

## Tuning Graphene Oxide Nano-Flakes Reduction Differently Affects Neuronal Networks in the Zebrafish

**Giuseppe Di Mauro**<sup>1</sup>, Rossana Rauti<sup>1</sup>, Raffaele Casani<sup>1</sup>, George Chimowa<sup>2</sup>,  
 Anne Marie Galibert<sup>2</sup>, Emmanuel Flahaut<sup>2</sup>, Giada Cellot<sup>1</sup>, Laura Ballerini<sup>1</sup>.

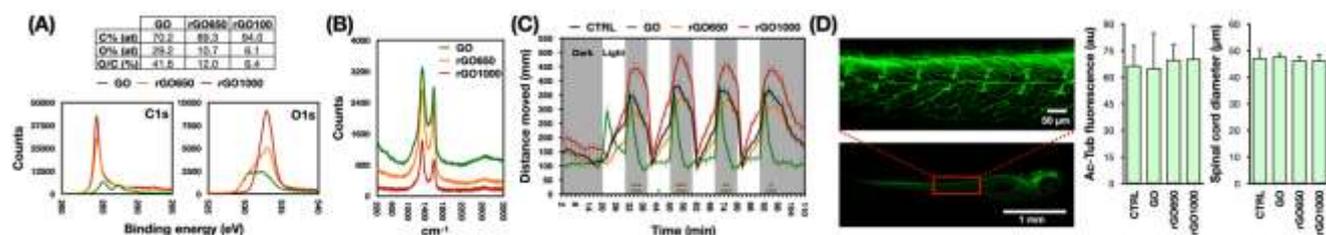
<sup>1</sup> Neuron Physiology and Technology Lab, International School for Advanced Studies (SISSA), Neuroscience, Via Bonomea 265, 34136, Trieste, Italy. <sup>2</sup> CIRIMAT, UMR CNRS 5085, Université Toulouse Paul Sabatier, Bat. CIRIMAT, 118 route de Narbonne, 31062 Toulouse CEDEX 9, France.  
 giuseppe.dimauro@sissa.it

Graphene related materials (GRMs) are characterized by diverse chemical and physical properties which may impact the nervous system. This ability has become a relevant issue following the increasing use of GRM's based technology in the design of neuro-biomedical devices [1-2]. In addition, the increased production of GRMs-based technologies might favor the dispersion in the environment of nanoparticles whose neurotoxicity needs investigation [3]. We exploited the thermal reduction of graphene oxide nanoflakes (GO) to generate materials with different oxygen/carbon ratio (Fig. A-B), we used a high throughput analysis of early stage-zebrafish locomotor behavior to investigate whether modifications of a specific GRMs chemical property influenced how these nanomaterials affect sensory motor neurophysiology. While zebrafish exposed to GO exhibited a quick reduction of locomotor activity, the reduced GO (rGO) induced an enhanced swimming performance (Fig. 1C). Histological analysis revealed that GRMs did not alter larval spinal cord morphology (Fig. 1D). Such modifications in locomotor behavior could emerge from GRMs direct interfacing of sensory/motor functions in the nervous system. We concluded that reducing the GO thermally is sufficient to produce differential effects on nervous system physiology, probably interfering with synaptic communication between neurons.

### References

- [1] Kostarelos et al., *Advanced Materials*, Graphene in the design and engineering of next-generation neural interfaces (2017) 29(42). 1700909.
- [2] Rauti et al., *Carbon*, Properties and behavior of carbon nanomaterials when interfacing neuronal cells: How far have we come? (2019) 143, 430-446.
- [3] Shareena et al., *Nano-micro letters*, A review on graphene-based nanomaterials in biomedical applications and risks in environment and health (2018) 10(3), 1-34.

### Figures



**Figure:** Characterization of GO and rGO 650-1000 °C using (A) XPS analysis and its oxygen/carbon quantification. (B) Raman spectroscopy. (C) Zebrafish locomotor activity measured as distance moved during light (white bars) and dark (grey bars) alternating periods. (D) Representative image of a whole mounted zebrafish larvae labelled with the neuronal marker acetylated-tubulin (Ac-Tub) and plots of spinal neurons fluorescence intensity and spinal cord diameter.