

Immunoinformatics Analysis Reveals Potential Immunogenic Cores of Experimentally Validated B-Cell Epitopes of the Human Amyloid-Beta Precursor Protein

Michelle Anne C. Duño, Arturo L. Gaitano III*

Immunoinformatics and Novel Synthetic Peptide Immunogens Research (INSPIRe) Group, Chemistry Department, College of Science, Adamson University, 900 San Marcelino Street, Ermita, Manila, 1000

*Author to whom correspondence should be addressed; email: arturo.gaitano.research@gmail.com

ABSTRACT

Synthetic peptide immunogens based on B-cell epitopes from protein antigens are key to peptide vaccine design. Experimentally validated epitopes can be quite long, and not all residues might be significant for binding. Immunogenic cores can be identified so synthesis will focus on the relevant amino acid sequences. An immunoinformatics approach was done using online servers such as AllerTOP v.2, VaxiJen, IEDB, and Protein-Sol to evaluate the allergenicity, antigenicity, flexibility, accessibility, hydrophilicity, and solubility of B-cell epitope sequences of HAPP (Human Amyloid-Beta Precursor Protein). The immunogenic sequences underwent docking using GalaxyPepDock and validation using MolProbity, while PRODIGY was used to determine the binding energies of the identified sequences with select antibodies. Five immunogenic cores were determined: ⁶⁸²EVHH⁶⁸⁵ was identified via structure similarity, ⁶⁷⁵FRHDSGY⁶⁸¹ and ⁶⁹⁴DVGSNKG⁷⁰⁰ via flexibility analysis, and ⁶⁷⁵FRHDSGYEVHH⁶⁸⁵ and ⁶⁹⁴DVGSNK⁶⁹⁹ identified via surface accessibility. All immunogenic cores exhibited spontaneous binding with antibodies, and also demonstrated comparable or even increased binding strength compared with the source epitope. These findings suggest that there are potential immunogenic cores found in HAPP, which can be potentially used for more efficient peptide vaccine design.

Keywords: HAPP, immunoinformatics, Alzheimer's Disease, immunogenic core, B-cell epitopes

INTRODUCTION

Dementia, as a disease, is characterized by a decline in the cognitive function and performance of a person to the point of interfering with their ability to fulfill their usual activities (Haque and Levey 2019). This condition can be characterized as either Alzheimer's Disease (AD) dementia, or all cause dementia, which covers all other types of dementia. AD represents the majority of dementia cases, and its frequency increases with age (Barker et al. 2002). Currently, the leading hypothesis explaining its pathophysiology is the formation of neurotoxic plaques from the amyloid β -protein ($A\beta$ hypothesis) caused by the imbalance between production and clearance of $A\beta_{42}$ and other related $A\beta$ proteins (Selkoe and Hardy 2016). Thus, the promotion of clearance of this protein is a potential therapeutic intervention for AD.

The sequential cleavage of the human amyloid- β precursor protein (HAPP) by β -secretase and γ -secretase results in the formation of the $A\beta$ peptide (Abubakar et al. 2022). All known dominant mutations that cause early-onset AD are found either in the protease (presenilin) of the reaction that generates $A\beta$ or on the substrate itself (HAPP) (Selkoe and Hardy 2016). As such, one way by which early-onset AD may be prevented is via the elimination of cells in the circulatory system (peripheral sink) in which HAPP is overexpressed in order to promote $A\beta$ clearance in both the brain interstitial fluid (central sink) and the cerebrospinal fluid (CSF sink), following the "three-sink therapeutic strategy" (Schreiner et al., 2022).

Clearance of HAPP, $A\beta$, and $A\beta$ related peptides may be achieved via small molecules and similar therapies (Cheng et al., 2020) or via immunotherapy (Song et al. 2022). Immunotherapy against Alzheimer's may be achieved via peptide-based vaccine constructs (Malonis et al., 2020) that elicit a more complete immune response involving T-cells and B-cells, or via the use of monoclonal antibodies produced by B-cells that can slow down the progression of AD and promote $A\beta$ clearance (Qiu et al. 2023). Eliciting the production of these antibodies requires the activation of B-cells via the interaction of B-cell receptors with specific sequences within antigens known as epitopes (Akbar et al. 2021). These epitopes, despite being part of the entire antigenic sequence, can be synthesized into discrete peptides that still elicit the same response as the whole protein in its native state (Camacho, et.al., 2008). This provides the groundwork for the use of epitopes in peptide-based vaccines and other peptide immunogens. B-cell epitopes, upon eliciting antibody production, eventually bind to complementary sequences in the antibody known as paratopes (Akbar et al. 2021).

B-cell epitopes have traditionally been identified by empirical means, particularly via immunoassays, and are archived thereafter in the Immune Epitope Database (IEDB) (Martini et al. 2020). The experimentally validated epitopes stored in this database have varying lengths, and not every residue found within them might be responsible for the observed immune response, because some sequences are more immunodominant than others (Abbott and Crotty 2020). This means that certain sequences are better at eliciting an immune response and may already be enough to elicit the production of antibodies. The synthesis and use of these sequences for immunotherapy and immunodiagnostics in lieu of their source epitopes could prove more efficient. In this study, we define such sequences as immunogenic cores.

Technological progress has enabled computer-aided tools to become widespread in biochemical research. Computational tools have aided rational and efficient drug design (Macalino et al. 2015), elucidated mechanisms on the actions of peptide immunogens (Duay et al. 2023), and enabled epitope mapping and vaccine design via immunoinformatics (De Groot et al. 2020). The use of these *in silico* tools have accelerated many aspects of research on different kinds of therapeutics and allows for the more efficient use of time and resources before experiments are carried out *in vitro*, *ex vivo*, and *in vivo*.

This study provides a biochemical exploration on the potential immunogenic cores found in HAPP via an immunoinformatics approach. Identified immunogenic cores are verified via molecular modelling and docking. These immunogenic cores should have comparable binding energy and dissociation constants with their source epitopes and could eventually provide a basis for in vivo immunogenicity using only those sequences.

METHODS

Materials and Software. The entirety of this study was done in silico using freely available and accessible webserver. Experimentally validated peptide epitopes were obtained from the Immune Epitope Database (IEDB) by using the search query “Linear Peptide + B-cell Positive Assays + Alzheimer’s Disease (ID: 10652). Focus was given on epitopes from HAPP (UniProt: P05067). The resulting peptide epitopes underwent further immunoinformatics analysis.

Testing for Allergenicity. Potential peptide immunogens for eventual vaccine design need to be non-allergenic in order to prevent severe side-effects. The potential allergenicity of candidate peptides was tested via AllerTOP v2.0 (Dimitrov et al. 2014) a webserver that determines allergenicity via comparison of the structure and sequence of a given peptide with known allergens. Peptides that are deemed allergenic did not undergo further analysis.

Testing for Water Solubility. Solubility in aqueous systems is necessary for potential peptide immunogens that will be used in physiological environments. Water solubility of these potential peptide immunogens was evaluated via Protein-Sol (Hebditch et al. 2017), a webserver that determines both the water solubility and isoelectric point of a peptide given its sequence. Peptides that are not soluble in water and have an isoelectric point equivalent to the physiological pH (7.35-7.45) (Hopkins, et. al., 2024) did not undergo further testing.

Identification of Potential Immunogenic Cores. There is a total of six linear B-cell prediction software found on the IEDB webserver, and another software found on a different webserver. Broadly, the algorithms they employ to determine immunogenicity can be grouped into three. The first group relies on structure similarity with known antigens. The second group utilizes flexibility analysis to determine immunogenic sequences, and the last group identifies immunogenicity via analysis of water accessibility. The remaining peptide epitopes that are non-allergenic and water-soluble were analyzed using these webserver to determine the potential immunogenic cores within each epitope by finding the consensus sequences in each group. In all of these tools, the default settings found in the webserver were used.

Via Structure Similarity. There are 3 algorithms that utilize structure similarity with known antigens to determine the antigenicity of a peptide sequence. These are: VaxiJen (Doytchinova and Flower 2007), BepiPred 2.0 (Jespersen et al. 2017), and Kolaskar-Tongaonkar Antigenicity Prediction (Kolaskar and Tongaonkar 1990).

Via Flexibility Analysis. Flexibility is an important parameter that determines the potential antigenicity of a given sequence since it provides the ability to adopt different conformations necessary for binding. This can be measured by the Chou & Fasman Beta-Turn Prediction Tool (Chou and Fasman 2006) and the Karplus & Schulz Flexibility Prediction Tool (Karplus and Schulz 1985).

Via Surface Accessibility. Antigenic sequences need to be on the surface of the antigen so that they are accessible by B-cell receptors. A good measure of surface accessibility is the degree of hydrophilicity of sequences on the surface of the protein. Tools to measure surface accessibility

include the Parker Hydrophilicity Scale (Parker, et.al., 1986) and the Emini Surface Accessibility Prediction (Emini et al. 1985).

Collection and Preparation of Antibody Structures. Currently, no tool exists that can predict the sequence and structure of the specific B-cell receptors that will bind to B-cell epitopes. This is circumvented by using the structures of experimentally verified antibodies, since the structures of antibodies closely resemble the B-cell receptors that triggered the maturation of a naïve B-cell into a mature plasma cell (Alberts et al. 2002). Five antibody structures developed against Alzheimer's disease (Qiu et al. 2023) were obtained from the Research Collaboratory for Structural Bioinformatics- Protein Data Bank (RCSB- PDB). These are Aducanumab (PDB ID: 6CNR), Crenezumab (PDB ID: 5VZX), Gantenerumab (PDB ID: 5CSZ), Ponezumab (PDB ID: 3U0T), and Solanezumab (PDB ID: 4XXD). The first two antibodies are both present in PDB as their free form, but the structures of the last three antibodies are in complex with some A β peptide fragments. These fragments were removed via iCn3D (Wang et al. 2020), and the energy of the modified structure was minimized using UCSF Chimera (Pettersen et al. 2004). This was done by clicking "Tools" and selecting "Minimize structure", with the parameters being as follows: a) Steepest descent steps: 100; b) Steepest descent step size (Å): 0.02; c) Conjugate gradient steps: 10; d) Conjugate gradient step size (Å): 0.02; e) Update interval: 10; and f) no fixed atoms and finally executing the "Minimize" command.

Modelling of Antibody-Peptide Interactions. The interactions between the identified potential immunogenic cores and the antibodies were modelled using the GalaxyPepDock webserver (Lee et al. 2015). This is a freely accessible tool that performs similarity-based docking by utilizing experimentally validated structures available in databases as templates, then uses energy-based optimization that permits structural flexibility. This enables the webserver to perform interaction modelling without expending too much computational power. The structures of the antibody-peptide complexes generated by GalaxyPepDock were validated by getting the percentages of Ramachandran favored residues using MolProbity (Williams et al. 2018). The Root Mean Square Deviation (RMSD) between the solved antibody protein structure and the antibody-peptide complex was generated via iCn3D.

Determination of Binding Energy and Dissociation Constants. The modelled protein-peptide interactions generated by GalaxyPepDock were then evaluated for binding energy through the PRODIGY webserver (Xue et al. 2016). This allows for the determination of the antibody-epitope pairs that exhibit the most spontaneous binding and forms the strongest complexes. It also verifies the presence of immunogenic cores by comparison of their binding strength compared with the source epitope. For immunotherapy, determination of binding energy was done at human body temperature (37°C), but since antibodies can also be used for immunodiagnostics (Tabll et al. 2024), measurement was also done at room temperature (25°C).

Residue Interaction Analysis. iCn3D was used to determine the specific residues within the antibody-epitope complex that interact with one another. The number and the type of interactions were also determined and illustrated via a 2-dimensional interaction map, and the numbers and types of residues were summarized for correlation with binding behavior. This entire workflow is summarized in Figure 1.

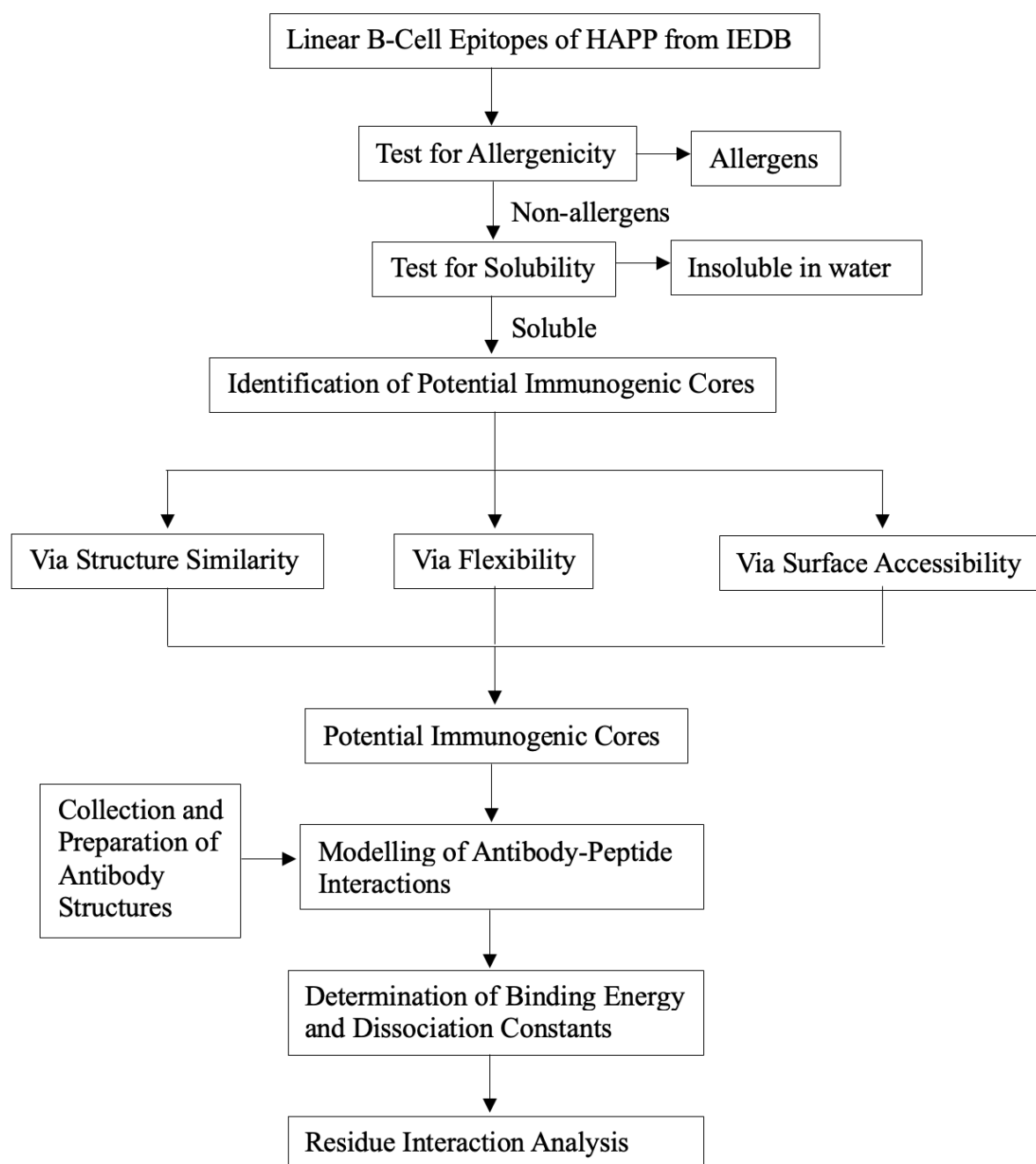


Figure 1. Overview of the immunoinformatics workflow employed in this study.

RESULTS AND DISCUSSION

Results of Allergenicity and Water Solubility Analysis of Linear B-cell Epitopes. Following the query indicated in the methods section, a total of 81 epitopes from 4 different antigens were obtained. Filtering further for those found only in *Homo sapiens* yields 45 epitopes, and limiting them to those found only in HAPP reduces it to 43 epitopes. Testing for allergenicity and water solubility reduces the count to the final 16 epitopes, which are summarized in Table 1. These are the epitopes that underwent further immunoinformatics analysis.

Table 1. Non-allergenic, water-soluble, experimentally validated linear B-cell epitopes from HAPP obtained from IEDB.

Epitope ID	Sequence
7493	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA
99921	DAEFRHDS
99922	DAEFRHDSGY
103097	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV
157148	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIATVIVITLVMLKKK
157151	DAEFRHDSGYEVHHQKLVFFAKDVGSNKGAIIGLMVGGVVIA
157152	DAEFRHDSGYEVHHQKLVFFAQDVGSNKGAIIGLMVGGVV
157191	EFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA
157223	EVHHQKLVFFAEDVGSNKGAIIGLMVGGVV
157224	EVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA
157292	GAIIGLM
100097	HDSGYEVHHQ
100216	KLVFFAEDVG
100217	KMDAEFRHDS
100606	VHHQKLVFFA
100611	VKMDAEFRHD

Potential Immunogenic Cores. Immunoinformatics analysis of these remaining epitopes revealed 5 potential immunogenic cores, summarized in Table 2. Structure similarity identified one epitope, while the analysis of both flexibility and surface accessibility identified 2 cores each. Their sequences, length, and location as part of the full tertiary structure of HAPP are also presented in Figure 2.

Table 2. Identified potential immunogenic cores found in the remaining epitopes.

Method	Start	End	Length	Sequence
Via Structure Similarity	682	685	4	EVHH
Via Flexibility	675	681	7	FRHDSGY
	694	700	7	DVGSNKG
Via Surface Accessibility	675	685	11	FRHDSGYEVHH
	694	699	6	DVGSNK

Models of Antibody-Peptide Interactions. The interactions of these epitopes made into discrete peptides with known antibody structures are illustrated in Figures 3-7. All of these show the expected appearance of 2 heavy chains and 2 light chains in the antibodies, with the epitopes binding between one heavy and one light chain. This makes sense because an antibody's complementarity-determining region (CDR) is often found within these areas (Goulet and Atkins 2020). These CDRs contain the hypervariable sequences that make up the antigen binding interface, and they are responsible for the high specificity of the binding behavior of these antibodies to pathogens. These models of antibody-peptide interactions were validated via an assessment of their Ramachandran-favored residues using MolProbity, and the results are summarized in Table 3. Complexes with Ramachandran-favored residues of 95% or higher are considered acceptable. Only the antibody-peptide complexes that meet this criterion are subjected to further analysis. The 3 antibodies that generated reliable antibody-peptide models were analyzed for their RMSD between their solved protein structures and the docked structures using iCn3D. The resulting RMSD data are summarized in Table 4.

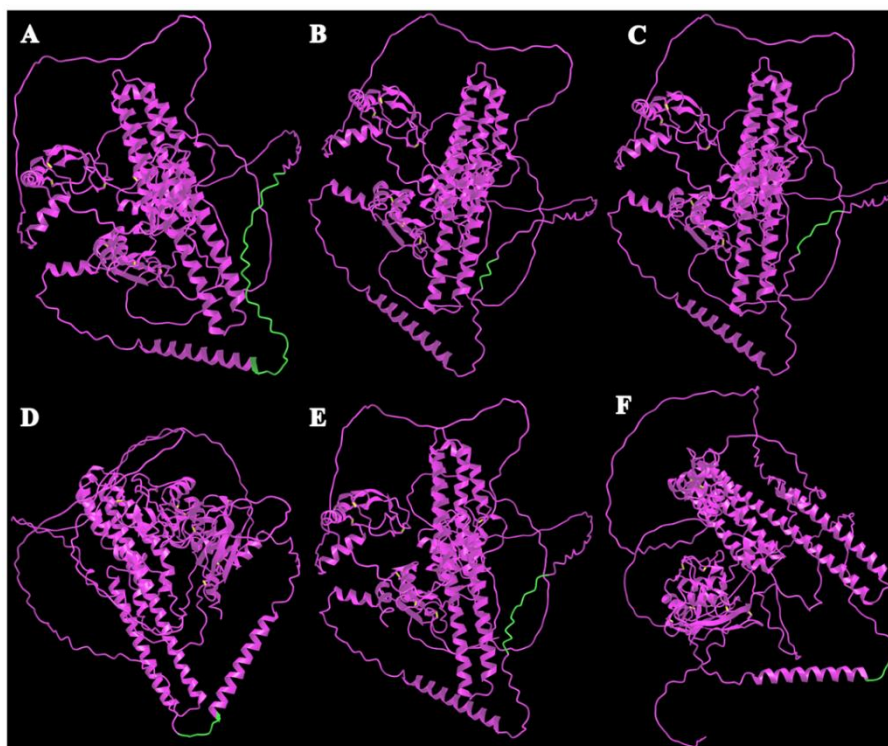


Figure 2. The location of each potential immunogenic core (green) in HAPP (magenta). The respective sequences are: (A) ⁶⁷²DAEFRHDSGYEVHHQKLFFFAEDVGSNKG⁷⁰¹; (B) ⁶⁸²EVHH⁶⁸⁵; (C) ⁶⁷⁵FRHDSGY⁶⁸¹; (D) ⁶⁹⁴DVGSNKG⁷⁰⁰; (E) ⁶⁷⁵FRHDSGYEVHH⁶⁸⁵; and (F) ⁶⁹⁴DVGSNK⁶⁹⁹. HAPP structure obtained from AlphaFold (Jumper et al. 2021) and viewed via iCn3D.

Binding Energy Determination. The spontaneity of the binding of a given ligand to a receptor can be determined by calculating the binding energy of the resulting complex. This is a method usually employed to provide supporting evidence for the immunogenicity of predicted epitopes via immunoinformatics since immunogenicity is predicated on whether spontaneous binding will occur (Fatoba et al. 2021). The results of these parameters at room temperature are given in Table 5, which will be relevant if the epitopes are intended to be used for diagnostic purposes with HAPP as a biomarker. On the other hand, the results for the binding energy at human body temperature are given in Table 6, data that will be relevant if the epitopes are intended to be used for therapeutic purposes. The data show that all epitope-antibody pairs bind spontaneously and form stable antibody-peptide complexes as indicated by exergonic binding energies.

Table 3. Results for Antibody-Peptide Model Validation using MolProbity

Epitope	Percent of Ramachandran-Favored Residues				
	Aducanumab	Crenezumab	Gantenerumab ¹	Ponezumab ¹	Solanezumab
⁶⁷² DAEFRHDSGYEVHHQKLFFFAEDVGSNKG ⁷⁰¹	97.12	98.87	76.57	77.60	98.15
⁶⁸² EVHH ⁶⁸⁵	98.36	98.95	77.95	75.66	98.45
⁶⁷⁵ FRHDSGY ⁶⁸¹	97.67	99.30	79.11	76.03	98.21
⁶⁹⁴ DVGSNKG ⁷⁰⁰	97.67	98.72	78.79	78.02	98.10
⁶⁷⁵ FRHDSGYEVHH ⁶⁸⁵	97.46	98.73	79.10	76.90	98.70
⁶⁹⁴ DVGSNK ⁶⁹⁹	98.60	98.72	77.98	76.49	98.45
No Bound Epitope	97.64	95.33	98.56	98.09	96.89

¹ Antibodies that did not exhibit sufficient Ramachandran-favored residues when bound to an epitope.

Table 4. Results for RMSD Analysis using iCn3D

Epitope	Root Mean Square Deviation (Angströms)					
	Aducanumab ¹		Crenezumab		Solanezumab	
	Heavy Chain	Light Chain	Heavy Chain	Light Chain	Heavy Chain	Light Chain
672DAEFRHDSGYEVHHQKLVFFAEDVGSNKG ⁷⁰¹	0.7466	0.7408	1.215	1.142	1.684	1.566
682EVHH ⁶⁸⁵	0.7813	0.7539	1.132	0.8646	1.419	1.581
675FRHDSGY ⁶⁸¹	0.7423	0.7658	1.157	1.157	1.636	1.746
694DVGSNKG ⁷⁰⁰	0.7355	0.7325	1.218	0.9650	1.536	1.397
675FRHDSGYEVHH ⁶⁸⁵	0.7586	0.7586	1.460	0.9723	1.581	1.611
694DVGSNK ⁶⁹⁹	0.7974	0.8123	1.117	0.8686	1.661	1.481

¹ Antibody that exhibited lowest RMSD between solved protein structure and docked protein structure.

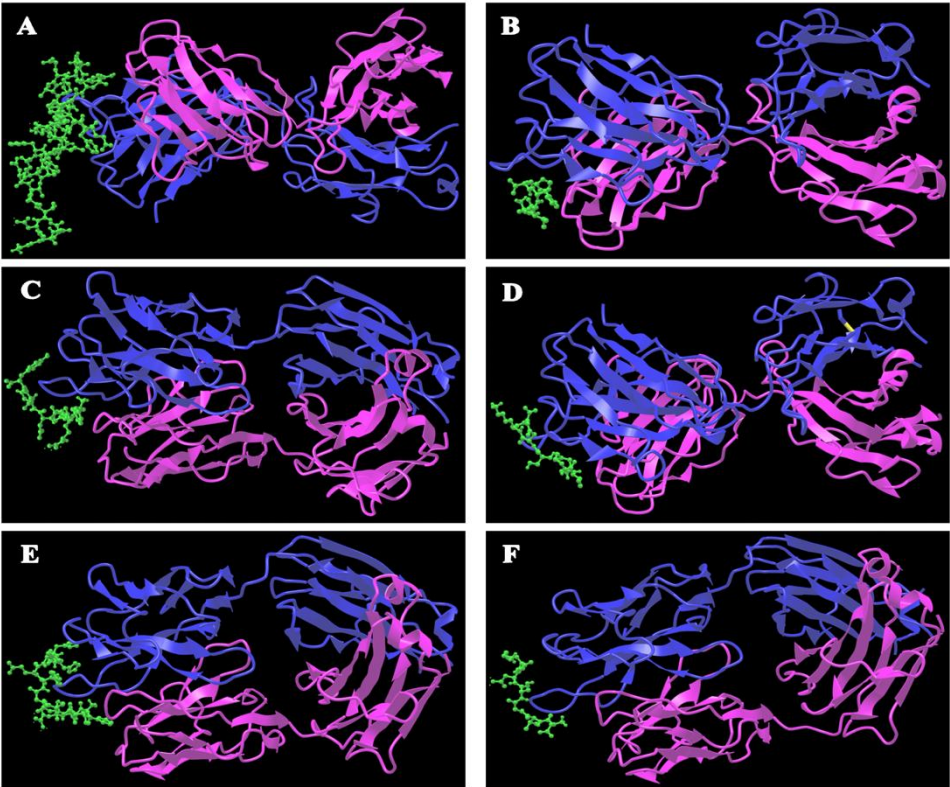


Figure 3. Interactions of the heavy chains (blue) and light chains (magenta) of Aducanumab with the epitopes as discrete peptides (green). The respective epitopes are: (A) 672DAEFRHDSGYEVHHQKLVFFAEDVGSNKG⁷⁰¹; (B) 682EVHH⁶⁸⁵; (C) 675FRHDSGY⁶⁸¹; (D) 694DVGSNKG⁷⁰⁰; (E) 675FRHDSGYEVHH⁶⁸⁵; and (F) 694DVGSNK⁶⁹⁹. Structures viewed via iCn3D.

Table 5. Gibbs free energy change for each epitope-antibody pair at room temperature.

Epitope	Binding Energy (kcal mol ⁻¹) at 25°C		
	Aducanumab ¹	Crenezumab	Solanezumab
672DAEFRHDSGYEVHHQKLVFFAEDVGSNKG ⁷⁰¹	-17.9	-15.8	-15.2
682EVHH ⁶⁸⁵	-18.5	-17.4	-15.4
675FRHDSGY ⁶⁸¹	-18.0	-17.0	-15.0
694DVGSNKG ⁷⁰⁰	-17.9	-15.4	-14.5
675FRHDSGYEVHH ⁶⁸⁵	-18.9 ²	-16.4	-14.5
694DVGSNK ⁶⁹⁹	-17.8	-17.1	-16.0

¹ Antibody that exhibited the strongest binding across all epitopes.

² Epitope with the highest binding strength to Aducanumab.

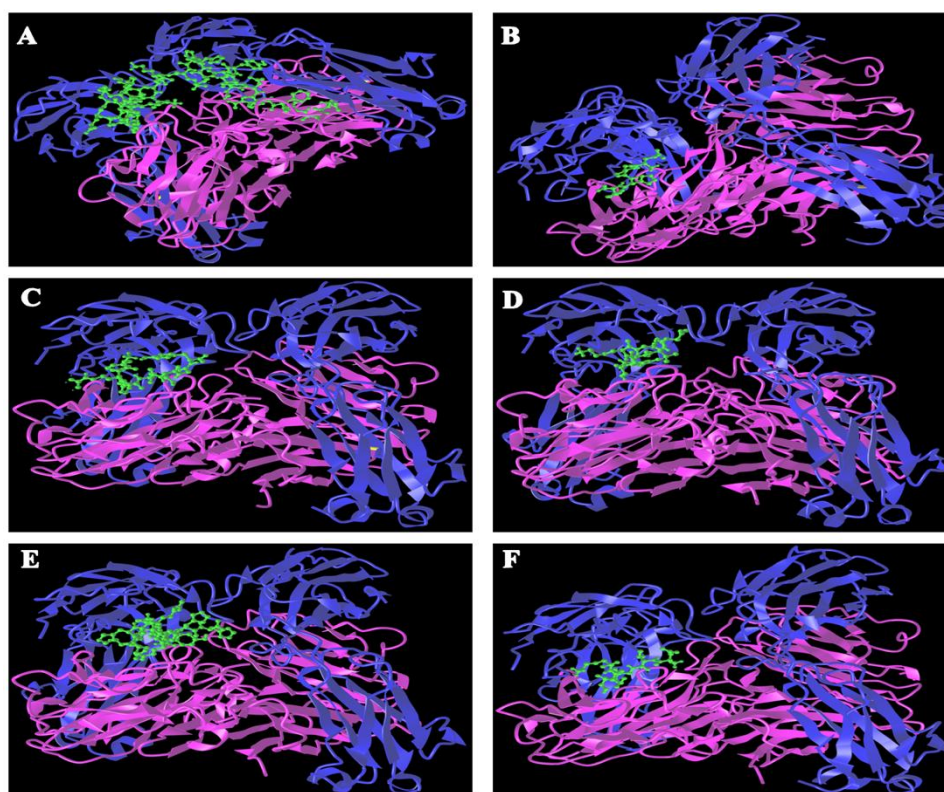


Figure 4. Interactions of the heavy chains (magenta) and light chains (blue) of Crenezumab with the epitopes as discrete peptides (green). The respective epitopes are: (A) $^{672}\text{DAEFRHDSGYEVHHQKL VFFAEDVGSNKG}^{701}$; (B) $^{682}\text{EVHH}^{685}$; (C) $^{675}\text{FRHDSGY}^{681}$; (D) $^{694}\text{DVGSNKG}^{700}$; (E) $^{675}\text{FRHDSGYEVHH}^{685}$; and (F) $^{694}\text{DVGSNK}^{699}$. Structures viewed via iCn3D.

Table 6. Gibbs free energy change for each epitope-antibody pair at body temperature.

Epitope	Binding Energy (kcal mol ⁻¹) at 37°C		
	Aducanumab ¹	Crenezumab	Solanezumab
$^{672}\text{DAEFRHDSGYEVHHQKL VFFAEDVGSNKG}^{701}$	-17.9	-15.8	-15.2
$^{682}\text{EVHH}^{685}$	-18.5	-17.4	-15.4
$^{675}\text{FRHDSGY}^{681}$	-18.0	-17.0	-15.0
$^{694}\text{DVGSNKG}^{700}$	-17.9	-15.4	-14.5
$^{675}\text{FRHDSGYEVHH}^{685}$	-18.9 ²	-16.4	-14.5
$^{694}\text{DVGSNK}^{699}$	-17.8	-17.1	-16.0

¹ Antibody that exhibited the strongest binding across all epitopes.

² Epitope with the highest binding strength to Aducanumab.

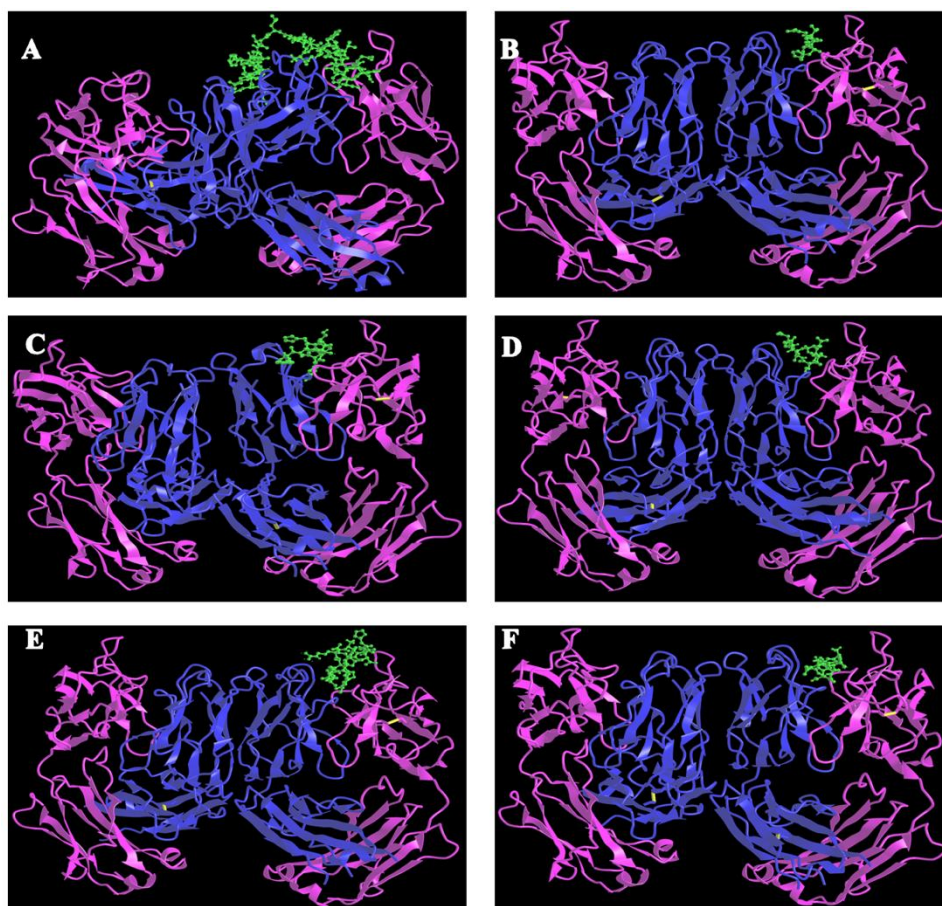


Figure 5. Interactions of the heavy chains (blue) and light chains (magenta) of Solanezumab with the epitopes as discrete peptides (green). The respective epitopes are: (A) $^{672}\text{DAEFRHDSGYEVHHQKL VFFAEDVGSNKG}^{701}$; (B) $^{682}\text{EVHH}^{685}$; (C) $^{675}\text{FRHDSGY}^{681}$; (D) $^{694}\text{DVGSNKG}^{700}$; (E) $^{675}\text{FRHDSGYEVHH}^{685}$; and (F) $^{694}\text{DVGSNK}^{699}$. Structures viewed via iCn3D.

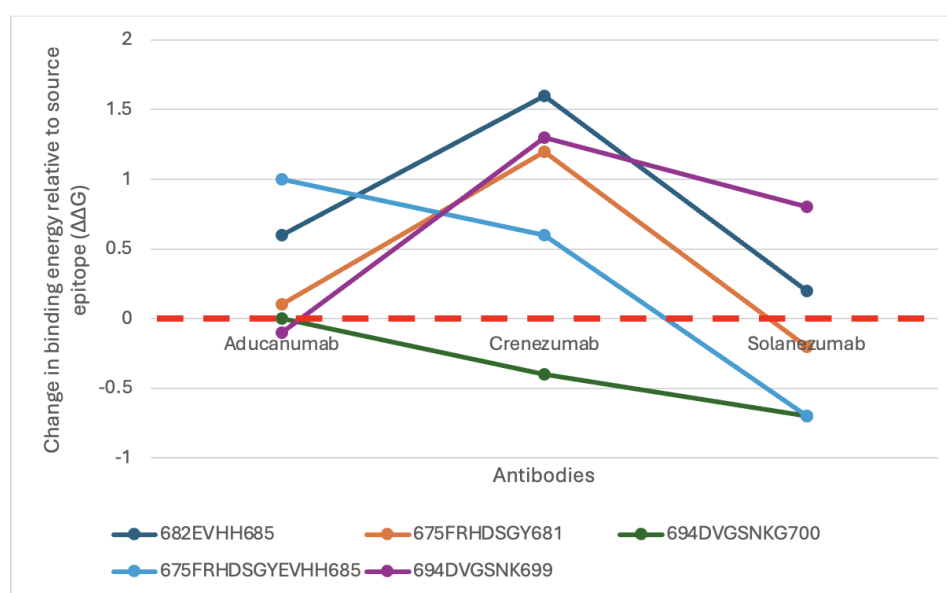


Figure 6. Comparison of the binding strength of potential immunogenic cores with source epitope (broken red line).

The calculated binding energy is the same at both 25°C and 37°C. The dissociation constants determined from PRODIGY vary slightly, although the difference is less than 2 orders of magnitude, which is not a statistically significant difference and, as such, are not included in this paper. This indicates that there is no significant difference between the binding of the peptides with the antibodies at these 2 relevant temperatures, and as such, the peptides may potentially be equally effective for therapeutic and diagnostic purposes, with binding strength as the sole criterion. Another thing that can be surmised from this data is the nature of the interactions between the epitopes and the antibodies. The variability of the binding energies of the different epitopes with each antibody indicates differences in the number and the types of interactions for each antibody-epitope pair (Reverberi and Reverberi 2007). This warrants additional analysis and will be explored in greater detail in another section. Other studies also suggest that the number of hydrogen bond interactions, and therefore the binding strength, shows variability with respect to the degree of disorder present in identified epitopes (Badaya and Sasidhar 2022). This potential disorder present in the identified epitopes and its correlation with binding energies is a topic that may be explored further in future studies.

To validate the identified potential immunogenic cores, the binding strength of each was compared with that of the source epitope. A positive result is indicated if the binding energies of these cores are comparable with that of the source. To better illustrate this, the binding strength of each potential immunogenic core was compared with the binding strength of the source epitope by getting the difference between their values of Gibbs Free Energy change ($\Delta\Delta G$), with the results graphed in Figure 6. The use of such a parameter is often used for predicting the effects of mutations on protein-protein binding interactions (Brender and Zhang 2015), but is applied here to determine differences in ΔG values between the potential immunogenic cores and the source epitope. Values appearing above the broken red line (which denotes zero, for the change in the ΔG value of the source with respect to itself) indicate that there is an increase in the binding strength of the immunogenic core with respect to the source epitope (has a more negative ΔG value compared with the source). This means that the identified immunogenic cores, despite being shorter than the source, bind more strongly to the antibodies compared with the epitope from which they were obtained. This thermodynamic behavior, in theory, should indicate that these immunogenic cores, when synthesized as discrete peptides, should also stimulate an antibody-based immune response (Camacho et al., 2008).

It is interesting to note that almost all epitopes in Figure 6 displayed at least one epitope-antibody interaction that exhibited a stronger binding than the source epitope, except ⁶⁹⁴DVGSNKG⁷⁰⁰, which showed only equal or weaker binding energies. The next fewest number of increases is exhibited by epitope ⁶⁷⁵FRHDSGY⁶⁸¹, interacting more favorably only with Crenezumab. This is followed by epitope ⁶⁷⁵FRHDSGYEVHH⁶⁸⁵, which shows increased binding strength to Aducanumab and Crenezumab. Epitope ⁶⁹⁴DVGSNK⁶⁹⁹ bound more favorably to all antibodies except Aducanumab, while ⁶⁸²EVHH⁶⁸⁵, despite being the shortest among the immunogenic cores, exhibited increased binding strength to all antibodies.

Aducanumab, a recombinant human antibody developed as a potential therapeutic to promote amyloid-beta (A β) plaque clearance (Söderberg et al. 2023), exhibited the strongest binding across all epitopes, despite having relatively modest increases among the immunogenic cores. It functions by binding to amino acids 3-7 of the A β peptide and was approved by the US Food and Drug Administration (FDA) in 2021 (Arndt et al. 2018). This ²AEFRHD⁷ fragment of the A β peptide (as shown in UniProt) is an experimentally validated sequence that is also found in the ⁶⁷²DAEFRHDSGYEVHHQKLFFAEDVGSNKG⁷⁰¹ epitope of HAPP found in IEDB, and fragments of it are included in ⁶⁷⁵FRHDSGY⁶⁸¹ and ⁶⁷⁵FRHDSGYEVHH⁶⁸⁵. Though these two immunogenic cores did not exhibit the greatest number of increases in favorability of binding, it is worth noting that epitope ⁶⁷⁵FRHDSGYEVHH⁶⁸⁵ binding with Aducanumab displayed the strongest binding energy out of all epitope-antibody pairs. The overlap of the FRHD sequence on both the A β peptide and HAPP that binds to aducanumab supports this strong binding energy obtained via computational

means. Additionally, despite B-cell receptors and free antibodies possessing differences in both structure and kinetics, they are both immunoglobulin (Ig) isoforms (García-Sánchez, et.al., 2023). As such, it can reasonably be said that the B cell receptors to which these immunogenic cores could potentially bind resemble Aducanumab. Further details on what these entails can be obtained from residue interaction analysis of the epitope-antibody interactions.

Residue Interaction Analysis. The webserver iCn3D was used to determine the specific residues within the antibody-epitope complex that interact with one another. The results are shown in Figures 7-9 as 2D interaction maps. Though to varying degrees, the epitopes interact with both the heavy and light chains of the antibodies, since the antigen-binding fragment (Fabs) require interactions with both, and even show increased potency of the immune response when the heavy and light chain signatures of each chain are paired instead of being partial to one of the chains (Banach et al. 2021). Additionally, for most functional antibodies, the heavy chain determines the light chain, so interactions of an epitope with both may indicate greater functionality of the antibody (Jaffe et al. 2022). Cross-referencing the interaction maps in Figures 7-9 with the models in Figures 3-5 visually illustrate the interactions of the epitopes with both heavy and light chains, with the epitopes often present in the cleft between these 2 chains. The antibody structures present in the PDB used for this study are humanized monoclonal antibodies which often do not contain the fragment crystallizable (Fc) stems present in antibodies naturally produced by the body during an infection. This Fc stem is composed exclusively of constant domains that are important in eliciting effector functions (Goulet and Atkins 2020). Besides this role, these constant regions are also relevant for the stabilization of the paratope found on the antibody and crucial in enforcing epitope specificity (McConnell and Casadevall 2024). This difference may affect the binding of the Fab with the epitopes, though this is not explored in the current study.

The number and type of interactions between the antibodies and the peptides are summarized in Tables 7-9. Cross-referencing the results of the residue interaction analysis with the results for binding energies indicates that it is not the mere number of contacts that is relevant for binding strength, but the number of relevant interactions such as hydrogen bonding, salt bridges, halogen bonds, π -cation interactions, and π - π stacking. For example, epitope ⁶⁷⁵FRHDSGYEVHH⁶⁸⁵ exhibits the strongest binding to Aducanumab (-18.9 kcal/mol) despite the source epitope ⁶⁷²DAEFRHDSGYEVHHQKLVFFAEDVGSNKG⁷⁰¹ having more contacts since the former has the same number of H-bonds as the latter but has more π - π stacking. However, this does not fully explain the binding strengths of all peptides with the antibodies. For example, ⁶⁸²EVHH⁶⁸⁵ exhibits the strongest binding with Crenezumab, and ⁶⁹⁴DVGSNK⁶⁹⁹ shows the strongest binding to Solanezumab despite both having less relevant interactions than the source epitope. The reason for this may be the topic of future studies, especially involving molecular dynamics simulations but is outside the scope of the current study. A possible hypothesis is that these epitopes, because of their shorter length, may be able to adopt spatial arrangements that lead to more stable binding confirmations despite having a lower number of interactions (Parisi et al. 2024). Finally, the relevant FRHD sequence highlighted earlier shows interactions with most antibodies, showing its significance.

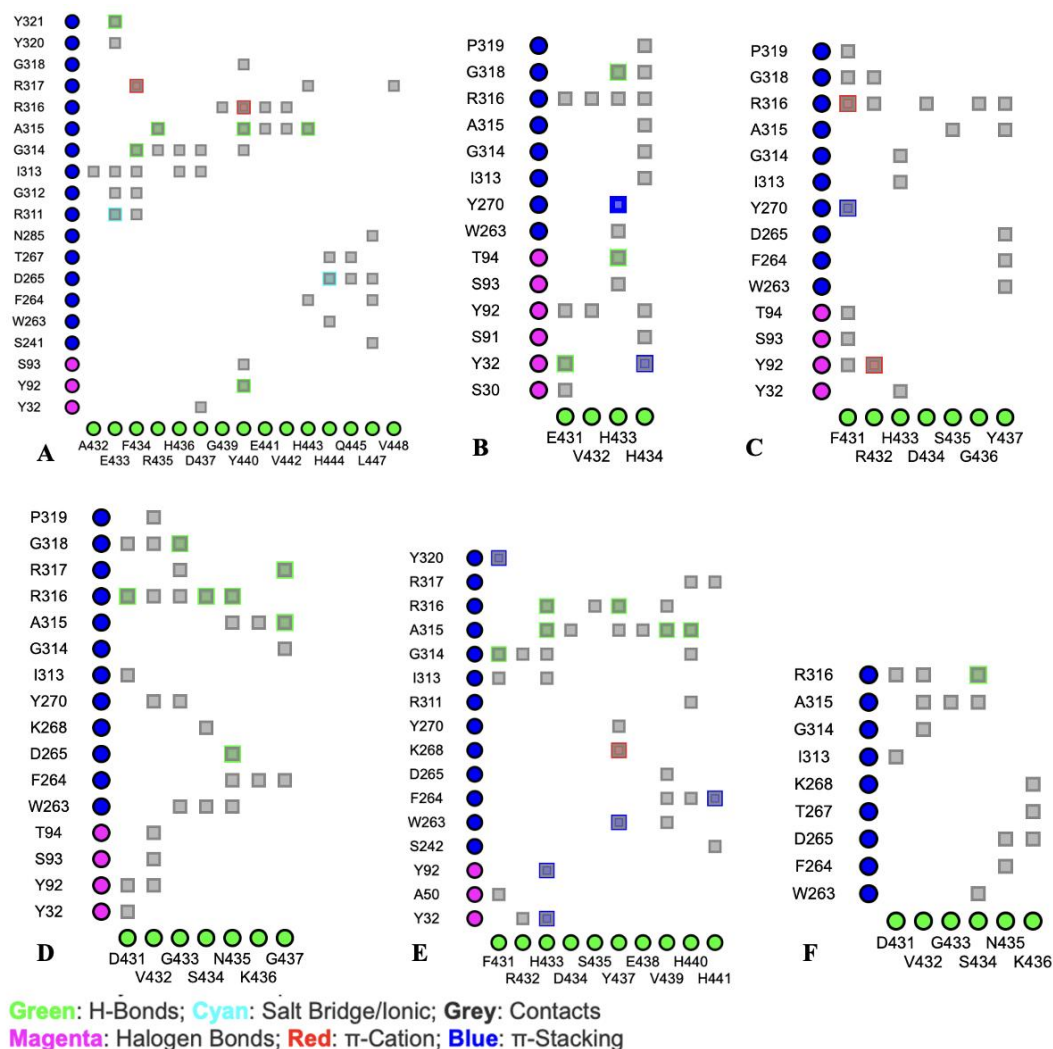


Figure 7. 2D interaction map of the epitopes (green) with the heavy chains (blue) and light chains (magenta) of Adcanumab generated via iCn3D. The respective epitopes are: (A) ⁶⁷²DAEFRHDSGYEVHHQKLVFFAEDVGSNKG⁷⁰¹; (B) ⁶⁸²EVHH⁶⁸⁵; (C) ⁶⁷⁵FRHDSGY⁶⁸¹; (D) ⁶⁹⁴DVGSNKG⁷⁰⁰; (E) ⁶⁷⁵FRHDSGYEVHH⁶⁸⁵; and (F) ⁶⁹⁴DVGSNKG⁶⁹⁹

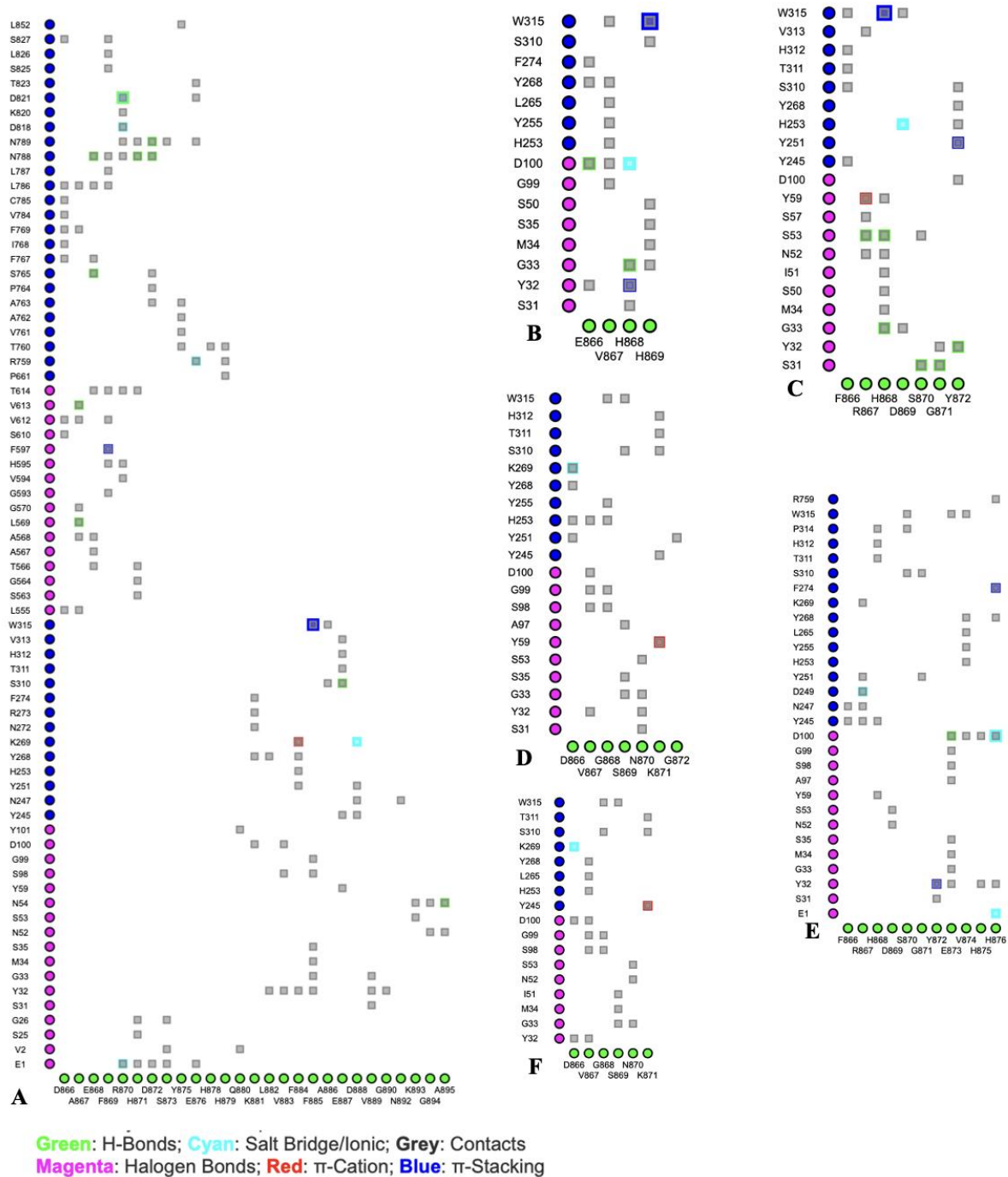


Figure 8. 2D interaction map of the epitopes (green) with the heavy chains (magenta) and light chains (blue) of Crenezumab generated via iCn3D. The respective epitopes are: (A) ^{672} DAEFRHDSGYEVHHQKLVFFAEDVGSNKG ^{701} ; (B) ^{682} EVHH ^{685} ; (C) ^{675} FRHDSGY ^{681} ; (D) ^{694} DVGSNKG ^{700} ; (E) ^{675} FRHDSGYEVHH ^{685} ; and (F) ^{694} DVGSNKG ^{699} .

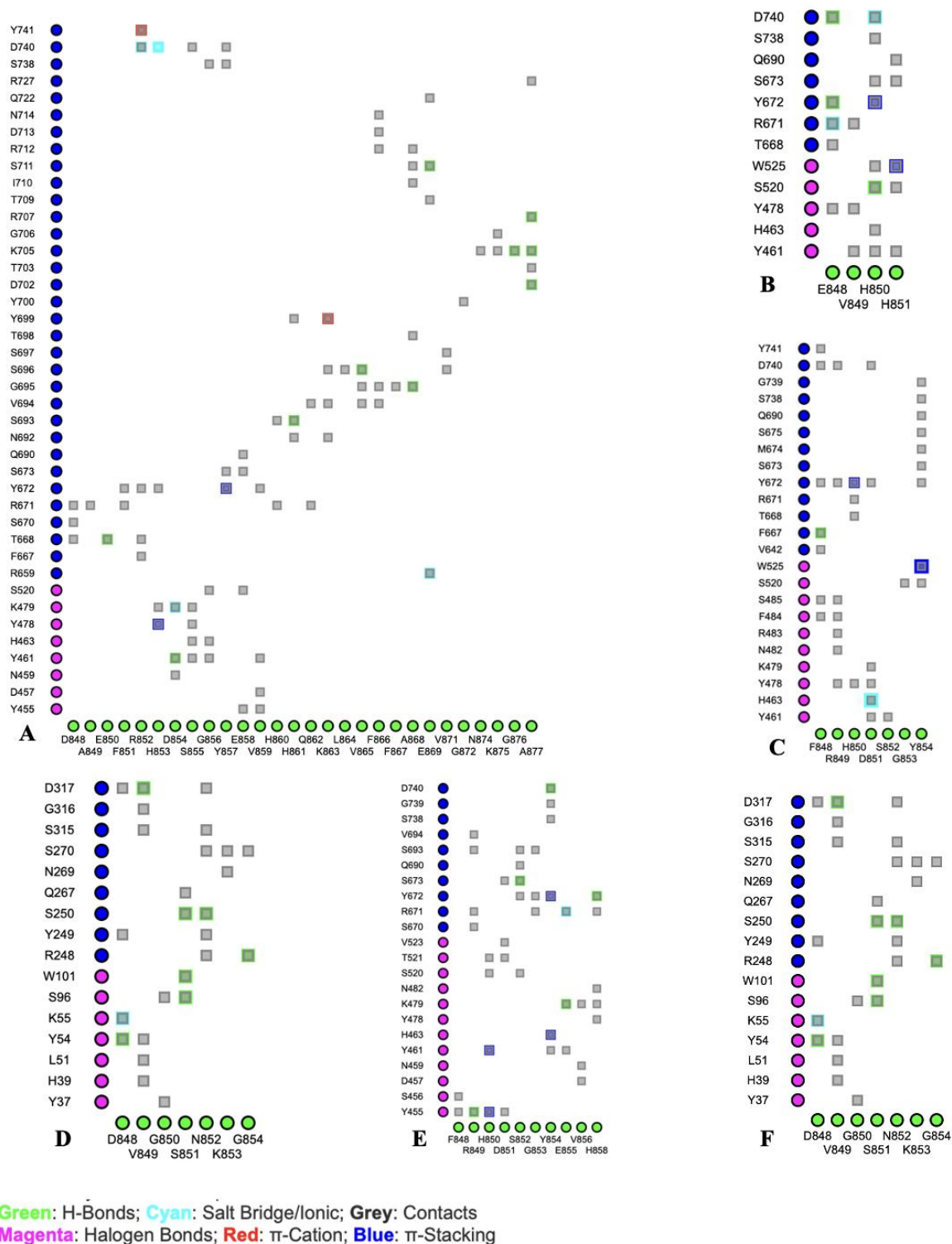


Figure 9. 2D interaction map of the epitopes (green) with the heavy chains (blue) and light chains (magenta) of Solanezumab generated via iCn3D. The respective epitopes are: (A) ^{672} DAEFRHDSGYEVHHQKLVFFAEDVGSNKG ^{701} ; (B) ^{682} EVHH ^{685} ; (C) ^{675} FRHDSGY ^{681} ; (D) ^{694} DVGSNKG ^{700} ; (E) ^{675} FRHDSGYEVHH ^{685} ; and (F) ^{694} DVGSNKG ^{699} .

Table 7. Summary of the Number and Types of Interactions Between Aducanumab and the Peptides

Epitope	Number of Each Type of Interaction					
	H-bonds	Salt bridge	Contacts	Halogen bonds	π -Cation	π -stacking
⁶⁷² DAEFRHDSGYEVHHQKLVFFAEDVGSNKG ⁷⁰¹	6	2	42	0	2	0
⁶⁸² EVHH ⁶⁸⁵	3	0	21	0	0	2
⁶⁷⁵ FRHDSGY ⁶⁸¹	0	0	21	0	2	1
⁶⁹⁴ DVGSNKG ⁷⁰⁰	7	0	31	0	0	0
⁶⁷⁵ FRHDSGYEVHH ⁶⁸⁵ ¹	6	0	33	0	1	5
⁶⁹⁴ DVGSNK ⁶⁹⁹	1	0	14	0	0	0
¹ Epitope that exhibited the strongest binding with Aducanumab.						

Table 8. Summary of the Number and Types of Interactions Between Crenezumab and the Peptides

Epitope	Number of Each Type of Interaction					
	H-bonds	Salt bridge	Contacts	Halogen bonds	Pi-Cation	Pi-stacking
⁶⁷² DAEFRHDSGYEVHHQKLVFFAEDVGSNKG ⁷⁰¹	10	4	95	0	1	2
⁶⁸² EVHH ⁶⁸⁵ ¹	2	1	20	0	0	2
⁶⁷⁵ FRHDSGY ⁶⁸¹	6	1	31	0	1	2
⁶⁹⁴ DVGSNKG ⁷⁰⁰	0	1	29	0	1	0
⁶⁷⁵ FRHDSGYEVHH ⁶⁸⁵	1	3	44	0	0	2
⁶⁹⁴ DVGSNK ⁶⁹⁹	0	1	24	0	1	0

¹ Epitope that exhibited the strongest binding with Crenezumab.

Table 9. Summary of the Number and Types of Interactions Between Solanezumab and the Peptides

Epitope	Number of Each Type of Interaction					
	H-bonds	Salt bridge	Contacts	Halogen bonds	Pi-Cation	Pi-stacking
⁶⁷² DAEFRHDSGYEVHHQKLVFFAEDVGSNKG ⁷⁰¹	10	4	82	0	2	2
⁶⁸² EVHH ⁶⁸⁵ ¹	3	2	21	0	0	2
⁶⁷⁵ FRHDSGY ⁶⁸¹	1	1	35	0	0	2
⁶⁹⁴ DVGSNKG ⁷⁰⁰	0	1	26	0	1	0
⁶⁷⁵ FRHDSGYEVHH ⁶⁸⁵	5	1	40	0	0	4
⁶⁹⁴ DVGSNK ⁶⁹⁹ ¹	7	1	26	0	0	0

¹ Epitope that exhibited the strongest binding with Solanezumab.

CONCLUSIONS

Immunoinformatics analysis revealed 16 non-allergenic, water-soluble, experimentally validated epitopes from IEDB, and identified 5 potential immunogenic cores present in those epitopes. These are ⁶⁸²EVHH⁶⁸⁵, ⁶⁷⁵FRHDSGY⁶⁸¹, ⁶⁹⁴DVGSNKG⁷⁰⁰, ⁶⁷⁵FRHDSGYEVHH⁶⁸⁵, and ⁶⁹⁴DVGSNK⁶⁹⁹. All of these exhibited comparable binding strength and affinity with the source epitope ⁶⁷²DAEFRHDSGYEVHHQKLVFFAEDVGSNKG⁷⁰¹ when docked with 3 monoclonal antibodies developed against Alzheimer's disease. These immunogenic cores displayed more favorable binding with at least one antibody compared with the source except for ⁶⁹⁴DVGSNKG⁷⁰⁰ which displayed only equal or weaker binding. Aducanumab showed the most exergonic binding energy

across all immunogenic cores, indicating that the antibodies that these cores may elicit when used as immunogens would most likely resemble Aducanumab. The epitope ⁶⁸²EVHH⁶⁸⁵, despite being the shortest, showed increased binding strength with all antibodies compared with the source epitope, indicating its versatility. Molecular modelling also showed that the immunogenic cores situated themselves between one heavy and one light chain of the antibodies, potentially showing the Fab region of the antibody. The antibody-epitope complexes demonstrated similar binding strengths at 25°C and 37°C, indicating that they might be equally effective for therapeutic and diagnostic purposes based solely on binding strength. The Aducanumab-⁶⁷⁵FRHDSGYEVHH⁶⁸⁵ complex displayed the strongest binding out of all complexes, indicating that this immunogenic core may be the best epitope to synthesize as a discrete peptide to elicit the production of an antibody resembling Aducanumab. This also contains the FRHD sequence present in the Aβ fragment ²AEFRHD⁷ that is experimentally validated to be in complex with Aducanumab, supporting its binding strength. These results could lead to *in vitro* and *in vivo* testing of these potential immunogenic cores.

REFERENCES

- Abbott RK, Crotty S. Factors in B cell competition and immunodominance. *Immunologic Rev.* 2020 July; 296(1):120–131. <https://doi.org/10.1111/imr.12861>
- Abubakar MB, Sanusi KO, Ugusman A, Mohamed W, Kamal H, Ibrahim HN, Khoo CS, Kumar J. Alzheimer's disease: An update and insights into pathophysiology. *Front Aging Neurosci.* 2022 March; 14:742408. <https://doi.org/10.3389/fnagi.2022.742408>
- Akbar R, Robert PA, Pavlović M, Jeliaskov JR, Snapkov I, Slabodkin A, Weber CR, et al. A compact vocabulary of paratope-epitope interactions enables predictability of antibody-antigen binding. *Cell Rep.* 2021 March; 34(11):108856. <https://doi.org/10.1016/j.celrep.2021.108856>
- Alberts B, Johnson S, Lewis J, Raff M, Roberts K, Walter P. B cells and antibodies. In *Molecular Biology of the Cell*. 4th Edition. Garland Science; 2002. <https://www.ncbi.nlm.nih.gov/books/NBK26884/>
- Arndt JW, Qian F, Smith BA, Quan C, Kilambi KP, Bush MW, Walz T, et al. Structural and kinetic basis for the selectivity of aducanumab for aggregated forms of amyloid-β. *Sci Rep.* 2018 Apr; 8(1):6412. <https://doi.org/10.1038/s41598-018-24501-0>
- Badaya A, Sasidhar YU. The role of temperature in the binding of the disordered epitope region of human thrombopoietin to antibody: A molecular dynamics simulations study. *J Mol Graph Model.* 2022 March; 111:108098. <https://doi.org/10.1016/j.jmgm.2021.108098>
- Banach BB, Cerutti G, Fahad AS, Shen C-H, De Souza MO, Katsamba PS, Tsybovsky Y, et al. Paired heavy- and light-chain signatures contribute to potent SARS-CoV-2 neutralization in public antibody responses. *Cell Rep.* 2021 Oct; 37(1):109771. <https://doi.org/10.1016/j.celrep.2021.109771>
- Barker WW, Luis CA, Kashuba A, Luis M, Harwood DG, Loewenstein D, Waters C, et al. Relative frequencies of Alzheimer disease, Lewy body, vascular and frontotemporal dementia, and hippocampal sclerosis in the state of Florida brain bank. *Alzheimer Dis Assoc Disord.* 2002 Oct; 16(4): 203-212. <https://doi.org/10.1097/00002093-200210000-00001>
- Brender JR, Zhang Y. Predicting the effect of mutations on protein-protein binding interactions through structure-based interface profiles. *PLOS Comput Biol.* 2015 Oct; 11(10):e1004494. <https://doi.org/10.1371/journal.pcbi.1004494>

Camacho CJ, Katsumata Y, Ascherman DP. Structural and thermodynamic approach to peptide immunogenicity. *PLoS Comput Biol* 4. 2008 Nov; (11):e1000231. <https://doi.org/10.1371/journal.pcbi.1000231>

Cheng Y, Tian D-Y, Wang Y-J. Peripheral clearance of brain-derived A β in Alzheimer's disease: pathophysiology and therapeutic perspectives. *Transl Neurodegener*. 2020 May; 9(1):16. <https://doi.org/10.1186/s40035-020-00195-1>

Chou PY, Fasman GD. Prediction of the secondary structure of proteins from their amino acid sequence. In Meister A. *Advances in Enzymology - and Related Areas of Molecular Biology*, 45–148. Hoboken, NJ, USA: John Wiley & Sons, Inc. 2006, Nov; <https://doi.org/10.1002/9780470122921.ch2>

De Groot AS, Moise L, Terry F, Gutierrez AH, Hindocha P, Richard G, Hoft DF, et al. Better epitope discovery, precision immune engineering, and accelerated vaccine design using immunoinformatics tools. *Front Immunol*. 2020 April; 11:2020. <https://doi.org/10.3389/fimmu.2020.00442>

Dimitrov I, Bangov I, Flower DR, Doytchinova I. AllerTOP v.2—a server for in silico prediction of allergens. *J Mol Model*. 2014 June; 20(6):2278. <https://doi.org/10.1007/s00894-014-2278-5>

Doytchinova IA, Flower DR. VaxiJen: A server for prediction of protective antigens, tumour antigens and subunit vaccines. *BMC Bioinformatics*. 2007 Jan; 8(1):4. <https://doi.org/10.1186/1471-2105-8-4>

Duay SS, Yap RCY, Gaitano AL, Santos JAA, Macalino SJY. Roles of virtual screening and molecular dynamics simulations in discovering and understanding antimalarial drugs. *Int J Mol Sci*. 2023 May; 24(11):9289. <https://doi.org/10.3390/ijms24119289>

Emini EA, Hughes JV, Perlow DS, Boger J. Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. *J Virol*. 1985 Sept; 55(3):836–839. <https://doi.org/10.1128/jvi.55.3.836-839.1985>

Fatoba AJ, Maharaj L, Adeleke VT, Okpeku M, Adeniyi AA, Adeleke MA. Immunoinformatics prediction of overlapping CD8⁺ T-Cell, IFN- γ and IL-4 inducer CD4⁺ T-cell and linear B-cell epitopes based vaccines against COVID-19 (SARS-CoV-2). *Vaccine*. 2021 Feb; 39(7): 1111–1121. <https://doi.org/10.1016/j.vaccine.2021.01.003>

García-Sánchez M, Castro M, Faro J. B cell receptors and free antibodies have different antigen-binding kinetics. *Proc Natl Acad Sci*. 2023 Aug; 120(35):e2220669120. <https://doi.org/10.1073/pnas.2220669120>

Goulet DR, Atkins WM. Considerations for the design of antibody-based therapeutics. *J Pharm Sci*. 2020 Jan; 109(1):74–103. <https://doi.org/10.1016/j.xphs.2019.05.031>

Haque RU, Levey AI. Alzheimer's disease: A clinical perspective and future nonhuman primate research opportunities. *Proc Natl Acad Sci*. 2019 Dec; 116(52):26224–26229. <https://doi.org/10.1073/pnas.1912954116>

Hebditch M, Carballo-Amador MA, Charonis S, Curtis R, Warwicker J. Protein-sol: A web tool for predicting protein solubility from sequence. *Bioinformatics*. 2017 Oct; 33(19):3098–3100. <https://doi.org/10.1093/bioinformatics/btx345>

Hopkins E, Sanvictores T, Sharma S. Physiology, acid base balance. In StatPearls. Treasure Island (FL): StatPearls Publishing. 2024. <http://www.ncbi.nlm.nih.gov/books/NBK507807/>

Jaffe DB, Shahi P, Adams BA, Chrisman AM, Finnegan PM, Raman N, Royall AE, et al. Functional antibodies exhibit light chain coherence. *Nature*. 2022 Nov; 611(7935):352–357. <https://doi.org/10.1038/s41586-022-05371-z>

Jespersen MC, Peters B, Nielsen M, Marcatili P. BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. *Nucleic Acids Res*. 2017 Jul; 45(W1):W24–29. <https://doi.org/10.1093/nar/gkx346>

Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, et al. Highly accurate protein structure prediction with AlphaFold. *Nature*. 2021 Aug; 596(7873):583–589. <https://doi.org/10.1038/s41586-021-03819-2>

Karplus PA, Schulz GE. Prediction of chain flexibility in proteins: A tool for the selection of peptide antigens. *Naturwissenschaften*. 1985 Apr; 72(4):212–213. <https://doi.org/10.1007/BF01195768>

Kolaskar AS, Tongaonkar PC. A semi-empirical method for prediction of antigenic determinants on protein antigens. *FEBS Lett*. 1990 Dec; 276(1–2):172–174. [https://doi.org/10.1016/0014-5793\(90\)80535-Q](https://doi.org/10.1016/0014-5793(90)80535-Q)

Lee H, Heo L, Lee MS, Seok C. GalaxyPepDock: A protein–peptide docking tool based on interaction similarity and energy optimization. *Nucleic Acids Res*. 2015 Jul; 43(W1):W431–435. <https://doi.org/10.1093/nar/gkv495>

Macalino SJY, Gosu V, Hong S, Choi S. Role of computer-aided drug design in modern drug discovery. *Arch Pharm Res*. 2015 Sept; 38(9):1686–1701. <https://doi.org/10.1007/s12272-015-0640-5>

Malonis RJ, Lai JR, Vergnolle O. Peptide-based vaccines: current progress and future challenges. *Chem Rev*. 2020 Mar; 120(6): 3210–3229. <https://doi.org/10.1021/acs.chemrev.9b00472>

Martini S, Nielsen M, Peters B, Sette A. The immune epitope database and analysis resource program 2003–2018: reflections and outlook. *Immunogenet*. 2020 Nov; 72(1–2):57–76. <https://doi.org/10.1007/s00251-019-01137-6>

McConnell SA, Casadevall A. Immunoglobulin constant regions provide Stabilization to the paratope and enforce epitope specificity. *J Biol Chem*. 2024 Jun; 300(6):107397. <https://doi.org/10.1016/j.jbc.2024.107397>

Parisi G, Piacentini R, Incocciati A, Bonamore A, Macone A, Rupert J, Zacco E, et al. Design of protein-binding peptides with controlled binding affinity: The case of SARS-CoV-2 receptor binding domain and angiotensin-converting enzyme 2 derived peptides. *Front Mol Biosci*. 2024 January; 10(2023):1332359. <https://doi.org/10.3389/fmolb.2023.1332359>

Parker JMR, Guo D, Hodges RS. New hydrophilicity scale derived from high-performance liquid chromatography peptide retention data: correlation of predicted surface residues with antigenicity and x-ray-derived accessible sites. *Biochem*. 1986 Sept; 25(19):5425–5432. <https://doi.org/10.1021/bi00367a013>

Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. UCSF chimera--a visualization system for exploratory research and analysis. *J Comput Chem*. 2004 Oct; 25(13):1605–1612. <https://doi.org/10.1002/jcc.20084>

Qiu Y-H, Liu M, Zhan J, Liu L-L, Zheng J-Y, Wu D, Wu G-L, Cai Y-F, Zhang S-J. N-terminal-targeted anti-amyloid monoclonal antibodies illuminate the therapy for Alzheimer’s disease: A systematic

review and comprehensive meta-analysis. medRxiv. 2023 Nov; <https://doi.org/10.1101/2023.11.20.23298794>

Reverberi R, Reverberi L. Factors affecting the antigen-antibody reaction. *Blood Transfus.* 2007 Oct; 5(4):227–240. <https://doi.org/10.2450/2007.0047-07>

Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 Years. *EMBO Mol Med.* 2016 Jun; 8(6):595–608. <https://doi.org/10.15252/emmm.201606210>

Schreiner TG, Menéndez-González M, Popescu BO. The 'cerebrospinal fluid sink therapeutic strategy' in Alzheimer's disease—from theory to design of applied systems. *Biomed.* 2022 Jul; 10(7):1509. <https://doi.org/10.3390/biomedicines10071509>

Söderberg L, Johannesson M, Nygren P, Laudon H, Eriksson F, Osswald G, Möller C, Lannfelt L. Lecanemab, aducanumab, and gantenerumab — binding profiles to different forms of amyloid-beta might explain efficacy and side effects in clinical trials for Alzheimer's disease. *Neurotherapeutics.* 2023 Jan; 20(1):195–206. <https://doi.org/10.1007/s13311-022-01308-6>

Song C, Shi J, Zhang P, Zhang Y, Xu J, Zhao L, Zhang R, Wang H, Chen H. Immunotherapy for Alzheimer's disease: targeting β -amyloid and beyond. *Transl Neurodegener.* 2022 Mar; 11(1):18. <https://doi.org/10.1186/s40035-022-00292-3>

Tabll AA, Shahein YE, Omran MM, Hussein NA, El-Shershaby A, Petrovic A, Glasnovic M, Smolic R, Smolic M. Monoclonal IgY antibodies: advancements and limitations for immunodiagnosis and immunotherapy applications. *Therapeut Adv Vaccines Immunotherapy.* 2024 Jan; 25151355241264520. <https://doi.org/10.1177/25151355241264520>

Wang J, Youkharibache P, Zhang D, Lanczycki CJ, Geer RC, Madej T, Phan L, et al. 2020. iCn3D, a web-based 3D viewer for sharing 1D/2D/3D representations of biomolecular structures. *Bioinformatics* (Oxford, England). 2020 Jan; 36(1):131–135. <https://doi.org/10.1093/bioinformatics/btz502>

Williams CJ, Headd JJ, Moriarty NW, Prisant MG, Videau LL, Deis LN, Verma V, et al. MolProbity: more and better reference data for improved all-atom structure validation. *Protein Sci: Pub Protein Soc.* 2018 Oct; 27(1):293–315. <https://doi.org/10.1002/pro.3330>

Xue LC, Rodrigues JP, Kastritis PL, Bonvin AM, Vangone A. PRODIGY: A web server for predicting the binding affinity of protein–protein complexes. *Bioinformatics.* 2016 Dec; 32(23):3676–3678. <https://doi.org/10.1093/bioinformatics/btw514>