Supporting Information

Amyloid engineering – how terminal capping modifies morphology and secondary structure of supramolecular peptide aggregates

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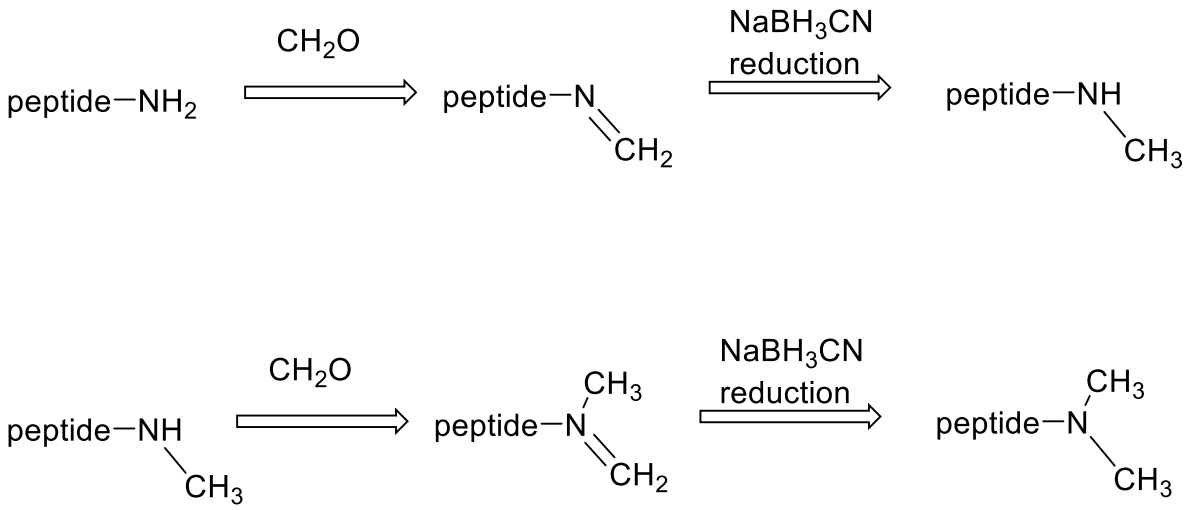


Figure S1. Chemical steps to obtain N,N-dimethylated peptides.

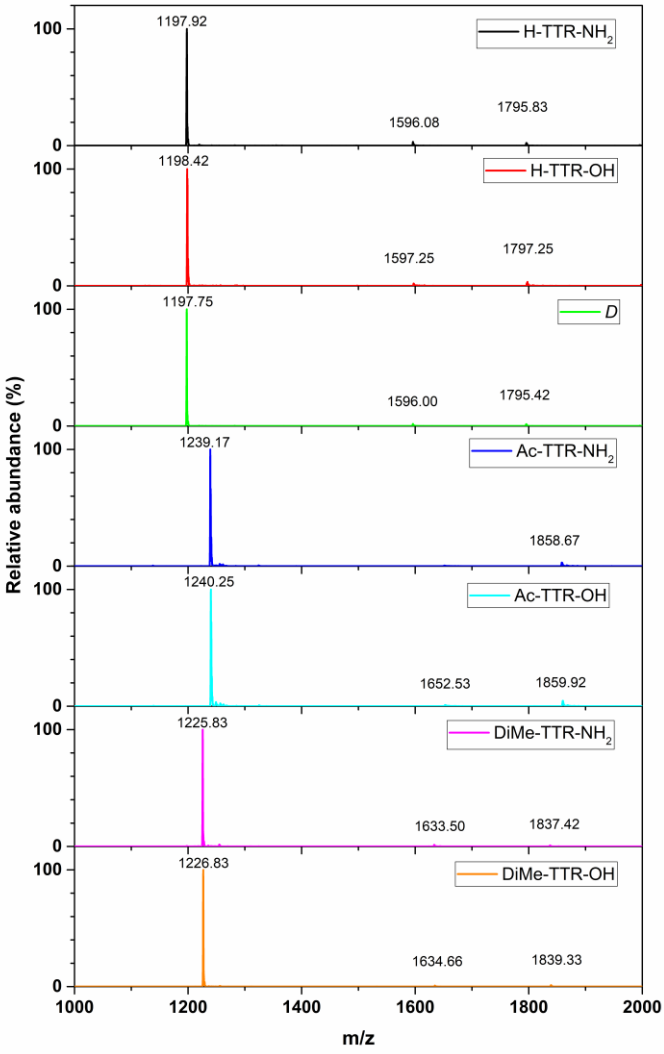


Figure S2. ESI MS spectra of the investigated peptides, namely H-TTR-NH_2 (black), H-TTR-OH (red), *D* (green), Ac-TTR-NH_2 (blue), Ac-TTR-OH (cyan), DiMe-TTR-NH_2 (magenta), DiMe-TTR-OH (orange). The most abundant peaks correspond to hydrogen ion adducts ($M+H^+$). The less intense peaks are the result of ion cluster formation ($4M+3H^+$ and $3M+2H^+$).

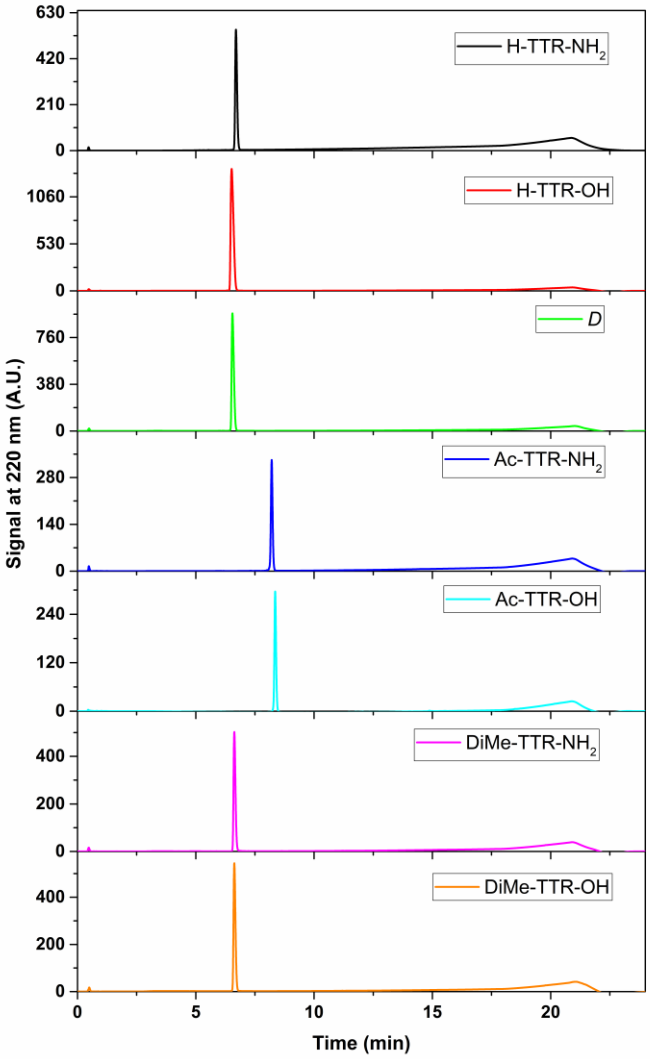


Figure S3. Analytical RP-HPLC traces confirming purity (>98%) of the studied peptides, namely H-TTR-NH_2 (black), H-TTR-OH (red), *D* (green), Ac-TTR-NH_2 (blue), Ac-TTR-OH (cyan), DiMe-TTR-NH_2 (magenta), DiMe-TTR-OH (orange).

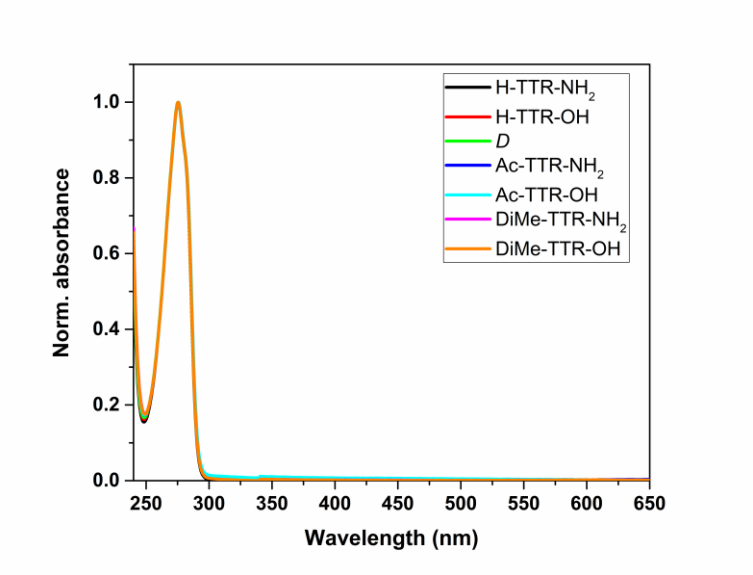


Figure S4. Normalized absorption spectra of the examined peptides, namely: H-TTR-NH_2 (black), H-TTR-OH (red), *D* (green), Ac-TTR-NH_2 (blue), Ac-TTR-OH (cyan), DiMe-TTR-NH_2 (magenta), and DiMe-TTR-OH (orange).

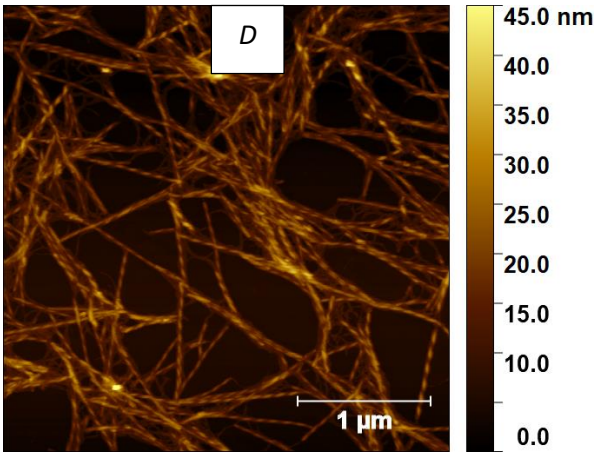
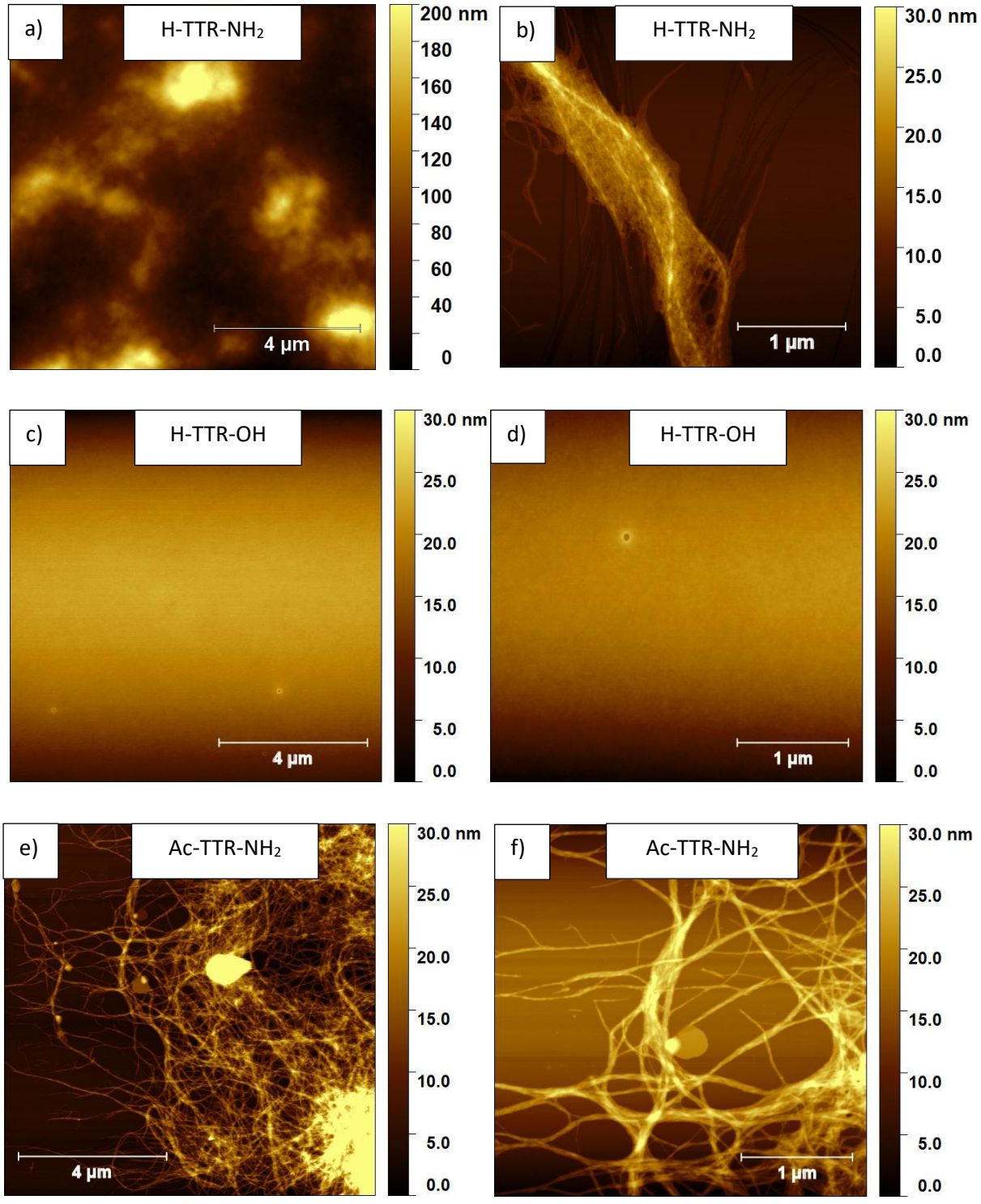


Figure S5. Morphology of peptide aggregates at t_1 of *D*-enantiomer of TTR(105-115), capped by α-amino and amide groups at N- and C-terminus, respectively. For the AFM imaging the sample was not diluted.

Table S1. Analysis of the average width, height and cross-over distance of the peptide supramolecular structures at t_1, formed upon incubation of the *L*-peptides and their quasi-racemic mixtures.

Sample	Width (nm)	Height (nm)	Cross-over distance (nm)
H-TTR-NH_2	22.1 ± 2.7	11.7 ± 2.4	98.0 ± 6.5
H-TTR-OH	22.2 ± 3.9	8.5 ± 2.7	104.4 ± 11.7
D	23.3 ± 2.8	11.9 ± 2.7	96.8 ± 6.9
DiMe-TTR-NH_2	16.4 ± 2.5	2.5 ± 1.0	-
DiMe-TTR-OH	20.3 ± 8.0	3.6 ± 2.2	-
D + H-TTR-NH_2	103.7 ± 39.8	5.3 ±1.3	-
D + H-TTR-OH	18.0 ± 3.6	6.9 ± 1.7	-
D + Ac-TTR-NH_2	104.6 ± 56.3	5.1 ± 1.3	-
D + Ac-TTR-OH	21.8 ± 3.4	7.4 ± 1.4	-
D + DiMe-TTR-NH_2	63.6 ± 33.5	7.5 ± 2.4	-
D + DiMe-TTR-OH	17.0 ± 3.2	7.3 ± 1.3	-


a)
H-TTR-NH2
4 µm
200 nm
180
160
140
120
100
80
60
40
0
b)
H-TTR-NH2
1 µm
30.0 nm
25.0
20.0
15.0
10.0
5.0
0.0
c)
H-TTR-OH
4 µm
30.0 nm
25.0
20.0
15.0
10.0
5.0
0.0
d)
H-TTR-OH
1 µm
30.0 nm
25.0
20.0
15.0
10.0
5.0
0.0
e)
Ac-TTR-NH2
4 µm
30.0 nm
25.0
20.0
15.0
10.0
5.0
0.0
f)
Ac-TTR-NH2
1 µm
30.0 nm
25.0
20.0
15.0
10.0
5.0
0.0

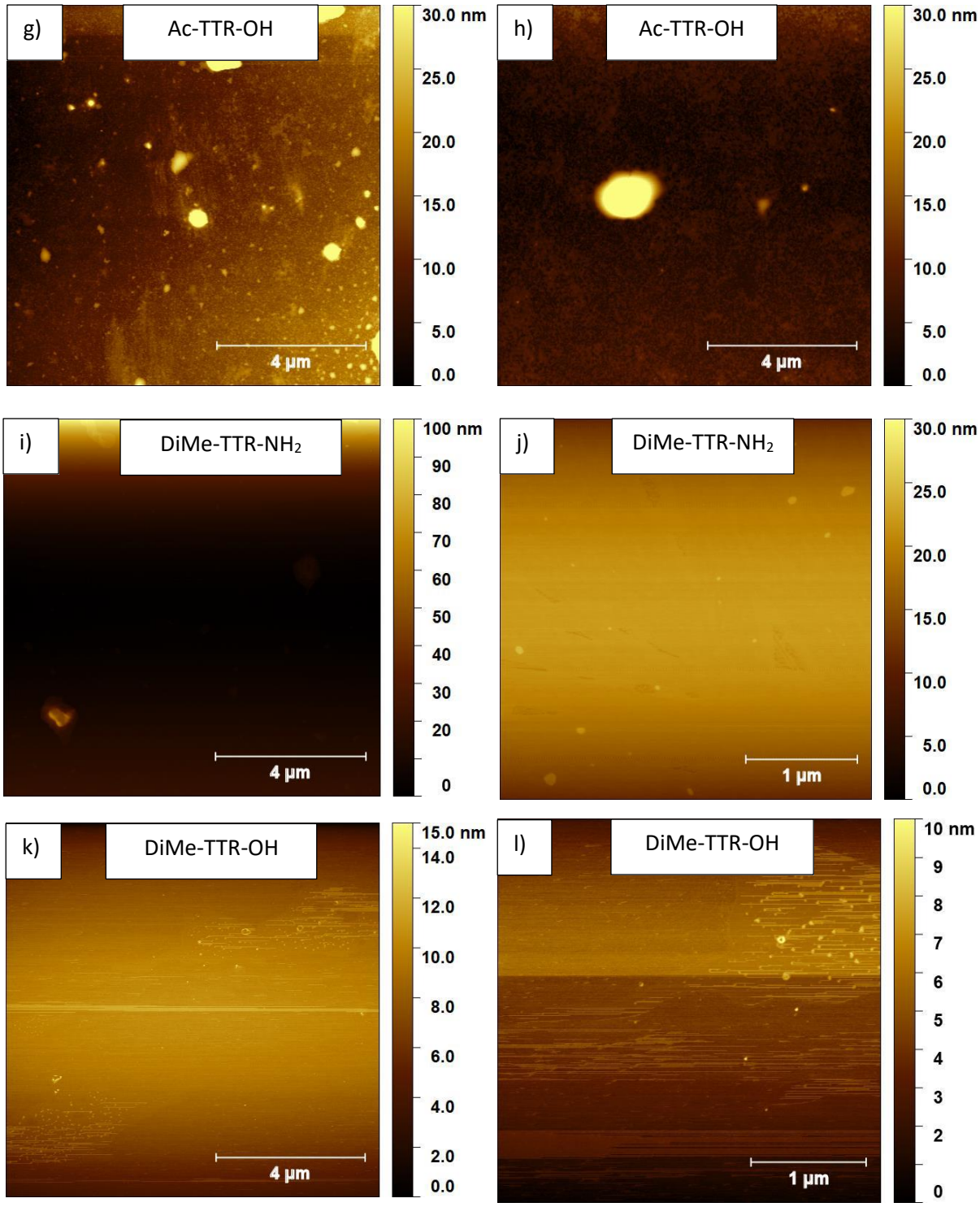


Figure S6. Morphology of assemblies of *L*-peptides: (a, b) H-TTR-NH_2, (c, d) H-TTR-OH, (e, f) Ac-TTR-NH_2, (g, h) Ac-TTR-OH, (i, j) DiMe-TTR-NH_2, and (k, l) DiMe-TTR-OH observed at t_0. For the AFM imaging the samples were not diluted.

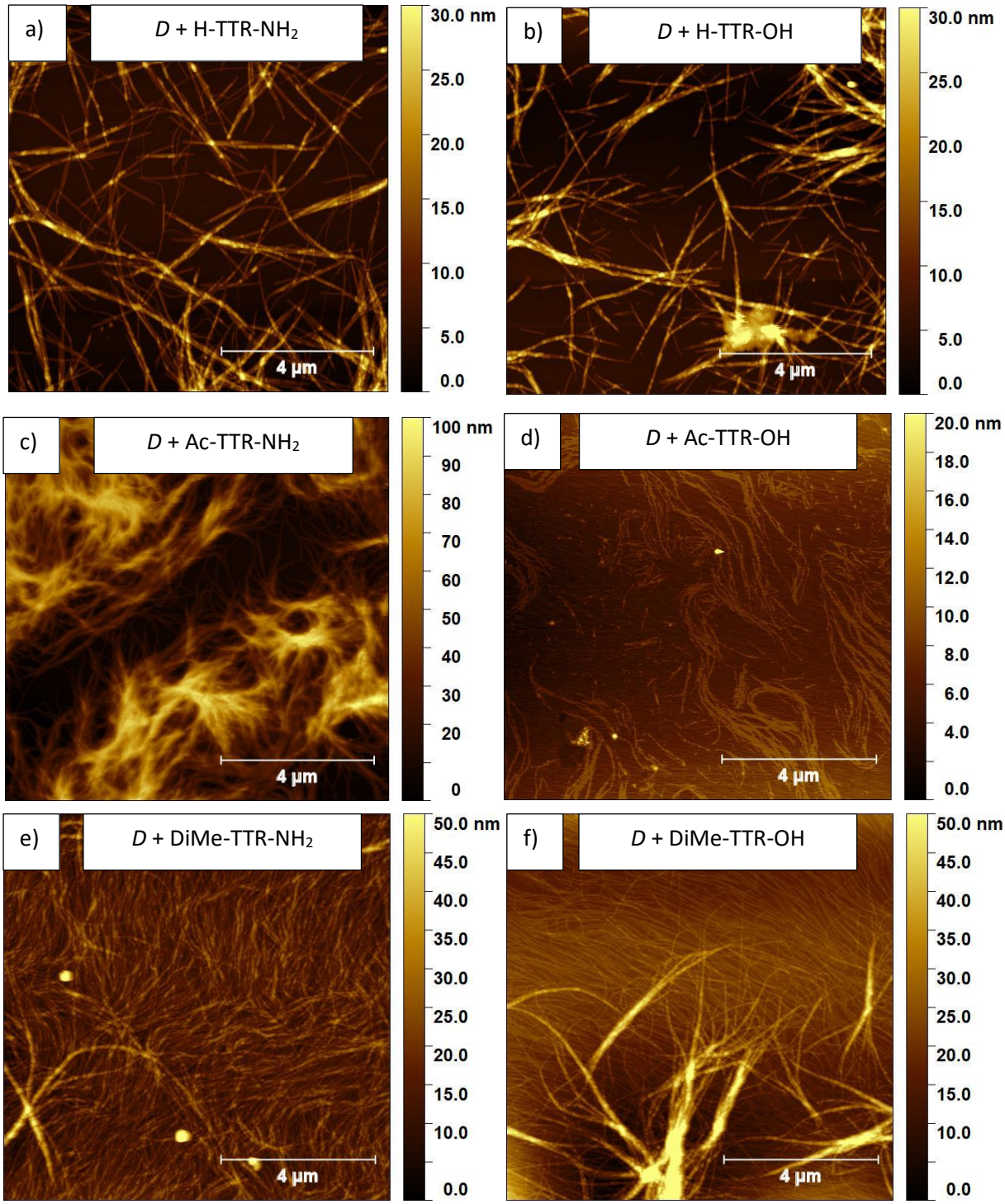
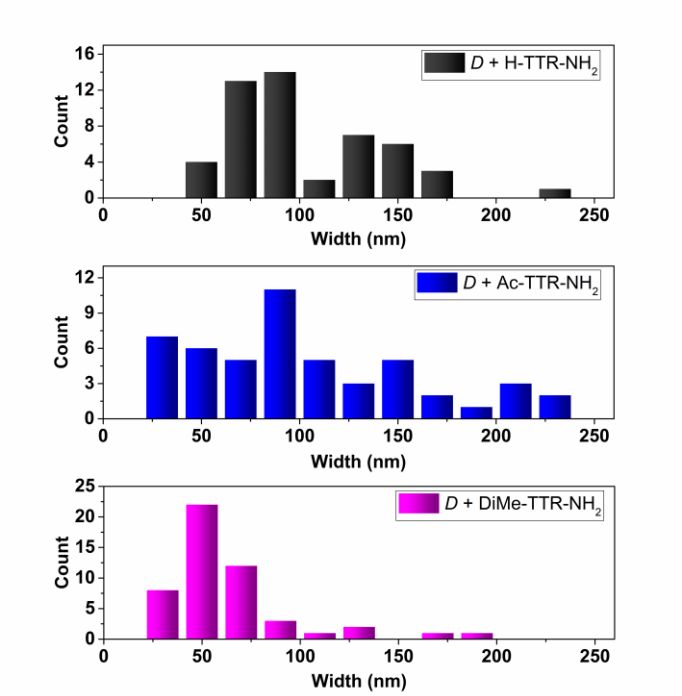


Figure S7. Morphology of assemblies of quasi-racemic peptide mixtures, namely of *D* with: (a) H-TTR-NH_2, (b) H-TTR-OH, (c) Ac-TTR-NH_2, (d) Ac-TTR-OH, (e) DiMe-TTR-NH_2, and (f) DiMe-TTR-OH. The images represent morphology observed at t_0. For the AFM imaging the samples (a, b, e, f) were 10 times diluted; the samples (c, d) were 1 000 times diluted.

a)



b)

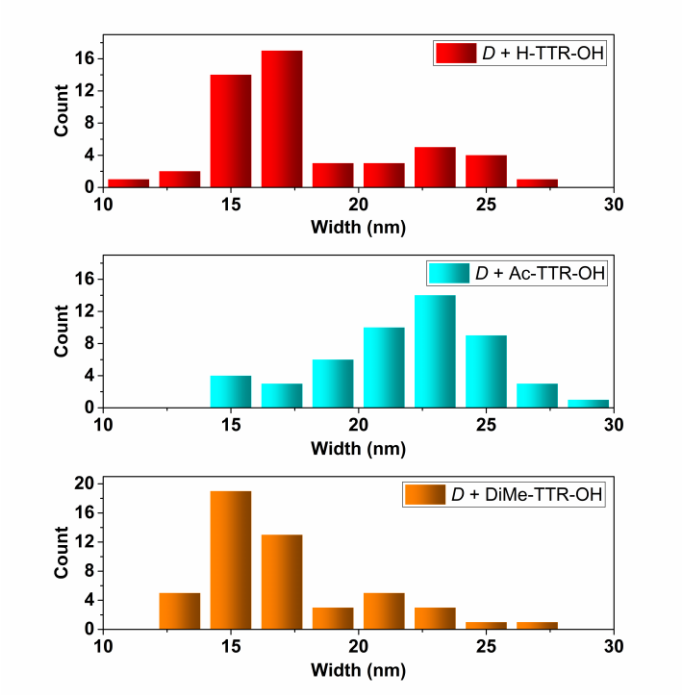
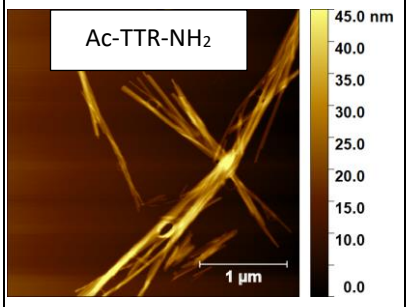
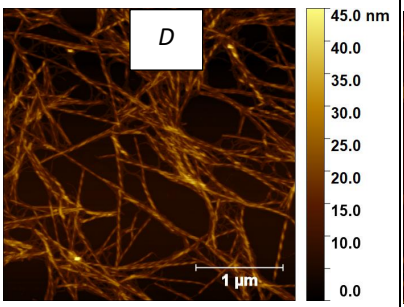
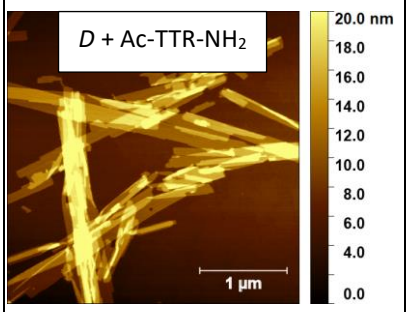
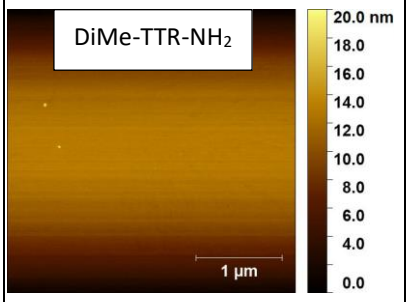
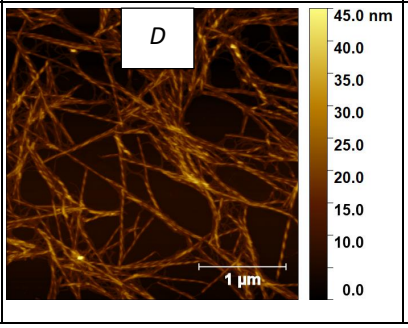
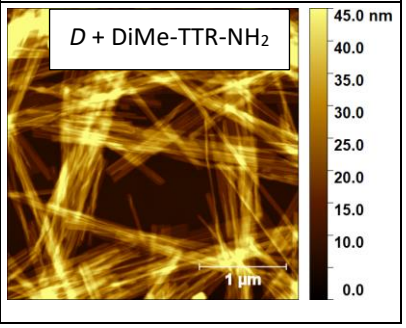


Figure S8. Width distribution at t_1 among fibrils formed upon co-incubation of *D* with peptides C-terminally capped by (a) -NH_2 and by (b) -OH group.

Table S2. Morphology observed for enantiomers *L* (left), enantiomer *D* (middle) and the corresponding quasi-racemic mixtures (right).

Enantiomer *L*	Enantiomer *D*	Quasi-racemic mixture
		
		

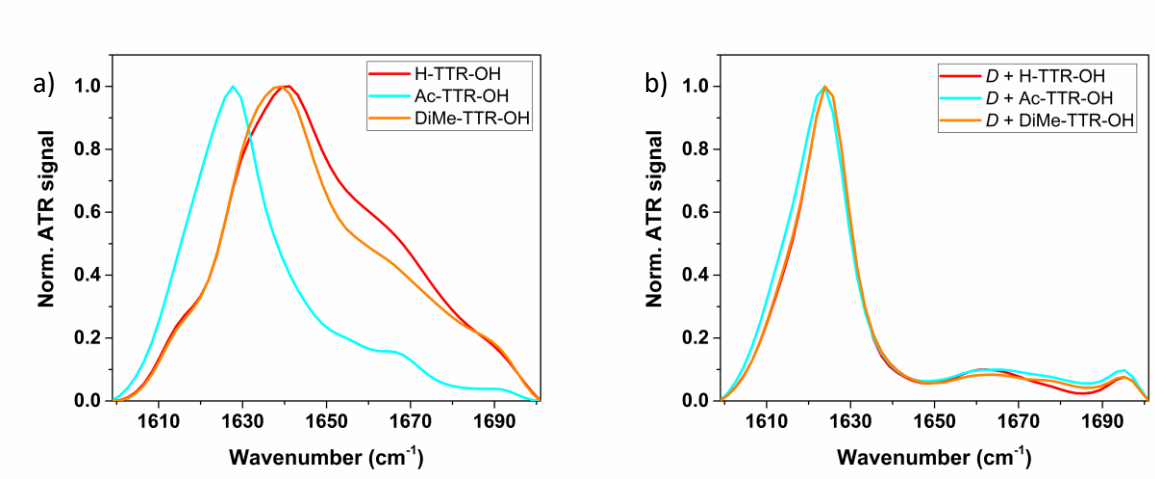


Figure S9. Normalized ATR-FTIR spectra of the studied samples after the incubation period; (a) of *L*-peptides C-terminally capped by carboxyl group and (b) of their quasi-racemic mixtures with *D*-enantiomer. The spectra were recorded at t_1.

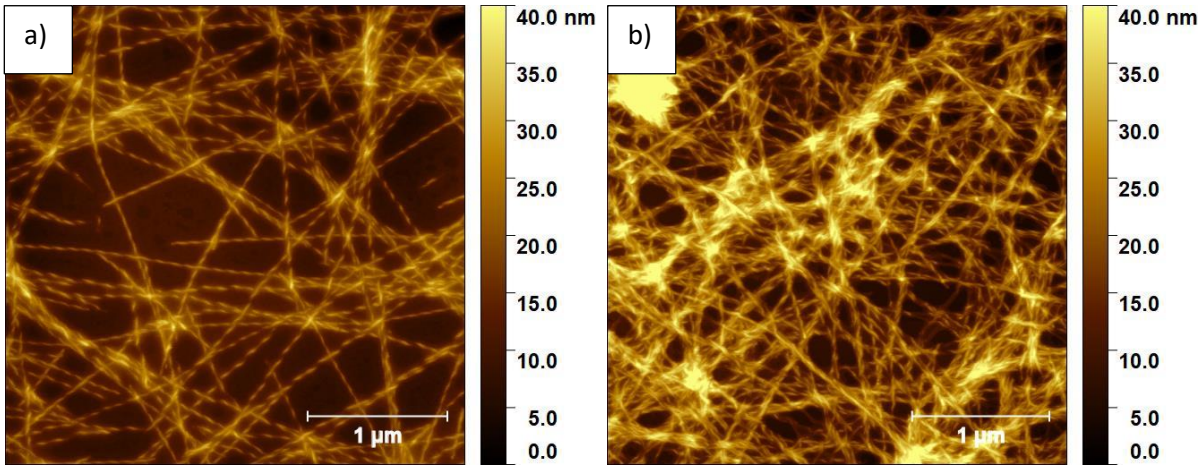


Figure S10. Morphology of fibrils formed from H-TTR-OH at t_2. For the AFM imaging the samples were not diluted.

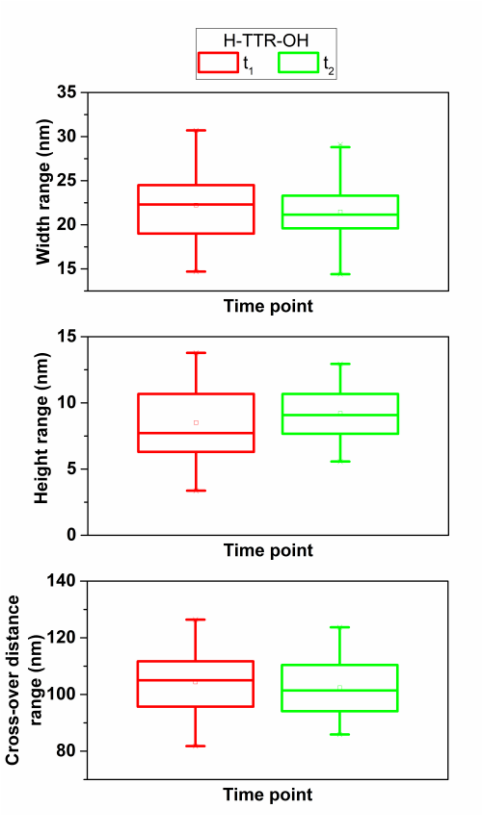


Figure S11. Box charts demonstrating width (nm), height (nm) and cross-over distance ranges (nm) calculated for H-TTR-OH fibrils at t_1 (red) and t_2 (green).

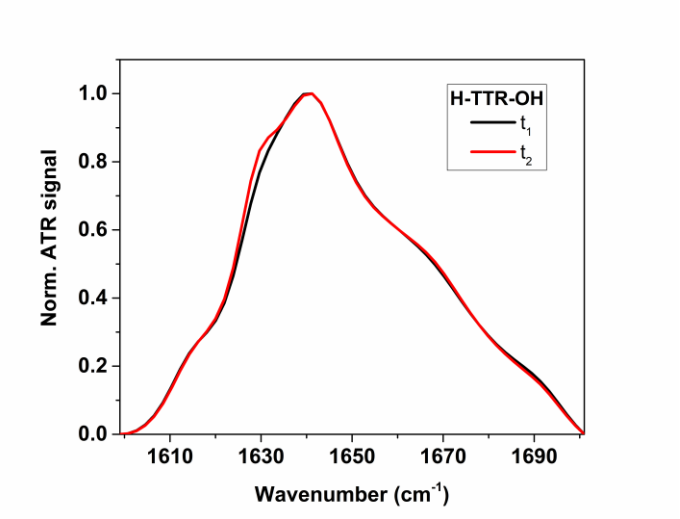
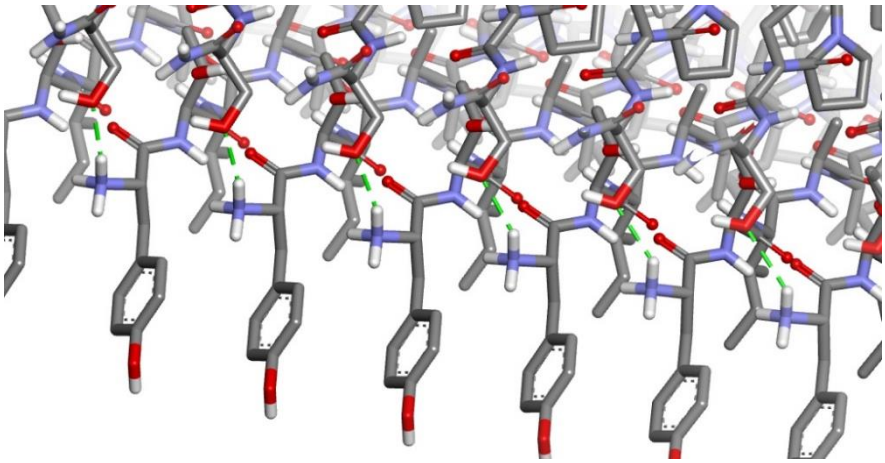
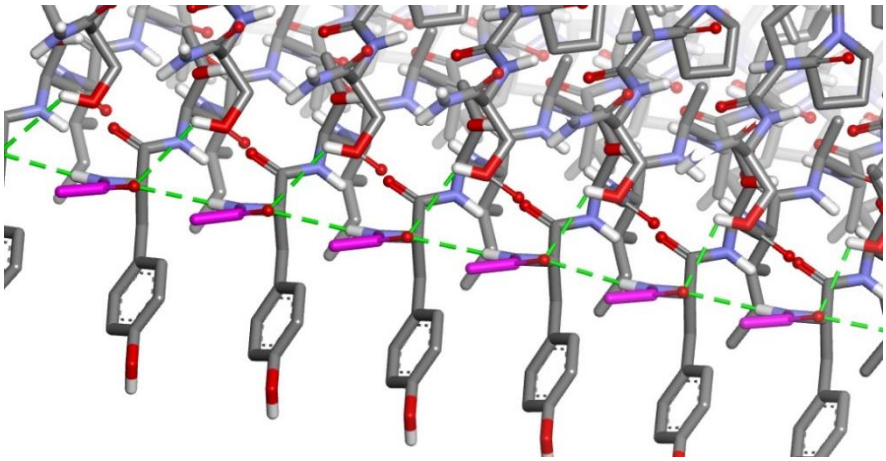


Figure S12. Normalized ATR-FTIR spectra obtained for fibrils formed from H-TTR-OH after 3 weeks of incubation (t_1) (black) and after 7 weeks of incubation, indicated as t_2 (red).

a)



b)



c)

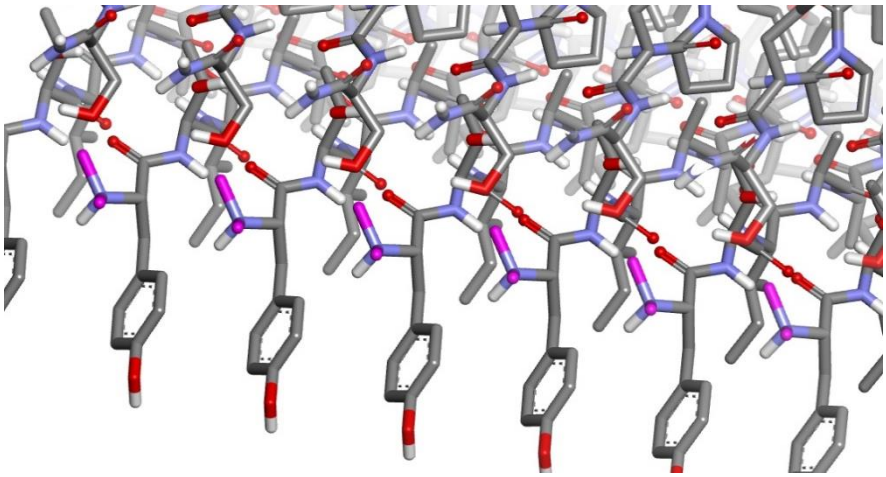
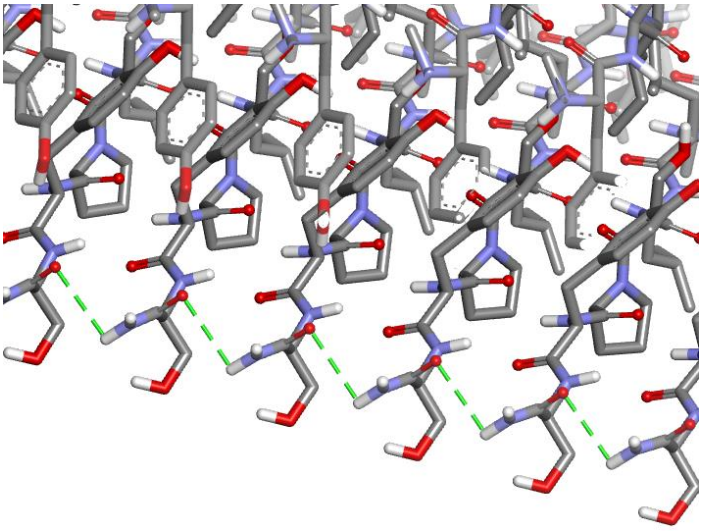


Figure S13. Molecular models of bilayers formed by (a) H-TTR-NH_2, (b) Ac-TTR-NH_2, and (c) DiMe-TTR-NH_2 peptides showing their N-termini (N-acetyl-α-amino and N,N-dimethyl-α-amino groups are highlighted in magenta). Green dotted lines represent hydrogen bonds.

a)



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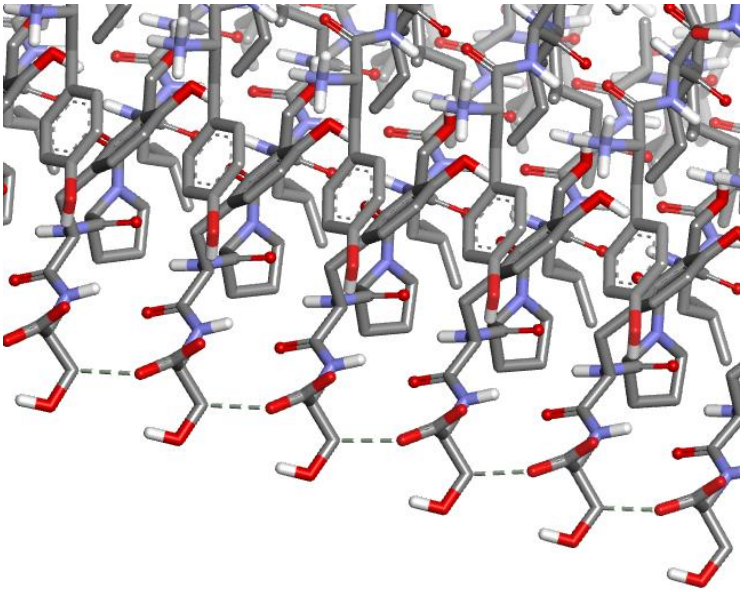

Figure S14. Molecular models of bilayers formed by (a) H-TTR-NH_2, (b) H-TTR-OH peptides showing their C-termini. Green dotted lines represent conventional hydrogen bonds, while weak carbon-oxygen hydrogen bonds are indicated by gray dotted lines.