Electronic Supplementary Information

Multicomponent Hydrogels from Enantiomeric amino acid derivatives: Helical Nanofibers, Handedness and Self-Sorting

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Preparation of hydrogel.

In a closed glass vial (inner diameter 1.1 cm, height 4.5 cm) equimolar mixture of Fmoc-(L/D)Glu and (L/D)Lysine/Arginine/Ornithine and the required amount of water were taken. The mixture was heated and shaken until these components dissolved in water. When the mixture was cooled to room temperature a self-supporting hydrogel was formed within a few minutes. Sometimes sonication assists the hydrogel formation. The formation of hydrogel was confirmed by vial inversion method. Minimum gelation concentration of two component hydrogel was determined using a glass vial with inner diameter 1.1 cm and height 4.5 cm.

pH stability test of two component hydrogel:

The stability of the two component hydrogels were examined through out the pH range 1−11 using phosphate buffer solutions. For this purpose, we have prepared phosphate

buffers (50 mmol strength) of pH range 5–8.5 using NaH_2PO_4 and Na_2HPO_4 solution, basic phosphate buffers of pH range 8.5–11 using Na_2HPO_4 and 0.1 (M) NaOH/0.1 (M) HCl solution and acidic phosphate buffers of pH range 1–4 using NaH_2PO_4 and 0.1 (M) HCl solution. In this study the two component hydrogel is stable in the pH range 2–9. Actually, this pH stability has been stated in the sense that these gels were prepared using buffer solutions of corresponding pH values. The final pH values after mixing both components in all the cases were changed. In each of these cases within the pH range 2–9 using phosphate buffer solutions, after hydrogel formation the pH was determined using a pH meter. After gel formation using buffer solution at pH 7.4 and 9.0, hydrogels show a decrease in pH values to pH 5.20 and 5.38 respectively. There is an increase in pH value from 2.0 to 4.16 is observed, when the gel was obtained using buffer solution at pH 2.0. Therefore, in this study the two component gelators (one having acidic moiety and other having basic moiety) itself acts as a buffer system. Using buffer below pH 2.0, two component system was very hardly soluble even at high temperature and it was precipitate out from the medium when the hot solution was cooled down to room temperature. Using buffer higher than pH 9 (up to 10) they produce viscous solution and higher than 10 they remain in soluble form.

pK_a and degree of ionization determination:

In this study, degree of ionization/protonation of each species within two component hydrogel has been determined on the basis of the pK_a values of these species. Glutamic acid alone (N-terminally free) has pK_a values 2.10 for α-COOH, 4.3 for side chain-(γ)-COOH and 9.47 for α-NH_2. However, the N-terminally hydrophobic Fmoc protected glutamic acid (Fmoc-Glu) should have pK_a values greater than that of the glutamic acid alone. The pKa values for Fmoc-Glu have been calculated using SPARC web calculator to be found at http://ibmlc2.chem.uga.edu/sparc.[S1] These calculated pKa values are 3.59 for α-COOH, 4.80 for side chain-COOH. In this study, these pKa values have also been experimentally calculated using titration method. For titration, 40 mg of Fmoc-Glu was taken into 20 ml distilled water (Milli Q) and it was dissolved completely by the slow addition of diluted NaOH solution. After complete dissolution, the final pH of this basic aqueous Fmoc-Glu solution was set at pH 10.26 using excess NaOH solution. At this pH,

almost all the Fmoc-Glu molecules are expected to be ionized. The titration experiment was performed by step wise addition of small volumes of dilute HCl. After each addition of HCl, mixture was shaken well and pH values were recorded using a pH meter. Then the obtained pH values have been plotted against the corresponding volume of HCl added and this plot has been shown in the Figure S13 of the 'Supplementary Information (ESI)' in the revised version of the manuscript. From this titration curve, two pKa values for two carboxylic acid of Fmoc-Glu have been calculated. These experimentally obtained pKa values are 3.60 for α-COOH, 5.05 for side chain-(γ)-COOH. It can be noted that these experimentally obtained pKa values are greater than theoretically obtained pK_a values calculated using SPARC web calculator. Saiani and coworkers, and Adams and coworkers, separately reported that the pK_a value of COOH of Fmoc-conjugated dipeptide is higher than the expected. [S1,S2]

[S1.C. Tang, A. M. Smith, R. F. Collins, R. V. Ulijn, and A. Saiani, *Langmuir* 2009, **25**, 9447–9453. S2. L. Chen, K. Morris, A. Laybourn, D. Elias, M. R. Hicks, A. Rodger, L. Serpell and D. J. Adams, *Langmuir* 2010, **26**, 5232–5242.]

Using these pK_a values (obtained from titration experiment), degree of ionizations of Fmoc-Glu and lysine at the final pH values of these hydrogel have been calculated. For the calculation of degree of ionization, we have selected three cases: One is the hydrogel prepared using the buffer of pH 7.45, and this has been frequently use for different experiments for characterization of the hydrogel, other two are the two limiting cases where the gel is stable i.e., hydrogel prepared using the acidic buffer at pH 2.0 and basic buffer of pH 9.0. The final pH values of these hydrogels were determined to be 5.2, 4.12 and 5.38 when these hydrogels were prepared using phosphate buffers at pH 7.4, 2.0 and 9.0 respectively.

(a) at final pH value 5.2:

Fmoc-Glu:

For α-COOH, degree of ionization or degree of deprotonation= 0.9754

For side chain-(γ)-COOH, degree of ionization or degree of deprotonation = 0.585

Lysine:

For α-COOH, degree of ionization or degree of deprotonation= 0.99991

For α-NH_2, degree of protonation=0.99968

degree of deprotonation=0.0003159

For side chain-(ε)-NH_2, degree of protonation=0.999995

degree of deprotonation=0.00000467

[Lysine has pKa values 2.18 for α-COOH, 10.53 for side chain-COOH and 8.95 for α-NH_2.]

From degree of ionization values it can be stated that there is strong electrostatic interaction between Fmoc-Glu and Lysine at a final pH 5.20.

(b)At final pH value 4.16:

Fmoc-Glu:

For α-COOH, degree of ionization or degree of deprotonation= 0.784

For side chain-(γ)-COOH, degree of ionization or degree of deprotonation = 0.0988

Lysine:

For α-COOH, degree of ionization or degree of deprotonation= 0.9896

For α-NH_2, degree of protonation=0.99998

degree of deprotonation=1.62×10^{-5}

For side chain-(ε)-NH_2, degree of protonation=0.99999957

degree of deprotonation=4.3×10^{-7}

(c) at final pH value 5.38:

Fmoc-Glu:

For α-COOH, degree of ionization or degree of deprotonation= 0.98

For side chain-(γ)-COOH, degree of ionization or degree of deprotonation= 0.681

Lysine:

For α-COOH, degree of ionization or degree of deprotonation= 0.99939

For α-NH_2, degree of protonation=0.99973

degree of deprotonation=2.69×10^{-4}

For side chain-(ε)-NH_2, degree of protonation=0.99999929

degree of deprotonation=7.079×10^{-6}

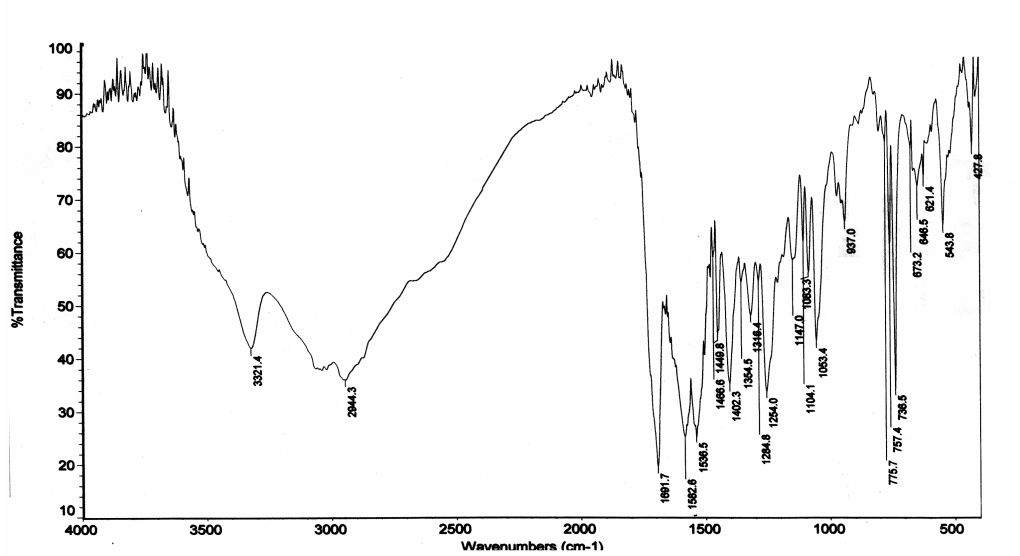

Figure S1. FT-IR spectrum of dried hydrogel obtained from {Fmoc-(L)Glu+(L)Lys}. The characteristics peak at 3321 cm^{-1} for N–H stretching and 1536 cm^{-1} for N–H bending suggest that presence of intermolecular hydrogen bonds in the gel state.

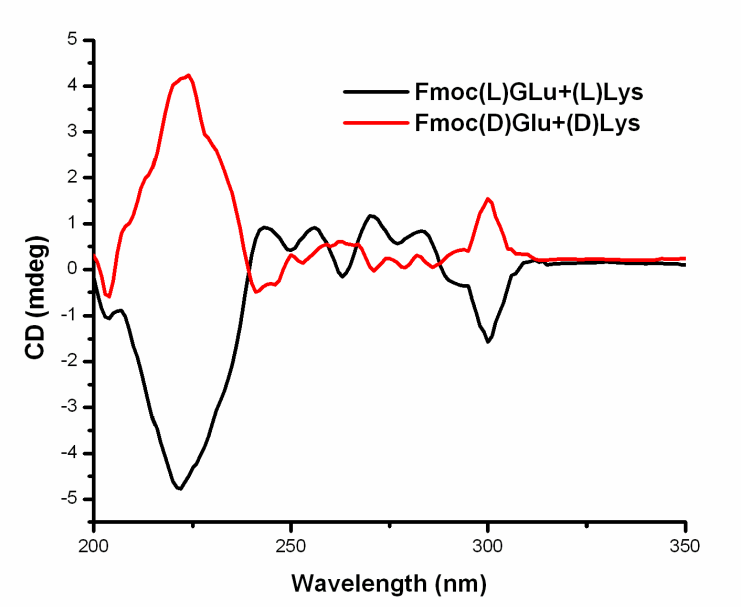

Figure S2. CD spectra of two component systems in the solution state with 0.006 (M) concentration of gelators showing a prominent peak at 223 nm.

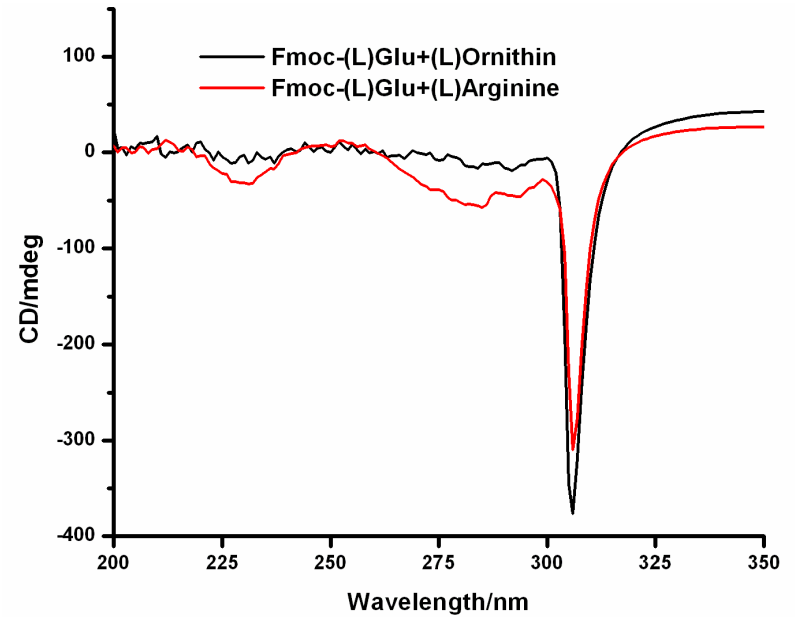

Figure S3. CD spectra of two component hydrogels obtained from {Fmoc-(L)Glu +(L)Ornithin} and {Fmoc-(L)Glu+(L)Arginine} separately as indicated in the figure.

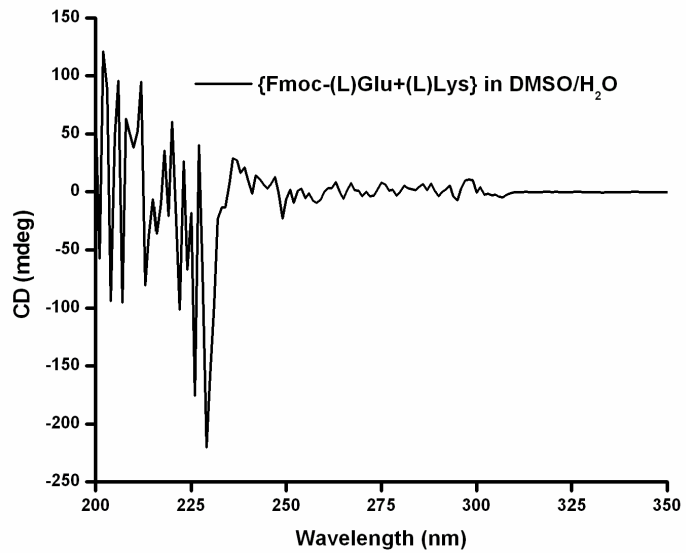

Figure S4. CD spectrum of the equimolar mixture of two component {Fmoc-(L)Glu +(L)Lys} in DMSO/water mixture at solution state.

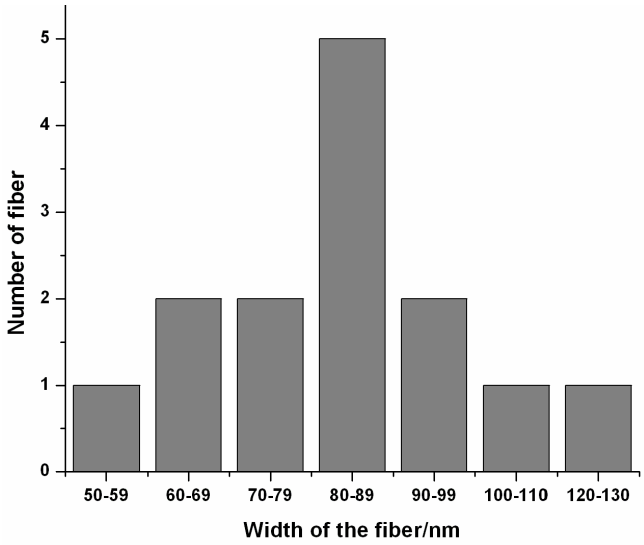

Figure S5. Size distribution of width of fibers obtained from AFM image of {Fmoc-(L)Glu +(L)Lys}.

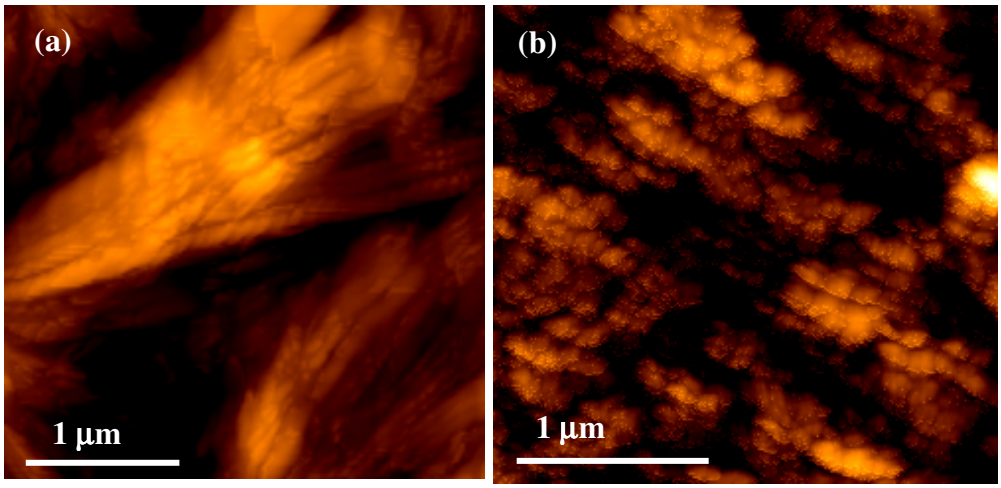

Figure S6. AFM image of two component hydrogel (a) [Fmoc-(L)Glu+(L)Arginine] and (b) [Fmoc-(L)Glu+(L)ornithin] showing left handed helical fibers.

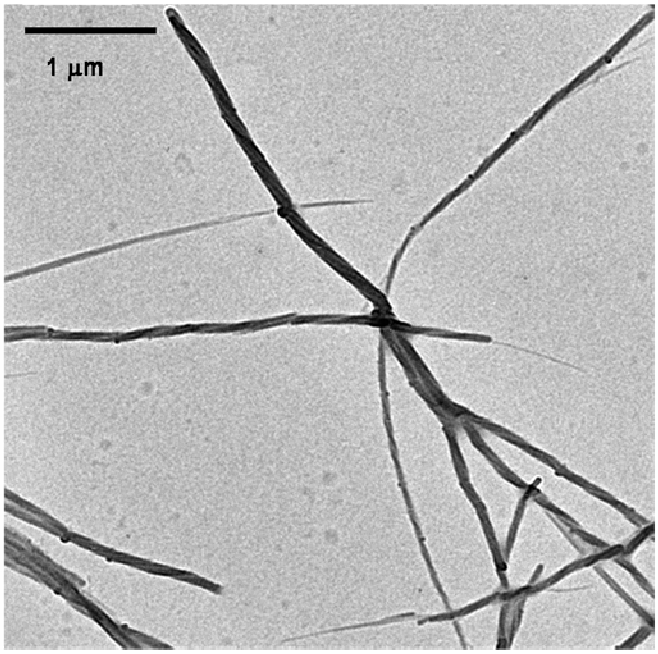

Figure S7. TEM image of two component hydrogel obtained from {Fmoc-(L)Glu +(L)Lys}.

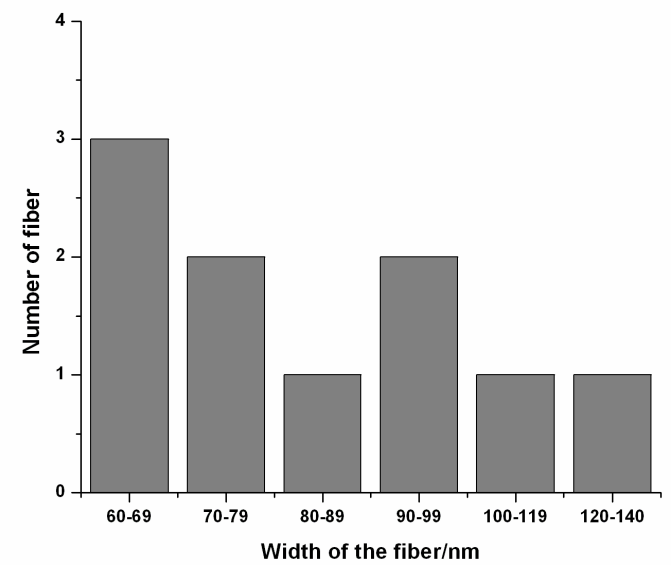

Figure S8. Size distribution of widths of these fibers obtained from the AFM image of {Fmoc-(D)Glu +(D)Lys}.

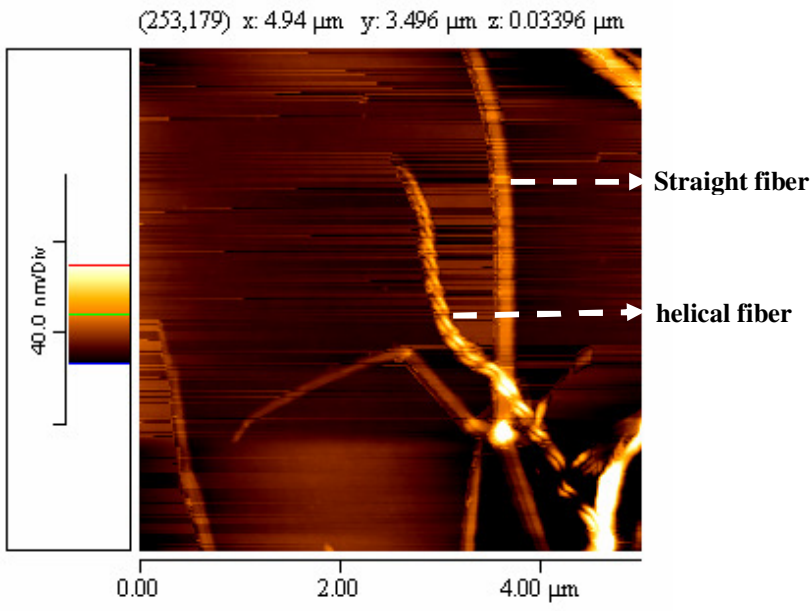

Figure S9. AFM image of two component hydrogel {Fmoc(L)Glu+(L)Lys} in presence of 0.5 equimolar Ca^{2+} showing the presence of both helical fiber (left handed) and straight fiber.

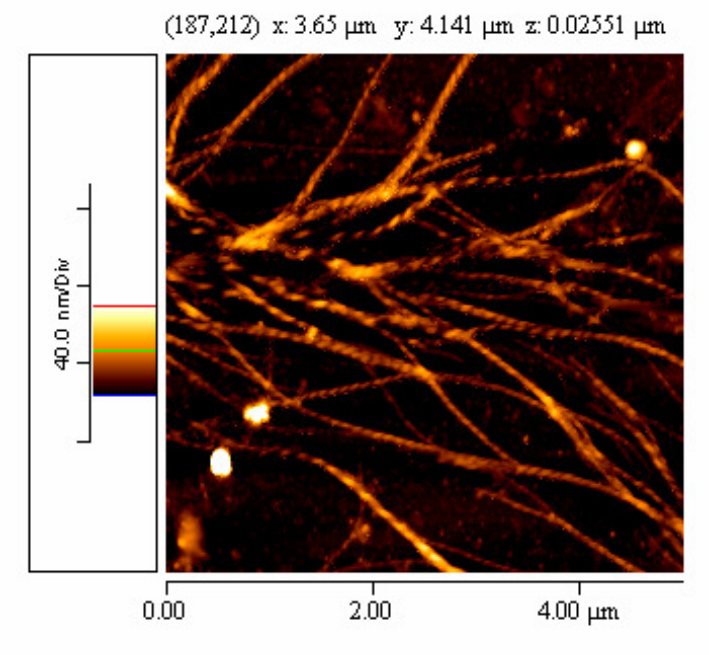

Figure S10. AFM image of two component hydrogel {Fmoc(L)Glu+(L)Lys} in presence of mono-valent Na^+ and K^+ showing the left handed helical fibers clearly.

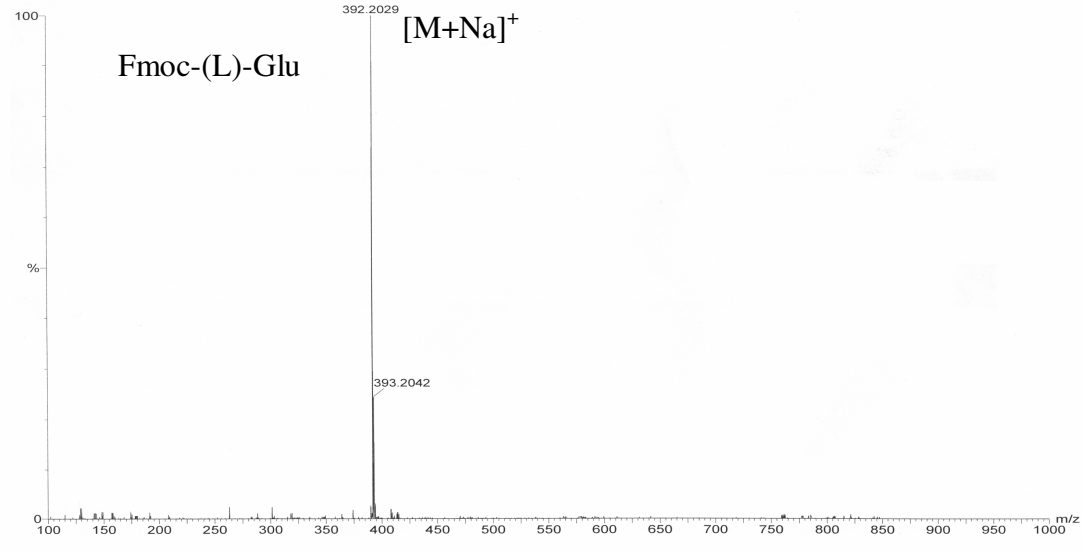

Figure S11. High resolution mass spectrum (HRMS) of Fmoc-(L)Glu.

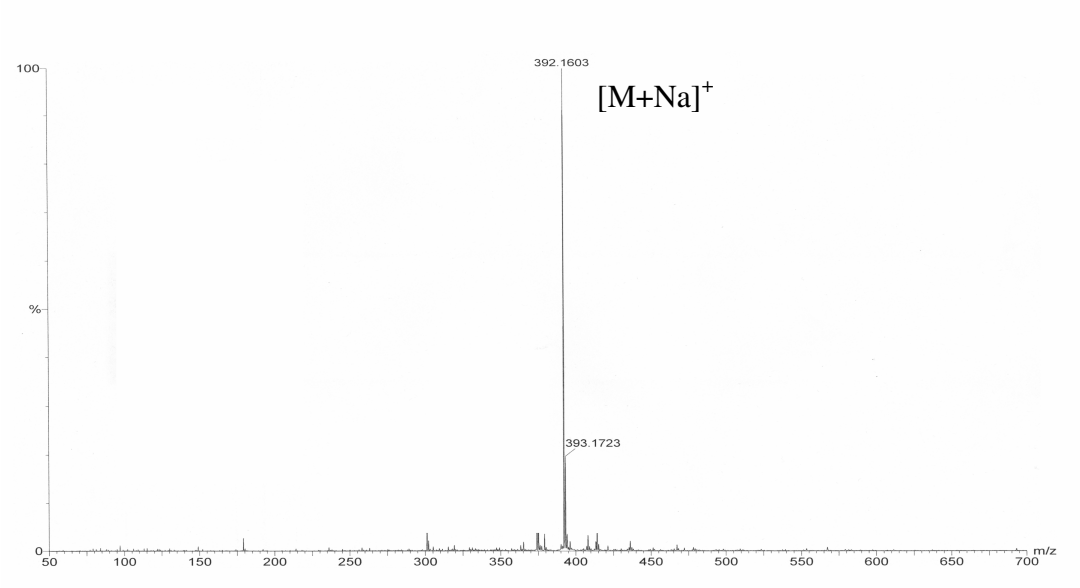

Figure S12. High resolution mass spectrum (HRMS) of Fmoc-(D)Glu.

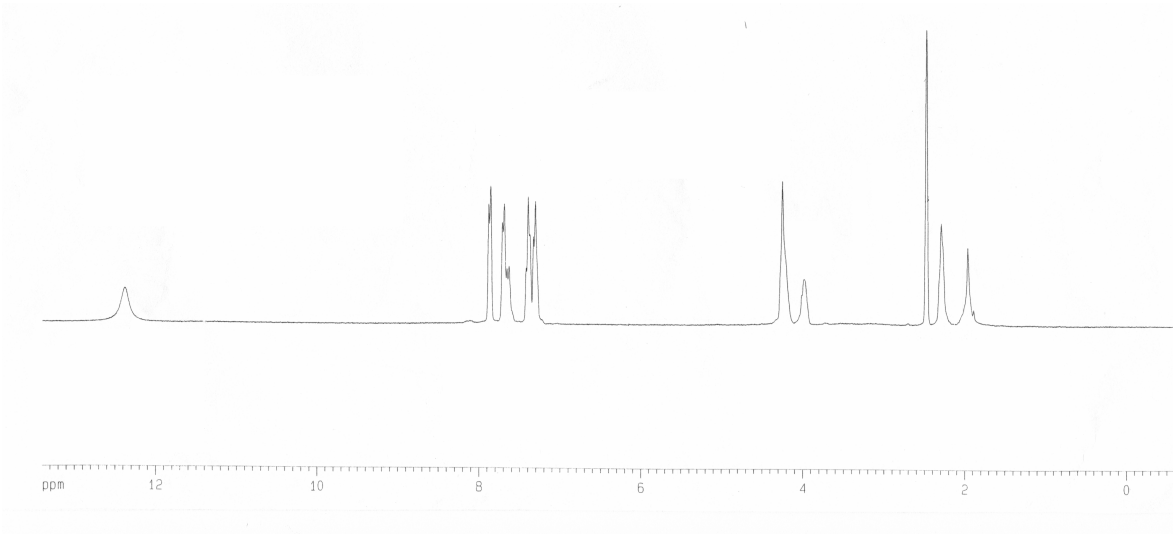

Figure S13. ^{1}H NMR spectra of Fmoc-(L)Glu.

^{1}H NMR (300 MHz, [D_6]DMSO, 25° C): δ12.37 (br, 2H, H of COOH), δ7.88- 7.86 (m, 2H; aromatic CH), δ7.71- 7.63 (m, 2H; aromatic CH and 1H, NH), δ7.42- 7.37 (m, 2H; aromatic CH), δ7.33- 7.31 (m, 2H; aromatic CH), δ4.25 (m, 3H; CH and CH_2), δ3.89-3.98 (m, 1H; αCH), δ2.29 (m, 2H; γCH_2), δ1.17 (m, 2H; βCH_2).

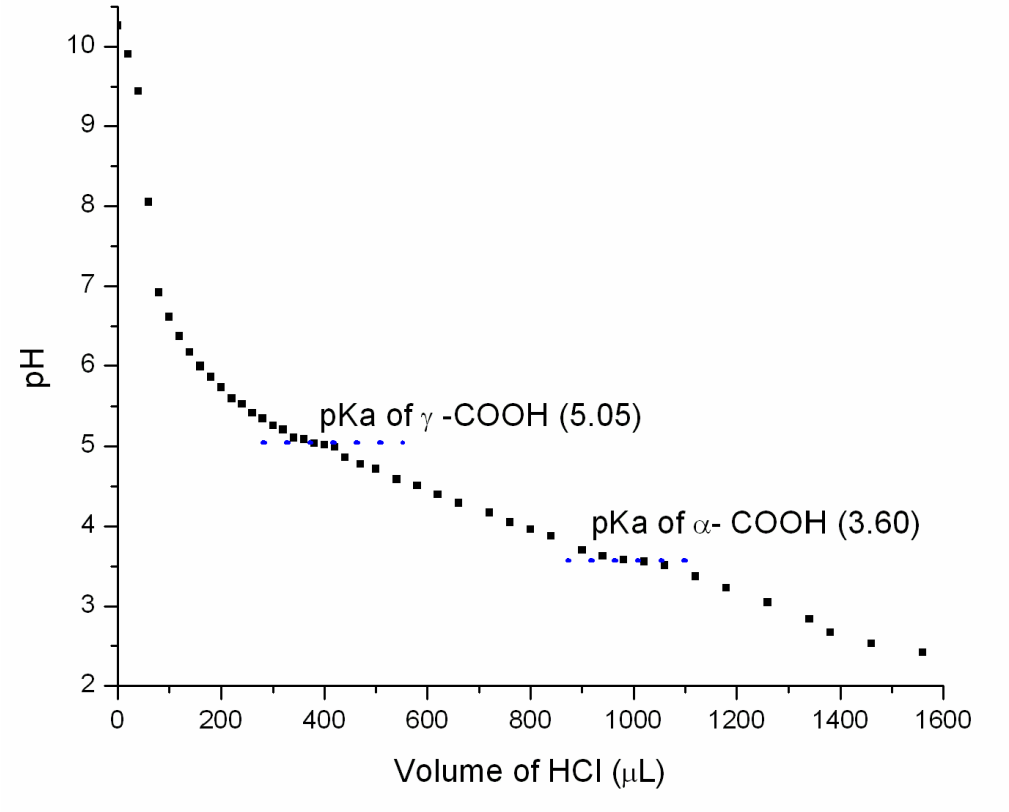

Figure S14. Titration curve of Fmoc (L)-Glu for pK_a value determination.

S1. C. Tang, A. M. Smith, R. F. Collins, R. V. Ulijn, and A. Saiani, *Langmuir* 2009, **25**, 9447–9453.

S2. L. Chen, K. Morris, A. Laybourn, D. Elias, M. R. Hicks, A. Rodger, L. Serpell and D. J. Adams, *Langmuir* 2010, **26**, 5232–5242.