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Metal-chelating nanoparticles as selective fluorescent sensor for Cu^{2+}†

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A fluorescent sensor for Cu^{2+} at the nanomolar level in water has been designed by associating a BODIPY fluorophore and a selective ligand (cyclam) in ultrafine polymer nanoparticles.

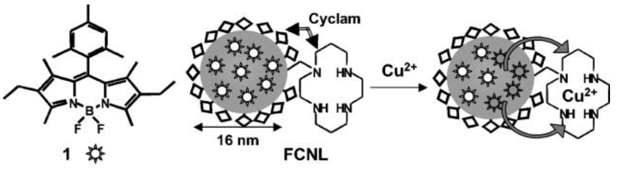
The design of sensors that can selectively recognize and signal the presence of a specific analyte is an important goal and there is nowadays a great demand for new sensors that can be used in complex aqueous media encountered in biology and environmental monitoring.[1] Fluorescent, highly sensitive, chemosensors have been widely studied. They typically combine two covalently linked components: a recognition site that binds the target substrate and a transducer (chromophore) that signals the binding.[2,3] Multi-fluorophoric macromolecules are attracting increasing attention because the fluorescence modulation upon recognition of a given target can be greatly improved in organized structures when cooperative photophysical processes can occur.[4,5] The assembly of fluorophores or fluorophores and probes in close proximity in micellar aggregates,[6] monolayers[5] and very recently at the surface of silica nanoparticles[7] have been found to ensure communication between active components without the need for any covalent link between them. Our goal was to design selective chemosensors, usable in complex aqueous media, by assembling a fluorophore and a probe in nanometer-sized polymer particles. The choice of ultrafine polymer particles, in the 15–20 nm diameter range, as three-dimensional scaffolds originates from the following reasons: (1) Dye molecules entrapped within a polymer matrix are usually protected from side reactions or quenching and conventional fluorescent-polymer microspheres are widely used for a variety of applications.[8,9] Moreover, owing to their small size, polymer nanoparticles are suitable for intracellular measurements and sensing.[9] (2) Polymer nanoparticles are readily synthesized by polymerisation in microemulsions, a versatile technique that allows one to control the size and permits the introduction of various chemical functionalities.[10,11] (3) The high surface-to-volume ratio of nanometer-sized particles provides a remarkable ligand accessibility[10] and their restricted volume should ensure multiple interactions between the sensing components. (4) Aqueous suspensions of nanoparticles are translucent (very weak light scattering) and remarkably stable, two points that are of great importance for optical sensing applications.

Herein we describe the concept of associating an efficient fluorescent reporter, a BODIPY derivative **1**, and an excellent metal-chelating receptor, cyclam,‡ in polymer nanoparticles (Scheme 1). The hydrophobic dye is entrapped within the particle core and the receptor, covalently linked to the polymer backbone, is mainly located near the surface. The overall intention is to produce supramolecular sensors that selectively and rapidly respond to metal ions in aqueous medium. The transduction is based on the quenching of fluorescence by energy transfer from the dye to the receptor-target complex that occurs when the absorption band of the complex overlaps the emission band of the dye. Since the absorption of ligand–metal complexes depends on the nature of the coordinated ion, this modular approach is expected to be quite general and applicable for a wide range of targets provided that the dye is properly chosen. In this study, the two components have been chosen to design a selective sensor for Cu^{II}.

The mesityl BODIPY dye **1** was chosen in order to impart high fluorescence quantum yields and an appropriate emission wavelength.[3] Cyclam-functionalized nanoparticles were readily prepared in one-step by copolymerisation of vinylbenzylcyclam in an oil-in-water microemulsion.[12] The obtained aqueous colloidal suspension, so-called nanolatex **CNL**, is stable and translucent with an average particle diameter of 16 nm. The affinity of cyclam for transition metal ions is retained after anchoring onto nanoparticles and **CNL** readily binds Zn^{II}, Ni^{II}, Co^{II} and Cu^{II} with a marked selectivity for cupric ions.‡§ **CNL** has a Cu^{II} binding capacity of 0.54 mmol g^{-1} of polymer (about 700 Cu per particle). Spectrophotometric titrations of the violet Cu^{II}-cyclam complex upon addition of a dilute copper nitrate solution indicate that about 85% of the overall cyclam moieties (*i.e.* 580–600 per particle) are readily accessible for complexation in a rapid solution-like process.[12]

The fluorophore **1** was entrapped in the polymer particles by impregnation. Spectroscopic studies were performed on a fluorescent metal-complexing nanolatex **FCNL** containing 59 μmol dye **1** g^{-1} of polymer; that is about 75 molecules per particle, and an overall content of polymer of 40 mg L^{-1} (*i.e.* 2.2×10^{-5} mol cyclam L^{-1}). The doped, red-colored, **FCNL** is colloidally stable and no leakage of **1** is observed over a long period of time. The fluorescence properties of **FCNL** (λ_{abs} = 528.5 nm, $\lambda_{emission}$ = 540 nm, fluorescence yield 77%) are very close to those of **1** in dichloromethane (λ_{abs} = 526 nm, $\lambda_{emission}$ = 535 nm, fluorescence yield 72%) thus indicating that the dye is embedded within the hydrophobic polymer matrix. The fluorescence lifetime in **FCNL** is 6.5 ns, compared with 6.1 ns in CH_2Cl_2 or 5.8 ns in EtOH. Moreover the comparison of the fluorescence decay curves of **FCNL** and **1** in aqueous micellar solution, shown in Fig. 1, clearly indicates that the dye is protected against water and intermolecular quenching when encapsulated within the particle (the mean lifetime is 2.9 ns in micellar solution).

Cyclam-functionalized nanoparticles **CNL** bind cupric ions with an absorption band at 535–550 nm, characteristic of the Cu^{II}/ligand complex, that overlaps the emission band of the entrapped dye **1**. Upon addition of Cu^{2+} to **FCNL**, an instantaneous decrease of the fluorescence intensity is observed, thus indicating that quenching by energy transfer between the complex and the dye readily occurs in



Scheme 1

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† Electronic supplementary information (ESI) available: experimental procedures; characteristics of nanolatex **FCNL**. See http://www.rsc.org/suppdata/cc/b4/b407766k/

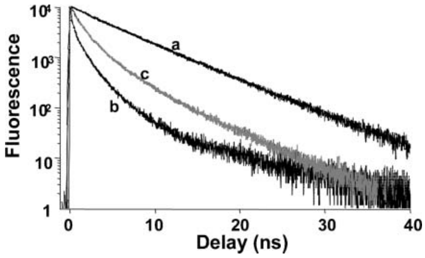

Fig. 1 Fluorescence decay curves (**a**) **FCNL**; (**b**) **1** in aqueous micellar solution (80 µl of a 5 g L^{-1} solution of **1** in CH_2Cl_2 in 2 ml of a 15 wt% solution of DTAB in water); (**c**) **FCNL** plus $Cu(NO_3)_2$, 10^{-6} M.

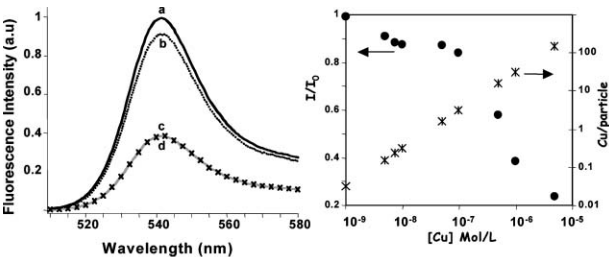

Fig. 2 Fluorescence emission spectra, λ_{exc} = 495 nm, left. (**a**) **FCNL**, (**b**) **FCNL** plus $Cu(NO_3)_2$, 5×10^{-9}M, (**c**) **FCNL** plus $Cu(NO_3)_2$, 10^{-6}M, line (**d**) **FCNL** plus $Cu(NO_3)_2$ 10^{-6}M and $Ni(NO_3)_2$, 10^{-3}M, crosses. Variation of I/I_0 upon addition of $Cu(NO_3)_2$ to **FCNL**, right.

the particles (Fig. 2).¶ The intensity decreases as the concentration of Cu^{2+} is increased. **FCNL** detects Cu^{2+} in the nanomolar range: 10% quenching is observed in the presence of 5×10^{-9} M Cu^{2+}, which corresponds to less than one Cu^{II} per particle (statistically only 16% of the particles contain a Cu^{2+} ion). At this concentration, about 44 dyes are estimated to be quenched per copper-containing particle, thus indicating that cooperative processes take place in nanoparticles. A sharp decrease of intensity is observed when the copper concentration is raised above one Cu^{2+} per particle (*i.e.* $>3 \times 10^{-8}$ M). The fluorescence intensity is reduced to 50% of its initial value for submicromolar concentrations of about 0.5 µM Cu^{2+}. A plateau is reached for high copper concentrations (>20 µM) that correspond to a complete complexation of the cyclam residues. The I/I_0 value at the plateau, about 0.20, indicates that 80% of dye molecules can be quenched (accessible dyes) and thereby that only 20% of the fluorophores embedded within the particles are not accessible for energy transfer. These results clearly shed light on the great interest in nano-sized scaffolds, with a very small inner volume fraction, for cooperative photophysical processes. The fluorescence decay curves confirm that quenching occurs upon addition of cupric ions and can be fitted by a bi-exponential function with a long lifetime (6.5 ns, unquenched fluorophores) and a short lifetime (1.1 ns, quenched fluorophores) (Fig. 1). The mean fluorescence lifetimes decrease with increasing Cu^{2+} concentration: 6 and 2.5 ns respectively at 10^{-9} M and 5×10^{-6} M.

Moreover, **FCNL** selectively responds to Cu^{II} since the absorption band of complexes formed with other metal ions does not overlap the emission of the dye.§ Although **FCNL** readily binds Zn^{II} and Ni^{II},§ the fluorescence intensity of **FCNL** is not affected in the presence of high concentrations of Zn^{2+} or Ni^{2+} (1 mM) even after several hours. Most importantly, these cations do not interfere with Cu^{2+}: as shown in Fig. 2, in the presence of copper, similar emission spectra are obtained with or without added competitive ions. Cu^{II} is selectively detected even in the presence of a large excess of other metal ions. **FCNL** acts as a specific Cu^{2+} sensor at nanomolar level and may find various applications for sensing of copper in complex aqueous media; for example in sea-water or in biological samples.

In conclusion, nano-sized polymer particles are useful scaffolds for assembling fluorophores and probes in close proximity, thereby allowing energy transfer between the components without the need for covalent linkage. The dye/probe system depicted here has been specially designed for Cu^{2+} ions and leads to a sensor that is able to compete in sensitivity and selectivity with other more sophisticated systems. This modular approach allows for the easy variation of the components: other commercially available dyes may be inserted into the nanoparticle core, thus giving access to sensors for other metal ions. Moreover, a variety of functionalized nanoparticles are readily accessible using the technique of polymerisation in microemulsions and hence the concept can be extended to a wide range of ligands and receptors for the sensing of other ions or neutral molecules.

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Notes and references

‡ Cyclam, tetraazacyclotetradecane, has a high affinity for transition metal ions. Association constants are (log *K*): 27, 20, 20, 13, 11 respectively with Cu^{II}, Ni^{II}, Zn^{II}, Co^{II}, Cd^{II}.[13]

§ The complexation yields are about 70–80% with Zn^{II}, Ni^{II} and Co^{II}. Almost complete complexation (>95%) is achieved in the presence of Cu^{II}. Absorption wavelengths (nm) of the cyclam-metal complexes: 465 (Ni), 300 (Co), 540 (Cu), no absorption above 300 nm for Zn.

¶ Cu^{2+} ions do not quench fluorescence of **1** in the absence of cyclam.

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