Analysis of *Candida albicans* cell adhesion proteins for amyloid-like association through quantitative computational methods

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***Abstract*– Amyloids are fibrous protein structures that play a key role in many serious diseases, including Alzheimer's and Parkinson's, by blocking cell synapses and triggering inflammation. They are formed from proteins that stack together through backbone-backbone and hydrophobic amino acid interactions in the process known as amyloid-like association [1]. We studied the propensity and extent of amyloid-like association in Als3, a *Candida albicans* (yeast) cell adhesion protein. When Als3 is used to adhere the cell to the host, it can cause Candidiasis (yeast infection), an often fatal disease in immuno-compromised patients. We conclude that based on the radius of gyration (R_g) values of amyloid-forming regions in Als3 multimeric systems, Als3 has strong potential for amyloid-like association. Our work lays the foundation for a broader understanding of the mechanisms that cause these debilitating diseases, and may lead to more advanced therapies that target amyloid-forming proteins similar to the ones we study.**

I. Methods

We used Desmond, a software package by D.E Shaw Research, to run molecular dynamics simulations that allow us to determine conformations of protein structures [2]. We built multimeric (4-peptide) systems of Als3 peptides with the following amyloid-forming region (AFR) sequences:

TABLE I. Simulation Setup

Peptides			
Protein	***Species***	***Sequence (AFR)***	***Propensity[a]***
Als3	*Candida albicans*	IVIVA	Strong
Aß	*Homo sapiens*	LVFFA	Medium
--	--	AAAAA[b]	Weak
FLO1	*Saccharomyces cerevisiae*	TVIVA	Strong

a. Qualitative flag for the propensity of the peptide to aggregate (weak-strong).

b. An all Alanine sequence was used for negative control.

We created a Java-based tool to calculate the radius of gyration (R_g), in angstroms (Å), for the protein structures in each simulation, as in equation (1).

$$R_g = \sqrt{\frac{\sum_{k=1}^{N} \left(r_k - r_{mean}\right)^2}{N}} \quad (1)$$

N is the number of coordinates in a given structure, and r_{mean} is the average coordinate of the system. R_g is a measure of the average distance between a set of coordinates and their mean coordinate in a structure, essentially a measure of how compact a protein structure is, which makes it a reliable quantitative measure of amyloid-like association, where the proteins form a compact structure. We used similar aggregates to Als3 in *Candida albicans* as a potential method of comparison using models with confirmed secondary structure, as well as for points of reference.

II. Results & Conclusions

We plotted the R_g values (Å) over time to visualize the aggregation of each peptide, as shown in Fig 1. A lower R_g value corresponds to a tighter and more compact protein structure, which indicates amyloid formation.

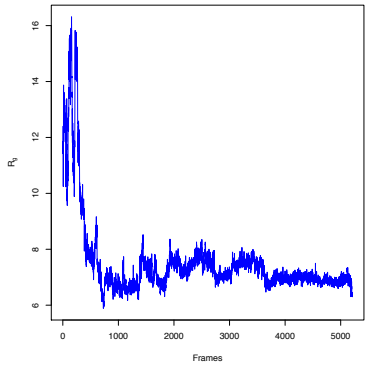

Figure 1. Example of an R_g plot over 5000 frames (50 ns).

Based on the R_g values of the simulations in Fig 1, we find that Als3 associates within 6 ns of the starting frame (5.89 Å R_g), and stays in the aggregated position for the remainder of the simulation. Aß also aggregates within 6 ns of the starting position (7.23 Å R_g), and remains aggregated for the duration of the simulation. The Alanine sequence (negative control) did not aggregate during the simulation. FLO1, a *Saccharomyces cerevisiae* cell adhesion protein, aggregates at the 6 ns mark, and eventually reaches an R_g value lower than that of Als3. We conclude that Als3 is a strong candidate for aggregation and amyloid formation, based on the R_g values of the simulation. We plan to use both qualitative and quantitative methods to further analyze the structure of the aggregates, which is vital for the eventual design of drugs that target the amyloid-forming mechanisms.

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References

[1] Fitzpatrick et al., "Atomic structure and hierarchical assembly of a cross-amyloid fibril.", *Proc. Natl. Acad. Sci. U.S.A.*, 2013, 5468-73

[2] Bowers, Kevin J. et al "Scalable Algorithms for Molecular Dynamics Simulations on Commodity Clusters", 2006.