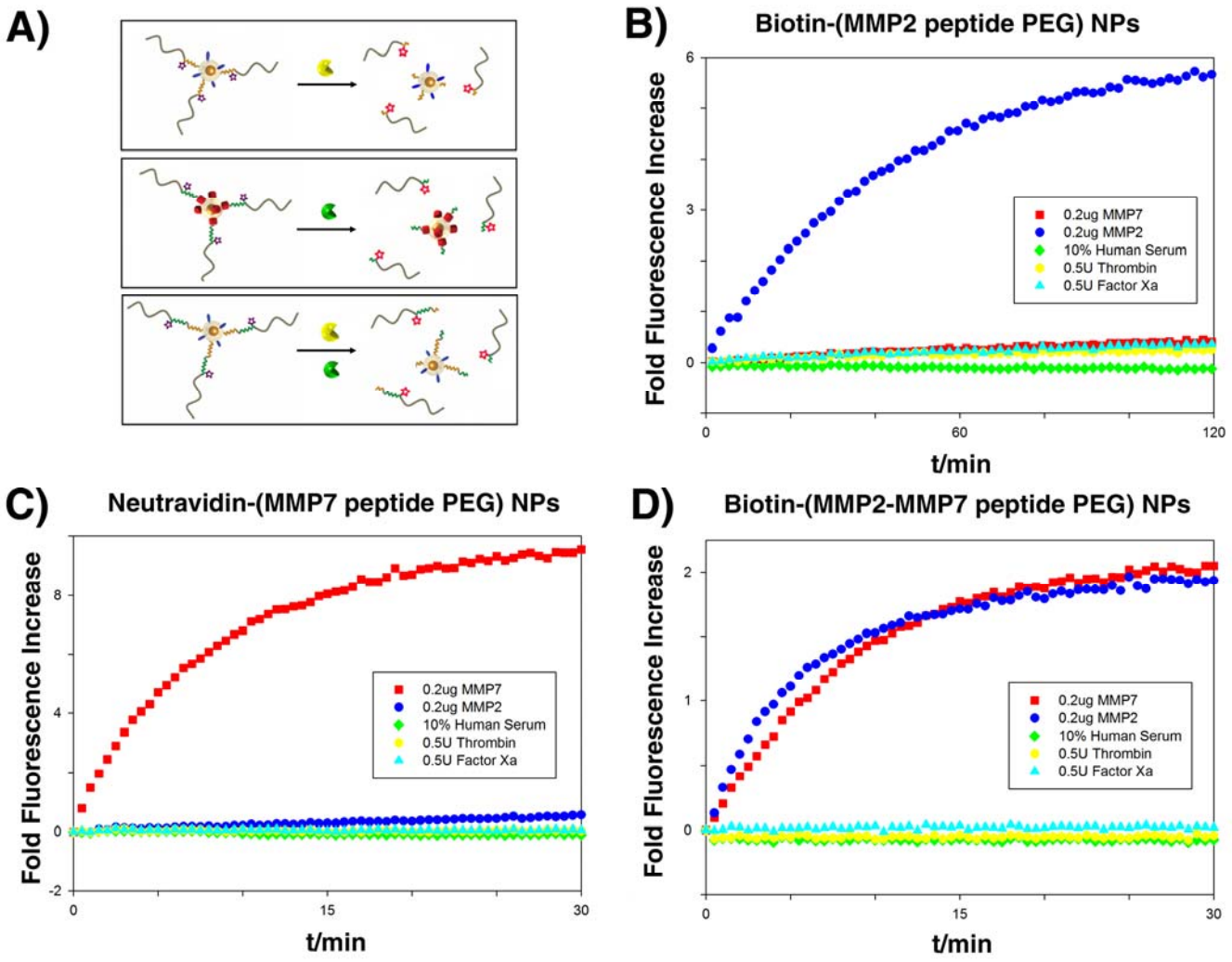

Figure S1. Protease activation of substrate-linked TAMRA fluorescence. A) Biotin-MMP2-PEG NPs, Neutravidin-MMP7-PEG NPs, and Biotin-MMP2-MMP7-PEG NPs (OR NPs) are incubated with proteases which, upon cleavage of substrates, relieve TAMRA fluorochrome quenching by NP core and increase solution fluorescence. B-D) NPs (40ug/ml; 50ul) were incubated with 0.2ug MMP2, 0.2 ug MMP7, purified human enzyme controls, or 10% human serum. Thrombin and factor Xa concentrations (10U/ml) were chosen to approximate 10x the activity present in human serum.

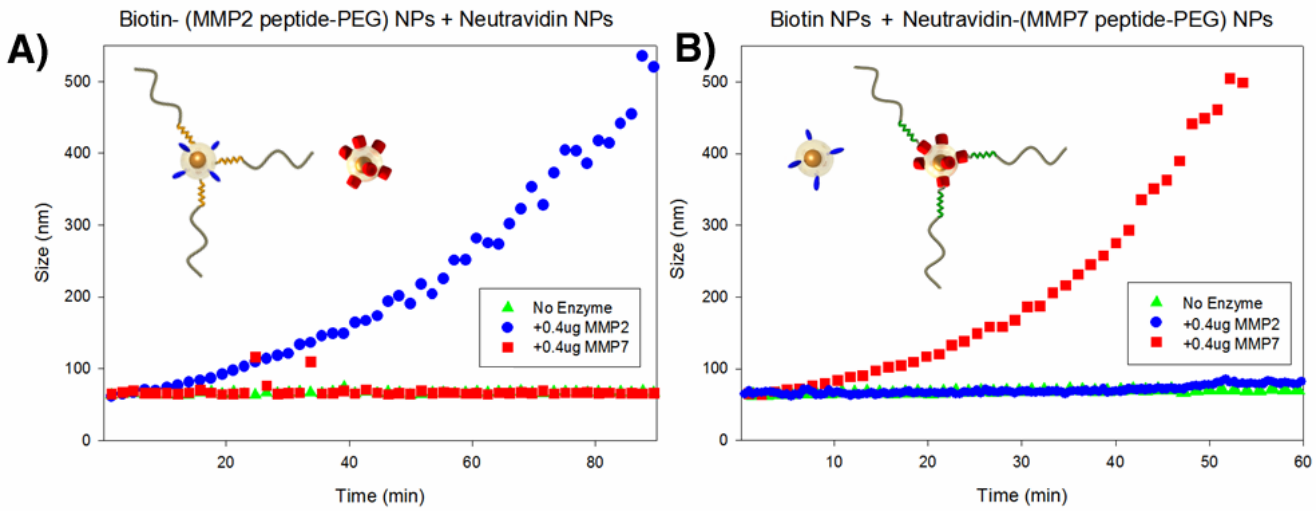

Figure S2. Probing nanoparticle latency and specificity using dynamic light scattering. a) Ligand-nanoparticles were masked with MMP2-PEG to inhibit assembly with unmodified receptor nanoparticles (40ug Fe/ml). Addition of 0.4ug MMP2 actuates nanoparticle assembly, while 0.4ug MMP7 or no enzyme is insufficient. b) Receptor-nanoparticles were masked with MMP7-PEG to inhibit assembly with unmodified ligand nanoparticles (40ug Fe/ml). Here, addition of 0.4 ug MMP7 induces assembly, while 0.4ug MMP2 cannot.

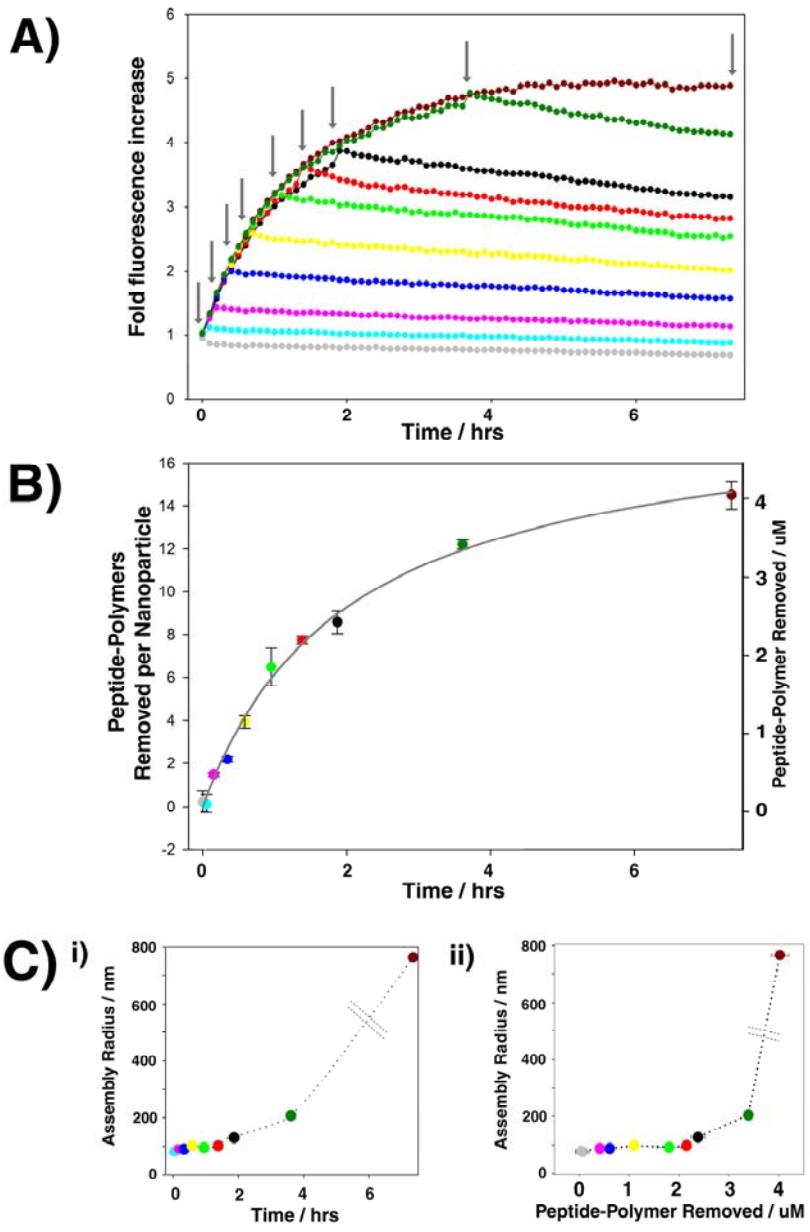

Figure S3. Investigating assembly dependence on polymer removal. A) Biotin-MMP2-PEG NPs (200ug Fe/ml) were incubated with MMP2 and TAMRA fluorescence was recorded over time. At various time points, reactions were quenched with addition of 20mM EDTA (quenching times indicated by arrows). B) The amount of free MMP2-PEG released in samples from (A) was quantified by TAMRA absorbance in solution after ultracentrifugation of NPs. Peptide-Peg removal is plotted vs MMP2 incubation time with data colors corresponding to those in (A). Polymer removal kinetics are consistent with the fluorescence de-quenching data in (A), suggesting that TAMRA quenching was largely mediated by fluorochrome-NP interactions over fluorochrome-fluorochrome interactions. C) i)

Quenched ligand-MMP2-PEG samples from (A) were incubated with unmodified receptor NPs (each at 40ug/ml) for 24 hours at which time size was measured with DLS. The 7.5 hour MMP2 reaction precipitated during the incubation, indicating assembly is closely controlled by the degree of polymer removal between 4-6 hrs. ii) Assembly data is plotted vs. the polymer removal data of (B), demonstrating minimal assembly induction prior to removal of ~50% of NP polymers.

Methods:

Unless otherwise stated all reagents were purchased from Sigma-Aldrich and all reactions were performed at room temperature.

Superparamagnetic iron oxide nanoparticles were synthesized according to the published protocol[1-3]. Briefly, dextran-coated iron oxide nanoparticles were synthesized, purified, and subsequently cross-linked using epichlorohydrin. After exhaustive dialysis, particles were aminated by adding 1:10 v/v ammonium hydroxide (30%) and incubated on a shaker overnight. Aminated-nanoparticles were subsequently purified from excess ammonia using a Sephadex G-50 column and concentrated using a high-gradient magnetic-field filtration column (Miltenyi Biotec).

Peptide-Polymer synthesis: Peptides were synthesized in the MIT Biopolymers core to sequentially contain a lysine (for the attachment of polyethylene glycol polymers), a MMP-cleavage sequence, and a terminal cysteine (for linkage onto amines in the dextran coat or lysines on neutravidin (Pierce) proteins. Peptide purity was verified with HPLC and mass spectrometry. Amine-reactive 20kDa mPEG-SMB reagents (methoxy-polyethylene glycol- succimidyl α methylbutanoate) were purchased from Nektar Therapeutics. The following sequences were used in this investigation: (N->C) MMP2 substrate: G-K(TAMRA)-G-P-L-G-V-R-G-C-CONH2; MMP7 substrate: G-K(TAMRA)-G-V-P-L-S-L-T-M-G-C-CONH2; MMP7-MMP2 tandem substrate: TAMRA-G-K-G-V-P-L-S-L-T-M-Ahx-G-P-L-G-V-R-G-C-

CONH2 where K(TAMRA) = Lys(DDE) substituted with 5(6)-TAMRA, TAMRA=5(6)-TAMRA, and Ahx= aminohexanoic acid. Peptides were reacted with polymers in PBS + 0.005M EDTA pH 7.2 at 500uM and 400uM, respectively, for >24 hours with shaking. Free peptide was removed by reducing with 0.1M TCEP and filtering using a G-50 Sepahdex column. The reduced polymer was then quantified using fluorochrome extinction and added to nanoparticle preparations as described below.

Ligand nanoparticle synthesis: Following each conjugation, nanoparticles were purified using a high-gradient magnetic-field filtration column (Miltenyi Biotec). Aminated nanoparticles (1mg Fe/ml) were simultaneously reacted with biotinamidohexanoyl-6-aminohexanoic acid N-hydroxysuccinimide ester and 4-Maleimidobutyric acid N-hydroxysuccinimide ester (.8mM and 1.2mM, respectively) in 0.1M HEPES 0.15M NaCl pH 7.2 buffer for 30 minutes. Purified nanoparticles (1 mg Fe/ml) were then combined with reduced peptide-polymers (1mM) in phospho-buffered saline + 0.005M EDTA pH 7.2 and incubated for >2hrs. Particles were again purified and used in subsequent assembly experiments.

Receptor nanoparticle synthesis: Aminated nanoparticles (1mg Fe/ml) were reacted with biotinamidohexanoyl-6-aminohexanoic acid N-hydroxysuccinimide ester (0.03mM) in 0.1M HEPES 0.15M NaCl pH 7.2 buffer for 30 minutes. Following filtration, nanoparticles (1 mg Fe/ml) were combined with a saturating concentration of neutravidin protein (Pierce, 5mg/ml) and incubated for >3hrs. The extinction of nanoparticle solutions at 600nm was monitored during neutravidin-coating to ensure cross-linking was not occurring. After purification, neutravidin particles were passed through a 0.2 micron filter to ensure removal of any aggregates. Neutravidin nanoparticles (1mg Fe/ml) were then reacted with 4-Maleimidobutyric acid N-hydroxysuccinimide ester (2mM) for 30 minutes, purified, and incubated with peptide-polymers (1mM) for >2hrs as before. Particles were finally purified from excess peptide-polymer and used in subsequent assembly experiments.

Dynamic light scattering studies: All dynamic light scattering experiments were performed in 100ul solutions of 0.1M HEPES 0.15M NaCl 0.005M $CaCl_2$ at 25ºC with nanoparticles at 40ug Fe/ml (added at equimolar concentrations). To begin experiment, catalytic domains of MMP2 and MMP7 (Biomol) were added in 5ul to 95ul of nanoparticles or 5ul control buffer was added. Kinetic dynamic light scattering intensity size measurements were taken using a Malvern ZS90 and hydrodynamic radius was plotted vs time.

Fluoresence activation experiments: NPs were incubated with various enzymes and TAMRA fluorescence was monitored (ex: 515nm/em: 580nm; cutoff 530nm) at 30sec intervals over time. Buffer was matched to DLS studies. Purified plasma proteins (Haematologic Technologies) were incubated with 40ug/ml NP samples in 40ul. For partial assembly studies, reactions were quenched with addition of 10% 0.2M EDTA.

Polymer quantitation: Triplicate partial activation samples were ultracentrifuged (50k, 30min) to precipitate nanoparticles. The supernatant was collected and TAMRA absorbance was quantified to determine peptide-polymer concentration.

MRI detection of nanoparticle self-assembly: MRI T2 mapping was performed using a 7 cm bore, Bruker 4.7T magnet. Nanoparticles were mixed together and serially diluted in 384-well plate, containing 95ul total sample/well. Recombinant MMP2 or MMP7 (Biomol) was pre-incubated at 37C for 30 minutes to activate and added in a total of 5 uL Tris-Cl (50 mM), CaCl2 (5 mM), Brij-35 (0.005%), pH 7.5 were added to each well. After 3 hr incubation, T2 relaxation maps were obtained. Data in each well was displayed by fitting images on a pixel by pixel basis to the equation $y=M*L10^{(-TE/T2)}$ using MATLAB.

[1] S. Palmacci, and Josephson, L, in *U.S. Patent Vol. 5*, **1993**, p. 176

[2] T. Shen, R. Weissleder, M. Papisov, A. Bogdanov, Jr., T. J. Brady, *Magn Reson Med* **1993**, *29*, 599.

[3] L. Josephson, C. H. Tung, A. Moore, R. Weissleder, *Bioconjug Chem* **1999**, *10*, 186.