Supporting Information

Tuning crosslink density in a physical hydrogel by supramolecular self sorting

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Table of Contents

Materials: Solvents used in synthesis were reagent grade. CH_2Cl_2, $CHCl_3$, Et_3N and Pyridine were distilled from CaH_2. All PEO derivatives were dried in vacuum over P_2O_5 during at least 12 h. The reagents 11-aminoundecanoic acid, poly(ethylene glycol)-monomethyl ether (M_n = 350), 1,4-diisocyanatobutane, 1,6-diisocyanatohexane and polyethylene oxide (average molecular weight = 8000) were purchased from Aldrich, Fluka, or Acros and were used without additional purification. 11-Aminoundecanoyl-(poly(ethylene glycol)-monomethylether)-ester[1] was prepared according to literature procedures.

General Methods: NMR spectra were acquired on a 400 MHz Varian Mercury Vx (400 MHz for ^{1}H-NMR, 100 MHz for ^{13}C-NMR). Proton and carbon chemical shifts are reported in ppm downfield of tetramethylsilane using the resonance of the deuterated solvent as internal standard. Splitting patterns are designated as singlet (*s*), doublet (*d*), triplet (*t*) and multiplet (*m*). Infrared spectra were measured on a Perkin Elmer 1600FT-IR. Matrix assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI-TOF) was performed on a Perseptive DE PRO Voyager MALDI-TOF mass spectrometer using α-cyano-4-hydroxycinnamic acid as the calibration matrix.

Samples for cryogenic transmission electron microscopy (cryo-TEM) were prepared in a 'Vitrobot' instrument4 (PC controlled vitrification robot, patent applied, Frederik et al 2002, patent licensed to FEI) at room temperature and a relative humidity >95%. In the preparation chamber of the 'Vitrobot' a 3 µl sample was applied on a Quantifoil grid (R 2/2, Quantifoil Micro Tools GmbH; freshly glow discharged just prior to use), excess liquid was blotted away and the thin film thus formed was shot (acceleration about 3 g) into liquid ethane. The vitrified film was transferred to a cryoholder (Gatan 626) and observed at -170 °C in a Tecnai microscope operating at 120 kV. Micrographs were taken at low dose conditions.

The Small-Angle X-Ray Scattering (SAXS) measurements were performed at the Dutch-Belgian BM26B beamline at the ESRF in Grenoble (France). A sample-to-detector distance of 4.53 m was used together with an X-ray photon energy of 12 keV. The observed q range was 0.04 nm^{-1} ≤ q ≤ 2.07 nm^{-1}, where q is the magnitude of the scattering vector $q = (4\pi/\lambda)\sin\theta$, and where λ is the x-ray wavelength and θ is half of the scattering angle.SAXS images were recorded using a 2D Pilatus 1M detector with 748×748; pixel dimension and with 260 µm2 pixel size. The 2D images were radially averaged in order to obtain the intensity $I(q)$ vs q profiles. The beam centre and the q range calibrations were achieved by using the position of the diffraction peaks of a silver behenate.

The liquid samples were contained in 2 mm borosilicate capillaries. Standard data reduction procedures, i.e. subtraction of the empty capillary contribution, correction for the sample absorption, were applied. Water has been used as secondary standard calibrants in order to perform intensity calibration on an absolute scale in cm^{-1}.

The SAXS intensity $I(q)$ scattered by an ensemble of monodisperse objects can be written as:

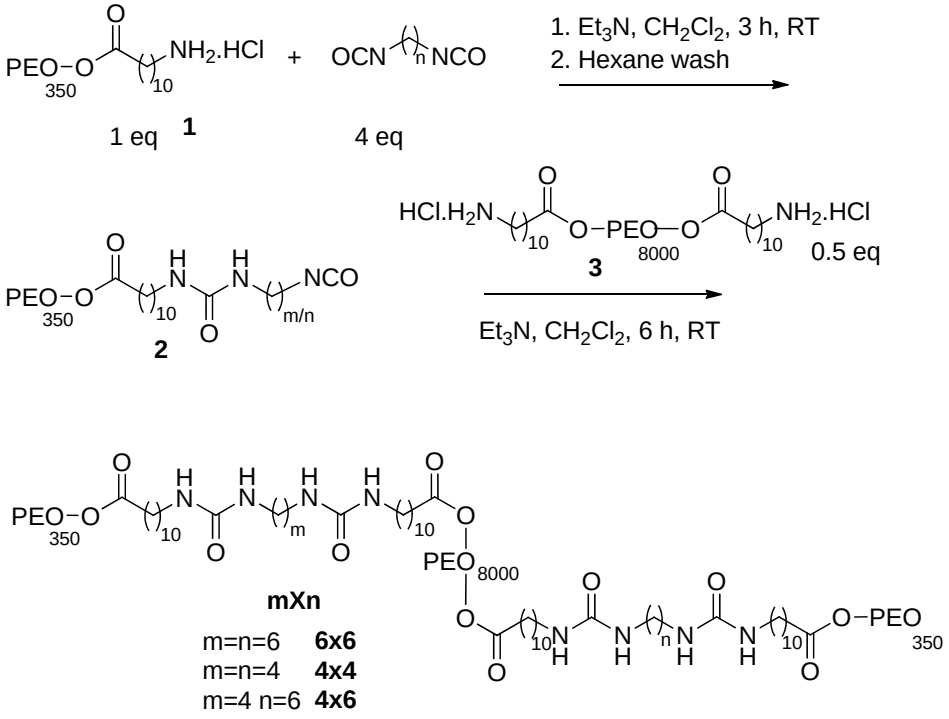
$I(q) = N_p (\Delta\rho)^2 V^2 P(q) S(q)$ where Np is the number density of scattering objects, Δρ is the electron densities difference between the object and the surrounding media (i.e. solvent), V is the object volume, $P(q)$ is the object form factor and $S(q)$ is the inter-particle structure factor which takes into account the correlation between the objects in solutions.

Mechanical properties of these hydrogels were tested by using rheology. Dynamic viscoelastic measurements were determined using a stress-controlled rheometer (Anton Paar, Physicia MCR501) equipped with a sand-blasted plate-plate geometry to prevent slippage. Measurement temperature was fixed at 20°C.

Synthesis of Tetraurea based crosslinkers (mXn):

A synthetic strategy was developed to prepare 'crosslinker' molecules that combine two terminal hydrophobic-bisurea blocks with a central hydrophillic PEO block (UmU-PEO8K-UnU, **mXn**) (Figure 2). The controlled synthetic strategy resulted in crosslinkers with the terminal-terminal sequence hydrophilic-hydrophobic- hydrophilic- hydrophobic- hydrophilic. The m/n values in the hydrophobic blocks have been varied to design crosslinkers with a single type of hydrophobic block or with a statistical mixture of different hydrophobic blocks, having either 4 or 6 methylene units between the urea groups. The crosslinker with a statistical mixtureof U4U and U6U hydrophobic segments is expected to contain 50% of U4U-PEO8K-U6U heterocrosslinker (**4X6**) and 25% of each homocrosslinker, U4U-PEO8K-U4U (**4X4**) and U6U-PEO8K-U6U (**6X6**) homocrosslinker.

The multistep synthesis (Scheme S1) was performed through reaction of the PEO derivative **1** with amine functionality with one of the isocyano groups of 1,6-diisocyanatoalkane. The excess 1,6-diisocyanatoalkane was separated from the product by precipitation of the reaction mixture with hexane. Intermediate **2** with one isocyanate group was reacted with PEO derivative **3** to give segmented copolymer, **6X6**.



Scheme S1: Stepwise synthesis of PEO-tetraurea crosslinkers, **mXn**.

General procedure:

A mixture of 11-Aminoundecanoyl-(poly(ethylene glycol)-monomethylether)-ester hydrochloride salt (71.6 mg 0.119 mmol) and triethylamine (20 mg, 0.2 mmol) in dry dichloromethane was added very slowly dropwise to an excess of 1,6-diisocyanatoalkane (80 mg, 0.47 mmol) over 30 min at 0 ^{o}C and was allowed to stir for 3 h. Then the solvent was evaporated in vacuum and was washed with dry hexane 3-4 times to remove excess of 1,n-diisocyanatoalkane. Then the monoderivatived isocyanate (**5**) was reacted with a mixture bis(11-Aminoundecanoyl-(poly(ethylene glycol)-ester hydrochloride salt) (**4**) (500 mg, 0.059 mmol) and triethylamine (20 mg, 0.2 mmol) in dichloromethane and stirred at room temperature for 6 h. Then the reaction mixture diluted with chloroform and was extracted with brine. The organic layer was the dried over anhydrous sodium sulphate and evaporated to give white solid which was recrystallized from diethylether and dichloromethane to yield final products.

U6U-PEO8K-U6U homo crosslinker (6X6):

Yield: 70%

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.12, 4.95 (bs, 8H, N*H*), 4.22 (t, 4H, ^{3}J(H,H) = 4.0 Hz, CH_2OCO), 3.71-3.46 (m, 376H, OCH_2), 3.38 (s, 6H, OCH_3), 3.20-3.09 (m, 16H, CH_2N), 2.32 (t, 8H, ^{3}J(H,H) = 8 Hz, CH_2CO), 1.65-1.58 (m, 8H, $CH_2CH_2CH_2NH$), 1.51-1.40 (m, 16H, $CH_2CH_2CH_2NH$), 1.27 (bs, 32H, CH_2). ^{13}C-NMR (100 MHz, $CDCl_3$, T=295K): δ = 173.82, 160.97, 72.54, 71.91, 70.82, 70.59, 70.55, 70.54, 70.30, 69.18, 63.35, 61.67, 59.01, 45.83, 40.38, 39.37, 34.18, 30.39, 29.82, 29.48, 29.38, 29.34, 29.21, 29.08, 26.94, 25.51, 24.87. FT-IR (cm^{-1}): 3333, 2879, 1733, 1615, 1579, 1466.

GPC (DMF; PS standards): M_n = 10.1 x 10^3 g/mol, 70%; M_n = 19.0 x 10^3 g/mol, 25%; 27.8 x 10^3 g/mol, 5%.

MALDI-TOF [M+Na+] = 10651 ± n*44

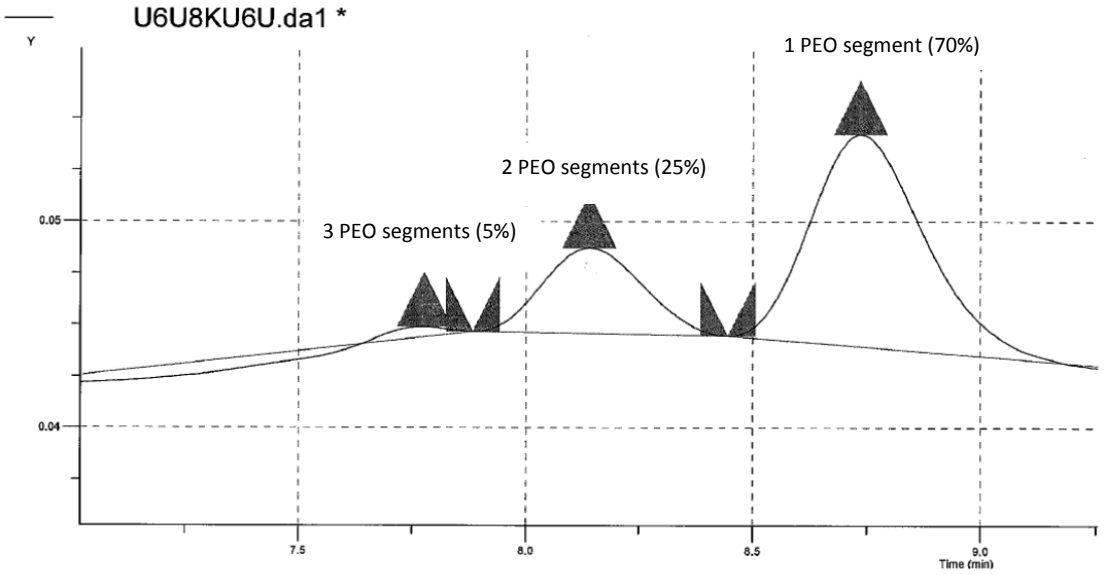


Figure S1 GPC trace of U6U-PEO8K-U6U homo crosslinker (6X6).

U4U-PEO8K-U4U homo crosslinker (4X4)

Yield: 75%

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 4.97, 4.75 (bs, 8H, N*H*), 4.22 (t, 4H, ^{3}J(H,H) = 4.0 Hz, CH_2OCO), 3.71-3.46 (m, 376H, OCH_2), 3.38 (s, 6H, OCH_3), 3.20-3.09 (m, 16H, CH_2N), 2.32 (t, 8H, ^{3}J(H,H) = 8 Hz, CH_2CO), 1.65-1.58 (m, 8H, $CH_2CH_2CH_2NH$), 1.51-1.40 (m, 16H, $CH_2CH_2CH_2NH$), 1.27 (bs, 32H, CH_2).

^{13}C-NMR (100 MHz, $CDCl_3$, T=295K): δ = 173.82, 158.85, 71.91, 70.60, 70.55, 70.49, 70.34, 69.18, 63.35, 59.01, 40.39, 39.74, 34.18, 30.40, 29.46, 29.36, 29.32, 29.22, 29.18, 29.06, 27.54, 26.92, 24.87. FT-IR (cm^{-1}): 3333, 2879, 1733, 1615, 1579, 1466.

GPC (DMF; PS standards): M_n = 9.6 x 10^3 g/mol, 82%; M_n = 18.9 x 10^3 g/mol, 17%

MALDI-TOF [M+Na+] = 10498 ± n*44.

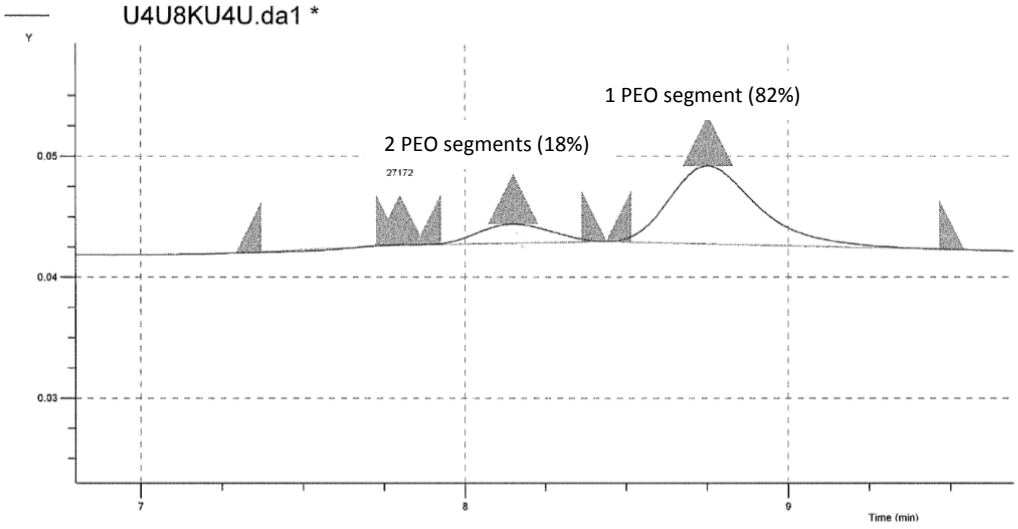


Figure S2 GPC trace of U4U-PEO8K-U4U homo crosslinker (4X4).

U6U-PEO8K-U4U statistical mixture

Yield: 65%

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.12, 4.95 (bs, 8H, N*H*), 4.22 (t, 4H, ^{3}J(H,H) = 4.0 Hz, CH_2OCO), 3.71-3.47 (m, 332H, OCH_2), 3.38 (s, 6H, OCH_3), 3.20-3.09 (m, 16H, CH_2N), 2.33 (t, 8H, ^{3}J(H,H) = 8 Hz, CH_2CO), 1.65-1.58 (m, 8H, $CH_2CH_2CH_2NH$), 1.51-1.40 (m, 14H, $CH_2CH_2CH_2NH$), 1.28 (bs, 32H, CH_2). ^{13}C-NMR (100 MHz, $CDCl_3$, T= 295 K): δ = 173.85, 158.76, 72.56, 71.92, 70.56, 70.44, 69.20, 63.37, 61.71, 59.03, 40.47, 39.76, 34.20, 30.35, 29.45, 29.31, 29.06, 27.46, 26.90, 24.88. FT-IR (cm^{-1}): 3333, 2879, 1732, 1614, 1578, 1466. GPC (DMF; PS standards): M_n = 9.9 x 10^3 g/mol, 80%; M_n = 19.1 x 10^3 g/mol, 20%.

MALDI-TOF [M+Na+] = 10315 ± n*44

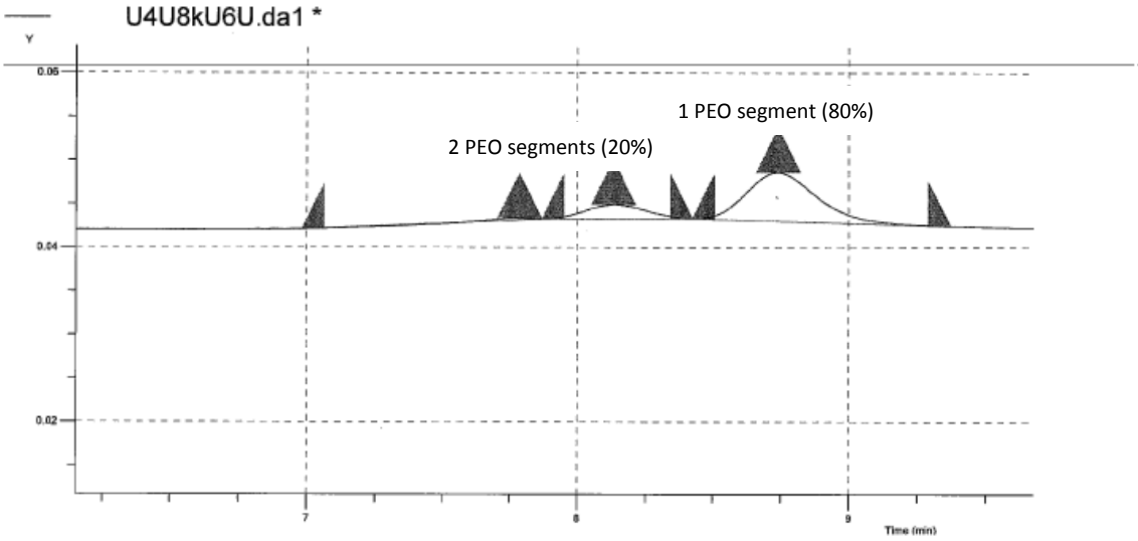


Figure S3 GPC trace of U6U-PEO8K-U4U ($\overline{4X6}$) heterocrosslinker.

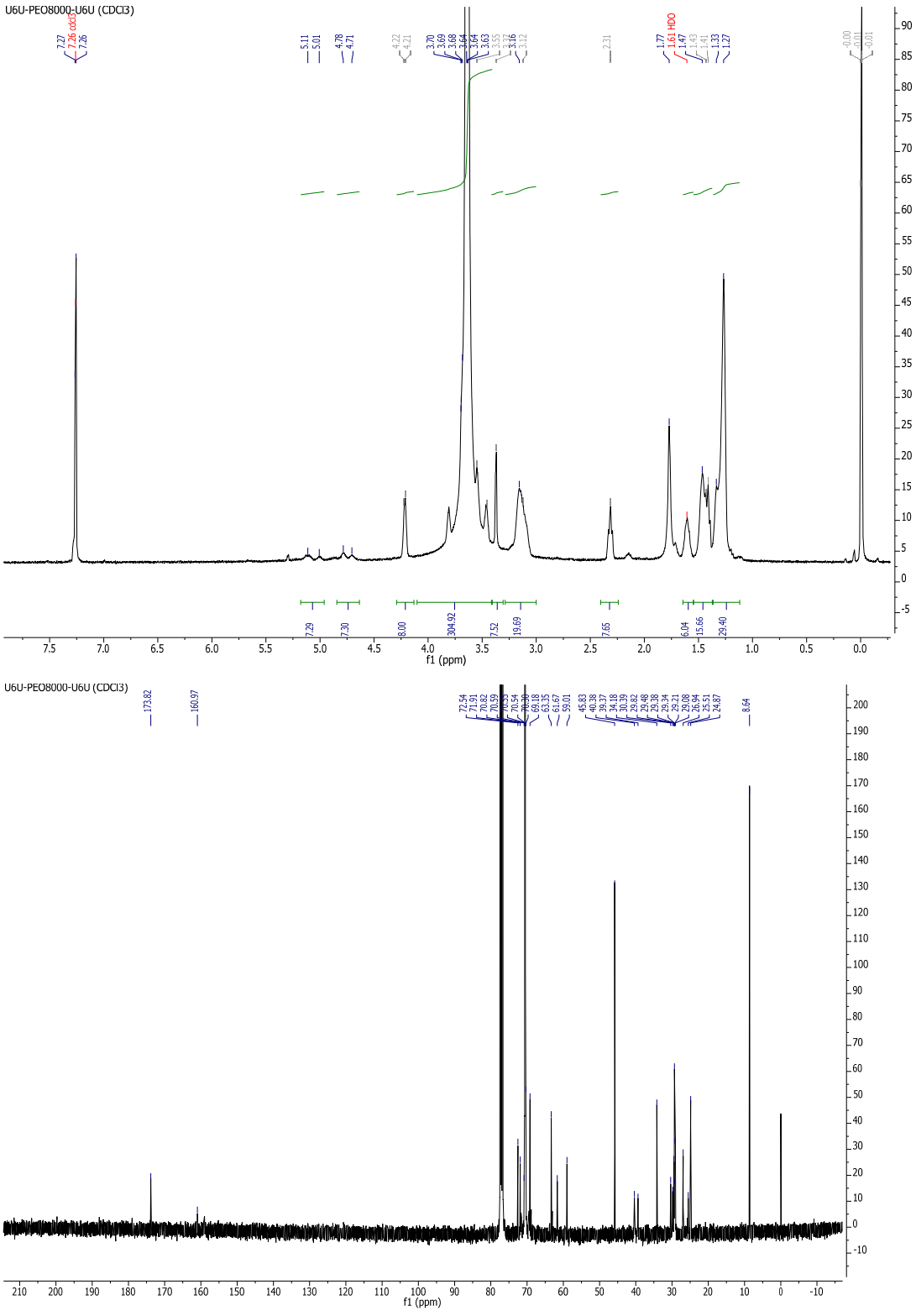


Figure S4: 1H and 13C spectra of U6U-PEO8000-U6U, **6X6**.

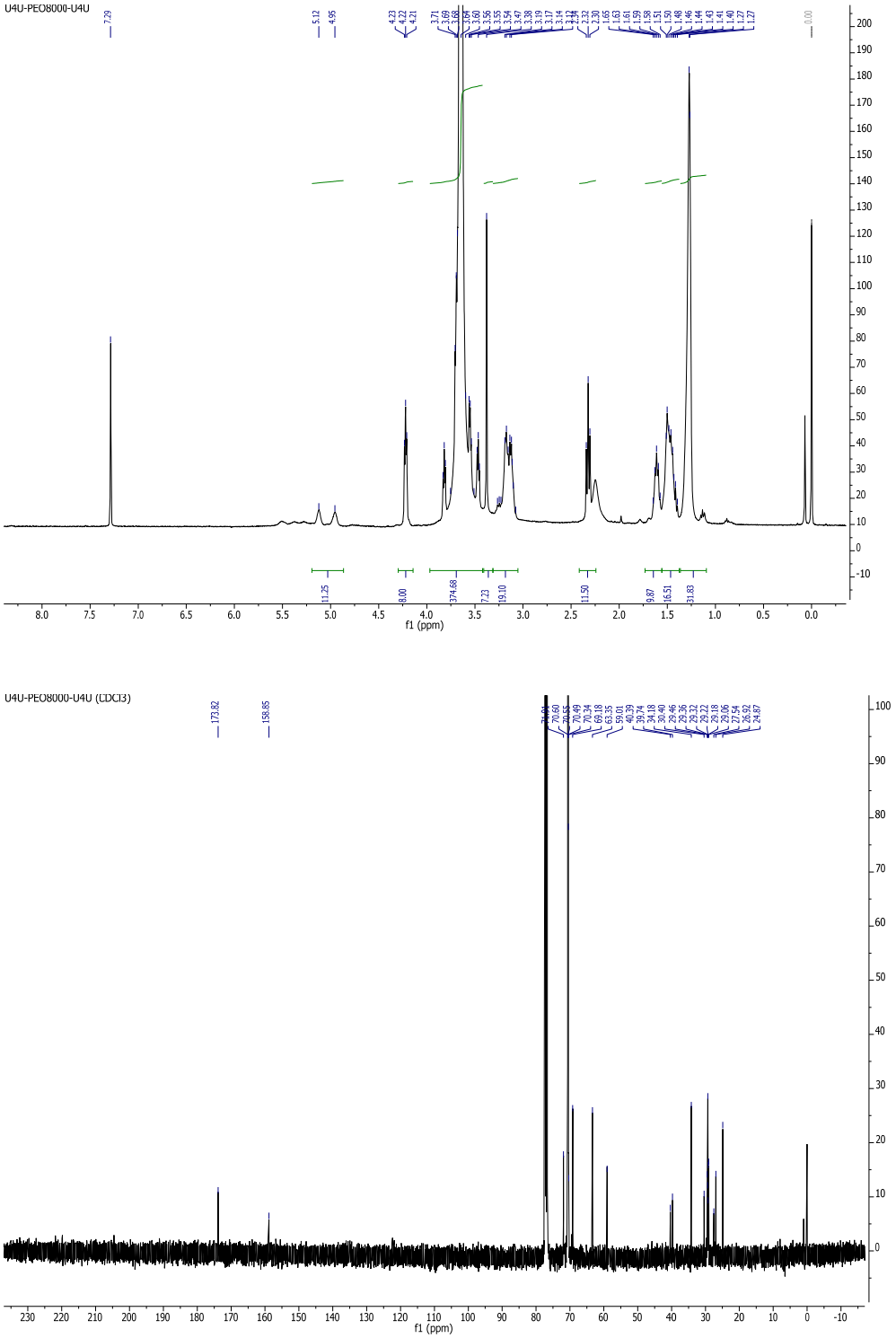


Figure S5: 1H and 13C spectra of U4U-PEO8000-U4U, **4X4**.

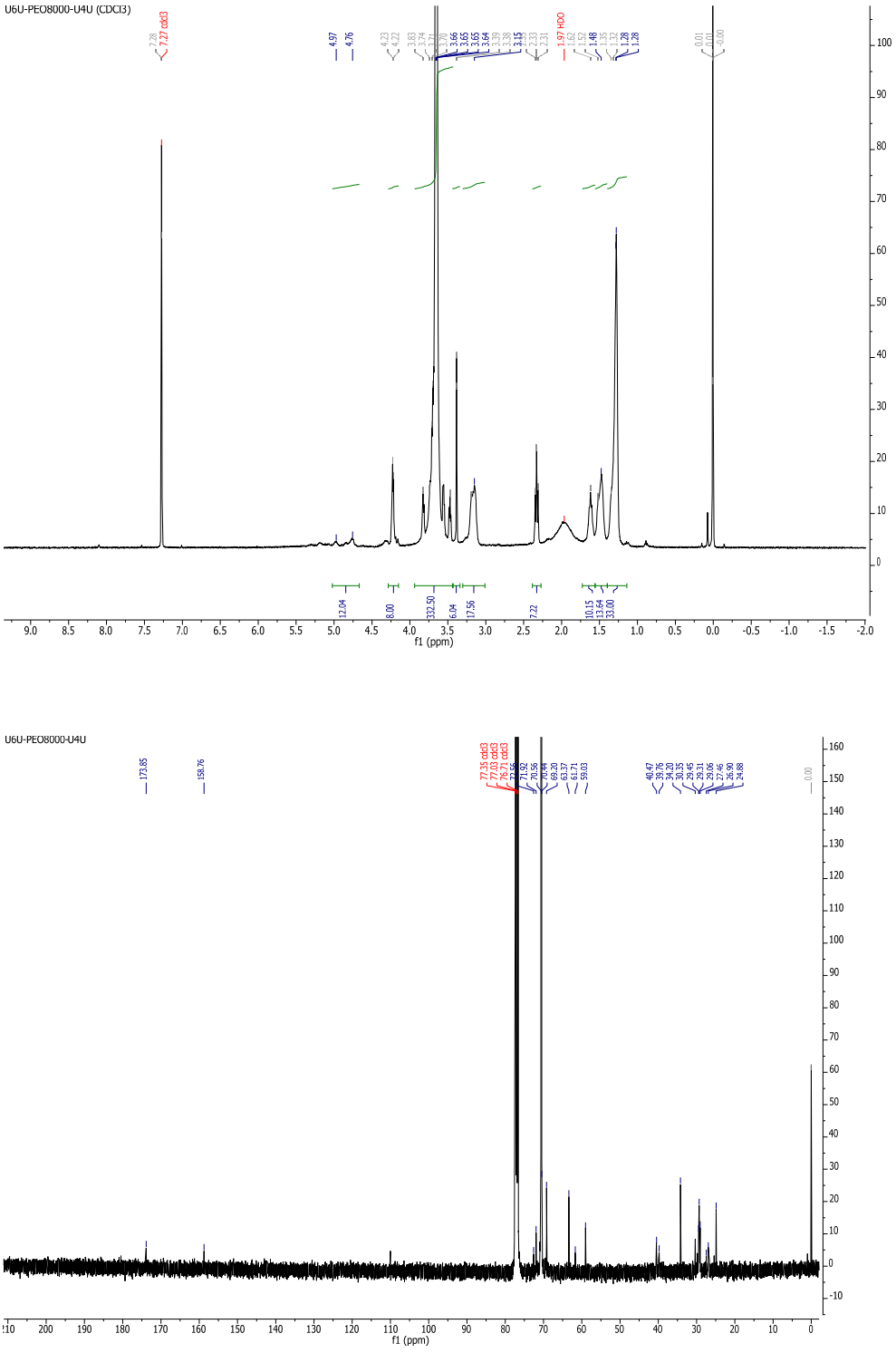


Figure S6: 1H and 13C spectra of U6U-PEO8K-U4U hetero crosslinker (4X6).

Cryogenic Transmission Electron Microscopy:

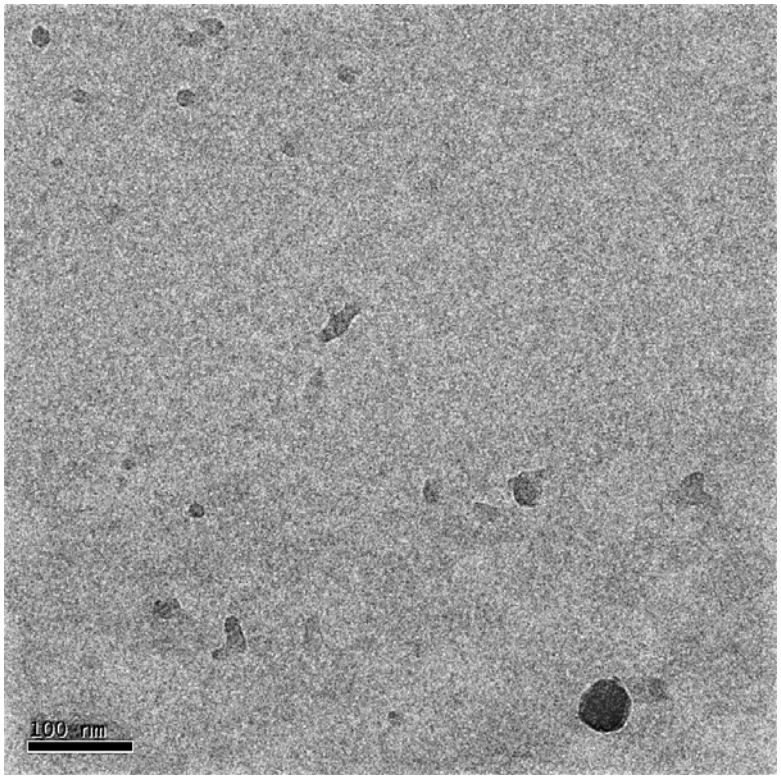


Figure S7: CryoTEM of **$\overline{\text{4X6}}$** (1 mg/ml). The round darker features are artifacts caused by non-vitrified water.

SAXS:

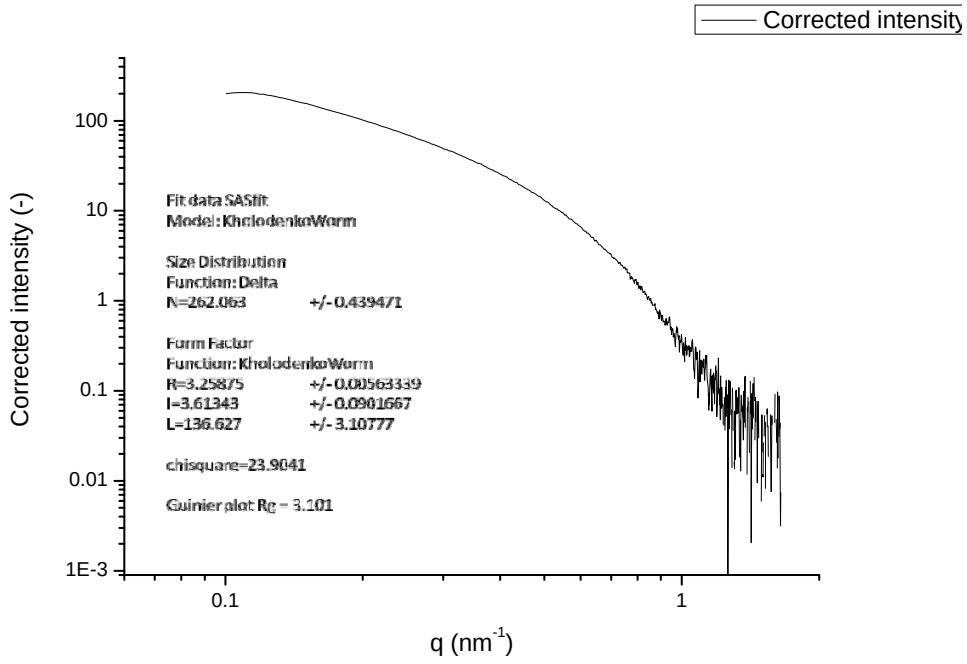


Figure S8. SAXS of U6U 0.5w%

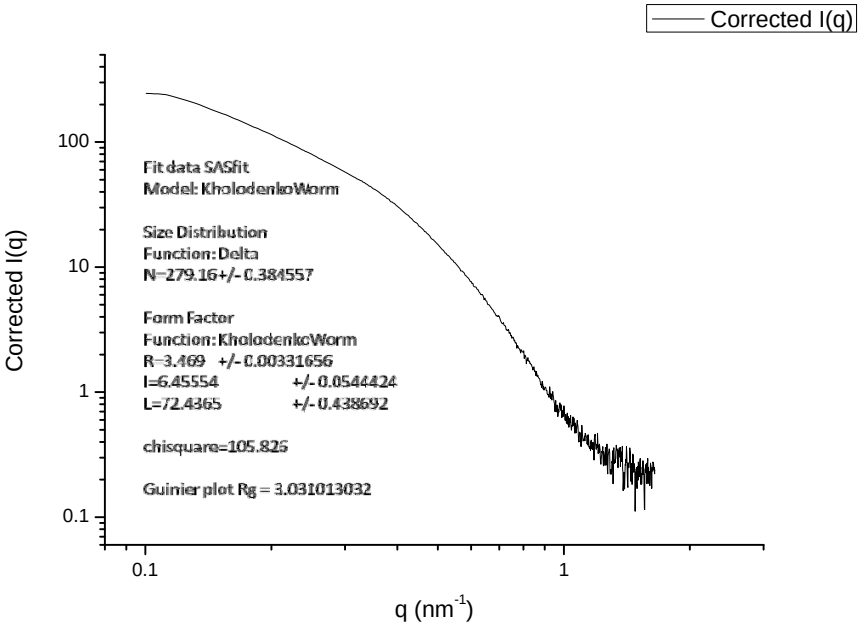


Figure S9. SAXS of U6U 0.25w% with 6X6 0.125w%.

Rheology:

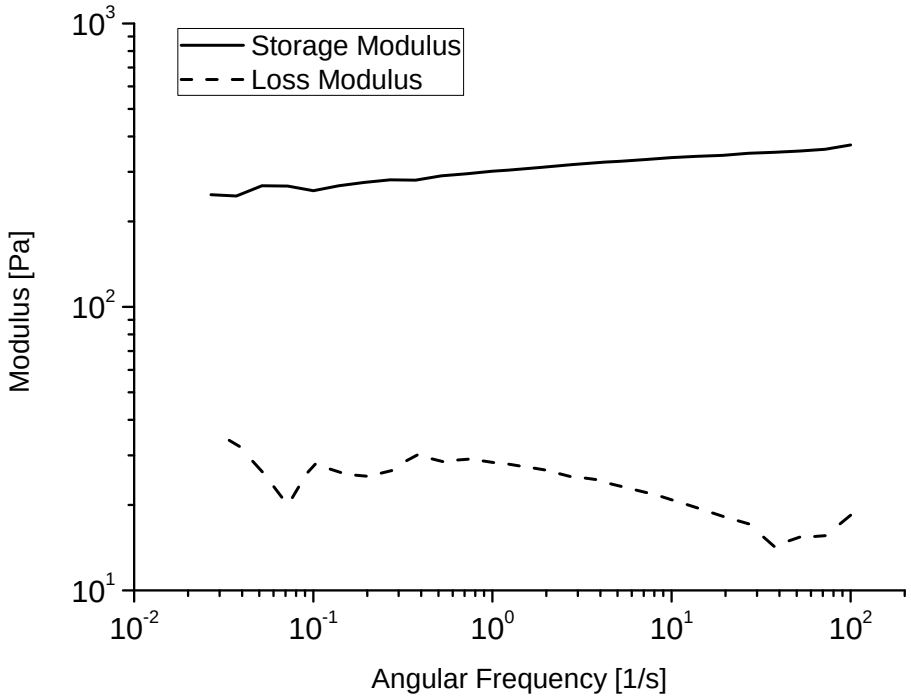


Figure S10. Frequency dependent storage and loss modulus, showing the effect of matching crosslinking at concentration U6U (1w%) and 6x6 (0.5w%).

Statistical Mechanical Calculations:

In this supplement, we present the method used to calculate the ratio of bridging probabilities used to estimate the ratio of moduli between the hetero-crosslinked and the homo-crosslinked systems.

Method

We consider a mixture of crosslinkers and rods. The rods, as described in the main text, self-sort into two distinct, pure types - we label them U4U and U6U. Linkers are either homotypic (both ends identical - their urea functional group containing 4 or 6 methylenes - we denote them 4X4 or 6X6) or heterotypical (their urea functional group containing one of each type, denoted 4X6). In the main text, we establish that the energetic penalty for a mismatched linking - i.e., the 6-end of a crosslinker incorporating into a U4U stack or vice versa - is approximately $2.6\,k_BT$. In our computation, we assume moreover that the energetic penalty of not linking at all is prohibitively large, and thus that all crosslinkers are bound to rods at both ends. In the following, we will symbolically represent the structures **as (Rod 1):(Linker):(Rod 2)** in the case of bridged configuration, and **(Rod 1)::(Linker)** in the case of a looped configuration.

To compute the equilibrium probabilities of bridging we enumerate all different states φ_i of the system in question, as well as their energies ε_i. In equilibrium, the probability of each state is given by

$$P(\varphi_i) = \frac{1}{\mathcal{Z}} e^{-\varepsilon_i/k_BT}$$

with $\mathcal{Z}$ the partition function

$$\mathcal{Z} = \sum_i e^{-\varepsilon_i/k_BT}$$

The probability of bridging may then be computed by summing the probabilities of all the bridged configurations, given a composition for the rod mixture and, separately, the crosslinker mixture. We now perform this calculation for the settings considered in the paper.

Homocrosslinker

The first system considered explicitly in the main text is one containing a 1:1 mixture of U4U and U6U rods, combined with 100% 6X6 linkers. The complete set of states is readily listed: two looped configurations **U4U::6X6** (double mismatch) and **U6U::6X6** (no mismatch), and

three distinct bridged configurations **U4U:6X6:U4U** (double mismatch), **U4U:6X6:U6U** (single mismatch, and carries a combinatorial factor of two due to the distinct rods) and finally **U6U:6X6:U6U** (no mismatch). The partition function is computed as

$$\mathcal{Z} = 2 + 2e^{-2.6} + 2e^{-5.2}$$

And the bridging probability is

$$P_{bridge,homo} = \frac{1 + 2e^{-2.6} + e^{-5.2}}{2 + 2e^{-2.6} + 2e^{-5.2}} \approx 0.53$$

Heterocrosslinker

Next, we consider the 1:1 mixture of U4U and U6U rods, combined with 100% 4X6 linkers. Again, we enumerate the complete set of states: 2 looped configurations **U4U::4X6** (1 mismatch) and **U6U::4X6** (single mismatch). The heterosystem has more bridged configurations: **U4U:4X6:U4U** (1 mismatch), **U4U:4X6:U6U** (no mismatch, combinatorial factor of two), **U6U:4X6:U4U** (double mismatch, combinatorial factor of two), **U6U:4X6:U6U** (single mismatch). The partition function is computed as

$$\mathcal{Z} = 2 + 4e^{-2.6} + 2e^{-5.2}$$

And the bridging probability is

$$P_{bridge,hetero} = \frac{2 + 2e^{-2.6} + 2e^{-5.2}}{2 + 4e^{-2.6} + 2e^{-5.2}} \approx 0.93$$

Statistical mixture of linkers

There is no difference, at least not in our approach here, between the mismatch energy penalty for a 6 linker into a U4U rod and vice versa. The statistical mixture of linkers considered in Fig. 6. of the main text is therefore described as a 50% hetero, 50% homo mixture. The change in bridging probability will be

$$\frac{P_{bridge,mixture}}{P_{bridge,homo}} = \frac{\frac{1}{2}(P_{bridge,hetero} + P_{bridge,homo})}{P_{bridge,homo}} \approx 1.37$$

This increase in bridging probability results in an increase in modulus of

$$\frac{G_{mixture}}{G_{homo}} = \left(\frac{P_{bridge,mixture}}{P_{bridge,homo}}\right)^7 \approx 9.2$$

References:

1. Kalyanasundaram K., Thomas, J. K., *J. Am. Chem. Soc*. **1977**, 99, 2039.