Nanoscale-Controlled Surface Materials, Bioanalysis, and Commercialization

- NANO KOREA 2007 -

Aug 28, 2007

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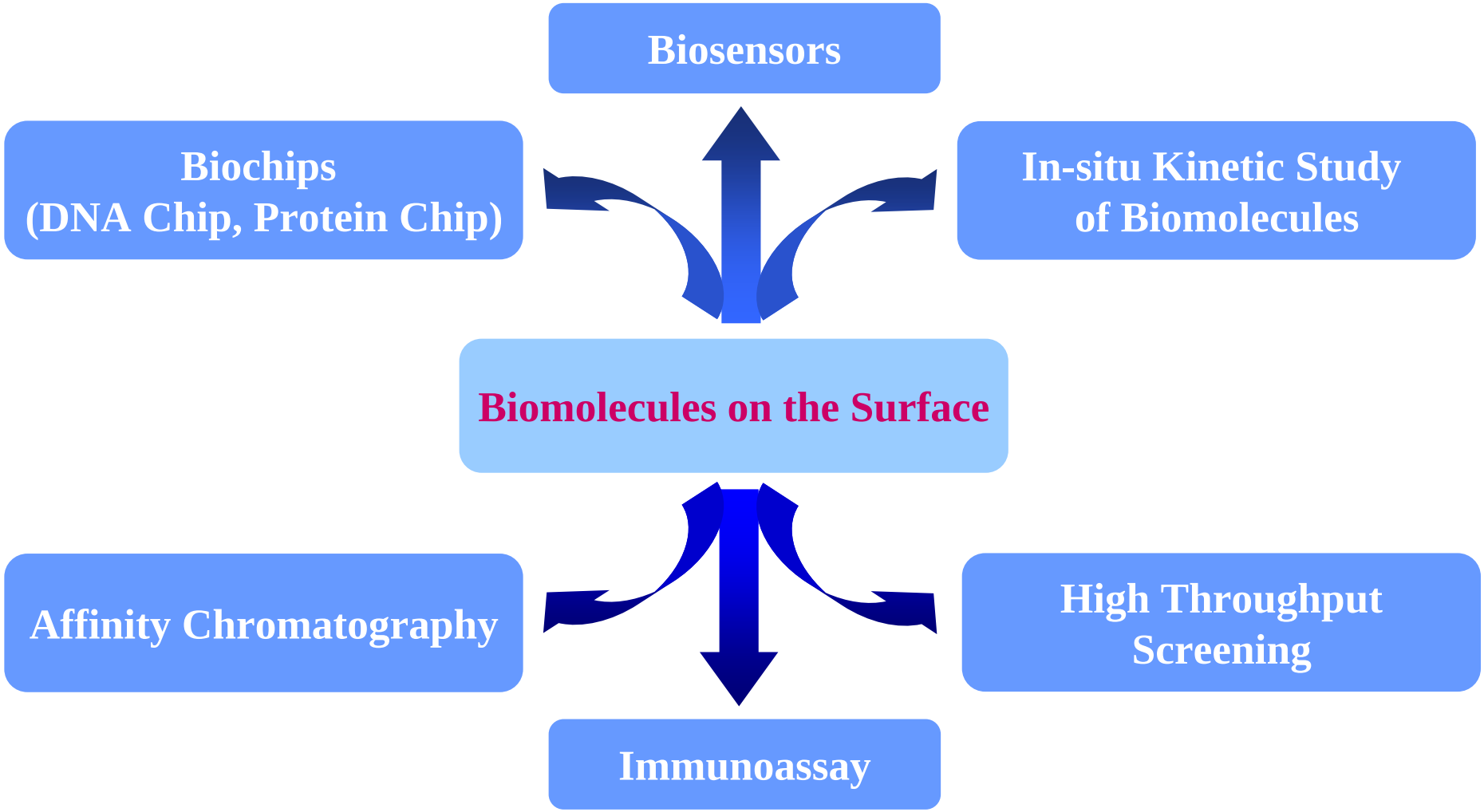
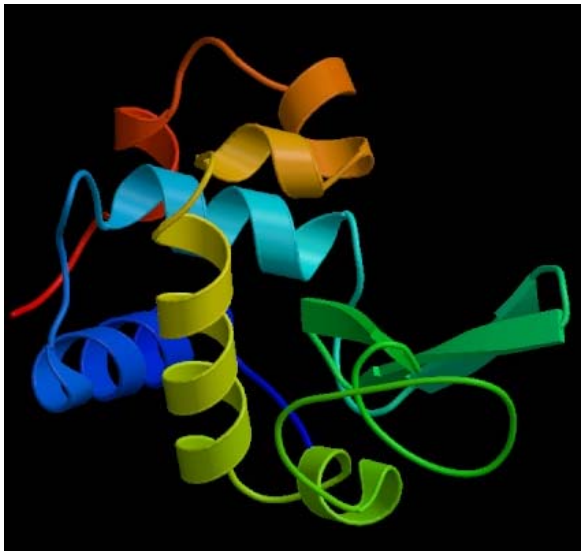
Biomolecules on the Surface in Various Applications



Biomolecules Require Mesospacing for Comfortable Anchoring on the Surface

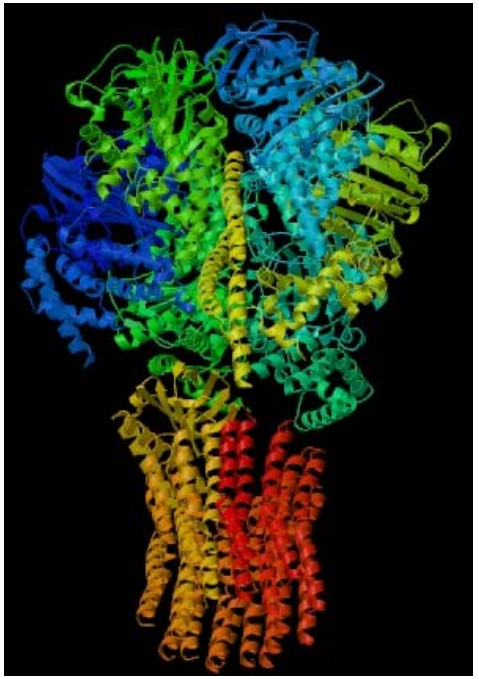
23.7 Å

B-DNA



45 Å

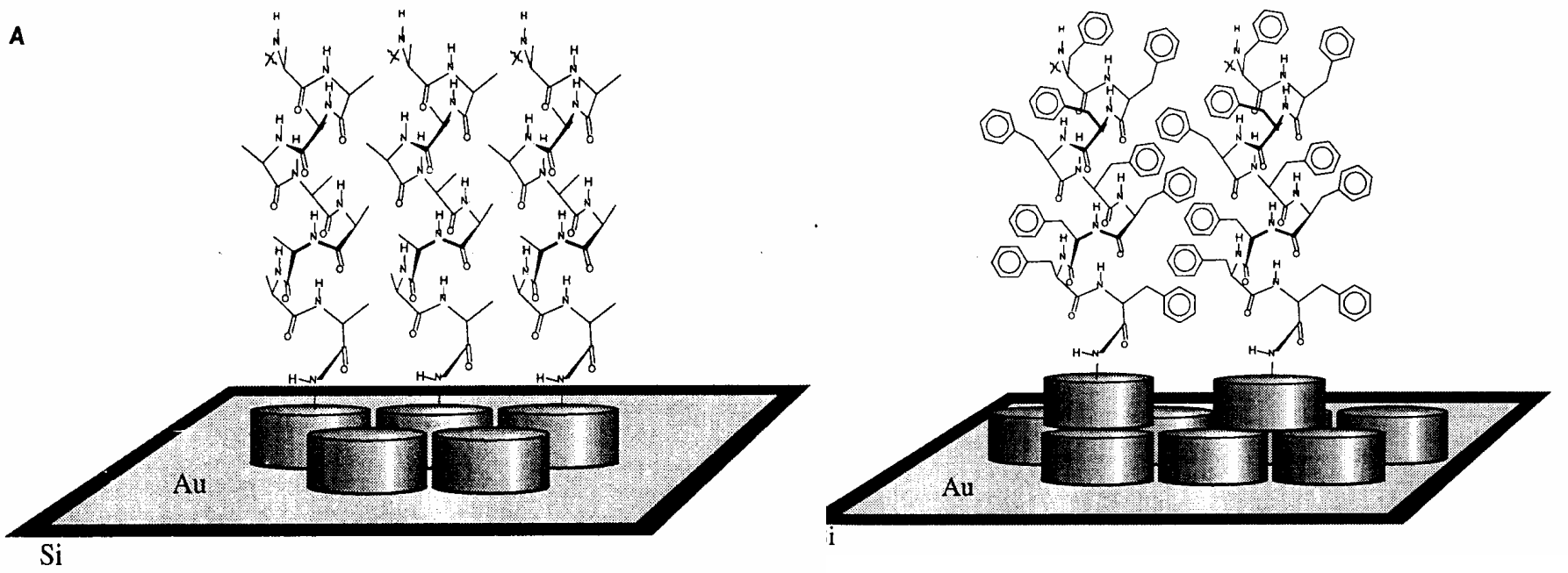
Lysozyme



139 Å

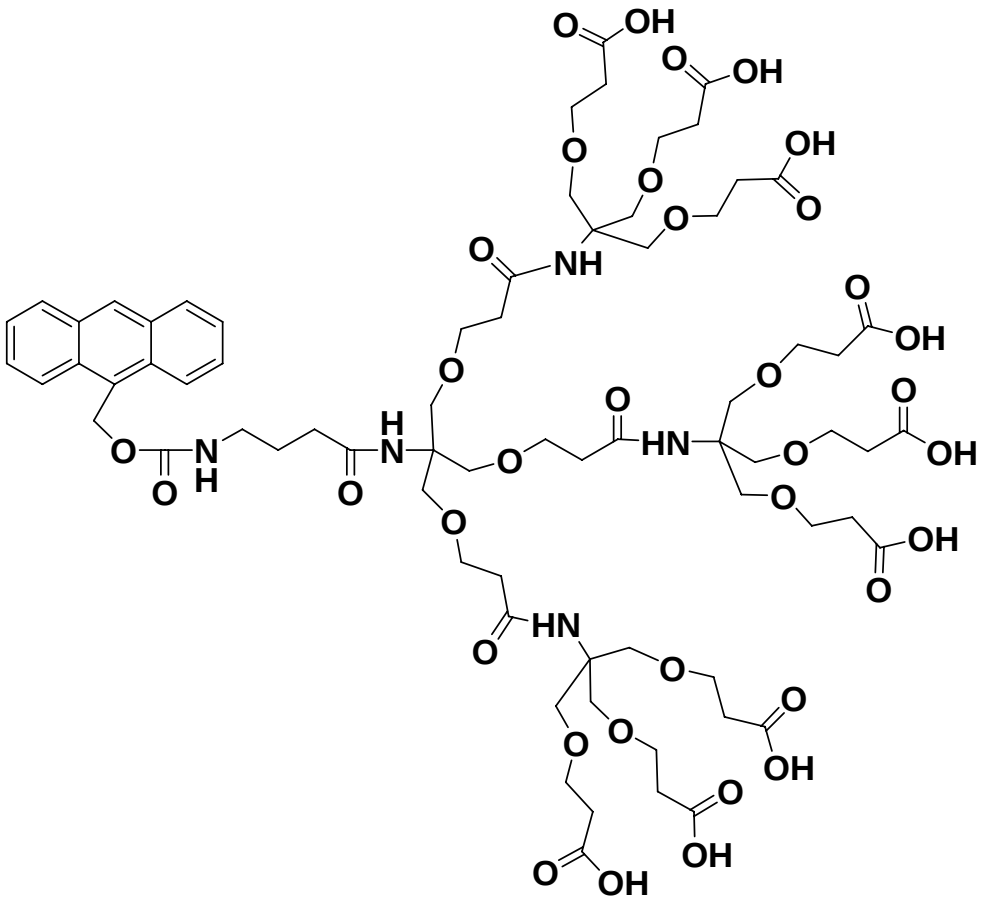
ATP synthase

Peptide Folding on the Spacing Controlled Gold Surface

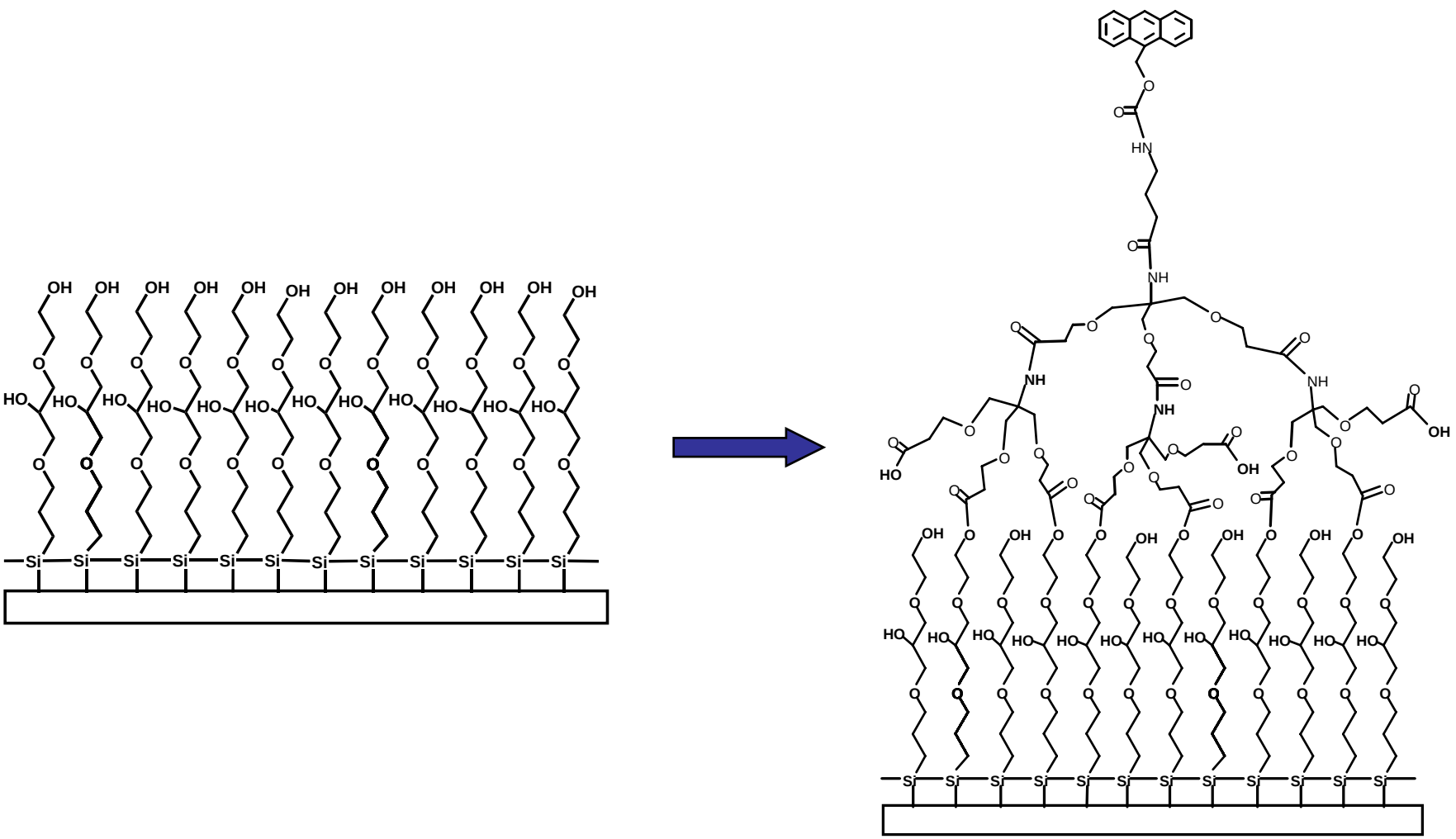


Whitesell et al. *Science* 261, 73 (1993)

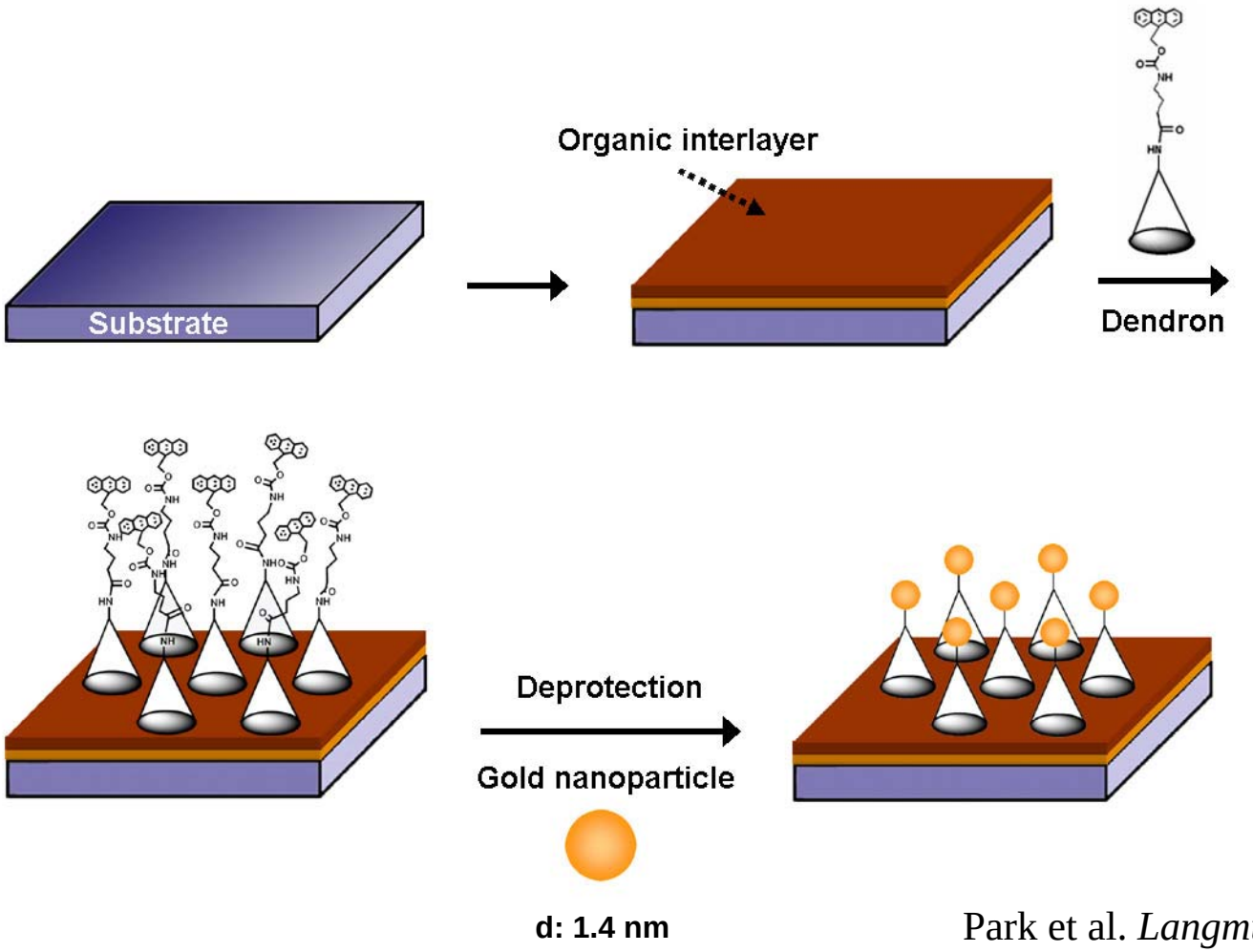
An Optimized Dendron



Inert Base Layer and Covalent Bonding



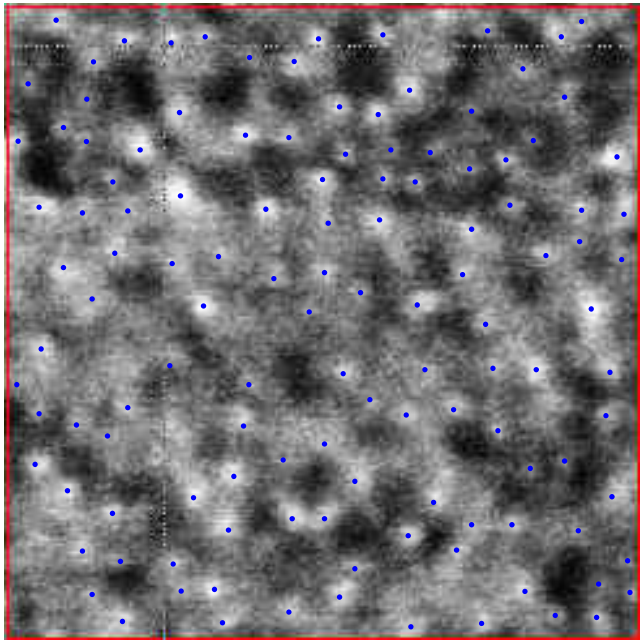
Immobilization of Gold Nanoparticles at the Dendron Surface



Park et al. *Langmuir* 4257 (2005)

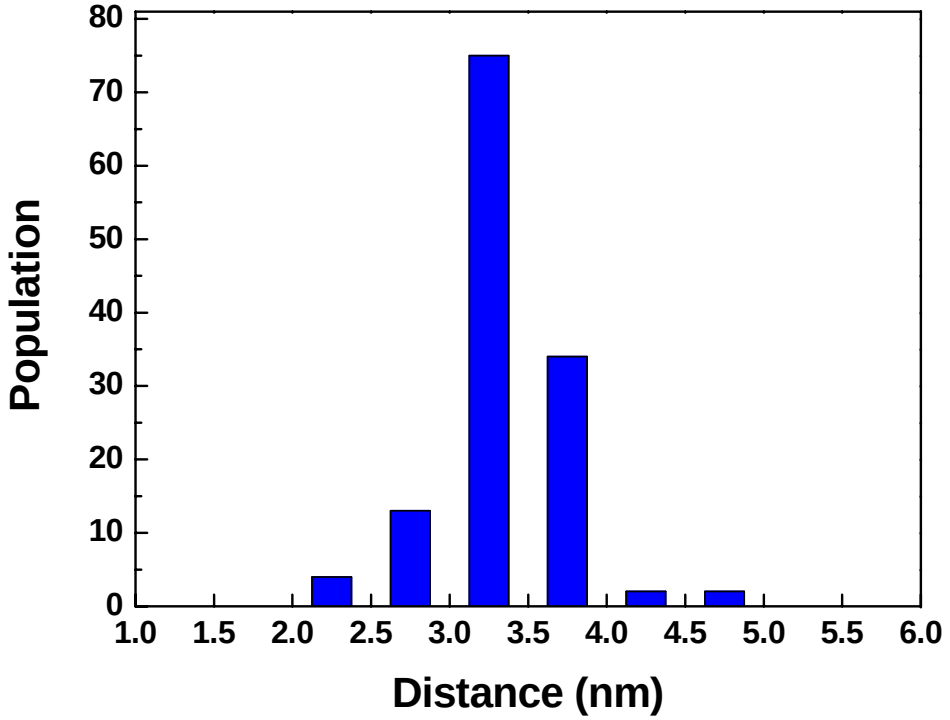
Spacing between Dendron Molecules on Surface

SEM Image



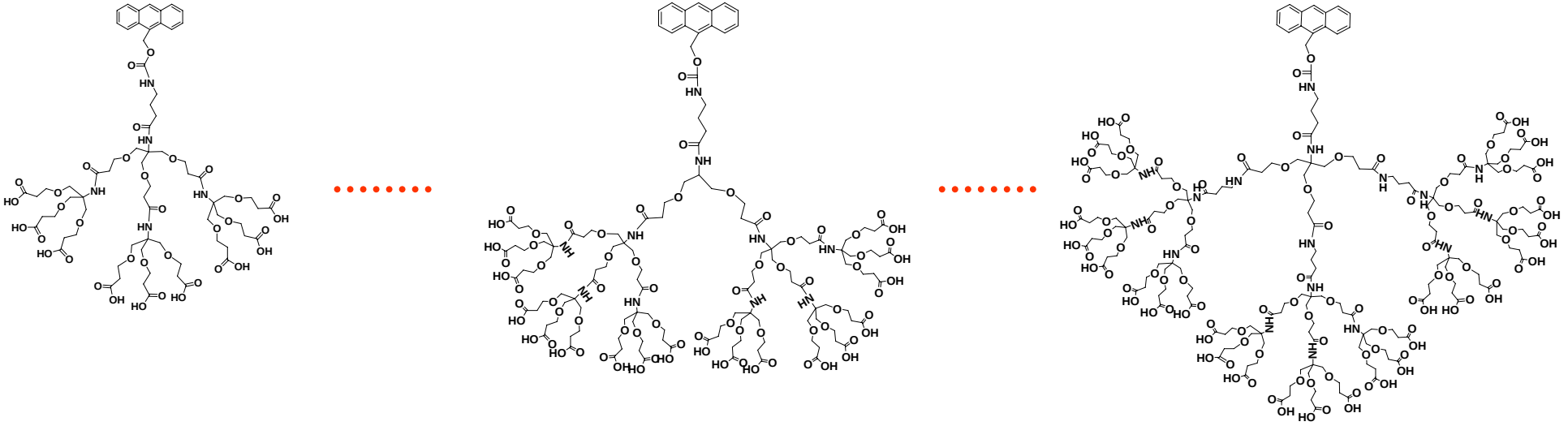
- Particle number / area
  : 130 ea / 50 x 50 nm^2
- Density: 0.05 – 0.06 ea/nm^2

Distance between Dendron Molecules



- Average distance: 3.2 nm
- Standard deviation: 0.4 nm

Various Spacing up to 10 nm



[9]-acid **[18]-acid** **[27]-acid, [81]-acid**

~ 3 nm **Spacing** **~ 10 nm**

Dendron-Coated Surface Improves Significantly SNP Discrimination Efficiency and Detection Limit of Gene Expression Profiling

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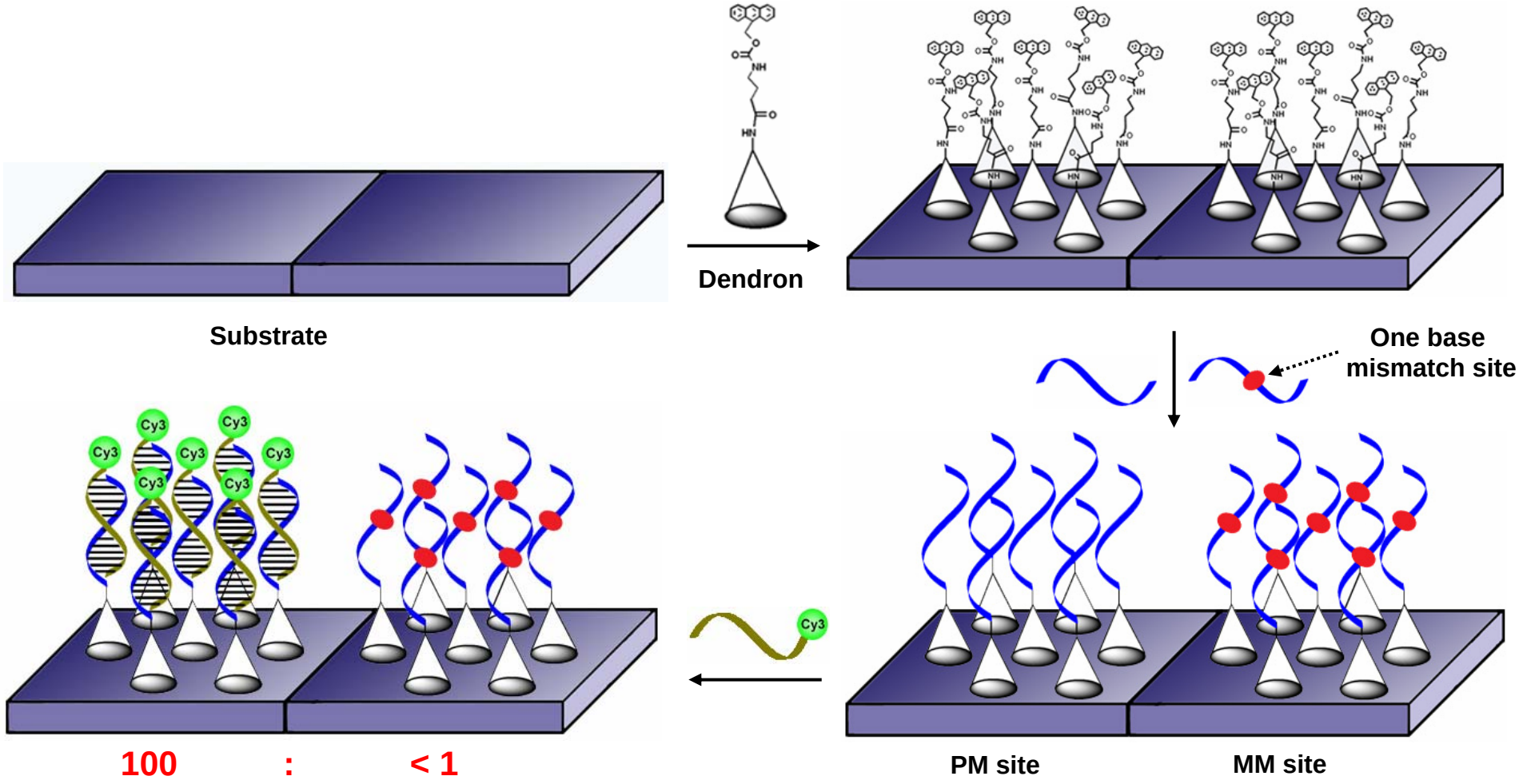
KOREA

Fabrication of DNA Microarray on Dendron-Modified Surface



US Patent Application (2004)

Probe & Target Oligonucleotides

Probe oligonucleotide

Probe 1: 5´-NH_2-C_6-CAT TCC G**X**G TGT CCA-3´

Probe 2: 5´-NH_2-C_6-$(T)_{30}$-CAT TCC G**X**G TGT CCA-3´

X = A (complementary), T, G, C (mismatched)

Target oligonucleotide

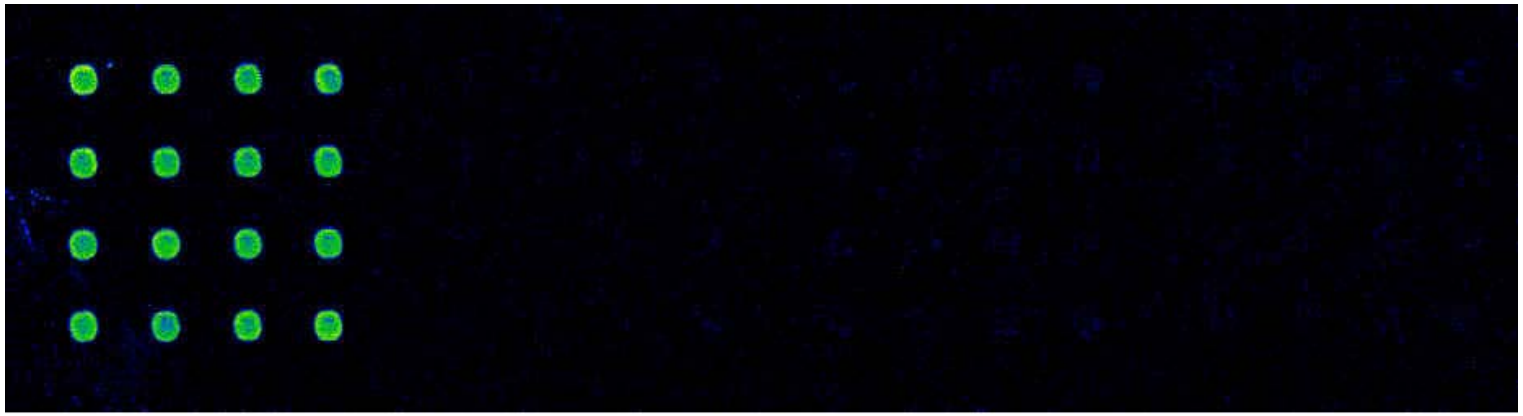
Target 1: 5´-Cy3-TGG ACA C**T**C GGA ATG-3´

Target 2: 5´-Cy3-CCT ACG AAA TCT ACT GGA ACG AAA TCT ACT TGG ACA C**T**C GGA ATG-3´

Fluorescence Image of the Microarrays (I)

15-Base oligonucleotide (Probe 1) & 15-Base oligonucleotide (Target 1)

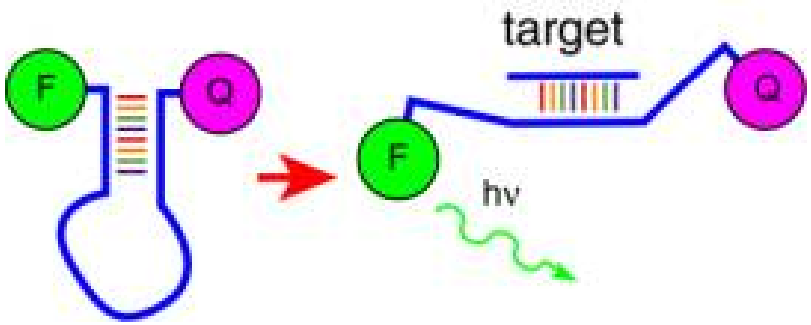
PM **IMM(TT)** **IMM(GT)** **IMM(CT)**

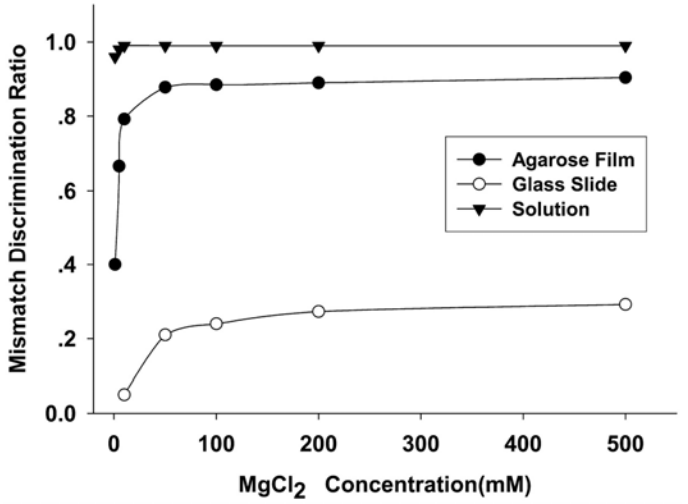


	PM(AT)	IMM(TT)	IMM(GT)	IMM(CT)
Normalized Signal Ratio	100	**0.5**	**0.8**	**0.6**

Hybridization condition: 1 nM target at 50 °C for 1 h

Molecular Beacon showed 100: 1 Ratio in Solution





Molecular beacon in solution: 100: ~1 SNP discrimination ratio (Wang, H. *et al.* *Nucleic Acids Res.* 2002, *30*, e61.)

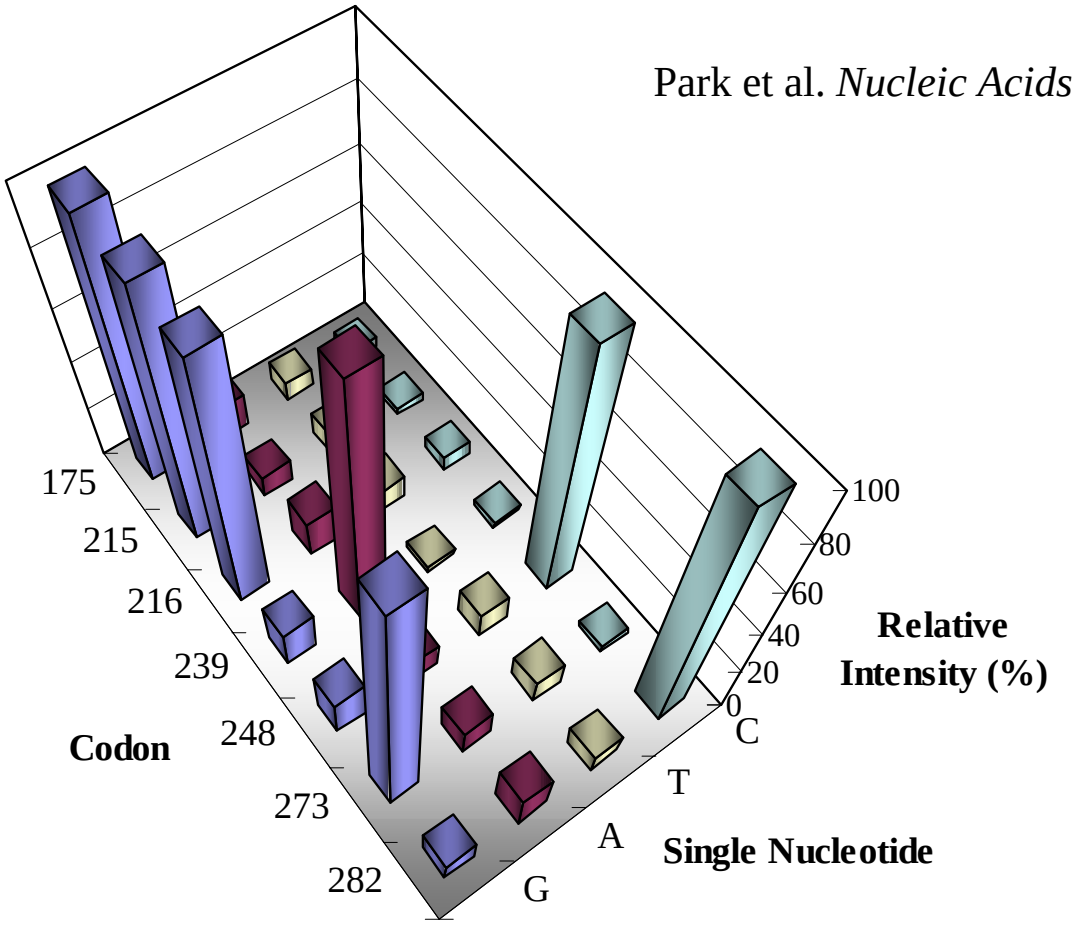
(MM/PM) in solution: 1 %

(MM/PM) on agarose film: 19 %

(MM/PM) on glass slide: 69 %

Simultaneous Detection of 7 Hotspots of p53 Gene

Park et al. *Nucleic Acids Research* e90 (1995)



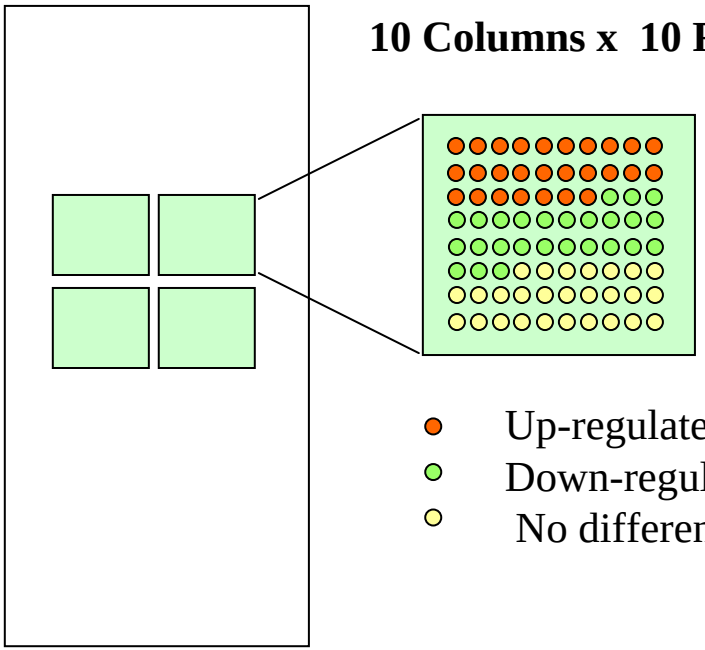
Intensity less than 16% was observed for the all mutations.

Characteristics of the Surface in RNA Expression Profiling

1-1) Chip Design

Chip design-4

10 Columns x 10 Rows x 4 Blocks

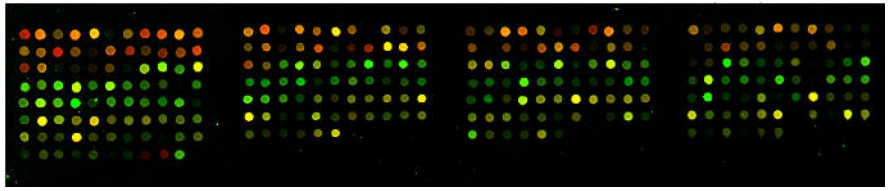


- Up-regulated genes
- Down-regulated genes
- No differentially expressed genes

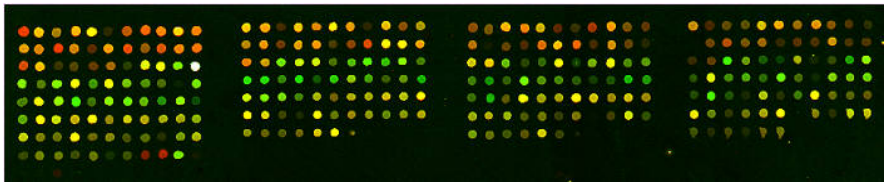
US patent application (2004)

1-2) Hybridization Condition:
Probe concentration of 50 pmole/μl

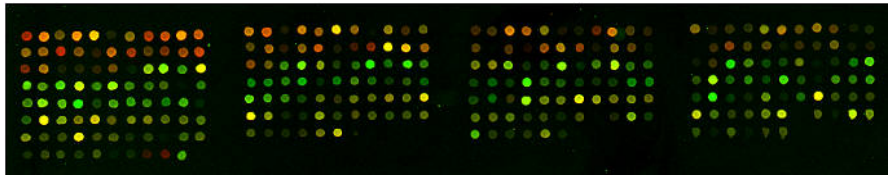
No blocking, 42°C with 30% DMF



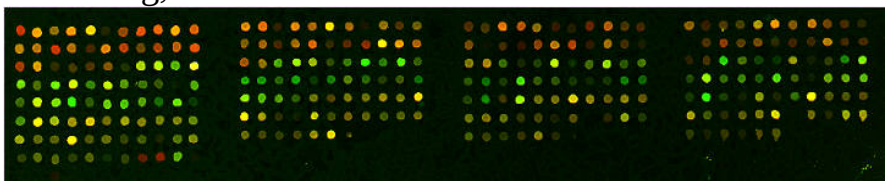
No blocking, 65°C



Blocking, 42°C with 30% DMF



Blocking, 65°C

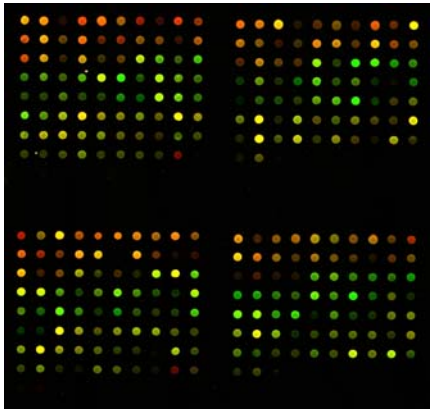


For gene expression analysis with NSB slides, blocking agent was not needed, while use of BSA was required for Corning slides.

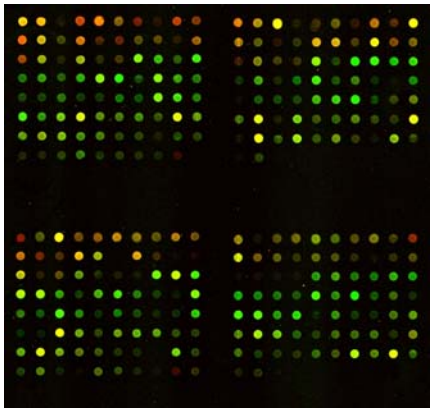
1-3) Detection Limit:
RNA from 293 Cell Line labeled with Cy3, HeLa Cell Line with Cy5

NSB POSTECH

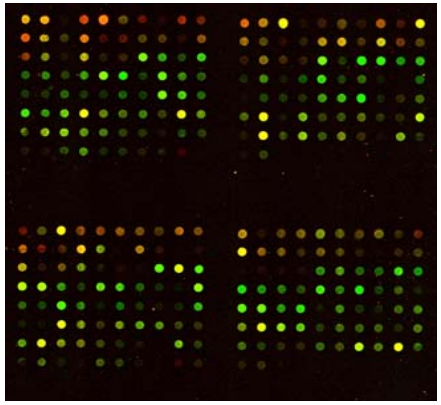
100 μg



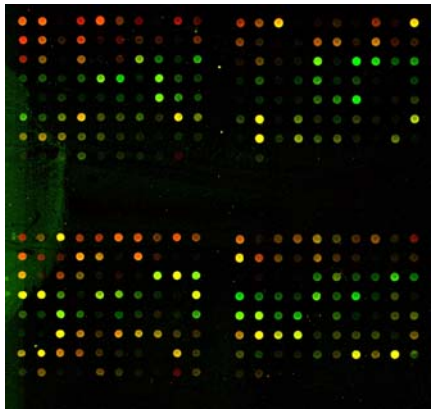
50 μg



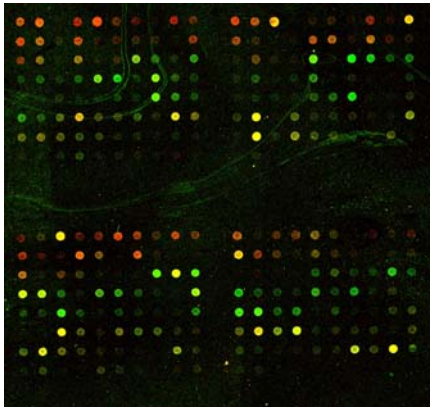
30 μg



10 μg

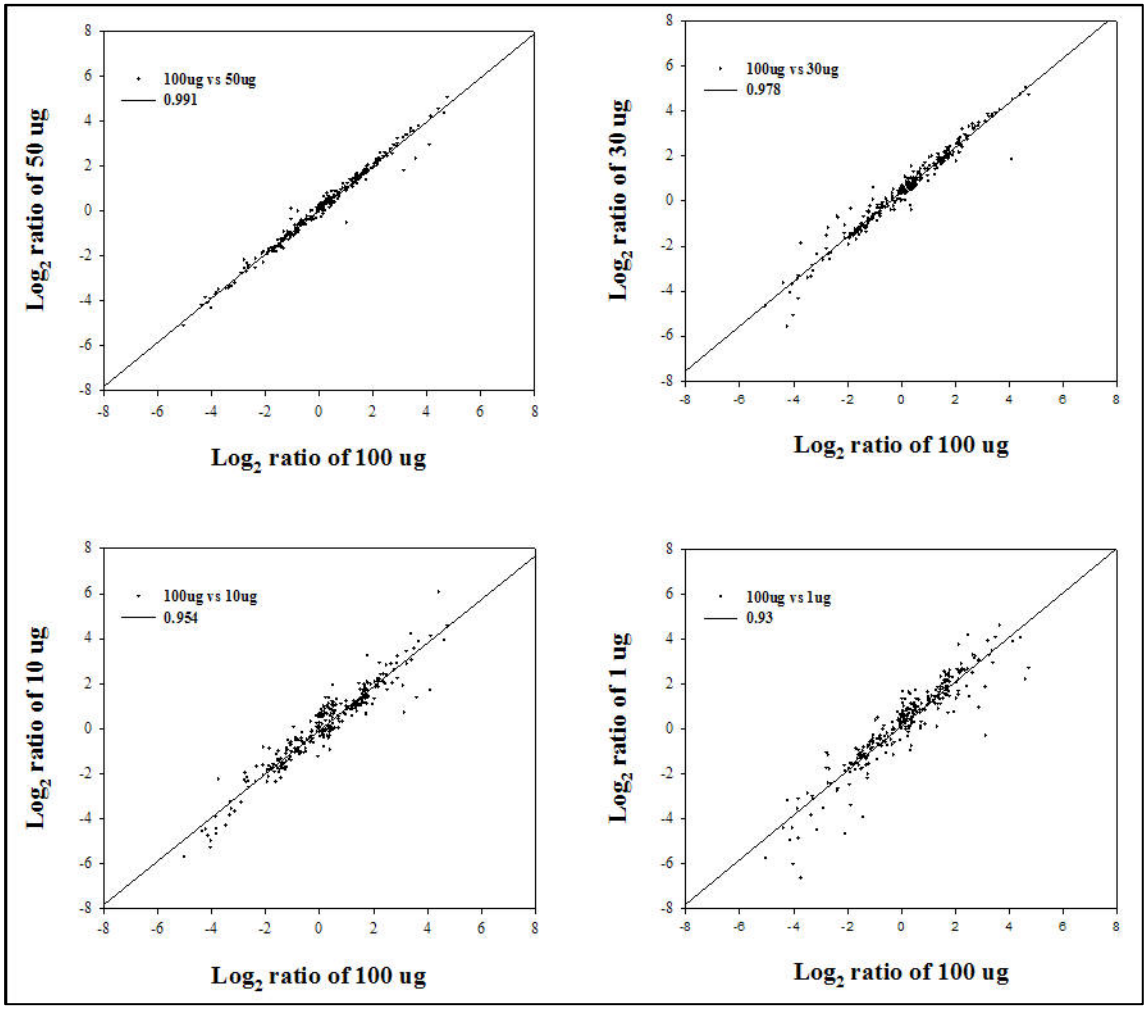


1.0 μg



- The signal intensity decreases as the target concentration decreases. It seems possible to detect the signal with very small amount of total RNA as low as 1.0 μg.

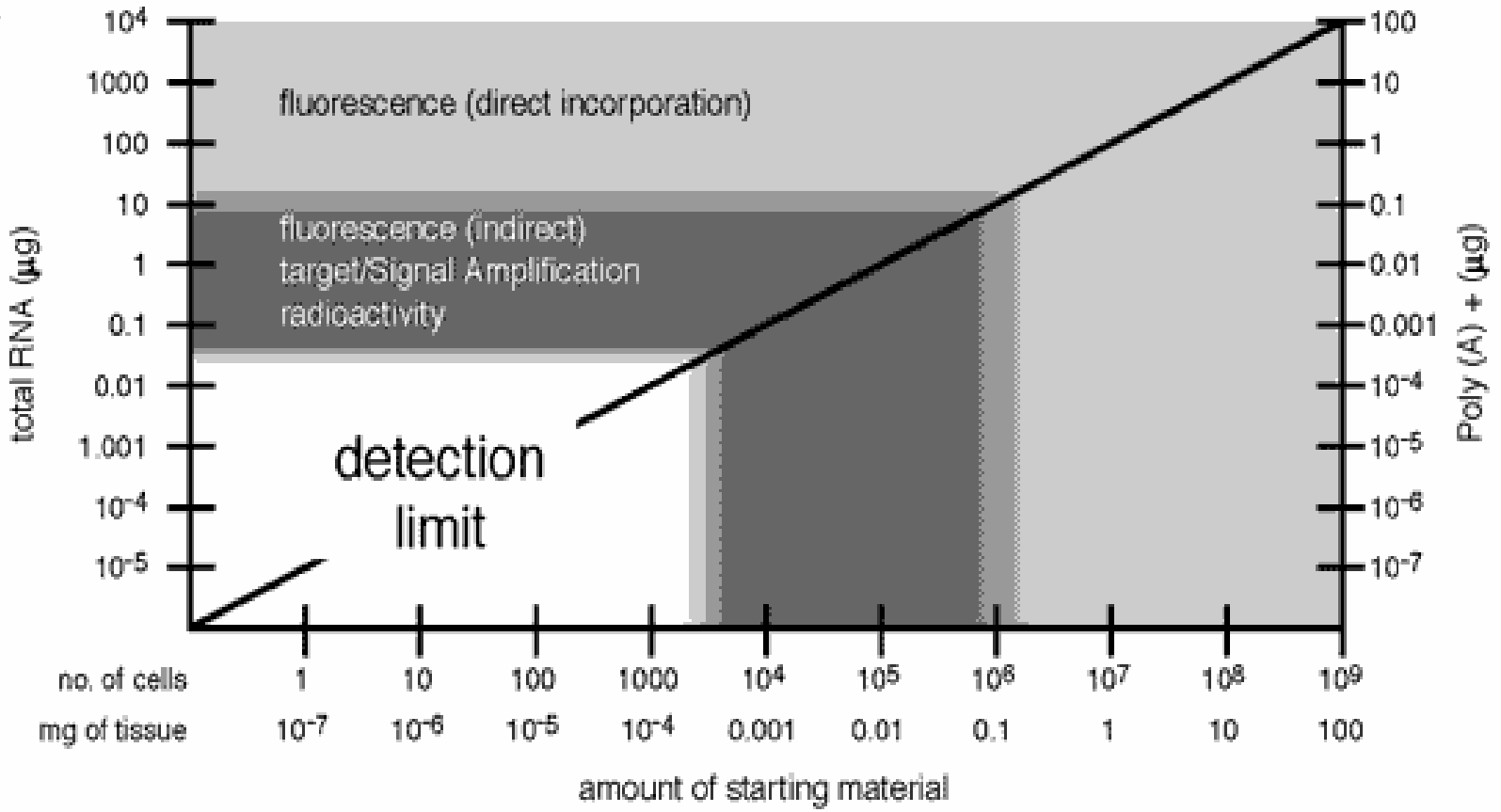
1-4) Correlation Analysis: Pearson's Correlation, p = 0.01



NSB POSTECH Slide

- **Superb correlation was maintained even with 1 μg of total RNA.**

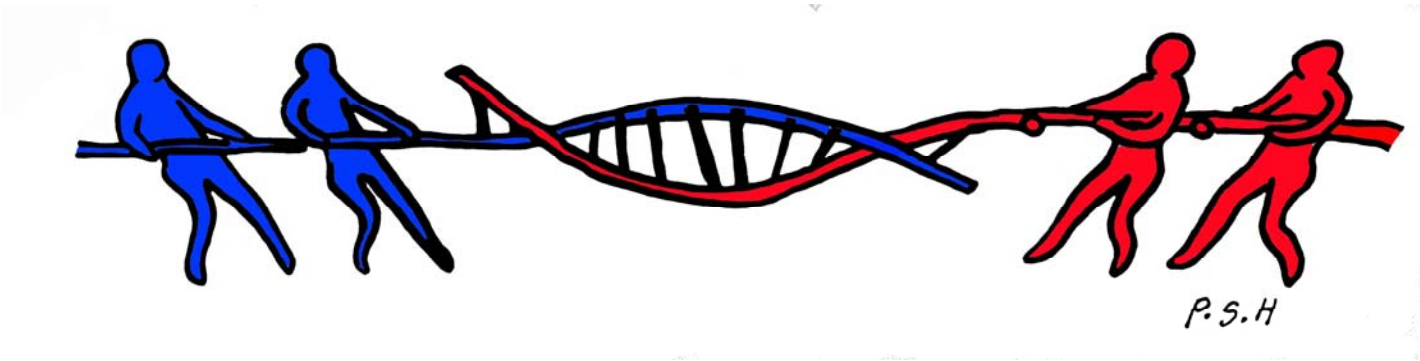
Detection Limit of Total RNA by Microarray



"For adequate fluorescence, the total RNA required …… per array, is 50-200 µg."

Nat. Genetics Suppl. 21, 10 (1999)

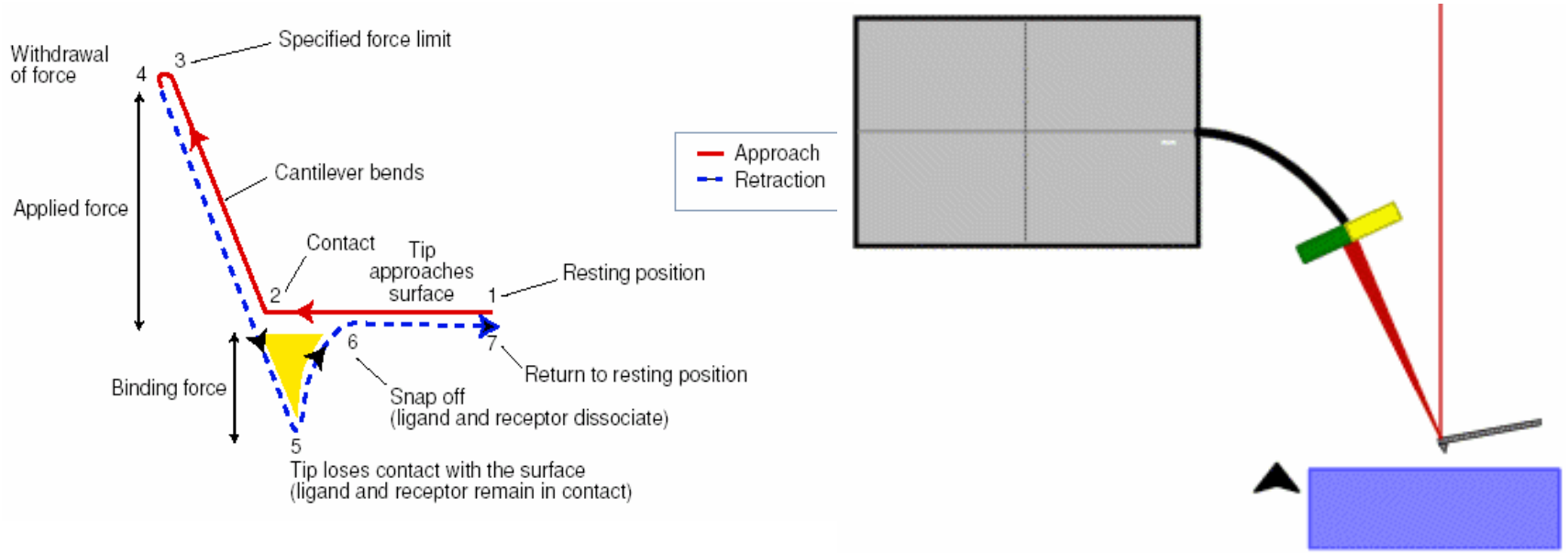
DNA-DNA Interaction Measured with Picoforce AFM



Yu Jin Jung and Joon Won Park

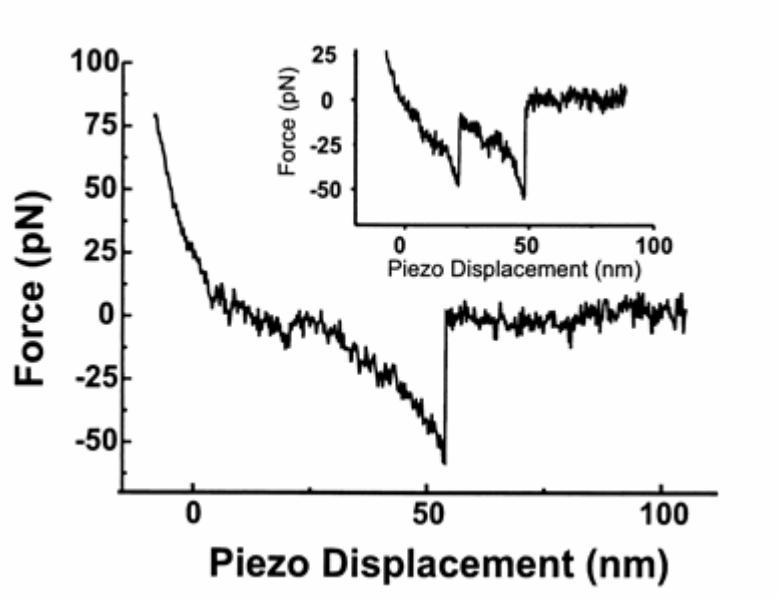
In collaboration with School of Pharmacy, Nottingham University

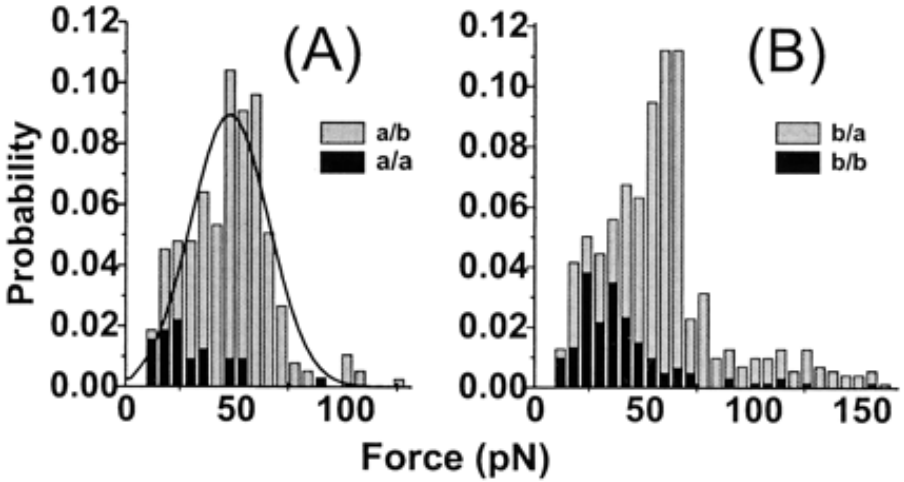
Force – Distance Curve from AFM



F = -kd (Hook's Law)

Force – Distance Curve for DNA-DNA



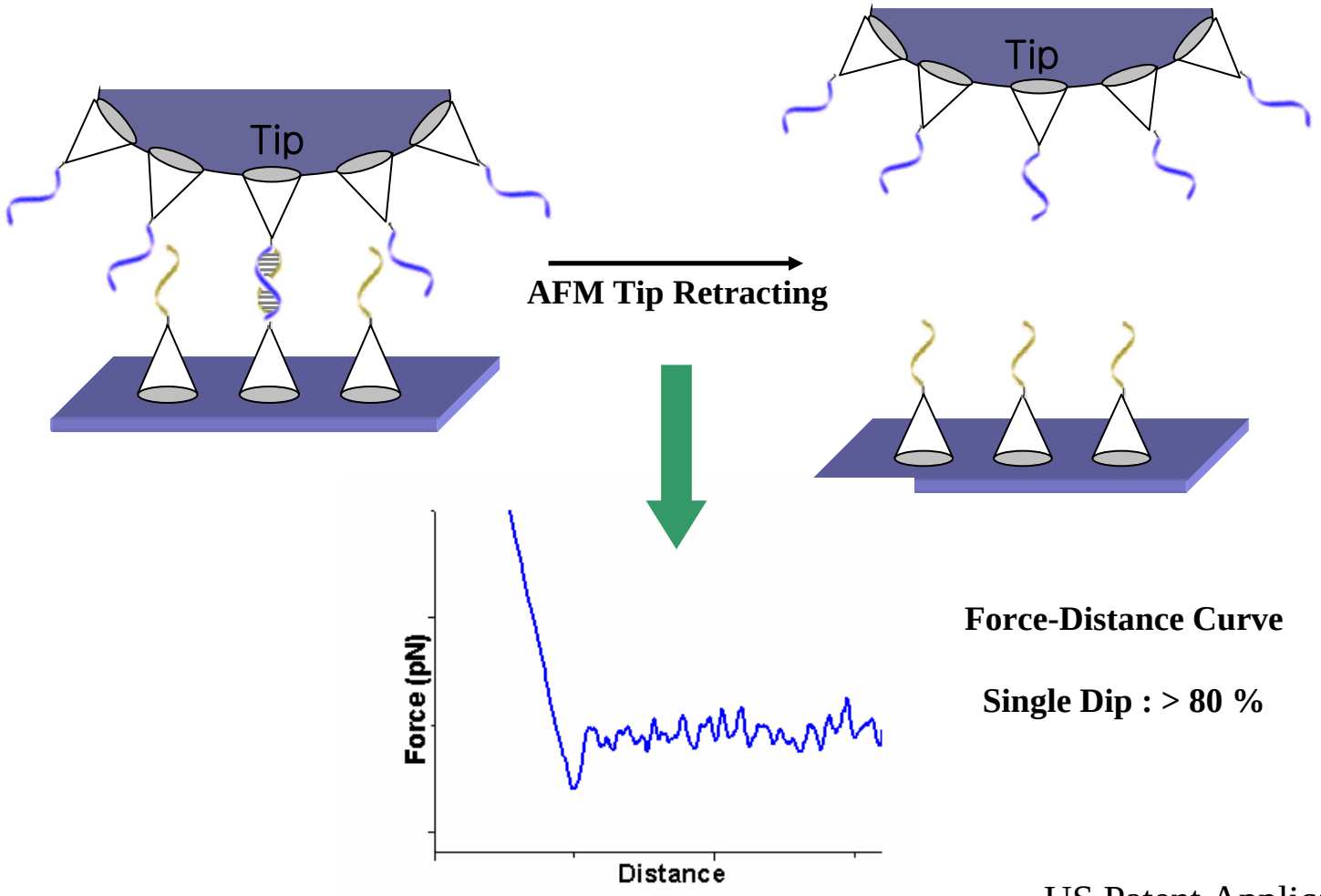


⟹ 48 ± 2 pN

a = 5'-G-G-C-T-C-C-C-T-T-C-T-A-C-C-A-C-T-G-A-C-A-T-C-G-C-A-A-C-G-G-3'
b = 3'-C-C-G-A-G-G-G-A-A-G-A-T-G-G-T-G-A-C-T-G-T-A-G-C-G-T-T-G-C-C-5'

Strunz et al. *Proc. Natl. Acad. Sci. USA* 96, 11277 (1999)

Proper Spacing Simplifies Force-Distance Curve

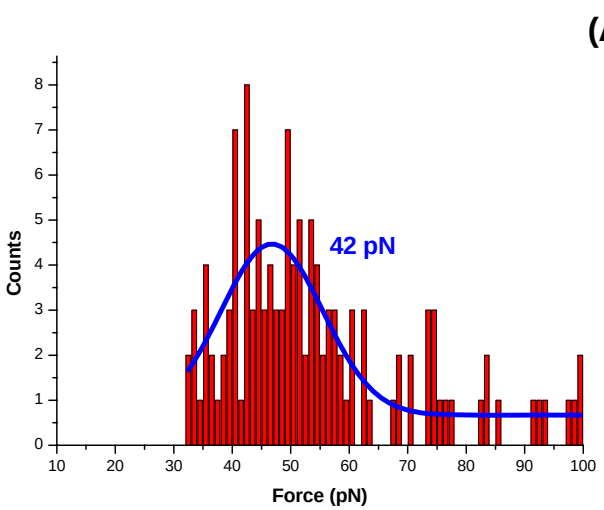


Force-Distance Curve

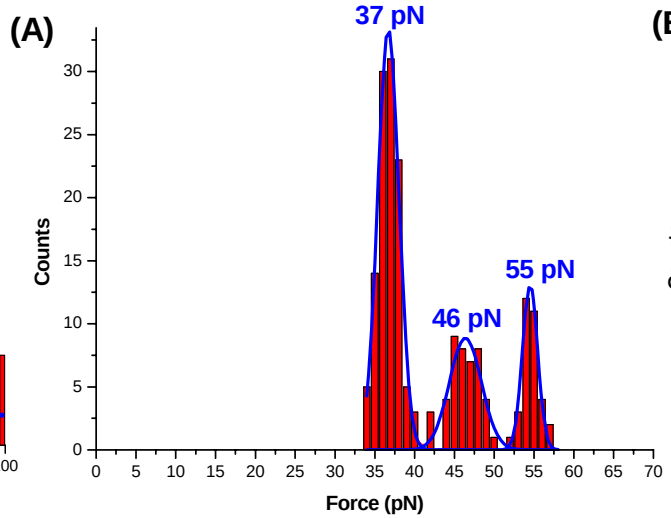
Single Dip : > 80 %

US Patent Application (2005)

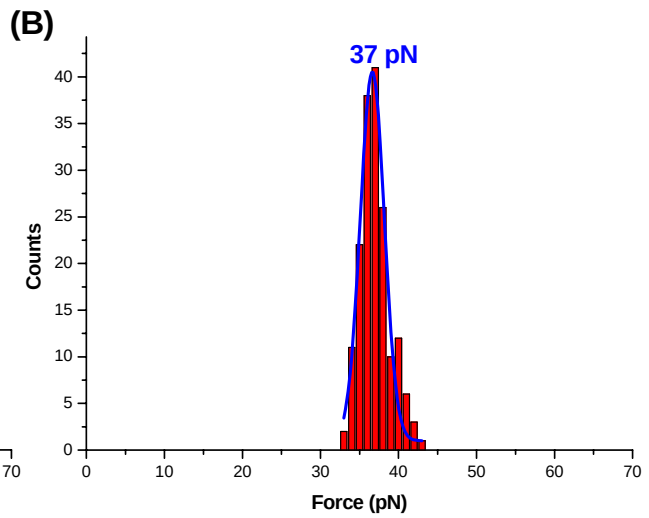
Optimized Spacing gives a Single and Sharp Histogram



30-base DNA immobilized on 3-acid/GPDES



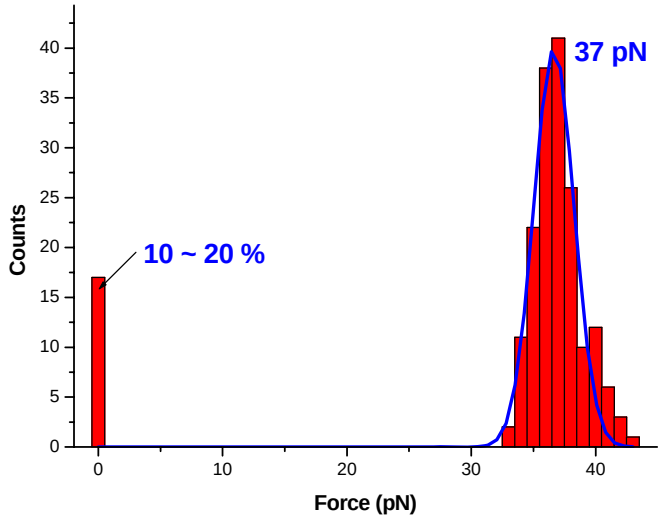
30-base DNA immobilized on 9-acid/GPDES



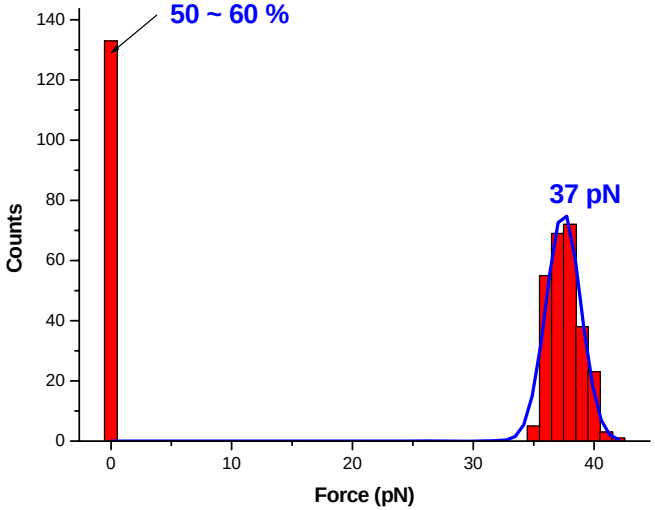
30-base DNA immobilized on 9-acid/TPU

A Bigger Spacing Reduces Chance of the Binding

30-base DNA immobilized on 9-acid/TPU

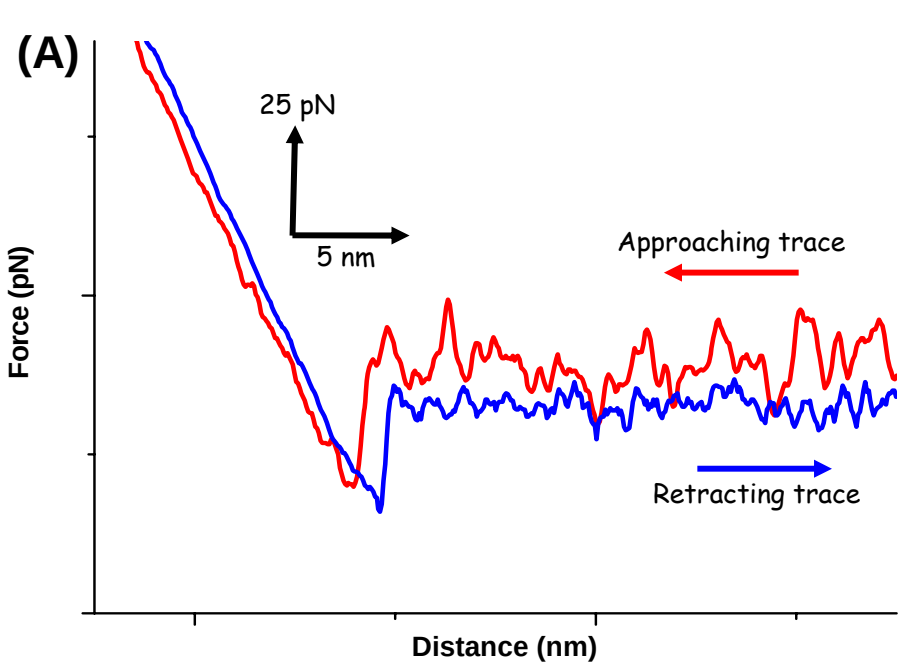


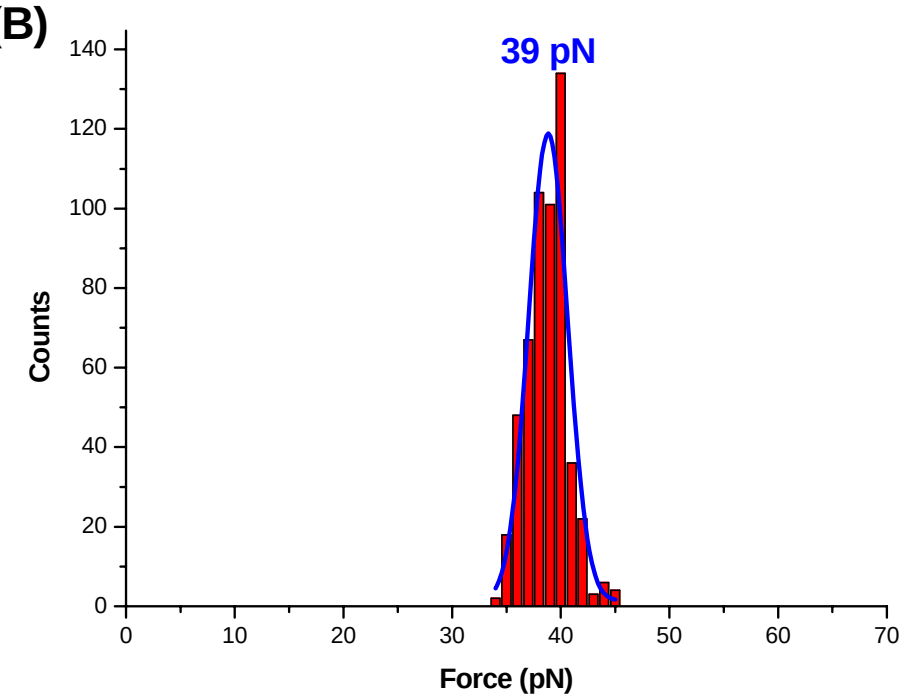
30-base DNA immobilized on 27-acid/TPU



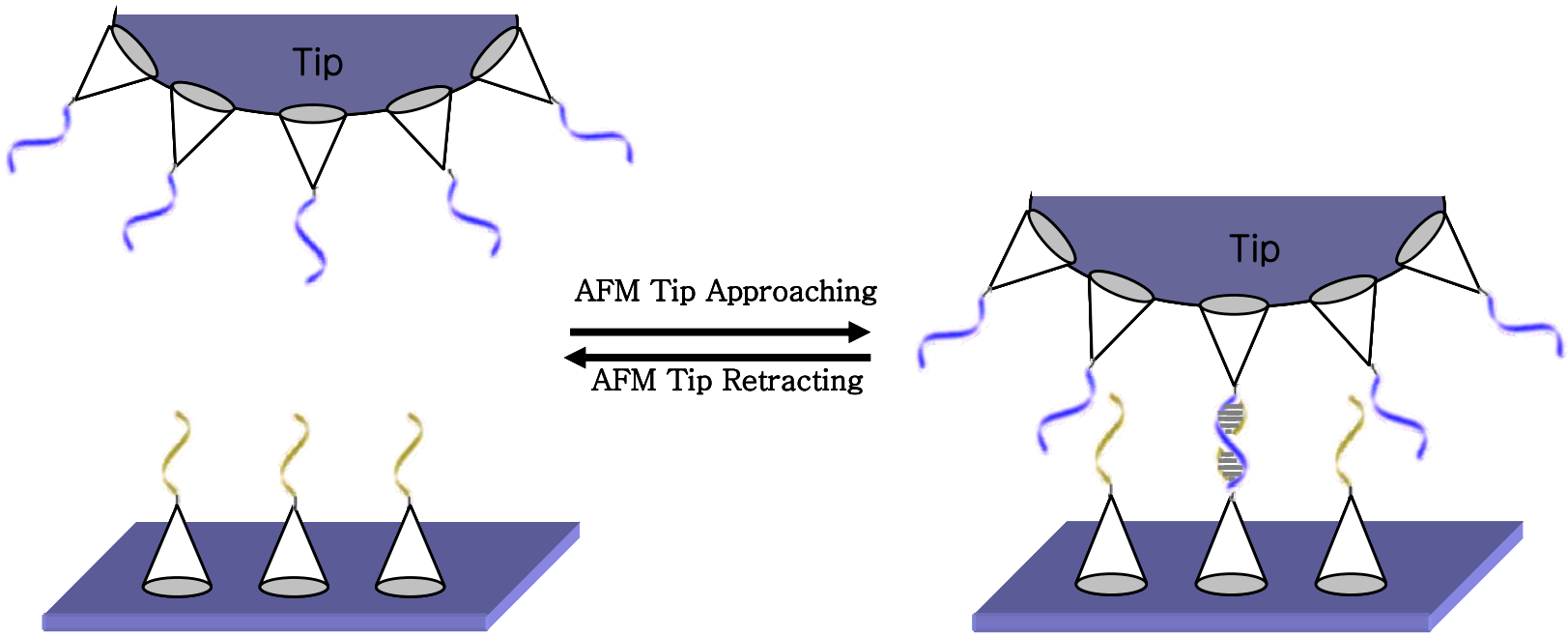
The observed binding force is constant with a bigger spacing, while chance of the binding event is reduced significantly.

Unprecedented Binding Event



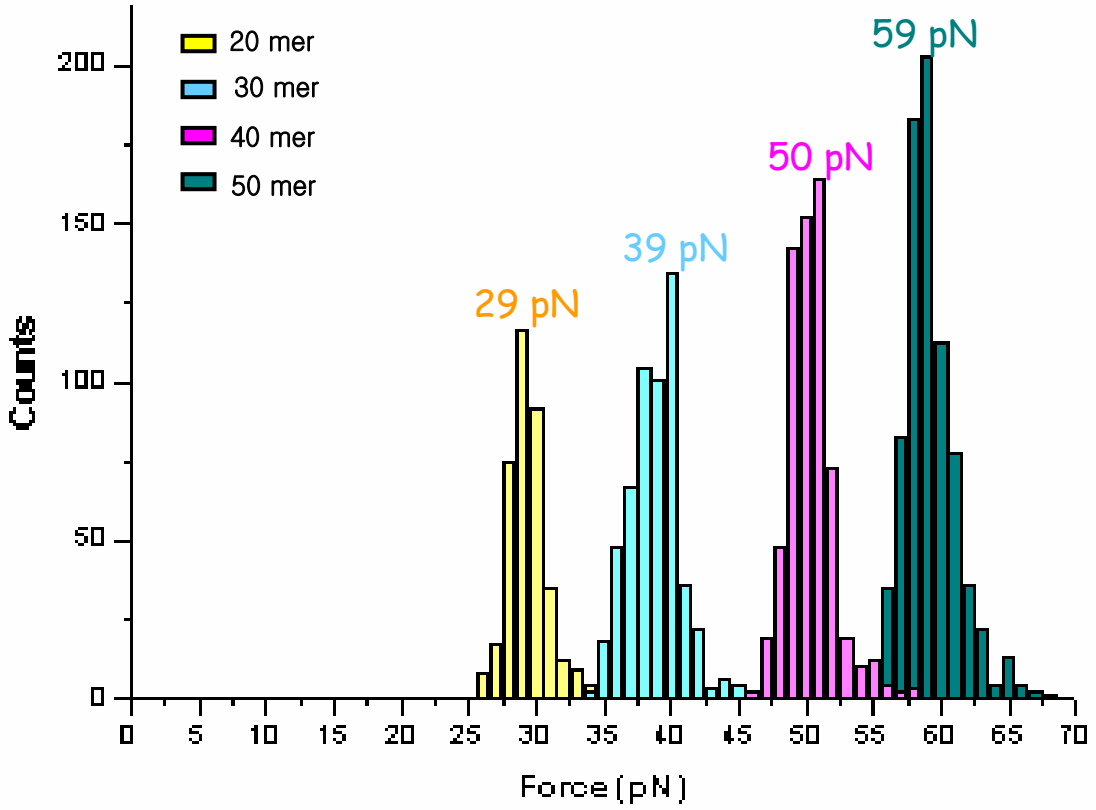


It is Now a Reversible Process



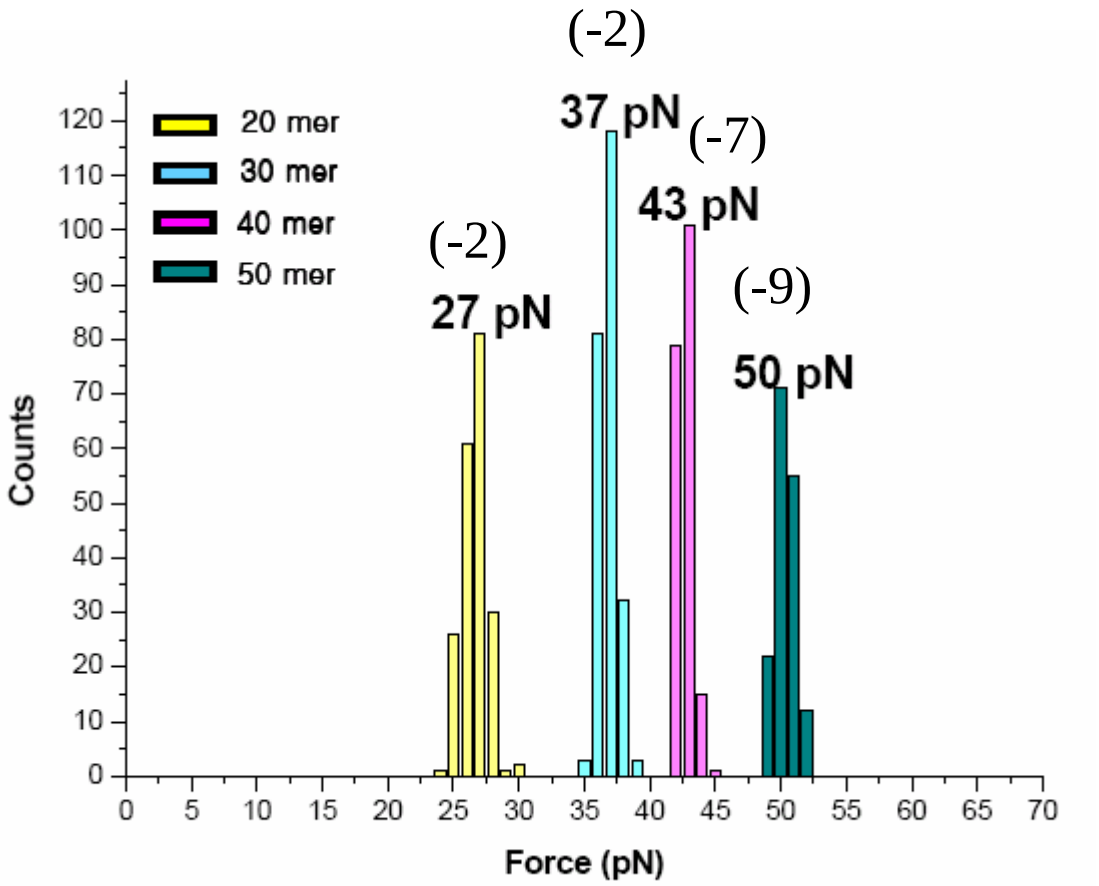
Park et al. *J. Am. Chem. Soc.* in print

Resolved Binding Force Histogram

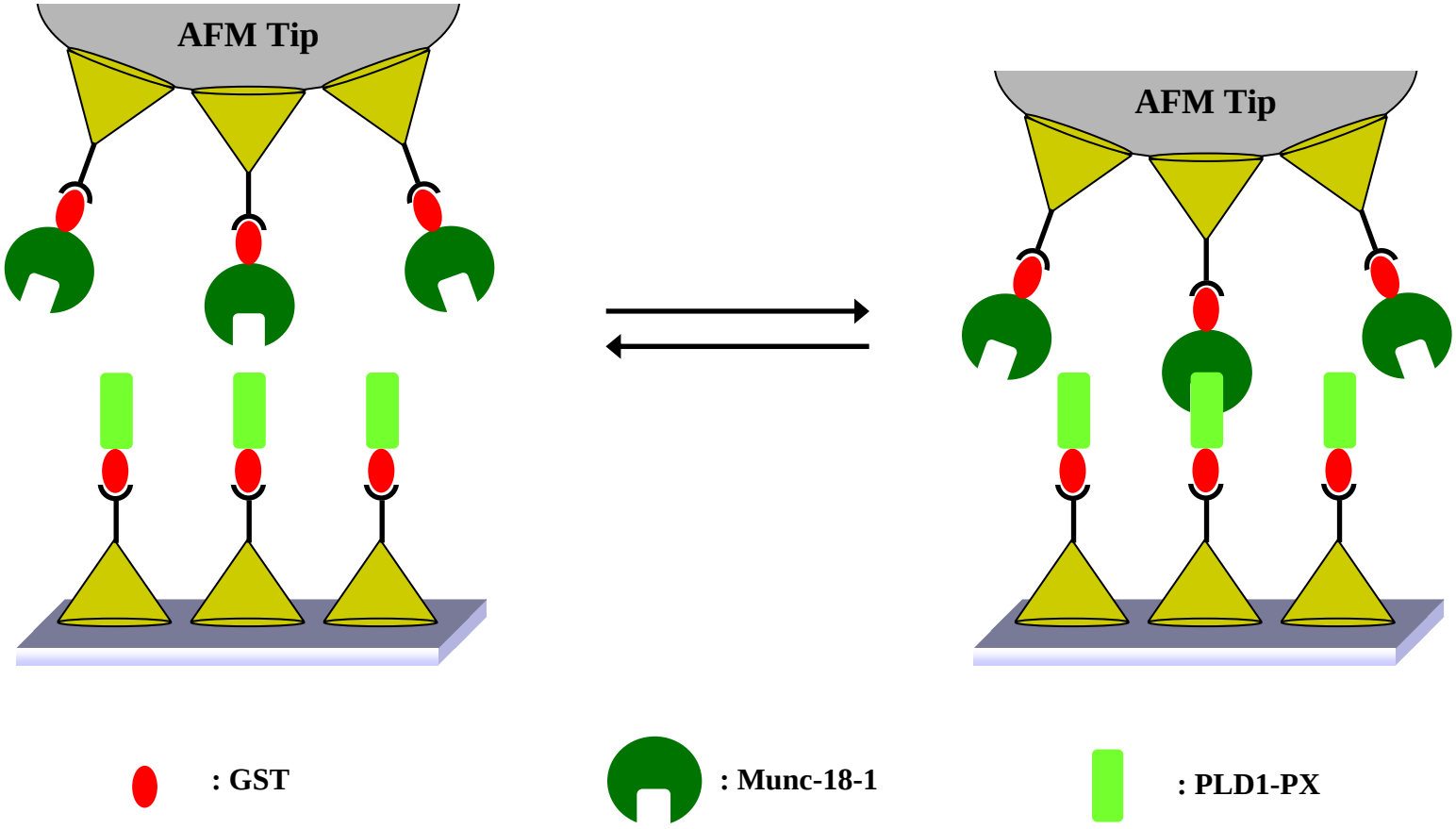


GC content = 60 %

Discrimination of Single Base Mismatched Pairs

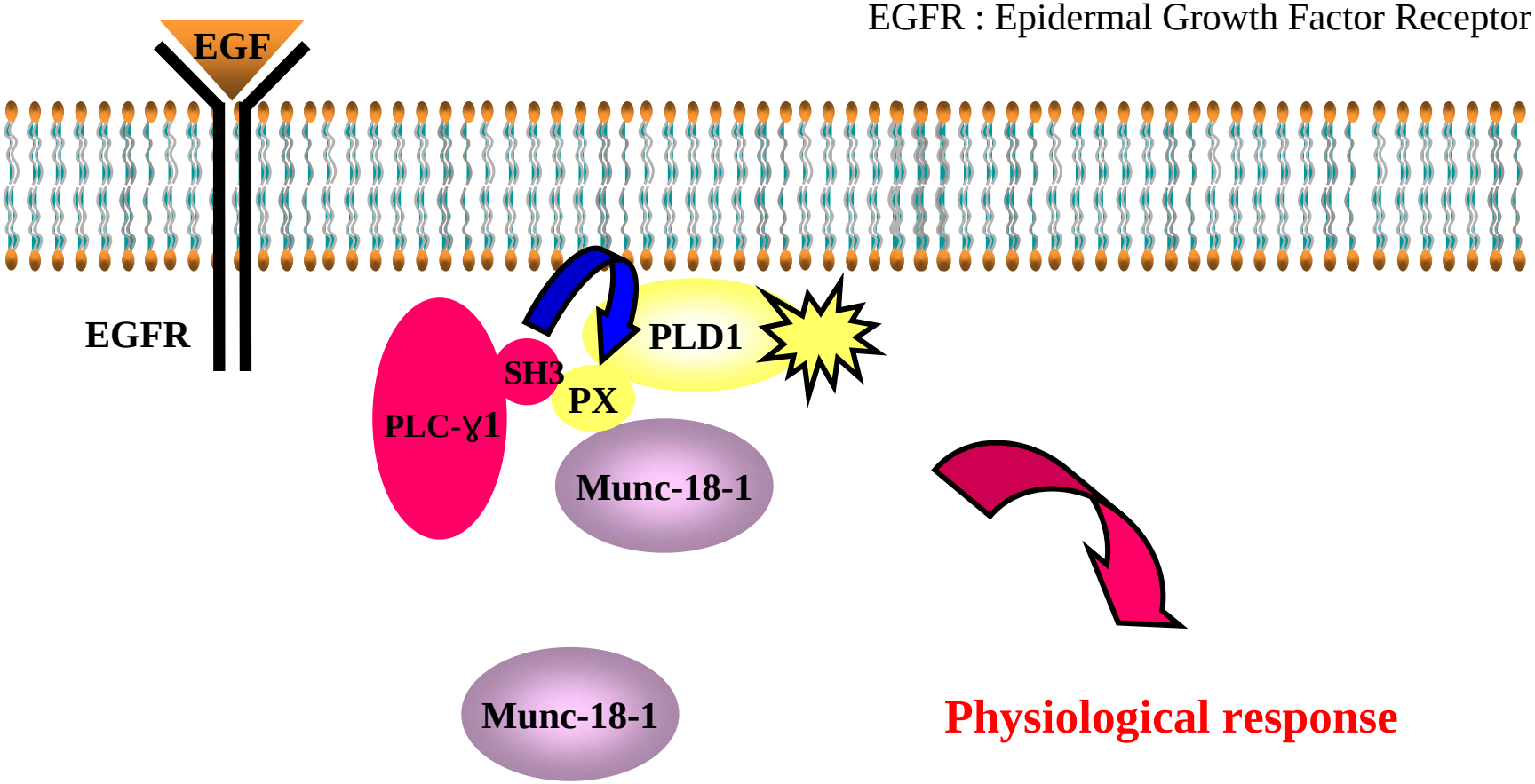


Interaction Force between Signal Transducing Proteins



Interaction between PLD1 and Munc-18-1

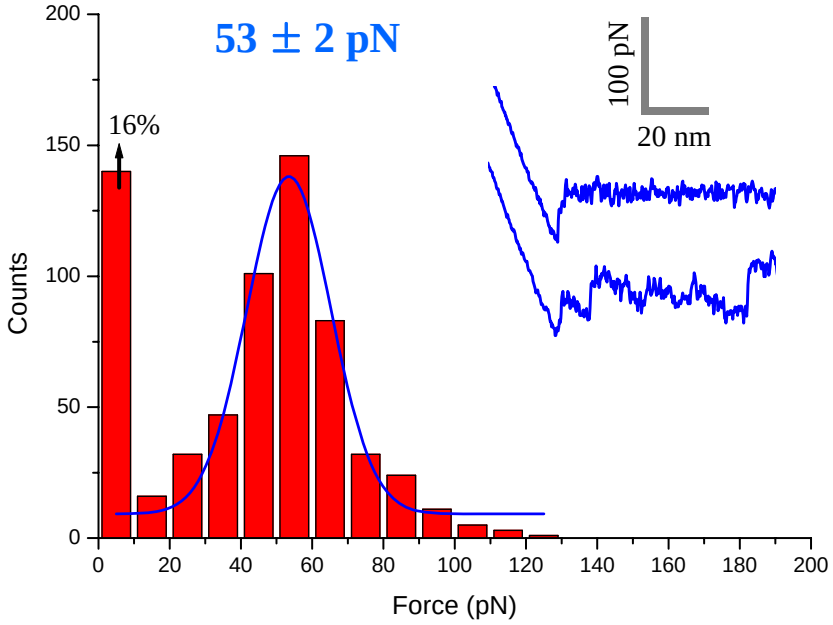
EGF : Epidermal Growth Factor
EGFR : Epidermal Growth Factor Receptor



Spacing Matters !

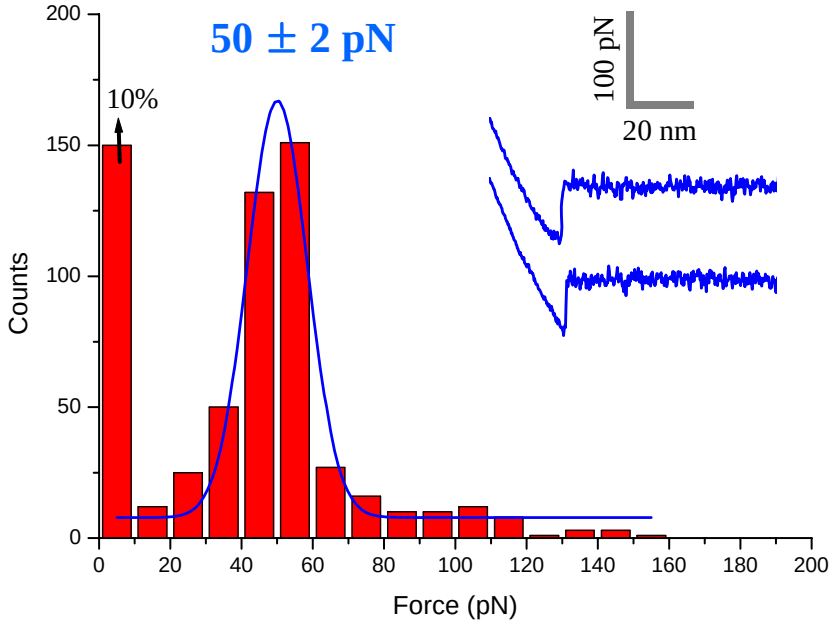
At a proper concentration

❖ **9-acid (~ 3-4 nm)**



Ratio of force-distance curve
Single : Multiple = **2.4 : 1**

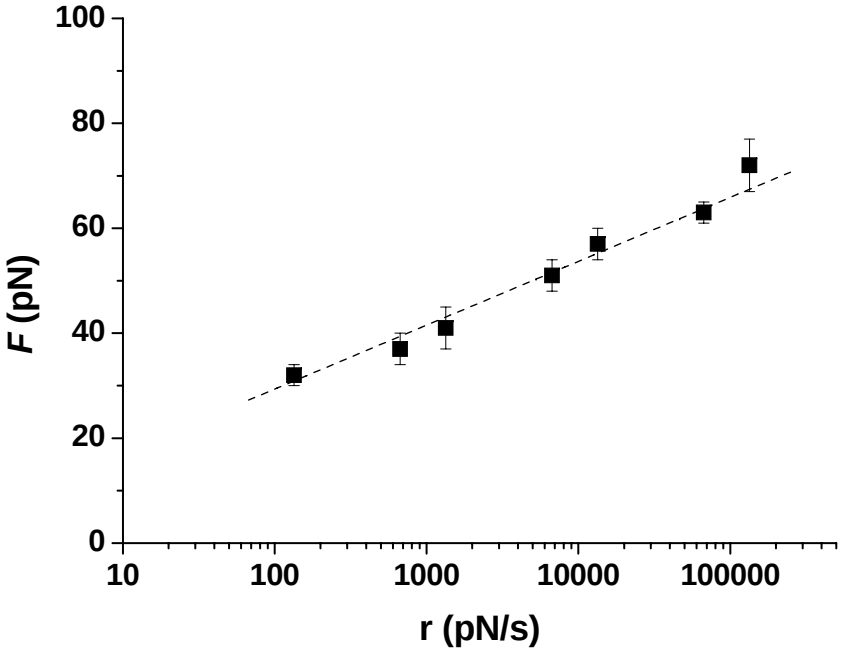
❖ **27-acid (~ 5-6 nm)**



Ratio of force-distance curve
Single : Multiple = **5.4 : 1**

Loading Rate Dependence of the Unbinding Force

PX-Munc-18-1 interaction



$$F = \frac{k_B T}{x_\beta} \cdot \ln\left(\frac{r \cdot x_\beta}{k_{off} \cdot k_B T}\right)$$

Slope : $k_B T / x_\beta$

$x_\beta = 0.77$ nm

Extrapolated to $F = 0$: $k_{off} = r \cdot x_\beta / k_B \cdot T$

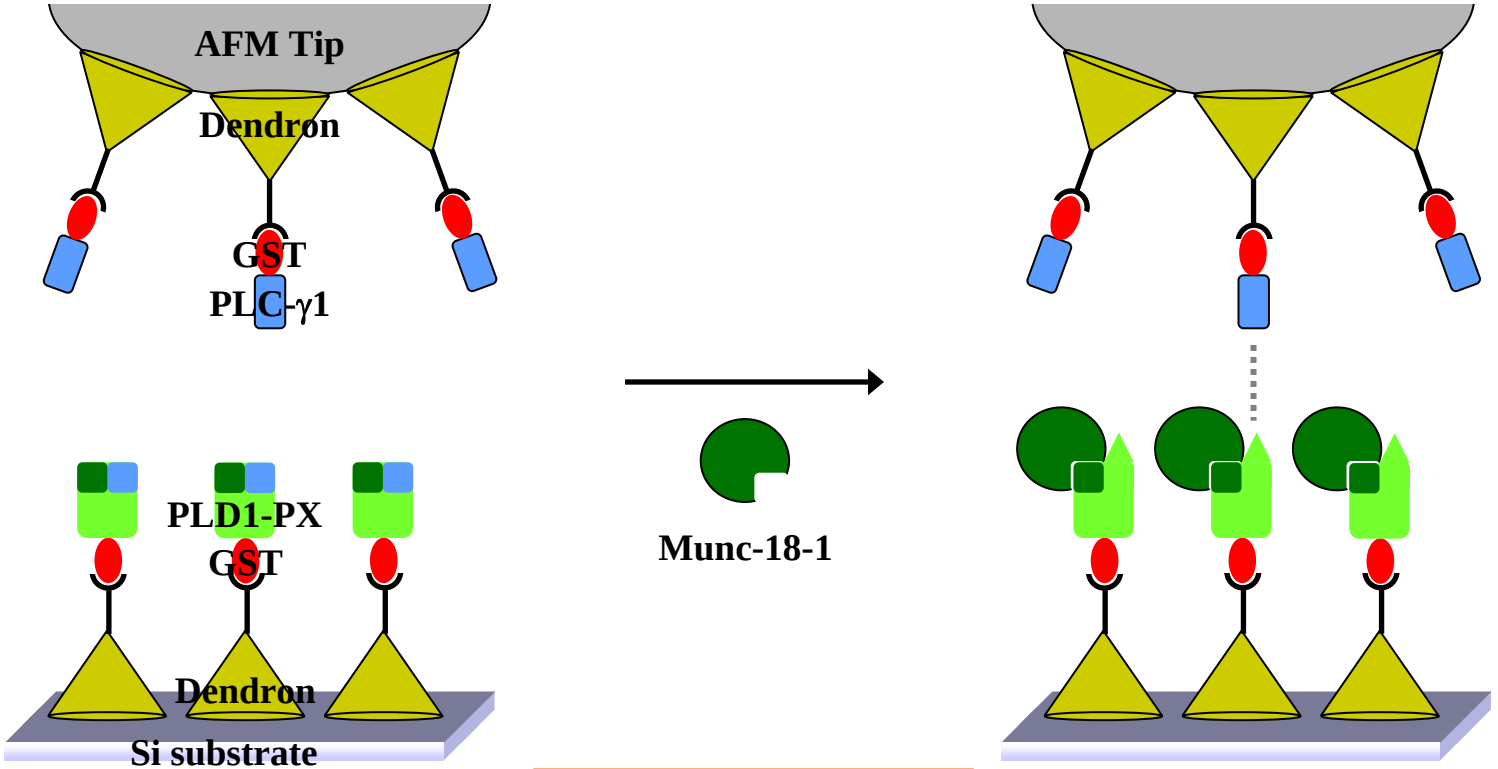
$k_{off} = 7.3 \times 10^{-2}\ s^{-1}$

$K_A = 1.3 \times 10^{9}\ M^{-1}$

$k_{on} = 9.5 \times 10^{7}\ M^{-1}s^{-1}$

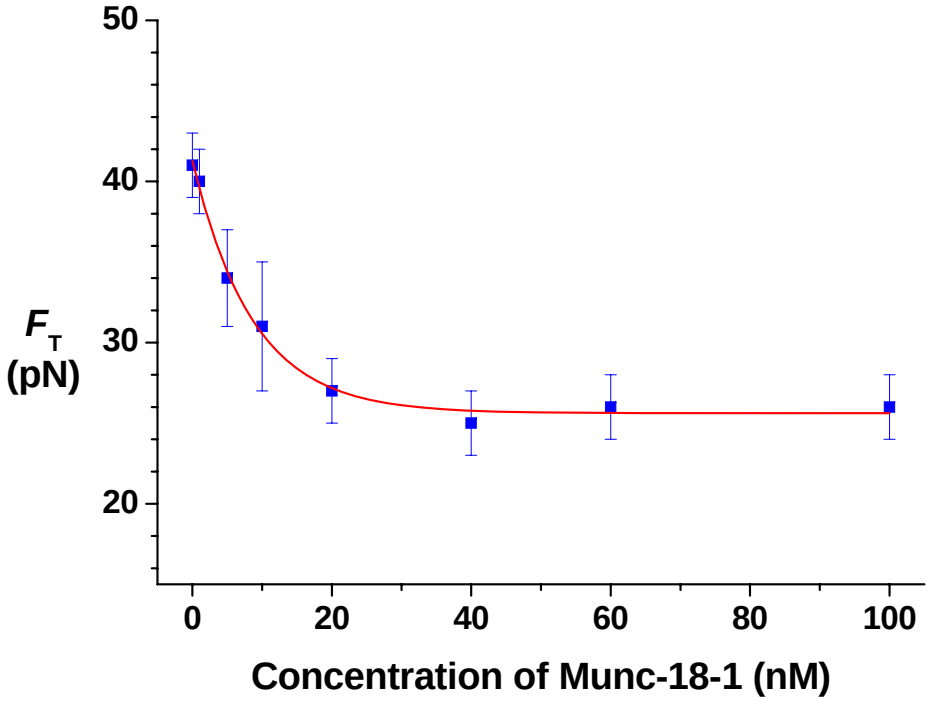
$K_D = 7.7 \times 10^{-9}\ M$

Modulation of the Force with Munc-18-1



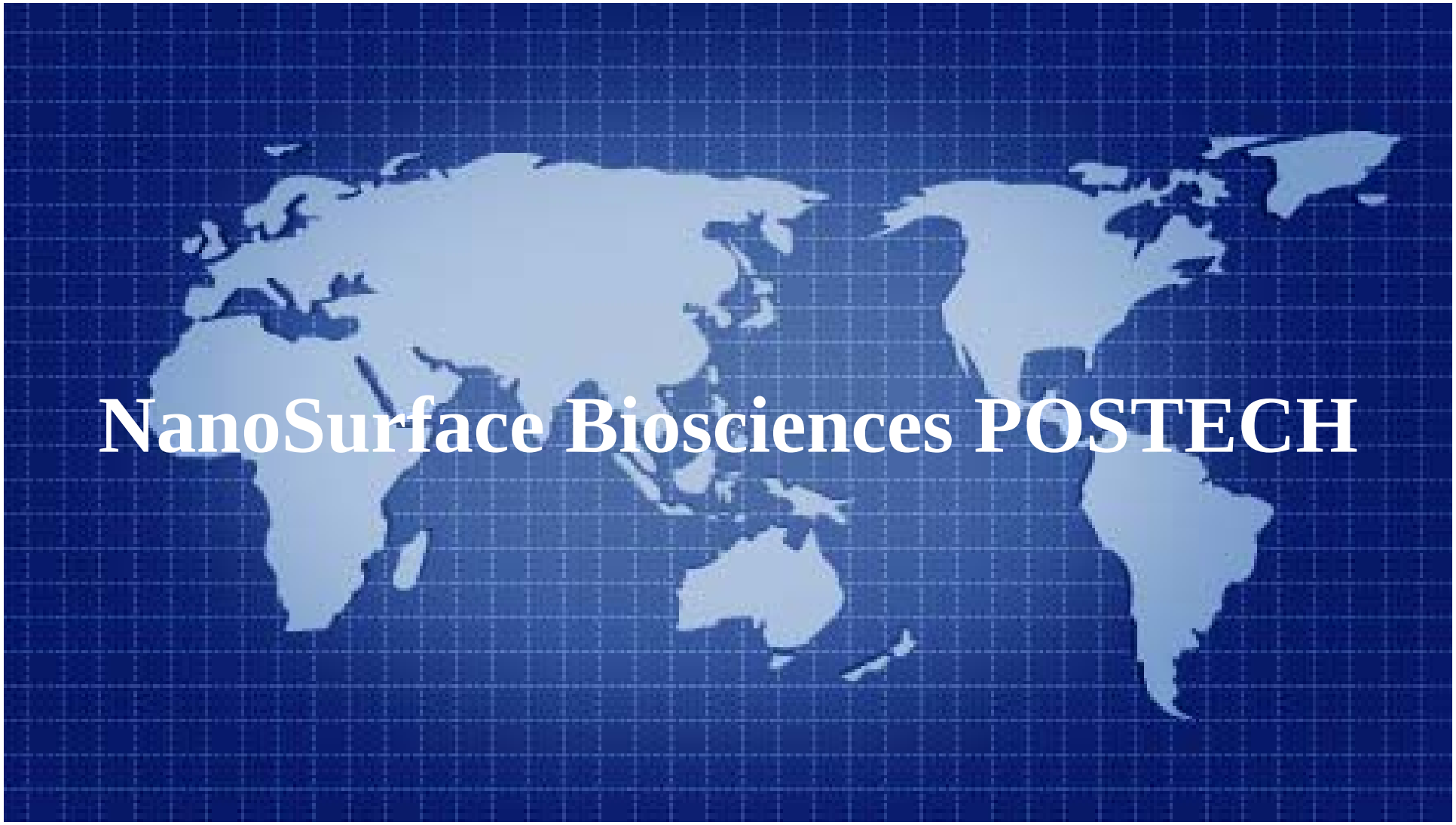
k = 10.2 ~ 12.2 pN/nm
r = 0.5 μm/s
At 15 ˚C in PBS

Titration Curve with Munc-18-1



Langmuir fitting shows that K_A between PLD1 and Munc-18-1 is 1.3 x 10^9 M^{-1}.

Manuscript in preparation



August 2007

www.nsbpostech.com

NSB POSTECH At a Glance

- The first POSTECH-founded company.
- NSB POSTECH specializes on surface science for biological application.
- The first commercial products, dendron-coated glass slides for biochips, have been launched in the 4th quarter of 2006.
- The second line of products, dendron-coated AFM tips, will be launched in the 3rd quarter of 2007.
- Efficacy of our technology has been confirmed internationally and over 10 US patents have been filed.
- Revenue > 1M $ in US in the second year.

Short-Term Goals

- **Sales of Biochip Plates and Coated AFM-Tips**
  - **Cash Cows**
  - **Expedite FDA Approval of Biochips**
- **Sales of Biochips (Non-FDA Approval Items)**
  - **Research Uses**
  - **Animal Diagnostics**
- **Biomarker Discovery**
  - **Collaboration with Renowned US Medical Institutes**

Homepage

주소(D) http://www.nsbpostech.com/

NSB POSTECH NanoSurface Biosciences

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- COMPANY
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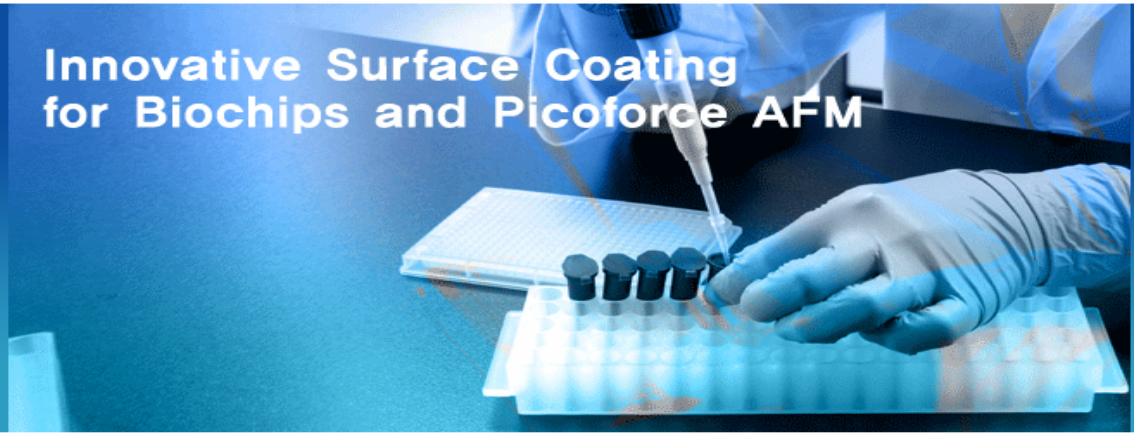
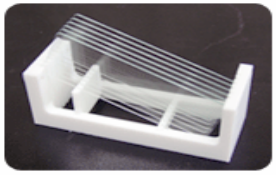


PRODUCTS



Control of regular spacing between the probes offers probe homogeneity and results in high accuracy and reproducibility
Free samples are available now!

NanoSurface Biosciences POSTECH

NSB POSTECH is the first POSTECH-owned enterprise dedicated to commercializing biochip and AFM tip products based on the proprietary NSB Technology, developed by Professor Joon Won Park and his coworkers at Pohang University of Science and Technology (POSTECH). It plans to spin off a start-up venture in 2007 in order to realize globalization of NSB POSTECH. Currently pilot production of NSB slide for DNA microarray has been completed successfully, and our first product, NSB amine slide is on sale. Efficacy of the slide has been confirmed by top players in diagnostic and DNA microarray-based service companies. In addition, beta test of NSB AFM tip is under progress and the product will be launched soon.

webmaster@nsbpostech.com | Address : San 31 Hyo-ja Pohang 790-784, Korea, POSTECH | President : Chan-Mo Park |
TEL : 82-54-279-8414~8 | FAX : 82-54-279-8419 | Business registration : 506-82-02384 | e-trade registration : Pohang 06-162 | User Agreement

Products Overview

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PRODUCTS
NanoSurface Biosciences

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Overview

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If you want the best result with your DNA microarray,
the NSB slide helps you.

The proprietary NSB technology of NSB POSTECH can enhance the performance of biomolecular interation on surface. This technology allows precise control of regular spacing between surface immobilized biomolecules and minimized steric hindrance and optimal distance between biomolecules make them mimic solution-phase behavior. Our first product, NanoCone slide can help you prepare a highly efficient DNA microarray by ensuring high selectivity and low background signal without any blocking agent. Especially, gene expression profiling studies can be performed using cDNAs reverse-transcripted out of 1 μg total RNA without further amplification process.

TOP

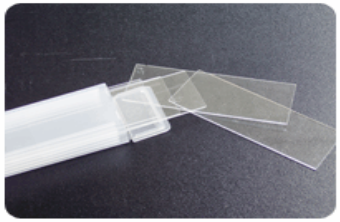
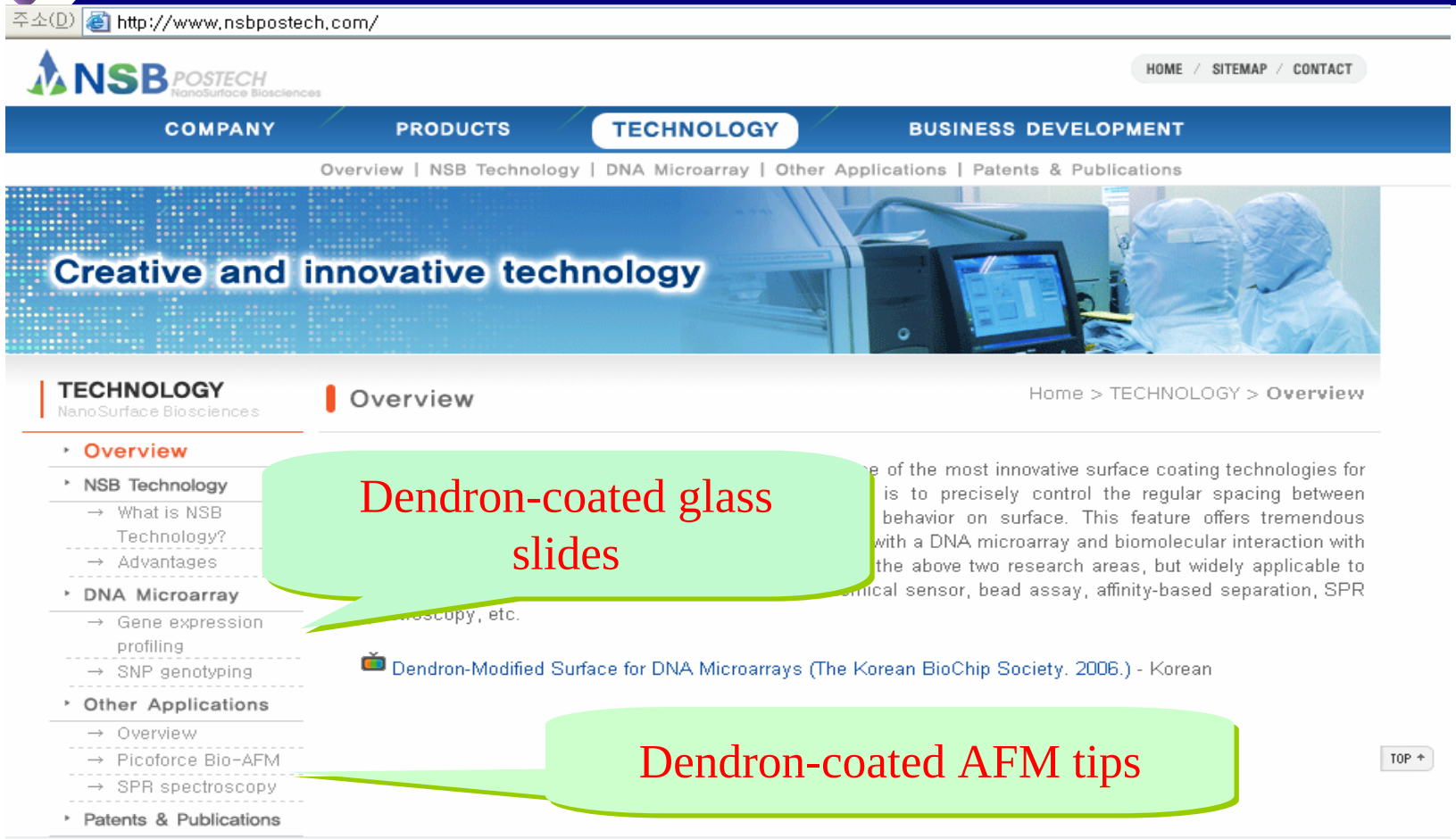


Table1. Type of slide products

Surface functional group	Lateral spacing between surface functional groups	
	3 - 4 nm	6 - 7 nm
Amine	NSB9 Amine Slide	NSB27 Amine Slide
Epoxy	NSB9 Epoxy Slide	NSB27 Epoxy Slide
Aldehyde	NSB9 Aldehyde Slide	NSB27 Aldehyde Slide

Technology Overview



Dendron-coated glass slides

Dendron-coated AFM tips