Detection of C- reactive Protein by Liquid-gated Carbon Nanotube Field Effect Transistors (LG-CNTFET): A promising tool against antibiotic resistance

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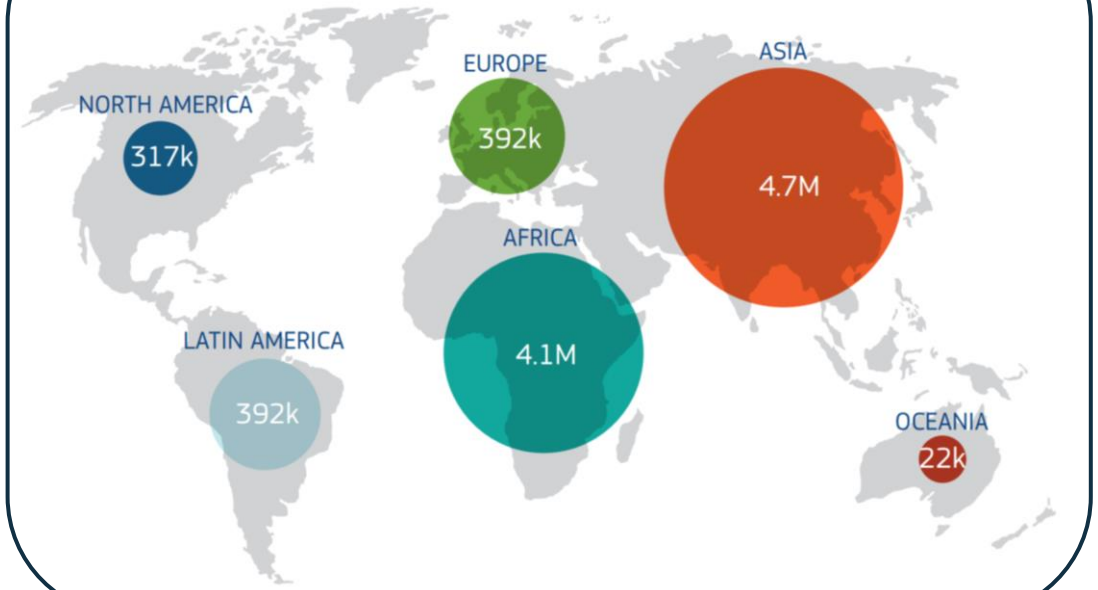
Antimicrobial Resistance (AMR)

One of the biggest threats in global health (WHO) !

700,000 Deaths / year

25,000 Deaths / year
1.5 billion € / year

Number of Deaths per Year by 2050



European Commission, 2019

Antibiotics Misuse

EU One Health Action Plan Against AMR

Development of Novel Diagnostics

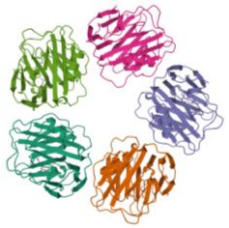
"Novel, rapid and reliable diagnostics are crucial for differentiating between bacterial and viral infections and identifying AMR, so that the most appropriate treatment can be given in a timely manner…"

Respiratory Track Infections

https://livescience.com/

- Highest antibiotic prescriptions
- Difficult diagnosis due to similar symptomatology

C-Reactive Protein (CRP) as Differentiation Biomarker



Infection Type	[CRP] mg/L
Healthy patient	0.5 (<10)
Viral	18.73
Influenza	25.65
Bacterial	135.96

Haran, J.P. et al. *Am. J. Emerg. Med.* (2013) **31**, 137–144

CRP Detection Technologies

- Particle-enhanced immunophelometry
- Immunoturbidimetry (IT)
- Fluorescent immunosorbent assay
- ELISA

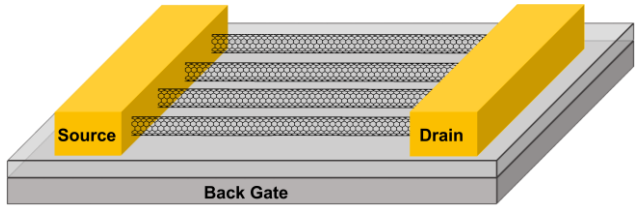
- Time consuming
- Bulky detection systems
- Trained personnel required
- Expensive reagents

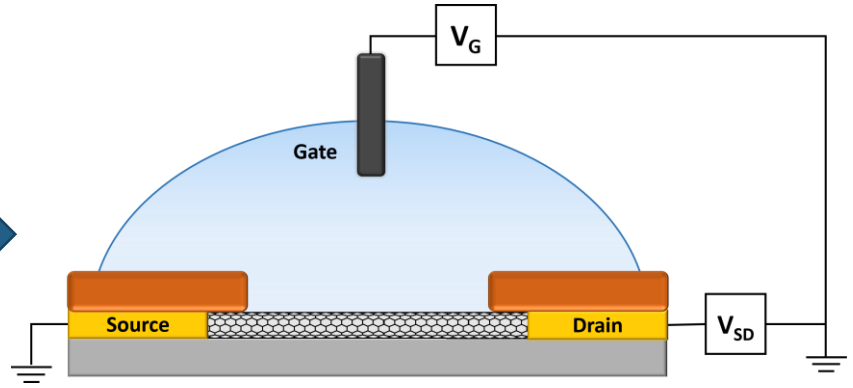
Electrical sc-SWCNTs based Biosensors

- High surface-to-volume ratio
- High carrier mobility
- High sensitivity and selectivity
- Miniaturization
- Low power consumption

Llobet, E. *Sensors and Actuators B* (2013) **179** 32-45

LG-SWCNTFET – Working Principle





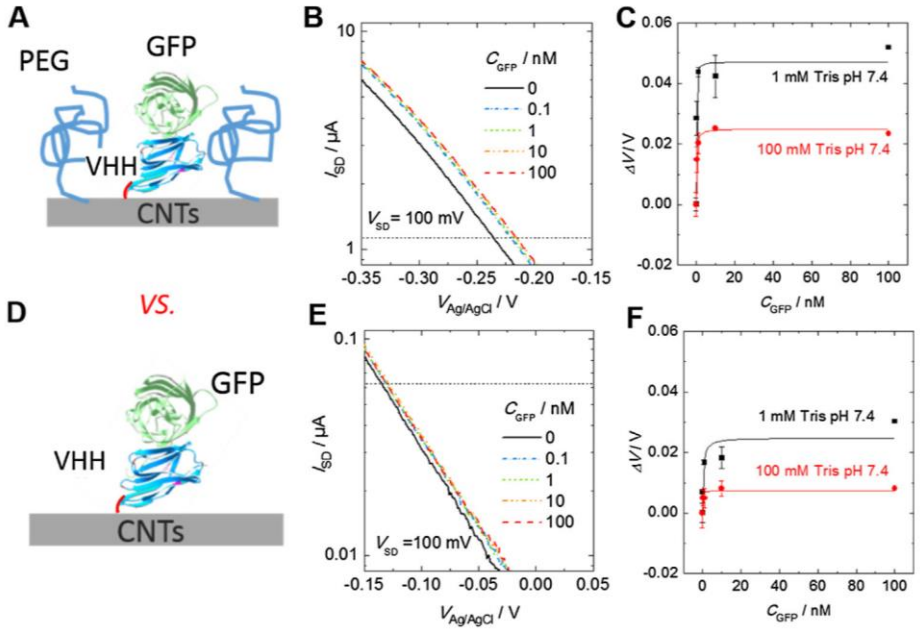
- Sample in contact with SWCNTs
- Electrical double layer
- Variation in the surface potential modulates the current flow

Real-time and label-free detection

LG-SWCNTFET as Biosensors

Main Challenges:

1. Non-specific adsorption
2. Debye Screening



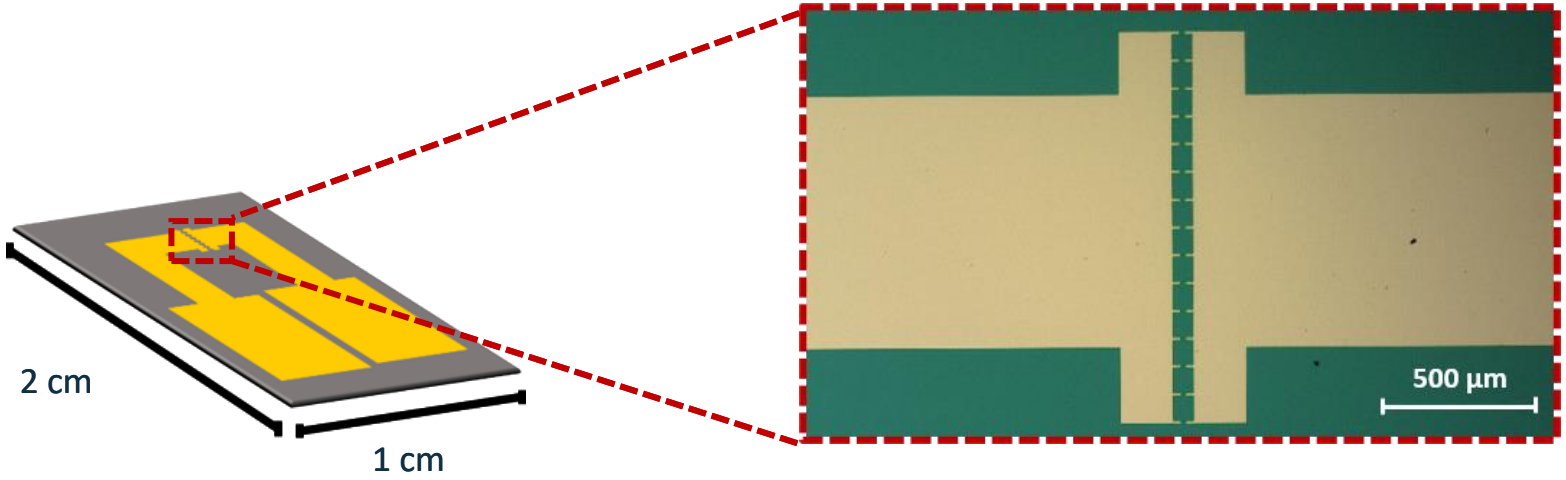
Filipiak, et al. *Sensors and Actuators B* (2018) **255** 1507-1516

Objective

Development of a CRP sensing platform based on SWCNTFETs using specific antibodies and polyethylene glycol (PEG) functionalization

Contact Electrodes Fabrication

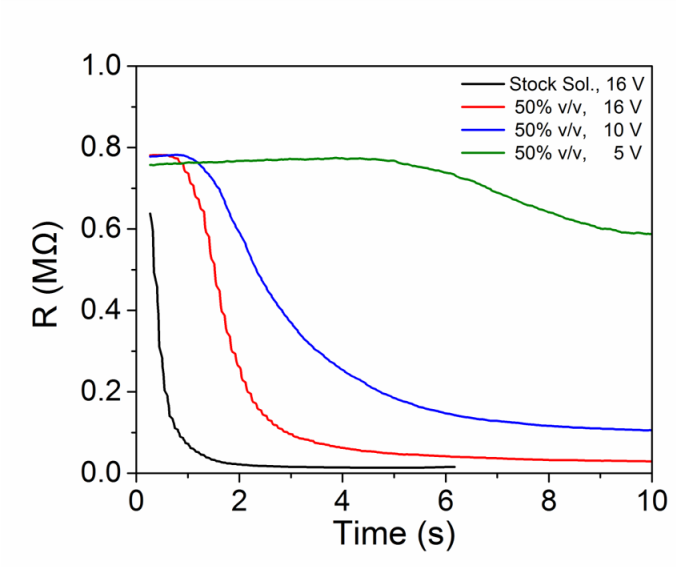
- Si/SiO_2 wafers
- UV lithography
- Metal Evaporation:
  3 nm Cr 50 nm Au
- Array of 15 electrode pairs
- 20 µm separation gap

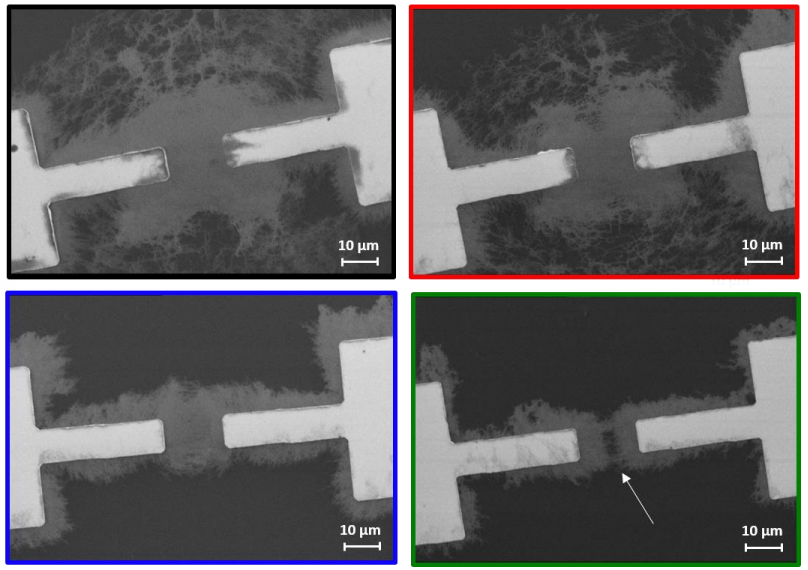


Controlled Dielectrophoretic Deposition of sc-SWCNTs

- 98% sc-SWCNTs
- N-methyl-2-pyrrolidone (NMP)
- Optimization process based on dispersion concentration and applied AC voltage.
- Optimal deposition using:

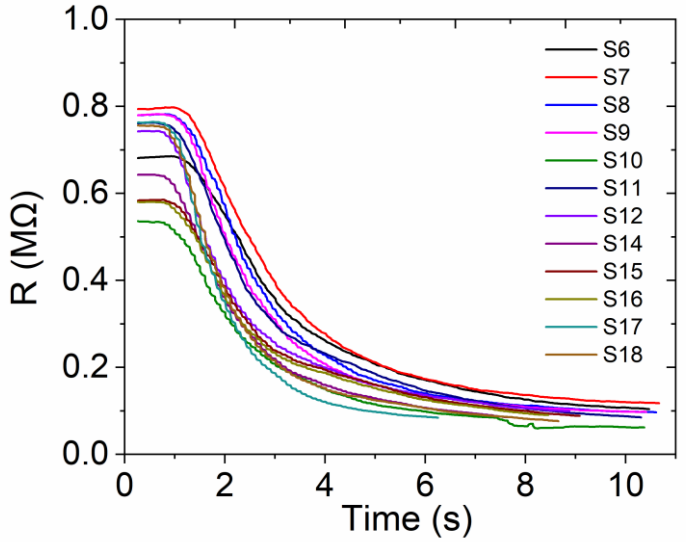
50% v/v , 10 V_{pp}

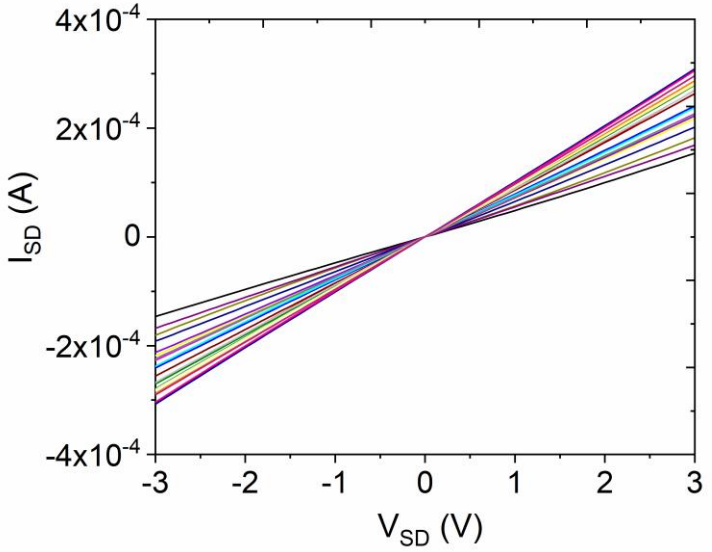




Controlled Dielectrophoretic Deposition of sc-SWCNTs

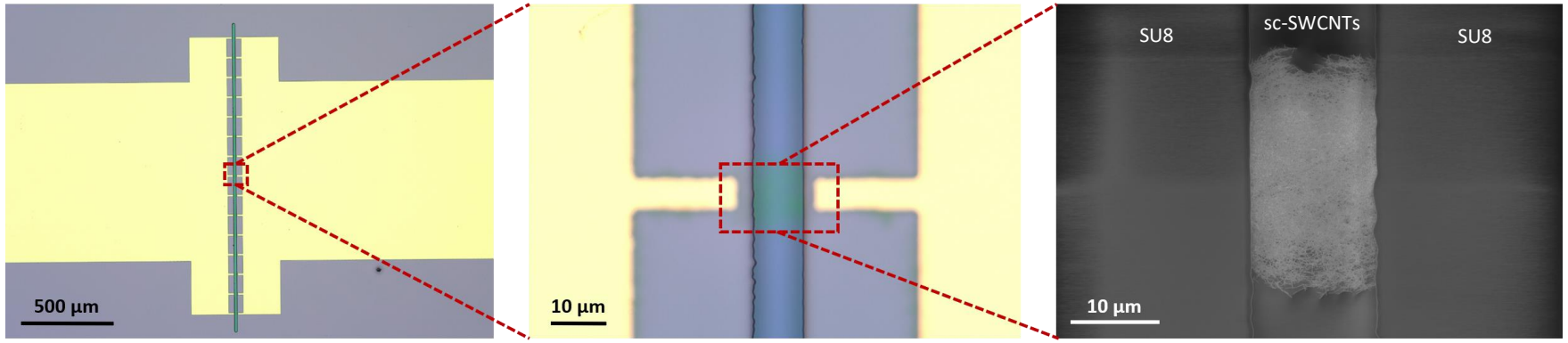
- Reproducible deposition
- Final resistances of ~20 kΩ





Electrical Passivation

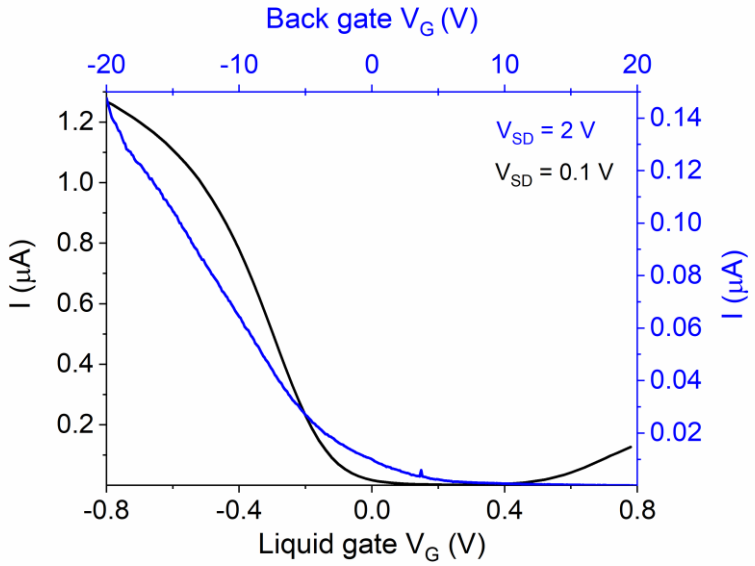
- 10 µm thick SU8 passivation layer
- Slit aperture of 16 µm width
- sc-SWCNT network open area of ~300 µm² / finger-pair

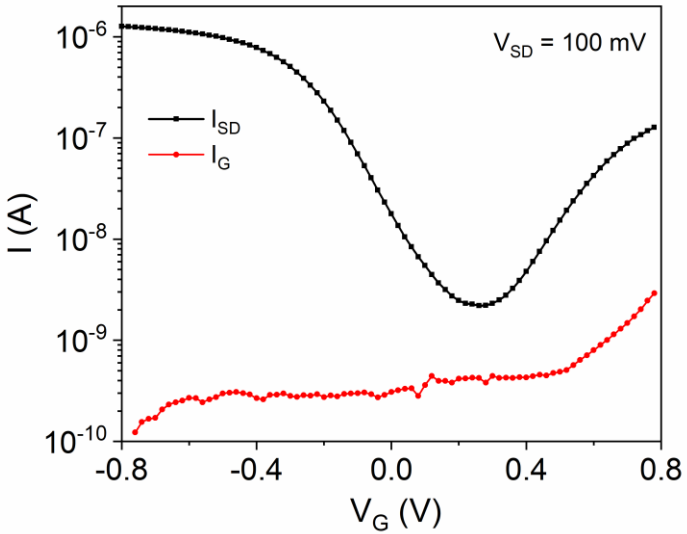


Electrical Characterization

- p-type semiconductor behavior using back-gate and liquid-gate systems

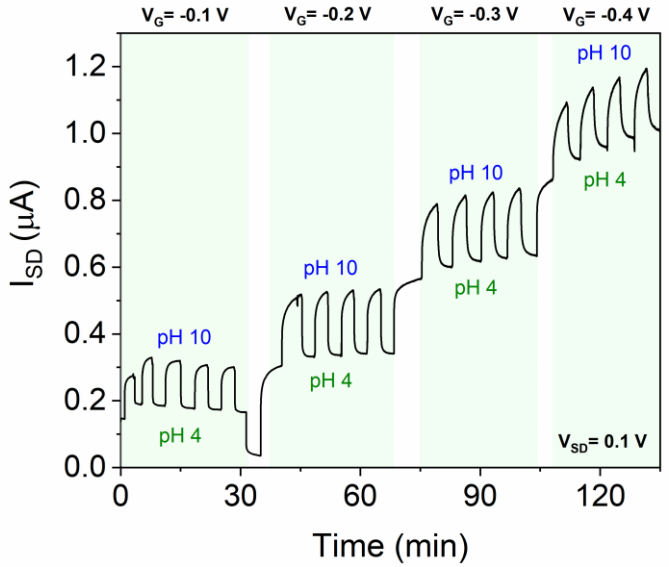
- On/off ratios of ~ 10^3 in PBS 150 mM and pH 7.8
- Subthreshold swings of ~ 180 mV/dec
- Low Gate-current leakage (I_G)

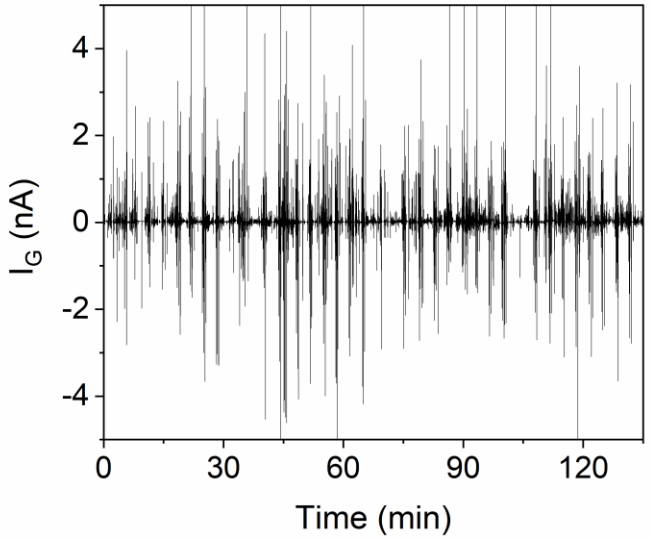




pH Sensing

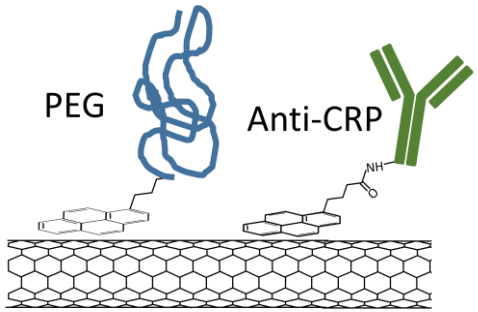
- Robust and stable detection of highly basic and acidic buffers at different V_G
- Neglectable gate-current leakage (successful electrode passivation)

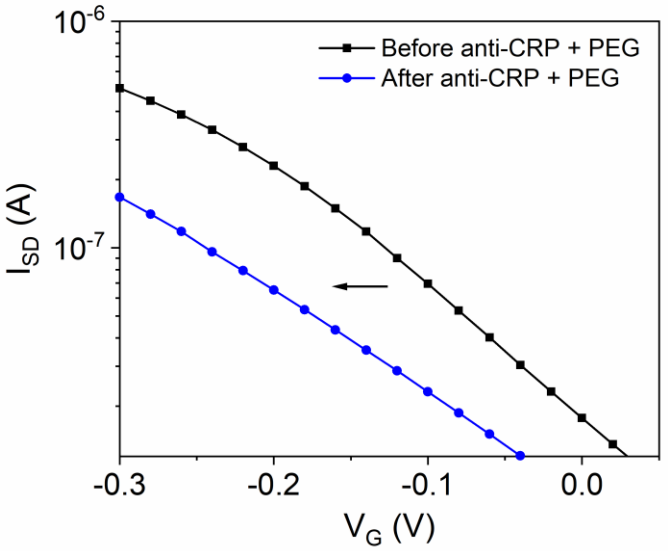




Non-covalent functionalization of sc-SWCNTs Networks

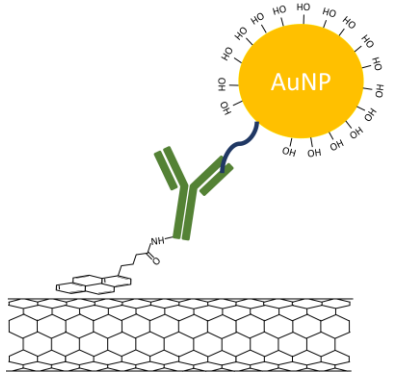
1. Pyrene-NHS + Pyrene-PEG (4:1) on sc-SWCNTs by π-π stacking
2. Anti-CRP coupling by NHS / EDC

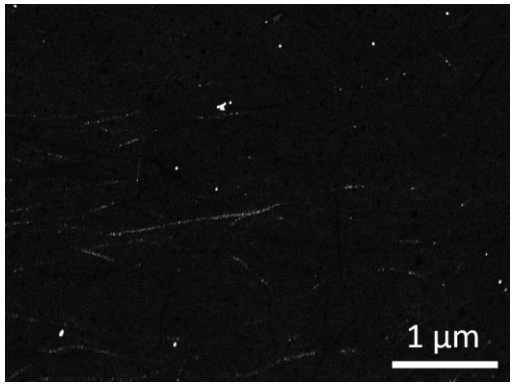


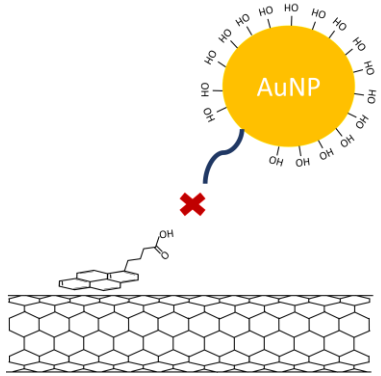


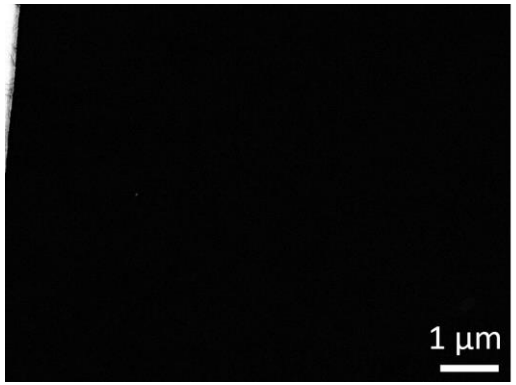
Biofunctionalization Validation

- AuNP thiol-functionalized with specific peptide
- AuNP deposition analysis by SEM BSD

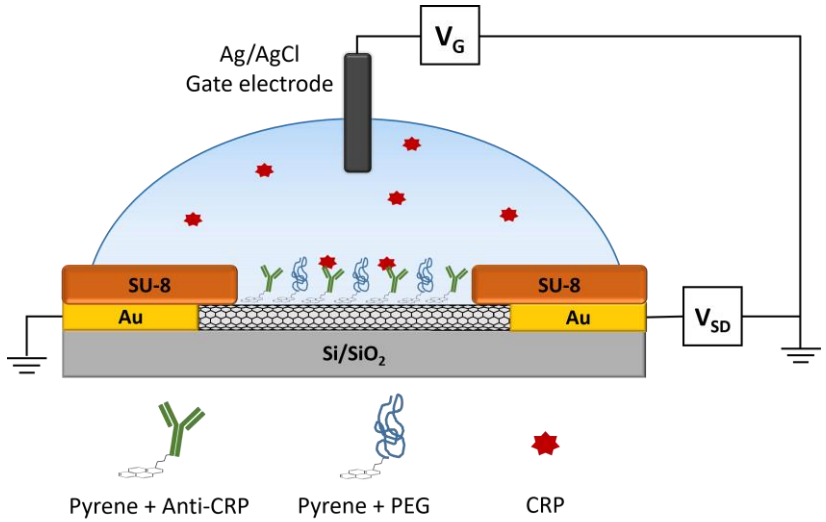




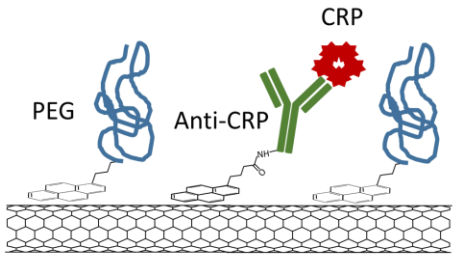


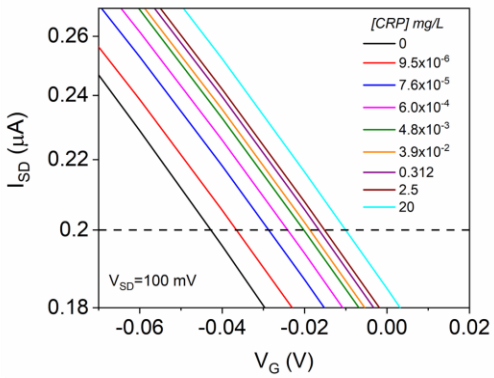


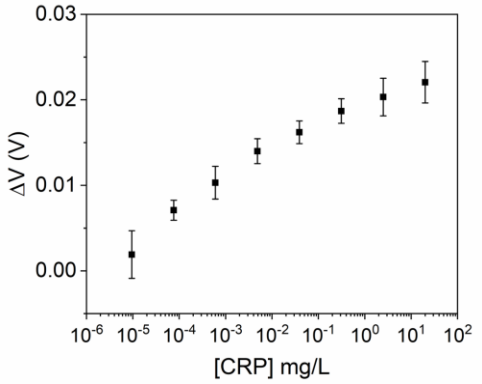
CRP Sensing in 150 mM PBS

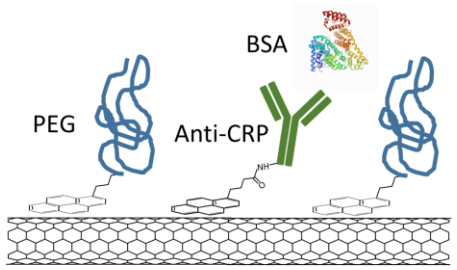


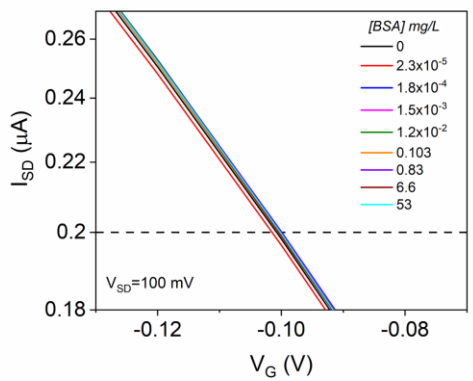
1. Sensor stabilization
2. 15 min incubation in each CRP concentration
3. Transfer Characteristics in PBS pH 7.8

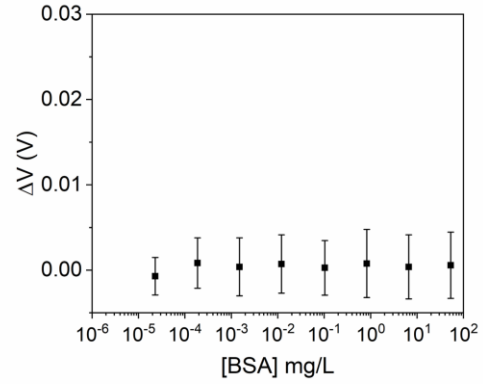


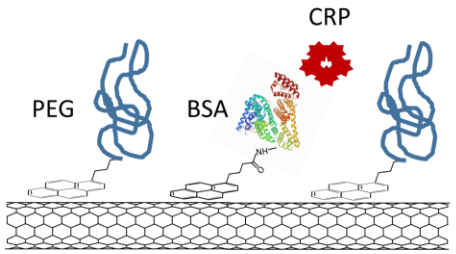


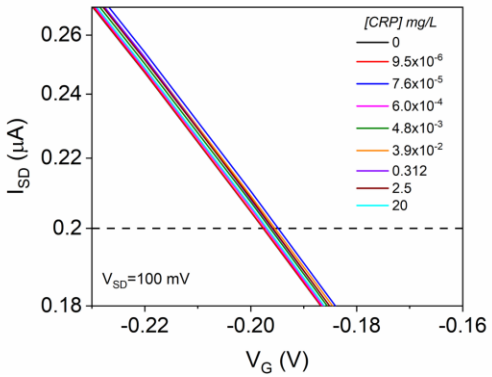


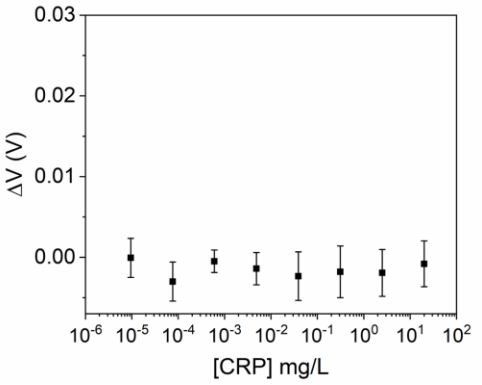






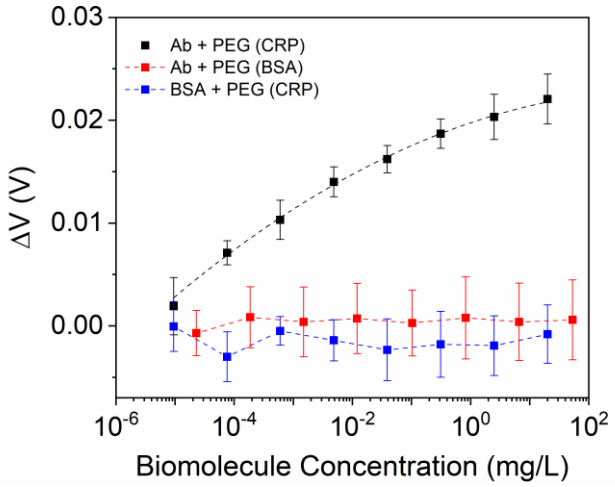






Conclusions

1. Successful fabrication of LG-SWCNTFETs by controlled DEP deposition of sc-SWCNTs.
2. Bio-functionalization of SWCNT-networks with a novel Anti-CRP antibody and PEG.
3. Selective detection of relevant CRP concentrations (**pg/ml**) in high ionic strength buffer without complicated microfluidics.
4. Potential use as fast diagnostic tool to address unnecessary antibiotic prescriptions in respiratory track infections.



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Influenza	25.65
Bacterial	135.96

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Outlook

1. Further miniaturization by gate electrode modification.
2. Implementation in a multiplexing platform.
3. CRP detection in more complex samples.

Acknowledgements

CONACYT

Thank you for your attention

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