Structural Basis for Inhibiting β–Amyloid Oligomerization by a Non-Coded β-Breaker Substituted Endomorphin Analogue

Supporting Information

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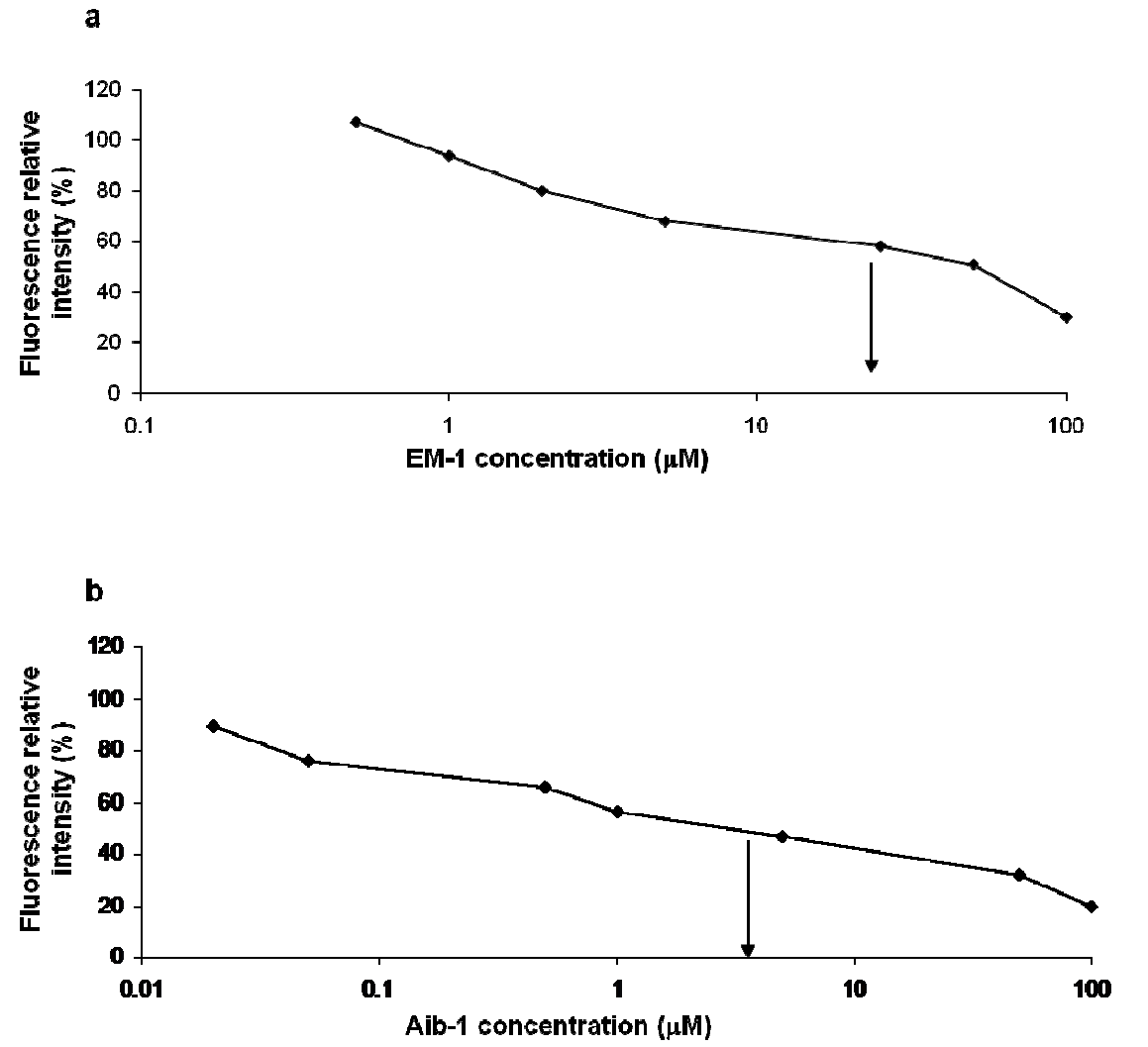
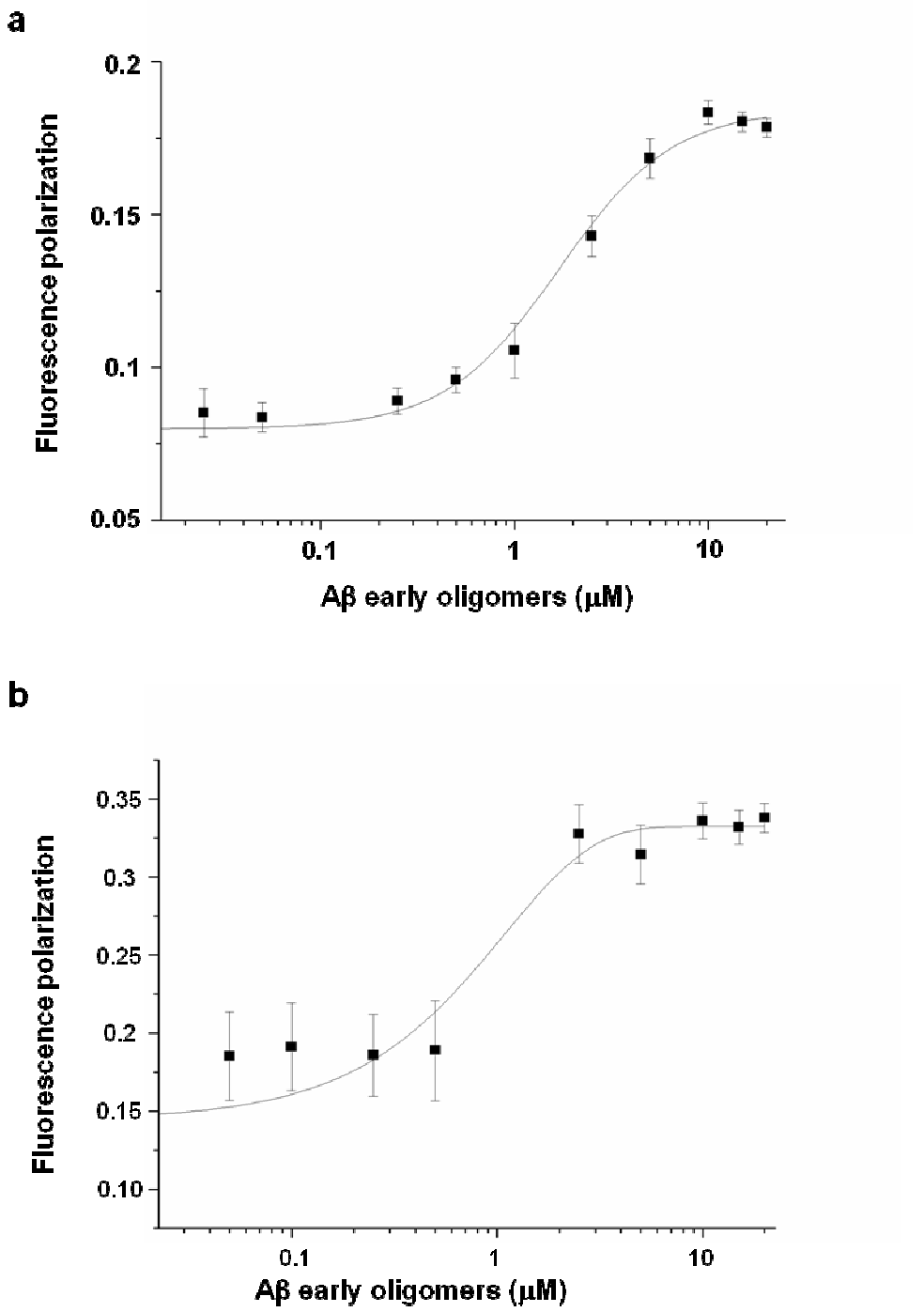
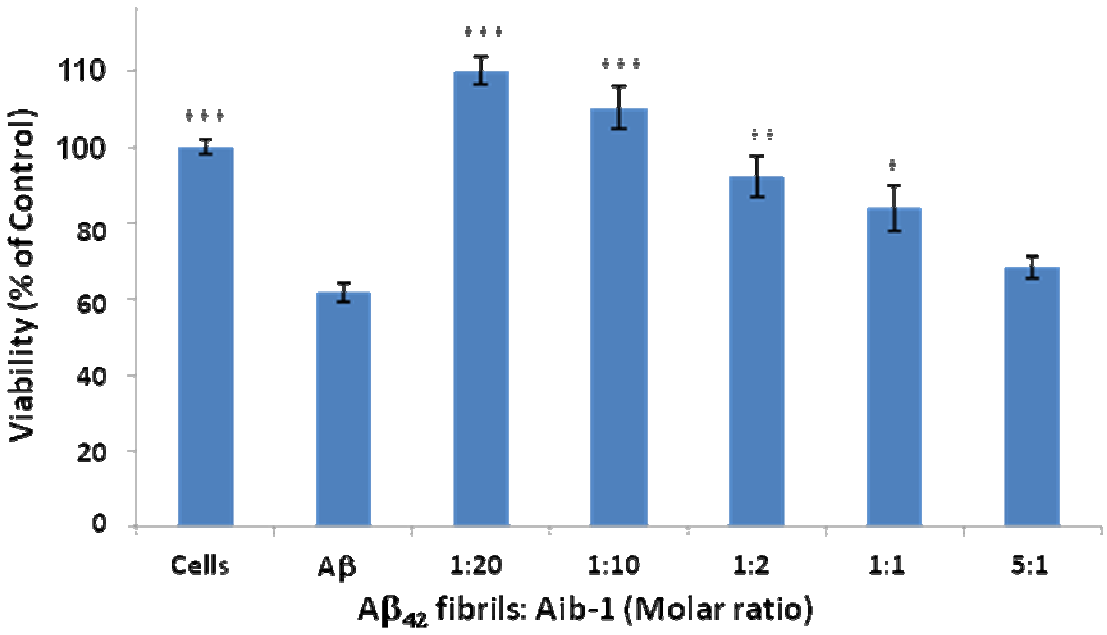


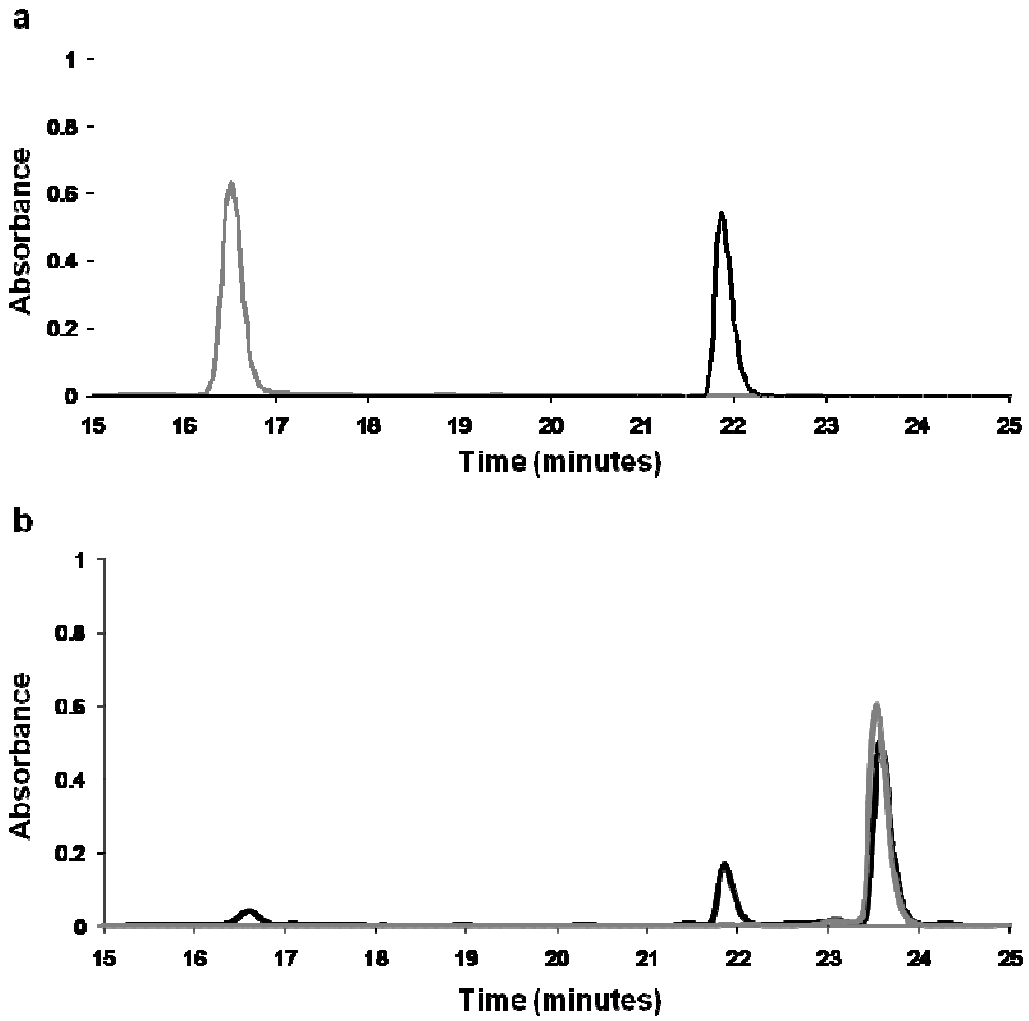
S1: Concentration-dependent inhibition of $A\beta_{1-42}$ fibrillogenesis. **a)** EM-1 and **b)** Aib-1 were added at different concentrations to a fixed amount of 5 μM $A\beta_{1-42}$. After incubation for 264 hours at 25°C, ThT fluorescence was monitored at an emission wavelength of 480 nm (excitation at 450 nm).



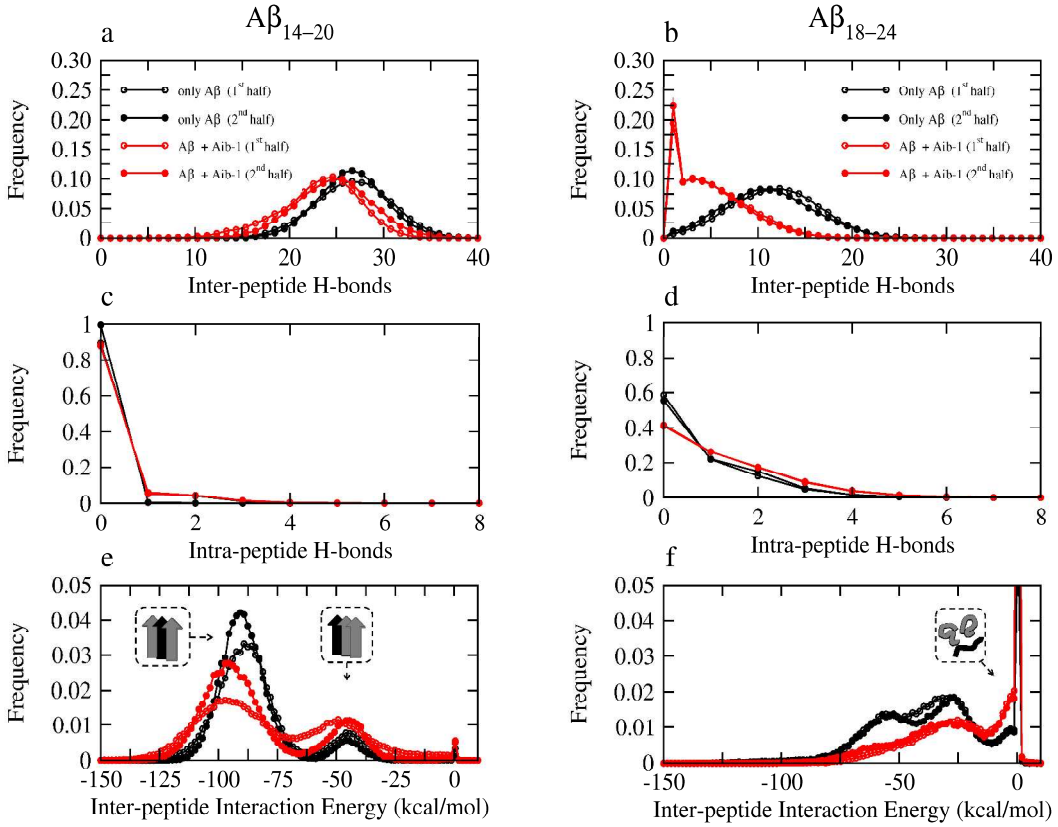
S2. The affinity of EM-1 and Aib-1 towards Aβ early oligomers. Fluorescence anisotropy binding curve of **a)** EM-1 and **b)** Aib-1 upon titration with Aβ early oligomers.



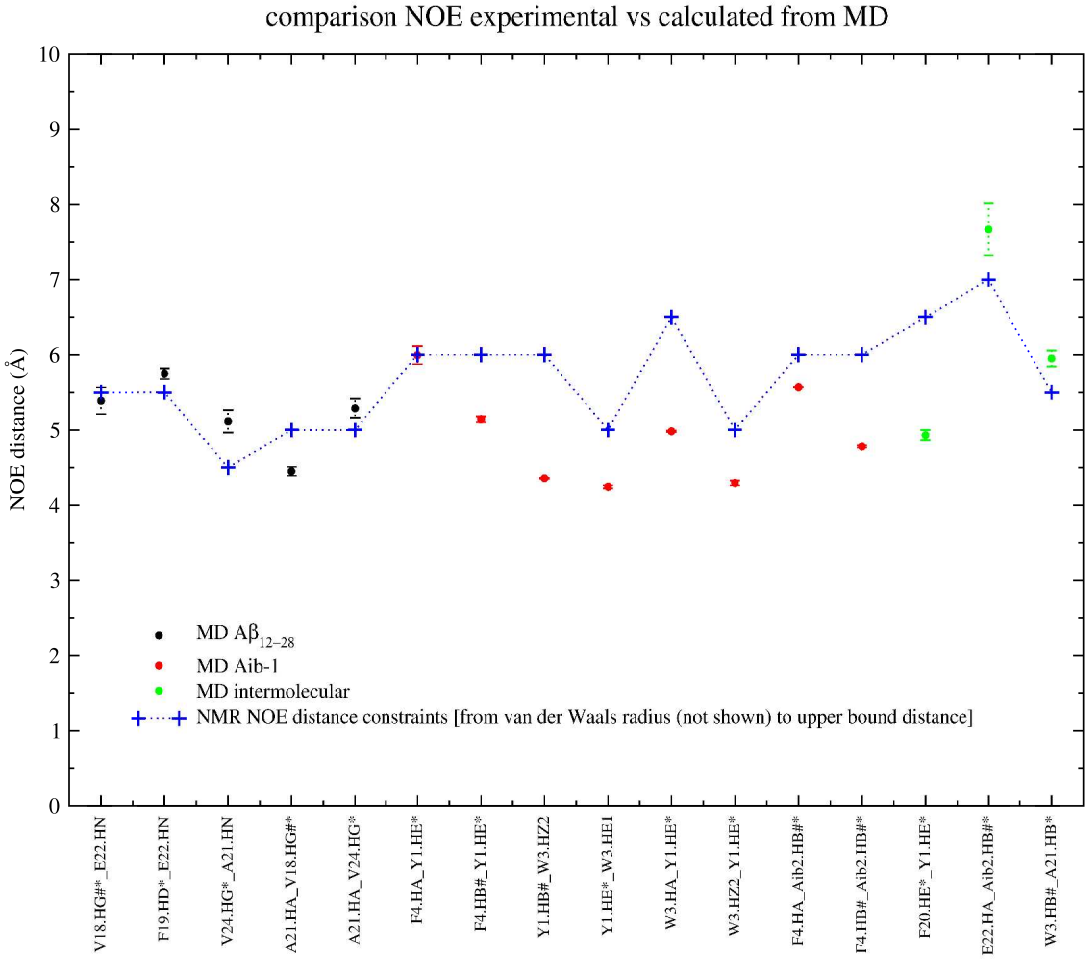
S3: Aib-1 alleviates toxic effects of Aβ fibrils towards PC12 cell line: Samples of $A\beta_{1\text{-}42}$ fibrils (1 μM) with or without various concentrations of Aib-1 were incubated for 24 hours with PC12 cells culture. Cells viability was determined using MTT viability assay. *** Pv< 0.001, ** Pv< 0.005, * Pv< 0.05.



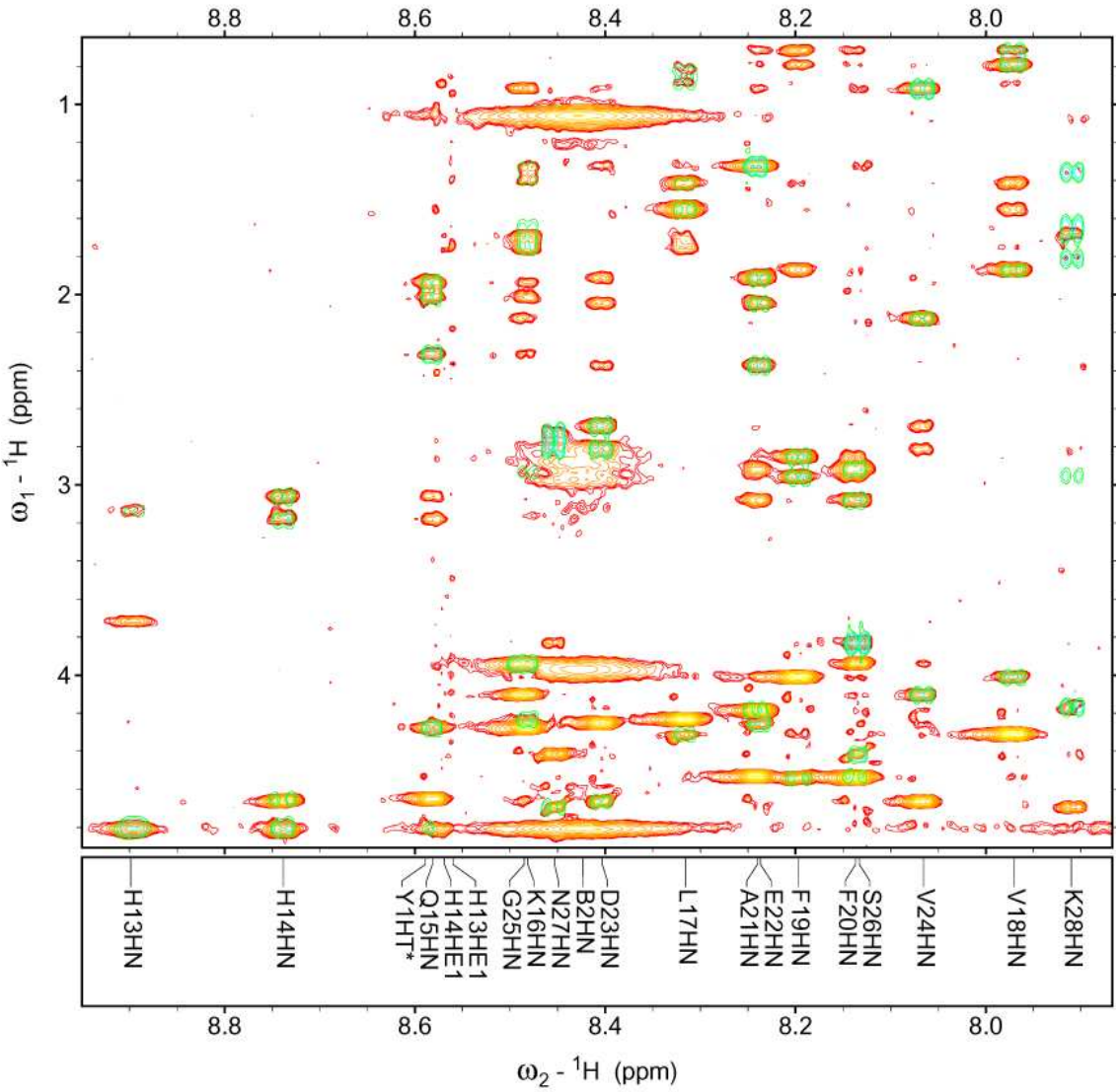
S4. Peptides stability in a mouse brain extract. A brain of WT ICR white mouse was extracted and incubated with peptides EM-1 and Aib-1 for two hours and loaded on a C18 HPLC column. **a)** Spectra of End−1 peptide (black) and End-1 peptide with brain extract (grey) showing almost complete disappearance of EM-1 signal. **b)** Spectra of Aib-1 peptide (black) and Aib-1 peptide with brain extract (grey) showing the persistence of the Aib-1 signal after this period of incubation.



S5. Analysis of the statistical significance of the distributions extracted from the MD sampling and shown in Figure 4. Each curve represents a block average over half of the simulation data, i.e., five of the ten 2 μs runs. Since the deviations between pairs of blocks of data are much smaller than the differences between the simulations with and without Aib-1, one can safely conclude that the latter differences are not an artifact due to limited sampling.



S6. Comparison between NMR NOE upper distance values and corresponding values extracted from the MD simulations. The NOE distances were calculated from the MD trajectories using the formula <r(t)-6>-1/6, where r(t) is the inter proton distance at the simulation time t and < > represents the average over the whole sampling of 15 µs. The error bars were obtained by block averaging over three 5 microsecond independent MD runs. The blue dashed line is only a guide.



S7. NMR TOCSY (greens) and NOESY (reds) overlay of HN-Hα fingerprint region of $A\beta_{12\text{-}28}$ with Aib-1 (1:4 molar ratio), where Aib-1 residue AIB is denoted B2 and amine resonances of W3 and F4 of Aib-1 are not shown in this range.

Supplementary Table 1 – ^{1}H Chemical shift assignment of Aib-1 and $A\beta_{12-28}$ complex. (UR – unresolved)

	HN	Hα	Hβ	Others
Aib-1				
Y1	8.59	3.97	2.97, 2.87	CHδ 7.05; CHε 6.82
B2	8.42		1.09, 1.06	
W3	7.54	4.41	3.07, 2.92	CHδ1 7.02; CHη2 7.12; CHε3 7.41; CHζ2 7.57; CHζ3 UR; NHε1 10.16
F4	7.14	4.60	3.25, 3.15	CHδ 7.05; CHε 7.22
Aβ(12-28)				
V12	3.72	3.72	0.82	
H13	8.90	4.66	3.14	CHδ2 7.25; CHε1 8.56
H14	8.74	4.65	3.17, 3.06	CHδ2 7.26; CHε1 8.57
Q15	8.58	4.28	2.01, 1.94	Hγ 2.31
K16	8.48	4.23	1.39, 1.33	Hγ 1.75, 1.70
L17	8.32	4.31	1.42	Hγ 1.55 CH_3δ 0.83
V18	7.97	4.01	1.87	Hγ 0.79, 0.72
F19	8.20	4.54	2.96, 2.85	CHδ 7.14; CHε 7.28
F20	8.14	4.53	3.08, 2.92	CHδ 7.21; CHε 7.31
A21	8.24	4.18	1.32	
E22	8.24	4.25	2.04, 1.91	Hγ 2.37
D23	8.40	4.66	2.81, 2.69	
V24	8.07	4.10	2.13	Hγ 0.92
G25	8.49	3.94		
S26	8.13	4.42	3.83	
N27	8.45	4.69	2.80, 2.73	
K28	7.91	4.18	1.68	Hγ 1.68

Supplementary Table 2 – $\Delta\delta^1$H of Aib-1 and $A\beta_{12-28}$, including aromatics (in Hz). (UR – unresolved)

	HN	Hα	Hβ	Others
Aib-1				
Y1	7.4	4.1	8.0	CHδ 1.4; CHε 0.5
B2	UR	--	8.8	
W3	17.0	UR	8.4	CHδ1 UR; CHη2 UR; CHε3 0.8; CHζ2 7.36; NHε1 8.2
F4	UR	UR	19.2	CHζ3 UR; CHε 0.3
Aβ(12-28)				
V12				
H13	2.7	0.7		CHδ2 6.8
H14	5.4	1.7		CHδ2 5.8
Q15	0.2	0.0		
K16	2.3	0.2		
L17	2.1	1.8		
V18	8.6	0.2		
F19	4.2	2.0		CHδ 2.6; CHε 1.2 CHζ 0.4
F20	0.5	1.5		CHδ 0.3; CHε 0.2
A21	4.2	1.0		
E22	16.0	4.2		
D23	9.3	7.5		
V24	2.7	0.7		
G25	3.9	0.9		
S26	3.6	0.0		
N27	1.0	0.4		
K28	17.7	9.0		

Supplementary Table 3 – NOE restraints used for structure calculation (XPLOR format)

```
!i.HN
!!Intra-residual restraints.
!i+1.HN
!!Restraints i,i+1.
  assign (resid 17 and name HN) (resid 16 and name HN) 5.5  3.7  0.0
  assign (resid 18 and name HN) (resid 17 and name HN) 4.5  2.7  0.0
  assign (resid 18 and name HN) (resid 19 and name HN) 4.5  2.7  0.0
  assign (resid 22 and name HN) (resid 23 and name HN) 5.0  3.2  0.0
  assign (resid 24 and name HN) (resid 23 and name HN) 4.0  2.2  0.0
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  assign (resid 26 and name HN) (resid 25 and name HN) 5.0  3.2  0.0
  assign (resid 28 and name HN) (resid 27 and name HN) 4.5  2.7  0.0
!i.FP
!!Intra-residual restraints.
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  assign (resid 13 and name HB*) (resid 13 and name HN) 5.5  3.7  0.0
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  assign (resid 14 and name HB#) (resid 14 and name HN) 4.5  2.7  0.0
  assign (resid 14 and name HB#) (resid 14 and name HN) 4.0  2.2  0.0
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  assign (resid 15 and name HB#) (resid 15 and name HN) 3.5  1.7  0.0
  assign (resid 15 and name HB#) (resid 15 and name HN) 3.5  1.7  0.0
  assign (resid 15 and name HG*) (resid 15 and name HN) 4.5  2.7  0.0
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  assign (resid 16 and name HB#) (resid 16 and name HN) 5.5  3.7  0.0
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  assign (resid 18 and name HG#*) (resid 18 and name HN) 3.0  1.2  0.3
  assign (resid 18 and name HG#*) (resid 18 and name HN) 4.5  2.7  0.0
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  assign (resid 19 and name HB#) (resid 19 and name HN) 3.5  1.7  0.0
  assign (resid 19 and name HB#) (resid 19 and name HN) 2.5  0.7  0.5
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  assign (resid 20 and name HB#) (resid 20 and name HN) 3.5  1.7  0.0
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  assign (resid 20 and name HN) (resid 20 and name HD*) 4.5  2.7  0.0
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  assign (resid 26 and name HB*) (resid 26 and name HN) 2.5  0.7  0.5
  assign (resid 27 and name HA) (resid 27 and name HN) 4.0  2.2  0.0
  assign (resid 27 and name HB#) (resid 27 and name HN) 3.5  1.7  0.0
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  assign (resid 27 and name HB#) (resid 27 and name HN) 3.5  1.7  0.0
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!i+1.FP
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  assign (resid 14 and name HB#) (resid 15 and name HN) 3.5  1.7  0.0
  assign (resid 14 and name HB#) (resid 15 and name HN) 5.0  3.2  0.0
  assign (resid 15 and name HA) (resid 16 and name HN) 2.5  0.7  0.5
  assign (resid 15 and name HB#) (resid 16 and name HN) 4.5  2.7  0.0
  assign (resid 15 and name HB#) (resid 16 and name HN) 5.0  3.2  0.0
  assign (resid 15 and name HG*) (resid 16 and name HN) 5.5  3.7  0.0
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  assign (resid 18 and name HB) (resid 19 and name HN) 3.5  1.7  0.0
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  assign (resid 18 and name HG#*) (resid 19 and name HN) 4.5  2.7  0.0
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  assign (resid 20 and name HB#) (resid 21 and name HN) 3.5  1.7  0.0
  assign (resid 20 and name HB#) (resid 21 and name HN) 4.0  2.2  0.0
  assign (resid 20 and name HD*) (resid 19 and name HN) 5.5  3.7  0.0
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  assign (resid 23 and name HB#) (resid 24 and name HN) 5.5  3.7  0.0
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  assign (resid 26 and name HB*) (resid 27 and name HN) 5.5  3.7  0.0
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  assign (resid 26 and name HA) (resid 28 and name HN) 5.5  3.7  0.0
!i+3.FP
!!Restraints i,i+3.
  assign (resid 19 and name HD*) (resid 22 and name HN) 5.5  3.7  0.0
  assign (resid 24 and name HG*) (resid 21 and name HN) 4.5  2.7  0.0
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!i-lr.FP
!!Long range (further than i+3).
  assign (resid 18 and name HG#*) (resid 22 and name HN) 5.5  3.7  0.0
!i.AL
!!Intra-residual restraints.
  assign (resid 13 and name HB*) (resid 13 and name HD2) 5.5  3.7  0.0
  assign (resid 13 and name HE1) (resid 13 and name HD2) 5.5  3.7  0.0
  assign (resid 14 and name HA) (resid 14 and name HD2) 5.5  3.7  0.0
  assign (resid 14 and name HB#) (resid 14 and name HD2) 5.0  3.2  0.0
  assign (resid 14 and name HB#) (resid 14 and name HD2) 4.5  2.7  0.0
  assign (resid 14 and name HE1) (resid 14 and name HD2) 5.5  3.7  0.0
  assign (resid 16 and name HA) (resid 16 and name HB#) 5.5  3.7  0.0
  assign (resid 18 and name HA) (resid 18 and name HG#*) 3.0  1.2  0.3
  assign (resid 18 and name HA) (resid 18 and name HG#*) 3.0  1.2  0.3
  assign (resid 18 and name HB) (resid 18 and name HA) 5.5  3.7  0.0
  assign (resid 19 and name HA) (resid 19 and name HD*) 4.0  2.2  0.0
  assign (resid 19 and name HB#) (resid 19 and name HD*) 3.0  1.2  0.3
  assign (resid 19 and name HB#) (resid 19 and name HE*) 4.5  2.7  0.0
  assign (resid 19 and name HB#) (resid 19 and name HD*) 3.5  1.7  0.0
  assign (resid 19 and name HB#) (resid 19 and name HE*) 4.5  2.7  0.0
  assign (resid 20 and name HA) (resid 20 and name HD*) 3.5  1.7  0.0
  assign (resid 20 and name HB#) (resid 20 and name HD*) 3.0  1.2  0.3
  assign (resid 20 and name HB#) (resid 20 and name HD*) 3.0  1.2  0.3
  assign (resid 20 and name HB#) (resid 20 and name HE*) 5.5  3.7  0.0
  assign (resid 21 and name HB*) (resid 21 and name HA) 4.5  2.7  0.0
  assign (resid 24 and name HB) (resid 24 and name HA) 5.0  3.2  0.0
!i+1.AL
!!Restraints i,i+1.
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  assign (resid 17 and name HA) (resid 18 and name HG#*) 5.5  3.7  0.0
  assign (resid 18 and name HA) (resid 19 and name HD*) 5.5  3.7  0.0
  assign (resid 19 and name HA) (resid 18 and name HG#*) 5.5  3.7  0.0
  assign (resid 20 and name HA) (resid 21 and name HB*) 5.0  3.2  0.0
  assign (resid 25 and name HA*) (resid 24 and name HG*) 5.5  3.7  0.0
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  assign (resid 20 and name HA) (resid 18 and name HG#*) 5.5  3.7  0.0
  assign (resid 21 and name HB*) (resid 19 and name HE*) 5.5  3.7  0.0
!i+3.AL
!!Restraints i,i+3.
  assign (resid 21 and name HA) (resid 18 and name HG#*) 5.0  3.2  0.0
  assign (resid 21 and name HA) (resid 24 and name HG*) 5.0  3.2  0.0
!i.FP
!!Intra-residual restraints.
  assign (resid 2  and name HB*) (resid 2  and name HN) 3.0  1.2  1.5
  assign (resid 3  and name HA) (resid 3  and name HN) 5.0  3.2  0.0
!i+1.FP
!!Restraints i,i+1.
  assign (resid 1  and name HA) (resid 2  and name HN) 3.5  1.7  0.0
  assign (resid 1  and name HD*) (resid 2  and name HN) 4.0  2.2  0.0
  assign (resid 1  and name HE*) (resid 2  and name HN) 4.5  2.7  0.0
  assign (resid 2  and name HN) (resid 1  and name HD*) 3.5  1.7  1.5
  assign (resid 2  and name HN) (resid 1  and name HE*) 3.5  1.7  1.5
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  assign (resid 3  and name HA) (resid 2  and name HN) 5.5  3.7  0.0
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  assign (resid 4  and name HA) (resid 3  and name HN) 5.0  3.2  0.0
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!!Restraints i,i+2.
  assign (resid 2  and name HB*) (resid 4  and name HN) 4.5  2.7  0.0
!i.AL
!!Intra-residual restraints.
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  assign (resid 1  and name HB#) (resid 1  and name HE*) 3.5  1.7  1.5
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  assign (resid 3  and name HE3) (resid 3  and name HE1) 3.5  1.7  1.5
  assign (resid 3  and name HZ2) (resid 3  and name HE1) 5.5  3.7  1.5
  assign (resid 3  and name HZ3) (resid 3  and name HE1) 4.0  2.2  1.5
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!!Restraints i,i+1.
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  assign (resid 1  and name HA) (resid 2  and name HB#*) 2.5  0.7  1.5
  assign (resid 1  and name HB#) (resid 2  and name HB#*) 5.0  3.2  0.0
  assign (resid 1  and name HB#) (resid 2  and name HB#*) 4.5  2.7  0.0
  assign (resid 1  and name HB#) (resid 2  and name HB#*) 4.5  2.7  0.0
  assign (resid 2  and name HB*) (resid 1  and name HD*) 3.5  1.7  1.5
  assign (resid 2  and name HB*) (resid 1  and name HE*) 2.5  0.7  1.5
  assign (resid 2  and name HB*) (resid 3  and name HE1) 4.0  2.2  1.5
  assign (resid 2  and name HB*) (resid 3  and name HE3) 4.5  2.7  1.5
  assign (resid 2  and name HB*) (resid 3  and name HH2) 4.5  2.7  1.5
  assign (resid 2  and name HB*) (resid 3  and name HZ2) 4.0  2.2  1.5
  assign (resid 2  and name HB*) (resid 3  and name HZ3) 4.5  2.7  1.5
  assign (resid 3  and name HA) (resid 2  and name HB#*) 5.0  3.2  0.5
  assign (resid 3  and name HA) (resid 2  and name HB#*) 4.5  2.7  0.5
  assign (resid 3  and name HZ2) (resid 4  and name HD*) 3.5  1.7  1.5
  assign (resid 4  and name HA) (resid 3  and name HE1) 5.0  3.2  1.5
  assign (resid 4  and name HA) (resid 3  and name HZ2) 4.5  2.7  1.5
  assign (resid 4  and name HB#) (resid 3  and name HE1) 5.0  3.2  1.5
  assign (resid 4  and name HB#) (resid 3  and name HE1) 5.5  3.7  1.5
  assign (resid 4  and name HB#) (resid 3  and name HZ2) 4.5  2.7  1.5
!i+2.AL
!!Restraints i,i+2.
  assign (resid 1  and name HB#) (resid 3  and name HZ2) 4.5  2.7  1.5
  assign (resid 1  and name HB#) (resid 3  and name HZ2) 5.0  3.2  1.5
  assign (resid 1  and name HE*) (resid 3  and name HE1) 5.0  3.2  1.5
  assign (resid 3  and name HA) (resid 1  and name HE*) 5.0  2.7  1.5
  assign (resid 3  and name HE1) (resid 1  and name HE*) 3.5  1.7  1.5
  assign (resid 3  and name HZ2) (resid 1  and name HE*) 3.5  1.7  1.5
  assign (resid 4  and name HA) (resid 2  and name HB#*) 4.5  2.7  1.5
  assign (resid 4  and name HA) (resid 2  and name HB#*) 4.0  2.2  1.5
  assign (resid 4  and name HB#) (resid 2  and name HB#*) 4.5  2.7  1.5
  assign (resid 4  and name HB#) (resid 2  and name HB#*) 5.0  3.2  1.5
  assign (resid 4  and name HB#) (resid 2  and name HB#*) 4.5  2.7  1.5
!i+3.AL
!!Restraints i,i+3.
  assign (resid 4  and name HA) (resid 1  and name HE*) 4.5  2.7  1.5
  assign (resid 4  and name HB#) (resid 1  and name HE*) 4.5  2.7  1.5
  assign (resid 4  and name HB#) (resid 1  and name HE*) 5.0  3.2  1.5
!i-lr.AL
!!Long range (further than i+3).
  assign (resid 20 and name HE*) (resid 1 and name HE*) 5.0  3.2  1.5
  assign (resid 22 and name HA) (resid 2  and name HB#*) 5.5  3.7  1.5
  assign (resid 3  and name HB1) (resid 21 and name HB*) 5.5  3.7  0.0
  assign (resid 3  and name HB2) (resid 21 and name HB*) 5.5  3.7  0.0
```