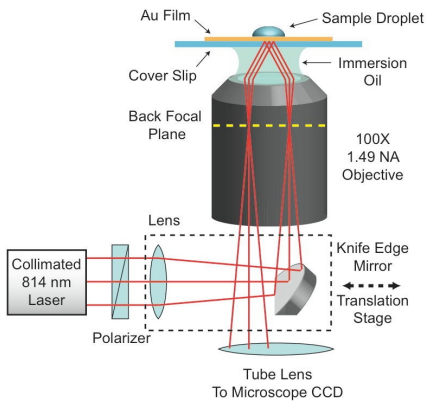
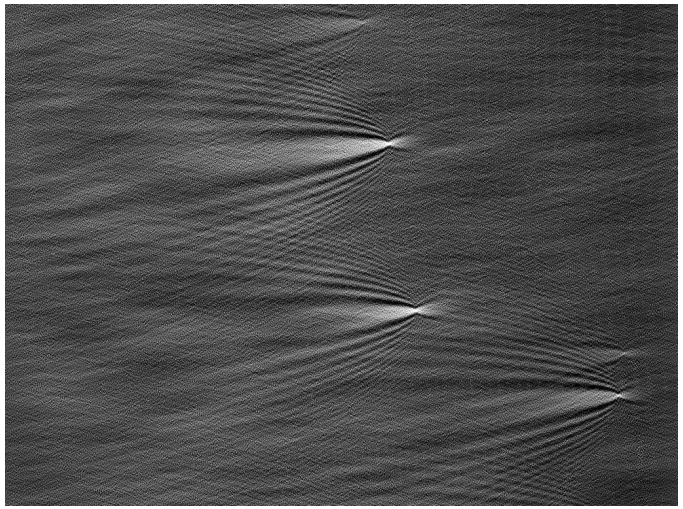
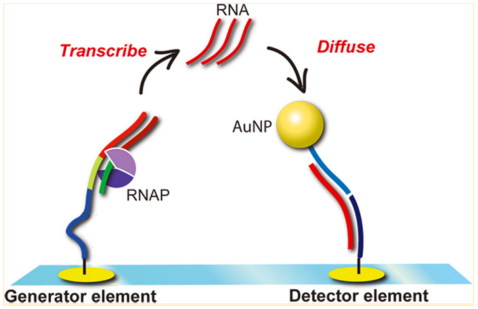
Single Nanoparticle Surface Plasmon Resonance Imaging Biosensing

Single Nanoparticle SPRI

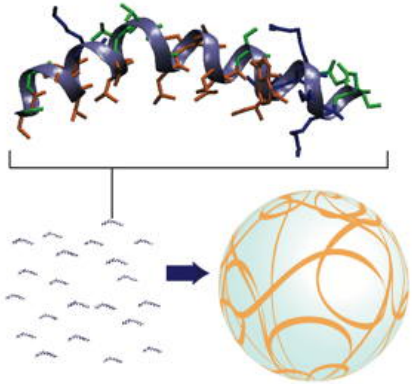


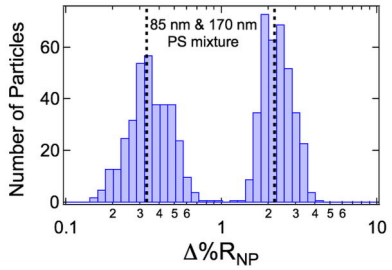


Surface Enzyme Chemistries



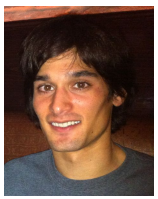
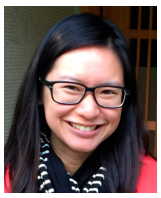
Bioaffinity Uptake into Single Nanoparticles

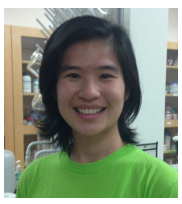




Robert M. Corn
UCI Department of Chemistry



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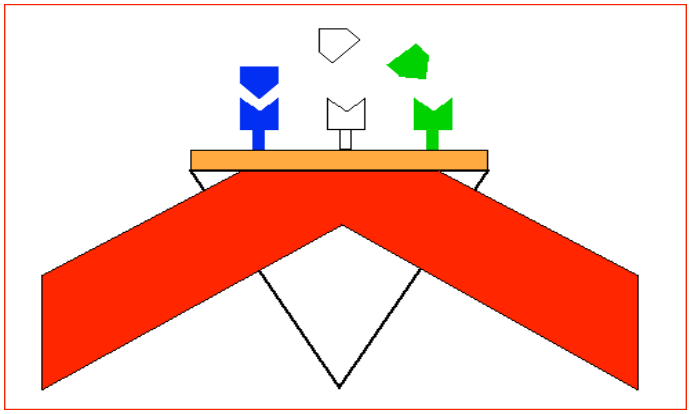
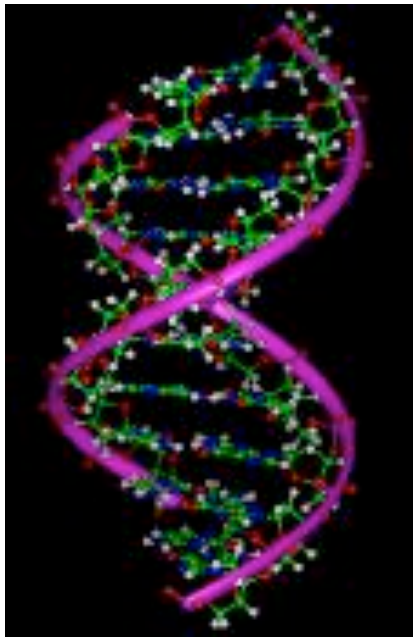
Adsorption Biosensors



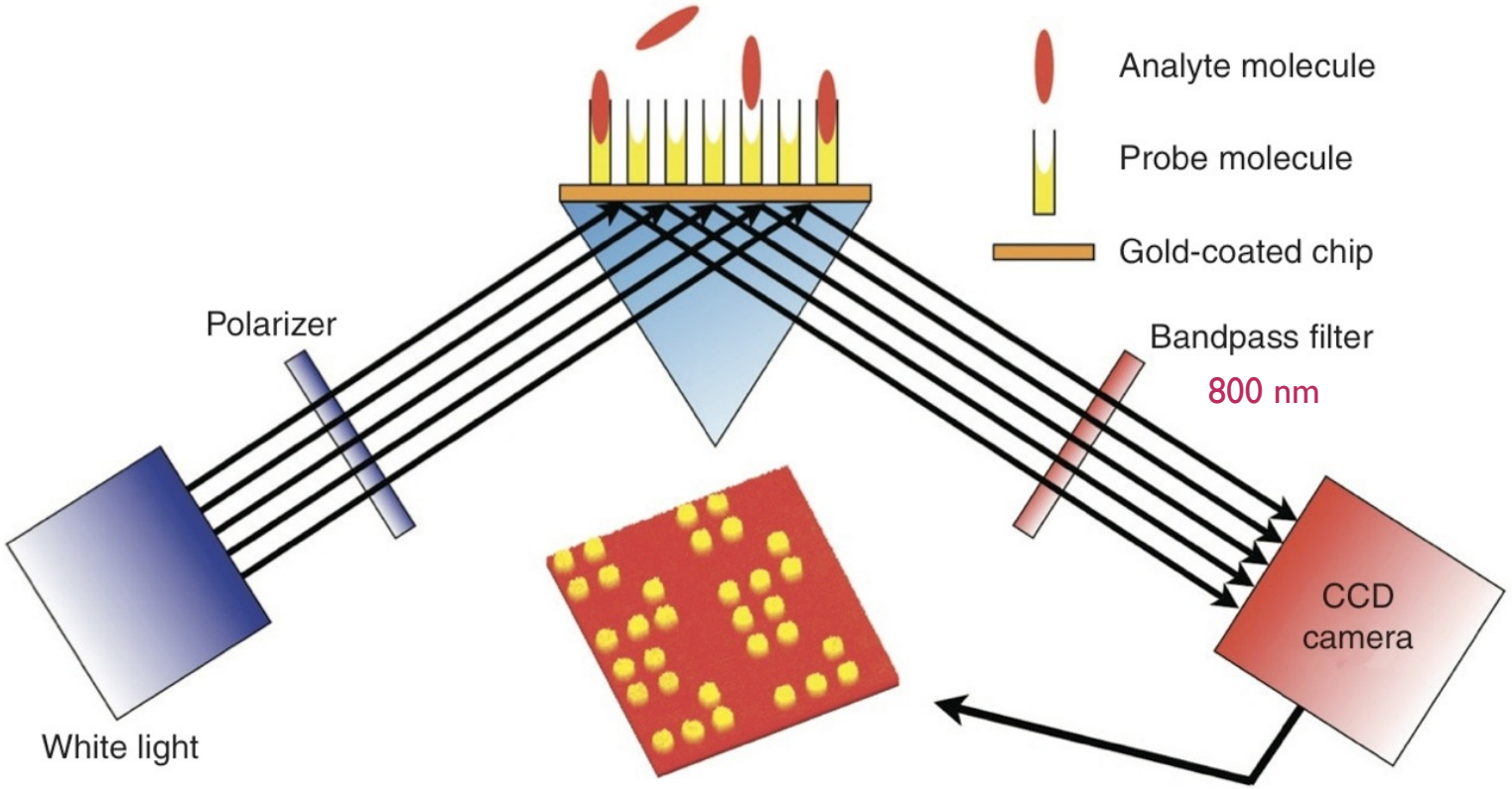


DNA-DNA
Binding

Target molecules (DNA, proteins, biomarkers) are detected when they adsorb to a surface.

Surface Plasmon Resonance Imaging (SPRI)

SPRI detects the adsorption of a biomolecule by its Refractive Index



DNA microarray

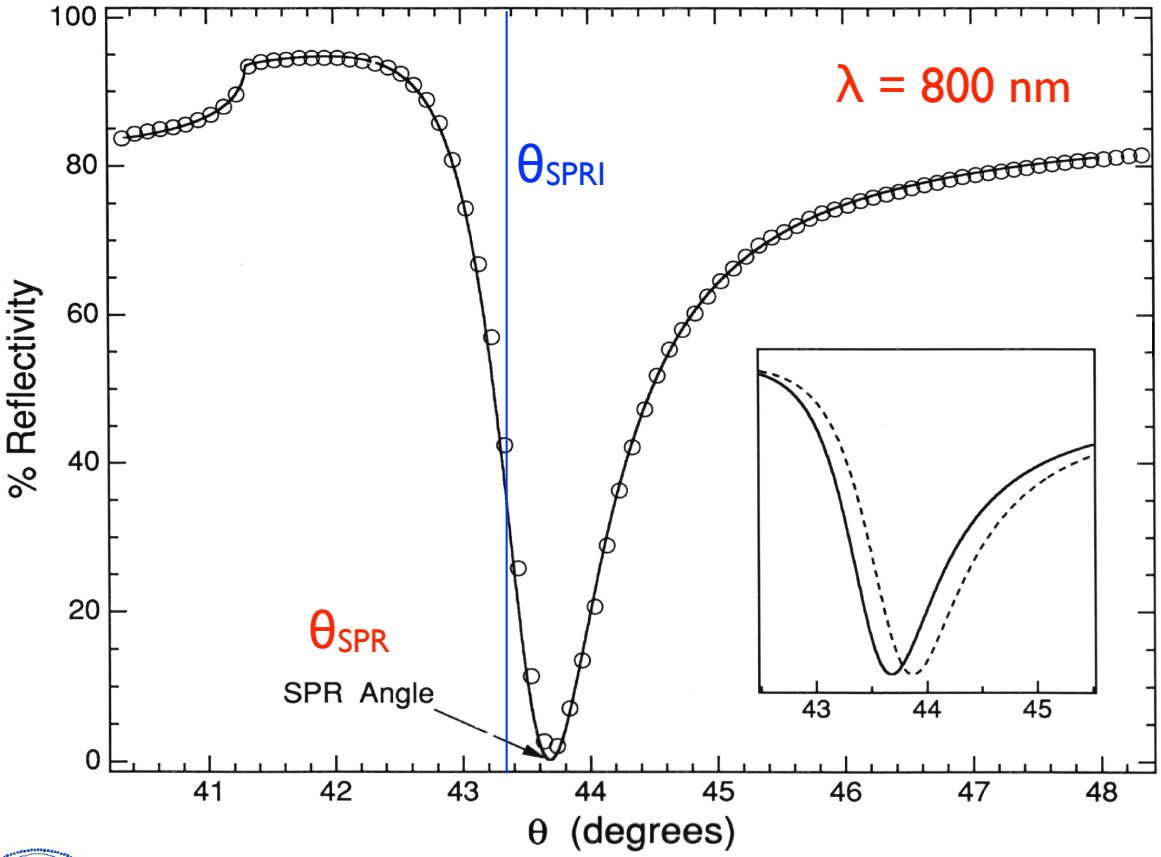
*B. Rothenäusler and W. Knoll, Nature **332**, 615-617 (1988).*
JM Brockman, AG Frutos, BP Nelson and RM Corn, Analytical Chem., 71 3928-3934 (1999).

A quick note on Surface Plasmons and SPRI versus SPR versus LSPR

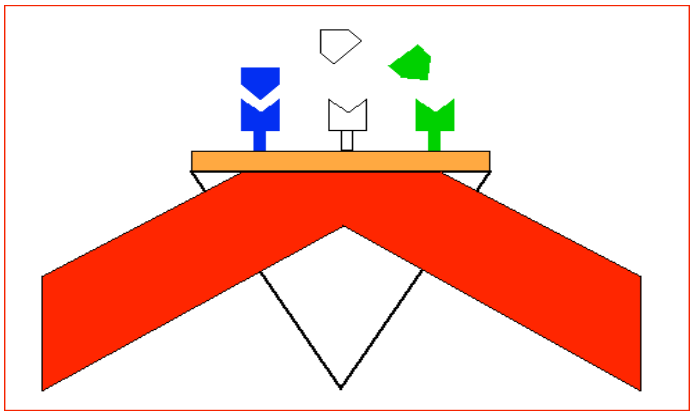
Surface plasmons (SPs) are localized propagating electromagnetic waves attached to a thin gold film (45 nm) attached to a prism surface.

Surface Plasmon Resonance (SPR)

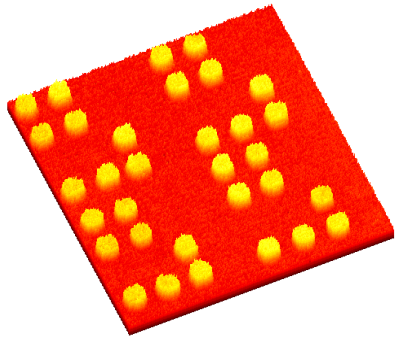
Changes in the resonant SPR angle (θ_{SPR}) can be used to study adsorption onto the gold surface.



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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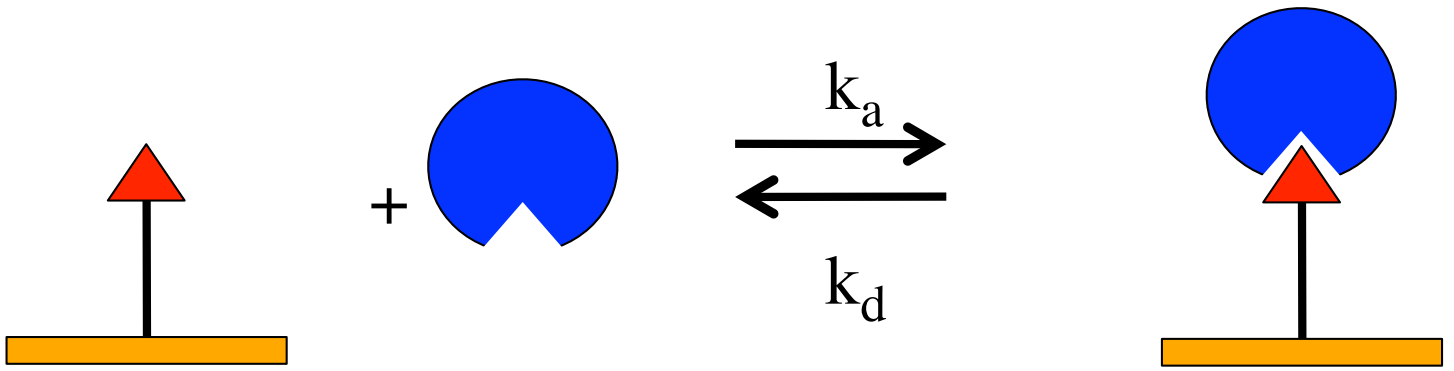
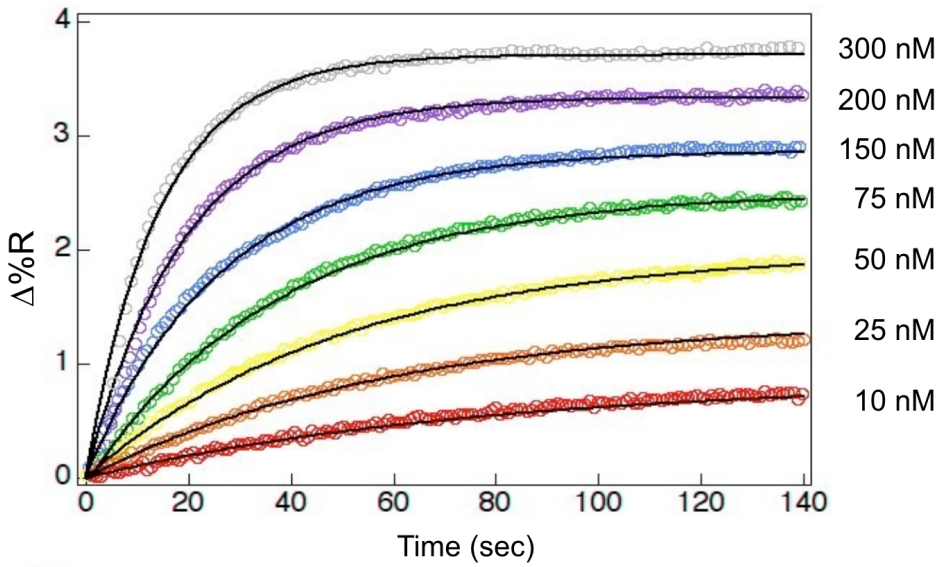
SPRI Measures Surface Adsorption Kinetics



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

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

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

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

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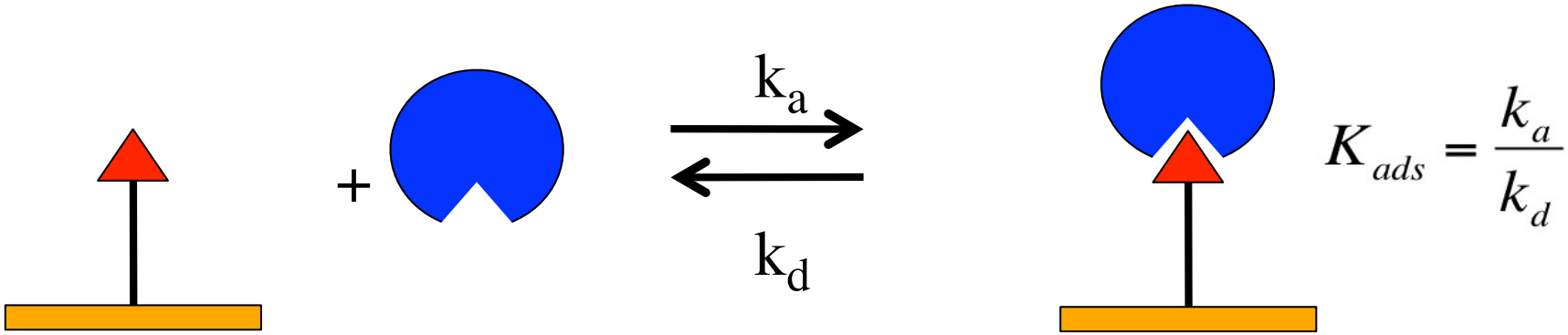
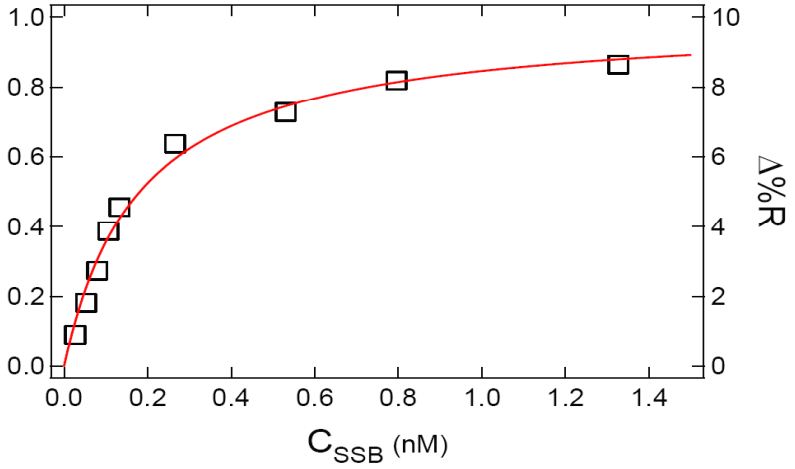
SPRI Equilibrium Measurements: Langmuir Adsorption Isothrem



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

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

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



Langmuir Adsorption Isotherm

Dr. Yulin Chen

Greta Wegner

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

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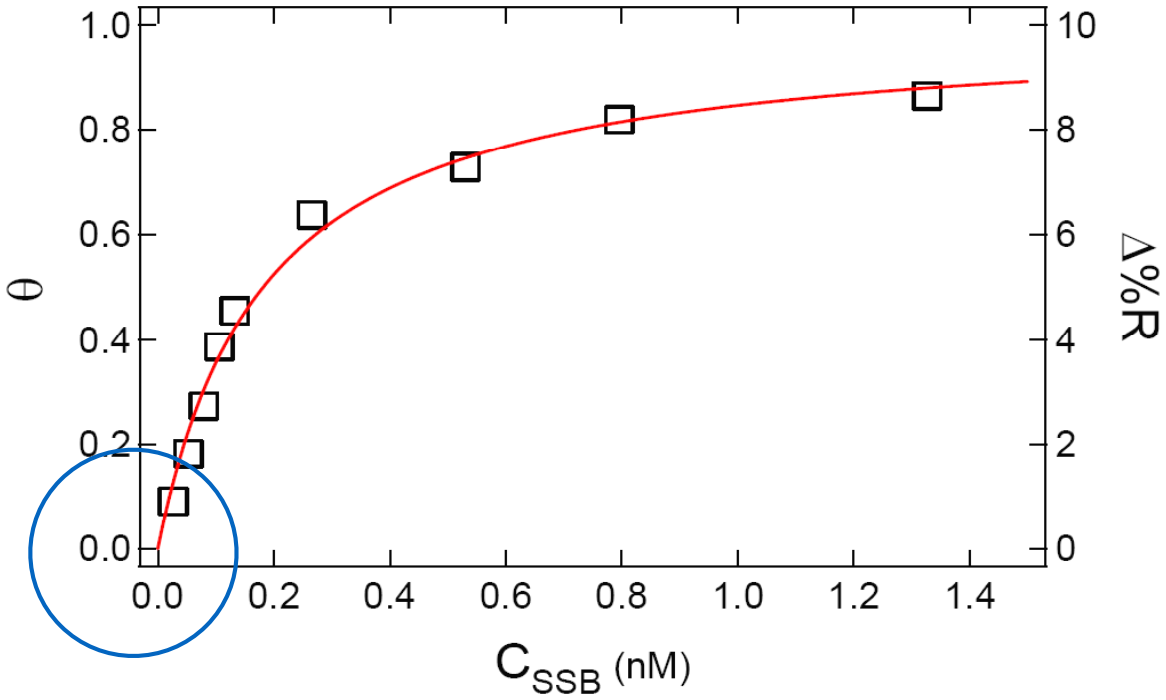
Femtomolar Detection

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

Dr. Yulin Chen

Langmuir Adsorption equilibrium

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



for 10 fM:

$\theta = 10^{-6}$

Must Use Surface Enzyme Chemistry + Nanoparticle-Enhanced SPRI Detection

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

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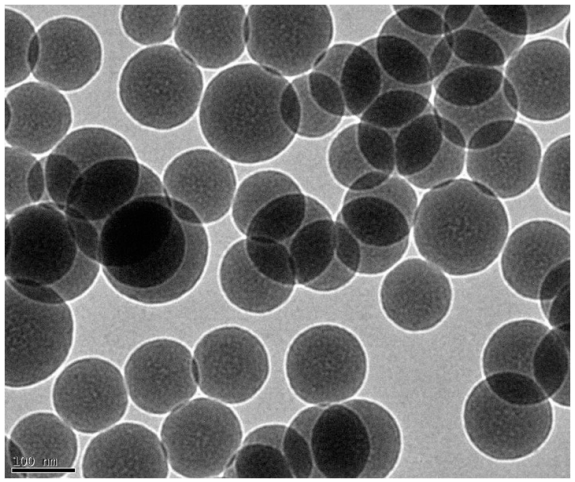
DNA-Functionalized Nanoparticles

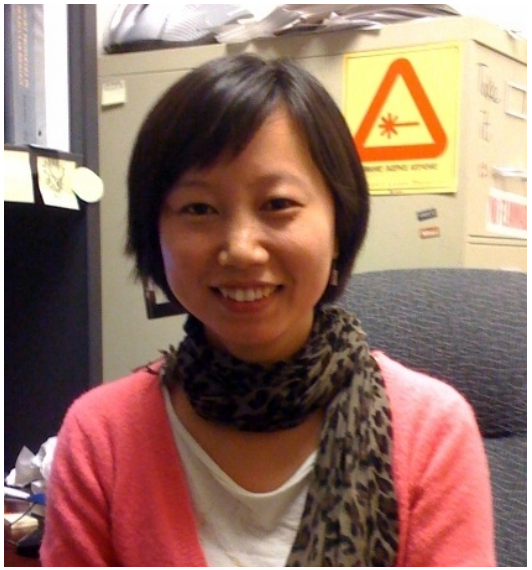
100 nm silica nanoparticles (SiNPs)



TEM

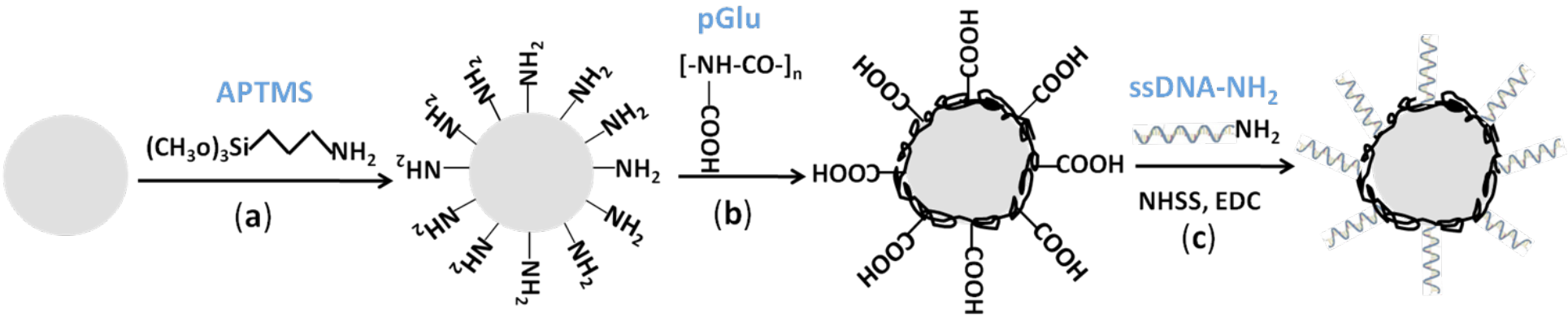
DNA-functionalized:

- *Silica Nanoparticles*
- *Gold Nanoparticles*
- *Polystyrene Nanoparticles*
- *Magnetite Nanoparticles*



Dr. WenJuan Zhou

pGlu DNA Attachment Chemistry on Silica Nanoparticles (SiNPs)



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

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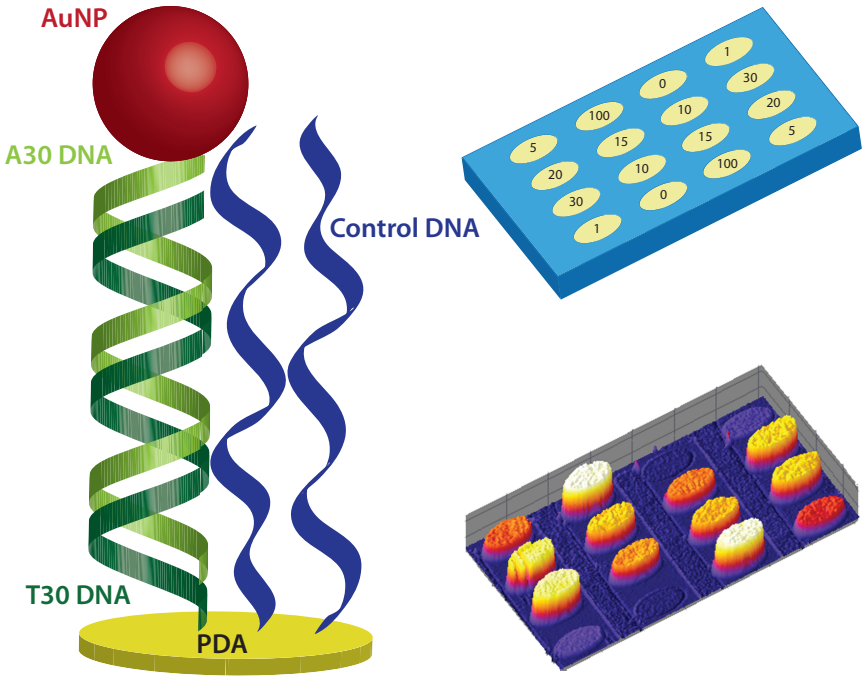
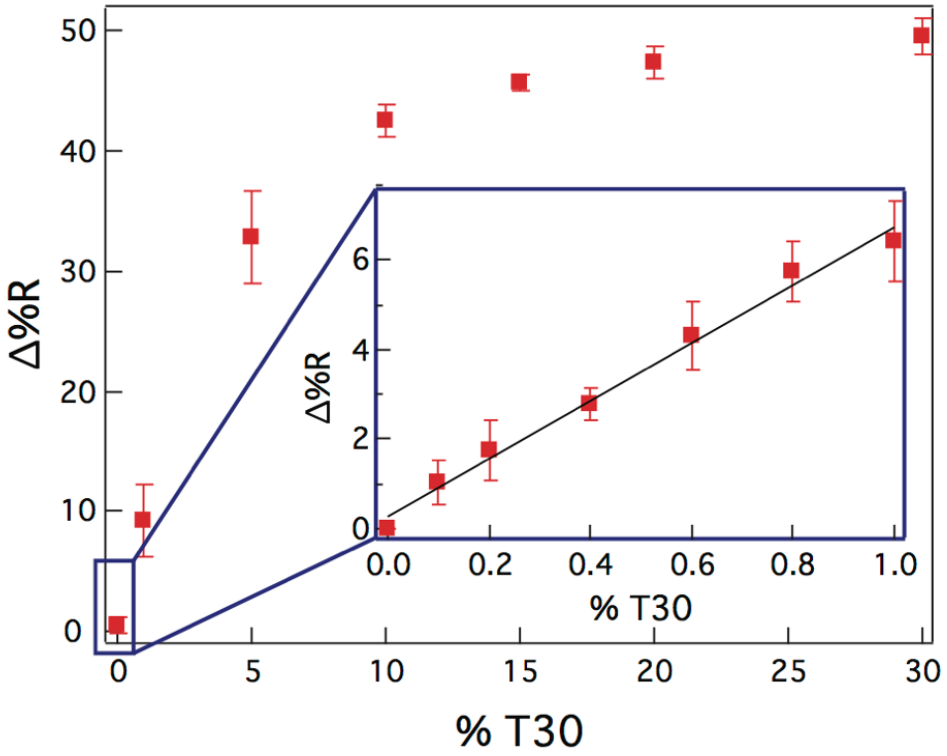
Nanoparticle-Enhanced SPRI Detection of Surface DNA

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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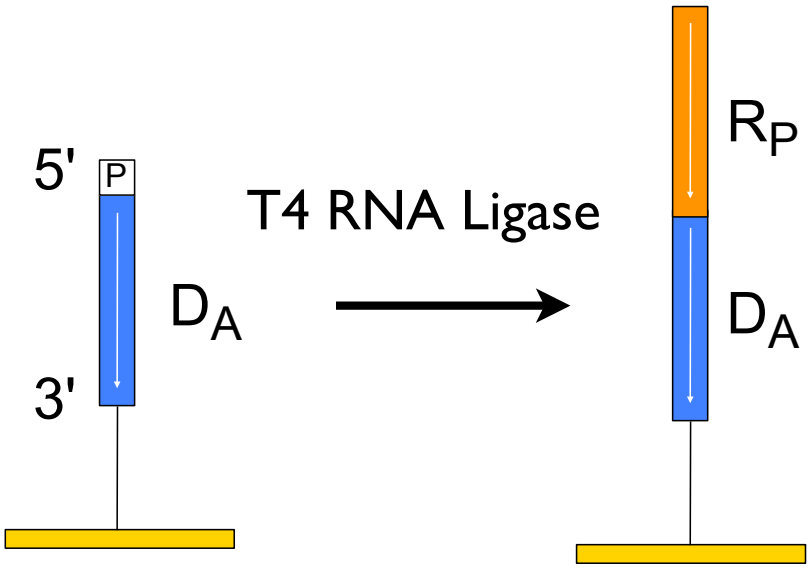
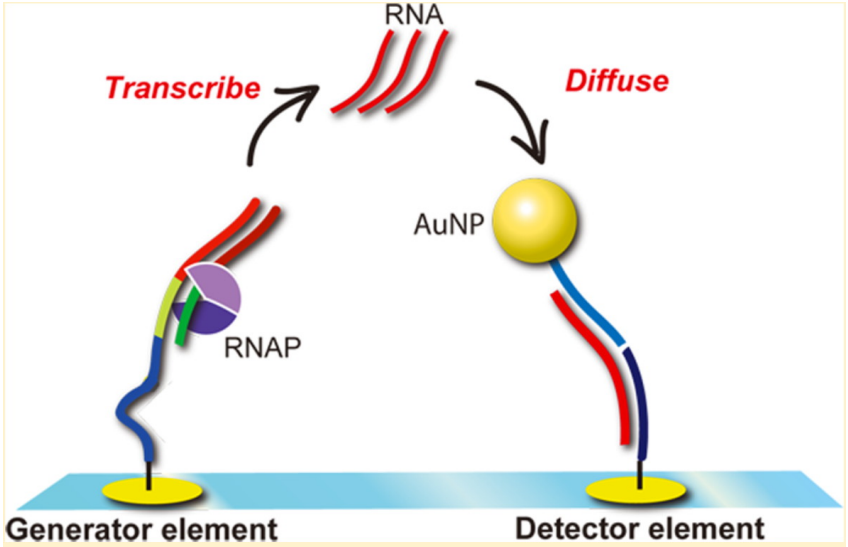
Surface Enzyme Chemistries for SPRI Biosensing

Surface Ligation Chemistry (capture)



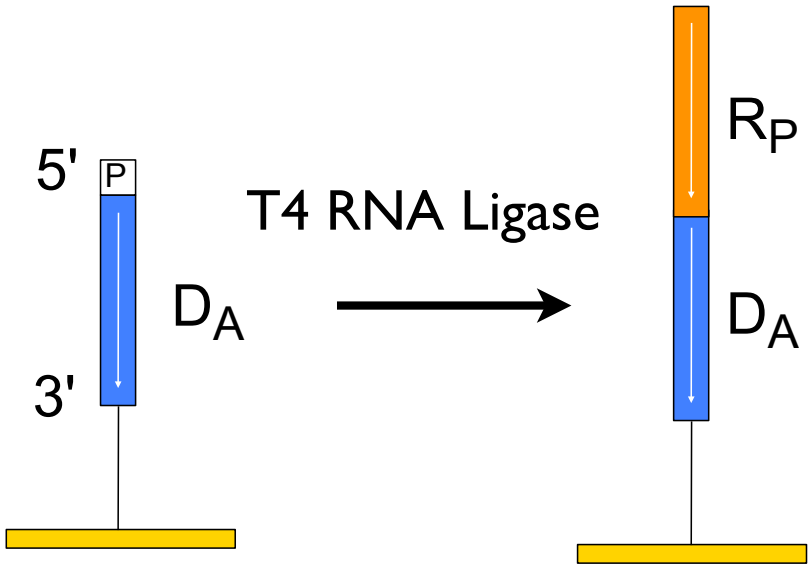


Surface Polymerase Chemistry (amplify)

Surface Enzyme Chemistry + Nanoparticle-Enhanced SPRI Enhanced Detection

Surface Attachment Chemistry: Enzymatic Ligation of ssRNA to ssDNA

T4 RNA Ligase: No template required.
Previously used to make RNA Aptamer arrays:



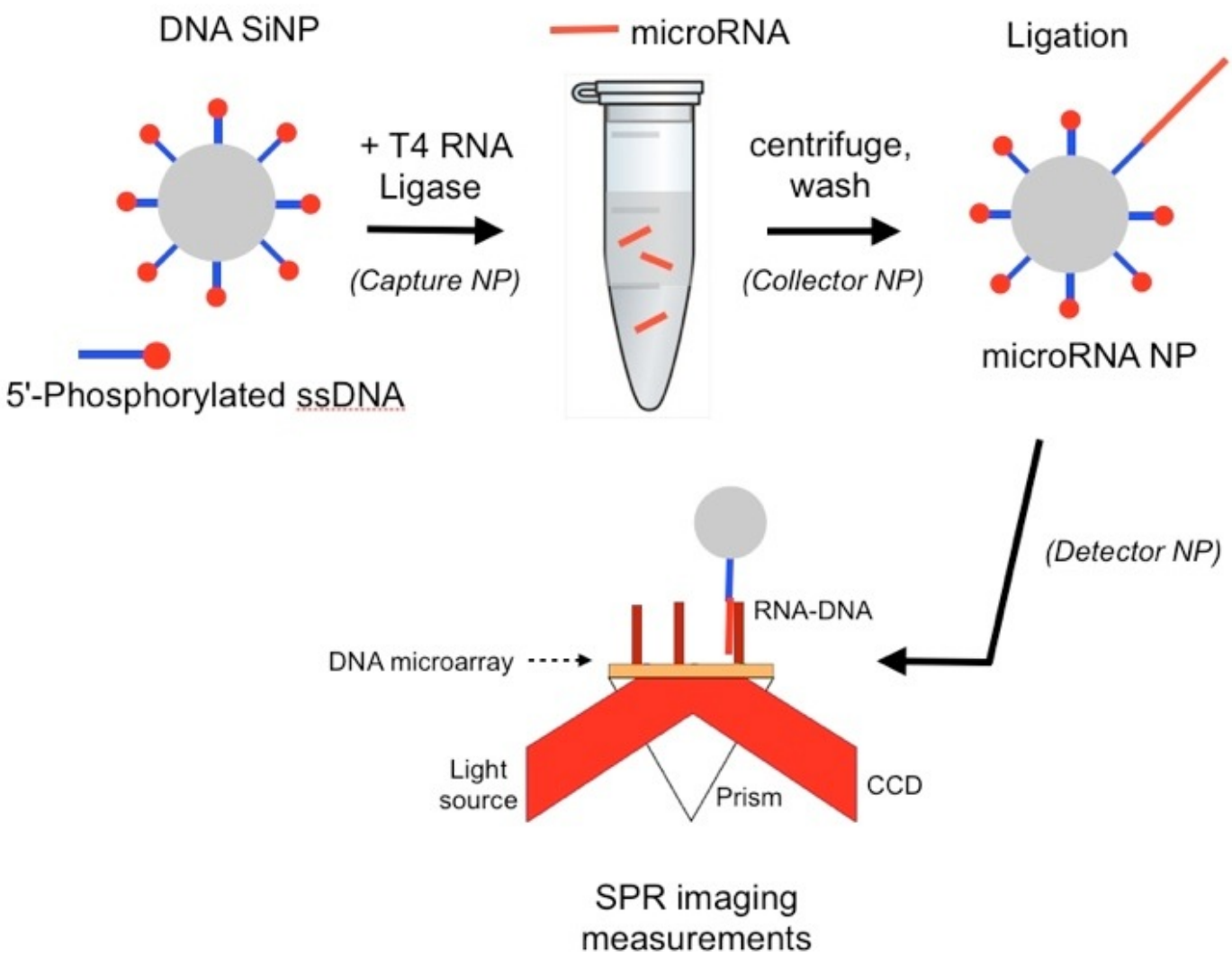
Ting Nico Hu Seefeld

Surface Enzyme Chemistries:
Attachment, Amplification, Selectivity

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

Surface Enzyme Chemistry + Nanoparticle-Enhanced SPRI

- *MicroRNA Detection with Enzymatic Nanoparticles: Ligation Capture*



Dr. Yulin Chen

Dr. WenJuan Zhou

T4 RNA Ligation for miRNA capture

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

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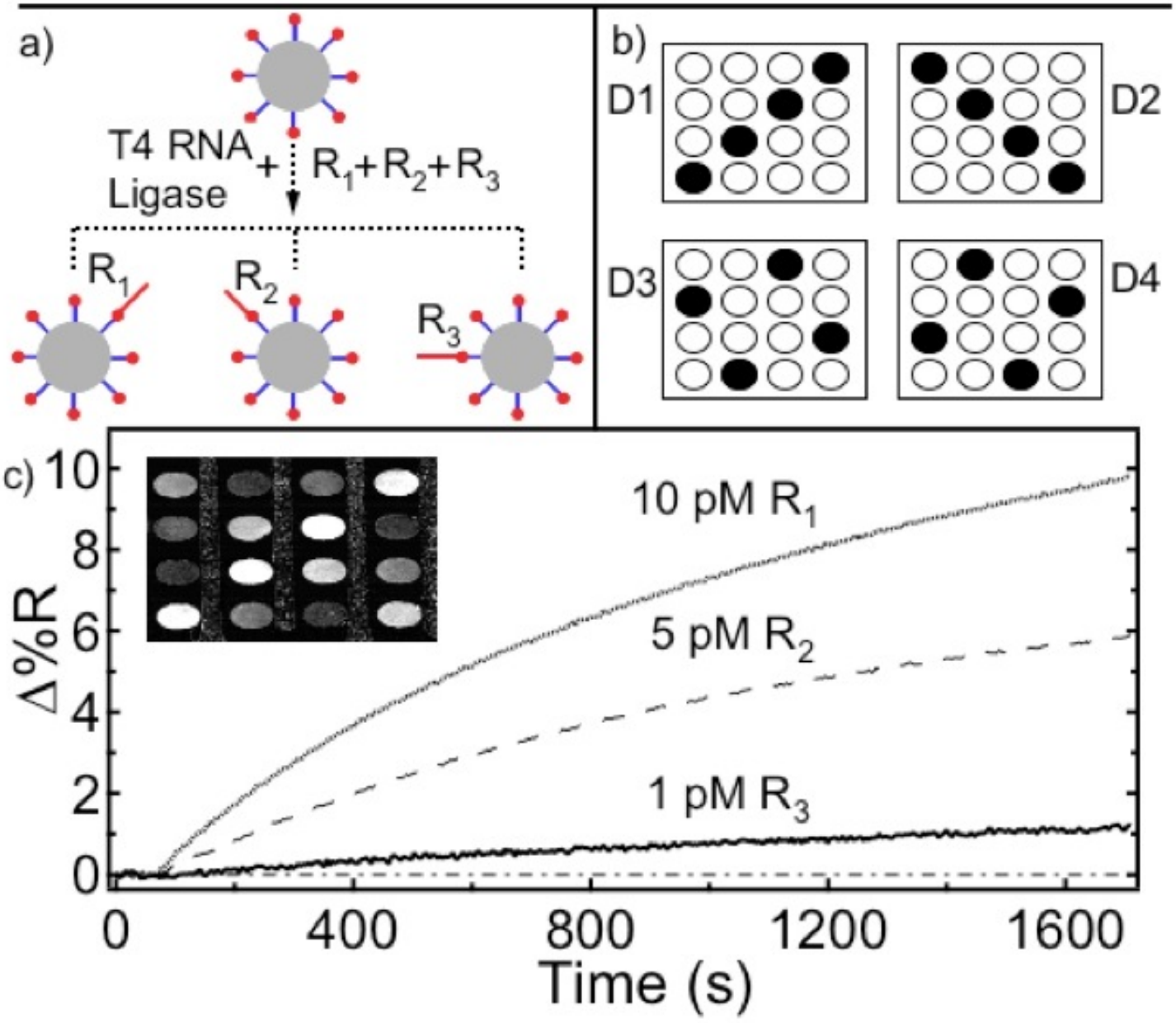
MicroRNA Detection with Enzymatic Ligation Capture Nanoparticles



Multiplex MicroRNA Detection!

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

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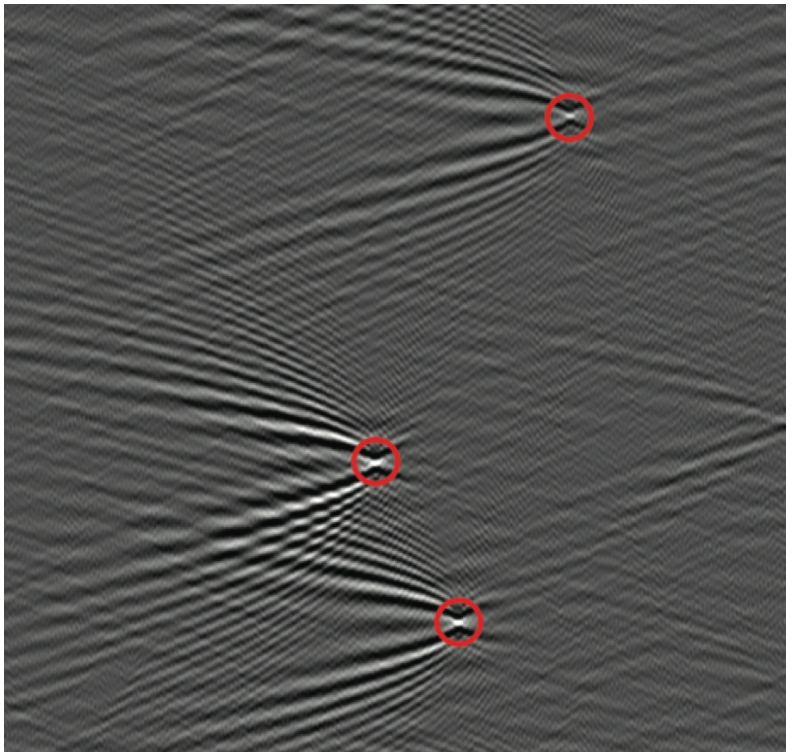
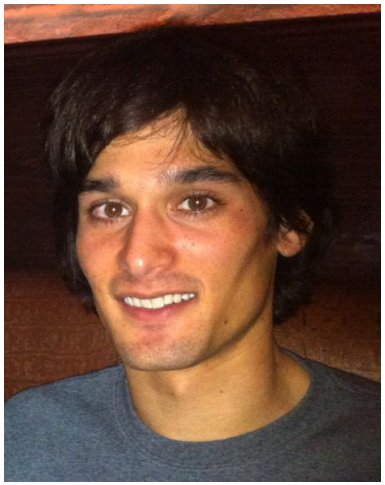
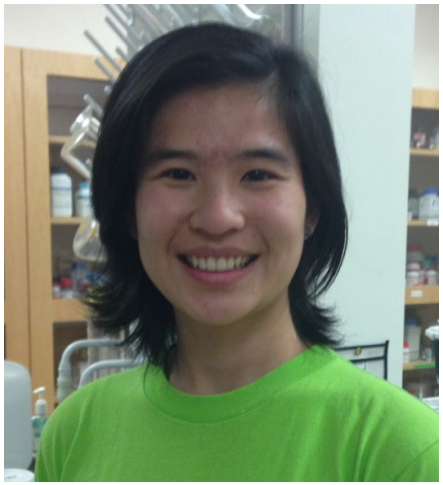
Single Nanoparticle SPRI Microscopy







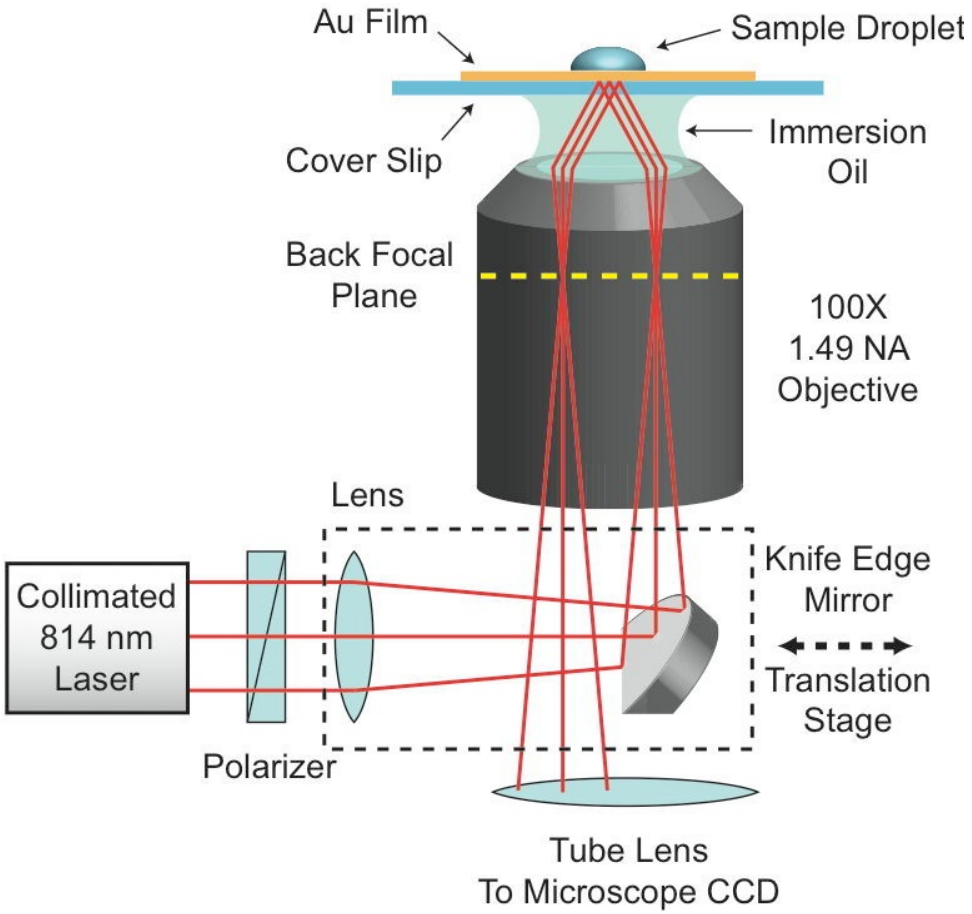
Dr. Aaron Halpern

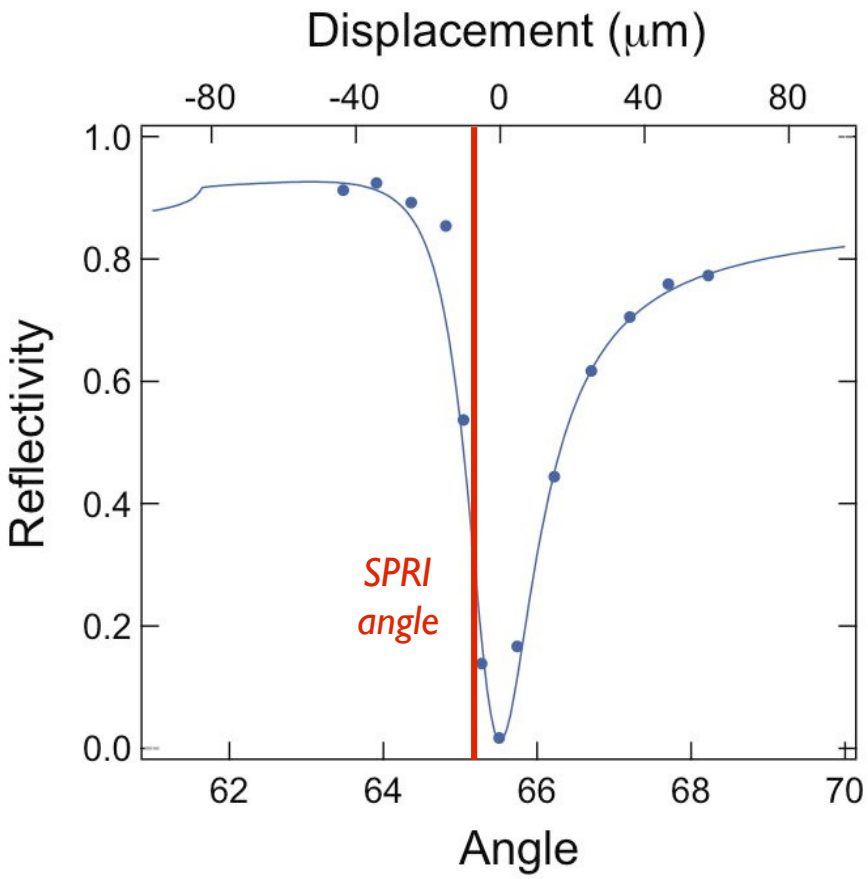
Adam Maley

Millie Fung

Robert M. Corn
UC Irvine

Near Infrared TIR SPR microscope: 814 nm

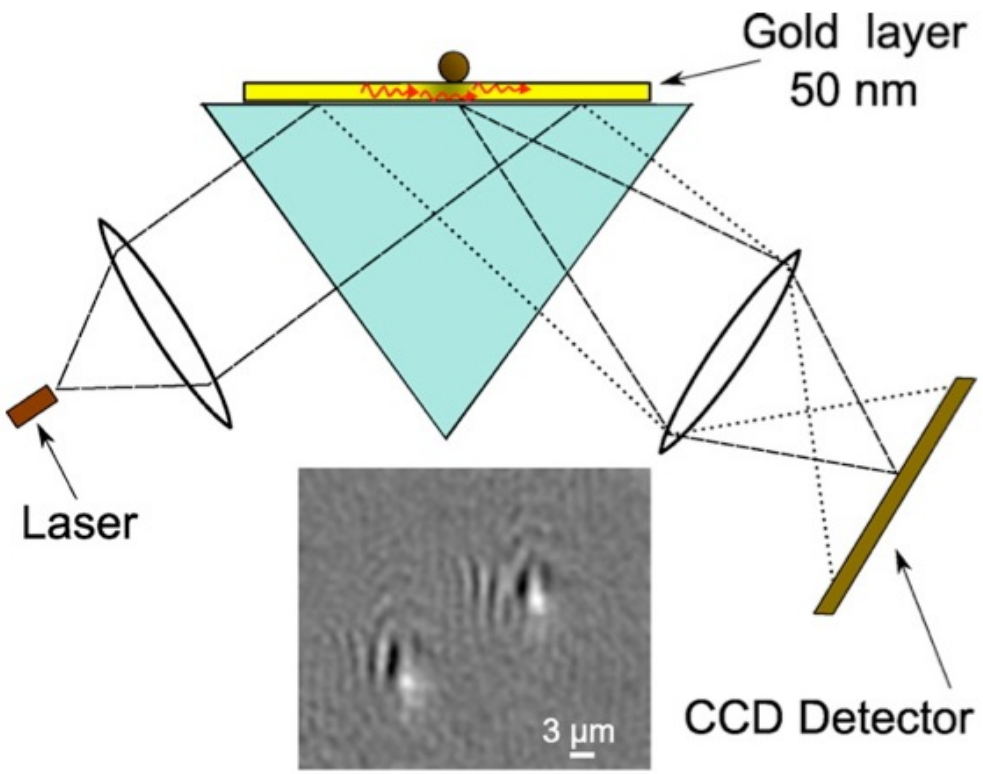




100x Objective
N.A. = 1.49
90 μm x 70 μm FOV

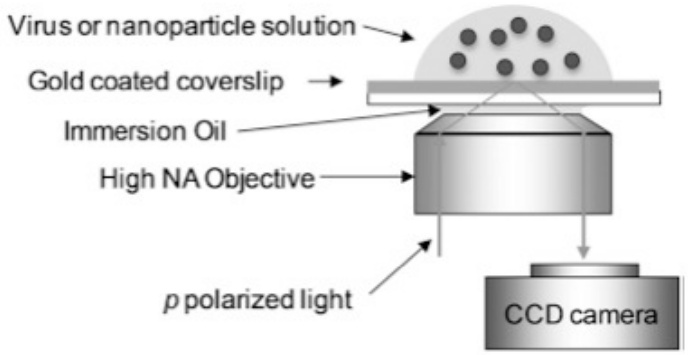
Control angle of incidence by varying d

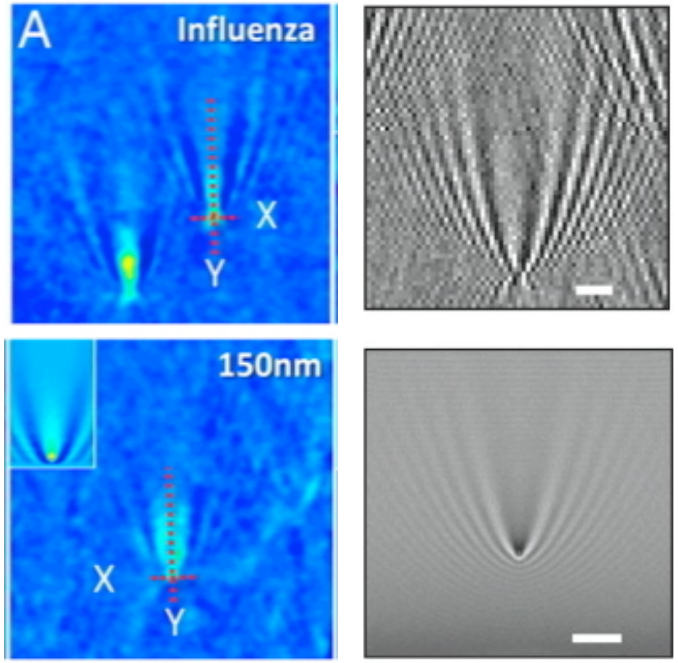
SPR microscopy of nanoparticles



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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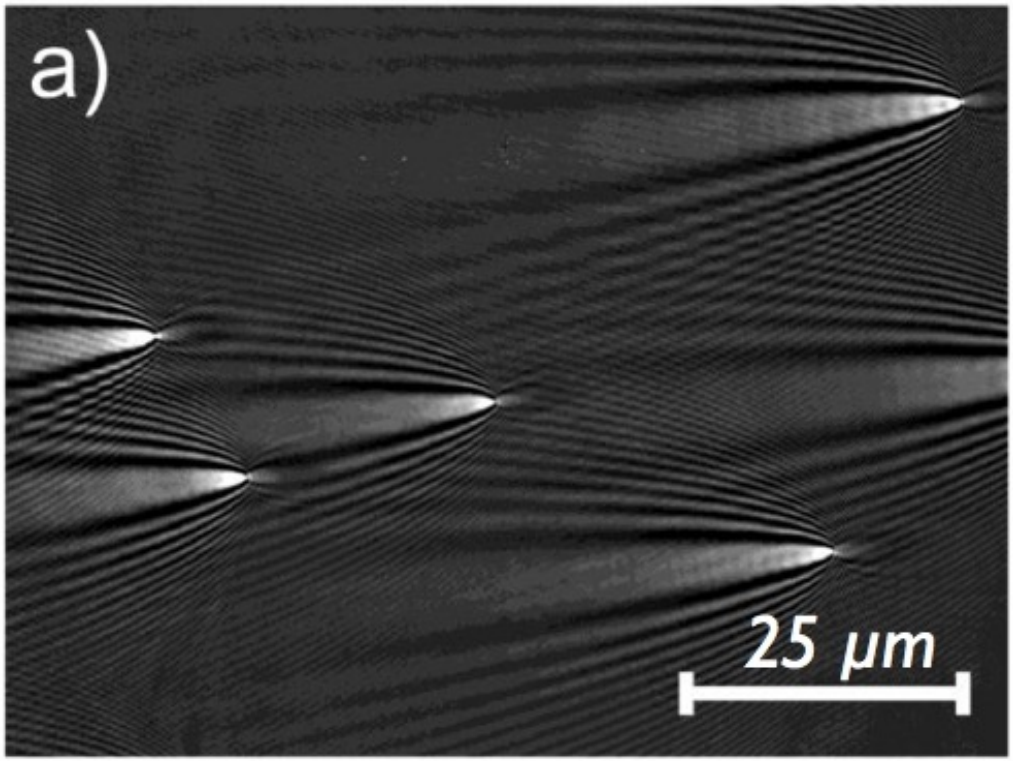
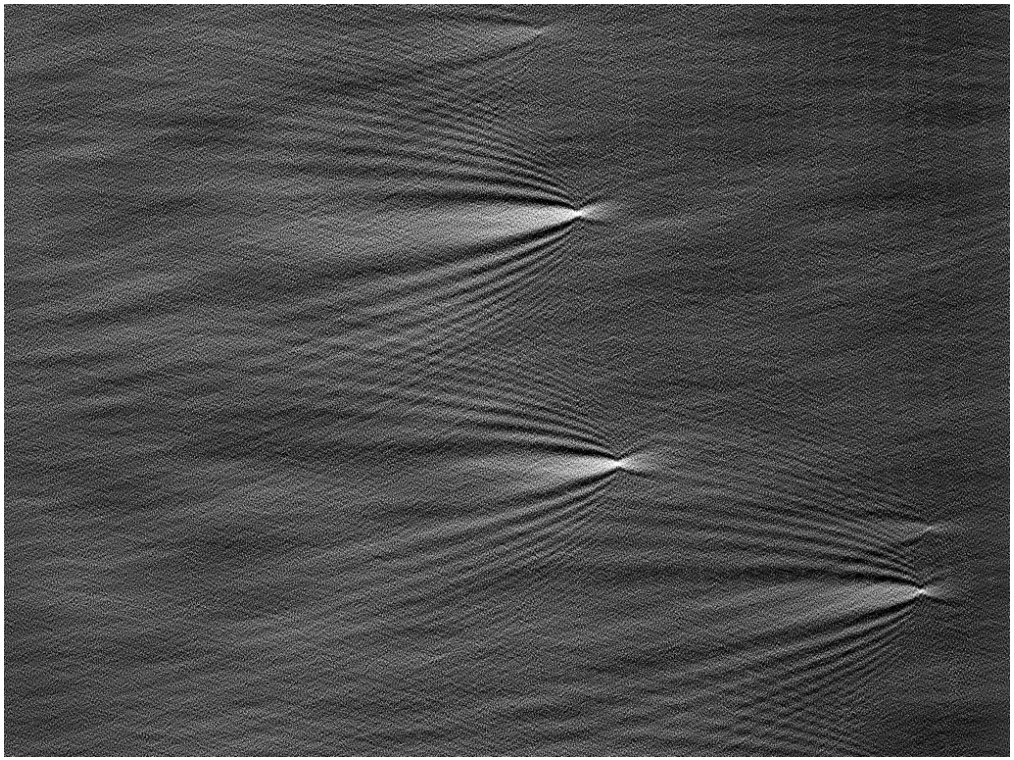
Near Infrared TIR SPRI microscope data:





200 nm polystyrene NPs

40 nm gold NPs

90 μm x 70 μm FOV

814 nm SPP excitation

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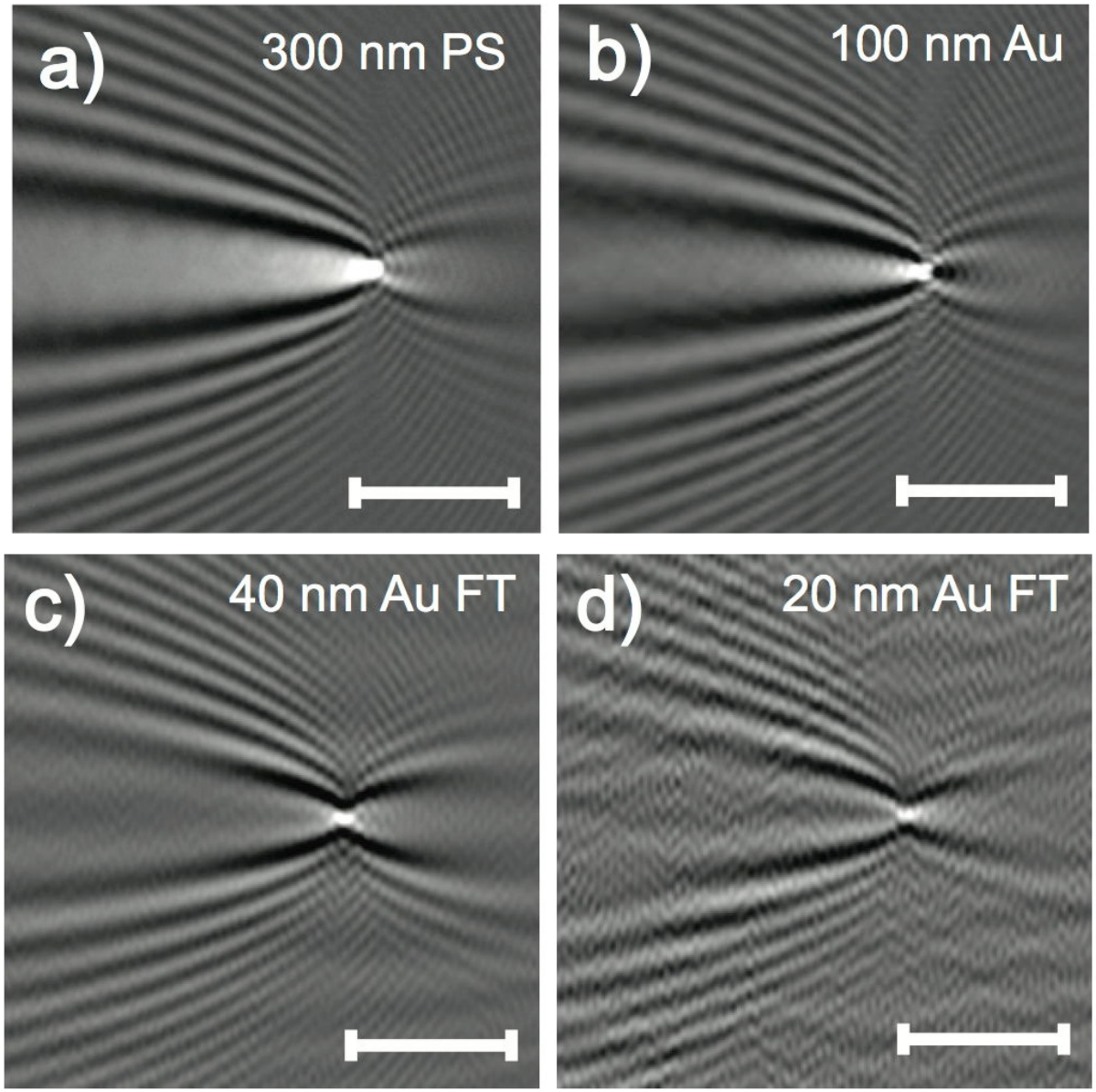
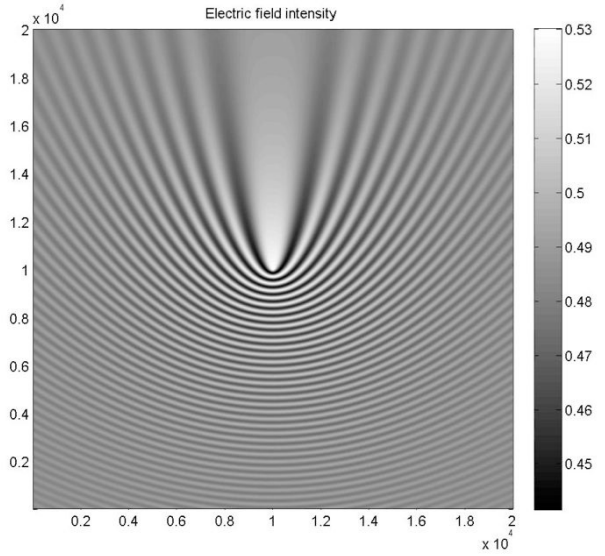
Near Infrared TIR SPR microscope: 814 nm SPP diffraction





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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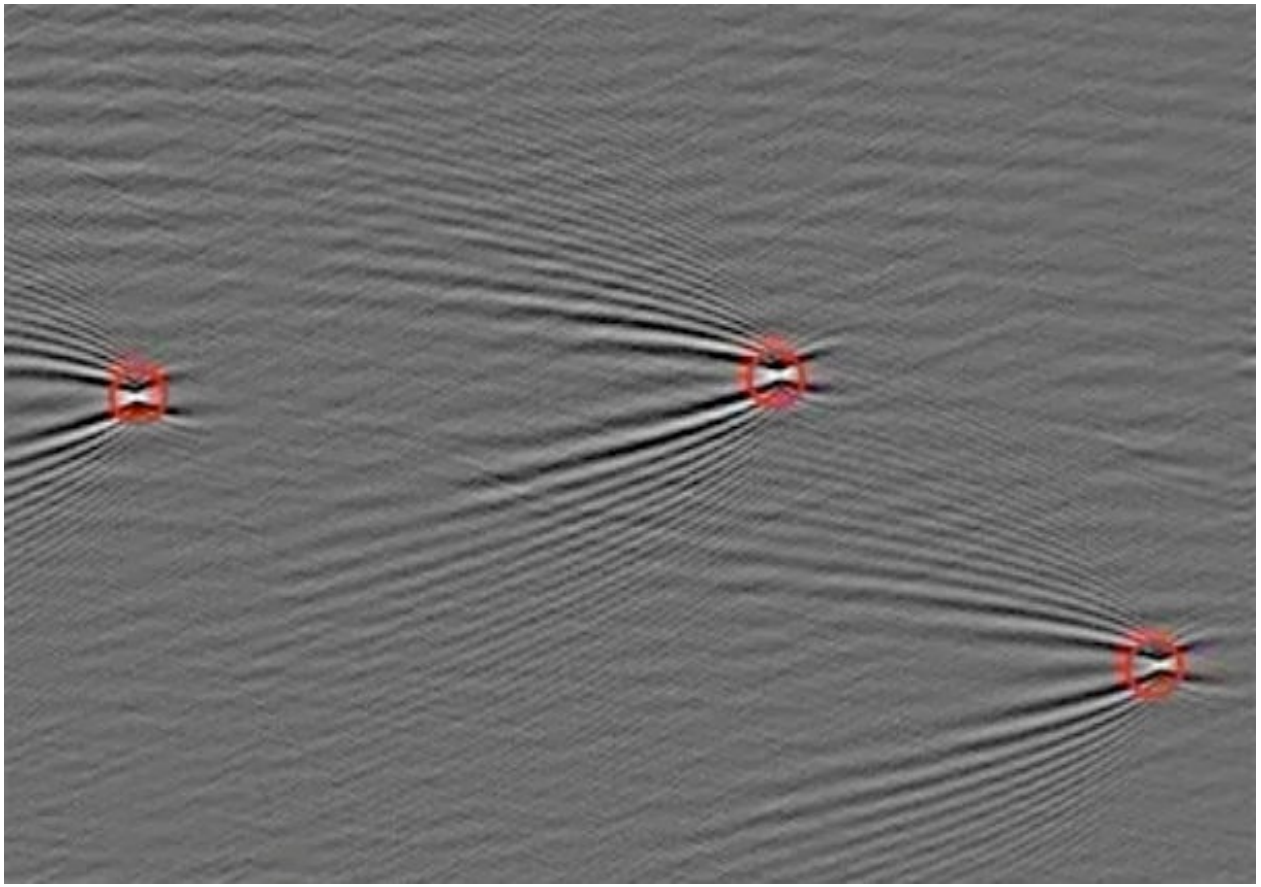
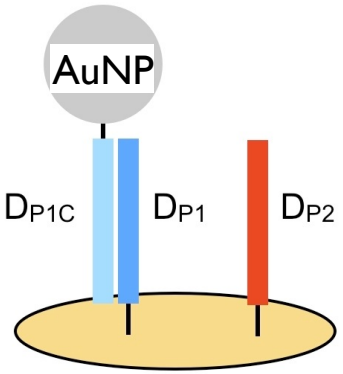
Bioaffinity Adsorption of 40 nm DNA-modified Au Nanoparticles



A 3 second frame image from a 1 pM solution with three Au NPs

100% complementary surface



Initial solution: 3 µL

Add 3 µL of AuNPs

Record SPR images every 3 seconds

Create difference image movie

Count NPs in each frame

Create Adsorption Kinetics Plot

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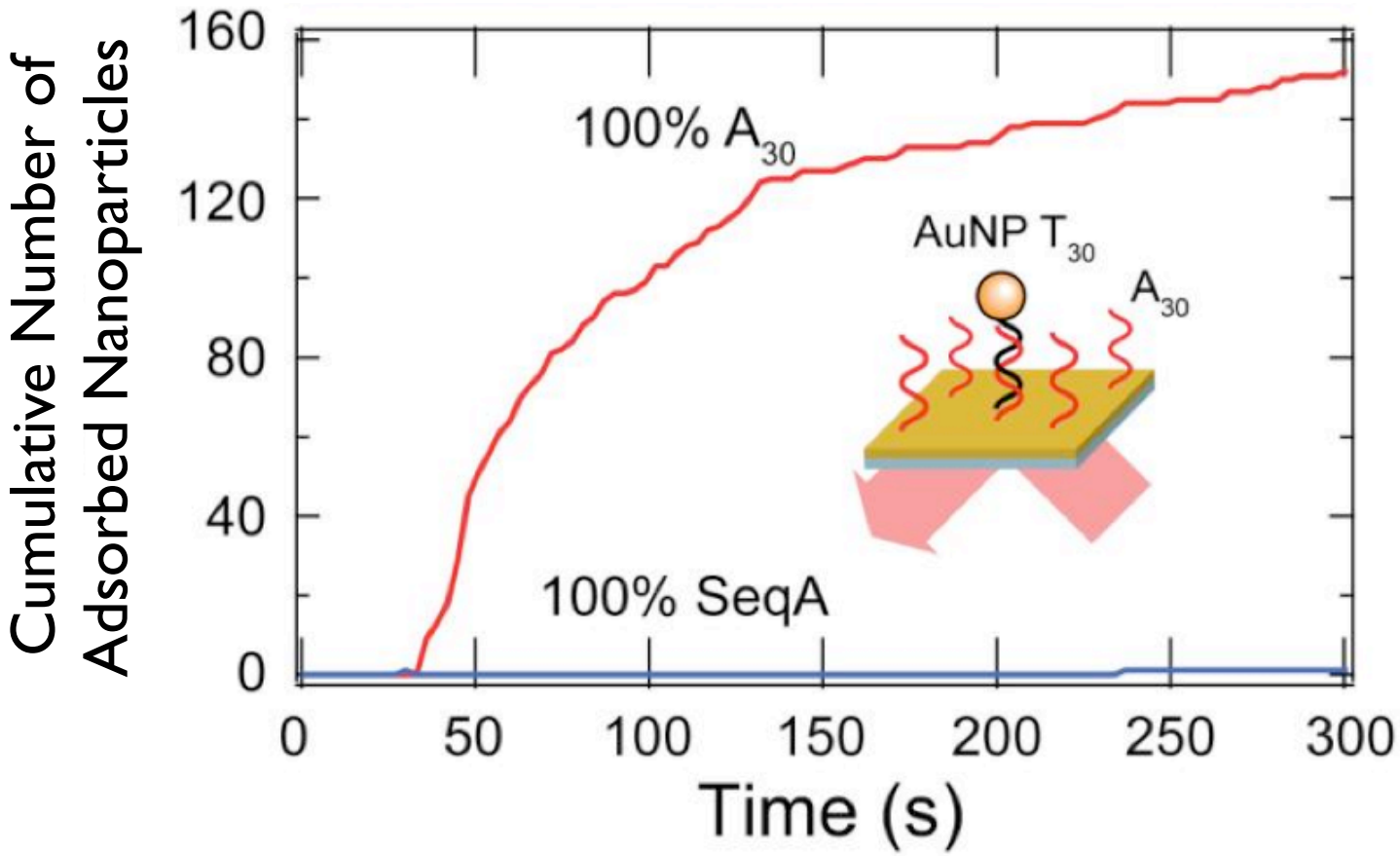
Bioaffinity Adsorption of 40 nm DNA-modified Au Nanoparticles



1 pM solution of 40 nm DNA-modified AuNPs

Noncomplementary DNA-modified surface shows negligble nanoparticle binding

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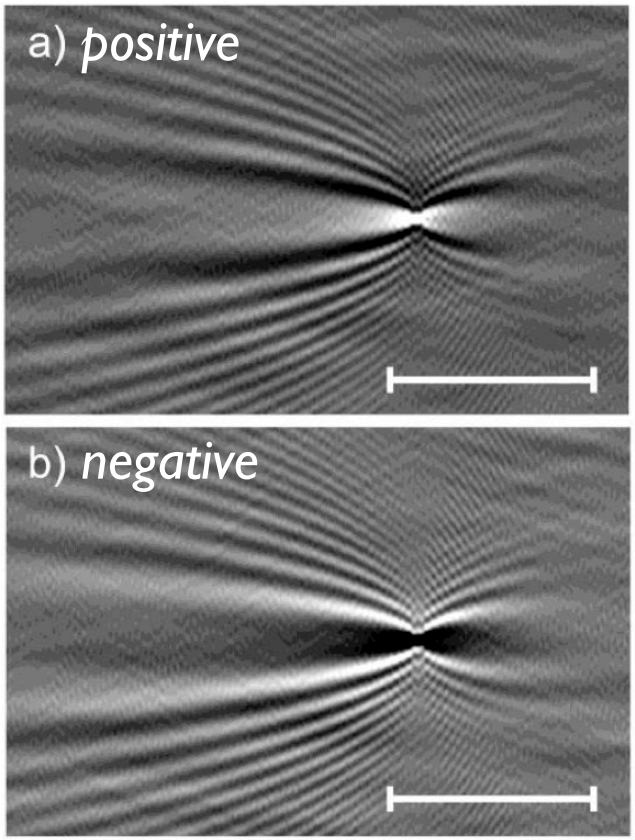
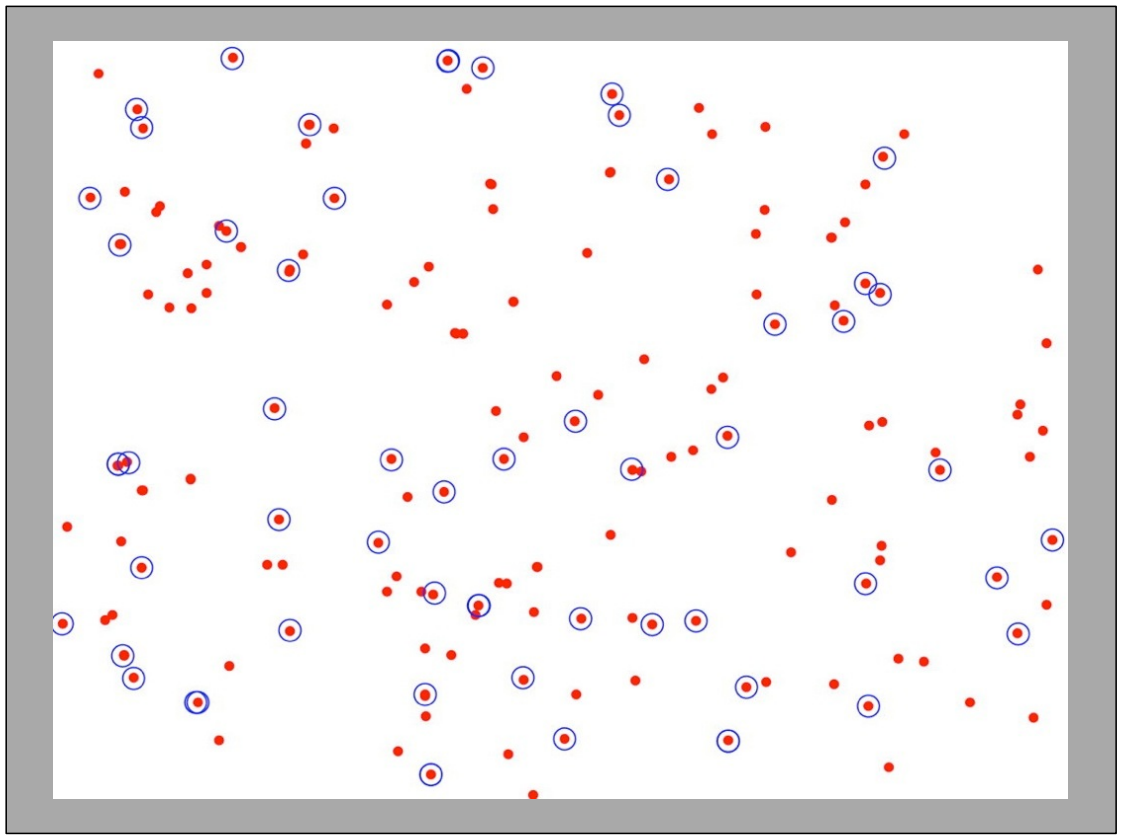
DNA nanoparticle adsorption onto single DNA sequences

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



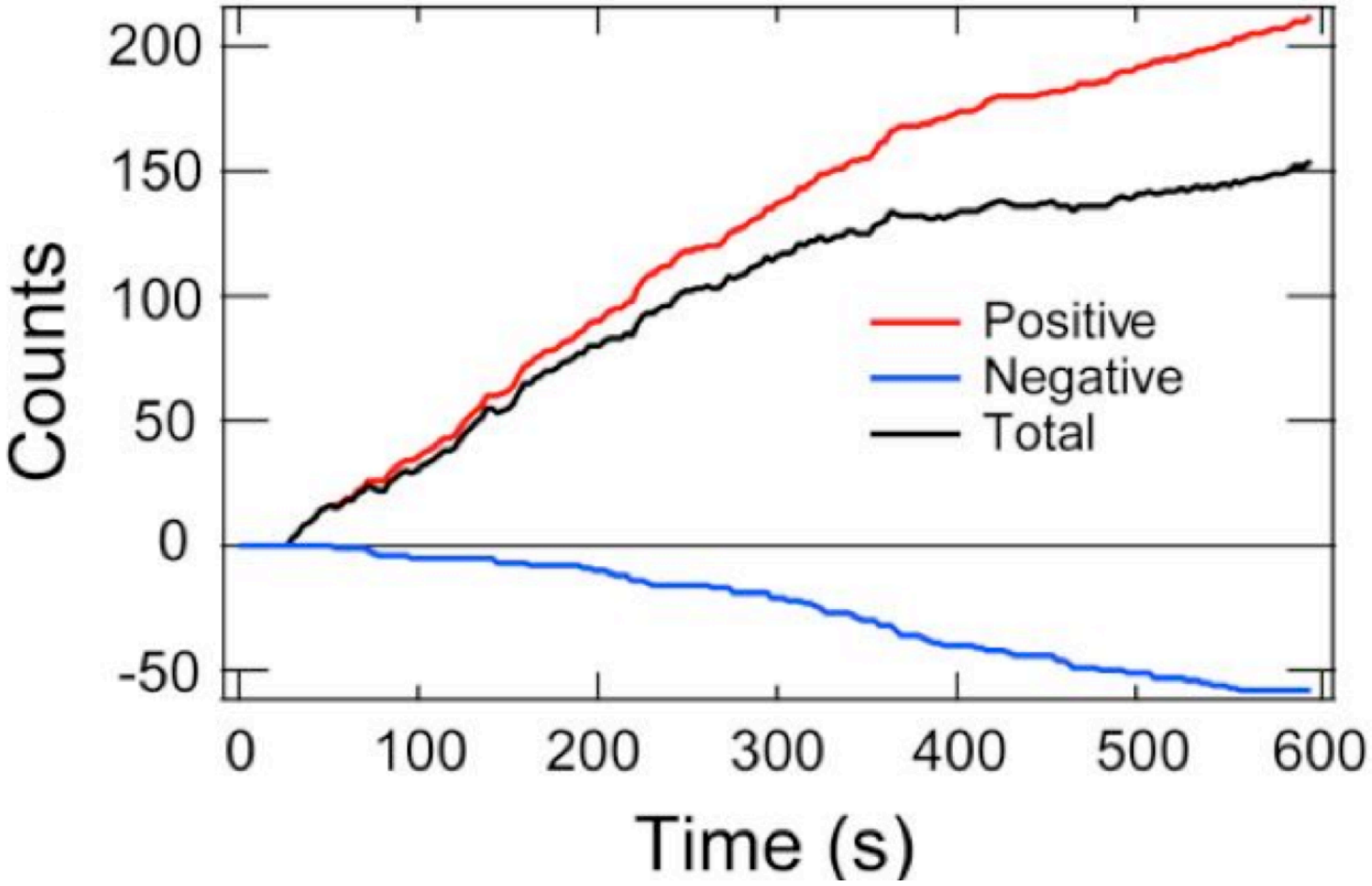


0.01% complementary monolayer

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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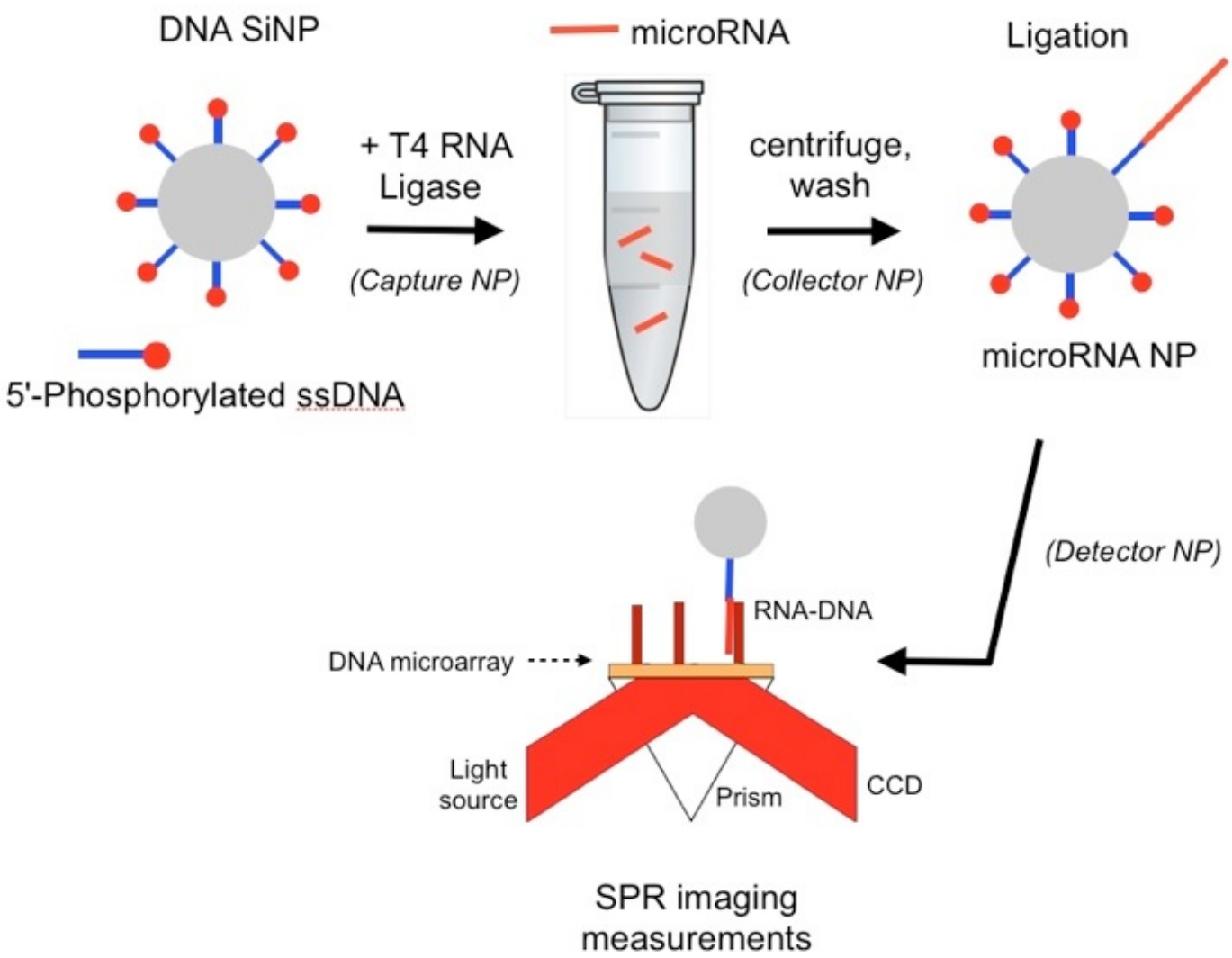
Surface Enzyme Chemistry + Nanoparticle-Enhanced SPRI

- *MicroRNA Detection with Enzymatic Nanoparticles: Ligation Capture*



Dr. Yulin Chen

Dr. WenJuan Zhou

T4 RNA Ligation for miRNA capture

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

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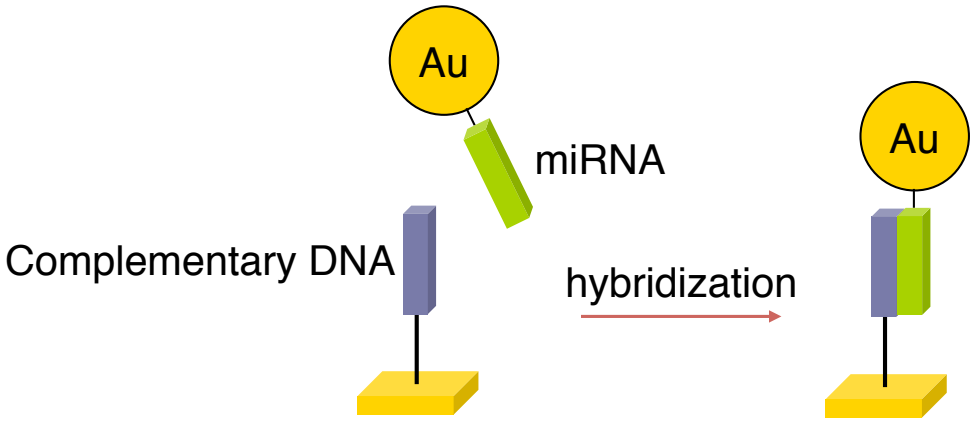
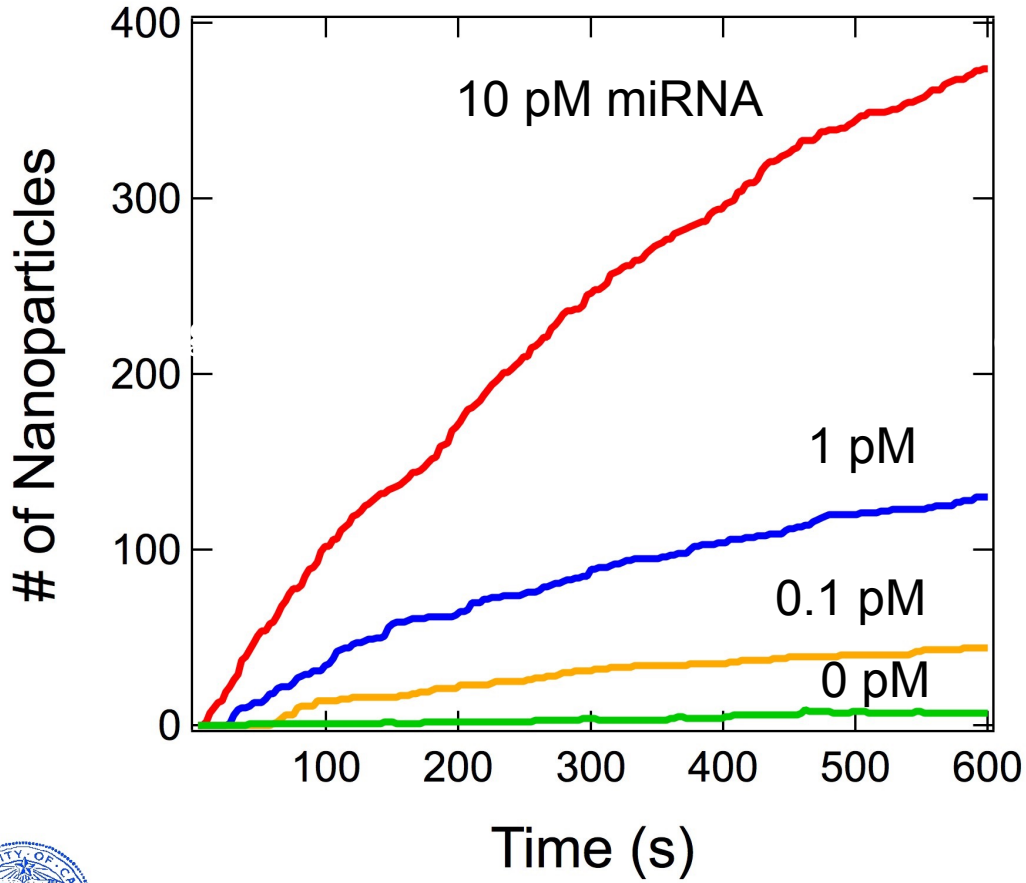
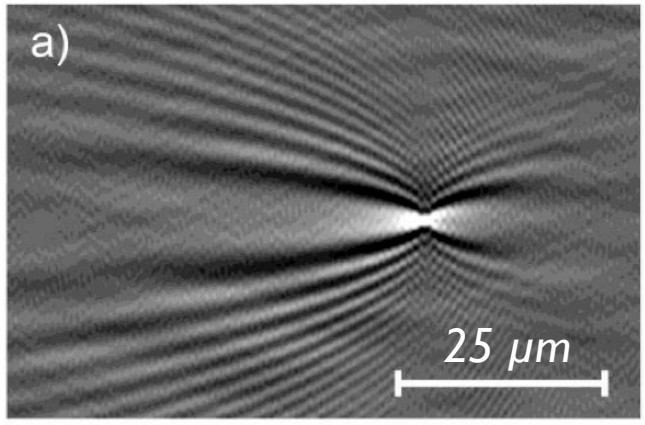
*Single NP Detection of miRNA-146a**

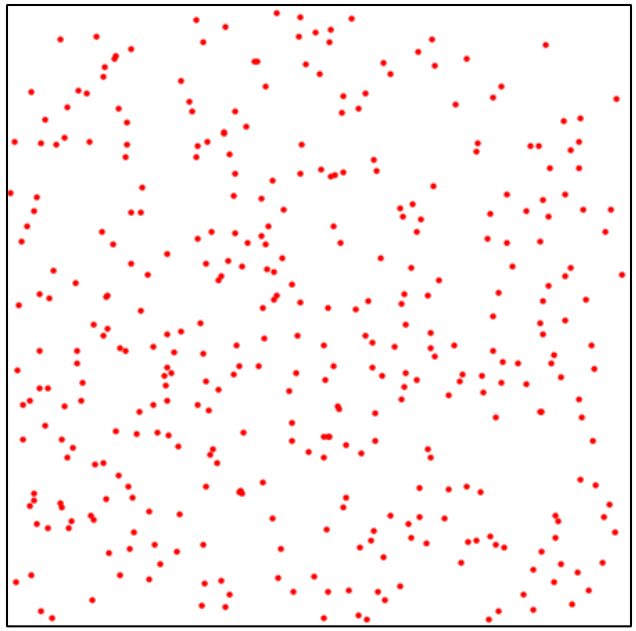




Total Concentration of AuNPs: 25 pM

Single NP adsorption event



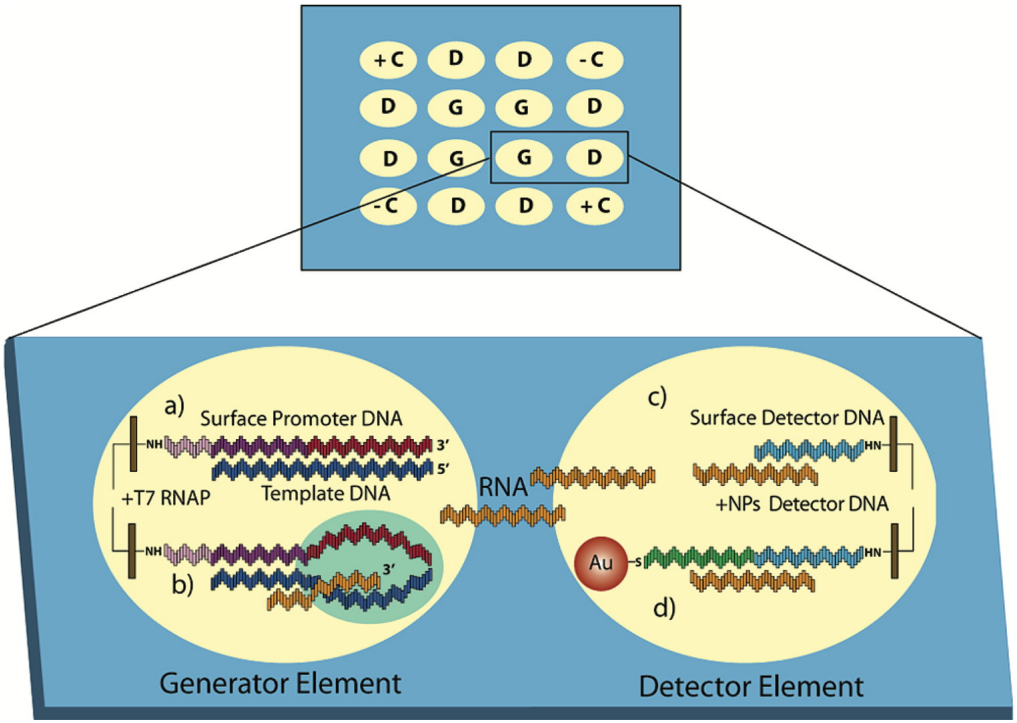


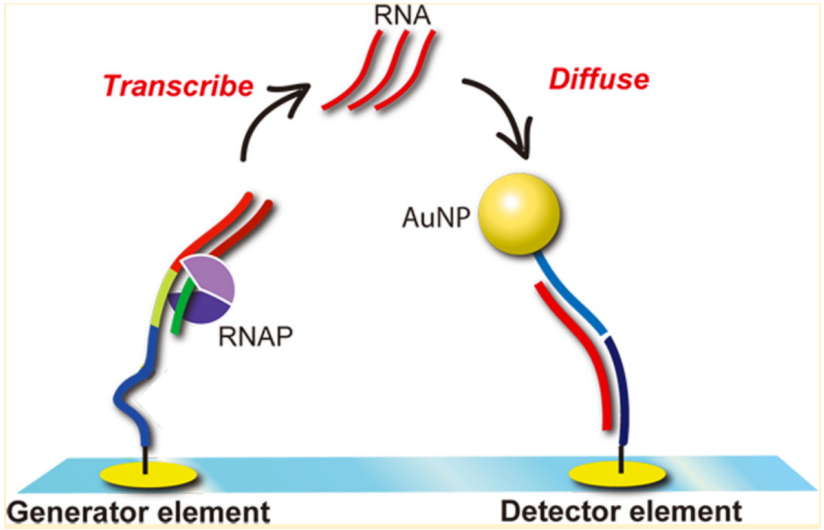
NP adsorption map (10 pM)

* UGAGAACUGAAUUCCAUAGGC

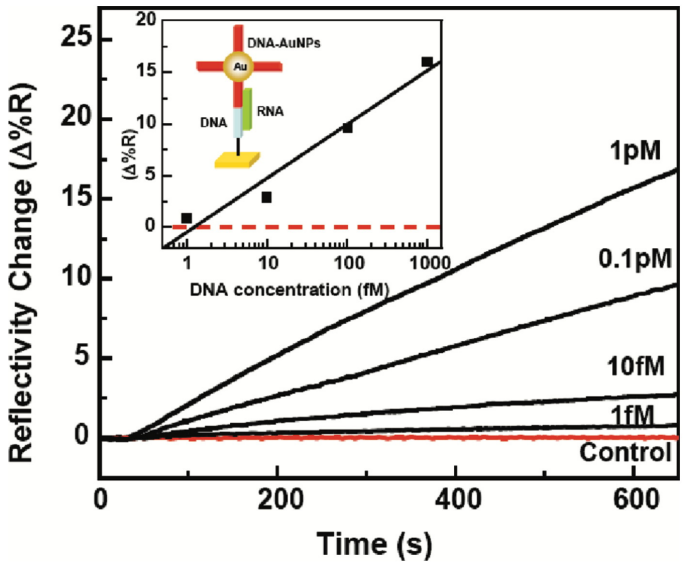
Surface Polymerase Chemistries: RNA Polymerase Amplification

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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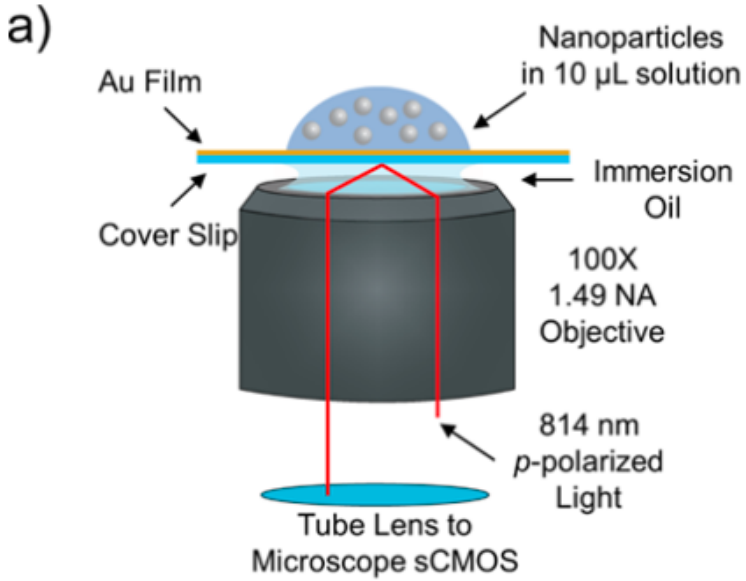
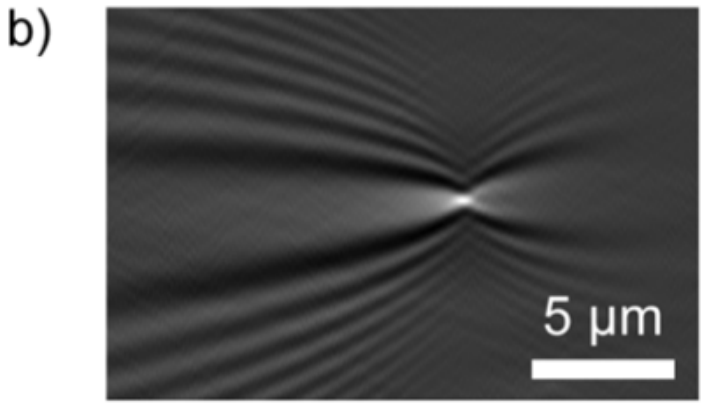
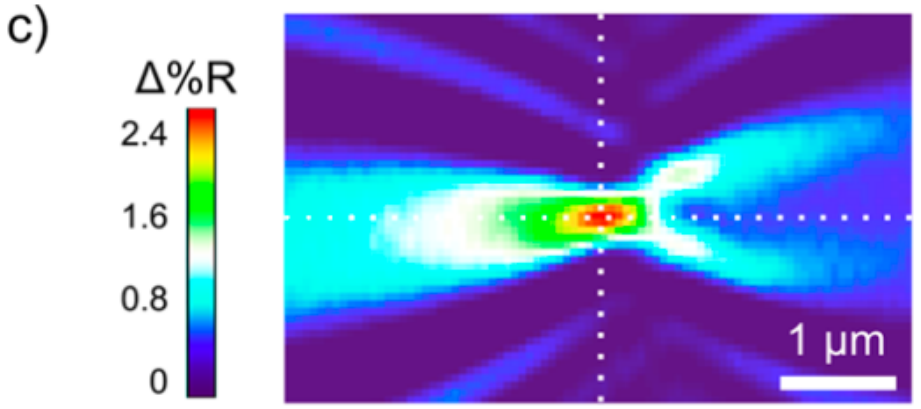
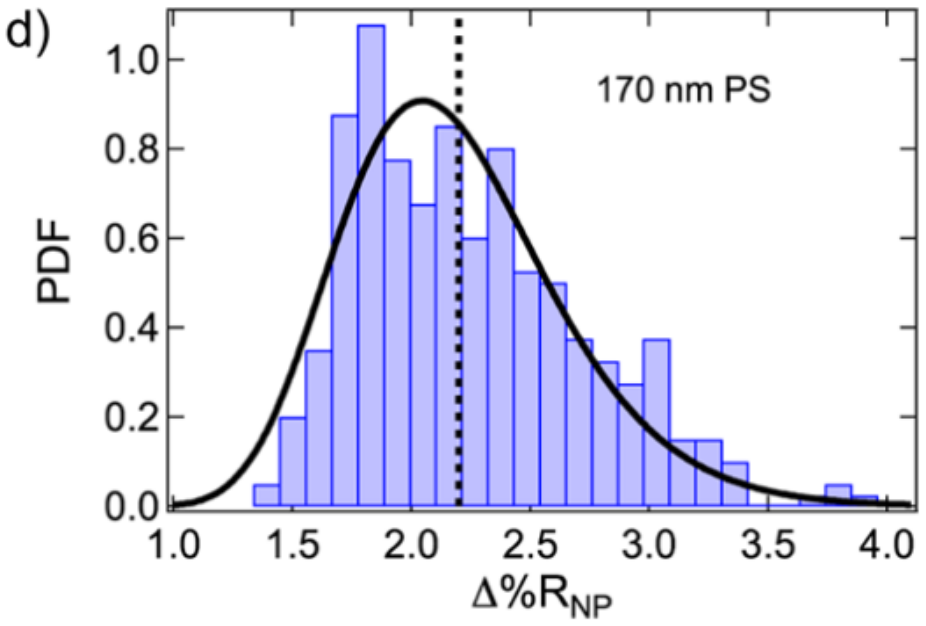
Single Nanoparticle Refractive Index Measurements









$\Delta\%R_{NP}$ *"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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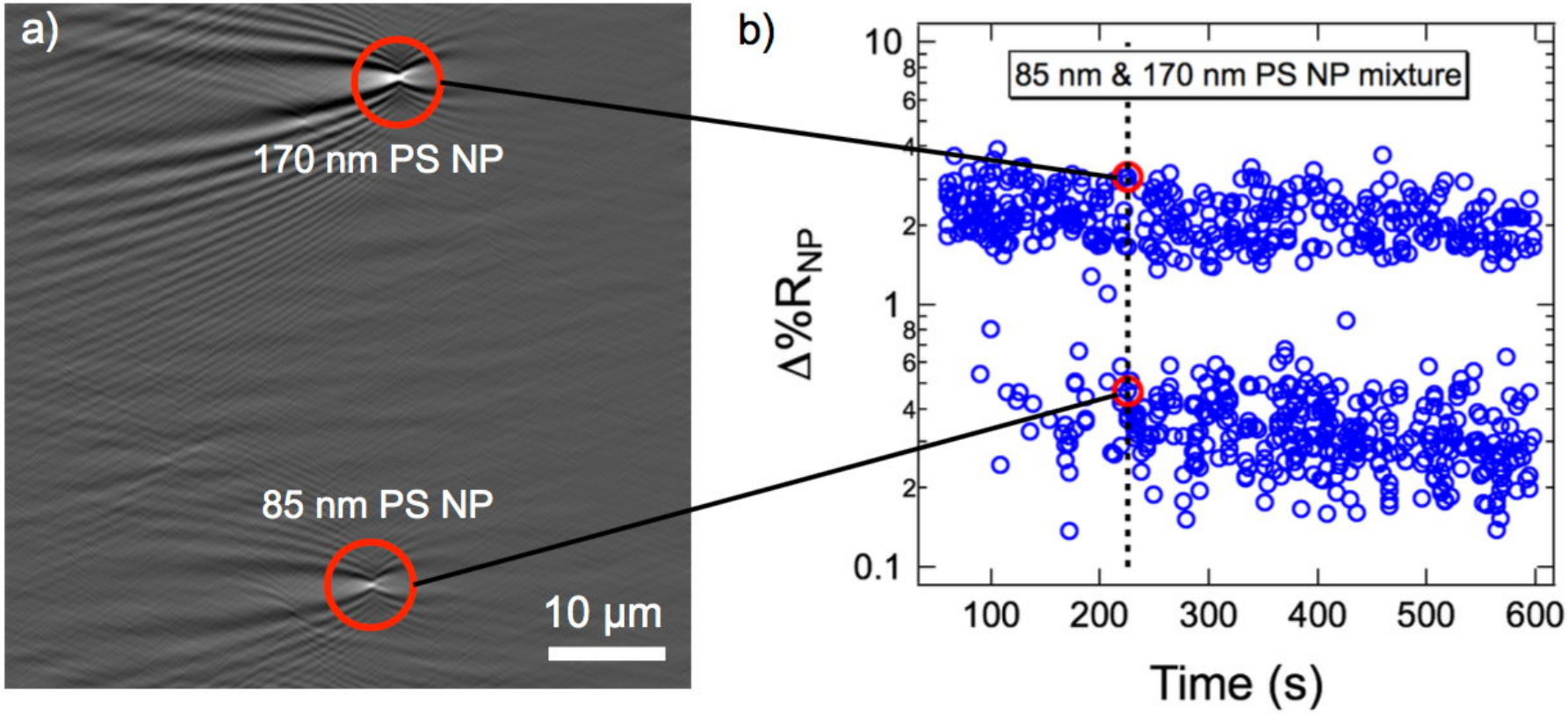
Single Nanoparticle Distribution Measurements

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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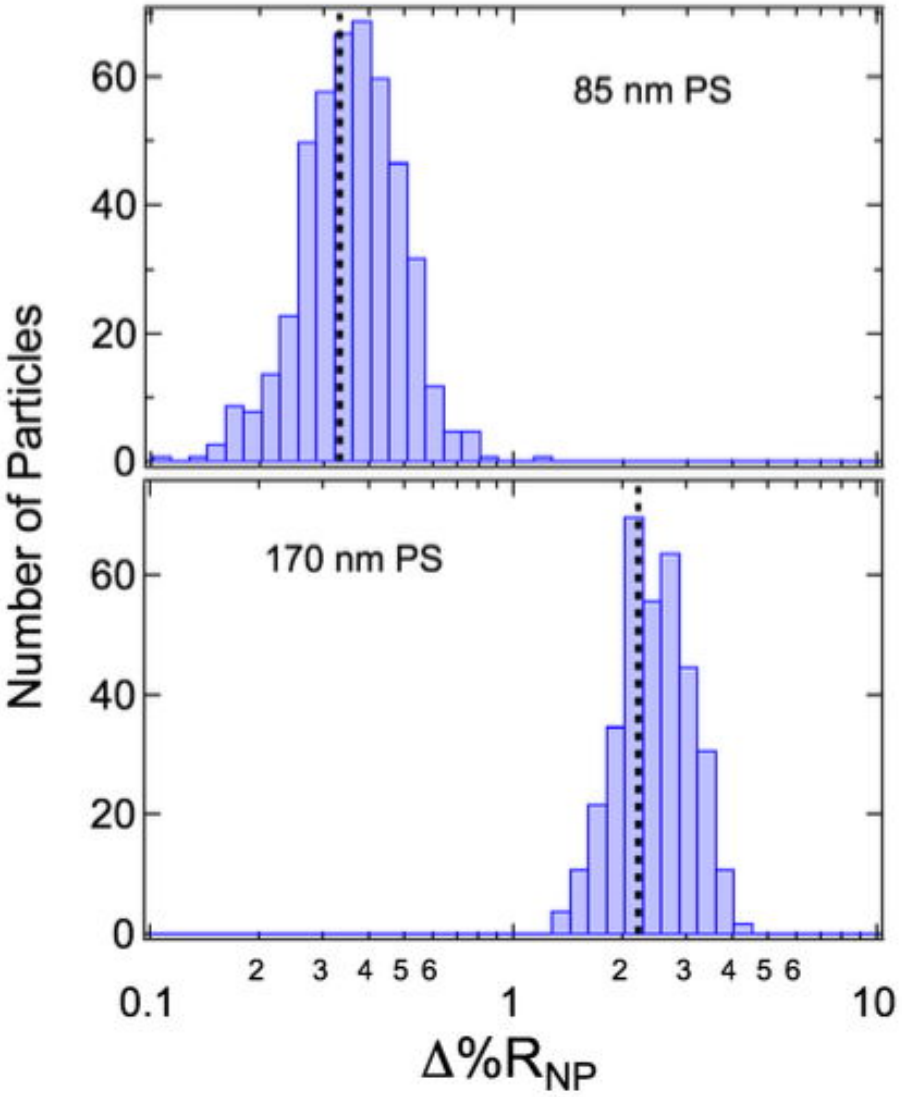
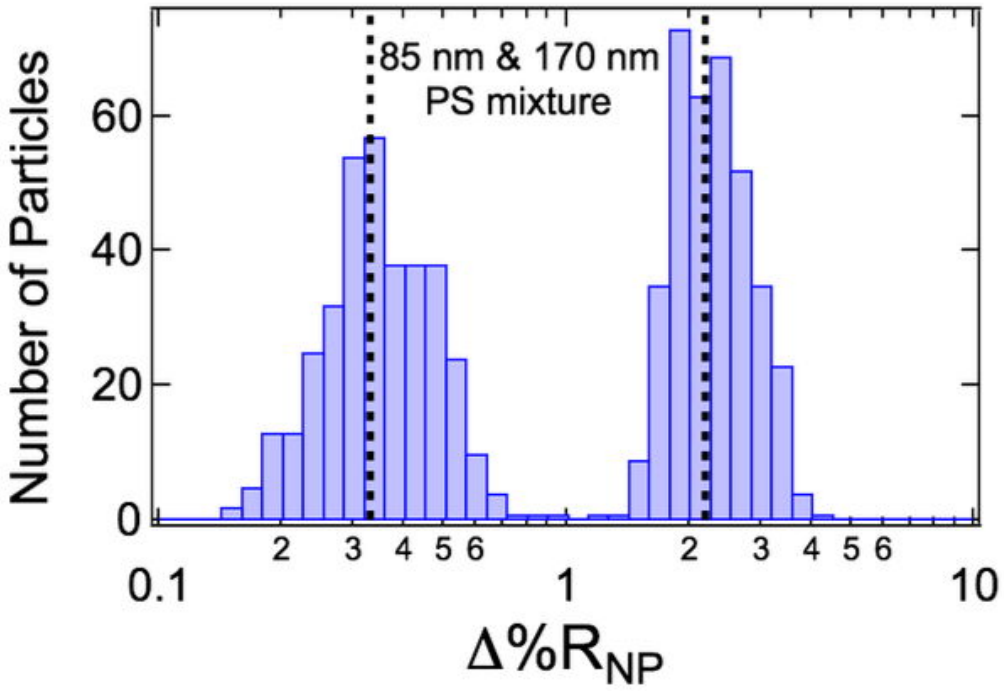
Single Nanoparticle Distribution Measurements



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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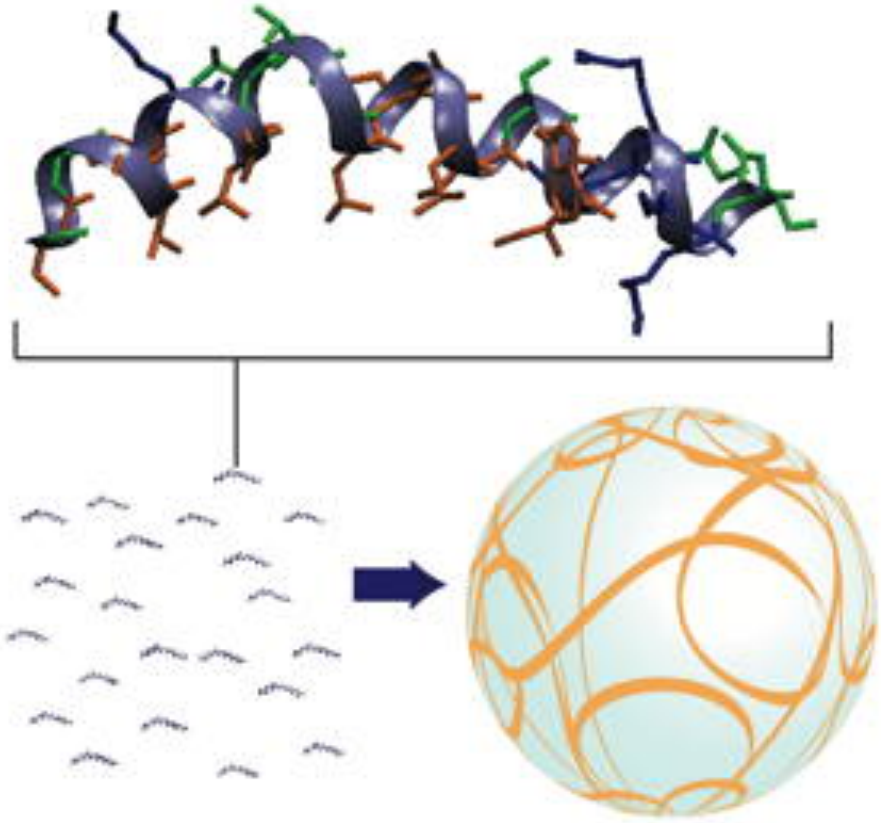
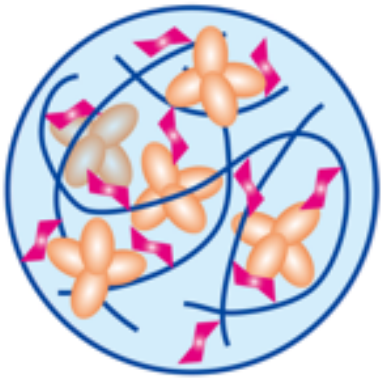
Bioaffinity Uptake in Nanoparticles



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

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



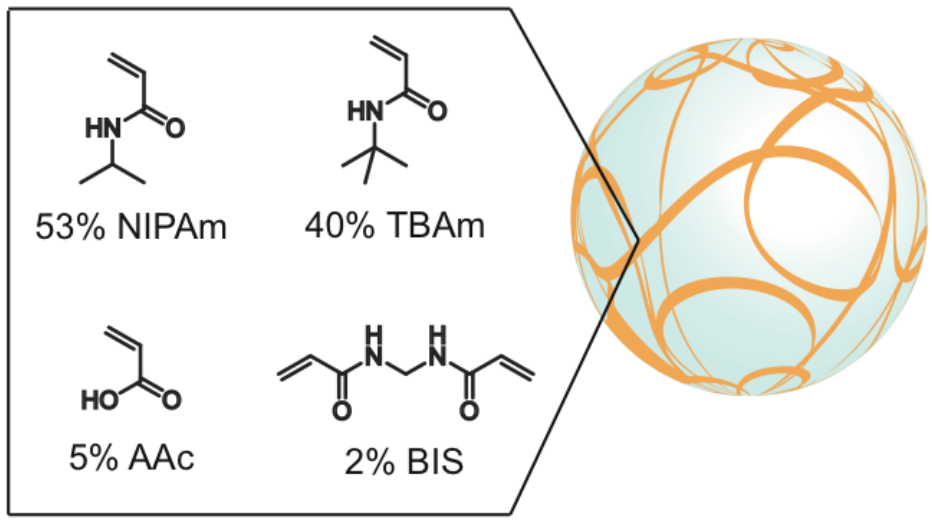
ConA and mannose (HNPs)

Melittin: GIGAVLKVLTTGLPALISWIKRKRQQ

NIPam-based Hydrogel Nanoparticles

220 nm HNPs

a.



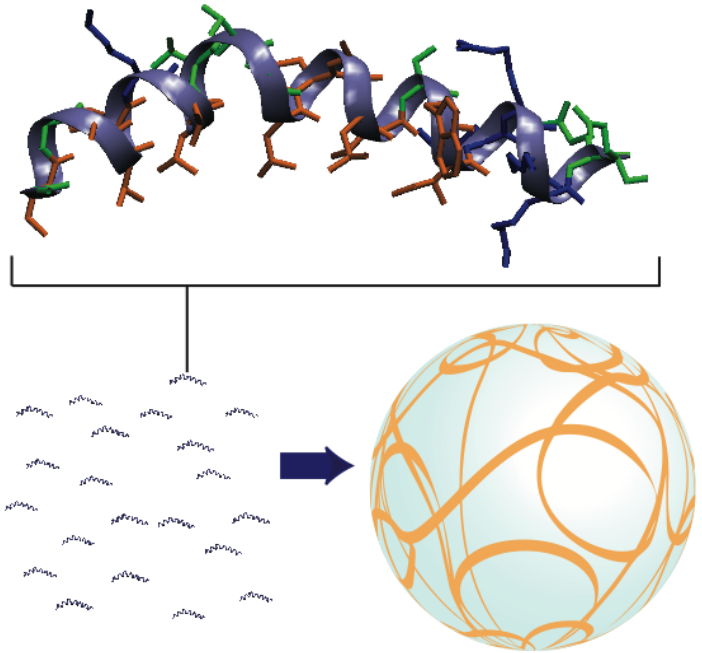
220 nm diameter (from DLS)

1.24 E09 Mol.Wt. (from MALS)

approx. 65% solvent (PBS) by volume

Melittin

b.



26 residue peptide
from bee venom

GIGAVLKVLTTGLPALISWIKRKRQQ

NIPam: N-isopropylacrylamide

Collaborator: Prof. Ken Shea

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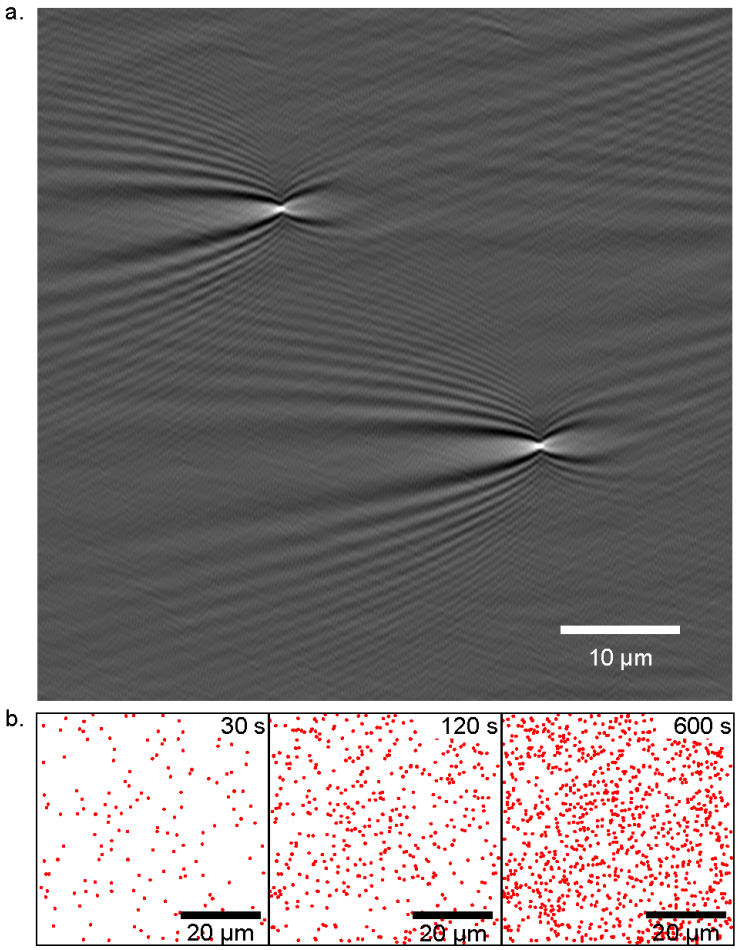
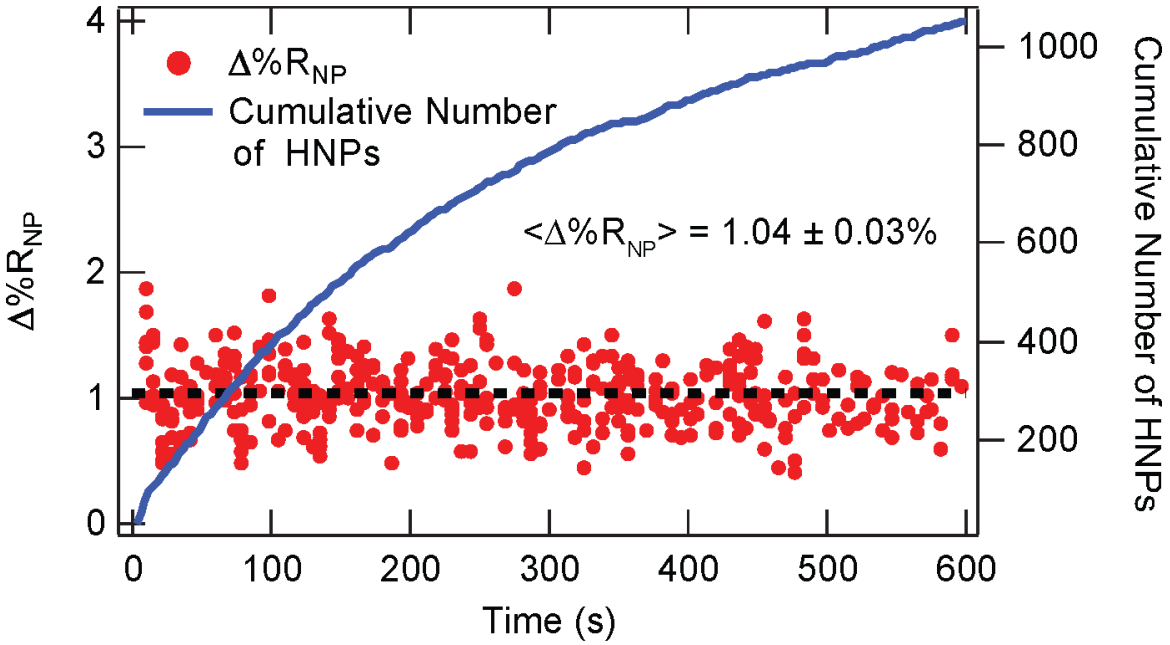
Single Nanoparticle SPRI 180 nm Hydrogel NPs





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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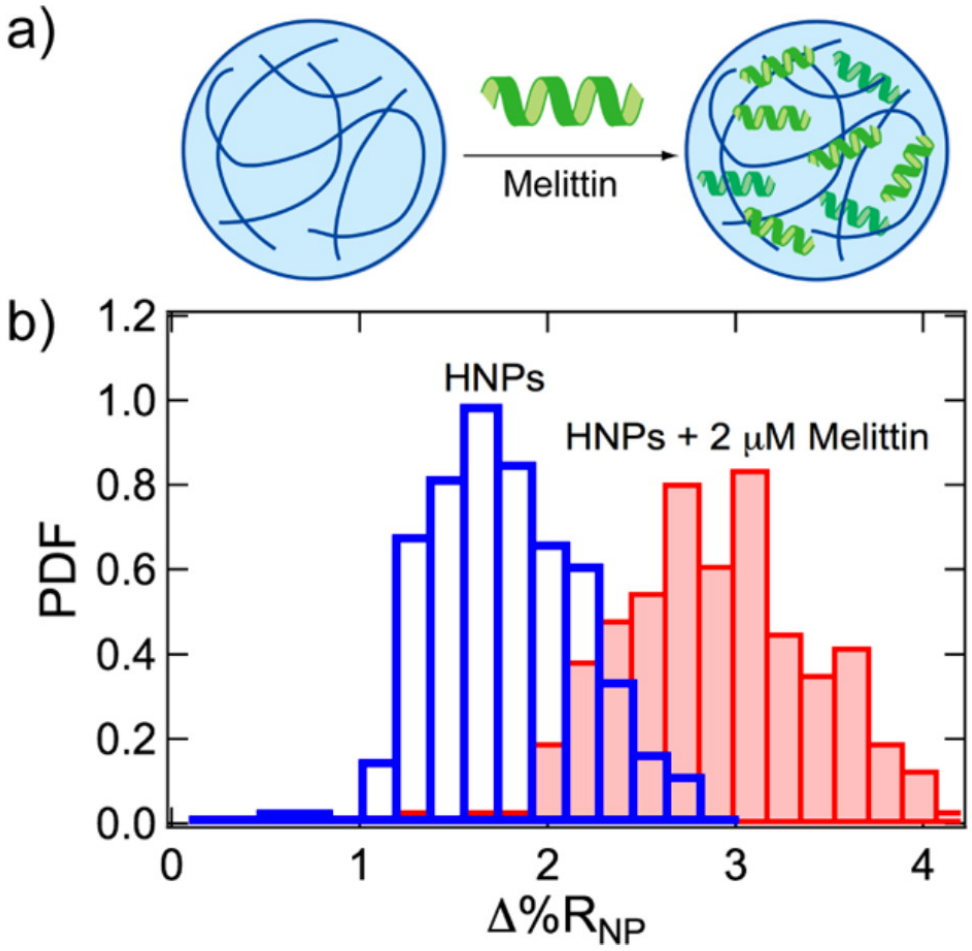
$<\Delta\%R_{NP}>$ for HNPs in the presence of Melittin



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

Mike Cho

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

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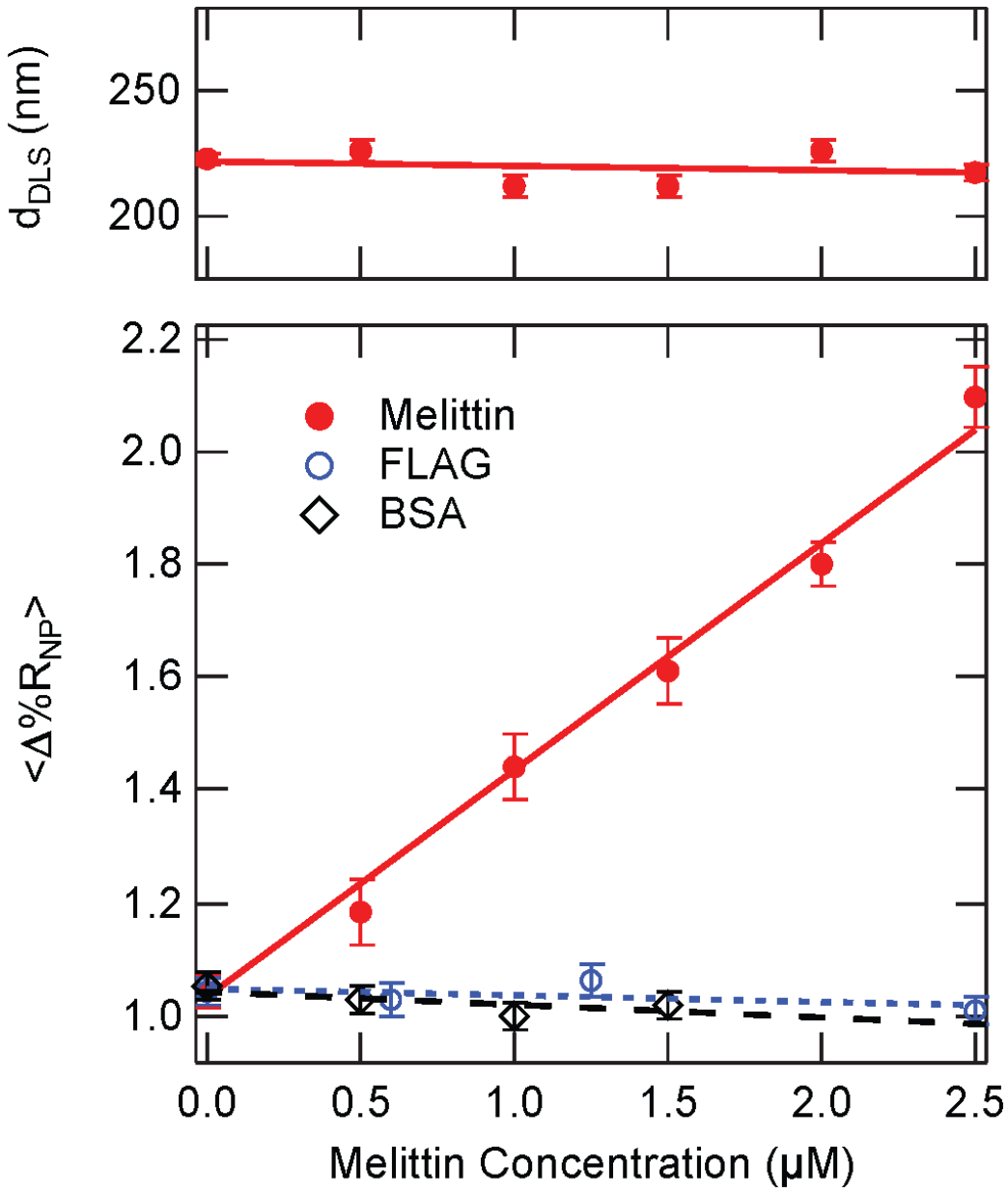
$<\Delta\%R_{NP}>$ for HNPs in the presence of Melittin



$<\Delta\%R_{NP}>$ 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 $<\Delta\%R_{NP}>$ 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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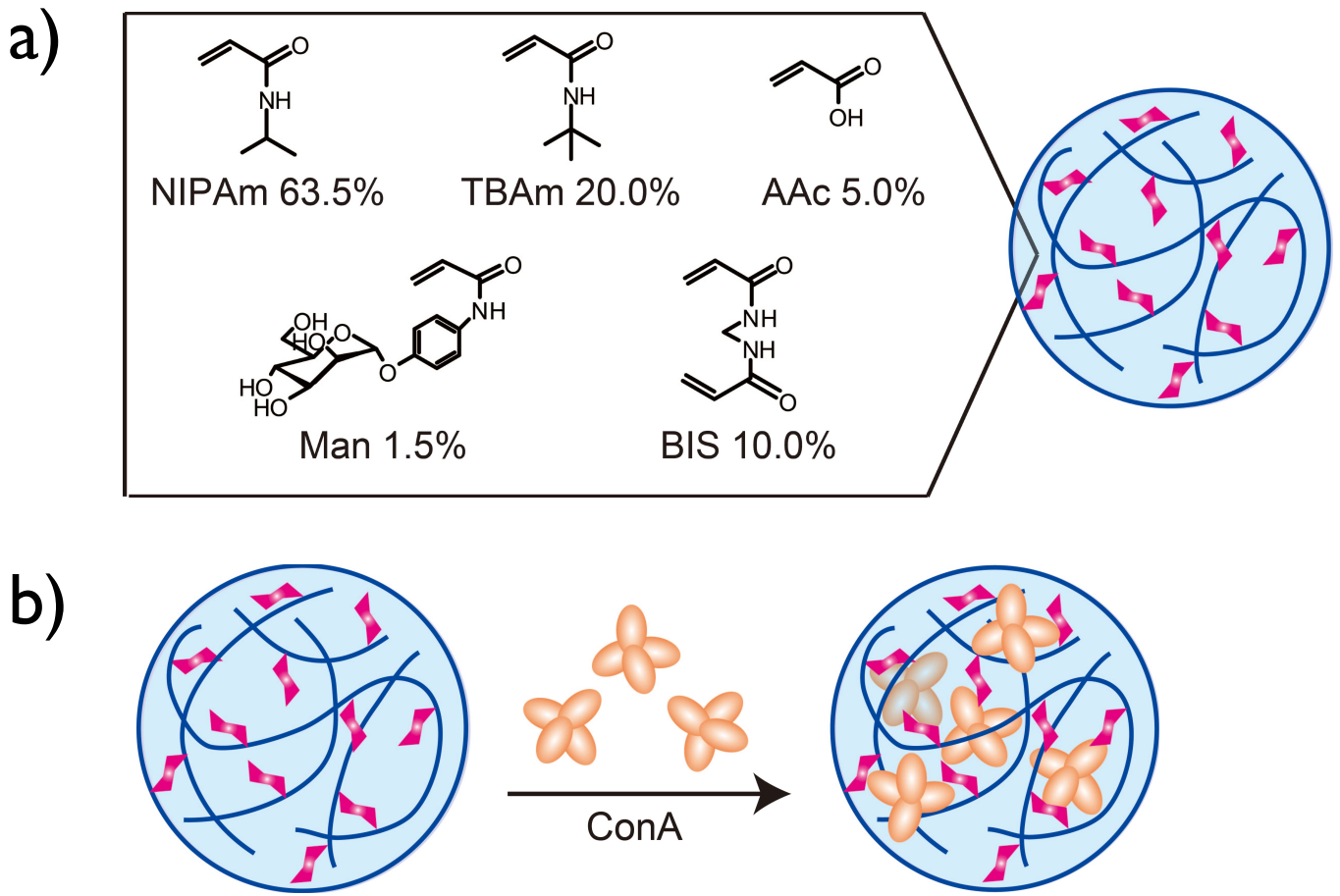
ConA uptake into HNPs



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).*

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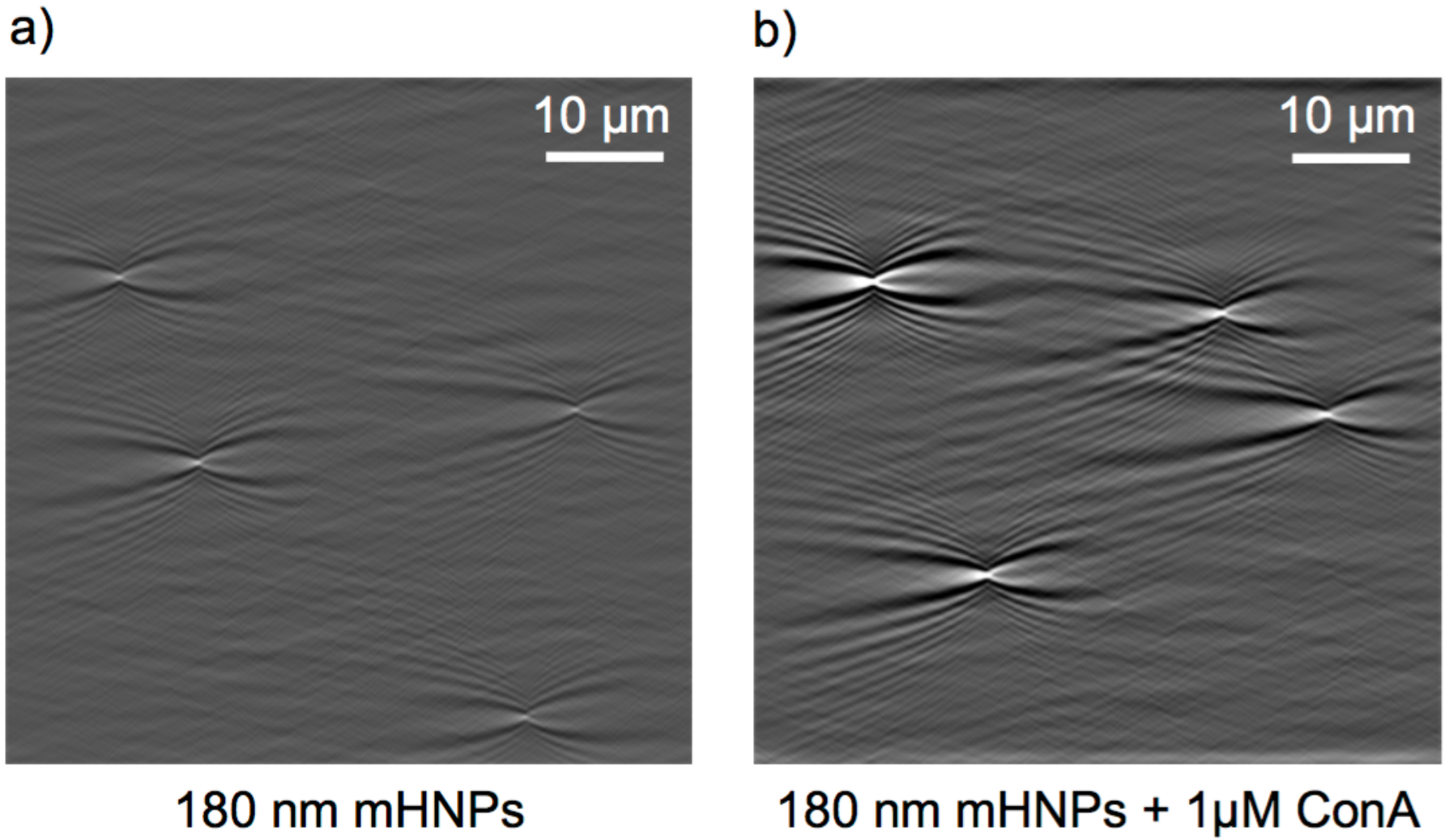
ConA uptake into HNPs



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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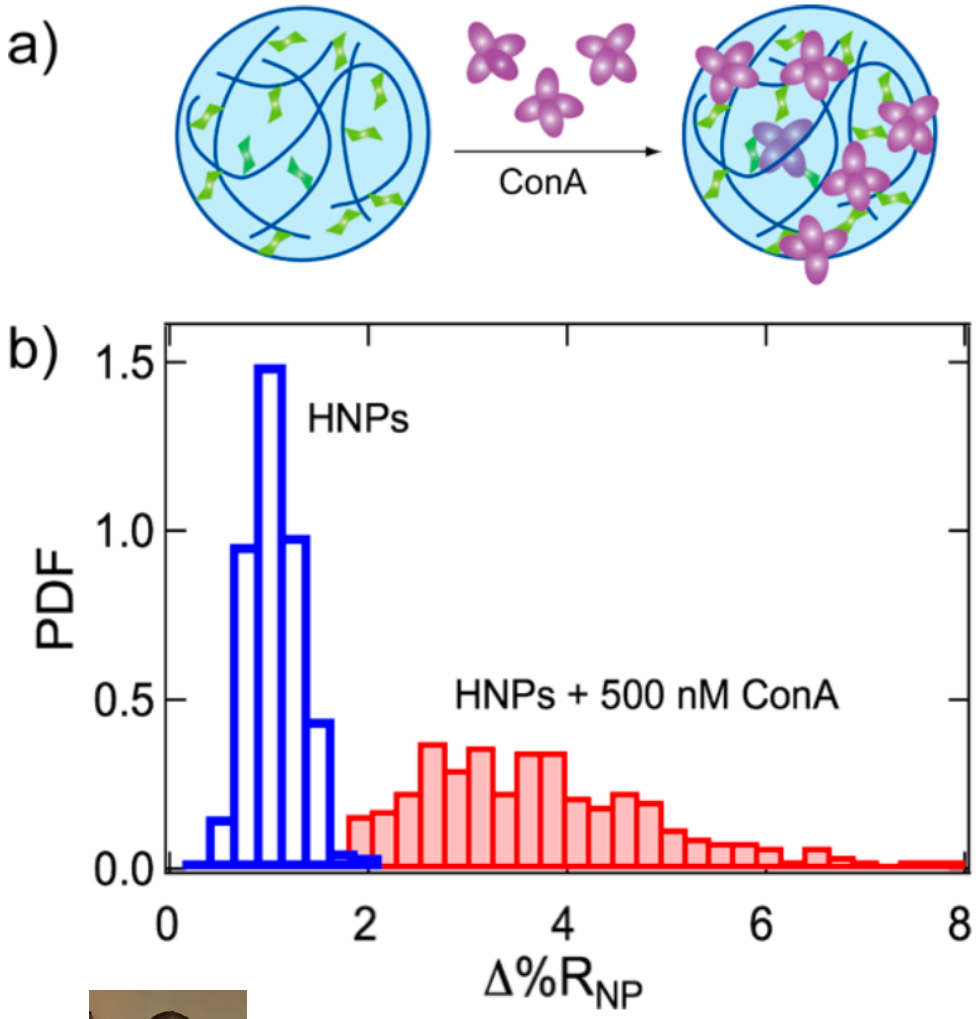
ConA uptake into HNPs



Both <Δ%R_{NP}> and the distribution width increases with ConA concentration as the uptake of ConA both increases the refractive index of the hydrogel nanoparticle and allows for inter-nanoparticle binding

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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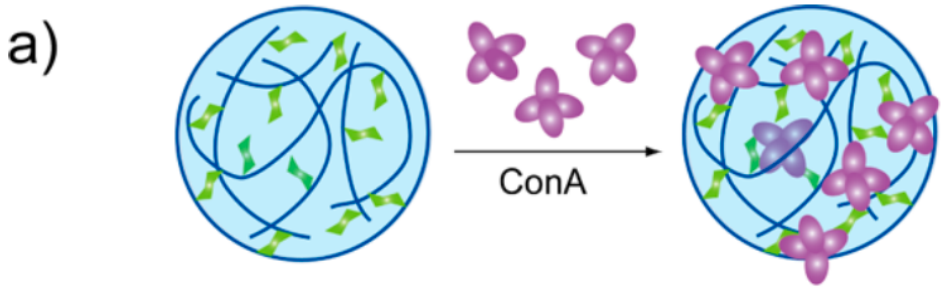
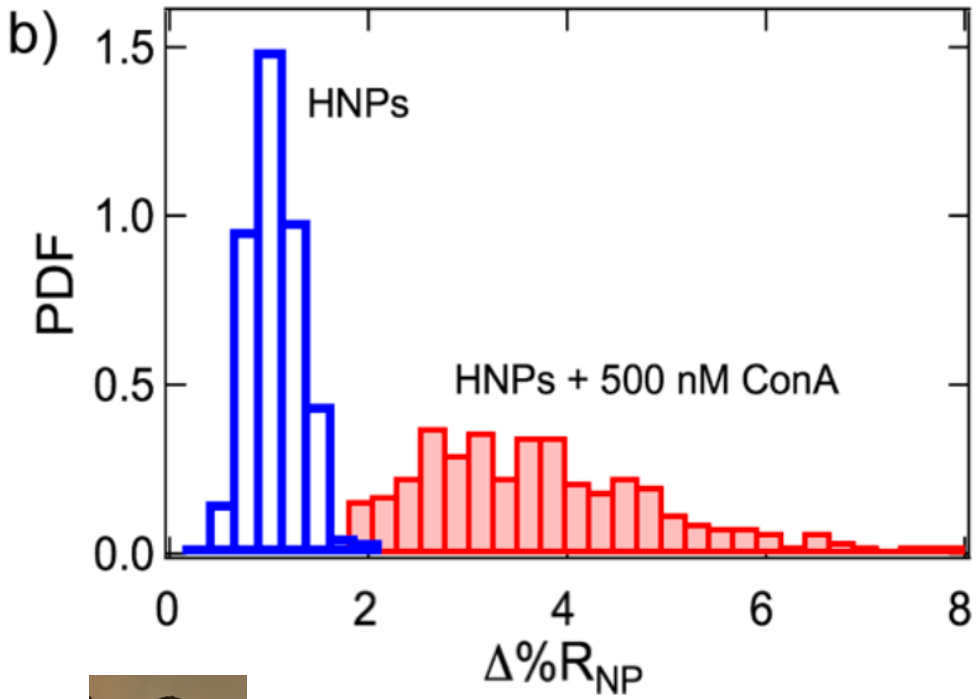
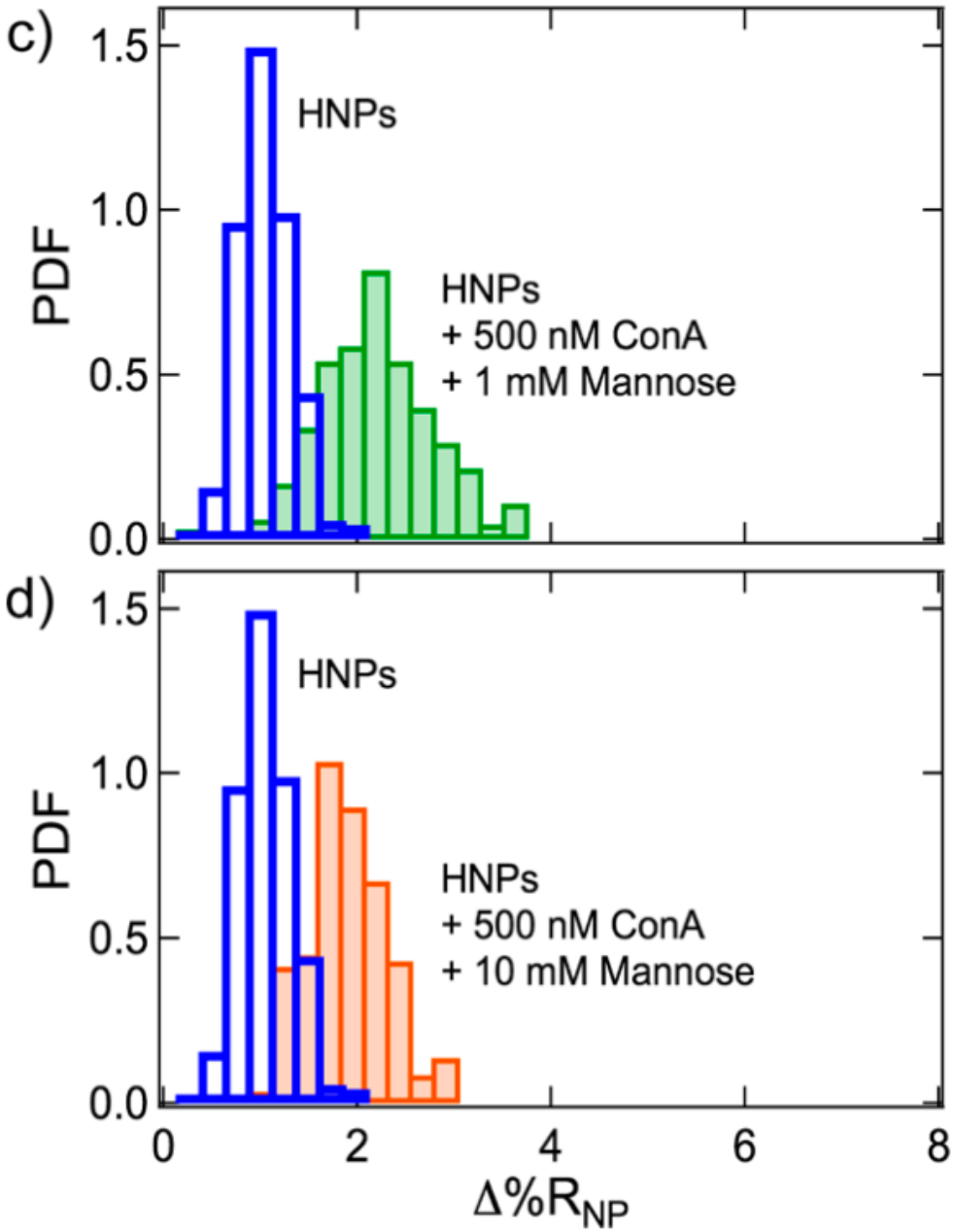
ConA uptake into HNPs

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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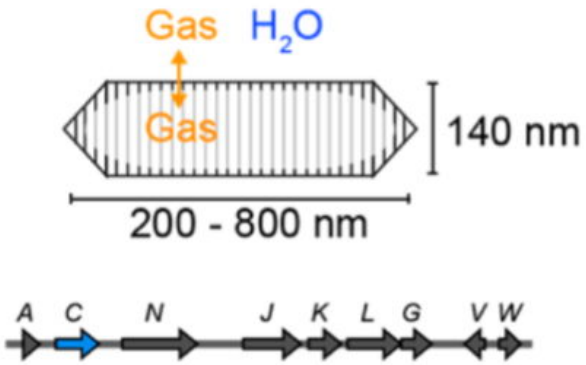
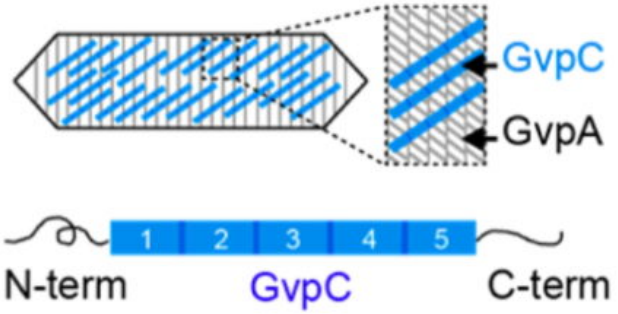
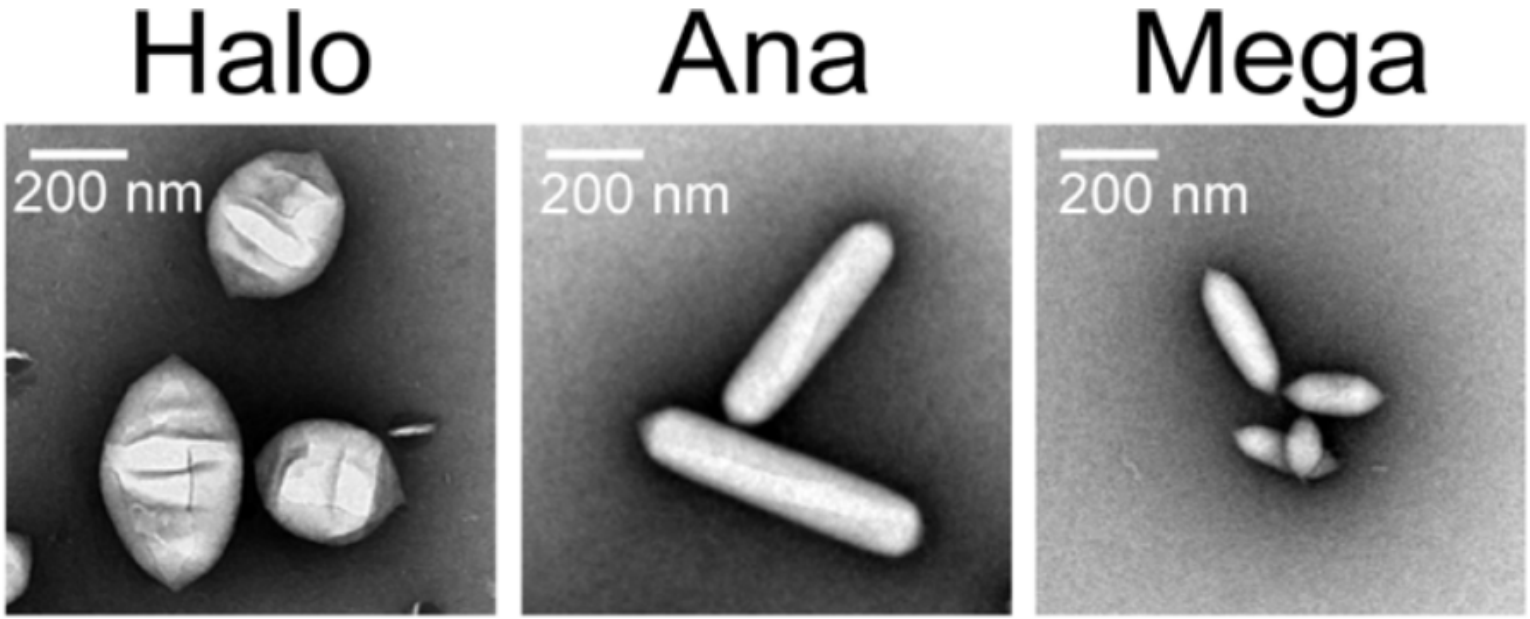
Biogenic Gas-Filled Protein Vesicles







With Prof. Mikhail G. Shapiro, Cal Tech.

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

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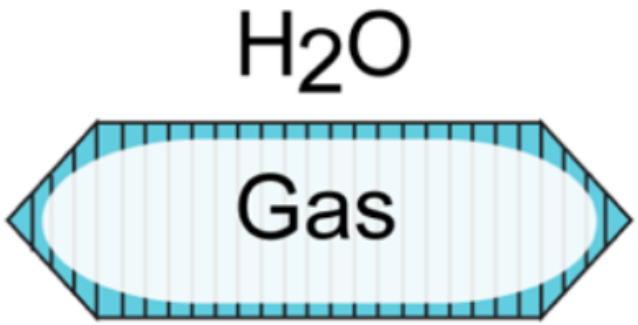
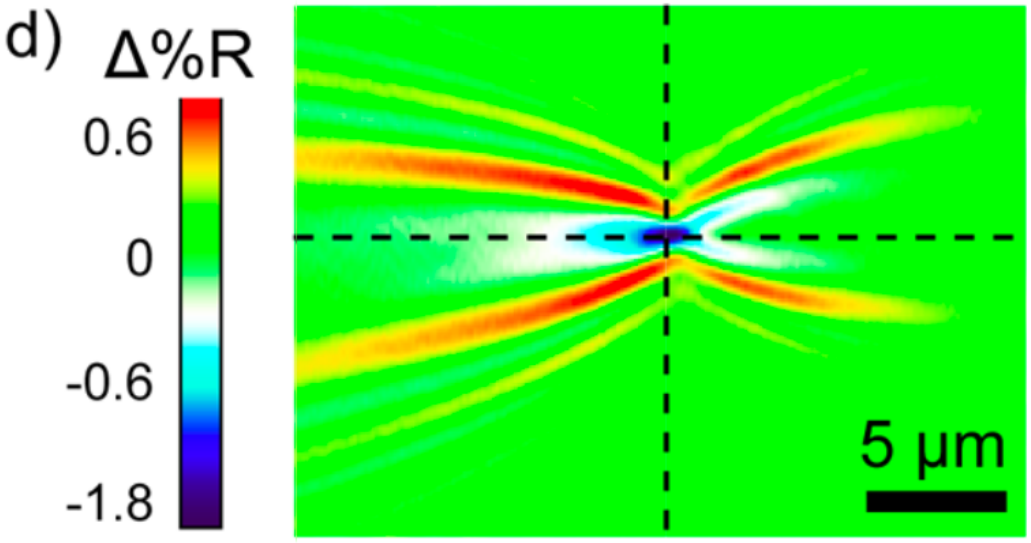
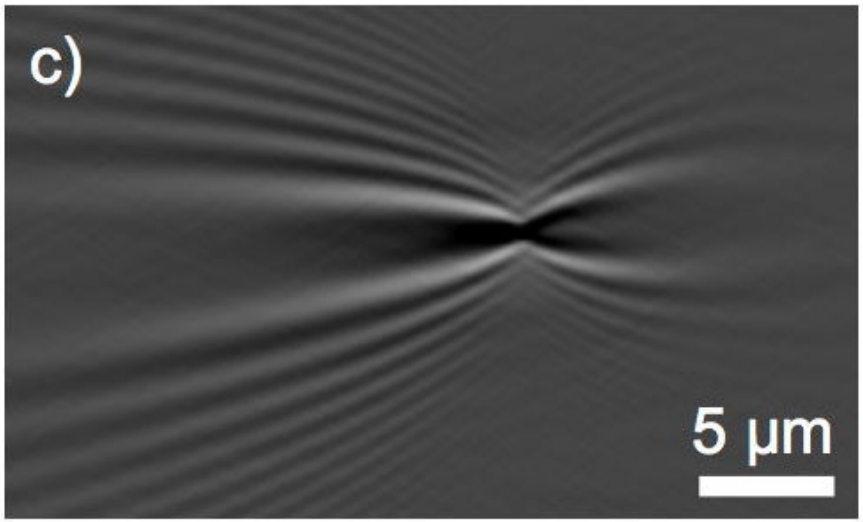
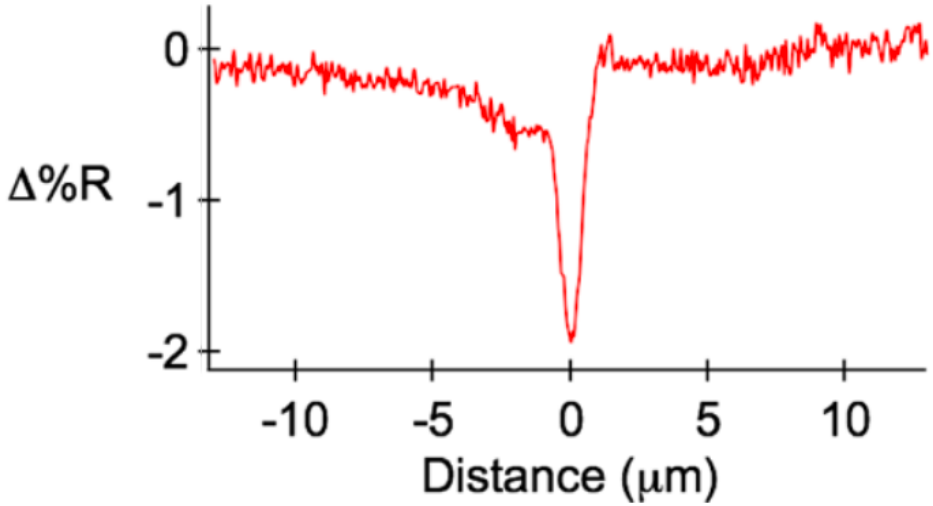
Biogenic Gas-Filled Protein Vesicles









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

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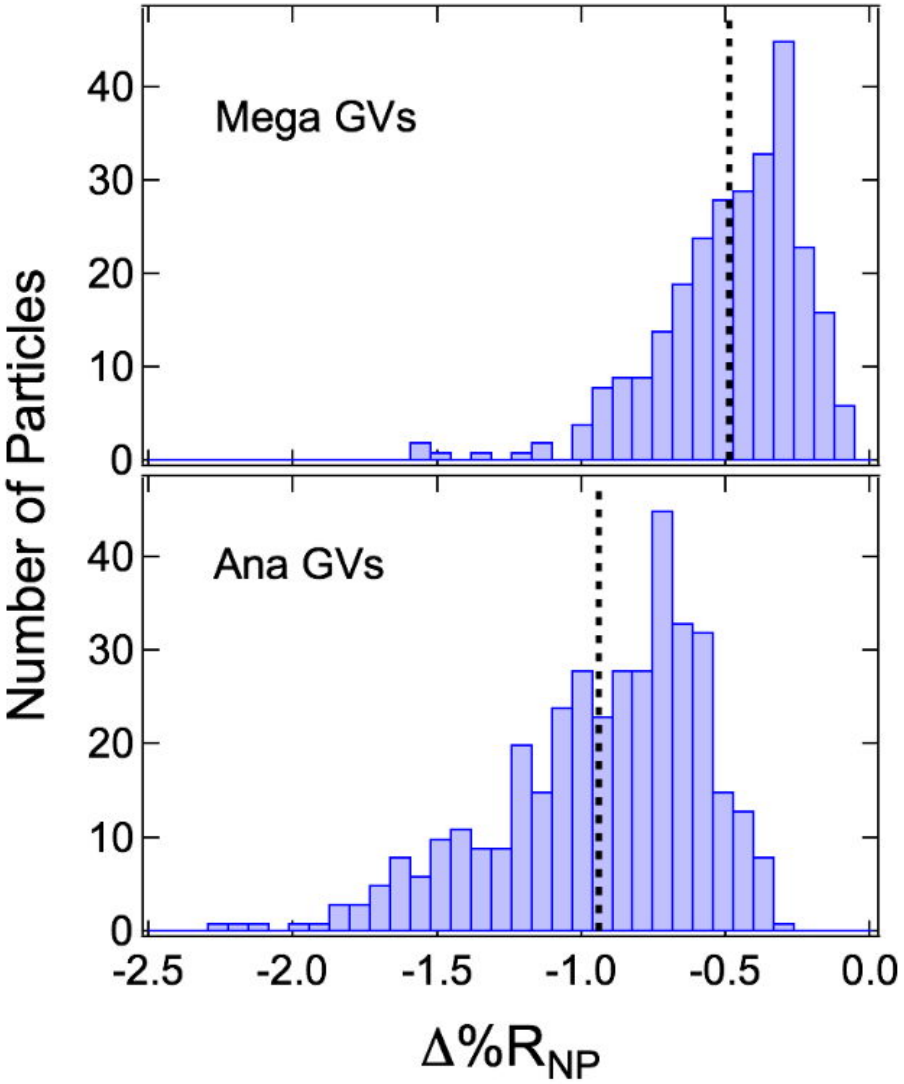
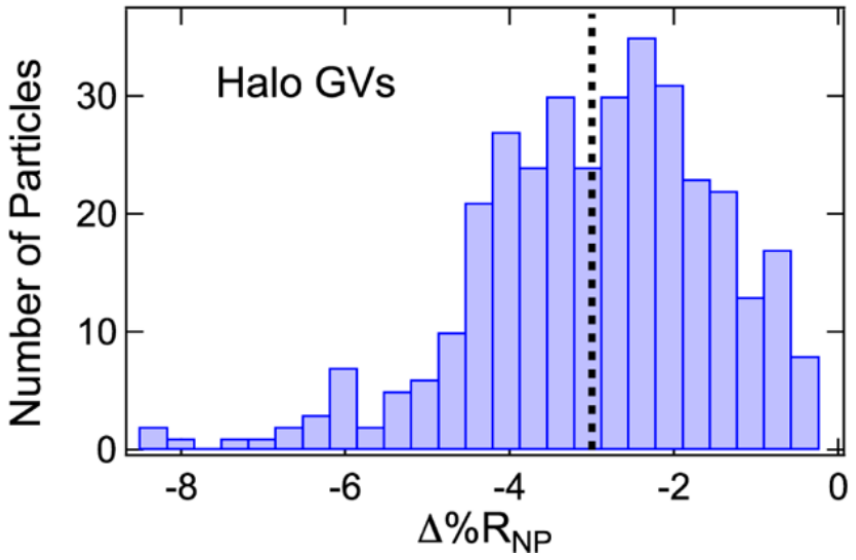
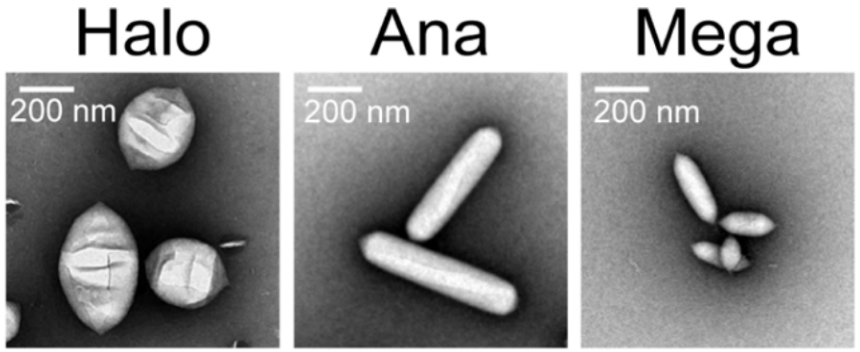
Biogenic Gas-Filled Protein Vesicles







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

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

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Millie Fung
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Kellen Kartub
Gerald Manuel
Anna Plett
Mike Cho
Jennifer Fasoli
Aaron Halpern
Megan Szyndler
Nico Hu
Yulin Chen
Yuhei Terada (Kyushu)

Dr. Seulgi So
Dr. Gabriel Loget
Dr. Mana Toma
Dr. WenJuan Zhou
Dr. Lifang Niu
Dr. Iuliana Sendroiu
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Dr. Alastair Wark
Dr. Hye Jin Lee

Funding: NIH
NSF
UC-CRCC

WenJuan

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

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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

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).

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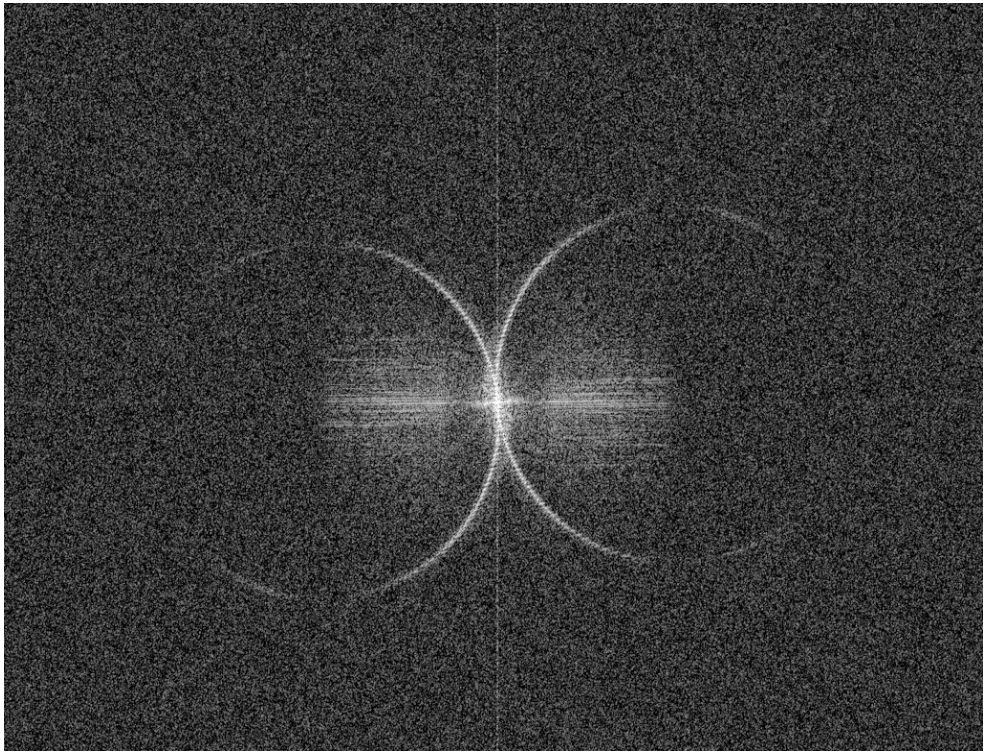
40 nm Gold Nanoparticles



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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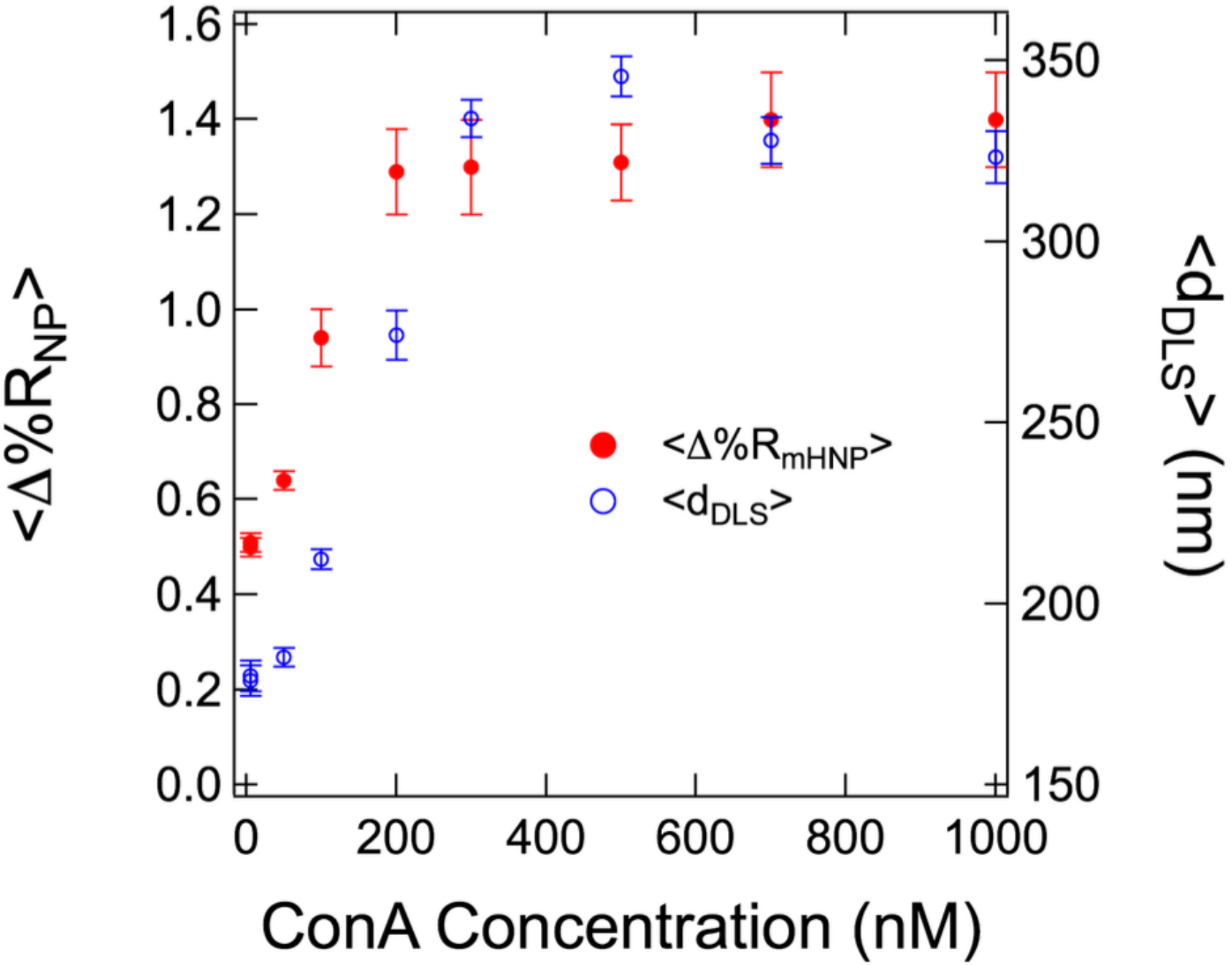
ConA uptake into HNPs



Average single nanoparticle response and DLS hydrodynamic diameter

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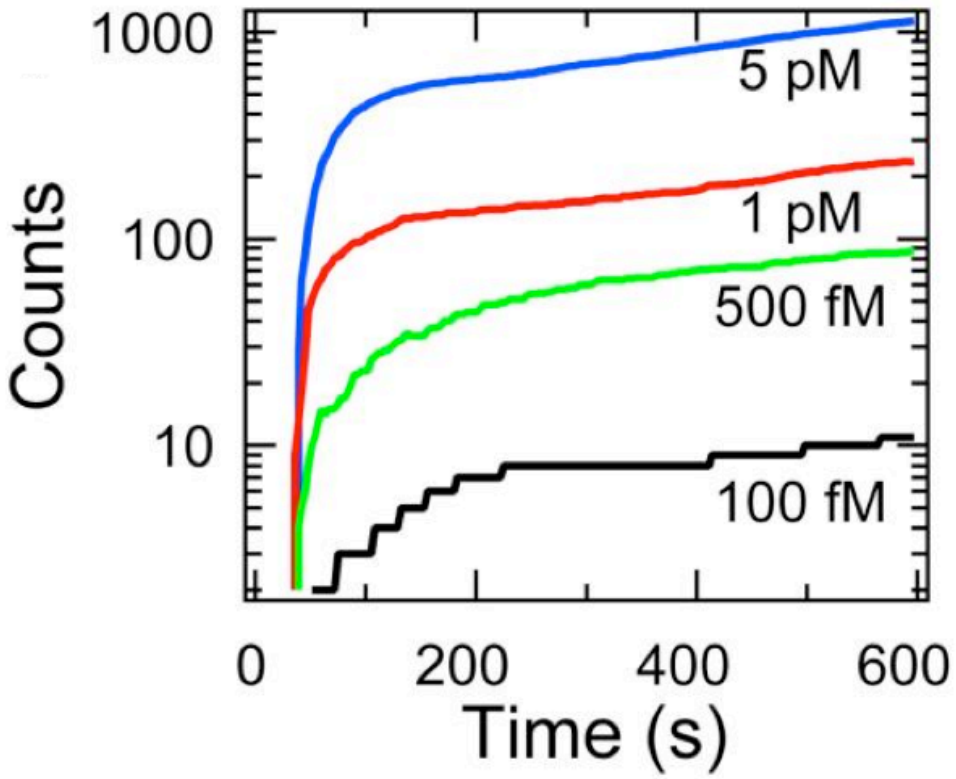
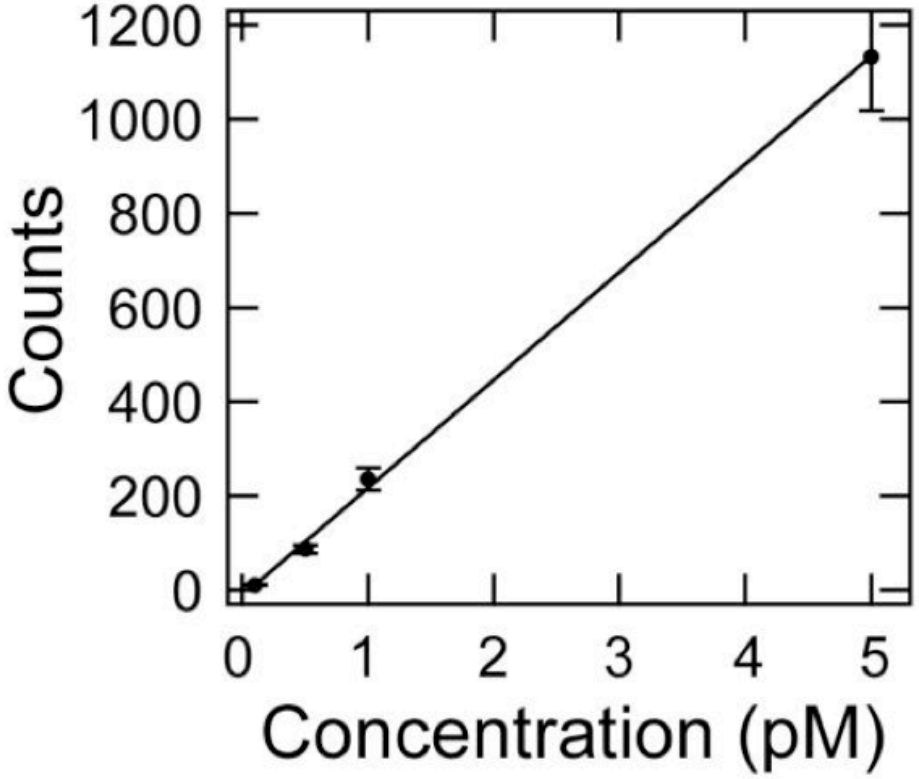
Bioaffinity Adsorption of 40 nm DNA-modified Au Nanoparticles





The number of binding events scale with Au NP concentration

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