Supplementary Information

Contrast Agent Synthesis and Characterization

The amide bond formation reaction was optimized for pH. The highest number ofGd(III) per molecule was achieved at pH 6 and 7 (Figure S1).

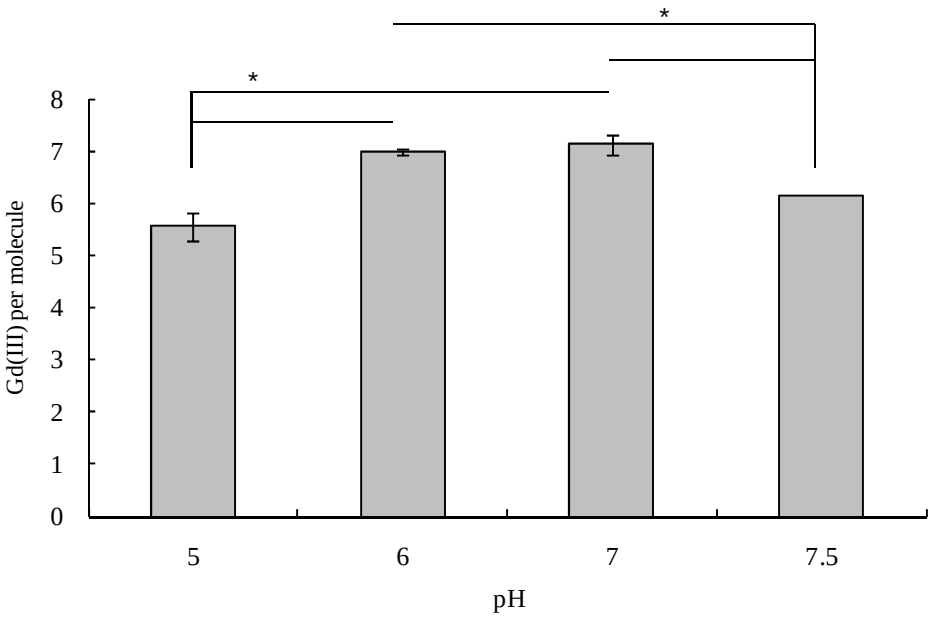

Figure S1. Number of Gd(III) chelates conjugated to a K8-30 backbone versus pH of the conjugation reaction in MES buffer with EDC and Sulfo-NHS. Reactions with pH 6 and pH 7 are statistically higher than pH 5 and pH 7.5. * indicates $p < .01$

Molecular weights of molecules close to or greater than 100 kDa are very difficult to obtain using traditional MALDI instruments because the high molar mass hinders the creation of gas-phase ions.[36] Very high laser powers are necessary to obtain these ions. We were only able to measure the molecular weight of one of the three K8-120 conjugations and thus used that molecular weight for calculating the conjugation efficiency.

Cation exchange chromatography of the PPCA elutes as a single peak, indicating the purity of the conjugate (Figure S2A). Although the PPCA does not have as low a polydispersity as the protein polymer itself, it nonetheless maintains a very low PDI of 1.003 (Figure S2B). This monodispersity agrees to earlier findings of these protein polymer-based conjugates where polydispersity increased from a PDI of 1.000 for the protein polymer alone to a maximum of 1.011.[32] Most of the conjugates had a PDI less than 1.005, similar to the K8-30 CA.

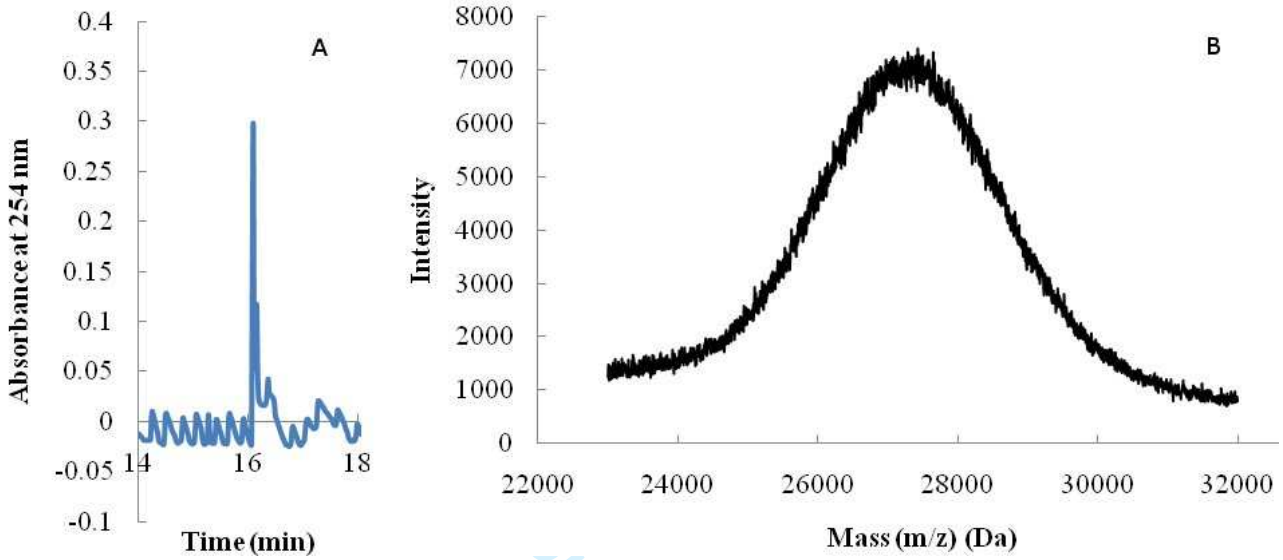

Figure S2. A) Cation exchange chromatography trace of K8-30 CA using a mono S cation exchange column. Peak indicates the elution of the contrast agent. B) MALDI-TOF trace of elution, confirming molecular weight. M_w = 27384 Da; M_n = 27299 Da; PDI = 1.003.

PPCA Degradation

In addition to degradation by plasmin, trypsin can digest the PPCAs (Figure S3).

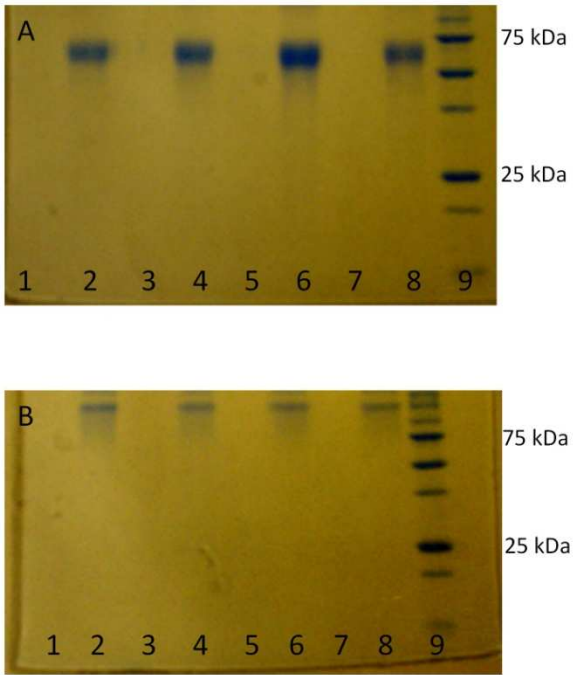

Figure S3. Degradation of K8-60 PPCA (A) and K8-120 PPCA (B) by trypsin. Lane 1) with trypsin, 30 minutes; Lane 2) without trypsin, 30 minutes; Lane 3) with trypsin, 60 minutes; Lane 4) without trypsin, 30 minutes; Lane 5) with trypsin, 3 hours; Lane 6) without trypsin, 3 hours; Lane 7) with trypsin, 6 hours; Lane 8) without trypsin, 6 hours; Lane 9) Molecular weight marker

Viability

MIN6 cells were also incubated for longer periods of time with PPCAs to assess viability. Viability at 24 and 48 hours of incubation with the K8-30 PPCA was greater than 95% (Figure S4).

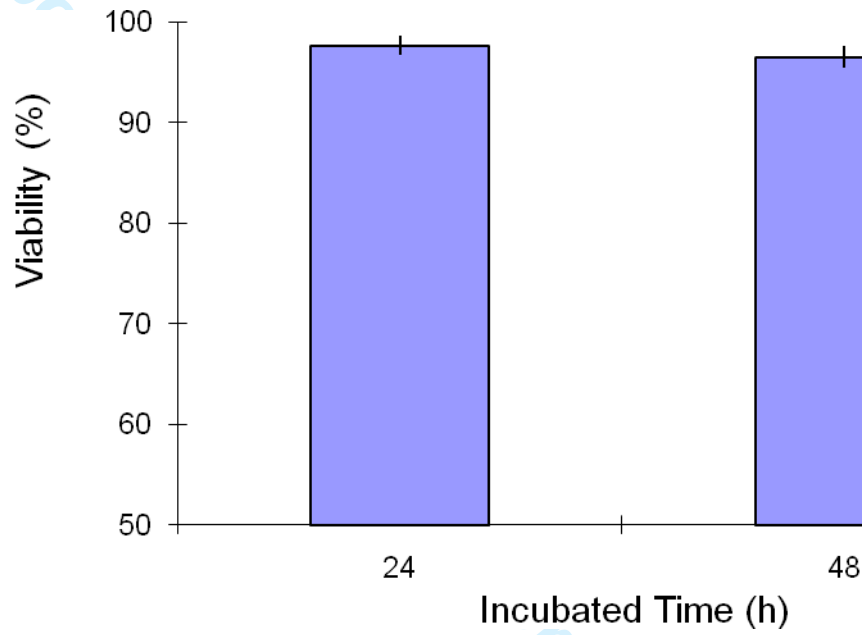

Figure S4. Incubation of MIN6 cells with the K8-30 PPCA for 24 and 48 hours.