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Computational Analysis of the *S. cerevisiae* Proteome Reveals the Function and Cellular Localization of the Least and Most Amyloidogenic Proteins

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ABSTRACT **Protein sequences have evolved to optimize biological function that usually requires a well-defined three-dimensional structure and a monomeric (or oligomeric) state. These two requirements may be in conflict as the propensity for β-sheet structure, which is one of the two most common regular conformations of the polypeptide chain in folded proteins, favors also the formation of ordered aggregates of multiple copies of the same protein (fibril, i.e., polymeric state). Such β-aggregation is typical of amyloid diseases that include Alzheimer's, Parkinson's, and type II diabetes as well as the spongiform encephalopaties. Here, an analytical model previously developed for evaluating the amyloidogenic potential of polypeptides is applied to the proteome of the budding yeast (*Saccharomyces cerevisiae*). The model is based on the physicochemical properties that are relevant for β-aggregation and requires only the protein sequence as input. It is shown that β-aggregation prone proteins in yeast are accrued in molecular transport, protein biosynthesis, and cell wall organization processes while they are underrepresented in ribosome biogenesis, RNA metabolism, and vitamin metabolism. Furthermore, β-aggregation prone proteins are much more abundant in the cell wall, endoplasmic reticulum, and plasma membrane than in the nucleolus, ribosome, and nucleus. Thus, this study indicates that evolution has not only prevented the selection of amyloidogenic sequences in cellular compartments characterized by a high concentration of unfolded proteins but also tried to exploit the β-aggregated state for certain functions (e.g. molecular transport) and in well-confined cellular environments or organelles to protect the rest of the cell from toxic (pre-)fibrillar species. Proteins 2007;68:273–278.** © **2007 Wiley-Liss, Inc.**

Key words: amyloid; molecular transport; protein biosynthesis; cell wall; endoplasmic reticulum; ribosome; nucleolus; yeast

INTRODUCTION

The β-aggregation tendency of many natural-occurring peptides and proteins originates from the formation of backbone-backbone hydrogen bonds[1] and favorable side chains interactions.[2,3,4] Although in eukaryotes aggregation can have a functional role,[5,6,7] the cytotoxicity of prefibrillar aggregates[8] and their association with diseases such as Alzheimer's, Parkisons's, Huntington's, prion disease, cystic fibrosis, and type II diabetes[9,10] indicates that ordered aggregation is potentially harmful.[1,11]

We have recently developed an analytical model to predict β-aggregation rates and β-aggregation prone segments in polypeptide chains.[12,13,14] In our model, the aggregation tendency of a protein is evaluated with a parameter-free formula that solely requires the polypeptide sequence as input. The β-aggregation profile along a sequence is computed considering the β-propensity, ratio of polar vs. non polar water-accessible surfaces, solubility, charge, and aromatic-content of the polypeptide. In a previous application we have shown that organism complexity anticorrelates with proteomic β-aggregation propensity.[15] Moreover, we have provided evidence that natural proteomes have a higher degree of polarization in both low and high β-aggregation prone sequences than proteomes with randomized sequences. Here, we use our analytical model to quantify the β-aggregation propensity of yeast proteins in individual biological processes and cellular components as annotated in the Gene Ontology (GO) database.[16]

METHODS

Our model is based on the physicochemical properties of the residues and takes into account both amino acid composition and positional information. The aggregation propensity $\pi_{il}(s)$ of an l-residue segment starting at posi-

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tion i in the sequence s is evaluated as:

$$\pi_{il}(s) = \phi_{il}(s)\Phi_{il}(s) \quad (1)$$

The factor Φ_{il} contains exponential functions and is mainly position-dependent:

$$\Phi_{il}(s) = e^{A_{il}+B_{il}+C_{il}} \quad (2)$$

where A_{il}, B_{il}, and C_{il} are functionals related to the aromaticity, β-propensity, and charge, respectively. The factor ϕ_{il} depends almost exclusively on the amino acid composition:

$$\phi_{il}(s) = \left[\prod_{j=i}^{i+l-1}\left(\frac{S_j^a}{\hat{S}_a}\theta^{\uparrow\uparrow} + \frac{S_j^p}{\hat{S}_p}\theta^{\uparrow\downarrow}\right)\frac{\hat{S}^t}{S_j^t}\,\frac{\hat{\sigma}}{\sigma_j}\right]^{1/l} \quad (3)$$

where S_i^{a}, S_i^{p}, $S_{\mathrm{i}}^{\mathrm{t}}$, and σ_i, weighted by their average over the 20 standard amino acids (hatted values), are the side-chain apolar, polar, total water-accessible surface area, and solubility, respectively. The functionals $\theta^{\uparrow\uparrow}$ and $\theta^{\uparrow\downarrow}$ include positional effects and reflect the parallel or antiparallel tendency to aggregate if the majority of residues is apolar or polar, respectively. Details of the method are presented in our previous work.[13]

For each protein sequence s the β-aggregation potential $\prod(s)$ is evaluated as:

$$\prod(s) = \frac{1}{L-l+1}\sum_{i=1}^{L-l+1}\pi_{il}(s) \quad (4)$$

where L is the protein length and $l = 5$.

For each GO-slims annotation g the β-aggregation propensity $\Gamma(g)$ is defined as:

$$\Gamma(g) = \frac{1}{N}\sum_{s=1}^{N}\left[\prod(s) - \prod(r(s))\right] \quad (5)$$

where N is the number of proteins classified with the GO annotation g, and $\prod[r(s)]$ is the β-aggregation potential of the sequence $r(s)$ obtained by shuffling the sequence s (i.e., having the same length and amino acid composition as s).

RESULTS AND DISCUSSION

We use GO-slims annotations (i.e., high-level terms from the Biological Process and Cellular Component ontologies) taken from the yeast genome server available at *http://www.yeastgenome.org/* selecting all chromosomal features. To measure the β-aggregation propensity, we generate the "amyloid spectrum" of each sequence in the proteome following the procedure introduced in our previous work.[13] The amyloid spectrum is then averaged over each sequence and over each GO-slims annotation and used to rank the terms in the ontologies "biological process" and "cellular component" of the GO database. Importantly, within each GO term (e.g. protein biosynthesis) the average β-aggregation propensity is normalized by subtracting the average value of randomly shuffled sequences of same length and amino acid composition. In this way, systematic sources of variation like protein size and residue composition are eliminated. As in our previous work,[13] we generate the amyloid spectrum using a window size of 5 amino acids. In our model we assume that a protein with a high number of β-aggregation prone segments is more likely to form an amyloid fibril than a protein that has only a few β-aggregation prone segments. Although this assumption is compatible with the "amyloid stretch hypothesis" that attributes to small β-aggregating peptides the capability of converting soluble nonamyloidogenic proteins into amyloidogenic prone molecules,[17] we do not make any assumption on the number of amyloidogenic stretches needed to form a fibril and use the amyloid spectrum only to quantify the amyloidogenic potential of a protein.

Protein Function

High β-aggregation propensity

Our analysis of biological processes indicates that proteins with transporter activity have the highest β-aggregation potential followed by those involved in protein biosynthesis (Fig. 1). The URE2 protein, whose 93-residues N-terminal segment is a prion-forming domain,[18] belongs to the class "transporter activity" and has ordered locus name YNL229C.[1]

Up to now also the yeast protein ERF2 (i.e., Eukaryotic peptide chain release factor GTP-binding subunit 2), is known to form amyloid aggregates as its 7-residue fragment GNNQQNY is the major component of SUP35 fibrils.[19] ERF2 belongs to the class "protein biosynthesis" and has ordered locus name YDR172W.[2]

Among the 10 transporters ranked with the highest β-aggregation potential we found HXT10 (locus name YFL011W), Ferric reductase transmembrane (locus name YOR381W), Golgin IMH1 (locus name YLR309C), and SEC23 (locus name YPR181C). The Hexose transport HXT10 and Ferric reductase transmembrane component 7 (iron transporter) share a sequence similarity of 75% with the amyloidogenic Aβ42 peptide involved in Alzheimer's disease[20] (stretches SNKGAIIGLMVGGVVI and RHDSGYEVHHQK, respectively). It is worth mentioning that the precursor of Aβ42, the Amyloid β A4, is

[1]The complete GO-term lineage is: Cell organization and biogenesis/cellular localization/establishment of cellular localization/intracellular transport/intracellular protein transport/protein targeting/protein import into nucleus/regulation of protein import into nucleus/negative regulation of protein import into nucleus/negative regulation of transcription factor import into nucleus/cytoplasmic sequestering of transcription factor (the full lineage, including "children terms" was determined using the server "amiGO" available at *http://www.godatabase.org/cgi-bin/amigo/go.cgi*).

[2]The complete GO-term lineage is: Cellular physiological process/cellular metabolism/cellular biosynthesis/macromolecule biosynthesis/protein biosynthesis/translation. Hence, the two prion-like proteins of the yeast proteome belong to the classes for which our model predicts the highest β-aggregation propensity.

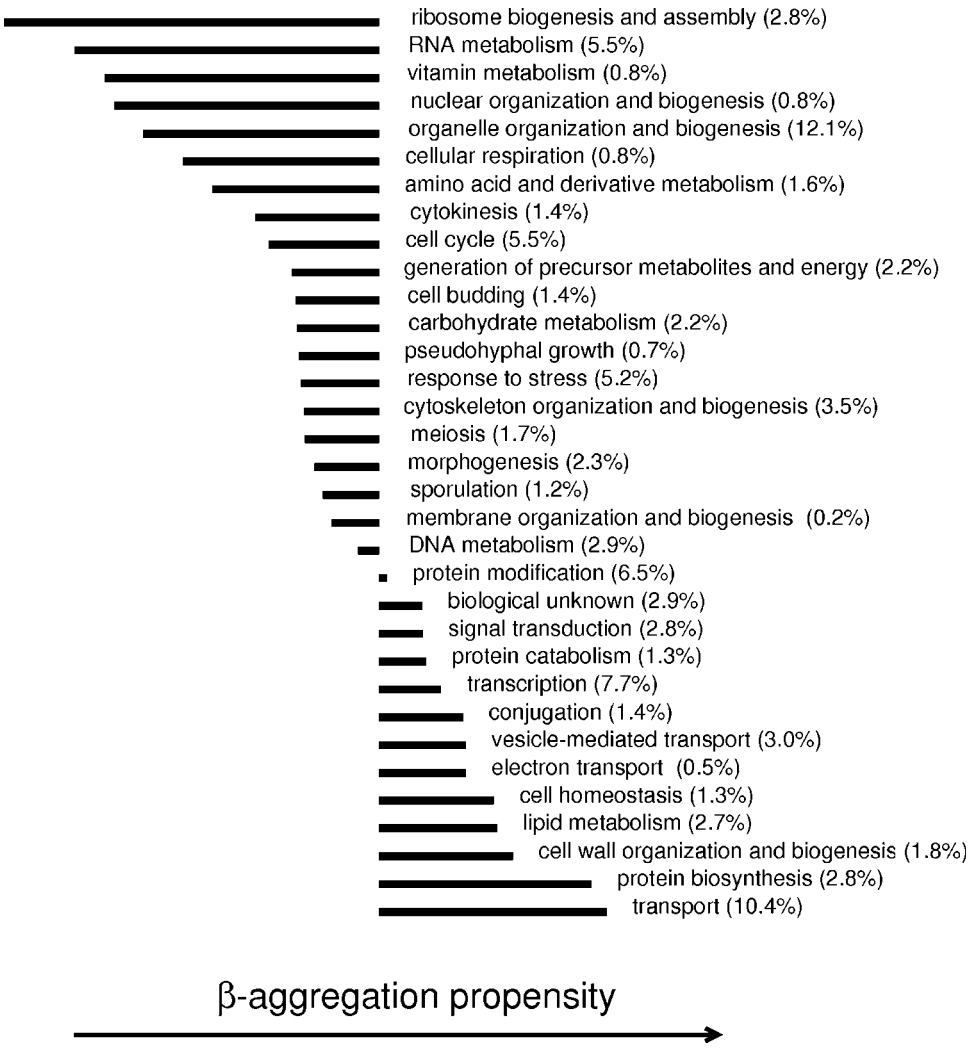

Fig. 1. Ranking of biological processes in yeast according to the average value of β-aggregation propensity of the proteins belonging to individual gene ontology terms (the number of proteins in each term is shown as percentage relative to the length of the complete proteome). The horizontal bars quantify the deviation from the average over randomized sequences with the same length and residue composition.

also a transporter and mediates the axonal transport of β-secretase and presenilin 1. We observed a similarity of 50% between the yeast Golgin IMH1 (vesicle-mediated transport) and the amyloidogenic Apolipoprotein A-I (lipid transporter). Also the yeast SEC23 (endoplasmic reticulum to Golgi vesicle-mediated transport) and the amyloidogenic Transthyretin (transporter of thyroxyne) show a sequence similarity of 50%. According to *Psiblast*,[21] the aforementioned yeast transporters have their most significant match with known amyloids when aligned to the human proteome, which not only indicates the ability of the method to detect amyloidogenic stretches, but also supports the view that transport proteins have the highest β-aggregation potential in a proteome.

Low β-aggregation propensity

The classes "ribosome biogenesis and assembly", "RNA metabolism", and "vitamin metabolism" show the lowest β-aggregation propensity. Recent investigations indicate that disordered regions are very frequent in both RNA and protein chaperones. In fact, the frequency of disorganized structure is higher for RNA chaperones than for any other protein class and is also very high for protein chaperones.[22] Interestingly, many ribosomal proteins are unfolded when isolated and become ordered when they associate with the RNA.[23] Ribosomal proteins are highly basic to bind ribosomal RNA which is negatively charged.[24] Although a high net charge is one of the properties that determine low aggregation in our model[13] other factors not related only to amino acid composition play a role because of the aforementioned normalization with respect to randomly shuffled sequences. Thus, we speculate that the low aggregation propensity of the proteins involved in "ribosome biogenesis and assembly", "RNA metabolism", and "vitamin metabolism", may arise from the presence of intrinsically disordered proteins

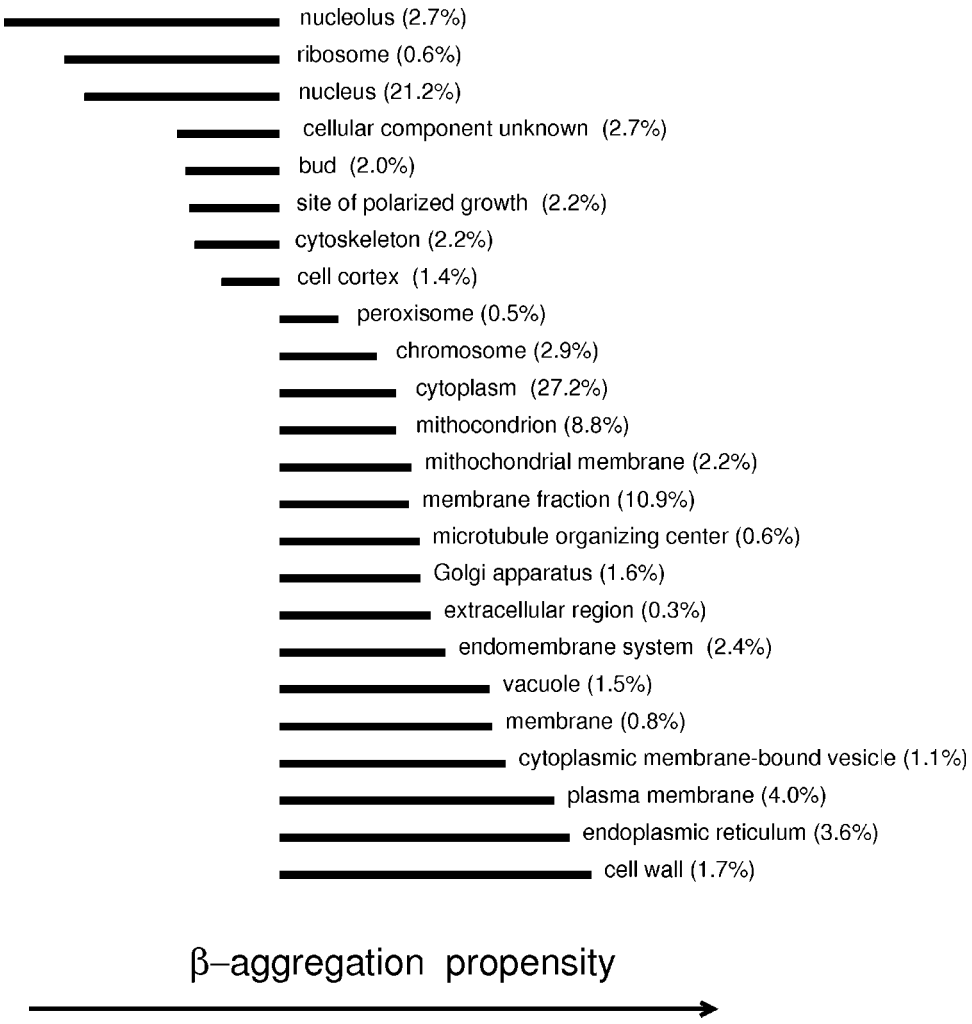

Fig. 2. Ranking of cellular localizations in yeast according to the average value of β-aggregation propensity of the proteins belonging to individual gene ontology terms. The horizontal bars quantify the deviation from the average over randomized sequences with the same length and residue composition.

which contain fewer aggregation prone segments than globular proteins.[25,26]

Cellular Localization

Low β-aggregation propensity

Proteins in the nucleolus, ribosome, and nucleus show a significantly reduced β-aggregation propensity with respect to extracellular and cytoplasmic proteins (Fig. 2). This observation can be explained by evolutionary pressure to avoid β-aggregation expected for the high protein concentrations in well-defined cellular organelles and compartments. The nucleolus coordinates the assembly of ribosomal subunits and its function is tightly linked to cell growth and proliferation as well as cell-cycle regulation and stress responses: β-aggregation in the nucleolus and nucleus would have lethal effects. This suggestion is supported by magnetic relaxation dispersion experiments, which show that in presence of high concentrations of inert macromolecules the self-association of proteins is strongly enhanced trough an entropic, excluded-volume effect called "macromolecular crowding" or depletion attraction.[27]

The amino acid composition of proteins associated to the nucleolus, ribosome, and nucleus is on average different from the amino acid composition of membrane and transport proteins, being the number of hydrophobic residues lower and the number of charges much higher. Nevertheless, we observed that for a few nuclear proteins the aggregation propensity is high because of the presence of hydrophobic stretches. Moreover, we found identity matches between the prion fragment TNMKHMAGAAAAGAVVGGLG, which is known to be amyloidogenic,[28] and the following nuclear proteins:

- TATA-binding protein-associated factor MOT1 (locus name YPL082C, best match: VGGLG).
- PAB1-binding protein 1 (locus name YGR178C, best match: AAAGA).
- Transcription elongation factor SPT5 (locus name YML010W, best match: AGAAA).

The aforementioned proteins are all involved in binding and contain hydrophobic stretches which are highly prone to aggregate. Moreover, as shown in Figure 1, proteins involved in transcription have aggregation propensity higher than their randomly shuffled counterparts. Taken together these findings indicate that the same factors that promote functional protein binding can also lead to ordered aggregation and that exposure to the solvent of hydrophobic binding sites can play a decisive role in protein aggregation.

High β-aggregation propensity

Endoplasmic reticulum is the site of translation, folding, and transport of proteins that are to become part of the cell membrane (e.g., transmembrane receptors and other integral membrane proteins) as well as proteins that are to be secreted or exocytosed from the cell (e.g., digestive enzymes). Our prediction of high β-aggregation propensity for endoplasmic reticulum and (trans)-membrane proteins is supported by a search on the "expasy" proteomics server that annotates as amyloid proteins the Amyloid β A4 (type I membrane protein), Integral membrane protein 2B (single-pass type II membrane protein), Lactadherin (peripheral membrane protein), Microtubule-associated protein τ (mostly found in the axons of neurons, in the cytosol and in association with plasma membrane components), Neprilysin (single-pass type II membrane protein), Peripheral plasma membrane protein CASK (plasma membrane), and tumor-associated hydroquinone oxidase (extracellular and plasma membrane-associated). Importantly, the increased sodium tolerance protein 2, which is a multi-pass membrane protein (locus name YBR086C), is predicted as one of the most amyloidogenic of the membrane proteins and contains among its aggregation prone stretches the segment FGAIL which is known to form ordered amyloid fibrils.[2] Moreover, the well-regulated intracellular amyloidogenesis of Pmel17 (the only functional amyloid known-up to now-in mammals) is orchestrated by the secretory pathway to protect the cell from toxic intermediates.[7] The membrane sequestration of Pmel17 is consistent with the presence of β-aggregation prone proteins in the membrane systems of yeast.

In addition, clathrin-coated pits and vesicles are cellular structures that are involved in both endocytosis of various extracellular macromolecules and in Golgi sorting and transport. The plasma membrane-associated clathrin AP-2 molcules can undergo an extensive self-association reaction that can be detected quantitatively by measuring sample absorbance because of turbidity.[29] Similarly, the "Tat" protein-export system serves to translocate folded proteins, often containing redox cofactors, across the bacterial inner membrane. The crystal structure of *Escherichia coli* formate dehydrogenase-N demonstrates that some "Tat" substrates are integral membranes. Moreover, the essential "Tat" components TatB and TatC are known to have a strong tendency to self-associate.[30] The magnetic relaxation dispersion technique has been used to characterize quantitatively the self-association of secreted and β-aggregating proteins, such as bovine pancreatic trypsin inhibitor (BPTI),[31] bovine β-lactoglobulin,[32] and hen lysozyme.[33]

The highest aggregation propensity is found for "cell wall" proteins (Fig. 2). Although the cell wall was initially considered an almost inert cellular structure that protected the protoplast against osmotic offense, more recent studies have demonstrated that it is a dynamic organelle. Cell wall proteins have been found implicated in adhesion to host tissues and ligands.[34,35,36] Thus, the high β-aggregation propensity may reflect a functional role of "cell wall" proteins which is missing in higher eukaryotes. Intriguingly, several extracellular matrix components as well as fibrinogen, which can form amyloid aggregates,[37] can bind to cell wall proteins.[35]

CONCLUSIONS

We have analyzed the aggregation propensity of the *S. cerevisiae* proteome and linked amyloidogenic propensity of proteins to GO annotations. As pointed out in our previous study, short stretches are preferable to long stretches for the analysis of β-aggregation propensities because the latter contain folding features that deteriorate the signal-to-noise ratio.[13] Nevertheless, in the present study the rank of GO annotations does not change if one uses full length proteins instead of short stretches, which indicates the robustness of our method (see Supplementary Material). Moreover, the use of stretches is extremely helpful to localize the amyloidogenic "hot spots," that is the fragments that have the highest β-aggregation potential within the protein sequence, and understand the role of specific amino acid mutations. It is worth mentioning that the sequence alignment of yeast proteins with their mammal homologues indicates a change of the aggregation propensities for these "hot spots." In fact, by focusing on the fragment showing the highest β-aggregation in each protein sequence, we found that 87% of the yeast "hot spots" correspond to stretches that have a lower aggregation propensity in man. The most relevant changes in aggregation propensities are found for proteins in the GO classes "membrane" and "ribosome," which suggests that pressure against amyloidogenic stretches is a general requirement for higher eukaryotes and pertain to all the proteins of complex organisms.[26] The analysis of changes in the aggregation propensity of homologue proteins will be the object of further investigations.

In conclusion, it is still a matter of debate if ordered aggregates might also fulfill specific functions or are only harmful species which evolution has not been able to eliminate. The latter hypothesis is consistent with the fact that protein-aggregation diseases are becoming increasingly prevalent as our societies age. On the other hand, recent experimental evidence suggests that ordered aggregates might have a functional reason to exist and are therefore confined in specific cellular compartments to prevent them to be toxic.[7] The highest β-aggregation tendency is observed for proteins involved in transport and polypeptide biosynthesis. This

finding suggests that β-aggregation prone sequences are the price to be paid not only for the existence of globular structure but also for the requirement to bind and transport other (macro-) molecules. Notably, it was shown recently by a large-scale, quantitative single-cell yeast proteomic approach that the abundance of proteins involved in polypeptide biosynthesis is almost constant without significant variations of copy numbers in response to environmental perturbations.[38] Such constant concentration might play a key role in preventing ordered aggregation of these proteins. Concerning localization, the nucleolar and ribosomal proteins have much less β-aggregation propensity than those in the cell wall, while cytoplasmic proteins show intermediate values of propensity. Since most nucleolar and ribosomal proteins are partially or completely unstructured in the isolated state,[39] the results of this analysis provide further evidence to the suggestion that the evolutionary pressure has reduced the β-aggregation tendency of proteins in cellular compartments characterized by a high concentration of unfolded molecules.[25,26]

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