JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

pubs.acs.org/JACS

"NMR Chemosensing" Using Monolayer-Protected Nanoparticles as Receptors

Barbara Perrone, Sara Springhetti, Federico Ramadori, Federico Rastrelli,* and Fabrizio Mancin*

Dipartimento di Scienze Chimiche, Università di Padova, via Marzolo 1, 35131 Padova, Italy

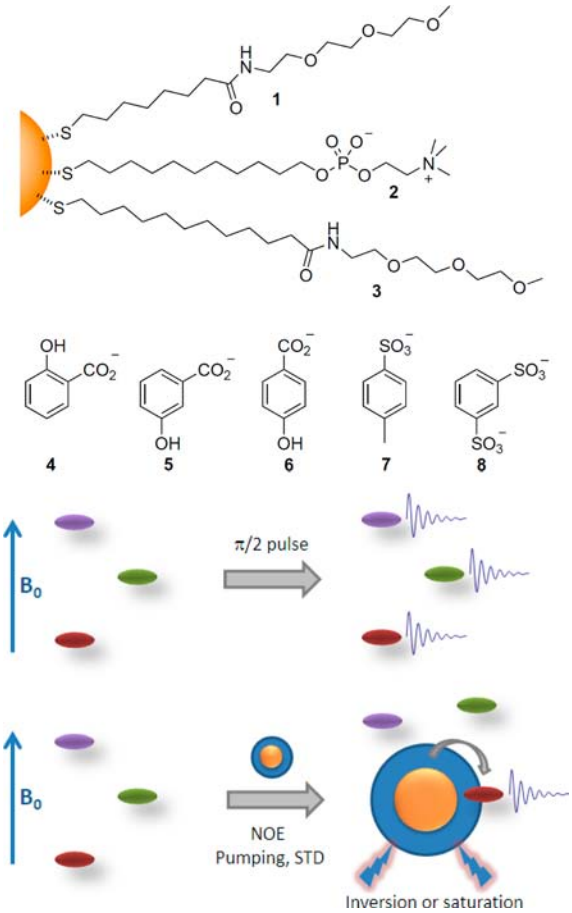
Supporting Information

ABSTRACT: A new sensing protocol based on NMR magnetization transfer sequences and the molecular recognition abilities of nanoparticles allows the detection and identification of organic molecules in complex mixtures.

According to its definition, a chemosensor is "a molecule of abiotic origin that signals the presence of matter or energy".[1] This concept has been commonly translated into a supramolecular receptor that, upon selective binding (recognition) of its target (analyte), undergoes a measurable change in its properties.[2] Such a change, properly monitored, reveals the presence of the analyte in the sample under investigation. Typical molecular properties used for signal generation include luminescence,[3] absorbance,[4] redox potential,[5] relaxivity,[6] and many others. Although several advantages justify the widespread interest in chemosensors, one intrinsic limitation of such an approach is that the reliability of a chemosensor response depends crucially on its selectivity. Indeed, the signal produced arises from a property of the chemosensor itself and therefore does not provide any direct information on the identity of the analyte detected. The user must presume that he is measuring the presence of the desired target rather than a known or unknown interferent because he trusts the recognition ability of the chemosensor. Indeed, the design of new sensing schemes that allow for direct individuation of the target in complex environments represent a major challenge. Our interest in understanding the structure and properties of monolayer-protected gold nanoparticles, particularly using new tools based on NMR spectroscopy,[7] led us to the development of a new sensing protocol that also allows analyte identification and quantification in complex mixtures. Moreover, the results reported here also address the more classic but still actual problem of realizing systems that can detect organic molecules in water, where most receptors fail in efficiently recognizing their targets.

As a test mixture, we selected a group of three water-soluble aromatic compounds with similar sizes and features, namely, sodium salicylate (**4**), sodium *p*-toluenesulfonate (**7**), and disodium benzene-1,3-disulfonate (**8**) (Chart 1). The identification of the single components from a ^{1}H NMR spectrum of this fairly simple mixture (Figure 1a) is not trivial at all, and this is a well-known drawback that heavily limits its usefulness in the case of complex mixtures. However, when we mixed the same sample with gold nanoparticles (2 nm gold core diameter) protected with thiol **1** (Chart 1), we were able to extract just those signals arising from salicylate by means of diffusion-assisted nuclear Overhauser effect (NOE) experiments (NOE pumping; Figure 1b).[8]

Chart 1. (top) Nanoparticle-Coating Thiols and Analytes Used in This Work; (bottom) Working Scheme for Nanoparticle-Based NMR Sensing with NOE Pumping Experiments



In designing this experiment, we had in mind two starting points. First, it is well-known that water-soluble monolayer-protected gold nanoparticles can incorporate hydrophobic molecules in the protecting monolayer.[10] Second, several NMR experiments have been devised to identify compounds with

Received: July 1, 2013
Published: July 26, 2013

   dx.doi.org/10.1021/ja406688a | J. Am. Chem. Soc. 2013, 135, 11768−11771

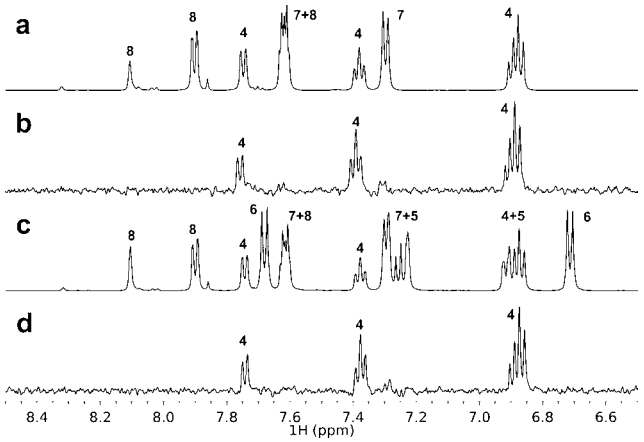


Figure 1. (a) ^{1}H NMR subspectrum of a mixture of molecules **4**, **7**, and **8** (7 mM in D_2O). (b) NOE-pumping subspectrum of the same sample in the presence of **1**-coated gold nanoparticles (70 μM). (c) ^{1}H NMR spectrum of a mixture of molecules **4**–**8** (7 mM in 100 mM carbonate buffer, pD 10).[9] (d) NOE-pumping subspectrum of the sample from (c) in the presence of **1**-coated gold nanoparticles (70 μM).

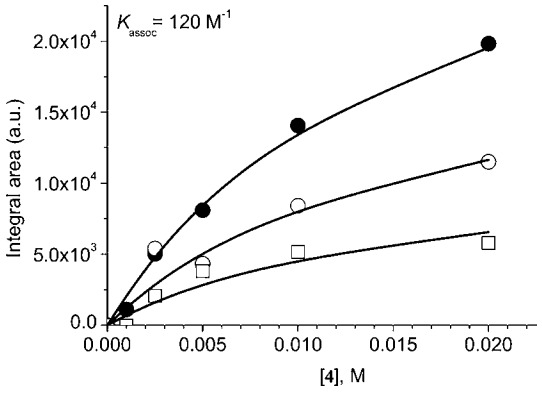


Figure 2. Relative areas of NMR signals of **4** (●, 6.8 ppm; ○, 7.4 ppm; □, 7.75 ppm) in the NOE-pumping experiment as functions of its concentration. Solid lines are best fits to the data. Conditions: [**1**-nanoparticles] = 70 μM, 100 mM carbonate buffer, pD 10.

binding affinity to proteins and other biomacromolecules on the basis of the transfer of net magnetization (or saturation) from large systems to small bound molecules.[8,11] Among these, the NOE-pumping experiment originally proposed by Shapiro[8] looked particularly suitable for our purposes. This experiment consists of two blocks: first, the signals of the small molecules in the sample are canceled (dephased) with a diffusion filter, and second, a NOE experiment is started immediately after. In this way, only the signals of the small molecules interacting with the macromolecule in the fast-exchange regime can be detected, as they arise from the magnetization transferred from the macromolecule, which survives the diffusion filter. Hence, in our experiment the nanoparticles can be considered as "self-organized" supramolecular receptors that recognize their substrate and label it by magnetization transfer, which in turn generates the detected response (Chart 1). It should be noted that the signal produced in this way does not arise from the chemosensor but from the analyte itself, and containing as much information as a NMR spectrum does, it allows not only the detection but also the unambiguous identification of the target.

The selectivity we found using this protocol is somehow surprising. NOE pumping of a water solution containing **1**-coated nanoparticles and the three isomers salicylate (**4**), 3-hydroxybenzoate (**5**), and 4-hydroxybenzoate (**6**), which differ only in the relative positions of the two functional groups, revealed the presence of solely the salicylate signals [see the Supporting Information (SI)]. This selectivity remained unaffected even when all of the compounds **4**–**8** so far studied were mixed together in a single tube with the nanoparticles. Again, only the salicylate signals emerged from the NOE-pumping experiment (Figure 1c,d), with a substantial simplification of the spectrum of the mixture and clear detection of the presence of salicylate in the sample.

It is important to note that besides selective detection, the method reported here also allows quantitative determination of the analyte. In a series of experiments performed with different concentrations of salicylate (Figure 2), we found that the integrals of the analyte signals increased according to a saturation profile, as expected for a binding process. Fitting of the relative intensity data with a 1:1 binding model provided an apparent association constant (K_{assoc}) of 120 ± 5 M^{-1}. This value is comparable to those reported by Pasquato and Lucarini[10] for the association of organic radicals to **1**-coated nanoparticles of similar size and confirms that analyte quantification, upon construction of a calibration curve, is possible with this method. In fact, recalculation of the concentration of salicylate in the independent experiment of Figure 1d using the parameters obtained from the fitting of the data in Figure 2 (see the SI) yielded a value of 6.8 ± 0.4 mM, which is very close to the analytical value (7.0 mM). Under the conditions used (4 h acquisition, 70 μM nanoparticle concentration), the experiment reported in Figure 2 also allowed a detection limit of 2.5 mM for sodium salicylate to be established.

The effective binding of salicylate to the nanoparticles was confirmed by selective NOE experiments (see the SI). Upon selective inversion of the nanoparticle resonances, we observed that the signals arising from salicylate displayed clear NOEs, confirming the direct interaction of the two species. The NOE intensities appeared to be larger when the signals relative to the alkyl chains of thiol **1** were inverted. This observation suggests that salicylate is probably localized in the inner, hydrophobic, region of the monolayer.

The remarkable selectivity obtained with **1**-coated nanoparticles should therefore arise from the different hydrophobicities of the five mixture components. As a matter of fact, computationally predicted *n*-octanol/water partition coefficients at pH 7.4 (logD) follow the order **4** (−1.14) > **6** (−1.35) ≈ **5** (−1.47) > **7** (−2.57) ≫ **8** (−7.14),[12] with **4** being the most hydrophobic of the series. A similar correlation was obtained by submitting the mixture of the five compounds to HPLC separation using a C18 reversed-phase column (see the SI). Here, the order of elution times was: **8** ≪ **7** < **6** < **5** ≪ **4**. These observations indicate that the **1**-coated nanoparticles act as selective receptors for hydrophobic molecules, with a threshold that can be set at calculated logD (pH 7.4) values around −1.2 or HPLC retention times around 9–10 min under the adopted conditions.

However, other structural parameters influence the recognition process. One of the main advantages brought about by the use of nanoparticles as detection agents is the ease of their modification. Indeed, the use of nanoparticles coated with a different thiol, namely, phosphorylcholine derivative **2**, led to a

dx.doi.org/10.1021/ja406688a | J. Am. Chem. Soc. 2013, 135, 11768–11771

different selectivity. When used to analyze the mixture of **4**, **7**, and **8** by NOE pumping, these nanoparticles were able to reveal the presence of not only **4** but also **7** and **8** as well as (with remarkable sensitivity) an impurity contained in the latter (Figure 3b). Hence, a change in the features of the

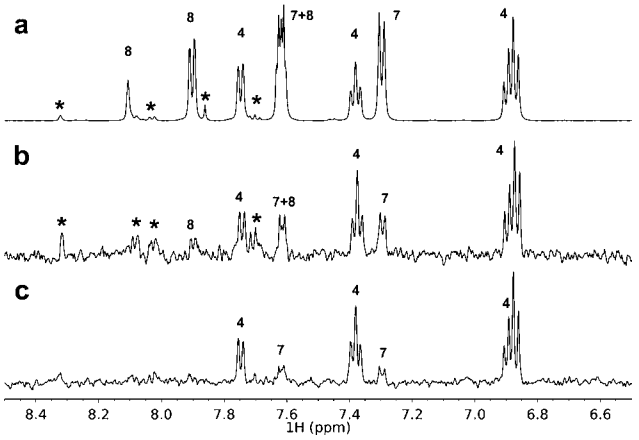


Figure 3. (a) ^{1}H NMR subspectrum of a mixture of molecules **4**, **7**, and **8** (7 mM in D_2O). (b) NOE-pumping subspectrum of the same sample in the presence of **2**-coated gold nanoparticles (70 μM). (c) NOE-pumping subspectrum of the same sample in the presence of **3**-coated gold nanoparticles (70 μM). The asterisks identify the signals of an impurity in compound **8**.

nanoparticle-coating monolayer led in this case to a broadening of the affinity toward different molecules. To identify the features of the coating thiol **2** that were responsible for such an affinity modification, as **2** features both a longer alkyl chain (11 carbon atoms) and a different headgroup (phosphoryl choline), we synthesized thiol **3** featuring an 11-carbon alkyl chain as in **2** and a triethyleneglycol headgroup as in **1**. The results of NOE-pumping experiments performed with **3**-coated nanoparticles (Figure 3c) revealed a selectivity in between that of the **1**- and **2**-coated nanoparticles, indicating that both the size of the hydrophobic region in the coating monolayer and the specific interactions with the thiol headgroup may affect the binding affinity, a well-known phenomenon for HPLC stationary phases. Such observations also indicate that hydrophobicity is not the only source of recognition. In fact, although hydroxybenzoates **5** and **6** are more hydrophobic than *p*-toluensulfonate **7**, they were not associated with the nanoparticles and hence were not revealed by the NOE-pumping experiment (see the SI). It is likely that an amphiphilic structure, with quite well-defined hydrophilic and hydrophobic regions, is required for the inclusion of the analyte in the monolayer.

Since there are no limitations on the chemical structures of the analytes and the nanoparticle-coating thiols, the NOE-pumping experiment has the advantage of very general applicability. However, we found that in some cases the experimental times and detection limits of the protocol can be substantially improved by using saturation transfer difference (STD) experiments.[11] While conceptually similar to an NOE experiment, the STD experiment provides stronger signals because saturation can be driven for longer periods compared with typical NOE mixing times. The final result is that the intensities of the signals stemming from interacting molecules decrease, allowing these signals to be revealed by subtraction from a reference spectrum. The use of STD is, however, limited by the requirement that there must be no overlap between the signals of the analyte and the macromolecule. Salicylate and **1**-coated nanoparticles meet such conditions, allowing us to cut the acquisition times down to 30 min and reduce the detection limit to 250 μM using STD experiments (see the SI).

Finally, we decided to test our nanoparticle-based NMR sensing protocol in a situation as challenging as the analysis of drug metabolites in urines. Indeed, NMR-based metabolomics is an area of utmost interest that could substantially benefit from any protocols that would expand the amount of information attainable.[13] In this framework, we added **1**-coated nanoparticles to a human urine sample containing 5 mM sodium salicylate, a concentration similar to that found in urines after medium-dose administration of acetylsalicylic acid.[14] Clean detection of salicylate in the ^{1}H NMR spectrum of the urine sample (Figure 4a) was quite difficult, since its

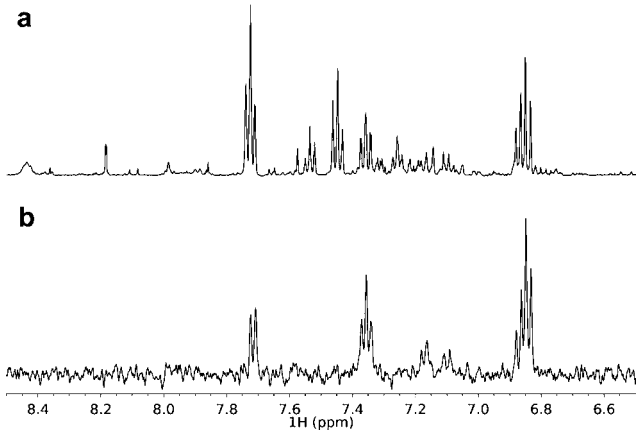


Figure 4. (a) ^{1}H NMR subspectrum of human urine containing 5 mM sodium salycilate. (b) NOE-pumping subspectrum of the same sample in the presence of **1**-coated gold nanoparticles (70 μM).

signals were mixed with many others having similar intensities and chemical shifts in the aromatic region (e.g., those of hippuric acid). On the other hand, the NOE-pumping experiment canceled out the whole bunch of matrix signals, leaving only those of salicylate (Figure 4b) and allowing for a clear and unambiguous detection of the target molecule. A similar experiment (see the SI) also allowed us to reveal and identify another metabolite of acetylsalicylic acid, salicyluric acid, that is excreted in urines under different physiological conditions.

To our knowledge, all of the NMR-based chemosensors reported to date follow the "classical" approach, in which the recognition causes modifications of a chemosensor property, such as the ability to affect the water relaxivity[6] or the chemical shift of a receptor's heteronucleus.[15] In this way, most of the meaningful information associated with NMR data is lost. More similar to our approach is "chromatographic NMR spectroscopy", where interactions with a stationary phase are used to perturb the diffusion coefficients of the sample components in such way that the NMR signals can be separated by means of a diffusion-ordered NMR spectroscopy (DOSY) experiment.[16] However, besides the intrinsic difficulty of spreading the diffusion rates with a chromatographic medium, this approach suffers from limitations such as the need for high resolution in both the frequency and diffusion domains as well as nontrivial spectral inversion problems. The "NMR chemosensing" approach introduced here retains all of the structural

dx.doi.org/10.1021/ja406688a | J. Am. Chem. Soc. 2013, 135, 11768−11771

information provided by NMR spectroscopy, can be very easily implemented on standard instruments, and can benefit from the features of monolayer-protected nanoparticles, which can be easily tailored to meet the recognition requirements of different classes of analytes.[17]

■ ASSOCIATED CONTENT

Supporting Information

Synthesis and characterization of the organic compounds and nanoparticles and results of additional NMR experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

federico.rastrelli@unipd.it; fabrizio.mancin@unipd.it

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was funded by the ERC Starting Grants Project MOSAIC (Grant 259014). The authors acknowledge Davide Zaramella and Massimo Bellanda for assistance with HPLC and STD measurements, respectively, and Moreno Lelli and Giulio Tognin for helpful discussions.

■ REFERENCES

(1) *Fluorescent Chemosensors for Ion and Molecule Recognition*; Czarnik, A. W., Ed.; ACS Symposium Series *538*; American Chemical Society: Washington, DC, 1993.

(2) Bissell, R. A.; De Silva, A. P.; Gunaratne, H. Q. N.; Lynch, P. L. M.; Maguire, G. E. M.; Sandanayake, K. *Chem. Soc. Rev.* **1992**, *21*, 187−195.

(3) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515−1566.

(4) Xu, Z.; Chen, X.; Kim, H. N.; Yoon, J. *Chem. Soc. Rev.* **2010**, *39*, 127−137.

(5) Oton, F.; Tarraga, A.; Espinosa, A.; Velasco, M. D.; Molina, P. *J. Org. Chem.* **2006**, *71*, 4590−4598.

(6) McRae, R.; Bagchi, P.; Sumalekshmy, S.; Fahrni, C. J. *Chem. Rev.* **2009**, *109*, 4780−4827.

(7) (a) Rastrelli, F.; Jha, S.; Mancin, F. *J. Am. Chem. Soc.* **2009**, *131*, 14222−14223. (b) Guarino, G.; Rastrelli, F.; Mancin, F. *Chem. Commun.* **2012**, *48*, 1523−1525. (c) Guarino, G.; Rastrelli, F.; Scrimin, P.; Mancin, F. *J. Am. Chem. Soc.* **2012**, *134*, 7200−7203.

(8) Chen, A.; Shapiro, M. J. *J. Am. Chem. Soc.* **1998**, *120*, 10258−10259.

(9) This experiment was performed using a carbonate buffer to prove that the particle selectivity is not affected under high-ionic-strength conditions.

(10) (a) Lucarini, M.; Franchi, P.; Pedulli, G. F.; Pengo, P.; Scrimin, P.; Pasquato, L. *J. Am. Chem. Soc.* **2004**, *126*, 9326−9329. (b) Lucarini, M.; Franchi, P.; Pedulli, G. F.; Gentilini, C.; Polizzi, S.; Pengo, P.; Scrimin, P.; Pasquato, L. *J. Am. Chem. Soc.* **2005**, *127*, 16384−16385. (c) Pezzato, C.; Lee, B.; Severin, K.; Prins, L. J. *Chem. Commun.* **2013**, *49*, 469−471.

(11) Mayer, M.; Meyer, B. *Angew. Chem., Int. Ed.* **1999**, *38*, 1784−1788.

(12) Data from the Chemspider database.

(13) DeFeo, E. M.; Wu, C. L.; McDougal, W. S.; Cheng, L. L. *Nat. Rev. Urol.* **2011**, *8*, 301−311.

(14) MacPherson, C. R.; Milne, M. D.; Evans, B. M. *Br. J. Pharmacol.* **1955**, *10*, 484−489.

(15) Kitamura, M.; Suzuki, T.; Abe, R.; Ueno, T.; Aoki, S. *Inorg. Chem.* **2011**, *50*, 11568−11580.

(16) (a) Pages, G.; Delaurent, C.; Caldarelli, S. *Angew. Chem., Int. Ed.* **2006**, *45*, 5950−5953. (b) Reddy, G. N. M.; Ballesteros-Garrido, R.; Lacour, J.; Caldarelli, S. *Angew. Chem., Int. Ed.* **2013**, *52*, 3255−3258.

(17) Perrone, B.; Springhetti, S.; Ramadori, F.; Rastrelli, F.; Mancin, F. Patent application PD2013A000153 (30/5/2013).