Basics of the iCn3D Program: A User-Friendly Tool for Biomolecular Modeling

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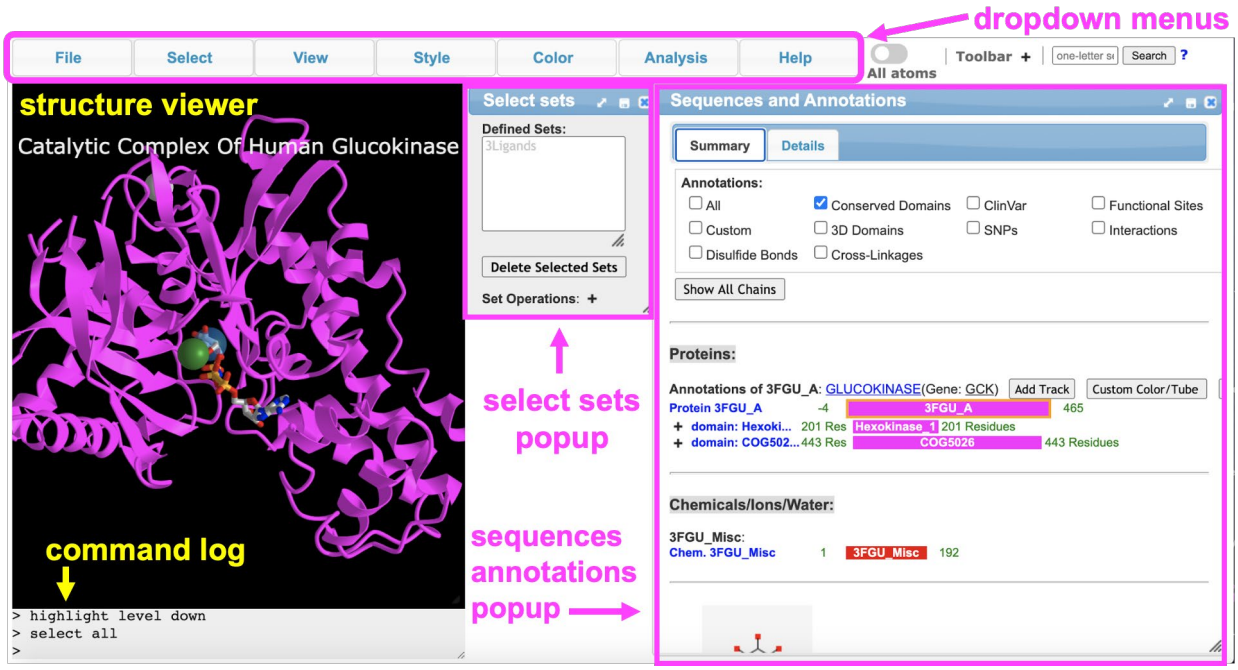
PART 1: Introduction & iCn3D Operation
PART 2: Open-ended modeling & a classroom activity

Table of Contents

Activity 1: Introduction to iCn3D, basic controls and menus

The instructions below will guide you through the iCn3D activity for this part of the workshop. If you get stuck, an iCn3D shareable link is provided at the end, which will bring you to a pre-rendered structure that you can manipulate and modify. iCn3D shareable links are a useful tool for collaborating with students and colleagues.

Figure: *Overview of the iCn3D graphical user interface*



Definitions:

- Structure: The 3D topology of a protein with each atom occupying a space defined by a single xyz coordinate
- Structure File: A file (ex: PDB file) that contains the x, y, and z coordinates of each atom in a given structure along with additional information describing the structure and function of the molecule
- Model: The display (computer, 3D printed) of a structure made using a structure file
- Render: A specific display of a model highlighting different structures features of the model to illustrate structure/function relationships

About this model

PDB ID: 1xww
Protein: Low molecular weight protein tyrosine phosphatase
Activity: hydrolyzes Tyr-OPO_3^{2-} phosphoester bond
Description: single chain, bound SO_4^{2-} (competitive inhibitor), bound glycerol (nonspecific stabilizer)

Load Structure and Mouse/Trackpad Controls

1. Open iCn3D - https://www.ncbi.nlm.nih.gov/Structure/icn3d/full.html
2. For a simple menu, use the dropdown: File > Customize Menus > Simple Menus.
3. In the *Please input MMDB or PDB*, enter 1xww. Press enter or click load biological unit.

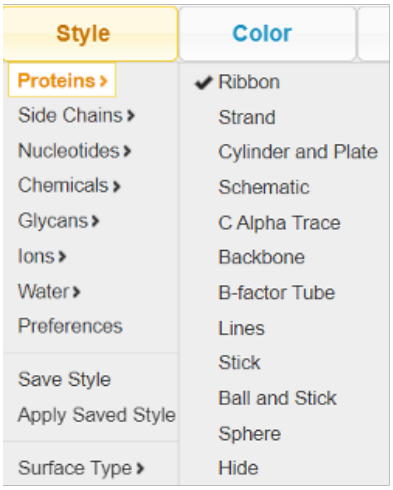
4. Default render is ribbon (cartoon) with black background and small molecules shown as sticks. Hover over objects with the mouse to reveal their identity.
5. Try the following mouse or trackpad controls to manipulate the structure

rotate: click and drag (*mouse*: left click and drag; *keyboard*: j, i, l, and m keys)
zoom: pinch and spread (*mouse*: rotate the scroll wheel; *keyboard*: x and z keys)
translate: two finger click and drag (*mouse*: right click and drag)
Re-center: left click View from the top menu bar, then select "Center Selection"

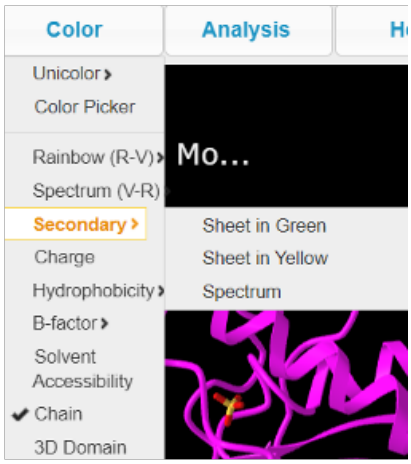
Note: ctrl click on a PC = command click on Mac
alt click on PC = option click on Mac

Alternative Rendering

6. From the top menu bar, choose **Style**, **Protein**, then try some of the available choices:

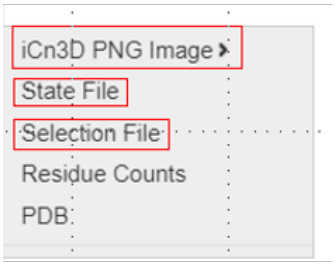


2. For your favorite protein styles, select **Color** by left-clicking on the top menu, then pick available choices. Try:
    - **Secondary**, **Sheets in Yellow**
    - **Charge**
    - **Hydrophobicity**



3. Under **Style**, choose **Protein → Ribbon**. Under **Color**, choose **Secondary, Sheets in Yellow** before the next step.
4. To view sidechains, **Style, Side Chains, Stick** (they will remain the same color as secondary structure for now)
5. Color for SO_4^{2-} and bound glycerol will default to CPK coloring (key below)
6. Convert back to cartoon (**Style, Side Chains, Hide**)

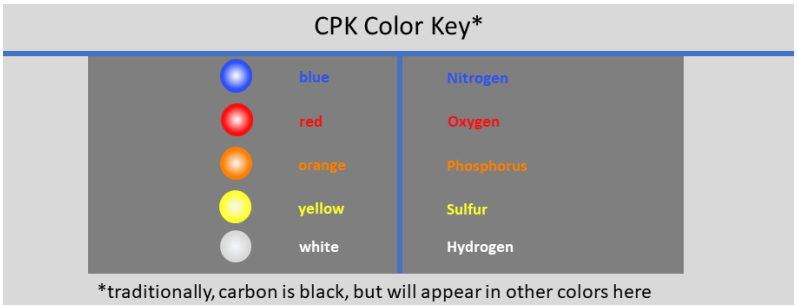
Saving Files



7. Style, Background, Transparent
8. Saving Files: There are several ways to save your work. The first option below saves a PNG image, the second creates a share link
    a. **File, Save Files, iCn3D PNG image, original size**; Give it a name. Can be reloaded in iCn3D with File, Load, iCn3D PNG IMAGE
    b. **File, Share Link, Save Lifelong Short URL**. Copy and paste this link to share your work.

Pre-Rendered Model Link

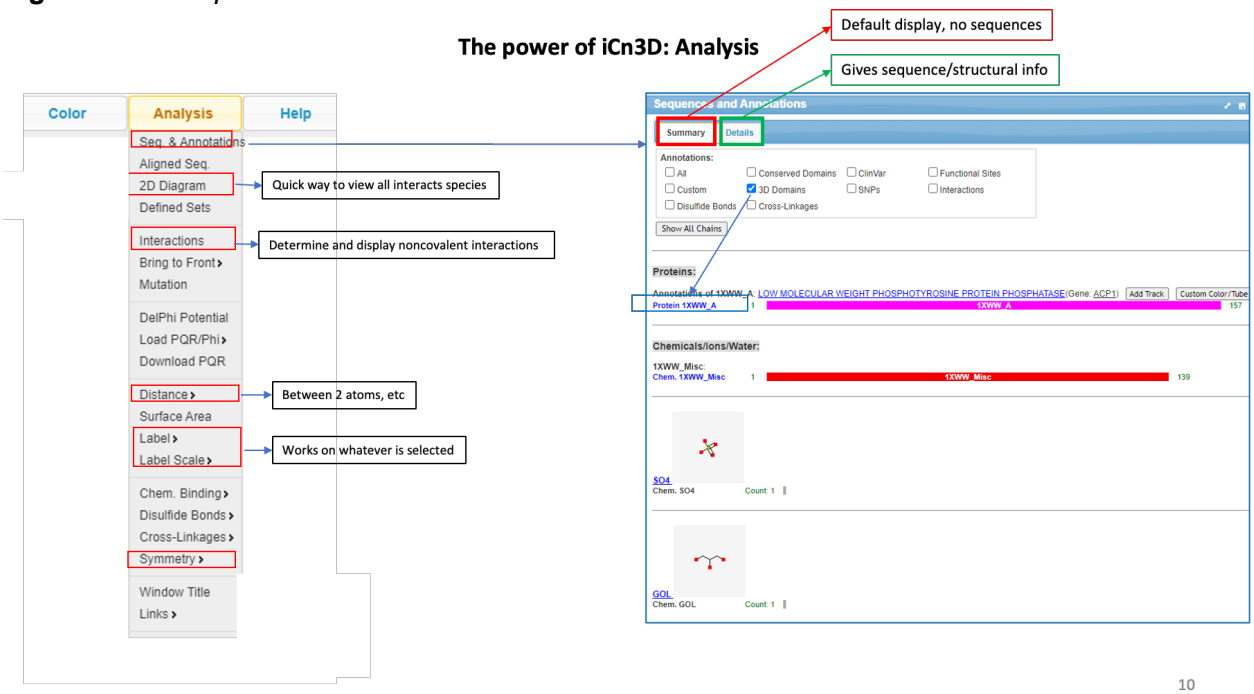
To check your work (or if you got stuck during any of the steps above) catch up using this link: https://structure.ncbi.nlm.nih.gov/icn3d/share.html?CgfEnF27TN7aYQpr6



Activity 2: Sequences & Annotations

The instructions below will guide you through the iCn3D activity for this part of the workshop. If you get stuck, an iCn3D shareable link is provided at the end, which will bring you to a pre-rendered structure that you can manipulate and modify.

Figure: *The Sequences and Annotations Menu*



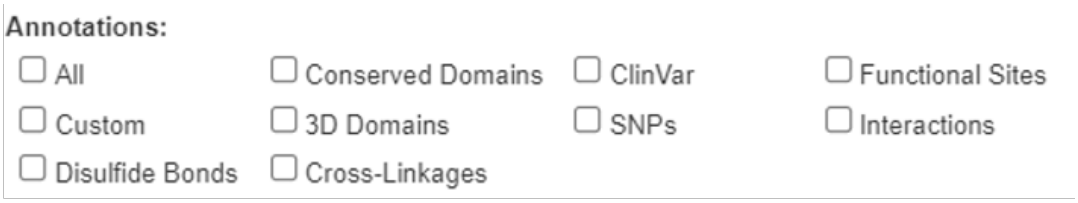
For this part, we will be using the model from Activity 1. To load the premade model from Part 1, use this link: https://structure.ncbi.nlm.nih.gov/icn3d/share.html?CgfEnF27TN7aYQpr6

From the literature, it is known that the active site is a nucleophilic cysteine (C12). It is part of the phosphate-binding loop (P-loop, AA 12-18: sequence CLGNICR). Let's find, select, and render these amino acids.

Modeling Instructions

1. Under **Analysis** (top menu bar), choose **Sequence and Annotations**
2. Choose **Details tab, uncheck Conserved Domains**

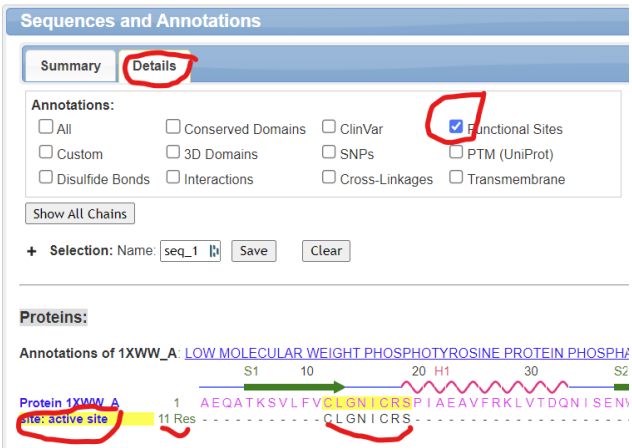
Before we continue, look at the built-in choices you have for selection:



3. In the Sequences and Annotation window, click Protein 1XWW_A
4. Under **Select** (top menu bar), choose **Toggle Highlights**
5. Hover over C12 in the sequence (in Seq and Annot window), click and hold down the mouse key, and sweep over C12-C18 to select the P loop
6. **Select, Save Selection**, name it: Ploop
7. Within this highlighted selection**, Style, Side Chains, Sticks**
8. **Color, Atom**
9. **Analysis, Label, Per Residue & Number**
10. **Analysis, Label Scale,** pick number that works for you
11. **Analysis, Label, Change Label Color** (globally). Click in the text box and a Color box will pop up, choose from a palette, then **Display**. (Alternatively, pick a hex code**).**
12. In the Sequences and Annotation window, click SO4
13. **Style, Chemicals, Sphere** to change the sulfate to a space filling rendering
14. Files, Share Link, Copy Short URL

Pre-Rendered Model Link

To check your work (or if you got stuck during any of the steps above) catch up using this link: https://structure.ncbi.nlm.nih.gov/icn3d/share.html?QhtGuE8pkaJpGs1X9



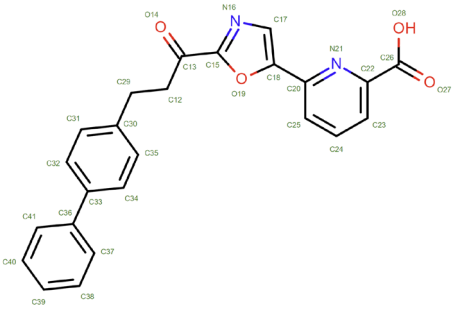
Note: For some enzymes, iCn3D can automatically display key active site and binding residues. These can be seen as shown by selecting the items indicated in the left figure.

Return to the Table of Contents

Activity 3: iCn3D analysis of noncovalent interactions

The instructions below will guide you through the iCn3D activity for this part of the workshop. If you get stuck, an iCn3D shareable link is provided at the end, which will bring you to a pre-rendered structure that you can manipulate and modify.

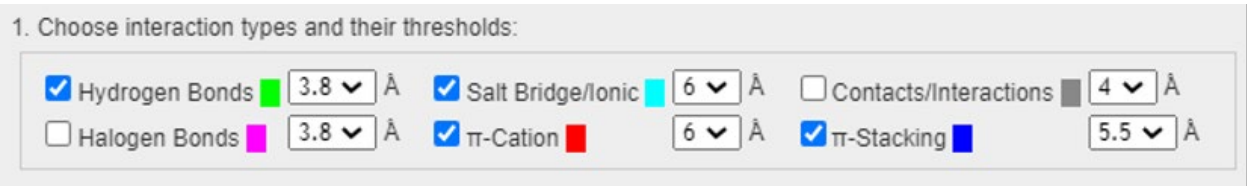
About this model:
PDB ID: 3K83
Protein: Inhibitor (right, abbreviated F278458) bound to a humanized variant of Fatty Acid Amide Hydrolase (FAH)
Activity: FAH Catalyzes the hydrolysis of endogenous amidated lipids like the sleep-inducing lipid oleamide, the endocannabinoid anandamide, and other fatty amides, regulating the signaling functions of these molecules
Description: The functional unit is a dimer, each with an active site. A chloride binds between the two subunits



In this activity you will see key noncovalent interactions between the inhibitor and (FAH). You will pick one inhibitor and view its noncovalent interactions with one subunit

Modeling Instructions

1. Open a new iCn3D window, 3K83 at: https://www.ncbi.nlm.nih.gov/Structure/icn3d/full.html
2. **Select, Select on 3D** to ensure default is **Residue**; With mouse, zoom and center to Alt-Click the inhibitor (F278458) in the magenta subunit (option-click on a Mac)**.**
3. **Select, Save Selection,** name it **Drug**
4. **Analysis, Interactions.**
5. Under 1. Choose interaction types and their thresholds, Check only the noncovalent interactions shown below. Make sure the Contacts/Interactions is unchecked as it shows all interactions between contacting Van der Waals surfaces including many hydrophobic interactions, so it is quite cluttered.



6. Select the Drug as the first set (under 2. Select the first set); choose 3K83 (under 3. Select the second set)
5. Click on the box: 4. 3D Display Interactions. This will display interacting side chains. Drag the HBonds/Interactions window to the bottom right away from the molecular display. Note the coloring of the dotted lines:
   Green - hydrogen bonds
   Red - π-cation
   Blue - π stacking
6. **Select, Save Selection,** name it **Interactions**
7. **Style, Sidechains, Stick** (so the next step will color side chains)
8. **Color, atom (*Note*:** this drug is covalently bound to Serine241)
9. In defined sets Ctrl click Drug and Interaction to highlight all
10. **View, View Selection** (to only see Drug and Interactions)
11. **Select, Toggle Highlight** (to remove highlights if necessary)

Note: Once you run interactions, iCn3D adds many new additions to the Defined sets window. Explore these to learn different ways to examine the interactions.

Optional (if you'd like to add labels, recolor the background, and obtain the share link as in the previous activity)

12. **Analysis, Label, Per Residue & No**
13. **Analysis, Label Scale,** pick a number that works for you
14. **Style, Background, Transparent**
15. **File, Share Link,** copy short link

Pre-Rendered Model Link

To check your work (or if you got stuck during any of the steps above) view the model using this link: https://structure.ncbi.nlm.nih.gov/icn3d/share.html?pDr5EBZmo3TyAbTP6

Note: You can alter interactions on the 3D model, or examine 2D displays of them. Try:

- Click **5. Reset** at the bottom of the HBonds/Interactions window. Add Contacts/ Interactions (mostly nonpolar) by clicking on the box. Choose Drug as the first set. Choose 3K83 as the second set. Then click **4. 3D Display Interactions** to show.
- Next, try **4. 2D Interaction Network**. In the popup that opens, click a colored line in the 2D window to highlight a specific interaction.
- If using the Pre-Rendered model: From the top menu, select: **Analysis** → **Interactions**. In the popup choose **5. Reset**, select 3K83 (full protein) as set 1 and drug as set 2, then **4. 2D Interaction Network**. Click colored lines in the 2D window.

Return to the Table of Contents

Activity 4: Creating and saving selections

The instructions below will guide you through the iCn3D activity for this part of the workshop. If you get stuck, an iCn3D shareable link is provided at the end, which will bring you to a pre-rendered structure that you can manipulate and modify.

About this model:

PDB ID: 3UBB
Protein: Rhomboid intramembrane serine protease GlpG (3UBB) with phosphonofluoridate inhibitor
Activity: Integral membrane serine protease
Description: Single chain transmembrane protease from *E. coli* bound to a phosphonofluoridate inhibitor, which is covalently bonded to the catalytic serine. Red and blue dots ("dummy" atoms) indicate extracellular and intracellular membranes, respectively. Uses a catalytic **dyad** composed of serine (S201) and histidine (H254).

Load Structure

1. Open iCn3D - https://www.ncbi.nlm.nih.gov/Structure/icn3d/full.html
2. In the *Please input MMDB or PDB*, enter 3UBB. Press enter or click load biological unit.

Selecting using: 1. The structure viewer window with the Mouse and 2. The sequences and annotations menu (Key Learning Objectives)

This section will show how you can select residues, chains, etc in the Modeling window.

3. **Select, Select on 3D** to ensure default is **Residue**; With cursor, hover over the inhibitor (name 3UB) and Alt Click (option click on Mac) it. A yellow halo will appear around it.
4. **Select, Save Selection**, name it Inhibitor.
5. Now, use the top menu to open the sequences and annotations tab: **Analysis, Seq. and Annotations**
6. In the sequences and annotations window, uncheck "Conserved Domains," and then click the **Details tab**. Click individually on the one letter code for S201 and H254. On selection, they will turn yellow.
   (Note: To see the scroll bar in sequences/annotations in a Mac, choose Systems preferences in your operating system [not the iCn3D settings], General, show scroll bars and check always; see iCn3D About notes).
7. **Select, Save Selection,** name CatDyad

Rendering (Optional, can use the highlighted link below instead)

8. **Select**, **defined sets** (Note that "defined sets" brings up a complete list of objects, many selections are pre-built into iCn3D to get you started with a model.)
9. In Selected Sets, click 3UBB_A.
10. **Color, Unicolor, Gray, Light Gray**
11. In Selected Sets, click CatDyad
12. We want to show the side chain of the catalytic dyad, so use the top menu: **Style, SideChains, Sticks**
13. Recolor to CPK coloring: **Color, Atom**
14. **Analysis, Label, Per Residue and Number**
15. **Analysis, Label Scale,** pick number that works for you
16. **Style, Background, Transparent**
17. Remove any active selections (yellow glow) by Select, Toggle highlight

Pre-Rendered Model Link

https://structure.ncbi.nlm.nih.gov/icn3d/share.html?fhT3dwckYg8i5XJj8
The short URL above may be used to catch up for the next section of the tutorial

Selecting sphere within around 5Å of the inhibitor (Key Learning Objective)

Our goal is to find all atoms with 5Å from the inhibitor, this time without showing interactions. In the next step, we will designate 2 sets of objects. Set 1 will be the inhibitor. Set 2 will be nearby residues/bound molecules. In our case, Set 2 will be defined as the protein.

18. **Select, by Distance**
19. For the first set, select inhibitor; for Set 2 click 3UBB
20. For **Set 2. Sphere with a radius to 5 Å**
21. **Click Display; Close the Select by distance window**
22. Now save the highlighted groups 5Å from the inhibitor through **Select, Save Selection,** Name 5AfromInhib
23. **Style, Side chains, Stick**
24. **Color, Atom**
25. Select both the inhibitor and the surrounding residues: In **Defined Sets,** Click Inhibitor, and Ctrl+Click 5AfromInhib (Command+Click on a Mac)
26. Display only the active site for clarity: **View, View Selection**
27. **Analysis, Label, Per Residue & Number**
28. To see water, **Style, Water, Sphere**
29. **File, Share Link,** copy short link

Pre-Rendered Model Link

To check your work (or if you got stuck during any of the steps above) view the model using this link: https://structure.ncbi.nlm.nih.gov/icn3d/share.html?sqZf4Zvqn5zWK21d9 This link shows the membrane. Choose **View, Toggle Membrane** to hide it.

Return to the Table of Contents

Activity 5: A Classroom Activity (time permitting)

The instructions below will guide you through the iCn3D activity for this part of the workshop. This involves a proposed classroom activity for students.

About this model:
PDB ID: 4PH9
Protein: Ibuprofen (IBP) bound to cyclooxygenase-2
Activity: COX-2 catalyzes the conversion of arachidonic acid(AA) to prostaglandin G2 (PGG2), and is a target of non-steroidal anti-inflammatory drugs (NSAIDs) and COX-2 selective inhibitors (coxibs). Arachidonic acid (AA), not shown in this structure, binds in a "L" shape

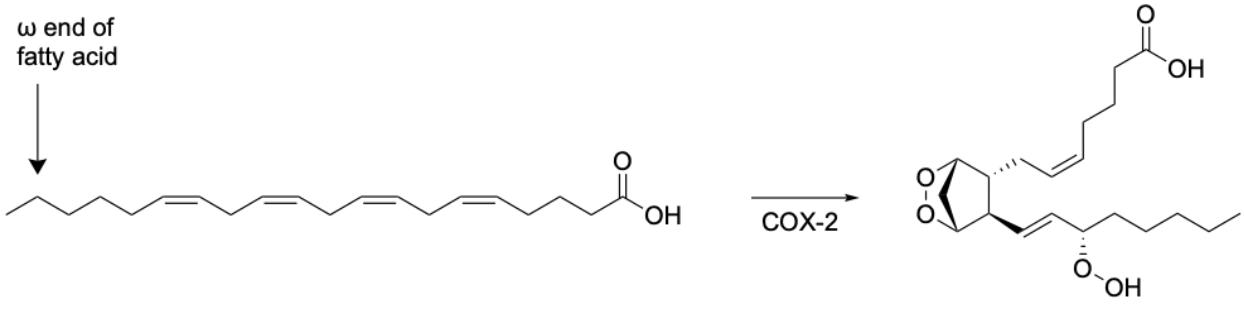
Key amino acids:

- Arg-121 and Tyr-356 are close to the carboxylate of AA
- Phe205, Phe209, Val228, Val344, Phe381, and Leu534 form a hydrophobic groove for the ω-end of AA.
- Ser 530, which is above this, gets acetylated by aspirin
- Tyr 385, near C13 in AA, forms a free radical which removes a single electron from C13

For more information on the mechanism of COX-2, scroll down to the end of the chapter section in Fundamentals of Biochemistry.

Description: Biological dimer with heme, ibuprofen (IBP), and many other ligands bound. The initial display is messy! Your group task is to clearly render a specific structural feature.

Assessment: The enzyme cyclooxygenase-2 (COX-2) produces arachidonic acid from prostaglandin G2, as shown in the reaction below. Prostaglandin G2 is an important metabolite in inflammation, so inhibition of (COX-2) reduces inflammation.



arachidonic acid

Prostaglandin G2

Potential visualization activities for students:

A. Identify the noncovalent interactions of COX-2 with a heme in 1 subunit
B. Model and describe the noncovalent interactions of COX-2 with ibuprofen
C. Model the interactions at the dimer interface of the protein; identify two amino acids on different subunits that are participating in a [hydrogen bond / ionic interaction]
D. Show the key active site residues of the enzyme

Steps for Discussion and/or Modeling

STEP 1: As a table, or small group at one table, choose one from A-D above.

STEP 2: Load the model 4PH9 at https://www.ncbi.nlm.nih.gov/Structure/icn3d/full.html
With your group, broadly discuss the steps students would need to perform to accomplish the activity you chose in STEP 1. Consider:

- For your course, would it make sense for them to start with the model as it is loaded?
- If you were to pre-render the model and provide them a shared link, which steps would you perform ahead of time? Which steps of modeling are important to their learning

STEP 3: Open the sequences and annotations tab:
Analysis → Sequences & Annotations
Uncheck "conserved domains," and then check "functional sites"

STEP 4: Discuss how the built in iCn3D features may help you create a model quickly.

Pre-Rendered Model Links from Workshop Leaders

1. Identify the noncovalent interactions of COX-2 with a heme in 1 subunit
   https://structure.ncbi.nlm.nih.gov/icn3d/share.html?ffnfemgp1ZJZxa4u9 w/o preset
   https://structure.ncbi.nlm.nih.gov/icn3d/share.html?8PaBfJKGZoKR5y5g8 w/preset

2. Model and describe the noncovalent interactions of COX-2 with ibuprofen
   https://structure.ncbi.nlm.nih.gov/icn3d/share.html?XHxMrn48zrVbKKCy6 w/o presents
   https://structure.ncbi.nlm.nih.gov/icn3d/share.html?sJHhAqhPDgT57kTV8 w/preset

3. Model the interactions at the dimer interface of the protein; identify two amino acids on different subunits that are participating in a [hydrogen bond / ionic interaction]
   https://structure.ncbi.nlm.nih.gov/icn3d/share.html?V3Zs4zCAoTM2Mk8A8 w/preset
   https://structure.ncbi.nlm.nih.gov/icn3d/share.html?K27jkv5n25rhXm9B8 surface

4. Show the key active site residues of the enzyme
   https://structure.ncbi.nlm.nih.gov/icn3d/share.html?A3mmUMd7KznoymrN6 (w/o preset)
   https://structure.ncbi.nlm.nih.gov/icn3d/share.html?fznyAtJnTkTmuNreA (3KRK with bound arachadonic acid)

Return to the Table of Contents

An Invitation to Connect with BioMolViz

Join us for our spotlight talk, right after this workshop!

(Abstract ID 1536) Evaluating Biomolecular Visual Literacy: A Library of Classroom-Tested Assessments for Instructor Use. Monday, March 27, 2023, 2:45 p.m. in Room 6C.

About the BioMolViz Project

Since 2012, the BioMolViz group has been working to advance BMV instruction. In 2017, with broad contributions from the BMB educator community, we authored a Framework for assessing visual literacy (Dries et al.). The most current, browseable version of the Framework is available at https://biomolviz.org/framework/.

Through workshops and remote working groups, we train instructors to use the Framework for backward design of assessments to evaluate students' visual literacy in the classroom. We recently built a repository to house these assessments, the BioMolViz Library, available at library.biomolviz.org (https://doi.org/10.15781/6mcy-8m69)

We invite instructors to use the assessments in the Library to evaluate visual literacy in their courses. You may request an account for the BioMolViz Library using this form.

Connect with our Community

To keep up with BioMolViz news and events, please sign up to receive our newsletter. Our activities are also posted to the events page of our website. We welcome you to reach out to us at info@biomolviz.org, or directly connect with any of the workshop leaders by email.

Return to the Table of Contents

Some molecular models from Fundamentals of Biochemistry

The table below lists some models from Fundamentals of Biochemistry, a LibreText OER.

Click on the links to go to a pre-rendered iCn3D model. To see the text from the book associated with the model, search for the PDB ID here: Fundamentals of Biochemistry: SEARCH . Open iCn3D to create a similar model of your own.

Topic	Models
Carbohydrates and Glycobiology	Human Galectin-1 in Complex with Type 1 N-acetyllactosamine (Gal(β1,3)GlcNAc) (4XBL) Simian immunodeficiency virus (SIV) gp120 core glycoprotein (3fus) P-selectin lectin/EGF domains complexed with SLeX (1g1r) (Choose **Style, Glycans, Show Cartoon or Style, Preferences,Show Glycan Cartoon** to shown SNFG cartoons)
Carbohydrate Metabolism	Pyruvate dehydrogenase E3 bound to both FAD (noncovalently) and NADH (NAI) (1ZMD) Pig citrate synthase bound to CoASH and citrate (2CTS)
Enzymes	A methyltransferase ribozyme (7V9E) Rhomboid intramembrane serine protease GlpG (4QO2) The NSAID ibuprofen bound to cyclooxygenase-2 (4PH9) Dihydrofolate reductase with bound NADP and folate (7DFR)
Lipid and Membrane Structure	Bovine mitochondrial ADP-ATP carrier protein (1OKC) Human cannaboid receptor with bound cholesterol and THC (5xra) Lac permease (1PV7)
Lipid Metabolism	Human Serum Albumin Complex to Arachidonic Acid (1gnj) Nascent HDL particle (3k2s) Mouse cyclooxygenase 2 (5COX) Catalytic domain of human HMG-CoA reductase with bound HMG-CoA (1DQ9)

Topic	Models
Nucleic Acid Struct/Metabolism	Adenine mispaired with 8-oxoguanine by MutY adenine DNA glycosylase (1RRQ) Class I E. Coli Cysteinyl-tRNA synthetase -tRNACys (1U0B) E. Coli Trp repressor - operator complex (1TRO) Human Argonaute2 Bound to a Guide and Target RNA (4W5O) N-terminal fragment of the yeast transcriptional activator GAL4 bound to DNA (1D66)
Photosynthesis and Plant CHO Synthesis	Barley starch synthase I in complex with maltooligosaccharide (4HLN) Heavy and light chain of ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCo) from Synechococcus PCC6301 (1RBL)
Protein Structure/Function	Alpha-Beta Three layer sandwich (aba) - Human biliverdin IX beta reductase (1HDO) C3 Symmetry - porin (2POR), homo 3-mer Cation-pi or ion-induced dipole interaction in hen egg white lysozye (1LPI) HenEggWhiteLysozome_FabComplex (3HFM) RNase with four intrachain disulfide bonds (1KF5) Mouse Toll-like receptor 3 ectodomain complexed with double-stranded RNA (3CIY)
Signaling Molecules	CBD-bound full-length rat TRPV2 in nanodiscs (6U88) Crystal structure of the beta2 adrenergic receptor-Gs protein complex (3SN6) Holo-calmodulin with 4 bound Ca2+ ions (1CLL) Human Cas 9 AlphaFold model (P55211) Human low molecular weight protein tyrosine phosphatase bound to sulfate (1XWW) Ras-GAP complex (1WQ1) Ras and SOS (a GEF) complex (1BKD)

Popup models for all iCn3D structures in Fundamentals of Biochemistry. To see a model in a full iCn3D window, click on = (menu, top left) and select File, Share Link, and copy/paste the Lifelong Short URL in a new browser window. Return to the Table of Contents