Pioneering Anti-Inflammatory Therapy

The Path to Precision Therapy with C3aR Peptide Libraries

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*All figures were created by the author unless otherwise indicated.

Introduction

The Body's Immune System's Function

- Helps to **defend the body against infections** and maintains a steady homeostasis in one's body
- Triggers inflammation to combat harmful agents and promote tissue repair

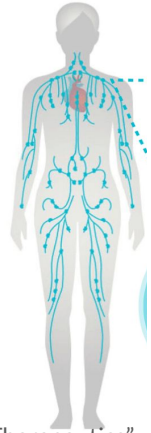
The Complement System

- An integral component of the immune system
- Includes an array of protein complexes that work together to **orchestrate its function** as a mediator against inflammation and other diseases
    - These protein complexes includes anaphylatoxin C3a that would bind with the C3a Receptor (C3aR)

C3aR and C3a

- Anaphylatoxin C3a could bind with the C3a Receptor (C3aR)
- Important in the immune/complement system
- Acts as a chemotactic mediator and is a dual-pathway
- Can perform **anti-inflammatory and pro-inflammatory functions** depending on the cells and diseases

Immune System



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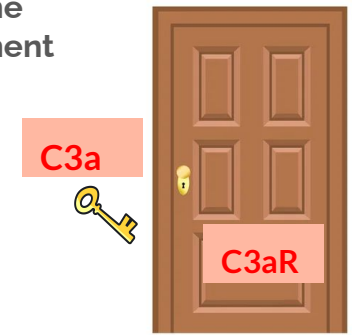
Part of Immune and Complement System



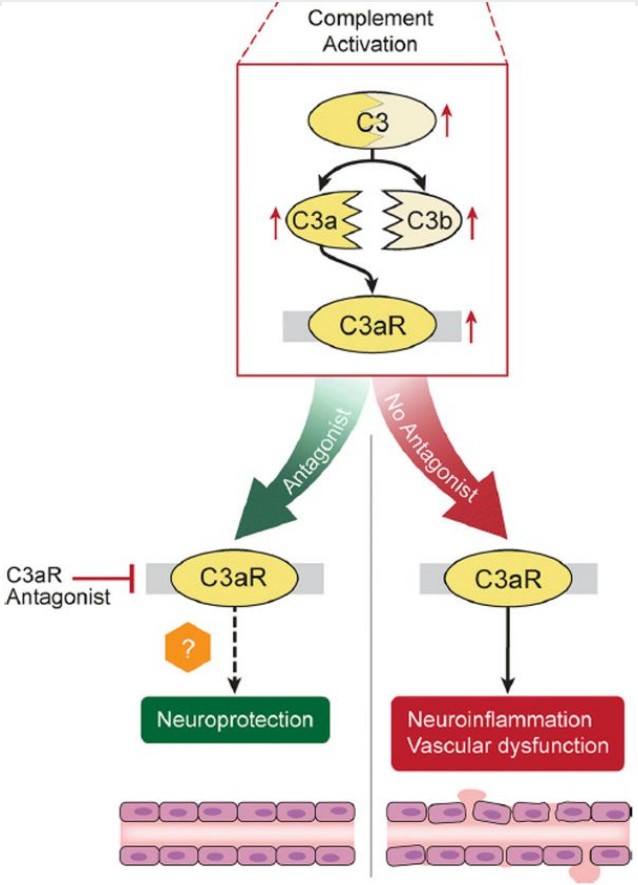
Introduction

Purpose

- Design peptide with higher-affinity binding to C3a Receptor to **enhance itself anti-inflammatory functions**
- Enhance the function of the receptor C3aR in the body, as this receptor controls inflammation

Finding

- **Double mutation** on residue **Glycine 74 and Alanine 76 to strengthen bond between C3a/C3aR; enhancing the receptor's anti-inflammatory function**



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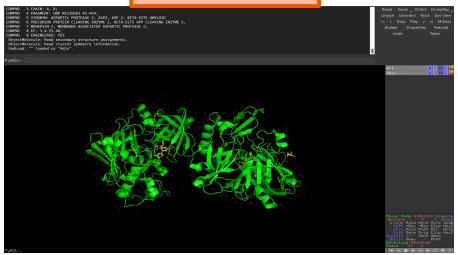
Preparation and Methodology

Structure Preparation	Stability Test	Affinity Test	Single Mutations	Double Mutations
Extracted C3aR and C3a from the 8ig1 complex in PDB and analyzed it in PyMol	Used **select residues** from C3a ligand to run **mutations' stability test**	Used residue scan in MOE to **determine the affinities of the mutations** of the select residues from C3a	Picked mutations with highest binding affinity scores and used MOE to **create mutated ligand** -> put ligand into HDock to run for docking and confidence scores	Picked single mutations with best binding scores and **paired up two mutations in one ligand** -> created mutated ligand in MOE -> tested for docking in HDock

PyMOL



Pymol is a user-friendly molecular visualization system for **creating 3D representations of molecules and proteins.**

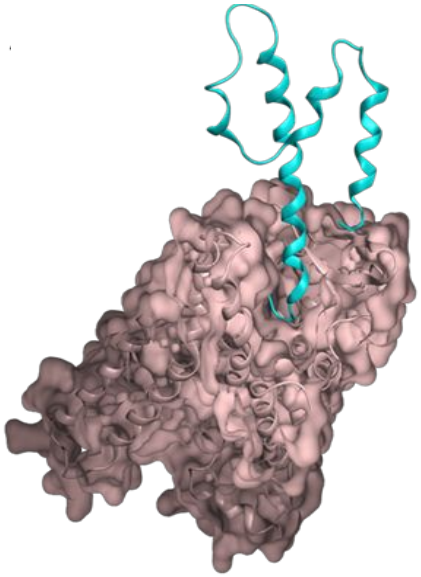
MOE

Molecular Operating Environment (MOE) is a software for **drug discovery, modeling**, **simulations**, and **methodology development**.

Result - structure preparation

- **Compared active site residues** found via HDock Server with Cryo-EM research findings.
- Similar residues **confirmed** our method's accuracy.

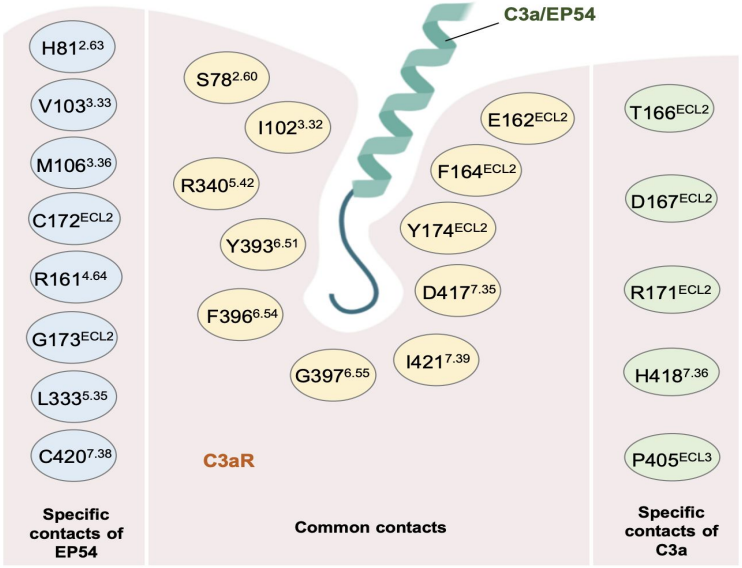
Pymol figure of C3a and C3aR -> helped visualize active/binding site



HDock results of important residues involved in active site

Receptor-C3aR	Ligand-C3a	Distance (Å)
H81	L73	2.767
R161	F74	2.744
R161	R77	2.714
T165	Q3	2.769
D167	Q3	2.541
Y174	R69	2.962
Y174	R77	2.904
T331	R69	2.938
R340	R77	2.764
R393	A76	2.897
R393	R77	2.743
D404	R65	2.83
E406	R64	2.83
E406	R65	2.851
D417	R77	2.783
H418	H72	2.756

Results from research that used Cryo-EM pictures to study residues involved in active site



Retrieved from Yadav, M. K., Yadav, R., Sarma, P., Maharana, J., Soni, C., Saha, S., Singh, V., Ganguly, M., Saha, S., & Khant, H. A. (2023). Structural insights into agonist-binding and activation of the human complement C3a receptor. *bioRxiv*, 2023.2002. 2009.527835.

Result - Stability

Identified key focus residues in C3a protein for this study: 63-77.

Method

Ran mutations in MOE and got the **dStability scores** (stability scores compared to the wild type score)

Results Interpretation

More red = **more negative**

= **Better stability**

What are stability scores?

Indicates interaction and stability among ligand's residues.

Mutations' dStability Table

Mutation	Leu63	Arg64	Arg65	Gln66	His67	Ala68	Arg69	Ala70	Ser71	His72	Leu73	Gly74	Leu75	Ala76	Arg77
A	2.12	0.37	1.15	1.06	0.72	0.00	2.20	0.00	0.27	0.53	2.26	-0.01	2.02	0.00	1.67
C	1.72	0.20	1.62	0.76	0.63	0.55	2.00	0.74	0.37	0.43	2.17	0.12	2.15	0.84	1.55
D	2.11	0.76	0.84	1.09	1.10	0.94	2.82	1.11	1.32	0.95	2.54	0.12	2.47	1.08	2.49
E	1.71	0.84	0.70	0.91	0.29	0.62	2.02	0.85	1.11	0.51	1.94	0.39	1.85	1.70	2.13
F	0.87	-0.49	0.08	0.56	-0.69	0.39	0.24	-0.94	0.02	-0.76	1.48	-0.90	0.54	-0.37	-0.65
G	2.34	0.96	2.33	1.93	1.47	1.48	2.96	1.61	0.72	0.98	3.90	0.00	2.86	1.60	2.38
H	1.64	0.66	1.07	1.63	0.00	1.44	1.85	0.49	0.59	0.00	1.76	-0.58	1.99	0.65	1.40
I	0.43	-0.22	0.17	0.57	-0.66	-0.14	0.61	-0.84	0.31	-0.32	1.25	-1.04	0.67	0.49	0.46
K	1.82	1.10	1.18	1.01	1.04	1.44	1.54	-0.30	0.97	0.12	1.70	-0.38	1.22	1.06	0.49
L	0.00	-0.26	-0.26	0.07	-0.76	-0.33	0.55	-1.08	-0.18	-0.87	0.00	-0.89	0.00	0.01	0.31
M	1.47	-0.05	0.37	0.31	-0.42	-0.10	1.03	-0.22	0.40	-0.13	1.63	-0.44	0.92	0.04	0.23
N	1.67	0.42	1.08	0.51	0.64	0.77	2.10	0.62	0.40	0.67	2.31	0.20	2.01	1.12	1.70
P	2.34	1.45	2.35	2.53	1.27	1.82	2.80	1.76	1.08	0.84	2.77	-0.01	2.03	1.10	1.06
Q	1.51	0.56	0.90	0.00	0.13	0.31	1.46	-0.72	0.65	0.56	2.11	0.59	1.74	0.84	1.43
R	0.72	0.00	0.00	1.03	-0.03	1.05	0.00	-0.25	-0.31	-0.38	0.89	-0.92	0.29	0.14	0.00
S	2.50	0.38	1.79	1.53	0.96	0.75	2.43	0.83	0.00	0.61	2.59	0.46	2.47	1.13	1.78
T	0.82	0.20	0.56	0.86	0.56	0.62	1.67	0.16	0.35	0.43	1.82	0.26	1.63	0.70	1.08
V	0.72	-0.11	0.59	0.94	-0.05	0.19	1.08	-0.32	-0.15	-0.06	1.52	-0.34	0.73	0.62	1.00
W	1.39	-0.70	0.46	-0.06	-0.36	0.09	0.38	-1.57	-0.39	-1.25	0.81	-1.69	0.73	-0.08	-0.84
Y	0.80	-0.18	0.14	0.28	-0.21	0.17	0.91	-0.53	-0.02	-0.13	1.59	-1.31	0.88	-0.49	-0.31

Result - Affinity

What are affinity scores?

Indicates interaction strength and receptor activation between two proteins.

Methods: Same method of using MOE software but ran for affinity scores instead; all of the possible mutations for the key C3a residues (63-77)

Results Interpretation: More Red -> Stronger Affinity for the mutated ligand compared to wild type unchanged ligand -> Stronger Affinity

Most Red and Bolded: Exceptionally negative dAffinity scores = mutation yields high affinity for the ligand when interacted with C3aR

Mutations' dAffinity Table

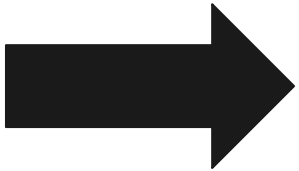
Mutation	Arg64	Arg65	Gln66	His67	Ala68	Arg69	Ala70	Ser71	His72	Leu73	Gly74	Leu75	Ala76	Arg77
A	-0.72	0.32	2.96	5.54	0.00	5.09	0.00	-0.11	4.12	3.44	-0.56	8.40	0.00	7.46
C	-0.69	-1.10	2.67	-1.31	2.09	3.19	-0.48	-2.55	4.65	2.29	-1.52	4.32	-0.85	5.82
D	-1.89	2.26	-0.18	1.44	-0.97	1.34	-0.68	0.70	3.33	1.74	-1.07	7.66	-0.39	5.54
E	-4.02	1.74	-4.22	-3.06	-3.03	2.26	-6.13	-0.60	1.43	-0.36	-4.36	0.45	-3.91	9.09
F	-3.47	-1.65	1.21	-0.75	**-10.34**	-1.41	-0.43	-4.33	3.19	2.28	-2.55	0.18	**-7.44**	-0.26
G	-1.16	1.91	2.84	-0.49	-4.72	5.78	2.50	0.15	5.42	-0.99	0.00	10.49	3.46	8.70
H	-4.24	2.62	-3.11	0.00	-4.90	4.11	-1.23	-1.95	0.00	6.45	**-7.17**	1.84	-6.99	1.57
I	2.46	2.51	3.11	4.71	1.45	3.28	-6.27	0.49	3.62	1.63	-5.62	1.02	-4.04	2.10
K	1.62	-0.07	3.49	-1.71	4.50	-0.02	**-7.71**	0.75	4.80	-0.59	-2.70	-1.16	-6.93	4.43
L	-3.82	-3.16	3.01	0.08	-0.59	0.63	-2.54	-2.24	0.64	0.00	-3.68	0.00	-0.54	1.57
M	-1.15	-3.64	-0.91	0.40	-2.09	1.51	-2.38	-2.74	-1.57	-0.63	-5.33	0.62	-4.34	2.36
N	-2.92	0.29	3.58	1.37	-0.62	2.66	-3.27	0.87	1.33	0.38	-4.34	2.44	-3.22	3.59
P	-3.68	-0.40	3.74	2.46	1.60	5.17	1.58	1.69	5.27	0.88	-3.74	4.09	-0.67	5.07
Q	-1.49	-0.72	0.00	-0.30	3.67	1.75	-6.75	-0.27	-1.20	1.53	**-7.35**	-0.18	-5.68	6.47
R	0.00	0.00	0.34	-1.69	-2.45	0.00	-6.59	-2.83	0.49	-2.32	**-9.76**	2.57	**-12.24**	0.00
S	-2.17	4.30	4.97	4.96	2.28	6.35	-1.05	0.00	5.30	5.31	-2.15	4.10	-2.07	8.07
T	-2.06	3.36	2.08	2.95	1.48	3.12	-3.70	-0.77	3.53	1.69	-1.92	3.58	0.08	4.38
V	-2.11	-0.57	4.33	3.42	0.94	4.79	-0.01	0.83	5.80	2.57	-1.34	2.73	**-7.32**	3.55
W	-3.37	-3.09	-3.75	-2.90	0.94	-1.88	-0.84	-4.70	-1.29	5.52	-6.79	-1.44	3.50	-1.91
Y	1.39	3.17	0.06	-1.58	-2.14	-2.25	-2.30	-1.41	-1.65	-3.22	-3.86	3.34	**-10.34**	0.29

Result - Affinity Summary

Summary of all mutations with exceptionally well affinity from all trials that attained affinity scores

No.	Mutation	Affinity	dAfffinity
1	Wild	-109.88	0
2	A68F	-119.96	-9.56
3	A70Q	-111.39	-7.43
4	A70K	-111.45	-7.71
5	S71W	-116.25	-8.80
6	G74H	-122.80	-7.18
7	G74Q	-123.32	-7.71
8	G74R	-125.48	-9.87
9	A76F	-120.63	-7.44
10	A76R	-125.30	-11.64
11	A76W	-123.95	-9.82
12	A76V	-120.51	-7.32
13	A76Y	-123.53	-10.34

Input mutated ligand from table and receptor into HDock -> **Got binding and confidence scores**



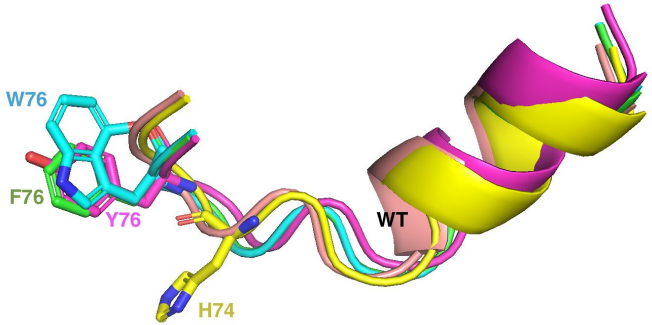
Same table but with docking and confidence scores; highlighted cells are cells with **exceptionally outstanding docking scores** from HDock Server

No.	Mutation	Docking Score	Confidence Score
1	Wild	-226.59	0.9115
2	A68F	-263.82	0.9069
3	A70Q	-275.3	0.9246
4	A70K	-226.75	0.8227
5	S71W	-276.71	0.9265
6	G74H	-309.7	0.9606
7	G74Q	-299.66	0.9523
8	G74R	-292.6	0.9454
9	A76F	-334.36	0.9756
10	A76R	-288.5	0.941
11	A76W	-334.01	0.9754
12	A76V	-284.82	0.9368
13	A76Y	-328.41	0.9726

Result - Single Mutations

Analysis: The mutated ligands, just by looking at it visually, are more bulky and all of them seem to have these aromatic rings. Especially W76, the mutated ligand is extremely bulky with lots of new aromatic rings.

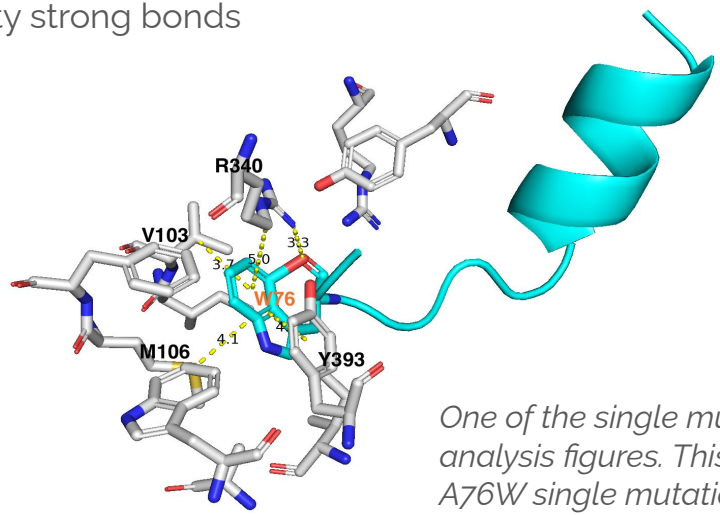
This figure was created in Pymol where all of the mutated ligands with exceptionally high docking scores are aligned together: G74H, A76F, A76W and A76Y.



Method:

Open mutated ligand and C3aR file in MOE → Scan for residues from the C3aR receptor protein nearby the mutated residue → Create bonds from that mutated residue

Analysis: Dotted lines: bonds from the mutated residues. Bonds are all pretty short too (around 3-4 angstrom) so pretty strong bonds



One of the single mutations analysis figures. This one is the A76W single mutation figure

Result - Double Mutations

No.	Mutation	Docking Score	Confidence Score
1	Wild	-226.59	0.9115
2	G74H_A76F	-392.35	0.9922
3	G74H_A76W	-392.21	0.9922
4	G74H_A76Y	-400.56	0.9934

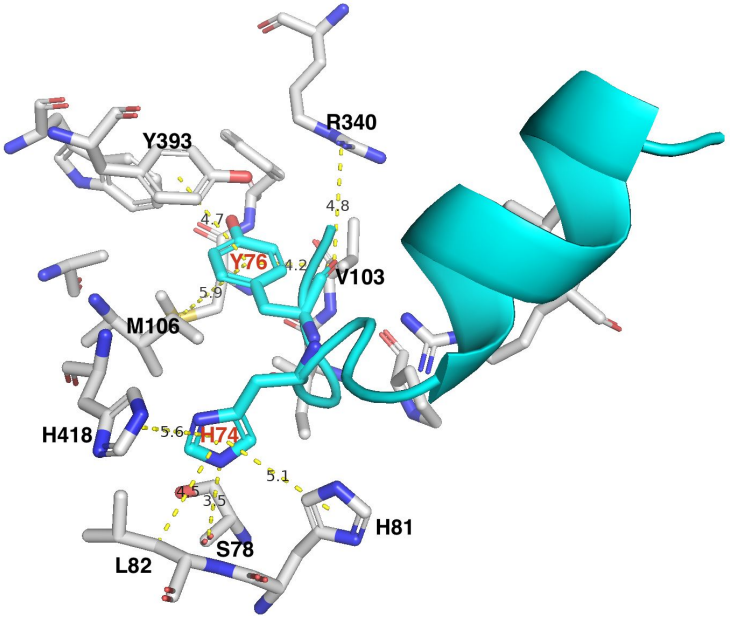
Method:

Pair up single mutations for a mutated ligand ⇨ Double Mutation Strategy (One ligand has 2 mutated residues) ⇨ Input mutated ligand into HDock Server for docking and confidence scores

Scores Analysis: The improved docking and confidence scores over the wild type demonstrate that **double mutations enhance binding strength.**

Method: Exactly the same method as the single mutation bonding analysis; just with **two mutated residues** this time

Analysis: Lots of **new strong bonds** created



Three Main Findings

- Generated a library including **247 peptides** and among those, **13** were tested with higher affinities when binded with receptor especially residues: A68f, A70Q, A70K, S71W, G74H, G74Q, G74R, A76F, A76R, A76W, A76V and A76Y.
- Amongst those mutations, **4** stood out with their docking and confidence scores when ran in HDock paired with C3aR as its receptor: **G74H, A76F, A76W and A76Y**.
- Double Mutations formed from those 4 single mutations created new ligands that had really strong bindings with the receptor C3aR thus further **enhancing the protein complexes anti inflammatory function.**

Further Contributions

- The best mutations and their combinations could lead to potential future compounds for anti-inflammatory therapy or drugs
- Provides a methodology for future target drug discoveries

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