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Supplementary Information for:

Cleavable β-cyclodextrin nanocapsules incorporating Gd^{III}-chelates as bioresponsive MRI probes

Jonathan Martinelli, Marianna Fekete, Lorenzo Tei and Mauro Botta

Università del Piemonte Orientale "Amedeo Avogadro"

Dipartimento di Scienze dell'Ambiente e della Vita

Viale T. Michel 11, 15121, Alessandria, Italy.

Fax: +39 0131 360250; e-mail: *mauro.botta@mfn.unipmn.it*

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1. General experimental conditions

- All reactants were used as supplied from commercial sources unless stated otherwise.
- Reactions requiring exclusion of moisture were carried out under a nitrogen atmosphere.
- Water refers to high purity water with conductivity of 0.04 μS cm^{-1}, obtained from the "MILLI-Q" purification system.
- Dialysis tubing (MWCO 12000 a.m.u.) was soaked in distilled water (30 min) and thoroughly rinsed prior to use.
- Measurements of pH were performed using a Hanna 211 pH-meter and Aldrich Chemical Company micro-pH combination electrode, calibrated using pH 4, pH 7 and pH 10 buffer solutions.
- TEM analyses were performed on a Philips EM201 transmission electron microscope. Samples of nanocapsules from dilute aqueous dispersions were evaporated over a measurement grid. The instrument operated at an accelerating voltage of 60 kV, for the acquisition of section images. The selected acquired photographs were finally scanned and digitalised.
- The amount of Gd^{III} in the final materials was determined by inductively coupled plasma mass spectrometry (ICP-MS, Element-2, Thermo-Finnigan, Rodano (MI), Italy). Sample digestion was performed with concentrated HNO_3 (70%, 2 mL) under microwave heating (Milestone MicroSYNTH Microwave lab station equipped with an optical fiber temperature control and HPR-1000/6M six position high pressure reactor, Bergamo, Italy).
- Dynamic Light Scattering experiments were carried out by using a Zetasizer NanoZS, Malvern, UK, operating in a particle size range from 0.6 nm to 6 μm and equipped by a laser He-Ne with λ = 633nm.
- ^{1}H and ^{13}C NMR spectra were recorded on JEOL ECP 400 (^{1}H at 399.968, ^{13}C at 100.572 MHz) spectrometer.
- Mass spectra with electrospray ionization (ESI) were recorded on a 3100 Mass Detector (Waters), operating in positive or negative ion mode, with methanol as the carrier solvent.

- The proton $1/T_1$ NMRD profiles were measured on a fast field-cycling Stelar Smart Tracer relaxometer over a continuum of magnetic field strengths from 0.01 MHz to 10 MHz (0.00024 to 0.25 T). The relaxometer operates under computer control with an absolute uncertainty in $1/T_1$ of ± 1%. Additional data points in the range 15-70 MHz (0.37-1.75 T) were obtained on a Stelar Spinmaster console connected to WP-80 magnet (80 MHz (2 T)).
- MR images at 1 T were acquired with an Aspect MRI System (Aspect Magnet Technologies Ltd., Netanya, Israel) consisting of a NdFeB magnet, equipped with a solenoid coils 35 mm inner diameter. MR images were acquired using a standard T_1-weighted spin-echo sequence, using the following parameters: TR/TE/NEX 100/7/8, FOV 30 mm, matrix 128x128, slice thickness 5 mm.

2. Synthesis

Per-6-iodo-β-cyclodextrin was synthesized from commercial β-cyclodextrin according to the procedure reported by Gadelle *et al.*[1] The synthesis of per-6-thio-β-cyclodextrin was accomplished according to Rojas *et al.*[2]

β-cyclodextrin nanocapsules were prepared following a modification of the procedure described by Jones *et al.*,[3] as reported below.

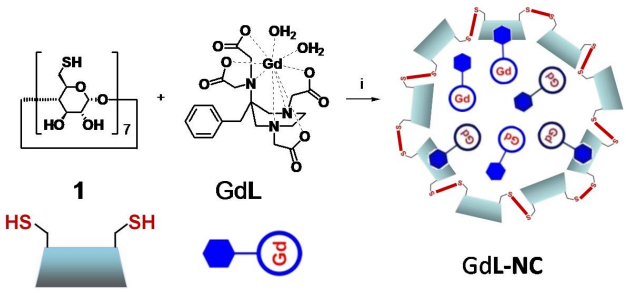

Fig. S1 Reaction scheme for the synthesis of Gd-loaded β-CD nanocapsules.

Synthesis of Gd**L-NC**

Per-6-thio-β-cyclodextrin **1** (100 mg, 0.08 mmol) was dissolved in NaOH 0.1 M (40 ml), and the mixture was stirred until complete dissolution. Gd**L** (255 mg, 0.40 mmol) was added and the solution was sonicated for 30 min. The reaction mixture was vigorously stirred at open air and RT for 24 h. After acidification to pH 8 with 1 M HCl, the suspension was transferred into a dialysis tube and dialysed against water (1 L). The water was changed every 24 h, until no free Gd^{3+} was detected by relaxometric analysis. The dialysed mixture was filtered and freeze-dried, yielding the desired nanocapsules (176 mg, 176 w% with respect to the initial **1**).

ICP-MS (of a 4.6 mg/ml solution): [Gd] = 0.65 mM, corresponding to 0.14 $mmol_{Gd}$ / $g_{GdL\text{-}NC}$.

[1] A. Gadelle and J. Defaye, *Angew. Chem. Int. Ed. Engl.*, 1991, **30**, 78.

[2] M. T. Rojas, R. Königer, J. F. Stoddart and A. E. Kaifer, *J. Am. Chem. Soc.*, 1995, **117**, 336.

[3] L. C. Jones, W. M. Lackowski, Y. Vasilyeva, K. Wilson and V. Chechik, *Chem. Commun.*, 2009, 1377.

3. TEM images

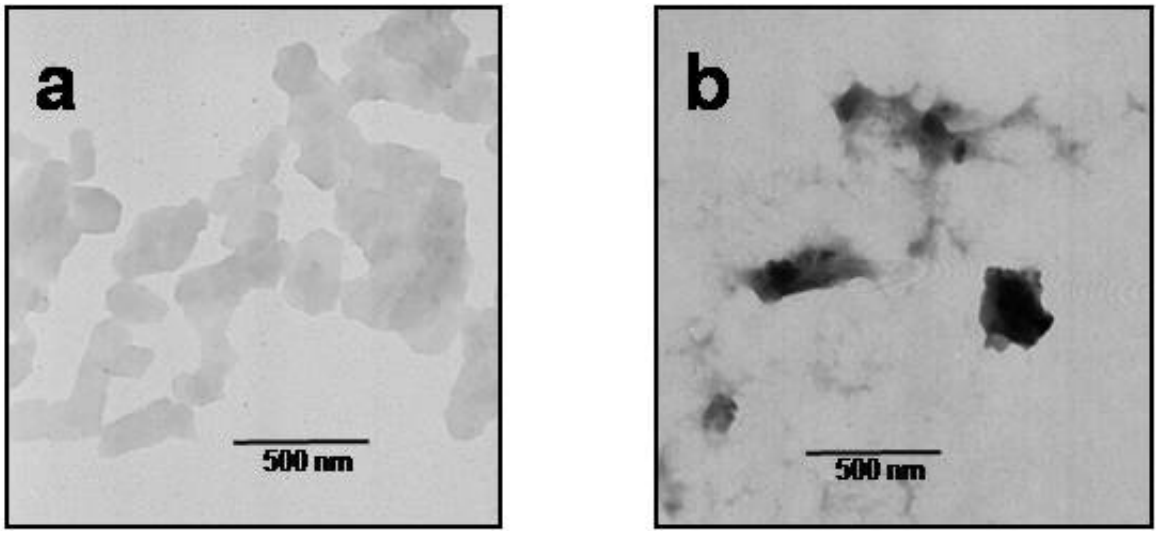

Fig. S2. TEM images: a) empty nanocapsules (**NC**) and b) Gd**L**-loaded nanocapsules (Gd**L**-**NC**).

4. DLS measurements

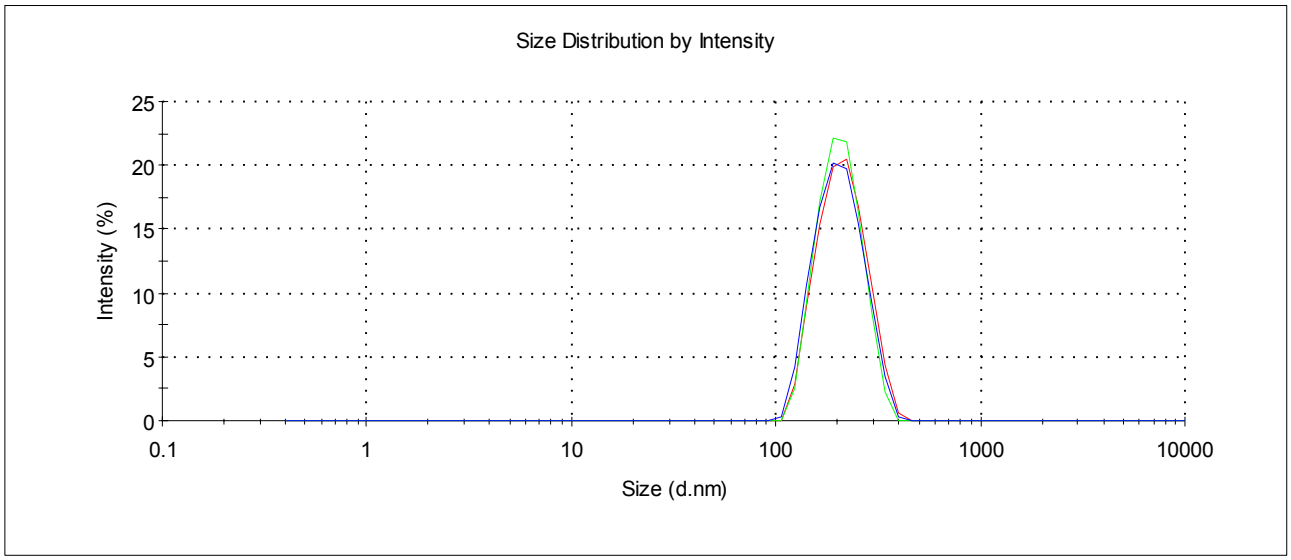

Fig. S3 DLS measurements for the empty capsules **NC** (0.1 mg/L aqueous solution, average diameter size 233±2 nm, PDI 0.27±0.01).

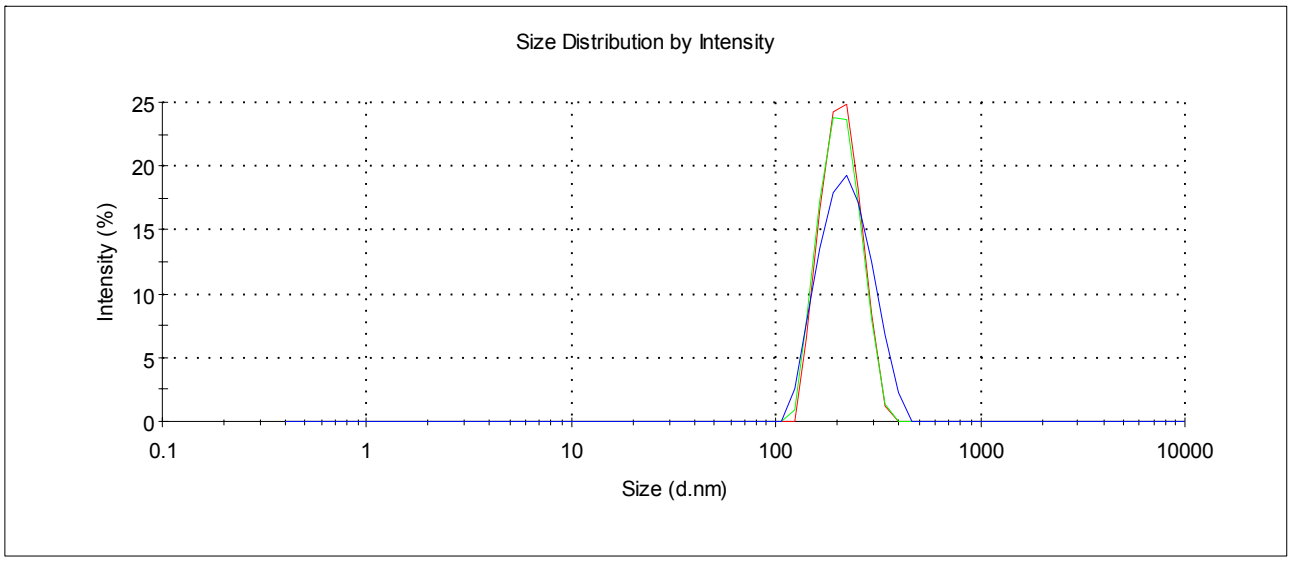

Fig. S4 DLS measurements for Gd**L-NC** (0.1 mg/L aqueous solution, average diameter size 238±6 nm, PDI 0.27±0.02).

5. Relaxometric characterizations and analyses

5.1. Gd**L**

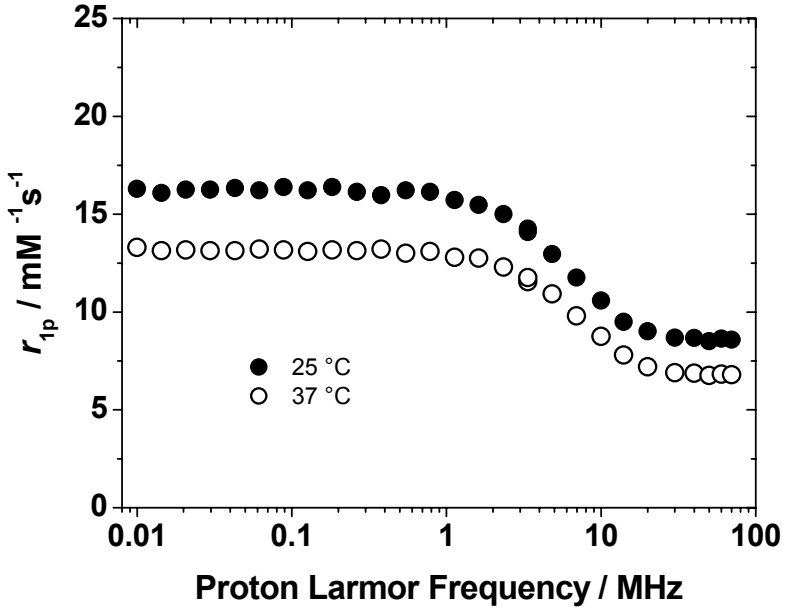

Fig. S5 ^{1}H NMRD profiles at 25 and 37 °C of the complex Gd**L** at pH = 7.5.

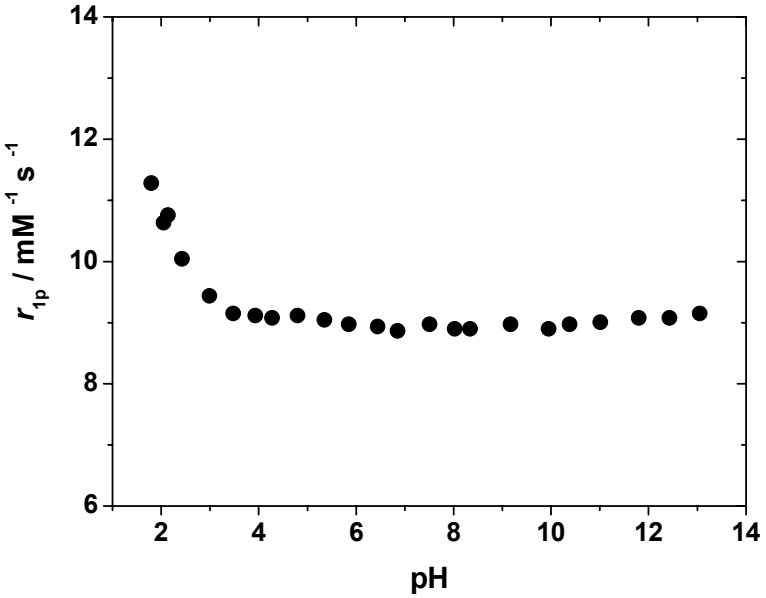

Fig. S6 pH-dependence of r_{1p} of the complex Gd**L** at 25 °C and 20 MHz.

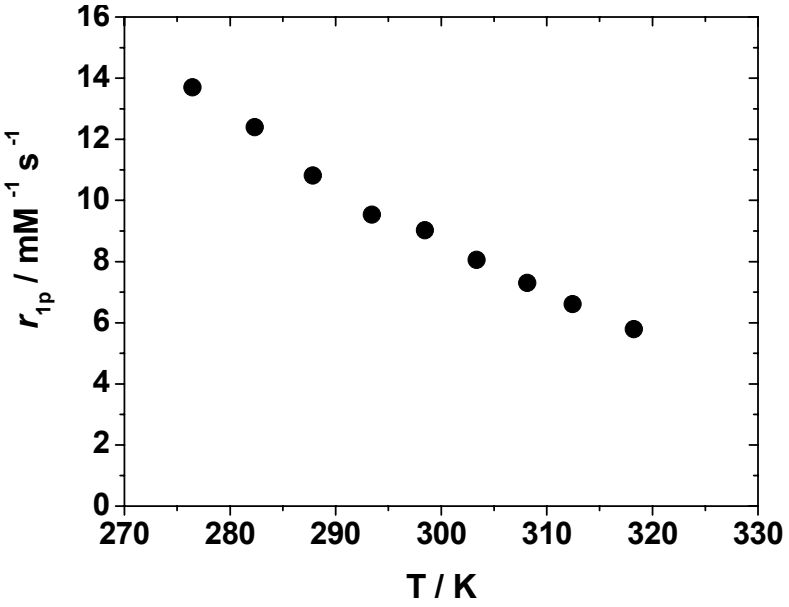

Fig. S7 Temperature-dependence of r_{1p} of the complex Gd**L** at 20 MHz and pH 6.7.

5.2. Gd**L** + β-CD

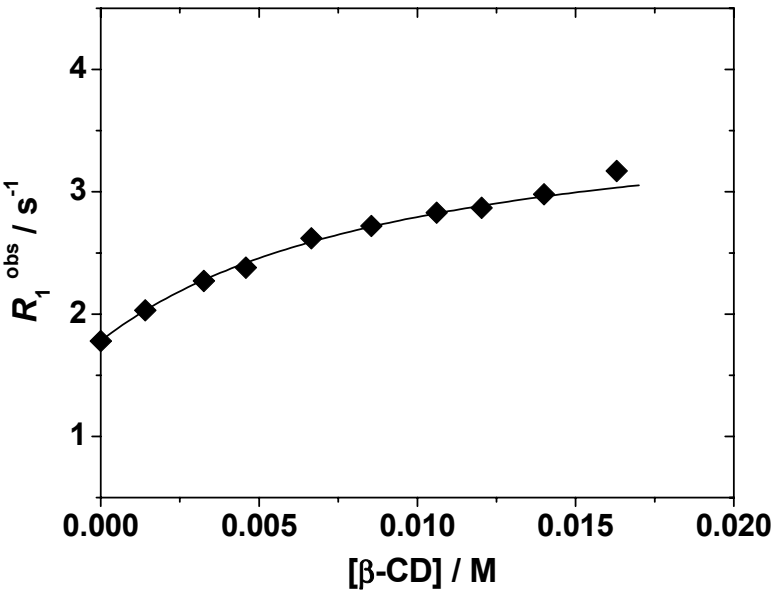

Fig. S8 Titration of Gd**L** (0.154 mM) with β-CD (0-16.3 mM) at 25 °C, 20 MHz and pH 7.8. Fitting of the data provides the binding constant (K_a = 103±1 M^{-1}) and the relaxivity of the adduct (r_b = 22±0.6 mM^{-1} s^{-1}).

5.3. Gd-loaded nanocapsules Gd**L-NC**

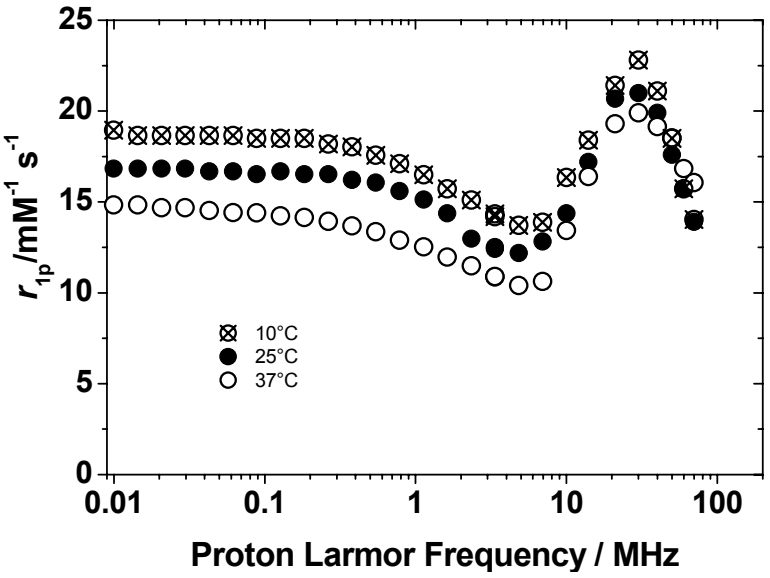

Fig. S9 ^{1}H NMRD profiles at 10, 25 and 37 °C of Gd**L-NC** (4.6 mg/mL aqueous solution) at pH = 8.5.

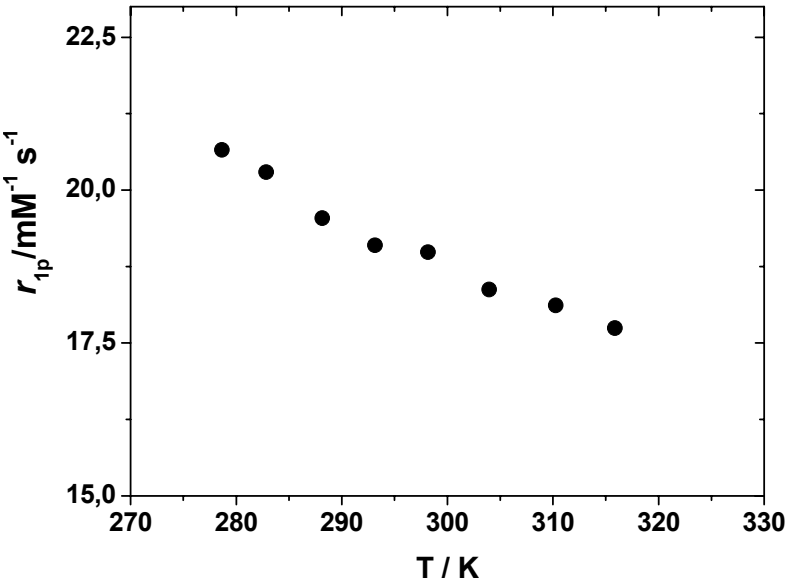

Fig. S10 Temperature-dependence of r_{1p} of Gd**L-NC** (4.6 mg/mL aqueous solution) at 20 MHz and pH = 8.6.

5.4. Reduction kinetics experiments

To an aqueous solution of Gd**L-NC** (1.1 mL, 4.6 mg/mL) at 37 °C and pH 7 was added TCEP (0.028 mmol, 57 µL of a 0.5 mM aqueous solution at pH 7). The mixture was left under magnetic stirring at 37 °C. The R_1 data were measured at 30 MHz and 37 °C.

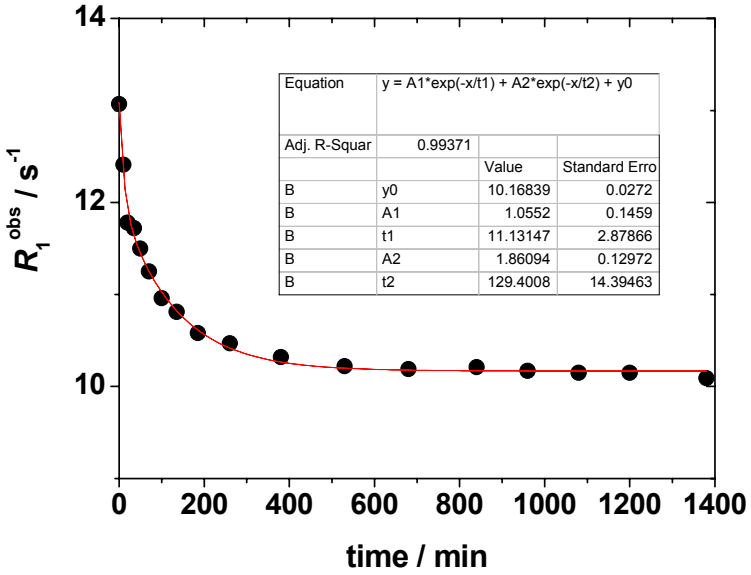

Fig. S11 Reduction kinetics experiment of Gd**L-NC** (4.6 mg/mL aqueous solution) with TCEP. [Gd] = 0.65 mM, 37 °C, 20 MHz and pH = 7.

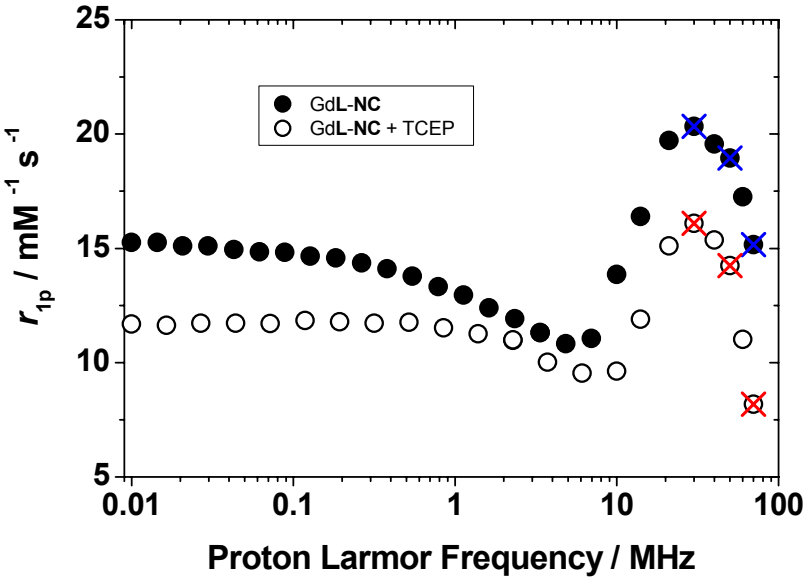

Fig. S12 ^{1}H NMRD profiles at 37 °C of Gd**L-NC** (4.6 mg/mL aqueous solution) before and after reduction with TCEP (pH = 8.5). Marked values refer to data at 0.70, 1.17 and 1.64 T compared in Fig. 3.

The profile acquired after several hours from the addition of the reducing agent TCEP shows a peak at about 20 MHz that is due to the interaction of Gd**L** not only with monomeric cyclodextrin but also with some oligo-CD fragments present in the final mixture. The relatively high molecular weight of the adducts between such species and Gd**L** is responsible for the slightly higher relaxivity than that obtained for a 1:6 Gd**L**/β-CD solution.

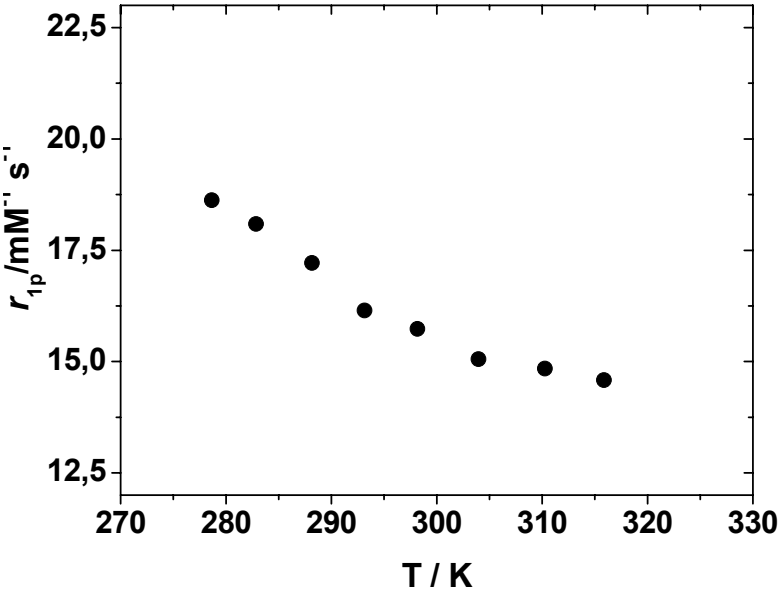

Fig. S13 Temperature-dependence of r_{1p} of a water solution (4.6 mg/mL) of Gd**L-NC** after reduction with TCEP, at 20 MHz and pH = 8.5.