Supplementary Information

Unravelling the role of amino acid sequence order in the assembly and function of amyloid-β core

Santu Bera,^a Elad Arad,^b Lee Schnaider,^a Shira Shaham-Niv,^a Valeria Castelletto,^c

Yossef Peretz,^b Dor Zaguri,^a Raz Jelinek,^b Ehud Gazit,^{*a} and Ian W. Hamley^{*c}

a. Department of Molecular Microbiology and Biotechnology,

George S. Wise Faculty of Life Sciences,

Tel Aviv University, Tel Aviv 69978, Israel;

E-mail: ehudg@post.tau.ac.il

b. Department of Chemistry &, Ilse Katz Institute (IKI) for Nanoscale Science and Technology,

Ben Gurion University of the Negev,

Beer Sheva 8410501,

Israel

c. Department of Chemistry,

University of Reading, Reading RG6 6AD,

United Kingdom.

E-mail; I.W.Hamley@reading.ac.uk

Materials and methods

Materials. All peptides were synthesized by DGpeptides Co., Ltd. (Hangzhou, China). The peptides were purified to at least 95%, and their identity was confirmed by mass spectrometry (ESI Figure S8-S11). Thioflavin T (ThT) and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), were purchased from Sigma-Aldrich (Rehovot, Israel). 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), sphingomyelin (brain, porcine) and cholesterol (ovine wool, >98%) were purchased from Avanti Polar Lipids.

Preparation of peptide assemblies. The self-assembled peptide solutions were prepared according to a previously reported method.¹ Stock solutions were prepared by dissolving the corresponding peptide in 4:6 acetonitrile/water at concentration of 2.5 mM and slowly titrating with 200 mM NaOH to a final pH 7. The samples were first vortexed for 2 mins, followed by sonication until the peptides dissolved completely. Except for kinetics studies, the peptide solutions were incubated for 2 weeks at 4 °C with frequent shaking to complete the self-assembly process.

Transmission electron microscopy (TEM). The stock peptide solutions were diluted 10x with water. A 10 μ l aliquot of the peptide solution was placed on a 400-mesh copper grid. After 1 min, excess fluids were removed. For negative staining, the grid was stained with freshly prepared 2% uranyl acetate in water, and after 2 min, excess fluid was removed from the grid. Samples were viewed using a JEOL 1200EX electron microscope operating at 80 kV.

Atomic force microscopy (AFM). The stock solutions were diluted 10x with water. AFM images were obtained by depositing 5 μ l solutions onto freshly cleaved V1 grade mica (Ted

Pella, Redding, CA, USA). The drops were allowed to dry under ambient conditions overnight. The AFM images were obtained with an AIST-NT Smart AFM system in non-contact (tapping) mode using 100 µm long silicon nitride cantilevers (OMCL-RC800PSA-W, Olympus, Japan) with resonance frequency of 70 kHz. The images were analyzed and visualized using the WSxM imaging software (Nanotec Electronica S.L, Madrid, Spain).

Fourier-transform infrared (FTIR) spectroscopy. A 30 μ l aliquot of the stock peptide solution was deposited onto disposable KBr infrared sample sheets (Sigma-Aldrich, Rehovot, Israel), which were then allowed to dry under vacuum overnight. The samples were saturated twice with 30 μ l D₂O and vacuum dried. FTIR spectra were collected using a nitrogen purged Nicolet Nexus 470 FTIR spectrometer (Nicolet, Offenbach, Germany) equipped with a deuterated triglycine sulfate (DTGS) detector. Measurements were performed at 4 cm⁻¹ resolution and by averaging 64 scans. The absorbance maxima values were determined using an OMNIC analysis program (Nicolet). The background was subtracted using a control spectrum.

Circular dichroism (CD) spectroscopy. The experiments were performed without further dilution of the peptide stock solutions. CD spectra were collected using a Chirascan spectrometer (Applied Photophysics, Leatherhead, UK) fitted with a Peltier temperature controller set to 25 °C, using quartz cuvettes with an optical path length of 0.1 mm (Hellma Analytics, Müllheim, Germany). The absorbance of the sample was kept within the linear range of the instrument during measurement. Data acquisition was performed in steps of 1 nm at a wavelength range of 190 to 250 nm with a spectral bandwidth of 1.0 nm and an averaging time of 3 s. The spectrum of each sample was collected three times and averaged.

Baseline spectra were similarly recorded for phosphate buffer and subtracted from the sample spectra. Data processing was performed using the Pro-Data Viewer software (Applied Photophysics, Leatherhead, UK).

Small-angle X-ray scattering (SAXS). Synchrotron SAXS experiments were performed on beamline B21 at Diamond (Didcot, UK). Solutions were loaded into the 96 well plate of an EMBL BioSAXS robot, and then injected via an automated sample exchanger into a quartz capillary (1.8 mm internal diameter) in the X-ray beam. The quartz capillary was kept in a vacuum chamber, in order to avoid parasitic scattering. After the sample was injected in the capillary and reached the X-ray beam, the flow was stopped during the SAXS data acquisition. Beamline B21 was operated with a fixed camera length (3.9 m) and fixed energy (12.4 keV). The images were captured using a PILATUS 2M detector. Data processing was performed using the dedicated beamline software ScÅtter.

Cell cytotoxicity experiments. SH-SY5Y cells (2×10^5 cells/mL) were cultured in 96-well tissue microplates (100 µL/well) and allowed to adhere overnight at 37 °C. Half of each plate was seeded with cells, with the other half serving as a solution-only control. The two peptides were dissolved at 90 °C in DMEM:Nutrient Mixture F12 (Ham's) (1 :1) (Biological Industries, Israel) at a concentration of 1.25 mM, followed by gradual cooling of the solution. The control experiment was performed with medium only, without any peptide, and treated in the same manner. The next day, 100 µL aliquots of medium with or without peptides was added to each well. Following incubation for 6 hours at 37 °C, cell viability was evaluated using the 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay kit (Biological Industries, Israel) according to the manufacturer's instructions. Briefly, 10 µl of 5 mg/ml MTT dissolved in PBS were added

to each well. After a 4 h incubation at 37 °C, 100 μ l extraction buffer (20% SDS dissolved in a 1:1 mixture of DMF and DDW (pH 4.7)) were added to each well, and the plates were incubated again at 37 °C for 30 min. Finally, color intensity was measured using an ELISA reader at 570 nm. The data are presented as mean \pm SEM.

Stability study in presence of human serum. The experiment has been performed according to previous reported procedure (ref. 15). 10% (v/v) human serum was supplemented in 1 ml DMEM media (Sigma-Aldrich) and incubated at 37 °C for 15 min. Then 10 μ l of peptide solution in DMSO (15 mg/ml) was added and incubated at 37 °C. In different time interval, 100 μ l of the aliquot was taken out from the mixture and added to 200 μ l of ethanol solution, kept at 4 °C for 10 min for complete precipitation of the serum. Then the mixture was centrifuged at 12000 rpm for 4 min. The supernatant was collected and analyzed by LC-MS spectroscopy.

Lipid binding.

Peptide and sample preparation. Peptides were dissolved in HFIP at a concentration of 4 mM and stored at -20 °C until use, to prevent aggregation. For each experiment, the solution was thawed, and the required amount was dried by evaporation for four hours to remove the HFIP. The dried peptide sample was dissolved in water and phosphate buffer at pH 7.4 (final concentration 40 mM). Peptide stock solutions were prepared at 1 mM and diluted to the required concentration.

Vesicles consisting of DOPC, sphingomyelin and cholesterol were prepared by dissolving the lipid components in chloroform/ethanol (1:1, v/v) and drying together in vacuo. Small unilamellar vesicles (SUVs; DOPC/sphingomyelin/cholesterol 0.67:0.08:0.25, mole ratio)

S5

were prepared in phosphate buffer (40 mM, pH 7.4) by probe sonication of the aqueous lipid mixtures at room temperature for 10 min in 1 min pulses. Vesicle suspensions were stored for 1 h at room temperature prior to use.

ThT fluorescence kinetics assay. The fluorescence of Ac-KLVFFAE-NH2 and Ac-EAFFVLK-NH2 samples (total volume of 100 μ L, 0.5 mM peptide concentration) was measured in the absence or presence of lipid vesicles (0.5 mM total lipid concentration) in 96-well cell culture plates using a Biotek Synergy H1 plate reader (Biotek, Winooski, VT, USA) at 25 °C. ThT aqueous solution was added to a final concentration of 10 μ M per well. Fluorescence was recorded every 5 min over 20 hours at excitation λ ex = 440 and emission λ em = 490 nm. For each sample, the self-fluorescence of lipid vesicles and buffer was subtracted from the measured fluorescence.

Transmission electron microscopy (TEM). Cryo-TEM aliquots were prepared by a 12hour incubation of the peptides (0.5 mM) and lipid vesicle solution (0.5 mM). A 3 μ L droplet of the sample was deposited on a glow-discharged TEM grid (300 mesh lacey Cu substrate grid; Ted Pella). The excess liquid was blotted with a filter paper. Negatively stained samples were then stained with 1% uranyl acetate solution for 20 seconds and then blotted with filter paper. For cryogenic samples, the specimen was rapidly plunged into liquid ethane precooled with liquid nitrogen in a controlled environment (Leica EM GP). The vitrified samples were transferred to a cryo-specimen holder (Gatan mode 626) and examined at -181 °C using a FEI Tecnai 12 G2 TWIN TEM operated at 120 kV in lowdose mode. Grids were imaged a few micrometers under focus to increase phase contrast. The images were recorded with a Gatan charge-coupled device camera (model 794). **Circular dichroism (CD) spectroscopy.** CD spectra were recorded in the range of 190–260 nm at room temperature on a Jasco J-715 spectropolarimeter, using 1 mm quartz cuvettes. Spectra were obtained for solutions composed of 400 μ L containing 0.5 mM peptide in the absence or presence of lipid vesicles (0.5 mM lipids) after incubation for 12 hours. CD signals resulting from vesicles and buffer alone were subtracted from the corresponding spectra.



Fig. S1 Temperature-dependent CD spectra of (a) $A\beta(16-22)$ and (b) $A\beta(22-16)$ showing partial unfolding upon increasing the temperature for both peptides.



Fig. S2 Temperature-dependent CD spectra of (a) $A\beta(16-22)$ and (b) $A\beta(22-16)$ showing transition towards the original conformation after cooling down to the initial temperature indicating their high conformational flexibility.



Fig. S3 Synchrotron SAXS data. Bottom: diamonds data for $A\beta(16-22)$ (1 wt% solution) and blue line, model form factor fit. Top: circles, data for $A\beta(22-16)$ (0.5 wt% solution) and red line, model form factor fit. The lower curves have been displaced by division by 100 for ease of visualization. For convenience, only every 3rd measured data point is shown.



Fig. S4 MTT cell viability assay using PC12 cells grown in the presence of either peptide for 6h. The results showed the toxicity of the native peptide and the non-toxic nature of reverse sequence, as also observed for the SH-SY5Y cell line (Fig. 3b).



Fig. S5 Stability kinetics of the native peptide in the presence of human serum as monitored by LC-MS.



Fig. S6 Stability kinetics of the reverse peptide in the presence of human serum as monitored by LC-MS.



Fig. S7 Cryo-TEM images of $A\beta(22-16)$ in the presence of lipid vesicles.



Fig. S8 HPLC trace of $A\beta(16-22)$.

MASS SPECTROMETRY REPORT

Fig. S9 Mass spectrum of $A\beta(16-22)$.

HPLC								
Sample: Sequence:	T-2117 Ac-EAFFVLK	-NH2		Analyzed date:	2018.6.12			
Lot. No.:	DG-69356							
Column:	Symmetrix ODS-R, 4.6*250mm, 5µm							
Solvent A	A: 0.1% Trifluoroacetic Acid in 100% Acetonitrile							
Solvent B	B: 0.1% Trifluoroacetic Acid in 100% Water							
Gradient:		Α	в					
	0.0min	32%	68%					
	25.0min	57%	43%					
	25.1min	100%	0%					
	30.0min	Stop						
Volume:	20µ1							
Wavelength:	220nm							
Flow rate:	1.0ml/min							
aW								
- 1000								
-900			22					
-800			Ī					
-100								
- 40								
- 500								
-400								
-300								
-200								
0			<u>141 - 144</u>					
-100 2	1 1	1 P	P	N N	r.	2P 22		
Rank Time	Conc. Area	Height						
1 10.611	1.72 210470	30204						
2 10.873	95.65 117073	17 849187						
3 11.194	2.052 251153	22009						
4 11.654	0.5779 70730	4605						
Total 10	00 12239670	906005						

Fig. S10 HPLC trace of $A\beta(22-16)$.

MASS SPECTROMETRY REPORT

Fig. S11 Mass spectrum of $A\beta(22-16)$.

Parameter	Αβ(16-22)	Αβ(22-16)
t (thickness) (Å)	25.0	42.0
Polydispersity in t	23.0	9.7
σ_{out} (Å) ^a	2.6	2.4
$\eta_{out} (a.u.)^b$	5.5x10 ⁻⁷	4.8x10 ⁻⁷
σ_{in} (Å) ^c	5.2	8.9
$\eta_{in} (a.u.)^d$	8.4x10 ⁻⁸	1.5x10 ⁻⁷
$D^{\mathrm{e}}(\mathrm{\AA})$	500	500
BG ^f	6.8x10 ⁻⁴	-1.0x10 ⁻⁵

Table S1 SAXS Fitting Parameters using a Gaussian bilayer model² fitted with SASfit.³

^a Width of outer Gaussians, ^b Scattering contrast of outer Gaussians, ^c Width of inner Gaussian, ^d Scattering contrast of inner Gaussian, ^e Layer diameter (fixed), ^f Constant background.

References

- T. O. Omosun, M.-C. Hsieh, W. S. Childers, D. Das, A. K. Mehta, N. R. Anthony, T. Pan, M. A. Grover, K. M. Berland and D. G. Lynn, *Nat. Chem.*, 2017, 9, 805.
- (a) G. Pabst, M. Rappolt, H. Amenitsch and P. Laggner, *Phys. Rev. E*, 2000, 62, 4000;
 (b) V. Castelletto, R. J. Gouveia, C. J. Connon and I. W. Hamley, *Faraday Discuss.*, 2013, 166, 381.
- I. Bressler, J. Kohlbrecher and A. F. Thünemann, J. Appl. Crystallogr., 2015, 48, 1587.