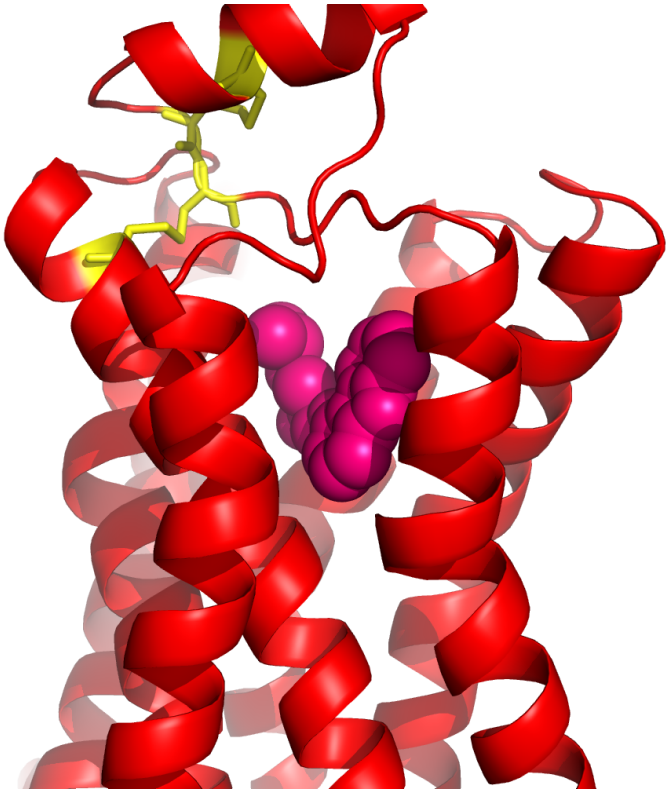
Structural Biology 2017
Assignment 2

Investigation of protein ligand binding sites.



Notes

The assignment is to be carried out individually. The files that you need are available from the course Moodle page, and you will also find the tutorial document on protein structure prediction and the matlab code that we worked through in the tutorial helpful for this assignment.

Due: via Moodle by 5.45pm on Thursday 15th June 2017. This is a fixed deadline and there will be no extensions.

Page limit: There is a limit of 6 pages, including figures, this is the absolute maximum and any additional pages will not be read. Minimum font size is 11pt.

Please keep answers short, but avoid simple yes/no answers and use figures and tables where appropriate. It is in your interests to be lucid and concise, and to think about the most informative way to present your findings.

Submission: write-ups must be submitted via Moodle as one single PDF file. If you have any technical difficulties with the assignment please email Lucy Colwell (ljc37@cam.ac.uk).

Aim

The focus of this assignment is to explore the binding sites of ligand-protein complexes and their mutual interactions in more detail. Throughout the course, we have discussed the different type of physico-chemical interactions that are important for interactions between proteins and small molecules. To help you complete this assignment, in addition to the course lecture slides a comprehensive description of these interactions is provided in this paper, which is available on the moodle site:

Bissantz, Caterina, Bernd Kuhn, and Martin Stahl. "A medicinal chemist's guide to molecular interactions." *Journal of medicinal chemistry* 53.14 (2010): 5061-5084.

Receptor Ligand structures and corresponding papers (recall your number from class):

1	1U19	2.20 Å	2004-10-12	bovine rhodopsin with 11-cis-retinal	Okada, T.; Sugihara, M.; Bondar, A. N.; Elstner, M.; Entel, P.; Buss, V. J. Mol. Biol. 2004, 342, 571
1	2HPY	2.80 Å	2006-08-22	bovine lumirhodopsin with all-trans-retinal	Nakamichi, H.; Okada, T. Proc. Natl. Acad. Sci. U.S.A. 2006, 103, 12729
1	3PXO	3.00 Å	2011-03-09	metarhodopsin II.	Choe, H. W.; …….Ernst, O. P. Nature 2011, 471, 651
2	2Z73	2.50 Å	2008-05-13	squid rhodopsin with 11-cis-retinal	Murakami, M.; Kouyama, T. Crystal structure of squid rhodopsin. Nature 2008, 453, 363
2	3AYN	2.70 Å	2011-08-17	batho intermediate of squid rhodopsin	Murakami M, Kouyama T. J Mol Biol. 2011 Oct 28;413(3):615-27.
2	4WW3	2.8 Å	2015-06-17	Crystal structure of the lumi intermediate of	Murakami M, Kouyama T. PLoS One. 2015 May 29;10(5):e0126970.

				squid rhodopsin	
3	2RH1	2.40 Å	2007-10-30	human B2AR with the inverse agonist carazolol	Cherezov, V.,..., Stevens, R. C. Science 2007, 318, 1258
3	2R4R	3.40 Å	2007-11-06	human B2AR with the inverse agonist carazolola	Rasmussen, S. G.,....., Kobilka, B. K. Nature 2007, 450, 383
3	3NY9	2.84 Å	2010-08-11	human B2AR with a novel inverse agonist	Wacker, D.,....., Stevens, R. C. J. Am. Chem. Soc. 2010, 132, 11443
4	3D4S	2.80 Å	2008-06-17	human B2AR with the inverse agonist timolol	Hanson, M. A, ..., Stevens, R. C. Structure 2008, 16, 897
4	3NY8	2.84 Å	2010-08-11	human B2AR, inverse agonist ICI 118,551	Wacker, D; Stevens, R. C. J. Am. Chem. Soc. 2010, 132, 11443
4	3PDS	3.50 Å	2011-01-12	human B2AR with an irreversible agonist	Rosenbaum, D. M.,....., Kobilka, B. K. Nature 2011, 469, 236
5	3NYA	3.16 Å	2010-08-11	human B2AR with the antagonist alprenolol	Wacker, D.; Stevens, R. C. J. Am. Chem. Soc. 2010, 132, 11443
5	3P0G	3.50 Å	2011-01-19	nanobody-stabilized active state of B2AR	Rasmussen, S. G.; ……Kobilka, B. K. Nature 2011, 469, 175
5	4LDL	3.10 Å	2013-09-25	B2AR bound to hydroxyl-benzylisoproterenol	Ring AM, ...Kobilka BK., Nature. 2013 Oct 24;502(7472):575-9.
6	2R4S	3.40 Å	2007-11-06	human B2AR with the inverse agonist carazolola	Rasmussen, S. G.,....., Kobilka, B. K. Nature 2007, 450, 383
6	4LDO	3.20 Å	2013-09-25	B2AR bound to adrenaline and an engineered nanobody	Ring AM, ...Kobilka BK., Nature. 2013 Oct 24;502(7472):575-9.
6	4QKX	3.30 Å	2014-07-23	hman B2AR bound to a covalent agonist	Weichert D,…… Gmeiner P. Proc Natl Acad Sci U S A. 2014 Jul 8
7	2VT4	2.70 Å	2008-06-24	turkey B1AR with the antagonist cyanopindolol	Warne, T.;…. Schertler, G. F. Nature 2008, 454, 486
7	2Y00	2.50 Å	2011-01-12	turkey B1AR with the partial agonist dobutamine	Warne, T.; .....Tate, C. G. Nature 2011, 469, 241
7	4BVN	2.10 Å	2014-04-02	beta1-adrenoceptor (B1AR) with cyanopindolol bound	Miller-Gallacher JL, Tate CG. PLoS One. 2014 Mar 24;9(3):e92727
8	2Y02	2.60 Å	2011-01-12	turkey B1AR with the agonist carmoterol	Warne, T.; .....Tate, C. G. Nature 2011, 469, 241
8	2YCW	3.00 Å	2011-06-01	turkey B1AR with the antagonist carazolol	Moukhametzianov, R.; Schertler, G. F. Proc. Natl. Acad. Sci. U.S.A. 2011, 108, 8228
8	4AMJ	2.30 Å	2012-05-23	turkey B1AR with bound biased agonist carvedilol	Warne T, Tate CG. Structure. 2012 May 9;20(5):841-9.
9	3EML	2.60 Å	2008-10-14	human A2AR with the antagonist ZM241385	Jaakola, V. P.; Stevens, R. C. Science 2008, 322, 1211
9	3UZA	3.27 Å	2012-03-21	Thermostabilised A2AR in complex with T4G	Congreve M, Marshall FH. J Med Chem. 2012 Mar 8;55(5):1898-903.

9	4UG2	2.6 Å	2015-04-08	Thermostabilised human A2a Receptor with CGS21680	Lebon G, Tate CG. Mol Pharmacol. 2015 Jun;87(6):907-15.
10	3QAK	2.71 Å	2011-03-09	human A2AR with the agonist UKA	Xu, F.; Stevens, R. C. Science 2011, 332, 322
10	3PWH	3.30 Å	2011-09-07	thermostabilized A2AR	Dore AS, , Marshall FH. Structure. 2011 Sep 7;19(9):1283-93.
10	4EIY	1.80 Å	2012-07-25	chimeric protein of A2aAR-BRIL in complex with ZM241385	Liu W, Stevens RC. Science. 2012 Jul 13;337(6091):232-6.
11	3ODU	2.50 Å	2010-10-27	human CXCR4 with the small ligand antagonist IT1t	Wu, B.; Stevens, R. C.. Science 2010, 330, 1066
11	4RWS	3.1 Å	2015-02-11	CXCR4 and viral chemokine antagonist vMIP-II complex	Qin L, Stevens RC, Handel TM. Science. 2015 Mar 6;347(6226):1117-22.
11	3OE0	2.90 Å	2010-10-27	human CXCR4 with the peptide antagonist CVX15	Wu, B.; Stevens, R. C.. Science 2010, 330, 1066
12	4DAJ	3.40 Å	2012-02-22	human M3 muscarinic acetylcholine receptor	Kruse AC, Kobilka BK. Nature. 2012 Feb 22;482(7386):552-6. doi: 10.1038/nature10867
12	4U15	2.80 Å	2014-11-26	rat M3 muscarinic acetylcholine-mT4L receptor bound to tiotropium	T.S.THORSEN, B.K.KOBILKA, Structure 22: 1657-1664, 2014
12	4U16	3.70 Å	2014-11-26	rat M3 muscarinic acetylcholine-mT4L receptor bound to NMS	T.S.THORSEN, B.K.KOBILKA, Structure 22: 1657-1664, 2014
13	4GRV	2.8 Å	2012-10-17	Neurotensin receptor	White JF, Grisshammer R. Nature. 2012 Oct 25;490(7421):508-13.
13	4BUO	2.75 Å	2014-01-29	Thermostable Agonist-bound Neurotensin Receptor 1 Mutant	Egloff et al, Proc Natl Acad Sci USA. 2014 Feb 11;111(6):E655-62
13	3ZEV	3.00 Å	2014-01-29	thermostable agonist-bound neurotensin receptor 1 mutant	Egloff et al, Proc Natl Acad Sci USA. 2014 Feb 11;111(6):E655-62
14	3UON	3.00 Å	2012-02-01	human M2 muscarinic acetylcholine receptor bound to an antagonis	Haga K, Kobayashi T. Nature. 2012 Jan 25;482(7386):547-51.
14	4MQT	3.70 Å	2013-11-27	active human M2 muscarinic acetylcholine receptor bound to the agonist iperoxo and allosteric modulator LY2119620	Haga K, Kobayashi T. Nature. 2012 Jan 25;482(7386):547-51.
14	4MQS	3.50 Å	2013-11-27	active human M2 muscarinic acetylcholine receptor bound to the agonist iperoxo	Kruse AC, Kobilka BK., Nature 2013; 504:101–106

15	4JKV	2.45 Å	2013-05-29	human smoothened 7TM receptor in complex with an antitumor agent	Wang C, Stevens RC. Nature. 2013 May 16;497(7449):338-43.
15	4N4W	2.80 Å	2014-01-22	human smoothened receptor in complex with SANT-1	Wang C, Stevens RC. Nat Commun. 2014 Jul 10;5:4355
15	4QIM	2.60 Å	2014-07-23	human smoothened receptor in complex with ANTA XV	Wang C, Stevens RC. Nat Commun. 2014 Jul 10;5:4355
16	4IB4	2.70 Å	2013-03-13	chimeric protein of 5-HT2B-BRIL in complex with ergotamine	Wacker D, Stevens RC. Science. 2013 May 3;340(6132):615-9.
16	4IAQ	2.80 Å	2013-03-13	chimeric protein of 5-HT1B-BRIL in complex with di-hydroergotamine	Wang C, Xu HE. Science. 2013 May 3;340(6132):610-4.
16	4NC3	2.80 Å	2013-12-18	5-HT2B receptor	Liu W, Cherezov V. Science. 2013 Dec 20;342(6165):1521-4.
17	4NTJ	2.62 Å	2014-03-26	human P2Y12 receptor in complex with an antithrombotic drug	Zhang K, Zhao Q. Nature. 2014 May 1;509(7498):115-8
17	4PXZ	2.50 Å	2014-04-30	P2Y12 receptor in complex with 2MeSADP	Zhang J, Zhao Q. Nature. 2014 May 1;509(7498):119-22.
17	4PY0	3.10 Å	2014-04-30	P2Y12 receptor in complex with 2MeSATP	Zhang J, Zhao Q. Nature. 2014 May 1;509(7498):119-22.
18	2YDO	3.00 Å	2011-05-18	human A2AR with the agonist adenosine	Lebon, G.; Tate, C. G. Nature 2011, 474, 521
18	3VG9	2.70 Å	2012-02-01	human A2AR with an allosteric inverse-agonist antibody	Hino T, Murata T. Nature. 2012 Jan 29;482(7384):237-40
18	4UHR	2.6 Å	2015-04-08	Thermostabilised human A2a Receptor with CGS21680 bound	Lebon G, Tate CG. Mol Pharmacol. 2015 Jun;87(6):907-15.

Instructions

In this assignment, you will each explore the interactions between a particular protein receptor and one or more ligands, by analysing receptor ligand co-crystal structures and carefully reading the corresponding publications.

Start by using the PDBe or PDB RCSB sites

Search using the PDBID given in the table above to find the entry for each of your three structures. Using the web portal, check whether there are any mutations (AA insertions or deletions) within the secondary structural elements surrounding the binding site.

Using a molecular viewer, VMD (see your previous practicals /assignments), PyMOL or Avagadro, identify the domain(s) complexed with your specified ligand. If the protein is too big – find a smaller one that is manageable to visualise. You don't have to extract or do anything, just inspect the complexed binding site, and make a list of the residues facing the ligand or interacting via the polypeptide backbone.

Using the scPDB website (below), use Database Search to identify the PDB id you have collected. Some PDB ids might not be in there. If not, there is similar information on the PDB website. The scPDB file might not be bang-up-to-date or synchronised with the latest structures deposited in the PDB. Write a brief summary for each of your three structures describing what scPDB tells you about the complexed binding site.

Using the original publication in which the structure was released, briefly summarise the argument or suggestions made by the authors about which residues are important for ligand binding.

Finally, write a brief paragraph comparing the information for each structure that you obtained from scPDB to the description found in the associated publication for each structure.

Collection of Tools that might be useful

From http://www.openchemistry.org/projects/
Avogadro - a molecular editor and visualisation tool
https://sourceforge.net/p/avogadro/news/2015/08/avogadro2-080-released/
Documentation with tutorial
http://avogadro.cc/wiki/Category:Documentation
From BIOVIA (Accelrys)
BIOVIA Studio Visualizer (**Windows and Linux only**)
http://accelrys.com/products/collaborative-science/biovia-discovery-studio/visualization.html

The PDB in Europe (from EMBL-EBI) also called PDBe

We will be using PDBe and the RSCB PDB site
For the search for protein-ligand complexes try both of them
http://www.ebi.ac.uk/pdbe/
http://www.rcsb.org/pdb/

Secondary Database of Binding Sites

We will be using this

sc-PDB – An annotated Database of Druggable Binding Sites from the PDB
http://bioinfo-pharma.u-strasbg.fr/scPDB/
this database contains approximately 140,000 receptor-ligand pharmacophore models.