Fmoc-diphenylalanine Hydrogels: Understanding the Variability in Reported Mechanical Properties

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SUPPORTING INFORMATION

Ref	G'	G''	Gelation method	Geometry
2a	~1000 Pa (2mg/mL) ~10000 Pa (5mg/mL) ~30000 Pa (10mg/mL)	Not obtained	FmocFF (2 mg/mL, 5 mg/mL, 10 mg/mL) Gel formed from diluting FmocFF/HFIP stock solution 100 mg/mL and 25 mg/mL to certain concentration by water	Cone-plate 60mm/1^{o} and 40mm/4^{o} AR 2000 (TA instrument, UK)
2b	A weak flowing gel is formed between 0.22 and 1.07 wt%, pH < 8		FmocFF Gels can be formed 0.22-2.14 wt%, by adding HCl.	No rheology measurements
2c	The gels could be stable, unstable , firm and soft depending on the buffers used.		FmocFF (0.5 wt-1.0 wt%) gels formed by diluting FmocFF/DMSO 100 mg/mL with H_2O . At 20^{o}C	No rheology measurements
2d	~10000 Pa (neutral pH)	~1000 Pa (neutral pH)	FmocFF (20 mM). At 25 ^{o}C by adding HCl to the initial pH 9 stock solutions	Cone-Plate 40mm/4^{o} Bohlin C-CVO
2e	~21.2 kPa(pH 7.5)	G'/G''= 4.8 (pH 7.5)	FmocFF (20 mM), Gels were formed in standard culture medium (DMEM) incubated for 1 h at 37 ^{o}C in a humidified atmosphere with 5% CO_2	Cone-Plate 40mm/4^{o} Bohlin C-CVO
2f	~10000 Pa	Not obtained	FmocFF (5 mg/mL) Gels formed from diluting FmocFF/DMSO 100mg/mL or 25 mg/mL stock solution to 5 mg/mL by water. Room Temperature	Parallel-plate (gap 0.6mm) and 210 uL sample used for measurements. AR G2 (TA Instrument, UK)
2g	~1.9 kPa(pH 7.2)	Not obtained (pH 7.2)	FmocFF (10-20 mM) pH switch by adding HCl. Gelation at 20 or 37^{o}C with and without DMEM.	Cone-Plate 20mm/2^{o} Bohlin C-VOR
2h	A transparent gel shown in figure		FmocFF (0.5-5 mg/mL) Gels are formed by diluting FmocFFOH/DMSO 100mg/mL stock solution by water containing 0.2mM HPTS .	No rheology measurement
2i	~ 1 Pa(at pH 9.0) ~0.01 Pa(at pH 7.6)	~0.8 Pa (at pH 9.0) ~0.05 Pa (at pH 7.6)	FmocFF (10 mM) pH switch by adding HCl to pH 10.5 stock solution. Heating up to 75-80 ^{o}C for 1 min and then incubated at 4 ^{o}C for gelation	Parallel plate with a diameter 40mm. Bohlin C-CVO
2j	Transparent Gel		FmocFF 2 mg/mL Diluting 100 mg/mL FmoFFOH/HFIP stock solution with deionised water.	No rheology measurements
2k	Not determined . Gels at pH 7-8 (opaque at low pH)		FmocFF ($3.654X10^{-5}$mol in 2.5 mL), GdL triggered	Vane-Cup Rheometric Scientific ARES

				Vane-Cup
2l	No gel description.		FmocFF (10 mg/mL) Gels formed by diluting 25 mg/mL FmocFFOH/DMSO solution by water containing 0.01 M QDS and 1 mg/mL Enzyme (GOX or HRP)	No rheology measurements
2m	~2000 Pa (Low shear, pH 6) ~800 Pa (High shear, pH 6)	~200 Pa (Low shear, pH 6) Not obtained (high shear)	FmocFF (20 mM, 1 wt%). pH switch by adding HCl to pH 10.5 stock solution . Incubation at 37 ^{o}C for gelation	Parallel Plate 25mm in diameter, gap=100 mm Malvern UK
2n	~7500 Pa (pH 7.4) Gels are not stable at 37^{o}	G'/G''=7-12 (pH 7.4)	FmocFF (4 mg/mL) Gels formed from diluting 100 mg/mL FmocFF/DMSO solution by H_2O.	AR-1000 (TA,UK) Cone-plate 40mm in diameter, 52 um gap and 2^{o} cone

Table S1. Rheological data for FmocFF gels available from the literature. Reference refers to the reference number in the paper.

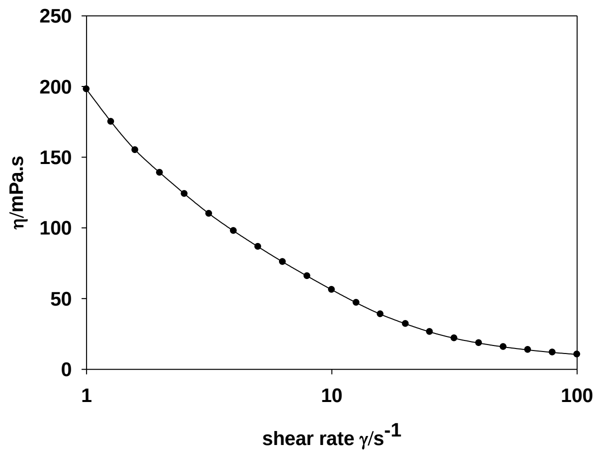

Figure S1. Viscosity data for FmocFF at pH 11.7 and a concentration of 5 mg/mL.

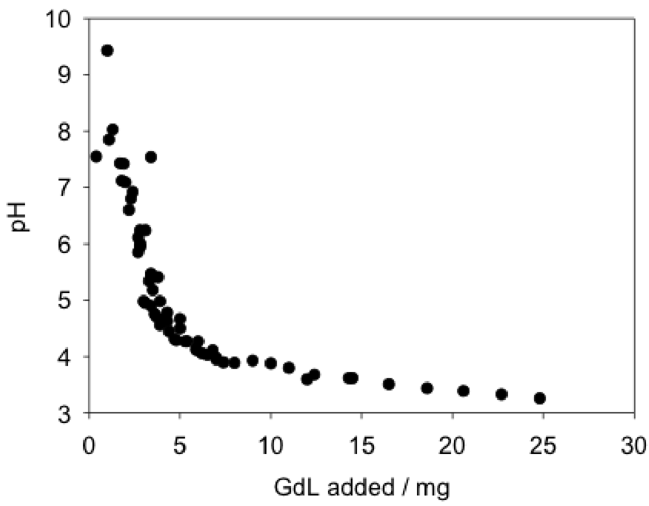

Figure S2. Final pH 24 hours after adding different quantities of GdL to a solution of FmocFF (0.5 wt%) at pH 11.

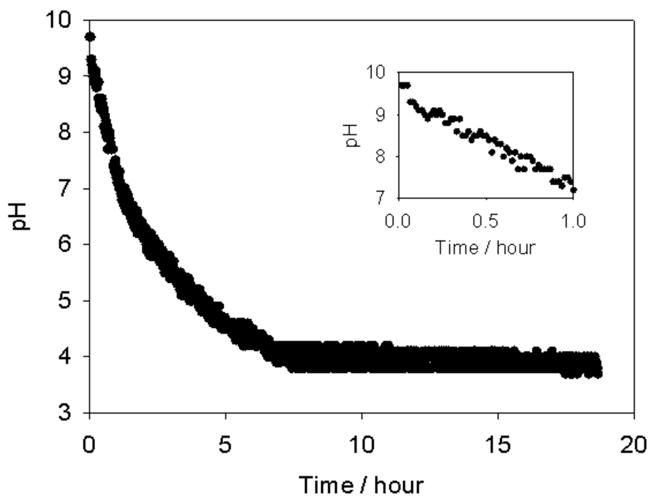

Figure S3. Change in pH with time on adding 8.2 mg GdL to a solution of FmocFF (0.5 wt%) at an initial pH of 10. Insert shows changes over the first hour.

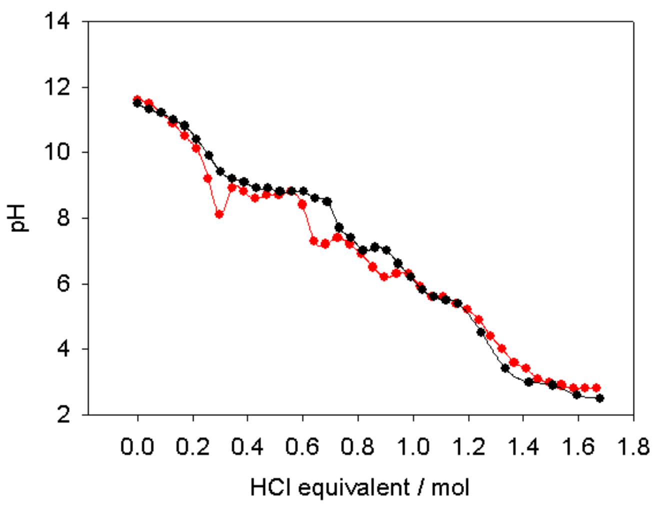

Figure S4. Titrations of FmocFF (0.5 wt%) solutions starting from a pH of approximately 11.7 using HCl. In-house synthesized (red data) and commercial FmocFF were titrated (black data). From this, an apparent p*K*a of 8.9 was extracted.

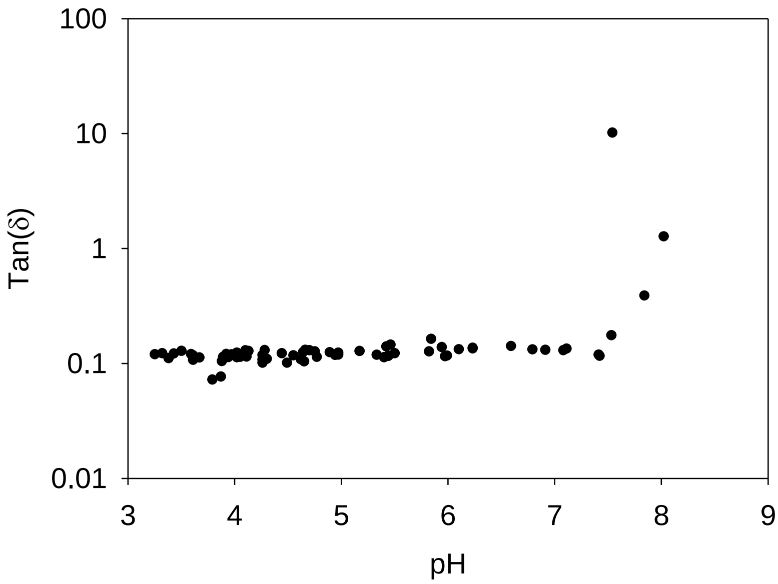

Figure S5. Tan δ for FmocFF gels prepared by the pH method using GdL. Gels were not formed above a pH of 8.

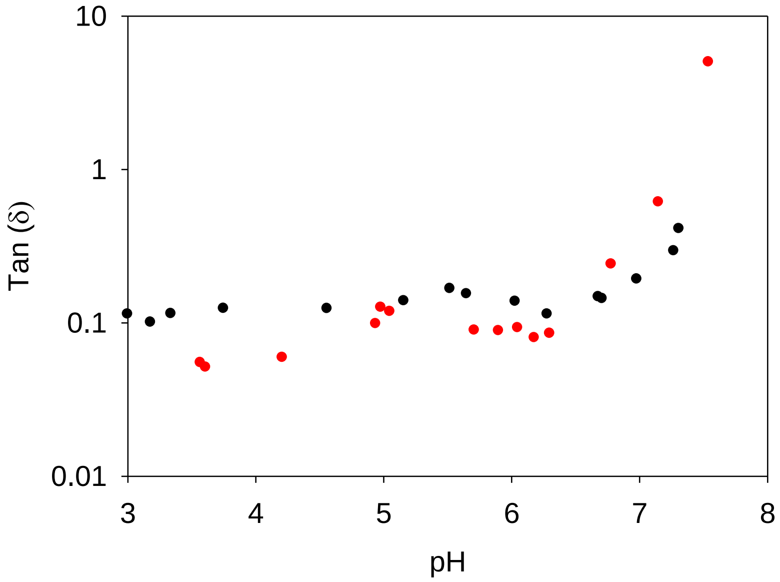

Figure S6. Tan δ for FmocFF gels prepared by the pH method using HCl (data for vortexed samples in red and without vortexing in black). Gels were not formed above a pH of 8.

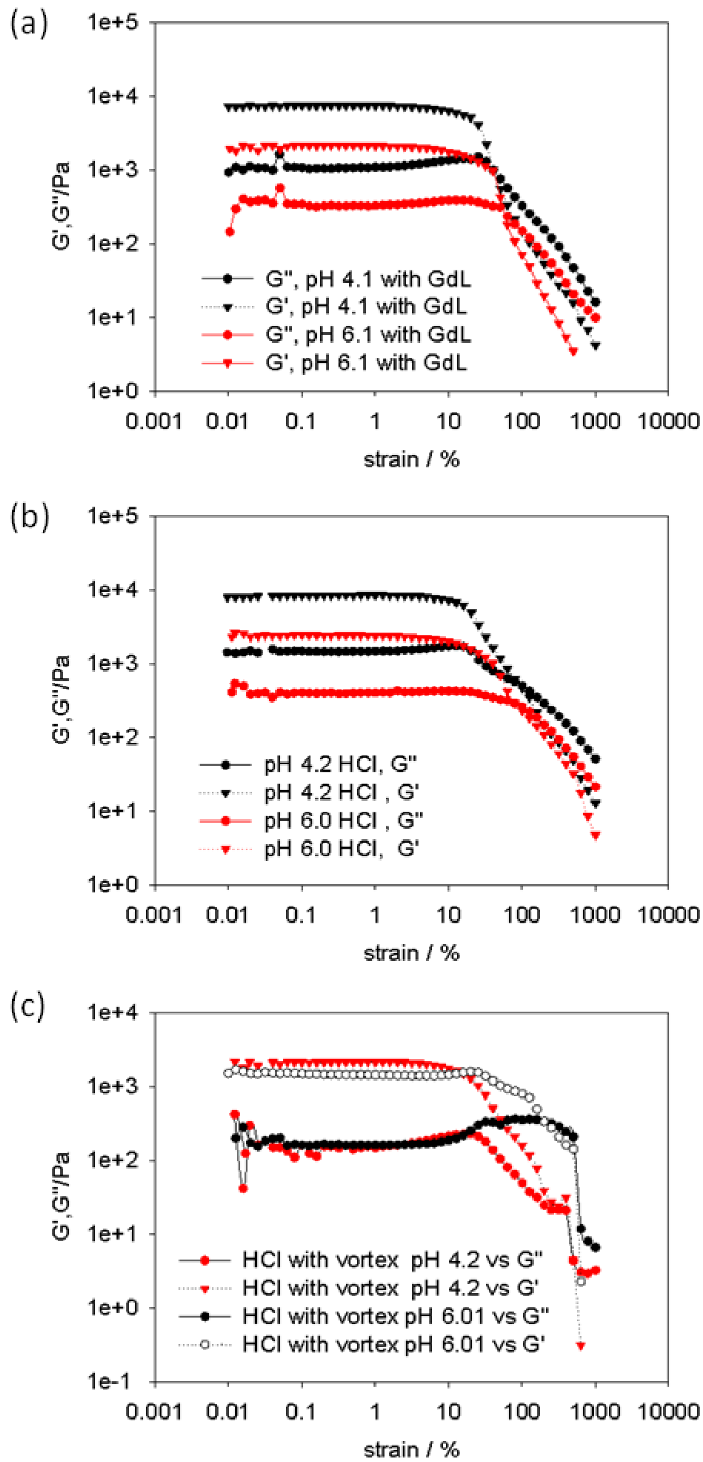

Figure S7. Strain sweeps for FmocFF gels (0.5 wt%) prepared using (a) GdL (red, pH 6.1 and black, pH 4.1) or (b) HCl (red, pH 6.0 and black, pH 4.2) or (c) HCl with vortex mixing(red, pH 6.0 and black, pH 4.2).

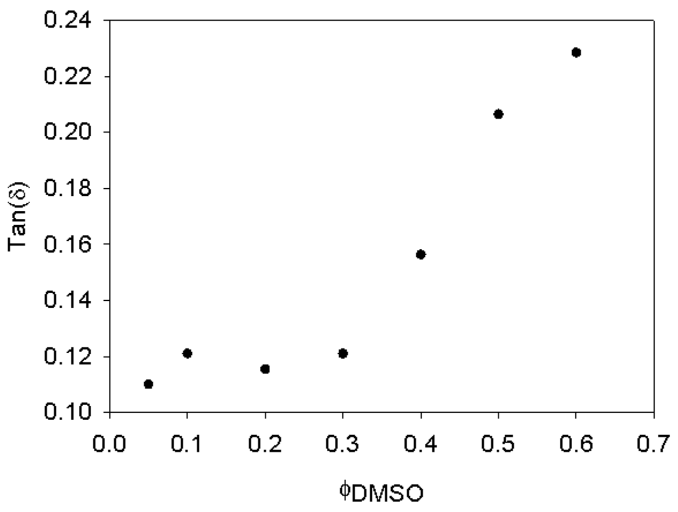

Figure S8. Tan δ for FmocFF gels prepared by the solvent switch method.

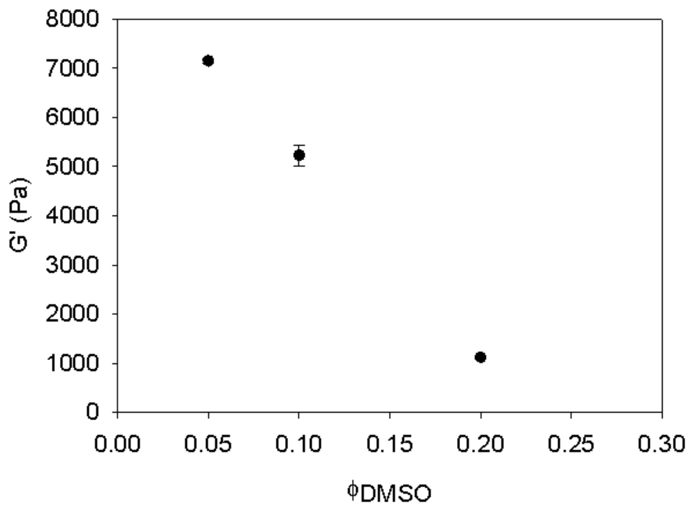

Figure S9. G′ as a function of ϕ_{DMSO} for FmocAG. FmocAG is more hydrophilic than FmocFF or FmocLG and gels are only formed at a ϕ_{DMSO} of 0.20 or below. FmocAG was prepared as described previously.[1]

Discussion regarding the pH of solvent-switch systems.

There are three possible explanations as to why the pH of the systems are around 4. First, the presence of DMSO may affect the pH reading. However, pH measurements in DMSO/water combinations in the absence of FmocFF are as expected (data not shown).

Second, there could be acidic impurities in the peptide. However, when preparing a solution at high pH, we do not need to add excess sodium hydroxide above that expected from the molar concentration of the peptide, implying that this is not the case. During the titration, 25mg FmocFFOH (0.0468mmol) was used and dissolved in 5mL H_2O, followed by adding 0.6 mL NaOH (0.1M), which is 0.06 mmol NaOH, to the solution to result in a clear solution. Equal moles of NaOH will be used to deprotonate the FmocFFOH to $FmocFFO^-$. The excess of NaOH will contribute to the final pH of the solution, (excess is 0.0132 mmol NaOH in 5.6 mL total solution). The final pH should therefore be 11.4 by calculation. This is close to the experimental value of 11.7, within reasonable experimental errors. Hence, there is no acidic impurities in the FmocFF that are contributing to the fact that the solvent switch systems are at pH 4.

Hence, we interpret the lower than anticipated pH to the fact the FmocFF is a weak acid. Despite the lack of acidic imprities, the final pH of gels formed by a solvent switch method at a constant ϕ_{DMSO} of 0.05 is affected by the concentration of FmocFF used, Fig. S10.

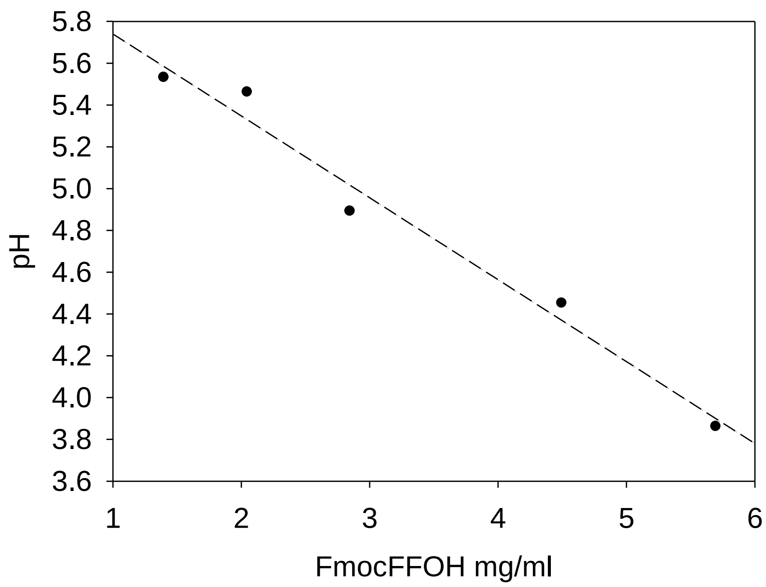

Figure S10. Final pH of gels formed at ϕ_{DMSO} of 0.05 depends on the concentration of FmocFF. The line is a guide to the eye.

References

1. D.J. Adams, L.M. Mullen, M. Berta L. Chen and W.J. Frith, *Soft Matter*, **2010**, *6*, 1971-1980.