Supplementary information

Materials and Methods

Aβ peptide sample preparation

Human Lysozyme(*L1667*) was purchased from Sigma Inc., while 40 aa long Aβ peptide was bought either unlabeled, ^{15}N-labeled, or $^{13}C^{15}N$-labeled from AlexoTech AB (Umeå, Sweden) and prepared according to previously described protocols[1].

CD spectra

5μM Aβ peptide(40) was incubated in an Eppedorf tube in a shaker either with or without human Lysozyme in 2 mM phosphate buffer with 150 mM NaCl (pH 7.4) at 37°C between 0 h and 24 h at 200 rpm. Subsequently, CD spectra were recorded in a Jasco J-810 Spectropolarimeter (Jasco Co., Tokyo, Ja-pan) at 37°C. Far UV CD spectra were recorded using a cuvette with a 1 mm path length at 0.5 nm intervals be-tween 195 and 260 nm. The spectra were taken as the average of 4 scans recorded at a speed of 20 nm/min.

ThT assay

A 10 mM ThT stock solution was prepared in 50 mM tris buffer pH 7.4 and filtered to remove ThT particles. This solution was combined with Aβ40 sample and Lysozyme to yield a mixture containing 5 μM ThT, 10 μM Aβ40, 50 mM tris buffer, and either 40nM 200nM, 1μM, 5μM, 20μM or 100μM Lysozyme. The mixture was pipetted into a plate with 384 wells holding 45 μl each, set to a temperature of 37 ˚C. Fluorescence measurements were recorded with a Tecan Safire2 plate reader every 15 minutes, with excitation and emission wavelengths of 446 nm and 490 nm, respectively. The plate was set to automatically shake the wells 30 seconds before each measurement. Each sample was prepared in duplicate, and average fluorescence signals were calculated after the baseline fluorescence of control samples (i.e. samples without Aβ40) had been subtracted from each measurement. The experiments performed in triplicate. The lag time and transition time of amyloid aggregation were

calculated using a Boltzmann function[2,£].

$$F(\mathrm{t}) = F_0 + A/(1 + \exp(-k(t - t_{1/2}))) \qquad (1)$$

$$t_{\mathrm{lag}} = t_{1/2} - 2/k \qquad (2)$$

AFM

Samples of 50 µM Aβ peptide with and without 100µM Lysozyme were incubated in a shaker at 30°C for 12 h at 200 rpm to make aggregates and the obtained aggregates were diluted 1:1 with Tris buffer (50 mM pH7.4) and deposited on freshly cleaved mica for 5 min. Then the excess liquid was shaken off, the substrate rinsed once with 50 mM Tris buffer (pH 7.4) and then dried in a stream of dry nitrogen. Specimens were mounted on a Multi-Mode atomic force microscope. Silicon cantilevers were used, and imaging was carried out in air. The images were collected in tapping mode at frequencies of about 70 kHz.

NMR spectroscopy

A Bruker Avance 500 MHz spectrometer was used to record $^{1}H^{15}N$-HSQC spectra at 5 ^{o}C of 100 µM ^{15}N-labeled Aβ(1-40) peptide in 20 mM sodium phosphate at pH 7.3 (90/10 H_2O/D_2O), both in the absence and presence of 200 µM Lysozyme. The spectrometer was equipped with a triple-resonance cryogenically cooled probe-head, and the spectra were referenced to the water signal.

Molecular modeling

It is impossible to resolve the random coil structure of amyloid beta peptide in normal buffer by NMR or X-ray due to the heterogeneity and conformational instability of the peptide. So far, the monomeric Aβ peptide could only be determined while dissolved in an in SDS solution by liquid NMR. It has a helical structure (pdb ID: 1BA4), even though the Aβ peptide has a random coil structure in water or tris buffer. We used the SDS dissolved structure as a starting model for our docking simulations. The three-dimensional structure of human lysozyme was also taken from the Protein Data Bank

---

£Where ($t_{1/2}$) is the half-time of each kinetic assay, (F_0) is the sloping baseline, (A) is the amplitude and (k) is the elongation rate constant. The lag time (t_{lag}) and transition time (t_{trans}) are calculated using equation 2 and $t_{trans}=4/k$.

(www.rcsb.org/pdb, PDB entries 1REX). The programs Patchdock and Firedock3,4 were used to determine the interaction between Aβ peptide and Lysozyme. PatchDock determined the best starting candidate solutions based on shape complementarity of soft molecular surfaces. The clustering RMSD was set to 4.0 Å as recommended by the software developer for larger molecules and the complex type was set to default. The higher the value of clustering RMSD, the smaller the number of the results we can get. From the protocol of patchdock, the recommended values are 4 Å for protein-protein docking and 1.5 Å for protein-small molecule docking (http://bioinfo3d.cs.tau.ac.il/PatchDock/help.html). Since the Aβ peptide is a peptide (protein), we decided to use the value of 4.0 for the clustering of the RMSD. The PatchDock algorithm divides the connolly dot surface representation of the molecules into concave, convex and flat patches. Then, complementary patches are matched in order to generate the candidate transformations of docked complex (the candidate transformations are the docked complexes of specified receptor and ligand molecule based on the patchdock theory). Patchdock generated the 1000 best-docked candidate transformations, which were selected by global energy, aVdW, rVdW, atomic contact energy, and insidedness measurements. The 1000 best-docked candidate transformations from patchdock were used in FireDock (http://bioinfo3d.cs.tau.ac.il/). The best docking conformations as identified by FireDock were selected for the molecular dynamics simulation.

MD simulations on Aβ and human Lysozyme were performed by the GROMACS suite (version 4.5.5) using the amber03 force field[5] (AMBER-03 is a variant of the AMBER-99 potential and its charges and main-chain torsion potentials have been re-derived based on QM+continuum solvent calculations and each amino acid is allowed unique main-chain charges. Thus, we used it for our modeling). The complex of Aβ peptide and Lysozyme was solvated in a cubic box of 12 Å cutoff with TIP3P water. The conjugated gradient algorithms were implemented for energy minimization with 1000 steps. In order to reduce close contacts between Lysozyme and Aβ peptide, the solvent system was heated to 300 K and equilibrated for 100 ps by a restrained simulation. In the unrestrained molecular dynamics simulation, the particle-mesh Ewald method was used to calculate long-range electrostatic interactions. The temperature coupling and pressure coupling were conducted in the NpT ensemble by using a Berendsen thermostat of 300 K and 0.1 ps relaxation time, and a pressure of 0.5 bar with 0.000045 compressibility and 1 ps relaxation time, respectively. The LINCS algorithm was applied for all bond constraints. The molecular dynamics simulations at 300 K were applied by 173,529 seeds used to

initialize random generator for random velocities. In the analysis, GROMACS 4.5.5 subroutines including g_traj, dssp were used. We analyzed the secondary structure of the proteins using the DSSP algorithm, which is the standard method for assigning secondary structure to the individual amino acids of a protein. From DSSP analysis, we identified secondary structural transition of individual amino acids after lysozyme- Aβ interaction over the whole simulation (we also discussed them in the last paragraph of page2). All molecular representations in this study were generated using Pymol(www.pymol.org/) and UCSF chimera (UCSF Chimera--a visualization system for exploratory research and analysis[6].

Reference:

1. J. Luo, C.-H. Yu, H. Yu, R. Borstnar, S. C. L. Kamerlin, A. Gräslund, J. P. Abrahams, and S. K. T. S. Wärmländer, ACS chemical neuroscience, 2013, 4, 454-62.
2. E. Hellstrand, B. Boland, D. M. Walsh, and S. Linse, ACS chemical neuroscience, 2010, 1, 13-8.
3. D. Schneidman-Duhovny, Y. Inbar, R. Nussinov, and H. J. Wolfson, Nucleic acids research, 2005, 33, W363-7.
4. N. Andrusier, R. Nussinov, and H. J. Wolfson, Proteins: Structure, Function, and Bioinformatics, 2007, 69, 139-159.
5. B. Hess, C. Kutzner, D. van der Spoel, and E. Lindahl, Journal of Chemical Theory and Computation, 2008, 4, 435-447.
6. E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng, and T. E. Ferrin, Journal of computational chemistry, 2004, 25, 1605-12.

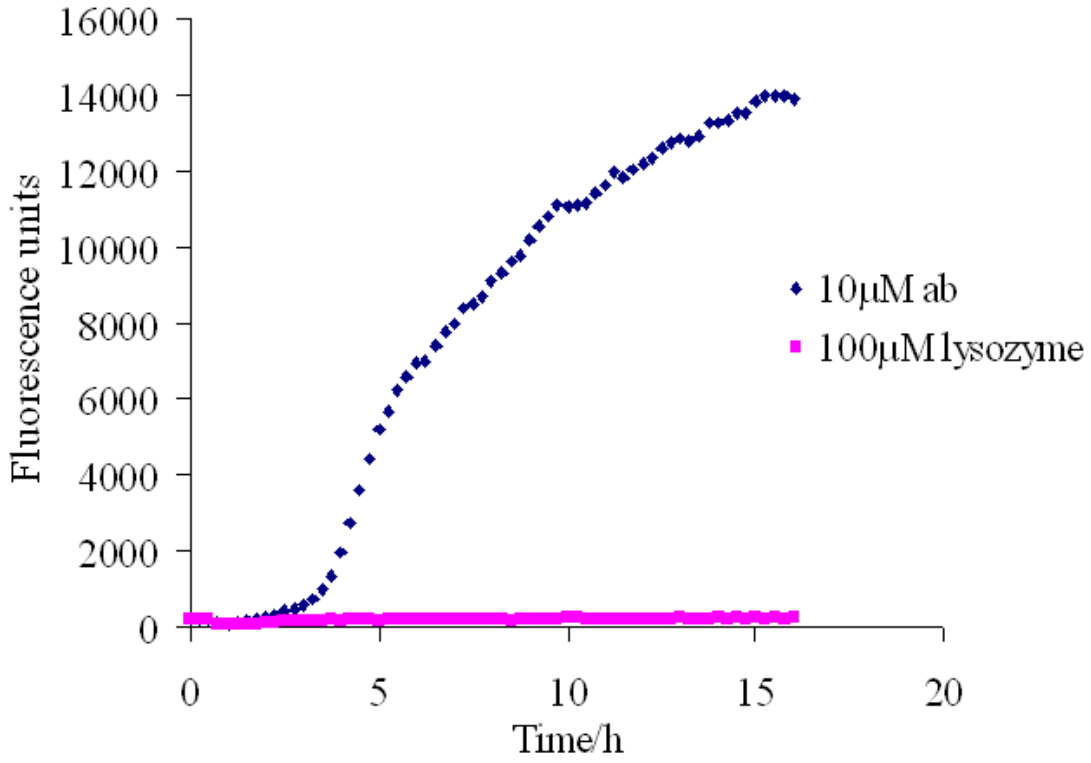

Figure S1. ThT kinetics of Aβ and Lysozyme at 37 °C.

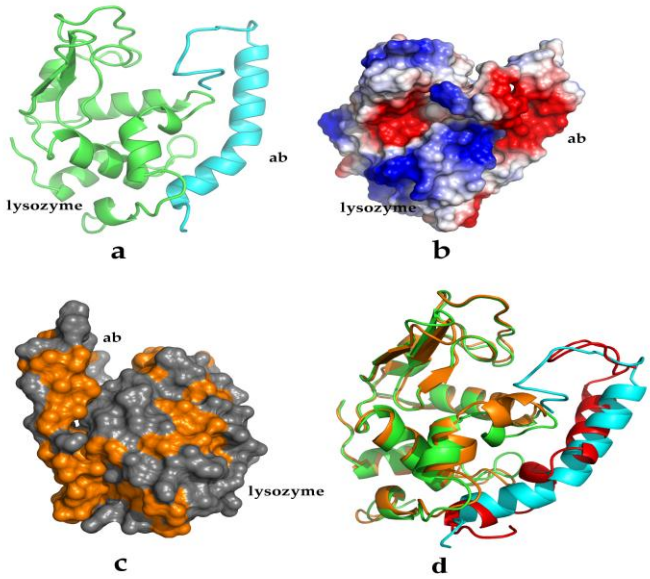

Figure S2. (a) The proposed complex of Aβ peptide(green) and Lysozyme(cyan) after docking. (b-c) Potential and hydrophobic surface of the Aβ peptide - Lysozyme complex: red and blue represent negative and positive charged residues and orange/gray denote hydrophobic/hydrophilic residues, respectively. (d) Superimposition of Lysozyme-Aβ complex before (Lysozyme in green color and Aβ in cyan color) and after (Lysozyme in orange color and Aβ peptide in red color) 100ns molecular dynamic simulations.

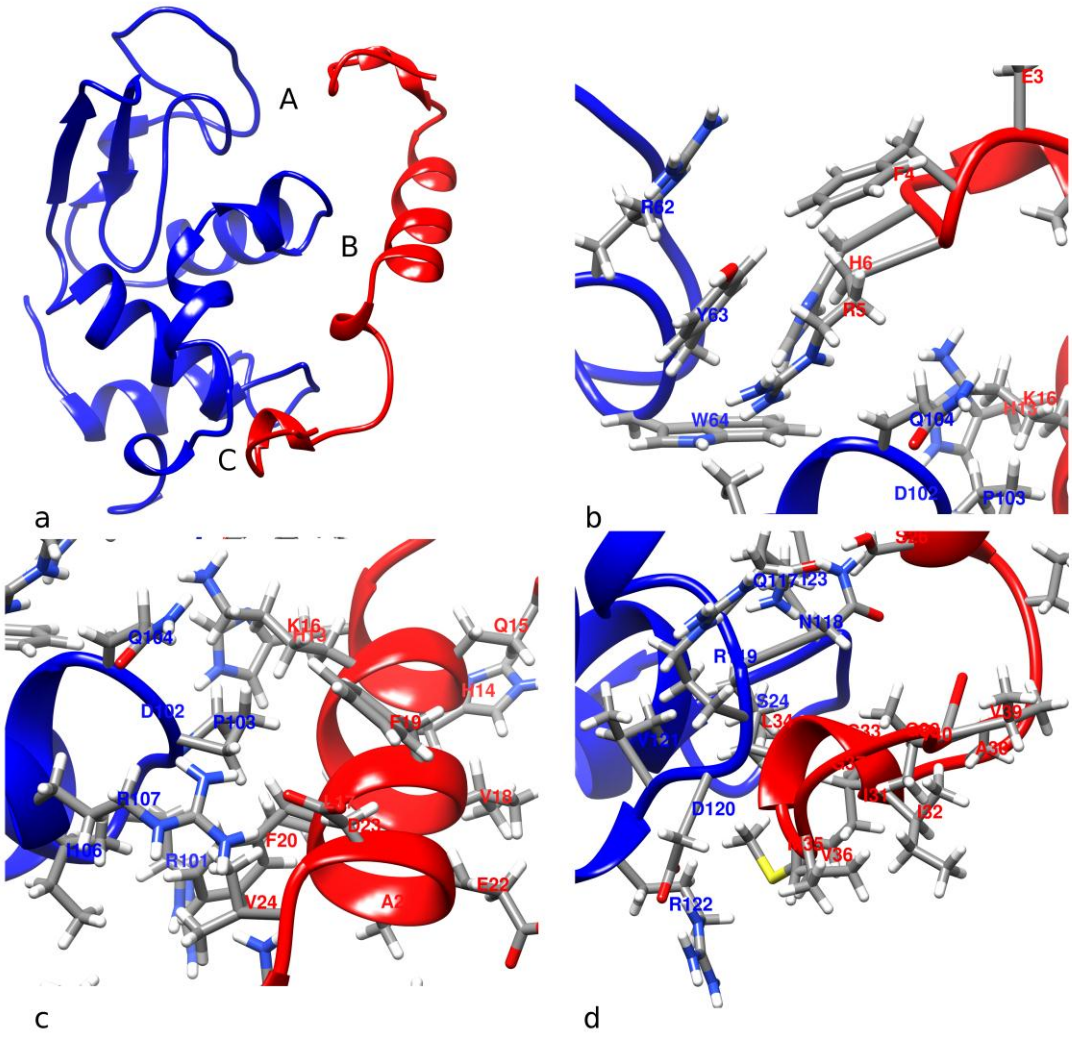

Figure S3. Three different sites of interaction between Aβ40 (red) and human lyszoyme(blue). The A, B and C binding sites are shown in more detail in panels b, c and d, respectively, and the residues1-14, 15-24 and 25-40 from Aβ40 are involved into three different binding sites A, B, C, respectively.

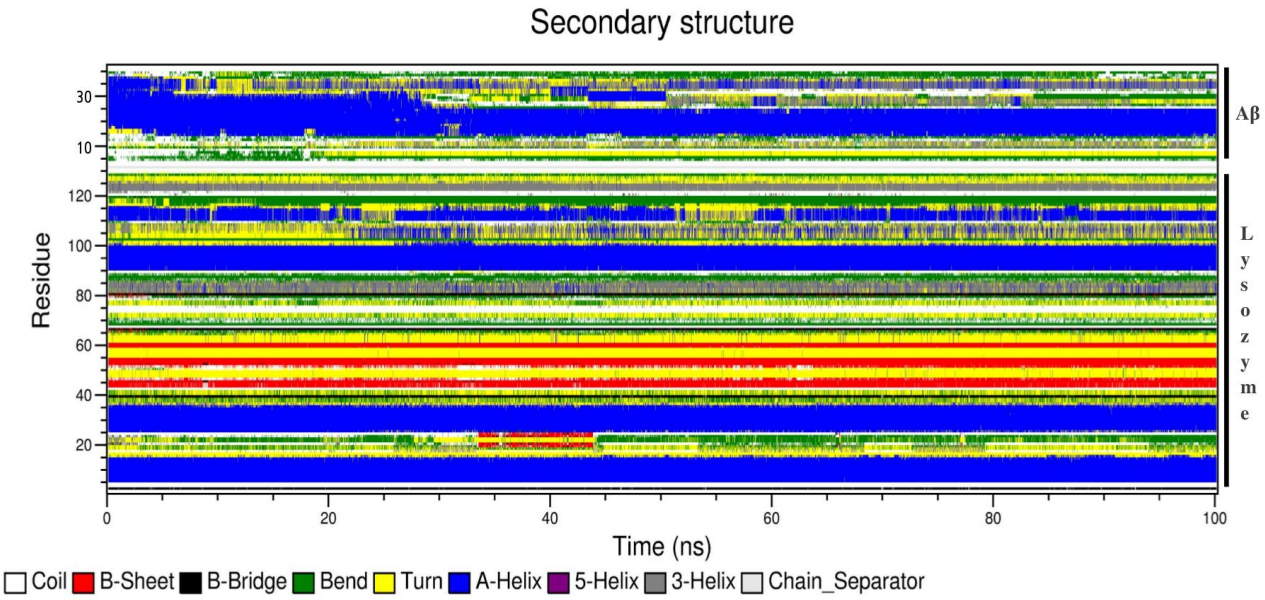

Figure S4. The components of secondary structure of the Aβ-lysome from 100ns MD simulation.