Computational Study of Diseases Associated with Protein Aggregation

Mai Suan Li[1] and Ngo Son Tung[1,2]

[1] Institute of Physics, Polish Academy of Sciences, Al. Lotnikow 32/46, 02-668 Warsaw, Poland
[2] Institute for Computational Science and Technology, Quarter 6, Linh Trung Ward, Thu Duc District, Ho Chi Minh City, Vietnam

Abstract — **A number of diseases such as the Alzheimer's disease, Creutzfeldt-Jakob disease, type-2 diabetes are believed to be associated with the aggregation of proteins and amyloid peptides. In this mini-review we discuss general factors that govern the aggregation of polypeptide chains. It is shown that the extent of population of an ensemble of fibril-prone structures in the spectrum of conformations of an isolated protein, is the major determinant of fibril formation rates. Presently, available drugs help to mask the symptoms, but do not treat aggregation-associated diseases making it vital to develop drugs to cope them. Recent progress on design of top-leads for the Alzheimer's disease will be covered.**

Keywords — **Aggregation, Alzheimer's disease, drug design, polyphenols.**

I. Introduction

Proteins that are unrelated by sequence or structure aggregate to form amyloid-like fibrils with a characteristic cross b -structures, which are linked to a number of diseases such as Alzheimer's and prion-disorders [1]. The observation that almost any protein could form fibrils seemed to imply that fibril rates can be predicted solely based on sequence composition and the propensity to adopt global secondary structure. Such a conclusion has limited validity because it does not account for fluctuations that populate aggregation-prone structures. Despite the common structural characteristics of amyloid fibrils [1] the factors that determine the fibril formation tendencies are not understood. In this review we consider the main factors that control the aggregation process. We highlight the role of aggregation-prone ensemble of **N*** structures [2] in the folding landscape of the monomer in determining τ_{fib} and the propensity of sequences to form fibrils.

The design of one new drug approved by FDA takes about 15 years and it costs about one billion USD. In order to shorten this process and make it less expensive one can use the computer simulations to identify leads and validate them as potential drugs. We will discuss recent progress on designing new leads for Alzheimer's disease (AD) with the main focus on natural products.

II. Factors governing fibrillogenesis of proteins

Protein folding and function take place in the environment crowded with biologicalmacromolecules. As a result proteins are exposed to intermolecular interactions that may lead to aggregation [3]. In all, about 20 proteins and polypeptides such as polylysine or polyglutamic acid peptides, myoglobin, SH3 e al are now implicated in amyloid formation in vivo [1]. In many cases protein aggregates take the form of amyloid fibrils, which appear as unbranched rod-like nanostructures with the diameter of an order of 10 nm and varying length [4]. A large body of evidence suggests that amyloid fibrils and associated oligomeric intermediates are related to a number of diseases, including Alzheimer's, Parkinson's, Huntington's, and prion diseases [1]. For example, in the case of the Alzheimer's disease the memory decline may result from the accumulation of the amyloid β (Aβ) peptide present in two forms - 40 (Aβ_{40}) and 42 (Aβ_{42}) amino acids of which are produced through endoproteolysis of the β-amyloid precursor transmembrane protein.

Although amyloid forming proteins and peptides exhibit no obvious sequence or structure homology, the common structural element shared by all amyloid fibrils is an extensive cross-b structure stabilized by backbone hydrogen bonds oriented parallel to the fibril axis (Fig. 1). Then an important question emerges is what are general principles that govern the fibril formation process? Experiments on fibril formation times (τ_{fib}) have been shown that global factors such as the hydrophobicity of side chains [5], net charge [6], patterns of polar and non-polar residues [7], frustration in secondary structure elements [8], and aromatic interactions [9] are very important. The initial hypothesis about the role of aromatic interactions in amyloid fibril formation, e.g., was based on the remarkable occurrence of aromatic residues in many amyloid-related proteins and short peptide fragments [9] as well as on the well-known role of aromatic stacking in processes of self-assembly in chemistry and biochemistry [10]. This hypothesis led to the suggestion that stacking of aromatic residues may play a role in acceleration of the assembly process in many cases of amyloid fibril formation. However, recent experiments

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suggest that aromatic pi-stacking interactions are not critical for Aβ aggregation or for the inhibition of Aβ aggregation [11].

Using the simple lattice model [12] we have shown that the population of the fibril-prone state **N*** (Fig. 2A), $\boldsymbol{P}_{N^*}$, is a key factor that governs aggregation of polypeptide chains [2] (the fibril-like structure of the six-chain system is shown in Fig. 2B). Namely, fibril formation time $\tau_{\rm fib}$ measured in Monte Carlo steps (MCS), depends on $\boldsymbol{P}_{N^*}$ exponentially,

$$\tau_{\rm fib} = \tau^0_{\rm fib}\exp(-cP_{N^*}) \qquad (1)$$

For 8-bead sequences the prefactor $\tau^0_{\rm fib} \approx 1.014\times10^{10}$ MCS and 3.981×10^{11} MCS, and $c \approx 0.9$ and 1.0, for number of chains $N = 6$ and 10, respectively [2] (Fig. 2C). The important role of the population of **N*** is also revealed by all simulations by different force fields [13,14]. Enhancement of $\boldsymbol{P}_{N^*}$ either by mutation or chemical cross linking should increase fibril formation rates. Indeed, a recent experiment [15] showed that the aggregation rate of Aβ_{1-40}-lactam[D23-K28], in which the residues D23 and K28 are chemically constrained by a lactam bridge, is nearly a 1000 times greater than in the wild-type. Since the salt bridge constraint increases the population of the **N*** conformation in the monomeric state [14], it follows from Eq. 1, $\tau_{\rm fib}$ should decrease. One can show that those molecules that have $\boldsymbol{P}_{N^*}$ less than a few percents have low propensity to oligomerization [2,13]. From this prospect, our result (Eq. 1) is useful for elucidating the fibrillogenesis at the single-monomer level. This is of paramount importance because the fibril formation is an extremely slow process which is difficult for numerical study.

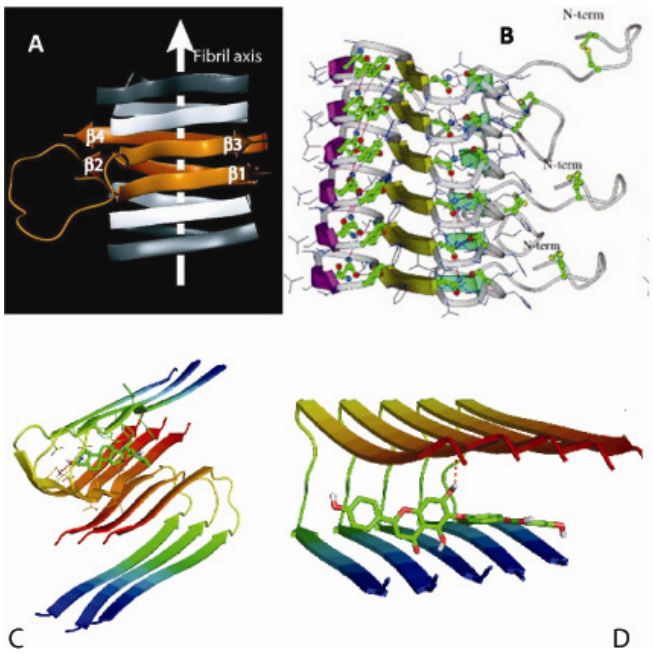

Fig. 1 (A) The fibril from the C-terminal domain 218-289 of the prion protein HET-s (PDB ID: 2RNM). (B) The same as in (A) but for amylin which is associated with the type-2 diabetes. (C) The same as in (A) but for 6 truncated peptides Aβ_{9-40}. The structure was kindly provided by R. Tycko. (D) The same as in (C) but for 5Aβ_{17-42} (PDB ID: 2BEG).

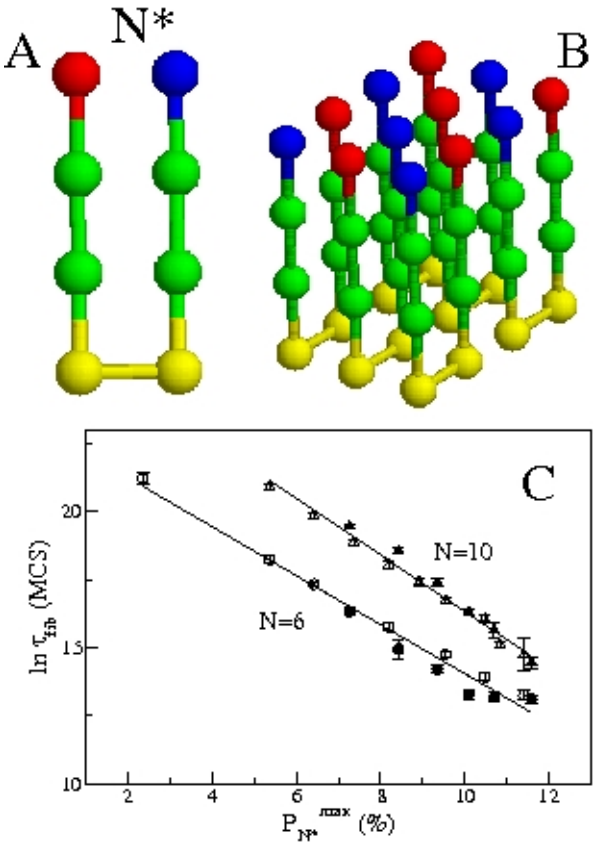

Fig. 2 (A) The 8-bead **N*** conformation in the lattice models.12 (B) The fibril-like structure for $N = 6$ monomers. (C) Dependence of $\tau_{\rm fib}$ on $\boldsymbol{P}_{N^*}$ for N = 6 and 10. $\tau_{\rm fib}$ is measured in MCS and $\boldsymbol{P}_{N^*}$ in %. The correlation coefficient for all fits $R \approx 0.98$. (C) is taken from Ref [2].

III. Recent Progress on Design of New Leads for AD

Two major hypotheses about the pathogenesis of AD have been proposed: Aβ peptide and tau protein. According to the Aβ hypothesis this peptide forms extra-cellular fibrils, which in turn aggregate to form senile plaques, one of the two major morphological hallmarks of AD. The tau protein hypothesis assumes that this protein, in its hyper-phosphorylated form, is the main component of the neurofibrillary tangles, the other hallmark lesion of AD [16].

The amyloid hypothesis states that the generation of Aβ is a key event of AD, and inhibiting this process may affect

the disease progression. Aβ peptides are generated from the posttranslational processing of a large transmembrane protein, the amyloid precursor protein (APP), by two proteases, β- and γ-secretase, respectively [17]. From a structure-based perspective, β- rather than γ-secretase has appeared to be a suitable target for drug design purposes because the structure of β-secretase has been experimentally resolved, while γ-secretase has not been crystallized yet. The β-secretase APP-cleaving enzyme (BACE-1) catalyzes the rate-limiting step in the production of Aβ. BACE-1 inhibition represents a possible therapeutic strategy to drastically reduce Aβ levels [18]. Indeed, huge progress has been made in search for BACE-1 inhibitors as potential anti-AD drug candidates using structure-based approaches [19,20].

On the another hand, since AD is presumably associated with oligomerization of Aβ peptides [16], the second of strategy to cope with this disease is to develop compounds able to promote Aβ anti-aggregation and clearance. In this case Aβ peptides and their fibrils (Fig. 1C and D) serve as drug targets. Here we focus on inhibitors of this class of receptors.

Because Aβ is self-assembling, one can use short peptide fragments homologous to the fulllength wild-type protein [21–25] or N-Methylated peptides [26] as inhibitors. The binding affinity of beta-sheet breaker peptides KLVFF and LPFFD have been studied by experiment [21,22] and simulation [27] in detail. The binding free energy is presumably related to hydrophobicity of ligands in such a way that the higher is hydrophobicity the lower inhibitory capacity. The use of specific peptides to inhibit Aβ oligomerization and toxicity, although intriguing, has yet to progress beyond vivo models of amyloidosis.

Carbohydrate-containing compounds, polyamines, chaperones, metal chelators etc may be used to interfere Aβ fibrillogenesis [28,29]. One of the most important classes of potential leads is polyphenols [30] that represent a large group of natural and synthesized small molecules. They are composed of one ormore aromatic phenolic ringsmaking them susceptible to Aβ peptides. Natural polyphenols are phytochemicals found in high concentrations in wine, tea, nuts, and a wide variety of other plants. Some nutraceuticals, as shown by pre-clinical and certain clinical studies, may be of value as AD therapeutic [31,32]. Among them Curcumin (diferulomrthane) [33] (Fig. 3), ginkgo biloba [34] and (-)-Epigallocatechin-3-Gallate (EGCG) (green tea) [35] from the traditional Chinese and Indian medicines are reported to inhibit Aβ aggregation and to be capable against Aβ -inducedtoxicity. Ginkgo biloba tree extract includes many compounds including ginkgolides A, B, C and J (see Fig. 3 for chemical structure of ginkgolide A) which may protect against Aβ-induced synapse damage. Clinical trials are going in phase II and III for curcumin [36] and ginkgo biloba [37], respectively. Tannic acid (Fig. 3) is the most potent inhibitor of Aβ fibril formation [38] among polyphenols having the inhibition constant $IC_{50} \approx 0.01\mu$m, while Curcumin has $IC_{50} \approx 0.8\mu$m.

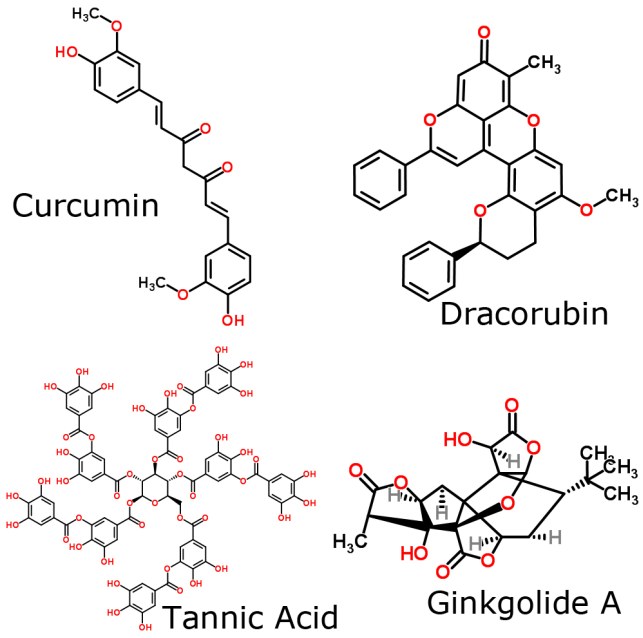

Fig. 3 Chemical structures of Curcumin, ginkgolide A, Tannic acid and Dracorubin (ID: 160270)

Recently we have conducted search for possible leads among compounds derived from Vietnamese herbs and plants for anti-aggregation of amyloid peptides [39]. This problem is of interest because although the traditional Vietnamese medicine shares common features with Chinese and Indian ones, it has many specific herbs due to difference in geography and soil quality [40]. The binding affinity of 342 compounds to full-length Aβ_{40} and Aβ_{42} peptides and their mature fibrils have been studied using Autodock Vina version 1.1. [41] Results obtained by the docking have been then refined by the more accurate Molecular Mechanic-Poisson Boltzmann Surface Area (MM-PBSA) method [42]. We have found that the champion lead is Dracorubin which has ID number 160270 in the Pubchem and Chemspider database (see http://pubchem.ncbi.nlm.nih.gov and http:// http://www.chemspider.com/) (Fig. 3). This compound shows even higher binding affinity to Aβ pepides than Curcumin having the binding free energy $\Delta G_{bind} = -15.59$ kcal/mol [39]. Recall that Curcumin has the binding free energy $\Delta G_{bind} \approx -13.3$ kcal/mol [43].

IV. Conclusions

We have reviewed the key factors such as charge, hydrophobicity, aromatic rings and population of the fibril-prone state in controlling aggregation of polypeptide chains. They are intrinsic and dependent on protein sequences. External conditions like temperature, pH, salt concentration, environment crowding etc also affect aggregation but they are not mentioned here. The recent *in-silico* and *in-vitro* development on search for potential drugs for AD has been briefly discussed. Further clinical tests for potent leads would be an important step in this direction.

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The address of the corresponding author:

Author: Mai Suan Li
Institute: Institute of Physics, Polish Academy of Sciences
Street: Al. Lotnikow 32/46, 02-668
City: Warsaw
Country: Poland
Email: masli@ifpan.edu.pl