# Supplementary information

# Introducing hyaluronic acid into supramolecular polymers and hydrogels

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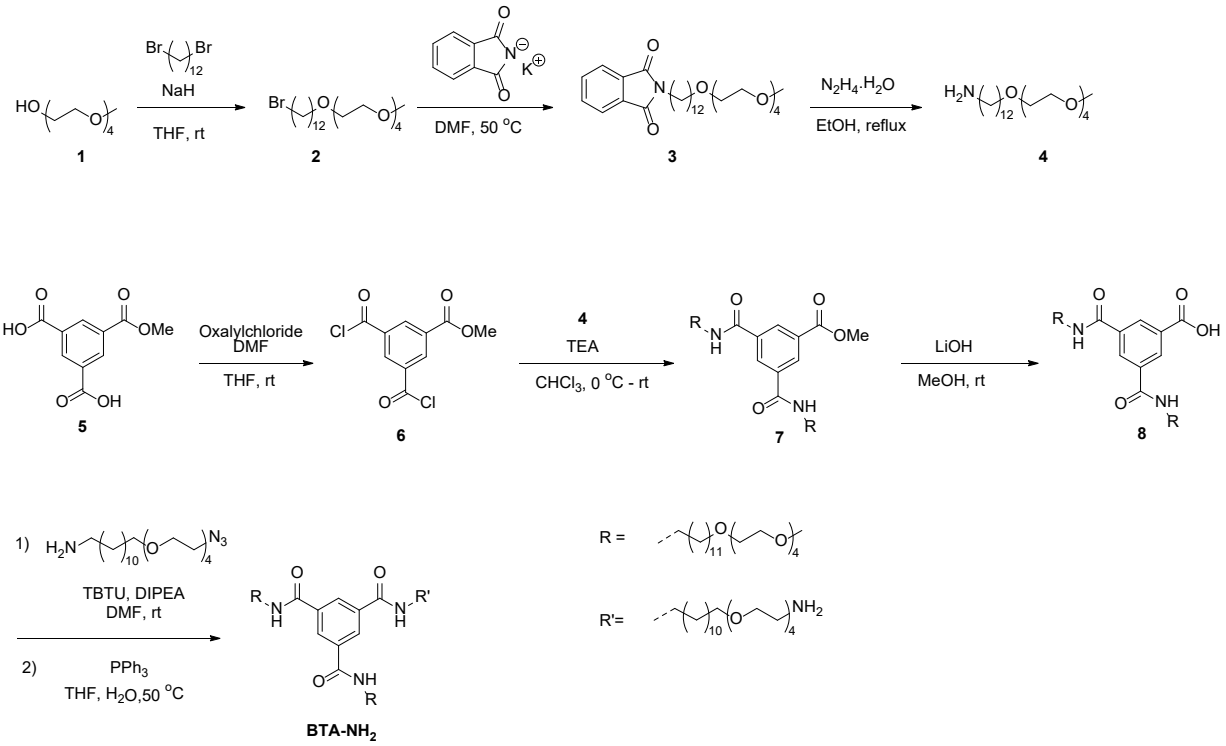
# Synthesis

**Scheme S1.** Synthesis of asymmetric **BTA-NH$_2$**.




### *26-Bromo-2,5,8,11,14-pentaoxahexacosane (2)*

A stirring mixture of NaH (60% dispersion in mineral oil, 772.1 mg, 19.3 mmol) in anhydrous THF (10 mL) was cooled to 0 °C and subsequently a solution of tetraethyleneglycol monomethyl ether (2.01 g, 9.65 mmol) was added dropwise. Another stirring solution containing 1,12-dibromododecane (6.46 g, 19.3 mmol) in anhydrous THF (20 mL) was placed in an ice bath. Under constant stirring, the mixture of tetraethyleneglycol monomethyl ether **1** and sodium hydride in THF was added dropwise during 15 minutes and the reaction mixture was allowed to stir overnight at room temperature under an argon atmosphere yielding a light milky yellow solution. To this solution water (100 mL) was added. The mixture was extracted four times with hexane (100 mL). The organic layers were combined, concentrated and dried with $MgSO_4$. Removal of the solvent in *vacuo* yielded a colorless oil that was purified by column chromatography (heptane/ethyl acetate 95/5-50/50) giving product **2** in 13% yield (570.0 mg, 1.3 mmol). $^1$H NMR (400 MHz, $CDCl_3$) δ 3.69 – 3.61 (m, 12H), 3.60 – 3.52 (m,

4H), 3.44 (t, 2H), 3.40 (t, 2H), 3.38 (s, 3H), 1.85 (q, 2H), 1.61 – 1.52 (m, 2H), 1.47 – 1.37 (m, 2H), 1.34 – 1.25 (m, 16H). NMR data was in good agreement with the literature.[1]

***2,5,8,11,14-Pentaoxahexacosan-26-phthalimide (3)***

A round bottom flask (50 mL) was equipped with a condenser and charged with 26-bromo-2,5,8,11,14-pentaoxahexacosane **2** (550.0 mg, 1.2 mmol), potassium phthalimide (273.9 mg, 1.5 mmol) and anhydrous DMF (30 mL). The mixture was heated to 80°C for 30 minutes and subsequently allowed to stir overnight at 50 °C. The solvent was removed in *vacuo*, and a yellow paste was obtained. Deionized water (50 mL) was added and the mixture was extracted three times with diethylether (50 mL). The organic layers were combined, dried over $MgSO_4$ and the solvent was evaporated under reduced pressure yielding product **3** as a yellow oil in 93% yield (588.0 mg, 1.1 mmol). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.82 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.72 (dd, *J* = 5.4, 3.1 Hz, 2H), 3.71 – 3.60 (m, 14H), 3.60 – 3.51 (m, 4H), 3.44 (t, *J* = 6.7 Hz, 2H), 3.37 (s, 3H), 1.66 (p, *J* = 7.3 Hz, 2H), 1.57 (p, *J* = 6.8 Hz, 2H), 1.32 – 1.23 (m, 16H). NMR data was in good agreement with the literature.[1]

***2,5,8,11,14-Pentaoxahexacosan-26-amine (4)***

To a stirring solution of 2,5,8,11,14-pentaoxahexacosan-26-phthalimide **3** (550.0 mg, 1.1 mmol) in ethanol (15 mL), hydrazine monohydrate (1.0 mL, 13.7 mmol) was added and the mixture was heated to reflux overnight. After solvent removal, chloroform (15 mL) was added and the mixture was extracted three times with NaOH (1 M, 15 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated in *vacuo* yielding product **4** as a yellow oil in 94% yield (388.0 mg, 990.8 μmol). $^1$H NMR (400 MHz, $CDCl_3$) δ 3.68 – 3.60 (m, 12H), 3.56 (m, 4H), 3.44 (t, *J* = 6.8 Hz, 2H), 3.37 (s, 3H), 2.67 (t, *J* = 7.0 Hz, 2H), 1.61 – 1.52 (m, 2H), 1.43 (dd, *J* = 8.3, 5.6 Hz, 2H), 1.33 – 1.24 (m, 16H). NMR data was in good agreement with the literature.[1]

***Methyl-3,5-bis-chlorocarbonyl-benzoate (6)***

5-Methoxycarbonyl-benzene-1,3-dicarboxylic acid[2] **5** (240.0 mg, 1.1 mmol) was dissolved in 7 mL of anhydrous THF under an argon atmosphere. A catalytic amount of DMF (one droplet) was added to the solution. Oxalyl chloride (0.2 mL, 2.4 mmol) was dissolved in 5 mL anhydrous THF and added dropwise to this solution in an ice bath. The solution was stirred for 3 hours at

room temperature. Subsequently, THF was removed and a yellowish suspension was obtained. The excess of oxalyl chloride was removed by co-evaporation with toluene (twice). The product **6** was obtained as a yellow solid in 95% yield (266.0 mg, 1.0 mmol). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.98 (d, *J* = 13.6 Hz, 2H), 8.85 (d, *J* = 7.1 Hz, 2H), 4.11 (s, 3H). NMR data was in good agreement with the literature.[1]

***Methyl-3,5-bis(2,5,8,11,14-pentaoxahexacosan-26-ylcarbamoyl)benzoate (7)***

To a stirring solution of methyl-3,5-bis(chlorocarbonyl)benzoate **6** (266.7 mg, 1.0 mmol) in anhydrous DCM (10 mL), dry triethylamine (0.7 mL, 4.1 mmol) was added. 2,5,8,11,14-pentaoxahexacosan-26-amine **4** (880.1 mg, 2.3 mmol) was dissolved in dry DCM (5 mL) and added dropwise to the stirring reaction mixture at 0 °C. After addition the reaction mixture was stirred overnight at room temperature under argon. After, the reaction mixture was concentrated, the solids were dissolved in chloroform (10 mL) and extracted twice with HCl (1 M, 20 mL) and with brine (20 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated in *vacuo* and further purified by column chromatography (heptane/dimethoxy ethane 90/10-40/60) giving product **7** in 42% yield (414.7 mg, 1.0 mmol). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, *J* = 1.6 Hz, 2H), 8.46 (t, *J* = 1.6 Hz, 1H), 7.21 (t, *J* = 5.6 Hz, 2H), 3.92 (s, 3H), 3.64 – 3.61 (m, 24H), 3.56 – 3.52 (m, 8H), 3.44 – 3.40 (m, 8H), 3.35 (s, 6H), 1.61 – 1.53 (m, 8H), 1.33 – 1.24 (m, 32H). NMR data was in good agreement with the literature.[1]

***Methyl-3,5-bis(2,5,8,11,14-pentaoxahexacosan-26-ylcarbamoyl)benzoic acid (8)***

To a stirring solution of methyl-3,5-bis(2,5,8,11,14-pentaoxahexacosan-26-ylcarbamoyl)benzoate **7** (414 mg, 427 μmol) in methanol, lithium hydroxide monohydrate (55.8 mg, 1.3 mmol,) and $H_2O$ (0.1 mL) were added. The mixture was stirred overnight at room temperature. After solvent removal, HCl (10 mL, 1 M) was added and extracted three times with chloroform (10 mL). The organic layers were combined, dried with $MgSO_4$, filtered and concentrated in *vacuo* yielding product **8** in 90% yield (366.5 mg, 382.9 μmol) as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.64 (s, 1H), 8.54 (s, 2H), 3.68 – 3.61 (m, 24H), 3.59 – 3.52 (m, 8H), 3.43 (t, *J* = 6.8 Hz, 8H), 3.36 (s, 6H), 1.70 – 1.59 (m, 4H), 1.58 – 1.49 (m, 4H), 1.36 – 1.20 (m, 32H). NMR data was in good agreement with those previously reported.[1]

***$N^1$-(1-Amino-3,6,9,12-tetraoxatetracosan-24-yl)-$N^3$,$N^5$-di(2,5,8,11,14-pentaoxahexacosan-26-yl)benzene-1,3,5-tricarboxamide (BTA-$NH_2$)***

To a stirring solution containing methyl-3,5-bis(2,5,8,11,14-pentaoxahexacosan-26-ylcarbamoyl)benzoic acid **8** (264 mg, 275 μmol) and DIPEA (96 μL, 552 μmol) in DMF (1 mL), TBTU (97.4 mg, 303.3 μmol) was added and let react for 5 minutes at room temperature. Another solution containing an excess of 1-azido-3,6,9,12-tetraoxatetracosan-24-amine[3] (121 mg, 1.7 mmol) in 1 mL of DMF was added to the mixture and allowed to stir for 1 hour at room temperature. The solvent was evaporated and the crude was re-dissolved in 10 mL of chloroform and extracted three times with water. The organic phase was combined, dried over $MgSO_4$ and evaporated under reduced pressure.

Without purification, the mixture was dissolved in THF (10 mL) and water (4 mL). Triphenylphosphine (52.4 mg, 0.2 mmol) was added to the solution, and the reaction was allowed to stir overnight at 50 °C. The solvent was evaporated in *vacuo* and the reaction mixture was purified by column chromatography ($CHCl_3$/MeOH 9/1-$CHCl_3$/MeOH/isopropyl amine 90/7/3) giving product **BTA-$NH_2$** as a yellow oil in 40% yield over two steps (140.5 mg, 106.8 μmol). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.80 – 8.70 (m, 3H, $CH_2N\underline{H}C{=}O$), 8.45 (s, 3H, Ar), 3.54 – 3.41 (m, 46H, O-($C\underline{H}_2$)$_2$-O), 3.40 – 3.25 (m, 14H, $CH_2C\underline{H}_2NHC{=}O$, $CH_2CH_2C\underline{H}_2O$, $CH_2C\underline{H}_2NH_2$), 3.24 (s, 6H, $OC\underline{H}_3$), 2.87 (t, *J* = 5.4 Hz, 2H, $CH_2CH_2N\underline{H}_2$), 1.56 – 1.42 (m, 12H, $CH_2C\underline{H}_2CH_2O$, $C\underline{H}_2CH_2NHC{=}O$), 1.31 – 1.21 (m, 48H, aliphatic). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 165.80, 135.48, 128.80, 71.75, 70.78-69.95, 67.13, 58.51, 39.04, 29.68-29.28, 26.98, 26.13. MALDI-TOF-MS: calculated MW = 1314.97 g/mol, observed m/z = 1315.98 [$MH^+$], 1337.95 [$Na^+$ adduct].

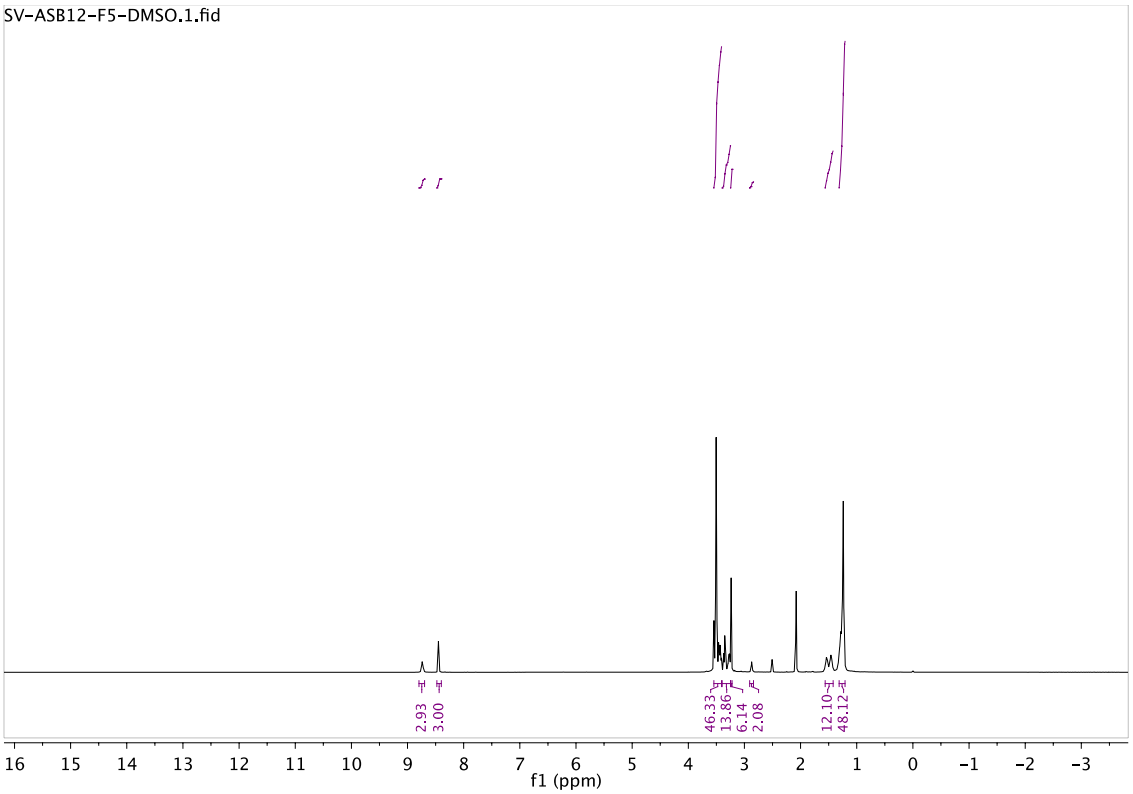



**Figure S1**. $^{1}$H NMR of BTA **9** (400 MHz, DMSO-$d_6$)

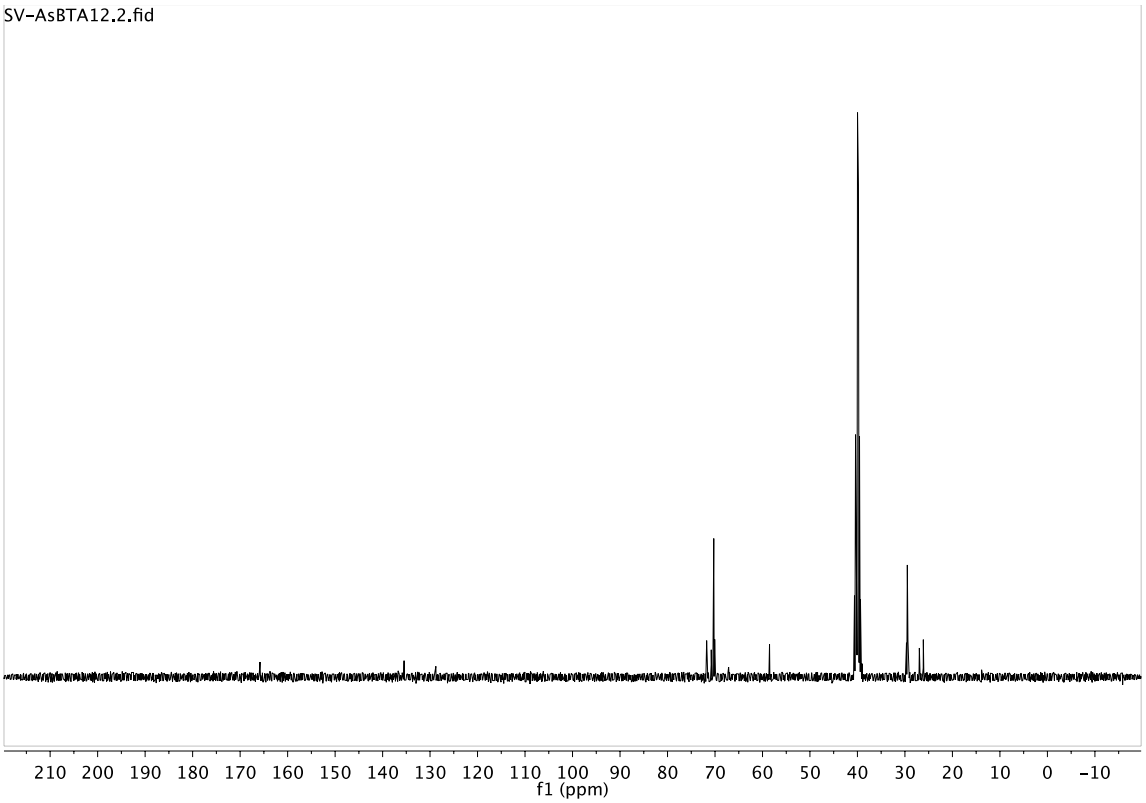


**Figure S2**. $^{13}C$ NMR of BTA **9** (100 MHz, DMSO-$d_6$)

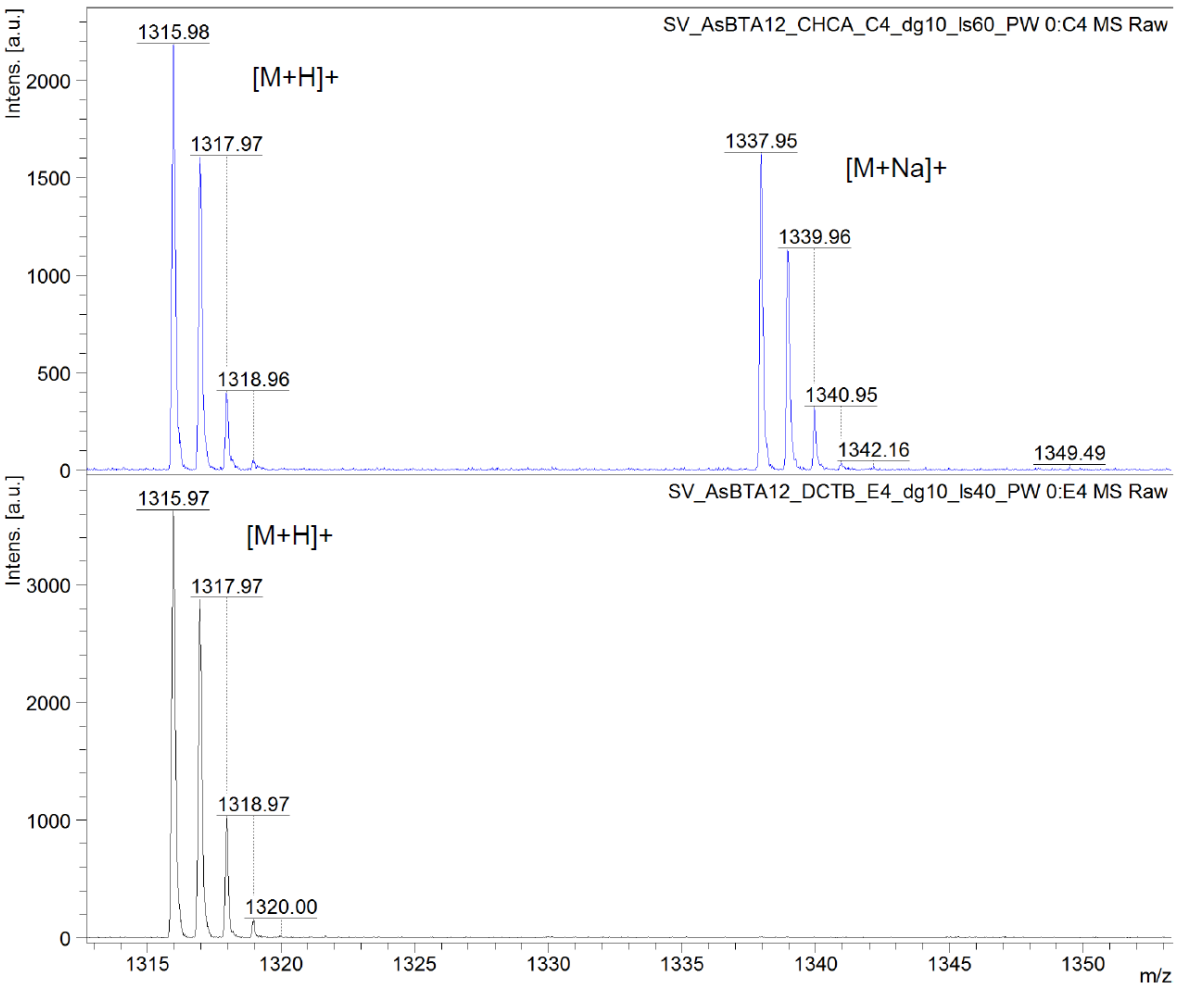



**Figure S3**. MALDI-TOF-MS of BTA **9**

## Supporting figures

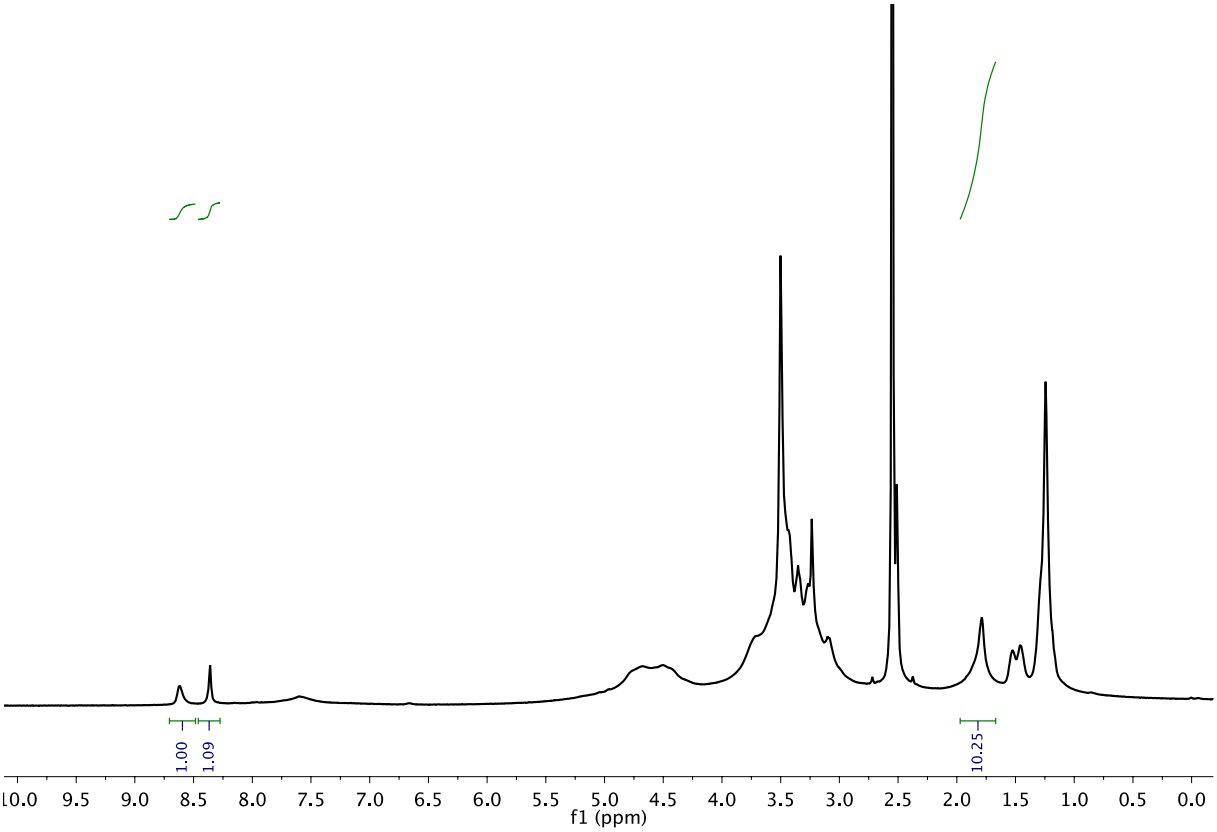



**Figure S4**. $^{1}$H-NMR (400 MHz) of **HA-BTA** in DMSO-$d_6$ shows the typical BTA peaks at 8.4 ppm (aromatic protons), 8.6 ppm (amide protons) and the typical peak of HA at 1.8 ppm (methyl protons). Integration of these peaks was used to calculate the BTA loading (10%).

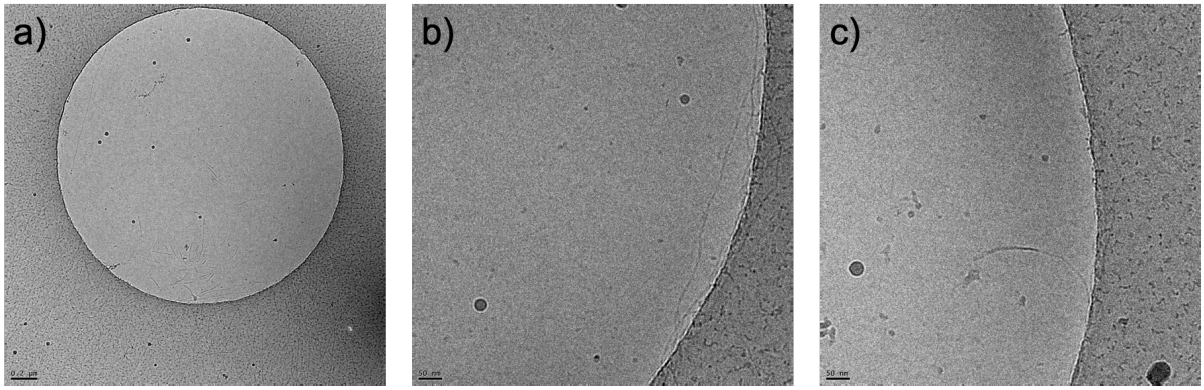



**Figure S5**. CryoTEM imaging of HA co-assembled with **BTA-OEG$_4$** (1:2) show both long and short fiber formation; a) scale bar = 200 nm, b) and c) scale bar = 50 nm. Dark grey objects are crystalline ice particles.

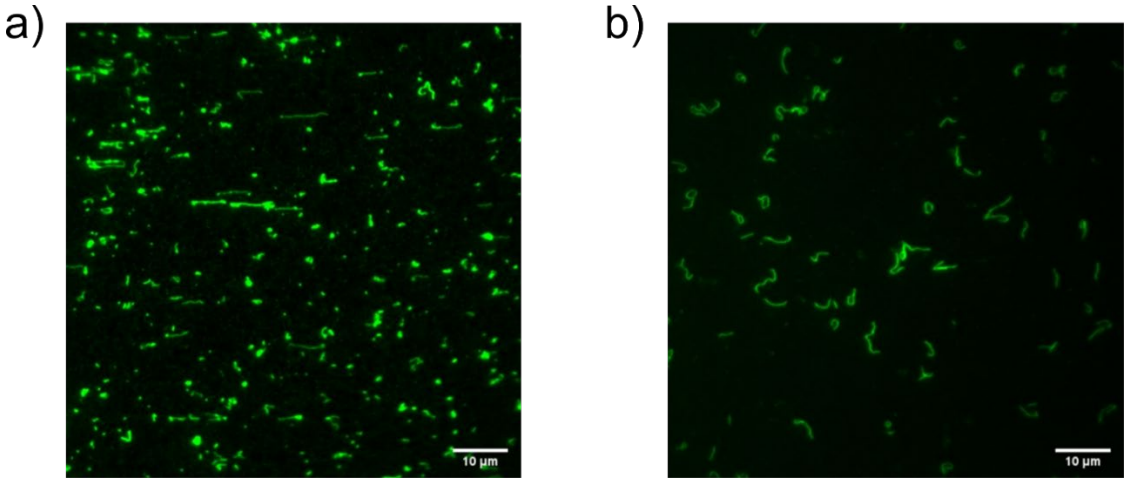



**Figure S6.** TIRF imaging on a) HA + **BTA-OEG$_4$-Cy3** at 2:2 HA:**BTA-OEG$_4$-Cy3** weight ratio forms a mixture between long and short fibers and small aggregates; b) **BTA-OEG$_4$-Cy3**, as control forms 1D fibers.

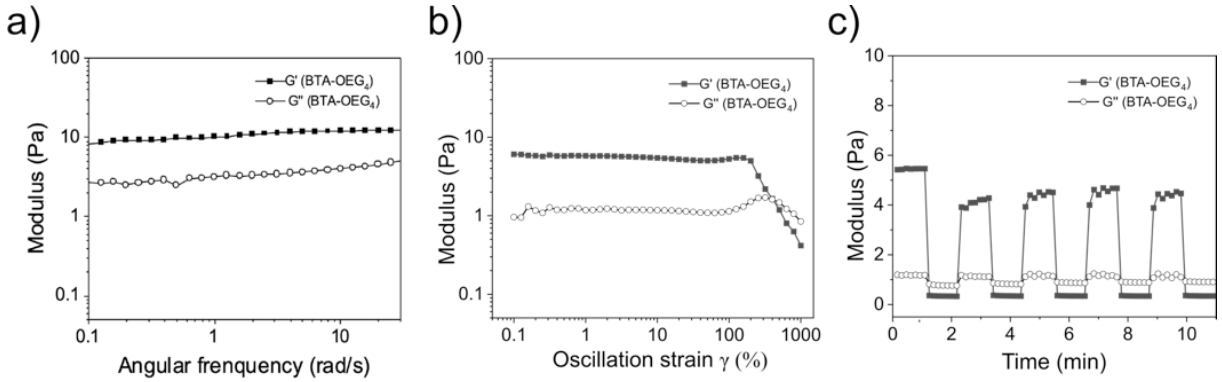



**Figure S7**. a) Frequency sweep rheology measurements at 37 °C (1% fixed applied strain) of **BTA-OEG$_4$** (2 wt%) in water; b) Strain sweep oscillatory rheology (fixed angular frequency of 1 rad/s) of **BTA-OEG$_4$** (2 wt%) in water shows a sol-to gel transition upon 300% strain; c) Oscillatory rheology measurements of **BTA-OEG$_4$** (2 wt%) in water, alternating between 1 and 1000% strain for 30 s periods at 37 °C (1 rad/s fixed angular frequency) show self-healing properties over at least 5 cycles.

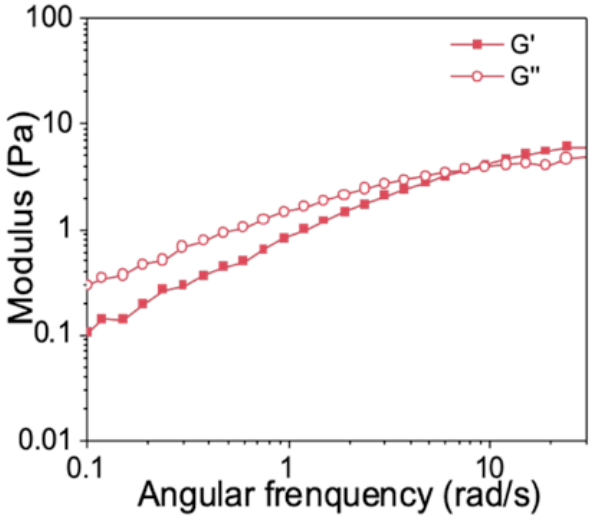



**Figure S8**. Frequency sweep rheology measurements at 37 °C (1% fixed applied strain) of **HA-BTA** 5 wt% in water shows a higher loss modulus (G") than storage modulus (G') in most of the regime, indicating the formation of a viscoelastic fluid.

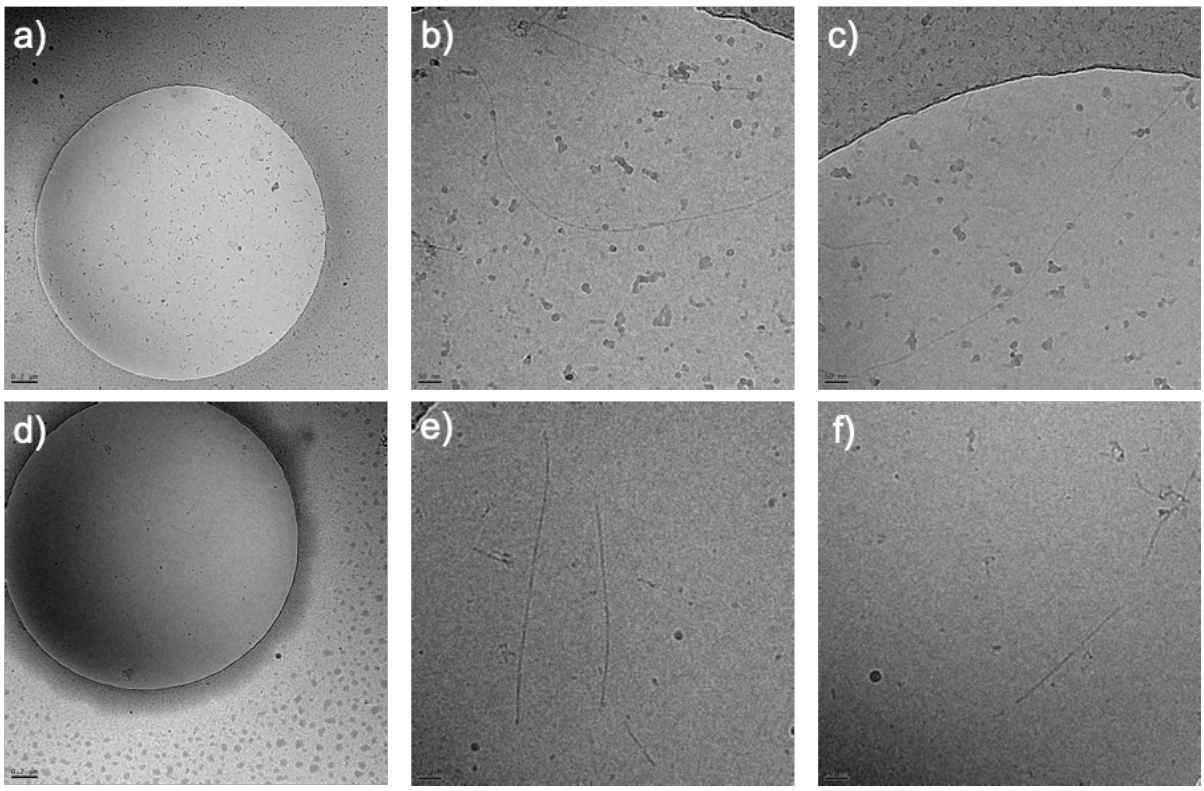



**Figure S9**. CryoTEM image of **HA-BTA** co-assembled with **BTA-OEG$_4$** (**HA-BTA/BTA-OEG$_4$**, 0.5:2 HA:BTA) a) scale bar = 200 nm, b) and c) scale bar = 200 nm. CryoTEM image

of **HA-BTA** co-assembled with **BTA-OEG$_4$** (**HA-BTA/BTA-OEG$_4$**, 2:2 HA:BTA) d) scale bar = 200 nm, e) and f) scale bar = 50 nm. Dark grey objects are crystalline ice particles.

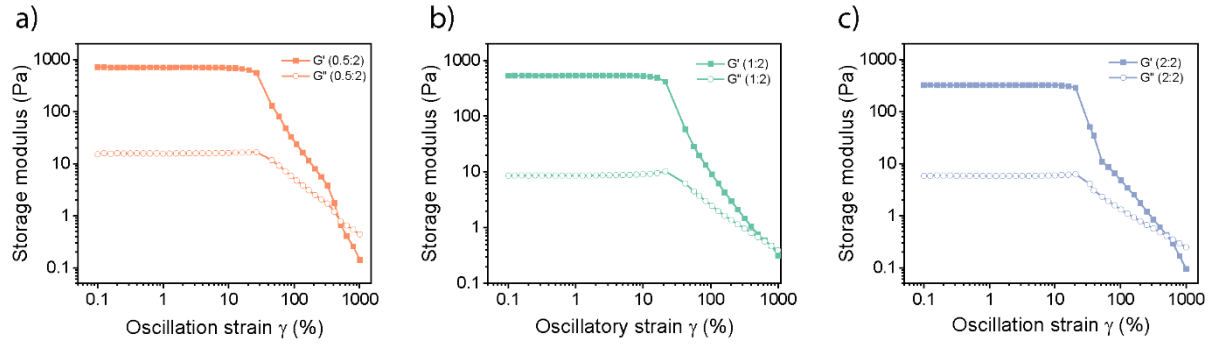



**Figure S10**. Strain dependent oscillatory rheology at 37 ºC (fixed angular frequency of 1 rad/s) of **HA-BTA/BTA-OEG$_4$** at three different HA:BTA ratios in water a) 0.5:2 (2.5 wt%); b) 1:2, (3 wt%); c) 2:2, (4 wt%) show a sol-to-gel transition upon 500% strain.

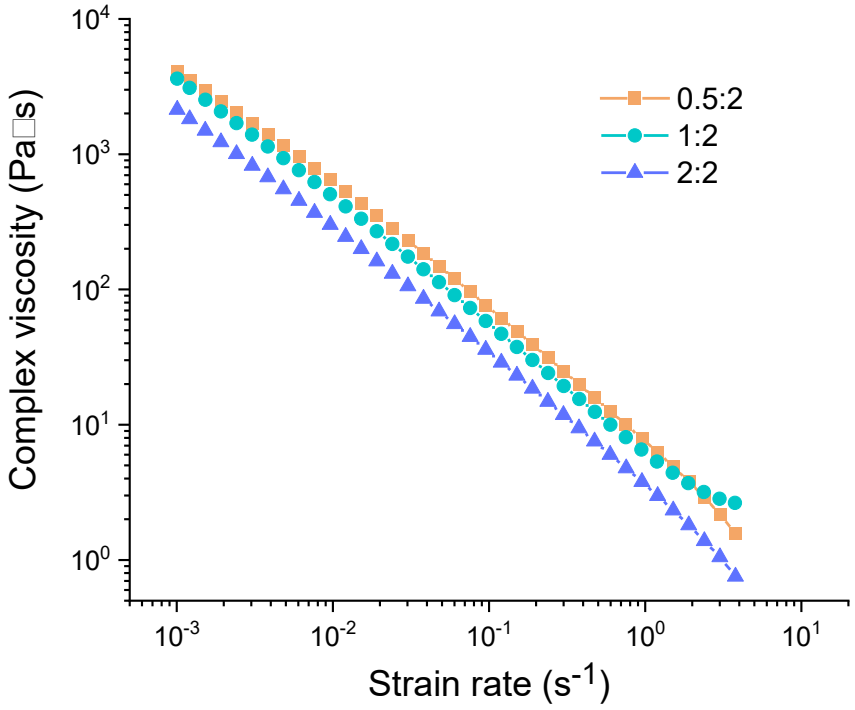



**Figure S11.** Shear-thinning behavior of **HA-BTA/BTA-OEG$_4$** samples at three different HA:BTA ratios in water 0.5:2 (2.5 wt%), 1:2, (3 wt%) and 2:2 (4 wt%).

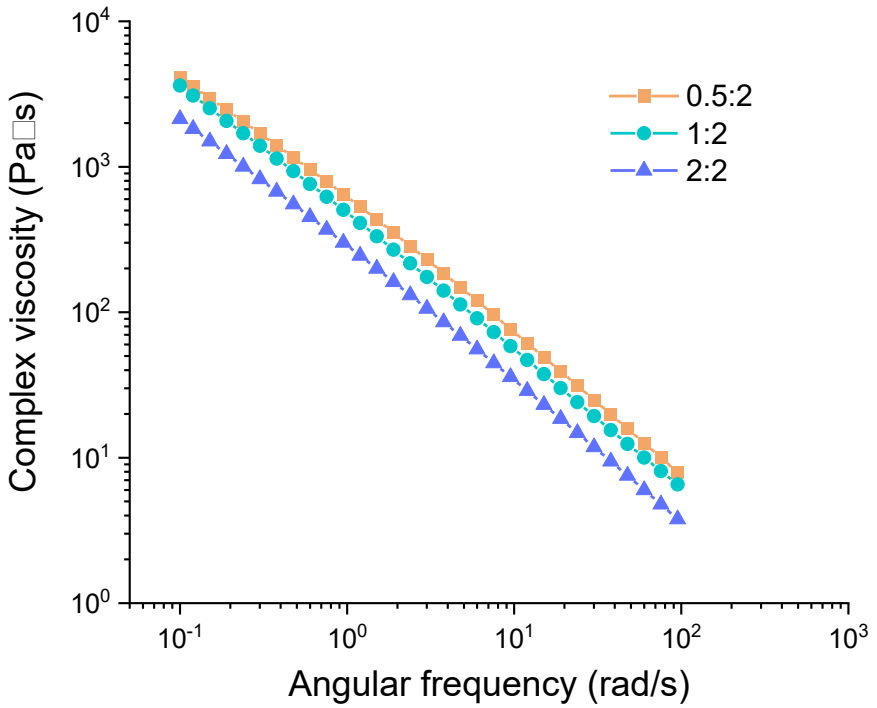



**Figure S12**. Frequency-dependent dynamic properties of the **HA-BTA/BTA-OEG4** hydrogels at three different HA:BTA ratios in water 0.5:2 (2.5 wt%), 1:2, (3 wt%) and 2:2 (4 wt%).

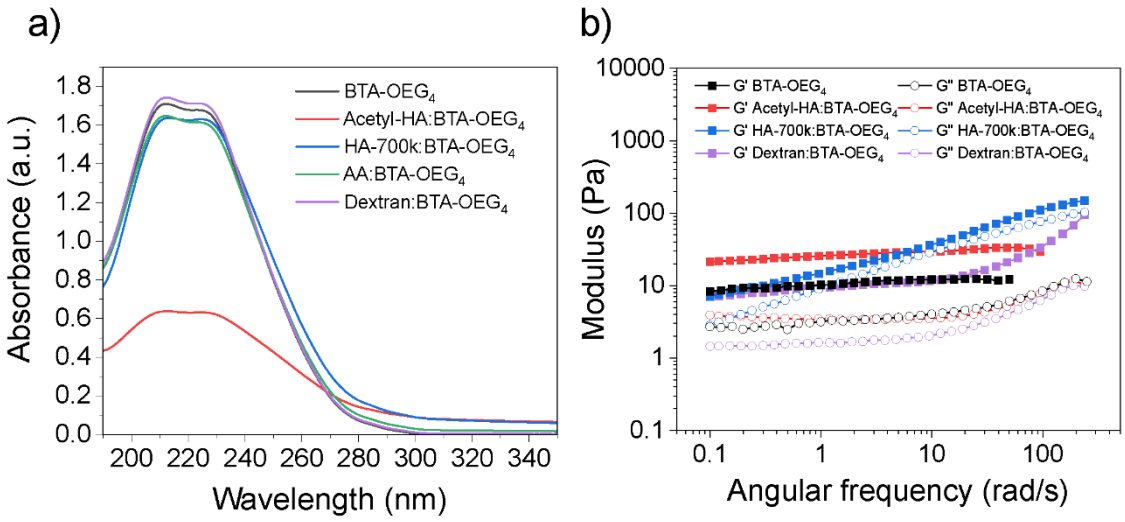



**Figure S13**. a) UV-Vis spectra of **BTA-OEG4** (500 μM) in water co-assembled with acetyl-HA, HA 700 kDa, AA and dextran at 1:2 (polysaccharide:BTA) weight ratio; b) Frequency

sweep rheology at 37 ºC (fixed angular frequency of 1 rad/s) of **BTA-OEG$_4$** (2 wt%) alone or co-assembled with Acetyl-HA, HA-700k and dextran (1 wt%) in water.

# References


(1) Leenders, C. M. A.; Mes, T.; Baker, M. B.; Koenigs, M. M. E.; Besenius, P.; Palmans, A. R. A.; Meijer, E. W. From Supramolecular Polymers to Hydrogel Materials. *Mater. Horiz.* **2014**, *1* (1), 116–120. https://doi.org/10.1039/C3MH00103B.

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(3) Albertazzi, L.; Martinez-Veracoechea, F. J.; Leenders, C. M. A.; Voets, I. K.; Frenkel, D.; Meijer, E. W. Spatiotemporal Control and Superselectivity in Supramolecular Polymers Using Multivalency. *Proc. Natl. Acad. Sci.* **2013**, *110* (30), 12203 LP – 12208. https://doi.org/10.1073/pnas.1303109110.