### SUPPLEMENTARY INFORMATION



**Figure S1**. (A) Controlled hetero-assembly of KLVFWAK and ELVFWAE and (B) Creation of functional amyloid assemblies through controlled hetero-assembly between the peptide variants fused to functional domains. In (A), KLVFWAK is biotinylated and immobilized on streptavidin-coated beads. Hetero-assembly between KLVFWAK and ELVFWAE can occur by incubating KLVFWAK-bound beads in ELVFWAE solution, and then in KLVFWAK solution. Amyloid assemblies grow during this repetitive process in combination with intermittent removal of unbound peptides by washing. The step-wise peptide addition with intermittent washing can offer more uniform assemblies of specific size, which can be adjusted by the number of cycles of alternating between the peptide pairs. The immobilized hetero-assemblies can be released from beads by addition of excess biotin. In (B), the peptides are fused to functional domains (e.g., X&Y). The fused functional domains can be incorporated into amyloid assemblies at specific locations through similar hetero-assembly.

NaCl Concentration - (mM)	Assembly onset time (min) <sup>a</sup>		
	KLVFWAK <sup>b</sup>	ELVFWAE <sup>b</sup>	KLVFWAK + ELVFWAE <sup>b</sup>
25	> 1,000	> 1,000	$3 \pm 1$
150	> 1,000	> 1,000	$11 \pm 2$
1000	> 1,000	> 1,000	$3 \pm 1$

Table S1. The time for assembly onset of peptides at room temperature

<sup>a</sup> Assembly onset time was determined by laser light scattering as incubation time needed until samples exhibited light scattering intensities greater than 10 SD of those from buffer. Individual peptide solutions of KLVFWAK and ELVFWAE showed light scattering intensity no stronger than that of buffer during incubation.

<sup>b</sup> Total peptide concentration of each sample was 200  $\mu$ M. For mixtures, two peptides were mixed at an equimolar ratio, 100  $\mu$ M each.

Errors : 1SD from two independent experiments

Table S2. The time required for precipitation during hetero-assembly of KLVFWAK and ELVFWAE at room temperature in the presence of 150 mM NaCl

Total peptide concentration (µM) <sup>a</sup>	Precipitation time <sup>b</sup>	% soluble fraction of peptides upon precipitation <sup>c</sup>
40	> 2 weeks	100
100	5 day	82
150	1 day	34
200	3 hour	28

<sup>a</sup> Total peptide concentration. The two peptides were mixed at an equimolar ratio.

<sup>b</sup> Precipitation time was determined by size exclusion chromatography (SEC) as incubation time needed for the loss of soluble fractions of peptides.

 $^{\circ}$  The percent soluble fraction was determined by measuring SEC peak areas. For samples at 40  $\mu$ M total peptide concentration, % soluble fraction was measured after 2 weeks of incubation.

Table S3. The fractions of soluble peptides remaining in supernatant during hetero-assembly at room temperature in the presence of 150 mM NaCl.

Incubation	KLVFWAK	+ ELVFWAE	Capped-KLVFWAK -	+ Capped-ELVFWAE
time (day)	(200 µM total, 100 µM each)		(400 µM total, 200 µM each)	
	KLVFWAK	ELVFWAE	Capped-KLVFWAK	Capped-ELVFWAE
1	20 %	27 %	90%	78%
2	20 %	26 %	46%	51%

The mixture samples were incubated for designated time periods, and then aliquoted for centrifugation to obtain supernatants. The supernatants were subsequently injected into a SEC column, and the fractions of soluble peptides remaining in supernatant were determined based on SEC peak areas. The rest of peptides hetero-assembled to precipitates.

Additional evidence of equimolar incorporation with FITC-tagged peptides

To further confirm that the hetero-assemblies consisted of both KLVFWAK and ELVFWAE, Nterminal FITC-tagged peptides were introduced. An equimolar mixture of 200  $\mu$ M total peptide with 5% FITC-KLVFWAK was incubated for 1 day at room temperature in the presence of 150 mM NaCl. The mixture was centrifuged, and the supernatant was analyzed for FITC fluorescence. A similar experiment was performed with FITC-ELVFWAE. The extent of incorporation of FITC-KLVFWAK and FITC-ELVFWAE into insoluble hetero-assemblies was 80 and 82%, respectively, further confirming that the both peptides were incorporated at ~ 1:1 ratio during hetero-assembly.



**Figure S2**. A TEM image of hetero-assemblies formed by an equimolar mixture of KLVWAK and ELVFWAE (200  $\mu$ M total peptide concentration) in the presence of 150 mM NaCl after 3 hour incubation at room temperature. Small globules are indicated by white arrows. Scale bar: 100 nm



**Figure S3**. A TEM image of hetero-assemblies formed by an equimolar mixture of KLVWAK and ELVFWAE (150  $\mu$ M total peptide concentration) in the presence of 150 mM NaCl after 8 day incubation at room temperature. Scale bar: 100 nm

	KLVFWAK + ELVFWAE	Capped-KLVFWAK + Capped-ELVFWAE
α-helix	11	2
β-sheet	61	85
β-turn	19	12
Random coil	9	1

Table S4. Secondary structures of hetero-assembled fibrils as determined by deconvolution of FT-IR spectra.

The role of balanced hydrophobic and electrostatic interactions in hetero-assembly between KLVFWAK and ELVFWAE: modulation of hetero-assembly by varying temperature and NaCl concentration

Based on our peptide design, we reasoned that hydrophobic and electrostatic interactions may play a major role in the hetero-assembly. Aggregation of many amyloid polypeptides, including  $A\beta$ , is accelerated at elevated temperatures, characteristic of hydrophobic interaction-driven self-assembly.<sup>1-3</sup> Interestingly, hetero-assembly of KLVFWAK and ELVFWAE was substantially suppressed at an elevated temperature. When the mixture at 200 µM total peptide concentration was incubated for 1 day at 80 °C, neither precipitation nor structural changes were observed (Fig. S4). In contrast, visible aggregates were observed, accompanied by CD spectrum changes when the incubation temperature was set at  $\leq \sim 50^{\circ}$ C (Fig. S4). To further modulate a balance between electrostatic and hydrophobic interactions between KLVFWAK and ELVFWAE, NaCl at varying concentrations was added to peptide mixtures. Compared to 150 mM NaCl, the hetero-assembly onset of the equimolar peptide mixture occurred ~4 times faster (3 min vs 11 min) at 25 mM NaCl, as judged by LLS (Table S1). The low salt concentration condition produced fibrillar aggregates after 3 hour incubation (Fig. S5), whereas only globular aggregates were detected at 150 mM NaCl under the otherwise same incubation condition (Fig. S2). The implication is that the kinetics of heteroassembly to form fibrils can be accelerated at lower NaCl concentrations enhancing electrostatic interactions relative to hydrophobic interactions. The hetero-assembly onset time of the equimolar mixture was also reduced at 1 M NaCl compared to 150 mM NaCl (3 min vs. 11 min), as determined by LLS (Table S1). However, the accelerated aggregation at 1 M NaCl seems to produce only globular aggregates, as no fibrillar aggregates were detected from this sample after 1 day incubation (Fig. S6) in contrast to tape-like fibrils observed at 150 mM NaCl under the otherwise same incubation condition (Fig. 3B). Overall, the results suggest that a proper balance between electrostatic and hydrophobic interactions between KLVFWAK and ELVFWAE is required for hetero-assembly to form fibrillar assemblies.



**Figure S4**. CD spectra of an equimolar mixture of KLVFWAK and ELVFWAE each at 100  $\mu$ M (total 200  $\mu$ M peptide concentration) during cooling from 80 °C. Individual peptides solutions were initially heated to 80 °C in aqueous buffer and subsequently mixed at final concentrations of 100  $\mu$ M each. The mixture was then incubated for 1 day, followed by cooling at a rate of 1°C/min, and CD spectra acquired every 5 minutes to observe structure changes.



Figure S5. A TEM image of hetero-assemblies formed by an equimolar mixture of KLVWAK and ELVFWAE (200  $\mu$ M total peptide concentration) in the presence of 25 mM NaCl after 3 hour incubation at room temperature. Scale bar: 100 nm



Figure S6. A TEM image of hetero-assemblies formed by an equimolar mixture of KLVWAK and ELVFWAE (200  $\mu$ M total peptide concentration) in the presence of 1 M NaCl after 1 day incubation at room temperature. Scale bar: 100 nm

	Assembly onset time (min) <sup>a</sup>		
NaCl Concentration (mM)	Capped-KLVFWAK <sup>b</sup>	Capped-ELVFWAE <sup>b</sup>	Capped-KLVFWAK + Capped-ELVFWAE <sup>b</sup>
25	> 1,000	> 1,000	$10 \pm 1$
150	> 1,000	> 1,000	$6 \pm 1$
1000	> 1,000	> 1,000	$12 \pm 1$

Table S5. The time for assembly onset of capped peptides at room temperature

<sup>a</sup> Assembly onset time was determined by laser light scattering as incubation time needed until samples exhibited light scattering intensities greater than 10 SD of those from buffer. Individual peptide solutions of capped-KLVFWAK and capped-ELVFWAE showed light scattering intensity no stronger than that of buffer.

 $^{b}$  Total peptide concentration of each sample was 400  $\mu M.$  For mixtures, two peptides were mixed at an equimolar ratio, 200  $\mu M$  each.

Errors : 1SD from two independent experiments

Computational models of uncapped- vs. capped-KLVFWAK + ELVFWAE hetero-assemblies

Based upon CD, tryptophan fluorescence, ATR-FTIR, and XRD data, we propose structural models for the orientation of the peptides in hetero-assemblies (**Figs. 6 and S7**), although exact residue locations remain unknown. Due to the exciton effect observed on CD (**Fig. 4A**) and the significant tryptophan fluorescence quenching (**Fig. 4B**) for uncapped KLVFWAK + ELVFWAE, we concluded that these heteroassemblies must have tryptophans that interact in close proximity. Due to the tryptophan fluorescence quenching, the tryptophans may also be shielded from the aqueous environment. Additionally, the uncapped peptide mixtures resulted in hetero-assemblies with anti-parallel  $\beta$ -sheet configuration and typical amyloid fibril *d*-spacing of ~4.7 and ~10Å, as determined by ATR-FTIR (**Fig. 5A**) and XRD (**Fig. 5B**), respectively. Thus, the model in **Figs. 6A** and **S7A** shows uncapped peptides arranged in anti-parallel  $\beta$ -sheet configuration, with all tryptophans interacting in the core of the amyloid hetero-assembly.

Conversely, hetero-assemblies of capped-KLFVWAK + capped-ELVFWAE exhibited no exciton effect on CD (**Fig. 4C**) and limited to no tryptophan fluorescence quenching (**Fig. 4D**). This result indicates the tryptophans are further apart in the hetero-assemblies and likely solvent exposed. However, similar to those of uncapped peptides, hetero-assemblies of capped peptides also exhibited anti-parallel  $\beta$ -sheet configuration and typical amyloid fibril *d*-spacing (**Fig. 5A** and **Fig. 5C**, respectively). Thus, the model in **Figs. 6B** and **S7B** shows capped peptides also arranged in anti-parallel  $\beta$ -sheet configuration; however, the tryptophans are on opposite faces of the assembly, resulting in a lower local tryptophan density and greater spacing between neighboring tryptophans.

In both models, KLVFWAK and ELVFWAE peptides are alternating on the anti-parallel  $\beta$ -sheet, which is the most energetically favorable state satisfying all electrostatic interactions. In the uncapped peptide model (**Figs. 6A** and **S7A**), salt-bridges are formed between lysine residues of KLFVWAK and glutamic acid residues of ELVFWAE of opposite  $\beta$ -sheets in the core of the assembly. However, in the capped peptide model (**Figs. 6B** and **S7B**), the salt-bridges are formed between neighboring strands of the same  $\beta$ -sheet on the face of the assembly. Based on the TEM images of uncapped (**Fig. 3B,C**) hetero-assemblies, the width of the flat nanotapes and mature fibrils (~40-60 nm) suggests that the peptides in **Figs. 6A** and **S7A** further assemble along the fibril axis as well as in a lateral direction, likely due to the charged termini. The narrower fibrils of the capped hetero-assemblies (**Fig. 3E,F**) suggests the fibril strand shown in **Figs. 6B** and **S7B** further assembles along the fibril axis with minimal lateral association.



**Figure S7**. Structural models of hetero-assemblies (A) between KLVFWAK (blue ribbon) and ELVFWAE (purple ribbon) and (B) between capped-KLVFWAK (blue ribbon) and capped-ELVFWAE (purple ribbon) viewed from top. Tryptophans are shown as spheres.

## **EXPERIMENTAL PROCEDURES**

### Materials

All peptides (KLVFWAK, ELVFWAE, Ac-KLVFWAK-NH<sub>2</sub> referred to as capped-KLVFWAK, Ac-ELVFWAE-NH<sub>2</sub> referred to as capped-ELVFWAE) were synthesized using solid-phase chemistry and purified using reverse-phase HPLC by GenScript (Piscataway, NJ, USA). Peptides with fluorescein iso-thiocyanate (FITC) labeled at the N-terminal  $\alpha$ -amine (referred to as FITC-KLVFWAK and FITC-ELVFWAE) were similarly synthesized by GenScript. A precision column prepacked with Superdex 75 was purchased from GE Healthcare (Piscataway, NJ, USA). All other chemicals were purchased from Fisher Scientific (Pittsburgh, PA) unless otherwise stated.

### Peptide sample preparation

Lyophilized KLVFWAK and ELVFWAE peptides were dissolved at  $\leq 1 \text{ mg/ml}$  in phosphate buffered saline (PBSA, 1X contains 20 mM Na<sub>2</sub>HPO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub>, 150 mM NaCl, 0.02% (w/v) NaN<sub>3</sub>, pH 7.4). Lyophilized capped-KLVFWAK and capped-ELVFWAE peptides were similarly dissolved at  $\leq 1 \text{ mg/ml}$  in phosphate buffer (PBA, 1X is 20 mM Na<sub>2</sub>HPO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub>, 0.02% (w/v) NaN<sub>3</sub>, pH 7.4). Final salt concentrations were adjusted by adding 5M NaCl to individual peptide solutions prior to incubation of the peptides. The peptide solutions were filtered through Millipore 0.45 µm syringe filters (EMD Millipore, Billerica, MA, USA). The concentration of the filtered peptide solutions was measured by UV absorbance at 280 nm. FITC-Labeled peptides were prepared similarly.

### *Time-course peptide assembly*

KLVFWAK and ELVFWAE were mixed at a 1:1 ratio at concentrations of 100  $\mu$ M each, unless otherwise specified. The samples were incubated at room temperature without shaking for up to 8 days. KLVFWAK and ELVFWAE controls (200  $\mu$ M) were incubated separately. The samples were incubated in siliconized tubes, unless otherwise specified. For time course experiments, stock samples were aliquoted and incubated in several vials, each of which was then subjected to characterization after specified time periods. Hetero-assembly of capped-KLVFWAK and capped-ELVFWAE at a 1:1 ratio was studied similarly except that concentration of each peptide was 200  $\mu$ M, unless otherwise mentioned.

### Incorporation of FITC-labeled Peptides

The hetero-assembly of KLVFWAK and ELVFWAE was analyzed utilizing FITC-labeled peptides at their respective N-termini. In one mixture, 100  $\mu$ M of 10% FITC-KLVFWAK was mixed with 100  $\mu$ M unlabeled ELVFWAE. In a second mixture, 100  $\mu$ M of 10% FITC-ELVFWAE was mixed with 100  $\mu$ M unlabeled KLVFWAK. After 1 day of incubation, the aggregates were separated by centrifugation at 15,000 rpm for 5 minutes, and fluorescence intensity of the supernatant was measured with excitation/emission wavelengths of 495/525 nm. Concentration of soluble FITC labeled peptides remaining in the supernatant was calculated from calibration curves of individual solutions of labeled peptide.

# Thioflavin T (ThT) fluorescence

One hundred  $\mu$ l of peptide solutions were mixed with 80  $\mu$ l of dH<sub>2</sub>O and 20  $\mu$ l of 0.1 mM ThT solution. The ThT fluorescence of the samples was immediately measured on a Photon Technology QuantaMaster QM-4 spectrofluorometer (Photon Technology International, Edison, NJ, USA). The excitation wavelength was 440 nm, and the emission was monitored between 460-500 nm with a maximum at ~485 nm. For all samples of the equivalent total peptide concentration, the maximum ThT fluorescence

signal was assigned a value of 1 and all other values were normalized accordingly. Experiments were repeated in triplicate, and the average normalization value was reported with error bars representing 1 SD.

## Transmission electron microscopy (TEM)

Transmission electron microscopy was used to analyze the morphology of the peptide assemblies. Five  $\mu$ l of the samples were pipetted on copper grids and then negatively stained with 1% uranyl acetate solution. The samples were washed 3 times, 5 minutes each with uranyl acetate. The samples were then imaged on a Phillips CM12 Transmission Electron Microscope (FEI Corp., Hillsboro, OR, USA) at 120 kV with a 4k × 2.67k GATAN digital camera, located at the Skirball Institute for Biomolecular Medicine at the NYU Langone Medical Center.

# Size exclusion chromatography (SEC)

Assembled state of the peptides in solution was analyzed with size exclusion chromatography (SEC) using a precision column prepacked with Superdex 75 (separation range from 3 to 70 kDa, GE Healthcare) on a GE FPLC system, as described previously.<sup>4, 5</sup> Briefly, the mobile phase (PBSA) flow rate was set to 0.1 ml/min and elution peaks were detected by UV absorbance at 280 nm. When necessary, samples were centrifuged at 15,000 rpm for 5 minutes to separate insoluble aggregates from soluble peptides, and the soluble fraction was injected. Soluble peptide concentrations were determined by areas of peaks resolved on SEC. The column was calibrated using the following proteins as molecular weight standards: aprotinin (6.5 kDa), ribonuclease A (13.7 kDa), carbonic anhydrase (29 kDa), ovalbumin (44 kDa), and conalbulmin (75 kDa) (GE Healthcare). A column with separation range for smaller polypeptides (e.g., Superdex peptide with a separation range from 0.1 to 7 kDa) was not used for SEC in our study because KLVFWAK was found to stick to the column materials without eluting from the column.

# Laser light scattering (LLS)

Assembly of the peptides in solution was also monitored by laser light scattering (LLS) using the Zetasizer Nano-S system (Malvern Instruments Ltd., Malvern, UK). Samples were mixed and incubated in a quartz cuvette and intensities of scattered light at 633 nm were then measured at 90° relative to the incident light at the same wavelength.

### *Circular dichroism (CD) spectroscopy*

Secondary structure elements of the assemblies in solution were estimated using circular dichroism (CD). Spectra were collected on a Jasco J-815 spectropolarimeter in the far-UV range with a 0.1 cm path length cuvette. The spectrum of the respective background buffers was subtracted from the sample spectrum.

### Intrinsic tryptophan fluorescence

The Tryptophan fluorescence of the samples was measured on a Photon Technology QuantaMaster QM-4 spectrofluorometer. The excitation wavelength was 295 nm, and the emission was monitored between 320-400 nm with a maximum at  $\sim 353$  nm.

Tryptophan fluorescence intensities of samples containing 25  $\mu$ M of KLVFWAK or capped-KLVFWAK (receptor) were measured during incubation with 0-200  $\mu$ M ELVFWAE or capped-ELVFWAE (ligand), respectively. The degree of fluorescence quenching was fit using least mean square regression to Equation 1:

$$\theta = \frac{L}{K_d + L}$$
 Equation 1

where  $\theta$  is fraction of the receptor protein concentration bound to the ligand, *L* is the concentration of free, unbound ligand, and  $K_d$  is the apparent dissociation constant.  $\theta$  was calculated as the normalized fluorescence quenching, and assigned a value of 1 when fluorescence quenching reached its maximum. A free ligand concentration (*L*) was calculated from  $\theta$  and initial ELVFWAE or capped-ELVFWAE concentrations, with the assumption of 1:1 binding.

## Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR)

Attenuated total reflectance-FTIR (ATR-FTIR) spectroscopy was used to analyze the secondary structure components of the insoluble aggregates. The Nicolet-6700 ATR-FTIR system (ThermoFisher Scientific, Waltham, MA, USA) was utilized with a Smart iTR with single reflection diamond plate HATR, located at the NYU Chemistry Shared Instrument Facility. Samples were loaded onto the diamond plate and an average of 128 scans was taken for wavenumbers 1800 to 1000 cm<sup>-1</sup>. Scans of buffer were subtracted from the sample spectra. The Amide I region of the spectra was deconvoluted using Fityk<sup>6</sup> software by fitting Gaussian curves at wavelengths corresponding to known secondary structure elements, and peak areas were compared to the total sum.

### X-Ray Diffraction (XRD)

X-ray diffraction was performed on a Bruker AX8 DISCOVER with GADDS diffractometer. Peptide assemblies were centrifuged for 5 minutes at 15,000 rpm, and washed three times with distilled water. The resulting pellet was vacuum centrifuged overnight in siliconized microcentrifuge tubes to create a dry film. The dried film was mounted on a 20  $\mu$ M thick Nylon Cryoloop with a small amount of immersion oil type B for analysis.

### Structural modeling of hetero-assesmbly

Hetero-peptide amyloid assemblies were modeled initially by threading cationic and anionic peptide sequences onto the 40-residue D23N A $\beta$  solid state NMR structure (PDB ID 2LNQ<sup>7</sup>).  $\beta$ -strand geometry was sampled using rigid-body transformations while simultaneously optimizing sidechain rotamer conformations using the BBDEP2010 rotamer library.<sup>8</sup> Calculations were run in using the protCAD modeling platform.<sup>9</sup>

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