Enhancement of electrochemical biosensor performances using redox cycling at 3D sub-micrometer scale electrode architectures

Heungjoo Shin

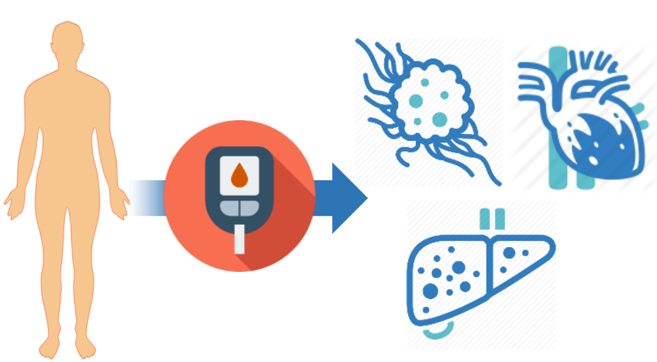
School of Mechanical and Nuclear Engineering

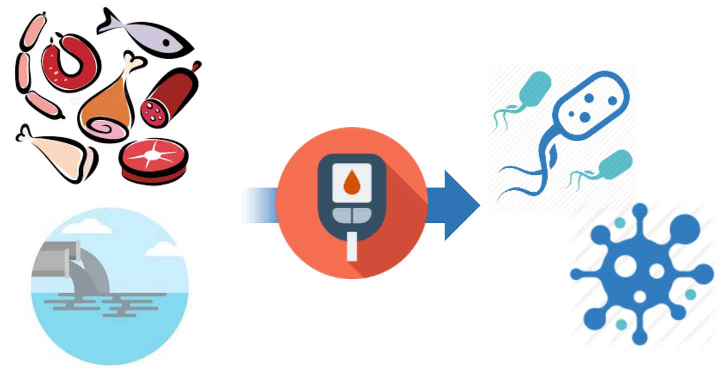
UNIST

Contents

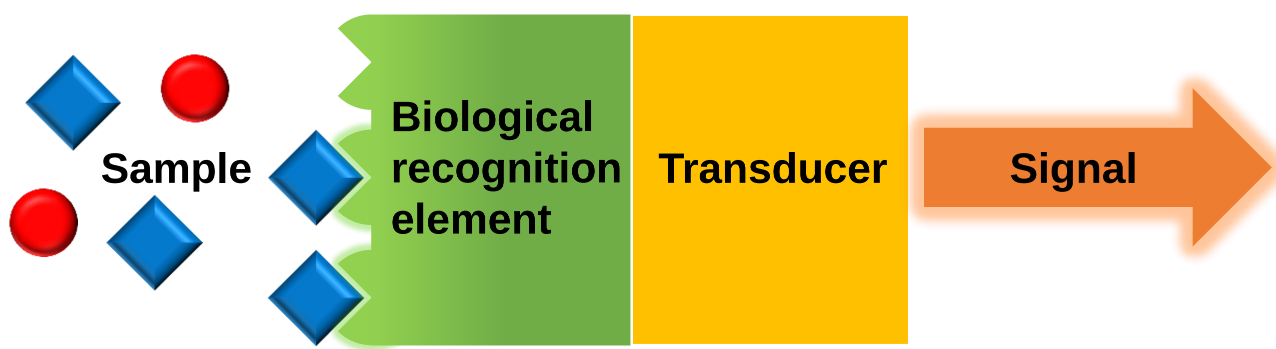
Electrochemical Biosensors: Applications

- **Health & safety**
  - Clinical diagnosis, food control, environmental screening





- **Biosensor classification**
  - Transducer types
    - Electrochemical, Optical, Electrical, Piezoelectric, Calorimetric



Electrochemical vs. Optical sensors

	Optical Sensor	Electrochemical Sensor
Sensitivity	Excellent	High
Selectivity	High	Good
Sample	Clear sample only	No limitation
Device configuration	Complex	Simple
Hand-held & Disposable	Difficult	Feasible
Cost	Relatively expensive	Cheap

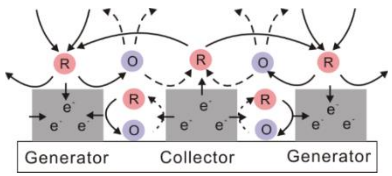
- Amperometry
- Voltammetry
- Coulometry
- EIS

Enhancement of electrochemical sensing

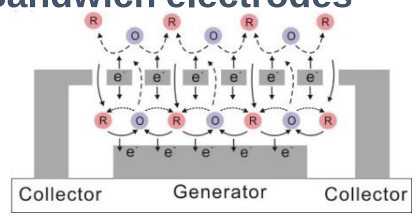
3D sub-micrometer scale electrodes

➢ Redox sensor signal enhancement

- 3D carbon IDEs



- Sandwich electrodes

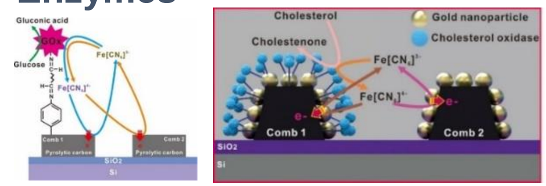


Redox cycling at 3D micro/nano-scale electrode architecture

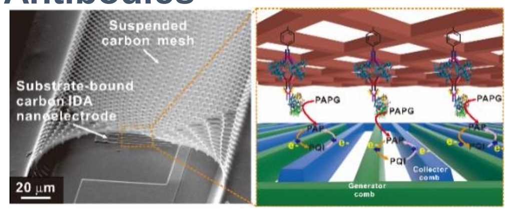
✓ Large S/V
✓ Linear diffusion
⇨ Signal amplification
> 30 in bulk
> 800 in μ-channel

➢ Selective bioreceptor immobilization

- Enzymes



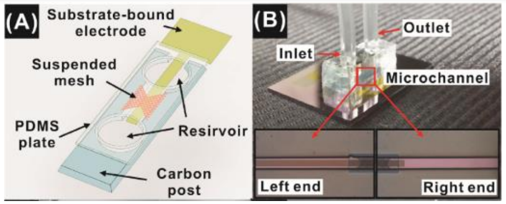
- Antibodies

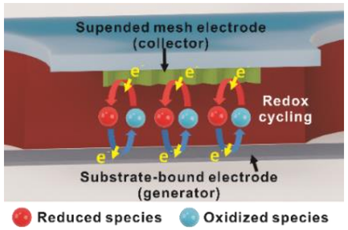


Electrochemical reduction of aryl diazonium salt

✓ Bioreceptors
⇨ Selectivity ↑
✓ Efficient redox reaction
⇨ Sensitivity ↑

➢ Simple microchannel integration





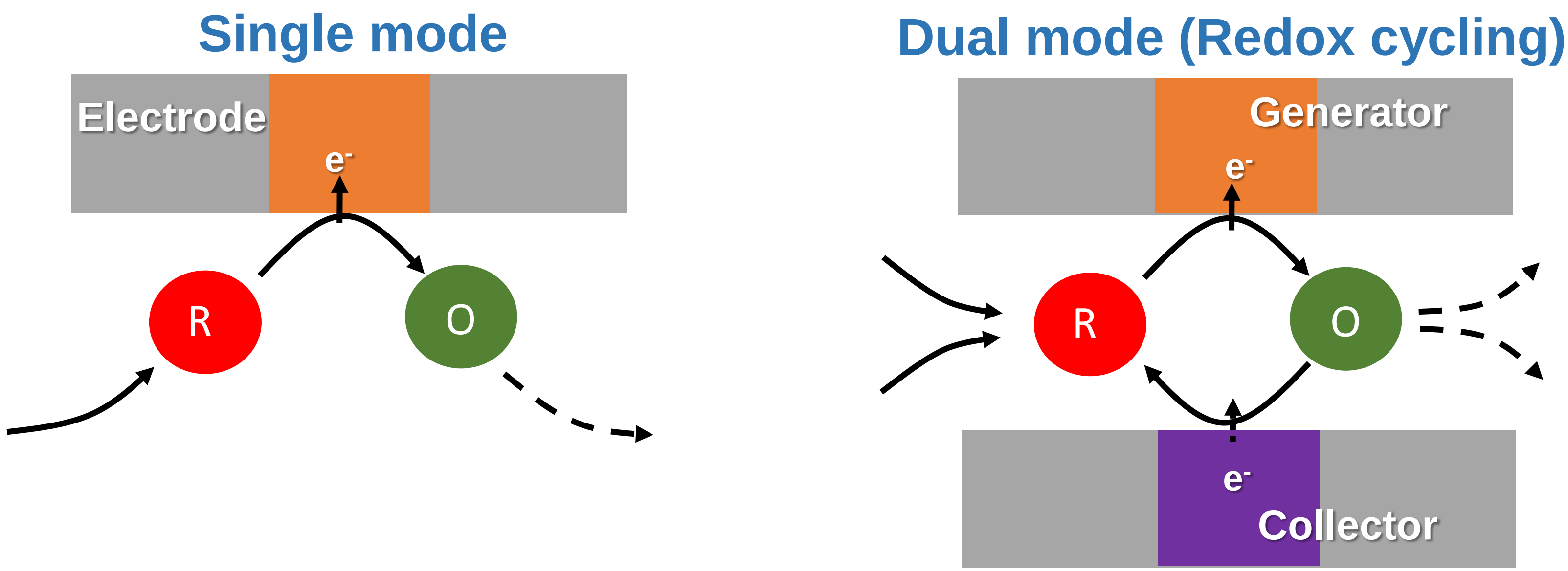
Redox in confined environment

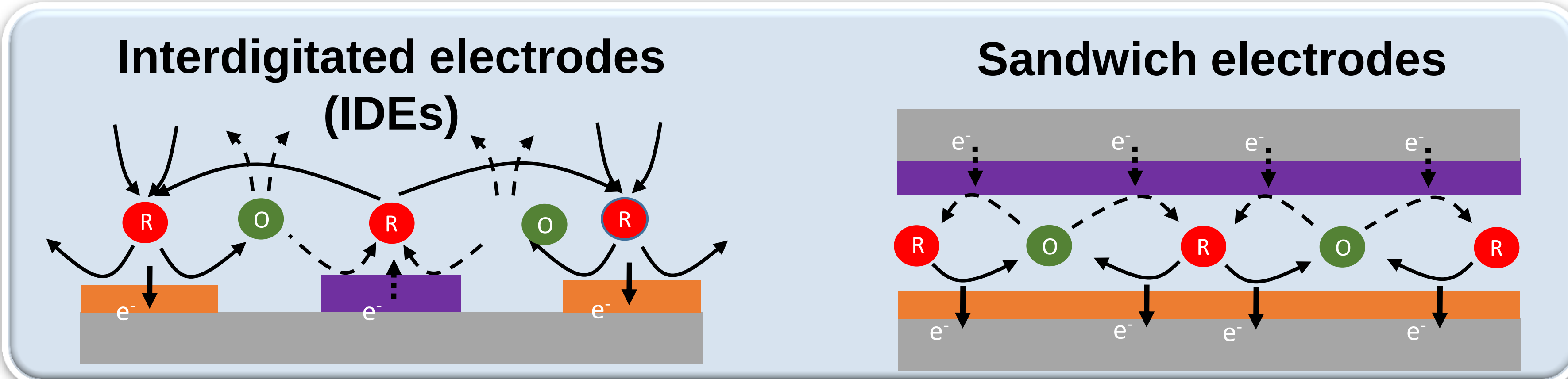
✓ Irreversible species
⇨ Depletion
✓ Reversible species
⇨ Redox cycling

Selectivity enhancement

Redox cycling

- **Sensitivity enhancement**
  - Amplifying Faradaic current signal



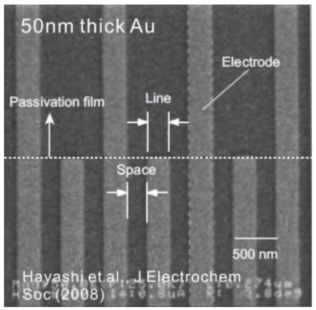


Improvement of Redox cycling effect

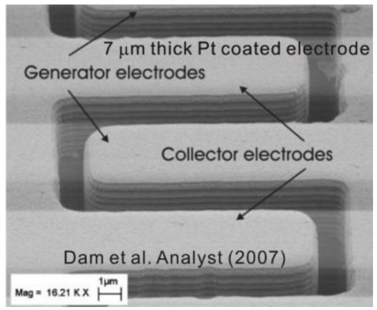
- **Diffusion enhancement via electrode reconfiguration**

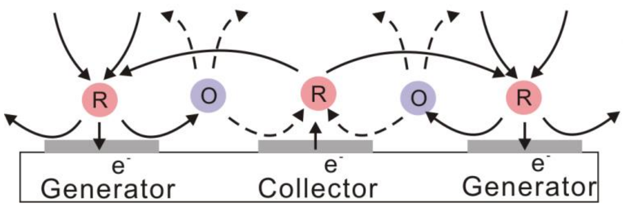
Interdigitated electrodes (IDEs)

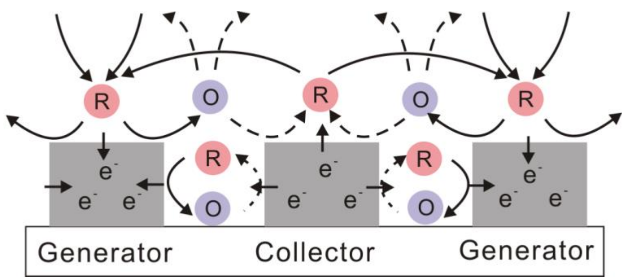
- **Nano-gap**



- **High aspect ratio**

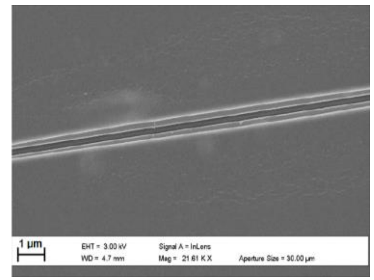




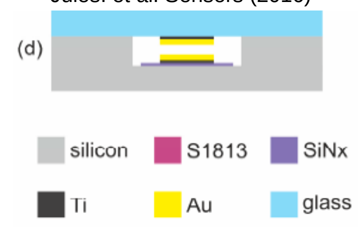


Sandwich electrodes

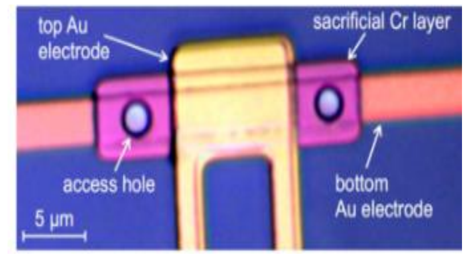
- **Large surface**



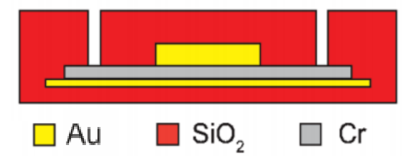
Jules. et al. Sensors (2016)

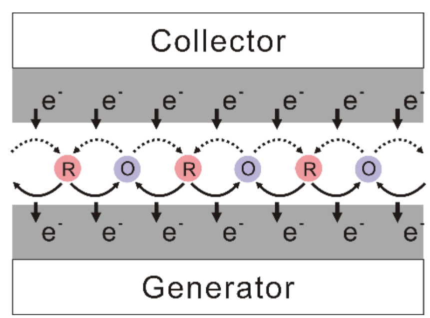


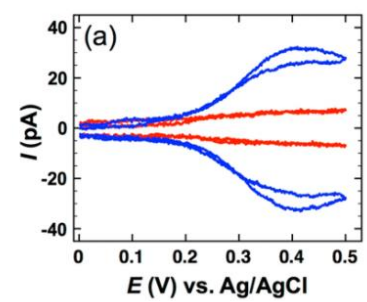
- **Nano-gap**



M. A. G. et al. J. Am. Chem. Soc. (2009)







Improvement of Redox cycling effect

- Limitations of previous approaches

Interdigitated electrodes (IDEs)

- ✓ Nano-gap:
  - ➡ Expensive nanofabrication
- ✓ High aspect ratio:
  - ➡ Complex MEMS process
- ✓ Limitation in electrode gap reduction with high aspect ratio

Sandwich electrodes

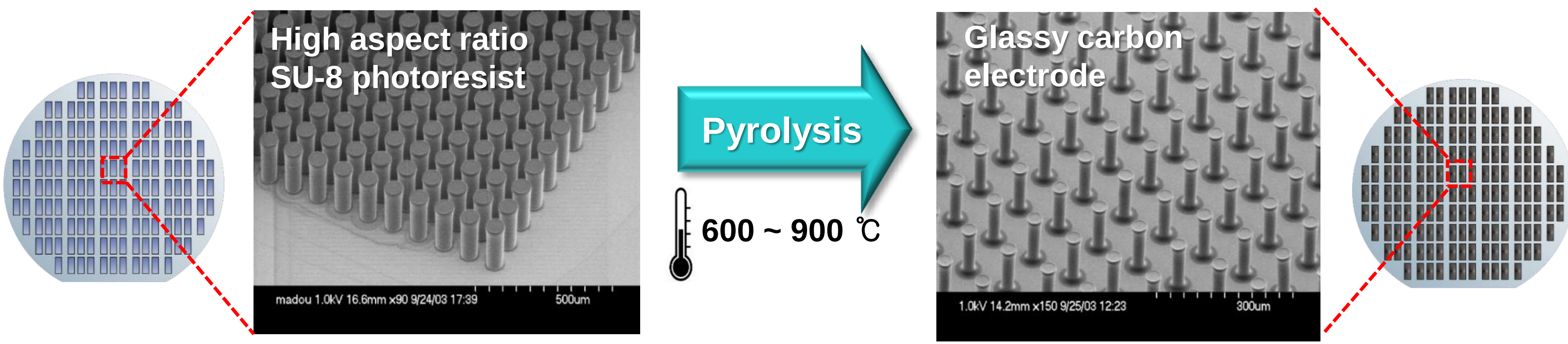
- ✓ Large electrode surface:
  - ➡ Hassle alignment process
- ✓ Nano-gap:
  - ➡ Sacrificial layer removal
  - ➡ Difficulty in chip integration
  - ➡ Small current signal

Carbon-MEMS-based nanoelectrodes

- ✓ Simple two-step nanoelectrode fabrication
- ✓ 3D architecture with complex design
- ✓ Electrode gap control via pyrolysis
- ✓ Comparable or even better current amplification

Carbon-MEMS

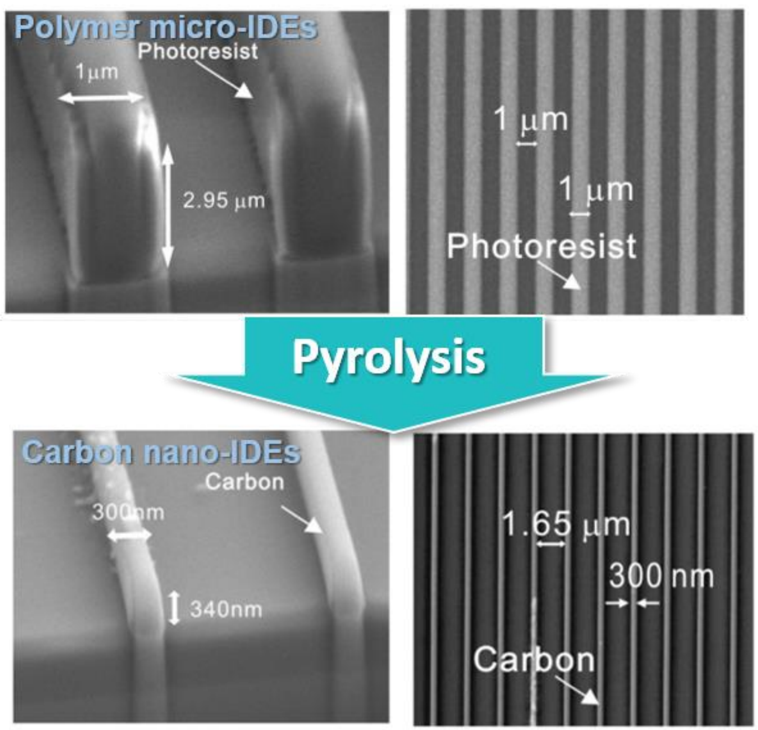
- **Polymer patterning + Pyrolysis**
  - Wafer-level simple fabrication of micro/nano carbon 3D structures
    - Conversion from polymer to glassy carbon
    - Conversion from insulator to conductor
    - Overcome limited manufacturability (brittleness)
    - Controllable geometry: photolithography, nano-imprint, e-beam lithography, electrospinning.



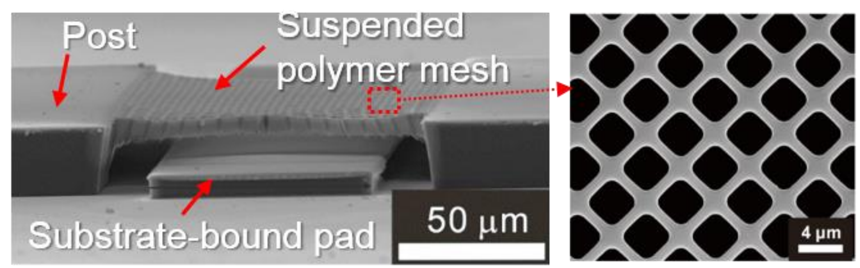
Carbon-MEMS

- **Polymer patterning + Pyrolysis**
  - Dramatic volume reduction up to 90%
    - Conversion from microstrucutres to nanostructures
    - Simple and easy fabrication of 1D carbon nanostructures

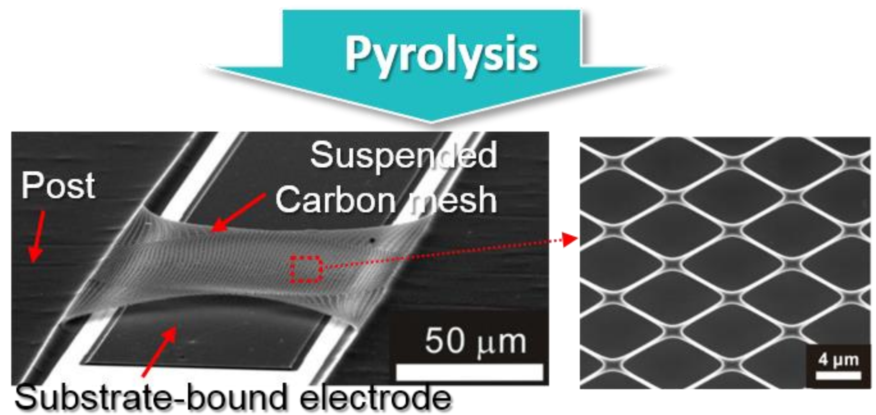
Interdigitated electrodes (IDEs)



Sandwich electrodes



Post height: 10 ~ 20 µm; Electrode gap: 4 ~ 14 µm



Post height: 3 ~ 7 µm; Electrode gap: 2 ~ 4 µm

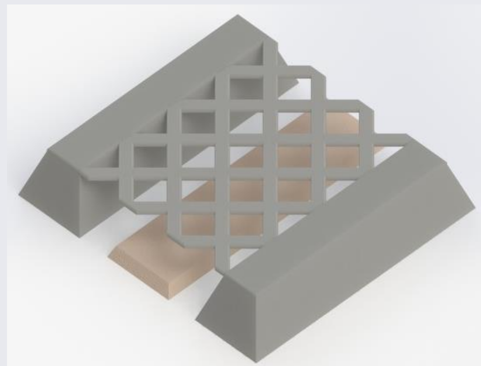
Electrode configurations

3D sub-microscale electrode sets

Dual electrodes

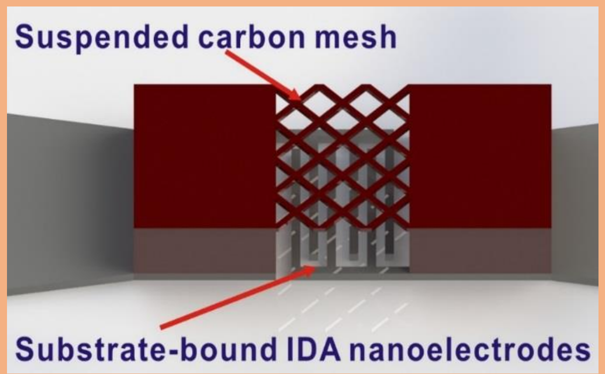
1:1 aspect ratio IDEs
- Generator: Comb 1
- Collector: Comb 2

Sandwich electrodes
- Generator: Planar E
- Collector: Mesh



Triple electrodes

Suspended mesh (Bioreceptors) + IDEs (Redox cycling)



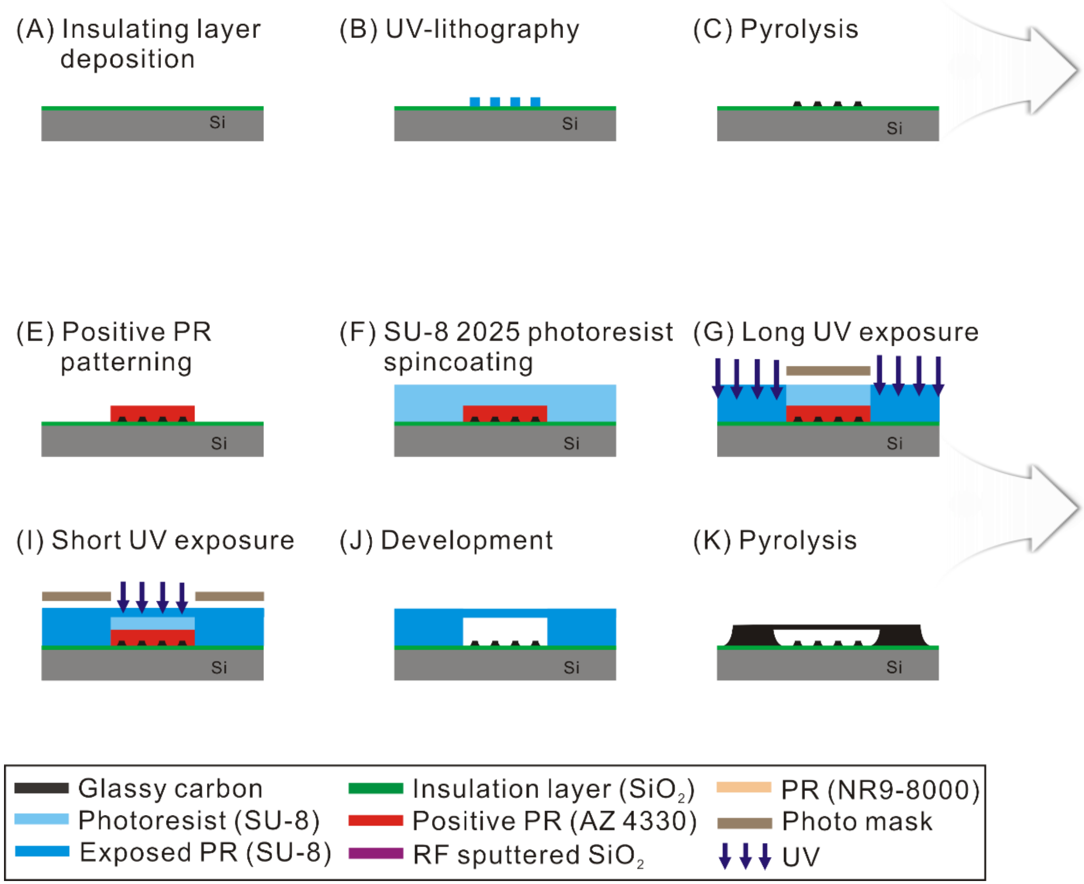
Advantages

- Simple fabrication
- Large surface area
- Efficient Redox cycling
- Biocompatible material

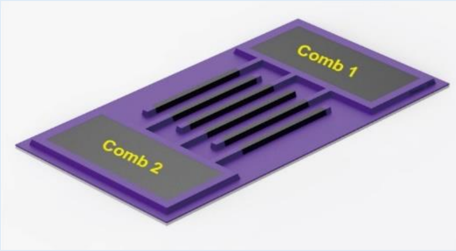
Fabrication

- # Carbon-MEMS

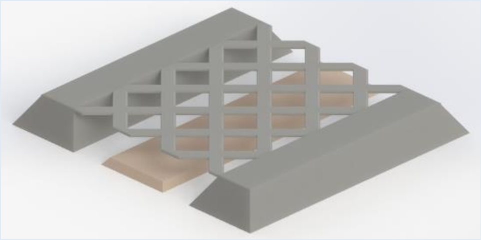
  ### 3D sub-micro scale architecture



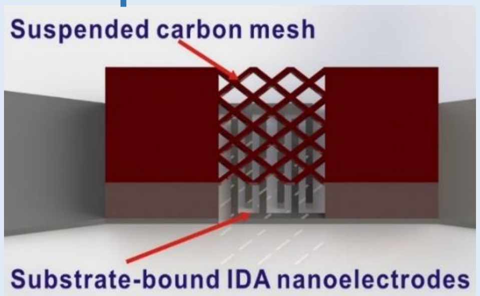
1:1 aspect ratio IDEs



Sandwich electrodes

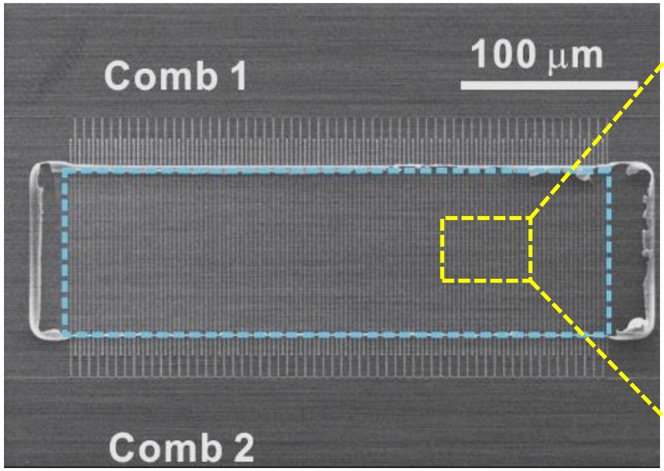


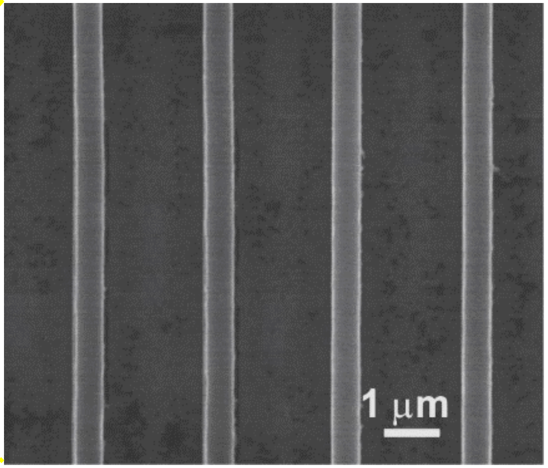
Triple electrodes



Electrode morphology

- **Carbon-MEMS ➡ Dual electrodes**
  - **Interdigitated Array (top view)**

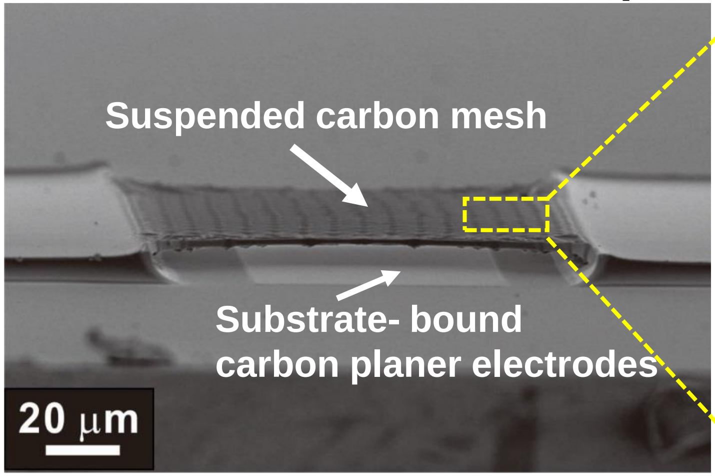


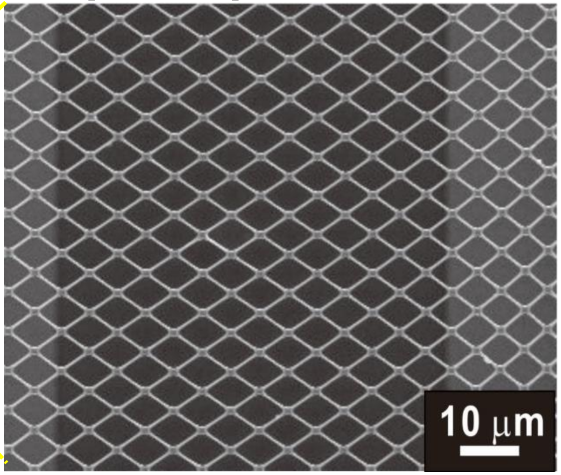


Dimensions:

- Length: 100 µm
- Width: 620 nm
- Thickness: 650 nm
- Electrode gap ~ 1.9 µm

  - **Sandwich electrodes (side & top view)**





Dimensions

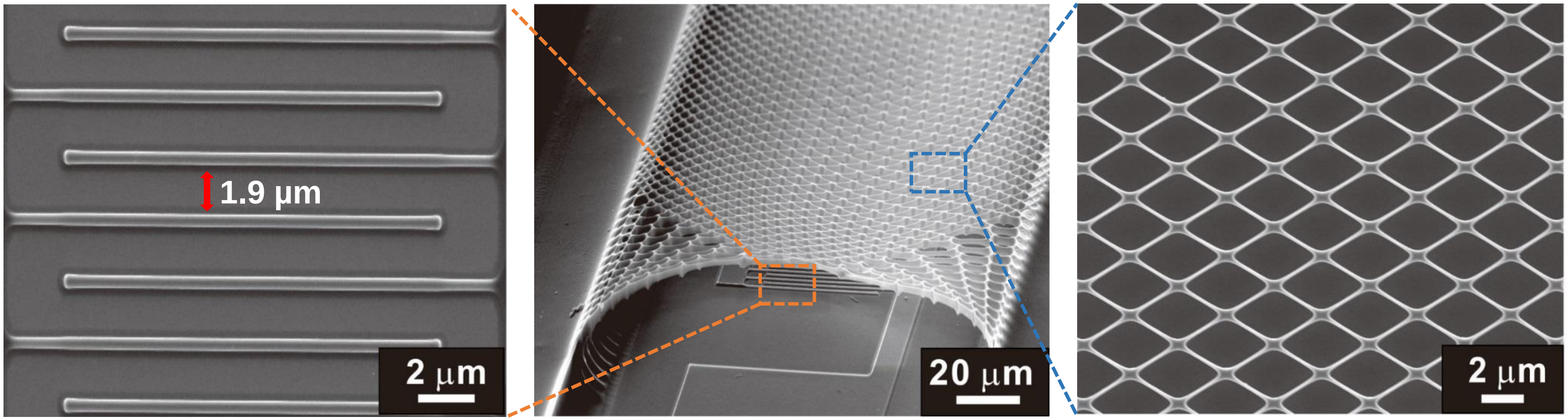
- Post height: ~ 4 µm
- Mesh thickness ~ 1 µm
- Mesh width ~ 300 nm
- Pad thickness ~ 600 nm
- Electrode gap ~ 2.4 µm

Electrode morphology

- **Carbon-MEMS ➡ Triple electrodes**

Substrate- bound carbon IDEs

Suspended carbon mesh



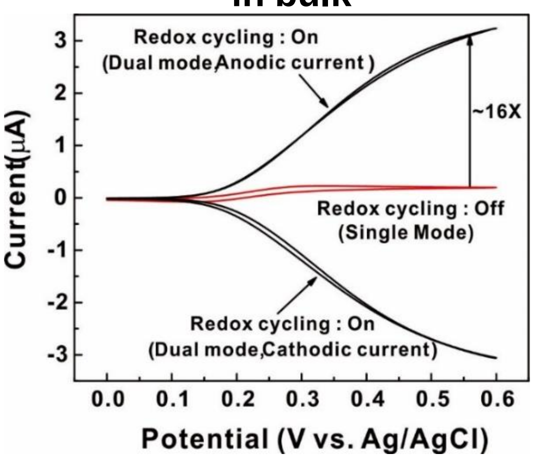
Substrate-bound IDEs	Suspended mesh
620 nm (line width)	300 nm (line width)
650 nm (thickness)	1.2 mm (thickness)

Signal amplification via redox cycling

- **Signal amplification**
  - Amplification factor $= \dfrac{I_{\text{Dual mode}}\ \text{(Current signal with Redox cycling)}}{I_{\text{Single mode}}\ \text{(Current signal without Redox cycling)}}$
  - Linear diffusion between generator and collector
  - Bulk vs Microchannel
    - Volume confinement effect in microchannel
    - Cyclic voltammetry (CV) vs Chronoamperometry

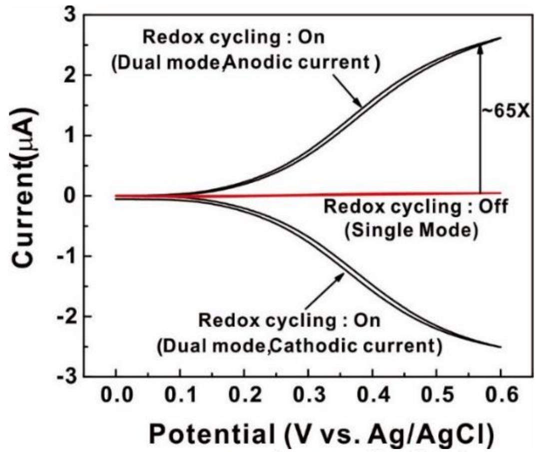
Carbon nano-IDEs

Cyclic voltammetry in bulk

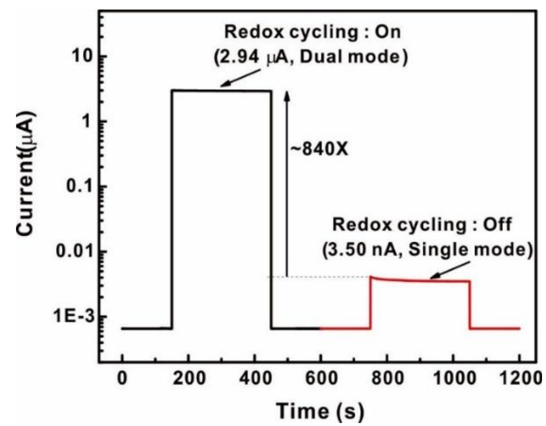


Sandwich electrodes

Cyclic voltammetry in microchannel



Chronoamperometry in microchannel

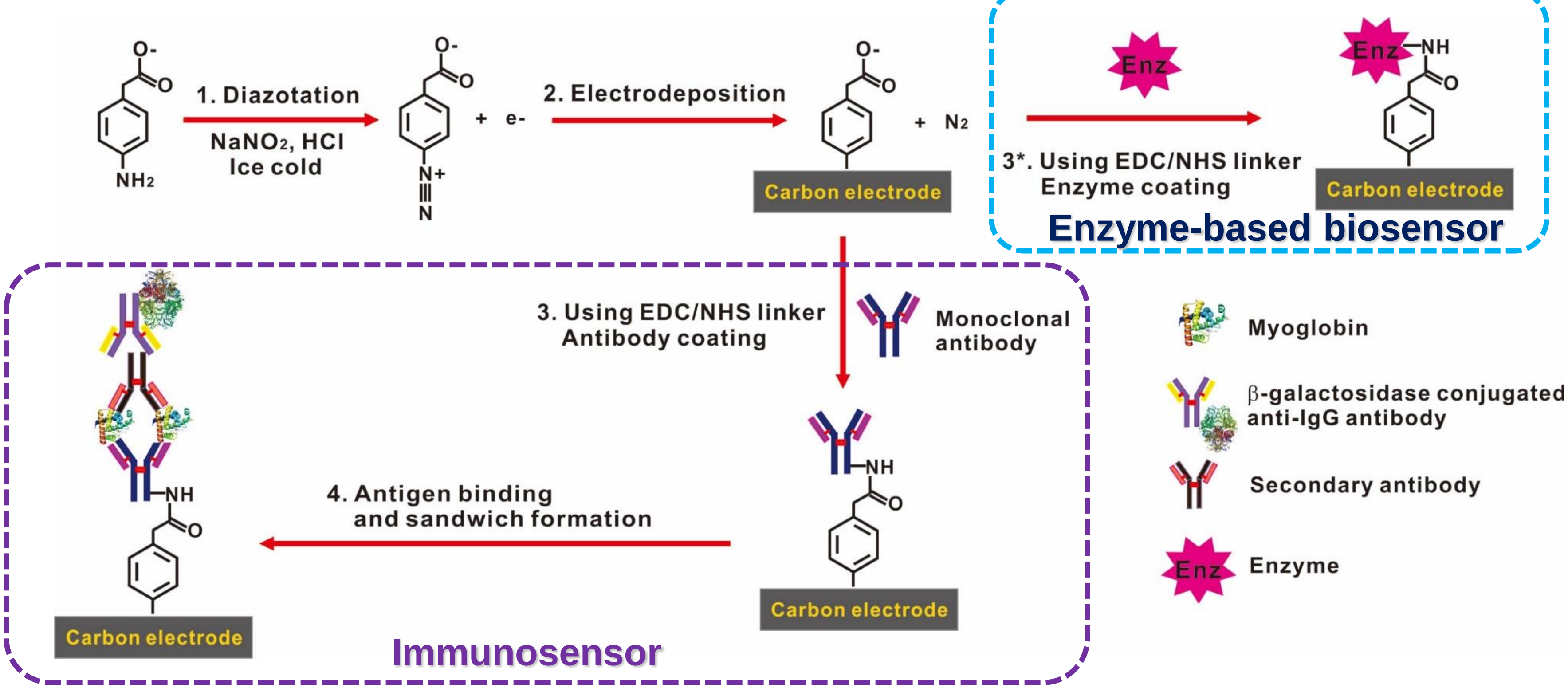


Approaches for selective modification

3D sub-microscale electrode sets

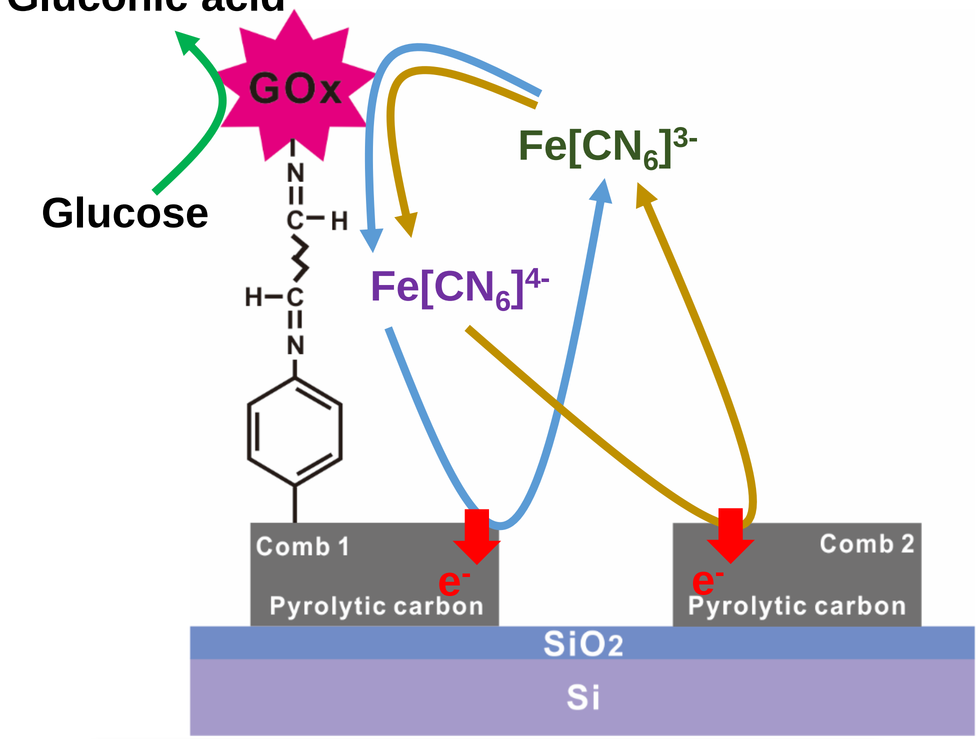
Enzyme-based biosensor | Immunosensor

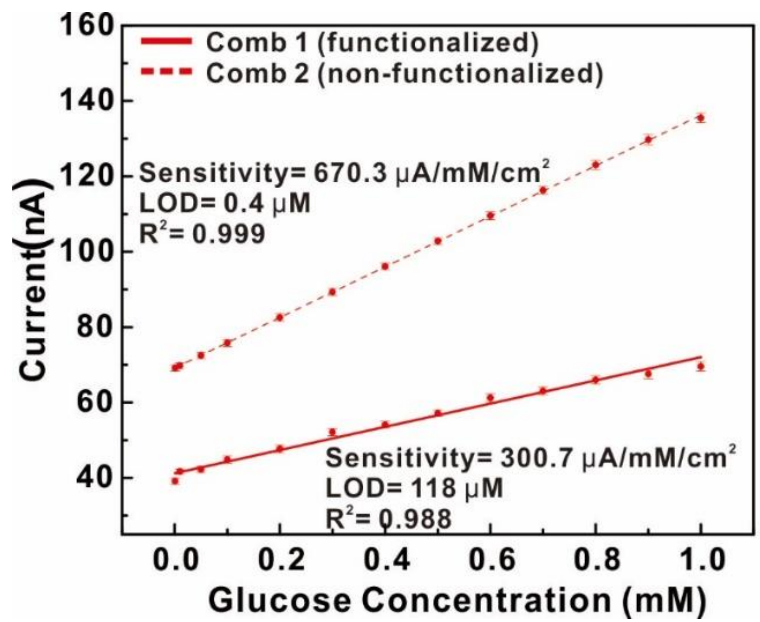
- Diazonium salt reduction ➡ Selective covalent modification



Enzyme-based biosensors

- **Carbon IDEs**
  - Glucose biosensor



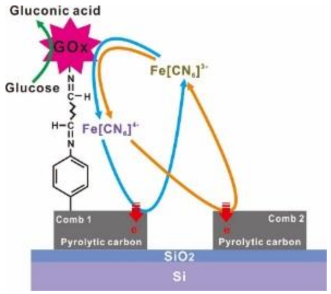


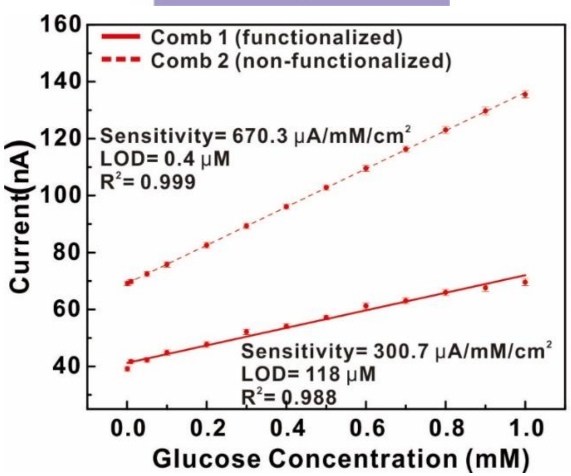
Sensitivity enhancement at non-functionalized comb (Comb 2)

- ✓ More electrochemically active area
- ✓ Efficient Redox cycling
- ✓ 2-fold higher sensitivity vs Comb 1
- ✓ 295-fold better LOD vs Comb 1

Enzyme-based biosensors

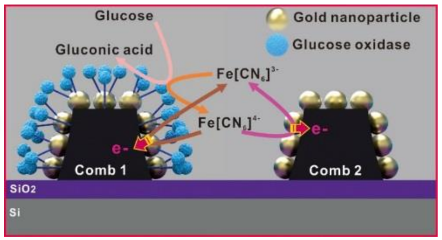
- **Carbon IDEs**
  - Glucose biosensor

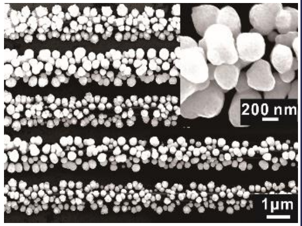


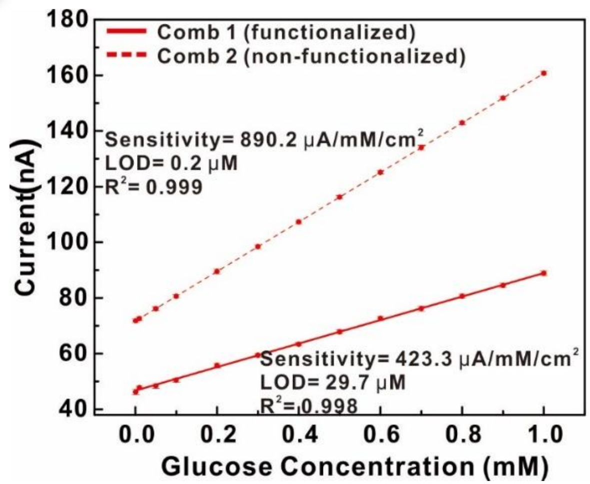


AuNP electrodeposition

- **Carbon IDEs with AuNPs**
  - Glucose biosensor







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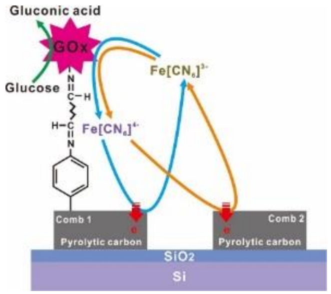
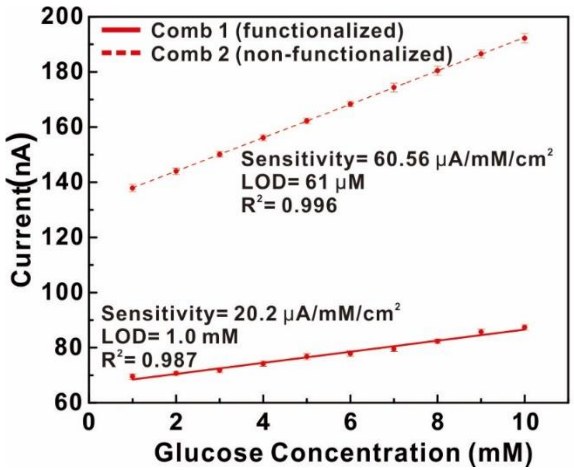
Sensitivity enhancement with electrode modification (AuNPs)

- ✓ More electrochemically active
- ✓ Larger surface area
- ✓ 30% better sensitivity vs carbon IDEs
- ✓ 2-fold better LOD vs carbon IDEs

Enzyme-based biosensors

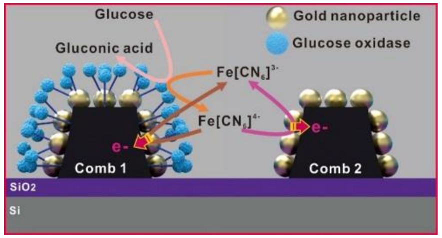
- **Carbon IDEs**
  - Glucose biosensor

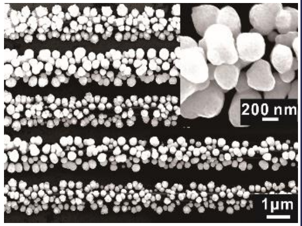


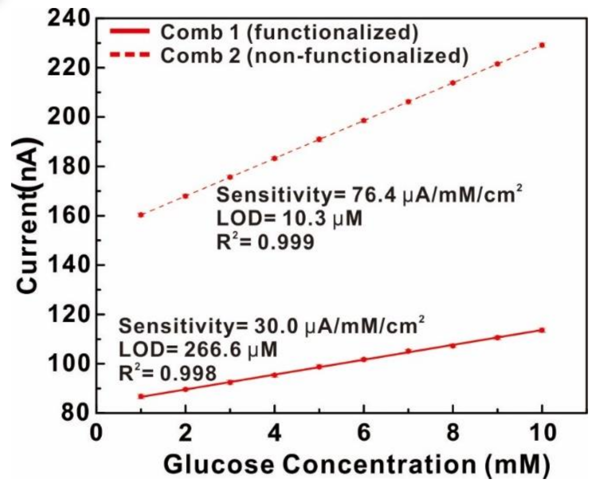


AuNP electrodeposition

- **Carbon IDEs with AuNPs**
  - Glucose biosensor







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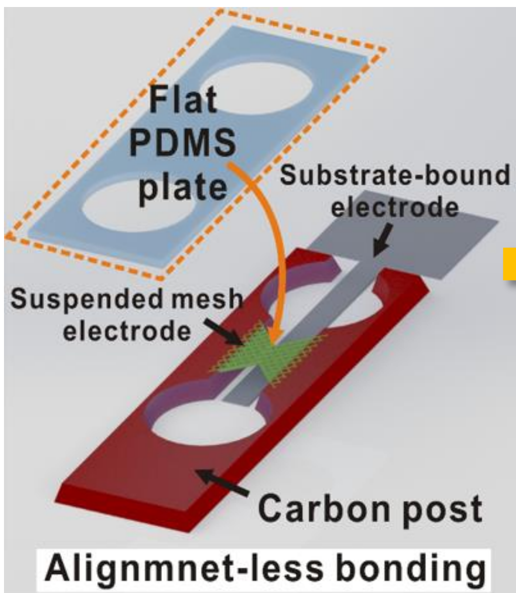
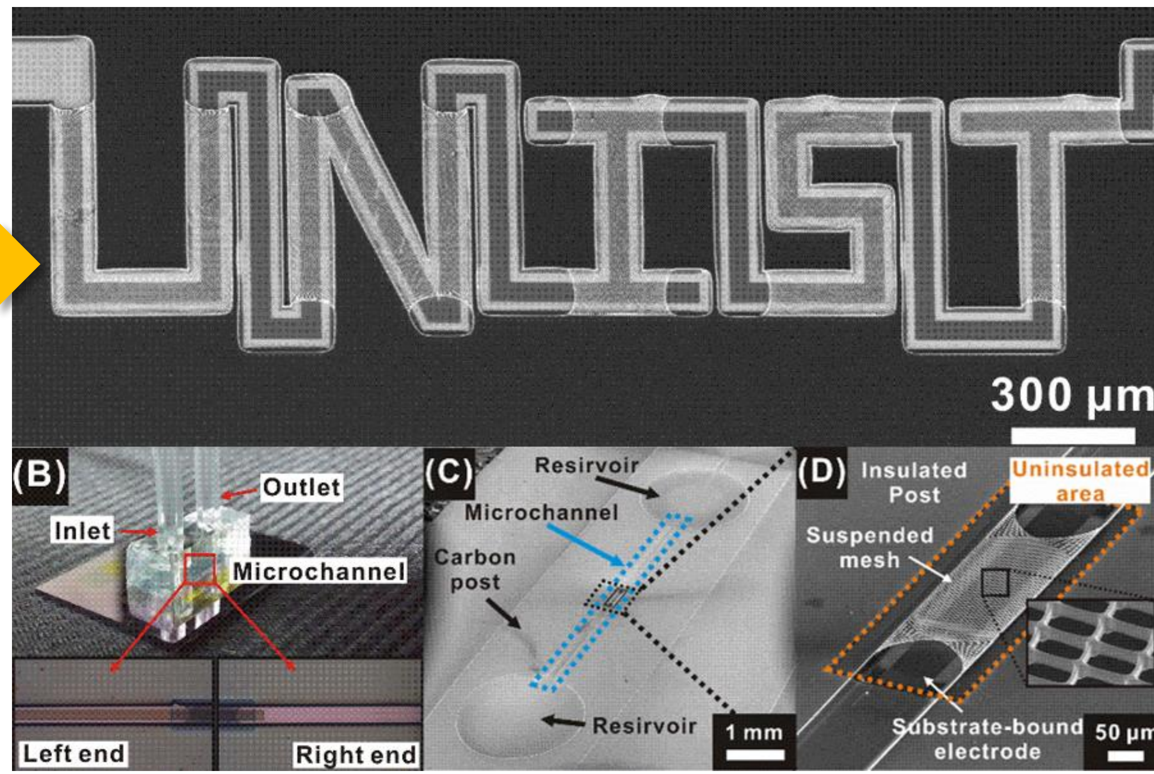
Sensitivity enhancement with electrode modification (AuNPs)

- ✓ More electrochemically active
- ✓ Larger surface area
- ✓ 30% better sensitivity vs carbon IDEs
- ✓ 2-fold better LOD vs carbon IDEs

Selectivity enhancement in microchannel

- **Microchannel-integrated sandwich electrodes**
  - Alignment-less process
    - Flat PDMS plate + Sandwich electrodes
    - Carbon posts work as channels walls

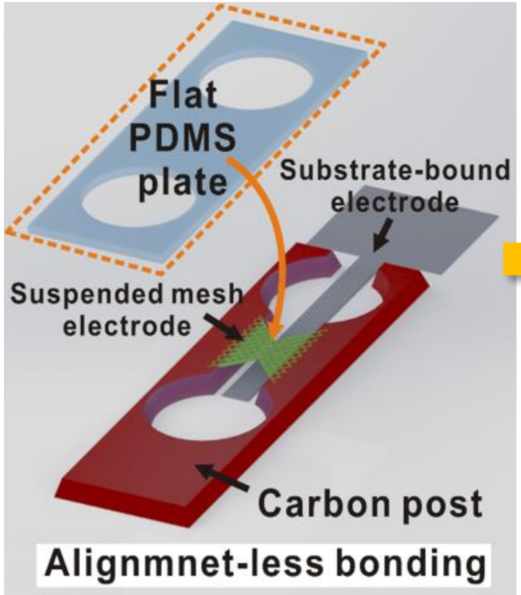


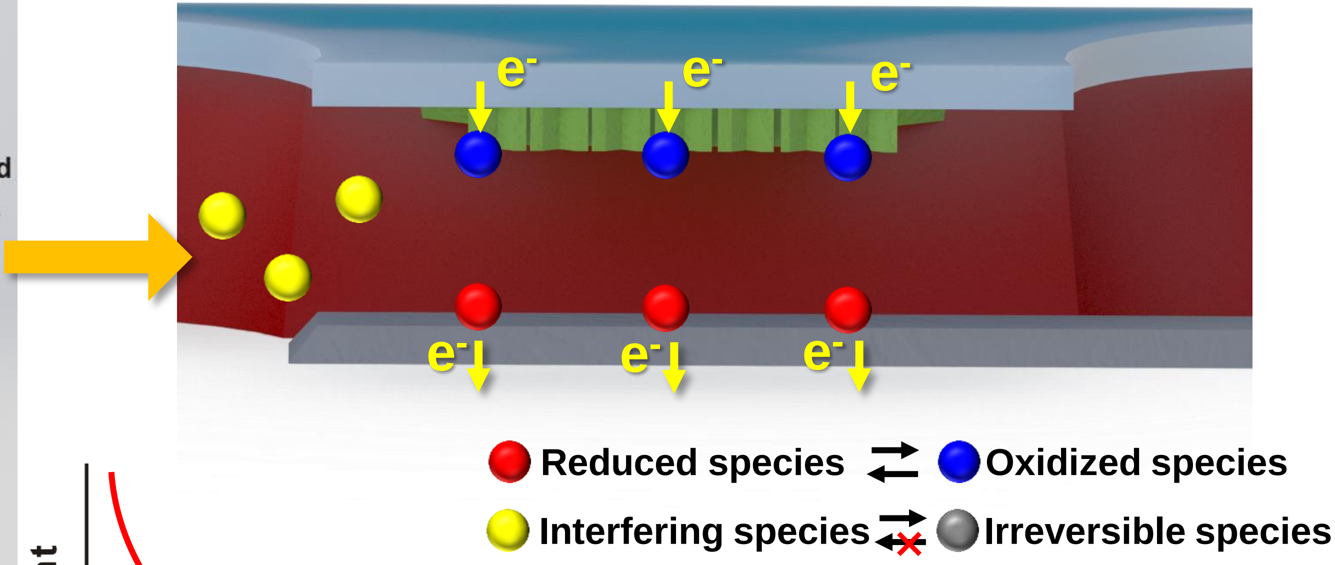


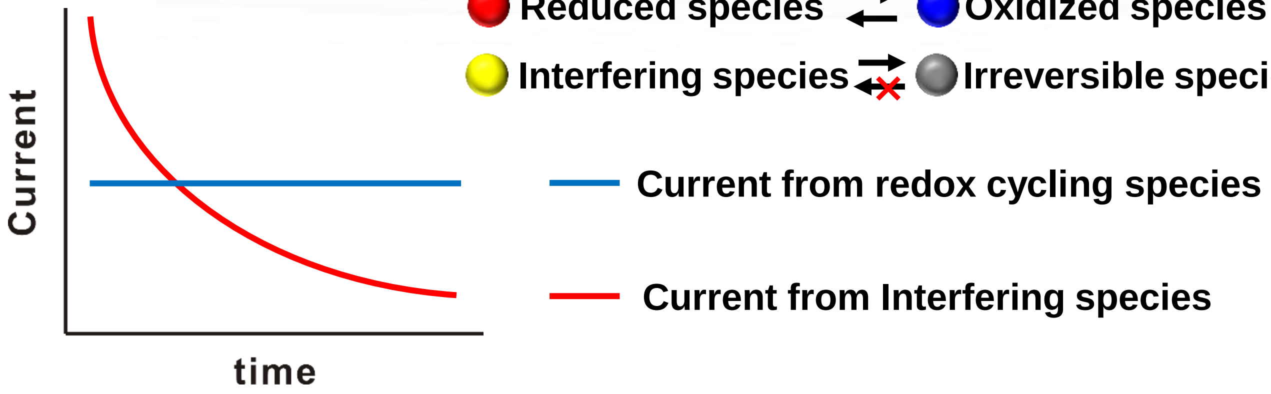
Selectivity enhancement in microchannel

- **Confined volume in microchannel**
  - Electrochemically reversible species ➡ Redox cycling
  - Electrochemically irreversible species ➡ Depletion

➡ Reduction of interference from irreversible spieces



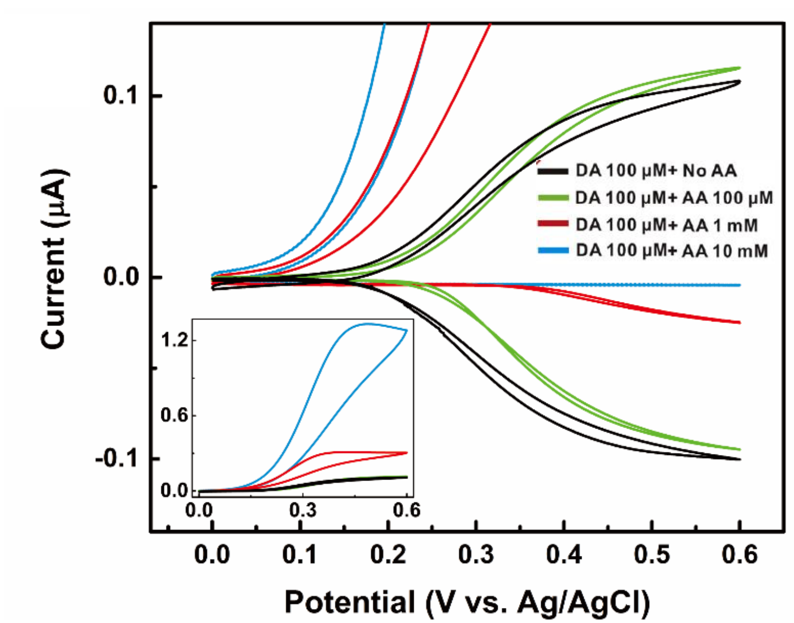




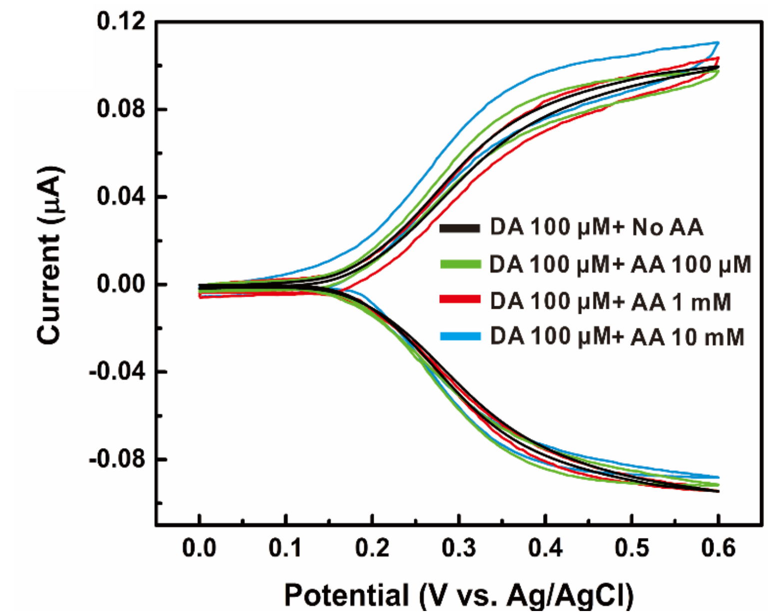
Selectivity enhancement in microchannel

- **Selective detection of dopamine**
  - Electrochemically reversible species: Dopamine (DA)
  - Electrochemically irreversible species: Ascorbic acid (AA)

(A) Bulk solution (w/o PDMS ceiling)



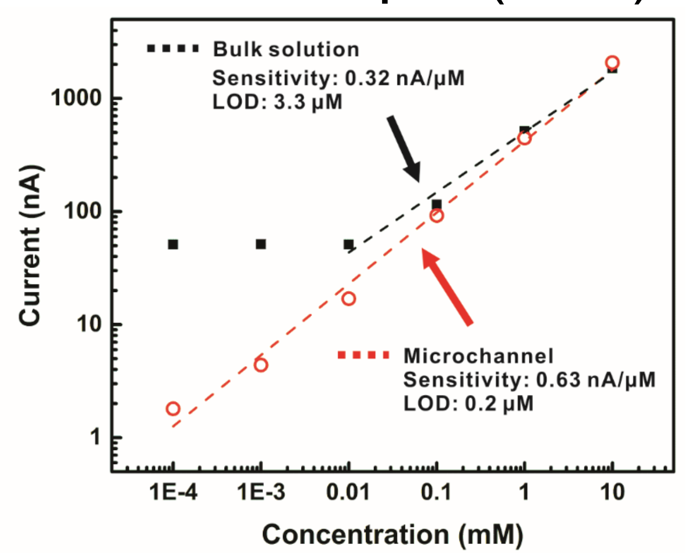
(B) Microchannel (w/ PDMS ceiling)



Interfering current signal from AA ⇒ **No interference effect**

Selectivity enhancement in microchannel

- **Selective detection of dopamine**
  - Electrochemically reversible species: Dopamine (DA)
  - Electrochemically irreversible species: Ascorbic acid (AA)
- **Dopamine sensing (Bulk vs Microchannel)**
  - LOD enhancement in microchannel
    : 3.3 μM (Bulk) → 0.2 μM (17 fold)
  - Sensitivity enhancement in microchannel
    : 0.32 nA/μM (Bulk) → 0.63 nA/μM (2 fold)

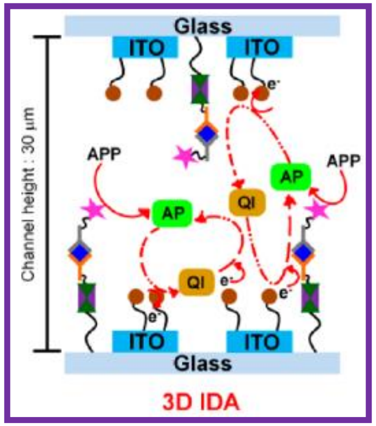


Dopamine sensing in a microchannel
- ✓ Dopamine: 100 nM – 10 mM
- ✓ Ascorbic acid: 1 mM
- ✓ PBS: 0.1 M

Triple electrode-based immunosensors

- **Electrochemical immunosensors**
  - Sandwich immunoassay
  - Redox cycling
  - Generation of redox substrate near the adjacent electrode

(a) Parallel IDA electrodes



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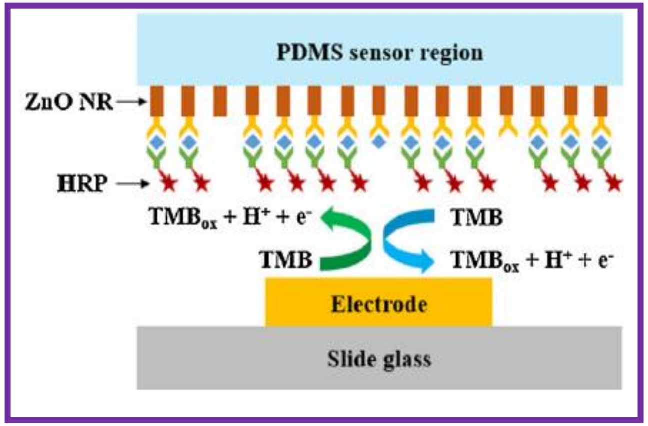
(b) PDMS with electrodes



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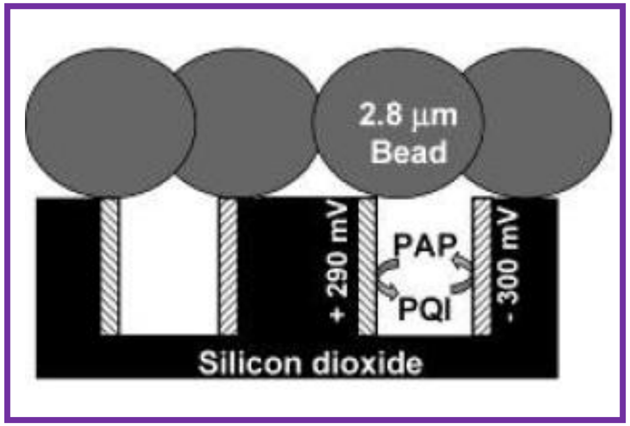
(c) Magnetic-beads



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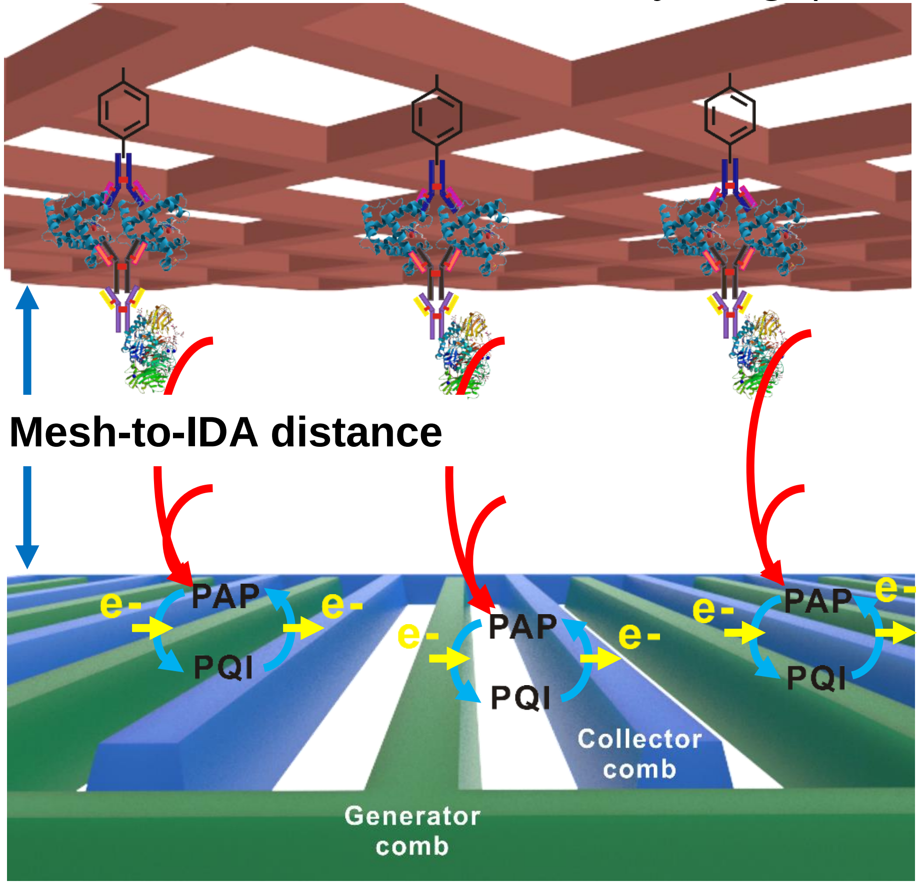
Limitations of reported immunosensing schemes

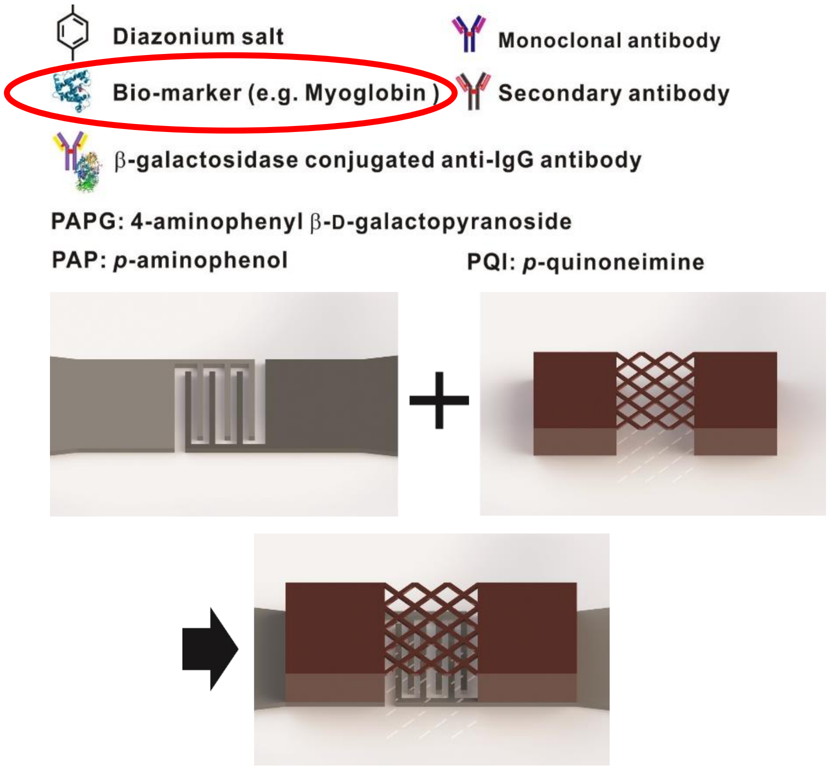
1. Large gap between the bottom and ceiling electrodes; (a) and (b)
2. Required proper alignment; (a) and (b)
3. Beads can block and inhibit the diffusion of electroactive species; (c)

Require new sensor configurations

Triple electrode-based immunosensors

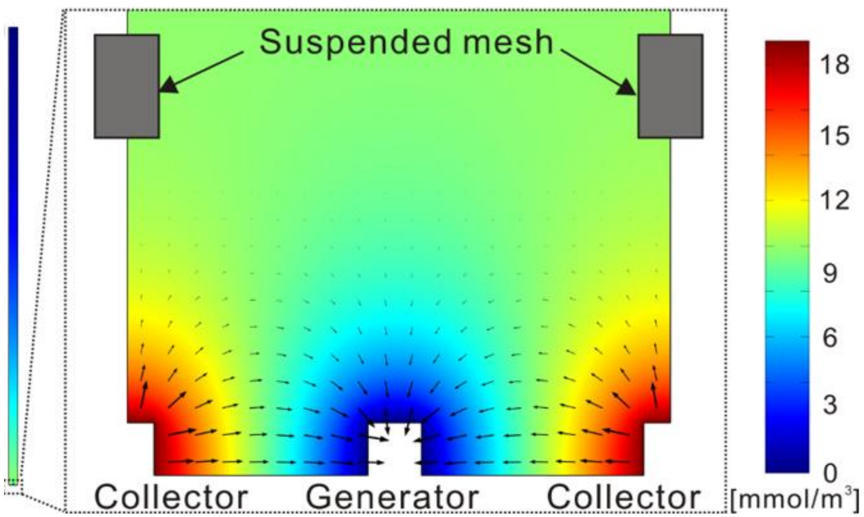
- **Suspended mesh + Substrate-bound IDEs**
  - Selective sandwich formation at suspended mesh
    - Generation of redox substrate (PAP) near carbon IDEs
    - Efficient Redox cycling (PAP ↔ PQI) at IDEs

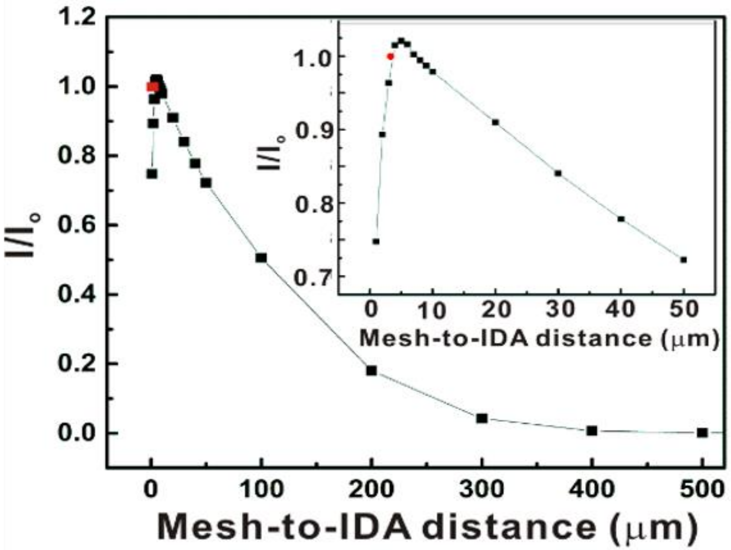




Triple electrode-based immunosensors

- **Suspended mesh + Substrate-bound IDEs**
  - Effect of mesh-to-IDE distance
    - Simulation on diffusion-limited current signal

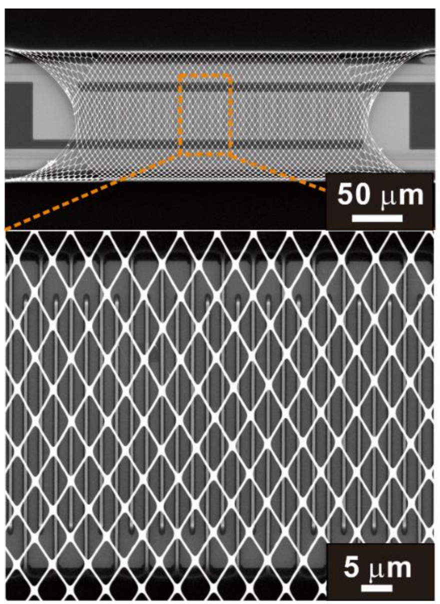


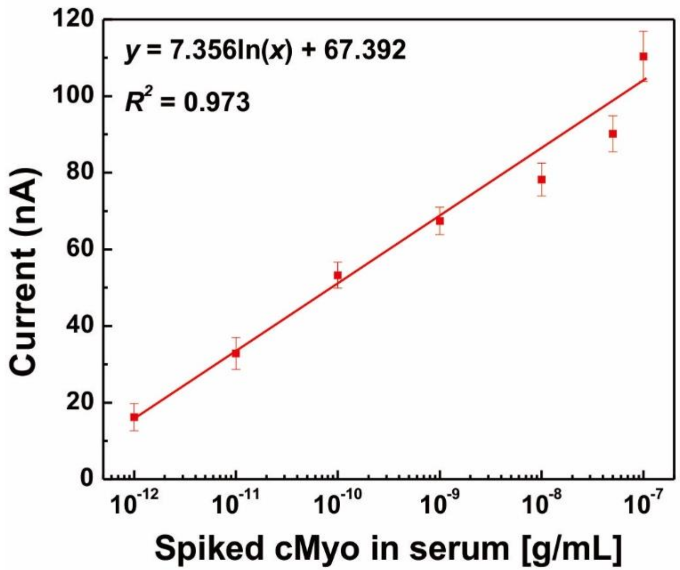


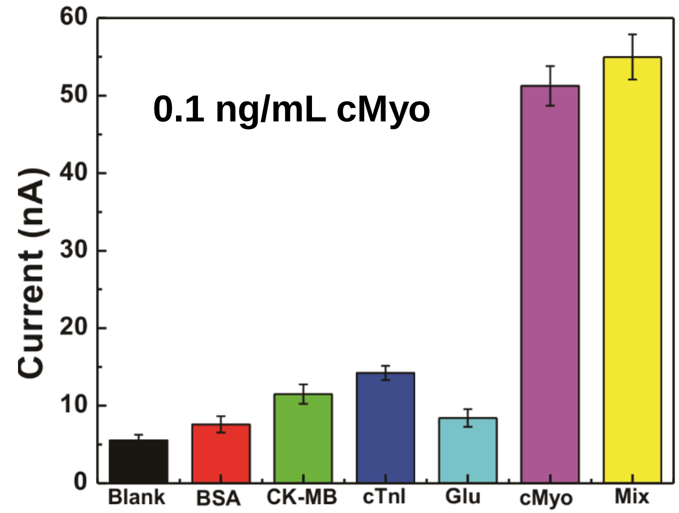
- ✓ Mesh-to-IDEs distance > 5 μm → small distance enhances diffusion of PAP
- ✓ Maximum current at 5 μm
- ✓ Mesh-to-IDEs distance < 5 μm → disturb diffusion among IDEs

Triple electrode-based immunosensors

- **Suspended mesh + Substrate-bound IDEs**
  - Mesh-to-IDEs distance ~ 3.3 μm
    - Efficient production of redox species near IDEs
  - Cardiac biomarker (Myoglobin) in human serum:
    - LOD ~ 0.48 pg/mL
    - High selectivity against interfering species





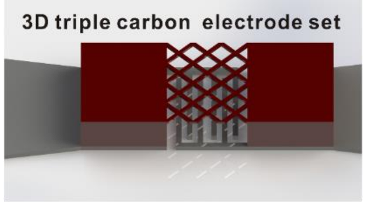


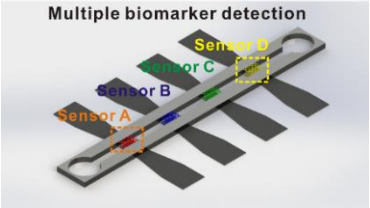
BSA, CK-MB, cTnI $\overset{10x}{>}$ cMyo

Glucose $\overset{900,000x}{>}$ cMyo

Summary & Future works

- **Summary**
  - Wafer-level batch fabrication via C-MEMS
    - 3D sub-micrometer scale electrode architectures
    - Simple and cost-effective processes
  - Electrochemical sensor performance enhancement
    - Sensitivity
      - Selective bioreceptor immobilization near sensing area
        ➡ Efficient Redox cycling at 3D electrodes
      - Electrode surface modification with AuNPs
    - Selectivity
      - Bioreceptors (Enzymes, Antibodies)
      - Depletion effect in microchannel





- **Future works**
  - Multiple biomarker detection in a single chip
    - Microchannel integration
    - Integration of multiplex sensor array
    - Cholesterol, Glucose, CK-MB, Myoglobin, Troponin, etc.

Acknowledgements

- **Collaborator**
  - Prof. March Madou (UC Irvine)

- **MNIS members**
  - Dr. Deepti Sharmar
  - Dr. Yeongjin Lim
  - Jongmin Lee
  - Sanghee Jung

- **Technical supports**

UNIST — UCRF UNIST Central Research Facilities

- **Funding sources**

NRF 한국연구재단 National Research Foundation of Korea

산업통상자원부 Ministry of Trade, Industry and Energy

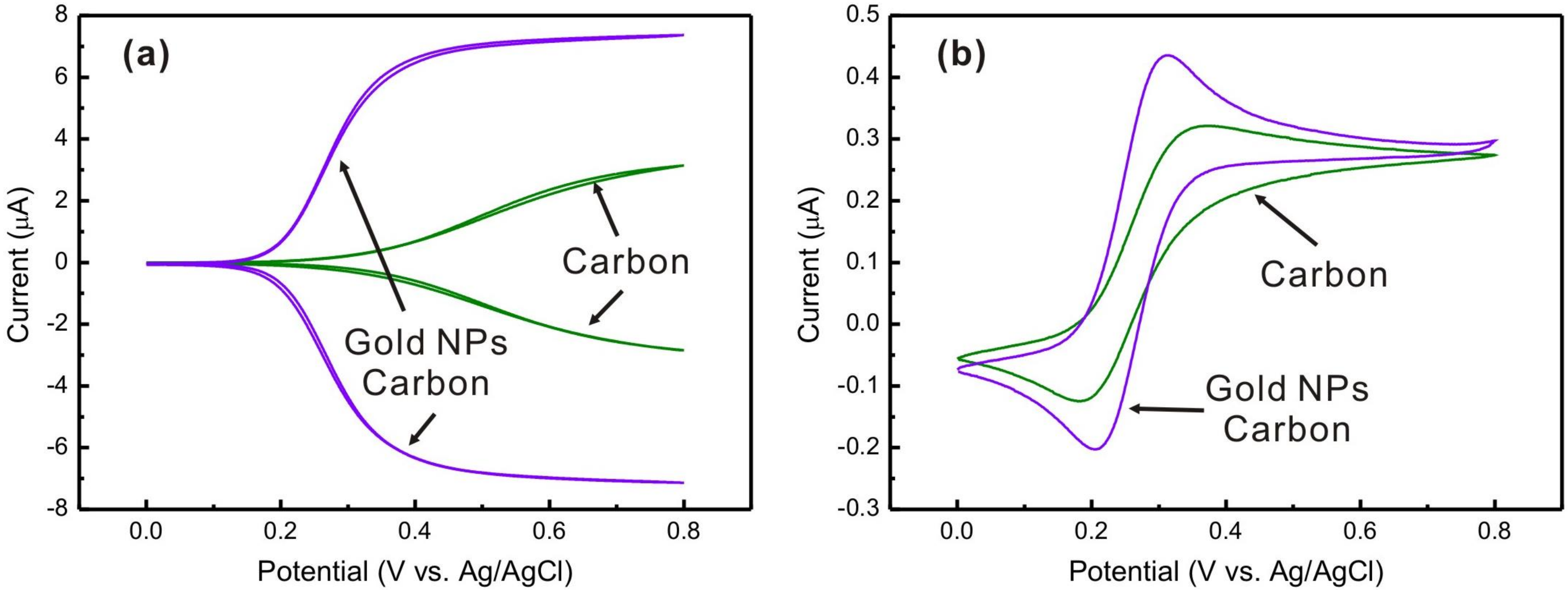
과학기술정보통신부 Ministry of Science and ICT

SK innovation Institute of Technology Innovation

Thank you
for your attention

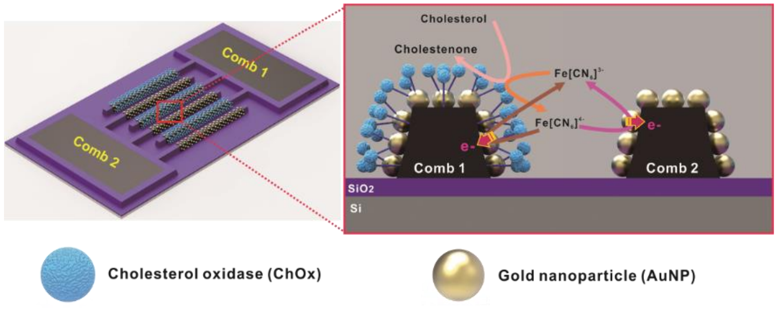
AuNPs/Carbon IDEs

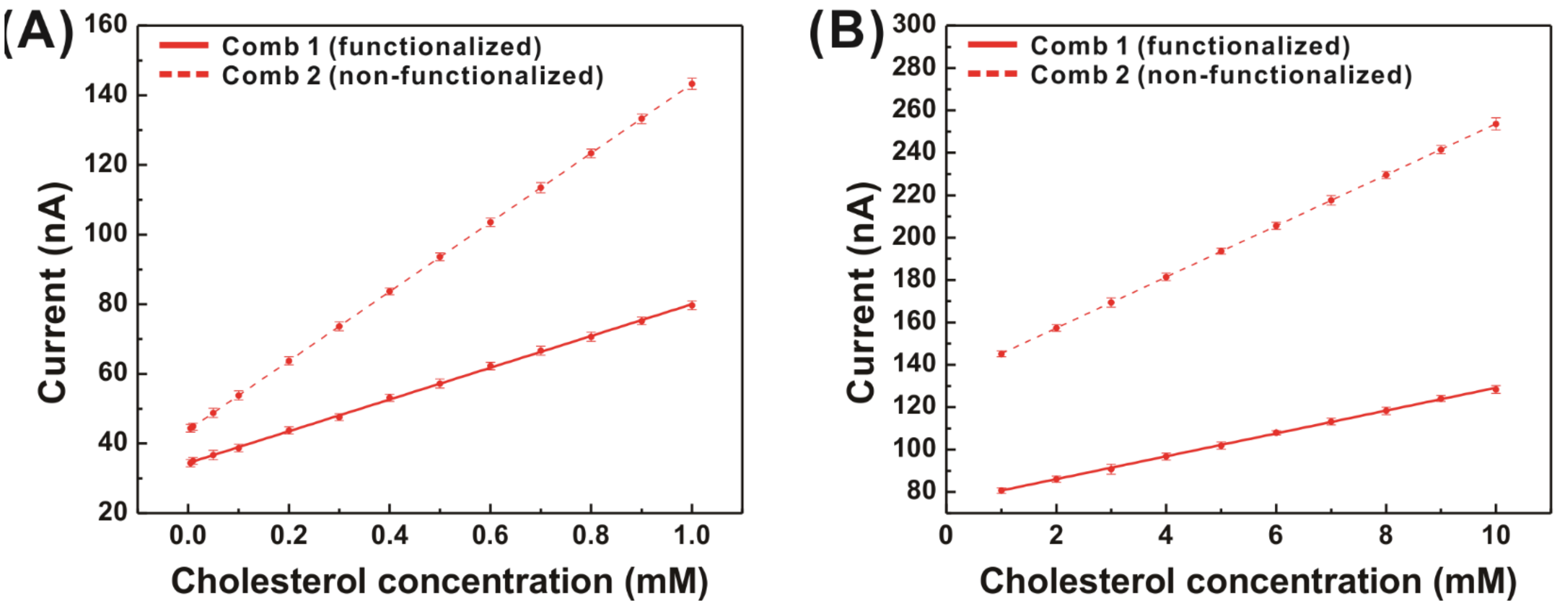
- Electrochemical characterization
  - Cyclic voltammetry
    - 10 mM $[Fe(CN)_6]^{2+}$ in 0.1 M KCl
  - Carbon nanoelectrode
    - Width = 650 nm; Thickness = 650 nm
  - Current signal enhancement in dual mode



Triple electrode-based immunosensors

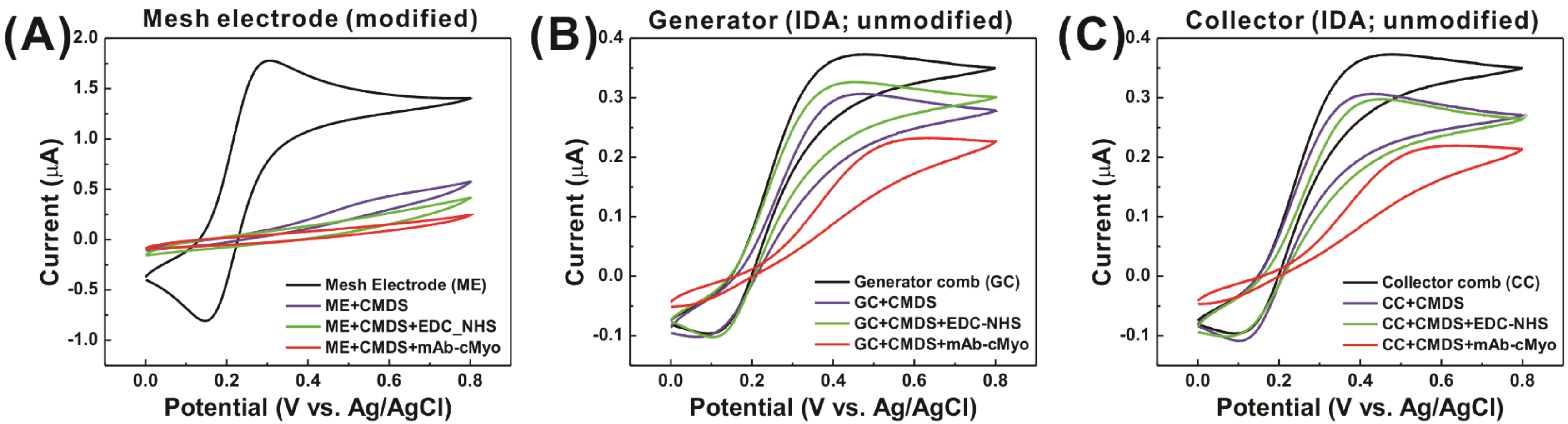
- **AuNPs/carbon IDEs**
  - Cholesterol sensors
    - Sensing range: 0.005-10 mM
    - Sensitivity ~994 μA mM^{-1} cm^{-2}
    - LOD ~1.28 μM



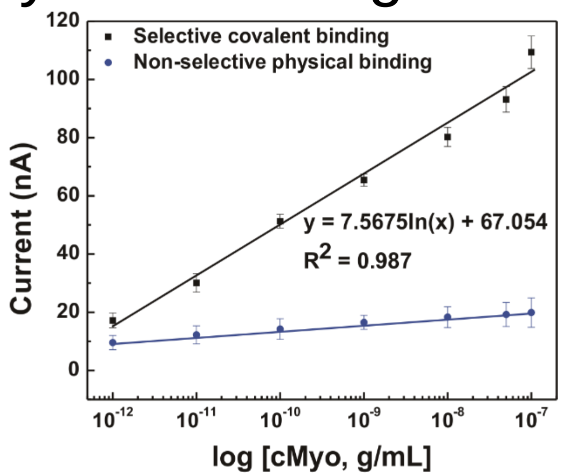


Triple electrode-based immunosensors

- **Effect of selective immobilization**
  - Low current signal deviation after immobilization (IDEs)

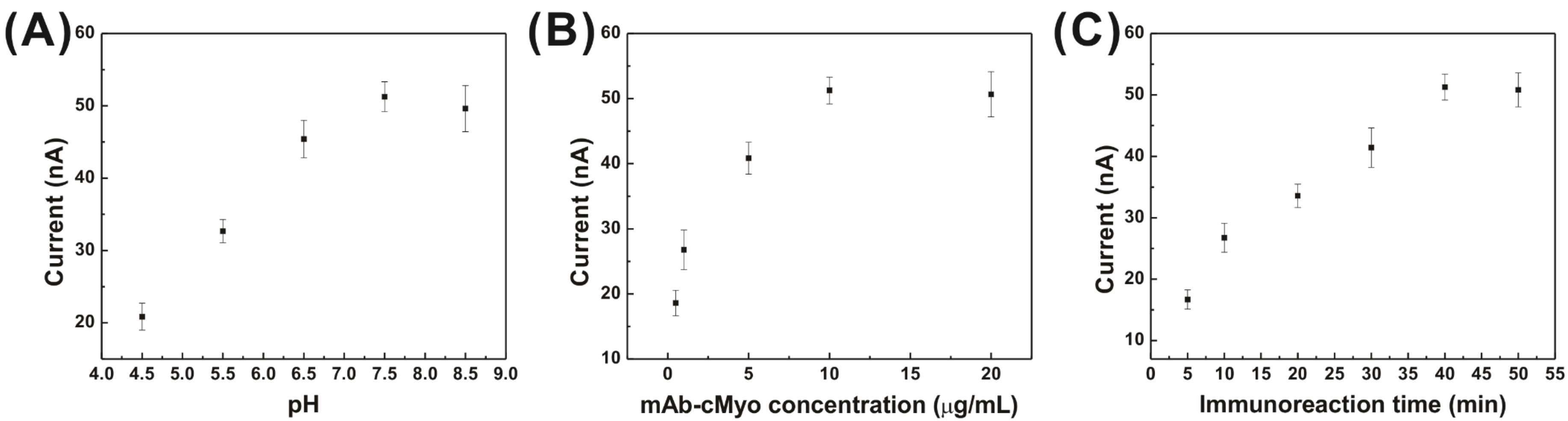


  - Spontaneous physical binding reduces signal significantly.



Triple electrode-based immunosensors

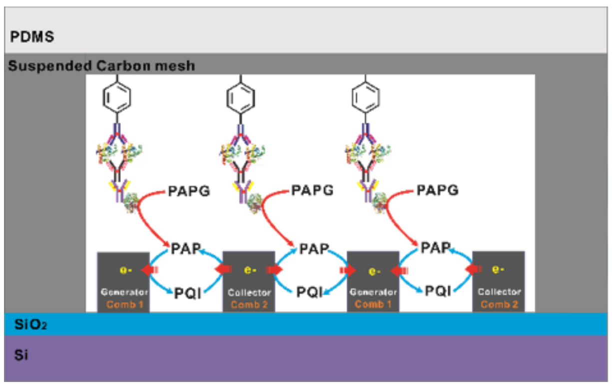
- **Effects of various sensor preparation conditions on the detection of 0.1ng/mL cMyo**



Immunosensing performance

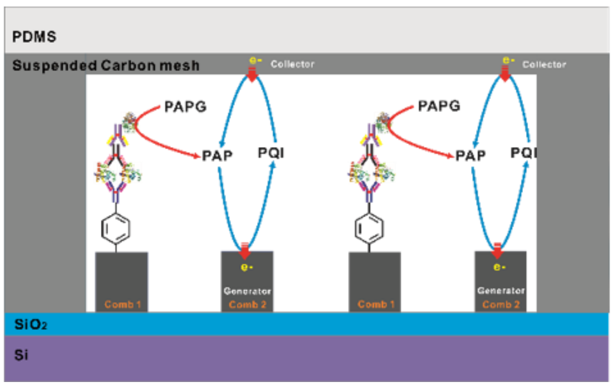
- **Carbon 3D triple electrode**
  - 3D triple electrode set + selective immobilization
    - Reversible redox cycling (PAP ↔ PQI)
    - Generation of redox substrate (PAP) near the IDA electrode
  - Characterization according to antibody binding site

Configuration (I)



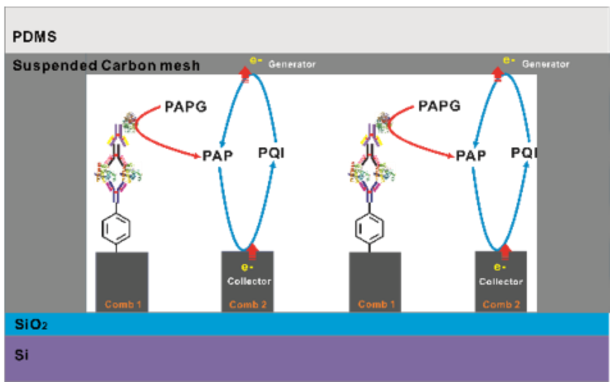
Antibody binding site
: suspended mesh
generator & collector
: IDA nanoelectrodes

Configuration (II)



Antibody binding site
: one comb of IDA
Generator
: the other comb of IDA
Collector
: suspended mesh

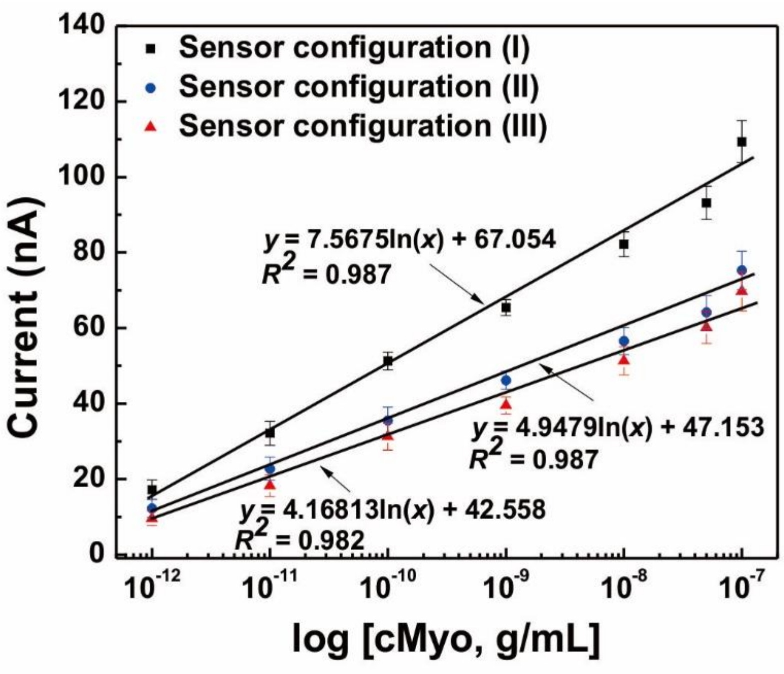
Configuration (III)



Antibody binding site
: one comb of IDA
Generator
: suspended mesh
Collector
: the other comb of IDA

Immunosensing performance

- **Carbon 3D triple electrode**
  - 3D triple electrode set + selective immobilization
    - Reversible redox cycling (PAP ↔ PQI)
    - Generation of redox substrate (PAP) near the IDA electrode
  - Characterization according to antibody binding site



Sensitivity enhanced at sensor configuration (I)

- ✓ Small inter-electrode gap between IDEs
- ✓ Efficient redox cycling
- ✓ Large surface area of mesh enabling substantial biomolecule binding
- ✓ Efficient mass transfer

(I) Antibody site: suspended mesh, generator and collector: IDA nanoelectrodes;
(II) Antibody site: one comb of IDA nanoelectrodes, generator: the other comb of IDA nanoelectrodes, collector: suspended mesh;
(III) Antibody site: one comb of IDA nanoelectrodes, generator: suspended mesh, collector: the other comb of IDA nanoelectrodes)