

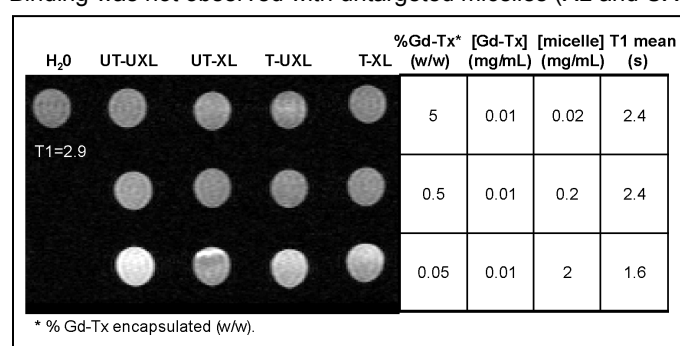
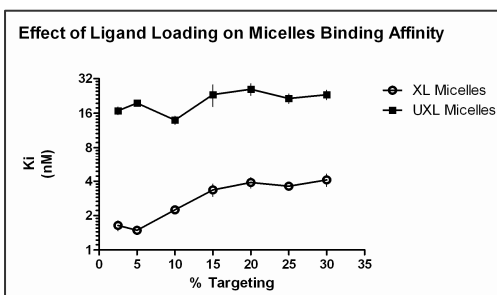
# Development and In-vivo Magnetic Resonance Imaging of Polymer Micelles Targeted to the Melanoma-Specific Marker MC1R

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**Introduction:** Rationally-designed, polymer-based micelle carriers offer a promising approach to the delivery of therapeutic and/or diagnostic payloads. Systemic toxicity can be reduced by targeting micelles with cancer-specific ligands, and circulation time can be enhanced through the use of stabilized (cross-linked) micelles. Our target, the melanocortin 1 receptor (MC1R) has been extensively evaluated as a target for melanoma therapies. We have previously reported the development of a ligand specific to MC1R and we have shown that the conjugation of this ligand a triblock polymer micelle system does not result in a significant decrease in binding affinity. MC1R targeted and stabilized Gd-Tx micelles demonstrated tumor-specific MR contrast in vivo with clearance from the kidney and liver. **Materials and Methods:** An alkyne functionalized MC1R ligand was conjugated to the terminal azide of a triblock polymer by copper-assisted click chemistry. A new gadolinium texaphyrin (Gd-Tx) was encapsulated for MR contrast. Gd-Tx micelle formulation was carried out in DMSO at 5%, 0.5% and 0.05% Gd-Tx weight loading with a sheer mixer and microfluidizer. Following formation, micelles were stabilized with a pH-sensitive Fe(III) crosslinking (XL) reaction. Binding affinity was determined through time-resolved Eu-fluorescence competition assays. In vitro MR phantoms were performed on all micelle formulations to determine relaxivity. In vivo studies were conducted on female SCID/beige mice injected with MC1R-expressing cells on the right and left flanks. MC1R-targeted XL micelles (T-XL, 0.5% Gd-Tx w/w) were injected via tail vein into mice when the tumors reached ca. 500 - 800 mm<sup>3</sup>. The uncrosslinked (UXL), as well as XL and UXL untargeted (UT) micelles were injected as a control. Mice were imaged on a Varian 7T small animal imaging system using a SEM sequence (te=8.62ms, tr=180ms).

**Results:** Optimal ligand loading was determined to be 5% w/w through Eu binding assays (lowest K<sub>i</sub>, 1.49 ± 0.12 nM, n = 4) and XL micelles bound to MC1R-expressing cells with significantly higher avidity than UXL micelles at all loading levels (p < 0.001). The binding avidity of the XL micelles at 5% ligand loading is 4 times greater than that of the UXL micelles (2.9 ± 0.42 Ki and 12 ± 2.6 Ki respectively, n = 4). Binding was not observed with untargeted micelles (XL and UXL).



In-vitro phantoms revealed that higher relaxivity (lower T<sub>1</sub>) was obtained with lower Gd-Tx percent weight loadings. This is most likely attributed the T<sub>2</sub><sup>\*</sup> affects of gadolinium.

Positive enhancement throughout the tumor was observed only in mice receiving the T-XL micelles. The T-XL group is significantly different from all other groups at 4h to 48h (p < 0.001 for 4h – 24; p < 0.05 at 48h). There were no statistical differences (t-test) among the UT-XL, T-UXL or UT-UXL groups at any time point. Contrast enhancement for the T-XL micelles peaks at 4h in the kidneys and 1h

in the liver, and steadily decreases thereafter. This suggests that the micelles do not accumulate in these organs.

**Conclusion:** Herein, we have described the synthesis and characterization of MC1R targeted triblock polymer micelles with encapsulated gadolinium texaphyrin (Gd-Tx) and Fe(III) crosslinking for stabilization. These micelles selectively targeted MC1R expressing xenograft tumors in-vivo. To the best of our knowledge, this represents the first report of a targeted micelle system that is able to effectively, and selectively, accumulate in the tumor relative to other tissues.

