Dynamics of Plaque Formation in Alzheimer Disease

B. Urbanc[*,§], L. Cruz[*], S. V. Buldyrev[*], S. Havlin[*,†], M. C. Irizarry[‡], H. E. Stanley[*] & B. T. Hyman[‡]

[*]Center for Polymer Studies and Department of Physics, Boston University, Boston, MA 02215;

[†]Gonda–Goldschmied Center and Department of Physics, Bar-Ilan University, Ramat-Gan, Israel;

[‡]Neurology Service, Massachusetts General Hospital, Boston, MA 02114

Classification: Physical and Biological Sciences.

Corresponding author:

H. Eugene Stanley Ph.D.
Boston University
Department of Physics
Center for Polymer Studies
590 Commonwealth Avenue
Boston, Massachusetts 02215

Telephone: 617/353-8000
Telefax: 617/353-3783 or 617/353-9393

e-mail: hes@buphyk.bu.edu

Manuscript information: 12 text pages (including title page, references and figure legends) with 4 figures.

Character count: 44,456 characters.

Abbreviations: AD, Alzheimer disease; Aβ, amyloid–β.

[§]On leave from the J. Stefan Institute, Jamova 39, 1001 Ljubljana, Slovenia

ABSTRACT

Plaques that form in the brains of Alzheimer patients are made of deposits of the amyloid–β peptide. We analyze the time evolution of amyloid–β deposition in immunostained brain slices from transgenic mice. We find that amyloid–β deposits appear in clusters whose characteristic size increases from 14 μm in 8–month old mice to 22 μm in 12–month old mice. We show that the clustering has implications on the biological growth of amyloid–β by presenting a growth model that accounts for the experimentally observed structure of individual deposits as well as predicts the formation of clusters of deposits and their time evolution.

Alzheimer Disease (AD) is a progressive neurodegenerative disorder of the central nervous system [1], experienced by an increasing number of the elderly. AD is associated with plaques, which are primarily extracellular deposits of the amyloid–β (Aβ) peptide (40 to 42 amino acids long) that is derived from the larger amyloid precursor protein (APP). Although the role of plaques in AD is not understood [2], compelling genetic and biochemical evidence suggests that Aβ is central to the pathological process in AD [3–5].

The physical and biological basis of Aβ aggregation is unknown. Unfortunately, experiments do not allow for a systematic study of the time development of plaques in AD patients because brain tissues can only be studied *post mortem*. The new technology of transgenic mice [6], however, makes it possible to study temporal development of Aβ deposits in diseased brains because mice can be sacrificed at any stage of development. Figs. 1a and 1b present two photomicrographs from brains of 8– and 12– month transgenic mice, respectively. The Aβ deposits (dark connected areas in Fig. 1) seem to cluster together into larger formations that typically consist of a larger deposit surrounded by many smaller ones. This is surprising because the distribution of APP throughout the cortex of transgenic mice is fairly uniform [7] and thus one would have expected a uniform spatial distribution of Aβ deposits at any stage of aggregation.

In this paper we focus on the time progression of Aβ aggregation and its implications on how Aβ forms in the brain. The question that we address in particular is why are deposits not uniformly distributed throughout the cortex but tend to be close to one another in clusters [1] and what are the growth mechanisms [8–11] that account for the observed spatial distribution of deposits. The conclusions of the paper will be stated in terms of time evolution of the deposits and the clusters of deposits.

In order to quantify the experimentally observed clusters of deposits and their time

[1]In this paper we use a term cluster to mean not a connected entity (as usually used) but rather a correlated yet not connected set of objects (like e.g. a star galaxy).

evolution (Figs. 1a and 1b), we examine photomicrographs from the temporal neocortex (hippocampus) of transgenic mice of 8 and 12 months of age, and calculate the correlation function, $C(r)$, between deposits. By definition, $C(r)$ is proportional to the probability of finding the center of mass of a deposit at a given distance r from a reference deposit at $r = 0$. In practice, we determine $C(r)$ by first identifying the centers of mass for each connected region (deposit), and then calculating the histogram of distances between pairs of deposits. $C(r)$ is normalized such that it approaches the average number density of deposits at large distances r. Results for the average $\langle C(r) \rangle$ are presented in Figs. 2a and 2b (solid lines) for all photomicrographs from 8– and 12–month mice, respectively.

As a control we randomly shuffle the deposits by placing a disk with the area of the initial deposit in place of the original deposit, and then randomize the positions of the disks in a non–overlapping way. For the control case, $\langle C(r) \rangle$ (dashed lines in Figs. 2a and 2b) is constant (non–correlated) everywhere except at very small distances, where it decreases due to the exclusion area of the deposits.

¿From inspection of Figs. 2a and 2b we see that $\langle C(r) \rangle$ shows a dramatically increased probability for finding a deposit at small r, in comparison to $\langle C(r) \rangle$ for the shuffled controls. This means that we can define ξ_{cl}, the characteristic size of clusters, as the size where $\langle C(r) \rangle$ reaches the value of $\langle C(r) \rangle$ for shuffled controls. For mice of age 8 months, the curves join at about $\xi_{cl} = 14 \mu m$, whereas for mice of age 12 months, ξ_{cl} is increased to $22 \mu m$. To confirm the results from $\langle C(r) \rangle$ we determine ξ_{cl} of each individual $C(r)$ calculated from individual photomicrograph. Histograms of individual ξ_{cl} (Fig. 2c) show peaks at $14 \mu m$ and $22 \mu m$ for 8–month and 12–month mice, respectively.

The natural question that follows is: How does ξ_{cl} increase in time? One possible mechanism is that the clusters grow (ξ_{cl} increases) because the deposits that form them grow. We show that this is not the case and that instead the deposits—whose characteristic size does *not* change with time—cluster together, increasing ξ_{cl}. To show this, we calculate ξ_{dep}, the characteristic size of deposits, by first calculating the average deposit area B from the size dis-

tribution of deposit areas and then computing $\xi_{dep} = 2\sqrt{B/\pi}$. We find $\xi_{dep} \approx 1.3\mu m \pm 0.5\mu m$ for both the 8 and 12 month mice. This result is in accord with the observation that the typical diameter of an Aβ deposit in AD brain does not vary as the illness progresses [12,14]. Because ξ_{dep} does not change in time and ξ_{cl} increases from roughly $11 \times \xi_{dep}$ in 8–month mice to about $17 \times \xi_{dep}$ in 12–month mice (Figs. 2a and 2b), we conclude that *clusters grow because more deposits cluster together.*

In order to study the implications of the above results to Aβ aggregation we propose a phenomenological model that is based on several experimental findings. From earlier studies we know that a successful model of the experimentally determined size distribution of Aβ has to consider growth that is proportional to the volume of the growing aggregate [12]. Further work that revealed the porosity of individual Aβ deposits, using the confocal microscopy [13], clarified that the model should take into account disaggregation, that competes with aggregation, as well as surface diffusion. [2] Starting from these elements, the challenge is to test the time evolution of the model against the experimental results of Aβ deposition.

The model is defined as follows. Starting from a random arrangement of occupied sites (particles) in a discrete three–dimensional lattice, each particle is chosen with equal probability 0.5 to duplicate or to be eliminated. This initial state of randomly distributed "seeds" corresponds to the fact that APP is uniformly distributed through the cortex [7] and therefore the growth of deposits is equally probable at any position. Additionally, a particle chosen to duplicate (aggregation) will do so with probability P_{agg}, and, if chosen to be eliminated (disaggregation), it is removed with probability P_{dis}. In the aggregation process, a

[2] In order for a deposit to acquire a typical porous structure with well defined pores the model additionally takes into account surface diffusion—after every step each particle in the lattice is allowed to move to one randomly chosen neighboring empty sites only if the new position has more nearest–neighboring particles. However, the model does not need surface diffusion to exhibit the clustering and its further time evolution, as presented in this paper.

newly–created particle performs a random walk from the original occupied site until the first empty site is encountered. This yields a growth proportional to the volume in agreement with experimental data [12]. The equation for an average number of particles (fluctuations due to a probabilistic nature of the model are not taken into account), N_{t+1}, at time $t+1$ is

$$N_{t+1} = N_t + \frac{1}{2}(P_{agg} - P_{dis})N_t. \tag{1}$$

The two probabilities P_{agg} and P_{dis} can be easily interpreted as the aggregation and disaggregation rates, respectively. If both P_{agg} and P_{dis} are kept constant, Eq. (1) yields either exponential growth (if $P_{agg} > P_{dis}$) or exponential decrease (if $P_{agg} < P_{dis}$) of the number of particles. This model can be mapped onto percolation on the Cayley tree lattice [15]. Even for $P_{agg} = P_{dis}$ the fluctuations eventually lead to the extinction of particles since the probability that the descendants of any given particle survive until time step t decreases as $1/\sqrt{t}$. Because in the brain of AD patients a relatively fixed percentage of cortical surface area is covered by Aβ deposits regardless of duration or severity of dementia (no exponentially unstable behavior) [3], we postulate a steady state. To achieve a dynamical steady state in the model we introduce a feedback that acts upon the disaggregation process such that at every time step, P_{dis} is changed in proportion to the change in the coverage N_t/V (where V is the total number of sites in the lattice). Hence

$$P_{dis}(t) = P_{dis}(t-1) + w\frac{N_{t-1} - N_{t-2}}{V}, \tag{2}$$

where $w > 0$ parametrizes the feedback strength. The feedback mechanism as introduced here postulates a *global response* of the brain: a *change* in the disaggregation rate due to the *change* in the overall amount of Aβ. This response is such that it tends to minimize the changes occurring in the brain, thus naturally leading to a steady state. At this point the model is defined completely.

---

[3] Aβ can occupy as much as 15–20% of the surface area of the cortex of the AD brain, see e.g. [12,13,18].

Some remarks about the above two equations are in order. The equation for the average number of particles in the model with feedback is defined by Eq. (1) with $P_{dis} = P_{dis}(t)$ as given by Eq. (2). The solution of the combined recurrent Eqs. (1,2) can be found by numerical iterations. However, by approximating the differences in both equations by first derivatives (thus neglecting the time delays), we can find an analytic solution that is fairly close to the exact one. From Eq. (2) we first find P_{dis} to be a linear function of N_t, then we insert the solution $P_{dis}(t)$ into Eq. (1), solve it for N_t and find

$$N_t = \frac{N_\infty}{1 + [(N_\infty - N_0)/N_0] \exp(-At)}, \tag{3}$$

where $N_0 = N_{t=0}$ is the number of initial "seeds", $N_\infty = N_0 + V(P_{agg} - P^0_{dis})/w$ is the steady–state number of particles as $t \to \infty$ (total coverage), that depends only on the initial conditions (N_0 and $P^0_{dis} = P_{dis}(t = 0)$), and $A = [P_{agg} - P^0_{dis} + wN_0/V]/2$ is the rate of approaching the steady state. How do we understand this solution? Let us say that the deposition starts with a small number of seeds (small N_0) randomly placed in the lattice and assume that $P^0_{dis} = 0$ (no disaggregation initially). Then N_t (the total amount of deposited Aβ) will keep increasing with t as well as P_{dis} until $P_{dis}(t)$ is on average equal to P_{agg}. At the same time that $P_{dis}(t)$ stabilizes, the total number of particles N_t saturates as well, and the system is in a dynamical steady state. The necessity of disaggregation and feedback processes in the model highlights the importance of biological modifiers (e.g. feedback clearance mechanisms such as microglia [16]) in shaping the topology and microarchitecture of Aβ deposits.

We now proceed to test the model by examining whether the model provides an insight into the clustering phenomenon and whether it is able to explain also *time dependence* of Aβ deposition. Figs. 3a and 3b with the two magnified inserts show the time evolution of our simulation (to be compared with Figs. 1a and 1b). Within the time scale of 300 steps the deposits form. The clustering of deposits, however, is not very pronounced until the time that is roughly one order of magnitude larger, i.e. around 4000 steps. At 4200 steps (Fig. 3b), well–defined clusters occupy an area that is small compared to the system size and

that depends on the total number of occupied sites in the lattice [17]. As time progresses the model predicts typically big deposits that are surrounded by many smaller ones, thus forming clusters similar to the ones observed in transgenic mice.

Similarly to the experiment, we quantify the degree of clustering in the model by considering cross sections from the simulations at different times, up to 4200 simulation steps, and calculating $\langle C(r) \rangle$ (Figs. 4a and 4b with corresponding shuffled controls). As in the experimental case, $\langle C(r) \rangle$ increases at small r. Also, ξ_{cl} increases with time (Fig. 4c), in agreement with the results of the analysis of photomicrographs of transgenic mice of age 8 and 12 months. On the other hand, ξ_{dep} reaches a saturation value on the time scale of 1000 steps, while ξ_{cl} continues to increase beyond 4000 steps (compare the two curves in Fig. 4c). The model therefore predicts that after about 3000 steps the deposits of fixed sizes assemble into clusters, and the size of clusters, ξ_{cl}, increases with time in agreement with the experimental observations above.

The increase of ξ_{cl} can be understood within the model by considering the asymmetry between aggregation and disaggregation [17]. We know that in the dynamical equilibrium the average disaggregation rate, $\langle P_{dis} \rangle$, is equal to the aggregation rate P_{agg}, which means that equal amounts of particles are on average created and destroyed. Let us consider the symmetric case first. If the growth rule would allow the duplicate particle to appear at any empty site in the lattice, the distribution of particles would be uniform, independent of time, and neither deposits nor clusters would form. The nature of the asymmetry in our model is attained because the duplicate particle occupies the *nearest* empty site it encounters, while the disaggregation is uniform (independent of the position). This asymmetry thus permits small deposits to occur only very close to a big deposit. As time progresses, the asymmetry leads to a higher effective disaggregation probability for particles that are isolated and far from the big deposit as opposed to the particles close to it.

The experimental part of our analysis leads to the following conclusions. Aβ deposits in the brain of transgenic mice are not randomly distributed throughout the cortex as expected. Rather, they appear in clusters whose characteristic size increases with the duration of

illness. This study sheds light on the evolution of Aβ deposits from the initially uniformly distributed APP. In the second part we give an interpretation of the observed clustering in transgenic mice within the phenomenological model, which has strong implications on the understanding of pathological changes in Alzheimer disease. Previously, diffuse, amorphous amyloid deposits observed in the brain of Alzheimer patients have been called "primitive" or "immature" plaques, implying that they "mature" into compact classical senile plaques later in the disease process [19]. Our phenomenological model, data from transgenic mice, and preliminary results from human AD cases argue against this paradigm, and instead suggest that the amorphous deposits are not the direct precursor of the more compact senile plaques seen later in the disease. Instead, the model predicts the time evolution of the morphological appearance of Aβ deposits from compact to bigger and more diffuse plaques. Moreover, the model presented here supports the idea that Aβ deposition may be a reversible process, with aggregation *and* disaggregation as its essential components, a feature of critical importance for therapeutic strategies aimed at resolving Aβ deposition.

ACKNOWLEDGMENTS. We thank Drs. Dora Games and Dale Schenk, Athena Neurosciences, for providing the PDAPP mouse tissue used in this work. We also appreciate helpful discussions with T. Gómez-Isla, R. Knowles, M. R. Sadr-Lahijany, D. Wolf and C. Wyart. This paper was partly supported by NIH Grant AG08487, NSF, and by the Adler Foundation.

REFERENCES

[1] Kandel, E. R., Schwartz, J. H., & Jessel, T. M., eds. (1991) *Principles of Neural Science*, (Appleton and Lange, Norwalk).

[2] Beyreuther, K. & Masters, C. L. (1997) *Nature* **389**, 677–678.

[3] Selkoe, D. (1994) *J. Neuropath. Exp. Neurol.* **53**, 438–447.

[4] Goate, A., Chartier-Harlin, M.-C., & Mullan, M. (1991) *Nature* **349**, 704–707.

[5] Cai, X.-D., Golde, T. E., & Younkin, S. G. (1993) *Science* **259**, 514–516.

[6] Games, D., Adams, D., Alessandrini, R., Barbour, R., Berthelette, P., Blackwell, C., Carr, T., Clemens, J., Donaldson, T., Gillespie, F., Guido, T., Hagoplan, S., Johnson-Wood, K., Khan, K., Lee, M., Leibowitz, P., Lieberburg, I., Little, S., Masliah, E., McConlogue, L., Montoya-Zavala, M., Mucke, L., Paganini, L., Penniman, E., Power, M., Schenk, D., Seubert, P., Snyder, B., Soriano, F., Tan, H., Vitale, J., Wadsworth, S., Wolozin, B., & Zhao, J. (1995) *Nature* **373**, 523–527.

[7] Irizarry, M. C., Soriano, F., McNamara, M., Page, K. J., Schenk, D., Games, D., & Hyman, B. T. (1997) *J. Neurosci.* **17**, 7053–7059.

[8] Vicsek, T. (1992) *Fractal Growth Phenomena*, 2nd Edition (World Scientific, New York).

[9] Herrmann, H. J. (1986) *Phys. Rept.* **136**, 153–227.

[10] Takayasu, H. (1990) *Fractals in the Physical Sciences* (Manchester University Press, Manchester).

[11] Meakin, P. (1998) *Fractals, Scaling and Growth Far From Equilibrium* (Cambridge University Press, Cambridge).

[12] Hyman, B. T., West, H. L., Rebeck, G. W., Buldyrev, S. V., Mantegna, R. N., Ukleja, M., Havlin, S. & Stanley, H. E. (1995) *Proc. Natl. Acad. Sci.* **92**, 3586–3590.

[13] Cruz, L., Urbanc, B., Buldyrev, S. V., Christie, R., Gómez-Isla, T., Havlin, S., McNamara, M., Stanley, H. E., & Hyman, B. T. (1997) *Proc. Natl. Acad. Sci.* **94**, 7612–7616.

[14] Arriagada, P. V., Growdon, J. H., Hedley-Whyte, E. T., & Hyman, B. T. (1992) *Neurology* **42**, 631–639.

[15] Harris, T. E. (1989) *The Theory of Branching Processes* (Dover Publications, New York).

[16] Paresce, D., Ghosh, R., & Maxfield, F. R. (1996) *Neuron* **17**, 553–565; El-Khoury, J. (1996) *Nature* **382**, 716–719.

[17] Meyer, M., Havlin, S., & Bunde, A. (1996) *Phys. Rev. E* **54**, 5567–5570.

[18] Hyman, B. T., Marzloff, K., & Arriagada, P. V. (1993) *J. Neuropathol. Exp. Neurol.* **52**, 594–600.

[19] Terry, R. D. & Davies, P. (1980) *Annu. Rev. Neurosci.* **3**, 77–95.

FIGURES

FIG. 1. Images taken using an optical microscope from samples of transgenic mice at ages of (a) 8 and (b) 12 months (magnification $16X$, size $100\mu m \times 100\mu m$ and depth $50\mu m$). A prominent feature of the upper left quadrant of (b) is a large cluster formed by many smaller deposits.

FIG. 2. Depicted by solid lines are $\langle C(r) \rangle$ of deposits from transgenic mice of ages of (a) 8 months (21 micrographs) and (b) 12 months (22 micrographs). Dashed lines are the corresponding $\langle C(r) \rangle$ from the shuffled controls. (c) Histograms of cluster sizes extracted from each $\langle C(r) \rangle$ corresponding to individual micrographs.

FIG. 3. Snapshots from the growth model simulation after (a) 300 (b) 4200 steps with two magnified inserts. The simulation, on a lattice of size $512 \times 512 \times 32$, is started with 2.5% of scattered sites occupied. The aggregation and disaggregation probabilities are $P_{agg} = P_{dis} = 0.80$. Although surface diffusion is not needed for the model to exhibit the clustering, we use in this simulation a surface diffusion corresponding to 10 relaxation steps per particle at every time step.

FIG. 4. Depicted by solid lines are $\langle C(r) \rangle$ averages over 16 different cross sections of the simulation and by dashed lines the corresponding averages $\langle C(r) \rangle$ of the shuffled controls after (a) 300 and (b) 4200 simulation steps. (c) Time dependence of the characteristic deposit size ξ_{dep} and the characteristic cluster size ξ_{cl} as predicted by the model.