

ChemComm

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Investigation of the Interaction Modes between Nonpolar Organic Pollutants with Ionizable Functional Groups and Natural Organic Matter via AuNPs-Based Colorimetric Assays†

Hongyun Niu,^a Saihua Wang,^a Yixin Tan,^{a,b} and Yaqi Cai^{*a}

Received (in XXX, XXX) Xth XXXXXXXXXX 20XX, Accepted Xth XXXXXXXXXX 20XX

DOI: 10.1039/b000000x

For the first time, natural organic matter (NOM) modified AuNPs are used as sensors to “observe” the specific interactions (such as hydrogen-bonds and halogen-bonds) between functional groups of organic compounds and NOM using the colorimetric assays.

Nonpolar and moderately polar organic compounds with ionizable functional groups (such as hydroxyl- and amino-substituted aromatics) are usually environmentally relevant.¹ The binding on dissolved natural organic matter (NOM) directly influences their bioavailability, fate, and behavior in aquatic ecosystems.^{2,3} It is necessary to investigate the affinity ability and binding mechanisms of these kinds of contaminants on NOM, which usually proceeds by measuring the partition coefficient (K_{DOC}) through adsorption isotherms of organic pollutants on humic substance. However, the adsorption isotherms are insufficient to estimate the interactions between NOM and functional groups of nonpolar and moderately polar organic compounds. It is vital to introduce new method to probe the interaction mechanisms between these chemicals and NOM.

AuNP-based colorimetric assays are of particular interest because molecular events are easily transformed into color changes, which can be observed by the naked eye. The color change is highly sensitive to the size, shape, capping agents, medium refractive index, as well as the aggregation state of AuNPs. Most assays designed for compounds rely on the binding of ligands modified on AuNPs with target analytes, which is defined as “interparticles crosslinking aggregation”.⁴ In this study, for the first time, we use NOM modified AuNPs as sensors to investigate the interactions between organic contaminants and NOM through color change of AuNPs suspension. According to the results of colorimetric assays, we then infer backward the interaction modes between NOM and organic targets.

The AuNPs are obtained by reduction of HAuCl_4 (0.025 mM) by NaBH_4 in the presence of the stabilizer Suwannee River fulvic acid (SRF) (15 mg L^{-1}). Detailed information about the synthesis and characterization of SRF-AuNPs is presented in ESI.† The synthesized SRF-AuNPs are about 5 nm in-diameter and well-dispersed in neutral and alkali solution (Fig. 1A and Fig. S1C). According to the study of Huo,⁵ the extinction coefficient ϵ of SRF-AuNPs solution is calculated to be $1.03 \times 10^7 \text{ M}^{-1}\text{cm}^{-1}$. The absorbance of the as-prepared SRF-AuNPs suspension at $\lambda = 514 \text{ nm}$ is 0.51 (RSD=5%), therefore, the molar concentration of

SRF-AuNPs solution is $4.95 \times 10^{-8} \text{ mol L}^{-1}$. The element composition on the surface of SRF-AuNPs sensors are investigated with XPS. Fig. S1E describes that SRF-AuNPs mainly consist of C, O, and Au atoms. The high resolution O 1s XPS line of sensors shows that phenolic groups/ester, carboxylic groups, carbonyl groups are the main oxygen-containing species on the surface of sensors (Fig. S1F). Due to the intra- and intermolecular H-bonding among these oxygen-containing groups in acid solution, the SRF-AuNPs show slight aggregation at pH 3.5-5.5 (Fig. S1A, 1B and 1D). In the presence of monovalent cations (Na^+) or at different temperature, the dispersion of the sensors remains unchanged (Fig. S2A). However, the addition of 50 and 100 μM of Ca^{2+} lead to aggregation of sensors in the whole pH range because Ca^{2+} can bind with the carboxylic or phenolic groups of NOM. In the UV-Vis spectra, the location of surface plasmon resonance (SPR) of sensors suspension at pH 5.5-9.5 shifts from 514 nm to 530-534 nm (Fig. S2B-2D). Anyway, the as-prepared SRF-AuNPs sensors are stable with and without Ca^{2+} , and the color and location of SPR of sensors suspension do not change in several months. We measure the concentrations of residual NOM in mixture using a fluorescence spectrophotometer according to the fluorescence intensity at Ex/Em=340nm/460 nm. As a result, about 5 mg L^{-1} of free NOM remain in AuNPs colloid. The unbound NOM can enhance the solubility of hydrophobic chemicals and reduce the particle-particle interaction between AuNPs.⁶

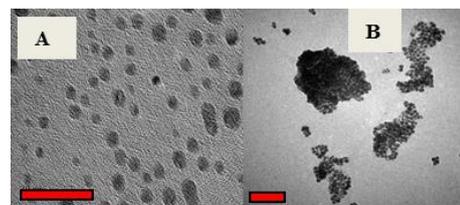


Fig. 1 TME images of the as-prepared SRF-AuNPs sensors (A) and sensors in the presence of PBP (B) at pH 7.5.

The SRF-AuNPs sensors are used to investigate the interaction modes between NOM and several highly hydrophobic and moderately hydrophobic phenols, bisphenol A (BPA), tetrabromobisphenol A (TBBPA), pentabromophenol (PBP), pentachlorophenol (PCP), and tetrachlorobisphenol A (TCBPA). As a result, only TBBPA and PBP trigger the aggregation of SRF-AuNPs sensors at $\text{pH} \leq 5.5$, notable color change and red-

shift of SPR peaks in UV-Vis spectra of sensors suspension are seen in Fig. S3 and S4. At pH 7.5-9.5, the two targets do not cause aggregation of sensors. The SRF-AuNPs sensors show no response to the chlorinated phenols and BPA across the pH range.

The influence of coexisting cations (Na^+ and Ca^{2+}) on the interactions between targets and NOM can be explored by the NOM-AuNPs sensors as well. The presence of 0.2, 1.0 and 10 mM of Na^+ does not affect the response of sensors to these compounds (Fig. S5-S7). The addition of Ca^{2+} cations (5, 10, 50 and 100 μM) shows no influence on the response of PCP and TCBPA to SRF-AuNPs sensors at pH 3.5-9.5. Slight aggregation of sensors in the presence of BPA is observed at $\text{pH} > 5.5$ as the concentration of Ca^{2+} increases to 100 μM . When the concentration of Ca^{2+} reaches to 50 μM , aggregation of SRF-AuNPs sensors caused by PBP and TBBPA is promoted especially at pH 7.5-9.5 (Fig. 3 and Fig S8). The color of sensors solutions changes from rosy to pink red or blue. Heavy aggregation of sensors can be seen in TEM image after reaction with PBP in Ca^{2+} solution at pH 7.5 (Fig. 1B). This result suggests that the influence of Ca^{2+} on the sorption of BPA and two brominated phenols to NOM can be detected obviously even when the concentration of Ca^{2+} is as low as 50 μM or 100 μM .

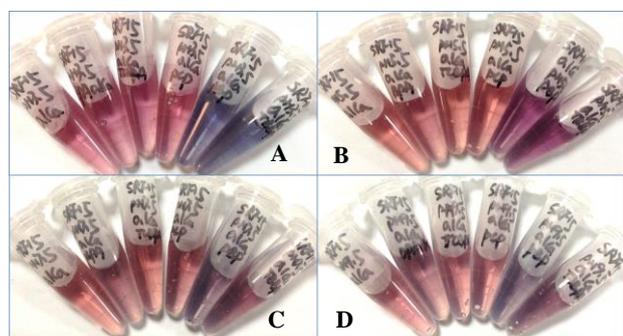


Fig. 2 Photograph of SRF-AuNPs suspension in the presence of targets with 0.1 mM Ca^{2+} at pH 3.5 (A), pH 5.5 (B), pH 7.5 (C), pH 9.5 (D), from left to right: Control, BPA, TCBPA, PCP, PBP, and TBBPA, Concentration of targets: 10 μM .

We detect the solution pHs of the sensor suspension after the sorption of these targets. The fluctuation of pH is negligible in all cases, indicating that the aggregation of sensors triggered by BPA, PBP and TBBPA is not related with change of solution pH. Previous studies suggest that the affinity of BPA and halogenated phenols to NOM proceed via van der Waals interactions, π - π stacking interactions or hydrophobic interactions.^{2,3} For the AuNPs based colorimetric assay, the sensors can be linked together by these nonspecific interactions among the sorbed targets or between sorbed targets and ligands on AuNPs surface.⁷ In the current study, the surface of SRF-AuNPs sensors are highly negative (ζ -potentials of sensors suspension range from -17.5 to -62.2 mV at pH 1.6-11, Fig. S9). The repulsive interactions among the negatively charged oxygen-containing groups of NOM will prevent the sensors from aggregation in water if the affinity of targets hardly shields the negative charges of SRF-AuNPs. On the other hand, the behavior of SRF-AuNPs sensors in acid and Ca^{2+} solution indicates that the interactions

with functional groups of NOM can trigger the aggregation of sensors. Therefore, we deduce that sensors aggregation is caused by specific interactions between target and oxygen-containing groups of NOM.

The molecular structures of SRF and targets are listed in Fig. 3. The lack of response of sensors to PCP and TCBPA suggests the absence of specific interactions with NOM. To verify the interaction mechanisms between the selected targets with NOM, SRF-AuNPs before and after interaction with these targets are analyzed using XPS. In the O1s XPS peaks of sensors with the sorbed phenols, the intensities of C-OH species increase and the sorbed H_2O decrease obviously, suggesting the strong sorption of these hydrophobic targets to SRF-AuNPs sensors (Fig. 4A, O1s peaks of BPA, PCP and PBP sorbed sensors is shown in Fig S10). Compared with the O1s lines of the as-prepared AuNPs sensors and Cl2p lines of TCBPA powder, the binding energies of O=C-O and C=O species and C-Cl bonds do not shift (Fig. 4A and 4B), indicating that the oxidation state of these species is unchanged during sorption, that is, neither the phenolic groups nor the Cl atoms in their molecules bind with polar moieties of NOM.

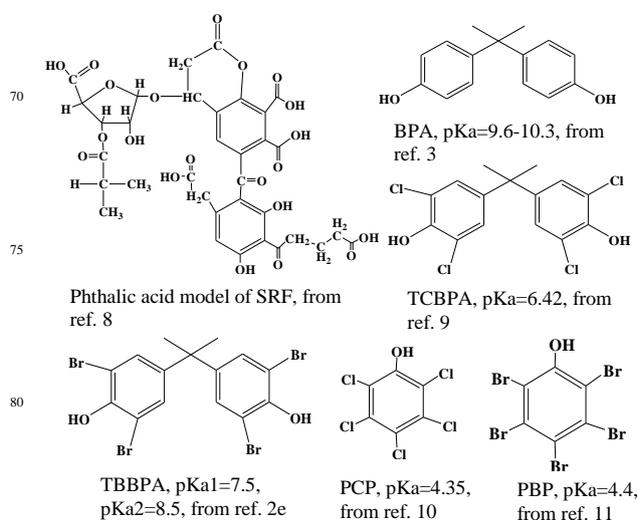


Fig. 3 Molecular structure of Suwannee River fulvic acid and selected targets.

The aggregation of SRF-AuNPs sensors in acid solution caused by PBP and TBBPA implies the possible specific interactions between the brominated phenols and NOM. With the rising of solution pH, more O-containing groups of NOM deprotonate, increasing the negative charges of NOM. The phenolic groups of PBP ($\text{pK}_a=4.4$) and TBBPA ($\text{pK}_a1=7.5$, $\text{pK}_a2=8.5$) begin to deprotonate at $\text{pH} > 4.4$ and 7.5, respectively. Thereby, the sensors show no response to the two targets at $\text{pH} \geq 7.5$ due to the strengthened electrostatic repulsion between anionic brominated phenols and NOM. Ca^{2+} cations can chelate negatively charged carboxylic and phenolic groups of NOM, and the value of ζ -potentials of sensors at pH 6.5-11 increases clearly in 100 μM Ca^{2+} (Fig S9). The reduced repulsive interactions between the negatively charged targets and sensors facilitate the sorption of targets on the surface of sensors in neutral and alkali solution. Therefore, the response of the sensors to PBP and TBBPA regains at pH 7.5-9.5.

The formation of hydrogen bonds (H-bonds) between these brominated phenols and NOM can be ruled out, because the H-bonds are absent between the corresponding chlorinated phenols and SRF. In the O1s core-level lines of sensors sorbed with PBP and TBBPA, the binding energies (BEs) of the C=O and O=C-O species show clear upshift, while the Br-C bonds in the Br 3d signal shift to lower BEs (Fig 4A and 4C). These results imply that the Br atoms in brominated phenols might bind polar moieties of NOM, and the formers and latters serve as Lewis acid and Lewis base, respectively. We deduce that halogen bonding (XB), a non-covalent interaction, occurs between the Br atoms and carbonyl and carboxylic groups of NOM by forming C-Br \cdots O=C bonds, which has been reported the existence by Auffinger and De Moliner in protein and nucleic acid structures.¹² Since most XB is unstable in water, this reaction may only happen between sorbed NOM on solid (such as soil and minerals) and brominated phenols. The halogen bonds of charged systems are stronger than those of neutral ones,¹³ therefore, aggregation of SRF-AuNPs sensors caused by the two targets is still significant at pH7.5-9.5.

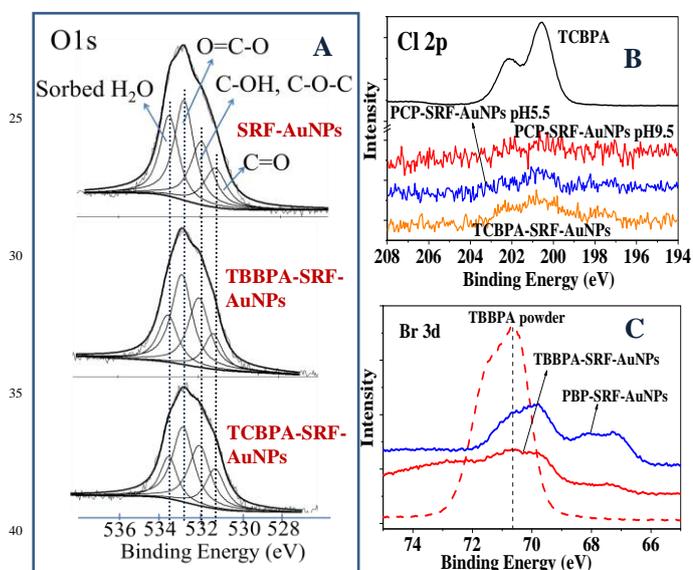


Fig. 4. Fitted XPS O 1s spectra of the as-prepared SRF-AuNPs sensors and sensors after reaction with TBBPA and TCBPA at pH 5.5 (A), Cl 2p spectra of TCBPA powder and TCBPA and PCP sorbed SRF-AuNPs sensors (B), Br 3d core-level lines of TBBPA and TBBPA and PBP sorbed SRF-AuNPs sensors at pH 5.5 (C), Concentration of targets and Ca²⁺: 10 μ M and 0.1 mM, respectively.

To further confirm the specific interactions between brominated phenols and NOM, we detect the response of SRF-AuNPs sensors to monobromophenol (4-BP), dibromophenol (DBP), and tribromophenol (TBP). In 0.1 mM of Ca²⁺ solution, the three phenols can not trigger aggregation of sensors at pH 3.5 (Fig. 5A). Aggregation of sensors caused by TBP, DBP and 4-BP begins at pH 5.5, 7.5 and 9.5, respectively (Fig. 5B-D). This result shows that the response of SRF-AuNPs sensors to brominated phenols is related with the number of substituted Br atoms in molecules. Fig. 5 also proves that the formation of XB between brominated phenols

and NOM is more favorable at higher solution pH.

In acid solution, the absent response of sensors to BPA implies that BPA affinity to NOM takes place via nonspecific interactions. In neutral and weak alkali solution, the slight aggregation of SRF-AuNPs sensors caused by BPA (pKa=9.6-10.3) in the presence of Ca²⁺ suggests the existence of specific interactions (H-bonds) between BPA and polar groups of NOM, which can be confirmed by the BEs shift of O=C-O species in O1s spectra of BPA sorbed sensors (Fig S10). This result is consistent with the assumption of Zhu et al. that the interaction between BPA and dissolved organics occurs primarily by H-bonds and not aromatic interactions in neutral solution.³

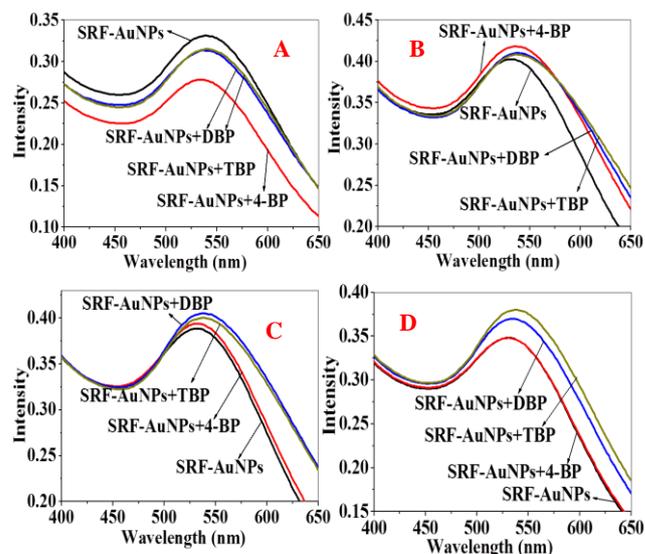


Fig. 5 UV-Vis spectra of the SRF-AuNPs suspension in response to 4-BP, DBP and TBP with 0.1 mM Ca²⁺ at pH 3.5 (A), pH 5.5 (B), pH 7.5 (C), and pH 9.5 (D), concentration of target 10 μ M.

Suwannee River humic acid (SRH), Suwannee River fulvic acid (SRF), Pahokee peat humic acid (PPH), Pahokee peat fulvic acid (PPF), and Lenoardtic humic acid (LH) are also used to modify AuNPs to study the interactions between different kinds of NOM and targets. PPF-AuNPs sensors collides exhibit red-shift in the presence of BPA, PBP, and TBBPA and no change to PCP and TCBPA. The SRH-AuNPs, PPH-AuNPs and LH-AuNPs sensors only respond to PBP and TBBPA, but the shift degree of SPR peaks of the three kinds of sensors is less than those of fulvic acid coated sensors (Fig. S11 and S12). The less sensitivity of the humic acid modified AuNPs sensors to their interactions with targets is possibly due to the high aromaticity which provides more nonspecific interactions than specific interactions to targets.

To investigate the sensitivity of the colorimetric assay to the interactions of these targets with NOM, the concentration of targets change in 0.1-100 μ M. The results show that BPA, TBBPA and PBP respond sensitively to SRF-AuNPs sensors, and color changes of sensors suspension can be observed as the concentration of BPA, PBP, and TBBPA is as low as 1.0, 1.0 and 0.5 μ M, respectively (Fig. S13 and S14). The sensors show no response to PCP and TCBPA as their concentrations

range in 0.1-100 μM .

In summary, we have established a new method to study the possible interaction modes between organic pollutants and NOM using colorimetric assays based on NOM modified AuNPs. The interactions between NOM and targets can be “observed” sensitively according to the color change of AuNPs colloids. Through the colorimetric assays, effect of solution pHs, coexisting cations, molecule structure of targets, and NOM aromaticity on organic pollutants affinity to NOM is detected sensitively. For the first time, we find the possible existence of halogen bonds between NOM and brominated phenols. Overall, the colorimetric assays based on AuNPs sensors may be a potential alternative to monitor the interactions, especially the specific interactions, (including H-bonds, halogen-bonds, cation binding) between targets and ligand on AuNPs surface.

We thank the National Basic Research Program of China (2014CB441102), National High Technology Research and Development Program of China (2013AA065201), National Natural Science Foundation of China (21537004, 21477140, 21321004), and Strategic Priority Research Program of the Chinese Academy of Sciences (XDB01020300) for financial support.

Notes and references

- ^a State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, China. Tel: +86-10-62849182; E-mail: caiyaqi@rcees.ac.cn;
- ^b Department of Resources Environmental and Chemical Engineering of Nanchang University, Jiangxi, Nanchang Province, 330031, China
- † Electronic Supplementary Information (ESI) available: Experimental details and additional figures and tables. See DOI:10.1039/b000000x/
- (1) Chen, W.; Duan, L.; Wang, L. L.; Zhu, D. Q. *Environ. Sci. Technol.* **2008**, *42*, 6862.
- (2) (a) Fei, Y. H.; Li, X. D.; Li, X. Y. *Mar. Pollut. Bull.* **2011**, *63*, 578; (b) Tian, C.; Wang, J. T.; Song, X. L. *Water Air Soil Pollut.* **2009**, *199*, 301. (c) Sun, Z. H.; Yu, Y. J.; Mao, L.; Feng, Z.; Yu, H. X. *J. Hazard. Mater.* **2008**, *160*, 456. (d) Paolis, F. D.; Kukkonen, J. *Chemosphere* **1997**, *34* (8), 1693; (e) Han, W.; Luo, L.; Zhang, S. Z. *Colloid Surf. A: Physicochem. Eng. Asp.* **2013**, *428*, 60.
- (3) Zhu, F.-D.; Choo, K.-H.; Chang, H.-S.; Lee, B. *Chemosphere* **2012**, *87*, 857.
- (4) (a) Deng, J. J.; Jiang, Q.; Wang, Y. X.; Yang, L. F.; Yu, P.; Mao, L. Q. *Anal. Chem.* **2013**, *85*, 9409; (b) Zeng, Z. H.; Mizukami, S.; Kikuchi, K. *Anal. Chem.* **2012**, *84*, 9089; (c) Deng, J. J.; Yu, P.; Wang, Y. X.; Yang, L. F.; Mao, L. Q. *Adv. Mater.* **2014**, *26*, 6933; (d) Zhang, M.; Liu, Y. Q.; Ye, B. C. *Chem. Commun.* **2011**, *47*, 11849; (e) Kim, C.-J.; Lee, D.-I.; Kim, C.; Lee, K.; Lee, C.-H.; Ahn, I.-S. *Anal. Chem.* **2014**, *86*, 3825.
- (5) Liu, X.; Atwater, M.; Wang, J. H.; Hou, Q. *Colloid Surf. B-Biointerfaces* **2007**, *58*, 3.
- (6) Furman, O.; Usenko, S.; Lau, B. L. T. *Environ. Sci. Technol.* **2013**, *47*, 1349.
- (7) (a) Niu, H. Y.; Wang, S. H.; Zhou, Z.; Ma, Y. R.; Ma, X. F.; Cai, Y. Q. *Anal. Chem.* **2014**, *86*, 4170; (b) Shirman, T.; Arad, T.; van der Boom, M. E. *Angew. Chem. Int. Ed.* **2009**, *48*, 1.
- (8) Nantsis, E. A.; Carper, W. R. *J. Mol. Struct.-Theochem* **1998**, *468*, 51.
- (9) Bastos, P. M. Comparison of experimentally and theoretically determined oxidation and photochemical transformation rates of some organohalogenes to promote prediction of persistence. Ph.D. Dissertation, Stockholm University, Stockholm, Sweden, 2009.
- (10) Luo, L. P.; Liu, F. X.; Yue, Q. K.; Chen, F.; Yang, Q.; Hu, B. L.; Chen, Y. X. *Appl. Geochem.* **2013**, *33*, 76.

- ⁶⁵ (11) Kuramochi, H.; Maeda, K.; Kawamoto, K. *Environ. Toxicol. Chem.* **2004**, *23*(6), 1386.
- (12) (a) Auffinger, P.; Hays, F. A.; Westhof, E.; Shing Ho, P. *Proc. Natl. Acad. Sci. USA* **2004**, *101*(48), 16789; (b) De Moliner, E.; Brown, N. R.; Johnson, L. N. *Eur. J. Biochem.* **2003**, *270*, 3174;
- ⁷⁰ (13) Carlsson, A. C. C.; Veiga, A. X.; Erdélyi, M. *Top. Curr. Chem.* **2015**, *359*, 49.