Protein Structure Comparison

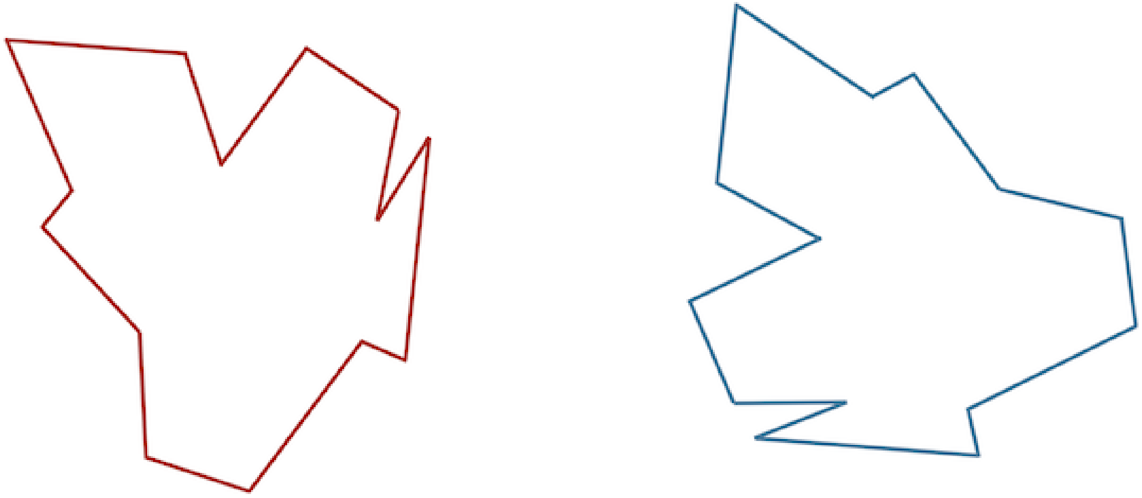
biologicalmodeling.org/coronavirus/accuracy

In this lesson, we will compare the results of the SARS-CoV-2 spike protein prediction from the previous lesson against each other and against the protein's empirically validated structure. To do so, we need a method of comparing two structures.

Comparing two shapes with the Kabsch algorithm

Comparing two protein structures is intrinsically similar to comparing two shapes, such as those shown in the figure below.

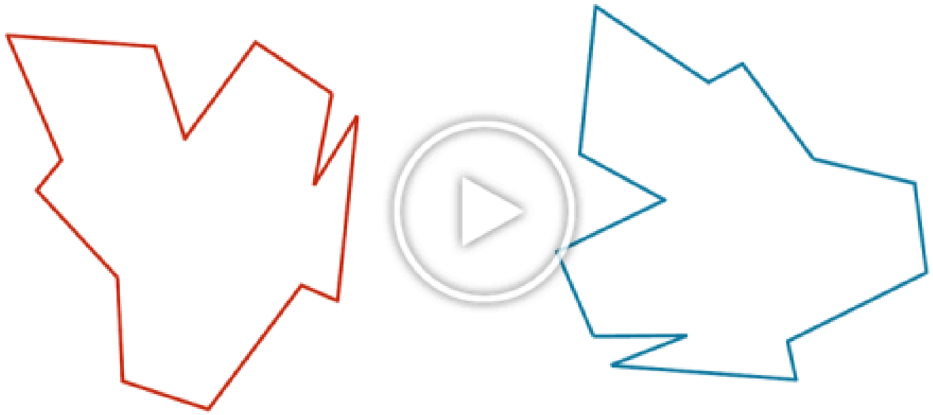
STOP: Consider the two shapes in the figure below. How similar are they?



If you think you have a good handle on comparing the above two shapes, then it is because humans have very highly evolved eyes and brains. As we will see in the next module, training a computer to detect and differentiate objects is more difficult than you think!

We would like to develop a distance function $d(S, T)$ quantifying how different two shapes S and T are. If S and T are the same, then $d(S, T)$ should be equal to zero; the more different S and T become, the larger d should become.

You may have noticed that the two shapes in the preceding figure are, in fact, identical. To demonstrate that this is true, we can first move the red shape to superimpose it over the blue shape, then flip the red shape, and finally rotate it so that its boundary coincides with the blue shape, as shown in the animation below. In general, if a shape S can be translated, flipped, and/or rotated to produce shape T, then S and T are the same shape, and so $d(S, T)$ should be equal to zero. The question is what $d(S, T)$ should be if S and T are not the same shape.



We can transform the red shape into the blue shape by translating it, flipping it, and then rotating it.

Our idea for defining $d(S, T)$, then, is first to translate, flip, and rotate S so that it resembles T "as much as possible" to give us a fair comparison. Once we have done so, we will devise a metric to quantify the difference between the two shapes that will represent $d(S, T)$.

We first translate S to have the same **center of mass** (or **center of mass**) as T. The center of mass of S is found at the point (x_S, y_S) such that x_S and y_S are the respective averages of the x-coordinates and y-coordinates on the boundary of S.

The center of mass of some shapes can be determined mathematically. But for irregular shapes, we will first sample n points from the boundary of S and then estimate x_S and y_S as the average of all the respective x- and y-coordinates from the sampled points.

After finding the center of mass of the two shapes S and T that we wish to compare, we translate S so that it has the same center of mass as T. We then wish to find the rotation of S, possibly along with a flip as well, that makes the shape resemble T as much as possible.

Imagine that we have found the desired rotation; we are now ready to define $d(S, T)$ in the following way. We sample n points along the boundary of each shape, converting S and T into **vectors s** = $(s_1, \ldots, s_n)$ and **t** = $(t_1, \ldots, t_n)$, where s_i is the i-th point on the boundary of S. The **root mean square deviation (RMSD)** between the two shapes is the square root of the average squared distance between corresponding points in the vectors,

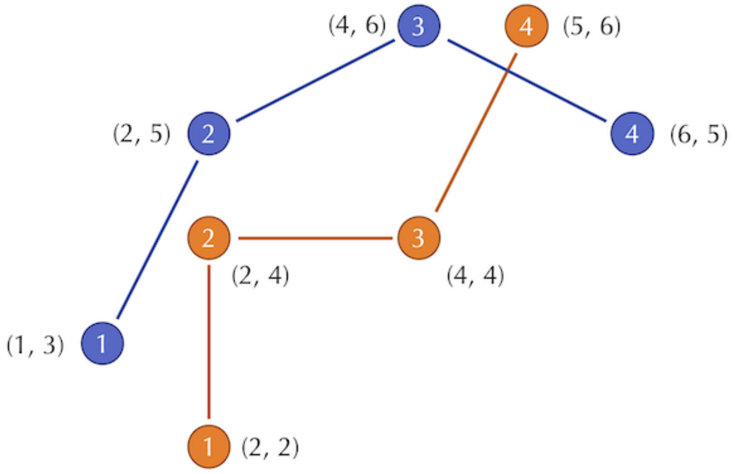
RMSD(s,t)=1n·(d(s1,t1)2+d(s2,t2)2+⋯+d(sn,tn)2)−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−−√.

RMSD(s,t)=1n·(d(s1,t1)2+d(s2,t2)2+⋯+d(sn,tn)2).

In this formula, $d(s_i, t_i)$ is the distance between the points s_i and t_i.

Note: RMSD is a very commonly used approach across data science when measuring the differences between two vectors.

For an example two-dimensional RMSD calculation, consider the figure below, which shows two shapes with four points sampled from each. (Note: for simplicity, the shapes do not have the same center of mass.)



Two shapes with four points sampled from each.

The distances between corresponding points in this figure are equal to 2–√2, 1, 4, and 2–√2. As a result, we compute the RMSD as

RMSD(s,t)=14·(2–√2+12+22+2–√2)—————————————————————√=14·9————√=94——√=32

RMSD(s,t)=14·(22+12+22+22)=14·9=94=32

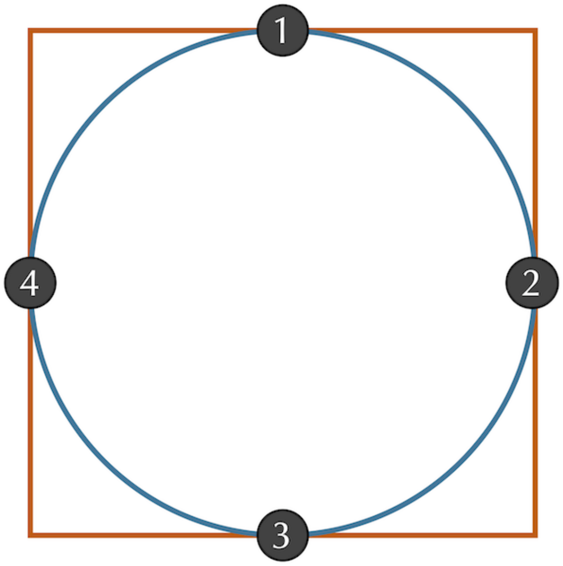
STOP: Do you see any issues with using RMSD to compare two shapes?

Even if we assume that two shapes have already been overlapped and rotated appropriately, we still should ensure that we sample enough points to give a good approximation of how different the shapes are. Consider a circle inscribed within a square, as shown in the figure below. If we happened to sample only the four points indicated, then we would sample the same points for each shape and conclude that the RMSD between these two shapes is equal to zero. Fortunately, this issue is easily resolved by making sure to sample enough points from the shape boundaries.

A circle inscribed within a square. Sampling of the four points where the shapes intersect will give a flawed estimate of zero for the RMSD.

However, we have still assumed that we already rotated (and possibly flipped) *S* to be as “similar” to *T* as possible. In practice, after superimposing *S* and *T* to have the same center of mass, we would like to find the flip and/or rotation of *S* that *minimizes* the RMSD between our vectorizations of *S* and *T* over all possible ways of flipping and rotating *S*. It is this minimum RMSD that we define as *d*(*S*, *T*).

The best way of rotating and flipping *S* so as to minimize the RMSD between the resulting vectors *s* and *t* can be found with a method called the **Kabsch algorithm**. Explaining this algorithm requires some advanced linear algebra and is beyond the scope of our work but is described here.

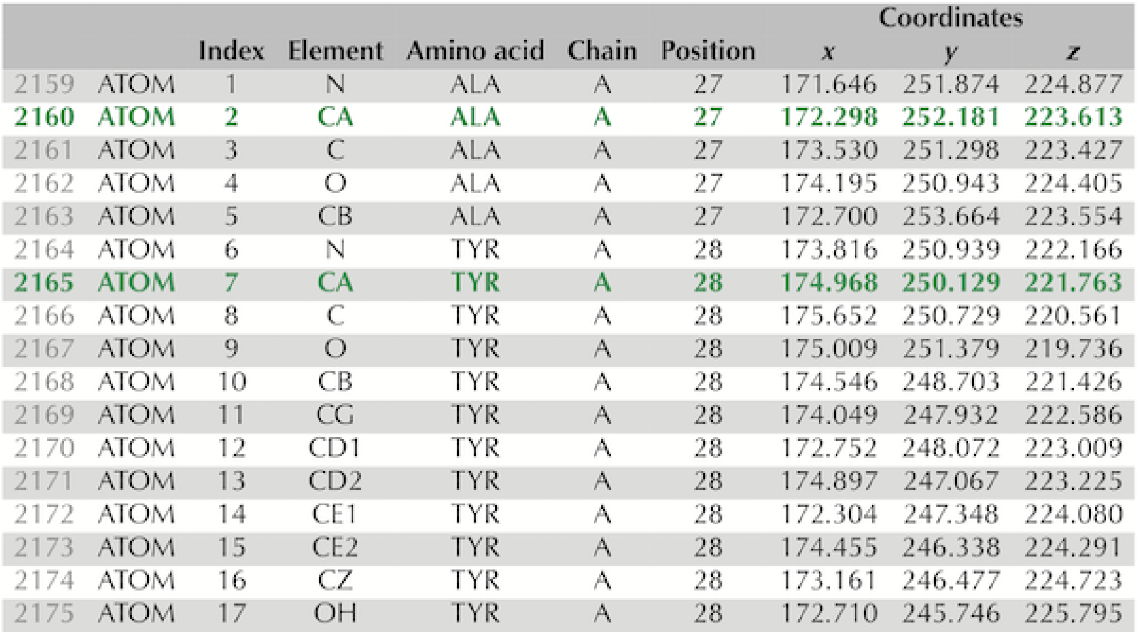


PDB format represents a protein’s structure

The Kabsch algorithm offers a compelling way to determine the similarity of two protein structures. We can convert a protein containing *n* amino acids into a vector of length *n* by selecting a single representative point from each amino acid. We typically use the alpha carbon, the amino acid’s centrally located carbon atom.

Whether a protein structure is experimentally validated or predicted by an algorithm, the structure is often represented in a unified file format used by the PDB called `.pdb` format. In this format (see the figure below), each atom in the protein is labeled according to several different characteristics, including:

1. the element from which the atom derives;
2. the amino acid in which the atom is contained;
3. the chain on which this amino acid is found;
4. the position of the amino acid within this chain; and
5. the 3D coordinates (x, y, z) of the atom in angstroms (10^{-10} meters).



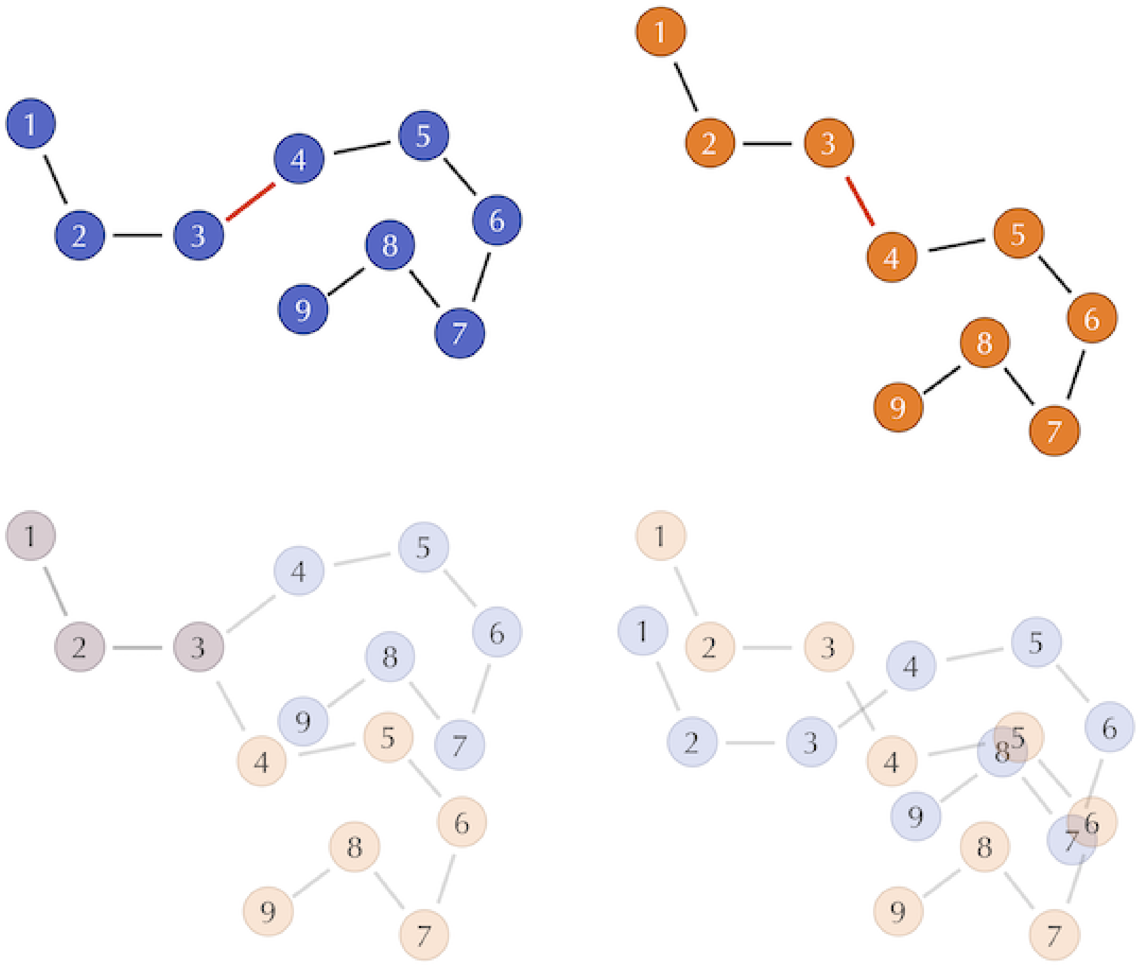
							Coordinates		
		Index	Element	Amino acid	Chain	Position	*x*	*y*	*z*
2159	ATOM	1	N	ALA	A	27	171.646	251.874	224.877
2160	ATOM	2	CA	ALA	A	27	172.298	252.181	223.613
2161	ATOM	3	C	ALA	A	27	173.530	251.298	223.427
2162	ATOM	4	O	ALA	A	27	174.195	250.943	224.405
2163	ATOM	5	CB	ALA	A	27	172.700	253.664	223.554
2164	ATOM	6	N	TYR	A	28	173.816	250.939	222.166
2165	ATOM	7	CA	TYR	A	28	174.968	250.129	221.763
2166	ATOM	8	C	TYR	A	28	175.652	250.729	220.561
2167	ATOM	9	O	TYR	A	28	175.009	251.379	219.736
2168	ATOM	10	CB	TYR	A	28	174.546	248.703	221.426
2169	ATOM	11	CG	TYR	A	28	174.049	247.932	222.586
2170	ATOM	12	CD1	TYR	A	28	172.752	248.072	223.009
2171	ATOM	13	CD2	TYR	A	28	174.897	247.067	223.225
2172	ATOM	14	CE1	TYR	A	28	172.304	247.348	224.080
2173	ATOM	15	CE2	TYR	A	28	174.455	246.338	224.291
2174	ATOM	16	CZ	TYR	A	28	173.161	246.477	224.723
2175	ATOM	17	OH	TYR	A	28	172.710	245.746	225.795

Lines 2,159 to 2,175 of the `.pdb` file for the experimentally verified SARS-CoV-2 spike protein structure, PDB entry 6vxx. These 17 lines contain information on the atoms taken from two amino acids, alanine and tyrosine. The rows corresponding to these amino acids' alpha carbons are shown in green and appear as "CA" under the "Element" column. We have labeled the columns to make it clear what each column corresponds to: "Index" refers to the number of the amino acid; "Element" identifies the chemical element to which this atom corresponds; "Chain" indicates which chain the atom is found on; "Position" identifies the position in the protein of the amino acid from which the atom is taken; "Coordinates" indicates the x, y, and z coordinates of the atom's location (in angstroms).

Note: The above figure shows just part of the information needed to fully represent a protein structure. For example, a `.pdb` file will also contain information about the disulfide bonds between amino acids. For more information, consult the official PDB documentation).

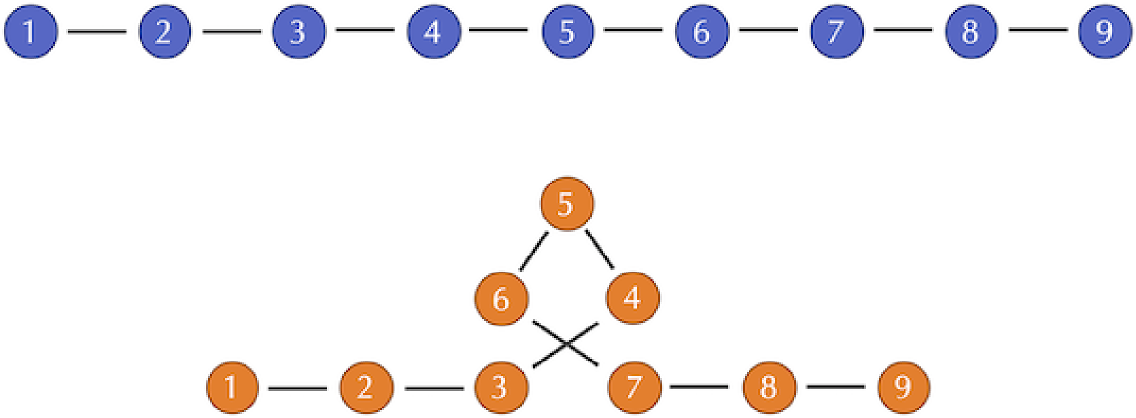
The Kabsch algorithm can be fooled

Although the Kabsch algorithm is powerful, we should be careful when using it. Consider the figure below, which shows two toy protein structures; the orange structure (S) is identical to the blue structure (T) except for a change in a single bond angle between the third and fourth amino acids. And yet this tiny change in the protein's structure causes a significant increase in $d(s_i, t_i)$ for every i greater than 3, which inflates the RMSD.



(Top) Two hypothetical protein structures that differ in only a single bond angle between the third and fourth amino acids, shown in red. Each circle represents an alpha carbon. (Bottom left) Superimposing the first three amino acids shows how much the change in the bond angle throws off the computation of RMSD by increasing the distances between corresponding alpha carbons. (Bottom right) The Kabsch algorithm would align the centers of gravity of the two structures in order to minimize RMSD between corresponding alpha carbons. This alignment belies the similarity in the structures and makes it difficult for the untrained observer to notice that the two proteins only differ in a single bond angle.

Another way in which the Kabsch algorithm can be tricked is in the case of an appended substructure that throws off the ordering of the amino acids. The following figure shows a toy example of a structure into which we incorporate a loop, thus throwing off the natural order of comparing amino acids. (The same effect is caused if one or more amino acids are deleted from one of the two proteins.)



Two toy two protein structures, one of which includes a loop of three amino acids. After the loop, each amino acid in the orange structure will be compared against an amino acid that occurs farther long in the blue structure, thus increasing $d(s_i, t_i)^2$ for each such amino acid.

To address this second issue, biologists will often align the sequences of two proteins first, discarding any positions that do not align well when it comes time to perform the RMSD calculation. We will soon see an example of a protein sequence alignment when comparing the coronavirus spike proteins.

In short, if the RMSD of two proteins is *large*, then we should be wary of concluding that the proteins are very different, and we may need to combine RMSD with other methods of structure comparison. But if the RMSD is *small* (e.g., just a few angstroms), then we can have confidence that the proteins are indeed similar.

We are now ready to apply the Kabsch algorithm to compare the structures that we predicted for human hemoglobin subunit alpha and the SARS-CoV-2 spike protein against their experimentally validated structures.

Visit tutorial

Assessing the accuracy of our structure prediction models

In the tutorials occurring earlier in this module, we used publicly available protein structure prediction servers to predict the structure of human hemoglobin subunit alpha (using *ab initio* modeling) and the SARS-CoV-2 spike protein (using homology modeling). We will now see how well our models performed by showing the values of RMSD produced by the Kabsch algorithm when comparing each of these models against the validated structures.

Ab initio (QUARK) models of Human Hemoglobin Subunit Alpha

The table below shows the RMSD between each of the five predicted structures returned by QUARK and the validated structure of human hemoglobin subunit alpha (PDB entry: 1si4). We are tempted to conclude that our *ab initio* prediction was a success. However, because human hemoglobin subunit alpha is so short (141 amino acids), researchers would consider this RMSD score to be high.

Quark Model	RMSD
QUARK1	1.58
QUARK2	2.0988
QUARK3	3.11
QUARK4	1.9343
QUARK5	2.6495

It is tempting to conclude that our *ab initio* prediction was a success. However, because human hemoglobin subunit alpha is such a short protein (141 amino acids), researchers would consider this RMSD score high.

Homology models of the SARS-CoV-2 spike protein

In the homology tutorial, we used SWISS-MODEL and Robetta to predict the structure of the SARS-CoV-2 spike protein, and we used GalaxyWeb to predict the structure of this protein's receptor binding domain (RBD).

GalaxyWEB

First, we consider the five GalaxyWEB models produced for the spike protein RBD. The following table shows the RMSD between each of these models and the validated SARS-CoV-2 RBD (PDB entry: 6lzg).

GalaxyWEB	RMSD
Galaxy1	0.1775
Galaxy2	0.1459
Galaxy3	0.1526
Galaxy4	0.1434
Galaxy5	0.1202

All of these models have an excellent RMSD score and can be considered very accurate. Note that their RMSD is more than an order of magnitude lower than the RMSD computed for our *ab initio* model of hemoglobin subunit alpha, despite the fact that the RBD is longer (229 amino acids).

SWISS-MODEL

We now shift to homology models of the entire spike protein and start with SWISS-MODEL. The following table shows the RMSD between each of three structures produced by SWISS-MODEL and the validated structure of the SARS-CoV-2 spike protein (PDB entry: 6vxx).

SWISS MODEL	RMSD
SWISS1	5.8518
SWISS2	11.3432
SWISS3	11.3432

The first structure has a lowest RMSD over the three models, and even though its RMSD (5.818) is significantly higher than what we saw for the GalaxyWEB prediction for the RBD, keep in mind that the spike protein is 1281 amino acids long, and so the sensitivity of RMSD to slight changes should give us confidence that our models are on the right track.

Robetta

Finally, we produced five predicted structures of a single chain of the SARS-CoV-2 spike protein using Robetta. The following table compares each of them against the validated structure of the SARS-CoV-2 spike protein (PDB: 6vxx).

Robetta	RMSD
Robetta1	3.1189
Robetta2	3.7568
Robetta3	2.9972
Robetta4	2.5852
Robetta5	12.0975

STOP: Which do you think performed more accurately on our predictions: SWISS-MODEL or Robetta?

SSGCID

The SSGCID models of the S protein released by Rosetta@Home used large amounts of computational resources. Therefore, we might expect to see RMSD scores lower than those of our models. Like before, we will compare the models to the validated structure of (PDB: 6vxx). This time, we will assess the accuracy of predictions of a single chain as well as of the entire spike protein.

SSGCID	RMSD (Full Protein)	RMSD (Single Chain)
SSGCID1	3.505	2.7843
SSGCID2	2.3274	2.107
SSGCID3	2.12	1.866

SSGCID	RMSD (Full Protein)	RMSD (Single Chain)
SSGCID4	2.0854	2.047
SSGCID5	4.9636	4.6443

As we might expect due to having access to thousands of users' computers, the SSGCID models outperform our SWISS-MODEL models. Yet a typical threshold for whether a predicted structure is accurate is if its RMSD compared to a validated structure is smaller than 2.0 angstroms, a test that the models in the above table do not pass.

The inability of even powerful models to obtain an accurate predicted structure may make it seem that protein structure prediction is a lost cause. Perhaps biochemists should head back to expensive experimental validations and ignore the musings of computational scientists. In the conclusion to part 1, we will find hope.

Next lesson

Comments