

A fluorescence nanosensor for Cu²⁺ on silica particles†Elena Brasola,^a Fabrizio Mancin,^a Enrico Rampazzo,^a Paolo Tecilla*^b and Umberto Tonellato*^a^a Dipartimento di Chimica Organica and Istituto CNR di Tecnologia delle Membrane – Sezione di Padova, Università di Padova, via Marzolo 1, I-35131 Padova, Italy. E-mail: umberto.tonellato@unipd.it; Fax: +39 0498275239; Tel: +39 0498275269^b Dipartimento di Scienze Chimiche, Università di Trieste, via Giorgieri 1, I-34127, Italy. E-mail: tecilla@dsch.univ.trieste; Fax: +39 0405583903; Tel: +39 0405583925

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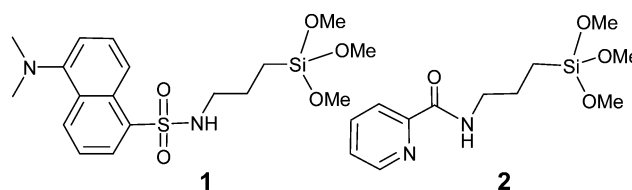
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A fluorescence nanosensor for Cu²⁺ ions has been obtained by surface functionalization of silica particles with trialkoxysilane derivatized ligand and fluorescent dye.

The interest in fluorescence chemosensors is continually growing, as they provide a sensitive and selective method to recognize and evaluate the concentration of different substrates.¹ In their typical design, these systems are composed of a substrate binding unit and one or more photoactive components which generate the fluorescence signal. However, the synthetic efforts involved in their realization can be demanding. Self-organization of receptors and fluorescent dyes to form organized assemblies can, at least partially, overcome this problem by providing an efficient strategy for the easy realization and optimization of fluorescence chemosensors. After our first report on a self-organized fluorescent chemosensor for Cu²⁺ in micellar aggregates,² analogous strategies, based on Langmuir–Blodgett films³ and trialkoxysilane self-assembled monolayers on quartz surfaces,⁴ have been successfully explored.⁵ However, the real applicability of such systems is limited by several factors. In particular, surfactant aggregates and Langmuir–Blodgett films are delicate objects which form only in particular conditions, while the limited surface area of quartz glasses does not allow the generation of a strong fluorescence signal. Modification of nanoparticle surfaces offers an attractive approach to the realization of organized assemblies of functional subunits and chemosensors supported on nanoparticles would not suffer from the previous limitations.⁶ Silica nanoparticles are particularly suitable for the realization of fluorescence chemosensors as they are transparent and photophysically inert. Moreover, a recent study on dansyl functionalized silica nanoparticles reported evidence that collective processes can arise from the organization of the components in an extended network.⁷

We here describe the first example of a self-organized fluorescence chemosensor for Cu²⁺ ions obtained by surface functionalization of silica nanoparticles.⁸ Mixed dye and ligand coated silica nanoparticles (CSNs) in different ratios were prepared, following reported procedures,⁷ via the reaction of commercially available silica nanoparticles (Aldrich AS-30) with the trimethoxysilane derivatized dansylamide **1** and picolinamide **2**. The picolinamide subunit was chosen as metal binding subunit as it was reported to act as selective Cu²⁺ ligand via the deprotonation of the amidic group.⁹ The coated nanoparticles are dispersible in non-polar organic solvents (CH₂Cl₂, CHCl₃) and in 10% water/DMSO mixtures. ¹H-NMR analysis showed the signal broadening typical of immobilized subunits on the nanoparticles surfaces and allowed to determine the ratio of the two components (see Supplementary Information†).

CSNs characterized by different **1** : **2** ratios, hereafter defined by the ligand molar fraction $\chi = 2/(1+2)$, are simply obtained by modulating the concentrations of **1** and **2** in the reacting



mixture used to functionalize the silica nanoparticles. The UV-vis and fluorescence spectra are typical of the dansyl chromophore (see Supplementary Information†). Fluorescence anisotropy values, measured in CHCl₃, were in the range 0.04–0.08 and indicate a slow rotational relaxation of the fluorophore due to the immobilization on the nanoparticle surface.⁷ The particle size (average 18 nm, with $\sigma = 3$ nm) was investigated by transmission electron microscopy (TEM, Fig. 1 and Supplementary Information†). As a rough estimate, each coated nanoparticle may bear up to 10⁴ attached subunit molecules.

Fig. 2a shows the variation of the fluorescence emission of solutions of nanoparticles with different χ values (0.003 mg ml⁻¹ in 1:9 buffered water (HEPES pH 7.0)/DMSO solutions) after addition of Cu(NO₃)₂. In any case, the metal ion induces an almost complete (down to a plateau of about 5% of the initial emission) quenching of the fluorescence emission of CSNs.

Fluorescence quenching is due to the binding of the Cu²⁺ ions to the nanoparticles, as no changes of the emission are observed after addition of Cu²⁺ to a CSNs solution in the presence of

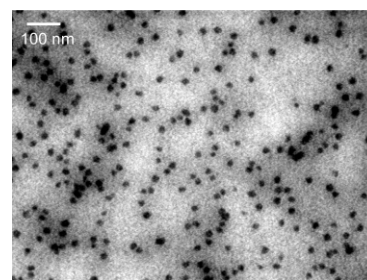


Fig. 1 TEM image of the CSNs with a χ value of 0.93.

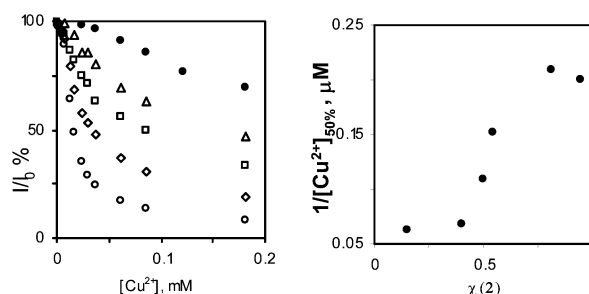


Fig. 2 (a) Spectrofluorimetric titration of CSNs (0.03 mg ml⁻¹) with Cu(NO₃)₂ in 10% water/DMSO, HEPES buffer 0.01 M pH 7, 25 °C, $\lambda_{exc} = 340$ nm, $\lambda_{em} = 520$ nm (●: $\chi = 0$; △: $\chi = 0.15$; □: $\chi = 0.4$; ◇: $\chi = 0.55$; ○: $\chi = 0.81$). (b) $1/[Cu^{2+}]_{50\%}$ as a function of **2** molar fraction on the CSNs ($[2] = 2 \mu\text{M}$, 10% water/DMSO, HEPES buffer 0.01 M pH 7, 25 °C).

† Electronic supplementary information (ESI) available: experimental procedure; TEM images; NMR, UV-vis and fluorescence spectra; fluorescence titration. See <http://www.rsc.org/suppdata/cc/b3/b310582b/>

excess of EDTA as a competitive ligand. In the case of CSNs made with only **1**, a modest fluorescence decrease is observed after Cu²⁺ addition (Fig. 2a, ●). A similar effect is obtained by adding Cu²⁺ to a *N*-propyl-dansylamide solution indicating that in this solvent system the dansylamide unit alone can provide weak Cu²⁺ binding sites. However, the presence of ligand **2** is necessary to achieve a strong binding of the substrate and authenticate the CSNs as sensors. Indeed, it is the grafting of the sensor components on the nanoparticles surfaces that ensures the spatial proximity necessary to the communication between the subunits. This is clearly shown by the fact that addition of Cu²⁺ to solutions containing both *N*-propyl-dansylamide and *N*-propyl-picolinamide leads to a fluorescence quenching profile identical to that obtained for the *N*-propyl-dansylamide alone.

The sensitivity of the CSNs depends on both the pH control (use of buffer) and, most notably, on the ligand to dye ratio on the nanoparticles surface. Addition of Cu²⁺ to unbuffered CSNs solutions results in incomplete fluorescence quenching and this is related to the increase of the acidity of the solution. In fact, the uptake of the metal ion by the sensor implies the release of protons and this, in turn, hampers the deprotonation of the amidic group of the **2** subunit and weakens its ligand ability. On the other hand, inspection of Fig. 2a clearly reveals that the sensitivity of the systems increases as the χ value increases. CSNs with a **2** to **1** ratio close to unity can detect Cu²⁺ in the micromolar range: using nanoparticles with a χ value of 0.86, a 10% quenching of the fluorescence was observed in the presence of a 4 μ M concentration of Cu²⁺. This behavior is expected by taking into account that, at a constant nanoparticle concentration, the increase of the χ value implies an increase of the total concentration of ligands in solution and, as a consequence, a larger extent of binding of the metal ions.

However, cooperative effects also seem to play an important role. This is highlighted by the results of experiments carried out on nanoparticles solutions with different χ values but with constant overall concentration of ligand **2**. If the Cu²⁺ concentration necessary to reduce the fluorescence emission at 50% of its initial value is taken as a reference value, the reciprocal $1/([Cu^{2+}]_{50\%})$ is a quantitative indication of sensitivity. The plot of such quantity against χ (Fig. 2b) shows that the CSNs with large χ values have the best efficiency as sensors of the metal ion. The sigmoidal shape of the profile clearly indicates cooperation between the ligands subunits. In fact, the Cu²⁺ affinity of the ligand subunits improves as their relative density on the CSNs surface increases. Quite likely, this can be ascribed to the surface-organization of the **2** subunits that may lead to the formation of multivalent binding sites (e.g. with 2 : 1 or 3 : 1 ligand to metal stoichiometries) with a greater Cu²⁺ affinity.¹⁰

The selectivity of the CSNs with regard to other divalent metal ions, such as Ca²⁺, Mg²⁺, Zn²⁺, Cd²⁺, Co²⁺, Ni²⁺, Fe²⁺ and Pb²⁺, was evaluated in competition experiments as reported in Fig. 3. In the presence of a 50 μ M concentration of the different cations, no effect on the fluorescence of the CSNs is observed, with the only exception being Ni²⁺, which brings

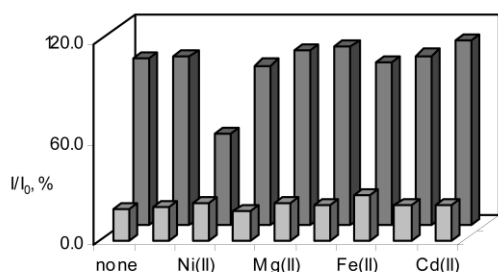


Fig. 3 Relative fluorescence intensity of CSNs ($\chi = 0.86$, 0.03 mg ml^{-1}) in the presence of the indicated metal ions alone ($50 \mu\text{M}$, back row) and with Cu²⁺ ($50 \mu\text{M}$, front row). 10% water/DMSO, HEPES buffer 0.01 M pH 7, $25 \text{ }^\circ\text{C}$, $\lambda_{\text{exc}} = 340 \text{ nm}$, $\lambda_{\text{em}} = 520 \text{ nm}$.

about a 45% decrease of the emission.¹¹ In contrast, $50 \mu\text{M}$ of Cu²⁺ quenches the emission of CSNs to 20% of the initial value. The same effect is observed after the addition of Cu²⁺ to the solutions containing each other metal ion, thus showing that they do not interfere with the Cu²⁺ determination. Moreover, titration with Cu²⁺ of solutions containing the CSNs and all the metal ions (but not Ni²⁺) produces a quenching profile very close to that obtained in the absence of any ion (Supplementary Information†).

In summary, we have shown that silica nanoparticles can be effectively employed as template for the self-organization of fluorescence chemosensors. Grafting of the ligand and the dye subunits to the nanoparticles surface not only ensures the inter-component communication in the sensor, but also induces cooperative processes in the binding of the substrate. Tuning of the sensor sensitivity can be easily obtained by changing the ligand to dye ratio and allows the determination of the substrate in different concentration intervals. Moreover, with respect to other systems^{3,4} used for the realization of self-organized chemosensors CSNs are easier to prepare and to handle. Particularly, they are chemically and physically stable and, therefore, more suited for real practical applications. A large number of chemosensors with different substrate affinity or photophysical properties can be realized by simply changing the trimethoxysilane derivatives employed in the functionalization of CSNs. Work is in progress in our laboratory.

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Notes and references

- (a) Special Issue, Luminescent Sensors, *Coord. Chem. Rev.*, 2000, **205**; (b) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515–1566; (c) *Fluorescent Chemosensors for Ion and Molecule Recognition*; A. W. Czarnik, Ed.; ACS Symposium Series 538; American Chemical Society, Washington DC, 1993.
- (a) P. Grandini, F. Mancin, P. Tecilla, P. Scrimin and U. Tonellato, *Angew. Chem. Int. Ed.*, 1999, **38**, 3061–3064; (b) M. Berton, F. Mancin, G. Stocchero, P. Tecilla and U. Tonellato, *Langmuir*, 2001, **17**, 7521–7528.
- Y. Zheng, J. Orbulescu, X. Ji, F. M. Andreopoulos, S. M. Pham and R. M. Leblanc, *J. Am. Chem. Soc.*, 2003, **125**, 2680–2686.
- M. Crego-Calama and D. N. Reinhoudt, *Adv. Mater.*, 2001, **13**, 1171–1174.
- An alternative approach to the realization of self-assembled chemosensors, called “chemosensing ensemble”, has been proposed and involves the displacement of a non covalent dye-receptor complex by the substrate. See for example: S. L. Wiskur, H. Ait-Haddou, J. J. Lavigne and E. V. Anslyn, *Acc. Chem. Res.*, 2001, **34**, 963–972; M. A. Hortalà, L. Fabbrizzi, N. Marcotte, F. Stomeo and A. Taglietti, *J. Am. Chem. Soc.*, 2003, **125**, 20–21.
- Selected reviews: (a) R. Shenhar and V. M. Rotello, *Acc. Chem. Res.*, 2003, **36**, 549–561; (b) C. M. Niemeyer, *Angew. Chem. Int. Ed.*, 2001, **40**, 4128–4158; (c) For examples of sensors supported on microparticles see: P. Buhlmann, E. Pretsch and E. Bakker, *Chem. Rev.*, 1998, **98**, 1593–1687; (d) S. Peper, I. Tsagkatakis and E. Bakker, *Anal. Chim. Acta*, 2001, **442**, 25–33.
- M. Montalti, L. Prodi, N. Zaccheroni and G. Falini, *J. Am. Chem. Soc.*, 2002, **124**, 13540–13546.
- Monolayers formed by trialkoxysilane derivatives on glass or silica surfaces are not strictly “self-assembled” or “self-organized”, as the grafting to the surface is covalent and not reversible, but they are included in this category since they share many features with the “true” self-assembled monolayers (see for example: A. Ulman, *Chem. Rev.*, 1996, **96**, 1533–1554).
- H. L. Conley and R. B. Martin, *J. Phys. Chem.*, 1965, **69**, 2914–2923.
- For a recent study on the “surface chelate effect” in the binding of Cu²⁺ to 16-mercaptohexadecanoic monolayers on gold surfaces see: R. C. Major and X.-Y. Zhu, *J. Am. Chem. Soc.*, 2003, **125**, 8454–8455.
- The ability of picolinamide to bind Ni²⁺ with lower affinity than Cu²⁺ has been described. See ref. 9.