexed DNA Detection (HIV, Ebola Virus, Small Pox, Hepatitis B): Nucleic Acid Markers

Target is present

Target is not present
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).

# Field Defining Technologies

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

The table above illustrates the concentration ranges and corresponding molecule counts (in quadrillions, trillions, billions, millions, thousands, and tens) for various technologies in the field. Detection targets and diseases associated with different concentration ranges are also listed.
Bio-barcode Assay Detects PSA Levels Undetectable by ELISA (450 patient study)

ELISA LOD 100 pg/mL
Acknowledgements

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DARPA, and ONR