Supplementary Materials for

Rapid Discovery of Self-Assembling Peptides with One-Bead One-Compound Peptide Libraries

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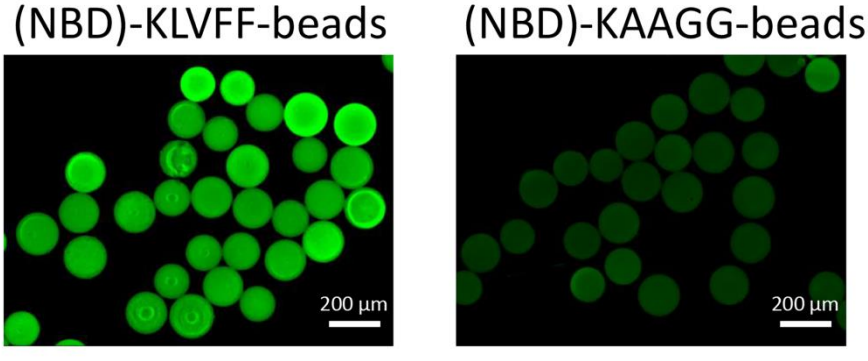

Figure S1. Under aqueous condition, TentaGel beads displaying self-assembling peptide KLVFF N-capped by NBD was found to fluoresce strongly. TentaGel beads displaying negative control non-assembling peptide KAAGG did not fluoresce, thus validating the screening assay for self-assembling peptide discovery.

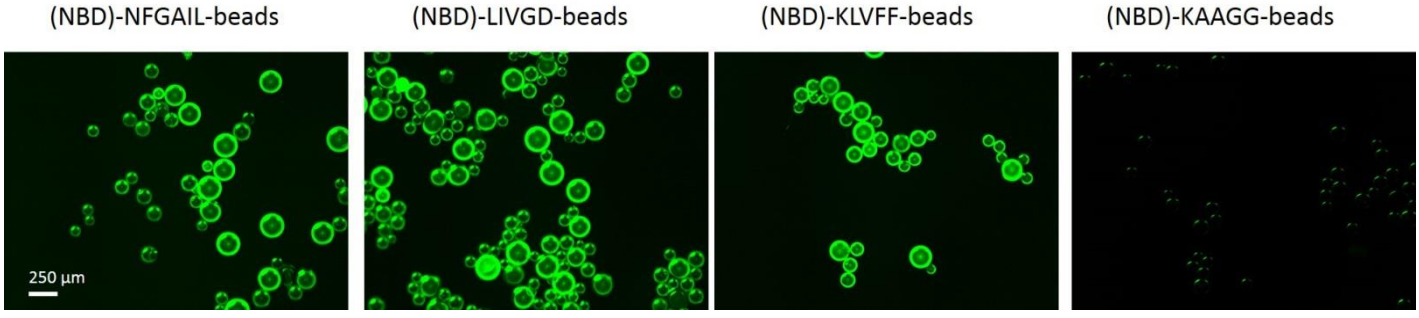

Figure S2. Fluorescent micrograph of NFGAIL-, LIVGD-, KLVFF-, and KAAGG-beads N-capped with NBD. Limiting level (5% substitution) of NBD was used during the N-capping, resulting in preferential coupling of NBD to the peptides on the outer shell of the beads. As a result, fluorescent activation was higher at the outer shell. NFGAIL, LIVGD, KLVFF are known self-assembling peptides under aqueous condition.

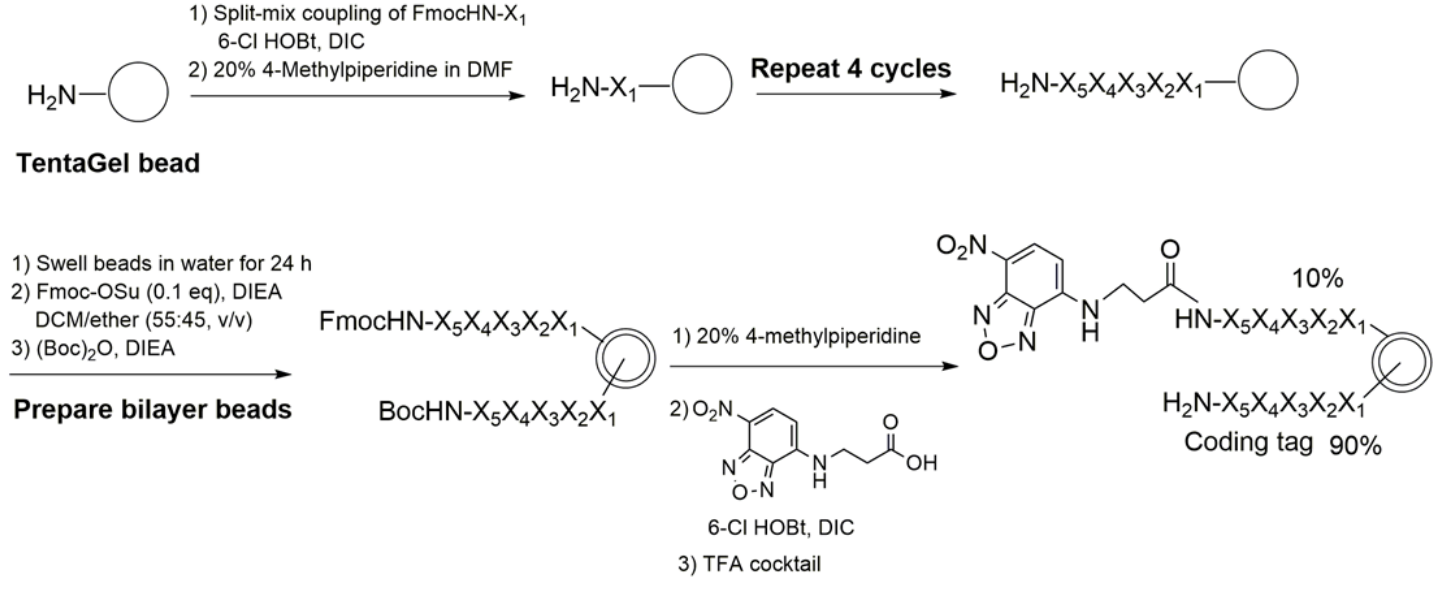

Figure S3. Synthetic scheme of OBOC peptide library N-capped with nitro-1,2,3-benzoxadiazole (NBD), for self-assembling peptide discovery. Only the outer layer of the random peptides was N-capped with the dye. The peptides in the bead interior remained N-terminally free, which was needed for Edman sequencing.

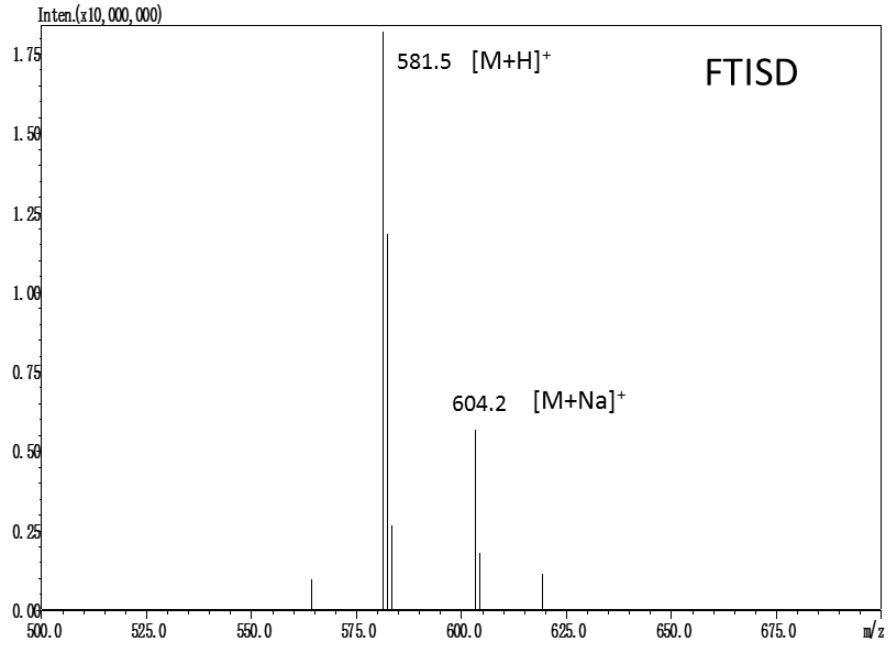

Figure S4. The MALDI-TOF spectrum of peptide FTISD

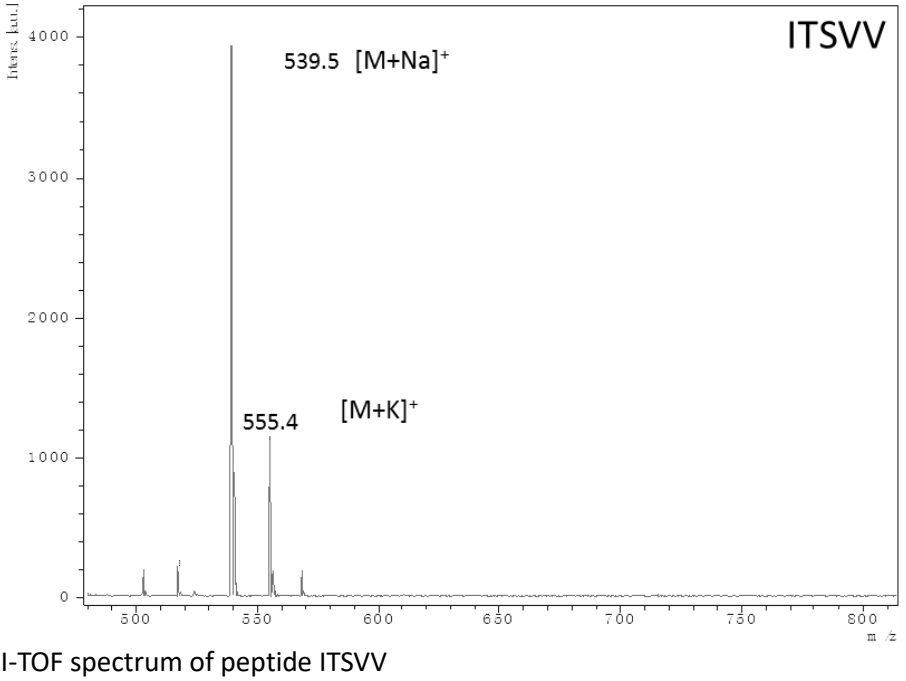

Figure S5. The MALDI-TOF spectrum of peptide ITSVV

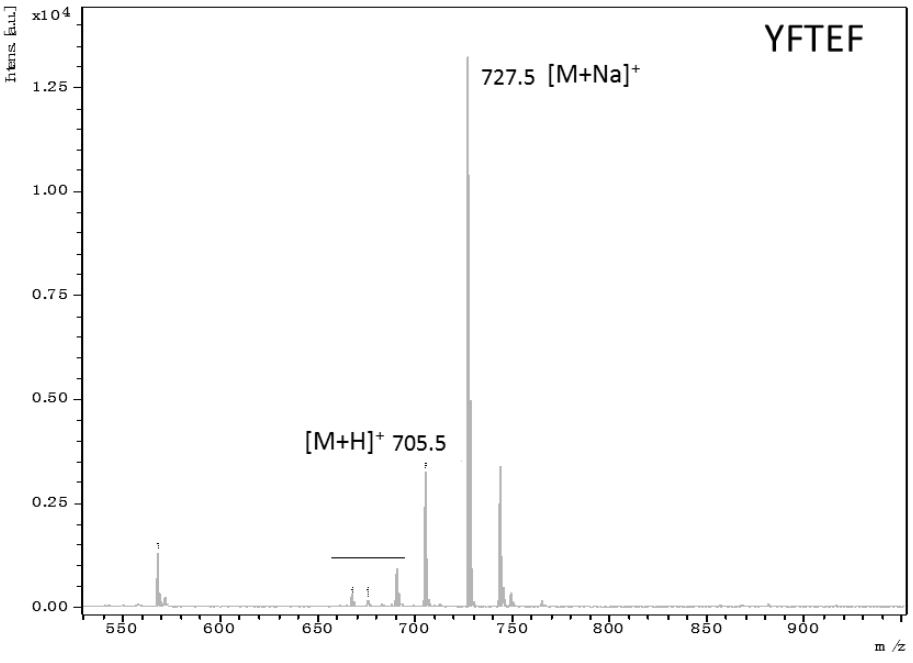

Figure S6. The MALDI-TOF spectrum of peptide YFTEF.

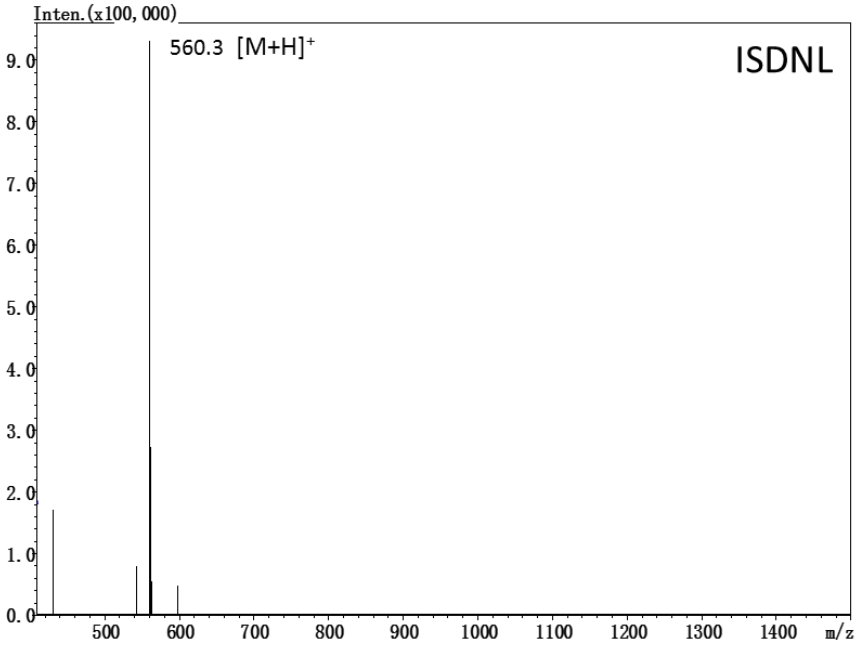

Figure S7. The MALDI-TOF spectrum of peptide ISDNL.

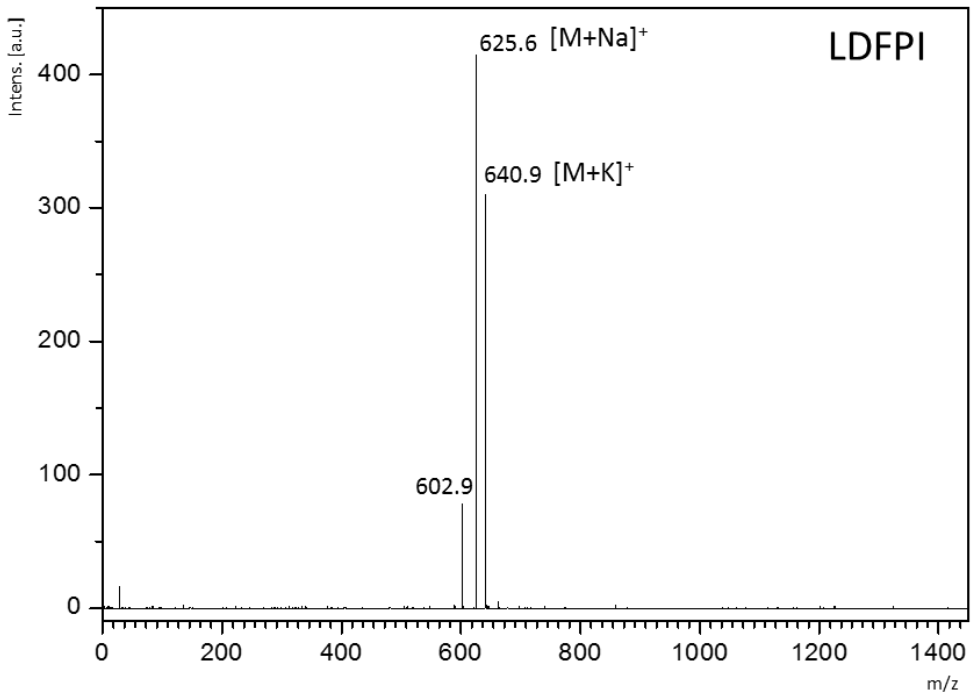

Figure S8. The MALDI-TOF spectrum of peptide LDFPI.

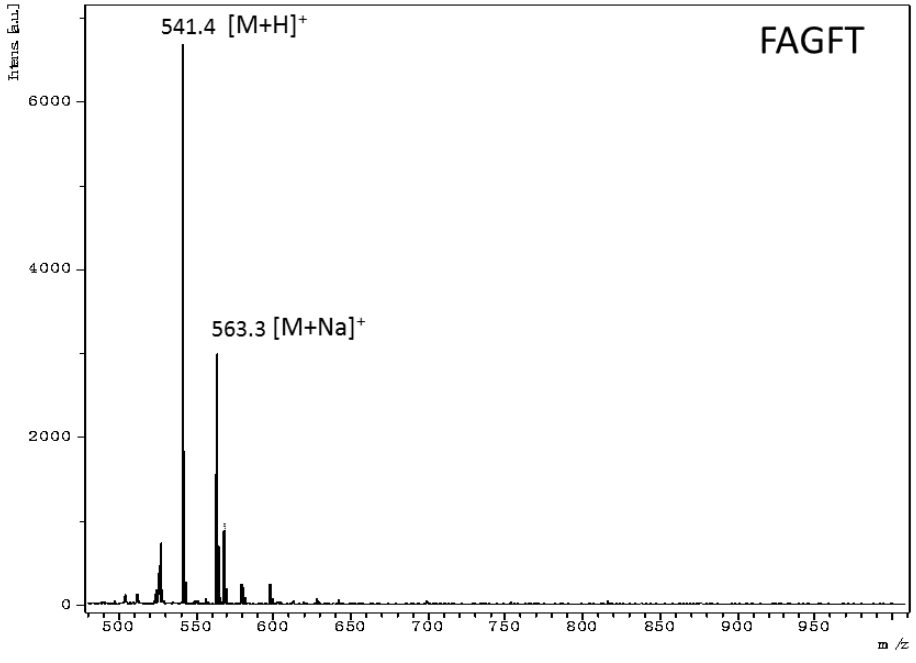

Figure S9. The MALDI-TOF spectrum of peptide FAGFT.

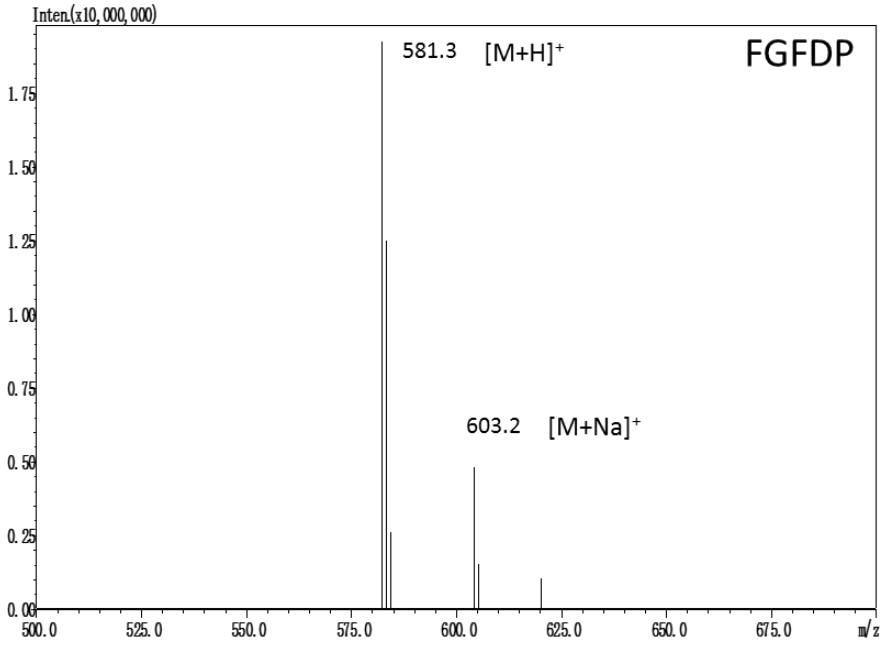

Figure S10. The MALDI-TOF spectrum of peptide FGFDP.

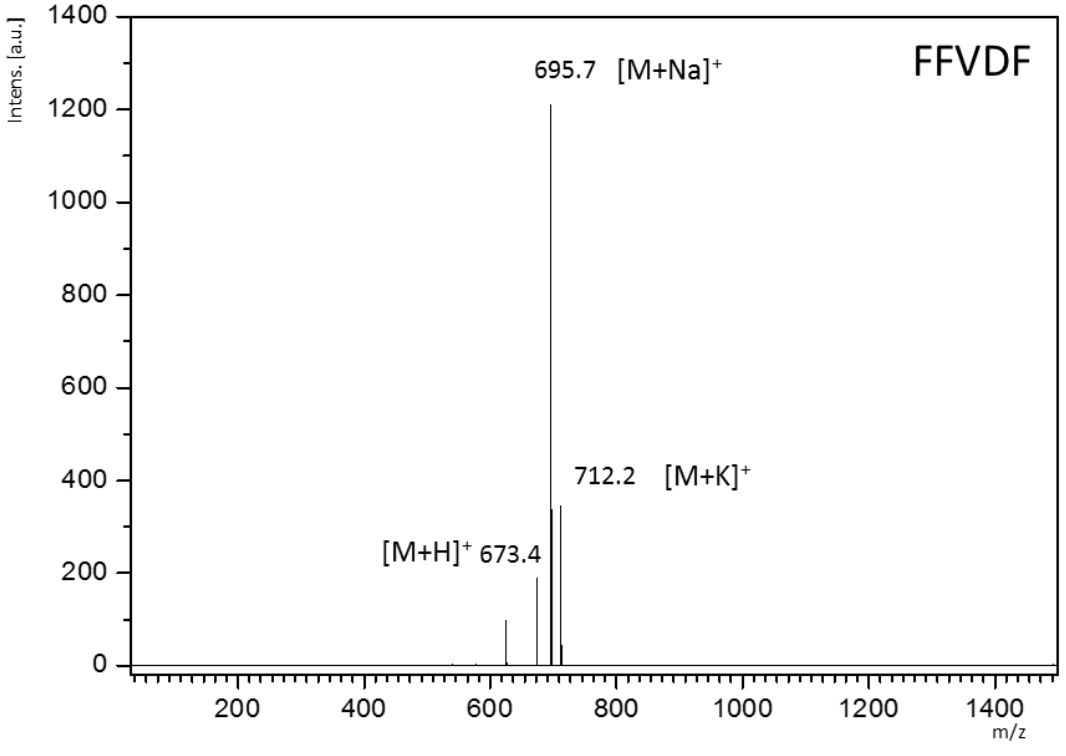

Figure S11. The MALDI-TOF spectrum of peptide FFVDF.

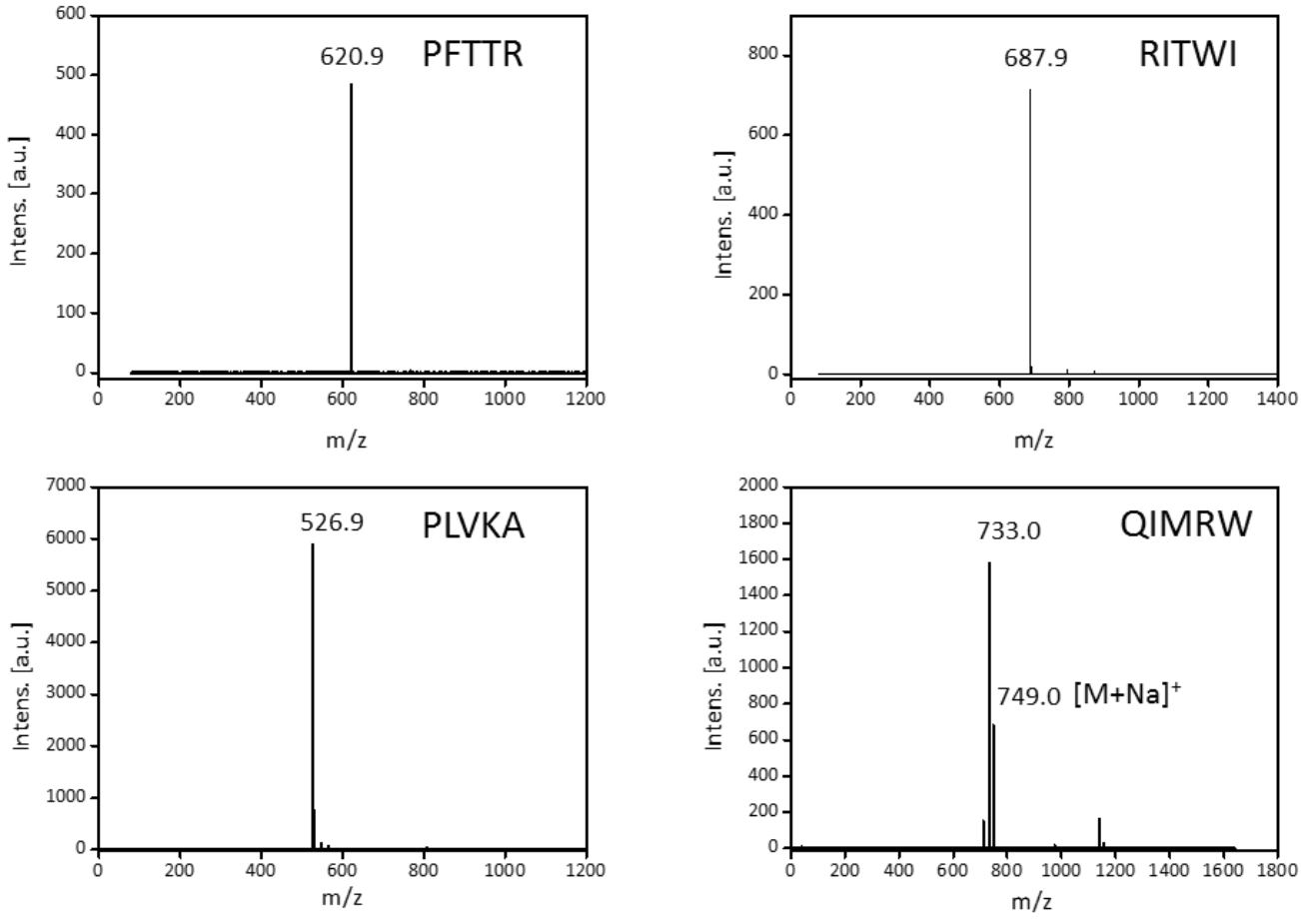

Figure S12. The MALDI-TOF spectra of negative non-assembling pentapeptides identified from screening OBOC library.

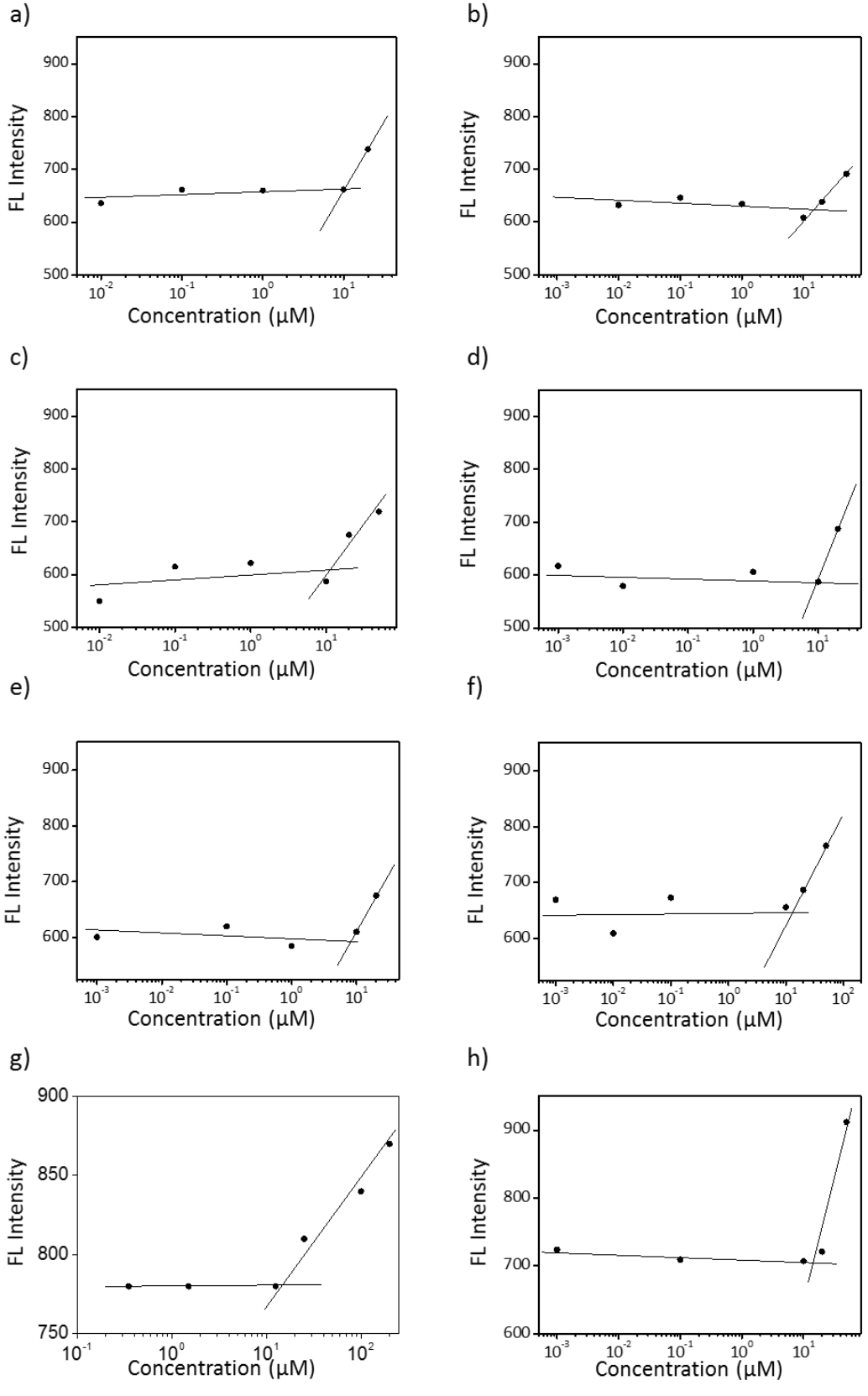

Figure S13 The CMC values of the peptide a)-h) FTISD, ITSVV, YFTEF, ISDNL, LDFPI, FAGFT, FGFDP and FFVDF in PBS with 1% DMSO.

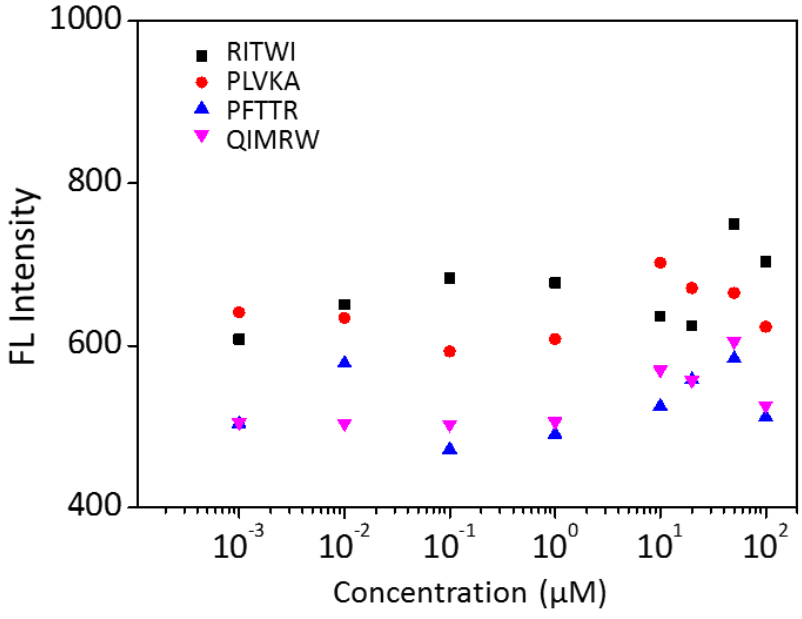

Figure S14 The CMC experiment of non-assembling peptide RITWI, PLVKA, PFTTR, and QIMRW in PBS with 1% DMSO.

Table 1 *q* values, Miller indexes, observed *d*-spacings (d_{obs}, nm) and calculated *d*-spacings (d_{cal}, nm) of FFVDF crystals in the nanofibers.

q(nm^{-1})	*h*	*k*	*l*	d_{obs} (nm)	d_{cal} (nm)
1.81	0	1	0	3.47	3.53
2.77	0	0	2	2.27	2.28
3.41	0	1	2	1.84	1.92
5.64	0	0	4	1.11	1.14
7.24	0	4	0	0.87	0.88
8.94	0	4	4	0.70	0.70
10.21	1	3	4	0.61	0.62
11.49	0	2	8	0.55	0.54
13.20	2	0	0	0.48	0.47
13.73	2	0	2	0.46	0.47
14.15	2	2	2	0.44	0.45
15.54	0	8	4	0.40	0.41
15.75	2	4	4	0.40	0.39
17.24	2	4	6	0.36	0.37
20.22	2	8	4	0.31	0.31

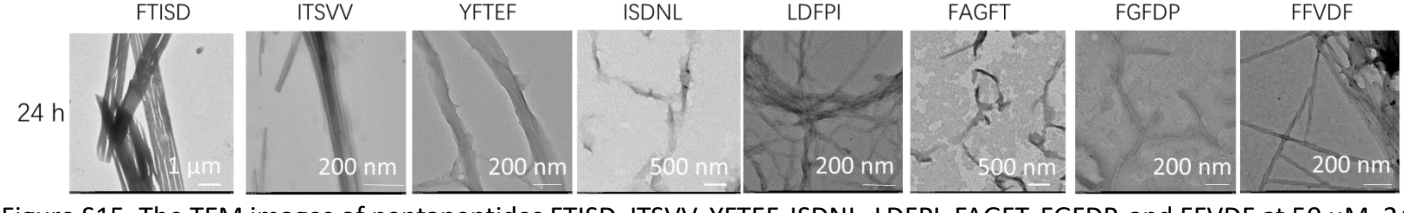

Figure S15. The TEM images of pentapeptides FTISD, ITSVV, YFTEF, ISDNL, LDFPI, FAGFT, FGFDP, and FFVDF at 50 μM, 24 h after solubilized in water with 1% DMSO. The scale bar: 1 μm.

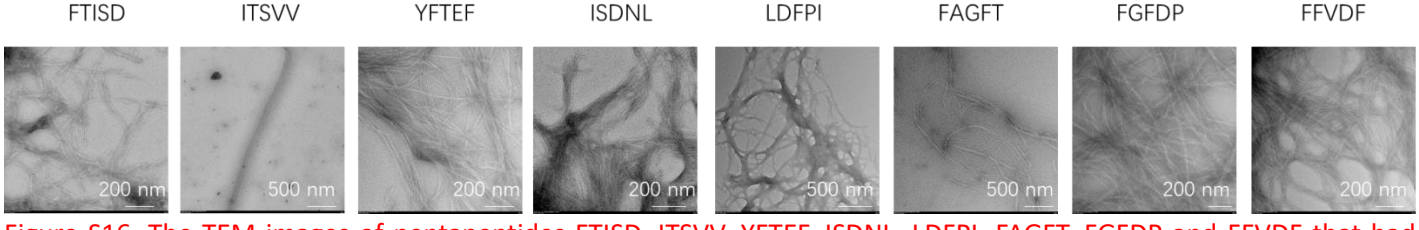

Figure S16. The TEM images of pentapeptides FTISD, ITSVV, YFTEF, ISDNL, LDFPI, FAGFT, FGFDP and FFVDF that had undergone thermal annealing at 90 °C for 5 h.

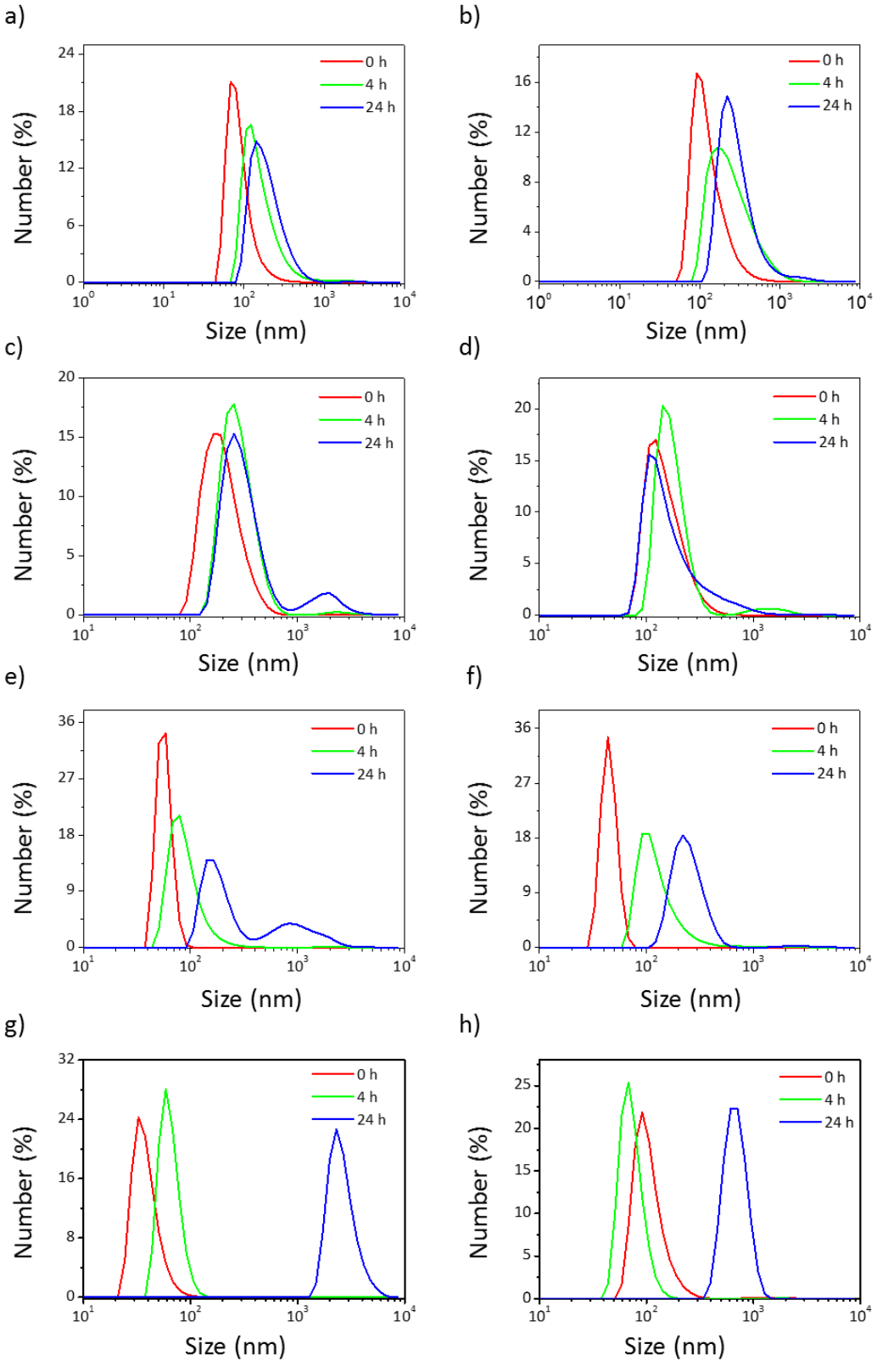

Figure S17. The DLS spectra of the pentapeptides a) - h) FTISD, ITSVV, YFTEF, ISDNL, LDFPI, FAGFT, FGFDP, and FFVDF at 50 µM in water with 1% DMSO, obtained at 0, 4 and 24 h.

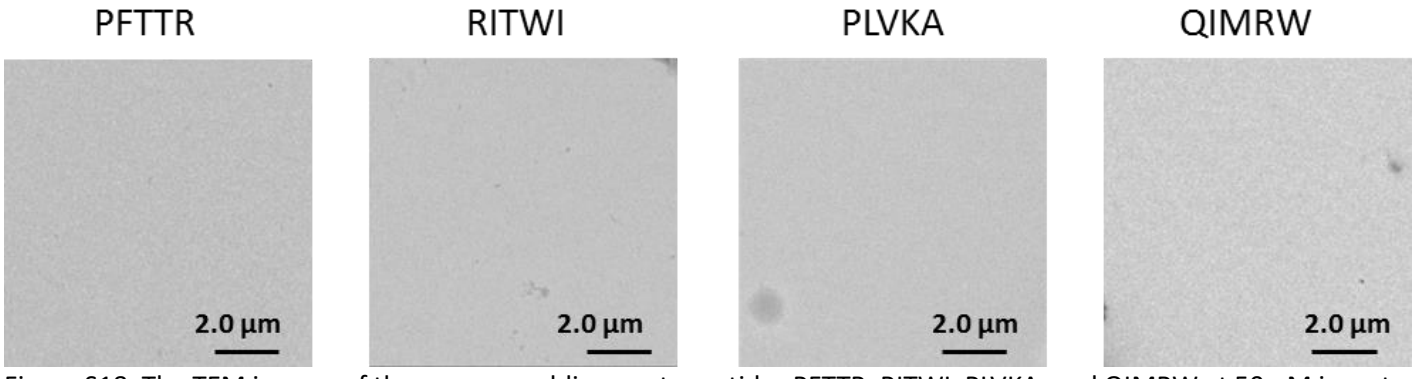

Figure S18. The TEM images of the non-assembling pentapeptides PFTTR, RITWI, PLVKA, and QIMRW at 50 µM in water with 1% DMSO after 24 h. No nanostructure was detected.

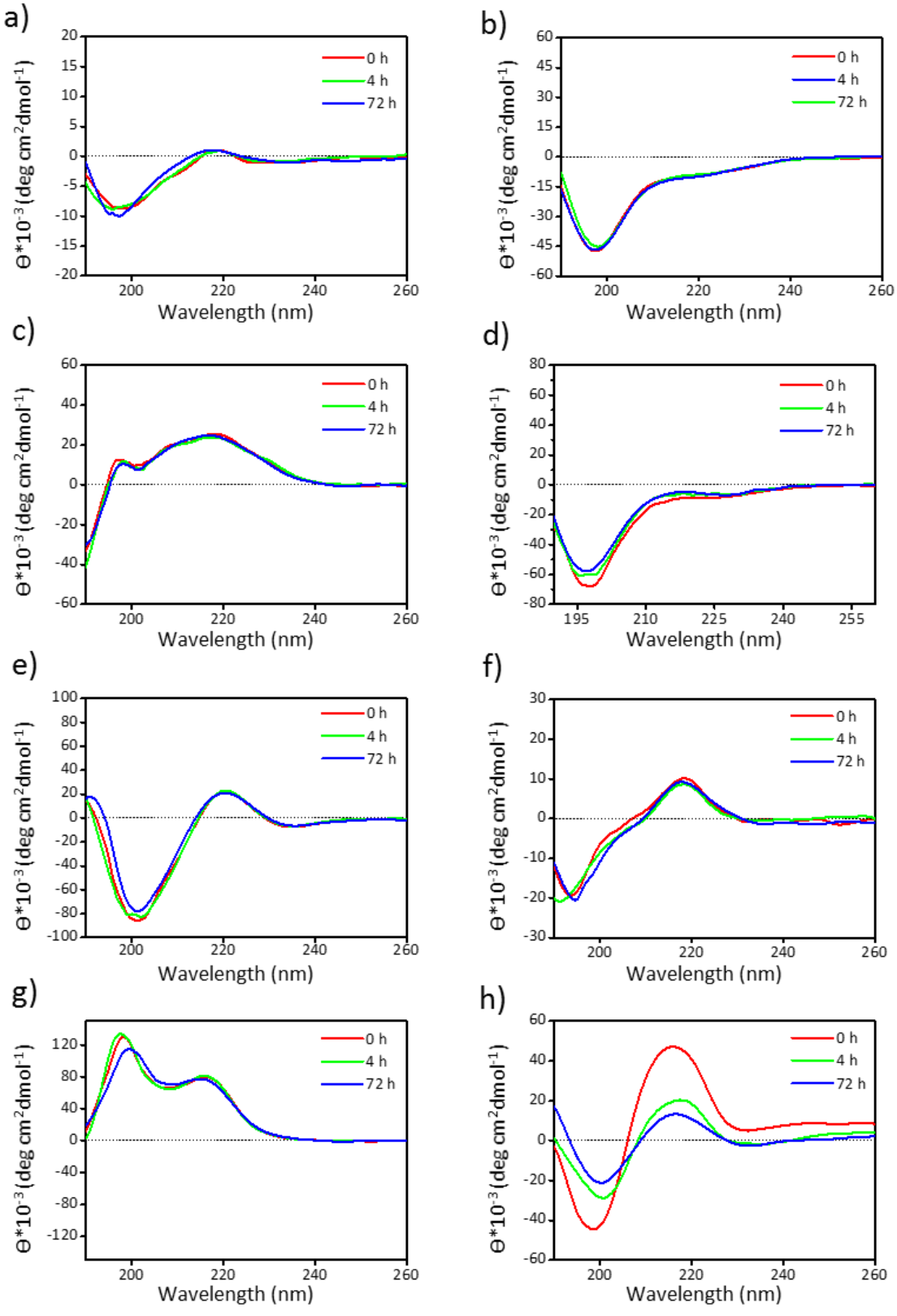

Figure S19. The time-dependent CD spectra of the pentapeptides FTISD, ITSVV, YFTEF, ISDNL, LDFPI, FAGFT, FGFDP, FFVDF at 40 µM corresponding to a)-h) in water with 1% DMSO. Significant changes in CD-spectra were observed over time in FFVDF peptide.

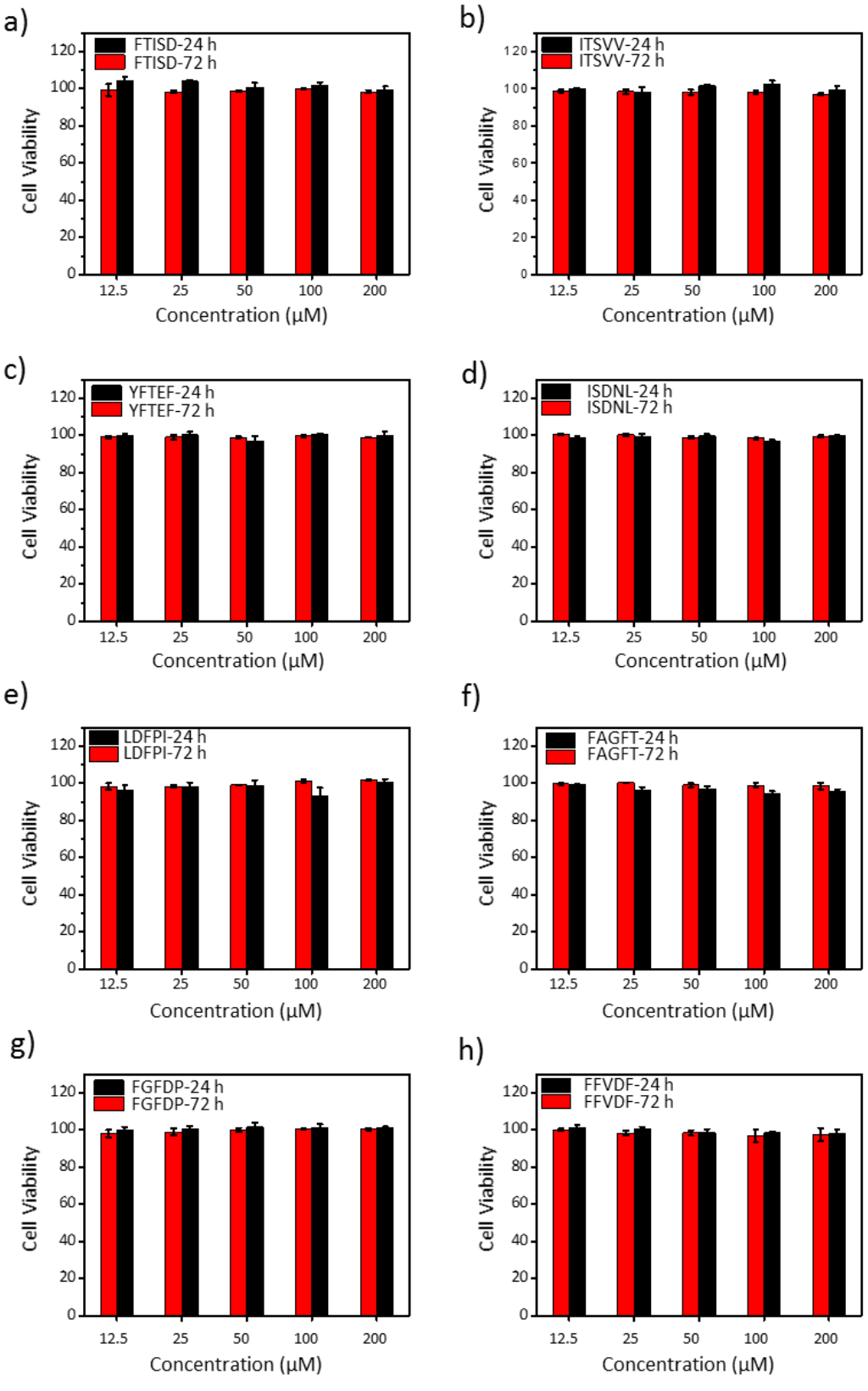

Figure S20. CCK-8 cell viability assay for 8 pentapeptides (FTISD, ITSVV, YFTEF, ISDNL, LDFPI, FAGFT, FGFDP, FFVDF) after incubation with HeLa cells for 24 and 72 h. No cell toxicity was observed.

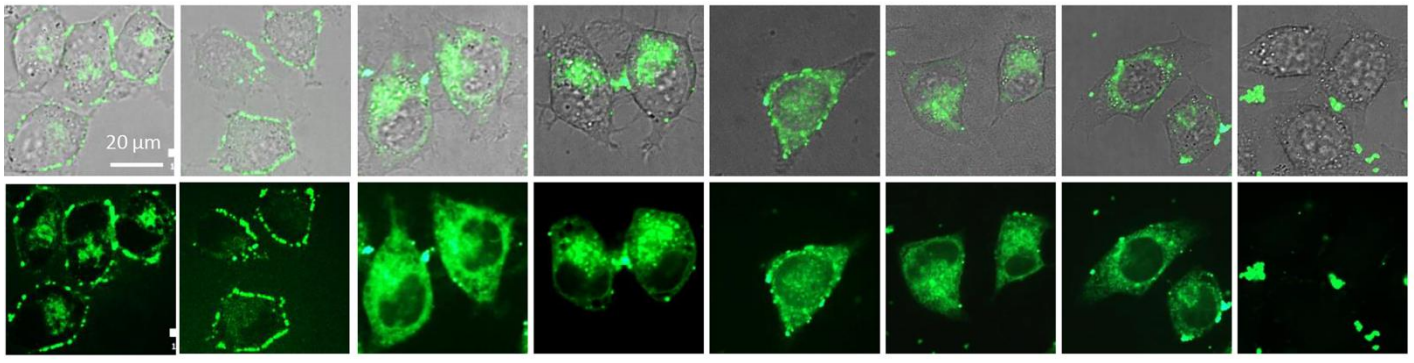

Figure S21. Confocal microscopy of HeLa cells after incubation with FITC-labeled pentapeptide assemblies (from left to right: FTISD, ITSVV, YFTEF, ISDNL, LDFPI, FAGFT, FGFDP, FFVDF) for 4 h; peptide concentration used was 50 µM. 3% of the assembled peptides was N-terminally labeled with FITC.

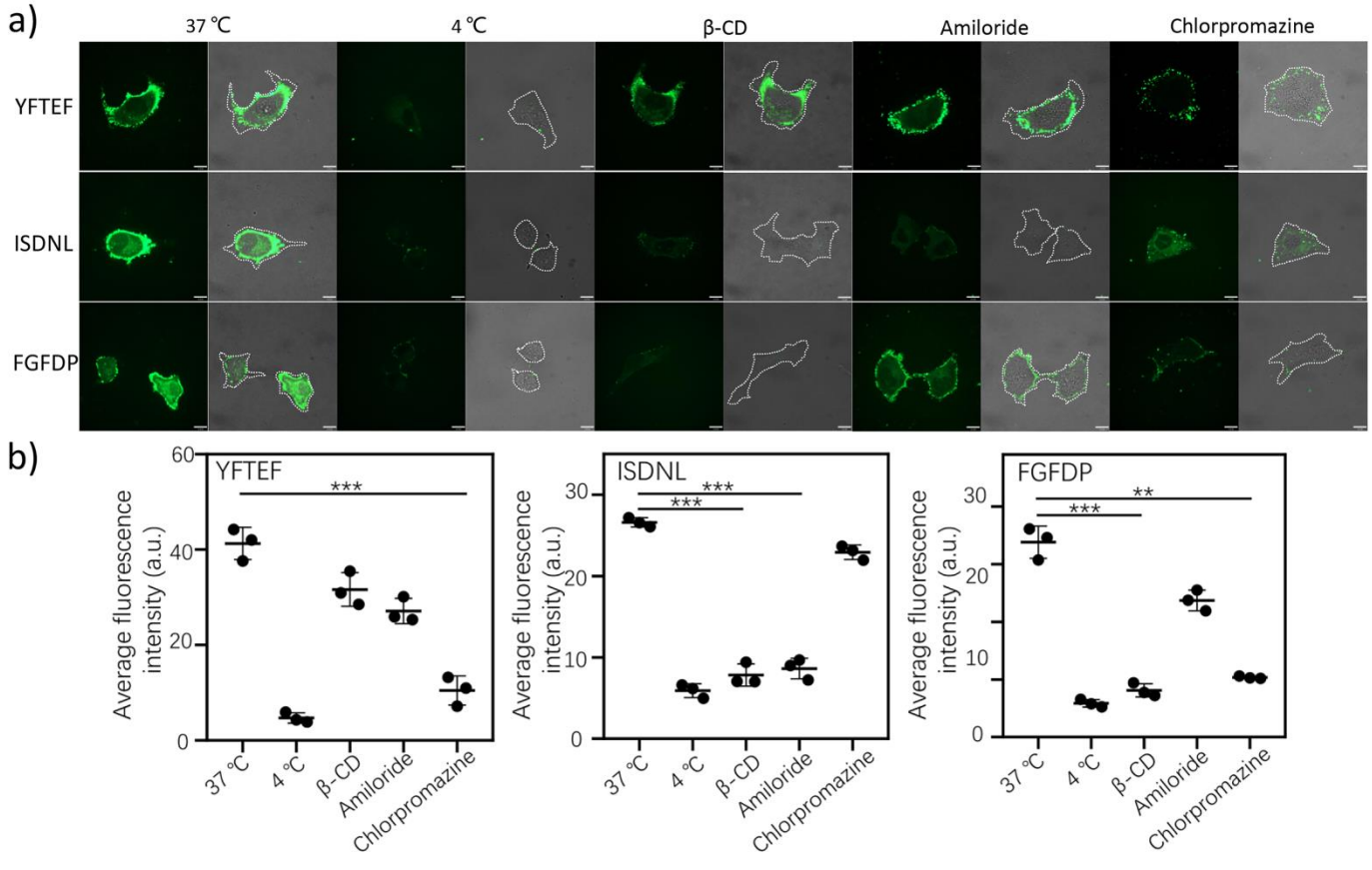

Figure S22. Cell uptake pathway analysis for FITC-labeled pentapeptide assemblies YFTEF, ISDNL and FDFDP. a) CLSM images of HeLa cells incubated with pentapeptides (50 µM) at different temperatures (37 or 4 °C) and in the presence of various endocytosis inhibitors, such as amiloride (2 mM), M-β-CD (5 mM), and chlorpromazine (50 µM). The scale bar is 10 µm. b) Relevant quantitative analysis of images from 'a'. 3% of the assembled peptides was N-terminally labeled with FITC.

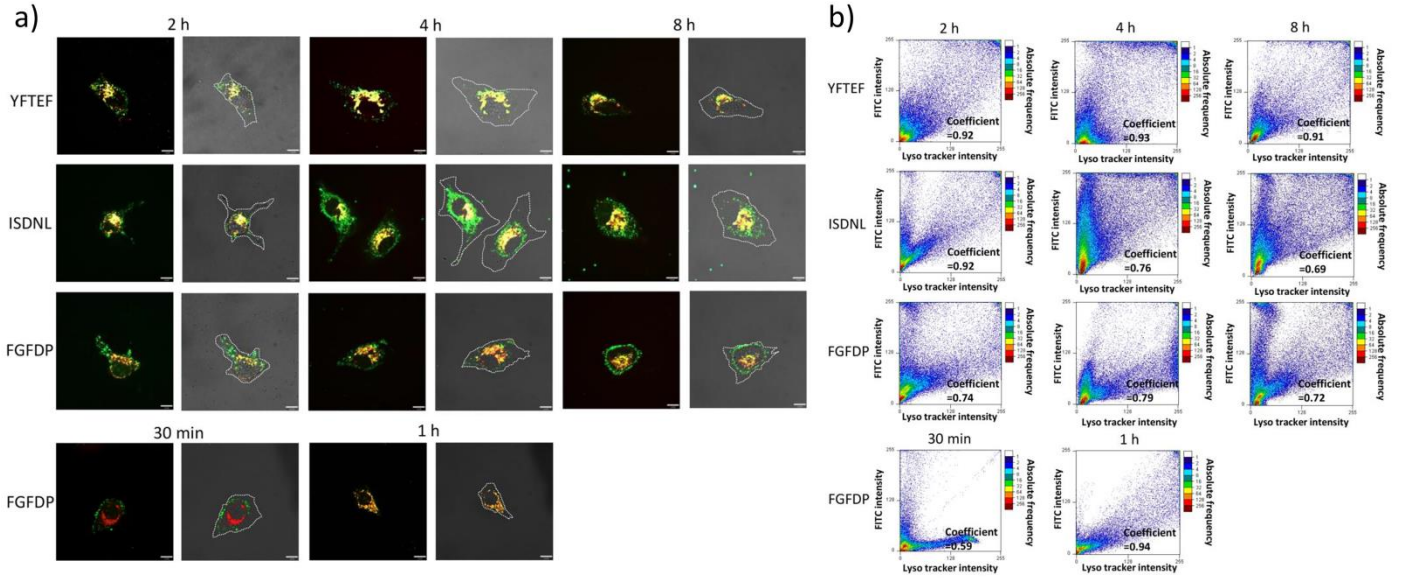

Figure S23. a) Time-dependent (2, 4, 8 h) CLSM images for monitoring and quantitatively analyzing uptake of fluorescent peptide assemblies (YFTEF, ISDNL, and FGFDP at 50 µM) by HeLa cells. The scale bar is 10 µm. b) Corresponding colocalization analysis of images from 'a'.

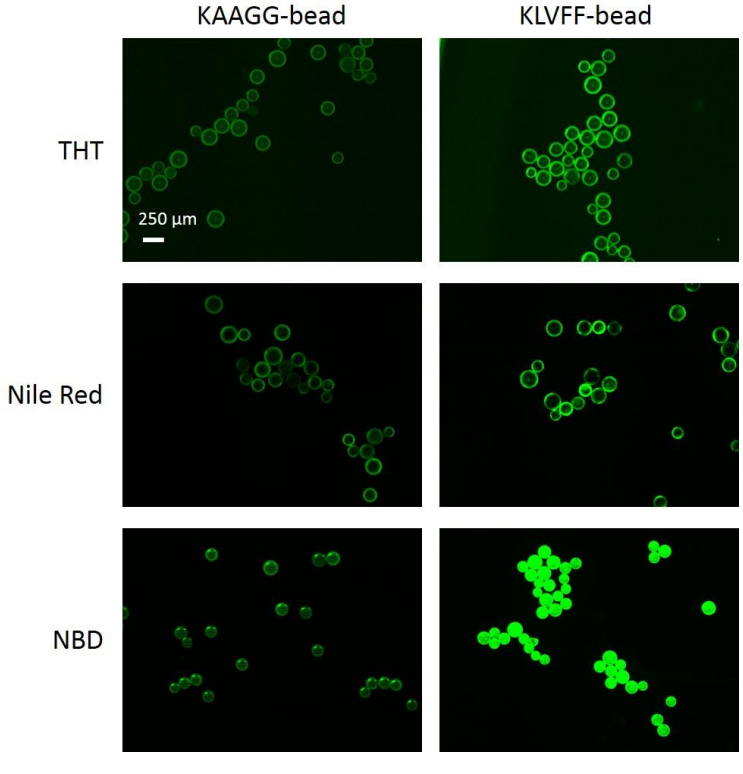

Figure S24. Fluorescent microscopy of KLVFF and KAAGG beads after incubation with free ThT, Nile Red, or NBD in water for 2 hours.