Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information

Prostate-specific membrane antigen targeted protein contrast agents for molecular imaging of prostate cancer by MRI

Fan Pu[a,b,#], Mani Salarian[a,#], Shenghui Xue[a,#], Jingjuan Qiao[a,b], Jie Feng[a], Shanshan Tan[a], Anvi Patel[a,b], Xin Li[c], Kenza Mamouni[c], Khan Hekmatyar[d], Juan Zou[a], Daqing Wu[c] and Jenny J. Yang[a,*]

[a.] *Departments of Chemistry, Center for Diagnostics & Therapeutics, Georgia State University, Atlanta, GA 30303, USA.*
[b.] *Inlighta Biosciences LLC, Marietta, GA 30068, USA*
[c.] *Georgia Regents University Cancer Center, Augusta, GA 30912, USA.*
[d.] *BioImaging Research Center, University of Georgia, Athens, GA, 30602, USA.*
[#] *These authors contributed equally to this work.*

Supplementary Figures

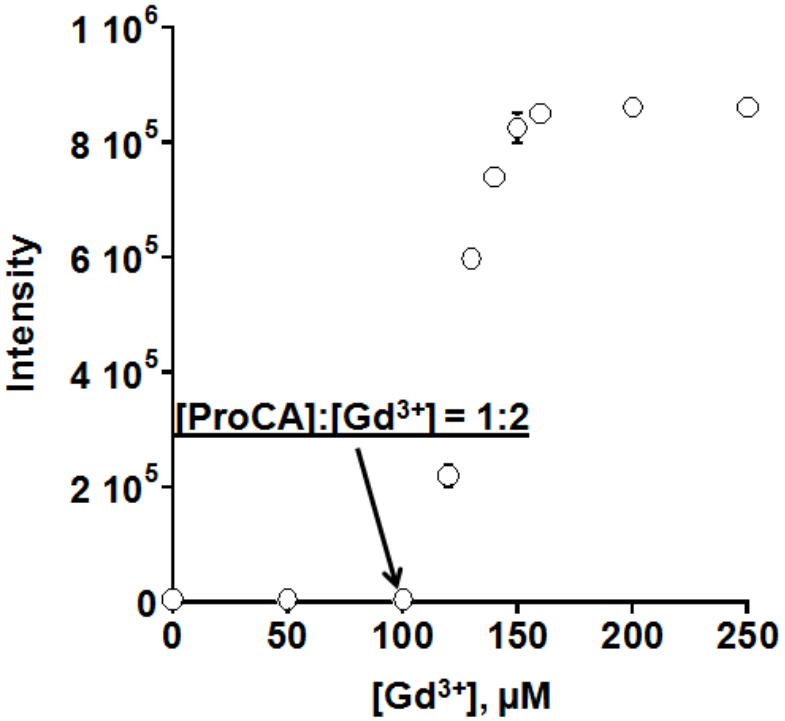

Fig. S1. Determination of Gd^{3+} loading stoichiometry and loading efficiency to protein MRI contrast agent (ProCA) using fluorescence titration. A Gd^{3+} responsive dye with low affinity, Rhod-5N, along with 50 µM ProCA were added in 100 mM Tris/HCl, 100 mM KCl at pH 7.4. The Rhod-5N emission fluorescence spectrums between 560 and 650 nm were collected under different concentration of total Gd^{3+}. Any free Gd^{3+} in the solution can bind to Rhod-5N and cause fluorescence signal increase. There was no fluorescence change at total Gd^{3+} concentration of 100 µM or below indicating that all the Gd^{3+} was loaded to ProCA with high efficiency when the ProCA to Gd^{3+} ratio was at 1:2 or above. The Rhod-5N fluorescence immediately increased when the total Gd^{3+} concentration was higher than 100 µM, indicating that 50 µM ProCA was saturated by 100 µM Gd^{3+} and additional free Gd^{3+} (with total Gd^{3+} concentration higher than 100 µM) bound to Rhod-5N causing fluorescence increase. In summary, this experiment demonstrated that Gd^{3+} can be loaded into ProCA at 2:1 ratio with high efficiency.

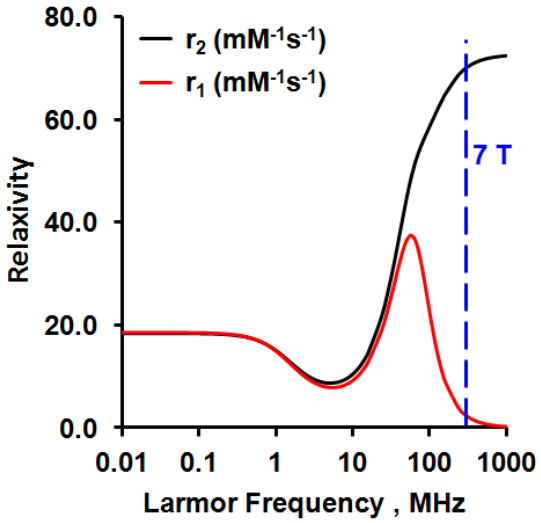

Fig. S2. Simulation of per Gd r_1 and r_2 relaxivities at different magnetic field strengths (0.01-1000 MHz). r_1 and r_2 were simulated using the given τ_R (5 ns), τm (100 ns), τv (10 ps), and Δ^2 (5×10^{19} s^{-2}).

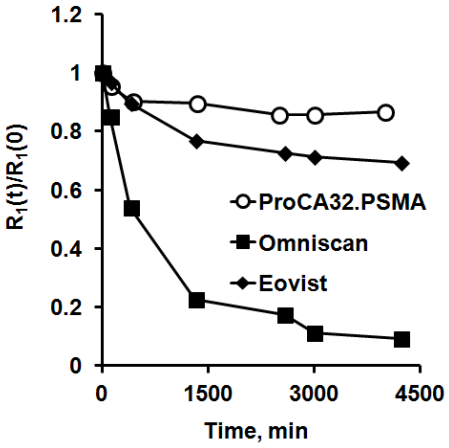

Fig. S3. Transmetallation study of ProCA32.PSMA in the presence of phosphate and $ZnCl_2$. The relaxation rates changes of clinical contrasts in phosphate buffer supplemented with $ZnCl_2$ were measured according to previously reported method[1]. The relaxation rates change of ProCA32.PSMA was monitored with 110 µM of ProCA32.PSMA loaded with 100 µM Gd^{3+}, 100 µM $ZnCl_2$, and 1.2 mM PO_4^{3-}.

Fig. S4. PSMA expression on LNCaP and PC3 cells were identified by Western Blot using antibody against PSMA. PC3 cells do not have PSMA expression and LNCaP cells have PSMA expression.

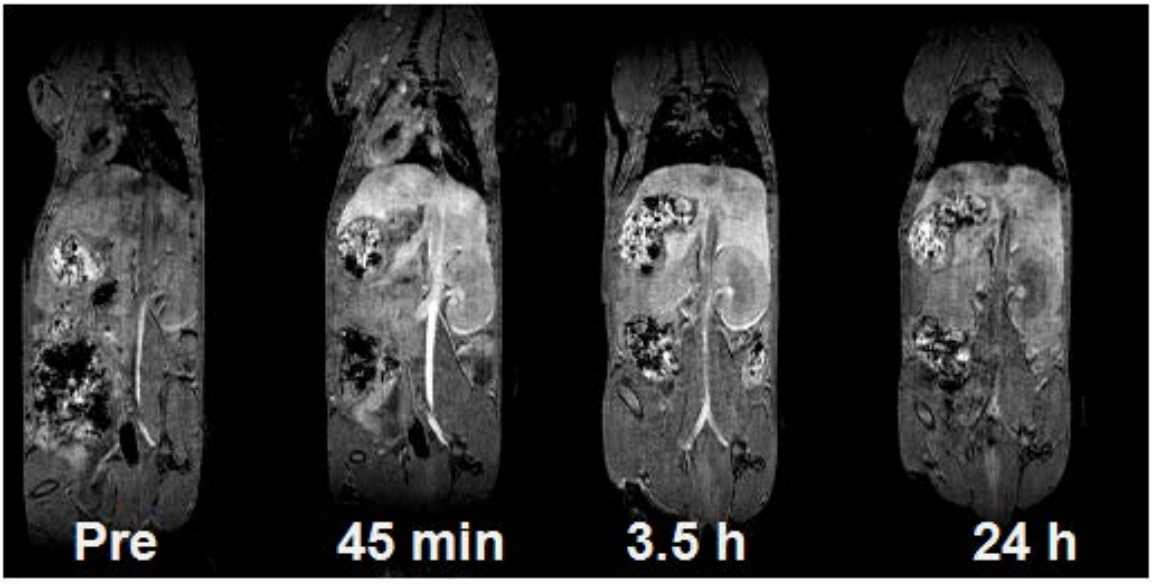

Fig. S5. Three dimensional T_1-weighted MRI of normal mice before and after injection of ProCA32. ProCA32 is mainly distributed in the liver, kidney and blood at 45 min and 3.5 hours post injection of ProCA32.

Supplementary Table

Table S1. Competitive binding assay to compare the Gd^{3+} selectivity between ProCA32 and clinical contrast agent, Omniscan.

Omniscan	+	+	+	+
heat	-	+	+	+
Zn^{2+}	-	-	+	+
ProCA32	-	-	-	+
r_1, $mM^{-1}s^{-1}$	5.8	6.7	5.8	21.2

The per Gd r_1 was measured at four different conditions. 1) the r_1 of Omniscan (containing 20 μM Gd-DTPA-BMA and 1 μM Na[Ca-DTPA-BMA]) was determined at 37 °C. 2) Omniscan was heated at 95 °C for 30 min before measuring r_1 at 37 °C. 3) Omniscan was incubated with 100 μM $ZnCl_2$, then heated at 95 °C for 30 min before measuring r_1 at 37 °C. 4) Omniscan was incubated with 100 μM $ZnCl_2$ and 20 μM of ProCA32, heated at 95 °C for 30 min before measuring r_1 at 37 °C. In condition 4, the r_1 of the mixture increased to 21.2 $mM^{-1}s^{-1}$, indicating that most of the Gd^{3+} in Omniscan was competed out by ProCA32 and Zn2+, and the released Gd^{3+} bound to ProCA32 to generate high r_1.

Supplementary Reference

1. S. Laurent, L. V. Elst, F. Copoix and R. N. Muller, *Investigative radiology*, 2001, 36, 115-122.