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Effects of Cations on HPTS Fluorescence and Quantification of Free Gadolinium Ions in Solution; Assessment of Intracellular Release of Gd3+ from Gd-Based MRI Contrast Agents

Angelo Scarciglia, Enza Di Gregorio *, Silvio Aime and Giuseppe Ferrauto

Molecular Imaging Center, Department of Molecular Biotechnologies and Health Sciences, University of Torino (IT), Via Nizza 52, 10126 Turin, Italy; angelo.scarciglia@edu.unito.it (A.S.); silvio.aime@unito.it (S.A.); giuseppe.ferrauto@unito.it (G.F.)

* Correspondence: enza.digregorio@unito.it; Tel.: +39-0116708459

Table S1. Parameters of the sigmoidal fitting for pH variation in different media

		Value	Standard Error
HEPES	A1	1.49×10^{5}	2.23×10^{6}
	A2	7.87×10^{7}	5.07×10^{5}
	x0	7.04	0.89×10^{-2}
	dx	0.44	0.73×10^{-2}
R^2		0.99852	
H_2O	A1	1.56×10^{5}	8.92×10^{5}
	A2	6.61×10^{7}	1.96×10^{6}
	x0	7.87	0.39×10^{-1}
	dx	0.40	0.04
R^2		0.99701	
BSA	A1	-1.48×10^{-5}	4.76×10^{4}
	A2	5.89×10^{7}	8.65×10^{5}
	x0	7.75	0.03
	dx	0.55	0.02
R^2		0.99907	

		Value	Standard Error
HEPES/NaCl	A1	-2.89×10^{5}	4.76×10^{5}
	A2	7.41×10^{7}	8.65×10^{5}
	x0	7.78	0.27×10^{-1}
	dx	0.42	0.02
R^2		0.99981	
NaCl	A1	-1.24971×10^{6}	2.89×10^{6}
	A2	7.38×10^{7}	3.33×10^{6}
	x0	7.15	0.08
	dx	0.57	0.08
R^2		0.98655	
PBS	A1	3.65×10^{6}	1.58×10^{6}
	A2	7.21×10^{7}	3.48×10^{5}
	x0	7.15	0.24×10^{-1}
	dx	0.44	1.46×10^{-2}
R^2		0.99957	
EQUATION		$y=(A2 + (A1-A2)) / (1+ e^{((x-x0)/dx)})$	

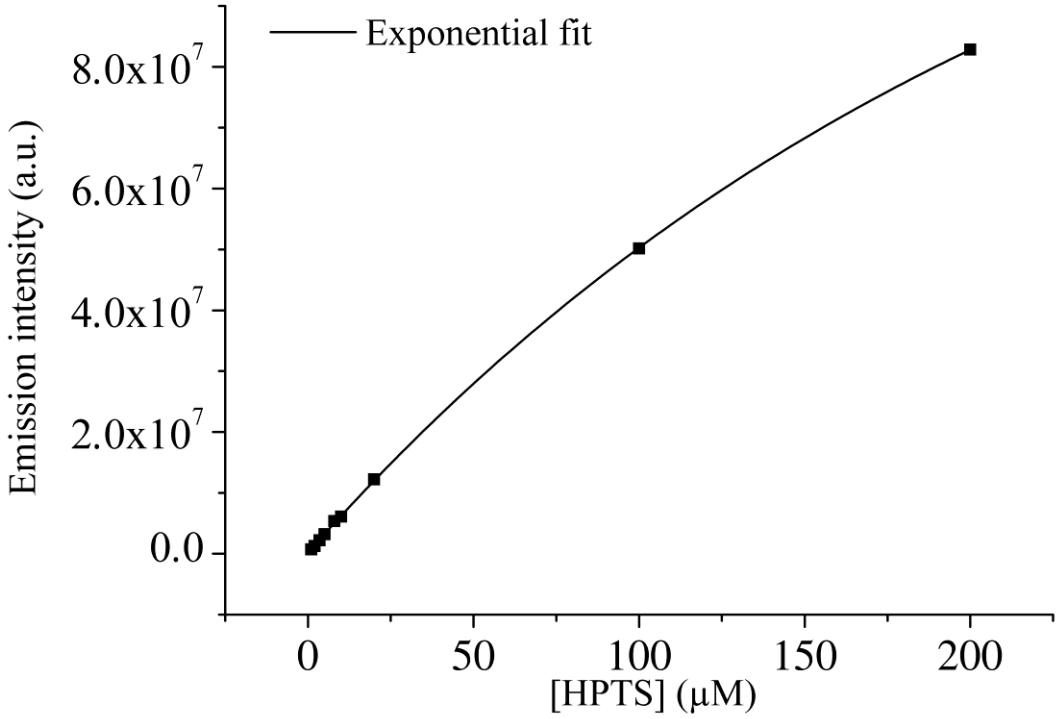

Figure S1. Exponential fit on variable HPTS concentration.

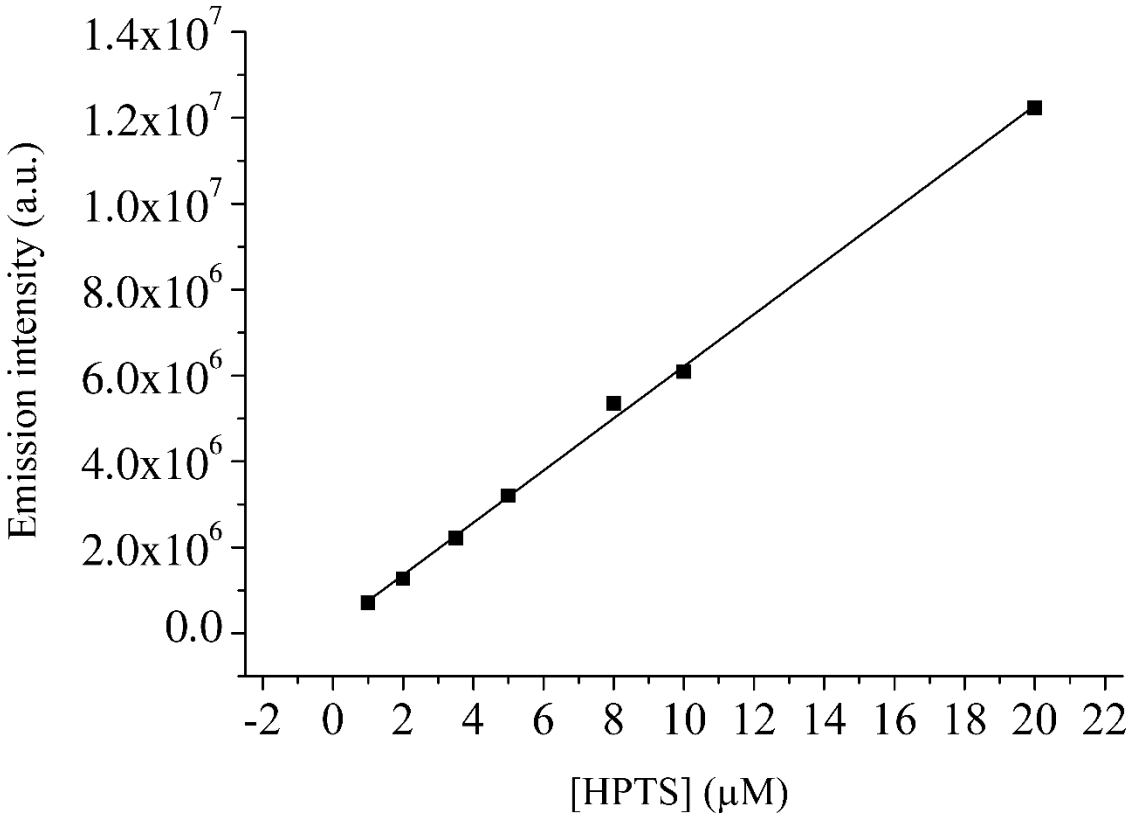

Figure S2. Focus on the range 1μM to 20μM.

Table S2. Parameters of the exponential fitting.

	yo	A	R0	Reduced Chi-Sqr	Adj. R-Square
Value	$1.43x10^{8}$	$-1.43x10^{8}$	$-4.32x10^{-3}$	$3.66x10^{10}$	0.99996
Standard error	$2.49x10^{6}$	$2.45x10^{6}$	$1.12x10^{-4}$		
Equation	$Y = y0 + Ae^{R0x}$				

Table S3. Parameters of the exponential fittings in Fig. 2A.

		Value	Standard Error
Na^{+}	y0	$1.45x10^{7}$	$1.19x10^{6}$
	A	$-1.15x10^{7}$	$1.13x10^{6}$
	R0	-6.62298	$2.33x10^{11}$
R^2		0.98248	
Ca^{2+}	y0	$2.48x10^{7}$	$1.93x10^{6}$
	A	$-2.15x10^{7}$	$2.05x10^{6}$
	R0	-9.5933	2.3314
R^2		0.96894	
La^{3+}	y0	$2.41x10^{7}$	$3.29x10^{5}$
	A	$-2.14x10^{7}$	$6.41x10^{5}$
	R0	-30.324	2.748
R^2		0.9956	
EQUATION	$y= y_0 + A*e^{(R0*x)}$		

Table S4. Parameters of the exponential fitting in Fig. 3B.

	yo	A	R0	Reduced Chi-Sqr	Adj. R-Square
Value	1.94×10^{7}	-1.55×10^{7}	-0.00538	2.45×10^{10}	0.99886
Standard error	252784.97	2.36×10^{5}	2.03×10^{-4}		

Table S5. Parameters of the linear fitting in Fig. 3C.

	Intercept	Slope	Adj. R-Square
Value	16.5678	-0.0055	0.99905
Standard error	0.01027	5.67×10^{-5}	

able S6. Parameters of the exponential fitting to determine HPTS concentration in solution of the sample.

	yo	A	R0	Reduced Chi-Sqr	Adj. R-Square
Value	6.84×10^{7}	-6.83×10^{7}	-0.71	4.64×10^{10}	0.99991
Standard error	2.56×10^{5}	2.57×10^{5}	6.45×10^{-3}		
Equation	$Y = y0 + Ae^{R0x}$				

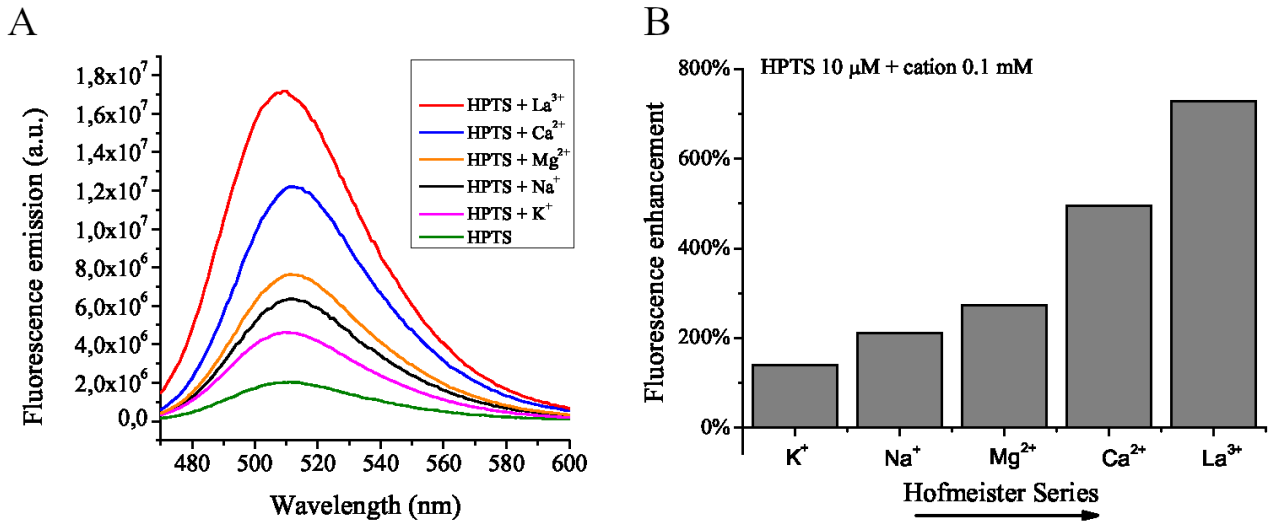

Figure S3. (A) Comparison of emission spectra for 0.1 mM of cations and 10 µM HPTS (pH=6.2±0.1), (B) Enhancement of fluorescence emission at λ=511 nm upon excitation at λ=450 nm.

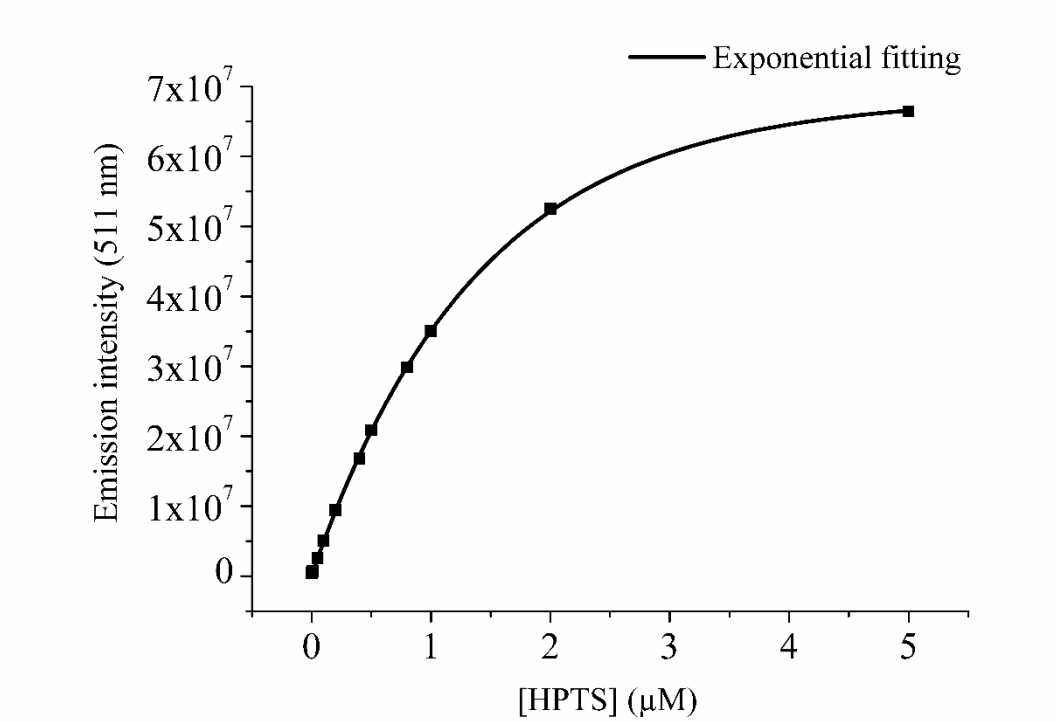

Figure S4. Calibration curve and exponential fitting for the quantification of the concentration of HPTS in the samples

Figure S5. Spectra acquired after cell lysis

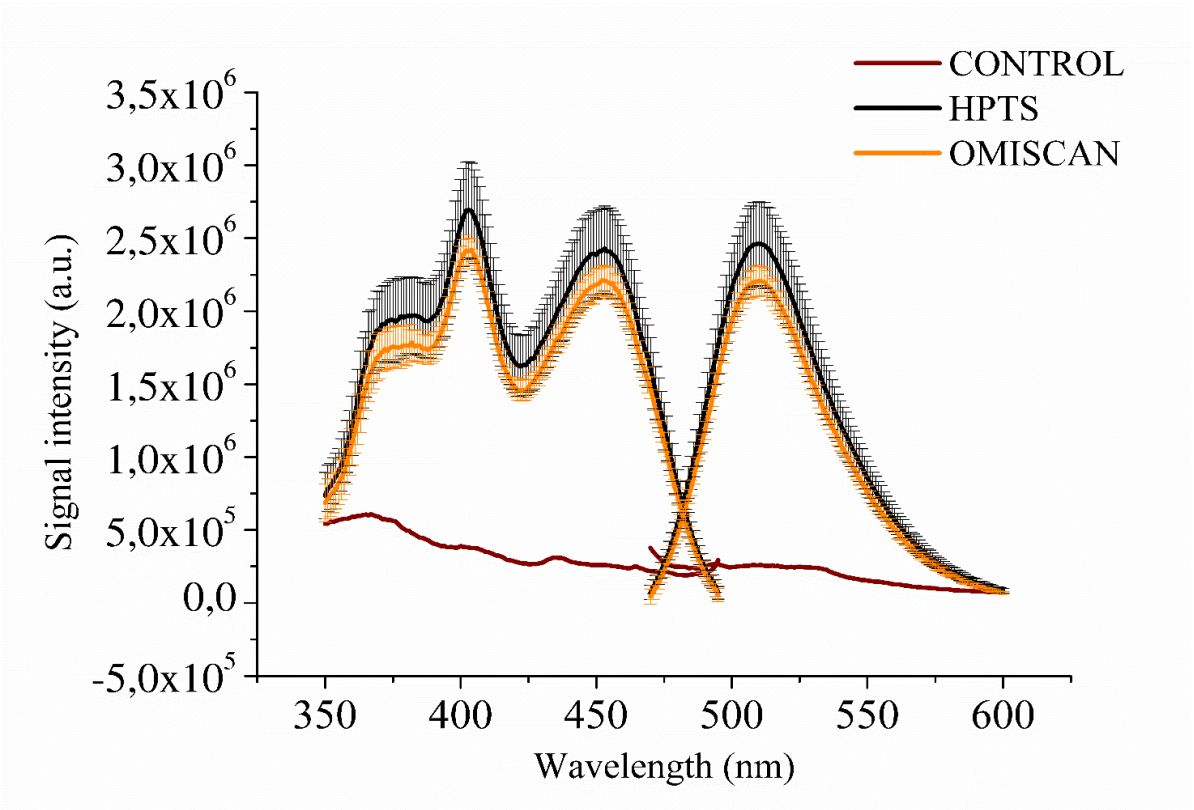

Table S7. Calculated HPTS concentration and the *per* cell content.

SAMPLE	Emission (511 nm)	Cell number	[HPTS] (μM) in 3ml solution	Moles in 3ml solution	Number of HPTS molecules per cell	Moles of HPTS molecules per cell
WHITE	$2.59x10^{5}$	$2.20x10^{6}$	0.0018	10^{-12}	-	-
HPTS	$2.46x10^{6}$	$1.82x10^{6}$	0.047	$1.41x10^{-10}$	$4.66x10^{7}$	$7.74x10^{-17}$
MAGNEVIST	$1.49x10^{6}$	$8.68x10^{5}$	0.027	$8.1x10^{-11}$	$5.61x10^{7}$	$9.33x10^{-17}$
OMNISCAN	$2.20x10^{6}$	$1.34x10^{6}$	0.042	$1.26x10^{-10}$	$5.67x10^{7}$	$9.42x10^{-17}$
PROHANCE	$1.64x10^{6}$	$1.24x10^{6}$	0.03	$9x10^{-11}$	$4.37x10^{7}$	$7.26x10^{-17}$

Table S8. Calculated total Gadolinium concentration and the *per* cell content.

SAMPLE	Cell number	[Gd] (mM) in 3ml solution	R1	Error	Moles in 3ml solution	Number of Gd atoms/molecules per cell	Moles of Gd atoms/molecules per cell
HPTS	$1.82x10^{6}$	0.018	0.62	$1.25x10^{-3}$	-	-	-
MAGNEVIST	$8.68x10^{5}$	0.28	2.44	$4.5x10^{-3}$	$5.67x10^{-8}$	$3.93x10^{10}$	$6.53x10^{-14}$
OMNISCAN	$1.34x10^{6}$	0.49	3.88	$7.6x10^{-3}$	$9.88x10^{-8}$	$4.45x10^{10}$	$7.39x10^{-14}$
PROHANCE	$1.24x10^{6}$	0.54	4.22	$9x10^{-3}$	$1.08x10^{-7}$	$5.27x10^{10}$	$8.76x10^{-14}$

Table S9. SMILES code of the molecules.

MOLECULE	SMILES CODE
HPTS	c1cc2c(cc(c3c2c4c1c(cc(c4cc3)S(=O)(=O)[O-])O)S(=O)(=O)[O-].[Na+].[Na+].[Na+]
MAGNEVIST®	[Gd+3].OC(=O)CN(CCN(CCN(CC(O)=O)CC([O-])=O)CC([O-])=O)CC([O-])=O
OMNISCAN®	[Gd+3].CNC(=O)CN(CCN(CCN(CC([O-])=O)CC(=O)NC)CC([O-])=O)CC([O-])=O
PROHANCE®	[Gd+3].CC(O)CN1CCN(CC([O-])=O)CCN(CC([O-])=O)CCN(CC([O-])=O)CC1