SUPPLEMENTARY INFORMATION

Therapeutic implication of L-phenylalanine aggregation mechanism and its modulation by D-phenylalanine in phenylketonuria

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Solubilization and Concentration Determination. 1 mg mL^{-1} L-Phenylalanine solution in water was prepared and UV absorbance was measured at 258 nm in 1 cm path length cuvette. Concentration was calculated using extinction coefficient[1] 195 M^{-1} cm^{-1} and found to be 5.94 mM.

ANS (8-Anilino-1-Naphthalenesulfonic acid) Binding Assay. The experiment was performed on Perkin Elmer LS55 Fluorescence Spectrometer. The scan was done at excitation wavelength of 350 nm with slit width of 10 nm and Emission wavelength from 400 – 660 nm with slit width of 10 nm and voltage of 650 V. 10 mM ANS stock was prepared in 1 M NaOH and final concentration in the sample was 165 µM. The average of three scans is reported here.

N-Acetyl-L-Phenylalanine Methyl Ester Sample Preparation. Sample was prepared by heating in water bath at 65 ^{o}C for two hour. Analysis was performed after cooling sample at room temperature.

Critical Concentration & Free Energy. Method defined earlier was used for calculating critical concentration, CrC and ΔG[2, 3]. The critical concentration was calculated from HPLC sedimentation assay. The concentration of soluble Phe remained at the end of the aggregation reaction represents dynamic equilibrium state (CrC). This amount was obtained after 24 h of incubation. The CrC was calculated from average of three values and reported as mean ± sd. Free energy of elongation of aggregates of L and DL-Phe was calculated based on CrC using equation E1: $\Delta G = -RT\, lnk$ where k = 1/CrC, CrC is critical concentration, R is gas constant (2 cal K^{-1} mol^{-1}) and T is temperature (298 K).

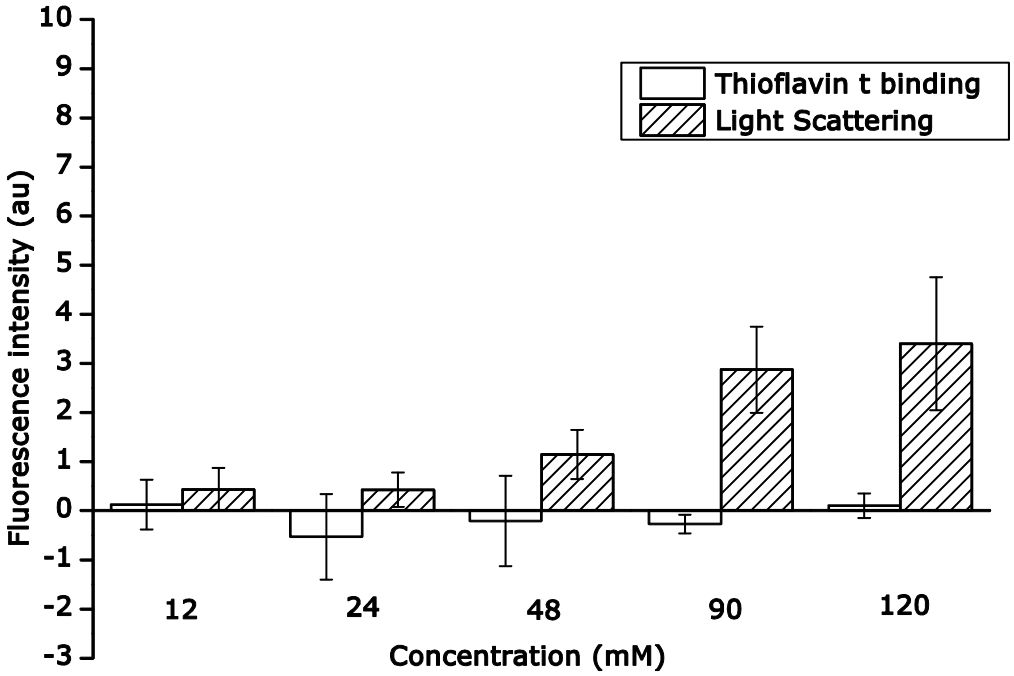

Figure S1. Thioflavin T binding and light scattering assays of L-Glutamine at different concentrations.

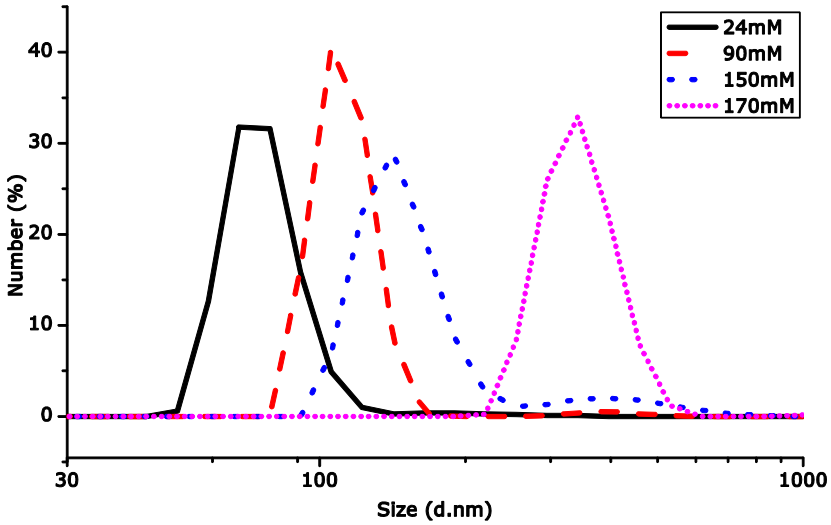

Figure S2. Dynamic light scattering of L-Phe at different concentrations. Particle size in nm on log scale is plotted against number %.

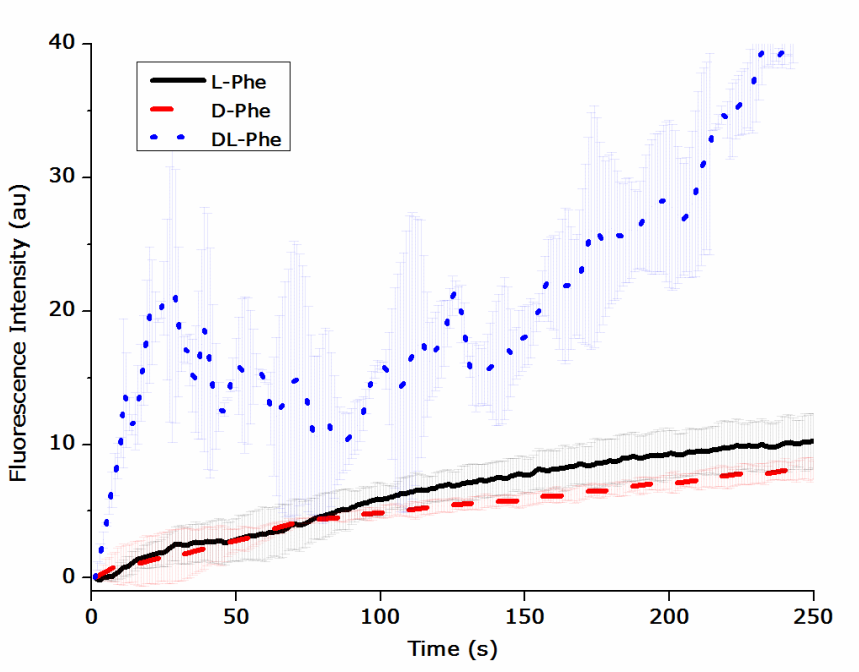

Figure S3. Self-assembly kinetics of L, D and DL-Phe in phosphate buffer saline (PBS) monitored by thioflavin T (ThT) binding fluorescence assay.

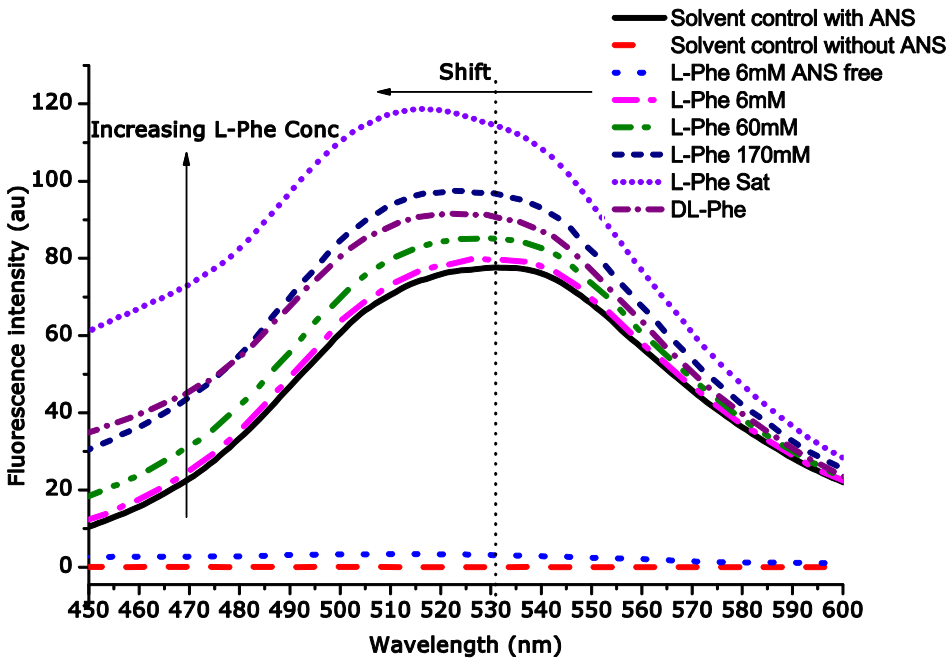

Figure S4. ANS binding fluorescence assay of L-phenylalanine. Upward arrow indicates increase in concentration from 6 mM to 300 mM (saturated L-Phe) and DL-phenylalanine.

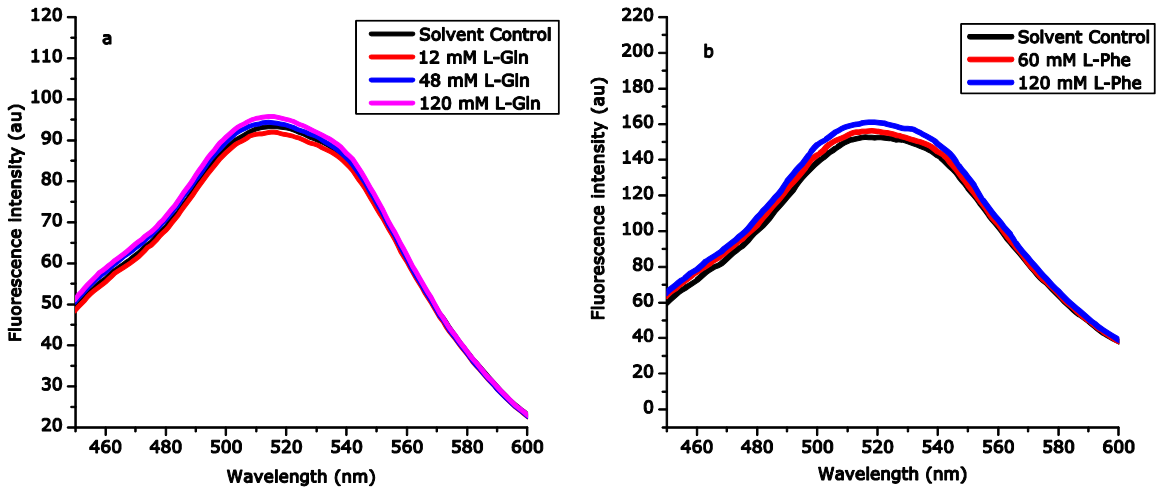

Figure S5. ANS binding fluorescence assay a) L-Glutamine with increasing concentration from 12 mM to 120 mM. b) L-phenylalanine in presence of Guanidium Hydrochloride with increasing concentration from 60 mM to 120 mM.

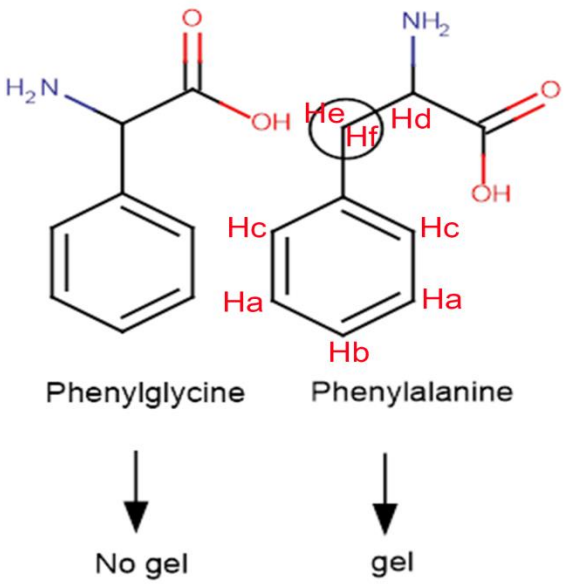

Figure S6. Comparison of alpha-phenylglycine and L-phenylalanine. Difference in structure highlighted by circle (MarvinSketch 5.12.3).

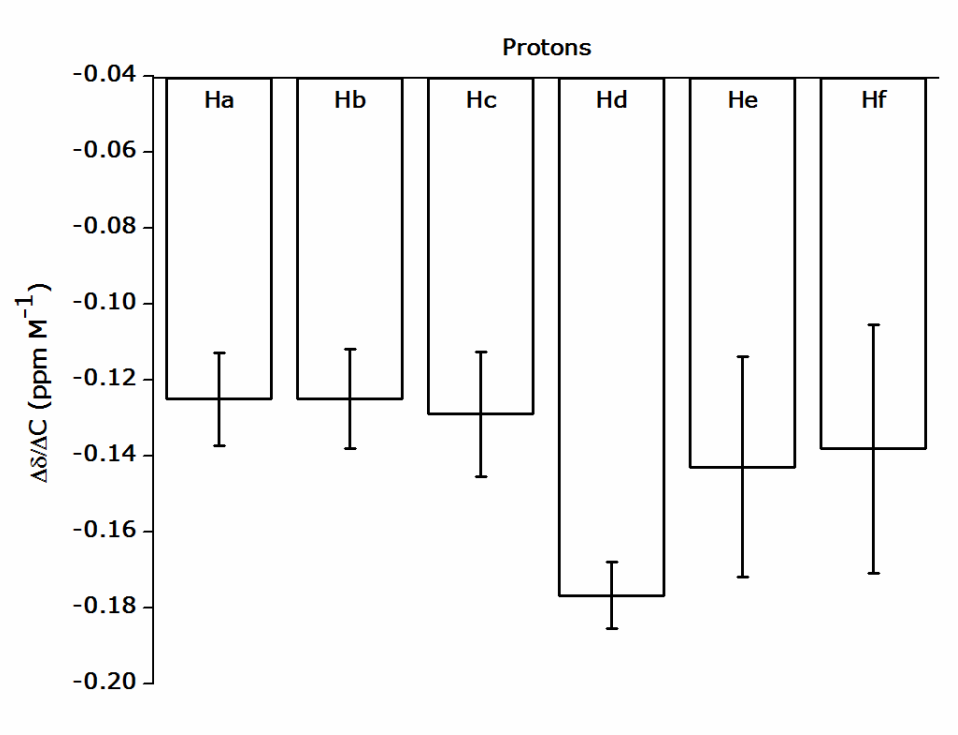

Figure S7. Rate of change of chemical shift with respect to change in concentration ($\Delta\delta/\Delta C$) for protons of L-Phe (mean ± s.e.m.).

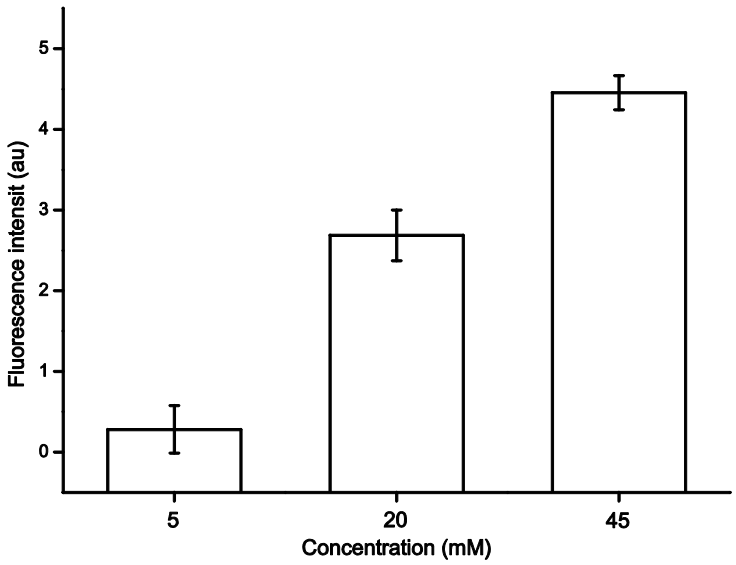

Figure S8. Thioflavin T binding assays of N-acetly-L-phenylalanine methyl ester at different concentrations.

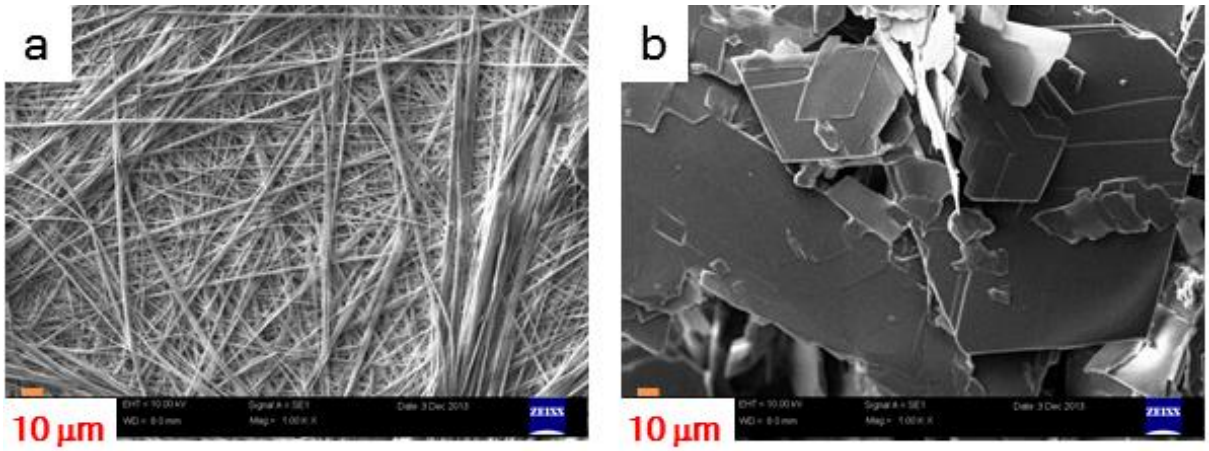

Figure S9. Scanning Electron microscope images of phenylalanine assemblies formed in PBS (a) D-Phe (b) DL-Phe.

Critical Concentration & Free Energy calculation. In amyloid forming reactions such as in Aβ 1-40, the stability of the fibers can be calculated by elongation free energy from the critical concentration (CrC) achieved at dynamic equilibrium state where the rate of aggregation and dissociation becomes equal[3, 4]. The Critical concentration for Phe gel formation and DL-Phe flake formation was determined by RP-HPLC sedimentation assay as described earlier[2, 3].The Initial soluble concentration of L-Phe, D-Phe and DL-Phe solutions at 24 mM to 300 mM was determined and CrC at the end of aggregation reaction after 24 h was calculated and plotted (Figure 6). A linear correlation was observed till 177 mM of L-Phe and 90 mM of DL-Phe (Figure S8), which also correlates well with the reported solubility of Phe in water at room temperature[5].The CrC was found to be 184 ± 3 mM for L-Phe and 99 ± 5 mM for DL-Phe as shown by horizontal dotted lines (Figure S8). Free energy of fiber elongation in gel and flake state (ΔG) was calculated by substituting values of respective critical concentration in equation E1 and found to be -1.01 k cal mol^{-1} for L-Phe and -1.38 k cal mol^{-1} for racemate DL-Phe.

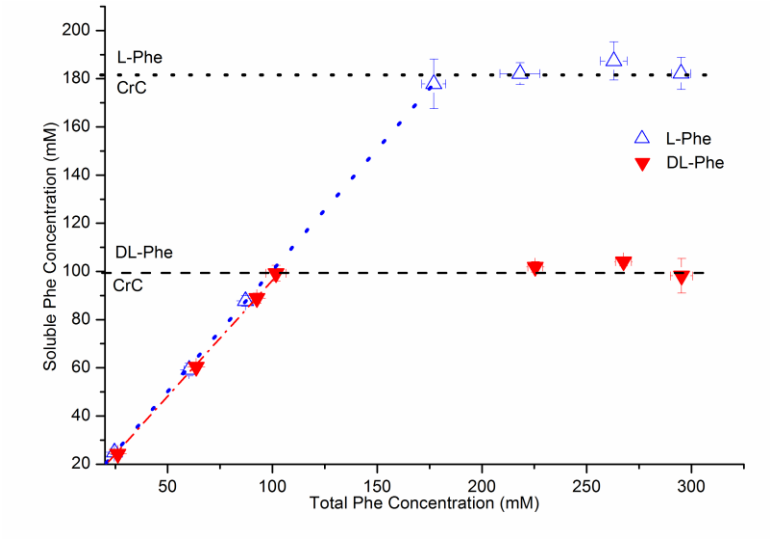

Figure S10. Critical concentration determination. Plot of initial Phe concentration versus final concentration left after the completion of aggregation.

Table S1. Titration of saturated solution of L-Phe with increasing mole fraction of D-Phe.

S. No.	Mole Fraction of enantiomer		Observation
	L-Phe	D-Phe added	+ Gel; - flake; ± partial gel
1	0.5	0.5	-
2	0.8	0.2	-
3	0.85	0.15	-
4	0.90	0.10	-
5	0.92	0.08	-
6	0.95	0.05	±
7	0.98	0.02	+
8	0.99	0.01	+
Positive Control	100	0	+

Table S2. Seeding of L-Phe saturated solution with preformed DL-Phe flakes.

S. No.	L-Phe (%)	DL-Phe seed (%)	Observation
			+ Gel; - flake; ±Mixture
1	100	0	+
2	98	2	±
3	95	5	±
4	92	8	±
5	90	10	-
6	80	20	-

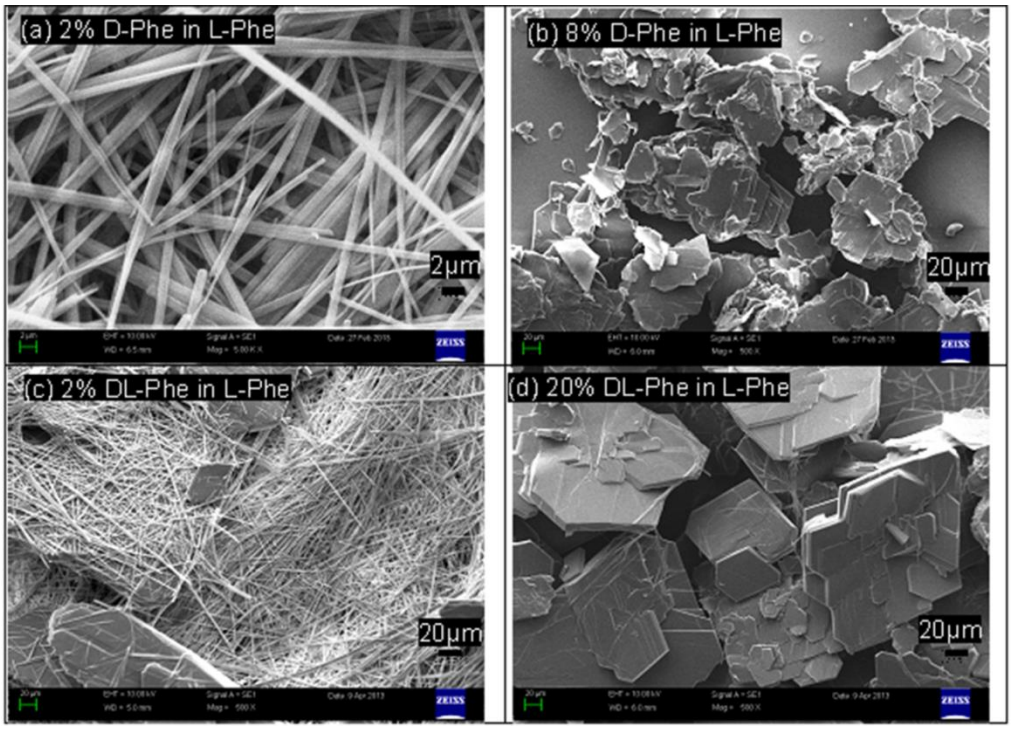

Figure S11. Scanning Electron microscope images of L-phenylalanine with D-Phe and DL-Phe.

1. Fasman, G.D. In *Handbook of Biochemistry and Molecular Biology*, 3rd ed.; Proteins. CRC Press, 1976; **Vol. I***,* p 183-203.
2. Kar, K., Jayaraman, M., Sahoo, B., Kodali, R. & Wetzel, R. Critical nucleus size for disease-related polyglutamine aggregation is repeat-length dependent. *Nat. Struct. Mol. Biol.* **18,** 328-336 (2011).
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4. Williams, A. D. *et al.* Mapping Aβ amyloid fibril secondary structure using scanning proline mutagenesis. *J. Mol. Biol.* **335,** 833-842 (2004).
5. Gonnade, R. G. *et al.* Observation of efficient preferential enrichment phenomenon for a cocrystal of (DL)-phenylalanine and fumaric acid under nonequilibrium crystallization conditions. *Cryst. Growth Des.* **11,** 607-615 (2011).