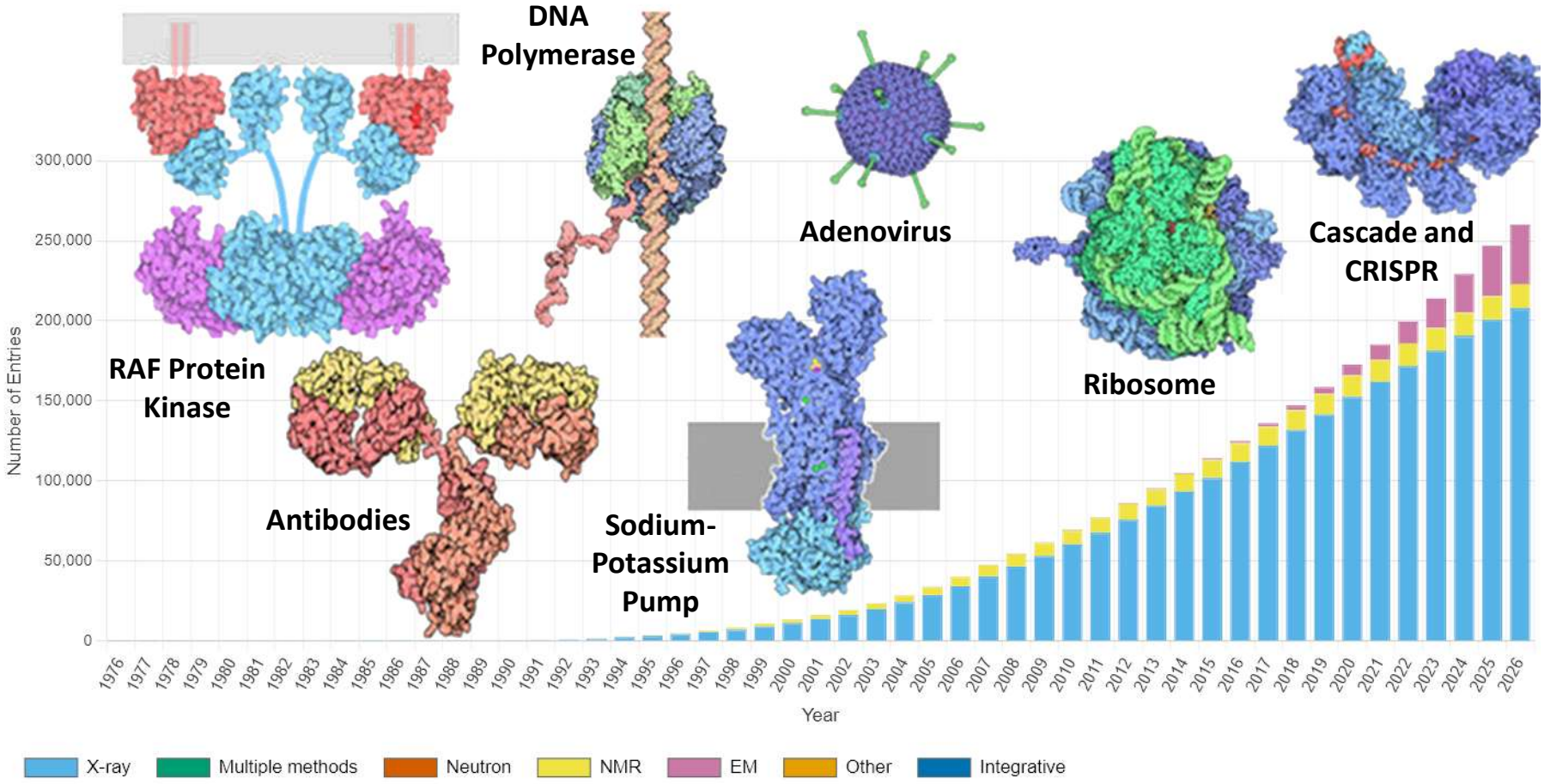
Biocrystallography and Electron Microscopy

Introduction: Why structural studies?



As of 29/9/2026, number of structures in PDB: 260,383

from X-ray 207,615

from EM 37,131

rdezorzi@units.it

Schedule of the lectures

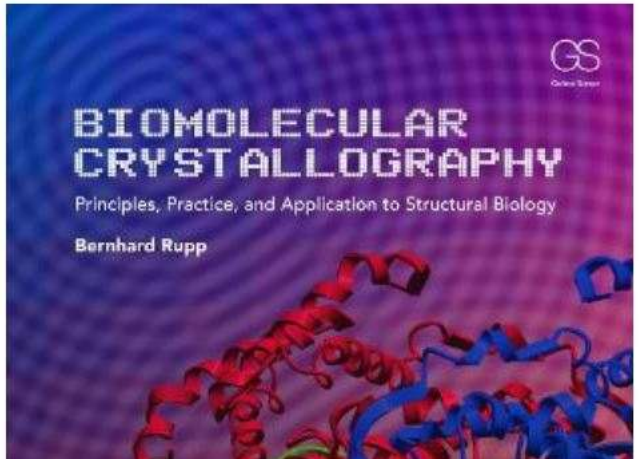
- **Wednesday 11-13** – Room A8
- **Friday 11-13** – Room A8

Books

Bernhard Rupp

Biomolecular Crystallography: Principles, Practice, and Application to Structural Biology

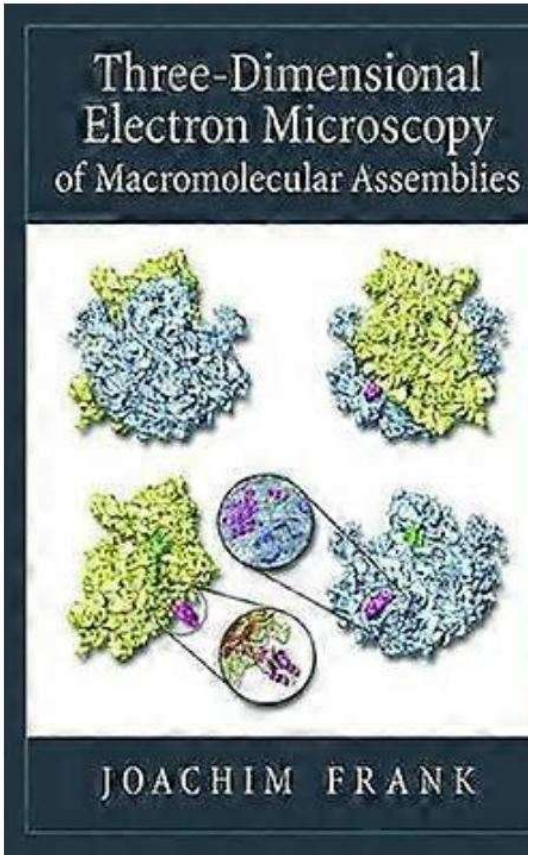
Garland Science



Joachim Frank

Three-Dimensional Electron Microscopy of Macromolecular Assemblies: Visualization of Biological Molecules in Their Native State

Oxford University Press



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

Practicals
- Crystallization of a protein sample
- Data analysis, structure solution and refinement from X-ray diffraction data
- Image preparation

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:

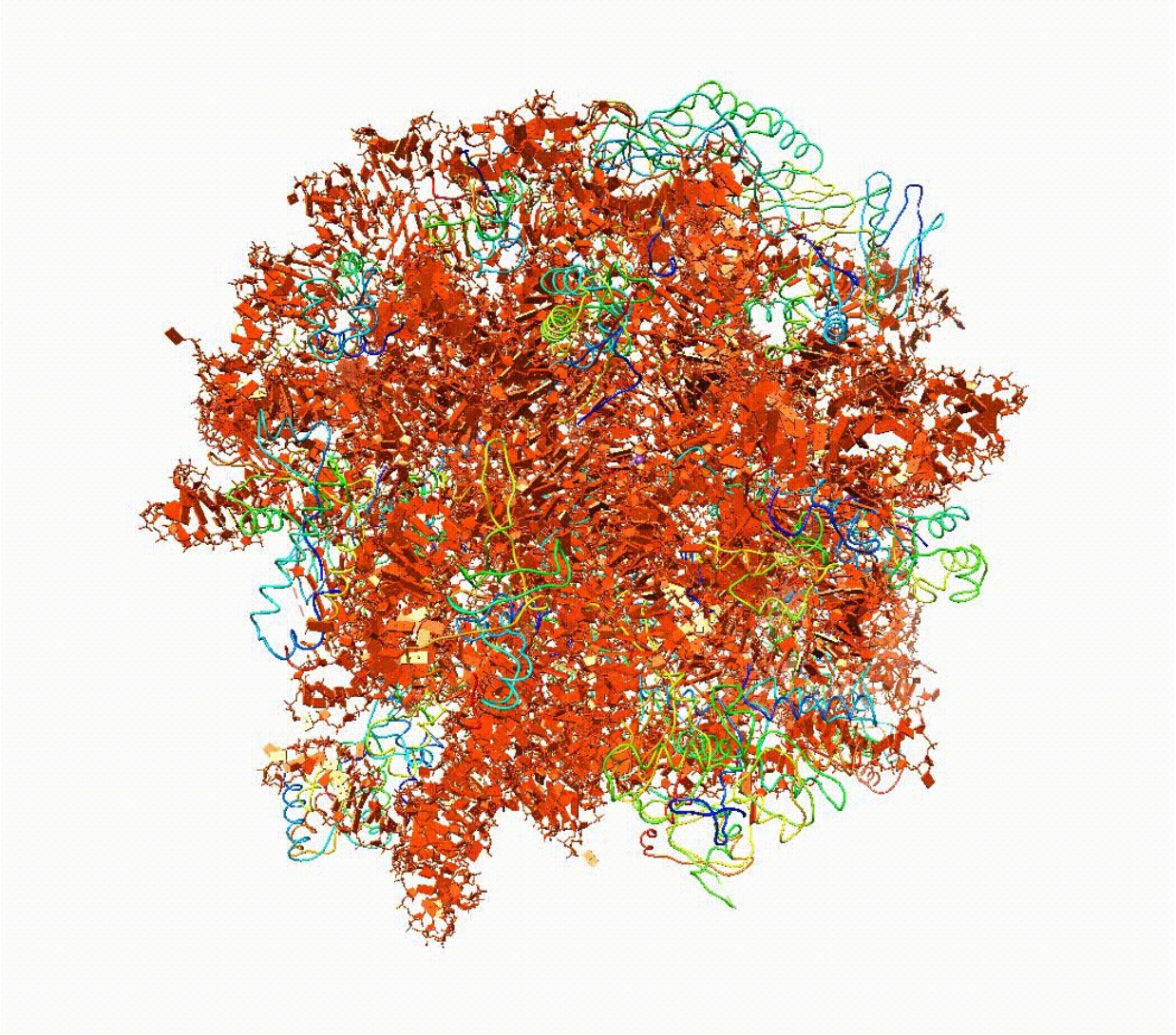
- Describe the 3D structure of a protein
- Plan a X-ray diffraction or electron microscopy experiment, from expression to structure
- Highlight main reasons to chose a structural biology technique over the others
- Recognize crucial factors that can improve/hamper a structural biology experiment
- Identify 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

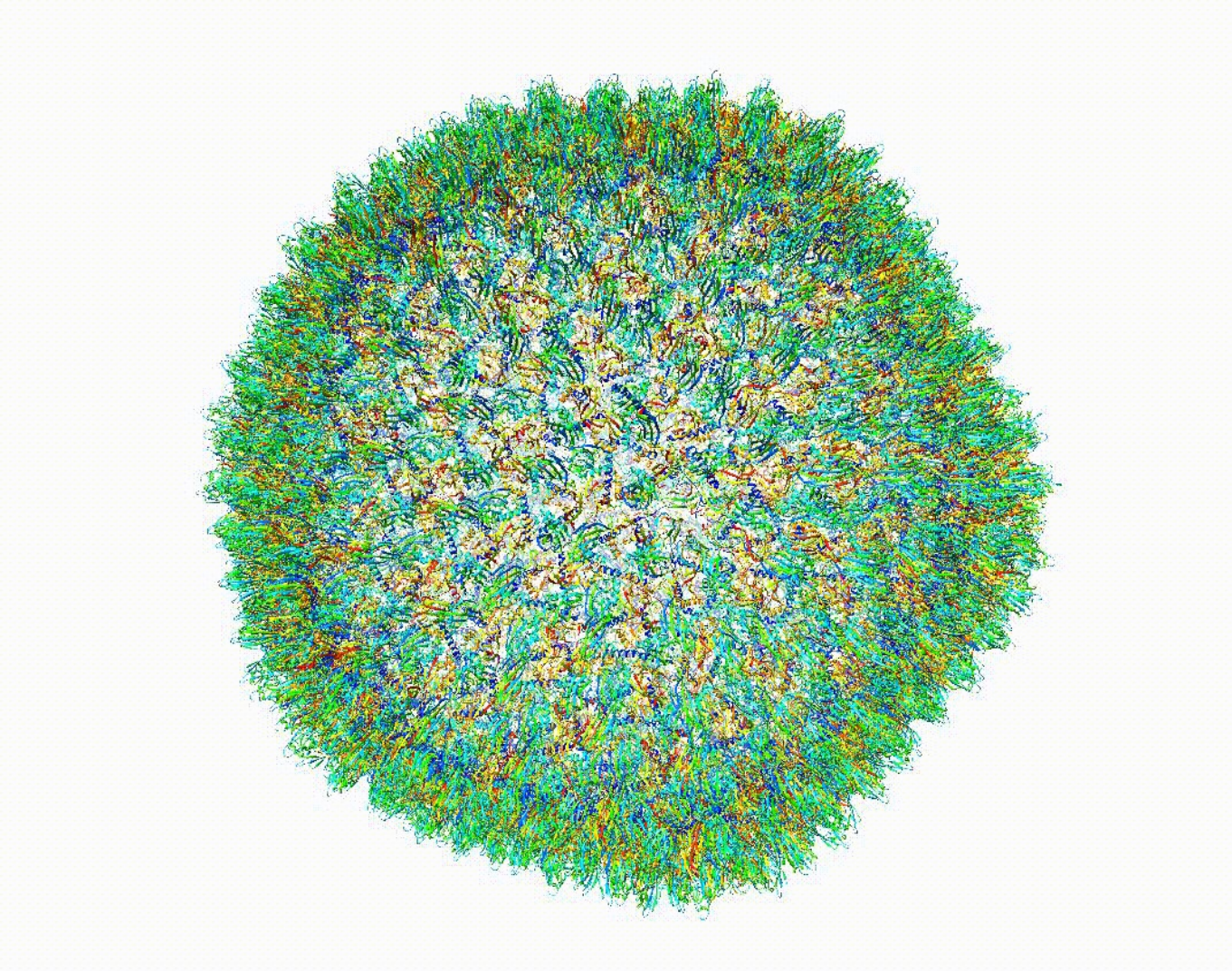
- Mechanisms of biologically relevant proteins and complexes: e.g. ribosome



PDB: 1ffk

Structure-function relationship

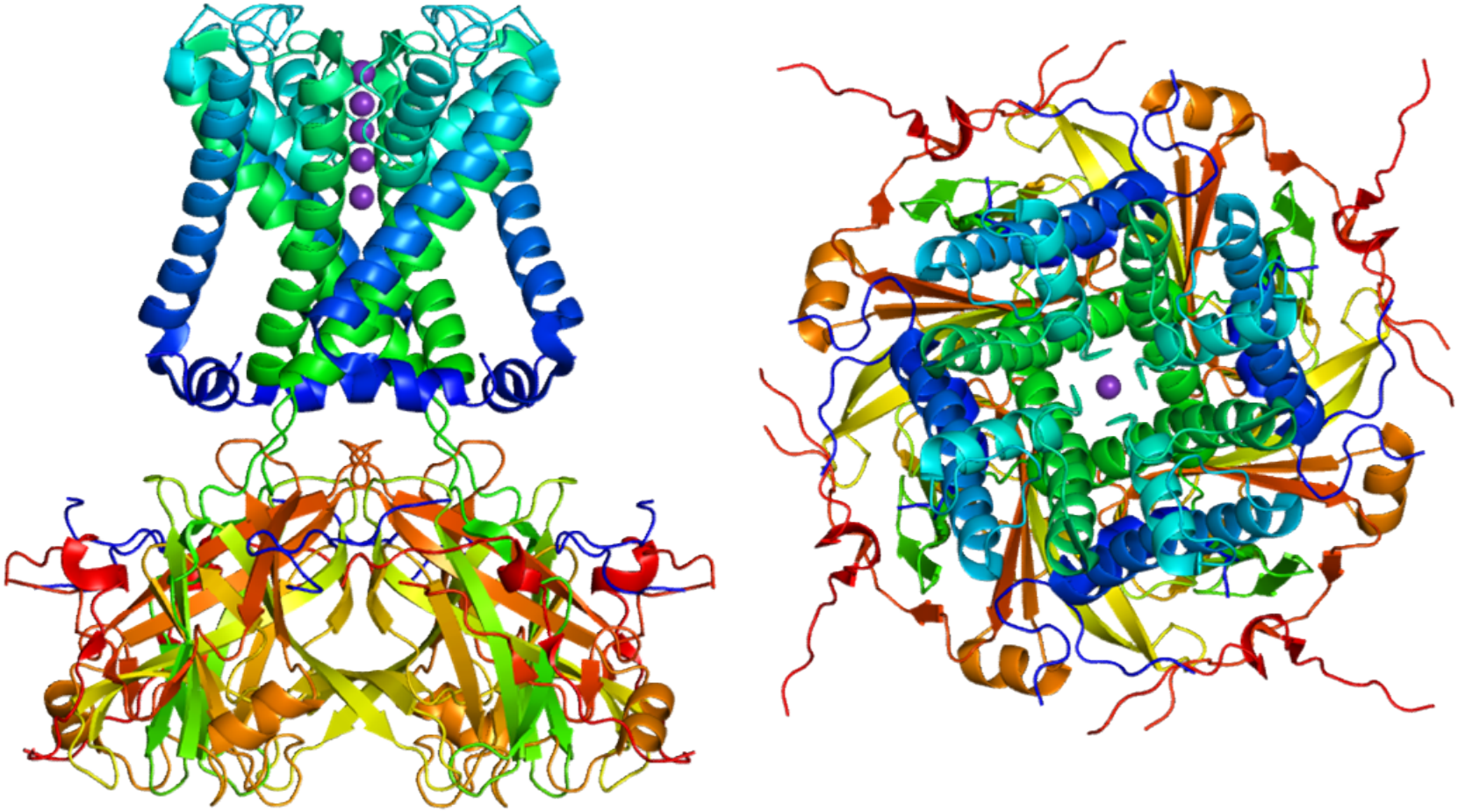
- Mechanisms of biologically relevant proteins and complexes: e.g. virus



PDB: 1w8x

Structure-function relationship

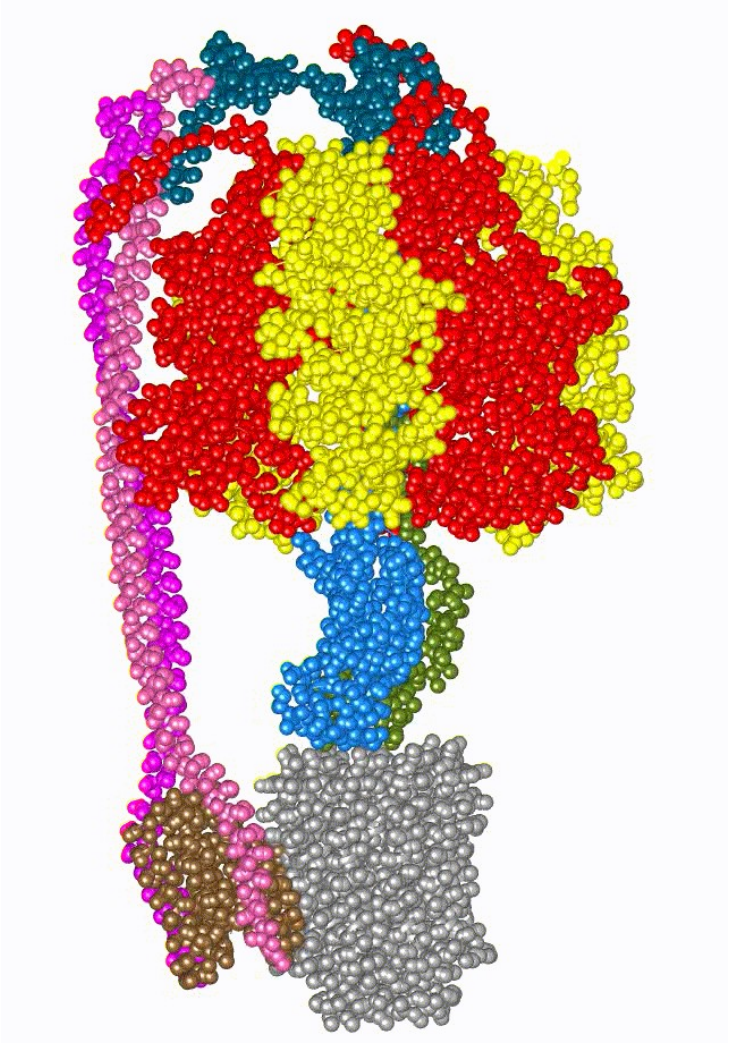
- Mechanisms of biologically relevant proteins and complexes: e.g. Potassium channel



PDB: 3zrs

Structure-function relationship

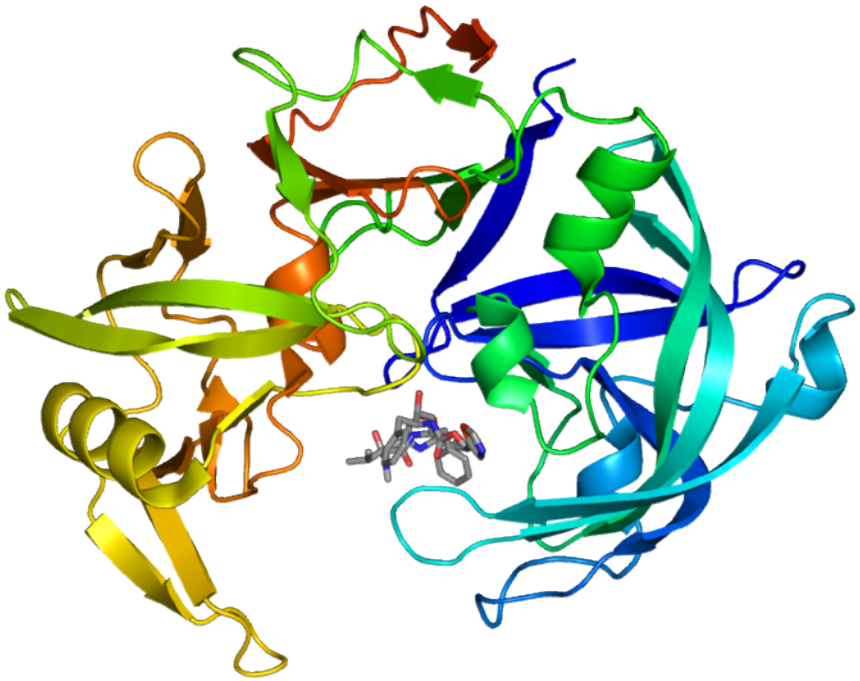
- Mechanisms of biologically relevant proteins and complexes:
  e.g. ATP-synthase



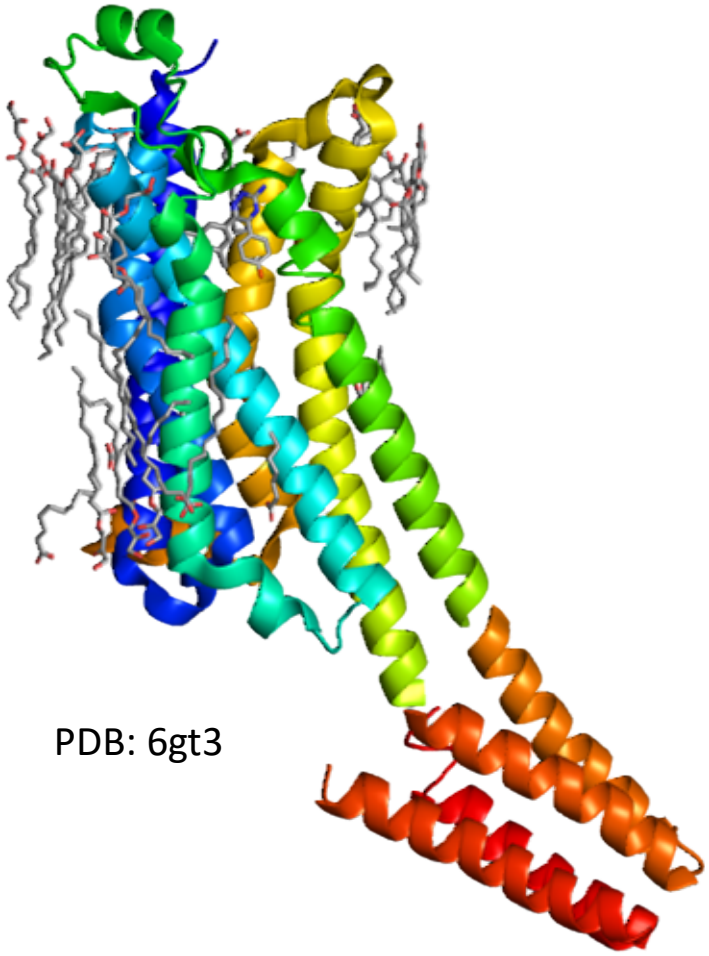
PDB: 5are, 5fil, 5fij, 5fik, 5ara, 5ari, 5arh

Structure-function relationship

- Drug design: e.g. HIV protease inhibitors, G-protein Coupled Receptor (GPCR)



PDB: 3q70



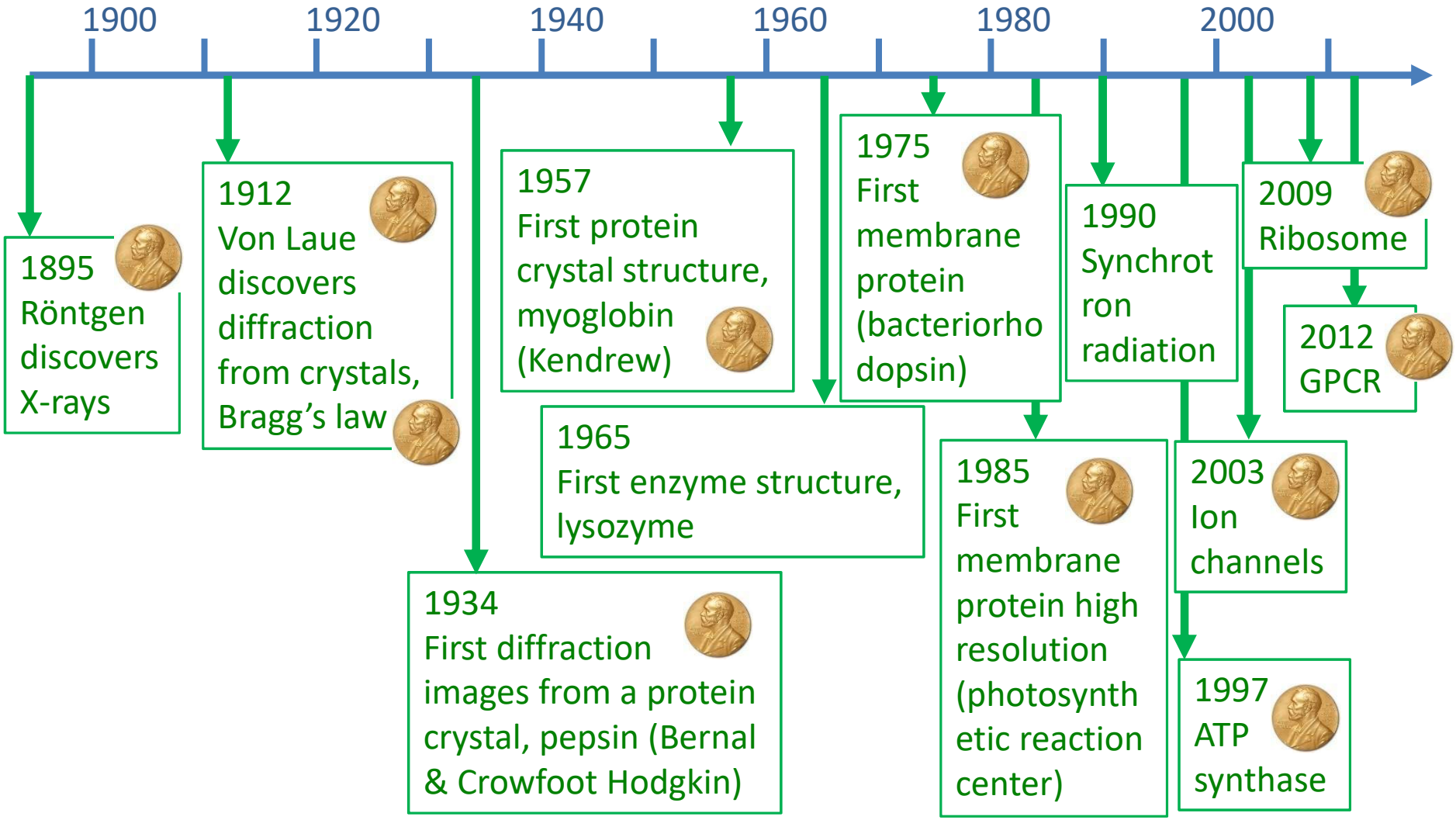
PDB: 6gt3

Structural biology: techniques

	X-ray crystallography	**NMR**	**Electron Microscopy**
Protein size	No limitation	< 80 KDa	> 70 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 (Validation)

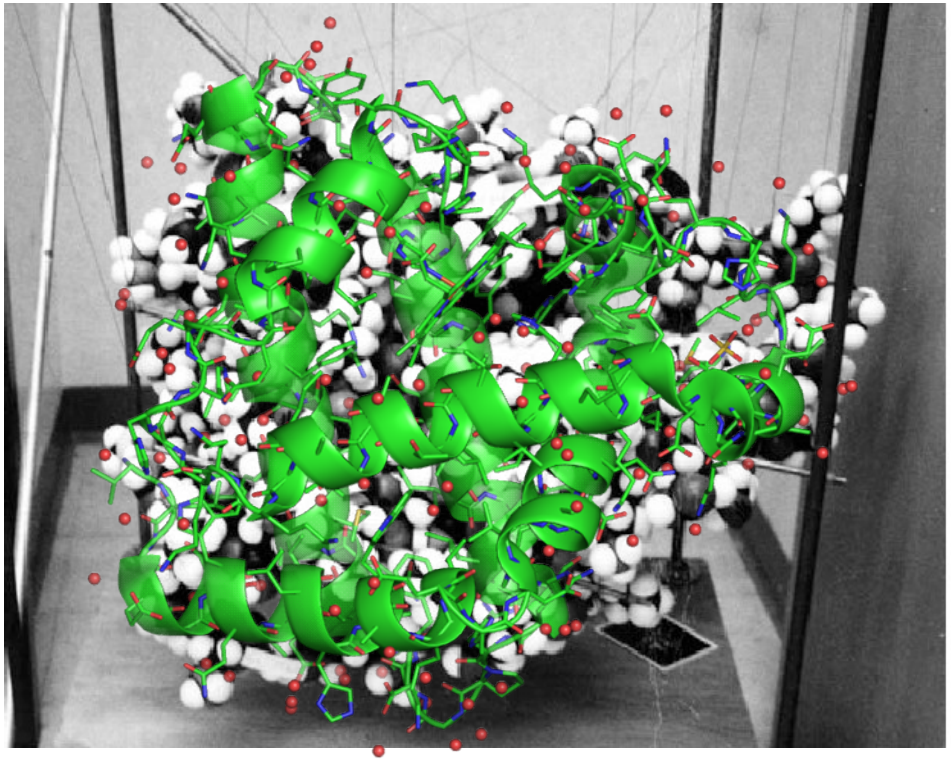
Different structural biology techniques give complementary results!!

Biocrystallography



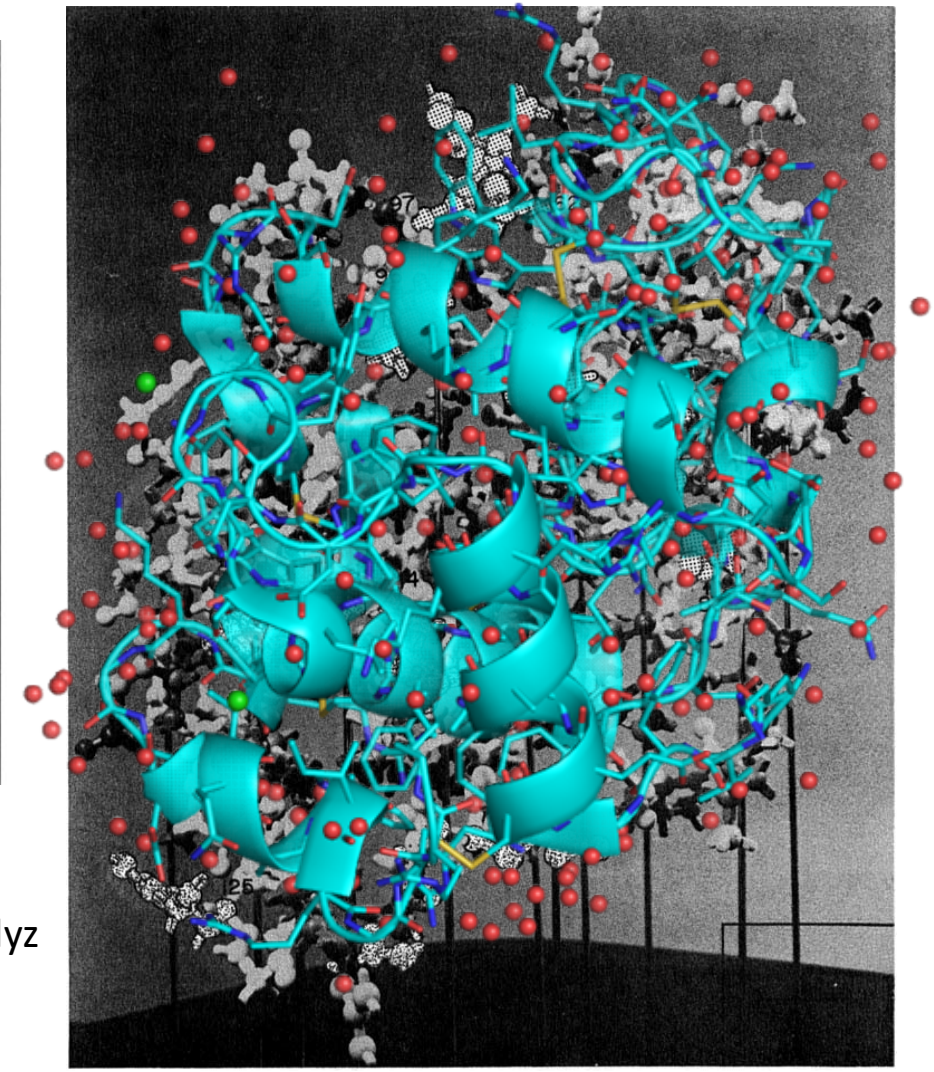
First protein structures determined by X-ray crystallography

- Myoglobin from whale sperm



PDB: 1vxa

- Lysozyme from chicken egg whites

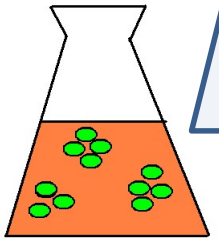


PDB: 1lyz

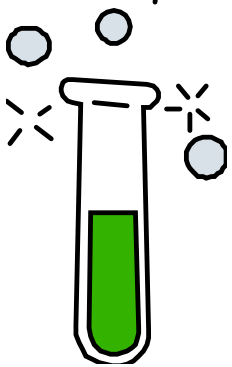
A biocrystallography experiment

Bioinformatics

Molecular biology

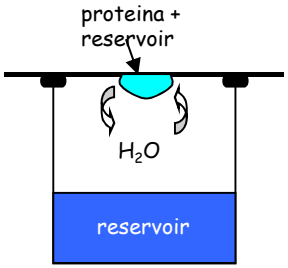


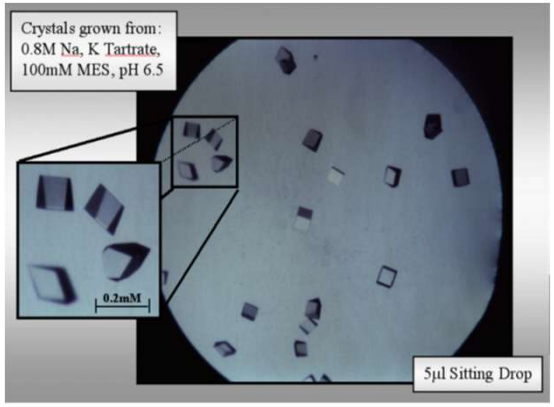
Expression and purification

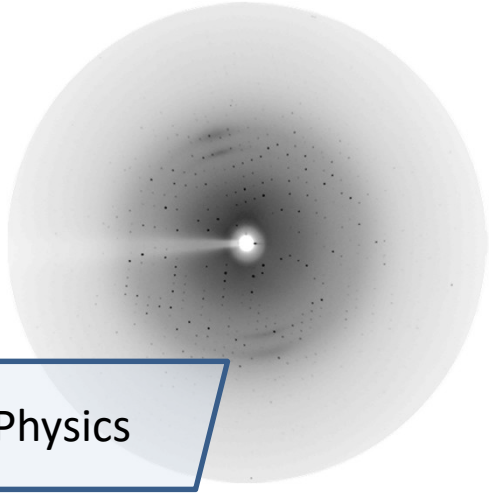


Chemistry

Crystallization







Physics

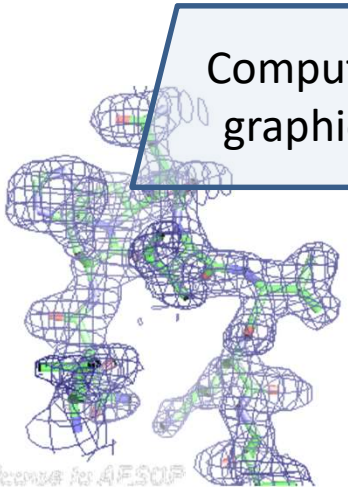
Diffraction data collection

Software development

Mathematics

Statistics

Phasing



Computer graphics

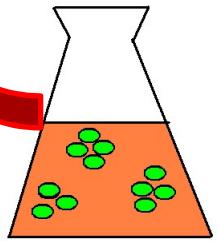
Structure refinement

Biology

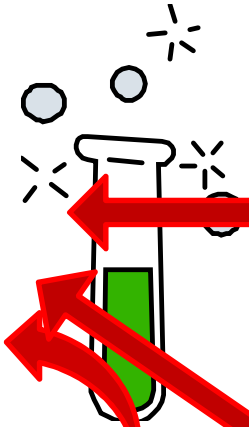
Biochemistry

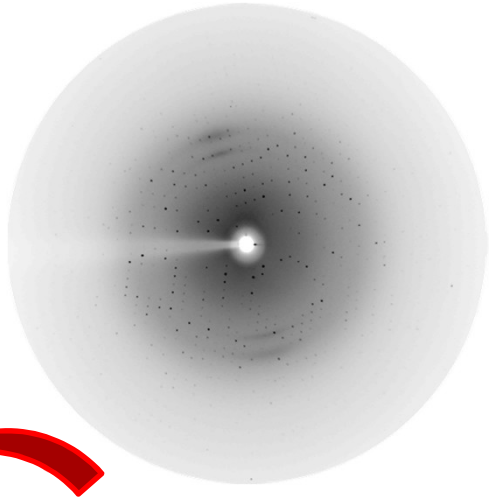
10 days to
10 years

A biocrystallography experiment

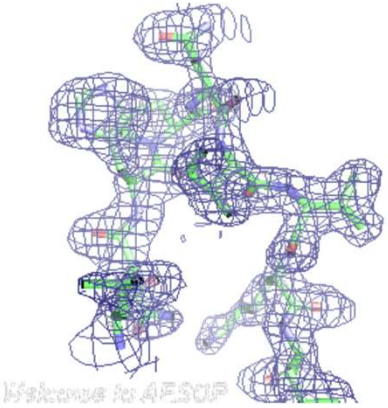


Expression and purification



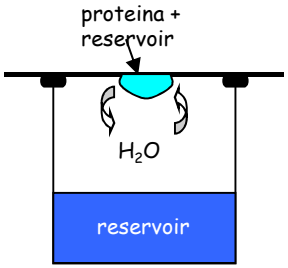


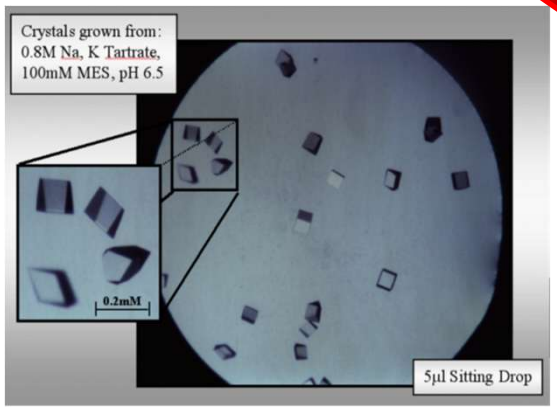
Diffraction data collection

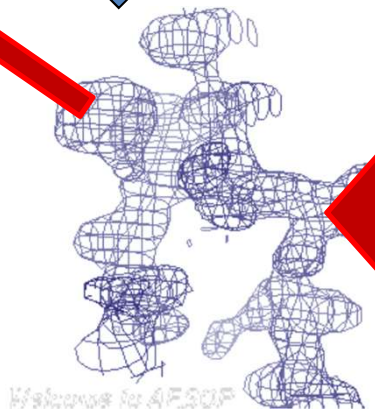


Structure refinement

Crystallization







Phasing

The structure needs to be confirmed by biochemical and functional studies

Databank resources: PDB (www.rcsb.org)

RCSB PDB Deposit Search Visualize Analyze Download Learn More MyPDB

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

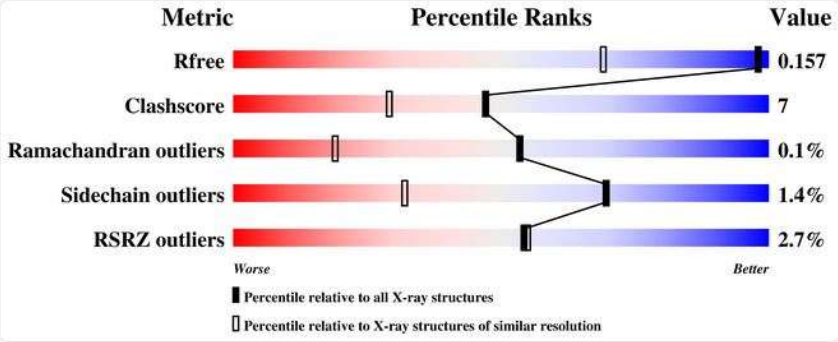
Software Package:

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



Contact Us

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

EMBL-EBI

Services | Research | Training | About us

Protein Data Bank in Europe
Bringing Structure to Biology

Search

Examples: hemoglobin, BRCA1_HUMAN

Advanced search

Feedback

EMDB › EMD-0632

Single particle reconstruction
2.7Å resolution

Rotavirus A-VP3 (RVA-VP3)

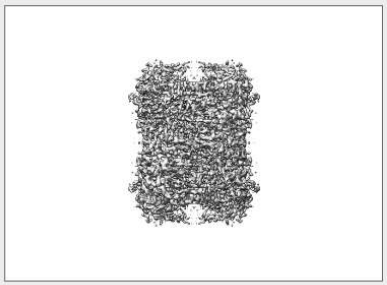
Source organism: *Rotavirus A* [28875]

Fitted atomic model: 6o6b

3Dbionotes: available for this entry

To be published

Map released:
2020-03-11



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)

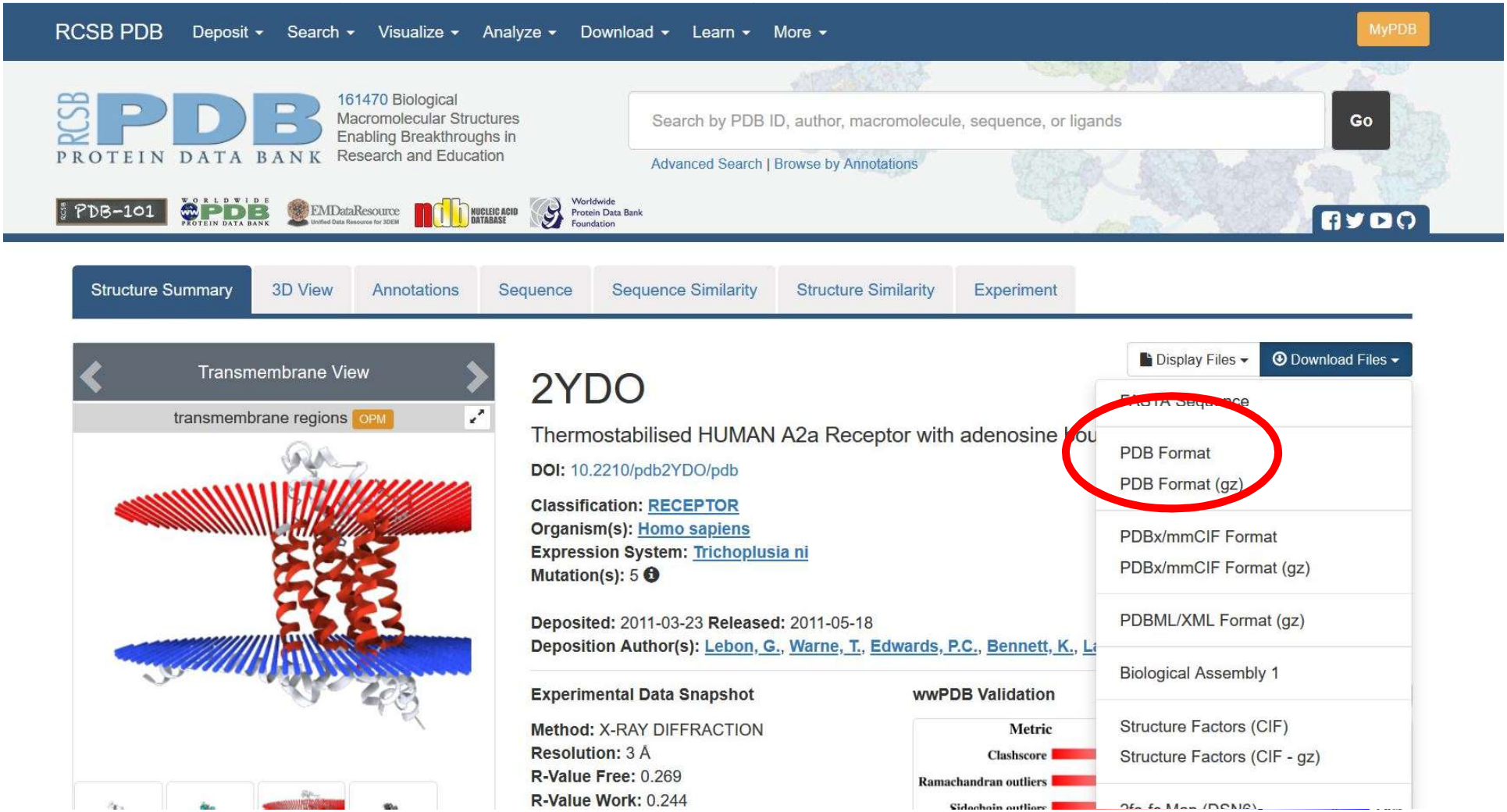
Quick links

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

Related entries

- By authors
- By sample
- By organism

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

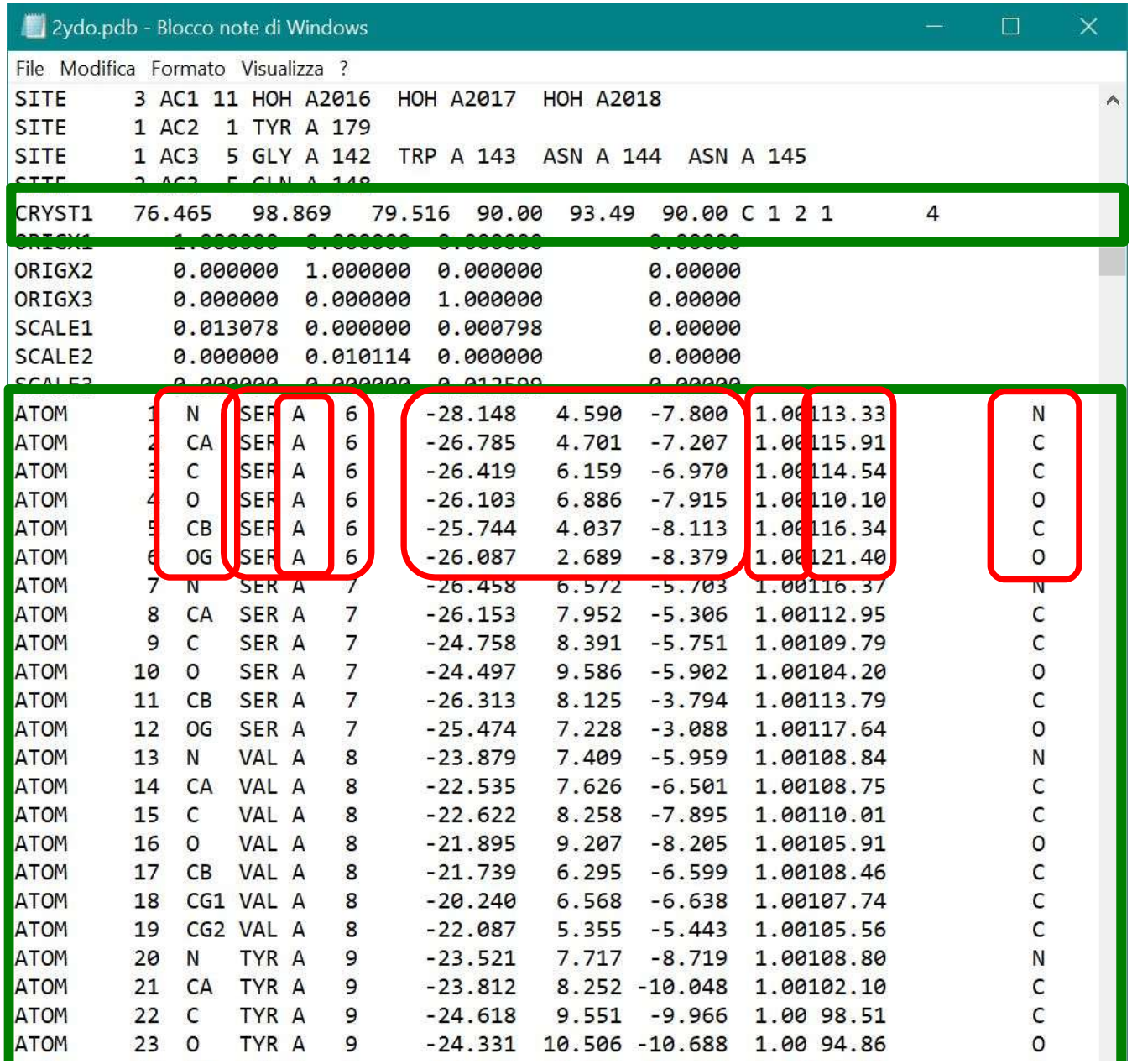
2ydo.pdb - Blocco note di Windows

File Modifica Formato Visualizza ?

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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 ?

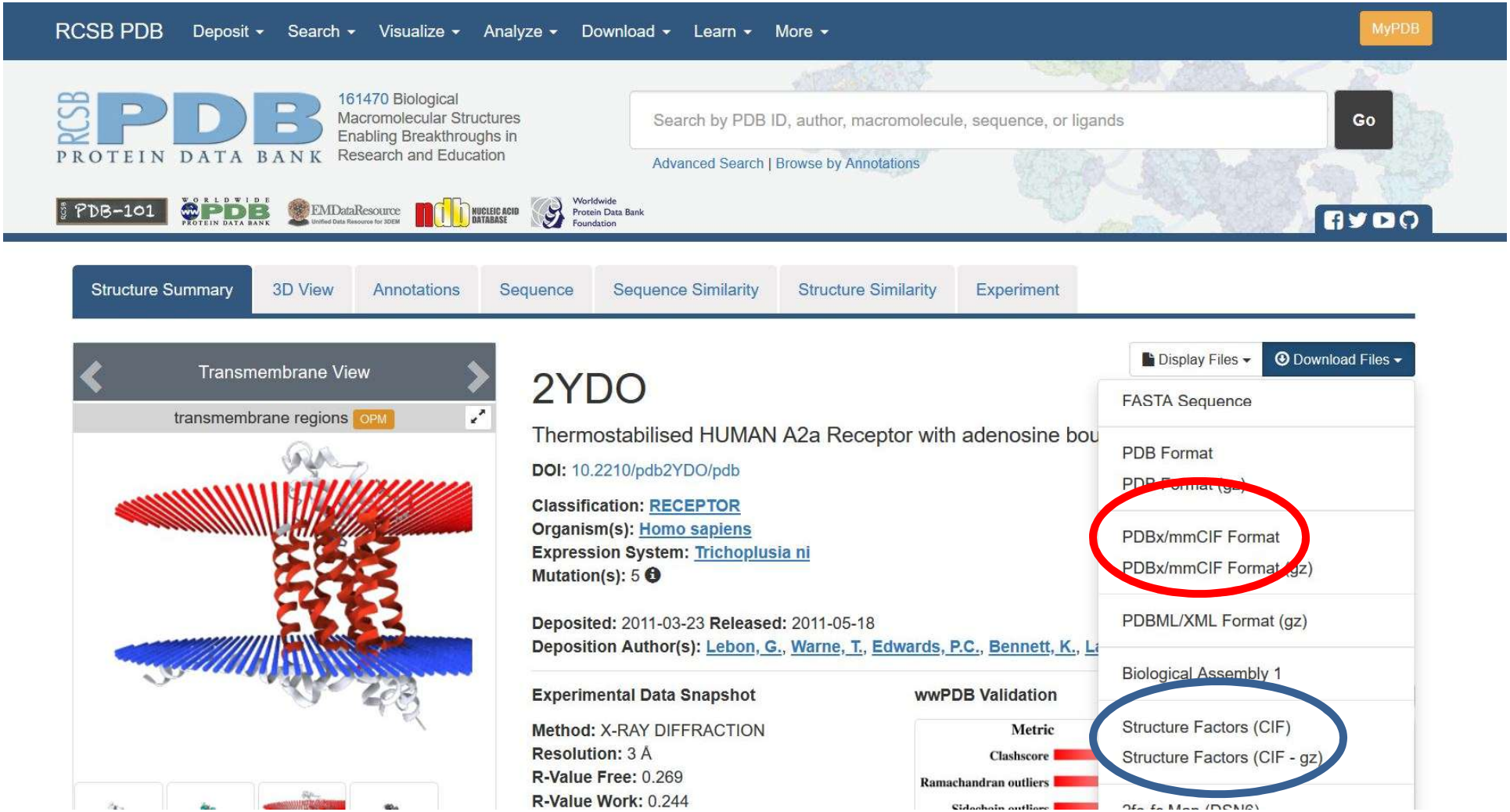
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SITE     1 AC2  1 TYR A 179
SITE     1 AC3  5 GLY A 142  TRP A 143  ASN A 144  ASN A 145
SITE     2 AC3  5 GLN A 148
CRYST1   76.465   98.869   79.516  90.00  93.49  90.00 C 1 2 1       4
ORIGX1      1.000000  0.000000  0.000000        0.00000
ORIGX2      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
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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
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Unit cell and symmetry

List of atoms with:

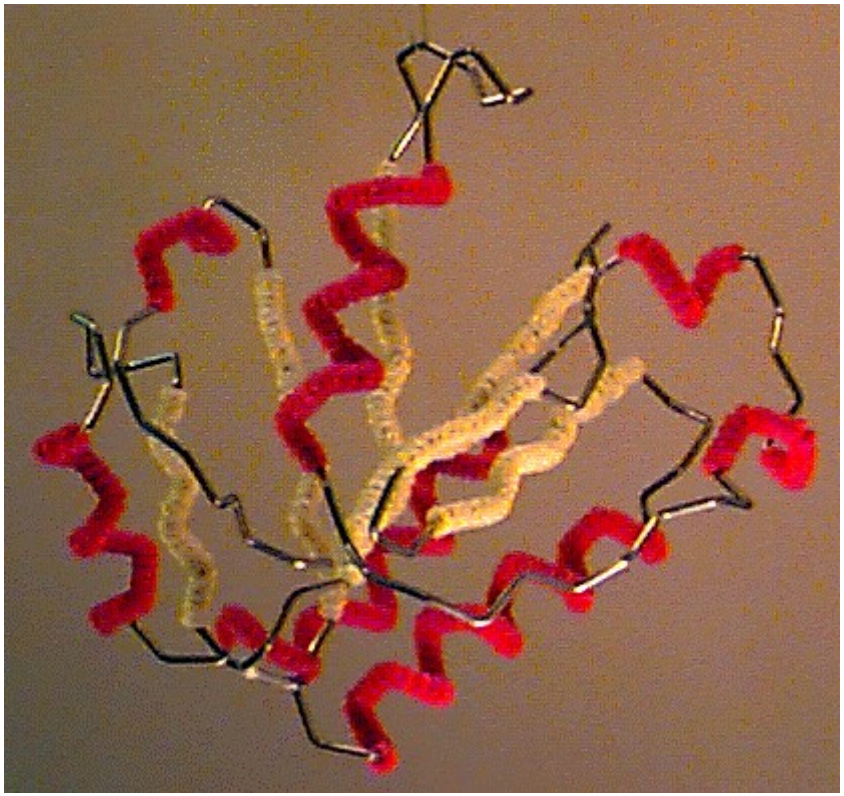
1. **Atom name**
2. **Residue type and number**
3. **Chain**
4. **Cartesian coordinates**
5. **Occupancy**
6. **Thermal factor**
7. **Atom type**

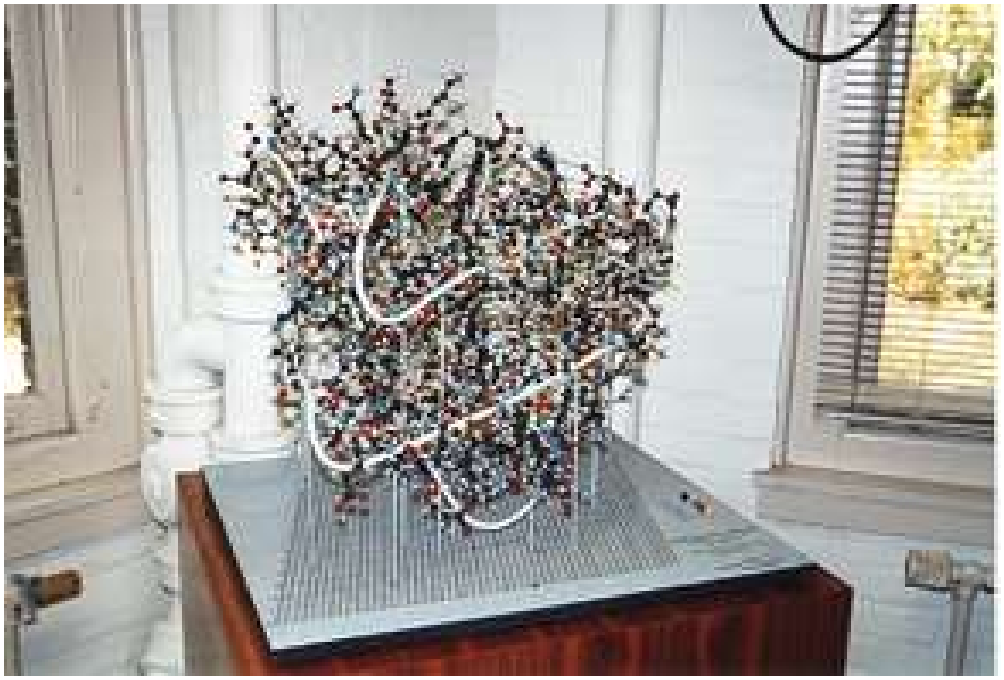
Structure atomic coordinates: mmCIF file



for the electron density!!!

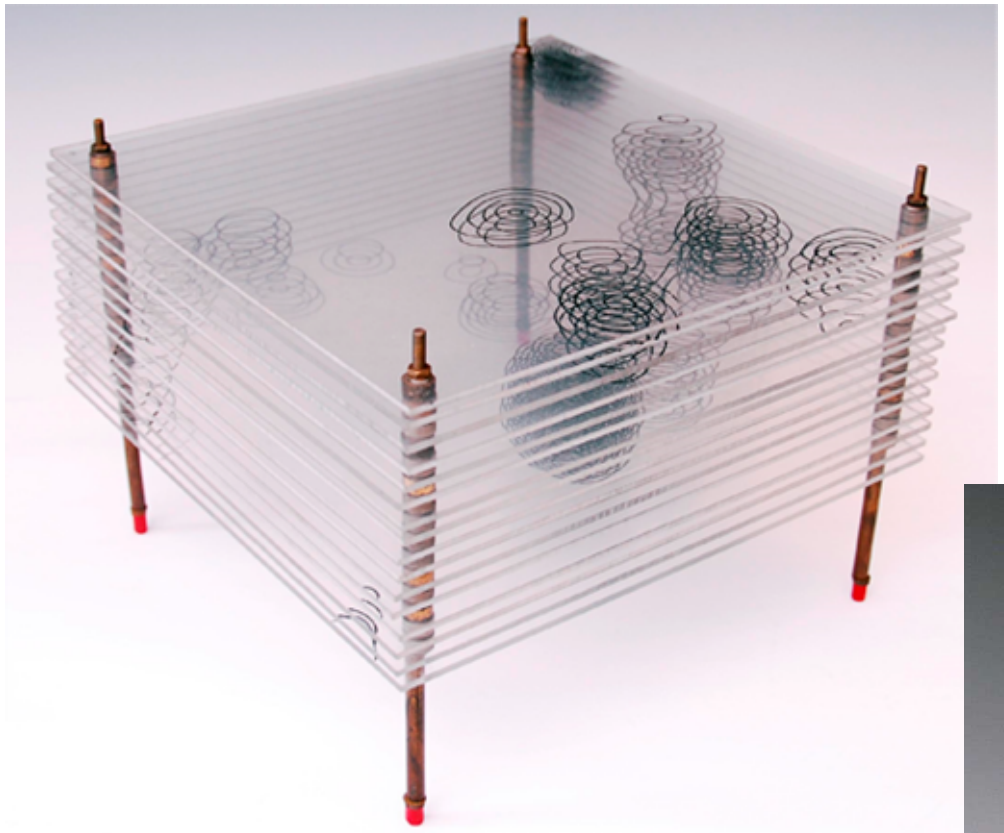
Graphical software: in the old days...

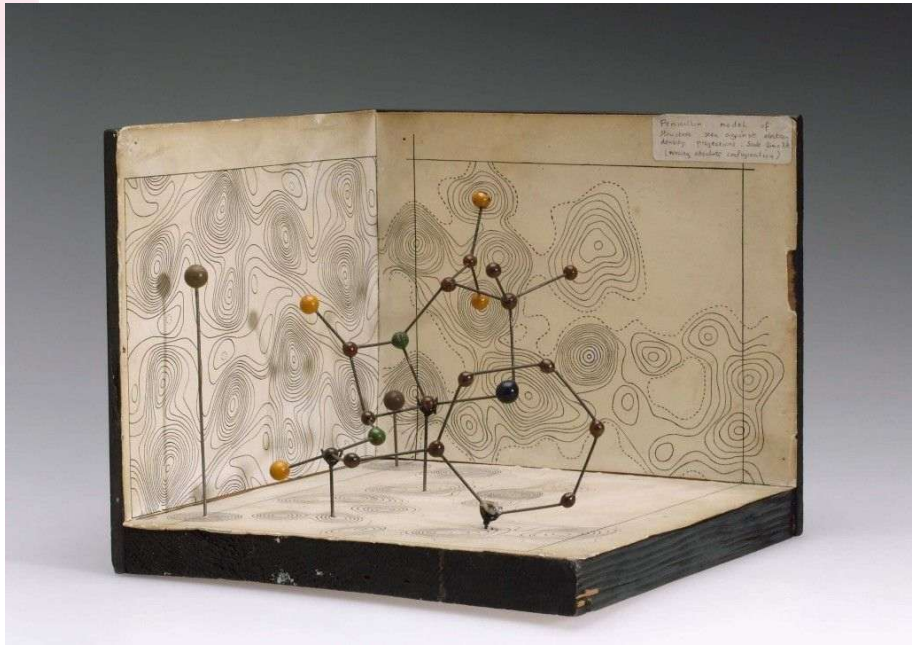






Graphical software: in the old days...





Graphical software: in the old days...



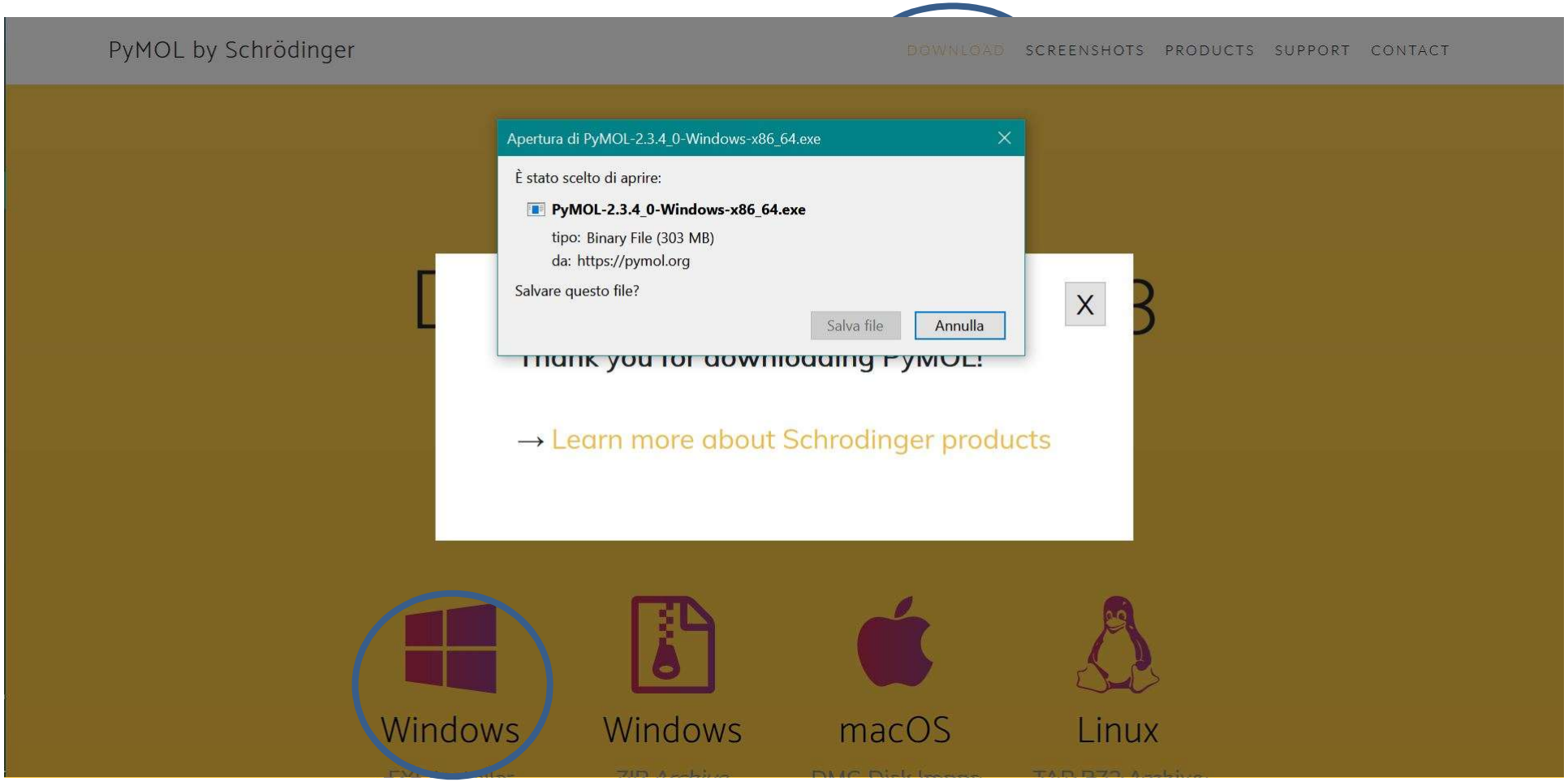


Graphical software:... today

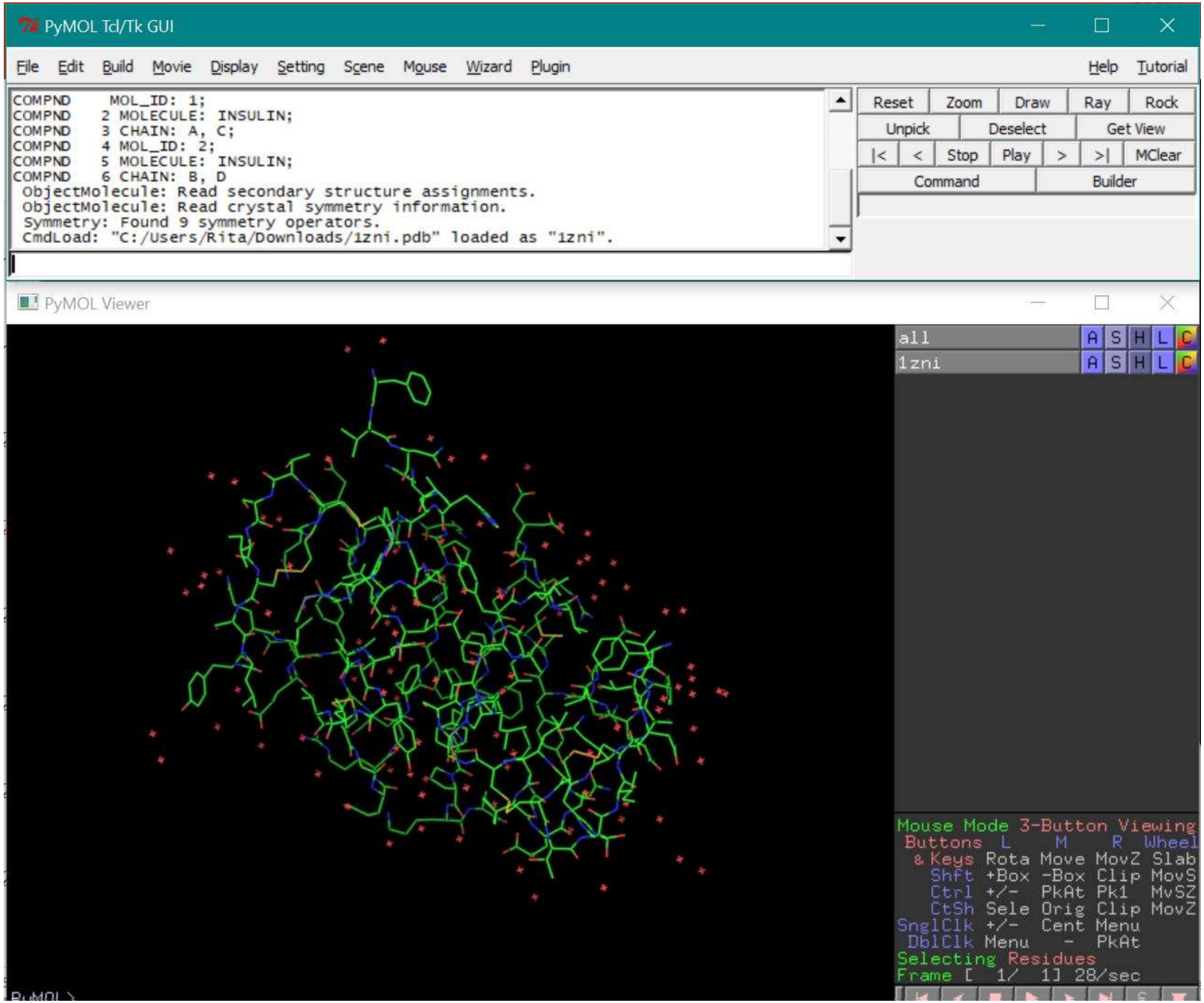


Graphical software: Pymol

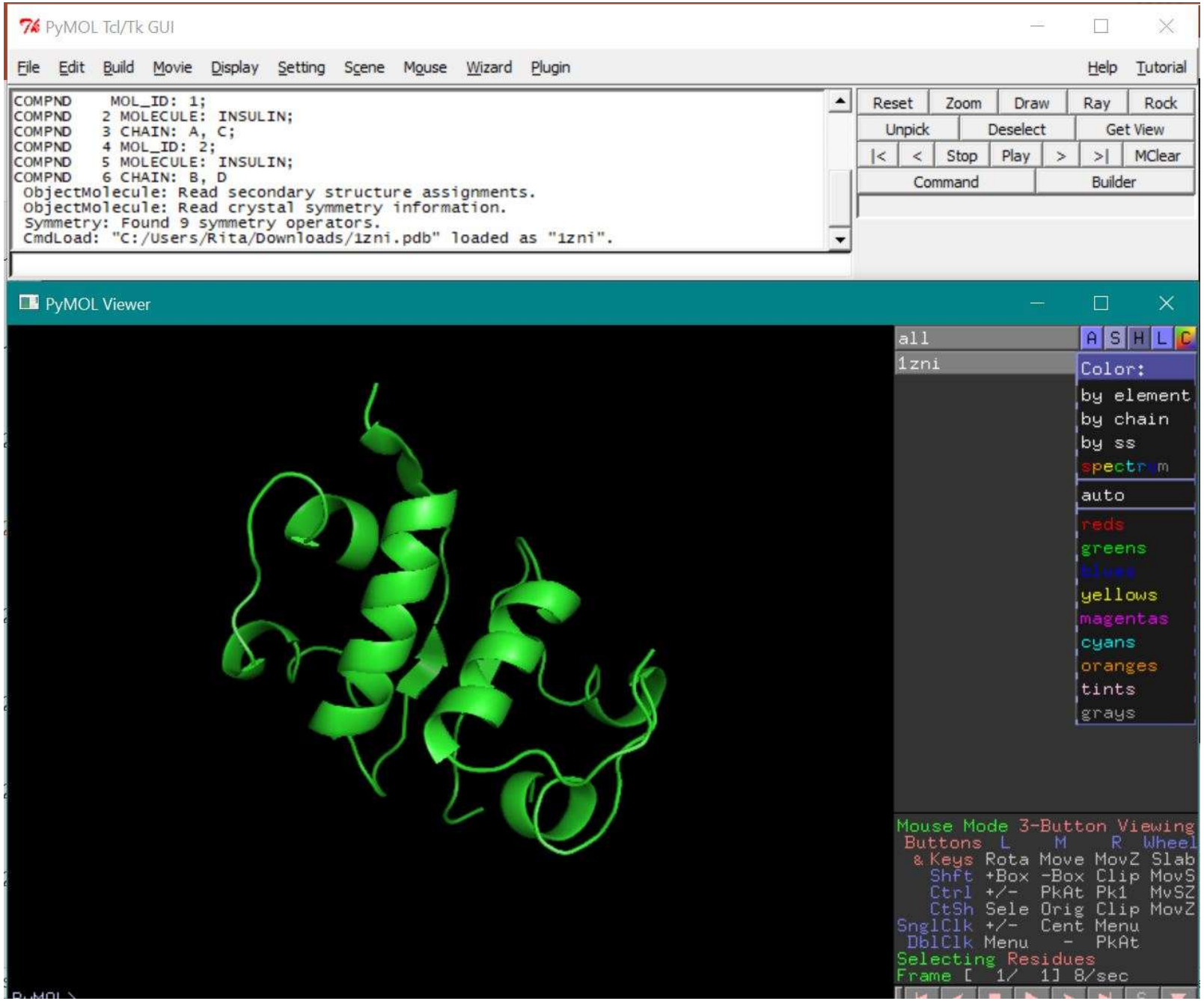
1. Download: https://pymol.org/2/



2. Open and load pdb file

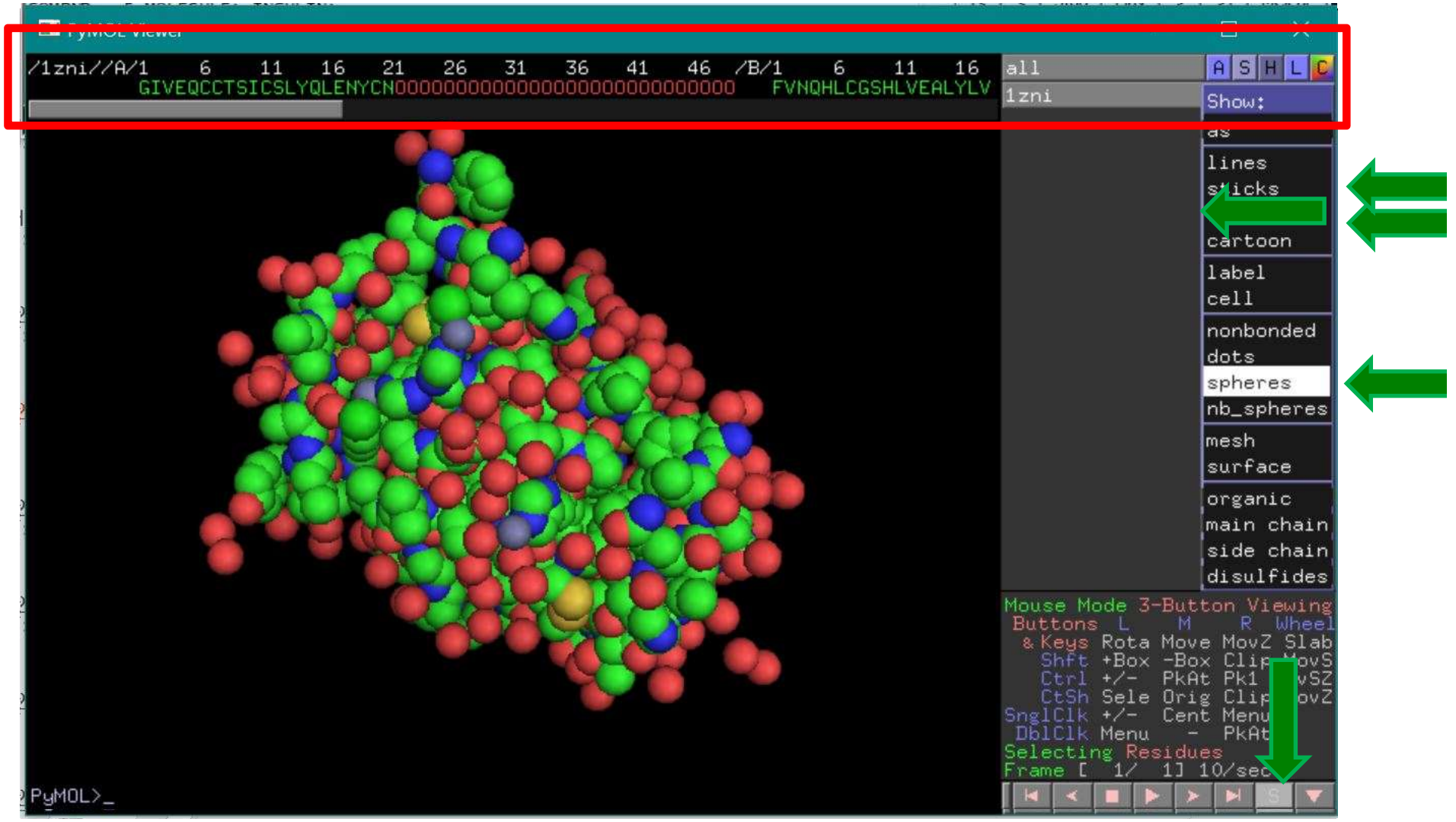


3. Menu options



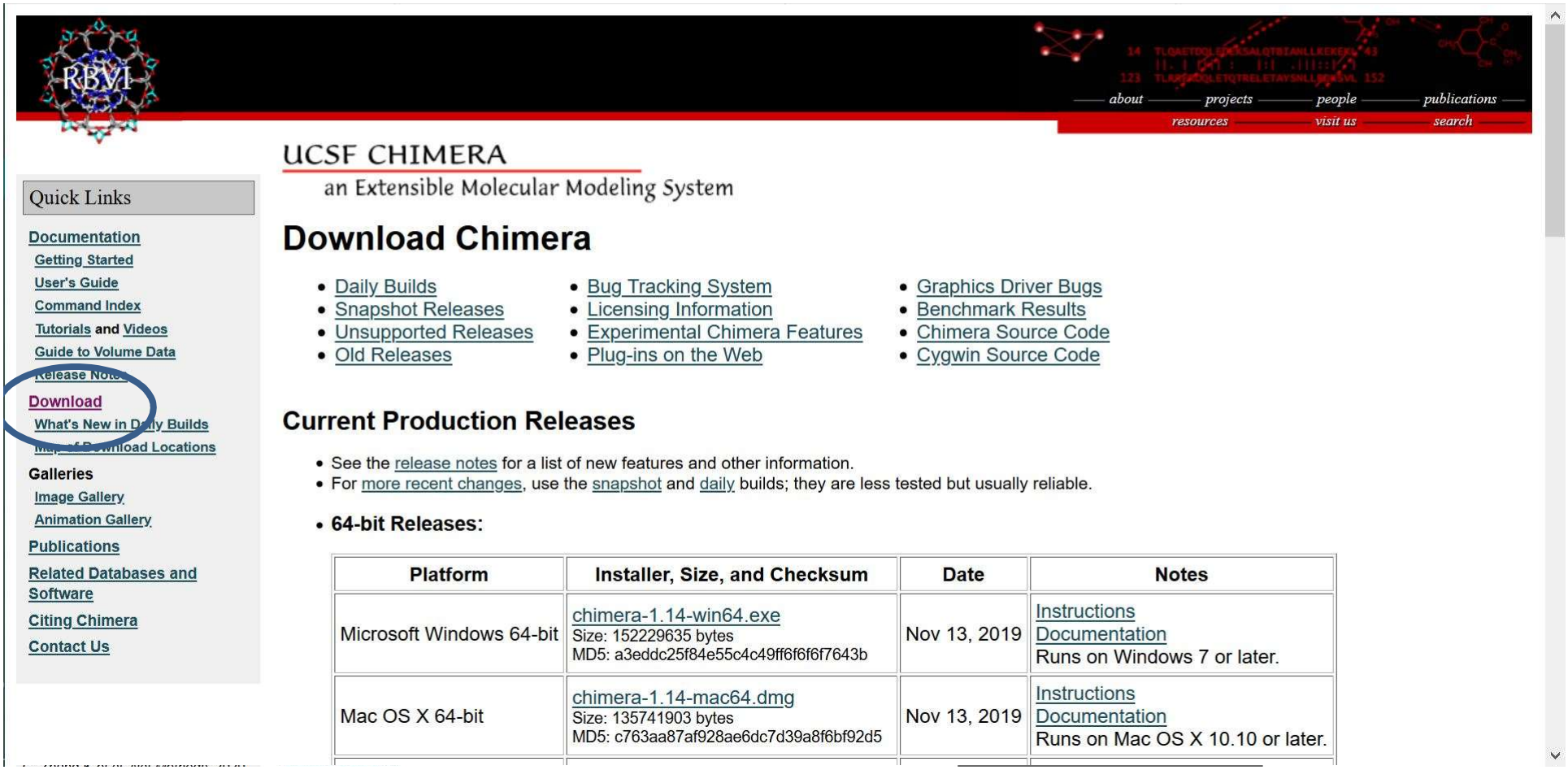
Visualization options:

spheres

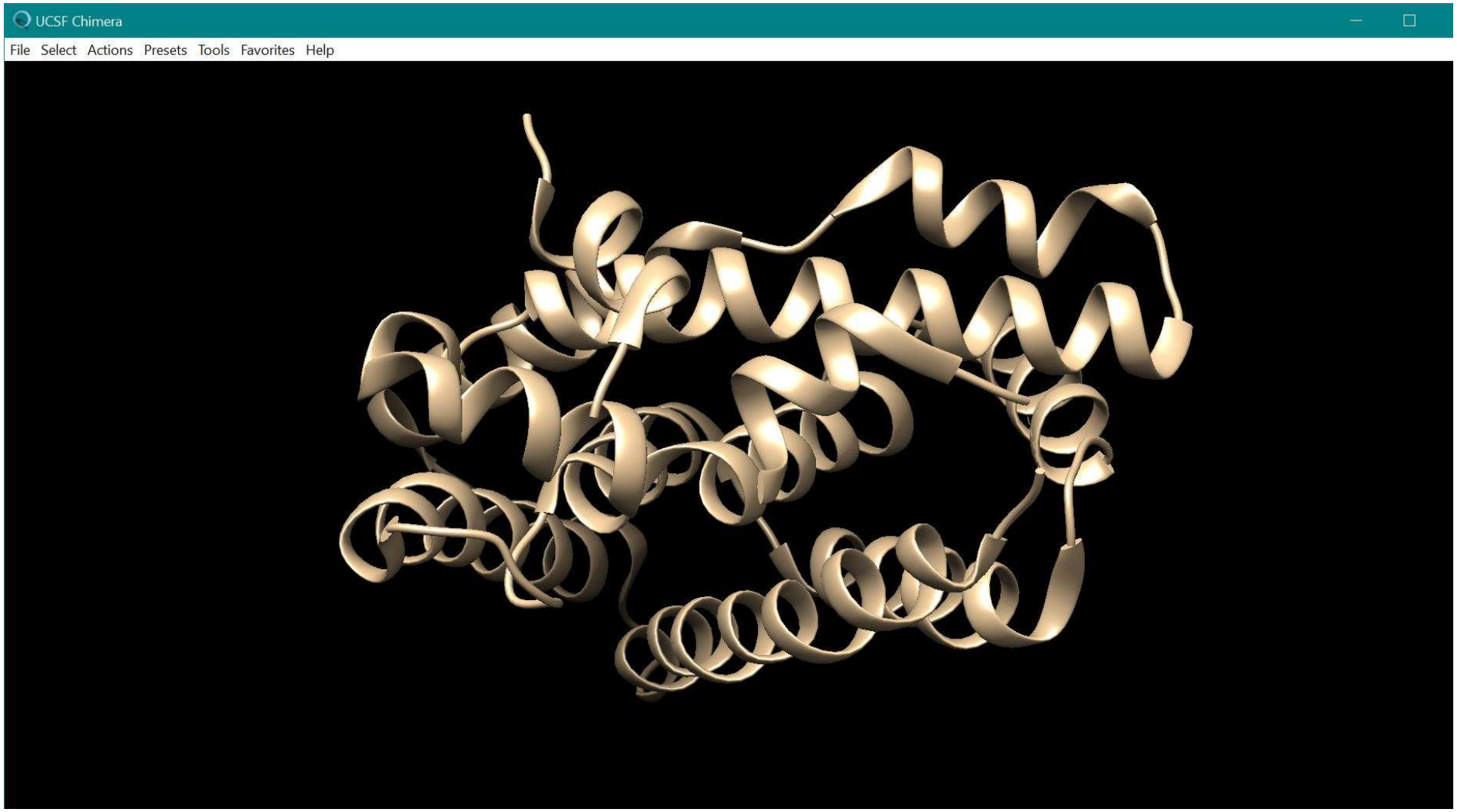


Graphical software: UCSF Chimera

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

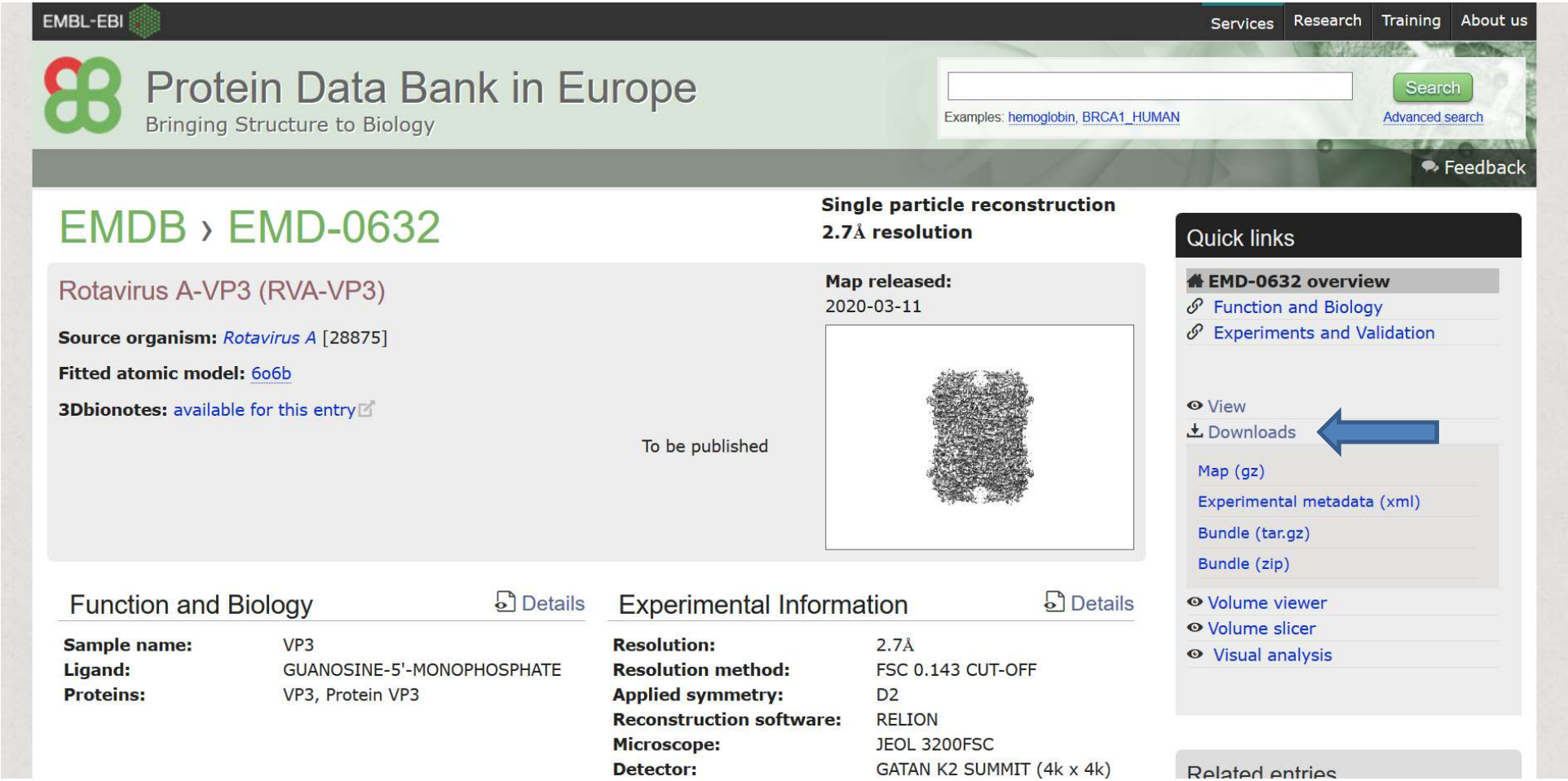


2. Open and load pdb file

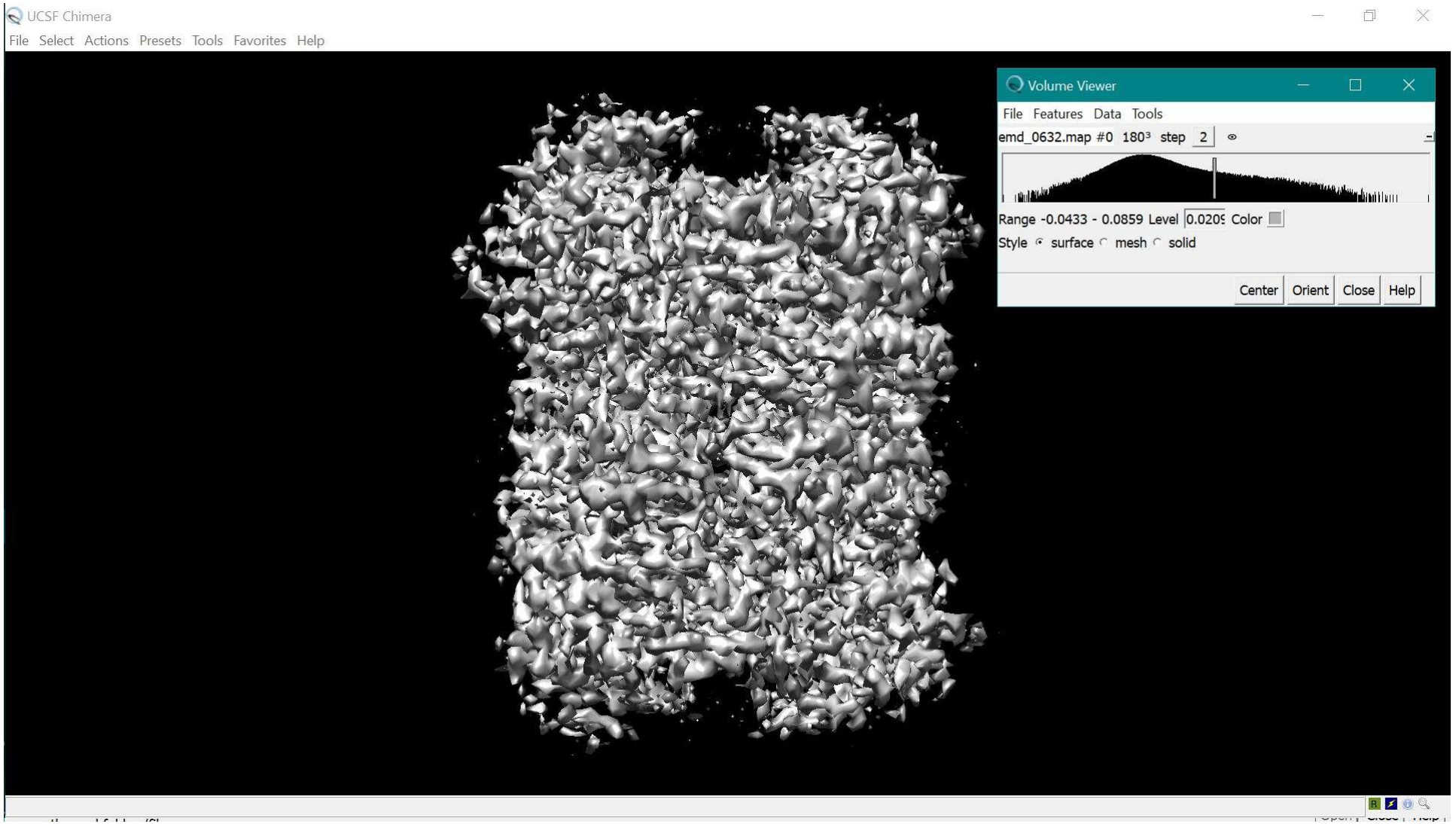


3. Load a map file (electron density!)

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



3. Load a map file (electron density!)



99.Animation!

