Controlled nucleation and formation rate of self-assembled peptide nanofibers

Lei Lu, [a, b] Devlin Morrison [b] and Larry D. Unsworth [* b]

[a] School of Life Science and Engineering, Southwest Jiaotong University, Chengdu, Sichuan, 611756, China

[b] Department of Chemical and Materials Engineering, University of Alberta, Edmonton, Alberta, T6G 1H9, Canada

*** Corresponding author:**

Larry D. Unsworth

Tel.: +1(780) 492 6020; Fax: +1(780) 492 2881,

Email address: larry.unsworth@ualberta.ca

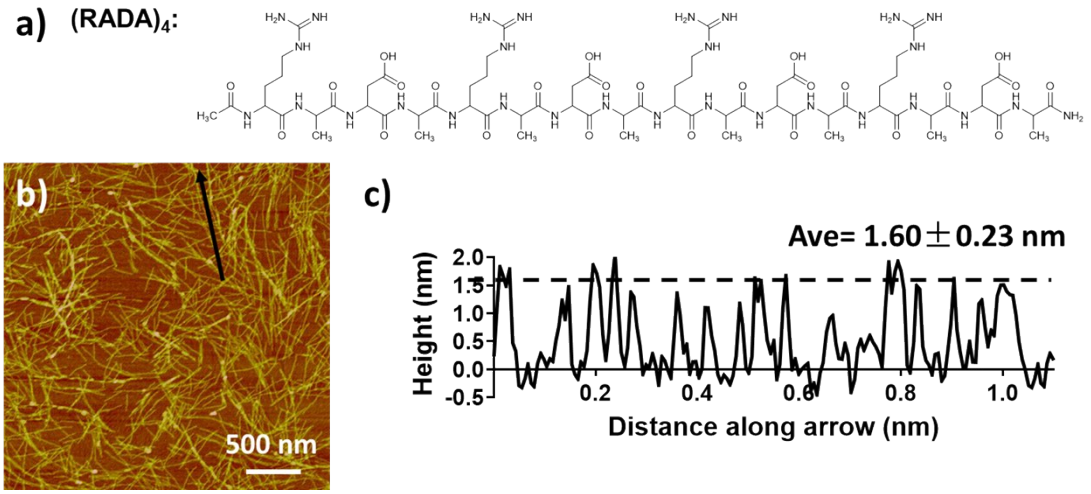

Fig. S1 (a) Chemical structure of $(RADA)_4$ self-assembling peptide. (b) Representative AFM images of 0.1% w/v $(RADA)_4$ nanofibers with 100x dilution. (c) The section height analysis of $(RADA)_4$ nanofibers (b, black arrow). The average height of nanofibers was collected and analyzed at random points for each sample. Data are represented as mean ± SEM, n≥50.

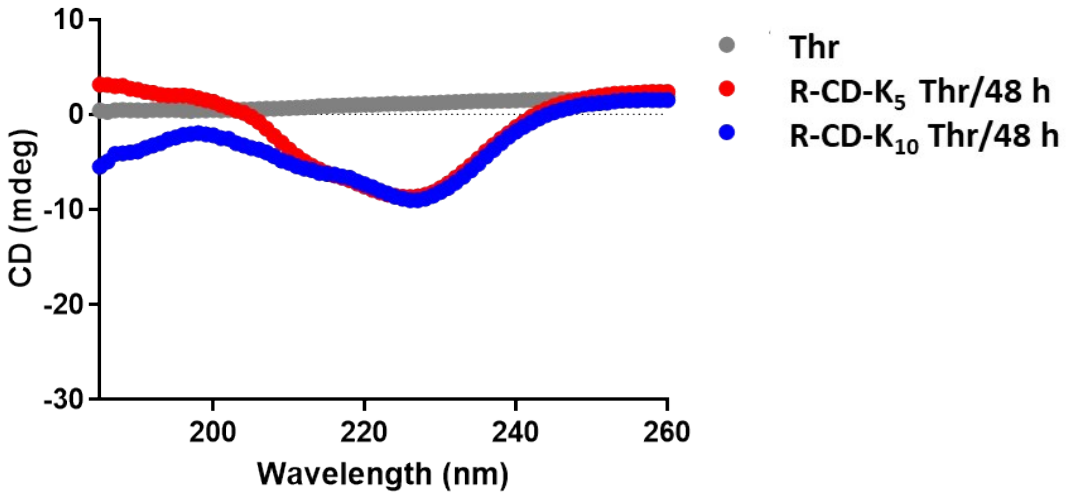

Fig. S2 The circular dichroism raw data of thrombin (Thr); R-CD-K_5 and R-CD-K_{10} incubation with thrombin for 48 h (Thr/48 h) in 5 mM PB.

Table S1. Estimated structure fractions of peptides at 25°C.

Items	Secondary-structure fractions (%)					β-Sheet Increase Rate*
	Helix	β-Sheet	β-Turn	Rndm. Coil	Total Sum	
R-CD	6.1	52.7	16.6	29.1	104.5	0.91
R-CD Thr/48 h	7.2	48.5	18.3	32.5	106.5	
R-CD-K_5	9.4	44.4	22.5	29.6	105.9	1.18
R-CD-K_5 Thr/48 h	8.8	54.1	18.4	28.3	109.5	
R-CD-K_{10}	9.9	35.5	24.2	32.5	102.1	1.30
R-CD-K_{10} Thr/48 h	8.9	48.1	19.3	30.2	106.5	

*β-Sheet increase=(Thr/48 h: β-Sheet/Total Sum)/(β-Sheet/Total Sum); At the current state of the trained networks, the RMS error (%) for the prediction of one of these protein structures is ~2.1

Materials and Methods

Materials

Human thrombin was purchased from Sigma-Aldrich (USA). R-CD (Ac-$(RADA)_4$-GG-NleTPR-COOH), R-CD-K_5 (Ac-$(RADA)_4$-GG-NleTPR/SFL-$(K)_5$-$CONH_2$) and R-CD-K_{10} (Ac-$(RADA)_4$-GG-NleTPR/SFL-$(K)_{10}$-$CONH_2$) peptides (≥90% purity by HPLC) were purchased from RS Synthesis (Louisville, KY, USA). The chemical structures of peptides are shown in Figure 1a. Peptide stock solutions (1.0% w/v) were prepared by dissolving peptide powder in syringe filtered (0.2 μm) Milli-Q water. Peptide stock solutions were sonicated for 30 min to avoid bulk aggregates.

Nanoparticle Size and Zeta-potential

The size (by dynamic light scattering, DLS) and Zeta-potential of the nanostructures in 5 mM phosphate buffer (PB, pH 7.4) were measured using the Malvern Zetasizer Nano-S (Malvern Instruments, UK).

Thrombin Cleavage Test

Peptides stock solution was diluted into 0.1% w/v with 5 mM PB (pH 7.4), with or without human thrombin (1 U/μL), and incubated at 25 ^oC for 1, 12, 24 and 48 h.

Atomic Force Microscopy (AFM)

The morphology of the nanoparticles and nanofibers were measured using Dimension 3100 Nanoman Atomic Force Microscopy (AFM, Veeco Metrology, LLC) with tapping mode. Peptide solutions used in AFM studies were prepared by 100

times dilution with Milli-Q water. A drop (5 µL) of each solution was placed on freshly cleaved mica substrate then rinsed with water. The surfaces were air dried overnight at room temperature before being imaged.

Circular Dichroism Measurement

The secondary structure of peptides was investigated using circular dichroism spectroscopy in 5 mM PB. Circular dichroism data was collected with a Jasco J-810 Circular Dichroism Chiroptical Spectrometer using a quartz cuvette with a 0.1 mm path length. A background spectrum was first collected for each sample using same concentration of PB or thrombin in PB. All circular dichroism measurements were repeated at least three times from 185 to 260 nm. The raw data was converted to mean residue ellipticity (θ).

Statistical Analysis

All data was measured in at least triplicate with independent repeats and presented as average ± standard deviation (S.D.). The statistical significance of differences between mean values was determined using one-way ANOVA followed by two-tailed Student's t-test for analysis of variance, where significance was evaluated for $p<0.05$, $p<0.01$, $p<0.001$.