Antibody Modeling

Choice of Antibodies For Lateral Flow Assay

A functional Lateral Flow Assay (LFA) requires two antibodies that recognize non-overlapping epitopes on the target biomarker to serve as the detector and receptor. We used Rosetta modeling to identify suitable antibody pairs from commercially available candidates.

Modeling Procedure

Our wet lab team created a list of commercially available antibodies for our target biomarkers. We found the 3D structure of antibody-biomarker complex on the Protein Data Bank (PDB) for some of them. For antibodies whose structures are not found on PDB, we used the Kotai Antibody Builder to predict their 3D structure from the amino acid sequence (Yamashita et al, 2014). We then predicted their binding sites using Rosetta SnugDock, whose algorithm compensates for errors in computationally predicted antibody structures (Sircar & Gray, 2010).

SnugDock requires the user to provide an initial pose of the antibody-biomarker complex, from which it randomly changes the position of each molecule to generate a user-defined number of new poses (Weitzner et al, 2017). Rosetta calculates the potential energy of each new pose, and the pose with the lowest energy is chosen as the most likely binding configuration (Weitzner et al, 2017). For each antibody, we compared 200 new poses, as recommended to us by Colleen Maillie - a computational biology PhD student at the University of Rochester with expertise in using Rosetta to find protein complex configurations.

We had to provide Rosetta with a specific initial pose as input for this model. For this pose, we identified the segment of antibody variable region - the antibody region that binds to biomarkers - that connects to the non-binding antibody constant region using the IMGT database (Lefranc, 2001). We positioned the antibody such that this connection segment, and consequently the constant region, faces away from the biomarker (Figure 1a). Since the commercial antibodies are intended to inhibit biomarker binding to its biological receptor, it is likely that they bind to the biomarkers at the same site as the biological receptor. Therefore, we found the configuration of the biomarker-receptor complex from the RCSB Protein Data Bank (Berman et al, 2000). We positioned the biomarker such that its antibody epitope (Figure 1a) aligns with the receptor epitope (Figure 1b).

Since we only guessed the binding site on the biomarker in the initial pose, we made SnugDock randomize the orientation of the biomarker for each new pose. Since we were certain of the binding region on the antibody, we only made Rosetta spin the antibody around an axis that would keep the same binding region for each new pose (Figure 1a).

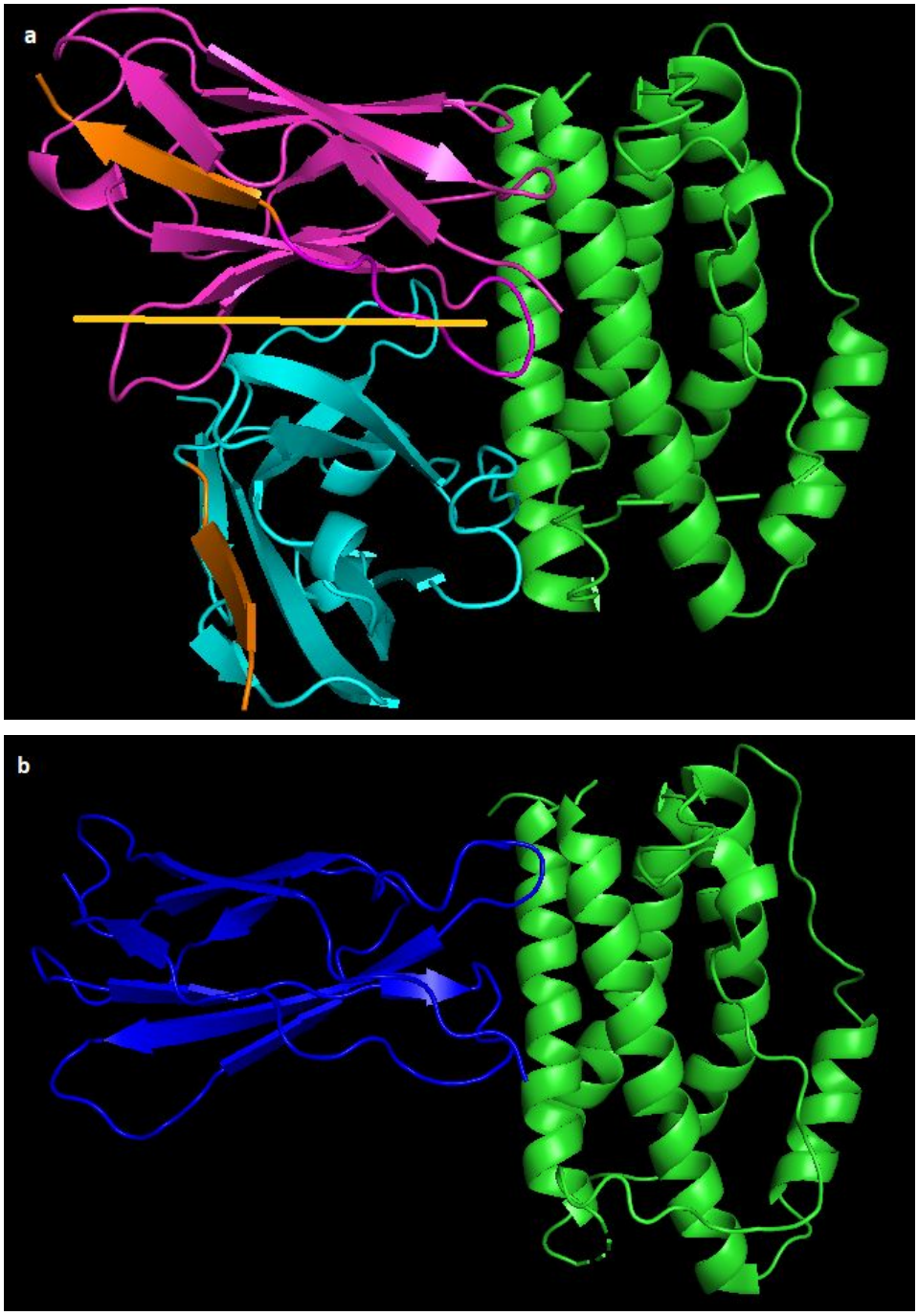

Figure 1: (a) The initial pose of IL-6 (green) and siltuximab variable region (magenta - heavy chain, cyan - light chain). The siltuximab part that connects to its constant region (orange) is faced away from IL-6. Siltuximab is spun around an axis (orange line) for each new pose, such that the same region on siltuximab faces IL-6 in every pose. (b) IL-6 (green) is in a complex with its biological receptor (blue), found from the Protein Data Bank (Boulanger et al, 2003). In the initial pose the biomarker is positioned such that siltuximab has the same epitope on the biomarker as the receptor.

Rosetta SnugDock outputs a PDF of the biomarker-antibody complex, which we visualized in PyMOL. In PyMOL, we identified the residues on the biomarker that are within 4.0 Angstrom to the antibody (Figure 2), which we identified these residues as the binding site. Because 4.0 Angstrom is the length of Van der Waals interactions - the weakest molecular force, contacts within 4.0 Angstrom would include all interactions between the biomarker and antibody, including covalent bonds (Batsanov, 1995). If the binding sites of two antibodies did not share any common residues, they were identified as having non-overlapping epitopes. For each biomarker, we selected an antibody pair with non-overlapping binding sites as the detector-receptor pair.

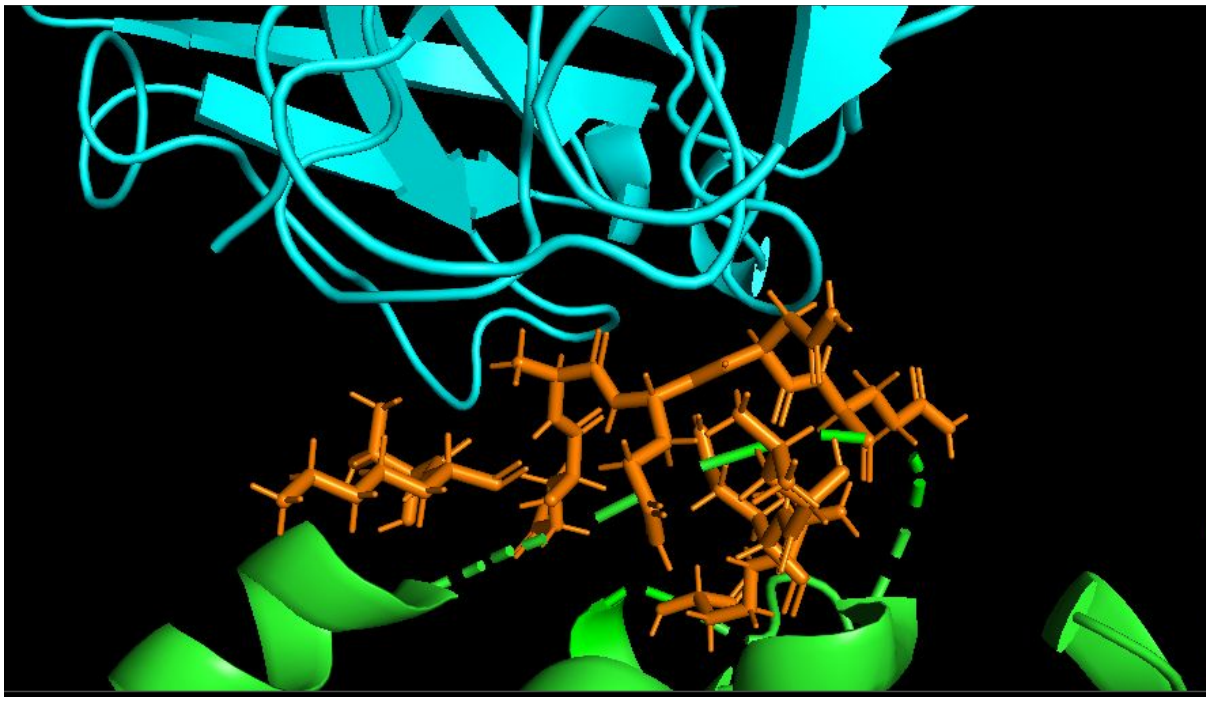

Figure 2: Binding site (orange) of IL-6 (green) to siltuximab (blue). IL-6 residues within 4 Angstrom of siltuximab were found and chosen to be the binding site (orange sticks).

Results

We used modeling to pick detector-receptor pairs for three different biomarkers: IL-6, IL-1B, TNFα (Table 1). No antibody pair with non-overlapping epitopes was found for IGF-1, so IGF-1 was excluded from our test panel. The two commercially available antibodies of CA125 have been reported to have non-overlapping binding sites, so they were not modeled (Marcos-Silva et al, 2014). The binding sites of candidate antibodies are reported in Table 2.

Table 1: Modeled antibodies and chosen detector-receptor pairs for IL-6, IL-1B, and TNFα.

Biomarker	**Antibody**	**Selected**
IL-6	Siltuximab	X
	Olokizumab	
	Clazakizumab	
	Sirukumab	
	Ziltivekimab	X
IL-1B	Gevokizumab	X
	Canakinumab	X
TNFα	Infliximab	
	Certolizumab	X
	Golimumab	
	Adalimumab	X
	Adalimumab Beta	
IGF-1	Dusigitumab	
	Xentuzumab	

Table 2: Predicted binding sites of candidate antibodies: protein residues on the biomarker that have a polar bond with the antibody. The letter denotes the amino acid, and the number denotes the position in the amino chain.

Antibody	**Structure**	**Binding Residues**	**Overlap with**
Siltuximab	Predicted	R104, T149-Q159	Sirukumab Olokizumab
Olokizumab	PDB	S47-N48, L57, N103-E106, A153-D160	Siltuximab Sirukumab
Clazakizumab	Predicted	E69-T82, Q183	
Sirukumab	Predicted	N103, T142-A153	Siltuximab Olokizumab

Ziltivekimab	Predicted	K27-K41, E51, K171-L178	
Gevokizumab	PDB	V72-D75, Q81-R98, A115-P118	
Canakinumab	PDB	V19-K27, Q32-V41, E64-N66, D86-K88, N129	
Infliximab	PDB	Q67-T77, I97, T105-A111, N137-Y141	Certolizumab Golimumab Adalimumab Adalimumab Beta
Certolizumab	PDB	G24, R44D45, I72H73, I83-K90, R131-D140	Adalimumab Golimumab Infliximab
Golimumab	PDB	E23G24, K65-S71, T105-P113, R138-Y141	Infliximab Certolizumab Adalimumab Adalimumab Beta
Adalimumab	PDB	P20-E23, K65, E110-Y115, Y141-S147	Adalimumab Beta Golimumab Infliximab
Adalimumab Beta	Predicted	G24Q25, Q67-H73, R103-A111, R138-Y141	Infliximab Certolizumab Adalimumab Golimumab
Dusigitumab	Predicted	Y24-S34, R55-M59	Xentuzumab
Xentuzumab	Predicted	E3-T4, S33-S34, T41-D45, R50-R56	Dusigitumab

The biomarkers in complex with the antibody pair were visualized in PyMOL (Figure 3, 4, 5). In all three complexes, the detector and receptor bind to distant sites on the biomarker, such that the binding of one antibody to the biomarker does not obstruct the binding of another.

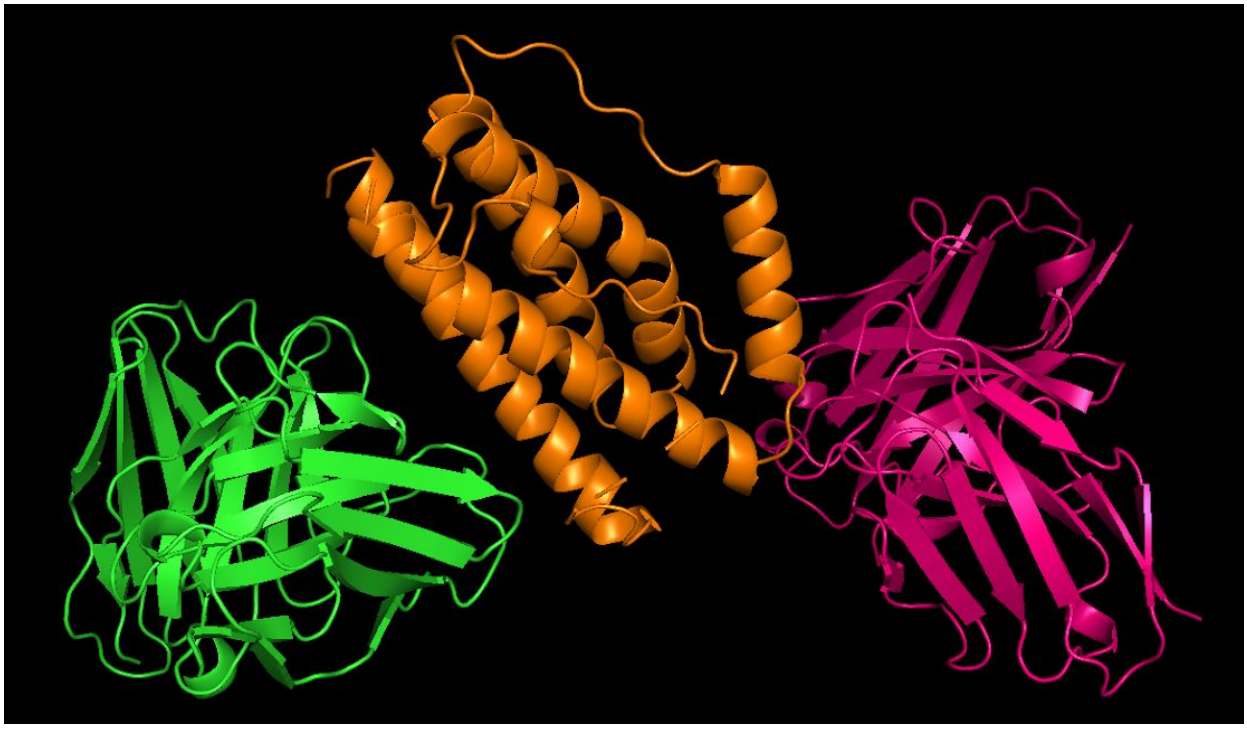

Figure 3: The biomarker is IL6 (orange). We chose our detector-receptor pair to be Ziltivekimab (green) and Siltuximab (pink).

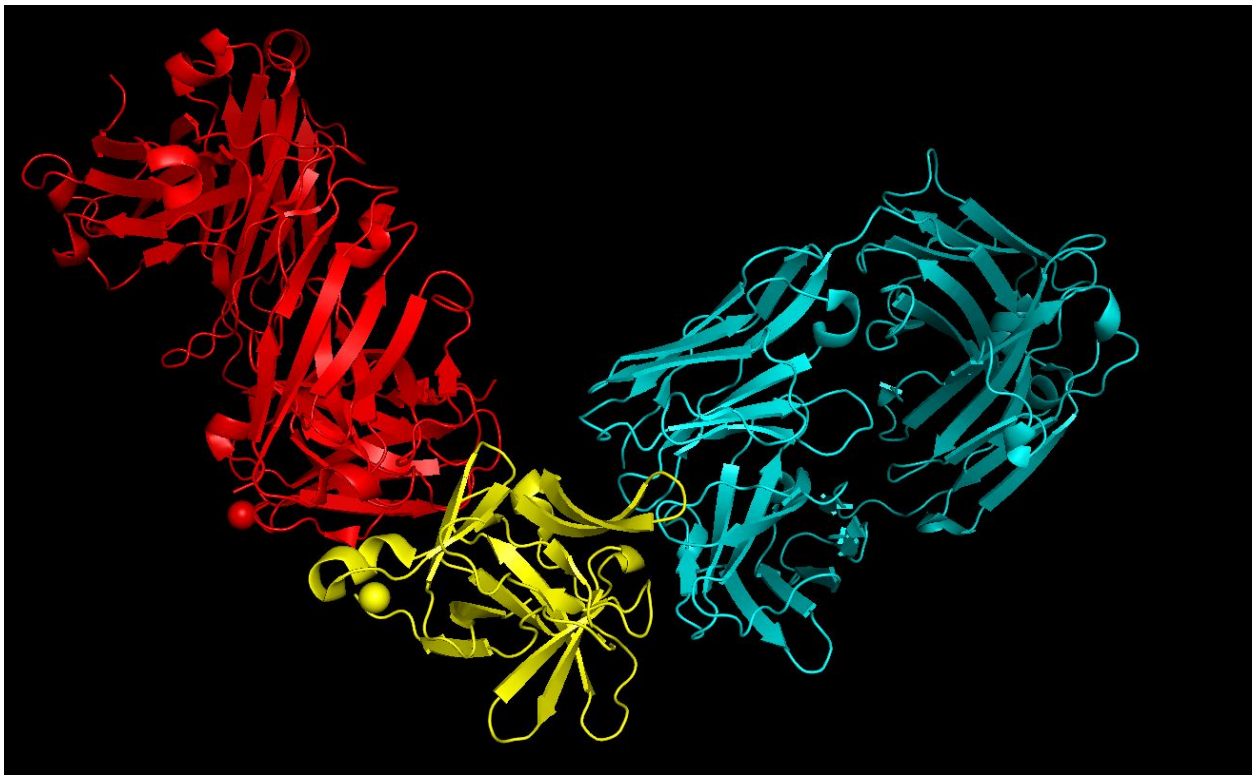

Figure 4: The biomarker is IL1B (yellow). We chose our detector-receptor pair to be Gevokizumab (teal) and Canakinumab (red).

Figure 5: The biomarker is TNFa (magenta). We chose our detector-receptor pair to be Adalimumab (teal) and Certolizumab (dark blue).

Conclusion

Using Rosetta software for structural protein modeling, we identified three suitable pairs of antibodies to be used in our LFA test for three different biomarkers of endometriosis: IL-6, IL-1B, TNFα. Our modeling results can be tested with a sandwich ELISA assay, which measures the ability of two antibodies to bind to an antigen simultaneously (Borel et al, 2017).

References

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