Supporting Information for:

β-sheets not required: combined experimental and computational studies of self-assembly and gelation of the ester-containing analog of an Fmoc-dipeptide hydrogelator

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Supplemental Methods

Fmoc-L-Ala-L-Lac synthesis and purification

The carboxyl terminus of lactic acid was benzyl-protected using the method of Fan et al.[1] After purification by distillation, the benzyl-lactate product was dissolved in dichloromethane (DCM) with 1.5 equivalents of Fmoc-L-Ala-OH (EMD Chemicals, Gibbstown, NJ), and the mixture was chilled to 0° C, at which time 1.5 equiv dicyclohexylcarbodiimide (DCC) and 0.01 equiv 4-dimethylaminopyridine (DMAP) were added to initiate the esterification reaction. The reaction was allowed to slowly warm to room temperature and was stirred overnight. The crude mixture was filtered to remove dicyclohexylurea (DCU) crystals, then concentrated *in vacuo* and redissolved in ethyl acetate for aqueous washes (2x 0.1 M HCl, 2x DI water, 1x brine). The organic layer was dried over $MgSO_4$, filtered, and then purified via silica column chromatography using 20% ethyl acetate in hexane as the mobile phase. Fmoc-Ala-Lac-Bn was recovered with a yield of 80-90%.

Fmoc-Ala-Lac-Bn was benzyl-deprotected using the transfer hydrogenolysis method of Bajwa, et al.[2] Fmoc-Ala-Lac-Bn was dissolved in dry methanol and a small amount of dry DCM, and a 1:1 mass ratio Pd/C (10 wt% Pd) was added to the mixture. The mixture was stirred vigorously and 10 equiv of 1,4-cyclohexadiene was added to the mixture. After four hours the mixture was filtered over Celite and triturated with dry DCM, then concentrated *in vacuo*. The crude product was purified by silica column chromatography using a 1-5% methanol in DCM gradient. The pure Fmoc-Ala-Lac-OH was lyophilized and recovered at a 30-40% yield. Purity was assessed using LC-MS and NMR and was determined to be > 96%. An ^{1}H NMR spectrum and HPLC chromatogram of the purified product are provided in Figure S1.

^{1}H NMR (500 MHz, DMSO-d_6, δ): 7.89 (d, J = 7.6 Hz, 2H, H_{Ar}), 7.85 (d, J = 7.32, 1H, -N*H*CHCH$_3$), 7.73 (t, J = 6.7 Hz, 2H, H_{Ar}), 7.42 (t, J = 7.4 Hz, 2H, H_{Ar}), 7.34 (t, J = 7.4 Hz, 2H, H_{Ar}), 4.96 (q, J = 7.0 Hz, 1H, -OC*H*CH$_3$), 4.35-4.27 (m, 2H, CHC*H*$_2$), 4.24 (t, J = 6.8 Hz, 1H, -C*H*CH$_2$), 4.17 (quin, J = 7.3, 1H, -NHC*H*CH$_3$), 1.41 (d, J = 7.1 Hz, 3H, -OCHC*H*$_3$), 1.36 (d, J = 7.3 Hz, 3H, -NHCHC*H*$_3$).

^{13}C NMR (500 MHz, DMSO-d_6, δ): 172.43 (1C, -CH(*C*=O)O or –CH$_3$(*C*=O)OH), 171.57 (1C, -CH(*C*=O)O or –CH$_3$(*C*=O)OH), 155.79 (1C, -O(*C*=O)NH), 143.73 (2C, $C_{Ar,\ quaternary}$), 140.66 (2C, $C_{Ar,\ quaternary}$), 127.57 (2C, $C_{Ar,5}$), 127.01 (2C, $C_{Ar,6}$), 125.15 (2C, $C_{Ar,7}$), 120.04 (2C, $C_{Ar,1}$), 68.59 (1C, -O*C*HCH$_3$), 65.62 (1C, CH*C*H$_2$), 48.99 (1C, -NH*C*HCH$_3$), 46.54 (1C, -*C*HCH$_2$), 16.68 (1C, -NHCH*C*H$_3$), 16.64 (1C, -OCH*C*H$_3$).

ESI MS (*m/z*): $[M+H]^+$ calculated for $C_{21}H_{21}NO_6$, 384.14; found, 384.0.

References for Supplemental Methods:

(1) Fan, W.; Li, X.; Qian, S.; Wang, S.; Wu, Y. Enhanced Brain Targeting of Tegafur Using Novel Lactyl Cholesterol Liposome as a Carrier. *Lett. Drug Des. Discov.* **2009**, *6*, 542–547.

(2) Bajwa, J. S. Chemoselective Deprotection of Benzyl Esters in the Presence of Benzyl Ethers, Benzyloxymethyl Ethers and N-Benzyl Groups by Catalytic Transfer Hydrogenation. *Tetrahedron Lett.* **1992**, *33*, 2299–2302.

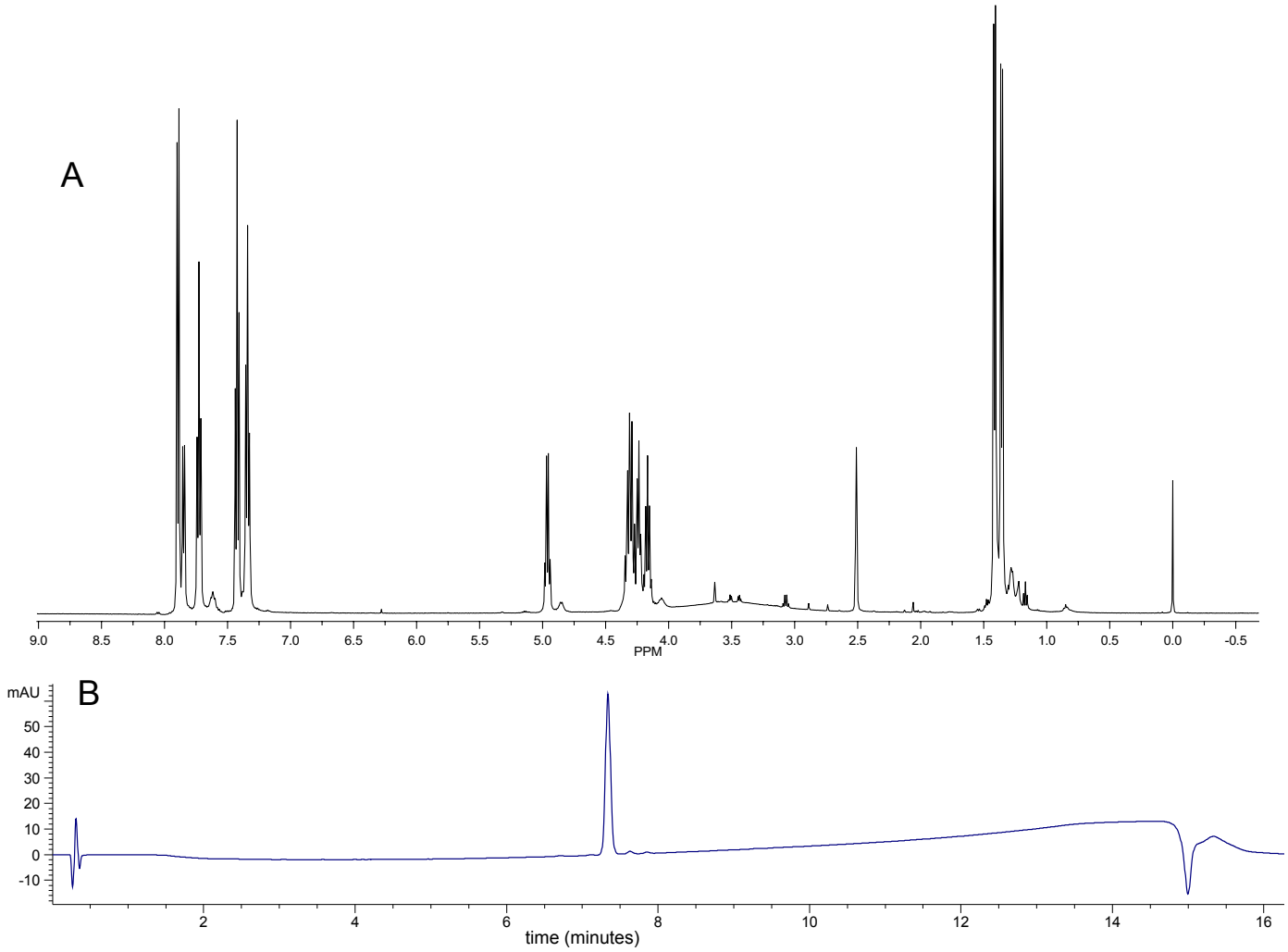

Figure S1. NMR spectrum (a) and HPLC-MS trace (b) of column-purified Fmoc-Ala-Lac sample. Percent area of product peak (7.4 min) is >98%.

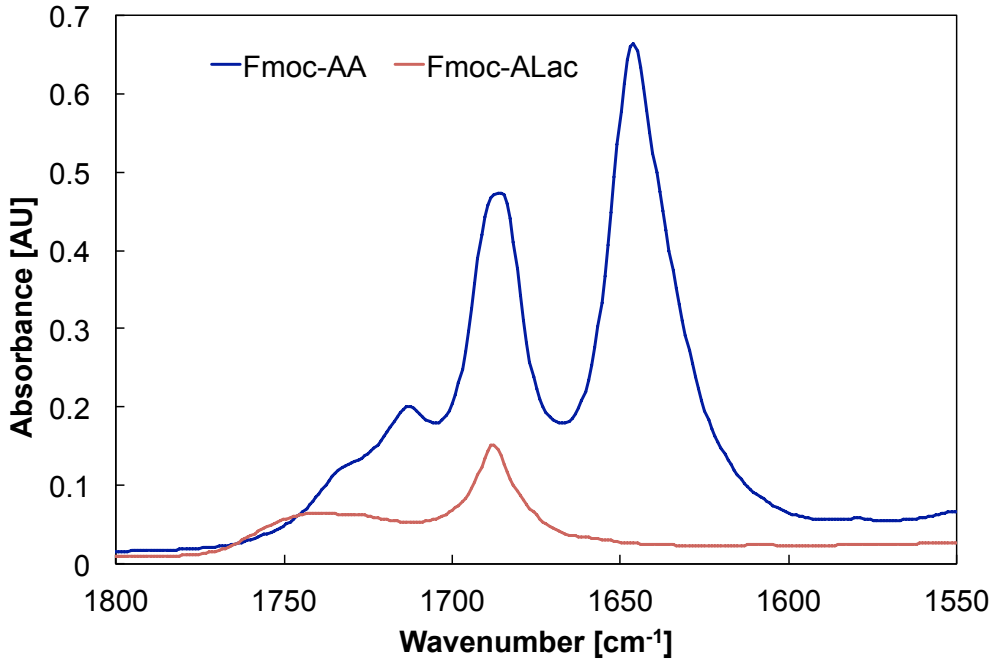

Figure S2. Non-normalized version of bottom plot in Figure 2B.

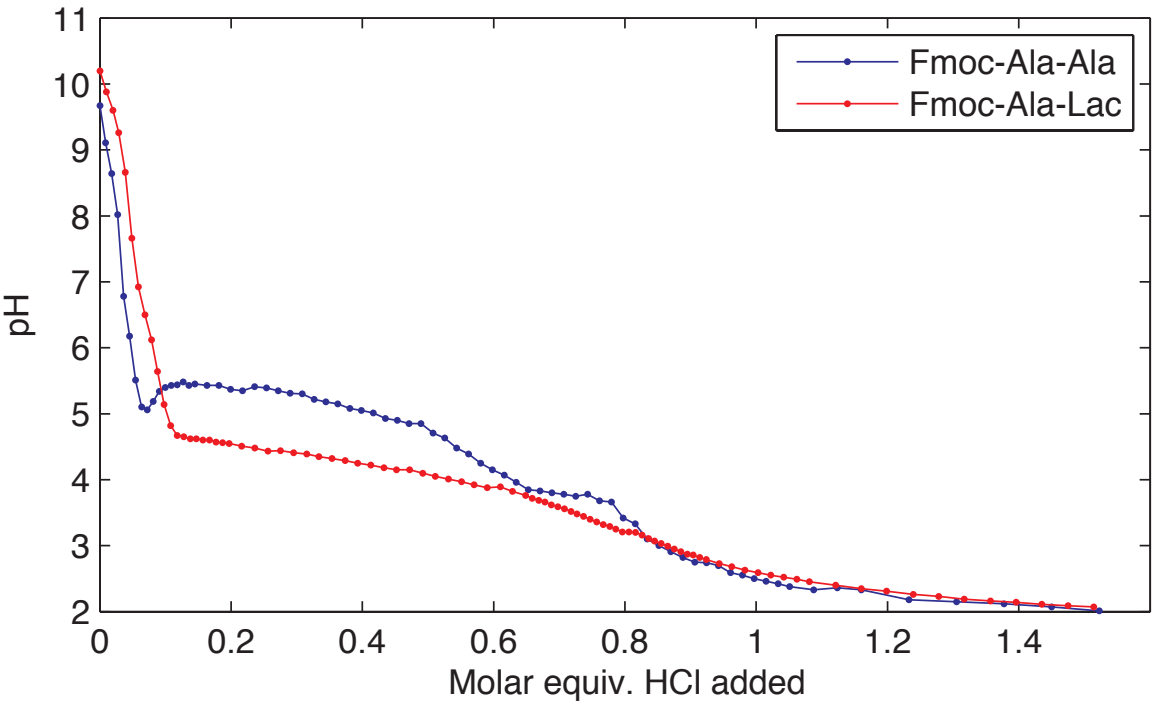

Figure S3. Titration curves show that Fmoc-Ala-Ala has a higher apparent pKa (~5.1) (indicated by midpoint of the buffering region between ~0.1 – 0.5 eq HCl) than Fmoc-Ala-Lac (pKa ~4.3).

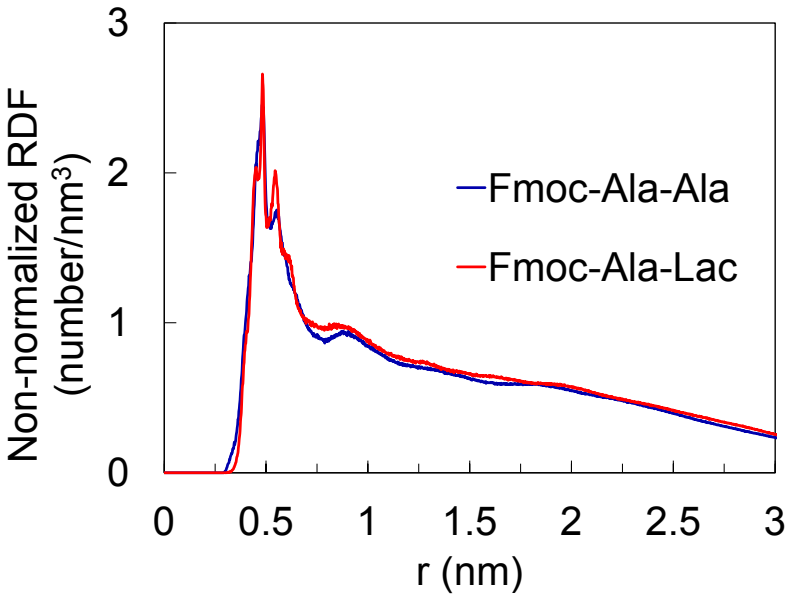

Figure S4. Radial distribution function (RDF) plot for strand-to-strand (where strand refers to peptide backbone) distance in simulated fibrils of Fmoc-Ala-Ala and Fmoc-Ala-Lac shows marked overlap, indicating similar interaction distances independent of amide-amide hydrogen bonding ability.