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Functionalized graphene oxide as nanocatalyst in dephosphorylation reaction: pursuing artificial enzymes

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The present study reports for the first time the use of thiol-functionalized graphene oxide nanocatalyst with impressive activity (>10^5-fold) in dephosphorylation reactions. The innovative and recyclable nanocatalyst has potential in designing artificial enzymes with targeted multifunctionalities and in detoxification of organophosphorus agents.

Dephosphorylation reactions are vital in biological systems, such as in regulatory and signaling processes, which are mediated by extraordinarily efficient enzymes. Particularly, reversible phosphorylation of protein dictates many cell life functionalities. Herein, the thiol group, mainly involved in oxidative thiol-disulfide pathways,[1] also participates in the rarely occurring thiol phosphorylation reactions, important in cysteine-based phosphatases, *e.g.*, bacterial antibiotic resistance.[2] Moreover, many aspects regarding mechanistic elucidation and detection of intermediates in protein phosphorylation reactions are still not clear.[3] Therefore, mimicking enzymatic active sites is strategic for designing novel artificial enzymes and potential enzymatic inhibitors for therapeutic purposes.

Carbon-based nanomaterials, such as graphene, are multifunctional and versatile, hence, the complex enzymatic architecture can be more readily mimicked, *e.g.*, by anchoring different functionalities on the backbone of these materials. The two dimensional sheets with sp^2 carbons of graphene comprise many optimum chemical, mechanical and electrical properties,[4] thus prompting promising applications in nanoelectronics, biomedicine, sensors and artificial enzymes.[5] A common graphene precursor is graphene oxide (GO), which is obtained by oxidizing graphite, leading to a highly oxygenated surface, followed by chemical reduction, yielding graphene (or the so-called reduced-graphene oxide, rGO). Hence, targeted covalent functionalization of the oxygenated groups on GO can enhance the materials properties and extend further applications.[6] Recently, we reported the thiolation of GO, by anchoring cysteamine (CA) by amide bonds on the carboxylate sites, under mild conditions.[7] The modified GO, namely GOSH, was thoroughly characterized and showed impressive size controllable features in nanocomposites with silver nanoparticles. Insofar, the freely available thiol groups on GOSH can efficiently act as nucleophiles in many reactions, although GOSH cannot be dispersed in any solvent, especially in water. In this context, we report GOSH as nanocatalyst in heterogeneous dephosphorylation reactions, using the activated triester diethyl 2,4-dinitrophenyl phosphate (DEDNPP) as a model (Fig. 1).[8] The proposed nanocatalyst can be readily separated and recycled consecutively, maintaining its catalytic activity.

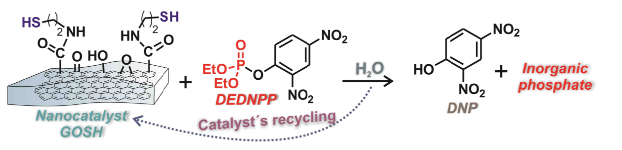

Fig. 1. Reaction of DEDNPP with the nanocatalyst GOSH.

Solid GOSH was added to a buffered solution at different pH values, kept under controlled temperature (20°C) and constant magnetic stirring and the reaction initiated by adding an aliquot of DEDNPP. Periodically, stirring was interrupted, the solid was decanted and the remaining solution was monitored by UV-Vis spectroscopy, following the appearance of the product 2,4-dinitrophenolate (DNP) at 400 nm. Rate constants (k_{obs}) were obtained from the pseudo-first order kinetic profiles and correlated with the mass of catalyst used and the local concentration of reactive thiol groups on the GO surface, obtained by thermogravimetric data (TGA, Supplementary Information). It should be noted that the highly reproducible pseudo first-order profiles are consistent with heterogeneous catalytic reactions where kinetic control overcomes diffusion contributions.[9, 10] Post-reaction characterization were carried out using TGA (under Ar) infrared (FTIR) and Raman spectroscopy. Full description of the characterization techniques are given in the Supplementary Information. Table 1 presents the most representative kinetic data obtained, along with comparatives to assign the catalytic efficiency of the reaction proposed.

Table 1 – Kinetic parameters obtained for reactions of DEDNPP with GOSH

	Rate constant, k_{GOSH}		Comparative [c]	
	$M^{-1}s^{-1}$, [a]	$g^{-1}s^{-1}$ [b]	k_{GOSH}/k_{H2O}	k_{GOSH}/k_{CA}
pH 8	1.73×10^{-2}	1.46×10^{-2}	3×10^{5}	14
pH 9	2.33×10^{-2}	1.96×10^{-2}	2×10^{5}	8
pH 10	3.17×10^{-2}	2.67×10^{-2}	9×10^{4}	4

[a] Second order rate constant: ratio of the pseudo-first order rate constant and the concentration of thiol groups 8.42×10^{-4} mol L^{-1} /mg of solid GOSH, calculated by TGA analysis; [b] Considering the total mass of catalyst used; [c] Comparative using the second order rate constant ($M^{-1}s^{-1}$) for the reaction of DEDNPP with GOSH, H_2O (spontaneous reaction) [8] and CA.

Results show impressive second order constants (k_{GOSH}, $M^{-1}s^{-1}$), which increase with higher pH. Indeed, the alkaline hydrolysis of phosphate esters[11] contributes to the overall reaction at higher pH, although results show that solely GOSH acts proficiently, regardless of the parallel reaction with ^{-}OH, as observed by the high rate constants at pH 8 (k_{GOSH}). Moreover, mild reaction condition (*e.g.* lower pH) is desirable when promoting greener catalytic reactions, as performed by our proficient biological enzymes. It should be noted that the reaction conversion is 100%, although no selectivity can be accounted, since only one substrate was evaluated. In order to infer catalytic activity, rate constants in the presence of GOSH were compared to the analogous spontaneous hydrolysis reaction (k_{H2O}) of DEDNPP, giving rate enhancements (k_{GOSH}/k_{H2O}) over 10^5-fold, which are among the highest increments reported for heterogeneous dephosphorylation reactions.[12, 13] Comparing the proposed heterogeneous catalysis with an analogous homogenous reaction, DEDNPP was reacted solely with CA, *i.e.*, the reactive thiol group present on GOSH. Surprisingly, rate constants in the presence of the nanocatalyst GOSH are 14-fold higher than with CA (k_{GOSH}/k_{CA}). Normally, a homogenous reaction is more efficient than its analogous heterogeneous reaction,[12] thus corroborating the prominent catalytic efficiency of the proposed nanocatalyst. Also, one would expect CA (with same concentration of thiol groups on GOSH) to promote similar enhancements. These results indicate synergistic effects on the multifunctional nanocatalyst.

We propose that the additional effects contributing to the overall catalysis can be due to attractive interactions of DEDNPP by the nanocatalyst, but further studies are needed to corroborate this proposition. The substrate could adsorb on the GO domains, common for carbon derived materials[14, 15] and this process concentrates reactants, thus optimizing the catalysis, as in micellar catalysis.[16] Comparing the rate constant per mass of catalyst (k_{GOSH}, g^{-1} s^{-1}) with similar studies is even more impressive[12] due to the low mass of catalyst used (1-4 mg), ideally desired in heterogeneous reaction.

Regarding the mechanism of the reaction, we propose that DEDNPP can adsorb on the GOSH surface and react with its functional groups, as shown in Fig. 2: the highly nucleophilic thiolate active species can attack the phosphorus atom leading to an unstable intermediate (**Int.**, Fig. 2) and the leaving group DNP.

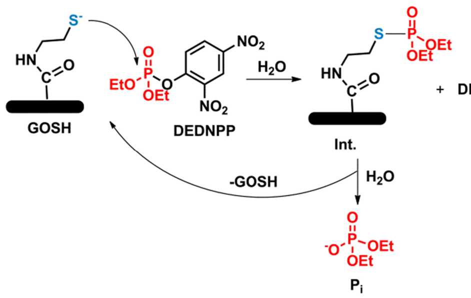

Fig. 2. Proposed mechanism for the reaction of GOSH with DEDNPP.

Potentiometric titration was also carried out to determine the pK_a value for the thiol group on GOSH (see Supplementary Information). Results indicate an unexpected low pK_a of 6.46±0.02 for the thiol moiety of GOSH, in contrast to CA with pK_a of 8.35.[17] Although, this lower pK_a is common in complex systems (proteins),[18] with neighboring groups that can stabilize the thiolate, such as the alcohol groups available on GO surfaces. Hence, over the pH range studied, thiolate species are present predominantly on GOSH, consistent with the proposed mechanism. As in similar nucleophilic reactions,[8] the putative phosphorylated surface of GOSH (**Int.**, Fig. 2) easily hydrolyses, regenerating the thiol sites for successive reactions. Another possible mechanism is the nucleophilic attack by thiolate on the aromatic carbon (C-O cleavage). Nevertheless, this path should not contribute significantly to the overall reaction, since the absorbance variation profile shows early formation of DNP, consistent with attack on phosphorus atom.[19] Indeed, on-going mechanistic studies involving the reaction of CA with DEDNPP by mass spectrometry confirms the presence of the phosphorylated intermediate formed via the thiolate attack, that should analogously occur herein.[20] It should be noted that in the reaction conditions carried out, only the thiol groups of CA are reactive, since the nitrogen atom is protonated (pK_a^{NH3+}=10.86), hence, unavailable as a nucleophile.[17] Due to this higher pK_a of the potentially nucleophilic amine site, CA reactivity increases more upon increasing pH, compared to GOSH. Additionally, the formation of disulphide bond, which would inhibit the reactivity of CA, was discarded since for GOSH, previous characterizations indicate freely available thiol groups.[7] For CA, the disulphide formation does not seem to contribute significantly during the reaction time followed.[20]

In order to infer recycling features, GOSH was recovered by filtration and reused as catalyst in new reactions with DEDNPP (three cycles). Fig. 3 illustrates with photos how recycling was carried out, along with rate constants for each cycle evaluated.

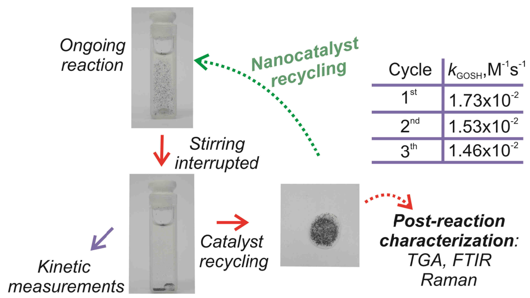

Fig. 3. Nanocatalyst recycling efficiency.

Results confirm that the highly efficient nanocatalyst GOSH maintains its catalytic property over several reaction cycles, suggesting its reactive groups maintain intact. The slight decrease in reactivity upon recycling can be attributed do adsorbed species such as the phosphate inorganic product. Although, even after the third cycle, GOSH is very effective: 2.6×10^5-fold increment, compared to the spontaneous reaction, in contrast to 3×10^5-fold observed in the first cycle. Overall, the nanocatalyst is easily recovered from reaction medium and can be recycled consecutively, which is desired in catalytic heterogeneous reactions.

After recovering the reused GOSH, the preservation of its overall properties and functionalities were confirmed by various characterization techniques: TGA, FTIR and Raman spectroscopy. FTIR spectra for GOSH taken before and after reaction with DEDNPP are given in Fig. 4.

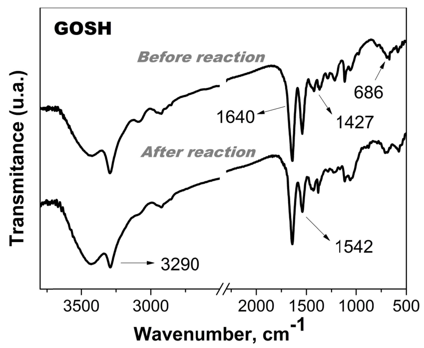

Fig. 4 FTIR spectra for GOSH before and after reaction with DEDNPP.

Results conclusively show typical bands for GOSH that are preserved upon catalyst recycling: C=O stretching (1640 cm^{-1}), N–H bending (3292 cm^{-1}), N–H stretching (1542 cm^{-1}), C–N stretching (1427 cm^{-1}) and N–H wagging (686 cm^{-1}). Other observed bands are attributed to GO domains.[7] The weak band due to S–H stretching (2569 cm^{-1}) is hardly distinguishable after recycling GOSH, which is attributed to the predominance of deprotonated thiolate (reaction pH > pK_a,). Even so, the amide bond preservation confirms the thiol group on GOSH.

TGA analysis (Supplementary Information) confirms the characteristic mass losses of GOSH before and after recycling: (i) 120-230°C, with ~15% mass loss due to oxygenated groups; (ii) 250-400°C, with ~ 25% mass loss attributed to thiol groups attached to GO domains and (iii) 400-550°C, with ~50% mass loss due to GO carbon backbone. Hence, the thiol moieties maintain intact after reaction, reiterating that the proposed recycling is legitimate.

Lastly, Raman spectra were obtained mainly to confirm GO-like nature. Indeed, results show (Supplementary Information) intense bands, characteristic for this kind of carbonaceous materials: D (1350 cm^{-1}), G (1580 cm^{-1}) and D´ (1607 cm^{-1}) bands.[7] After recycling, these bands are maintained and the intensity ratio between D and G bands (I_D/I_G), associated to the defective nature of the material (functionalized) is retained ~2.2, and no further major change occurs on GO surface. Also, the band at 2920 cm^{-1} related to the CH_2 asymmetric stretch of the thiol group was detected after reaction.[7] Therefore, post-reaction characterizations confirm the recycling features of GOSH, with no indication of thiol leaching, which is expected since it is covalently attached to the GO surface by highly stable amide bonds.

In conclusion, the present study reports for the first time the use of GO-based materials with impressive catalytic activity in dephosphorylation reactions. Additionally, the nanocatalyst comprising reactive thiol groups, GOSH, can effectively be recycled consecutively, without losing its catalytic activity significantly. All characterization carried out confirmed that the catalyst maintained its overall functionalities after reuse. A mechanism involving nucleophilic attack by thiolate was proposed, mimicking enzymatic reactions involving thiol-based groups. Therefore, the nanocatalyst proposed has certainly innovative features, particularly promising in designing artificial enzymes, by exploring its multifunctionalities, *e.g.*, coupling GOSH with therapeutic agents, envisioning advances in genetic therapy. In this sense, we believe that targeted functionalization of GO and overall engineering of carbon nanomaterials broadens the field of novel complex multifunctional catalyst which can combine assembly characteristics and multiple catalyses (nucleophilic, acid-base). It is noteworthy that these catalysts are also very promising in the detoxification of chemical warfare and pesticides from the phosphate ester family.[21] There is a great interest in developing new and efficient methods to detoxify these agents to eliminate stocks, treat population and contain attacks. These concerns are evident by the 2013 Peace Nobel Prize awarded to the Organisation for the Prohibition of Chemical Weapons for its efforts in eliminating chemical weapons.[22] The nanocatalyst GOSH can be readily used for detoxification purposes, since it effectively cleaved DEDNPP, a substrate similar to known toxic agents (*e.g.* paraoxon). This attribution is not unprecedented since it is common to evaluate detoxification properties in model substrates. Finally, studies that involve dephosphorylation processes by a clear-cut catalysis are promising for both development of artificial enzymes and detoxification of organophosphorus agents.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Potentiometric titration data, kinetic profiles, TGA curves and Raman spectra. See DOI: 10.1039/c000000x/

1. C. E. Paulsen and K. S. Carroll, *Chem Rev*, 2013, **113**, 4633-4679.
2. A. Salmeen and D. Barford, *Antioxid Redox Sign*, 2005, **7**, 560-577.
3. P. Cohen, *Nat Cell Biol*, 2002, **4**, E127-E130.
4. V. Singh, D. Joung, L. Zhai, S. Das, S. I. Khondaker and S. Seal, *Prog Mater Sci*, 2011, **56**, 1178-1271.
5. H. J. Jiang, *Small*, 2011, **7**, 2413-2427.
6. T. Kuila, S. Bose, A. K. Mishra, P. Khanra, N. H. Kim and J. H. Lee, *Prog. Mater Sci.*, 2012, **57**, 1061-1105.
7. E. S. Orth, J. E. S. Fonsaca, S. H. Domingues, H. Mehl, M. M. Oliveira and A. J. G. Zarbin, *Carbon*, 2013, **61**, 543-550.
8. E. S. Orth, E. H. Wanderlind, M. Medeiros, P. S. M. Oliveira, B. G. Vaz, M. N. Eberlin, A. J. Kirby and F. Nome, *J Org Chem*, 2011, **76**, 8003-8008.
9. S. Sawadjoon, A. Lundstedt and J. S. M. Samec, *Acs Catal*, 2013, **3**, 635-642.
10. J. A. Widegren and R. G. Finke, *J Mol Catal a-Chem*, 2003, **198**, 317-341.
11. W. W. Cleland and A. C. Hengge, *Chem. Rev.*, 2006, **106**, 325-3278.
12. Y. C. Chang and D. H. Chen, *React Funct Polym*, 2009, **69**, 601-605.
13. V. Chandrasekhar, A. Athimoolam, S. G. Srivatsan, P. S. Sundaram, S. Verma, A. Steiner, S. Zacchini and R. Butcher, *Inorg Chem*, 2002, **41**, 5162-5173.
14. D. R. Dreyer, S. Park, C. W. Bielawski and R. S. Ruoff, *Chem Soc Rev*, 2010, **39**, 228-240.
15. F. M. Koehler, A. Jacobsen, K. Ensslin, C. Stampfer and W. J. Stark, *Small*, 2010, **6**, 125–1130.
16. T. Dwars, E. Paetzold and G. Oehme, *Angew Chem Int Edit*, 2005, **44**, 7174-7199.
17. H. Fleischer, Y. Dienes, B. Mathiasch, V. Schmitt and D. Schollmeyer, *Inorg Chem*, 2005, **44**, 8087-8096.
18. S. Vohnik, C. Hanson, R. Tuma, J. A. Fuchs, C. Woodward and G. J. Thomas, *Protein Sci*, 1998, **7**, 193-200.
19. E. S. Orth, P. L. F. da Silva, R. S. Mello, C. A. Bunton, H. M. S. Milagre, M. N. Eberlin, H. D. Fiedler and F. Nome, *J Org Chem*, 2009, **74**, 5011-5016.
20. E. S. Orth, T. A. Golin, R. C. Campos and A. J. G. Zarbin, *Unpublished work.*, 2013.
21. Y.-C. Yang, J. Baker and J. R. Ward, *Chem. Rev*, 1992, **92**, 1729-1743.
22. , http://www.nobelprize.org/. (Accessed Fev 26, 2014).

