Filamentous aggregates of tau proteins fulfil standard amyloid criteria

Dawid Dulak¹, Małgorzata Gadzała², Mateusz Banach¹, Magdalena Ptak³, Zdzisław Wiśniowski³, Leszek Konieczny⁴ and Irena Roterman*²

1. Introduction

The origin of amyloid transformation has attracted scientific attention for more than 35 years – at least since being acknowledged as the cause of various neurodegenerative disorders [1]. The coexistence and mutual relations between Aβ amyloids and tau tangles, resulting in damage and destruction of synapses, is believed to provoke behavioural changes associated with cognitive impairment [2,3]. It is often stated that effective therapy should rely on arresting the formation of tau fibrils [4]. The search for genetic causes singles out APOE4 as the most important risk factor affecting Aβ pathogenesis, mediated by tau proteins [5,6]. The natural capability of tau to bind to tubulin – a hallmark of its biological activity – is due to phosphorylation. Pathological processes associated with Alzheimer’s disease, evidenced by abnormal phosphorylation, result not only in
disorganization of microtubulin, but also in the appearance of intracellular tau aggregates, referred to as neurofibrillary tangles [7]. Tauopathies are defined as clinically, morphologically and biochemically heterogeneous neurodegenerative diseases characterized by the deposition of abnormal tau protein in the brain [8].

Comprehensive structural analysis, based on the function of shielding tensors of the Ca atoms which relates binding affinities and folding rate constants to secondary conformational preferences, may reveal common patterns of backbone density distribution in amyloidogenic regions of Alzheimer’s amyloid β and tau, Parkinson’s α-synuclein and prions [9]. Emergence of fibrillar structures is also thought of as the result of involvement of intrinsically disordered proteins, especially at early phases of the folding process [10].

Tau amyloid fibrils are regarded as peculiar due to the existence of two distinct superfibrillar forms: straight filament and paired helical filament [11]. Two individual protofilaments may form different structures depending on their mutual arrangement in the dimer. The authors of [11] refer to the conformation of the protofibril as “C-shaped”. The dimer (superfibril), when analyzed under cryo-EM imaging, resembles two arched C-shaped structures bound back to back. In one form, the structure is symmetrical (with the same residues in both unit molecules involved in complexation) while the other form lacks such symmetry (in this case, the central fragment of one chain makes contact with a fragment which is somewhat closer to the N terminus of the adjacent chain). The structure of this type of amyloid fibril is revealed thanks to cryo-electron microscopy [12].

Due to the unusual nature of tau amyloids, certain occurrences of Alzheimer’s disease are characterized as tauopathies [13]. The cause behind these pathologies is thought to be associated with chemical processes including hyperphosphorylation, acetylation, glycation, ubiquitination, nitration and proteolytic cleavage [14]. An interesting property of protein-based fibrillar filaments, differentiating them from other structures, is the low density of β-strands (which tend to dominate in other types of amyloid fibrils, such as Aβ(42)).

According to the analysis presented in [11], dimerization of protofilaments occurs by way of hydrogen bonds forming between adjacent fragments. In its native form, tau in complex with a microtubule adopts a conformation referred to as “natively unfolded”. This conformation is highly resistant to spikes in temperature and acidity [15].

The presented here analysis focuses on microtubule-associated tau neurofibrillary tangle protein, paired helical filaments (consisting of two individual fibrils), individual proto-fibrils as well as individual chains.

Identification and characterization of amyloid structures, as presented in this work, bases on comparative analysis of the structure of globular and fibrillar proteins. The proposed fibrillarization model for globular structures is also discussed in [16, 17]. At the core of the fuzzy oil drop (FOD) model lies the concept of applying a 3D Gaussian form to express the idealized distribution of hydrophobicity in a globular protein. Such distribution has a distinct peak at the geometric center of the globule and then falls off along with distance from the center, reaching almost zero at the surface (i.e. at a distance of 3σ from the center). We have identified proteins whose structure closely corresponds to this theoretical distribution [18,19]. Any local deviations are usually associated with the protein’s intended function: local excess of hydrophobicity, if occurring on the surface, usually marks a complexation interface, while hydrophobicity deficiencies tend to
correspond to binding cavities, capable of housing ligands (or substrates, in the case of enzymes) [20]. The universality and ubiquity of hydrophobic cores is attested to by a study of a large number of proteins with varying secondary and supersecondary structural characteristics [21]. A specific type of discordance vs. the idealized (monocentric) distribution of hydrophobicity is observed in the case of proteins which contain so-called solenoid fragments, including some antifreeze proteins [22]. Such fragments deviate from a centralized core in favor of a distribution comprising alternating bands of high and low hydrophobicity, propagating along solenoids long axis [22]. However, in addition to solenoids, biologically active proteins also include fragments whose purpose is to restrict unchecked propagation of such linear sequences (thereby preventing dimerization or polymerization), as well as to ensure solubility. The special “caps” are identified in such proteins [22]. Structures which strongly deviate from the monocentric distribution and lack suitable “caps” are prone to amyloid aggregation [16, 17]. In the context of the fuzzy oil drop model such structures can be likened to ribbonlike micelles, capable of unrestricted propagation [23]. A thorough description of the fuzzy oil drop model can be found in [24].

In the fuzzy oil drop model, the emergent structure of the hydrophobic core is thought to result from interactions between the protein and the aqueous solvent, which directs hydrophobic residues towards the center of the globule and favors exposure of hydrophilic residues on the surface. As already indicated, local discords are often associated with the presence of external factors. The fact that amyloids forms do not require mutations to emerge suggests that misfolding is not caused by factors internal to the polypeptide itself. This hypothesis is further supported by the observation that amyloids are dominated by linear patterns of hydrophobicity, themselves dependent on the intrinsic hydrophobicity of each residue, rather than by the cooperative tendency to form a hydrophobic core [16, 17, 21]. Shaking is known to promote amyloidogenesis – and it can hardly be called a chemical factor. Perhaps shaking disrupts the structure of the solvent in such a way as to prevent it from guiding “natural” conformational changes within the protein chain. Alternatively, shaking is notable for aerating the solvent. The resulting increase in the area of the liquid/gas interface may produce structural changes within the solvent itself.

In addition to analysis of the tau protein amyloid, as listed in PDB [25], this work proposes an in silico experiment, which involves determining alternative structures which the tau amyloid sequence may attain (using specialized protein folding software, such as Robetta [26, 27] and I-Tasser [28, 29]), and performing folding simulations based on the fuzzy oil drop model. It turns out that the sequence is indeed capable of producing a globular form with a single, monocentric hydrophobic core. Subjecting globular structures to FOD characterization enables us to track changes which result in amyloidogenesis. The work focuses on three distinct structures: 1. the superfibril (seeking the causes behind its structural variability), 2. the protofibril (identifying the characteristic properties of amyloid structures), 3. a single chain participating in the fibril. The presented research is based on observations rooted in the fuzzy oil drop model, specifically, the linear propagation of hydrophobicity in amyloids (which prevents a shared hydrophobic core from forming). As discussed in [16, 17], the presence of alternating bands of high and low hydrophobicity can be regarded as one of the principal indicators of amyloid transformation.
2. Results

3.1. Superfibril

This analysis concerns the amyloid form listed in PDB as 5O3O, 5O3L and 5O3T (pronase-treated paired helical filament in alzheimer’s disease brain neurofibrillary tangle protein, paired helical filament-tau, phf-tau, Homo sapiens). Fragment: residues 623-695 of tau protein (306-378 according to PDB numbering) Chains A, C, E, G, I, along with their counterparts (B, D, F, H, J) make up the proto-fibrils [30]. In order to characterize individual chains in the context of the superfibril and proto-fibrils, we have singled out chains E and F. These two chains are located in the central part of the fibril and can be regarded as representative of an arbitrarily long structure. This selection also minimizes edge effects caused by the finite width of the complex.

3.1.1. Properties of superfibrils and interfaces – What is the source of different isoforms of tau aggregates?

Table 1 presents the status of amyloid tau structures in terms of RD values, revealing large discordances between T and O profiles in both models (T-O-R and T-O-H). This means that the distribution of hydrophobicity does not involve a central hydrophobic core. Further analysis will reveal that amyloids are dominated by a pattern which consists of alternating bands of high and low hydrophobicity. High values of RD further indicate that the folding process is driven by the intrinsic properties of each residue rather than by a global force field – this is also typical for amyloids [16, 17]. Regarding the hydrophobicity profile correlation coefficients, HvT and TvO lag behind HvO. This is also due to the absence of a central hydrophobic core, which is replaced by linear propagation of narrow “bands”. In further sections we will specifically describe locations which exhibit these properties.

Visual comparison of T and O (Figure 1) highlights major differences between these distributions. It should be noted that the chart consists of many overlapping profiles, which means that the distribution of local minima and maxima is replicated in each adjacent chain, resulting in a set of narrow bands, as suggested above.

The fuzzy oil drop model may also be used to predict the properties of shared hydrophobic cores in protein complexes [31]. In order to properly characterize a given complex, it is important to assess the status of its interface. With regard to proto-fibrils, the distribution observed in 5O3T differs from those exhibited by the remaining structures. However the difference is limited only to the structure of interface, which is discussed in this paper. In 5O3O and 5O3L the status is similar and suggests that the superfibril emerges as a result of factors consistent with the fuzzy oil drop model, i.e. under the influence of the aqueous solvent. This interpretation is supported by high values of all correlation coefficients. We may conclude that the interface is shaped by all factors which determine the structure of the complex itself, with major involvement of water.

The picture changes, however, when dealing with 5O3T. Its high value of RD (T-O-R), coupled with negative values of HvT and TvO coefficients and a high value of the HvO coefficient, suggest that in this case the solvent does not play a significant role in complexation.

It should be noted that the status of the interface is computed by taking into account all interface residues in the entire fibril (following protein-protein contact distance criteria of PDBsum [32]). When all three correlation coefficients adopt strongly positive values, we may assume that the structure of the interface represents a compromise between all three hydrophobicity profiles (observed, intrinsic and theoretical). In contrast, negative values of HvT and TvO are understood to
mean that the interface folds “in spite of” fuzzy oil drop model and in consequence in spite of environmental effects which act upon the proto-fibril complex.

Table 1. -RD values and correlation coefficients computed for the superfibril, proto-fibrils and individual chains treated as components of the superfibril.

<table>
<thead>
<tr>
<th>PDB ID</th>
<th>RD T-O-R</th>
<th>RD T-O-H</th>
<th>Correlation coefficient HvT</th>
<th>Correlation coefficient TvO</th>
<th>Correlation coefficient HvO</th>
</tr>
</thead>
<tbody>
<tr>
<td>5O3O</td>
<td>0.745</td>
<td>0.687</td>
<td>0.012</td>
<td>0.259</td>
<td>0.675</td>
</tr>
<tr>
<td>5O3L</td>
<td>0.731</td>
<td>0.669</td>
<td>0.013</td>
<td>0.296</td>
<td>0.646</td>
</tr>
<tr>
<td>5O3T</td>
<td>0.724</td>
<td>0.641</td>
<td>0.008</td>
<td>0.301</td>
<td>0.716</td>
</tr>
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</table>

Inter-fibril interface

<table>
<thead>
<tr>
<th>PDB ID</th>
<th>RD T-O-R</th>
<th>RD T-O-H</th>
<th>Correlation coefficient HvT</th>
<th>Correlation coefficient TvO</th>
<th>Correlation coefficient HvO</th>
</tr>
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<tbody>
<tr>
<td>5O3O</td>
<td>0.401</td>
<td>0.550</td>
<td>0.666</td>
<td>0.754</td>
<td>0.950</td>
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<tr>
<td>5O3L</td>
<td>0.368</td>
<td>0.587</td>
<td>0.696</td>
<td>0.772</td>
<td>0.929</td>
</tr>
<tr>
<td>5O3T</td>
<td>0.527</td>
<td>0.386</td>
<td>-0.164</td>
<td>-0.003</td>
<td>0.850</td>
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</tbody>
</table>

Chains in superfibril

5O3O

<table>
<thead>
<tr>
<th>Chain</th>
<th>RD T-O-R</th>
<th>RD T-O-H</th>
<th>Correlation coefficient HvT</th>
<th>Correlation coefficient TvO</th>
<th>Correlation coefficient HvO</th>
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</thead>
<tbody>
<tr>
<td>Chain E</td>
<td>0.741</td>
<td>0.700</td>
<td>0.011</td>
<td>0.237</td>
<td>0.731</td>
</tr>
<tr>
<td>Chain F</td>
<td>0.761</td>
<td>0.722</td>
<td>0.010</td>
<td>0.224</td>
<td>0.731</td>
</tr>
<tr>
<td>Chains E+F</td>
<td>0.752</td>
<td>0.711</td>
<td>0.013</td>
<td>0.230</td>
<td>0.731</td>
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5O3L

<table>
<thead>
<tr>
<th>Chain</th>
<th>RD T-O-R</th>
<th>RD T-O-H</th>
<th>Correlation coefficient HvT</th>
<th>Correlation coefficient TvO</th>
<th>Correlation coefficient HvO</th>
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</thead>
<tbody>
<tr>
<td>Chain E</td>
<td>0.728</td>
<td>0.675</td>
<td>0.017</td>
<td>0.274</td>
<td>0.696</td>
</tr>
<tr>
<td>Chain F</td>
<td>0.747</td>
<td>0.698</td>
<td>0.012</td>
<td>0.265</td>
<td>0.696</td>
</tr>
<tr>
<td>Chains E+F</td>
<td>0.741</td>
<td>0.690</td>
<td>0.014</td>
<td>0.268</td>
<td>0.606</td>
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5O3T

<table>
<thead>
<tr>
<th>Chain</th>
<th>RD T-O-R</th>
<th>RD T-O-H</th>
<th>Correlation coefficient HvT</th>
<th>Correlation coefficient TvO</th>
<th>Correlation coefficient HvO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chain E</td>
<td>0.732</td>
<td>0.666</td>
<td>0.034</td>
<td>0.400</td>
<td>0.769</td>
</tr>
<tr>
<td>Chain F</td>
<td>0.728</td>
<td>0.666</td>
<td>-0.018</td>
<td>0.136</td>
<td>0.785</td>
</tr>
<tr>
<td>Chain E+F</td>
<td>0.730</td>
<td>0.666</td>
<td>0.008</td>
<td>0.273</td>
<td>0.777</td>
</tr>
</tbody>
</table>

The presented characterization concerns chains E and F, which are located centrally and therefore representative of an unrestricted fibril. The calculated values are typical for amyloid forms, and include high values of RD and HvO, along with very low (sometimes even negative) values of HvT and TvO. In 5O3T both individual chains as well as the interface fragment are shaped by intrinsic hydrophobicity rather than by the external environment, which would favor the formation of a monocentric hydrophobic core.

As the status of individual chains (in the context of the superfibril) is largely similar in all structures, we will limit their presentation to 5O3O and 5O3T (Table 2).

Table 2. Status of individual chains treated as components of the superfibril. The presentation of 5O3L is omitted since due to its similarity to 5O3O.

<table>
<thead>
<tr>
<th>Chain</th>
<th>RD T-O-R</th>
<th>RD T-O-H</th>
<th>Correlation coefficient HvT</th>
<th>Correlation coefficient TvO</th>
<th>Correlation coefficient HvO</th>
</tr>
</thead>
</table>
When analyzing individual chains as components of the superfibril, we arrive at similar RD values. In contrast, when the same chains are analyzed as components of individual proto-fibrils (5O3T), their values differ due to differences in the orientation of each proto-fibril. Negative values of correlation coefficients for HvT and TvO with high value of correlation coefficient for HvO relation suggest that 5O3T is a typical amyloid form.

Figure 1 provides a graphical representation of the superfibril and both chains (E and F) treated as components of the superfibril.

Figure 1. Theoretical (T-blue) and observed (O-red) distributions of hydrophobicity in: A – the 5O3O superfibril; B, C – chains E and F as components of the 5O3O superfibril. Orange highlighting marks residues which comprise the inter-fibril interface area (331-338).
The status of 5O3O – superfibril is visualized in Figure 1.A, which shows that the theoretical distribution involves two local maxima, along with hydrophilic fragments exposed on the surface. Neither maximum is evident in the observed distribution, however O includes other local maxima, located in areas where low hydrophobicity is expected. It should be noted that each of these local maxima (as well as minima) represents an entire band stretching along the fibril’s long axis. The overlap is due to the repeating pattern present in each individual chain, with only the outlying chains exhibiting slightly lower hydrophobicity. On the other hand, the differences between theoretical distributions are readily apparent since this distribution predicts that hydrophobicity should decrease along with distance from the center. The degree of discordance between T and O can be analyzed by comparing theoretical charts with the observed distributions for chains E and F (which are centrally located and therefore representative for the entire fibril – see Figure 1.B, C).

The interface fragment appears to be consistent with the fuzzy oil model. Given the central location of the interface, a hydrophobicity peak is expected and – to a certain extent – present in the actual complex. Comparing O with T reveals that two outlying residues exhibit relatively low hydrophobicity, while the central section corresponds to a major spike. Consequently, we rate the interface fragment as accordant with the model.

A characteristic feature of amyloids is the presence of numerous local maxima in areas where low hydrophobicity (and vice versa) is predicted by the theoretical model. However, it is important to remember that unlike globular proteins (which may also exhibit this phenomenon), the complexed chains form here bands which stretch along the entire long axis of the fibril. These observations are confirmed by analysis of T and O for chains E and F (treated as components of the superfibril). The discordance between T and O distribution in most of proteins is of local character.

### 3.2. Properties of proto-fibrils

Table 3 presents the hydrophobicity parameters for each proto-fibril. In this case, each proto-fibril is treated as a distinct structural unit. This means that a separate Gaussian is constructed for each proto-fibril (in the preceding section, a shared Gaussian form was computed for the entire superfibril). Results are indicative of an amyloid form: high values of RD and HvO along with very low (even negative) values of HvT and TvO. The correlation coefficients reveal that the structure is dominated by the intrinsic properties of its component residues – an observation supported by the observed high values of RD in both models (T-O-R and T-O-H). Thus, the observed distribution is more closely aligned with R (or H) rather than T.

The status of chains E and F, treated as components of their respective proto-fibrils confirms that they adopt amyloid-like forms, although this effect is less pronounced than in the case of the superfibril. (lower values of T-O-H RD and HvO – see Table 3).
Table 3. Status of individual chains treated as components of their respective proto-fibrils. The table lists only values obtained for the ACEGI proto-fibril. Differences with regard to the other proto-fibril are negligible.

<table>
<thead>
<tr>
<th>PDB ID</th>
<th>RD</th>
<th>T-O-R</th>
<th>T-O-H</th>
<th>HvT</th>
<th>TvO</th>
<th>HvO</th>
</tr>
</thead>
<tbody>
<tr>
<td>5O3O</td>
<td>Chain E</td>
<td>0.661</td>
<td>0.607</td>
<td>-0.012</td>
<td>0.089</td>
<td>0.772</td>
</tr>
<tr>
<td></td>
<td>Chain F</td>
<td>0.679</td>
<td>0.430</td>
<td>-0.027</td>
<td>0.095</td>
<td>0.548</td>
</tr>
<tr>
<td>5O3L</td>
<td>Chain E</td>
<td>0.664</td>
<td>0.595</td>
<td>-0.022</td>
<td>0.082</td>
<td>0.767</td>
</tr>
<tr>
<td></td>
<td>Chain F</td>
<td>0.674</td>
<td>0.410</td>
<td>-0.039</td>
<td>0.091</td>
<td>0.545</td>
</tr>
<tr>
<td>5O3T</td>
<td>Chain E</td>
<td>0.673</td>
<td>0.602</td>
<td>-0.017</td>
<td>0.118</td>
<td>0.773</td>
</tr>
<tr>
<td></td>
<td>Chain F</td>
<td>0.683</td>
<td>0.415</td>
<td>-0.033</td>
<td>0.098</td>
<td>0.550</td>
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</tbody>
</table>

Figure 2 provides a visual representation of these results, showing T and O distributions for one proto-fibril (ACEGI), and the status of the E chain within this structural unit.

An asymmetrical distribution of local maxima is observed in the proto-fibril as a result of significant displacement of the system’s central point compared to the superfibril. Numerous local maxima are present in areas where low hydrophobicity is expected. The involvement of a local maximum in the interface fragment indicates that complexation of proto-fibrils is generated as the effect of the influence of environment (according to FOD model).
3.3. Properties of individual chains treated as distinct structural units

Our analysis also covers individual chains treated as distinct structural units, with a separate Gaussian plotted for each chain (under the assumption that each chain folds in separation from other chains). To determine the causes of the discordance between the observed and theoretical distributions, we have singled out fragments for which this discordance is particularly evident. Note that we are not dealing with isolated deviations – in many areas, both distributions strongly oppose each other, indicating that the chain does not produce a globule and is likely insoluble due to the lack of a polar surface.

Figure 3 illustrates the status of chains E and F treated as individual structural units.

Figure 3. Theoretical (T-blue) and observed (O-red) distributions for 5O3O: A – chain E treated as a distinct structural unit; B – chain F treated as a distinct structural unit. Orange highlighting marks residues which comprise the inter-fibril interface (331-338).

It is clear that even when analyzed as distinct units, the presented chains still diverge from the theoretical distribution of hydrophobicity (Figure 3). No C-terminal maxima (predicted by T) are present in the observed distributions.

Regarding 5O3L, both distributions are similar to those calculated for 5O3O, with only the interface being somewhat different. The observed distribution, while discordant, does not resemble an amyloid (which would appear as a sinusoidal pattern consisting of similar local maxima).

Taking into account the presented distributions it is easy to pinpoint fragments where O deviates from T (see Figure 4).
Table 4. RD values in both models (T-O-R and T-O-H), along with HvT, TvO and HvO correlation coefficients for chains E and F analyzed as part of the superfibril, as part of a proto-fibril and on their own. The corresponding values for 5O3L and 5O3R may be found in the Supplementary Materials section. Figure 4 illustrates the division of the chain into individual fragments.

### 5O3O

#### Chain E – superfibril

<table>
<thead>
<tr>
<th>Fragment</th>
<th>RD</th>
<th>Corr coeff</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>T-O-R</td>
<td>T-O-H</td>
</tr>
<tr>
<td>1-6</td>
<td>0.521</td>
<td>0.405</td>
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<tr>
<td>16-26</td>
<td>0.820</td>
<td>0.771</td>
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<tr>
<td>23-30</td>
<td>0.496</td>
<td>0.495</td>
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<tr>
<td>39-49</td>
<td>0.575</td>
<td>0.544</td>
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<tr>
<td>50-61</td>
<td>0.851</td>
<td>0.802</td>
</tr>
<tr>
<td>61-72</td>
<td>0.563</td>
<td>0.491</td>
</tr>
<tr>
<td>CHAIN</td>
<td>0.741</td>
<td>0.700</td>
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</table>

#### Chain F – superfibril

<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>T-O-R</td>
<td>T-O-H</td>
</tr>
<tr>
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<td>16-26</td>
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<td>23-30</td>
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<td>39-49</td>
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#### Chain E – proto-fibril

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<tr>
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<tr>
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<td>0.668</td>
<td>0.553</td>
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<td>16-26</td>
<td>0.615</td>
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</tr>
<tr>
<td>23-30</td>
<td>0.571</td>
<td>0.573</td>
</tr>
<tr>
<td>39-49</td>
<td>0.657</td>
<td>0.623</td>
</tr>
<tr>
<td>50-61</td>
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<td>61-72</td>
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<td>CHAIN</td>
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#### Chain F – proto-fibril

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<tbody>
<tr>
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<td>T-O-H</td>
</tr>
</tbody>
</table>
Figure 4. Theoretical (T – blue) and observed (O – red) distribution for the chain E from 5O3O (the
distribution is similar in all structural units). Fragments where O significantly deviates from T are
highlighted by different colors (see Table 4) that match the colors of 3D presentations on Figure 5.
Figure 4 provides a sample set of distributions (T and O) for a single chain – E from 5O3O. As already noted, the chains differ in detail, while the overall pattern remains largely identical regardless of the structural unit in question (superfibril, proto-fibril or individual chain). The highlighted fragments have been singled out on the basis of visual inspection, supplemented with correlation coefficients computed for successive five-residue segments. Fragments for which HvT and TvO are negative while HvO assumes a large value will be subjected to further analysis. Table 4 summarizes the results obtained for all structural units in 5O3O.

As shown in Table 4, the status of selected fragments is quite similar regardless of the structural unit in question – in all cases these fragments are strongly discordant vs. the theoretical distribution.

3.4. Comparative analysis involving theoretical models

As previously noted, we have carried out an in silico experiment which consisted of predicting the conformation of a tau protein whose sequence matches the presented amyloids. Our analysis concerned the entire molecule as well as fragments highlighted in Figure 4.

Figure 5. 3D presentation of structures obtained using I-Tasser (upper row B-F), Robetta (central row – G-K) and the fuzzy oil drop model (bottom row – L-P). The top structure (A) is the structure 5O3O as appears in PDB database. The colors used correspond to highlights on Figure 4. Models whose status is consistent with FOD predictions (RD < 0.5) have been additionally underlined in blue (D, M, N).
Figure 5 presents 3D models of tau polypeptides obtained using software packaged described in the Materials and Methods section. Visual inspection reveals the possible emergence of globular forms: I-Tasser produces four such structures (out of five input cases), while Robetta produces one (out of five). The tendency of the fuzzy oil drop model to produce globular forms should come as no surprise given the model’s propensity to direct hydrophobic residues towards the center of the molecule (due to interactions with the aqueous solvent).

One of the models produced by I-Tasser appears to involve a hydrophobic core (in the sense of the fuzzy oil drop model – cf. underlined structures in Figure 5). None of the models produced by Robetta satisfies this criterion. Regarding the fuzzy oil drop model, despite its natural tendency to generate hydrophobic cores, only two 500 structures analyzed in the course of the study contain a hydrophobic core (i.e. satisfy the RD < 0.5 condition).

Table 5. RD values obtained using I-Tasser, Robetta and FOD. In addition to RD values (for T-O-R and T-O-H) the table also lists HvT, TvO and HvO correlation coefficients. The division into fragments is consistent with the one presented in Figure 4. Values in bold distinguish the status accordant with FOD model (RD < 0.5).

<table>
<thead>
<tr>
<th>I-Tasser</th>
<th>RD</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fragment</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1-6</td>
<td>0.411</td>
<td>0.142</td>
</tr>
<tr>
<td>16-26</td>
<td>0.509</td>
<td>0.200</td>
</tr>
<tr>
<td>23-30</td>
<td>0.641</td>
<td>0.357</td>
</tr>
<tr>
<td>39-49</td>
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<td>0.283</td>
</tr>
<tr>
<td>50-61</td>
<td>0.614</td>
<td>0.618</td>
</tr>
<tr>
<td>61-72</td>
<td>0.627</td>
<td>0.232</td>
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<tr>
<td>CHAIN</td>
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<td>23-30</td>
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<tr>
<td>1-6</td>
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<td>16-26</td>
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<td>23-30</td>
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</tr>
<tr>
<td>39-49</td>
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</tr>
<tr>
<td>50-61</td>
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<td></td>
</tr>
<tr>
<td>61-72</td>
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<tr>
<td>CHAIN</td>
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<tr>
<td></td>
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<td></td>
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<td>0.179</td>
</tr>
<tr>
<td></td>
<td>-0.438</td>
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<tr>
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<td>0.416</td>
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**IT-4 RD Correlation coefficient**

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<th>TvO</th>
<th>HvO</th>
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<tbody>
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**IT-5 RD Correlation coefficient**

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<th>TvO</th>
<th>HvO</th>
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**Robetta**

**ROB-1 RD Correlation coefficient**

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<th>TvO</th>
<th>HvO</th>
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**ROB-2 RD Correlation coefficient**

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<th>TvO</th>
<th>HvO</th>
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<td>TvO</td>
<td>HvO</td>
</tr>
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</tr>
<tr>
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</tr>
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</tr>
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**ROB-5**

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<td>0.809</td>
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<td>0.375</td>
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**FOD**

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<td>-0.291</td>
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<td>61-72</td>
<td>0.568</td>
<td>0.217</td>
<td>-0.269</td>
<td>0.279</td>
<td>0.200</td>
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<td>0.563</td>
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**FOD – 2**

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<th>HvO</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Analysis of numerical values listed in Table 5, along with visual inspection of 3D forms reveals that some of these fragments adopt structures consistent with the Gaussian distribution. Of particular note is the fragment marked in red on Figure 5 (residues 50-61, numbered 356-357 according to PDB), which does not conform to theoretical predictions in any model. In interpreting this fact we may refer to the dominant role of the fragment whose sequence does not adapt to the centralized distribution of hydrophobicity. We can speculate that this fragment (GSLDNITHVPGG) is therefore the most amyloidogenic sequence in the presented set. This suggestion is supported by the data.
shown in Table 5, particularly RD values which are either highest or second highest in the entire set.

### 3.5. Other fragments of tau proteins

In order for our comparative analysis to be as comprehensive as possible, we also include tau proteins (or fragments of tau amyloids) which PDB lists as capable of adopting non-amyloid conformations. 1I8H represents the 541-553 fragment of the previously described tau protein, in complex with a microtubule – specifically, pin1 ww domain complexed with human tau phosphothreonine peptide microtubule-associated protein tau. In this complex the A chain comprises residues 541-553 (so-called phf-tau), while chain B represents the ww domain (6-44) [33]. The 1I8H sequence does not fully match 5O3O (and the others), however we have included it in our analysis due to functional similarities.

Table 6. Parameters describing the 541-553 fragment (chain A) of the tau protein 1I8H in complex with the ww domain (residues 6-44).

<table>
<thead>
<tr>
<th>1I8H</th>
<th>RD</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T-O-R</td>
</tr>
<tr>
<td>Complex</td>
<td>0.680</td>
<td>0.546</td>
</tr>
<tr>
<td>Chain A (tau) in complex</td>
<td>0.452</td>
<td>0.152</td>
</tr>
<tr>
<td>Chain A(tau) individual</td>
<td>0.630</td>
<td>0.240</td>
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</tbody>
</table>

Results shown in Table 6 and on Figure 6 reveal that the 1I8H complex does not conform to the fuzzy oil drop model. The status of chain A (tau), when analyzed on its own, is also discordant. On the other hand, the same chain conforms to the model when analyzed as part of the complex. This means that chain B creates suitable conditions for chain A to produce a shared hydrophobic core consistent with the 3D Gaussian.

Considering individual fragments of 1I8H, it turns out that the fragment 27-29 of chain B is the most discordant, and that eliminating this fragment from calculations lowers the RD value of the complex. This may indicate conformational alignment between chain B and chain A (given that the presence of chain A disrupts the distribution of hydrophobicity in chain B).
Figure 6. Distribution of hydrophobicity in chain A in the 1I8H complex (T-blue, O-red, H-green): A – when treated as a distinct structural unit; B – when treated as a component of the complex. Good alignment between O, T and H can be observed in the latter case.

Analysis of the presented results indicates the need of a “chaperone”, which chain A requires to reach together a conformation consistent with the fuzzy oil drop model.

2MZ7 is another protein related to the presented tau structure. Its sequence matches the short fragment at 306-312 in tau as it is present in 5O3O. According to [34] this fragment (267-312) of tau protein is bound to microtubules.

Figure 7. T (blue) and O (red) hydrophobicity distributions in 2MZ7, revealing overall strong discordance.

The status of the tau chain in 2MZ7 reveals strong discordance with regard to the theoretical model (see Figure 7), with RD=0.680 (T-O-R) and 0.527 (T-O-H). Correlation coefficients are -0.049, -0.042 and 0.673 for HvT,TvO and HvO respectively. These values indicate that the structure of the chain is dominated by the conformational tendencies of individual residues rather than by the external hydrophobic force field. Interaction with microtubules is likely the driving force behind conformational adaptation. The structure of the entire complex is not known, however information regarding the interaction of individual residues with the microtubule might...
explain the discordance observed throughout the chain. Only the helical fragment at 295-299 appears accordant with the fuzzy oil drop model (with RD values and correlation coefficients of 0.264, 0.170, 0.876, 0.921 and 0.965 respectively, showing good alignment between the theoretical and observed distribution).

3.6. Peptides

To complete our study of tau-derived structures listed in PDB we also need to consider peptides capable of amyloid transformation. The possible mechanism driving this process, discussed in [16, 17], remains applicable in the presented case.

Peptides which match the tau protein sequence are mostly related to the fragment at 306-311 – the short N-terminal fragment of the tau protein present in 5O3O, 5O3L and 5O3T. A short peptide which does not produce a globular form should not, in principle, be analyzed using the fuzzy oil drop model. Nevertheless, for the sake of completeness, we will present RD (T-O-R) values calculated for such peptides – see Table 7.

Table 7. Properties of peptides which match the N-terminal fragment of the presented tau protein.
The table lists PDB ID, sequences (indicating which fragments are identical to the tau chain) and RD (T-O-R) values.

<table>
<thead>
<tr>
<th>PDB ID</th>
<th>Sequence</th>
<th>Position</th>
<th>RD</th>
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<tbody>
<tr>
<td>2ON9</td>
<td>VQIVYK</td>
<td>306-311</td>
<td>0.164</td>
</tr>
<tr>
<td>3Q9G</td>
<td>VQIVYK + LA</td>
<td>306-311 + 2 AA</td>
<td>0.332</td>
</tr>
<tr>
<td>3OVL</td>
<td>VQIVYK</td>
<td>306-311</td>
<td>0.160</td>
</tr>
<tr>
<td>4E0M</td>
<td>AS + VQIVYK+AEFYK</td>
<td>2AA + 306-311 + 5AA</td>
<td>0.707</td>
</tr>
<tr>
<td>4E0O</td>
<td>AS + VQIVYK+AEFYK</td>
<td>2AA + 306-311 + 5AA</td>
<td>0.543</td>
</tr>
<tr>
<td>4NP8</td>
<td>VQIVYK</td>
<td>306-311</td>
<td>0.174</td>
</tr>
<tr>
<td>5K7N</td>
<td>VQIVYK</td>
<td>306-311</td>
<td>0.170</td>
</tr>
</tbody>
</table>

The values shown in Table 7 only reveal the type of hydrophobicity distribution, with no assessment of the hydrophobic core structure. Low values indicate that hydrophobic residues are located in the central part of the chain, surrounded by N- and C-terminal hydrophilic residues. As shown, the short VQIVYK fragment, despite including an outlying Val residue, is a good match for the centralized hydrophobic core structure. When additional neighboring residues (adjacent to the 306-311 fragment) are included in analysis, the value of RD increases significantly.

Peptides identified as capable of amyloidogenesis appear to adopt amyloid-like conformations themselves. As discussed in [16, 17], the distribution of hydrophobicity in a peptide may – regardless of its accordance with the theoretical distribution – give rise to amyloid formation, as long as the environment favors linear complexation of additional peptides, with alternating bands of high and low hydrophobicity emerging along the axis of the fibril. This is visualized in Figure 8, which compares two fringe cases (in terms of RD values).
Figure 8. H (green), T (blue) and O (red) distributions for selected peptides: A – 4E0M, B – 3OVL; C (for A) and D (for B) – pseudo-3D view, presenting the observed hydrophobicity of a theorized structure of fibrillary tangles formed by linear propagation of the corresponding peptides. Dashed lines on A represent deleted residue number 11 in the PDB structure of 4E0M.

Figure 8 evidences the appearance of structures characterized by linear propagation of hydrophobicity peaks/troughs, which is a precondition of amyloid formation. As highlighted by to-date observations and interpretations, the environment must “support” the creation of such forms. It is thought that under natural conditions the structure of water does not favor formation of amyloid fibrils.

The value of RD computed for a short peptide implies how many local maxima are present. A low value indicates that hydrophobicity is concentrated in the central part of the peptide (e.g. 3OVL), while a high value suggests the presence of numerous local maxima (e.g. 4E0M).

3. Discussion

The presented comparative analysis of proteins associated with amyloid tau confirms the previously stated hypothesis concerning the structural properties of the amyloid. According to this hypothesis, the amyloid is characterized by the presence of alternating bands of variable hydrophobicity. It seems that linear propagation – which can be regarded as contrary to the emergence of a centralized hydrophobic core (as seen in globular proteins) – is a characteristic property of amyloids. A similar phenomenon can be observed in Aβ amyloids [35] as well as amyloids formed by synuclein [36]. The network of hydrogen bonds discussed in numerous studies [11, 37] favors this type of conformation and is thought to be associated with the linear properties of beta folds. In the tau amyloid, however, β-strands play a much smaller role than in other known amyloids. This suggests that while hydrogen bonds are important, their role is not necessarily linked to β-structures.

Hydrophobicity is capable of binding together proximate charged residues, however, electrostatic interactions should, in principle, prevent such clustering. Under such conditions only hydrophobic forces can result in the presented arrangement. Thus, a conformation which is driven by intrinsic
hydrophobicity (and does not generate a central hydrophobic core) may be regarded as both the cause and the mechanism of amyloid transformation.

The fuzzy oil drop model recognizes several possible forms for the tau superfibril. This diversity is likely caused by interactions between the solvent and the emerging amyloid. We suggest that while 5O3O and 5O3L emerge as the effect of the influence of surrounding water, in 5O3T the structure is driven by the specific band-like arrangement of hydrophobicity in the amyloid itself.

The tau protein, whose task is to mediate interaction with microtubules, must align itself to the complexed object. When the protein is subjected to folding on its own, in an independent manner, it may adopt a globular conformation and remain soluble. An open question is why the same protein undergoes complexation in a form which does not resemble a globule. As shown, a chain which is sequentially identical to the amyloid fragment of the tau chain cannot produce a globular structure.

In this context, microtubules may be viewed as a „chaperone” which ensures that the protein adopts its intended conformation, required for biological activity.

Conclusions related to the process of amyloidogenesis and the role of the fuzzy oil drop model in explaining this process, all point to the need for further research into the properties of the aqueous solvent. While we possess good knowledge of the properties of ice, the corresponding “normal” (or physiological) condition of liquid water is poorly understood – for example, we are still unsure why the density of water peaks at 4 degrees C. This may explain the recent uptick in investigations which aim to explain such phenomena [37-42]. We believe that these studies may also cast a new light on the process of amyloidogenesis, which – in all likelihood – is associated with the (heretofore unknown) influence of the force field exerted by the surrounding water. This field should be modeled as a continuum rather than (as is common practice in modern molecular dynamics packages) as a collection of distinct molecules. The fuzzy oil drop model provides a good baseline for such research.

Presented analysis allows distinguishing of critical short sequences especially resistant to adopt the conformation accordant with the expected uni-centric hydrophobic distribution. This phenomenon is also observed in other amyloids especially Aβ(1-42) amyloid [35].

4. Materials and Methods

4.1. Data

The analysis concerns tau protein amyloids listed in PDB as capable of forming highly ordered superfibrils. In addition, we also consider selected fragments of the tau protein, including short peptides. Table 8 gives the full list of structures subjected to analysis.

Table 8. Set of proteins subjected to analysis, along with an indication of chain length and complexation capabilities. The rightmost column provides references.

<table>
<thead>
<tr>
<th>PDB ID</th>
<th>Characteristics</th>
<th>Length</th>
<th>Complex</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>5O3O</td>
<td>Microtubule-associated protein tau</td>
<td>73 aa</td>
<td>10 chains</td>
<td>[30]</td>
</tr>
<tr>
<td>5O3L</td>
<td>Microtubule-associated protein tau</td>
<td>73 aa</td>
<td>10 chains</td>
<td>[30]</td>
</tr>
<tr>
<td>5O3T</td>
<td>Microtubule-associated protein tau</td>
<td>73 aa</td>
<td>10 chains</td>
<td>[30]</td>
</tr>
</tbody>
</table>
Table 8 includes tau superfibrils (5O3L, 5O3O, 5O3T), smaller structural units (including individual chains – 2MZ7), as well as complexes with other proteins (1I8H). We also consider individual peptides which are widely characterized as capable of forming amyloid structures (2ON9, 3Q9G, 3OVL, 4E0M, 4NP8, 5K7N).

All the above structures are subjected to FOD characterization in the context of the superfibril, the protofibril and the individual chain. Our analysis further extends to peptides whose composition is similar or identical to PDB sequences. The status of such molecules is determined by computing their RD coefficients. It should be noted that seeking proper hydrophobic cores in very short peptides (<15 aa) makes little sense – such peptides are characterized using FOD criteria only in order to provide a coherent platform for comparative studies. The fuzzy oil drop model provides useful information regarding the relationship between each residue’s intrinsic hydrophobicity and its placement in a fully folded chain.

4.2. Folding of peptides – components of amyloid structures

Peptide sequences which form parts of the tau amyloid (e.g. 306-378, as listed under 5O3O) have been subjected to folding simulations using Robetta [26, 27] and I-Tasser [28, 29], as well as to simulations based on the fuzzy oil drop model [49]. This operation can be regarded as an in silico experiment whose aim is to provide alternatives to structures generated by specialized 3D structure prediction software. Our goal is to identify theoretical opportunities for alternative folds (unlike those listed under 5O3O and similar entries). The globular forms generated by the fuzzy oil drop model may provide clues regarding the discordance between the theoretical distribution of hydrophobicity and actual location of hydrophobicity maxima/minima. A ranking list of the resulting structures may be composed in order to identify factors which increase similarities between the theorized conformation and the corresponding amyloid form.
Robetta is a software package aimed at modeling and analysis of protein structures [26, 27]. It is a strong performer in successive editions of the CASP challenge, which focuses on predicting the 3D conformations of input residue sequences [50]. Robetta works in the following manner: the user is asked to input a sequence of amino acids comprising a given protein chain. This sequence is then subdivided into fragments (called domains) using the “Ginzu” hierarchical scanning algorithm. The algorithm searches for fragments homologous to sequences for which the preferred secondary conformation has been established on the basis of experimental studies. Such homologous areas are detected by (in the order of accuracy) BLAST, PSI-BLAST [51], FFAS03 [52] and 3D-Jury [53] taking as input the sequences produced in the preceding step. The identified domains are modeled by applying a comparative modeling protocol, while all other chain fragments are treated as linkers (if they consist of fewer than 50 residues) or assigned to structural families as defined in the Pfam-A database [54] using HMMER [55]. Fragments and sequences which have not been recognized as putative domains, are analyzed via MSA of the full-length target derived from a PSI-BLAST search against the NCBI non-redundant (NR) protein sequence database [56]. Putative domains identified through Pfam-A and MSA are modeled using de novo structure prediction. Finally, following assembly, side chains are modeled by applying Monte Carlo algorithms [57]. The presented description is based on [56].

I-Tasser (Iterative Threading ASSEmbly Refinement) is a software package which can predict the structure of a protein given its sequence. In this application, prediction bases on querying PDB for templates using the multiple threading approach. I-Tasser is a strong contender in CASP challenges, topping the ranking in editions 7 through 12 [58-60]. The user submits a sequence of amino acids, which is then compared (by LOMETS [61]) to template proteins with similar structural characteristics. An optimal template is then selected and overlapping fragments are assembled into an output model using replica-exchange Monte Carlo simulations [62], while differing fragments are modeled ab initio. If LOMETS is unable to identify a suitable template, the entire structure is subjected to ab initio modeling. The next step involves a search for low energy states (using SPICKER [63]) in the resulting chain via clustering simulation decoys. This is followed by reassembly of the template protein starting with SPICER cluster centroid, however this time the simulation is guided by spatial constraints provided by TM-align on the basis of LOMETS templates and PDB data. The purpose of the second iteration is to remove steric clashes as well as to refine the global topology of the cluster centroids. The decoys generated in the second simulations are clustered and the lowest energy structures are selected. [64] The final step involves construction of a detailed model from the available structures via optimization of the hydrogen bond network using REMO. Further information can be found in [64].

Robetta computations were carried out using the publicly available service [27]. I-Tasser computations were carried out using [64]. Fuzzy oil drop model computations were carried out using PL-Grid software [65], a detailed description of which can be found in [66]. The fuzzy oil drop model involves two intermediate folding stages: the early-stage intermediate [67-70] and the late-stage intermediate [68-70]. The initial step, which is meant to generate a starting structure for further optimization, is omitted since the conformation listed under 5O3O is taken as the starting structure (treated as early-stage in this case). This chain is then immersed in an aqueous solvent, whose effects are modeled using a 3D Gaussian (as an external force field). In line with the fuzzy oil drop model, hydrophobic residues tend to congregate at the center of the protein body.
while hydrophilic residues are exposed on its surface. The process produces a prominent hydrophobic core encapsulated by a hydrophilic “shell” (with near-zero values of hydrophobicity on the surface). Optimization of hydrophobic interactions and optimization of nonbinding internal interactions is carried out in an alternating fashion, with each step repeated several times. Nonbonding interactions are optimized using Gromacs [71] software suite, available on the PL-Grid infrastructure at ACK Cyfronet AGH Kraków [65]. Fuzzy oil drop-based optimization aims to minimize differences between the idealized (3D Gauss function) and observed distribution of hydrophobicity in the target protein. The workflow interleaves both procedures in order to converge on the final conformation.

Folding simulations which rely on the fuzzy oil drop model are relevant since they acknowledge the effects exerted by the aqueous solvent, and treat them as a global phenomenon (i.e. external force field producing a molecule-wide hydrophobic core).

4.3. Comparative analysis

All tertiary conformations produced by the modeling algorithms, as well as structures listed in PDB, were analyzed with regard to the status of their hydrophobic cores, which is described by the RD (relative distance) coefficient. Comparing RD values brings the information about the degree of disorder in respect to ideal distribution. In consequence the approach to amyloid form can be assessed. RD expresses the degree of order present in the protein’s hydrophobic core and indirectly indicates whether the protein is globular or not. Generating a globular structure with a prominent hydrophobic core (hydrophobicity peaking at the center of the molecule and decreasing along with distance from the center, becoming very low on the surface) suggests that the given amyloid peptide may, under certain circumstances, adopt a globular conformation. The presented ranking of protein structures, sorted in the order of decreasing globularity, reveals changes which cause proteins to forfeit their centralized hydrophobic cores and which may – in extreme cases – produce amyloid forms.

The RD coefficient can be computed for two independent cases: T-O-R and T-O-H. The former case expressing the relative distance between the observed distribution (O) and two boundary distributions: theoretical (T) which is given by the 3D Gaussian, and uniform (R, random) where each residue is ascribed a hydrophobicity value of 1/N (N being the number of residues in the input chain). R-distribution represents the case of uniform (absence of any local hydrophobicity concentration) distribution which is the opposite one versus the centralized distribution. In the latter case the uniform distribution is replaced by a distribution corresponding to the intrinsic hydrophobicity of each residue in the input chain (H). Comparing both values reveals factors which guide the folding process (this is particularly true in the T-O-H case). A high value of RD (T-O-H) indicates that folding is dominated by the intrinsic properties of each residue with no regard to cooperative generation of a shared hydrophobic core. When this type of distribution is repeated in successive fragment of the polypeptide, the result is a linear sequence of alternating bands of high and low hydrophobicity. This, in turn, enables unrestricted elongation of the fibril. An interpretation of this phenomenon (referred to as “ladders”) can also be found in [73].

A comparative assessment of T-O-R and T-O-H coefficients in fibrillary/amyloid structures as well as the structures produced by various folding algorithms may enable us to identify the “seeds” of linear propagation. Fuzzy oil drop criteria have previously been used to assess the distribution of hydrophobicity in structures published by the CASP project [74].

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