Supplementary Information for:

Unique photophysical behavior of coumarin-based viscosity probes during molecular self-assembly

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Supplementary information

S1. Materials

Reagents

Raspberry ketone glucoside was provided by Beijing Brilliance Bio, and vinyl esters were purchased from TCI America. Lipase acrylic resin (Novozymes 435) from *Candida Antarctica* (≥5,000 U/g), recombinant, expressed in Aspergillus niger was provided by Novozymes. 9-Formyl-8-hydroxyjulolidin, diethyl malonate, piperidine, 4-ethynylphenylacetonitrile, 1-Azido-1-deoxy-β-D-glucopyranoside, 8-bromoctanoic acid, and Poly(ethylene glycol) methyl ether methacrylate (average MW 950 Da) were purchased from Sigma-Aldrich (St Louis, MO) and used as provided.

Sodium azide, Silica Gel (200-300 Mesh), toluene, hexanes, ethyl acetate, ethanol and acetone were purchased from Thermofisher (NY, NY). The following solvents were dried with the indicated desiccant and distilled before use under dry N₂: triethylamine with CaH₂, THF with Na, dichloromethane with P₂O₅.

Bruker ScanAssyst-AFM tips (70 kHz 70, 0.4 N/m, silicon nitride), Ted Pella, Inc. AFM specimen discs (12 mm diameter, stainless steel)

NMR Spectrometer Varian XL-300 (300 MHz); Mass spectrometer 4000 QTRAP (Applied Biosystems, Foster City, CA); HPLC system: Shimadzu Prominence HPLC (Shimadzu USA, Canby, OR); UV/Vis Spectrophotometer Thermo scientific Evolution 300; Fluorimeter HORIBA Jobyn Yvon FluoroMax-3; J-810 Cricular Dichroism Spectropolarimeter Jasco; Atomic Force Microscope Bruker MultiMode 8 AFM; Stimulated Emission Depletion microscope Leica TCS SP8 STED 3X; Ubbelohde Viscometer (k = 0.332 and k = 1.055)

S2. Methods

**Raspberry Ketone Glucoside Fatty Acid Ester Synthesis.**

In a 500 mL screw-cap Erlenmeyer flask, solid Novozymes 435 lipase catalyst (0.3 g/mmol glucoside) was added to mixture of raspberry ketone glucoside (2.0 mmol, 0.652 g), and vinyl octanoate (3:1 mmol acyl donor/glucoside ratio) containing 50 mL of dried acetone. The reaction proceeded in an orbital shaker at 250 rpm and 50 °C. The reaction was monitored by thin-layer chromatography (TLC) with an ethyl acetate eluent and visualized using 5% sulfuric acid solution in water and gentle heating. After 24 h, the bottom glucoside spot (Rf = 0.4) faded and a product spot appeared (Rf = 0.1). Before the solution was allowed to cool to room temperature, the enzymes were filtered out and rinsed with acetone until the washings showed no further product on TLC before they were air-dried and stored for reuse. Acetone was evaporated under vacuum from the filtrate, leaving behind a crude solid mixture of glucoside–ester product. The solid mixture was triturated thrice with 50 mL of hexanes at 50 °C to remove the excess fatty acid and derivatives from the opaque light yellow bulk solid. To remove trace elements of unreacted sugar, ester, or acids, the product (0.85 g) was dissolved in 25 mL of methanol and coated by evaporation onto 5.0 g of silica gel before being spread onto a short silica plug (40.0 g). The column was twice eluted to dryness with 200 mL ethyl acetate, and the solvent was evaporated from the second fraction via rotary evaporation to afford the pure fatty acid glucoside ester product (95% yield).

**Rheological Characterization of Gels**
Oscillatory rheological measurements were performed on a stress-controlled rheometer (AR 2000 ex, TA Instruments) with a cone and plate geometry (1° 58’ 47” angle and 40 mm diameter with a truncation gap of 45 μm). 1 mL of gel was loaded onto the plate, and the cone was lowered to minimize the truncation gap. Precautions were taken to minimize shear-induced disruption of the gel network: before experiments samples were equilibrated within the geometry for 10 minutes. Excess gel was trimmed away from the cone to ensure optimal filling. Thixotropic/repeating yield strain (σy) was examined for toluene gels by performing oscillatory strain sweep measurements from 0.01 to 100% deformation at a fixed frequency of 1 Hz and then ramping through this cycle several times.

S3. Synthetic protocols

**Synthesis of C1, Oct-C1 and Glu-C1**

The synthetic scheme is reported in Supplementary Fig. 2.

2,3,6,7-tetrahydro-1H,5H,11H-pyrido[2,3-f]pyrido[3,2,1-ij]quinolin-11-one (3)

In a 25 ml two necked round bottom flask equipped with condenser, stirrer and CaCl₂ tower, containing julolidine derivative (1, 1.00 g, 4.6 mmol) and diethylmalonate (1.47 g, 1.4 ml, 2 eq) dissolved in ethanol (15 ml), before piperidine (0.4 g, 0.46 ml, 1 eq) was added. The mixture was stirred under reflux for 6 h. After confirmation by TLC that the starting material (1) had been fully consumed, the solvent was carefully removed under reduced pressure.

The intermediate 2 (deep orange solid) was suspended in diluted HCl (20 ml) and glacial acetic acid (2 ml) and the mixture was kept under reflux for 12 h. After this time the mixture was left to cool down to RT and the pH was adjusted to 4.5 by adding small amount of NaHCO₃(sat). Afterwards, the crude product was extracted with dichloromethane (3 x 20 ml) and the combined organic layers were dried with Na₂SO₄, filtered and concentrated under reduced pressure. The resultant green solid was purified by flash chromatography (silica gel; petroleum ether / ethyl acetate, 3:2) to give 3 (1.29 g) in 74% yield as a dark yellow solid.

1H-NMR (CDCl₃, 298 K) δ (ppm): 7.74 (d, 1H, J = 9.20 Hz), 6.83 (s, 1H), 5.99 (d, 1H, J = 9.20 Hz), 3.24 (q, 4H, J = 5.60 Hz), 2.87 (t, 2H, J = 6.40 Hz), 2.74 (t, 2H, J = 6.40 Hz), 1.95 (m, 4H).

13C-NMR (CDCl₃, 298 K) δ (ppm): 162.70, 151.70, 145.90, 144.00, 125.00, 118.31, 108.43, 106.77, 50.02, 49.63, 27.53, 21.59, 20.66, 20.35

ESI-MS (m/z): 242.3 (M+H⁺)

11-oxo-2,3,6,7-tetrahydro-1H,5H,11H-pyrido[2,3-f]pyrido[3,2,1-ij]quinolin-10-carboxaldehyde (4)

In a 50 ml three necked bottom flask, equipped with condenser, stirrer, CaCl₂ tower and N₂ inlet tube, containing POCl₃ (0.3 ml), anhydrous DMF (0.3 ml) was added dropwise to yield a scarlet homogenous solution which was stirred for 30 min at 45 °C. Afterwards, 3 (220 mg, 0.91 mmol) was dissolved in DMF (2 ml) and introduced dropwise after which the mixture was stirred at 60 °C for 6 h. After cooling to RT, the solution was slowly poured in cracked ice (10 g) and vigorously stirred for further 15 min. The pH was adjusted to about 5.5 by adding a small amount of NaOHconc. The large amount of yielded solid was filtrated in vacuum, washed with cold water, dried and recrystallized with ethanol to give 4 (216 mg) in 88 % yield as iridescent dark green crystals.

1H-NMR (CDCl₃, 298 K) δ (ppm): 9.85 (s, 1H), 8.23 (s, 1H), 7.25 (s, 1H), 3.36 (m, 4H), 2.70 (m, 4H), 1.86 (m, 4H, J = 5.90 Hz).

13C-NMR (CDCl₃, 298 K) δ (ppm): 187.35, 153.93, 150.12, 146.10, 129.40, 120.40, 108.43, 105.80, 50.50, 50.11, 27.45, 21.11, 20.12
ESI-MS (m/z): 270.3 (M+H+)

10-((2’Z)-2’-(4’-ethynyl)phen-1-yl-prop-2’-en-3’-yl)-2,3,6,7-tetrahydro-1H,5H,11H-pyran-2,3,6,7-tetrahydro-1H,5H,11H-pyrido[2,3-f]pyrido[3,2,1-ij]quinolin-11-one (C1)

In a 25 ml two necked round bottom flask equipped with condenser and stirrer, containing 4 (180 mg, 0.67 mmol) and 4-ethyl phenyl acetonitrile (0.104 g, 1.1 eq) dissolved in MeOH (13 ml), a solution of piperidine (12 mg, 14 μl, 0.2 eq) in MeOH (1 ml) was added. The mixture was stirred under reflux for 6 h. After evaporation of the solvent the crude product was recrystallized with acetone and diethyl ether, filtered in vacuum with an Hirsch funnel, carefully washed with cold ether and dry in vacuum to yield C1 (126 mg) in 48% yield as a dark red crystalline solid.

1H-NMR (DMSO, 298 K) δ (ppm): 1.75 (2H, ddd), 1.97 (2H, ddd), 2.82-3.01 (5H, m), 3.59-3.77 (4H, m), 7.21 (1H, s), 7.49-7.60 (4H, dd), 8.13 (1H, s)

13C-NMR (DMSO, 298 K) δ (ppm): 20.3, 21.1, 21.8, 27.5, 40.1, 50.0, 80.7, 107.4, 113.7, 117.5, 118.2, 120.9, 122.8, 127.2, 128.2, 131.6, 133.0, 134.1, 140.1, 149.4, 153.7, 159.3

ESI-MS (m/z): 393.2 (M+H+)

Oct-C1

4.5 mmol of 8-bromoocatanoic acid (1.00 g) and 1.6 eq of sodium azide were added to 6 ml of DMSO. The solution was stirred at RT for 24 hours after which 70 ml of H2O were added and the product was extracted 2x40 ml of diethyl ether. The combined organic phases were washed 3x50 ml H2O and 2x50 ml NaCl sat. The solvent was evaporated via rotavapor to yield 650 mg of the crude product as a clear liquid. 10 mg of crude 8-azido caprylic acid (5, 0.05 mmol) were added to 1 eq of C1 in 0.4 ml of DCM and 0.3 ml of H2O. After complete dissolution, 1.5 mg of CuSO4 pentahydrate (1.5 eq) and 3 mg of sodium ascorbate (2 eq) dissolved in 0.1 ml of H2O were added. The biphasic reaction was stirred vigorously for 4 hours. 20 ml of H2O and 40 ml of DCM were added. The organic phase was recovered via separatory funnel, dried on anhydrous MgSO4 and the solvent removed via rotavap to yield 66 mg of Oct-C1 as a dark orange solid (2.4%).

1H-NMR (CDCl3, 298 K) δ (ppm): 1.12-1.36 (6H, m), 1.55 (2H, tt), 1.64-1.95 (4H, m), 2.02 (2H, tt), 2.88-3.11 (4H, dd, dd), 3.44 – 3.64 (4H, dd, dd), 4.27 (2H, t), 7.13 (1H, s), 7.17 (1H, s), 7.39-7.46 (4H, dd), 7.95 (1H, 1), 8.08 (1H, s)

13C-NMR (CDCl3, 298 K) δ (ppm): 20.1, 21.4, 21.9, 25.0, 26.1, 26.8, 27.5, 29.2, 34.9, 49.4, 50.0, 107.4, 113.7, 117.5, 118.2, 120.9, 125.4, 127.2, 127.3, 127.7, 128.2, 128.3, 133.0, 134.1, 140.1, 147.3, 149.4, 153.7, 159.3, 177.0

ESI-MS (m/z): 576.6 [M-H]−

Glu-C1

2 mg of C1 (0.02 mmol) was dissolved in 0.4 ml of DCM and 0.3 ml of H2O in a 15 ml-brown glass vial. 4 mg of 1-azido-1-deoxy-β-D-glucopyranoside (0.02 mmol, 1 eq) dissolved in 50 μl of H2O were added to the biphasic C1 solution. 1.5 mg of CuSO4 pentahydrate (1.5 eq) and 3 mg of sodium ascorbate (2 eq) were dissolved in 0.1 ml of H2O and finally added to the reaction vial. The reaction was stirred at RT for 48 hours. The product was purified via flash chromatography on silica gel by using pure ethyl acetate followed by 1:1 ethyl acetate:methanol to recover the product in 30% yield as a dark orange solid.
$^1$H-NMR (CDCl$_3$, 298 K) $\delta$ (ppm): 1.62-1.96 (4H, m), 2.86-3.01 (2H, m), 3.12 (1H, ddd), 3.31 (1H, dd), 3.34-3.66 (6H, m), 3.80-3.83 (2H, m), 4.19 (1H, dd), 5.95 (1H, d), 7.14 (1H, s), 7.17 (1H, s), 7.41-7.48 (4H, m), 7.98 (1H, s), 8.10 (1H, s).

$^{13}$C-NMR (CDCl$_3$, 298 K) $\delta$ (ppm): 20.4, 21.2, 21.6, 27.4, 50.2, 61.1, 69.9, 71.6, 76.4, 79.0, 87.0, 107.4, 113.7, 117.5, 118.2, 120.9, 125.5, 127.5, 127.9, 128.4, 133.4, 134.2, 140.5, 147.2, 149.8, 153.8, 159.2
ESI-MS ($m/z$): 619.8 (M+Na$^+$)
S4. Supplementary Figures I

**SI Figure 1:** $^1$H spectrum of C1 in CDCl$_3$

**SI Figure 2:** $^{13}$C NMR spectrum of C1 in CDCl$_3$
SI Figure 3: $^1$H NMR spectrum of Oct-C1 in CDCl$_3$

SI Figure 4: $^{13}$C NMR spectrum of Oct-C1 in CDCl$_3$
SI Figure 5: $^1$H NMR spectrum of Glu-C1 in CDCl$_3$

SI Figure 6: $^{13}$C NMR spectrum of Glu-C1 in CDCl$_3$
**SI Figure 7:** Synthetic scheme for the preparation of C1, Oct-C1 and Glu-C1.

**SI Figure 8:** Calibration curves of C1, Glu-C1 and Oct-C1 in mixtures of toluene-PEG methyl ether methacrylate (viscosity values in cSt: 0.7, 53, 100, 200, 280, 440).
**SI Figure 9:** Rheological characterization of 1.0% R8 toluene gel demonstrating a thixotropic response to periodic pauses to its deformation.

**SI Figure 10:** Left: 216 R8 aggregate from CG MD simulations. Lipid tails are shown in yellow and glucose ring in red. Phenyl tails are omitted for clarity. The zoom shows a local structure with stacked glucose rings. Right: Radial distribution function of the aggregate components during the simulation.
**SI Figure 11:** top a) and b) lateral and top view of the "double-helix" aggregate. c) The R8 quintuplet. Red triangles correspond to R8 Glu ring (beads 5-7), yellow sticks to R8 lipid tails (beads 9-10), thin green sticks phenyl ring and ketone (beads 1-4).

**SI Figure 12:** Gelation process monitored with C1. The response of C1 towards gel formation is minimal.
**SI Figure 13:** a. Top and lateral view of the “symmetrical” stack, before (left) and after (right) 50ns MD simulation. b. 100 equally spaced snapshots from the 50ns MD simulation at 300K are superimposed by fitting on the glucose ring atoms. c. Root mean square deviation (RMSD) from starting structure of “symmetrical”, in red, vs non-symmetrical stack (the one in Fig. 1, main text), in black. The RMSD was calculated on the glucose ring atoms.

**SI Figure 14:** Confocal image showing Glu-C1-containing R8 gel. Oct-C1 results in a completely indistinguishable staining of the fibers (data not shown, scale bar: 10 μm).
Figure 15: Lateral (a) and top (b) views of representative snapshots from the simulations of Oct-C1 (left) and Glu-C1 (right) within the R8 fibril. Oct-C1 and Glu-C1 beads are represented as spheres, in green ones the coumarin part (beads 1-7), in gray the phenyl and triazol rings (beads 8 to 11), in red Glu-C1 glucose (beads 12-14), in yellow Oct-C1 beads 12-13. (c) Radial distribution functions of various groups of the investigated molecules with respect to the R8 fibril axis, from the coarse grained simulations of a single fibril. In the lower panel, the solid lines refer to the Oct-C1 simulation and the dotted lines to the Glu-C1 simulation, showing no appreciable difference between the R8 arrangement in the two simulations.

Figure 16: Two configurations of Oct-C1 within the R8 fibril corresponding to C15-C14-C16-C17 dihedral at -150 and -60 respectively. At -60, a H-bond with a nearby sugar hydroxyl group stabilizes the configuration.
**SI Figure 17:** Fluorescence spectra showing the variation of the s-cis/s-trans Oct-C1 ratio at different temperatures.

**SI Figure 18:** RP-HPLC-MS (gradient of water/acetonitrile with 0.1% formic acid) of Oct-C1 dissolved in ethanol performed on a C18 achiral HPLC column showing that the two atropisomers are chemically distinguishable (retention times are 13.57 min and 14.86 min) and have the same m/z (576.3 m/z for the mono-deprotonated adduct, [M-H]).
SI Figure 19: Potential of mean force for torsion around the dihedral describing Z-s-cis and Z-s-trans isomers.

SI Figure 20: Representative snapshots from the simulations of Oct-C1 (left) and Glu-C1 (right) within the R8 fiber (double helix). Oct-C1 and Glu-C1 beads are represented as spheres, in green ones the coumarin part (beads 1-7), in gray the phenyl and triazol rings (beads 8 to 11), in red Glu-C1 glucose (beads 12-14), in yellow Oct-C1 octanoic acid (beads 12-13).
SI Figure 21: Views of the R8 quadruplet stack with Glu-C1 glucose ring inserted in the glucose core. Upper left: top view from a minimized MD snapshot (R8 molecules above Glu-C1 are omitted for clarity). Upper right: Top view. 60 equally spaced snapshots from the last 20ns of the 50ns MD simulation at 310K are superimposed by fitting on the glucose ring atoms. Lower left: lateral view.
S5. Quantum Mechanics Calculations

Supplementary Table 1 reports the ground-state energies for the various C1-triazol conformers Z-s-cis and Z-s-trans (See Figure 4 of main text), evaluated at the DFT-PBE0 level, with the inclusion of solvent effects by the Polarizable Continuum Method (PCM). Excitation energies were calculated by TD-DFT CAM-B3LYP, both in the gas phase and using PCM.

|          | Dipole (Debye) | Delta Ground State Energy (kJ/mol) | Excitation wavelength (nm) |
|----------|---------------|---------------------------------|---------------------------|
|          | (PBE0/6-31G*) | (PBE0/6-31G*)                   | Z-s-cis                  |
|          |               |                                  | Z-s-trans                 |
| gas phase| 7.8           | 12.9                             | 29.6                      |
|          | 2.7           | 27.5                             | 417                       |
|          | 4.7           | 444                              | 408                       |
| toluene  | 9.8           | 15.7                             | 25.3                      |
| methanol | 12.3          | 19.9                             | 16.7                      |
| water    | 12.5          | 20.2                             | 12.9                      |

SI Table 1: Ground state energies, permanent dipole moments and excitation wavelengths of s-cis and s-trans conformers of C1-triazol in the gas phase and in various solvents.

In all examined cases the Z-s-cis conformer is the most stable, though the ΔE decreases (i.e. Z-s-trans gets less unstable) in more polar solvents, because of the sizably larger dipole moment of the Z-s-trans conformer. The excitation energy of Z-s-trans is ~ 30-35 nm blue shifted w.r.t. Z-s-cis in all solvents examined. Both display a slight blue shift in going from apolar to polar solvents.

In the Z-s-cis structures the CN groups is coplanar with the coumarin ring system. By contrast in Z-s-trans the C15-C14-C16-C17 dihedral angle is ~30 deg, because of steric clash with the coumarin carbonyl.

Coarse-grained MD simulations

Preparation of the system and formation of the R8 “double helix”

Aggregation of the R8 gelator in toluene was first studied by coarse-grained simulations with the Martini force field (for the parameterization procedure see Methods). To speed up aggregation we used model 1 of Toluene (with C4/SC4 beads, at 350K to prevent toluene freezing). Indeed this model better segregates the R8 molecules, resulting in faster formation of a single cluster.

We began with 216 R8 molecules, evenly spaced in a 16.3-nm cube with 23544 toluene molecules. After 200ns of simulation time, three large clusters formed. We then reduced the number of toluene molecules in the system to 8000, and a single cluster formed (Figure S1). In this aggregate the R8 molecules present the lipid tails both to the external solvent and to the interior of the cluster, the glucose rings being halfway between the hydrophobic regions (see radial distribution function in left panel of Supplementary Fig. 4). Already at this early stage, the cluster features glucose-ring stacking.

We switched to semi-isotropic pressure coupling in order to guide the formation of a protofiber along the z axis. This was done by increasing the pressure along the z-axis to 30 bar while leaving that on the x-y plane to 1 bar, in a 10-ns simulation. As a result the box z side decreased and the cluster interacted with its image to form a continuous aggregate along z. The squeezing along z was stopped when the lateral width of the aggregate was compatible with the diameter.
of the fiber from the experimental measurement (i.e. ~ 6nm). The simulation was then switched to isotropic pressure coupling. Due to the over-aggregating tendency of model 1 of toluene, keeping semi-isotropic coupling with equal 1-bar pressure on z and x-y resulted in further reduction of the box z length, and a thicker aggregate. Model 2 of toluene preserves the formed aggregate, but considerably slows the aggregation. Indeed only around 85% of R8 molecules are aggregated, while the remaining 15% exchange with the solvent.

The emerging structure consists of segments of stacked R8 molecules, arranged in a way similar to a double helix (Supplementary Fig. 5). The stacking is made of 5 staggered R8 molecules, with the glucose rings at the center and the lipid and benzenoid tails on the outside (Supplementary Fig. 5). Such structure is stable over the µs simulated timescale at 310K with model 2 of toluene.

**Interaction of Glu-C1 and Oct-C1 with the double helix fiber**

In order to study the interaction of the fluorophores (Glu-C1 and Oct-C1) with the R8 supramolecular structure we started with the molecules in the solvent part of the simulation box. We then partially melted the double helix by increasing the temperature to 350K for 100ns. We then decreased the temperature to 310K so as to let the double helix re-nature. As a result of the process both Oct-C1 and Glu-C1 get inserted in the double helix (Supplementary Fig. 14) and remain stacked between the various R8 molecules in the 3µs time of the simulations. By visual inspection it is apparent that the glucose ring of Glu-C1 is constantly stacked among the R8 glucose rings, while Oct-C1 octanoic acid stays in contact with R8 lipid tails. This arrangement causes a different positioning of the coumarin in the two cases, though the twisted geometry of the double helix prevents a simple assessment of these features.

**Insertion of Glu-C1 and Oct-C1 in the single fibril**

To better characterize the interaction of Glu-C1 and Oct-C1 with the R8 supramolecular structure, we isolated a single fibril from the double helix structure. This was done by taking two stacked quintuplets and replicating them 4 times in the z direction. The resulting structure is stable over the µs-simulated timescale.

Glu-C1 and Oct-C1 were initially put in the solvent and inserted in the fibril by pulling their center of mass toward the axis of the fibril by a constant 500 kJ mol⁻¹nm⁻¹ force for 10 ns. Then the two systems were run for 4 µs and the analysis performed on the last 2 µs. The resulting positioning of the two molecules is analogous to the spontaneous insertion in the double helix. With respect to the axis of the fibril, the different arrangement of the two derivatives emerges very clearly (Supplementary Fig. 9), and is quantified by the radial distribution function with respect to the fibril axis. The glucose of Glu-C1 is aligned with the R8 glucose rings within the central region of the fibril. The coumarin part prevalently points outwards, in contact with R8 lipid tails (shown in Supplementary Fig. 15). By contrast, Oct-C1 coumarin is more internal to the fibril and Oct-C1 C1 octanoic acid tails project outwards (Supplementary Fig. 16).

**All Atom MD simulations**

**Structure of the R8 fibril**

The local ordering from the CG simulations was used as a template for the all-atom model of the R8 aggregate structure. This was achieved by using the coordinates of the stacked glucose-
ring beads (the most stable groups within the aggregate) as restraints for the corresponding atoms in the all-atom representation. More in detail, 10 R8 molecules in toluene were simulated with increasingly strong restraints on their glucose ring atoms, forcing them to a configuration of two stacked R8 quintuplets taken from the CG simulation. The system was then replicated four-fold along the z axis in order to obtain a (periodic) 8 R8 quintuplet stack.

The system was then simulated with no constraints. During a 100ns simulation a regular structure emerges with 4 instead of 5 R8 molecules per stack (Figure 1e-g in the main text). This difference between the CG and all-atom prediction stems from the less directional and “softer” description of inter bead interactions in the CG representation. It comes as no surprise that the CM method is able to capture the general architecture of the assembly but may fail in the fine details, for which the all-atom description is clearly more reliable. As a consequence, we extracted one quadruplet and replicated it 6 times along z (24 total R8 molecules). The system was then simulated for 50ns at 300K and other 50ns at 310K (with semi isotropic pressure coupling, in order to allow for adjustment along z). The resulting aggregate structure is remarkably stable during the simulation (see RMSD in Supplementary Fig. 7c). Such stability is the result of the tightly packed sugar rings, interacting with each other by establishing several H-bonds. In addition, the phenyl rings are rather regularly π-stacked. The alkyl chains are less ordered though some degree of mutual contact is present along the z-axis.

The discovered quadruplet arrangement is not symmetric, insofar as it does not maximize the in-plane inter phenyl tail and inter alkyl chains contact. We tried a more symmetrical quadruplet structure, in which the phenyl tails and the alkyl chains are paired (Supplementary Fig. 7). However such an arrangement is much less stable during the simulated 50ns at 310K, and in particular the regular stacking is lost at the end of the simulation (see comparison between rmsd, ~0.1 vs ~0.16 nm, Supplementary Fig. 7c).

**Insertion of Glu-C1 and Oct-C1**

Again, the results of the CG simulations in terms of the interaction of Glu-C1 and Oct-C1 with the fibril were used to guide the initial structures for the all-atom simulations.

The CG simulations predicted two possible arrangements in the Glu-C1 case, both having Glu-C1 glucose blocked within the sugar core of the fibril, sandwiched with the other glucose rings of the stack. In the first, the C1 is positioned out of the sugar core, in contact with phenyl and/or alkyl chains. In the second it is midway between the sugar core and the external part of the fiber. The second case is not feasible in the all-atom case, because the C1 part is sizably tilted w.r.t. Glu-C1 glucose ring, so it cannot be accommodated between the stacks once the glucose ring intercalates with the other glucoses. (The structure was feasible in the CG case because of the softer and less directional glucose-glucose interactions).

We started from the regular R8-quadruplet stack from the previous simulations, and substituted one R8 molecule with Glu-C1, in such a way as to fill the left void with Glu-C1 glucose ring. Due to the presence of the C1 substituent the glucose ring of C1-Glu cannot be positioned in the same orientation of the missing R8 glucose. Nonetheless, several H-bonds are established with the rest of the R8 matrix, and the structure is stable over the 50ns simulation at 310K (Supplementary Fig. 15).

In the CG simulation Oct-C1 is positioned with the C1 part within the sugar core of the fibril, and its octanoic acid tail points outward and is in contact with the other alkyl chains and phenyl ring tails. This arrangement was translated into the all-atom simulation by removing two R8 molecules and filling the empty space with Oct-C1 and then letting the system relax for 50ns (Supplementary Figure 10).
Free-energy calculations

We studied how the different arrangement of C1 in the two cases (Glu-C1 and Oct-C1) impacts the relative population of the two C1 isomers (Z-s-cis and Z-s-trans). Indeed the polarity experienced by C1 in the two cases (low for Glu-C1 and high for Oct-C1) would point to spectral shifts that are opposite \( w.r.t \) the measured spectra. However, we show that the interaction of Glu-C1/Oct-C1 with the R8 stack modifies the population of the two isomers leading to agreement with the experiments.

Supplementary Fig. 13 shows the free-energy profile (potential of mean force) for rotation around the dihedral characterizing Z-s-cis and Z-s-trans isomers (torsion around C14-C16), evaluated by metadynamics simulations (see Methods). The different positioning of Glu-C1 and Oct-C1 within the fibril results in a markedly different free energy profile. In Glu-C1 the Z-s-cis isomer is the stable one (with dihedral C15-C14-C16-C16 at ~ -150/+150). The same stabilization is present in toluene. By contrast, in Oct-C1 the Z-s-trans isomer (dihedral at ~ 60, Supplementary Fig. 10 and 13) is lower in free energy.

Methods

QM calculations

Optimization of the structures was performed at the DFT PBE0 6-31G(d) level [1]. The excitation energies were calculated on the optimized structure using TD-DFT CAM-B3LYP [2] with the 6-31+G(d) basis set. Solvent effects were introduced within an implicit solvent treatment by the Polarizable Continuum Model (PCM). Gaussian in the g09 version was used [3].

Molecular dynamics simulations

All atom simulations were performed using a 2 fs timestep, with constraints on the bond lengths by the LINCS algorithm. Constant temperature and pressure (1 bar) ensemble was forced by respectively the v-rescale thermostat (with separate thermostats for toluene and the rest of the system) and the Parrinello-Rahman barostat (or Berendsen in the initial equilibration stages). In some cases, semi-isotropic pressure coupling was used, in which changes in the z direction are uncoupled to those in the x-y plane. Temperature and pressure time couplings were 0.2 and 5.0 ps respectively. The Verlet cutoff scheme was used with a 1.2-nm cutoff for VdW and Coulomb short-range interactions. The long-range Coulomb interactions were treated with the usual PME scheme.

In the coarse-grained case, a 20fs time step was used. Temperature and pressure time couplings were 1 and 10ps respectively. The interactions were treated as appropriate for the Martini 2.0 force field. In detail, the Verlet cutoff scheme was used with a 1.1-nm cutoff for Lennard-Jones and Coulomb interactions. A reaction field was used for electrostatic with \( \epsilon = 15 \).

The Gromacs software [4] was used for both all-atom and coarse-grained simulations and for the analysis.

Free energy calculation

The potential of mean force along the chosen reaction coordinate (in this case dihedral torsion) was evaluated using the metadynamics method [5], in which the potential energy along the coordinate is modified by adding Gaussian-shaped “hills” centered on the instantaneous value of the reaction coordinate at regular intervals during the MD simulation. The idea is that, at
convergence, when the distribution of the coordinate is flat, the sum of all the hills gives the (opposite of) free energy as a function of the coordinate. Hills were deposited each 1000 MD steps, and a bias factor of 9 for hills height was used. Plumed patch to Gromacs [6].

**Force Field parameterization**

The parameters used in the MD simulations are listed at the end of this document. The paragraphs below describe the parameterization procedures.

**All Atom Force-Field parameters**

Charmm-compatible force field parameters for R8, Oct-C1 and Glu-C1 were obtained using CgenFF [7] available on line (https://cgenff.paramchem.org/). CgenFF assigns both partial charges and molecular mechanics atom types to each atom in the molecule, also giving a “penalty” higher than 10 for charges and/or parameters in need of validation/re-parameterization.

In the R8 case, the partial charges assigned by CgenFF have a penalty lower than 8, so there was no need for additional validation (Supplementary Table 1). The only parameter with high penalty is the dihedral potential around the glycosidic bond linking the glucose to the phenyl ring (torsion around O7-C14). A QM scan validates the dihedral torsion around that bond. The PBE0/6-31G(d) QM scan was performed on a smaller model molecule (phenyl glucopyranoside).

The C1 part of Glu-C1 and Oct-C1 needed extensive validation on the charges. We started from the RESP HF 6-31G(d) charges and adjusted them in order to get a reasonable comparison with the HF 6-31G(d) dipole moment of the molecule (C1 part capped at N5 with a methyl group). The target QM values were X=5.7, Y=3.6, Z=1.6 Debye, and the adjusted partial charges gave X=6.3, Y=5.9, Z=-0.8. Additional parameterization was required for the torsion around the N5-C27 bond and for the angles between triazole and glucose rings.

**Martini coarse-grained parameters**

The coarse grained model of R8 was made in analogy with the Martini 2.0 force field [8] for similar molecules/chemical groups. The assignment of the bead types follows ref. [9] for carbohydrates and [10] for glycolipids.

For C1 we made use of the auto-Martini script [11], for an automatic assignment of the bead type. In auto-martini the bead center coincides with an atom center rather than in the center of mass of the coarse grained atoms.

The bead-type assignment specifies all non-bonded interactions. Bond, angle and dihedral interactions were parameterized by comparison with the distributions sampled from a 10ns all-atom simulation. The center of mass of the atoms included in each bead was considered.
Supplementary Figures and Tables II

SI Table 1: Partial Charges for R8 Gelator

| Atom Name          | Atom Type   | Partial charge |
|--------------------|-------------|----------------|
| C1                 | CG331       | -0.270         |
| C2, C3, C4, C5, C6 | CG321       | -0.380         |
| C7                 | CG321       | -0.219         |
| C8                 | CG2O2       | 0.900          |
| O1                 | OG2D1       | -0.632         |
| H1-H12, H30-H33    | HGA2        | 0.090          |
| H13-H15, H34-H36   | HGA3        | 0.090          |
| O2                 | OG302       | -0.489         |
| C9                 | CG321       | 0.082          |
| C10                | CG311       | 0.101          |
| C11                | CG311       | 0.148          |
| C12                | CG311       | 0.150          |
| C13                | CG311       | 0.152          |
| C14                | CG311       | 0.277          |
| O3                 | OG3C61      | -0.390         |
| O4                 | OG311       | -0.656         |
| O5, O6             | OG311       | -0.657         |
| H16, H17           | HGA2        | 0.090          |
| H18                | HGP1        | 0.419          |
| H19-H25            | HGA1        | 0.090          |
| O7                 | OG301       | -0.398         |
| C15                | CG2R61      | 0.219          |
| C16                | CG2R61      | -0.108         |
| C17, C19           | CG2R61      | -0.117         |
| C18                | CG2R61      | -0.002         |
| C20                | CG2R61      | -0.108         |
| C21                | CG321       | -0.182         |
| C22                | CG321       | -0.137         |
| C23                | CG2O5       | 0.393          |
| O8                 | OG2D3       | -0.481         |
| C24                | CG331       | -0.229         |
| H26-H29            | HGR61       | 0.115          |

SI Figure 22: R8 all-atom parameters

SI Figure 23: Potential Energy Scan for R8

Potential energy scan (with optimization for each angle value) for torsion around O3-C14-O7-C15 from force field MM and DFT-PBE0.
### SI Table 2: Partial charges of Glu-C1 and Oct-C1

| Atom Name | Atom Type | Partial Charge |
|-----------|-----------|----------------|
| C1        | NG301     | -0.300*        |
| C1        | CG321     | -0.100         |
| C2,C8     | CG321     | -0.070*        |
| C3,C7     | CG321     | -0.050*        |
| C4,C6     | CG2R61    | -0.050*        |
| C5        | CG2R61    | +0.150*        |
| C9        | CG321     | -0.100         |
| C10       | CG2R61    | -0.250*        |
| C11       | CG2R62    | +0.010         |
| C12       | CG2R62    | +0.200*        |
| C13,C14   | CG2R62    | -0.150*        |
| C15       | CG2R63    | +0.700*        |
| O1        | O2D4      | -0.650*        |
| O2        | O3G60     | -0.340         |
| C16       | CG2DC1    | -0.155*        |
| C17       | CG2DC1    | -0.055*        |
| C18       | CG1N1     | +0.390         |
| C19       | CG2R61    | +0.020         |
| C20,C21,C23,C2 | CG2R61 | -0.120*        |
| C22       | CG2R61    | -0.050*        |
| C25       | CG2R51    | -0.330*        |
| N3        | NG2R50    | -0.245*        |
| N4        | NG2R50    | -0.335*        |
| N5        | NG2R51    | +0.400*        |
| C26       | CG2R51    | -0.435*        |

* differ more than 0.025 from cgenFF assignment
SI Figure 25: Comparison of Cgenff, RESP and final adjusted partial charges for the C1 part (the molecule and the atom numbering are shown).
SI Figure 26: Dihedral scan around N5-C27 (calculations on triazole glucose only).

| SI Table 3: Parameters for the N5-C27 dihedral |
|-----------------------------------------------|
| **Atoms types (names)** | **phase** | **V (kJ/mol)** | **n** |
| CG311/OG3C61(C28/O3) | CG311(C27) | NG2R51 (N5) | CG2R51 | 0.0 | 14.00 | 1 |
| CG311/OG3C61 | CG311 | NG2R51 | CG2R51 | 0.0 | 6.15 | 2 |
| CG311/OG3C61 | CG311 | NG2R51 | CG2R51 | 0.0 | 6.15 | 2 |
| CG311/OG3C61 | CG311 | NG2R51 | CG2R51 | 0.0 | 6.15 | 2 |
| CG311/OG3C61 | CG311 | NG2R51 | NG2R50(N4) | 180.0 | 14.00 | 1 |
| CG311/OG3C61 | CG311 | NG2R51 | NG2R50 | 0.0 | 6.15 | 2 |
| CG311/OG3C61 | CG311 | NG2R51 | NG2R50 | 180.0 | 1.70 | 3 |
| CG311/OG3C61 | CG311 | NG2R51 | NG2R50 | 0.0 | 3.90 | 4 |

| SI Table 4: Angle parameters |
|-------------------------------|
| **Atoms types (names)** | **Reference angle (deg)** | **Force constant (kJ mol⁻¹ rad⁻²)** |
| CG311 (C28) | CG311 (C27) | NG2R51 (N5) | 112.0 | 633.4576 |
| CG311 (C28) | CG311 (C27) | OG3C61 (O3) | 111.5 | 376.5600 |
| NG2R51 (N5) | CG311 (C27) | OG3C61 (O3) | 110.4 | 376.5600 |
SI Figure 27: Martini model and parameters for R8

| SI Table 5: Bead index (included atoms) | Bead type | Mass (a.u.) |
|----------------------------------------|-----------|-------------|
| 1 (C22,C23,C24,O8)                    | Na        | 72.0        |
| 2 (C17,C18,C21)                       | SC5       | 45.0        |
| 3 (C19,C20)                           | SC5       | 45.0        |
| 4 (C15,C16)                           | SC5       | 45.0        |
| 5 (O3,O7,C14)                         | Na        | 72.0        |
| 6 (O5,O6,C12,C13)                     | P4        | 72.0        |
| 7 (O4,C10,C11)                        | P2        | 72.0        |
| 8 (O1,O2,C8)                          | Na        | 72.0        |
| 9 (C4,C5,C6,C7)                       | C1        | 72.0        |
| 10 (C1,C2,C3)                         | C1        | 72.0        |

| SI Table 6: Angle | Reference angle | Force constant (kJ mol⁻¹ deg⁻²) |
|-------------------|-----------------|-------------------------------|
| 1 2 3             | 100.0           | 50.0                          |
| 1 2 4             | 130.0           | 50.0                          |
| 2 4 5             | 170.0           | 50.0                          |
| 3 5 6             | 140.0           | 50.0                          |
| 3 5 7             | 140.0           | 50.0                          |
| 4 5 6             | 140.0           | 50.0                          |
| 4 5 7             | 160.0           | 50.0                          |
| 5 7 8             | 70.0            | 25.0                          |
| 6 7 8             | 130.0           | 50.0                          |
| 7 8 9             | 140.0           | 25.0                          |
| 8 9 10            | 180.0           | 25.0                          |

| SI Table 7: Bond | Reference length (nm) | Force constant (kJ mol⁻¹ nm⁻²) |
|------------------|-----------------------|-------------------------------|
| 1 2              | 0.37                  | 7500                          |
| 3 5              | 0.35                  | 2500                          |
| 4 5              | 0.25                  | 7500                          |
| 5 8              | 0.47                  | 7500                          |
| 7 8              | 0.35                  | 5000                          |
| 8 9              | 0.37                  | 2500                          |
| 9 10             | 0.45                  | 2500                          |

| Constraints      | Reference angle       | Force constant (kJ mol⁻¹ deg⁻²) |
|------------------|-----------------------|-------------------------------|
| 2 3              | 0.23                  |                               |
| 2 4              | 0.27                  |                               |
| 3 4              | 0.20                  |                               |
| 5 6              | 0.28                  |                               |
| 6 7              | 0.30                  |                               |
| 5 7              | 0.28                  |                               |
**SI Figure 28:** Inter bead distance distributions from coarse-grained and all-atom simulations for R8. In the all-atom distributions the center of mass of the atoms included in the bead is considered.
SI Figure 29: Angle distribution from coarse-grained and all-atom simulations for R8. The involved atoms are indicated in the graphs.
### SI Table 8 – Martini model and parameters for C1

| Bead index (atom) | Bead type |
|-------------------|-----------|
| 1 (N1)            | N0        |
| 2 (C4)            | SC4       |
| 3 (C6)            | SC4       |
| 4 (C11)           | SC5       |
| 5 (O2)            | SNa       |
| 6 (C14)           | SC5       |
| 7 (C18)           | Na        |
| 8 (C19)           | SC5       |
| 9 (C21)           | SC5       |
| 10 (C23)          | SC5       |
| 11 (C25)          | SP1       |
| 12 (N4)           | SP1       |

### SI Table 10 – Bond

| Bond | Reference length (nm) | Force constant (kJ mol⁻¹ nm⁻²) |
|------|-----------------------|-------------------------------|
| 4 7  | 0.44                  | 7500                          |
| 6 8  | 0.38                  | 7500                          |
| 7 9  | 0.43                  | 7500                          |

### SI Table 9 – Angle

| Angle | Reference value (deg) | Force constant (kJ mol⁻¹ rad⁻²) |
|-------|-----------------------|---------------------------------|
| 1 2 4 | 120.6                 | 25.0                            |
| 1 3 4 | 119.8                 | 25.0                            |
| 1 3 5 | 177.6                 | 25.0                            |
| 5 6 7 | 150.0                 | 50.0                            |
| 5 6 8 | 160.0                 | 50.0                            |
| 6 7 8 | 89.5                  | 50.0                            |
| 6 8 9 | 150.0                 | 25.0                            |
| 6 8 10| 150.0                 | 25.0                            |
| 7 8 9 | 120.0                 | 50.0                            |
| 7 8 10| 160.0                 | 25.0                            |
| 8 9 11| 122.0                 | 25.0                            |
| 8 10 11| 122.0              | 25.0                            |
| 9 11 12| 130.0               | 50.0                            |
| 10 11 12| 170.0           | 50.0                            |

### SI Table 11 – Improper Dihedrals

| Improper Dihedrals | Reference angle (deg) | Force constant (kJ mol⁻¹ rad⁻²) |
|--------------------|-----------------------|---------------------------------|
| 6 4 2 3            | 0.0                   | 10.0                            |
| 6 4 3 2            | 180.0                 | 10.0                            |
| 6 4 3 5            | 0.0                   | 10.0                            |
| 6 4 5 3            | 180.0                 | 10.0                            |
| 6 5 3 4            | 0.0                   | 10.0                            |
| 4 6 5 3            | 0.0                   | 10.0                            |
| 4 2 1 3            | 0.0                   | 10.0                            |
| 4 2 3 1            | 180.0                 | 10.0                            |
| 4 2 3 5            | 0.0                   | 10.0                            |
| 4 3 2 1            | 180.0                 | 10.0                            |
| 4 3 3 2            | 0.0                   | 10.0                            |
| 2 4 3 1            | 0.0                   | 10.0                            |
| 2 4 3 5            | 180.0                 | 10.0                            |
| 2 4 5 3            | 0.0                   | 10.0                            |
| 2 3 4 5            | 180.0                 | 10.0                            |
| 1 2 4 3            | 180.0                 | 10.0                            |
| 1 2 4 5            | 0.0                   | 10.0                            |
| 1 3 4 5            | 0.0                   | 10.0                            |
| 6 4 3 2            | 180.0                 | 10.0                            |
| 6 4 3 5            | 0.0                   | 10.0                            |
| 6 4 5 3            | 180.0                 | 10.0                            |
| 6 5 4 2            | 180.0                 | 10.0                            |

### SI Table 10 – Bond

| Bond | Reference length (nm) | Force constant (kJ mol⁻¹ nm⁻²) |
|------|-----------------------|-------------------------------|
| 4 7  | 0.44                  | 7500                          |
| 6 8  | 0.38                  | 7500                          |
| 7 9  | 0.43                  | 7500                          |

### SI Table 9 – Angle

| Angle | Reference value (deg) | Force constant (kJ mol⁻¹ rad⁻²) |
|-------|-----------------------|---------------------------------|
| 1 2 4 | 120.6                 | 25.0                            |
| 1 3 4 | 119.8                 | 25.0                            |
| 1 3 5 | 177.6                 | 25.0                            |
| 5 6 7 | 150.0                 | 50.0                            |
| 5 6 8 | 160.0                 | 50.0                            |
| 6 7 8 | 89.5                  | 50.0                            |
| 6 8 9 | 150.0                 | 25.0                            |
| 6 8 10| 150.0                 | 25.0                            |
| 7 8 9 | 120.0                 | 50.0                            |
| 7 8 10| 160.0                 | 25.0                            |
| 8 9 11| 122.0                 | 25.0                            |
| 8 10 11| 122.0               | 25.0                            |
| 9 11 12| 130.0               | 50.0                            |
| 10 11 12| 170.0             | 50.0                            |

### SI Table 11 – Improper Dihedrals

| Improper Dihedrals | Reference angle (deg) | Force constant (kJ mol⁻¹ rad⁻²) |
|--------------------|-----------------------|---------------------------------|
| 6 4 2 3            | 0.0                   | 10.0                            |
| 6 4 3 2            | 180.0                 | 10.0                            |
| 6 4 3 5            | 0.0                   | 10.0                            |
| 6 4 5 3            | 180.0                 | 10.0                            |
| 6 5 3 4            | 0.0                   | 10.0                            |
| 4 6 5 3            | 0.0                   | 10.0                            |
| 4 2 1 3            | 0.0                   | 10.0                            |
| 4 2 3 1            | 180.0                 | 10.0                            |
| 4 2 3 5            | 0.0                   | 10.0                            |
| 4 3 2 1            | 180.0                 | 10.0                            |
| 4 3 3 2            | 0.0                   | 10.0                            |
| 2 4 3 1            | 0.0                   | 10.0                            |
| 2 4 3 5            | 180.0                 | 10.0                            |
| 2 4 5 3            | 0.0                   | 10.0                            |
| 2 3 4 5            | 180.0                 | 10.0                            |
| 1 2 4 3            | 180.0                 | 10.0                            |
| 1 2 4 5            | 0.0                   | 10.0                            |
| 1 3 4 5            | 0.0                   | 10.0                            |
| 6 4 3 2            | 180.0                 | 10.0                            |
| 6 4 3 5            | 0.0                   | 10.0                            |
| 6 4 5 3            | 180.0                 | 10.0                            |
| 6 5 4 2            | 180.0                 | 10.0                            |
SI Figure 30: Inter-bead distance from coarse-grained and all-atom simulations for C1.

SI Figure 31: Angle distribution from coarse-grained and all-atom simulations for C1.
SI Figure 32: Martini model and parameters for Glu-C1 and Oct-C1

| SI Table 12: Bead index (atom) | Bead type |
|-------------------------------|-----------|
| Oct-C1                        |           |
| 13 (C27-C32)                  | C1        |
| 14 (C33,C34,O3,O4)            | P3        |
| Glu-C1                        |           |
| 13 (C27,C28,O4)               | P1        |
| 14 (C28,C30,O5,O6)            | P4        |
| 15 (C31,C32,O3,O7)            | P1        |

| SI Table 13: Angle Reference angle (deg) | Force constant(kJ mol\(^{-1}\) deg\(^{-2}\)) |
|-----------------------------------------|---------------------------------------------|
| Oct-C1                                  |                                             |
| 1 12 13                                 | 113.0                                       | 50.0                                       |
| 12 13 14                                | 150.0                                       | 25.0                                       |
| Glu-C1                                  |                                             |
| 12 13 14                                | 130.0                                       | 50.0                                       |
| 9 11 13                                 | 150.0                                       | 150.0                                      |
| 11 12 13                                | 110.0                                       | 100.0                                      |
| 11 12 15                                | 110.0                                       | 100.0                                      |
| 10 11 13                                | 140.0                                       | 50.0                                       |
| Dihedrals                               |                                             |
| 12 13 14 15                             | 30.0                                        | 10.0                                       |
SI Table 14: Harmonic constraints

| Oct-C1 | Reference length (nm) | Force constant (kJ mol⁻¹ nm⁻²) |
|--------|-----------------------|-------------------------------|
| 4 12   | 1.23                  | 1000                          |
| 5 12   | 1.25                  | 1000                          |
| 9 13   | 0.77                  | 1000                          |

| Glu-C1 | Reference length (nm) | Force constant (kJ mol⁻¹ nm⁻²) |
|--------|-----------------------|-------------------------------|
| 4 12   | 1.23                  | 1000                          |
| 5 12   | 1.25                  | 1000                          |
| 6 13   | 1.20                  | 1000                          |
| 8 13   | 0.84                  | 1000                          |
| 8 14   | 1.10                  | 1000                          |
| 8 15   | 1.00                  | 1000                          |
| 9 15   | 0.80                  | 1000                          |
| 10 14  | 0.90                  | 1000                          |
| 11 14  | 0.67                  | 1000                          |

SI Table 15: Bond

| Oct-C1 | Reference length (nm) | Force constant (kJ mol⁻¹ nm⁻²) |
|--------|-----------------------|-------------------------------|
| 12 13  | 0.43                  | 2500                          |
| 13 14  | 0.50                  | 2500                          |

| Glu-C1 | Reference length (nm) | Force constant (kJ mol⁻¹ nm⁻²) |
|--------|-----------------------|-------------------------------|
| 12 13  | 0.30                  | 7500                          |
| 12 15  | 0.46                  | 7500                          |

| Constraints | Reference length (nm) | Force constant (kJ mol⁻¹ nm⁻²) |
|-------------|-----------------------|-------------------------------|
| 13 14       | 0.28                  |                               |
| 13 15       | 0.39                  |                               |
| 14 15       | 0.29                  |                               |

SI Figure 33: Inter-bead distance for Glu-C1 (Glu part only).

SI Figure 34: Angles for Glu-C1 (Glu part only).
SI Figure 35: Inter-bead distance for Oct-C1 (Oct part only).

SI Figure 36: Angles for Oct-C1 (Oct part only).

SI Figure 37: Martini model and parameters for toluene

| SI Table 16: Bead index | SI Table 17: Reference length (nm) |
|-------------------------|-----------------------------------|
| Bead type               | Constraint                       |
| Model 1                 |                                   |
| 1                       |                                   |
| C4                      |                                   |
| SC4                     |                                   |
| 2                       |                                   |
| SC4                     |                                   |
| 3                       |                                   |
| SC4                     |                                   |
| Model 2                 |                                   |
| 1                       |                                   |
| C5                      |                                   |
| SC5                     |                                   |
| 2                       |                                   |
| SC5                     |                                   |
| 3                       |                                   |
| SC5                     |                                   |
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