A Computational Method to Predict Disease-Specific Epitopes in Aβ, and Its Application to Oligomer-Selective Antibodies for Alzheimer's Immunotherapy

Xubiao Peng, PhD[1]; Judith M. Silverman, PhD[2]; Ebrima Gibbs, MD[2]; Neil R. Cashman, MD[2]; Steven S. Plotkin, PhD[1];
[1]Departments of Physics and Genome Sciences, University of British Columbia, [2]Department of Medicine, University of British Columbia

ABSTRACT

Oligomer-specific epitope predictions are presented for Aβ. Cyclic peptides of these epitope primary sequences are both computationally and experimentally generated, which constitute antigenic targets. Clustering analysis, curvature, exposure to solvent, solubility, dihedral angle distribution, and Ramachandran angle distributions are all used to characterize the conformational properties of predicted epitopes, which quantify the distinction between the antigenic profile when presented in the context of the oligomer from that in either the monomer or fibril.

A set of five oligomer-specific epitopes are proposed for Aβ. The computational epitope discovery approach has produced multiple Aβ oligomer-specific antibodies (see also poster #12185, session P4-14).

CONTACT

Steven S. Plotkin
Associate Professor, Depts of Physics
and Genome Sciences & Technology
University of British Columbia
Email: steve@phas.ubc.ca
or steven.plotkin@promisneurosciences.com
Phone: 604-822-8813
Website: www.ubc.ca/~steve

INTRODUCTION

Treatment for Alzheimer's disease (AD) has faced the difficult challenge of effectively targeting and clearing toxic and/or propagative species of protein, which together lead to pathology and neurodegeneration. Current evidence points to a cascade of aberrant proteostasis, generally involving cytotoxic tau pathology that is induced by misfolded Aβ oligomer [1,2,3]. A method for identifying antibodies to Aβ that are conformationally-selective to the toxic oligomer, and which also have relatively low affinity to either Aβ monomer or fibril, is thus a highly desired goal that holds significant promise for AD therapy [4,5].

Antibody	Epitope	Epitope Structure	Conformations Recognized			Aria-E
			Monomer	Oligomer	Fibril	
Solanezumab	AA 16-26	Helix-β coil	Yes	?	?	Low
Crenezumab	AA 16-26	Helix-β	Yes	Yes	Yes	Low
Bapineuzumab	AA 1-7	Helix	Yes	?	Yes	High
Gantenerumab	AA 1-11	Linear	?	Yes	Yes	Low
Aducanumab	AA 3-6	Linear	No	?	Yes	High

Table 1. Antibody therapeutics

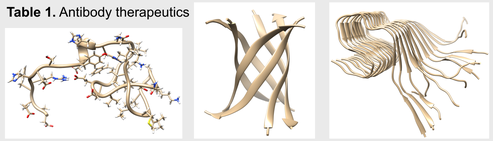


Figure 1. Schematics of Aβ monomer (left), oligomer (centre), and fibril (right)

METHODS AND MATERIALS

We employ computational simulations, using molecular dynamics with standardized force-fields. An experimentally-validated structural model of a protofibrillar aggregate is globally biased away from its "native" conformation to be partially unfolded, using molecular dynamics with collective coordinates to yield a set of regions of contiguous primary sequence that are prone to be disordered upon an external challenge in an anomalous cellular environment.

Rationale

We hypothesize these weakly-stable regions are likely to be exposed in nascent protofibrils or oligomers, and that in this context they are present in an alternate conformation than they are either in the free monomer ensemble or in the context of the fibril. They thus constitute oligomer-specific epitope predictions.

RESULTS

Toxic Oligomer Models

•We seek a candidate therapeutic target for Aβ toxic oligomer; we thus need a model for the Aβ oligomer.
•We seek disrupted regions in the Aβ protofibril under the hypothesis that these will tend to be exposed in the oligomer, but will be conformationally distinct from the same regions in the linear peptide.

RESULTS

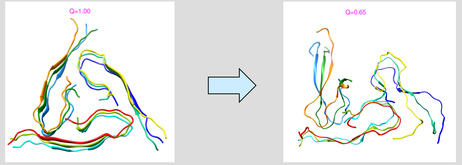


Figure 2. Computational method for epitope discovery

- Note: The oligomer model does not need to be perfect to give good predictions! E.g. "catalytic" sites of aged fibrils.
- Goal: Predict regions of low thermodynamic stability in the oligomer model.
- Method: Use a "collective coordinate" to globally bias the protein to unfold, say, to be 1/3 unfolded
- The protein "decides" where to unfold
- Example: Applying the method to Superoxide dismutase (SOD1) yields 7 epitopes:
- The output is regions that show increased exposure to solvent
- These regions would become accessible to abnormal interactions when the protein is challenged

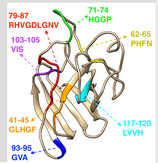


Figure 4. Epitopes in SOD1.

At least 5 of these predicted regions are consistent with known misfolding-specific epitopes (41-45, 62-65, 79-87, 93-95, and 117-120)

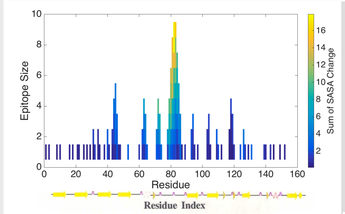


Figure 3. Contiguous regions in SOD1 that show an increase in solvent accessible surface area.

- We also tend to see that epitopes emerge persistently across different proto-fibril morphologies, or "strains" of Alzheimer's.
- This implies that Abs raised to these epitopes may be generally useful across AD cases.

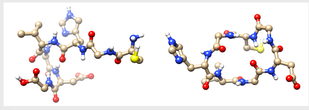
Figure 5. Predicted epitopes persist across several fibril morphologies.

RESULTS

Oligomer-specific Epitopes

- Validate that the cyclic peptide equilibrium ensemble is distinct from the fibril or linear peptide ensembles, so that Abs raised to it will be conformationally-selective.

Figure 6. Linear and cyclic peptides of sequence HVGD (80-83 in SOD1) with linker amino acids.



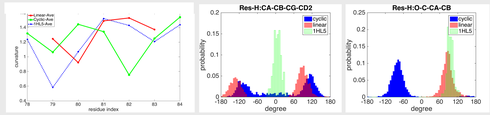


Figure 7. (**top left**) Curvature alone often doesn't distinguish cyclic from linear or fibril. (**top middle**) Some dihedrals discriminate from the fibril, but not the linear monomer. (**Top right**) Other angles discriminate the cyclic peptide from both linear and fibril.

Clustering analysis

- Take all the conformations of the linear peptide ensemble, and structurally align them to each other, i.e. by RMSD (least squares fit)
- The conformations are not all completely dissimilar from each other, instead they tend to cluster around "centroid structures", which give hints of the peptide's structural preference
- Find the centroid structures of the largest clusters for linear, fibril, and cyclic; plot RMSD1, RMSD2, RSMD3 for one configuration.
- This gives a set of points in 3D space. Do this for all configurations, in the equilibrium ensembles of linear, cyclic, and fibril

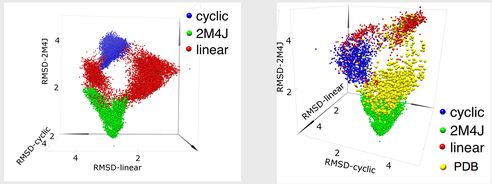


Figure 8. (**Epitope 1, Above left**) The ensemble overlap between the cyclic and fibril conformations is essentially zero; between the cyclic and linear it is minimal, < 3%. (**Epitope 2, Above right**). Too distinct is unphysical, yet physical conformations can lead to proteomic target distraction for proteins displaying the epitope. Scanning for the sequence across the PDB, analyzing the structural overlap, addresses this issue.

RESULTS

Oligomer-selective Antibodies

- Make cyclic peptides of the predicted epitopes
- Conjugate to immunogens (KLH), extract monoclonal antibodies.
- Use ELISA and Surface Plasmon Resonance (SPR) to screen clones against cyclic and linear epitopes, as well as in vitro Aβ oligomers and monomers.

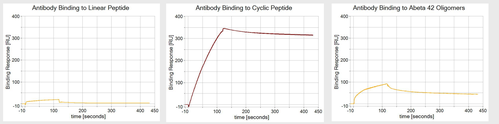
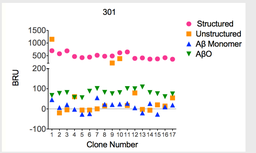


Figure 9. SPR results for the binding of mAbs to linear peptide of a predicted Aβ epitope sequence (above left), cyclic peptide (above center), and in vitro $A\beta_{42}$ oligomers (above right).

Figure 10. The most promising antibody clones have shown selective binding to both structured peptide, and to *in vitro* oligomer, over binding to either linear peptide epitope, or unstructured Aβ monomer (See Silverman et al. poster #12185, session P4-14).



CONCLUSIONS

- We have predicted 5 *conformational* epitopes in Aβ.
- Computational analysis of these epitopes shows that these epitopes are conformationally distinct from the same sequence in either Aβ monomer or in the fibril.
- We have now raised over 300 antibody clones designed to be oligomer-selective; this has yielded about 50 lead antibodies, roughly 10 for each epitope.
- Next steps will involve screening in cadaveric brain homogenates and CSF of AD patients and healthy controls. Successful products will then be assessed for clinical development.

REFERENCES

1. Hadley, KC et al. *eLife*(2015).
2. Ittner LM, Gotz J, Nat Rev Neurosci (2011).
3. Vasconcelos B et al. Acta Neuropathol (2016).
4. Sevigny J, et al. Alzheimers Dement (2015).
5. Alzforum.org "Shape of a Hug: How the Embrace of a Therapeutic Aβ Antibody Really Matters"