Supplementary Information

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

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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 $\tau_R$ (5 ns), $\tau_m$ (100 ns), $\tau_v$ (10 ps), and $\Delta^2$ ($5 \times 10^{19}$ 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\textsuperscript{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 T₁-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.

<table>
<thead>
<tr>
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<th>Omniscan</th>
<th>heat</th>
<th>Zn(^{2+})</th>
<th>ProCA32</th>
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<tr>
<td>+</td>
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<td>r(_1), mM(^{-1})s(^{-1})</td>
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<td>6.7</td>
<td>5.8</td>
<td>21.2</td>
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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 Zn\(^{2+}\), and the released Gd\(^{3+}\) bound to ProCA32 to generate high \(r_1\).

Supplementary Reference