**ChE293**

**Binding Efficiencies of GM1 Ganglioside to Influenza and Coronavirus Matrix and Membrane Proteins**

**Grant Proposal**

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Abstract

As the world becomes increasingly globally connected, epidemics that once would have only affected a region, are capable of becoming pandemics that effect the entirety of the globe. As such the emergence of coronaviruses have become an increasing concern, in particular SARS-CoV-2 has impacted billions of lives and has led to more than 150 million cases [1]. The importance of better understanding coronaviruses and elucidating mechanisms of suppression has become more than obvious and has garnished massive interest from the pharmaceutical industry. Here we show that GM1 ganglioside has favorable binding energies with both SARS and SARS-CoV-2 Membrane proteins (M Proteins). Previous work on GM1 has shown to inhibit proliferation of Haemophilus influenzae [2]. Additionally, the SARS and SARS-CoV-2 M proteins are relatively conserved with multiple regions of high conservation, this suggests that the M Protein could be a potential candidate for drug targeting in not only current strains of coronaviruses but also future strains [3].

* 1. Introduction
  2. Significance

Acute respiratory syndrome (SARS) is a highly pathogenic novel coronavirus that emerged from southern China [4]. While the origin of SARS is still unknown it is largely believed to be zoonotic [5]. The virus largely targets angiotensin converting enzyme 2 (ACE2) receptors, in particular human airway epithelia cells and lung parenchyma [6]. SARS emerged in late 2002 and caused more than 8,000 cases and over 700 deaths [4]. SARS is approximately 30kb long and is comprised of six main domains of interest, ORF1a, ORF1b, spike protein (S protein), envelope protein (E protein), membrane protein (M protein), and nucleocapsid protein (N protein) [7] [8].

Similar to SARS, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is highly pathogenic novel coronavirus that emerged from Wuhan China [9]. The origins of SARS-CoV-2 has been a target of misinformation however the true origins are still unknown [10]. Bioinformatics and sequencing research have indicated that the virus likely originated from Bat SL-CoVZC45, a strain of bat corona virus, with a recombination event at the S protein region [11]. Like most coronaviruses, SARS-CoV-2 is approximately 30kb and comprised of ORF1a, ORF1b, S protein, E protein, M protein, and N protein [12]. While the S protein of SARS and SARS-CoV-2 have differences, both still largely target ACE2 receptors in respiratory cells [13]. Unlikely SARS which has an incubation period of less than a week, SARS-CoV-2 can have an incubation time of 12-13 days and can spread asymptomatically [14] [15]. This has caused SARS-CoV-2 to be especially problematic and due to the speed of modern day travel a sick individual can spread the disease worldwide. Previous work done on coronavirus M proteins has shown that coronavirus M proteins tend to be well conserved with SARS-CoV-2 being between 90-99% similar to other corona viruses ranging from bat to pangolin strains [16].

In SARS the M protein is comprised of 221 amino acids with the transmembrane glycoprotein composing of almost one third of the residues and is the most abundant structural protein of the SARS virion [17]. The M protein in SARS-CoV-2, like in SARS, is the most abundant protein of SARS-CoV-2 [18]. While its role is still not fully discovered, it spans the membrane bilayer of the virion and leaves an NH2-terminal domain on the exterior of the virion with a COOH terminus in the interior [19]. Additionally, the M protein binds with all of the virions structural proteins and helps stabilize the N protein [20].

Interestingly a structural analysis of SARS M protein has been shown it to be analogous to influenzae matrix protein [21]. While no such analysis has been conducted on SARS-CoV-2, due to their conservation it is reasonable to assume that a similar result is to be expected. Influenza matrix protein (M1 protein) is a matrix protein that forms a layer under the nucleocapsid protein and plays multiple roles in the virus [22]. Primarily M1 is responsible for shuttling RNA nucleoproteins to the exterior of the viral membrane [23]. As mentioned previously Gangliosides GM1, GM2, and GM3 all have an effect on inhibiting influenza adherence to cells and as such decrease infection rates of the cells [2]. This presents Gangliosides as a potential candidate for inhibiting coronavirus adherence like it does with influenza.

Gangliosides are acidic glycosphingolipids that consist of an oligoglycosyl backbone which is linked to a ceramide base [24]. Gangliosides, with few exceptions, are found at the exterior of the plasma membrane [25]. They are primarily found in nerve cells however they are still widely found throughout mammalian cells, they often coalesce into lipid rafts and play roles in ion transportation, G protein coupled receptors, neuronal cell differentiation, immune system response and more [26] [27].

Because of the relatively recent emergence of coronaviruses into the human viral pool, crystallography of SARS and SARS-CoV-2 M proteins has not yet been conducted. This requires us to conduct our work on predicted structures of the SARS and SARS-CoV-2 M proteins. Specifically, in this proposal we use Iterative Threading ASSEmbly Refinement (I-Tasser) generated models to conduct our research. I-Tasser is based on sequence-to-structure-to-function paradigm to generate 3D models, first it uses multiple threading alignments and iterative structures to generate a 3D structure, then it uses a native pre-cataloged data base of known protein structures in order to infer the function of the model and to refine the output [28]. Finally, a confidence score is assigned to the model after its generation, this is done in order to ensure accuracy of the final model.

This research is a vital step in not only elucidating the underlying mechanisms of coronaviruses but also in identifying conserved binding domains across the strains. Better understanding such viral interactions can lead to everything ranging from faster turnaround time in drug development, targeted pharmaceuticals that decrease the off-target effects, and treatments with higher success rates than current anti-viral medication. While the implications produced from this field could potentially spans multiple species of viruses, it is even more relevant to coronaviruses in particular. As we have seen in the past decade, there has been a trend towards novel coronaviruses epidemics, and in the case of SARS-CoV-2 pandemic. This makes research, such as this, our first and best response to future outbreaks of future novel coronavirus.

* 1. Aims

**Aim 1:**

Previous work conducted on GM1/2/3 gangliosides show an inhibiting ability of the influenza virus to adhere to cells however we have yet to determine if gangliosides interact with influenza matrix proteins. Additionally, work conducted by Dr. Lustig’s lab indicates that the B-chain from our isolation of a GM1 ganglioside model might have the highest binding affinity, as such in Aim 1, we will be using that B-chain pentasaccharide. **Aim 1A:** Determine if the highest inhibiting ganglioside, GM1, has a binding affinity to influenza M1 protein. **Aim 1B:** If GM1 ganglioside has a binding affinity to the influenza M1 protein then determine if GM1 ganglioside has a binding affinity to SARS M protein which has been previously established in this report to be analogous to influenza M1 protein. **Aim 1C:** If SARS M protein has a binding affinity to GM1 then determine if the highly conserved SARS-CoV-2 M protein has a binding affinity to GM1.

**Aim 2:**

**Aim 2A:** Determine if one of the other GM1 pentasaccharide candidates, I-chain and J-chain, have favorable binding affinities. **Aim 2B:** While less significant in the scope of our research, we should still determine the binding efficiencies of the remaining two non-ganglioside tetrasaccharide chains, A-chain, and C-chain.

**Aim 3:**

If Aims 1 and 2 produce favorable results then we would also like to investigate other gangliosides, in particular GM2 and GM3.

**Aim 4:**

If Aims 1 and 2 produce favorable results we would like to expand our list of coronaviruses tested and compare their binding affinities. A few notable targets of interest are human beta-coronaviruses MERS, OC43, HKU1, and animal beta-coronaviruses Bat-SL-CoVZC45, and Pangolin-CoV-GX/P2V

**Aim 5:**

If Aim 4 produces favorable results, **Aim 5A**: Compare ganglioside binding regions across various coronaviruses and determine their level of sequential and structural conservation. **Aim 5B:** If binding regions are conserved, then determine if more highly conserved regions have a higher likelihood of producing similar binding affinities.

1. Methods

PDB files of GM1 ganglioside pentasaccharide chains and tetrasaccharide chains were acquired by isolating saccharide chains bound to Cholera Toxin B-Pentamer Complex [29]. Influenza M1 proteins 1AA7 and 7JM3 were taken from previous work conducted with X-Ray Diffraction [30] [31]. PDB files for SARS M protein were taken from Group A-2 generated I-Tasser model and SARS-CoV-2 M protein was taken from the Zhamg Lab who generated the SARS-CoV-2 M protein model using I-Tasser [32]. Binding sites of M1 and M proteins were predicted using HotSpot Wizard, PrankWeb, and CASTp [33] [34] [35]. Binding sites which were predicted by more than one prediction tool were then selected to be used in Autodock Vina during binding predictions as seen in Tables. 1, 3, 4 [36]. M1 protein influenza A: 7JM3 binding predictions did not produce any overlapping binding site predictions as shown in Table. 2, all predicted binding sites were used in Autodock Vina for this protein. PyMOL was then used in order to generate 3D models of predicted binding position results from Autodock Vina [37].

1. Preliminary Results

Table 1. Binding Site predictions for M1 protein influenza A: 1AA7

|  |  |  |
| --- | --- | --- |
| **Binding Site predictions for M1 protein influenza A: 1AA7 (https://www.rcsb.org/structure/1AA7)** | | |
| **Tools** | **Chain** | **Residue** |
| HotSpot Wizard | Chain A | A107, A108, A141, A3, A6 |
| Prankweb | Chain A | A\_120 A\_121 A\_122 A\_125 A\_150 A\_154 A\_55 A\_82 A\_83 A\_85 A\_86 A\_87 A\_92 A\_44 A\_47 A\_77 A\_78 A\_79 B\_275,  A\_131 A\_132 A\_134 A\_136 A\_137 A\_138 A\_140 A\_143 A\_62 A\_65 A\_66 A\_71 A\_76 |
| CASTp | Chain A | A55, A83, A85, A86, A87, A121, A125, A150, A154 |

Three protein binding site predicting tools, HotSpot Wizard, Prankweb, and CASTp, were used for M1 protein influenza A: 1AA7, the overlapping predicted proteins are highlighted.

Table 2. Binding Site predictions for M1 protein influenza A: 7JM3

|  |  |  |
| --- | --- | --- |
| **Binding Site predictions for M1 protein influenza A: 7JM3 (https://www.rcsb.org/structure/7JM3)** | | |
| **Tools** | **Chain** | **Residue** |
| HotSpot Wizard | Chain C | 121, 231, 97, 101 |
| Prankweb | Chain C | C\_132 C\_135 C\_39 C\_40 C\_43 C\_65 C\_73 C\_74 C\_75 C\_76 C\_77, C\_121 C\_122 C\_125 C\_150 C\_154 C\_55 C\_83 C\_86, C\_135 C\_138 C\_139 C\_140 C\_65 C\_66 C\_70 C\_71 |
| CASTp | Chain C | SER17, LYS21, HIS162, MET165, ASN 177, VAL180, LEU 181, THR184, THR185, ALA 188, LYS 230, ARG250, PHE251, LYS252 |

Three protein binding site predicting tools, HotSpot Wizard , Prankweb, and CASTp , were used for M1 protein influenza A: 7JM3, No overlapping binding site were predicted.

Table 3. Binding Site predictions for M protein: Severe acute respiratory syndrome coronavirus

|  |  |  |
| --- | --- | --- |
| **Binding Site predictions for M protein: Severe acute respiratory syndrome coronavirus (SARS-CoV)** | | |
| **Tools** | **Chain** | **Residue** |
| HotSpot Wizard | A | 64 71 72 74 75 78 82 |
| Prankweb | A | 43, 45 49, 109, A\_53 A\_54 A\_56 A\_57 A\_60 A\_84 A\_85 A\_87 A\_88 A\_91 A\_92 A\_95, A\_110 A\_111 A\_114 A\_38 A\_49 A\_50, 153 A\_53 A\_91 A\_94 A\_95 A\_98, 112, 113, 115 |
| CASTp | A | 41 42 43 45 46 49 109, 112 113 115 131 153 154 |

Three protein binding site predicting tools, HotSpot Wizard, Prankweb, and CASTp, were used for M protein: Severe acute respiratory syndrome coronavirus, the overlapping predicted proteins are highlighted.

Table 4. Binding Site predictions for M protein: Severe acute respiratory syndrome coronavirus 2

|  |  |  |
| --- | --- | --- |
| **Binding Site predictions for M protein: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)** | | |
| **Tools** | **Chain** | **Residue** |
| HotSpot Wizard | A | VAL10, ILE73, LYS14, ARG72, THR77, GLU11, LEU13, GLU18, PHE65, ALA68, TYR71, ASP3 |
| Prankweb | A | 21, 22,25,28,29,61,65,84,88,81, \_114  \_116  \_132  \_134  \_154  \_155  \_39  \_40  \_41  \_42  \_44  \_47,   \_14  \_17  \_18  \_21  \_64  \_65  \_68  \_73  \_77  \_80  \_81,  \_100  \_103  \_109  \_112  \_55  \_96  \_99,   \_105  \_121  \_123  \_169 |
| CASTp | A | ALA38, TYR39, ALA40, ASN41, ARG42, ARG44, TYR47, PRO114,THR116, THR130, ARG131, PRO132, LEU 133, LEU134, GLY153, HIS154, HIS155, LEU156 |

Three protein binding site predicting tools, HotSpot Wizard, Prankweb, and CASTp, were used for M protein: Severe acute respiratory syndrome coronavirus 2, the overlapping predicted proteins are highlighted.

**Aim 1A:**

Figure 1. Predicted Binding Position of Ligand GM1 pentasaccharide B-Chain to Influenza A M1 protein (1AA7)

**A picture containing coelenterate, coral

Description automatically generated**

Figure 1. Predicted Energy of Ligand GM1 B-Chain to Influenza A M1 protein (1AA7). Ball and stick binding of highest predicted affinity binding site generated using PyMOL

Table 5. Predicted Energy of Ligand GM1 pentasaccharide B-Chain to Influenza A M1 protein (1AA7)

**Table

Description automatically generated**

Nine best predicted bindings using overlapping sites taken from Table 1. Generated using Autodock Vina.

Figure 2. Predicted Binding Position of Ligand GM1 pentasaccharide B-Chain to Influenza A M1 protein (7JM3)

**A picture containing plant

Description automatically generated**

Figure 2. Predicted Energy of Ligand GM1 B-Chain to Influenza A M1 protein (7JM3). Ball and stick binding of highest predicted affinity binding site generated using PyMOL

Table 6. Predicted Energy of Ligand GM1 pentasaccharide B-Chain to Influenza A M1 protein (7JM3)

**mode |   affinity | dist from best mode**

**| (kcal/mol) | rmsd l.b.| rmsd u.b.**

**-----+------------+----------+----------**

**1         -6.8      0.000      0.000**

**2         -6.4      8.809     15.056**

**3         -6.4      4.578     12.028**

**4         -6.3     15.211     19.622**

**5         -6.3     14.279     17.994**

**6         -6.2     27.635     31.424**

**7         -6.2     27.721     31.424**

**8         -6.2     26.966     30.821**

**9         -6.2      3.001     11.032**

**Writing output ... done.**

Nine best predicted bindings using all predicted binding sites taken from Table 2. Generated using Autodock Vina

**Aim 1B:**

Figure 3. Predicted Binding Position of Ligand GM1 pentasaccharide B-Chain to SARS M Protein Model

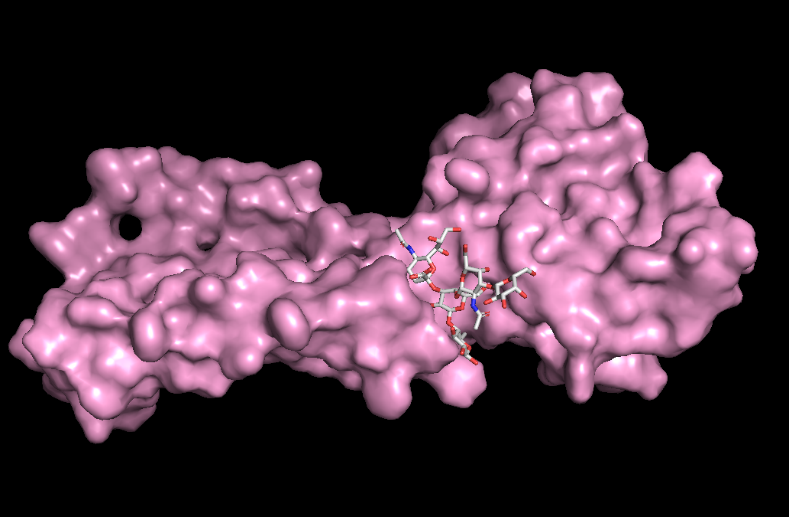
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Figure 3. Predicted Energy of Ligand GM1 B-Chain to SARS Matrix Protein Model. Ball and stick binding of highest predicted affinity binding site generated using PyMOL

Table 7. Predicted Energy of Ligand GM1 pentasaccharide B-Chain to SARS Matrix Protein Model

**mode |   affinity | dist from best mode**

**| (kcal/mol) | rmsd l.b.| rmsd u.b.**

**-----+------------+----------+----------**

**1         -7.3      0.000      0.000**

**2         -7.0      4.052      9.092**

**3         -7.0      1.586      2.654**

**4         -6.9      3.913      9.061**

**5         -6.7      4.038      9.803**

**6         -6.5      1.817      3.283**

**7         -6.4      4.233      9.865**

**8         -6.4      2.071      3.463**

**9         -6.3      2.372     10.101**

**Writing output ... done.**

nine best predicted bindings using overlapping sites taken from Table 3. Generated using Autodock Vina

**Aim 1C:**

Figure 3. Predicted Binding Position of Ligand GM1 pentasaccharide B-Chain to SARS-CoV-2 M Protein Model

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Figure 3. Predicted Energy of Ligand GM1 B-Chain to SARS-CoV-2 Matrix Protein Model. Ball and stick binding of highest predicted affinity binding site generated using PyMOL

Table 8. Predicted Energy of Ligand GM1 pentasaccharide B-Chain to SARS-CoV-2 Matrix Protein Model

**Table

Description automatically generated**

Nine best predicted bindings using overlapping sites taken from Table 4. Generated using Autodock Vina

**Aim 2A:**

Table 9. Binding Energies of GM1 pentasaccharide I-Chain and J-Chain to Target Protein

|  |  |  |
| --- | --- | --- |
|  | **Ligand GM1 pentasaccharide**  **I-Chain** | **Ligand GM1 pentasaccharide**  **J-Chain** |
| **Influenza A**  **M1 protein (1AA7)** | **Table  Description automatically generated** | **Table  Description automatically generated** |
| **Influenza A**  **M1 protein (7JM3)** | **mode |   affinity | dist from best mode**  **| (kcal/mol) | rmsd l.b.| rmsd u.b.**  **-----+------------+----------+----------**  **1         -6.7      0.000      0.000**  **2         -6.3      7.716     14.500**  **3         -6.1      1.343      2.541**  **4         -6.0      8.123     13.043**  **5         -5.9     15.112     18.375**  **6         -5.9     15.246     19.995**  **7         -5.9     15.599     19.642**  **8         -5.8     28.207     31.598**  **9         -5.8     14.630     18.689**  **Writing output ... done.** | **mode |   affinity | dist from best mode**  **| (kcal/mol) | rmsd l.b.| rmsd u.b.**  **-----+------------+----------+----------**  **1         -6.6      0.000      0.000**  **2         -6.5      4.733     10.419**  **3         -6.4     27.600     30.971**  **4         -6.3      2.237      9.869**  **5         -6.3     26.794     30.195**  **6         -6.3     23.948     27.600**  **7         -6.2     27.221     30.559**  **8         -6.2     27.182     30.547**  **9         -6.2     27.615     30.847**  **Writing output ... done.** |
| **SARS**  **M protein Models** | **mode |   affinity | dist from best mode**  **| (kcal/mol) | rmsd l.b.| rmsd u.b.**  **-----+------------+----------+----------**  **1         -7.6      0.000      0.000**  **2         -6.8      2.865      9.421**  **3         -6.7      2.746     10.875**  **4         -6.7      1.639      2.645**  **5         -6.6      2.779      9.484**  **6         -6.5      3.961      8.678**  **7         -6.5      3.077      8.715**  **8         -6.4     10.460     14.926**  **9         -6.4      7.843     13.293**  **Writing output ... done.** | **mode |   affinity | dist from best mode**  **| (kcal/mol) | rmsd l.b.| rmsd u.b.**  **-----+------------+----------+----------**  **1         -7.1      0.000      0.000**  **2         -7.1      1.628      2.382**  **3         -7.0      1.637      2.731**  **4         -6.5      3.925     10.102**  **5         -6.4      2.897     10.523**  **6         -6.3      2.259      7.461**  **7         -6.3      6.432     10.468**  **8         -6.3      4.547     10.490**  **9         -6.2      4.190     10.215**  **Writing output ... done.** |
| **SARS-CoV2**  **M protein Models** | Table  Description automatically generated | **Table  Description automatically generated** |

**Aim 2B:**

Table 10. Table 9. Binding Energies of tetrasaccharide A-Chain and C-Chain to Target Protein

|  |  |  |
| --- | --- | --- |
|  | **Tetrasaccharide A-Chain** | **Tetrasaccharide C-Chain** |
| **Influenza A**  **M1 protein (1AA7)** |  |  |
| **Influenza A**  **M1 protein (7JM3)** | **mode |   affinity | dist from best mode**  **| (kcal/mol) | rmsd l.b.| rmsd u.b.**  **-----+------------+----------+----------**  **1         -6.9      0.000      0.000**  **2         -6.5      3.432     11.027**  **3         -6.5      3.275      6.918**  **4         -6.4     27.964     32.408**  **5         -6.2     28.861     33.005**  **6         -6.1     12.201     16.970**  **7         -6.1      5.051      8.427**  **8         -6.1      3.406      7.169**  **9         -6.1     27.703     32.232**  **Writing output ... done.** | **mode |   affinity | dist from best mode**  **| (kcal/mol) | rmsd l.b.| rmsd u.b.**  **-----+------------+----------+----------**  **1         -6.0      0.000      0.000**  **2         -5.9      2.028      7.538**  **3         -5.8      3.069     10.612**  **4         -5.7     23.420     28.479**  **5         -5.6     28.216     32.462**  **6         -5.6     25.670     28.730**  **7         -5.6     28.245     32.531**  **8         -5.5     27.573     32.052**  **9         -5.5     27.928     30.852**  **Writing output ... done.** |
| **SARS**  **M protein Models** | **mode |   affinity | dist from best mode**  **| (kcal/mol) | rmsd l.b.| rmsd u.b.**  **-----+------------+----------+----------**  **1         -6.6      0.000      0.000**  **2         -6.5      1.604      3.116**  **3         -6.5      3.410      9.383**  **4         -6.1      1.547      2.113**  **5         -6.1      3.213      8.901**  **6         -6.1      3.028      9.487**  **7         -6.0      2.788      8.774**  **8         -5.9      3.258      8.932**  **9         -5.9      4.079      7.578**  **Writing output ... done.** | **mode |   affinity | dist from best mode**  **| (kcal/mol) | rmsd l.b.| rmsd u.b.**  **-----+------------+----------+----------**  **1         -7.0      0.000      0.000**  **2         -6.9      3.324      6.430**  **3         -6.6      3.540     11.737**  **4         -6.4      4.592     11.138**  **5         -6.4      4.399     11.057**  **6         -6.3      3.548     11.640**  **7         -6.2      4.695      8.536**  **8         -6.0      3.931     10.518**  **9         -6.0      5.490      9.603**  **Writing output ... done.** |
| **SARS-CoV2**  **M protein Models** |  |  |

1. Personal Contributions

Helped lead the team and alocate tasks and responsablities to my peers, ivolved in preliminary background research on the topic at hand, helped isolate GM1 ganglioside chains for use in experiments as well as find other PDB files needed. Ran receptor binding site predictions using HotSpot Wizard and helped identify overlapping resedues across multiple binding site predicting programs. Ran binding afinities for SARS Matrix Protein Model using saccharide chains A, B, C, J and Influenza A M1 protein (7JM3) using saccharide chains B, C, I, J.

1. Proposed Tasks

The preliminary results look promising, this suggests a potential target for inhibiting both SARS and SARS-CoV-2 adhesion to cells. Because of the high binding affinity of the tested pentasaccharide and the similarity between binding affinities, we are interested in further exploring the binding interactions of GM1 ganglioside with various other strains of coronavirus and their M proteins as stated in Aim 4. Now that we have streamlined our methods, we expect a significantly shorter turn over time for generating these results and we believe we could accomplish it within one to three weeks depending on the availability of M protein 3D structures.

Similarly, to Aim 4 we believe that due to our streamlined methods the limiting time variant will be to procure other ganglioside 3D models and depending on their availability we estimate about a three-week period to generate data. However, if we meet our goals for both Aim 3 and 4 than we expect to run energy binding calculations on the entire array of gangliosides and M proteins and as such we expect an additional two weeks to generate these results.

Finally, we are especially interested in better understanding the conservation of these coronaviruses M protein ganglioside binding regions on both the sequential and structural level. These results are often time consuming and especially so with the array of gangliosides and M proteins we anticipate. Generating and interpreting these results could take approximately two months.

1. References

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