

Electrical and fluorescence based detection for biospecific interactions using SWNTs

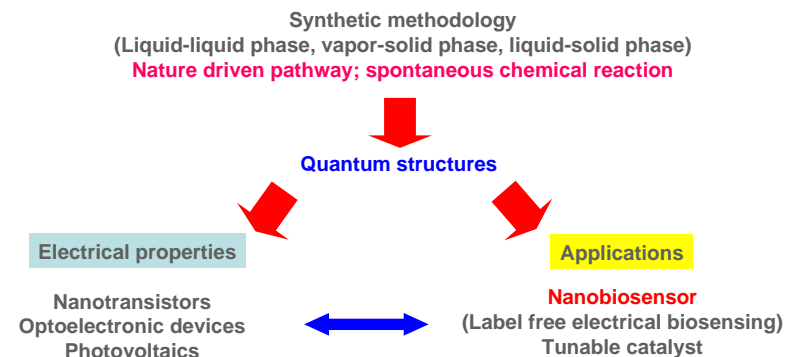
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Hee Cheul Choi
Department of Chemistry
Nanoscale Materials Research Laboratory (NMRL)
Pohang University of Science and Technology (POSTECH)

NMRL's interests

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Contents

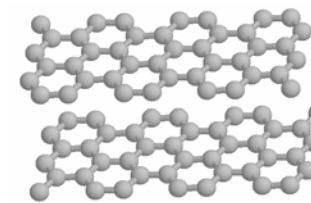
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- I. Electronic CNT-FET devices for biosensing application
- II. Fluorescence-microarray based carbon nanotube protein chip

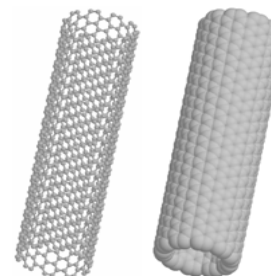
Structure of carbon nanotubes

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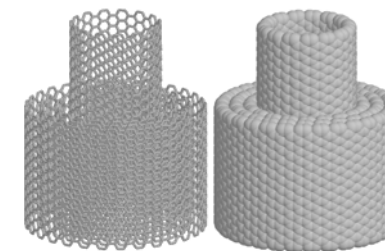
- Nanotubes consist of graphene sheets of carbon
- Rolled into a cylinder
- Some with multiple concentric cylinders



Graphite



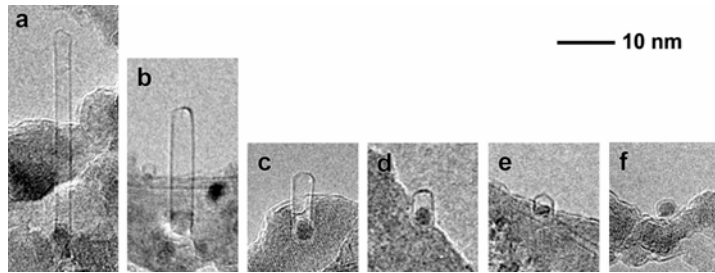
Single-walled nanotube (SWNT)



Multi-walled nanotube (MWNT)

Nanoparticle assisted growth of CNT

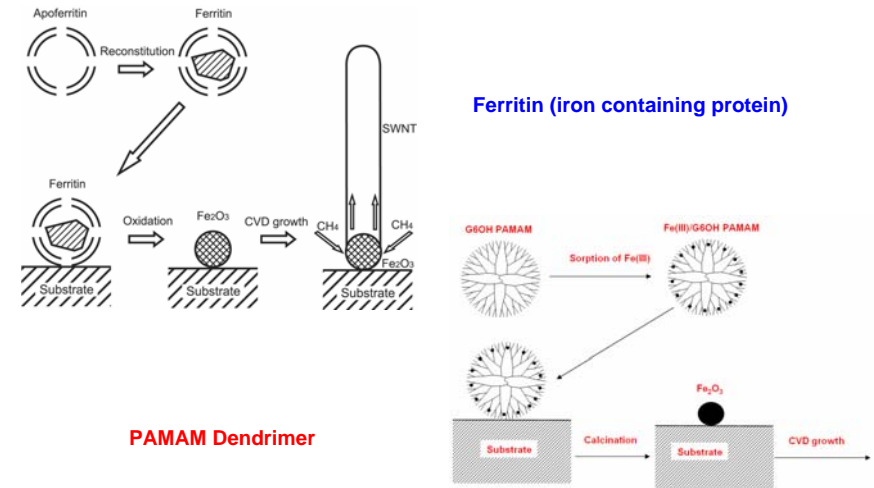
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- Particle size is corresponding to the nanotube diameter
- Catalytic particles (active end) remain on support
- The other end is dome-closed
- Base growth (differs from the VLS growth mode)

Diameter controlled catalyst nanoparticles

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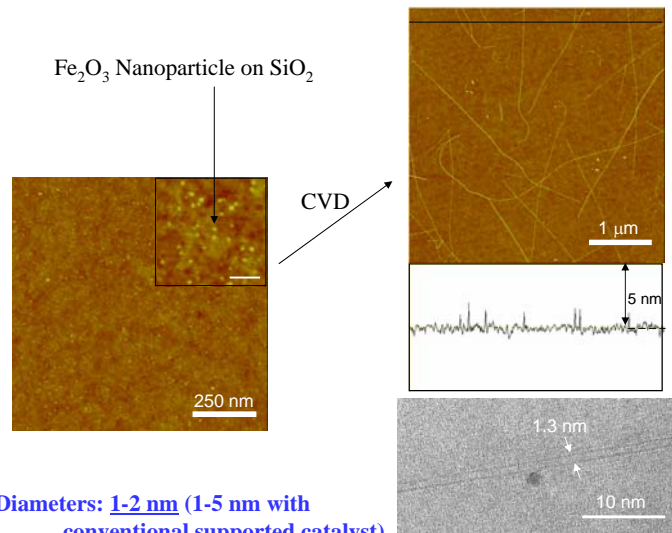


Choi, H. C. et al. *J. Phys. Chem. B* **2002**, 106, 12361.

Y. Li, et al., *J. Phys. Chem. B*, **105**, 11424, 2001

Nanotube growth using dendrimer driven nanoparticles

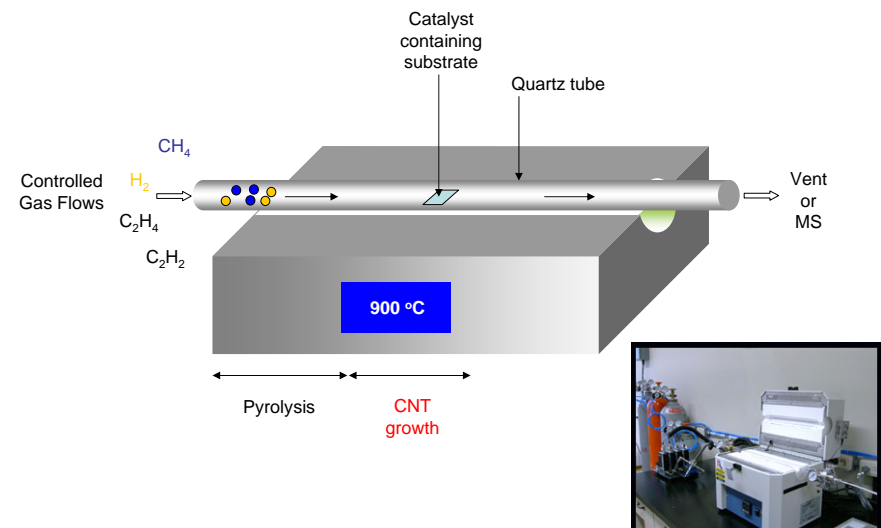
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- **Diameters: 1-2 nm (1-5 nm with conventional supported catalyst)**

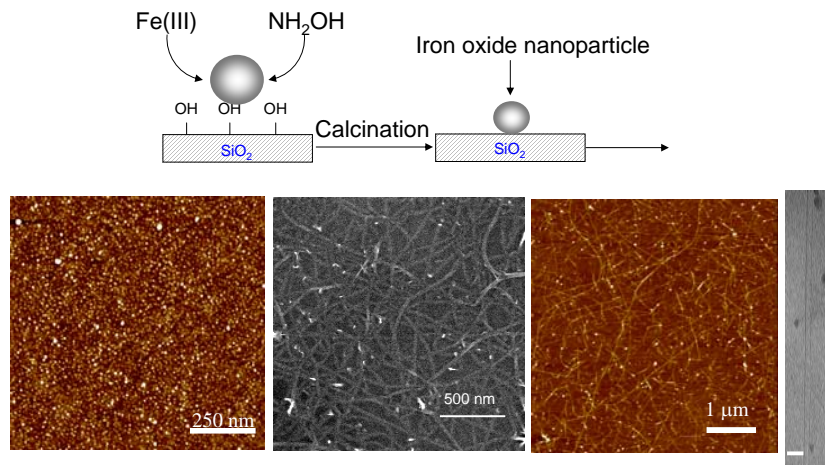
Chemical Vapor Deposition (CVD)

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Thin film of pure SWNT by CVD – New catalyst NPs

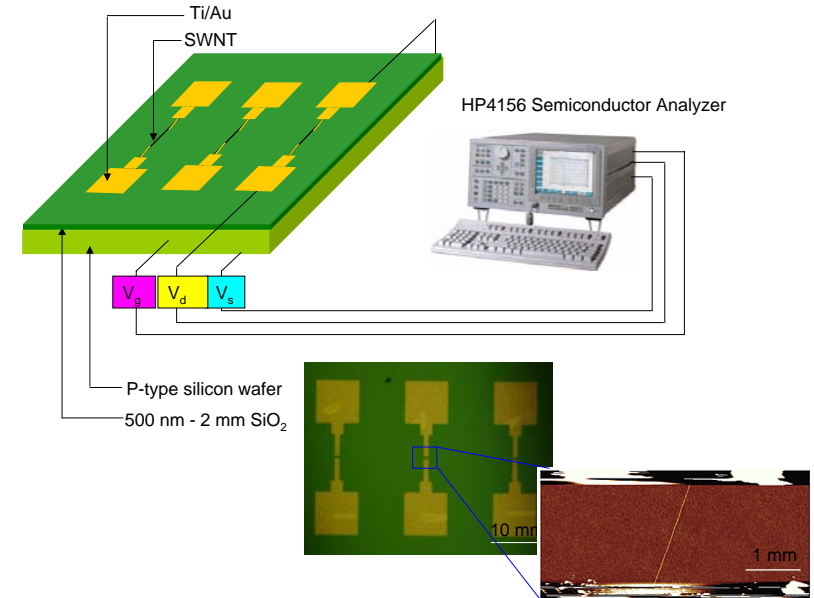
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Choi, H. C. et al., *Nano. Lett.* **2003**, 3, 157.

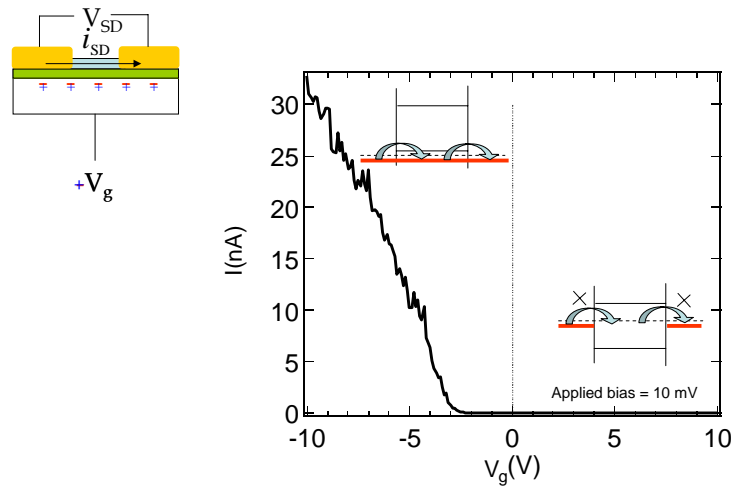
Process for the fabrication of CNT-FET

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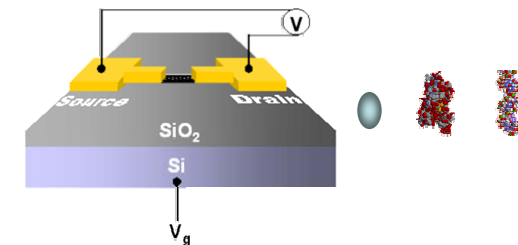
I-V_g characteristics of CNT-FET

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CNT-FET as a smart sensor

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Conventional CNT-FET

- Change of I_{DS} by the effect of V_{GS}
- V_{GS} by electric field

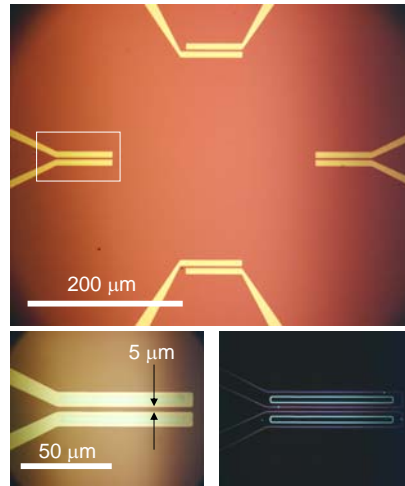
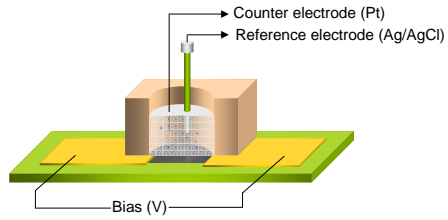
CNT-Chemical Effect Transistor (CET)

- Change of I_{DS} NOT by the effect of V_{GS}
- Why not by chemical effects?
 - * Charge transfer from molecules to CNT

CNT-FET device for biosensor applications

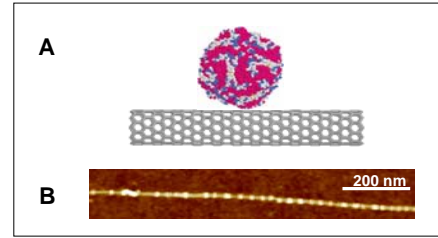
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-Teflon based electrochemical cell-

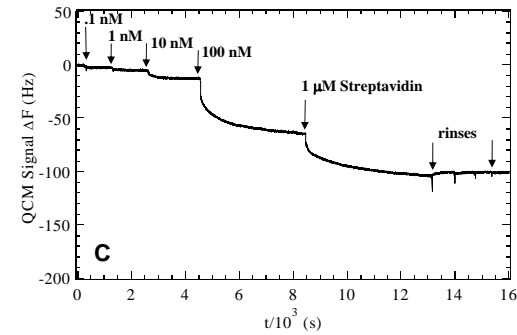


Non-specific interaction of SWNT with proteins

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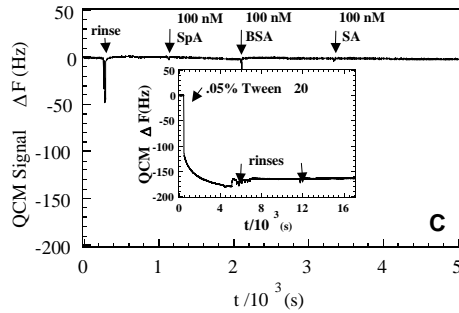
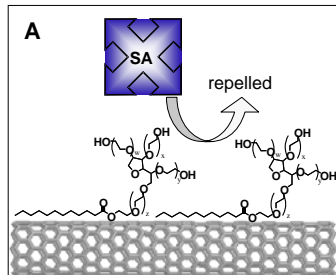
Turns out to be generic:
Streptavidin, Protein A,
Glucosidase, Bovine
Serum Albumin, IgG...



Chen et al, *PNAS* 2003, 100, 4984

Hydrophobic/vdW anchoring of Tween20/PEG

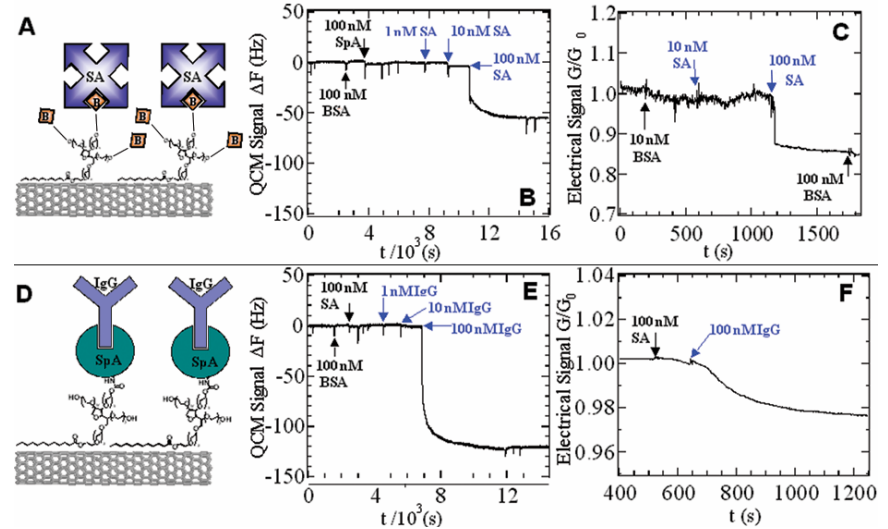
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- ❖ Non-covalent irreversible adsorption
- ❖ Water solubility, highly stable
- ❖ Protein resistant
- ❖ Tween 20 & Pluronic block copolymer P103 are the best

Selective electronic biosensor

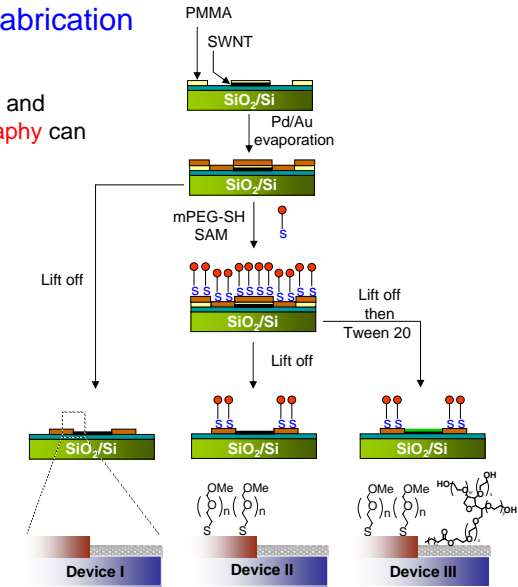
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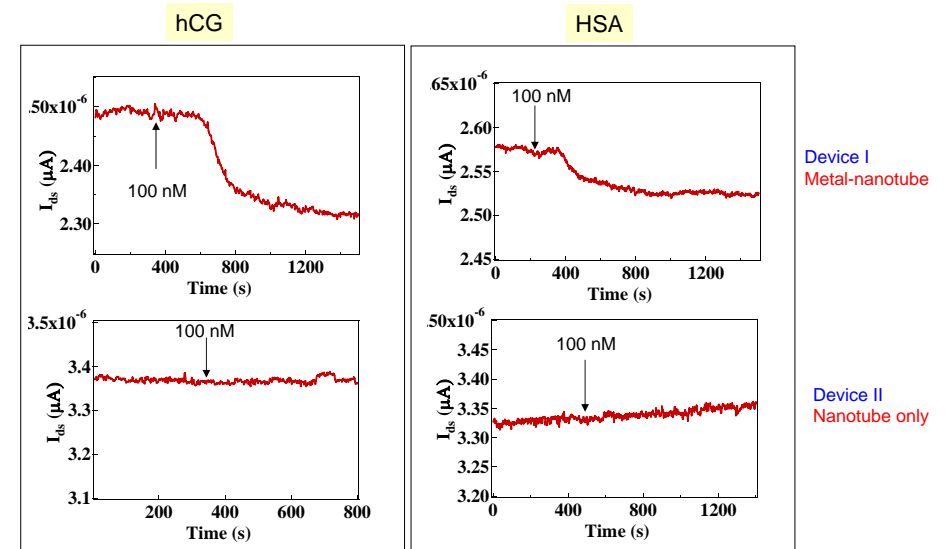
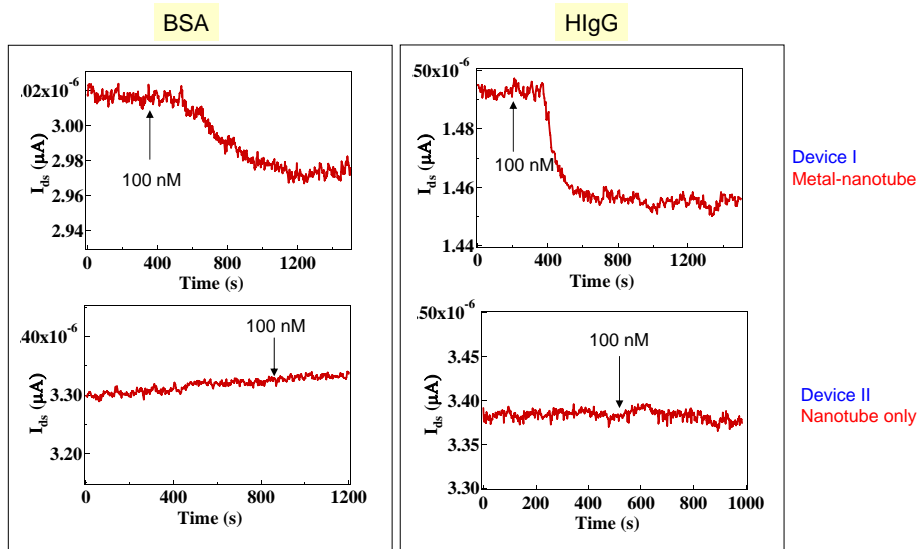
Where does the conductance change come from?

- Nanotube aspects:
 - Charge injection from biomolecules
 - Electric double layer field modulation caused by biomolecules
- Metal-nanotube contact aspect:
 - Adsorbed chemical species may modulate work function level of contact metals, which consequently change the Schottky barrier height resulting in the conductance change.

Both photolithography and electron-beam lithography can be used

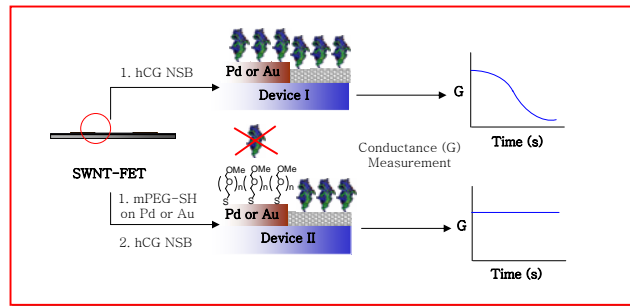


Chen, Choi et al. *J. Am. Chem. Soc.* **2004**, 126, 1563



Summary of biomolecule sensing mechanism

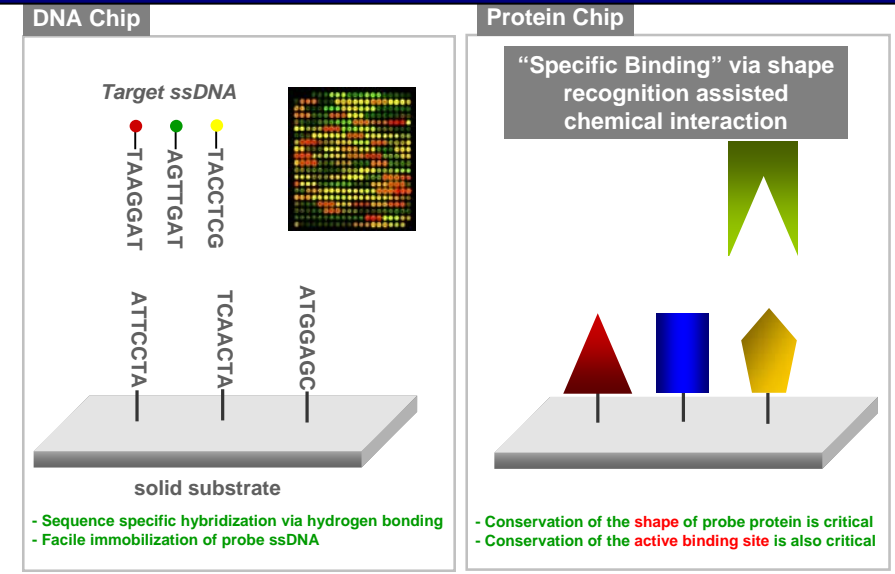
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Effective functionalization of metal surface with appropriate chemical species will lead high sensitive and selective nanotube-biosensor.

Biochip: DNA vs. Protein Chip

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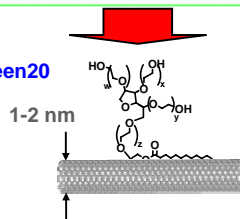
Importance of density control of probe biomolecules

Hurdles for protein chip applications

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- Preservation of active sites
 - Introducing orientation directing linker molecules
- Preservation of protein shapes
 - Requires smart substrates providing only limited (minimum contact surface area)
 - Highly porous inorganic, organic structures (polymers, porous silicon)
- Effective protection of the chip from nonspecific binding (NSB)
 - Bovine serum albumin (BSA) generally used by space filling and repelling proteins
 - Protein resisting polymers (PEG, Triton X100, Tween20, etc.)

Spontaneous coating of Tween20

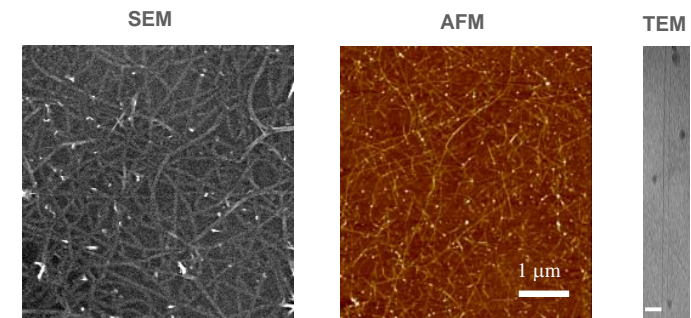


MacBeath, and Schreiber *Science* 2000, 289, 1760
Kim, Y. D. *et al, Biotechnol Bioeng.* 2001, 73, 331
Ressine, A. *et al, Anal. Chem.* 2003, 75, 6968

High yield SWNT for protein immobilization

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How about carbon nanotubes?

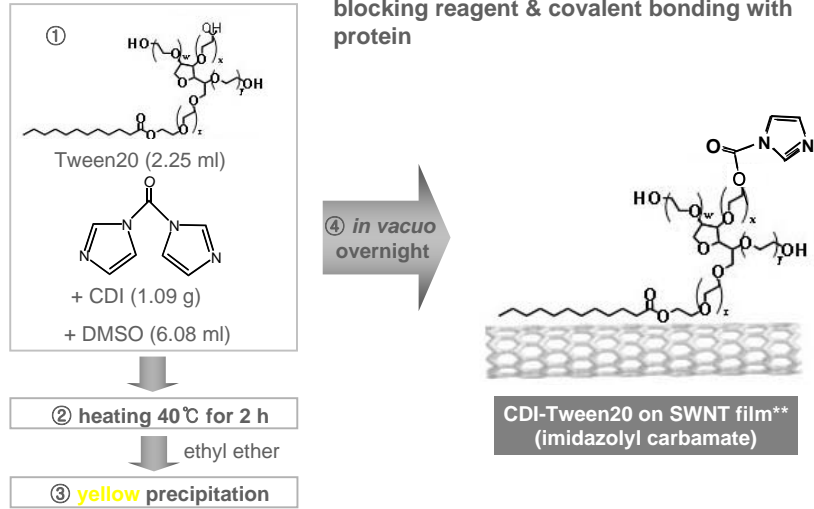


Nanotube strings formed as a pseudo-3D structures
: providing minimum contact surface area

Choi, H. C. *et al., Nano. Lett.* 2003, 3, 157.

Non-covalent functionalization of SWNT

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* 1,1'-carbonyldiimidazole

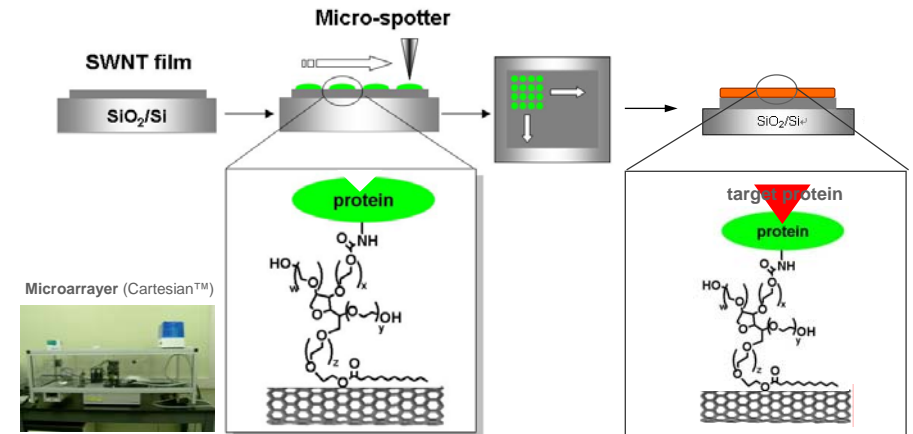
** CDI-Tween20 solution (1wt% in water) soaked SWNT film

Chen, et al, *PNAS* 2003, 100, 4984

Probe protein spot arrays formed using microarrayer

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1. Growth High Yield SWNTs & anchor CDI-Tween20
2. Spotting Probe Protein using Microarrayer, then 3 h incubation with 80% humidity
3. Reaction with target protein for 1 h



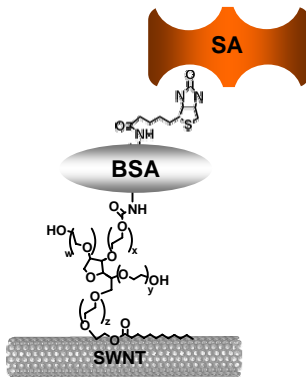
* with PBS (Phosphate Buffered Saline) buffer, pH=7.4 and DI water for 1m

Specific binding and cross-reactivity

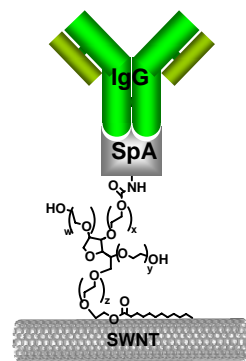
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Representation of specific bindings

biotin-Streptavidin (SA)



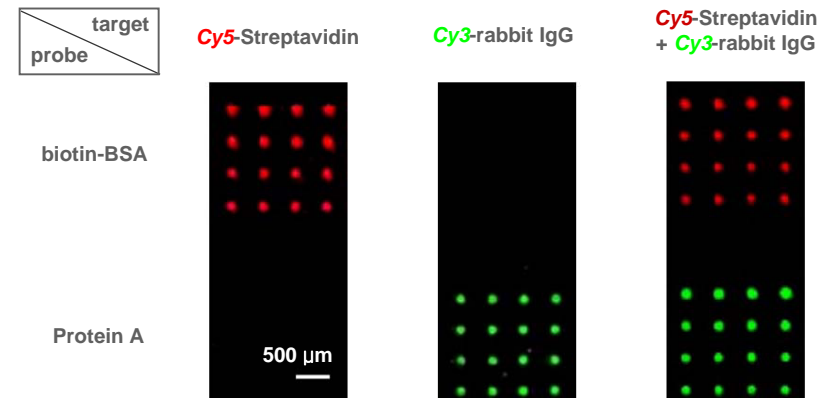
Protein A (SpA)-rabbit IgG



* diluted with PBS buffer, pH = 7.4

Specific binding and cross-reactivity

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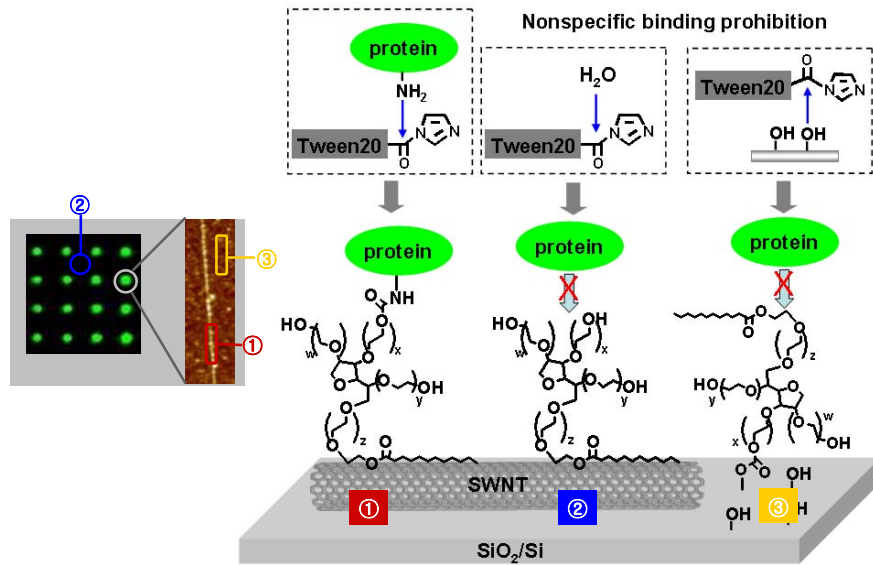


		Target Protein*		
		Cy5-Streptavidin	Cy3-rabbit IgG	Cy5-Streptavidin + Cy3-rabbit IgG
Probe	biotin-BSA	biotin-Streptavidin	no binding	biotin-Streptavidin
Protein*	Protein A	no binding	Protein A-rabbit IgG	Protein A-rabbit IgG

Byun et al, submitted (2004)

Dual function of Tween20

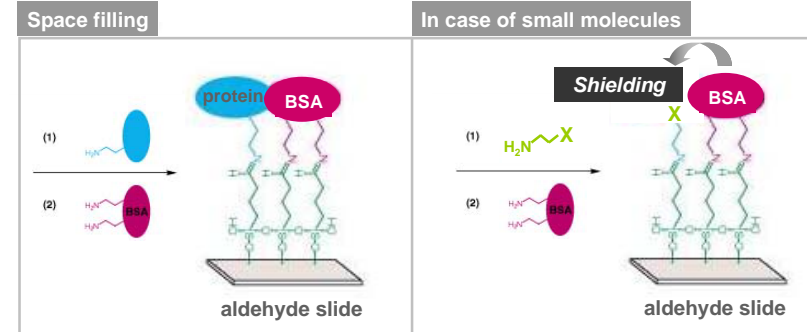
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BSA-free protein chip

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- Non-specific binding blocking agents: BSA vs. **Tween20**

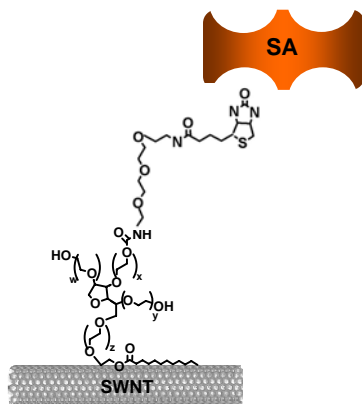


MacBeath, G. *et al*, *Science* **2000**, 289, 1760

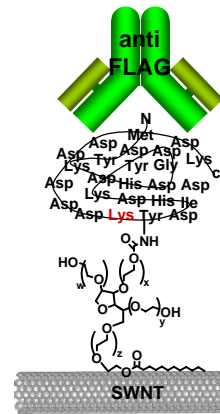
Selective sensing of small biomolecules

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biotin-Streptavidin (SA)

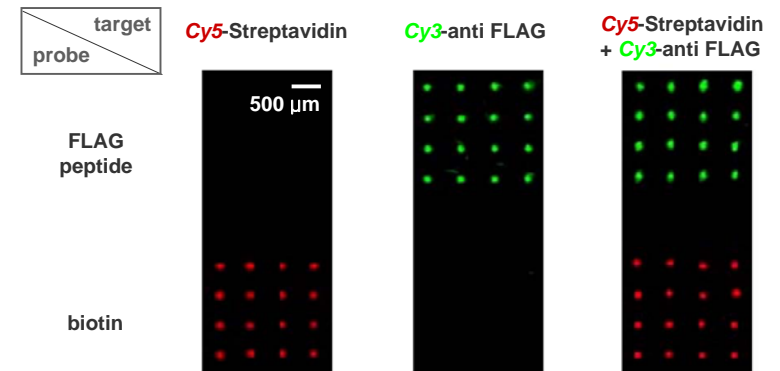


3X FLAG[®] peptide-anti FLAG



Selective sensing of small biomolecules

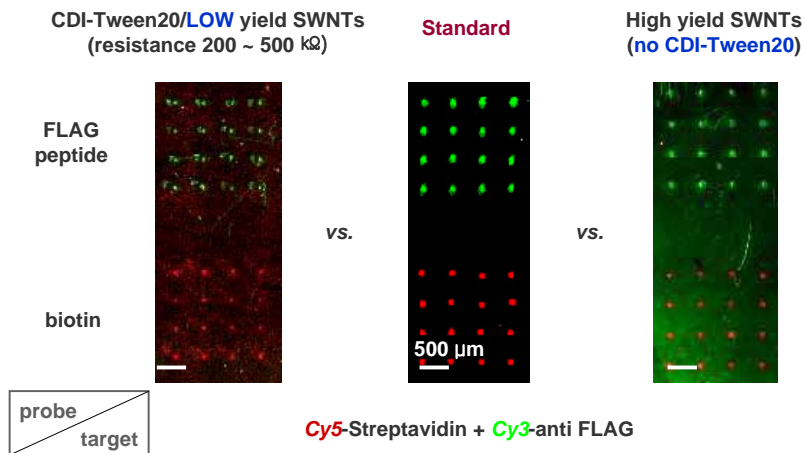
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		Target molecule*		
		Cy5-Streptavidin	Cy3-anti FLAG	Cy5-Streptavidin + Cy3-anti FLAG
Probe	3X FLAG [®] peptide	no binding	3X FLAG-anti FLAG	3X FLAG-anti FLAG
molecule*	biotin	biotin-Streptavidin	no binding	Biotin-Streptavidin

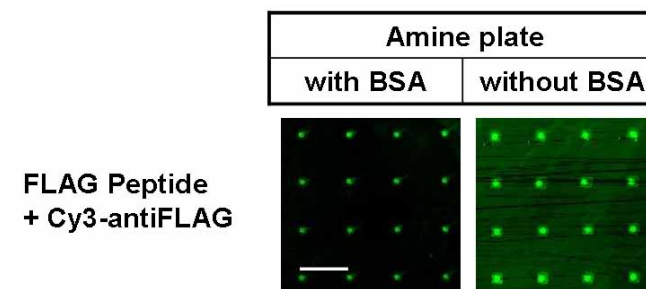
Control experiments

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Control experiments with state-of-the-art substrates

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- Increased background intensity in the absence of BSA
- Decreased spot size in the presence of BSA (~75%)

Summary

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Successful demonstration of **CVD grown SWNT film substrate** for fluorescence based microarray protein chip

- Reproducible formation micro-spots of proteins on SWNT film
- High specific/non-specific binding discrimination
- BSA-free protein chip system respecting to Tween20



Acknowledgements

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Graduate Students

Ms. Hye Ryung Byun

Ms. Yoonmi Lee
Mr. Seok Min Yoon
Mr. Hyunjae Song
Ms. Hynjin Yang

Undergraduate Students

Mr. Hyunsub Lim
Mr. Yungjin Na

Dr. Bong Jin Hong
Prof. Joon Won Park
Prof. Yong Song Gho
(Department of Life Science)



This work was supported by

Air Force Office of Scientific Research (AFOSR, US)
Basic Research Program of the Korea Science & Engineering Program
Center for Integrated Molecular Systems (CIMS)