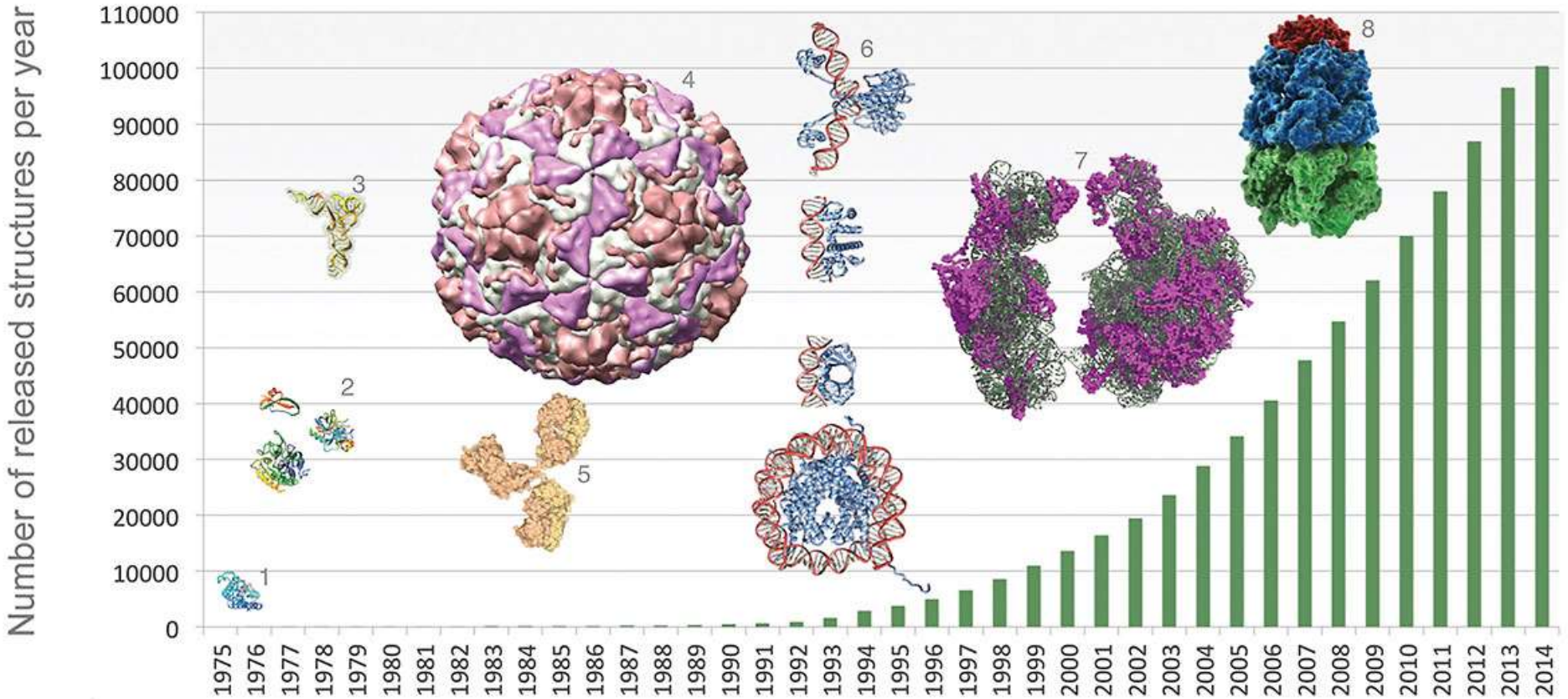
Introduction:
Why structural studies?

Corso di Biocristallografia e Microscopia Elettronica
rdezorzi@units.it



Contents

Introduction
- Structure function relationships
- Elements of protein structure
- Visualization software and databanks of protein structures

Protein samples for structural studies
- Recombinant proteins
- Expression and purification
- Evaluation of sample quality and purity
- Analysis of conformational stability

Biocrystallography
- Crystallization of proteins
- Symmetry in crystals
- Non-crystallographic symmetries
- Basic concepts of diffraction physics
- Instruments
- Diffraction data collection techniques
- Phase problem in crystallography
- Refinement of the structural model
- Validation

Transmission electron microscopy
- Basic concepts of optics for TEM
- Instruments
- Single particle techniques
- Image analysis
- 3D reconstruction from images
- Validation
- Electron crystallography

Practical experiences
- Crystallization of a protein sample
- Sample preparation for data collection
- Data collection at the Elettra Synchrotron
- Data analysis, structure solution and refinement

Objectives of the course

Knowledge of:

- Basic elements of 3D structure of proteins
- Main characteristics of crystals and crystallization techniques
- Physical basis of X-ray diffraction
- X-ray data collection techniques, structural determination and refinement
- Techniques of structural determination by electron microscopy
- Electron microscopy data analysis up to structure validation

Apply knowledge to:

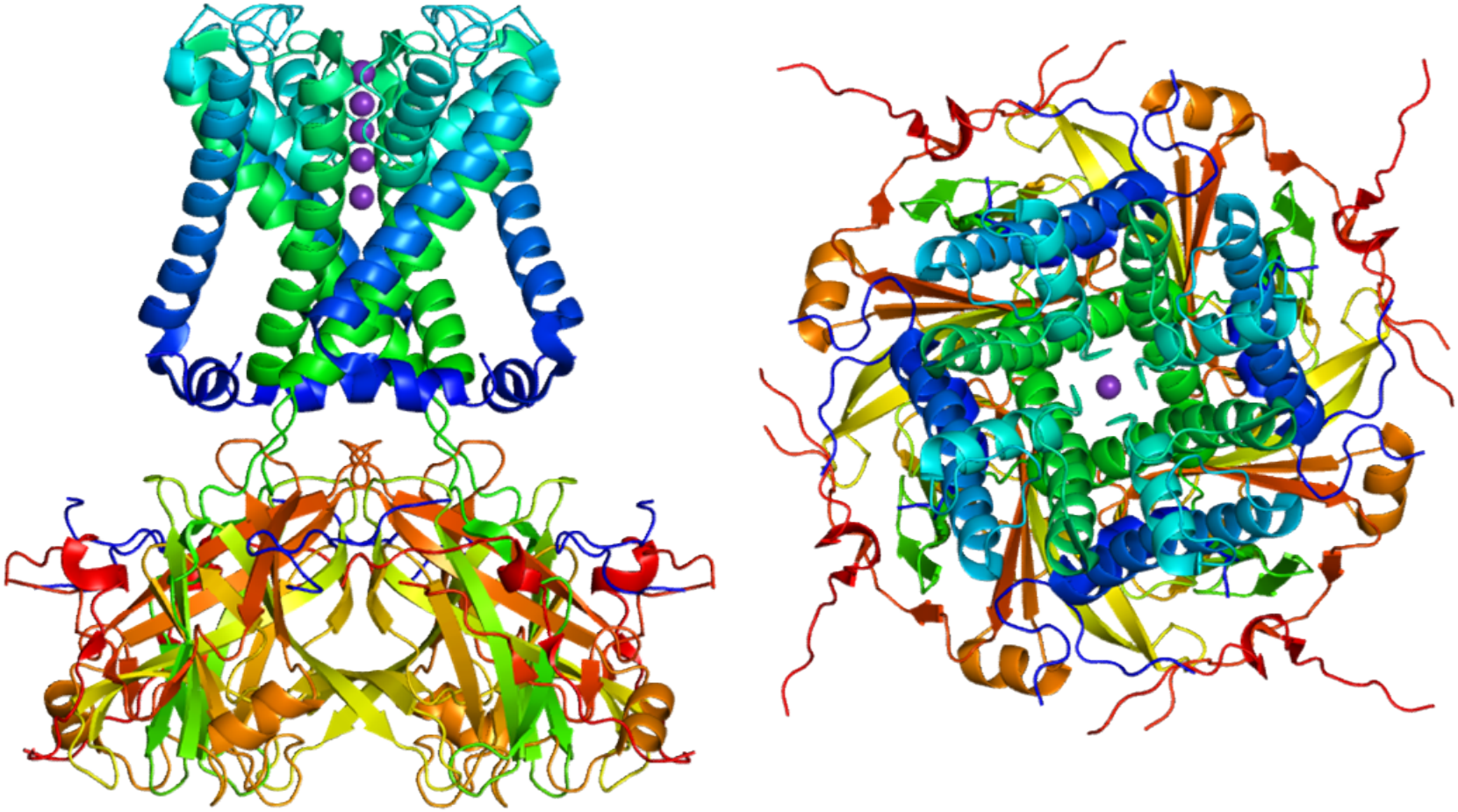
- Describe the 3D structure of a protein
- Plan a X-ray diffraction or electron microscopy experiment, from expression to structure
- Highlight main reasons to chose a structural biology technique over the others
- Recognize crucial factors that can improve/hamper a structural biology experiment
- Identify significant information that can be obtained from a protein structure
- Evaluate quality and reliability of a protein structure obtained through crystallography or EM

Communicate:

- Read and understand a structural biology paper, discussing critical aspects
- Obtain useful information from the available online databases of protein structures
- Present a structural biology study, highlighting useful information obtained from structures
- Obtain images of a protein structure to describe its main features
- Highlight structure-function relationships

Structure-function relationship

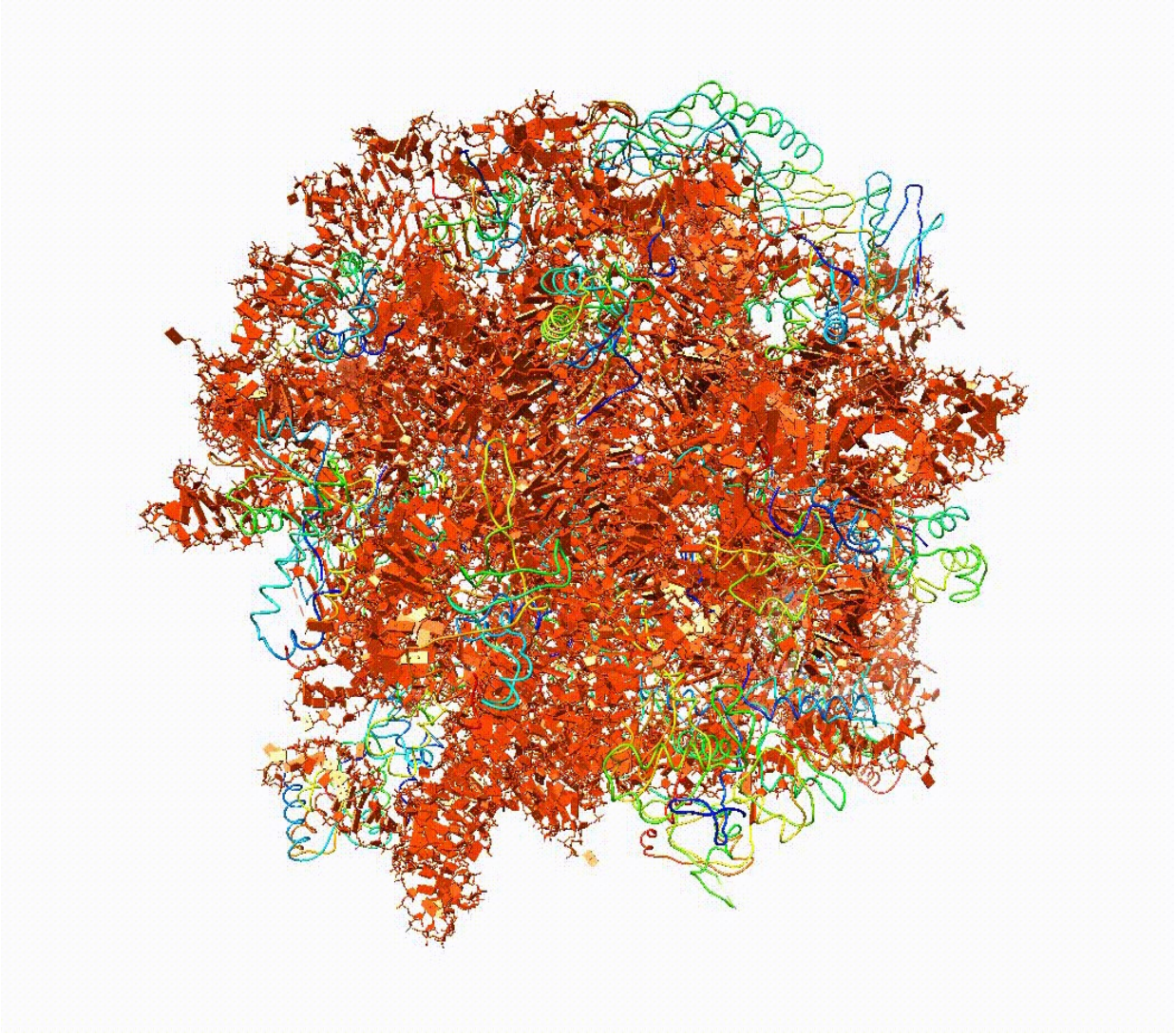
- Mechanisms of biologically relevant proteins and complexes: e.g. Potassium channel



PDB: 3zrs

Structure-function relationship

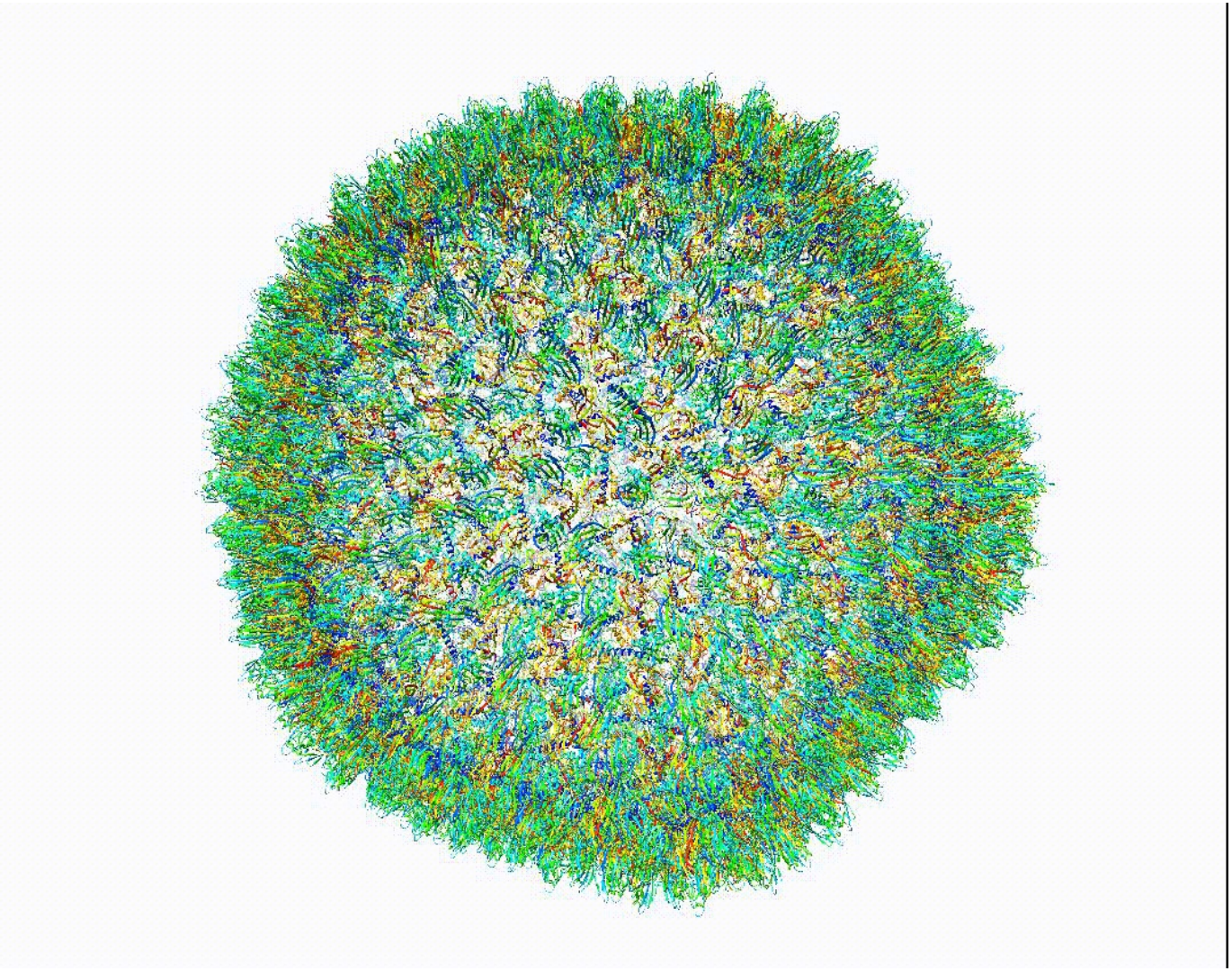
- Mechanisms of biologically relevant proteins and complexes: e.g. ribosome



PDB: 1fft

Structure-function relationship

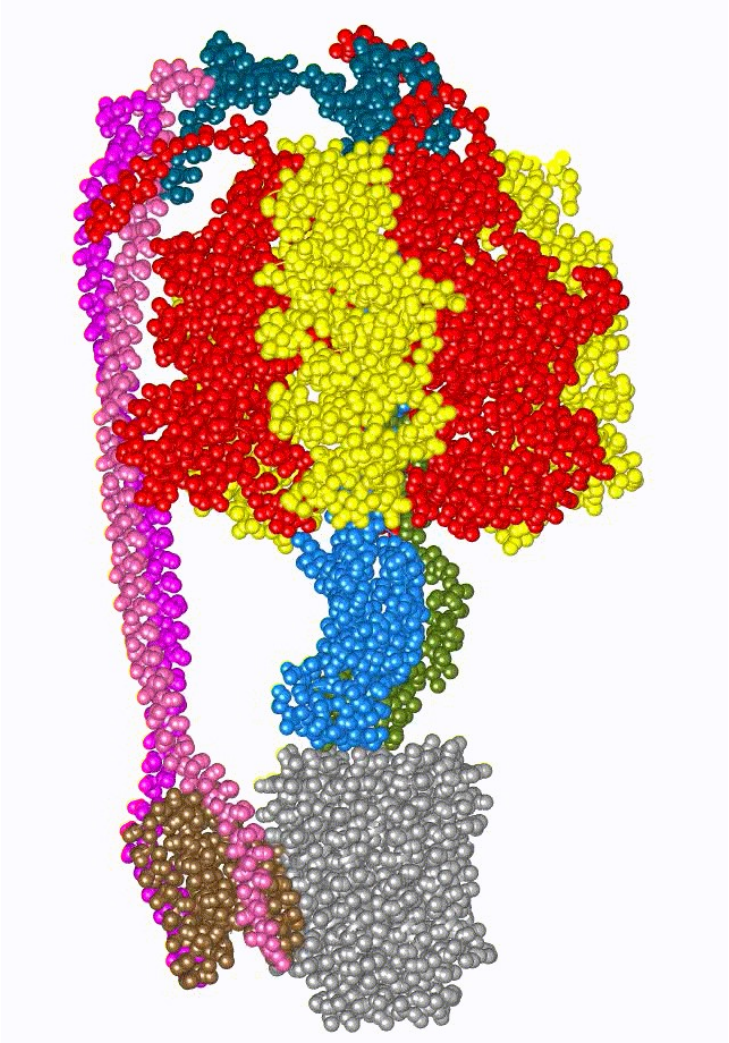
- Mechanisms of biologically relevant proteins and complexes: e.g. virus



PDB: 1w8x

Structure-function relationship

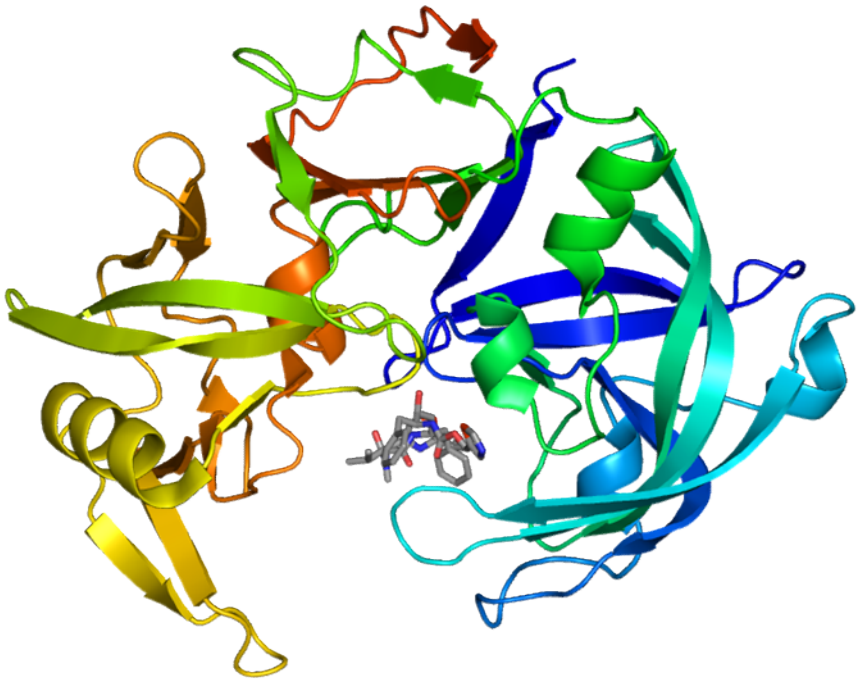
- Mechanisms of biologically relevant proteins and complexes: e.g. ATP-synthase



PDB: 5are, 5fil, 5fij, 5fik, 5ara, 5ari, 5arh

Structure-function relationship

- Drug design: e.g. HIV protease inhibitors, GPCR receptors



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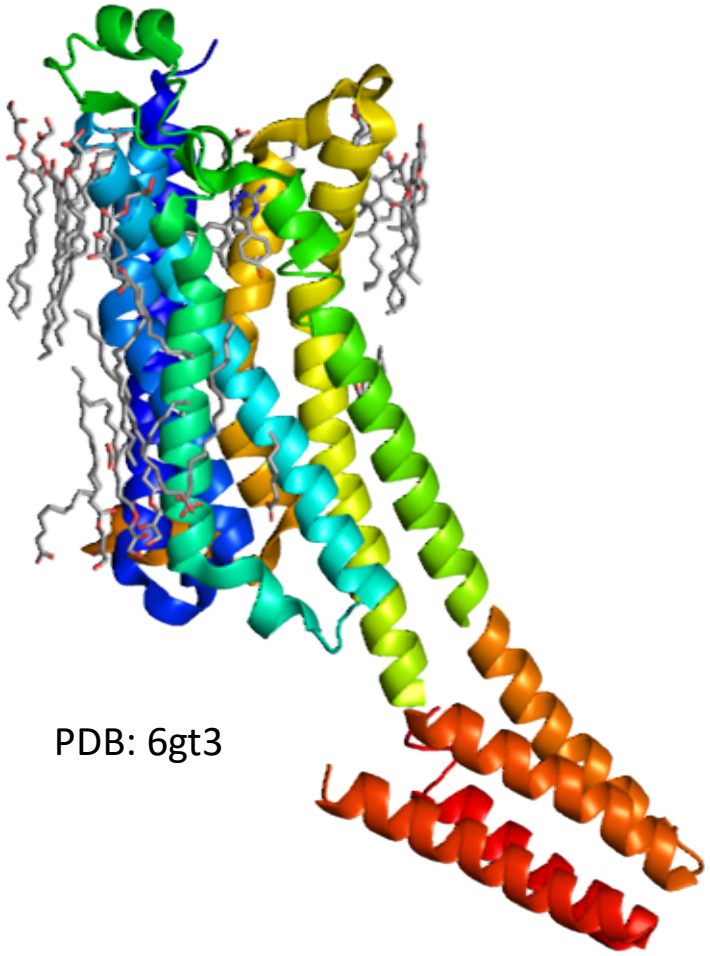


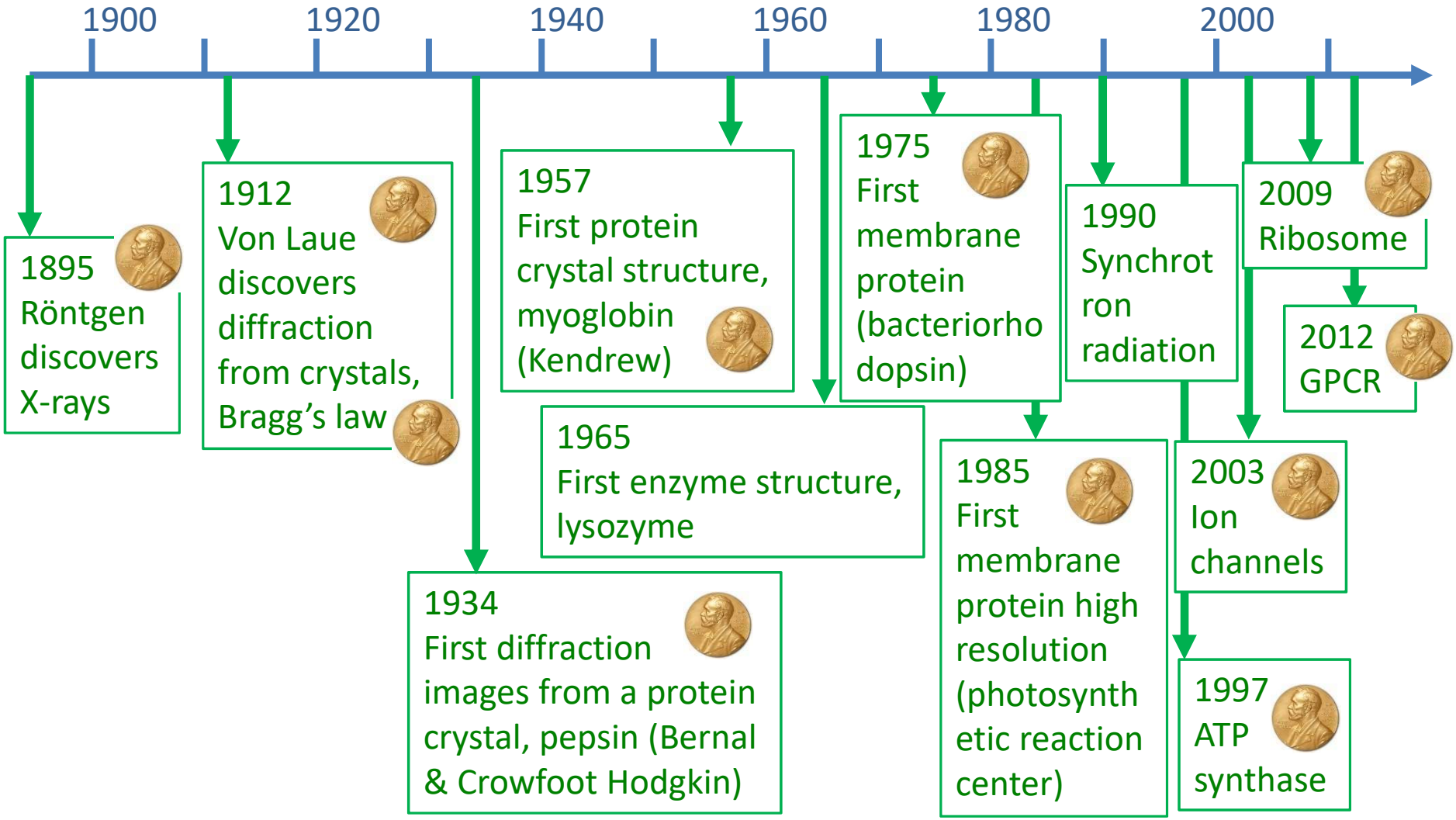
PDB: 6gt3

Structural biology: techniques

	X-ray crystallography	**NMR**	**Electron Microscopy**
Protein size	No limitation	< 80 KDa	> 100 kDa
Resolution	Atomic	Atomic	> 2 Å
First structure	1957	1985	Low res: 1975 Medium res:1990 High res: 2015
Dynamics	No	Yes	(Yes)
Main bottlenecks	Crystallization	Isotope enrichment	Image analysis, model validation

Different structural biology techniques give complementary results!!

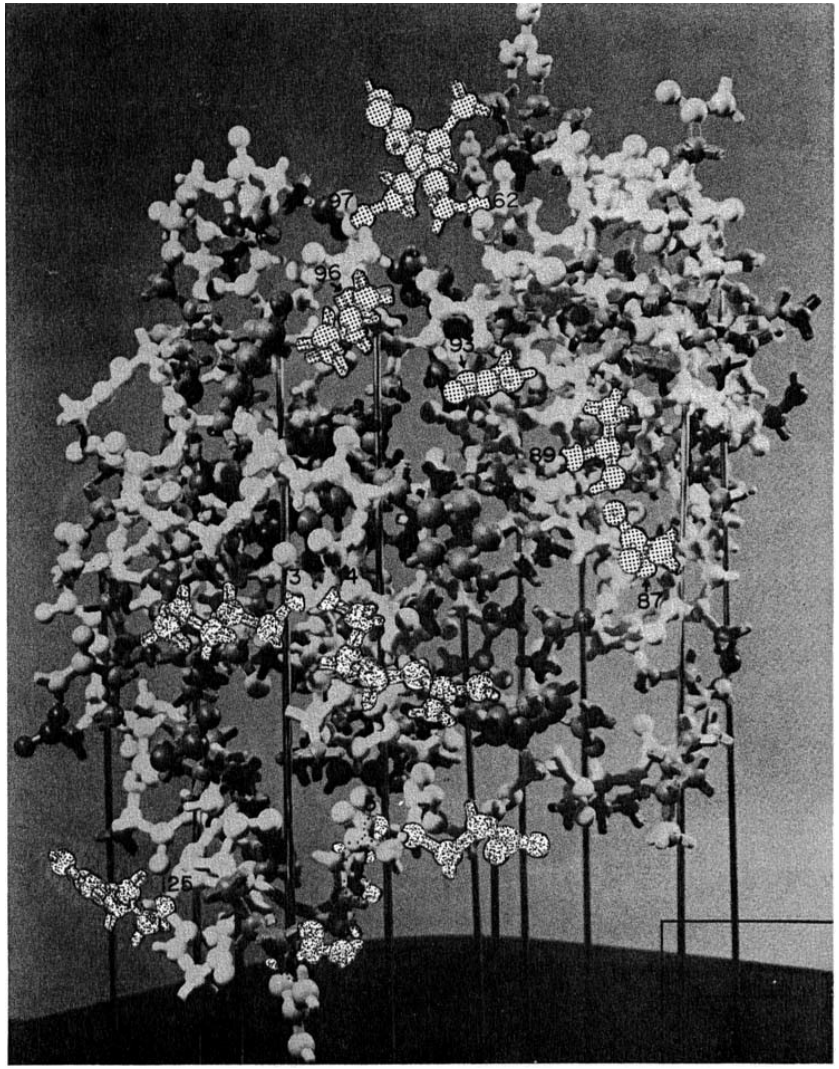
Biocrystallography



First protein structures determined by X-ray crystallography

- Myoglobin from sperm whale

- Lysozyme from chicken egg whites



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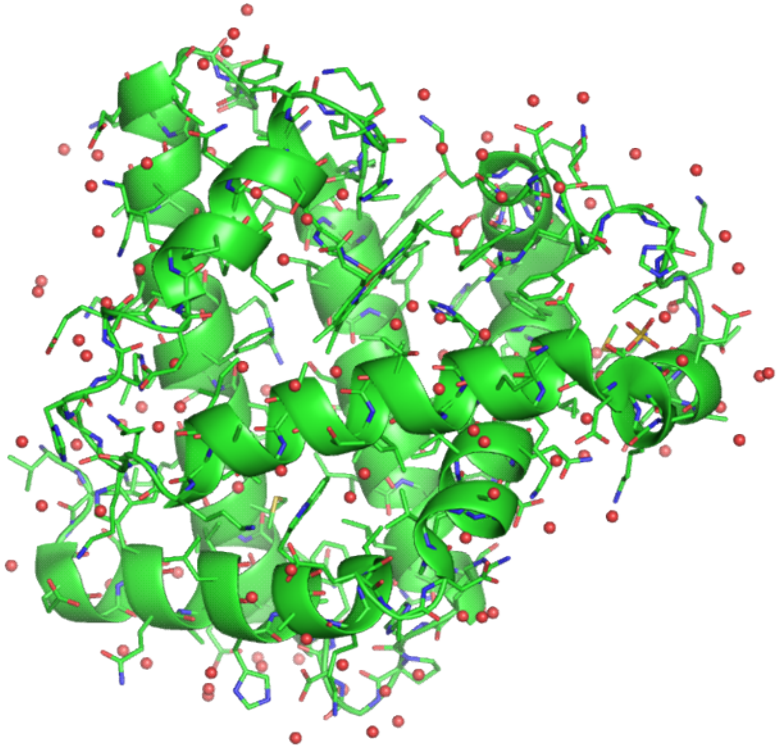
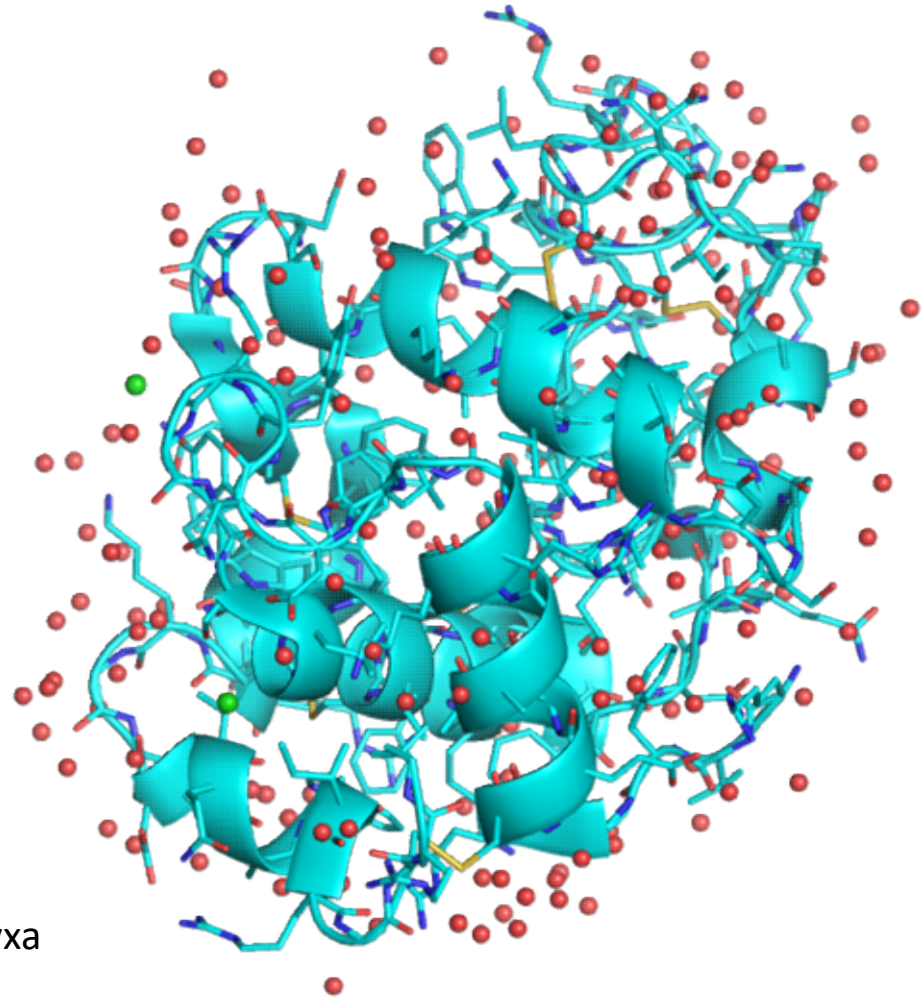


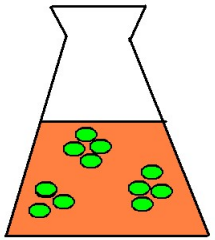
PDB: 1vxa

- Lysozyme from chicken egg whites

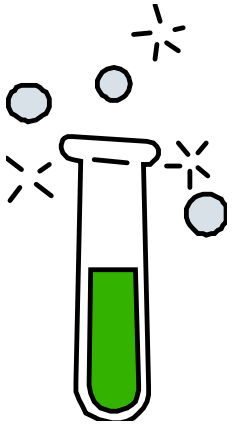


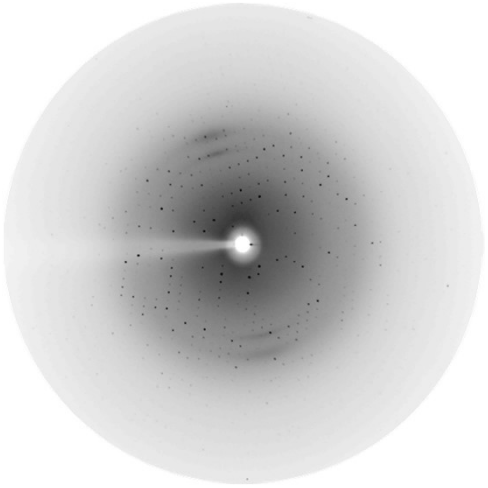
PDB: 1vxa

A biocrystallography experiment

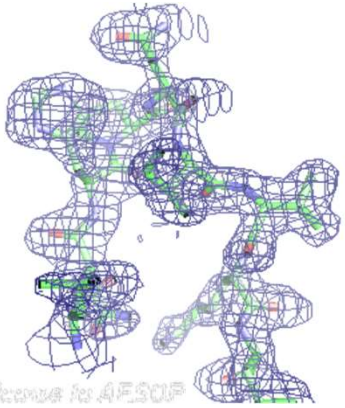


Expression and purification



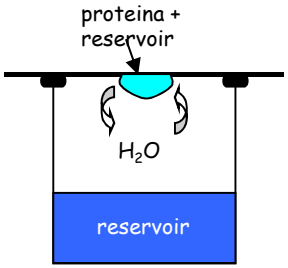


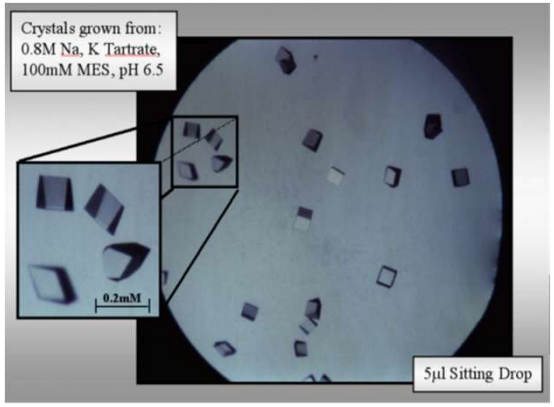
Diffraction data collection

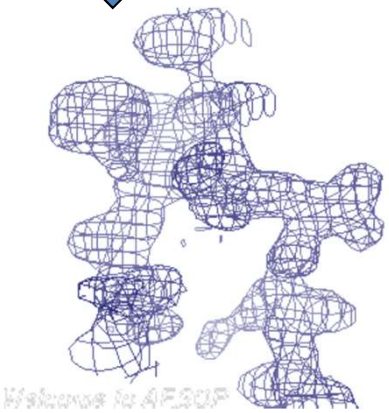


Structure refinement

Crystallization







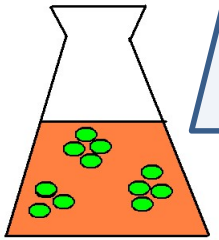
Phasing

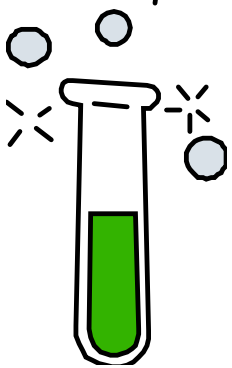
10 days to
10 years

A biocrystallography experiment

Bioinfor matics

Molecular biology

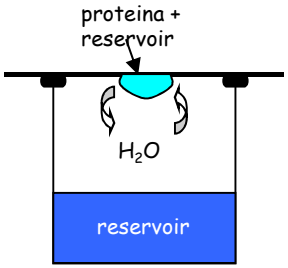


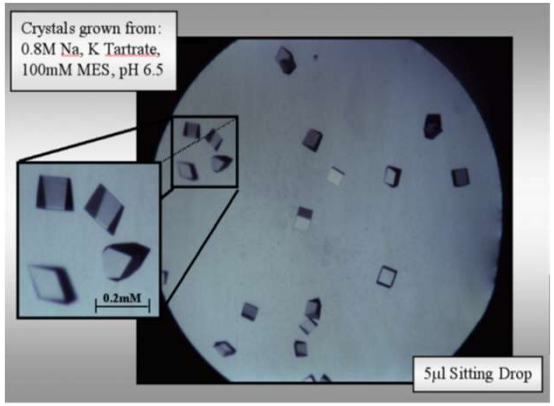


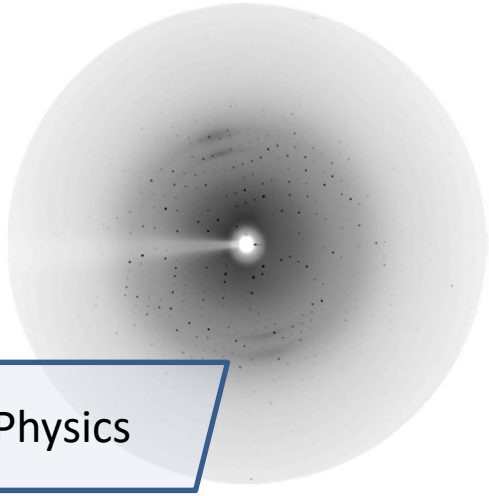
Expression and purification

Chemistry

Crystallization







Physics

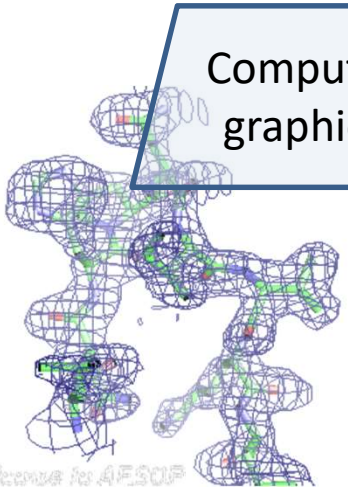
Diffraction data collection

Software develop ment

Mathematics

Statistics

Phasing



Computer graphics

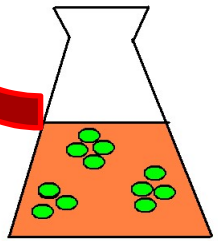
Structure refinement

Biology

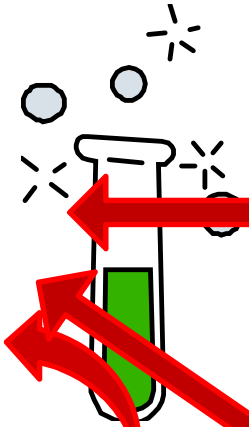
Biochemistry

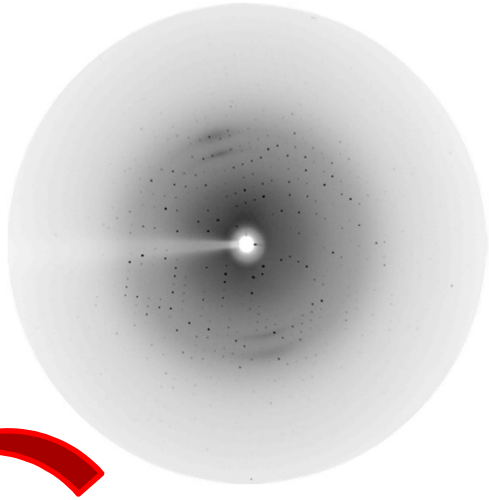
10 days to
10 years

A biocrystallography experiment

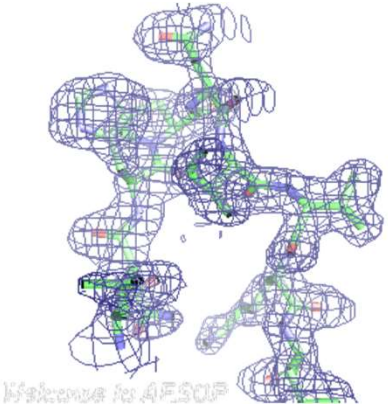


Expression and purification



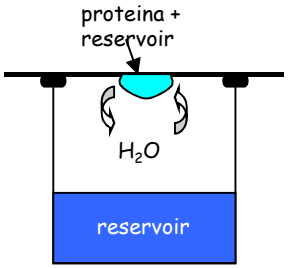


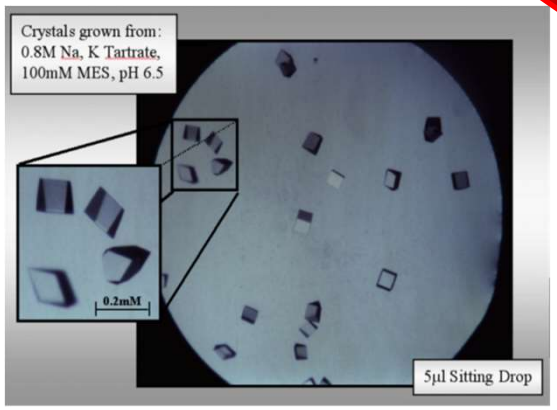
Diffraction data collection

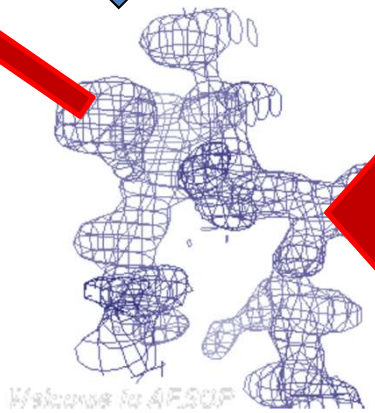


Structure refinement

Crystallization



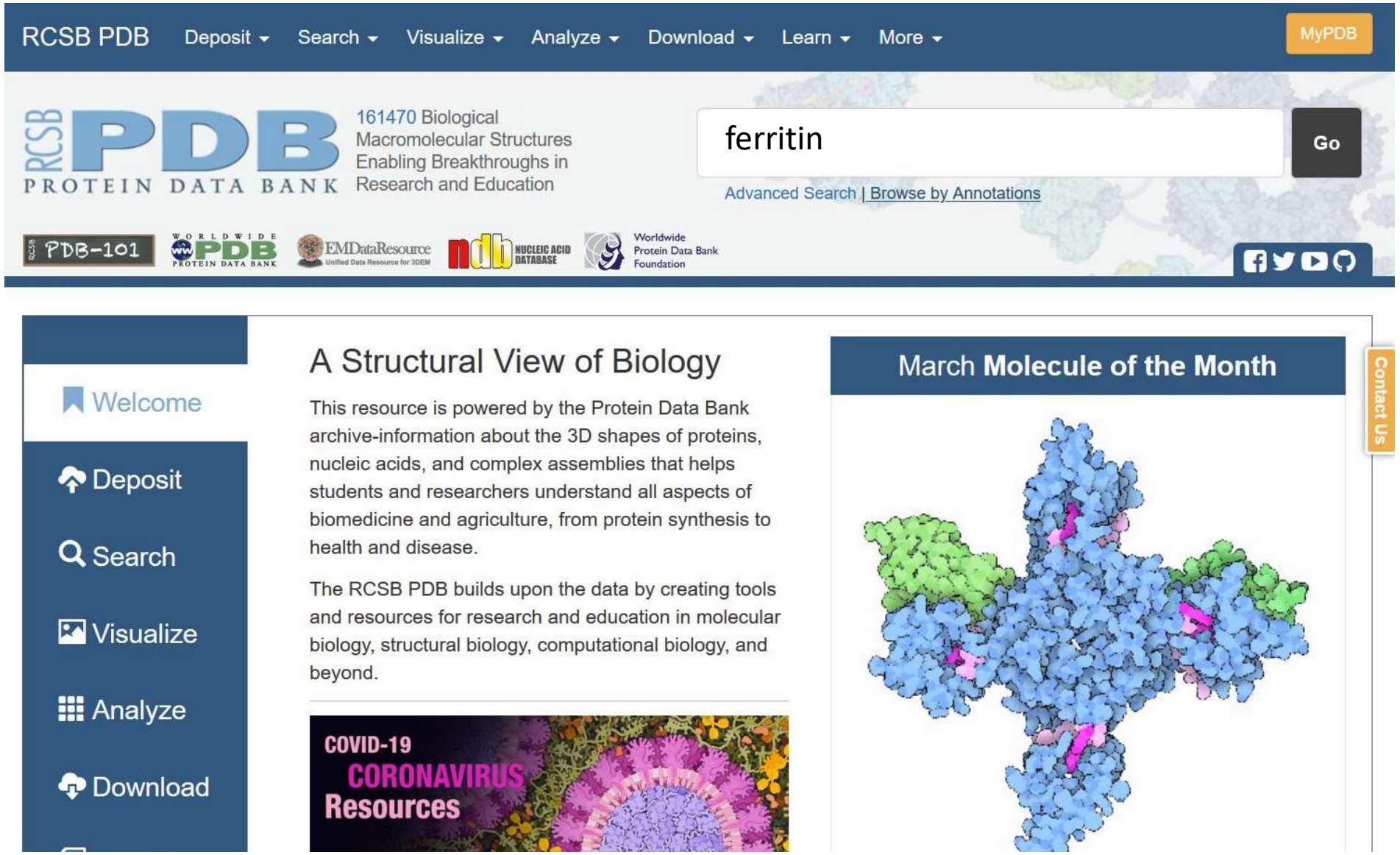




Phasing

The structure needs to be confirmed by biochemical and functional studies

Databank resources: PDB (www.rcsb.org)



Databank resources: PDB (www.rcsb.org)

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419 Structures | 41 Unreleased Structures | 151 Citations | 112 Ligands | 2 News & PDB-101 Articles

Search Parameter:

Text Search for: ferritin

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Refinements

ORGANISM

Equus caballus (87)
Homo sapiens (76)
Lithobates catesbeianus (47)
Escherichia coli (24)
Pseudo-nitzschia multiseries (16)
Mycolicibacterium smegmatis (12)
Synechococcus sp. CC9311 (11)
Other (146)

UNIPROT MOLECULE NAME

Ferritin light chain (98)

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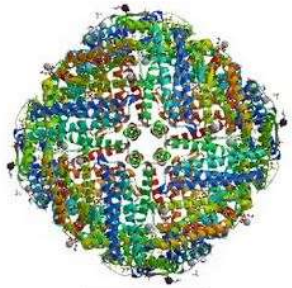

3D View

6A4U

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The first crystal structure of crustacean ferritin that is a hybrid type of H and L ferritin

Masuda, T., Zang, J., Zhao, G., Mikami, B.

(2018) Protein Sci **27** 1955-1960

Released: 8/22/2018
Method: X-ray Diffraction
Resolution: 1.16 Å

Macromolecule:
Ferritin (protein)
Unique Ligands: CL, EDO, MG, SO4

Databank resources: PDB (www.rcsb.org)

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RCSB PDB PROTEIN DATA BANK

161470 Biological Macromolecular Structures Enabling Breakthroughs in Research and Education

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PDB-101 WORLDWIDE PDB PROTEIN DATA BANK EMDataResource Unified Data Resource for 3DEM ndb NUCLEIC ACID DATABASE Worldwide Protein Data Bank Foundation

Structure Summary | 3D View | Annotations | Sequence | Sequence Similarity | Structure Similarity | Experiment

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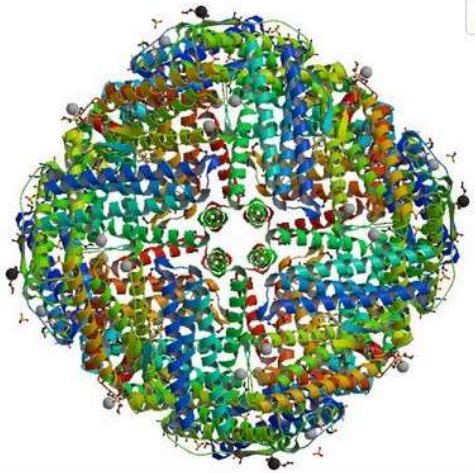
Biological Assembly 1



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6A4U

The first crystal structure of crustacean ferritin that is a hybrid type of H and L ferritin

DOI: 10.2210/pdb6A4U/pdb

Classification: METAL BINDING PROTEIN
Organism(s): Penaeus japonicus
Expression System: Escherichia coli BL21(DE3)
Mutation(s): 1

Deposited: 2018-06-21 **Released:** 2018-08-22
Deposition Author(s): Masuda, T., Mikami, B., Zang, J., Zhao, G.
Funding Organization(s): Japan Society for the Promotion of Science

Databank resources: PDB (www.rcsb.org)

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Funding Organization(s): Japan Society for the Promotion of Science

3D View: Structure | Electron Density | Ligand Interaction

Standalone Viewers
Protein Workshop | Ligand Explorer

Global Symmetry: Octahedral - O (3D View)
Global Stoichiometry: Homo 24-mer - A24

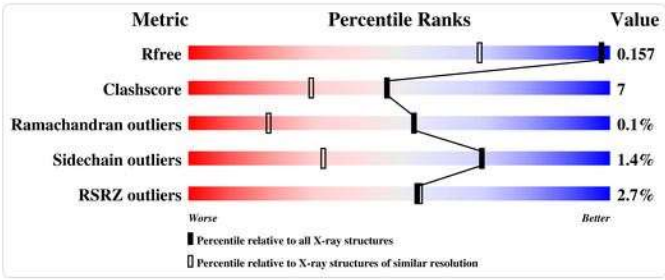
Biological assembly 1 assigned by authors and generated by PISA (software)

Biological Assembly Evidence: gel filtration

Experimental Data Snapshot

Method: X-RAY DIFFRACTION
Resolution: 1.16 Å
R-Value Free: 0.157
R-Value Work: 0.138

wwPDB Validation 3D Report Full Report



This is version 1.1 of the entry. See complete history.

Macromolecule Content

- Total Structure Weight: 120841.39
- Atom Count: 9619
- Residue Count: 1014
- Unique protein chains: 1

Literature

Download Primary Citation

The first crystal structure of crustacean ferritin that is a hybrid type of H and L ferritin

Masuda, T., Zang, J., Zhao, G., Mikami, B.
(2018) Protein Sci. **27**: 1955-1960

PubMed: 30099791 Search on PubMed Search on PubMed Central
DOI: 10.1002/pro.3495

PubMed Abstract:

Ferritin, a ubiquitous iron storage protein, has a crucial role in innate immunity in arthropods,

files.rcsb.org/pub/pdb/validation_reports/a4/6a4u/6a4u_full_validation.pdf

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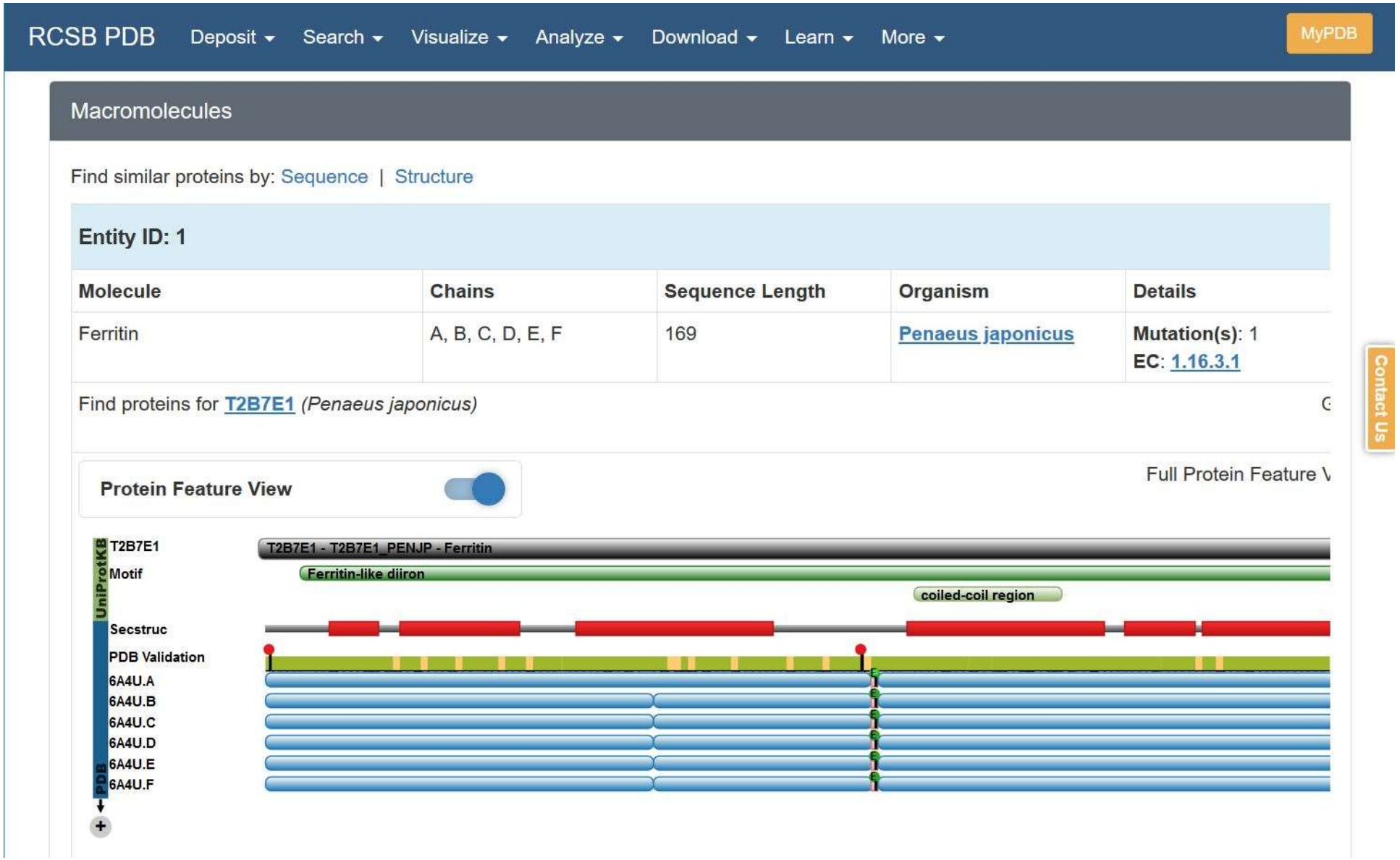
Databank resources: PDB (www.rcsb.org)



Databank resources: PDB (www.rcsb.org)

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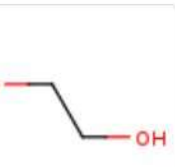
Small Molecules

Ligands 4 Unique

ID	Chains	Name / Formula / InChI Key	2D Diagram & Interactions	3D Interactions
SO4 **Query on SO4** Download SDF File Download CCD File	A, B, C, D, E, F	**SULFATE ION** O_4 S QAOWNCQODCNURD-UHFFFAOYSA-L		Ligand Interaction
CL **Query on CL** Download SDF File Download CCD File	B	**CHLORIDE ION** Cl VEXZGXHMUGYJMC-UHFFFAOYSA-M		Ligand Interaction
EDO **Query on EDO** Download SDF File Download CCD File	A, B, C, D, E, F	**1,2-ETHANEDIOL** *ETHYLENE GLYCOL* C_2 H_6 O_2 LYCAIKOWRPUZTN-UHFFFAOYSA-N		Ligand Interaction
MG **Query on MG**	C, D, F	**MAGNESIUM ION** Mg		Ligand Interaction

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Databank resources: PDB (www.rcsb.org)

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Experimental Data & Validation

Experimental Data

Method: X-RAY DIFFRACTION
Resolution: 1.16 Å
R-Value Free: 0.157
R-Value Work: 0.138
Space Group: I 4

Unit Cell:

Length (Å)	Angle (°)
a = 124.872	α = 90.00
b = 124.872	β = 90.00
c = 175.683	γ = 90.00

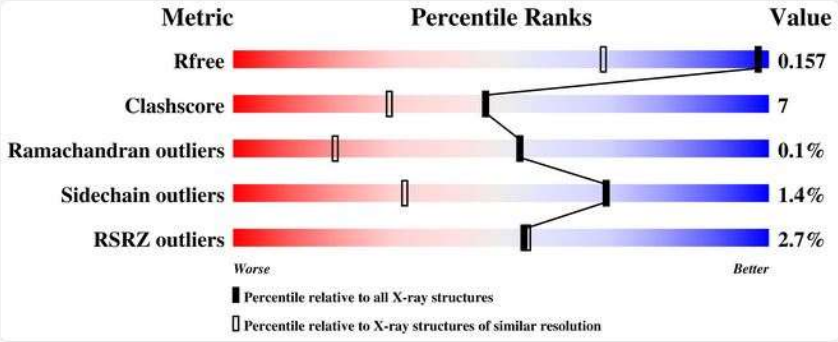
Software Package:

Software Name	Purpose
MOLREP	phasing
HKL-2000	data reduction
PHENIX	refinement
HKL-2000	data scaling

View more in-depth experimental data

Structure Validation

View Full Validation Report or Ramachandran Plots



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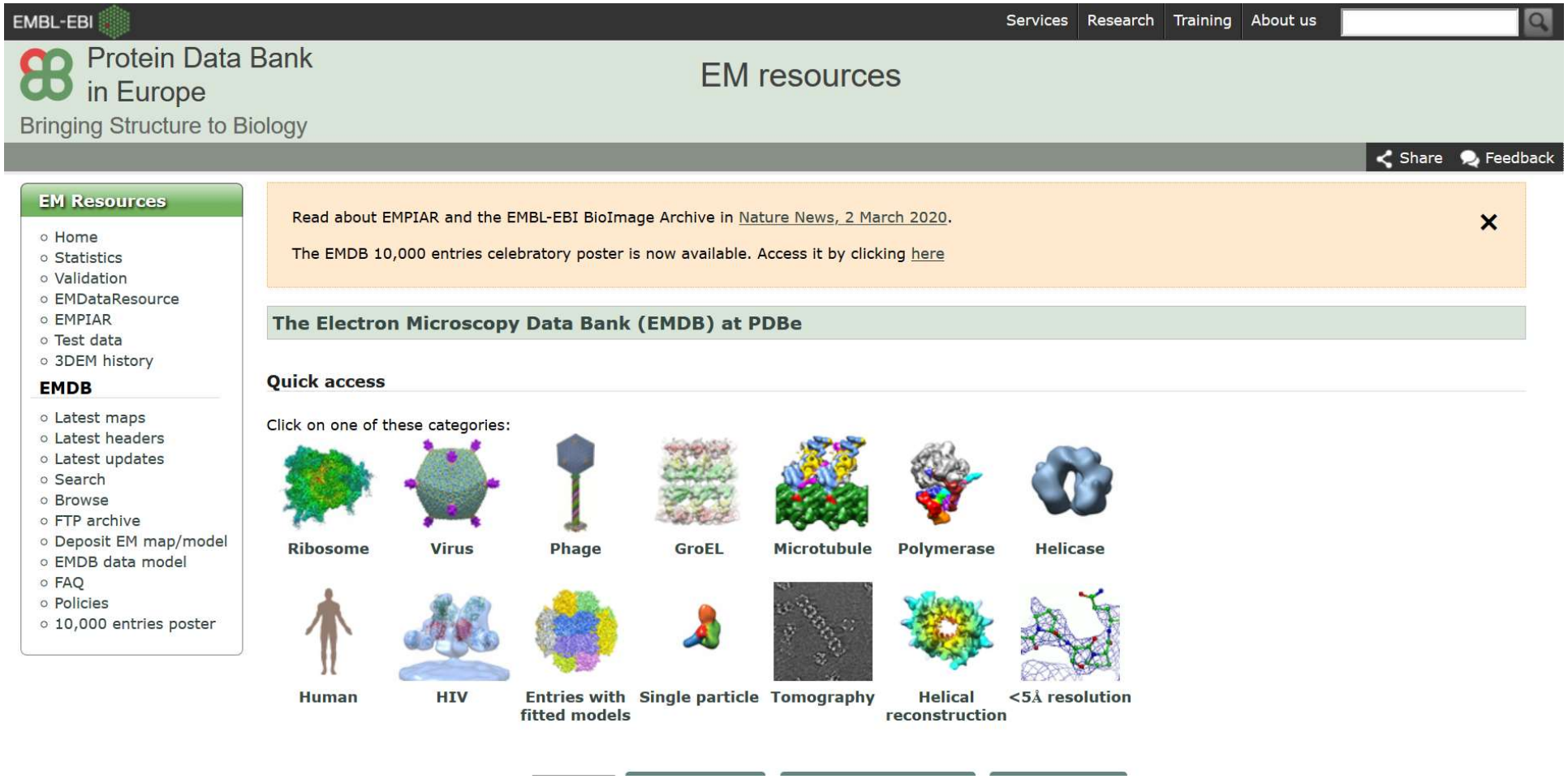
Databank resources: EMDB (www.ebi.ac.uk/pdbe/emdb/)



Databank resources: EMDB (www.ebi.ac.uk/pdbe/emdb/)

EMBL-EBI

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Protein Data Bank in Europe
Bringing Structure to Biology

Search

Examples: hemoglobin, BRCA1_HUMAN

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EMDB › EMD-0632

Single particle reconstruction
2.7Å resolution

Rotavirus A-VP3 (RVA-VP3)

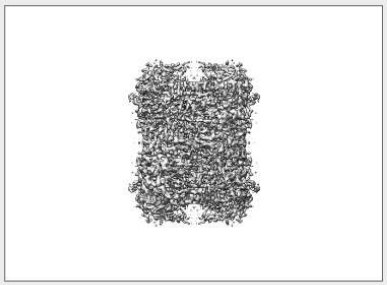
Source organism: *Rotavirus A* [28875]

Fitted atomic model: 6o6b

3Dbionotes: available for this entry

To be published

Map released:
2020-03-11



Quick links

- **EMD-0632 overview**
- Function and Biology
- Experiments and Validation
- View
- Downloads
- Volume viewer
- Volume slicer
- Visual analysis

Function and Biology

Details

Sample name:	VP3
Ligand:	GUANOSINE-5'-MONOPHOSPHATE
Proteins:	VP3, Protein VP3

Experimental Information

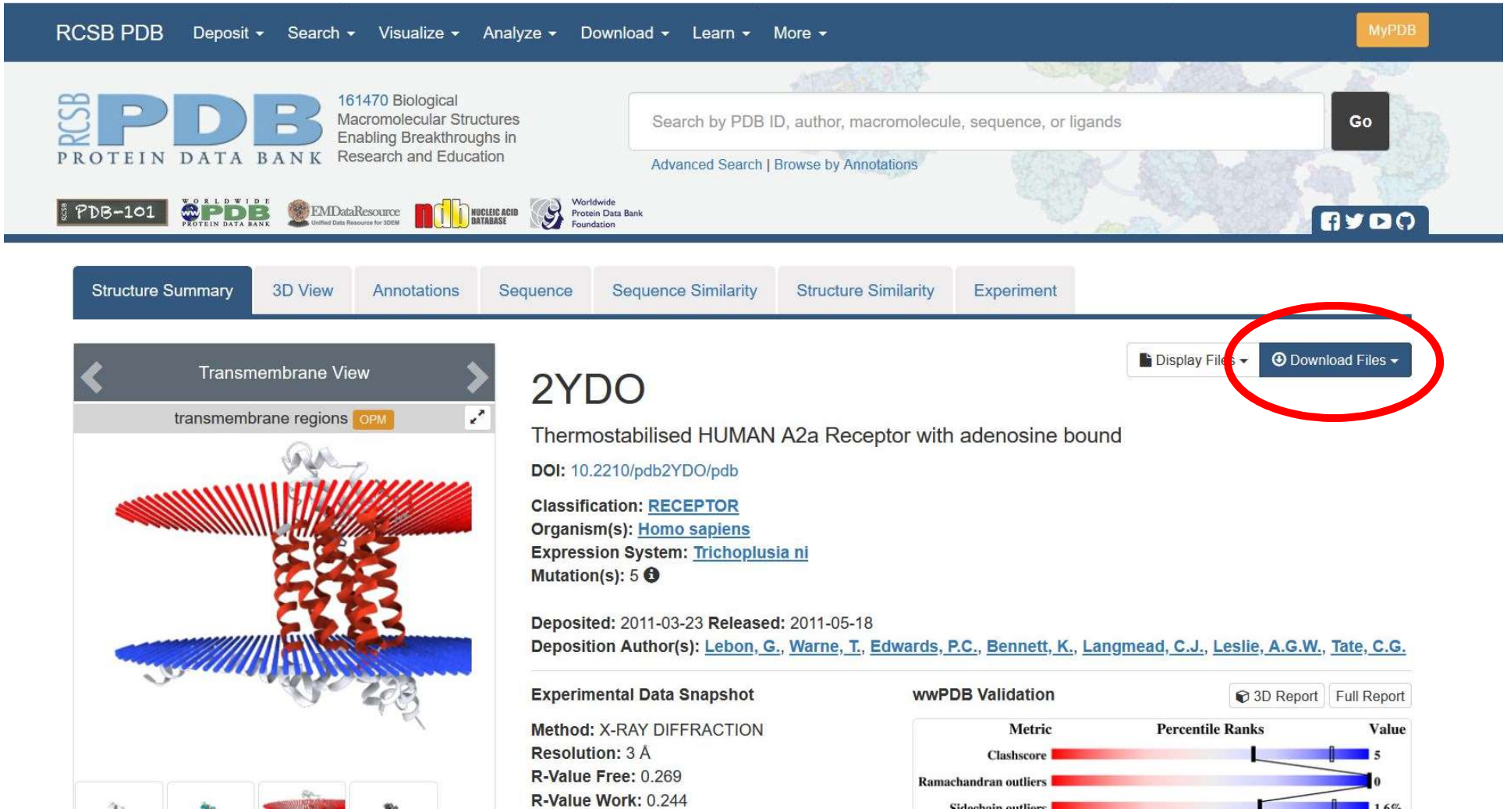
Details

Resolution:	2.7Å
Resolution method:	FSC 0.143 CUT-OFF
Applied symmetry:	D2
Reconstruction software:	RELION
Microscope:	JEOL 3200FSC
Detector:	GATAN K2 SUMMIT (4k x 4k)

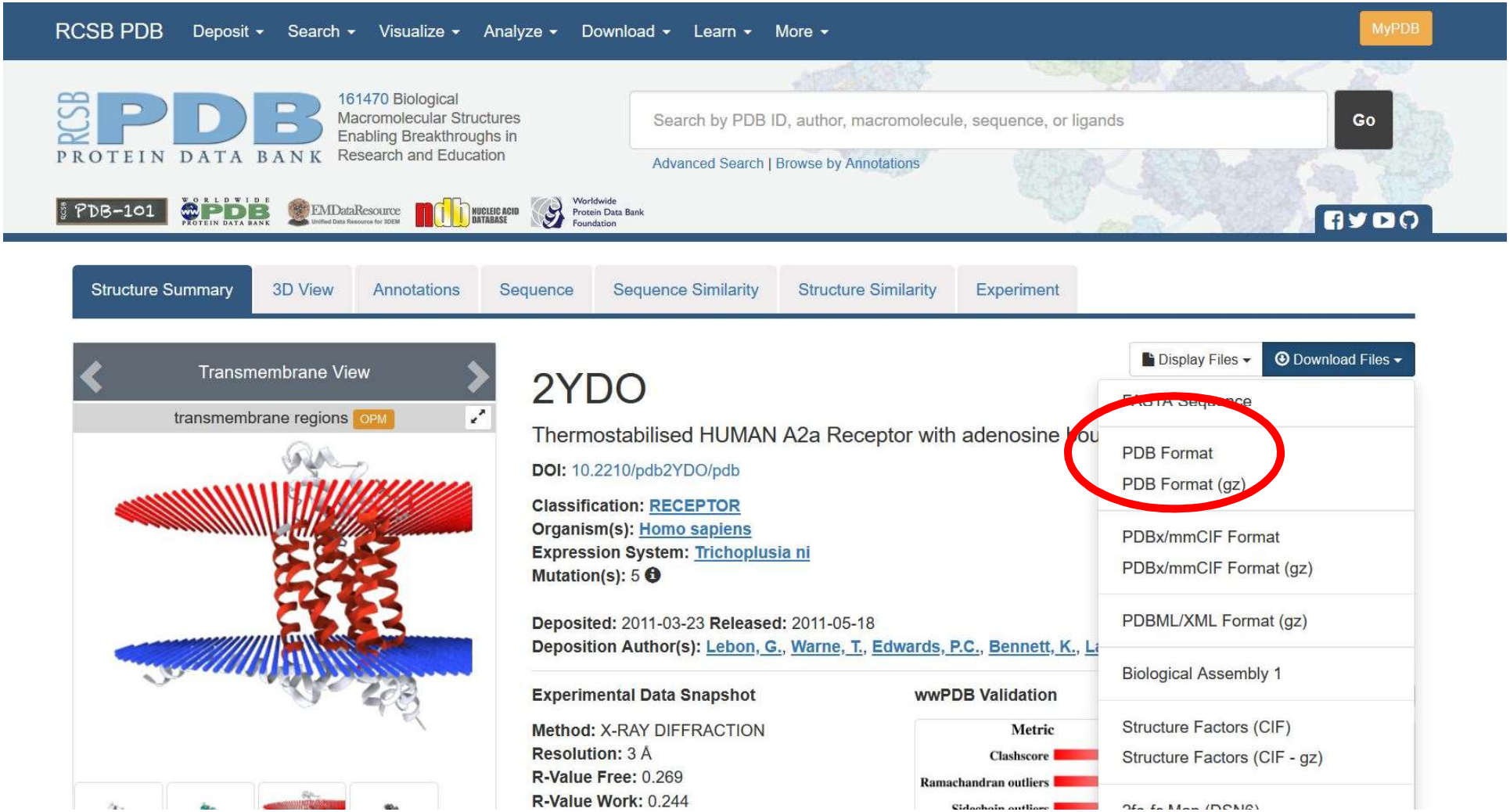
Related entries

- By authors
- By sample
- By organism

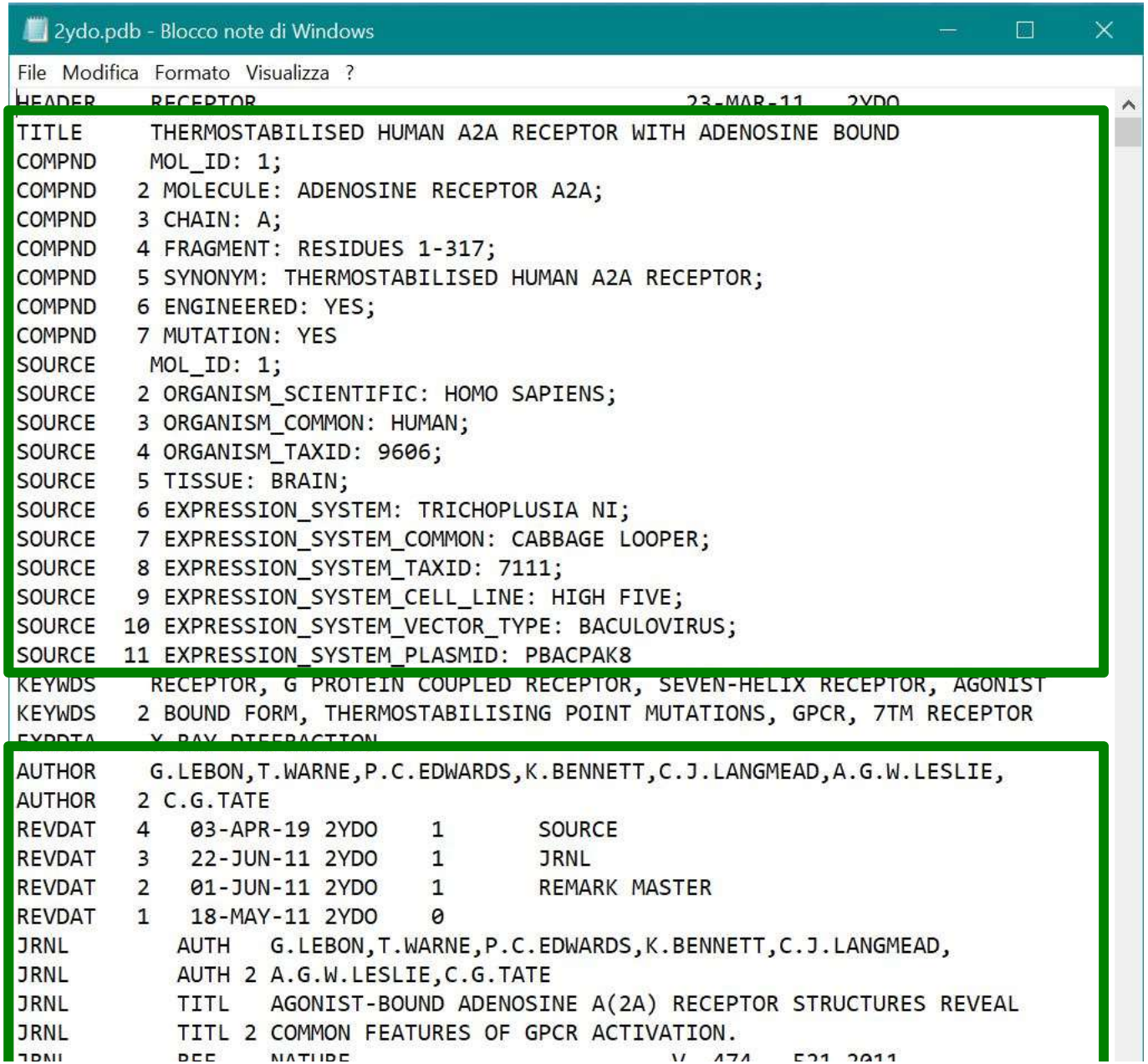
Structure atomic coordinates: pdb file



Structure atomic coordinates: pdb file



Structure atomic coordinates: pdb file



2ydo.pdb - Blocco note di Windows

File Modifica Formato Visualizza ?

```
HEADER    RECEPTOR                                23-MAR-11   2YDO
TITLE     THERMOSTABILISED HUMAN A2A RECEPTOR WITH ADENOSINE BOUND
COMPND    MOL_ID: 1;
COMPND   2 MOLECULE: ADENOSINE RECEPTOR A2A;
COMPND   3 CHAIN: A;
COMPND   4 FRAGMENT: RESIDUES 1-317;
COMPND   5 SYNONYM: THERMOSTABILISED HUMAN A2A RECEPTOR;
COMPND   6 ENGINEERED: YES;
COMPND   7 MUTATION: YES
SOURCE    MOL_ID: 1;
SOURCE   2 ORGANISM_SCIENTIFIC: HOMO SAPIENS;
SOURCE   3 ORGANISM_COMMON: HUMAN;
SOURCE   4 ORGANISM_TAXID: 9606;
SOURCE   5 TISSUE: BRAIN;
SOURCE   6 EXPRESSION_SYSTEM: TRICHOPLUSIA NI;
SOURCE   7 EXPRESSION_SYSTEM_COMMON: CABBAGE LOOPER;
SOURCE   8 EXPRESSION_SYSTEM_TAXID: 7111;
SOURCE   9 EXPRESSION_SYSTEM_CELL_LINE: HIGH FIVE;
SOURCE  10 EXPRESSION_SYSTEM_VECTOR_TYPE: BACULOVIRUS;
SOURCE  11 EXPRESSION_SYSTEM_PLASMID: PBACPAK8
KEYWDS    RECEPTOR, G PROTEIN COUPLED RECEPTOR, SEVEN-HELIX RECEPTOR, AGONIST
KEYWDS   2 BOUND FORM, THERMOSTABILISING POINT MUTATIONS, GPCR, 7TM RECEPTOR
EXPDTA    X-RAY DIFFRACTION
AUTHOR    G.LEBON,T.WARNE,P.C.EDWARDS,K.BENNETT,C.J.LANGMEAD,A.G.W.LESLIE,
AUTHOR   2 C.G.TATE
REVDAT   4   03-APR-19 2YDO    1       SOURCE
REVDAT   3   22-JUN-11 2YDO    1       JRNL
REVDAT   2   01-JUN-11 2YDO    1       REMARK MASTER
REVDAT   1   18-MAY-11 2YDO    0
JRNL        AUTH   G.LEBON,T.WARNE,P.C.EDWARDS,K.BENNETT,C.J.LANGMEAD,
JRNL        AUTH 2 A.G.W.LESLIE,C.G.TATE
JRNL        TITL   AGONIST-BOUND ADENOSINE A(2A) RECEPTOR STRUCTURES REVEAL
JRNL        TITL 2 COMMON FEATURES OF GPCR ACTIVATION.
JRNL        REF    NATURE                        V. 474   521 2011
```

Information about the protein

Information about the publication

Structure atomic coordinates: pdb file

2ydo.pdb - Blocco note di Windows

File Modifica Formato Visualizza ?

```
REMARK   3
REMARK   3  DATA USED IN REFINEMENT.
REMARK   3   RESOLUTION RANGE HIGH (ANGSTROMS) : 3.00
REMARK   3   RESOLUTION RANGE LOW  (ANGSTROMS) : 60.42
REMARK   3   DATA CUTOFF            (SIGMA(F)) : NULL
REMARK   3   COMPLETENESS FOR RANGE        (%) : 93.5
REMARK   3   NUMBER OF REFLECTIONS             : 10556
REMARK   3
REMARK   3  FIT TO DATA USED IN REFINEMENT.
REMARK   3   CROSS-VALIDATION METHOD           : THROUGHOUT
REMARK   3   FREE R VALUE TEST SET SELECTION   : RANDOM
REMARK   3   R VALUE     (WORKING + TEST SET) : 0.246
REMARK   3   R VALUE           (WORKING SET) : 0.244
REMARK   3   FREE R VALUE                     : 0.269
REMARK   3   FREE R VALUE TEST SET SIZE   (%) : 5.200
REMARK   3   FREE R VALUE TEST SET COUNT      : 575
REMARK   3
REMARK   3  FIT IN THE HIGHEST RESOLUTION BIN.
REMARK   3   TOTAL NUMBER OF BINS USED           : 20
REMARK   3   BIN RESOLUTION RANGE HIGH       (A) : 3.00
REMARK   3   BIN RESOLUTION RANGE LOW        (A) : 3.08
REMARK   3   REFLECTION IN BIN     (WORKING SET) : 745
REMARK   3   BIN COMPLETENESS (WORKING+TEST) (%) : 91.87
REMARK   3   BIN R VALUE           (WORKING SET) : 0.3360
REMARK   3   BIN FREE R VALUE SET COUNT          : 35
REMARK   3   BIN FREE R VALUE                    : 0.2600
REMARK   3
REMARK   3  NUMBER OF NON-HYDROGEN ATOMS USED IN REFINEMENT.
REMARK   3   PROTEIN ATOMS            : 2410
REMARK   3   NUCLEIC ACID ATOMS       : 0
REMARK   3   HETEROGEN ATOMS          : 59
REMARK   3   SOLVENT ATOMS            : 18
REMARK   3
```

Information about crystal structure and refinement

Structure atomic coordinates: pdb file

2ydo.pdb - Blocco note di Windows

File Modifica Formato Visualizza ?

```
REMARK 200
REMARK 200 EXPERIMENTAL DETAILS
REMARK 200  EXPERIMENT TYPE                : X-RAY DIFFRACTION
REMARK 200  DATE OF DATA COLLECTION        : 09-DEC-10
REMARK 200  TEMPERATURE           (KELVIN) : 100
REMARK 200  PH                             : 7.6
REMARK 200  NUMBER OF CRYSTALS USED        : 2
REMARK 200
REMARK 200  SYNCHROTRON              (Y/N) : Y
REMARK 200  RADIATION SOURCE               : DIAMOND
REMARK 200  BEAMLINE                       : I24
REMARK 200  X-RAY GENERATOR MODEL          : NULL
REMARK 200  MONOCHROMATIC OR LAUE    (M/L) : M
REMARK 200  WAVELENGTH OR RANGE        (A) : 0.9778
REMARK 200  MONOCHROMATOR                  : NULL
REMARK 200  OPTICS                         : NULL
REMARK 200
REMARK 200  DETECTOR TYPE                  : PIXEL
REMARK 200  DETECTOR MANUFACTURER          : DECTRIS PILATUS 6M
REMARK 200  INTENSITY-INTEGRATION SOFTWARE : MOSFLM
REMARK 200  DATA SCALING SOFTWARE          : SCALA
REMARK 200
REMARK 200  NUMBER OF UNIQUE REFLECTIONS   : 10556
REMARK 200  RESOLUTION RANGE HIGH      (A) : 3.000
REMARK 200  RESOLUTION RANGE LOW       (A) : 79.340
REMARK 200  REJECTION CRITERIA  (SIGMA(I)) : 0.000
REMARK 200
REMARK 200 OVERALL.
REMARK 200  COMPLETENESS FOR RANGE     (%) : 93.9
REMARK 200  DATA REDUNDANCY                : 2.600
REMARK 200  R MERGE                    (I) : 0.10000
REMARK 200  R SYM                      (I) : NULL
REMARK 200  <I/SIGMA(I)> FOR THE DATA SET  : 7.1000
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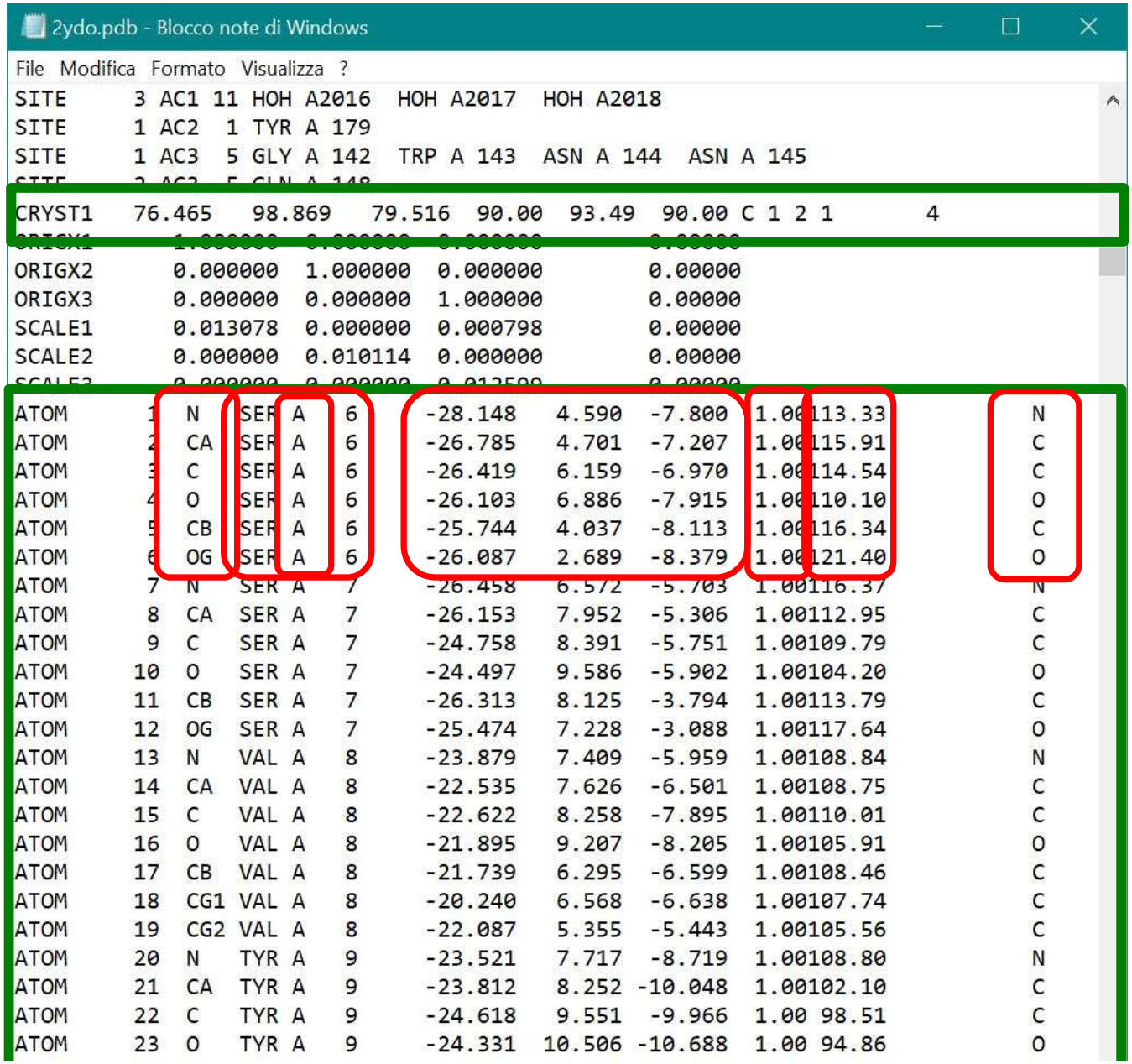
Information about the crystallographic experiment

Structure atomic coordinates: pdb file



2ydo.pdb - Blocco note di Windows

File Modifica Formato Visualizza ?

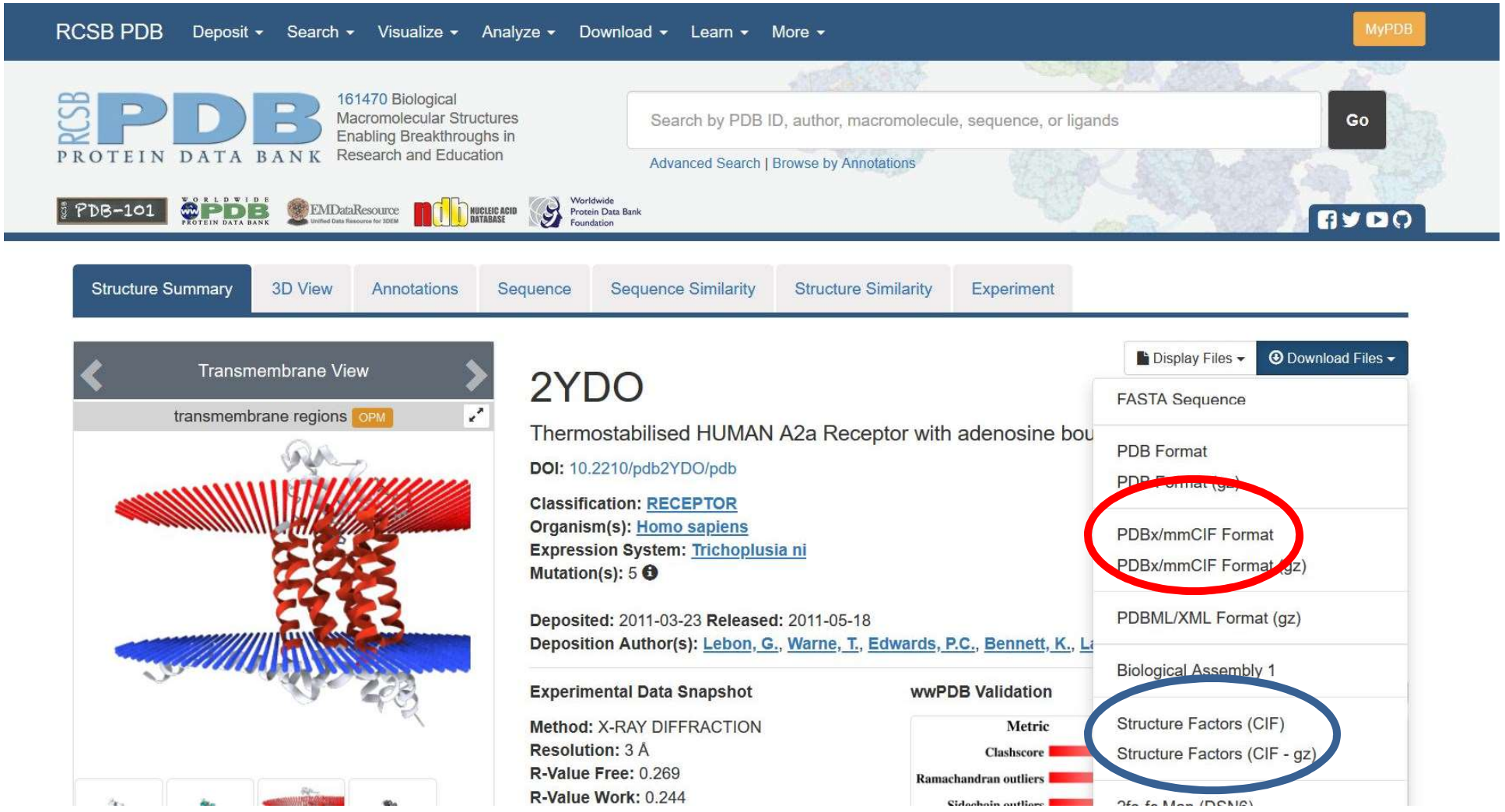
```
SITE     3 AC1 11 HOH A2016  HOH A2017  HOH A2018
SITE     1 AC2  1 TYR A 179
SITE     1 AC3  5 GLY A 142  TRP A 143  ASN A 144  ASN A 145
SITE     2 AC3  5 GLN A 148
CRYST1   76.465   98.869   79.516  90.00  93.49  90.00 C 1 2 1       4
ORIGX1      1.000000  0.000000  0.000000        0.00000
ORIGX2      0.000000  1.000000  0.000000        0.00000
ORIGX3      0.000000  0.000000  1.000000        0.00000
SCALE1      0.013078  0.000000  0.000798        0.00000
SCALE2      0.000000  0.010114  0.000000        0.00000
SCALE3      0.000000  0.000000  0.012599        0.00000
ATOM      1  N   SER A   6     -28.148   4.590  -7.800  1.00113.33           N
ATOM      2  CA  SER A   6     -26.785   4.701  -7.207  1.00115.91           C
ATOM      3  C   SER A   6     -26.419   6.159  -6.970  1.00114.54           C
ATOM      4  O   SER A   6     -26.103   6.886  -7.915  1.00110.10           O
ATOM      5  CB  SER A   6     -25.744   4.037  -8.113  1.00116.34           C
ATOM      6  OG  SER A   6     -26.087   2.689  -8.379  1.00121.40           O
ATOM      7  N   SER A   7     -26.458   6.572  -5.703  1.00116.37           N
ATOM      8  CA  SER A   7     -26.153   7.952  -5.306  1.00112.95           C
ATOM      9  C   SER A   7     -24.758   8.391  -5.751  1.00109.79           C
ATOM     10  O   SER A   7     -24.497   9.586  -5.902  1.00104.20           O
ATOM     11  CB  SER A   7     -26.313   8.125  -3.794  1.00113.79           C
ATOM     12  OG  SER A   7     -25.474   7.228  -3.088  1.00117.64           O
ATOM     13  N   VAL A   8     -23.879   7.409  -5.959  1.00108.84           N
ATOM     14  CA  VAL A   8     -22.535   7.626  -6.501  1.00108.75           C
ATOM     15  C   VAL A   8     -22.622   8.258  -7.895  1.00110.01           C
ATOM     16  O   VAL A   8     -21.895   9.207  -8.205  1.00105.91           O
ATOM     17  CB  VAL A   8     -21.739   6.295  -6.599  1.00108.46           C
ATOM     18  CG1 VAL A   8     -20.240   6.568  -6.638  1.00107.74           C
ATOM     19  CG2 VAL A   8     -22.087   5.355  -5.443  1.00105.56           C
ATOM     20  N   TYR A   9     -23.521   7.717  -8.719  1.00108.80           N
ATOM     21  CA  TYR A   9     -23.812   8.252 -10.048  1.00102.10           C
ATOM     22  C   TYR A   9     -24.618   9.551  -9.966  1.00 98.51           C
ATOM     23  O   TYR A   9     -24.331  10.506 -10.688  1.00 94.86           O
```

Unit cell and symmetry

List of atoms with:

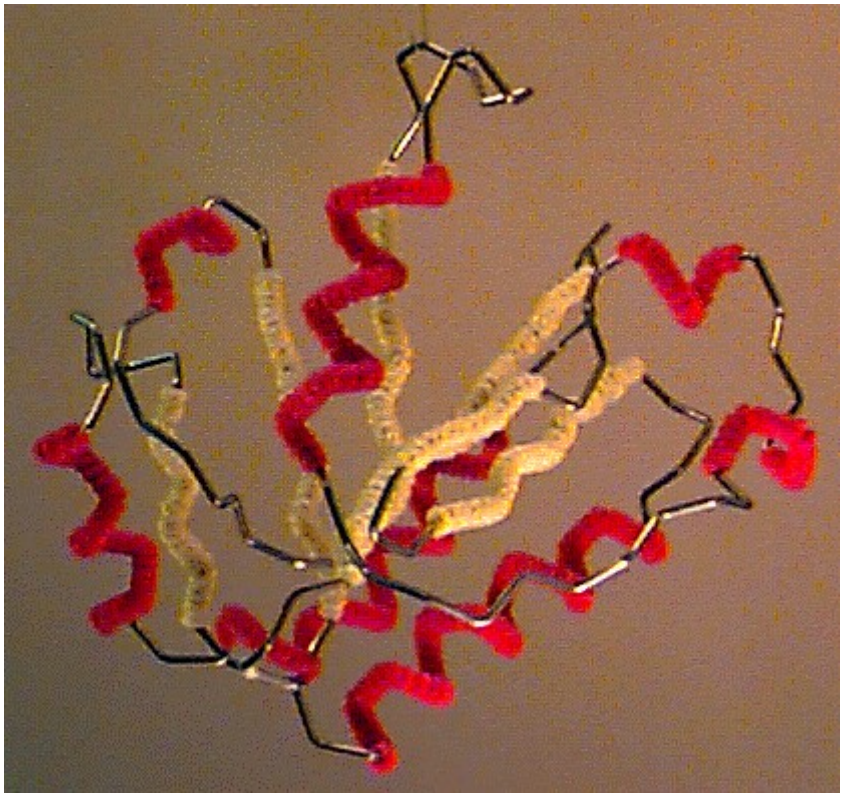
1. **Atom name**
2. **Residue type and number**
3. **Chain**
4. **Cartesian coordinates**
5. **Occupancy**
6. **Thermal factor**
7. **Atom type**

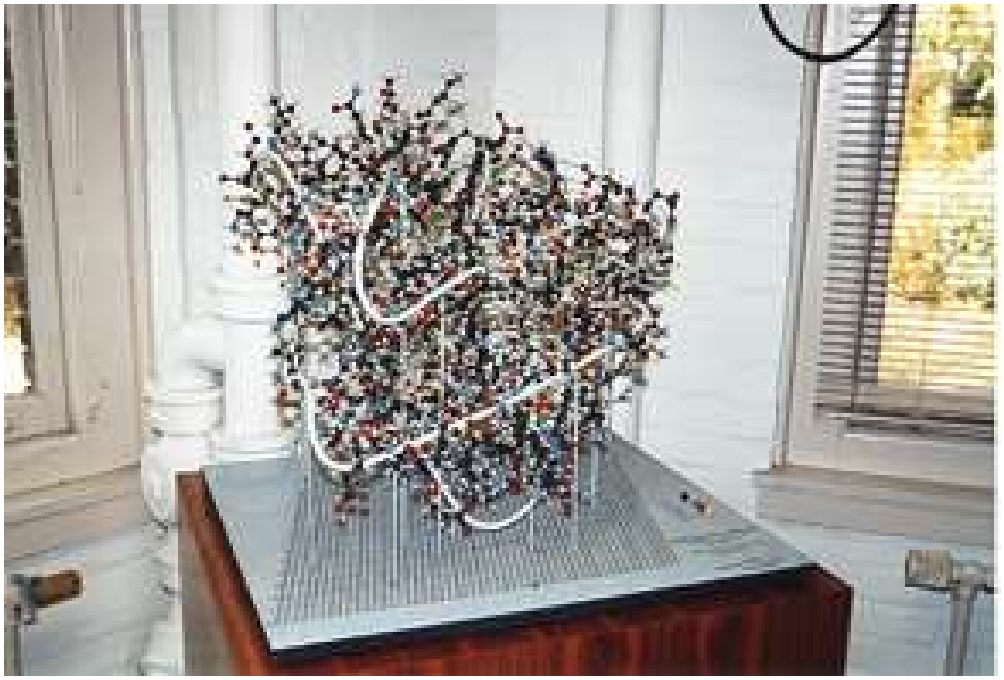
Structure atomic coordinates: mmCIF file



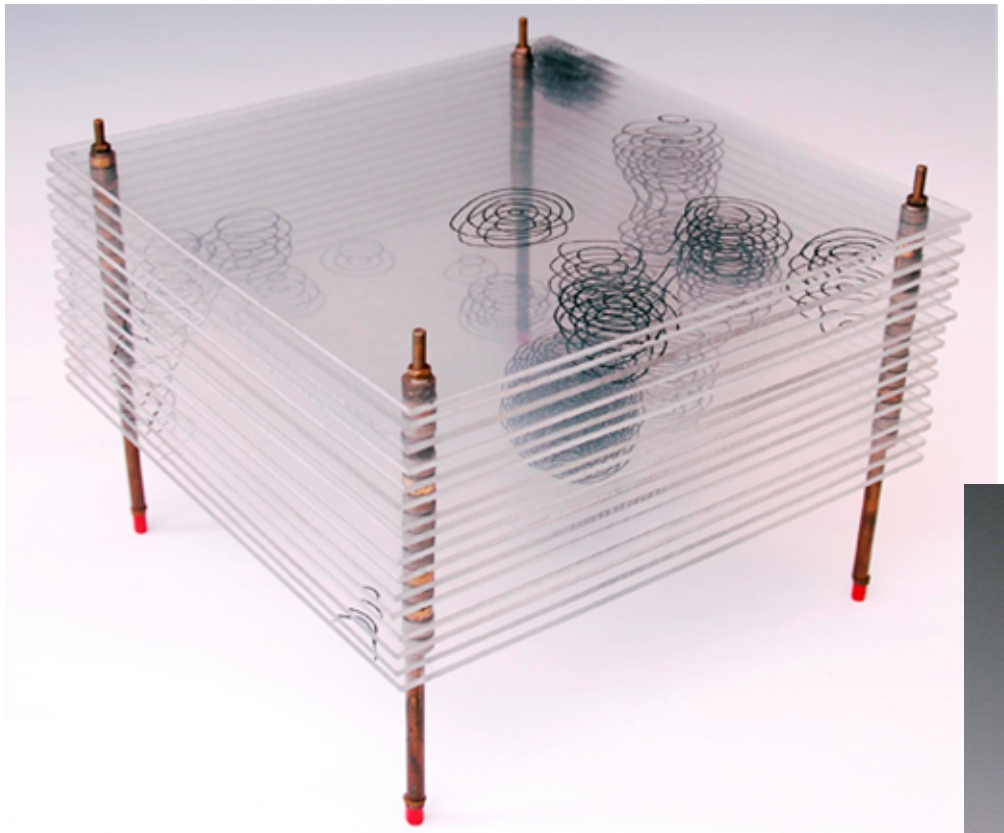
for the electron density!!!

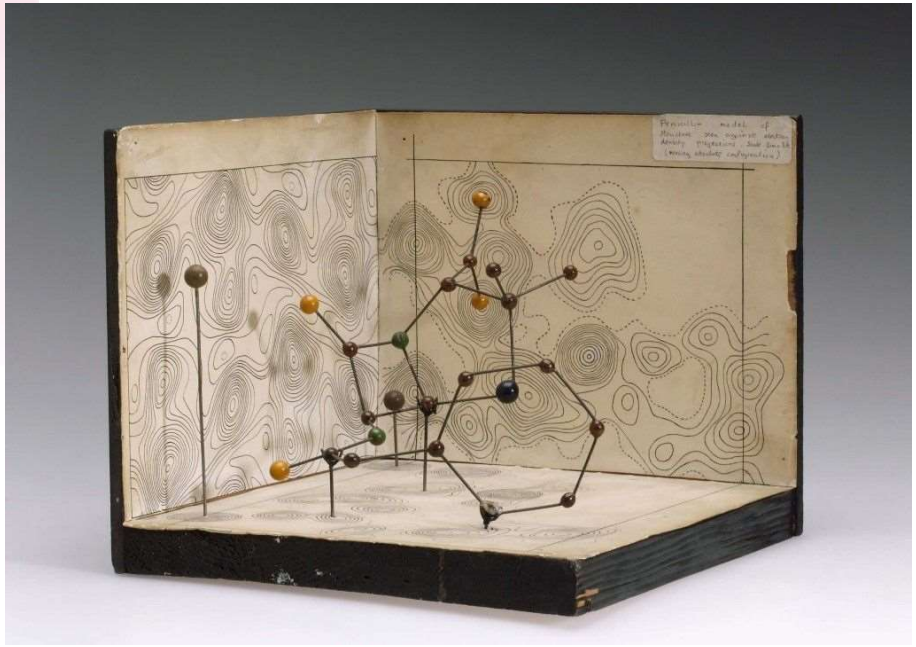
Graphical software: in the old days...





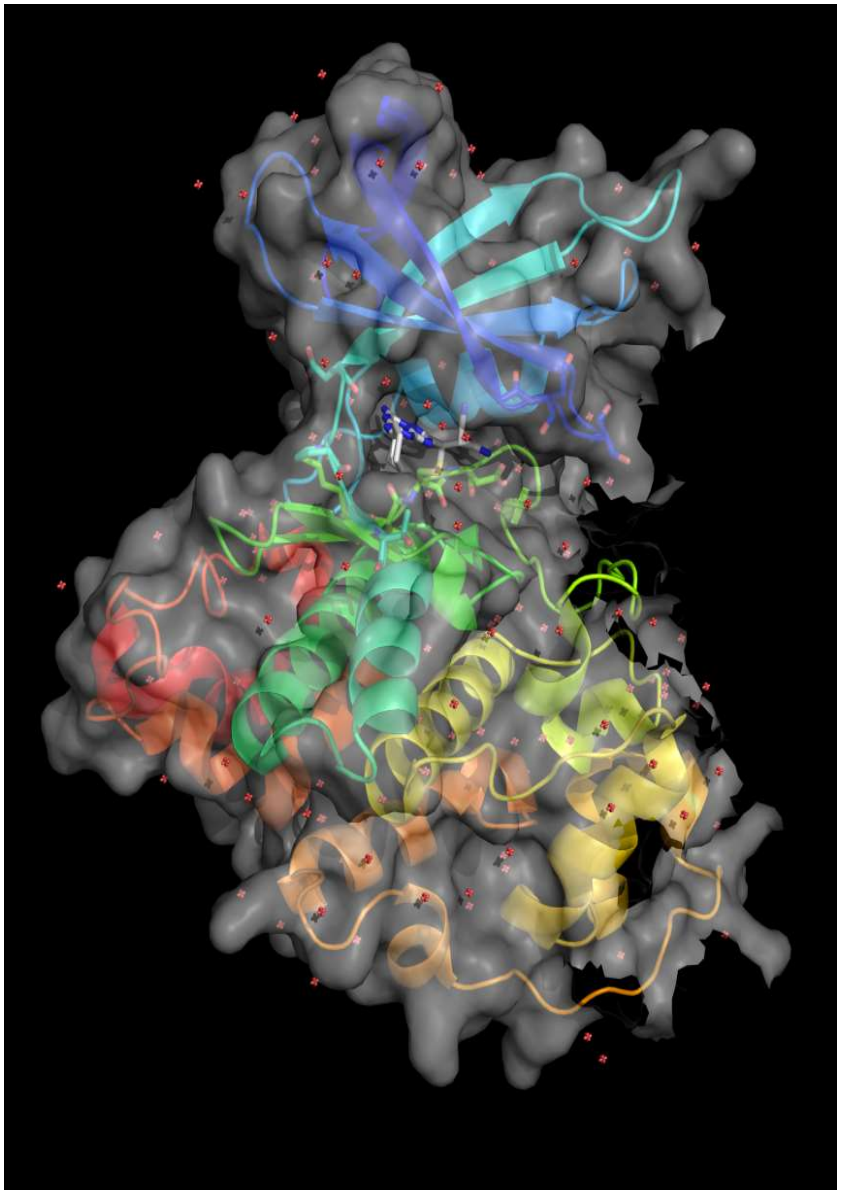
Graphical software: in the old days...

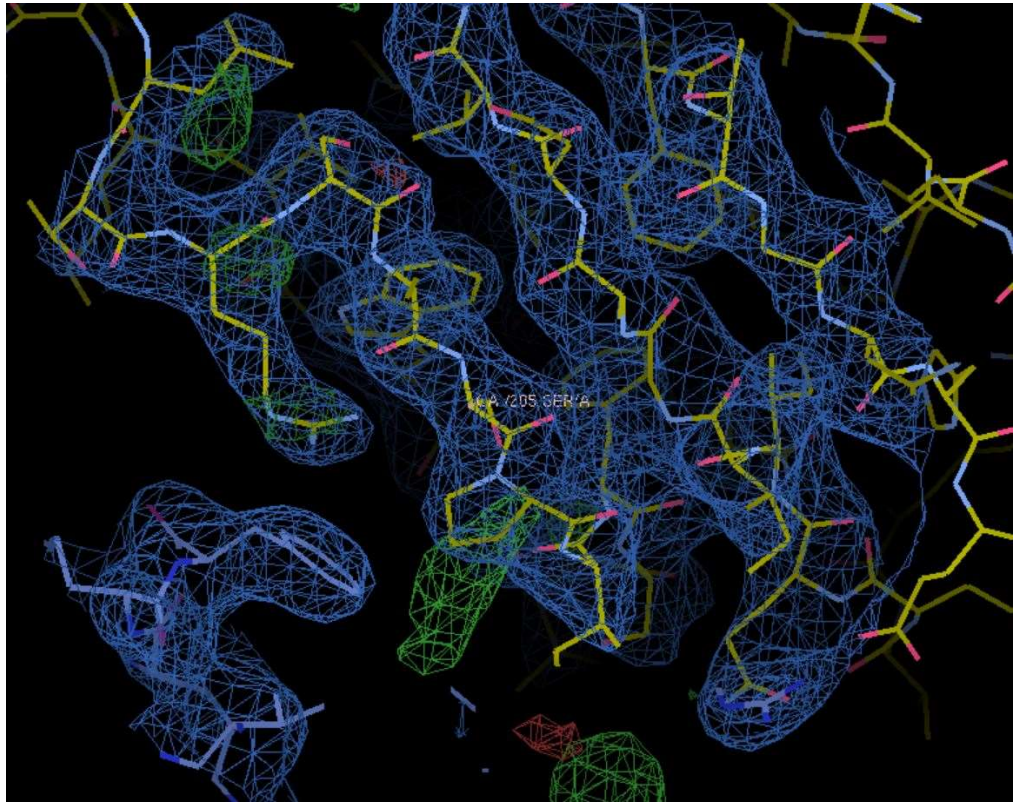




Graphical software: in the old days...

Graphical software:... today





Graphical software:... today

Graphical software: Pymol

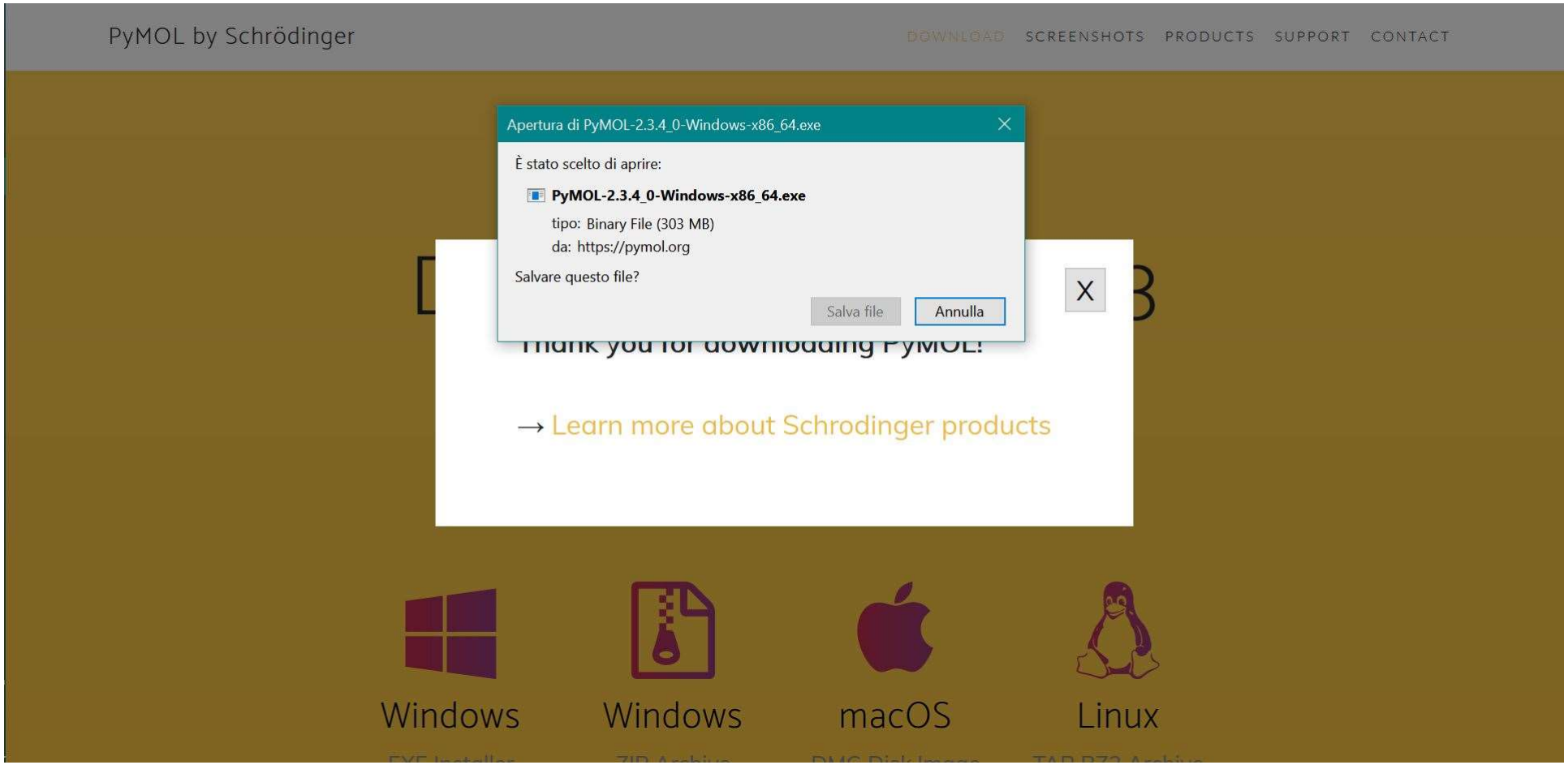
1. Download: https://pymol.org/2/

Graphical software: Pymol

1. Download: https://pymol.org/2/

Graphical software: Pymol

1. Download: https://pymol.org/2/



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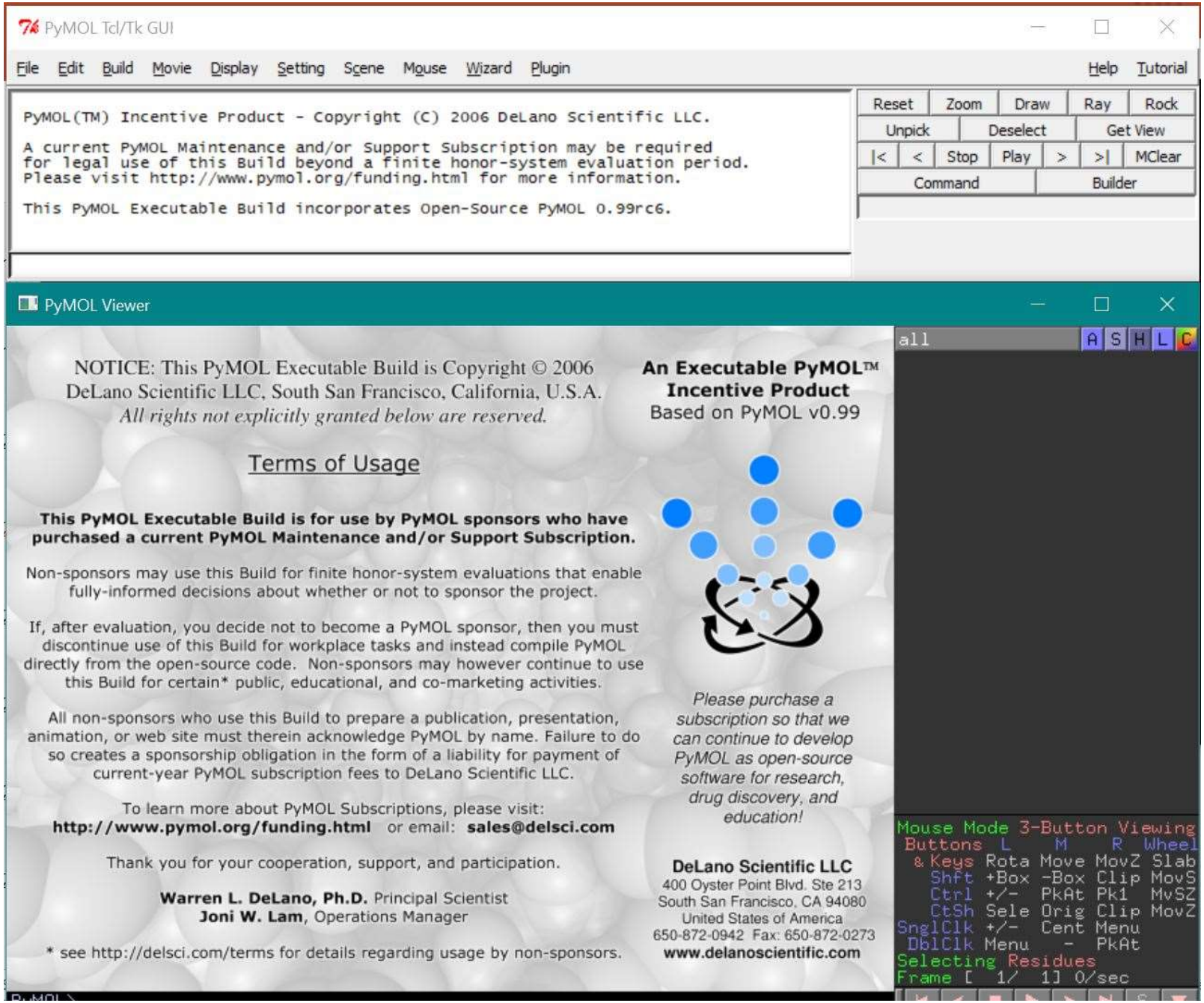

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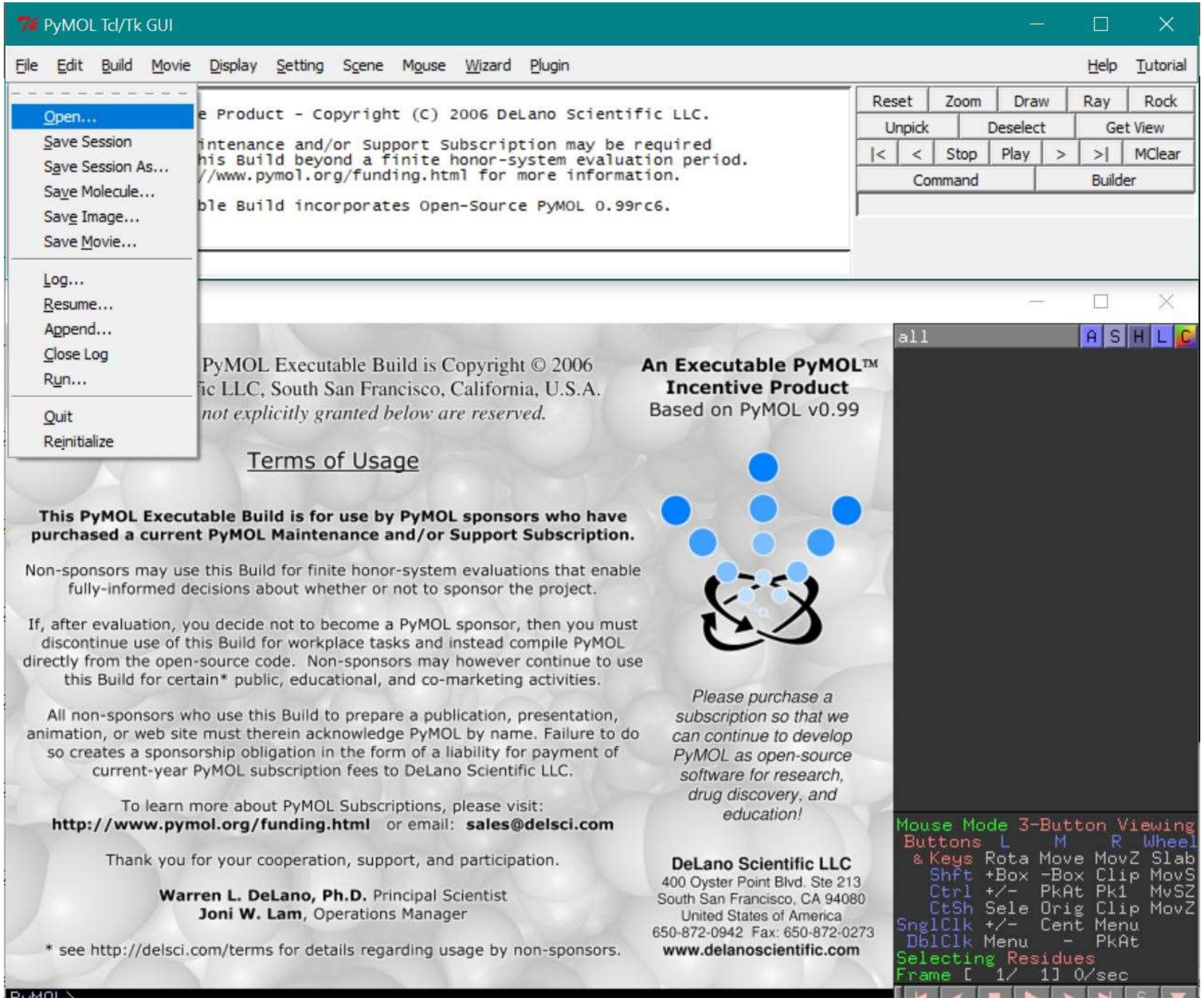

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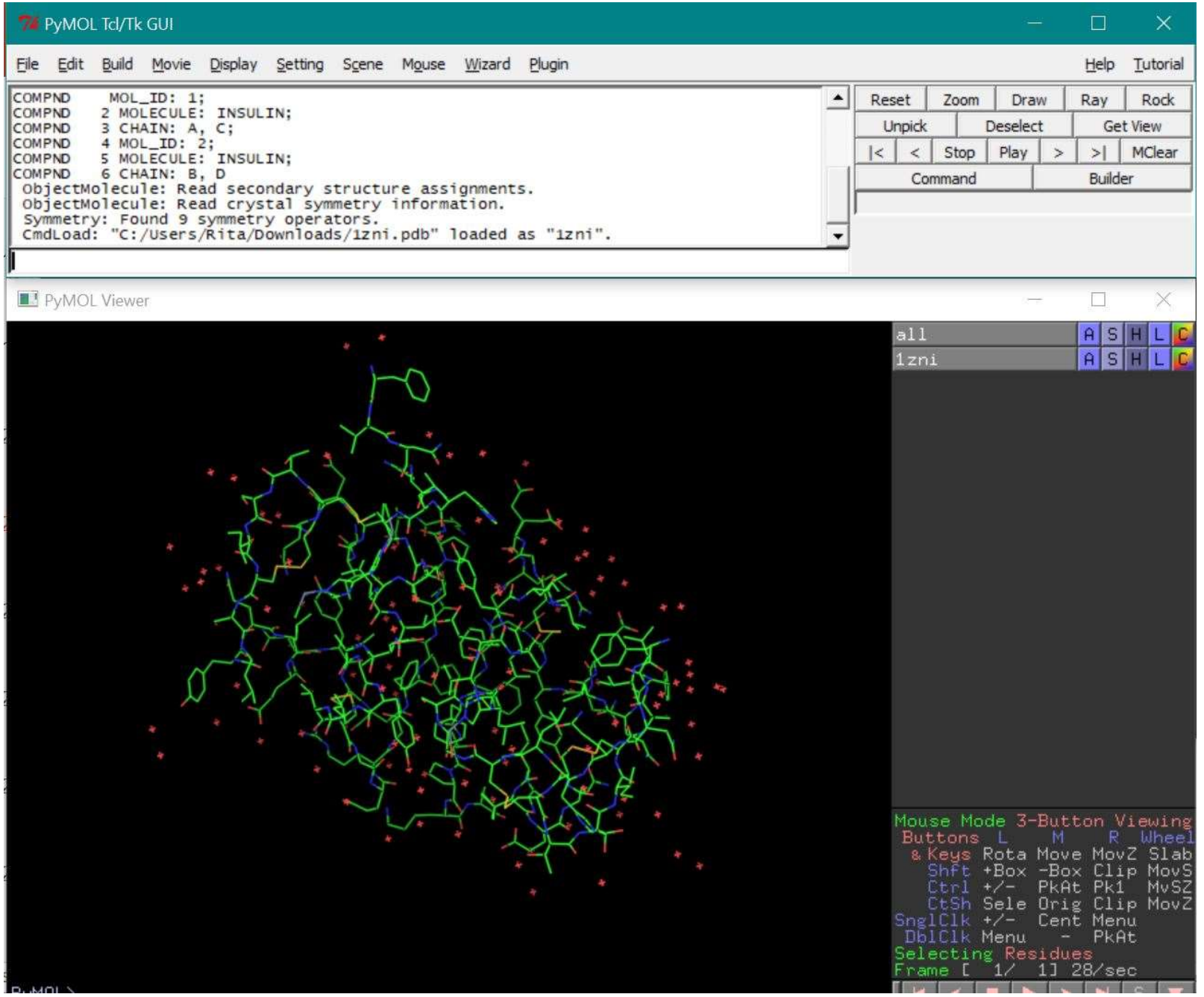

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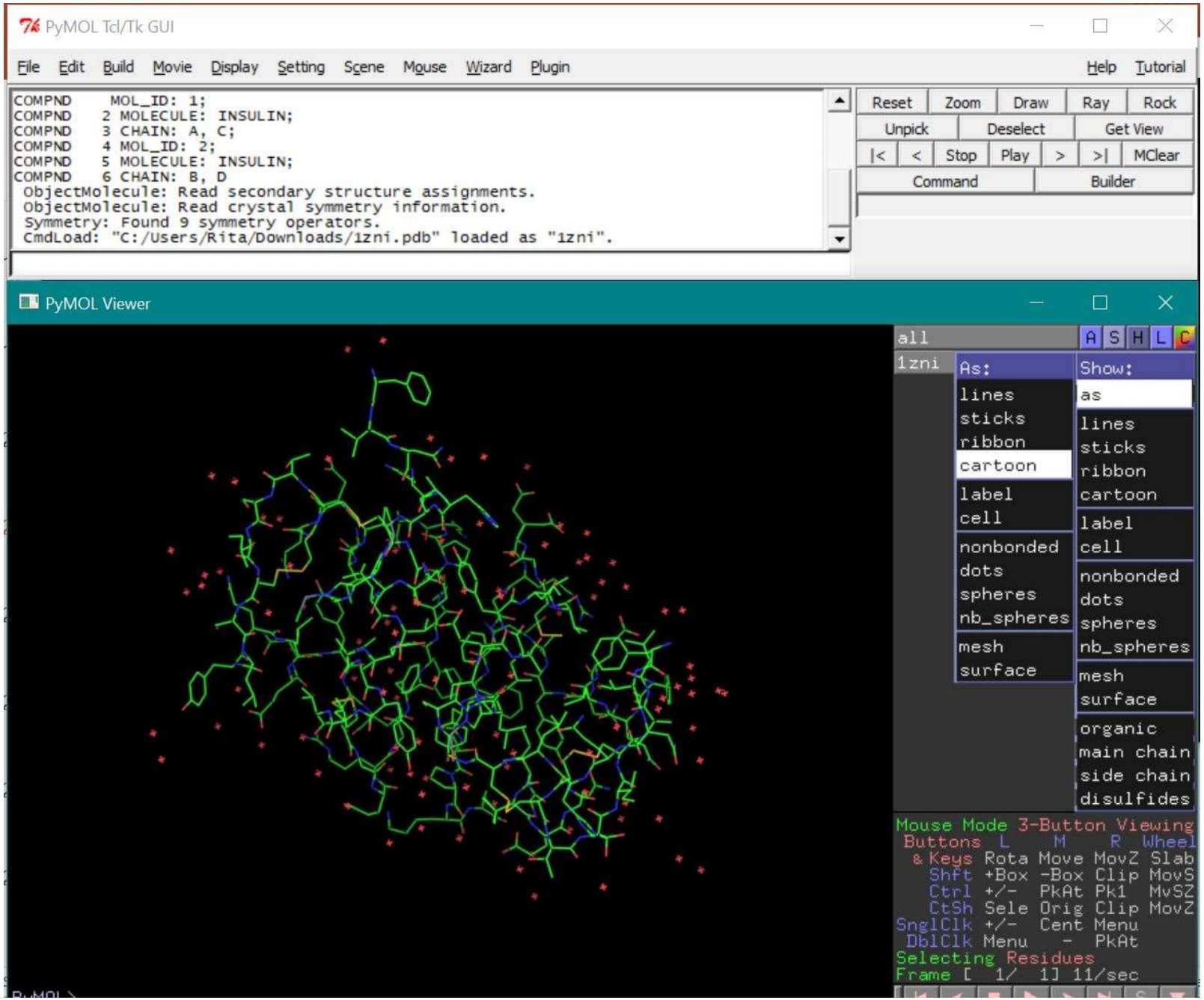
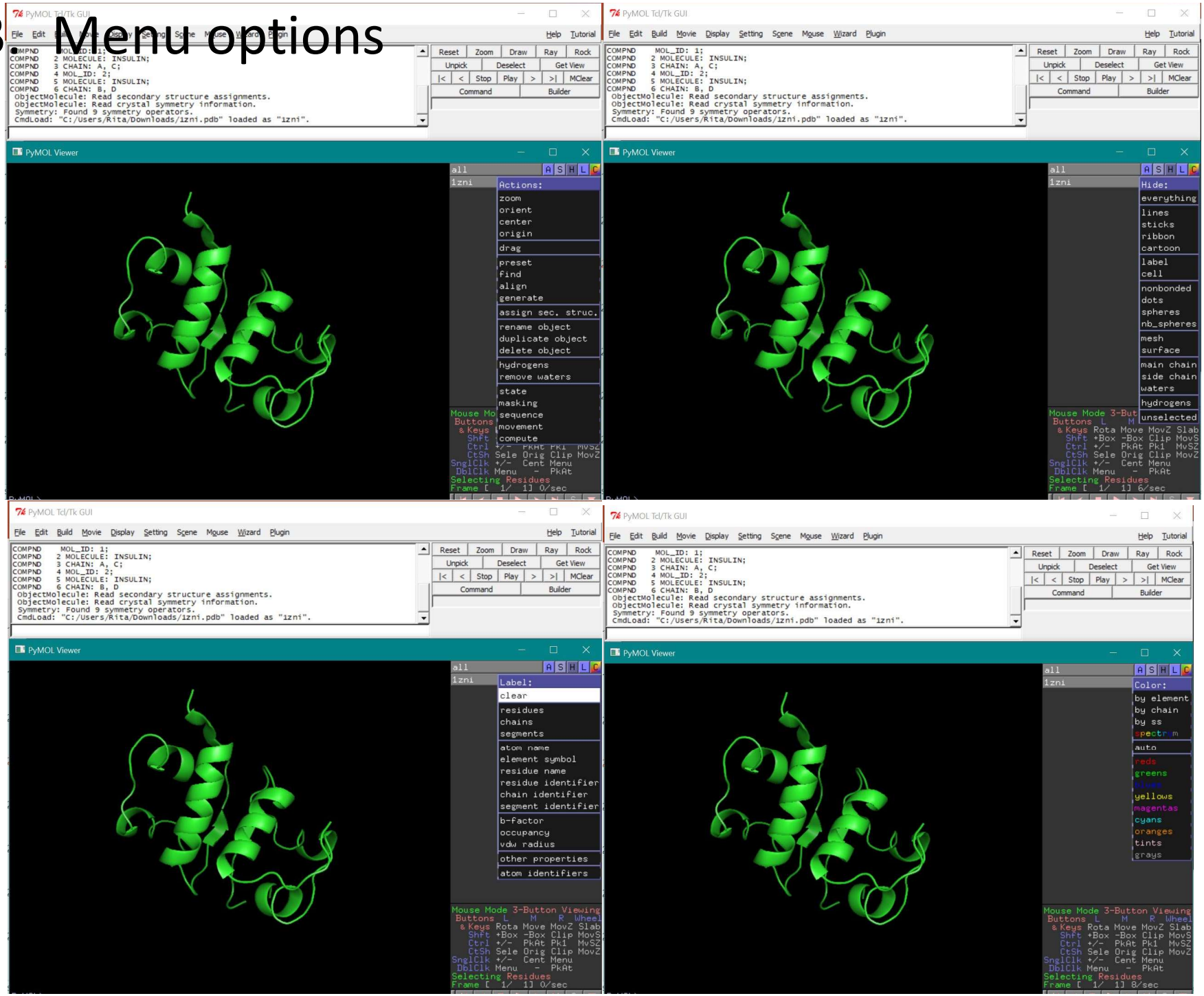
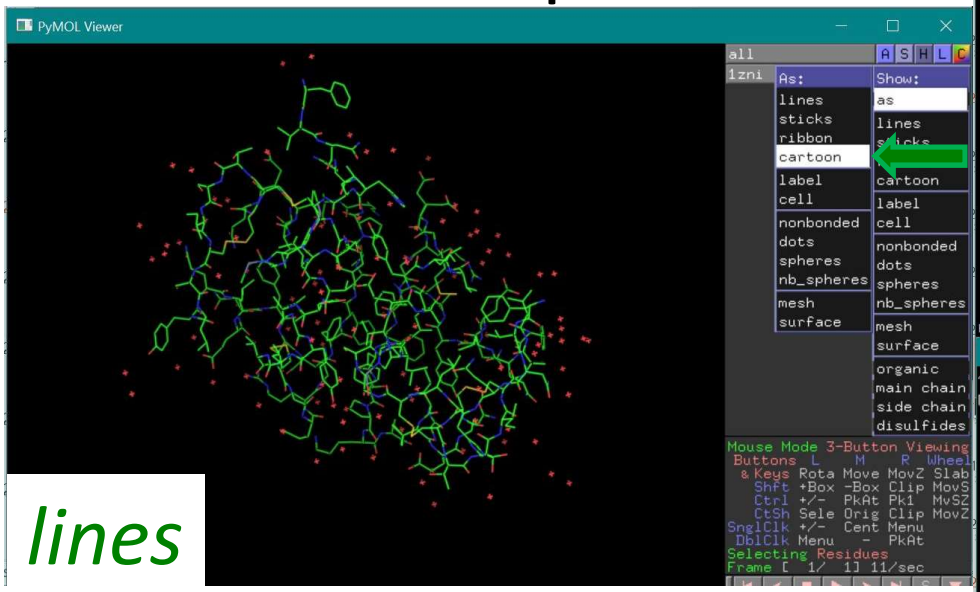
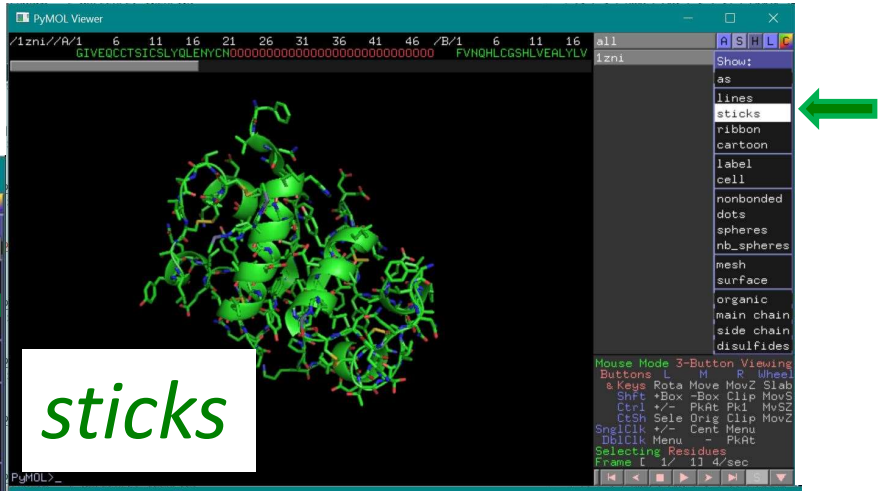


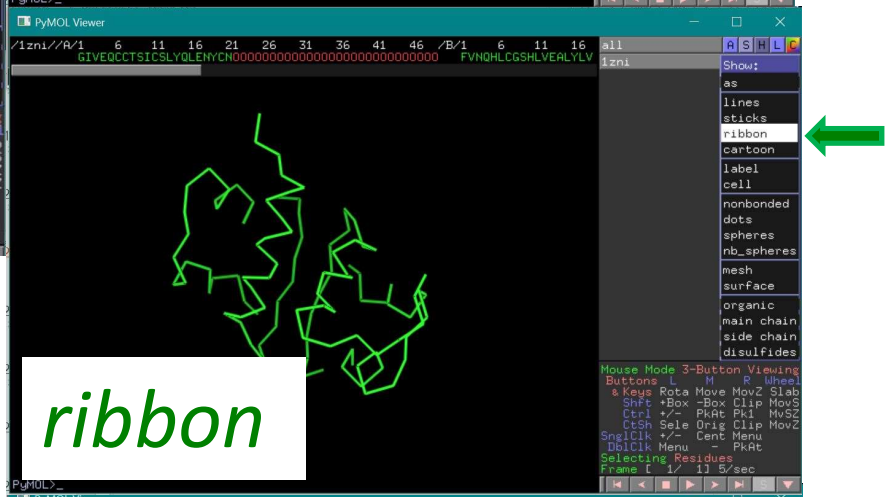
3 Menu options

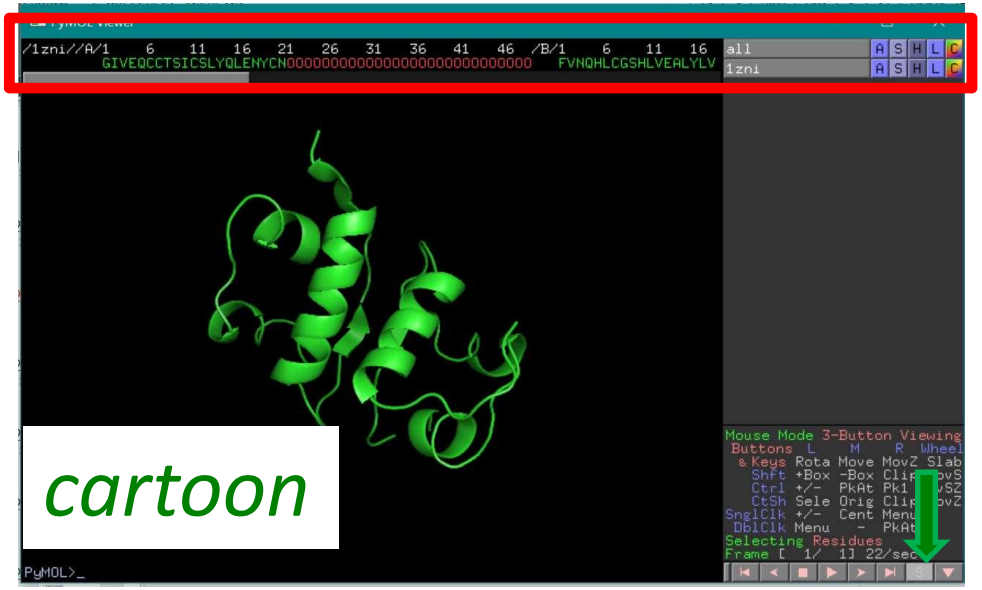


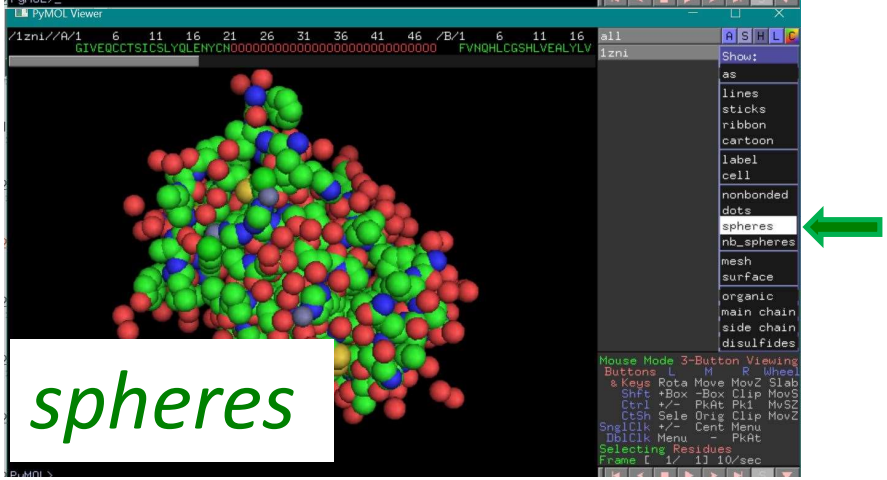
Visualization options:





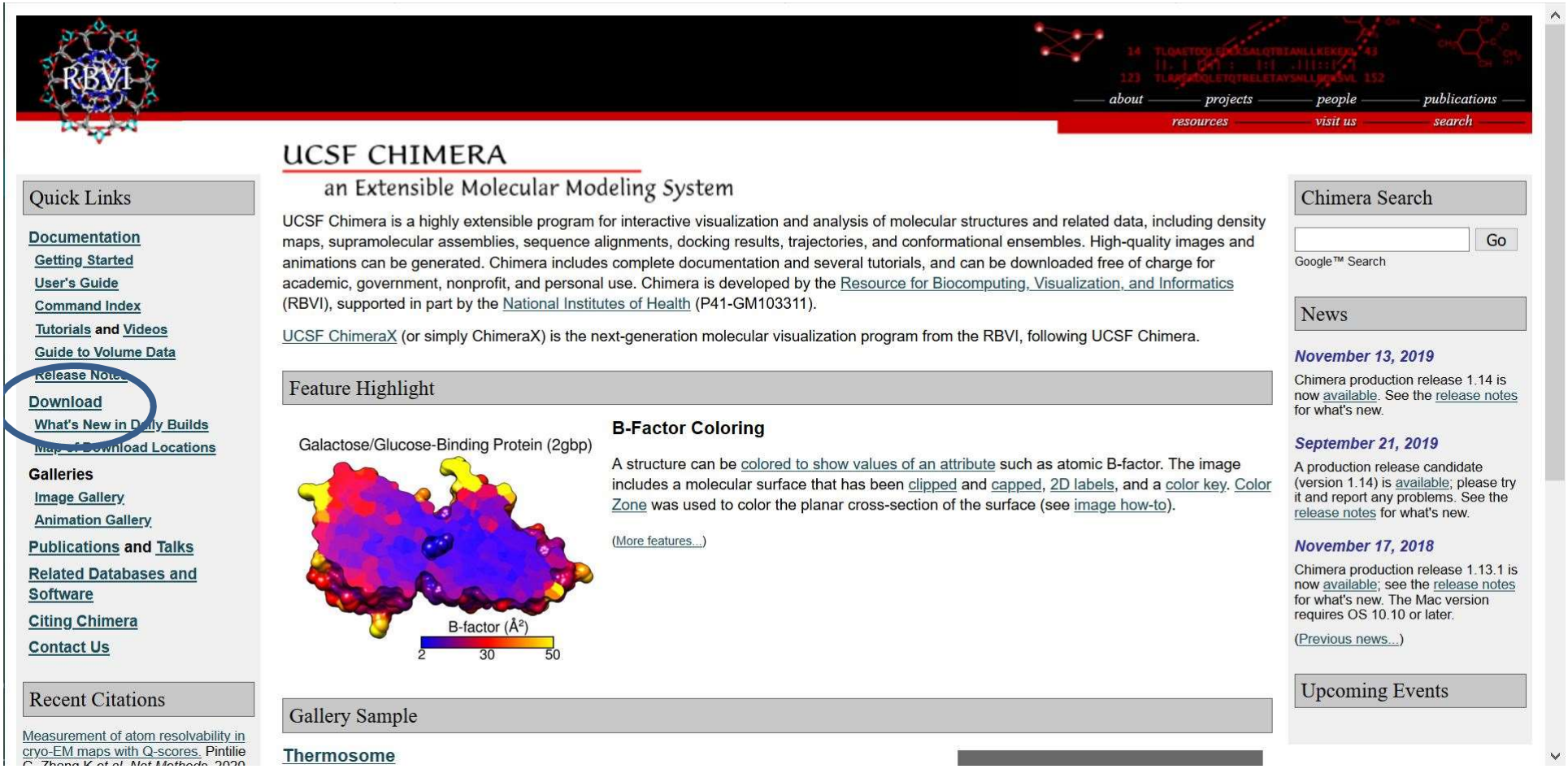






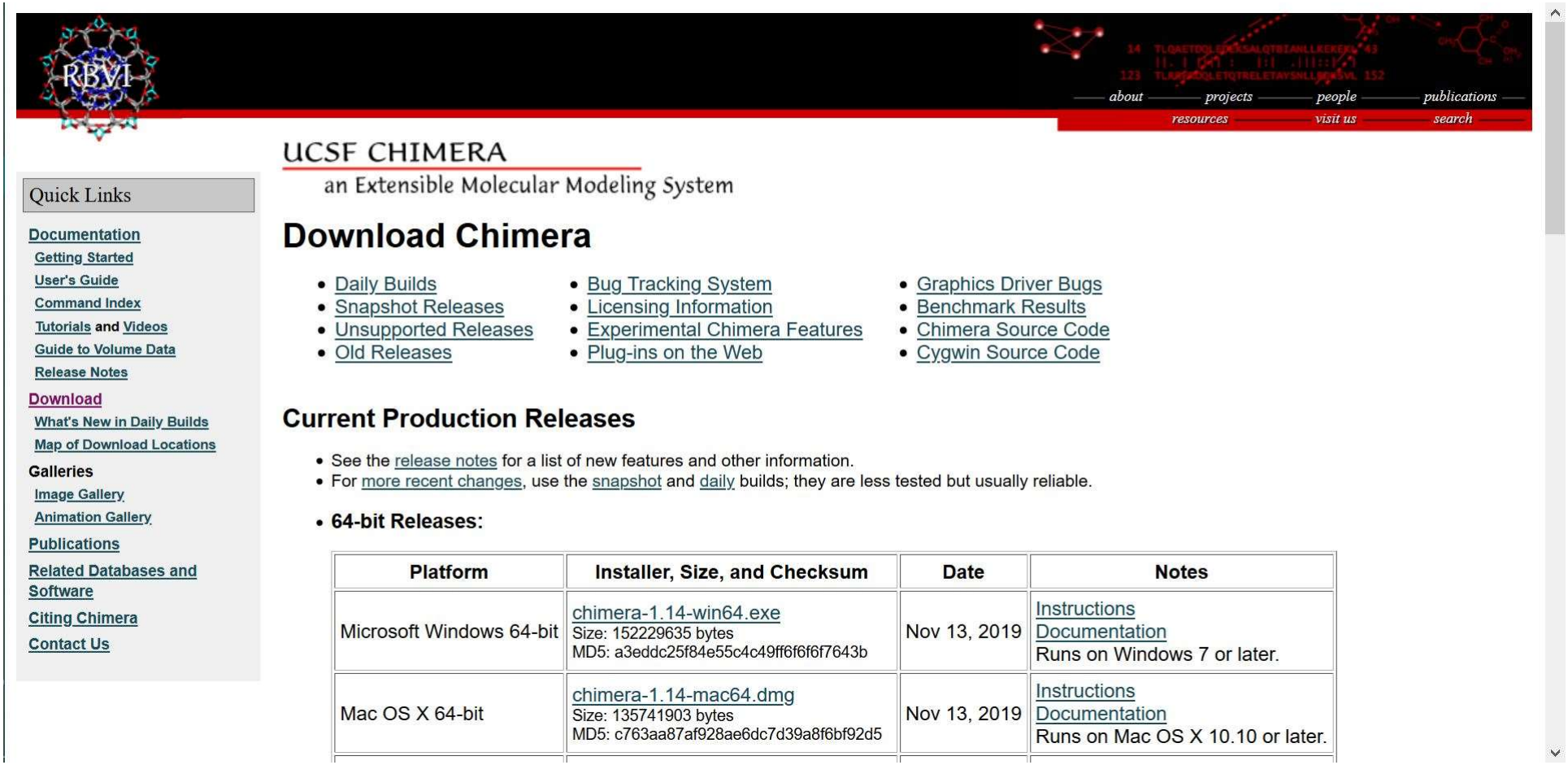
Graphical software: UCSF Chimera

1. Download:www.cgl.ucsf.edu/chimera/

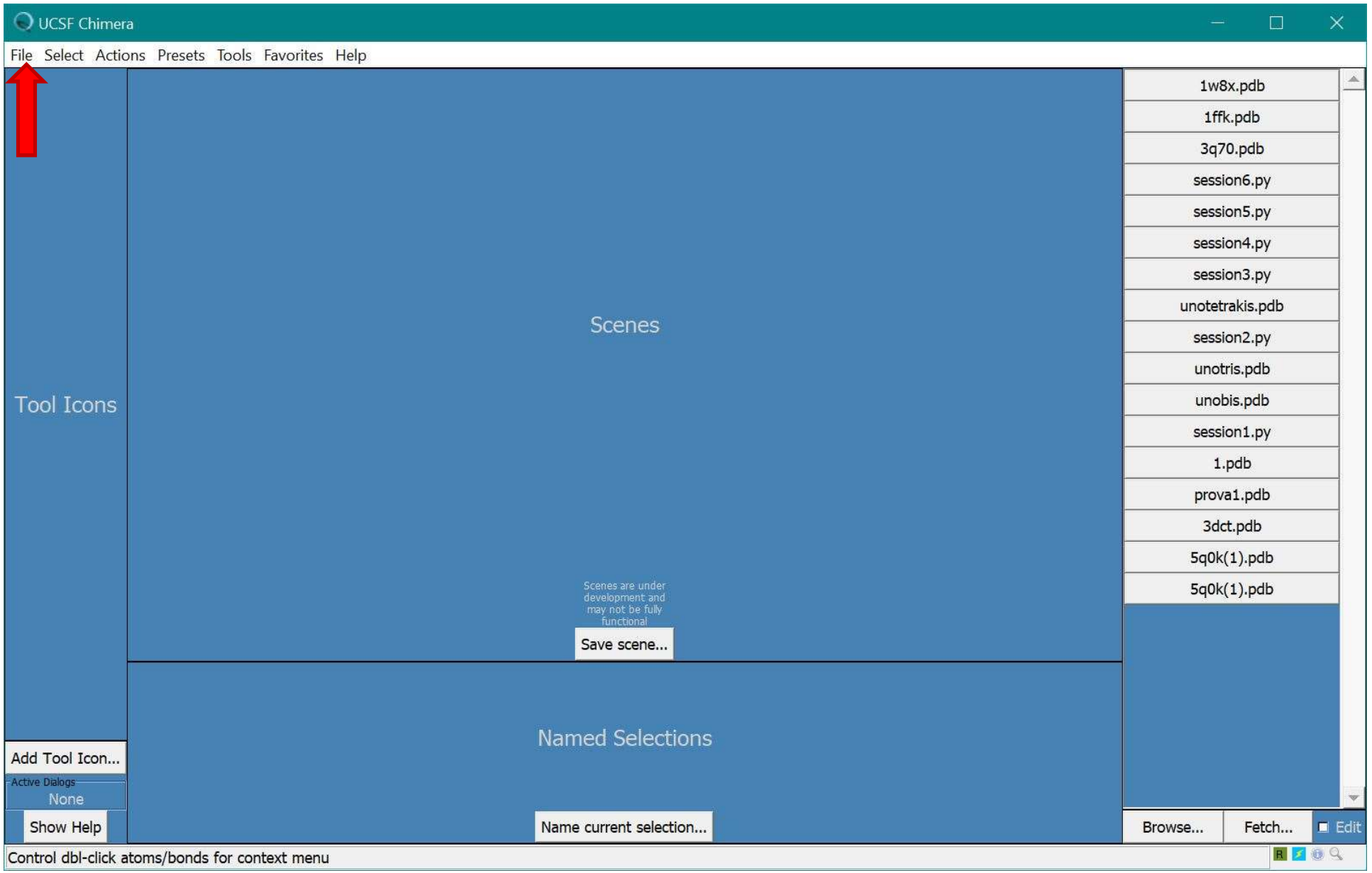


Graphical software: UCSF Chimera

1. Download:www.cgl.ucsf.edu/chimera/



2. Open and load pdb file



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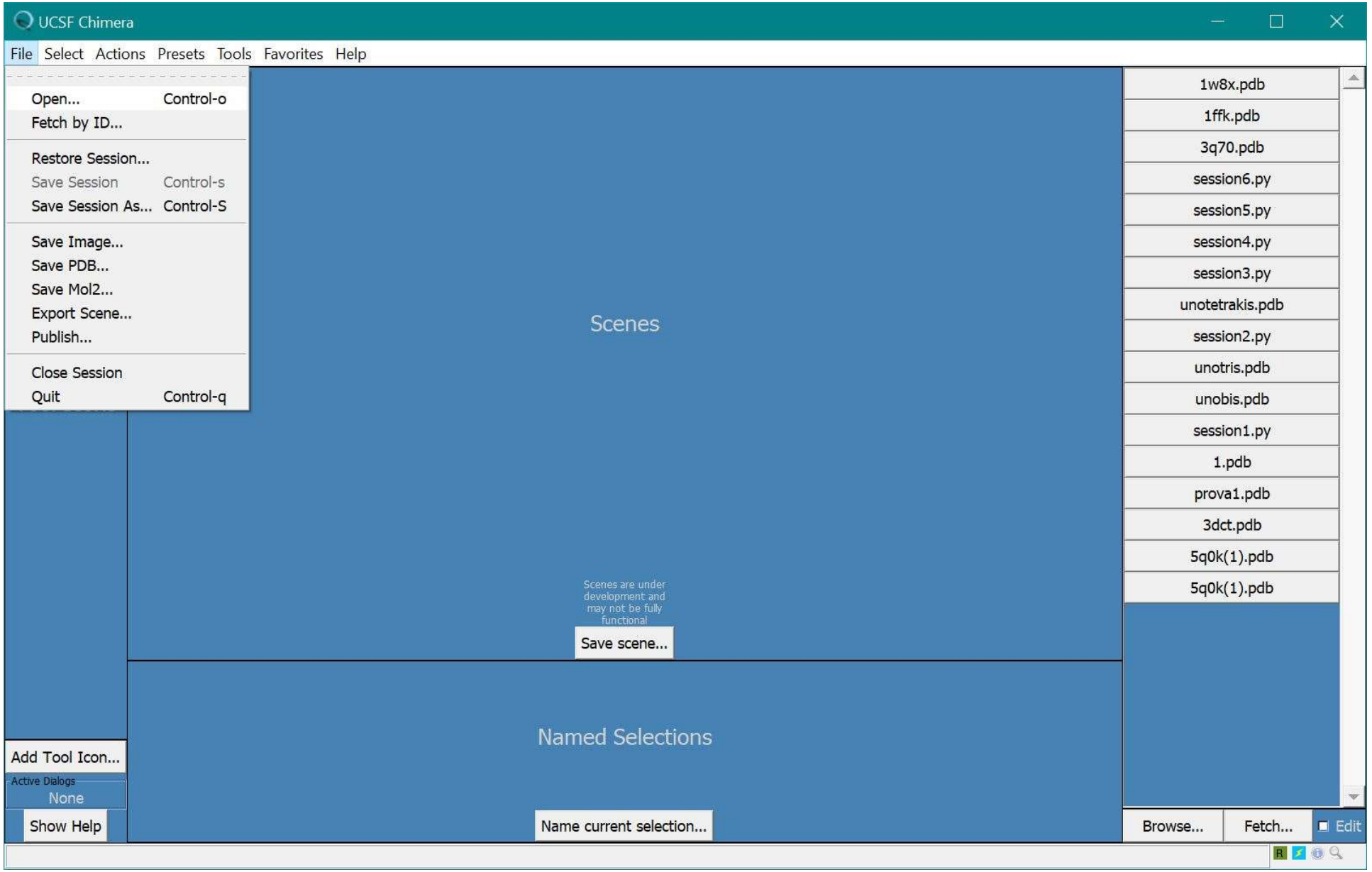
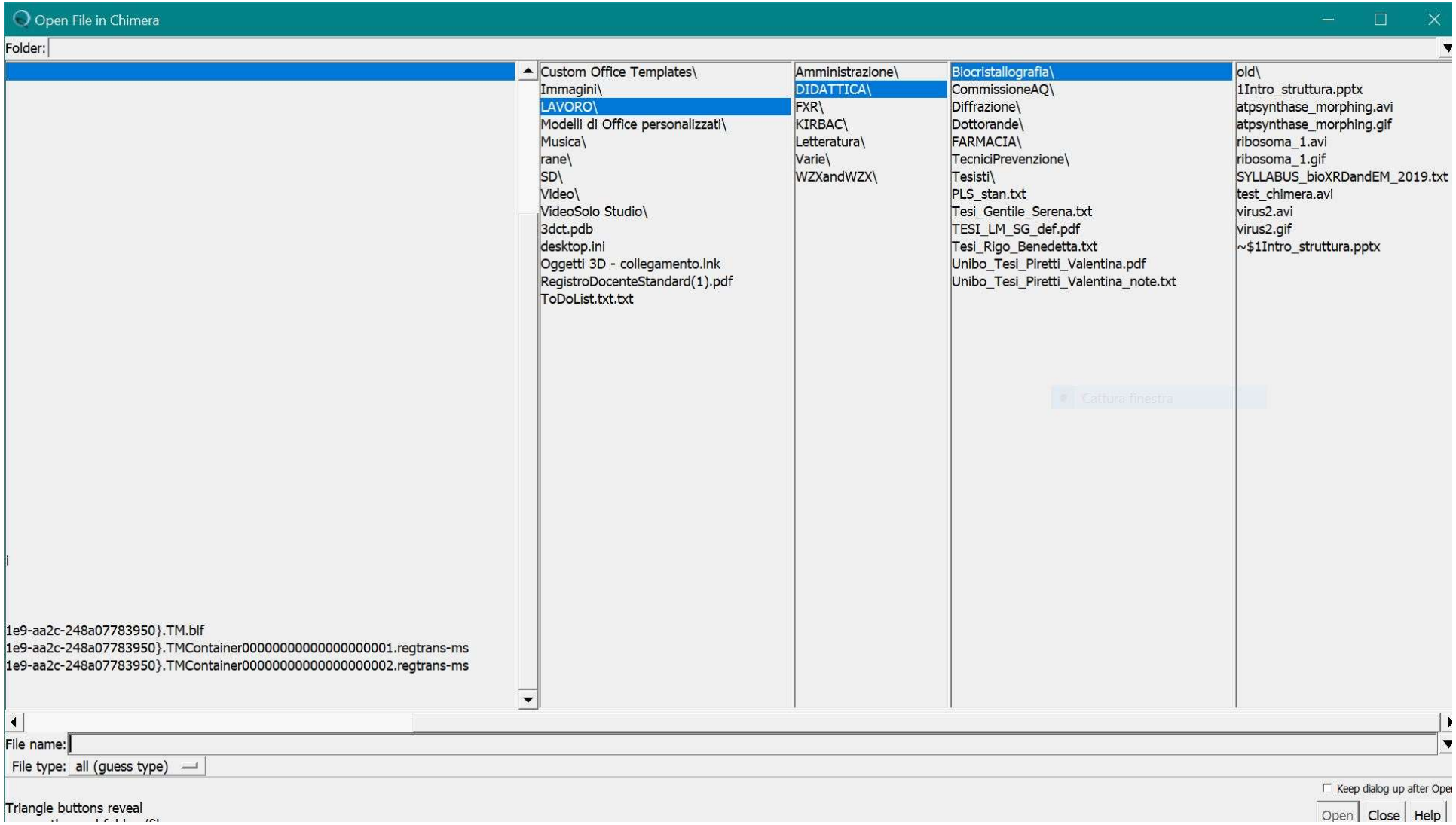


2. Open and load pdb file



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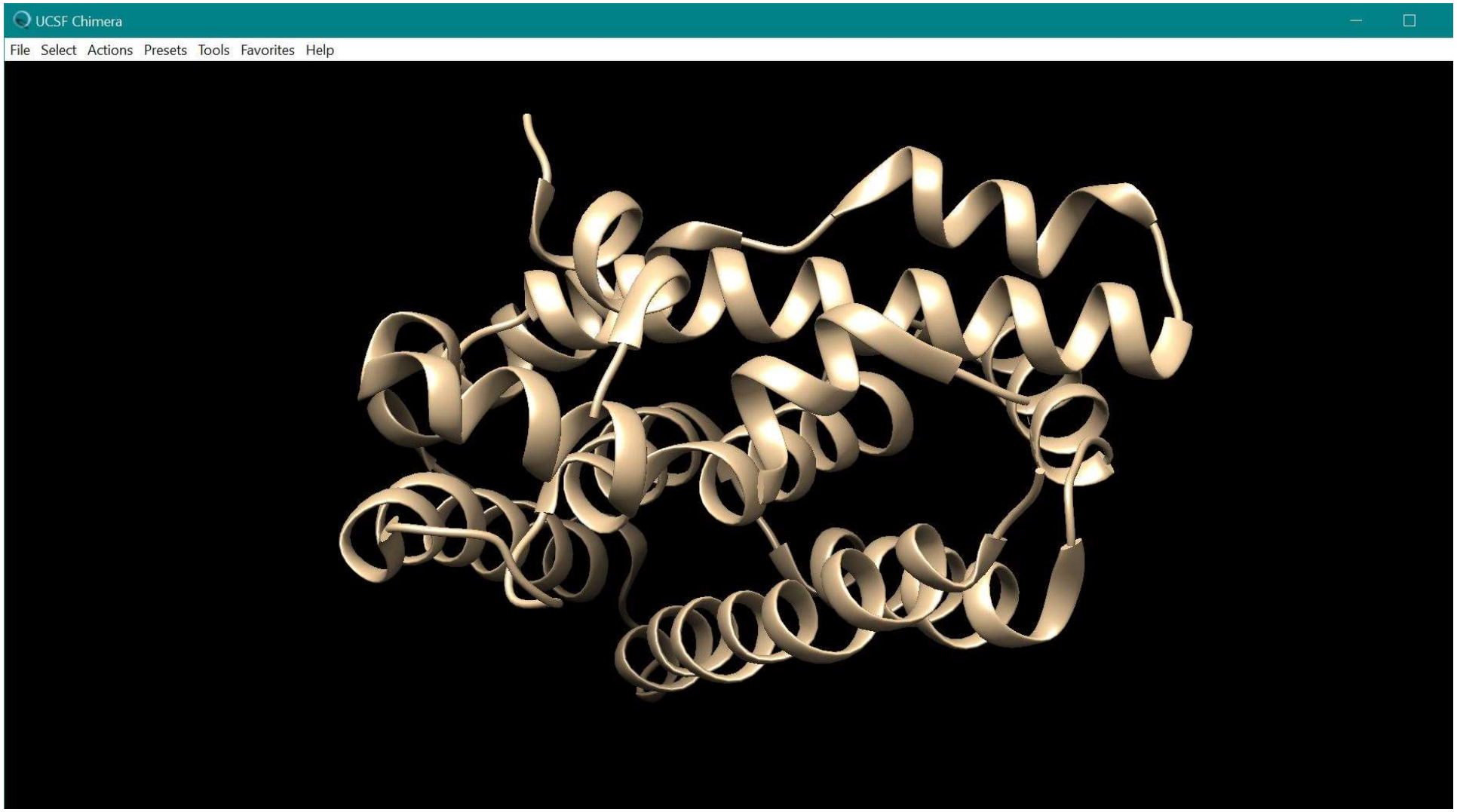
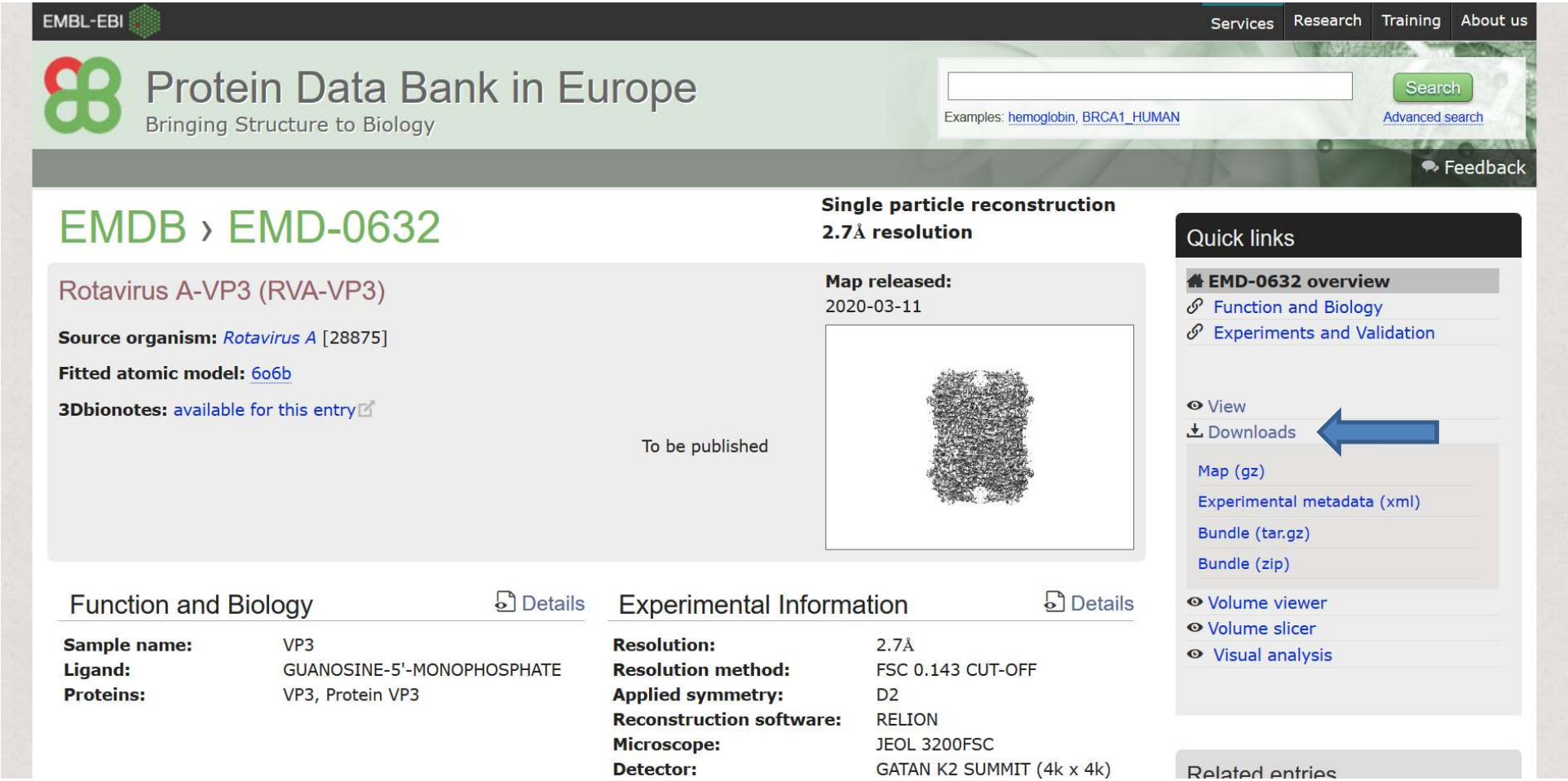
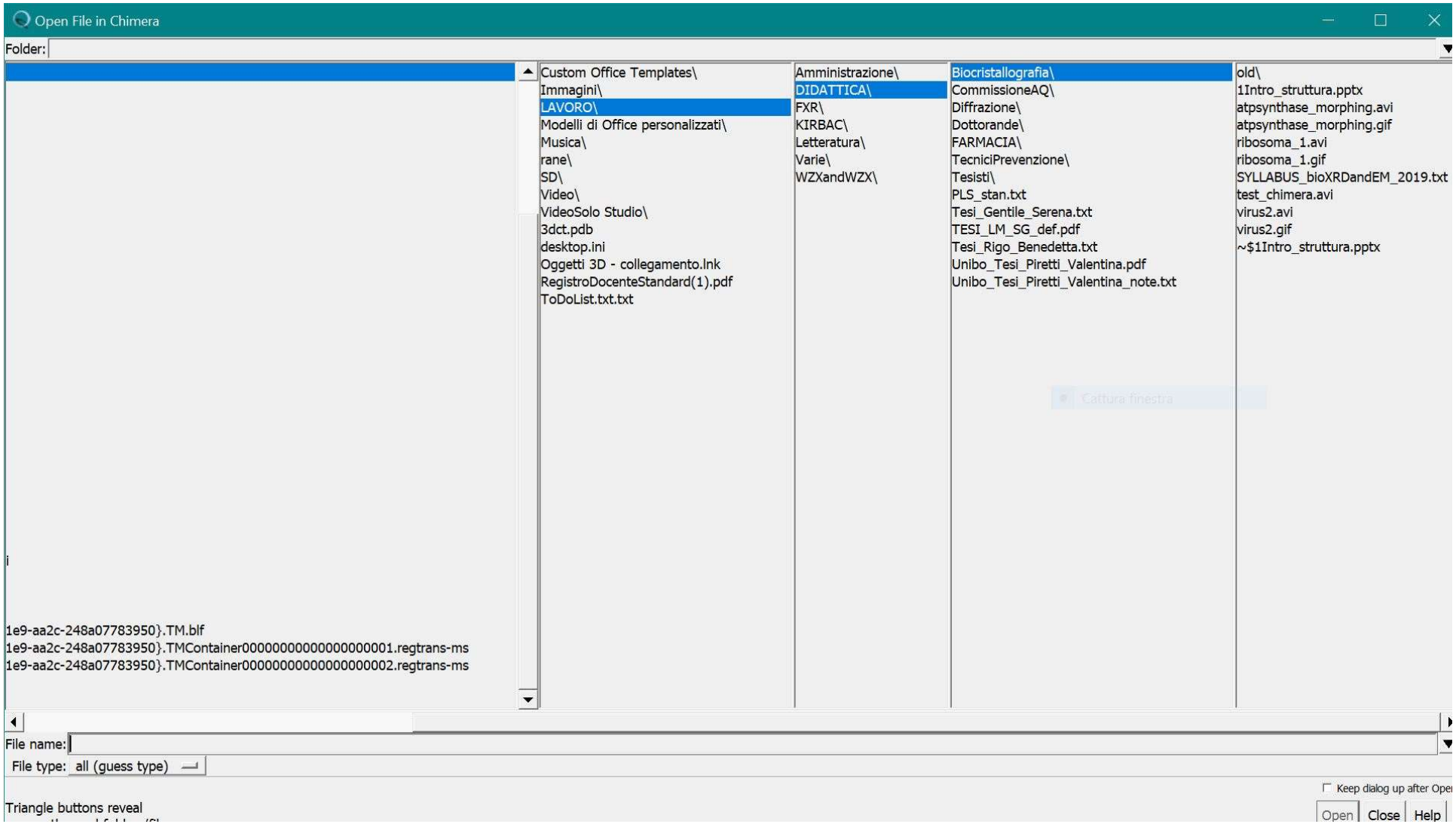


3. Load a map file (electron density!)

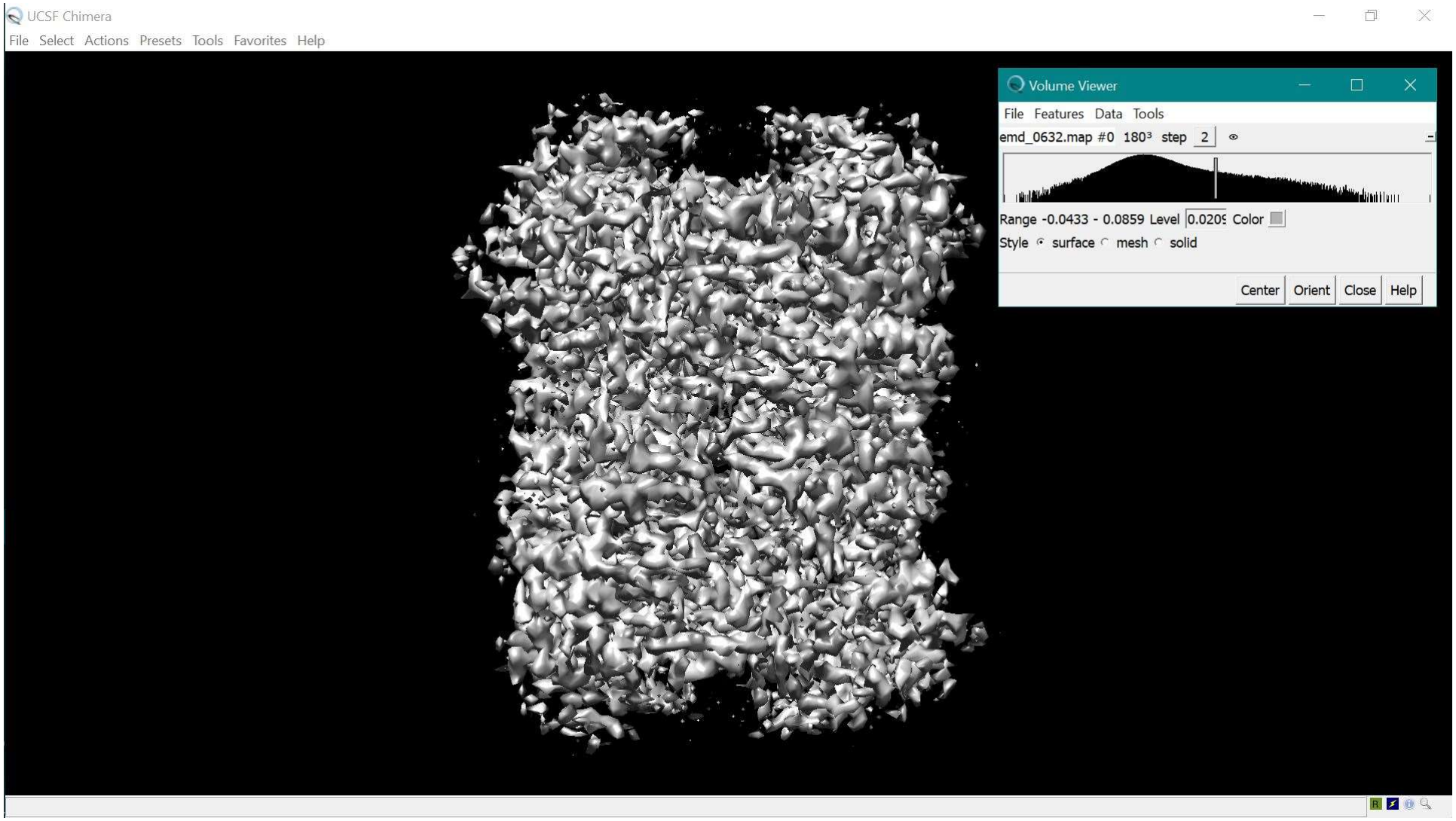
(www.ebi.ac.uk/pdbe/emdb/)



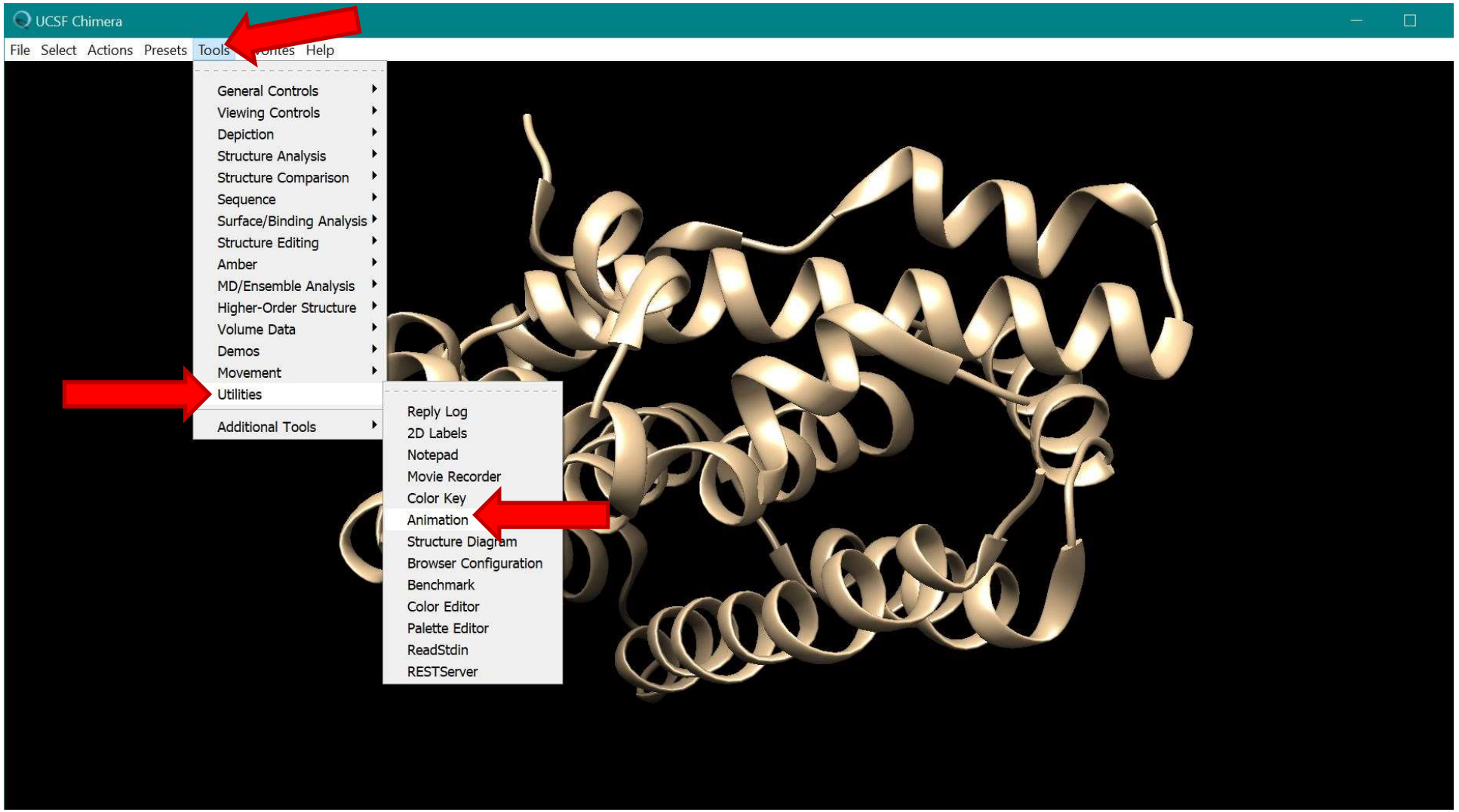
3. Load a map file (electron density!)



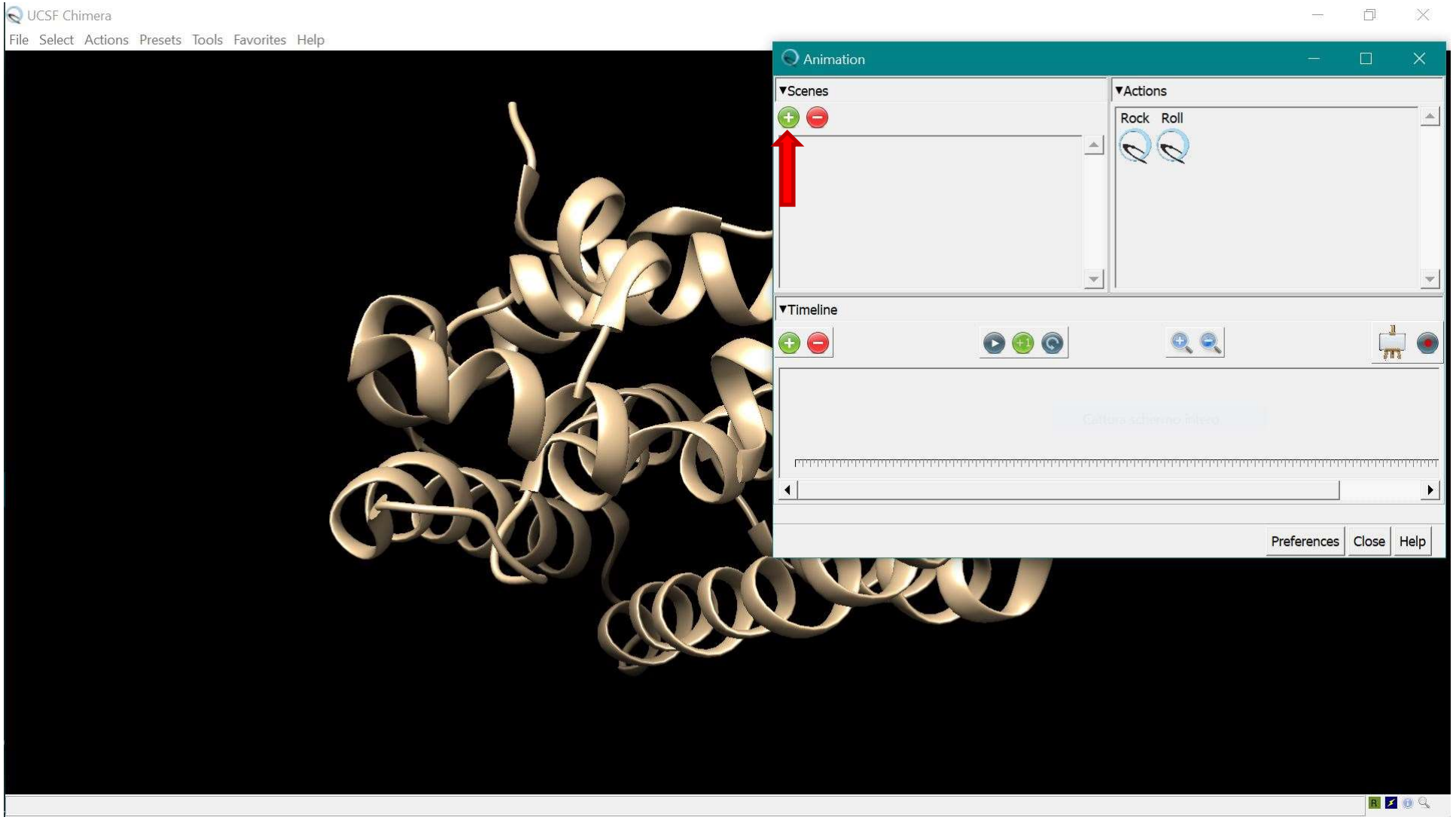
3. Load a map file (electron density!)



99.Animation!



99.Animation!



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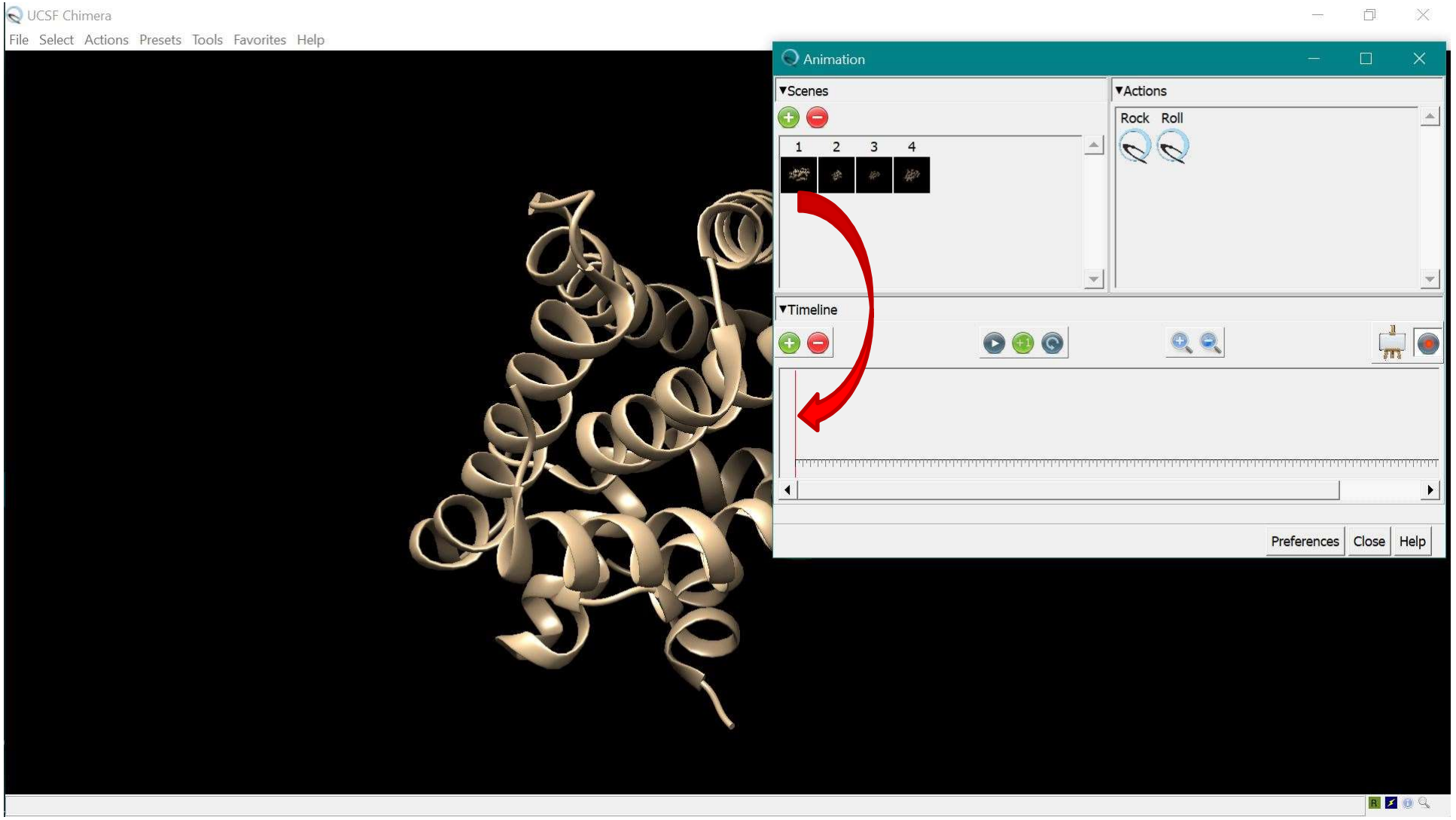

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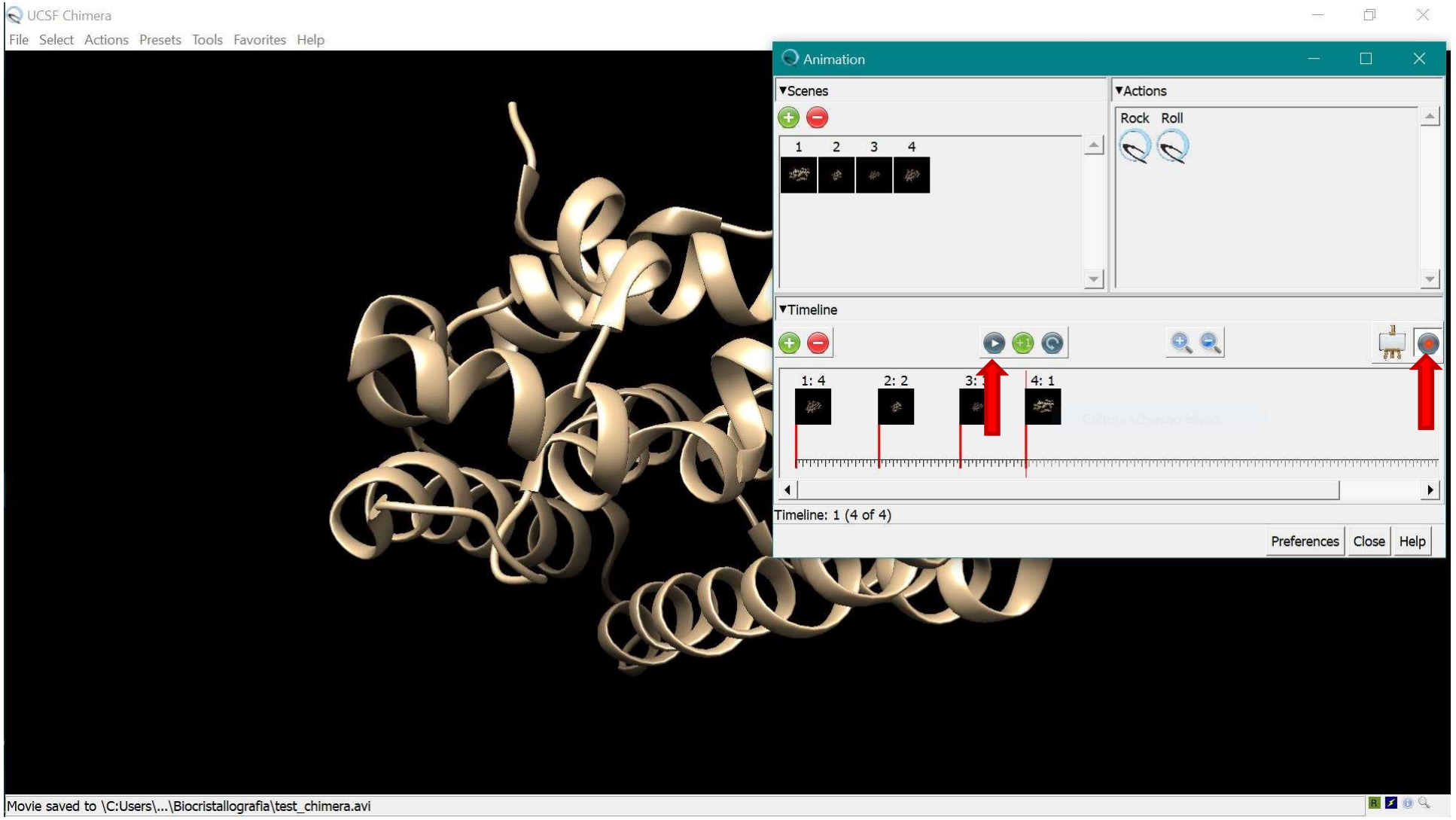

99.Animation!