A multi-ion particle sensor

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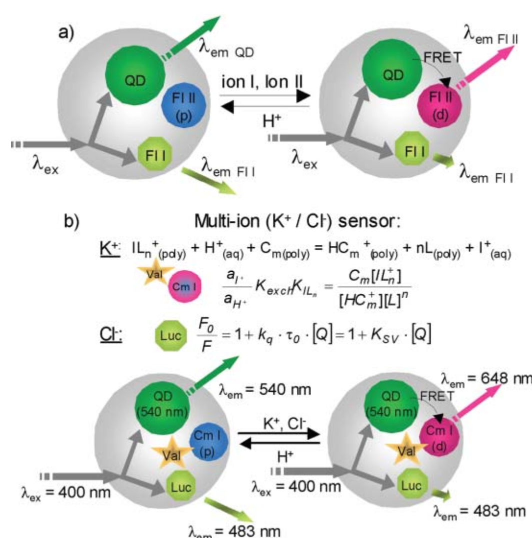
The first sub-micron polyacrylic sensor containing two independent ion-sensing systems is shown, that uses a single excitation wavelength and separates signals by using quantum dot donors to form FRET pairs with other fluorophores.

Organic ion-sensitive dyes may be used to generate ion-sensitive signals, each corresponding to a particular target molecule,[1–3] but due to their narrow absorption and red-tailed emission spectra, simultaneous detection of multiple signals is complicated, often requiring several excitation sources and complex filtering to produce independent channels with reduced signal overlap.[4,5] However, in this work we show how the efficiency of fluorescence resonance energy transfer (FRET) between quantum dots (QDs) and proximal organic dyes[6–8] can be exploited to separate ion sensitive emission signals, even using a single excitation wavelength. This exploits the broad absorption spectra of QDs and the pairing of their narrow emission spectra with the absorption spectra of acceptor dyes. For example, (Scheme 1(a)) excitation at the correct wavelength for fluorophore I (Fl I) will simultaneously excite QD. QD is chosen such that its narrow emission spectrum coincides with the absorption of fluorophore II (Fl II). The requirements are that λ_{em}(Fl I) is adequately separated from λ_{em}(Fl II) or that λ_{em}(QD) is separated from λ_{em}(Fl I). There are many QD/dye combinations for a wide range of multi-ion sensors, using single or multiple QDs, since simultaneous excitation of several QD populations, generating a distinct emission characteristic for each population,[9–11] could excite different ion-sensitive dyes.



Scheme 1 (a) General FRET-multi-ion sensing configuration: (p) and (d) represent the protonated Fl II and deprotonated Fl II respectively. (b) FRET-multi-ion sensing configuration in this study: Cl^--sensitive lucigenin (Luc), K^+-selective-valinomycin (Val) with chromoionophore I (Cm I), and QDs were immobilised in acrylic nanospheres. In the presence of K^+ and Cl^- fluorescence of Luc is quenched, and QDs act as donors interacting with deprotonated Cm I (acceptor) in FRET effect. The response mechanisms and their equations are also shown.

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A combination that could extend the ion-sensing capabilities, using a non-fluorescent ionophore (Scheme 1(b)) could be especially significant since it would offer the possibility to transfer directly many optrode recipes, already well explored by us[12] and by others.[13–15] This requires ionophore/fluorophore cation/proton extraction chemistry linked with an H^+-sensitive Fl II, while the other ion is detected directly by selective quenching of Fl I (Scheme 1(b)). However, the challenge is to spatially separate the two analytical reaction pathways, so that the cation/proton equilibria that determine Fl II fluorescence are not influenced by the ion that quenches Fl I.

We show herein that this can be achieved for a K^+/Cl^- analyte pair, tested initially using valinomycin as non-fluorescent selective ionophore for K^+, combined with chromoionophore I (ETH 5294, Fl II) as the indicator of the ion exchange, and KTClPB as an ionic additive to reduce co-extraction of counter-ion. In contrast, for chloride determination, the sensing principle was based on the dynamic quenching of the fluorescence of lucigenin (Fl I)[16,17] where the decrease of the fluorescence intensity in the presence of aqueous chloride ions is described by the Stern–Volmer equation. These two sensing principles thus have completely independent mechanisms and different sensing locations, which is essential for simultaneous ion detection without interference.

To create these dual-ion sensing particles, QDs and other reagents were embedded into the polyacylic matrix through photo-initiated suspension polymerization. Briefly, the mixture of monomer *n*-butylacrylate (*n*BA, 3.35 mmol), cross linker hexanedioldiacrylate (HDDA, 0.98 mmol), initiator azoisobutyronitrile (AIBN, 0.06 mmol) and CdSe/ZnS QDs with maximum emission at 540 nm (25 nmol, Evident Technologies) were mixed as the dispersed phase. An aqueous 1% (v/v) poly(vinyl alcohol) (PVA) solution (5 mL) was used as the continuous phase. The final isolated latex spheres were less than 1 μm size, measured using a Zetasizer Nano (Malvern). K^+ and Cl^- sensing components were diffused into the QD-embedded sub-microspheres and remained incorporated, producing a stable signal, through hydrophobic interaction: typically lucigenin–chromoionophore I (ETH 5294)–KTClPB–valinomycin in the mole ratio 0.03 : 2.6 : 40 : 10 was used with polymer nanosphere embedded CdSe–ZnS QDs. The final ratio of QDs (donor) to chromoionophore I (acceptor) was controlled around 1 : 50.

Fig. 1 shows the individual absorption and emission spectra of all the fluorophores as well as the QD/chromoionophore

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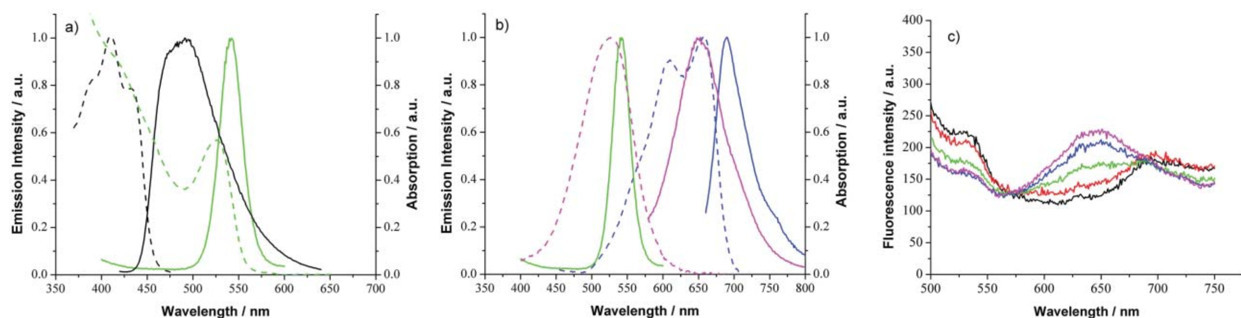

Fig. 1 Emission (solid lines) and absorption (dashed lines) spectra of: (a) lucigenin (black) and QDs (green). (b) deprotonated chromoionophore I (pink), and protonated chromoionophore I (blue) and QD (green). (c) Emission spectra of QD/chromoionophore I-embedded acrylic particles after exposure to different K^+ concentrations: 0 M (black), 0.001 M (red), 0.01 M (green), 0.1 M (blue) and 1 M (pink).

I-embedded particles. It can be seen that both QDs and lucigenin have strong absorption at 400 nm while chromoionophore I does not (Fig. 1(a) and (b)). Additionally, above the pK_a of chromoionophore I, the predominant form is the red deprotonated (Cm) form with a strong emission maximum at 660 nm and an absorption peak at 540 nm, which overlaps the emission of QD_{540}, whereas below the pK_a the protonated chromoionophore I (HCm^+) only absorbs at longer wavelength with two peaks at 610/660 nm. This results in a much smaller overlap with QD_{540} emission compared with Cm, and gives a weak emission maximum at 700 nm (Fig. 1(b)). When the QD/chromoionophore I-embedded particles are excited at 400 nm the dual emission peaks at 540/648 nm support the FRET mechanism proposed between QDs and Cm. Furthermore, in the presence of K^+-ionophore, valinomycin, the emission at 540 nm (QD, donor) and 648 nm (Cm, acceptor) shows a systematic change (Fig. 1(c)) when the concentration of potassium is varied, which is consistent with the change in the Cm/HCm^+ ratio resulting from the consequent proton extraction (Scheme 1(b)). Taken together with the [Cl^-] sensitive lucigenin, which gives an emission maximum at 483 nm (Fig. 2(b)), with a red-tailed overlap with the sharp QD emission (Fig. 1), some of the enabling features of the dual ion sensing system are that:

Only absorption of deprotonated chromoionophore I overlaps efficiently with the QD emission to allow FRET. Thus the change in QD fluorescence intensity with [K^+] is a result of different FRET efficiency linked to the $^+$HCm/Cm ratio (see scheme, Fig. 1(b) and (c)).

There is no evidence of FRET from lucigenin to QD_{540} although a spectral overlap is observed (Fig. 1(a)), which is consistent with reports by Mattoussi and co-workers, attributing this to the dominance of the fast radiative decay channels of the donor dye relative to the slow nonradiative FRET decay pathways to the QD acceptor.[18] Thus, the internal cross talk of the sensing components is avoided. Direct fluorescent signals are measured for the lucigenin and Cm emission, without the need for deconvolution analysis, due to the large separation of the emission peaks.

Since the QD emission is sharp the direct fluorescent emission for lucigenin is measured without deconvolution analysis in the blue-tail of the lucigenin emission peak.

In the dual ion system, the experimental value obtained for the equilibrium exchange constant, pK_{exch}, was 6.25 $\pm$ 0.02 (Fig. 2(a), calculated from Cm emission at 648 nm), which is very similar to the pK_{exch} obtained for the single potassium ion sensing nanospheres,[12] as well as the linear response range. This is important since increasing anionic site concentration has been shown previously to result in a decrease in the observed ion extraction, reducing the change in degree of protonation of chromoionophore I, and decreasing the response range.[12] The apparent diffusion coefficient for chloride in this matrix has not been measured, but the strong correlation in [K^+] response between the dual-ion nanospheres and the single-ion K^+-nanospheres suggests that co-extraction of Cl^- is not significant, so the presence of a Cl^- sensitive fluorophore in the nanosphere has not influenced the K^+-measurement.

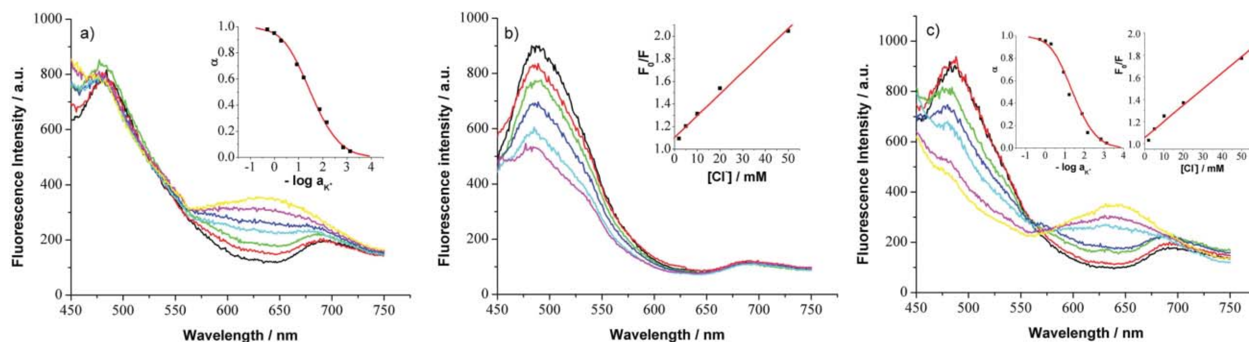

Fig. 2 Emission spectra of acrylic/QDs nanospheres acting as: (a) single K^+-sensors after addition of $KHCO_3$ solutions. (b) Single Cl^- -sensors after addition of NaCl solutions. (c) K^+/Cl^- multi-ion sensors after addition of KCl solutions. Concentrations: 0 mM (black), 2 mM (red), 5 mM (green), 10 mM (blue), 20 mM (cyan), 50 mM (pink) and 200 mM (yellow). Fluorescent signals were recorded between 450 and 750 nm (λ_{ex} = 400 nm) with a spectrofluorimeter (Cary Eclipse, Varian) in a 96-well microplate. In all samples the pH was fixed at 7.0 by using phosphate buffer.

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When only concentration of Cl^- was varied, a significant change in the fluorescence intensity of lucigenin was observed (Fig. 2(b), monitored at 483 nm), which can be described by the Stern–Volmer equation indicating that the quenching is collision controlled with a good linear relationship (I_0/I = 0.9625 + 0.0516[Cl^-]; R^2 = 0.9982) observed for [Cl^-] < 50 mM. Interestingly, even when very high chloride concentrations were used, the nanosphere fluorescence was not totally quenched. This background fluorescence is most likely to be due to the lucigenin in the core of the acrylic material and, if co-extraction of Cl^- is not significant, this leads to the conclusion that just the lucigenin on the outside of the nanosphere is responsible for the Cl^--sensitive signal. Thus, this hybrid nanosphere system separates both the location of the analytical reaction pathway and the wavelength for the analytical signals.

The simultaneous detection of both ions is shown in Fig. 2(c) in response to KCl. It can be seen that with increasing KCl, the quenching of lucigenin and increase of deprotonated chromoionophore I emission can be monitored independently, even though the decrease of the narrow QD emission overlaps the lucigenin fluorescence. Indeed, the calibration curves for K^+ and Cl^- obtained through the dual ion measurement were identical to those from each single ion detection. However, consistent with the different reaction locations discussed above: for K^+ determination (reaction within the nanosphere), the response time was 2–3 min; whereas for Cl^- determination (surface reaction) the response time was decreased to 20–30 s.

Acrylic/QDs nanospheres acting as K^+/Cl^- multi-ion sensors also demonstrated rather good selectivity for most of the tested interferent ions. For K^+ determination, the nanosensors showed negligible response toward Na^+, Li^+ and Mg^{2+}, although NH_4^+ and Ca^{2+} could also be complexed by the ionophore, but only at high concentrations (>1 and 0.5 M, respectively). The quenching of the fluorescence of lucigenin by chloride ion is also reasonably selective (as expected for lucigenin): PO_4^{3-}, NO_3^- and SO_4^{2-} produced minimal quenching of fluorescence of lucigenin, acting rather weakly compared with HCO_3^-, which is an interfering agent only at concentrations higher than 0.5 M. Br^-, I^- and SCN^-, in contrast, act as stronger quenchers than chloride ion. However, for applications of these nanosensors in cell and physiological samples the selectivity is appropriate for the intended purpose, since the concentration of these other ions in cells or other physiological tissues is always lower than the concentrations of K^+ and Cl^-.

The multi-ion sensors were applied to determine K^+/Cl^- concentrations in a model system, mimicking the intracellular environment (Dulbecco's Modified Eagle's Medium (DMEM D-6171, Sigma)), containing different inorganic salts, amino acids, vitamins, glucose, and another cell components. The concentrations of potassium and chloride ions were simultaneously measured and showed satisfactory RSDs. The results showed a good agreement between experimental and actual values of each ion concentration, indicating the utility of the proposed sensors, and the potential for their successful use in cells and another physiological samples (Table 1).

In conclusion, we have demonstrated that two different ions can be simultaneously detected using an optical multi-ion sensor, where each sub-micron polyacrylic sphere contains two independent sensing systems and 'deconvolution' of fluorescent signals is achieved using QDs as energy donors to form FRET pairs with other fluorophores. This consequently allows the simultaneous excitation of multi-fluorophores using a single excitation wavelength. As another closely related example, the concentrations of the Na^+/Cl^- ion pair have also been successfully measured and in principle replacement of the ionophore with any other cation-selective ionophore should produce other cation/Cl^- pairs. However, the potential of the method can be extended to a wide range of multi-analyte determination through careful selection of the non-interfering or spatially separated sensing systems and adjustment of the fluorophores and QDs to achieve the required analyte pair and wavelength separation in the measured signal.

Table 1 Applications of proposed multi-ion K^+/Cl^- sensors to the simultaneous ion determination in samples with simulated physiological medium

Sample	Analyte	Activity actual/mM	Activity found ± SD[a]/mM
1	K^+	9.1	7.7 ± 1.4
	Cl^-	4.2	4.0 ± 1.2
2	K^+	9.1	12.9 ± 3.5
	Cl^-	8.4	10.3 ± 1.5
3	K^+	9.1	9.8 ± 1.4
	Cl^-	12.8	15.7 ± 0.5

[a] Average of three determination ± standard deviation

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