Rapid, Label-Free, Electrical Nanobiosensor Systems

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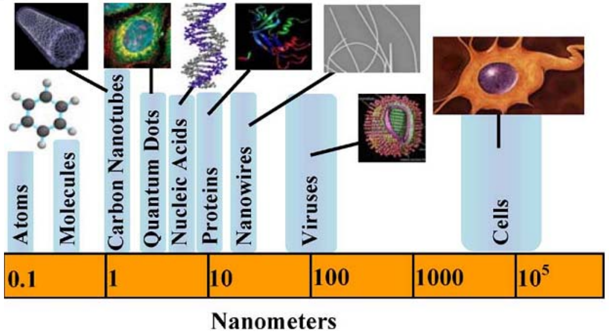
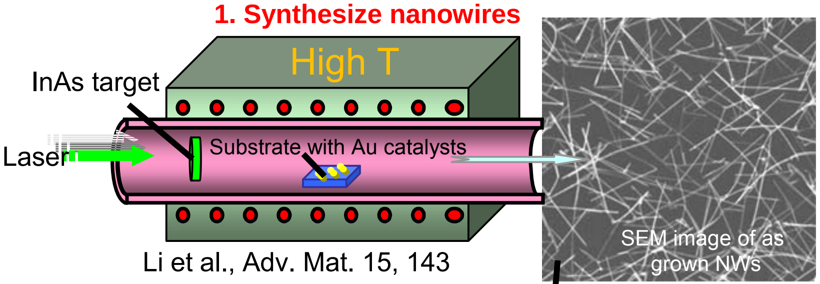
Project Goal



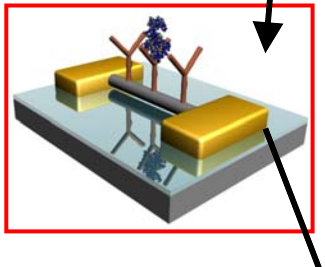
Curreli et al., IEEE Trans. Nanotechnol. 7, 6:651

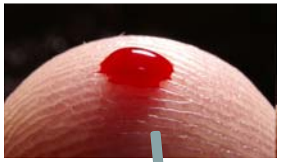
1. Synthesize nanowires

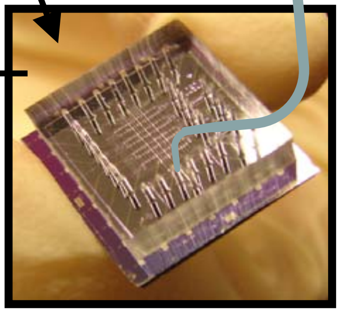


Li et al., Adv. Mat. 15, 143

2. Make transistors with chemically modified surface

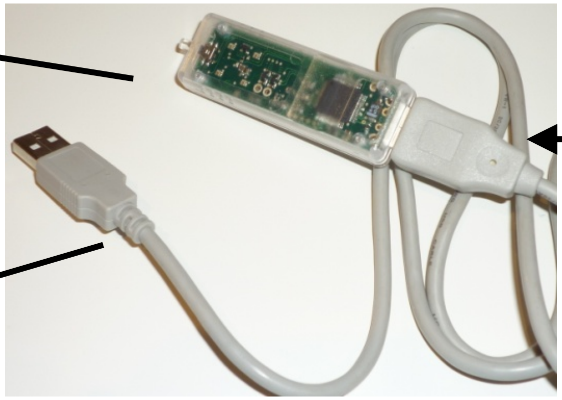






3. On-chip microfluidic processing

4. Packaging and User Interface



Outline

- **Introduction to nanobiosensor systems and indium oxide nanowire (In_2O_3 NW) nanobiosensors**
- **Prostate specific antigen (PSA) detection**
- **SARS detection using antibody mimic proteins (AMPs) as a receptor molecule for nanobiosensors**
- **Rapid, Label-free detection in physiological whole blood environment using ovarian cancer biomarkers**
- **Conclusion**

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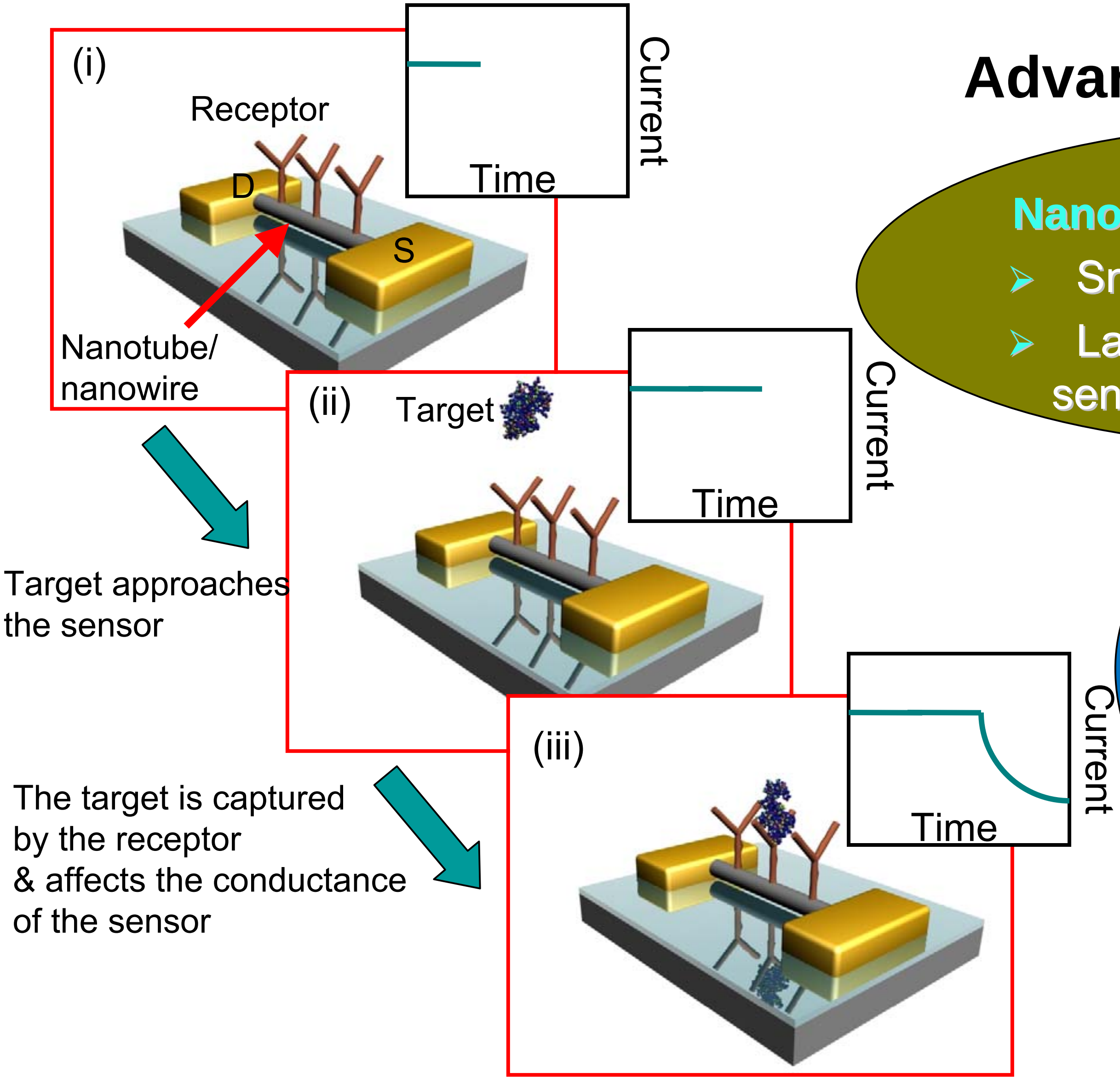
Introduction: Nanobiosensor Background



Target approaches the sensor

The target is captured by the receptor & affects the conductance of the sensor

Advantages

Nanoscale

- Small, nanoscale;
- Large surface to volume ratio; sensitive

Electrical sensor

- Rapid; *in-situ*
- *Label-free*
- Can be easily integrated to electronic components

Introduction: Sensing Mechanism

$$I_{ds} = e\underset{\overset{\nwarrow}{(1)}}{\mu}\varepsilon\underset{\overset{\uparrow}{(2)}}{\varepsilon}_{r}\frac{A}{d}\frac{V_{ds}}{L}\left(V_{g} - V_{\underset{\overset{\nwarrow}{(3)}}{T}}\right)$$

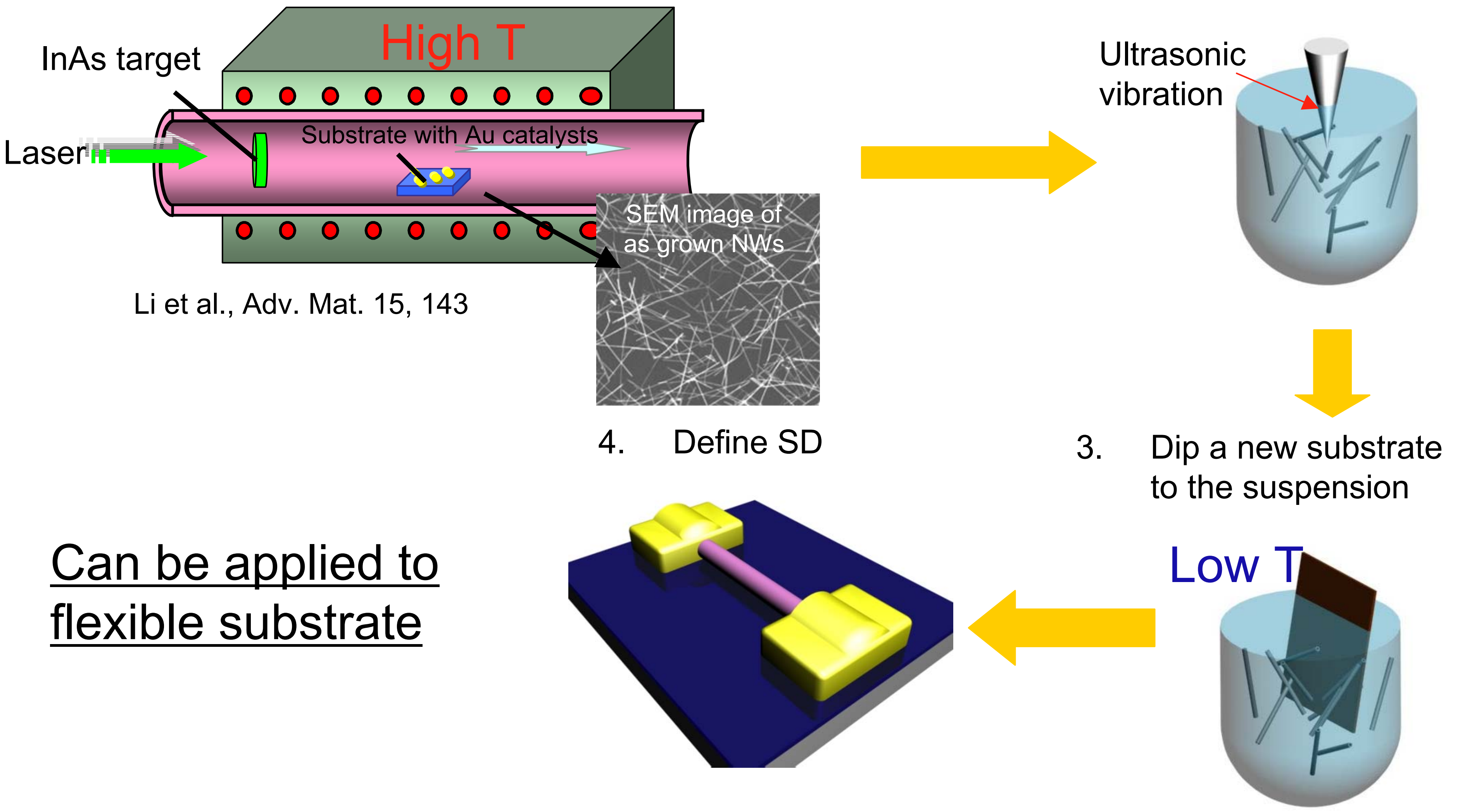
(1) Device mobility change
   1. Bulk mobility
   2. Schottky barrier resistance (barrier height)

(2) Dielectric constant change

(3) Threshold voltage change
   1. Charge transfer
   2. Electrostatic interaction ⟵ Proposed to be dominant

Heller, Nano Lett, 2007,
Stern, Nano Lett., 2007
Nair, Nano Lett., 2008

Introduction: In_2O_3 Nanowire Device Fabrication



1. Synthesize nanowires

2. Disperse nanowires

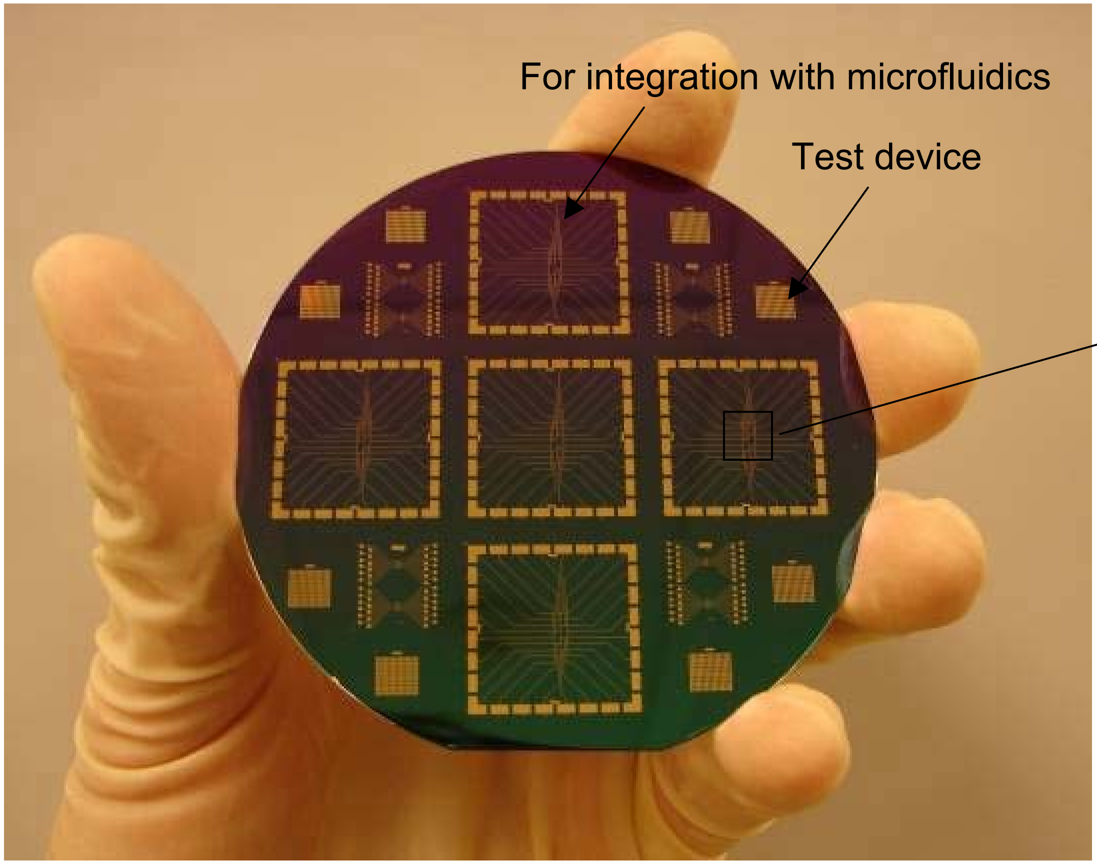
3. Dip a new substrate to the suspension

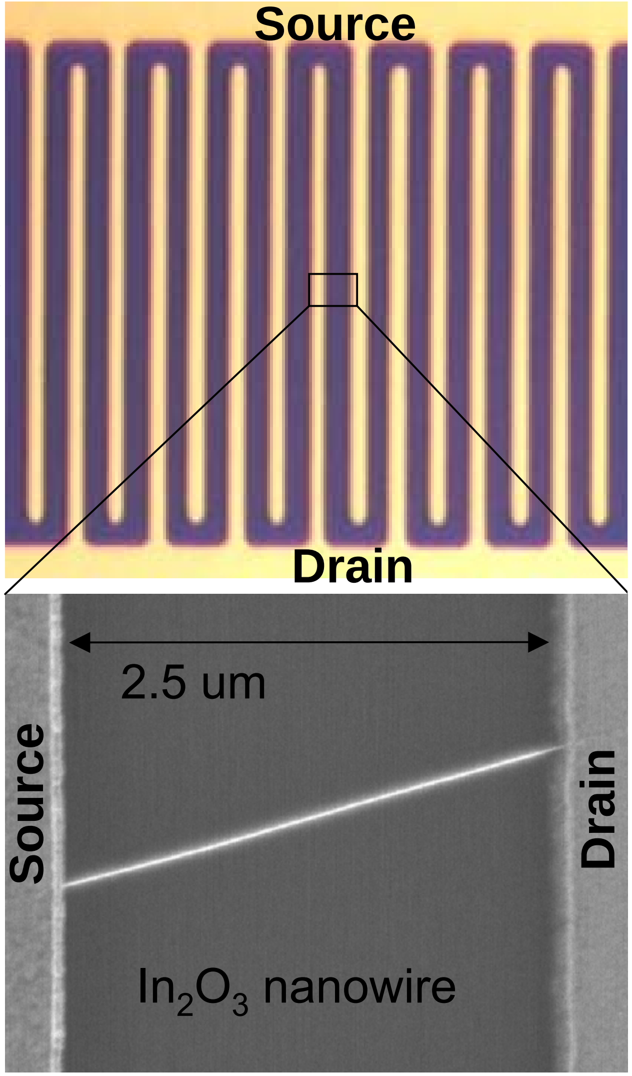
4. Define SD

Li et al., Adv. Mat. 15, 143

Can be applied to flexible substrate

Introduction: In_2O_3 Nanowire Devices on a 3" Wafer





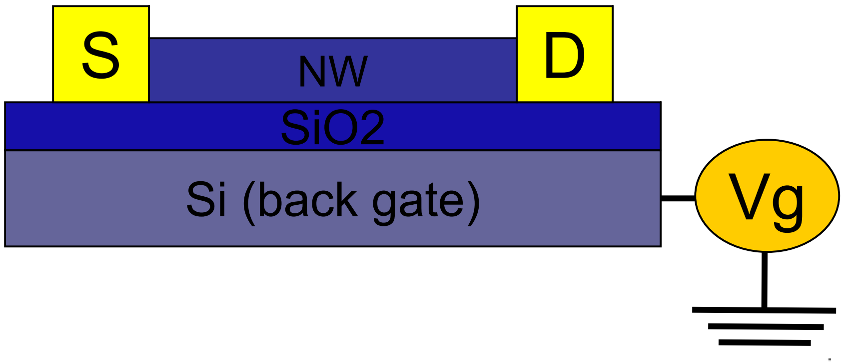
Effective channel width 400 ~ 2600 um within 100 x 100 um^2 by interdigitated electrodes

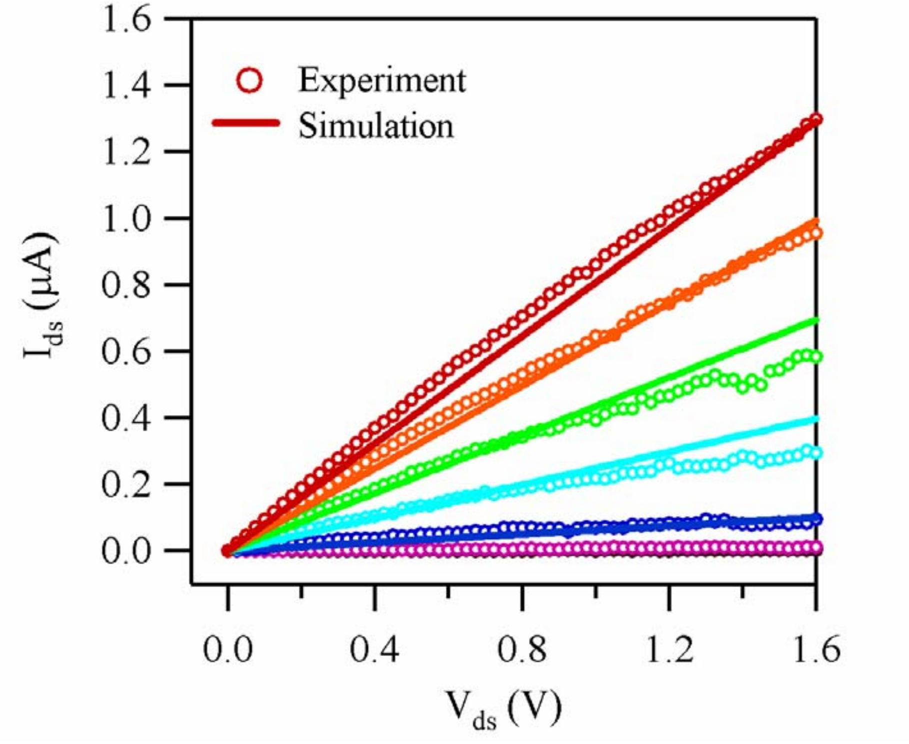
More nanowires in the channel
More uniform performance

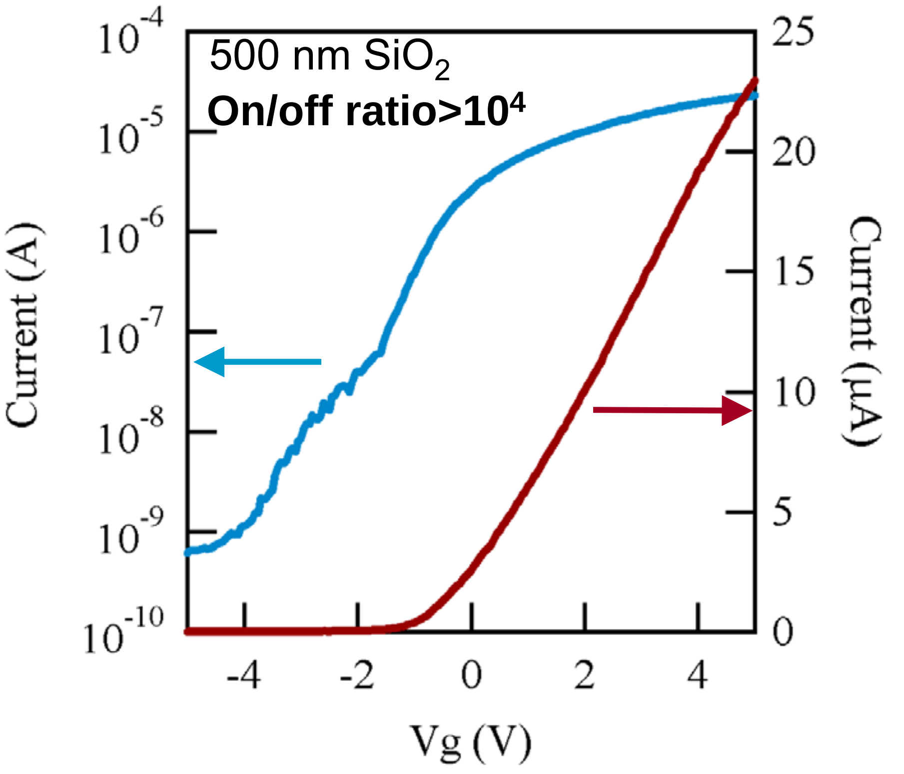
Introduction: Transistor Performance Using Back Gate

Remarks
- 70% devices exhibited on/off > 10^2
- Can be described by conventional MOSFET equation

$$I_{ds} = e\mu\varepsilon\varepsilon_r \frac{A}{d}\frac{V_{ds}}{L}\left(V_g - V_T\right)$$

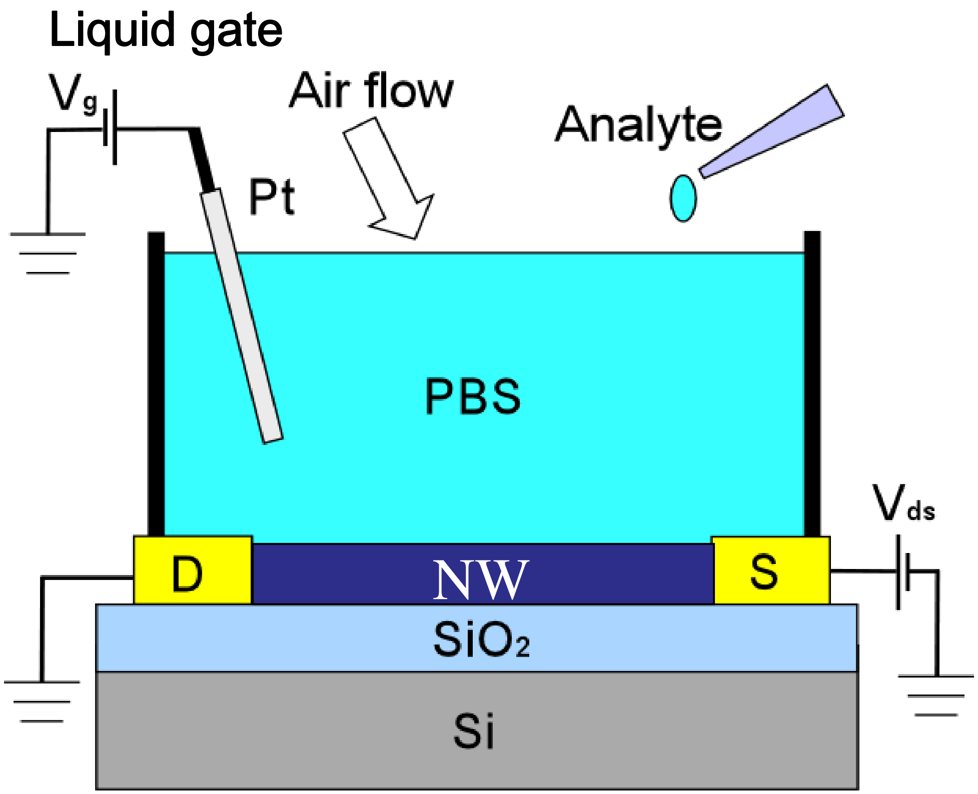


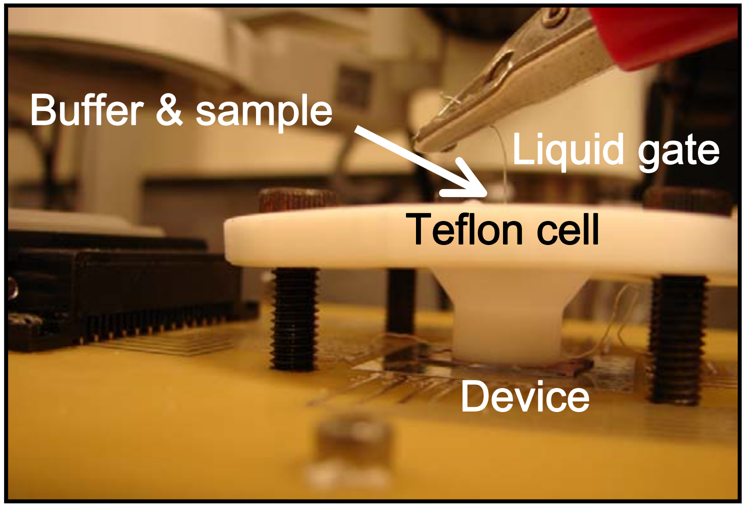



500 nm SiO_2

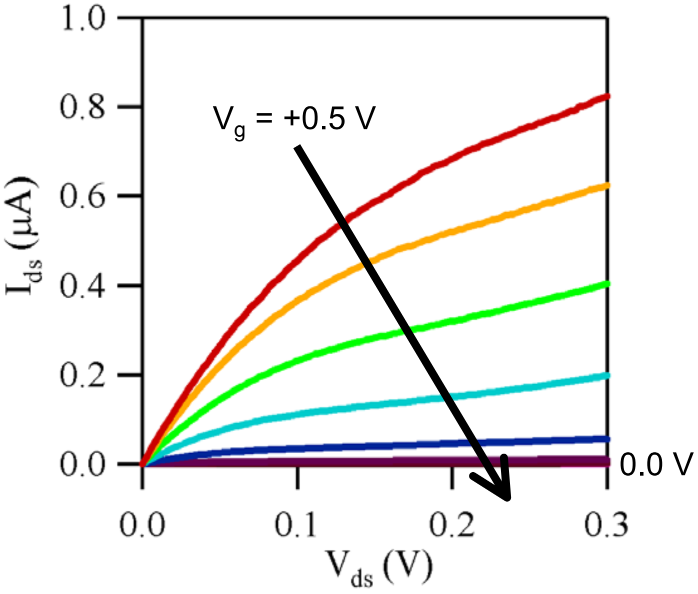
On/off ratio>10^4

Introduction: Biosensing Setup & Liquid Gate





FET operation of a NW device in buffer using a liquid gate



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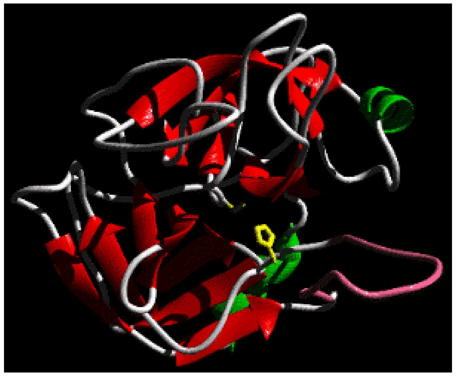
Prostate Specific Antigen (PSA) Detection

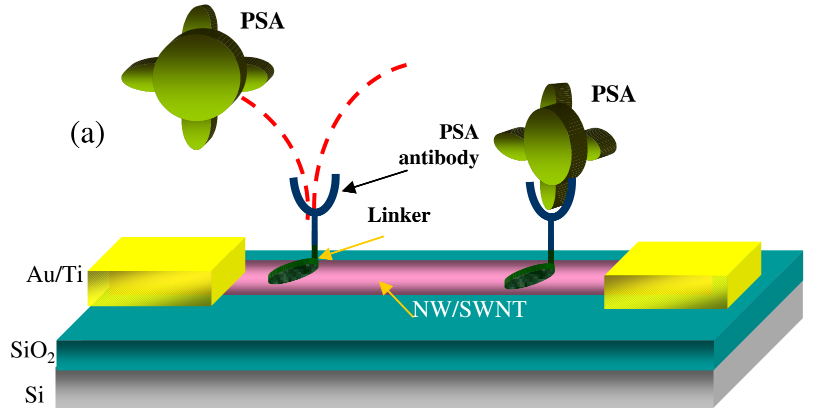
PSA is a bio marker for the presence of prostate cancer, which is the most frequently diagnosed cancer among men in the US.



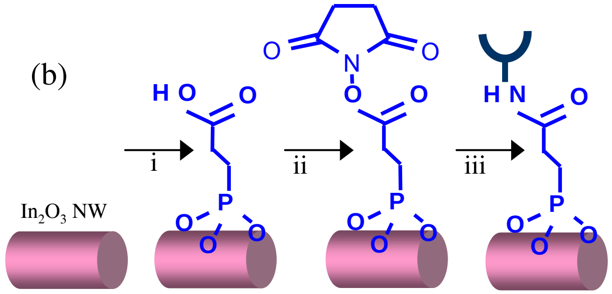
Standard PSA	**Probability of cancer**
0-2 ng/mL	1%
2-4 ng/mL	15%
4-10 ng/mL	25%
>10 ng/mL	>50%

In collaboration with Richard Cote (U of Miami, Pathology) and Mark Thompson (USC, Chemistry)

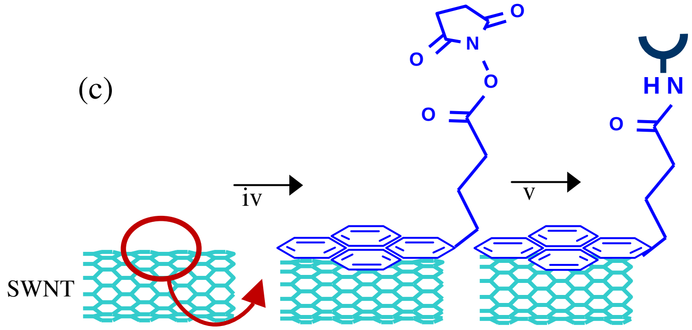
Li et al., JACS. 15, 127, 12484



In_2O_3 Nanowire functionalization

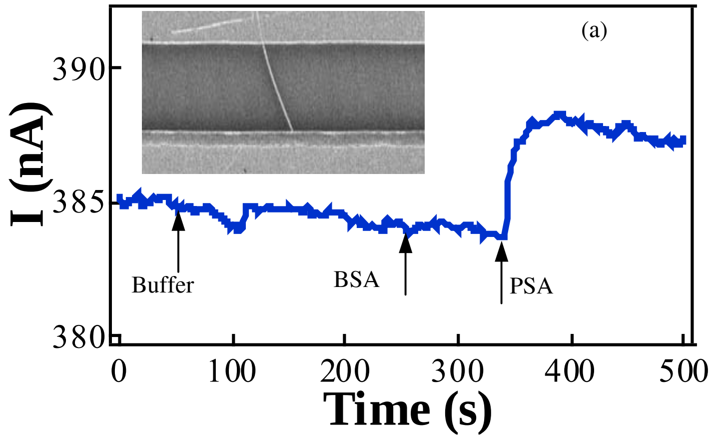


Carbon Nanotube functionalization

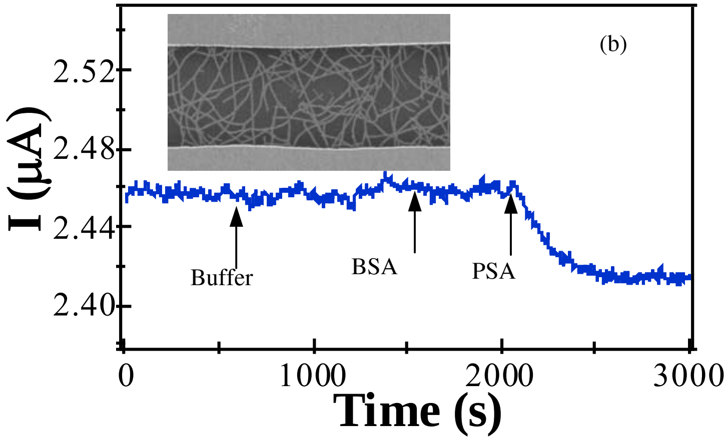


PSA Detection: Selective Detection in PBS Buffer

Individual In_2O_3 Nanowire: Enhanced Conduction



Carbon Nanotube Mat: Reduced Conduction



1. **No response when BSA was added;**
2. **Detection of PSA down to 5 ng/mL achieved in PBS buffer.**

Real-time detection of PSA in aqueous environment.

Li et al., JACS. 15, 127, 12484

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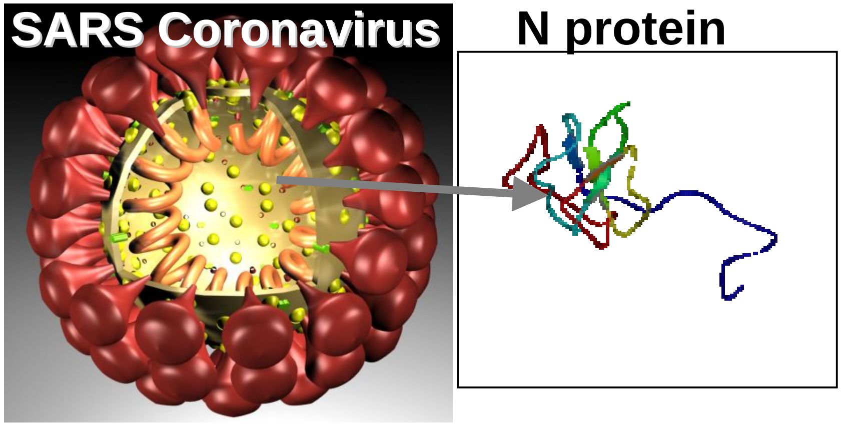
SARS Detection: Background

SARS (severe acute respiratory syndrome)

- SARS epidemic beginning in China, Nov 2002
- Transmitted by aerosol droplets created by coughing and sneezing
- Mortality rate ~ 5 to 20%

Neucleocapsid protein (N protein)



- 47kD phosphoprotein
- Most abundant structural protein in SARS Coronavirus

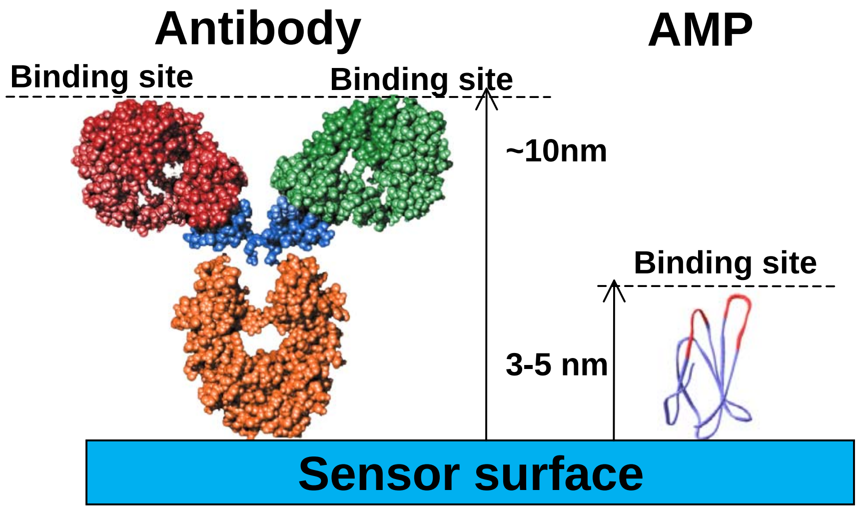
↓

Can be used as a biomarker for SARS diagnosis

SARS Detection: Antibody Mimic Proteins (AMPs) as a Receptor Molecule for N Protein

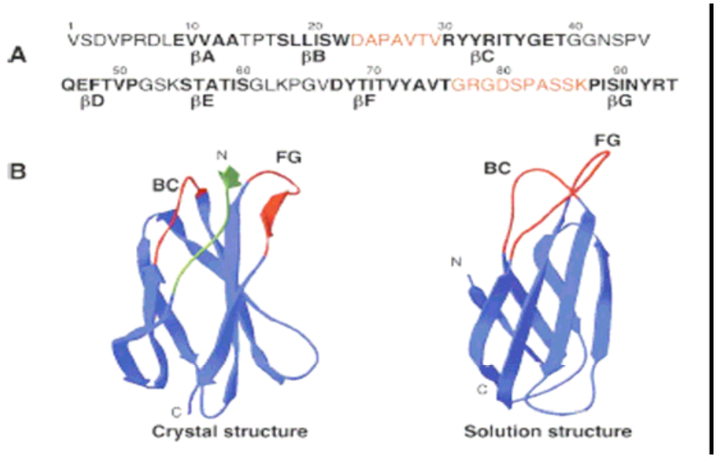
AMPs over antibodies

- Cheaper
- More stable
- Smaller (better electrostatic gating effect)
- Easier to evolve, manipulate, and engineer



Fibronectin (Fn)

- ➢ An antibody mimic protein (AMP)
- ➢ An extracellular matrix glycoprotein
- ➢ Developed for targeting SARS biomarker (N protein) by Prof. Roberts (USC) and Sun (UCLA)

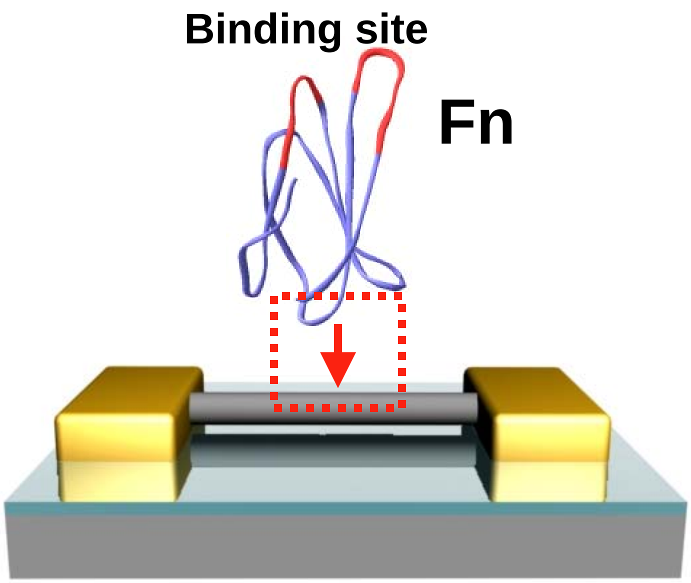


Binding affinity by SPR:

Fn	Kd	Domain	matured Fn	final Kd
Fn-N17	72nM	NTD	Fn-N17-602	3nM
Fn-N22	1.7nM	CTD	Fn-N22-460	300pM

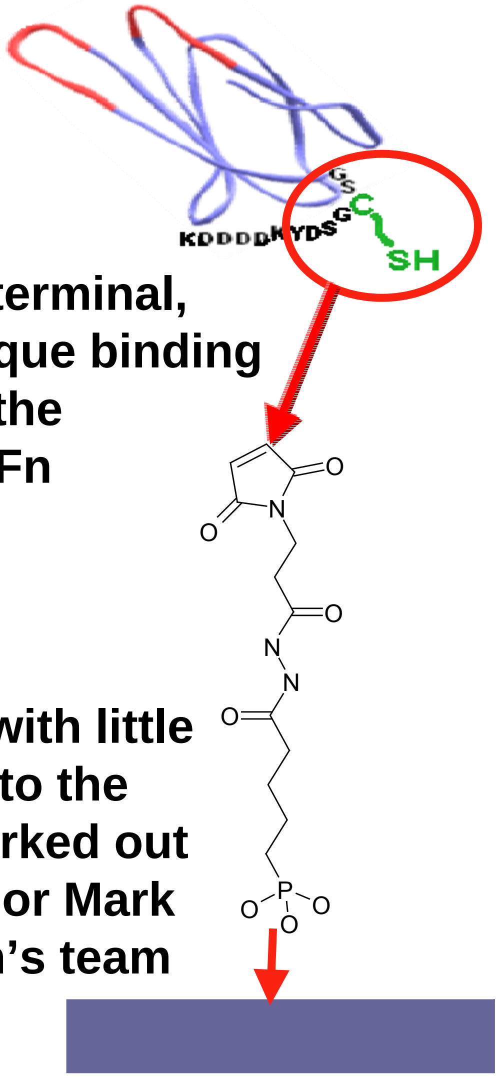
Affinity maturation

SARS Detection: In_2O_3 Nanowire Biosensor
Functionalization with Fn via Engineered Terminal Groups



Ideal functionalization scheme

- ➢Maintain structural information of Fn
- ➢Highly efficient/reliable
- ➢No damage to the devices



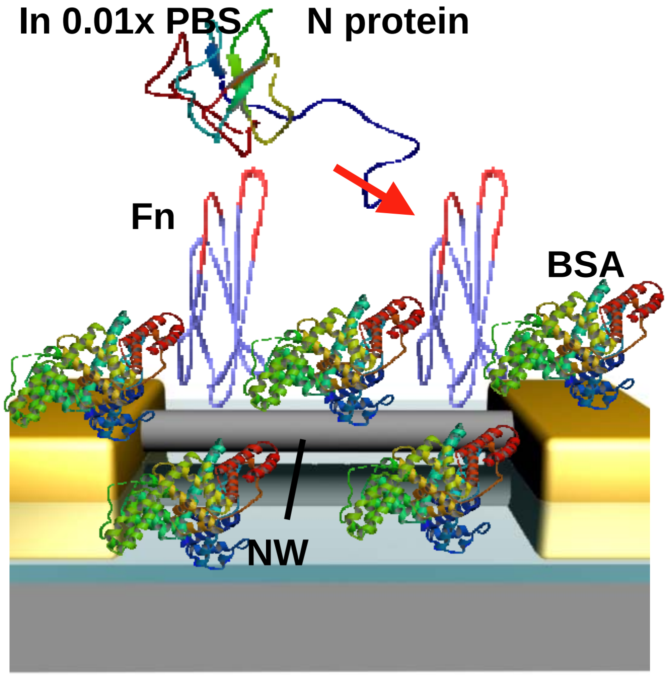
Engineered terminal, allowing unique binding site to keep the structure of Fn

Chemistry with little damage to the devices worked out by Professor Mark Thompson's team

In_2O_3 nanowire

SARS Detection: Selective N Protein Detection with In_2O_3 NW Devices

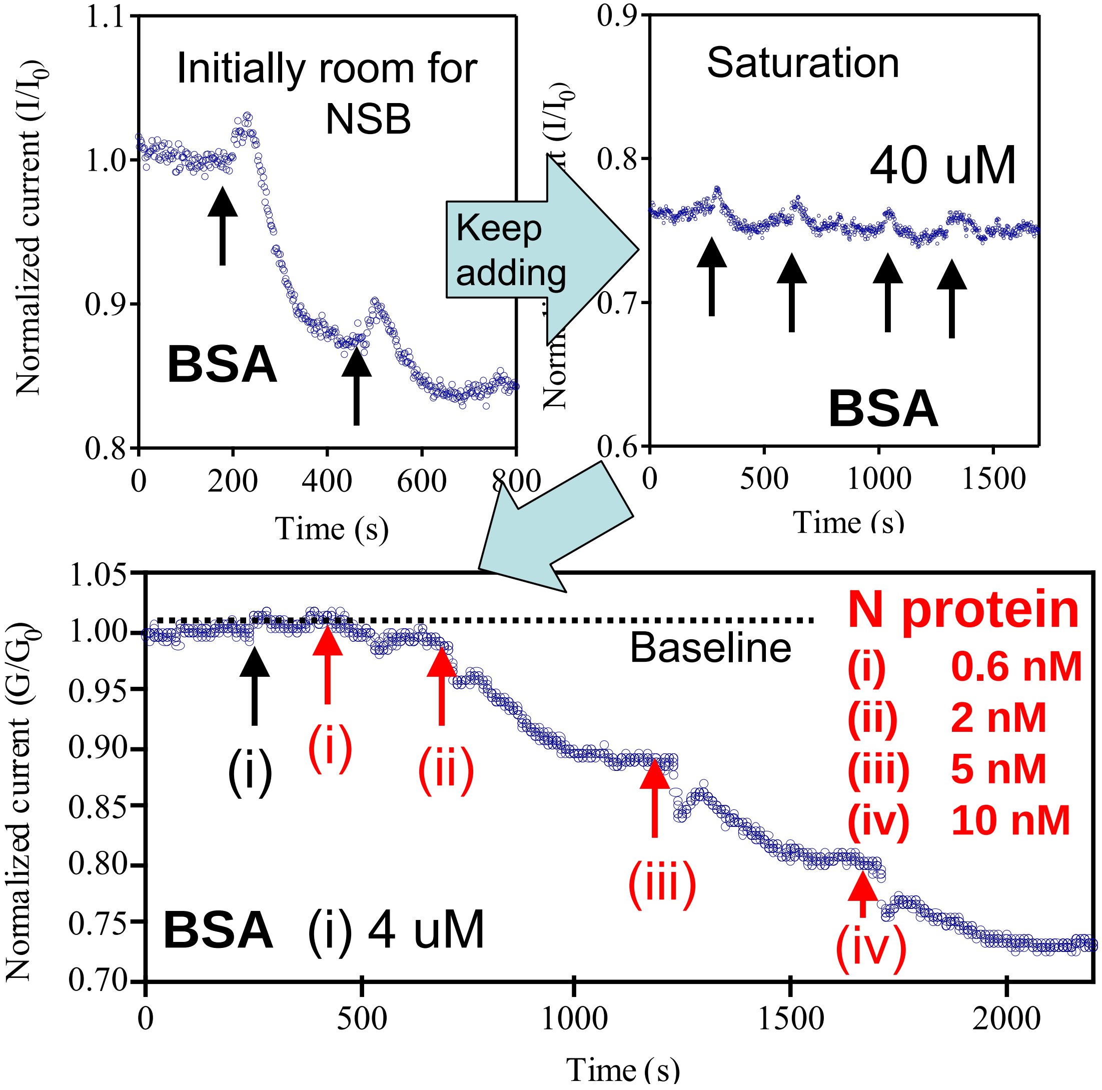
Device structure



- ➢ After Fn immobilization, rest of the sensor surface was saturated/passivated with BSA to block non-specific binding
- ➢ This method has been used for ELISA

Ishikawa et al., ACS Nano. 3, 5:1219

Selective N protein sensing



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Whole Blood Detection of Ovarian Cancer: Motivation and Challenges

Ovarian Cancer Detection Motivation

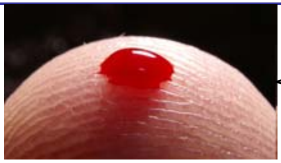
- Why detection in blood?
  - Prevention/health monitoring; Diagnosis/biomarker detection; Treatment/therapeutic efficatcy monitoring
  - Direct detection from blood minimizes external processing steps
  - Good for portable devices
  - Minimizes amount of sample needed
- Why ovarian Cancer?
  - 5th highest cancer mortality rate in women
  - Low survival rate largely due to lack of early stage detection
  - Only 1 approved biomarker (CA125): creates false positive
  - Other detection methods (CT Scans, sonograms) are costly and cumbersome
- Possible solution needs:
  - Portable (small), Cheap, Quick (label-free), Small sample consumption
  - Many characteristics of the nanowire nanobiosensor platform!

Challenges

- Detection from whole blood
  - Other blood proteins causes nonspecific binding on sensor surface
  - Ionic concentration of blood shields charges from molecules
- False positive signals
- Sensor to sensor variations

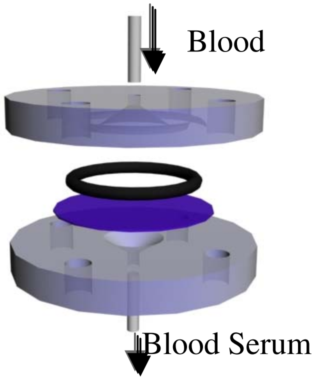
Whole Blood Detection: Nanobiosensor Bioassay Setup

Start: Whole blood sample collected by a finger prick

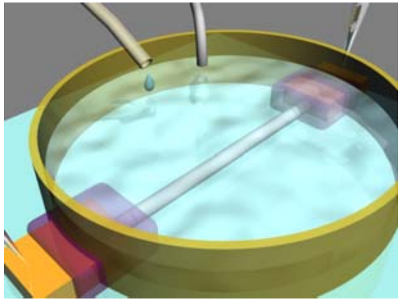


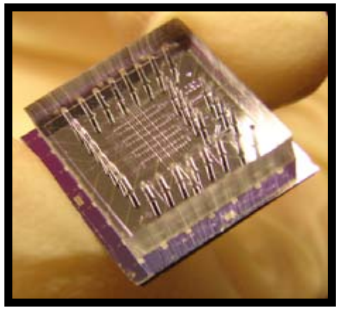
Time-efficient

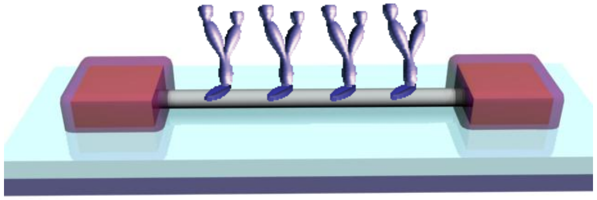
Negligible invasion & pain



Liquid gate





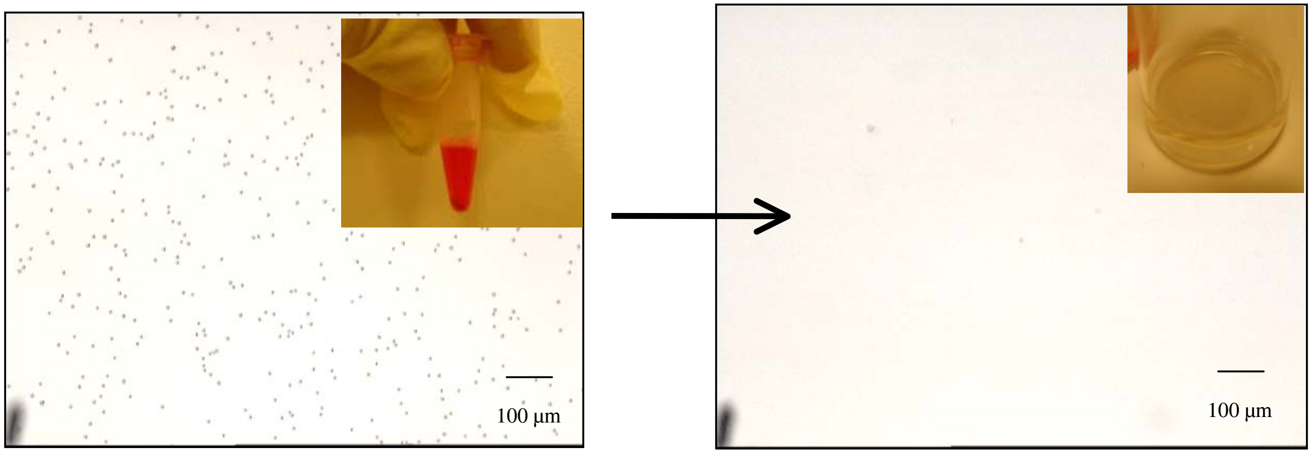


	Methods	Functions
Sample pre-treatment	✧Microfilter	➢Purification
Sample handling	✧Mixing cell ✧Microfluidic chip	➢Multiplexing ➢Automation
Detection	✧Nanobiosensor	➢Label-free detection ➢Sensitive detection

Our goal: real-time biomarker detection in blood with clinically relevant sensitivity

Chang et al., ACS Nano. 5, 12:9883

Whole Blood Detection: Results on Blood Filtration



- Cell count reduced by 99.5% after filtration
- Filtered whole blood is essentially serum, but other blood proteins still exist

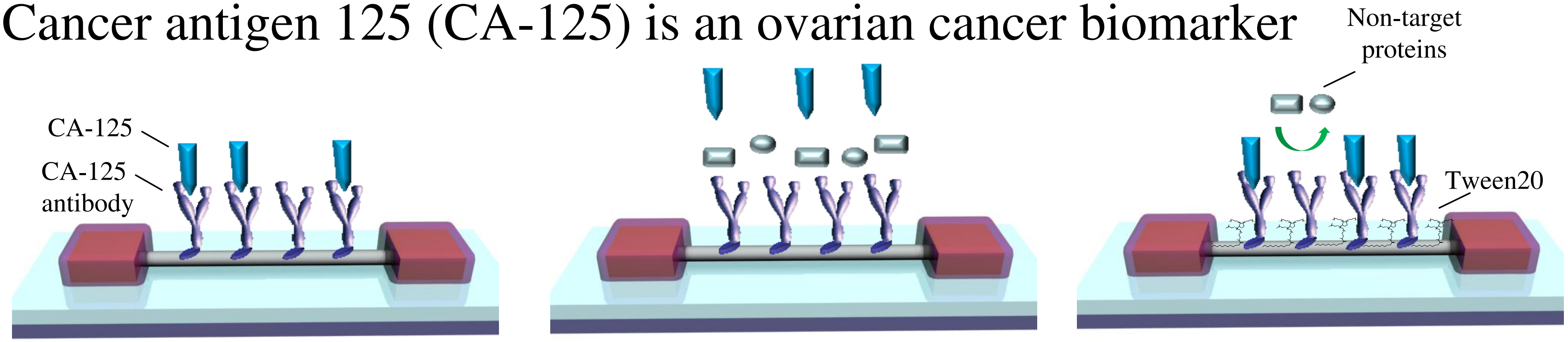
Serum ion concentration reduction

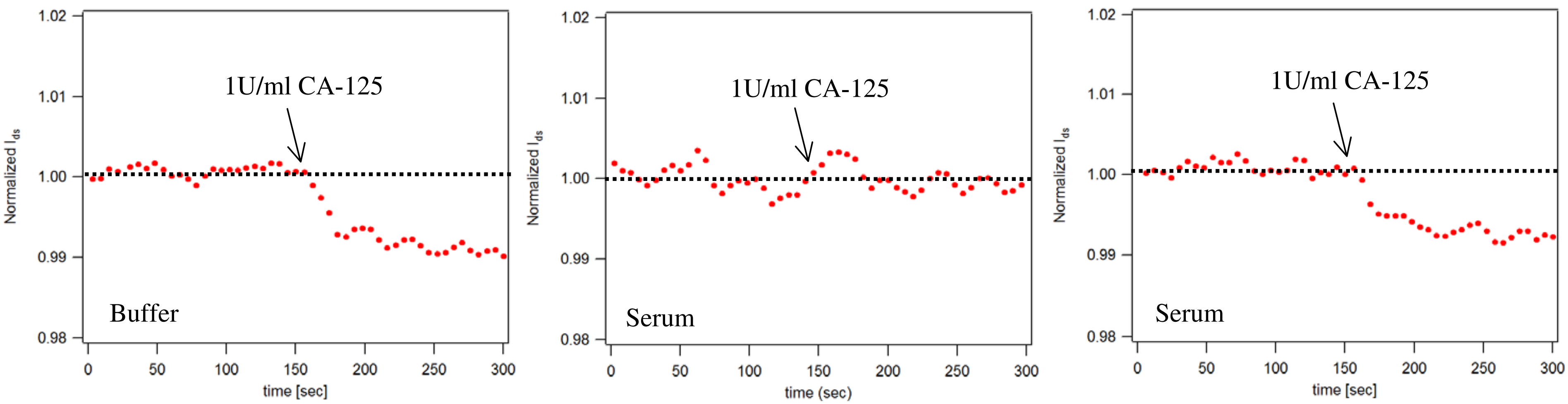
- **Dilution in buffer**
  - **No equipments required, but also dilutes sample content**
- **Desalting columns**
  - **Simply, commercially available, but easy to integrate into electronics?**
- **On-chip process**
  - **Better integration, but more fabrication steps**

Chang et al., ACS Nano. 5, 12:9883

Whole Blood Detection: Effect of Tween 20 Surface Passivation on Non-specific Binding – CA125 Detection

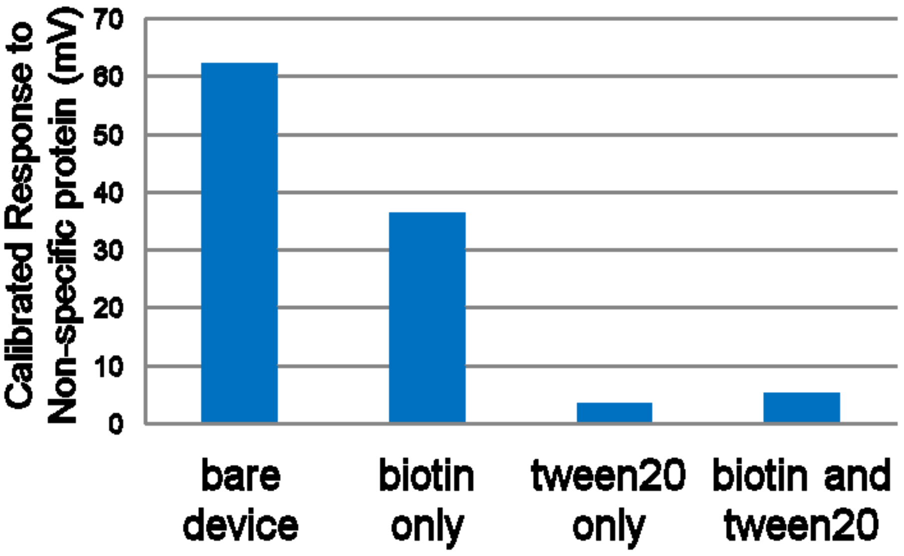
Cancer antigen 125 (CA-125) is an ovarian cancer biomarker



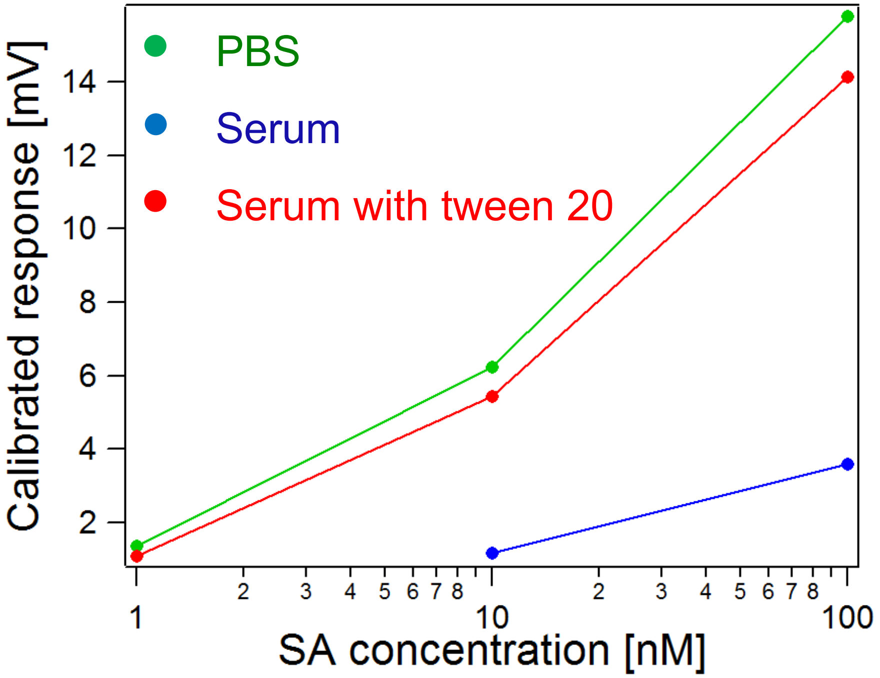


Chang et al., ACS Nano. 5, 12:9883

Whole Blood Detection: Effect of Tween 20 Surface Passivation on Streptavidin Detection



- ➢ Passivated device showed **reduced non-specific binding** to serum proteins

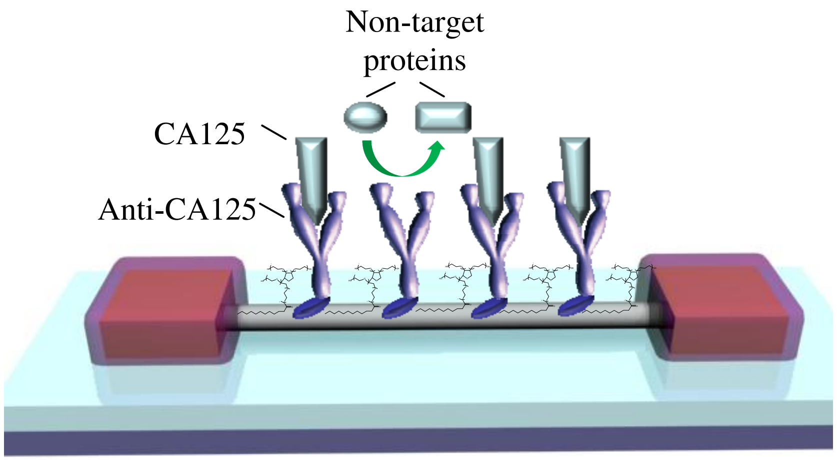


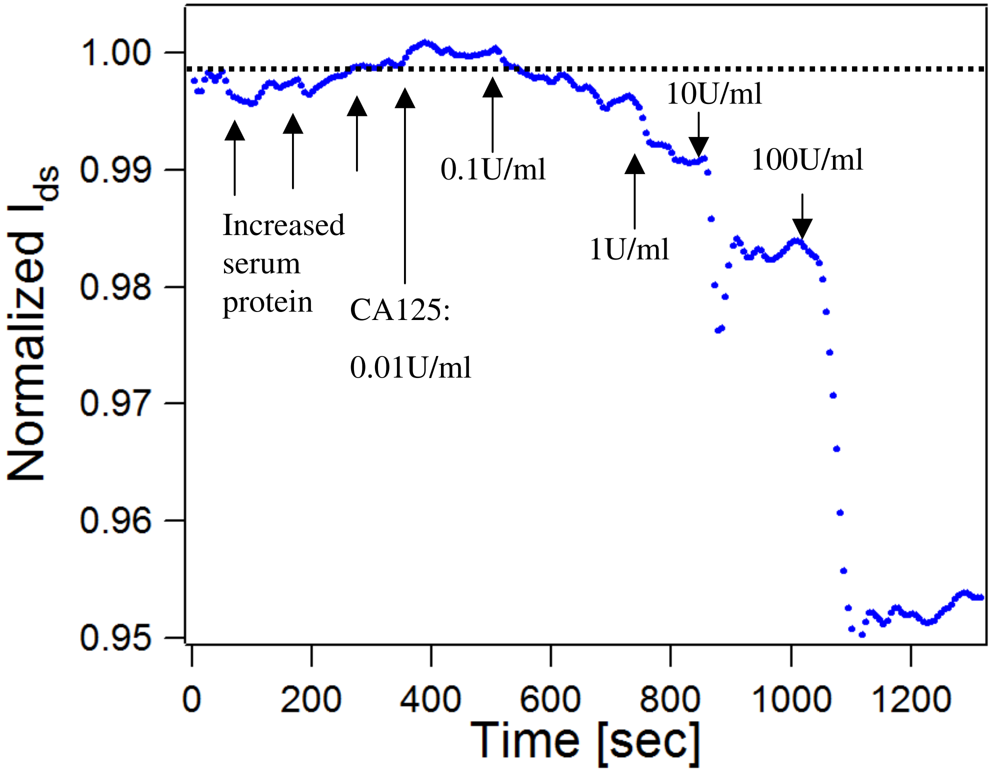
- ➢ Passivated device showed **enhanced signal to analyte** in complex media

Chang et al., ACS Nano. 5, 12:9883

Whole Blood Detection: CA-125 Detection Results

Concentration dependent CA-125 detection in blood:





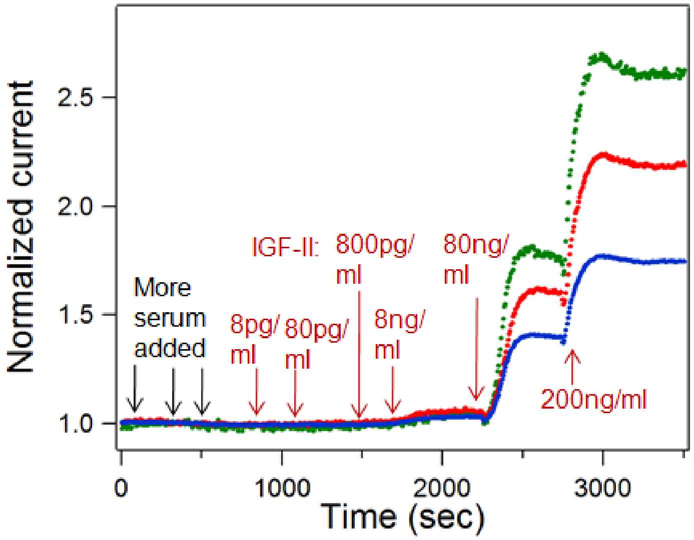
- ➢ **Detection limit: 0.1U/ml (500fM)**
- ➢ CA125 concentration in patient's blood: 275U/mL (1375 pM)

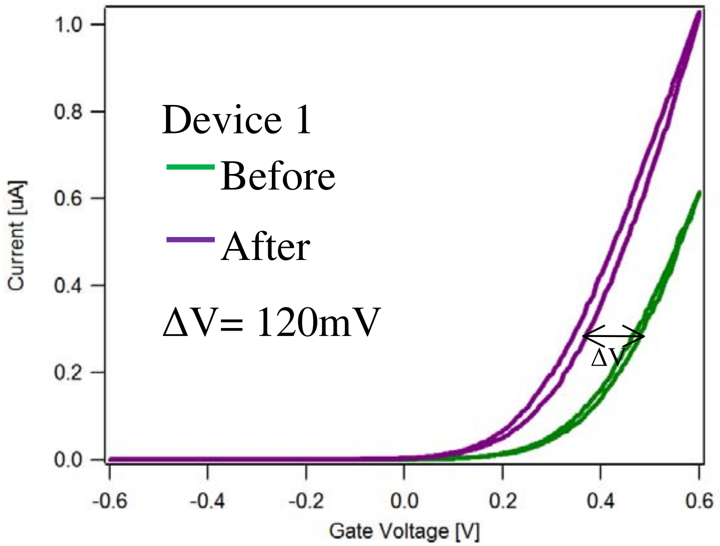
Chang et al., ACS Nano. 5, 12:9883

Whole Blood Detection: Insulin Growth Factor-II (IGF-II) Results

IGF-II is an ovarian cancer biomarker

EOC Serum Marker	Serum Level of EOCBiomarkers (ng/mL)		
	Healthy control	Cancer patient	Trend
CA-125	<35,000	>100,000	↑
Prolactin	5.8-6.0	68-78	↑
OPN	1.1-1.4	9.2-13.4	↑
IGF-II	800-900	150-190	↓
MIF	0.07-0.08	860-1180	↑
Leptin	13.6-15.2	6.5-9	↓





Chang et al., ACS Nano. 5, 12:9883

Comparing Calibration with Conventional Normalization Method

$$I_{before} = e\mu_1\varepsilon\varepsilon_{r1}\frac{A}{d}\frac{V_{ds}}{L}\left(V_g - V_{T1}\right)$$

Ids before sensing

$$I_{after} = e\mu_1\varepsilon\varepsilon_{r1}\frac{A}{d}\frac{V_{ds}}{L}\left(V_g - \left(V_{T1} + \Delta V\right)\right)$$

Ids after sensing*

$$\frac{dIds}{dVg} = e\mu_1\varepsilon\varepsilon_{r1}\frac{A}{d}\frac{V_{ds}}{L}$$

Conventional normalization

Device dependent

$$\frac{\Delta I}{I_0} = \left(\frac{e\mu_1\varepsilon\varepsilon_{r1}\frac{A}{d}\frac{V_{ds}}{L}\left(V_g - V_{T1}\right) - e\mu_1\varepsilon\varepsilon_{r1}\frac{A}{d}\frac{V_{ds}}{L}\left(V_g - \left(V_{T1} + \Delta V\right)\right)}{e\mu_1\varepsilon\varepsilon_{r1}\frac{A}{d}\frac{V_{ds}}{L}\left(V_g - V_{T1}\right)}\right) = \frac{\Delta V}{\left(V_g - \boxed{V_{T1}}\right)}$$

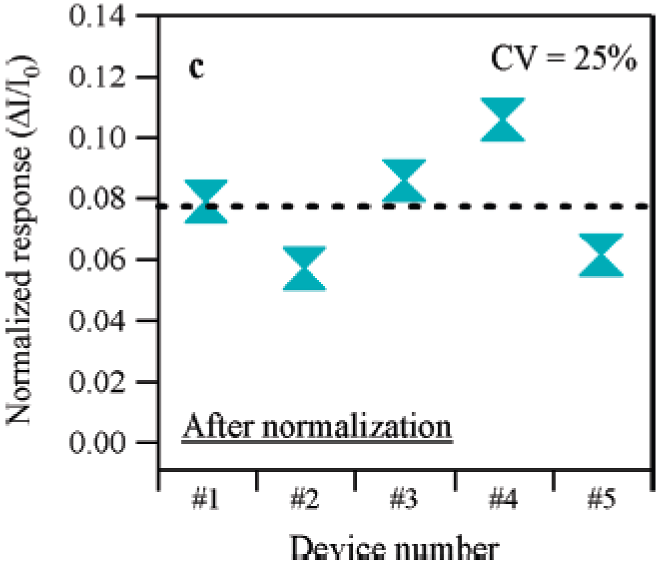
Proposed calibration method

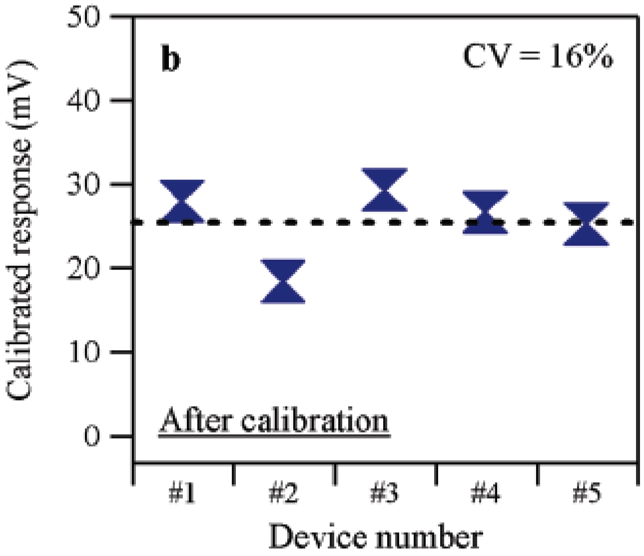
Device independent

$$\frac{\Delta I}{\frac{dIds}{dVg}} = \left(\frac{e\mu_1\varepsilon\varepsilon_{r1}\frac{A}{d}\frac{V_{ds}}{L}\left(V_g - V_{T1}\right) - e\mu_1\varepsilon\varepsilon_{r1}\frac{A}{d}\frac{V_{ds}}{L}\left(V_g - \left(V_{T1} + \Delta V\right)\right)}{e\mu_1\varepsilon\varepsilon_{r1}\frac{A}{d}\frac{V_{ds}}{L}}\right) = \Delta V$$

ΔV: equivalent gate potential induced by electrostatic doping

Ishikawa et al., ACS Nano. 3, 12:3969

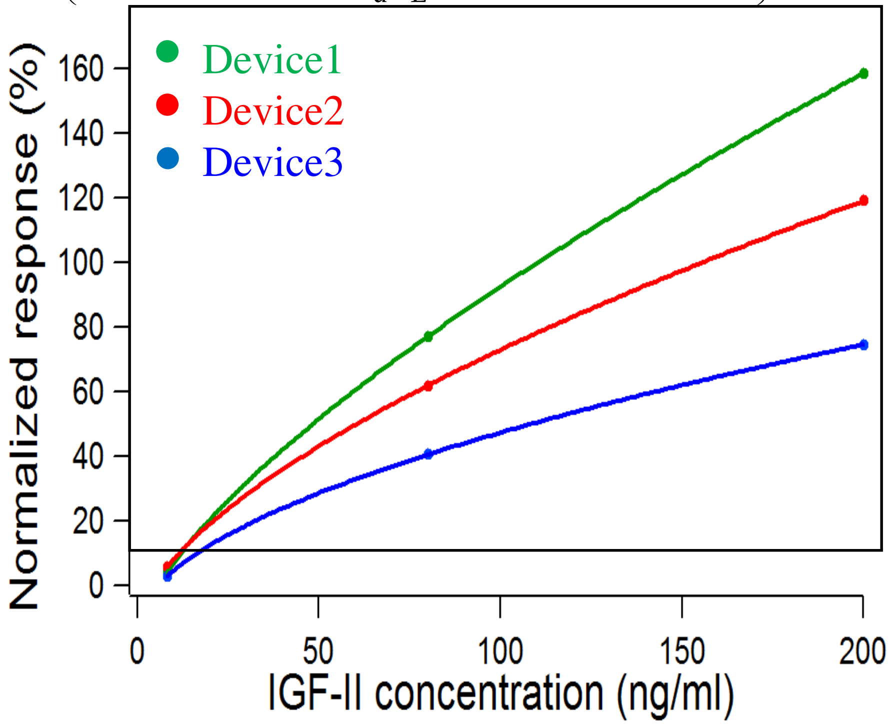




Whole Blood Detection: Calibrated vs Normalized IGF-II

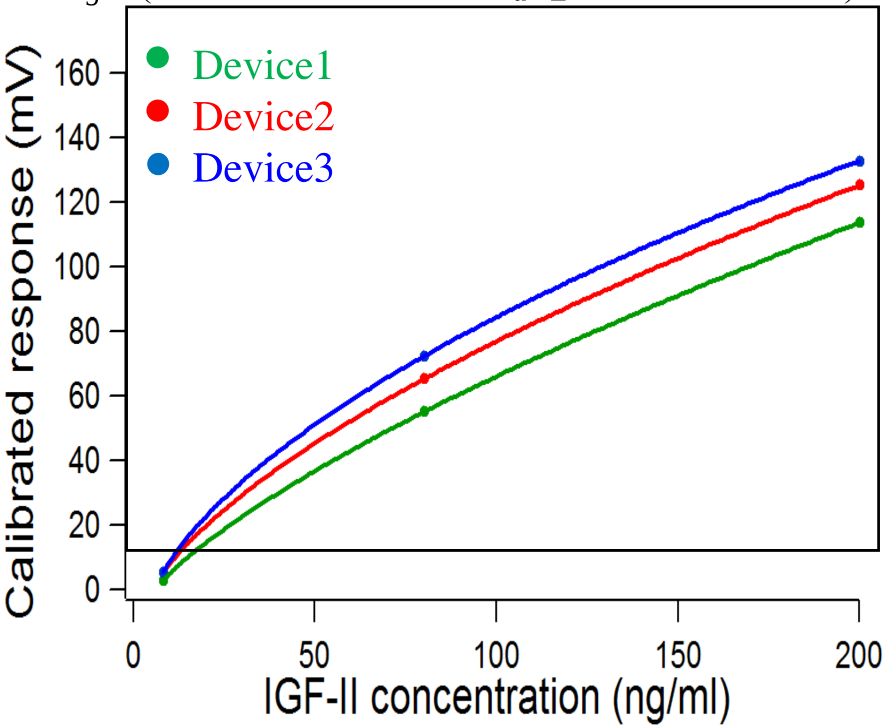
Normalization

$$\frac{\Delta I}{I_0}=\left(\frac{e\mu_1\varepsilon\varepsilon_{r1}\frac{A}{d}\frac{V_{ds}}{L}\left(V_g-V_{T1}\right)-e\mu_1\varepsilon\varepsilon_{r1}\frac{A}{d}\frac{V_{ds}}{L}\left(V_g-\left(V_{T1}+\Delta V\right)\right)}{e\mu_1\varepsilon\varepsilon_{r1}\frac{A}{d}\frac{V_{ds}}{L}\left(V_g-V_{T1}\right)}\right)=\frac{\Delta V}{\left(V_g-V_{T1}\right)}$$



Calibration

$$\frac{\Delta I}{\frac{dIds}{dVg}}=\left(\frac{e\mu_1\varepsilon\varepsilon_{r1}\frac{A}{d}\frac{V_{ds}}{L}\left(V_g-V_{T1}\right)-e\mu_1\varepsilon\varepsilon_{r1}\frac{A}{d}\frac{V_{ds}}{L}\left(V_g-\left(V_{T1}+\Delta V\right)\right)}{e\mu_1\varepsilon\varepsilon_{r1}\frac{A}{d}\frac{V_{ds}}{L}}\right)=\Delta V$$



- Response can be fitted with Langmuir isotherm model
- Calibrated response exhibit suppressed device-to-device variation compared to normalized response

Chang et al., ACS Nano. 5, 12:9883

Conclusion

- **Demonstration of selective detection of PSA antigen using In_2O_3 nanowire sensor platform**
- **SARS N protein was selectively detected in real time with AMP as capture probes**
- **Multiple ovarian cancer biomarker detection in serum is performed with enhanced signal and reduced non-specific binding utilizing nanowire surface functionalization**
- **Device-to-device variation suppressed for ovarian cancer biomarker IGF-II using calibration method**

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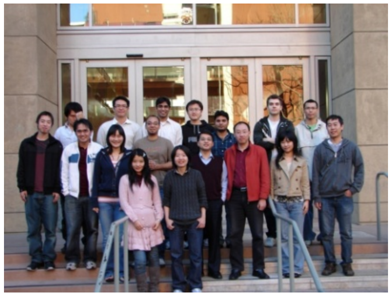

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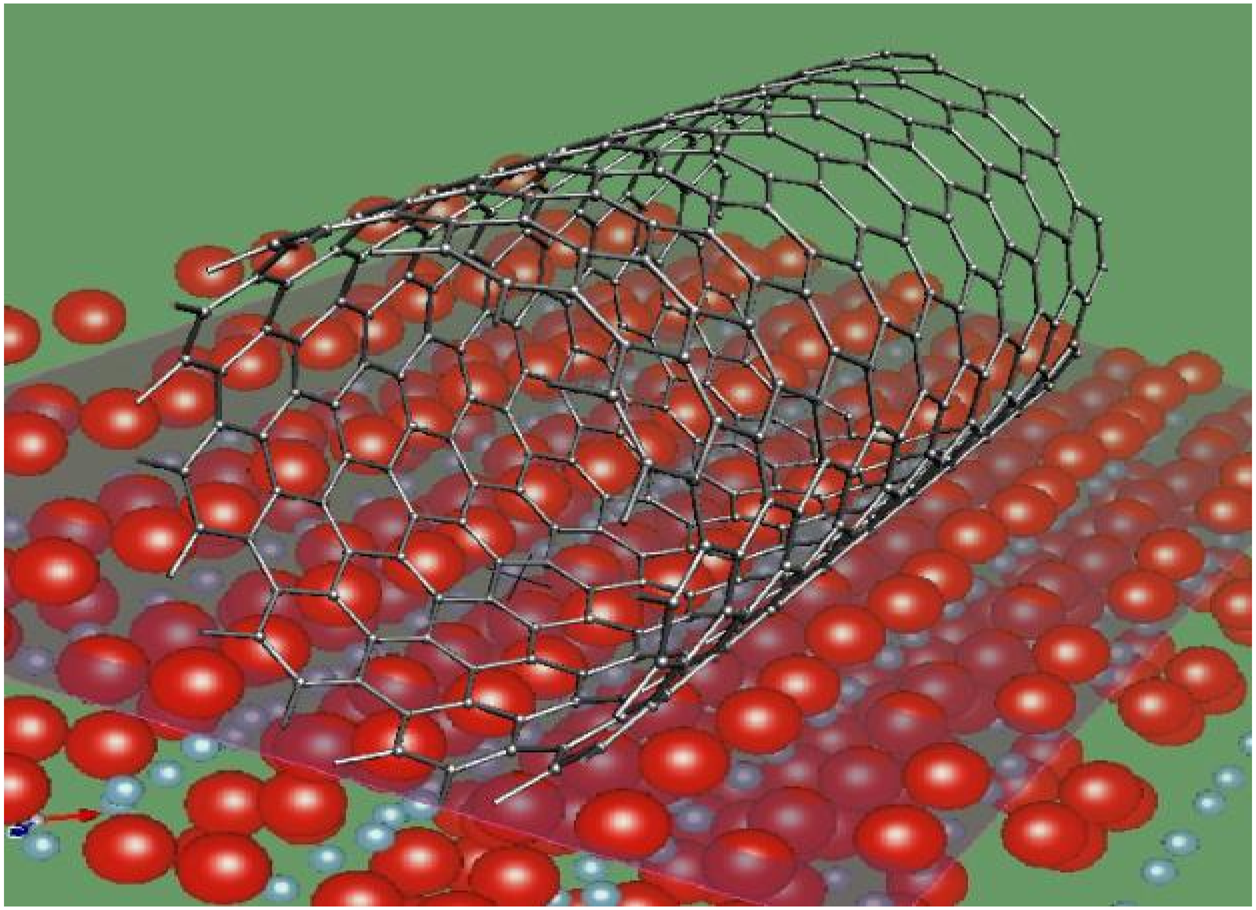
THANK YOU !



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