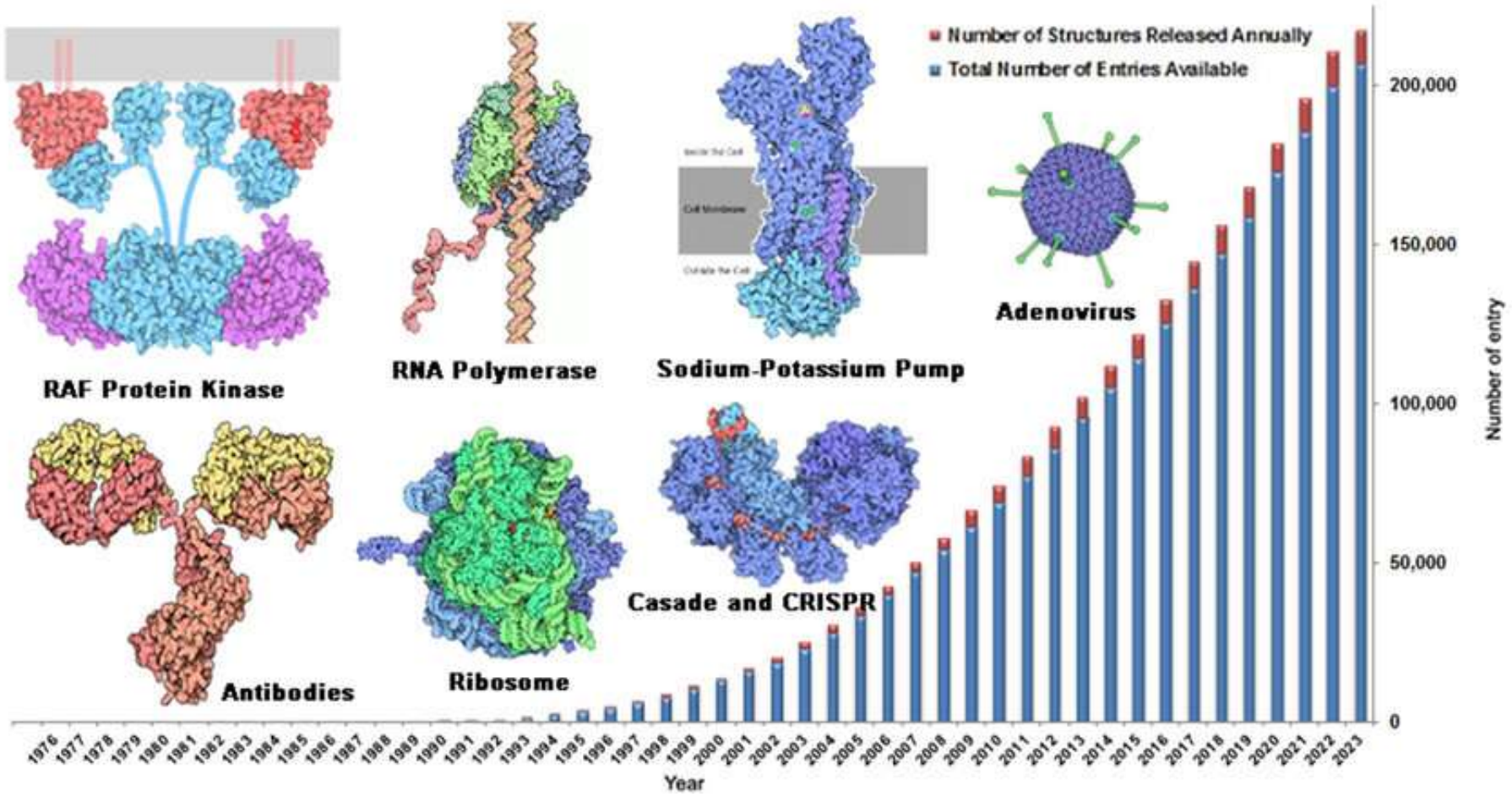
Corso di Biocristallografia e Microscopia Elettronica

Introduction: Why structural studies?



As of 2/3/2025, number of structures in PDB: 232,059

from X-ray 192,025

from EM 25,199

rdezorzi@units.it

Timetable of the lectures

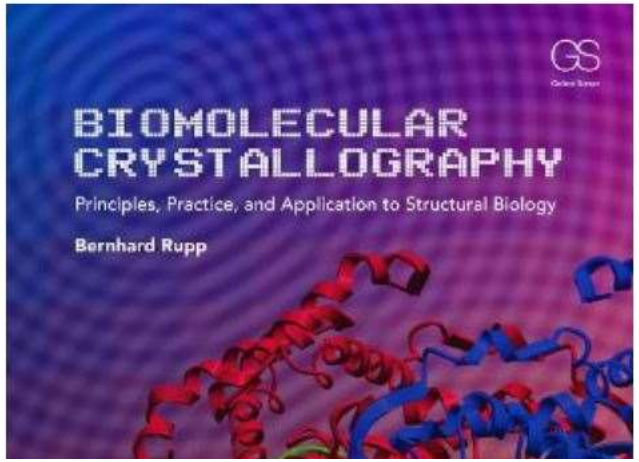
- **Monday 11-12** – Room A9
- **Tuesday 15-17** – Room A9
- **Wednesday 9-11** – Room A9

Books

Bernhard Rupp

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

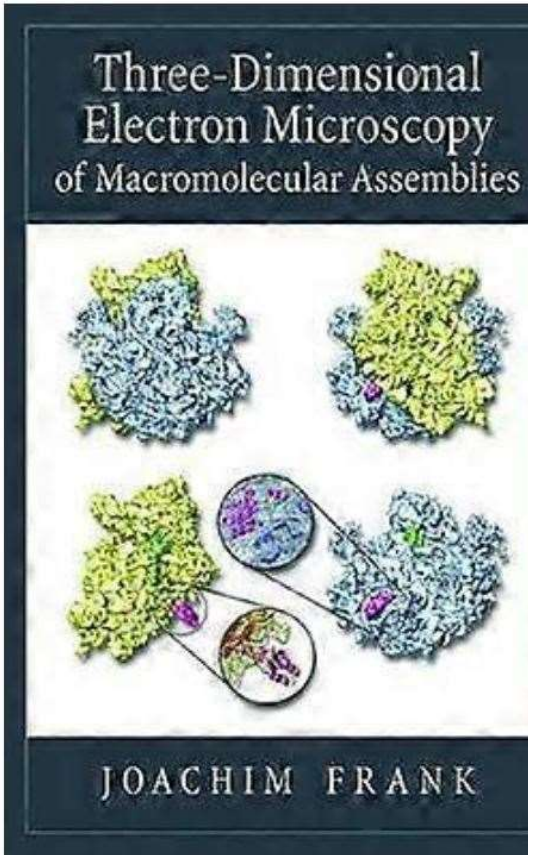
Garland Science



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
- 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:

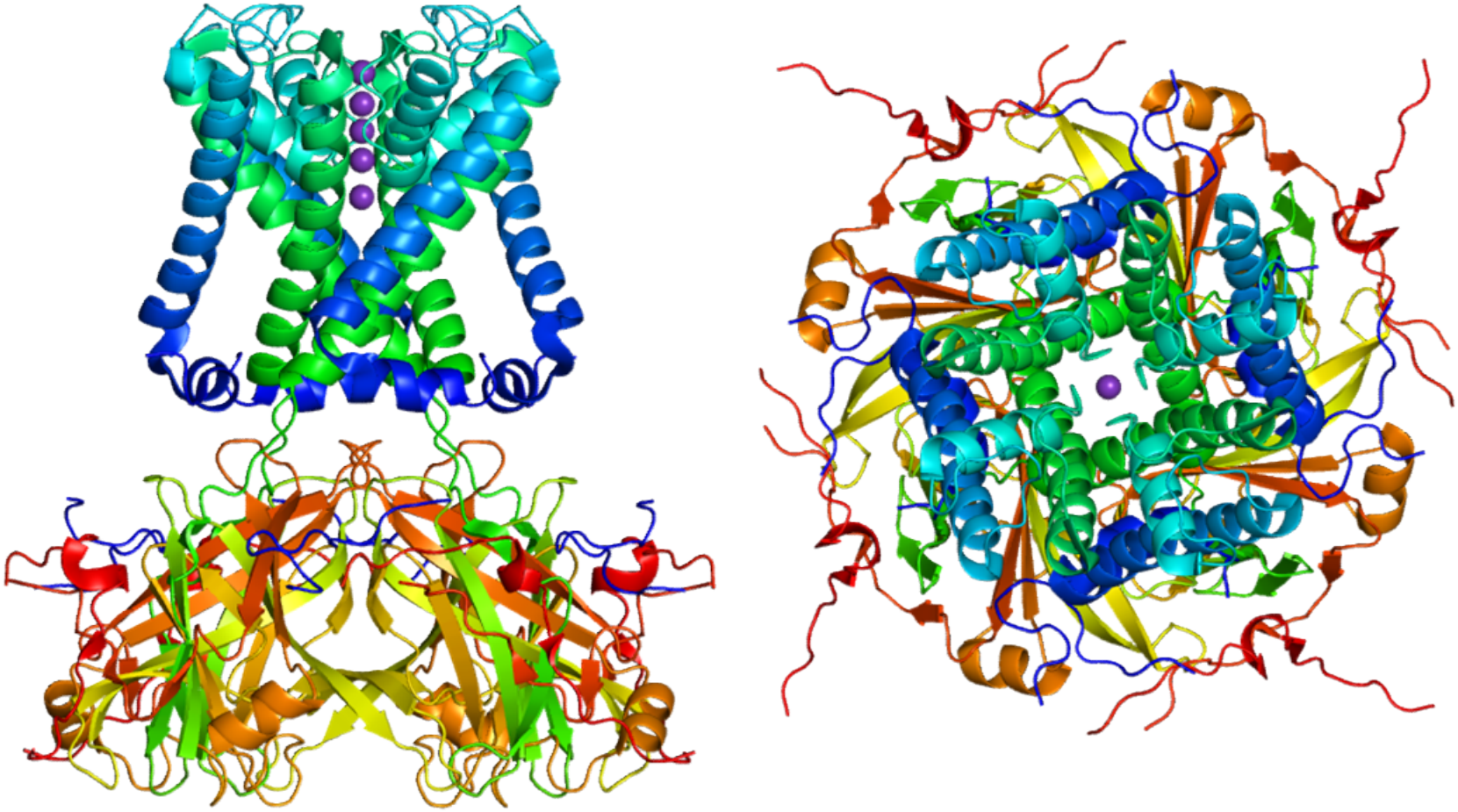
- Describe the 3D structure of a protein
- Plan a X-ray diffraction or electron microscopy experiment, from expression to structure
- Highlight main reasons to chose a structural biology technique over the others
- Recognize crucial factors that can improve/hamper a structural biology experiment
- Identify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

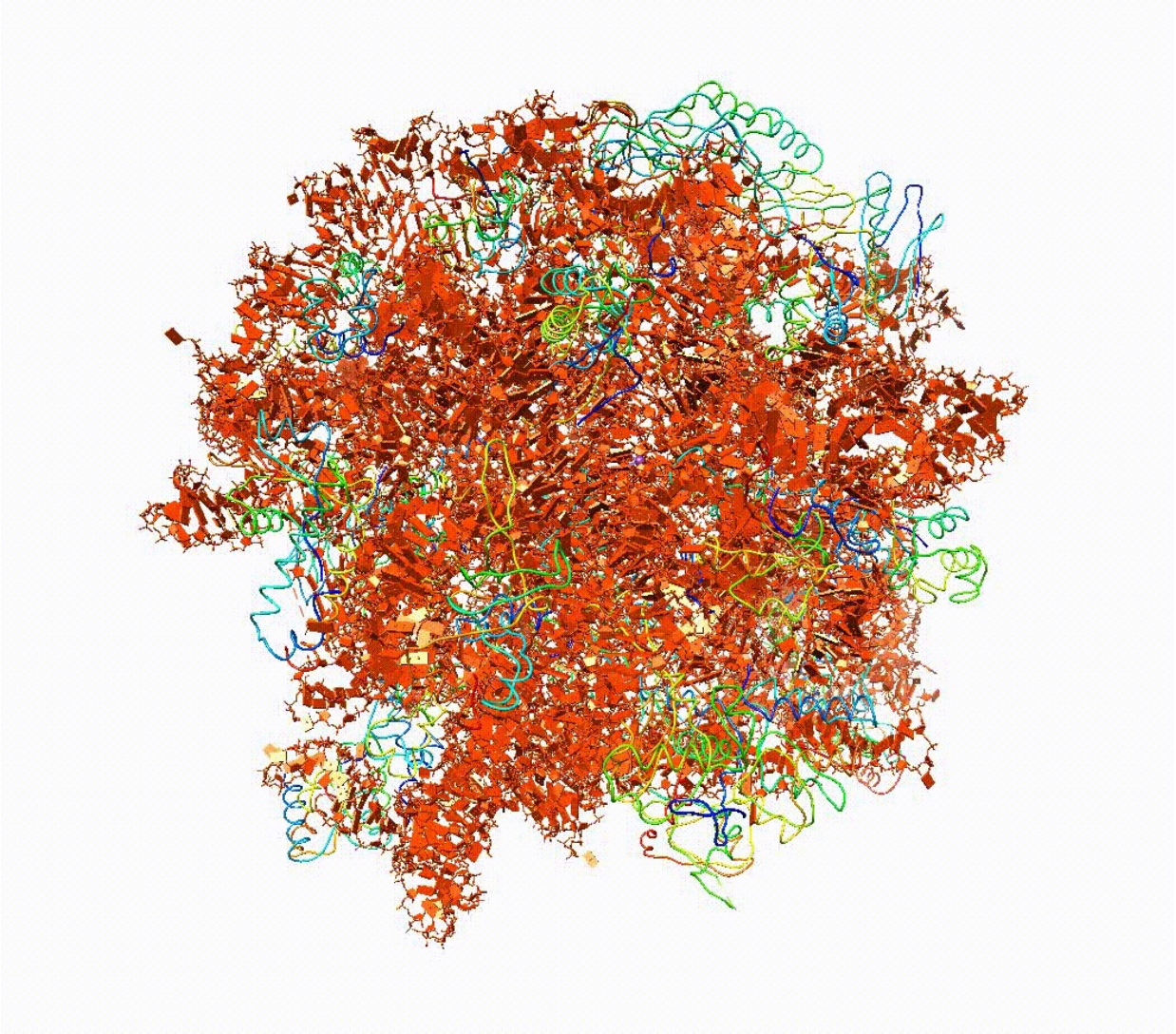
- Mechanisms of biologically relevant proteins and complexes: e.g. Potassium channel



PDB: 3zrs

Structure-function relationship

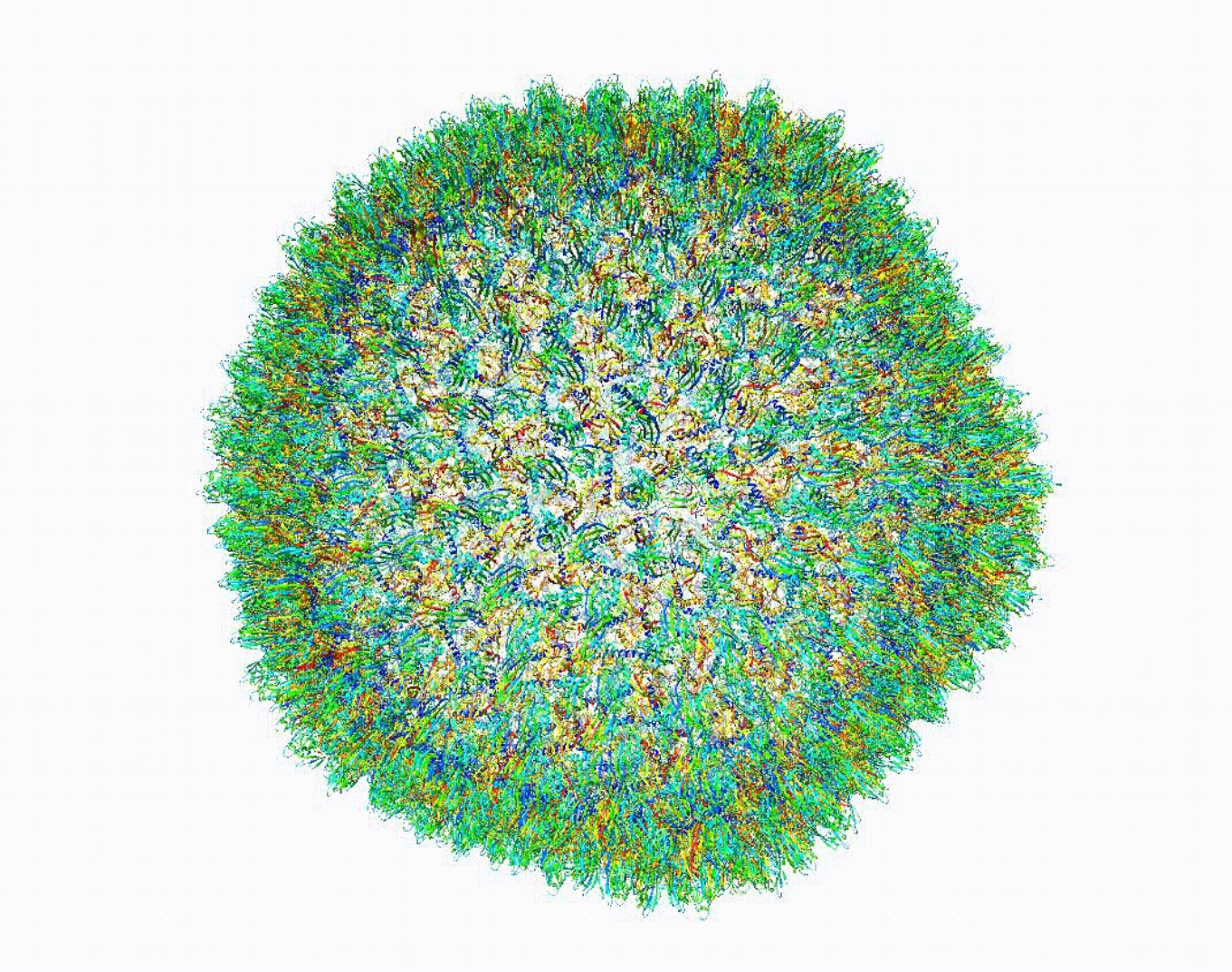
- Mechanisms of biologically relevant proteins and complexes: e.g. ribosome



PDB: 1ffk

Structure-function relationship

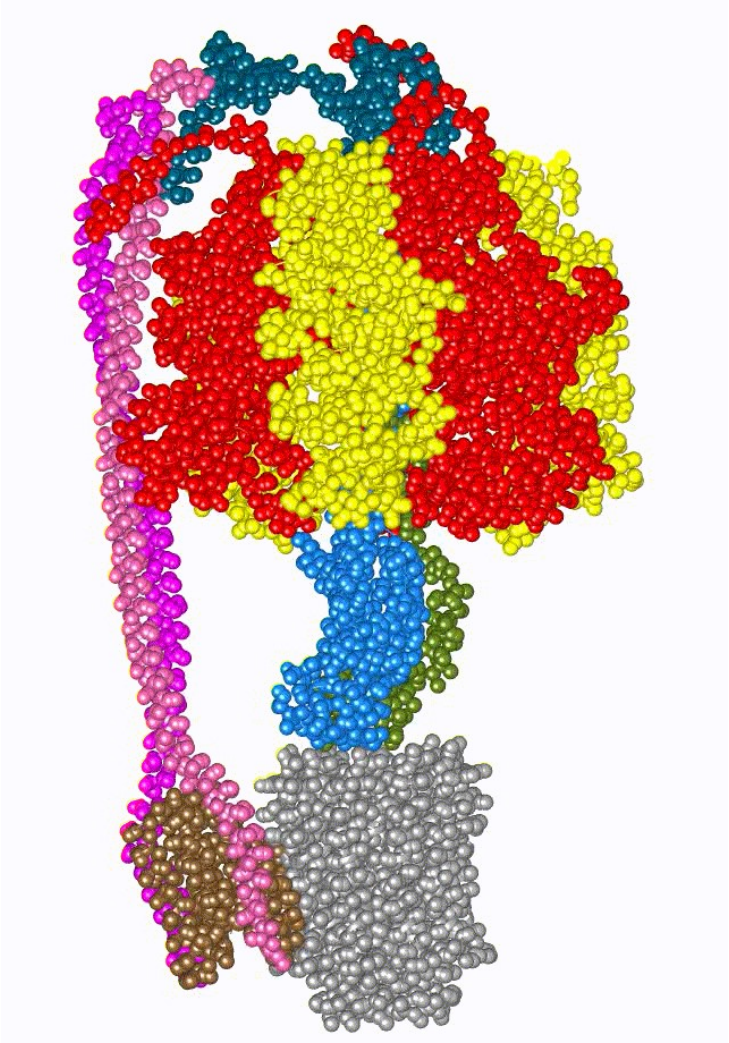
- Mechanisms of biologically relevant proteins and complexes:
  e.g. virus



PDB: 1w8x

Structure-function relationship

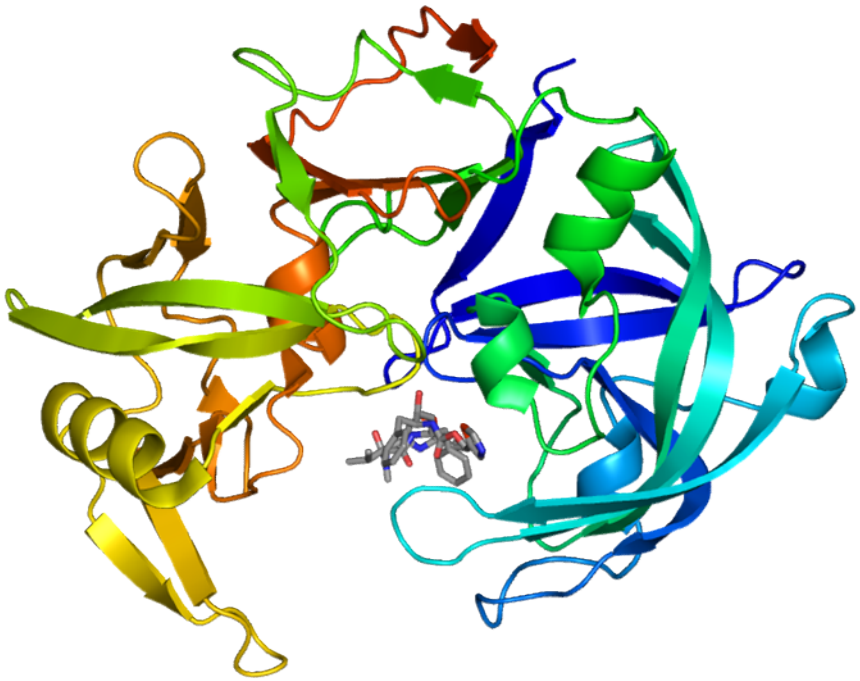
- Mechanisms of biologically relevant proteins and complexes:
  e.g. ATP-synthase



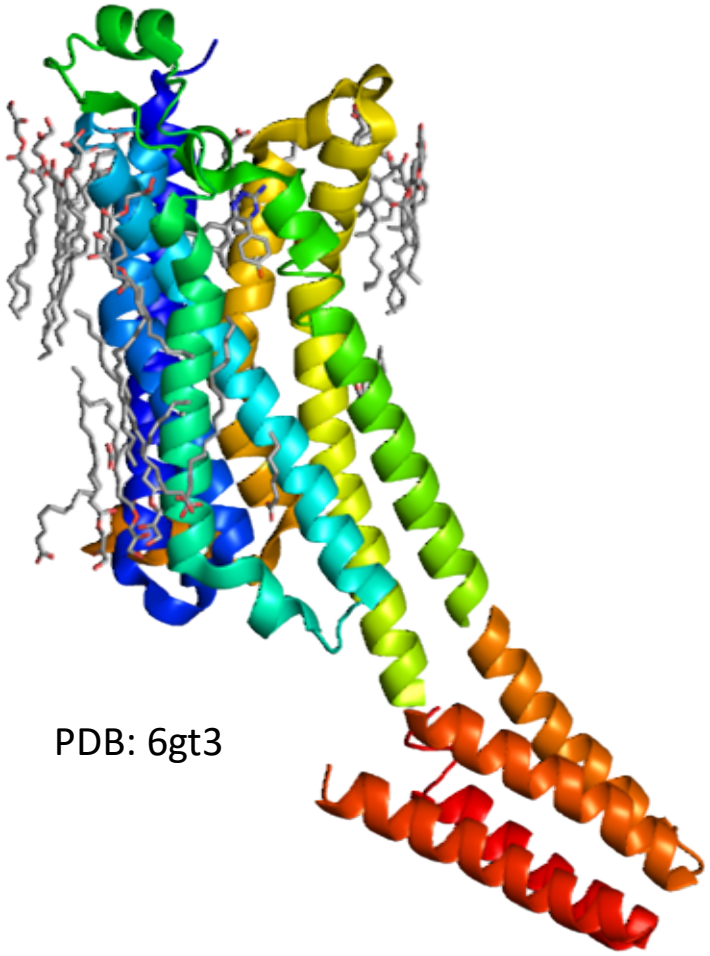
PDB: 5are, 5fil, 5fij, 5fik, 5ara, 5ari, 5arh

Structure-function relationship

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



PDB: 3q70



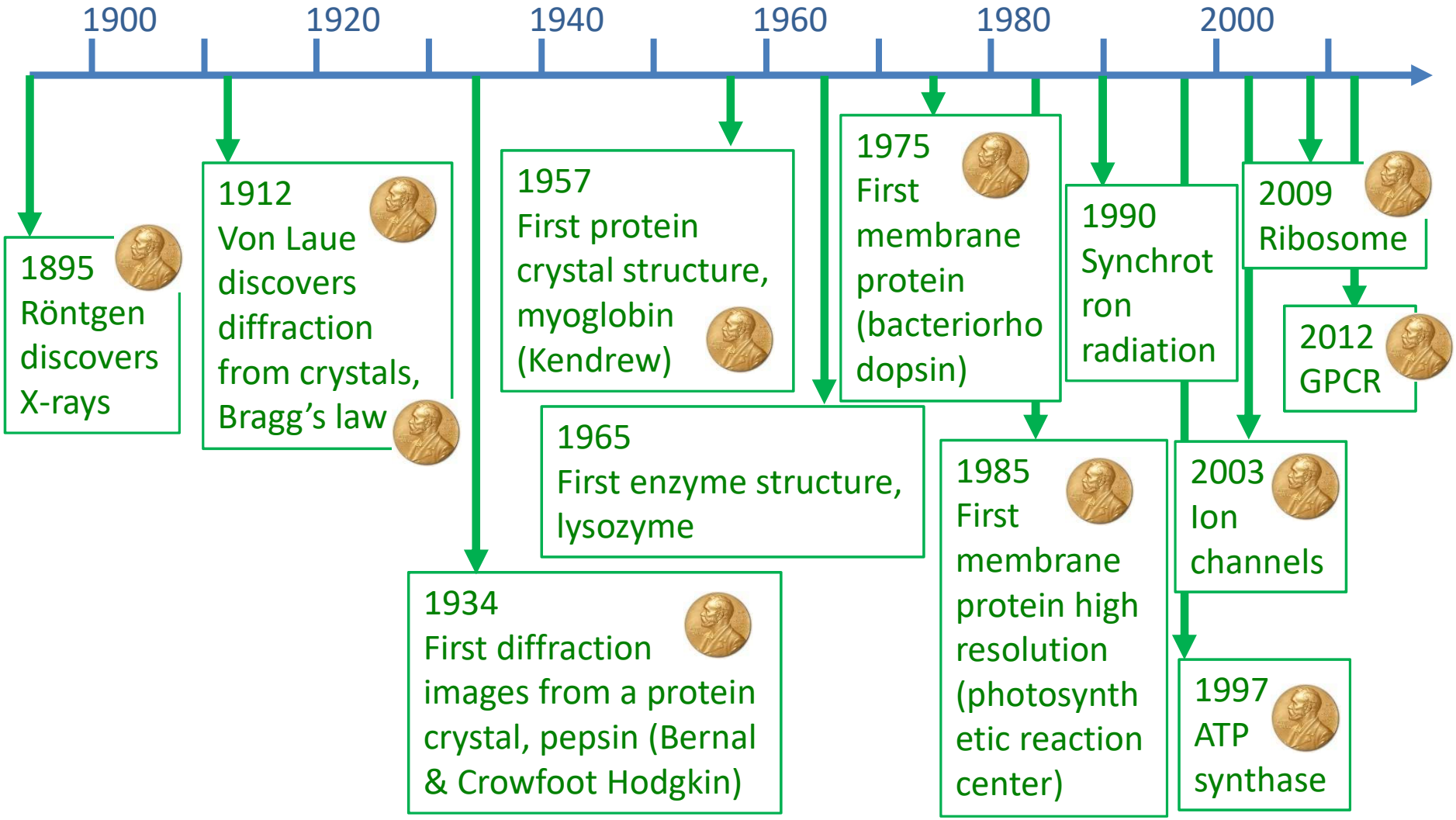
PDB: 6gt3

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, model validation

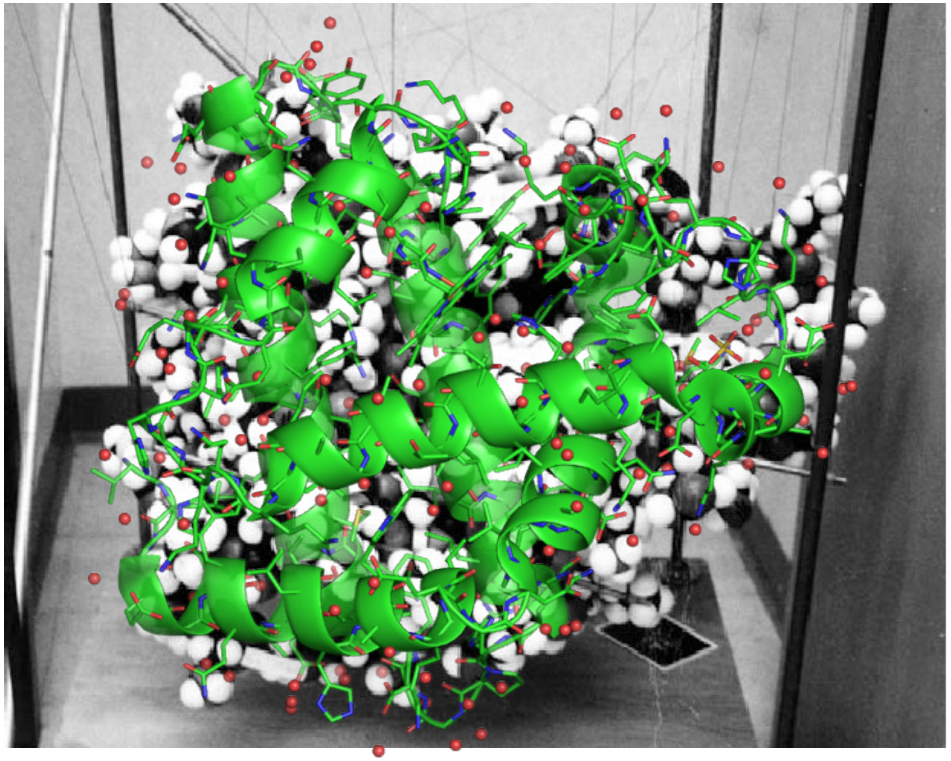
Different structural biology techniques give complementary results!!

Biocrystallography



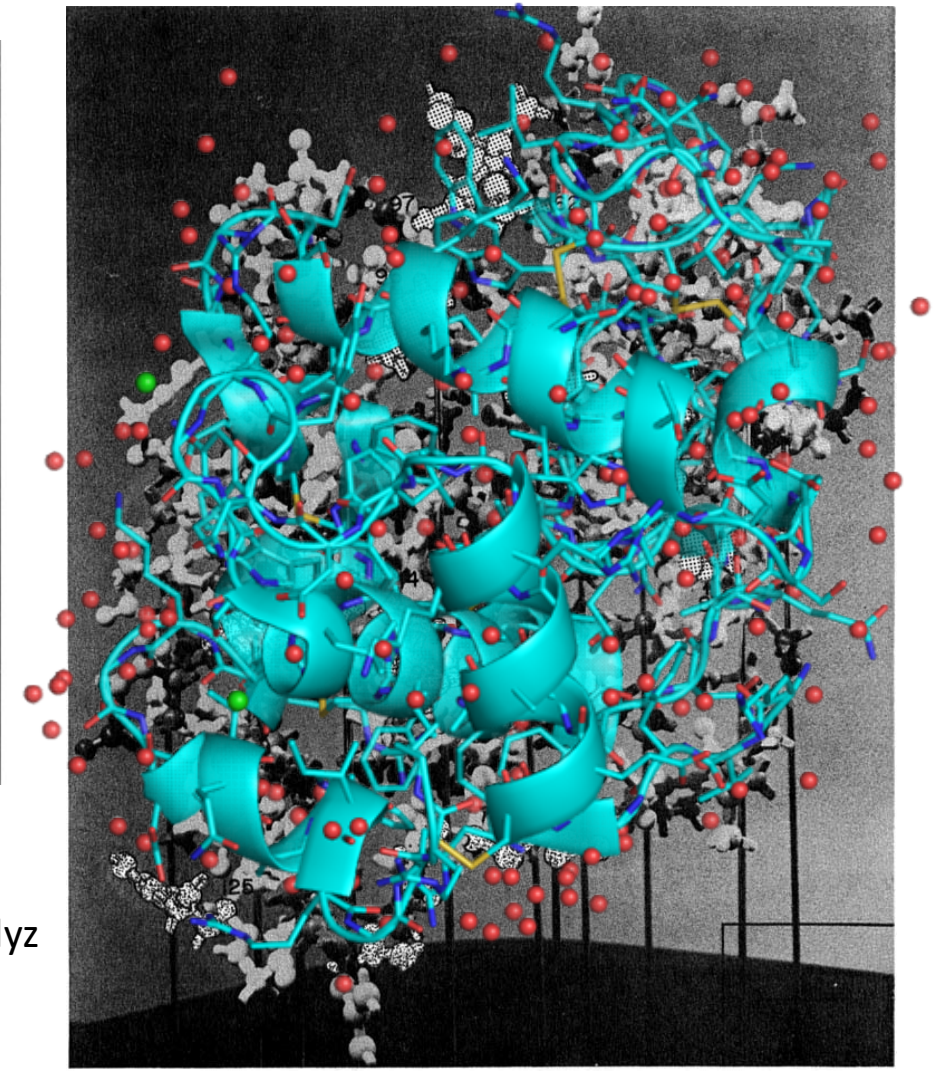
First protein structures determined by X-ray crystallography

- Myoglobin from sperm whale



PDB: 1vxa

- Lysozyme from chicken egg whites

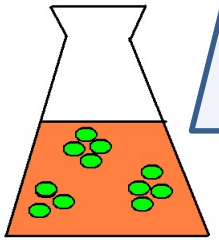


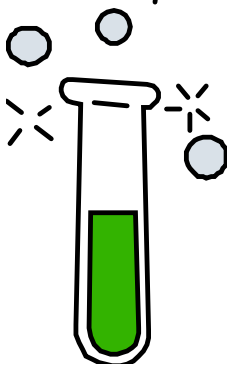
PDB: 1lyz

A biocrystallography experiment

Bioinformatics

Molecular biology

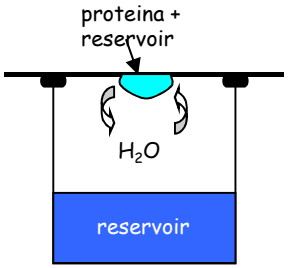


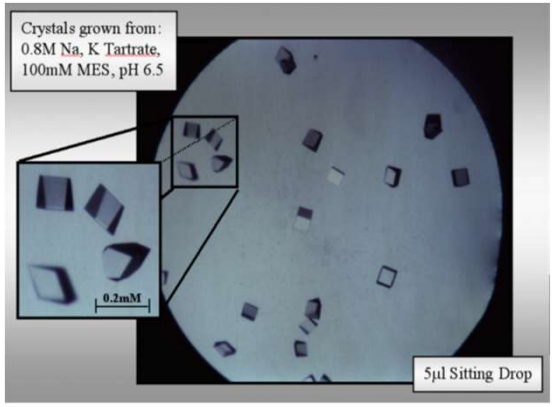


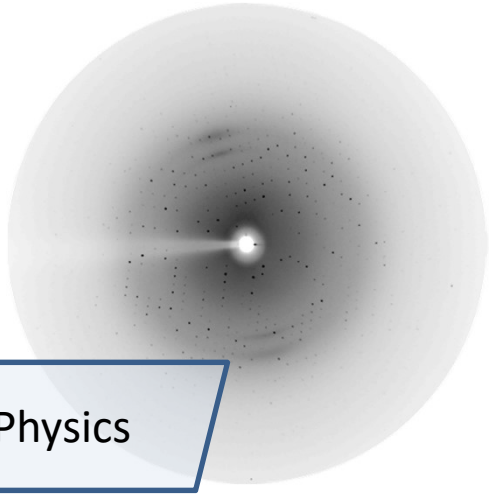
Expression and purification

Chemistry

Crystallization







Physics

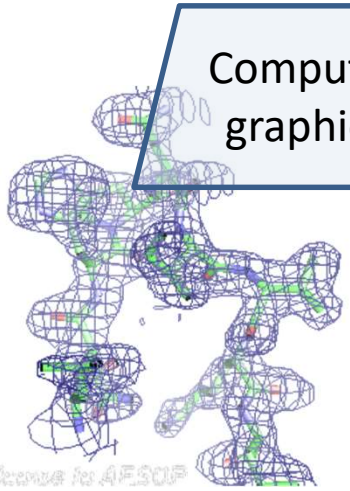
Diffraction data collection

Software development

Mathematics

Statistics

Phasing



Computer graphics

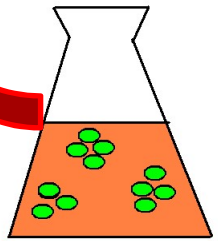
Structure refinement

Biology

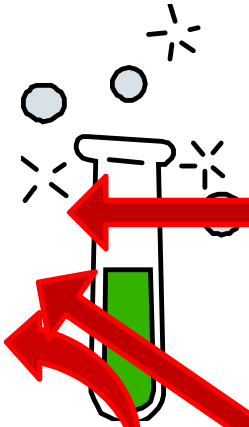
Biochemistry

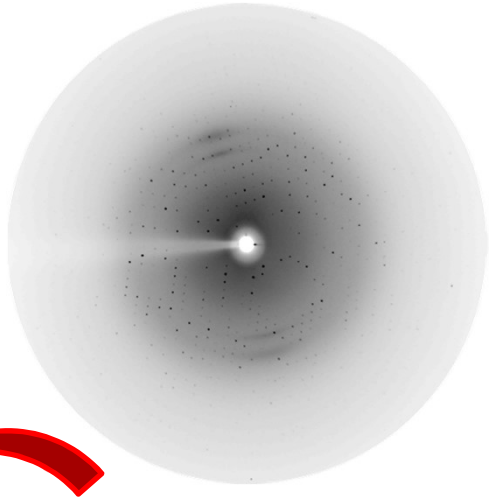
10 days to
10 years

A biocrystallography experiment

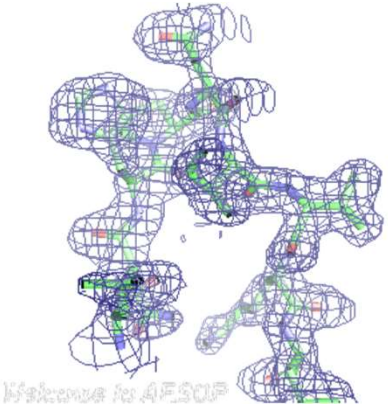


Expression and purification



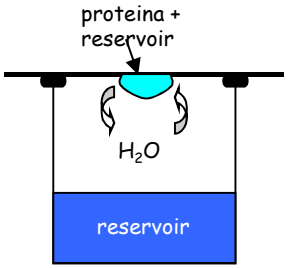


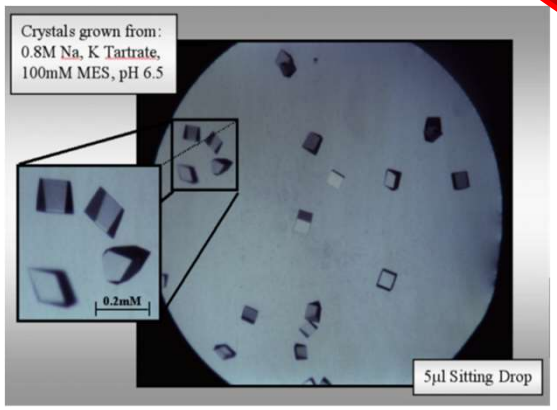
Diffraction data collection

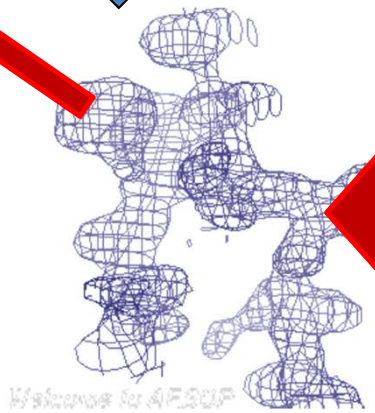


Structure refinement

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

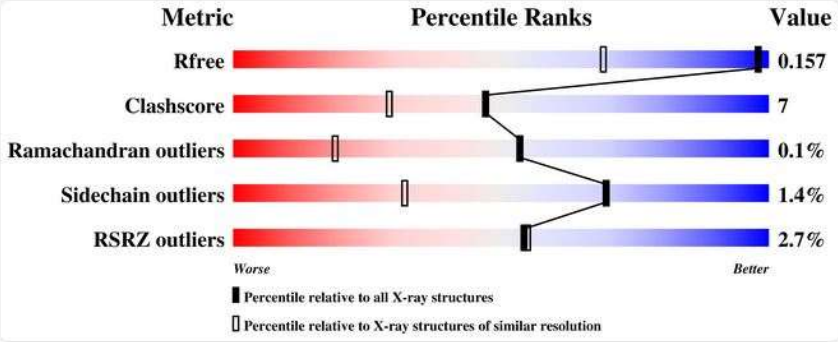
Software Package:

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)

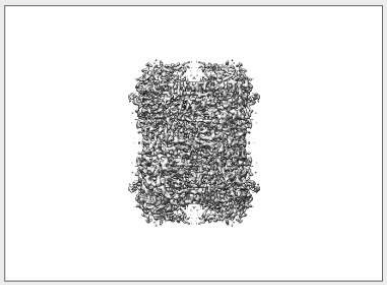
Source organism: *Rotavirus A* [28875]

Fitted atomic model: 6o6b

3Dbionotes: available for this entry

To be published

Map released:
2020-03-11



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

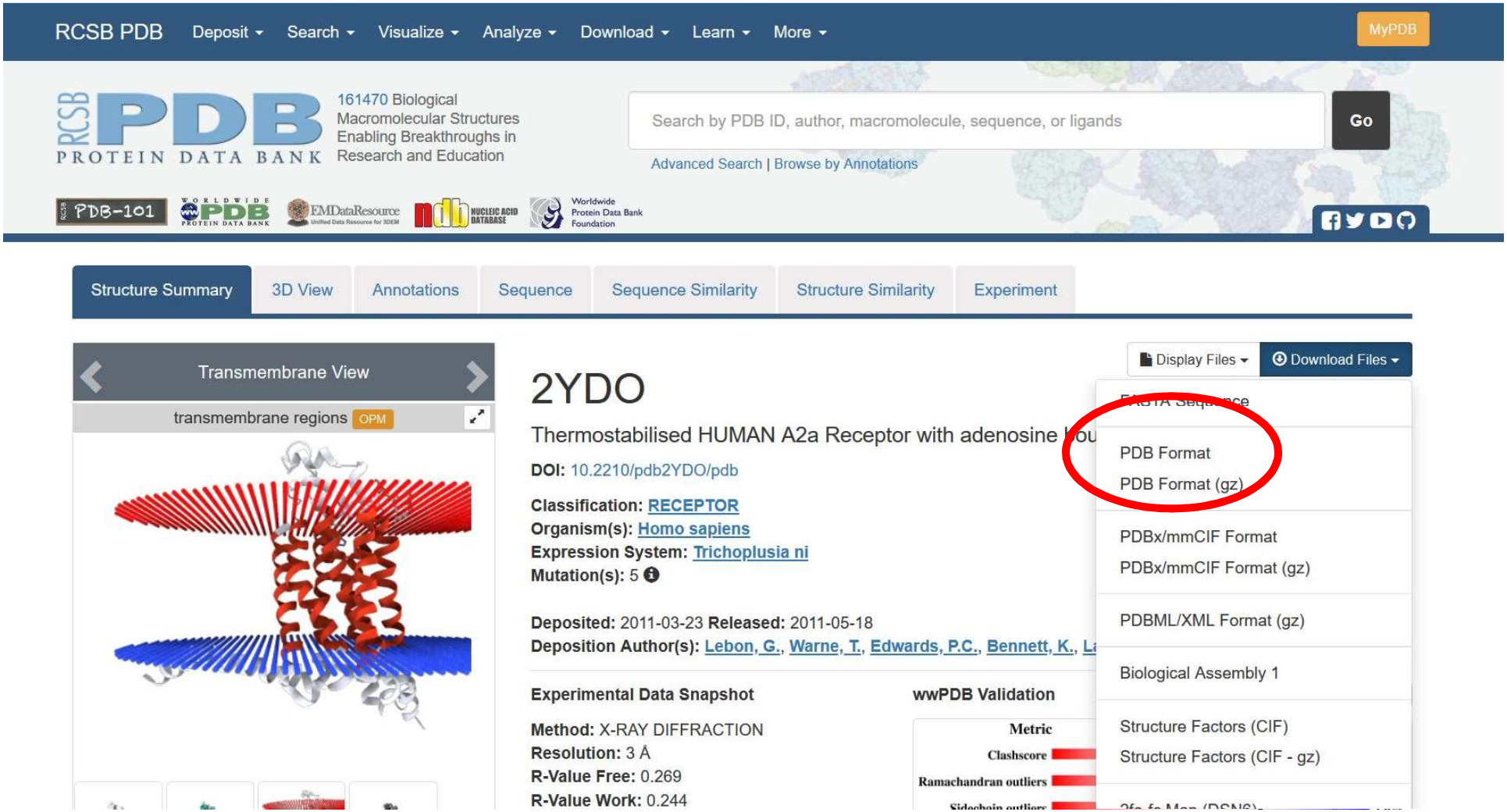
Details

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)

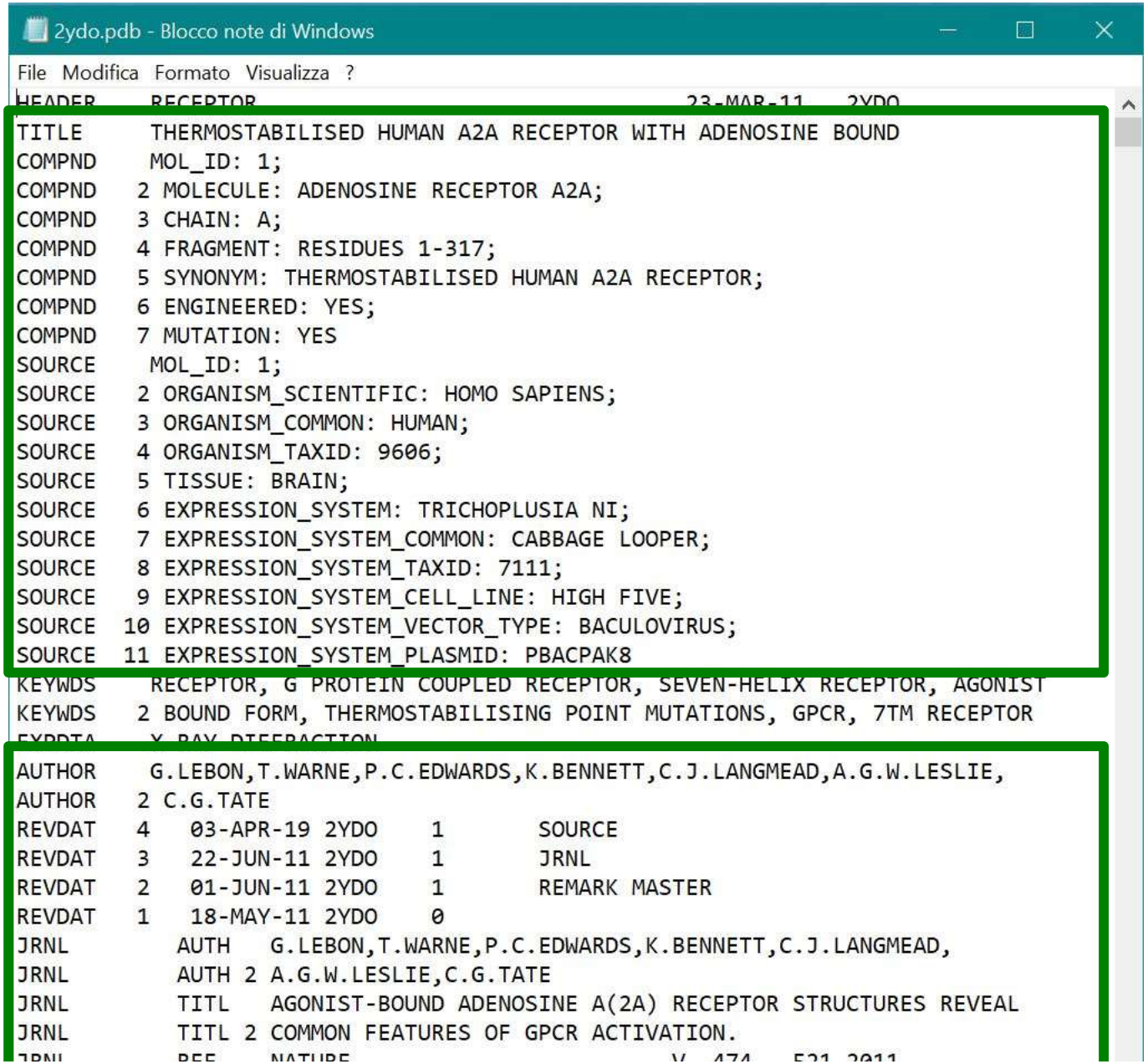
Related entries

- By authors
- By sample
- By organism

Structure atomic coordinates: pdb file



Structure atomic coordinates: pdb file



2ydo.pdb - Blocco note di Windows

File Modifica Formato Visualizza ?

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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
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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 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
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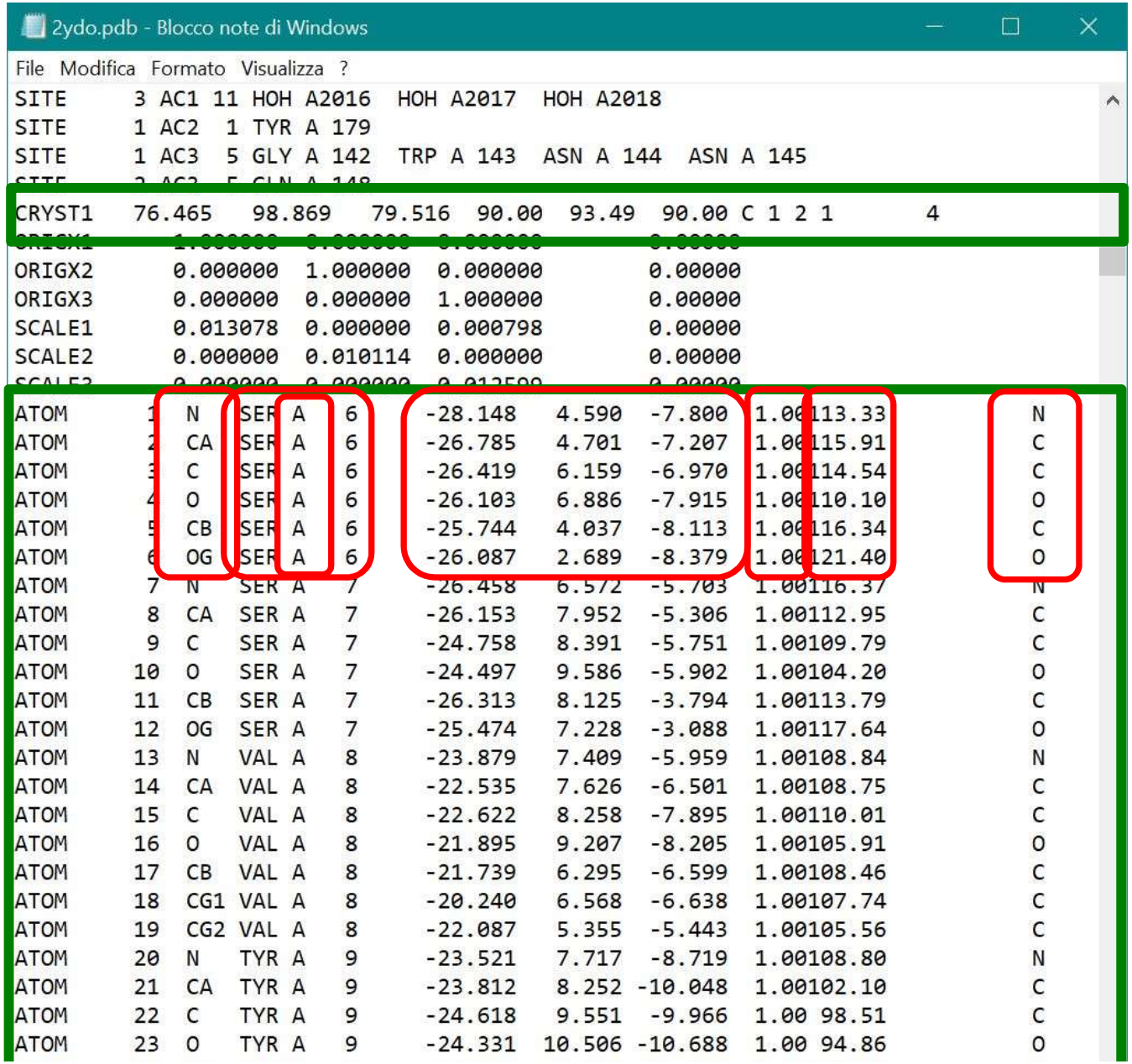
Information about the crystallographic experiment

Structure atomic coordinates: pdb file



2ydo.pdb - Blocco note di Windows

File Modifica Formato Visualizza ?

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SITE     1 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
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ORIGX2      0.000000  1.000000  0.000000        0.00000
ORIGX3      0.000000  0.000000  1.000000        0.00000
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SCALE2      0.000000  0.010114  0.000000        0.00000
SCALE3      0.000000  0.000000  0.012599        0.00000
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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
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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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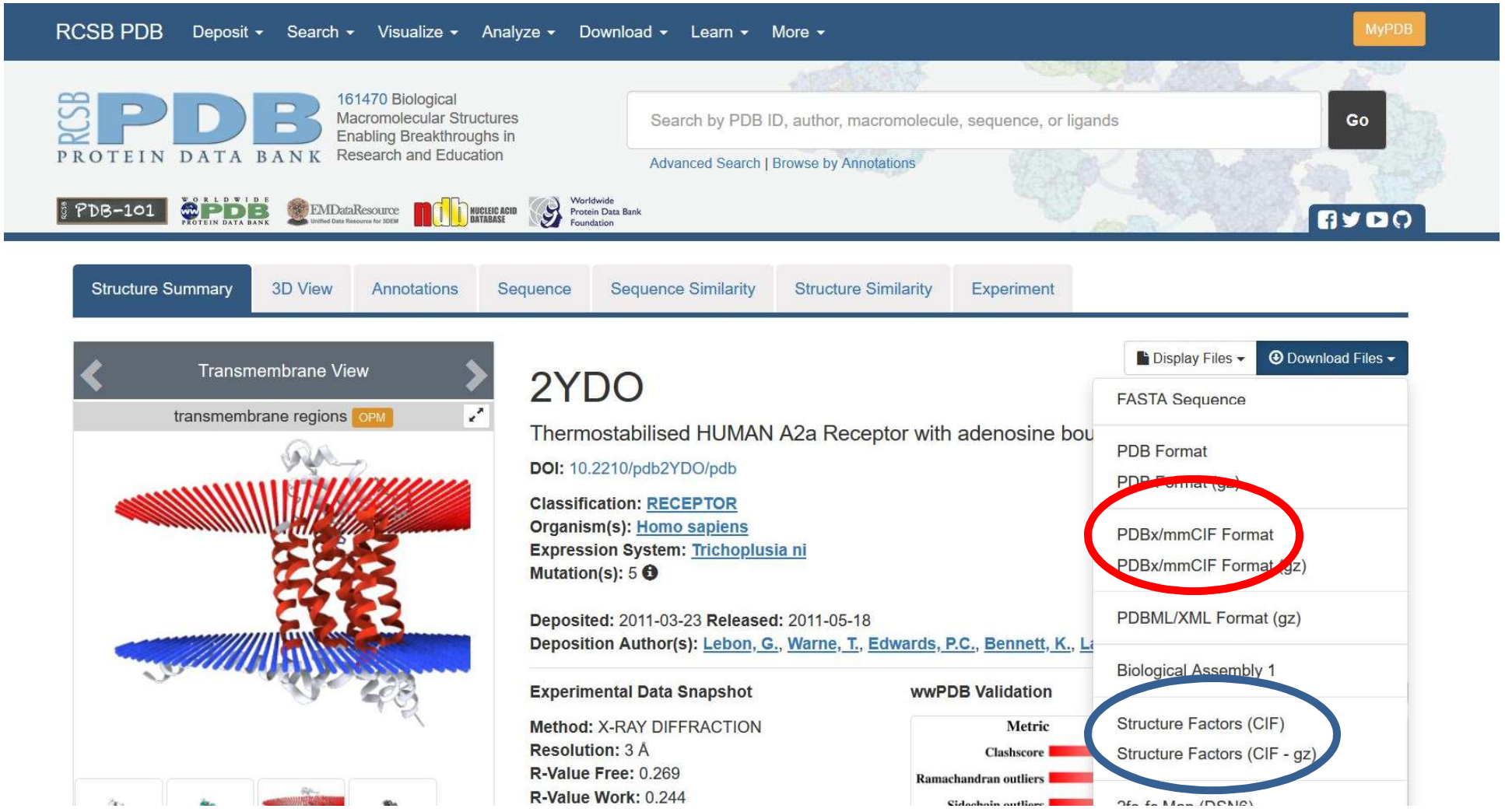
ATOM     15  C   VAL A   8     -22.622   8.258  -7.895  1.00110.01           C
ATOM     16  O   VAL A   8     -21.895   9.207  -8.205  1.00105.91           O
ATOM     17  CB  VAL A   8     -21.739   6.295  -6.599  1.00108.46           C
ATOM     18  CG1 VAL A   8     -20.240   6.568  -6.638  1.00107.74           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
```

Unit cell and symmetry

List of atoms with:

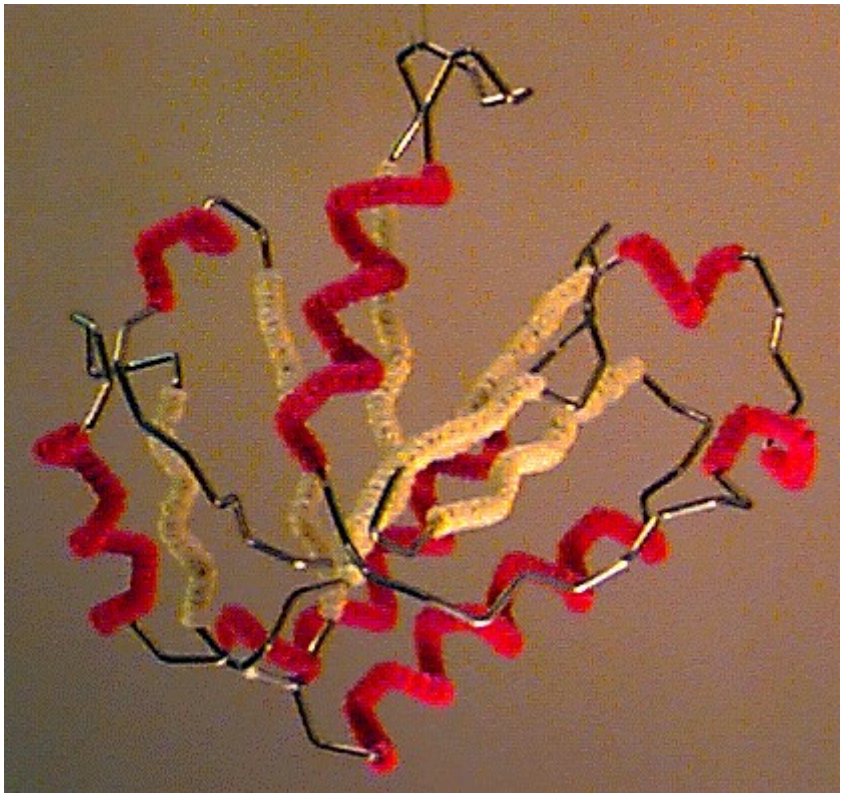
1. **Atom name**
2. **Residue type and number**
3. **Chain**
4. **Cartesian coordinates**
5. **Occupancy**
6. **Thermal factor**
7. **Atom type**

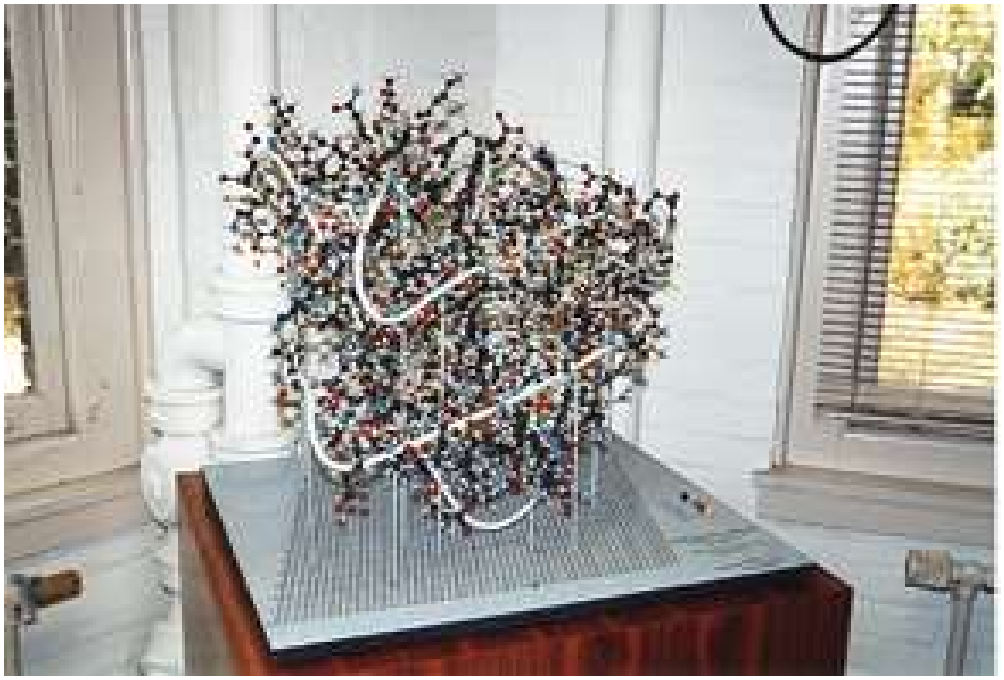
Structure atomic coordinates: mmCIF file



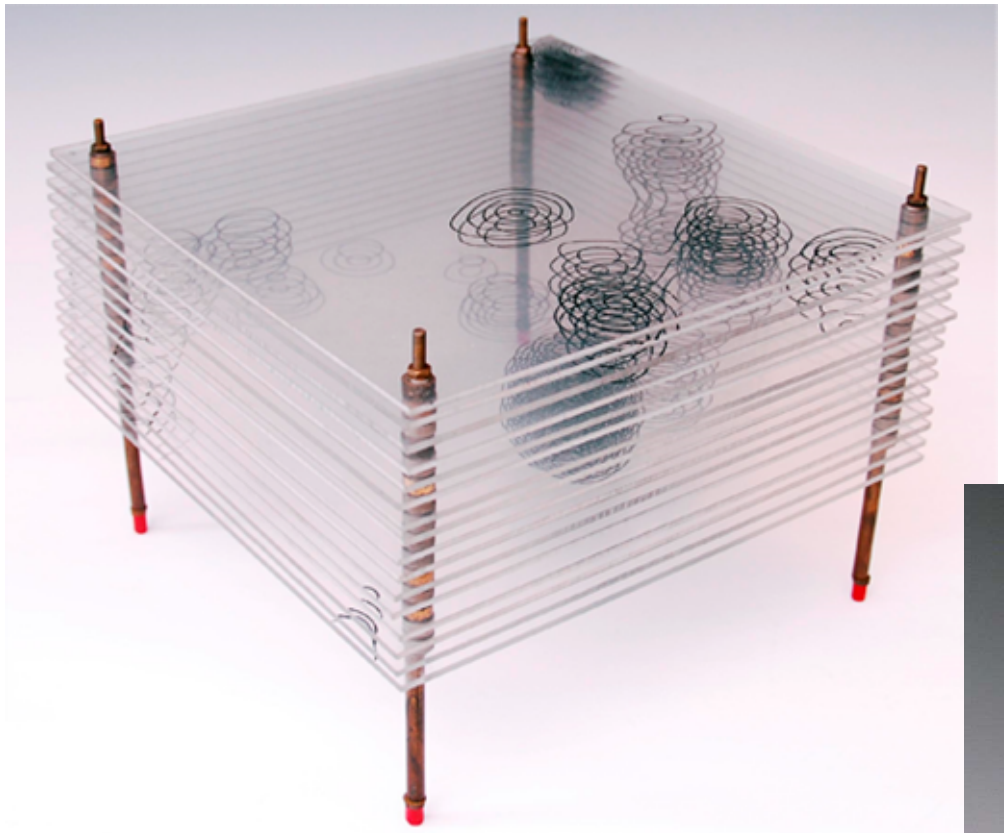
for the electron density!!!

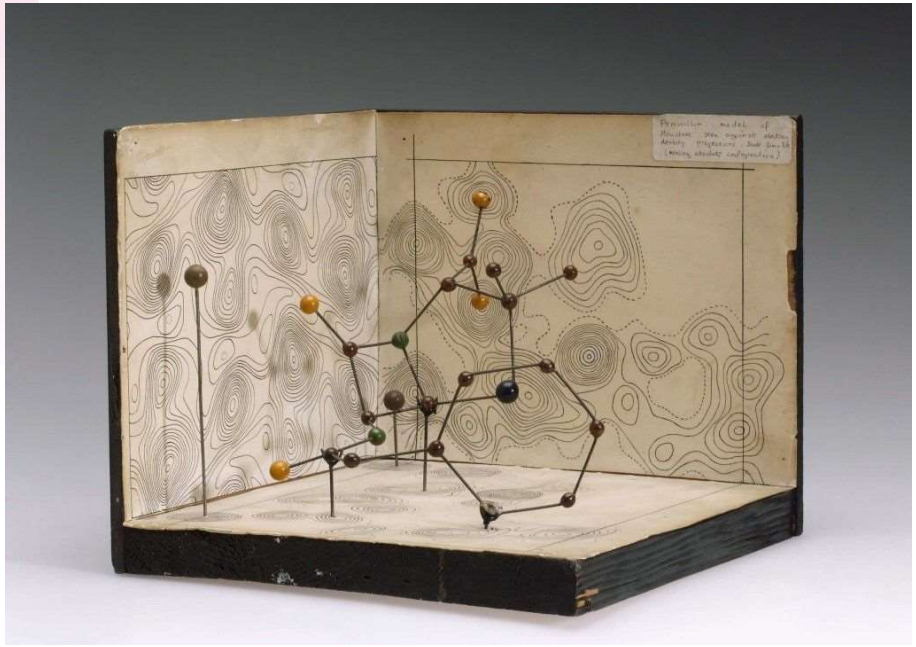
Graphical software: in the old days...





Graphical software: in the old days...



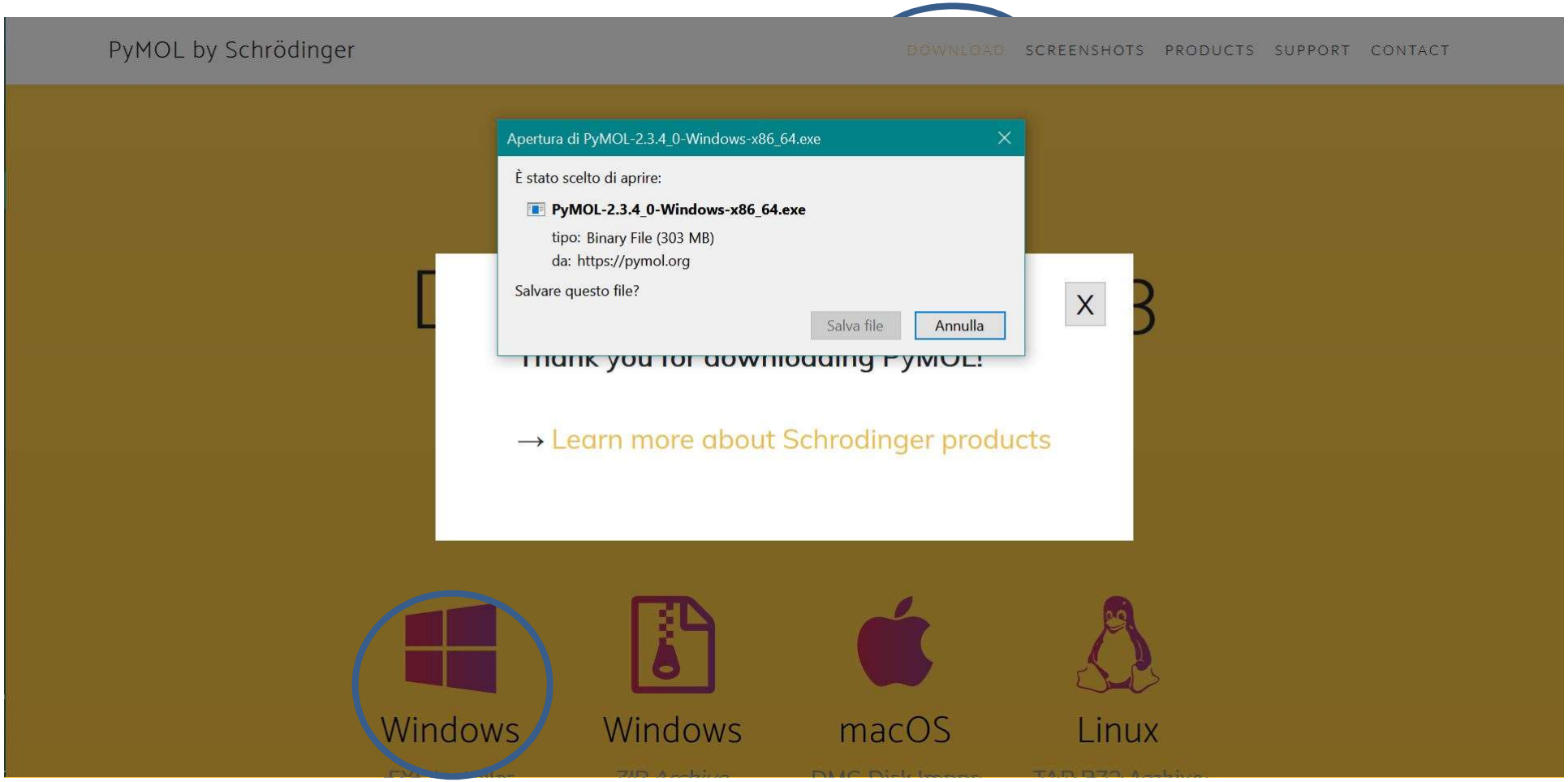


Graphical software: in the old days...

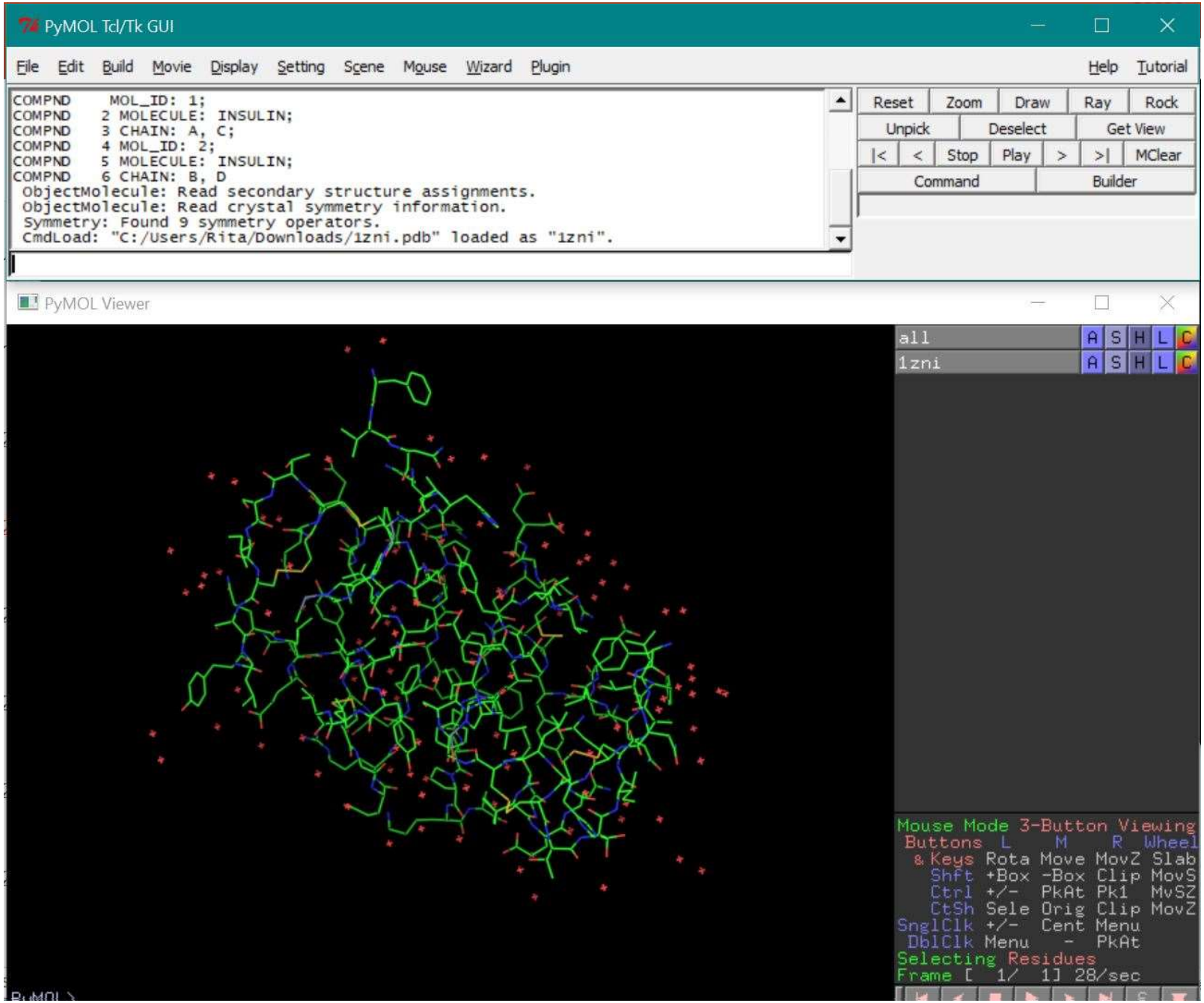
Graphical software:... today

Graphical software: Pymol

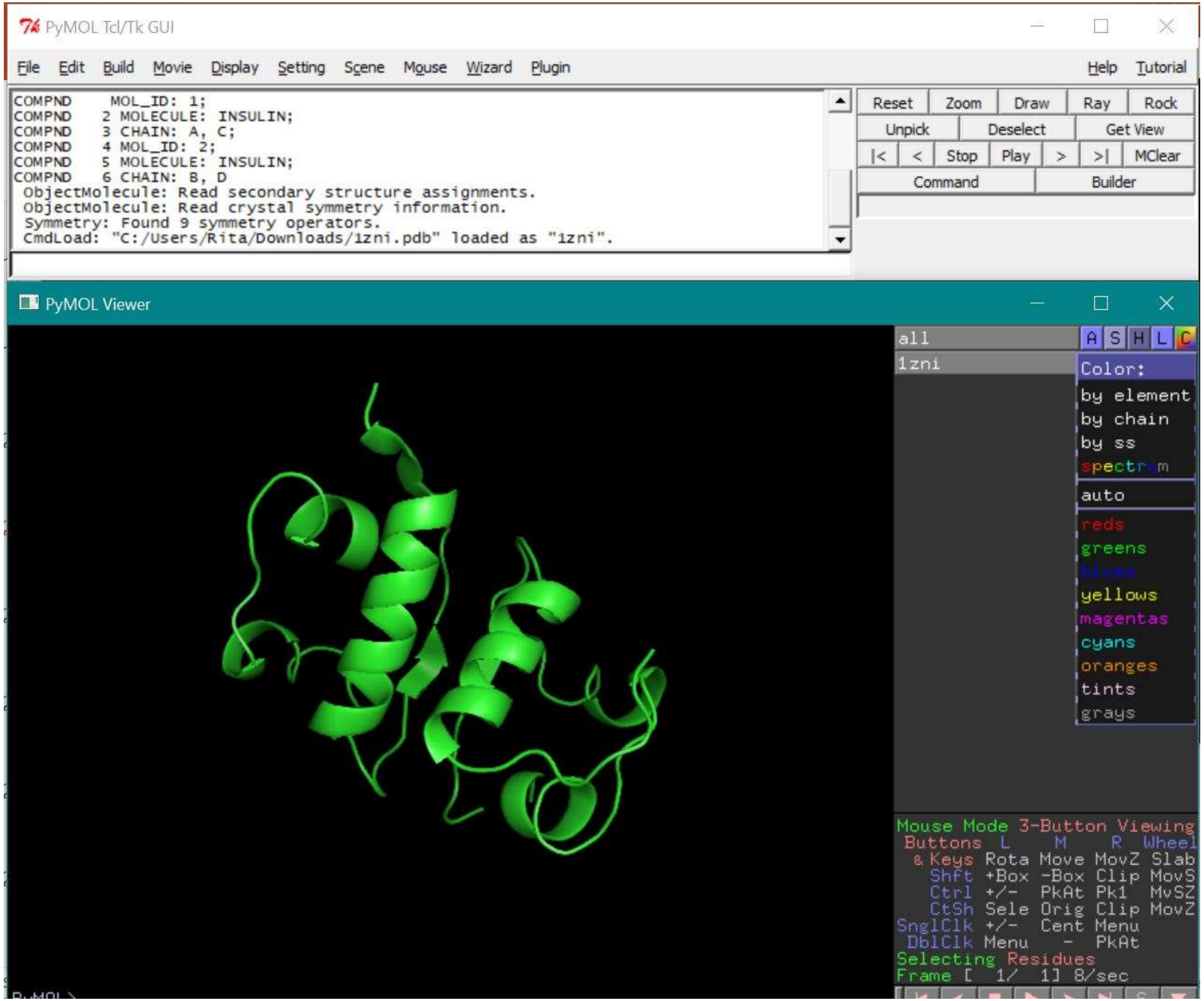
1. Download: https://pymol.org/2/



2. Open and load pdb file

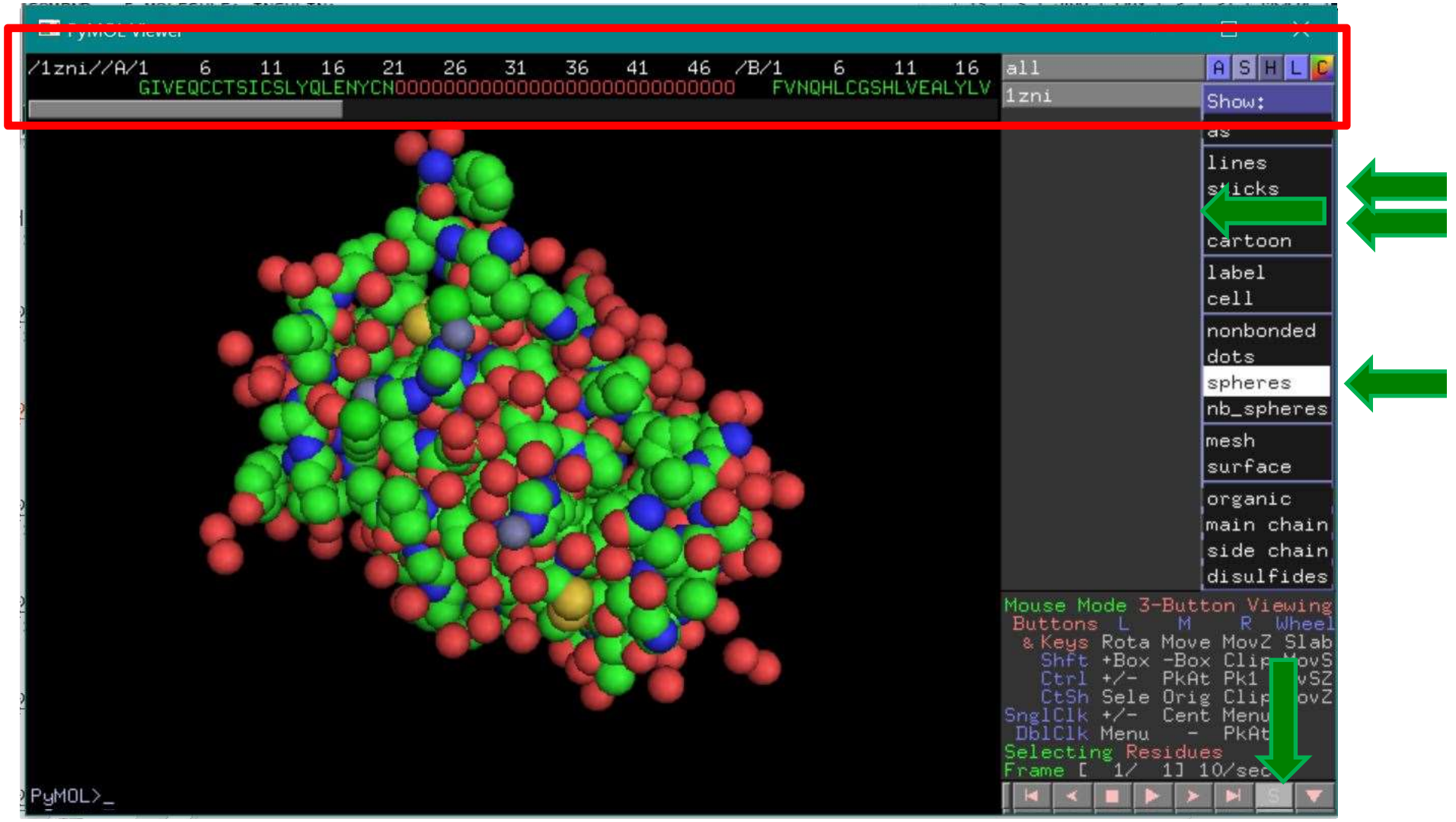


3. Menu options



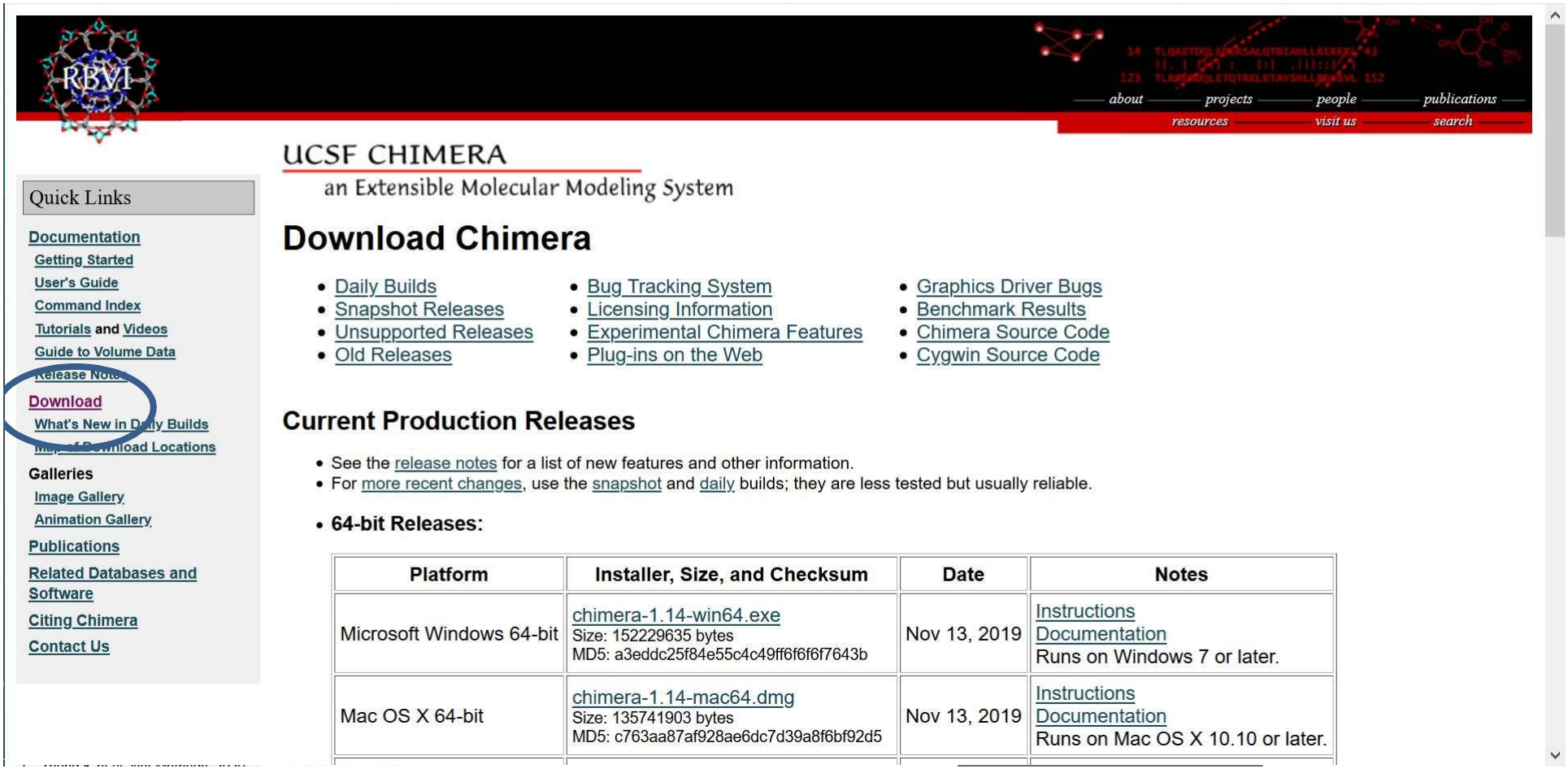
Visualization options:

spheres

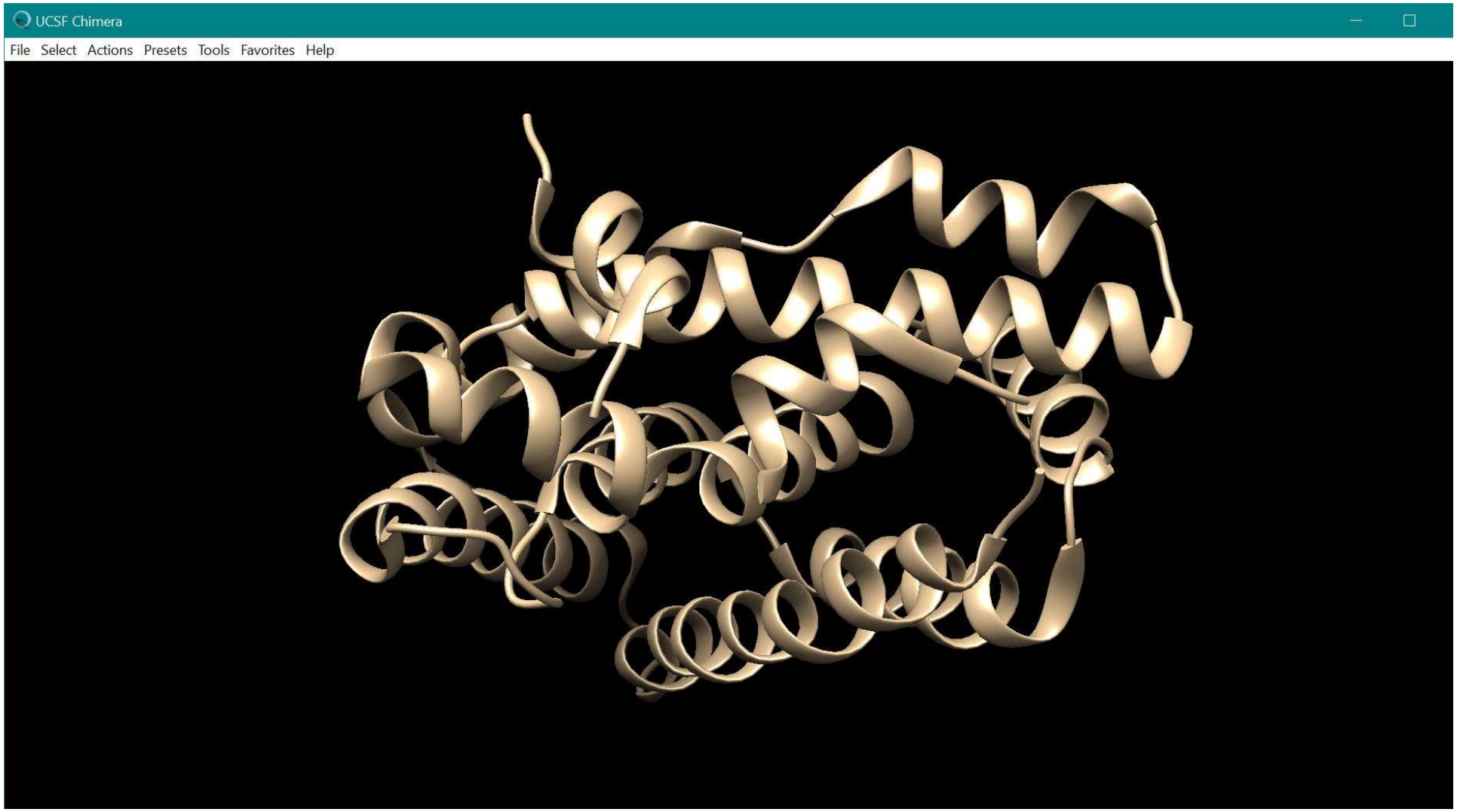


Graphical software: UCSF Chimera

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

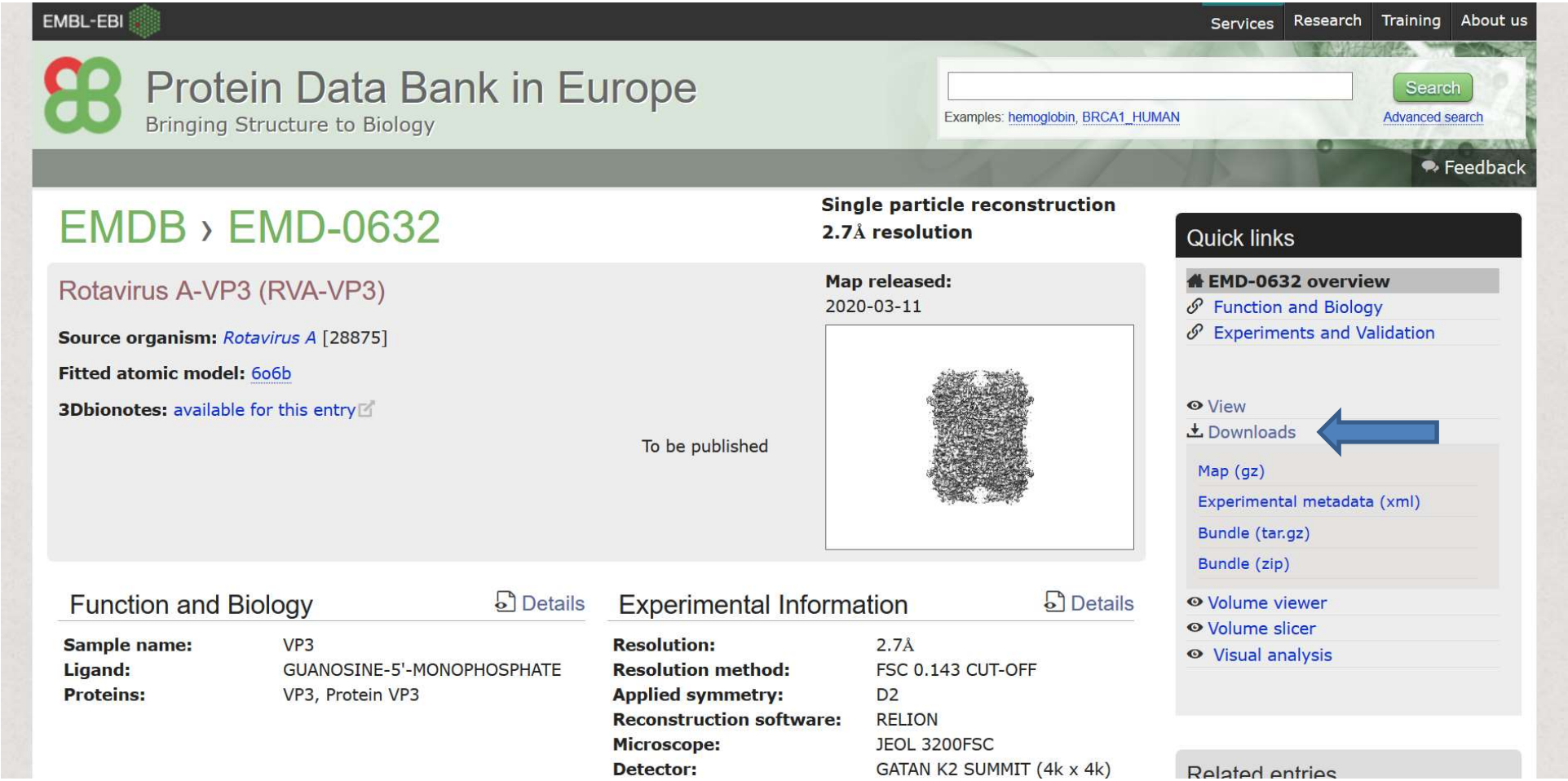


2. Open and load pdb file

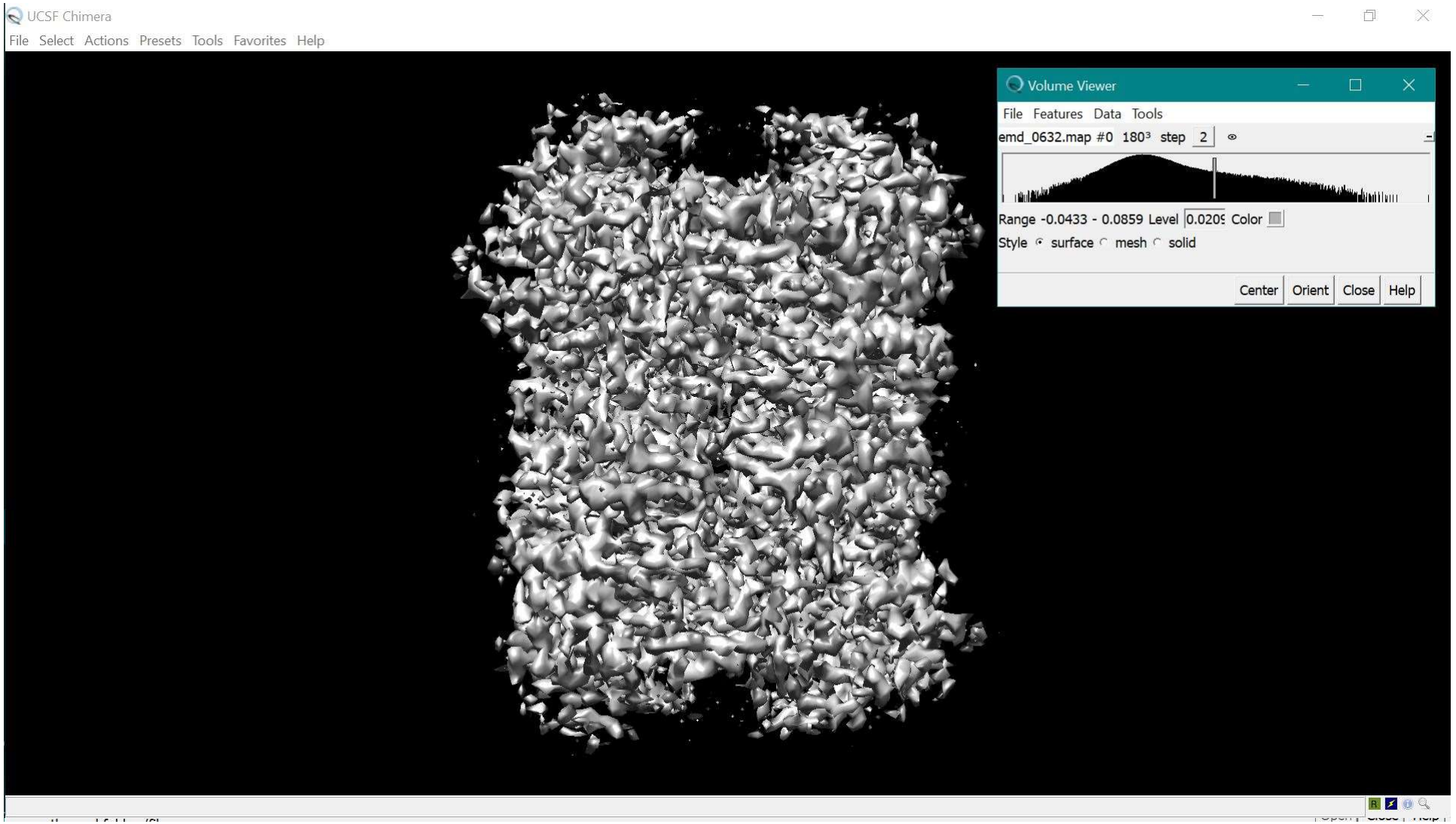


3. Load a map file (electron density!)

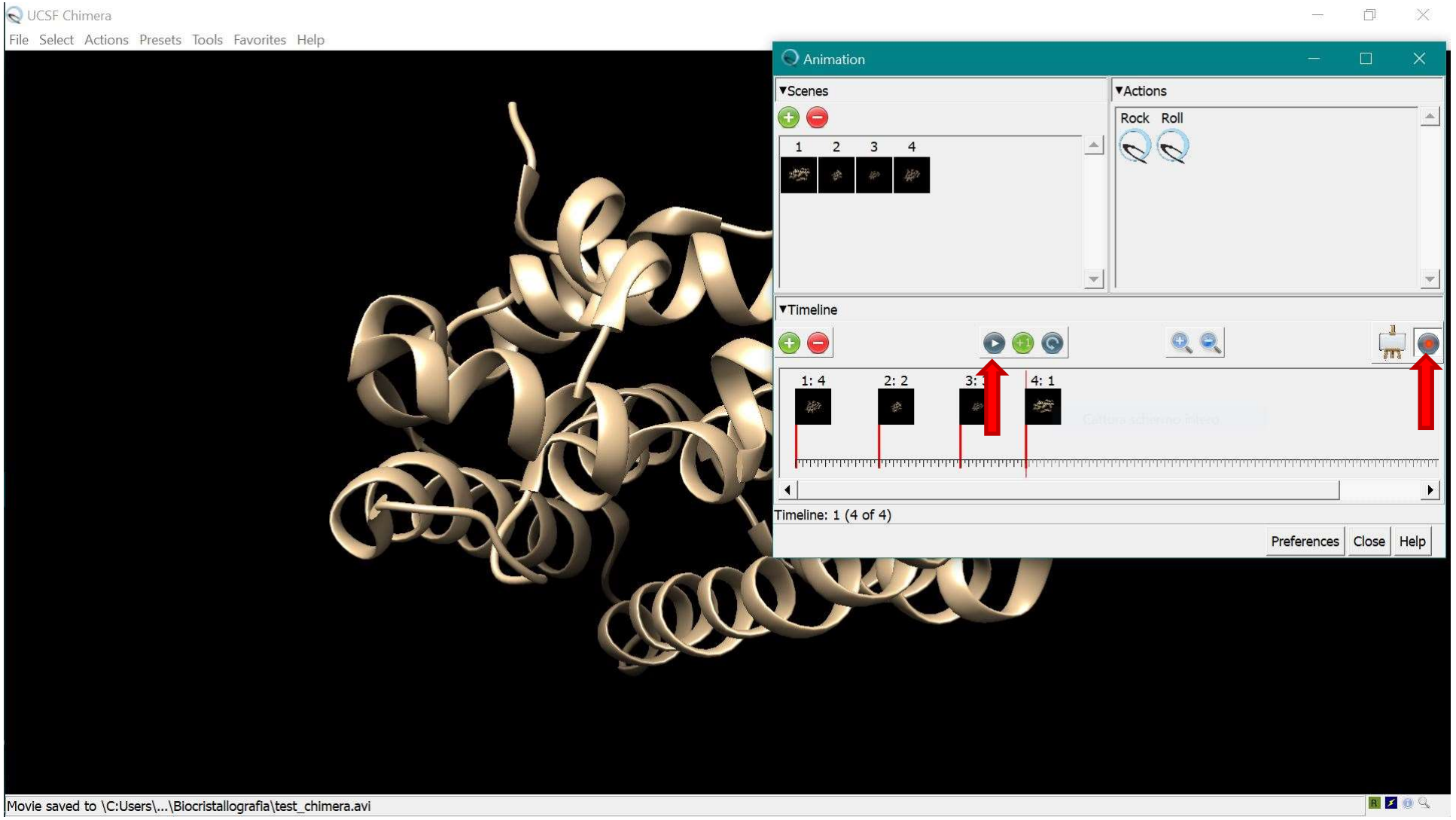
(www.ebi.ac.uk/pdbe/emdb/)



3. Load a map file (electron density!)



99.Animation!



99.Animation!