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Supporting information

Enantioselective binding of a lanthanide(III) complex to human serum albumin studied by ^{1}H STD NMR techniques

David M. Dias,[a] João M. C. Teixeira,[a] Ilya Kuprov[b], Elizabeth J. New,[b] David Parker[b] and Carlos F.G.C. Geraldes[a]

[a] Department of Life Sciences and Centre of Neurosciences and Cell Biology, Faculty of Science and Technology, University of Coimbra, P.O. Box 3046, 3001-401 Coimbra, Portugal; Tel: +351239853608; Fax: +351239853607; E-mail: geraldes@bioq.uc.pt

[b]Department of Chemistry, Durham University, South Road, Durham, UK DH1 3LE; Tel +1913342033.; Fax +1913844737 ; E-mail: david.parker@durham.ac.uk

Experimental Section

Materials. Chemicals were purchased from Sigma-Aldrich and used without further purification. *(SSS)*-**L** and *(RRR)*-**L** were synthesised as previously described.[1]

Synthesis of *(SSS)*-[Y.**L**]Cl_3: 1-(6-Methyl-10,11,12,13-tetrahydro-4,5,9,14-tetraazabenzo[b]triphenylene)-4,7,10-tris[*(S)*-1-(1-phenyl)ethylcarbamoylmethyl]-1,4,7,10-tetraazacyclododecane, ligand **L** (26 mg, 0.027 mmol) and yttrium (III) acetate (6.7 mg, 0.025 mmol) were dissolved in methanol:water (1:1, 5 mL) and the resulting solution was heated to reflux under argon overnight. The solution was then added dropwise to ether (20 mL), the precipitate centrifuged and the solvent decanted. The solid was redissolved in acetonitrile and the process repeated to yield a pale yellow solid. This solid was then converted to the chloride salt by stirring with Dowex 1×8 200-400 mesh Cl which had previously been washed with 1 M hydrochloric acid and neutralised with water. The solid resin was removed by filtration and the solvent removed by lyophilisation to yield the title compound as a yellow solid (23 mg, 0.019 mmol, 75%), m.p. > 200 ºC (dec).

^{1}H NMR (700 MHz, D_2O): 0.79 (3H, m, Me.1), 1.11 (3H, m, Me.2), 1.25 (1H, m, cyclen CH_2), 1.39 (1H, m, cyclen CH_2), 1.61 (3H, m, Me.3), 1.84 (2H, m, amide CH_2), 2.01 (5H, m, 5, 5', cyclen CH_2),

2.17 (3H, m, cyclen CH_2), 2.26 (1H, m, cyclen CH_2), 2.34 (1H, m, amide CH_2), 2.52 (2H, m, amide CH_2), 2.57 (1H, m, 1c), 2.75 (2H, m, cyclen CH_2), 2.97 (1H, m, cyclen CH_2), 3.20 (3H, m, cyclen CH_2), 3.46 (4H, m, 4, 4'), 3.33 (1H, m, amide CH_2), 3.68 (1H, m, cyclen CH_2), 4.17 (1H, m, 2c), 5.14 (1H, m, 3c), 6.37 (2H, br, amide NH), 6.51 (4H, m, 3e, 3d), 6.76 (1H, m, amide NH), 6.87 (3H, m, 1f, 2f, 3f), 7.33-7.50 (10H, m, 1e, 1d, 2e, 2d, aryl CH), 7.75 (1H, m, 2), 8.35 (1H, m, 2'), 9.30 (1H m, 1'), 9.53 (1H, m, 3), 9.59 (1H, m, 3'). HRMS (+ m/z): $[M]^{3+}$ calculated for $C_{57}H_{65}N_{11}O_3Y$, 347.4824; found 347.4816. Elemental analysis: found, C 55.7, H 6.35, N 12.4, Cl 8.40; $C_{57}H_{65}N_{11}O_3Cl_3Y.\ 4H_2O$ requires: C 56.0; H 6.19, N 12.6, Cl 8.71%. UV λmax (H_2O) 347 nm (ε 8,200 M^{-1} cm^{-1}).

(RRR)-[Y.**L**]Cl_3 was synthesised using an analogous procedure, using *(RRR)*-**L**. Spectroscopic characterisation was identical to that observed for *(SSS)*-[Y.**L**]Cl_3.

NMR studies. Solutions for NMR studies were prepared by mixing 99.6% D_2O (purchased from Sigma-Aldrich) solutions of each complex, HSA (defatted, from Fluka Biochemika, Switzerland) and *N*-dansyl sarcosine, in appropriate concentrations, to a total volume of 200 μL and then transferred to 3 mm NMR tubes. No buffer was used.

All ^{1}H NMR spectra were acquired on a Varian VNMRS 600 (14.09 T, 599.72 MHz) NMR spectrometer (Coimbra) using a 3 mm pulse field gradient (PFG) inverse probe and on Varian VNMRS-700 (16.44 T, 699.731 MHz) NMR spectrometer (Durham). ^{1}H chemical shifts (δ) are given in ppm relative to TSP as internal reference (^{1}H, δ 0.0). For each sample, one-dimensional (1D) ^{1}H spectra were obtained using a spectral window of 10 kHz, an acquisition time of 2 seconds, a repetition time of 5 seconds, 128 scans and a line broadening of 0.6 Hz. The proton 1D spectra were assigned using 2D g-COSY spectra. The ^{1}H saturation transfer difference (STD) NMR spectra were acquired on the Varian VNMRS 600 (Coimbra) spectrometer using the Double Pulse Field Gradient Spin Echo (DPFGSE) sequence[2] for water suppression. Since in this NMR system the STD NMR spectra are acquired directly from phase cycling, the ^{1}H 1D NMR spectra were used as off-resonance references in order to calculate the STD amplification factor.[3] All STD spectra were acquired using the same parameters: equal spectrometer gain value, the same acquisition parameters as for 1D spectra, except that 2048 scans were used. A selective Gaussian saturation pulse of 2.5 seconds was applied at protein resonances. A previously calibrated spin-lock filter ($T_{1\rho}$) was used to remove protein resonances. All spectra were analyzed using Mestre Nova Software v5.3.1-4825. In order to compare the reference spectra with the STD NMR spectra, the different number of acquisitions was normalized according to equation (1),

$$\text{Rel.STD}\,\% = \frac{I_{STD} \times 2 \times scans_{reference}}{I_0 \times scans_{STD}} \quad (1)$$

where I_{STD} is the peak intensity of the STD NMR spectra, I_0 is the intensity of the peaks in the ^1H reference spectra. Then, the peak intensities were normalized to the amplification factor STD (A_{STD}), equation (2),

$$A_{STD} = \text{Rel.STD}\,\% \times \text{Lig.Exc.} \quad (2)$$

DFT. The DFT calculations were performed by using the Gaussian 03[4] package Y^{3+} complexes; the structures of complexes of other lanthanides are likely to be nearly identical. Importantly, however, the use of diamagnetic La^{3+} and Y^{3+} for DFT calculations avoids a host of largely unresolved theoretical issues with spin-orbit coupling and zero-field splitting in open-shell lanthanides. The Y^{3+} results were found to be very similar to those obtained with La^{3+}, and given the similarity of the ionic radii of the central lanthanide ions, only Y^{3+} complex computations were undertaken. Gaussian 03[4] logs and checkpoints are available upon request. Molecular geometries were optimised *in vacuo* by using spin-restricted B3LYP exchange-correlation functional with a compound basis set (ccpVDZ for CHNOFS, Stuttgart ECP28MWB for La and WGBS for Y).

Docking. The molecular docking simulations were performed using Autodock Vina 1.1.1[5] from the Scripps Research Institute. Autodock Tools 1.5.4 revision 29 was used to prepare all the pdbqt files for Autodock Vina. The HSA structure used was the one with the entry 2XVQ.pdb, because this X-ray structure contains N-dansyl sarcosine, which is known to interact with drug site II, and therefore, is the adequate model to study the possible binding site to our ligands.

From the structure contained in 2XVQ.pdb, the chain A was selected and polar hydrogen atoms were added to the HSA structure and its non-polar hydrogen atoms were merged. The structures of the two enantiomeric complexes were obtained from DFT calculations performed with Gaussian 03. The structures of the complexes were prepared using Autodock Tools, which automatically added gasteiger charges, merged non-polar hydrogens, and detected the aromatic carbons and the rotatable bonds according to the stereochemistry of the molecule.

All calculations for fixed protein flexible ligand docking were done using the Lamarckian Genetic Algorithm (LGA) method. The GRID box parameters used were: center X = 0, center Y = 5, center Z = -24, dimension 30 x 30 x30 and spacing of 1 Angstrom, with a total of 29791 total grid points per map.

The grid box was set around the N-dansyl sarcosine binding site to fully cover the entire binding site and accommodate the ligands to move freely. The best pose was chosen with the lowest docked energy, after the docking search was completed.

USFC Chimera (version 1.5.2 build 32411) was used to read the Autodock data and to obtain the images/views presented in this work. For the 2D interactions, PoseviewWeb 1.97.0[6,7] was used to represent all possible interactions and fully characterize them. 2XVQ.pbd chain A was used with the best poses for all ligands in mol2 files that retain all the Cartesian coordinates like the pdb files.

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Supplementary Results

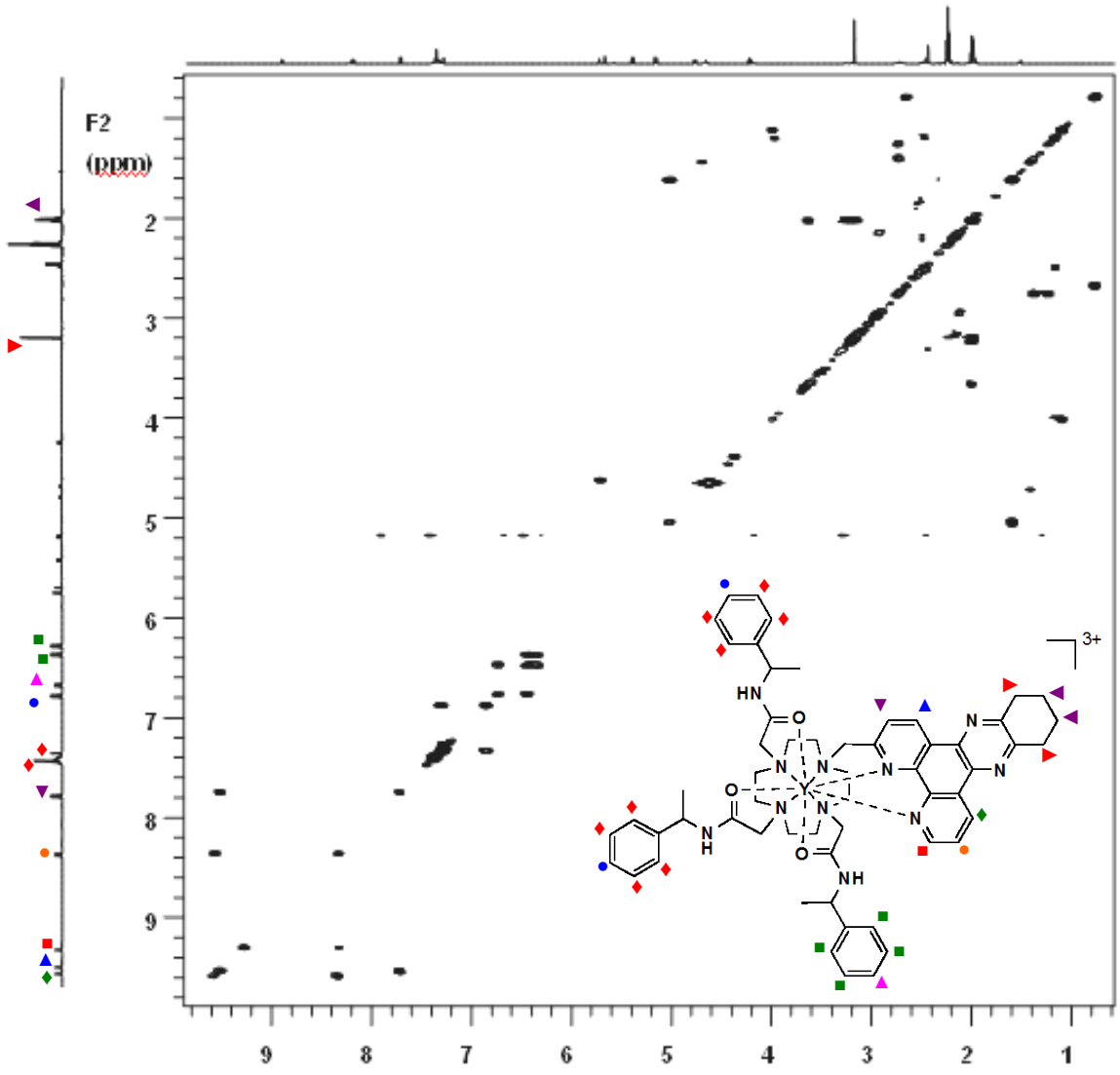

Figure S1. COSY NMR spectrum of *(SSS)*- Δ [Y.**L**] $^{3+}$ (600 MHz, pH 7.0, 295 K) showing partial assignments.

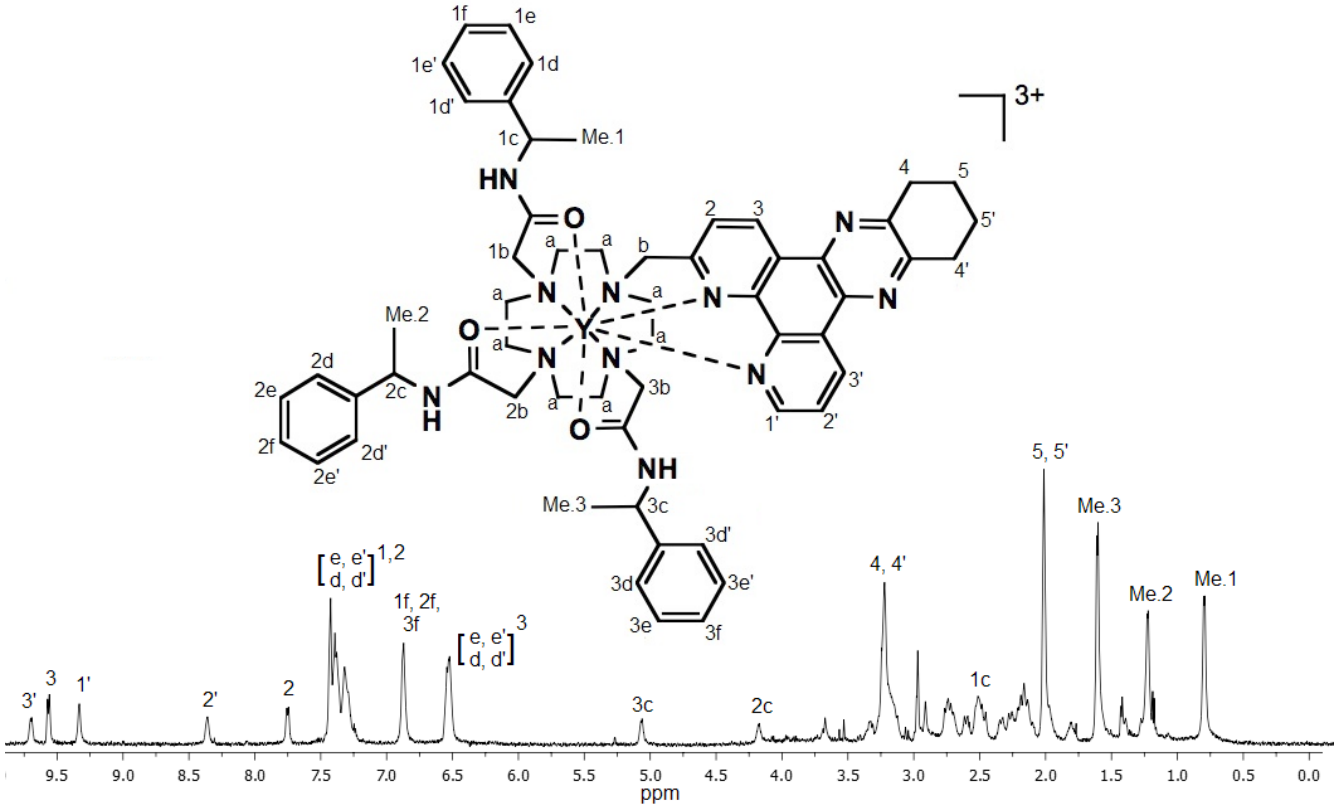

Figure S2. ^{1}H NMR assignments for the *(SSS)*- Δ [Y.**L**]$^{3+}$ complex.

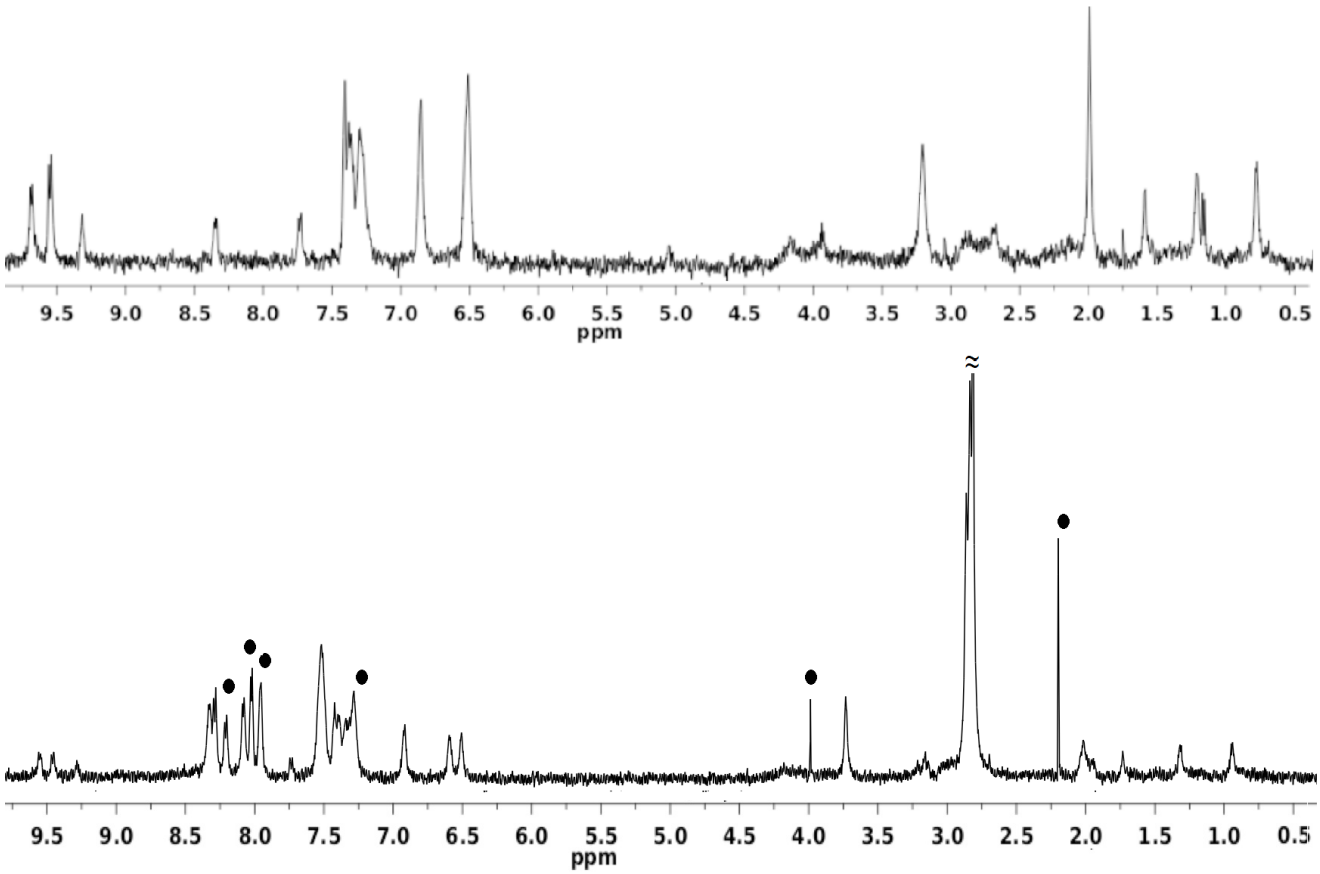

Figure S3. ^{1}H STD NMR of 30 μM HSA, 900 μM (*SSS*)- Δ [Y.**L**]$^{3+}$ in the absence (upper) and in the presence of 2mM *N*-dansyl sarcosine (● – N-Dansyl Sarcosine resonances) at 25ºC, D2O, 600 MHz, -0.5 ppm saturation.

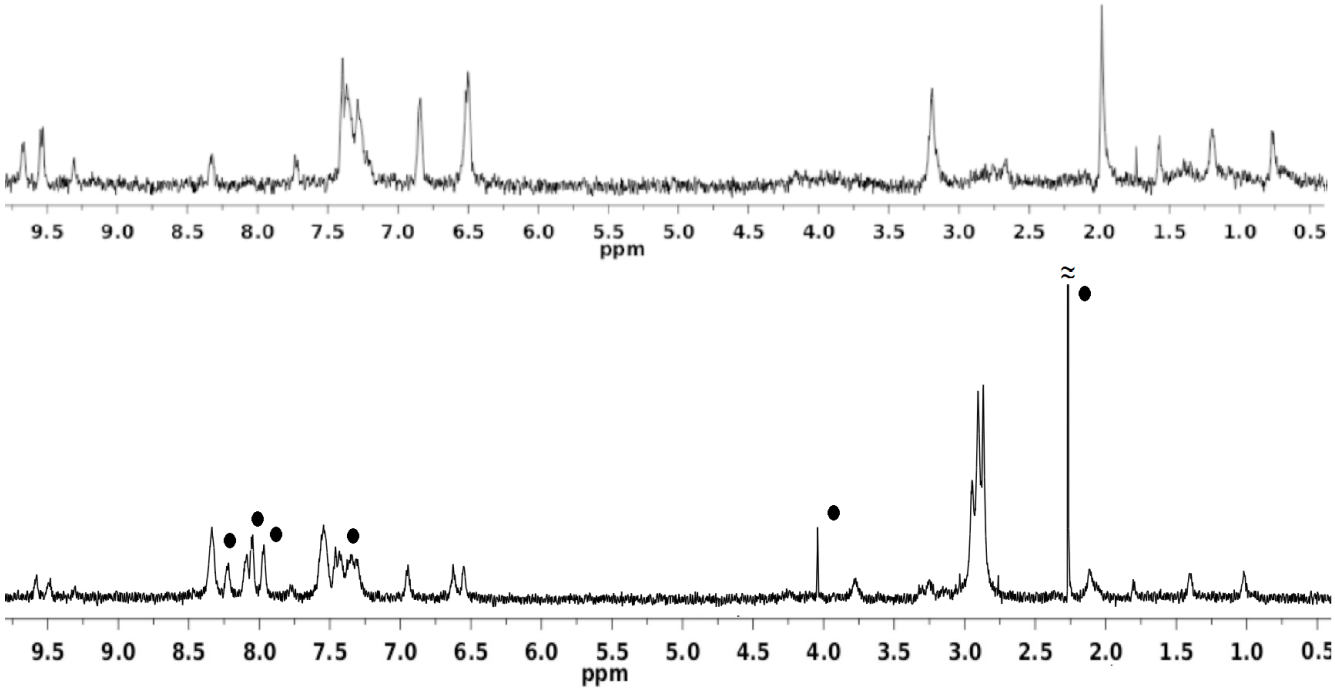

Figure S4. ^{1}H STD NMR of 30 μM HSA, 900 μM (*RRR*)- Λ [Y.**L**]$^{3+}$ in the absence (upper) and in the presence of 2mM *N*-dansyl sarcosine (● – *N*-dansyl Sarcosine resonances) at 25ºC, D_2O, 600 MHz, -0.5 ppm saturation.