A supramolecular two-photon-active hydrogel platform for direct bioconjugation under near-infrared radiation

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Experimental

\textbf{NMR data analysis: calculation of PEI methacrylation}

The extent of methacrylation of branched PEI was determined by the analysis of \textsuperscript{1}H NMR spectra using the following formulas (Forrest \textit{et al.}, \textit{Pharm. Res.}, 2004):

\[
\text{methacrylated secondary amines (\%)} = \left( \frac{E_{bb}}{4} \cdot \frac{3}{M'} \cdot N' \right)^{-1} \cdot 100\%
\]

\[
\text{methacrylated tertiary amines (\%)} = \left( \frac{E_{bb}}{4} \cdot \frac{3}{M''} \cdot N'' \right)^{-1} \cdot 100\%
\]

where $M'$ is the integration of $\delta$ 1.70-1.75 peaks corresponding to methacrylated secondary amines [-NH–COC(CH$_2$)CH$_3$], $M''$ is the integration of $\delta$ 2.0-2.1 peaks corresponding to methacrylated tertiary amines [\textgreater N–COC(CH$_2$)CH$_3$], and $E_{bb}$ is the integration of $\delta$ 2.5-3.0 peaks corresponding to ethylene backbone.

$N'$ and $N''$ are the mole fractions of PEI primary and secondary amines, 0.31 and 0.39 respectively, based on the analysis of the commercial PEI starting material by \textsuperscript{13}C NMR (von Harpe \textit{et al.}, \textit{J. Controlled Release}, 2000).
**Fig. S1** Small angle X-ray scattering (SAXS) profile in log-log plot of swollen PEIMA hydrogel obtained at room temperature, showing a steep intensity decay at lower q values (slope -3.5) and a broad peak at 0.7 nm\(^{-1}\).

**Fig. S2** Microscopy images showing control experiments for photopatterning of PEIMA supramolecular hydrogel. White light transmission images of PEIMA hydrogel in a solution without functional probes. No imprinting is visible before and after multiphoton laser irradiation at wavelengths of 680 nm (top) and 1000 nm (bottom). Scale bars are 100 µm.