Luminescent Core−Shell Imprinted Nanoparticles Engineered for Targeted Förster Resonance Energy Transfer-Based Sensing

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Abstract

Molecularly imprinted polymers (MIPs) are regarded as synthetic analogues of antibodies in that they bear cavities which are complementary in size, shape, and chemical functions to a target molecule.[1] Such biomimetic materials can be employed as adsorbents for selective binding of the latter, making them useful as stationary phases for smart chromatography-based analysis. However, alternative analytical methods based on fluorescent detection are needed to enable fast, sensitive, user-friendly, affordable sensing devices for health care, food, and environmental testing.

The task of coupling a binding event in a MIP with an optical signal is not straightforward. Here we show how this is possible to benefit from a *Förster resonance energy transfer* (FRET) process for signaling binding of our target analyte, enrofloxacin –a broad spectrum antibiotic– to a MIP layer. FRET is a photochemical distance-dependent process that can reveal the proximity of two species, an energy donor (D)−acceptor (A) pair, by way of their luminescence. An efficient electronic energy transfer from the photoexcited D to ground state A dyes occurs if D and A approach enough ($R < 10$ nm) and if there is significant spectral overlap between the emission of D and the absorption of A.[2]

The approach described herein involves FRET between near-infrared (NIR) labeled analyte molecules and *luminescent core−shell nanoparticles* (NPs). A cyanine-labeled enrofloxacin (NIR-ENR) has been chosen as the FRET acceptor and the $[Ru(phen)_3]^{2+}$ (phen: 1,10-phenantroline) complex is the energy donor (Figure 1).[3] In addition to detection in the NIR region, another advantage of this D−A pair is the long emission lifetime of the Ru(II) dye. Furthermore, the D molecules are encapsulated into silica NPs to bring about signal intensification[4] and minimize luminescence quenching by dissolved oxygen.[5] Finally, a thin polymer shell, imprinted with our target analyte, is grown around the silica NP core, providing selective binding sites for the antibiotic.

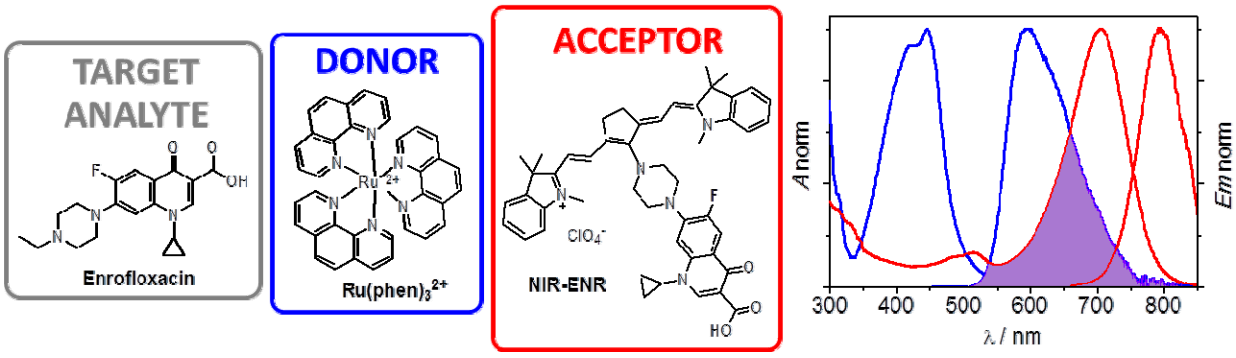

Figure 1. Chemical structures of the enrofloxacin (ENR) analyte, the FRET donor $(Ru(phen)_3^{2+})$, and the FRET acceptor fluorophore (NIR-ENR). The graph shows the absorption and emission spectra of D (blue) and A (red) in ethanol, with the region of the spectral overlap in violet.

In order to optimize the FRET signal output, we had to consider two parameters: i) the distribution of D dye molecules in the NPs –location of the dye in the NP can be controlled at will by adjusting the *time* elapsed before the luminophore is added to the reaction mixture;[6] and ii) the MIP shell *thickness*, that should not be too large in order to allow the adequate FRET to the bound acceptor. The last can be regulated by adjusting the concentration of monomers and cross-linkers in the polymerization mixture.[7]

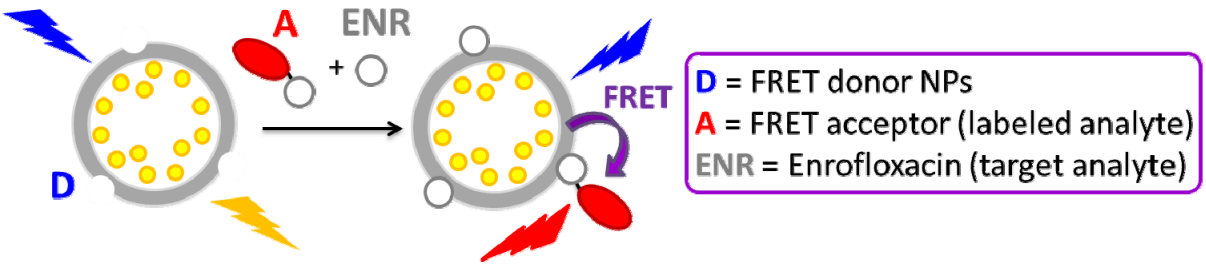

Figure 2. Schematic representation of the NPs FRET assay described here. In this assay, the target analyte ENR competes for the binding sites of the MIP shell in the NPs with the FRET acceptor dye. Consequently, the higher the concentration of ENR, the weaker the FRET signal.

The resulting core−shell NPs were evaluated in a competitive assay between NIR-ENR and ENR itself, and changes on the FRET signal were employed for determining the extent of enrofloxacin binding (Figure 2).

The *nanoanalytical platform* engineered in this way can be tuned to virtually any analyte to benefit from background-free emission intensity and lifetime measurements, the brightness of dyed nanoparticles, the fast kinetics of the assay and, remarkably, the advantage of performing the biomimetic assay in rich aqueous (buffered) media at neutral pH. In this way, the artificial system might become a cheaper more robust alternative to competitive fluorescence immunoassays.

References

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