**Salt-induced Hydrogelation of Functionalised-Dipeptides at high pH**

Lin Chen, a Guillaume Pont, a Kyle Morris, b Gudrun Lotze, c Adam Squires, c and Dave J. Adams a,*

a Department of Chemistry, University of Liverpool, Crown Street, Liverpool, L69 7ZD, U.K.
Email: d.j.adams@liverpool.ac.uk

b Ecole Nationale Superieure de Clermont Ferrand, Ensemble Scientifique des Cézeaux, 24 Avenue des Landais - BP 10187, 63174 AUBIERE Cedex - FRANCE

c Department of Chemistry and Biochemistry, School of Life Sciences, University of Sussex, Falmer, BN1 9QG, U.K.
d Department of Chemistry, University of Reading, Whiteknights, Reading, RG6 6AD

**SUPPORTING INFORMATION**

**Experimental Details**

**Materials.** All dipeptides were prepared as described elsewhere. 1 The final dipeptides used had a purity > 98% by NMR. All other materials were purchased from Sigma-Aldrich and used as received. De-ionised water was used throughout.

**Preparation of Dipeptide Solutions.** A 40 mL dipeptide stock solution at a concentration of 0.5wt% was prepared by adding 200 mg dipeptide into water. The pH was adjusted to 11.7 with the addition of 480 uL NaOH (1.0 M) solution into the stock solution to form a transparent, viscous solution after mild stirring for 2 hours.

**Preparation of Gels via Salt Addition.** Solutions of the salts were prepared at a concentration of 200 mg/mL, except for MgSO₄ due to the solubility limitation, where a concentration of 6 mg/ml was used. Aliquots of these salt solutions were added into 2 mL of the dipeptide stock solution and left to stand overnight. Transparent gels were formed. The final salt concentration was 0.05 M.

**Characterisation**

**Small Angle X-Ray Scattering.** SAXS experiments were carried out on beamline I711 at the MAX-lab synchrotron facility, Sweden. An x-ray beam of wavelength 1.2 Å was used, with a camera length of 1.6 m. The sample was sheared in a custom-built polycarbonate concentric cylinder couette device, at a shear rate of 400 s⁻¹.
**pH Measurement and pKa determination**

A FC200 pH probe (HANNA instruments) with a (6 mm X 10 mm) conical tip was employed for the pH measurements. The stated accuracy of the pH measurements is ± 0.1. The pH changes during the gelation process were recorded every 1 min for 24 h. All measurements were conducted at room temperature. The pKa values of dipeptide derivative (0.5 wt %) solution were determined by titration via the addition of aliquots of a 0.1 M HCl solution. pH values were also recorded every 5 min after equilibrium during the titration process. To prevent the formation of gel during the titration process, the solutions were gently stirred, thus keeping the solution liquid during the whole “titration” process.

**Rheology and Viscosity Measurements.**

All rheological experiments were performed using an Anton Paar Physica MCR101 rheometer. A cup and vane system was used to perform the frequency sweep. All gels were formed directly in 7 mL Sterilin cups and left overnight (at least 20 hours) at room temperature to gel before the measurements. Frequency scans were performed from 1 rad/s to 100 rad/s under a strain of 0.5 %. The shear moduli (storage modulus G’ and loss modulus G”) were measured at a frequency of 10 rad/s. The strain amplitude measurements were also performed within the linear viscoelastic region, where the storage modulus and loss modulus are independent of the strain amplitude. The cone and plate system was used to measure the viscosities of all high pH dipeptide solutions (0.5 wt %). The gap distance between two plates used was 0.05 mm. 2 mL dipeptide solutions were transferred onto the plate for measurement. The viscosity of each solution was recorded under the rotation shear rate varying from 1 to 100 s⁻¹. All the experiments were conducted at 20 °C.

**Cryo-TEM.** Sample preparation was carried out using a CryoPlunge 3 unit (Gatan Instruments) employing a double blot technique. 3 µL of sample was pipetted onto a plasma etched (15 s) 400 mesh holey carbon grid (Agar Scientific) held in the plunge chamber at approximately 90 % humidity. The samples were blotted, from both sides for 0.5, 0.8 or 1.0 s dependent on sample viscosity. The samples were then plunged into liquid ethane at a temperature of -170 °C. The grids were blotted to remove excess ethane then transferred under liquid nitrogen to the cryo TEM specimen holder (Gatan 626 cryo holder) at -170 °C. Samples were examined using a Jeol 2100 TEM operated at 200 kV and imaged using a Gatan Ultrascan 4000 camera; images captured using DigitalMicrograph software (Gatan).
Figure S1. Viscosity data for solutions of naphthelane-dipeptides at pH 11.7 and a concentration of 0.5 wt%. (▼) Dipeptide 1; (●) Dipeptide 2; (+) Dipeptide 3; (▲) Dipeptide 4; (○) Dipeptide 5.

Figure S2. Cryo-TEM of solution of 4 at pH 11. Scale bar represents 200 nm.
Figure S3. SAXS data for 1, 2, and 3 (top to bottom) with scale bars.
**Figure S4.** Storage moduli for gels formed using dipeptide 1 and different salts (salt concentration 0.05 M).

**Figure S5.** Tan δ ($G'' / G'$) for gels formed using 1 at pH 11.7 and different quantities of added calcium nitrate.
**Figure S6.** pH titration of dipeptide 1 using HCl from pH 11.7. An apparent pKa of 6.0 was extracted from this data.

**Figure S7.** Effect of pH on the G’ of gels formed using calcium nitrate at different pH. Without added calcium nitrate, no gels were formed (G’ < G’’ and G’ < 1 Pa).
**Figure S8.** G’ of hydrogels formed by pH switch with GdL only, GdL and calcium nitrate, or calcium nitrate alone. All gels with GdL were at a final pH of 3.4; the gel with no GdL was at pH 11.7.

**References**