Nanoscale-Controlled Surface Materials, Bioanalysis, and Commercialization

- NANO KOREA 2007 -

Aug 28, 2007

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NanoSurface Biosciences POSTECH
Pohang University of Science and Technology
Biomolecules on the Surface in Various Applications

Biosensors

Biochips (DNA Chip, Protein Chip)

In-situ Kinetic Study of Biomolecules

Biomolecules on the Surface

Affinity Chromatography

High Throughput Screening

Immuoassay
Biomolecules Require Mesospacing for Comfortable Anchoring on the Surface
Peptide Folding on the Spacing Controlled Gold Surface

An Optimized Dendron
Inert Base Layer and Covalent Bonding
Immobilization of Gold Nanoparticles at the Dendron Surface

Substrate → Organic interlayer → Dendron

Deprotection → Gold nanoparticle

d: 1.4 nm

Spacing between Dendron Molecules on Surface

SEM Image

Distance between Dendron Molecules

- Particle number / area: 130 ea / 50 x 50 nm²
- Density: 0.05 – 0.06 ea/nm²
- 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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Pohang University of Science and Technology
KOREA
Fabrication of DNA Microarray on Dendron-Modified Surface

Substrate

Dendron

One base mismatch site

PM site

MM site


100 : < 1
Probe & Target Oligonucleotides

**Probe oligonucleotide**

Probe 1: 5′-NH₂-C₆-CAT TCC GXG TGT CCA-3′
Probe 2: 5′-NH₂-C₆-(T)₃₀-CAT TCC GXG TGT CCA-3′

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

**Target oligonucleotide**

Target 1: 5′-Cy₃-TGG ACA CT C GGA ATG-3′
Target 2: 5′-Cy₃-CCT ACG AAA TCT ACT GGA ACG AAA
    TCT ACT TGG ACA CT C GGA ATG-3′
15-Base oligonucleotide (Probe 1) & 15-Base oligonucleotide (Target 1)

<table>
<thead>
<tr>
<th></th>
<th>PM(AT)</th>
<th>IMM(TT)</th>
<th>IMM(GT)</th>
<th>IMM(CT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normalized</td>
<td>100</td>
<td>0.5</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Signal Ratio</td>
<td></td>
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</tbody>
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Hybridization condition: 1 nM target at 50 °C for 1 h
Molecular beacon in solution: 100: ~1
SNP discrimination ratio (Wang, H. et al.  

(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

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

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

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

- **No blocking, 42°C with 30% DMF**
- **Blocking, 42°C with 30% DMF**
- **No blocking, 65°C**
- **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

- 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, $p = 0.01$

- Superb correlation was maintained even with 1 μg of total RNA.
“For adequate fluorescence, the total RNA required …… per array, is 50-200 μg.”

*Nat. Genetics Suppl. 21, 10 (1999)*
Yu Jin Jung and Joon Won Park

In collaboration with School of Pharmacy, Nottingham University
Force – Distance Curve from AFM

\[ F = -kd \text{ (Hook’s Law)} \]
Force – Distance Curve for DNA-DNA

\[ a = 5'\text{-}G\text{-}G\text{-}C\text{-}T\text{-}C\text{-}C\text{-}C\text{-}T\text{-}T\text{-}C\text{-}T\text{-}A\text{-}C\text{-}C\text{-}A\text{-}C\text{-}T\text{-}G\text{-}A\text{-}C\text{-}A\text{-}T\text{-}C\text{-}G\text{-}C\text{-}A\text{-}A\text{-}C\text{-}G\text{-}G\text{-}3' \]
\[ b = 3'\text{-}C\text{-}C\text{-}G\text{-}A\text{-}G\text{-}G\text{-}G\text{-}A\text{-}A\text{-}G\text{-}A\text{-}T\text{-}G\text{-}G\text{-}T\text{-}G\text{-}A\text{-}C\text{-}T\text{-}G\text{-}T\text{-}A\text{-}G\text{-}C\text{-}G\text{-}T\text{-}T\text{-}G\text{-}C\text{-}C\text{-}5' \]

\[ 48 \pm 2 \text{ pN} \]

Proper Spacing Simplifies Force-Distance Curve

AFM Tip retracting

Force-Distance Curve
Single Dip: > 80%

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

(A) Distance (nm)

(B) Force (pN)

(A)力（pN）

39 pN

(B) 计数

(B) 弹力（pN）

25 pN
It is Now a Reversible Process

AFM Tip Approaching

AFM Tip Retracting

Resolved Binding Force Histogram

GC content = 60 %
Discrimination of Single Base Mismatched Pairs

![Graph showing the force vs. counts for different mer sequences: 20 mer, 30 mer, 40 mer, 50 mer. Peaks at 27 pN, 37 pN, 43 pN, and 50 pN for different mismatches (-2), (-7), (-9)].
Interaction Force between Signal Transducing Proteins

AFM Tip

: GST

: Munc-18-1

: PLD1-PX
Interaction between PLD1 and Munc-18-1

EGF : Epidermal Growth Factor
EGFR : Epidermal Growth Factor Receptor

Physiological response
Spacing Matters!

At a proper concentration

- **9-acid (≈ 3-4 nm)**
  - 53 ± 2 pN
  - Ratio of force-distance curve: Single : Multiple = 2.4 : 1

- **27-acid (≈ 5-6 nm)**
  - 50 ± 2 pN
  - Ratio of force-distance curve: Single : Multiple = 5.4 : 1
Loading Rate Dependence of the Unbinding Force

PX-Munc-18-1 interaction

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

Slope: \( k_B T / x_\beta \)

\( x_\beta = 0.77 \text{ nm} \)

Extrapolated to \( F = 0 \): \( k_{\text{off}} = \frac{r \cdot x_\beta}{k_B \cdot T} \)

\( k_{\text{off}} = 7.3 \times 10^{-2} \text{ s}^{-1} \)

\( K_A = 1.3 \times 10^9 \text{ M}^{-1} \)

\( k_{\text{on}} = 9.5 \times 10^7 \text{ M}^{-1}\text{s}^{-1} \)

\( K_D = 7.7 \times 10^{-9} \text{ M} \)
Modulation of the Force with Munc-18-1

AFM Tip
Dendron
GST
PLC-γ1

PLD1-PX
GST
Dendron
Si substrate

Munc-18-1

\[ k = 10.2 \sim 12.2 \text{ pN/nm} \]
\[ r = 0.5 \text{ μm/s} \]
At 15 °C in PBS
Langmuir fitting shows that $K_A$ between PLD1 and Munc-18-1 is $1.3 \times 10^9$ M$^{-1}$.

Manuscript in preparation
NanoSurface Biosciences POSTECH

August 2007

www.nsbpostech.com
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
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's DNA microarray has been completed successfully, and its first product, NSB amino 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's AFM tip is under progress and the product will be launched soon.

Free samples are available now!
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 interaction 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-transcribed out of 1 pg total RNA without further amplification process.

Table 1: Type of slide products

<table>
<thead>
<tr>
<th>Surface functional group</th>
<th>Lateral spacing between surface functional groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.4 nm</td>
</tr>
<tr>
<td>Amine</td>
<td>NSB9 Amine Slide</td>
</tr>
<tr>
<td>Epoxy</td>
<td>NSB9 Epoxy Slide</td>
</tr>
<tr>
<td>Aldehyde</td>
<td>NSB9 Aldehyde Slide</td>
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</tbody>
</table>
Technology Overview

Dendron-coated glass slides

Dendron-coated AFM tips