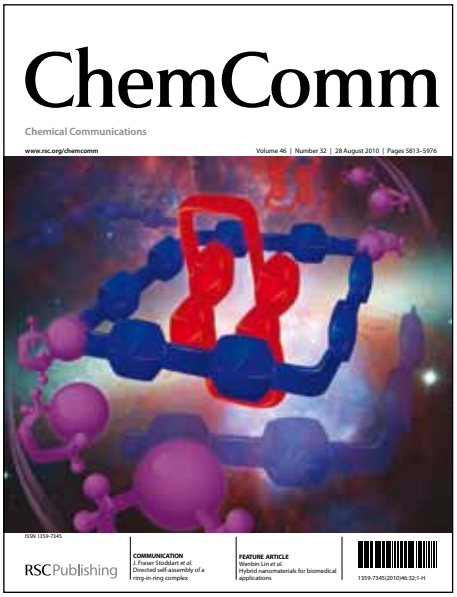
ChemComm

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the RSC Publishing peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, which is prior to technical editing, formatting and proof reading. This free service from RSC Publishing allows authors to make their results available to the community, in citable form, before publication of the edited article. This *Accepted Manuscript* will be replaced by the edited and formatted *Advance Article* as soon as this is available.

To cite this manuscript please use its permanent Digital Object Identifier (DOI®), which is identical for all formats of publication.

More information about *Accepted Manuscripts* can be found in the **Information for Authors**.

Please note that technical editing may introduce minor changes to the text and/or graphics contained in the manuscript submitted by the author(s) which may alter content, and that the standard **Terms & Conditions** and the **ethical guidelines** that apply to the journal are still applicable. In no event shall the RSC be held responsible for any errors or omissions in these *Accepted Manuscript* manuscripts or any consequences arising from the use of any information contained in them.

Journal Name

RSCPublishing

COMMUNICATION

Tuning, Inhibiting and Restoring the Enzyme Mimetic Activities of Pt-Apoferritin†

Cite this: DOI: 10.1039/x0xx00000x

Unai Carmona,[a] Lianbing Zhang,*[a] Le Li,[a] Wolfram Münchgesang,[bd] Eckhard Pippel,[b] and Mato Knez*[ac]

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Abstract: A selective and specific inhibition of the catalase mimetic activity but not SOD-like activity of Pt-apoferritin can be achieved through the choice of the inhibitor. The activity recovery with a reducing agent was explored and proven to successfully restore the surface-chemistry of NPs.

The catalytic properties of many nanoparticles (NPs) are key features with special interest for chemical or biological applications.[1,2] Often these catalytic reactions resemble the ones occurring with bioenzymes, including hydrogenation, oxidation and further reactions like C-C coupling, etc.[3,4] Although NPs often mimic biological enzymes, the reaction mechanisms which inorganic NPs and biomolecules undergo are apparently different. The catalytic activities of NPs are often not reaction specific. Pt NPs can mimic various enzymatic activities, such as oxidase, catalase and superoxide dismutase (SOD).[5-7] In contrast, biological enzymes have some natural inhibitors which regulate their catalytic activities. Heavy metals and many synthetic chemicals, such as sodium azide, can specifically or generally inhibit enzymatic activities.[8] In view of potential future applications in enzyme mimetics it is important to understand whether or not enzyme inhibitors act on NPs in a similar way as they do on molecular enzymes.

In this work the enzyme mimetic activities of the bioinorganic Pt-apoferritin (Pt-apo) hybrid compounds were analyzed with 3-amino-1,2,4-triazole (3AT), NaN_3 or Hg^{2+} as inhibitors. A selective and specific inhibition of the mimetic activities through the choice of the inhibitor provides a novel way to tune the activity of NPs and to suppress undesired reactions. The investigation of a strategy for recovery of the activity with a reducing agent was explored and proven successful. The recovery treatment restored the surface chemistry of the NPs and may even act as a general method for restoring numerous NPs.

Apoferritin it is a spherical protein complex with a hollow inner cavity. It is an active cellular protein with intrinsic impact on the iron metabolism and oxidative stress regulation and shows specific enzymatic activities.[9-11] Due to its structural features it is a widely used protein template for the synthesis of various nanomaterials with promising application potential.[12-16] In the case of NPs synthesized within apoferritin, the resulting hybrid composites combine the functions and activities of both the NPs and the apoferritin. It has been shown that composites combining the anti-oxidative activities of apoferritin and the catalase- and SOD-like activities of the Pt NPs can increase the cell viability under externally induced oxidative stress.[17] Apoferritin also provides a biological pathway for the cellular uptake of encapsulated NPs.[18]

Pt-NPs within apoferritin used in this work were prepared with well established methods (Supporting information (SI), Figure S1).[17, 18] The well-characterized enzyme mimetic catalase activity of Pt-NPs was used for further inhibition analysis with NaN_3 as general inhibitor and $HgCl_2$ as heavy metal based inhibitor for the biological catalase and SOD, and 3AT as catalase-specific inhibitor.[19] It was observed that in absence of inhibitors, 65% of the hydrogen peroxide was decomposed by Pt-apo within 30 min (Figure 1A). In presence of 10 mM NaN_3, Pt-apo decomposed less than 25% within 30 min. The inhibitory effect was even more pronounced with 10 mM Hg (II) or 3AT. The catalase activity of Pt-apo was almost completely inhibited with those two reagents. All three inhibitors exhibited a concentration-dependent inhibition (Figure S2). Pt-apo was incubated over night with 10 mM inhibitor and subsequently the treated Pt-apo was purified with a spin column before the catalase

activity was analyzed. Figure 1B shows that without further inhibitor added to the assay solution, the treated Pt-apo decomposed less than 40% H_2O_2. This indicates that presumably the strong interaction of the inhibitor with the surface of the Pt NPs is dominating the competitive adsorption, being the reason for the inhibitory effect. Our observations are concordant with the competitive adsorption mechanism of NaN_3 observed by its inhibition of the oxidase activity of Pt nanoparticles.[20]

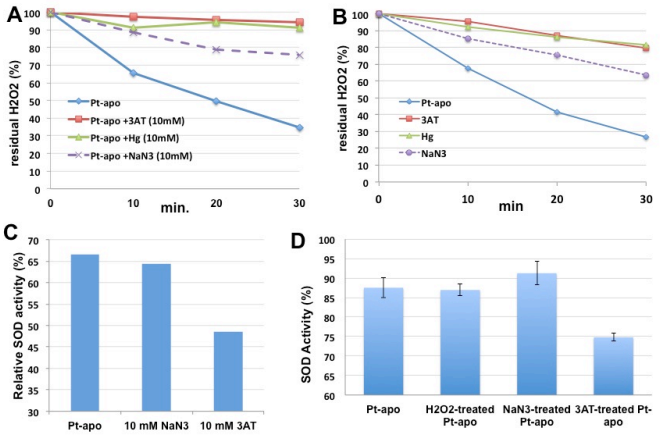


Figure 1. (A) Effects of the inhibitors on the hydrogen peroxide decomposition activity of Pt-apo. Assay conditions: 30 ºC, pH 7.4, 1.875 mg/ml Pt-apo, and 2 mM initial hydrogen peroxide. (B) The hydrogen peroxide decomposition activity of Pt-apo and inhibitor-treated Pt-apo. Assay conditions: 30 ºC, pH 7.4, 1.5 mg/ml Pt-apo or treated Pt-apo, and 2 mM hydrogen peroxide. (C) Inhibition effect on the SOD activity of the Pt-apo with 10 mM 3AT and NaN_3. (D) Inhibitory effect on the SOD activity of Pt-apo upon pretreatment with H_2O_2, NaN_3 and 3AT. The concentration of H_2O_2, NaN_3 and 3AT for the pretreatment: 8, 100 and 100 mM, respectively.

We further analyzed the inhibitory effect of NaN_3 or 3AT on the SOD activity of Pt-apo in order to verify whether or not the inhibition is reaction specific. Hg^{2+} could not be evaluated, since it readily inhibits the superoxide generation in the SOD assay system, even without addition of the nanocomposite. NaN_3 did not inhibit the SOD activity of Pt-apo, while 3AT inhibited 20% of the relative SOD activity (Figure 1C and Figure S3). In a further experiment, Pt-apo was treated with the aforementioned two inhibitors and H_2O_2 over night and the relative SOD activity was tested after the purification. While more than 20% of the SOD activity was inhibited upon 3AT treatment, the activity of NaN_3- and H_2O_2-pretreated Pt-apo did not significantly alter in comparison to untreated Pt-apo (Figure 1D). Together with the previously described catalase inhibition, these results indicate that inhibitors can influence the enzyme mimetic activities of NPs in a reaction-specific manner. This provides a novel combinatorial way to make NPs tunable and flexible catalysts by using inhibitors to selectively suppress their activity, inhibiting the catalase activity with NaN_3, or inhibiting both catalase and SOD activities with 3AT.

The competitive adsorption of 3AT on NPs can explain both its inhibition of catalase and SOD mimetic activities. However, the same theory alone cannot justify the selective inhibitory effects of NaN_3. It has been observed that superoxide can form singlet oxygen in absence of the enzyme.[21] NaN_3, but not 3AT, is a strong quencher of singlet oxygen.[22] The singlet oxygen may form in our case during the SOD-like reaction and react with NaN_3 adsorbed on the NPs surfaces, which will probably remove the adsorbed azide and abrogate the inhibition. Since superoxide and singlet oxygen are not involved in the catalase reaction, azide ions will remain absorbed and cause the inhibition.

Inhibition of biological enzymes is often irreversible, resulting in a permanent loss of activity.[23] Enzyme mimetic NPs, composed of a robust protein and even more robust inorganic core might be superior to their biological counterparts and the previously described inhibition of the activity might potentially be reversible. Many established methods for protein purification provide the possibility to easily recycle Pt-apo from a reaction mixture. This is in turn advantageous for investigating the activity recovery of Pt-based catalysts.

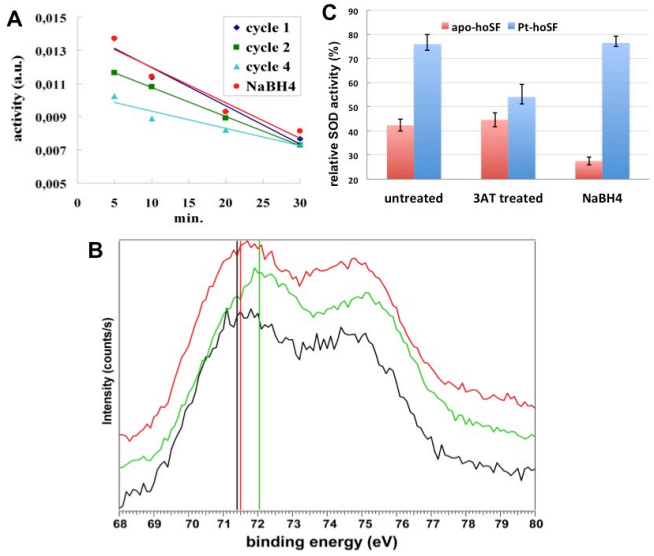


Figure 2. (A) Change of the catalytic activity of Pt-apo after multiple reaction cycles and the treatment with $NaBH_4$ (red line). Activity of Pt-apo was normalized with the Pt quantity (SI, Table S1). The normalization was done by calculating the percentage of decomposed H_2O_2 per minute per ppm Pt for the corresponding time. (B) XPS Spectra of initial Pt-apo (black), after the reaction with H_2O_2 (green), and after treatment with $NaBH_4$ (red). The spectra show the Pt $4f_{7/2}$ and the Pt $4f_{5/2}$ photoelectron spectral lines with the corresponding background. The corresponding Pt $4f_{7/2}$ position is indicated with vertical lines in the same color. (C) The SOD activity of untreated, 3AT-treated and $NaBH_4$-treated (after 3AT pre-treatment) Pt-apo. Protein concentration: 550 µg/ml apo-hoSF, 44 µg/ml Pt-apo.

The H_2O_2 decomposition activity of the Pt-apo decreased with each of the four serial reaction cycles (Figure S4). No significant change in the particle size was observed and most of the apoferritin still showed an intact protein shell (Figure S5). Since H_2O_2 is an oxidizing agent, the reason for the reduction of the catalytic efficiency could be an oxidation of the surface or adsorption of oxygen or some other oxygen species onto the Pt catalyst during the reaction.[24,25] An adsorption of corresponding species during storage could be excluded with a reference measurement of the bare sample after 4 weeks (Figure S6). If the assumption of poisoning in the oxidative environment holds, a removal of the adsorbed oxygen species would logically restore the catalytic activities. In order to prove this, Pt-apo was first treated with 1.32 mM H_2O_2 in sum (equal to four reaction cycles) and subsequently treated with the

reducing agent $NaBH_4$ for 5 min. Following this treatment, the peroxide decomposition activity of the Pt-apo was measured for comparison. The activity of Pt-apo after reduction indeed increased again as compared with the fourth cycle (Figure 2A and Figure S4). A similar effect was also observed if another common reducing agent, H_2 gas, was used (Figure S6).

The changes of the surface of the Pt induced by a potential adsorption or oxidation, as well as after treatment with $NaBH_4$, were investigated with XPS. The Pt $4f_{7/2}$ peak position of Pt-apo shifted from 71.4 eV to 71.9 eV after the H_2O_2 decomposition reaction and shifted back to 71.5 eV after treatment with $NaBH_4$ (Figure 2B). The XPS measurements demonstrate that the changes induced by the decomposition reaction could be recovered after treatment with $NaBH_4$. This indicated that a reversible surface chemistry is the origin of the restorable activity. The reducing agent was also able to restore the inhibited SOD activity of the 3AT- and NaN_3- treated Pt-apo (Figure 2C). The effects of the reducing agent on the protein part of the hybrid composite were clearly not the reason for the recovery (Figure 2C).

In summary, the enzyme mimetic activities of ferritin-encapsulated platinum nanoparticles can be inhibited with inhibitors typically active with biological enzymes. NaN_3 inhibited only the catalase activity of Pt NPs, but not the SOD activity, while 3AT inhibited both catalase and SOD activities, indicating the possibility to achieve selective and specific activity suppression with inhibitors. This opens the door towards controlling the activities of NPs in a reaction specific manner with further inhibitor screening, which is greatly interesting for NP engineering targeting enzyme mimetics. The demonstrated activity recovery of catalytic activities with $NaBH_4$, restoring the chemical properties of the nanoparticle´s surface, promise a general technique for regeneration of the NPs, which is not limited to catalytic applications.

Acknowledgements. M.K. and U.C. greatly acknowledge financial support through Marie Curie Actions (CIG) within project number 322158 (ARTEN).

Notes and references

[a] CIC nanoGUNE, Tolosa Hiribidea 76, 20018 Donostia-San Sebastian, Spain. E-mail: l.zhang@nanogune.eu; m.knez@nanogune.eu.

[b] Max Planck Institute of Microstructure Physics, Weinberg 2, 06120 Halle, Germany

[c] IKERBASQUE, Basque Foundation for Science, Alameda Urquijo 36-5, 48011 Bilbao, Spain

[d] Present address: Technische Universität Bergakademie Freiberg, Institut für Experimentelle Physik, 09596 Freiberg, Germany.

† Electronic Supplementary Information (ESI) available: Detailed materials and methods. Supplementary results. See DOI: 10.1039/c000000x/

1 X. Hu, J. Liu, S. Hou, T. Wen, W. Liu, K. Zhang, W. He, Y. Ji, H. Ren, Q. Wang, X. Wu, *Sci. China Phys. Mech. Astron.* 2011, **54**, 1749.

2 H. Wei, E. Wang, *Chem. Soc. Rev.* 2013, **42**, 6060.

3 M. Moreno-Mañas, R. Pleixats, *Accounts Chem. Res.* 2003, ***36***, 638.

4 D. Astruc, F. Lu, J. R. Aranzaes, *Angew. Chem. Int. Ed.* 2005, **44**, 7852.

5 S. R. Doctrow, M. Liesa, S. Melov, O. S. Shirihai, P. Tofilon, *Cur. Inorg. Chem.* 2012, **2**, 325

6 J. Liu, X. Hu, S. Hou, T. Wen, W. Liu, X. Zhu, J.-J. Yin, X. Wu, *Sensors Actuators B Chem.* 2012, **166**, 708.

7 X. Liu, W. Wei, C. Wang, H. Yue, D. Ma, C. Zhu, G. Ma, Y. Du, *J. Mater. Chem.* 2011, **21**, 7105.

8 Y. Yang, Y. Ding, X. Liao, Y. Cai, *Process Biochem.* 2013, **48**, 351.

9 T. Tosha, H-L. Ng, O. Bhattasali, T. Alber, E. C. Theil, *J. Am. Chem. Soc.* 2010, **132**, 14562.

10 S. R. Danielson, J. K. Andersen, *Free Radic. Biol. Med.* 2008, **44**, 1787.

11 A. Friedman, P. Arosio, D. Finazzi, D. Koziorowski, J. Galazka-Friedman, *Parkinsonism Relat. Disord.* 2011, **17**, 423.

12 M. Uchida, S. Kang, C. Reichhardt, K. Harlen, T. Douglas, *Biochim. Biophys. Acta Bba - Gen. Subj.* 2010, **1800**, 834.

13 L. Zhang, M. Knez, *J. Nanosci. Lett.* 2011, **2**, 6.

14 I. Yamashita, K. Iwahori, S. Kumagai, *Biochim. Biophys. Acta Bba - Gen. Subj.* 2010, **1800**, 846.

15 K. K. W. Wong and S. Mann, *Adv. Mater.* 1996, **8**, 928.

16 L. Zhang, J. Swift, C. A. Butts, V. Yerubandi and I. J. Dmochowski, *J. Inorg. Biochem.,* 2007, **101**, 1719.

17 L. Zhang, L. Laug, E. Münchgesang, E. Pippel, U. Göselle, M. Brandsch, M. Knez, *Nano Lett.* 2010, **10**, 219.

18 L. Zhang, W. Fischer, E. Pippel, G. Hause, M. Brandsch, M. Knez, *Small* 2011, **7**, 1538.

19 M. W. Hentze, *Proc. Natl. Acad. Sci.* 1987, **84**, 6730.

20 J. Liu, X. Hu, S. Hou, T. Wen, W. Liu, X. Zhu, X. Wu, *Chem. Commun.* 2011, **47**, 10981.

21 W. H. Koppenol, *Nature* 1976, **262**, 420.

22 R. D. Hall, C. F. Chignell, *Photochem. Photobiol.* 1987, **45**, 459.

23 R. Poulin, L. Lu, B. Ackermann, P. Bey, A.E. Pegg, *J. Biol. Chem.* 1992, **267**, 150.

24 M. J. Mummey, L. D. Schmidt, *Surf. Sci.* 1981, **109**, 29.

25 V. Stamenkovic, B. S. Mun, K. J. J. Mayrhofer, P. N. Ross, N. M. Markovic, J. Rossmeisl, J. Greeley and J. K. Norskov, *Angew. Chem. Int. Ed.* 2006, **45**, 2897.