Analysis of Anti-Amyloid Beta (Aβ) Antibodies Using Fragment-Based Docking and MMPBSA Binding Free Energy Calculations

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Abstract

Alzheimer’s Disease (AD) is a neurodegenerative disorder characterized by gradual memory and cognitive function loss. Currently, there are no effective treatments that can reverse or stabilize the symptoms of the disease. Anti-amyloid beta (Aβ) antibodies are the leading drug candidates to treat AD, but the results of clinical trials over the past two decades have been mostly disappointing. This was due to either low effectiveness or serious side effects that emerged during clinical trials. Introducing rational mutations into anti-Aβ antibodies to increase their effectiveness and remove harmful side reactions is a way forward, but the path to take is unclear since the key structural characteristics that determine the binding affinity and selectivity of anti-Aβ antibodies towards amyloid species are not well understood.

In this study, we have taken a computational approach to examine how an anti-Aβ antibody binds to one or more Aβ epitopes in its antigen-combining site. Our unbiased fragment-based docking method successfully predicted the emergence of the key EFRH epitope commonly observed when Aβ binds to anti-Aβ antibodies. MD simulations coupled with MMPBSA binding free energy calculations were used to analyze scenarios described in prior studies concerning the nature of anti-Aβ antibody binding to various Aβ species. Finally, based on observing our MD trajectories of the PFA1 antibody bound to Aβ2−7 and pE3-Aβ3−8, we introduced rational mutations into PFA1 in an effort to improve the calculated binding affinity of PFA1 towards the pE3-Aβ3−8 form of Aβ. Two out of our four proposed mutations stabilized binding. Our study demonstrates that a computational approach can predict beneficial mutations which may lead to an improved drug candidate for AD in the future.
**Introduction**

Alzheimer’s Disease (AD) is an incurable neurodegenerative disorder that leads to steady memory and cognitive function loss, culminating in death. AD is the leading cause of dementia, which affects an estimated 47 million people and incurs a total estimated worldwide cost of US$818 billion\(^1\). At present there is no cure for AD, and there is a notable absence of treatment options that can reverse or effectively slow progression of the disease.

At the level of brain tissue, AD is characterized by both the appearance of extracellular, fibrous plaques that are built up from the polymerization of amyloid beta (A\(\beta\)) peptides\(^2\) and the appearance of intracellular neurofibrillary tangles that consist of hyperphosphorylated tau proteins\(^3\). Amyloid fibril deposits are hallmarks of several neurodegenerative diseases\(^4\), and the amyloid hypothesis states that an excessive buildup of A\(\beta\) plaques in the brain is responsible for the cognitive decline observed in AD patients. It suggests that clearing A\(\beta\) plaques from the brain would help inhibit or reverse progression of the disease.

The amyloid hypothesis has been the leading theory driving therapeutic approaches for the treatment of AD for over two decades\(^5\). The most common therapeutic approach to AD treatment is immunotherapy\(^6\textendash}^8\). Several active and passive anti-A\(\beta\) immunotherapies that target A\(\beta\) species in the brain have advanced to clinical trials, but the results thus far have been mostly disappointing. After obtaining promising results in animal models, the vaccine AN1792, which targeted a full-size A\(\beta\) 1-42 peptide, advanced to human clinical trials in 2001\(^9\). In the phase I clinical trial, patients who responded to the treatment exhibited reduced cognitive
decline compared to control patients, and safety concerns were minimal\textsuperscript{10}. However, the phase II trial was terminated after 6\% of the treated patients developed meningoencephalitis\textsuperscript{11}.

The result of the AN1792 trial led to the development of several passive immunization approaches over the next several years. An advantage to passive immunization is that the treatment can be halted immediately if any severe side effects arise. In addition, it was believed that the A\textsubscript{\beta\textsubscript{1-42}} immunogen used in AN1792 led to an immune response triggered by autoreactive T cells against the C-terminus portion of the peptide\textsuperscript{11}. Shorter regions of the A\textsubscript{\beta} sequence were subsequently used to develop monoclonal antibodies (mABs) that target different cell types in the immune system. The N-terminus A\textsubscript{\beta\textsubscript{1-15}} sequence was used for A\textsubscript{\beta} specific B cell epitopes while A\textsubscript{\beta\textsubscript{16-42}} was used for A\textsubscript{\beta} specific T cell epitopes\textsuperscript{12}. Additionally, the choice of the epitope has a crucial effect on the ability of the antibody to bind to its amyloid target species. The N-terminus is accessible to antibody binding in aggregated forms of A\textsubscript{\beta} while the central and C-terminal epitopes are only able to bind to the antibody in monomeric, or perhaps small oligomeric, forms of A\textsubscript{\beta} due to the central and C terminal epitopes being inaccessible in mature fibril structures.

Initially, two anti-A\textsubscript{\beta} mABs advanced to clinical trials targeting distinct epitopes/species of A\textsubscript{\beta}. Bapineuzumab primarily targets insoluble amyloid plaques via the hydrophilic N-terminal epitope of A\textsubscript{\beta\textsubscript{1-5}}. Initial results looked promising, but again during phase II of the clinical trial a serious side effect appeared as 10\% of patients developed vasogenic edema. This side effect was found to be much more common in apoE4 carriers than in non-carriers\textsuperscript{13}. Currently, AAB-003 is a second generation bapineuzumab variant where the Fc domain has been mutated to remove Fc\gamma R and C1q binding. These mutations were predicted to reduce the incidences of
microhaemorrhage and phagocytosis for apoE4 carriers, and recent clinical results confirmed the desired effect for AAB-003\textsuperscript{14}. Clinical trials for bapineuzumab and AAB-003 are ongoing although phase III results for bapineuzumab have not improved its outlook\textsuperscript{15-18}.

Solanezumab targets soluble monomeric A\(\beta\) peptides via the hydrophobic central A\(\beta\)\textsubscript{16–24} epitope. Although it is unable to bind to amyloid plaques directly, it is proposed that solanezumab can indirectly prevent A\(\beta\) fibrillization through a peripheral sink mechanism\textsuperscript{19}. In the peripheral sink mechanism, solanezumab binds to the monomeric A\(\beta\) peptide, indirectly shifting the equilibrium away from plaque formation towards producing more A\(\beta\) monomers. In clinical trials, solanezumab had a much-improved safety profile as adverse side effects such as meningoencephalitis, microhemorrhage, and vasogenic endema were not observed\textsuperscript{20-21}. On the other hand, questions about solanezumab’s efficacy in reducing neuritic plaque burden arose during early clinical trials as it failed to meet its phase II clinical endpoints, and solanezumab advanced to late clinical trials as a potential treatment option for mild-to-moderate cases of AD only\textsuperscript{22}. Recently, it was announced that the phase III trials for solanezumab had failed to show a significant benefit in slowing cognitive decline for mild-to-moderate AD as well\textsuperscript{23-25}.

Although solanezumab’s safety profile was desirable, the question of how to improve the efficacy of a mAB such as solanezumab to treat AD is not straightforward. To complicate matters further, A\(\beta\) species exhibit a high degree of structural polymorphism, and several other A\(\beta\) species have emerged as potential disease-causing agents that would presumably need to be removed in an effective AD treatment\textsuperscript{26}. Small, soluble A\(\beta\) oligomers have been shown to be sufficient to induce cognitive defects in animal studies\textsuperscript{27-30}. It has been demonstrated that
oligomers can alter membrane permeability\textsuperscript{31-34} and induce a hyperphosphorylation state in tau proteins\textsuperscript{35} which are both linked to neurotoxicity.

In addition, normally rare, N-terminal truncated variants of A\(\beta\) have been found in much higher concentrations in the stable, neurotoxic A\(\beta\) aggregates that are found in severe AD cases. The most prominent of these are the pyroglutamate modified forms of A\(\beta\) (pE3-A\(\beta\) and pE11-A\(\beta\)) which are more hydrophobic than A\(\beta\) and resist degradation. Both of these features facilitate the formation of stable A\(\beta\) aggregates, and plaques can be enriched by as much as 50\% with the pE3-A\(\beta\) form of A\(\beta\)\textsuperscript{36-37}. In light of this, the pE3-A\(\beta\) form of A\(\beta\) has become a target in antibody development\textsuperscript{38-40}. A study by Gardberg et al. involving the co-crystallization of anti-protofibril antibody 1 (PFA1) bound to various N-terminal A\(\beta\) species revealed that the bound peptide-antibody complex was very similar when comparing A\(\beta\)\textsubscript{2-7} and pE3-A\(\beta\)\textsubscript{3-8} bound to PFA1 in the two crystal structures; the C\(\alpha\) RMSD for residues 3-8 between the two peptides was only 0.24 Å. Nevertheless, there is a rather large reduction in binding affinity for pE3-A\(\beta\)\textsubscript{3-8} in comparison to A\(\beta\)\textsubscript{2-7} (\(K_D = 60\) nM for A\(\beta\)\textsubscript{2-7} bound to PFA1 versus \(K_D = 3000\) nM for pE3-A\(\beta\)\textsubscript{3-8} bound to PFA1)\textsuperscript{41}. The existence of such a wide variety of potentially pathogenic amyloid targets implies that targeting a single epitope associated with a single A\(\beta\) species might not be enough for an antibody to treat AD effectively.

In light of this, next generation mABs that can bind to multiple A\(\beta\) epitopes and species are currently in clinical trials. Gantenerumab binds at nanomolar affinity to several A\(\beta\) species (with a \(K_D\) of 0.6 nM for A\(\beta\) fibrils, 1.2 nM for A\(\beta\) oligomers, and 17 nM for A\(\beta\) monomers), and it recognizes two epitopes within A\(\beta\): the N-terminal EFRHDGYEV sequence and a central region from the sequence VFFAEDVGSN\textsuperscript{42}. Similarly, crenezumab, despite being generated by
immunization with the N-terminal $\text{A}\beta_{1-16}$ epitope$^{43-44}$, has been shown to bind to monomeric and oligomeric forms of $\text{A}\beta$ via the central $\text{A}\beta$ epitope while having a reduced capacity to bind to FcyR, which should help diminish the inflammation response$^{8,44-45}$. Due to cross reactivity patterns that it shares with solanezumab, the ability to bind to the central epitope of $\text{A}\beta$ has been emphasized in studies of crenezumab$^{46}$, and a co-crystal structure of crenezumab (more specifically, CreneFab) was recently obtained bound to an $\text{A}\beta$ peptide containing the central epitope$^{45}$. Nevertheless, crenezumab has also been shown to bind to amyloid fibril species$^{44-45}$, which is puzzling since the central epitope is not readily accessible for binding in mature fibril structures. Recently, aducanumab was heralded as possibly the first “successful” anti-$\text{A}\beta$ antibody as it was able to clear $\text{A}\beta$ plaques thoroughly at the highest dosage and was shown to reduce cognitive decline in an early Phase III clinical trial that took place over the course of a year$^{47}$. Aducanumab has been reported to bind to both N-terminal and central epitopes of $\text{A}\beta$, accounting for binding to both fibril and oligomeric forms of $\text{A}\beta$$^{48}$. Phase III trials for aducanumab are ongoing.

The ability of antibodies to recognize more than one epitope appears to be important for effective clearance of $\text{A}\beta$, but the actual structural characteristics that allow an antibody to favor binding to one specific epitope, or to bind to more than one epitope with high affinity, are not well understood$^{49}$. Conformational selection has recently been put forth as a general explanation for epitope preference of $\text{A}\beta$ binding to various antibodies$^{50-53}$, but the difference in charge characteristics between the N-terminal and central $\text{A}\beta$ epitopes coupled with a general lack of sequence similarity between the two regions is a confounding factor.
In this study, we have taken a computational approach to examine the question of how an anti-Aβ antibody binds to one or more Aβ epitopes in its antigen-combining site. This overarching question can be broken up into several sub questions including: 1) which residues in Aβ_{1-42} are most important in the initial binding event of Aβ to a given antibody structure?, 2) how might antibodies like gantenerumab, crenezumab, and aducanumab bind to both the hydrophillic N-terminal and hydrophobic central epitopes?, and 3) how can an antibody prefer a specific epitope of Aβ to another in cases where their sequence, charge characteristics, and binding pose seem quite similar to one another?

To address the first question, we have developed an unbiased fragment-based docking method to probe the antigen-combining site of various anti-Aβ antibodies. Our goal in carrying out an unbiased search across the entire surface of the antibody is to see how easily a certain amino acid residue finds the correct binding site and to see how strong the binding interaction is at that location. Our approach borrows heavily from current ideas being used in computational fragment-based drug design. In these methods, potential binding sites are located by probing the surface of a large protein receptor using small fragments of a ligand as opposed to attempting to dock the entire ligand. A potential disadvantage to using small fragments to probe the receptor is a decrease in the binding selectivity for the ligand; additional binding sites other than the primary site of interest are often identified as potential binding sites. On the other hand, conformational possibilities for a small fragment are much lower than for a larger ligand, and it has been shown that the leading hot spots identified using computational fragment-based methods correlate well with actual ligand binding sites. In the
present case, we were fortunate to be examining antibodies where the location of the ligand binding site was already known beforehand.

To address the second question, we ran molecular dynamics (MD) simulations using the available crystal structures of gantenerumab and crenezumab bound to short A\beta peptides\(^{42,45}\) (a crystal structure of aducanumab was not available for us to analyze at this time). We then calculated the binding free energy of the A\beta peptide towards the antibody in question using the Molecular Mechanics Poisson Boltzmann Surface Area (MMPBSA) module in Amber 16\(^57\). Proceeding from there, we produced homology models for gantenerumab and crenezumab where we changed the A\beta peptide to a peptide containing the other prominent epitope that was not found in the original crystal structure (i.e. we changed the N-terminal epitope to the central epitope for gantenerumab and vice versa for crenezumab). We used knowledge gained from our unbiased fragment-based docking study to initially align the new peptide model in the antigen-combining site with the strongest binding amino acid, and we ran MD simulations with the peptide model oriented in both forward and reverse orientations across the antigen-combining site. MMPBSA binding free energy calculations were then carried out for all of the homology models, and the free energy results and structures were compared to identify key contacts for the potentially stable binding of both A\beta epitopes to the antigen-combining site of each antibody.

For the third question, we built on the study of PFA1 bound to A\beta_{2-7} and pE3-A\beta_{3-8} that was carried out previously by Gardberg et al.\(^{41}\) The authors in that study wanted to know how PFA1 could bind so much more tightly to A\beta_{2-7} than to pE3-A\beta_{3-8} despite such a similar binding pose, and they suggested that a computational approach might provide an answer.
Here, we ran MD simulations of both PFA1 bound to Aβ_{2−7} and PFA1 bound to pE3-Aβ_{3−8}, and we observed both trajectories visually to compare the differences. Based on what we saw, we engineered two mutations into PFA1 in an attempt at improving the binding affinity of pE3-Aβ_{3−8} towards PFA1.

A deeper understanding of the structural binding characteristics of the antigen-combining site for an anti-Aβ antibody is a critical step towards the rational improvement of antibody drug candidates for AD treatment. The ability to rationally modify an existing mAB to bind to additional Aβ species, or alternatively, to reduce affinity towards a species of Aβ that leads to undesirable autoimmune side effects, may be key to producing the most effective drug candidate for this disease in the long term.

**Materials and Methods**

**Computational docking of amino acid residues to anti-Aβ antibodies**

We carried out an unbiased, fragment-based computational docking study to examine the initial binding characteristics of the antigen-combining site on various anti-Aβ antibodies. To minimize conformational effects from the polypeptide ligand, we chose to dock single amino acid residue fragments to the antibody surface. 16 amino acids were individually docked to each antibody, comprising the full Aβ 1-42 monomer sequence.

Each amino acid residue was generated using the sequence command in xleap from Amber 16^{57}. Three types of amino acid residue fragments were initially tested using our docking protocol: 1) the default amino acid residue that contained charged N- and C-terminal groups on the backbone, 2) a neutralized version where methyl groups were attached to both the N- and
C-terminal groups to remove the backbone charges, and 3) a non-physiological fragment where two hydrogens on the N-terminus and an oxygen on the C-terminus were omitted from the structure. We compared the results of the docking for a few test residues using each method above to the actual binding sites observed in the PFA1 and PFA2 crystal structures and also to the leading ligand-free hotspots found by submitting the PFA1 and PFA2 apo structures to the FTMap server\textsuperscript{54-55}. Of the three options, we found that method 3 worked the best. We noted that the zwitterionic backbone charges in method 1 were capable of binding to antibody hot spots in place of the side chain functional groups, and method 2 was unable to bind to certain hot spots due to steric issues brought about by the attached methyl groups. Therefore, residues were generated using method 3 for our full analysis.

After obtaining an amino acid residue, Open Babel\textsuperscript{58} was used to convert pdb files to pdbqt files for both the antibody and the residue, and then the residue was docked to the antibody using Autodock Vina/SMINA\textsuperscript{59}. The residue was allowed to search the entire antibody surface within a 100 Å\textsuperscript{3} box that was centered on the antigen-combining site with a search exhaustiveness of 128. Using the default settings in Autodock Vina/SMINA, the top nine docked results for each residue, ranked by their most stable binding free energy values, were subsequently used for our analysis.

To carry out a comparison study with a variety of anti-amyloid antibodies, this method was performed on the previously published crystal structures of bapineuzumab, solanezumab, gantenerumab, crenezumab, ponezumab, PFA1, and PFA2 in both the holo and apo forms (if available) of each structure (PDB IDs: 4OJF, 4XXD, 5CSZ, 5VZX, 5VZY, 3UOT, 2IPU, 2IPT, 2R0W,
and 2IQ9. The PDB file for each antibody complex was edited prior to docking to remove everything except for the residues of the isolated antibody structure.

**Molecular dynamics simulations**

To prepare the various complexes that were used in this study for MD simulations, the program Modeller\textsuperscript{60} was used to accomplish two purposes: 1) to model in any missing residues that were not present in the original PDB file, and 2) if applicable, to generate a homology model containing a new Aβ peptide that was not present in the original PDB file. Prior to using Modeller, the PDB file was processed to remove everything except for the Aβ peptide-antibody complex. After using Modeller, the structure was further processed using the program xleap in Amber 16 to add in hydrogens, water, counter ions, and disulfide bonds. For the pE3-Aβ\textsubscript{3−8} model (PDB ID: 3EYS), antechamber was used to model in the pyroglutamate (PCA) moiety.

For each MD run, a 1000 step minimization was carried out with 500 steps of steepest descent followed by 500 steps of conjugate gradient using a non-bonded cutoff of 8.0 Å. The system was then heated up to a constant 300 K over a period of 50 ps employing the Langevin thermostat. The density was equilibrated over an additional 50 ps, and an equilibration with all restraints removed was undertaken for approximately 250 ns. Finally, a 50 ns production run was carried out to bring the total simulation time to 300 ns. Due to the size and high conformational flexibility of both the amyloid peptide and the antibody, and the extensive use of homology modeling, a long equilibration/production run was needed in order to achieve acceptable convergence for our MMPBSA binding free energy calculation (see **MD method validation** in Results and Discussion). Afterwards, the MD trajectory of our production run was visualized using UCSF Chimera\textsuperscript{61}. In addition, to characterize alterations in binding patterns in
the antigen-combining sites of our peptide-antibody structures, residue-to-residue percent occupancy calculations were carried out between select residues in the Aβ peptide and on the antibody surface using a cutoff distance of 10 Å over 5000 frames collected at equal intervals from the 50 ns production run. This relatively high cutoff distance was chosen due to the high conformational flexibility of certain ligands in the antigen-combining site which led to a difficulty in identifying specific binding interactions; as a result, we do not use this measure to indicate such binding interactions per say, but rather, a low percent occupancy indicates a decreased likelihood that the two residues in question can participate in such a binding interaction.

**MMPBSA binding free energy calculations**

5000 frames, taken at equal intervals over the 50 ns production run, were used to calculate the MMPBSA binding free energy$^{62-67}$ for each Aβ peptide-antibody complex. All PBSA calculations were conducted with the PBSA program$^{68-76}$ in the AMBER 16 package.$^{57,77}$ For this calculation, inp = 2 was used for the non-polar solvation model,$^{78-79}$ radiopt=0 was used for the intrinsic atomic radii, and the ionic strength was set to 100 mM. All other settings were kept at the default values used in Amber 16.$^{57,77}$ Due to the rather high uncertainties in normal mode analysis, the entropy contribution was neglected in our binding affinity calculations. Experimental binding affinities were compared to our calculated binding affinities by converting $K_D$ values into binding free energies using:

$$\Delta G = RT \ln (K_D)$$

where $R = 1.987 \times 10^{-3}$ kcal mol$^{-1}$ K$^{-1}$, $T = 300$K, and $K_D$ is the dissociation constant in units of M.
Results and Discussion

Computational docking of amino acid residues to anti-Aβ antibodies

To address the question of which amino acids are key to the initial binding of an Aβ peptide to an anti-amyloid antibody, we carried out an unbiased fragment-based computational docking search using all 16 unique amino acid residues that appear in the Aβ₁⁻⁴² sequence. Two criteria from the output were considered to be relevant in determining the likelihood that an amino acid will bind with high affinity to the antigen-combining site of the antibody: 1) which residues have the strongest binding interaction at the antigen-combining site?, and 2) which residues find the antigen-combining site most consistently?

TABLE 1 lists the most stable binding affinities for the top 10 ranked amino acid residues bound to the antigen-combining sites of ten anti-Aβ antibody structures. From TABLE 1, we observe that approximately 95% of the amino acid residues that are ranked in the top 5 appear in the Aβ 1-23 region while 13% appear in the Aβ 24-42 region. Furthermore, 57% are found in the N-terminal DAEFRH epitope while 39% appear in the central KLVFFAEDV epitope. In particular, nine out of the ten antibodies have at least three out of the four residues in the EFRH sequence appear in their top 5 ranking; the only exception was gantenerumab where E and H were tied for sixth place. These results are consistent with the noted tendency of the N-terminal DAEFRH epitope to bind to most anti-Aβ antibodies⁵³.

In TABLE 2, we examine the number of docked amino acids found at the antigen-combining site for each residue across the 10 different anti-Aβ antibodies. If we consider only the amino acids that were able to dock at the antigen-combining site more than half of the
time, 79% of the amino acid residues in \textbf{TABLE 2} appear in the $\alpha$-1-23 region while 47% appear in the $\alpha$-24-42 region. 39% are found in the N-terminal DAEFRH epitope while 46% appear in the central KLVEFAEDV epitope. In contrast to \textbf{TABLE 1}, which displayed similar results in the residue ranking for each antibody, \textbf{TABLE 2} displayed clear differences between the different highly ranked residues docked to different antibodies and even showed some noticeably large differences for the holo and apo forms of the same antibody. These latter differences are presumably due to differences in the specific conformation of the antibody in the holo and apo crystal structures. It is worth pointing out that while charged and aromatic residues appear to dominate the top ranks in \textbf{TABLE 1}, the ability of fragments to find the active site, as observed in \textbf{TABLE 2}, does not correlate as strongly with polarity as many polar and non-polar residues tend to locate the antigen-combining site with similar ease.

An examination of \textbf{TABLES 1 and 2} reveals an important trend in the binding pattern that appears to be consistent across the various antibodies that we have studied; the top two residues on $\alpha$-1-42 that give that most stable binding free energy are either phenylalanine or tyrosine in all cases, and both residues are also near the top of the residues that are consistently docked to the antigen-combining site. Since phenylalanine and tyrosine are structurally identical except for a hydroxyl group, the strong binding free energy points to the presence of an aromatic binding pocket that is important for binding $\alpha$. The existence of such a binding pocket has been pointed out before as a key binding site for phenylalanine by several anti-$\alpha$ antibody crystallographers\textsuperscript{42,45,53,80}. In addition, the two prominent $\alpha$ epitopes, the N-terminal DAEFRH epitope and the central KLVEFAEDV epitope, correspond to the only two locations in the $\alpha$-1-42 sequence where phenylalanine appears. Our docking results, taken
together with these observations, point towards phenylalanine as a leading candidate for the most important residue in the initial binding of $\text{A}\beta$ epitopes to an anti-amyloid antibody.

**MD method validation**

Although the computational docking of single amino acid residues may give us some insight as to which amino acids might bind first to the antigen-combining site of an anti-$\text{A}\beta$ antibody, it does not necessarily help us understand how binding affinity emerges for an extended polypeptide. After the first residue in a polypeptide binds to the antigen-combining site, a previously accessible, high affinity binding site now becomes unavailable to other residues, and the binding of the first residue also restricts the search space where other residues can bind. In addition, our docking protocol lacks many key factors, such as the presence of water and the conformational motion of the full polypeptide and antibody, which have both been identified as being important factors in the selectivity of $\text{A}\beta$ peptide-antibody binding$^{53}$.

Therefore, to study the binding affinity of extended $\text{A}\beta$ peptides to amyloid antibodies, we turned to MD simulations and MMPBSA binding free energy calculations using the Amber 16 software suite$^{57}$. First, we needed to validate our computational approach using previously published experimental data. To do this, we compared our MMPBSA binding free energy calculations to the full set of experimental binding affinities reported in the study carried out by Gardberg et al. on PFA1 and PFA2 bound to various $\text{A}\beta$ peptides$^{80}$. **FIGURE 1** shows the correlation of our MMPBSA calculations with the experimental data (the numerical data are given in **TABLE 3**). With a Pearson’s R value of 0.95, our MMPBSA calculations show very good agreement with the trend seen in the experimental binding affinity data. **FIGURE S1** shows the
convergence of our MMPBSA free energy values taken over the entire 50 ns production run. It is seen that our data set shows reasonable convergence over this time frame. One data point, for the $\text{A}^\beta_{1-8}$ peptide bound to PFA1, converged very slowly and underwent a substantial change in its binding affinity over the 50 ns MMPBSA calculation. To verify that the MMPBSA result for $\text{A}^\beta_{1-8}$ bound to PFA1 had converged properly, we collected 10 ns of additional MD simulation data and ran a 60 ns MMPBSA calculation using the simulation data from 250 ns to 310 ns. The 60 ns MMPBSA result showed that the 50 ns MMPBSA result had indeed already converged (see FIGURE S2). In general, we found that running MD simulations for a total simulation time of 300 ns, and using 5000 frames taken from the last 50 ns for MMPBSA calculations, was sufficient to produce acceptable convergence for our data. This became the standard protocol that we used for any subsequent analysis.

The importance of phenylalanine to the stable binding of $\text{A}^\beta_{2-7}$ to PFA1

To test the importance of phenylalanine to $\text{A}^\beta$ peptide-antibody binding, we studied an experimental scenario discussed by Gardberg et al. The authors carried out a binding assay for $\text{A}^\beta_{2-7}$ and several other $\text{A}^\beta_{2-7}$ sequence variants bound to PFA1. They demonstrated that binding affinity was lowered (from 60 nM to 3400 nM), but not completely abolished, when the wild type sequence, AEFRHD, was changed to the human glutamate receptor interacting protein 1 (or Grip1) sequence, AKFRHD. This was surprising since the charge characteristics completely changed from the negative glutamate residue to the positive lysine residue in the $\text{A}^\beta$ peptide. On the other hand, no binding to PFA1 was observed at all when AEFRHD was mutated to AEIRHD (the Position 4 or Pos4 mutant) despite the swapping of two non-polar hydrophobic residues. We constructed homology models for the Grip1 and Pos4 mutants,
carried out MD simulations, calculated the MMPBSA binding free energies for each, and compared the results to the $A\beta_{2-7}$ MMPBSA binding free energy from our method validation. TABLE 4 shows that the MMPBSA binding affinities qualitatively reproduce the experimental results from the Gardberg study quite well.

To examine why the Pos4 (AEIRHD) mutation is much more severe than the Grip1 (AKFRHD) mutation, we first constructed RMSD plots of the 50 ns production runs for each of the three relevant structures. The average RMSD values for the two mutants, Grip1 and Pos4, are clearly higher than the average RMSD for the $A\beta_{2-7}$ structure indicating a larger degree of structural change from the initial structure for the two mutants (see FIGURE S3).

Next, the MD trajectories of the three complexes were visualized and compared with one another. It was observed that both mutant residues were no longer able to bind to their original binding pockets in comparison to the wildtype $A\beta_{2-7}$ peptide. FIGURE 2 shows a representative frame, taken from the halfway point of the MD trajectory, to illustrate the situation for each structure. In the Grip1 structure, the binding pocket and the lysine residue have separated from each other indicating that the binding contact between the two has been disrupted (see FIGURE 2B). However, the rest of the residues in the $A\beta_{2-7}$ sequence remain bound in their proper orientations (in agreement with structural observations made by Gardberg$^{80}$), which suggests that the loss or alteration of the single glutamate binding interaction is the major cause of the decreased binding affinity of PFA1 for Grip1.

The Pos4 mutation is more complicated. We note first that the isoleucine residue is displaced outside of the deep phenylalanine binding pocket (FIGURE 2C). Another noteworthy difference is that, when the isoleucine residue was pushed out of the pocket, the front half of
the Aβ peptide chain rotated. The glutamate residue disassociated from its normal binding pocket and was found instead binding to a nearby binding pocket that normally binds to either aspartate or alanine, which appear at positions 1 and 2 of the full Aβ₁₋₈ peptide respectively (FIGURE 3). Our unbiased computational docking data predicted that the Pos4 mutant would have a larger destabilizing effect on the binding affinity compared to the Grip1 mutant. From TABLE 1, phenylalanine had a top binding affinity of -5.9 kcal/mol and -6.1 kcal/mol for the holo and apo forms of PFA1 respectively, while glutamate had a top binding affinity of -4.5 kcal/mol and -4.8 kcal/mol for the holo and apo forms. However, the change in orientation of the binding pose in the Pos4 mutant for the full peptide clearly could not be captured by calculating the binding affinity for a single amino acid residue docked to the antibody surface.

**Analysis of gantenerumab and crenezumab binding to multiple Aβ epitopes**

Using our prior observation that phenylalanine is a very important residue for the binding of Aβ peptides to anti-amyloid antibodies, we also examined the antigen-combining sites of gantenerumab and crenezumab with the aim of discovering how these two antibodies might bind to both N-terminal and central epitopes of Aβ peptides.

We first ran MD simulations and calculated MMPBSA binding free energies for both gantenerumab and crenezumab bound to the Aβ peptides observed in their crystal structures (PDB IDs: 5CSZ and 5VZY). Afterwards, we generated homology models containing the other prominent Aβ epitope that was not present in the original crystal structure for both gantenerumab and crenezumab. Four homology models that featured the new peptide were generated where: 1) each new Aβ peptide was modeled in both forward and reverse orientations across the antigen-combining site, and 2) the location of phenylalanine residues in
the Aβ epitopes were used for the initial alignment in the antigen-combining site. The calculated MMPBSA binding free energies for the entire set are given in TABLE 5 while the convergence for our MMPBSA values is demonstrated in FIGURES S4 and S5. It should be noted upfront that we did not have an extensive experimental data set to validate our method for crenezumab and gantenerumab like we had for our study of PFA1 and PFA2. As such, in this section we must be content with using the calculated binding affinities to establish the relative order of bound Aβ peptide-antibody structures for each antibody as opposed to having an independent experimental data set that would have allowed us to compare the binding data for gantenerumab and crenezumab in a more quantitative fashion.

The N-terminal peptide, DAEFRHDSGYE, bound to gantenerumab yielded a very stable calculated binding free energy of -33.8 kcal/mol in comparison to the experimental value reported for the Aβ monomer bound to gantenerumab which was -10.7 kcal/mol. However, modeling the central epitope, HHQKLVVFAEDV, into gantenerumab's antigen-combining site did not yield a similarly stable, negative binding free energy for any of the four homology models we tested. FIGURE 4 compares the initial pose of the peptide with the N-terminal epitope bound to gantenerumab (FIGURE 4A and 4B) to snapshots taken of our most stable Aβ peptide containing the central epitope bound to gantenerumab, with a calculated binding free energy of 1.8 kcal/mol, at the first, middle, and last frames of the MD production run (FIGURE 4C, 4D, 4E, and 4F). The source of the strong binding affinity for gantenerumab with the N-terminal Aβ epitope seems apparent from an examination of FIGURE 4B. The gantenerumab antigen-combining site exhibits several positive (blue) electrostatic sites on its surface that establish contact with the many negatively charged aspartate and glutamate (red) residues that appear
within the N-terminal epitope sequence on the Aβ peptide. A central phenylalanine binding pocket is also present where the phenylalanine residue is buried deep within the center of the antigen-combining site. Calculated percent occupancy values for various sites can be found in **TABLE S1**.

Comparing the original N-terminal Aβ peptide binding pose in **FIGURE 4B** to the binding pose of the alternate central Aβ peptide in **FIGURE 4D, 4E, and 4F**, we see that most of these electrostatic contacts have been lost as the central Aβ peptide simply does not contain as many charged residues as the N-terminal peptide. However, there appear to be at least two potential binding interactions when the central Aβ peptide is bound to gantenerumab: E22 to R57 and F19 to F119 (both from the VFFAED portion of the epitope) appear to be close enough to interact in both structures. The percent occupancy for E3 to R57 of 100% when the N-terminal Aβ peptide is bound only drops to 85.58% for E22 to R57 when the central Aβ peptide is bound, and the percent occupancy for F4 to F119 of 96.12% when the N-terminal Aβ peptide is bound actually increased to 99.92% for F19 to F119 when the central Aβ peptide is bound. However, it is worth noting that D23 appears to be competing to some extent with E22 for occupancy near the R57 binding site as indicated by a noticeable 45.54% occupancy for D23 to R57 when the central Aβ peptide is bound. In our figures this competition can even be seen, as E22 is clearly bound to the pocket in **FIGURE 4E** whereas D23 is bound to that same site in **FIGURE 4F**.

Outside of these two interactions, it is noteworthy to point out that a very likely binding interaction for the N-terminal peptide between R5 and Y93, with a percent occupancy of 100% (see **FIGURE 4B**), is clearly lost in **FIGURE 4D, 4E, and 4F** as the possible corresponding interaction between K16 and Y93 for the central Aβ peptide has a 0% occupancy.
The above observations suggest that the VFFAED region of the alternate Aβ epitope still may form at least a few binding contacts with the gantenerumab antigen-combining site. Electrostatic contacts outside of this sequence appear to be lost or at the very least greatly altered in comparison. This is in qualitative agreement with experimental observations for gantenerumab, which display an epitope in the VFFAEDVGSN region, but do not display an epitope emerging from neighboring regions of the central epitope sequence that include the sequence HHQKL for instance\textsuperscript{42}. The overall positive, unstable calculated binding free energy that we obtained for the central Aβ peptide bound to gantenerumab is also consistent with the observation that gantenerumab is unable to bind and alter soluble Aβ levels in contrast to what was observed for solanezumab, which preferentially recognizes the central Aβ epitope\textsuperscript{19,42}.

The central Aβ peptide bound to crenezumab yielded a calculated binding free energy of -15.2 kcal/mol. After modeling in the alternate N-terminal peptide, we observed a slightly stable binding free energy value for one of our four homology models with the other three displaying positive, unstable binding free energy values. A slightly stable N-terminal binding free energy of -3.1 kcal/mol for crenezumab was obtained when the phenylalanine residue in the reverse N-terminal epitope, SDHRFEAD, was aligned with F20 in the original central Aβ epitope, HHQKLVFFAEDV, prior to model building. Binding in this reverse sense in the antigen-combining site of an anti-Aβ antibody has been noted before as solanezumab binds to the central Aβ peptide in this reverse sense compared to the orientation adopted by the N-terminal peptide in gantenerumab.\textsuperscript{53} The binding pose observed for this N-terminal peptide bound to crenezumab during the production portion of our MD simulation is shown in FIGURE 5C, 5D, 5E, and 5F. These may be compared to the original binding pose of the central Aβ peptide bound to
crenezumab which is shown in FIGURE 5A and 5B. Compared to the initial binding pose of the central Aβ peptide bound to crenezumab, it is seen that the binding site of crenezumab has had to distort considerably to bind to the N-terminal Aβ peptide in an alternate conformation.

The potential binding interactions for crenezumab binding to the alternate N-terminal Aβ peptide are qualitatively similar to what we saw before with gantenerumab binding to its alternate central Aβ peptide, but in general the percent occupancy for the various sites that we examined is usually higher for crenezumab. For comparison, the results of percent occupancy calculations are available in TABLE S2. The central phenylalanine interaction appears to stabilize the bound N-terminal Aβ peptide as seen by its lack of movement in FIGURE 5D, 5E, and 5F and its high percent occupancy in both structures. The percent occupancy is 99.0% for F19 to V94 when the central Aβ peptide is bound and is 100% for F4 to V94 when the N-terminal Aβ peptide is bound. The negatively charged E3 residue also appears to be attracted to a nearby binding pocket as seen in FIGURE 5E and 5F; the percent occupancy for E22 to N52 is 100% when the central Aβ peptide is bound and only drops to 92.28% for E3 to N52 when the N-terminal peptide is bound. In addition, the D23 to N53 percent occupancy of 36.94% when the central Aβ peptide is bound has actually increased to 86.3% for D1 to N53 when the N-terminal Aβ peptide is bound. Also, similar to the situation for gantenerumab, the positively charged residue in the alternate peptide does not stably bind to crenezumab. The percent occupancy of 100% for K16 to D101 when the central Aβ peptide is bound drops to 0% for R5 to D101 when the N-terminal Aβ peptide is bound.

In an attempt to understand why we were able to obtain a slightly stable binding free energy for the alternate epitope bound to crenezumab, it is noteworthy to point out the
electrostatic similarities between the N-terminal DAEFRHD epitope and the reverse sequence of the central epitope KLVFFAED, which when written out in reverse becomes DEAFFV Lak. When both sequences are compared in this way, it becomes apparent that the first four amino acids in the N-terminal sequence and the first four amino acids in the reverse central epitope sequence are very similar to each other, with DAEF and DEAF differing only by switching the internal positions of the alanine and glutamate residues.

However, despite this similarity, the inability to bind to key residues elsewhere in the sequence of the reverse epitope may explain why crenezumab achieves its preference for the central Aβ peptide experimentally and in terms of our MMPBSA binding free energy calculations. In particular, R5 in the N-terminal epitope is unable to bind to D101 in the same way that D101 binds to K16 in the central Aβ peptide as mentioned earlier. In FIGURE 5D, 5E, and 5F, R5 appears to be outside and above the antigen-combining site throughout our MD simulation whereas in FIGURE 5B K16 appears to be stably bound to the negative electrostatic region in the binding pocket. The inability for lysine and arginine residues to cross bind to the same binding pocket for both gantenerumab and crenezumab is likely due to a difference in the positioning of these residues in three-dimensional space, and our results suggest that this interaction may play a large role in determining binding preference for one epitope over the other. Comparing the sequences of the two epitopes, the arginine residue appears next to the DAEF sequence in the N-terminal epitope (DAEFRHDS) whereas the lysine residue appears three residues away from the corresponding FAED sequence in the central epitope (HHQKLVFFAEDV).

One additional observation that is worth mentioning is that crenezumab apparently has a puzzling ability to bind to both soluble monomers, using the central epitope of Aβ, and to
insoluble amyloid plaques where it is believed that only the N-terminal epitope is readily accessible.\textsuperscript{44-45} This behavior contrasts sharply with solanezumab which can only bind to soluble monomers using the central epitope of A\(\beta\) and not to fibril structures via the N-terminal epitope. Ultsch et al. suggested that the A\(\beta\) fibril species may have defects that expose the central A\(\beta\) epitope along the fiber axis to allow crenezumab to bind to it in a few locations. While this is certainly possible, the slightly stable MMPBSA binding affinity that we observed for binding the N-terminal peptide to crenezumab presents an alternative explanation for the observed cross binding to fiber species. A weak binding affinity for the N-terminal sequence would give crenezumab a chance to bind to fibril structures to some extent, and although crenezumab’s binding affinity for the fiber form would be lower than for the monomer or oligomer forms that bind via the central epitope, the high effective concentration of potential N-terminal binding sites along a fiber axis could still account for the sporadic fiber binding pattern observed by Ultsch et al.\textsuperscript{45} It should be noted that other possibilities for cross binding to fibers exist. Ma et al. has pointed out in a recent study that crenezumab can recognize the A\(\beta\) 13-16 epitope which may also be exposed in fibers to allow the antibody to bind.\textsuperscript{81}

**Improving the binding affinity of PFA1 to pE3-A\(\beta\)\textsubscript{3–8}**

Finally, with an eye towards the future, we wanted to see if we could use a visual inspection of our MD simulations to rationally plan out single amino acid mutations that improve the calculated MMPBSA binding free energy between an anti-A\(\beta\) antibody and a polymorphic A\(\beta\) species. Such a computational approach has been successfully demonstrated before\textsuperscript{82}, and it is a cost-effective way to probe for antibody mutations that could potentially improve the binding strength and specificity of an antibody for an additional A\(\beta\) target. The
most promising mutants that are identified may then be produced and tested in a laboratory to confirm the predicted improvement in binding affinity at a later point.

In order to illustrate this approach, we examined the question posed by Gardberg et al. in their study of pE3-Aβ3−8 bound to the anti-protofibril antibody, PFA141. As mentioned previously, the pE3-Aβ3−8 amyloid peptide was shown to bind to PFA1 with less affinity (K<sub>D</sub> = 3000 nM) than the wild type Aβ<sub>2−7</sub> peptide (K<sub>D</sub> = 60 nM), but pE3-Aβ3−8 is still considered to be dangerous due to its prominent presence in Alzheimer’s plaques41. A mutant antibody that can bind to both Aβ species with high affinity would be more desirable as a potential drug candidate.

To probe for such a mutant, we first ran a preliminary MD simulation of PFA1 bound to pE3-Aβ<sub>3−8</sub> and compared it to a MD simulation of PFA1 bound to Aβ<sub>1−8</sub> that had been used previously in our method validation. Of particular interest in the MD simulation for Aβ<sub>1−8</sub> was that the glutamate residue was found to be localized fairly well inside of its binding pocket as would be expected from analyzing the initial structure (see FIGURE 3). In contrast, the MD simulation for pE3-Aβ<sub>3−8</sub> revealed that the terminal pyroglutamate (PCA3) residue was engaged in a tug-of-war of sorts between the glutamate binding pocket and another nearby binding pocket that normally belongs to either alanine or aspartate.

Based on the MD simulations, we proposed that the difference in the observed binding affinity was caused by either: 1) the PCA3 residue in pE3-Aβ<sub>3−8</sub> lacking the full negative charge of the glutamate residue which may weaken its attraction to the glutamate binding pocket, or 2) the loss of alanine in pE3-Aβ<sub>3−8</sub> opening up the possibility for PCA3 to be attracted away from the glutamate binding pocket towards the other nearby binding pocket.
On these grounds, we introduced two sets of single amino acid substitution mutations into the binding pocket of PFA1, carried out MD simulations, and calculated the MMPBSA binding free energies between the mutant structures and the pE3-Aβ₃₋₈ peptide. The first two mutants, Y59A and N60A on the H chain, were designed to weaken the attraction of PCA3 towards the other nearby binding pocket. The next two mutants, S92K and H93K on the L chain, were designed to strengthen the positive electrostatic character in the glutamate binding pocket.

The MMPBSA results are given in TABLE 6, convergence plots for the calculated MMPBSA binding free energies are shown in FIGURE S6, and the results of percent occupancy calculations are available in TABLE S3. Two out of the four proposed mutants were able to lower the calculated binding free energy by an appreciable amount indicating that these mutations stabilized the bound structure. The calculated binding free energy for pE3-Aβ₃₋₈ bound to the N60A PFA1 mutant was -10.2 kcal/mol, and the calculated binding free energy for the Y59A PFA1 mutant was -8.4 kcal/mol. These were both more favorable than the binding free energy of -3.9 kcal/mol calculated for pE3-Aβ₃₋₈ bound to the PFA1 wildtype structure, and the binding free energies were comparable to the -14.3 kcal/mol and -10.4 kcal/mol values of the original wild type Aβ₁₋₈ and Aβ₂₋₇ peptides bound to PFA1 respectively.

Examining the MD trajectories (FIGURE 6) revealed that our N60A mutation worked out more or less as we expected. The PCA3 residue was now localized close to the two histidine residues near the glutamate binding pocket for the majority of the simulation time. For the N60A mutant, the percent occupancy for PCA3 to H27D of 97.9% and for PCA3 to H93 of 99.9% were large increases over the corresponding wildtype values of 19.7% and 44.0% respectively.
In a comparison to the MD trajectory for the pE3-Aβ3−8 peptide bound to the wildtype PFA1, the difference in stability between the two is readily apparent. In the wildtype simulation, the violent movement of the PCA3 residue actually pulled phenylalanine out of its pocket for an extended period of time in the first half of our production run (see FIGURE 6), leading to a low overall percent occupancy of 35.0% between F4 and L96. In the latter portion of the run, phenylalanine settled back into its pocket, and PCA3 engaged in the same tug of war behavior we had seen previously in our test run with an appreciable percent occupancy of 50.9% calculated for PCA3 to S58, near the location where the N60A mutation appeared. The percent occupancy for PCA3 to S58 was reduced to 8.6% in the N60A mutant.

In a somewhat similar way, our Y59A mutant appeared to stabilize PCA3 by reducing its movement, but this time the PCA3 residue localized more towards the other nearby binding pocket on the central right side as indicated by an increase in percent occupancy from 50.9% to 81.0% for PCA3 to S58 (FIGURE 6). There was still a fair amount of back and forth motion with the Y59A mutant, but, unlike in the N60A mutant, the PCA3 residue showed only a modest increase in its localization towards the H27D and H93 residues with percent occupancy values of 25.8% and 62.4% compared to 19.7% and 44.0% in the wildtype respectively.

The other two mutations did not improve the binding affinity of pE3-Aβ3−8 towards the mutant antibody. The S92K mutant had a calculated binding free energy of 5.3 kcal/mol that indicated a strong destabilization of the bound structure. Surprisingly, the bound structure visually looked to be quite stable, exhibiting no violent back and forth motion like that observed with pE3-Aβ3−8 bound to the wildtype PFA1. Its calculated percent occupancy values for the most part even showed increases that we were hoping to see. However, in the S92K mutant we
note that the PCA3 to H27D percent occupancy decreased considerably from 19.7% to 1.0% and also that the PCA3 to L96 percent occupancy increased from 17.3% to 76.1% indicating that perhaps an increase in percent occupancy at this particular site is disruptive to binding.

The H93K mutant was also destabilized as its calculated binding free energy of -2.7 kcal/mol was a bit lower than that of the wild type pE3-Aβ3−8 peptide bound to PFA1. Percent occupancy calculations showed that our H93K mutant failed to pull PCA3 residues towards the H27D and H93 locations as we intended. The PCA3 to H27D percent occupancy was lowered from 19.7% in the wildtype to 0% in the H93K mutant while the PCA3 to K93 (the position which was previously H93) was lowered from 44.0% in the wildtype to 9.8% in the H93K mutant. Analysis of the MD trajectory also revealed that the pE3-Aβ3−8 peptide had undergone drastic changes in its binding pose for the H93K mutant. The entire backbone of pE3-Aβ3−8 was flipped outwards toward the solvent, which allowed the PCA3 residue to move into the deep central binding pocket, displacing phenylalanine. The PCA3 to L96 percent occupancy increased to 100% for the H93K mutant compared to 17.3% in the wildtype.

When these observations are taken together, our results indicate that single amino acid mutations, when introduced at key locations on the PFA1 surface, can alter both the stability and orientation of the binding pose of the pE3-Aβ3−8 ligand in sometimes surprising and unpredictable ways. Nevertheless, even with a wide variety in the actual binding poses adopted by pE3-Aβ3−8 towards our PFA1 mutants, we were able to obtain improved MMPBSA binding affinities for two out of four mutant PFA1 structures based on simple predictive reasoning from visually observing our MD trajectories.
Given the rather large changes in binding pose and affinity for the pE3-Aβ_{3-8} peptide towards our PFA1 mutants, it is also reasonable to ask what changes might take place for the binding of the original wild type Aβ_{1-8} and Aβ_{2-7} peptides to our two successful mutant antibodies. This is an important issue if we wish to find a single antibody that is capable of binding with high affinity to multiple Aβ species. We therefore also examined MD simulations and calculated MMPBSA binding free energies for Aβ_{1-8} and Aβ_{2-7} bound to our N60A and Y59A mutant forms of PFA1. The binding free energies are given in **TABLE 6**, representative snapshots from the MD trajectories are shown in **FIGURE 7**, and the results of percent occupancy calculations for both wild type and mutant forms are available in **TABLE S4** and **S5** for Aβ_{1-8} and Aβ_{2-7} respectively.

For both mutants, the binding affinity for Aβ_{1-8} was actually improved over the wildtype PFA1 antibody. The calculated binding free energy for Aβ_{1-8} bound to the N60A PFA1 mutant was -18.0 kcal/mol, and the calculated binding free energy for the Y59A PFA1 mutant was -16.7 kcal/mol. One possible explanation for such an increase in binding affinity is that the percent occupancy of D1 to N27 increased to 99.9% in the N60A mutant and to 83.8% in the Y59A mutant compared to 79.1% in the wildtype. In contrast, both mutations slightly destabilized the binding of the Aβ_{2-7} peptide, perhaps because the D1 to N27 interaction is completely missing for this case. The calculated binding free energy for Aβ_{2-7} bound to the N60A PFA1 mutant was -8.3 kcal/mol, and the calculated binding free energy for the Y59A PFA1 mutant was -9.7 kcal/mol. Nevertheless, if we compare the computed binding free energies of the wild type PFA1 for Aβ_{1-8}, Aβ_{2-7}, and pE3-Aβ_{3-8} species to that of our N60A and Y59A mutants, we see that we have generally improved the affinity for our various Aβ species albeit
with a modest sacrifice in affinity for $A\beta_{2-7}$. In practice, a compromise between the binding of $A\beta$ species for a given antibody can be circumvented entirely by going beyond the single antibody approach. Instead, a cocktail of similar antibodies can be used to target various key $A\beta$ species in a treatment regime. In this case for instance, both wildtype and N60A mutant forms could be used together in a proposed treatment option to maximize effectiveness.

**Conclusion**

Anti-$A\beta$ antibodies are currently the most advanced treatment option on the horizon for patients suffering from AD. Several are currently undergoing clinical trials, and although the results for such antibodies thus far have proven disappointing, the recent, more positive clinical result reported for adacanumab indicate the potential for these drugs to be effective at clearing plaque burden and reducing cognitive decline. The main issues at present are the serious autoimmune side effects associated with the stronger binding anti-$A\beta$ antibodies and the presence of important polymorphic forms of $A\beta$ that may not bind to an antibody drug candidate with the same high affinity as its primary $A\beta$ target.

Although it remains a possibility to obtain new antibody drug candidates from standard drug screening procedures, these are expensive and time-consuming endeavors to undertake, and there is no way of knowing what impact the drug will have on a human population until a very late stage of drug development. Alternatively, there exists the possibility to rationally modify and improve current anti-$A\beta$ antibody drugs that have already undergone clinical trials and whose strengths and weaknesses as a drug candidate are at least somewhat understood.
Here, we have outlined a computational approach for studying the antigen-combining site of anti-Aβ antibodies. Our approach employs two computational techniques: 1) unbiased fragment-based docking, and 2) full molecular dynamics simulations accompanied by MMPBSA binding free energy calculations. Computational methods are a cost-effective way to study the binding properties of anti-Aβ antibodies whose crystal structures have previously been made available for analysis.

The fragment-based docking method provided us with a ligand-free way to dock single amino acid residues in an unbiased fashion to the surface of anti-Aβ antibodies. In doing so, three out of the four residues in the well-known Aβ EFRH epitope were found to be present in the top five binding amino acid residues for nine out of the ten antibody structures we studied, including the apo forms. In addition, phenylalanine emerged as a dominant interaction, displaying the most stable binding free energy and very consistently docking into the antigen-combining sites of all ten antibodies. This observation that phenylalanine is a central binding residue is corroborated by experimental observations from various crystallographers\textsuperscript{42, 45, 53, 80}.

While the fragment-based approach can give us some idea as to which residues might be important to the initial binding of Aβ peptides to anti-Aβ antibodies, it cannot capture the detailed characteristics of the binding pose for an extended peptide. On the other hand, analysis of MD simulations of holo structures that already contain extended peptides do not have this limitation. We used MD simulations to study a variety of questions posed by structural biologists in prior work. In their study of PFA1, Gardberg et al. brought up two interesting questions: 1) compared to the Aβ\textsubscript{2−7} peptide with sequence AEFRHD, why is the Grip1 (AKFRHD) mutation less severe than the Pos4 (AEIRHD) mutation?, and 2) why does pE3-Aβ\textsubscript{3−8}
bind to PFA1 with much lower affinity compared to Aβ_{2−7} when the observed binding poses in the two crystal structures are so similar to one another?

For the first question, our MD simulations indicated that the Grip1 mutant lost an important binding interaction when glutamate was mutated to lysine. However, the majority of the Aβ residues remained bound in their original pose following the mutation, so the effect on the overall binding affinity was modest. In contrast, the Pos4 mutant not only lost a key binding contact in the location where the mutation took place, but nearby alanine and glutamate residues were also forced to adopt new binding poses as the front half of the peptide was rotated away from its normal orientation in the binding pocket.

For the second question, we note that while the initial crystal structures had similar binding poses for Aβ_{2−7} and pE3-Aβ_{3−8} bound to PFA1, the trajectories in our MD simulations were not similar to each other at all. Whereas the glutamate at position 3 was very stably positioned in its binding pocket throughout our MD simulations of Aβ_{1−8} and Aβ_{2−7} bound to PFA1, the corresponding pyroglutamate residue in our MD simulation of pE3-Aβ_{3−8} bound to PFA1 was far less stably bound to the glutamate pocket. The pyroglutamate residue spent part of its time being pulled back and forth between the glutamate pocket and another nearby binding pocket, and also spent a large part of its time outside of the binding pocket entirely as phenylalanine was dislodged from its normal binding pocket for an extended period of time during our production run of pE3-Aβ_{3−8} bound to wildtype PFA1 before eventually returning.

In addition, we also wanted to explore two questions concerning the binding of different Aβ epitopes/species to the same antibody: 1) how might antibodies such as gantenerumab and crenezumab bind to both the hydrophilic N-terminal and the hydrophobic central Aβ
epitopes?, and 2) can we use knowledge gained from visually observing MD trajectories to design rational mutations into PFA1 that can improve its binding affinity towards a polymorphic variant of Aβ such as pE3-Aβ?

For gantenerumab and crenezumab, we ran MD simulations and calculated MMPBSA binding free energy values using the Aβ peptides that were bound in their original crystal structures, and we also generated homology models of peptides containing the other relevant Aβ epitope in various orientations and initial alignments for the same analysis. Consistent with experimental results, our MMPBSA results show that gantenerumab and crenezumab greatly prefer binding to the Aβ peptide with the epitope observed in their original crystal structures. However, crenezumab also showed weak, stable binding to the alternate epitope when it was aligned in a reverse orientation across the antigen-combining site. The possibility that crenezumab can cross bind to both N-terminal and central Aβ epitopes may be related to aducanumab’s reported ability to bind to both oligomeric and fibril Aβ species.

Finally, we introduced rational mutations into the PFA1 antibody in an attempt to improve its binding affinity towards the pE3-Aβ species of Aβ. The approach that we have taken here illustrates the potential for computational analysis to assist in the rational design and improvement of anti-Aβ antibodies. Given the high conformational flexibility of both the Aβ peptide and the antibody itself, predicting useful mutations from static crystal structures would be difficult, if not impossible, to do. On the other hand, visual inspection of the MD simulations of the amyloid-antibody complex allowed us to rationally identify potential mutation hot spots on the antibody surface with relative ease. We then calculated MMPBSA binding free energies to test the impact of our four mutations compared to the wild type, and
two out of the four mutants were shown to stabilize the binding of pE3-Aβ_{3-8} to PFA1. Other possible mutations may exist that can impact the binding in a similar or even better way. If a computational pre-screening approach such as this is carried out, it should produce a list of potential hot spot mutations that can then be tested in the lab to confirm the predicted effectiveness. If the results from the lab correlate with the predicted computational results, an improved drug candidate with a greater potential to treat AD can subsequently be developed.

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### Tables

| RANK | PFA1 | PFA1 (apo) | PFA2 | PFA2 (apo) | BAPI | SOLA | GANT | CREN | CREN (apo) | PONE |
|------|------|-----------|------|-----------|------|------|------|------|-----------|------|
| 1    | -5.9(F) | -6.1(F) | -6.4(F) | -6.3(Y) | -6.2(Y) | -5.9(Y) | -6.1(Y) | -5.8(F/Y) | -6.2(Y) |
| 2    | -5.8(Y) | -6.1(Y) | -5.9(Y) | -6.1(F) | -5.9(F) | -5.6(F) | -5.7(F) | -5.9(F) | -4.9(H) | -6.0(F) |
| 3    | -5.0(R) | -5.2(R) | -5.1(R) | -5.2(H) | -5.2(H) | -5.2(H) | -4.8(K) | -5.3(H) | -4.7(R/Q) | -5.3(H) |
| 4    | -4.8(Q) | -4.9(H) | -5.1(H) | -4.9(Q) | -5.1(E) | -5.0(R) | -4.7(I) | -5.2(R) | -4.6(L) | -4.8(D/E/L) |
| 5    | -4.6(H) | -4.8(E/N/Q) | -4.8(E/Q) | -4.8(E) | -5.0(D) | -4.7(K) | -4.6(Q/R) | -4.7(E/Q) | -4.5(E) | -4.7(I/K/Q) |
| 6    | -4.5(E/N) | -4.7(D) | -4.6(D/L) | -4.6(I) | -4.9(R) | -4.6(Q) | -4.5(D/E/H/L/V) | -4.6(N) | -4.4(N/K/I) | -4.5(V) |
| 7    | -4.4(D/L) | -4.6(I/L) | -4.5(I) | -4.5(L/N) | -4.8(N) | -4.5(E) | -4.0(M) | -4.5(L) | -4.3(D/V) | -4.2(R) |
| 8    | -4.2(I) | -4.2(V) | -4.3(K) | -4.4(D/K) | -4.7(I) | -4.4(I/L) | -3.9(N) | -4.4(V) | -4.1(M) | -4.0(M) |
| 9    | -3.9(V) | -4.1(K) | -4.1(V) | -4.3(R/V) | -4.6(L) | -4.1(N/V) | -3.7(S) | -4.3(D/I) | -4.0(S) | -3.9(S) |
| 10   | -3.8(M) | -3.9(S) | -4.0(M/S) | -4.2(M) | -4.5(K) | -3.9(D) | -3.4(A) | -4.2(M) | -3.6(A) | -3.6(A) |

**TABLE 1. The top binding affinities for amino acid residues bound to the antigen-combining site of anti-Aβ antibodies.** Binding free energies are given in units of kcal/mol while the corresponding amino acid is indicated by using the standard single-letter amino acid code. All of the structures given are based on the holo crystal structure form of the antibody except for those marked “(apo)” which are based on the apo crystal structure. Antibody abbreviations: PFA1 = protofibril antibody 1, PFA2 = protofibril antibody 2, BAPI = bapineuzumab, SOLA = solanezumab, GANT = gantenerumab, CREN = crenezumab, PONE = ponezumab.
### TABLE 2. The number of docked structures found at the antigen-combining site of anti-\(\beta\) antibodies.

The number of docked structures for each amino acid residue that were found in the antigen-combining site of the antibody are reported in the table above. Each amino acid is indicated by using the standard single-letter amino acid code. Antibody abbreviations: PFA1 = protofibril antibody 1, PFA2 = protofibril antibody 2, BAPI = bapineuzumab, SOLA = solanezumab, GANT = gantenerumab, CREN = crenezumab, PONE = ponezumab.
### Tables

| Complex Structure     | MMPBSA | SEM | Experimental |
|-----------------------|--------|-----|--------------|
| PFA1 (Aβ₁₋₈)         | -14.3  | 0.1 | -10.2**      |
| PFA1 (Aβ₂₋₇)         | -10.9  | 0.1 | -9.7*        |
| PFA1 (pE3-Aβ₃₋₈)     | -3.9   | 0.1 | -7.6         |
| PFA1 (Grip1)          | -6.7   | 0.1 | -7.5         |
| PFA1 (Ror2)           | -17.7  | 0.1 | -10.5        |
| PFA2 (Aβ₁₋₈)         | -14.0  | 0.1 | -10.4**      |
| PFA2 (Aβ₂₋₇)         | -9.5   | 0.1 | -9.1*        |
| PFA2 (pE3-Aβ₃₋₈)     | -4.0   | 0.1 | -6.7         |
| PFA2 (Grip1)          | -1.4   | 0.1 | -6.9         |
| PFA2 (Ror2)           | -10.8  | 0.1 | -9.7         |

**TABLE 3.** Method validation of our MMPBSA calculations for Aβ peptides bound to PFA1 and PFA2. The results of MMPBSA binding free energy calculations and experimental values are given in units of kcal/mol. Experimental values were taken from Gardberg et. al. and were converted to free energy values as described in Materials and Methods. * denotes cases where the experimental binding affinity values were reported as a range, and we used the average value of that range as the experimental binding affinity. ** denotes the experimental value given for Aβ₁₋₄₀ binding to both PFA1 and PFA2. The SEM is the standard error of the mean given in units of kcal/mol.
TABLE 4. The importance of phenylalanine in the binding of $A\beta_{2-7}$ to PFA1. The results of MMPBSA binding free energy calculations, in units of kcal/mol, are given to demonstrate the severity of the phenylalanine Pos4 mutation (AEIRHD) in the binding of $A\beta_{2-7}$ to the antibody PFA1. The less severe Grip1 mutation (AKFRHD) was also included for comparison. The crystal structure of $A\beta_{2-8}$ bound to PFA1 (PDB ID: 2IPU) was used both for constructing the wildtype $A\beta_{2-7}$ peptide and for generating the homology models of the two other $A\beta$ peptide mutants. The SEM is the standard error of the mean given in units of kcal/mol. Experimental values were taken from Gardberg et al.\textsuperscript{41,80} and were converted to free energy values as described in Materials and Methods.
### Tables

| Complex Structure                        | MMPBSA | SEM | Experimental |
|-----------------------------------------|---------|-----|--------------|
| gantenerumab (DAEFRHDSGYE)              | -33.8   | 0.1 | -10.7        |
| gantenerumab forward (HHQKLVFFAEDV)    | 1.8     | 0.1 | -            |
| gantenerumab forward 2 (HHQKLVFFAEDV)  | 4.7     | 0.1 | -            |
| gantenerumab reverse (VDEAFFVLKQHH)    | 6.0     | 0.1 | -            |
| gantenerumab reverse 2 (VDEAFFVLKQHH)  | 24.1    | 0.1 | -            |
| crenezumab (EVHHQKLVVFAEDVG)           | -15.2   | 0.1 | -11.7        |
| crenezumab forward (DAEFRHDS)           | 2.3*    | 0.2*| -            |
| crenezumab forward 2 (DAEFRHDS)         | 5.3     | 0.1 | -            |
| crenezumab reverse (SDHRFEAD)           | 14.9    | 0.2 | -            |
| crenezumab reverse 2 (SDHRFEAD)         | -3.1    | 0.1 | -            |

**TABLE 5.** MMPBSA binding free energy results for N-terminal and central Aβ peptides bound to gantenerumab and crenezumab. The crystal structures of N-terminal Aβ (DAEFRHDSGYE) bound to gantenerumab (PDB ID: 5CSZ) and central Aβ (EVHHQKLVVFAEDVG) bound to crenezumab (PDB ID: 5VZY) were used both to calculate the MMPBSA binding free energy for the crystal structure-based complexes and for generating the four homology models of the transposed epitopes for each. The colored type for phenylalanine reveals the residue that was used to line up the phenylalanine residue in each homology model to the phenylalanine residues(s) in the original crystal structure (residues that are colored the same indicate the phenylalanine residue that was used for the alignment). Experimental binding free energies are given for the antibody structures bound to the monomer form of Aβ42, 45. The results of MMPBSA binding free energy calculations are given in units of kcal/mol. The SEM is the standard error of the mean given in units of kcal/mol. The * values are based on a 60 ns MMPBSA calculation, as opposed to the standard 50 ns MMPBSA calculation, due to slow convergence (see **FIGURE S5**).
### TABLE 6. MMPBSA binding free energy results for $A\beta_{1-8}$, $A\beta_{2-7}$, and $pE3-A\beta_{3-8}$ bound to the wildtype and several mutant forms of PFA1.

The crystal structure of $pE3-A\beta_{3-8}$ bound to PFA1 (PDB ID: 3EYS) was used both for the wildtype $pE3-A\beta_{3-8}$-PFA1 complex and for generating the homology models of the four $pE3-A\beta_{3-8}$-PFA1 mutant complexes. The crystal structure of $A\beta_{2-8}$ (PDB ID: 2IPU) was used to generate the homology models of the $A\beta_{1-8}$ and $A\beta_{2-7}$ peptides bound to the N60A and Y59A mutant forms of PFA1. The results of MMPBSA binding free energy calculations are given in units of kcal/mol. The SEM is the standard error of the mean given in units of kcal/mol.

| Complex Structure                        | MMPBSA | SEM |
|------------------------------------------|---------|-----|
| $A\beta_{1-8}$-PFA1 wildtype             | -14.3   | 0.1 |
| $A\beta_{2-7}$-PFA1 wildtype             | -10.9   | 0.1 |
| $pE3-A\beta_{3-8}$-PFA1 wildtype         | -3.9    | 0.1 |
| $pE3-A\beta_{3-8}$-PFA1 mutant (Y59A (H chain)) | -8.4 | 0.1 |
| $pE3-A\beta_{3-8}$-PFA1 mutant (N60A (H chain)) | -10.2 | 0.1 |
| $pE3-A\beta_{3-8}$-PFA1 mutant (S92K (L chain)) | 5.3  | 0.1 |
| $pE3-A\beta_{3-8}$-PFA1 mutant (H93K (L chain)) | -2.7 | 0.1 |
| $A\beta_{1-8}$-PFA1 mutant (N60A (H chain)) | -18.0 | 0.1 |
| $A\beta_{2-7}$-PFA1 mutant (N60A (H chain)) | -8.3  | 0.1 |
| $A\beta_{1-8}$-PFA1 mutant (Y59A (H chain)) | -16.7 | 0.1 |
| $A\beta_{2-7}$-PFA1 mutant (Y59A (H chain)) | -9.7  | 0.1 |
FIGURE 1. Method validation for MMPBSA free energy calculations of various Aβ peptides bound to the antibodies PFA1 and PFA2. The original PFA1, PFA2, and pE3-Aβ3–8 crystal structures (PDB IDs: 2IPU, 2R0W, and 3EYS) served as the basis for constructing homology models for all the other bound peptide structures. Our calculated MMPBSA binding free energy values were compared to the experimental binding affinity values reported by Gardberg et al. for their entire data set. All free energy values are given in units of kcal/mol.
FIGURE 2. Comparison of the antigen-combining sites for Aβ$_{2-7}$ peptide variants bound to PFA1. Three Aβ peptides are shown bound to PFA1: (A) Aβ$_{2-7}$, (B) Grip1, and (C) the Pos4 mutant. All three images were taken at the halfway point of the production portion of the MD simulation.
FIGURE 3. Electrostatic potential surface for Aβ₁₋₈ bound to PFA1. A surface map of PFA1 which shows the key electrostatic contacts made between the PFA1 antibody and charged residues in Aβ₁₋₈. Negatively charged regions are depicted in red while positively charged regions are shown in blue.
**Figures**

**FIGURE 4. Gantenerumab bound to both N-terminal and central Aβ peptides.** Gantenerumab is shown bound to the Aβ peptide containing the N-terminal epitope (PDB ID: 5CSZ) in the first frame of the MD simulation in (A) and (B). Structures (C), (D), (E), and (F) show the most stable central Aβ peptide bound to gantenerumab in the forward sequence (HHQKLVFFAEDV) across the gantenerumab antigen-combining site taken from the first frame (C and D), the middle frame (E), and the last frame (F) of the MD production run. In all structures, the N-terminus end of the peptide appears towards the bottom of the antigen-combining site while the C-terminus appears near the top. Specific electrostatic regions of both the peptide and antibody are color-coded with the positive regions colored blue while the negative regions are colored red.
Figures

**FIGURE 5. Crenezumab bound to both central and N-terminal Aβ peptides.** Crenezumab is shown bound to the Aβ peptide containing the central epitope (PDB ID: 5VZY) in the first frame of the MD simulation in (A) and (B). Structures (C), (D), (E), and (F) show the N-terminal Aβ peptide bound to crenezumab in the reverse sense (SDHRFEAD) across the crenezumab antigen-combining site as observed in the first frame (C and D), the middle frame (E), and the last frame (F) of the MD production run. In structures (A) and (B), the N-terminus end of the peptide appears towards the bottom of the antigen-combining site while the C-terminus appears near the top. For (C), (D), (E), and (F), the N-terminus end appears towards the upper left of the antigen-combining site while the C-terminus end appears towards the lower right of the antigen-combining site. Specific electrostatic regions of both the peptide and antibody are color-coded with the positive regions colored blue while the negative regions are colored red.
FIGURE 6. Snapshots from the MD trajectories of pE3-Aβ_{3-8} bound to wild type and mutant forms of PFA1. The structures here are visualized as snapshots taken at 10ns, 30 ns, and 50 ns during the production run of the MD simulation.
Figures

FIGURE 7. Snapshots from the MD trajectories of Aβ₁⁻８ and Aβ₂⁻⁷ bound to wild type and mutant forms of PFA1. The structures here are visualized as snapshots taken at 10ns, 30 ns, and 50 ns during the production run of the MD simulation.
Supporting Information

Analysis of Anti-Amyloid Beta (A\(\beta\)) Antibodies Using Fragment-Based Docking and MMPBSA Binding Free Energy Calculations

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|        | Aβ residue | gant residue | Chain | % Occupancy |
|--------|------------|--------------|-------|-------------|
| gant   | R5         | Y93          | L     | 100         |
| gant   | D7         | N94          | L     | 94.2        |
| gant   | E3         | R57          | H     | 100         |
| gant   | E11        | R57          | H     | 72.3        |
| gant   | F4         | F113         | H     | 96.1        |
| gant (forward) | K16        | Y93          | L     | 0.0         |
| gant (forward) | E22        | N94          | L     | 65.4        |
| gant (forward) | D23        | R57          | H     | 45.5        |
| gant (forward) | E22        | R57          | H     | 85.6        |
| gant (forward) | F19        | F113         | H     | 99.9        |

**TABLE S1.** Percent occupancy results for N-terminal and central Aβ peptides bound to gantenerumab. The residue-to-residue percent occupancy was calculated between each pair of residues using a 10 Å cutoff over 5000 frames collected at equally spaced intervals from the 50 ns production run. Aβ residues were labeled to match the standard Aβ₁⁻⁴² numbering sequence while gantenerumab residues and chain designations were labeled to match the original PDB file (PDB ID: 5CSZ). The N-terminal Aβ peptide was bound in its original pose from the crystal structure in gant while the most stable central Aβ peptide bound to gantenerumab (1.8 kcal/mol) was used for comparison in gant (forward).
### Tables

| Aβ residue | cren residue | Chain | % Occupancy |
|------------|--------------|-------|-------------|
| cren       | E11          | R54   | L           | 100          |
| cren       | F19          | V94   | L           | 99.0         |
| cren       | E22          | N52   | H           | 100          |
| cren       | E22          | N53   | H           | 100          |
| cren       | D23          | N52   | H           | 100          |
| cren       | D23          | N53   | H           | 36.9         |
| cren (reverse) | K16      | D101  | H           | 100          |
| cren (reverse) | D8        | N28   | L           | 100          |
| cren (reverse) | H6        | Y27D  | L           | 100          |
| cren (reverse) | F4        | V94   | L           | 100          |
| cren (reverse) | D1        | N52   | H           | 100          |
| cren (reverse) | D1        | N53   | H           | 86.3         |
| cren (reverse) | E3        | N52   | H           | 92.3         |
| cren (reverse) | E3        | N53   | H           | 1.5          |
| cren (reverse) | R5        | D101  | H           | 0.0          |

**TABLE S2.** Percent occupancy results for N-terminal and central Aβ peptides bound to crenezumab. The residue-to-residue percent occupancy was calculated between each pair of residues using a 10 Å cutoff over 5000 frames collected at equally spaced intervals from the 50 ns production run. Aβ residues were labeled to match the standard Aβ<sub>1-42</sub> numbering sequence while crenezumab residues and chain designations were labeled to match the original PDB file (PDB ID: 5VZY). The central Aβ peptide was bound in its original pose from the crystal structure in cren while the most stable N-terminal Aβ peptide bound to crenezumab (-3.1 kcal/mol) was used for comparison in cren (reverse).
### Tables

| pE3-AB 3-8 | Aβ residue | PFA1 residue | Chain | % Occupancy (WT) | % Occupancy (N60A) | % Occupancy (Y59A) | % Occupancy (H93K) | % Occupancy (S92K) |
|------------|------------|--------------|-------|------------------|-------------------|-------------------|-------------------|-------------------|
| pE3-AB 3-8 | PCA3       | H27D         | L     | 19.7             | 97.9              | 25.8              | 0                 | 1.0               |
| pE3-AB 3-8 | H6         | H27D         | L     | 74.9             | 100               | 100               | 98.3              | 99.9              |
| pE3-AB 3-8 | H6         | Y32          | L     | 62.5             | 100               | 100               | 100               | 100               |
| pE3-AB 3-8 | H6         | S92          | L     | 32.2             | 100               | 100               | 100               | 100               |
| pE3-AB 3-8 | PCA3       | H93          | L     | 44.0             | 99.9              | 62.4              | 9.8               | 98.2              |
| pE3-AB 3-8 | PCA3       | L96          | L     | 17.3             | 7.1               | 22.4              | 100               | 76.1              |
| pE3-AB 3-8 | F4         | L96          | L     | 35.0             | 100               | 100               | 100               | 100               |
| pE3-AB 3-8 | R5         | D54          | H     | 100              | 100               | 100               | 100               | 100               |
| pE3-AB 3-8 | PCA3       | S58          | H     | 50.9             | 8.6               | 81.0              | 100               | 76.6              |

**TABLE S3.** Percent occupancy results for pE3-Aβ<sub>3–8</sub> bound to PFA1 wild type and mutant antibodies. The residue-to-residue percent occupancy was calculated between each pair of residues using a 10 Å cutoff over 5000 frames collected at equally spaced intervals from the 50 ns production run. Aβ residues were labeled to match the standard Aβ<sub>1–42</sub> numbering sequence while PFA1 residues and chain designations were labeled to match the original PDB file (PDB ID: 3EYS).
**Tables**

| Aβ residue | PFA1 residue | Chain | % Occupancy (WT) | % Occupancy (N60A) | % Occupancy (Y59A) |
|------------|--------------|-------|------------------|---------------------|---------------------|
| Aβ 1-8     | D1           | L     | 79.1             | 99.9                | 83.8                |
| Aβ 1-8     | E3           | H27D  | 100              | 100                 | 100                 |
| Aβ 1-8     | H6           | H27D  | 100              | 100                 | 100                 |
| Aβ 1-8     | H6           | Y32   | 100              | 100                 | 100                 |
| Aβ 1-8     | H6           | S92   | 100              | 100                 | 100                 |
| Aβ 1-8     | E3           | H93   | 100              | 100                 | 100                 |
| Aβ 1-8     | E3           | L96   | 0.1              | 0                   | 0                   |
| Aβ 1-8     | F4           | L96   | 100              | 100                 | 100                 |
| Aβ 1-8     | R5           | D54   | 100              | 100                 | 100                 |
| Aβ 1-8     | E3           | S58   | 0                | 0                   | 0                   |

**TABLE S4.** Percent occupancy results for Aβ₁₋₈ bound to PFA1 wild type and mutant antibodies. The residue-to-residue percent occupancy was calculated between each pair of residues using a 10 Å cutoff over 5000 frames collected at equally spaced intervals from the 50 ns production run. Aβ residues were labeled to match the standard Aβ₁₋₄₂ numbering sequence while PFA1 residues and chain designations were labeled to match the original PDB file (PDB ID: 2IPU).
### Tables

| Aβ Residue | PFA1 Residue | Chain | % Occupancy (WT) | % Occupancy (N60A) | % Occupancy (Y59A) |
|------------|--------------|-------|------------------|--------------------|--------------------|
| Aβ 2-7     | E3           | L     | 100              | 100                | 100                |
| Aβ 2-7     | H6           | L     | 100              | 100                | 100                |
| Aβ 2-7     | H6           | L     | 100              | 100                | 100                |
| Aβ 2-7     | H6           | L     | 100              | 100                | 100                |
| Aβ 2-7     | E3           | L     | 100              | 100                | 100                |
| Aβ 2-7     | E3           | L     | 0.1              | 0                  | 0                  |
| Aβ 2-7     | F4           | L     | 100              | 100                | 100                |
| Aβ 2-7     | R5           | D     | 100              | 100                | 100                |
| Aβ 2-7     | E3           | S      | 0                | 0                  | 0                  |

**TABLE S5.** Percent occupancy results for Aβ<sub>2-7</sub> bound to PFA1 wild type and mutant antibodies. The residue-to-residue percent occupancy was calculated between each pair of residues using a 10 Å cutoff over 5000 frames collected at equally spaced intervals from the 50 ns production run. Aβ residues were labeled to match the standard Aβ<sub>1-42</sub> numbering sequence while PFA1 residues and chain designations were labeled to match the original PDB file (PDB ID: 2IPU).
**Figures**

**FIGURE S1.** Convergence plots of MMPBSA calculations for various $\alpha\beta$ peptides bound to the antibodies PFA1 and PFA2. Cumulative MMPBSA binding free energies were calculated at each 1 ns time step during the 50 ns production run of the MD simulation in order to validate our MD protocol.
FIGURE S2. MMPBSA convergence plot for the production run of $\text{A}\beta_{1-8}$ bound to PFA1. The convergence for $\text{A}\beta_{1-8}$ bound to PFA1 appeared questionable in FIGURE S1, and so we extended the total simulation time to 310 ns and ran a 60 ns MMPBSA calculation to verify that our result had indeed converged. The MMPBSA binding free energy for $\text{A}\beta_{1-8}$ bound to PFA1 is shown to converge just before 50 ns.
FIGURE S3. RMSD plots for Aβ<sub>2−7</sub> peptides bound to PFA1. The Grip1 and Pos4 mutants of Aβ<sub>2−7</sub> both have larger RMSD values on average in comparison to PFA1 bound to the normal Aβ<sub>2−7</sub> peptide. The RMSD plots shown above comprise the entire 50 ns production run and were calculated with reference to the initial structure/frame of the 300 ns MD simulation. The RMSD values are reported in units of Angstroms (Å).
FIGURE S4. Convergence plots of MMPBSA calculations for N-terminal and central Aβ peptides bound to gantenerumab and crenezumab. Cumulative MMPBSA binding free energies were calculated at each 1 ns time step during the 50 ns production run of the MD simulation.
**Figures**

**FIGURE S5.** MMPBSA convergence plot for the production run of cren(forward), an N-terminal Aβ peptide bound to crenezumab. The convergence for cren (forward) was very slow (see FIGURE S4), and so we extended the total simulation time to 310 ns and ran a 60 ns MMPBSA calculation to improve the convergence for this data point as shown above.
**FIGURE S6.** Convergence plots of MMPBSA calculations for $\alpha\beta_{1-8}$, $\alpha\beta_{2-7}$, and pE3-$\alpha\beta_{3-8}$ bound to wild type and mutant forms of PFA1. Cumulative MMPBSA binding free energies were calculated at each 1 ns time step during the 50 ns production run of the MD simulation.