LOSCHMIDT LABORATORIES

Macromolecular complexes and interactions

Outline

Macromolecular complexes

Structure of complexes

Prediction of 3D structures of complexes

Analysis of macromolecular complexes

What is a macromolecular complex?

Protein – small molecule ☒

Protein – protein ☑

Protein – nucleic acids ☑

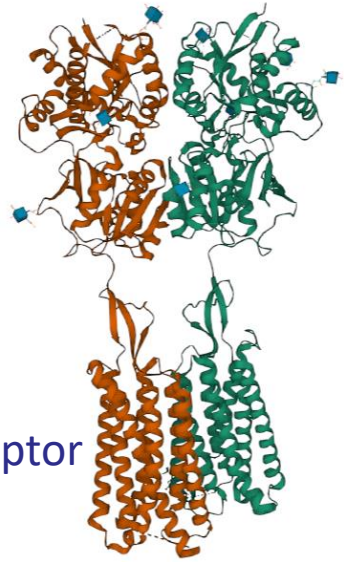
Nucleic acids – small molecule ☒

Protein-protein complexes

- Two or more polypeptide chains (protomers) may associate into an oligomer
- Protein-protein and protein-nucleic acid interactions are essential for every cellular process
  - Metabolism
  - Transport
  - Signal transduction
  - Genetic activity (transcription, translation, replication, repair, ...)
  - Membrane trafficking
  - Mobility
  - …

Protein-protein complexes

- **Obligate complexes**
  - Protomers (individual polypeptides) do not function as independent structures, only when associated
  - Examples: GABA receptors, ATP synthase, many ion channels, ribosome, etc.



$GABA_B$ receptor

- **Non-obligate complexes**
  - Protomers can exist and be functional as independent structures
  - Examples: hemoglobin, beta-2 adrenergic receptor, insulin receptor, etc.

Protein-protein complexes

Do individual subunits retain some activity?

YES → Non-obligate

NO → Obligate

Protein oligomerization

- Oligomerization is common
  - 75 % of proteins in a cell are oligomers
  - Homo-oligomers are the most common
  - Some proteins exists solely in the oligomeric state
- Often symmetric
- Oligomerization interfaces are complementary
- Favored by evolution

Advantages of oligomerization

Why do proteins form oligomers?

Advantages of oligomerization

- **Morphology**
  - More complex structures are often required for multiple functions

    (e.g. membrane pores)
- **Cooperativity**
  - Allostery (modulation of biological activity)
  - Multivalent binding
- **Stability against denaturation**
  - Smaller surface area
- **Redundancy and error control**
  - E.g. protein translation control

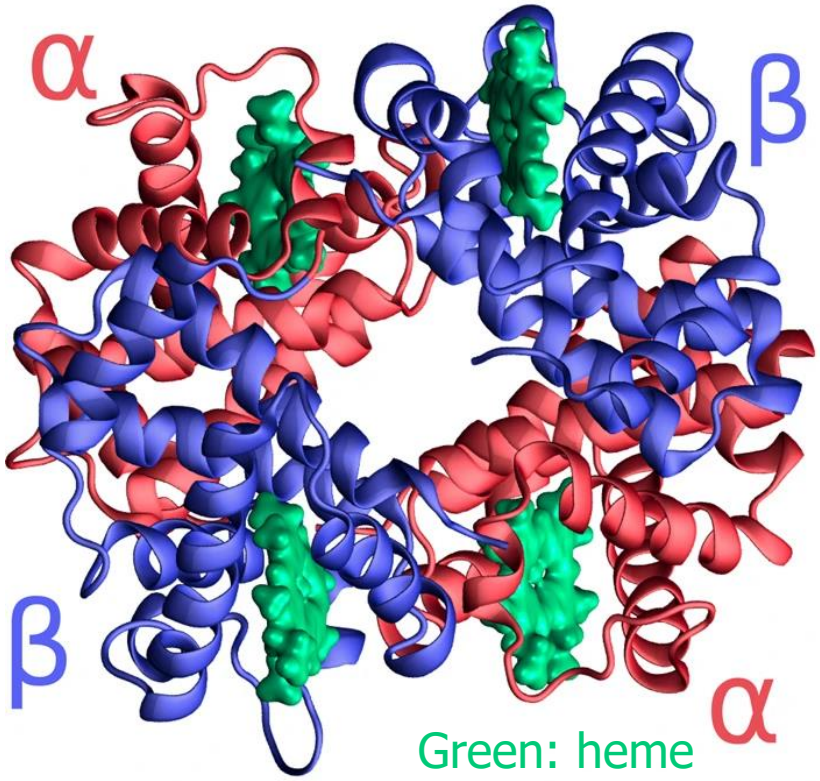
Oligomerization interface

- **Characteristics of oligomeric interface**
  - Large surface area (> 1400 Å^2)
  - Tendency to circular and planar shape (not for obligates)
  - Some residues protrude from the surface
  - More non-polar residues (about 2/3) than in other parts of surface
  - More polar residues (about 1/5) than in protein cores
  - About 1 H-bond per 200 Å^2
- **“Hot-spot” residues**
  - Responsible for most of the oligomeric interactions
  - More evolutionary conserved than other surface residues
  - Frequently polar residues, located about the center of the interface

Examples of macromolecular complexes

Metabolism

Hemoglobin



Tetramer

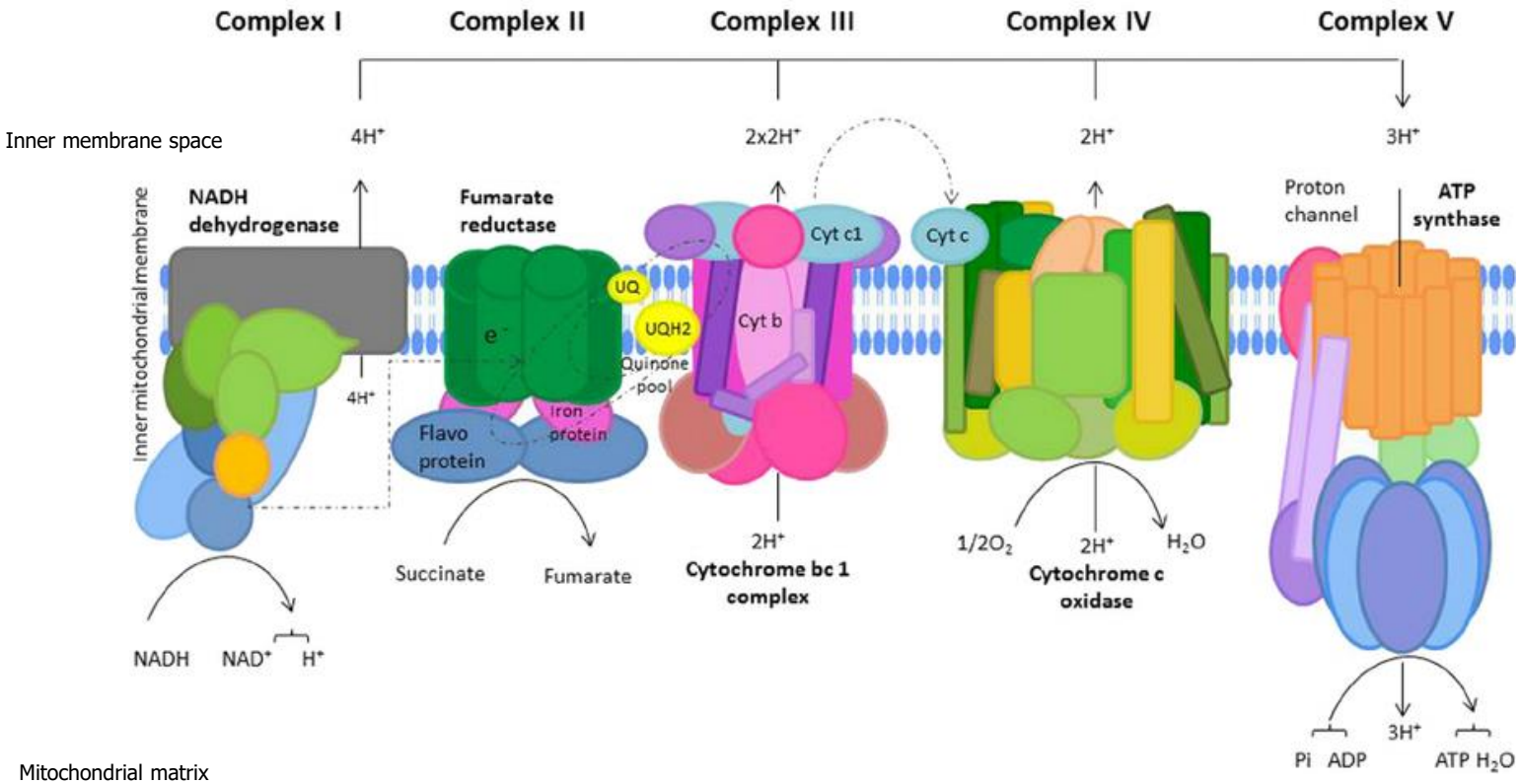
made of 2*2 subunits (α and β)

Bringas et al., 2017, Scientific Reports

Examples of macromolecular complexes

Metabolism

Oxidative phosphorylation complexes (mitochondria)

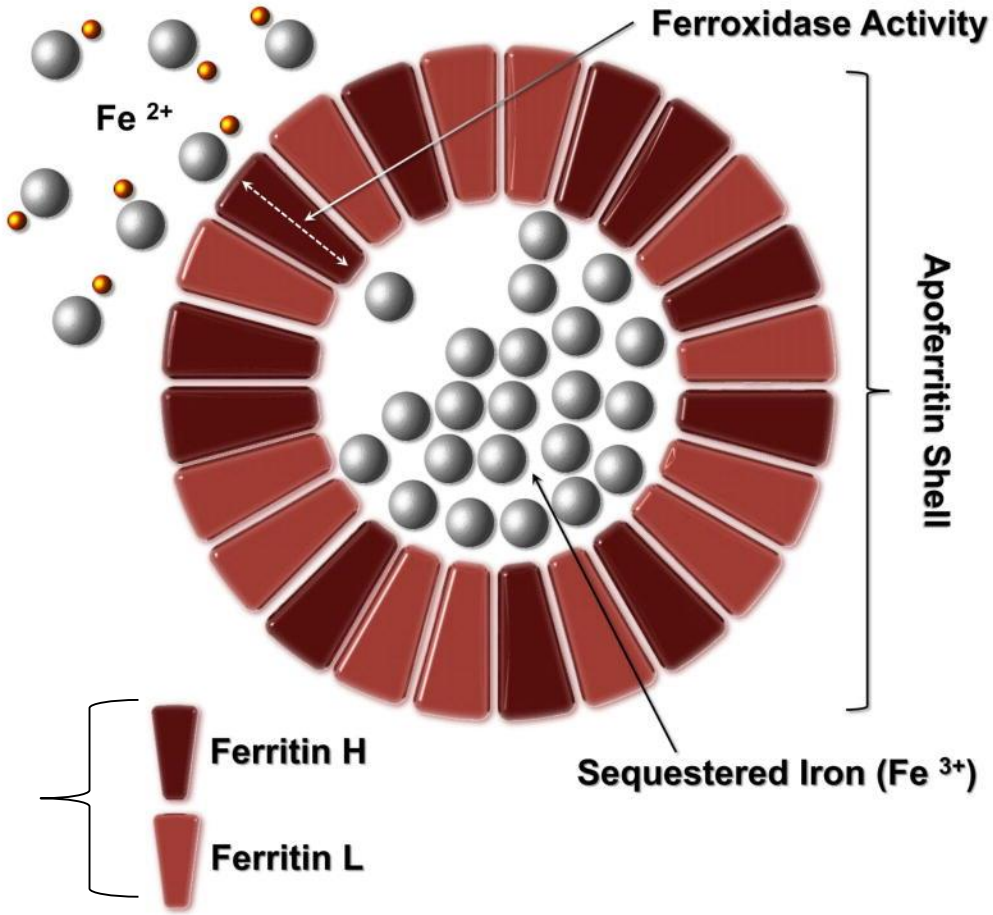


Granata et al., 2015, Nutrition & Metabolism

Examples of macromolecular complexes

Transport

Ferritine



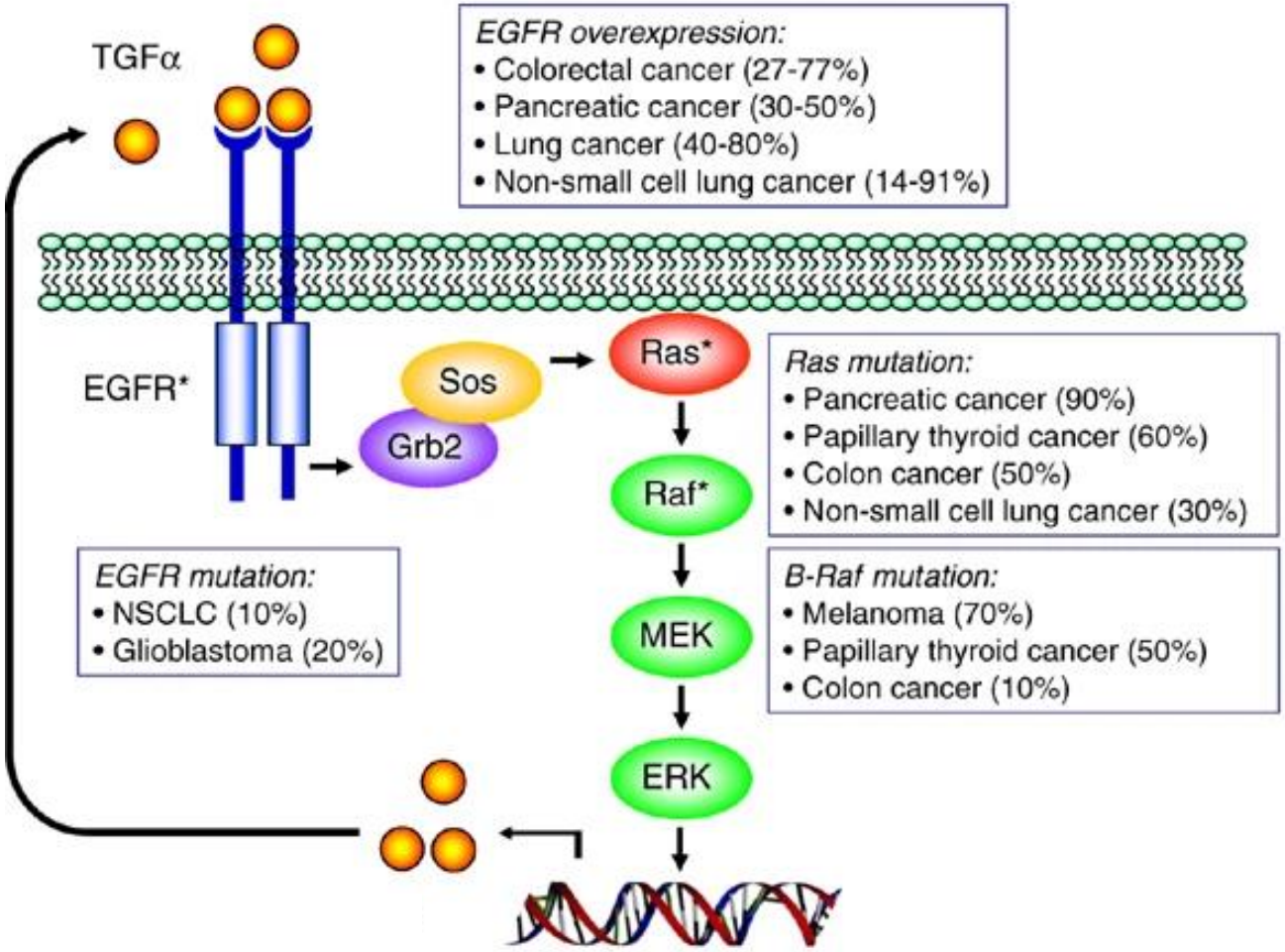
Hetero-oligomer

Knovich et al., 2009, Blood Rev

Examples of macromolecular complexes

Signal transduction

EGFR/RAS/RAF/MEK/ERK pathway

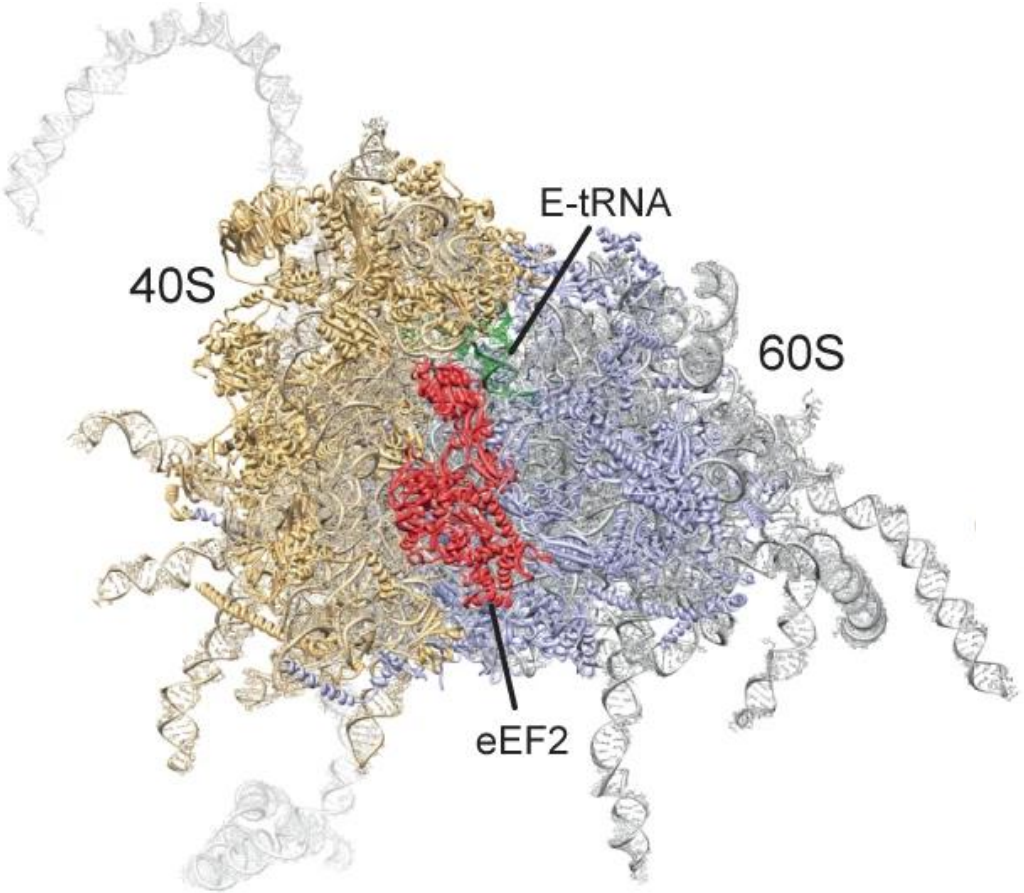


Roberts and Der, 2007, Oncogene

Examples of macromolecular complexes

Genetic activity

Ribosome

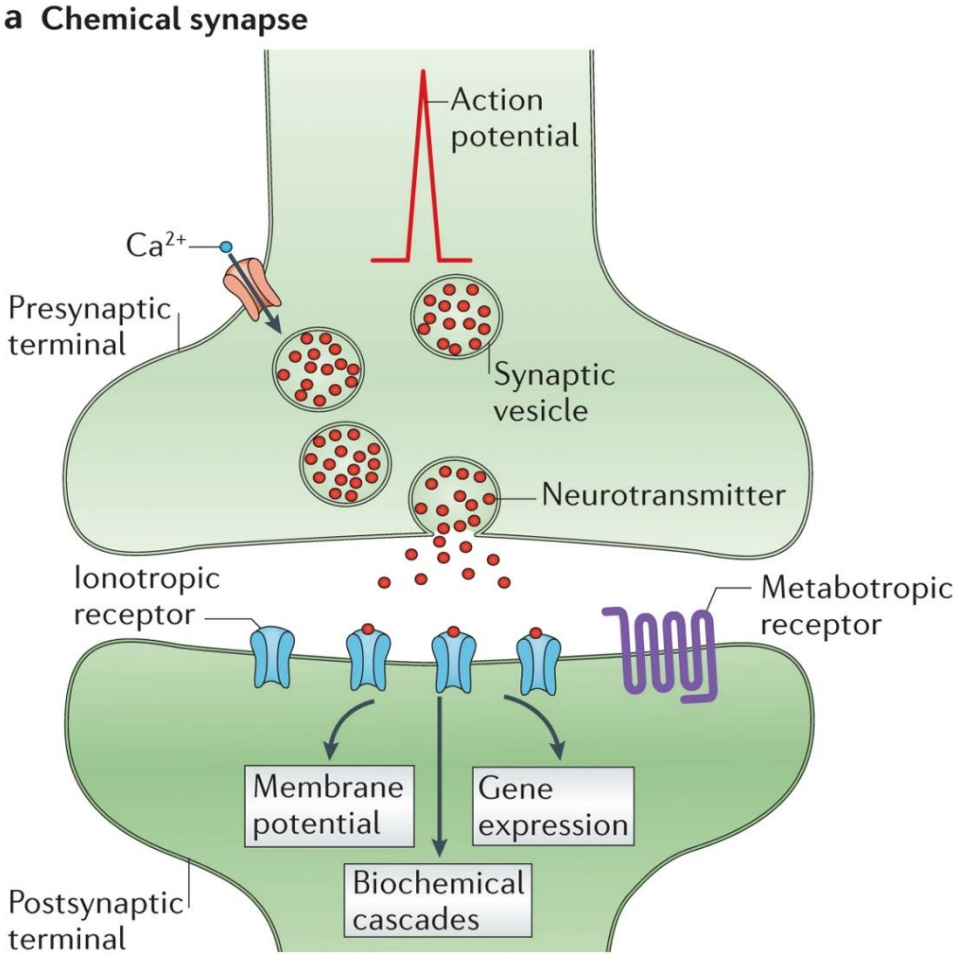


Anger et al., 2013, Nature

Examples of macromolecular complexes

Membrane trafficking

SNARE proteins



Examples of macromolecular complexes

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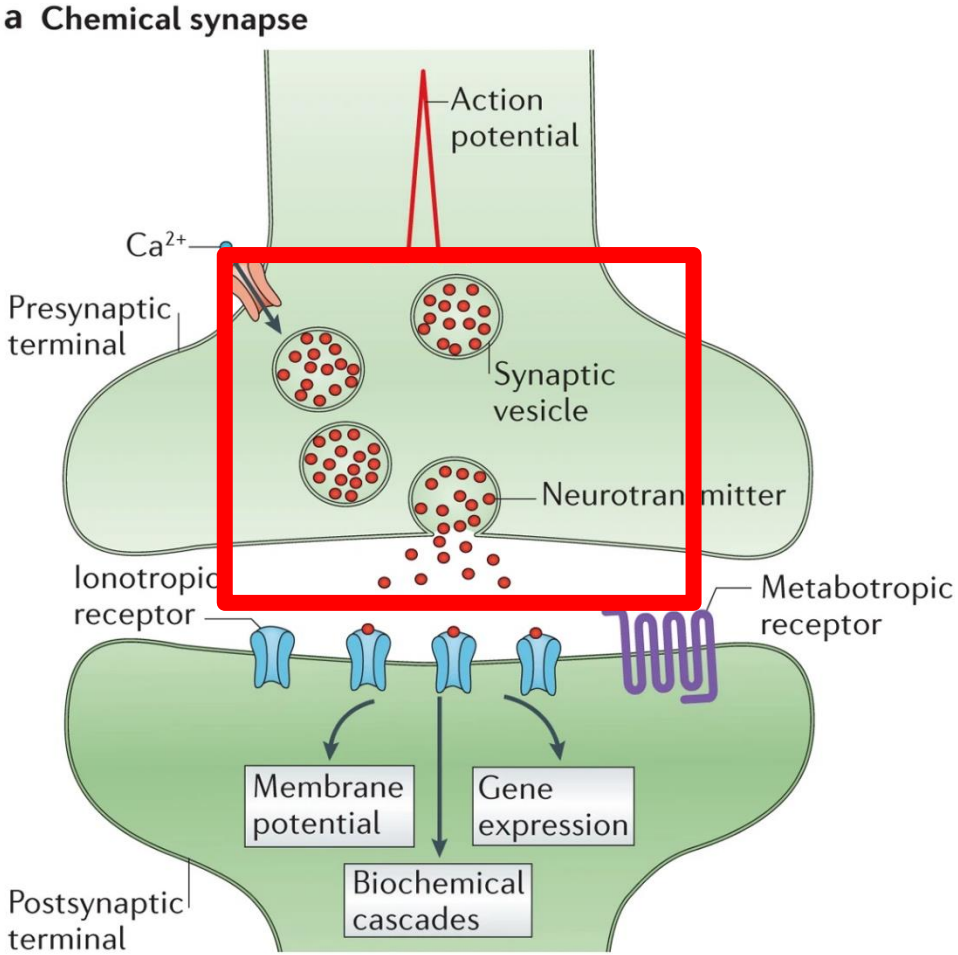

Perada, 2014, Nature Reviews Neuroscience

Palfreyman and Jorgensen, 2010, Molecular mechanisms of Neurotransmitter Release

Examples of macromolecular complexes

Membrane trafficking

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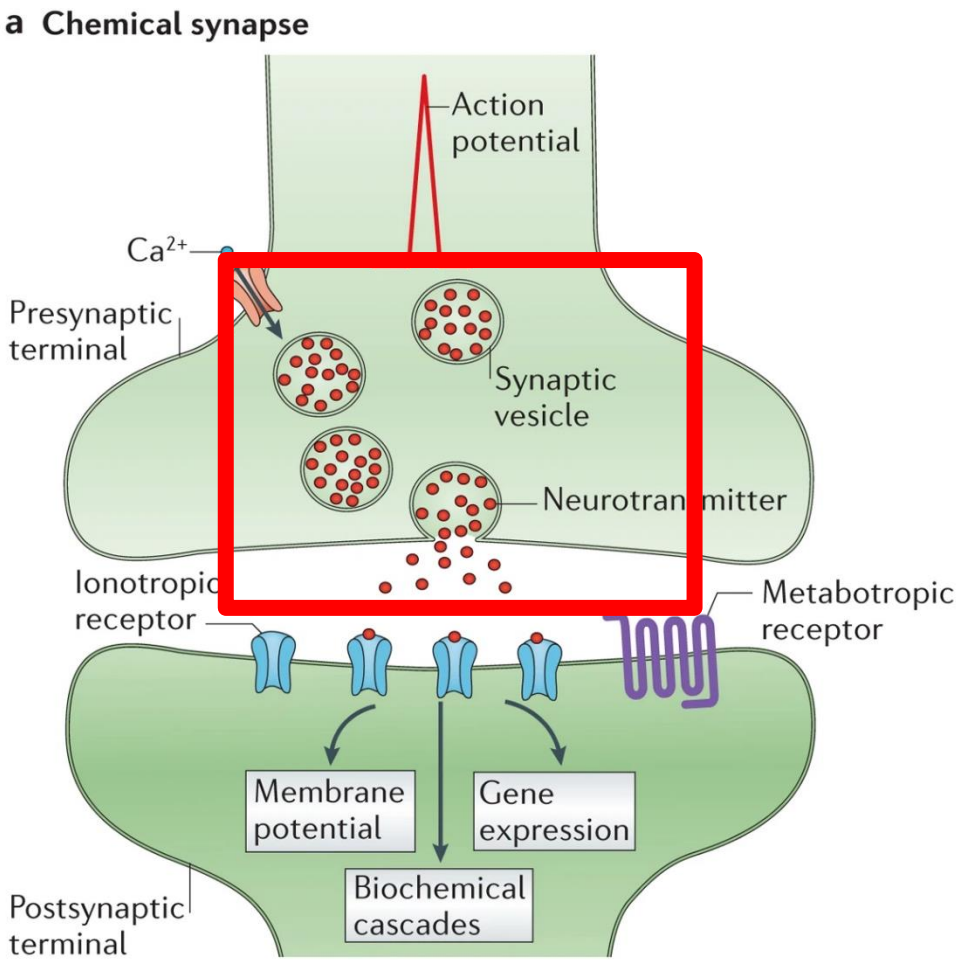
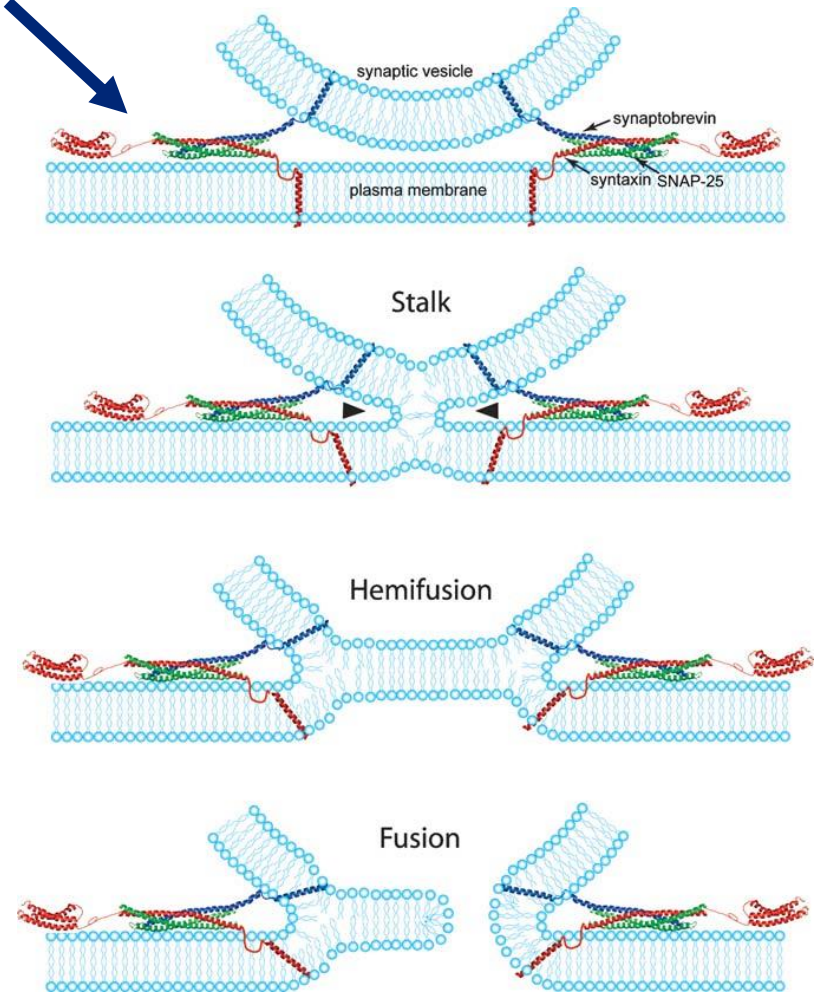




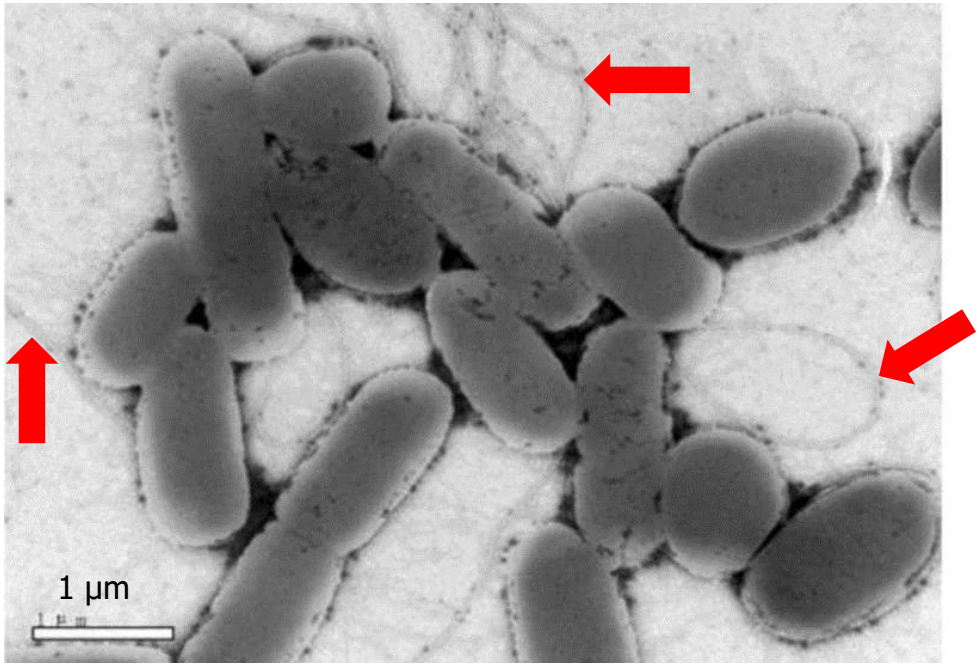
Perada, 2014, Nature Reviews Neuroscience

Palfreyman and Jorgensen, 2010, Molecular mechanisms of Neurotransmitter Release

Examples of macromolecular complexes

Mobility

Flagella (of *Salmonella*)



Yang et al., 2019, AMB Express

Examples of macromolecular complexes

Mobility

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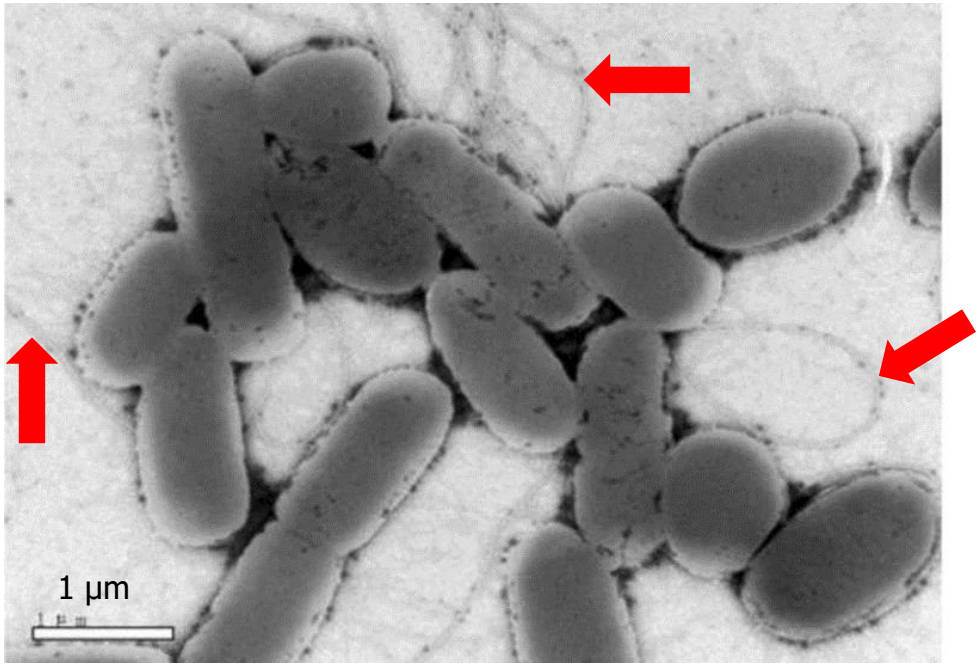
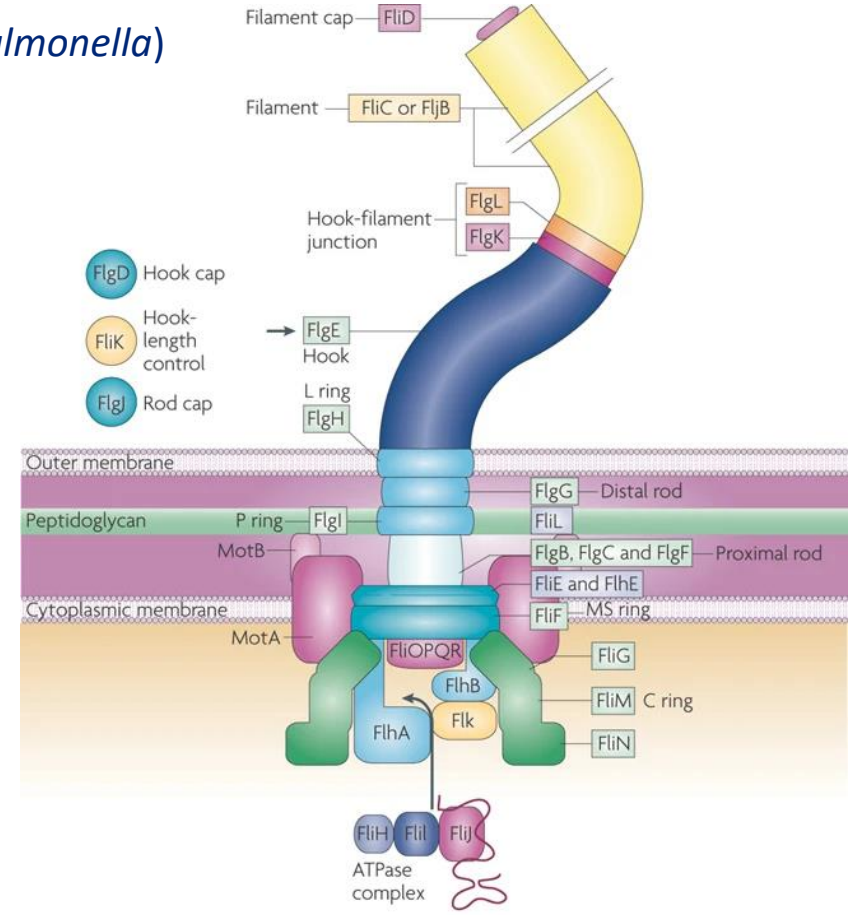


Yang et al., 2019, AMB Express

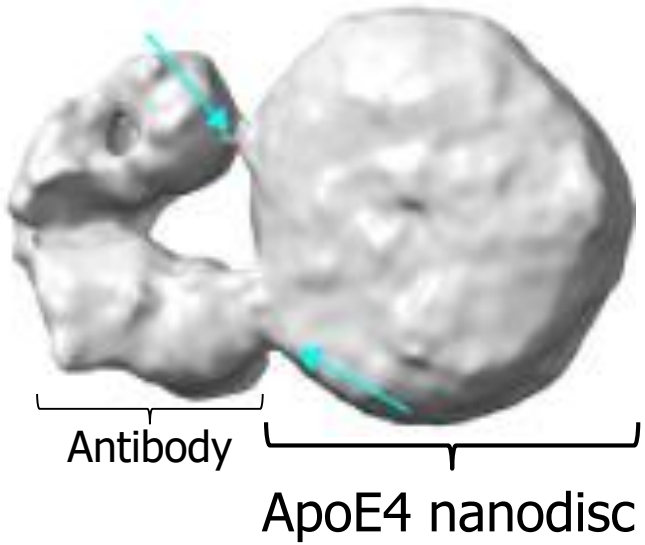


Chevance and Hughes, 2008, Nature Reviews Microbiology

Examples of macromolecular complexes

Protein-lipid nanoparticle

ApoE4



Density map from
cryo-electron microscopy

Strickland et al., 2024, Neuron

Examples of macromolecular complexes

Protein-lipid nanoparticle

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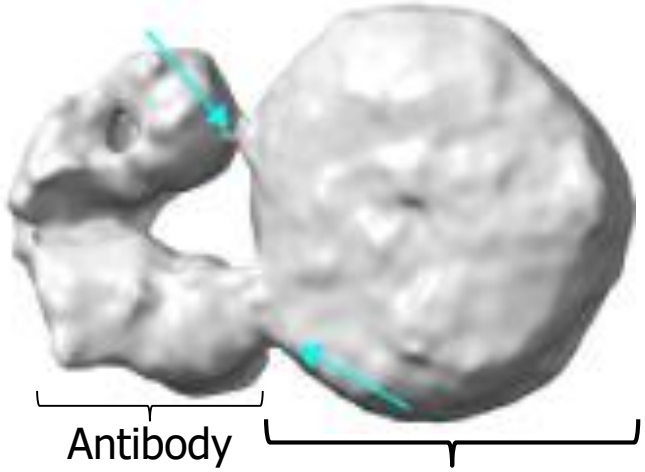

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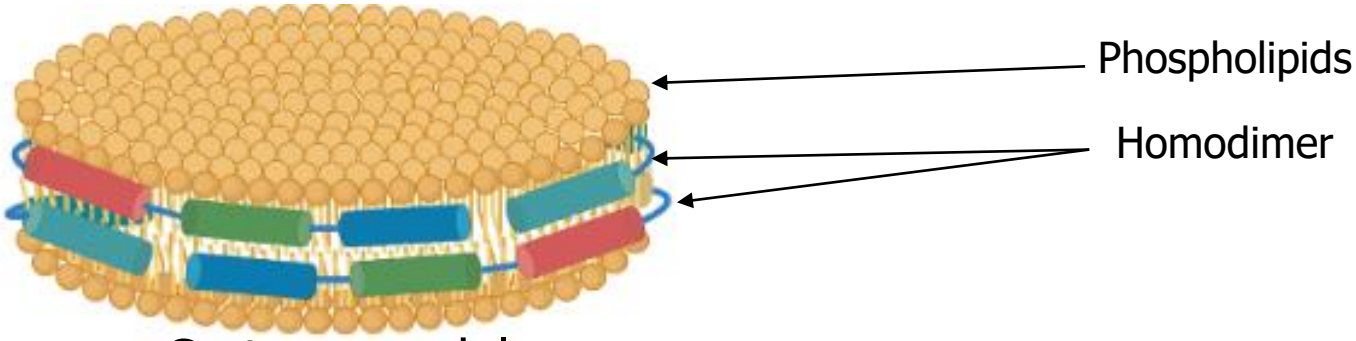


Cartoon model

Strickland et al., 2024, Neuron

Oligomerization vs Aggregation

Oligomerization

- Oligomers are soluble
- Precise fold
- Proteins are native (not denatured)
- Reversible (sometimes)

Aggregation

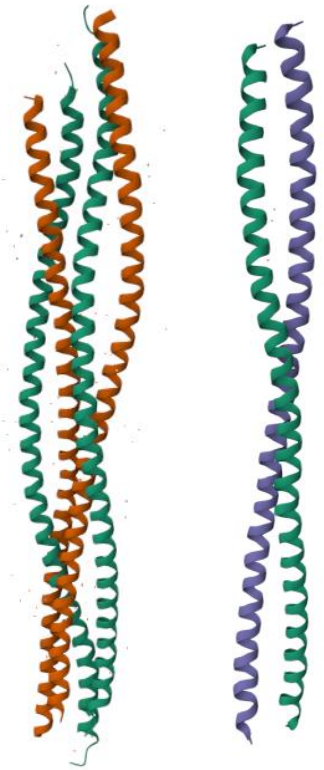
- Aggregates are insoluble
- Can be heterogenous
- Denatured proteins aggregate (temperature, pH, salt…)
- Irreversible

The function of some proteins **is** to aggregate.

Aggregates ≠ pathology

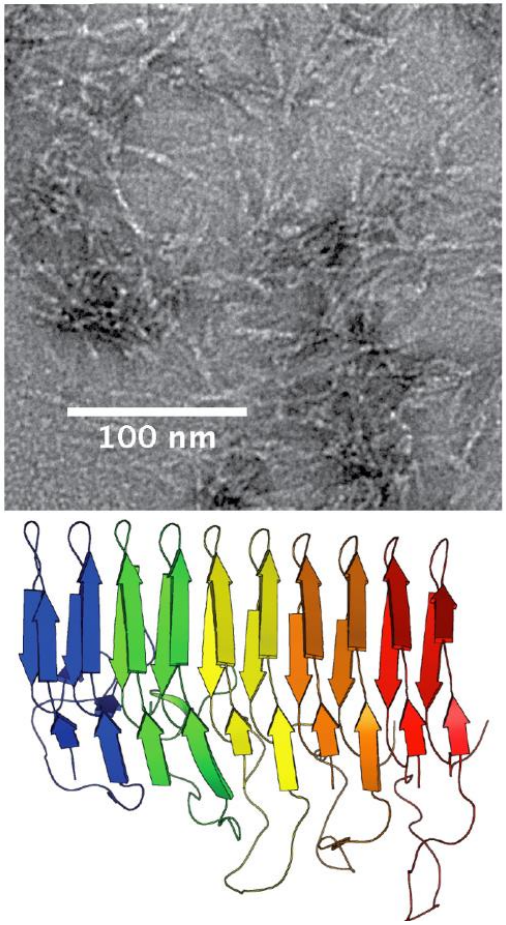
Non-pathological aggregates

Keratin filaments
(hair, skin, nails)



PDB code: 6EC0 6JFV

HET-s
(fungal reproduction and apoptosis)

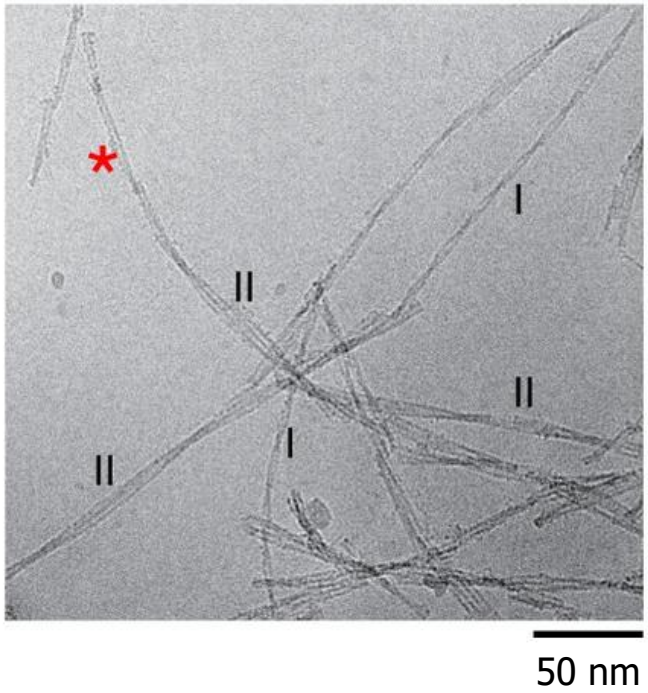


Daskalov et al., 2021, Front. Mol. Neurosci.

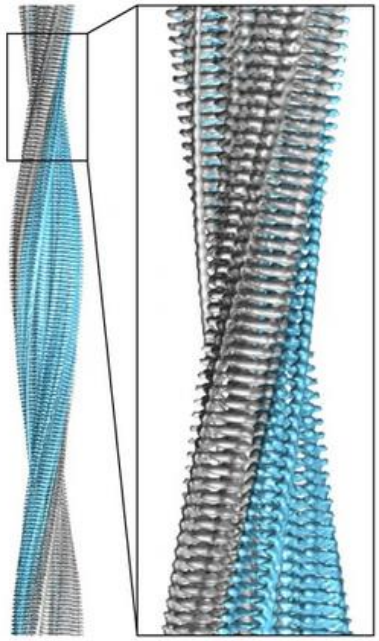
Pathological aggregates

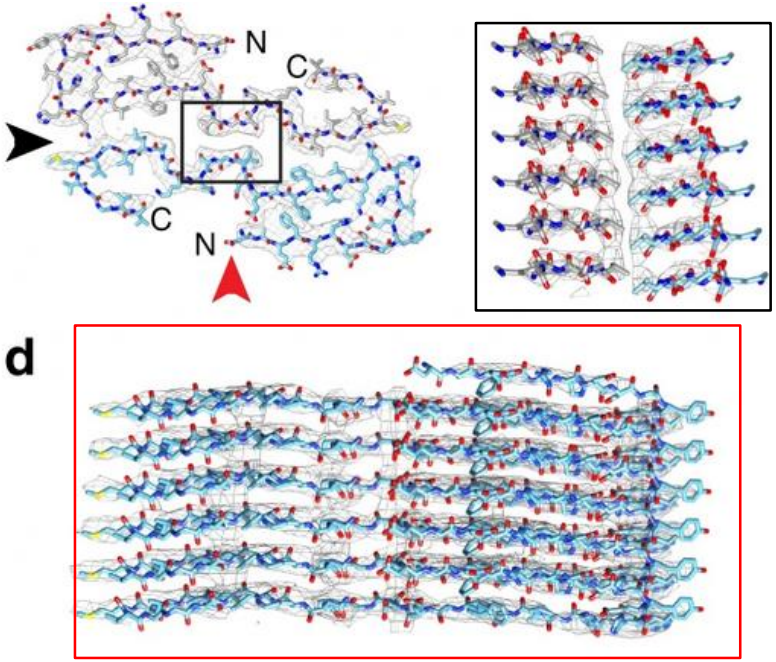
Amyloid β from human brain

(involved in Alzheimer's disease)



50 nm





β-solenoid

Two different morphologies (I and II)

* Transition from I to II

Kollmer et al., 2019, Nat Commun

Pathological aggregates

Amyloid β from human brain
(involved in Alzheimer's disease)

Has non-pathological functions too!

- ❑ Blood-brain barrier maintenance
- ❑ Anti-microbial peptide
- ❑ Synapse function
- ❑ …

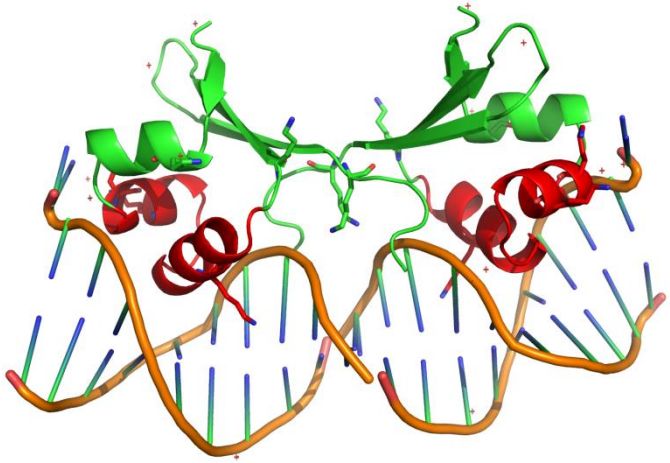
Bishop and Robinson, 2024, Drugs Aging.
Wang et al., 2021, Front. Cell. Neurosci.

Protein-nucleic acids complexes

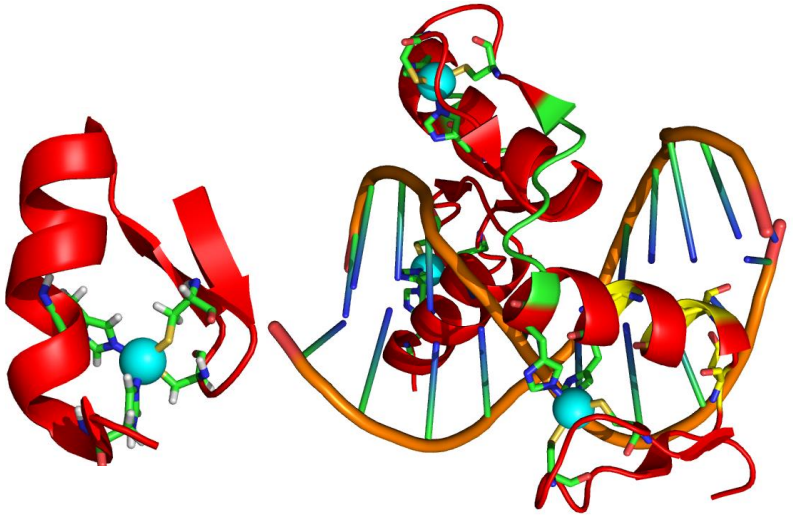
- **Protein-nucleic acid interactions**
  - Non-specific – electrostatic interactions with negative charge on the backbone of nucleic acid -> Lys and Arg residues
  - Specific – recognition of particular nucleotide sequences
    - Major groove – B-DNA
    - Minor groove – A-DNA or A-RNA
    - Single strand RNA
- **Typical interfaces/motifs**
  - DNA binding proteins
  - RNA binding proteins

Protein-nucleic acids complexes

- **DNA binding proteins**
  - Helix-turn-helix
    - (+)-sidechains
    - ≈ perpendicular helices
    - Recognises major groove

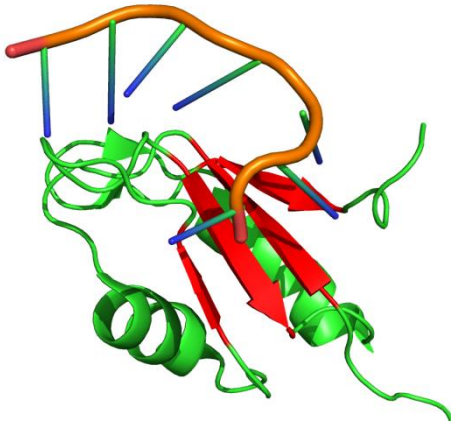


  - Zinc finger
    - Zn^{2+} stabilized by Cys and His residues
    - Zn^{2+} is essential for folding
    - Zn^{2+} mediates DNA binding

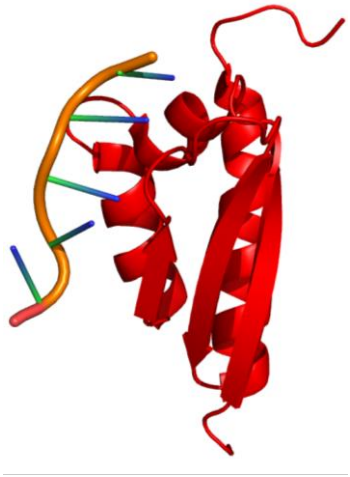


Protein-nucleic acids complexes

- **RNA binding proteins**
  - RRM: βαββαβ barrel-like arrangement, sequence-specific RNA recognition
  - KH domain: ssRNA/DNA binding through H-bonds, electrostatic and shape complementarity
  - PUF domain: each helix recognizes a single base



RNA recognition motif (RRM)



K-homology (KH) domain

Pumilio repeat domain (PUF)

How to detect macromolecular complexes?

How to detect macromolecular complexes

- Physics-based methods
  - Size
  - Molecular mass
  - Binding to a surface containing immobilised partner
  - Temperature shift upon binding
  - Binding of a fluorescent indicator
- Complementation of biological activity
  - Each partner has one half of a protein
  - If both partners interact, both halves also interact
  - Restoration of activity (e.g. critical enzyme for organism growth, fluorescence)
- Imaging
  - Fluorescence (need fluorescent tag)
  - Atomic force microscopy
  - Electron microscopy

How to resolve macromolecular complexes?

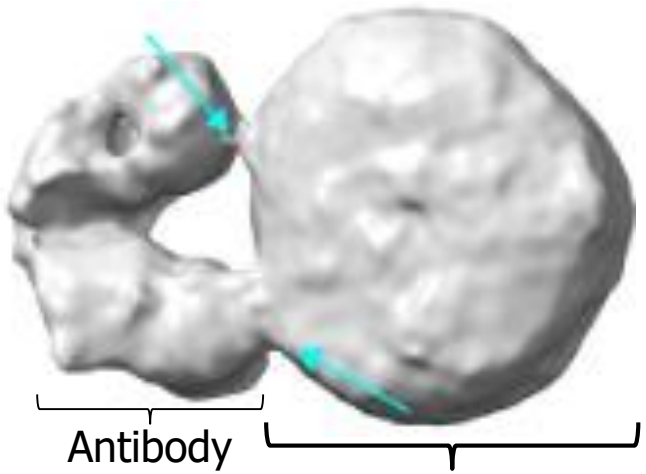
How to resolve macromolecular complexes

Electron microscopy

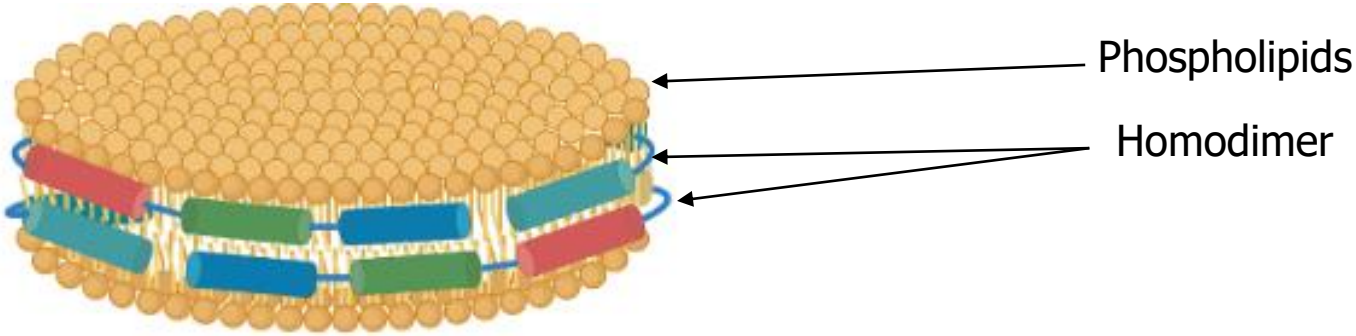
Nuclear magnetic resonance (NMR)

X-ray crystallography

Electron microscopy



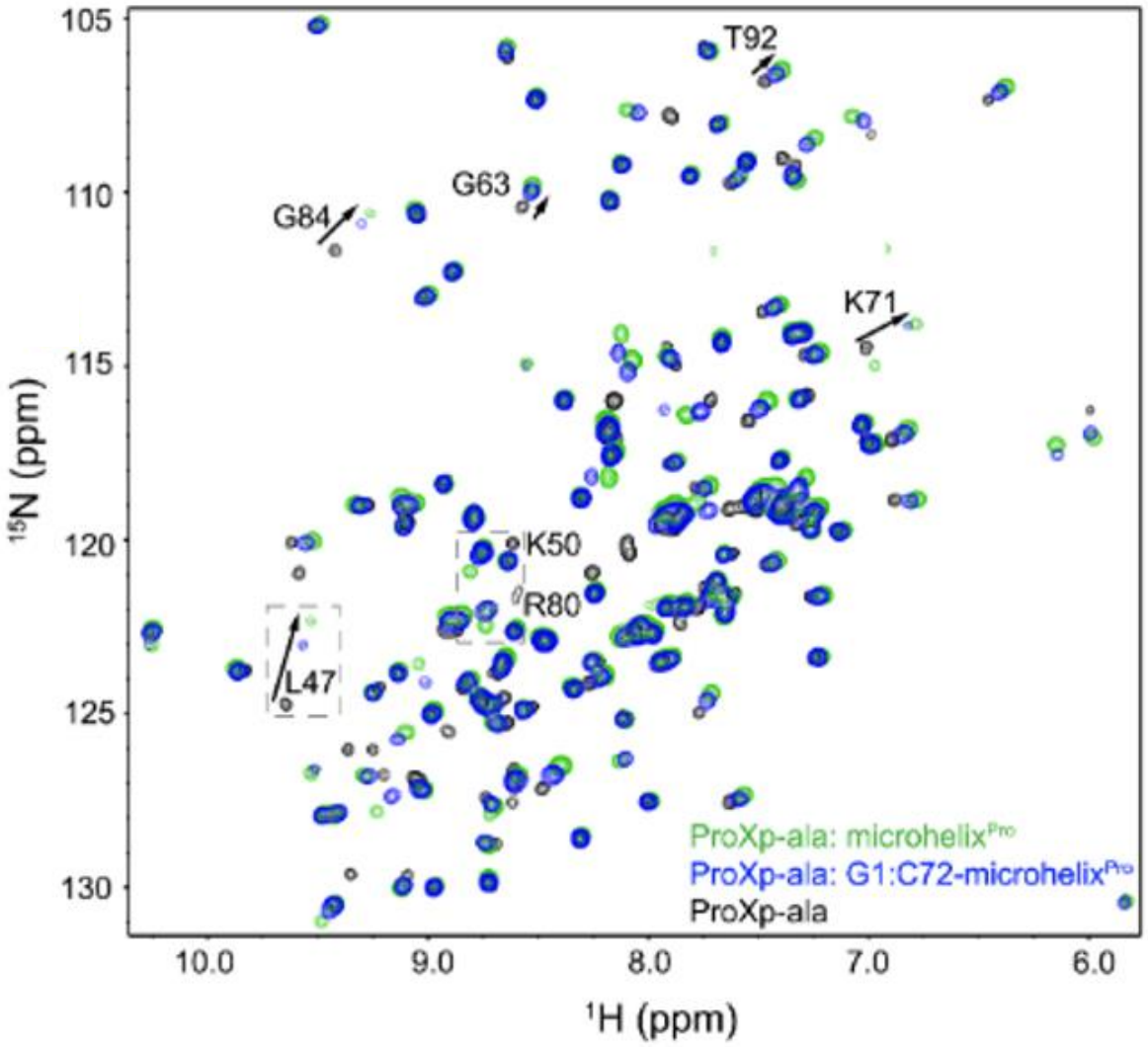
Density map from
cryo-electron microscopy



Cartoon model

Strickland et al., 2024, Neuron

NMR (Chemical Shift Perturbation, CSP)

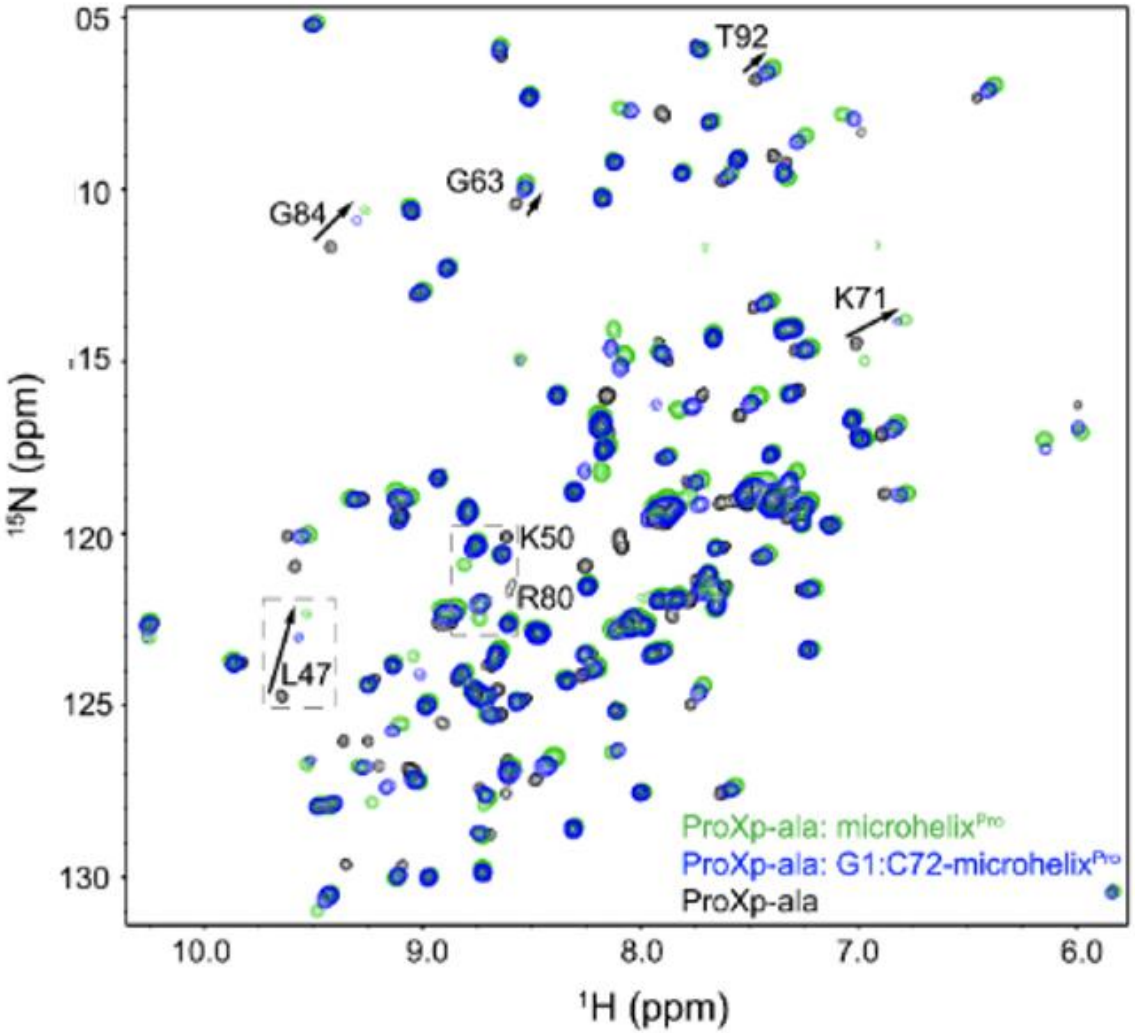


1 peak = 1 **protein** residue

Protein: **ProXp-ala**
+ tRNA: green or blue

Ma et al., 2023, Nucleic Acids Res.

NMR (Chemical Shift Perturbation, CSP)



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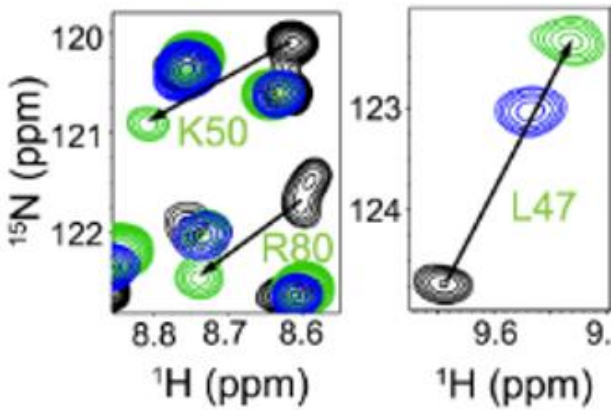
Protein: **ProXp-ala**
+ tRNA: green or blue

Upon interaction with tRNA, peaks are perturbed



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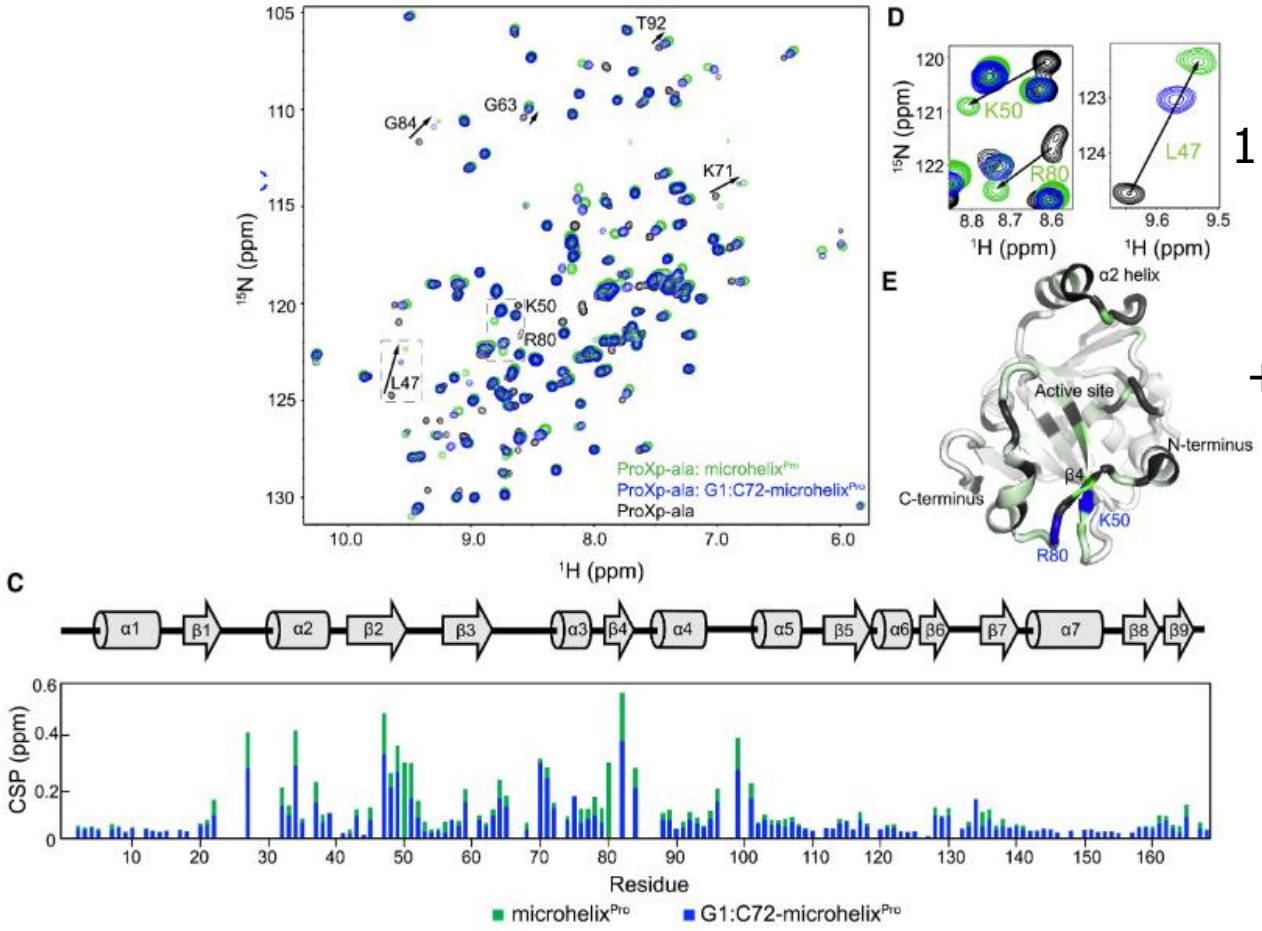

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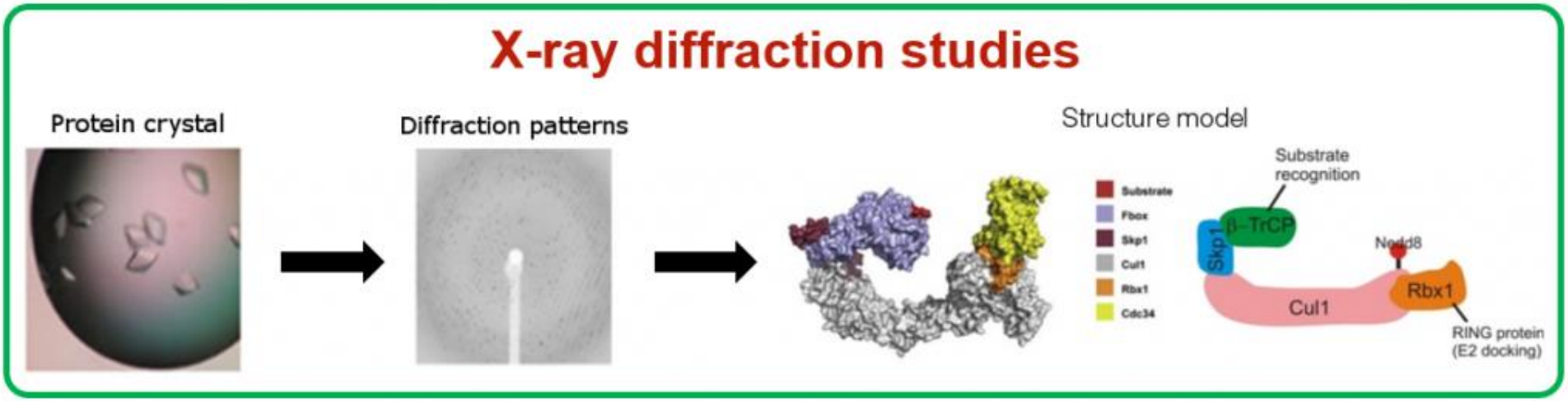
Mapping of interactions

Affinity measurement

Ma et al., 2023, Nucleic Acids Res.

X-ray crystallography



In Protein Data Bank (PDB, rcsb.org),

83% of structures come from X-ray crystallography.

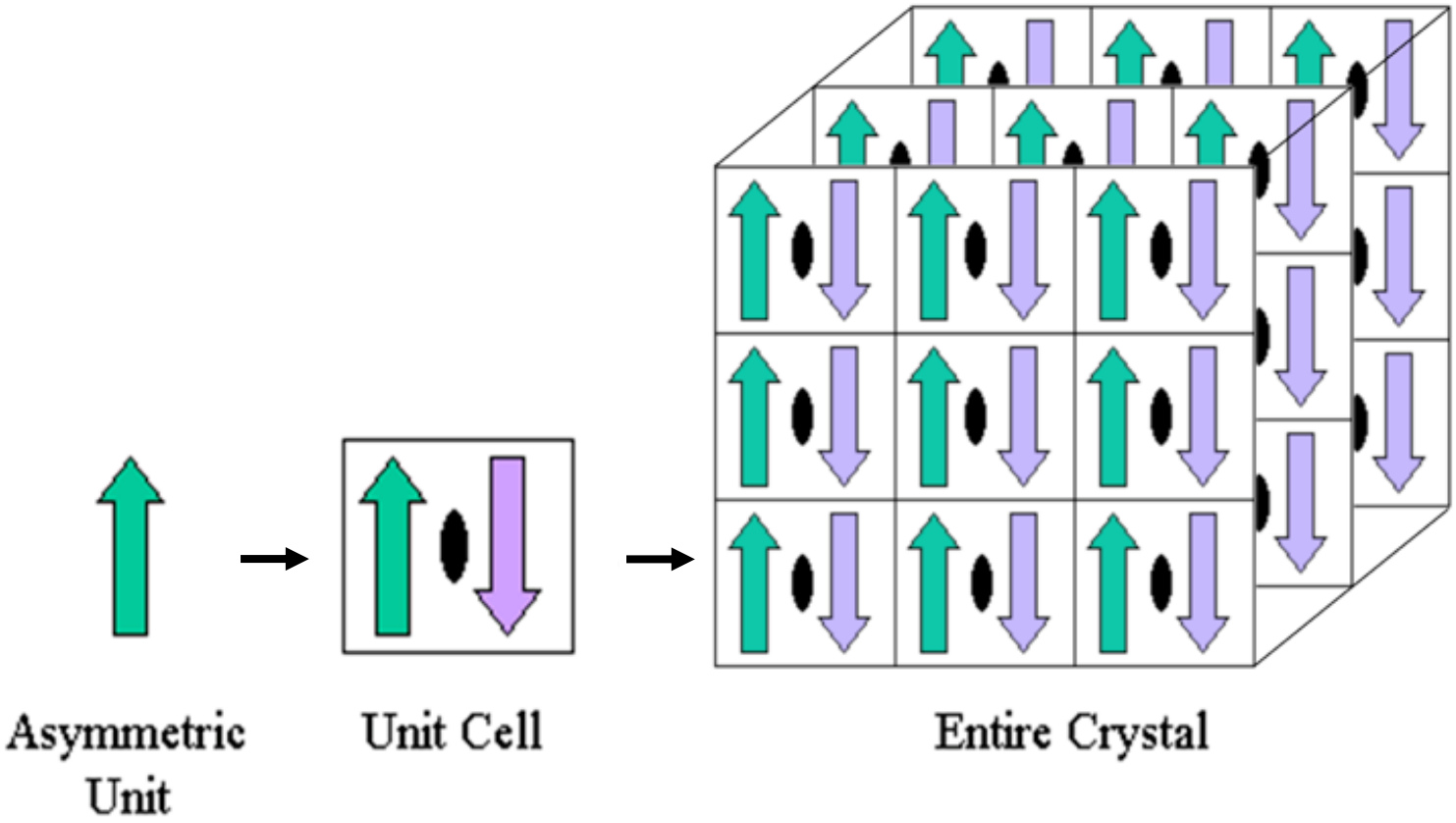
https://www.ebi.ac.uk/training/online/courses/protein-interactions-and-their-importance/where-do-the-data-come-from/x-ray-crystallography/

Quaternary structure in PDB database

- **Asymmetric unit (ASU)**
  - Macromolecular structures from X-ray crystallography deposited to PDB as a single asymmetric unit
  - The smallest portion of a crystal structure to which symmetry operations can be applied in order to generate the unit cell

- **Unit cell (crystal unit)**
  - The basic unit of a crystal that, when repeated in three dimensions, can generate the entire crystal

Quaternary structure in PDB database

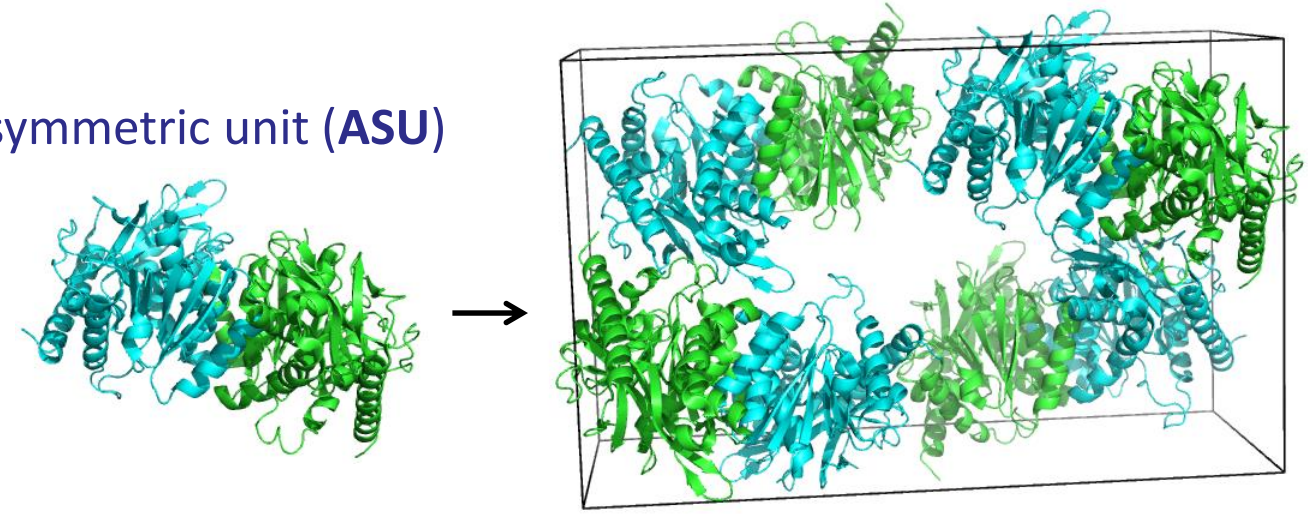


Crystalline environment

- **Crystal contacts**
  - Intermolecular contacts solely due to protein crystallization
    - Causes artifacts of crystallization
    - Crystal packing - complicates identification of native quaternary structure

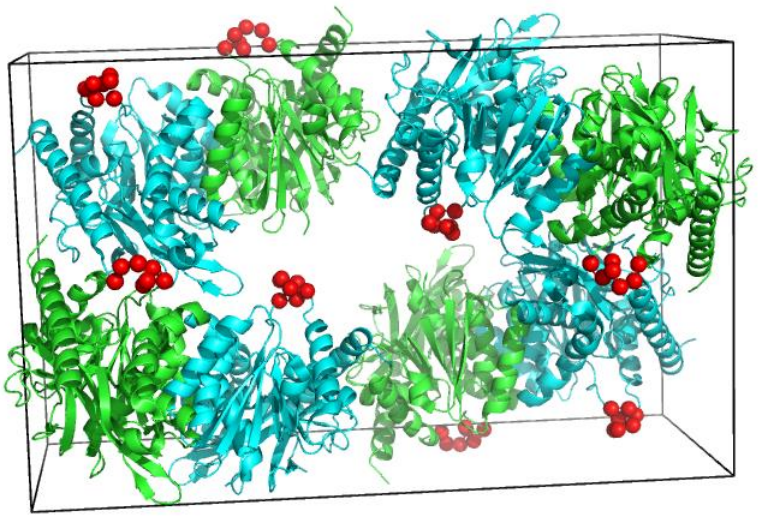
Crystal Unit (**CU**)

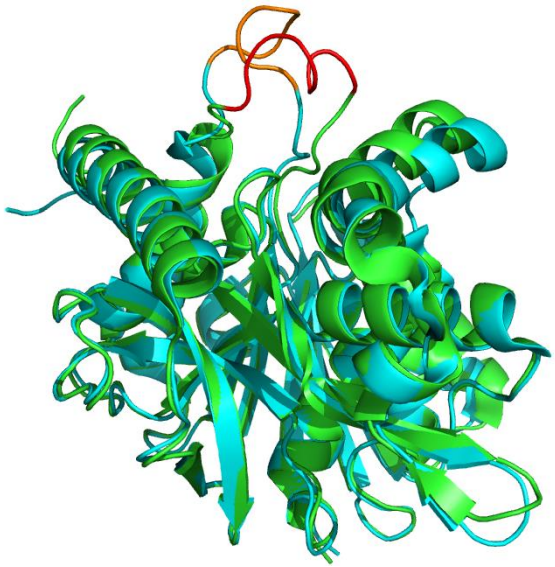
Asymmetric unit (**ASU**)



Crystalline environment

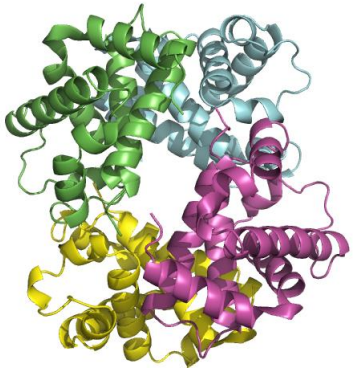
- **Artifacts of crystallization**
  - Concerns about conformation of some surface regions
  - Often loops or side chains are affected
  - Can complicate the evaluation of the effects of mutations





Quaternary structure in PDB database

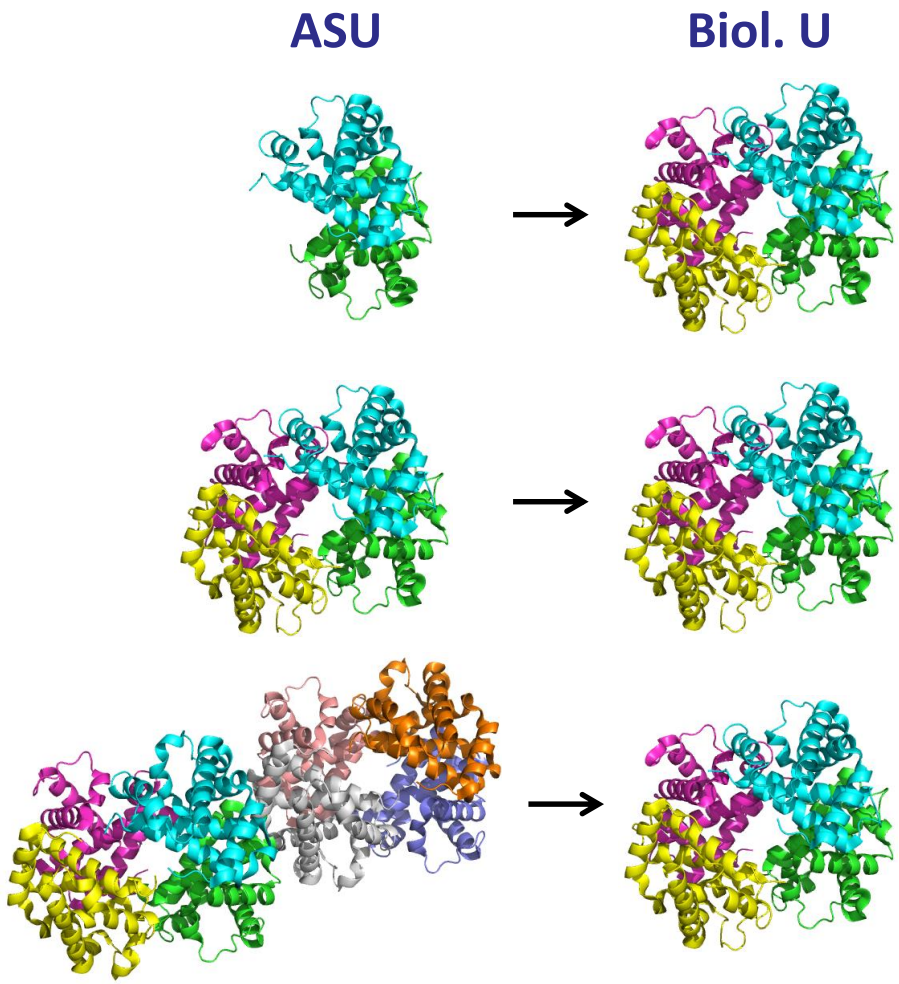
- **Biological unit**
  - The functional form of a protein in nature
  - Also called: functional unit, biological assembly, quaternary structure
  - Can depend on the environment, post-translational modifications of proteins and their mutations



Hemoglobin heterotetramer

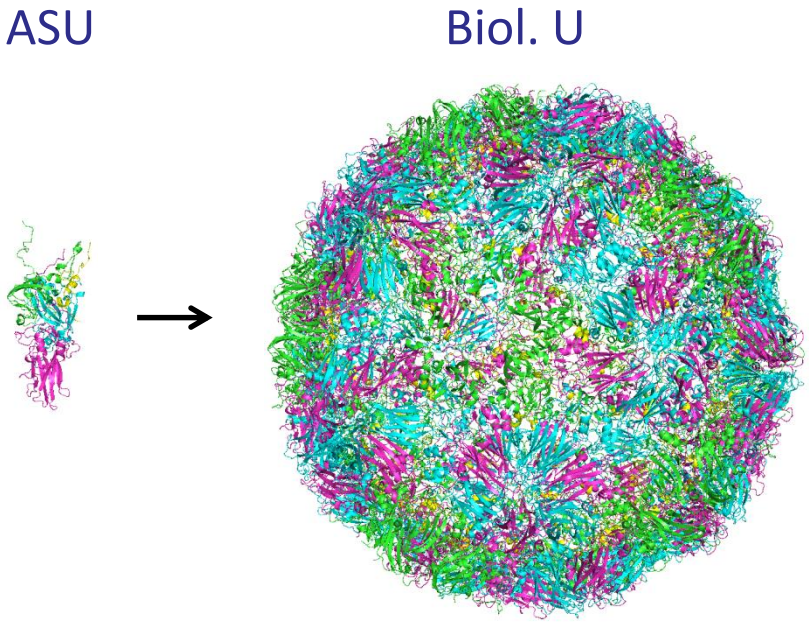
Biological *versus* asymmetric unit

- **Biological unit can consist of:**
  - Multiple copies of the ASU
  - One copy of the ASU
  - A portion of the ASU

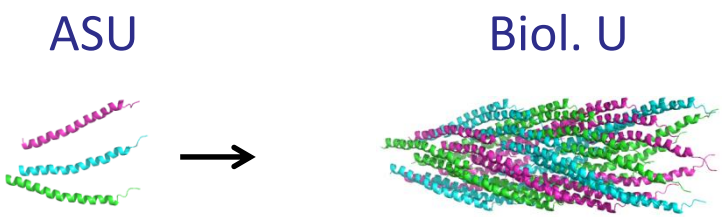


Biological *versus* asymmetric unit

- **Large assemblies**
  - Viral capsid

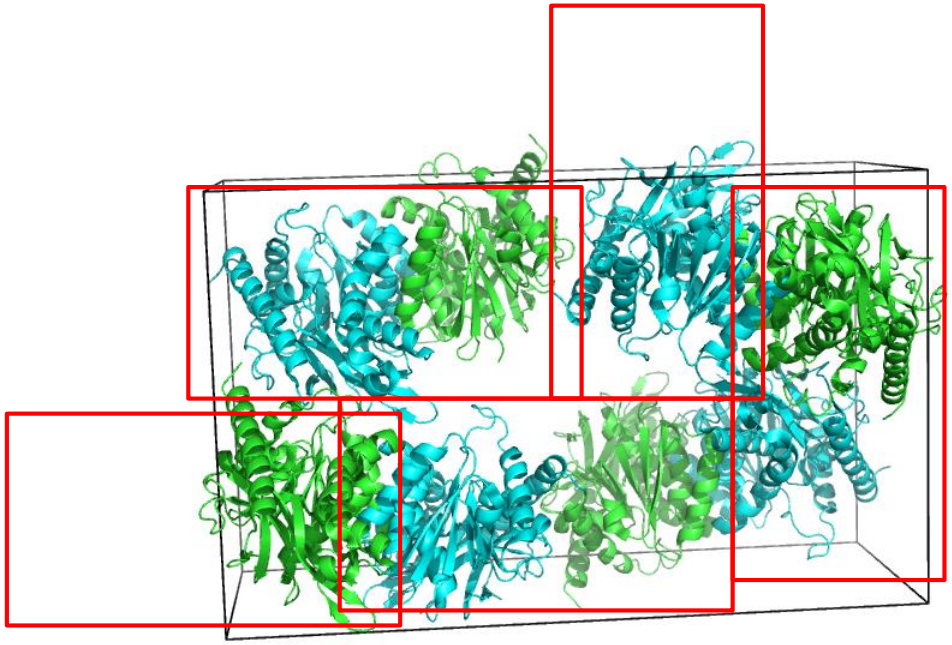


  - Filamentous bacteriophage PF1



Complex or artifact?

- **Problem**
  - Most proteins in the PDB have three or more crystal contacts that sum up to 30% of the protein solvent accessible surface area
  - How to recognize biologically relevant contacts from crystal one?



Complex or artifact?

- **Experimental knowledge of oligomeric state helps with identifying of the structure of native complex**
  - Search literature
  - Experimental methods
    - Gel filtration, static or dynamic light scattering, analytical ultracentrifugation, native electrophoresis, …
- **How to get the structure of a biological unit?**
  - Author-specified assembly
  - Databases
  - Predictive tools

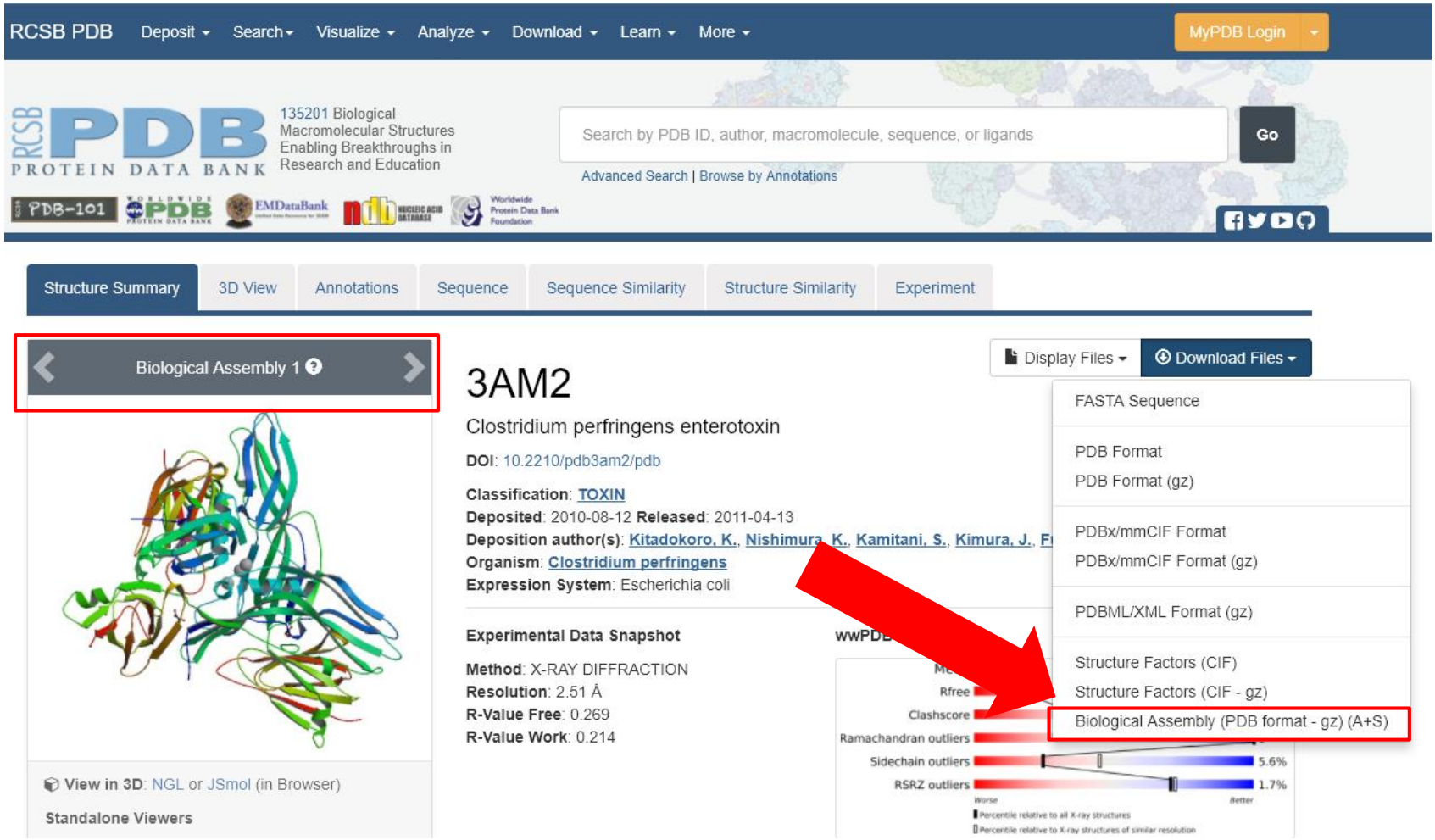
Author-specified assembly

- **REMARK 350 in headers of PDB file**
  - Contains symmetry operations to reconstruct biological unit, but…

  → Verify author-proposed biological unit by other means
    - Sometimes the specific oligomers were not known at the time the ASU was published
    - Some authors may have failed to specify the biological unit even when it was known
    - Rarely, the specified biological unit might be incorrect
- **Employed by**
  - RCSB PDB and other tools

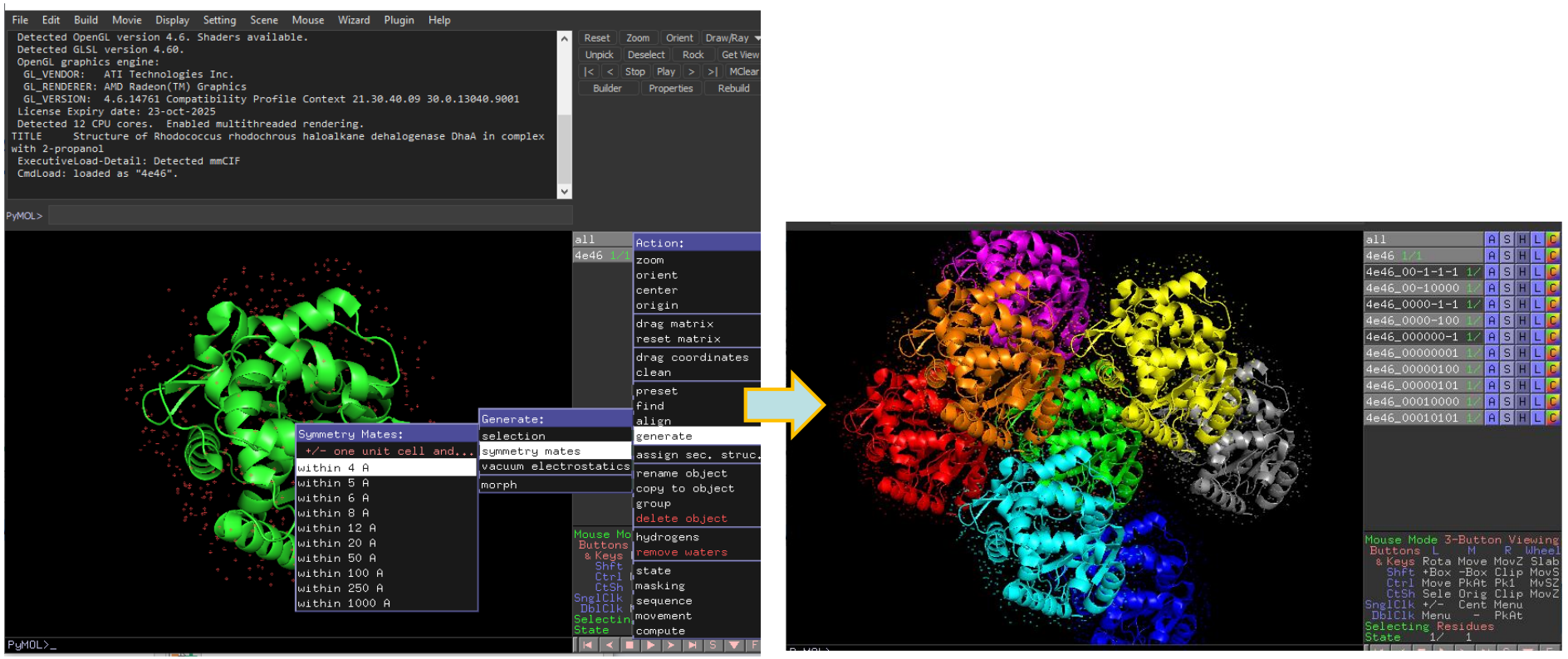
Author-specified assembly

- **RCSB PDB**



Crystal lattice

- **PyMOL**
  - Generate > Symmetry mates → to visualize nearest partners



Prediction of 3D structure of complexes

Discovering and characterising macromolecular complexes

requires heavy experimentation

How can we predict macromolecular complexes?

Prediction of 3D structure of complexes

Homology-based predictions

Machine learning-based predictions

Macromolecular docking

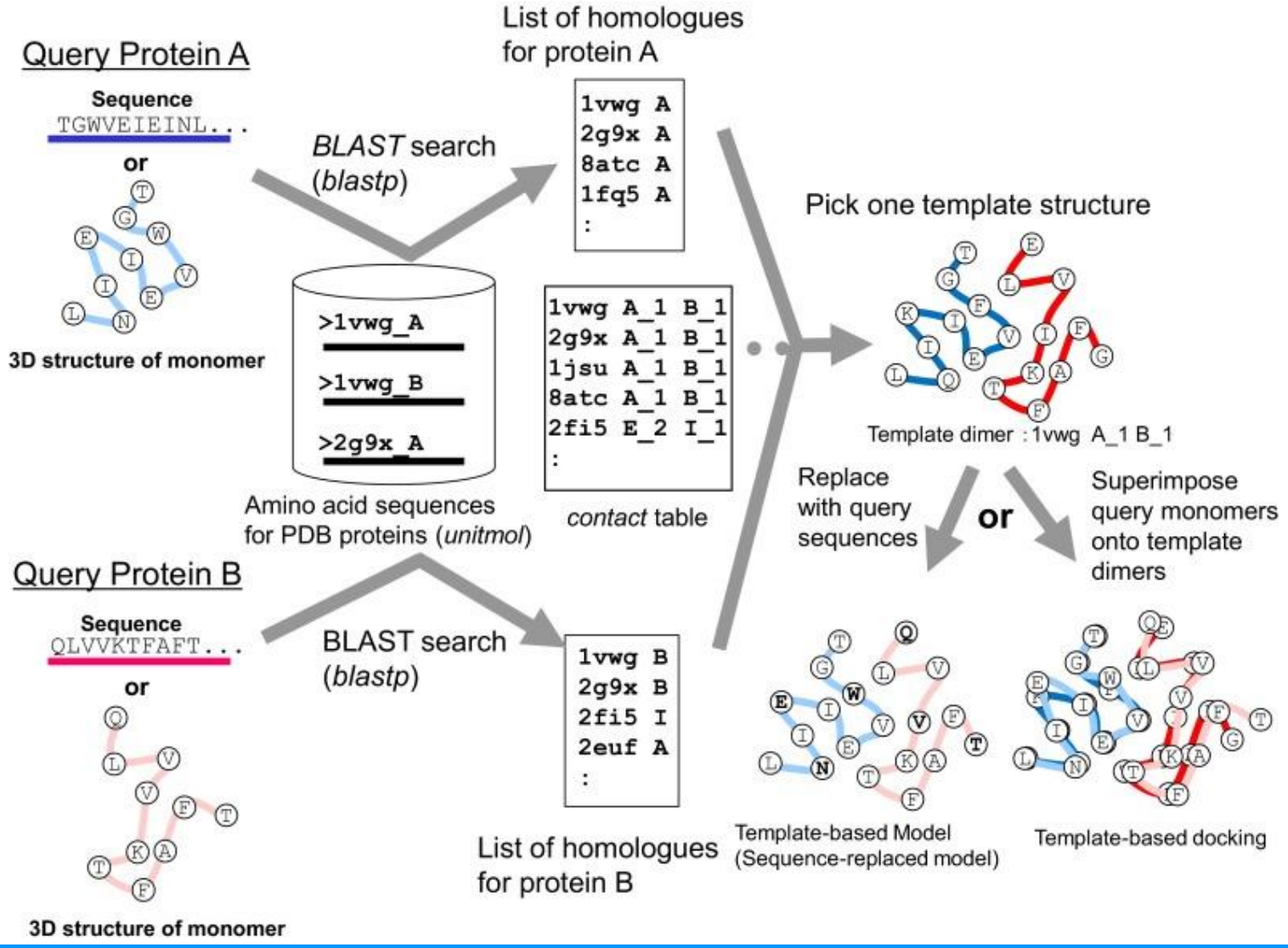
Homology based methods

- A protein complex is built based on a similar protein complex with a known 3D structure
- Assumes that the interaction information can be extrapolated from one complex structure to close homologs of interacting proteins
  - Close homologs (≥ 40% sequence identity) almost always interact in the same way (if they interact with the same partner)
  - Sequence similarity is only rarely associated with a similarity in interactions
- Limited applicability (low number of templates)

Homology based methods

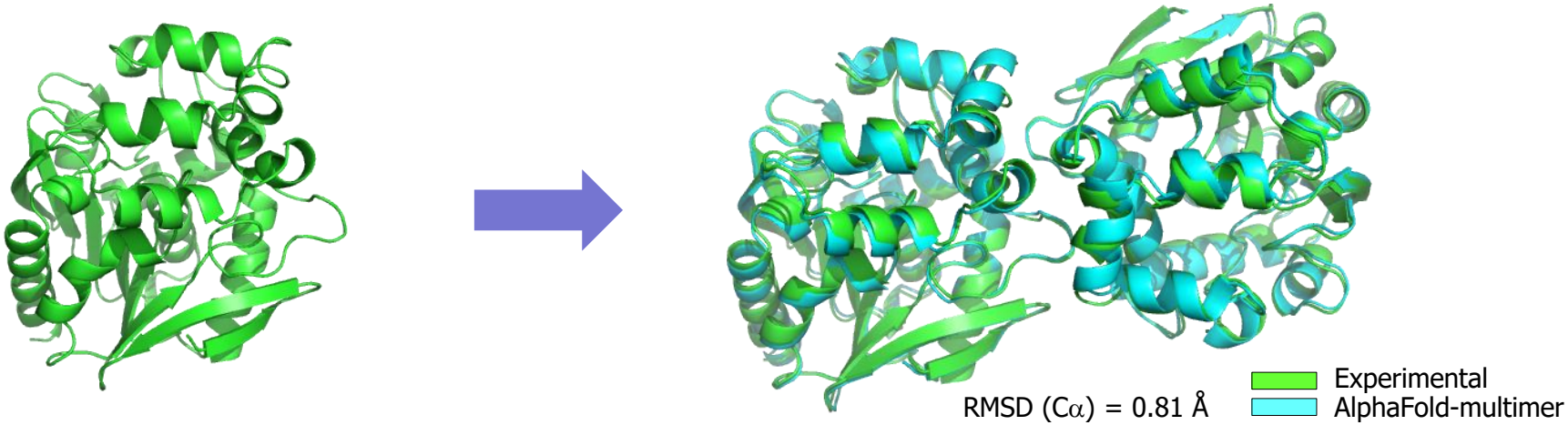
- HOMCOS (**Ho**mology **M**odeling of **Co**mplex **S**tructure)
  - https://homcos.pdbj.org/
  - Predicts 3D structure of homodimers and heterodimers by homology modeling
  - Optionally, identifies potentially interacting proteins
  - Steps:
    1. BLAST search to identify homologous templates
    2. Evaluation of the model validity by combination of sequence similarity and knowledge-based contact potential energy
    3. Generation of a full atomic model by MODELLER

Homology based methods



Machine learning-based predictions

- AlphaFold-Multimer
  - Variant of AlphaFold 2
  - Predicts 3D structure of multimers



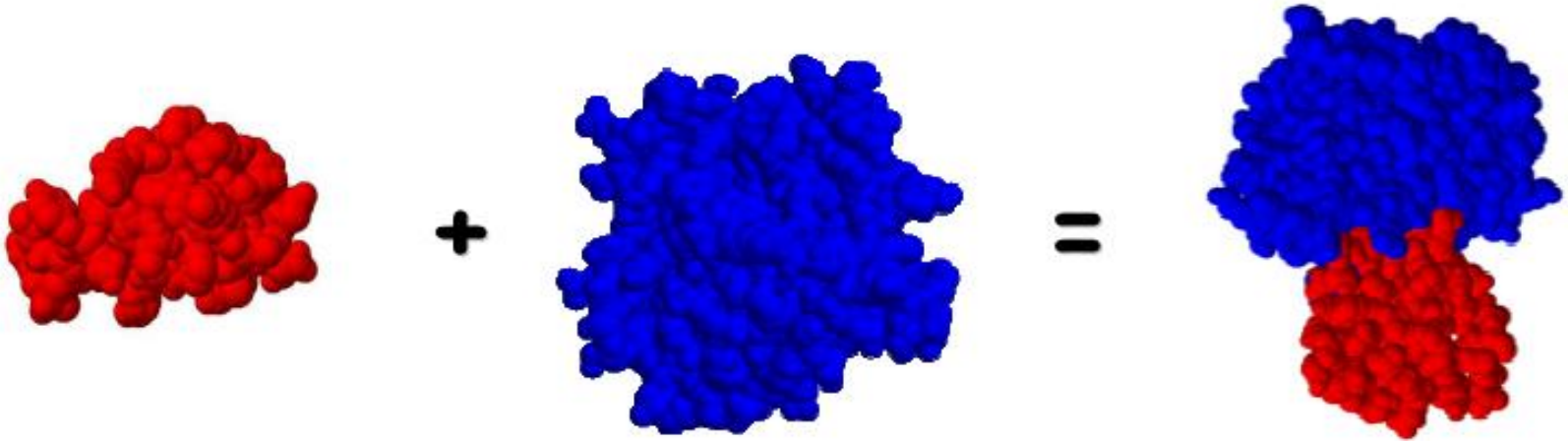
  - AlphaFold 3 equivalent just came out (Abramson et al., 2024, Nature)

Macromolecular docking

- Prediction of the best bound state for given 3D structures of two or more macromolecules
- Difficult task
  - Large search space - many potential ways in which macromolecules can interact
  - Flexibility of the macromolecular surface and conformational changes upon binding
- Can be facilitated by prior knowledge
  - Ex: known binding site → significant restriction of the search space
  - Distance constraints on some residues

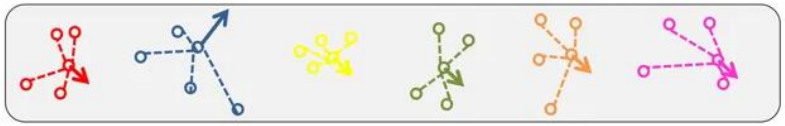
Macromolecular docking

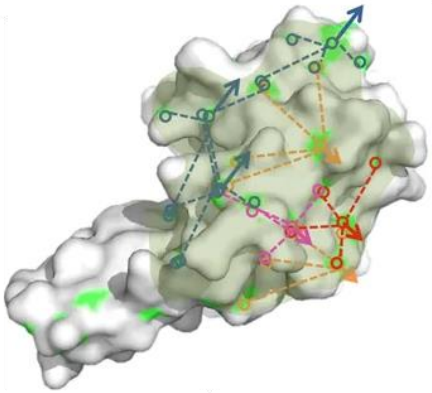
- 3 main parameters:
  - Macromolecule representation
  - Search algorithm
  - Scoring function

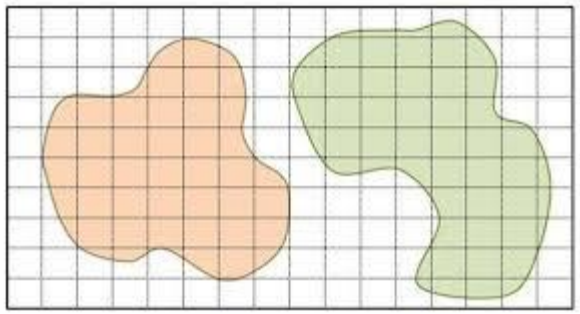


Macromolecule representation

- Representation of the macromolecular surface (applicable to both receptor and ligand)
  - Geometrical descriptors of shape (set of spheres, surface normals, vectors radiating from the center of the molecule,...)
  - Discretization of space: grid representation







Macromolecule representation

- Macromolecule flexibility
  - Fully rigid approximation
  - Soft docking – employs tolerant “soft” potential scoring functions to simulate plasticity of otherwise rigid molecule
  - Explicit side-chain flexibility – optimization of residues by rotating part of their structure or rotation of whole side-chains using predefined rotamer libraries
  - Docking to molecular ensemble of protein structure – composed from multiple crystal structures, from NMR structure determination or from trajectory produced by MD simulation

Macromolecule representation

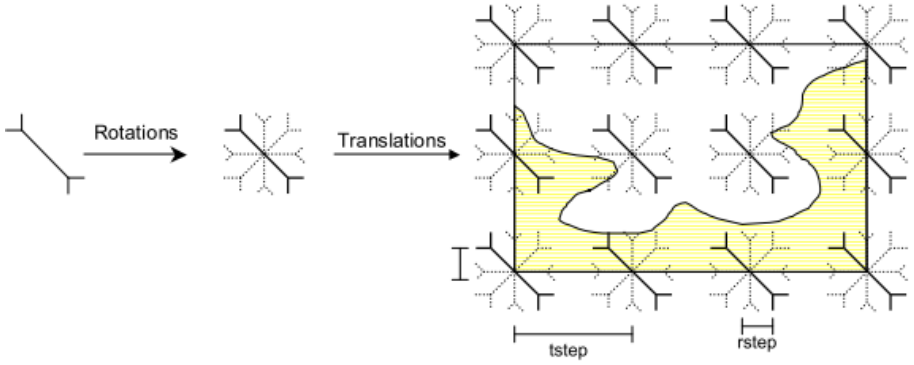
- Macromolecule flexibility
  - Rigid body docking – basic model that considers the two macromolecules as two rigid solid bodies
  - Semiflexible docking – one of the molecules is rigid, and one is flexible (typically the smaller one)
  - Flexible docking – both molecules are considered flexible

Macromolecular docking - search

- Generally based on the idea of complementarity between the interacting molecules (geometric, electrostatic or hydrophobic contacts)
- The main problem is the dimension of the conformational space to be explored:
  - Rigid docking: 6D (hard)
  - Flexible docking: 6D + N_{fb} (impossible!)
- Information on the rough location of the binding surface (experimental or predicted) → reduction of the search space

Macromolecular docking - search

- Exhaustive search
  - Full search of the conformational space: try every possible relative orientation of the two molecules
  - Computationally very expensive – 6 degrees of freedom for rigid molecules (translations + rotations)
  - Grid approaches



Macromolecular docking - search

- Stochastic methods
  - Monte Carlo
  - Genetic algorithms
  - Brownian dynamics
  - …

Macromolecular docking - scoring

- Scoring functions
  - Evaluation of a large number of putative solutions generated by the search algorithms

- Methods often use a two-stage ranking
  1. Approximate and fast-to-compute function – used to eliminate very unlikely solutions
  2. More accurate function – used to select the best among the remaining solutions

Macromolecular docking - scoring

- Scoring functions
  - Empirical
  - Knowledge-based
  - Force field-based
  - Clustering-based – the presence of many similar solutions is taken as an indication of correctness (all solutions are clustered, and the size of each cluster is used as a scoring parameter)

Macromolecular docking - scoring

- Good scores – a combination of several parameters:
  - Low free energy or pseudo-energy based on force field functions
  - Large buried surface area
  - Good geometric complementarity
  - Many H-bonds
  - Good charge complementarity
  - Polar/polar contacts favored
  - Polar/non-polar contacts are disfavored
  - Many similar solutions (large clusters)
  - …

Macromolecular docking - programs

Web server/software and link	Docking method	Filtering and refinement
BDOCK [152] http://www.biotec.tudresden.de/~bhuang/bdock/bdock.html	FFT correlation based on shape complementarity, degree of burial and conservation	Altering the docking solutions with a scoring function
ClusPro [110] http://nrc.bu.edu/cluster/	FFT correlation using DOT [109]	Filtering with empirical potential and clustering, refinement by SmoothDock [111]
DOT [109] http://www.sdsc.edu/CCMS/DOT/	FFT correlation based on electrostatics and shape complementarity	Refinement by energy minimization
FireDock [153] http://bioinfo3d.cs.tau.ac.il/FireDock/	None (refinement server)	Refinement using an energy function
GRAMMX [108] http://vakser.bioinformatics.ku.edu/resources/gramm/grammx	FFT correlation based on shape complementarity, hydrophobicity and smoothed potentials	Clustering and knowledge-based scoring
HADDOCK [154] http://www.nmr.chem.uu.nl/haddock/	Data-driven docking approach based on biochemical and/or biophysical interaction data	None
HEX [155] http://www.csd.abdn.ac.uk/hex/	Spherical polar Fourier correlations	None
MolFit [156] http://www.weizmann.ac.il/Chemical_Research_Support//molfit/home.html	FFT correlation based on chemical and shape complementarity	Clustering of the predicted conformations
PatchDock [114] http://bioinfo3d.cs.tau.ac.il/PatchDock/	Geometric hashing and pose-clustering	Ranking according to a geometric shape complementarity score
PyDock [157] http://mmb.pcb.ub.es/PyDock/	FFT based on electrostatics and desolvation energy	Ranking using an energy function
RosettaDock [115] http://rosettadock.graylab.jhu.edu/	Local docking by Monte Carlo search	Ranking using an energy function, clustering
ZDOCK [107] http://zlab.bu.edu/zdock/index.shtml	FFT correlation based on shape complementarity, desolvation energy and electrostatics	Refinement by energy minimization
3D-Dock [158] http://www.sbg.bio.ic.ac.uk/docking/	FFT correlation using FTDOCK [159]	Clustering, refinement of side-chains using Multidock [159]

Macromolecular docking - programs

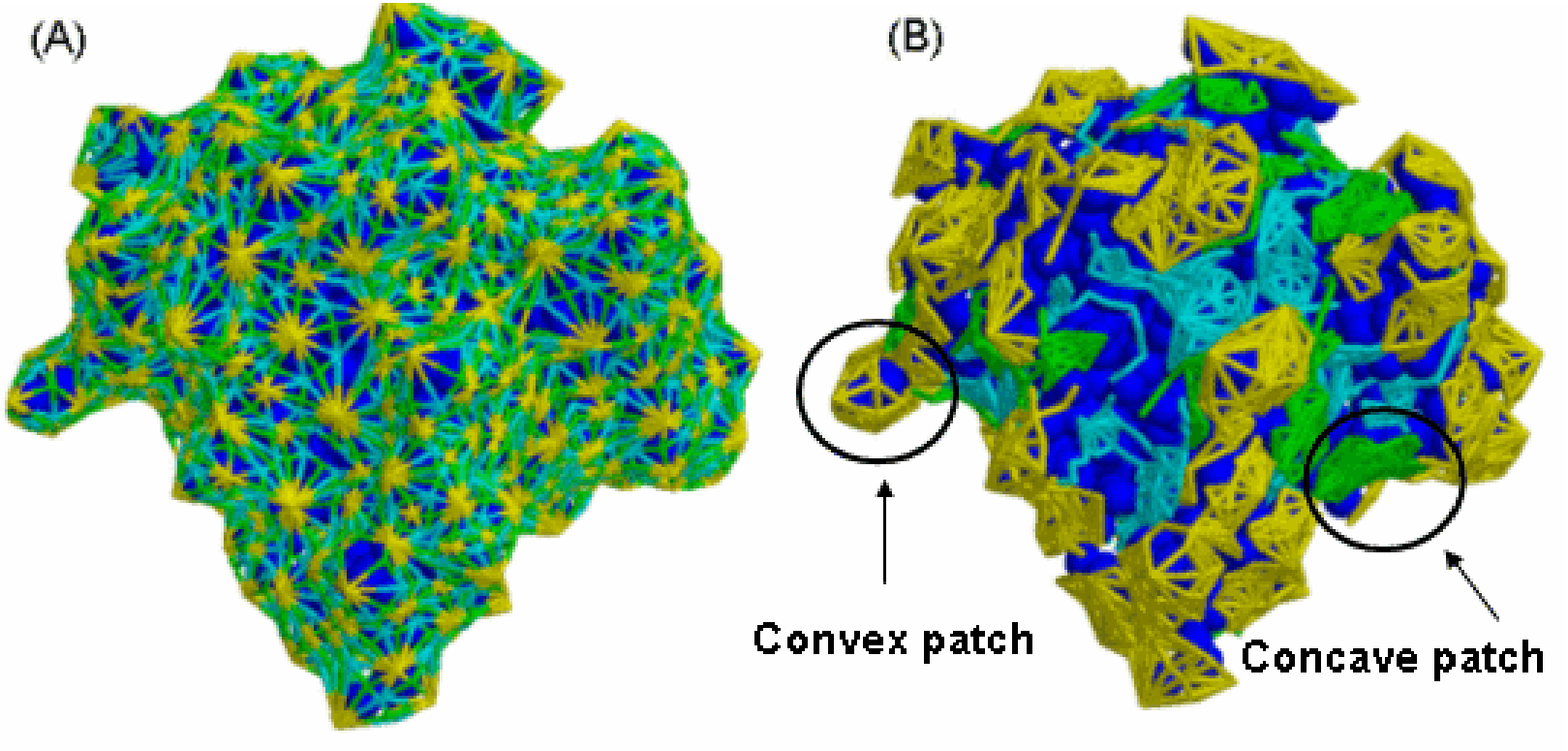
- ClusPro 2.0
  - http://cluspro.bu.edu/
  - Performs a global soft rigid-body search using PIPER docking program; employs knowledge-based potential
  - The top 1,000 structures are retained and clustered to isolate highly populated low-energy binding modes
  - A special mode for prediction of molecular assemblies of homo-oligomers

Macromolecular docking - programs

- PatchDock
  - http://bioinfo3d.cs.tau.ac.il/PatchDock/index.html
  - Performs a geometry-based search for docking transformations that yield good molecular shape complementarity (driven by local feature matching rather than brute force searching of the 6D space):
    1. The molecular surface is divided into concave, convex and flat patches
    2. Complementary patches are matched → candidate transformations
    3. Evaluation of each docking candidate by a scoring function considering both geometric fit and atomic desolvation energy
    4. Clustering of the candidate solutions to discard redundant solutions
  - Results can be redirected to FireDock for refinement and re-scoring

Macromolecular docking - programs

- PatchDock



Macromolecular docking - programs

- FireDock
  - http://bioinfo3d.cs.tau.ac.il/FireDock/index.html
  - Refines and re-scores solutions produced by fast rigid-body docking algorithms
  - Optimizes the binding of each candidate by allowing flexibility in the side-chains and adjustments of the relative orientation of the molecules
  - Scoring of the refined candidates is based on softened van der Waals interactions, atomic contact energy, electrostatic, and additional binding free energy estimations

Analysis of macromolecular complexes

- Binding energy
- Macromolecular interface
- Interaction hot spots

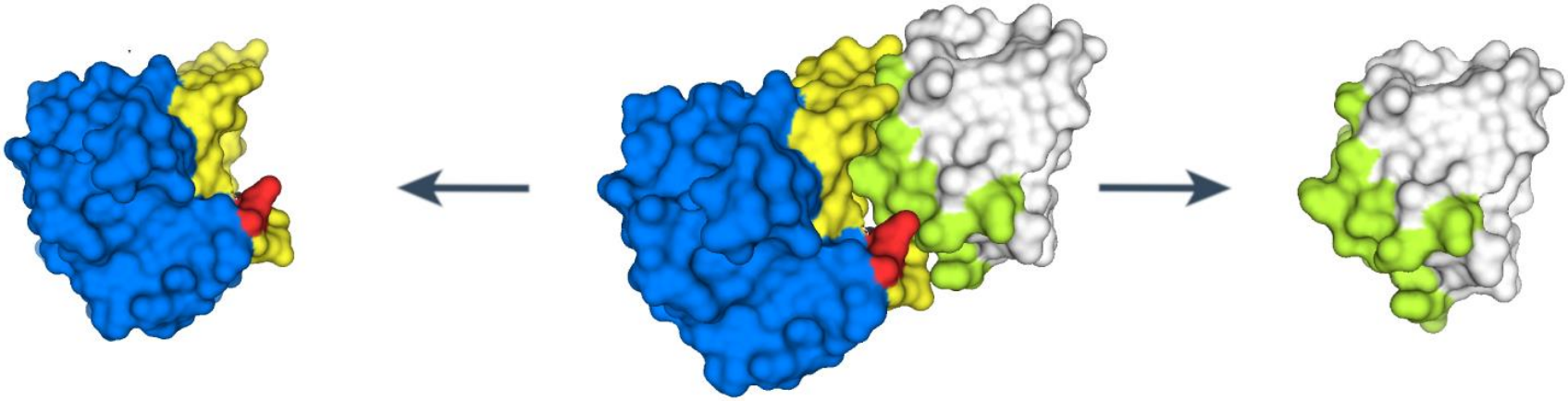
Binding energy

- FastContact
  - http://structure.pitt.edu/servers/fastcontact/
  - Rapidly estimates the electrostatic and desolvation components of the binding free energy between two proteins
  - Additionally, evaluates the van der Waals interactions using CHARMM and reports contribution of individual residues and pairs of residues to the free energy → highlight the interaction hot spots

```
----- SUMMARY ENERGIES -----------------------
 Electrostatic (4r) Energy:  -18.3684946 kcal/mol
 Desolvation  Free  Energy:   8.31365025 kcal/mol
 van der Waals (CHARMm19) :  -1734.5 kcal/mol
----------------------------------------------
Top 20 Min & Max ligand residues contributing to the binding free energy
 -2.628   89 ASN
 -2.586    6 LYS
 -2.209    9 TYR
 -2.135  125 LEU
 -2.114    2 PHE
 -1.832   45 ARG
 -1.684   87 ASN
```

Macromolecular interface

- The region where two protein chains or protein and nucleic acid chain come into contact
- Can be identified by the analysis of the 3D structure of the macromolecular complex



Interface analysis

- Provides information about basic features of macromolecular complexes interactions (e.g., shape complementarity, chemical complementarity,...)
- Provides information about interface residues
- Acquired information is useful for a wide range of applications
  - Design of mutants for experimental verification of the interactions
  - Development of drugs targeting macromolecular interactions
  - Understanding the mechanism of the molecular recognition
  - Computational prediction of interfaces and complex 3D structures
  - ...

Interface analysis

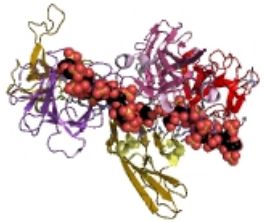
- Most common approaches for the definition of interfaces:
  - Methods based on the distance between interacting residues
  - Methods based on the change in the solvent accessible surface area (ASA) upon complex formation
  - Computational geometry methods (using Voronoi diagrams)
- All three approaches provide very similar results

Interface analysis - databases

- PDBsum (Pictorial database of 3D structures in the Protein Data Bank)
  - http://www.ebi.ac.uk/pdbsum/
  - Provides numerous structural analyses for all PDB structures and AlphaFold DB (human proteins), including information about protein-protein and protein-nucleic acid interfaces
  - Protein-protein interactions – schematic diagrams of all protein-protein interfaces and corresponding residue-residue interactions
  - Protein-nucleic acid interactions – schematic diagrams of protein-nucleic acid interactions generated by NUCPLOT

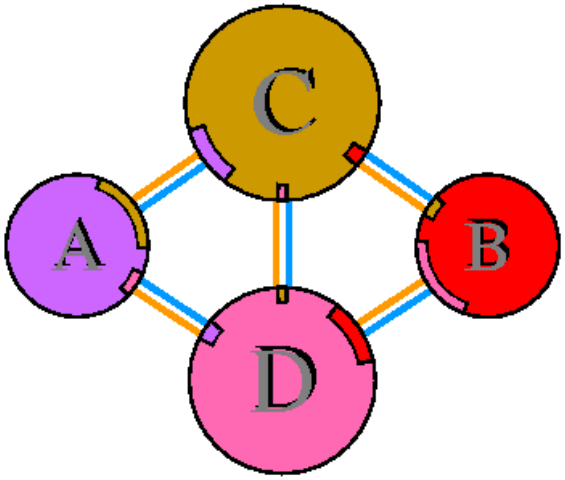
Interface analysis - databases

- PDBsum



Interfaces
- Interface summary
- A:C * (22:29 res)
- A:D * (6:10 res)
- B:C * (6:7 res)
- B:D * (23:25 res)
- C:D * (3:3 res)

Interfaces summary for 1fq9



Postscript version

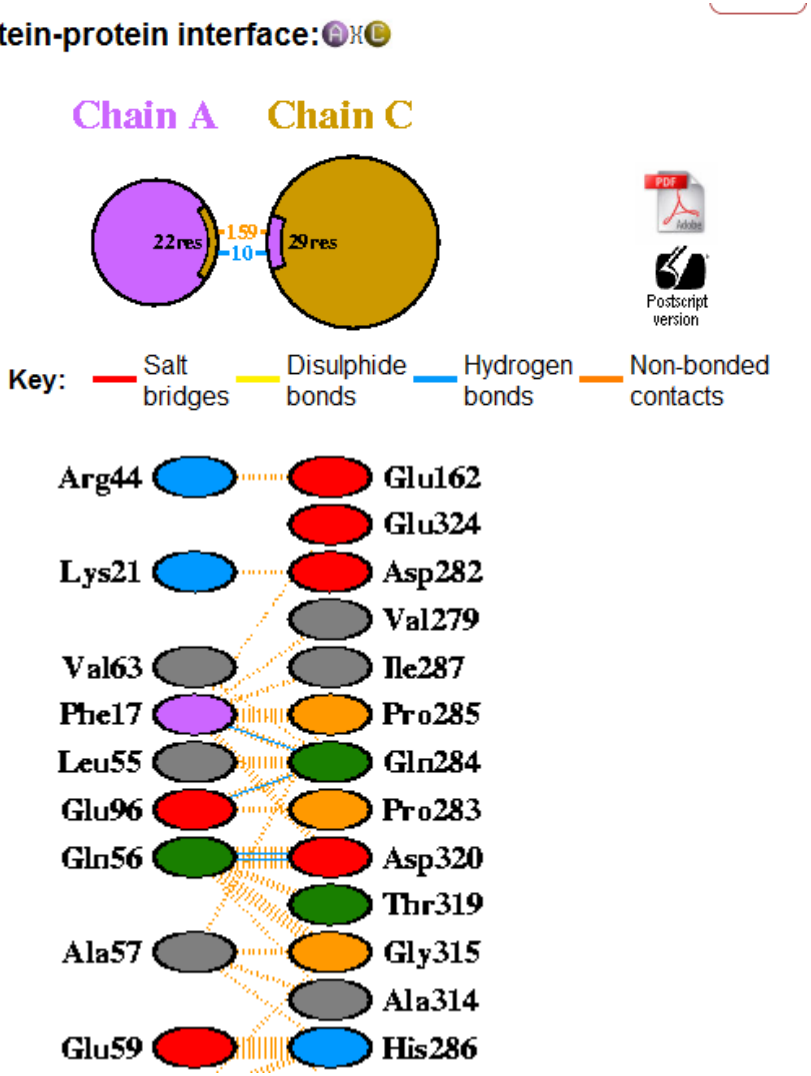
Key: Salt bridges | Disulphide bonds | Hydrogen bonds | Non-bonded contacts

Interface statistics

Chains	No. of interface residues	Interface area (A^2)	No. of salt bridges	No. of disulphide bonds	No. of hydrogen bonds	No. of non-bonded contacts
A:C	22:29	1344:1285	-	-	10	159
A:D	6:10	389:409	-	-	1	34
B:C	6:7	340:363	-	-	1	28
B:D	23:25	1369:1313	-	-	10	159
C:D	3:3	189:182	-	-	2	18

Interface analysis - databases

- PDBsum



Interface analysis - tools

- Analyze interface of a given macromolecular complex
  - PISA (Protein Interfaces, Surfaces and Assemblies)
  - MolSurfer
  - Contact Map WebViewer
  - PIC (Protein Interaction Calculator)
  - …

Interface analysis - tools

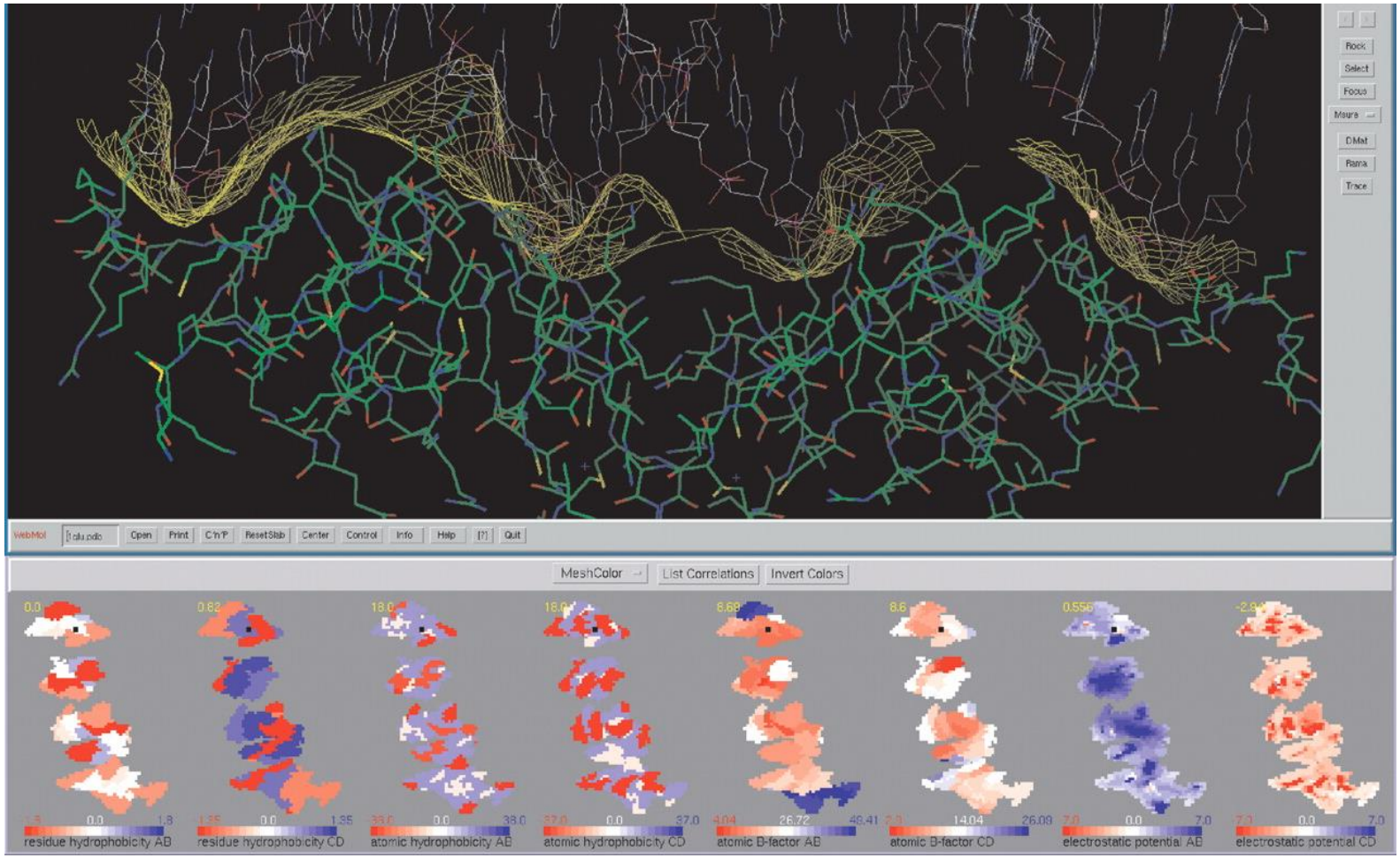
- PISA (**P**rotein **I**nterfaces, **S**urfaces and **A**ssemblies)
  - www.pdbe.org/pisa
  - An interactive tool for the exploration of macromolecular interfaces (protein, DNA/RNA and ligands), prediction of probable quaternary structures, database searches of structurally similar interfaces and assemblies
  - Overview and detailed characteristics of all interfaces found within a given structure (including those generated by symmetry operations)
  - Provides interface area, $\Delta^{i}G$, potential hydrogen bonds and salt bridges, interface residues and atoms, ...

Interface analysis - tools

- MolSurfer
  - http://projects.villa-bosch.de/dbase/molsurfer/index.html
  - Visualization of 2D projections of protein-protein and protein-nucleic acid interfaces as maps showing a distribution of interface properties (atomic and residue hydrophobicity, electrostatic potential, surface-surface distances, atomic distances,...)
  - 2D maps are linked with the 3D view of a macromolecular complex
  - Facilitates the study of intermolecular interaction properties and steric complementarity between macromolecules

Interface analysis - tools

- MolSurfer



Interface analysis - tools

- Contact Map WebViewer
  - http://cmweb.enzim.hu/
  - Represents residue-residue contacts within a protein or between proteins in a complex in the form of a contact map

- PIC (**P**rotein **I**nteraction **C**alculator)
  - http://pic.mbu.iisc.ernet.in/
  - Identifies various interactions within a protein or between proteins in a complex

Interaction hotspots

- Hot spots: the residues contributing the most to the binding free energy of the complex
- Knowledge of hot spots has important implications to:
  - Understand the principles of protein interactions (an important step to understand recognition and binding processes)
  - Design of mutants for experimental verification of the interactions
  - Development of drugs targeting macromolecular interactions
  - …

Interaction hotspots

- Hot spots are usually conserved and appear to be clustered in tightly packed regions in the center of the interface
- Experimental identification by alanine scanning mutagenesis → if a residue has a significant drop in binding affinity when mutated to alanine it is labeled as a hot spot
- Experimental identification of hot spots is costly and cumbersome → the computational predictions of hot spots can help!

Prediction of hotspots - tools

- Most of the available methods are based on the 3D structure of the complex
- Knowledge-based methods
  - Combination of several physicochemical features
  - Evolutionary conservation, ASA, residue propensity, structural location, hydrophobicity,...)
- Energy-based methods
  - Calculation of the change in the binding free energy ($\Delta\Delta G_{bind}$) of the complex upon *in silico* modification of a given residue to alanine

Prediction of hotspots - tools

- Robetta
  - http://old.robetta.org/alascansubmit.jsp
  - Energy-based method
  - Performs *in silico* alanine scanning mutagenesis of protein-protein or protein-DNA interface residues

1. The side chain of each interface residue is mutated to alanine
2. All side chains within 5 Å radius sphere of the mutated residue are repacked; the rest of the protein remains unchanged
3. For each mutant, $\Delta\Delta G_{bind}$ is calculated (residues with predicted $\Delta\Delta G_{bind} \geq +1$ kcal/mol = hot spot)

Prediction of hotspots - tools

- Robetta

Tue Nov 6 00:20:55 PST 2012
virtual alanine scanning, Minimized_PfTPR1_23_1.alascan

pdb#	chain	int_id	res#	aa	DDG(complex)	DDG(complex,obs)	DG(partner)
15	A	1	15	12	0.26	0.00	-0.11
18	A	1	18	5	1.99	0.00	1.27
45	A	0	45	16	-0.01	0.00	3.31
46	A	1	46	12	1.53	0.00	-0.07
53	A	1	53	16	-0.11	0.00	-0.57
80	A	1	80	15	2.52	0.00	4.85
83	A	1	83	2	-0.10	0.00	5.34
86	A	1	86	7	0.29	0.00	0.34
124	B	0	124	17	-0.02	0.00	0.60
125	B	1	125	8	1.75	0.00	0.08
126	B	1	126	4	-0.23	0.00	-0.41
127	B	1	127	4	0.02	0.00	-0.60
128	B	1	128	18	1.98	0.00	-0.45
129	B	1	129	3	-0.29	0.00	-0.81

Prediction of hotspots - tools

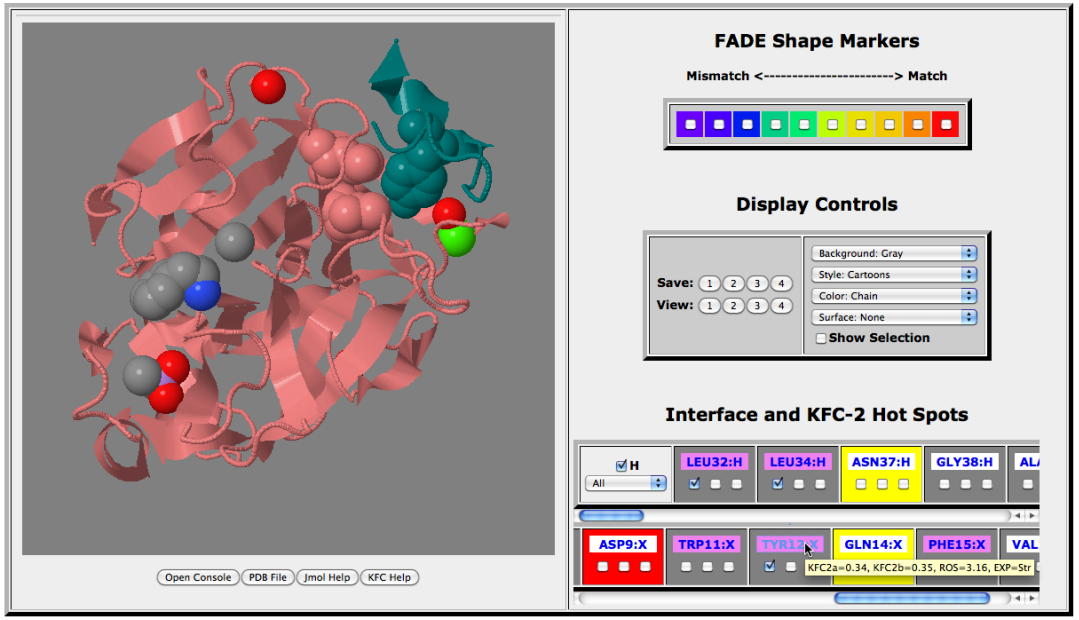
- KFC2 (**K**nowledge-based **F**ADE and **C**ontacts)
  - https://mitchell-web.ornl.gov/KFC_Server/
  - Knowledge-based method utilizing machine learning
  - Predicts hot spots in protein-protein interfaces by recognizing features of important binding contacts – solvent accessibility, residue position within the interface, packing density, residue size, flexibility and hydrophobicity of residues around the target residue
  - Optionally, user can provide data to improve the prediction (ConSurf conservation scores, Rosetta alanine scanning results or experimental data)

Prediction of hotspots - tools

- KFC2 (**K**nowledge-based **F**ADE and **C**ontacts)

KFC2 Hot Spot Prediction Server @mitchell-lab.org from Thu, 17 Mar 2011 12:18:45 CDT
JobId: 3748 JobName: Demo_22_1dva_kfc2

Chain	Res	Num	KFC2-A Class	KFC2-A Conf	KFC2-B Class	KFC2-B Conf	ConSurf Class	ConSu Value	Rosetta Class	Roset DDG	Exper Class	Exper Value
H	LEU	32	-------	-0.75	Hotspot	0.10	-------	2	-------	0.41	Hotspot	Str
H	LEU	34	-------	-0.71	Hotspot	0.11	-------	2	-------	1.25	Hotspot	Str
H	ASN	37	-------	-1.79	-------	-0.97	-------	1	-------	0.01	-------	Ins
H	GLY	38	-------	-0.15	-------	-0.61	-------	3	-------	---	-------	---
H	ALA	39	-------	-1.59	-------	-0.87	-------	1	-------	---	-------	---
H	GLN	40	-------	-1.53	-------	-0.98	-------	6	-------	0.01	-------	---
H	ASP	60	-------	-----	-------	-----	-------	1	-------	---	-------	---
H	ILE	65	-------	-0.77	-------	-0.40	-------	3	-------	0.73	-------	Ins
H	VAL	67	-------	-0.30	-------	-0.12	-------	5	-------	0.70	-------	Ins
H	GLU	70	-------	-1.28	-------	-0.73	Conserv	7	-------	1.02		
H	LEU	73	Hotspot	0.14	Hotspot	0.24	-------	2	-------	0.53		
H	SER	74	-------	-1.20	-------	-0.89	-------	5	-------	0.11		
H	GLU	75	-------	-1.83	-------	-0.98	-------	1	-------	0.00		
H	HIS	76	-------	-0.95	-------	-0.81	-------	1	-------	0.43		
H	GLU	80	-------	-1.26	-------	-0.65	Conserv	7	-------	0.01		
H	GLN	81	-------	-2.03	-------	-0.98	-------	2	-------	---		
H	SER	82	-------	-1.23	-------	-0.86	-------	1	-------	-0.01		



References I

- Liljas, A. *et al*. (2009). **Textbook Of Structural Biology**, World Scientific Publishing Company, Singapore.
- Goodsell, D. S. & Olson, A. J. (2000) Structural symmetry and protein function. *Annual Review of Biophysics and Biomolecular Structure* **29**: 105-153.
- Demachenko, A. P. (2001). Recognition between flexible protein molecules: induced and assisted folding. *Journal of Molecular Recognition* **14**: 42-61.
- Ali, M. H. & Imperiali, B. (2005) Protein oligomerization: How and why. *Bioorganic & Medicinal Chemistry* **13**: 5013-5020.
- Jahn, T. R. & Radford, S. E. (2008) Folding *versus* aggregation: Polypeptide conformations on competing pathways. *Archives of Biochemistry and Biophysics* **469**: 100-117.
- Csermely, P. *et al.* (2010) Induced fit, conformational selection and independent dynamic segments: an extended view of binding events. *Trends in Biochemical Sciences* **35**: 539-546.

References II

- Bujnicki, J. (2009). **Prediction of Protein Structures, Functions, and Interactions**, John Wiley & Sons, Ltd., Chichester, p. 302.
- Tramontano, A. (2005). **The Ten Most Wanted Solutions in Protein Bioinformatics**, CRC Press UK, London, p. 186.
- Tuncbag, N., *et al.* (2009). A survey of available tools and web servers for analysis of protein-protein interactions and interfaces. *Briefings in bioinformatics* **10**: 217-232.
- Ezkurdia, I., *et al.* (2009). Progress and challenges in predicting protein-protein interaction sites. *Briefings in bioinformatics* **10**: 233-246.
- Fernández-Recio, J. (2011). Prediction of protein binding sites and hot spots. *Computational molecular science* **6**: 680-698.
- Szilagyi, A., *et al.* (2005). Prediction of physical protein–protein interactions. *Physical biology* **2**: S1-S16.
- Moreira, I. S., *et al.* (2010). Protein-protein docking dealing with the unknown. *Journal of computational chemistry* **31**:317-342