

## Electronic Supplementary Information

### Nanoscale hydrophilic colloids with high relaxivity and low cytotoxicity based on Gd(III) complexes with Keplerate polyanions

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#### 1. Materials and methods

##### 1.1. Materials

Commercial chemicals Gd(NO<sub>3</sub>)<sub>3</sub>·6H<sub>2</sub>O (99.9wt%), NaOH (pellets, 98.5wt%), anhydrous (C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>O (99.7 wt%), N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>SO<sub>4</sub> (99 wt%), (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O, Pluronic F-127 (suitable for cell culture), Pluronic F-68 (suitable for cell culture), Pluronic P-123 were purchased from Sigma Aldrich. Absolute C<sub>2</sub>H<sub>5</sub>OH (99.5%), CH<sub>3</sub>COONH<sub>4</sub> (98 wt%), ammonium hydroxide (28-30 wt%), H<sub>2</sub>SO<sub>4</sub> (96 wt%) were obtained from Acros Organics. The purchased chemicals were used as received without further purification.

Cell culture WI-38 VA 13 subline 2RA (human embryo lung) was obtained from collection of the institute of cytology of Russian Academy of Sciences. The standard nutrient medium “Igla” with an addition of 10% calf serum and 1% of indispensable amino acids was purchased from Institute of poliomyelitis and viral encephalitis named after M.P. Chumakov (Moscow, Russia).

##### 1.2. Syntheses

###### 1.2.1. Synthesis of keplerate

The ammonium salt of  $[\{(Mo^{VI})Mo^{VI}_5O_{21}(H_2O)_6\}_{12}\{Mo^{V}_2O_4(CH_3COO)\}_{30}]^{42-}$  (Kp) was synthesized in accordance with the previously reported procedure [15]. The structure of Kp was verified by X-ray and Raman spectroscopy data (Fig. S1). Raman (cm<sup>-1</sup>): solid 956 (m), 942 (m), 870 (s), 829 (sh), 714 (w), 375 (s), 307 (m); aqueous solution 960 (m), 948 (m), 878 (s), 847 (sh), 712 (w), 371 (s), 311 (m).

###### 1.2.2. Synthesis of Gd<sub>14</sub>[{Mo<sub>6</sub>O<sub>21</sub>(H<sub>2</sub>O)<sub>6</sub>}]<sub>12</sub>{Mo<sub>2</sub>O<sub>4</sub>(CH<sub>3</sub>COO)}<sub>30</sub>·200H<sub>2</sub>O

The ammonium salt of Kp (0.500 g, 0.018 mmol) was dissolved in 50 mL of H<sub>2</sub>O to form dark solution. Solid gadolinium chloride (0.540 g, 1,450 mmol) was added, dark brown precipitate was formed immediately. The solution was stirred for 30 min at room temperature to complete precipitation. The product was isolated by filtration on a glass frit, washed successively with ethanol and diethyl ether and dried in air. Yield: 0.490g (98% based on Mo). Analysis for

$C_{60}H_{517}O_{704}Mo_{132}Gd_{14}$ : calcd: C 2.6, H 2.3, Mo 46.6, Gd 8.0; found: C 2.5, H 2.3, Mo 46.4, Gd 8.2. Raman ( $cm^{-1}$ ): solid 950 (w, br), 878 (s), 713 (w), 371 (m), 315 (w) (Fig. S2).

### 1.2.3. Synthesis of the $Gd_x(Kp)_y$ -based colloids.

The colloids were synthesized by simple mixing of the aqueous solutions of the triblock copolymers 9.92 mL for F-127 (0.081 mM), F-68 (0.121 mM) and P-123 (0.501 mM) with 0.07 mL of  $(NH_4)_4Kp$  (1 mM) with the following admixture of 0.015 mL of  $Gd(NO_3)_3$  (55.6 mM) under the stirring.

## 1.3. Methods

Raman spectra were recorded on a Triplemate spectrometer (Spex, USA) equipped with a multichannel detector LN-1340 PB (Princeton Instruments, USA). Elemental analyses for C and H were performed by the Analytical Laboratory of NIIC SB RAS (Novosibirsk) on a Eurovector EA3000 CHN analyzer. Inductively coupled plasma atomic emission spectroscopy for Mo and Gd was performed by the Analytical Laboratory of NIIC SB RAS (Novosibirsk) on an iCAP 6500 ICP Spectrometer (Thermo Scientific).

X-ray powder diffraction (PXRD) measurements were performed on a Bruker D8 Advance diffractometer equipped with Vario attachment and Vantec linear PSD, using Cu radiation (40 kV, 40 mA) monochromated by the curved Johansson monochromator ( $\lambda$  Cu  $K_{\alpha 1}$  1.5406 Å). Room-temperature data were collected in the reflection mode with a flat-plate sample. The sample was applied in liquid form on the surface of standard zero diffraction silicon plate. After drying the layer applied on top of it a few more layers were placed to increase the total amount of the sample. The sample was kept spinning (15 rpm) throughout the data collection. Patterns were recorded in the  $2\theta$  range between  $3^\circ$  and  $100^\circ$ , in  $0.008^\circ$  steps, with a step time of 0.1–4.0s.

Dynamic light scattering (DLS) measurements were performed by means of the Malvern Mastersize 2000 particle analyzer. A He–Ne laser operating at 633 nm wavelength and emitting vertically polarized light was used as a light source. The measured autocorrelation functions were analyzed by Malvern DTS software and the second-order cumulant expansion methods. The effective hydrodynamic radius (RH) was calculated by the Einstein–Stokes relation from the first cumulant:  $D = kBT/6\pi\eta RH$ , where  $D$  is the diffusion coefficient,  $kB$  is the Boltzmann constant,  $T$  is the absolute temperature, and  $\eta$  is the viscosity. The diffusion coefficient was measured at least three times for each sample. The average error in these experiments is approximately 4%. The samples for DLS study were prepared from deionized water, sonicated for 60 minutes and equilibrated at  $25.0\pm 0.1^\circ C$  before DLS and zeta-potential measurements.

Small angle X-Ray scattering (SAXS) data for samples were collected with the Bruker AXS Nanostar SAXS system using  $CuK_{\alpha}$  ( $\lambda$  1.5418Å) radiation from a 2.2 kW X-ray tube (35

kV, 40 mA) coupled with Gobbel mirrors optics and a HiStar 2D area detector. The beam was collimated using three pinholes with apertures of 800, 450 and 700  $\mu\text{m}$ . The instrument was operated with a sample-to-detector distance of 63.9 cm to provide data at angles  $0.1^\circ < 2\theta < 4.8^\circ$ , which correspond to  $0.007 \text{ \AA}^{-1} < q < 0.34 \text{ \AA}^{-1}$ . The value of  $q$  is proportional to the inverse of the length scale ( $q = (4\pi/\lambda)\sin(\theta)$  in units of  $\text{\AA}^{-1}$ ). Scattering patterns were obtained for the samples at  $23^\circ\text{C}$  in an evacuated chamber. The measurements were performed in transition mode with the use of glass capillaries filled by liquid samples. The capillaries (2 mm diameter) were sealed and put into evacuated chamber by means of the holders. For each sample, two experiments were performed, allowing to control the quality of the experiments. The results of two experiments are summarized, so that the total time of each experiment was equal to 50000 s. Integration of two-dimensional pictures scattering was performed using a software package SAXS [Small Angle X-ray Scattering, Version 4.0. Software Reference Manual, 2000, M86-E00005-0600, Bruker AXS Inc.]. Data analysis performed by programs SASView [S1] and PRIMUS [S2].

The UV-vis measurements were conducted on Lambda 35 spectrophotometer (Perkin-Elmer, USA) using a 10 mm cuvettes at room temperature.

pHs of the solutions were controlled with Microprocessor pH meter «pH 212» (Hanna Instruments, Germany) The pH-meter was calibrated with standard aqueous buffer solutions (pHs 7.01 and 4.01).

TEM images were obtained by use of Hitachi HT7700 (Japan) at an accelerating voltage of 100 kV.

#### *1.4. Cell viability evaluation*

Cell viability of human embryo lung cells (WI-38 VA 13 subline 2RA) towards studied nanoparticles was determined by means of multifunctional system Cytell Cell Imaging (GE Healthcare Life Sciences, Sweden) using application Cell Viability BioApp. The cells were dispersed in culture medium at the cell conc. 200000 cells per ml. Then, the cells were seeded into 96-well plates at  $150 \mu\text{l}$  or  $30 \times 10^3$  cells per well and cultivated in  $\text{CO}_2$ -incubator at  $37^\circ\text{C}$  for 24 hours. After removing the culture medium and their redispersion in the fresh culture medium the cells were exposed by 50  $\mu\text{l}$  of F-127- $\text{Gd}_x(\text{Kp})_y$ , P-123- $\text{Gd}_x(\text{Kp})_y$ , F-68- $\text{Gd}_x(\text{Kp})_y$  colloids in accordance with Cell Viability Bio App protocol, were Gd(III) and Kp concentrations were adjusted at 0.08, 0.04 and 0.02 mM for Gd(III), 0.018, 0.0088 and 0.0044 mM of Kp. Thus,  $30 \times 10^3$  cells per well were exposed by 0.02, 0.01 and 0.005 mM of Gd(III), 0.0045, 0.0022 and 0.0011 mM of Kp. The concentrations of the triblock copolymers in the cellular samples were varied within 0.02, 0.01 and 0.005 mM for F-127, 0.125, 0.0625, 0.0313 for P-123, 0.03, 0.015, 0.0075 mM for F-68. After 45 minute exposure the percentage of viable Wi-38 cells was

calculated with the use of Cytell Cell Imaging system. The experiments were repeated three times. The intact cells cultivated simultaneously with the studied ones served as a reference. The fraction of the grown-up cells was expressed in % vs. reference cells.

### 1.5. Relaxometric measurements

The transverse relaxation times  $T_2$  of water molecule protons in studied solutions were measured using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence [S3]. The longitudinal relaxation times  $T_1$  of water molecule protons were defined with the use of the analyzer's inversion-recovery pulse sequence with 20 data collected for fitting [S4]. The experiments were performed at  $25 \pm 0.5$  °C on an MQ20 Minispec NMR Analyzer (Bruker, Germany) at a field strength of 0.47 T (19.65 MHz). The classical inversion-recovery method using  $180^\circ$ - $\tau$ - $90^\circ$  pulse sequence was performed to determine  $T_1$ . The repetition delay was chosen to be at least five times  $T_1$ . The dead time was 7  $\mu$ s. Four scans were acquired for each measurement.  $T_2$  was measured with a Carr-Purcell-Meiboom-Gill (CPMG) sequence with a  $90$ – $180^\circ$  pulse gap of 1.0 ms. The  $90^\circ$  pulse was 2.64  $\mu$ s and  $180^\circ$  pulse was 5.16  $\mu$ s in length.

The relative measurement deviation for transverse and longitudinal relaxation times does not exceed 3%. Longitudinal and transverse relaxivities  $r_{1,2}$  are defined as:

$$r_i = [(1/T_i)_{\text{meas}} - (1/T_i)_{\text{dia}}]/C \quad i = 1, 2$$

where  $(1/T_i)_{\text{meas}}$  is the value measured for the sample with concentration  $C$  (mM) of magnetic centres,  $(1/T_i)_{\text{dia}}$  refers to the nuclear relaxation rate of pure water protons,  $r_1$ ,  $r_2$  are longitudinal and transverse relaxivities, respectively.

$T_1$  and  $T_2$  relaxation times of water protons were also measured by means of whole body 1.5 T scanner (Excel Art Vantage Atlas X, Toshiba, Japan) equipped with 65-cm horizontal bore size corresponding to a proton resonance frequency of 63.58 MHz. The special NMR sample tubes (Wilmad glass company, INC) were inserted in the centre of 20 cm cubic phantom filled with distilled water.

$T_1$  measurements were performed using inversion-recovery sequence with TE =10 ms (FSE+10\_slit) and various TR and TI : TR = 6000, 10000, 12000, 15000 ms and TI = 18, 30, 50, 100, 150, 200, 300, 500, 700, 1000, 1500, 2000, 3000, 5000, 10000 ms.

$T_2$  measurements were done with SE sequences with TR= 6000, 10000 ms TE= 15, 20, 30, 60,100, 120, 250, 500, 1000 ms, The Field of View was 200-mm, the matrix size was  $128 \times 256$ , and the slice thickness was 2 mm.

All  $T_1$  and  $T_2$  measurements were recorded at 23-25°C.

The dilution of the synthesized colloids was applied for the variation of Gd(III) concentration from 0.08 to 0.02 mM for plotting  $1/T_1$  and  $1/T_2$  versus Gd(III) concentration in the Fig.3.

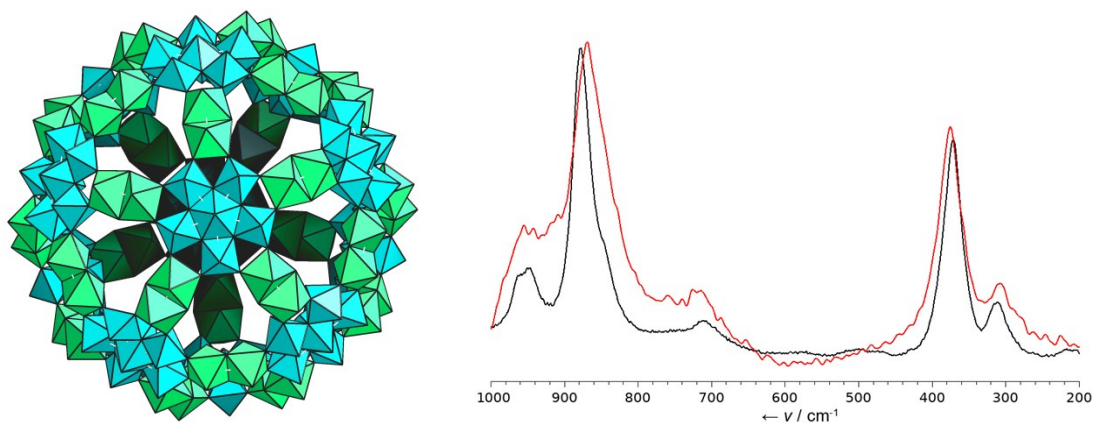


Figure S1. (*left*) The structure of Kp, estimated by X-ray. (*right*) Raman spectra of Kp – in solution (black) and in solid state (red).

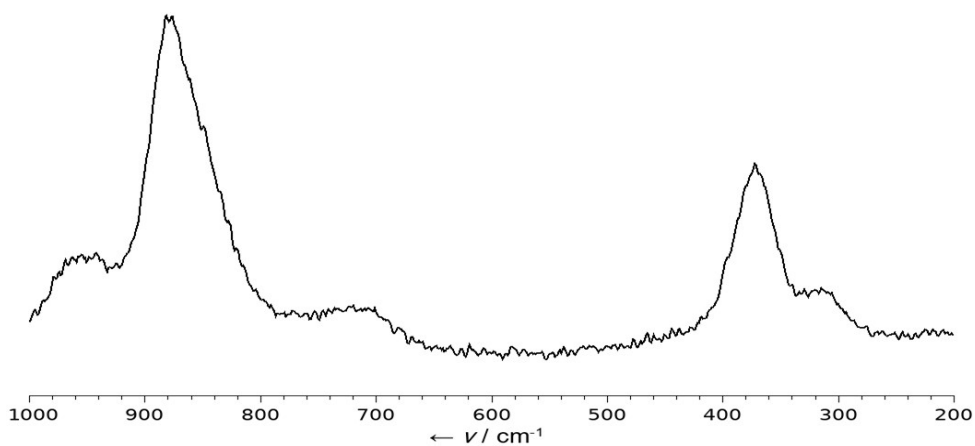


Figure S2. Raman spectrum of  $Gd_{14}Kp$  in solid state.

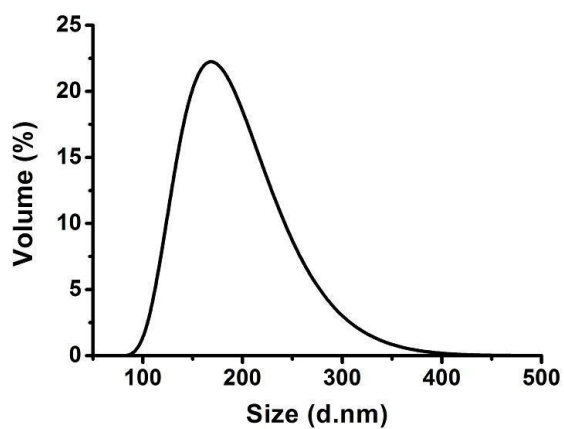


Figure S3. Dynamic light scattering (DLS) measurements of the colloids  $Gd_x(Kp)_y$  ( $\zeta = -22 \pm 1 \text{ mV}$ ) at 0.007 and 0.08 mM for Kp and  $Gd^{3+}$ , respectively.

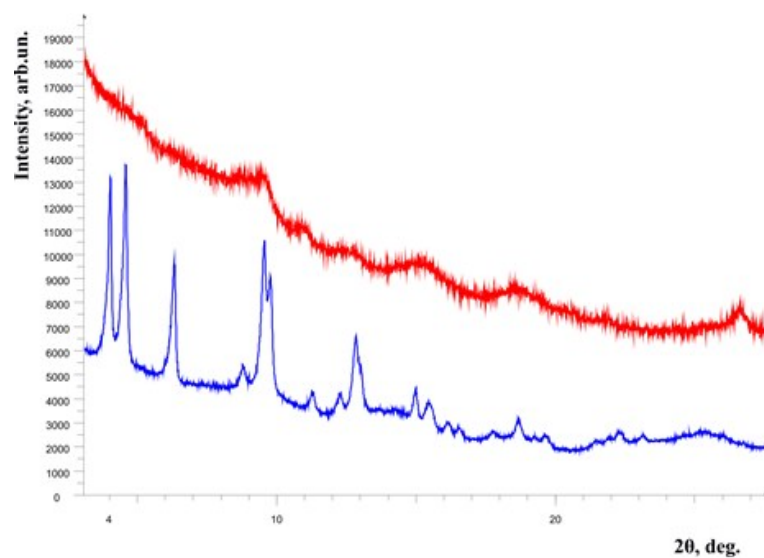
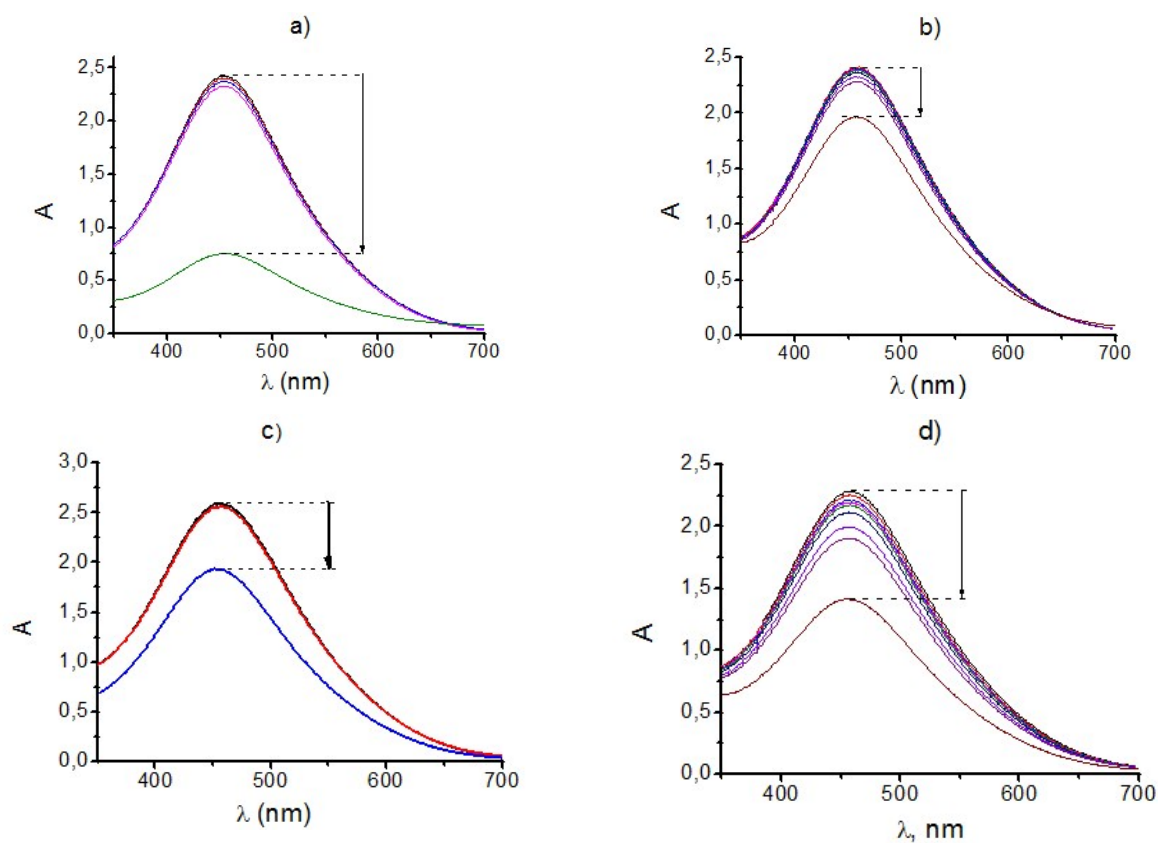


Figure S4. The experimental X-ray powder diffraction patterns of powder sample of ammonium salt of Kp (blue curve) and dried sample of the  $Gd_x(Kp)_y$  colloids (red curve).



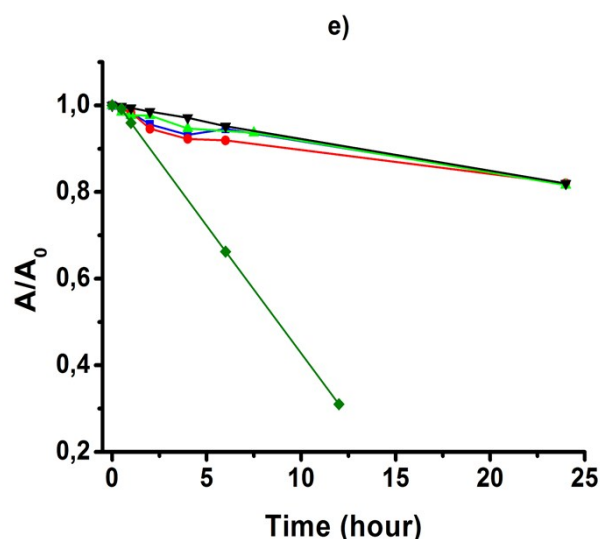


Figure S5. The UV-Vis spectra of the aqueous colloids  $Gd_x(Kp)_y$  (0.007 and 0.08 mM for Kp and  $Gd^{3+}$ , respectively) as a function of time, monitored within 24 hours: (a) without triblock copolymer; (b) F-127 (0,08 mM); (c) P-123 (0,5 mM); (d) F-68 (0,12 mM); e) The  $A/A_0$  values of the F-127- $Gd_x(Kp)_y$  colloids at different concentrations of the components as a function of time: Blue line - 0.01 Kp, 0.08 mM  $Gd^{3+}$ , 0.16 mM F-127; red line - 0.008 Kp, 0.06 mM  $Gd^{3+}$ , 0.16 mM F-127; green line - 0.007 Kp, 0.08 mM  $Gd^{3+}$ , 0.16 mM F-127; black line - 0.007 Kp, 0.008 mM  $Gd^{3+}$ , 0.08 mM F-127.

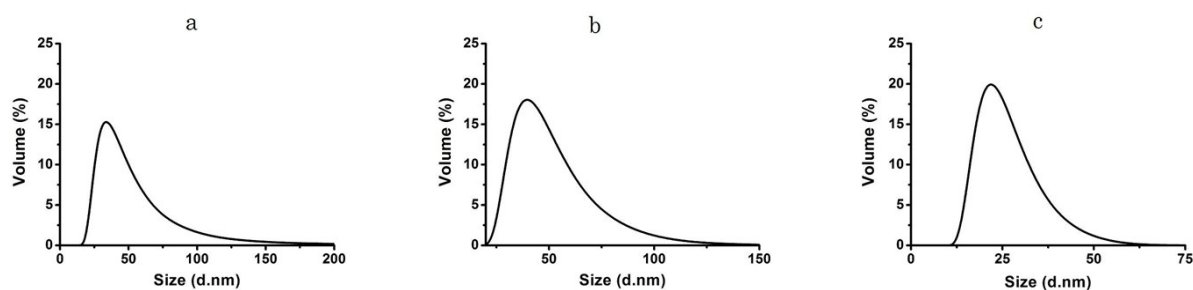


Figure S6. Size distribution from DLS measurements in the aqueous colloids  $Gd_x(Kp)_y$  (0.007 and 0.08 mM for Kp and  $Gd^{3+}$ , respectively) stabilized by: (a) F-127 (0,08 mM),  $d_{ev}$ =46.6, PDI=0.2; (b) F-68 (0,12 mM),  $d_{ev}$ =61.8 nm, PDI=0,15; (c) P-123 (0,5 mM),  $d_{ev}$ =24,5 nm, PDI=0.21.

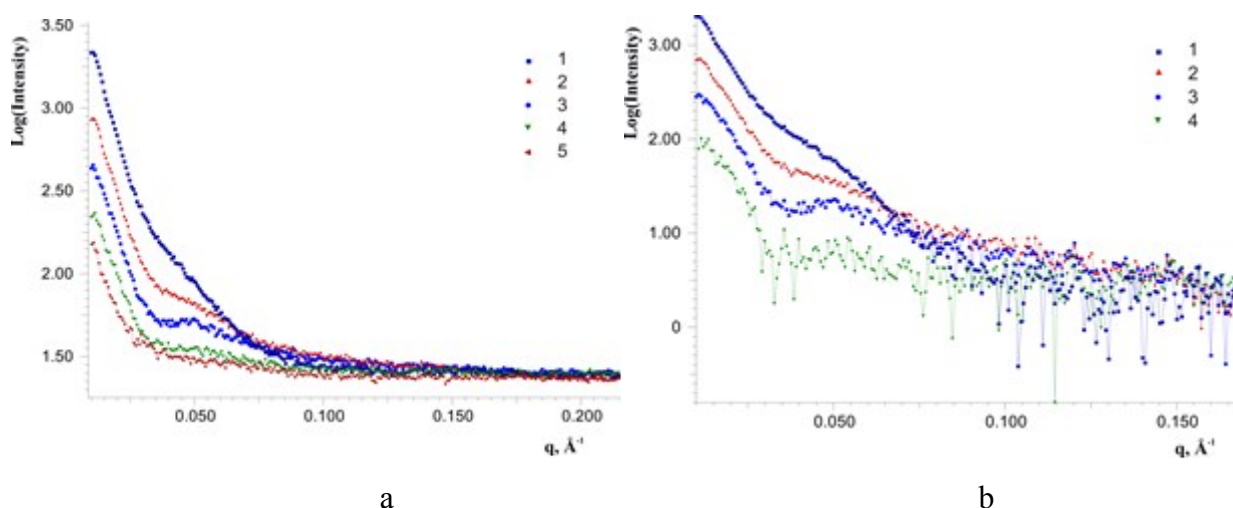


Figure S7. Experimental SAXS curves for aqueous micellar solutions, micellar solutions of  $(\text{NH}_4)_{42}\text{Kp}$  and P-123- $\text{Gd}_x(\text{Kp})_y$  colloids without (a) and after subtraction of water scattering (b):  $(\text{NH}_4)_{42}\text{Kp}$  (0.01mM) - P-123 (0.5mM) -  $\text{Gd}(\text{NO}_3)_3$  (0.06mM) (1);  $(\text{NH}_4)_{42}\text{Kp}$  (0.01mM) – P-123 (0.5mM) -  $\text{Gd}(\text{NO}_3)_3$  (0.03mM) (2);  $(\text{NH}_4)_{42}\text{Kp}$  (0.01mM) - P-123 (0.5mM) - $\text{Gd}(\text{NO}_3)_3$  (0.015mM) (3); aqueous P-123 (0.5mM) (4); water (5).

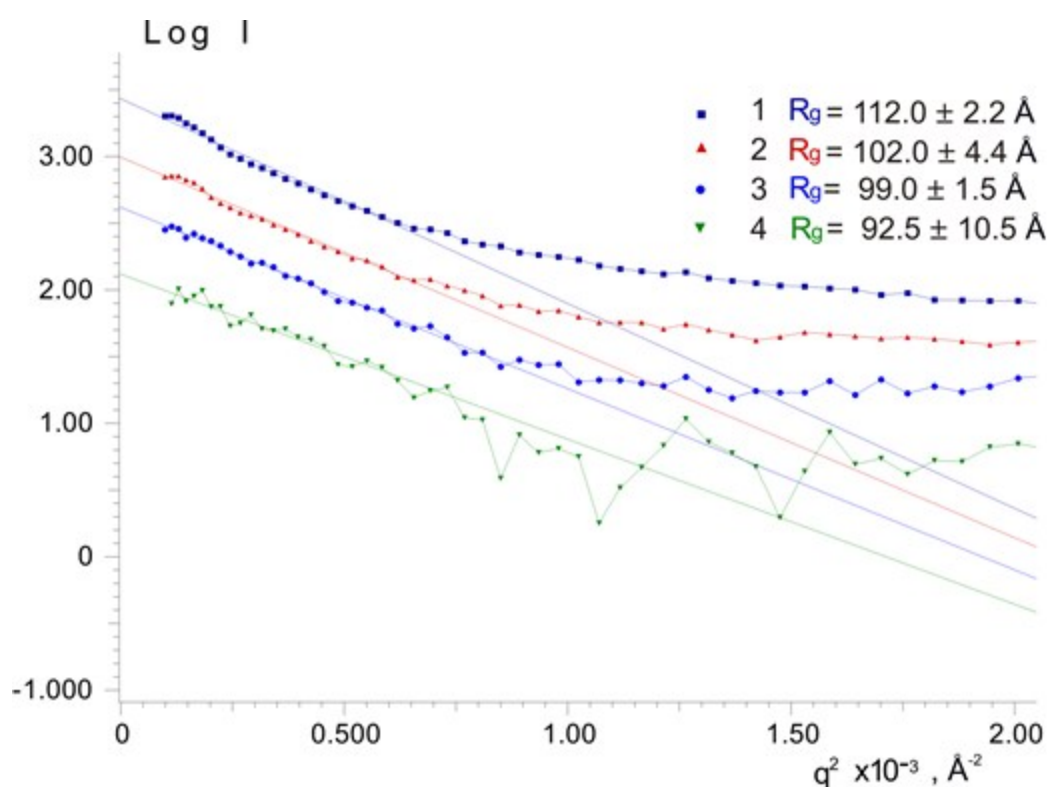


Figure S8. Guinier plot ( $\log I$  versus  $q^2$ ) of the SAXS data measured for the samples 1-4. Guinier plots ( $\log I$  versus  $q^2$ ) of the SAXS data (Fig.S8) indicates the presence of linear regions in all curves. The radius of gyration  $R_g$  was deduced from a weighted linear regression of the scattering curves. Under the assumption of the spherical symmetry of the particles, the average radii  $R_{\text{NP}}$  ( $R_{\text{NP}} = (\sqrt{5/3})R_g$ ) of the particles are 127.7, 131.6, 144.5 and 119.3Å for samples 1 to 4,



respectively (the samples designation is from Fig.S8). It is obvious that the increase in particle size and their interaction with each other is accompanied by the shifting to smaller angles of the peaks in the scattering curves (Fig. S7), which indicates the increased distances between the particles centers.

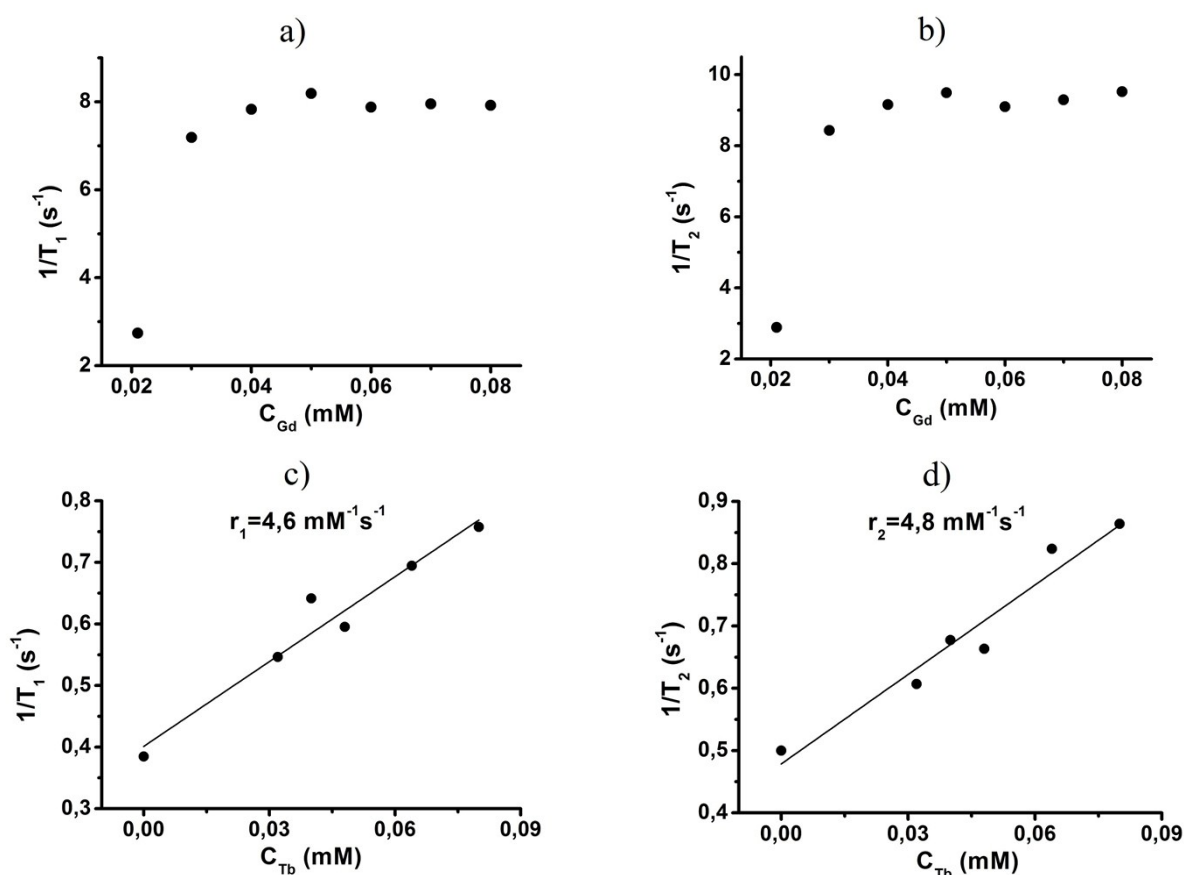


Figure S9.  $1/T_1$  (a) and  $1/T_2$  (b) values measured in F-127- $Gd_x(Kp)_y$  colloids at 0.08 mM F-127, 0.007 mM of Kp and various  $Gd^{3+}$  concentrations.

$1/T_1$  (c) and  $1/T_2$  (d) versus Tb(III) concentration measured in F-127- $Tb_x(Kp)_y$  aqueous colloids synthesized at 0.08 mM F-127, 0.007 mM Kp and 0.08 Tb(III).

The impact of the slowed down rotation regime in the high relaxivity of F-127- $Gd_x(Kp)_y$  is confirmed by low relaxivity ( $\sim 5 \text{ mM}^{-1} \text{ s}^{-1}$ ) for F-127- $Tb_x(Kp)_y$  (Fig.S9a, b, ESI), where electron spin reorientation ( $\tau_s$ ) is the correlation time.

### Notes and references

S1.UMD, UTK, NIST, ORNL, ISIS, ESS and ILL., 2009-2013, 39p.

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