# Protein induced self-assembly of GO

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Building three-dimensional (3D) graphene oxide (GO) as breakthrough precursor for next-generation devices is spreading worldwide. We accomplish this task by Peroxiredoxins (Prx), a family of multi-tasking enzymes with ring-like architectures. Taking advantage of either the protein's symmetric structure and function, 3D rGO-based composites are hereby built up. The "Janus" Prx rings adhere flat on single GO layers and induce partial reduction, driving their stacking into 3D multi-layer rGO-Prx composites, even when using very few amounts of GO. Further, protein engineering allows divalent metal ions to bind the Prx's lumen and this is exploited to capture pre-synthesized gold nanoparticles (AuNPs) and grow *in situ* palladium nanoparticles (PdNPs) using the protein ring as physical confinement, thus paving the way to straightforward and "green" routes to 3D rGO-metal composites.

GO quickly gets clumped in the presence of Prx during mixing experiments in solution. Such clumps progressively push together leading to a soft colloid which can be hanged as compact material. The colloid can form again even after breaking by shaking, hence suggesting that a reversible self-assembly process occurs (Fig. 1a). The interaction between GO and Prx is clearly proved by AFM showing that the roundish protein moieties adhere onto the GO layers and drive stacking into multi-layer complexes (Fig. 1b). Interestingly, such an interaction is coupled to partial reduction of GO as proved by XPS chemical state analysis (Fig. 1c)

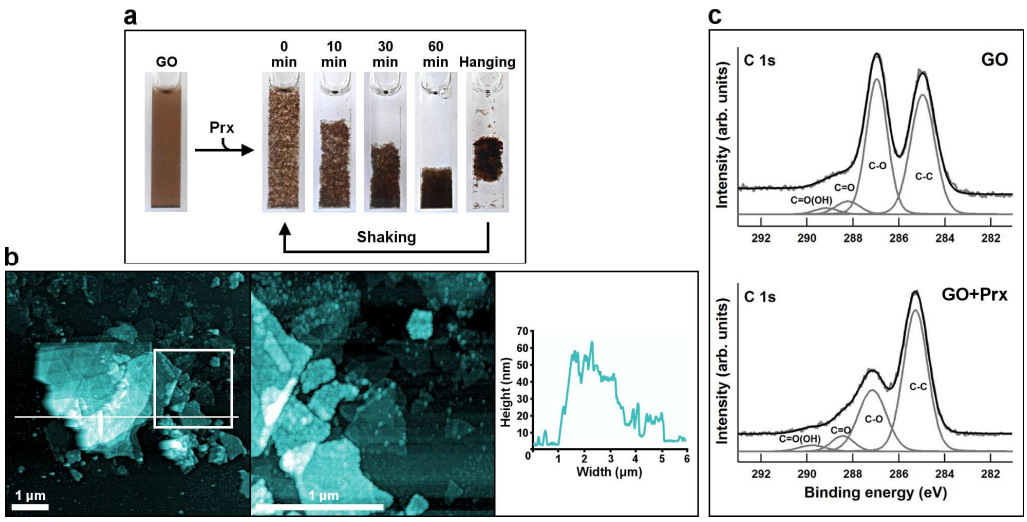


Figure 1: Self-assembly process (a). AFM (b) and XPS analysis (c) of GO after interaction with Prx.

The so-formed rGO-Prx colloid can be easily dried as a free-standing material by freeze-drying while keeping a microporous internal architecture as showed by SEM. This architecture encompasses several cross-linked multi-layer sheets forming the boundaries of 5-15 µm wide pores (Fig. 2a). The presence of Prx is proved by EDS microanalysis revealing

sulphur (S) and nitrogen (N), both unambiguously attributed to protein components. In addition, carbon (C) and oxygen (O) are also detected because of the presence of rGO (Fig. 2b).

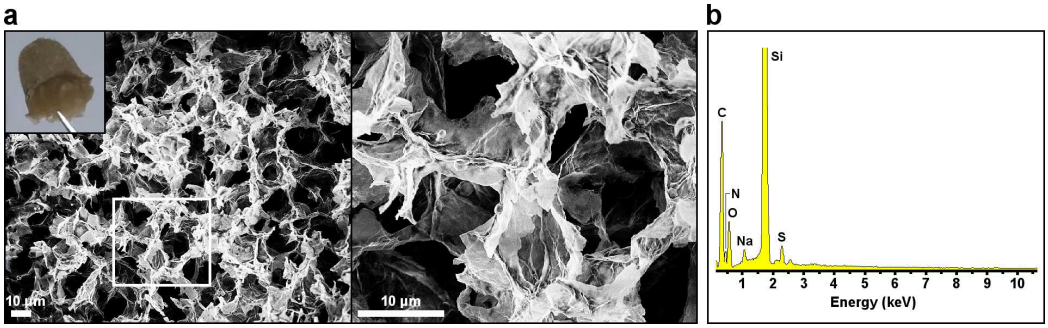


Figure 2: SEM imaging (a) and EDS microanalysis (b) of the freeze-dried rGO-Prx composite.

Addition by protein engineering of metal-binding sites on the lumen of the Prx ring allows the protein to bind divalent metal ions. By this way, Prx can trap Ni(II)-functionalized AuNPs with ~2 nm diameter and carrier them inside the multi-layer rGO-based composite as proved by STEM and EDX microanalysis (Fig. 3a). Similarly, Pd(II) ions are allowed to enter the protein's lumen, hence being encapsulated within the multi-layer rGO. It is estimated through the TGA-Pd(II) assays that up to 170 mg of Pd(II) are adsorbed per gram of Prx-conjugated GO; instead, GO alone is unable to stably capture ions. Further, the protein-bound Pd(II) ions can be metallized to Pd(0) by chemical reduction with $NaBH_4$ allowing a protein-dependent template synthesis of rGO-supported PdNPs with mean diameter of 3.3 nm (Fig. 3b). The versatility of the system is also demonstrated for Co(II) which is adsorbed up to 75 mg per gram of Prx-conjugated GO, against 41.3 mg Co(II) per gram of GO.

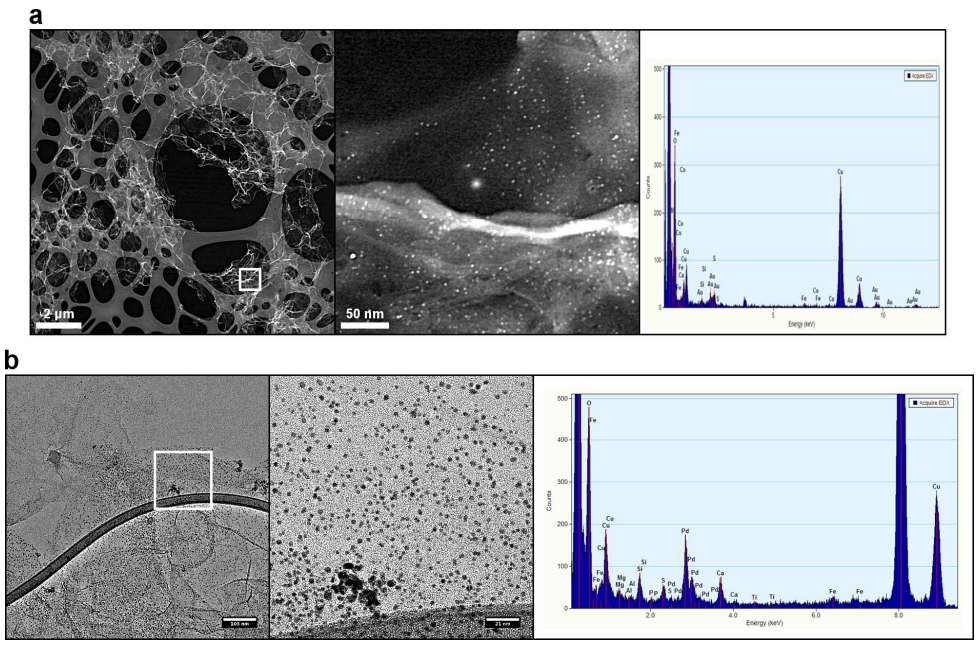


Figure 3: STEM-EDX of rGO-Prx-AuNPs (a) and TEM-EDX of rGO-Prx-PdNPs

## References

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