Cell-Permeable MR Contrast Agents with Increased Intracellular Retention

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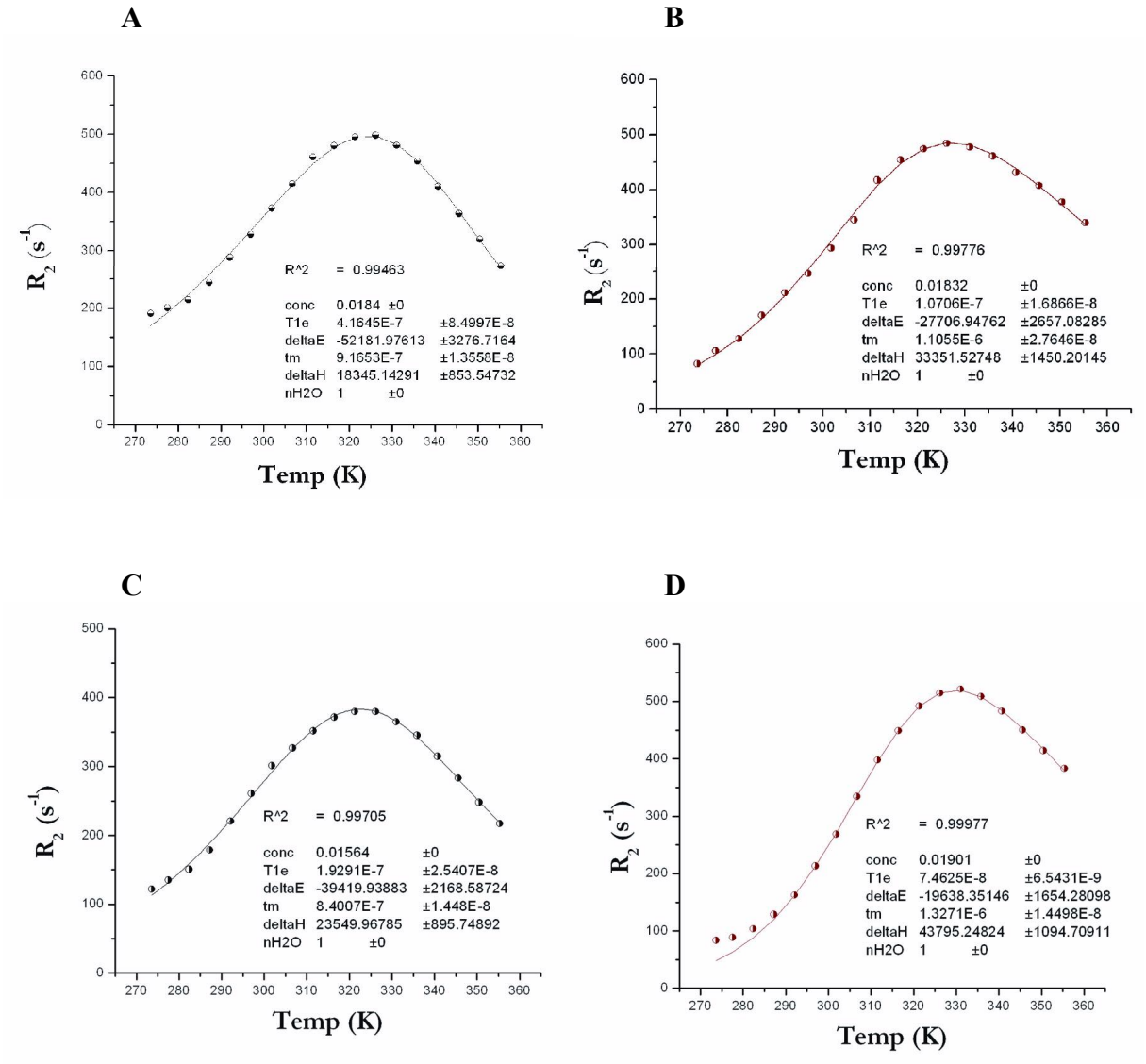
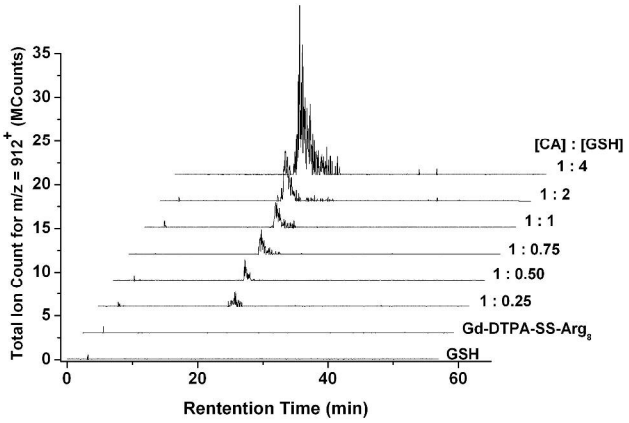
Determination of τ_m by ^{17}O Transverse Relaxation Rate Measurements. Samples of **1-4** were prepared at 15-20 mM concentrations in 1% ^{17}O enriched water (Medical Isotopes, Inc., Pelham, NH) adjusted to pH 7.40. Lock was achieved by means of an external D_2O standard. ^{17}O spectra were obtained at 54 MHz (number of averaged transients was 160 – 320 and relaxation delay was 400 ms) at temperatures ranging from 1 °C to 86 °C in 5 °C increments. The ^{17}O transverse relaxation rate was determined by obtaining the line width (in Hz) at half of the peak height, $\Delta\nu_{1/2}$, of the ^{17}O water signal and later fitting the data at 25 °C (references 22-24). This data and their respective fittings are shown below in Figure S1.



Supporting Figure S1. (A) Raw ^{17}O data and τ_m fitting of compound **1**. (B) Raw ^{17}O data and τ_m fitting of compound **2**. (C) Raw ^{17}O data and τ_m fitting of compound **3**. (D) Raw ^{17}O data and τ_m fitting of compound **4**. The relaxation data were fit to these four parameters: τ_m (water exchange rate), $\Delta H^{\ddagger}$ (activation enthalpy), T_{1e} (electronic relaxation rate), and ΔE_{T1e} (activation energy of T_{1e}).

In Vitro Cleavage. Determination of disulfide exchange using the total ion count (TIC) of **4** plus the addition of GSH with increasing amounts of GSH for 4 hours in PBS.



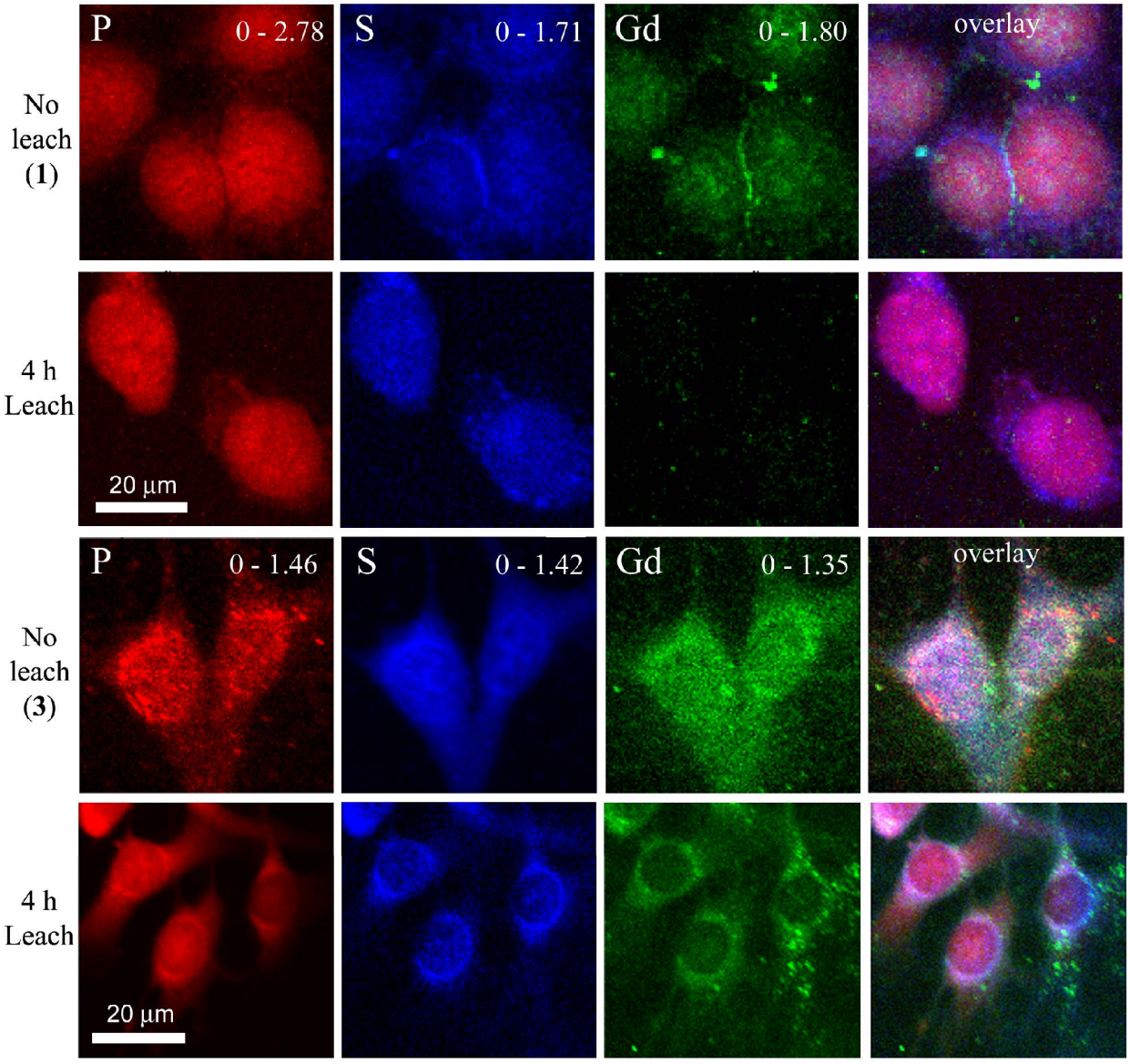
Supporting Figure S2. Treatment of **4** with increasing concentrations of GSH leads to disulfide exchange and subsequent product formation (Gd(III)-DTPA-GSH, $m/z = 912^{+}$).

Quantitation of Cell Uptake. Gadolinium ICP-MS data for complexes **1-4** associated with NIH/3T3 cells before (Initial Amt.) and after leaching. These data were used to produce Figure 3.

Complex	1	2	3	4
Initial Amt.(10^{-11} moles)	13.6 ±0.36[a]	15.3 ±1.0	2.45 ±0.22	2.23 ±0.04
Leach 1 (1 h)	3.39 ±0.03	3.87 ±0.15	1.18 ±0.09	1.12 ±0.16
Leach 2 (2 h)	3.04 ±0.21	3.58 ±0.03	1.16 ±0.02	1.06 ±0.02
Leach 3 (3 h)	2.77 ±0.26	3.27 ±0.10	1.05 ±0.02	0.97 ±0.07
Leach 4 (4 h)	3.56 ±0.22	3.26 ±0.24	1.03 ±0.05	0.94 ±0.04
Leach 5[b] (24 h)	2.35 ±0.08	2.59 ±0.06	0.97 ±0.06	0.92 ±0.03

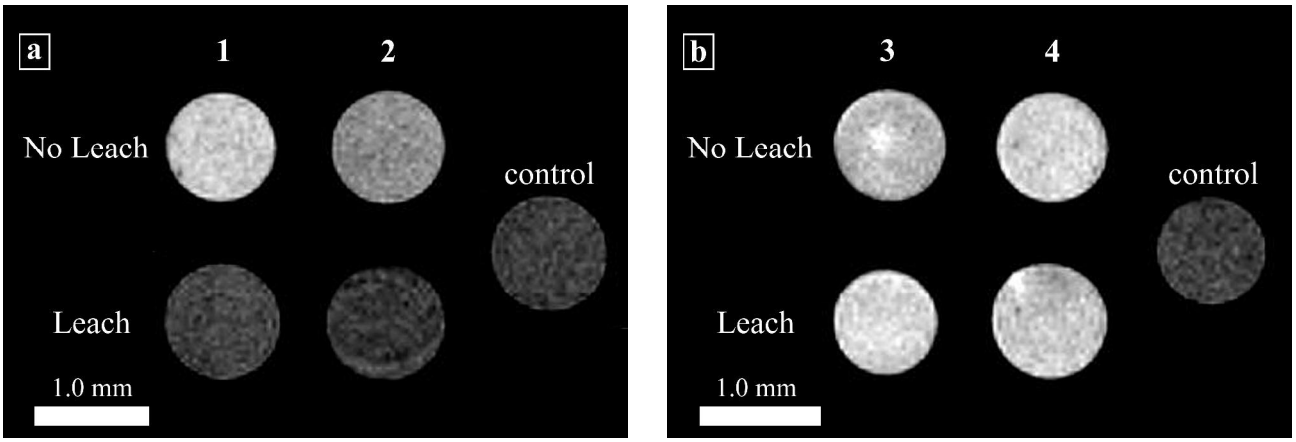
Supporting Table S1. All values given in 10^{-11} moles. [a]Error is given as +/- one standard deviation of triplicate runs. [b]A single wash allowed to leach for 24 hours.

SR-XRF Analysis. Synchrotron XRF analysis was performed to determine the cellular association of complexes **1**-**4**. These images are representative of the NIH/3T3 cells following the image acquisition procedure reported in the Experimental Procedures section. From our previous report, these high-resolution elemental maps provide pixel by pixel data sets that globally confirm, map, and quantify the Gd distribution within each sampled area *(24)*.



Supporting Figure S3. XRF images of NIH/3T3 cells treated with contrast agents **1** (top 8 images) and **3** (bottom 8 images) at time = 0 (No leach) and after 4 h (Leach). Phosphorus fluorescence is red, sulfur is blue, and gadolinium is green. Note that each column of images is scaled to its respective maximum value (displayed at the upper right corner and given in $\mu g/cm^2$).

MR Imaging and T_1 Analysis. To assess the feasibility of the disulfide linkage to increase cell retention and increase MR image contrast over an extended time period, NIH/3T3 cells were incubated with 1.0 mM of **1**-**4**. After the cells were harvested and placed in glass capillary tubes, they were visualized via MRI.



Sample name	Avg. T_1 time (ms)	Std. Dev. (ms)		*t* test comparison (value x *to* value y)	Meets 95% confidence level?
Fig. 7A: **1** *No Leach*	1847	94		contrast agent **1** No Leach *to* Leach	Yes
Fig. 7A: **1** *Leach*	2773	123		contrast agent **2** No Leach *to* Leach	Yes
Fig. 7A: **2** *No Leach*	2415	99		contrast agent **3** No Leach *to* Leach	No
Fig. 7A: **2** *Leach*	2824	138		contrast agent **4** No Leach *to* Leach	No
Fig. 7A: **control**	3017	111			
Fig. 7B: **3** *No Leach*	1687	63		contrast agent **1** Leach *to* Control	Yes
Fig. 7B: **3** *Leach*	1899	78		contrast agent **2** Leach *to* Control	Yes
Fig. 7B: **4** *No Leach*	1671	35		contrast agent **3** Leach *to* Control	Yes
Fig. 7B: **4** *Leach*	1834	59		contrast agent **4** Leach *to* Control	Yes
Fig. 7B: **control**	2912	72			

Figure 6 (in text) and accompanying table, **Supporting Table S2**. T_1 values and student *t*-tests confirming statistically significant differences. Student's *t*-tests were calculated in Origin 7 SR2 (Origin Lab, Northampton, MA) at a 95% confidence level with 8 degrees of freedom, independent testing, and a null hypothesis value (minimum acceptable time difference) of 100 ms. At least 5 slices per sample were averaged.