Molecular Modelling Virtual Chemistry Resource

PyMol Experiment Answer Sheet

Well done for completing our PyMol experiment! We really hope you enjoyed it!

Please make sure you have completed our post experiment questionnaire.
https://kings.onlinesurveys.ac.uk/post-questionnaire-for-outtheboxthinking-resources

Send us photos, comments, or thoughts to either our twitter or Instagram accounts using the hashtag #outtheboxthinking. We also encourage you to make a poster of your data and email it to us.

Exploring how proteins are formed

Press "Amino Acid"

Q. What is the R group in the side chain of this amino acid?

CH3- Methyl Group

Q. Which amino acid is shown here?

Alanine

Press the button "ALA_ASP" and an additional amino acid will appear

Q. What is the chemical formula of the side chain of the new amino acid?

CH_2COOH

Q. What is the name of the second, new amino acid?

Aspartic Acid or Aspartate

Press the button "Bonded_Ala_Asp"

Q. What colour(s) is the amide bond connecting the two amino acids?

Blue and green. It is important to highlight the bond between the nitrogen of one amino acid and the carbonyl of the second amino acid and not any other bond (Figure 1).

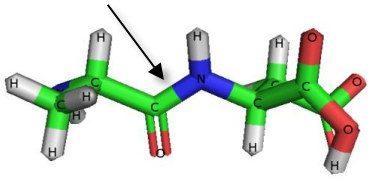


Figure 1: Arrow points to the amide bond formed between two amino acids.

Q. Which elements have been lost from each amino acid following their bonding?

Amino acid 1:: Alanine has lost an oxygen and a hydrogen

Amino acid 2: Aspartic Acid has lost a hydrogen

If you are unsure why this is correct look at figure 2 and open the PyMol document again to visualise the bonding

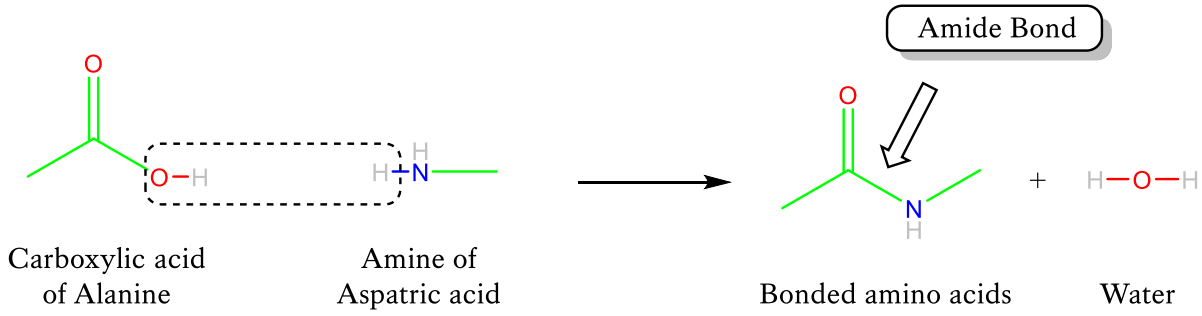


Figure 2: Reaction scheme for amino acid bonding, resulting in the formation of an amide bond and the loss of water.

Q. What molecule is lost as a side product of this reaction?

Water

Q. What is the general name for this type of reaction?

A condensation reaction

Click on the button "Peptide"

Q. Which 7 amino acids are present in this peptide

ALA = Alanine
ASP = Aspartic Acid / Aspartate
ASN = Asparagine
ARG = Arginine
CYS = Cysteine
GLN = Glutamine
HIS = Histidine

Click on the button "Protein".

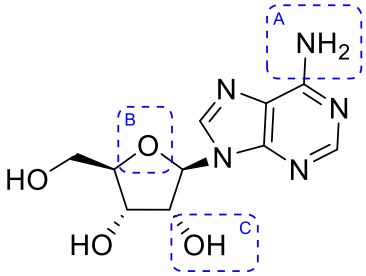
Q. Which protein do you think this structure might be? Hint – What protein is the drug trying to target?

Adenosine A2A receptor

Exploring how the drug molecule binds to the protein

Click on the button "Adenosine"

Q. Below is the skeletal structure of the chemical adenosine. Name all the functional groups highlighted.



A: Amine

B: Ether

C: Alcohol

Q. Which elements do you think are likely to be involved in the bonding of the adenosine molecule to the protein?

Elements that can be involved in hydrogen bonding – oxygen and nitrogen

Click the button "Adenosine_bound"

Q. **Record which 4 amino acids are involved in the binding of adenosine to the protein, and where in the protein sequence these amino acids come.**

SER-277 = Serine and it is the 277th amino acid in the protein sequence
HIS-278 = Histidine and it is the 278th amino acid in the protein sequence
ASN-253 = Asparagine and it is the 253rd amino acid in the protein sequence
GLU-169 = Glutamic acid/Glutamate and it is the 169th amino acid in the protein sequence

Q. Which elements within adenosine are facilitating its binding to the protein?

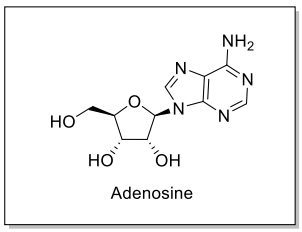
Nitrogen and oxygen

Q. What type of bonding do you think is likely to be occurring between these elements?

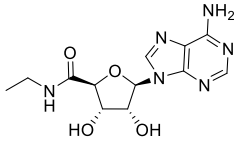
Intermolecular hydrogen bonding (oxygen as a H-bond acceptor, nitrogen as a H-bond acceptor and a H-bond donor).

Time to make your hypothesis

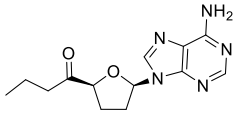
Look at the structure of adenosine compared to the drug molecule candidates below.



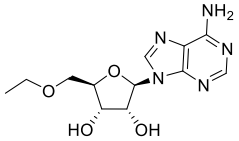
Adenosine



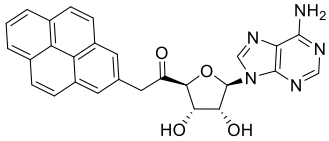
Candidate 1



Candidate 2



Candidate 3



Candidate 4

Q. Think about all the information learnt so far about the binding of adenosine to the receptor protein. Looking at the structure of adenosine and which elements are important for binding, which drug candidate would you predict will have the best binding to the adenosine binding pocket of the protein and why?

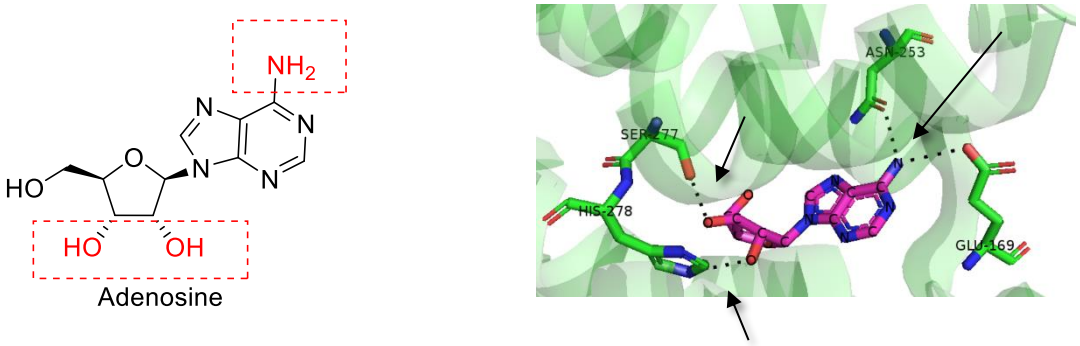
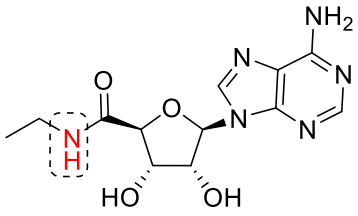


Figure 3: Skeletal structure of adenosine with key functional groups important for binding highlighted (left). PyMol image of adenosine binding to A_{2A} receptor, arrows show key interactions with the binding pocket (right).

Based on the binding of adenosine the key interaction sites are the amine and the two alcohol functional groups present at the base of the sugar ring (figure 4). All candidates except for candidate 2 have these key functional groups. Candidate 2 is missing the alcohols. This therefore suggests that all the candidates except for candidate 2 could show similar binding to the protein molecule, comparable to adenosine binding. Candidate 3 has the most similar structure to adenosine so could be the best.

However, knowing that nitrogen and oxygen are important in facilitating binding to the protein molecule, candidate 1 has the key functional groups essential for binding adenosine to the protein but also has the addition of an electron rich donor nitrogen. This may allow for additional binding of the molecule to the protein and therefore makes it the most likely candidate to have the best interactions with the binding pocket.



Candidate 1

The hypothesis is therefore that candidate 1 will have the best binding and candidate 2 will have the worst. Candidate 3 and 4 will likely show similar binding to adenosine

The experiment

Click through each of the candidate molecules one by one in the bound and the filled orientations. (eg. for candidate 1 look at "Candidate1_bound" and Candidate1_Filled") Fill out the answers to the questions in the table below. When rating the binding remember that we want the drug candidate to bind to the adenosine binding pocket ideally with a higher affinity than adenosine. Remember to scroll your wheel downward to remove the surface of the protein to look at how well the molecule fits into the binding pocket when looking at the candidate filled.

	1	2	3	4
How many amino acids are involved in the binding of this molecule to the protein?	6	2	5	4
Which amino acids are these, including their sequence number?	Histidine 278th Serine 277th Threonine 88th Histidine 250th Asparagine 253rd Glutamic acid 169th	Asparagine 253rd Glutamic acid 169th	Histidine 278th Serine 277th Threonine 88th Glutamic acid 169th Asparagine 253rd	Histidine 278th Serine 277th Glutamic acid 169th Asparagine 253rd
How many hydrogen donors/acceptors on the candidate molecule arefacilitating bindingto the protein?	5– 2 nitrogen, 3 oxygen	1- Nitrogen	4 - 1 nitrogen, 3 oxygen	4 – 2 nitrogen, 2 oxygen
Looking at the "candidate filled". Does the molecule fit into the binding pocket?	Yes	Yes	Yes	No
Rate the binding of each molecule from 1-4. With 1 being best and 4 being worst.	1st - best	3rd	2nd	4th - worst

Summarise your data

Q. Based on all the data you have gathered from your experiment in the table above, and everything else you have learnt throughout this worksheet, explain which candidate drug molecule you have chosen and why. You can use screen shots to help your audience understand your answer.

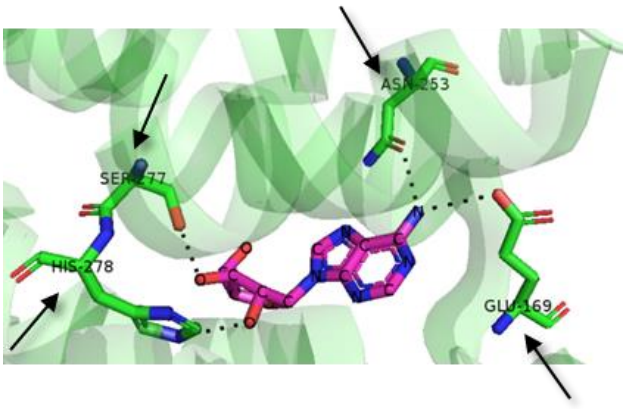
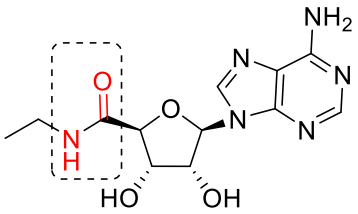


Figure 4: PyMol image of key amino acids in the binding of adenosine to the receptor protein

The endogenous (naturally occurring in the body) molecule is adenosine. The binding of adenosine is facilitated by Histidine 278, Serine 277, Asparagine 253, and Glutamic acid 169 in the adenosine binding pocket of the A_{2A} receptor (Figure 5). To recommend a drug molecule for biological testing it should therefore have similar or better binding than adenosine in this pocket



Candidate 1

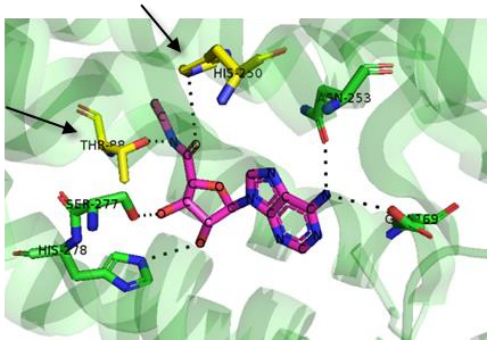


Figure 5: Skeletal structure of candidate 1 highlighting the addition of an amide (left). PyMol image of candidate 1 binding with arrows highlighting additional interactions with amino acids THR 88 and HIS 250 (right).

Candidate 1 has the best interaction with the protein molecule with 5 hydrogen donors and acceptors facilitating binding. The molecule has a higher affinity than adenosine when binding due to the addition of an amide, which results in further interaction occurring between the nitrogen of the amide and a threonine residue at position 88 on the amino acid chain, and the oxygen of the amide with the histidine at residue 250 (Figure 6). Looking at the molecule filled it fits nicely within the binding pocket of the protein (Figure 7).

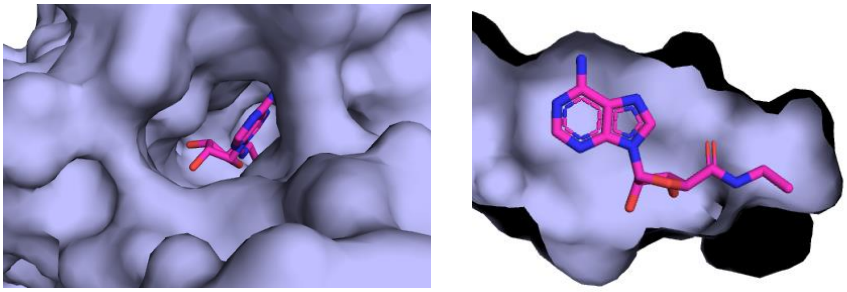


Figure 6: PyMol image of binding of candidate 1 in the adenosine binding pocket of the A_{2A} receptor protein.

This suggests candidate 1 would be a good fit for the adenosine binding pocket in the protein and could potentially elicit a response. Therefore, this candidate should be taken forward into testing.

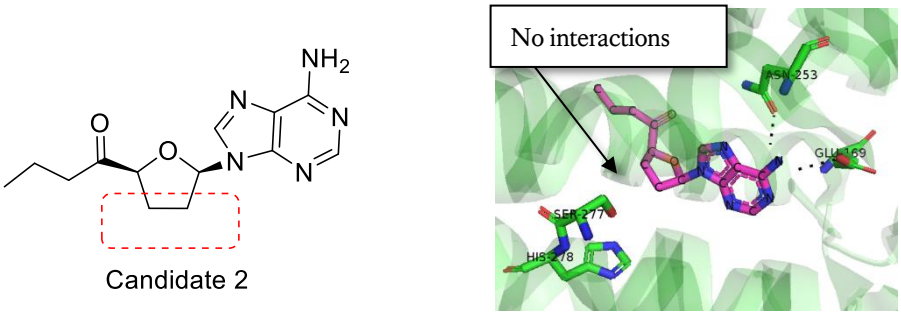


Figure 7: Skeletal structure of candidate 2 highlighting the loss of the two alcohol groups at the base of the sugar ring (left). PyMol image of candidate 2 binding, arrow highlights the absence of interaction between the amino acids SER277 and HIS278 due to the loss of the alcohol groups (right).

As predicted candidate 2 shows the worst binding due to the lack of the essential alcohol groups and therefore it is unable to form important hydrogen bonds with the protein. It has therefore lost interaction with amino acids Histidine 278, Serine 277 (Figure 8). It should therefore not be recommended for testing.

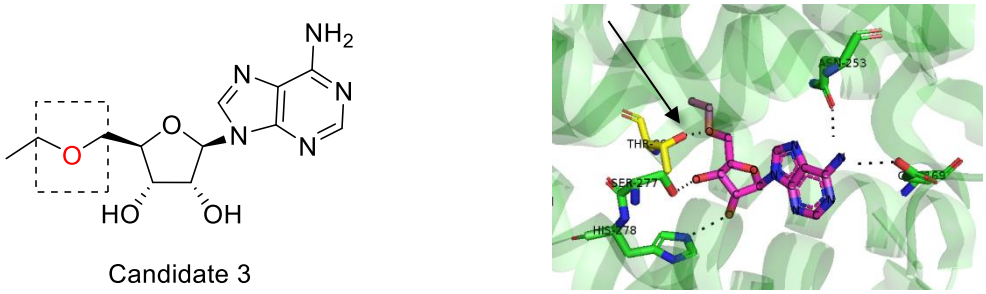


Figure 8: Skeletal structure of candidate 3 highlighting the addition of an ether (left). PyMol image of candidate 3 binding with arrows highlighting additional interactions with amino acid THR88 (right).

Candidate 3 should also be recommended to go through to biological testing. It has a higher affinity binding than adenosine with interaction with 5 amino acids facilitated by 4 hydrogen donor/acceptors. The addition of an ether has allowed for a further interaction site with a threonine residue at position 88 on the amino acid chain (Figure 9). Additionally, it also fits nicely within the binding pocket (Figure 10).

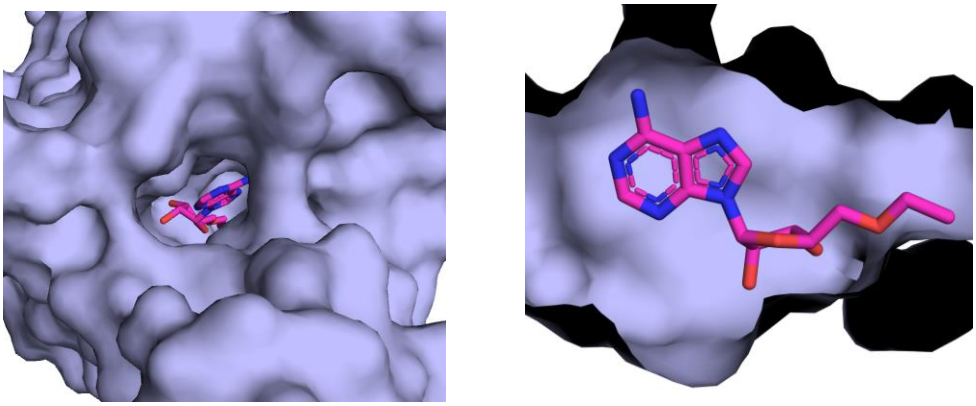
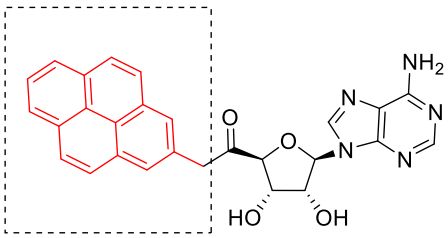


Figure 9: PyMol image of binding of candidate 3 in the adenosine binding pocket of the A_{2A} receptor protein

However, candidate 1 is better still and considering that sending multiple candidates to biological testing will accrue more costs, this candidate should only be recommended for testing if the budget allows.



Candidate 4

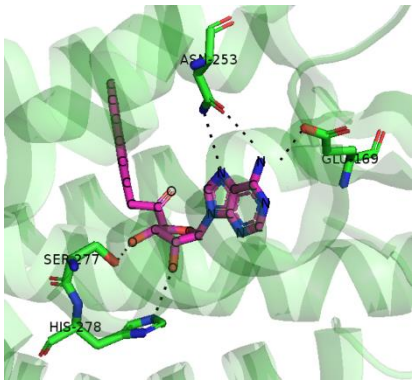


Figure 10: Skeletal structure of candidate 4 highlighting the addition of a large aromatic ring system (left). PyMol image of candidate 4 binding (right).

Candidate 4 has identical binding to adenosine with interactions occurring at Histidine 278, Serine 277, Asparagine 253, and Glutamic acid 169 in the adenosine binding pocket of the A_{2A} receptor (Figure 11). This would therefore suggest that it is a good candidate. However, when looking at this candidate in the filled orientation it is obvious that it does not fit into the adenosine binding pocket (Figure 12). This is due to its very bulky aromatic ring system. This addition results in a considerably larger molecule which does not fit in the adenosine binding pocket and therefore should not be recommended for testing.

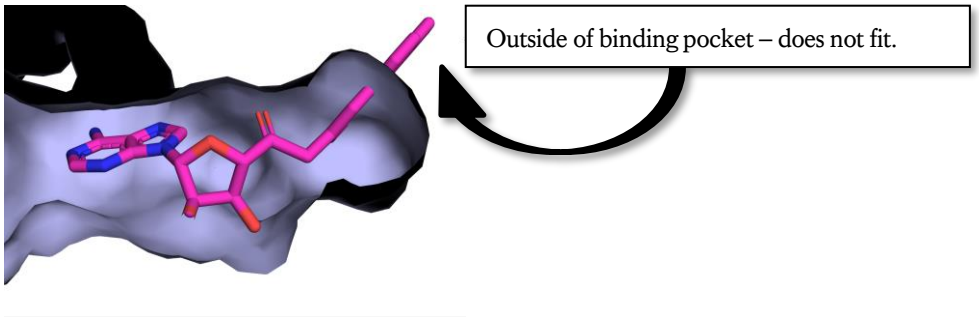


Figure 11: PyMol image of binding of candidate 4 in the adenosine binding pocket of the A_{2A} receptor protein. The large aromatic ring structure protrudes outside of the binding pocket showing that it does not fit.

Thank you for completing our PyMol resource, we hope you enjoyed it and learnt something new.