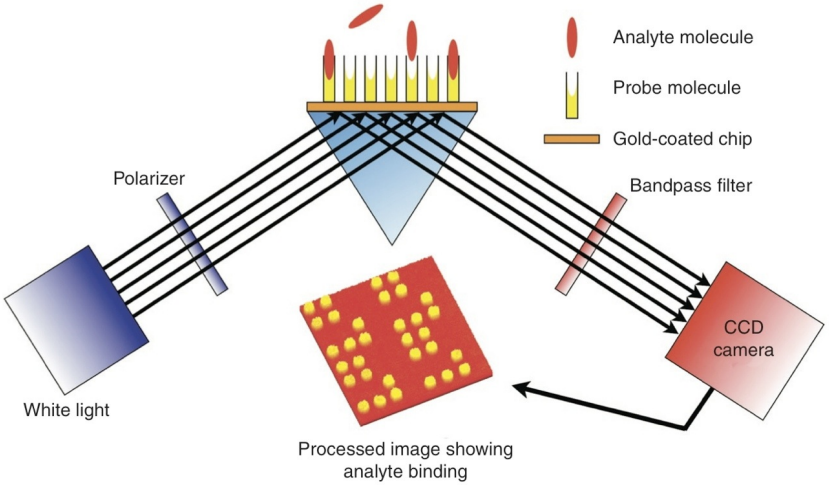
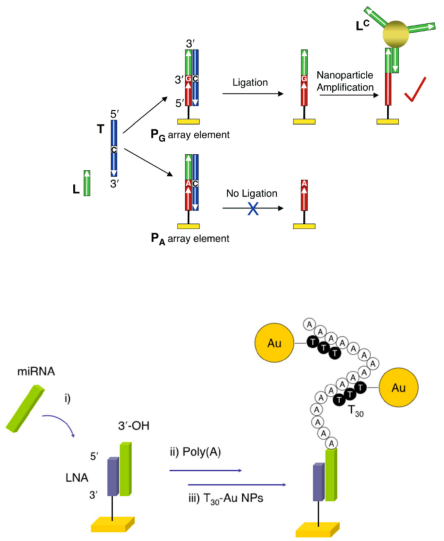
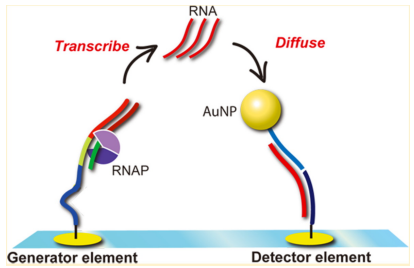
Isothermal Enzyme Chemistries for Ultrasensitive Multiplexed Biosensing of Proteins and Nucleic Acids

Surface Plasmon Resonance Imaging (SPRI) of Biopolymer Microarrays



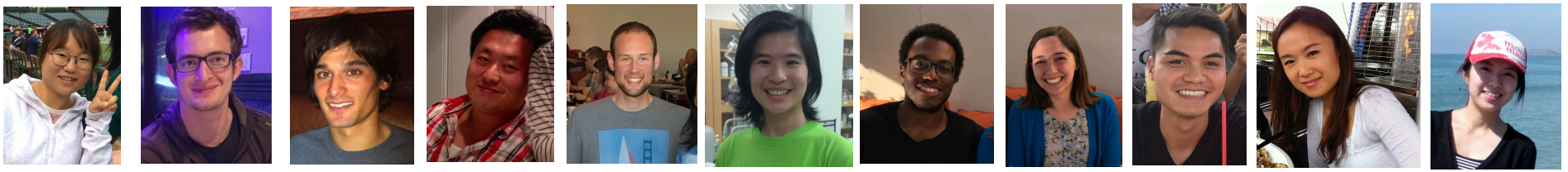
Surface Enzyme Chemistries





Nanoparticle-Enhanced SPRI

Robert M. Corn - UCI Department of Chemistry



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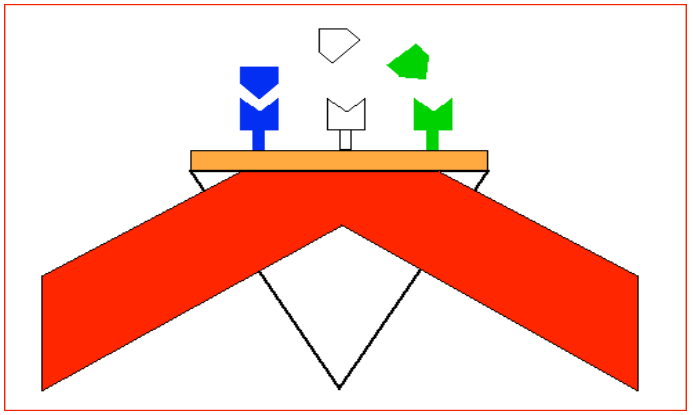
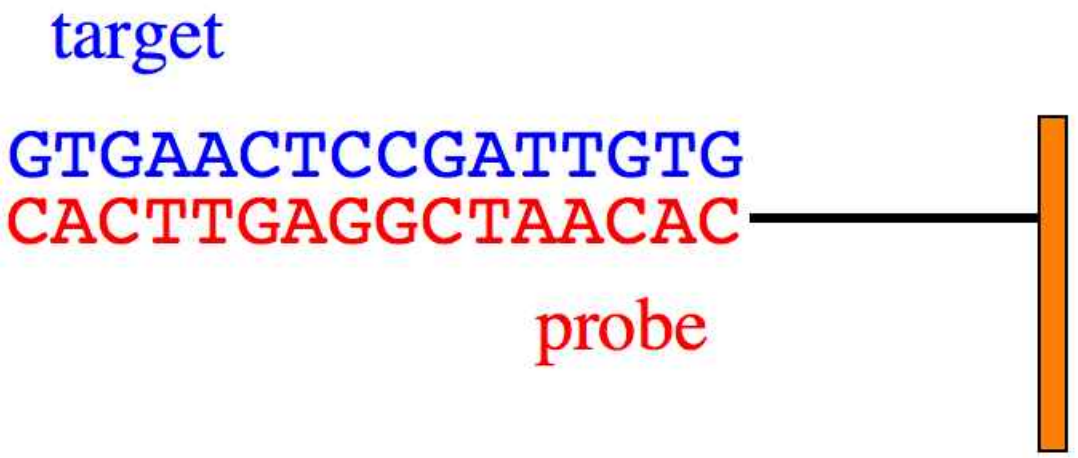
Adsorption Biosensors



DNA-DNA Binding

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



"hybridization adsorption"

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Ultrasensitive Adsorption Biosensor Requirements

- *Quantitative Multiplexed Detection Methodology: SPRI**
- *High Fidelity Surface Microarray Attachment Chemistry*
- *Surface Enzyme Chemistries for Enhanced Capture, Selectivity and Sensitivity*

  Surface Enzymatic Amplification
  Target Recycling
  RNA Aptamer-Based Biosensing
  Enzymatic Microarray Fabrication

**Surface Plasmon Polariton Momentum Resonance Imaging*
(Not localized nanoparticle SPR absorption)

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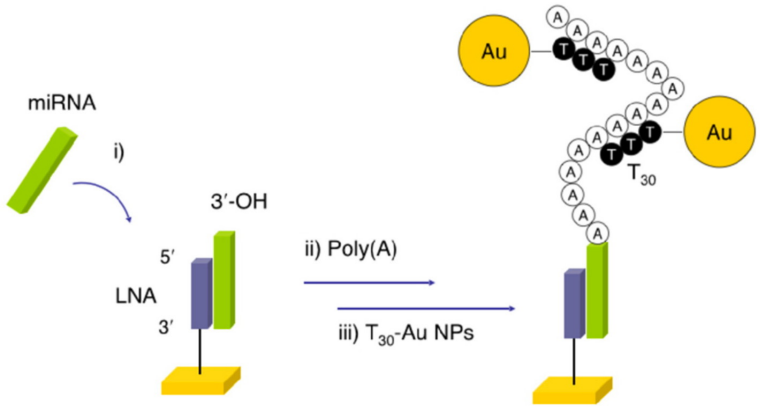
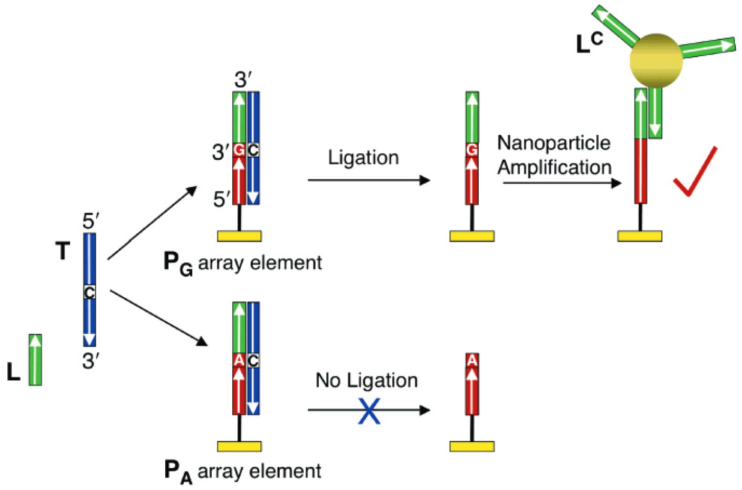
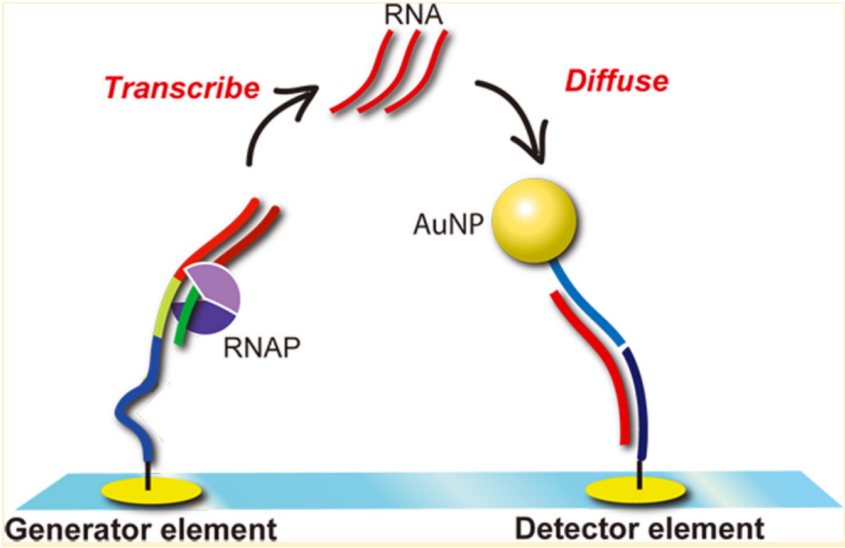
Surface DNA Enzyme Chemistry + Nanoparticle-Enhanced SPRI

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







J. B. Fasoli and R. M. Corn, Langmuir, 31 9527-9536 (2015).

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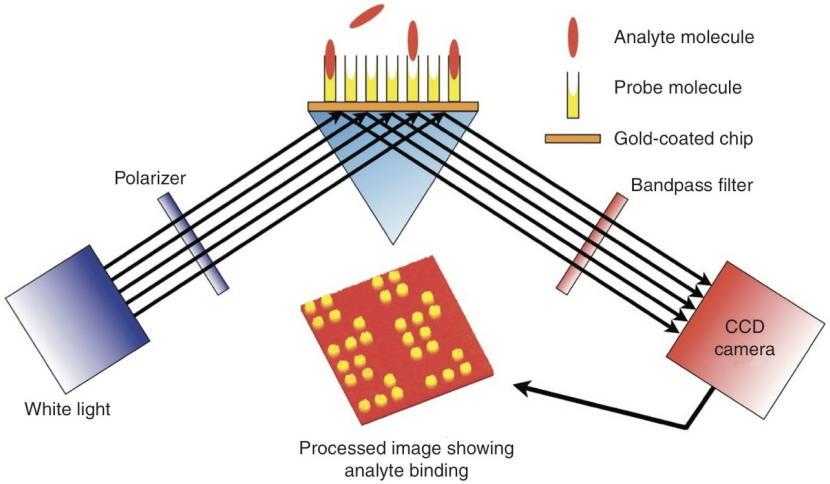
Quantitative Multiplexed Detection Methodology: SPRI

Surface Plasmon Resonance Imaging (SPRI)



800 nm SPRI

Prof. Hye Jin Lee
Kyungpook National Univ. (KNU)

In SPRI, bioaffinity adsorption changes the local interfacial refractive index which leads to an increase in reflectivity: Δ%R.

Advantages:

- *Works easily in a microarray format*
- *Sensitive to first 200 nm of solution near the gold surface*
- *Works with unmodified target proteins and nucleic acids*
- *Quantitative measure of sequential DNA, RNA, protein surface adsorption*
- *Bigger signal for larger proteins and nanoparticles*
- *"Nanoparticle-Enhanced SPRI"*

J. B. Fasoli and R. M. Corn, Langmuir, 31 9527-9536 (2015).

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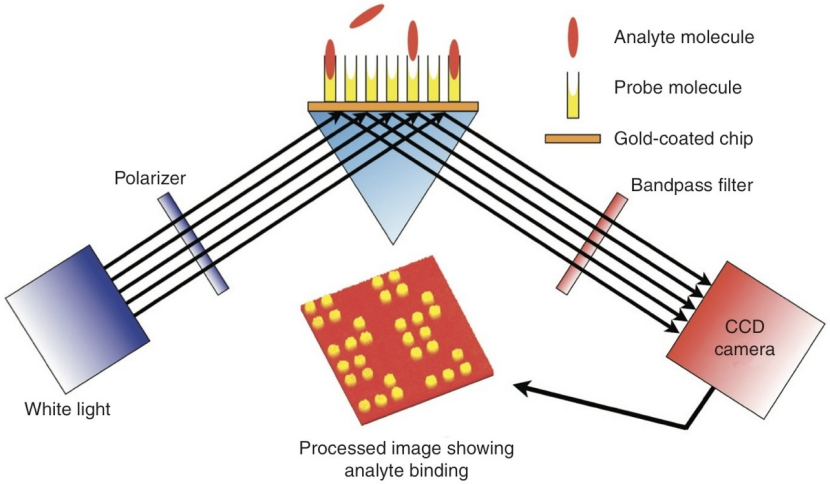
Quantitative Multiplexed Detection Methodology: SPRI

Surface Plasmon Resonance Imaging (SPRI)



800 nm SPRI

Prof. Hye Jin Lee
Kyungpook National Univ. (KNU)

In SPRI, bioaffinity adsorption changes the local interfacial refractive index which leads to an increase in reflectivity: Δ%R.

Disadvantages:
- *Sensitive to nonspecific adsorption*
- *SPRI useful for target concentrations >100 pM**

Nanoparticle-Enhanced SPRI

**Advanced SPRI methods for < 100 pm:*
Nanoparticle-Enhanced SPRI
SPRI Phase Imaging
Diffraction Grating SPRI
Long Range SPRI

J. B. Fasoli and R. M. Corn, Langmuir, 31 9527-9536 (2015).

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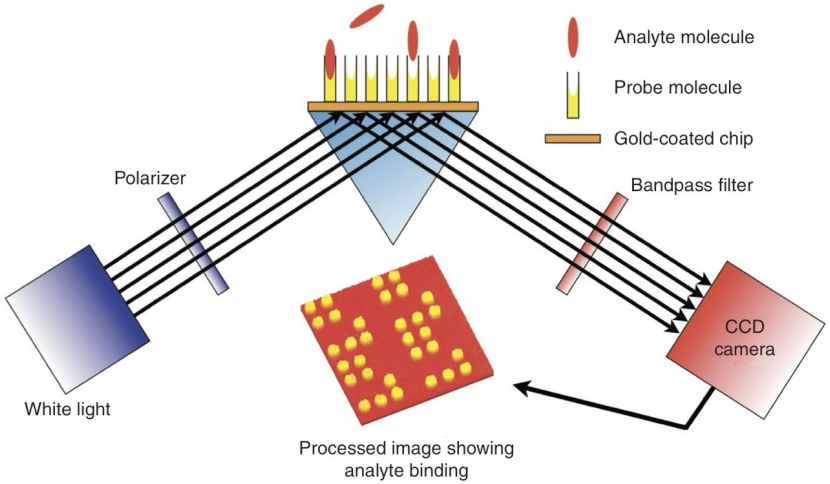
Quantitative Multiplexed Detection Methodology: SPRI

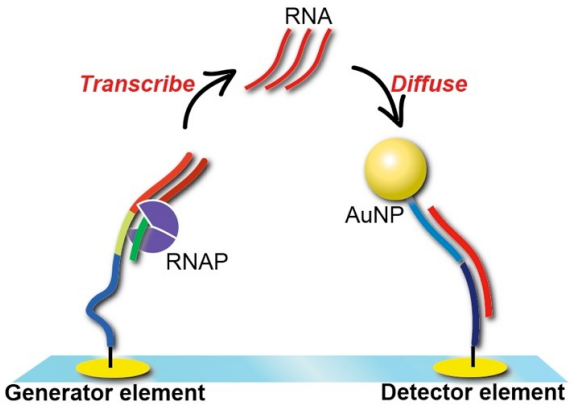
Surface Plasmon Resonance Imaging (SPRI)



800 nm SPRI

Prof. Hye Jin Lee
Kyungpook National Univ. (KNU)

Bioaffinity sensing with SPRI is now well-established around the world, with various commercially available instrumentation (e.g., Horiba, IBIS) and many new exciting extensions and applications.



Surface Plasmon Resonance Imaging: What Next?
G. Spoto and M. Minunni
J. Phys. Chem. Lett., 3, 2682–2691 (2012).

with Alastair Wark, Aaron Halpern, Yuan Li, Yulin Chen, Nico Hu, Wenjuan Zhou, Iuliana Sendroiu and others.

Reviews: S. Scarano et al., Biosensors and Bioelectronics 25, 957-966 (2010).
J. B. Fasoli and R. M. Corn, Langmuir, 31 9527-9536 (2015).

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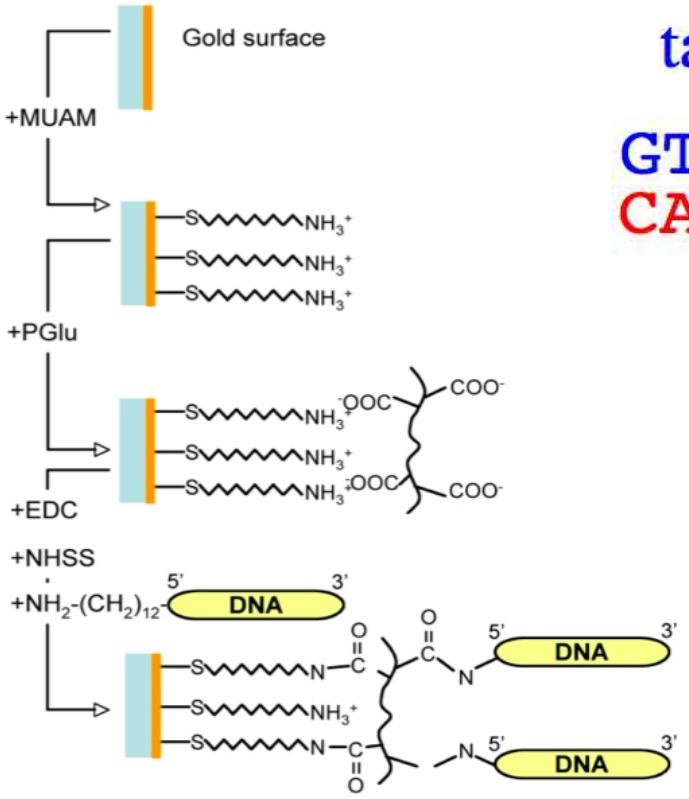
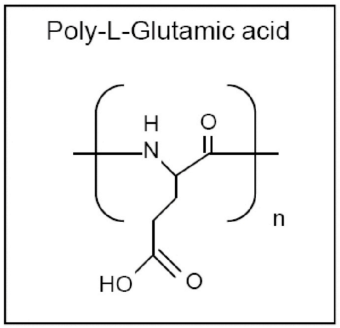
Surface Attachment Chemistry: MUAM-pGlu + NHSS/EDC coupling



DNA microarray surface attachment chemistry

pGlu



Dr. Yulin Chen

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

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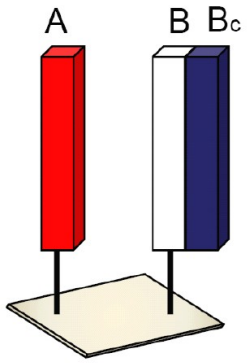
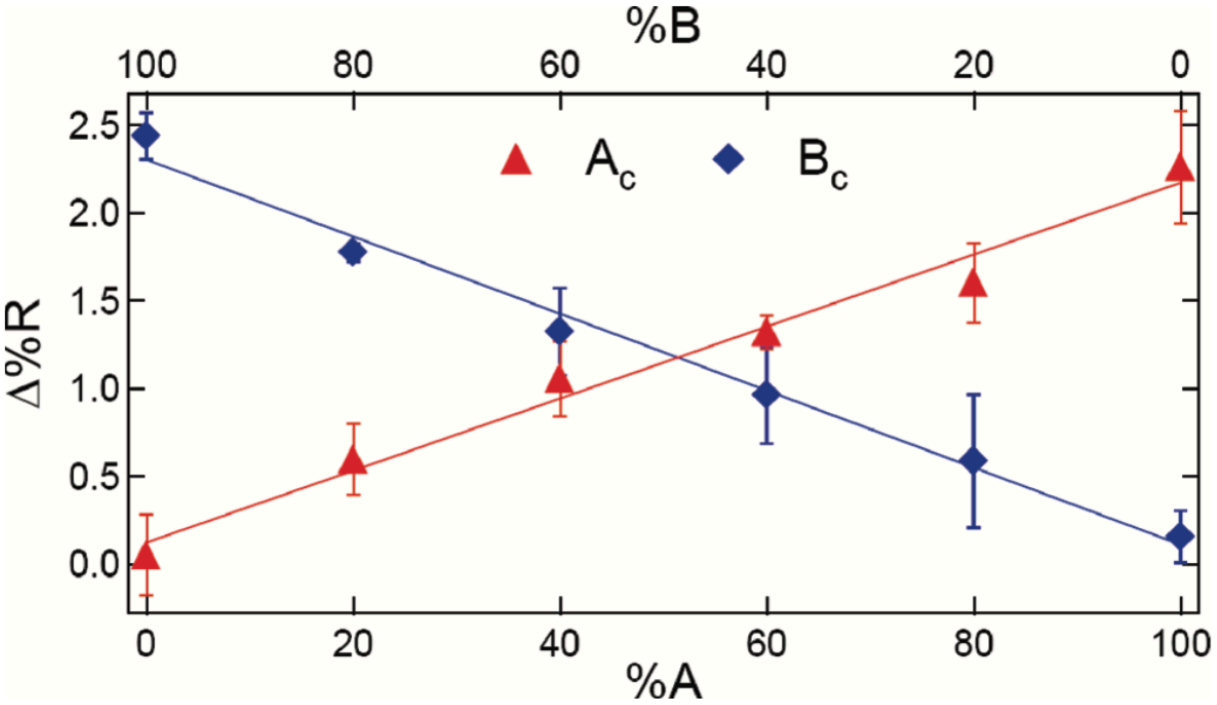
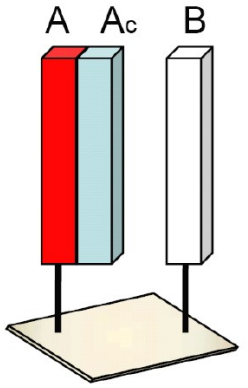
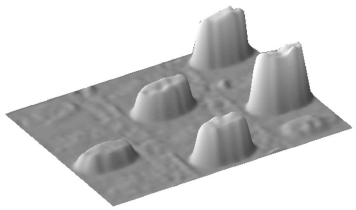
SPRI measurements of DNA microarrays: Quantitative Surface Chemistry

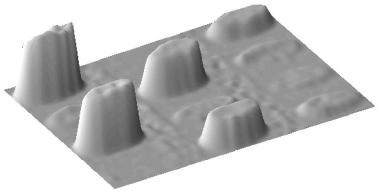
DNA Hybridization Adsorption on Binary (A/B) Mixed Monolayers











Quantitative Mixed Monolayer Measurements

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

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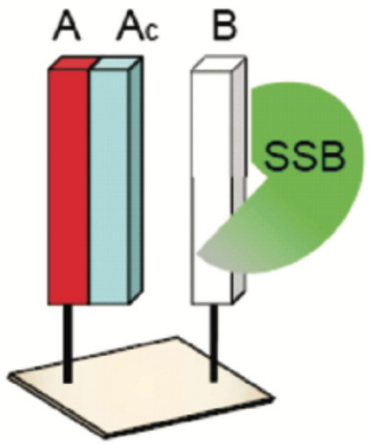
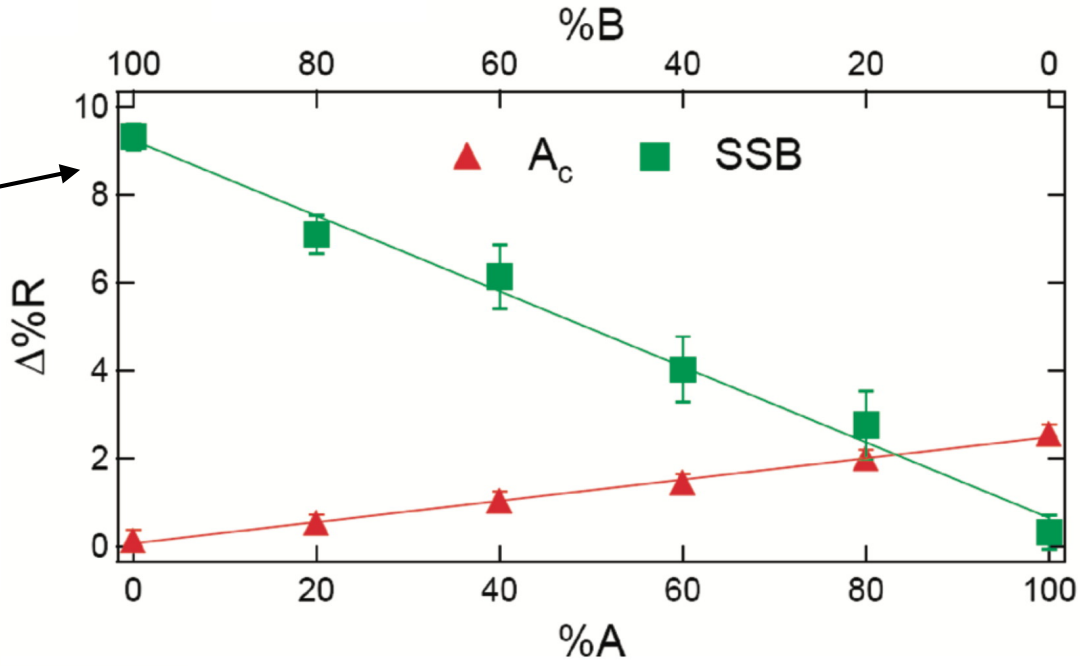
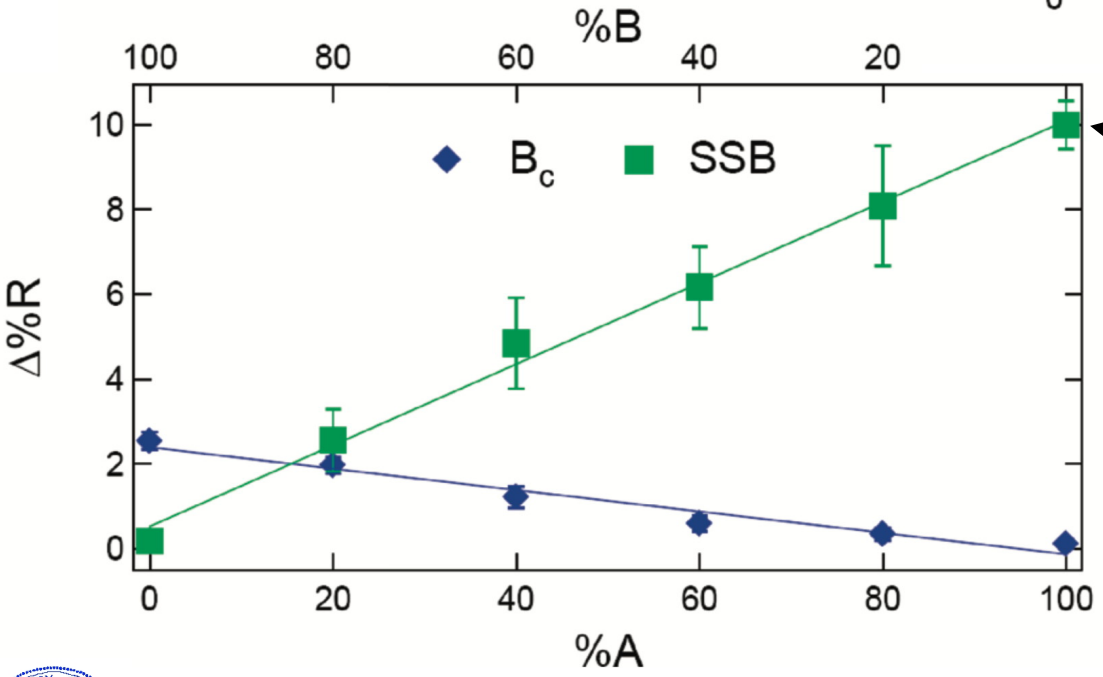
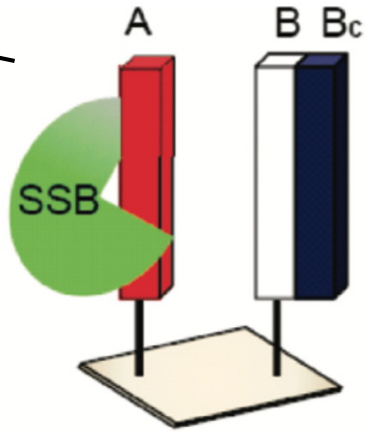
Single Strand Binding Protein (SSB) Adsorption

Protein Biosensing









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

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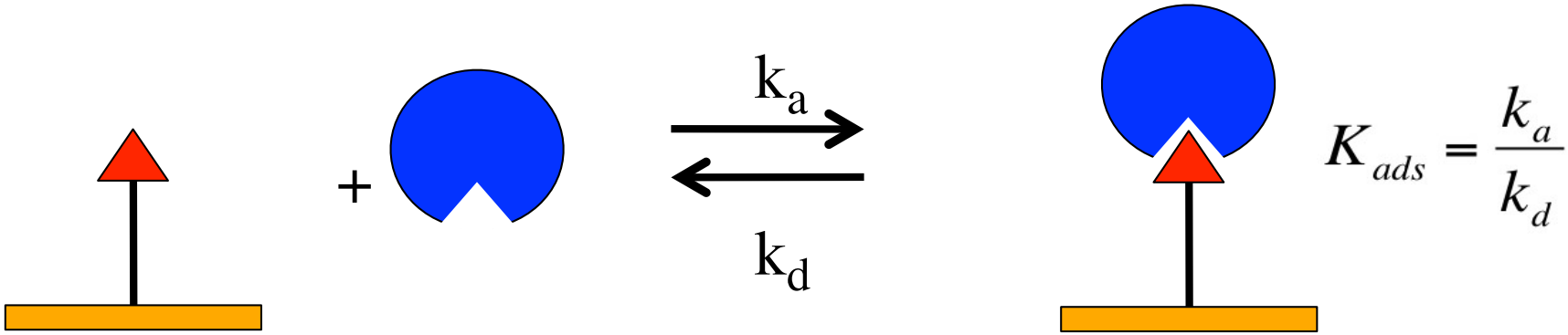
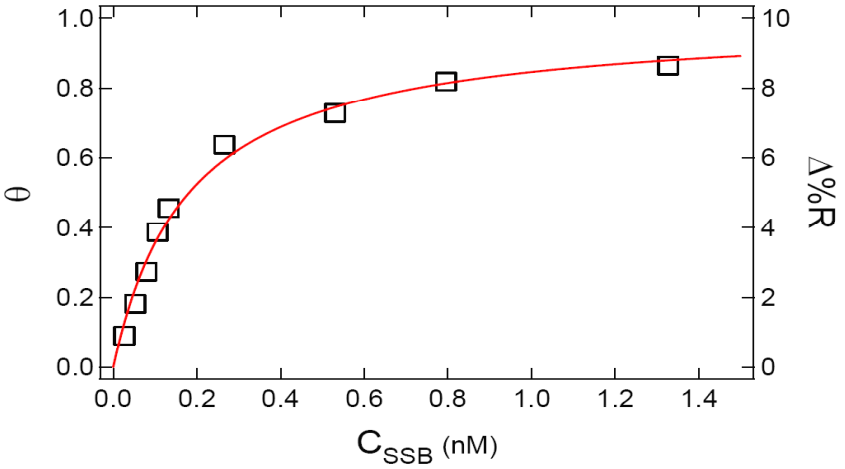
SPRI Equilibrium Measurements: Langmuir Adsorption Isothrem



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

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

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



Langmuir Adsorption Isotherm

Dr. Yulin Chen

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

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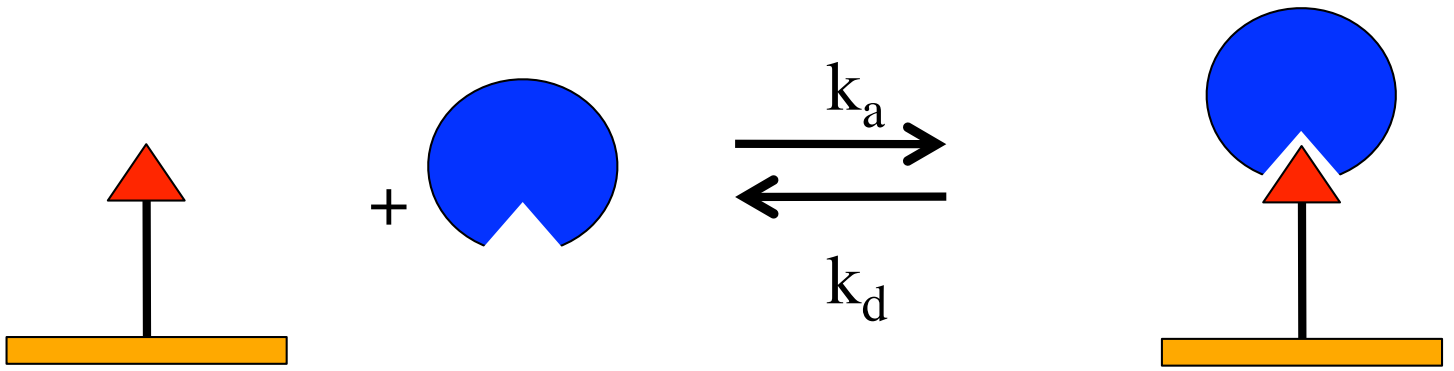
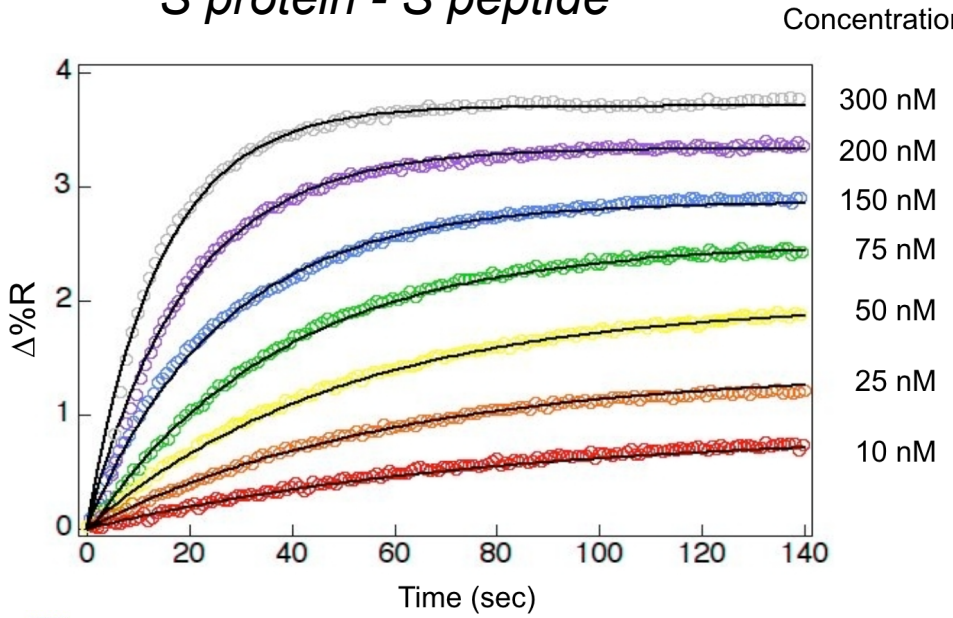
SPRI can be used to measure surface adsorption kinetics



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

S protein - S peptide



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

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

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

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

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

Greta Wegner

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

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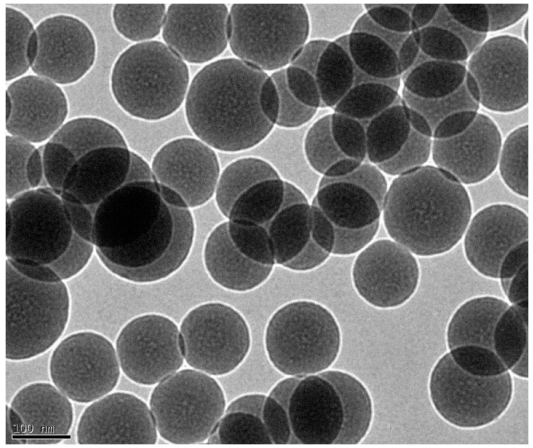
Nanoparticle-Enhanced SPRI

DNA-functionalized:

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

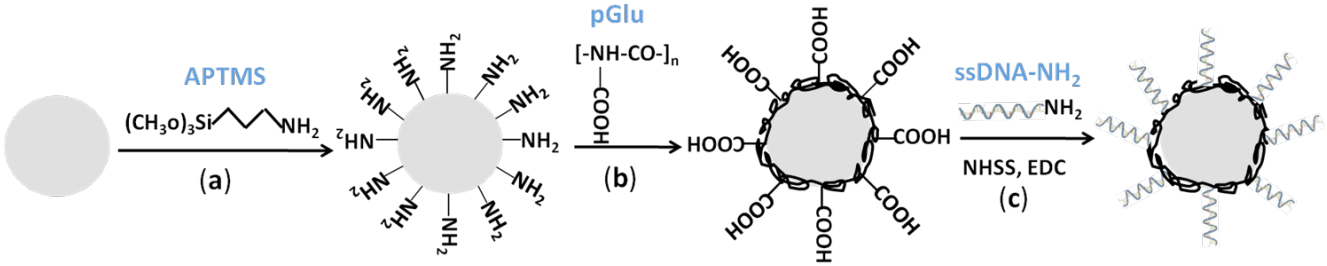
size range: 5 to100 nm



TEM

Dr. WenJuan Zhou

E.G., pGlu DNA Attachment Chemistry on Silica Nanoparticles (SiNPs)



100 nm silica nanoparticles (SiNPs)

** 800 nm SPRI avoids LSPR resonance*

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

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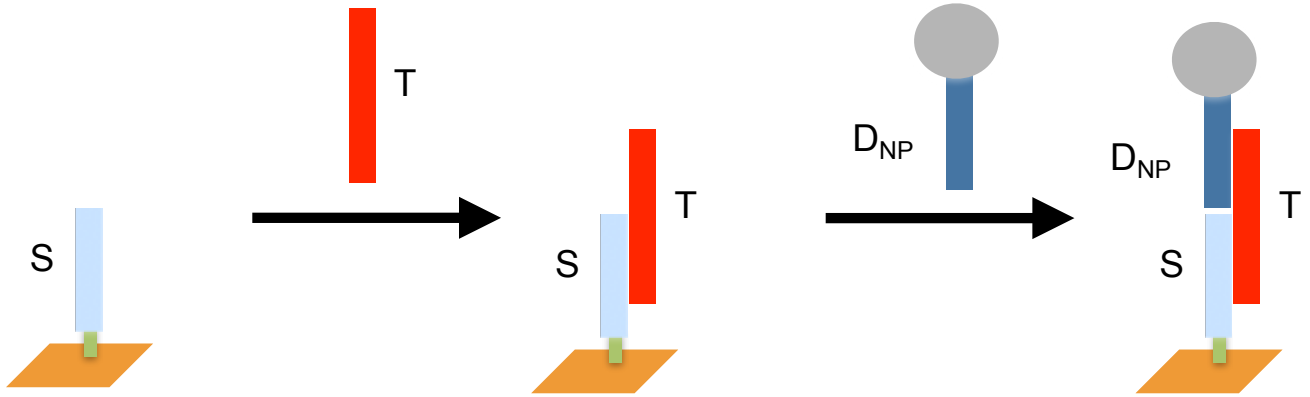
Nanoparticle-Enhanced SPRI: DNA Detection



Three sequence hybridization assay for unlabeled ssDNA target

10 pM LOD with SPR Imaging

L. He, M. J. Natan and C. D. Keating et al., J. Am. Chem. Soc., ***122*** *9071–9077 (2000).*

13 nm Au NPs

25 fM LOD with SPR Phase Imaging

W. Zhou, Y. Chen and R. M. Corn, Analytical Chem., ***84*** *440-445 (2012).*

100 nm Silica NPs

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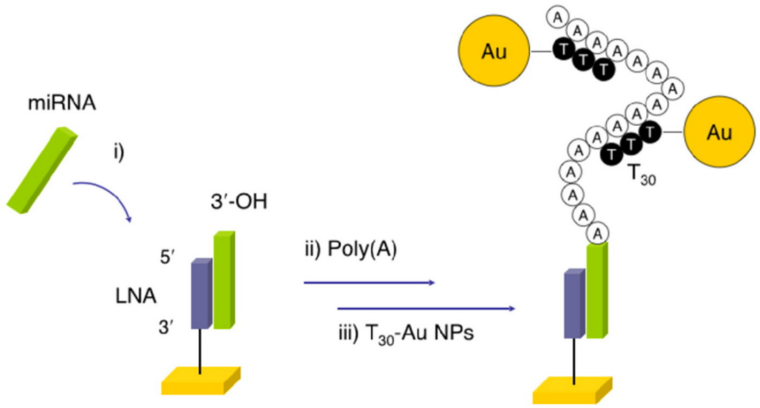
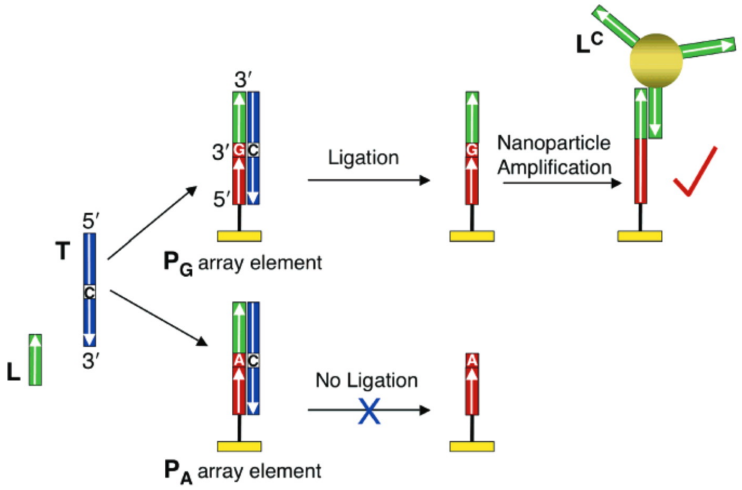
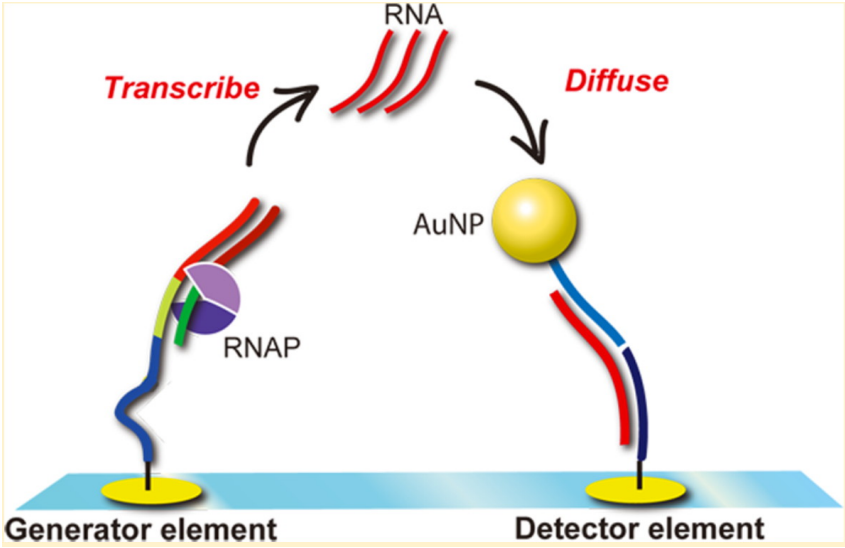
Surface DNA Enzyme Chemistry + Nanoparticle-Enhanced SPRI

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







J. B. Fasoli and R. M. Corn, Langmuir, 31 9527-9536 (2015).

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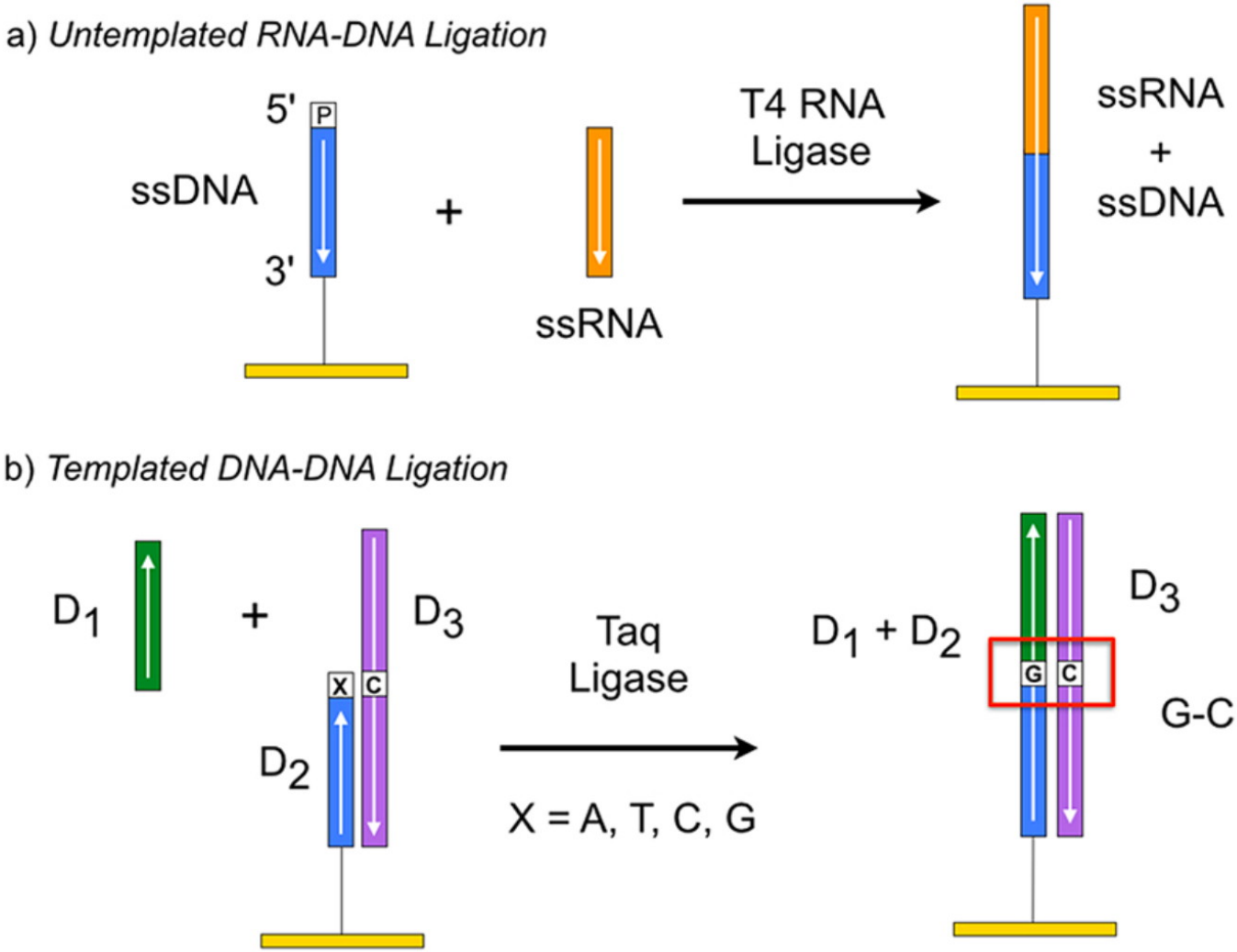
Surface Ligation Chemistries: T4 RNA Ligase and Taq Ligase



RNA microarrays

Dr. Nico Hu

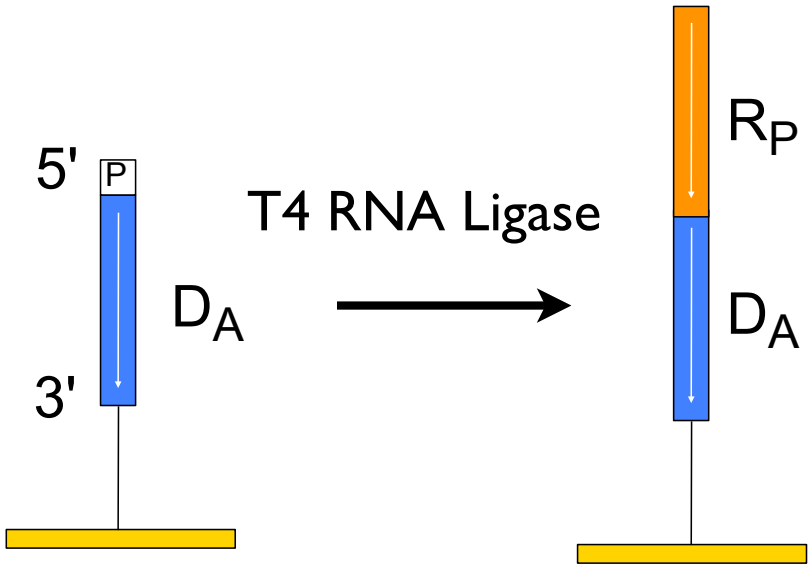
SNP detection

Dr. Yuan Li

UCIrvine
University of California, Irvine

Surface Attachment Chemistry: Enzymatic Ligation of ssRNA to ssDNA

T4 RNA Ligase: No template required.



Ting Nico Hu Seefeld

T4 RNA Ligase is used to:

- *create aptamer microarrays*
- *create arrays for DNA detection with RNase H target recycling*
- *capture miRNA onto DNA-modified NPs*

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

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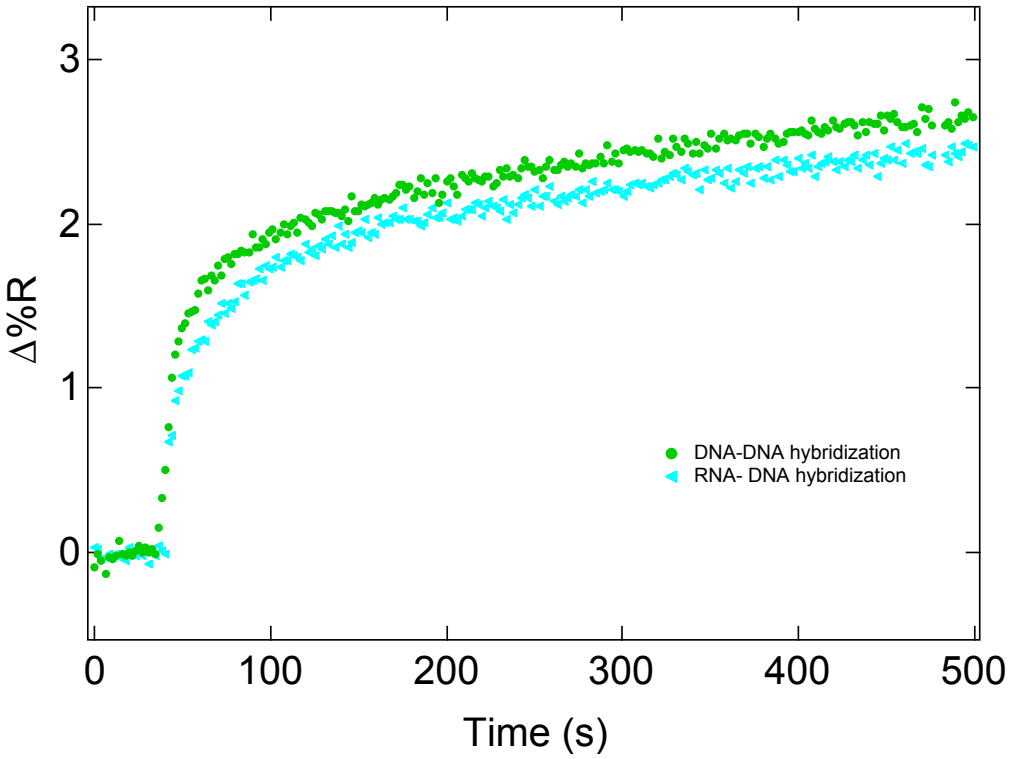
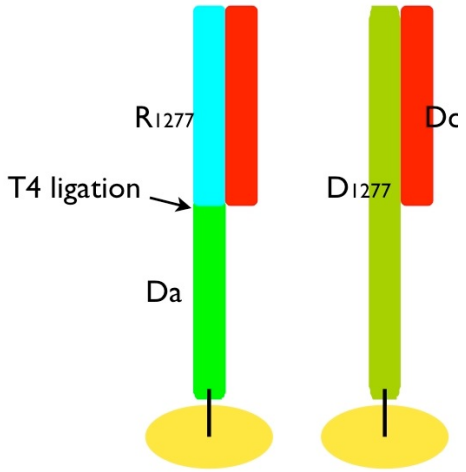
Surface Attachment Chemistry: Enzymatic Ligation of ssRNA to ssDNA



Ting Nico Hu Seefeld



T4 ligation reaction 90% efficiency!

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

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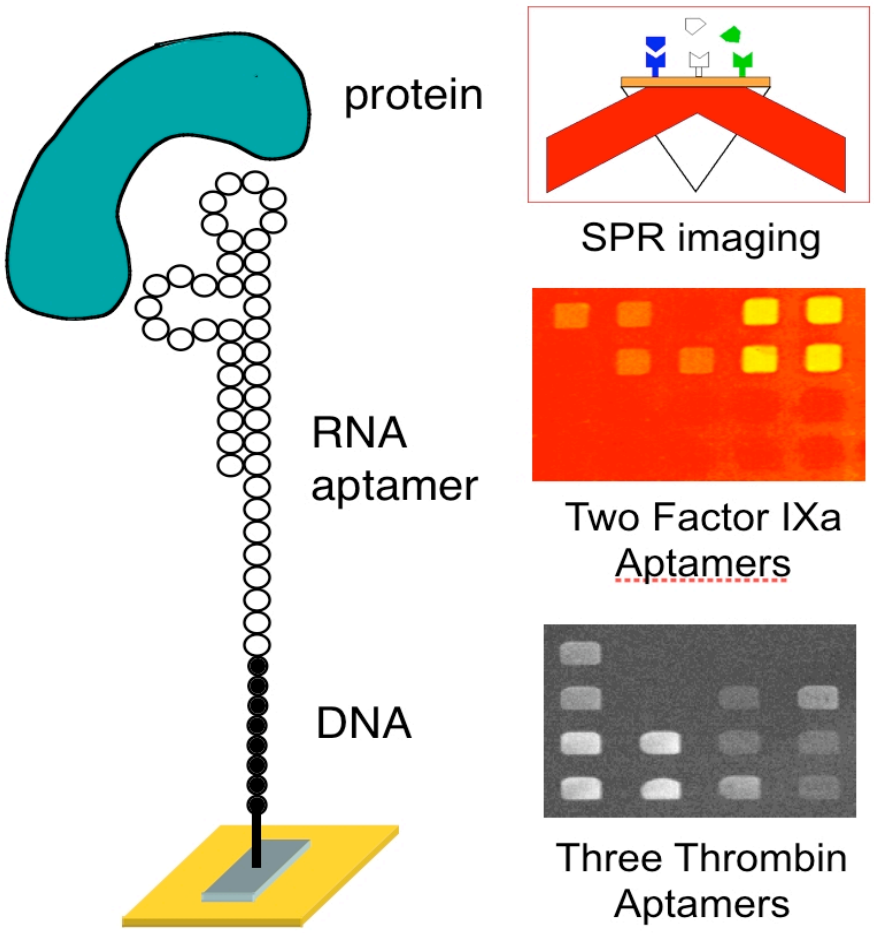
ssRNA Surface Attachment Chemistry: Enzymatic Ligation onto ssDNA

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



SPR imaging

Two Factor IXa Aptamers

Three Thrombin Aptamers

Dr. Ting Nico Hu Seefeld

Surface Enzyme Chemistries:
Attachment, Amplification, Selectivity

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

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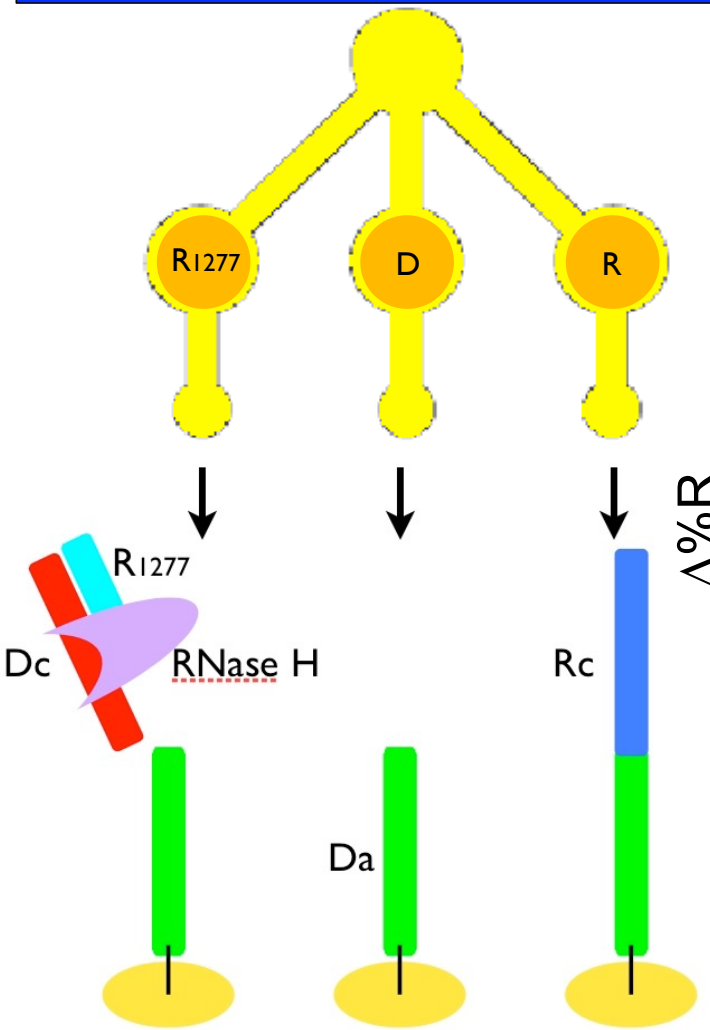
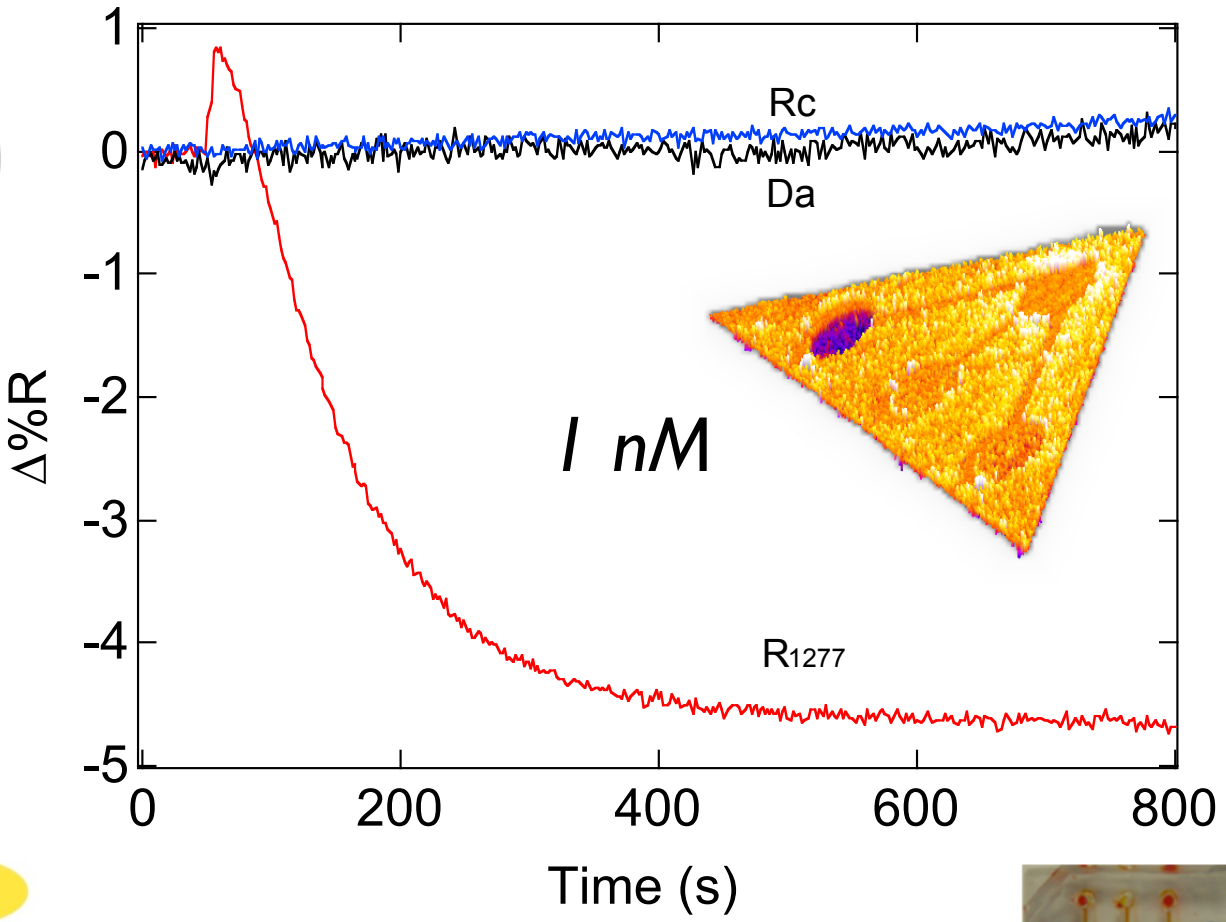
RNA microarrays and RNase H - Target Recycling for DNA detection

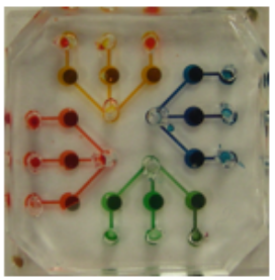


RNase H specifically cuts RNA when in a RNA-DNA duplex.



RNase H activity results in an SPRI reflectivity decrease

3 µL sample compartment



D_a and R_c controls insure no RNase A interference or nonspecific binding.

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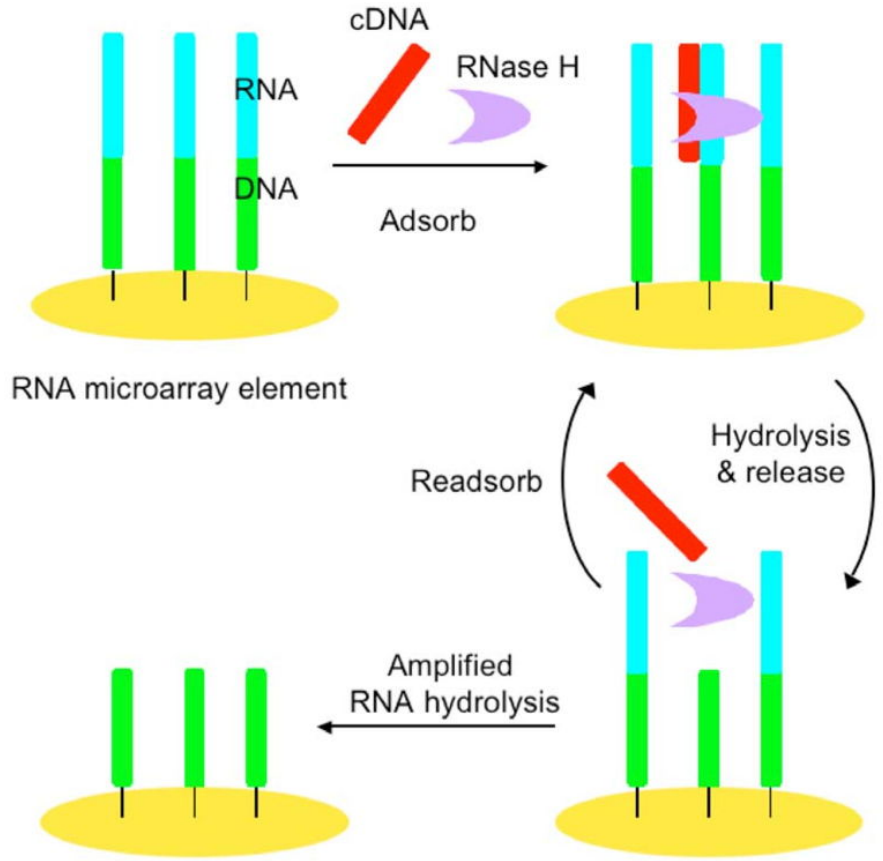
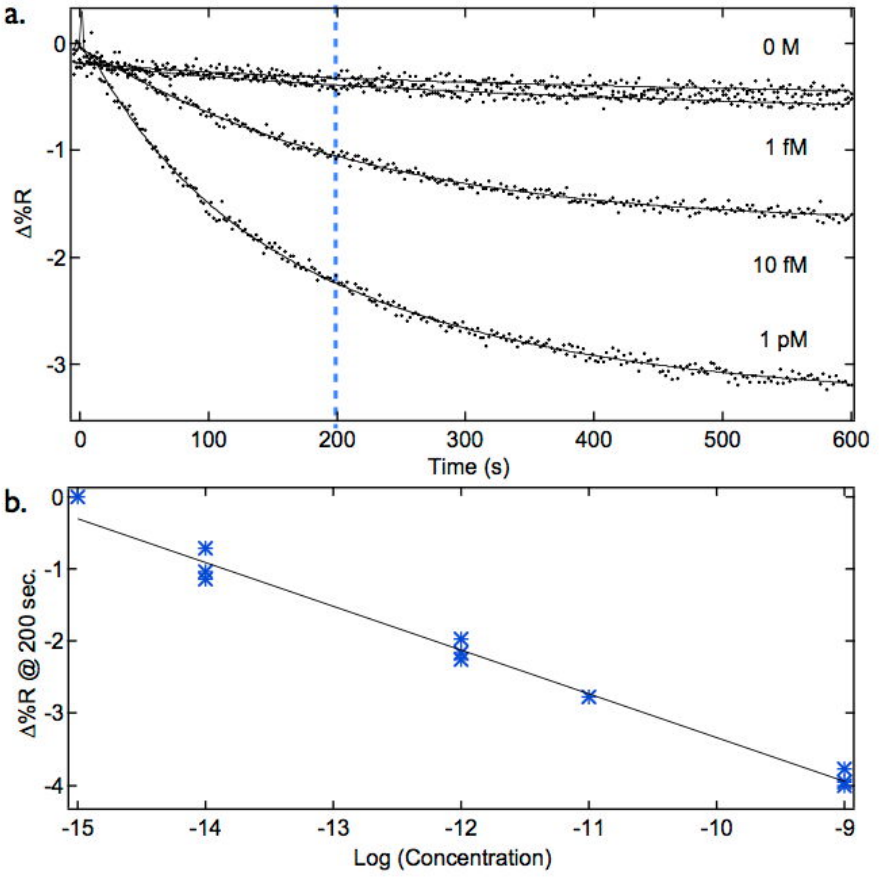
RNA microarrays and RNase H - Target Recycling for DNA detection

i)



ii)



10 fM in 3 μL = 18,000 molecules!

"Target Recycling"

Terry T. Goodrich, Hye Jin Lee, and Robert M. Corn,
"Direct Detection of Genomic DNA by Enzymatically Amplified SPR Imaging Measurements of RNA Microarrays,"
J. Am. Chem. Soc., 126 4086-4087 (2004).

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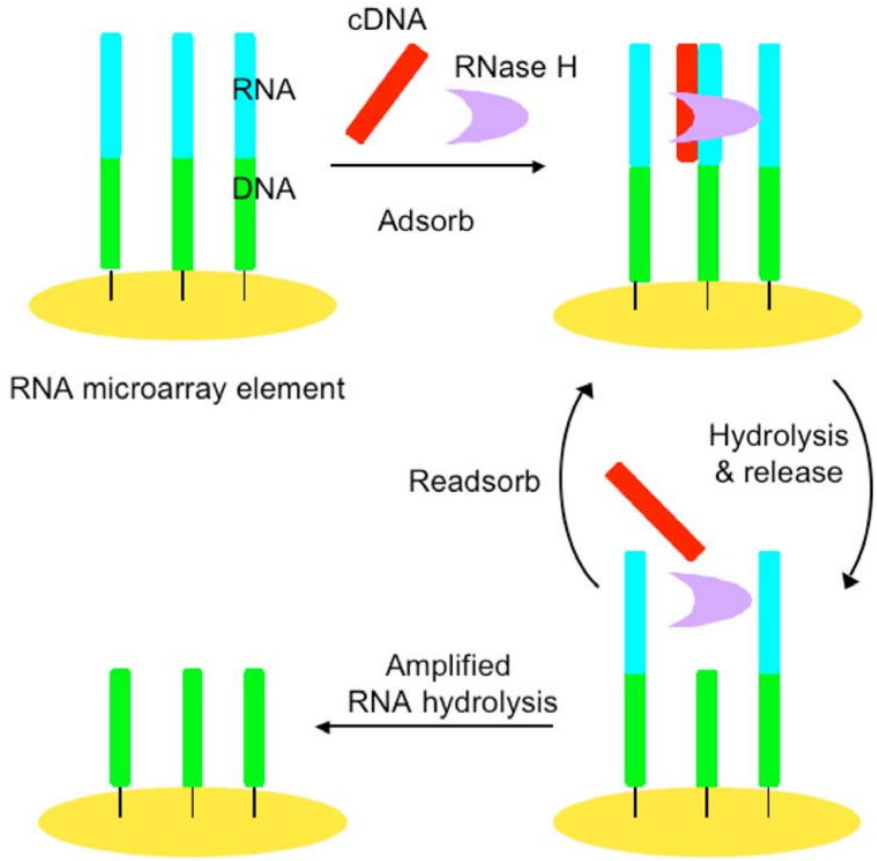
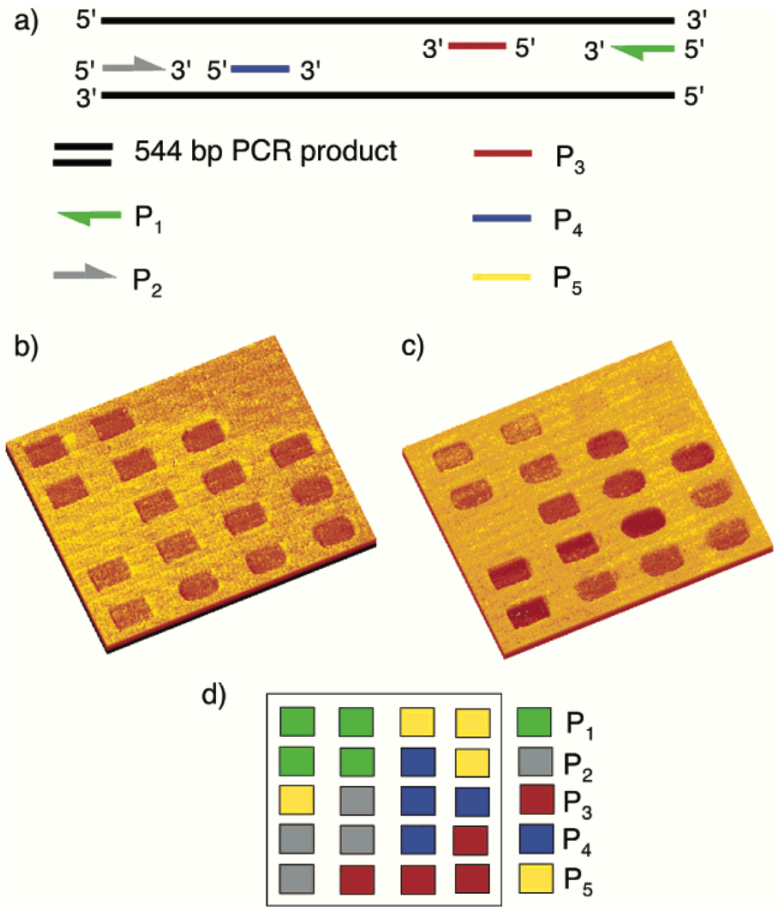
RNA microarrays and RNase H - Target Recycling for DNA detection





Multiplexed Femtomolar ssDNA Detection

Target Recycling = High Fidelity

Terry Terry T. Goodrich, Hye Jin Lee, and Robert M. Corn,
"Enzymatically Amplified Surface Plasmon Resonance Imaging Method Using RNase H and RNA Microarrays for the Ultrasensitive Detection of Nucleic Acids,"
Analytical Chem., **76** 6173-6178 (2004).

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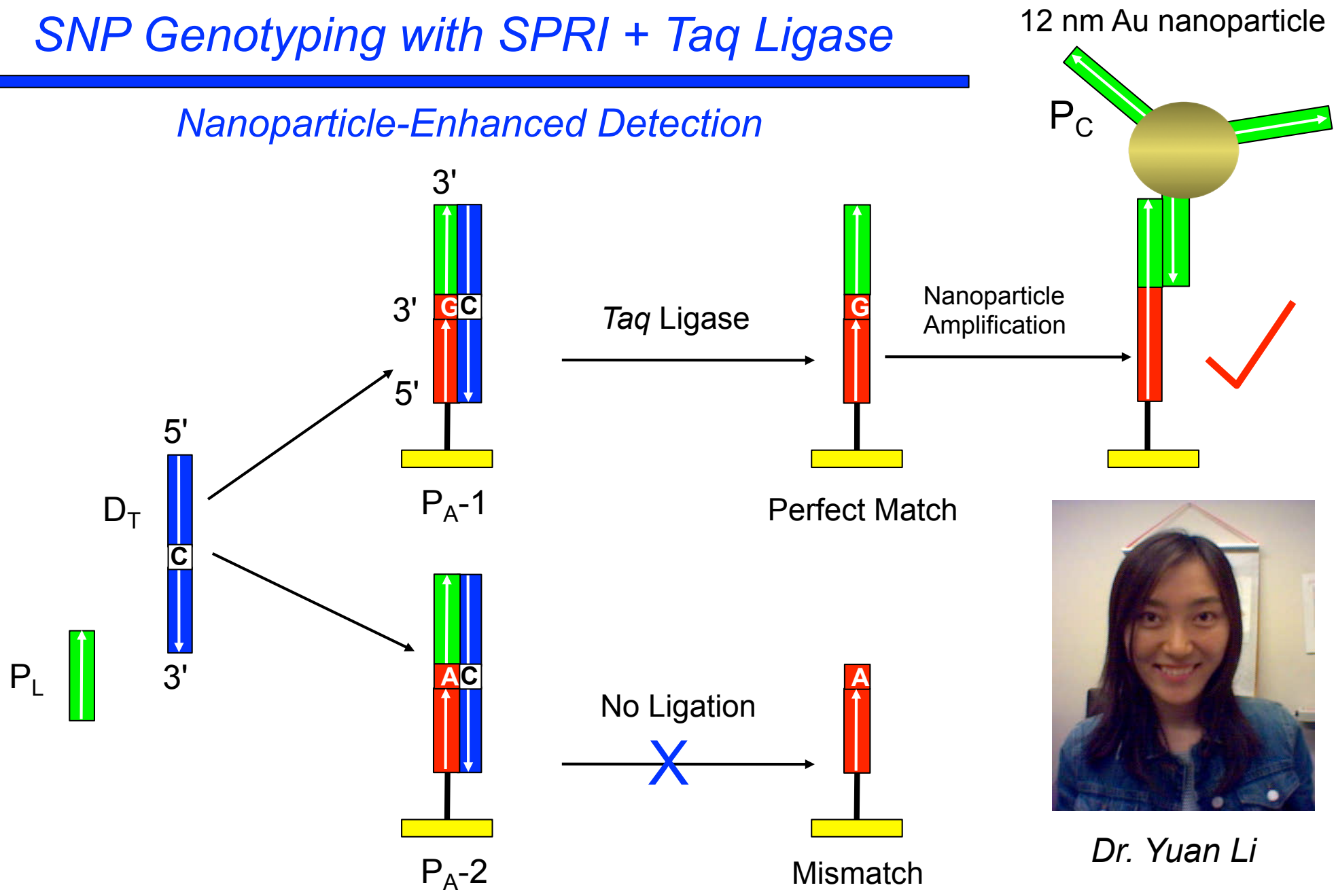
SNP Genotyping with SPRI + Taq Ligase

Nanoparticle-Enhanced Detection



Dr. Yuan Li

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

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BRCA1 Gene of Breast Cancer

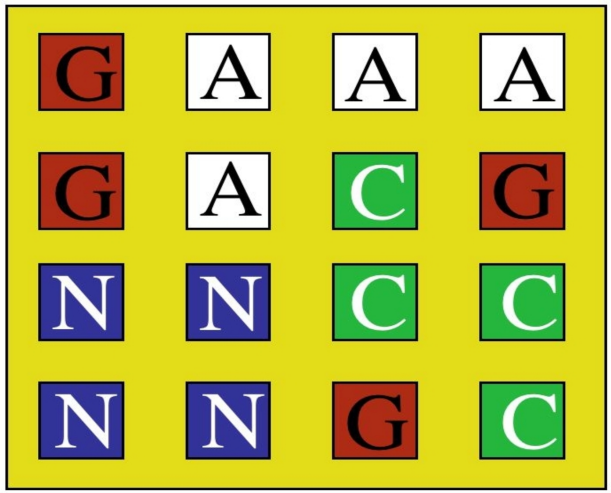
Breast cancer affects one in eight women during their lifetime. Mutations in BRCA1 gene are thought to be responsible for 45% of inherited breast cancer.

Mutations at nucleotide position 4446, codon 1443 located at exon 13

Wild Type: C - C

Arg1443ter: C - T (Coriell sample ID: NA14637)

Arg1443Gly: C - G (Coriell sample ID: NA13710)



Anchor probe sequence

Probe A: 5'-thiol TTT GTT CTG GAT TTC **A** -3'

Probe C: 5'-thiol TTT GTT CTG GAT TTC **C** -3'

Probe G: 5'-thiol TTT GTT CTG GAT TTC **G** -3'

Negative control: 5'-thiol $(T)_{20}$

Top ligation sequence

5'-phos CAG GTC CTC AAG G -3'

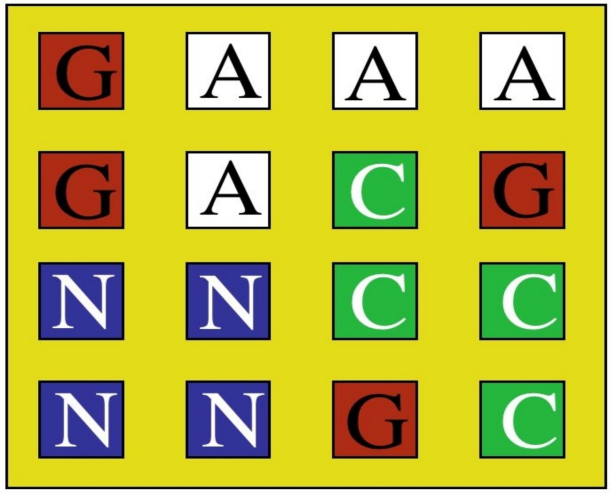
PCR primer for exon 13 of BRCA1:

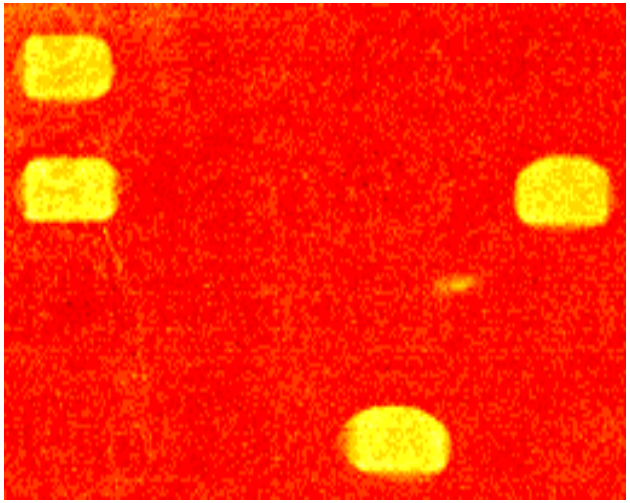
Forward primer: 5'-AAT GGA AAG CTT CTC AAA GTA -3'

Reverse primer: 5'-ATG TTG GAG CTA GGT CCT TAC -3'

T_M = 60 °C; size = 80 bp; 4303 - 4476

SNP Genotyping of a point mutation in the BRCA1 gene





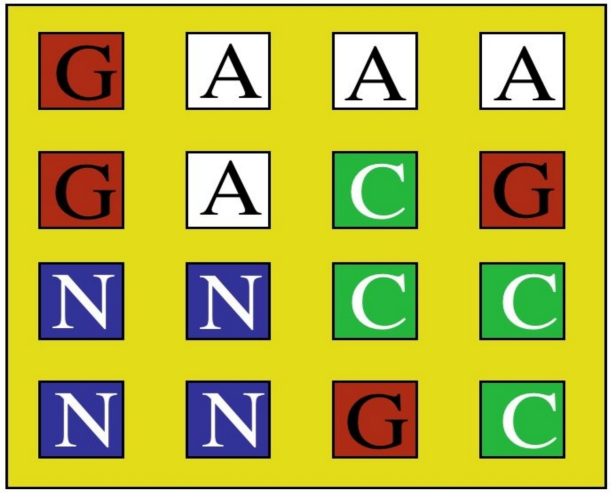
Sample: wild-type

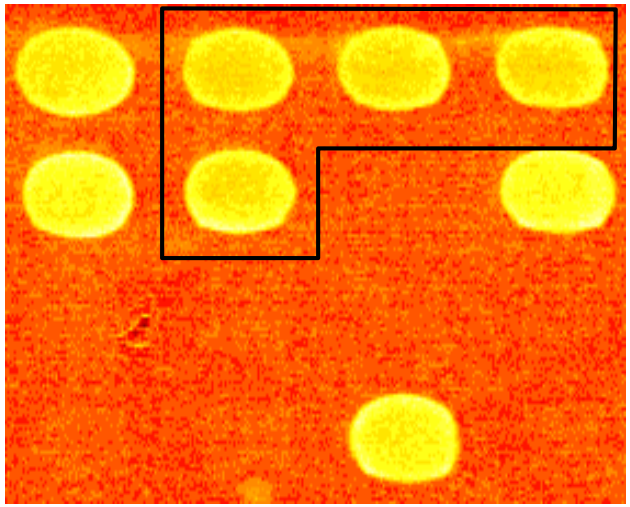
C - C Homozygote

Only G array elements light up.

Negative control insures no nonspecific DNA-enyzme adsorption.

SNP Genotyping of a point mutation in the BRCA1 gene



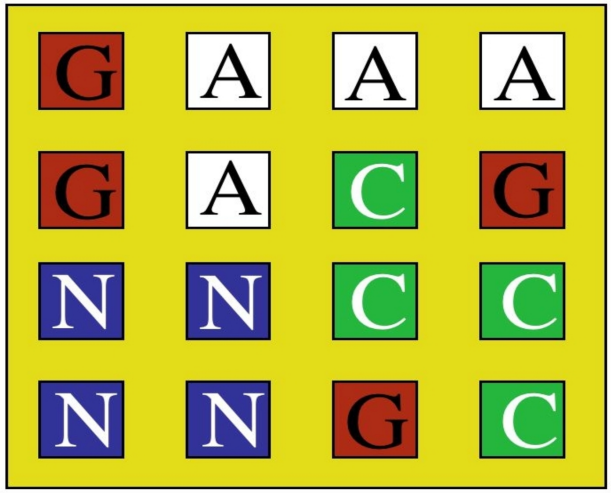


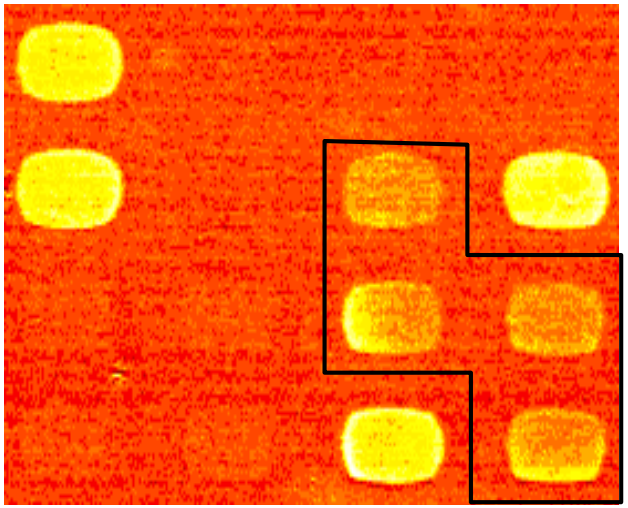
Sample: NA14637

C - T Heterozygote

Both G and A array elements light up.

SNP Genotyping of a point mutation in the BRCA1 gene





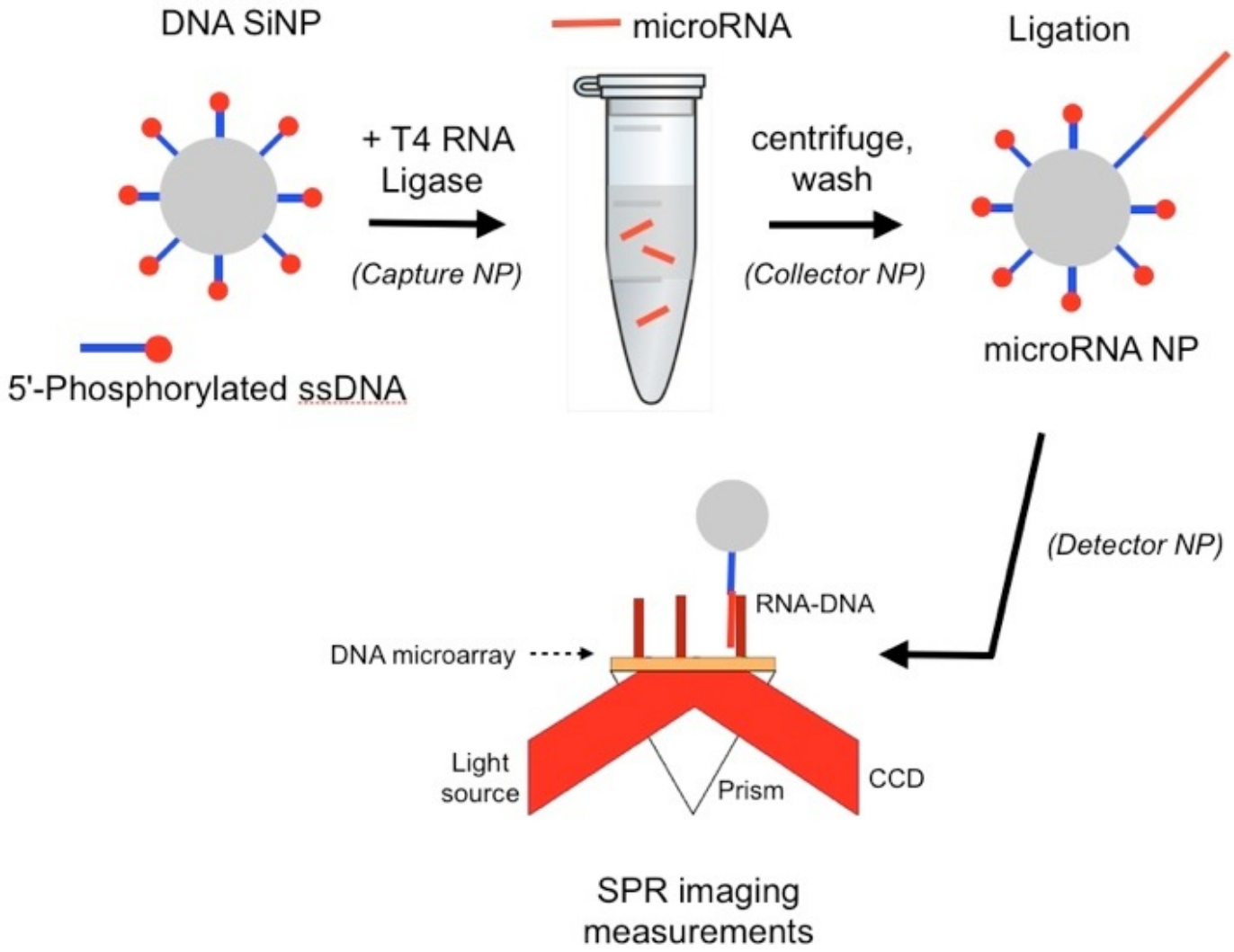
Sample: NA13710

C - G Heterozygote

Both G and C array elements light up.

T4 Ligase MicroRNA Capture + Nanoparticle-Enhanced SPRI

- *Multiplexed MicroRNA Detection with Nanoparticles: Enzymatic 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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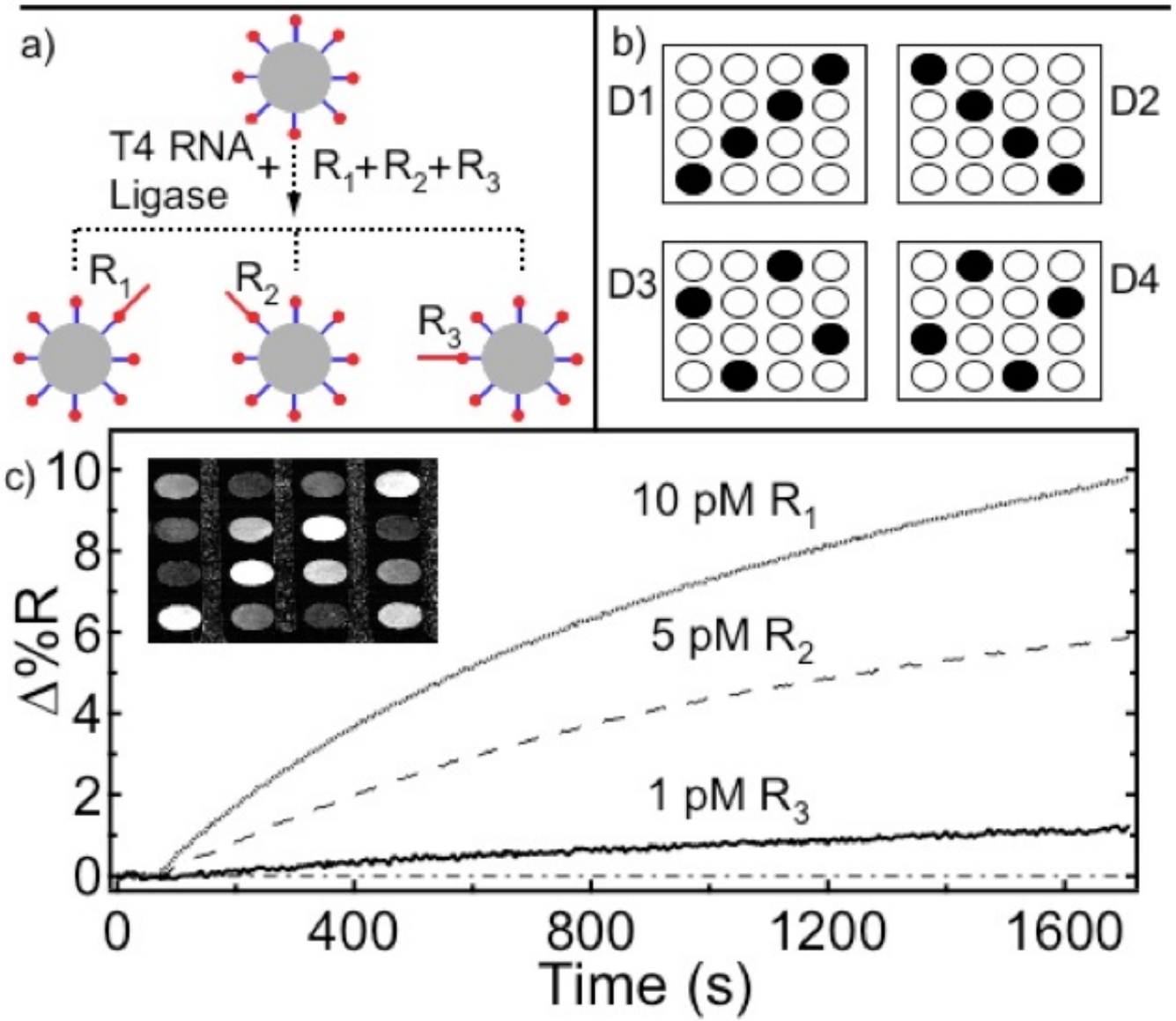
T4 Ligase MicroRNA Capture + Nanoparticle-Enhanced SPRI



Dr. Yulin Chen

Dr. WenJuan Zhou

Multiplexed MicroRNA Detection!

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

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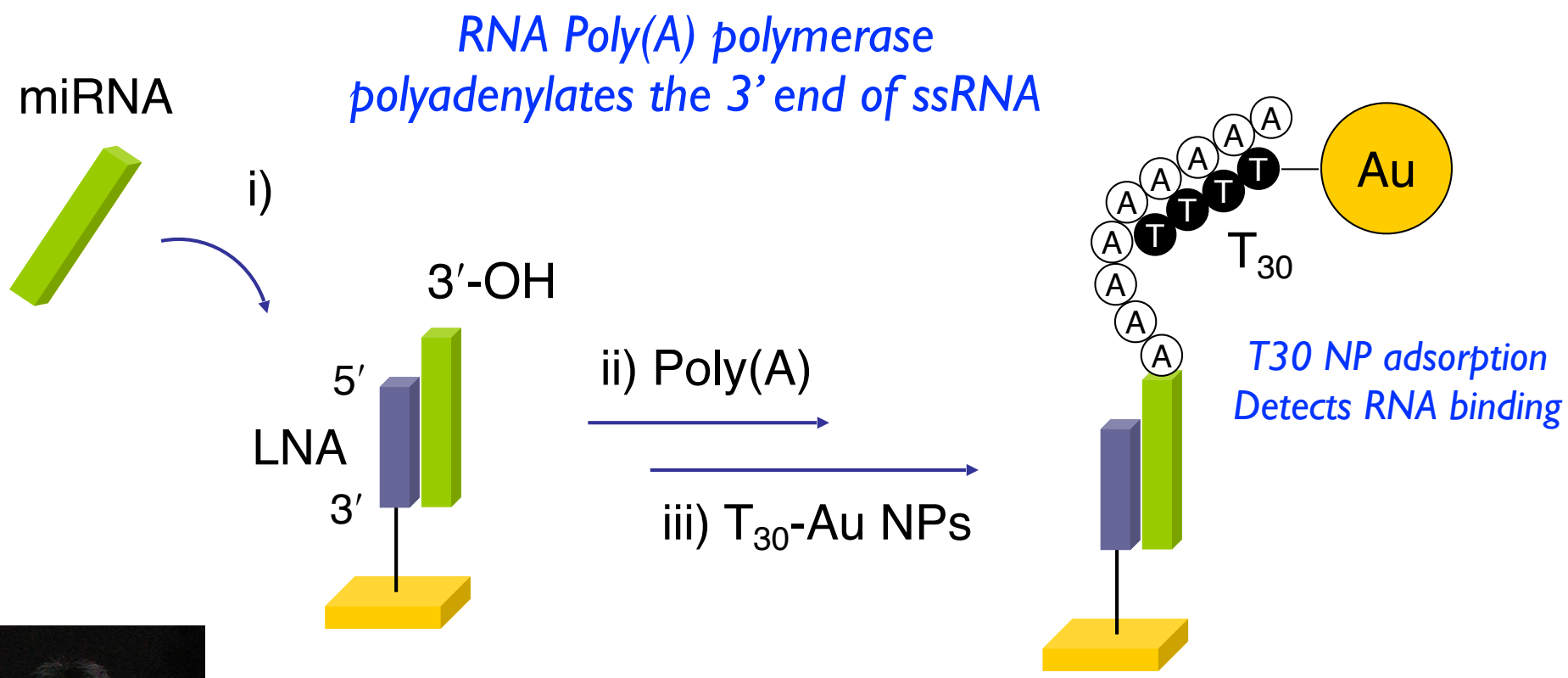
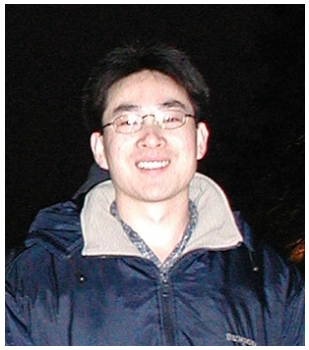
Ultrasensitive MicroRNA Biosensing with RNA Poly(A) polymerase





Dr. Shiping Fang

Femtomolar MicroRNA detection with enzymatic amplification and nanoparticle adsorption

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

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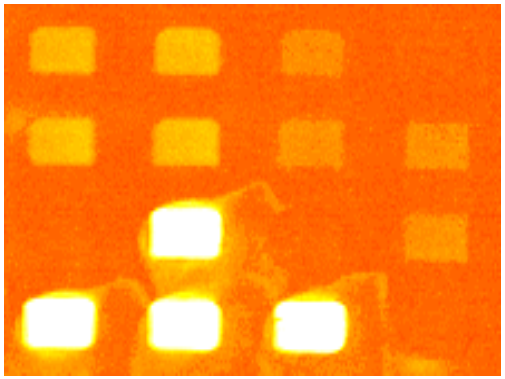
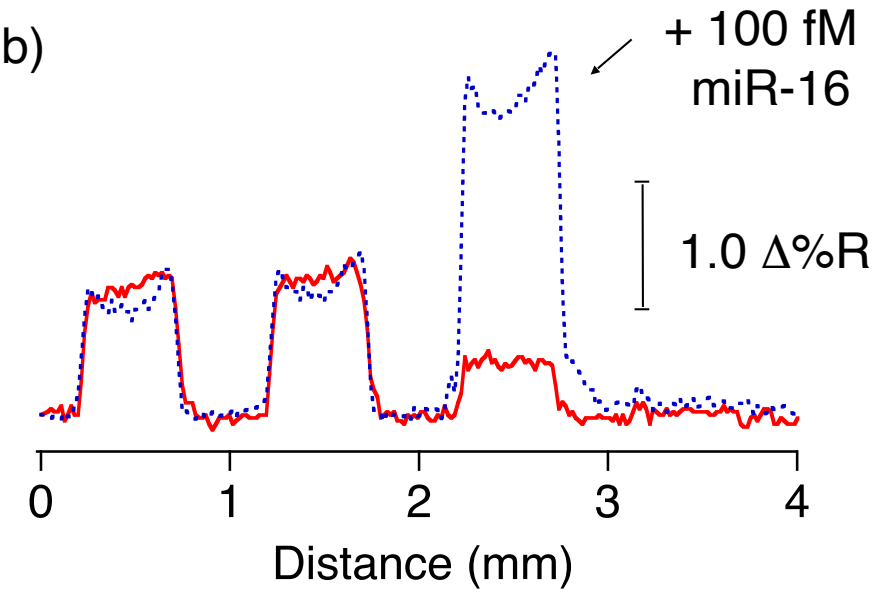
Surface Enzyme Chemistry + Nanoparticle-Enhanced SPRI

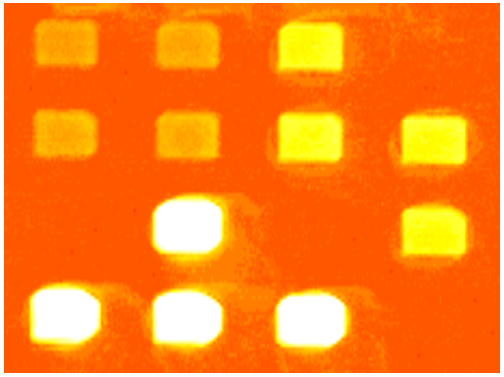
a) 250 ng total RNA



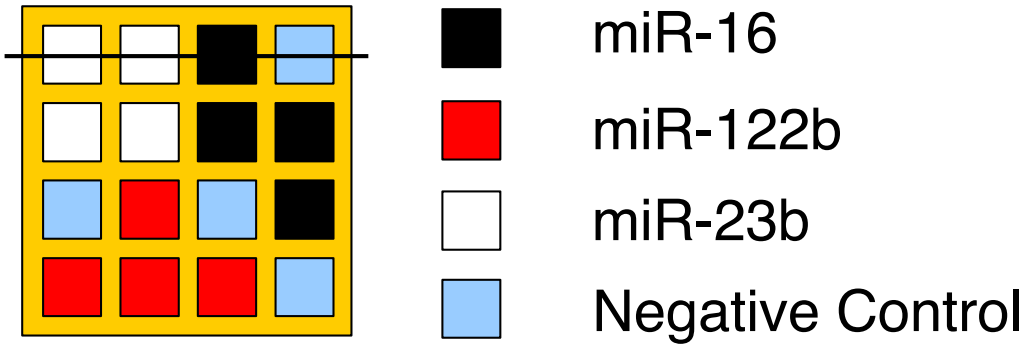
b)



c) 250 ng total RNA
+ 100 fM miR-16



d)



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

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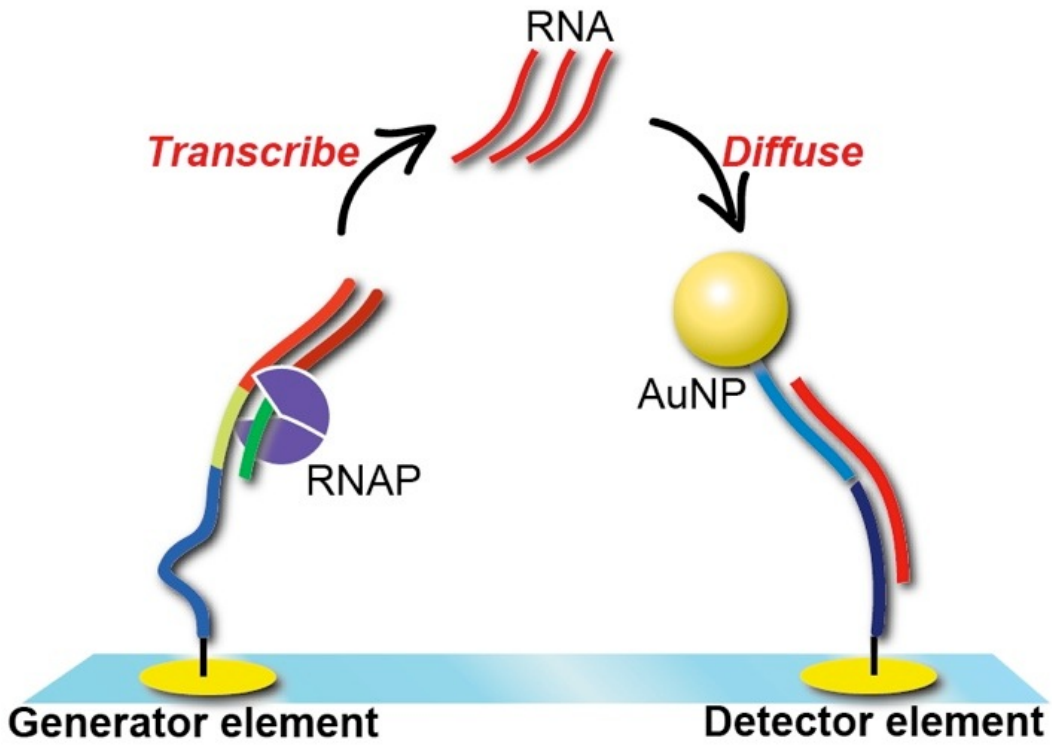
T7 RNA Polymerase Amplification + Nanoparticle-Enhanced SPRI



Dr. Iuliana Sendroiu

T7 RNA Polymerase reaction makes multiple RNA copies

Nanoparticle Adsorption Detects RNA binding

1 fM in 25µL with SPRI

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

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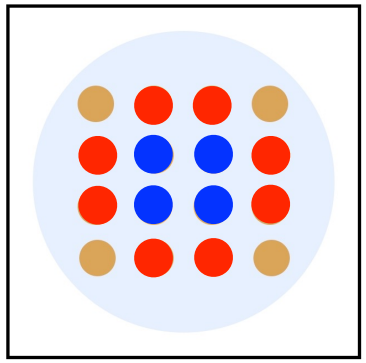
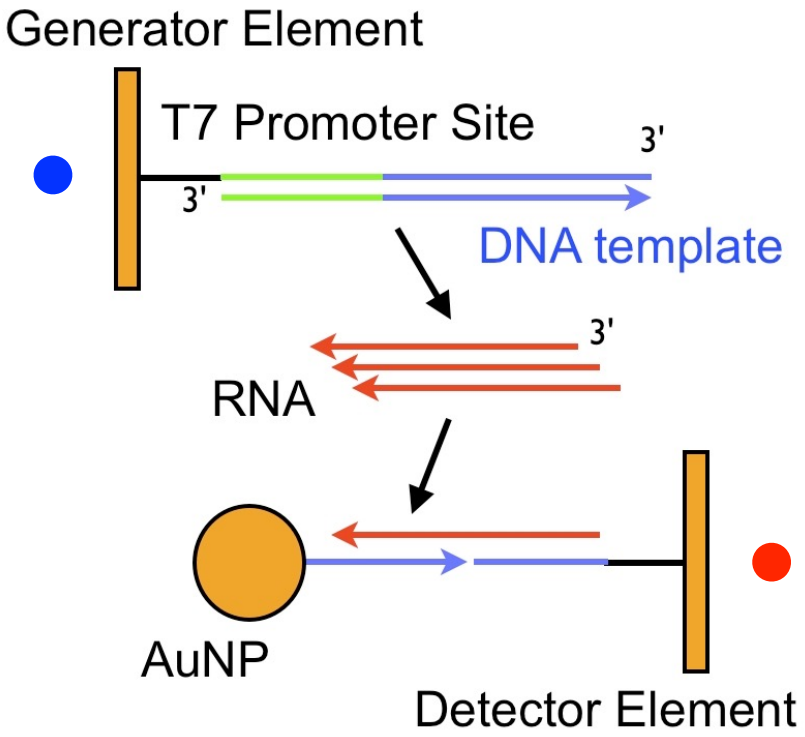
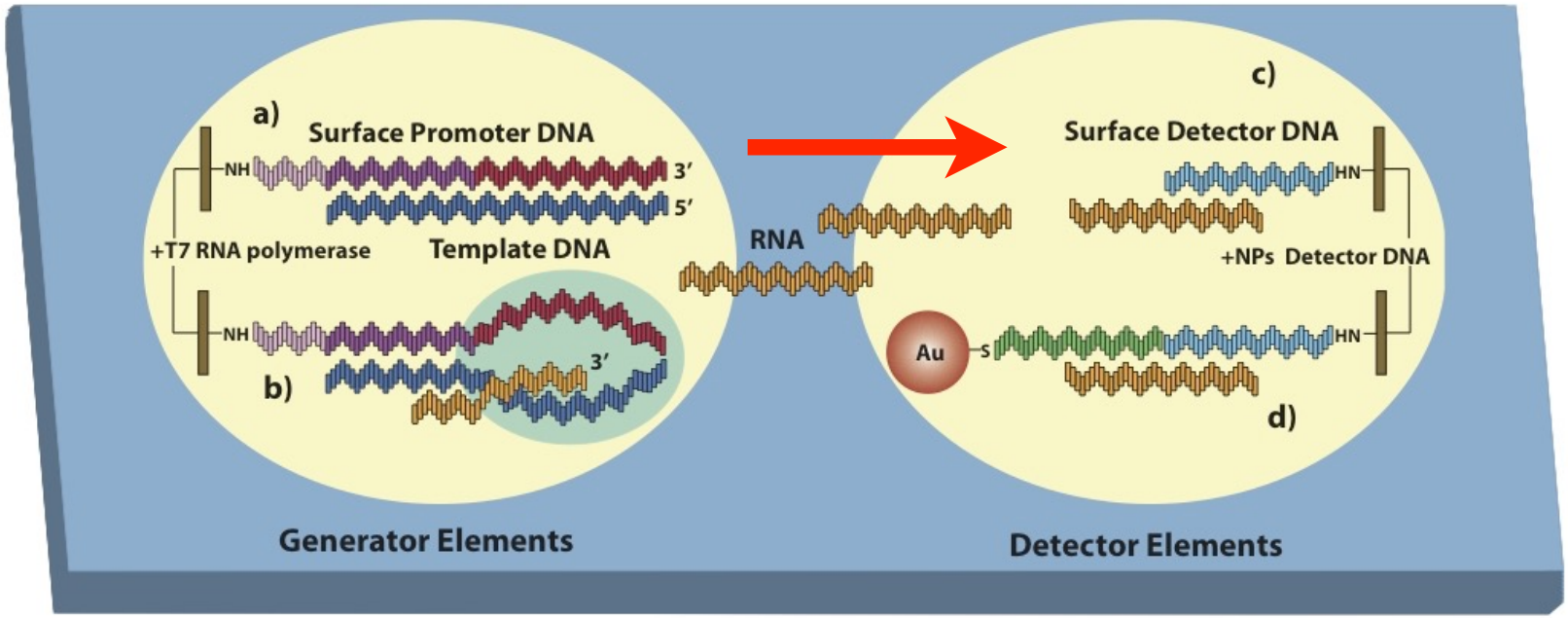
Detection of DNA with RNA Polymerase Surface Transcription and Nanoparticle-Enhanced SPRI

25 µL of DNA



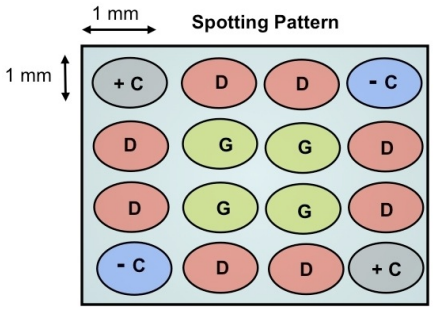
Surface microarray

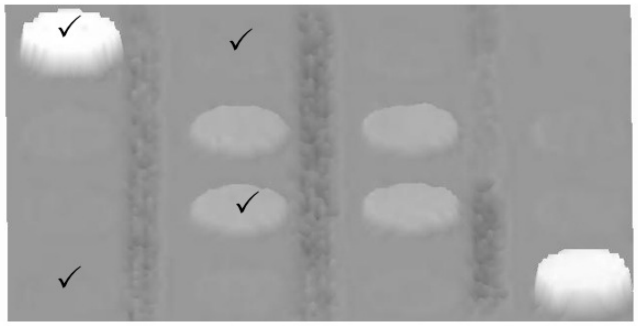




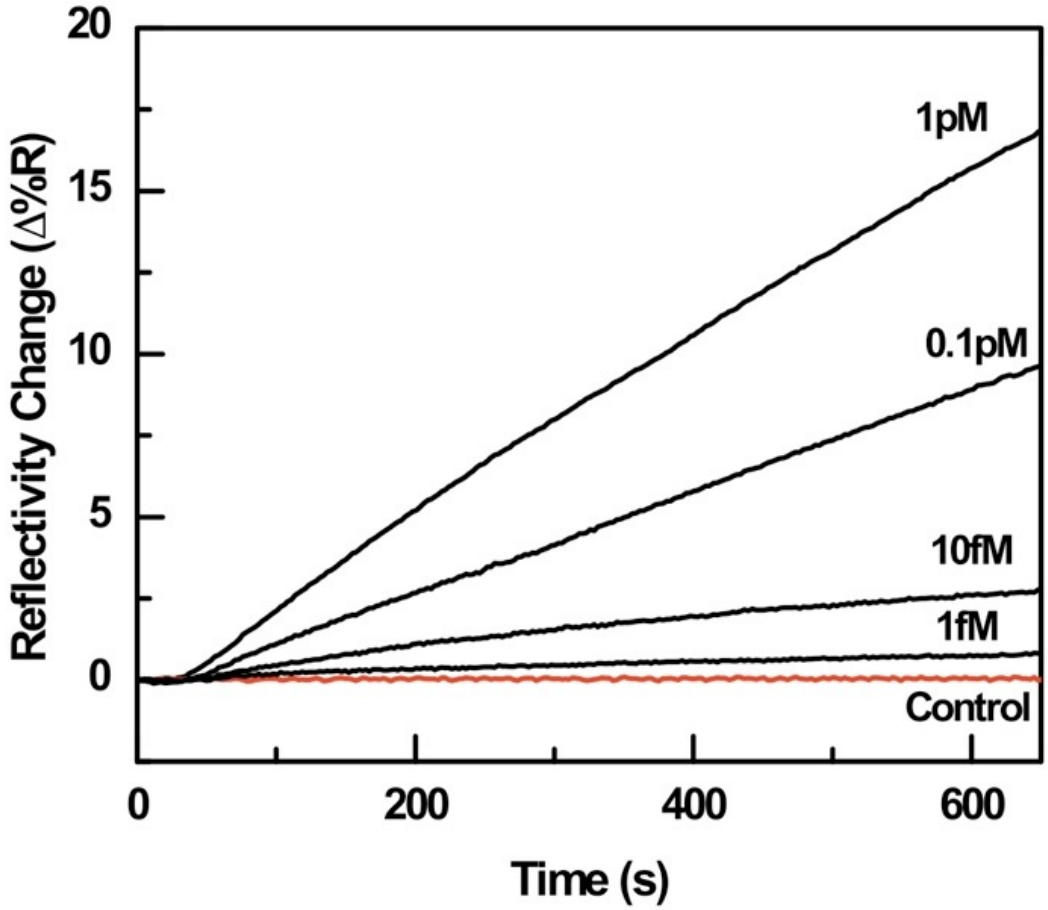
I. Sendroiu et al., JACS, **133** 4271-4273 (2011).

T7 RNA Polymerase Amplification + Nanoparticle-Enhanced SPRI





Control, No Template DNA



25 µL of 1 fM DNA = 25 zeptomoles or 15,000 molecules!

1 attomole of DNA produces 2 femtomoles of RNA

I. Sendroiu et al., JACS, **133** 4271-4273 (2011).

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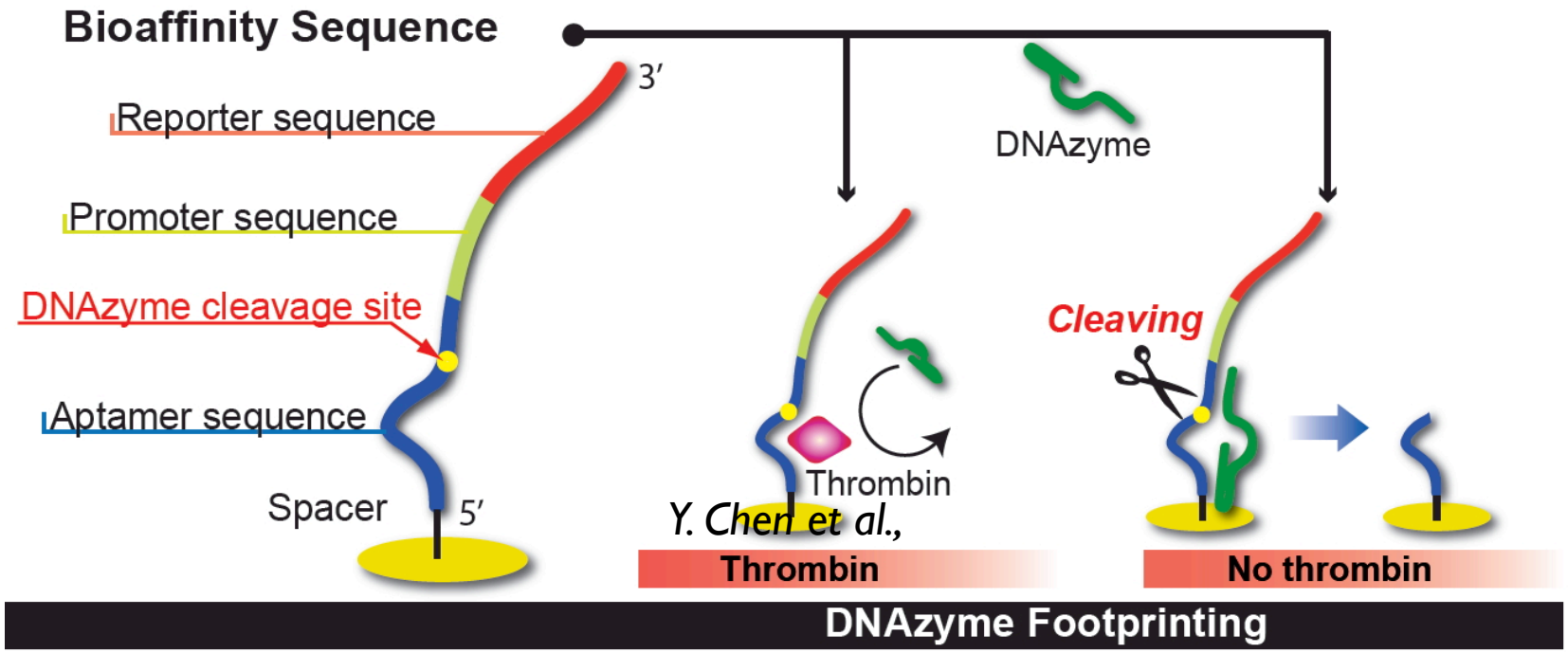
• DNAzyme Footprinting on Surfaces



Detecting Protein-Aptamer Complexation on Surfaces by Blocking DNAzyme Cleavage Activity

Y. Chen and R. M. Corn, J. Am. Chem. Soc., ***135*** *2072-2075 (2013).*

Dr. Yulin Chen

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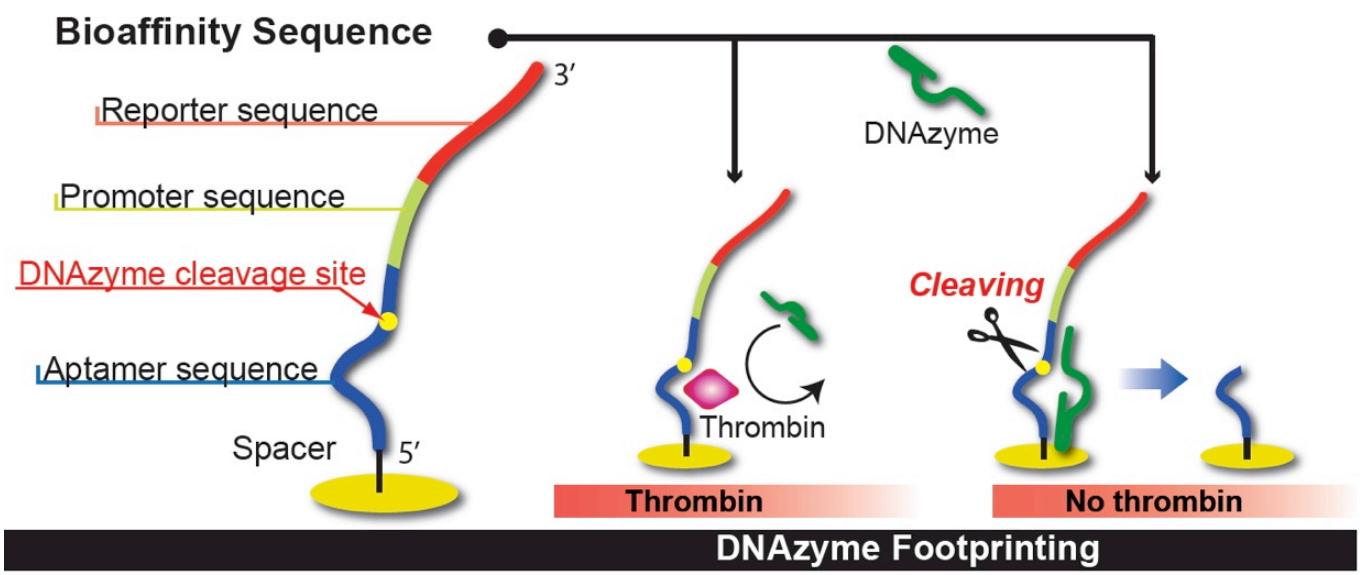
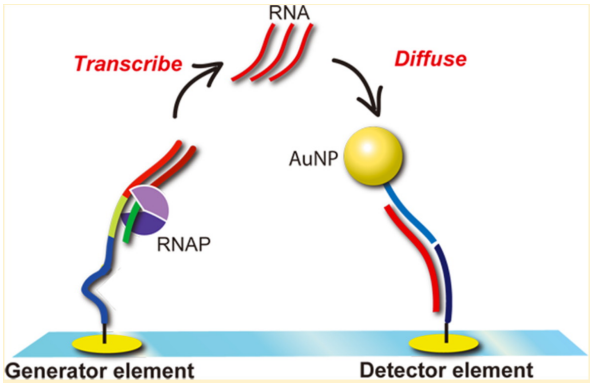
DNAzyme Footprinting on Surfaces: Enzymatically Amplified Protein Detection



Surface RNA Polymerase Reaction: RNA-amplified Detection



50 fM detection of thrombin

Y. Chen and R. M. Corn, J. Am. Chem. Soc., ***135*** *2072-2075 (2013).*

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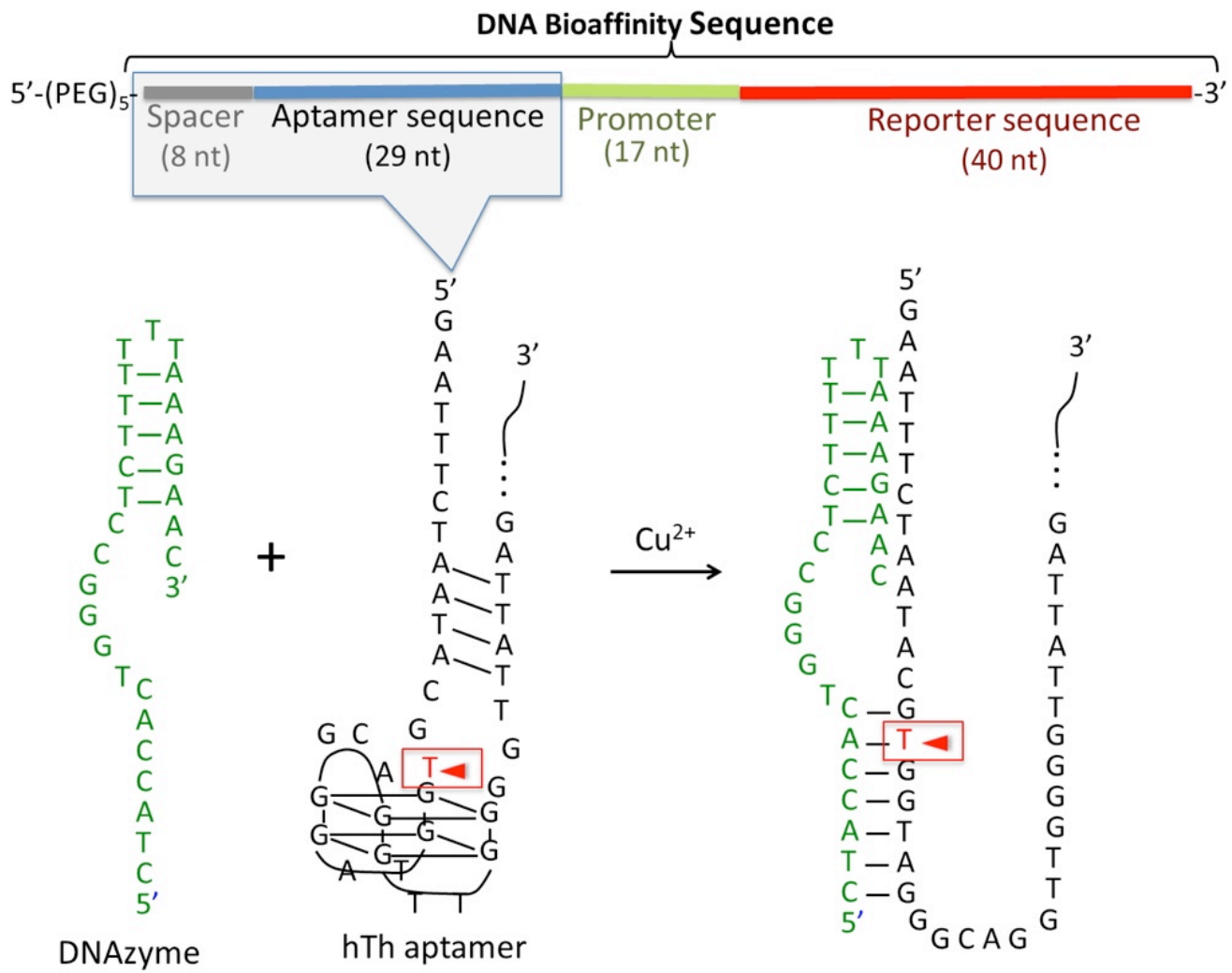
• DNAzyme Footprinting on Surfaces



Dr. Yulin Chen

Y. Chen and R. M. Corn, J. Am. Chem. Soc., ***135*** *2072-2075 (2013).*

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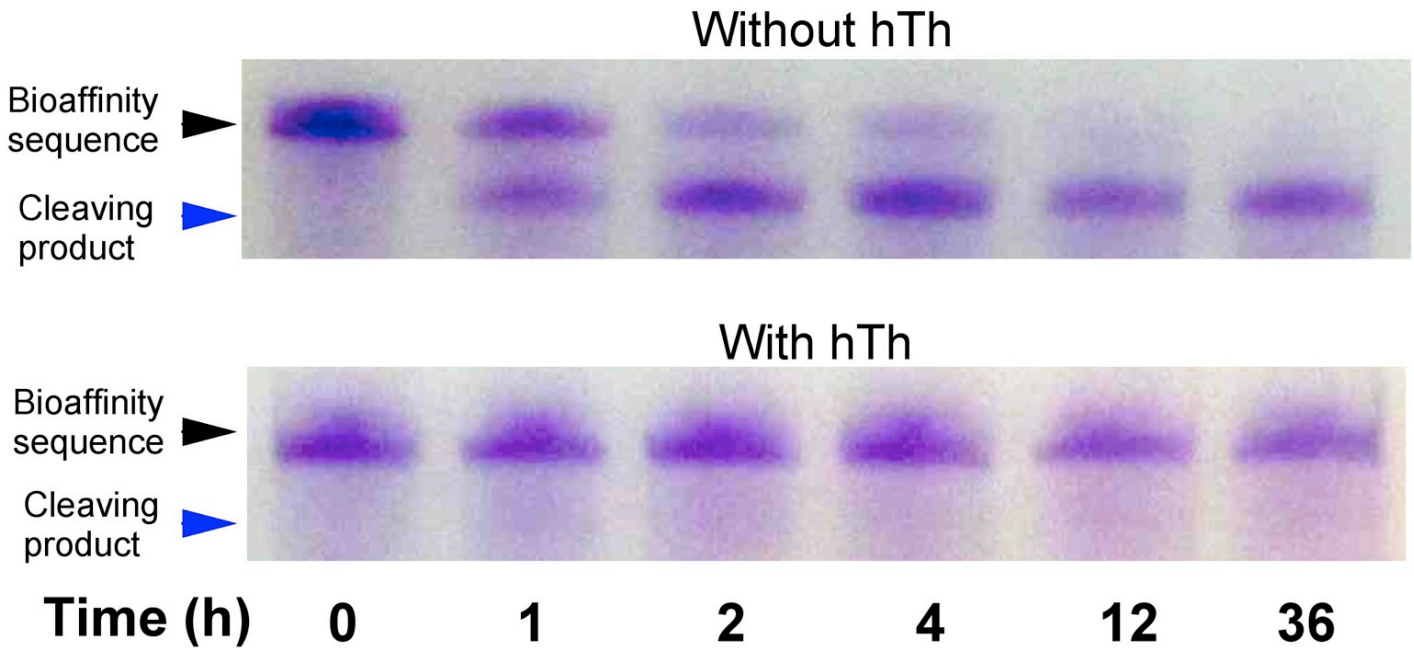
• *DNAzyme Footprinting on Surfaces*



Dr. Yulin Chen

Y. Chen and R. M. Corn, J. Am. Chem. Soc., ***135*** *2072-2075 (2013).*

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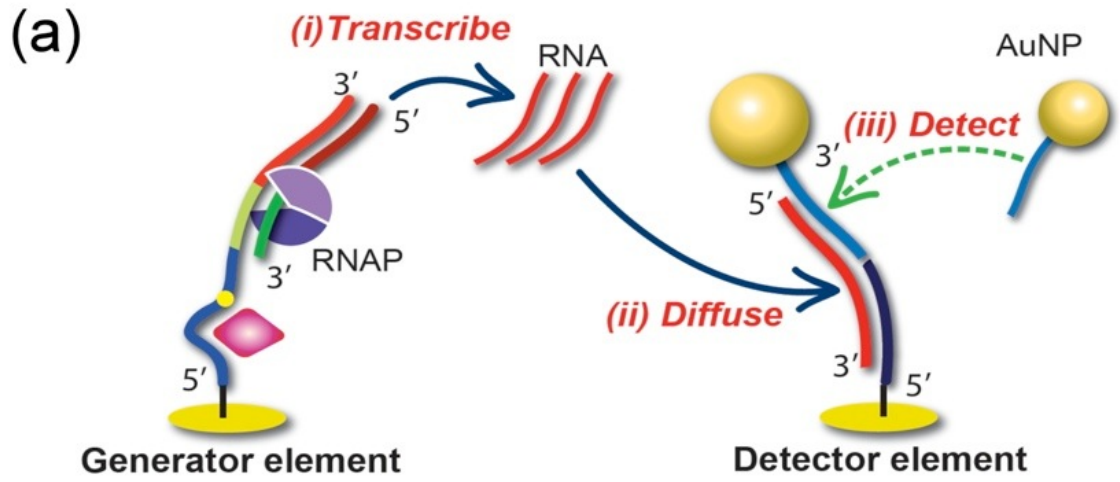
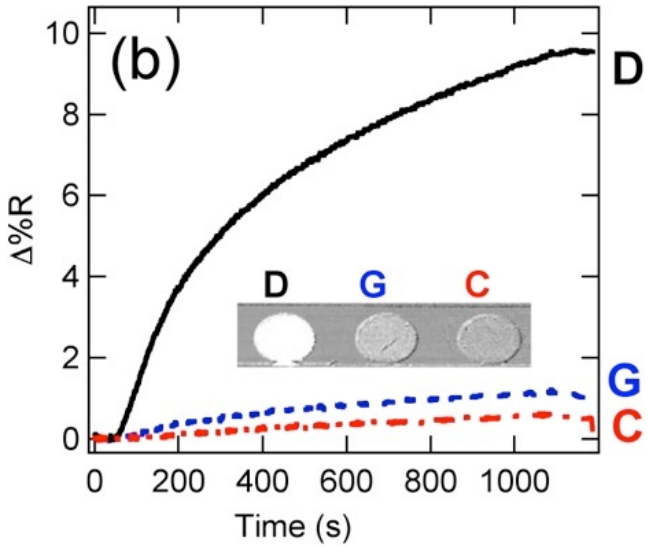
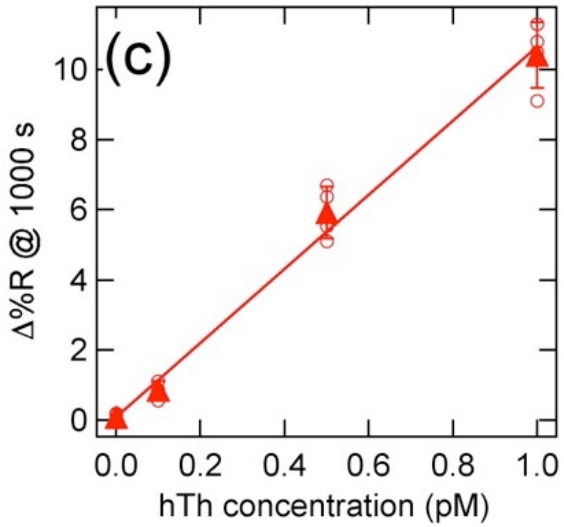
DNAzyme Footprinting on Surfaces: Enzymatically Amplified Protein Detection



Dr. Yulin Chen





50 fM detection of thrombin

Surface RNA Polymerase Reaction: RNA-amplified Detection

Y. Chen and R. M. Corn, J. Am. Chem. Soc., ***135*** *2072-2075 (2013).*

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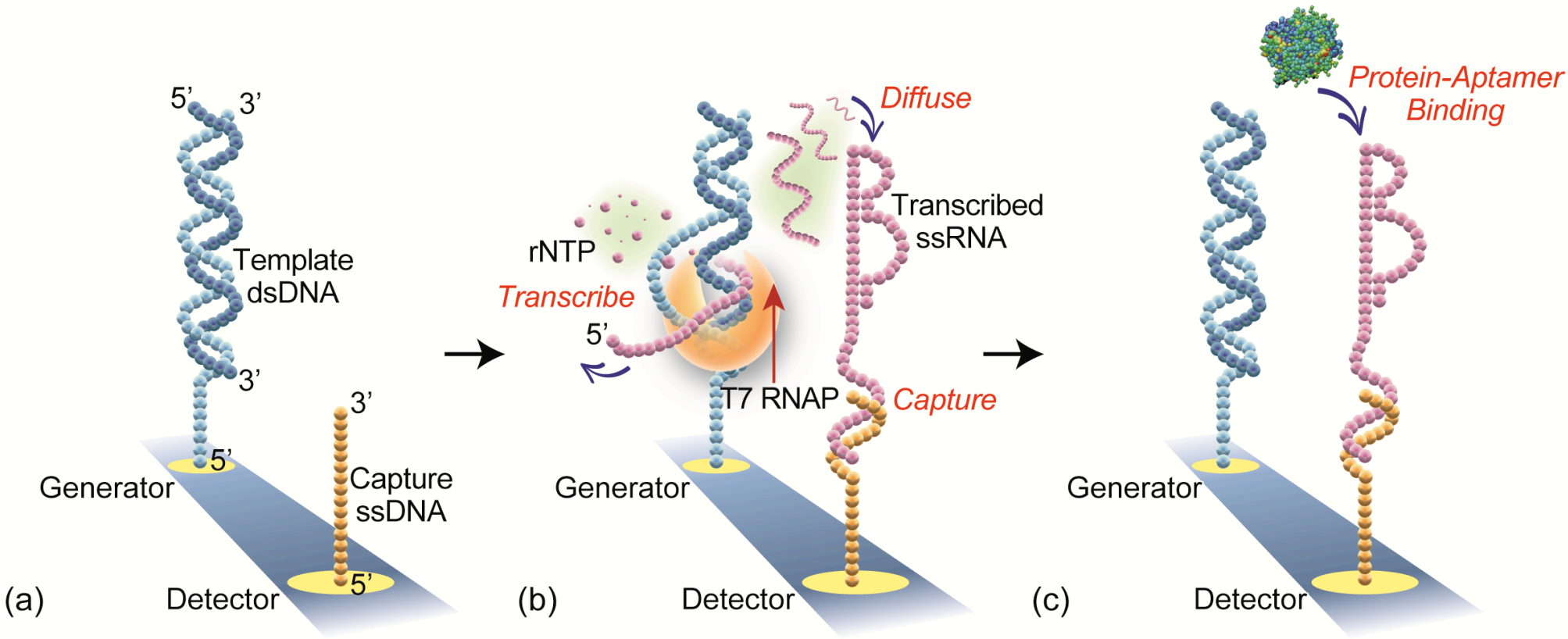
On-Chip Synthesis of RNA Aptamer Microarrays for Protein Biosensing



Dr. Yulin Chen

RNA Polymerase surface transcription followed by DNA hybridization capture

Y. Chen, K. Nakamoto, O. Niwa and R. M. Corn,
"On-Chip Synthesis of RNA Aptamer Microarrays for Multiplexed Protein Biosensing with SPR Imaging Measurements," Langmuir, 28 8281-8285 (2012).

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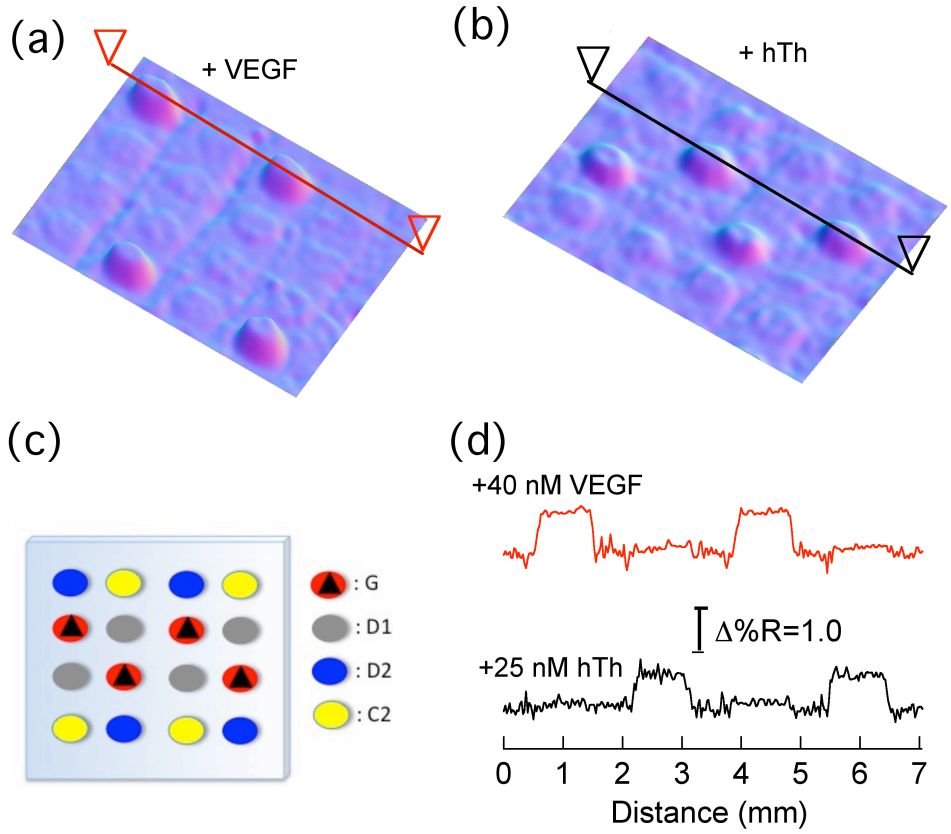
On-Chip Synthesis of RNA Aptamer Microarrays for Protein Biosensing



A microarray with two aptamers: VEGF and thrombin

Dr. Yulin Chen

RNA Polymerase surface transcription followed by DNA hybridization capture

Y. Chen, K. Nakamoto, O. Niwa and R. M. Corn, "On-Chip Synthesis of RNA Aptamer Microarrays for Multiplexed Protein Biosensing with SPR Imaging Measurements," Langmuir, 28 8281-8285 (2012).

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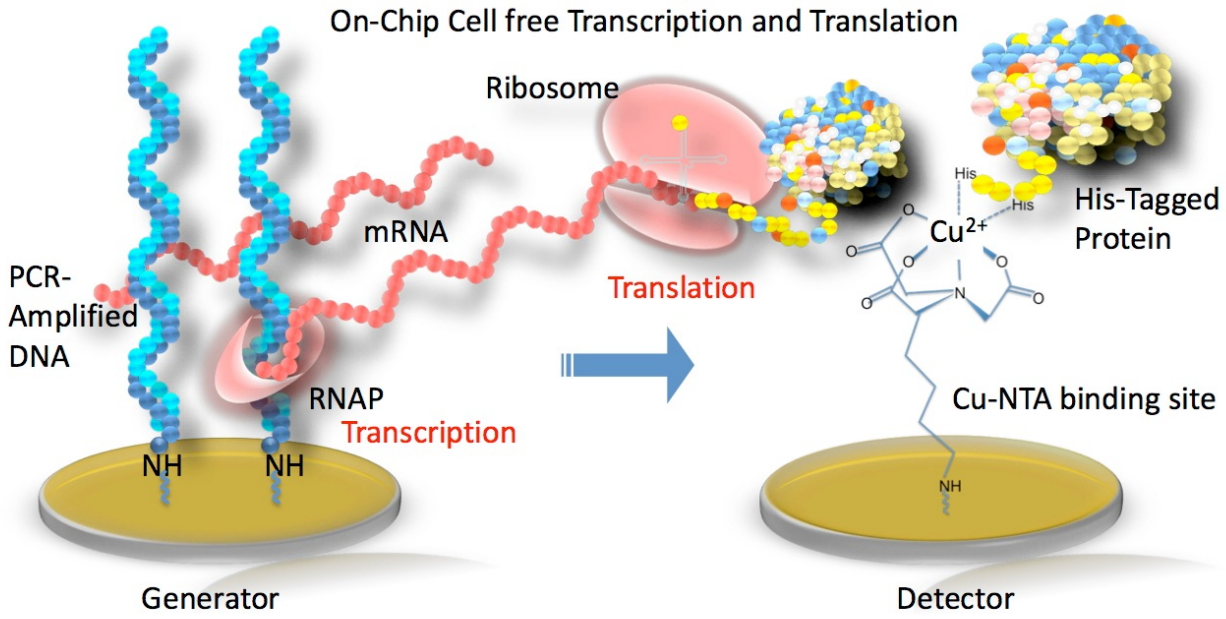
On-Chip Synthesis of Protein Microarrays from DNA Microarrays



Dr. Ting Hu (Nico)

On-Chip IVTT Synthesis; Histag Capture

T. H. Seefeld, A. R. Halpern and R. M. Corn,
"On-Chip Synthesis of Protein Microarrays from DNA Microarrays Via Coupled In Vitro Transcription and Translation for Surface Plasmon Resonance Imaging Biosensor Applications"
J. Am. Chem. Soc., 134 12358-12361 (2012).

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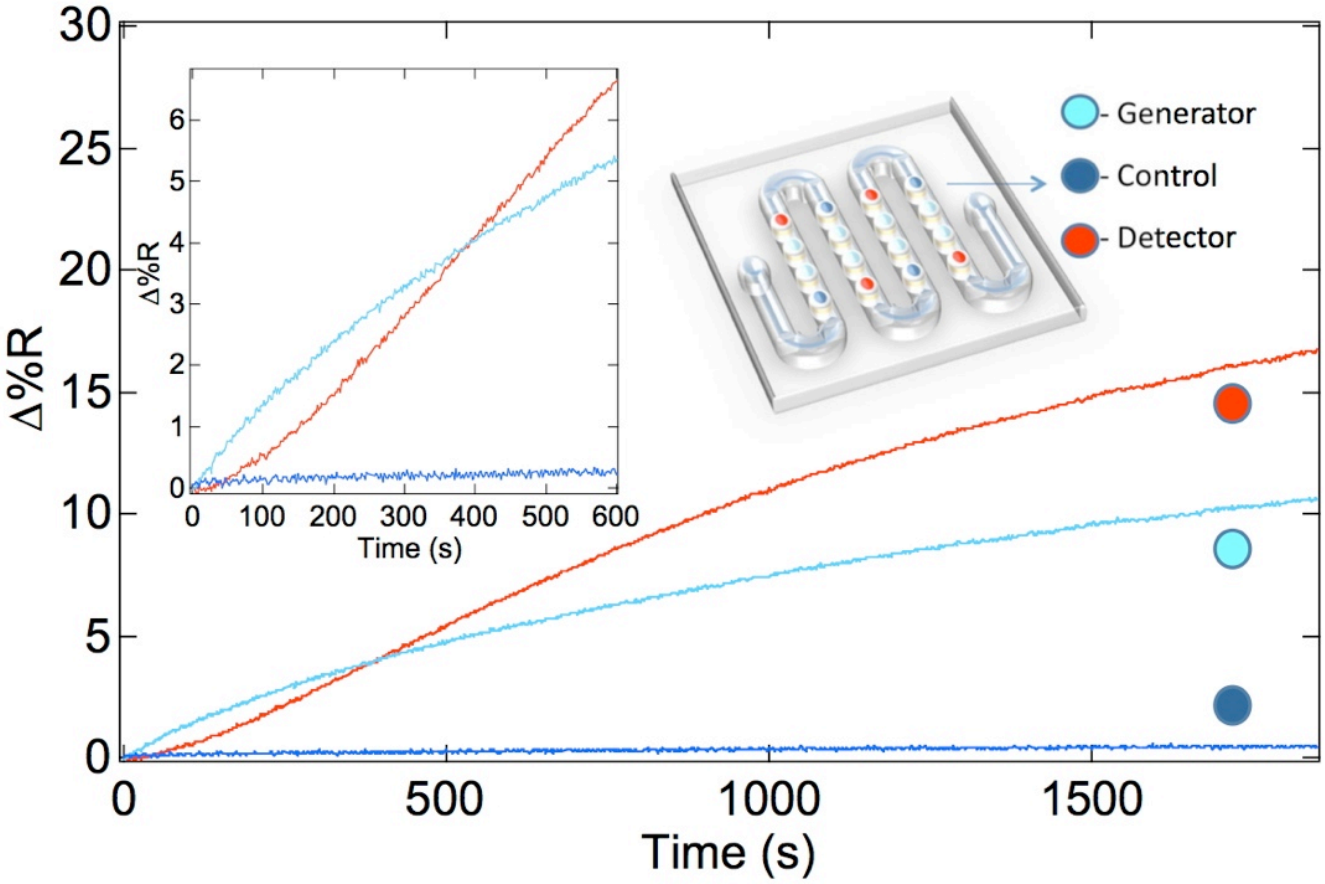
University of California, Irvine

• On-Chip Synthesis of Protein Microarrays from DNA Microarrays

Real-time SPRI of On-chip GFP Biosynthesis and Capture



Dr. Ting Hu (Nico)

Ting H. Seefeld et al., J. Am. Chem. Soc., ***134*** *12358-12361 (2012).*

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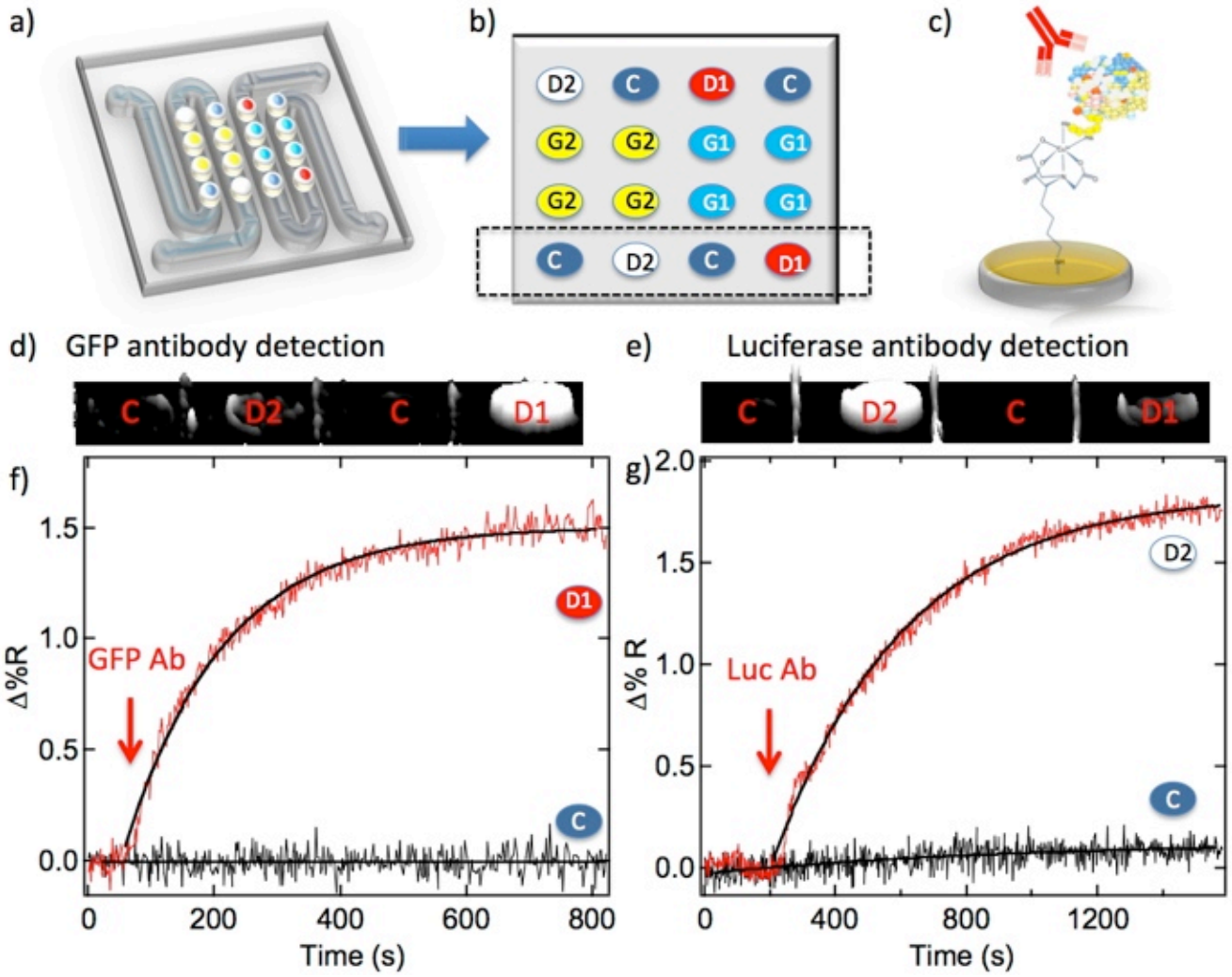
University of California, Irvine

• On-Chip Synthesis of Protein Microarrays from DNA Microarrays

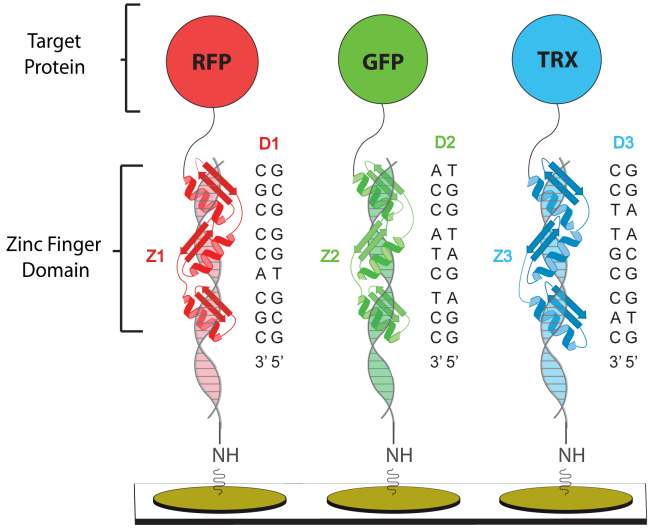


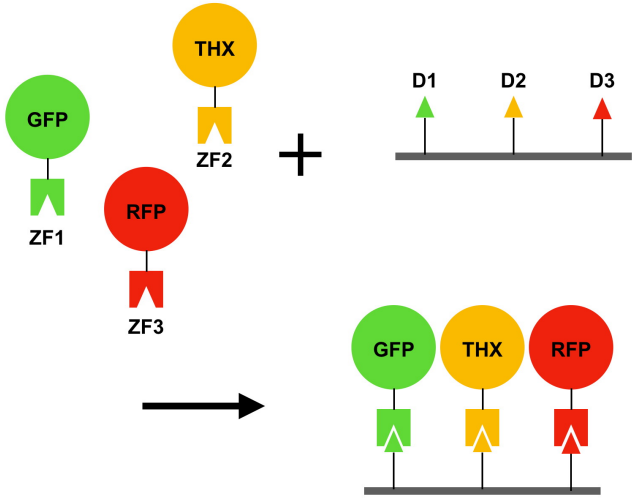
On-chip Biosynthesis of GFP and Luciferase

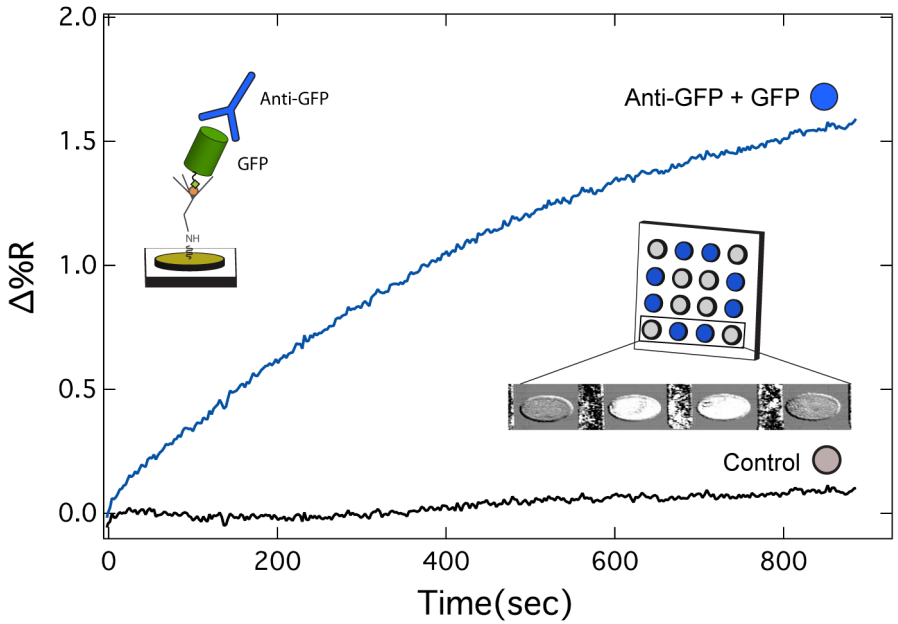
Dr. Ting Hu (Nico)

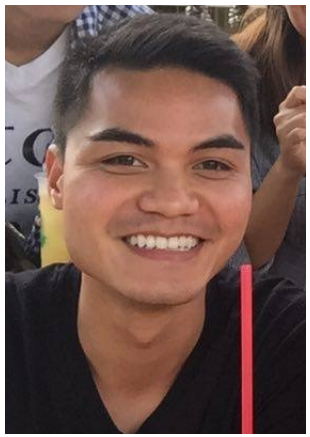
SPRI Detection of Anti-GFP and Anti-Luciferase

Self-Assembled Protein Microarrays: Zn Finger-directed microarray self assembly









Dr. Gerald Manuel

G. Manuel, A. Lupták, and R. M. Corn, J. Phys. Chem. C, 120 20984-20990 (2016).

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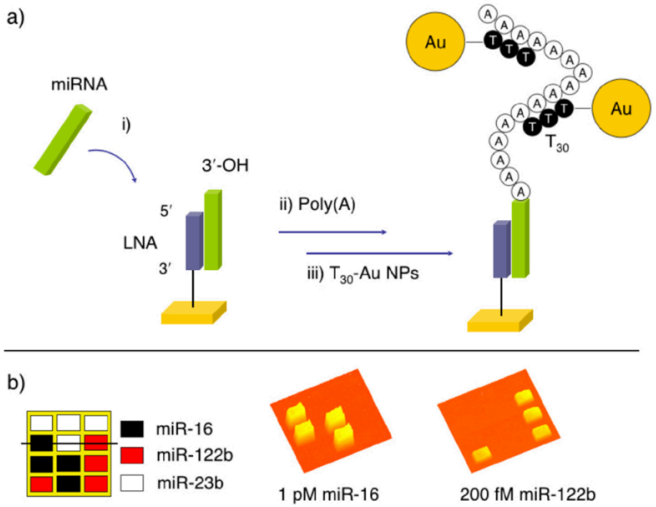
University of California, Irvine

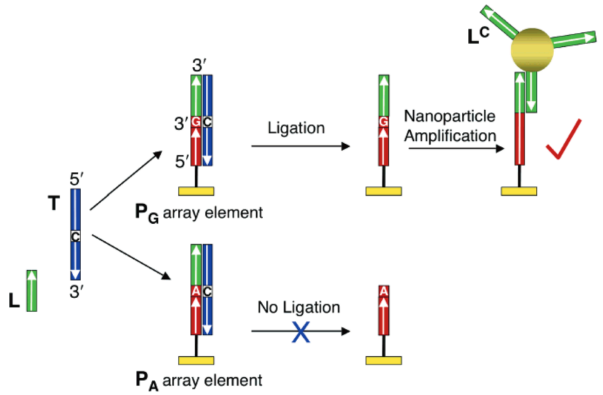
Isothermal Surface Enzyme Chemistries for Ultrasensitive Biosensing

Hye Jin Lee

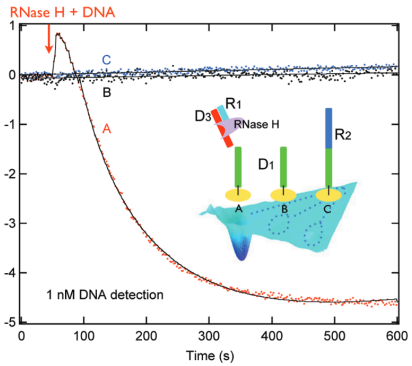
Poly-A Polymerase Amplification fM microRNA sensing

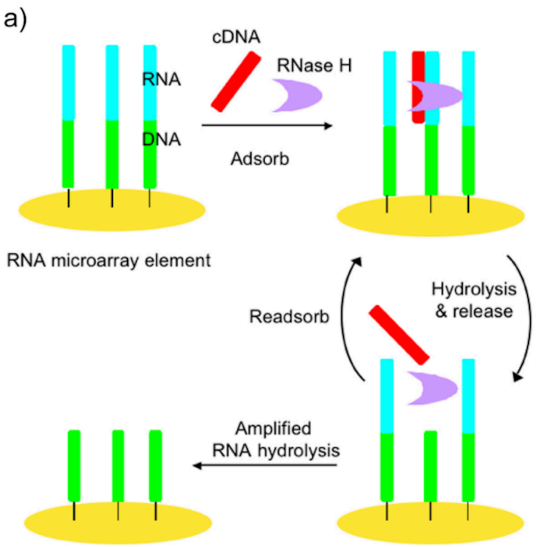


T4 Surface Ligation Chemistry Single Base Mismatch Identification SNP Genotyping

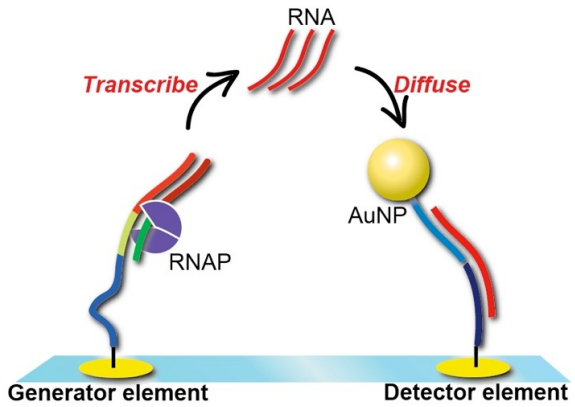


RNAse H Target Recycling genomic (fM) DNA biosensing





Surface T7 RNA polymerase chemistry fM ssDNA sensing



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

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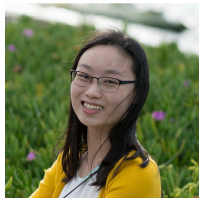
Funding: NIH
NSF

WenJuan



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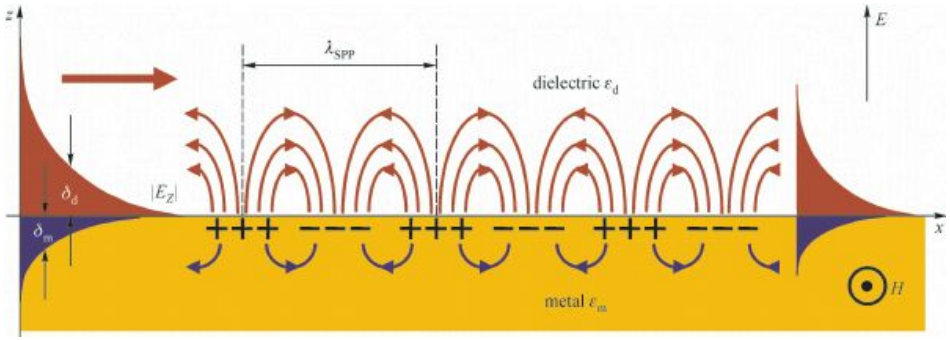
What are Surface Plasmon Polaritons (SPPs)?

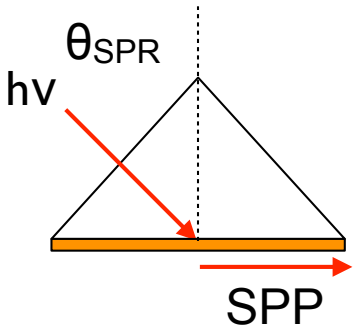
SPR uses SPPs that are traveling wave solutions to Maxwell's equations that are localized to the surface of plasmonic materials. SPPs can be created on gold thin films at all wavelengths longer than the SPP cutoff wavelength (540 nm for gold), propagate for tens of microns, and have fields that extend (decay exponentially) about 200 nm into solution.

Plasmonic materials have a negative real dielectric constant.

$$Re(\epsilon) < 0$$



"surface plasmon resonance"



SPPs can be created on gold thin films attached to a prism surface from photons at the SPR angle.

SPPs have be used for many spectroscopies: absorption, fluorescence, Raman scattering, SHG, CARS, and SPR imaging (SPRI).

SPR image: J. Wang, Front. Optoelectron. 7, 320-337 (2014)

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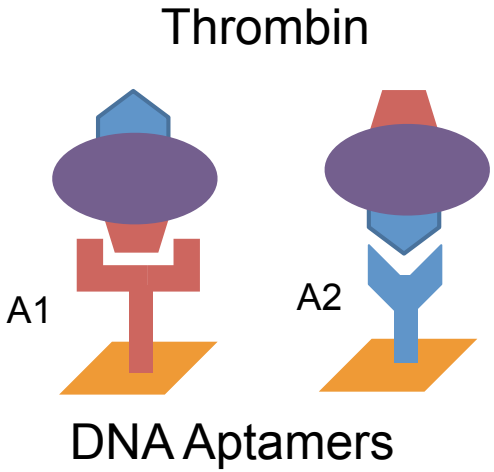
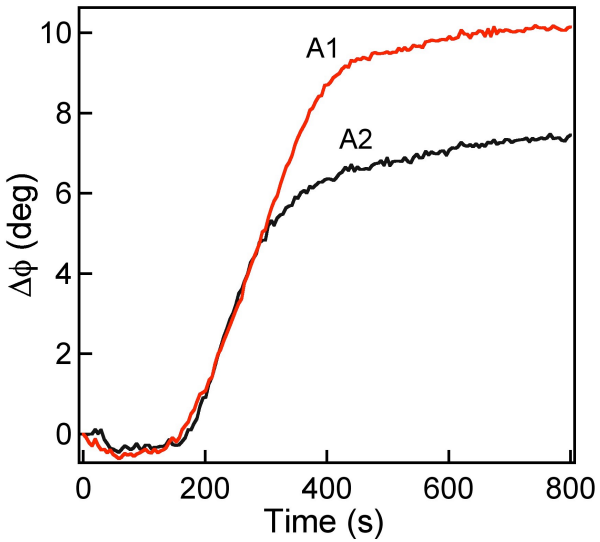
SPRI Phase Imaging Data: thrombin, a 36 kDa serine protease biomarker

Thrombin adsorption onto a thrombin aptamer microarray

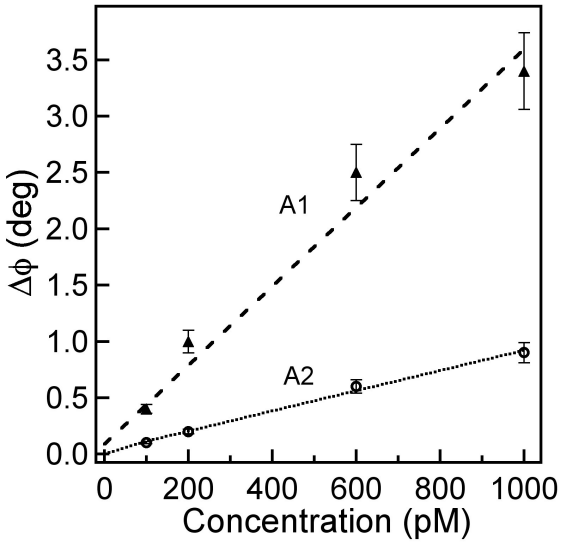


Real Time Adsorption Kinetics with SPR-PI



10 nM Thrombin

K_{A1}= 4.36E8
K_{A2}=1.24E8



K_{A1}/K_{A2} = 3.5

50 pM thrombin lod with SPR-PI

A2: 5-AGT CCGTGGTAG GGC AGGTTG GGGTGA C-3' ; A1: 5-GGTTGGTGT GGTTGG-3'
Ref: K. Edwards et al., Anal Bioanal Chem. 398 2645–2654, 2010.

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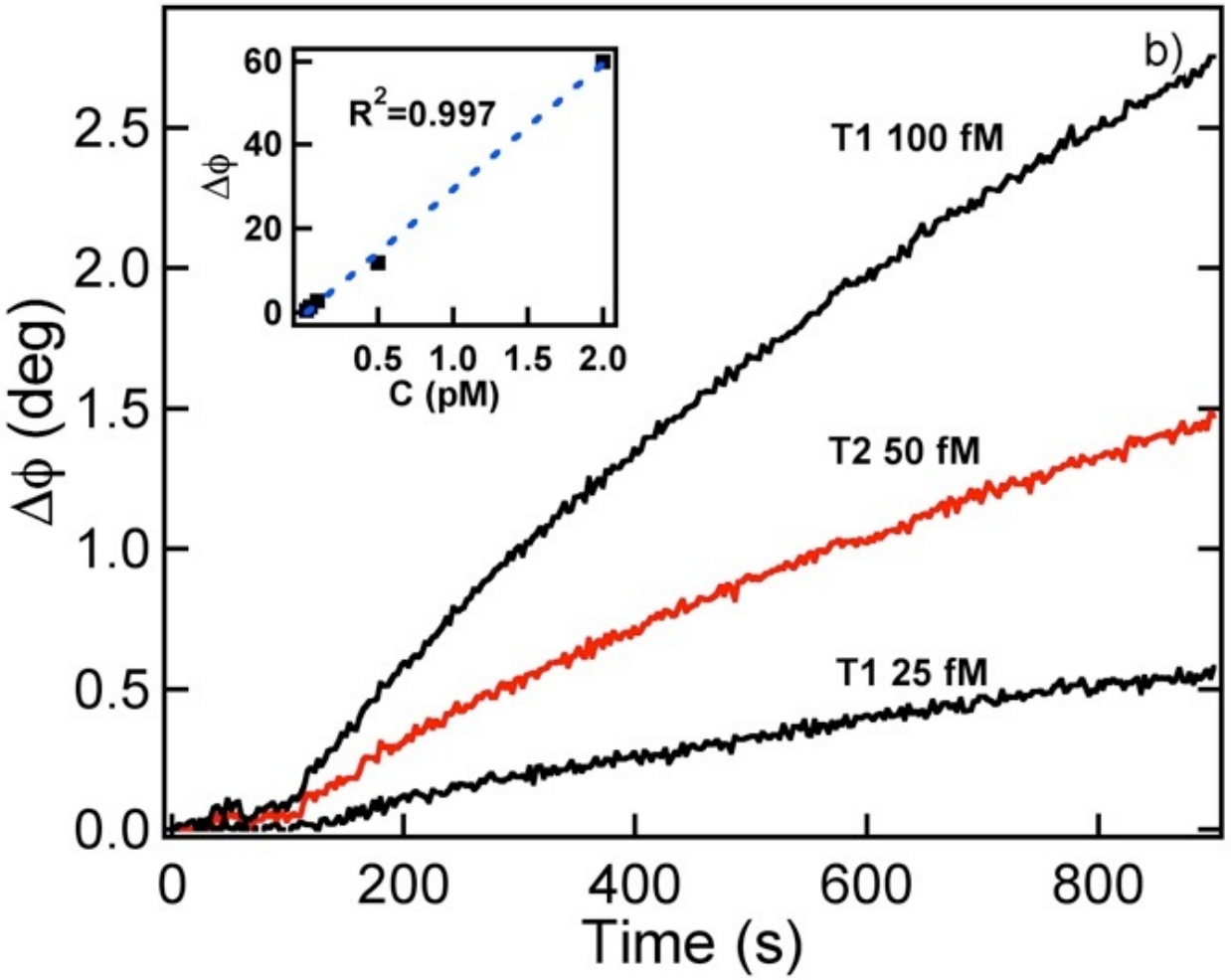
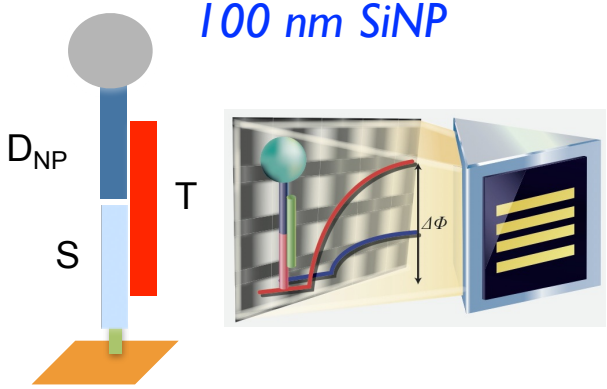
Nanoparticle-Enhanced SPR Phase Imaging with SiNPs

Three sequence hybridization assay with SPR-PI:





$\theta_{min} = 1 \times 10^{-6}$

$K_{ads} = 1 \times 10^{9}$

$C_{min} = \theta_{min} / K_{ads}$

$C_{min} = 1 \times 10^{-15}$ M

*W. Zhou et al., Analytical Chem., **84** 440-445 (2012).*

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