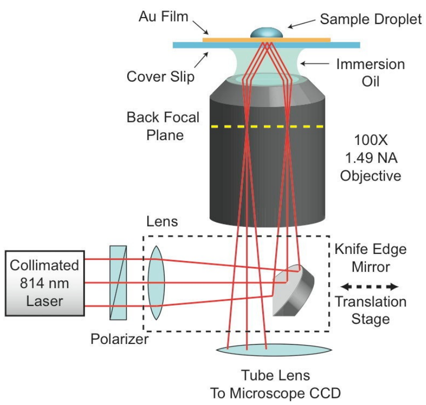
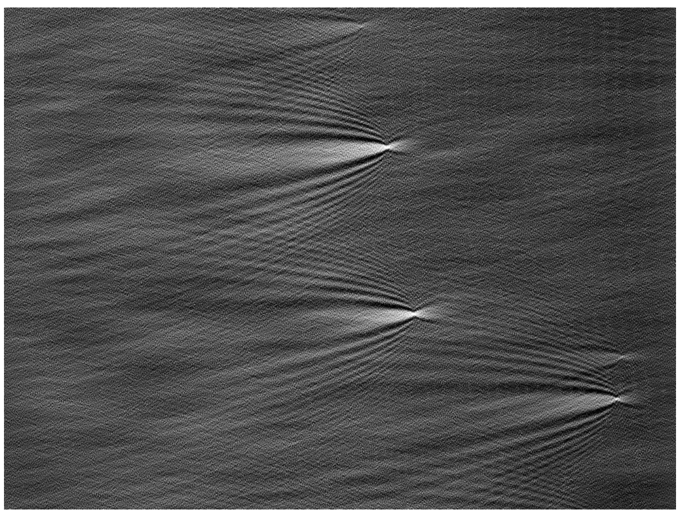
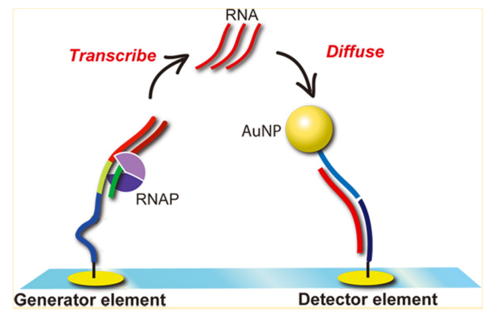
Single Nanoparticle Biosensing with NIR SPRI Microscopy

Single Nanoparticle SPRI

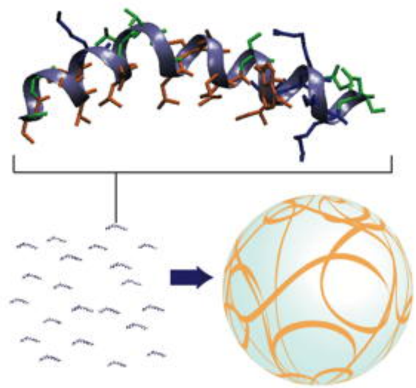


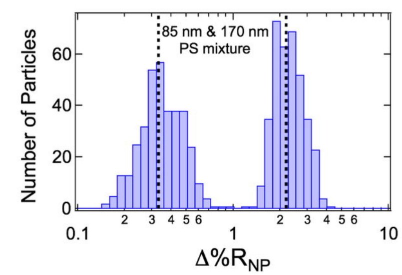


Surface Enzyme Chemistries

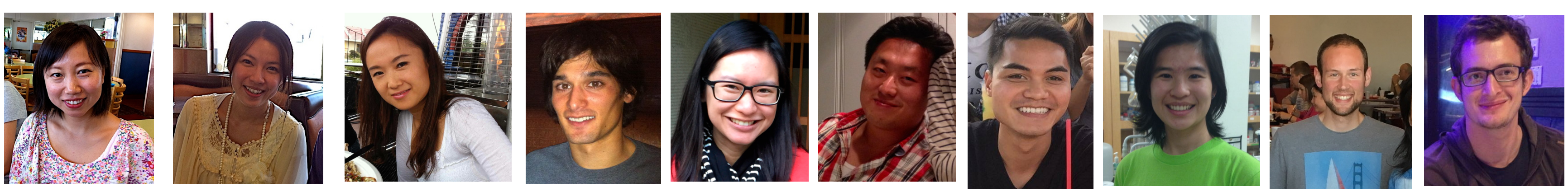


Bioaffinity Uptake into Single Nanoparticles





Robert M. Corn
UCI Department of Chemistry



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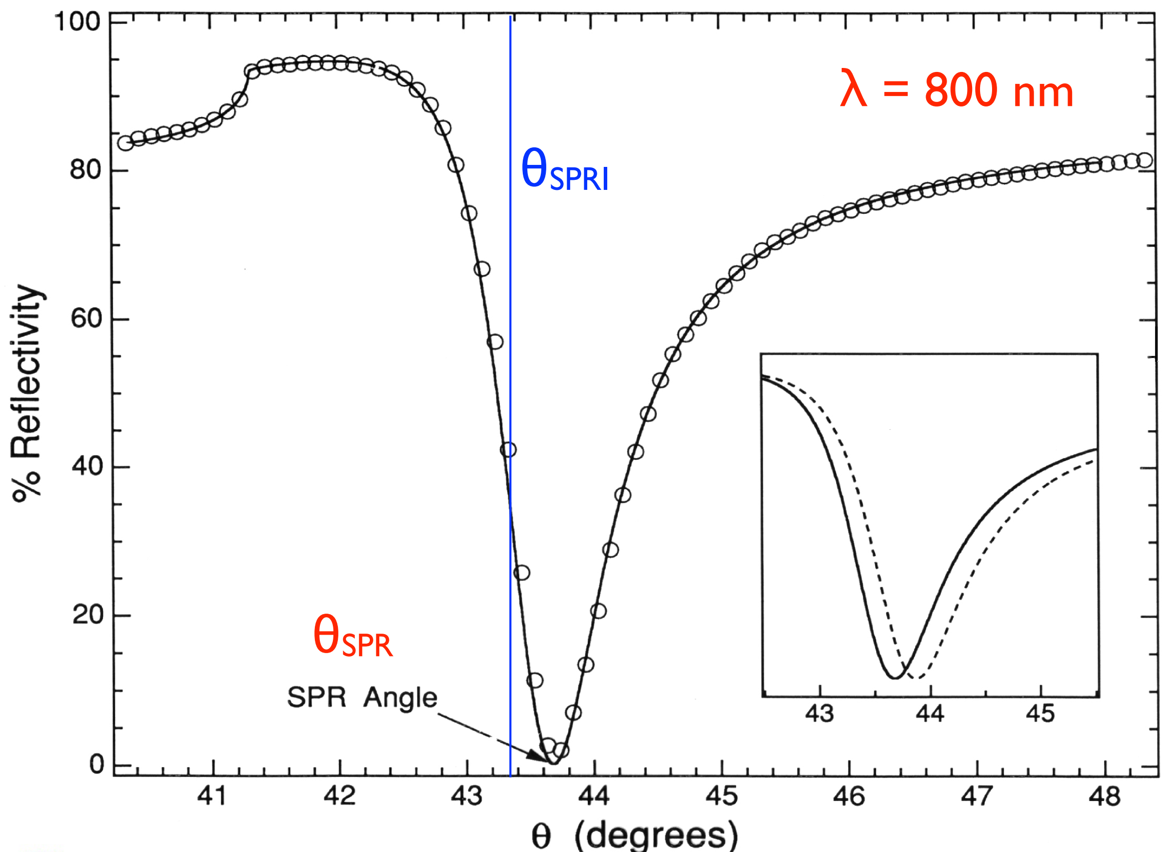
A quick note on Surface Plasmon Polaritons and SPRI

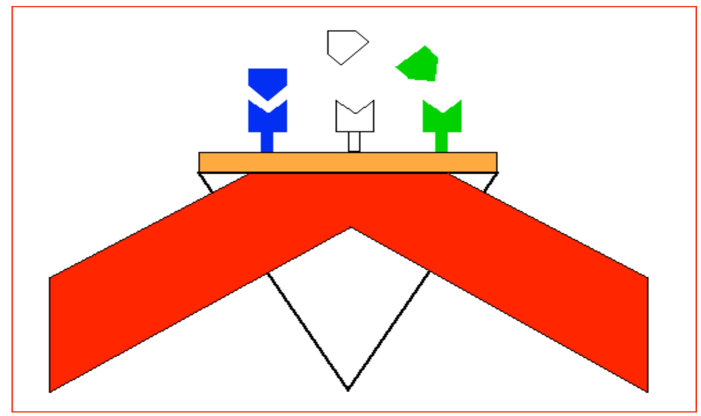
Surface plasmon polaritons (SPPs) are localized propagating electromagnetic waves that can be created on a thin gold film (45 nm) attached to a prism surface.

Surface Plasmon Resonance (SPR)

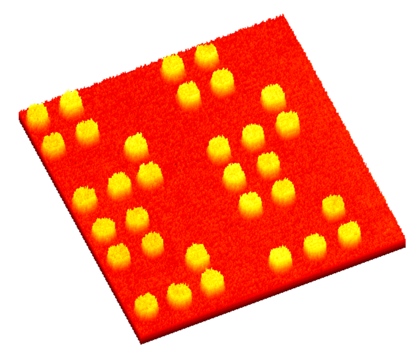
Changes in the resonant SPR angle (θ_{SPR}) can be used to study adsorption onto the gold surface.



NIR SPR Imaging (SPRI)



SPR imaging (SPRI) measures the change in reflectivity from a surface at the θ_{SPRI} upon adsorption.



JM Brockman, AG Frutos, BP Nelson and RM Corn, Analytical Chem., 71 3928-3934 (1999).

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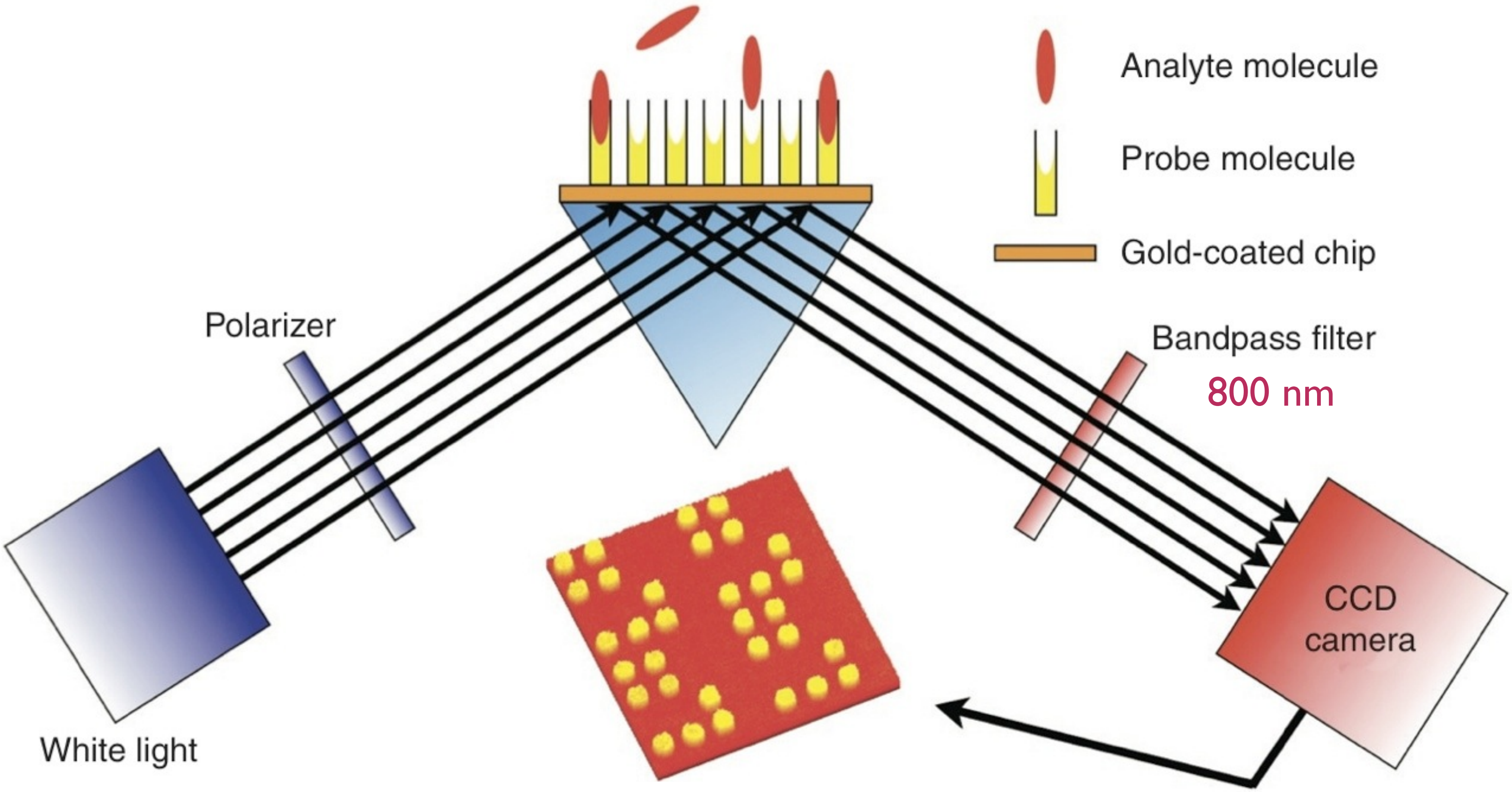
NIR Surface Plasmon Resonance Imaging (SPRI)

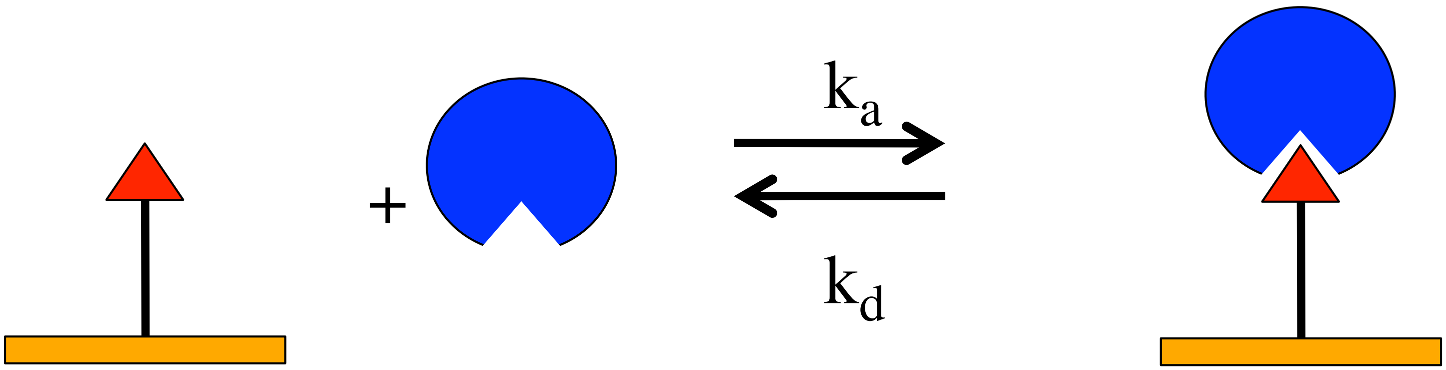


DNA microarray

Multiplexed Refractive Index Surface Bioaffinity Sensing

B. Rothenäusler and W. Knoll, Nature ***332****, 615-617 (1988).*
JM Brockman, AG Frutos, BP Nelson and RM Corn, Analytical Chem., 71 3928-3934 (1999).

Langmuir Surface Adsorption Kinetics

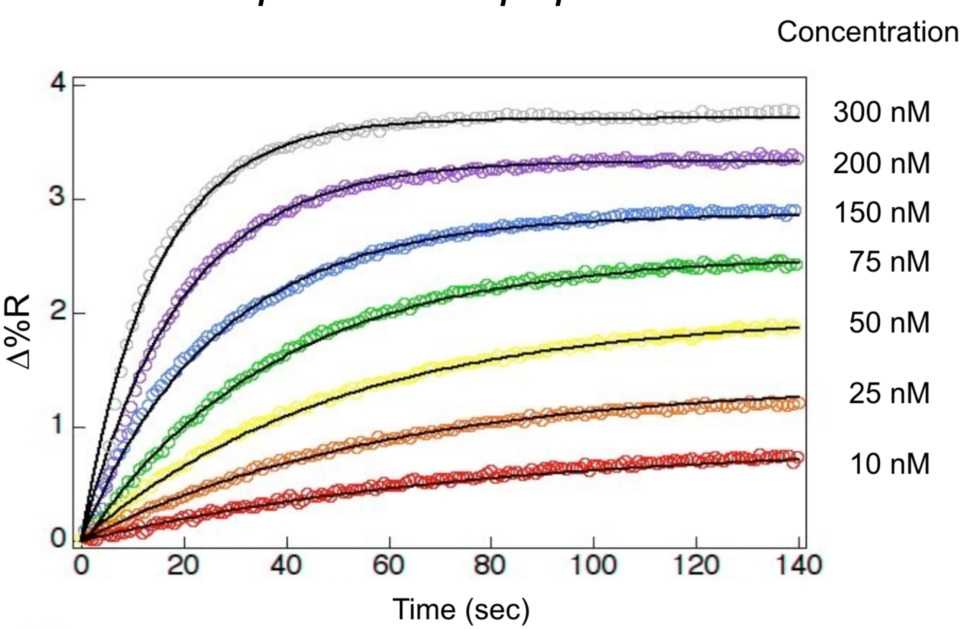


$$K_{ads} = \frac{k_a}{k_d}$$

$$\theta = \Gamma / \Gamma_{tot}$$

S protein - S peptide

$$\theta(t) = \theta^{eq}\left(1 - \exp(-(k_a C + k_d)t)\right)$$



$k_a = 1.9 \times 10^5\ M^{-1}\ s^{-1}$

$k_d = 1.1 \times 10^{-2}\ s^{-1}$

$K_{ads} = 1.7 \times 10^7\ M^{-1}$

Greta Wegner

G. J. Wegner et al. Analytical Chem., 76 5667-5684 (2004).

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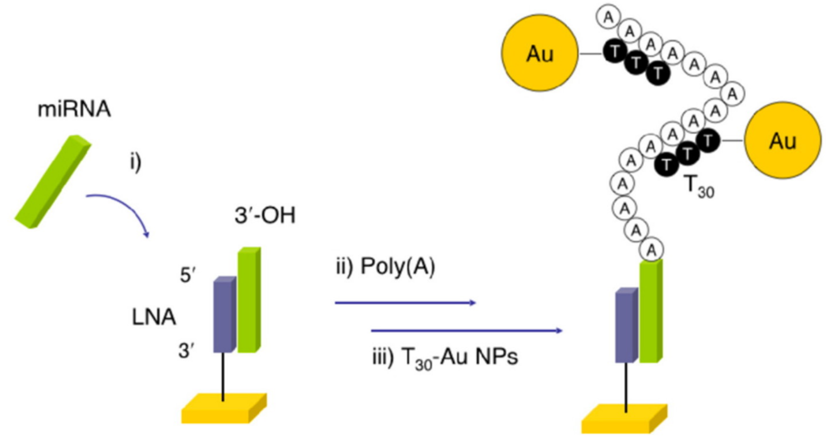
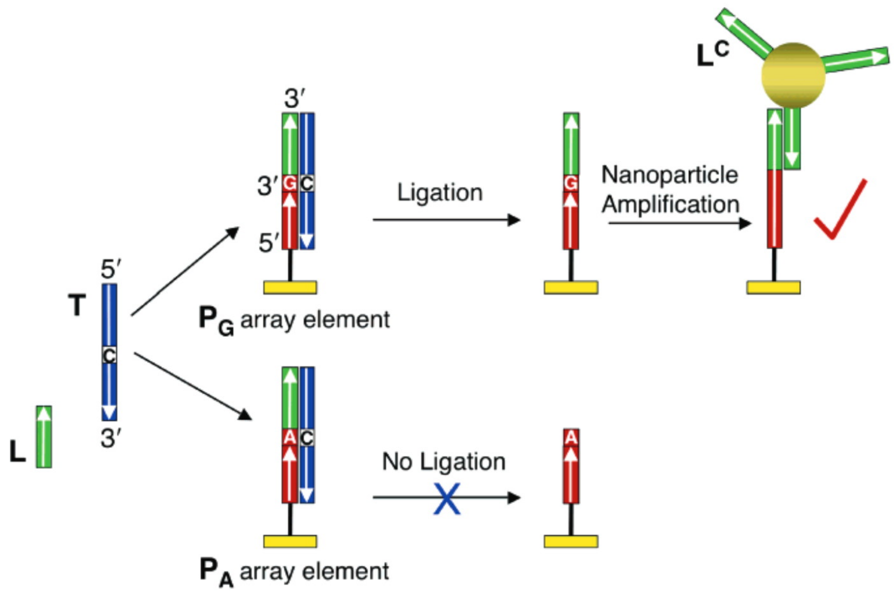
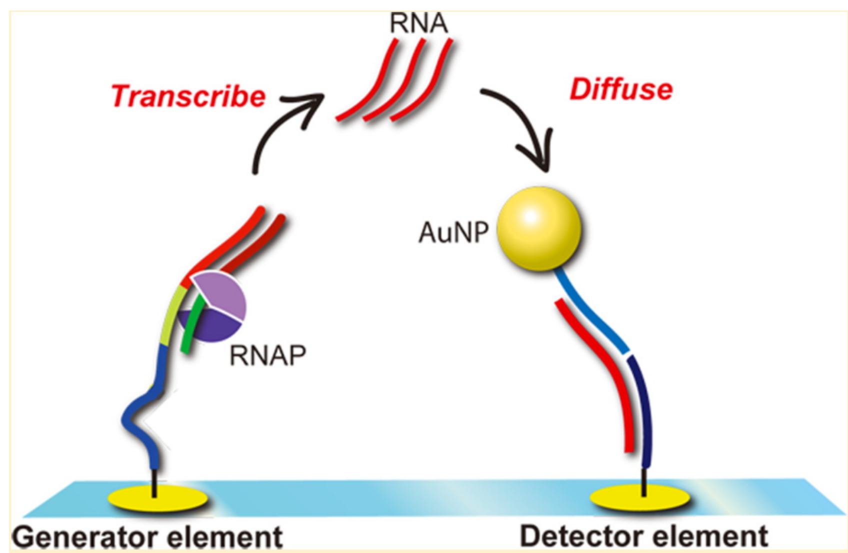
SPRI Measurements of Surface Enzyme Chemistries

- Surface Ligation Chemistry
- Surface Nuclease Chemistry
- Surface Polymerase Chemistry
- Surface DNAzyme Chemistry







Surface Enzyme Chemistry + Nanoparticle-Enhanced SPRI
Enhanced Detection

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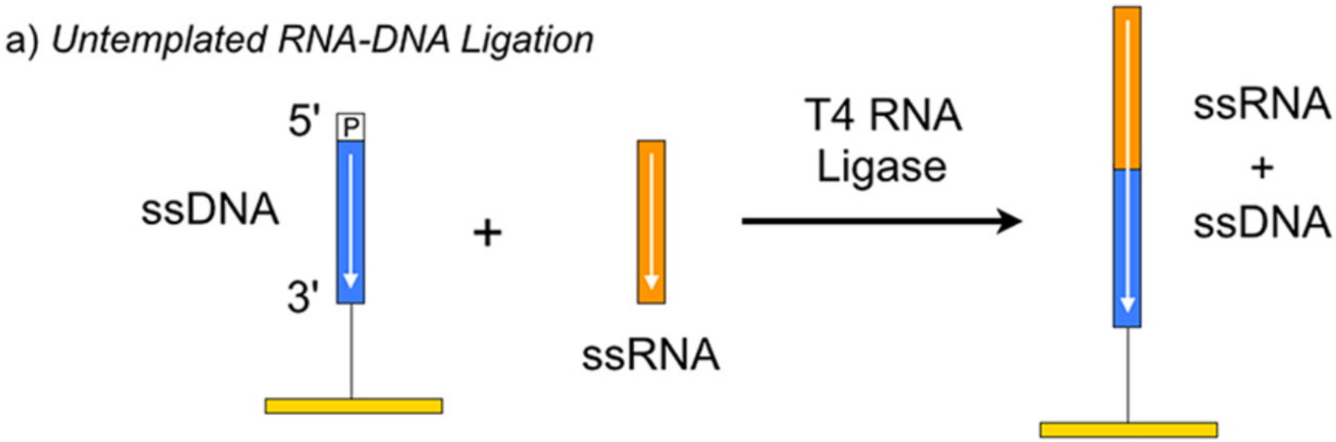
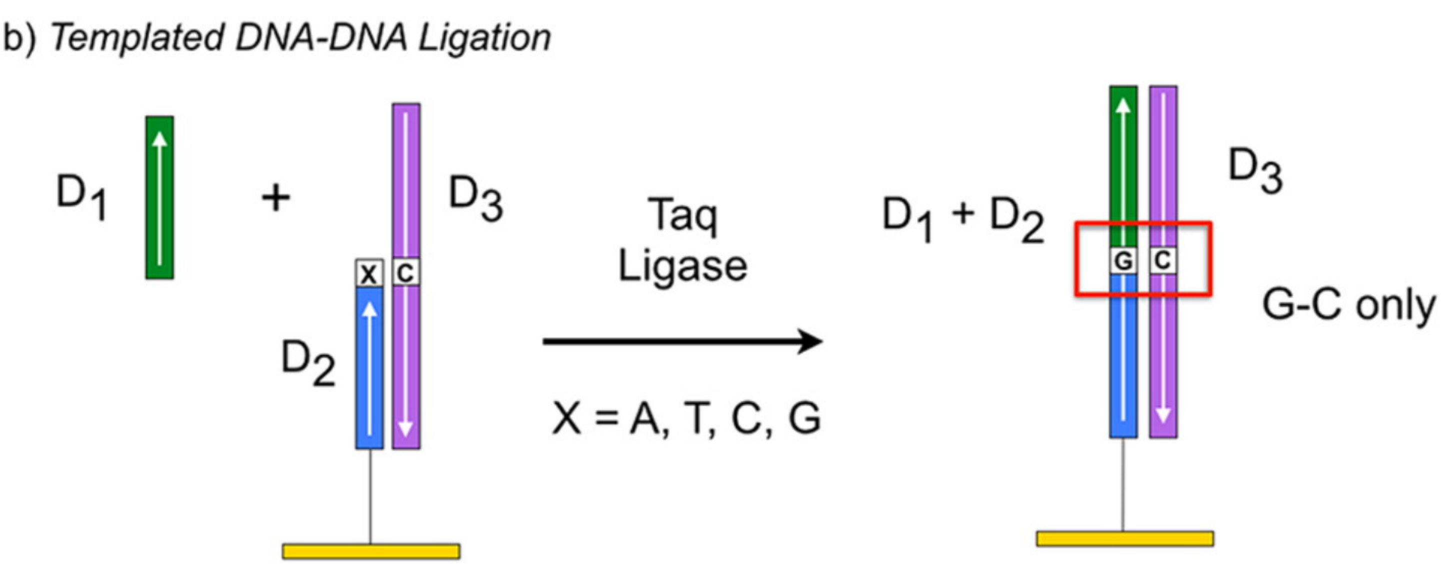
Surface Ligation Chemistries

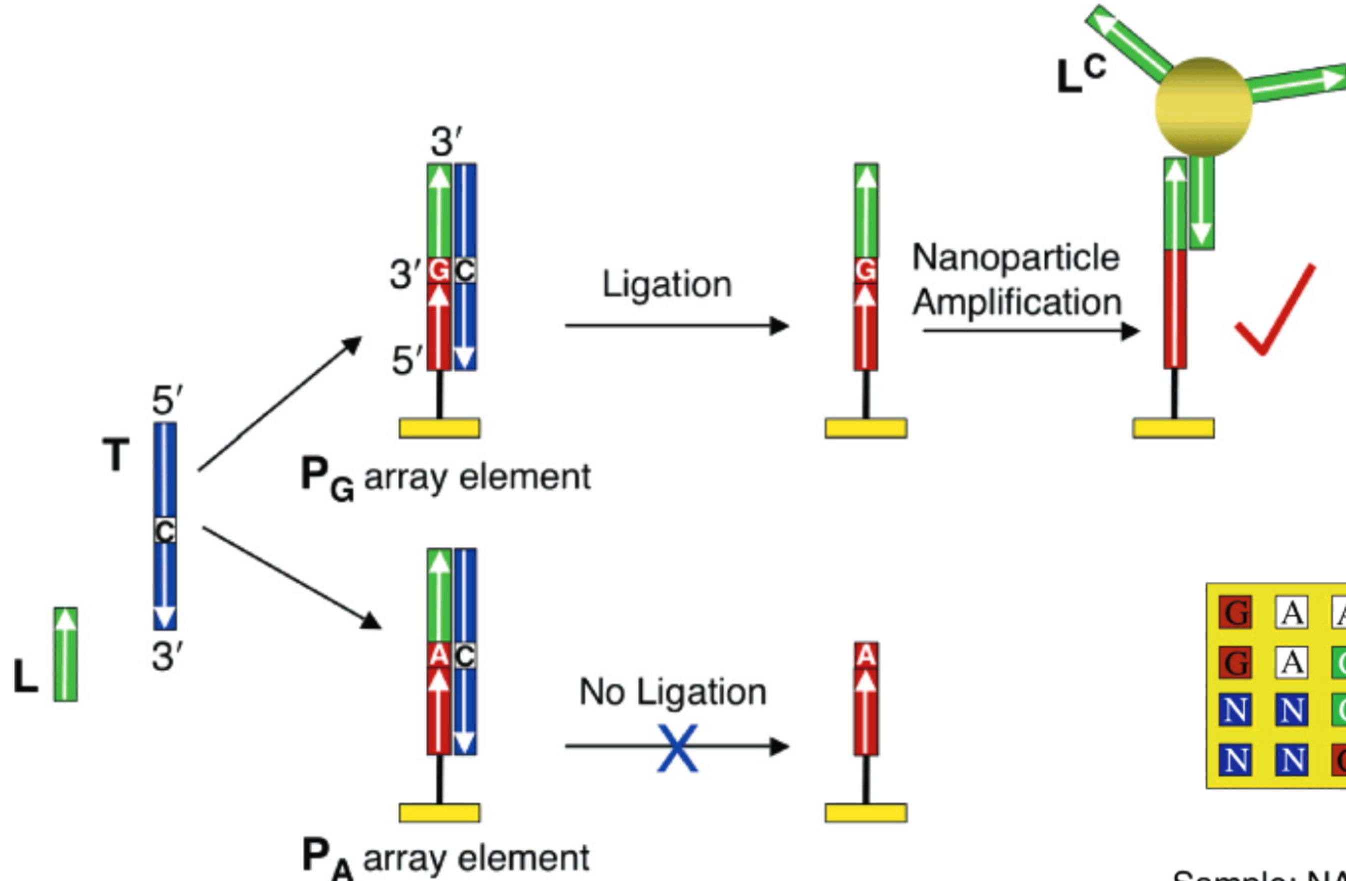


microRNA detection

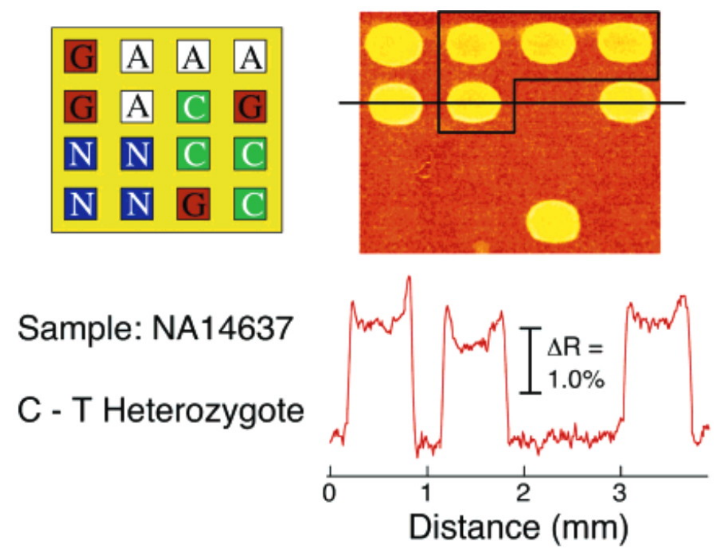


SNP detection

SNP Detection: Surface Ligation + Nanoparticle-Enhanced SPRI



SNP detection



Yuan Li

Y. Li et al., Analytical Chem., ***78*** *3158-3164 (2006).*

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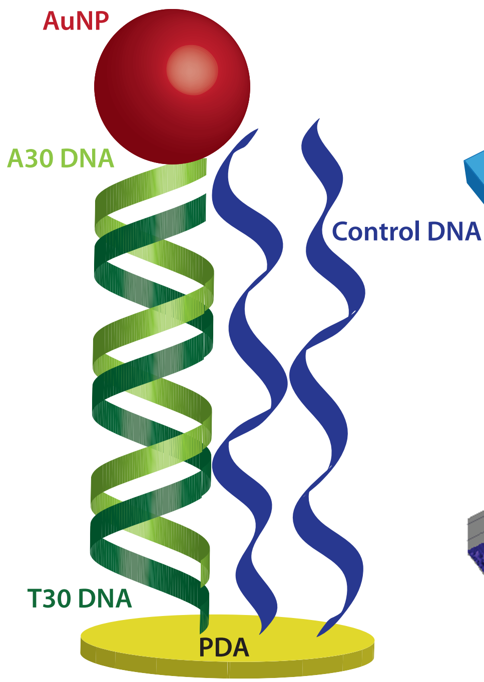
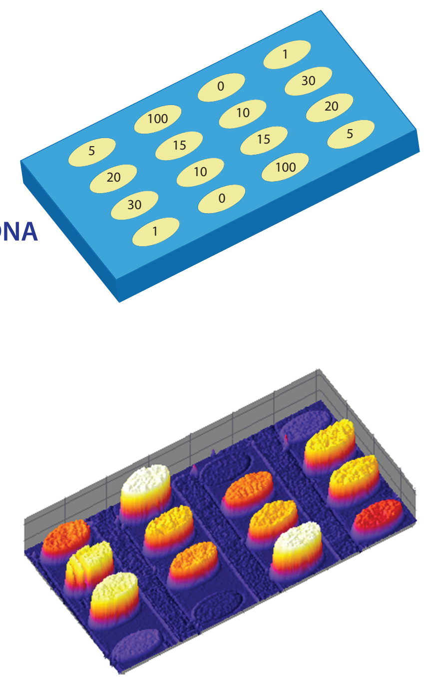
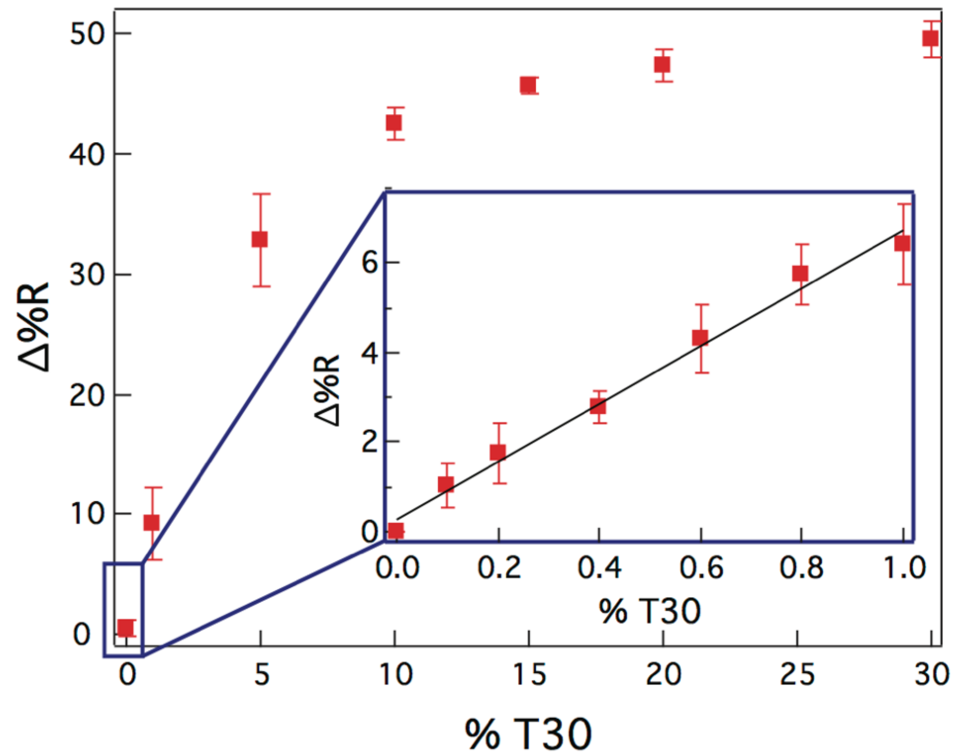
Nanoparticle-Enhanced SPRI Detection of Surface DNA

polydopamine + amino-functionalized ssDNA

$\Gamma_{max} = 10^{12}$ molecules cm^{-2}







0.1% ssDNA Detection in mixed ssDNA monolayers

Jennifer Wood Fasoli

J. Wood et al., Langmuir, ***29*** *10868-10873 (2013).*

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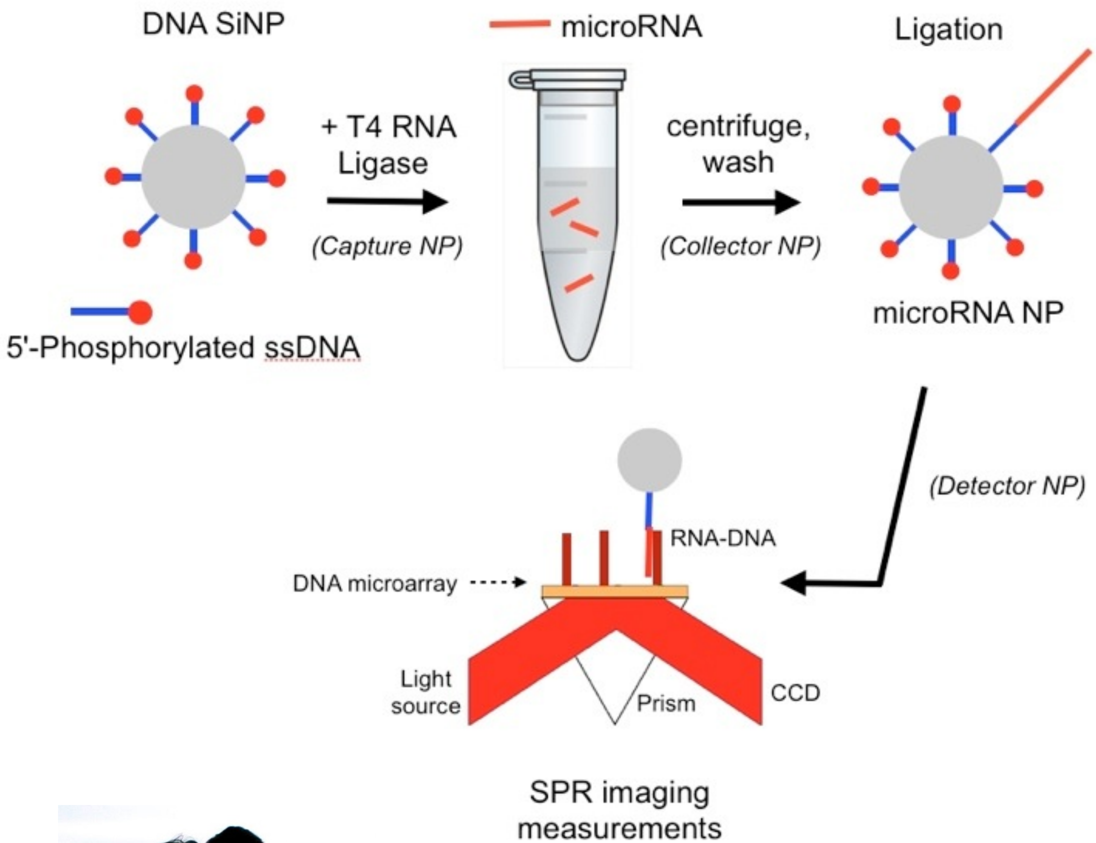
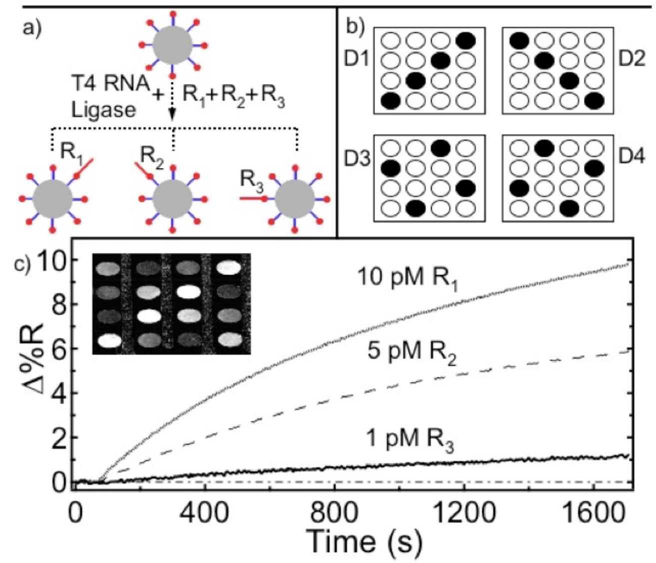
Surface Ligation Chemistry + Nanoparticle-Enhanced SPRI

MicroRNA Detection with Enzymatic Nanoparticles: Ligation Capture





T4 RNA Ligation
for miRNA capture

Dr. WenJuan Zhou

Yulin Chen

W. J. Zhou et al., Analytical Chemistry, ***83*** *3897-3902 (2011).*

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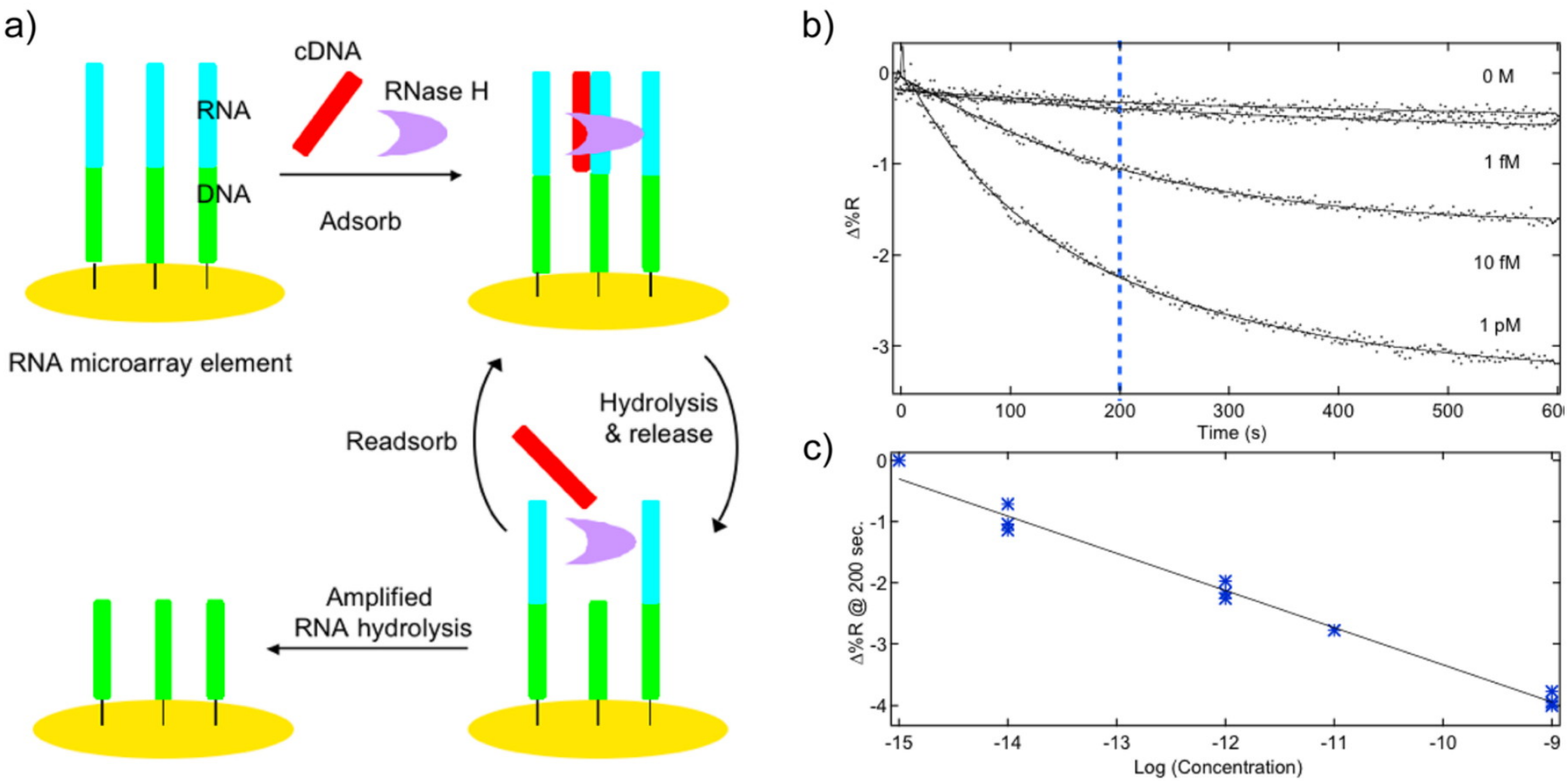
Surface Nuclease Chemistries: RNase H Digestion



Nico Hu
Seefeld

1 fM ssDNA detection

T. H. Seefeld et al., Langmuir, **27** 6534-6540 (2011).

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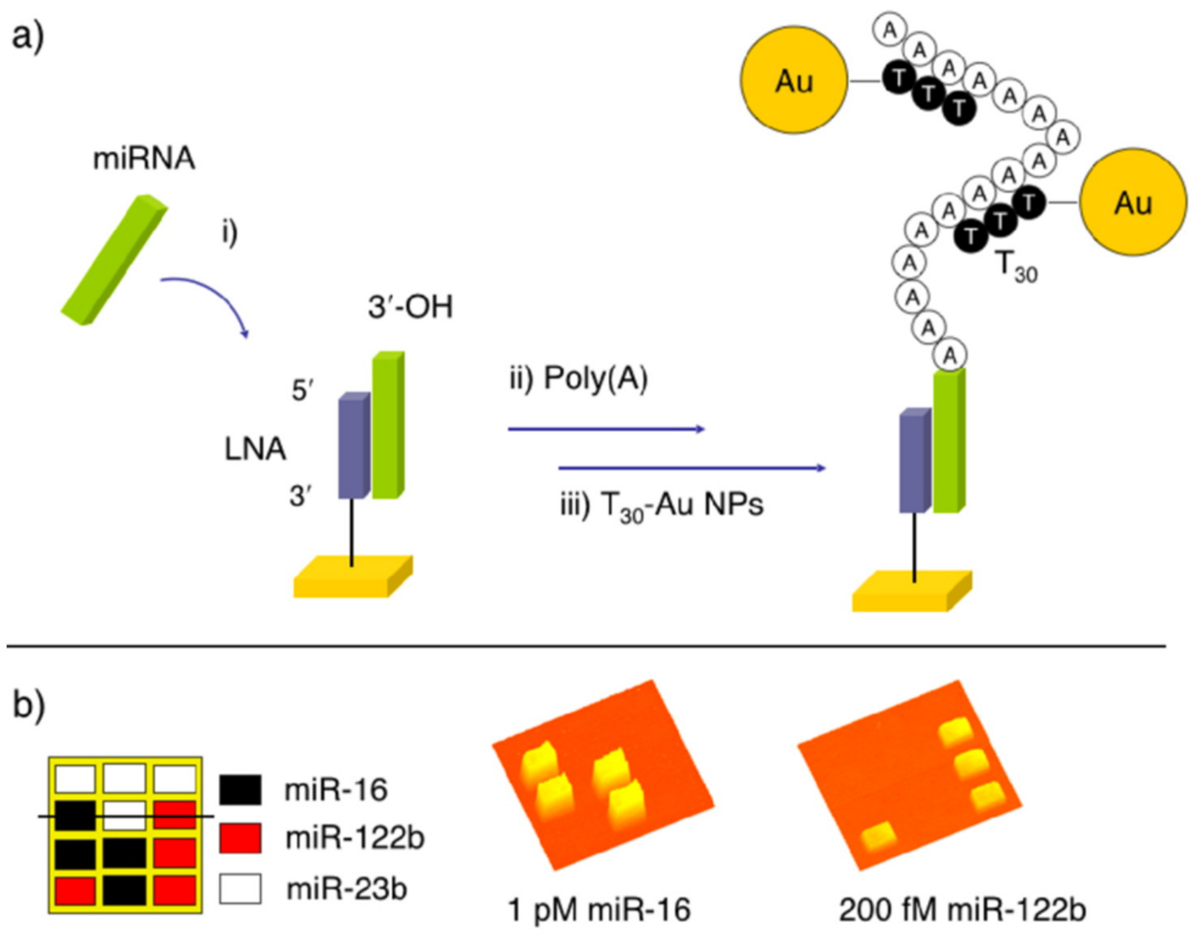
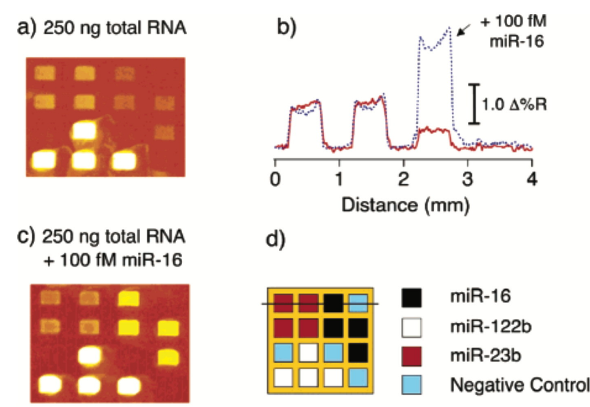
Surface Polymerase Chemistries: Poly A Polymerase miRNA Detection



Femtomolar miRNA Detection



S. Fang et al., J. Am. Chem. Soc., 128 14044-14046 (2006).

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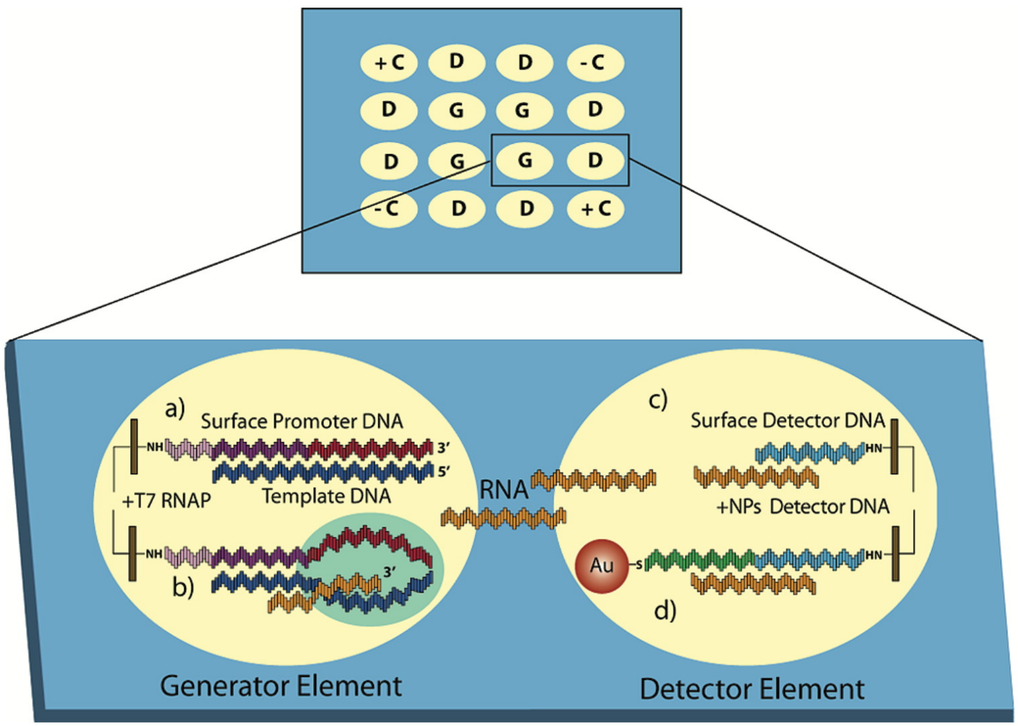
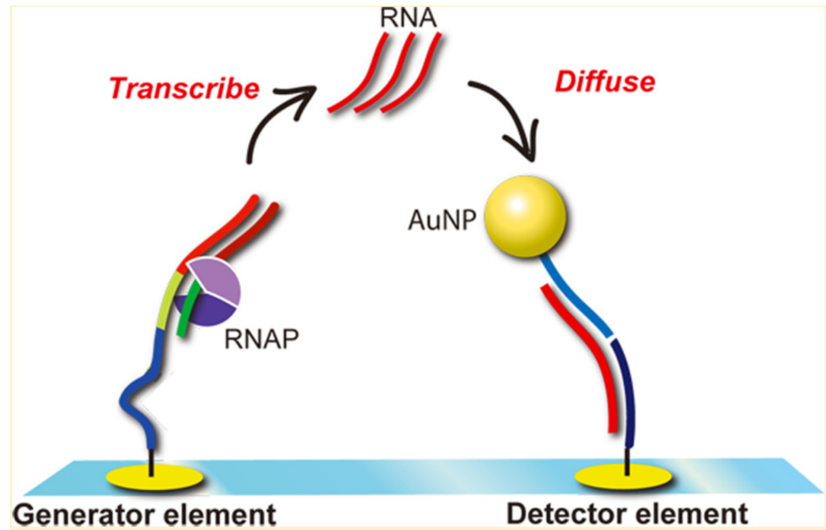
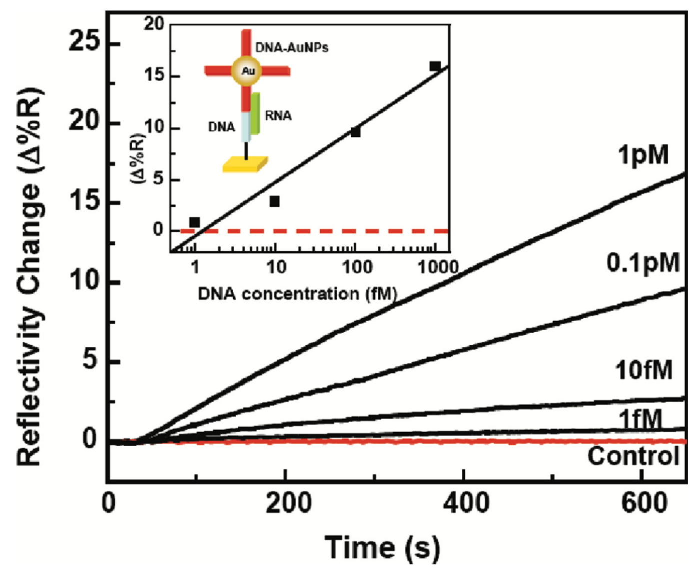
Surface Polymerase Chemistries: RNA Polymerase Amplification

Dual Element Methodology







1 fM ssDNA detection

Iuliana Sendroiu

I. Sendroiu et al., J. Am. Chem. Soc., 133 4271-4273 (2011)

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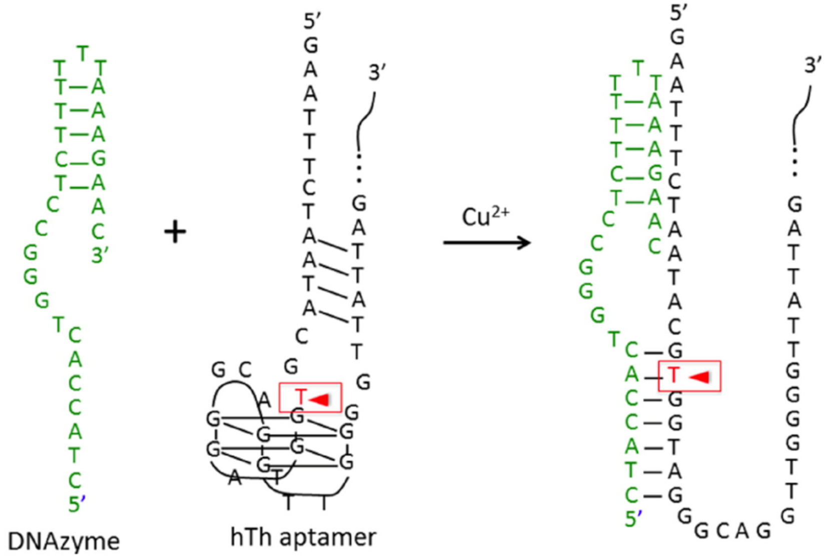
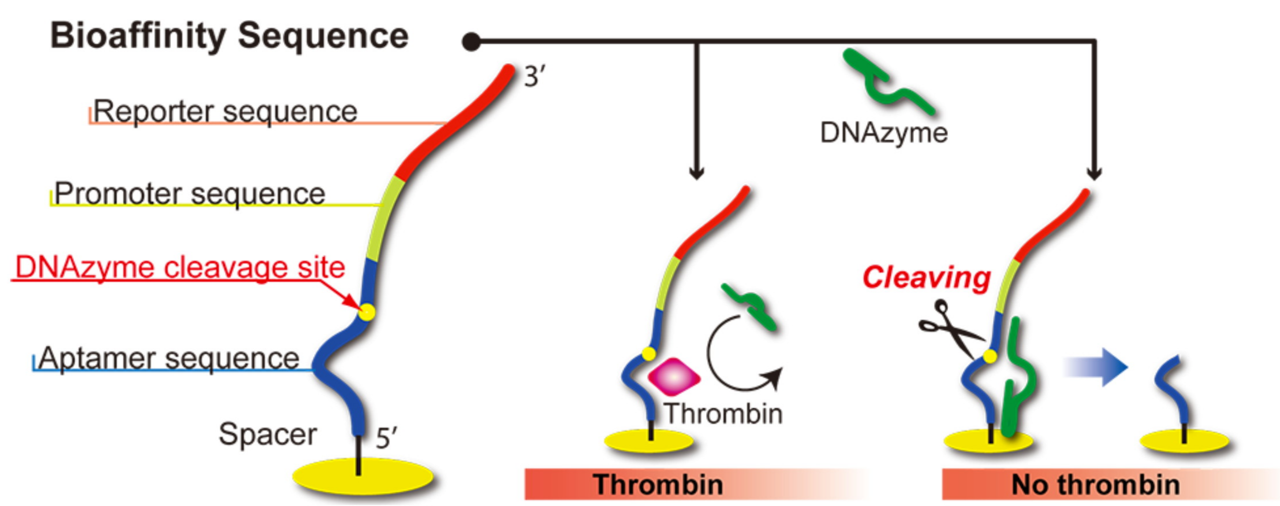
Surface DNAzyme Chemistries: DNAzyme Footprinting



Competitive binding of Thrombin and a DNAzyme designed to cut the aptamer sequence

DNAzyme Footprinting:



Y. Chen et al., J. Am. Chem. Soc., ***135*** *2072-2075 (2013).*

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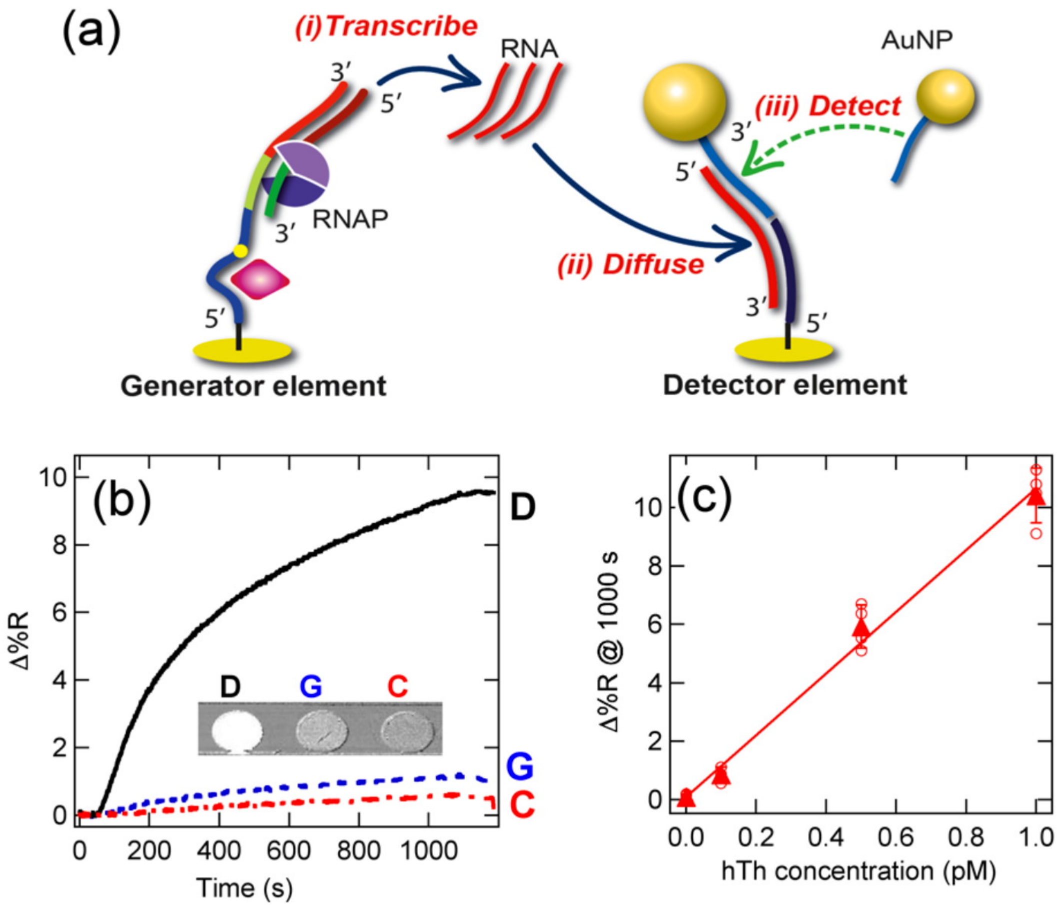
DNAzyme Footprinting: Femtomolar Biomarker Detection

Nanoparticle-Enhanced Detection



Femtomolar Biomarker Detection

Yulin Chen

Y. Chen et al., J. Am. Chem. Soc., ***135*** *2072-2075 (2013).*

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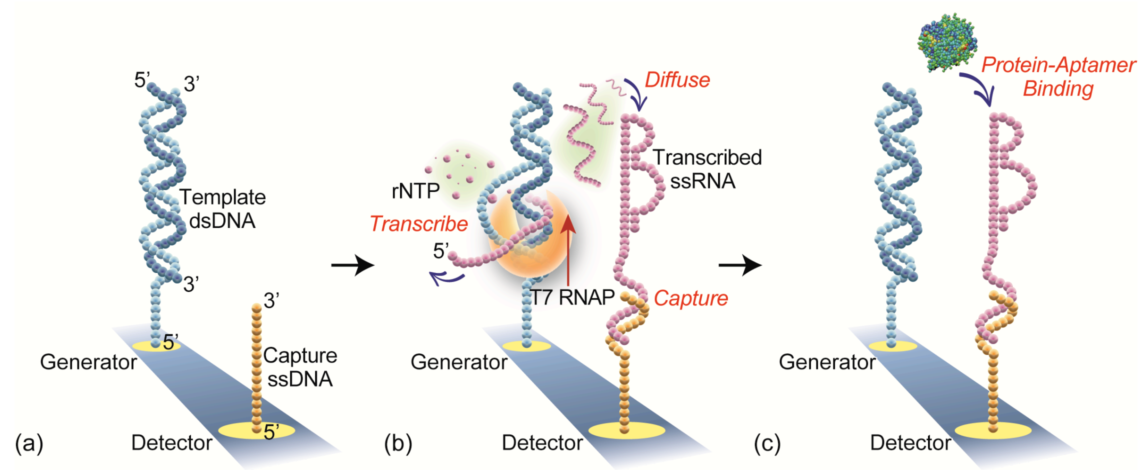
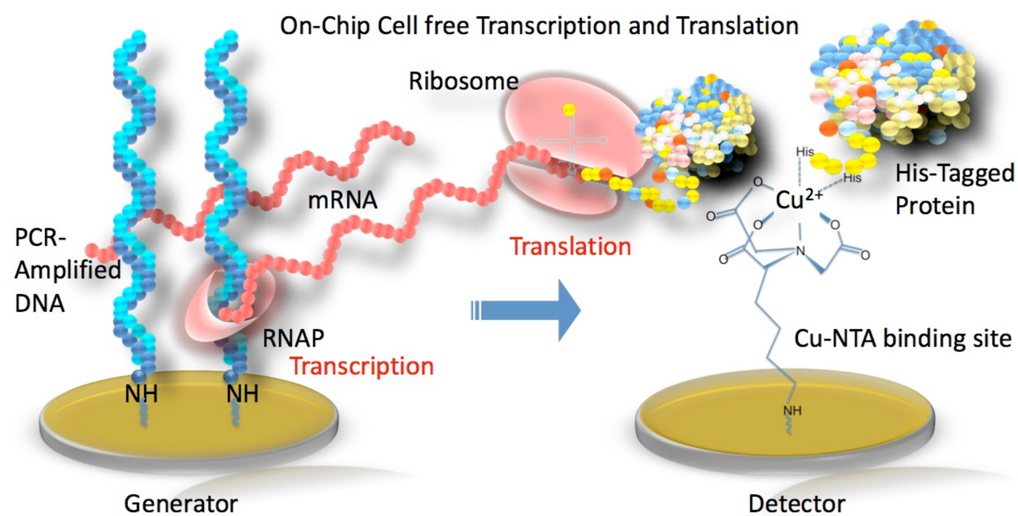
On-Chip Templated Biosynthesis of RNA and Protein Microarrays



On-Chip Synthesis of RNA aptamer microarrays



On-Chip Synthesis of Protein microarrays

Y. Chen, et al., Langmuir, ***28*** *8281-8285 (2012).*
T. H. Seefeld et al., J. Am. Chem. Soc., ***134*** *12358-12361 (2012).*

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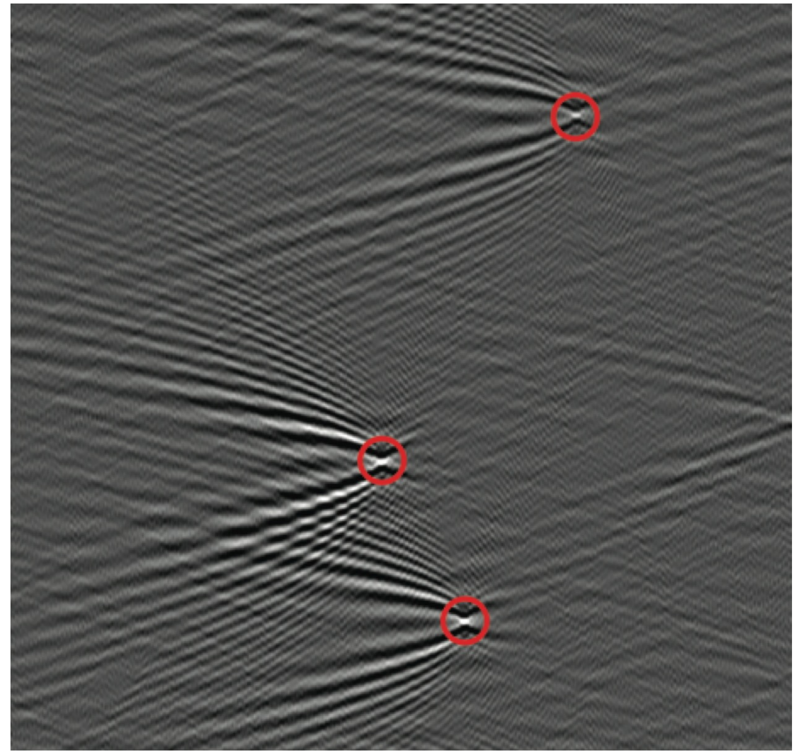
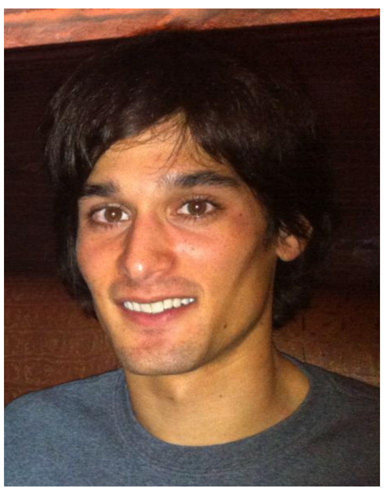
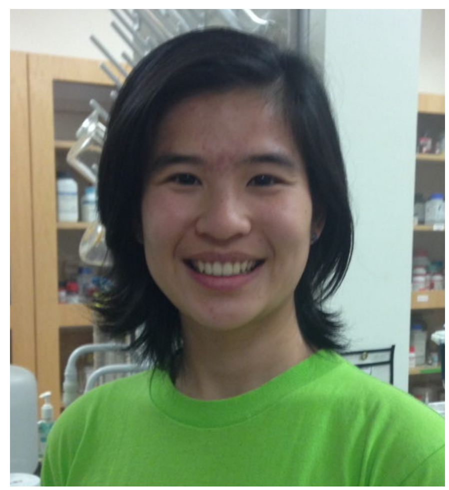
Single Nanoparticle SPRI Microscopy





Dr. Aaron Halpern

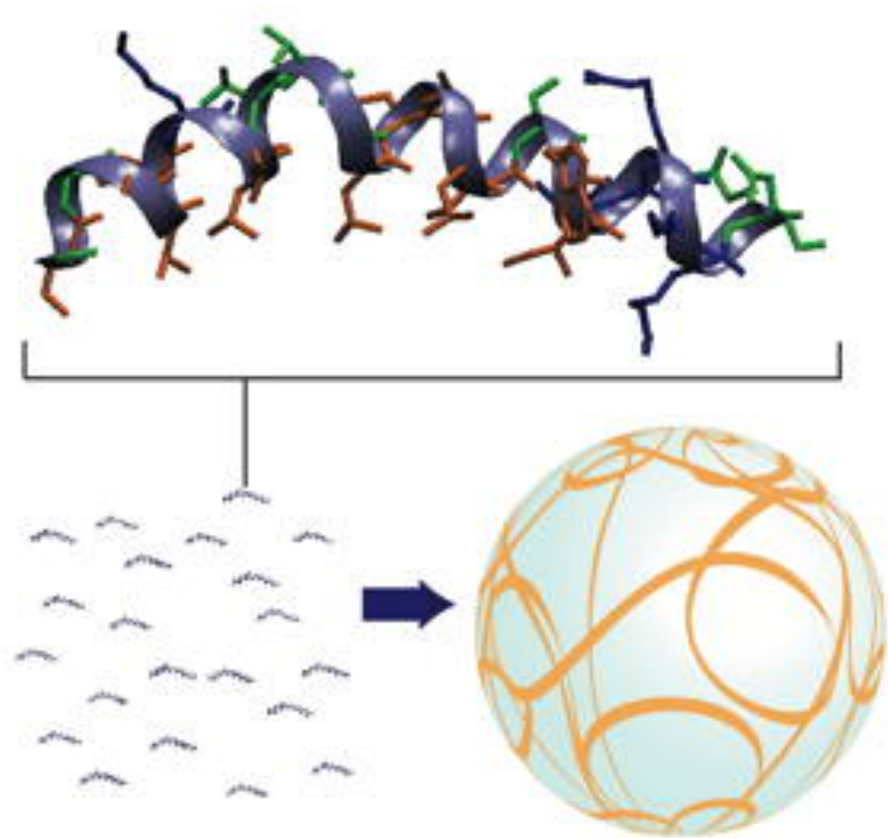
Adam Maley



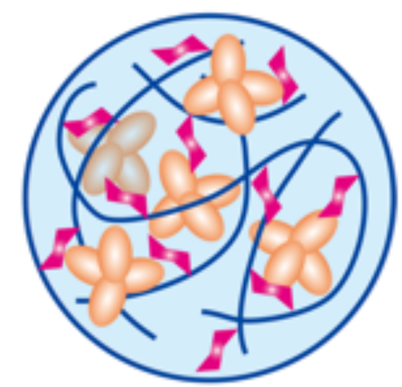
Millie Fung

Robert M. Corn
UC Irvine

Bioaffinity Uptake in Nanoparticles



Noncovalent bioaffinity interactions can also be used to absorb biomolecules (peptides, proteins, metabolites) into porous hydrogel, silica and liposomal nanoparticles.

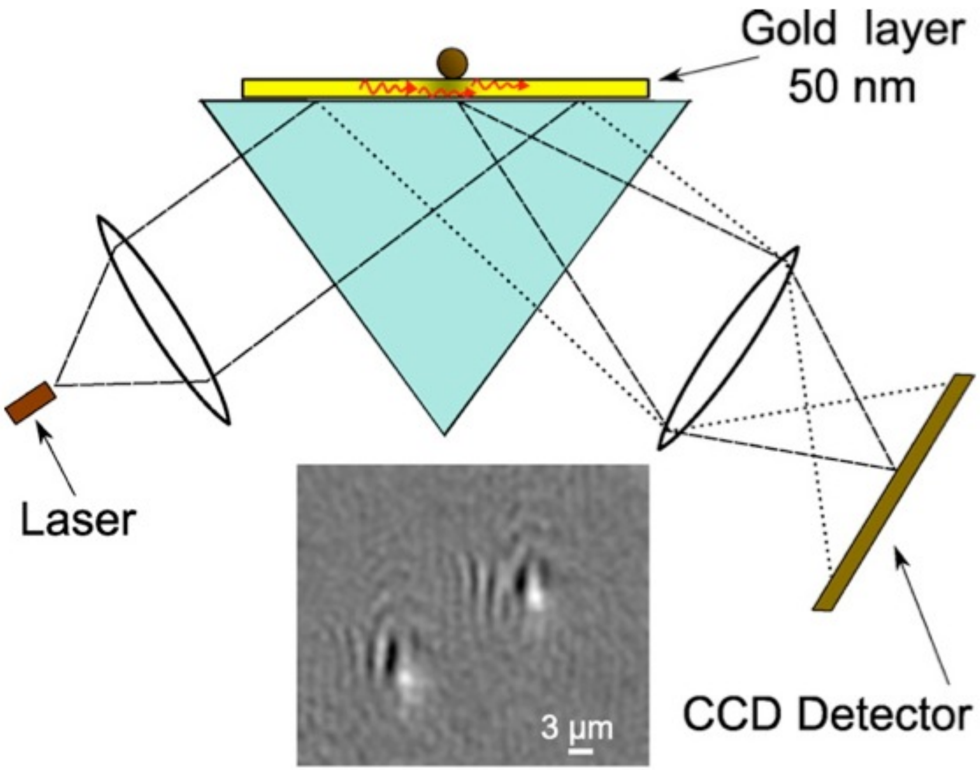


Melittin peptide uptake into hydrogel nanoparticles (HNP) for drug delivery

ConA and mannose (HNPs)

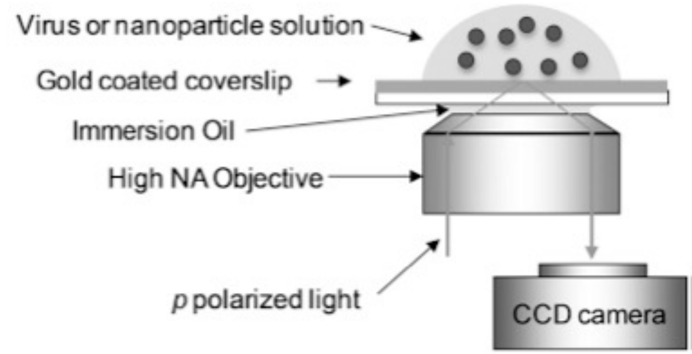
Melittin: GIGAVLKVLTTGLPALISWIKRKRQQ

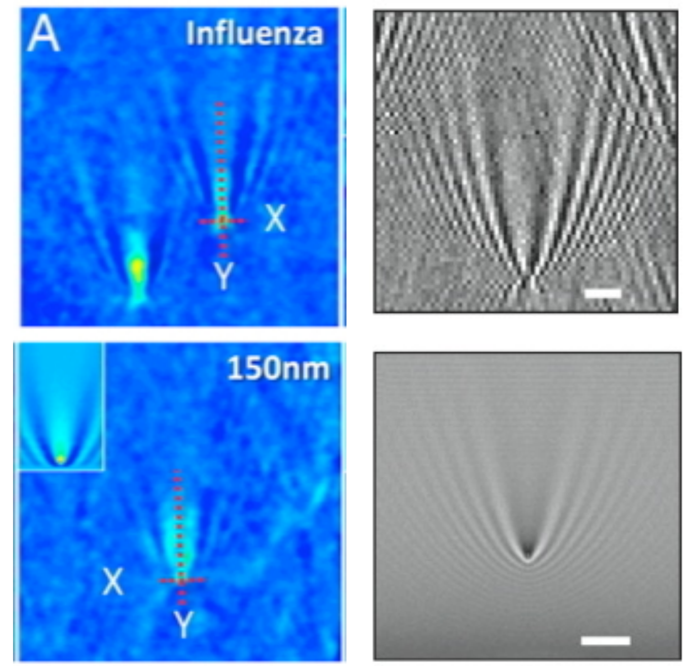
SPRI microscopy of nanoparticles



Viruses and polystyrene NPs (40-200 nm)

A. Zybin et al.,
*Plasmonics **5**, 31–35 (2010).*
*Sens. Actuators B, **151** 281–290 (2010).*





Silica NP *80 nm Pt NP*

N. Tao et al.,
*Proc. Natl. Acad. Sci. **107**, 16028−16032 (2010).*
*Nature Nanotech. **134** 668-672(2012)*

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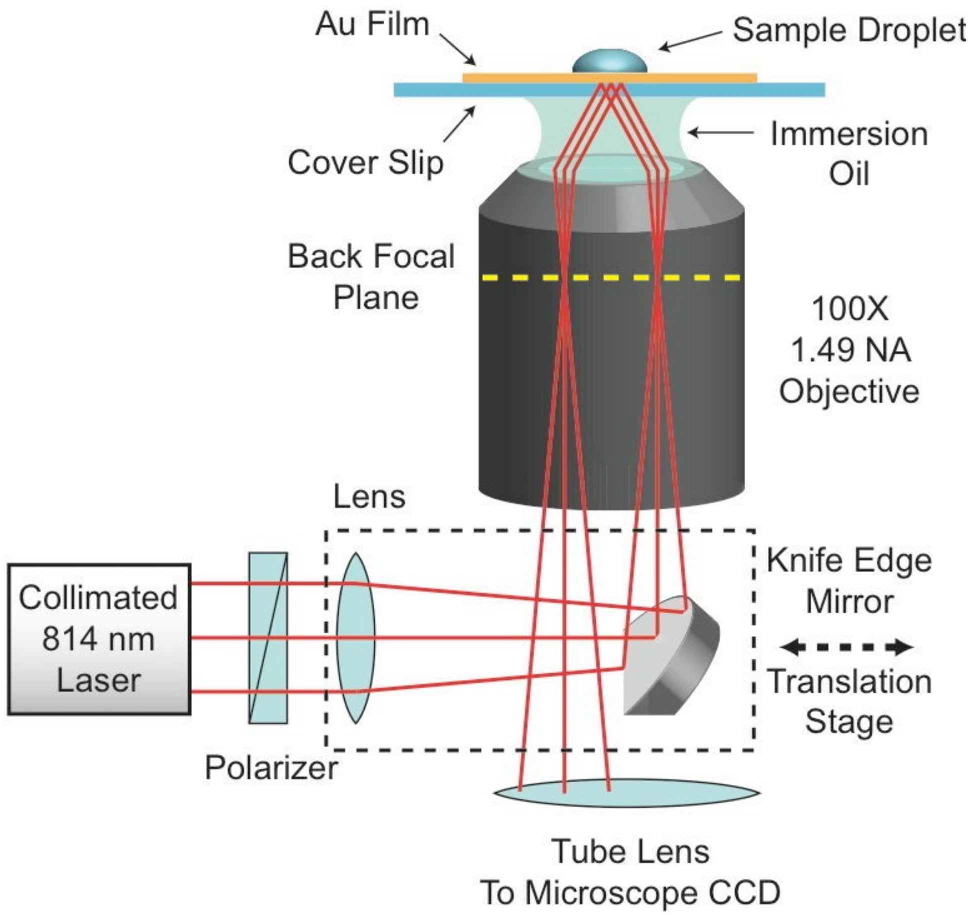
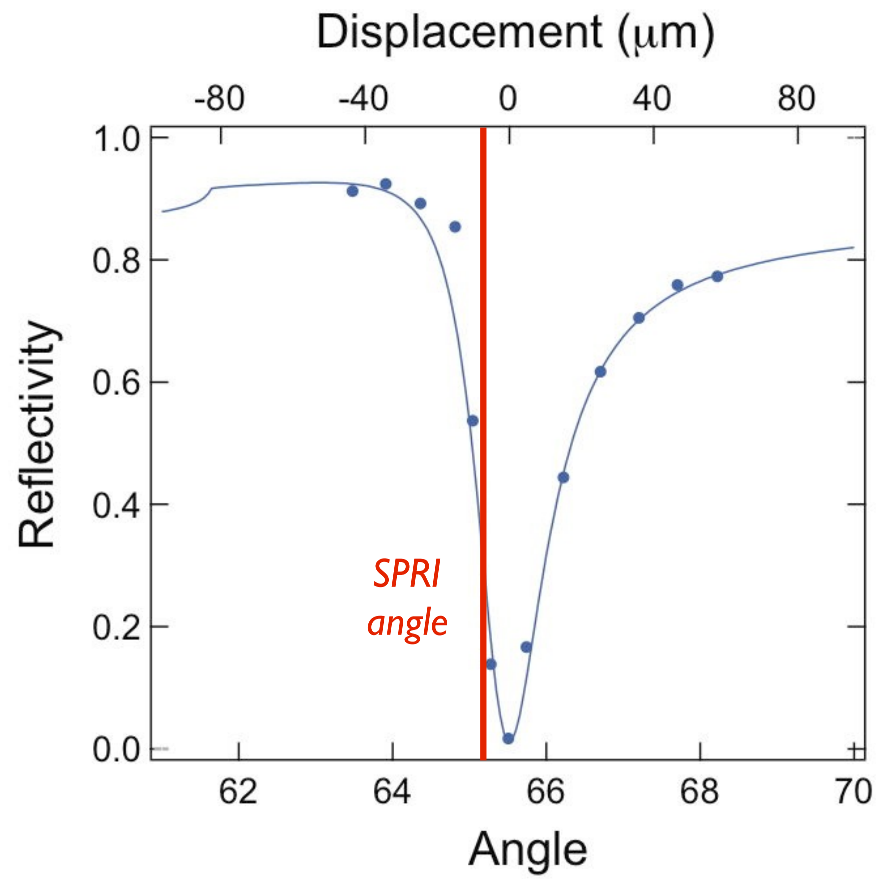
Near Infrared TIR SPRI microscope: 814 nm





100x Objective
N.A. = 1.49
90 μm x 70 μm FOV

Control angle of incidence by varying d

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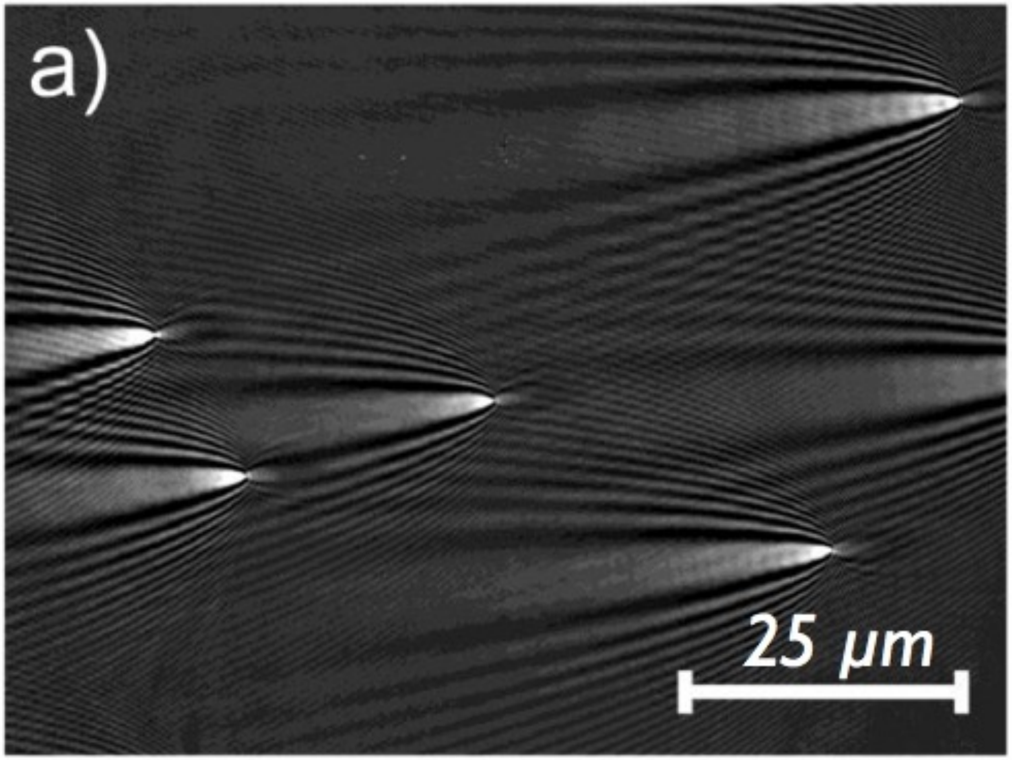
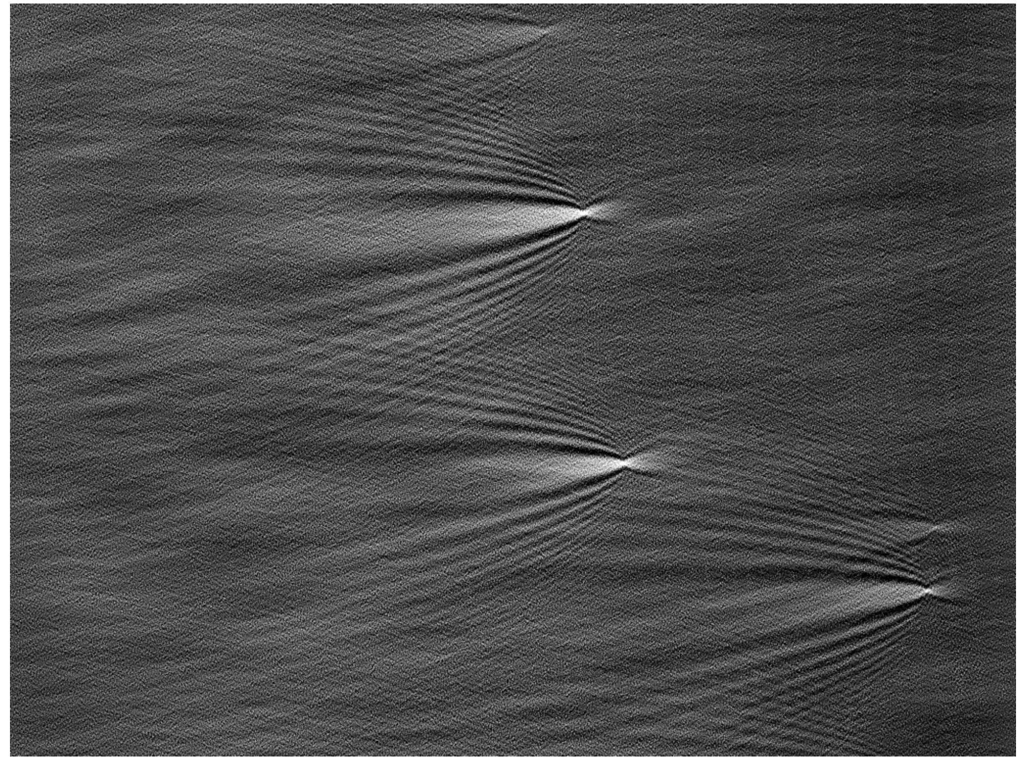
Near Infrared TIR SPRI microscope data:



200 nm polystyrene NPs

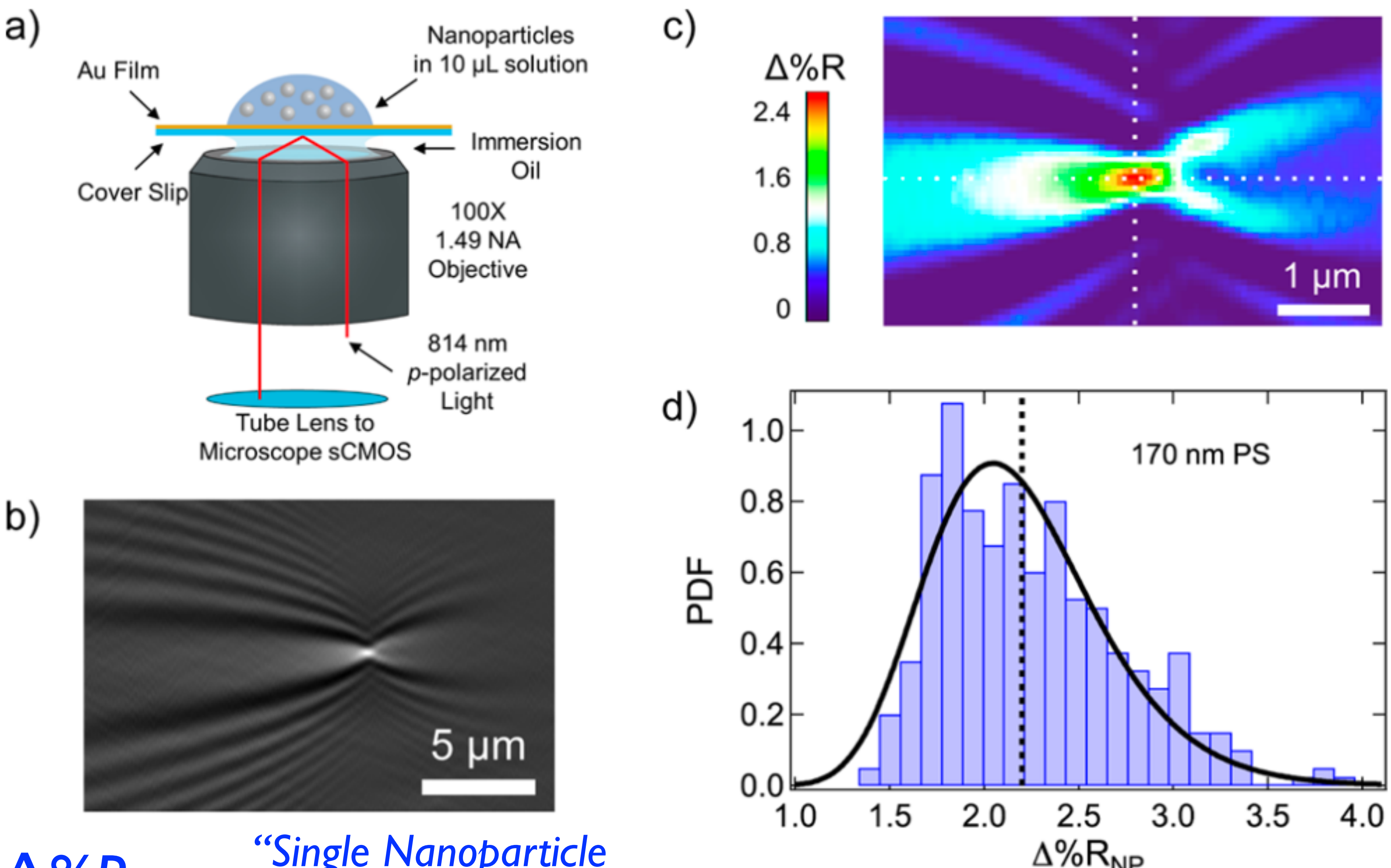


40 nm gold NPs

90 μm x 70 μm FOV

814 nm SPP excitation

Single Nanoparticle Distribution Measurements



$\Delta\%R_{NP}$ *"Single Nanoparticle SPRI Reflectivity Change"*

A.M. Maley, G.J. Lu, M.G. Shapiro and R.M. Corn, ACS Nano, ***11*** *7447-7456 (2017).*

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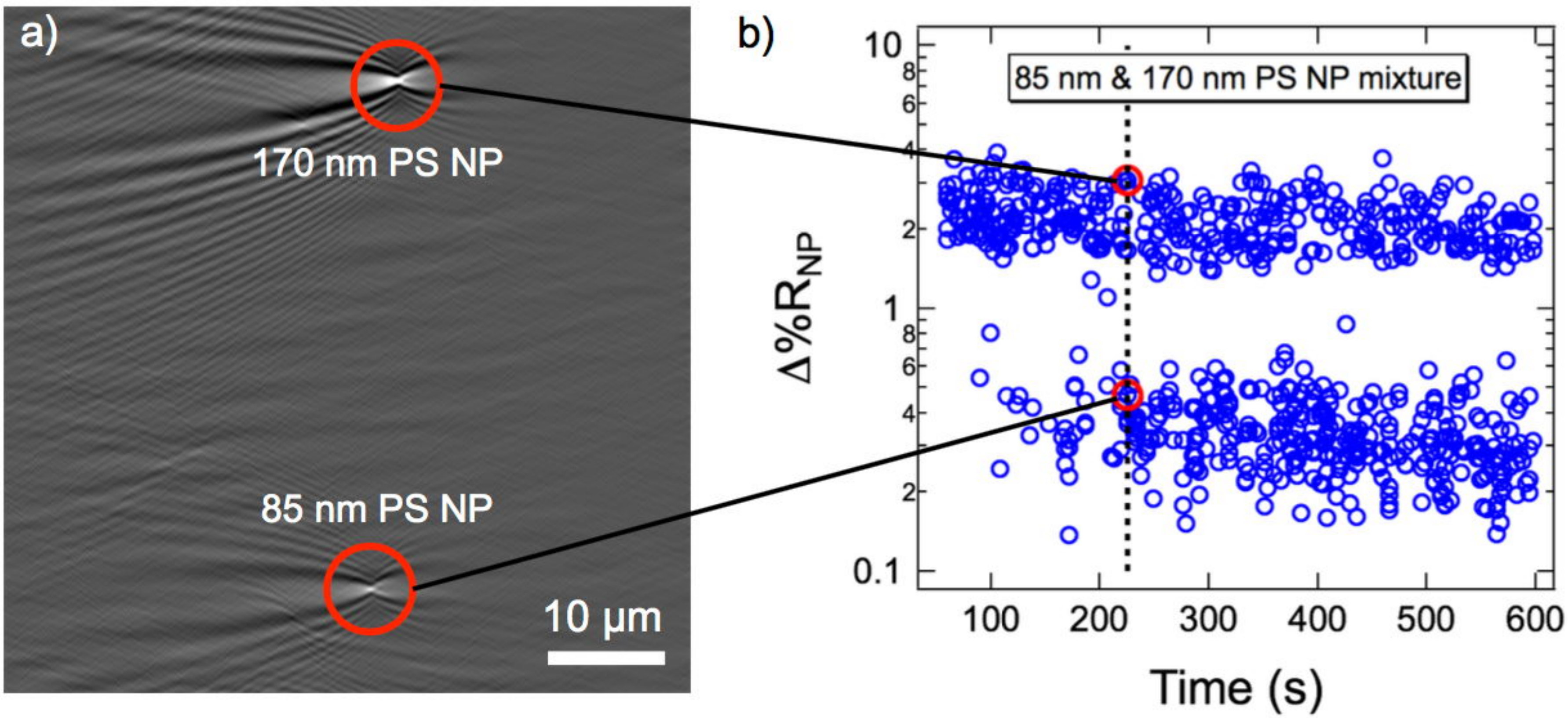
Single Nanoparticle Distribution Measurements

1:1 Mixture of 85 nm and 170 nm PS nanoparticles



Irreversible electrostatic adsorption of carboxy-modified PS NPs onto an amino-terminated alkanethiol monolayer (MUAM).

*A.M. Maley, G.J. Lu, M.G. Shapiro and R.M. Corn, ACS Nano, **11** 7447-7456 (2017).*

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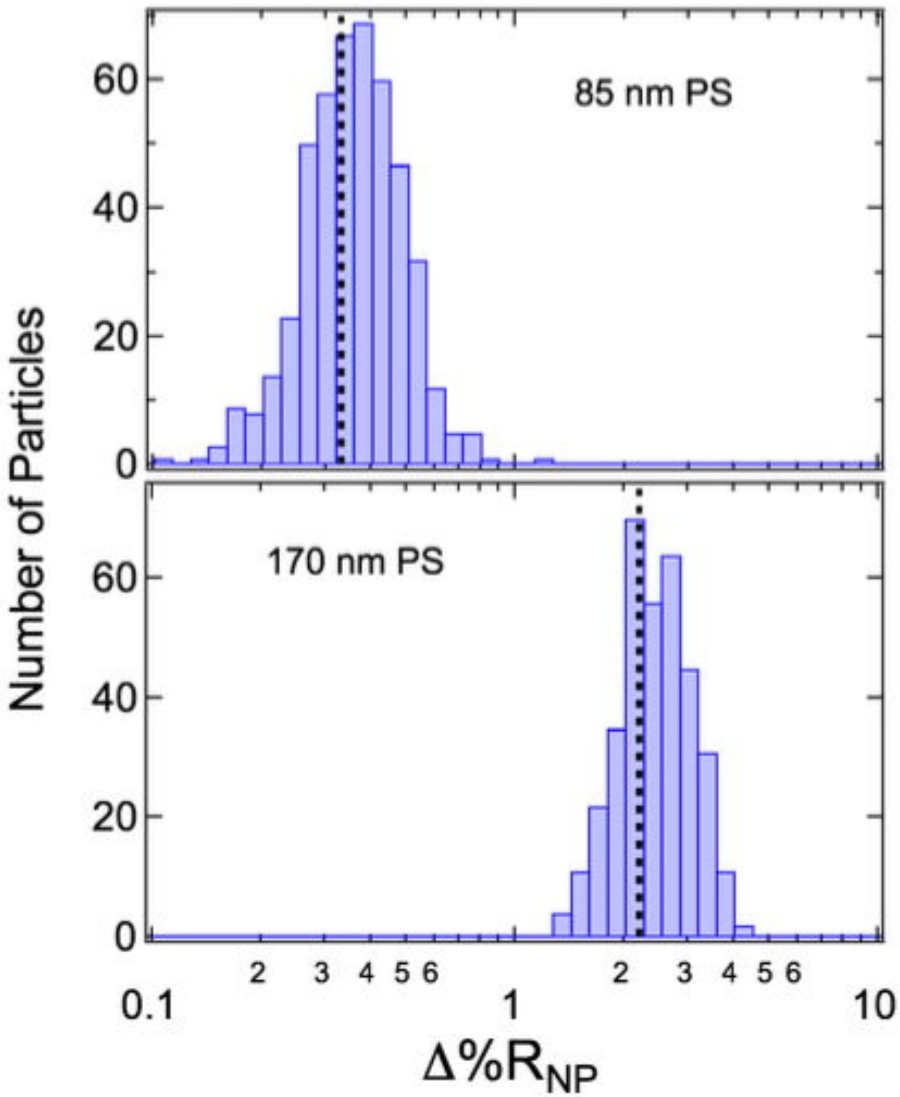
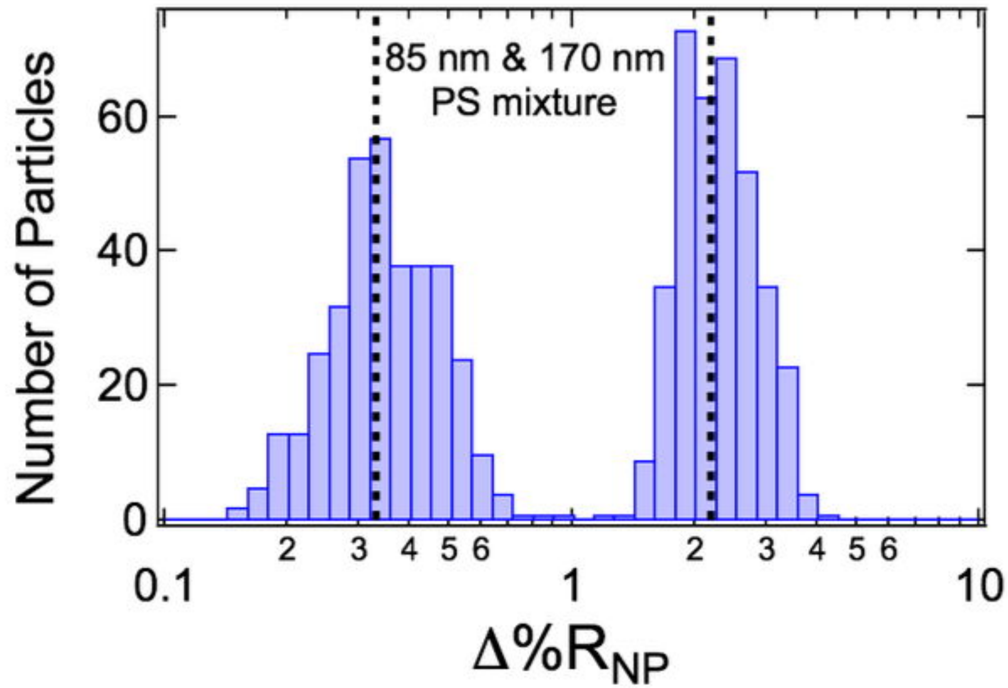
Single Nanoparticle Distribution Measurements



Mixture of Two Polystyrene NPs



A.M. Maley, G.J. Lu, M.G. Shapiro and R.M. Corn, ACS Nano, ***11*** *7447-7456 (2017).*

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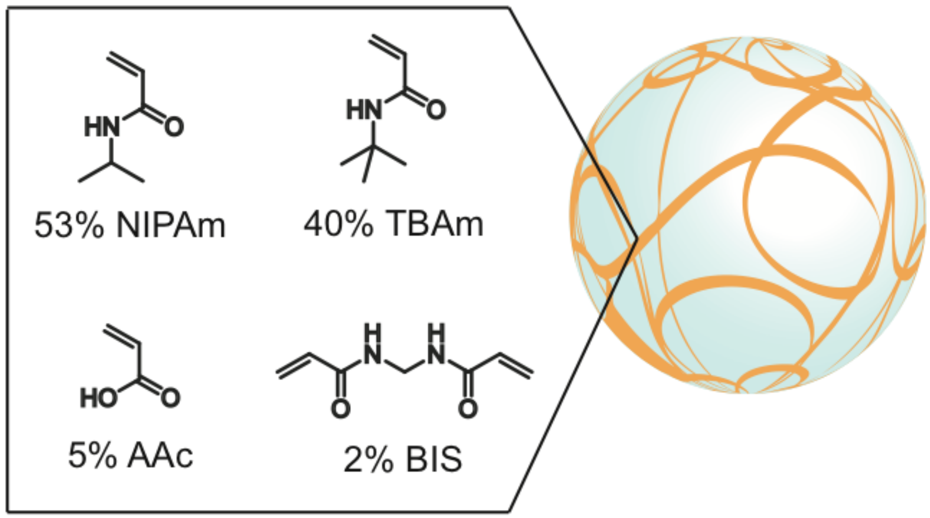
NIPam-based Hydrogel Nanoparticles

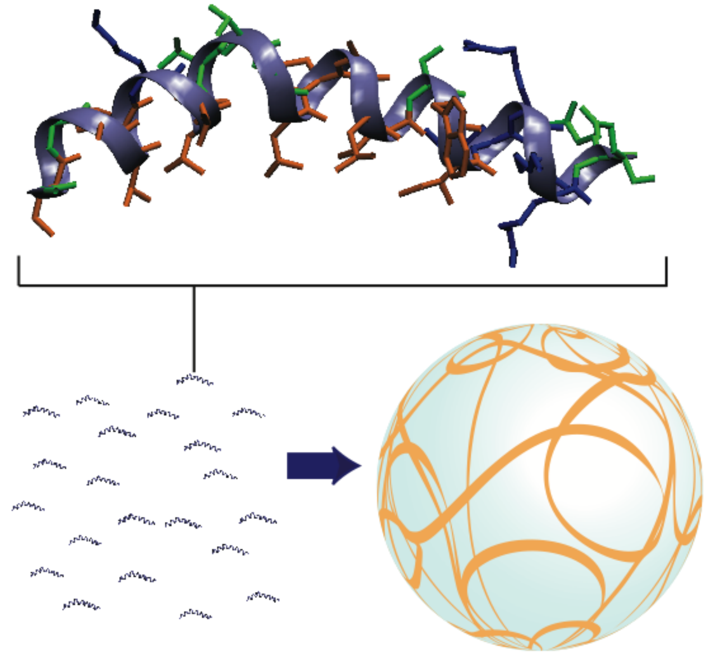
220 nm HNPs

Melittin

a.



b.



220 nm diameter (from DLS)

1.24 E09 Mol.Wt. (from MALS)

approx. 65% solvent (PBS) by volume

26 residue peptide
from bee venom

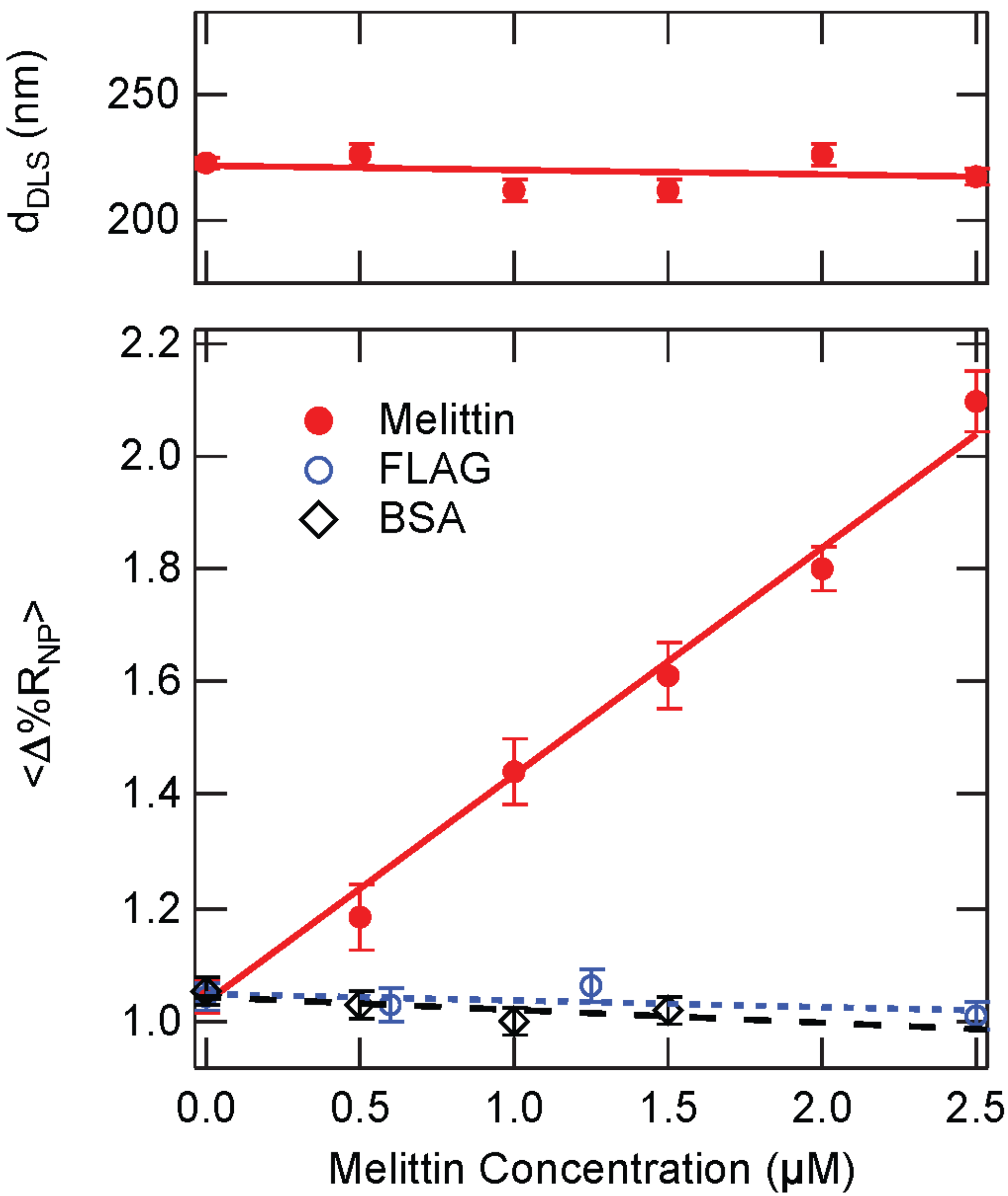
GIGAVLKVLTTGLPALISWIKRKRQQ

NIPam: N-isopropylacrylamide

Collaborator: Prof. Ken Shea

UCIrvine
University of California, Irvine

$\langle\Delta\%R_{NP}\rangle$ *for HNPs in the presence of Melittin*



$\langle\Delta\%R_{NP}\rangle$ for the HNPs increases with melittin concentration as the uptake of peptide increases the refractive index of the hydrogel nanoparticle.

Fluorescence measurements of the loss of melittin in solution are used to show that a 0.2% increase in $\langle\Delta\%R_{NP}\rangle$ corresponds to the uptake of 12,500 molecules per HNP on average.

Mike Cho

K. Cho et al. Analytical Chemistry, ***87*** *4973-4979 (2015).*

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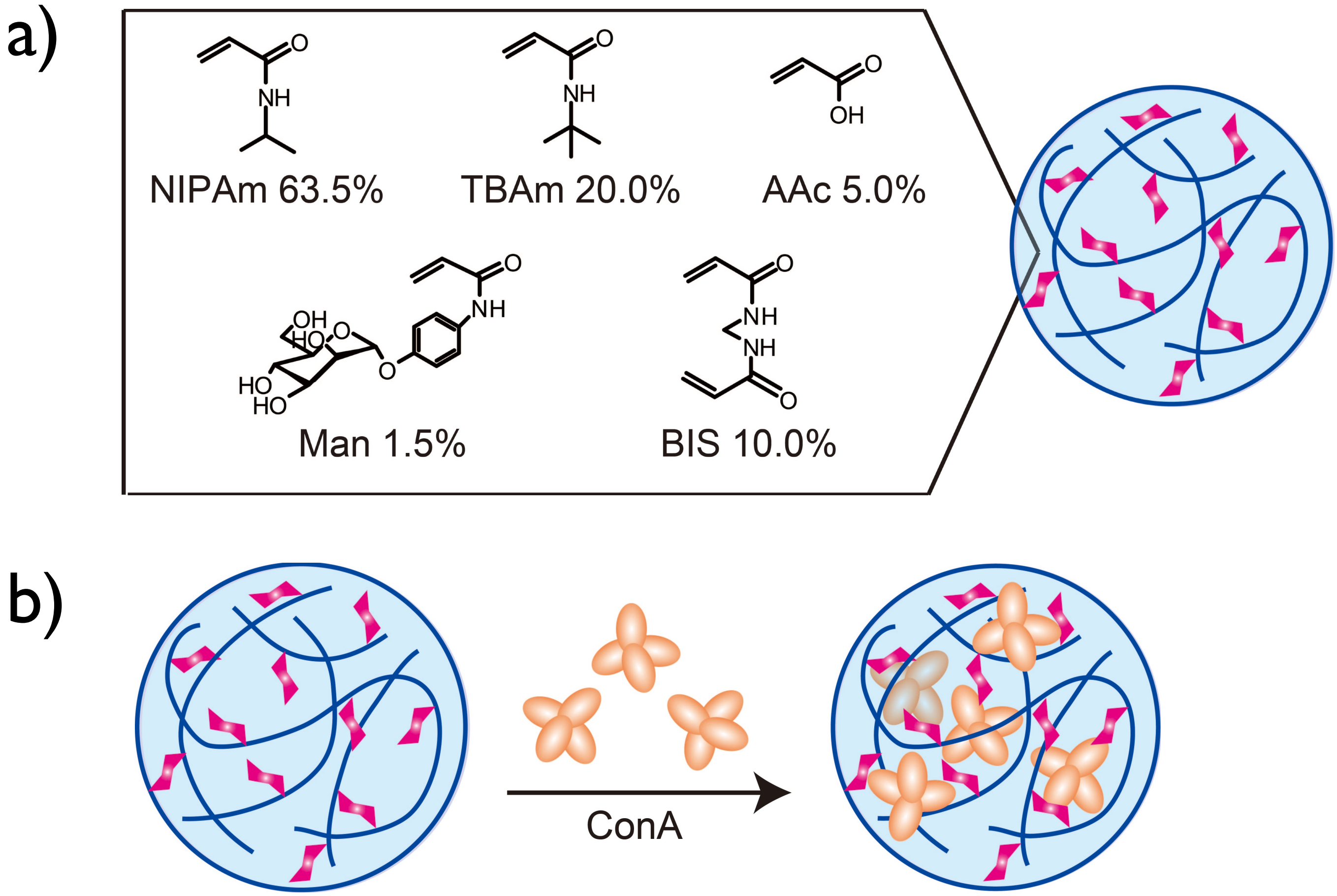
University of California, Irvine

ConA uptake into HNPs

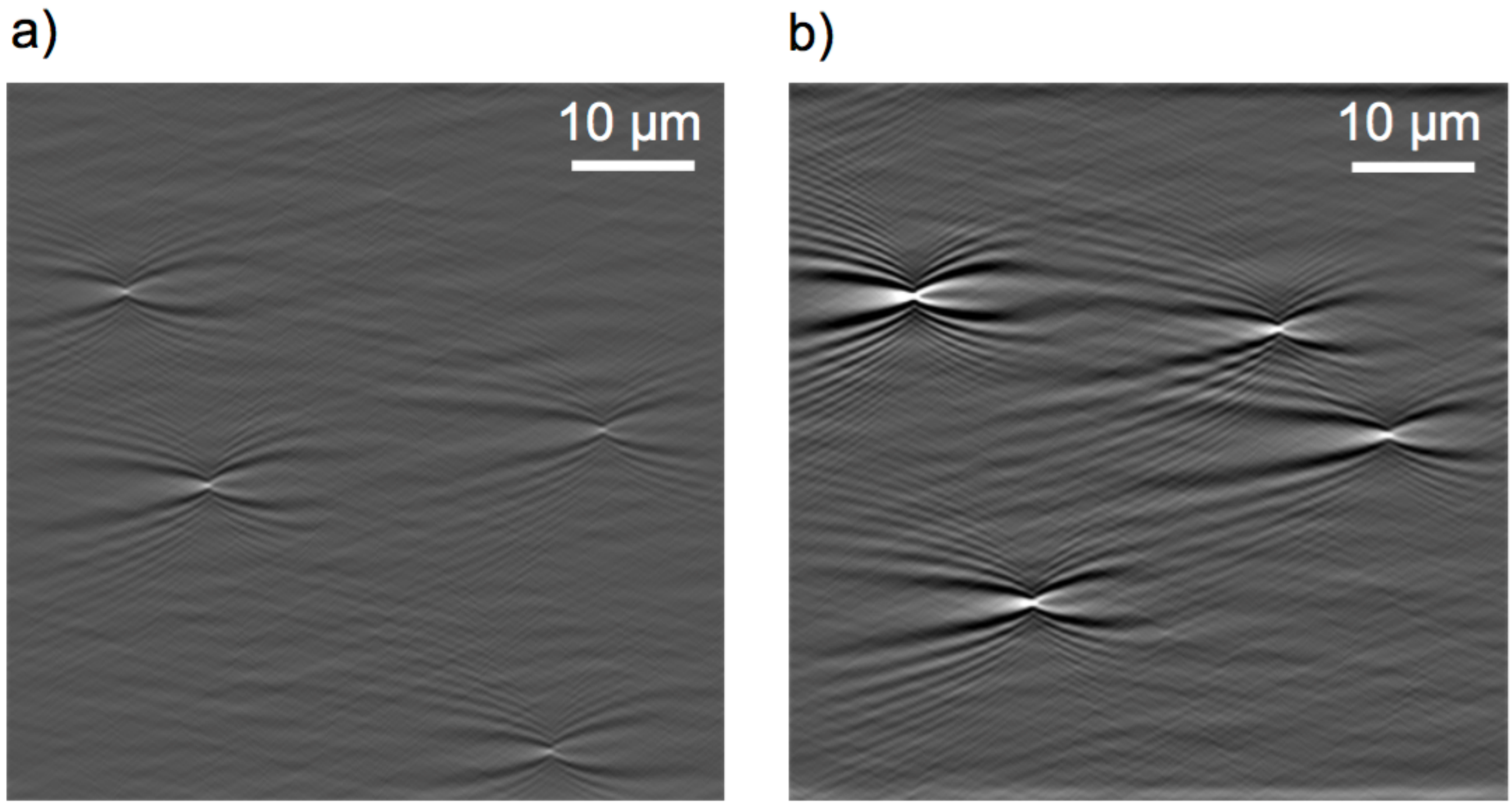


Yuhei

With Yuhei Terada and Prof. Yoshiko Miura (Kyushu Univ.)

A. M. Maley, Yuhei Terada et al., J. Phys. Chem. C, ***120*** *16843-16849 (2016).*

ConA uptake into HNPs



180 nm mHNPs | 180 nm mHNPs + 1μM ConA

Significant increase in the single nanoparticle response in the presence of ConA.

A. M. Maley, Yuhei Terada et al., J. Phys. Chem. C, ***120*** *16843-16849 (2016).*

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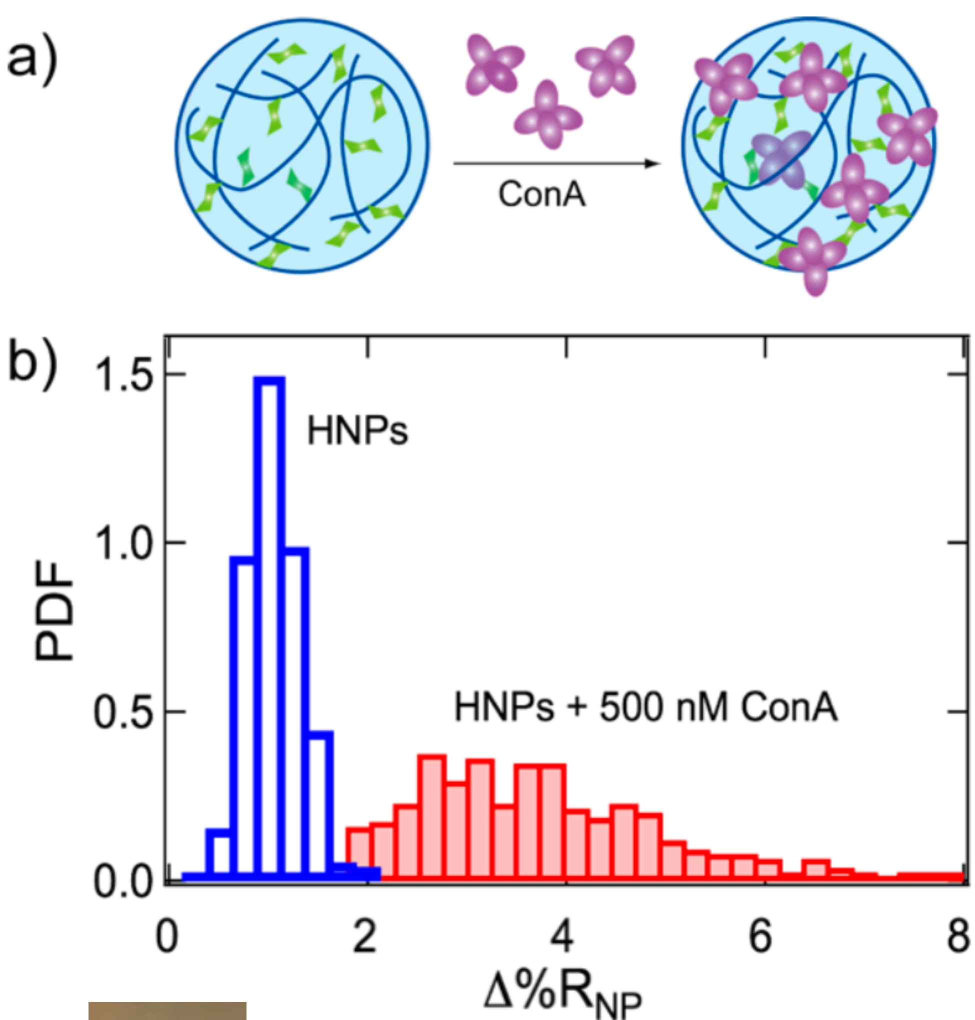
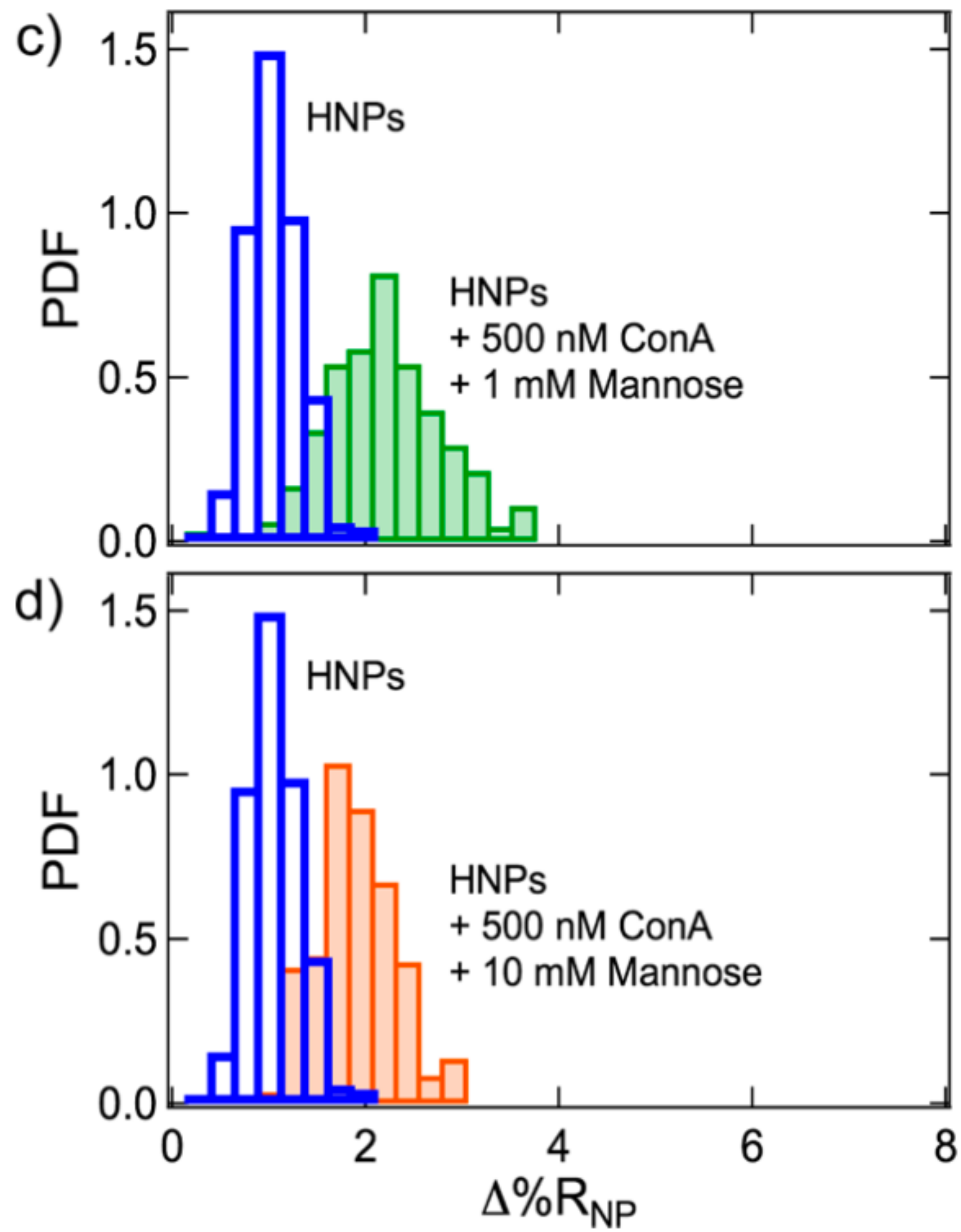
ConA uptake into HNPs

Mannose competition





Adam Maley

A.M. Maley, G.J. Lu, M.G. Shapiro and R.M. Corn, ACS Nano, **11** 7447-7456 (2017).

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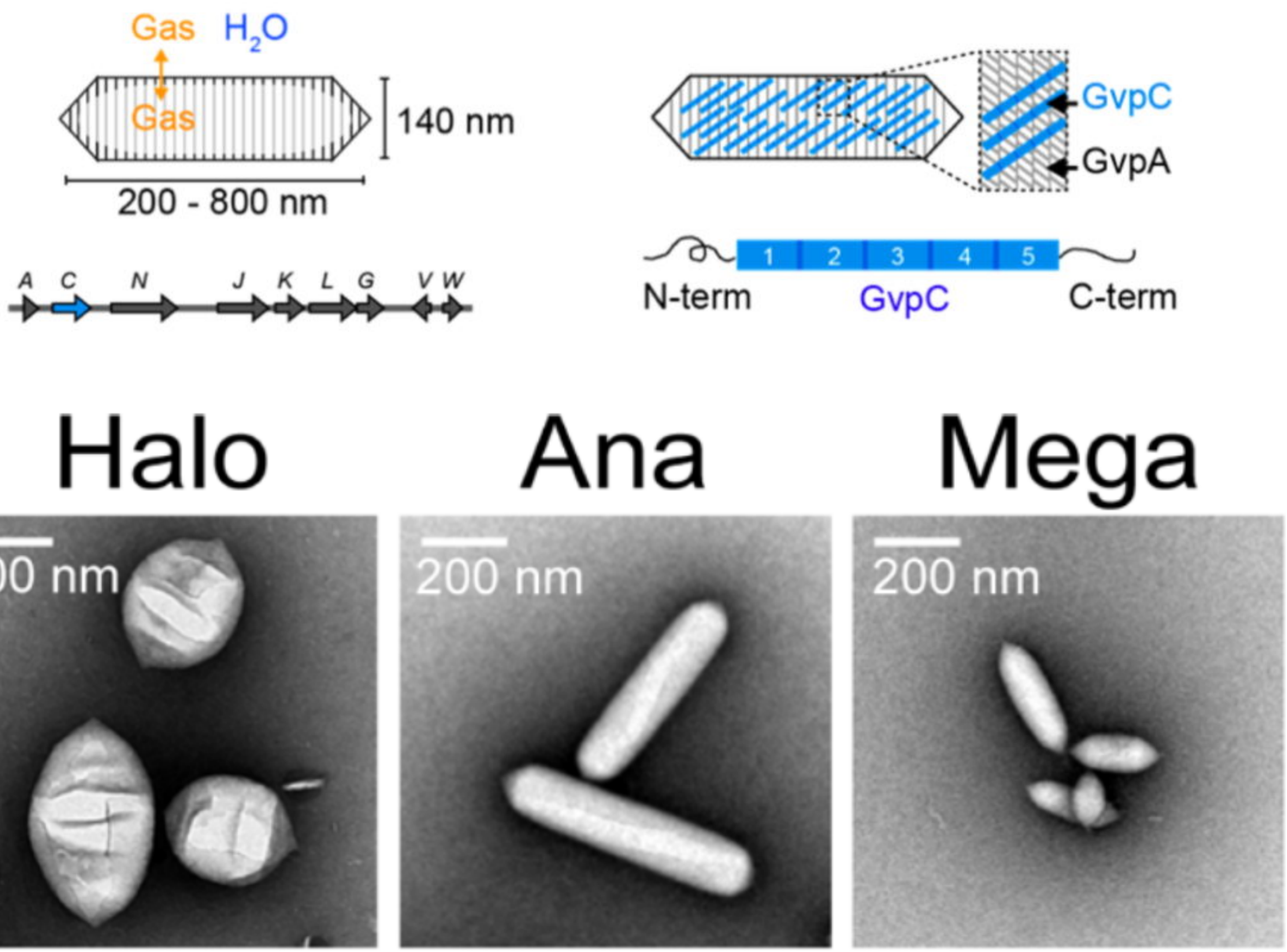
Biogenic Gas-Filled Protein Vesicles



With Prof. Mikhail G. Shapiro, Cal Tech.

*A. Lakshmanan et al., ACS Nano **10**, 7314–7322 (2016).*

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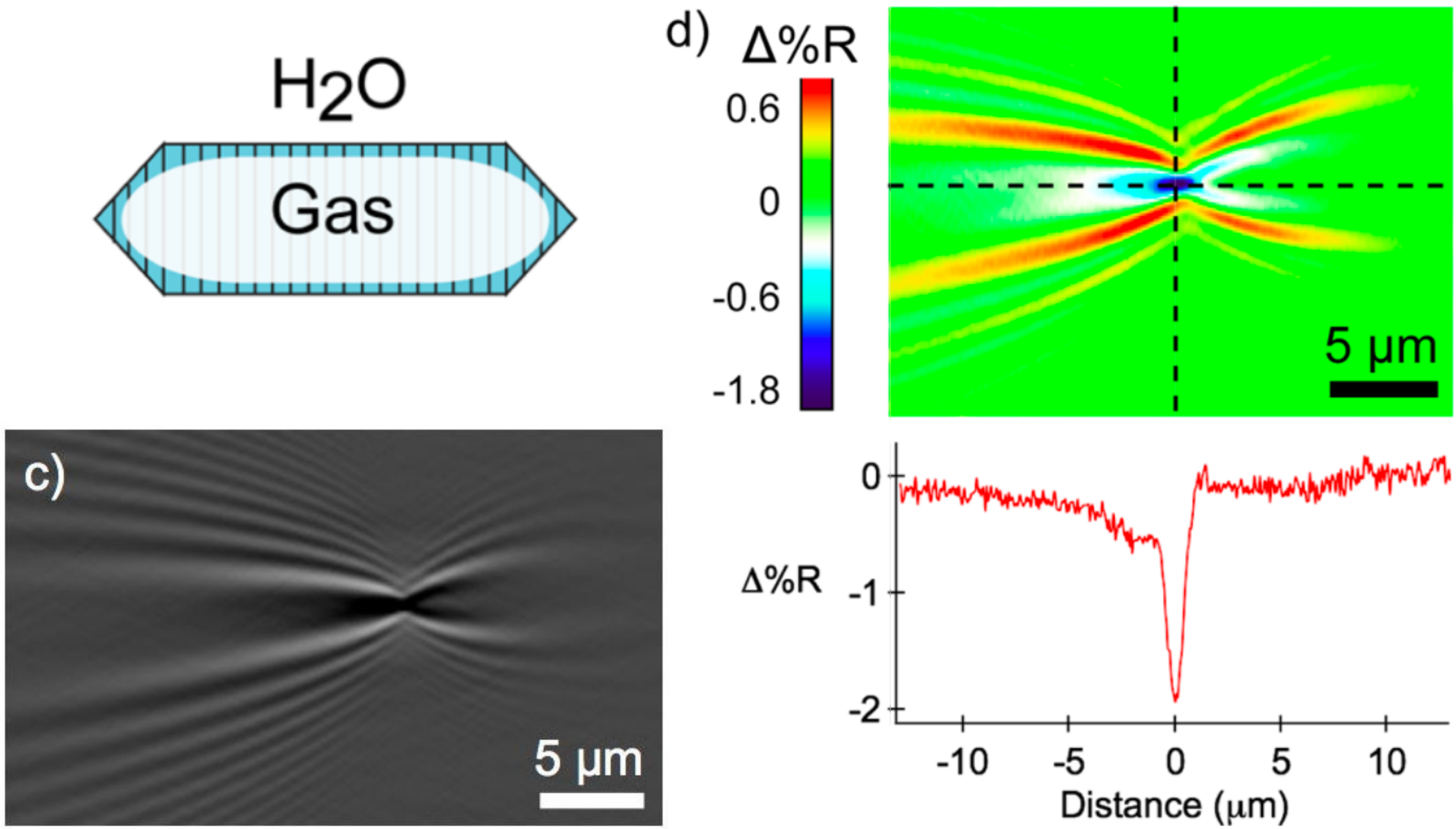
Biogenic Gas-Filled Protein Vesicles



A.M. Maley, G.J. Lu, M.G. Shapiro and R.M. Corn, ACS Nano, ***11*** *7447-7456 (2017).*

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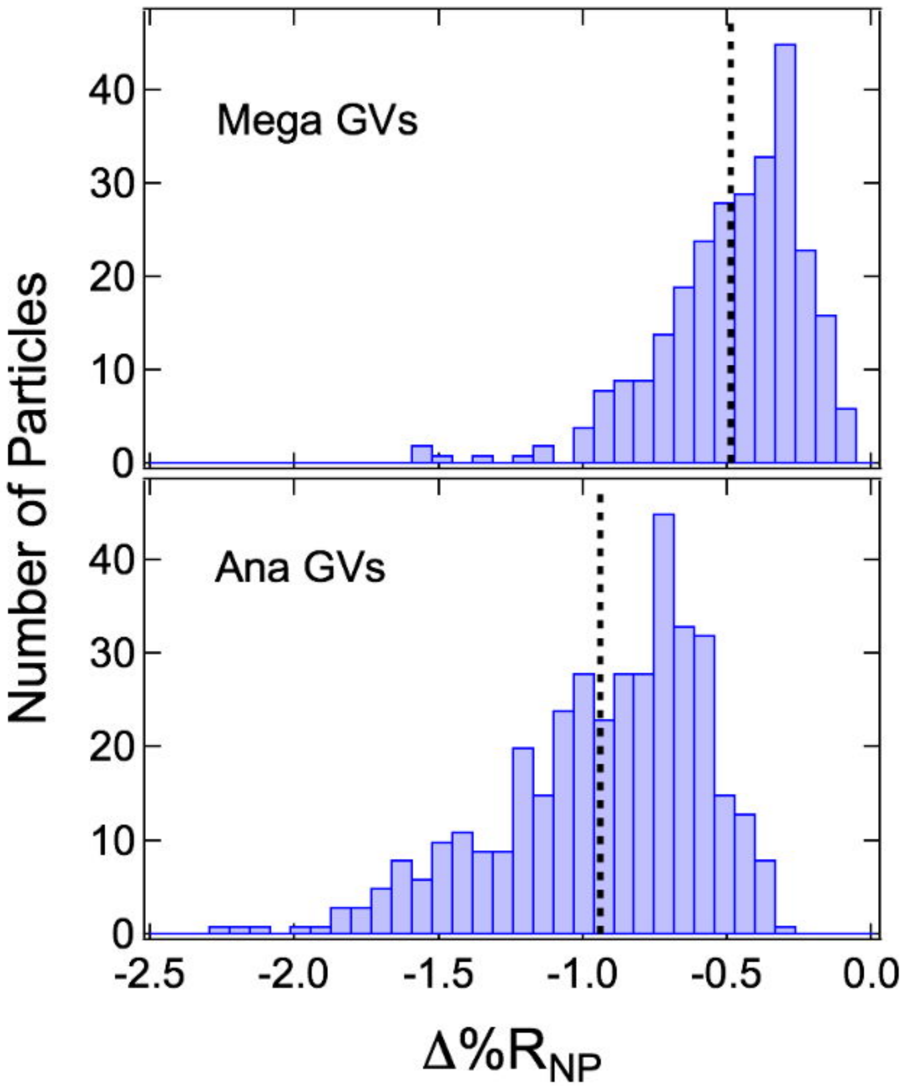
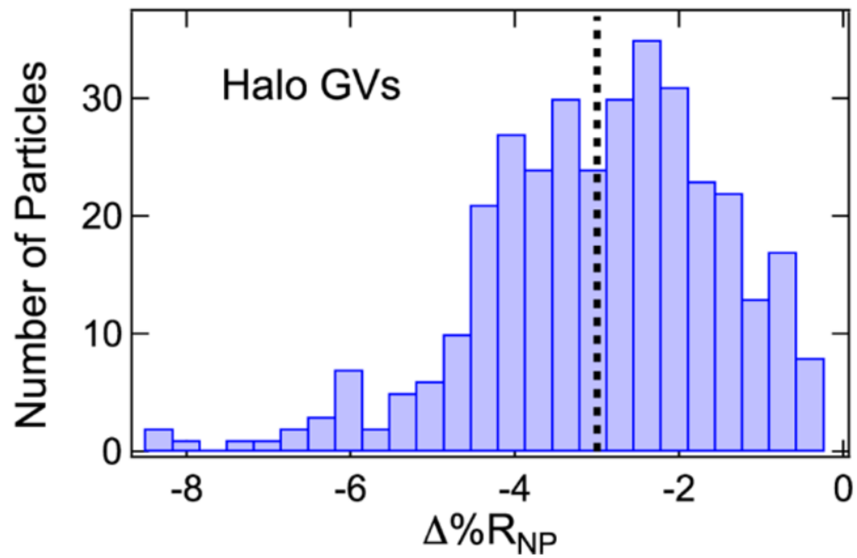
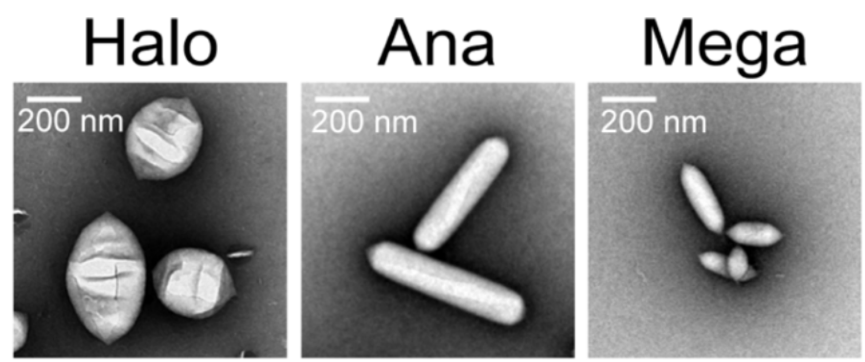
Biogenic Gas-Filled Protein Vesicles







A.M. Maley, G.J. Lu, M.G. Shapiro and R.M. Corn, ACS Nano, ***11*** *7447-7456 (2017).*

The Key Ingredient: Great Group Members and Collaborators!!!

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Dr. Alastair Wark
Dr. Hye Jin Lee

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NSF
UC-CRCC

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Prof. Yoshiko Miura (Kyushu)
Prof. Ken Shea (UCI)
Prof. Reg Penner (UCI)
Prof. Andrej Luptak (UCI)
Prof. Donghyun Kim (Yonsei)

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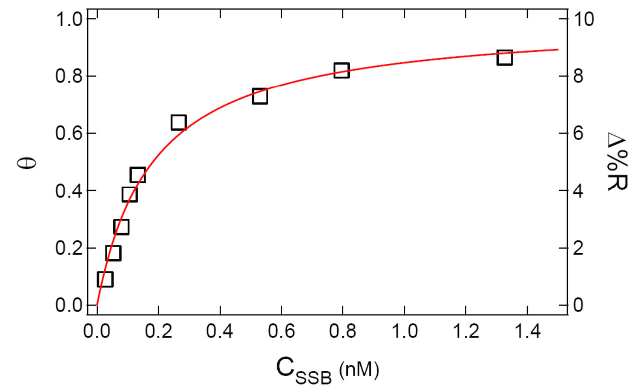
Langmuir Surface Adsorption Numbers: Femtomolar Detection

Stoichiometry: 1fM x 10μL = 10^{-20}moles = 6000 molecules

$$K_{ads} = \frac{k_a}{k_d} = \frac{\theta^{eq}}{C(1-\theta^{eq})}$$



Dr. Yulin Chen

$$\theta^{eq} = {}^{\Gamma}/_{\Gamma^{tot}} \approx K_{ads}C = (10^9 M^{-1})(10^{-15} M) = 10^{-6}$$

$$\Gamma^{tot} \approx 10^{12} molecules\ cm^{-2}$$

A = 100 μm x 100 μm

$$\Gamma A \approx (10^6 molecules\ cm^{-2})(10^{-4} cm^2) = 100\ molecules$$

Y. Chen et al., Langmuir, ***25*** *5054-5060 (2009).*

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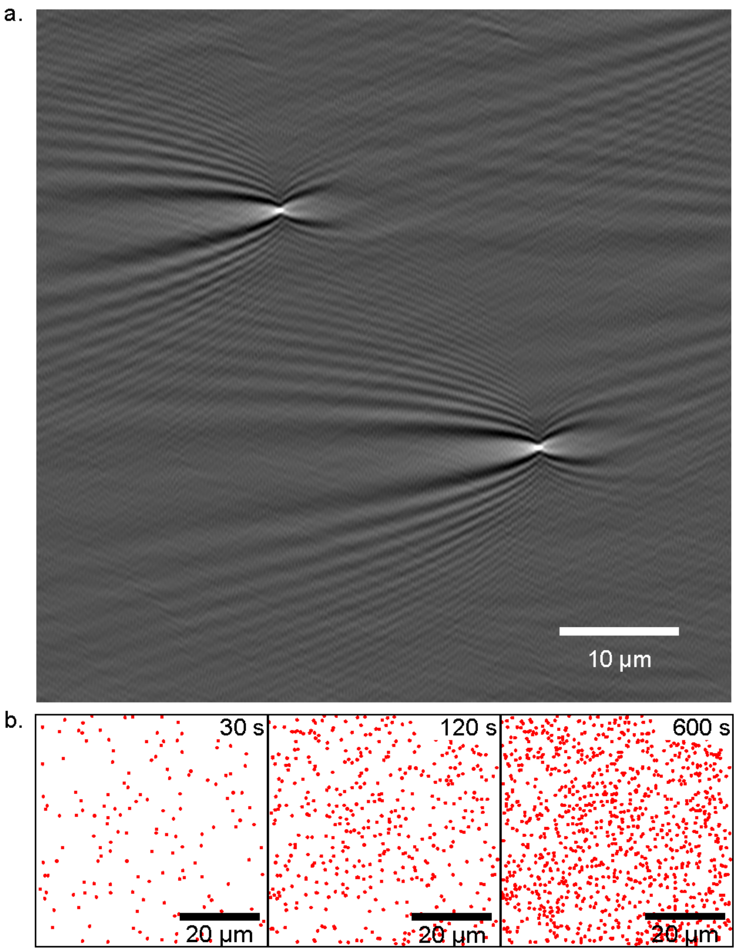
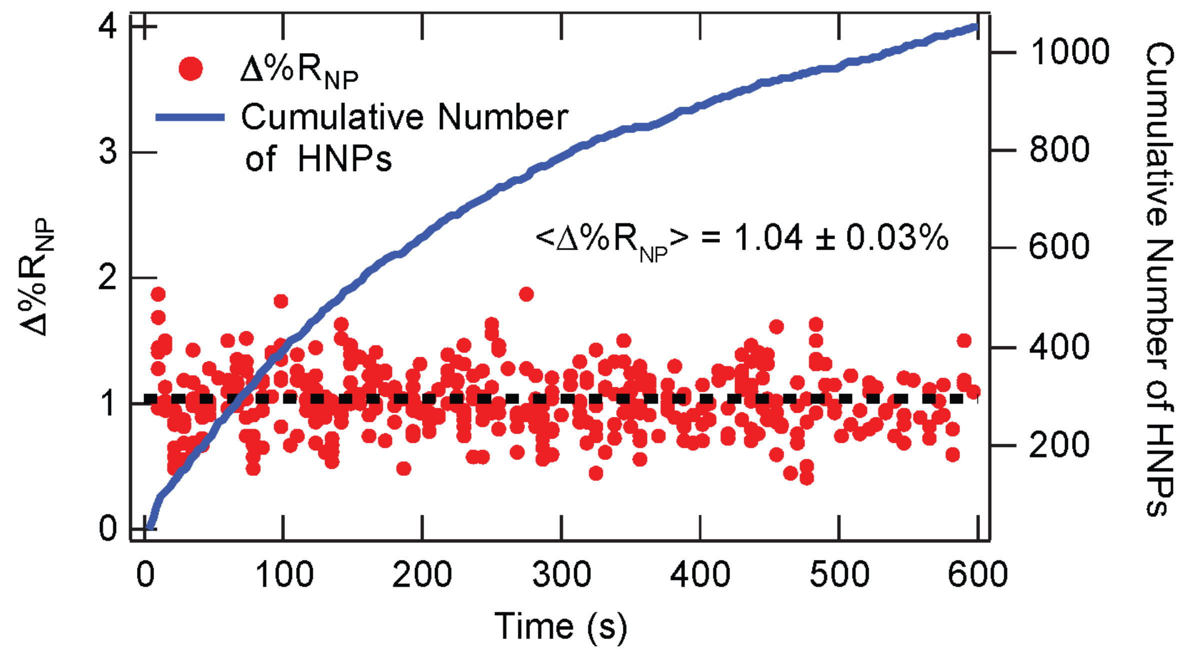
Single Nanoparticle SPRI 180 nm Hydrogel NPs





NIPam-based HNPs irreversibly adsorb onto C11-functionalized gold surfaces

2D maps of HNP adsorption after 30, 120 and 600 seconds

C11: undecanethiol

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Biogenic Gas-Filled Protein Vesicles

$$\mathrm{PDF} = \frac{1}{\Delta\%R_{\mathrm{NP}}\sigma\sqrt{2\pi}}\exp\left[-\frac{(\ln(\Delta\%R_{\mathrm{NP}}) - \mu)^2}{2\sigma^2}\right]$$

lognormal distribution function

$$g = \frac{m_3}{s^3} = \frac{\frac{1}{n}\sum(\Delta\%R_{\mathrm{NP}} - \langle\Delta\%R_{\mathrm{NP}}\rangle)^3}{s^3}$$

skewness

ACS Nano Article

Table 1. Hydrodynamic Size Measurements from DLS for Polystyrene and Hydrogel Nanoparticles and Statistics from Single-Nanoparticle SPRI Measurements for Polystyrene and Hydrogel Nanoparticles and Gas Vesicles

nanoparticle	diameter (nm)	standard deviation (nm)	$\langle\Delta\% R_{\mathrm{NP}}\rangle$	standard deviation (s)	95% CI	skewness (g)	μ[a]	σ[b]	no. of NPs
PS (A)	85	25	0.34	0.10	0.01	0.59	−1.13	0.31	354
PS (B)	170	40	2.19	0.48	0.05	0.68	0.76	0.21	365
HNP	271	55	1.67	0.43	0.05	0.60	0.48	0.27	324
HNP + 2 μM melittin	272	65	2.79	0.52	0.08	0.02	1.01	0.20	172
HNP	272	50	0.90	0.27	0.03	0.55	−0.15	0.31	289
HNP + 500 nM ConA	357	75	3.6	1.3	0.2	0.79	1.22	0.37	307
HNP + 500 nM ConA + 1 mM Man	338	65	2.04	0.60	0.07	0.05	0.66	0.36	270
HNP + 500 nM ConA + 10 mM Man	320	55	1.74	0.41	0.05	0.30	0.53	0.24	241
Mega GV	–[c]	–	−0.49	0.26	0.03	−1.28	−0.84	0.52	274
Ana GV	–	–	−1.07	0.44	0.04	−1.53	−0.0083	0.38	395
Halo GV	–	–	−3.0	1.5	0.2	−0.74	0.95	0.58	345

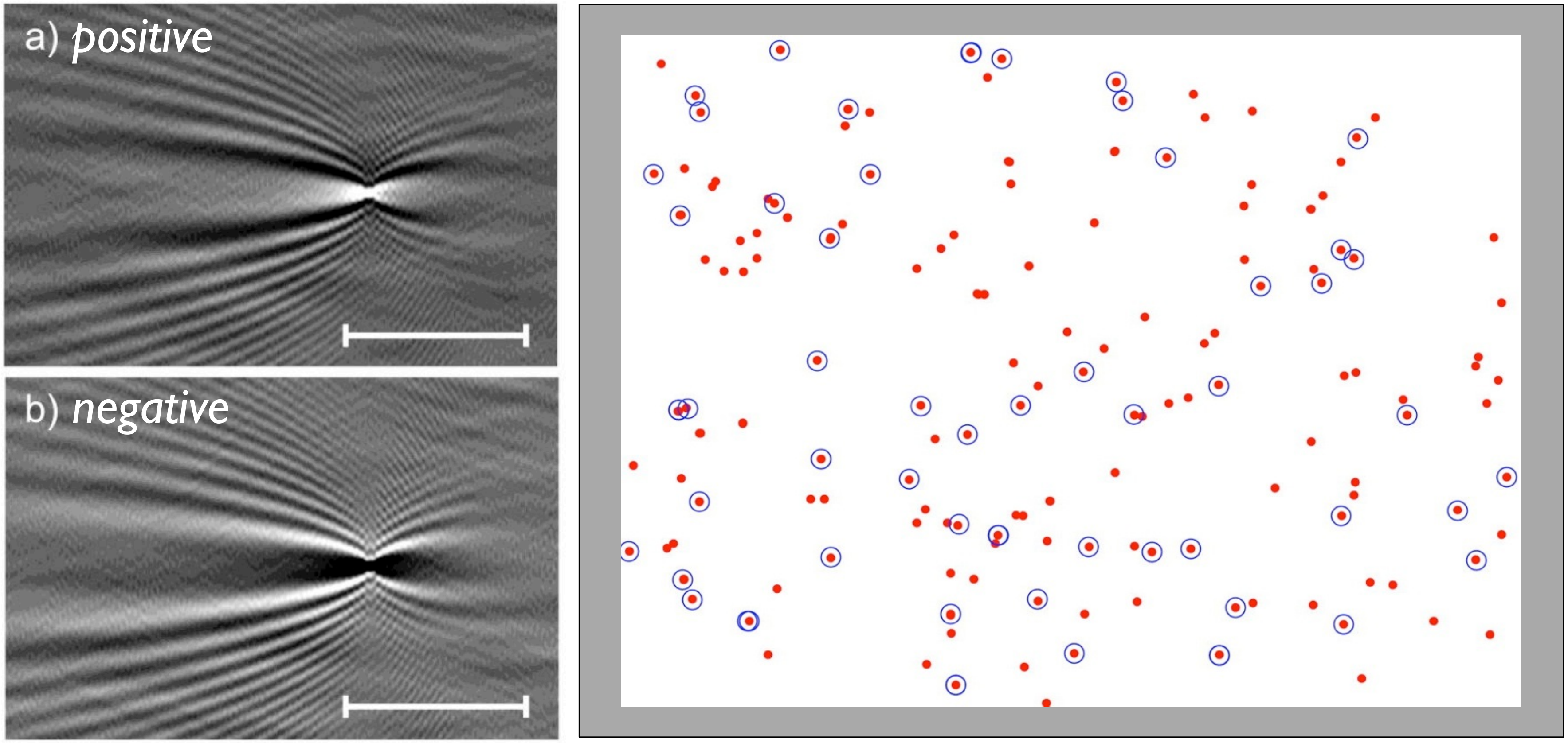
[a]Log-normal PDF location parameter. [b]Log-normal PDF scale parameter. [c]Size measurements for GVs are reported in Table 2.

*A.M. Maley, G.J. Lu, M.G. Shapiro and R.M. Corn, ACS Nano, **11** 7447-7456 (2017).*

DNA nanoparticle adsorption onto single DNA sequences

How much adsorption of 40 nm DNA-modified Au Nanoparticles do we see onto DNA-modified surfaces with only a small number of binding sites?

two component complementary/non-complementary monolayer



0.01% complementary monolayer

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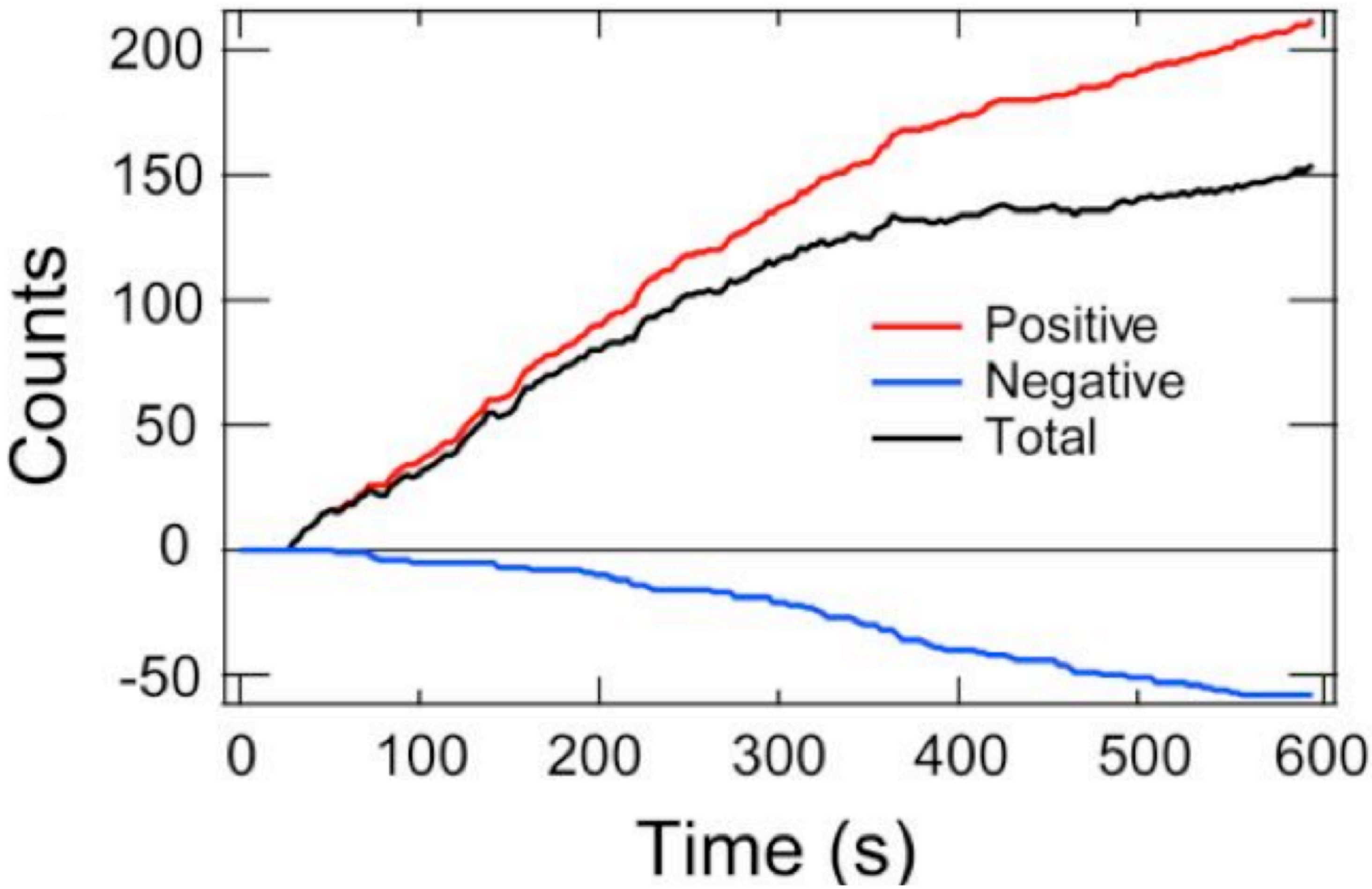
DNA nanoparticle adsorption onto single DNA sequences

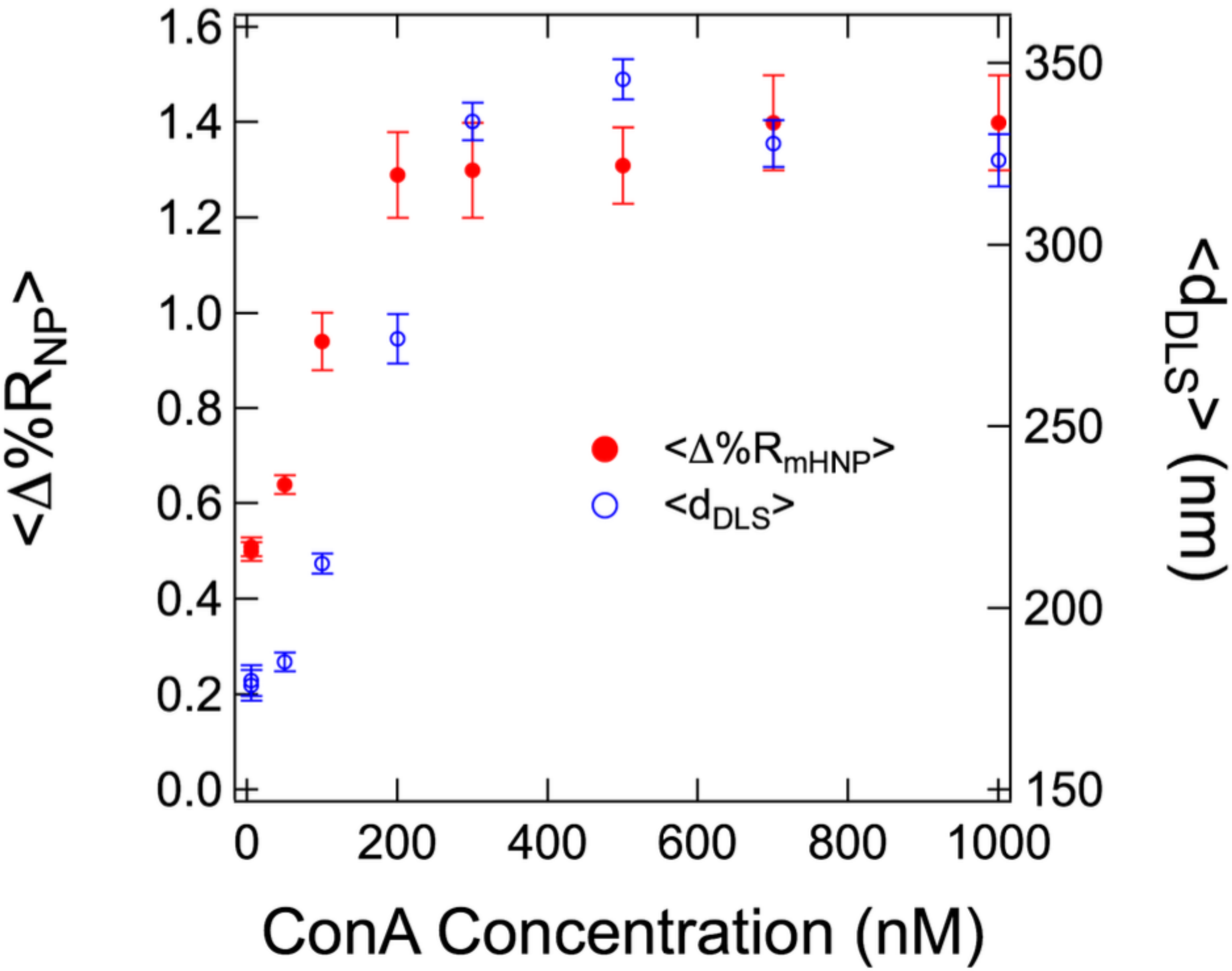
How much adsorption of 40 nm DNA-modified Au Nanoparticles do we see onto DNA-modified surfaces with only a small number of binding sites?

two component complementary/non-complementary monolayer



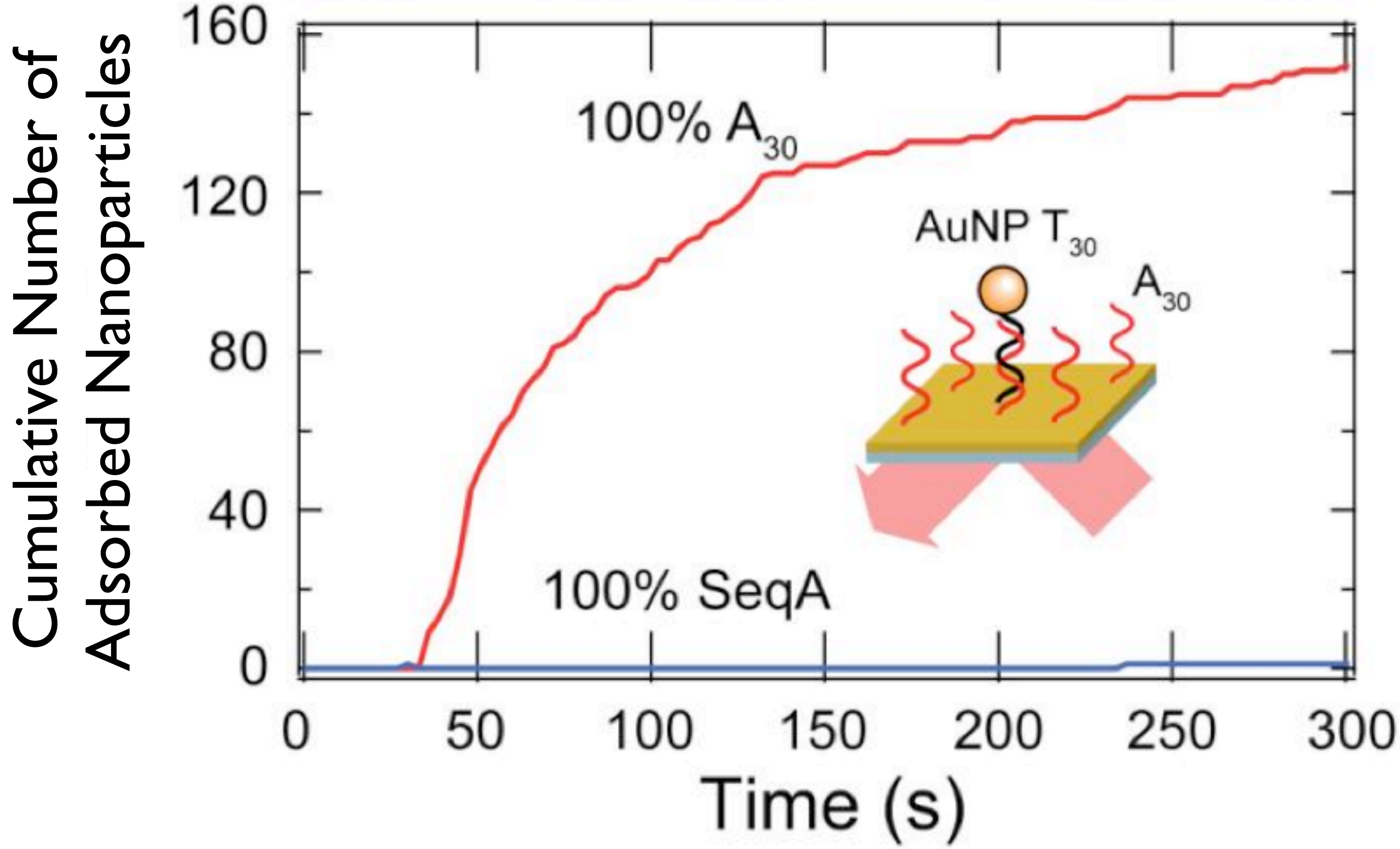
0.01% complementary monolayer

ConA uptake into HNPs



Average single nanoparticle response and DLS hydrodynamic diameter

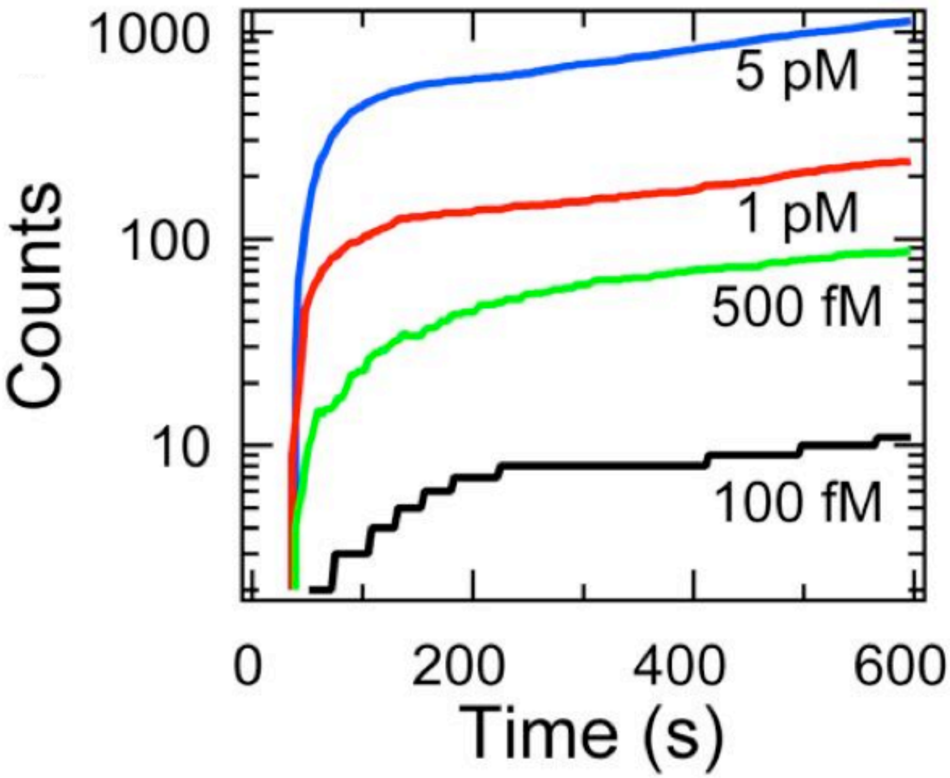
Bioaffinity Adsorption of 40 nm DNA-modified Au Nanoparticles

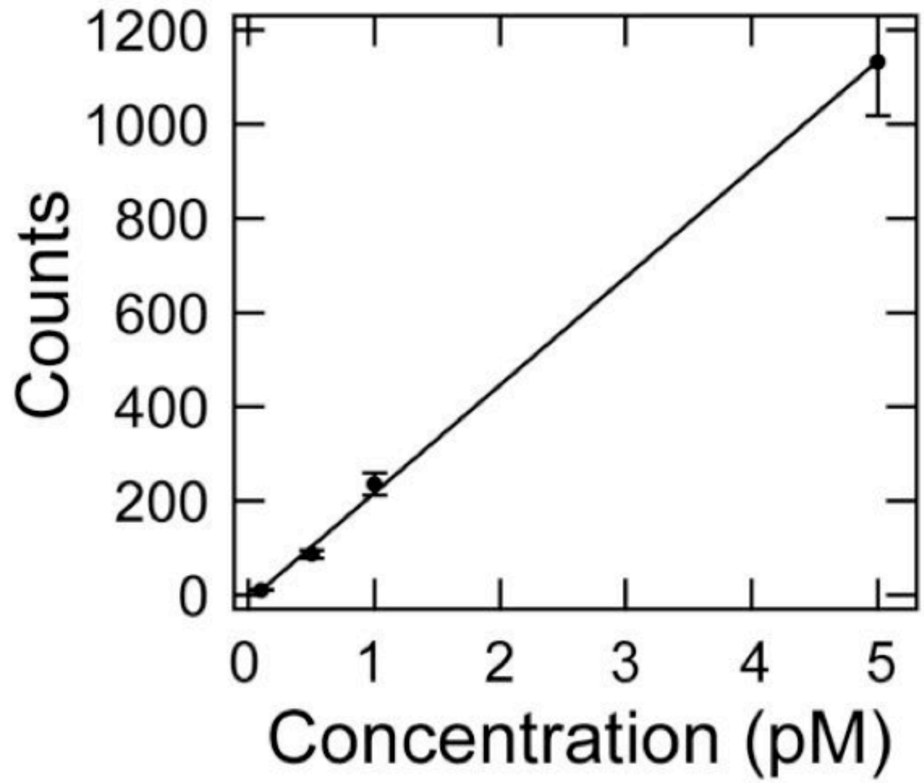


1 pM solution of 40 nm DNA-modified AuNPs

Noncomplementary DNA-modified surface shows negligble nanoparticle binding

Bioaffinity Adsorption of 40 nm DNA-modified Au Nanoparticles





The number of binding events scale with Au NP concentration

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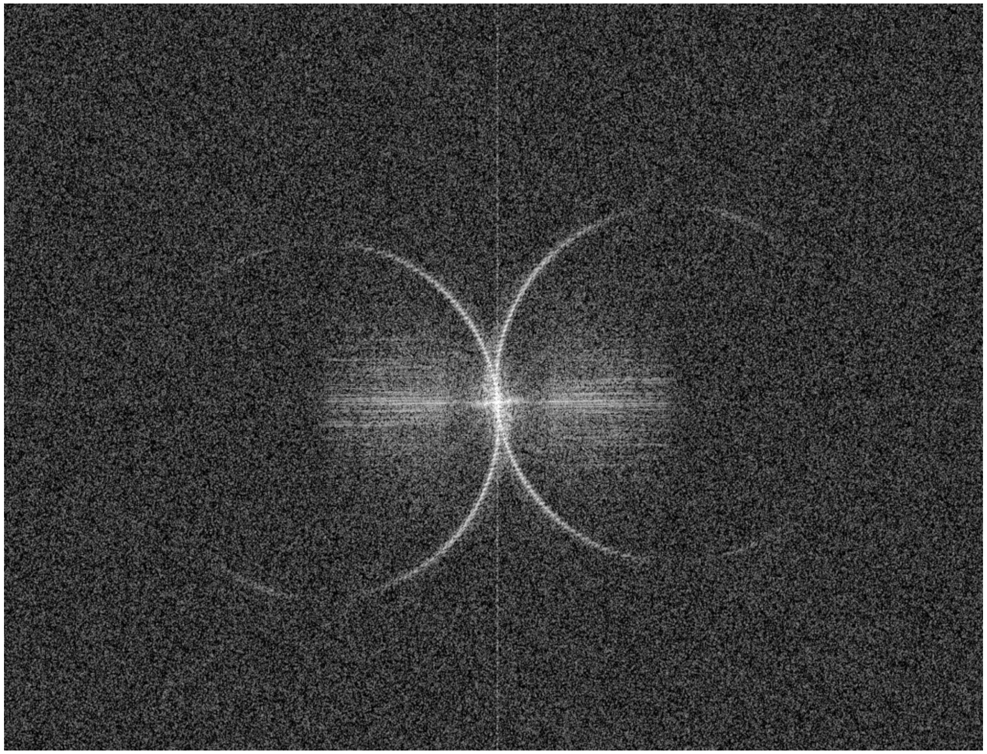
40 nm Gold Nanoparticles



2D FFT of CCD image

2D Helmholtz Equation
for wave propagation

$$(\nabla^2 + k_{sp}^2)u(x,y) = 0$$

$$(-\omega_x^2 + -\omega_y^2 + k_{sp}^2)u(\omega_x, \omega_y) = 0$$

Fourier Transform

$$\omega_x^2 + \omega_y^2 = k_{sp}^2$$

Resonance Condition

2D diffraction pattern of the surface plasmon polaritons off of the nanoparticle results in circles in the FFT of the image.

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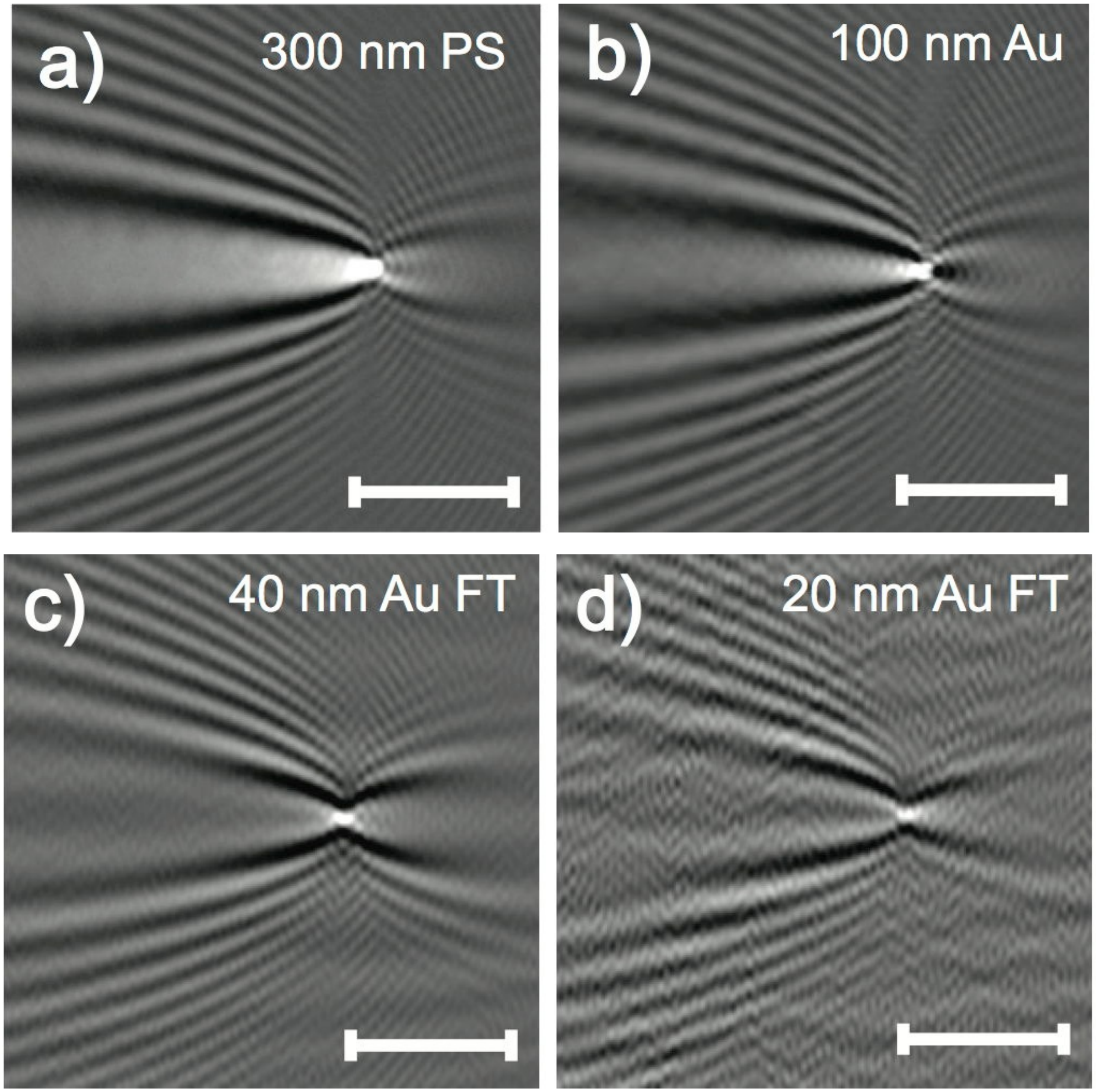
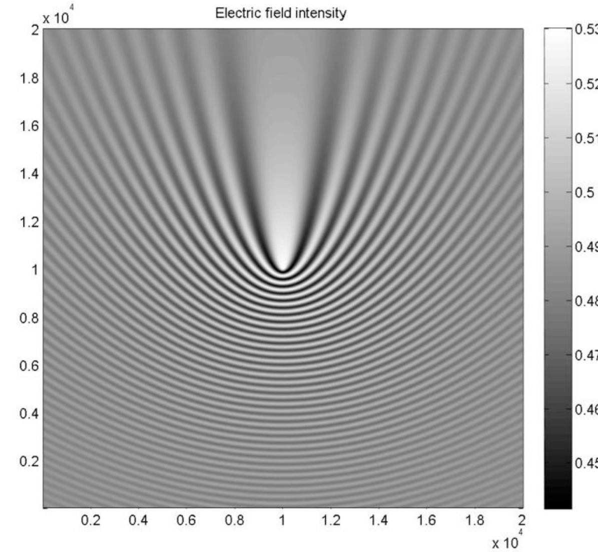
Near Infrared TIR SPR microscope: 814 nm SPP diffraction





Calculations from Prof. D. Kim
at Yonsei University

All particles have similar functional form

Tail periodicity due to SP propagation length at 814 nm

Difference Image in Aqueous Solution
3 sec integration time

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