# Supporting Information for:

**Unlocking a Caged Lysosomal Protein from a Polymeric Nanogel with a pH Trigger**

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**Synthesis:**

i) \( \text{HO-}O-\text{O-}O-\text{O-}O-\text{OH} + \text{Cl-}O \rightarrow \text{HO-}O-\text{O-}O-\text{O-}O-\text{O-}O-\text{O-}O-\text{O} \) **Monomer (M)**  

ii) \( \text{HO-}O-\text{O-}O-\text{O-}O-\text{O-}O-\text{O-}O-\text{O} + \text{HS-}SH + \text{HO-}O-\text{O-}O-\text{O-}O-\text{O-}O-\text{O-}O-\text{O} \rightarrow \text{HO-}O-\text{O-}O-\text{O-}O-\text{O-}O-\text{O-}O-\text{S-}S-\text{O-}O-\text{O-}O-\text{O-}O-\text{OH} \)  

\[ \text{HO-}O-\text{O-}O-\text{O-}O-\text{O-}O-\text{S-}S-\text{O-}O-\text{O-}O-\text{O-}O-\text{O-}O-\text{O} \rightarrow \text{Cross-linker (C)} \]  

iii) \( \text{HO-}O-\text{O-}O-\text{O-}O-\text{O-}O-\text{O-}O-\text{O} + \text{Cl-}O-\text{O-}O-\text{O-}O-\text{O-}O-\text{O-}O \rightarrow \text{HO-}O-\text{O-}O-\text{O-}O-\text{O-}O-\text{O-}O-\text{O-}O-\text{O-}O-\text{O} \) **Control cross-linker (CC)**

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**Reagents and Conditions:**  

- a) \( \text{Et}_3\text{N}, \text{DCM}, 0 \text{°C}-\text{rt}, 12 \text{ h} \)  
- b) \( \text{THF}, \text{Me}_3\text{PPh}, \text{rt}, 24 \text{ h} \)  
- c) \( \text{Acyloyl Chloride, DCM, Et}_3\text{N,0 °C-rt, 12 h} \)  
- d) \( \text{Et}_3\text{N, DCM, 0°C-rt, 12 h} \)

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**Scheme S1:** Synthesis of the monomer, crosslinker and control crosslinker.

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i) **Synthesis of Monomer (M):** Monomer was synthesized following a literature reported procedure.  

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S1
$^1$H-NMR (400 MHz, CDCl$_3$, TMS): $\delta$ (ppm) = 6.46 (d, 1H), 6.17 (m, 1H), 5.86 (d, 1H) 4.34 (t, 2H), 3.67 (m, 14H). HRMS (ESI): m/z calculated for $C_{11}H_{21}O_6$ (M + H)$^+$ = 249.1340, found 249.1350.

**ii) Synthesis of Crosslinker (C), Step 1:** 170 mg (1.80 mmol) of ethane dithiol and 980 mg (3.94 mmol) of tetra ethylene glycol acrylate monomer were taken in a round bottom flask along with 15 mL dry THF. Catalytic amount of dimethyl-phenylphosphine (6 mg, 0.044 mmol) was added to the reaction mixture and it was stirred for 24 h at room temperature under Argon atmosphere. The reaction was stopped and solvent was evaporated to get crude product as oil. It was purified by column chromatography using silica gel as stationary phase and hexane/Ethylacetate as eluent. Finally, pure product was separated as light yellow oil in 75 % yield.

$^1$H-NMR (400 MHz, CDCl$_3$, TMS): $\delta$ (ppm) = 4.30 (t, 4H), 3.75-3.62 (m, 28H), 2.84 (t, 4H), 2.76 (s, 4H), 2.67 (t, 4H). HRMS (ESI): m/z calculated for $C_{24}H_{47}O_{12}S_2$ (M + H)$^+$ = 591.2511, found 591.2510.

**Synthesis of Crosslinker (C), Step 2:** 650 mg (1.1 mmol) of the product obtained in the previous step and 15 mL dry CH$_2$Cl$_2$ was taken in a round bottom flask. To this solution 394 mg (3.9 mmol) triethylamine was added and the reaction mixture was stirred in an ice bath. To the reaction mixture a solution of acryloyl chloride (471 mg, 5.2 mmol) in 10 mL dry CH$_2$Cl$_2$ was added drop-wise with constant stirring under Argon atmosphere. After the addition was over the reaction mixture was stirred at room temperature for 12 h. The reaction was stopped and the solution was washed with H$_2$O ($2 \times 50$ mL) to remove triethyl amine hydrochloride salt and the unreacted acryloyl chloride which has been converted to acrylic acid in presence of water. The combined organic part was dried over anhydrous Na$_2$SO$_4$ and CH$_2$Cl$_2$ was removed under reduced pressure to get the crude product as brown color oil. For the complete removal of acrylic acid, the crude product was kept under vacuum for 24 h to get pure product as brown color semisolid in quantitative yield.
$^1$H-NMR (400 MHz, CDCl$_3$, TMS): $\delta$ (ppm) = 6.46 (d, 2H), 6.17 (m, 2H), 5.86 (d, 2H) 4.33-4.27 (m, 8H), 3.77-3.67 (m, 24H), 2.84 (t, 4H), 2.76 (s, 4H), 2.66 (t, 4H). $^{13}$C (CDCl$_3$) $\delta$ (ppm): 171.78, 166.16, 131.03, 128.28, 70.63, 70.58, 69.13, 69.07, 63.85, 63.67, 34.78, 32.11, and 26.98. HRMS (ESI): m/z calculated for $\text{C}_{30}\text{H}_{50}\text{O}_4\text{S}_2\text{Na}$ (M + Na)$^+$ = 721.2540, found 721.2517.

**iii) Synthesis of Control Crosslinker (CC):** 550 mg (2.21 mmol) of monomer, M and 253 mg (2.5 mmol) triethylamine were dissolved in 10 ml dry CH$_2$Cl$_2$ in a round bottom flask. The reaction mixture was stirred and cooled in an ice bath. Sebacoyl chloride (241 mg, 1.007 mmol) was dissolved in dry CH$_2$Cl$_2$ and was added drop wise to the cold solution of monomer and triethylamine mixture under argon atmosphere. After the addition was over reaction was carried out for another 12 h at room temperature. The reaction was stopped and the mixture was washed with H$_2$O (2 x 50 mL) to remove the triethylamine hydrochloride salt and the aqueous phase were treated with dichloromethane (2 x 30 mL) to extract the crude product. The combined organic layer was dried over anhydrous Na$_2$SO$_4$ and CH$_2$Cl$_2$ was removed under reduced pressure to get the crude product as colorless oil. It was purified by column chromatography using silica gel as stationary phase and ethylacetate/hexane as eluent. The pure product was separated as colorless oil in 50 % yield.

$^1$H NMR (300 MHz, CDCl$_3$, TMS): $\delta$ (ppm) = 6.46 (d, 2H), 6.17 (m, 2H), 5.86 (d, 2H) 4.33 (t, 4H), 4.23 (t, 4H), 3.76-3.66 (m, 24H), 2.33 (t, 4H), 1.62 (t, 4H), 1.3 (m, 8H). $^{13}$C (CDCl$_3$) $\delta$ (ppm): 173.76, 166.14, 131.00, 128.28, 70.64, 70.57, 69.21, 69.13, 63.67, 63.35, 34.16, 29.08, 29.06, and 24.85. HRMS (ESI): m/z calculated for $\text{C}_{32}\text{H}_{64}\text{O}_{14}\text{Na}$ (M + Na)$^+$ = 685.3412, found 685.3412.
**Figure S1**: Calibration curve using BCA assay; Absorbance monitored at 562 nm; Temperature = 25 °C.

**Figure S2**: Enzymatic activity of native GAA at pH 5.0 and pH 7.4; Temperature = 37 °C.
Figure S3: Activity assay of the released GAA from the nanogel (NG) and control nanogel (CNG) at pH 5.0 and at different time interval; a) 5 min, b) 30 min, c) 24 h, and d) after 48 h incubation. Temperature = 37 °C.

Figure S4: % Activity of the released GAA from the nanogel over 49 h.
**Figure S5**: a) Normalized emission spectra of the GAA loaded in the nanogel (black line) and native GAA (red line) at pH 7.4; b) Normalized emission spectra of GAA loaded in the nanogel (black line) and GAA released from nanogel after 48h incubation at pH 5.0

**Figure S6**: DLS profile (PDI = 0.44, 0.40, 0.45) of the GAA loaded nanogel at pH 7.4. Even after 10 days there was no significant size change.
Figure S7: *In vitro* cytotoxicities of degraded nanogel on 293T cell (black) and MDA-MB 231 cell (red).

Figure S8: Autocorrelation function for nanogel (NG) a) at pH 7.4 and b) at pH 5 (48 h incubation). For nanogel at pH 7.4 the smooth short decay time indicates one type of small size distribution. For swelled nanogel at pH 5.0 the slower decay indicates that larger aggregates are present in solution but also shows a slight bimodal distribution indicating the presence of other (larger) aggregates which can be correlated with DLS data and TEM data in Figure 3 in the main text.
**Figure S9:** Autocorrelation function for GAA loaded nanogel a) at pH 7.4 and b) at pH 5.0 (48 h incubation). For nanogel at pH 7.4 the smooth short decay time indicates one type of small size distribution. For swelled nanogel at pH 5.0 the slower decay indicates that larger aggregates are present in solution but also shows a slight bimodal distribution indicating the presence of other (larger) aggregates.

**Figure S10:** Additional TEM images of a) Nanogel; b) GAA encapsulated nanogel.
Figure S11: NMR of Crosslinker (CC). * indicates solvent peak. The chemical shift values are shown on the X axis.

Figure S12: NMR of Nanogel. * indicates solvent peak. The chemical shift values are shown on the X axis.
Calculation of % crosslinker present in the NG:

For crosslinker 10 protons = 0.49, so 1 proton = 0.049.

For ‘c’ protons, between 2 protons one is from crosslinker and another is from monomer.

Hence for ‘c’ protons, monomer contribution = (1 - 0.049) = 0.951

% of crosslinker in the nanogel = (0.049/1)*100 = 4.9 % ≈ 5 %

Figure S13: Left: Endpoint activity of the released GAA from the nanogel at different time point. Right: Size change of the nanogel at pH 5 at different time point.

Reference:

1. Dan, K.; Pan, R.; Ghosh, S. *Langmuir* 2011, 27, 612-617.