

Supporting information for

Manipulating the mechanical properties of biomimetic hydrogels with multivalent host-guest interactions

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1. Materials.

Acrylamide (AAm, 99%, contains 500 ppm monomethyl ether hydroquinone as inhibitor) monomers from Sigma-Aldrich was used after passing through an alumina column. All other chemicals were purchased at the highest purity and were used as received.

2. Methods.

The calculation of C_{HGM^*} . For easy comparison between the physical cross-links and chemical cross-links, the content of the host-guest macromers (HGM) was expressed as the effective concentration C_{HGM^*} , meaning that the content of cross-linkable acrylates originated from HGM is equivalent to that of MBA with the concentration of C_{HGM^*} . Since 2.7 wt/v% (27 mg/mL) of mono-Ac- β CD (Molecular weight: 1334 Da) was used for the preparation of HGM, the effective concentration of HGM can be calculated as:

$$2C_{\text{HGM}^*} = \frac{27 \text{ mg/mL}}{M_{\text{host}}}$$

Where M_{host} (1334 Da) is the molecular weight of the host monomer mono-Ac- β CD. Therefore,

$$C_{\text{HGM}^*} = 10 \text{ mM}$$

3. Experimental Section

3.1. Synthesis of AD_xHA .

The guest polymer AD_xHA (Mw. 74kD, substitution degree $x\% = 20\%$) was prepared as described elsewhere.¹ Briefly, to synthesis the guest polymer AD_xHA , sodium hyaluronic acid (HA-Na, 2.0 g) was treated by Dowex resin (6.0 g) for ion exchange in the water phase and then neutralized by tetrabutylammonium hydroxide (TBAOH) to produce the HA-TBA aqueous solution (pH = 7), which is soluble in DMSO after lyophilization. The obtained HA-TBA (1.0 g, 1.4 mmol disaccharide repeat units, 1 equiv), was then dissolved together with 1-adamantaneacetic acid (0.82 g, 4.2 mmol, 3 equiv.), and 4-dimethylaminopyridine (DMAP, 0.13 g, 1.05 mmol, 0.75 equiv.) in 100 mL of anhydrous DMSO at room temperature, and di-tert-butyl dicarbonate (BOC_2O , 0.5 equiv.) was added slowly under nitrogen protection at room temperature. The mixture was heated to 45 °C and kept for 24 h. After cooling down to room temperature, the reaction mixture was directly dialyzed against DMSO, NaCl(aq), and then DI water to remove all unreacted small organic compounds. The solution was then frozen and lyophilized to yield the product as a white solid.

3.2. Synthesis of mono-Ac- β CD.

The synthesis and characterization of the mono-acrylated β -cyclodextrin host monomers (mono-Ac- β CD) was reported elsewhere.¹ Briefly, the amino group of 6-monodeoxy-6-monoamino- β -cyclodextrin (β CD-NH₂) was converted to photopolymerizable acrylate by reacting with equivalent 2-(2-isothiocyanatoethoxy) ethylacrylate (Ac-NCS) in DMSO for 12 h at room temperature. The crude product was precipitated in acetone and purified by repeating the precipitation in acetone three times.

3.3. Preparation of hydrogels.

For the preparation of all the hydrogels, 400 μ L precursor solution was loaded on the rheometer (Malvern Kinexus Lap+) equipped with an UV curing system (OmniCure S1000), the disk like hydrogel objects (diameter = 20 mm, thickness = 1 mm) were obtained after the solution was exposed to UV light ($\lambda=365$ nm, 20 mW/cm²) for 30 minutes.

For the preparation of the HGM-containing hydrogels (MBA-HGM or HGM hydrogels), the HGM solution containing 4.0 wt/v% guest polymers (AD_xHA, $x = 20$) and 2.7 wt/v% host monomer (mono-Ac- β CD) was firstly prepared in PBS buffer (pH=7.4) and then mixed with *N,N*-methylenebisacrylamide (MBA), acrylamide (AAm, 2 M) and photo initiator 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (I2959, final concentration: 0.05 wt/v%) to give the precursor solution (the molar ratio between β -CD and AD was kept as 1:1.). For HGM-free hydrogels (MBA-*L* and MBA-*H* hydrogels), PBS buffer was used to dissolve MBA, AAm and I2959 for the preparation of precursor solutions as listed in Table 1.

3.4. Uniaxial tensile tests.

Uniaxial tensile tests were performed on the rheometer (Malvern Kinexus Lap+) with a deformation rate of 60 mm/min (in air). The hydrogel samples between the two clamps were 4.0 mm \times 4.0 mm \times 1.0 mm (width \times length \times thickness, W0 \times L0 \times T0).

3.5. Rheological tests.

Dynamic viscoelasticity of the hydrogels was measured by a Malvern Kinexus Lap+ rheometer equipped with a 20 mm plate-plate. For oscillatory time sweep experiments, the constant strain and frequency were fixed at $\gamma = 1\%$ and $f = 1$ Hz, respectively. For oscillatory frequency sweep experiments, the constant strain was fixed at $\gamma = 1\%$. For oscillatory strain sweep experiments, the constant frequency was fixed at $f = 1$ Hz. For the step-strain time-sweep experiment, the frequency was fixed at $f = 1$ Hz.

4. Supporting data

4.1. The calculation of the average crosslinker valency.

The chemical structures of the crosslinkers were illustrated in Scheme S1. The average crosslinker valency ($CV_{av.}$) is defined as the average number of double bonds on each crosslinker molecule:

$$CV_{av.} = \frac{2(C_{HGM^*} + C_{MBA})}{2C_{HGM^*}/N_{ADA} + C_{MBA}}$$

Where the content of the physical crosslinker (HGM) was expressed as the effective concentration C_{HGM^*} , meaning that the content of crosslinkable double bonds originated from HGM is equivalent to that of MBA with the concentration of C_{HGM^*} . C_{MBA} represents the concentration of the chemical crosslinker (MBA) and N_{ADA} represents the average number of adamantane functionalities on each AD_xHA ($x = 20$) polymer chain.

$$N_{ADA} = \frac{M_{HA}}{M_{ru}} \times x\%$$

The molecular weights of the HA polymer and its repeating unit are known as $M_{HA} = 74$ kD and $M_{ru} = 380$ D, respectively. Therefore, for MBA-*L* and MBA-*H* hydrogels that contain no physical crosslinkers ($C_{HGM^*} = 0$ mM), the average crosslinker valency can be calculated as

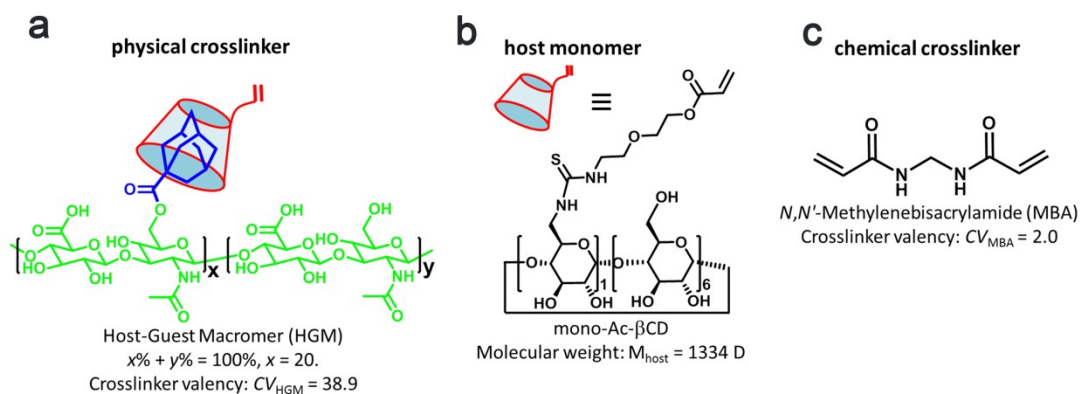
$$CV_{av.} = \frac{2(C_{HGM^*} + C_{MBA})}{2C_{HGM^*}/N_{ADA} + C_{MBA}} = \frac{2C_{MBA}}{C_{MBA}} = 2$$

For HGM hydrogels that contain no chemical crosslinkers ($C_{MBA} = 0$ mM), the average crosslinker valency can be calculated as

$$CV_{av.} = \frac{2(C_{HGM^*} + C_{MBA})}{2C_{HGM^*}/N_{ADA} + C_{MBA}} = N_{ADA} = \frac{74 \text{ kD}}{380 \text{ D}} \times 20\% = 38.9$$

For MBA-HGM hydrogels that contain both chemical crosslinkers and physical crosslinks ($C_{MBA} = 2$ mM, $C_{HGM^*} = 10$ mM), the average crosslinker valency can be calculated as

$$CV_{av.} = \frac{2(C_{HGM^*} + C_{MBA})}{2C_{HGM^*}/N_{ADA} + C_{MBA}} = 9.5$$



Scheme S1. The influence of the crosslinker valency on the gelation kinetics of PAAm hydrogels. The chemical structure of (a) the physical crosslinker, (b) the host monomer and (c) the chemical crosslinker.

4.2. The calculation of the energy dissipation efficiency.

The energy dissipation efficiency was calculated by $(A-B)/A = 22.8\%$, where A represents the integration area under the loading curve and B represents the integration area between the loading and unloading curves (Figure S1).

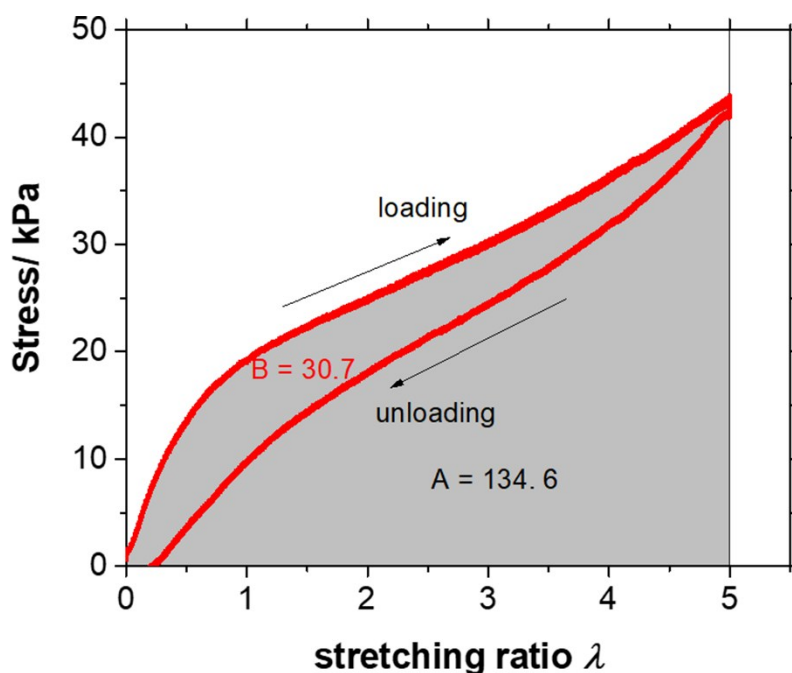


Figure S1. Cyclic tensile tests revealed that the MBA-HGM hydrogels can dissipate a large amount of the loading energy under large deformation (stretching ratio $\lambda = 5$) as evidenced by the substantial hysteresis between the loading and unloading curves.

Reference

1. K. Wei, M. Zhu, Y. Sun, J. Xu, Q. Feng, S. Lin, T. Wu, J. Xu, F. Tian and J. Xia, *Macromolecules*, 2016, **49**, 866-875.