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Dendrimers as fluorescent sensors with signal amplification

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In a fourth generation poly(propylene amine) dendrimer decorated with 32 dansyl units at the periphery and containing 30 aliphatic amine units in the interior, the strong fluorescence of all the dansyl units is quenched when a Co^{2+} ion is incorporated into the dendrimer.

In the last few years there has been a great development in the field of fluorescent chemosensors.[1] These compounds are usually made of a fluorescent unit connected with a receptor. Recognition of a substrate by the receptor affects the fluorescent properties of the fluorophore. For example [Fig. 1(a)], coordination of a metal ion to the receptor can cause quenching of the excited state of the fluorophore by energy or electron transfer, thereby switching off the fluorescent signal. In order to achieve sensory signal amplification, we have designed and synthesized compounds in which one transition metal ion is able to quench the excited state of a great number of fluorophores [Fig. 1(b)].

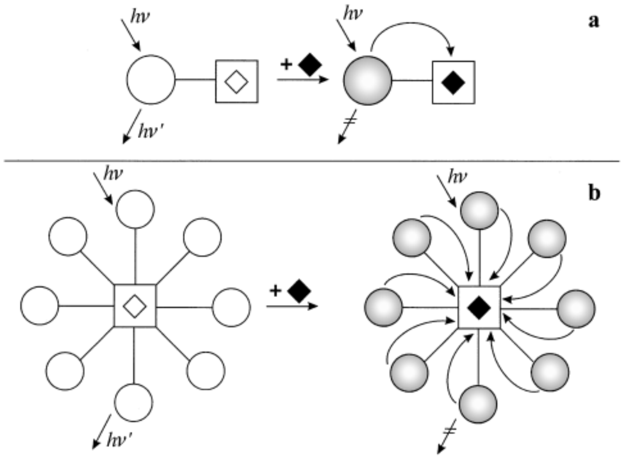

Fig. 1 Schematic representation of (a) a conventional fluorescent sensor and (b) a fluorescent sensor with signal amplification. The curved arrows indicate a quenching process. For more details, see text.

The described system belongs to the family of dendrimers, which are well defined macromolecules exhibiting tree-like structures.[2] By suitable design and synthetic strategies, it is possible to incorporate in a dendritic structure components exhibiting specific properties whose integration can lead to useful functions.[3] Continuing our investigations in the field of photoactive dendrimers,[4] we have prepared compounds that incorporate both receptor and fluorophore units, and we have shown that they can give rise to a strong signal amplification compared to fluorescence sensors carrying only one fluorescent unit (Fig. 1).

Because of its strong fluorescence, the dansyl group is often used as a fluorophore for sensory purposes.[5] When the dansyl unit is linked to a polyamine receptor,[6] coordination of a suitable metal ion by the receptor causes the quenching of the dansyl fluorescence. We have appended fluorescent dansyl units at the periphery of poly(propylene amine) dendrimers[7] which are able to coordinate metal ions by the aliphatic amine units contained in the interior of the structure. The formula of the fourth generation dendrimer **4D**, which contains 32 dansyl units at the periphery and 30 aliphatic amine units in the interior, is shown in Fig. 2.

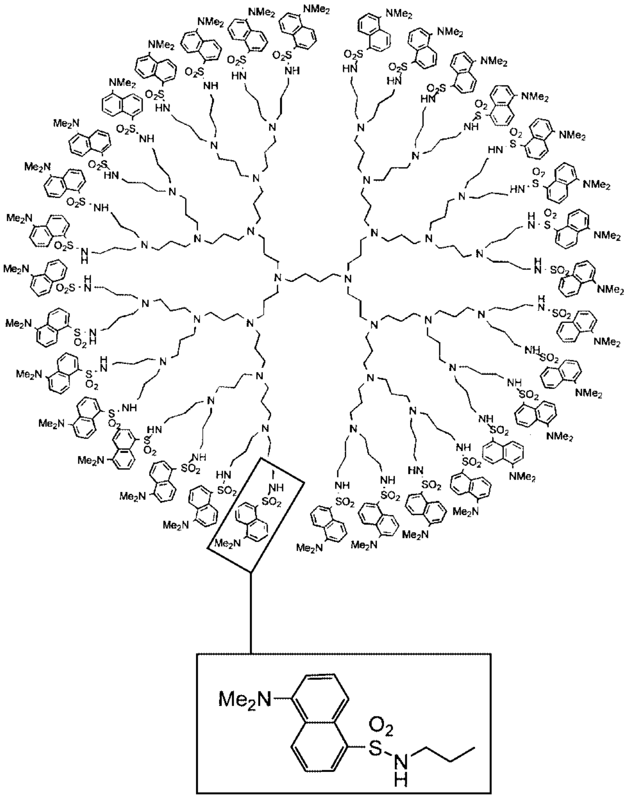

Fig. 2 Structure formula of the **4D** fourth-generation poly(propylene amine) dendrimer decorated with 32 dansyl units.

In acetonitrile–dichloromethane (5:1 v/v) solution, dendrimer **4D** exhibits intense absorption bands in the near UV spectral region (λ_{max} = 253 and 339 nm, ε_{max} = 357000 and 113000 M^{-1} cm^{-1}, respectively) and a strong fluorescence band in the visible region (λ_{max} = 514 nm, Φ = 0.25, τ = 13 ns).† Addition of Co^{2+} (up to 1.0×10^{-4} M, as $Co(NO_3)_2 \cdot 6H_2O$) to a 4.6×10^{-6} M solution of **4D** caused a strong quenching on the fluorescence intensity of the dansyl units appended at the periphery of the dendrimer, without affecting the absorption spectrum.

The observed quenching cannot be attributed to a dynamic process,‡ but it must originate from coordination of metal ions by the aliphatic amine groups which are present in the interior part of the dendrimer.[8] The **4D** dendrimer, in fact, can be considered as a polydentate ligand. In view of its size and of the 30 amine units, it can be expected that each dendrimer

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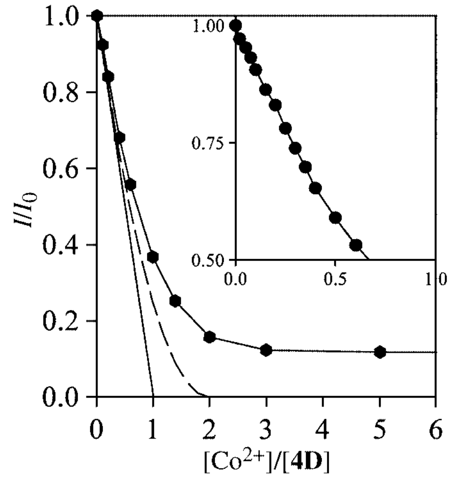

Fig. 3 Effect of addition of Co^{2+} ions on the fluorescence intensity of the **4D** dendrimer. The concentration of **4D** is 4.6×10^{-6} M. Inset shows the results of a detailed investigation at low Co^{2+} concentration.

coordinates several metal ions, giving rise to a complex titration curve (Fig. 3). The inset of Fig. 3 shows that for low Co^{2+} concentrations ([Co^{2+}]/[**4D**]$\leqslant$0.3) the fluorescence intensity decreases linearly with increasing [Co^{2+}]. More specifically, in this concentration range each Co^{2+} equivalent quenches 32 dansyl units, a number that corresponds to the dansyl units contained in each dendrimer. This means that at *low* metal ion concentration, 1 : 1 metal ion–dendrimer species are formed in which the dansyl fluorescence is completely quenched.§ Apparently, upon coordination of a metal ion the dendrimer shrinks around the metal, thereby allowing all the 32 dansyl units to be quenched when they are excited.

The titration curve (Fig. 3) is no longer linear on increasing Co^{2+} concentration further, which shows that species containing more than one metal ion per dendrimer are formed. The dashed curve in Fig. 3 corresponds to the behaviour expected if two metal ions can independently enter the dendrimer; the lack of fitting to this titration curve suggests that species containing more than two metal ions are also formed. Finally, it can be noted that the luminescence intensity does not fall to zero. Perhaps, since the metal ion is added as $Co(NO_3)_2{\cdot}6H_2O$, at very high Co^{2+} concentrations water molecules and/or counter ions may occupy part of the coordination sphere or even prevent complete dendrimer–Co^{2+} association. We have also found that, at high Co^{2+} concentrations, the titration curve is somewhat different when the metal ion is added as a chloride salt.

A most important property of a fluorescent sensor is, of course, sensitivity. By using dendrimer **4D** it is possible to amplify the sensitivity of a monodansyl compound by 32 times. The detection limit of Co^{2+} depends on the concentration used. Our results indicate that with a **4D** concentration of 4.6×10^{-6} M, a Co^{2+} concentration of 4.6×10^{-7} M causes a decrease in the fluorescence intensity of 5%. A signal amplification effect has already been obtained with polymeric chains of sensors.[9] Because of their well defined and fully programmable structures, dendrimers are more promising species for this kind of application.[10] Of course, several other requirements (*e.g.*, selectivity) are needed for useful sensory application.

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Notes and references

† The equipment used has been described elsewhere.[4]

‡ Since the lifetime of the fluorescent excited state of the dansyl unit is 13 ns, the metal ion concentration is too low to cause sizeable effects even in the case of a diffusion controlled quenching process.

§ The straight line shown in the inset of Fig. 3 extrapolates to $I/I_0 = 0$ at [Co^{2+}]/[**4D**] = 1, which confirms that at *low* metal ion concentration 1 : 1 metal ion/dendrimer species are formed in which the dansyl fluorescence is completely quenched.

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