**Waking Up Anna**

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**Part 4. GABA-A Receptor Function**

In this section we will explore the structure of GABA-A receptor with its natural ligand (GABA) bound to it. This will set the stage for understanding where the ligand binds and how it affects receptor function.

Explore the structure of GABA-A receptor in complex with GABA (RCSB - PDB ID 6dw0) and view in 3D. Rotate and explore the structure.

**Note**: As you proceed through this (and other) Mol\* explorations, you can save (use Camera icon) images as you proceed. The .png extension are still images. There is an option at the bottom of the camera window called State. This allows you to save an image in .molj, which has be ability to be opened (same pathway) as a 3D image in Mol\*. Periodically, you might wish to save a 3D version (.molj) if you wish to come back to the exploration.

Q1 (1 pt). Explore the structure summary page to learn about the contents of the structure and fill in the table. *Each of the structures has been deposited by different research groups – therefore, some of the subunit labeling is different.*

|  |  |
| --- | --- |
| PDB ID | 6dw0 |
| Title | Cryo-EM structure of the benzodiazepine-sensitive alpha1beta1gamma2S tri-heteromeric GABAA receptor in complex with GABA |
| Author(s) of entry | Phulera, S., Zhu, H., Yu, J., Yoshioka, C., Gouaux, E. |
| Year when the structure was published/ released | 2018-08-08 |
| Organism | Rat |
| # of entities | 7 |
| # of polymer chains | 3 |
| Small molecule (not carbohydrate) | ABU (GABA) |

Open the 3D image of the file.

In the top of the right hand toolbox, click structure and select Model.

* Color the polypeptide Entity with 3 different colors to distinguish the pentameric chains.
  + Toggle (click arrow) to see the bar above the 3D image
  + Select ‘Entity’ from the left hand pulldown (this selects all of the subunits of the same type)
  + Click on the subunit(s) that you wish to color
  + Click on the paintbrush, select a color and ‘Apply theme’
  + Repeat for other ‘Entities’
* Label the pentameric subunits as done previously.
  + Note: You may not be able to see all of the labels at the same time.
  + *Hint: Try labeling the pentameric subunits from a bottom view and then reposition the 3D image to view from the side.*
  + In the toolbar above the image, select ‘Chain’ from the left hand pulldown
  + Click to highlight the chain (subunit) that you wish to label
  + In the right hand tools, click Measurements > Add > Label
* Save a copy of your image in two formats
  + Using the Camera icon

Q2 (2 pts) Download a labeled image of the entire protein

Q3 (1 pt). Scrolling over each subunit, create a key to indicate which labels represent which subunits.

|  |  |
| --- | --- |
| **Subunit (chain)** | **Identity (Record the names of the subunits, not just the identifiers)** |
| A | Gamma subunit (structure information labels A and an alpha subunit) |
| B | Alpha subunit (structure information labels B as beta subunit) |
| C | Alpha subunit (correctly labeled in structure information) |
| D | Beta subunit (structure information labels D as the gamma subunit) |
| E | Beta subunit (correctly labeled in structure information) |

*Note: It is important to understand that in some cases, multiple PDB files have been uploaded for the same (or similar) structure by different groups. Each PDB file may have different subunit chain identifiers for the actual protein subunits. Make sure that you understand the subunit identification for the PDB structure that you are visualizing.*

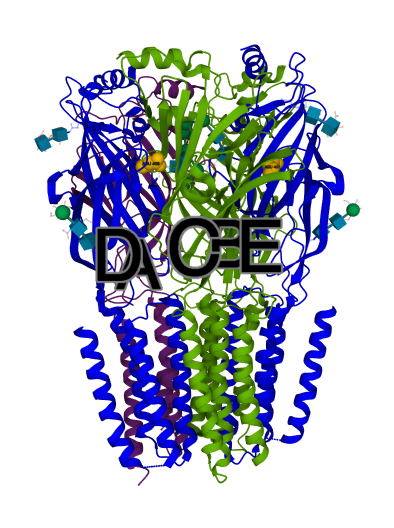
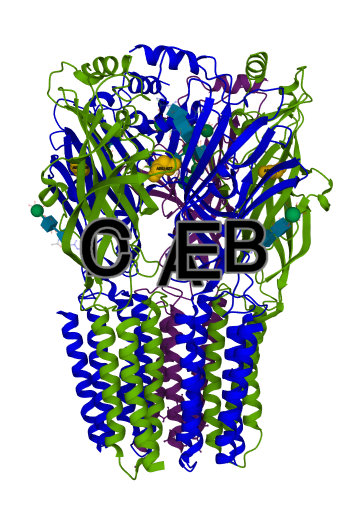
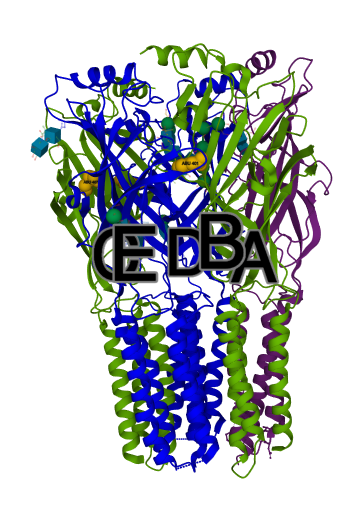
Where are the GABA molecules binding? There are three GABA molecules shown in the model (Entity ABU). To more clearly view the GABA binding sites:

* In the Sequence window at the top of the image screen, select the gamma-amino-butanoic acid. Click on the ABU (abbreviation for GABA).
  + To color, click Entity > paintbrush > yellow
* To make the GABA a space filling model
  + Ligand (right hand toolbox) > … > Add representation > Space filling
* To label the ABU (GABA) molecules
  + Chain (top toolbar) > Measurements (right hand toolbar) > Add > Label
  + Repeat for all 3 GABA molecules
    - *Note: if you did this all at one time (Entity), the labeling will not be as descriptive.*
    - *Note: Chains that have been labeled can be unlabeled by hiding (eyeball icon) or Remove under the Labels section in the right hand menu.*

Q4 (2 pts). To which GABA-A receptor chains are the GABA molecules bound (between which subunits)? Recalling the previous structure (or you can view on iCn3D), do you know if the GABA binding sites are intracellular or extracellular? Include one or more images of your protein that has been labeled to support your answer.

Ans: all are binding to extracellular domains

|  |  |  |
| --- | --- | --- |
| GABA | Between chains (letter) | Between Entities (record names) |
| ABU401 | Between E and B | Beta and alpha |
| ABU407 | Between C and E | Alpha and beta |
| ABU408 | Between D and C | Beta and alpha |



ABU401 ABU407 ABU408

Q5 (1 pt). Visualizing the GABA A receptor from the outside of the cell (end on), provide an image of the protein with the subunits labeled as well as the GABA molecules.



**Explore the binding environment of one of the GABA molecules as follows.**

Return to the Structure Summary page for PDB ID 6dw0.

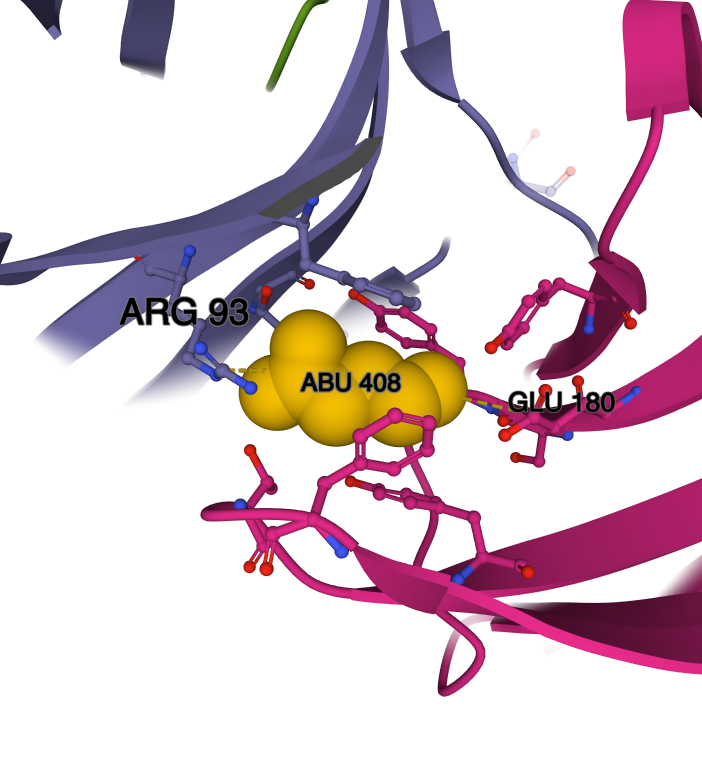
Scroll down to the Small Molecules section. In the model, there are three ABU (GABA) molecules indicated. You can explore the chemical interactions of one of these GABA molecules by clicking on the Ligand Interaction button on the right hand side. This takes you to ABU408 in the 3D model that you were previously looking at.

* To highlight the GABA molecule, click on the molecule (it will probably be highlighted from the previous page).
* To make this a space filling molecule, select the GABA, then go to Ligand > … > Add Representation > Spacefill.
* While highlighted, make the GABA yellow (use the paintbrush tool).
* Label the GABA (Toggle the Menu bar above the 3D image on, select the GABA, go to Measurements (right hand toolbox) > +Add > Label)
* You now should have the GABA (ABU408) as a spacefilled molecule that is yellow and labeled.
* If non-covalent bonds are not evident (dashed lines), zoom out and then zoom back in on the molecule.
  + Go to Components > Preset > Options >Non-covalent interactions
  + Turn on only Ionic (yellow dash) interactions
  + Label the amino acids that interact with ABU408 (GABA)
  + Make note of which protein subunits these amino acids interact.

The Option icon looks like .

Q6 (2 pts). List the names of 2 amino acids whose side chains for ionic bonds with GABA (ABU408) and identify which polypeptide chains these are from. Include a picture to support your answer.

Ans: ABU408 (GABA) forms ionic bonds with alpha subunit Arg93 and beta subunit Glu180.



* Turn off the ionic bonds and show the hydrogen bonds.
* Label the amino acids that interact with the GABA (ABU408) using hydrogen bonds. *You might wish to hide the labels on the amino acids that do not form H bonds but did form ionic bonds.*

Q7 (2 pts). List the names of 2 amino acids whose side chains form hydrogen bonds with GABA (ABU408) and identify which polypeptide (Entity) these are from. Include a picture to support your answer.

Ans: Thr227 (beta), Arg93 (alpha), Tyr122 (beta)

