Electronic Supplementary Information

PEG-urokinase nanogels with enhanced stability and controllable bioactivity

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Figure S1. ¹H NMR spectra of (a) uPA in D₂O; (b) mPEG2000-CHO in D₂O; (c) core-shell structural mPEG2000-uPA conjugates in D₂O at pH 7.4, where the aldehyde proton at 10 ppm is not visible indicating that unreacted mPEG-CHO chains were removed during the purification procedure; (d) mPEG2000-uPA in DCl/D₂O solution at pH 5.0, for an observation of the recovery of the aldehyde proton at 10 ppm in comparison with the spectrum recorded at pH 7.4 (c), which is an indication of the detachment of mPEG-CHO chains from the protein surface due to the hydrolysis of the benzoic-imine linkage.



Figure S2. Enlarged ¹H NMR spectrum of PEG600-uPA nanogels in D_2O (pH=7.4), where weak aldehyde proton peak can be observed at 10 ppm.



Figure S3. Circular dichroism (CD) spectra of the PEG-uPA nanogels (a, b) and the core-shell mPEG-uPA conjugates (c, d) in aqueous solution at pH 7.4, 37 °C and their spectra after the solution pH was changed to pH 5.0 for 4 h and then neutralized. Compared to the native uPA, the spectra indicate a slight change of the conformation of uPA upon the formation of PEG-protein conjugates. However this change of protein confirmation is reversible with the dissociation of the conjugates in acidic condition.



Figure S4. Confocal laser scanning microscopy (CLSM) observation on the internalization of PEG2000-uPA nanogel particles on MCF-7 cells. Channel: (a) DAPI, (b) FITC, (c) DOX, (d) merge. The uPA was label by FITC-isothiocyanate via a covalent bonding before the synthesis of PEG2000-uPA nanogel. Then the nanogels were labeled by doxorubicin (DOX) via Schiff's reaction between amino group of DOX and the residue aldehyde groups of OHC-PEG-CHO in the nanogel. The dual labeled nanogels were incubated with MCF-7 cells for 1 h before the observation. DAPI was used to stain the nucleus of the cells. It can be seen that the protein nanogels are efficiently taken up by the cells and majorly distributed in the cytoplasm. And from the merged image (d), it reveals that the fluorescent distribution of DOX and FITC is not completely overlapped which indicates the dissociation of the nanogel inside the cells possibly due to the acidic condition in the endosomal compartments.