Biosensing using wafer-scale electrolyte-gated graphene field-effect transistors

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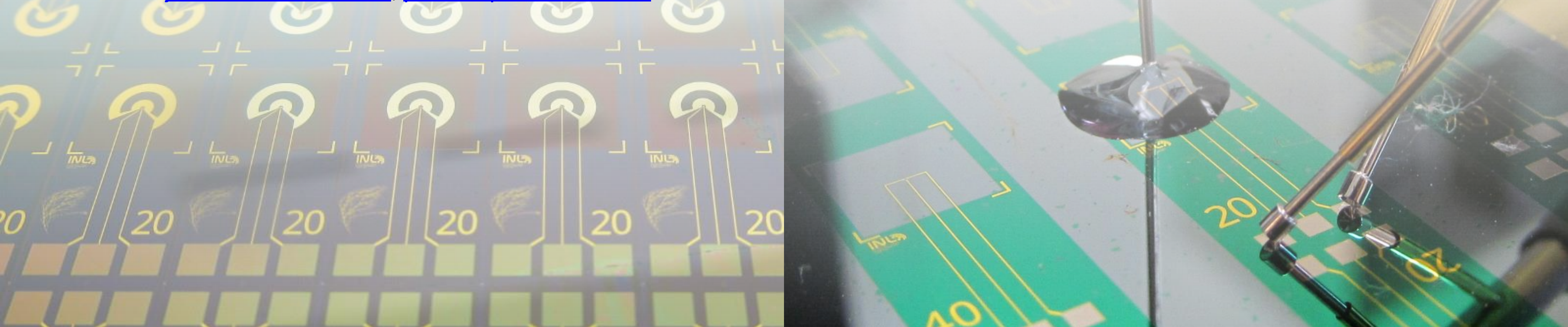

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Motivation

- Take advantage of graphene 2D **highly sensitive** electronic system for biosensing.
- Develop a **clean-room compatible process** for graphene.
- **Wafer-scale** fabrication of integrated devices.
- Access the process **uniformity**, device **performance** and the **repeatability** of the results

Graphene electrolyte-gated FETs

In a electrolyte-gated FET the solid state gate dielectric is replaced by na aqueous solution with a certain ionic strentgth.

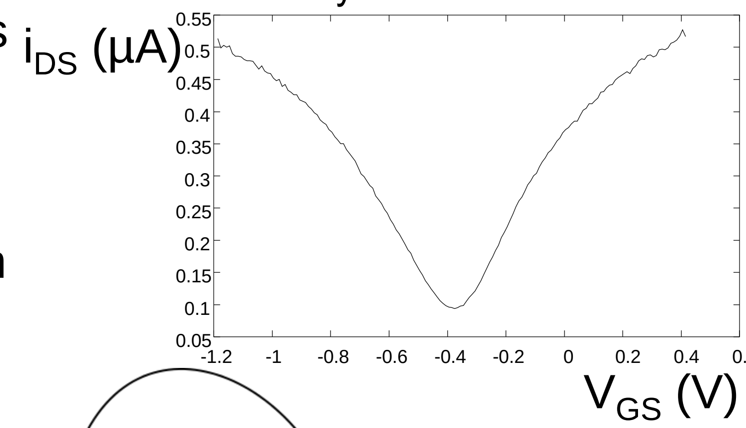
The gate voltage is transmitted through the **electrolyte** in graphene EGFETs

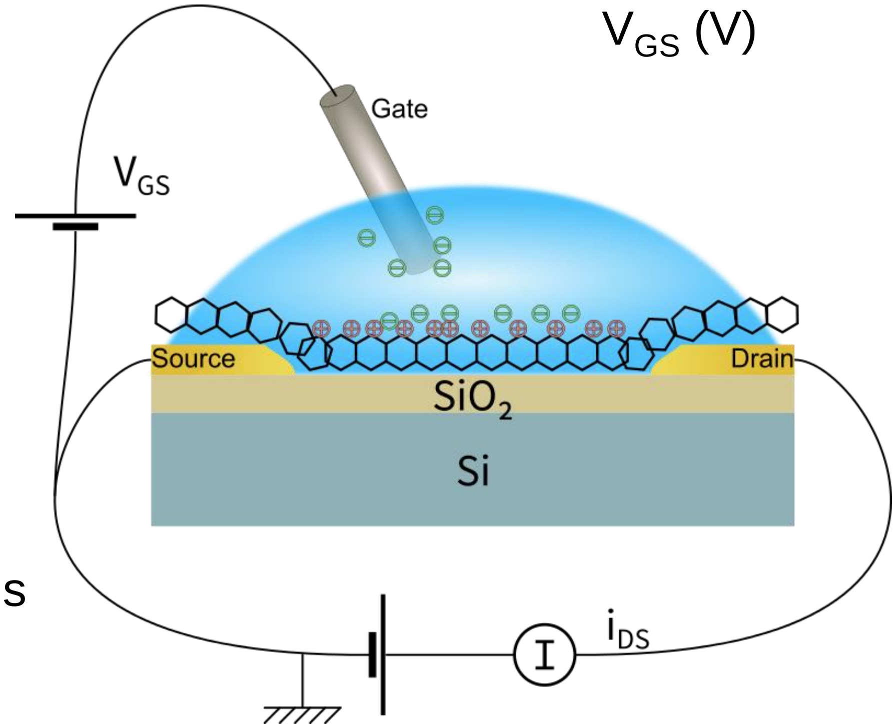
The **electrical double layer** acts as a capacitor
Its thickness of just a few nanometres makes a high capacitance, comparable in magnitude and in series to C_q of graphene.

$$V_G = \frac{ne}{C_{EDL}} + \frac{\hbar |v_F| \sqrt{\pi n}}{e}$$

Graphene is *very* sensitive to surface charge distributions
The presence of charged molecules within the Debye length will displace the electrostatic equilibrium.

mobility ≈ 1000 – 3000 cm² V⁻¹s⁻¹





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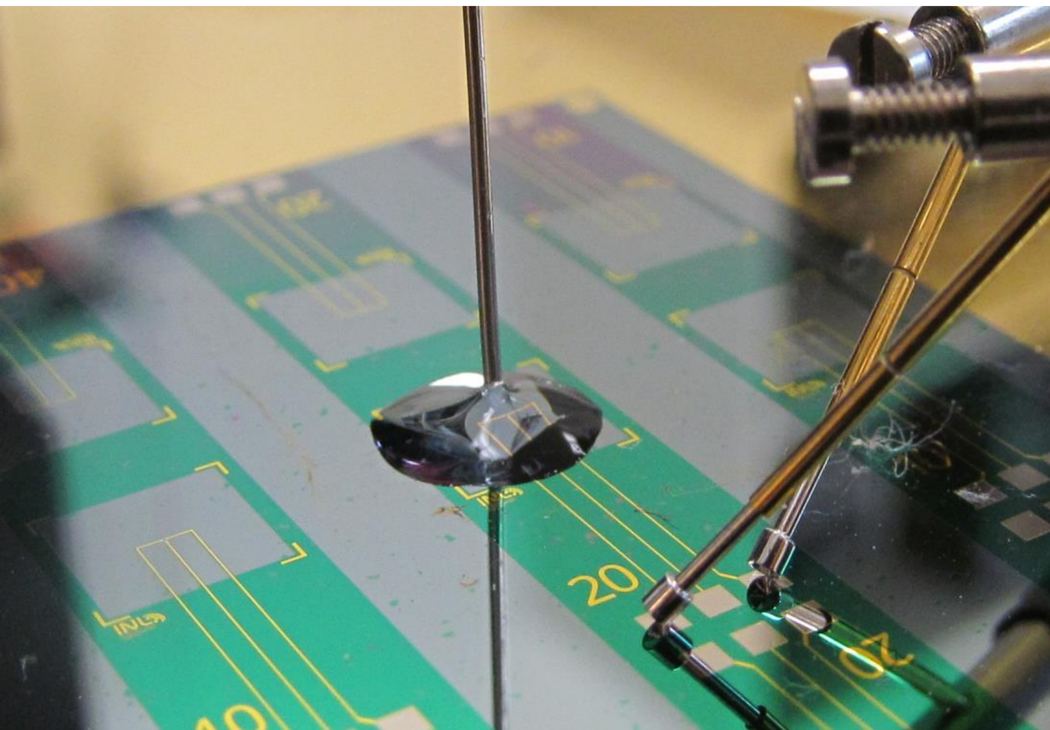
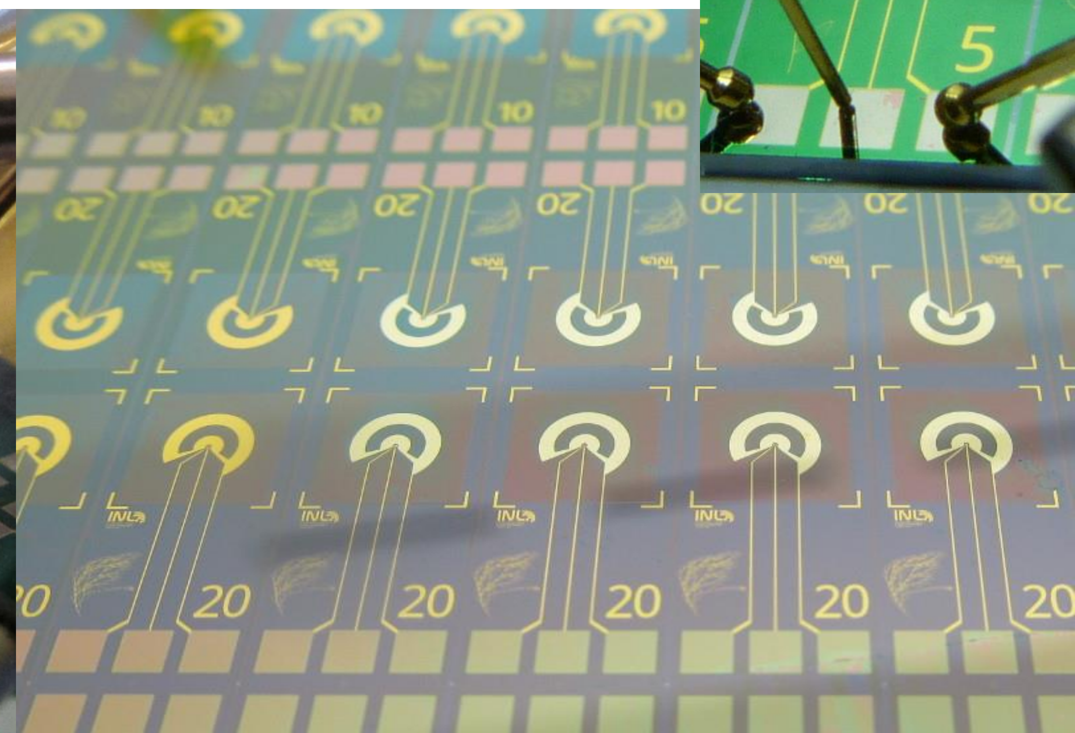
Planar SG-GFET

- Replace the cumbersome gate electrode by a receded, integrated gate
- Fabricate on 200 mm oxidized Si-wafer



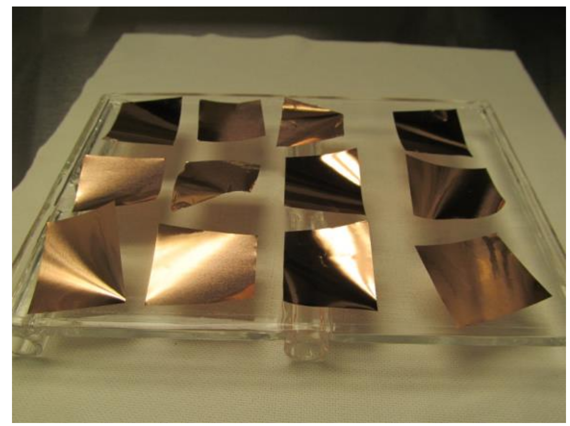
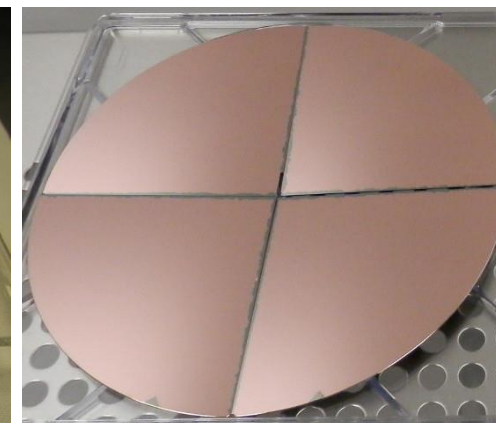


Graphene deposition and transfer

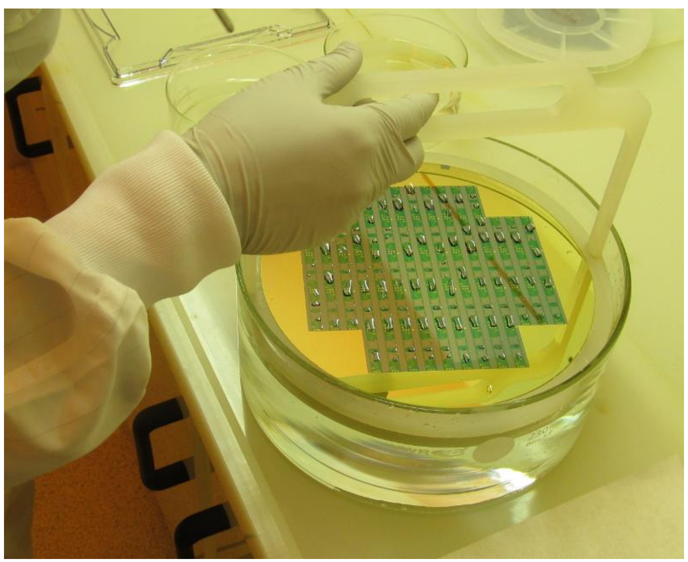
Easy Tube 3000, First Nano Corp.

Graphene CVD deposition in **100 mm** quartz tube on **copper catalyst**

- foil **25 µm**, 99.999 %
- sputtered **film 1.5 µm** (on Si wafer)
- **1020 °C**, H_2:CH_4 6:1, P = 0.5 Torr

(left) Cu (25 mm × 25 mm × 25 µm) foils
(right) 200 mm wafer with thin film Cu, cut in 100 mm quarters

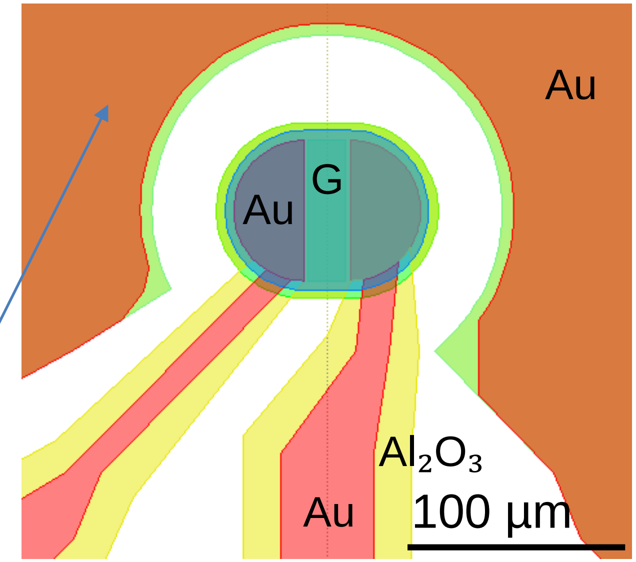


- Pre-transfer priming with HMDS
- Post-transfer anneal at 180 °C

Wafer treatment

Device fabrication

- Deposition of **Au 30 nm**/Cr 3 nm on SiO_2/Si
- Optical lithography (600 nm resist)
- **Ion mill, ∡40°** from normal incidence
- Optical lithography (2.2 µm resist)
- Deposition of **320 nm Al_2O_3**
- Lift-off in acetone
- **Transfer of graphene**



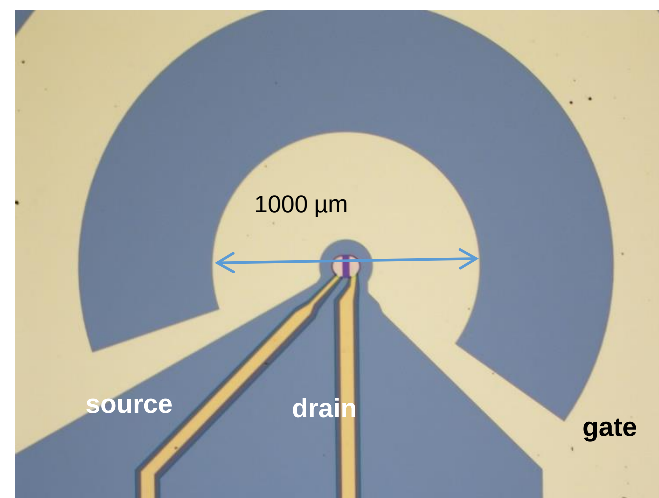
Graphene is patterned by exposure to **oxygen plasma**, where **noble metals sublimate** (through volatile oxides e.g. Au_2O_3).

→ One lithography step to protect gold with Al_2O_3 (10 nm)

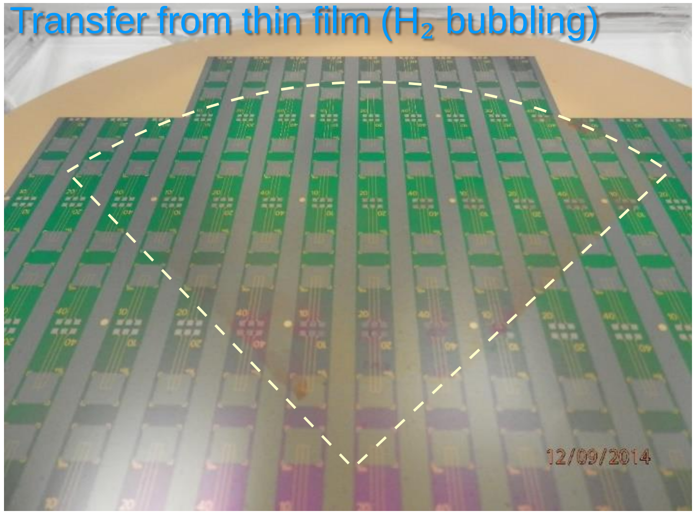
- Optical lithography on top of Au gate
- Deposition of **10 nm Al_2O_3** and lift-off (gate is now protected)

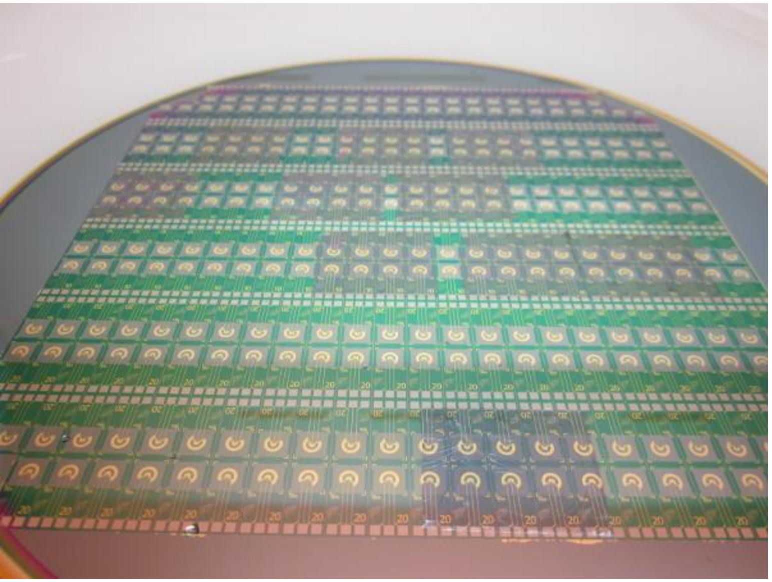
- Optical lithography to protect graphene FET area
- **O_2 plasma** (O_2:Ar 2:1, 0.9 bar, 250 W, 2 min)

- **Al_2O_3 wet etch** in standard photoresist developer



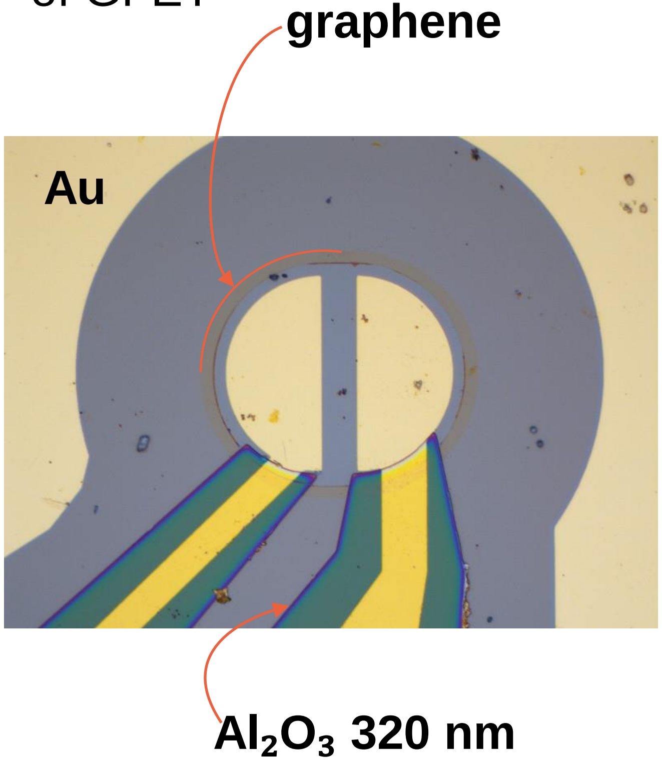
Patterned graphene



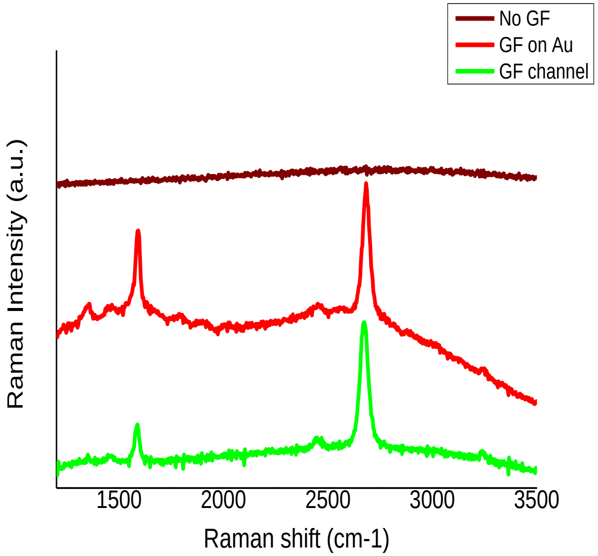


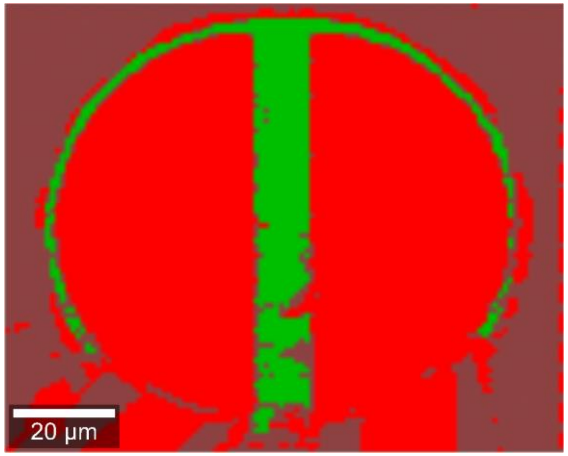
Channel length: 5, 10, 25 µm

Optical microscope image of GFET



Raman spectroscopy at 532 nm





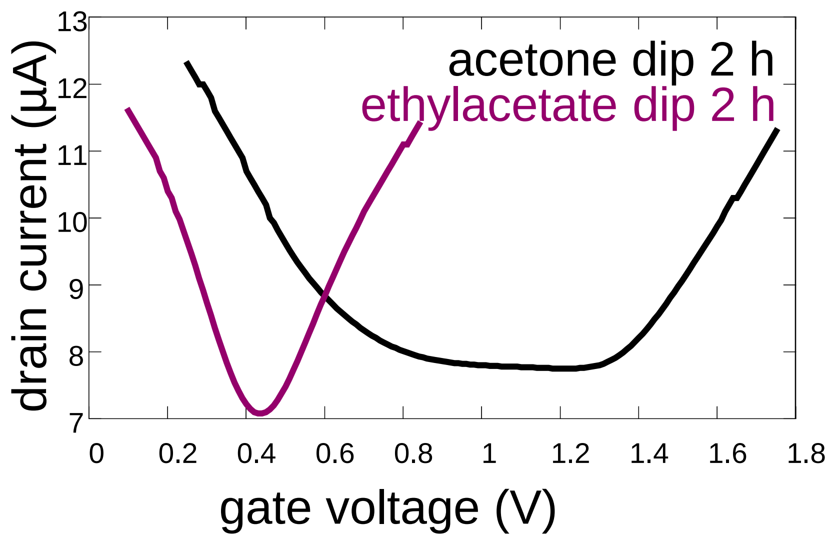
Graphene cleaning

- PMMA transfer lets organic residues on top of graphene
Oxygen from polymers provokes unintentional p-doping
- Acetone is a **not effective enough** to completely dissolve PMMA
- Among effective solvents, **ethyl acetate** is the safest

Solvent	% PMMA dissolved		
	40	90	120 min
Benzene	29.2	49.9	68.5
Toluene	18.7	29.7	40.0
o-Xylene	7.3	11.3	15.5
m-Xylene	16.7	26.2	27.3
Trichloromethane	1.4	3.4	4.0
Trichloroethylene (1)	96.0	–	–
1,4-Dioxane	17.2	27.2	37.9
Cyclohexanone	45.2	73.2	77.3
Acetophenone	21.0	31.9	45.6
Ethyl acetate (2)	56.7	89.5	–
Pentyl acetate	4.8	7.2	8.5
Dimethylformamide (3)	33.4	61.8	84.7

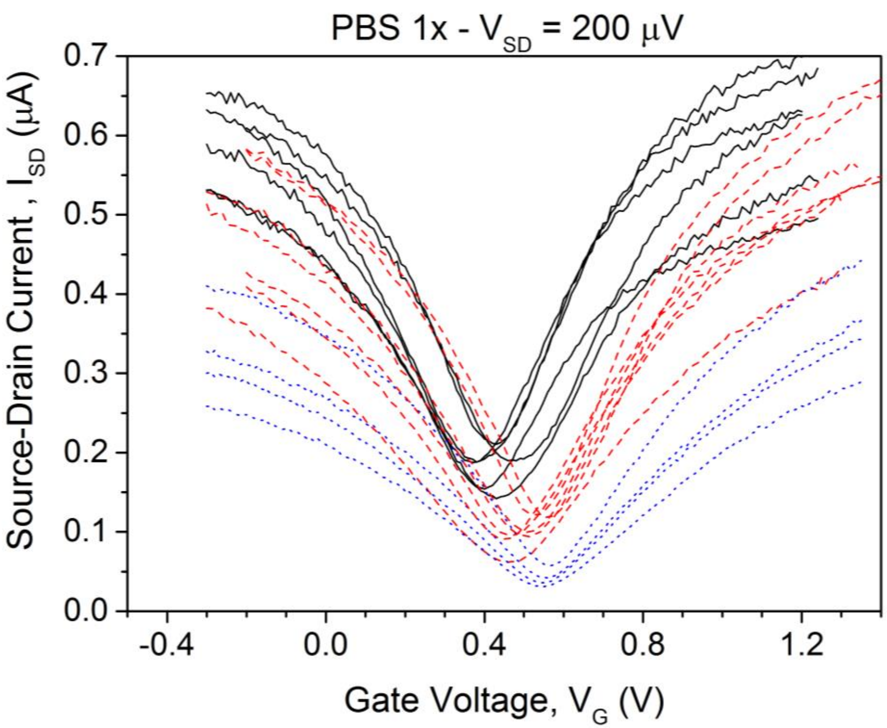
Solubility of polymethyl methacrylate in organic solvents
I. Yu. Evchuk *et al.* Russian J. Appl. Chem. **78**:10, pp. 1576-1580 (2005)
DOI: 10.1007/s11167-005-0564-9

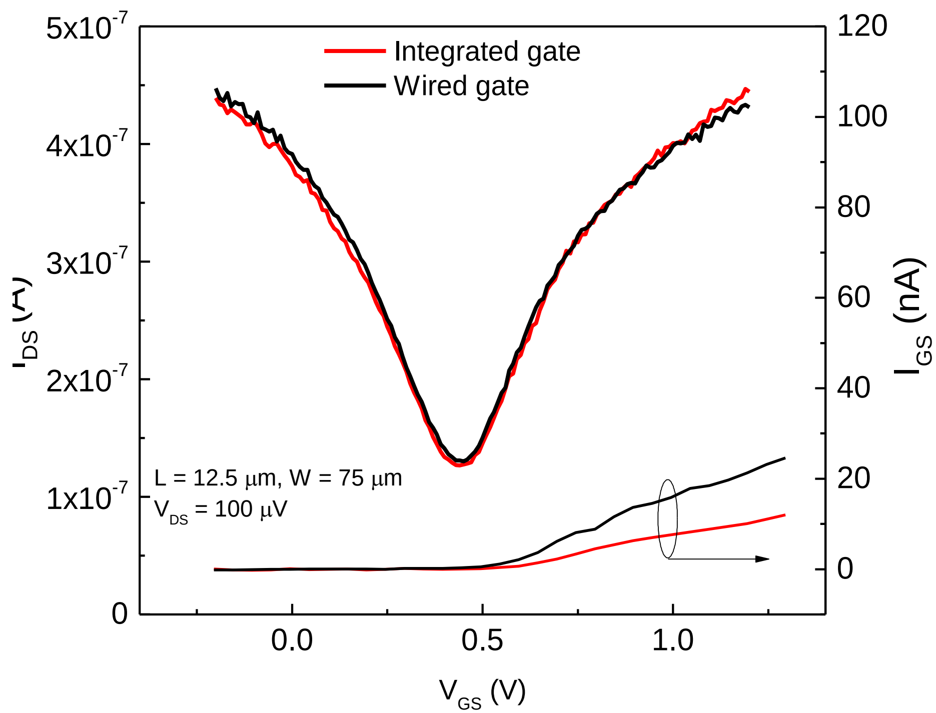
Transfer curve of a device cleaned in acetone followed by ethylacetate (unpatterned device)



Graphene electrolyte-gated FETs

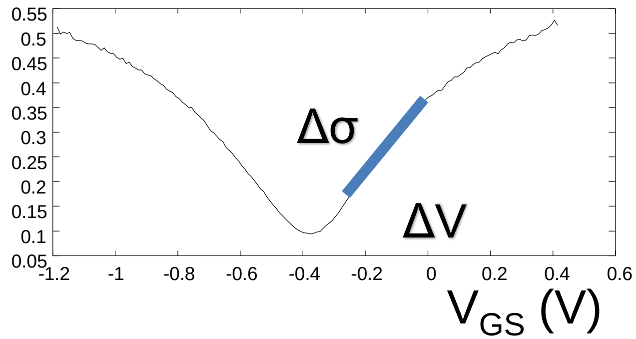
- Channel resistance: ≈ 400-2000 Ω (*W* / *L* = 75 / 5,10,25) (see below 17 transfer curves)
- The receded gate devices show similar performance when compared to wire-gated FETs
- **Leakage current** of integrated gate is smaller, although in absolute they are both **very small**

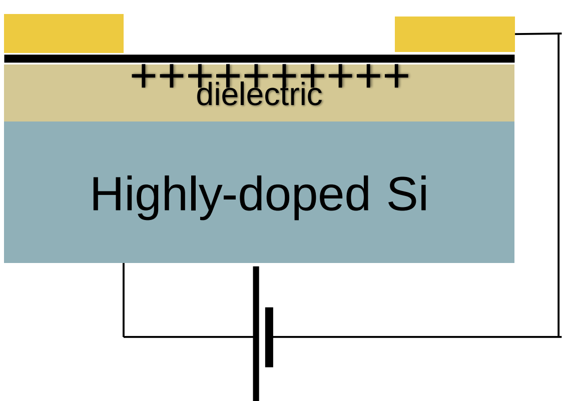




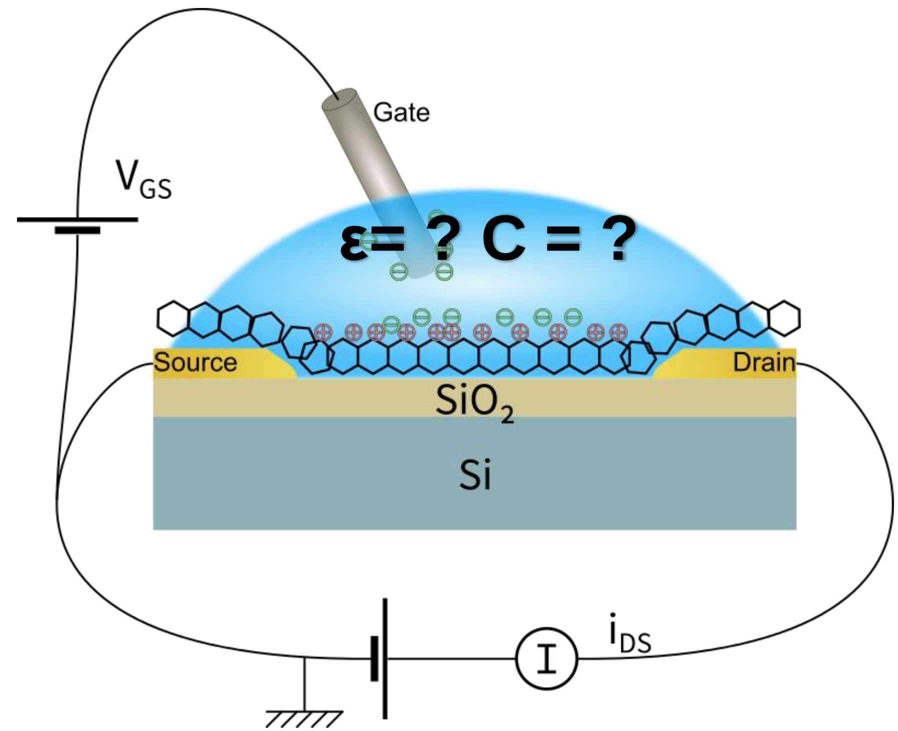
Extracting GFET parameters

In a solid state GFET, mobility can be extracted by fitting the linear range of the transfer curve to $\mu = \Delta\sigma / (C_g \Delta V_g)$

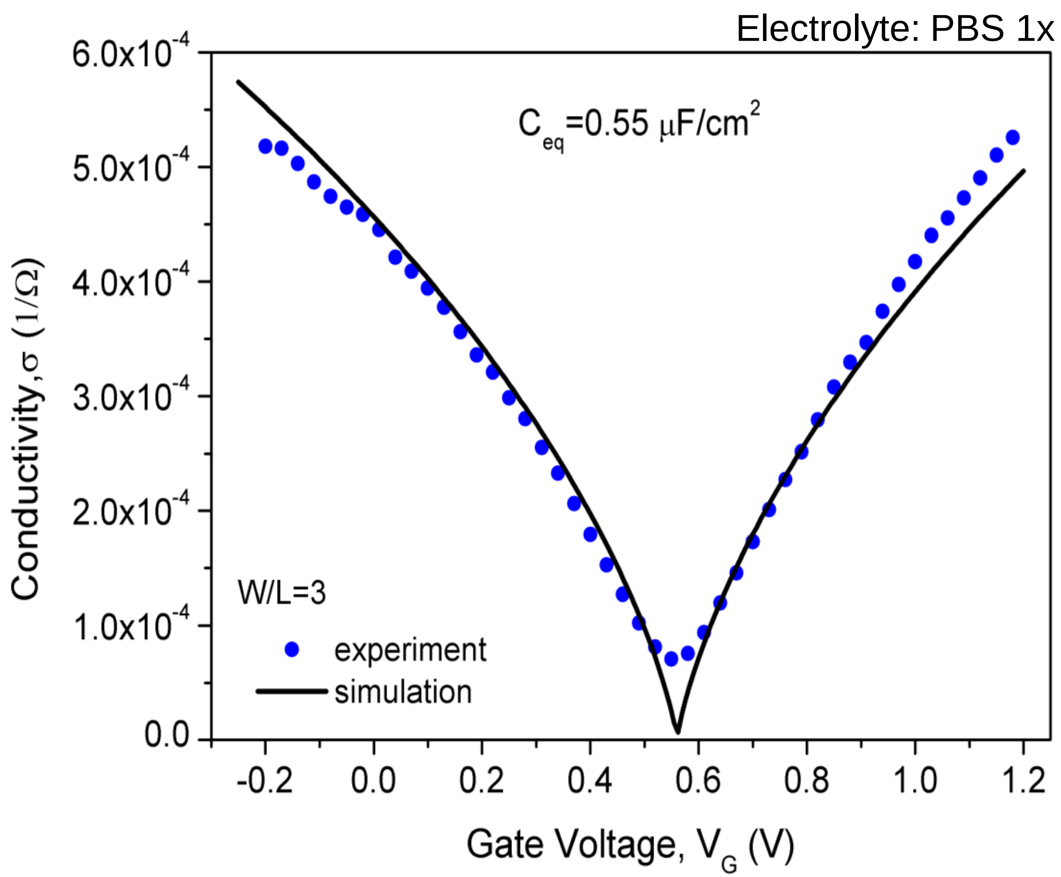




In a liquid state GFET the total capacitance is not easily known due to the electrical double layer in series



Extracting GFET parameters



Model for fitting the conductivity: **carrier resonant scattering** due to strong **short-range potentials** originated in impurities adsorbed at the graphene surface

$$\sigma = g_0 \frac{3\sqrt{3}}{4\pi}\frac{a_0^2\alpha}{n_i}|V_G| ln^2\left(\sqrt{\alpha\pi|V_G|}\cdot a_0\right)$$

g_0 – quantum of conductance

n_i – impurity concentration

$a_0 \approx 1.4\,\mathring{A}$ - range of scattering potential

$\alpha \cdot V_G = n$

μ_e = 1843 $cm^2V^{-1}s^{-1}$

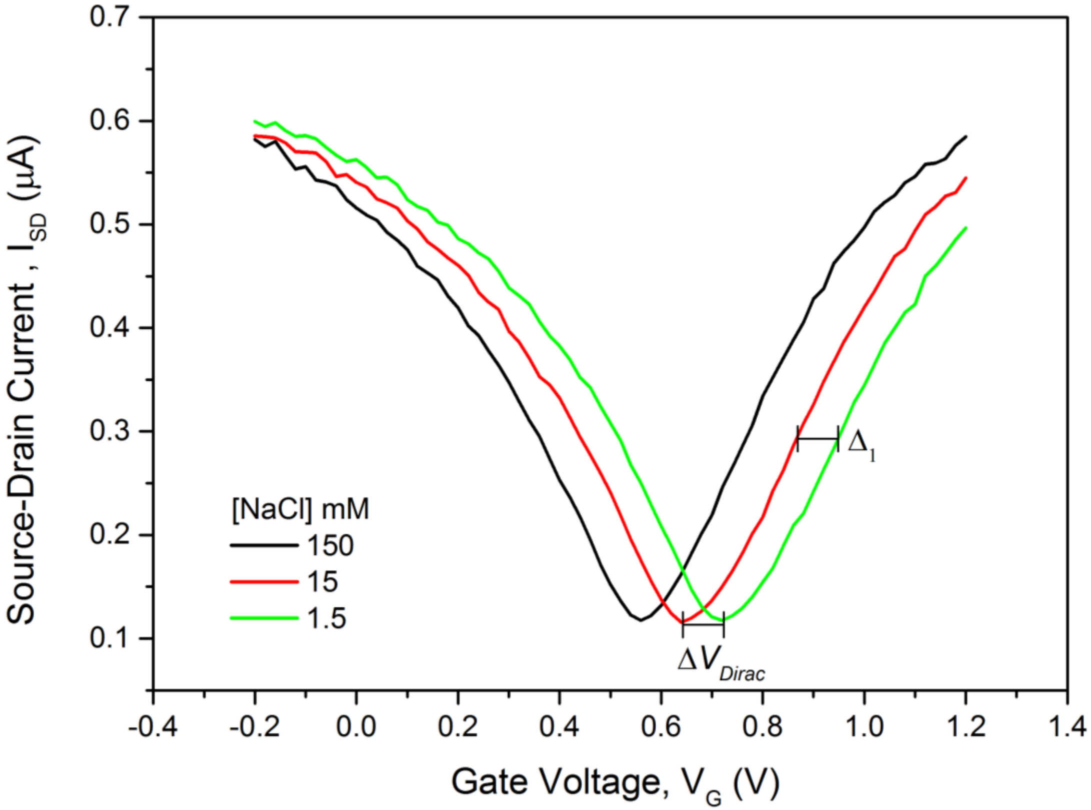
μ_h = 1833 $cm^2V^{-1}s^{-1}$

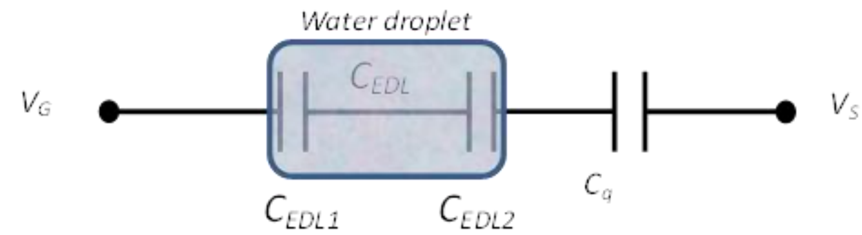
V_{ds} = 200 μV

$I_{ds} = C_{eq}\mu_{FE}\, W/L\, (V_{gs} - V_{Dirac})V_{ds}$

Aires Ferreira et al., Phys. Rev. B **2011**, 83, 165402-1

Effect of ionic strength on the transfer curve





$$\rho = \sqrt{8\varepsilon\varepsilon_0 k_B T I}\,\sinh\left(\frac{e\psi_0}{2k_B T}\right)$$

$\rho = ne$, is the surface charge density
ψ_0 is the surface potential
I is the ionic strength of the electrolyte

$$\Delta|\psi_0| = -0.06 \text{ V}$$

$$\Delta_1 = \Delta V_G \sim -0.08 \text{ V}$$

In DI water, at RT for 1:1 electrolytes

$$\lambda_D = \sqrt{\frac{\varepsilon\varepsilon_0 k_B T}{2N_A e^2 I}}$$

$$\lambda_D(nm) = \frac{0.304}{\sqrt{I(M)}}$$

However, because graphene has a hydrophobic surface, the dielectric constant of water is much lower than in the bulk (5 ≤ ε ≤ 80) !

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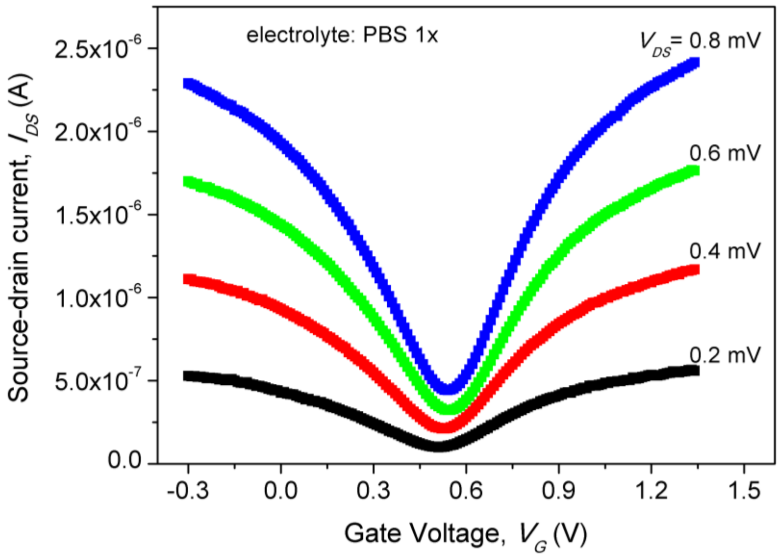
GFETs for biosensing

Transconductance is proportional to source-drain bias

V_{sd} > 5 mV → electrochemical regime (biological reactions)

We work at the lowest bias possible, $V_{sd} \approx 200\ \mu V$



Application	Sensitivity - reliability trade-off	Reusability
Environment monitoring	Low sensitivity (legal limits); Reliable	Continuous use
Medical diagnosis	Highly sensitive	Single-use

Biosensing by direct detection

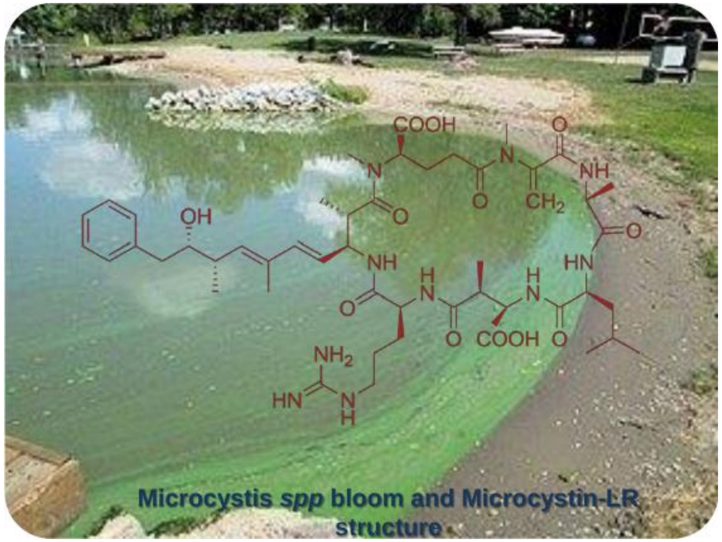
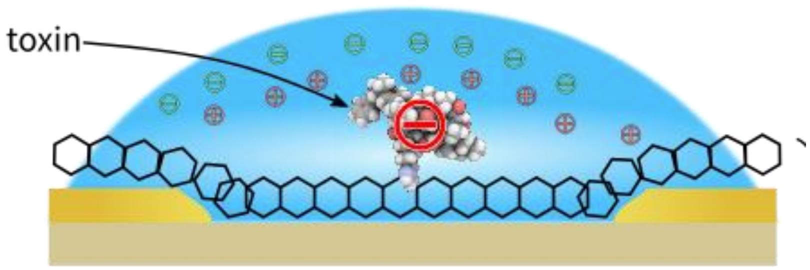
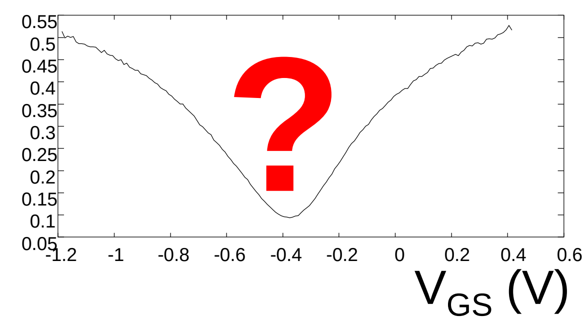


Image: courtesy of Begoña Espiña (INL)



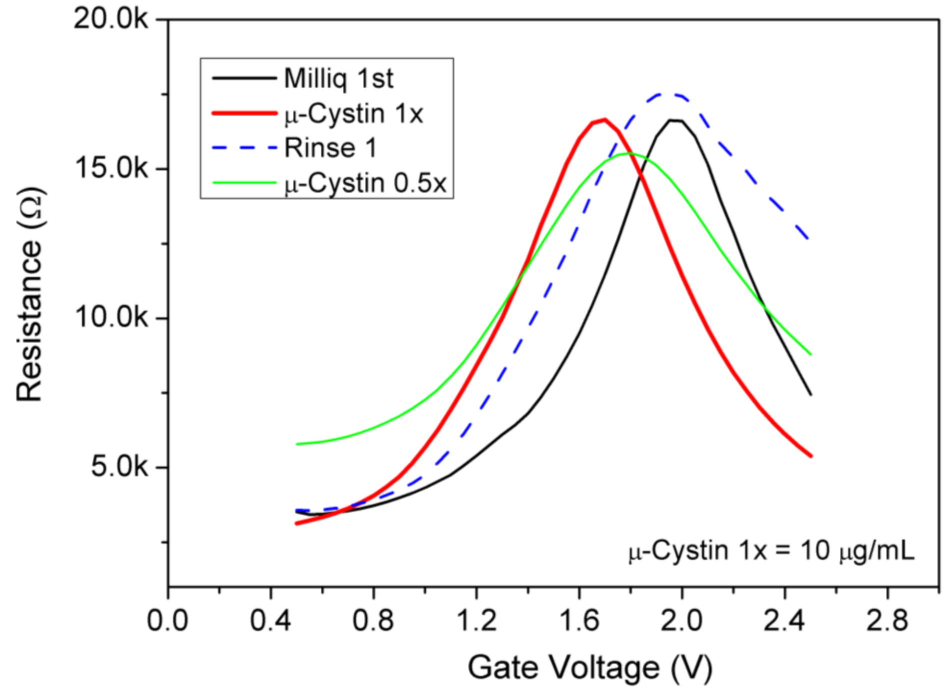
Microcystins: deadly toxins present in fresh water due to presence of *Microcystis* cyanobacteria blooms.



Biosensing (direct detection)

Graphene GFET is sensitive to the presence of microcystin-LR (can probably detect 1 μg/mL), a level ≈1000 times too high compared to recommended exposure limit (1 μg/L).

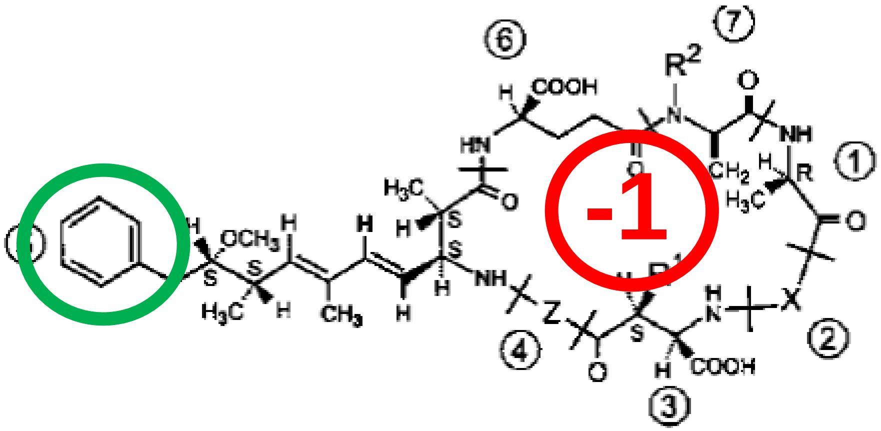
(current limit of detection: 0.1-1 μg/L in HPLC or commercial immunoassays kits)



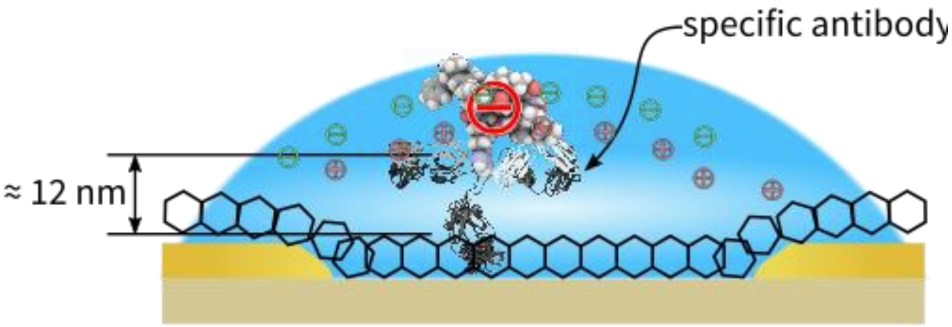
This scheme would work with target molecules:

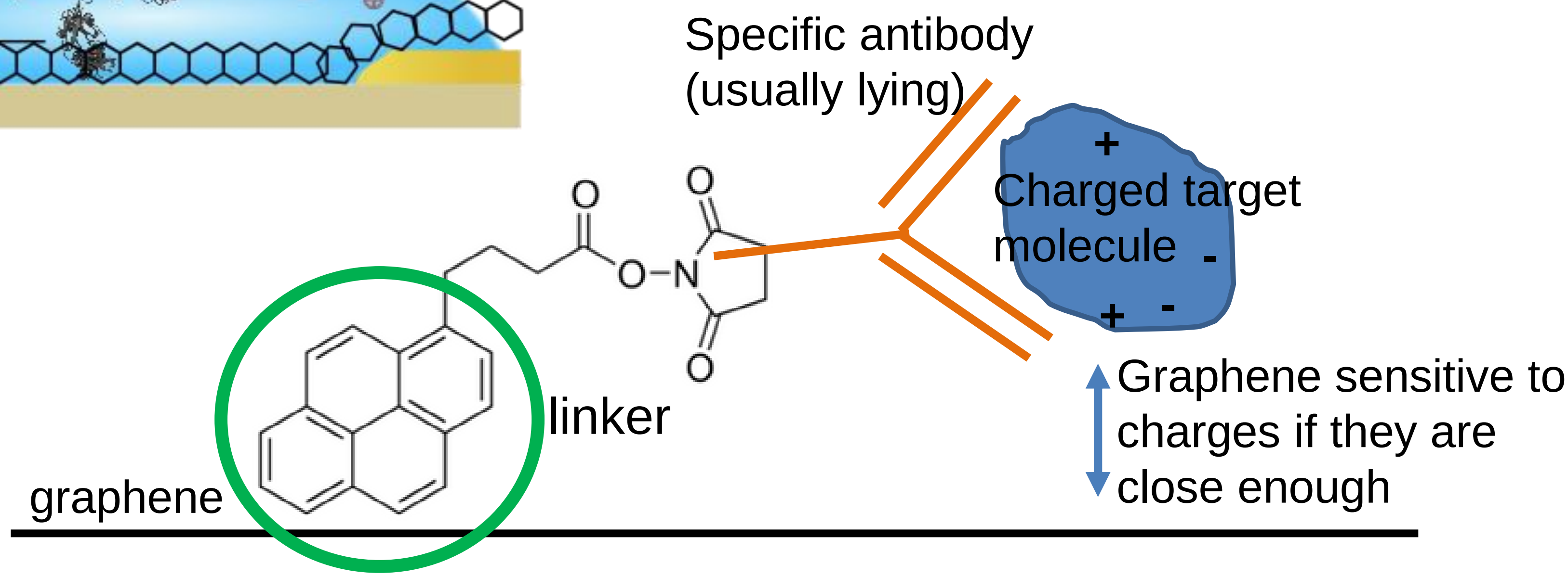
-- more affinity to graphene (more benzene rings)

-- more charges



Biosensing (with functionalization)





linker:
1-pyrenebuturic acid N-hydroxysuccinimide ester
CAS number: 114932-60-4

Biosensing: the target molecule

Blood Coagulation, Fibrinolysis and Cellular Haemostasis doi:10.1160/TH10-09-0621 © Schattauer 2011

The natural tissue plasminogen activator inhibitor neuroserpin and acute ischaemic stroke outcome

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Summary

Neuroserpin is a brain-derived natural inhibitor of tissue plasminogen activator (tPA) that has shown neuroprotective effects in animal models of brain ischaemia. Our aim was to investigate the association of neuroserpin levels in blood with functional outcome in patients with acute ischaemic stroke. Due to the potential effect of tPA treatment in- outcome (for each quartile decrease, adjusted odds ratio [OR] 15.0; 95% confidence interval [CI], 3.5 to 66). In the tPA-treated cohort, high neuroserpin levels before tPA bolus had the stronger effect on favourable outcome (for each quartile, OR 13.5; 95%CI, 3.9 to 47). Furthermore, for each quartile in neuroserpin levels before tPA bolus there was a 80% (95%CI, 48 to 92) reduction in the probability of subsequent

Neuroserpin levels during the first hours of **acute ischemic stroke** have strong correlation to a good or poor outcome. Current clinical analysis take 48 h, too long for **prevention of severe hemorrhagic transformation**.

Biosensing (functionalized graphene)

Rodríguez-González et al. Neuroserpin in ischaemic stroke

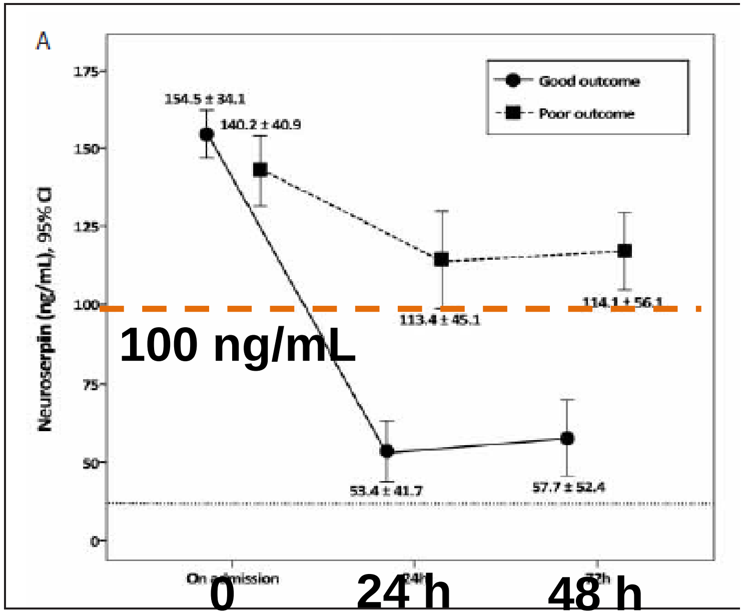
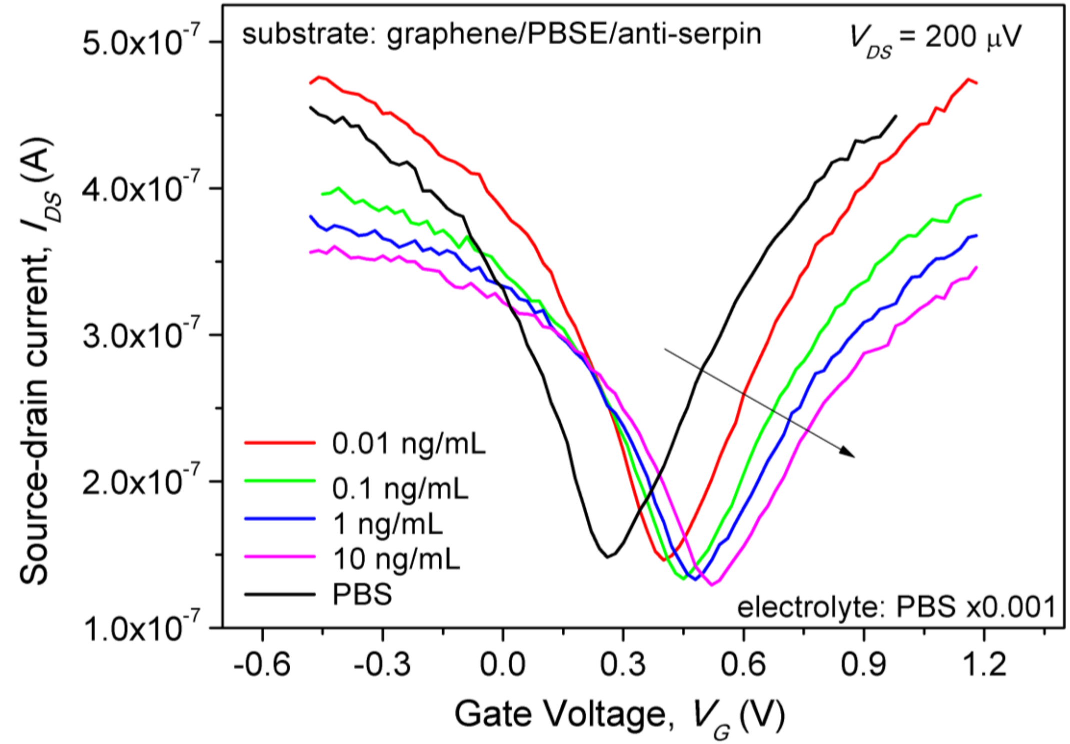


Figure 1: Temporal profile of serum neuroserpin levels in patients with acute ischaemic stroke. A) Mean (95%CI of mean) neuroserpin levels in non tPA-treated patients with good functional outcome were slightly higher on admission, but showed a greater decrease at 24 and 72 h than in the poor outcome group. In the MANOVA analysis, there was a significant group by time interaction on neuroserpin levels (F= 59.8, p<0.001). Contrast test showed no difference by time (F=0.032, p=858) and no time by group interaction (F=0.861, p=0.355) from 24 to 72 h. B) Mean (95%CI of mean)

doi:10.1160/TH10-09-0621

Functionalization with linker + antibody



Shift in the transfer curve as serpin concentration increased in the range from 0.01 ng/mL to 10 ng/mL

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Conclusion and outlook

Demonstration of

– a solution-gated graphene FET with an **integrated receded gate**.
– the ability to fabricate these devices at **wafer scale** (200 mm)
– the ability to transfer **graphene at large area** (100 mm)

The devices show

– **symmetric transfer curve** for electron and hole regions with good mobility (1500 – 3000 cm² V^{-1} s^{-1})
– **low leakage current** and are sensitive to the charge environment.

The devices perform

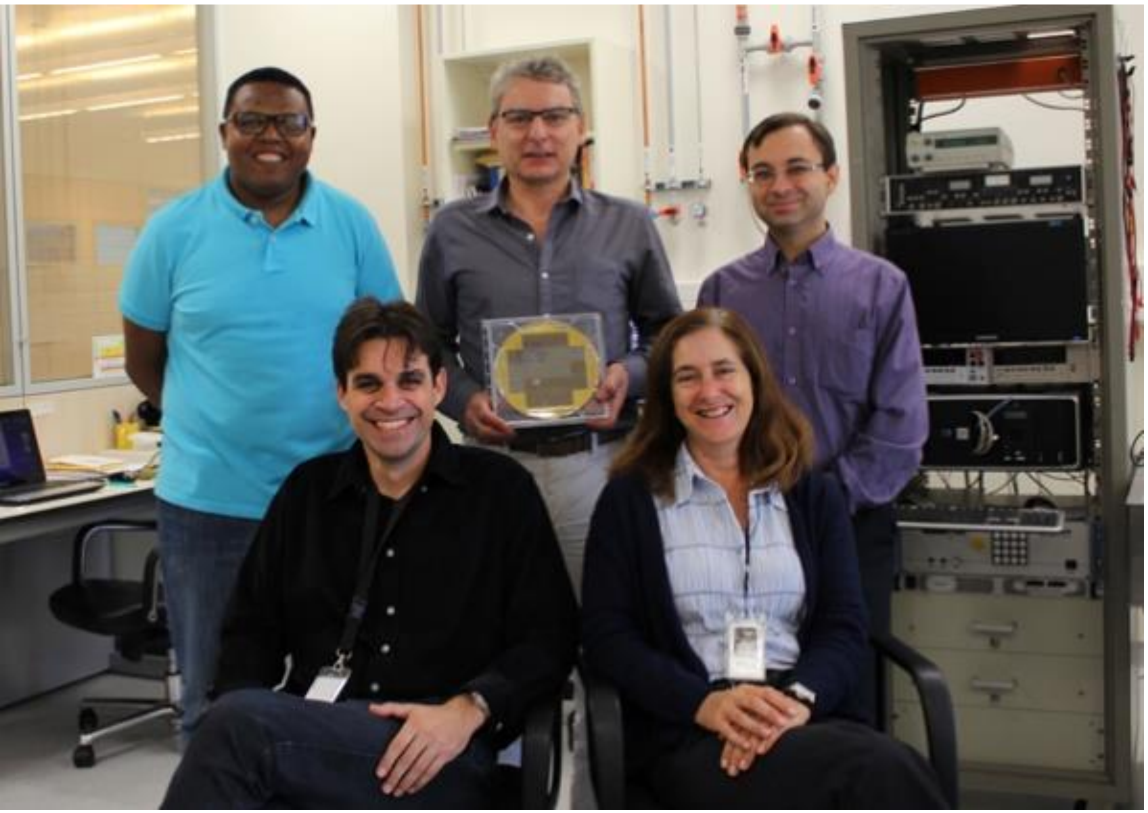
– poorly in the tested label-free detection scheme (**microsystin**-LR)
– excellent detection level and fast for protein detection (**neuroserpin**, with antibody functionalization)

Outlook

– **microfluidics** devices to enhance measurement stability and increase integration in the view of point-of-care applications
– **electrochemical** measurements to complement electrical (Vdirac) data

from carbon to life

Acknowledgment



The graphene team at INL

Clarissa Towle visiting student from INL-MIT summer scolarship program: Performed microcystine experiments

N.M.R. Peres from Universidade do Minho: developed the carrier resonant scattering model.

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Graphene EGFET as a biosensor

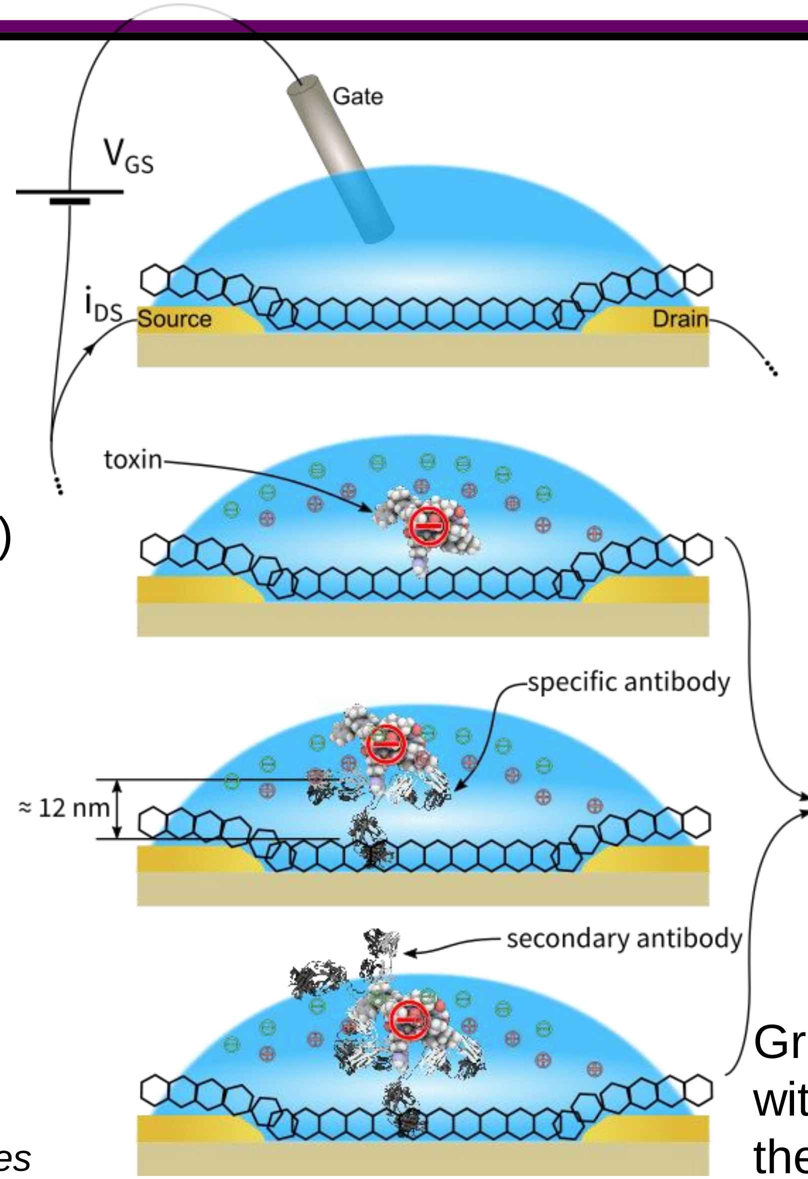
Graphene EGFET in a low-conductivity solution

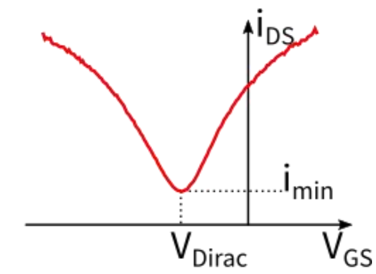
Label-free detection of charged molecules (e.g. toxins)

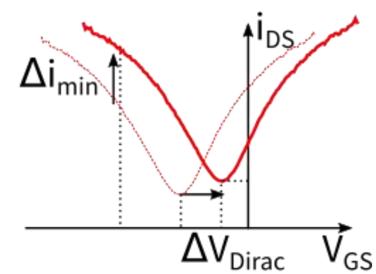
Detection of antigens using **antibody functionalization**
Biomolecular recognition
Preferred in most situations

Detection of antigens using **secondary antibodies** (sandwich assay)

To lower detection limits or with small analytes







Graphene senses charges within the **Debye length** of the solution.

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