

Supporting Material:

Complete Ref. 39:

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Analysis protocol:

Order score of a pair of associated peptides: We used the translation and rotation vectors to characterize the geometry relationship between two extended peptides. The translation vector \mathbf{t} between two peptides was calculated based on their center of mass. The relative rotation between two peptides was characterized by their reference frames which were unitary matrices. The unitary matrices of peptides one (\mathbf{U}_1) and two (\mathbf{U}_2) were calculated by the singular value decomposition of the correlation matrix ($\mathbf{A}^T \mathbf{B}$) as follows :

$$\mathbf{A}^T \mathbf{B} = \mathbf{U}_1 \mathbf{S}^2 \mathbf{U}_2^T \quad (3)$$

where $\mathbf{A}(n \times 3)$ and $\mathbf{B}(n \times 3)$ were the coordinates of the mainchain atoms from the peptide one and the peptide two respectively, $\mathbf{S} (3 \times 3)$ is a diagonal matrix where the singular values of the matrix \mathbf{A} or \mathbf{B} are in diagonal with a decreasing order, \mathbf{U}_1 (\mathbf{U}_2) consists of the orthonormal column vectors $\mathbf{h}_1, \mathbf{h}_2, \mathbf{h}_3$ ($\mathbf{k}_1, \mathbf{k}_2, \mathbf{k}_3$).

In this case, \mathbf{h}_1 and \mathbf{k}_1 , which were associated with the largest singular value (s_1), were parallel to β -strand direction; \mathbf{h}_2 and \mathbf{k}_2 associated with the second largest singular value (s_2) were almost parallel to the mainchain hydrogen bonding direction; \mathbf{h}_3 and \mathbf{k}_3 associated with the smallest singular value (s_3) were almost parallel to the norm of the β -sheet plane (hereafter defined as stacking direction).

The cosine values ($\cos\alpha, \cos\beta$ and $\cos\gamma$) of the angles between two corresponding vectors (($\mathbf{h}_1, \mathbf{k}_1$) ($\mathbf{h}_2, \mathbf{k}_2$) ($\mathbf{h}_3, \mathbf{k}_3$)) were calculated to characterize the rotation matrix from peptide one

to peptide two. The projections (a , b , c) of the translation vector \mathbf{t} on each column vectors of the matrix \mathbf{U}_1 were calculated to characterize the translation along each direction from the peptide one to the peptide two. Based on these six parameters, the association relation for a pair of associated peptides was further evaluated as follows:

There are two orderly association modes for a pair of peptides. One mode is β -sheet extension (edge-to-edge), which elongates the β -sheets along the hydrogen bonds. The other mode is β -sheet stacking (face-to-face). In order to classify a pair of peptides into either association mode, the cutoffs on the six parameters (a , b , c , α , β and γ) were empirically set. In β -sheet extension association mode, the cutoffs are $|\alpha| < 35^\circ$, $|\beta| < 60^\circ$, $|a| < 18 \text{ \AA}$ (corresponding to the largest registry-shift for two peptides to still be in contact), $|b| < 6 \text{ \AA}$ (roughly one hydrogen-bond distance), and $|c| < 4 \text{ \AA}$ (the largest deviation along the stacking direction allowed for the two peptides to still form a four-strand β -sheet). Similarly, the cutoffs for β -sheet stacking were defined as $|\alpha| < 45^\circ$, $|\beta| < 60^\circ$, $|a| < 18 \text{ \AA}$, $|b| < 3 \text{ \AA}$, and $|c| < 12.5 \text{ \AA}$. The motivation to relax the angle along the strand direction from no more than 35 degrees to 45 degrees was that the stacking of β -sheets was less ordered than the β -sheet extension which was mainly stabilized by cross-strand hydrogen bonds. Furthermore, there were two possible configurations (parallel or antiparallel) for each type of an ordered association (the β -sheet extension or the β -sheet stacking). The configuration type was defined by the directions of the two adjacent β -strands from the two associated peptides.

To facilitate direct comparison between two ordered association modes, score functions were developed. The equivalent between an orderly stacked peptide pair and a perfect hbonded peptide pair was assumed in term of the structure order. A score function for the β -sheet extension (when satisfying the cutoffs for β -sheet extension mode) was given by (3):

$$score = (18 - |a|) * |\cos \alpha| / 18 \quad (3)$$

The motivation for equation 3 is to consider the registry-shift defects of cross-strand hydrogen bonds, indicated by the relative shift along the β -strand direction and the relative rotation along the stacking direction. In this definition, the score is 1.0 when two peptides are aligned in-registry with perfect parallel or antiparallel orientation and becomes 0.0 when two peptides are either off-registry or orthogonal to one another. In a similar way, the β -sheet stacking (when satisfying the cutoffs for β -sheet stacking mode) score was given by (4)

$$score = |\cos \alpha| * (18 - |a|) * (3 - |b|) / 3 / 18 \quad (4)$$

The motivation for equation (4) was to consider the overlapping surface between the two peptides. The bigger the overlapping surface, the stronger the hydrophobic interaction between the two layers.

Order score of an oligomer

Since the oligomers are multi-layered β -sheets, both stacking and elongation must be considered for the structure order of the oligomer. Thus, the order score of an oligomer was defined as the summation of the order score of all unique pair of peptides in the oligomer. The average score over each set of simulations was calculated against the simulated time to monitor the formation of ordered oligomers. The scores can also interpreted as the number of ordered peptide pairs along the hbonding direction or along β -sheet stacking direction.

Free Energy Calculation

For every dodecamer formation event in last 50 ns of the restrained simulations, the numbers of ordered peptide pairs along hbonding direction and along β -sheet stacking direction were calculated as two order parameters of the dodecamer. Based the two order scores, the order space was divided into discrete grids with length 0.2. The total number of dodecamer events in

each grid were counted and normalized by the total event. The free energy in each grid state n was calculated by (7).

$$\Delta G_n = -RT \ln P_n \quad (7)$$

Where R is the Molar Gas Constant, T is the temperature and P_n is probability of dodecamer in the nth grid state.