Analysis of protein structures

Outline

❑ Residue solvent accessibility

❑ Protein solubility

❑ Molecular interactions

❑ Functional sites

 ▪ Binding sites

 ▪ Transport pathways

Residue solvent accessibility

❑ Solvent accessible surface area (ASA or SASA, in Å^2):
the extent to which a residue in a protein structure is
accessible to the solvent

❑ Typically calculated by rolling a spherical probe of a
particular radius over a protein surface and summing the
area that can be accessed by this probe on each residue
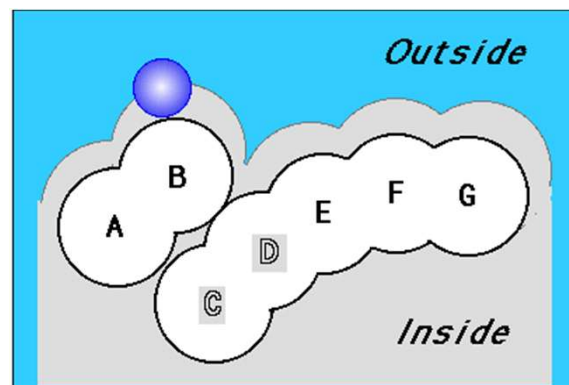
Residue solvent accessibility

- Solvent accessible surface area (ASA, SASA or SAS, in Å^2)

- Solvent excluded surface (SES) – also known as molecular surface, or Connolly surface area
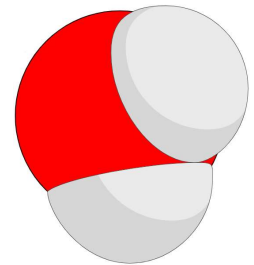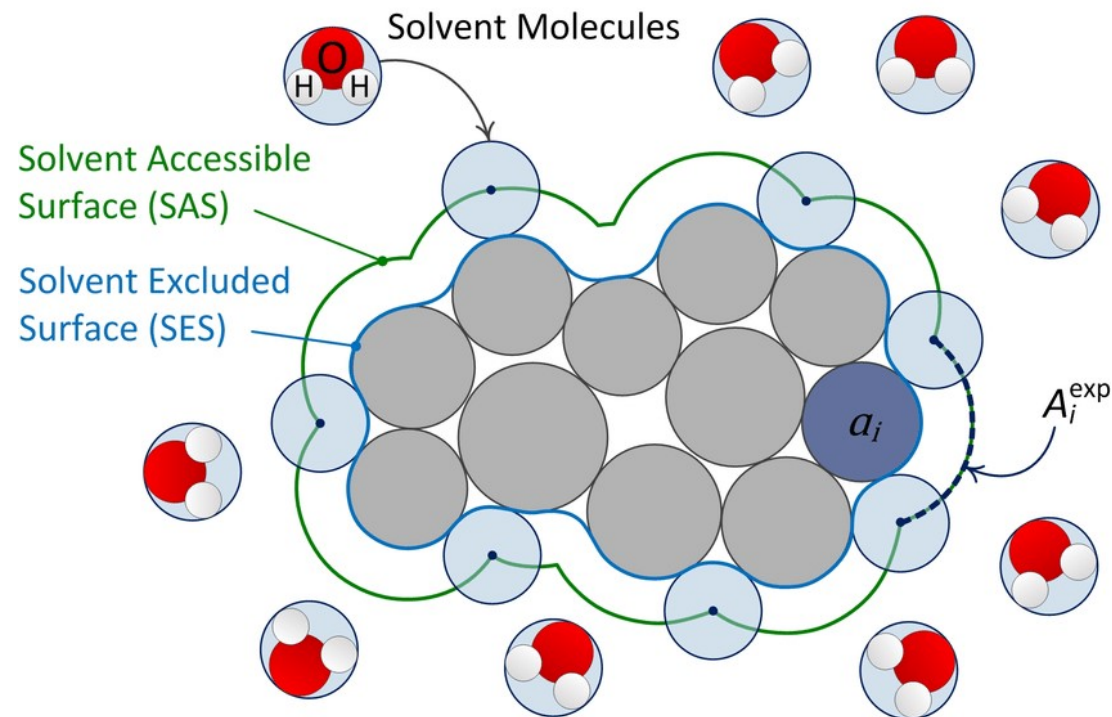
Water radius ≈ 1.4 Å

Residue solvent accessibility

- ❑ Relative accessible surface area (rASA)

  - ▪ Ratio of the actual accessible area of a given residue

    $$rASA = ASA / ASA_{MAX}$$

  - ▪ Enables comparison of accessibility of different amino acids (e.g., long extended vs. spherical amino acids)

- ❑ Simplified two state description

  - ▪ Buried vs. exposed residues

  - ▪ *threshold* for differentiating buried vs. surface residues is not well defined (usually 15–25 %)

  - ▪ rASA < *threshold* => buried; rASA ≥ *threshold* => exposed

Residue solvent accessibility – programs

❑ Naccess

  ▪ http://www.bioinf.manchester.ac.uk/naccess/

  ▪ Calculates atomic and residue ASAs and rASA for PDB files

  ▪ Adjustable settings (e.g., probe size, atomic radii,...)
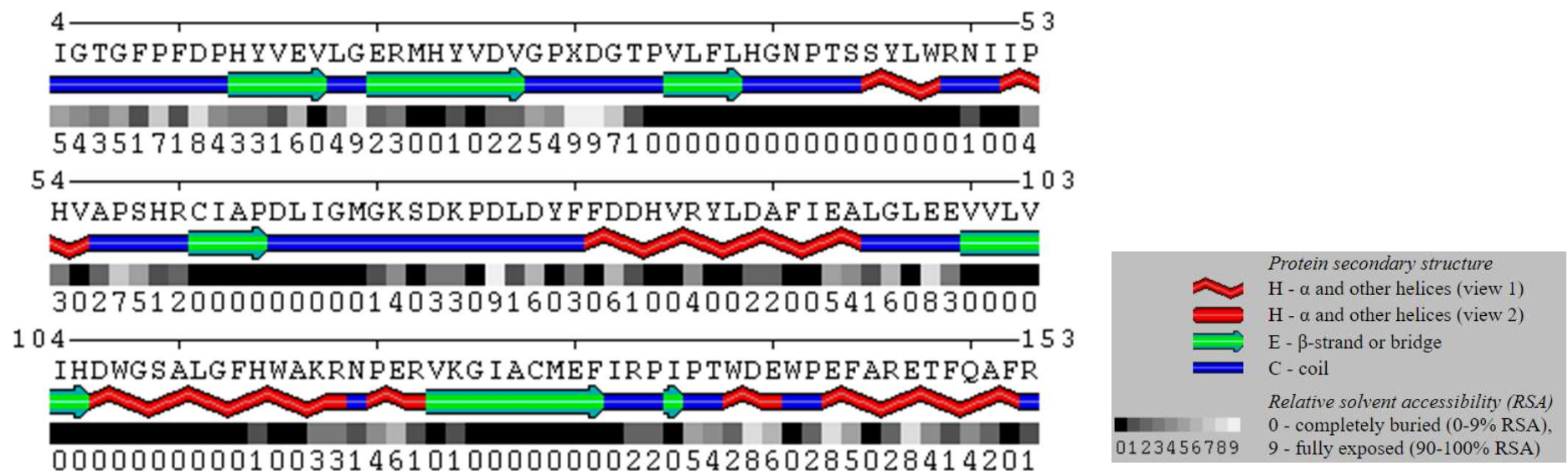
❑ DSSP

  ▪ https://swift.cmbi.umcn.nl/gv/dssp/

  ▪ Assignment of secondary structures and calculation of ASAs of residues

Residue solvent accessibility – programs

❑ POLYVIEW-2D

- https://polyview.cchmc.org/

- Visualization tool for structural and functional annotations of proteins, including solvent accessibility

- Residue SASA calculated by DSSP and transformed to rASA

Protein solubility

- Concentration of protein in saturated solution that is in equilibrium with solid phase

- For proteins expressed in the lab, it depends on
    - Hydrophilic/hydrophobic balance of the solvent-exposed residues
    - Aggregation-prone regions (APRs) – mainly hydrophobic residues prone to form beta-structures
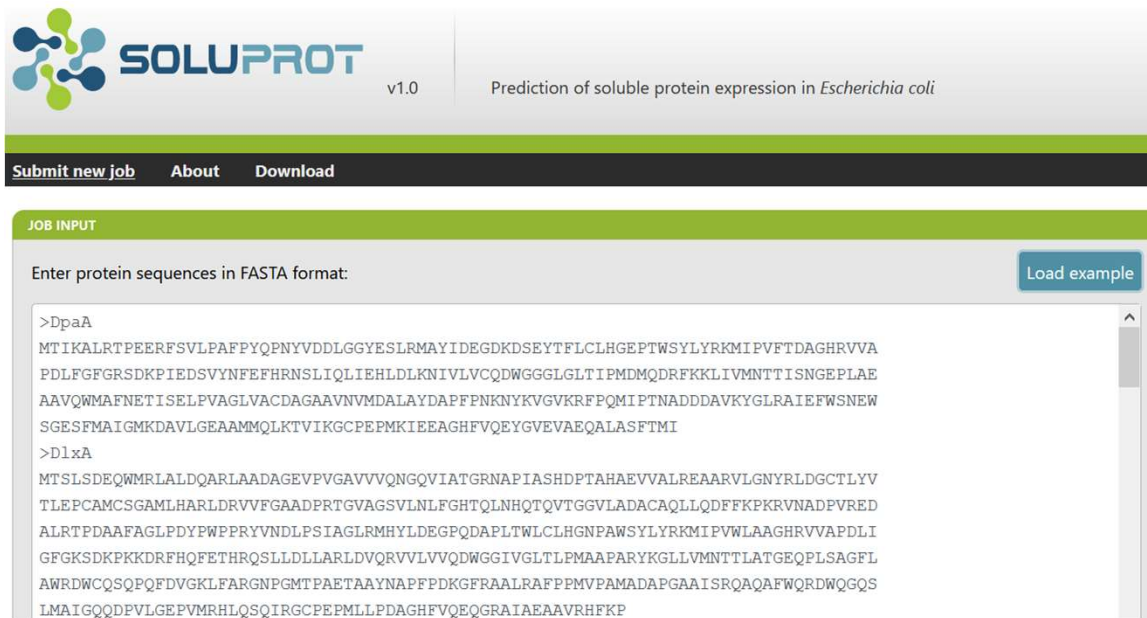   - Protein expressibility in the cells
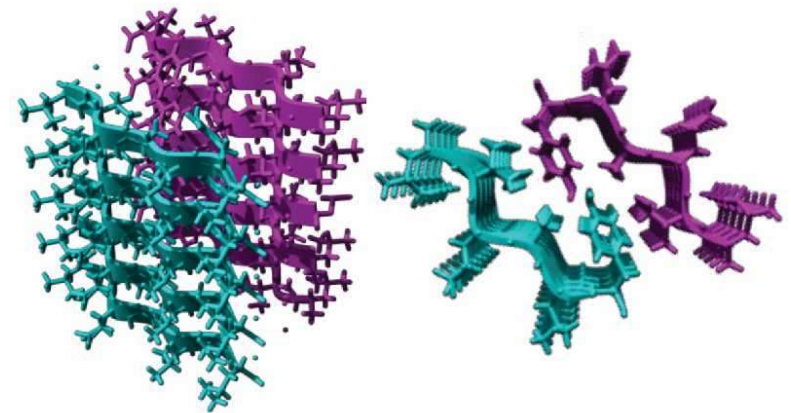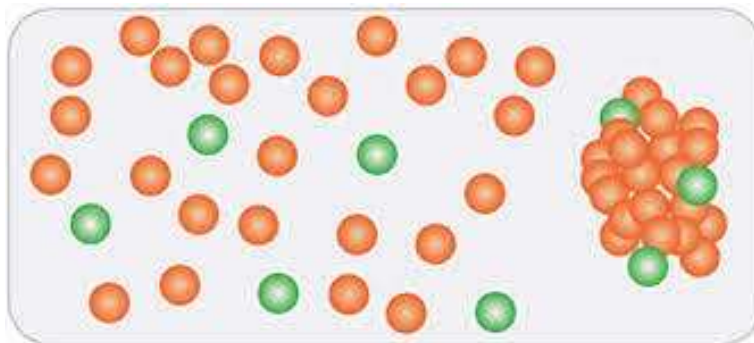
Cross-beta spines of amyloid fibrils

Protein solubility

- ❑ SoluProt

  - ▪ https://loschmidt.chemi.muni.cz/soluprot/

  - ▪ Soluble expression of protein sequences in *E.coli*

  - ▪ Based on machine learning

Input

Output

Protein solubility

- ❑ Aggrescan3D

  - ■ http://biocomp.chem.uw.edu.pl/A3D2/

  - ■ Predicts the aggregation propensities by identifying APRs

  - ■ Can introduce mutations and the predict impact on stability and aggregation-propensity

  - ■ Can account for protein flexibility ("dynamic mode")

Mutations

-2.5 -2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0 1.5

Aggrescan Score
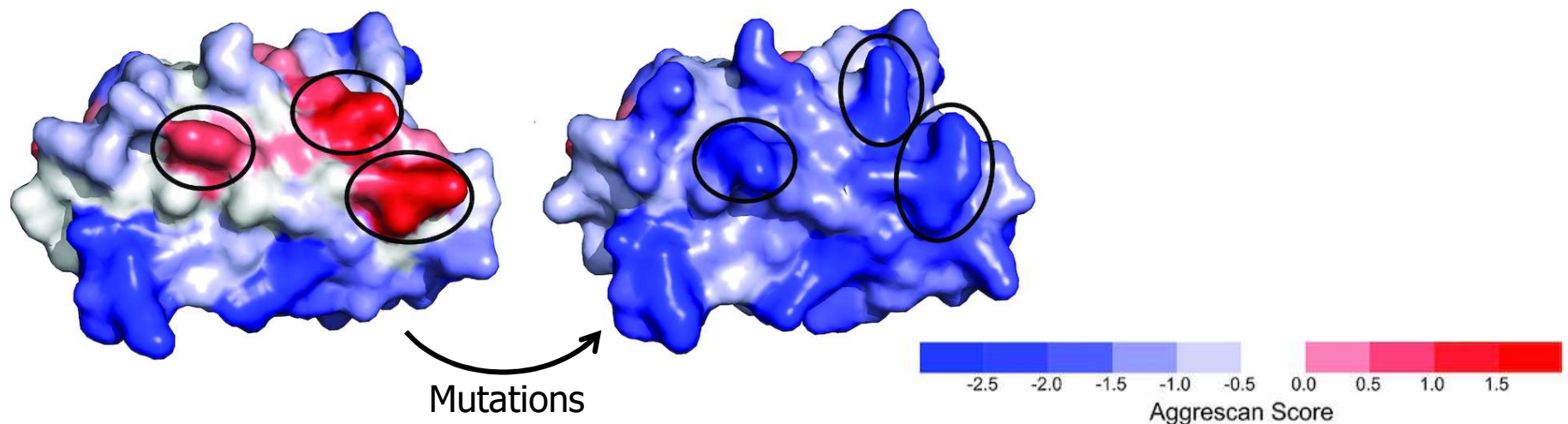
Molecular interactions
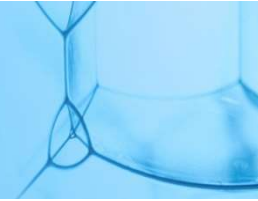
□ Assessment of the interactions within a protein structure and between proteins in an assembly is essential to understand the molecular basis of stability and function of proteins and their complexes

Types of interactions

- charge-charge (ionic) interactions

  - present in charged residues; e.g. salt bridges

- hydrogen bonds (H-bonds)

  - donor and acceptor atoms sharing hydrogen

- aromatic (π-π) interactions

  - attractive interaction between aromatic rings

- van der Waals (vdW) interactions

  - between any two atoms

- hydrophobic interactions

  - entropic origin; important for hydrophobic residues

Types of interactions

❑ **disulfide bonds (cysteine bridges)**

2 Cys $\text{R}-\text{SH}$, $\text{SH}-\text{R}$ $\xrightarrow{\text{oxidation}}$ $\text{R}-\text{S}-\text{S}-\text{R}$ $+$ $2H^+$ $+$ $2e^-$

polypeptide
cysteine
disulfide bridge
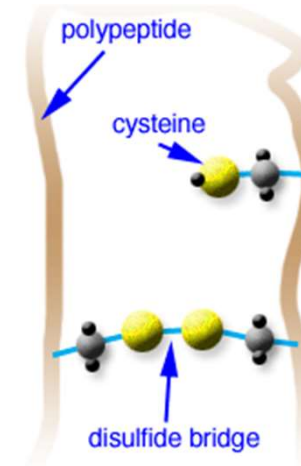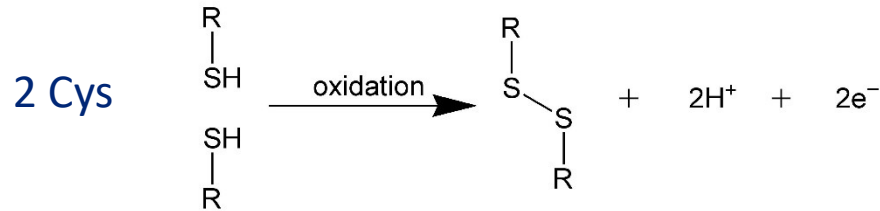
❑ **cation-π interactions**

▪ electrostatic interaction of a positively charged residue (Lys or Arg)

with an aromatic residue (Phe, Trp, or Tyr)

Cation
+
Aromatic ring

Lys

Trp
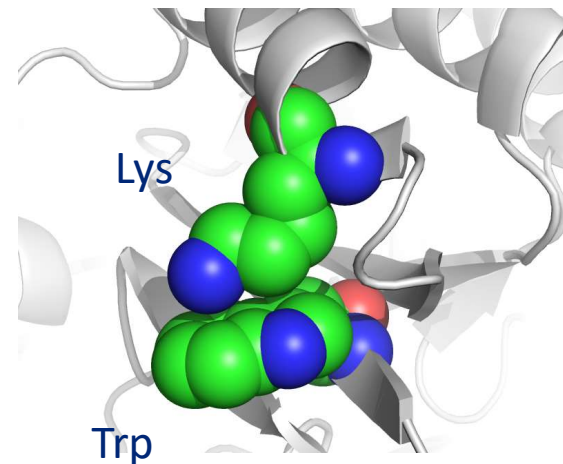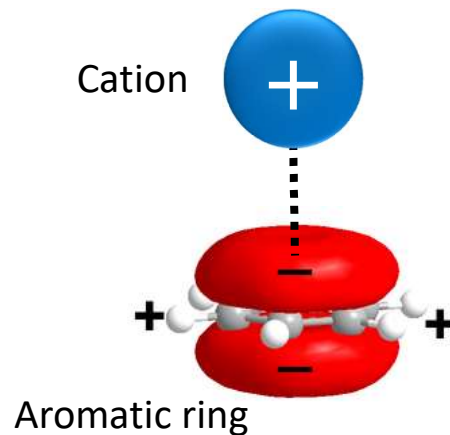
Molecular interactions – how to identify?

- criteria for recognizing various types of interactions

  - geometric rules (distances, angles)

  - atom types

  - energetics (physicochemical rules)

  - contact surface area between atoms

Sphere A   Sphere B

Contact surface of atom A with atom B
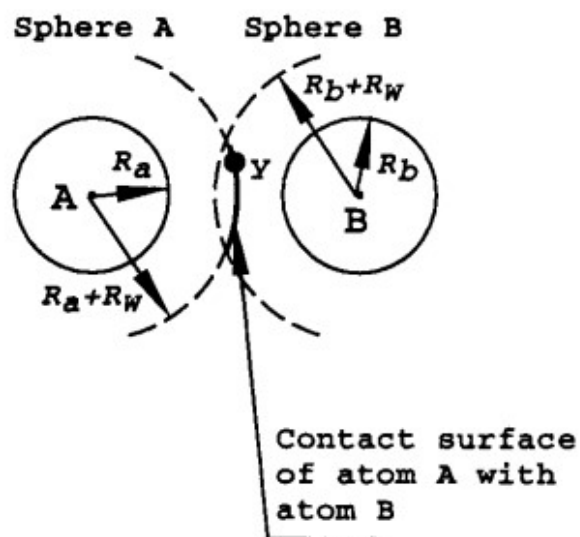
Molecular interactions – programs

❑ CMView

  ❑ https://www.bioinformatics.org/cmview/

  ▪ represents residue-residue contacts within a protein or between

    proteins in a complex in the form of a contact map

  ▪ 3D visualization using PyMol

Molecular interactions – programs

- PIC (**P**rotein **I**nteraction **C**alculator)

  - http://pic.mbu.iisc.ernet.in/
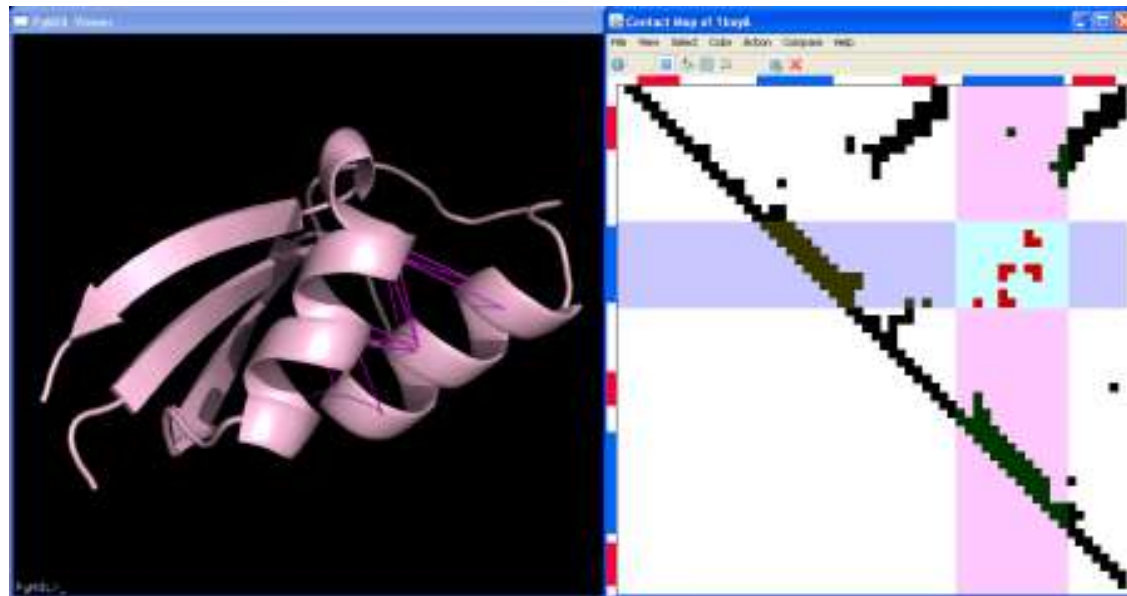
  - identifies various interactions – disulfide bonds, hydrophobic interactions, ionic (charged-charged) interactions, hydrogen bonds, aromatic–aromatic, aromatic–sulfur and cation–π interactions within a protein or between proteins in a complex

  - uses standard criteria (atom types and geometry)

Molecular interactions – programs

- ❑ PIC (**P**rotein **I**nteraction **C**alculator)

  - ▪ http://pic.mbu.iisc.ernet.in/

Molecular interactions – programs

❑ ESBRI (**E**valuating the **S**alt **BRI**dges in Proteins)

- ▪ http://bioinformatica.isa.cnr.it/ESBRI/introduction.html

- ▪ analysis of salt bridges interactions (ionic interaction + H-bond)

- ▪ checks if at least one Asp or Glu side-chain carboxyl oxygen atom and one side-chain nitrogen atom of Arg, Lys or His are within a distance ≤ 4.0 Å
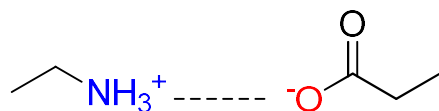
Salt bridge

Residue 1	Residue 2	Distance
NZ ALYS A 11	OD1 ASP A 62	3.86
NZ ALYS A 11	OD2 ASP A 62	2.78
NZ ALYS A 11	OD2 ASP A 68	2.85
NZ BLYS A 11	OD1 ASP A 62	3.79
NZ BLYS A 11	OD2 ASP A 62	2.74
NZ BLYS A 11	OD2 ASP A 68	2.75
NH1 ARG A 46	OE1 GLU A 276	3.61

Functional sites

- binding sites

  - binding sites for small molecules

  - binding sites for macromolecules

- transport pathways

  - overall void

  - tunnels

  - channels

Binding sites

❑ sites on the protein that provides the complementarity for

the bound molecule (ligand)

▪ binding site – its function is molecular recognition

▪ active/catalytic site – special case of the binding site – its function is

to promote chemical catalysis (break/formation of covalent bonds)

❑ binding involves the formation of non-covalent interactions

between the protein and the bound molecule

❑ bound molecule – small molecule or macromolecule

❑ binding is usually very specific – complementarity in shape

and charge distribution between the site and bound molecule

Binding sites
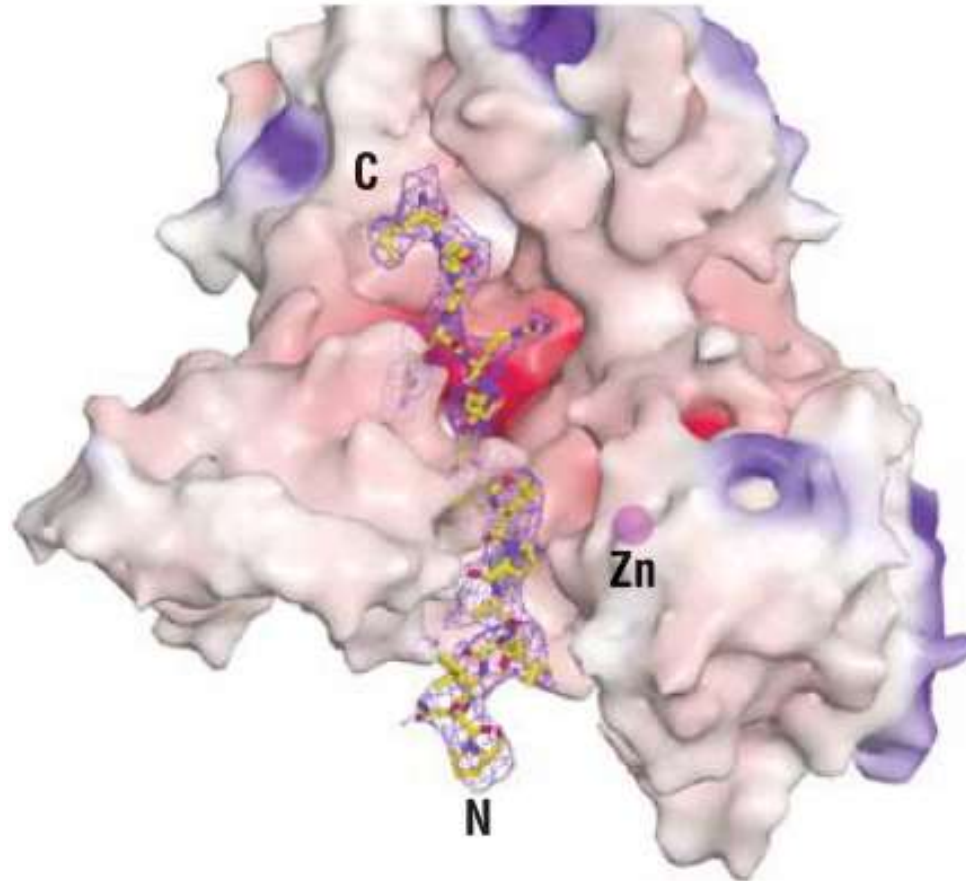
Complementarity in shape and charge distribution
between the active site and substrate

Binding sites for small molecules

- typically internal cavities, or surface pockets or clefts
  - concave regions
  - provide microenvironment different from that of the bulk solvent (e.g., many residues with negative charge → very strong electrostatic field enabling binding of highly charged ligands)
  - often identifiable by a simple examination of the protein structure
- highly conserved by evolution
- low desolvation energy
- characteristic physicochemical properties

Binding sites for small molecules
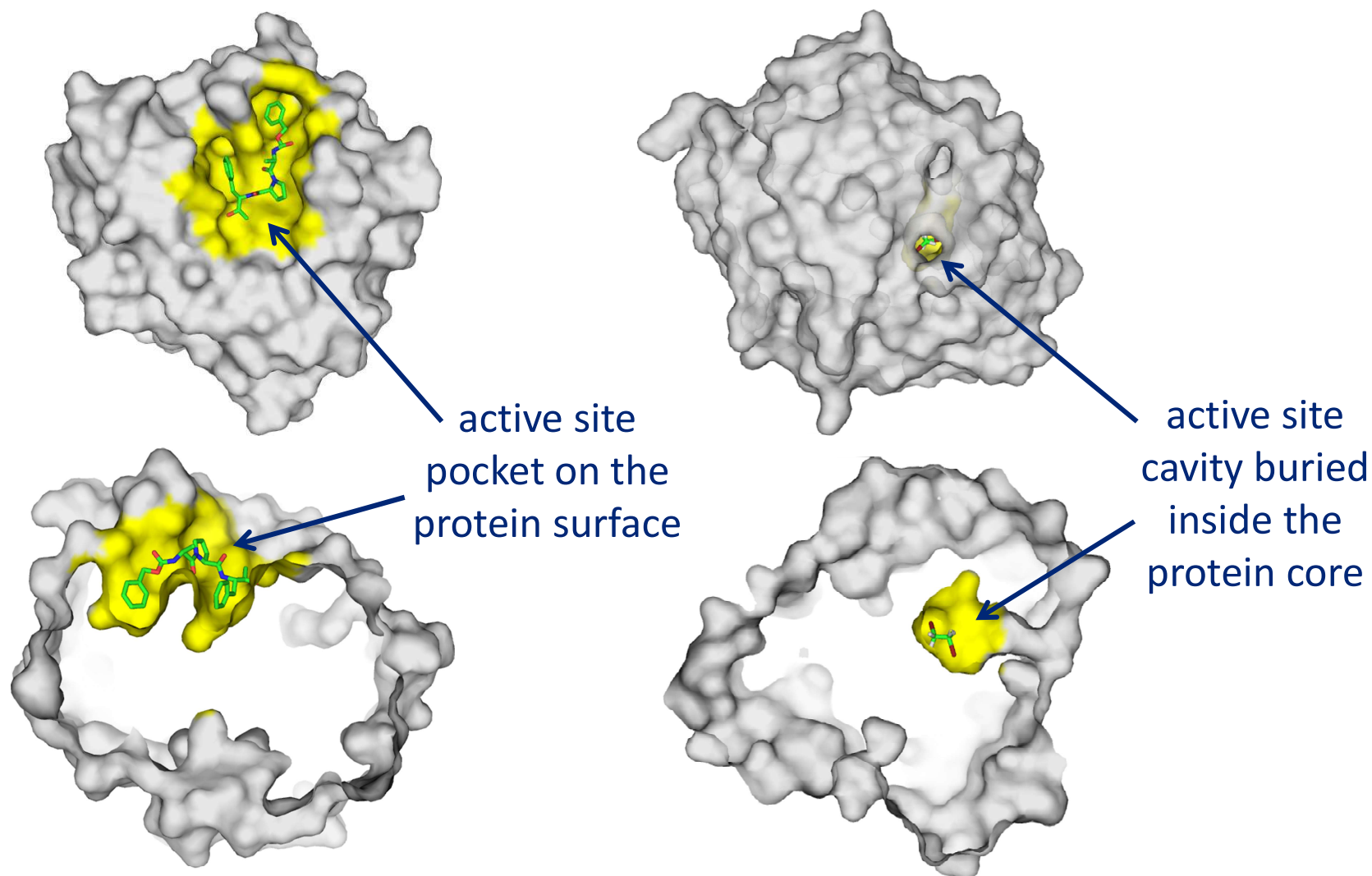
active site pocket on the protein surface

active site cavity buried inside the protein core

Binding sites for small molecules

❑ can be very ligand-specific

active site pocket   ligand

Binding sites for small molecules

- ❏ can be very ligand-specific

To be mutated

Binding sites for small molecules

❑ can be very ligand-specific

Before mutation

Binding sites for small molecules

- can be very ligand-specific

Before mutation

Binding sites for small molecules

- can be very ligand-specific

After mutation

Binding sites for small molecules

❑ can be very ligand-specific

After mutation

No longer a good fit!

Binding sites for small molecules

- approaches to identify binding sites:

  - evolutionary conservation

  - "pocket" detection

    - geometry based methods

    - energy based methods

  - binding site similarity

    - template-based methods

    - microenvironment-based methods

Evolutionary conservation
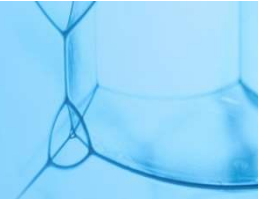
- residues important for protein function or stability tend to be highly conserved over evolution

- residue conservation in a set of related proteins can be derived from a multiple sequence alignment

- mapping of conservation on structure can reveal patches of conserved surface residues – potential binding sites

- protein interior usually more conserved than surface – not suitable for prediction of buried cavities

- not very specific – better to combine with other features

Evolutionary conservation

phylogenetic analysis

conservation scoring
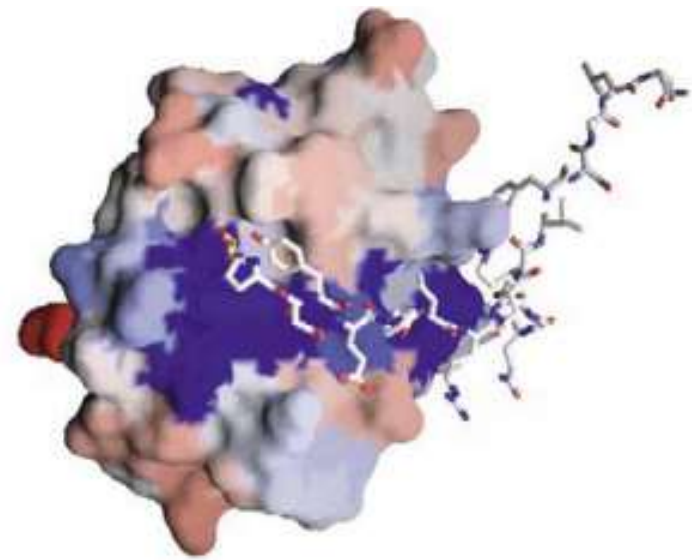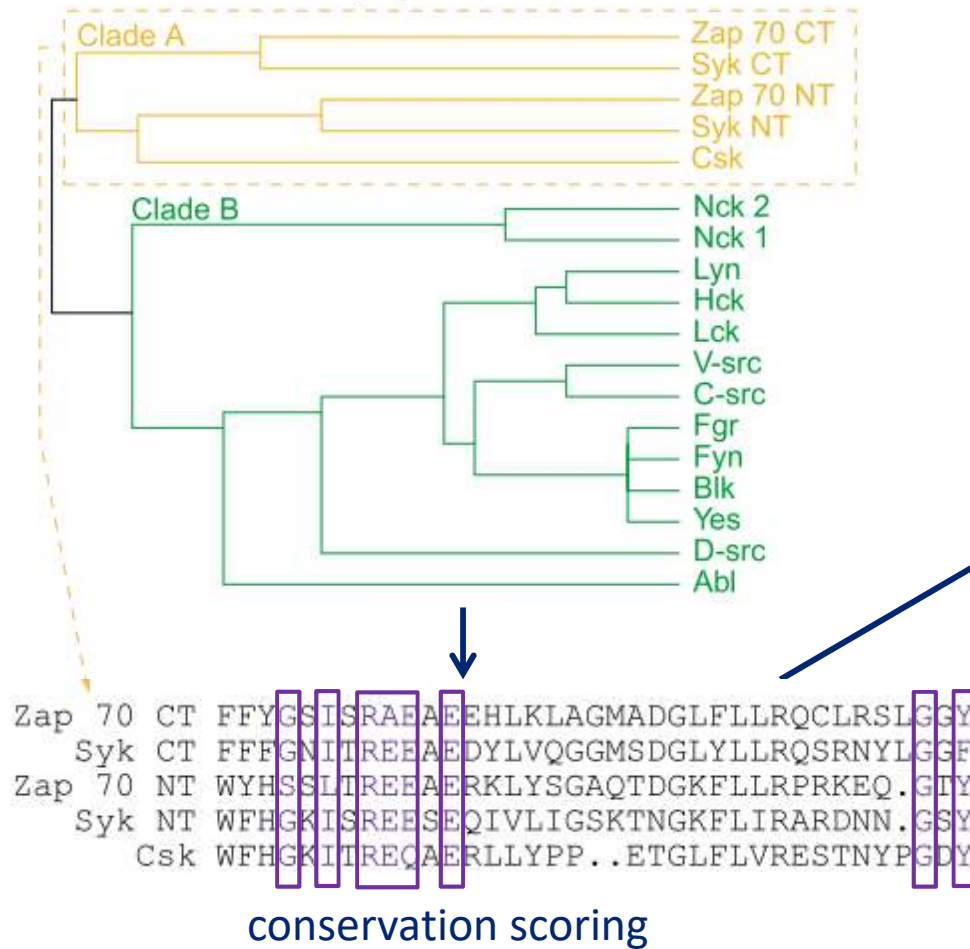
map evolutionary data on the structure

Evolutionary conservation

- ❑ ConSurf

  - ▪ http://consurf.tau.ac.il/

  - ▪ estimates the level of evolutionary conservation of individual positions in protein and maps this information onto its 3D structure

  - ▪ conservation score is derived based on the site-specific evolutionary rates calculated for each position by Rate4Site program

  - ▪ ConSurfDB – pre-calculated conservation scores for all structures from wwPDB

Evolutionary conservation

❑ ConSurf

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

Variable   Conserved
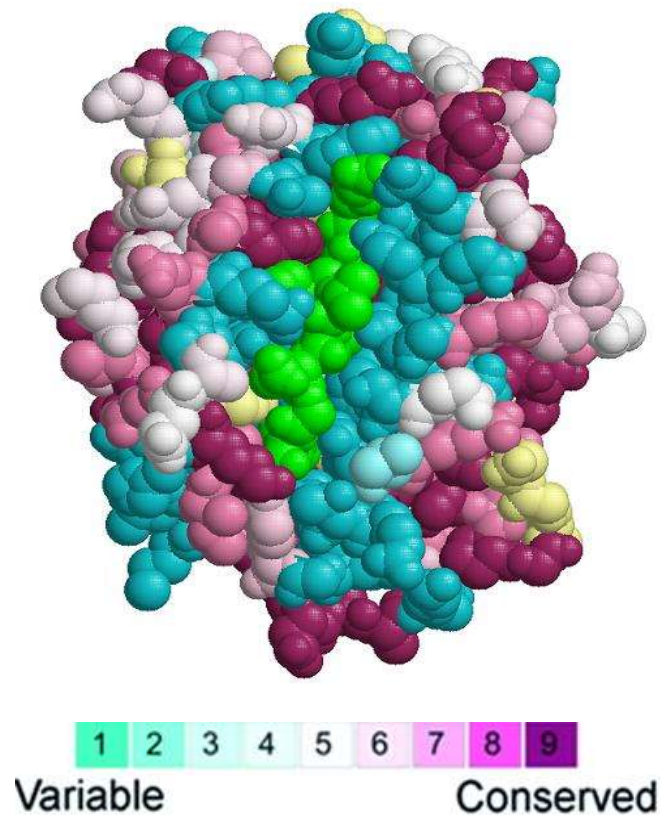
"Pocket" detection

❑ analyze the protein surface for pockets (clefts, cavities)
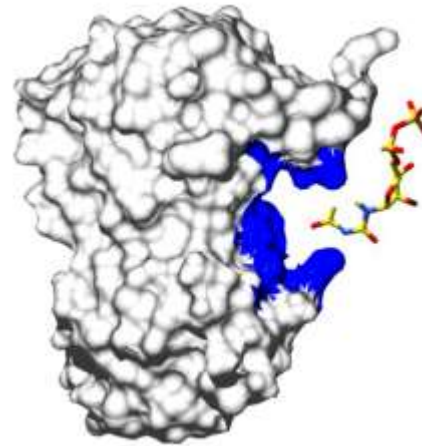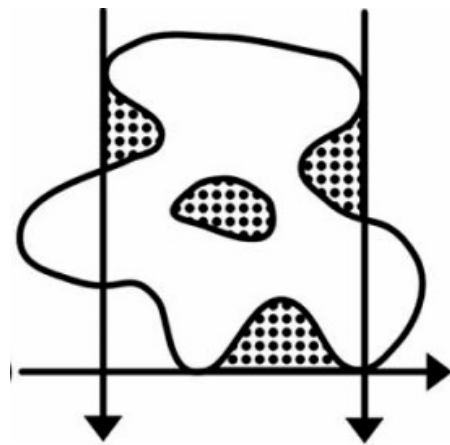
❑ **geometry-based** methods

▪ define favorable cleft regions based on sterical assessments

❑ **energy-based** methods

▪ define favorable cleft regions based on energetic evaluations

Geometry-based methods

❑ Computed Atlas of Surface Topography of proteins (CASTp)

- http://sts.bioe.uic.edu/castp

- uses computational geometry methods including Delaunay triangulation, alpha shape and discrete flow theory

- measures the volume and surface area of each pocket and cavity using the ASA model and molecular surface (Connolly) model
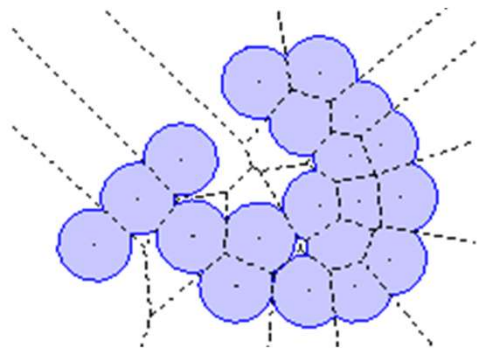
Voronoi
diagram
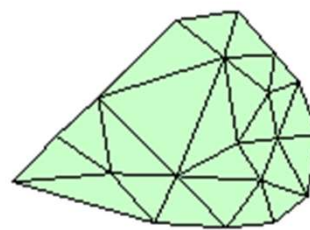
Delaunay
triangulation
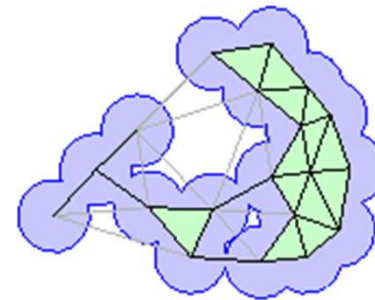
alpha shape

Geometry-based methods

- SURFNET

  - http://www.ebi.ac.uk/thornton-srv/software/SURFNET/

  - probe sphere filling method

- fPocket

  - http://fpocket.sourceforge.net/

  - uses alpha shape and Voronoi tessellation

  - mdpocket – version for analysis of pocket dynamics

- POCASA (POcket-CAvity Search Application)

  - http://altair.sci.hokudai.ac.jp/g6/Research/POCASA_e.html

  - combination of the grid system with probe sphere filling method

- ....

Energy-based methods

- pockets are defined by energetic criteria

- evaluate the interaction energy between the protein and a molecular fragment – probe (e.g., a methyl, hydroxyl, or amine groups) to locate energetically favorable binding sites

- recently combined with other methods to assess the *ligandability* (ability of a cavity to bind ligands)

Note: 'druggability' is usually referred to the likelihood of finding orally bioavailable small molecules that bind to a particular target in a disease-modifying way. Ligandability is a necessary but not sufficient condition for druggability.

Energy-based methods

❑ Cavity Plus

- http://www.pkumdl.cn/cavityplus

- applies Cavity program to detect the potential binding sites and rank them with ligandability and druggability scores

- extracts pharmacophore features within the cavities

Energy-based methods

- ❑ Cavity Plus
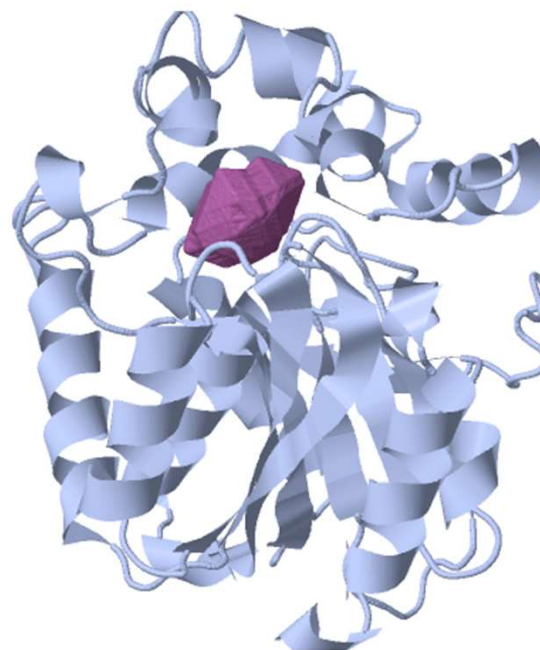
▾ **Cavity Results**

No.	Pred. Max pKd ?	Pred. Avg pKd	Drug Score	Druggability ?	Surface ?	Residues ?
1	10.19	6.11	493.00	less druggable	☑	More
2	8.87	5.66	-745.00	Undruggable	☐	More
3	8.16	5.42	-420.00	Undruggable	☐	More
4	7.87	5.32	-750.00	Undruggable	☐	More
5	7.11	5.06	-1105.00	Undruggable	☐	More
6	6.54	4.86	-992.00	Undruggable	☐	More
7	5.90	4.64	-1123.00	Undruggable	☐	More

Binding site similarity

❑ prediction of binding site is based on its similarity to other (known) binding site

❑ template-based methods

- binding sites are represented by 3D templates
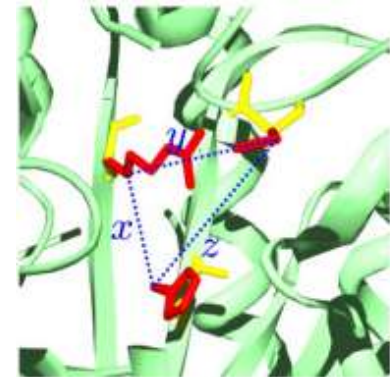
❑ microenvironment-based methods

- similar "microenvironments" associated with binding residues and their distances, physicochemical and evolutionary properties
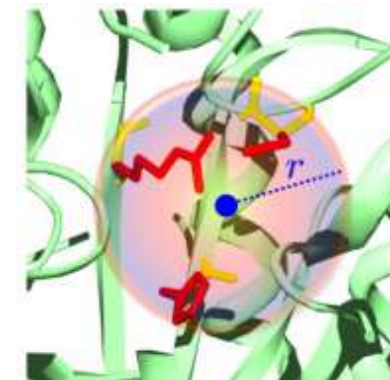
Template-based methods

- definition and construction of 3D templates

  - local structural motifs, patterns or descriptors that characterize the binding sites (e.g., functional groups)

  - capture essence of the binding site in protein

  - usually constraints on atom type and, occasionally, sequential relationships

- search in database for structures using template as a query

  - identification of structures with a given binding site

- compare the query structure against a 3D template database

  - identification of potential binding sites in the query structure

Template-based methods

- ❑ PINTS

  - ▪ http://www.russelllab.org/cgi-bin/tools/pints.pl

  - ▪ enables to compare a protein structure against a database of 3D templates as well as a 3D templates against a database of protein structures

  - ▪ additionally allows comparison of two structures

  - ▪ the 3D template database includes ligand-binding sites and SITE annotations from PDB files

Template-based methods

❑ ProFunc

- http://www.ebi.ac.uk/thornton-srv/databases/profunc/

- aims to identify the most likely function of a protein from its 3D structure

- uses series of methods, including fold matching, residue conservation, surface cleft analysis, and functional 3D templates (templates for enzyme active sites, ligand-binding templates, DNA-binding templates, reverse template comparison vs. structures in wwPDB)

Template-based methods

❑ Mechanism and Catalytic Site Atlas

- https://www.ebi.ac.uk/thornton-srv/m-csa/

- provides information about the active sites, catalytic residues and mechanisms in enzymes with experimentally determined 3D structure

- defines catalytic residues as the residues directly involved in some aspect of the enzymatic reaction

- provides 3D templates for catalytic sites in the database

Binding sites for macromolecules

- ❑ typically protruding loops, large surface clefts but also flat binding sites – flatter than binding sites for small molecules

  - ▪ recognition of a macromolecule involves interactions over a large continuous surface area or several discrete binding regions

  - ▪ difficult to identify by a simple examination of the protein structure

- ❑ high evolutionary conservation

- ❑ low desolvation energy

- ❑ characteristic physicochemical properties

- ❑ DNA binding sites have characteristic motifs and positive charged electrostatic patches

Binding sites for macromolecules

protein-protein complex

protein-DNA complex
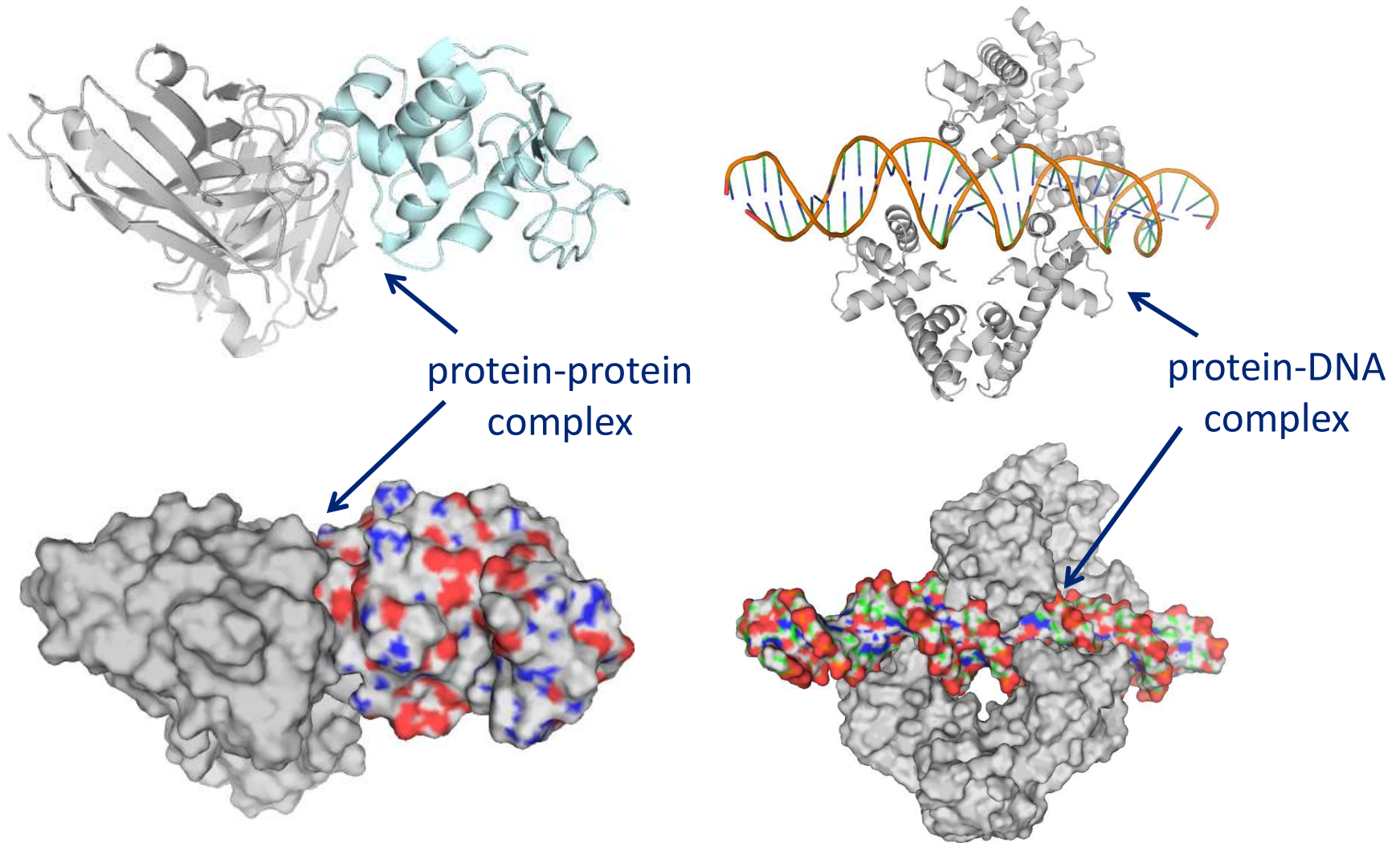
Binding sites for macromolecules

- ❑ approaches to identify binding sites

    - ▪ evolutionary conservation

    - ▪ knowledge-based

- ❑ meta-servers (combine several tools in a workflow)

Evolutionary conservation methods

- □ same principles as for binding sites of small molecules

- □ WHISCY

  - ▪ https://wenmr.science.uu.nl/whiscy/

  - ▪ predicts protein-protein interface using conservation and structural information (interface propensities for each surface residue are used to adjust the score)
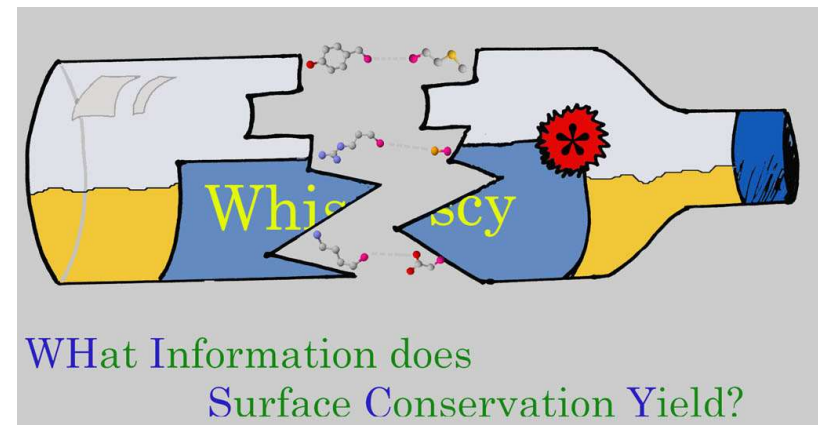
Knowledge-based methods

❑ combine multiple interface features

- conservation

- residue propensity for protein-protein interface

- physicochemical properties

- structural properties

❑ use known binding sites for parameterization or training →

empirical scoring functions and machine learning methods

Knowledge-based methods

- ❑ cons-PPISP (consensus Protein Protein Interaction Site Predictor)

  - ▪ http://pipe.scs.fsu.edu/ppisp.html

  - ▪ utilizes machine learning to predict protein binding sites

  - ▪ trained on position-specific sequence profiles and solvent accessibilities of each residue and its spatial neighbors

- ❑ Patch Finder Plus

  - ▪ http://pfp.technion.ac.il/

  - ▪ utilizes machine learning primarily to find DNA binding regions

  - ▪ identifies the largest positive electrostatic patch on a protein surface – combination of residue frequency, composition and conservation, surface concavity, accessible area and H-bond potential

Meta-servers

- ❑ combine multiple methods to improve prediction accuracy

- ❑ meta-PPISP (Protein Protein Interaction Site Predictor)

    - ▪ http://pipe.scs.fsu.edu/meta-ppisp.html

    - ▪ combines cons-PPISP, ProMate and PINUP

- ❑ PI^{2}PE (Protein Interface/Interior Prediction Engine)

    - ▪ http://pipe.scs.fsu.edu/

    - ▪ pipeline to use five different predictors including cons-PPISP, meta-PPISP and DISPLAR
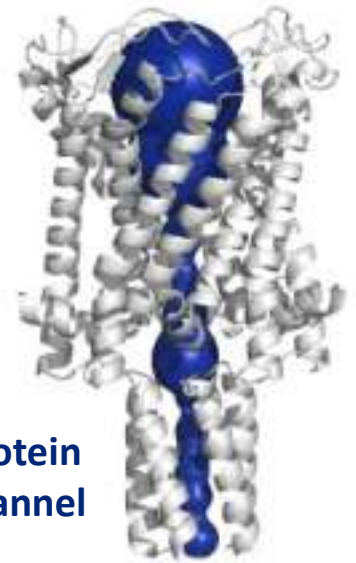
Transport pathways

❑ mediate transport of ions and small molecules in proteins – an essential role in functioning of large variety of proteins

- ▪ channels/pores – transport of substances across membranes

- ▪ tunnels – exchange of ligands between the active/binding site and bulk solvent in proteins with buried active/binding site cavities

- ▪ intramolecular tunnels – transport of reaction intermediates between two distinct active sites in bifunctional enzymes

❑ their permeability to different substances depends on the pathway size (radii), shape, length, amino acid composition (physicochemical properties) and dynamics
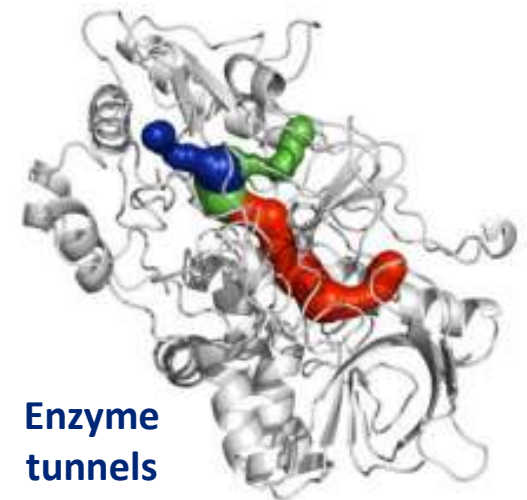
Transport pathways

- pocket/cleft/groove
- channel
- tunnel
- bottleneck
- cavity

Protein channel
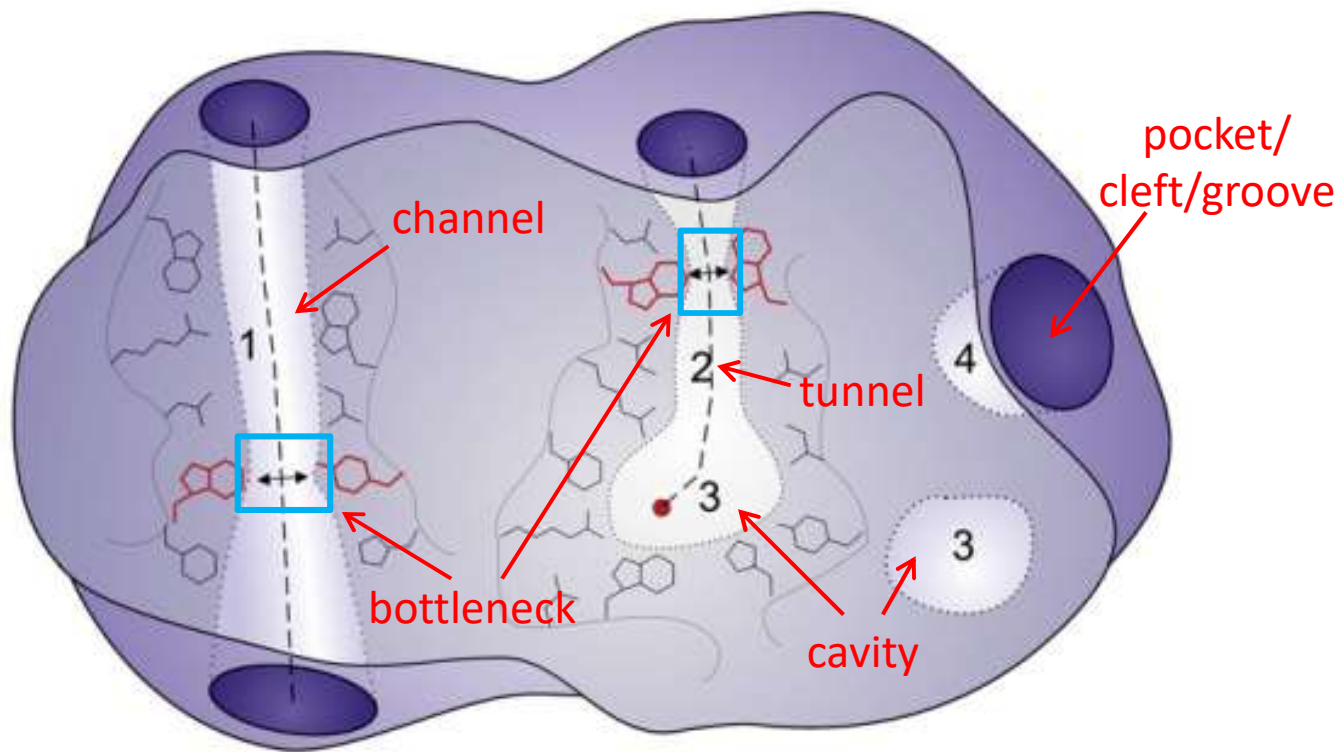
Enzyme tunnels

- **bottleneck** – the narrowest part of the tunnel/channel; it has critical importance to the selectivity

Transport pathways

active site pocket

ligand
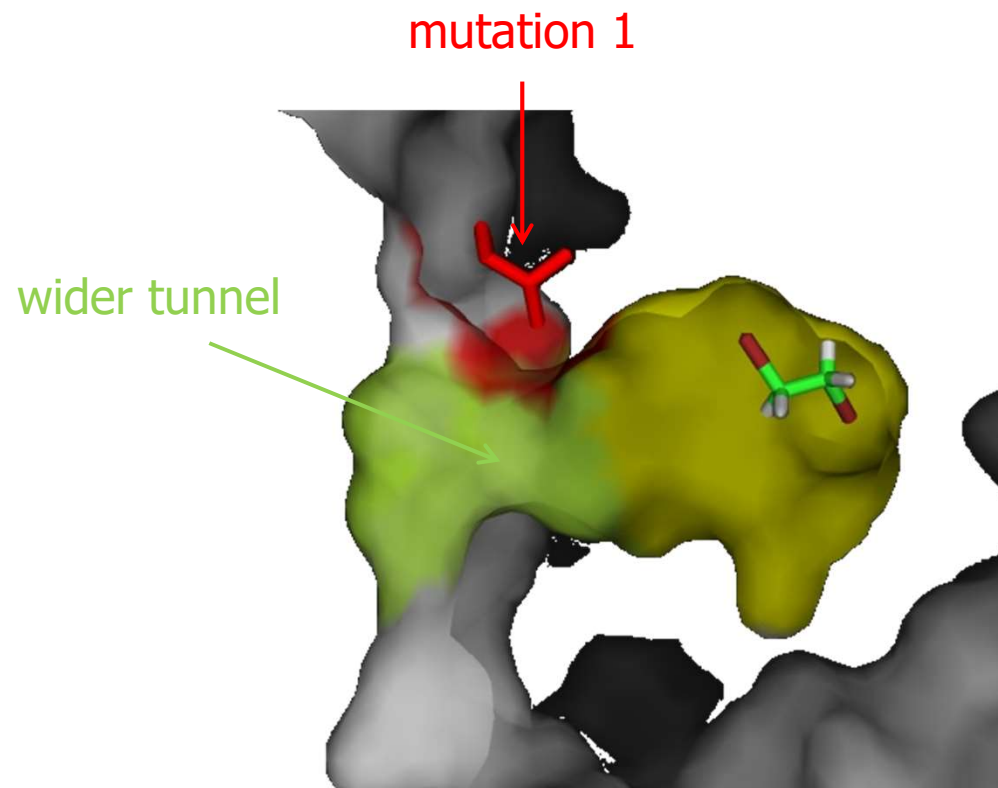
tunnel

Transport pathways

residue on the bottleneck (to be mutated)

active site pocket

ligand

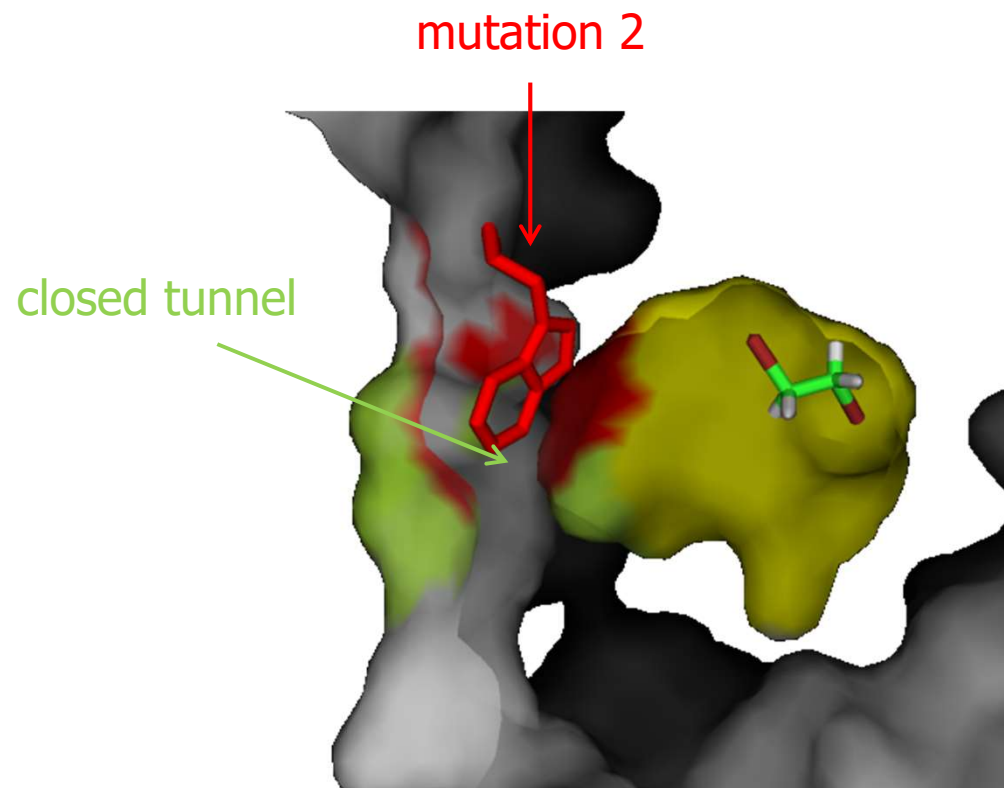tunnel

Transport pathways

mutation 1

wider tunnel

Transport pathways

mutation 2

closed tunnel
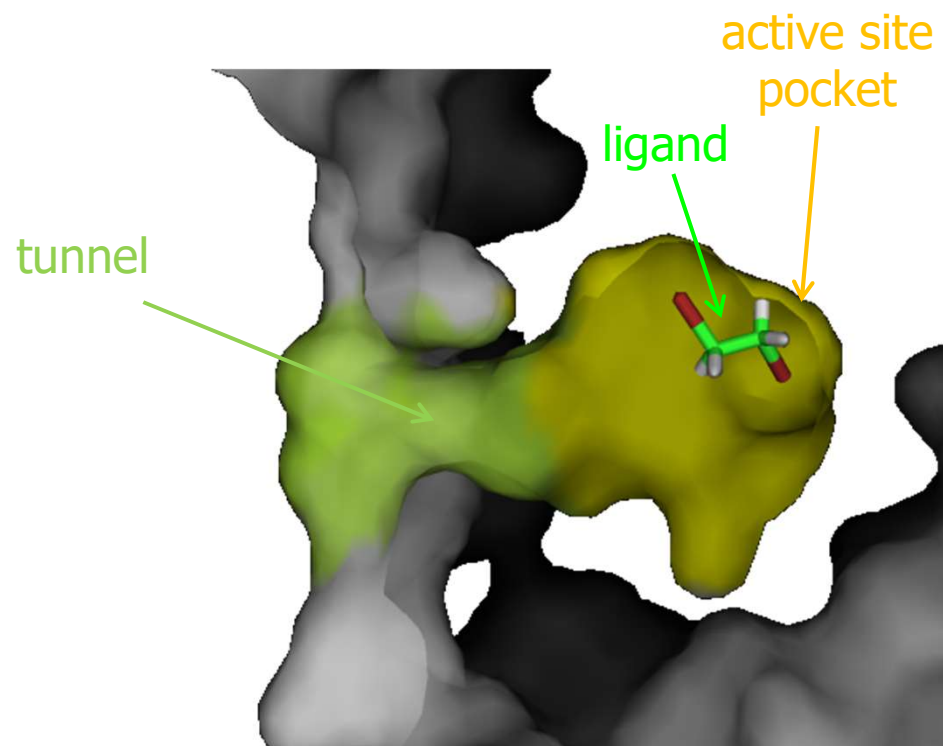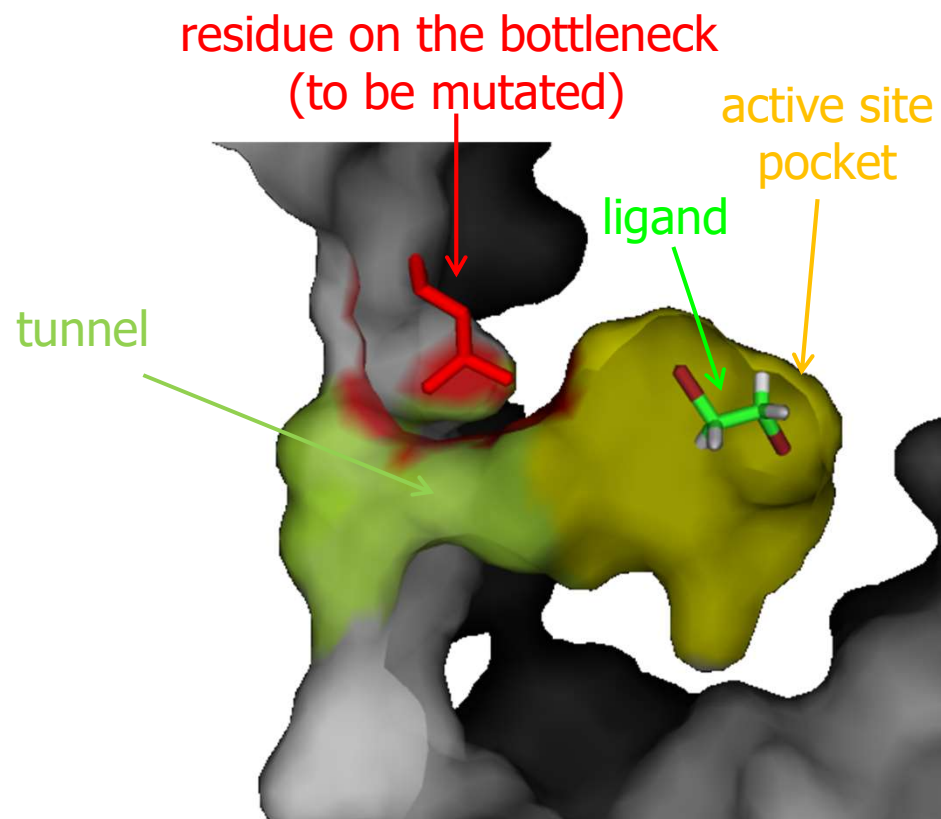
Prediction of transport pathways

- identification of overall voids in proteins

- identification of tunnels

- identification of channels

Identification of overall voids

- ❑ methods that aim to accurately represent all types of voids in a protein structure, including channels, tunnels, surface clefts, pockets as well as internal cavities

- ❑ usually provide very limited information on tunnel and channel characteristics – the identified voids have to be separated from each other

- ❑ geometry-based methods for pocket detection

    - ▪ HOLLOW – http://hollow.sourceforge.net/

    - ▪ 3V – http://3vee.molmovdb.org/

   - ▪ LIGSITE^{csc}, PASS, CASTp, fPocket, SURFNET, POCASA …

Identification of tunnels

- methods that calculate tunnels connecting occluded cavities with the surrounding bulk solvent

- identify the pathways from a cavity to the protein surface

- Voronoi diagrams described by the skeleton of voids between atoms to find all theoretically possible pathways connecting the starting point with the bulk solvent

- diagram is searched for optimal pathways using Dijkstra's algorithm, based on the criteria defined by a cost function

- the probe size defines the lowest radius threshold

- tunnel geometry is approximated by a sequence of spheres

Identification of tunnels

atom

tunnel origin

tunnel mouth

Voronoi diagram
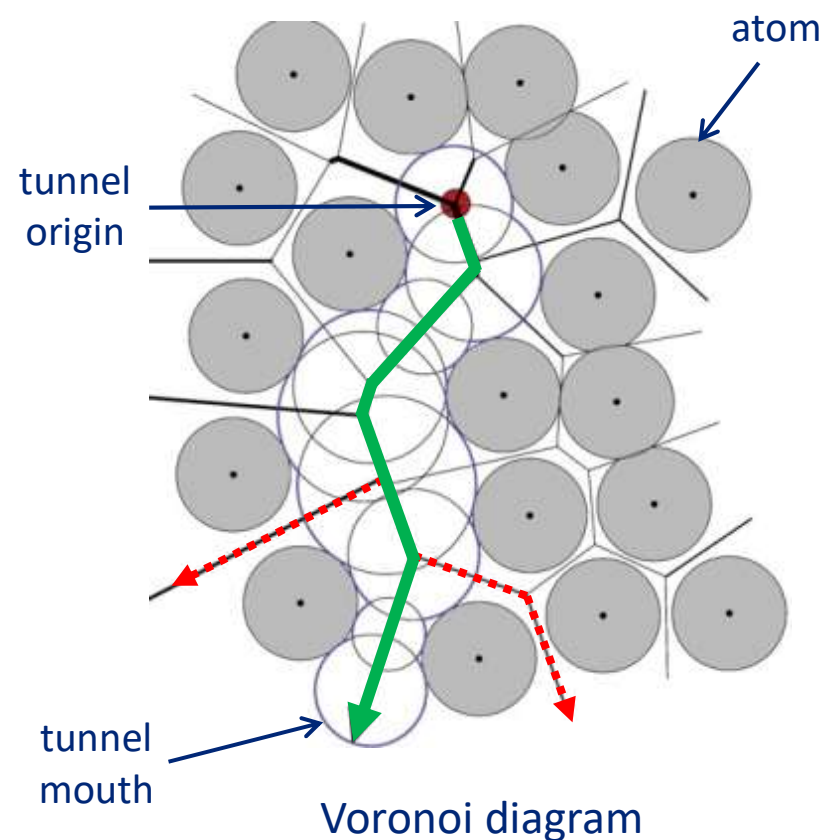
⊙ **Probe size**: the minimum radius specified for the tunnel search

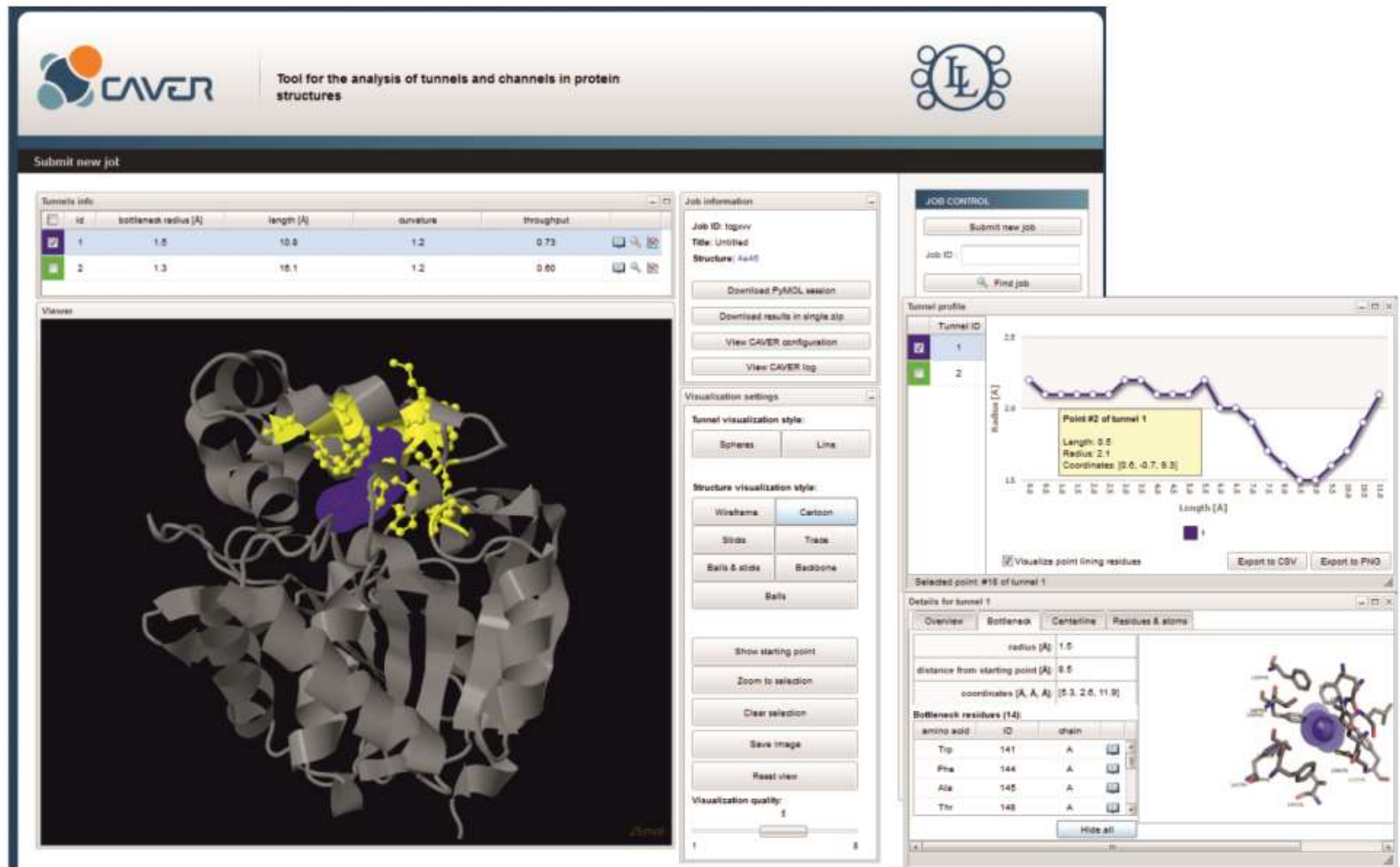➡ Allowed pathway according to the selected probe

▸ Disallowed pathways
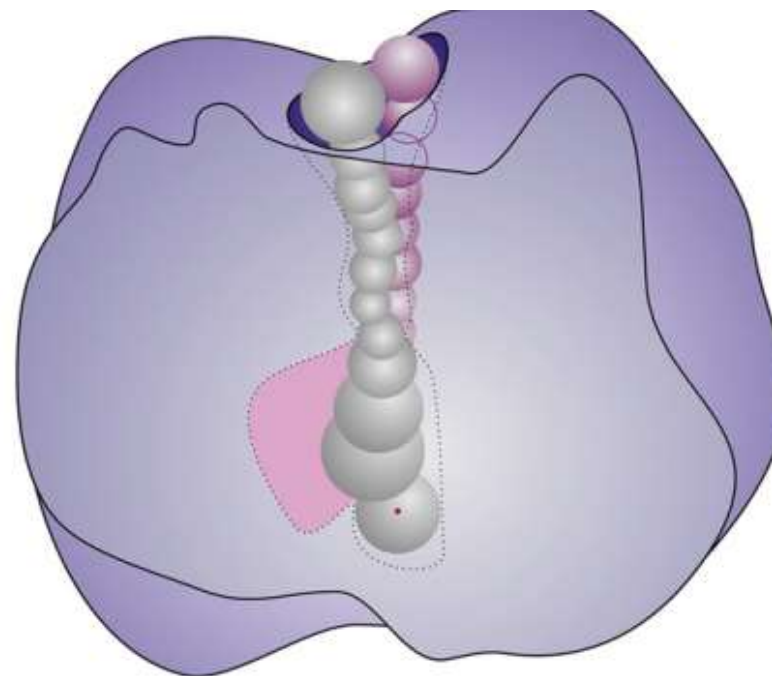
Common limitation: the tools identify two spherical tunnels instead of one asymmetric tunnel

Identification of tunnels - programs

- CAVER 3.0

  - http://caver.cz/

  - Command-line stand-alone

  - PyMOL plugin

  - GUI with CAVER Analyst 2

- CAVER Web

  - http://loschmidt.chemi.muni.cz/caverweb/

  - interactive guide-through web server

  - optimized protocol for detection of biologically relevant tunnels

  - based on CAVER 3.0 program

Identification of tunnels - programs

Identification of tunnels - programs
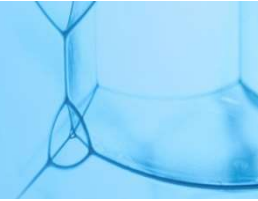
- MolAxis

  - http://bioinfo3d.cs.tau.ac.il/MolAxis/

- MOLE 2.0

  - http://mole.upol.cz/

- ChExVis

  - http://vgl.serc.iisc.ernet.in/chexvis/

- BetaCavityWeb

 - http://voronoi.hanyang.ac.kr/betacavityweb/

- …

Identification of channels

- methods calculate channels (pores) penetrating throughout proteins

- not convenient for identifying tunnels leading from occluded cavities

- usually analyze just one channel per structure

- usually need information about approximate position and direction of the channel (channel axis) – user-provided or automatically identified

Identification of channels - programs

❏ POREWALKER

- http://www.ebi.ac.uk/thornton-srv/software/PoreWalker/

- identifies channel axis by heuristic iterative approach (based on the axes of transmembrane secondary structures)

- protein is divided into equally-spaced slices perpendicular to the axis; the largest spheres fitting the channel are identified
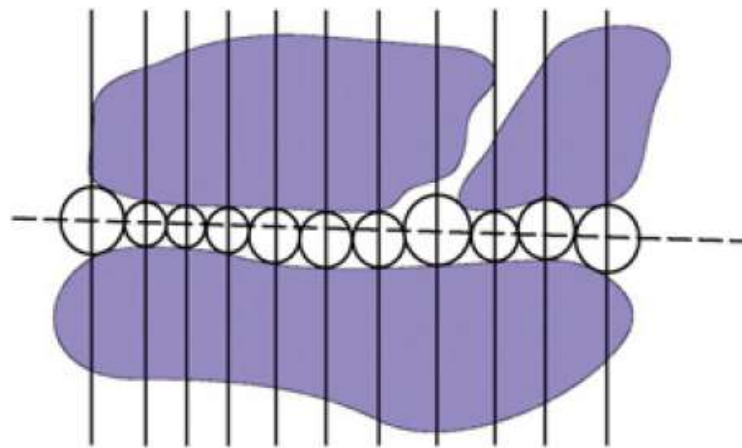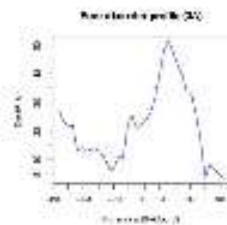
Identification of channels - programs
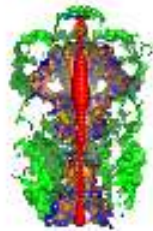
❑ POREWALKER

Pore analysis results

Overview of the available results:

Pore shape

Slideshow of the pore cross-sections

Pore visualization

Features of the cavity

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