화학생물공학부 나노바이오공학특론 2016년 11월 22일(화)

Programmable Assembly of Nanomaterials Using Biopolymers : Basic Structure

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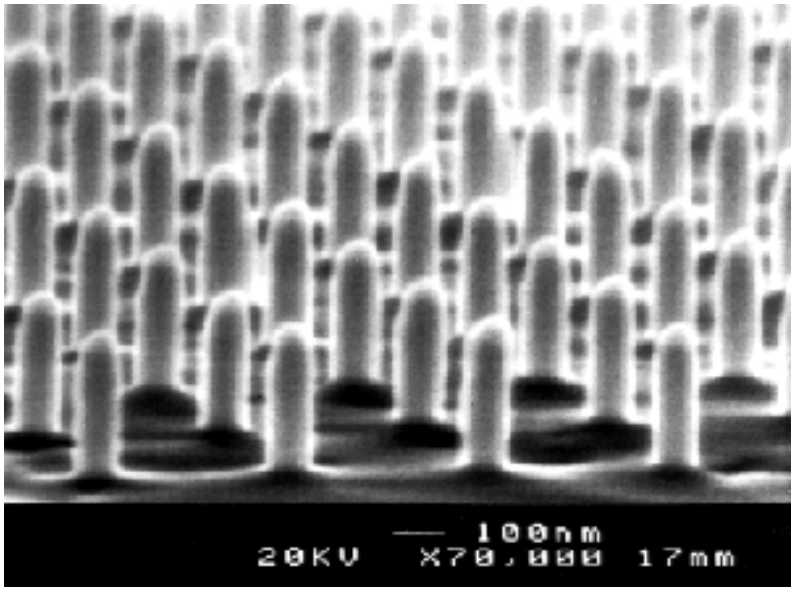
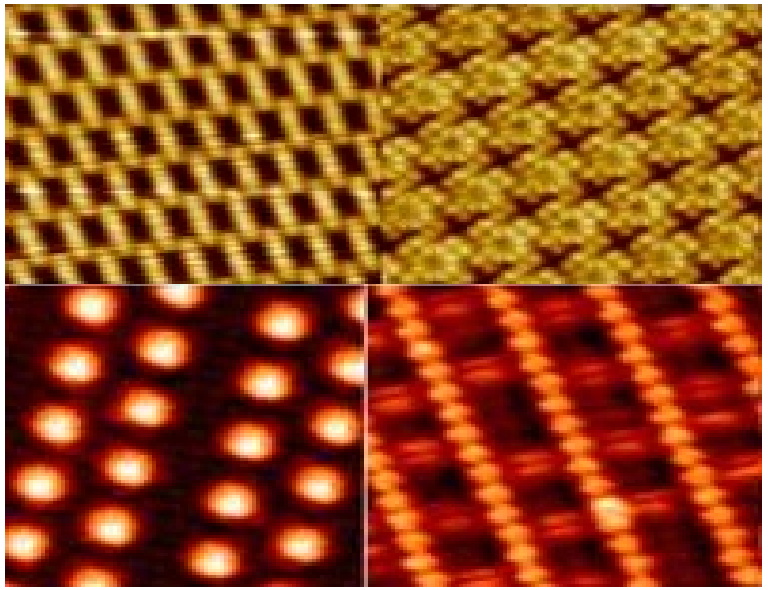
- Materials and Methods: Overview
- Hybrid Biopolymers for Molecular Building Blocks
- Applications of Nanoassembly: Nano-Optics and Beyond
- Future Research Directions

Introduction

- What Is Nanostructure?
- How to Make Nanostructures?
- Top-Down vs. Bottom-Up Approaches



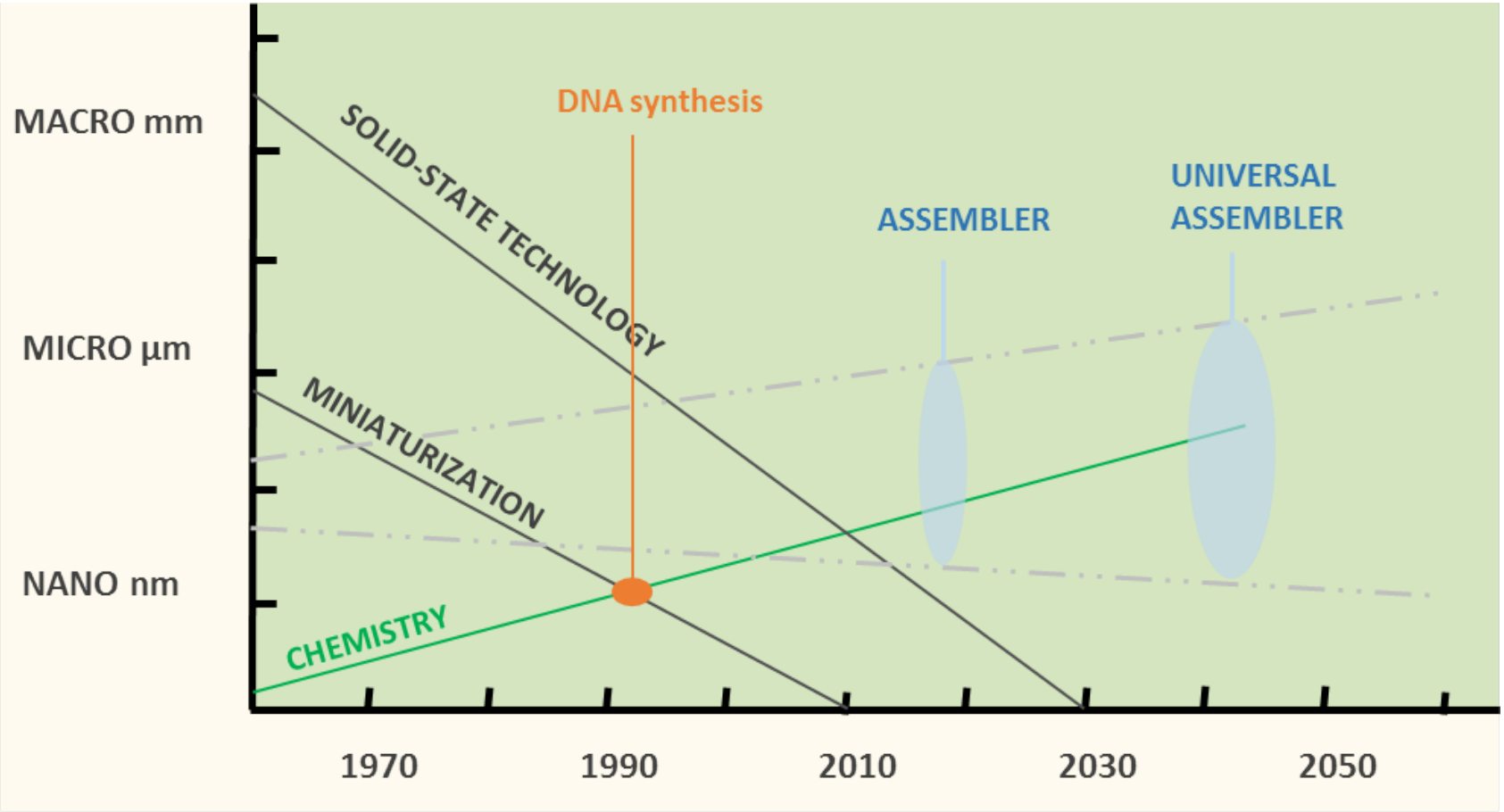
Array of GaAs nanoposts fabricated by RIE



Self Assembled array of ST molecules

Introduction

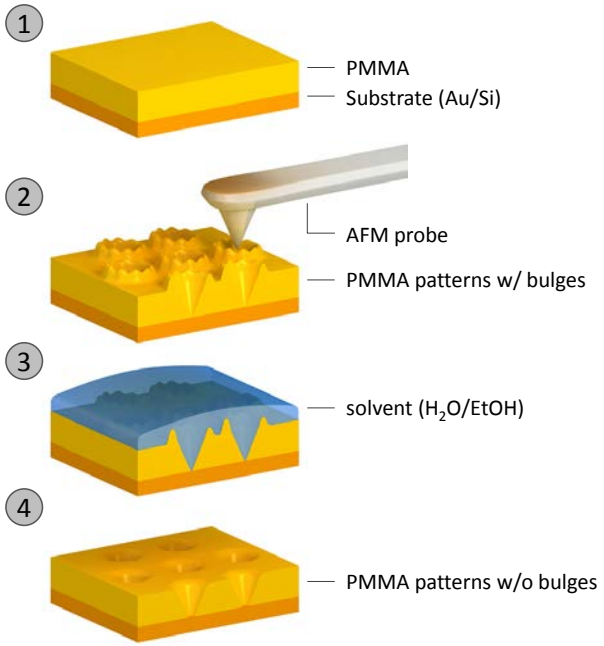
- **How to Make Nanostructures?**

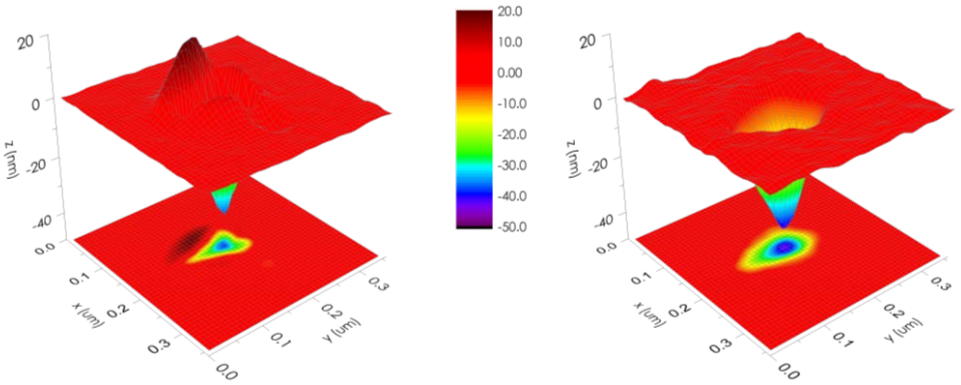


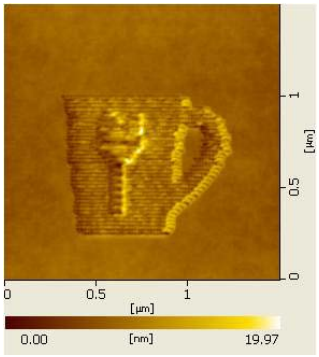
Note: Solid-state technology also adopts bottom-up processes.

Introduction

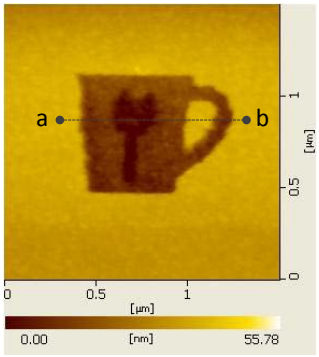
- **(Unconventional) Top-Down Approach: Nano Indentation Lithography**



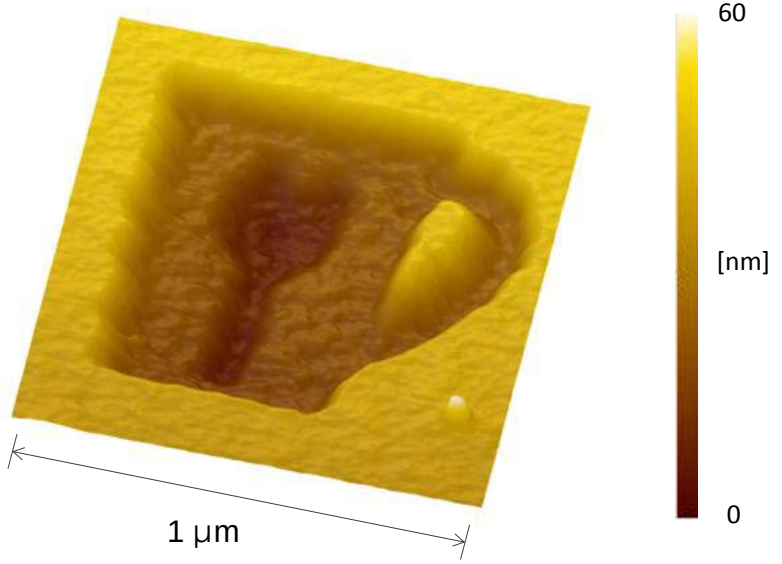




Before bulge removal

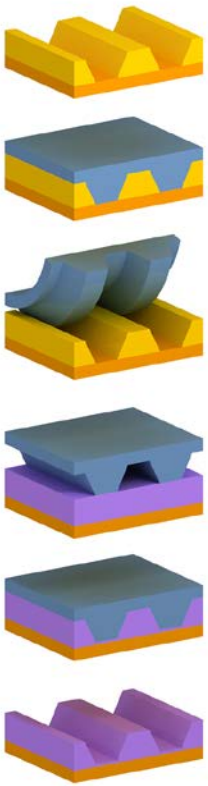


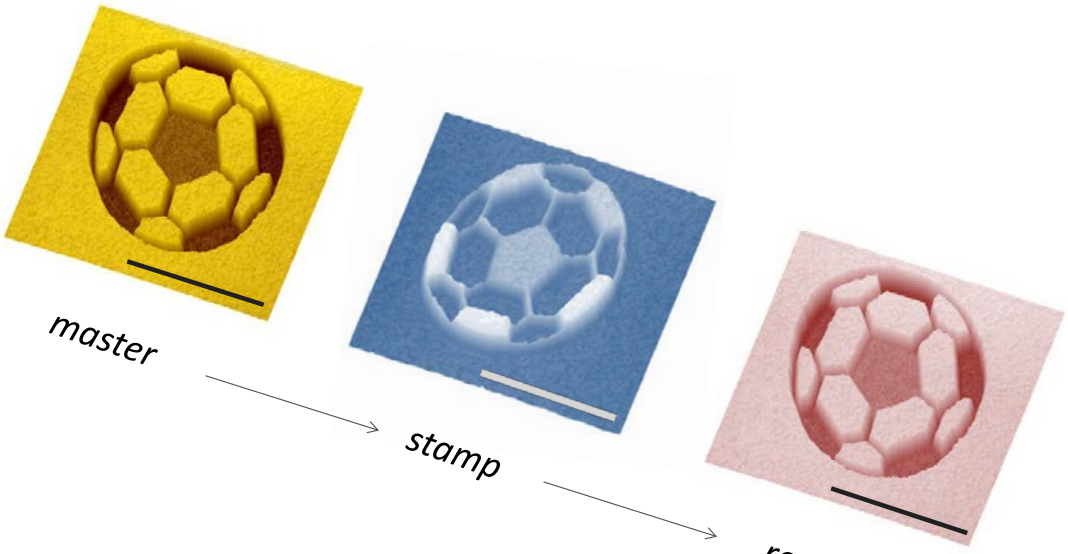
After bulge removal

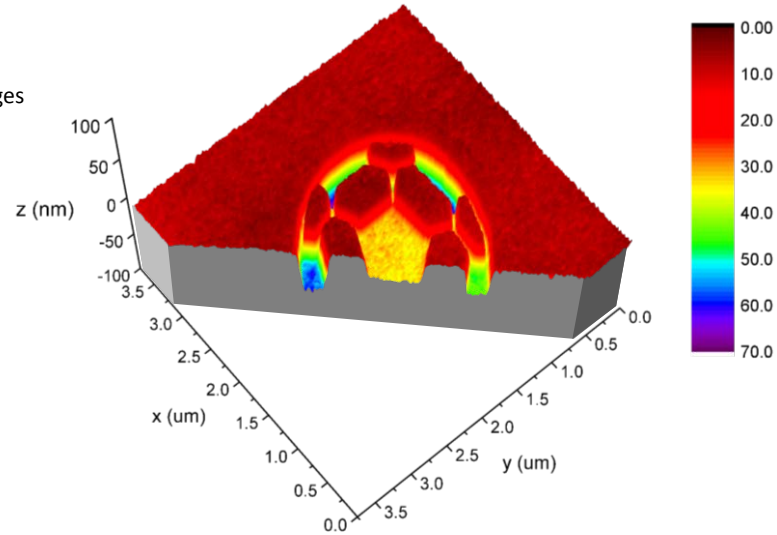


Introduction

- **(Unconventional) Top-Down Approach: Nano Indentation Lithography**

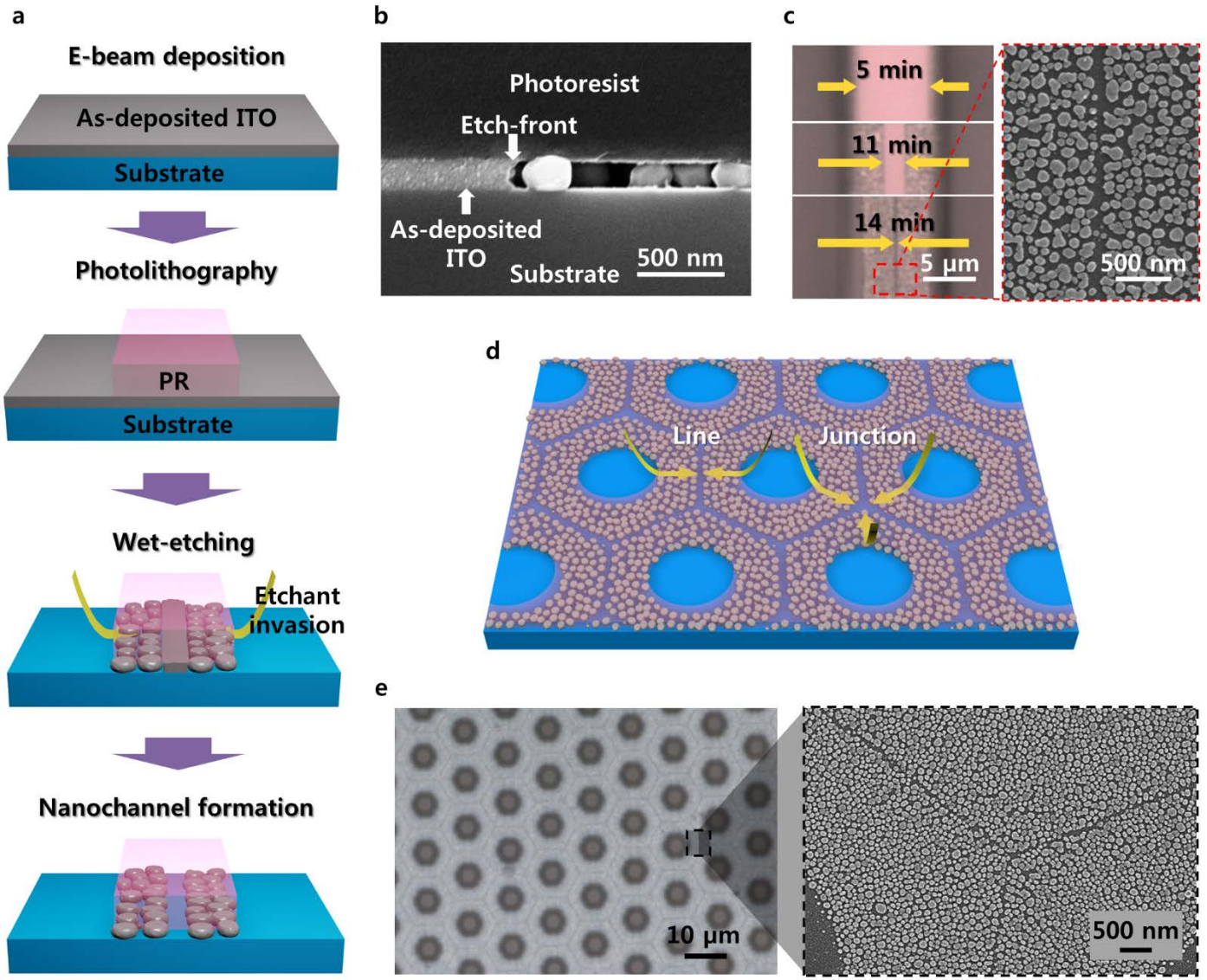






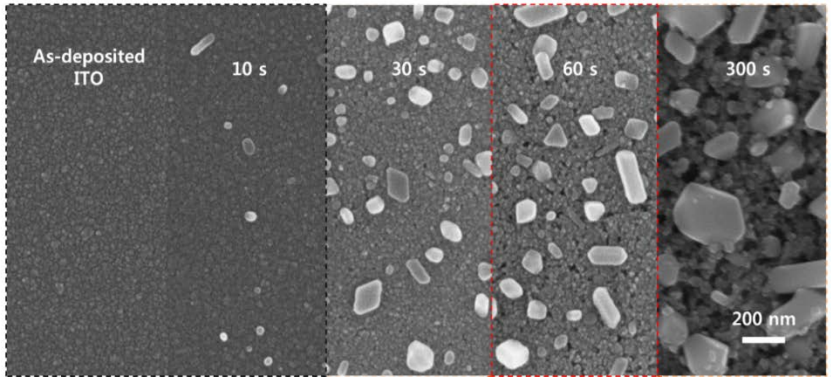
Introduction

- **Top-Down + Bottom-Up Approach: Crystallized Nano Islands**

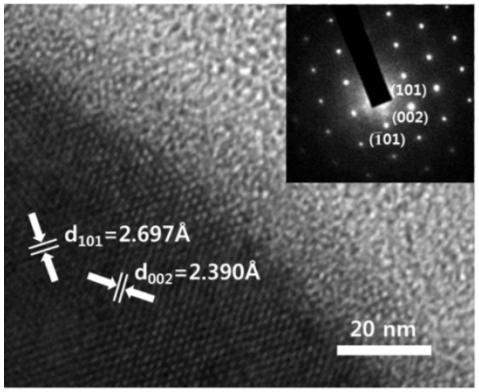


Introduction

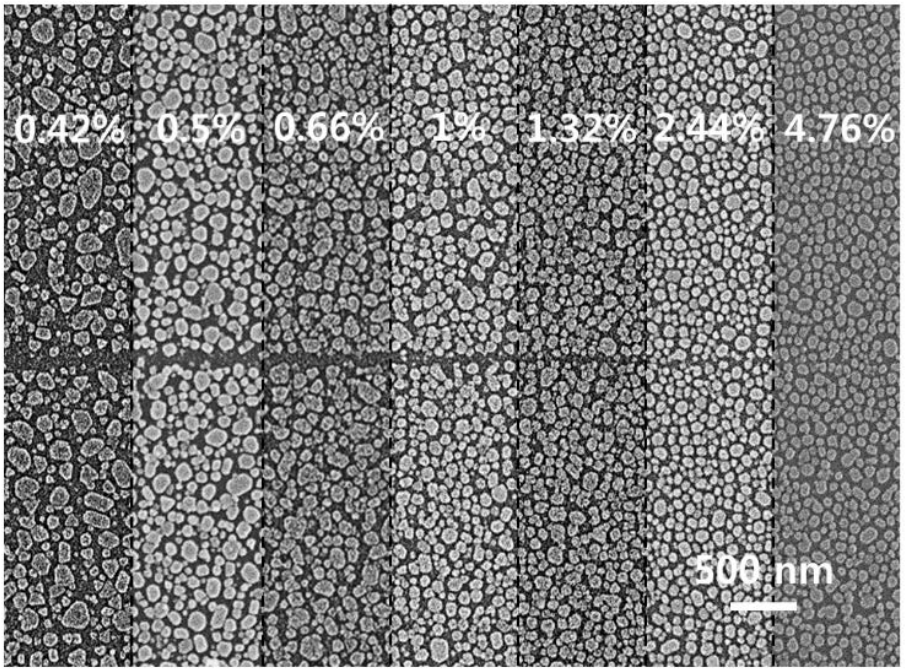
- **Top-Down Approach: Nano Indentation Lithography**



time evolution



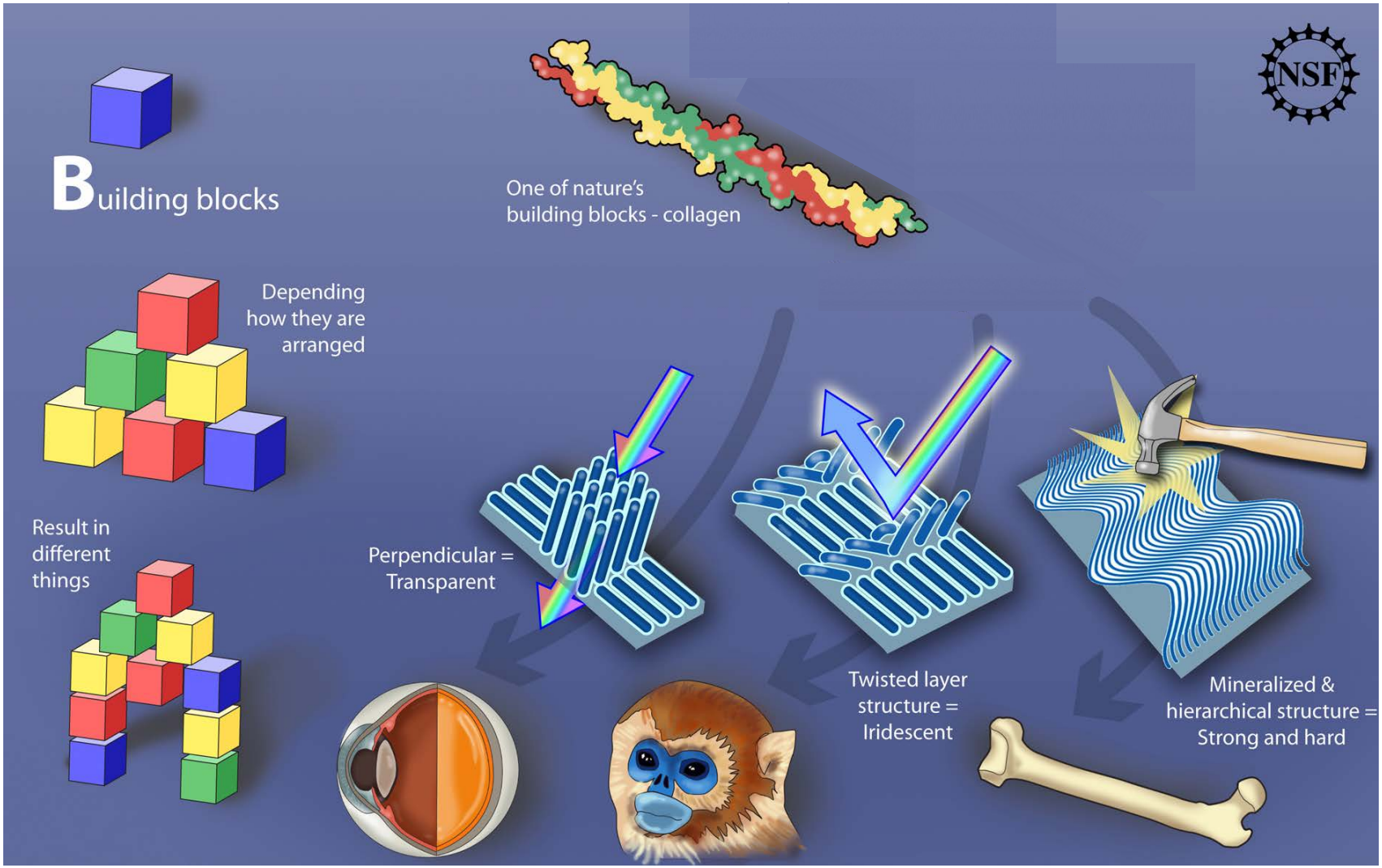
crystal structure



Interesting Point:
Nanochannels are created via bottom-up process from microstructures defined by top-down techniques

Introduction

- **(Supra-) Molecular Building Blocks and Their Assemblies in Nature**



Modified image from NSF (2013)

Introduction

- **Important Molecular Building Blocks in Nature**

Table 2–2 The Types of Molecules That Form a Bacterial Cell

	PERCENT OF TOTAL CELL WEIGHT	NUMBER OF TYPES OF EACH MOLECULE
Water	70	1
Inorganic ions	1	20
Sugars and precursors	1	250
Amino acids and precursors	0.4	100
Nucleotides and precursors	0.4	100
Fatty acids and precursors	1	50
Other small molecules	0.2	~300
Macromolecules (proteins, nucleic acids, and polysaccharides)	26	~3000

Introduction: Four Classes of Building Blocks in Life

1 **Lipids** ----------

- Amphipathic Macromolecule
- Cellular membranes
- Endo/exocytosis, Intracellular transport through vesicles

2 **Sugar – Polysaccharides** ----------

- Glycosidic bonds
- Energy storage
- Receptor on cytoplasmic membrane (glycolipids, glycoproteins)

3 **Nucleotides – Nucleic Acids (DNA/RNA)** ----------

- Phosphodiester bonds
- Sugar phosphate (backbone) and Purine/Pyrimidine base
- Genetic information store/express/transfer

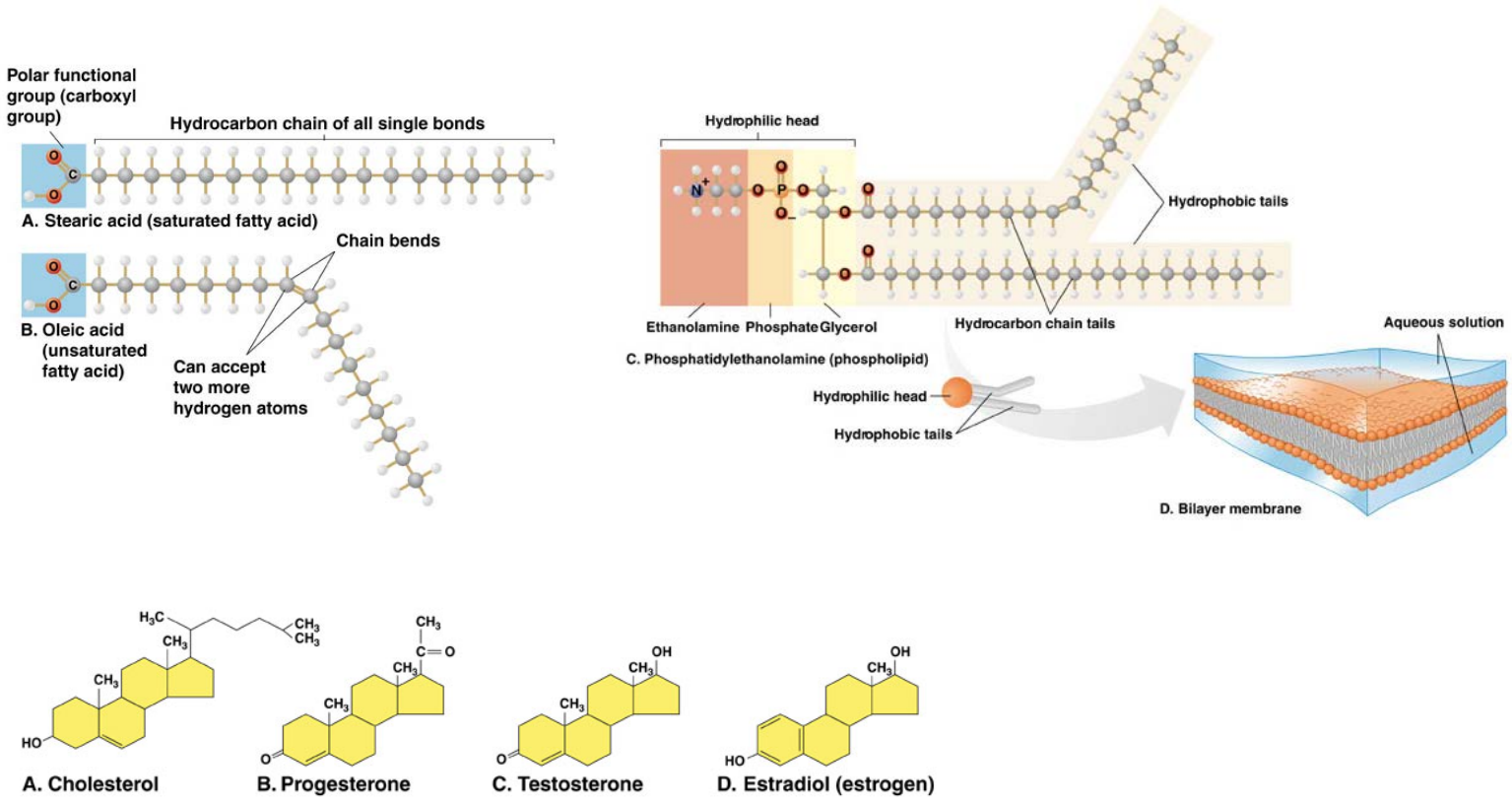
4 **Amino acids – Protein** ----------

- Peptide bonds
- Most abundant macromolecules
- Numerous structural and functional roles in life

Introduction: Four Classes of Building Blocks in Life

1 Lipids ----------

- Amphipathic Macromolecule
- Cellular membranes
- Endo/exocytosis, Intracellular transport through vesicles
- Functional regulation of organs (e.g. hormones)

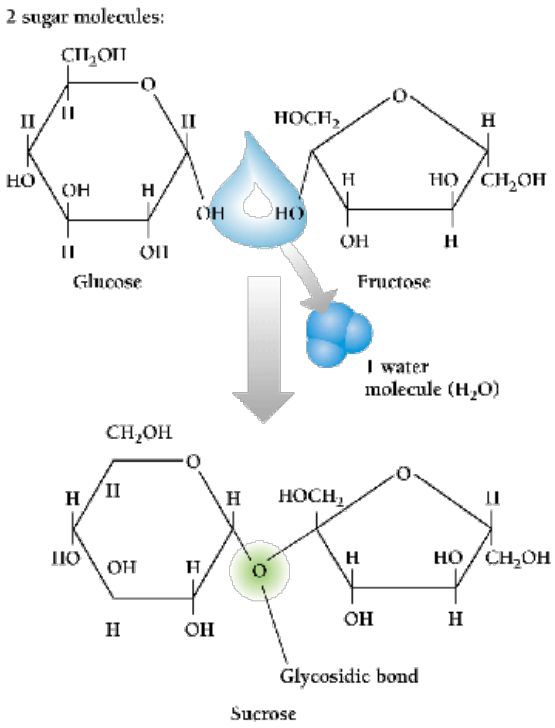


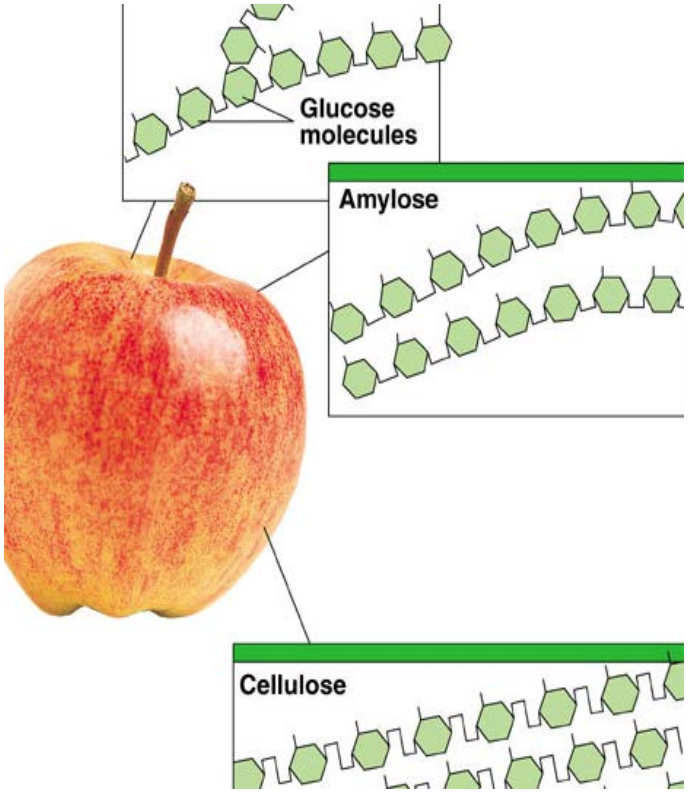
Various functional lipids

Introduction: Four Classes of Building Blocks in Life

2 Sugar – Polysaccharides ----------

- Glycosidic bonds
- Carbohydrate ($C:H_2O = 1:1$)
- Energy storage
- Receptor on cytoplasmic membrane (glycolipids, glycoproteins)

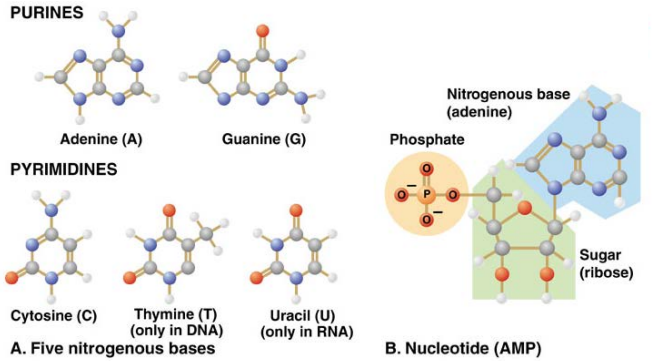


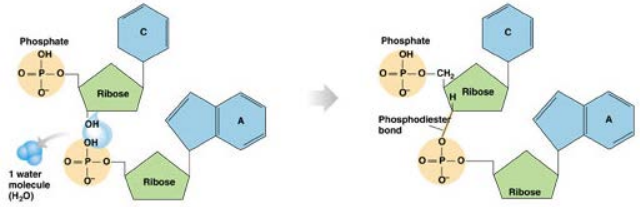


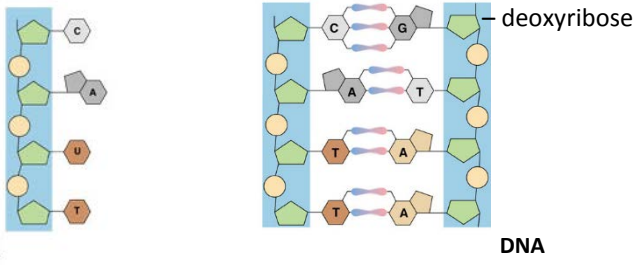
Introduction: Four Classes of Building Blocks in Life

3 Nucleotides – Nucleic Acids (DNA/RNA) ----------

- Phosphodiester bonds
- Sugar phosphate (backbone) and Purine/Pyrimidine base
- Genetic information store/express/transfer



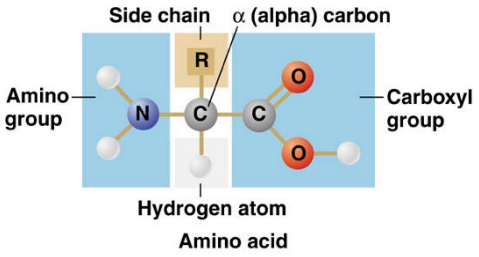


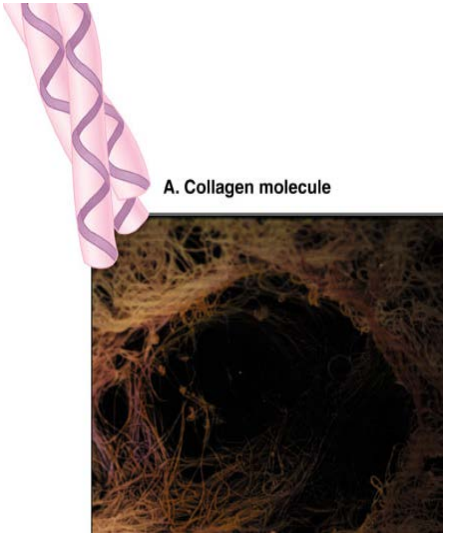


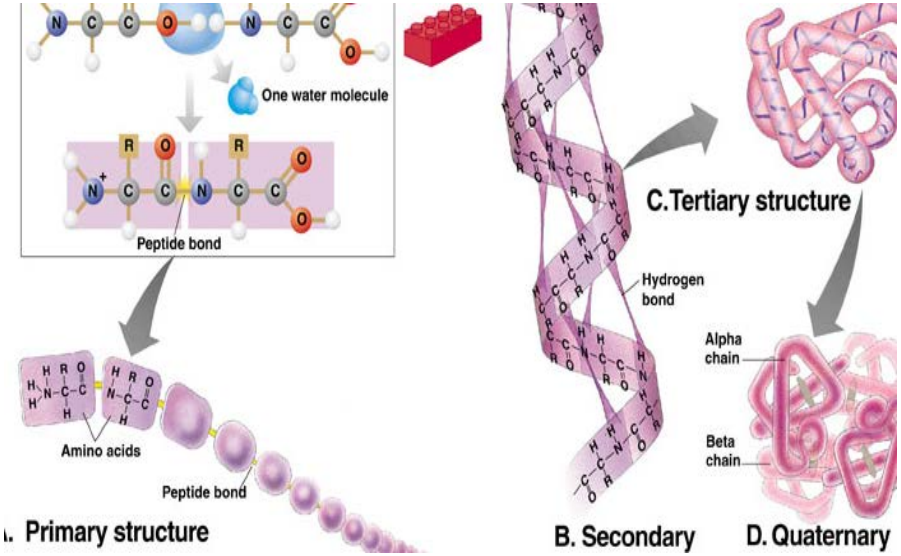
Introduction: Four Classes of Building Blocks in Life

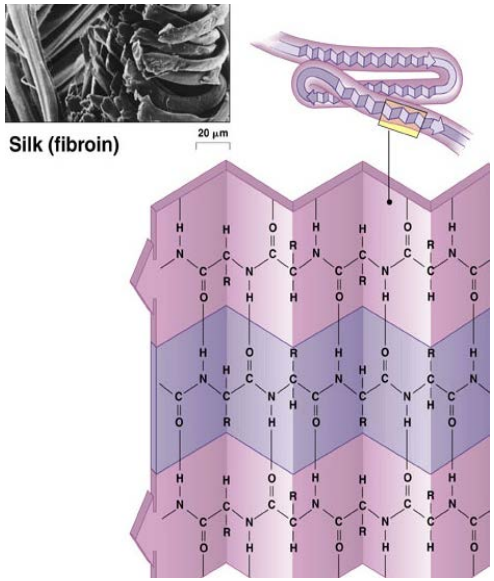
4 Amino acids – Protein ----------

- Peptide bonds
- Most abundant macromolecules
- Numerous structural and functional roles in life



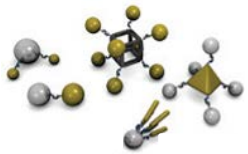


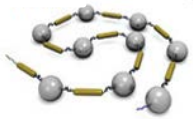


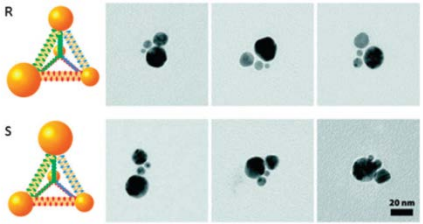


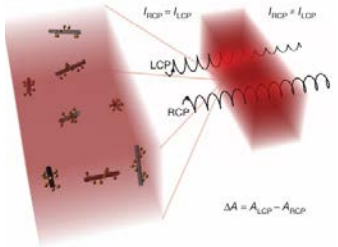
Programmable Nano Assembly: *Using Biopolymers*

1 Complex Plasmonic Nanomaterials

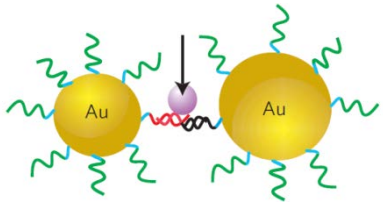




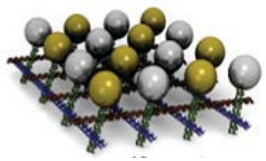


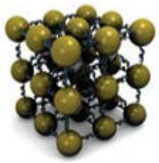


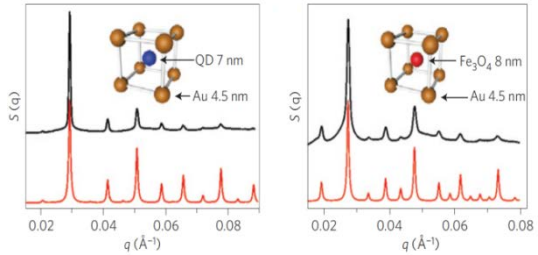
2 Multi-Functional Nanoprobes



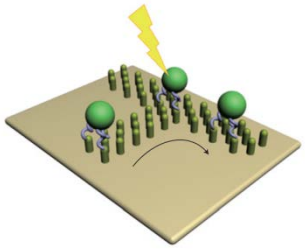
3 Highly Ordered Nanostructures

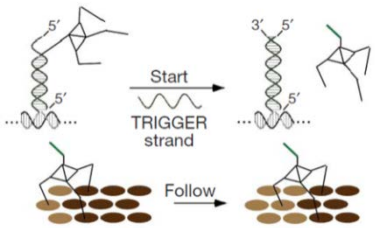


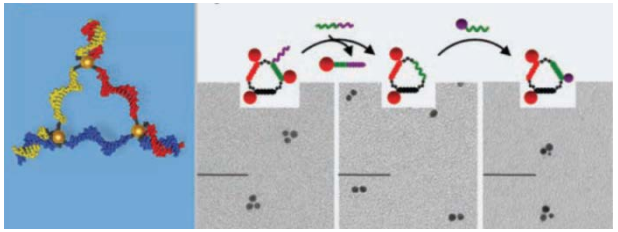




4 Nano Motors and Reconfigurable Nanostructures





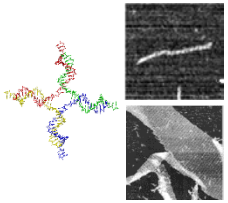
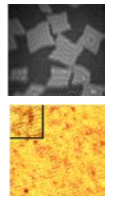
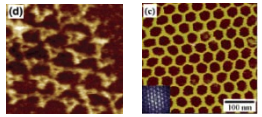
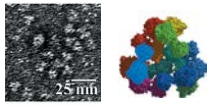
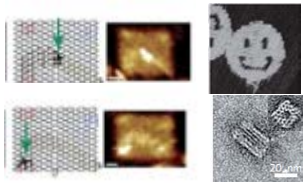
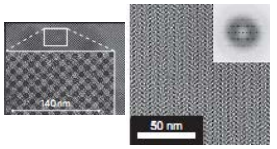
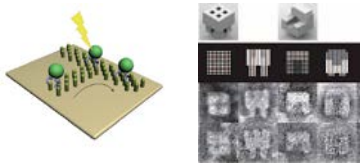
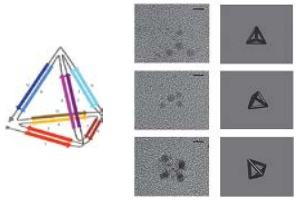


Biopolymers: DNA and Protein

- **Two Most Abundant Biopolymers in Nature**
- **Information Carrying Molecules : Programmable**
- **Controllable Assembly**
- **Provide Various Functionalities in Life**

	DNA	Protein
Pros	✓ Narrow range of functions ✓ Limited binding ✓ Highly negative charge ✓ High cost of synthetic DNA ✓ High error rate of self-assembly ✓ Thermally unstable	✓ Difficult handling ✓ Slow production ✓ Unpredictable process ✓ Low design freedom
Cons	✓ Easy design (4 bases) ✓ Fast production ✓ Easy assembly	✓ Broad range of functions ✓ Versatile binding ✓ Molecular recognition ✓ Precise alignment with symmetry

Molecular Building Blocks: DNA vs. Protein

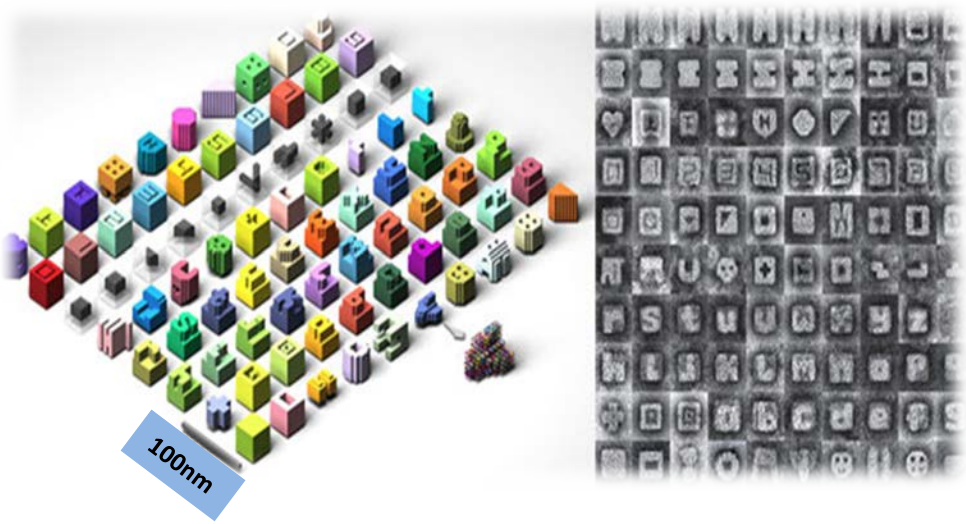
DNA		Protein	
▪ Passive DNA nanostructures ▪ Hetero-elements for Functionality Seeman		▪ Primitive Protein Nanostructures Alice P. Gast	
▪ Template & Algorithm Method ▪ Periodic & 3D Nanostructure Rothemund Winfree		▪ Symmetric/Asymmetric Pattern Todd O. Yeates	
▪ DNA Origami ▪ Nano-robot Rothemund Hao Yan		▪ Fusion Protein ▪ Computational Design Martin E. M. Noble	
▪ DNA Bricks ▪ Molecular Biophysics Peng Yin		▪ Coiled-Coil Protein Origami Roman Jerala	

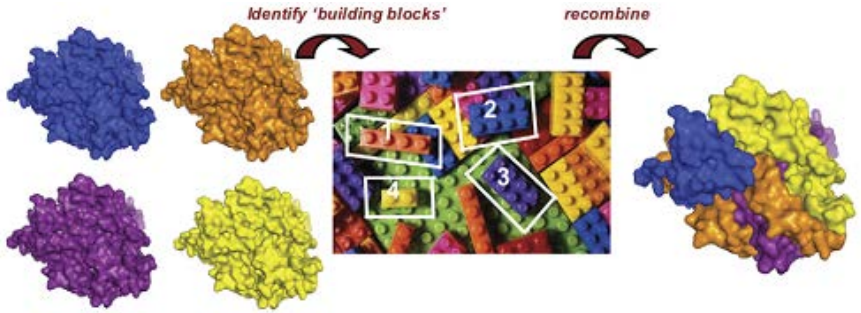
2000~ 2005~ 2010~ 2015~

DNA & Protein Hybrid Building-block?

Molecular Building Blocks: DNA *plus* Protein

Biocompatible | Smart Molecule | Controllable

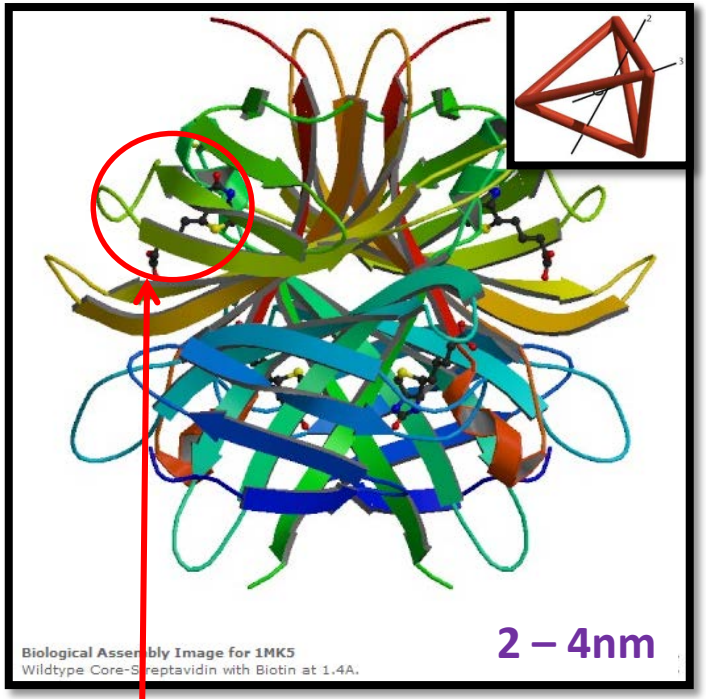




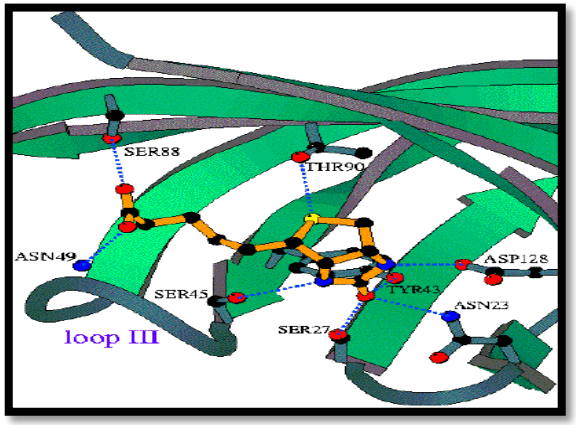
SCIENCE 2012, VOL.338, 30

DNA	Protein
-Watson-Crick base pairing -Directly synthesize -Freedom of Design	- Conformational variability - Biological recognition - Effective scaffold structure

Need a Glue? – Avidin-Biotin System



Biotin



Hydrogen bonding network of streptavidin-biotin:
One of the strongest non-covalent bindings in nature

Protein

- Conformational variability
- Effective scaffold structure
- Various properties

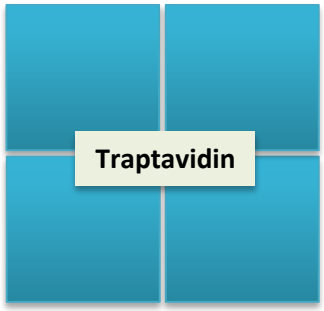
+

DNA

- Directly synthesized
- Freedom of Design
- Watson-Crick base pairing

DNA Base Paring: "Multivalent" Building Blocks

Building Block Design



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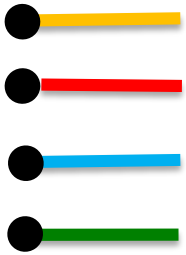

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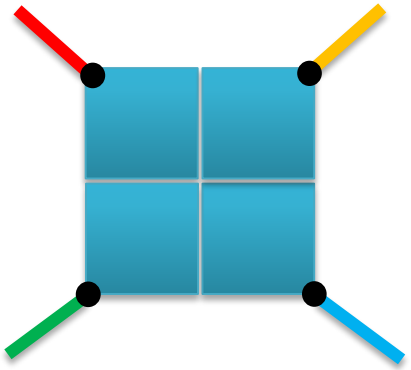


Identical subunit tetramer
- 4 biotin binding sites

Four "different" biotinylated-DNA

Distinguishable Tetravalent TV-bDNA complex

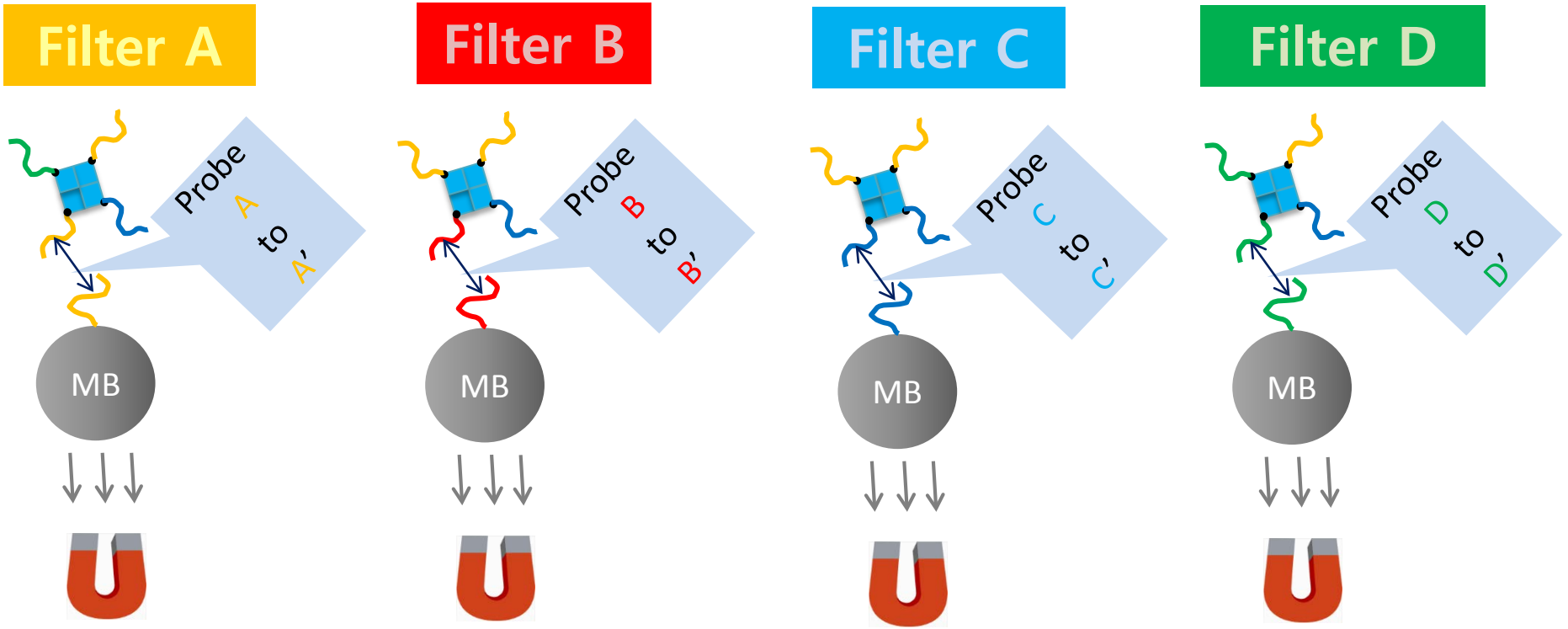
Strategy for Synthesizing Building Blocks

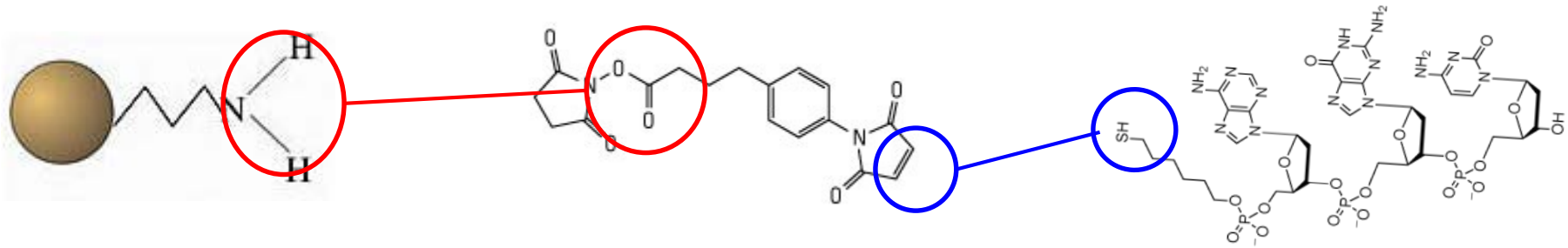
Randomly Mixed Samples

$$\frac{4!}{4^4} \fallingdotseq 9.4\%$$

Filter A → Filter B → Filter C → Filter D

Extraction of Programmable Units: Magnetic Separation





Dynabeads® M-270 Amine (Invitrogen™)

Succinimidyl 4-(p-maleimidophenyl) butyrate

Thiol modified ssDNA

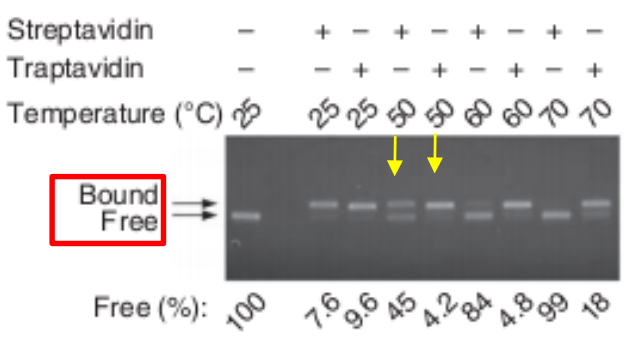
Issues with Magnetic Separation

Mutated
Avidin

Traptavidin

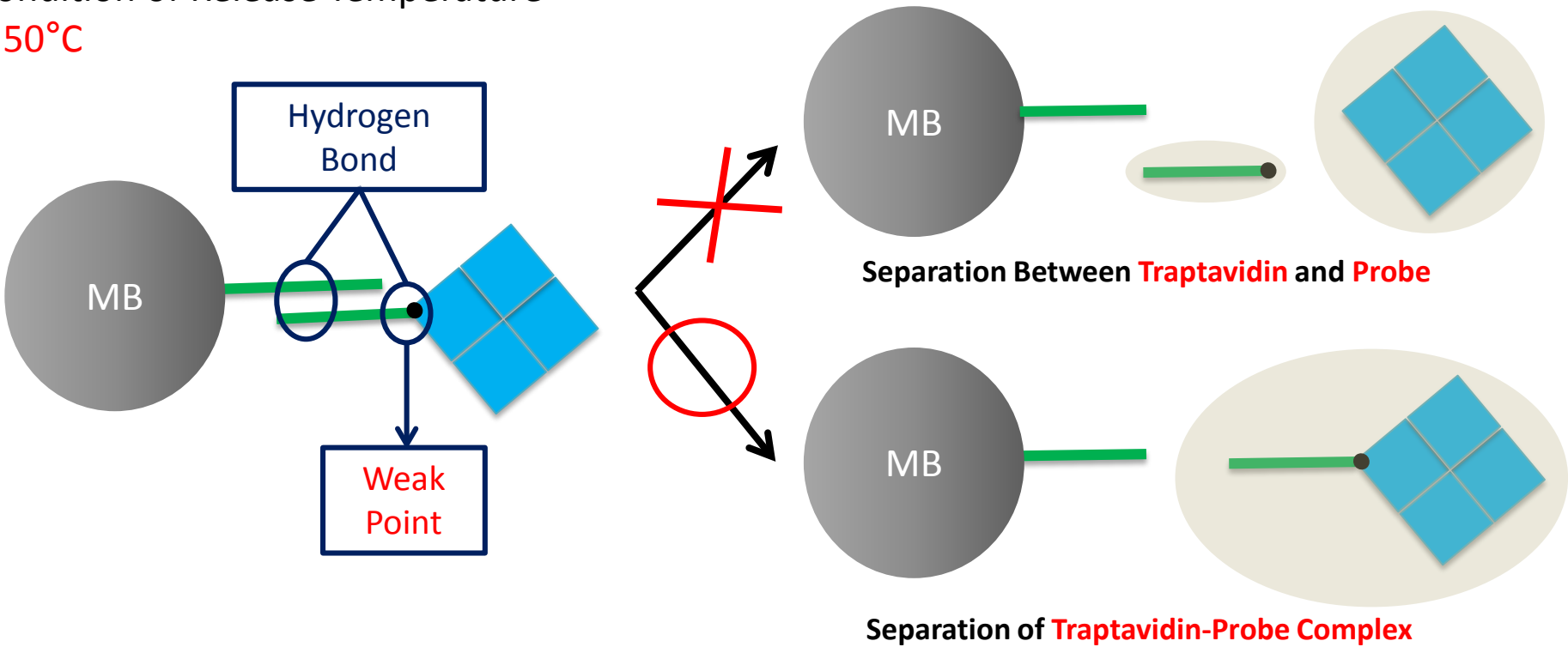
- **Enhanced** Thermal & Mechanical Properties
- **Low** Biotin-Traptavidin **Off-Rate**

a

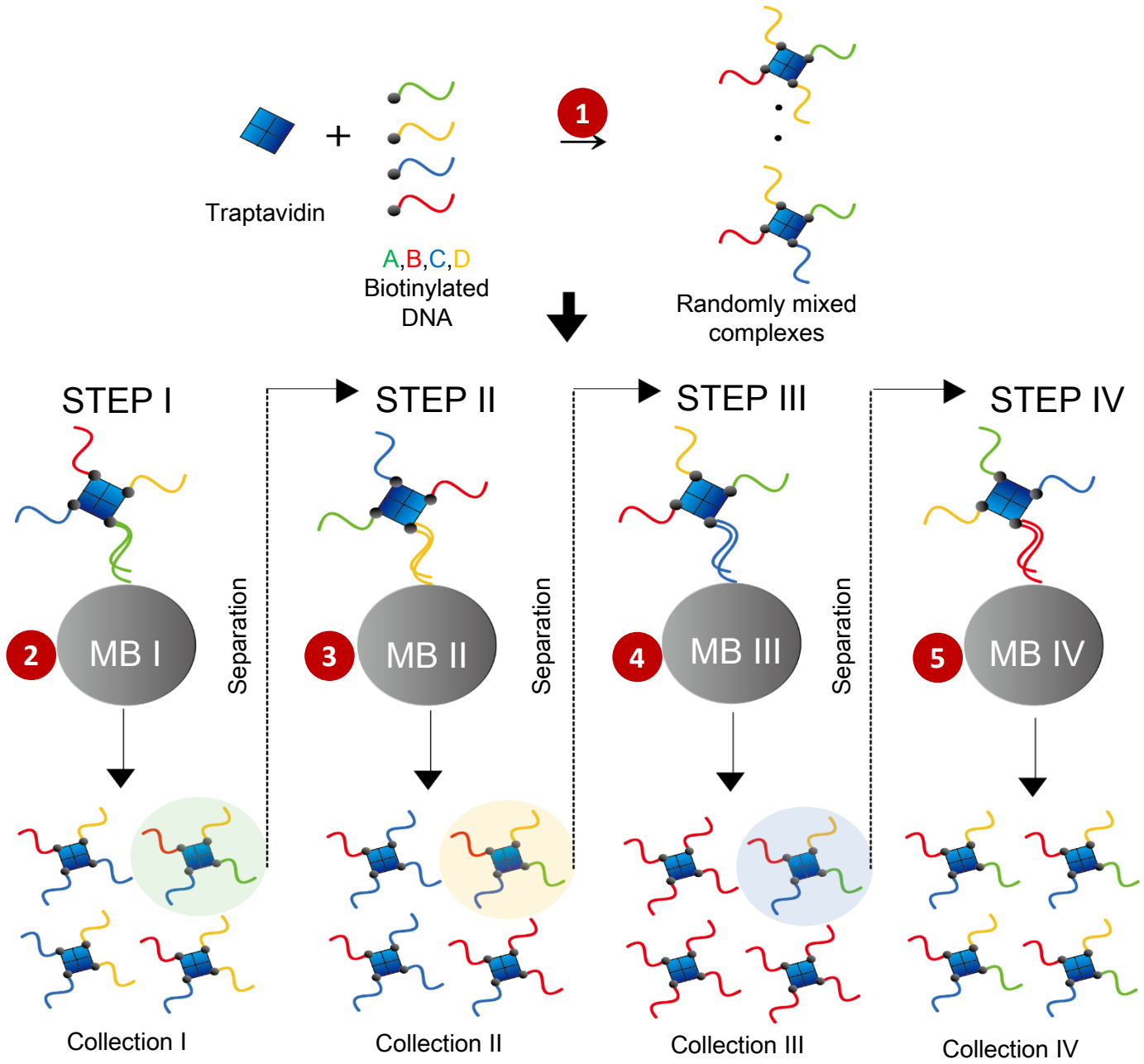


Nature Method 2010, VOL.7 , 5

Condition of Release Temperature
: 50°C

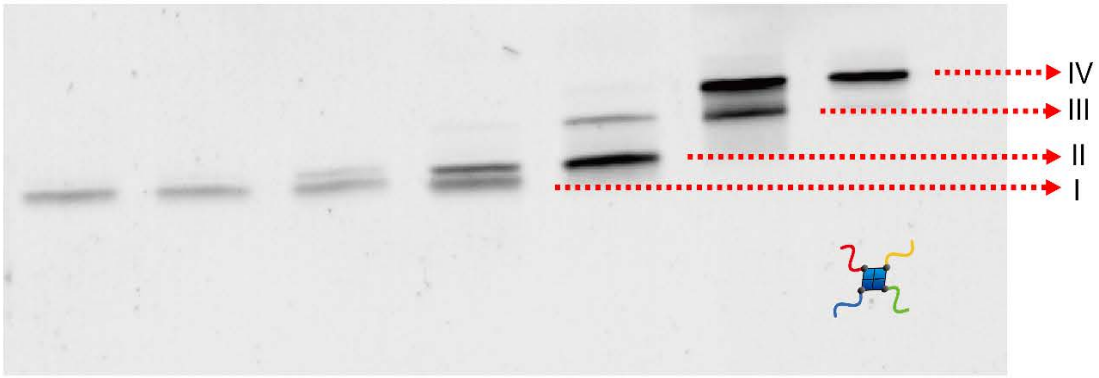


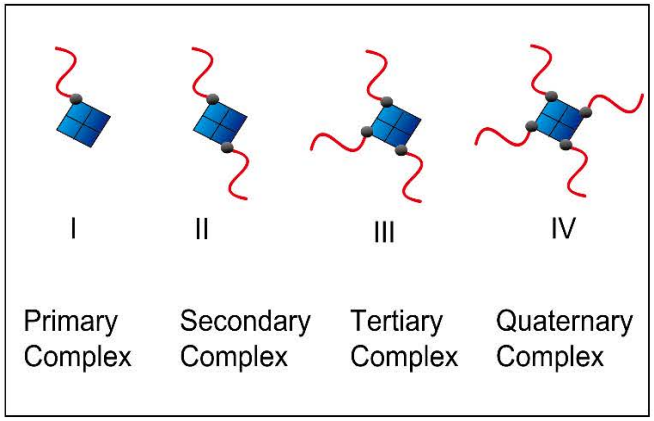
Synthesis of Multivalent TV-DNA Conjugates



PAGE Ananlysis of Tetravalent DNA-TA Complexes

a





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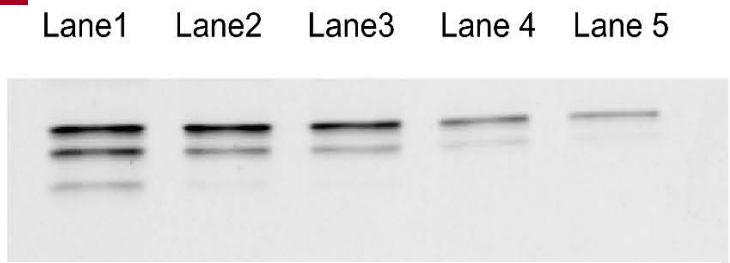
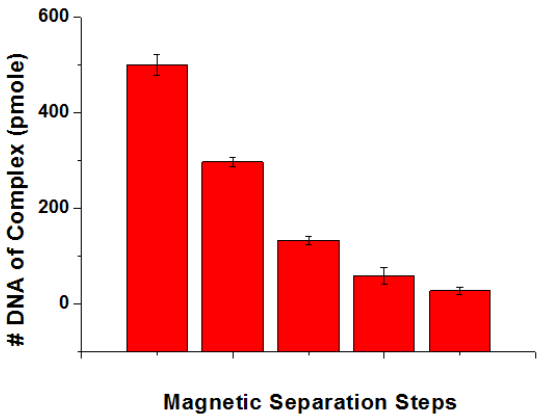
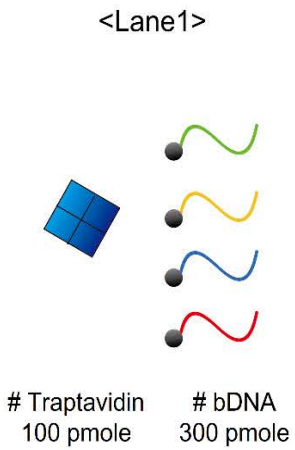
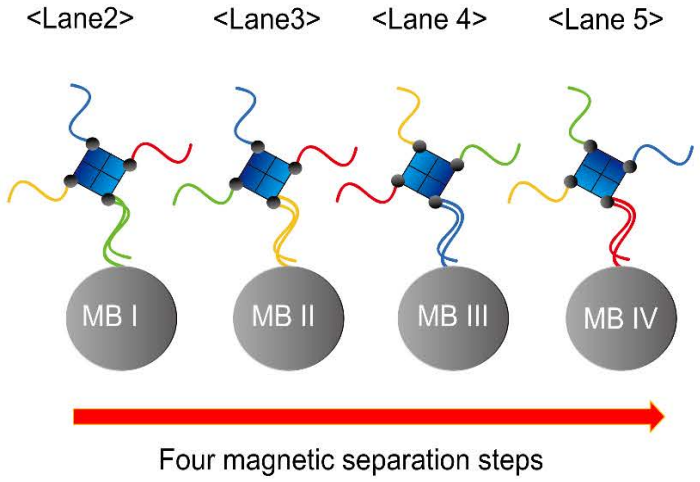
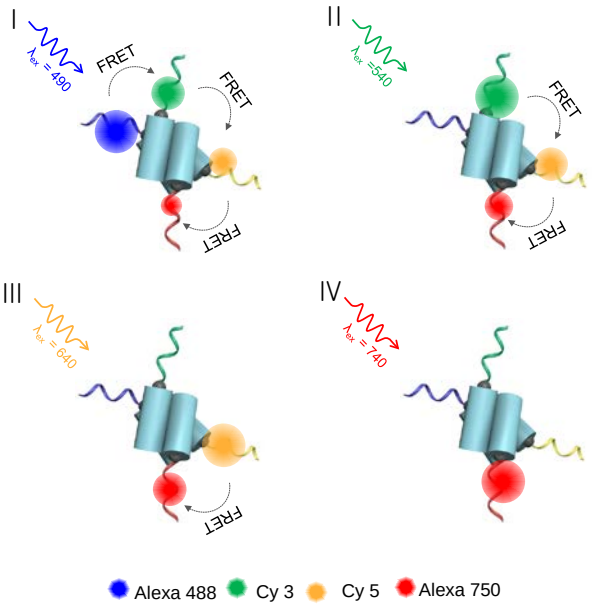




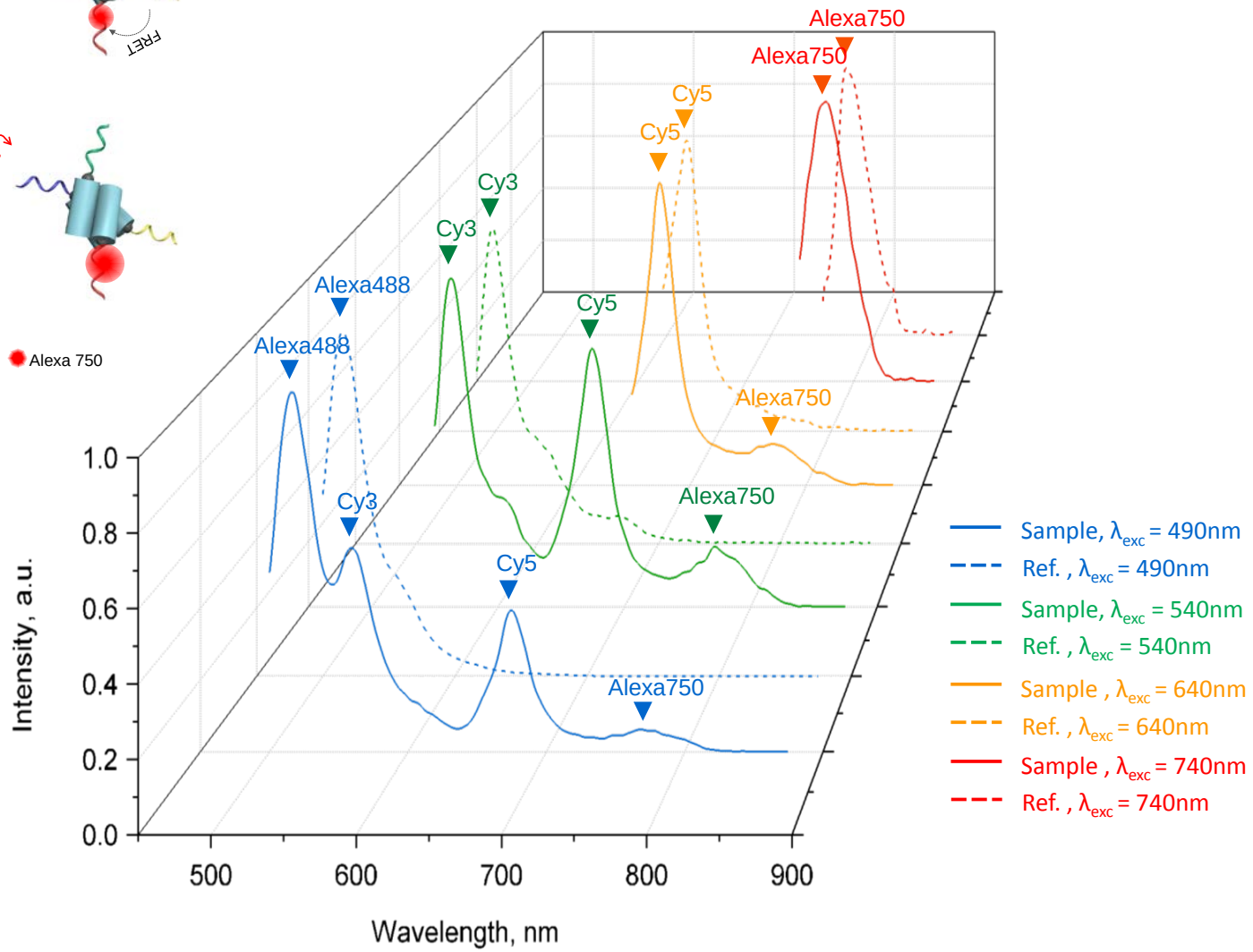




Spectroscopic Ananlysis of Tetravalent Complexes



- FRET based characterization of tetravalency

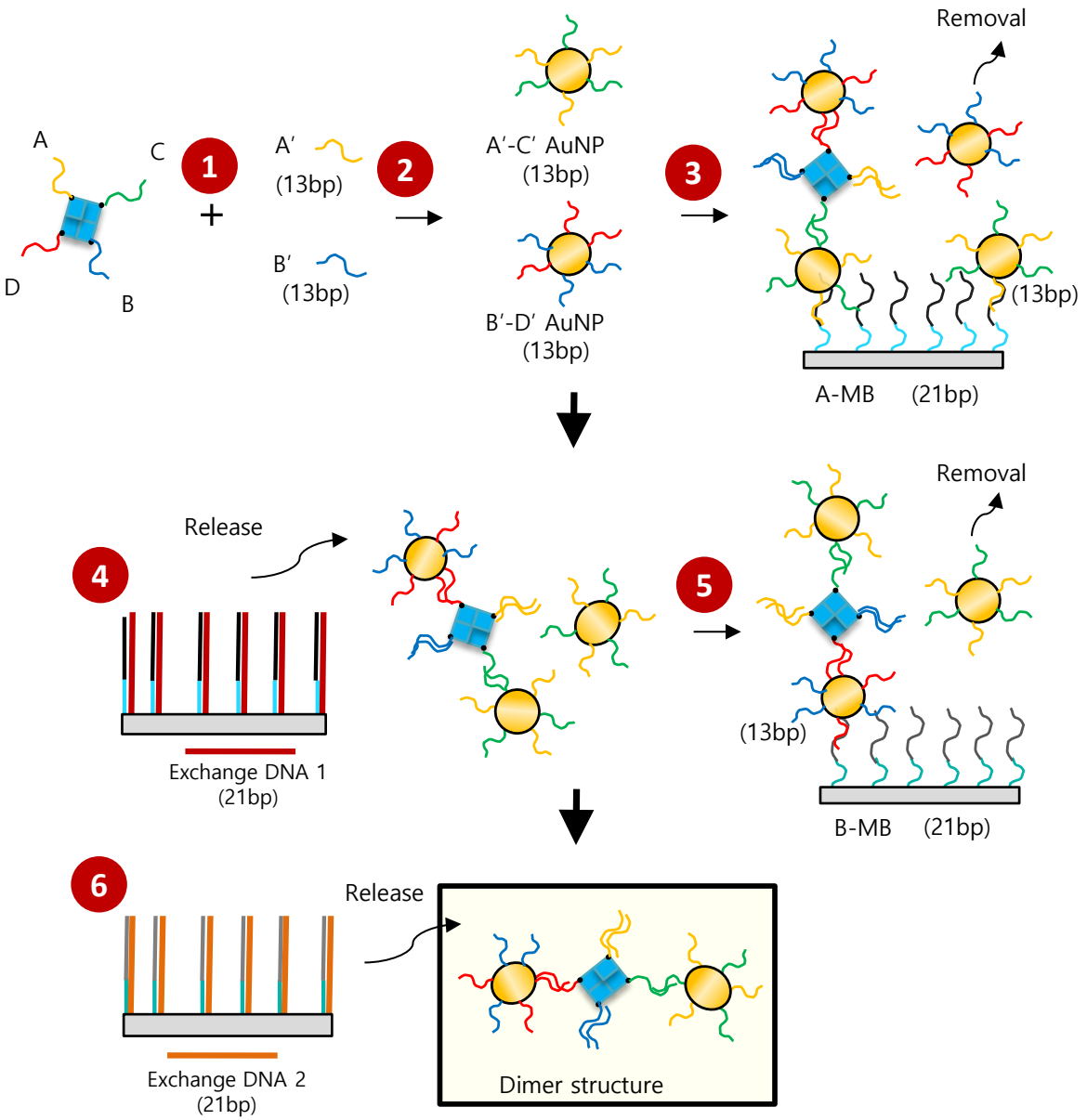


Schemes for Fabricating (Plasmonic) Nano Assembly

- Passivation to Dead-Probe
- Conjugation to AuNP I & II
- Collecting AuNP I and Dimer
- Remove AuNP II

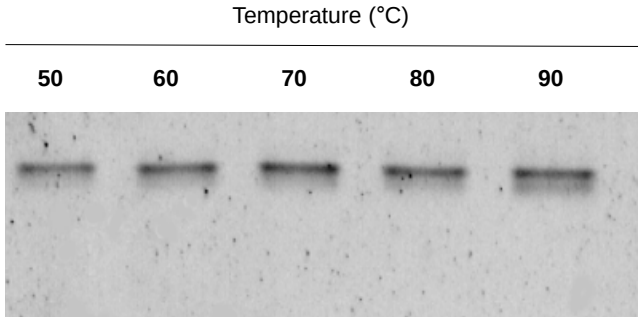
- Release from Magnetic Bead
- Conjugation to Dimer
- Remove AuNP I

- Release from Magnetic Bead
- Purification of Dimer Structure

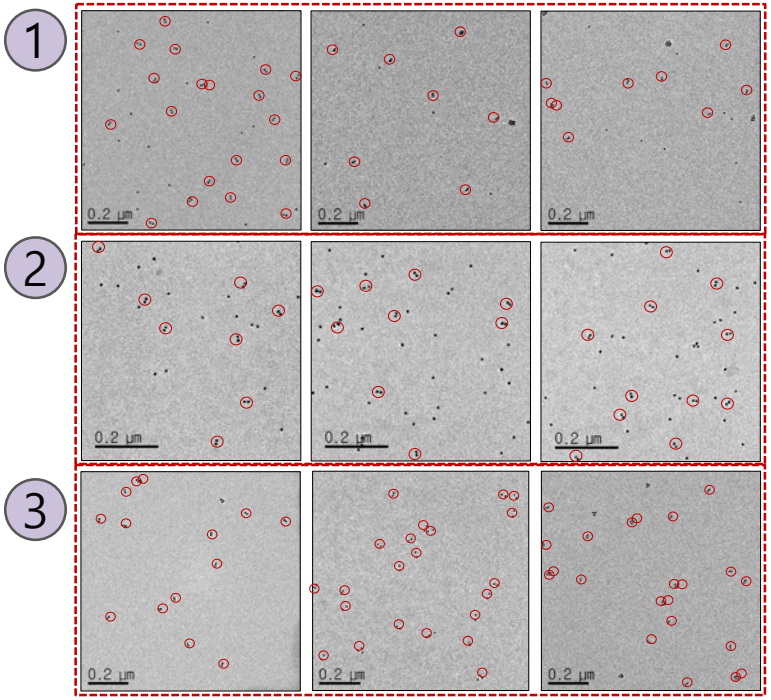


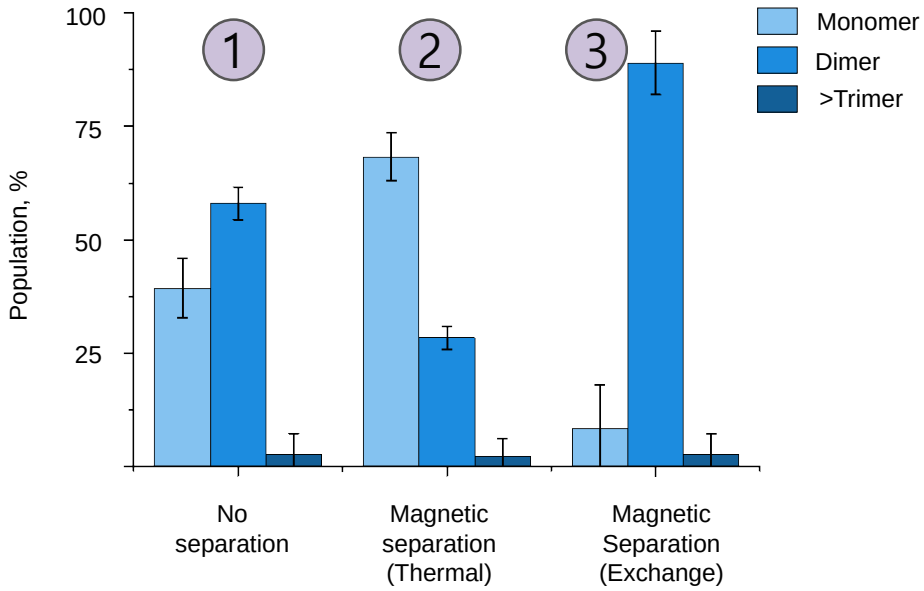
Fabrication Yield of (Plasmonic) Nano Assembly

a Thermal Stability of Traptavidin

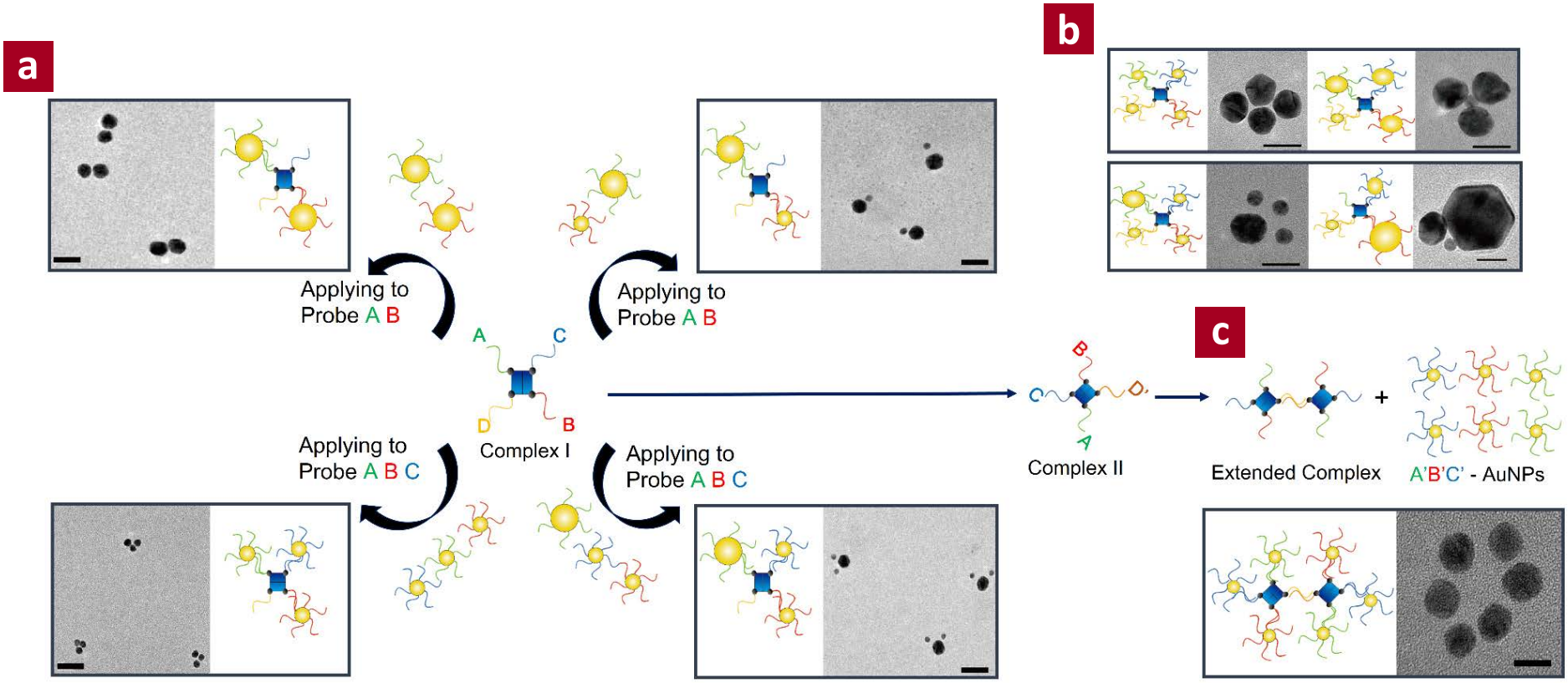


b Thermal vs. Exchange Release





Various Plasmonic Nanostructures

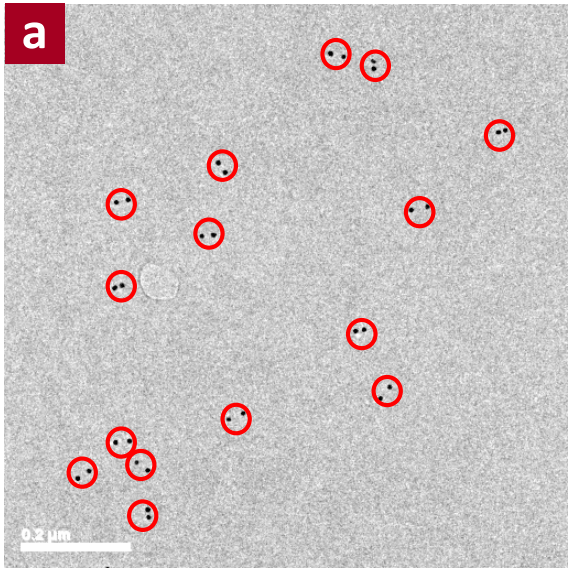


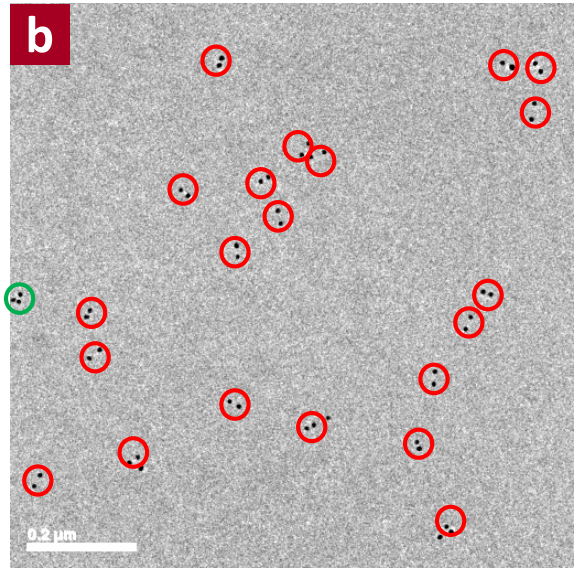
a Symmetric/asymmetric dimer clusters and symmetric/asymmetric trimer clusters

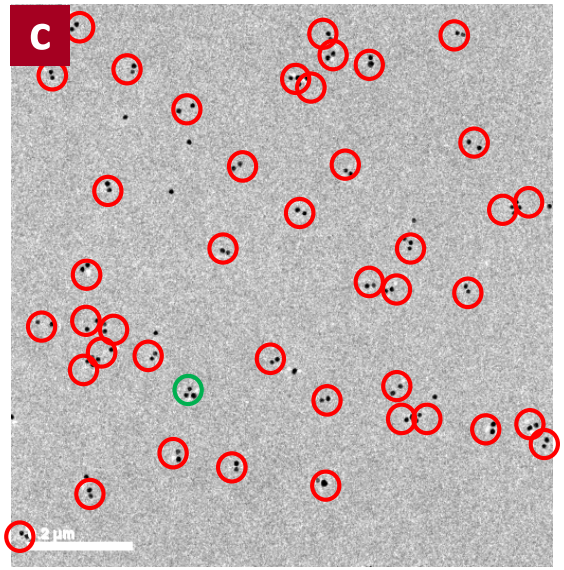
b Various plasmonic structures using four binding sites of the multivalent conjugates

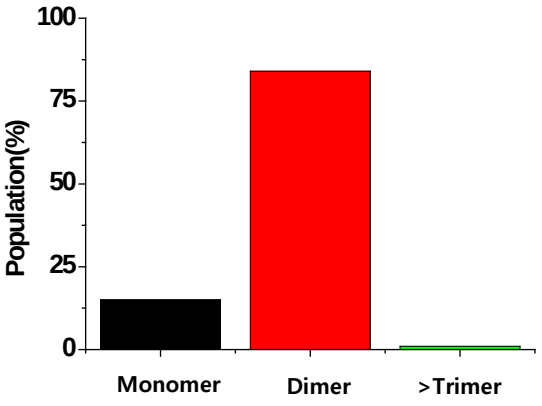
c A hexagonal plasmonic structure by connecting pre-programmed building blocks I & II

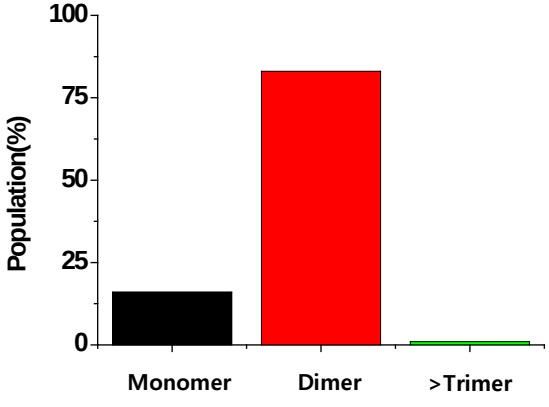
Plasmonic Homo-Dimers





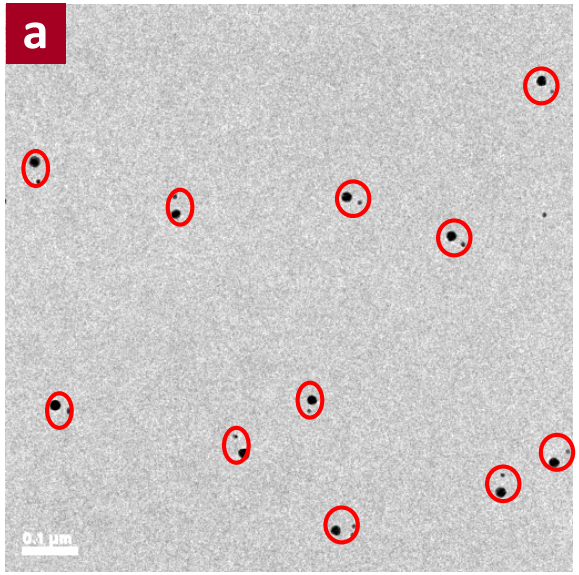


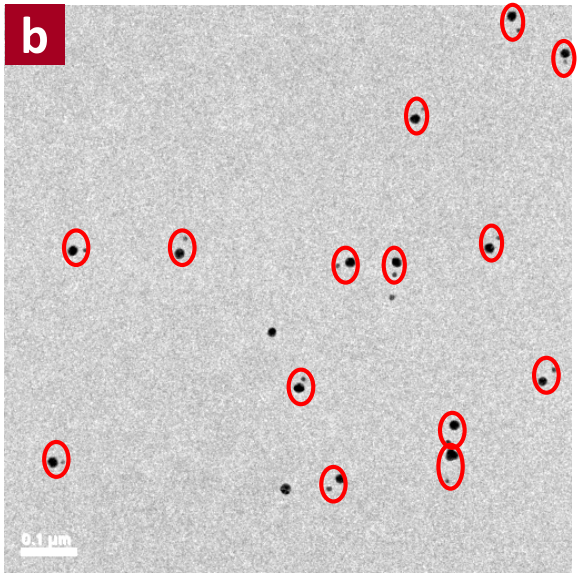


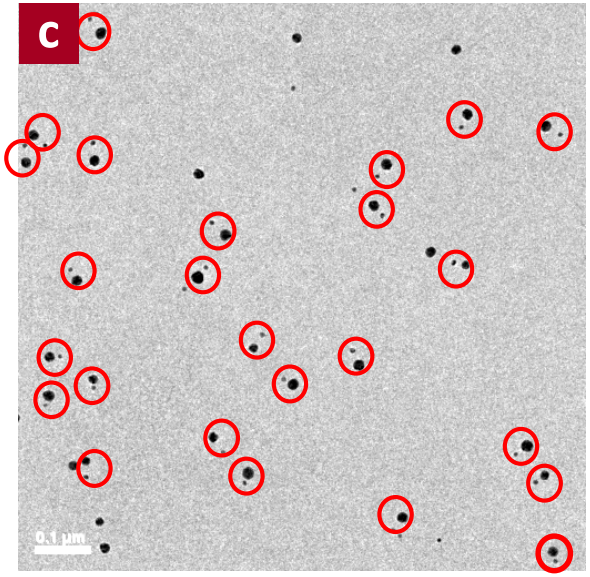


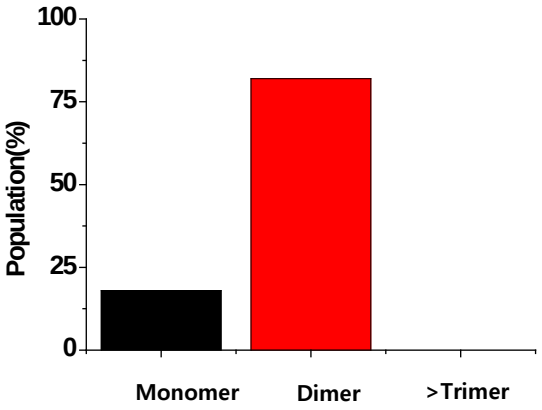
- Accurate number of binding sites for Traptavidin (**4**!!)
- Extra two binding sites

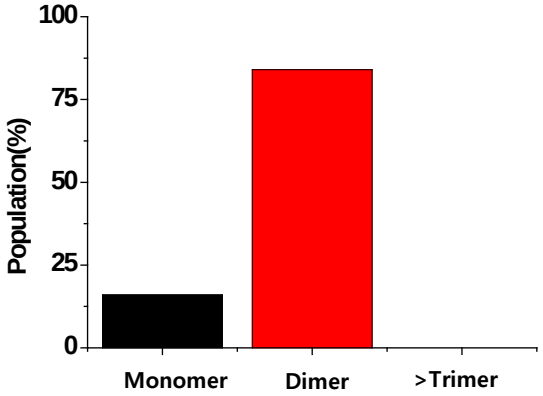
Plasmonic Hetero-Dimers

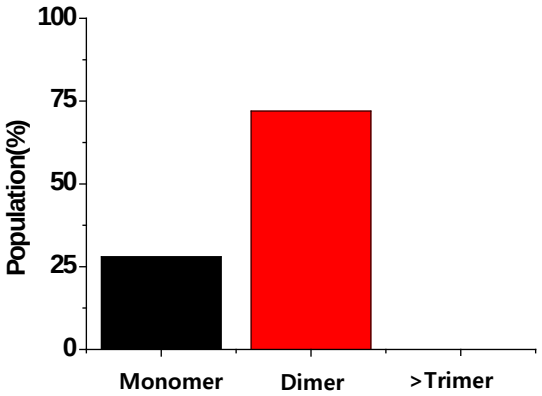






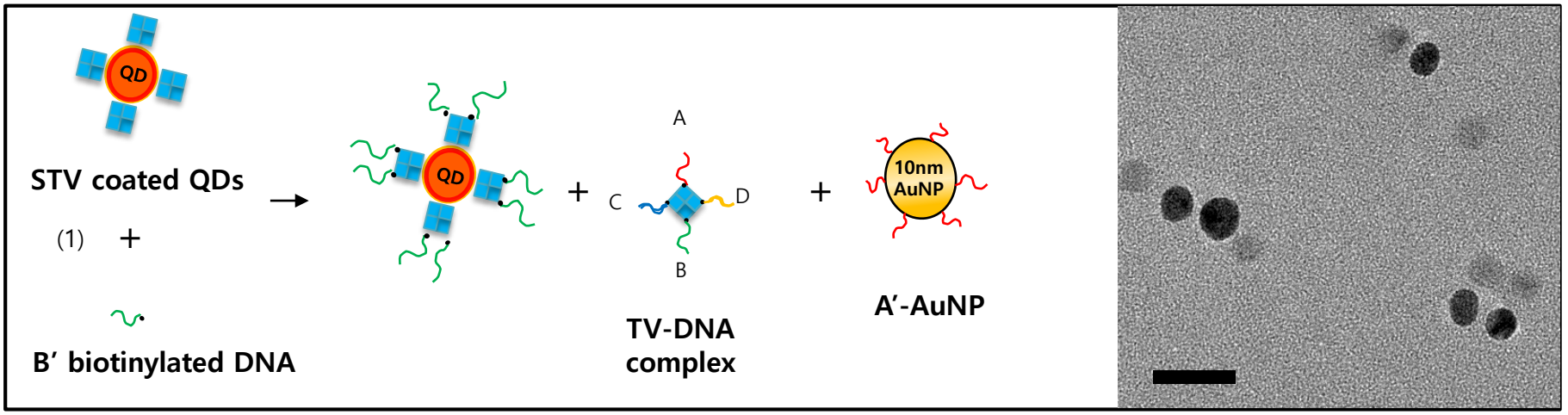




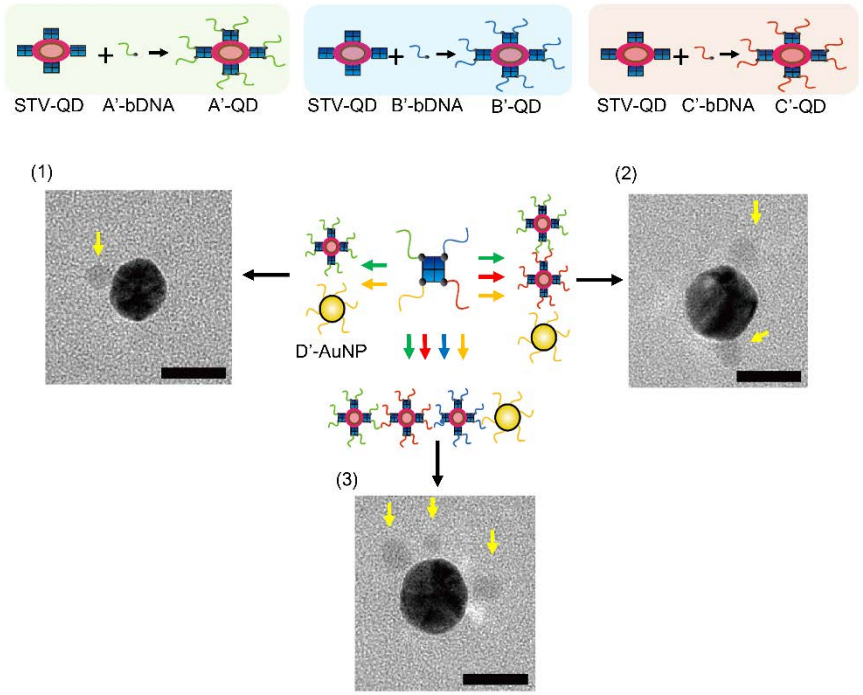


- Accurate number of binding sites for Traptavidin (**4**!!)
- Extra two binding sites

AuNP-Qdot Hetero-dimers, -trimers, -tetramers



Scale bar : 20nm



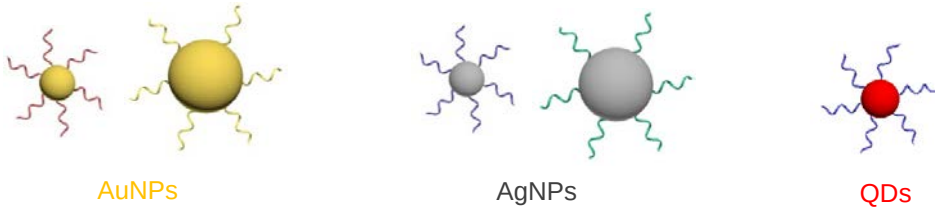
1) **Single QD and AuNP** bound to multivalent complex

2) **Double QD and AuNP** bound to multivalent complex

3) **Triple QD and AuNP** bound to multivalent complex

Various Plasmonic Nanostructures

Plasmonic atoms



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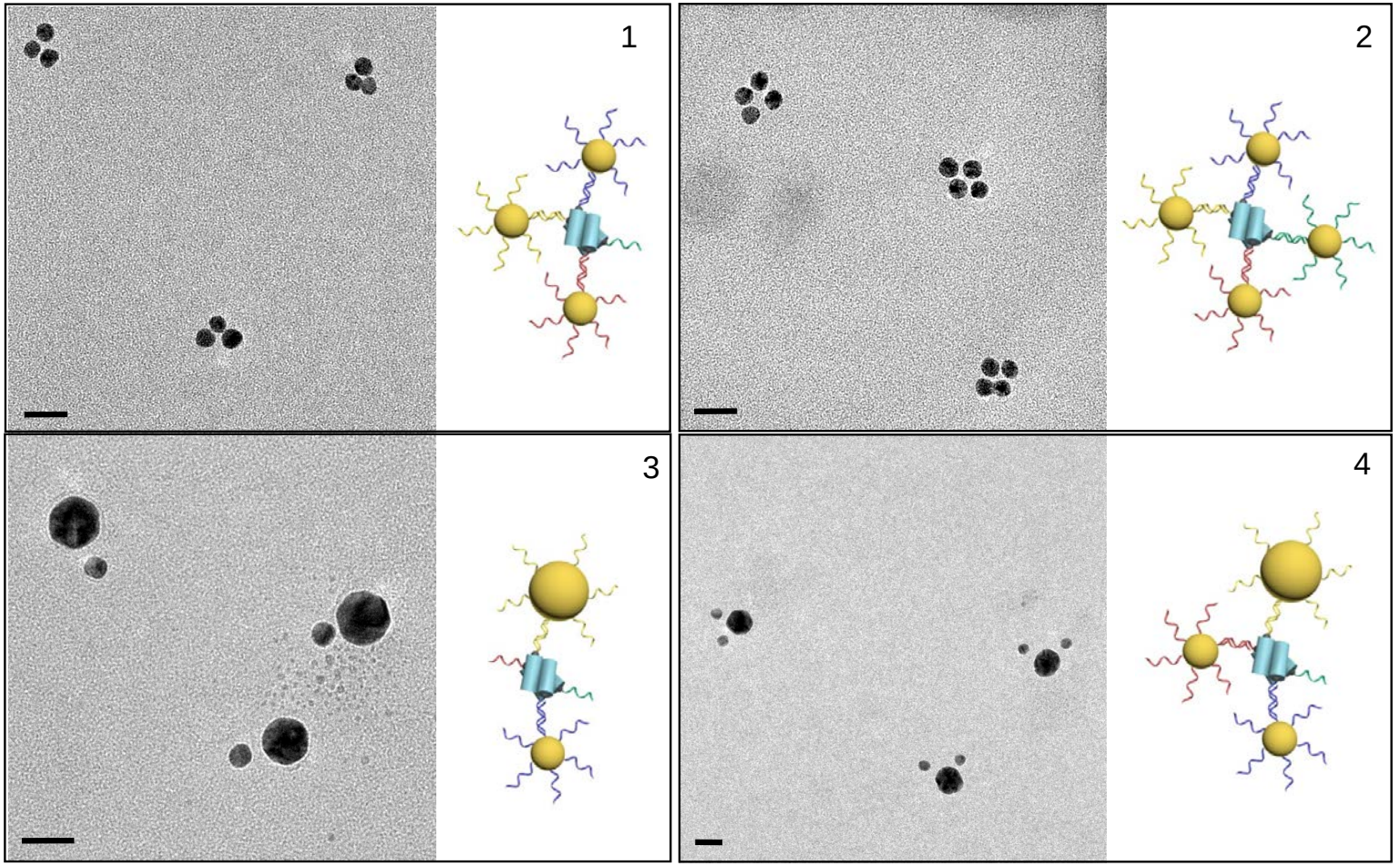
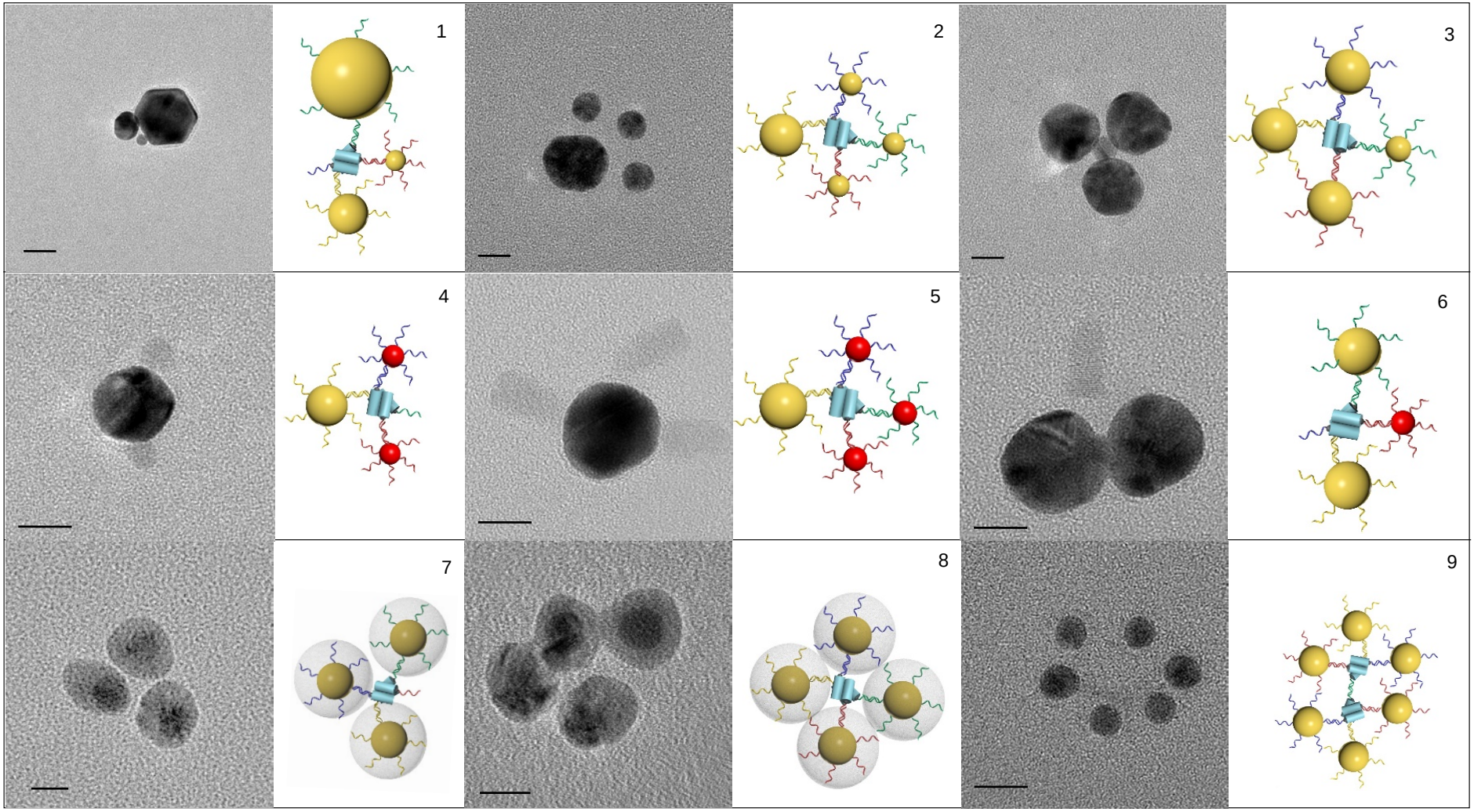


Various Plasmonic Nanostructures



Thank you for your attention.

Questions?

화학생물공학부 나노바이오공학특론 2016년 11월 24일(목)

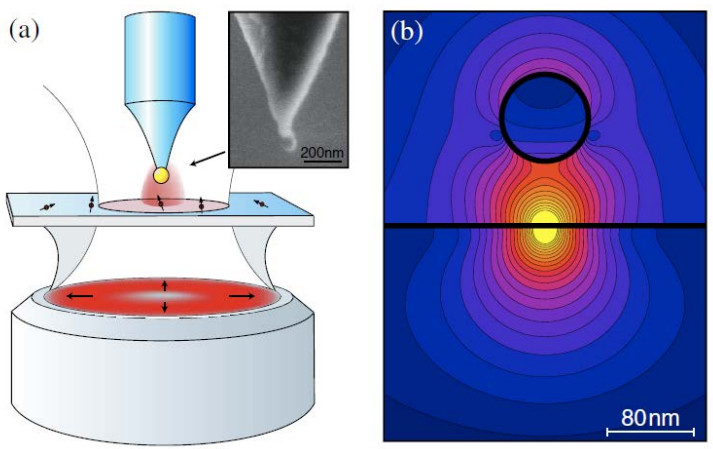
Programmable Assembly of Nanomaterials Using Biopolymers : Applications

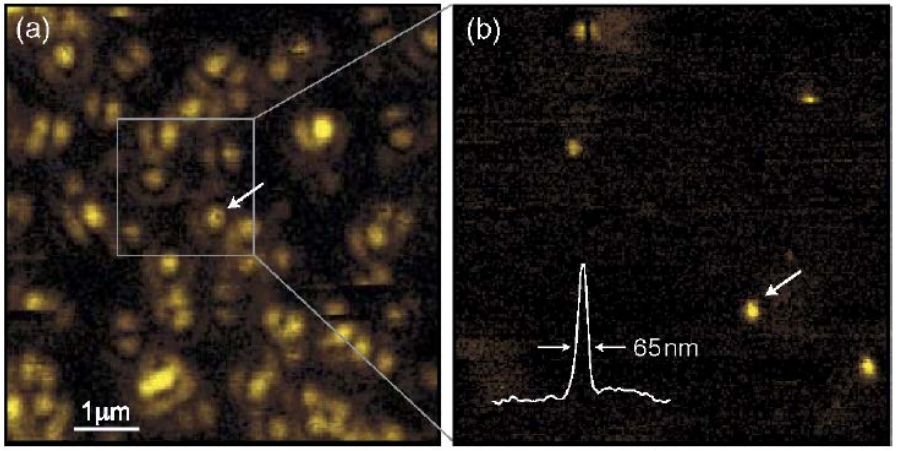
Yoon-Kyu Song

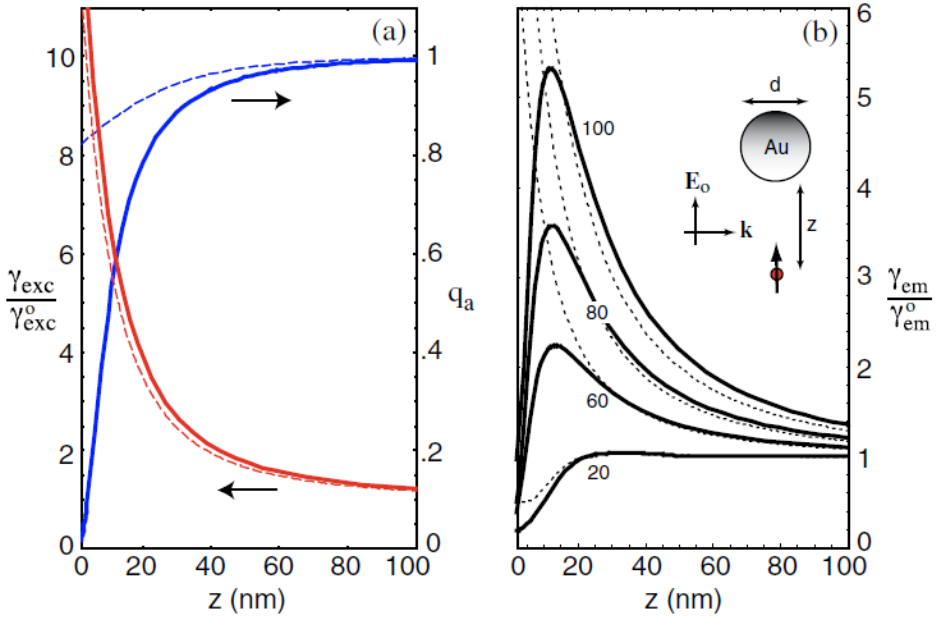
Graduate School of Convergence Science and Technology (GSCST)
Seoul National University

서울대학교 융합과학기술대학원

Fluorescent Enhancement in Plasmonic Nanoparticles





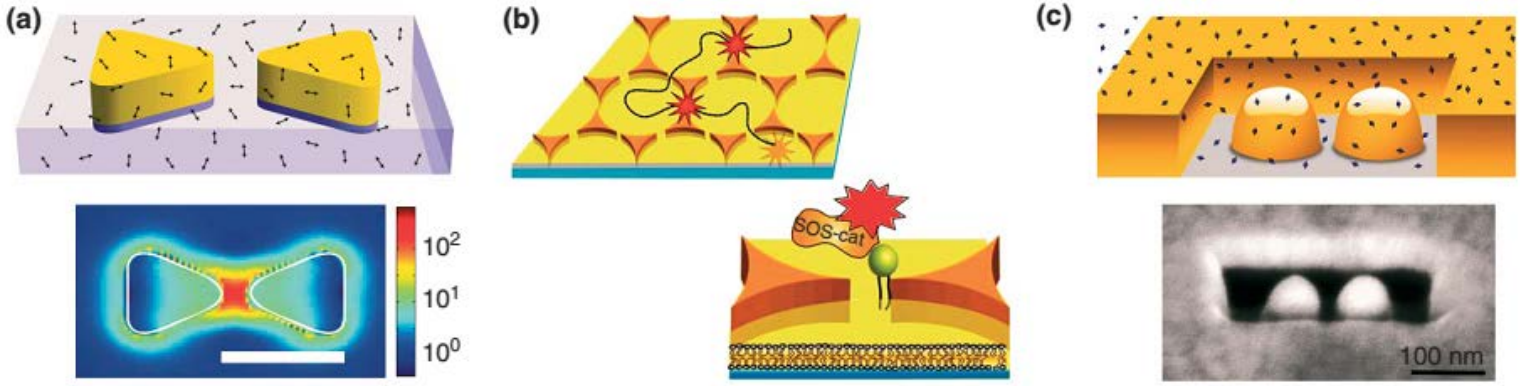


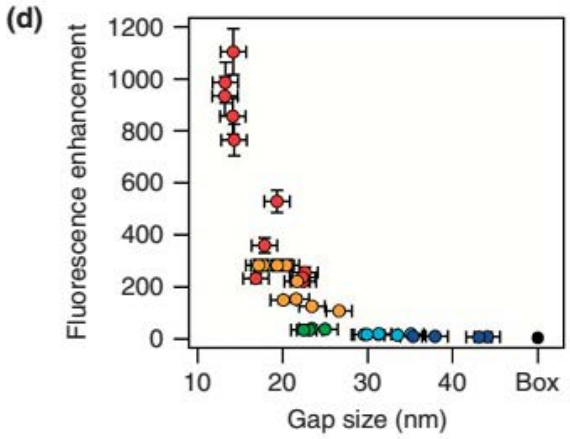
- Novotny et. al., PRL (2006)
- Enhancement vs. quenching
- Optimized for fluorescence enhancement
- More dramatic in Ag nanoparticles (x50)

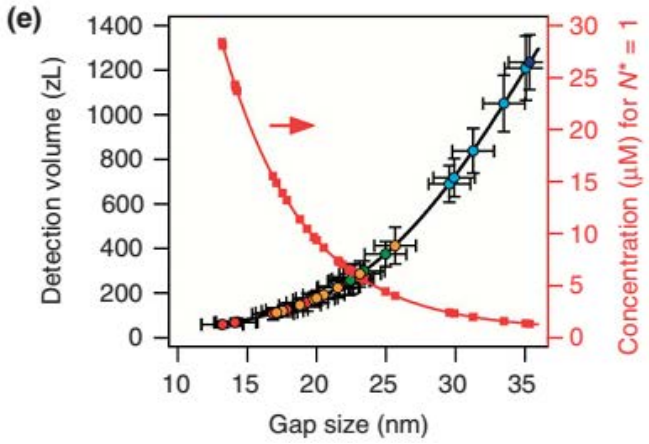
Plasmonic Antenna : Enhanced Optical Field at Nano Gap

- Increased optical field at nano-gap may enhance fluorescence and scattering
- Application to biosensors with ultra-high sensitivity

Top-Down Approach

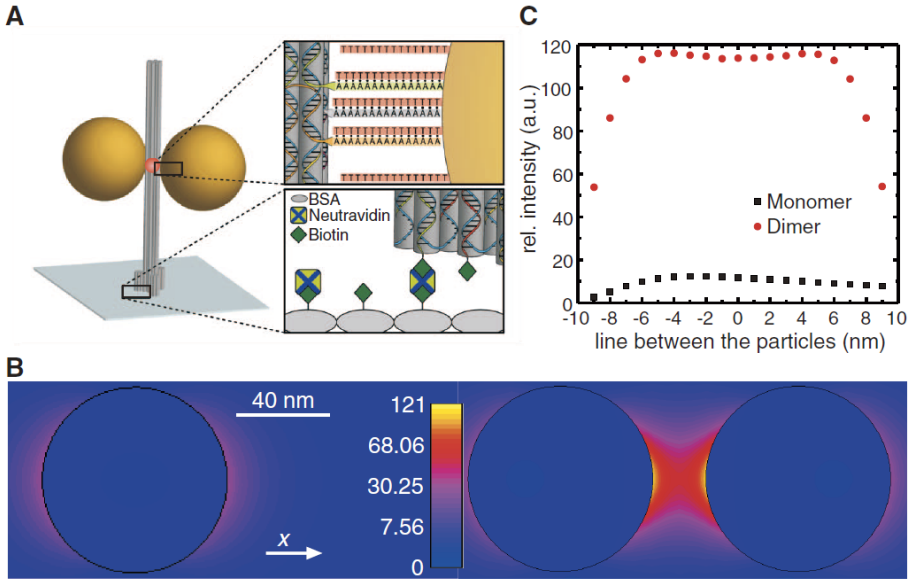




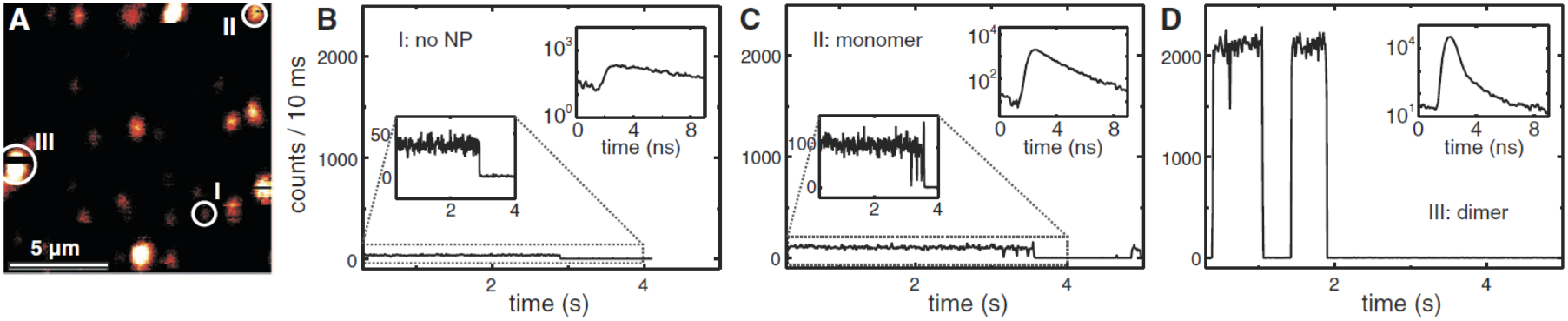


Plasmonic Antenna : Enhanced Optical Field at Nano Gap

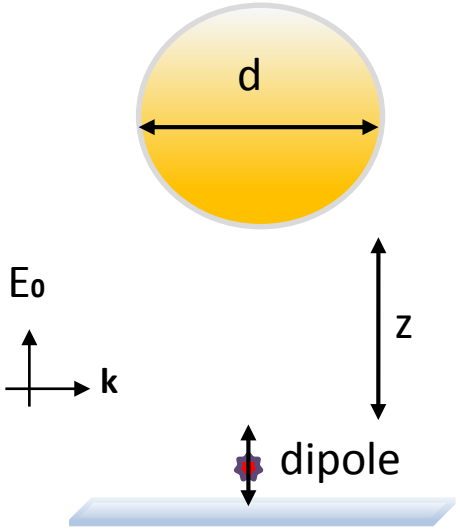
Bottom-Up Approach



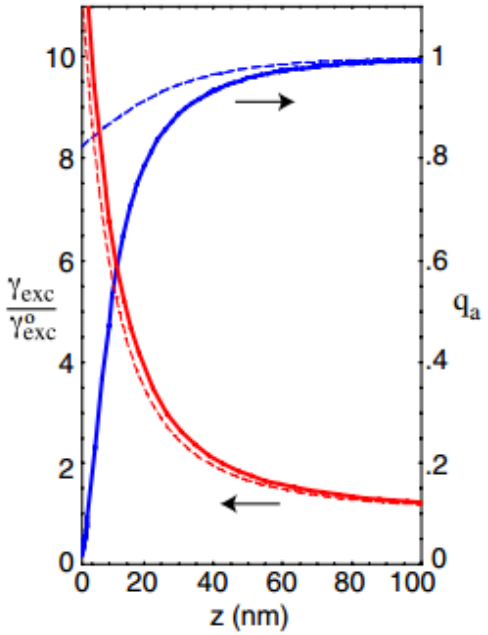
- Tinnefeld et. al., Science (2012)
- AuNP dimers on a DNA origami scaffold based “nano-post”
- 117-fold fluorescence enhancement
- Fluorescence lifetime measurements
- Application to DNA binding assay

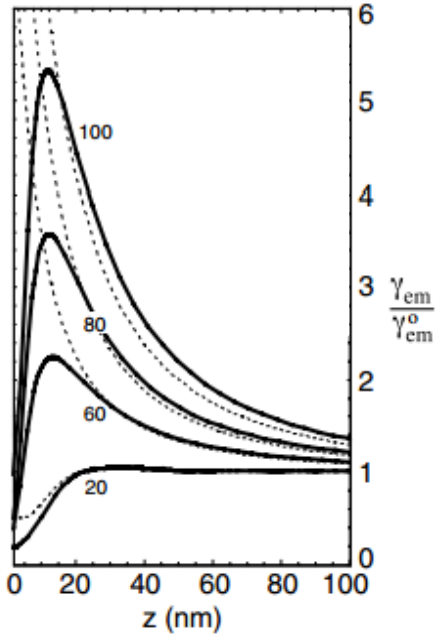


FDTD Simulation



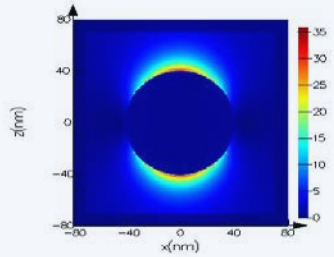
Phys. Rev. Lett. 96, 113002





$$\frac{\gamma_{em}}{\gamma^0_{em}} = \frac{\gamma_{exc}}{\gamma^0_{exc}} \frac{q}{q^0}$$

γ_{exc} : The excitation rate of the product
γ_{em} : The fluorescence rate
q : quantum yield



γ_{exc} ∝ Electric field

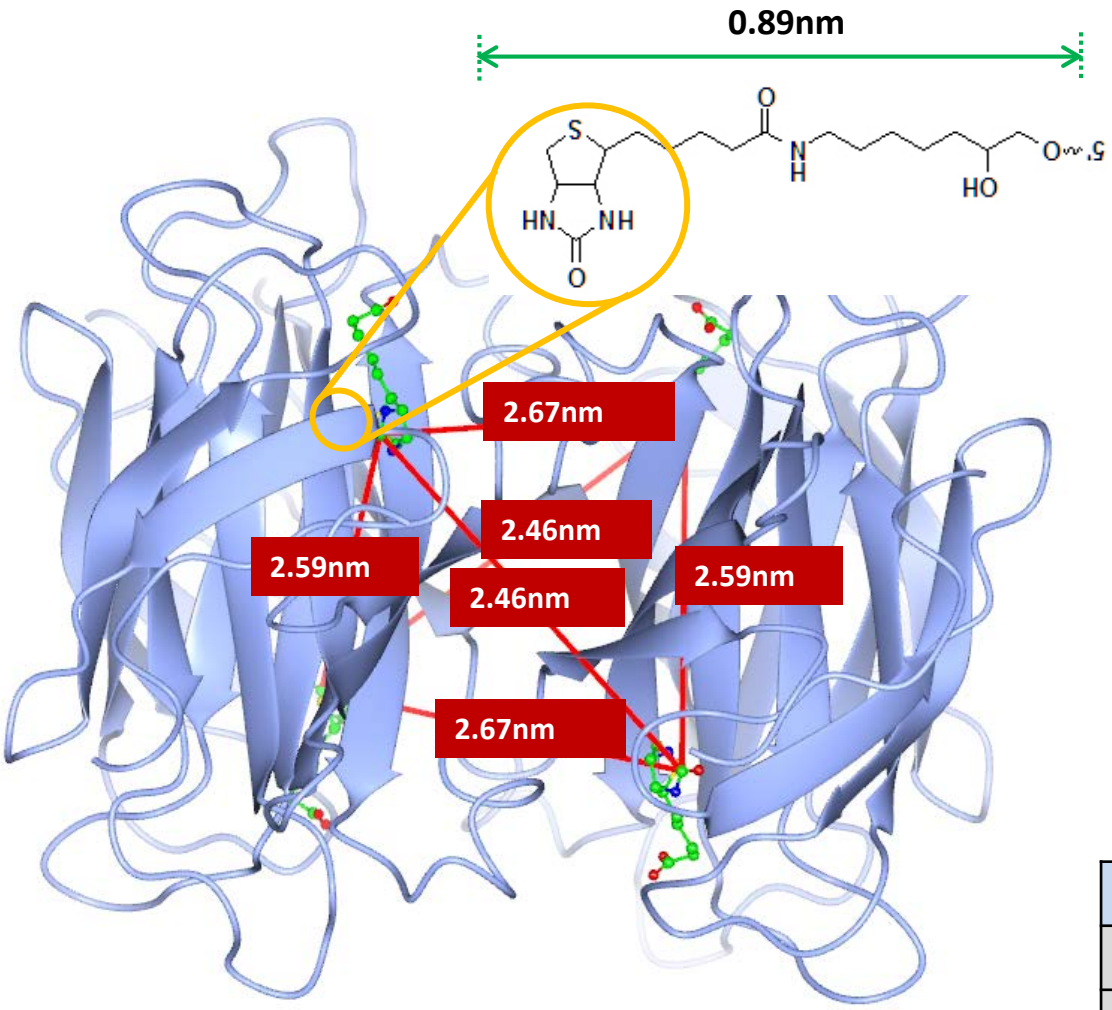
$$q^o = \gamma^o_r / (\gamma^o_r + \gamma^o_{nr})$$

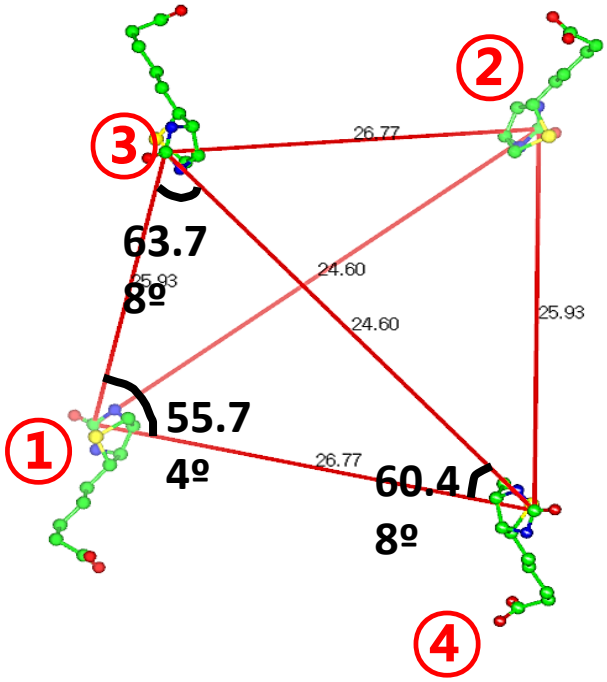
Quantum yield

$$q = \gamma_r / (\gamma_r + \gamma_{nr} + \gamma_{abs})$$

In the presence of the particle antenna

Dimensions of Traptavidin-Biotinylated DNA System





Tetrahedron with average side length of 4.36nm

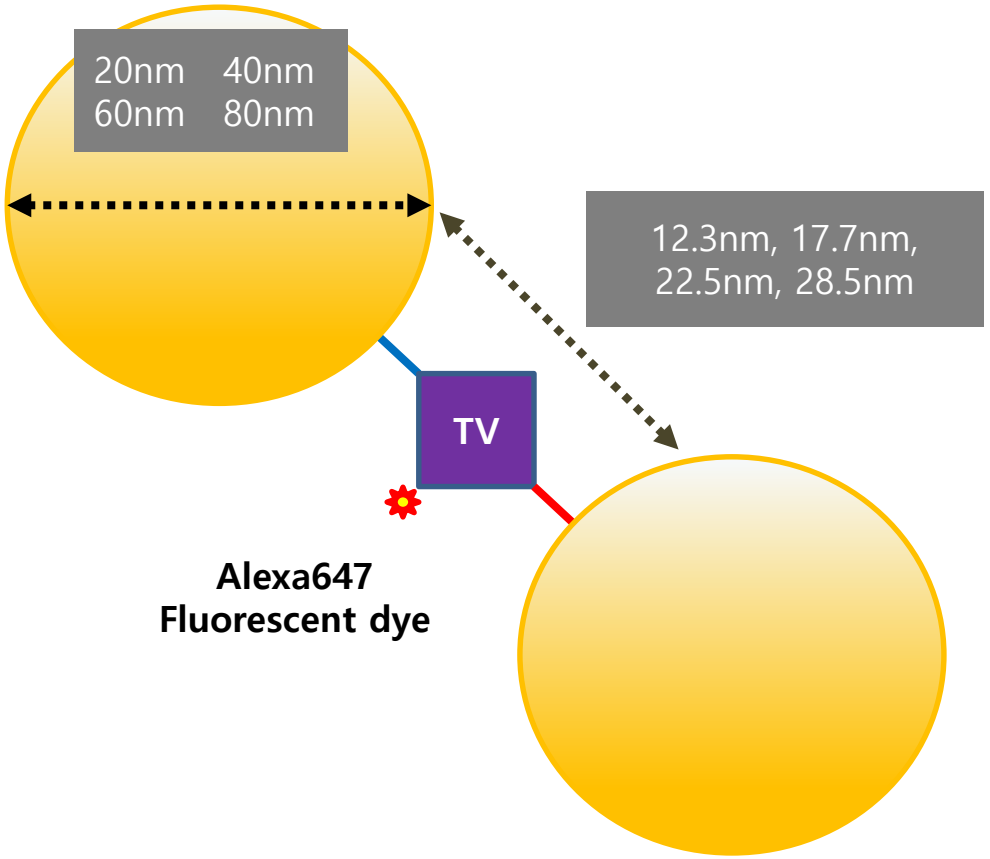
(Simulated by CCP4MG version 2.9.0.)

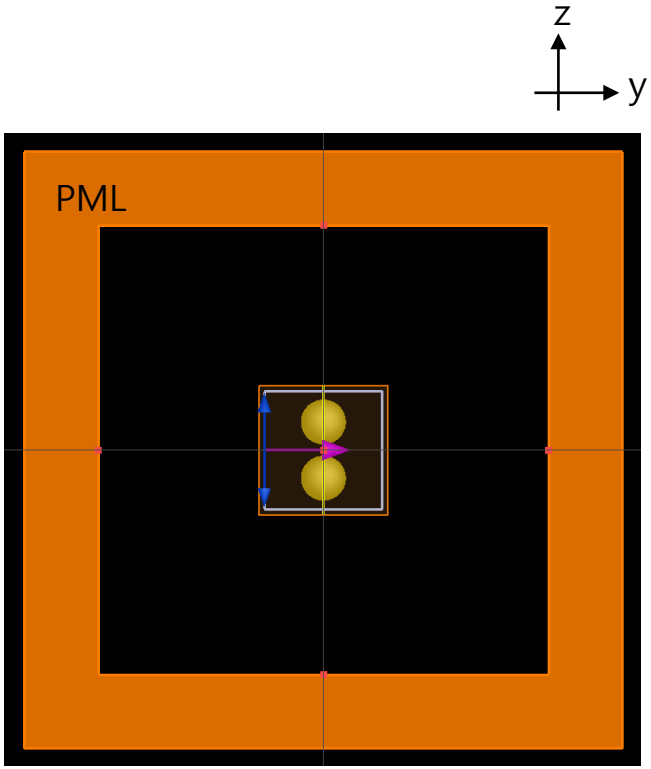
Gap size	Distance
12.3nm	4.5nm + (DNA 15bp)
17.7nm	4.5nm + (DNA 22bp)
22.5nm	4.5nm + (DNA 30bp)
28.5nm	4.5nm + (DNA 40bp)

** DNA 10bp = 3nm*

Simulation : Excitation Rate & Quantum Yield

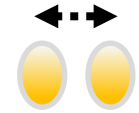
- Lumerical FDTD
- Diameter = 20nm, 40nm, 60nm, 80nm AuNP
- Gap size = 12.3nm, 17.7nm 22.5nm 28.5nm gap
- Surrounding medium = water (n=1.33)
- Incident light = 642nm plane wave (z-polarization, y-propagation)



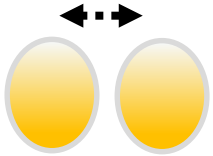


Simulation Results : Electric Field of Dimer AuNPs

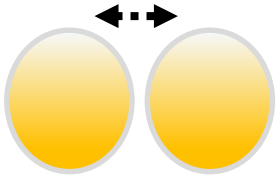
12.3nm, 17.7nm, 22.5nm, 28.5nm



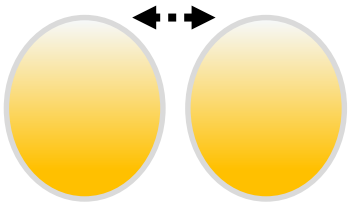
20nm



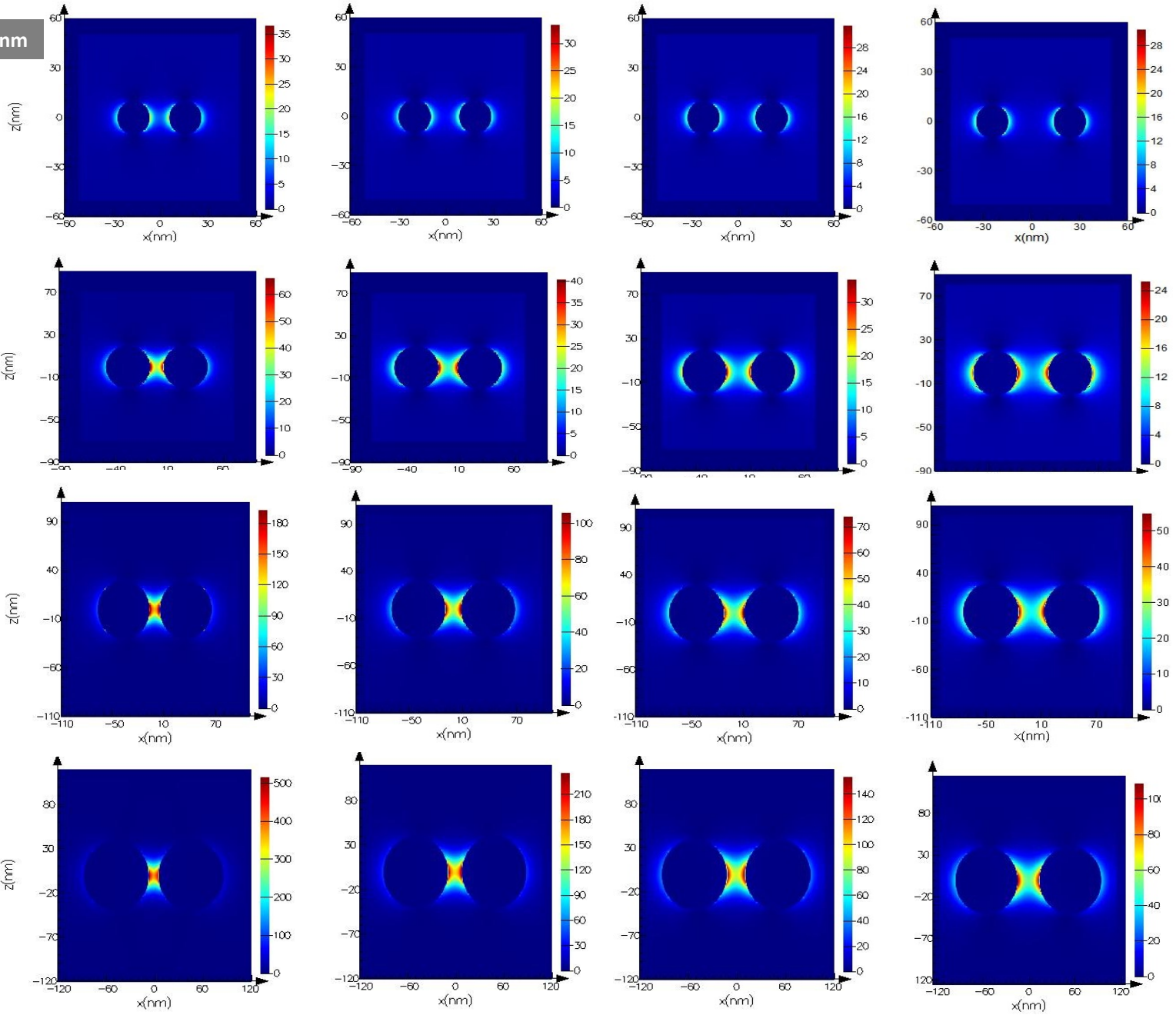
40nm



60nm



80nm



Simulation Results : Quantum Yield of Dipole

Scattering & Absorption Cross-section

$$C_{scat} = \frac{1}{6\pi}\left(\frac{2\pi}{\lambda}\right)^4 |\alpha|^2; \quad C_{abs} = \frac{2\pi}{\lambda}\mathrm{Im}[\alpha]$$

$$\alpha = 3V\frac{\omega_p^2}{\omega_p^2 - 3\omega^2 - i\gamma\omega} = 3V\left[\frac{\varepsilon_p/\varepsilon_m - 1}{\varepsilon_p/\varepsilon_m + 2}\right]$$

α polarizability of the particle

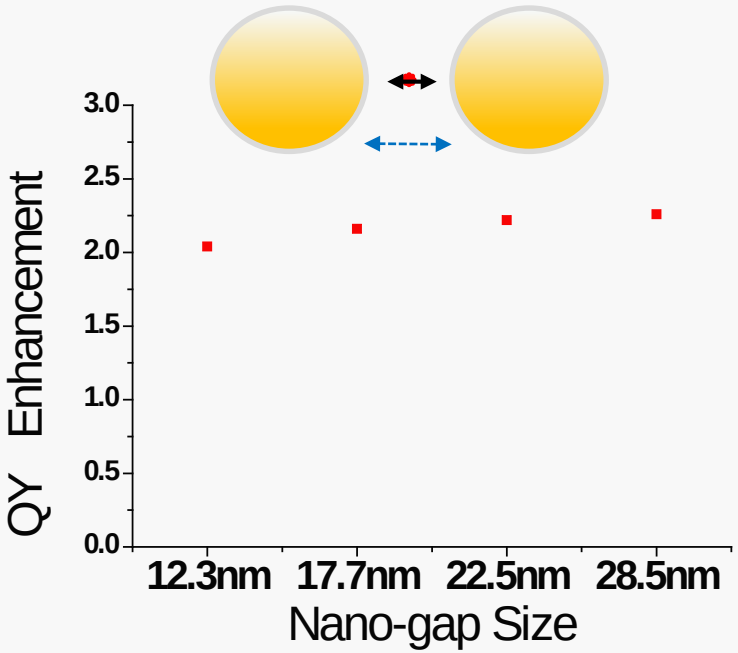
V particle volume

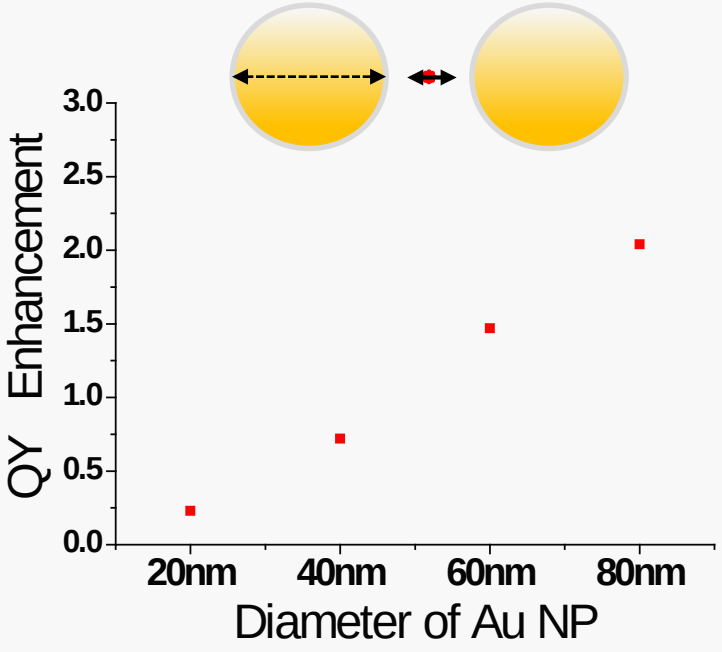
ε_p dielectric function of the particle

ε_m dielectric function of the medium

$$q = \gamma_r/(\gamma_r + \gamma_{nr} + \gamma_{abs})$$

In the presence of the particle antenna



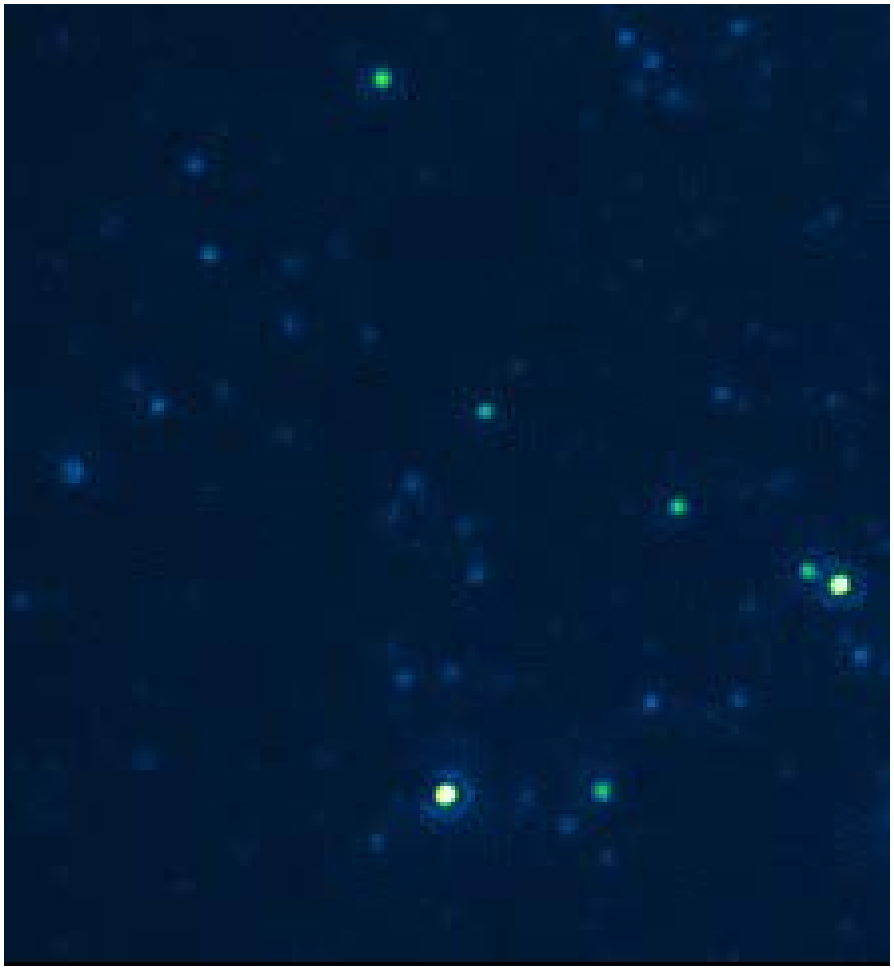


Experimental Results :: Dimer AuNPs

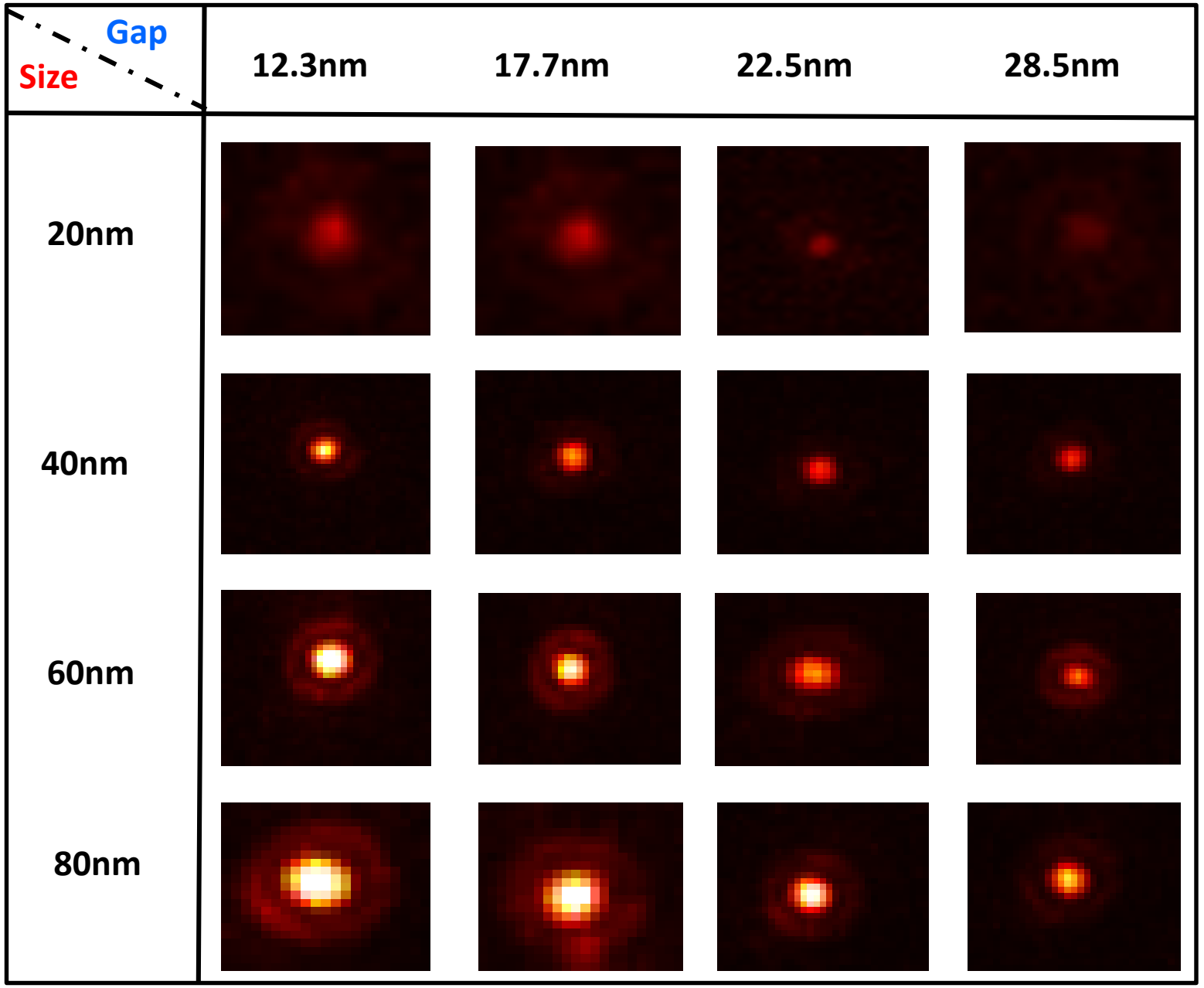
642nm laser (150mW)

TIRF(total internal reflection fluorescence)

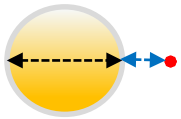
Blinking buffer (Oxygen Scavenger System)

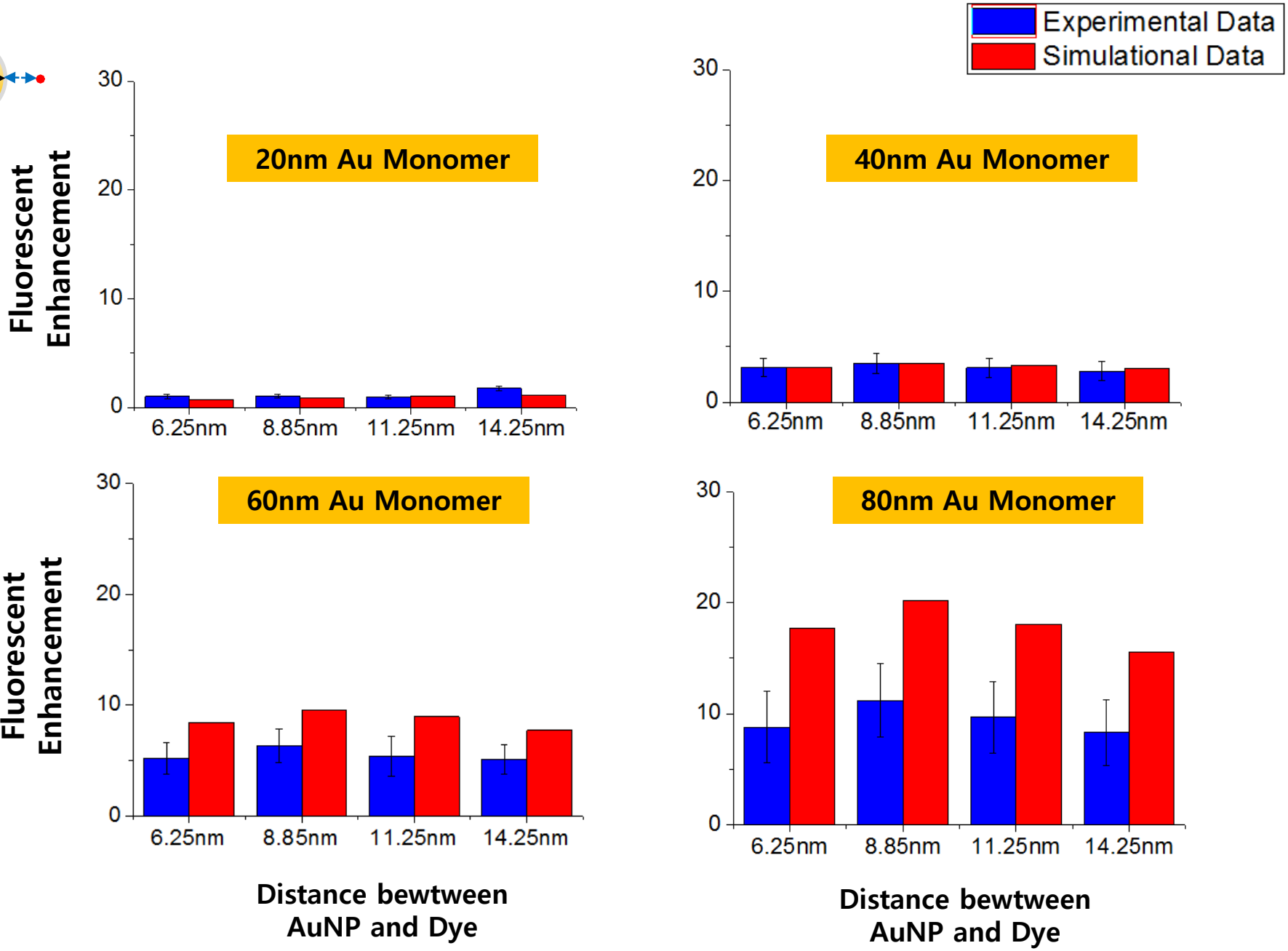


Experimental Results :: Dimer AuNPs

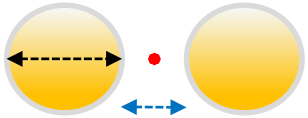


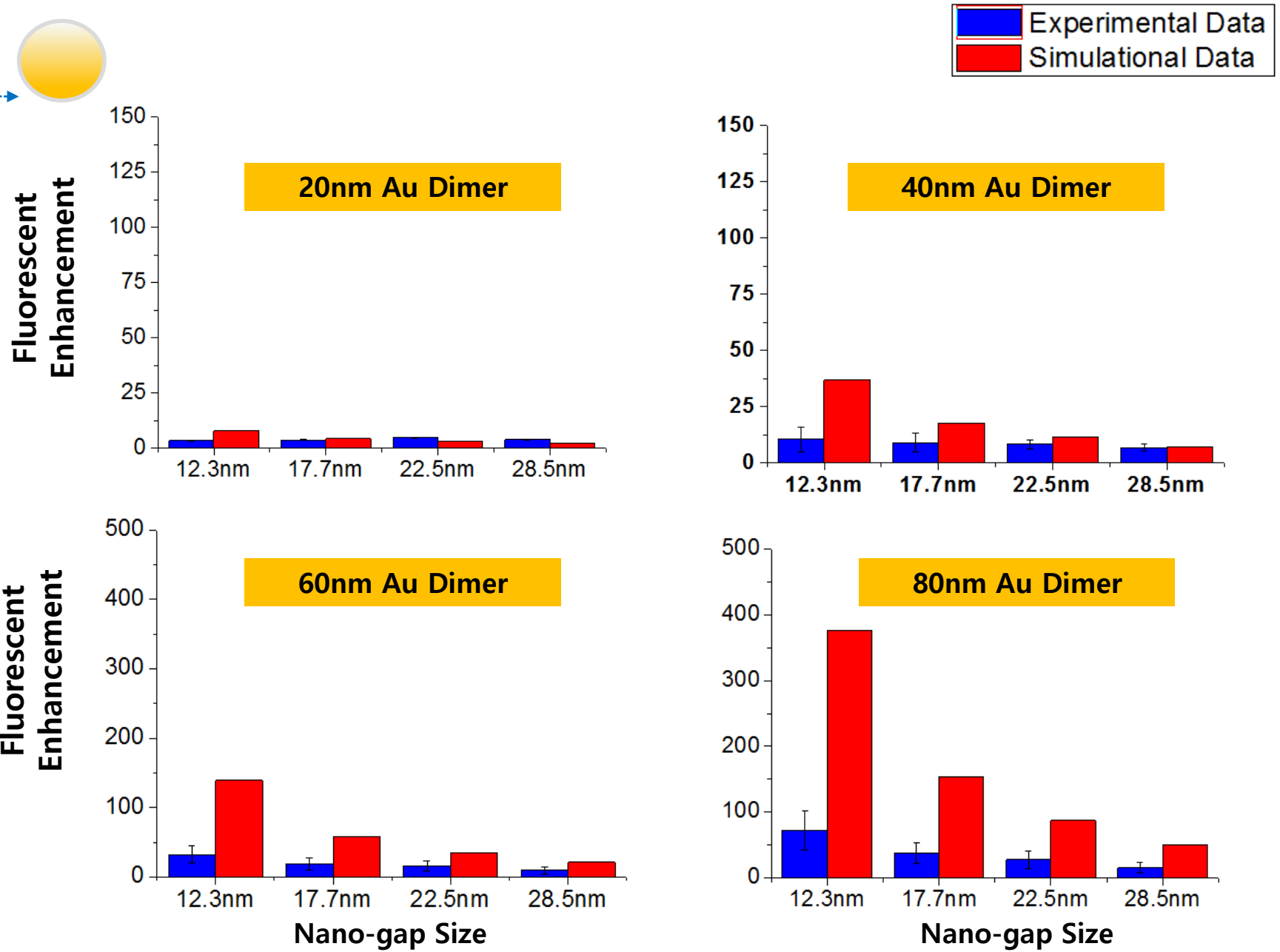
Fluorescent Enhancement : Monomers





Fluorescent Enhancement : Dimers



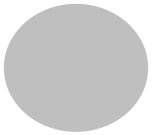


Discussion : Applications and Future Directions

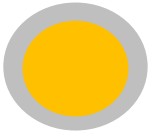
- **Molecular Building Blocks for Nano-Photonic Applications**

Fluorescent Enhancement Platform of Nano-gap Antenna

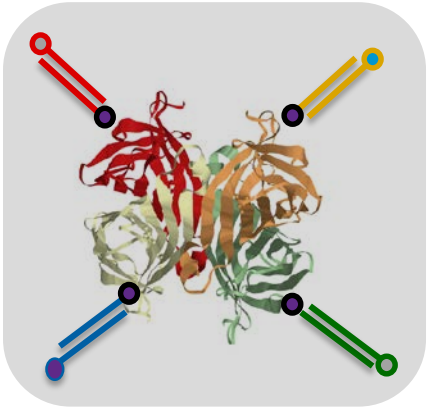
Short wave legnth



Silver Nanoparticle



Core-shell Nanoparticle

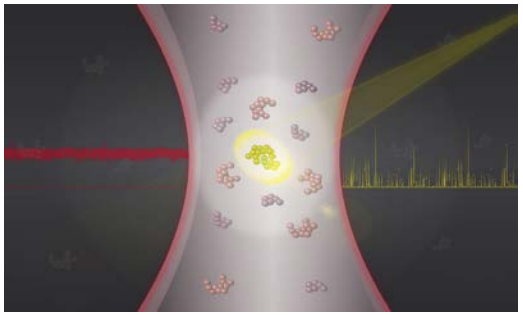


Long wave length

Au Nanorod

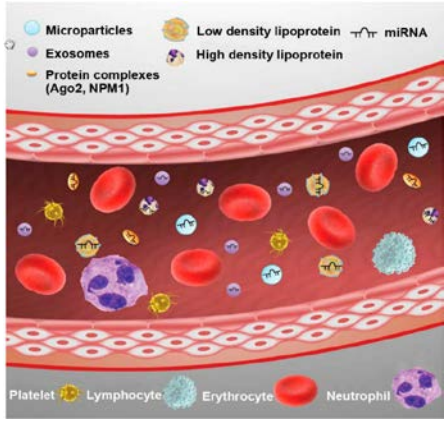
Au Ring

Single Molecule Detection



- Research on single molecule biophysics
- Dynamics of biological mechanism

Biosensor (Low Concentration)



Circulating miRNA detection

⬇

Very Low Concentration (pM , fM)

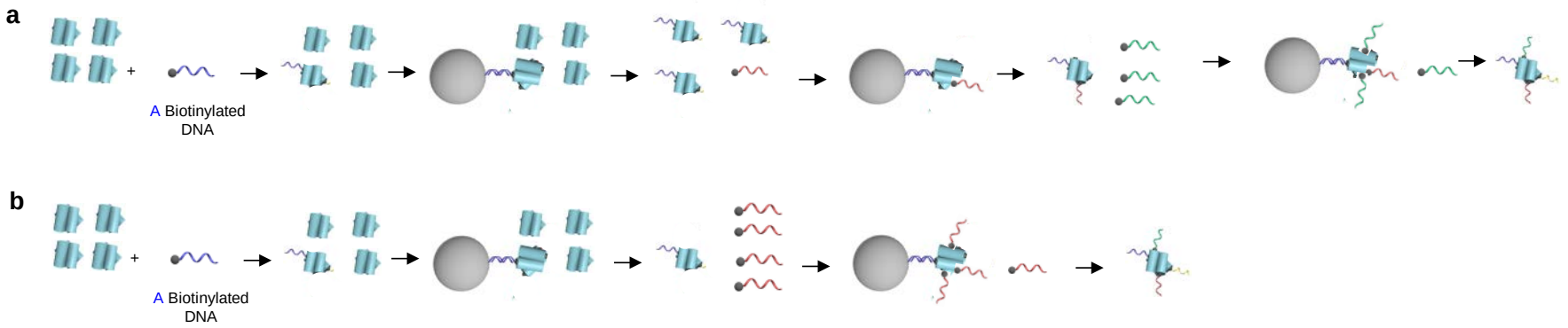
⬇

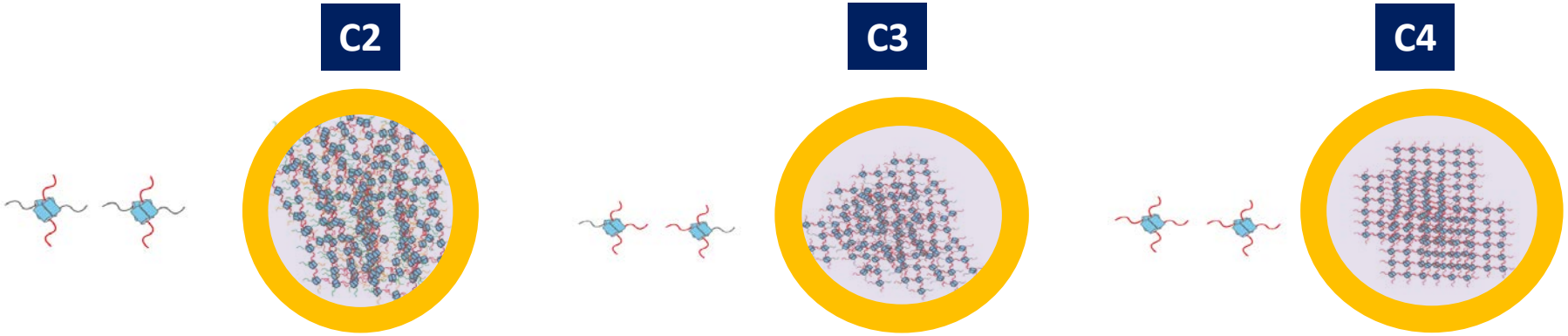
Fluorescence Enhancement with Nano-gap

Application of Nanoassembly: Carbon-Like Structures

- C1 structure: One DNA with complimentary pairing – *dimer*
- C2 structure: Two DNAs with complimentary pairing – *linear chain*
- C3 structure: Three DNAs with complimentary pairing – *denrimeric particle*
- C4 structure: Four DNAs with complimentary pairing – *extended aggregates (?)*

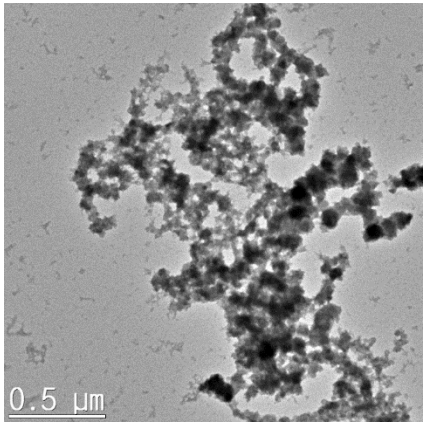
Examples of valency controls

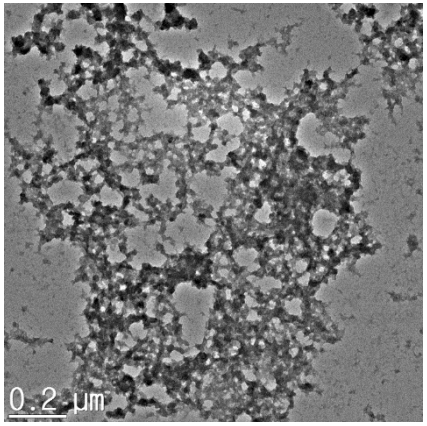


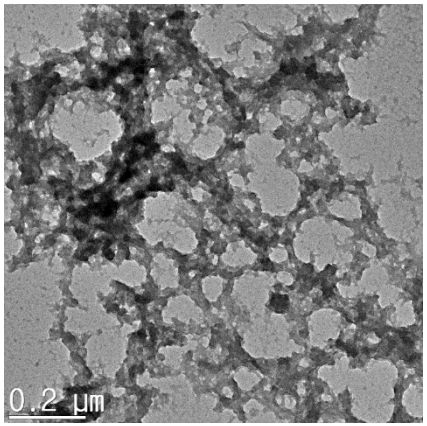


Application of Nanoassembly: Carbon-Like Structures

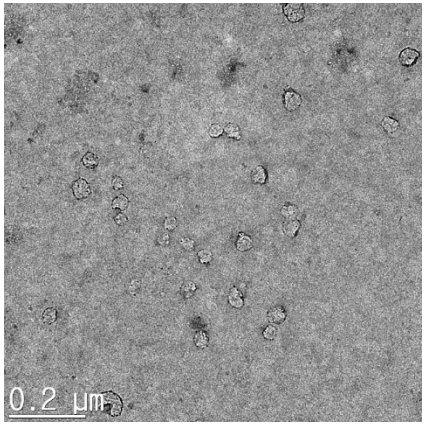
C2

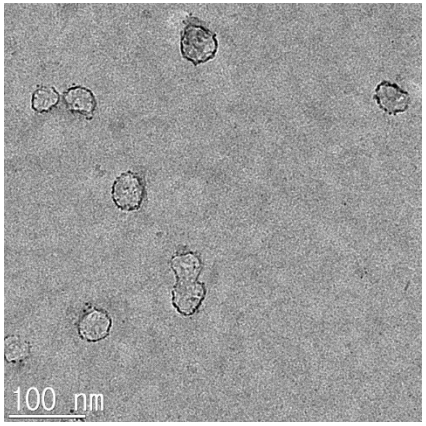


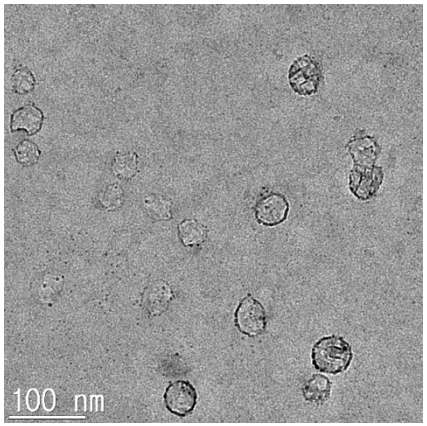




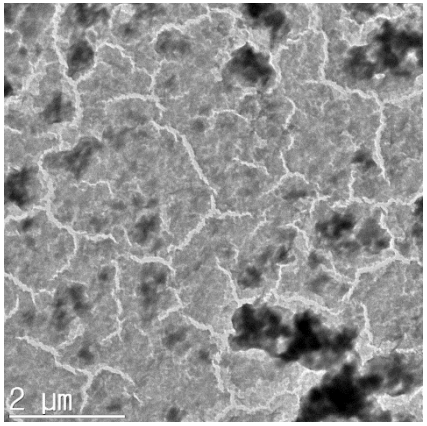
C3

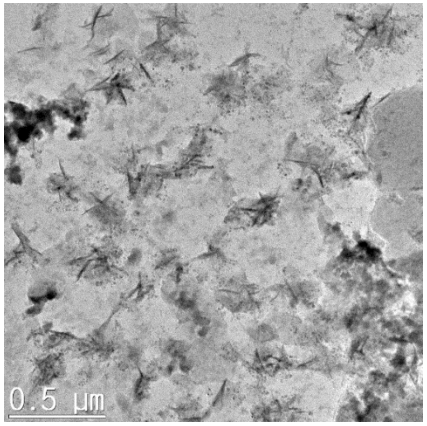


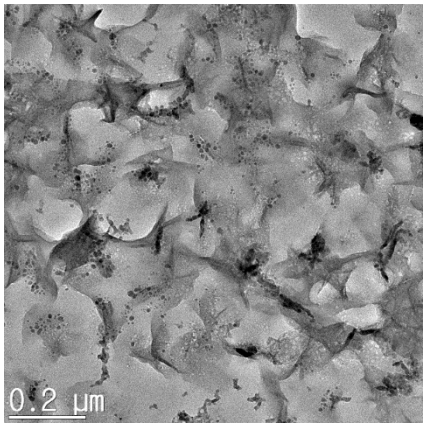




C4



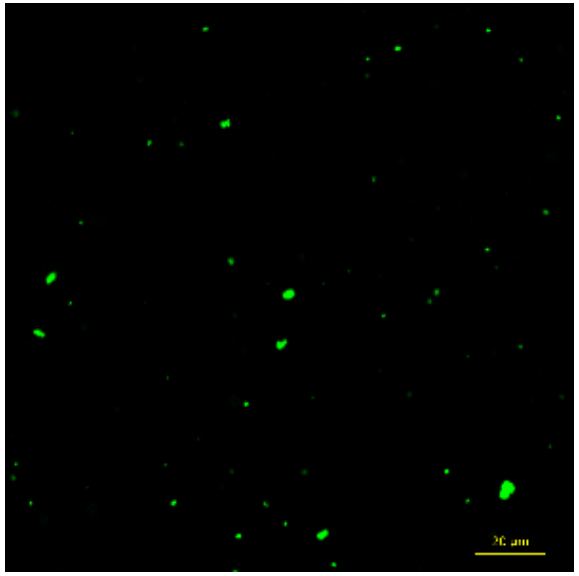




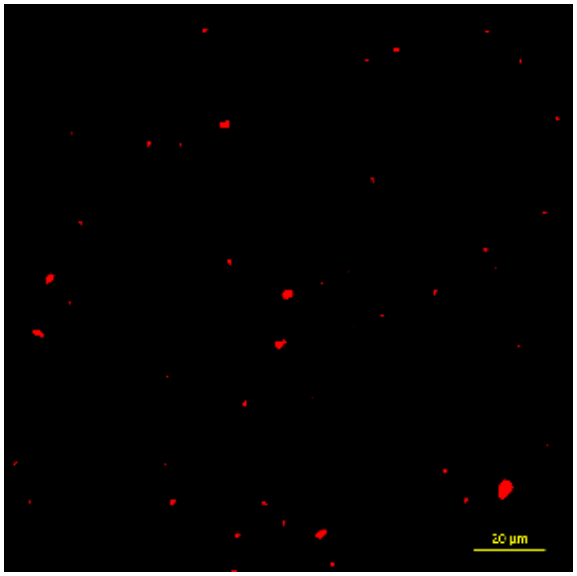
Application of Nanoassembly: Carbon-Like Structures

- FRET Analysis on C3 Structures

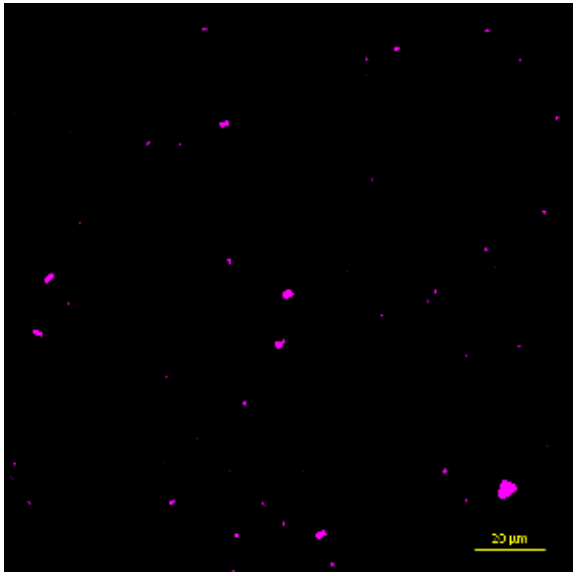
  Verification of coexistence of complimentary TA-DNA molecules using FRET



Cy3



Cy5

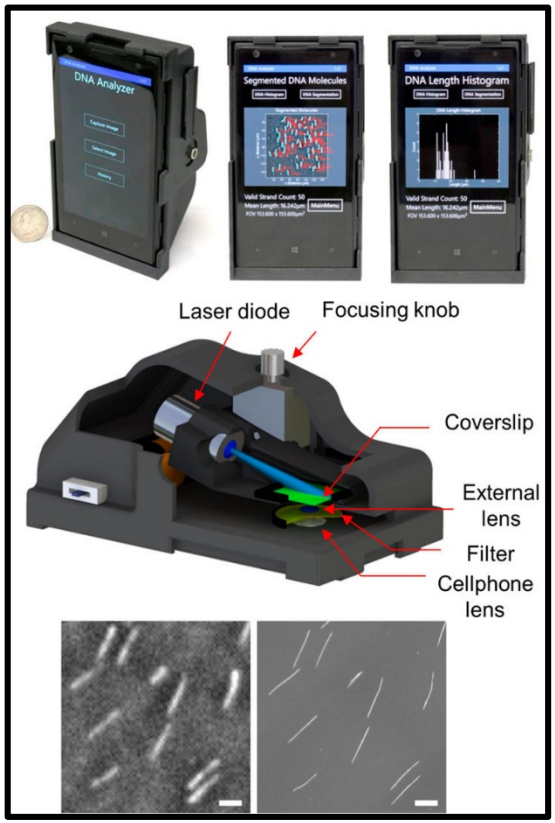


Merge

High degree of cross membrane penetration in C3 structure applicable to drug delivery system

Applications to POC Diagnostics Using Smart Phone

DNA Imaging



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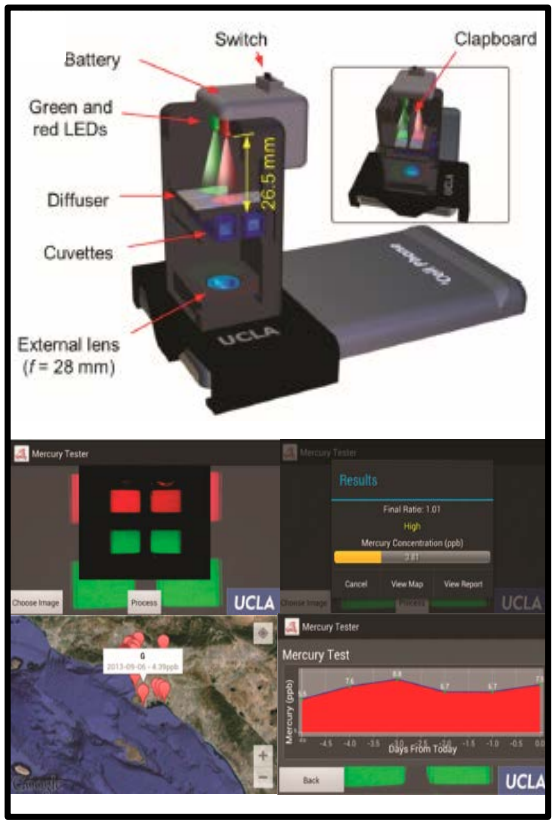
- Optical image of single λ-DNA (~20μm)
- Size dependent detection
- Low magnification

Hg Detection



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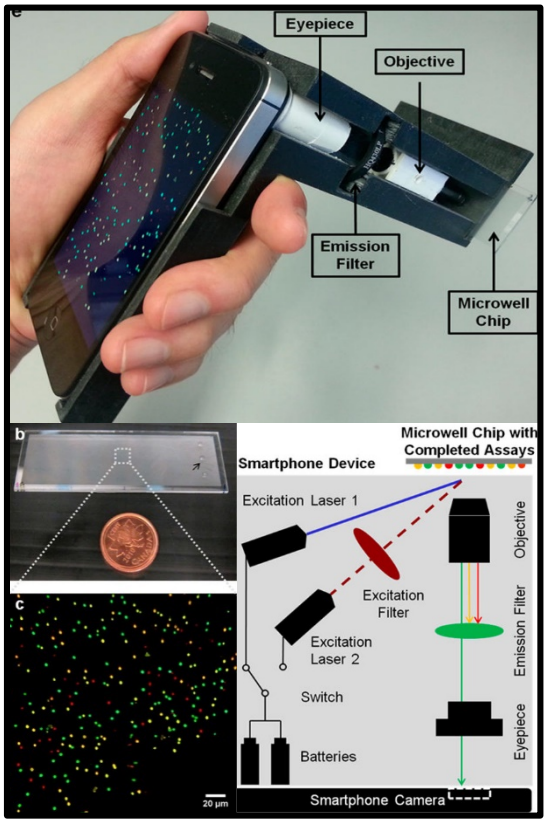
- Hg^{2+}detection using colorimetric sensing
- Quantitative detection of Hg^{2+}
- Mapping contaminated area using smartphones

Virus Detection



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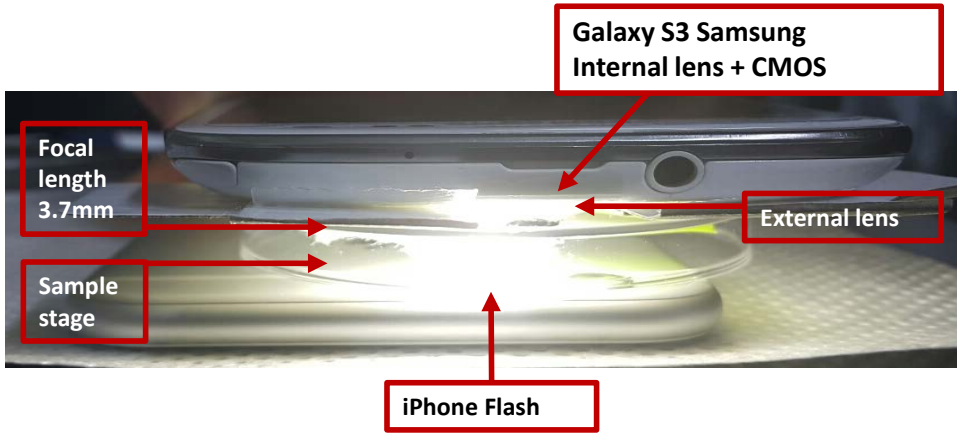
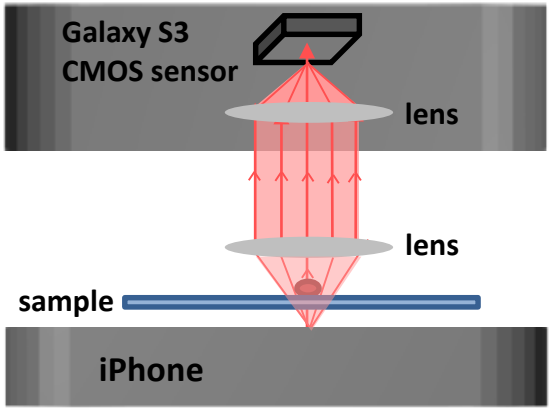
- QD cluster bar-code
- Multiple detection of 3 analytes simultaneously
- Requires pre-purification/ amplification steps

Applications to POC Diagnostics Using Smart Phone

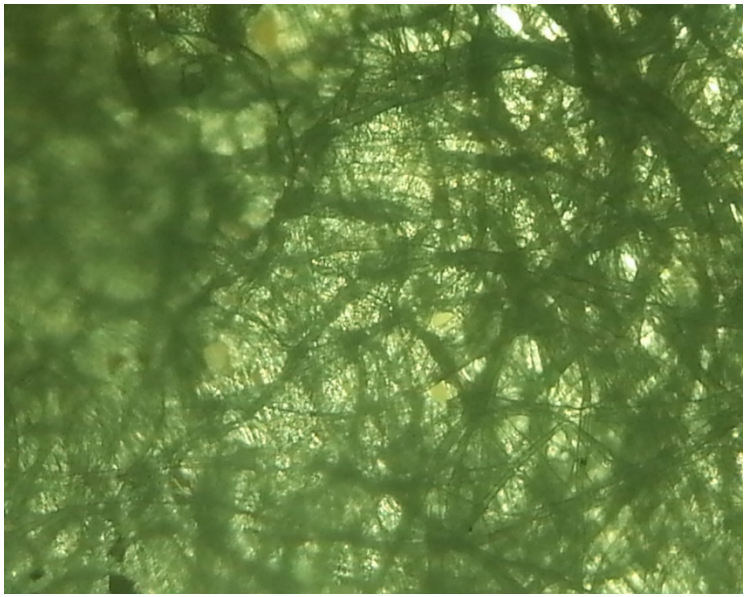
- Fluorescence Microscope Using Smart Phones





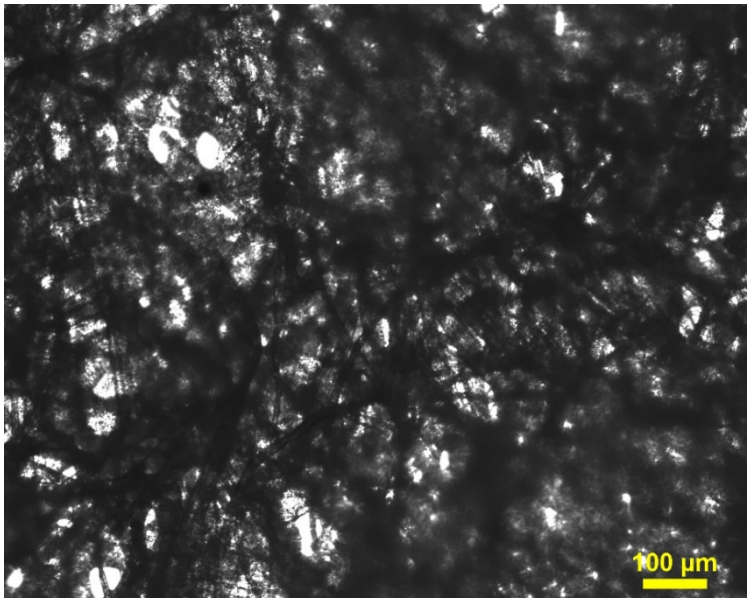
A smart phone microscopy

Galaxy S3 Samsung CMOS sensor



A conventional microscopy

CFI Plan Fluor 10x (NA=0.30) objective lens

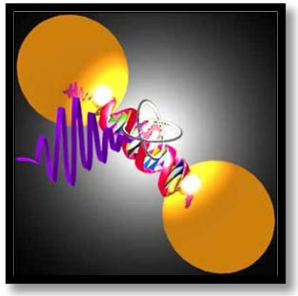


Applications to POC Diagnostics Using Smart Phone

- POC Detection System Based on Smart Phone Microscope

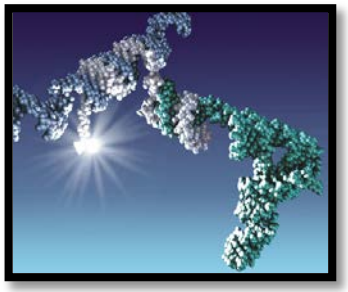
나노안테나 - 형광증폭

- 고감도 형광신호를 통해 스마트폰 CMOS 센서에서 감지할 수 있는 기술 확보

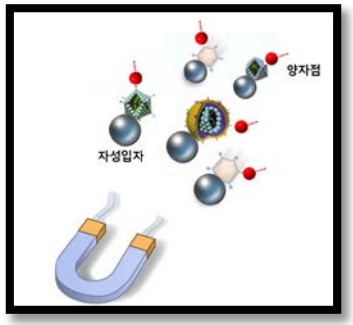


자성입자 분리기술

- 바이러스에 접합 된 자성 입자를 통해 선택적인 분리 기술 확보



BT NT IT 융합기술



바이오 분자 합성기술

- DNA 상보결합을 통한 나노안테나 구조 설계

스마트폰 형광센서

- 양자점이 접합 된 바이러스를 스마트폰을 통해 시공간의 제약없이 검지 기술 확보

Summary and Discussion

- Nanoparticles in assembly offer **structural and functional characteristics**
- **Pros and cons** for two different classes of biopolymers
- **Strategy to combine DNA with protein** – molecular building blocks
- **Various nanostructures** based on DNA-protein MBB platform
- **Fluorescent enhancement** in plasmonic dimers for biosensor applications
- **Future directions** include various scientific and technological applications

Thank you for your attention.

Questions?