SUPPORTING INFORMATION

for

Experimental and Computational Studies Reveal an Alternative Supramolecular Structure for Fmoc-dipeptide Self-assembly

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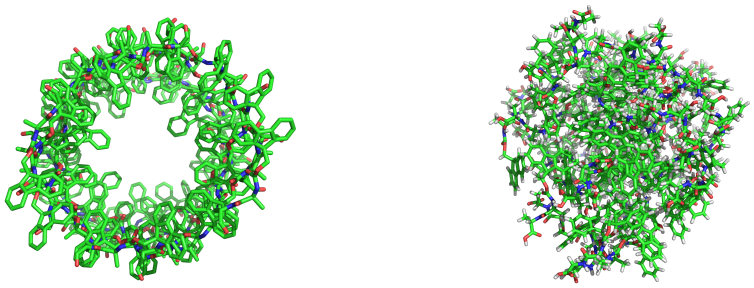
(1) A preliminary 300 ns simulation of Fmoc-AA fibril

A fibril proposed by Smith *et al*.[1] was used as the starting structure for our Fmoc-AA self-assembly preliminary simulation. However, the center hollow was not maintained and the tube shrunk into a more compact aggregate after 300 ns simulation, as shown in Figure S1.



(A) Starting structure

(hydrogens are not shown)

(B) After 300 ns simulation

Figure S1. "Top down" views of Fmoc-AA fibril structure before and after preliminary simulation.

(2) Analysis of GdL hydrolysis by Circular Dichroism

To slow down the process of gelation so that time course measurements of gels could be made easily using circular dichroism, we acidified solutions of Fmoc-AA with 1 eq NaOH using ~2.5 eq glucono-δ-lactone (GdL), after Adams et al.[2] GdL is a lactone ring that hydrolyzes in solution to yield gluconic acid, which then acidifes the solution. To understand what extent the hydrolysis of GdL contributed to the overall increase in dichroism during the gelation process, we took CD spectra of a GdL solution. A hypothetical concentration of 5 mg/ml Fmoc-AA was used to calculate the total solution volume (3 mL), the volume of 1 eq NaOH (78.5 μL), and the mass of ~2.5 eq GdL (16.2 mg). The NaOH was added to DI water, and a spectrum was obtained. Then the GdL was added, and the solution was vortexed and a small amount placed in

a cylindrical quartz cuvette with a path length of 0.1 mm. Spectra were taken every 10 minutes, as shown in Figure S2 below.

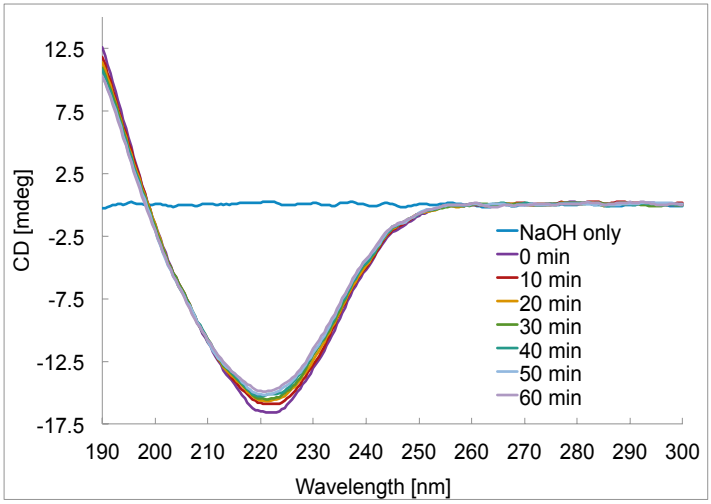


Figure S2. Time course CD spectra of glucono-δ-lactone. Note that peak intensity decreases over time as hydrolysis proceeds.

Interestingly, the magnitude of dichroism decreases slightly over time due to hydrolysis of GdL. Therefore, the increase in dichroism seen in Fmoc-AA solutions acidified with GdL must be due to the self-assembly process and not the hydrolysis of GdL to gluconic acid.

(2) Detailed information of models

In Table S1 below, geometry information and simulation conditions for each model have been provided.

Table S1. Detailed information of models.

system	Number of molecules	Box size (nm)	Water molecules	Final fibril size (nm)	Simulation time (ns)
Cubic 4	80 (20 layers)	13×13×3	71,801	3×3×7	160
Cubic 5	100 (20 layers)	13.3×13.3×13.3	77,377	3×3×7.3	160
Cubic 6	120 (20 layers)	13.2×13.2×13.2	71,584	3×3×7.5	160
400 ns	120 (30 layers)	7×7×17.2	25,793	3×3×10	400
SA	60 (15 layers)	6×6×11	11,966	----	40
REMD	60 (15 layers)	6×6×11	11,966	----	100×120

Temperatures used for REMD (K)

280.00	281.65	283.32	285.00	286.70	288.42	290.16	291.91	293.68	295.47
297.28	299.11	300.95	302.81	304.69	306.58	308.50	310.43	312.37	314.34
316.32	318.33	320.34	322.38	324.44	326.51	328.60	330.70	332.83	334.97
337.13	339.31	341.50	343.72	345.95	348.20	350.46	352.75	355.05	357.37
359.70	362.06	364.43	366.82	369.22	371.65	374.09	376.55	379.03	381.52
384.04	386.57	389.11	391.68	394.26	396.86	399.48	402.12	404.77	407.44
410.13	412.84	415.56	418.31	421.07	423.84	426.64	429.45	432.28	435.13
437.99	440.88	443.78	446.70	449.63	452.59	455.56	458.55	461.55	464.58
467.62	470.68	473.75	476.85	479.96	483.09	486.24	489.40	492.59	495.79
499.01	502.24	505.49	508.77	512.05	515.36	518.68	522.03	525.38	528.76
532.16	535.57	539.00	542.44	545.91	549.39	552.89	556.41	559.95	563.50
567.07	570.66	574.26	577.89	581.53	585.19	588.86	592.56	596.27	600.00

(3) Quantum Mechanical Characterization of Fmoc group

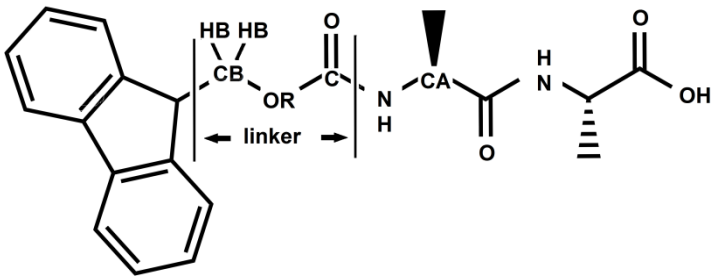


Figure S2. Chemical formula of the Fmoc-AA in MD simulations.

The parameters of the "linker" part and on Fmoc-AA (shown in Figure S2) originally were not included in GROMACS OPLS-AA force field or any available force fields. Therefore, we performed *ab initio* quantum mechanical (QM) calculations for the "linker" using the Gaussian 03 and 09 packages.[3,4] The partial charges for new atom types were derived and fitted according to the electrostatic potential (ESP) from QM single point calculations at the HF/6-31G* level. For each missing torsional angle, we scanned the torsion from -180°to 180° in 30° increments while constraining other angles; by comparing with our QM calculations, the parameters of missing torsional angles were obtained. The derived OPLS-AA/L[5-7] partial charges and torsional parameters for Fmoc group are summarized in Tables S2 and S3. Other parameters for the Fmoc-AA were transferred from OPLS-AA, OPLS-AA/L and MM3[8] force fields and are provided in part 3.

Table S2. Atomic Partial Charges

atom	charge (electron)
CB	0.23
OR	-0.36
C	0.80
O	-0.68
HB	0.002

Table S3. Parameters for Ryckaert-Bellemans torsional potential (kJ mol^{-1})

torsion angle	C_0	C_1	C_2	C_3	C_4	C_5
CB-OR-C-O	26.48	0	-26.48	0	0	0
CB-OR-C-N	5.68	1.09	-6.77	0	0	0
O-C-N-H	20.50	0	-20.50	0	0	0
O-C-N-CA	25.48	0	-25.48	0	0	0
OR-C-N-H	-4.65	-6.76	2.40	9.01	0	0
OR-C-N-CA	31.77	-5.27	-17.60	-8.91	0	0
HB-CB-OR-C	0.62760	-0.62760	0	0	0	0
CG-CB-OR-C	0	0	0	0	0	0

GROMACS uses the Ryckaert-Bellemans potential, which is given by: $V_{rb}(\phi_{ijkl}) = \sum_{n=0}^{5} C_n (\cos(\psi))^n$.

(5) Topology files for GROMACS 4.5.4 OPLS-AA force filed

To run an example of our simulation, readers may download files of our final Cubic 5 fibril structure ("cubic5.pdb") and our topology package for GROMACS 4.5.4 (FMOC_topology_files_for_GROMACS_OPLSAA). In order to implement the topology files, readers should name the Fmoc group according to our *.pdb, and paste our topology files to the ends of relevant GROMACS 4.5.4 OPLS-AA files respectively.

References

(1) Smith, A. M.; Williams, R. J.; Tang, C.; Coppo, P.; Collins, R. F.; Turner, M. L.; Saiani, A.; Ulijn, R. V. Advanced Materials 2008, 20, 37.

(2) Adams, D. J.; Butler, M. F.; Frith, W. J.; Kirkland, M.; Mullen, L.; Sanderson, P. Soft Matter 2009, 5, 1856.

(3) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Laham, A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. 2003.

(4) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G.

A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. 2009.

(5) Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. J. Am. Chem. Soc. 1996, 118, 11225.

(6) Jorgensen, W. L.; Tirado-Rives, J. P Natl Acad Sci USA 2005, 102, 6665.

(7) Zhang, J.; King, M.; Suggs, L.; Ren, P. Biomacromolecules 2007, 8, 3015.

(8) Allinger, N. L.; Yuh, Y. H.; Lii, J. H. J. Am. Chem. Soc. 1989, 111, 8551.