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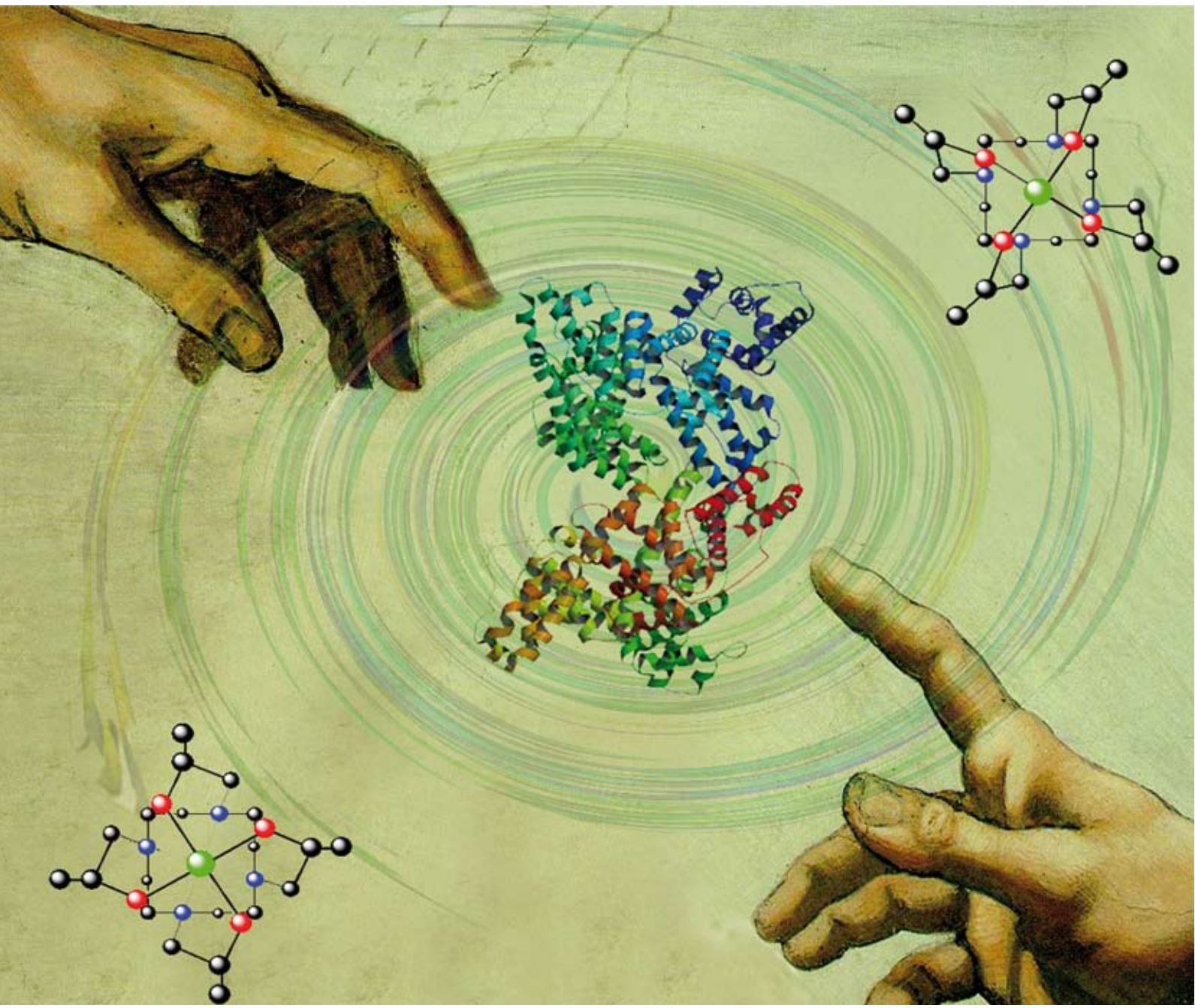

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Enantioselective regulation of a metal complex in reversible binding to serum albumin: dynamic helicity inversion signalled by circularly polarised luminescence†

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The helicity of the (*SSS*)-Δ enantiomer of a terbium and europium(III) complex is inverted on reversible binding to 'drug site II' of serum albumin, signalled by a switch in its circularly polarised emission; no such behaviour occurs with the (*RRR*)-Λ complexes, thereby defining a unique chiroptical probe of albumin binding.

In molecular recognition, examples of an 'induced-fit' process characterise innumerable processes.[1] Most commonly, the process is considered in terms of a change to a structured binding site upon recognition of a partner—like a glove altering shape to fit a hand. Cases with chiral systems in which this process leads to helicity inversion are much less common, although the inversion of helicity may play an important role in various cellular process and in gene expression.[2] Thus, right-handed *B*-DNA may be transformed into left-handed *Z*-DNA following binding to spermine or various cationic metal complexes.[3] There has been considerable interest recently in synthetic systems exhibiting helicity inversion following exposure to an external stimulus, including a variety of P and M helical organic compounds[4] and several types of Δ and Λ metal coordination complexes.[5] In this context, the binding of a slowly epimerising ruthenium complex to *N*-ethylguanine and *B*-DNA is notable and leads to high diastereoselectivity in the derived adducts, by virtue of switching of configuration at Ru and N stereogenic centres.[6]

The regulation of the structure of an enantiopure guest following non-covalent binding with a chiral host is a common feature of enantioselective recognition processes. However, there appear to be no well-defined examples where this process involves the selective inversion of the helicity of an enantiopure guest species.[7] Modulation of the chirality of a guest species is most conveniently signalled by optical methods, involving either differential absorption of left- or right-handed circularly polarised light (CD), or differential emission with luminescent systems (CPL). An induced CD has been observed using racemic $Nd(dpa)_3^{3-}$ (existing as equilibrating Δ and Λ enantiomers), following addition of vitamin B12 derivatives,[8] and several CPL studies have examined enantioselective quenching of non-racemic lanthanide(III) excited states, following addition of proteins or chiral transition metal complexes.[9]

In seeking to develop a chiroptical probe that can signal a binding event by modulation of the circular polarisation of emitted light, the class of enantiopure emissive Eu and Tb(III) complexes is pre-eminent.[10] The lanthanide(III) ions are pure spherical emitters, avoiding problems of anisotropy, possess millisecond excited state lifetimes and give rise to large emission dissymmetry factors (g_{em}) values, typically of the order of 0.1 to 0.4.[10,11] Recently, several examples of highly emissive enantiopure complexes have been devised that are stable in aqueous media and can be excited in the range 330–405 nm.[11,12] These include the complexes $[Ln.L^1]^{3+}$, in which an (*S*)-stereogenic centre at carbon gives rise to a Δ-complex. The enantiomeric complex, (*RRR*)-Λ (δδδδ)-$[Ln.L^2]^{3+}$, gives rise to mirror image CD and CPL spectra.[12a] In solution, exchange between Δ and Λ isomers for $[Ln.L^1]^{3+}$ may occur *via* ring inversion (δ/λ exchange, typically at 50 Hz (298 K))[10b] followed by concerted reorientation of the layout of the ring pendant arms (Δ/Λ exchange), possibly *via* a tricapped trigonal prismatic intermediate. Such processes (Scheme 1) allow the two monocapped square-antiprismatic isomers of a given enantiomeric complex to undergo exchange, with the position of equilibrium being determined by the relative free energies of these conformers.

Incremental addition of human (or bovine) serum albumin to (*SSS*)-Δ-$[Tb.L^1]^{3+}$ led to a reduction in both the intensity and lifetime of the terbium emission *via* dynamic quenching of the Tb excited state[13] (λ_{exc} 350 nm, Fig. 1). The binding isotherm was interpreted in terms of an apparent 1 : 1 binding constant (log K = 5.1, 298 K, pH 7.4, HEPES 0.1 M). A parallel experiment with (*RRR*)-Λ-$[Tb.L^2]^{3+}$ gave very different behaviour, (Fig. 1), consistent with stepwise formation of various adducts of lower affinity. Circularly polarised emission spectra for Δ-$[Tb.L^1]^{3+}$ and Λ-$[Tb.L^2]^{3+}$ were measured in the presence of added HSA or BSA. With the Λ isomer, no change in emission polarisation and spectral form was observed over the protein concentration range 0.03–0.3 mM. On the other hand, with the Δ-isomer an inversion of the sign of the emission was evident, in addition to the 35% intensity reduction (Fig. 2), and was most well-defined in the magnetic dipole-allowed $^5D_4$–$^7F_5$ transitions around 545 nm.† Such behaviour is consistent with an inversion of the helicity of the complex in the protein-bound form, for the Δ-isomer only. This striking effect was exhibited by the Δ terbium complexes of L^{1a} and L^{1b}, but was not shown by the structurally related analogues, Δ-$[Tb.L^3]^{3+}$ and $[Tb.L^4]^{3+}$.

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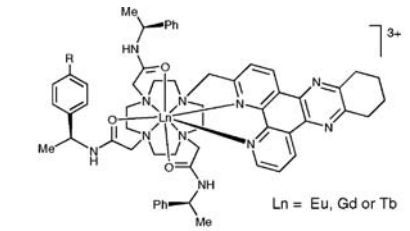
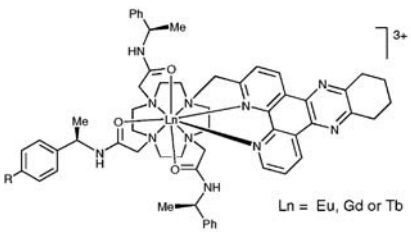
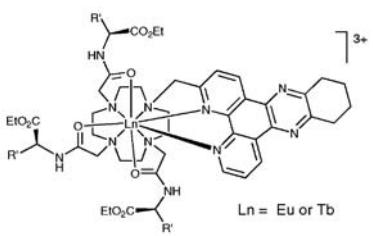
† Electronic supplementary information (ESI) available: Selected details of CPL spectra for enantiomeric complexes; relaxivity titrations for Gd complexes as a function of protein; effect of added warfarin and dansylsarcosine on observed relaxivities for enantiomeric complexes. See DOI: 10.1039/b810978h



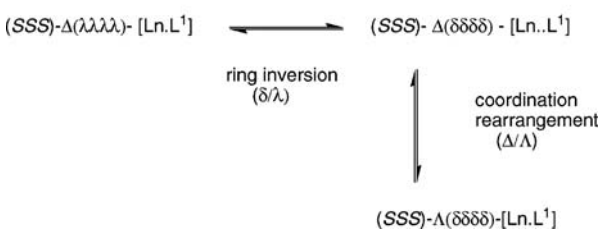
(SSS)-Δ-[Ln.L^1]$^{3+}$ 1a: R = H
1b: R = CO_2Me



(RRR)-Λ-[Ln.L^2]$^{3+}$ 2a: R = H
2b: R = CO_2Me



(SSS)-Δ-[Ln.L^n]$^{3+}$ n = 3, R' = Me
n = 4, R' = CH_2Ph



Scheme 1

Parallel sets of experiments were carried out with europium(III) complexes. Protein addition led to a 50% reduction in overall emission intensity in each case, with *no* change in CPL spectral form and sign for the Λ-complexes and for Δ or Λ Eu(III) complexes of L^3 and L^4.† With (*SSS*)-Δ-[Eu.L^1]$^{3+}$, addition of protein dramatically changed the sign and form of the CPL spectrum, (Fig. 3), suggesting that in the protein adduct, regulation of complex structure may involve both helicity inversion and changes in the relative orientation of ligand donors around the metal centre.[14]

Information on details of protein binding may be gleaned by following changes in proton relaxivity of aqueous solutions containing gadolinium(III) complexes.[15] Serum albumin contains two major well-defined drug binding sites, for which warfarin

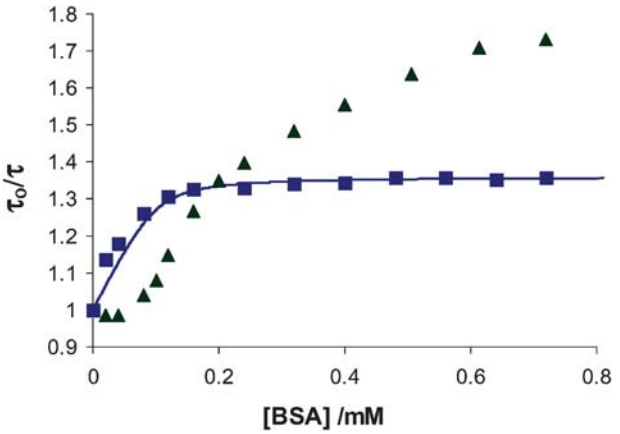


Fig. 1 Quenching of the observed terbium emission lifetime, τ, (τ_o – no added protein) as a function of added BSA for (*SSS*)-Δ-[Tb.L^1]$^{3+}$ (squares, showing the fit to the observed data for a 1 : 1 complex; log K = 5.1) and (*RRR*)-Λ-[Tb.L^2]$^{3+}$ (triangles) (298 K, pH 7.4, 10 mM NaCl, 0.1 M HEPES, 30 μM [complex]).

(K_d 4 μM) and *N*-dansylsarcosine (K_d 6 μM) are competitive[15a,16] probes of drug sites I and II, respectively. The proton relaxivity, r_{1p}, of Λ- and Δ-[Gd.L^1]$^{3+}$ is 3.1 mM^{-1} s^{-1} (60 MHz, 310 K), and increased to 13 mM^{-1} s^{-1} in each case, in the presence of 0.4 mM human serum albumin (HSA). Incremental addition of warfarin to these solutions (5% MeOH was added to solubilise warfarin; up to 3 mM warfarin was added) containing protein caused <10% change in the observed relaxivity. In contrast, incremental addition of dansylsarcosine to Δ-[Gd.L^1]$^{3+}$, over the same concentration range, caused a reduction of r_{1p}, restoring the value of the free complex, (Fig. 4), whereas addition to protein-bound Λ-[Gd.L^1]$^{3+}$ led to less than a 15% reduction. Taken together, this behaviour is consistent with the selective binding of Δ-[Ln.L^1]$^{3+}$ to 'drug site II' of serum albumin; the Λ isomer must possess at least an order of magnitude lower affinity for this site. Crystallographic studies of complexes of HSA have suggested[16b] that 'drug site II' is the most stereo-differentiating protein binding site in serum albumin.

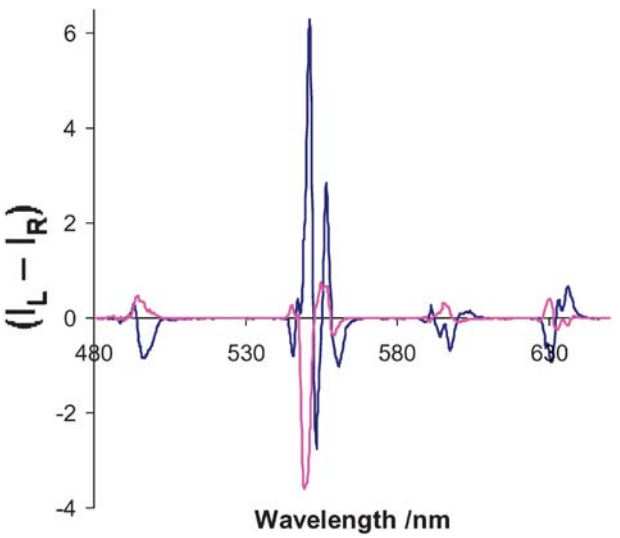


Fig. 2 CPL spectra for (*SSS*)-Δ-[Tb.L^{1b}]$^{3+}$ (blue) and in the presence of added BSA (red) (295 K, D_2O, λ_{exc} 348 nm, 15 μM complex, 30 μM protein; relative (I_L + I_R) = 60 on this scale).

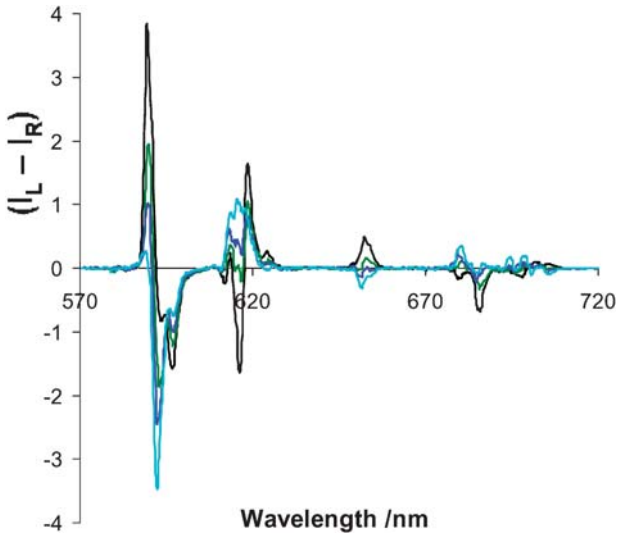


Fig. 3 CPL spectra for (*SSS*)-Δ-[Eu.L^{1a}]$^{3+}$ (*black*) and in the presence of added BSA (0, 2, 5 and 10 μM (*green*/*blue*) , 295 K, D_2O, λ_{exc} 348 nm, 15 μM complex; relative (I_L + I_R) = 60 on this scale).

In conclusion, the Δ-Eu, Gd and Tb complexes of ligand L^1 bind selectively to 'drug-site II' in serum albumin. During this process, their structure is regulated and their helicity reversed to maximise binding. This process occurs reversibly and is fast with respect to the experimental timescale. The Λ-enantiomers do not show this behaviour.

Binding of the Δ-enantiomer to a defined binding site on serum albumin is signalled by inversion of the lanthanide circularly polarised luminescence. This is the first report of chiral inversion following non-covalent protein association of an emissive probe and defines these lanthanide complexes as unique chiroptical probes for albumin binding, potentially allowing protein association to be tracked *in vitro* and in living cells, in real time.[17]

Thus, Faraday's early comment that 'polarised light was a most subtle and delicate investigator of molecular condition' has been vindicated again.

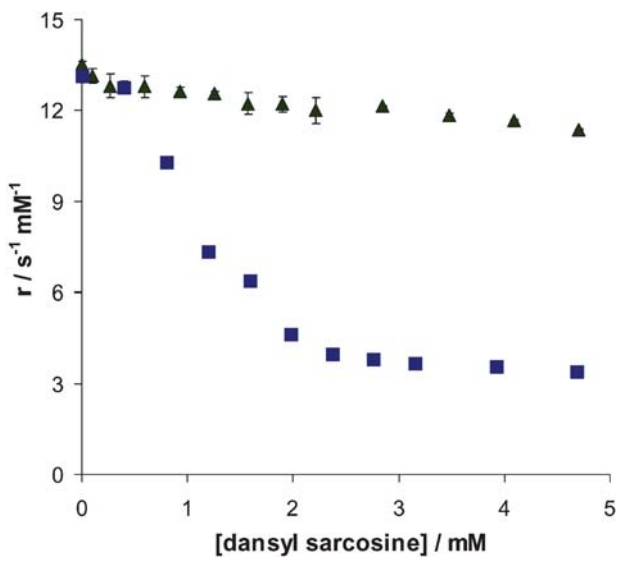


Fig. 4 Variation of proton relaxivity (310 K, 60 MHz, 0.4 mM HSA, 1.7 mM [Gd.L^1]Cl$_3$) as a function of added *N*-dansylsarcosine for (*SSS*)-Δ-[Gd.L^{1a}] (squares) and (*RRR*)-Λ-[Gd.L^{1a}] (triangles).

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