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# Grapevine Varieties Identification using a Miniaturized Graphene-based Sensor

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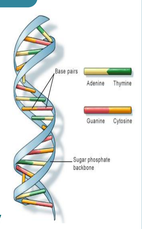
## ABSTRACT

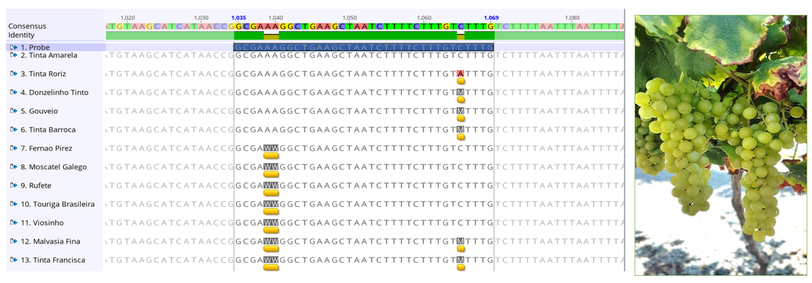
Wine is a highly valued product due to its premium quality and authenticity. However, the adulteration of wines, typically with a mixture of cheaper ingredients and even harmful chemicals and sweeteners, remains a significant issue in the winery industry [1]. Among analytical strategies, the identification of grapevine varieties through DNA analysis paves the way towards a new robust counterfaction strategy due to DNA high specificity and stability even under high temperatures, low pH, or chemical treatments during wine processing [2]. In this study, we develop a miniaturized DNA detection platform based on a single-layer graphene field-effect transistor (FET). The graphene chip can be easily operated using a miniaturized output reader directly connected to a laptop. The graphene-FET channel, modulated through a liquid-gating setup, is modified with a pyrene-based linker via π- π stacking, which preserves the electronic properties of graphene [3]. The integrated system monitors the shifting of Dirac voltage ($V_{Dirac}$) due to the probe DNA's immobilization and its hybridization with target DNA. The miniaturized system is applied to identify a series of sequences of nucleotides, representing a set of a fragment from F3H gene of the *V. vinifera* (grapevine), where some varieties show single nucleotide polymorphism (SNP) [4], to identify varieties from the Portuguese Douro protected Designation of Origin (PDO). Our miniaturized platform is able to detect and clearly distinguish between the hybridization of complementary or mismatched DNA sequences. The signal attenuation corresponding to the higher mismatched nucleobase(s) indicates the good specificity and selectivity of the sensor. Our results show the potential of graphene-FET technology for grape variety identification and multiplex detection on a chip for a future integration on a micro total analysis system (μTAS) for decentralized analysis.

## BACKGROUND & MOTIVATION




- The wine sector requires rapid and reliable methods for *Vitis vinivera L.* varietal detection
- DNA has been noted as a robust biomarker, stable under different soil composition, weather conditions, wine aging, etc
- The genes involved in anthocyanins profile is potentially interesting for varietal identification: FH3 Gene where SNP represents genetic sequence variations --» varietal discrimination
- **Research objectives:** Using the miniaturized graphene-FET sensor to detect and distinguish between the hybridization of complementary or mismatched DNA sequences.

### SNP(s) in FH3 Fragment shown by some varieties





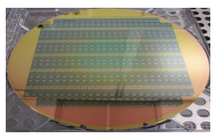

## GRAPHENE-FET CHIP FABRICATION & MEASUREMENT SETUP

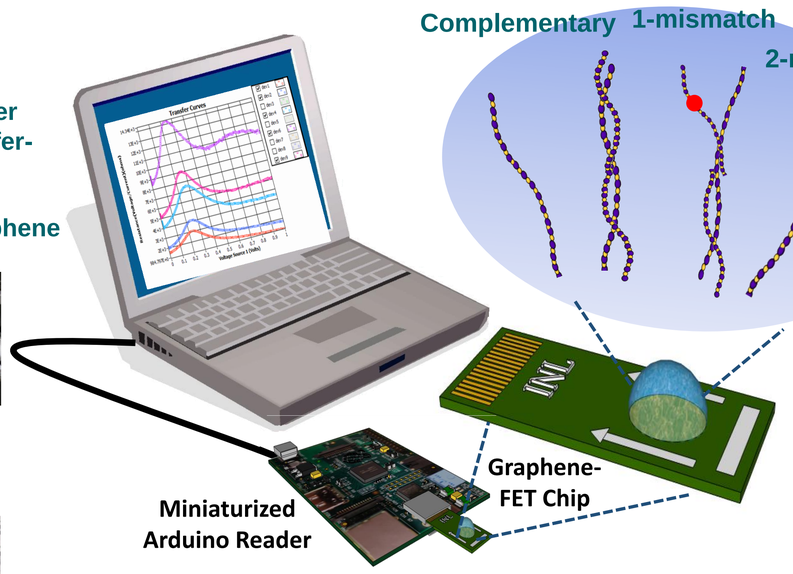
**Chip dicing and packaging in a PCB**

**Graphene transfer to transistor, wafer-scale testing**

**Single-layer graphene growth by CVD**

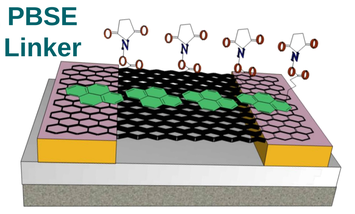
**Transistor fabrication on 200 mm Si wafer**

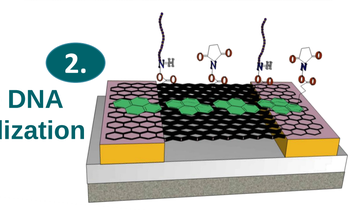


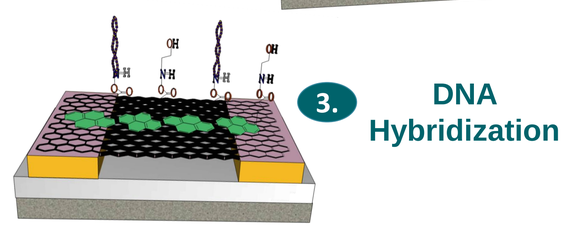



**Biorecognition strategy on Graphene-FET Sensor**



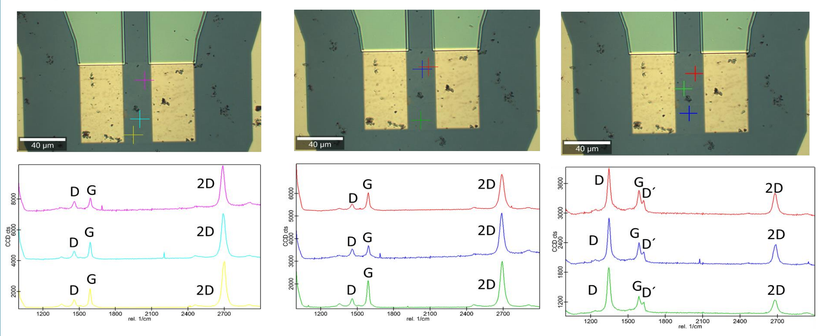



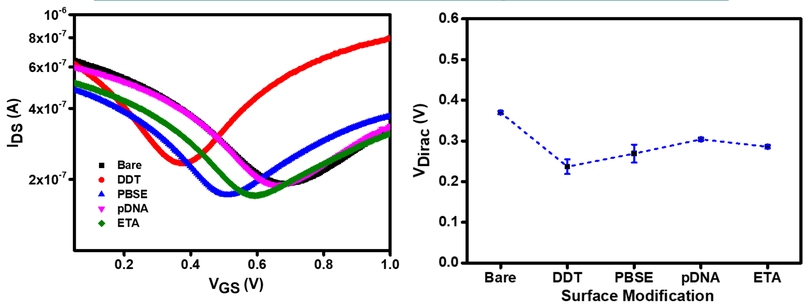




## RESULTS: SURFACE CHARACTERIZATION

### Surface Characterization of Graphene-FET Sensor by Raman Spectroscopy

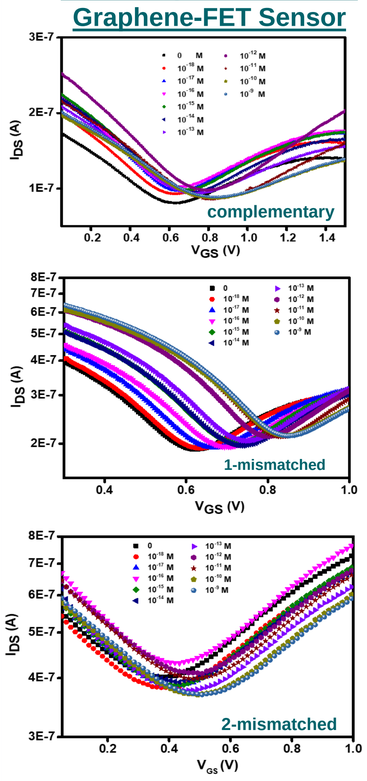



### Transfer Curve Characteristics of Surface Functionalization on Graphene-FET Sensor

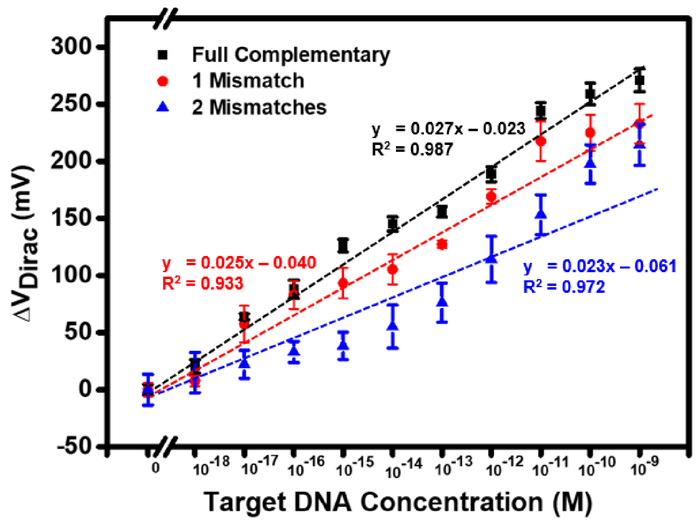



## RESULTS: DNA HYBRIDIZATION DETECTION

### Transfer Curve Characteristics of DNA Hybridization on Graphene-FET Sensor




### Calibration Plots of DNA Hybridization Signal from Complementary, Single and Double Mismatched Target




DNA sequence used in this study:

F3H gene sequence model from *Vitis vinivera L.* (grapevine),

Probe: C6-amine-GCG AAA GGC TGA AGC TAA TCT TTT CTT TGT CTT TG

Compl Target : CAA AGA CAA AGA AAA GAT TAG CTT CAG CCT TTC GC

1-mismatch : CAA AGA CAA AGA AAA GAT TAG CTT CAG CCT ATC GC

2-mismatches: CAA AGA CAA AGA AAA GAT TAG CTT CAG CCA ATC GC

## CONCLUSIONS

- Successful surface functionalization is confirmed by Raman spectra showing single-layer graphene properties, non-disrupted graphene peaks by DDT passivation and immobilization of PBSE linker.
- Transfer curves also significantly monitor the “step-by-step” surface functionalization protocol as shown by the $V_{Dirac}$ shifts.
- The hybridization of DNA resulted in a transfer curve shift toward more positive values due to the presence of negatively charged nucleotides at the interface.
- Our integrated miniaturized platform shows a clear distinction in the hybridization of complementary or mismatched DNA sequences.
- Overall results denote the potential of graphene-FET technology for grapevines variety identification and multiplex detection on a chip for a future integration on a micro total analysis system (μTAS) for decentralized analysis.

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## REFERENCES

[1] C. Villano *et al.*, *Food Control*, vol. 80, pp. 1–10, 2017.

[2] V. Catalano *et al.*, “*J. Agric. Food Chem.*, vol. 64, no. 37, pp. 6969–6984, 2016.

[3] R. Campos *et al.*, *ACS Sensors*, vol. 4, no. 2, pp. 286–293, 2019.

[4] S. Gomes *et al.*, *Sci. Rep.*, vol. 8, no. 1, pp. 1–12, 2018.

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