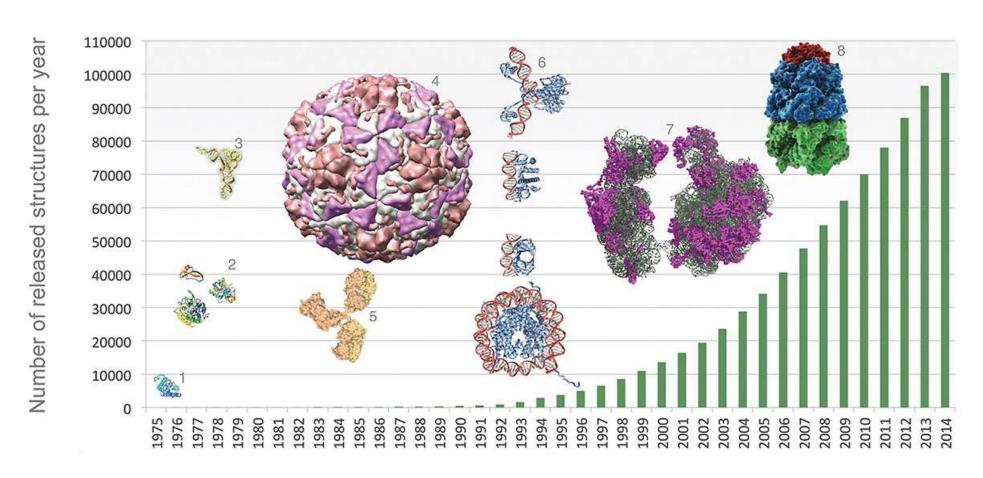
Introduction: Why structural studies?

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

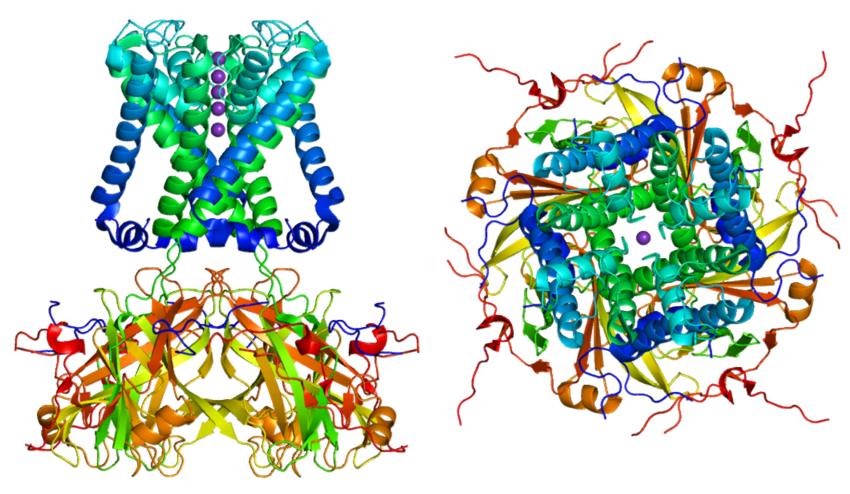
Apply knowledge to:

- 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

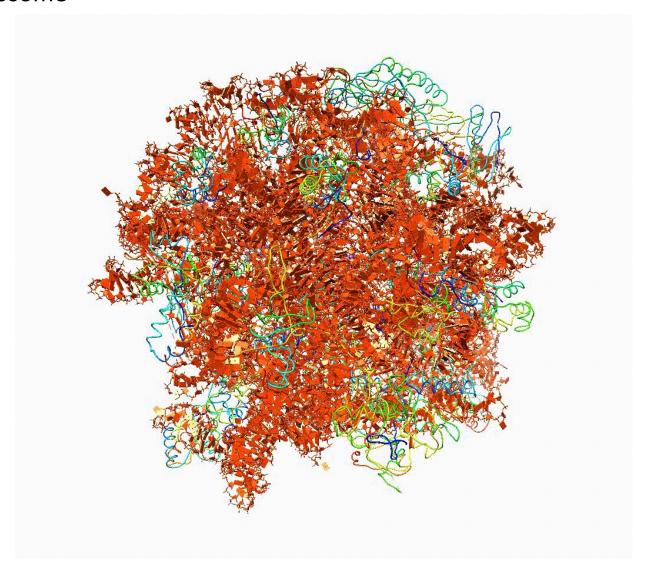
Communicate:

- 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

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

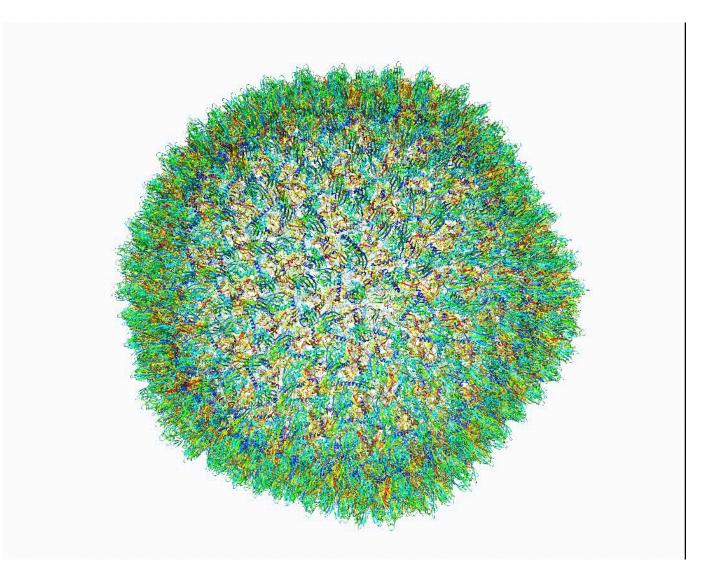


Mechanisms of biologically relevant proteins and complexes: e.g. ribosome



PDB: 1fft

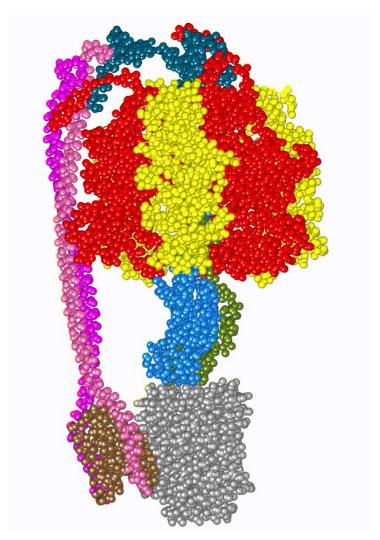
• Mechanisms of biologically relevant proteins and complexes: e.g. virus



PDB: 1w8x

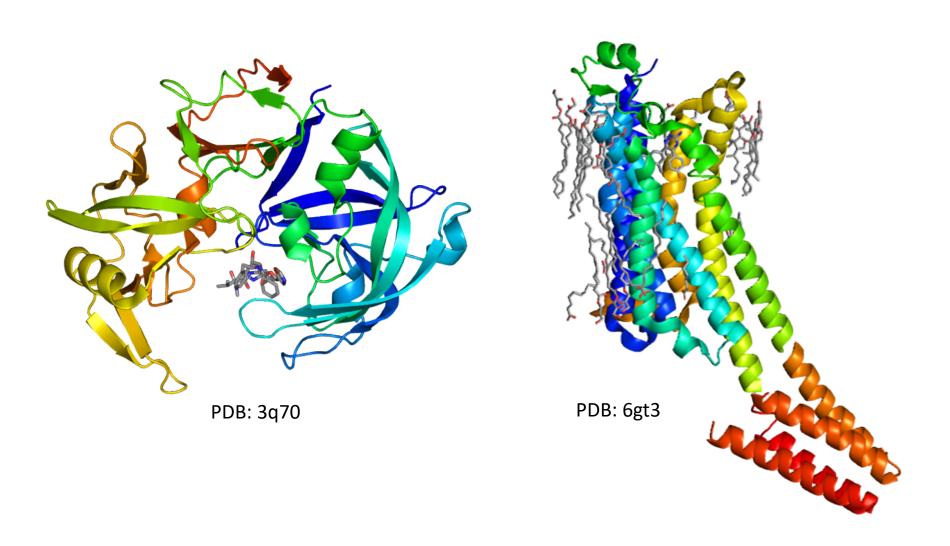
Mechanisms of biologically relevant proteins and complexes: e.g. ATP-

synthase



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

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

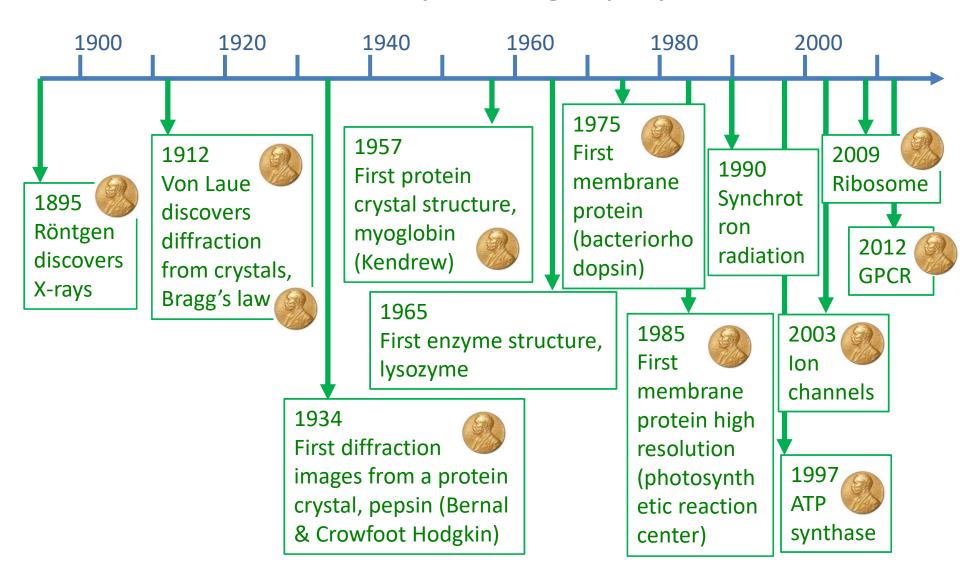


Structural biology: techniques

	X-ray crystallography	NMR	Electron Microscopy
Protein size	No limitation	< 80 KDa	> 50 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

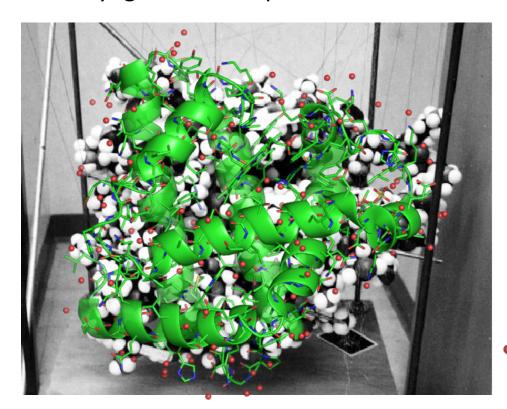
Different structural biology techniques give complementary results!!

Biocrystallography



First protein structures determined by X-ray crystallography

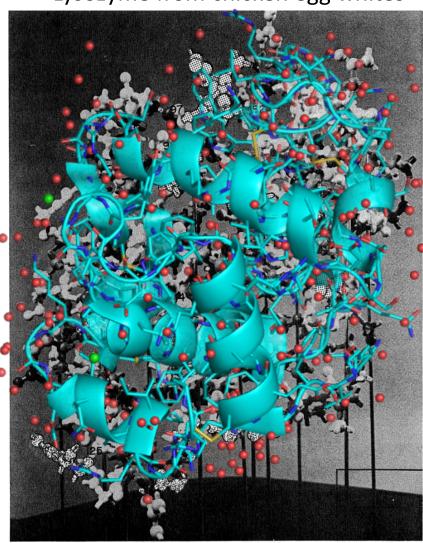
Myoglobin from sperm whale



PDB: 1vxa

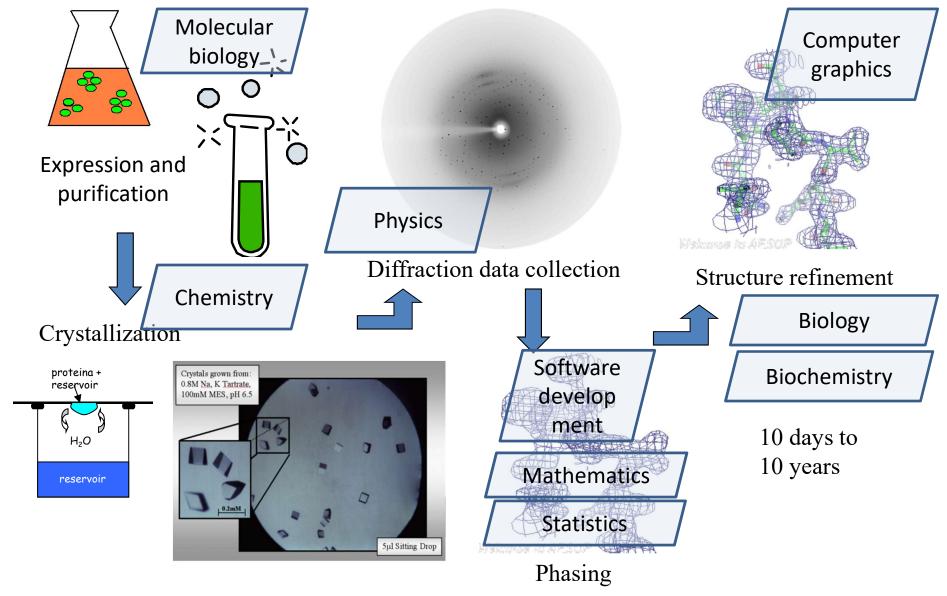
PDB: 1vxa

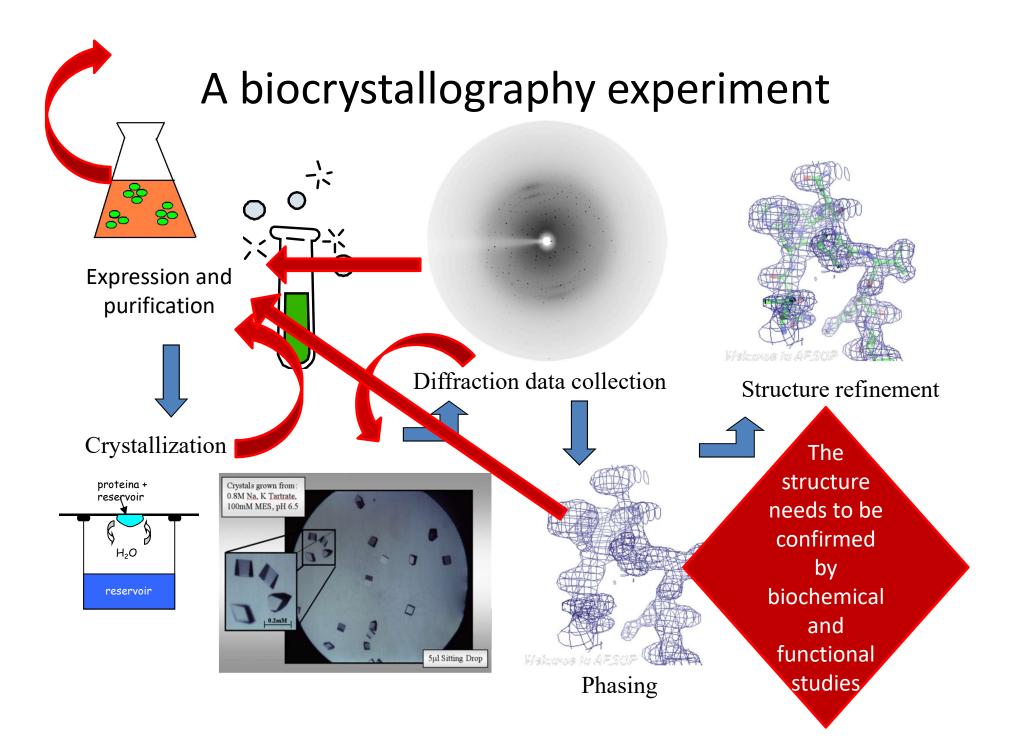
• Lysozyme from chicken egg whites



Bioinfor matics

A biocrystallography experiment





Databank resources: PDB (www.rcsb.org)

RCSB PDB Deposit ▼ Search ▼ Visualize ▼ Analyze ▼ Download ▼ **Experimental Data & Validation Experimental Data** Structure Validation View Full Validation Report or Ramachandran Plots Method: X-RAY DIFFRACTION Unit Cell: Resolution: 1.16 Å Length (Å) Angle (°) Percentile Ranks Metric Value R-Value Free: 0.157 Rfree 0.157 a = 124.872 $\alpha = 90.00$ R-Value Work: 0.138 Clashscore Space Group: | 4 b = 124.872 $\beta = 90.00$ Ramachandran outliers c = 175.683y = 90.00Sidechain outliers RSRZ outliers Software Package: Percentile relative to all X-ray structures Software Name Purpose Percentile relative to X-ray structures of similar resolution MOLREP phasing HKL-2000 data reduction

View more in-depth experimental data

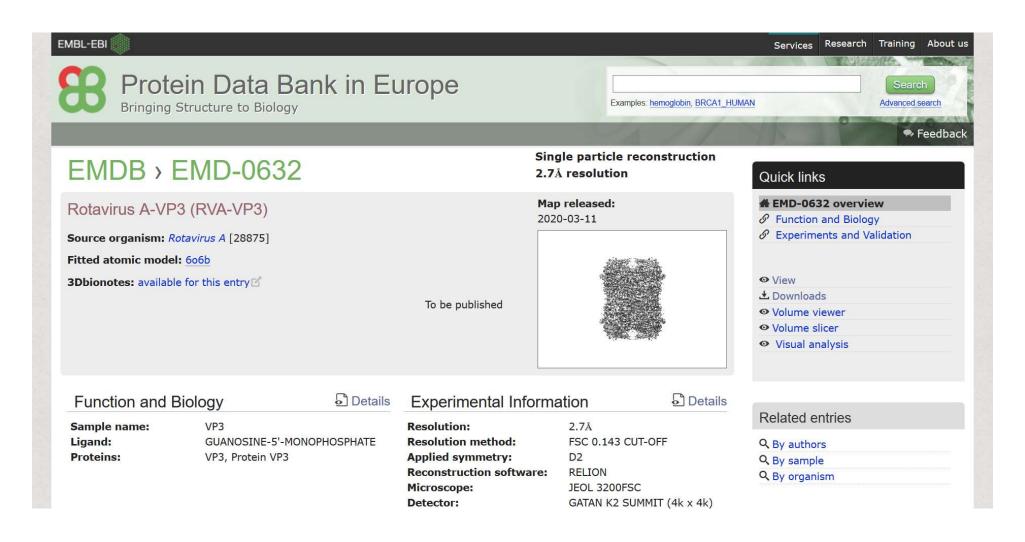
refinement

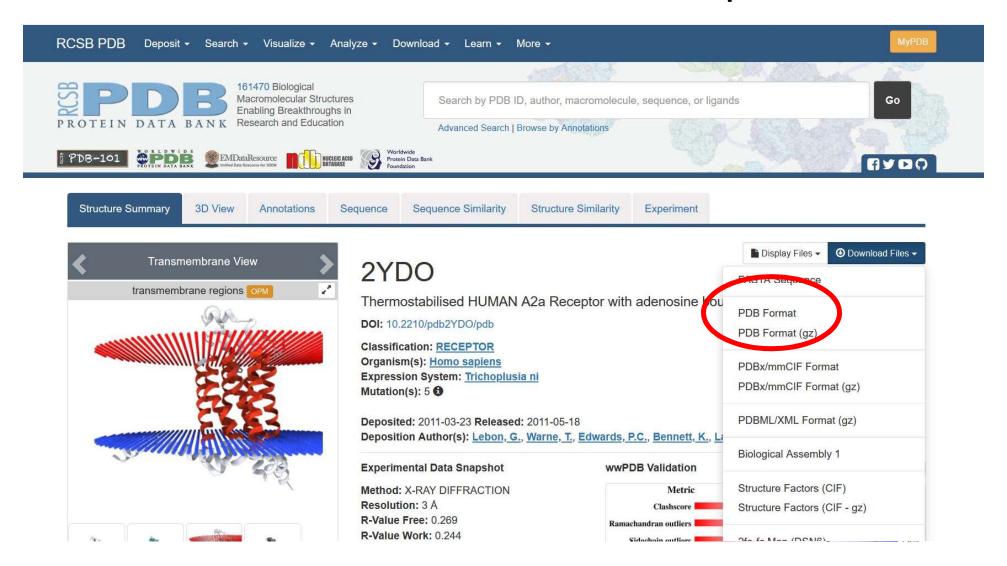
data scaling

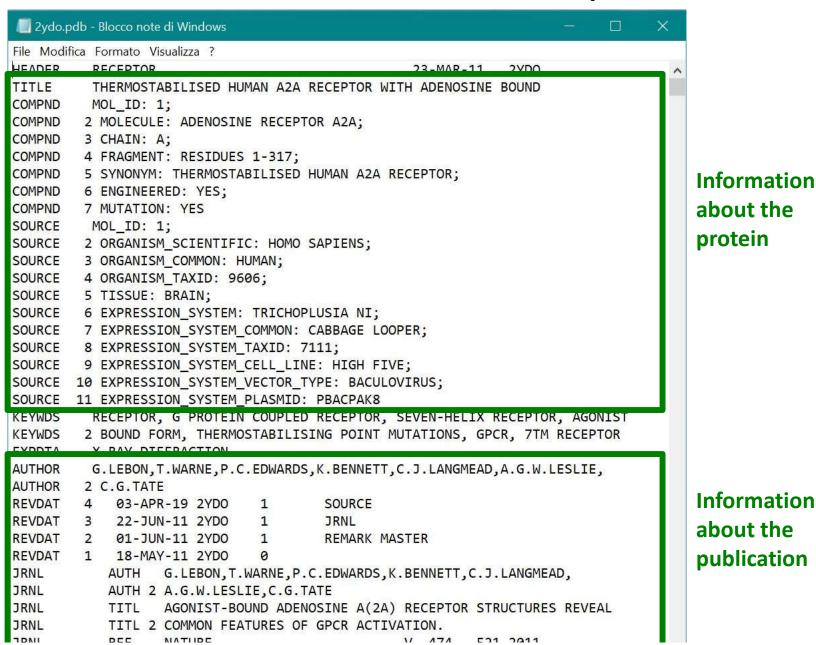
PHENIX

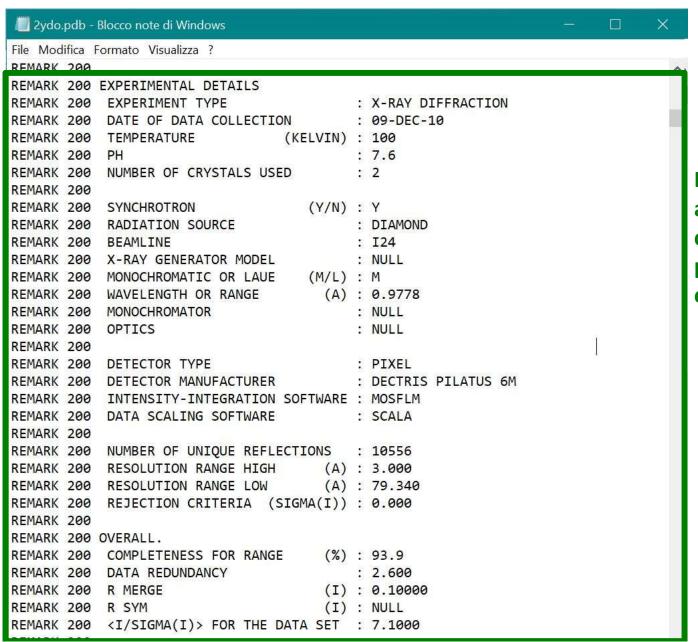
HKL-2000

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

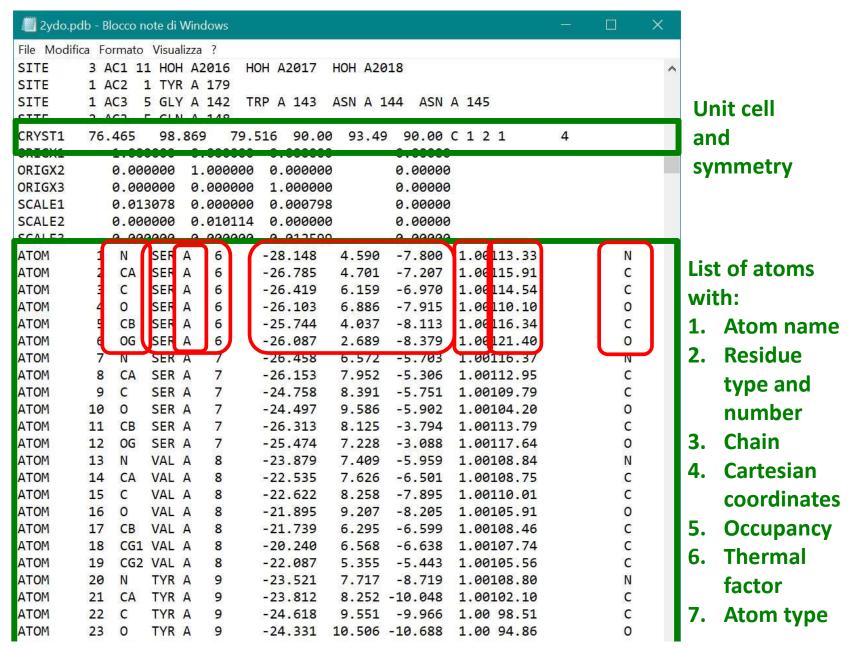




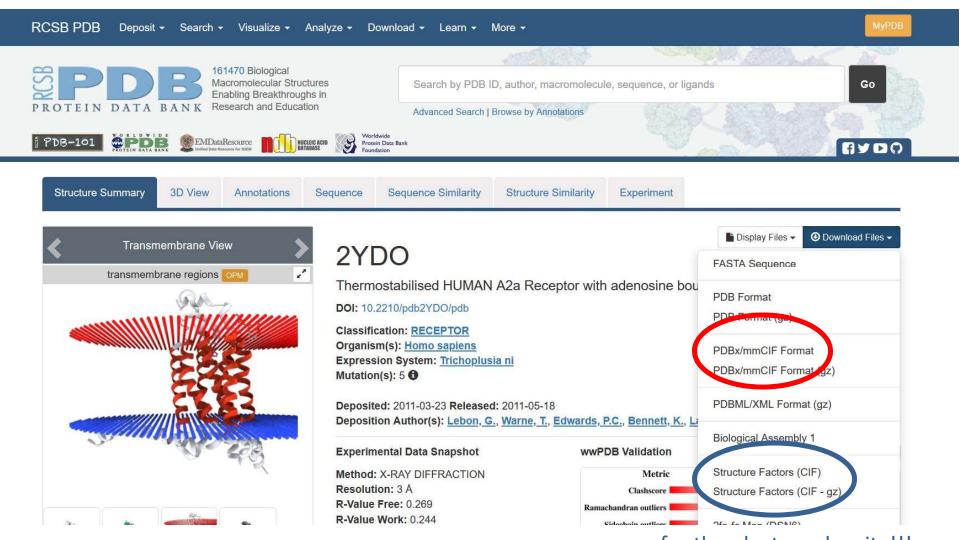




Information about the crystallogra phic experiment

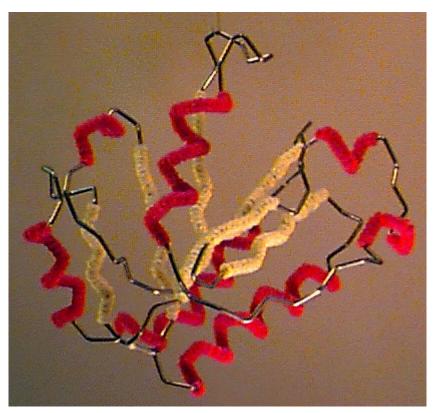


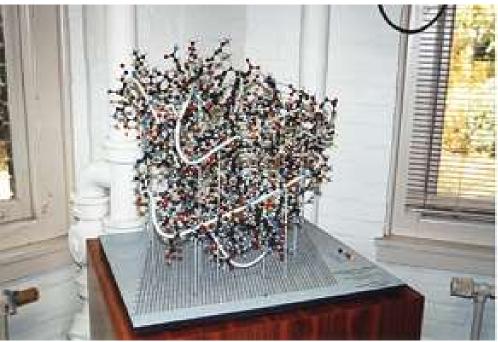
Structure atomic coordinates: mmCIF file



for the electron density!!!

Graphical software: in the old days...





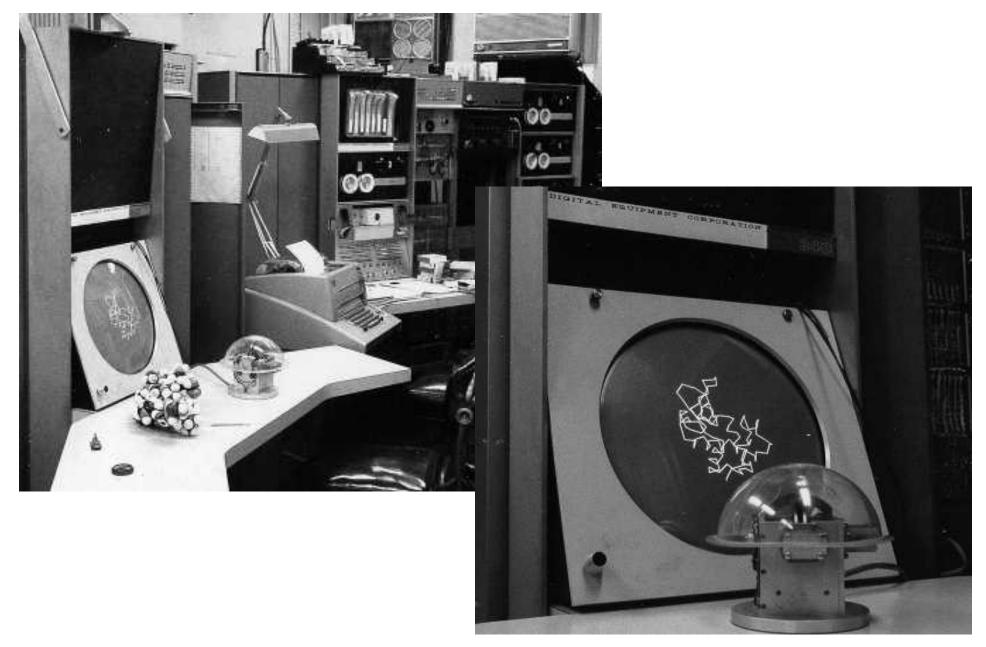


Graphical software: in the old days...

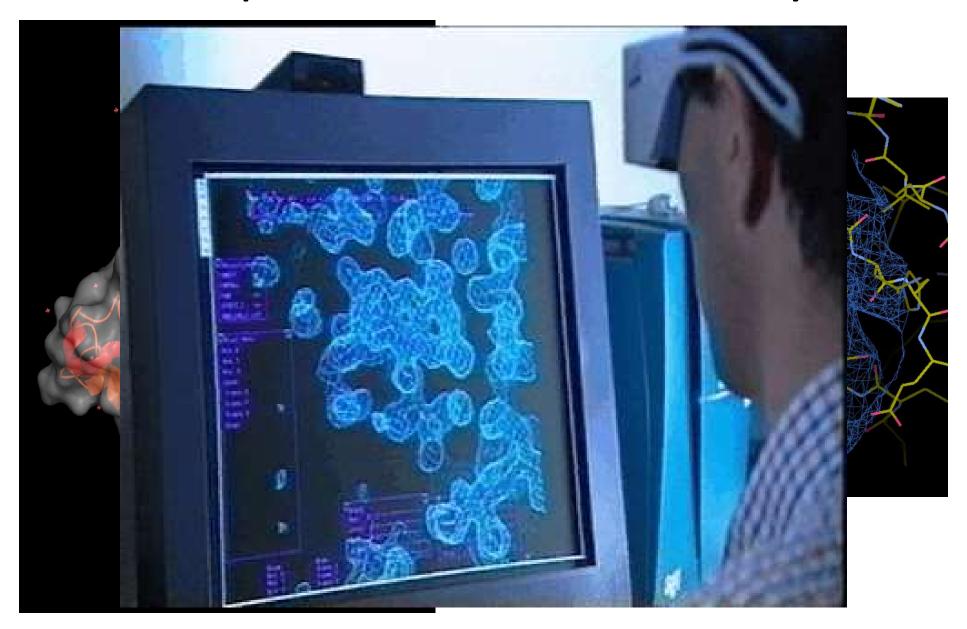




Graphical software: in the old days...

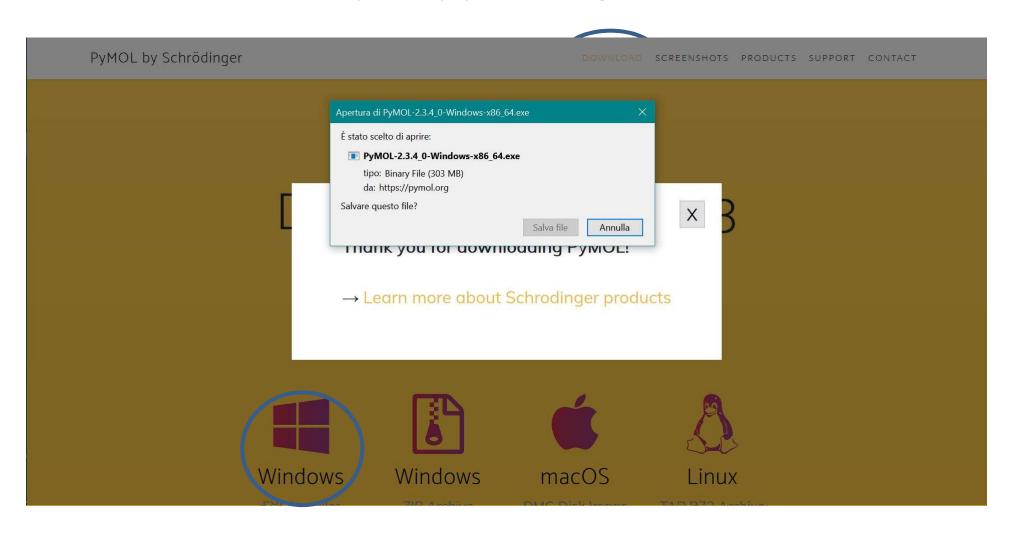


Graphical software:... today

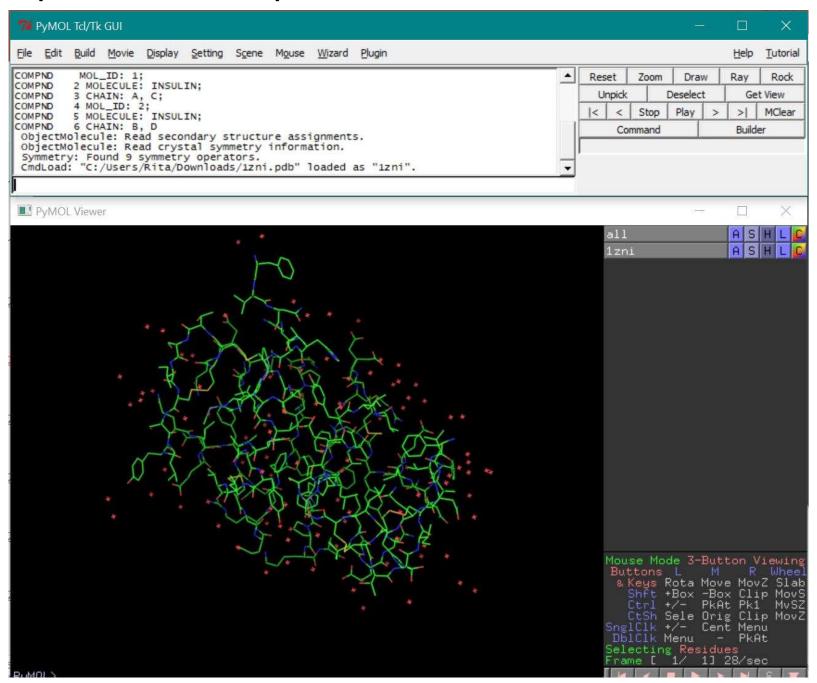


Graphical software: Pymol

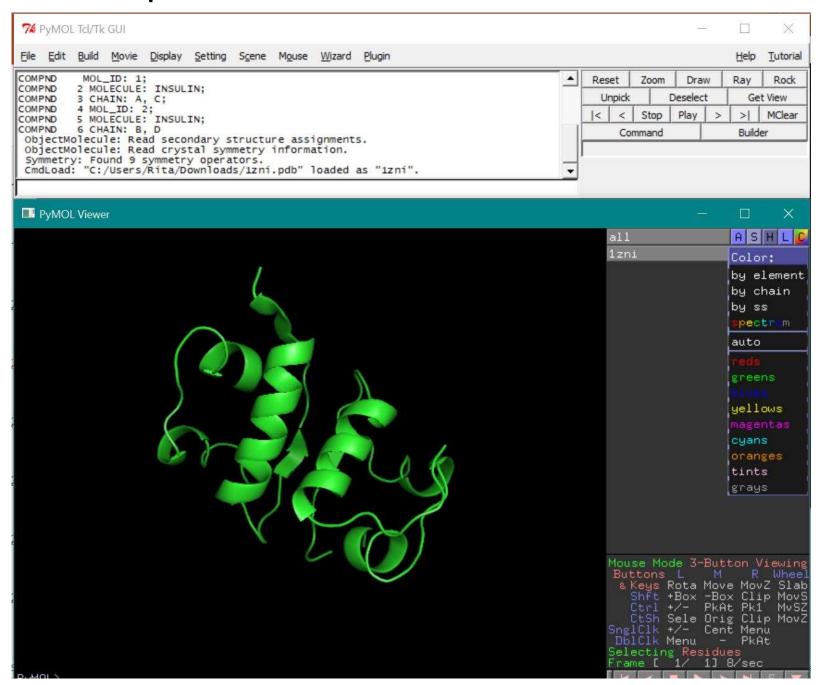
1. Download: https://pymol.org/2/



2. Open and load pdb file

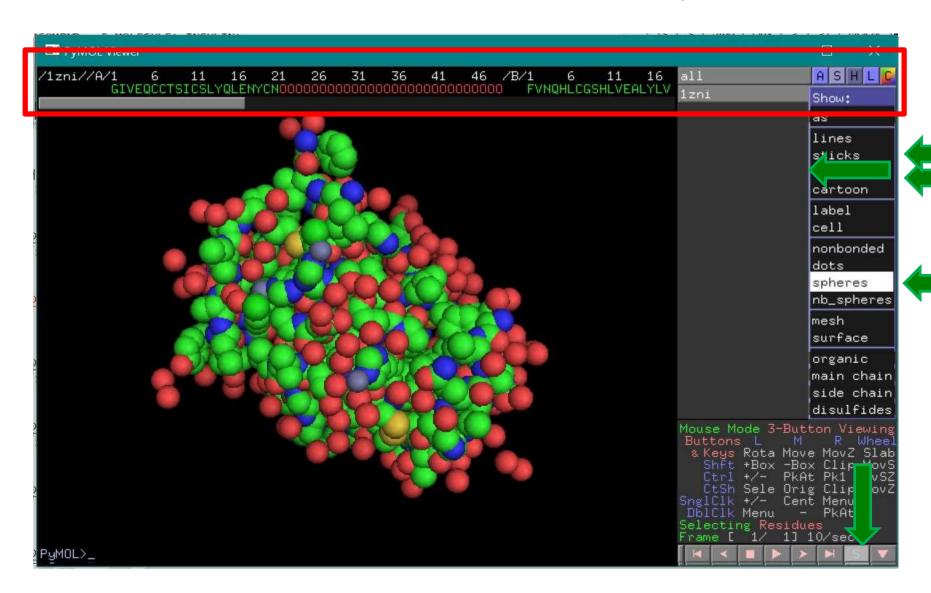


3. Menu options



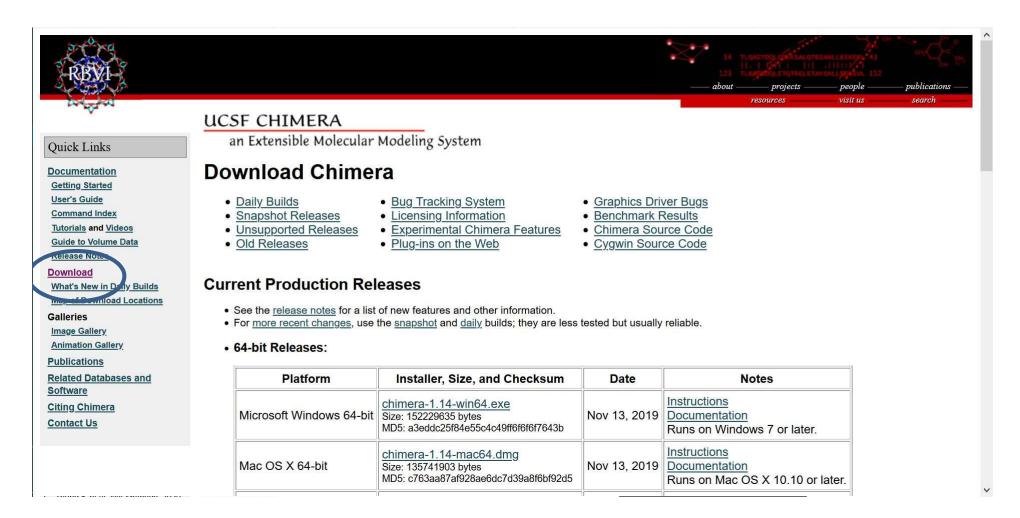
Visualization options:

spheres



Graphical software: UCSF Chimera

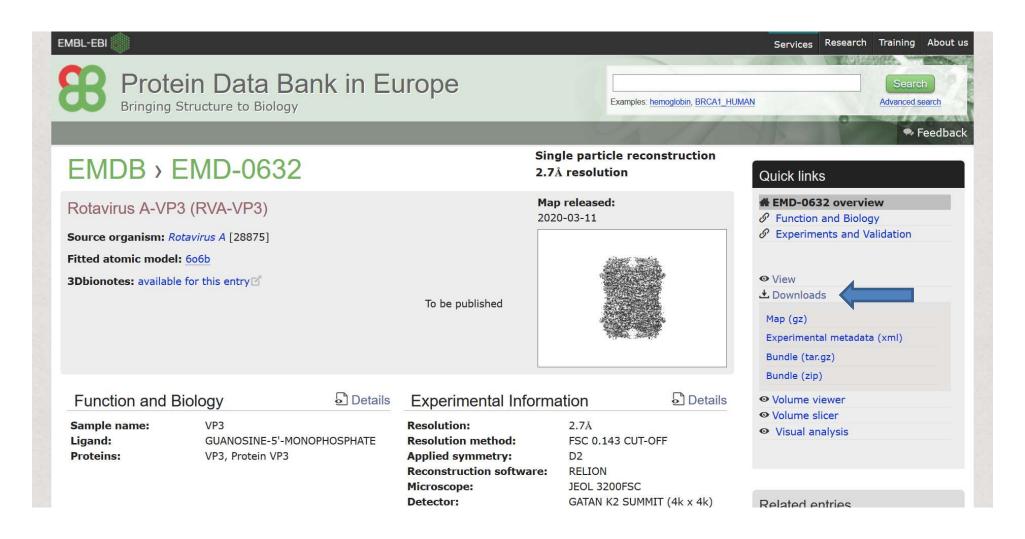
1. Download:www.cgl.ucsf.edu/chimera/



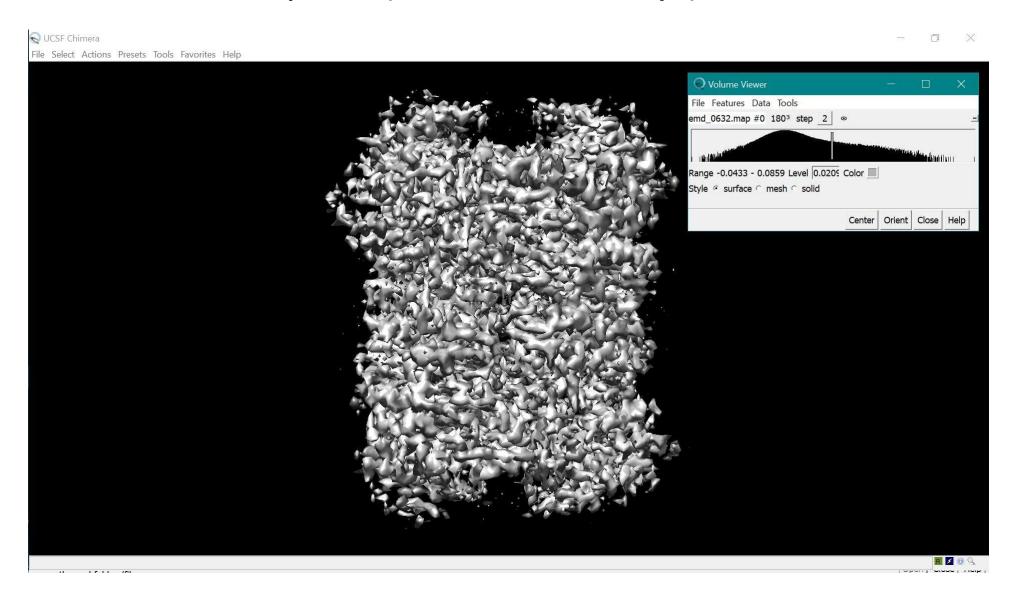
2. Open and load pdb file



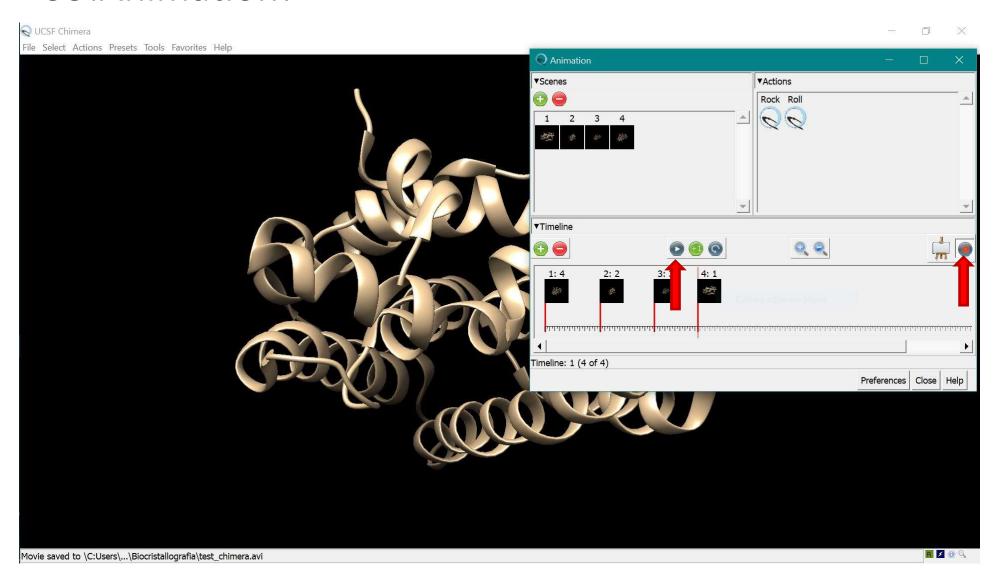
Load a map file (electron density!)(www.ebi.ac.uk/pdbe/emdb/)



3. Load a map file (electron density!)



99.Animation!



99.Animation!

