Appendix A

Materials and Instrumentation

**General remarks.**
All amino acids where obtained from Bachem, 1,3,5-cis,cis-cyclohexanetricarboxylic acid was obtained from TCI, all other chemicals were from Sigma Aldrich and used without further purification. Solvents were of reagent grade. Water used for gelation studies was doubly distilled.

**Gelation by heating and cooling cycle.**
In a typical gelation experiment a weighed amount of the compound under investigation and 1.0 mL of the solvent are placed in a closed 4 mL vial (D 12 mm). The vial was heated using a heating gun for 10 second intervals with shaking until the solid had dissolved. The cap of the vial was wrapped with tissue to check for any leaks during heating. The solution was allowed to cool to room temperature and subsequently examined. Gelation was considered to have occurred when a homogeneous substance was obtained that did not exhibit gravitational flow over 24 h.

**Gelation by pH jumping.**
In a typical gelation experiment a weighed amount of the compound under investigation was placed in a 4 mL vial (D 12 mm) and 100 µL of 1 M NaOH(aq) solution was added to dissolve the gelator. This solution was diluted with 800 µL of water and acidified by 100 µL of a 1 M HCl(aq) solution for a total of 1.0 mL. Gelation was considered to have occurred when a homogeneous substance was obtained that did not exhibit gravitational flow over 24 h.

**Gelation of with Au or Ag nanoparticles.**
Preparation of hydrogels: 800 µL of Ag or Au colloid containing solution was added to 100 µL of CH-Met (20 mg mL⁻¹) in 1 M NaOH(aq). Addition of 100 µL of 1 N acid(aq) (HNO₃, H₂SO₄, H₃PO₄ or HCl) to the mixture with gentle agitation resulted in a change in colour (from yellow to grey in the case of the Ag colloid and red to blue in the case of the Au colloid) concomitant with the formation of a hydrogel. For experiments in microtitre (96 well) plates, lower volumes were used with the same volume ratios.

**NMR**
¹H-NMR and ¹³C-NMR spectra were recorded on a Varian AMX400 (operating at 400 and 100 MHz, respectively) spectrometer or a Bruker Ascend™ 600 MHz NMR spectrometer (operating at 600 and 150 MHz, respectively).

**FTIR**
FTIR spectra were recorded on a Perkin Elmer Spectrum 400 spectrometer or on a JASCO FT/IR-4700.

**UV-Vis**
UV-vis absorption spectra were recorded using an Analytik Jena Specord 210 plus or on a JASCO.

**Circular Dichroism**
CD spectra were recorded using a JASCO 810 CD spectrometer.
Appendix A

**Fluorescence**

Fluorescence spectra were recorded with 266 nm excitation either with continuous wave excitation (0.1 mW at sample, with at 532 nm pumped SpectraPhysics Wavetrain) with emission detected using a Shamrock500 spectrograph (500 nm blaze 150 l/mm grating) and idus-420BU CCD camera or with pulsed excitation wave excitation (0.1 ml per pulse, 4th harmonic of a Innolas Spitelight200 Nd:YAG laser, at 3 Hz repetition rate) with emission detected using a Zolix Omni-300 spectrograph (500 nm blaze 150 l/mm grating) and iStar-740 iCCD camera in gated mode.

**Raman**

Raman spectra were recorded on a Perkin Elmer Raman station 400F with a RamanMicro 300 microscope at 785 nm or on an Olympus BX51M upright microscope with excitation at 632.8 nm (Thorlabs Hrr 120-1 HeNe laser, 10 mW at sample, with laser line clean up filter from Semrock). Excitation was delivered using a dichoric mirror (Semrock) and light collected via a round to line multicore fibre (which acted as slit) and delivered to a Shamrock 163 spectrograph and dispersed with a SRT-SHT-9003 grating onto a iDus-418 CCD detector (Andor Technology). Calibration was performed using the spectrum of polystyrene.

**Dropping Ball**

Gels with a volume of 1.0 mL were prepared as described above. A stainless steel ball (63 mg; d 2.5 mm) was placed on top of the gel and the vial was closed. A series of these samples were placed in a heating block that was heated at a 10 °C h⁻¹ while observing the positions of the balls with a video camera and concurrently monitoring the temperature by means of a thermocouple placed in the heating block. The melting temperature of the gel was taken as the temperature at which the steel ball reached the base of the vial. The upper temperature was limited to 130°C.

**Rheology**

The stress strain behaviour of the gels was analysed using an Anton Paar parallel plate rheometer. Gels were prepared on the bottom plate by pH jumping and the top plate was lowered to a 1 mm gap. Strain scans were performed with a 50 mm plate from 0.1 % to 100 % with a frequency of 1 rad/s. The critical strain was quoted as the point that G’ starts to deviate for linearity and ultimately crosses over the G", resulting in gel breakdown.

**Transmission Electron Microscopy**

TEM images of gels were recorded on a Phillips CM10 with a LaB₆ emitter. Samples were prepared on a carbon coated copper grid by either forming the gel on the grid by pH jumping or by pipetting 4 µL out of heat/cool formed gels on the grid. Both type of samples were blotted off after 1 min. The grid was stained with 2 µL of Uranylacetate and blotted off after 1 min.

TEM images of colloidal solutions were recorded on a Phillips CM12 with a LaB₆ emitter. Colloidal samples were prepared by pipetting 2 µL of colloidal solution on a carbon coated copper grid and bloting off after 1 min. Gel samples were prepared on a carbon coated copper grid by forming the gel on the grid by pipetting 2 µL out pH jump forming gels before fully set on the grid. Both type of samples were blotted off after 1 min. The grid was stained with 2 µL of Uranylacetate and blotted off after 1 min.

**Cryogenic Transmission Electron Microscopy**

Cryo-TEM images were taken with a slow scan CCD camera (Gatan) under low-dose conditions on a Tecnai T20 TEM microscope (FEI) operating at 200keV using a Gatan model 626 cryo-stage sample holder. Samples were prepared by mixing the gel on a 400 mesh holey carbon TEM grid (quantifoil 3.5/1). The time between mixing and blotting and freezing in liquid ethane, using a Vitrobot (FEI), was varied to get snapshots of different states of the gel.
Single Crystal X-ray
A single crystal of compound CH-Abu was mounted on top of a cryoloop and transferred into the cold nitrogen stream (100 K) of a Bruker-AXS D8 Venture diffractometer. Data collection and reduction was done using the Bruker software suite APEX3. The final unit cell was obtained from the xyz centroids of 9951 reflections after integration. A multiscan absorption correction was applied, based on the intensities of symmetry-related reflections measured at different angular settings (SADABS). The structures were solved by direct methods using SHELXT and refinement of the structure was performed using SHELXL. The hydrogen atoms were generated by geometrical considerations, constrained to idealised geometries and allowed to ride on their carrier atoms with an isotropic displacement parameter related to the equivalent displacement parameter of their carrier atoms.

Dark field microscope
Dark field microscope videos were acquired using a Nikon TI eclipse inverted microscope equipped with a 60 times magnification objective. Samples were illuminated with a Nikon Halogen 12V 100W lamp and the light was focused using a Nikon dry dark field condenser. Scattered light was collected by an Andor Clara CCD camera. Wilco wells glass bottom dish (0.17 mm thick) for dark field microscopy

Small Angle X-ray Scattering
SAXS was measured on the BL16B1 and BL19U2 (beamline of National Facility of Protein Science Shanghai (NFPS)) at Shanghai Synchrotron Radiation Facility. Samples were prepared in 1.5 mm diameter quartz capillaries (Hampton research). Best fit of the SAXS experimental data was performed using the Sasfit program. The following equations have been used to fit the experimental data. The Debye-Anderson-Brumberger model has been used to describe the scattering of spherical inhomogeneity’s without spatial arrangement:

\[ I(q) = I(0) / \left( 1 + (q\xi)^2 \right)^2 \]

where is a scaling factor depending on the electron density difference between the scattering object and the surrounding solution and is the average characteristic dimension of the objects.

Polarized Raman
Polarization dependent Raman spectra at 532 nm were recorded on a Olympus BX51M microscope equipped with a long working distance 100 times magnification objective. Excitation was with at 532 nm (25 mW Samba Laser from Cobolt). Estimated spot size was less than 1 microns (diameter). Laser power was controlled by rotation of its polarization using a half-wave plate retarder (Thorlabs) followed by a polarizing 50/50 beam splitter. The plane of the linearly polarized output was rotated using a second half-wave plate retarder. The laser was reflected into the optical path of the microscope using a long pass dichroic mirror (Semrock) and the collimated reflected Raman scattering was passed through a long pass filter and focused into a 50 micron diameter fibre to a Shamrock 163 spectrograph with a 500 nm blaze 1200 l/mm grating and an Andor iVac-OE CCD camera. UV-vis absorption spectra were recorded using an Analytik Jena Specord 210 plus and CD spectra using a JASCO 810 CD spectrometer.

Polarization dependent Raman spectra at 785 nm were recorded on an Olympus BX51M microscope equipped with a long working distance 100 times magnification objective. Excitation was with at 785 nm (75 mW, Ondax). Estimated spot size was less than 1 microns (diameter). Laser power was controlled by rotation of polarization using a half-wave plate retarder (Thorlabs) followed by a polarizing 50/50 beam splitter. The plane of the linearly polarized output was rotated using a second half-wave plate retarder. The laser was reflected into the optical path of the microscope using a long
pass dichroic mirror (Semrock) and the collimated reflected Raman scattering was passed through a long pass filter and focused into a 50 micron diameter fibre to a Kymera-163 spectrograph with a 500 nm blaze 1200 l/mm grating and a Andor iDus-DU416A-LDC-DD camera.
Appendix B

Synthesis and Characterisation

The synthesis of all compounds was based on a literature procedure. The synthesis of colloidal solutions of Silver and gold nanoparticles was based on literature procedure.

**1s,3s,5s)-cyclohexane-1,3,5-tricarbonyl trichloride**

A white suspension of 9.74 g (45.07 mmol) of 1,2,5-cis,cis-cyclohexane and 26 mL of SOCl\(_2\) was heated at reflux for 20 h. The formed clear yellow solution was cooled to room temperature (20 °C), and excess SOCl\(_2\) was removed by evaporation in vacuo. The resulting liquid was stored at 4 °C overnight, yielding solids which were isolated by filtration, yielding 11.77 g (43.35 mmol, 96%) of a pale yellow powder. 1H-NMR (300 MHz, CDCl\(_3\)) \(\delta\) (ppm) 2.87 (tt, \(J = 12.6, 3.4\) Hz, 3H), 2.75 – 2.61 (m, 3H), 1.69 (dt, \(J = 13.7, 12.6\) Hz, 3H). 13C NMR (75 MHz, CDCl\(_3\)) \(\delta\) (ppm) 174.2 (COCl), 52.2 (CH), 30.3 (CH2).

**CH-Gly**

Trimethylamine (9 mL) was added to a cooled (0 °C) solution of 4.28 g (24.05 mmol, 3.1 eq.) of glycine methyl ester hydrochloride in 300 mL of DCM. 3 g (11.05 mmol, 1 eq.) of 2.91 in 30 mL of DCM was added to the solution. Upon mixing the two clear solutions formed an opaque white solution which was stirred at room temperature (20 °C) for 24 h. The solids were isolated by filtration and suspended in 200 mL ethanol. The solids isolated by filtration and dried in vacuo, yielding 5.5 g of the methyl ester as a white powder (80%). The powder was suspended in 60 mL of methanol cooled to 0 °C and 30 mL of 2M NaOH(aq) was added. The suspension was allowed to reach room temperature (20 °C) slowly and stirred for 20 h. A clear solution was obtained and diluted with 100 mL of water. The solution was acidified to pH < 3 using 2M HCl(aq). Gelation was observed and the resulting gel was filtered and washed with 2 x 100 mL water. The solids were dried in vacuo (40 °C, 20 mBar) for 1 h and lyophilized overnight, yielding 1.70 g (4.39 mmol, 50%) of CH-Gly as a white powder. Decomposition above 235 °C. 1H-NMR (400 MHz, DMSO-d\(_6\)) \(\delta\) (ppm) 12.47 (s, 3H), 8.13 (t, \(J = 6.0\) Hz, 3H), 3.71 (d, \(J = 6.0\) Hz, 6H), 2.28 (t, J = 12.6 Hz, 3H), 1.94 (d, J = 12.6 Hz, 3H), 1.39 (q, J = 12.6 Hz, 3H). 13C-APT NMR (101 MHz, DMSO-d\(_6\)) \(\delta\) (ppm) 175.0 (COOH), 171.7 (C=O), 42.7 (CH), 31.8 (CH2). FTMS (ESI-TOF) m/z: [M + H]+ Calcd for C15H22N3O9 388.1351; Found 388.1348. Anal. Calcd for C15H21N3O9: C, 46.51; H, 5.46; N, 10.85. Found: C, 38.13; H, 4.89; N, 8.92.

**CH-Ala**

The synthesis was analogous to the synthesis of CH-Gly, using 4.76 g (24.05 mmol) of alanine methyl ester hydrochloride, yielding 1.68 g (3.91 mmol, 35%) of CH-Ala as a white powder. 1H NMR (400 MHz, DMSO-d\(_6\)) \(\delta\) (ppm) 12.41 (s, 3H), 8.04 (d, \(J = 7.4\) Hz, 3H), 4.15 (q, J = 7.3 Hz, 3H), 2.23 (t, J = 12.5, 3H), 1.70 (d, J = 12.6 Hz, 3H), 1.37 (q, J = 12.6 Hz, 3H), 1.23 (d, J = 7.3 Hz, 9H). 13C NMR (101 MHz, DMSO-d\(_6\)) \(\delta\) (ppm) 174.7, 174.5, 47.7, 42.7, 40.0, 31.7, 17.6. FTMS (ESI-TOF) m/z: [M + H]+ Calcd for C18H28N3O9 430.1820; Found 430.1815. Anal. Calcd for C18H27N3O9: C, 50.35; H, 6.34; N, 9.79. Found: C, 49.72; H, 6.20; N, 9.61. Melting point 150 °C degradation.

**CH-Val**

The synthesis was analogous to the synthesis of CH-Gly, using 5.71 g (24.05 mmol) of valine methyl ester hydrochloride, yielding 4.08 g (7.93 mmol, 71%) of CH-Val as a white powder. Decomposition
Appendix B

above 237 °C. 1H-NMR (400 MHz, DMSO-d6) δ (ppm) 12.53 (s, 3H), 7.93 (d, J = 8.7 Hz, 3H), 4.14 (dd, J = 8.6, 5.9 Hz, 3H), 2.44 (t, J = 12.0 Hz, 3H), 2.04 (h, J = 6.7 Hz, 3H), 1.68 (d, J = 12.4 Hz, 3H), 1.42 (q, J = 12.5 Hz, 3H), 0.86 (d, J = 6.8 Hz, 18H). 13C NMR (101 MHz, DMSO-d6) δ (ppm) 175.1, 173.6, 57.2, 42.4, 32.0, 30.2, 19.6, 18.4. FTMS (ESI-TOF) m/z: [M + H]+ Calcd for C24H40N3O9 514.2759; Found 514.2752. Anal. Calcd for C24H40N3O9; C, 56.13; H, 7.65; N, 8.18. Found; C, 50.34; H, 7.35; N, 7.27.

CH-Leu
The synthesis was analogous to the synthesis of CH-Gly, using 6.19 g (24.05 mmol) of leucine methyl ester hydrochloride, yielding 3.92 g (7.05 mmol, 64%) of CH-Leu as a white powder. Decomposition above 238 °C. 1H-NMR (400 MHz, DMSO-d6) δ (ppm) 12.45 (s, 3H), 8.02 (d, J = 8.0 Hz, 3H), 4.20 (ddd, J = 10.1, 8.1, 4.9 Hz, 3H), 2.28 (tt, J = 12.5, 3.4 Hz, 3H), 1.74 – 1.65 (m, 3H), 1.65 – 1.33 (m, 13H), 0.85 (dd, J = 22.3, 6.4 Hz, 18H). 13C-APT NMR (101 MHz, DMSO-d6) δ (ppm) 174.7, 174.6, 50.3, 42.8, 31.8, 24.8, 23.3, 21.6. FTMS (ESI-TOF) m/z: [M + H]+ Calcd for C27H46N3O9 556.3220; Found 556.3229. Anal. Calcd for C27H45N3O9: C, 58.36; H, 8.16; N, 7.56. Found: C, 55.66; H, 7.20; N, 7.88.

CH-Ile
The synthesis was analogous to the synthesis of CH-Gly, using 6.19 g (24.05 mmol) of isoleucine methyl ester hydrochloride, yielding 1.79 g (3.22 mmol, 29%) of CH-Ile as a white powder. Melting point 229-231 °C. 1H-NMR (400 MHz, DMSO-d6) δ (ppm) 12.51 (s, 2H), 7.93 (d, J = 8.6 Hz, 3H), 4.17 (dd, J = 8.6, 6.3 Hz, 3H), 2.40 (t, J = 12.5 Hz, 3H), 1.77 (bs, 3H), 1.67 (d, J = 12.5 Hz, 3H), 1.41 (q, J = 12.5, 11.6 Hz, 6H), 1.17 (h, J = 14.9, 7.5 Hz, 3H), 0.83 (t, J = 7.4 Hz, 18H). 13C-APT NMR (101 MHz, DMSO-d6) δ (ppm) 175.0, 173.6, 56.4, 42.4, 40.5, 40.3, 39.8, 39.6, 39.4, 36.7, 32.0, 25.1, 16.1, 11.6. FTMS (ESI-TOF) m/z: [M + H]+ Calcd for C27H46N3O9 556.3229; Found 556.3221. Anal. Calcd for C27H45N3O9: C, 58.36; H, 8.16; N, 7.56. Found: C, 56.54; H, 7.37; N, 7.91.

CH-Met
The synthesis was analogous to the synthesis of CH-Gly, using 6.80 g (34.05 mmol) of L-methionine methyl ester hydrochloride, yielding 1.86 g (2.86 mmol, 62%) of CH-Met as a white powder. Decomposition above 214 °C. 1H-NMR (400 MHz, DMSO-d6) δ (ppm) 12.57 (s, 3H), 8.06 (dd, J = 7.9, 3.2 Hz, 3H), 4.28 (tt, J = 8.2, 3.7 Hz, 3H), 2.45 (dt, J = 8.7, 4.6 Hz, 9H), 2.30 (d, J = 14.2 Hz, 3H), 2.03 (d, J = 3.3 Hz, 3H), 1.95 (dp, J = 12.3, 4.0 Hz, 3H), 1.84 (dp, J = 13.0, 4.8, 4.0 Hz, 3H), 1.74 (d, J = 12.2 Hz, 3H), 1.49 – 1.35 (m, 3H). 13C-APT NMR (101 MHz, DMSO-d6) δ (ppm) 174.9, 173.6, 56.4, 42.4, 40.3, 39.8, 39.6, 39.4, 36.7, 32.0, 25.1, 16.1, 11.6. FTMS (ESI-TOF) m/z: [M + H]+ Calcd for C24H40N3O9S3 610.1921; Found 610.1911. Anal. Calcd for C24H39N3O9S3: C, 47.27; H, 6.45; N, 6.89. Found: C, 46.63; H, 6.39; N, 6.73.

CH-Phe
The synthesis was analogous to the synthesis of CH-Gly, using 9.33 g (43 mmol) of L-phenylalanine methyl ester hydrochloride and 2.89 g (10.7 mmol) of (1s,3s,5s)-cyclohexane-1,3,5-tricarbonyl trichloride, yielding 2.68 g (4.1 mmol, 38%) of CH-Phe as a white powder. Decomposition above 245 °C. 1H-NMR spectrum (400 MHz, DMSO-d6) δ (ppm) 13.16 – 12.30 (m, 4H), 8.10 (d, 4H), 7.21 (dq, 18H), 4.38 (td, 4H), 3.05 (dd, 4H), 2.84 (dd, 4H), 2.18 (s, 2H), 1.46 (d, 3H), 1.21 (q, 4H). 13C-APT NMR (101 MHz, DMSO-d6) δ 132.2, 131.2, 129.4, 56.3, 45.2, 43.3, 43.1, 42.9, 42.7. HRMS (ESI) m/z: [M + Na]+ Calcd for C36H39N3O9Na 680.2579; Found 680.2569. Anal. Calcd for C36H39N3O9: C, 65.74; H, 5.98; N, 6.39. Found: C, 55.90; H, 5.39; N, 5.42.

CH-Trp
The synthesis was analogous to the synthesis of CH-Gly, using 5.38 g (21.1 mmol) of L-tryptophan methyl ester hydrochloride and 2.03 g (7.05 mmol) of (1s,3s,5s)-cyclohexane-1,3,5-tricarbonyl
trichloride, yielding 0.95 g (1.22 mmol, 17%) of CH-Trp as a white powder. Decomposition above 225 °C. 1H-NMR (400 MHz, DMSO-d6) δ 12.56 (s, 3H), 10.91 (s, 3H), 8.06 (s, 3H), 7.50 (s, 3H), 7.31 (s, 3H), 7.10 (s, 3H), 7.03 (s, 3H), 6.95 (s, 3H), 4.42 (s, 3H), 3.13 (s, 3H), 2.99 (s, 3H), 2.23 (s, 3H), 1.59 (s, 3H), 1.29 (s, 3H). 13C-APT NMR (101 MHz, DMSO-d6) δ 126.51, 123.97, 121.41, 121.23, 114.48, 55.91, 45.22, 42.88, 42.67, 42.34, 42.12. HRMS (ESI) m/z: [M + Na]+ Calcd for C42H42N6O9Na 797.2906; Found 797.2896. Anal. Calcd for C36H39N3O9: C, 65.11; H, 5.46; N, 10.85. Found: C, 54.46; H, 4.83; N, 9.02.

CH-Abu
The synthesis was analogous to the synthesis of CH-Gly, using 4.79 g (31.18 mmol) of abutaric acid methyl ester hydrochloride and 2.28 g (8.47 mmol) of (1s,3s,5s)-cyclohexane-1,3,5-tricarbonyl trichloride, yielding 2.84 g of CH-Abu as a white powder. Melting point 229-231 °C. 1H-NMR (400 MHz, DMSO-d6) δ (ppm) 12.50 (s, 3H), 8.04 (d, J = 7.9 Hz, 3H), 4.09 (td, J = 8.3, 5.1 Hz, 3H), 2.33 (s, 3H), 1.79 – 1.66 (m, 6H), 1.58 – 1.47 (m, 3H), 1.41 (q, J = 12.6 Hz, 3H), 0.86 (t, J = 7.4 Hz, 9H). 13C-APT NMR (75 MHz, DMSO-d6) δ (ppm) 175.0, 174.1, 53.5, 42.6, 31.9, 24.8, 10.9. FTMS (ESI-TOF) m/z: [M + H]+ Calcd for C21H34N3O9 472.2290; Found 472.2286. Anal. Calcd for C21H33N3O9: C, 47.27; H, 6.45; N, 6.89. Found: C, 46.63; H, 6.39; N, 6.73; Na, 5.05.

Single crystals of CH-Abu were grown from a saturated solution in 2 M NaCl (aq) by slow evaporation.

CH-Nva
The synthesis was analogous to the synthesis of CH-Gly, using 4.95 g (29.53 mmol) of L-norvaline methyl ester hydrochloride and 2.18g (8.03 mmol) of (1s,3s,5s)-cyclohexane-1,3,5-tricarbonyl trichloride, yielding 2.721 g (5.30 mmol, 66%) of CH-Nva as a white powder. Melting point 229-231 °C. 1H-NMR (400 MHz, DMSO-d6) δ (ppm) 12.43 (s, 3H), 7.97 (d, J = 7.9 Hz, 3H), 4.14 (td, J = 7.9, 5.2 Hz, 3H), 2.28 (t, J = 12.5 Hz, 3H), 1.72 – 1.58 (m, 6H), 1.58 – 1.47 (m, 3H), 1.39 (q, J = 12.5 Hz, 3H), 1.26 (tt, J = 13.7, 7.3 Hz, 6H), 0.84 (t, J = 7.3 Hz, 9H). 13C-APT NMR (101 MHz, DMSO-d6) δ (ppm) 174.8, 174.3, 51.7, 42.7, 40.0, 33.5, 31.8, 19.2, 13.9. FTMS (ESI-TOF) m/z: [M + H]+ Calcd for C24H39N3O9 514.2759; Found 514.2752. Anal. Calcd for C24H39N3O9: C, 56.13; H, 7.65; N, 8.18. Found: C, 52.51; H, 7.64; N, 7.55.

CH-Nle
The synthesis was analogous to the synthesis of CH-Gly, using 4.72 g (26 mmol) of L-norleucine methyl ester hydrochloride and 2.30 g (8.47 mmol) of (1s,3s,5s)-cyclohexane-1,3,5-tricarbonyl trichloride, yielding 2.22 g (4.00 mmol, 63%) of CH-Nle as a white powder. Melting point 220-221 °C. 1H-NMR (400 MHz, DMSO-d6) δ (ppm) 12.44 (s, 3H), 7.97 (d, J = 8.0 Hz, 3H), 4.18 – 4.07 (m, 3H), 2.34 – 2.22 (m, 3H), 1.73 – 1.60 (m, 6H), 1.55 (dq, J = 10.3, 6.1, 5.0 Hz, 3H), 1.40 (q, J = 12.6 Hz, 3H), 1.24 (t, J = 6.6, 4.2 Hz, 12H), 0.83 (h, J = 3.7, 3.1 Hz, 9H). 13C-APT NMR (101 MHz, DMSO-d6) δ (ppm) 174.8, 174.3, 51.9, 42.7, 31.8, 31.1, 28.0, 22.1, 14.2. FTMS (ESI-TOF) m/z: [M + H]+ Calcd for C27H46N3O9 556.3229; Found 556.3216. Anal. Calcd for C27H45N3O9: C, 58.36; H, 8.16; N, 7.56. Found: C, 55.35; H, 7.78; N, 7.17.

CH-Tyr
The synthesis was analogous to the synthesis of CH-Gly, using 14.4 g (62 mmol) of L-tyrosine methyl ester hydrochloride and 5.0 g (18.4 mmol) of (1s,3s,5s)-cyclohexane-1,3,5-tricarbonyl trichloride, yielding 4.0 g (5.7 mmol, 25%) of CH-Tyr as a white powder. ^1H NMR (400 MHz, DMSO-d6) δ 12.56 (s, 1H), 9.21 (s, 1H), 8.00 (d, J = 8.1 Hz, 1H), 6.99 (d, J = 8.2 Hz, 2H), 6.64 (d, J = 8.2 Hz, 2H), 4.28 (td, J = 8.1, 4.9 Hz, 1H), 2.90 (dd, J = 13.9, 4.9 Hz, 1H), 2.73 (dd, J = 13.9, 4.9 Hz, 1H), 2.21 (t, J = 12.6 Hz, 1H), 1.62 – 1.54 (d, J = 12.6 Hz 1H), 1.27 (q, J = 12.6 Hz, 1H). ^13C APT NMR (101 MHz, DMSO-d6) δ 177.3, 176.3, 159.0, 133.0, 130.8, 118.1, 56.8, 45.2, 39.0, 34.3. HRMS: 706.27783 (M+H:
706.72500). Elemental analysis, Calc C: 61.3H: 5.57 N: 5.95 Found C: 54.82 H: 5.43 N: 5.24

Single crystals of CH-Tyr were grown from a phosphate buffer (pH 5.7) by slow evaporation. For elemental analysis of the crystals, the crystals were filtered and washed 3 times with cold demineralized water to remove any residual phosphate buffer.

**Elemental Analysis**

The elemental analysis of the compounds is not as expected due to incomplete removal of NaCl during purification, however the C/N and C/H ratios are as expected showing that the only additional component is inorganic. The sodium content for CH-Abu was determined to be 5.05% by ICP- AA (Perkin Elmer). FTIR spectroscopy shows that the carboxylic acid groups are partially in the carboxylate form, consistent with salt formation. Hence the significant, but minor, deviations between expected and found elemental analyses is due to residual sodium content (typically one equivalent).

**BTA-Val**

3 g of benzene-1,3,5-tricarbonyl trichloride (11 mmol) was dissolved in 15 mL dichloromethane and added to a solution of 6.2 g L-Valine Methyl Ester Hydrochloride (37 mmol) and 6 mL triethylamine in 50 mL dichloromethane. The mixture was stirred overnight and the dichloromethane was removed in vacuo, the resulting white precipitate was washed with ethanol. The dried solids where dissolved in 75 mL 2 M NaOH and stirred overnight. The basic solution was acidified with concentrated HCl and the white precipitate was filtered and washed with water. The white solid was lyophilized yielding 1.4 g of BTA-Val (2.7 mmol, 25%). ¹H NMR (600 MHz, DMSO-d₆) δ 12.69 (s, 1H), 8.79 (d, J = 8.1 Hz, 1H), 8.41 (s, 1H), 4.33 (t, J = 7.5 Hz, 1H), 2.27 – 2.17 (m, J = 6.9, 6.5 Hz, 1H), 1.00 (t, J = 6.9 Hz, 6H). ¹³C NMR (151 MHz, DMSO-d₆) δ 173.49, 166.86, 134.95, 130.06, 58.99, 40.52, 40.40, 40.38, 40.26, 40.12, 39.98, 39.84, 39.70, 39.56, 30.01, 19.81, 19.29. Exact Mass: Calculated [M+H]+ 508.22896 Found: 508.22789 Elemental analysis: Calc C: 56.80 H: 6.55 N: 8.28 Found C: 55.79 H: 6.37 N: 8.04 Melting point degradation > 230 °C.

**BTA-Met**

BTA-Met was prepared as for BTA-Val by replacing the L-valine methyl ester hydrochloride by 7.4 g of L-methionine methyl ester hydrochloride (37 mmol). Yielding 1.43 g of BTA-Met (2.4 mmol, 22 %). ¹H NMR (600 MHz, DMSO-d₆) δ 12.73 (s, 1H), 9.11 (d, J = 7.6 Hz, 1H), 8.58 (s, 1H), 4.56 (ddd, J = 9.8, 7.6, 4.5 Hz, 1H), 2.65 (ddd, J = 13.5, 8.5, 5.2 Hz, 1H), 2.58 (dt, J = 13.3, 7.9 Hz, 1H), 2.07 (m, 5H). ¹³C NMR (151 MHz, DMSO-d₆) δ 173.8, 166.4, 134.8, 129.9, 52.3, 30.59, 30.50, 15.00. Exact Mass: Calculated [M+H]+ 604.14517 Found: 604.14374 Elemental analysis: Calc C: 56.80 H: 6.55 N: 8.28 Found C: 42.79 H: 5.25 N: 6.16 S: 13.68 Melting point degradation > 150 °C.

**Preparation of Gold colloid**

HAuCl₄ · 3 H₂O (58 mg) was added to a 1 L flask containing 500 mL water (UPLC grade filtered through a membrane filter) and was brought to boil while stirring rapidly. 7.5 mL of 1% aqueous sodium citrate was added. The solution was refluxed for 10 min during which the solution turned red and was allowed to cool to room temperature (20 °C). The colloidal solution was filtered using a 0.8 µm membrane filter and stored in a brown bottle wrapped in aluminium foil. UV-Vis absorption maximum at 520 nm. Particle size 5.7 nm ± 0.89 nm 100 particles sampled and 27.06 nm ± 5 nm 52 particles sampled by TEM.

**Preparation of Silver colloid:**

AgNO₃ (90 mg) was added to a 1 L flask containing 500 mL water (UPLC grade filtered through a membrane filter) and was brought to boil while stirring rapidly forming a grey solution. 10 mL of 1% aqueous sodium citrate was added. The solution was refluxed for 30 min during which the
solution turned yellow and was allowed to cool to room temperature (20 °C). The colloidal solution was filtered using a 0.8 µm membrane filter and stored in a brown bottle wrapped in aluminium foil. UV-Vis absorption maximum at 196 and 470 nm. Particle size 10.77 nm ± 1.14 nm 100 particles sampled and 134.3 nm ± 8 nm 3 particles sampled by TEM.

**Bibliography**

Appendix C

Appendix C
Structures of compounds

CH-Nva

CH-Nle

CH-Met

BTA-Val

BTA-Met

CH-Trp
Summary

Low molecular weight hydrogelators are a class of compounds that form supramolecular structures anisotropically, a so-called gel fibre network, and in so doing they ‘gel’ water. Gelation of water with small molecules (hydrogelators) sees continued interest ranging? from biological and medical applications to soft robotics. The design of hydrogelators is largely hit and miss and draws on the serendipitous balancing of multiple intra- and intermolecular interactions. Understanding why some compounds do and others do not form hydrogels is key to designing new gelators and to modifying existing gelators to have specific functions. A delicate interplay of anisotropic hydrophobic/hydrophilic, π-π stacking, ionic and hydrogen bond interactions determine the strength of hydrogelators and are considered key factors in their design. Achieving specific properties by design, however, is still well beyond the state of the art. Rational design of gelators requires understanding of both the interactions involved at multiple hierarchical levels and the actual dynamic processes during network formation. This thesis focusses on the understanding of what drives the class of C₃ symmetric cyclohexane triamides and benzene triamides to engage in solvent (water) gelation.

Chapter 2 focusses on the synthesis of a series of structural variants of cyclohexane triamide compounds and their analysis under various conditions. A couple of new gelator compounds were found by systemic variation of the side groups. The gelators show remarkable changes in behaviour, melting points and strength. Solvent deuteration and electrolytic strength affect the strength of hydrogels formed profoundly. Gels formed by self-assembly through heating/cooling of solutions or by pH switching show up to a 30 °C increase in their melting temperatures in D₂O compared to H₂O. The unusually large solvent isotope effect on gel formation and thermal properties indicates that, in contrast to expectations, hydrogen bonding is not the primary determinant of gel strength but instead hydrophobic interactions between the gelator molecules and the terminal carboxylic acid units are of greater importance. This conclusion is supported by a similarly large effect of electrolytes on gel strength.

In Chapter 3 the focus is shifted to understanding the underlying principles of gel formation. We report, through a combination of label free techniques, that the correspondence between crystal structures and molecular packing in the gel fibres is coincidental, and? that direct spectroscopic analysis using polarisation dependent Raman microspectroscopy can confirm the molecular packing state. Through a combination of techniques we probe the time dependence of hydrogelation by CH-Abu and CH-Leu, from the micron scale using darkfield microscopy to the nanoscale using cryo-TEM and SAXS. The combination of techniques used gives us direct evidence for a gelation mechanism and a total overview of all the processes that can happen during the switch from solution to gel (Figure 1). In the present system the key observation is that precipitation precedes the growth of the stable gel fibre bundles, which is counter to expectations for a kinetically controlled process. These data indicate that the anisotropy in the strength of intermolecular interactions can overcome precipitation and that gel formation can proceed through several stages before the final stable form is obtained.

Chapter 4 focusses on a gelator based on tyrosine (CH-Tyr). Adding functionality to a gelator can potentially introduce addressable/responsive properties in the gel state, but is still a major challenge. Gelation is achieved by balancing hydrophobic, hydrophilic and aromatic interactions. The addition of, for example, an aromatic fluorophore, can interfere with these interactions and potentially prevent the compound from gel formation. CH-Tyr forms weak gels on its own and has spectroscopic properties, i.e. UV-Vis absorption and fluorescence, which could be useful as a probe. The tyrosine unit retains its redox properties in solution and in gel form, which opens opportunities in crosslinking and metal binding. However, when mixed with the analogous gelators CH-Nle and
In Chapter 5 the gels studied in Chapters 2 and 3 acts as a scaffold for aggregated gold or silver nanoparticles to form a SERS active system. The rate of gel formation by a pH trigger matches well the rates of aggregation of Au or Ag colloids, allowing them to be trapped at the SERS-active point of the aggregation process. We show that the colloid-containing gels give SERS signals similar to the parent colloid but are stable over several months. Moreover, lyophilized gels can be stored as dry powders for subsequent use in the analysis of gases and dissolved analytes, by contact with either solutions or vapours. The present system shows how the combination of pH switchable low molecular weight gelators and pH induced colloid aggregation can be combined to make a highly stable and low-cost SERS platform for the detection of volatile organic compounds and microvolume analysis of solutions.

In Chapter 6 we use the knowledge gained in the field of hydrogelators to design and synthesise new hydrogelators. By combining the benzene triamide core and the amino acid groups from the

**Figure 1.** Model for all possible pathways during dissolving and gel triggers.
cyclohexane triamide hydrogels, new gelators were designed. We report two compounds that show promising gelation behaviour. Both BTA-Met and BTA-Val form opaque hydrogels. The gels from BTA-Met are unstable and precipitate within 24 h. By contrast, BTA-Val forms gels that are stable for weeks. From the polarisation dependence of the Raman spectrum of the formed fibres it is shown that the aromatic core is aligned as expected based on related crystal structures.

From the knowledge gained in the studies described in the chapters of this thesis we can now say that we have a clear understanding of what are the underlying driving forces for these compounds to form gels, and are able to design experiments to understand gelation on different length scales. The key structural motifs are the C$_3$-symmetry, combined with the amide stacking and peripheral acid groups. The properties of the compounds can be modified by changing the hydrophobicity of the amino acids. We are able to apply that knowledge in using them for applications such as SERS substrates and are able to combine this knowledge to design new gelators. However, rational design requires walking a tightrope of multiple interactions that are not necessarily orthogonal and the number of variables often exceeds the number of compounds in a given series. Hence, it will be a while before computer algorithms replace our chemical intuition in designing new hydrogelators.
**Samenvatting**

Hydrogelatoren met een laag moleculgewicht zijn een klasse van moleculen die anisotropisch supramoleculaire structuren vormen en tijdens dit proces water ‘gellen’. Het gelen van water met kleine moleculen (hydrogelatoren) krijgt steeds meer aandacht in biologische en medische toepassingen, zelfs tot aan zachte robotica. Het ontwerpen van hydrogelatoren is vaak een kwestie van *trial and error* en lukt alleen als onderzoekers (per ongeluk) de verschillende intra- en intermoleculaire interacties correct uitbalanceren. Om nieuwe gelatoren te ontwerpen of bestaande gelatoren nieuwe functionaliteiten te geven, is het belangrijk dat we begrijpen waarom sommige stoffen wel of geen hydrogels vormen. De sterkte van hydrogelatoren wordt bepaald door een delicaat samenspel van anisotropische hydrofobe/hydrofiele interacties, π-π stacking, ionische verbindingen en waterstoffbruggen. Hoewel we denken dat deze factoren de belangrijkste elementen zijn bij het ontwerpen van kleine moleculaire hydrogelatoren, is nog lang niet mogelijk om deze specifieke interacties te ontwerpen. Om dit voor elkaar te krijgen moeten we zowel de interacties tussen de verschillende levels van het netwerk als de dynamische processen tijdens de formatie van het netwerk in kaart brengen. In deze thesis proberen we te begrijpen wat de klasse van C₃-symmetrische cyclohexaantriamides en benzeentriamides tot geleren drijft.

**Hoofdstuk 2** focust op de synthese van een serie van structurele varianten op cyclohexaantriamide en de analyse van deze structuren onder verschillende condities. Systematische variatie van zijgroepen resulteerde in enkele nieuwe gelatoren. De gelatoren vertonen bijzondere veranderingen in gedrag, smeltpunten en sterkte. Deuterering van het oplosmiddel en de sterkte van het elektrolyt beïnvloedt de sterkte van de hydrogels. De hydrogels die gevormd zijn door verhitting en koeling of een pH sprong hebben in D₂O een smeltpunt dat wel dertig graden hoger ligt dan dat van de gels in H₂O. Dit onwaarschijnlijk grote isotoopeffect op de gelformatie en temperatuureigenschappen laat zien dat, in tegenstelling tot wat we dachten, waterstoffbindingen niet de bepalende factor zijn voor de sterkte van de gel. In plaats daarvan lijken de hydrofobe interacties tussen de gelator en de terminale carbonzuurgroepen van groter belang. Deze conclusie wordt onderbouwd door een vergelijkbaar groot effect van elektrolyten op de sterkte van een gel.

In **Hoofdstuk 3** verschuift de focus naar het begrijpen van de onderliggende principes van gelformatie. We rapporteren, door een combinatie van labelvrije technieken, dat de overeenkomst tussen de kristalstructuur van de hydrogelator en de moleculaire pakking in de vezels van de gel toevallig is. Directe spectroscopische analyse met gepolariseerde Raman microspectroscopie kan wel de moleculaire pakking bevestigen. Door een combinatie van technieken hebben we de tijdsafhankelijkheid van hydrogelering van CH-Abu en CH-Leu onderzocht, op de micron-schaal met donkerveldmicroscopie en op de nanoschaal met cryo-TEM en SAXS. De combinatie van technieken gaf ons direct bewijs voor een geleringsmechanisme en een totaaloeverzicht van alle processen die plaats kunnen vinden tijdens de overgang van oplossing naar gel (Figuur 1). In het huidige systeem is de belangrijkste observatie dat en neerslag vormt voordat een stabiele gelvezel groeit. Dit weerspreekt het idee dat gelering een kinetisch gecontroleerd proces zou zijn. De data laat zien dat de anisotropie in de sterkte van de intermoleculaire interacties de precipitatie kan overkomen en dat de gelformatie verschillende stadia succesvol kan doorlopen om uiteindelijk een stabiele netwerk te vormen.

**Hoofdstuk 4** kijkt naar een gelator op basis van tyrosine (CH-Tyr). Het toevoegen van dit soort functionaliteiten aan een gelator, kan er mogelijk voor zorgen dat de gel responsief wordt voor externe stimuli, maar het blijkt nog lastig voor elkaar te krijgen. Om de gel te laten gelen wordt een balans gezocht tussen de hydrofobe, hydrofiele en aromatische interacties. Het toevoegen van bijvoorbeeld een aromatisch molecuul, zoals een fluorofoor, kan deze interacties verstoren en mogelijk zelfs voorkomen dat een gel vormt. CH-Tyr vormt zwakke gels en heeft spectroscopische
Appendix E

**Crystallization**

**Precipitation**

**Trigger**

**Dissolve**

**Time**

**Gelation**

**Figuur 1.** Model van alle mogelijke paden die een gelator kan nemen na een trigger van gel formatie.

eigenschappen die mogelijk interessant zijn als probe, namelijk UV-Vis absorptie en fluorescencie. De tyrosinegroep behoudt zijn redoxeigenschappen in oplossing en in de gel, wat kansen biedt voor eventuele crosslinking en binding aan metalen. Echter wanneer CH-Tyr wordt gemixt met de analoge gelatoren CH-Nle en CH-Abu, zien we in de spectroscopische data dat de gelatoren geen gemengde vezels vormen, maar orthogonaal assembleren.

In Hoofdstuk 5 gebruiken we de gels uit hoofdstukken 2 en 3 als een matrix voor geaggregeerde gouden en zilveren nanodeeltjes om een SERS-actief systeem te vormen. De pH-geactiveerde zelfassemblage van de hydrogels gebeurt op een snelheid die overeenkomt met de aggregatiesnelheid van de goud- of zilvercolloïden, waardoor ze worden gevangen op het punt in het aggregatieproces dat ze SERS-actief zijn. We laten zien dat de colloidbevattende gels vergelijkbare SERS-signalen geven als de originele colloïden, maar dat ze tot wel maanden stabiel blijven. Bovendien kunnen gevriesdroogde gels bewaard worden als droge poeders en verder gebruikt worden in de analyse van gassen en oplossingen. Het huidige systeem laat zien dat de combinatie van pH-schakelbare gelatoren met een laag molecuulgewicht en pH-geïnduceerde colloidaggregaten een erg stabiel en goedkoop SERS-platform kan vormen voor de detectie van vluchtige organische moleculen en mi-
crovolumeanalyse van oplossingen.

In Hoofdstuk 6 gebruiken we de kennis die in het veld van hydrogelatoren is verzameld om nieuwe hydrogelatoren te ontwerpen en te synthetiseren. We combineren hiervoor de benzeentriamide kern en de aminozuurgroepen van de cyclohexaantriamide hydrogels om nieuwe hydrogelatoren te maken. We rapporteren twee moleculen die veelbelovende gelerende eigenschappen vertonen. Zowel BTA-Met als BTA-Val vormen ondoorzichtige hydrogels. De gels van BTA-Met zijn onstabiel en slaan binnen 24 uur neer. BTA-Val vormt daarentegen een gel die stabiel is voor meerdere weken. De polarisatiedichtheid van de Raman spectra van de gevormde vezels laat zien dat de aromatische kernen, zoals verwacht, op één lijn liggen.

Met de kennis die we hebben opgedaan in de hoofdstukken van deze thesis kunnen we zeggen dat we een duidelijk begrip hebben gekregen over wat de onderliggende drijfveren zijn waardoor deze moleculen gels vormen, en dat we in staat zijn om experimenten te ontwerpen waarmee we gelering op verschillende lenteschalen kunnen begrijpen. De belangrijkste structurele motieven zijn de C3-symmetrie, gecombineerd met de amide-H-bruggen en perifere zuurgroepen. De eigenschappen van de verbindingen kunnen worden gemedificeerd door de hydrofobiciteit van de aminozuren te veranderen. We zijn in staat deze kennis te gebruiken voor toepassingen zoals SERS substraten en het ontwerpen van nieuwe hydrogelatoren. Echter blijft het rationeel ontwerpen van dit soort moleculen balanceren op een slap koord. De verschillende interacties zijn niet altijd onafhankelijk van elkaar te beïnvloeden en het aantal variabelen is vaak groter dan het aantal moleculen in de reactie. Daarom zal het nog een tijd duren voor computeralgoritmes onze chemische intuïtie kunnen vervangen als het gaat om het ontwerpen van nieuwe hydrogelatoren.
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