

Christopher Arias, Ezekiel Buchert, Johana Cruz Lopez, Cesar Fernandez, Elizabeth Kowalski, Elisabet Tesla Nichols, Gabrielle Onnenga, Kellie Pierson, Jenny A. Cappuccio, and Frank E Cappuccio
Department of Chemistry, Cal Poly Humboldt, Arcata, CA, USA

Introduction

- RCSB PDB (RCSB.org) is the US data center for the global Protein Data Bank (PDB) archive of 3D structure data for large biological molecules (proteins, DNA, and RNA) essential for research and education in fundamental biology, health, energy, and biotechnology. (3)
- The Protein Data Bank (PDB) was the first open access digital data resource in biology and medicine. (3)

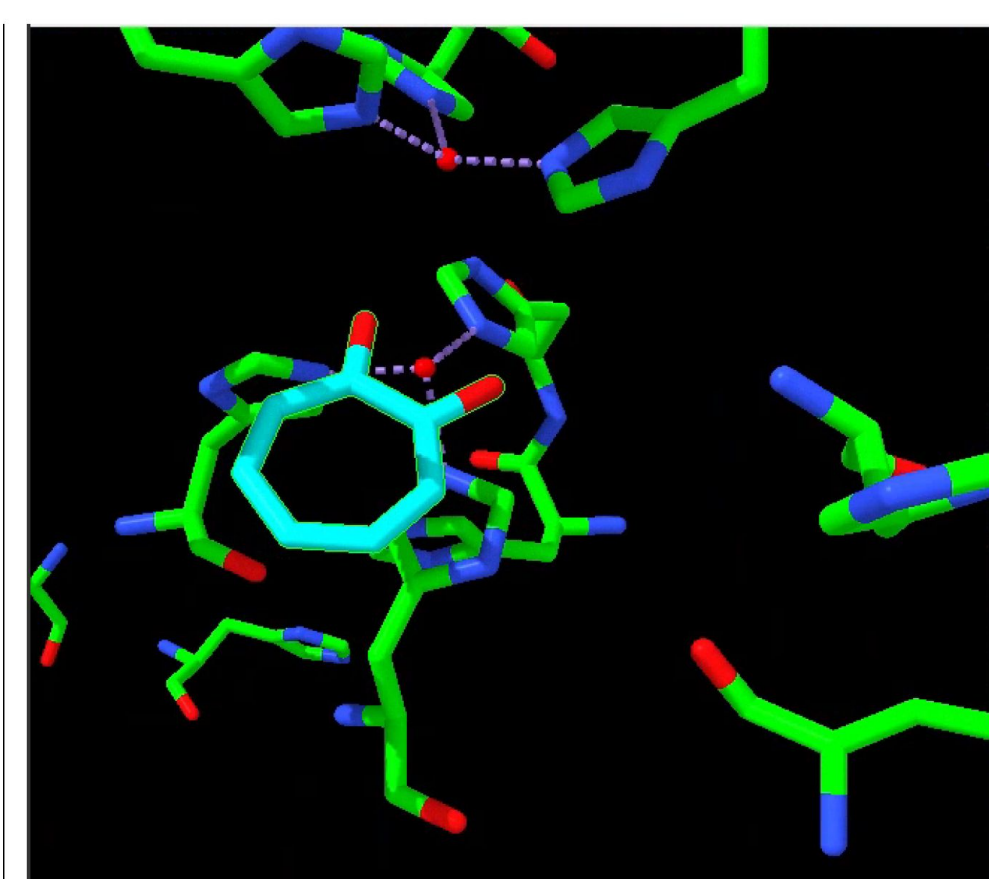


- Macromolecular structures are used in biotechnology, medicine and environmental studies to develop new cures for disease, and better biofuel production for example.
- Molecular visualization software ChimeraX was developed by UCSF faculty as a free program funded by the National Institutes of Health. (1, 2)

Methods

- Faculty developed hands-on student tutorial curriculum to demonstrate how to utilize PDB data.
- Students learned how to make 3D molecular representations of this data using UCSF ChimeraX software.
- A threaded curriculum biotechnology related protein (tyrosinase) was chosen to connect with previous knowledge in other courses.

Figure 1. Excerpt of student submissions for the hands-on PDB tutorial. This student utilizes their knowledge of charge to interpret the binding of an inhibitor to the tyrosinase enzyme active site, an enzyme causing browning in mushrooms. PDB 2Y9X. (5)



Inhibitor is the 7-membered cyan ring ^{AA}. It seems to be attracted to the Cu⁺ ions – which makes sense given the resonance and more negative Oxygen atoms in the ring being attracted to the Cu⁺.

- Students applied their knowledge to a protein of their choice creating both a Quad Chart presentation and a 3D printed surface model of the protein (4)
- Quad Chart presentations are used to pitch an idea or quickly and inform the audience for funding or further studies.

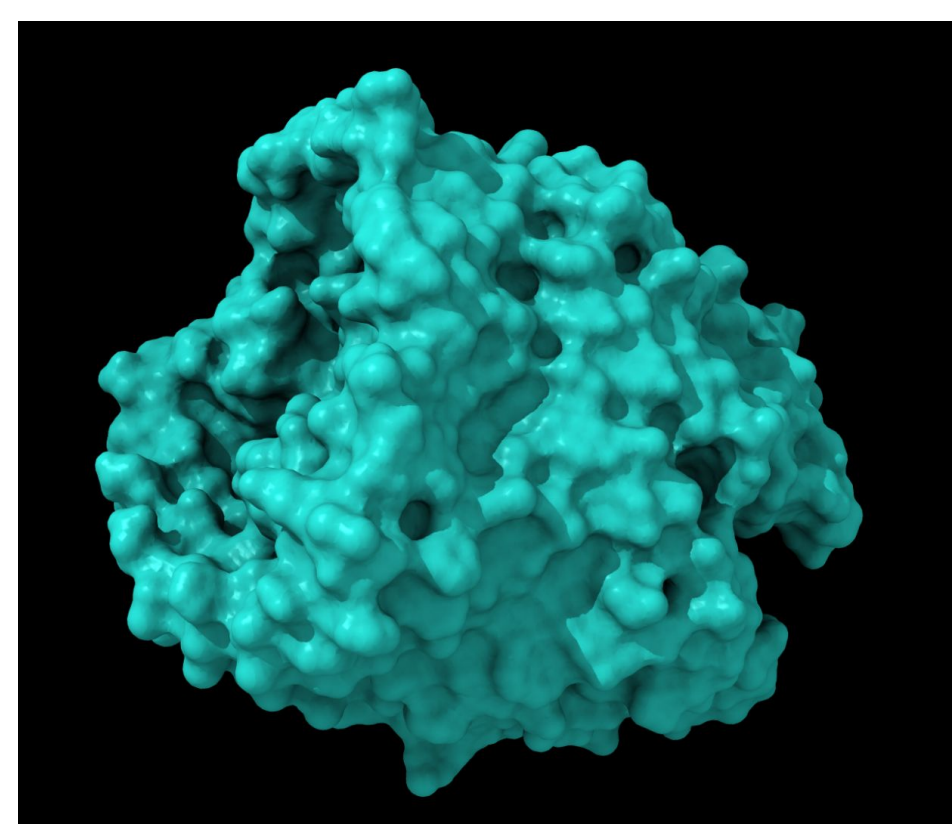


Figure 2. Student surface model submissions .stl file for the 3D printing of their protein choice, cobalamin (vit B12) transporter BtuB from the bacterium *E. coli* PDB 1NQH. (2003) Nat Struct Biol 10: 394-401

Student Products

Acetylcholinesterase PDB ID: 1ACJ

Elizabeth Kowalski
CHEM 425 - Intro. Biochem
3/6/2023

General & Structural Information

- Enzyme (esterase/hydrolase) that cleans up used acetylcholine¹
- acetylcholine carries signals from nerve → muscle cells; opens receptors triggers contraction
- breakdown: acetylcholine → acetic acid + choline
- Found in synapses between nerve/muscle cells²
- Structural motifs³
 - 2: 3' 10² α-helices, two β sheets, and numerous D loops connecting the two
 - 3: primarily connections by H bonds
 - 4: nonamide

Functional Information

- Active site: see the OctaH²
- Inhibitors: animal poisons/toxins, sarin gas, insecticides⁴
- No enzyme = too much acetylcholine = muscle paralysis
- ODDy: this can be good for AD patients
- Increased receptor stimulus stimulates memory loss⁵
- isoforms: AChE₁, AChE₂, AChE₃
- Generated by 3' end splicing of AChE gene⁶
- Some catalytic domain, but unique Chemical end
- Possible applications: development of anti-acetylcholinesterase drug⁷

Fun Facts

- 80 μs reaction time - one of the fastest of all enzymes⁸
- First isolated from electric fish (ex. Torpedo ray), which have extensive nervous connections including swimming organs
- Quaternary ligand binding to anionic residues in the active gorge of acetylcholinesterase. Heval et al. (2017). *Acta Med Chem* 36: 328-336
- Comprehensive regulation of glutamate release and glutamate uptake by nAChR⁹ and Ca²⁺ channels: acetylcholinesterase isoforms. Nasti et al. (2017). *Neurochem Res* 42: 1455-1464
- Du et al. (2005). Role of molecular function of acetylcholinesterase in learning and memory function. *Pharmacol Biochem Behav* 81: 89-92
- Crystal Structure of Acetylcholinesterase. Home Agent Reaction Products at the Atomic Level. Klotzel et al. (1999). *Biochemistry* 38: 7522-7526.

References

1. [Bark, R. C. S. B. P. D. IDEM: Protonase inhibitor homologues as potassium channel blockers. RCSB PDB](#)
2. [Gasparrini, S., Danesi, J. M., Lecocq, A., Pirkatchidze, S., Zinn-Justin, S., Young, L. C., de Medeiros, G. C. L., Rovati, L. G., Harary, A. L., and Menozzi, A. \(1999\). Definition of the functional site of α-dendrotoxin. *Journal of Biological Chemistry* 273: 25379-25403.](#)
3. [Klotzel, E., Winkler, H., and Hübner, K. \(2005\). Structural basis for the biological activity of dendrotoxin I, a potent potassium channel blocker. *Biopolymers* 54: 44-57.](#)

Dendrotoxin-I : PDB# 1DEM

Cesar Fernandez 3/9/23 PDB Assignment

MW: 7170 g/mol
2: 1 beta sheet and 2 alpha helix

A presynaptic neurotoxins which blocks potassium channels

(targeting certain subtypes of voltage-gated potassium channels found in neurons, is designed to boost the release of acetylcholine at neuromuscular junctions.)

Meant to destroy nerve tissue
Yet a useful protein for pharmacological research for ion channel proteins.

Produces Acetylcholine / and has anti protease functionality.

Gene source: Dendroaspis polylepis polylepis (Black mamba)

References

1. [Bark, R. C. S. B. P. D. IDEM: Protonase inhibitor homologues as potassium channel blockers. RCSB PDB](#)
2. [Gasparrini, S., Danesi, J. M., Lecocq, A., Pirkatchidze, S., Zinn-Justin, S., Young, L. C., de Medeiros, G. C. L., Rovati, L. G., Harary, A. L., and Menozzi, A. \(1999\). Definition of the functional site of α-dendrotoxin. *Journal of Biological Chemistry* 273: 25379-25403.](#)
3. [Klotzel, E., Winkler, H., and Hübner, K. \(2005\). Structural basis for the biological activity of dendrotoxin I, a potent potassium channel blocker. *Biopolymers* 54: 44-57.](#)

Human Hemoglobin 1CH4
Ezekiel Buchert

Total Structure Weight: 64.55 kDa
Atom Count: 4,997
Modelled Residue Count: 572
Deposited Residue Count: 574

The hemoglobin protein consists of two beta chains and two alpha chains. Each chain contains an iron atom. Oxygen binds these atoms of iron, and the molecule embeds into the red blood cells transports oxygen throughout the bloodstream. Carbon monoxide can irreversibly bond to the heme groups that contain the iron. This will poison the action of the molecule and can cause death.

This protein is capable of being synthesized by *E. coli*. This protein is capable of being identified via iron-60 in the heme group

References

1. [RCSB PDB: 1CH4: Modelled and deposited Human Hemoglobin Beta alpha \(1CH4\). RCSB PDB: <https://www.rcsb.org/structure/1CH4>. Accessed 6/4/2023](#)
2. [Shoji, T., Fujikake, M., Tamura, T., Inaba, C., Inohara, C., Morimoto, I. Crystal structure of a protein with an artificial iron-coordinating, non-heme MA substituted chelate hemoglobin beta alpha, at 2.4 Å resolution. *J Mol Biol*. 2008 Mar; 376\(2\):488-504. doi: 10.1016/j.jmb.2007.11.035. PMID: 18080893](#)
3. [Mason, T., Gorenflo, C., Wang, D., Hissinger, R., Gao, H., Mochly-Nesher, N., Bryant, D., Minko, G., and USTY: rationally designed antibodies between nucleocapsid proteins. *Nucleic Acid Res*. 2014 Jul; 42\(14\):4288-4295. doi: 10.1093/nar/nkt228. Epub 2013 Dec 6. PMID: 24252453; PMCID: PMC3626551](#)
4. [Mannervik, J. Carbon monoxide poisoning. *N Engl J Med*. 2002 Jun; 346\(23\):2172-6. doi: 10.1056/NEJMe0206066. PMID: 11936143; MEDLINE: 12061830](#)

Tau Tubulin Kinase I: PDB ID #4NFM
Kellie Pierson, 03/08/2023, PDB Assignment

General Features:

- Serine/Threonine Protein Kinase
- Part of the Casein Kinase 1 (CK1) family
- 1257 amino acids, 45 alpha-helices, 52 beta strands
- Conserved kinase domain
- MW: 126 kDa; N-terminal kinase MW: 41 kDa; C-terminal ubiquitin-associated domain MW: 85 kDa
- Isoforms: TTBK1A and TTBK1B (differ in N-terminal sequences)
- TBK1A is longer and the predominant form expressed in the brain
- Monomer; potential to dimerize
- Kinase domain: conserved catalytic core w/ ATP-binding site & catalytic residue for phosphorylation
- Localized to cytoplasm

TTBK1 Function & Substrate Specificity:

Phosphorylation
Implicated in Wnt signaling pathway; regulates cell growth, differentiation, and cell fate determination.

Known to phosphorylate a variety of substrates including tau proteins and tubulins; involved in microtubule assembly and stability; axonal transport, and neuronal development

Disease Implications:

- Spinocerebellar ataxia type 11; neurodegenerative disorder
- Alzheimer's disease; neurodegenerative disorder
- TTBK1 is involved in hyperphosphorylation of tau protein in both of these disorder
- Hyperphosphorylation of tau proteins contribute to formation of neurofibrillary tangles

References:

- 1. ["Human tau tubulin kinase 1 \(TTBK1\)". RCSB Protein Data Bank \(February 6, 2014\). DOI:10.2202/04NFM](#)
- 2. ["TTBK1 in complex with inhibitor". RCSB Protein Data Bank \(September 25, 2013\). DOI:10.2202/04NFM1949b](#)
- 3. [Hikins, et al. \(2021\). *Journal of medicine chemistry*, 64\(9\), 6358-6390. DOI: 10.1021/acs.jmedchem.1c00352](#)

Zika Virus :PDB ID # 5IRE
Christopher Arias, 03/08/23, PDB Assignment

General Features:

- Contains 504 amino acid residues
- Secondary structure:** contains 30 antiparallel beta sheets and 10 alpha helices
- Molecular weight:** 190.1 kDa
- Carbohydrate moiety functions as an attachment site of the virus to host cells.
- Is part of flaviviridae (flavivirus), which is a positive, single stranded RNA virus. Can be found in ticks and mosquitoes

Function:

- The virus is the causal agent of neurological disorders like the Guillain-Barre syndrome and microcephaly in newborns.
- The mechanism of Zika is to cross the placental barrier and the blood brain barrier.

"Fun" Facts:

- Zika virus was discovered in 1947, in an infected monkey in the Zika forest of Uganda
- Linked to birth defects
- Causes neurological symptoms
- Primary spread through infected mosquitoes

References

1. [Mason, T., Gorenflo, C., Wang, D., Hissinger, R., Gao, H., Mochly-Nesher, N., Bryant, D., Minko, G., and USTY: rationally designed antibodies between nucleocapsid proteins. *Nucleic Acid Res*. 2014 Jul; 42\(14\):4288-4295. doi: 10.1093/nar/nkt228. Epub 2013 Dec 6. PMID: 24252453; PMCID: PMC3626551](#)
2. [Mannervik, J. Carbon monoxide poisoning. *N Engl J Med*. 2002 Jun; 346\(23\):2172-6. doi: 10.1056/NEJMe0206066. PMID: 11936143; MEDLINE: 12061830](#)

Conclusions

- 3D modeling is a valuable interactive tool to understand the complexity of biological macromolecules informing funding.
- Student displayed in-depth understanding of protein structure and binding in hands-on activities and presentations of protein structures
- The knowledge of biological molecular structure in drug design and biotechnology is an essential skill set for modern scientists to meet the grand challenges of the future.

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References

1. [UCSF ChimeraX: Structure visualization for researchers, educators, and developers. Pettersen EF, Goddard TD, Huang CC, Meng EC, Couch GS, Croll TI, Morris JH, Ferrin TE. *Protein Sci*. 2021 Jan;30\(1\):70-82.](#)
2. [UCSF ChimeraX: Meeting modern challenges in visualization and analysis. Goddard TD, Huang CC, Meng EC, Pettersen EF, Couch GS, Morris JH, Ferrin TE. *Protein Sci*. 2018 Jan;27\(1\):14-25.](#)
3. [The Protein Data Bank H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne \(2000\) *Nucleic Acids Research*, 28: 235-242. <https://doi.org/10.1093/nar/28.1.235>](#)
4. [PDB-101: Educational resources supporting molecular explorations through biology and medicine. Christine Zardecki, Shuchismita Dutta, David S. Goodsell, Robert Lowe, Maria Voigt, Stephen K. Burley. \(2022\) *Protein Science* 31: 129-140 <https://doi.org/10.1002/pro.4200>](#)
5. [Crystal Structure of Agaricus Bisporus Mushroom Tyrosinase: Identity of the Tetramer Subunits and Interaction with Tropolone. *Jsmaya, W.T., Rozeboom, H.J., Weijn, A., Mes, J.J., Fusetti, F., Wichers, H.J., Dijkstra, B.W.* \(2011\) *Biochemistry* 50: 5477](#)