Putting molecules from the computer screen into students' hands using 3D printing

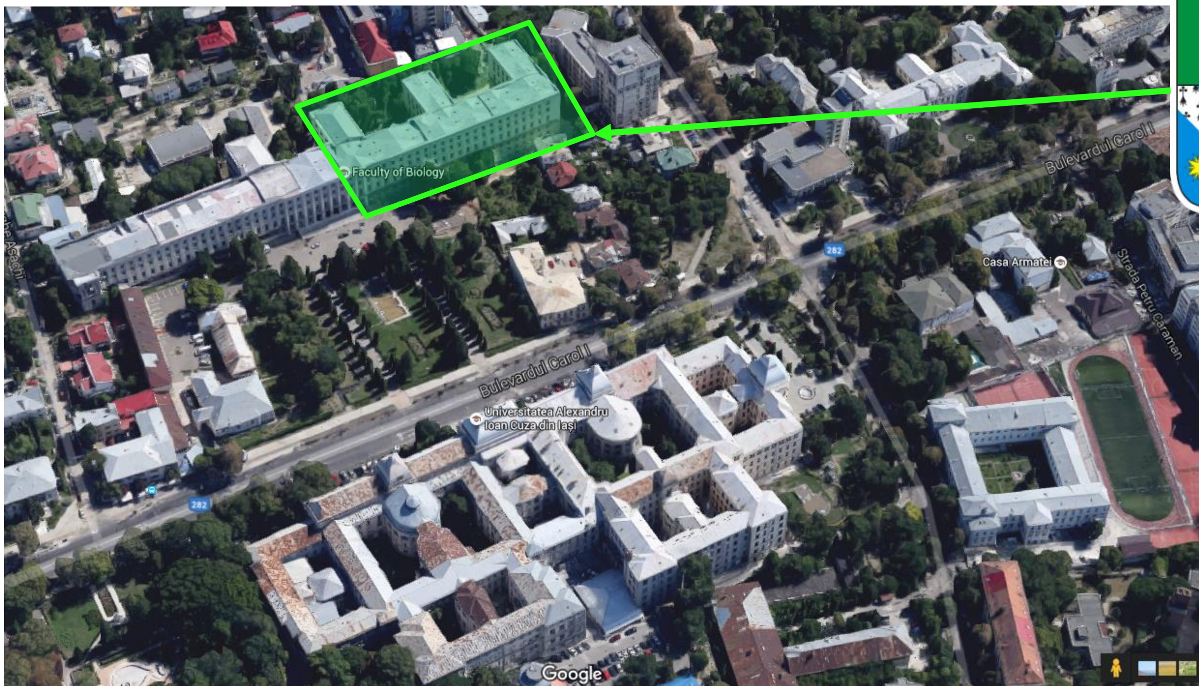
ALEXANDRU IOAN CUZA UNIVERSITY of IAŞI

www.uaic.ro

Marius Mihăşan, PhD.

Faculty of Biology
Alexandru Ioan Cuza University of Iaşi, Romania
E-mail: marius.mihasan@uaic.ro
https://3dprint.nih.gov/users/mariusmihasan
https://modelemoleculare.ro/

Department of Biology



Bulevardul Carol I nr. 20A,
Iaşi, Romania, 700505
Tel.:+40(0)232201072
Fax: 40(0)232201472
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Founded in **1948**

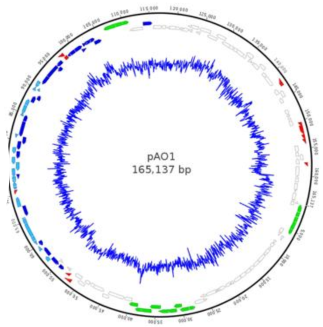
BioActive research group

- Isolation
- Identification
- Characterization
- Biological effects (neurological effects, citotoxycity, oxidative stress, antimicrobial activity

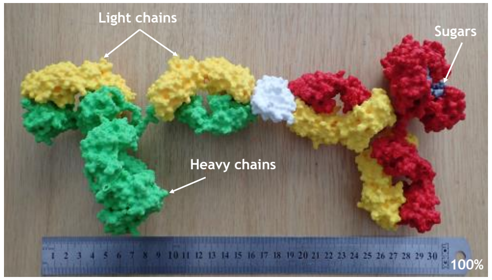
of biological active molecules with potential applications in biotechnology.

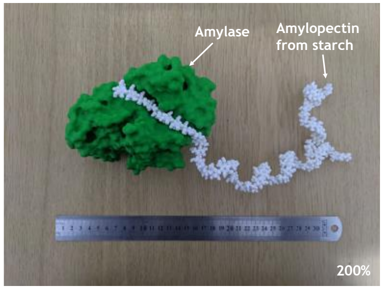
Main research subjects:

- molecular biology of pAO1 megaplasmid related to nicotine catabolism, stress induced by nicotine degradation and biotechnological applications..

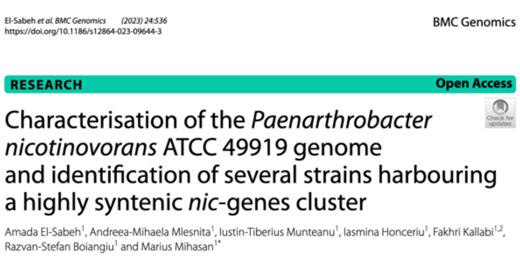


- using 3D printing for creating teaching materials to support molecular bioscience education.

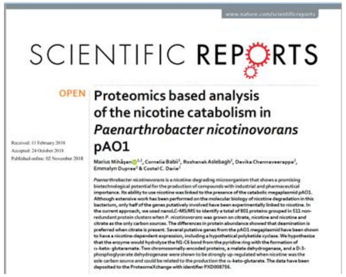




Latest paper:


El-Sabeh et al. BMC Genomics (2023) 24:536
https://doi.org/10.1186/s12864-023-09644-3
BMC Genomics
RESEARCH Open Access
Characterisation of the *Paenarthrobacter nicotinovorans* ATCC 49919 genome and identification of several strains harbouring a highly syntenic *nic*-genes cluster
Amada El-Sabeh[1], Andreea-Mihaela Mlesnita[1], Iustin-Tiberius Munteanu[1], Iasmina Honceriu[1], Fakhri Kallabi[1,2], Razvan-Stefan Boiangiu[1] and Marius Mihasan[1*]

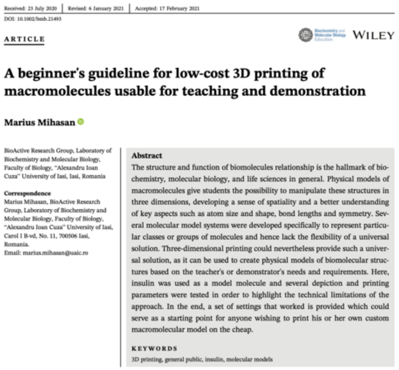
Most Important paper:


SCIENTIFIC REPORTS
OPEN Proteomics based analysis of the nicotine catabolism in *Paenarthrobacter nicotinovorans* pAO1

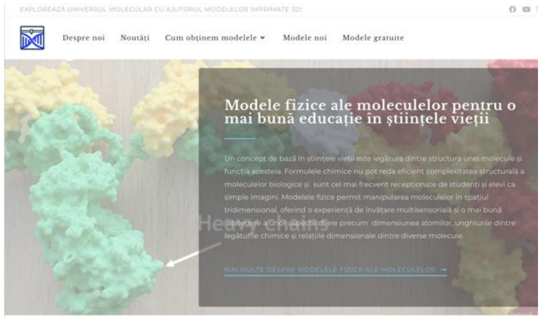
https://www.nature.com/articles/s41598-018-34687-y

https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-023-09644-3

Developed educational resources:


ARTICLE WILEY
A beginner's guideline for low-cost 3D printing of macromolecules usable for teaching and demonstration
Marius Mihasan

https://doi.org/10.1002/bmb.21493



https://modelemoleculare.ro/

The plan for today

Theoretical part:

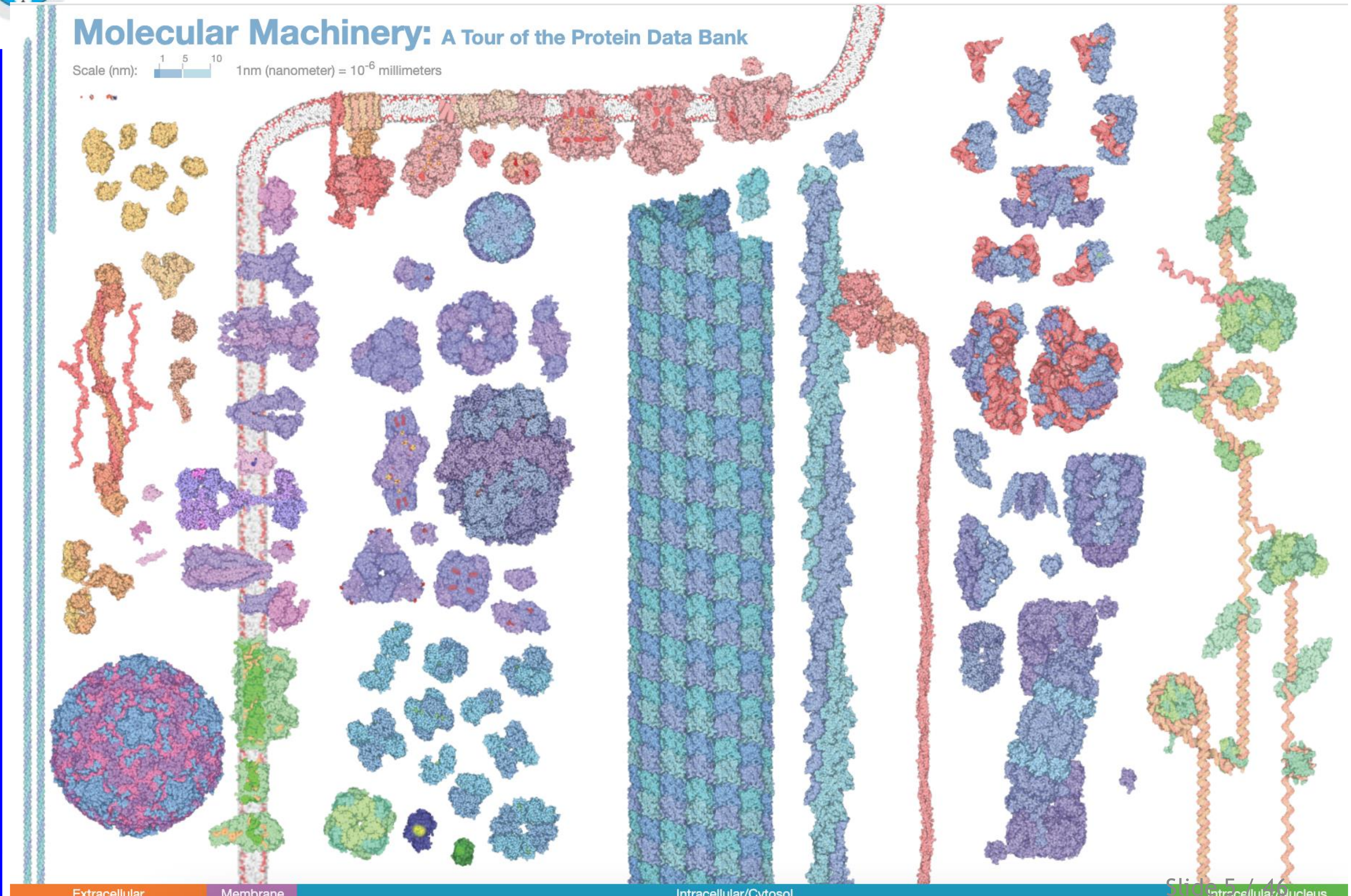
- **Why are 3D printed molecular models needed?**
- **How and from where can I get 3D printed models ?**
- **Are these models efficient?**

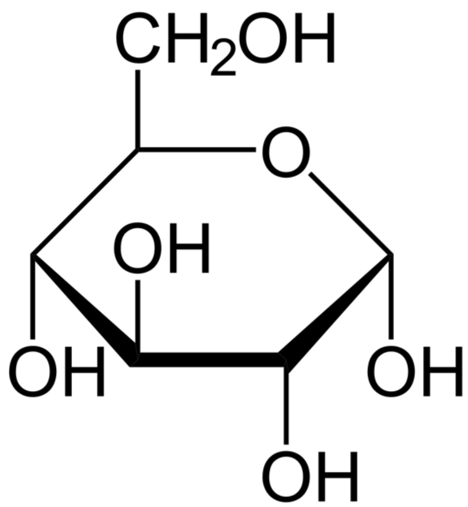
Hands-on part:

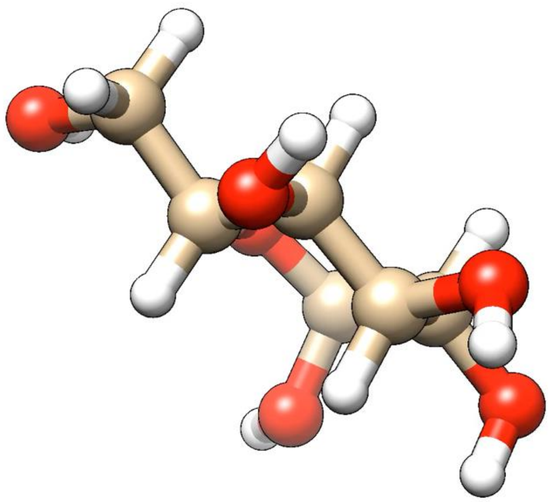
- **.pdb to .stl file - software and steps**
- **Practical considerations when 3D printing molecular models**

Understanding Life Sciences relies on understanding Structural Biology

https://cdn.rcsb.org/pdb101/molecular-machinery/

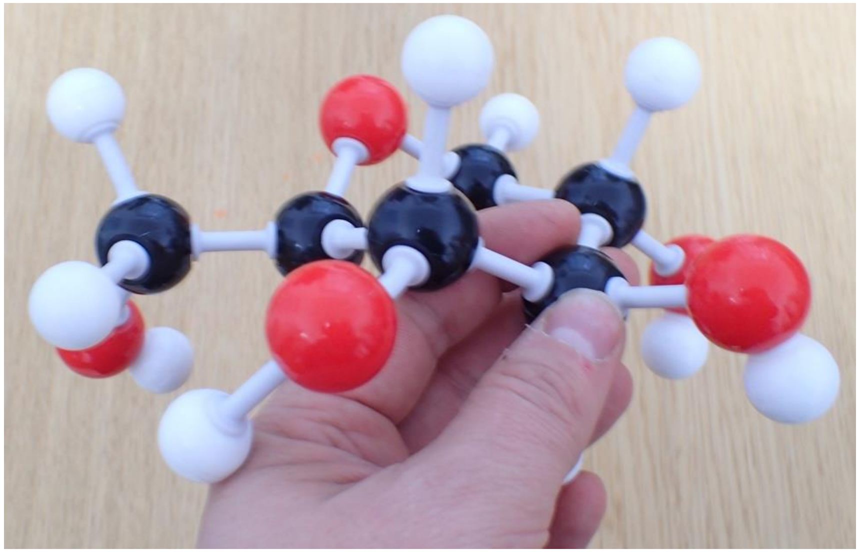






α-D-glucopyranose

Molecular models to aid teaching – Molymod

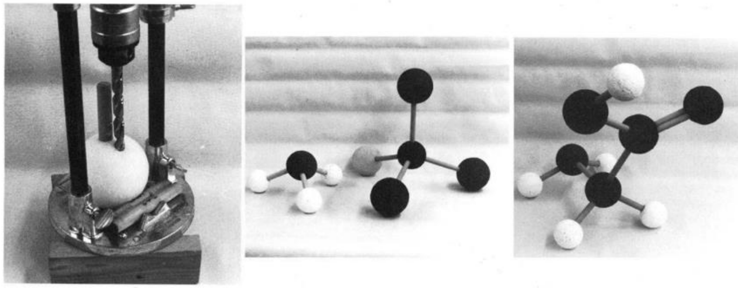


http://www.molymod.com/MMS-004_Inorganic__Organic_Teacher_Set.jpg

molymod®

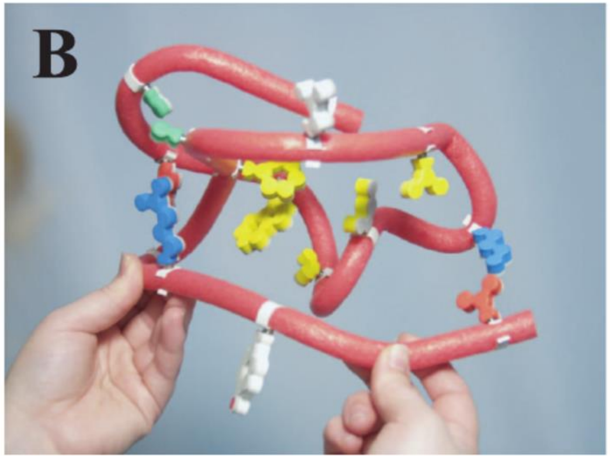
The *original* dual-scale system of molecular models

Molecular models to aid teaching - DIY



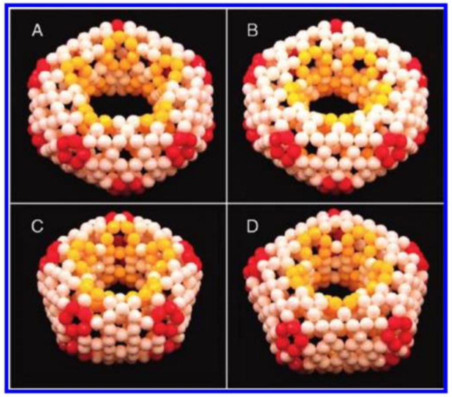
Styrofoam balls and copper wires

Birk, J. P.; Foster, J. Molecular models for the do-it-yourselfer. J. Chem. Educ. 1989, 66, 1015–1018.



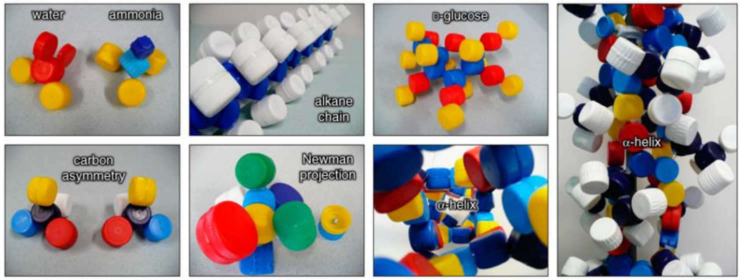
Flexible foam, wires and foam cut-outs

Herman T., et. al. Tactile teaching: Exploring protein structure/function using physical models. Biochem. Mol. Biol. Educ. **34**: 247–254.



Glass Beads

Chuang, C. et al. Molecular Modeling of Fullerenes with Beads. J. Chem. Educ. 2012, 89, 414−416



Screw-on bottle caps

Siodłak, D. Building Molecular Models Using Screw-On Bottle Caps. J. Chem. Educ. 2013, 90, 1247−1249.

Molecular models to aid teaching – Paper models


Antibody
(Paper Model)

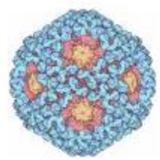

DNA
(Paper Model)


Dengue Virus
(Paper Model)


Green and Red Fluorescent Proteins
(Paper Model)


G Protein-Coupled Receptor (GPCR)
(Paper Model)


HIV Capsid
(Paper Model)

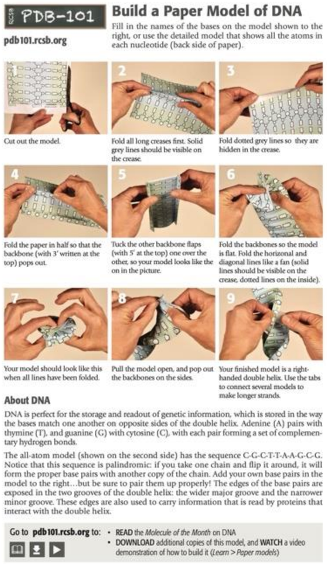

Human Papillomavirus (HPV)
(Paper Model)

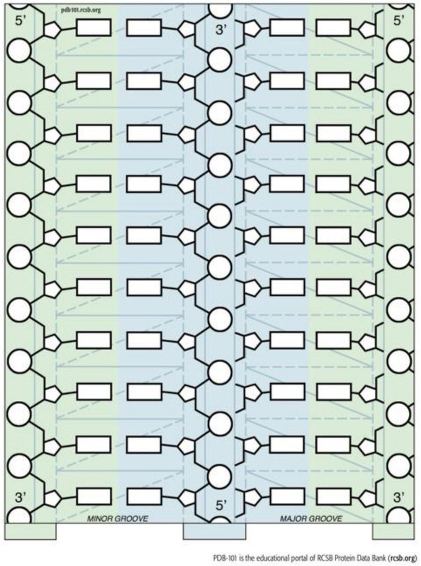

Insulin
(Paper Model)


Quasisymmetry in Icosahedral Viruses
(Activity Page)


tRNA
(Paper Model)

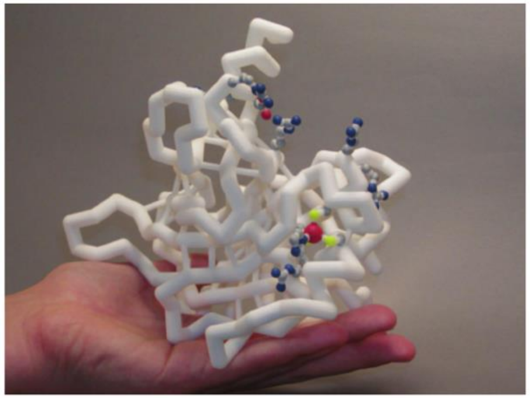

Zika Virus with and without antibodies
(Paper Model)





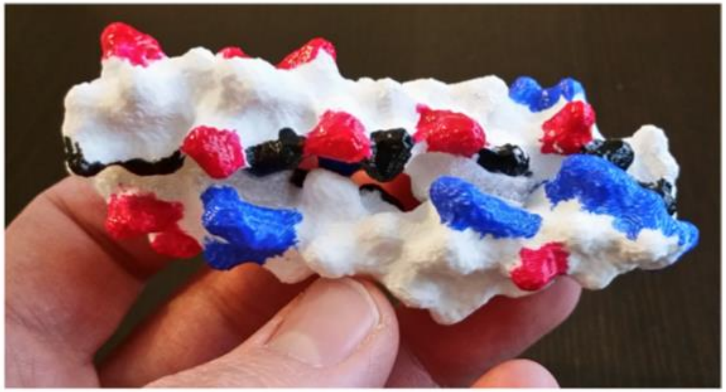
https://pdb101.rcsb.org/learn/paper-models

Molecular models to aid teaching – 3D printed models



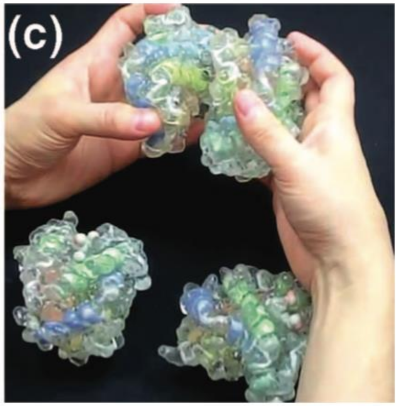
p53 tumor suppressor protein

Herman T., et. al. Tactile teaching: Exploring protein structure/function using physical models. Biochem. Mol. Biol. Educ. 34: 247–254.



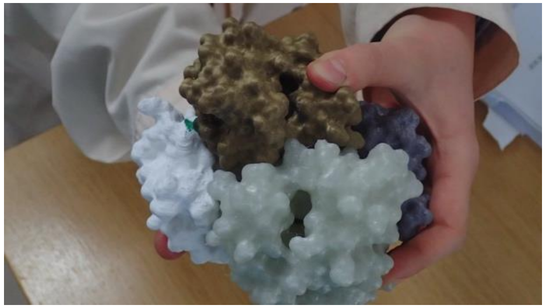
Leucine zipper

Meyer S.C. 2015. 3D Printing of Protein Models in an Undergraduate Laboratory: Leucine Zippers. J. Chem. Educ. 92: 2120–2125.

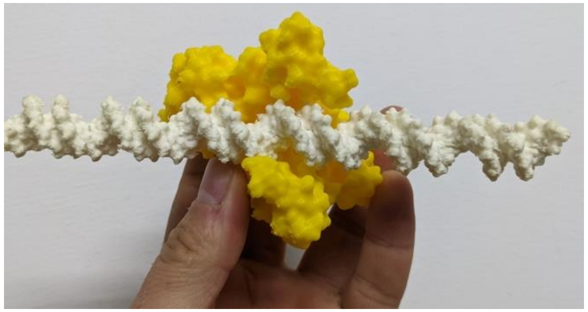


Human haemoglobin

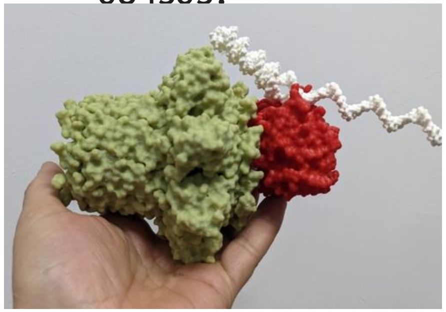
Kawakami M. A soft and transparent handleable protein model. Rev Sci Instrum. 2012; 83(8): 084303.



Human deoxyhaemoglobin

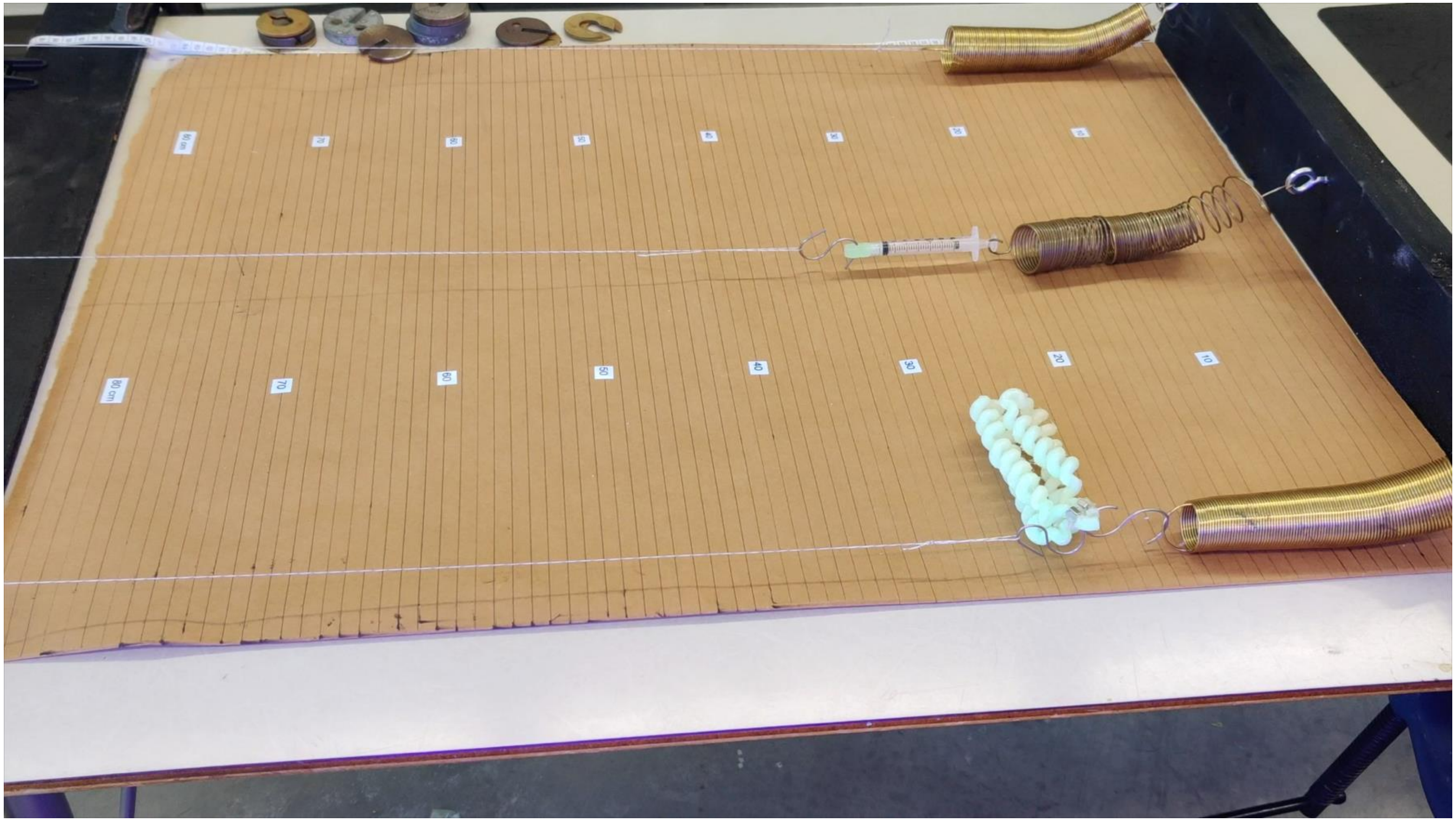


EcoRI endonuclease and DNA



Nanopore sequencing complex

Molecular models to aid teaching – 3D printed models



Ionel Popa and Florin Saitis, *Journal of Chemical Education* **2022** *99* (8), 3074-3082, DOI: 10.1021/acs.jchemed.2c00231

Custom macromolecular models, adapted to the teacher's/demonstrator's requirements are needed !!!

Custom macromolecular models for teaching are need it

The **custom macromolecular models** should be:

- Based on real scientific data;
- Depicted using standardized representations;
- Easy to edit and adapt to the outcomes of a specific lesson;
- Cheap to fabricate and reproduce;
- Easy to distribute

✓ 224 572 structures
1 068 577 CSM
freely available

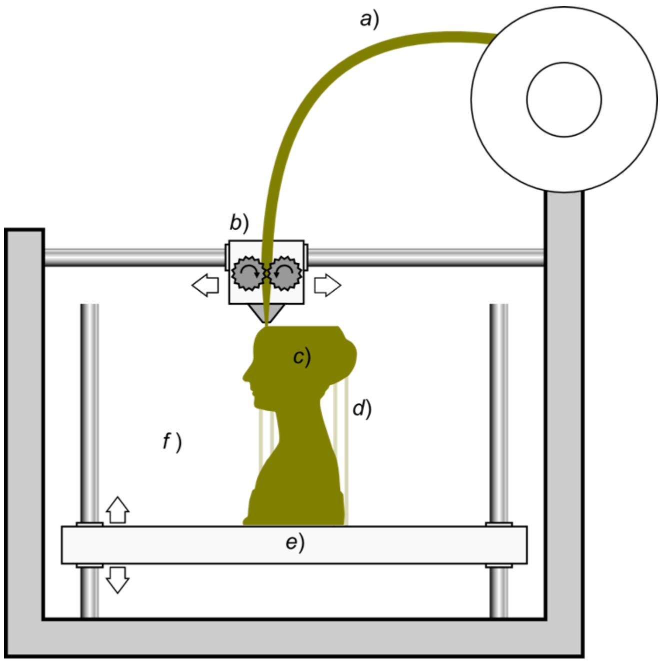
PDBe Protein Data Bank in Europe
RCSB PDB PROTEIN DATA BANK
PDBj Protein Data Bank Japan

✓ molecular visualization software
Chimera, Jmol, PyMol

✓ 3D printing can do that

3D printing - construction of a three-dimensional object from a digital 3D model. Also termed **additive manufacturing**.

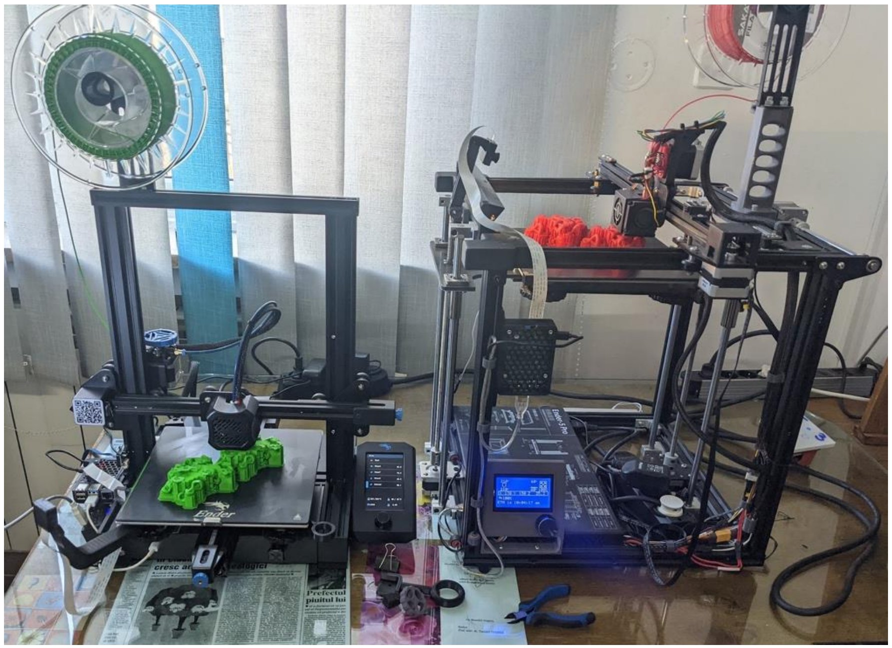
Material extrusion / Fused filament fabrication (FFF) / fused deposition modeling (FDM)



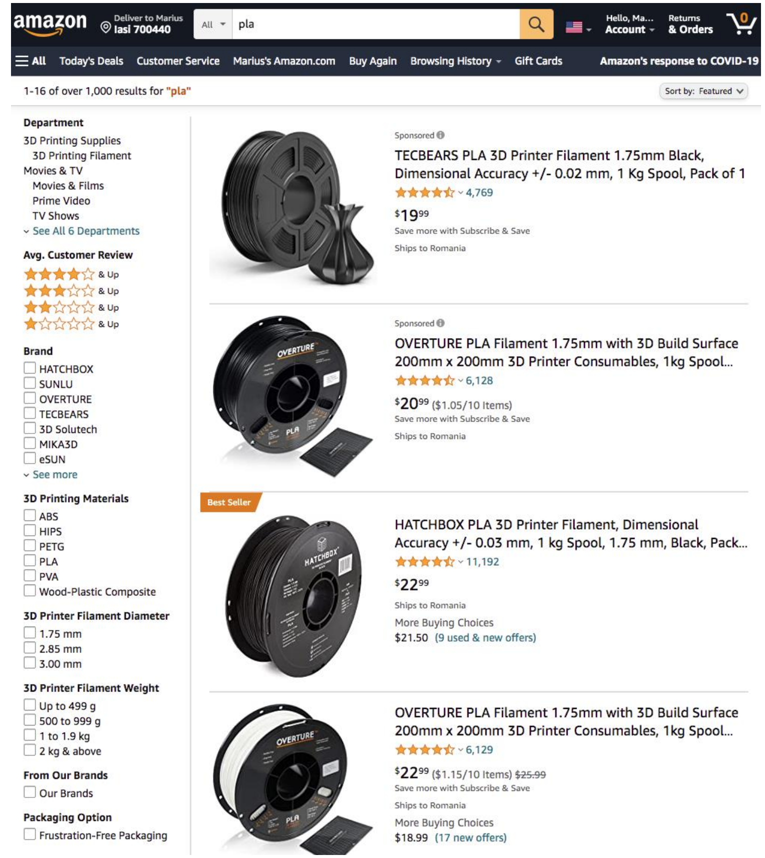
Scopigno R et al. (2017). "Digital Fabrication Techniques for Cultural Heritage: A Survey". Computer Graphics Forum 36 (1): 6-21

3D printing using FFF is accessible

Under 500$ printers



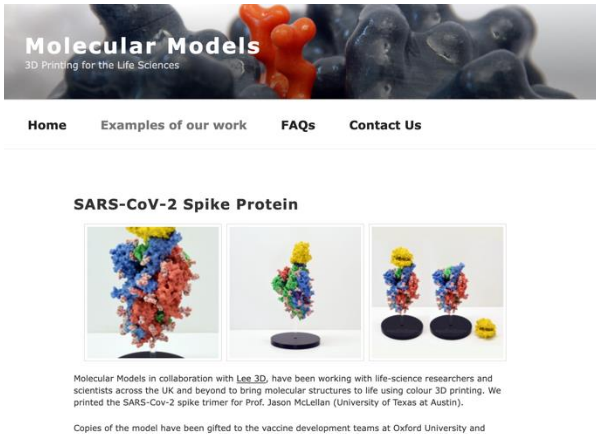
20$ - 40$ Kg of plastic

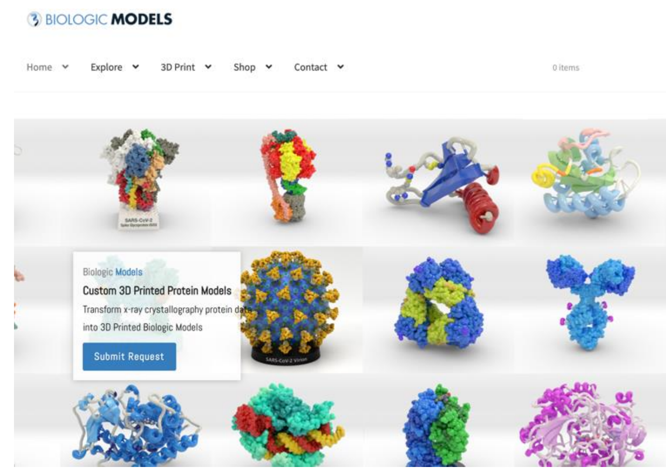


3D printing can pe used in high schools/universities from low-income countries to fabricate macromolecular models adapted to teachers needs

3D printed models - how to get them

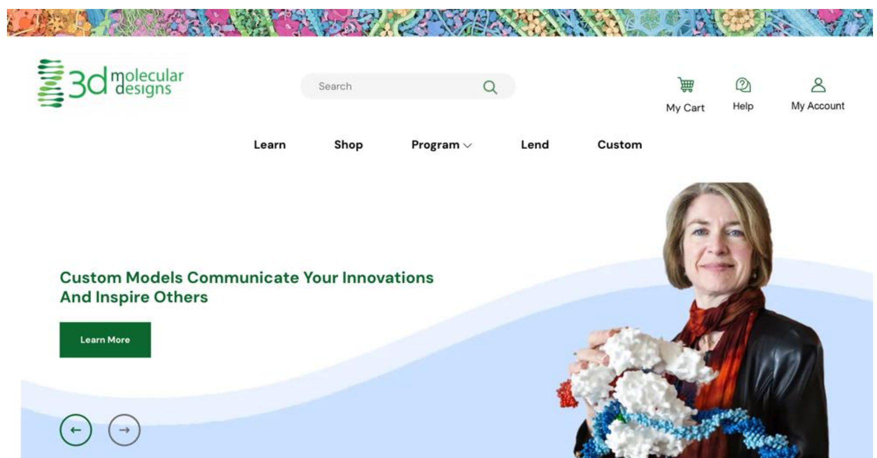
The easy, but not necessarily the cheap way:





http://www.molecmodels.co.uk/

https://biologicmodels.com/



https://3dmoleculardesigns.com/

3D printed models - how to get them

The almost easy, but a bit cheaper way:

A1. Find an already available model at:
https://3dprint.nih.gov/
https://modelemoleculare.ro/

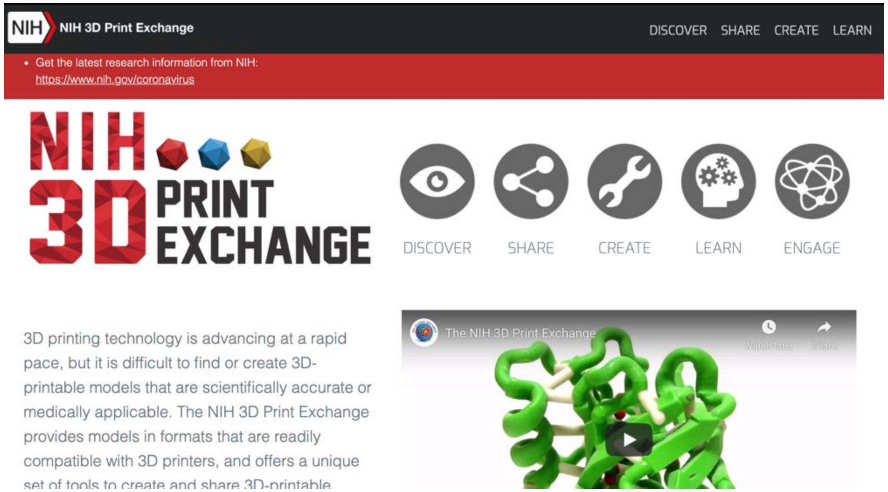
OR

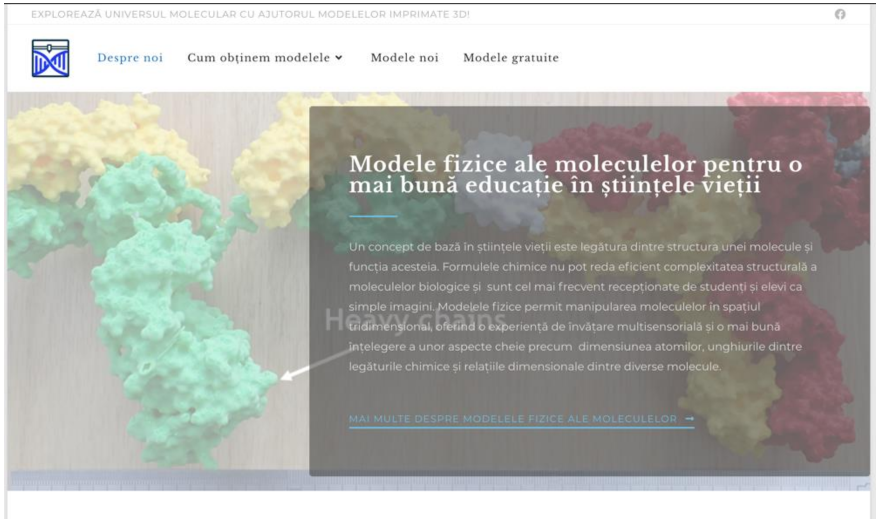
A2. Automatically create your own model at:
https://3dprint.nih.gov/create

OR

A3. Ask somebody else to do it such as:
https://modelemoleculare.ro/product-category/modele-la-cerere/

AND





B. Fabricate your model using your own 3D printer or access an on demand 3D printing service
printari-3d.ro 3dp.ro fablab.ro

Do these models make a difference in teaching?

A compensatory research study

	Week 1	Week 2			Week 3			Week 4 - Week 7
		Pre-test 1	Lecture 1 - Proteins Structure	Post-test 1	Pre-test 2	Lecture 2 - DNA structure	Post-test 2	
Group A	Announcement Recruitment Consent	2 days before lecture, 30 minutes, 13 questions	No intervention	2 days after lecture, 30 minutes, 13 questions	2 days before lecture, 30 minutes, 10 questions	**Intervention**	2 days after lecture, 30 minutes, 10 questions	Intervention and Feedback form
Group B			**Intervention**			No intervention		

The project was approved by the ethics committee at the Department of Phycology and Education Sciences, Alexandru Ioan Cuza University of Iași (no 186/29.01.2024). Students were informed prior to the start of instruction of the purpose and objectives of the investigation. Student participation was anonymous and voluntarily, and each student was presented with the opportunity to exclude him/herself from the study at any time. Information regarding data security, the type of information obtained, data storage procedures, and the measures taken to protect participants' anonymity was provided. Furthermore, students were assured that participation would have no bearing on any score assignment and that the results could be used for publication.

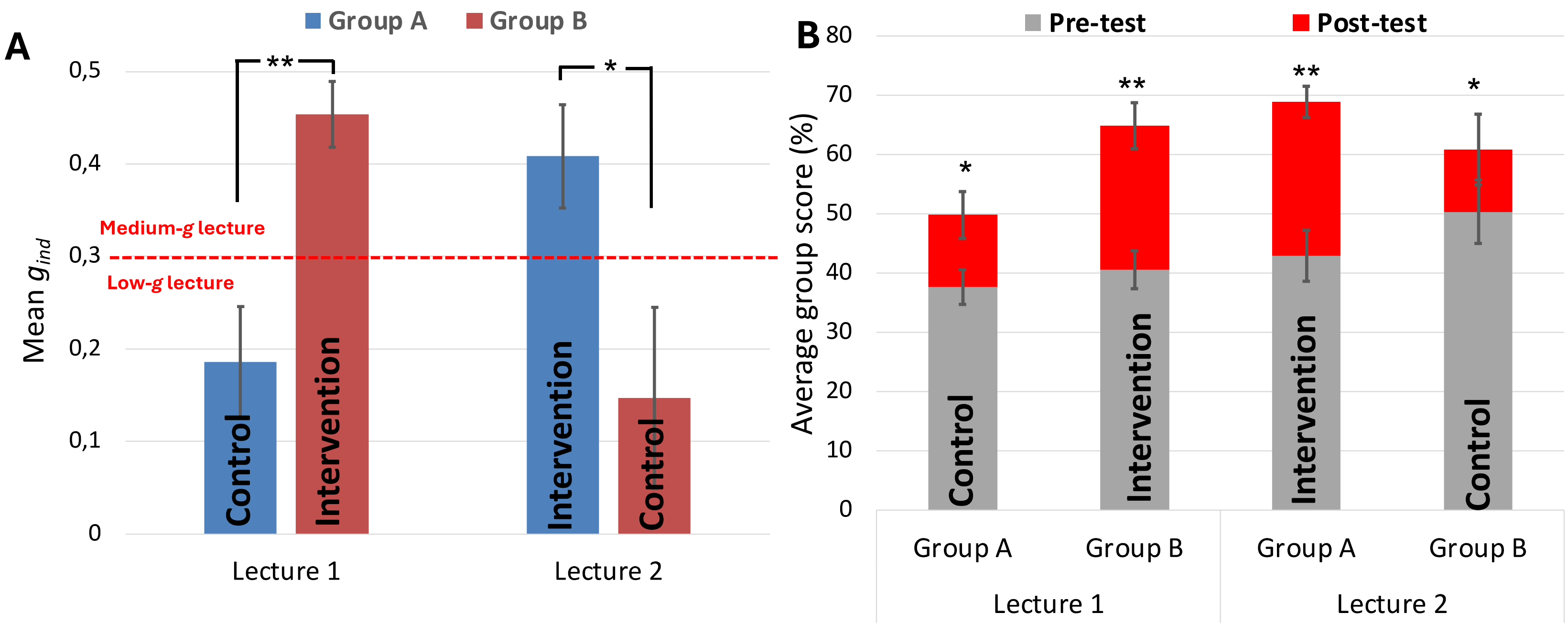
Do these models make a difference in teaching?

Evaluation of impact is key

Models	NIH 3D DOI:	Assessment questions	Learning objective	Biomolecular visualization learning goals
Amino acids, peptides and proteins				
4 amino acids (L-Glycine, L-Tryptophan, L-Proline, L-Arginine) in different representations	10.60705/3dpx/21049.1	Q2, Q4	Recognize a variety of molecular representations (i.e. stick and space fill).	AR2.02 students will describe the atoms that are represented in different renderings. (novice)
Two insuline chains (PDBID 4ins, chains C and D) in 4 representations: sticks, balls and sticks, cartoon and surface	10.60705/3dpx/21051.1	Q1, Q2, Q3, Q4, Q7	Recognize a variety of molecular representations (i.e. stick and space fill). Identify features of the peptide backbone, including the amino and carboxyl ends, peptide bonds, and alpha carbon. N to C direction	MR1.01 given a rendered structure of a biological polymer students will be able to identify the ends of a biological polymer. (novice, amateur, expert) MR1.02 given a rendered structure, students will be able to divide the polymer into its monomer units. (novice)
Quaternary structure of human deoxihaemoglobin with removable hem	10.60705/3dpx/14895.2	Q10, Q11	Describe why and how protein subunits interact to make the "quaternary structure"	TC2.06 Students can identify the levels of protein structure (e.g., parse a tertiary/quaternary structure into a series of secondary structures/motifs) and the ways in which they are connected from a three-dimensional structure. (Novice, Amateur, Expert)
Nucleotides and nucleic acids				
Deoxyribonucleotides and ribonucleotides in in different representations.	10.60705/3dpx/21050.1	Q2,	Recognize a variety of molecular representations (i.e. stick and space fill).	AR2.02 students will describe the atoms that are represented in different renderings. (novice)
B-DNA dodecamer printed in flexible	10.60705/3dpx/14893.2	Q5	Understand the flexibility of DNA due to the higher number of rotable bonds.	AG3.01 Students can identify a dihedral/torsion angle in a three-dimensional representation of a macromolecule. (Novice) AG3.02 Students can identify the planes between which a dihedral/torsion angle exists within a three-dimensional representation of a macromolecule. (Novice)

https://biomolviz.org/. Biochem Mol Biol Educ. 2017 Jan 2;45(1):69-75. doi: 10.1002/bmb.20991.

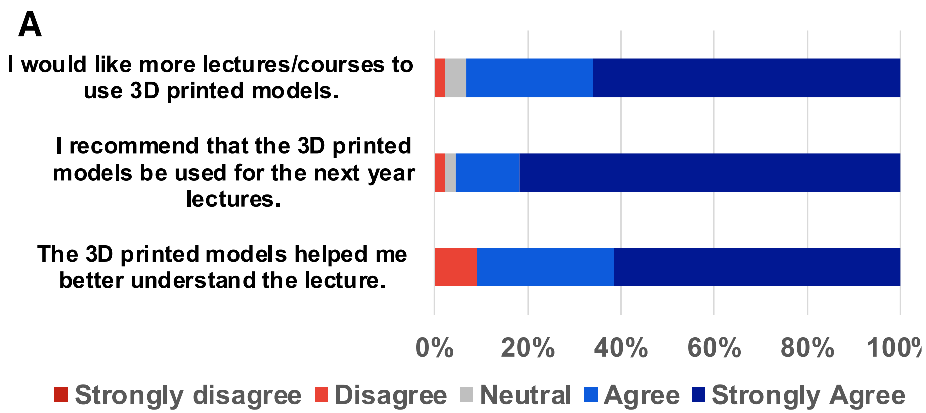
Individual learning gain

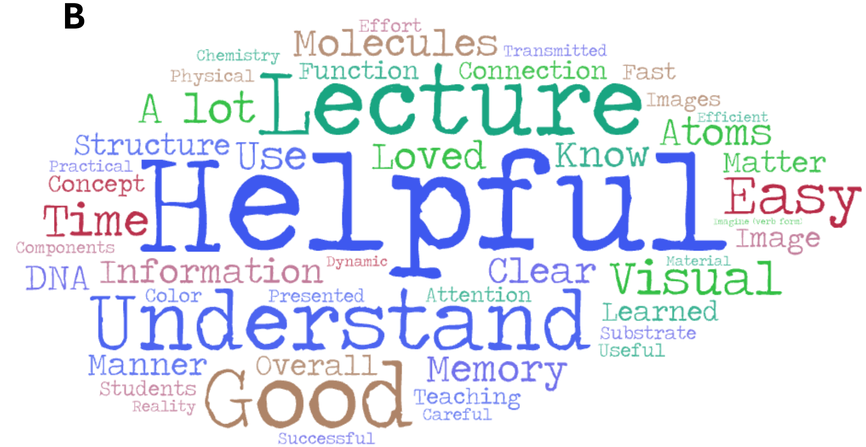


Boiangiu RS, Popa LN, Mihasan M. *Journal of Science Education and Technology*, submitted manuscript

Results

Free-form assessment





Boiangiu RS, Popa LN, Mihasan M. *Journal of Science Education and Technology*, submitted manuscript

"The 3D models were **verry useful** as the **information and images were transformed into something physical that I could touch**. And this helped me better understand the content presented. **It is easier to understand a notion or a concept if one can hold it in its hand and turn it around to evaluated it from all the angles**".

Other works reporting similar impact

Received: 13 July 2019 | Revised: 1 April 2020 | Accepted: 27 April 2020
DOI: 10.1002/bmb.21362

ARTICLE

Biochemistry and Molecular Biology Education
WILEY

Interactive learning modules with 3D printed models improve student understanding of protein structure–function relationships

Michelle E. Howell[1,2,3] | Christine S. Booth[2] | Sharmin M. Sikich[4] | Tomáš Helikar[2] | Karin van Dijk[2] | Rebecca L. Roston[2] | Brian A. Couch[3]

[1]LCC International University, Klaipėda, Lithuania
[2]Department of Biochemistry, University of Nebraska, Lincoln, Nebraska, USA
[3]School of Biological Sciences, University of Nebraska, Lincoln, Nebraska, USA
[4]Department of Chemistry, Doane University, Crete, Nebraska, USA

Correspondence
Dr. Rebecca L. Roston, Department of Biochemistry, University of Nebraska, N123 Beadle Center, Lincoln, NE 68588-0664.
Email: rroston@unl.edu
Dr. Brian A. Couch, School of Biological Sciences, University of Nebraska, 204 Manter Hall, Lincoln, NE 68588-0118.
Email: bcouch2@unl.edu

Funding information
National Science Foundation, Grant/Award Number: DUE-1625804

Abstract
Ensuring undergraduate students become proficient in relating protein structure to biological function has important implications. With current two-dimensional (2D) methods of teaching, students frequently develop misconceptions, including that proteins contain a lot of empty space, that bond angles for different amino acids can rotate equally, and that product inhibition is equivalent to allostery. To help students translate 2D images to 3D molecules and assign biochemical meaning to physical structures, we designed three 3D learning modules consisting of interactive activities with 3D printed models for amino acids, proteins, and allosteric regulation with coordinating pre- and post-assessments. Module implementation resulted in normalized learning gains on module-based assessments of 30% compared to 17% in a no-module course and normalized learning gains on a comprehensive assessment of 19% compared to 3% in a no-module course. This suggests that interacting with these modules helps students develop an improved ability to visualize and retain molecular structure and function.

KEYWORDS
3D printing, allosteric regulation, amino acids, model-based learning, molecular visualization, protein structure–function, student misconceptions, undergraduate

1 | INTRODUCTION

Protein structure and function is fundamental to biochemistry. Biochemistry textbooks and classes begin with a unit on protein structure and function because of their role in nearly all biochemical processes. Because of the versatility of these macromolecules (including enzymes and structural, transport, motility, and signaling proteins), undergraduate life science students must develop proficiency in foundational intramolecular structure–function relationships in order to understand complex macromolecular interactions and higher-order processes. Moreover, designing drugs, antibiotics, or pesticides and responding to disease-causing mutations represent practical applications that rely on comprehending how protein structure directly drives biochemical function.

Unfortunately, teaching protein structure and function with traditional two-dimensional (2D) methods results in crucial misunderstandings (Table) identified from the literature and polling instructors.[1–8] From chemical or stick representations of amino acids and peptides, biochemistry undergraduates develop inaccurate

Roston and Couch contributed equally to this manuscript.

https://doi.org/10.1002/bmb.21362

Article

Student Understanding of DNA Structure–Function Relationships Improves from Using 3D Learning Modules with Dynamic 3D Printed Models[S]

Michelle E. Howell†‡
Christine S. Booth†
Sharmin M. Sikich§
Tomáš Helikar†
Rebecca L. Roston†
Brian A. Couch‡*
Karin van Dijk†*

From the †Department of Biochemistry, University of Nebraska, Lincoln, Nebraska, 68588-0664, ‡School of Biological Sciences, University of Nebraska, Lincoln, Nebraska, 68588-0118, §Department of Chemistry, Doane University, Crete, Nebraska, 68333

Abstract

Understanding the relationship between molecular structure and function represents an important goal of undergraduate life sciences. Although evidence suggests that handling physical models supports gains in student understanding of structure–function relationships, such models have not been widely implemented in biochemistry classrooms. Three-dimensional (3D) printing represents an emerging cost-effective means of producing molecular models to help students investigate structure–function concepts. We developed three interactive learning modules with dynamic 3D printed models to help biochemistry students visualize biomolecular structures and address particular misconceptions. These modules targeted specific learning objectives related to DNA and RNA structure, transcription factor-DNA interactions, and DNA supercoiling dynamics. We also designed accompanying assessments to gauge student learning. Students responded favorably to the modules and showed normalized learning gains of 49% with respect to their ability to understand and relate molecular structures to biochemical functions. By incorporating accurate 3D printed structures, these modules represent a novel advance in instructional design for biomolecular visualization. We provide instructors with the materials necessary to incorporate each module in the classroom, including instructions for acquiring and distributing the models, activities, and assessments. © 2019 International Union of Biochemistry and Molecular Biology, 47(3):303–317, 2019.

Keywords: *DNA; RNA; student misconceptions; 3D printing; model-based learning; nucleic acid structure and function; molecular visualization*

Introduction

Understanding the complex interdependence of macromolecular structure and function represents a central goal of undergraduate life science education, particularly within biochemistry [1–3]. However, life science students frequently struggle to visualize and translate between the static two-dimensional (2D) images displayed in textbooks and the dynamic three-dimensional (3D) concepts they represent [4–8]. Hence, many students leave life sciences classrooms with misconceptions about structure–function relationships [8]. One fundamental biological concept with which students struggle is the relationship of DNA structure to its functions. For example, students have misconceptions about the way DNA bases are stacked and accessible to DNA binding proteins, the continuity of and information presented in DNA grooves, the flexibility and dynamic nature of DNA molecules, and the enzymes that cleave and repair DNA [9–12]. For example, students fail to realize that although DNA bases lie between the DNA backbones, they are accessible to proteins [9]. As a result, students do not realize that the presented chemical information varies between the major and minor grooves of a specific DNA segment. Moreover, many students do not realize that transcription factors can interact with a

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**To whom correspondence should be addressed. Tel.: (402) 472-8130; Fax: (402) 472-2083. E-mail: bcouch2@unl.edu.*
and
Tel.: (402) 472-2948; Fax: (402) 472-7842. E-mail: kvandijk2@unl.edu.
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How difficult it is to create your own 3D printable molecular model?

Let's walk through the process together, and judge yourself

Overview of the steps involved in fabricating a macromolecular model

Download structure from

PDBe Protein Data Bank in Europe | RCSB PDB PROTEIN DATA BANK | PDBj Protein Data Bank Japan

PDB or CIF file

Visualize and prepare the model in

UCSF ChimeraX

STL file

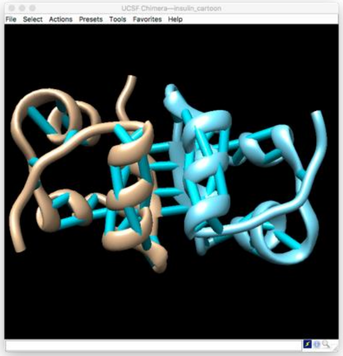
Prepare the file for printing using

PrusaSlicer

GCODE file

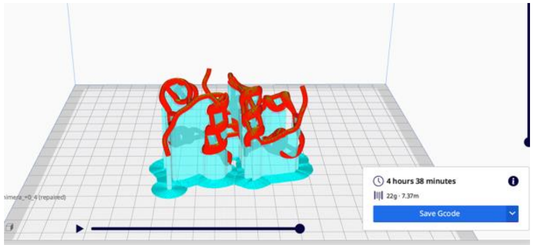
Print

1. Chose or combine **visualization styles;**
2. **Add H bonds** or **create struts to make the model more sturdy** (mandatory for cartoon and balls and sticks models, not required for surface);
3. **Increase the thickness** of each printed element and/or **improve the smoothness** for molecular surfaces.



A. Generate the computer model

1. Set the printing **scale**;
2. **Orient** the model on printing bed;
3. Set printing **resolution**;
4. Set shell **wall thickness** and **infill** %;
5. Automatically **add support**;
6. **Slice** the model;
7. **Send** the resulting gcode to printer (via SD-Card, USB or WiFi)



B. Print the model

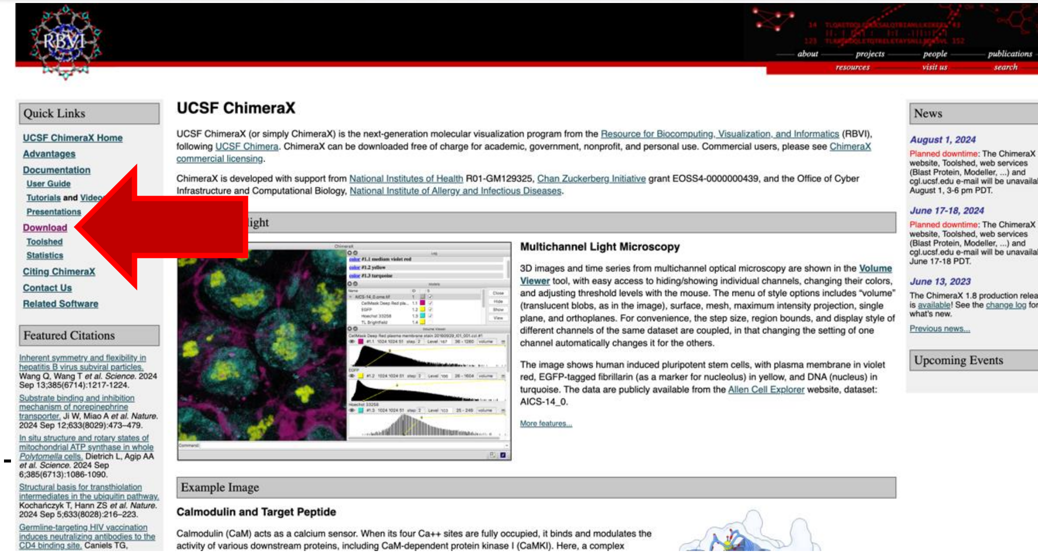
Support material removal

C. Clean up and finalize the physical model

A. Generation of the computer model (or stl file)

1. Download and install UCSF ChimeraX;

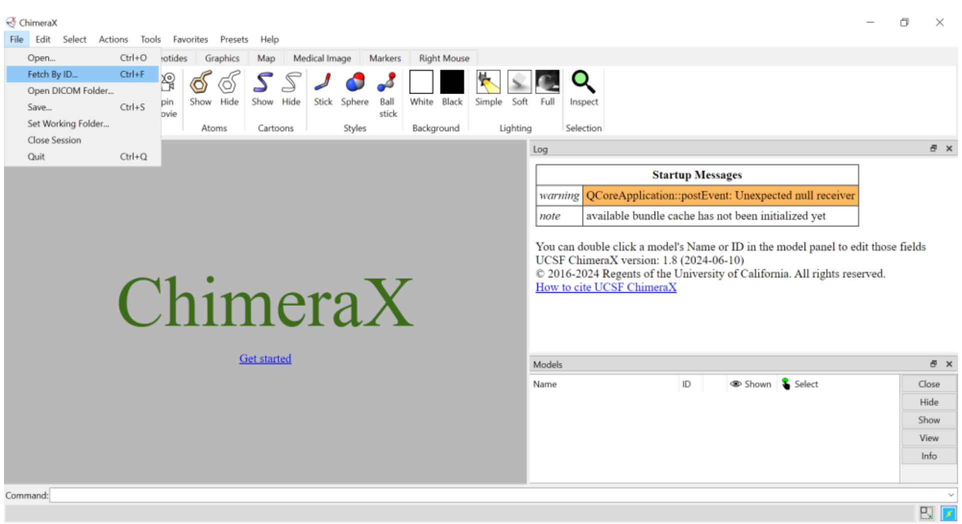
https://www.cgl.ucsf.edu/chimerax/

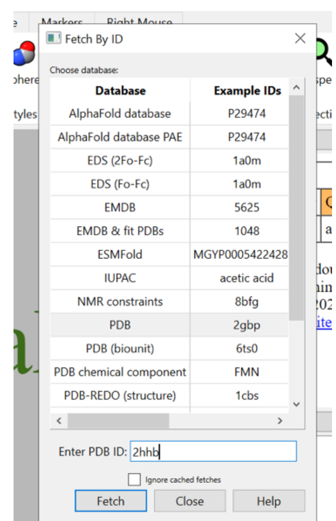


Alternative molecular visualization programs that will work but are not covered here:

1. **Jmol** - https://jmol.sourceforge.net/
2. **PyMOL** – https://www.pymol.org/ some extra steps in CAD software are required
3. **Molecular Maya** -https://clarafi.com/tools/mmaya/ - the plugin is free, but the Maya software is not

2. Open the .pdb file of your choice via File > Fetch by ID… > Select PDB and Enter PDB ID





If unsure, use one these PDB IDs:

1BDNA
1TRA
2HHB
4INS
1AON
6VYB
2FG4
3KXU
1EMA
1LYD

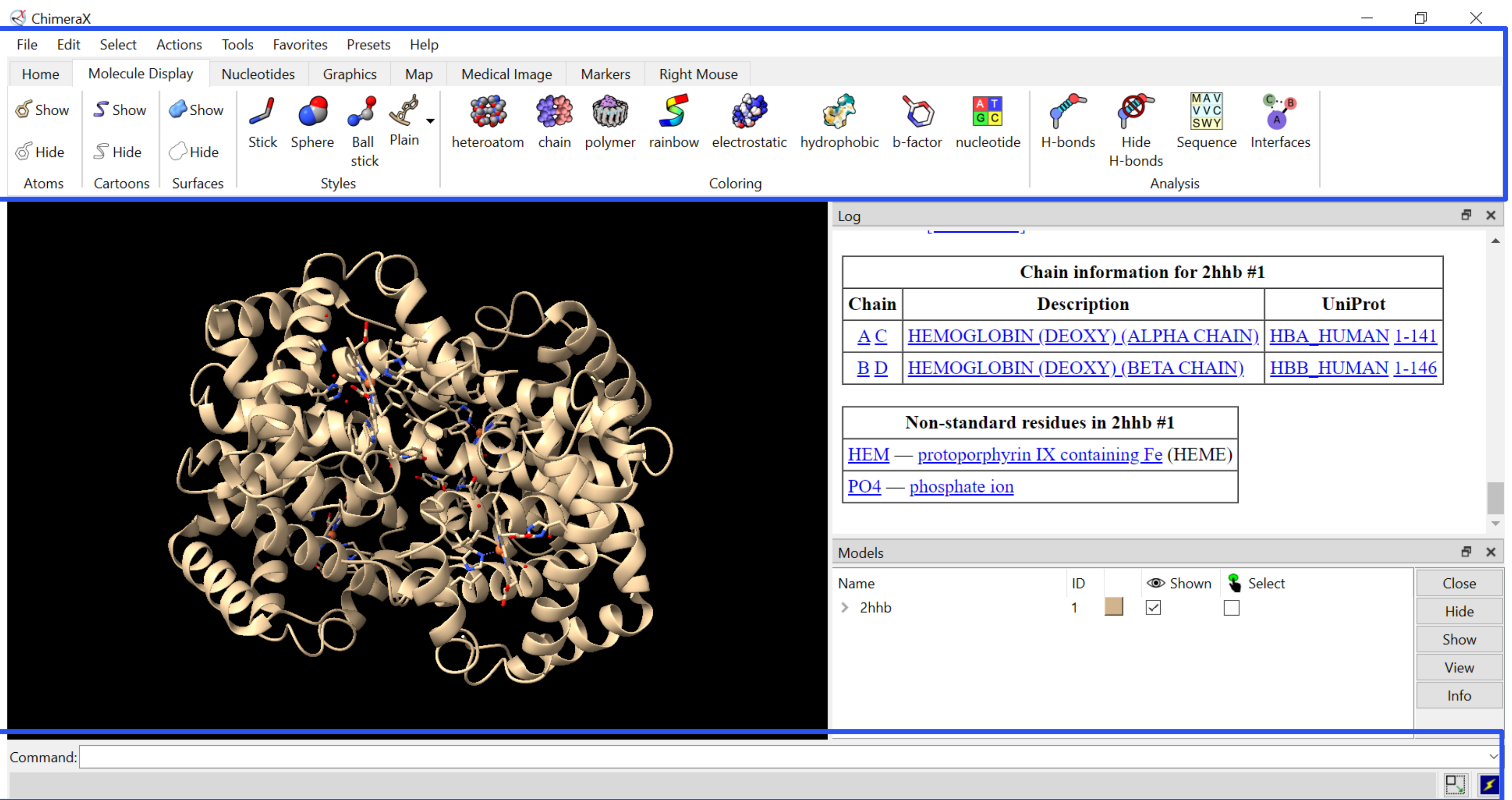
A. Generation of the computer model (or stl file)

3. Edit/apply a visualization style to make the molecule 3D printable

Default visualization styles in all molecular visualization software are not compatible with 3D printing

Specific visualization styles need to be applied via

1. Buttons and menus

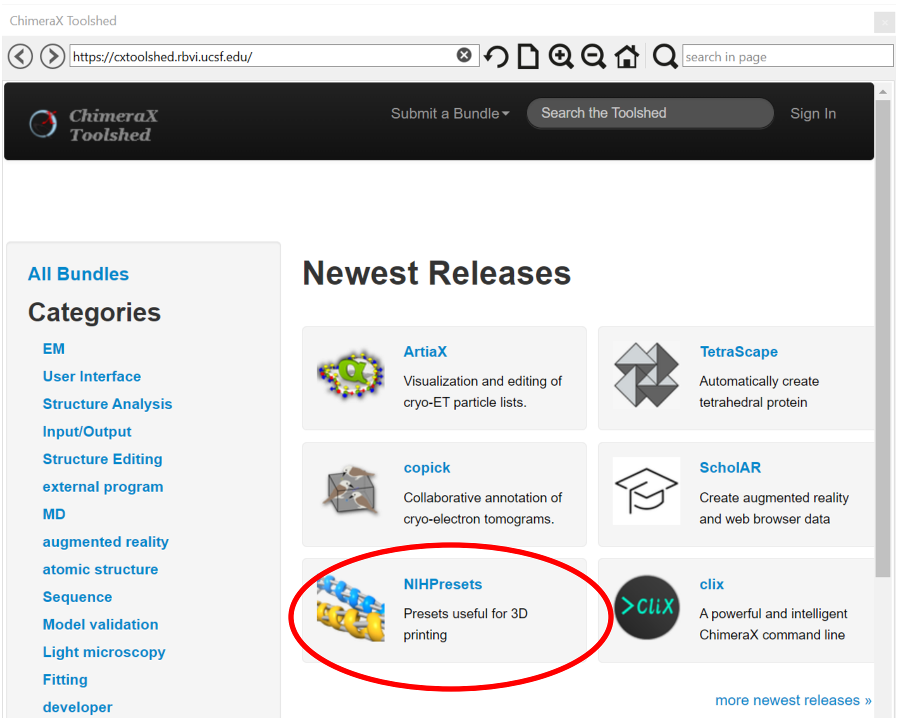


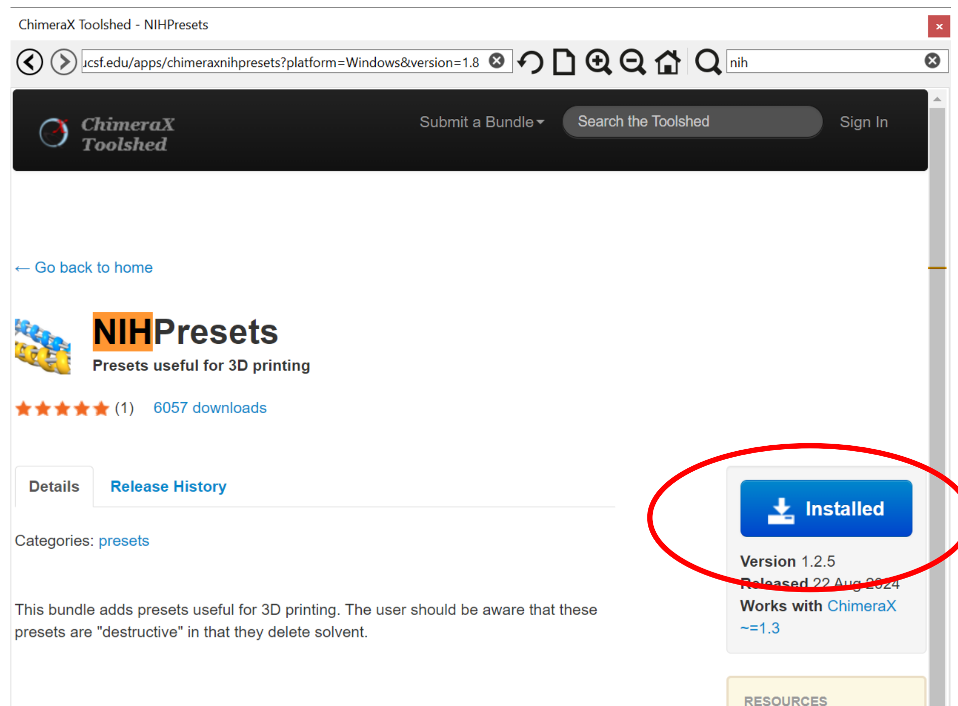
2. CLI

A. Generation of the computer model (or stl file)

The fastest and easiest way to have good-enough printable models:
NIHPresets from the ChimeraX Toolshed

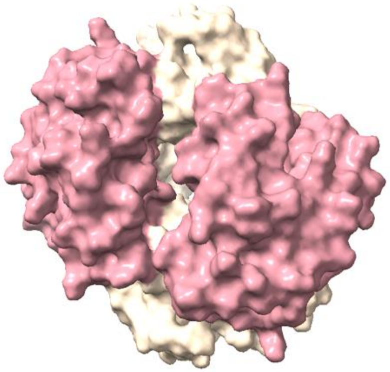
In ChimeraX install via Tools > More tools… > In the new window, search for NIHPresets, Click on it and hit Install





New visualization style presets are available in ChimeraX via the menu Presets > NIH3D>

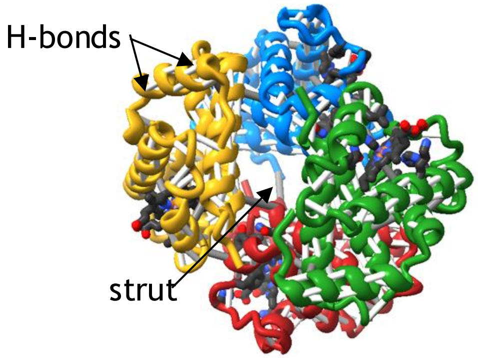
Frequently used visualization styles



In ChimeraX Menu Presets > NIH3D>

Surface by chains (Printable)

Great for arguing the complementary of molecular shapes
Extremely easy to print without any processing
Works well also for large macromolecular complexes

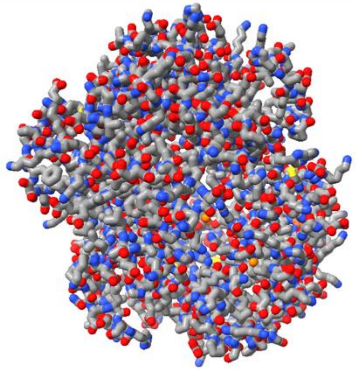


Ribbon by chains (Printable)

H-bonds and struts have been added
All elements are thicker
Works for small proteins (one chain of hemoglobine)
Can be difficult to print at times

Sticks (Printable)

Applicable **only** to small molecules or ligands



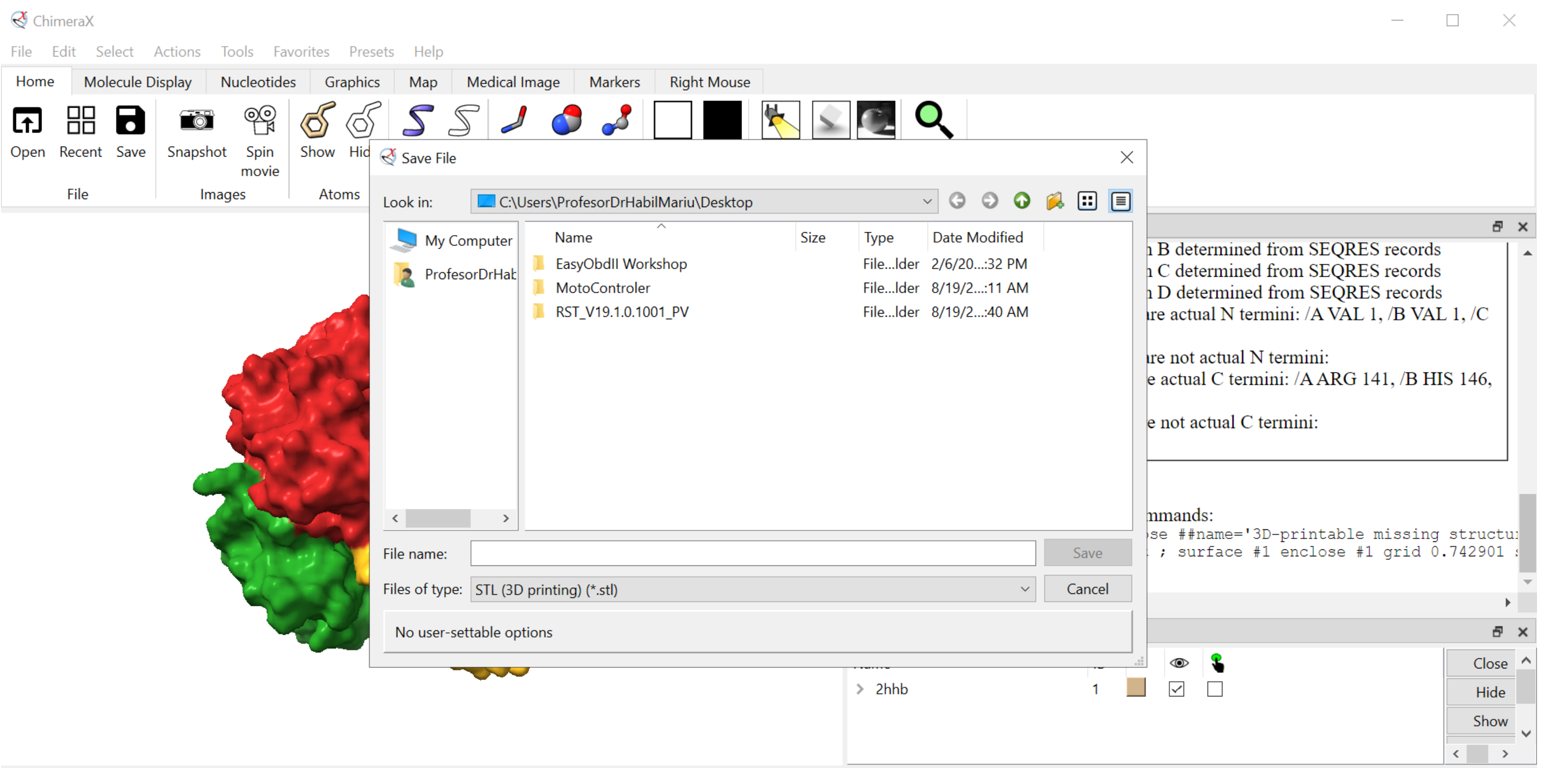
4. Final step

In ChimeraX Menu File > Save ...

In the new window under File name: **type your preferred name**

under Files of type: **STL (3D printing) (*.stl)**

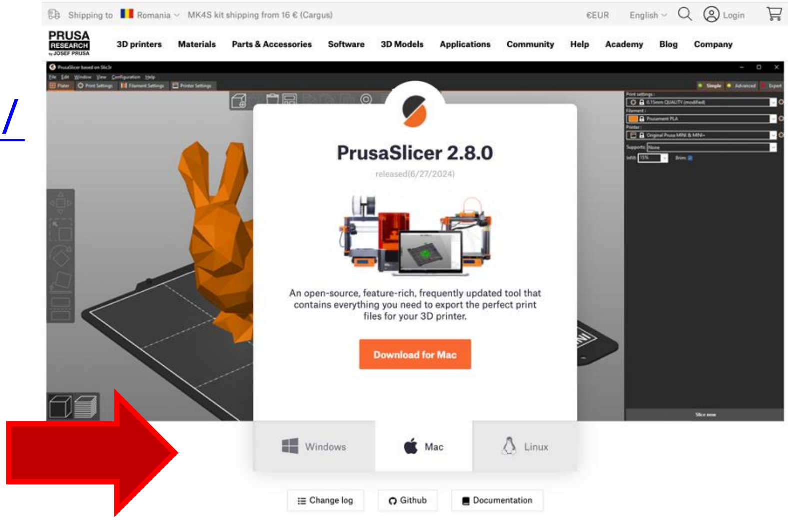
And hit **Save**



B. 3D Printing the computer generated model

1. Download and install PrusaSlicer;

https://www.prusa3d.com/page/prusaslicer_424/



Alternative slicer programs used to control 3D printers:

1. **Ultimaker Cura** - https://ultimaker.com/software/ultimaker-cura/
2. **Slic3r** – https://slic3r.org/
3. **Simplify3D** - https://www.simplify3d.com/ not free

Choosing one or another depends on the printer one has available

2. Open PrusaSlicer and Cancel the configuration Wizard.

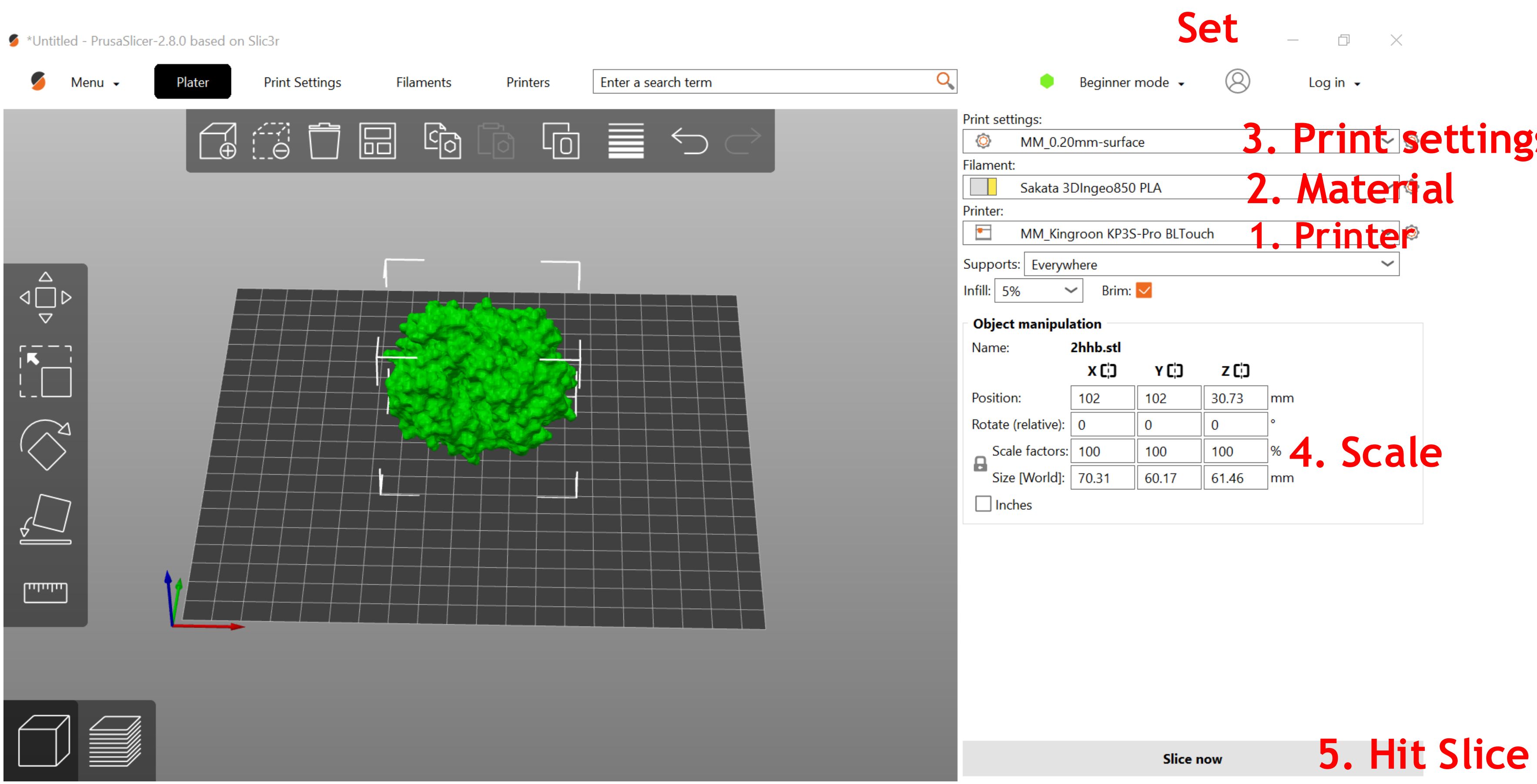
3. Download the configuration bundle. Link on the event website.
https://drive.google.com/file/d/1YM3sqMOEYSa7XxrpmzS5EqOsNFwNfaT8/view

4. In PrusaSlicer navigate to Menu > File > Import > Import Config Bundle…
Point to the newly downloaded file and hit Open

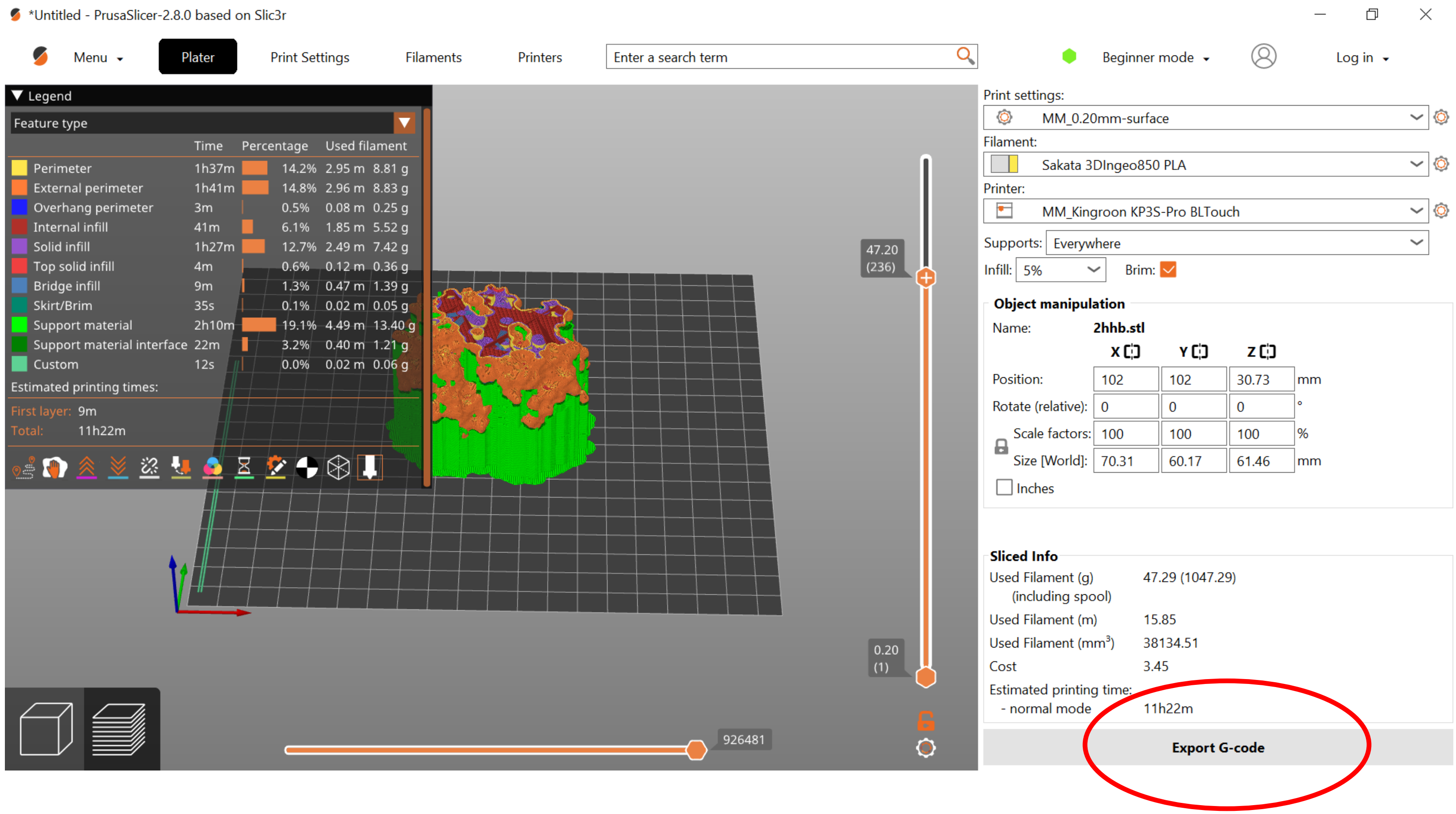
What we just did is to install the printer control software and configs required to run my printer

B. 3D Printing the computer generated model

4. Load the created .stl file - In PrusaSlicer navigate to Menu > File > Import > Import STL/3MF/STEP… Point to the .stl file and hit Open



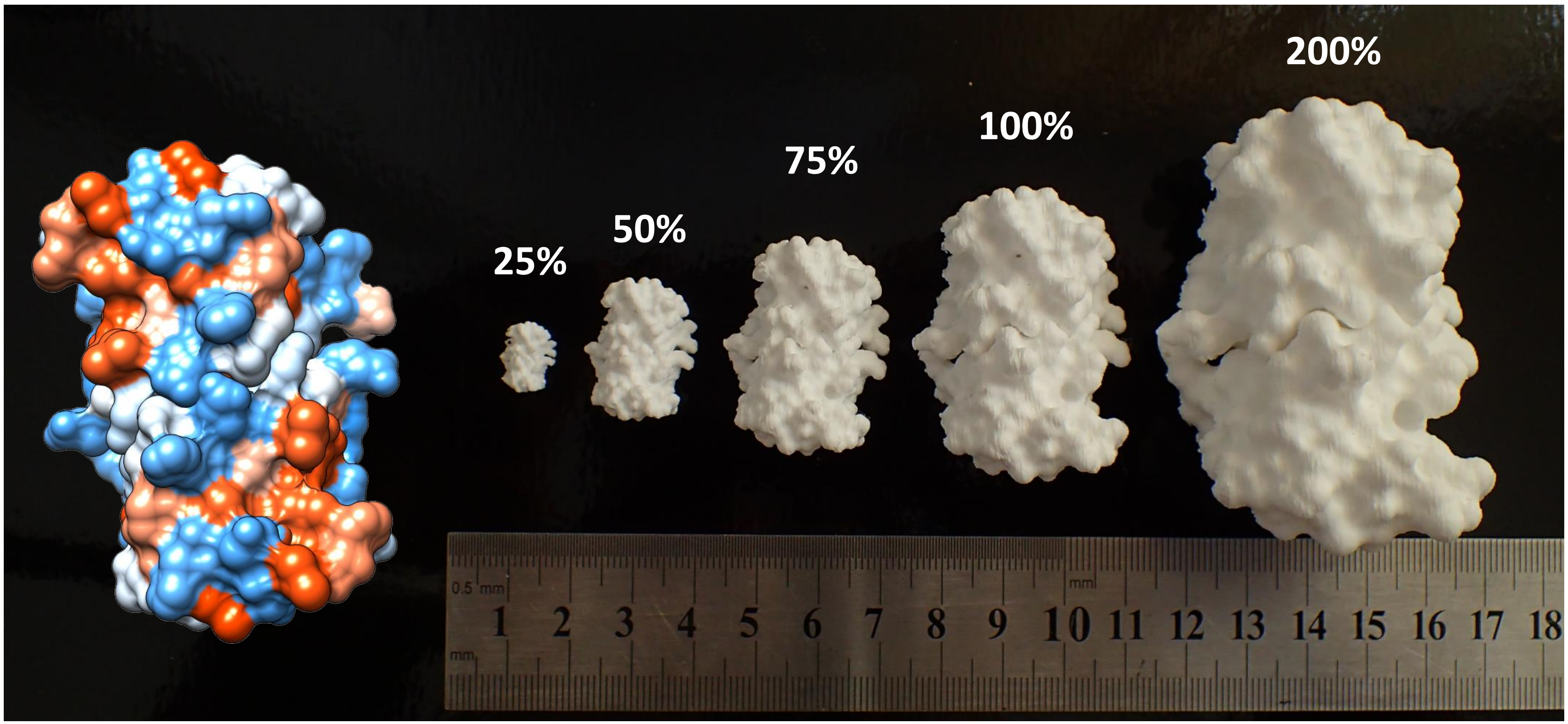
5. Export .gcode file and send it to the printer via a SD-Card



A few rules of thumb when printing computer models generated in Chimera

A. Models printed as molecular surface

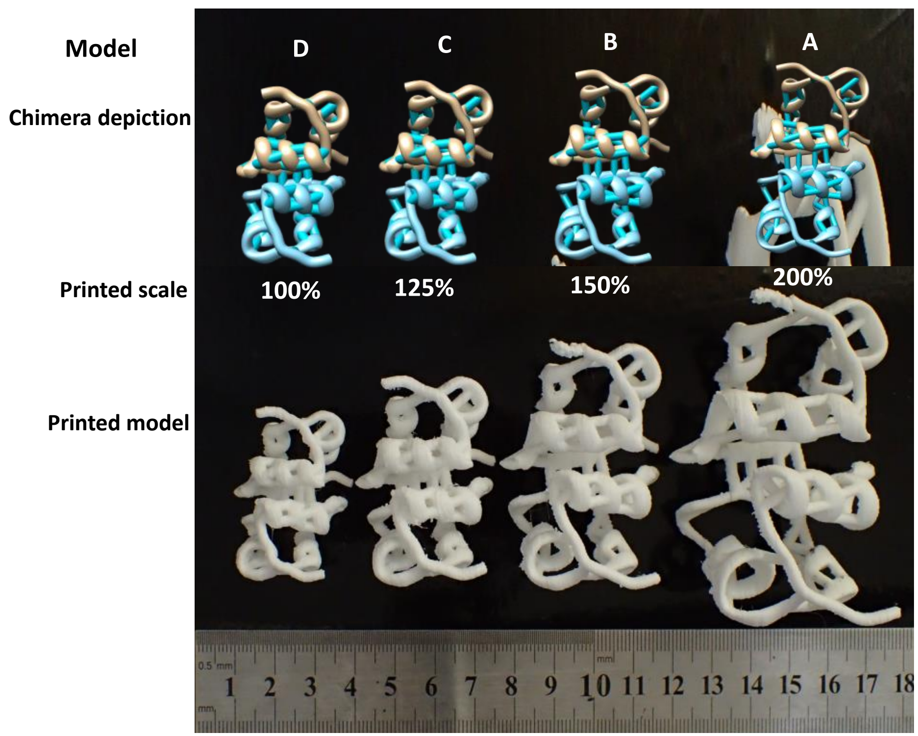
- Can be printed at scales as low as 50% with good details on atoms;
- Wall thickness of 0.8 mm (2 layers for walls, 4 mm nozzle) is suffice



Insulin model in Chimera — Physical models of insulin printed at various scales

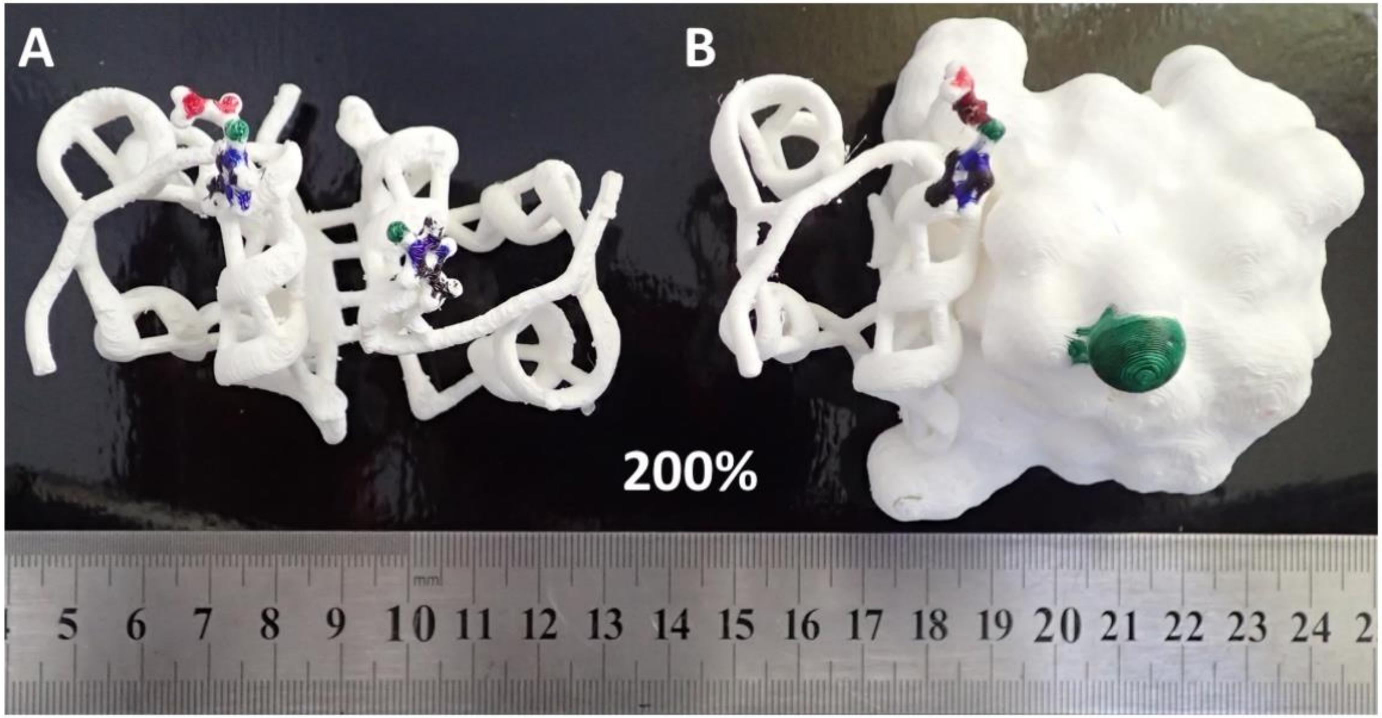
B. Models printed as cartoons

- H bonds and struts need to be added to increase rigidity of the model;
- Chimera default rendering parameters are not suitable for generating printable models. Rendering parameters need to be tinkered so that the thinnest elements (coils and H bonds) are printed at at least **2.6 mm in diameter;**
- Wall thickness of 1.6 mm (4 layers for walls, 4 mm nozzle) provides enough rigidity;
- Minimum recommended scale is **200x**



C. Models printed as balls and sticks

- Macromolecular models are complicated and difficult to print;
- Extensive support material is required and sometimes impossible to remove;
- Chimera rendering parameters need to be tinkered so that the thinnest elements (bonds) are printed at at least **2.6 mm in diameter;**
- Wall thickness of 1.6 mm (4 layers for walls, 4 mm nozzle) provides enough rigidity;
- Unusable for proteins, but can be combined with other visualization modes.



Guidelines and more details are available

jove Journal of Visualized Experiments www.jove.com

Video Article

3D Printing of Biomolecular Models for Research and Pedagogy

Eduardo Da Veiga Beltrame[1], James Tyrwhitt-Drake[2], Ian Roy[3], Raed Shalaby[4], Jakob Suckale[4], Daniel Pomeranz Krummel[5]

[1]Department of Physics, Brandeis University
[2]Bioinformatics and Computational Biosciences Branch (BCBB), NIH/NIAID/OD/OSMO/OCICB
[3]Library/LTS/MakerLab, Brandeis University
[4]Interfaculty Institute of Biochemistry (IFIB), University of Tübingen
[5]Winship Cancer Institute, Emory University School of Medicine

Correspondence to: Jakob Suckale at jakob.suckale@uni-tuebingen.de, Daniel Pomeranz Krummel at dapk@brandeis.edu

URL: https://www.jove.com/video/55427
DOI: doi:10.3791/55427

Keywords: Engineering, Issue 121, 3D printing, molecular biology, education, structure, biomolecules, models, extrusion printers

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JOURNAL OF CHEMICAL EDUCATION Communication
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Rapid Access to Multicolor Three-Dimensional Printed Chemistry and Biochemistry Models Using Visualization and Three-Dimensional Printing Software Programs

Ken Van Wieren,[†] Hamel N. Tailor,[‡] Vincent F. Scalfani,[§] and Nabyl Merbouh*[,‡]

[†]Science Technical Center and [‡]Department of Chemistry, Simon Fraser University 8888 University Drive Burnaby, British Columbia V5A 1S6, Canada
[§]University Libraries, Rodgers Library for Science and Engineering, The University of Alabama, Tuscaloosa, Alabama 35487, United States

Supporting Information

JOURNAL OF CHEMICAL EDUCATION Article
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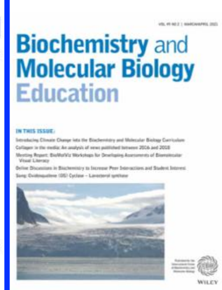
A Simplified Method for the 3D Printing of Molecular Models for Chemical Education

Oliver A. H. Jones*[,†] and Michelle J. S. Spencer*[,‡]

[†]Australian Centre for Research on Separation Science (ACROSS), School of Science, RMIT University, GPO Box 2476, Melbourne, Victoria 3001, Australia
[‡]School of Science, RMIT University, GPO Box 2476, Melbourne, Victoria 3001, Australia



Biochemistry and Molecular Biology Education

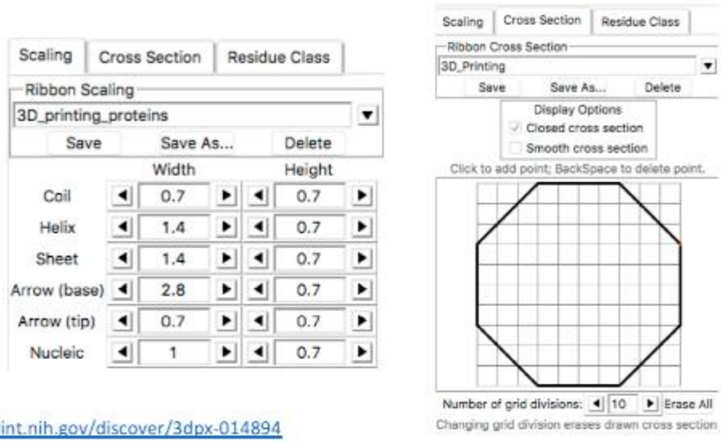
A beginner's guideline for low-cost 3D printing of macromolecules usable for teaching and demonstration

Marius Mihasan

First published: 23 March 2021 | https://doi.org/10.1002/bmb.21493

Secondary structure of lysozyme

Relevant Chimera model depiction settings*

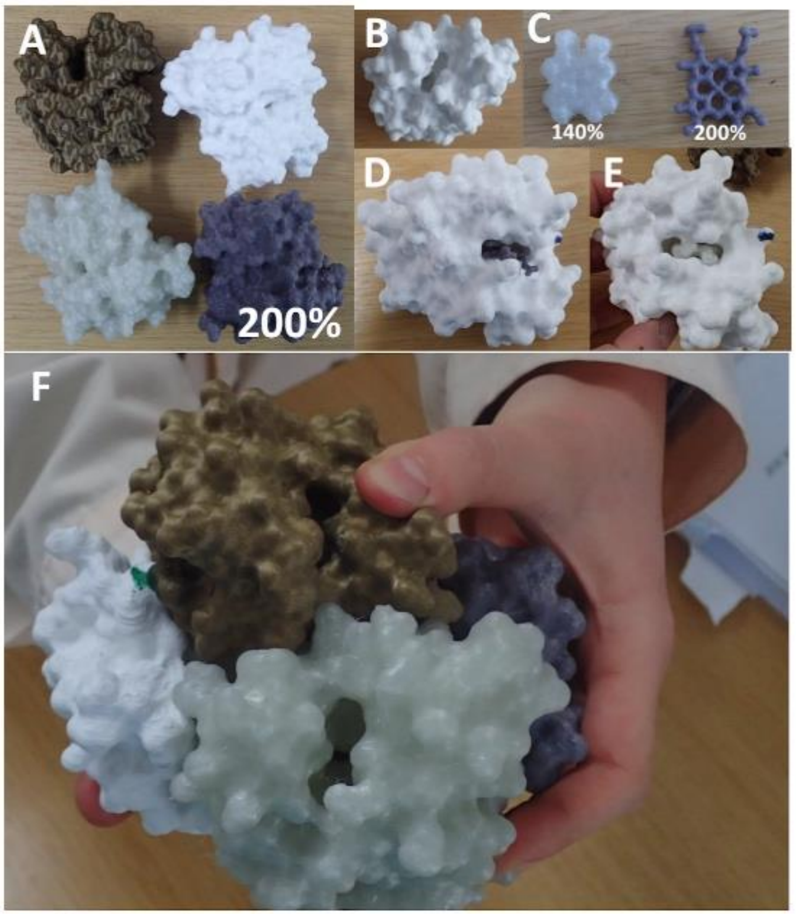


https://3dprint.nih.gov/discover/3dpx-014894

Printed scale and physical model

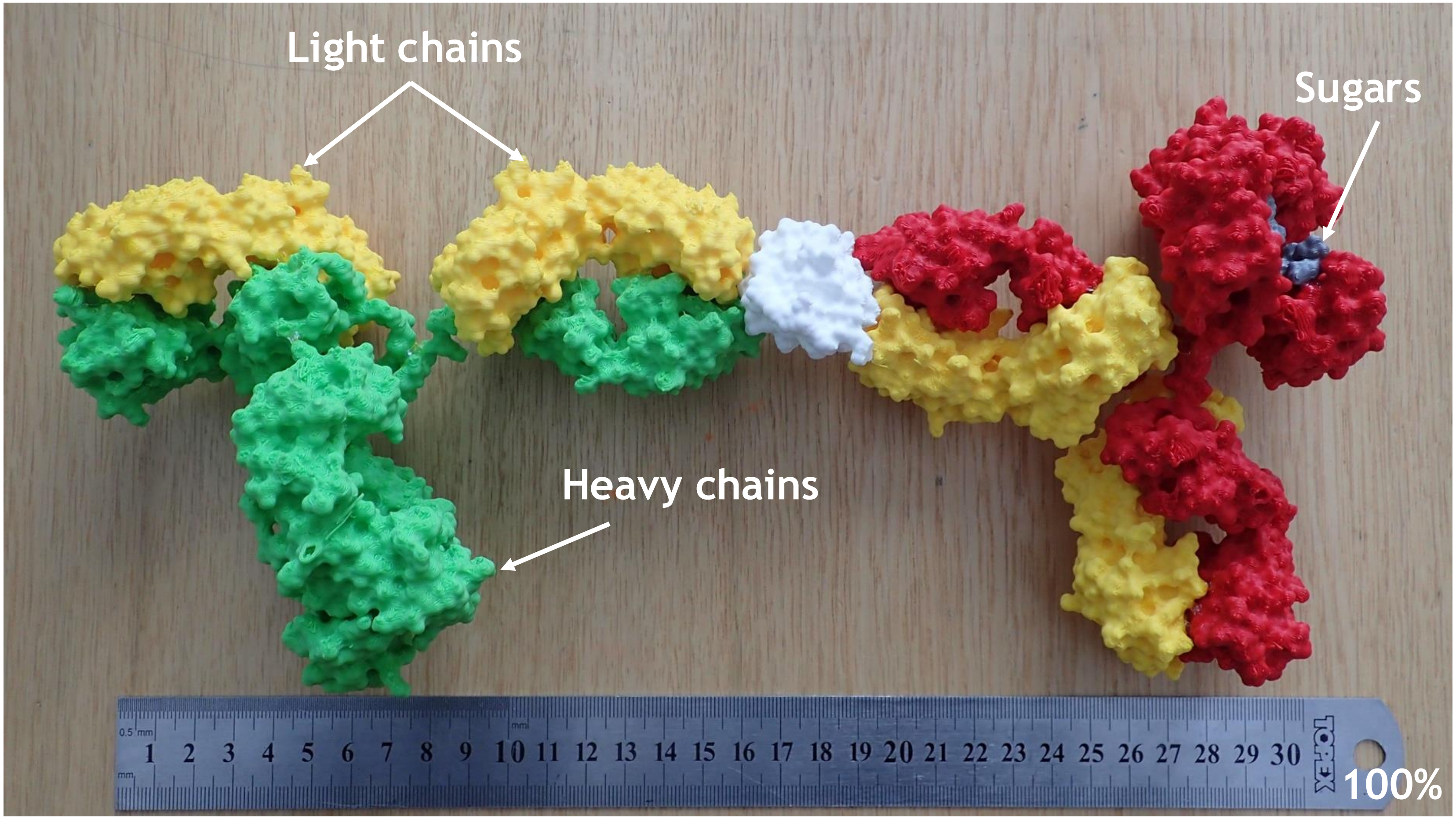
Quaternary structure of human deoxyhemoglobin

Printed scale and physical models



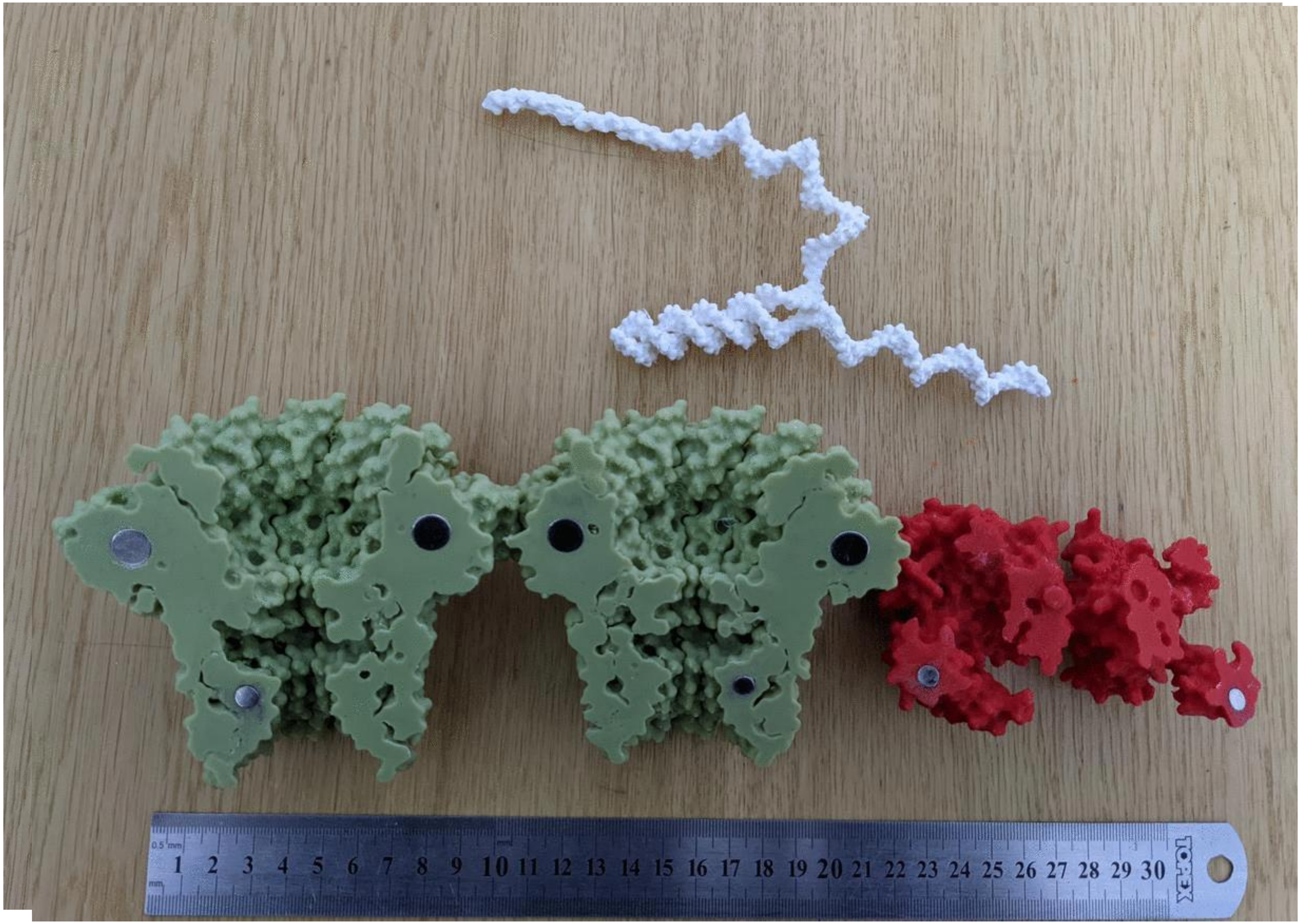
https://3dprint.nih.gov/discover/3dpx-014895

Antibodies interacting with an antigen (lysozyme)

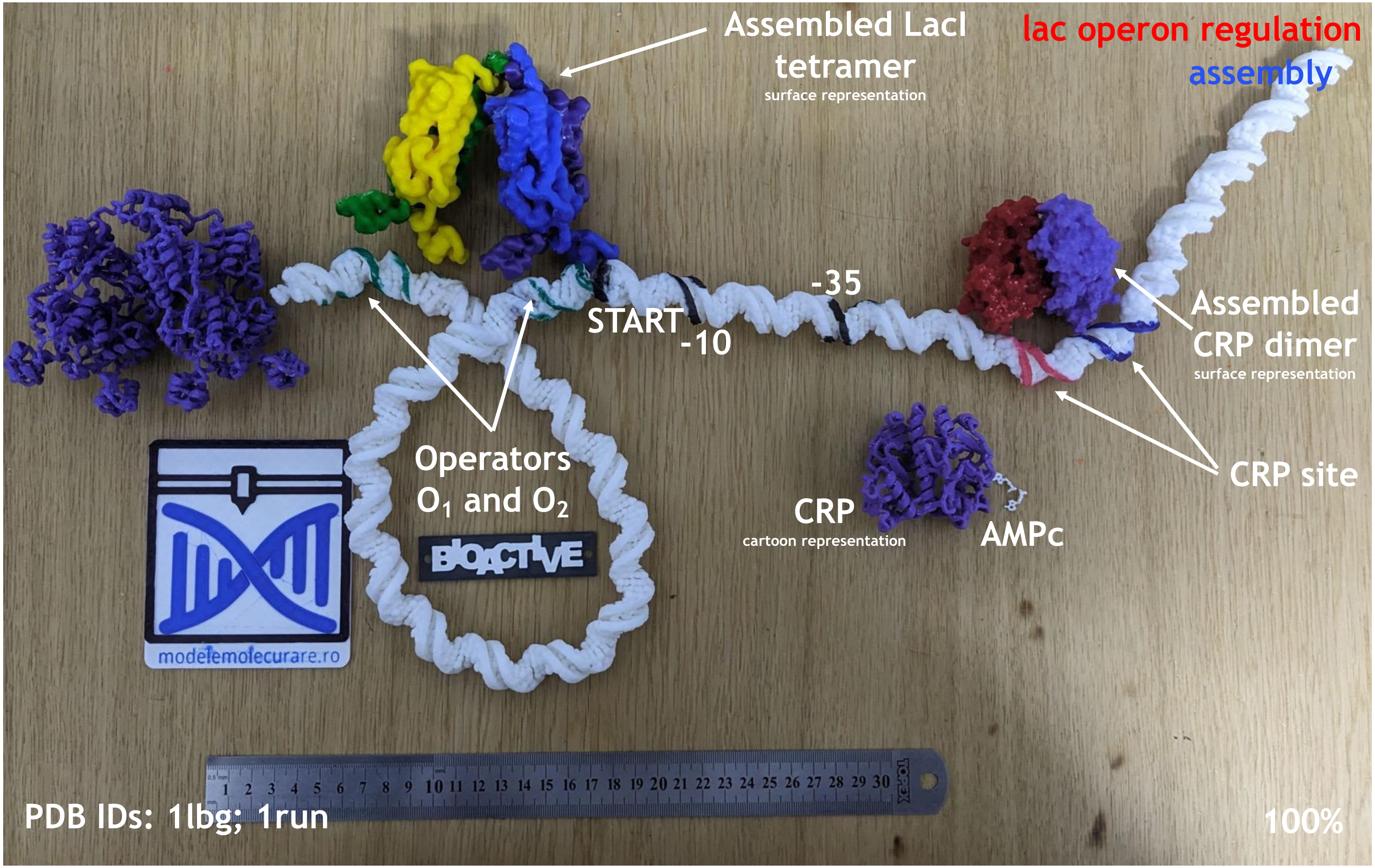


https://3dprint.nih.gov/discover/3dpx-015554

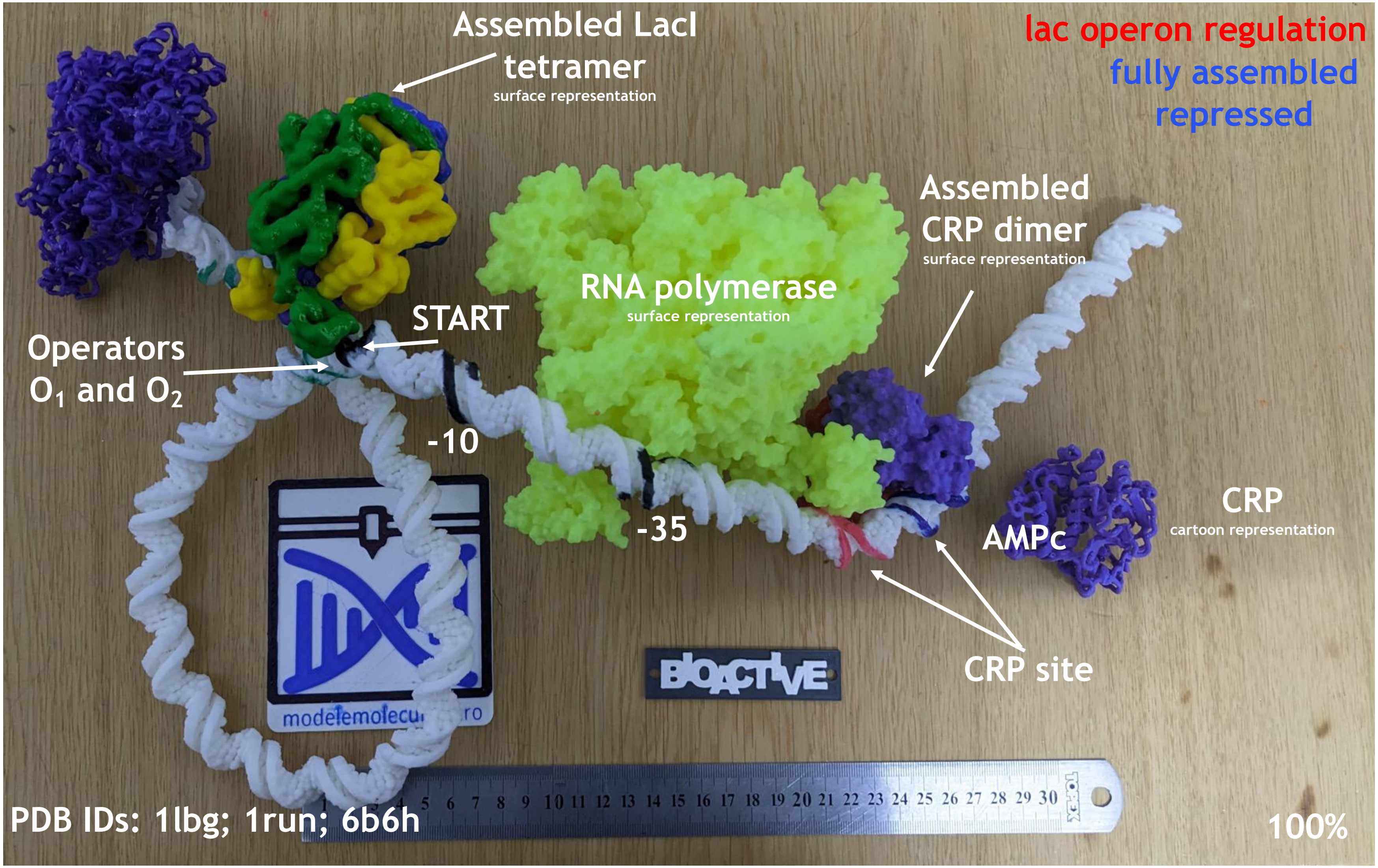
A protein nanopore sequencing DNA



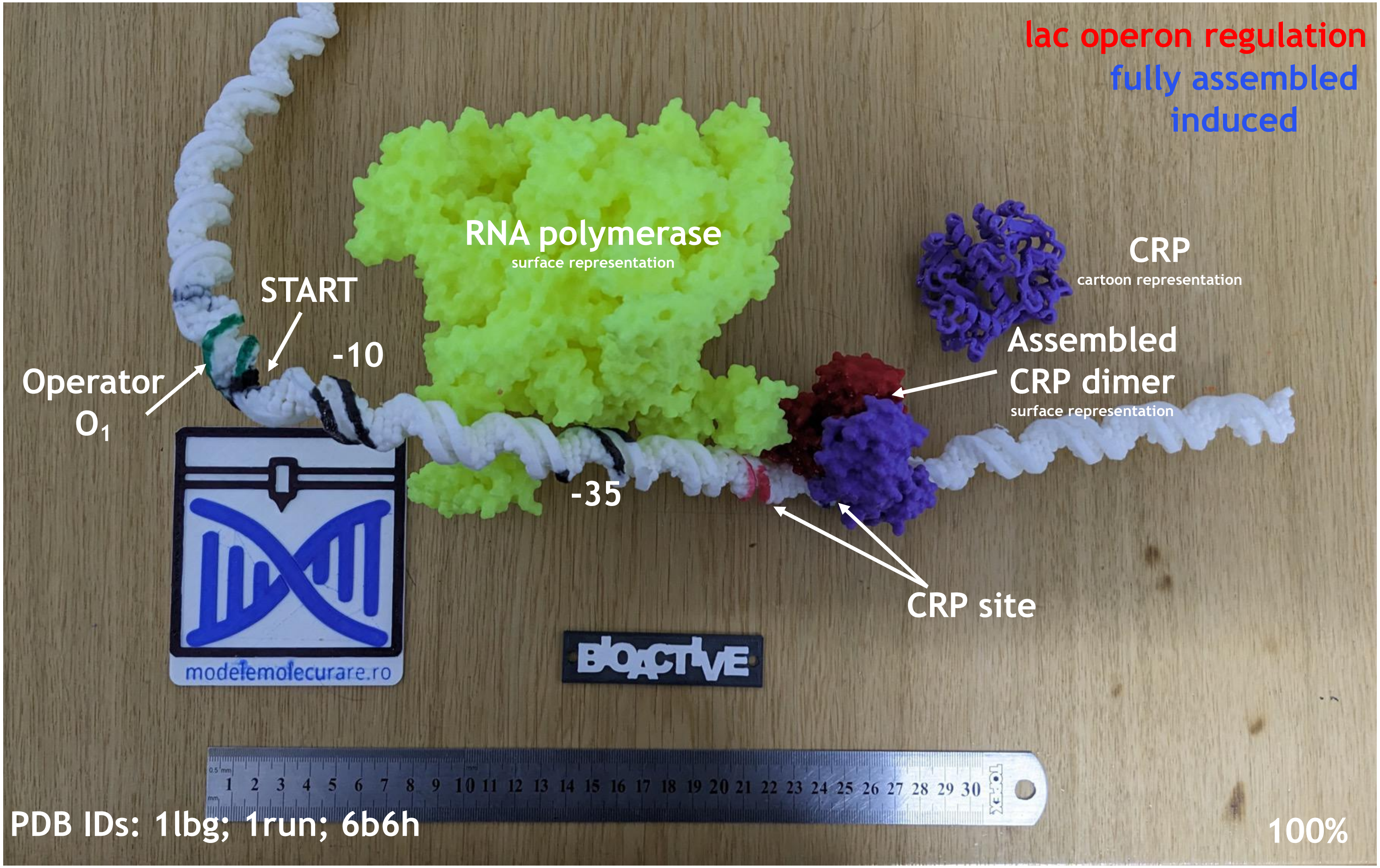
Physical model for teaching lac operon regulation



Physical model for teaching lac operon regulation



Physical model for teaching lac operon regulation



Alternative ways of using the models for teaching

Asking students to paint the models in order to recognize different structures

Summary

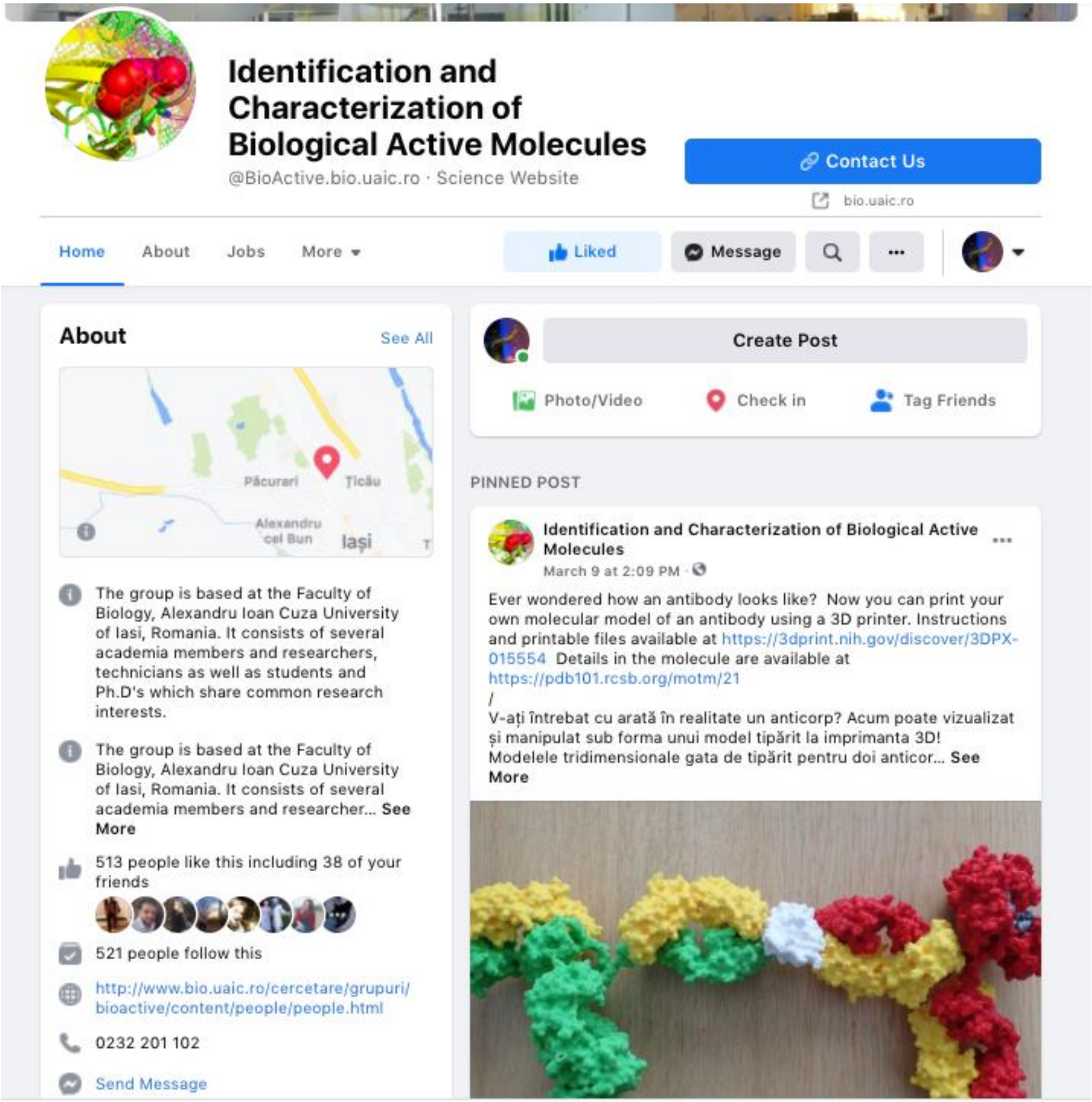
Usage of physical models of (macro)molecules improves learning outcomes, but need to be tailored to teachers needs

3D printing offers a cheap way of fabricating and distributing molecular models applicable in low income countries

Workflows for printing macromolecular models from PDB are available and are based on free software

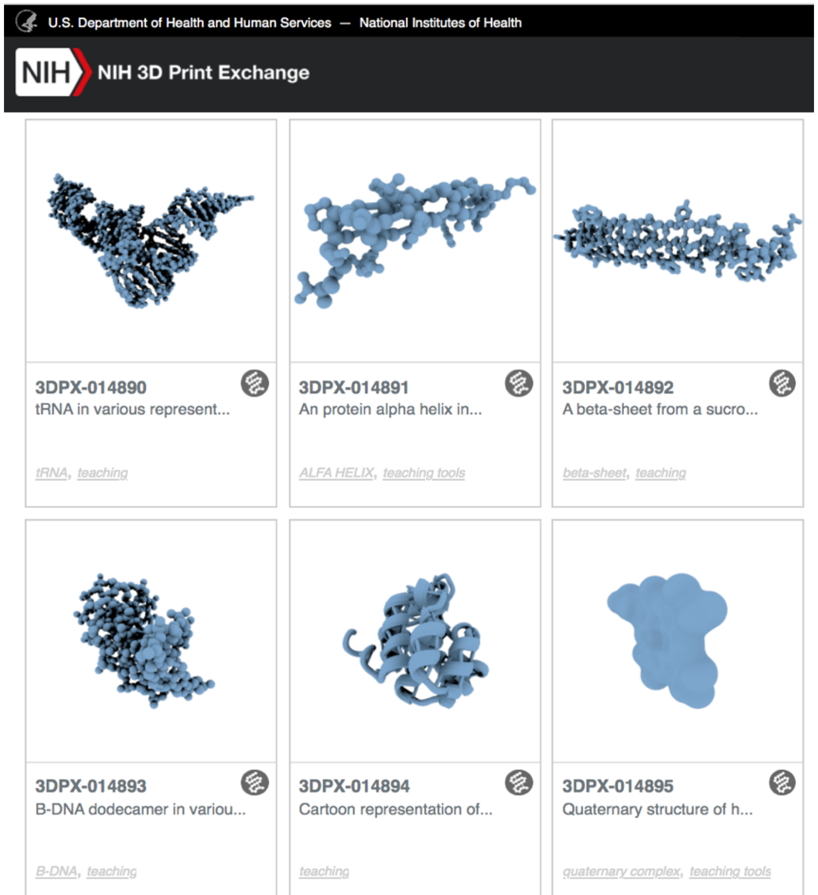
Models were received by students as being helpful as it provided a hands-on advantage. Allowing students 3-5 minutes to handle models converted a low-g lecture into a medium-g lecture.

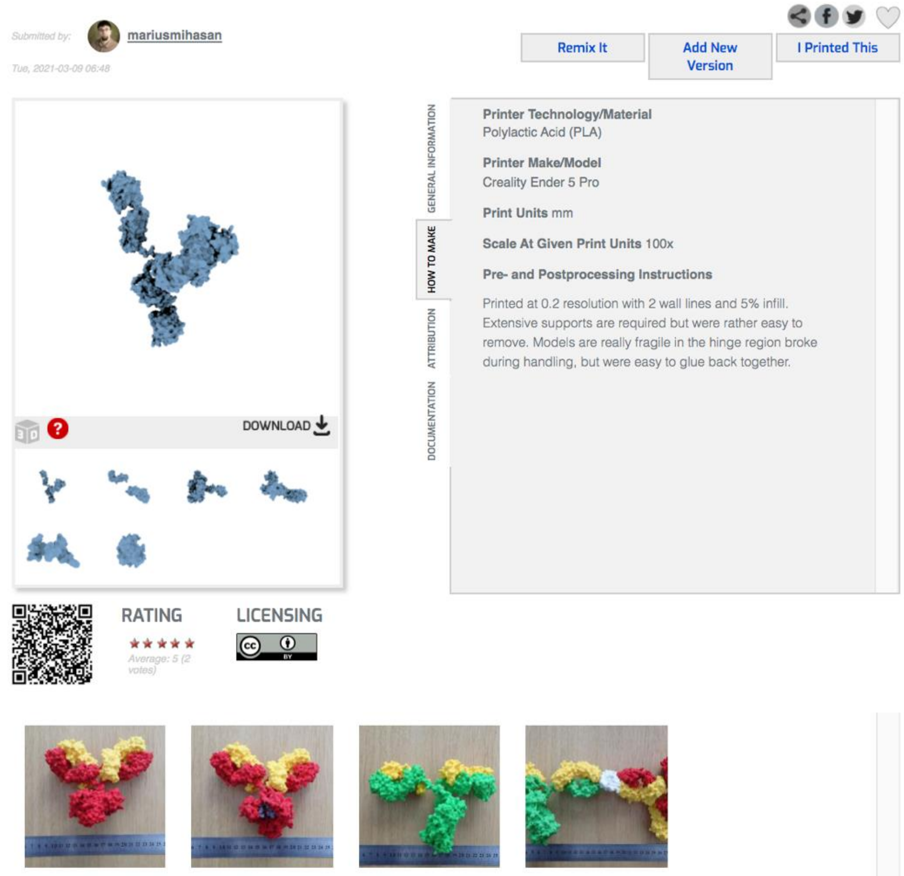
Updates and new printed models



Find us on Facebook @BioActive.bio.uaic.ro

Most models and instructions on how to print are available under CC BY license





https://3dprint.nih.gov/users/mariusmihasan/model