Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVIII

Enhancing Graphene Field-Effect Transistor Biosensors with DNA-mediated Protein Orientation

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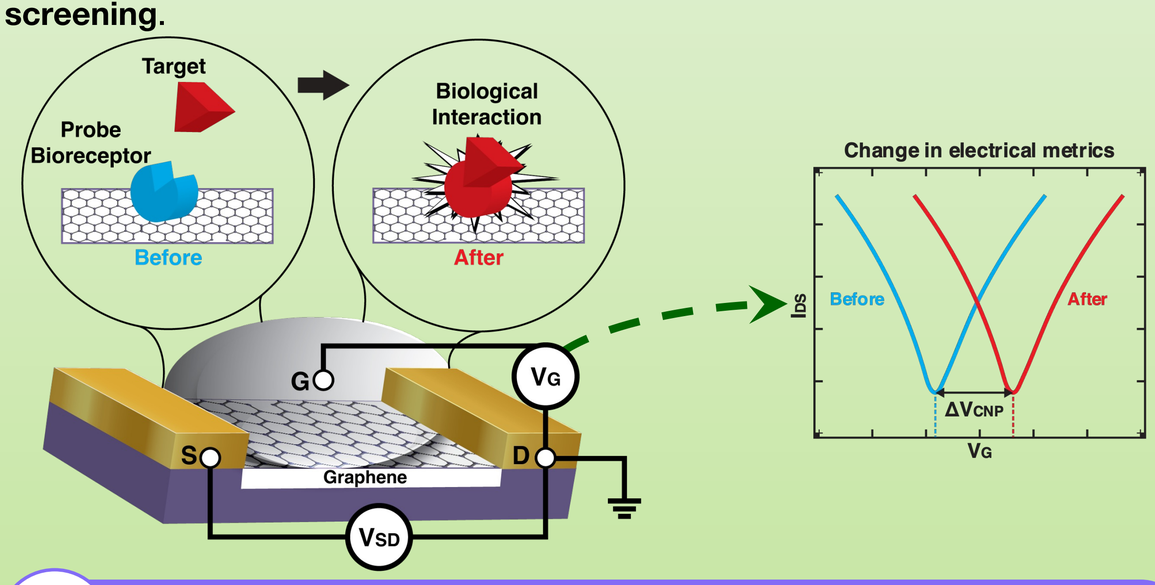
1 BACKGROUND

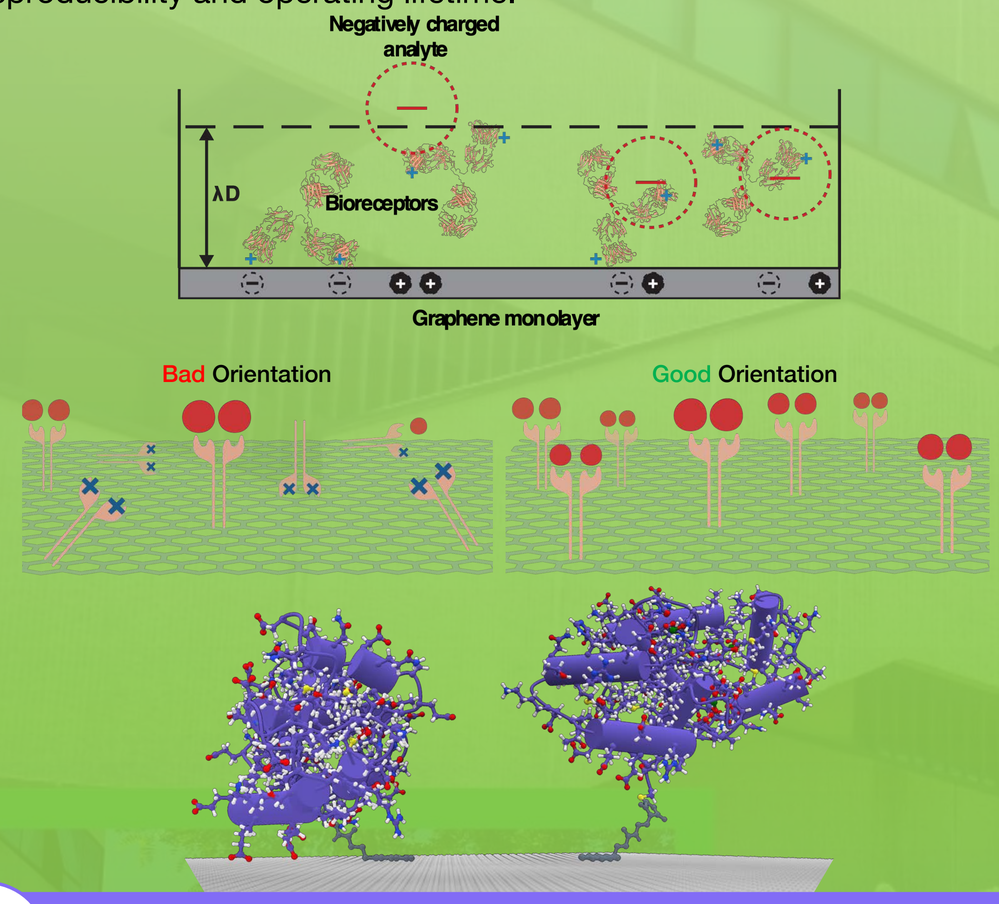
Graphene field-effect transistor (**GFET**) biosensors exploit the atomically thin, high-mobility, and low-noise characteristics of graphene to transduce **biorecognition events** directly into an electrical signal. When a bioreceptor is immobilized on the graphene channel, binding of target analytes perturbs the local charge distribution and shifts the GFET transfer curve, enabling label-free, real-time detection with sub-picomolar sensitivity. Because graphene is mechanically robust, chemically inert, and compatible with wafer-scale microfabrication, GFET platforms are being actively investigated for point-of-care **diagnostics**, **environmental monitoring**, and high-throughput **drug screening**.



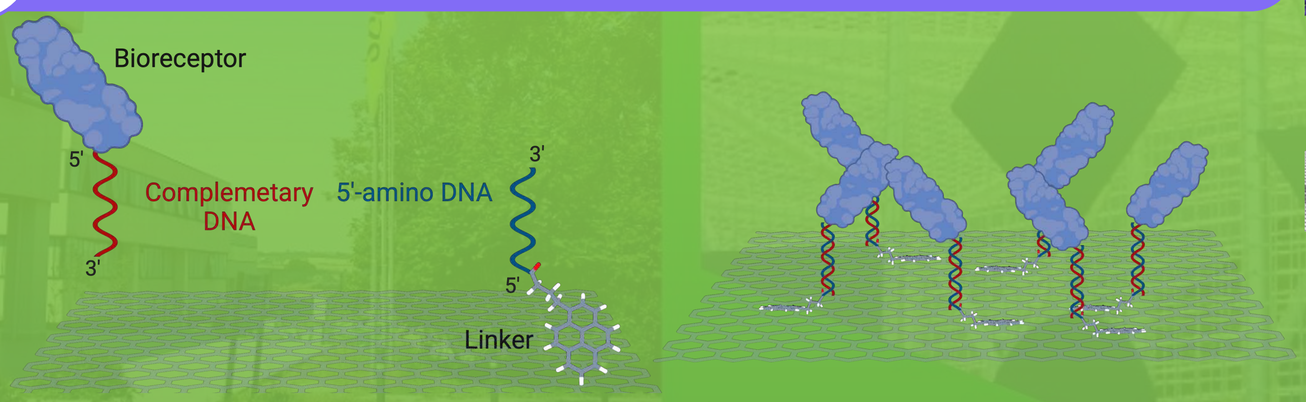
2 CHALLENGES

Despite these advantages, large-scale deployment of GFET biosensors remains limited by three inter-related phenomena:

1. **Debye Screening** – In physiological buffers (ionic strength ≈ 150 mM), the Debye length is < 1 nm, severely attenuating the electrostatic signature of most biomolecular interactions that occur a few nanometres away from the graphene surface.
2. **Random Protein Orientation** – Conventional physisorption or NHS-mediated coupling yields heterogeneous orientations, masking active sites and diminishing signal-to-noise ratios.
3. **Non-specific Adsorption** – Hydrophobic π–π stacking between aromatic residues and graphene promotes irreversible fouling, degrading device reproducibility and operating lifetime.



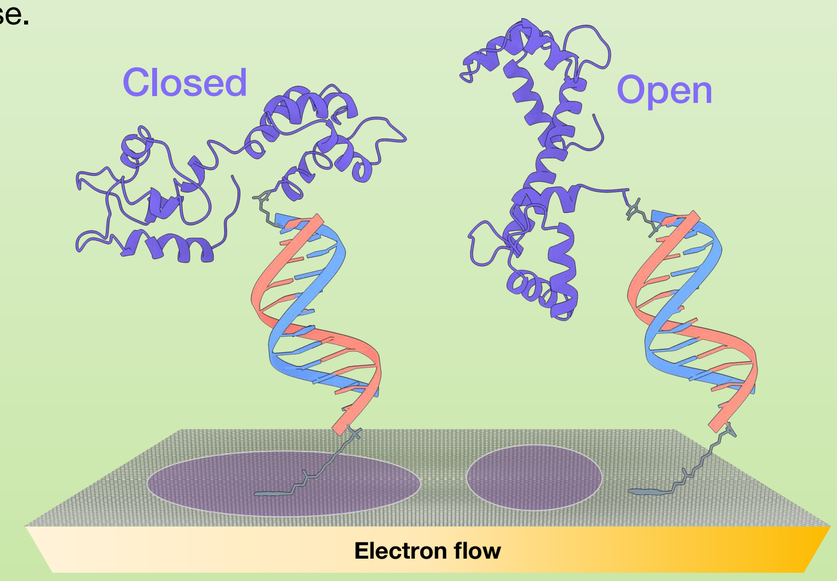
3 SOLUTION



We engineer a **bifunctional**, double-stranded DNA (**dsDNA**) that simultaneously (i) tethers proteins at a precisely defined distance from the graphene surface and (ii) enforces a uniform, upright orientation. One strand terminates in an amine-modified 5′ end that forms a stable covalent bond with a pyrene–NHS linker on graphene, while its 3′ end is complementary to a short oligonucleotide tag fused to the C-terminus of the protein. Theoretically, hybridization between the two strands—**orients the bioreceptor**, places its active site well within the Debye length, and minimizes non-specific π–π interactions. By varying dsDNA length (12 bp–28 bp) we can fine-tune the protein-to-graphene separation.

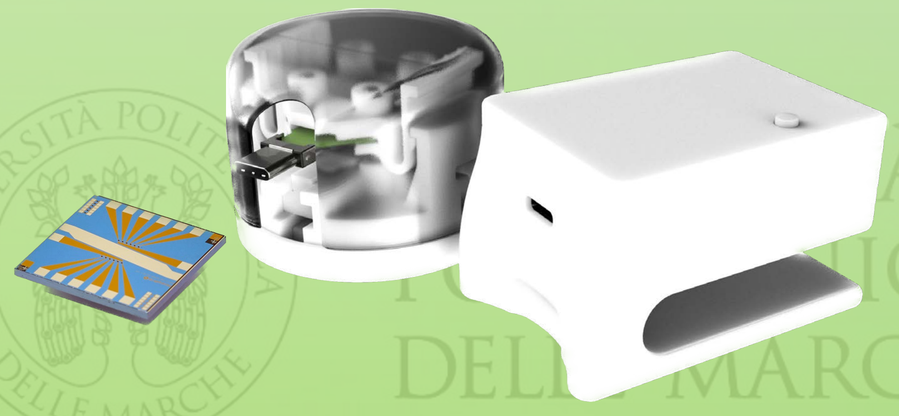
4 PROOF OF CONCEPT

To show that our idea really works, we chose a well-known test protein called **calmodulin**. Think of calmodulin as a tiny spring-loaded clamp that changes its shape the moment it grabs hold of a calcium ion. In its relaxed state the protein is compact, but as soon as calcium is present it swings open and exposes new surfaces. Because these **conformational shifts** are large and happen quickly, they act like a clear on-off signal that our graphene sensor can easily read. By watching the electrical response of the device as calmodulin opens and closes, we can confirm that our DNA-guided strategy puts the protein in just the right position to turn those motions into a measurable electronic pulse.



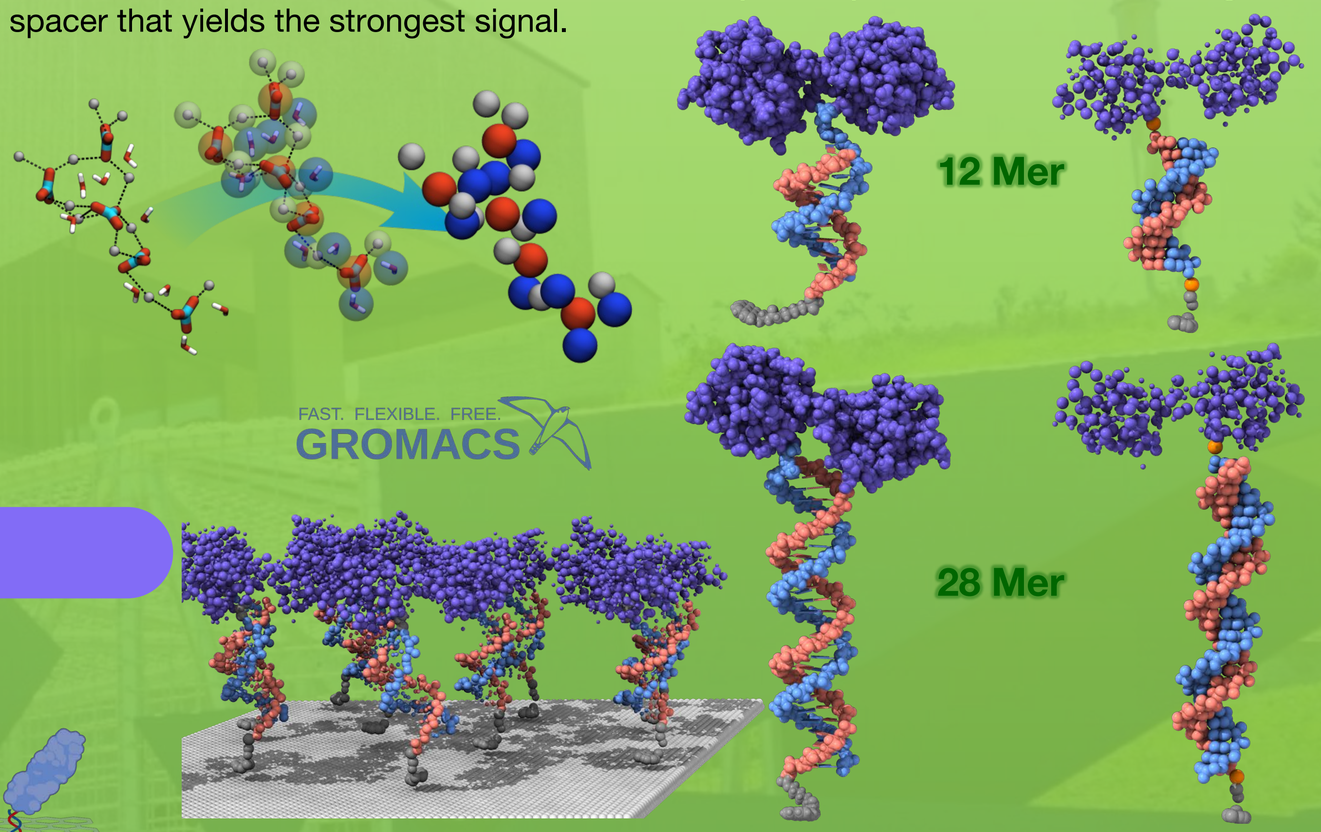
5 APPLICATION

The DNA-oriented GFET architecture can be seamlessly integrated into disposable chips for **point-of-care applications**.



6 COMPUTATIONAL PIPELINE

We built a step-by-step **modelling pipeline** to fine-tune the way DNA spacers hold a protein above the graphene surface. By running both detailed (**all-atom**) and simplified (**coarse-grained**) molecular dynamics simulations on biomolecules with double-stranded DNA spacers of 12, 16, 22, and 28 base pairs, we could observe how each length bends, tilts, and flexes. These simulations told us how often the attached protein remains within the sensor's sensitive electrical layer—key information for selecting the spacer that yields the strongest signal.



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