**Waking Up Anna**

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**Part 3. Molecular Basis of Sleep**

To better understand Anna’s story in molecular detail we need to understand the structure and function of the GABA-A receptor.

*A. GABA- A Receptor Structure*

In this section we will explore known structures of the GABA-A receptor to learn more about its shape and structure. The resource for accessing molecular structures of GABA-A receptors is the Protein Data Bank.

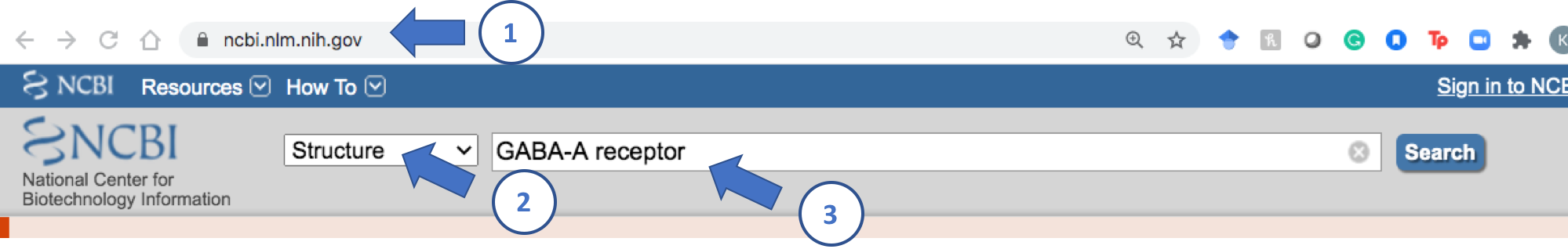
*Box 3: Resource*

RCSB Protein Data Bank (**RCSB PDB**, [www.rcsb.org](http://www.rcsb.org)) provides access to 3D structural data of biological macromolecules (proteins, nucleic acids, carbohydrates and their various complexes). In addition, it provides information about the experiment used to derive the data, details about the molecules included in the experiment, and links to bioinformatics resources that can provide additional information about the protein/molecule of interest. Each structure in the PDB has a unique identifier called PDB ID. Atomic coordinates form the PDB can be explored and analyzed using various visualization software (e.g., Jmol, Pymol, UCSF Chimera, iCn3D).

* Search for “GABA-A receptor” in the PDB ([www.rcsb.org](http://www.rcsb.org)) by typing in the protein name in the top search box.
* In the ‘Refinements’ window on the left side of the screen, select *Homo sapiens* and scroll down and select 2015-2019 as the ‘Release date’.

Q1 (1 pt). How many structures of the GABA-A receptor did you find in the archive? Why are there so many structures?

Ans: Since the PDB is a live archive, results of this search may be variable, based on when the search was done. As of Jun 2020, there are 69 structures in the results list. Note some of the structures are that of the GABA-A receptor related protein. These may have to be filtered out to get a correct number of results. The various GABA-A receptor structures have different combinations of alpha, beta, gamma proteins and also different drugs/ligands bound to them. These structures allow us to examine conformational changes when the receptor binds the various ligands. The search here gives >19,000 structures, so not sure how the original narrowed this down more.



Sometimes searches through RCSB are burdensome and yield many results to sift through. You can also perform a search at [NCBI](http://www.ncbi.nlm.nih.gov/). Go to the [NCBI website](http://www.ncbi.nlm.nih.gov/) (➀), select structure (➁) and enter ‘GABA-A receptor’ (➂). Perform the search.

Q2 (1 pt). Using the NCBI Structure database, how many structures of the GABA-A receptor did you find in the archive?

Ans: As of 3/9/21, there were 92 structures listed in NCBI using this keyword search.

Return to RCSB.org and search for PDB ID 6i53.

* Click on the **PDB ID 6i53** to open the structure summary page for this entry. This is the structure of a human GABA-A receptor. Review Box 4 and answer the following questions.

*Box 4: Navigating the Structure Summary Page*

1. **Title** - that tells you what the structure is about

2. **Snapshot** - of what the structure of the molecule/complex looks like.

3. **Authors** – who solved the structure

4. **Literature** –access the article that describes the structure. This section also includes links to PubMed page and the abstract of the article describing this structure, when available.

5. **Macromolecules** – All proteins and nucleic acids present in the structure are listed here. Each unique type of macromolecule or molecular chain is listed as a separate entity. There may be multiple copies of each molecule in the structure.

6. **Small molecules** – All ligands, ions, cofactors, inhibitors that are present in the structure are listed here. You can find links here to explore the interaction of this ligand with the target protein.

7. **Experimental details** – describe details about how the structure was determined

8. **Structure quality** – shows a slider that provides insights about the quality of the structure and its agreement with the experimental data and geometric standards.

See <http://pdb101.rcsb.org/learn/guide-to-understanding-pdb-data/introduction> for details

Q3 (1 pt). Explore the structure summary page to learn about the contents of the structure and fill in the table.

|  |  |
| --- | --- |
| PDB ID | 6i53 |
| Author(s) of entry | Laverty, D., Desai, R., Uchanski, T., Masiulis, S., Stec, W.J., Malinauskas, T., Zivanov, J., Pardon, E., Stevaert, J., Miller, K.W., Aricescu, A.R. |
| Year when the structure was published/ released | 2019 |
| Structure determination method | Cryo-Electron microscopy |
| # of macromolecular entities | 4 (8 total entities) |
| # of polymer chains | 6 |
| Names of proteins in these chains (chain ID) | Gamma-aminobutyric acid receptor subunit alpha-1 (A, D)  Gamma-aminobutyric acid receptor subunit beta-3 (B, E)  Gamma-aminobutyric acid receptor subunit gamma-2 (C)  Megabody38 (G) |
| # of different small molecule ligands in the structure and their identifiers | PIO, POV |

Q4 (1 pt). Why do some of the protein subunits list multiple chain IDs on the structure summary page?

Ans: They represent multiple copies of that protein in the structure. For example, there are 2 copies of the alpha and the beta subunits in the GABA-A receptor pentamer. Each subunit gets its own unique chain ID because it is located in a unique space.

* Before you explore the structures of the GABA-A receptor any further, review Box 5.

*Box 5: Vocab*

**Residues**: Building blocks of biological macromolecules are sometimes referred to as residues. Depending on the context, this may refer to amino acids (frequent use) or a nucleotide (less common use).

**Chains**: The term chain is used to refer to covalently linked amino acids (polypeptide). Some proteins structures contain more than one polypeptide - each subunit of this structure is referred to as a chain. To help locate amino acids in the structure, each chain is given an identifier (called Chain ID) and each amino acid in the chain is assigned a number.

**Domains** are conserved parts of a protein that can evolve, function, has a stable three-dimensional structure and often can stably fold and exist independently (of the full protein). On the other hand, loops and linker regions between domains are often flexible and cannot be clearly seen in experimental structures. Their atomic coordinates may be missing from the file because the large protein was cut into smaller pieces and only the relevant structures are included in the experiment OR they were present in the experiment, but its location could not be seen due to high mobility.

*Box 5: Continued*

**Complex assembly and stability**: Protein complexes can be assembled in vitro (outside the cell) to study the structure. In order to stabilize the assembly additional ligands or even polymer chains may be included in the experiment – for example

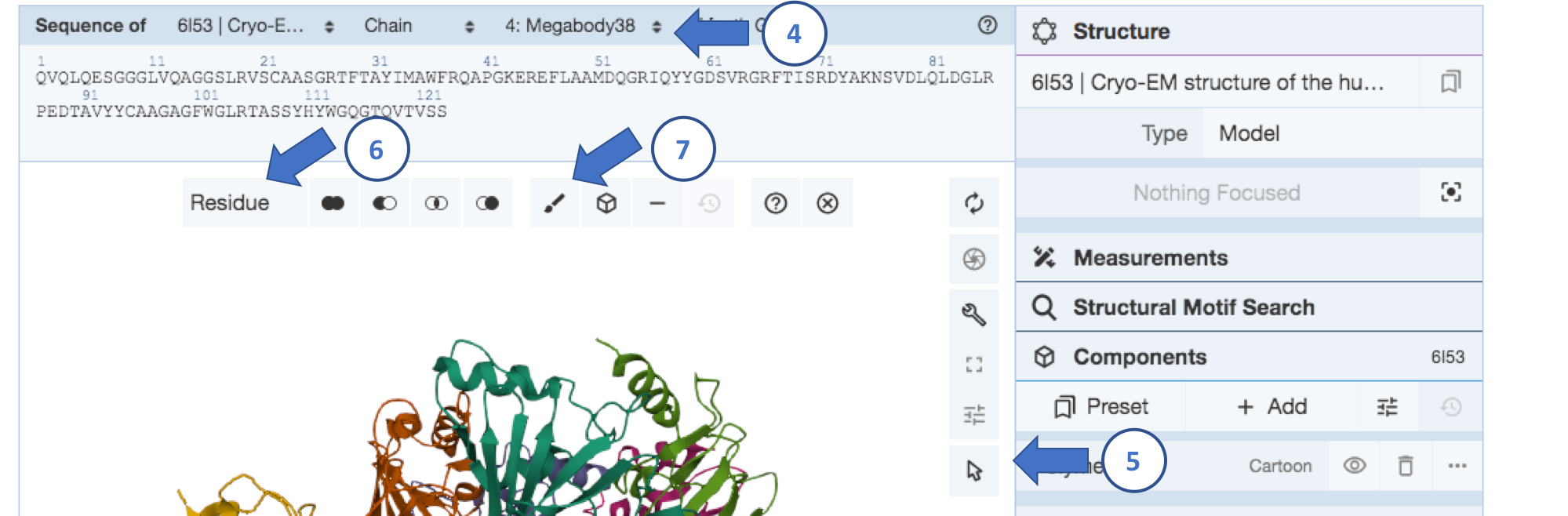
a. lipid-like or detergent molecules are included in membrane protein complexes to prevent them from aggregating or precipitating from the aqueous solutions

b. antibodies, parts of antibodies, nanobodies, megabodies, or other stable proteins/domains may be included in the experiment to facilitate assembly formation, enhance solubility of the complex, or trap the molecule/complex in a specific conformation.

* Click on the 3D View tab on the Structure summary page and view the molecular structure of the GABA-A receptor.
* Interactively rotate and reorient the structure to get acquainted with it.
* Spend a few minutes playing around with the different menus to see some of the different ways this protein view can be adjusted.

RCSB now uses a program called Mol\* to view the protein structure. Instructions have been altered to use Mol\* (apologies for any inconsistencies – please let me know).

In the 3D image, you should be able to see the window shown below.



* To gain orientation (there may be another way), highlight the Megabody (found outside the cell).
  + To do this, pull down the ‘Megabody’ selection from the ‘Sequence of’ window (➃). This changes the selection to that structure. The Megabody is found outside of the cell.
  + If the ‘Residue’ row is now visible above the 3D image, click on the arrow (➄).
  + Click on ‘Residue’ and pulldown ‘Entity’ (➅).
  + Click on the ‘Megabody’ portion of the structure in the 3D image (you may have to rotate to identify the Megabody and click on structures. *Hint: the structure protrudes from the remainder of the Model.*
  + Click on the paintbrush (➆) and select blue in the popup window and ‘Apply Theme’.
  + Click off of the image (but somewhere in the window) to view the colored chain.

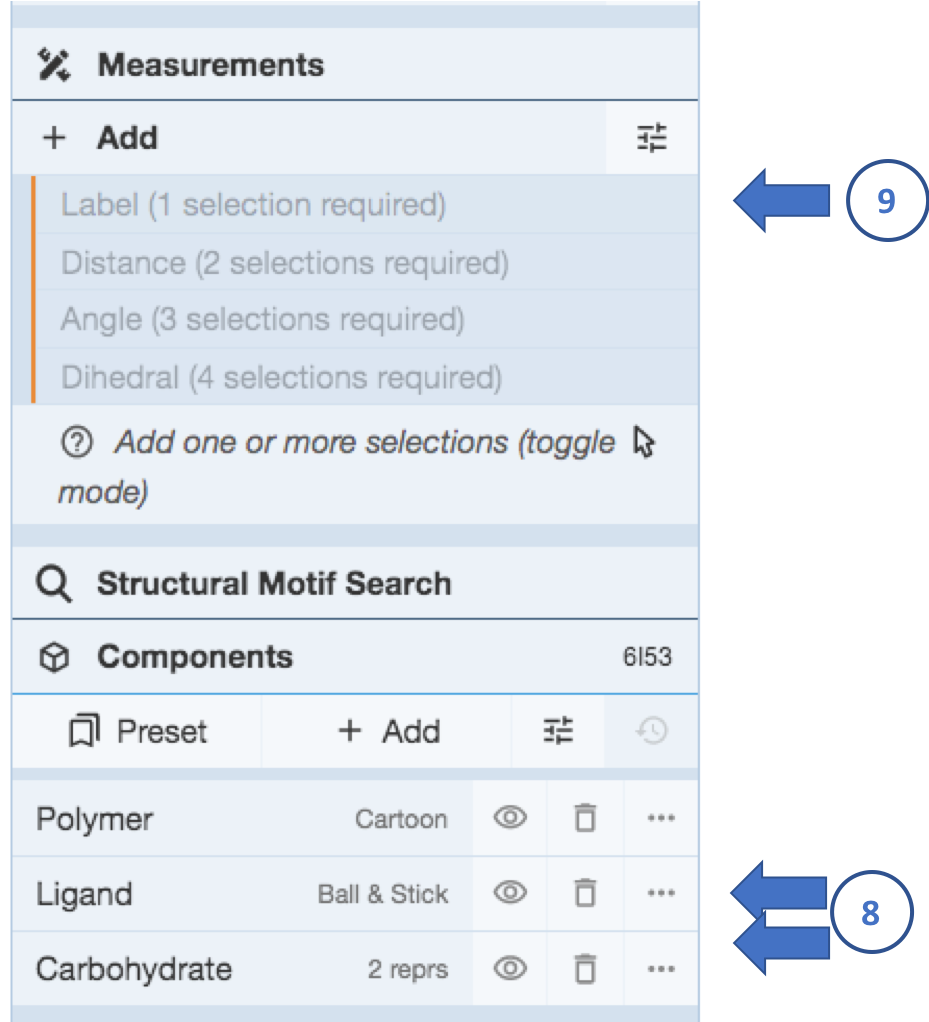
Q5 (1 pt). Using the extracellular Megabody, can you identify the extracellular ligand binding domains and transmembrane domains in the GABA-A receptor structure? Which type of secondary structural elements (helices or sheets) make up these domains?

Ans:

extracellular domain… beta sheets

Transmembrane domain… alpha helices.

Using the steps leading into Q4, repeat the coloration using different colors for the subunits of the GABA A receptor. *Look back to your answer for Q2 to remind yourself how many different polypeptide chains are present*.

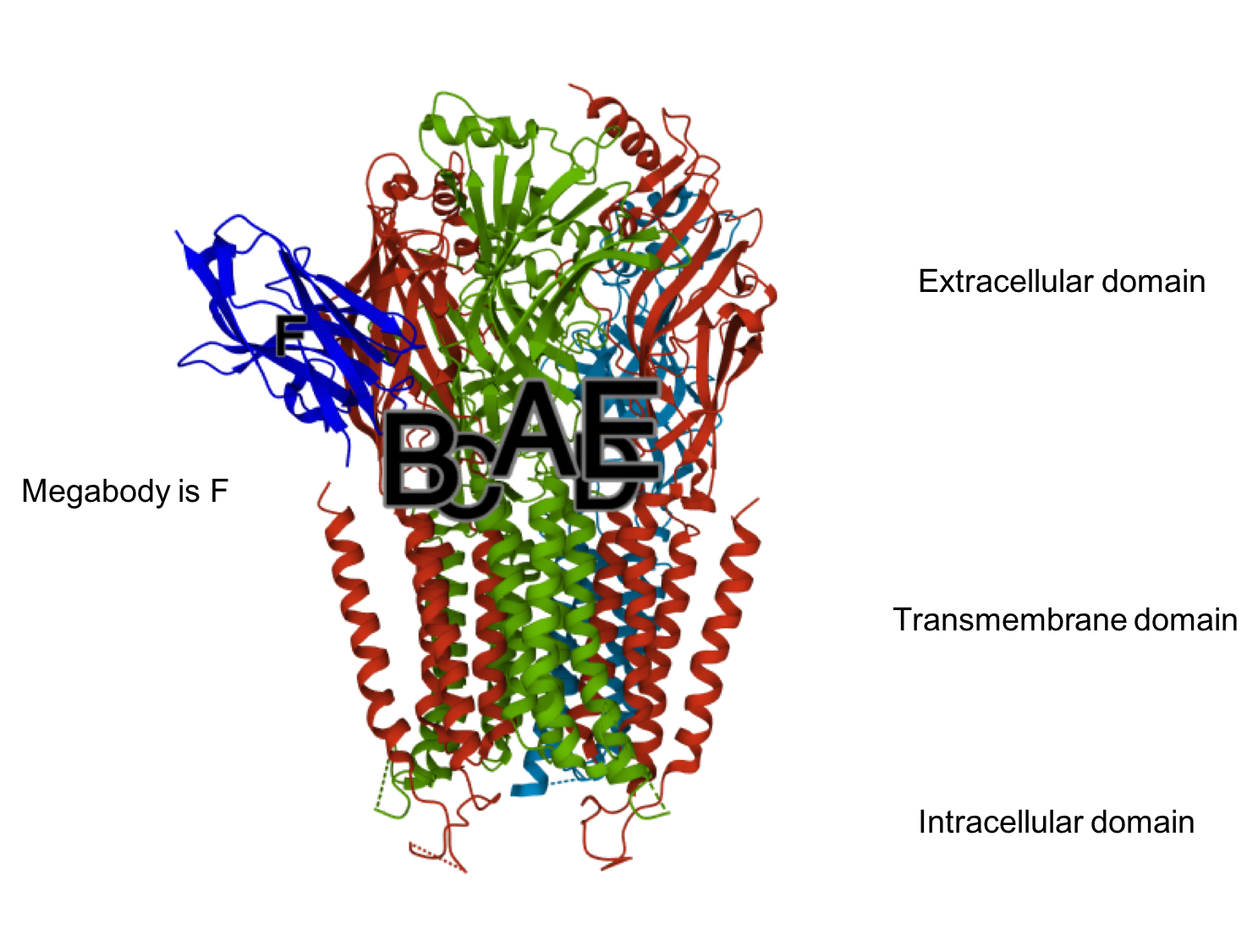


To hide non-protein structures, click the ‘Show component’ icon (eyeball) in the toolbar on the right (8). Click on both ‘Ligand’ and ‘Carbohydrate’.

To label the subunits (with the letters corresponding to the chains, click on ‘Measurements (9) in the toolbar on the right, click ‘Label’ and then click on one of the subunits of the protein. A letter (A-F) should appear. Repeat this process until all six letters are present (you may need to rotate the image to view all six). *Hint: Rotating the image so that you can view from the intracellular surface may facilitate labeling all of the subunits.*

Q6 (2 pt): Make an image with the extracellular domain on top. Make as many chain labels (A-F) visible in your image. Download the image (there is a tool icon to the right of the 3D image that looks like a camera lens). Label the extracellular domain, the transmembrane domain and the Megabody (F). Include the image below.

Ans: below

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You can (and should) also download a ‘state’ of this image, which is an image that you can reload if you happen to need to reload. When you download (Screenshot), scroll to the bottom and select ‘state’ and then ‘state’ again. This will download a .molj file (you may wish to rename it to identify the structure or state of the structure). You can come back and open this file at a later time if you wish. In this same box, you will also find an ‘Open’ option.

To visualize the secondary structures, select ‘Chain’ in the toolbar above the image. In the toolbar on the right, select ‘Component’ and to the right of ‘Polymer’ there will be … (click there). Now select ‘Set coloring’ > ‘Residue property’ > ‘Secondary structure’. Your image should show secondary structures for all of the subunits similarly.

Q7 (2 pts). Describe the distribution of the secondary structural features in this image. Upload an image of your results.

Ans: All the beta sheets are in the top part of the structure show here while a majority of the helices are located in the bottom part of the structure. A few helices are seen on the top of the structure too.

