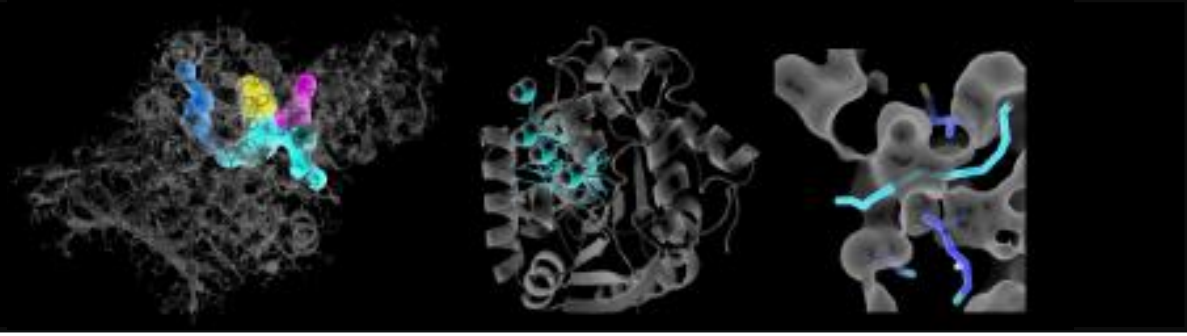


Exploring ligand-transport pathways in proteins

Structure, dynamics, function & dysfunction

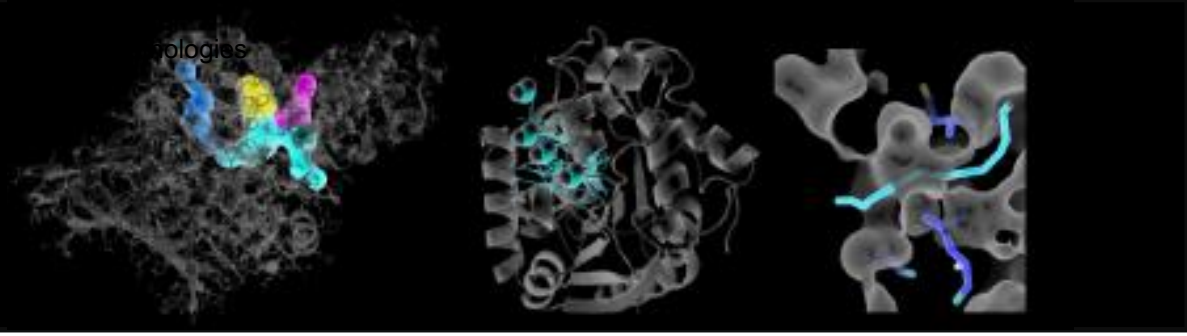
Jan Brezovsky, Ph.D., prof. IIMCB & UAM

Laboratory of Biomolecular Interactions and Transport

Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University

&

International Institute of Molecular and Cell Biology in Warsaw



Journey to the protein core and back

Jan Brezovsky, Ph.D., prof. IIMCB & UAM

Laboratory of Biomolecular Interactions and Transport

Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University

&

International Institute of Molecular and Cell Biology in Warsaw

Outline

- Transport pathways in soluble/globular proteins
- Bioinformatics methods for pathway analysis
- Effect of mutations in transport pathways
- Roles of transport pathways in pathology and drug discovery

Cellular environment

- Proteins
- Nucleic acids
- Membranes
- Metabolites
  - lipids, peptides & sugars
- Water & ions

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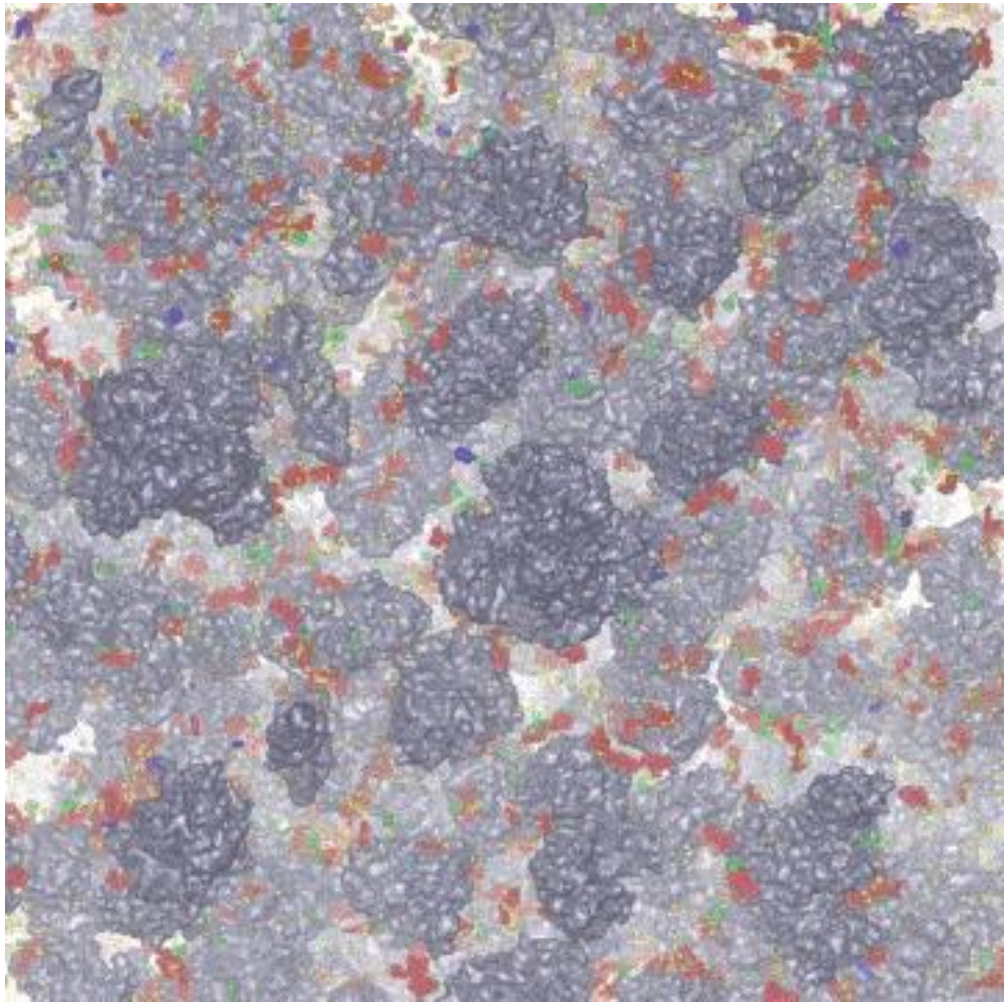
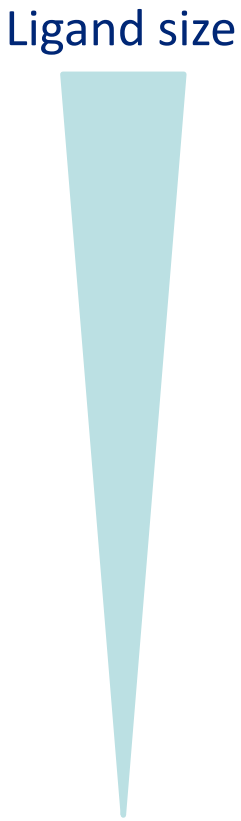


Yu et al. 2016, eLife 5: e19274.

Proteins in the crowded environment



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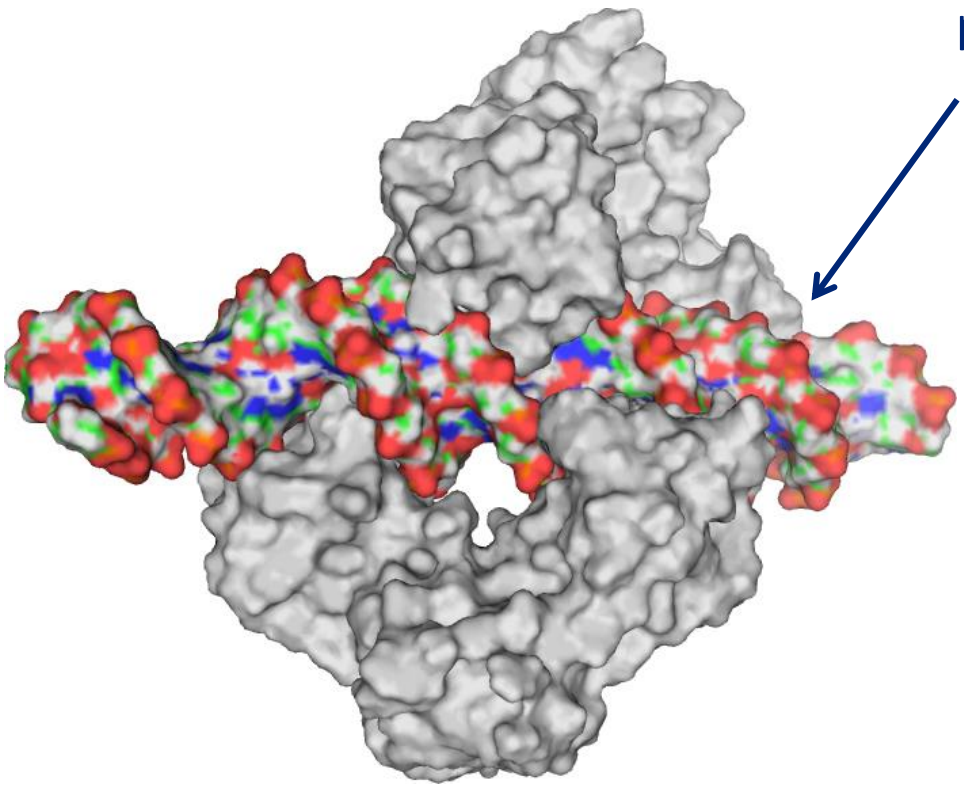
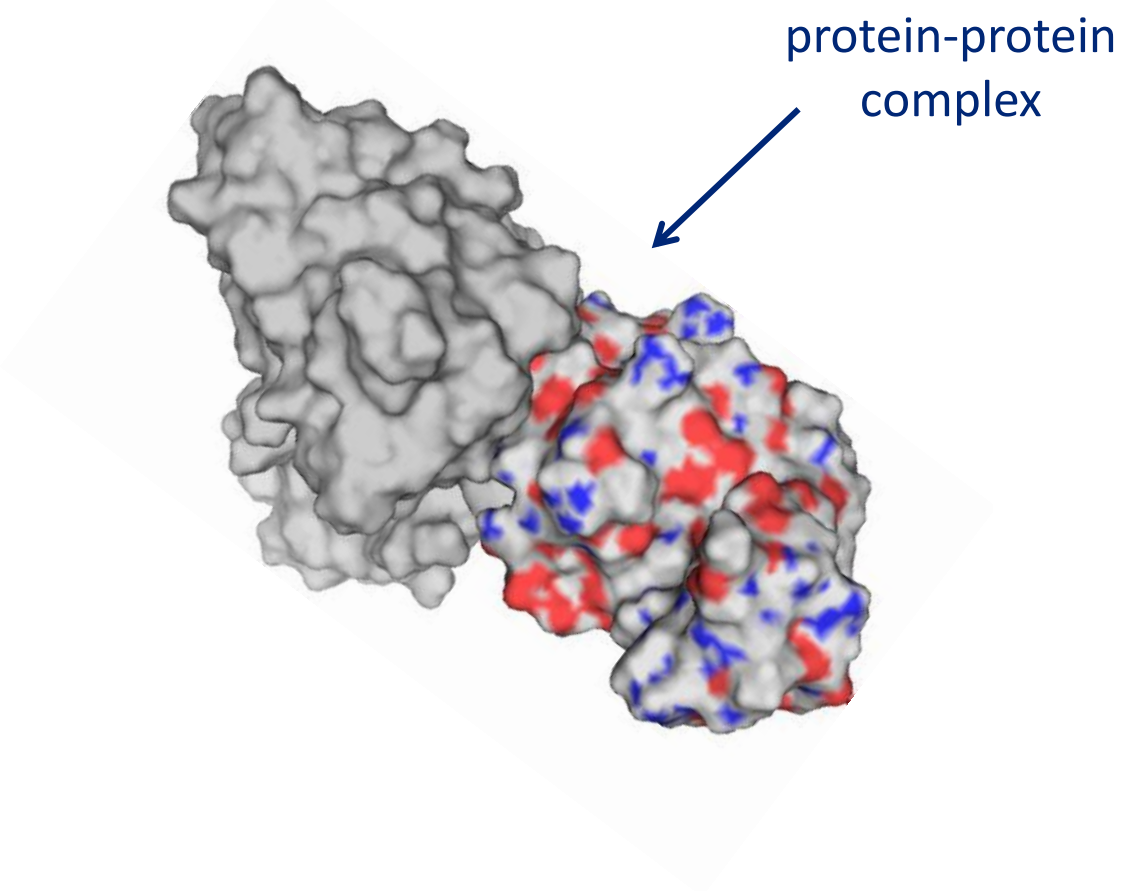
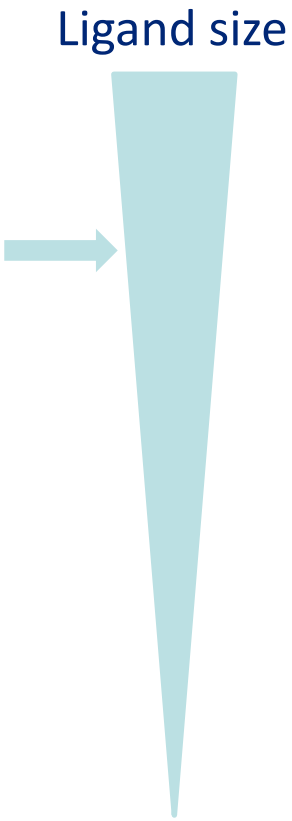


protein-NA complex

Ligand size

Proteins in the crowded environment





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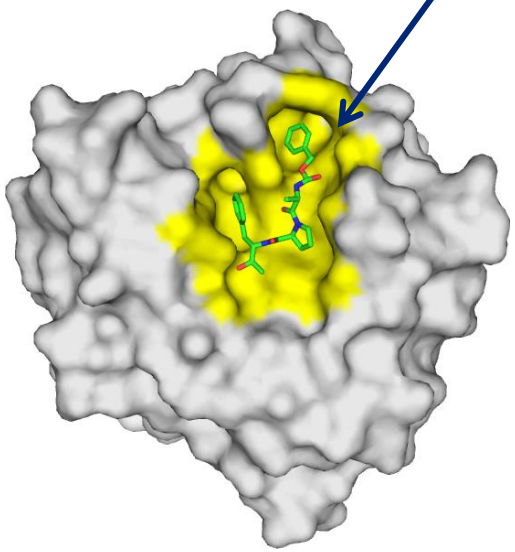

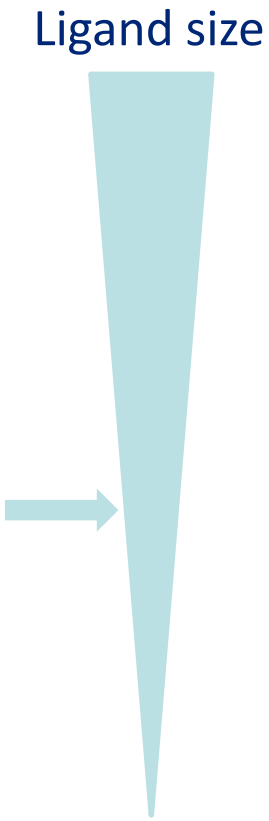

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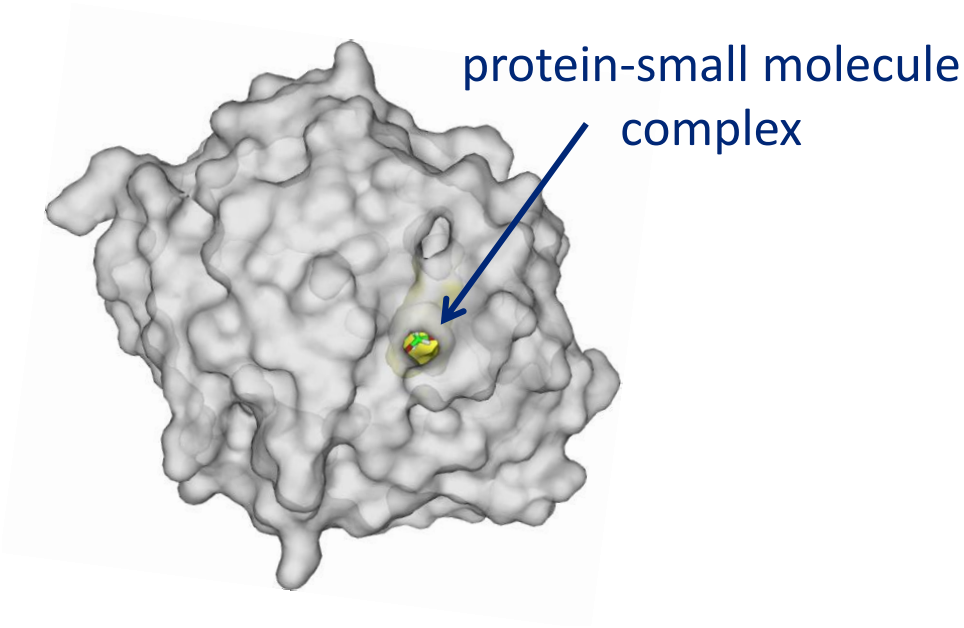
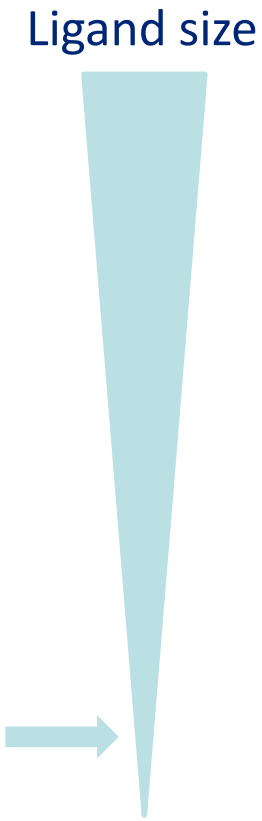
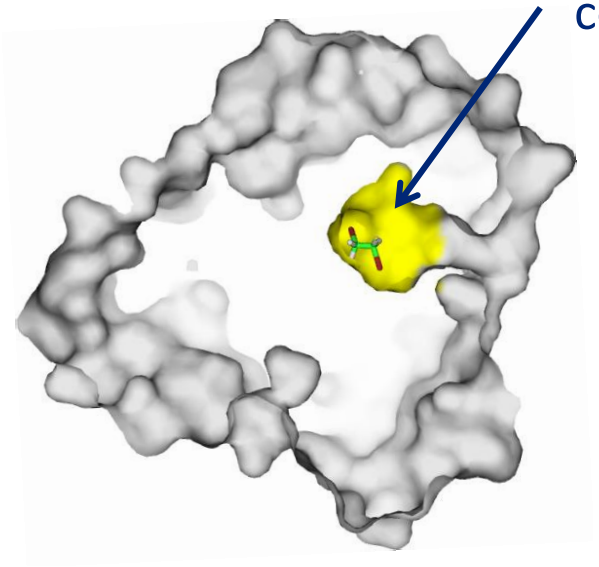
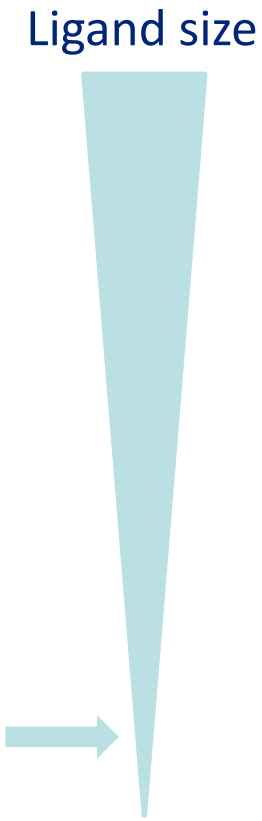




Proteins in the crowded environment





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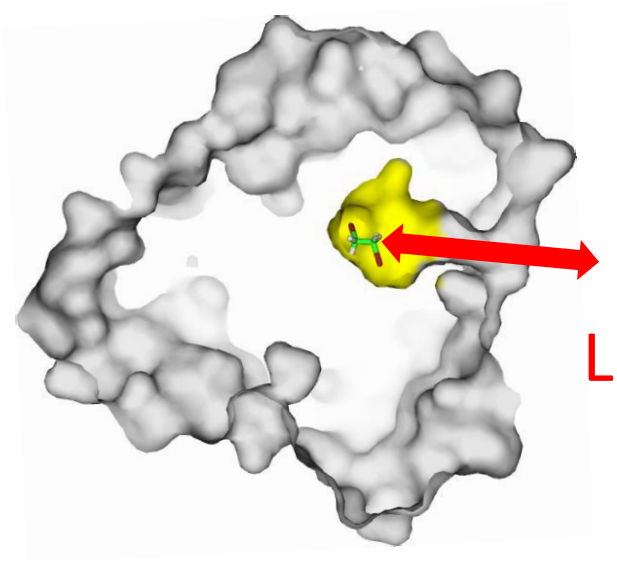
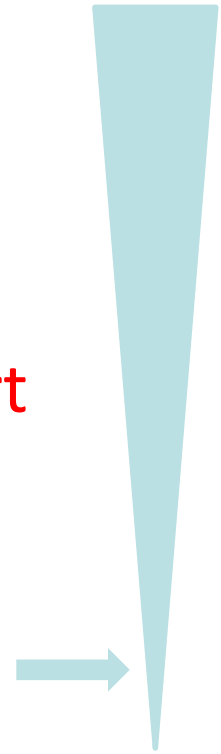


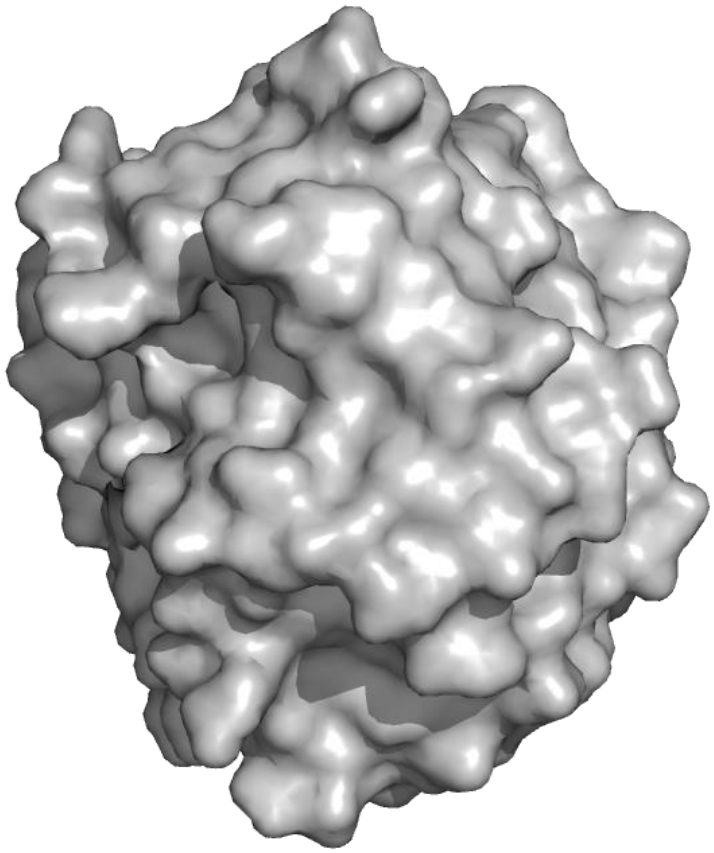
Ligand-transport pathways

Ligand size



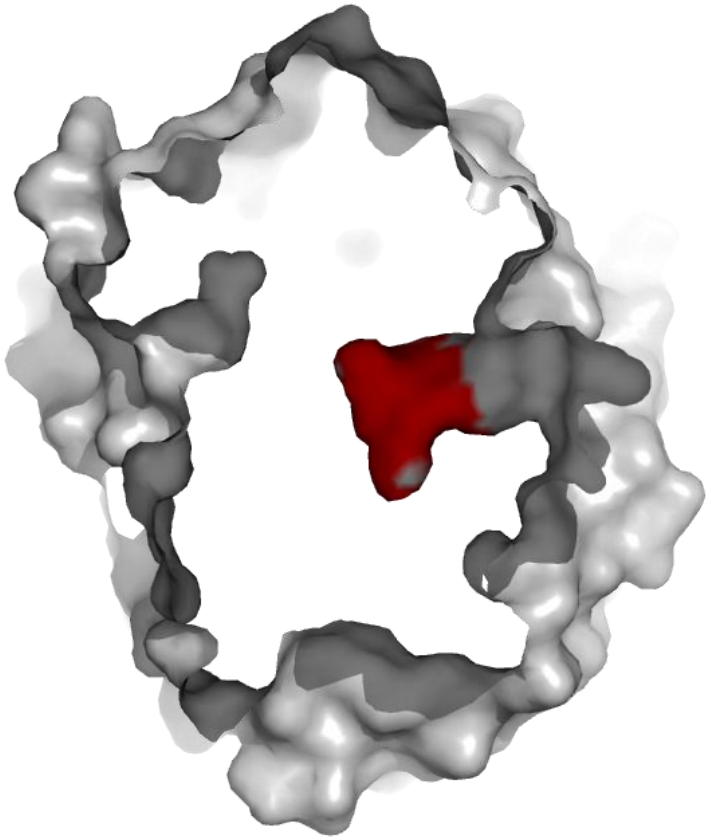
Ligand-transport pathways

- **Proteins with functional sites located in occluded cavities**



Ligand-transport pathways

- Proteins with functional sites located in occluded cavities
- Cognate ligands enter such sites via transport pathways – tunnels



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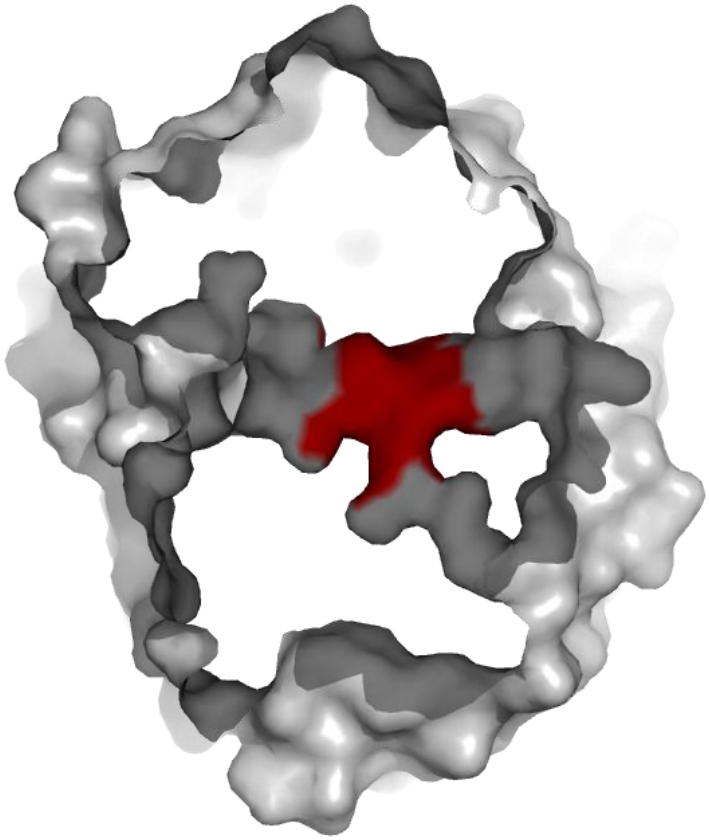

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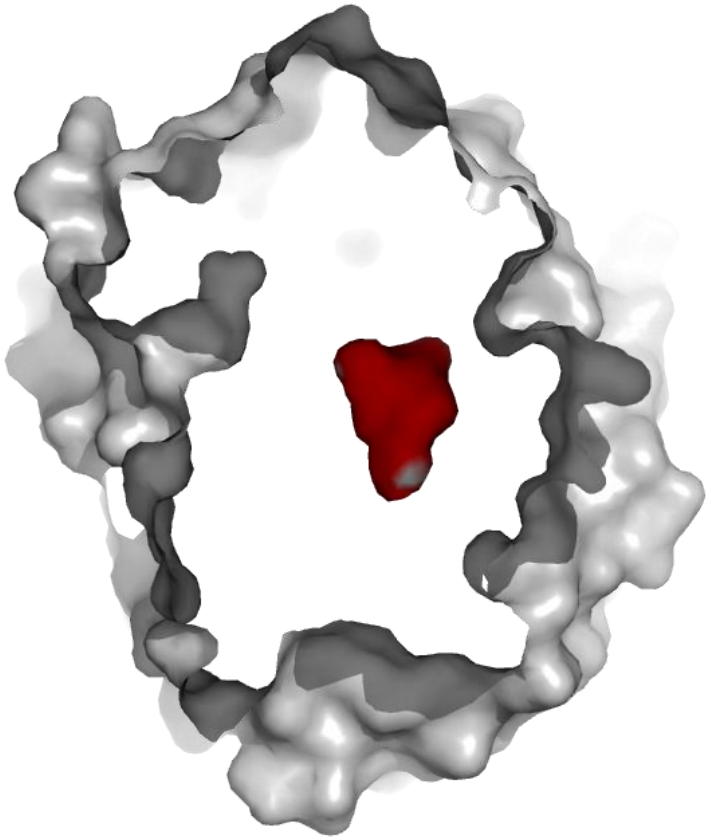

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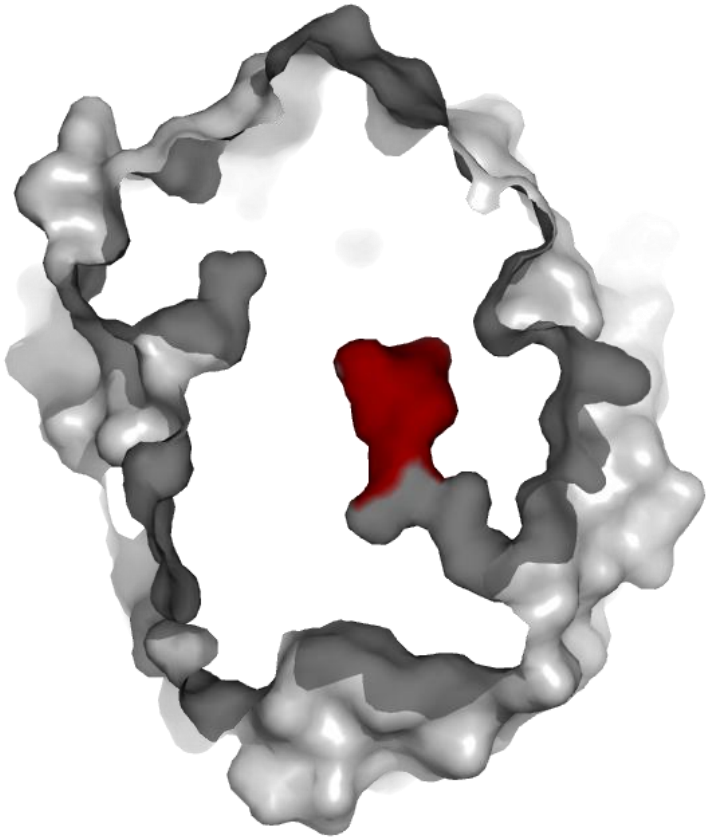

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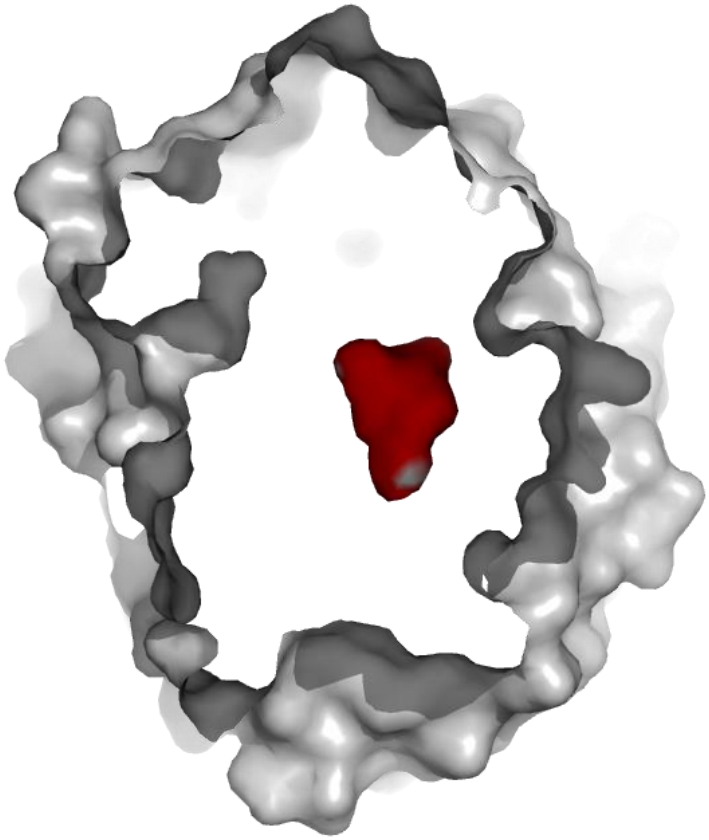

Ligand-transport pathways

- **Proteins with functional sites located in occluded cavities**
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- **Some basic questions**
  - How widespread are the tunnels?
  - What types of tunnels do exist?
  - What are functional roles of the tunnels?

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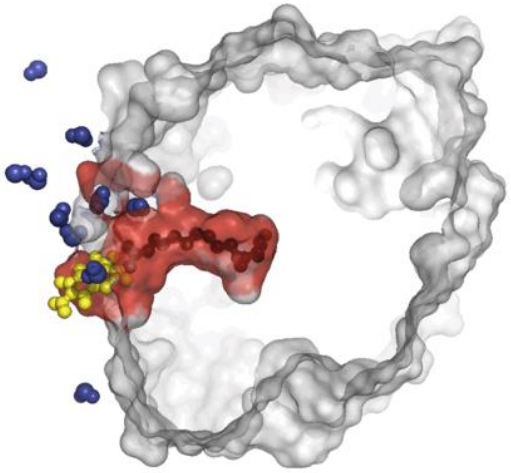

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- **Hard to study**
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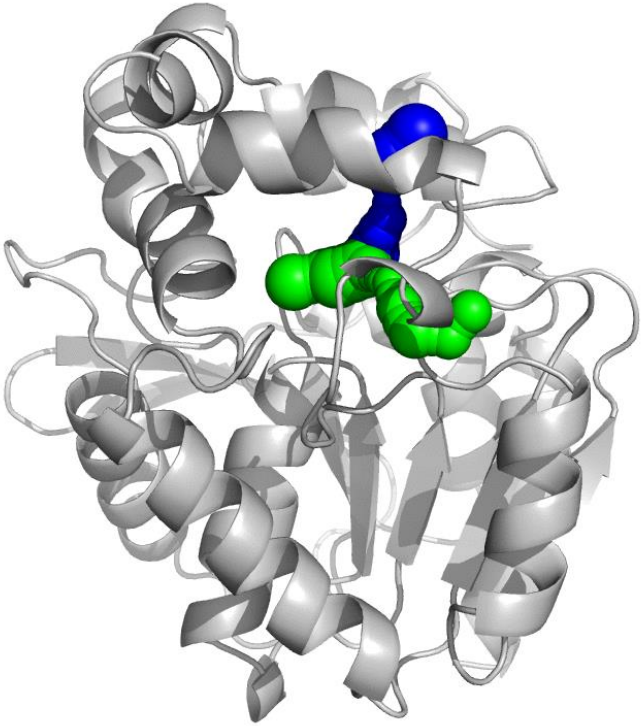
    => simulations needed
- **Need tools/methods to detect, evaluate and design the tunnels**

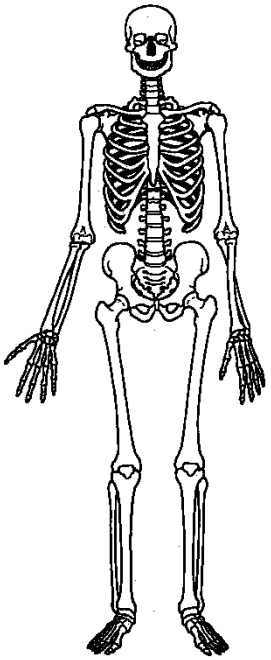
Ligand-transport pathways – software tools

- **Software tool that accounts for protein dynamics while analyzing tunnels**
  - to identify transient tunnels
  - to estimate importance of tunnels



Ligand-transport pathways – software tools

- **Software for analysis of tunnel dynamics**
  - analysis of pathways in Voronoi diagrams



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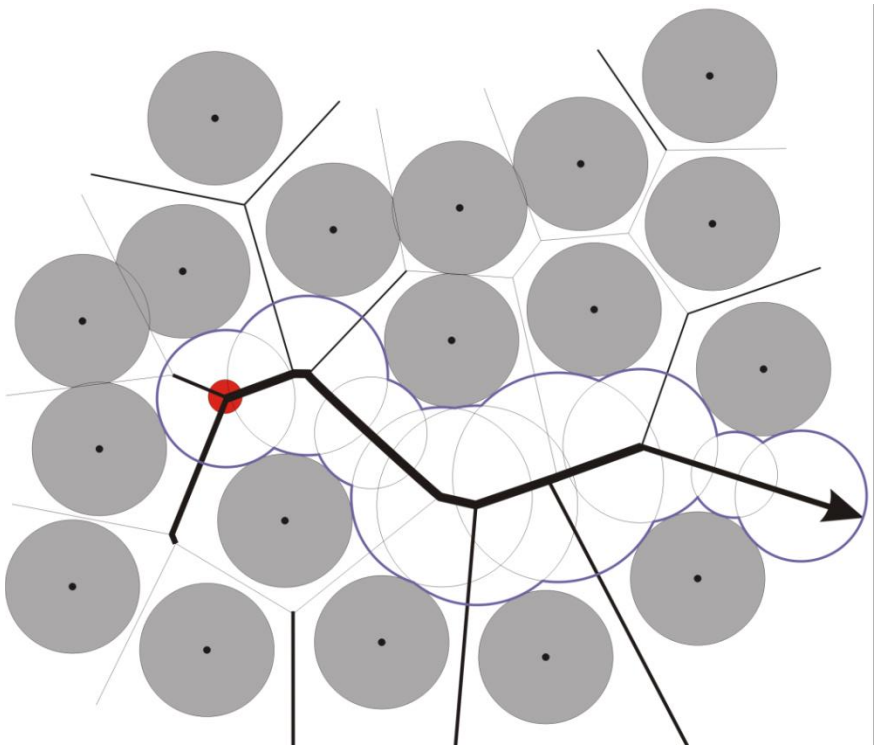
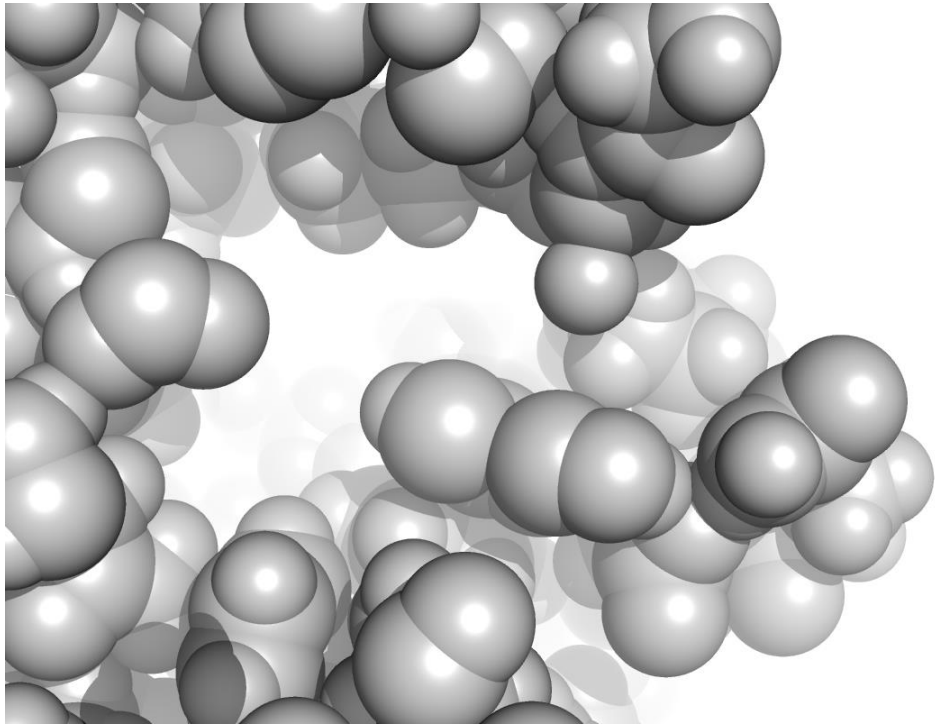


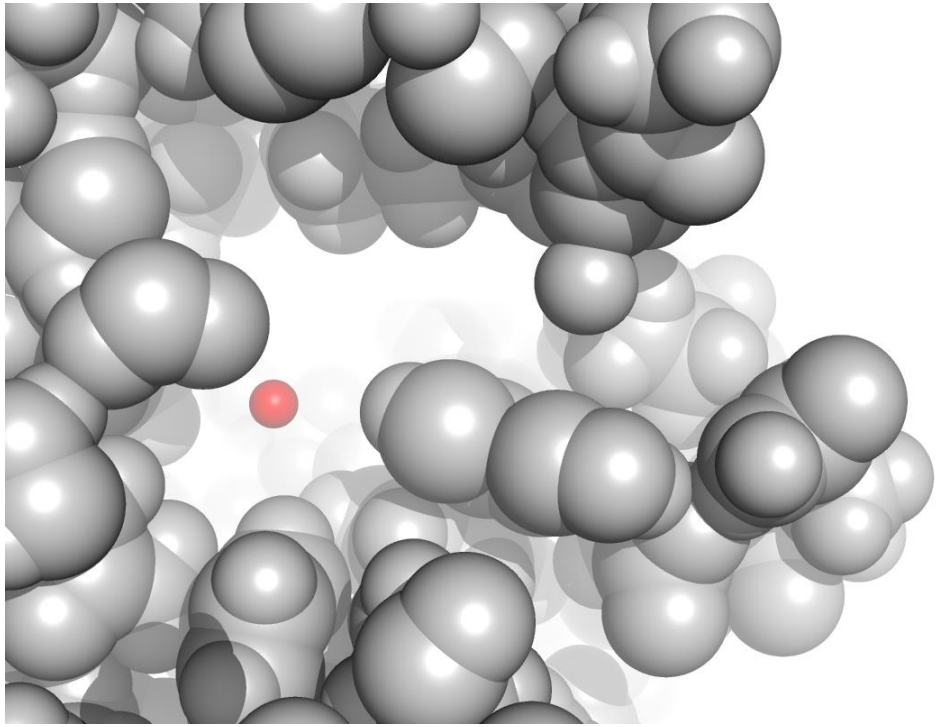
Ligand-transport pathways – software tools

- **Software for analysis of tunnel dynamics**
  - pathway from a cavity to the bulk solvent



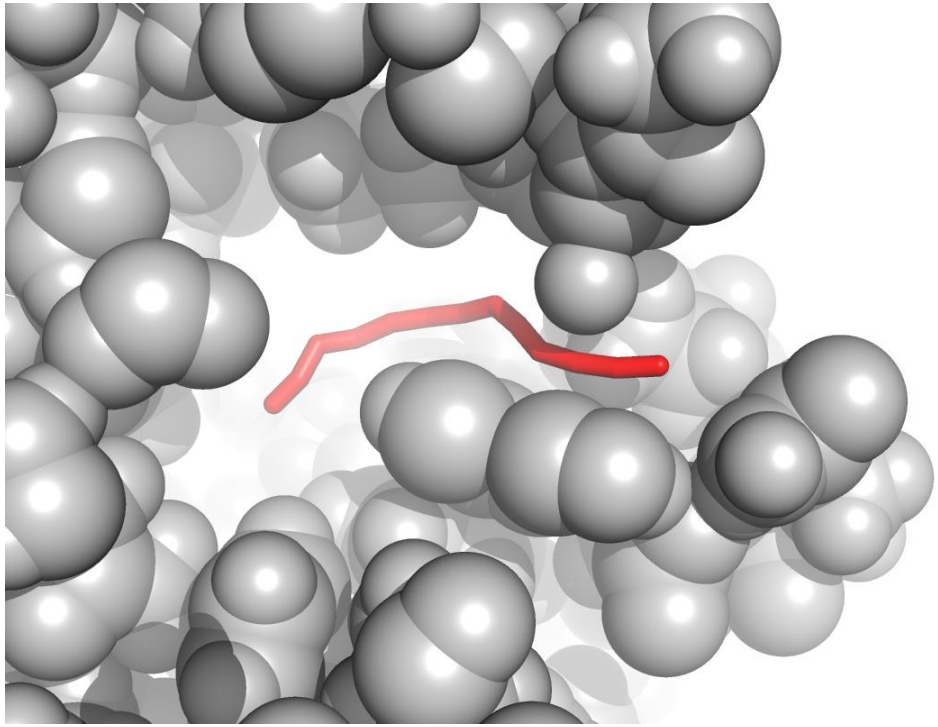
Ligand-transport pathways – software tools

- **Software for analysis of tunnel dynamics**
  - starting from a point in the cavity



Ligand-transport pathways – software tools

- **Software for analysis of tunnel dynamics**
  - the shortest and widest pathway identified



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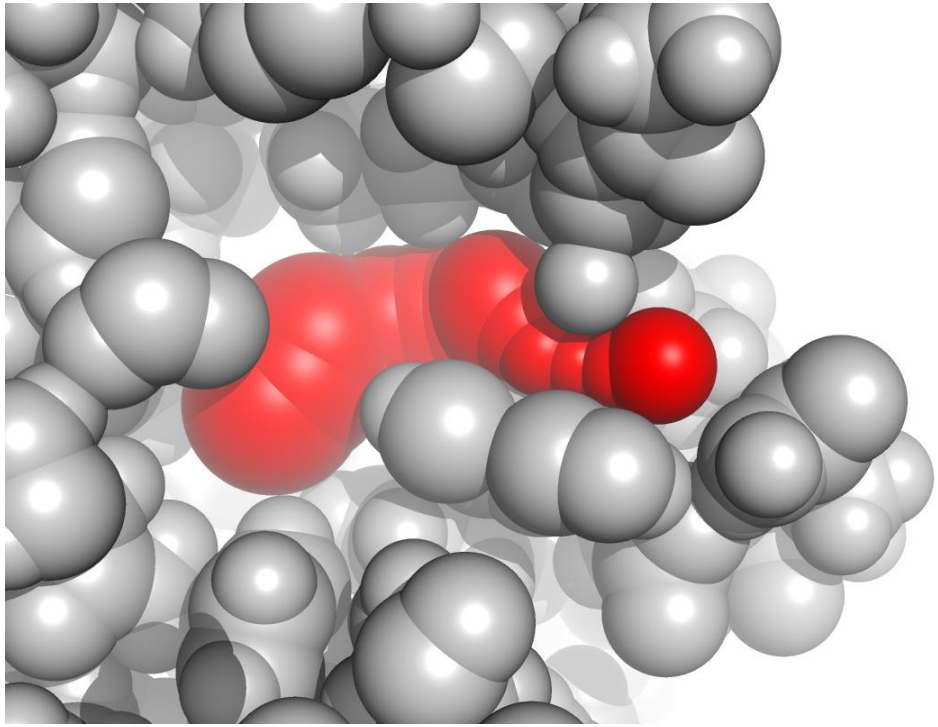
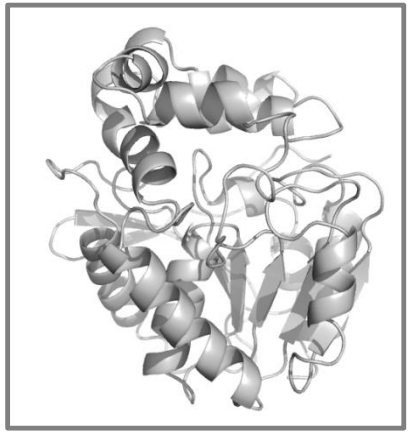


Ligand-transport pathways – software tools



identification of tunnels
in each structure

CAVER

Ligand-transport pathways – software tools

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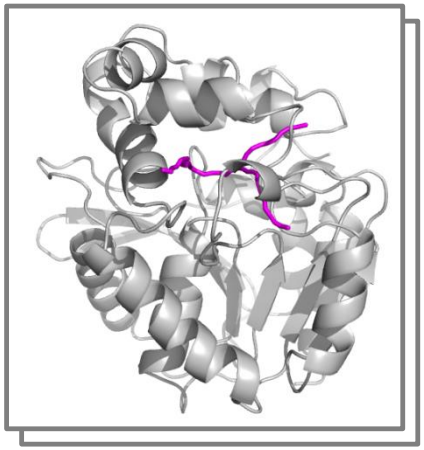
Ligand-transport pathways – software tools



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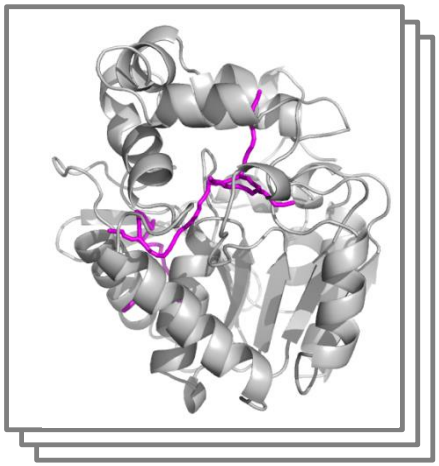

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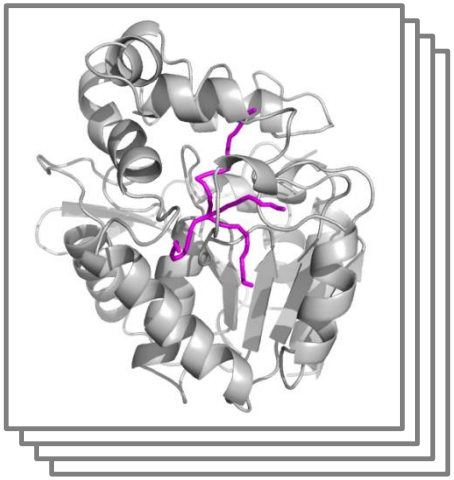

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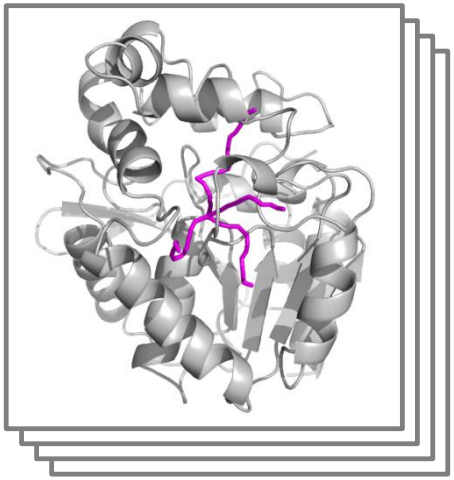

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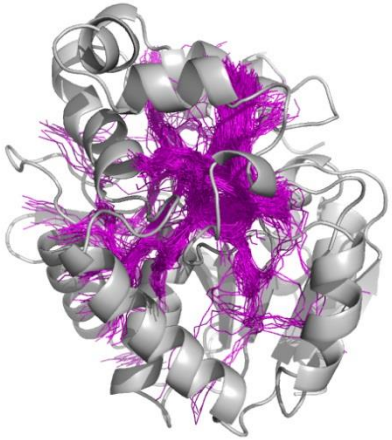
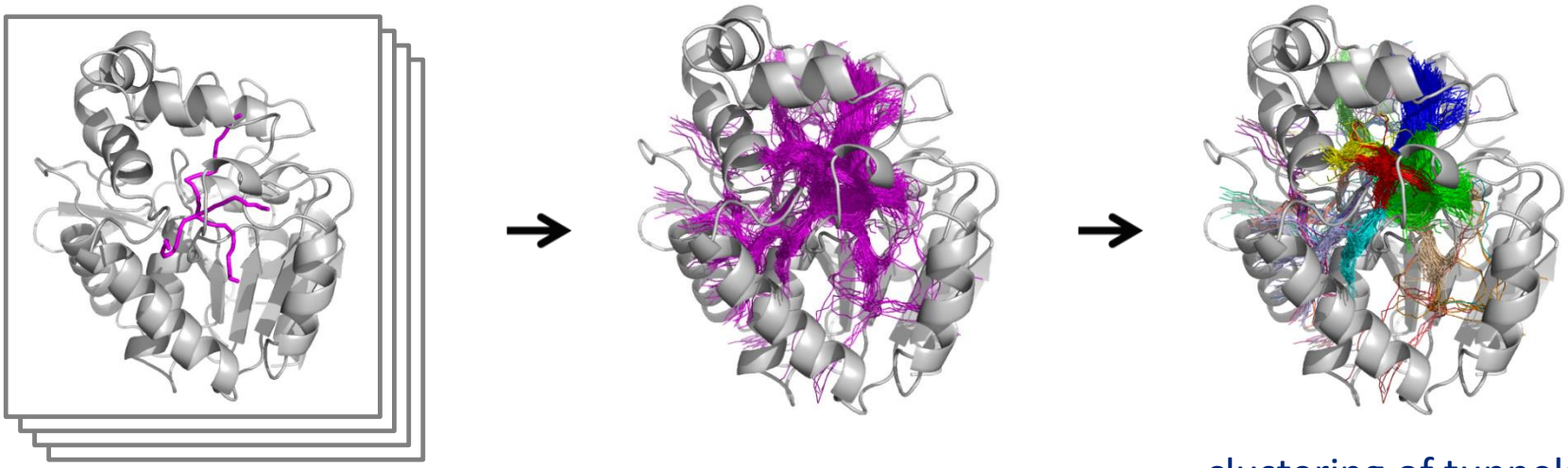


merging all identified tunnels

CAVER

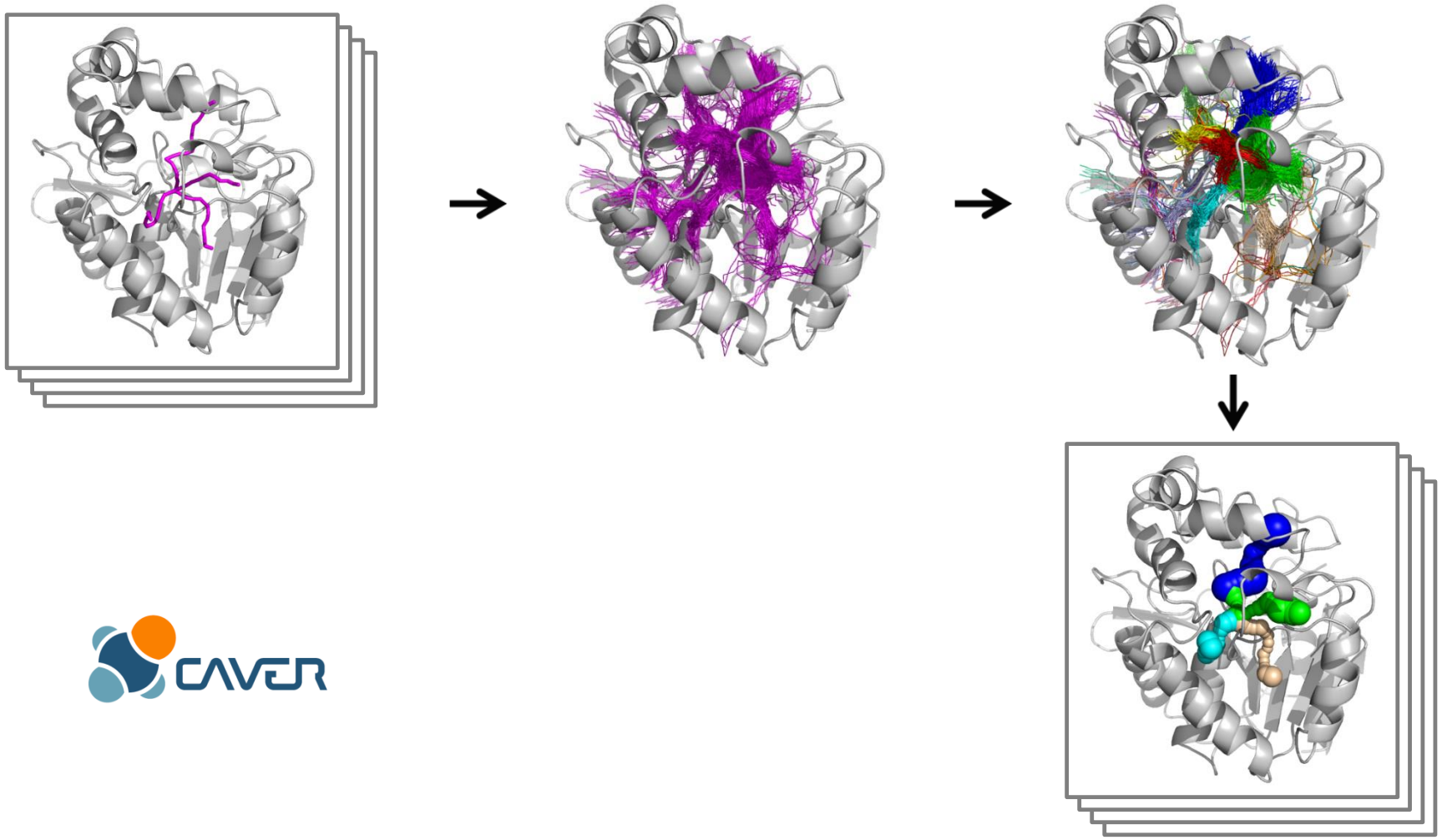
Ligand-transport pathways – software tools



clustering of tunnels

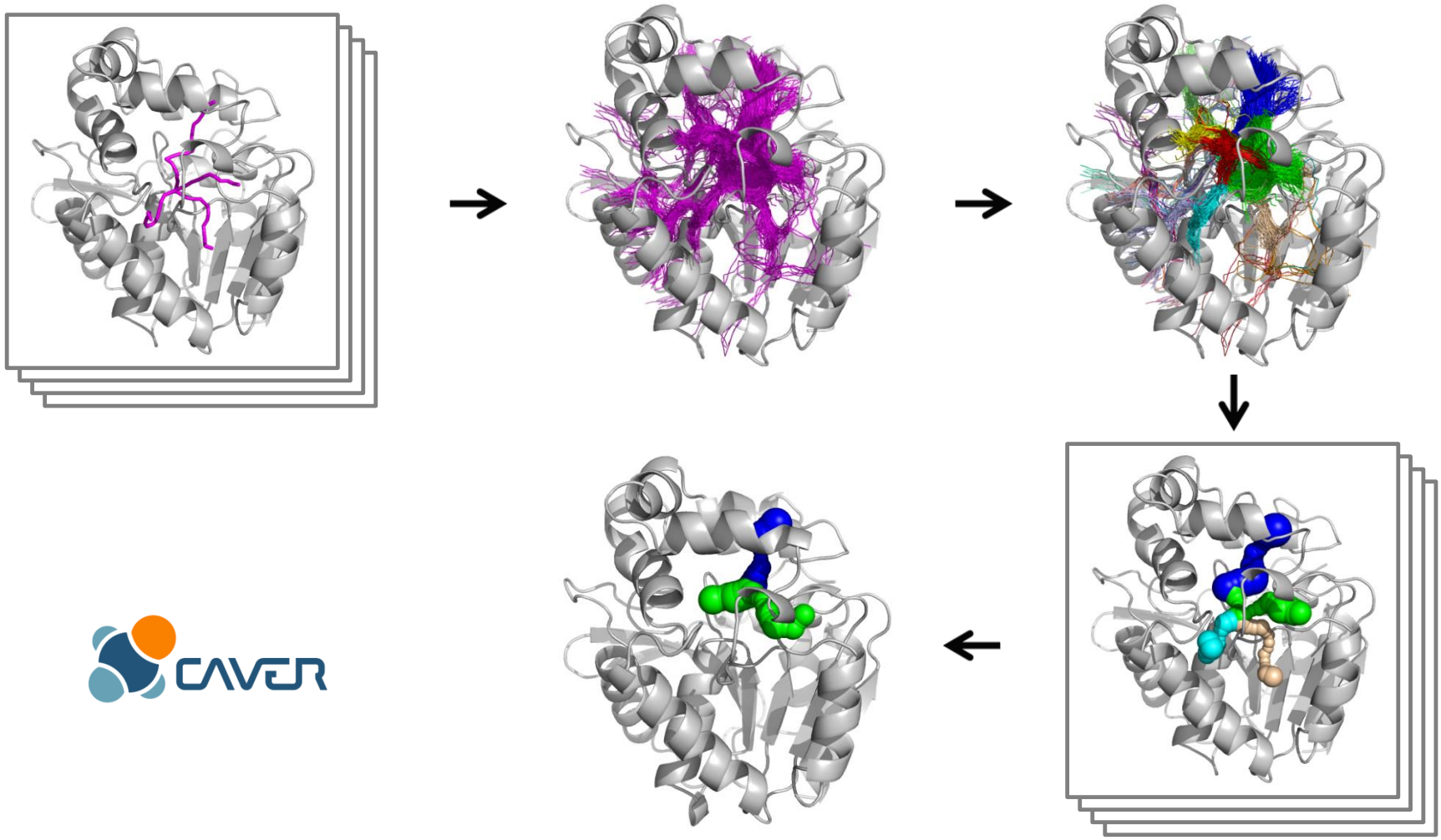
CAVER

Ligand-transport pathways – software tools



analysis of tunnels

Ligand-transport pathways – software tools

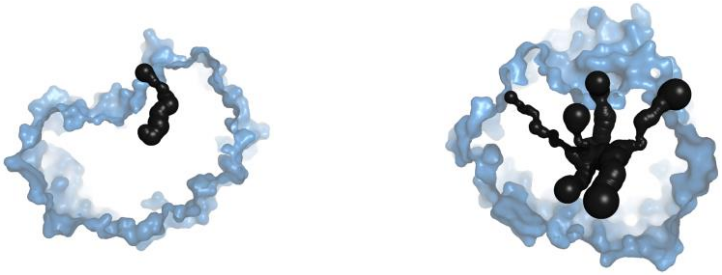


analysis of tunnel dynamics

Ligand-transport pathways – spread

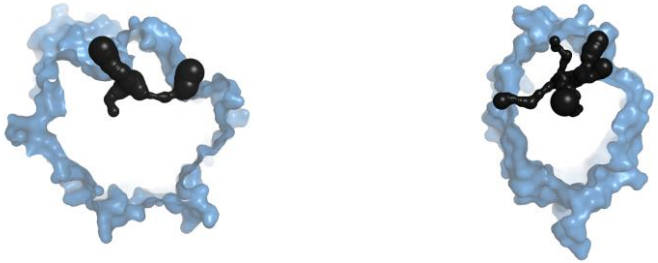
- **How widespread are the tunnels?**

OXIDOREDUCTASES



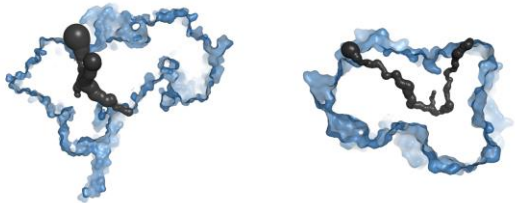
TRANSFERASES

HYDROLASES

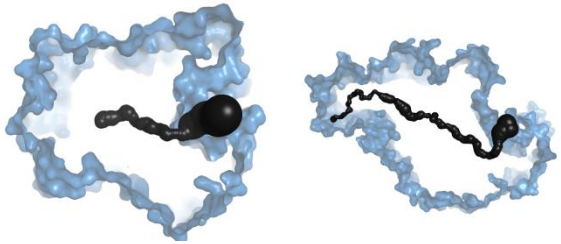


LYASES

ISOMERASES



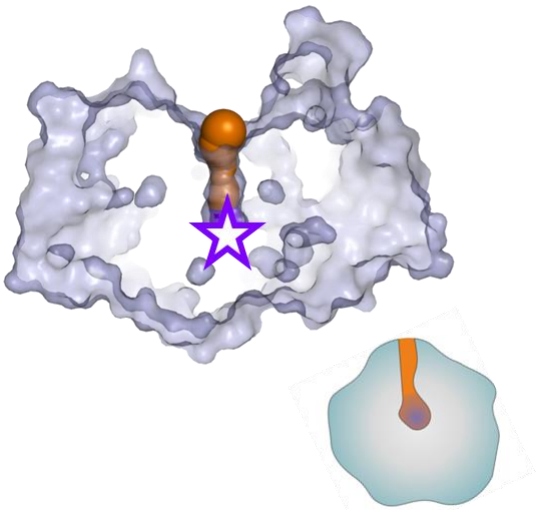
LIGASES



Prokop et al. 2012, Protein Engineering Handbook, Wiley-VCH, Weinheim, pp. 421-464.

Ligand-transport pathways – types

- **What types of tunnels exist?**
  - single tunnel connecting the active site cavity with the bulk solvent



1. ***Candida rugosa* lipase**
E.C. 3.1.1.3 *(PDB-ID 1CRL)*

Marques et al. 2016, Understanding Enzymes - Function, Design, Engineering and Analysis, PanStanford, pp. 421-464.

Ligand-transport pathways – types

- **What types of tunnels exist?**
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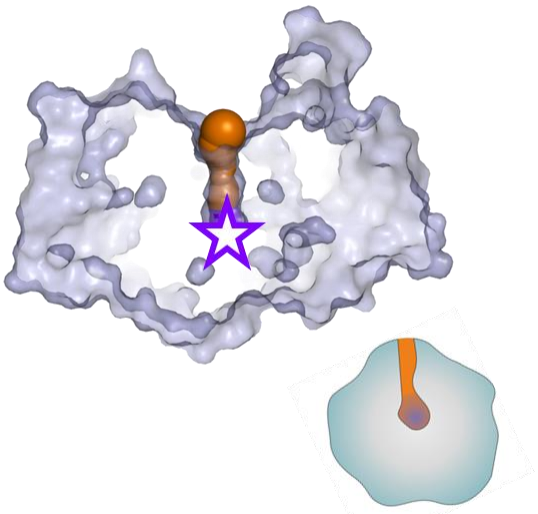

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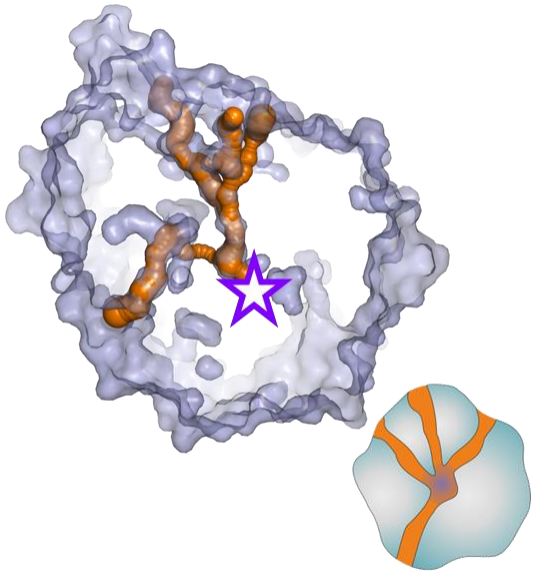


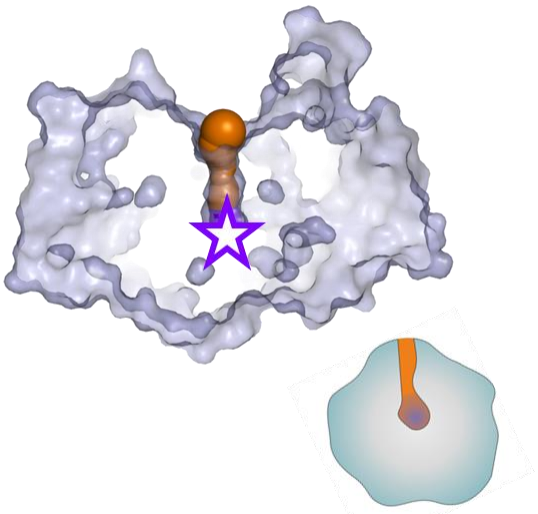
2. **[NiFeSe]-hydrogenase**
E.C. 1.12.99.6 *(PDB-ID 4KL8)*

Marques et al. 2016, Understanding Enzymes - Function, Design, Engineering and Analysis, PanStanford, pp. 421-464.

Ligand-transport pathways – types

❑ What types of tunnels exist?

- single tunnel connecting the active site cavity with the bulk solvent
- multiple tunnels connecting the active site cavity with the bulk solvent
- multiple tunnels connecting several active sites



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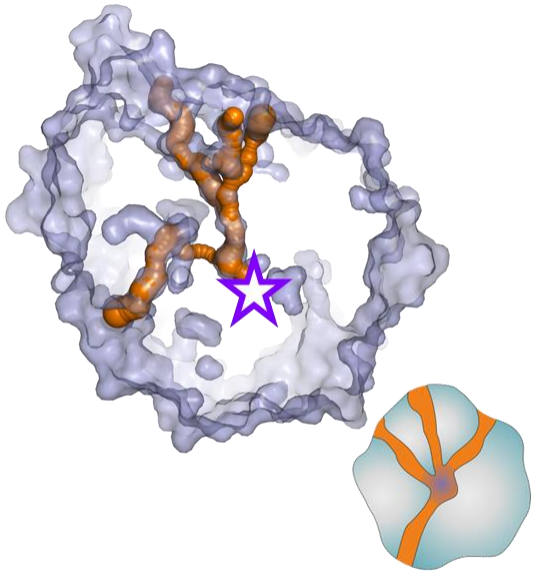

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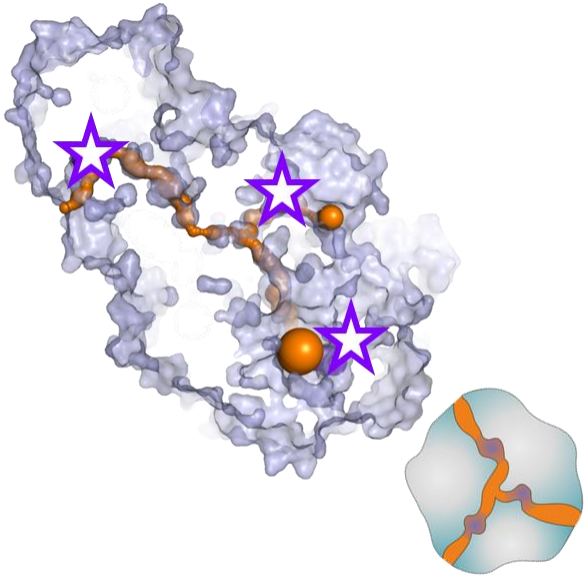
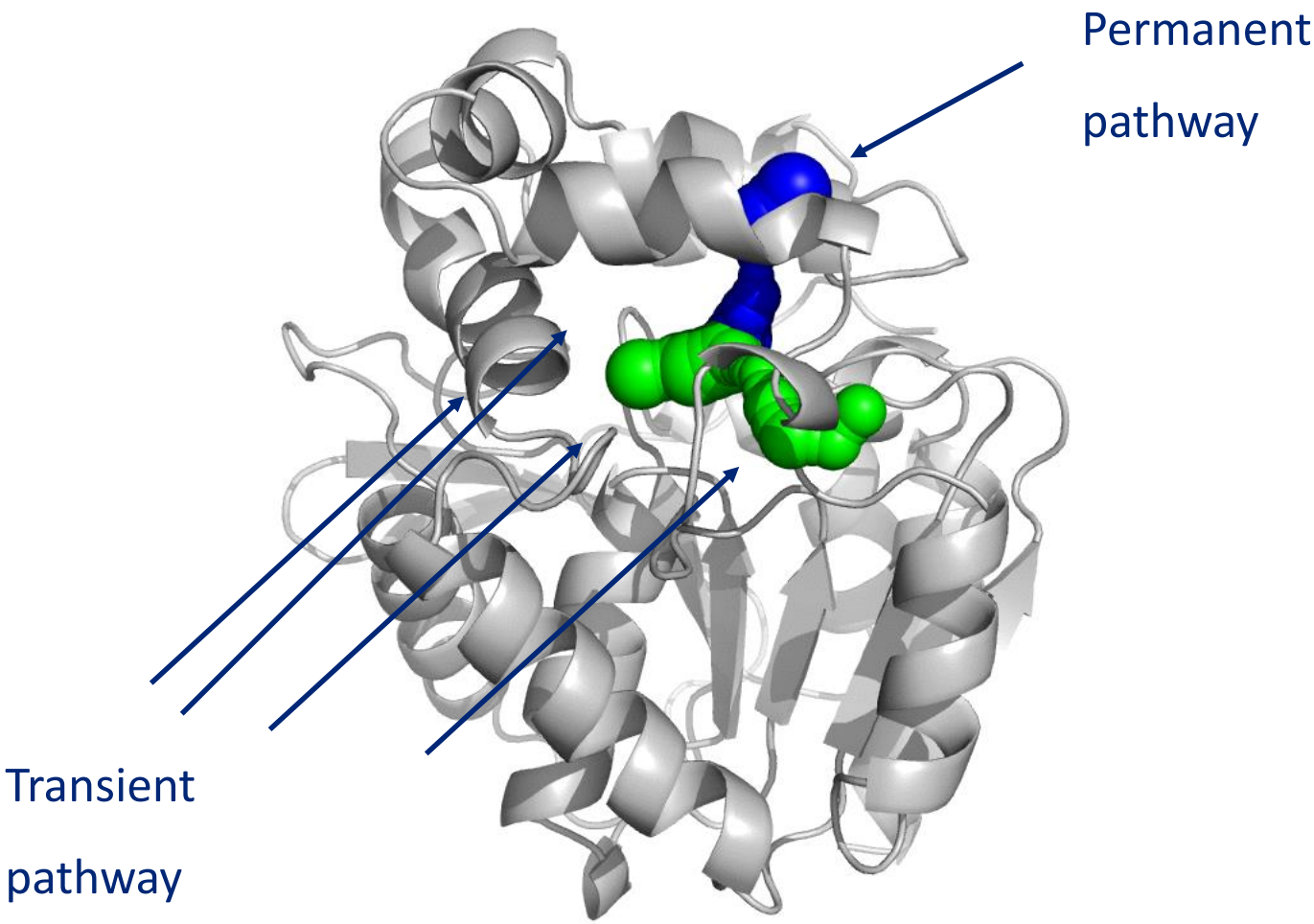


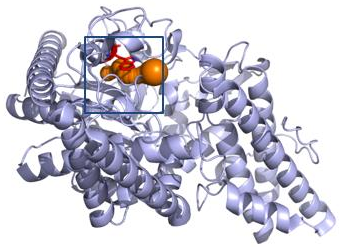
3. **Carbamoyl phosphate synthetase**
E.C. 6.3.5.5 *(PDB-ID 1A9X)*

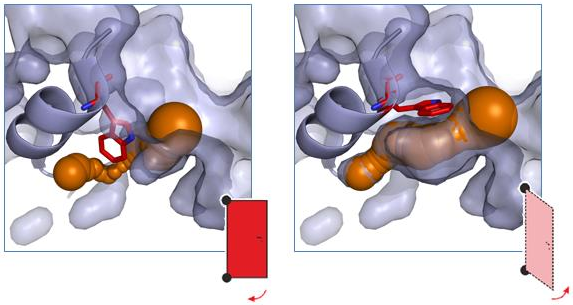
Marques et al. 2016, Understanding Enzymes - Function, Design, Engineering and Analysis, PanStanford, pp. 421-464.

Ligand-transport pathways – dynamics

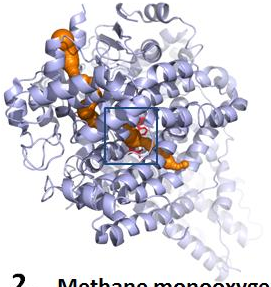


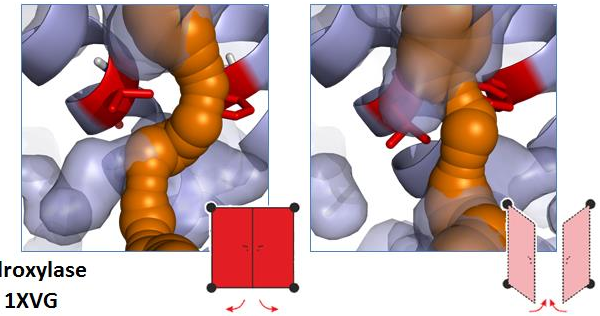
Ligand-transport pathways – gates



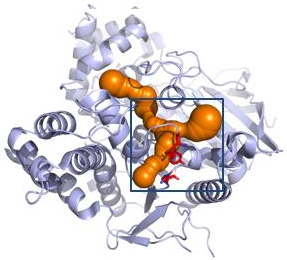


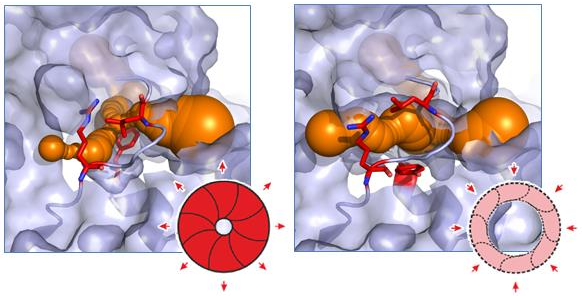
1. α-amylase
   E.C. 2.4.1.18; PDB-ID 3N98





2. Methane monooxygenase hydroxylase
   E.C. 1.14.13.25; PDB-ID 1MHY, 1XVG

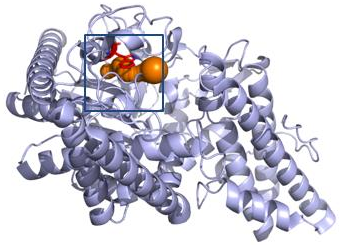


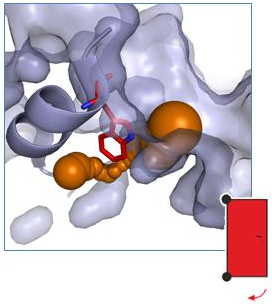


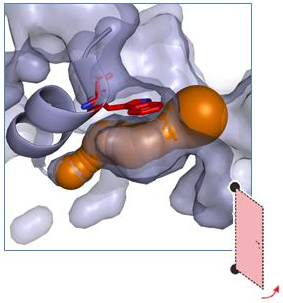
3. Acetylcholinesterase
   E.C. 3.1.1.7; PDB-ID 2XI4

Gora et al. 2013, Chem. Rev. 113: 5871–5923.

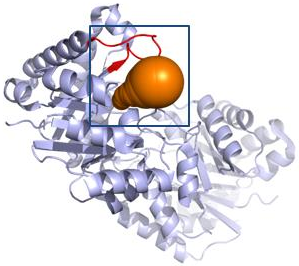
Ligand-transport pathways – gates

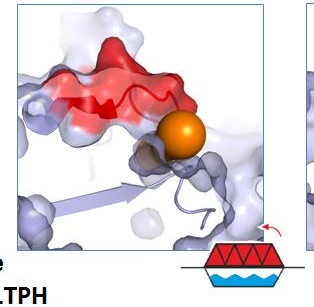


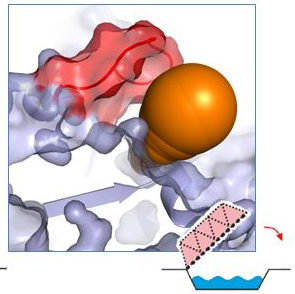




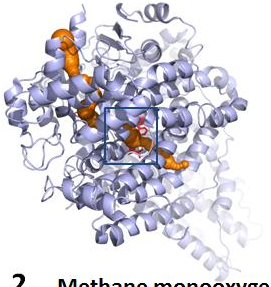
1. α-amylase
   E.C. 2.4.1.18; PDB-ID 3N98

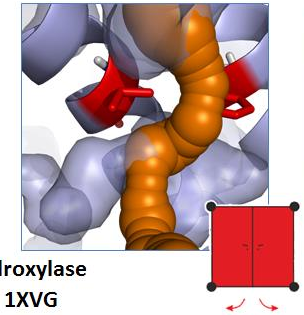


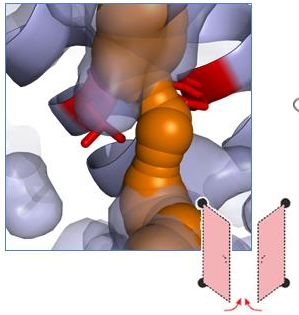




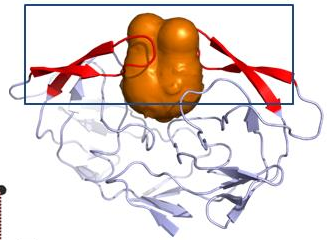
4. Triosephosphate isomerase
   E.C. 5.3.1.1; PDB-ID 1TIM, 1TPH

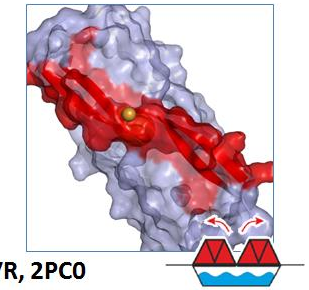


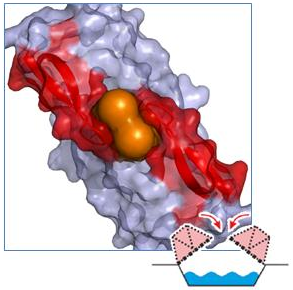




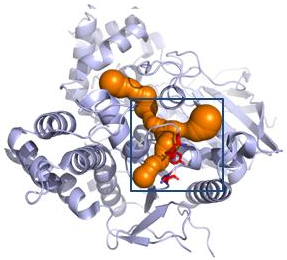
2. Methane monooxygenase hydroxylase
   E.C. 1.14.13.25; PDB-ID 1MHY, 1XVG

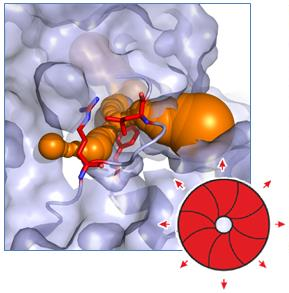


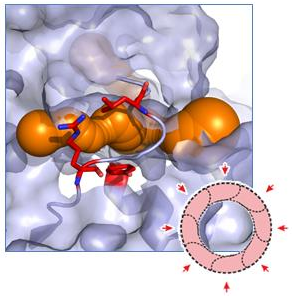




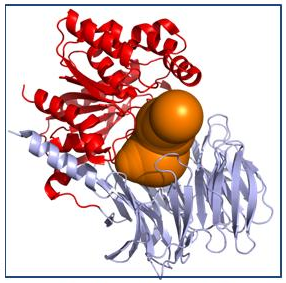
5. HIV Protease
   E.C. 3.4.23.16; PDB-ID 1HVR, 2PC0

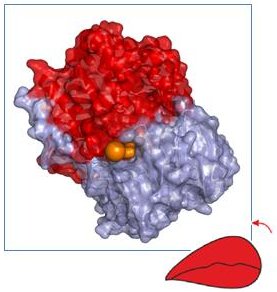


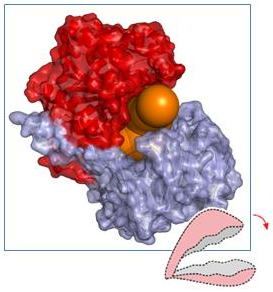




3. Acetylcholinesterase
   E.C. 3.1.1.7; PDB-ID 2XI4



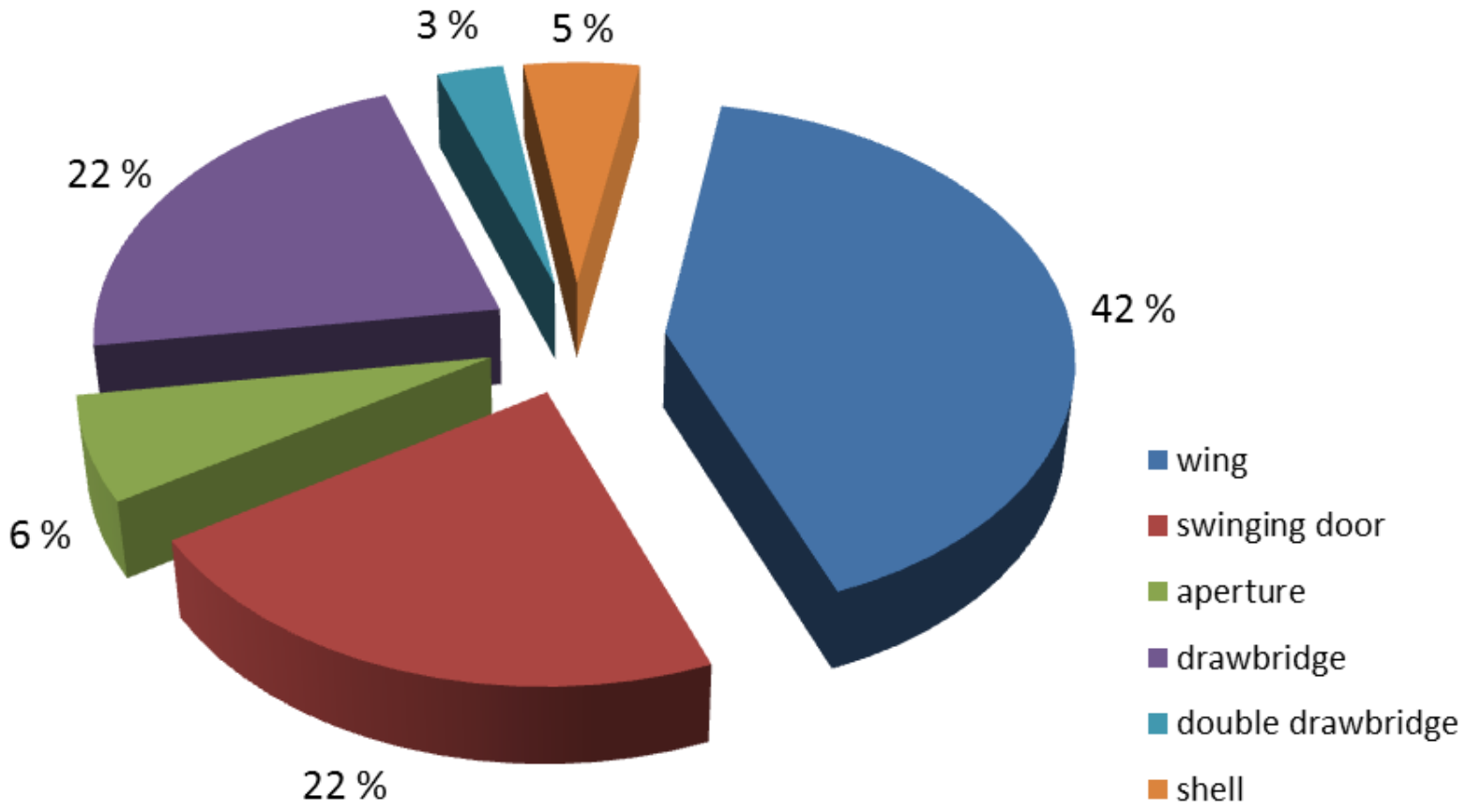




6. Acylaminoacyl peptidase
   E.C. 3.4.19.1; PDB-ID 3O4G

Gora et al. 2013, Chem. Rev. 113: 5871–5923.

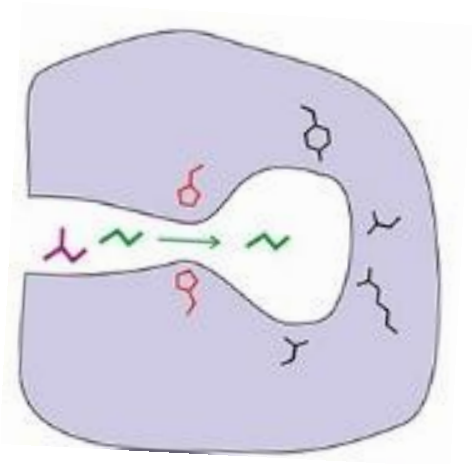
Ligand-transport pathways – gates



Gora et al. 2013, Chem. Rev. 113: 5871–5923.

Ligand-transport pathways – functions

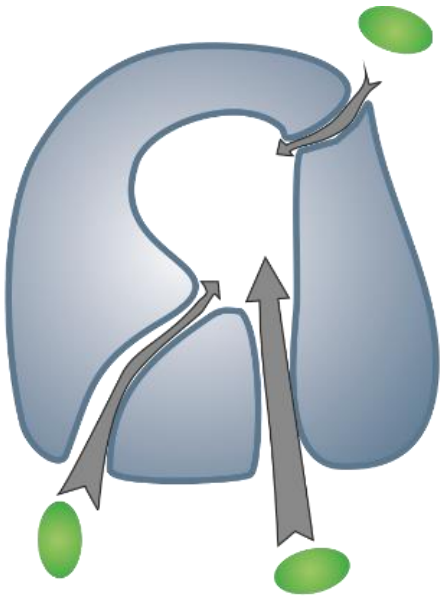
- **What are functional roles of the tunnels?**
  - control the ligands entry and release to/from the active site



Prokop et al. 2012, Protein Engineering Handbook, Wiley-VCH, Weinheim, pp. 421-464.

Ligand-transport pathways – functions

- **What are functional roles of the tunnels?**
  - control the ligands entry and release to/from the active site
  - synchronize reactions requiring contact of multiple substrates or cofactors



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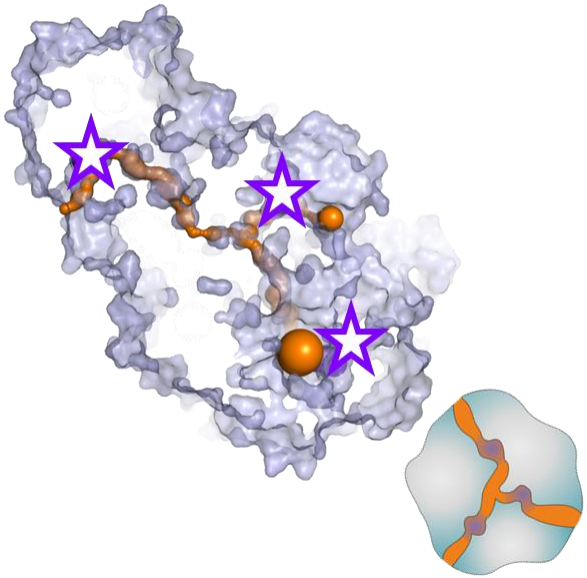
Ligand-transport pathways – functions

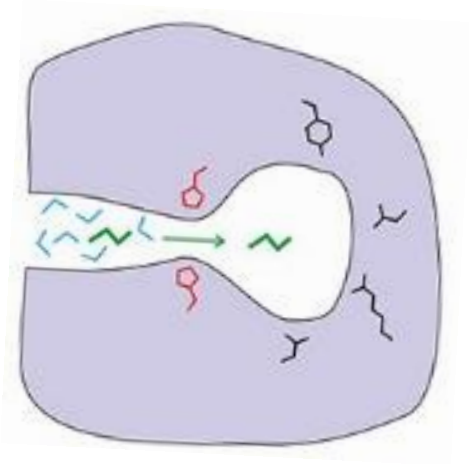
- **What are functional roles of the tunnels?**
  - control the ligands entry and release to/from the active site
  - synchronize reactions requiring contact of multiple substrates or cofactors
  - prevent potentially toxic intermediates to be released into the medium
  - avoid labile intermediates to be released into the medium and undergo side reactions



Prokop et al. 2012, Protein Engineering Handbook, Wiley-VCH, Weinheim, pp. 421-464.

Ligand-transport pathways – functions

- **What are functional roles of the tunnels?**
  - control the ligands entry and release to/from the active site
  - synchronize reactions requiring contact of multiple substrates or cofactors
  - prevent potentially toxic intermediates to be released into the medium
  - avoid labile intermediates to be released into the medium and undergo side reactions
  - control access of various solvents to the active sites
  - prevents dissipation of electrons by solvent



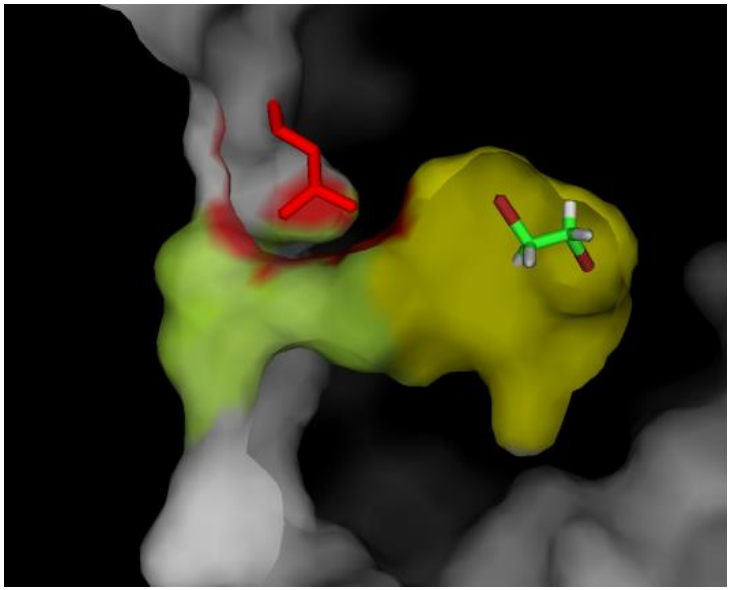
Prokop et al. 2012, Protein Engineering Handbook, Wiley-VCH, Weinheim, pp. 421-464.

Mutations in ligand-transport pathways

- **Mutations in existing pathways**

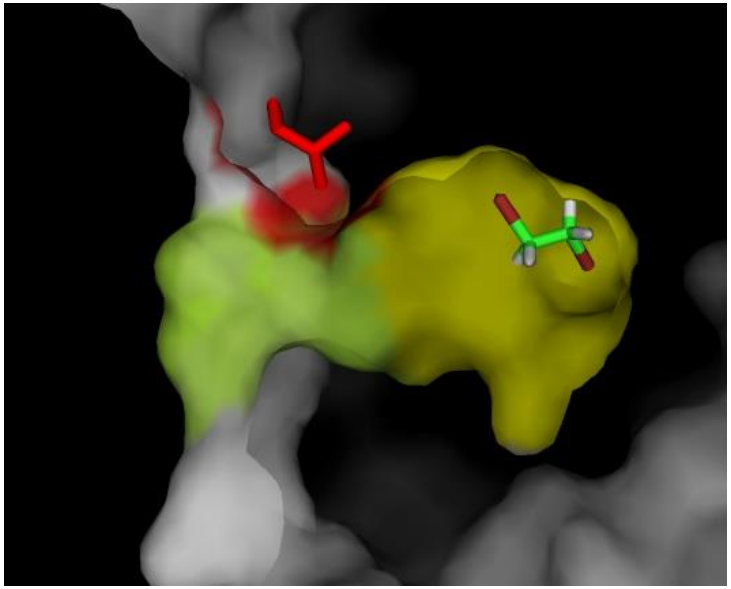
Mutations in ligand-transport pathways

- **Mutations in existing pathways**



Mutations in ligand-transport pathways

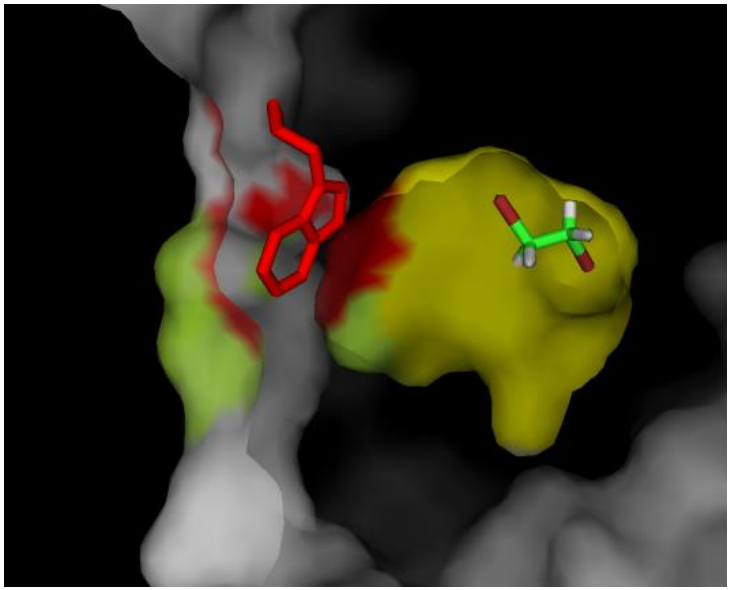
- **Mutations in existing pathways**
  - alter properties permanent pathways



Prokop et al. 2012, Protein Engineering Handbook, Wiley-VCH, Weinheim, pp. 421-464.

Mutations in ligand-transport pathways

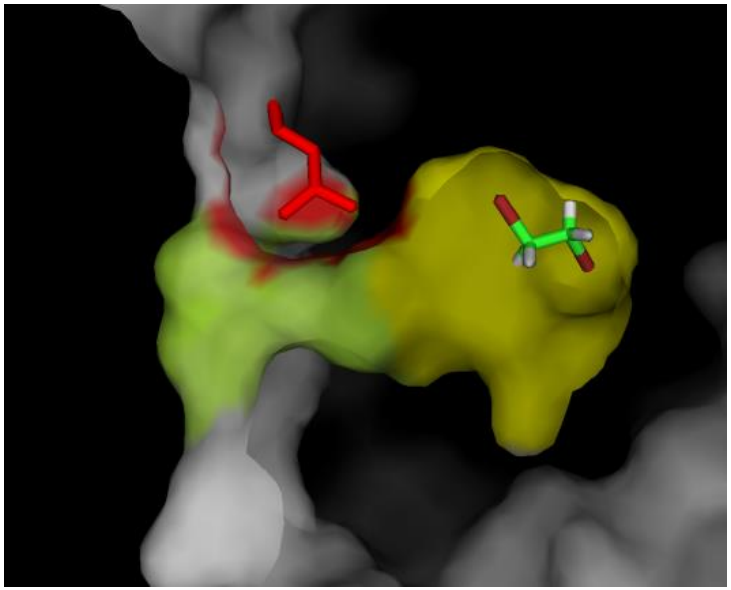
- **Mutations in existing pathways**
  - alter properties permanent pathways
  - introduce gate (permanent -> transient)



Koudelakova et al. 2013, Angew. Chem. Int. Ed Engl. 52: 1959–1963.
Marques et al. 2017, J. Chem. Inf. Model. 50: 1970–1989.

Mutations in ligand-transport pathways

- **Mutations in existing pathways**
  - alter properties permanent pathways
  - introduce gate (permanent -> transient)
  - remove gate (transient -> permanent)
  - modulate gating frequency or amplitude



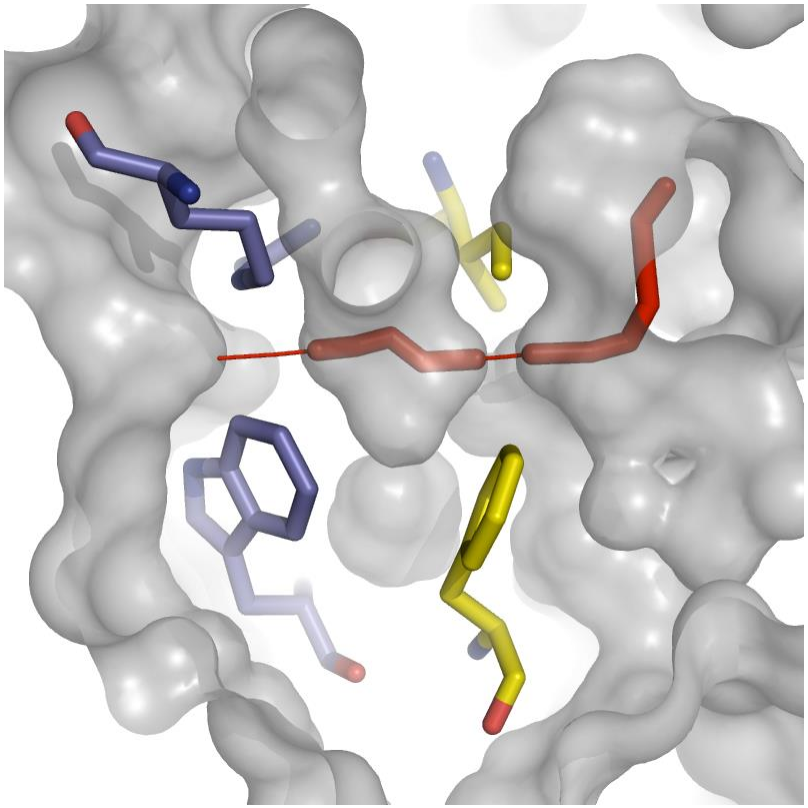
Liskova et al. 2015, ChemCatChem 7: 648–659.

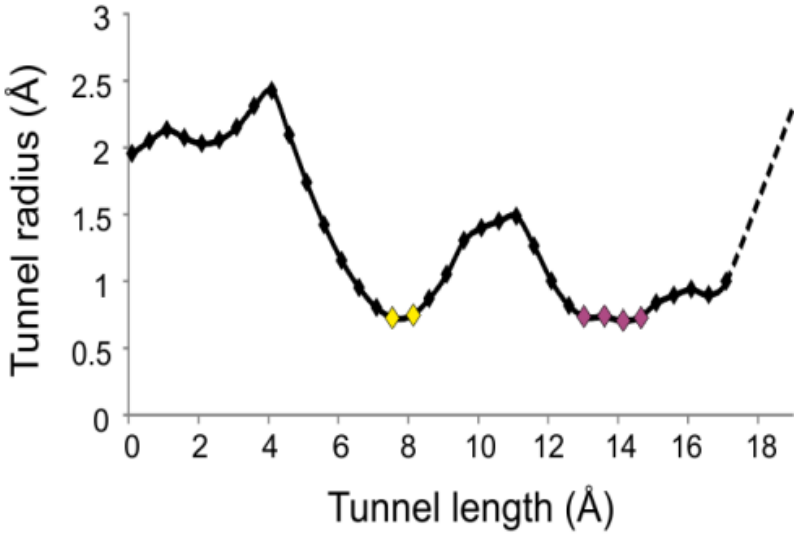
Mutations in ligand-transport pathways

- **Mutations in existing pathways**
  - alter properties permanent pathways
  - introduce gate (permanent -> transient)
  - remove gate (transient -> permanent)
  - modulate gating frequency or amplitude
- **Gain-of-function mutations in potential pathways**
  - activate new functional pathways

Gain-of-function mutations in potential pathways

- **Potential pathways – globular proteins**
  - leading through voids of proteins with only sub-Å dimension

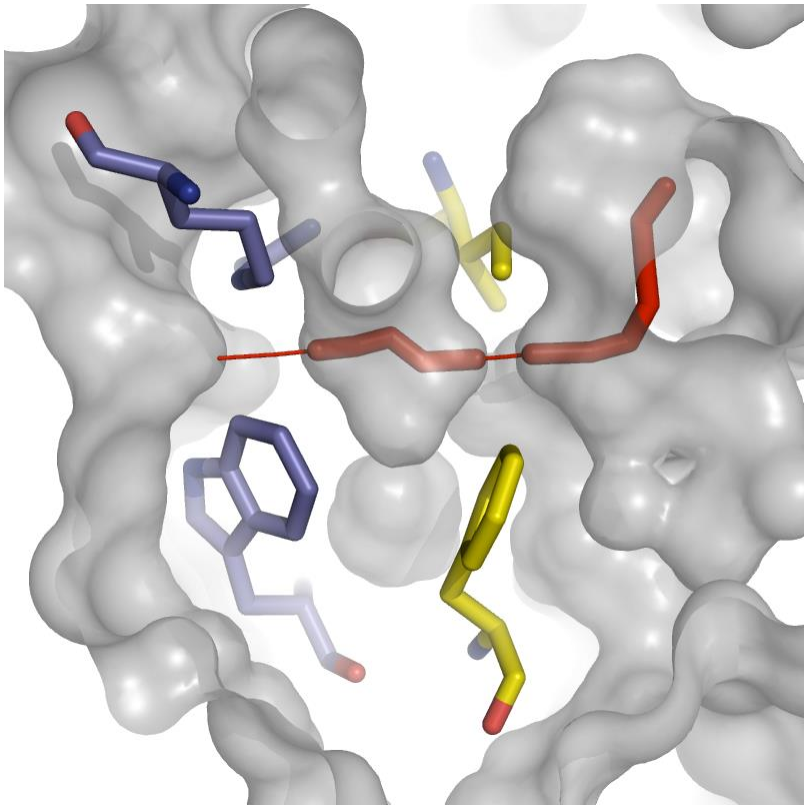




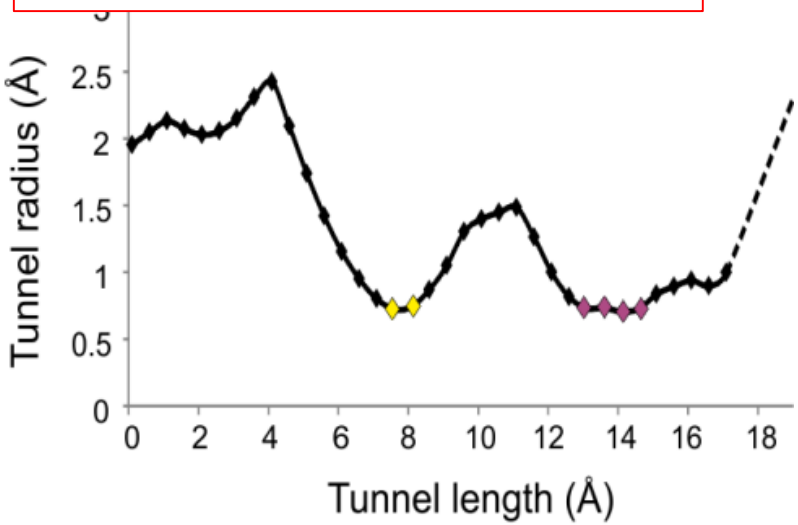
Brezovsky et al. 2016, ACS Catal. 6: 7597–7610.
Kokkonen et al. 2018, J. Am. Chem. Soc. 140: 17999-18008.

Gain-of-function mutations in potential pathways

- **Potential pathways – globular proteins**
  - leading through voids of proteins with only sub-Å dimension



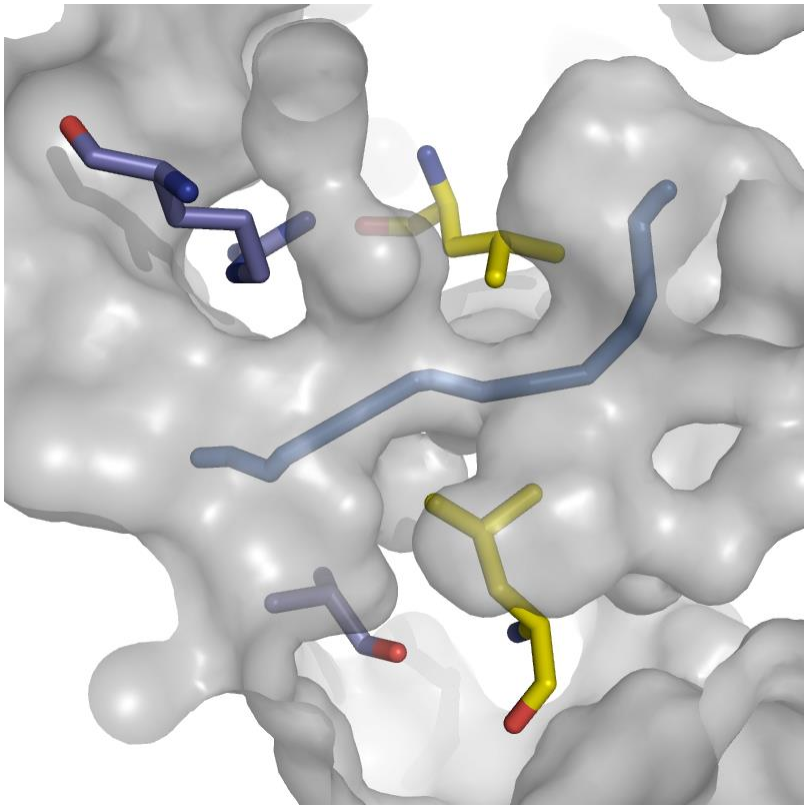
What is Å ???



Brezovsky et al. 2016, ACS Catal. 6: 7597–7610.
Kokkonen et al. 2018, J. Am. Chem. Soc. 140: 17999-18008.

Gain-of-function mutations in potential pathways

- **Potential pathways – globular proteins**
  - upon gain-of-function mutations, leading to well-defined tunnel



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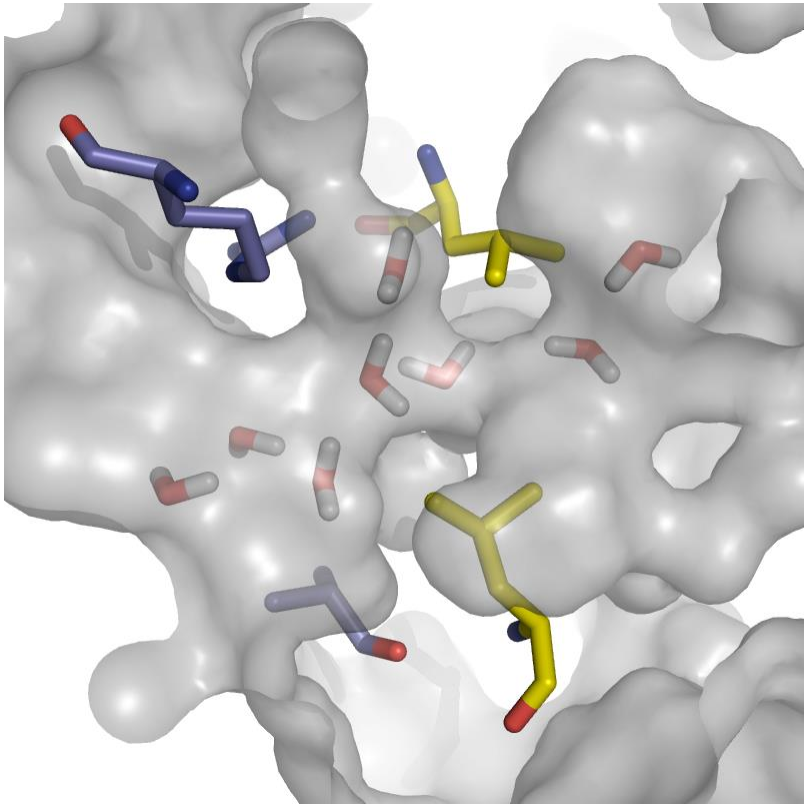
Kokkonen et al. 2018, J. Am. Chem. Soc. 140: 17999-18008.

Gain-of-function mutations in potential pathways

- **Potential pathways – globular proteins**
  - the open tunnel enables efficient transport of waters to the active site



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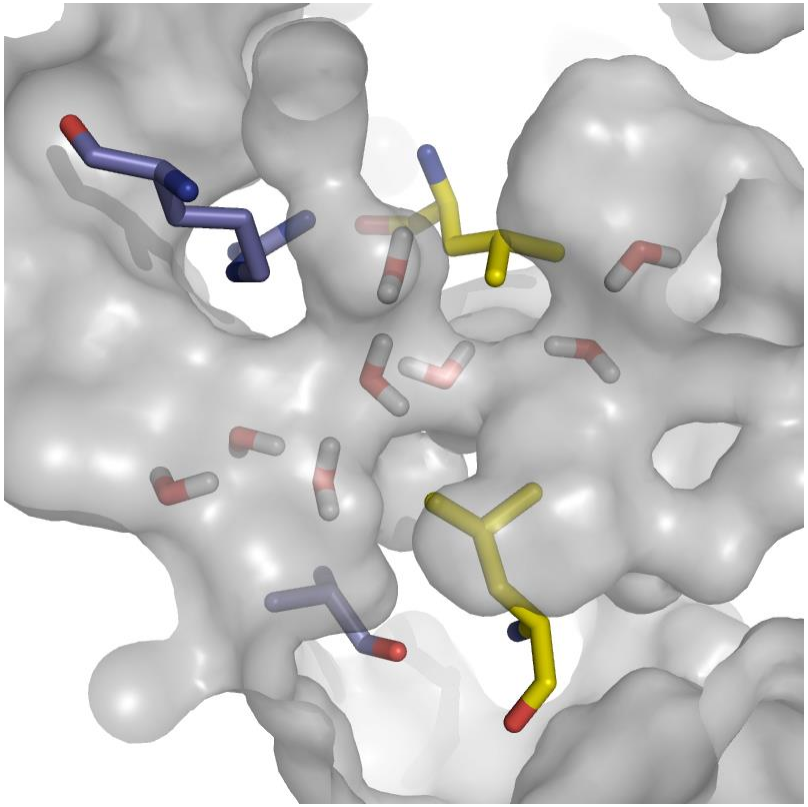
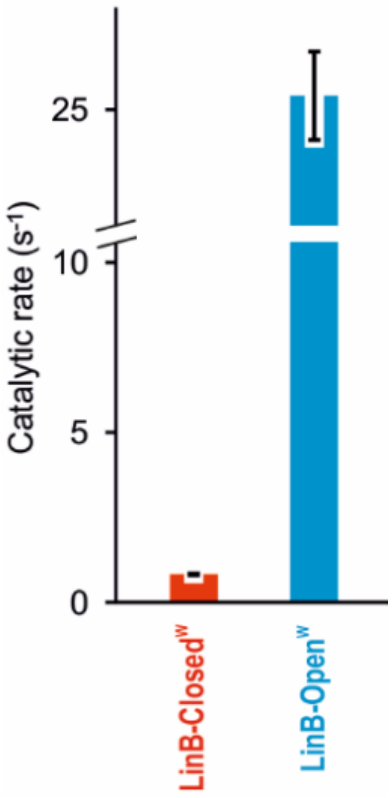
Kokkonen et al. 2018, J. Am. Chem. Soc. 140: 17999-18008.

Gain-of-function mutations in potential pathways

❑ Potential pathways – globular proteins

- the opening of the tunnel have profound functional consequences

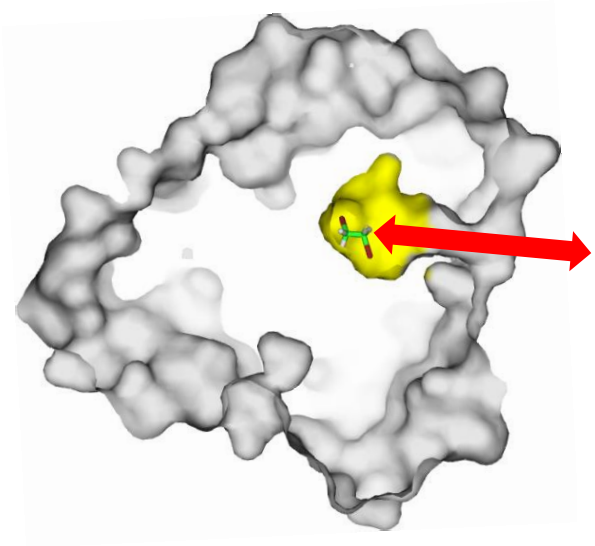




Brezovsky et al. 2016, ACS Catal. 6: 7597–7610.
Kokkonen et al. 2018, J. Am. Chem. Soc. 140: 17999-18008.

Analogies with ion-channels

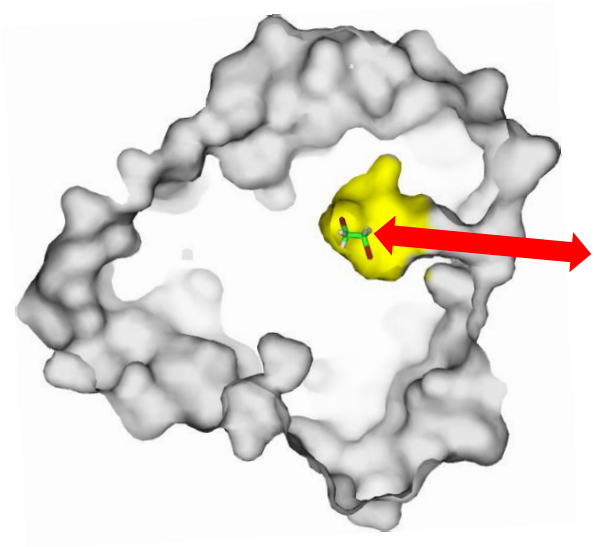
Soluble/globular proteins



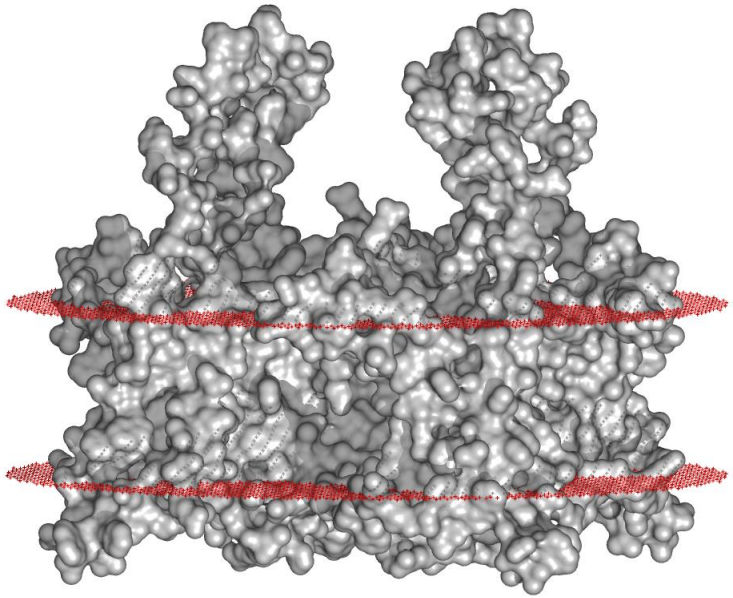
Ligand-transport pathways

Analogies with ion-channels

Soluble/globular proteins



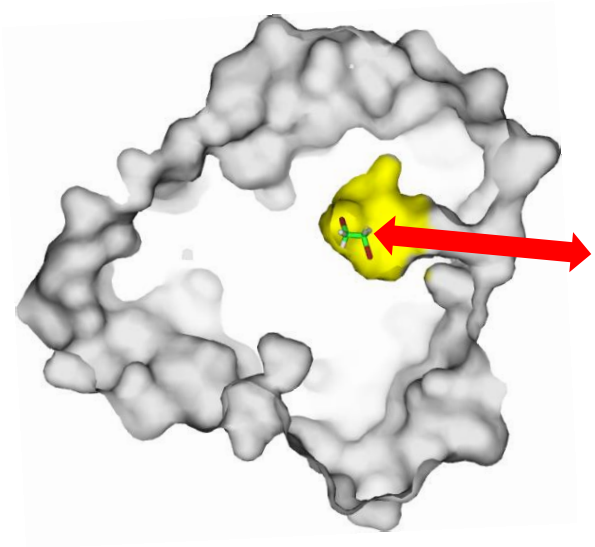
Transmembrane proteins



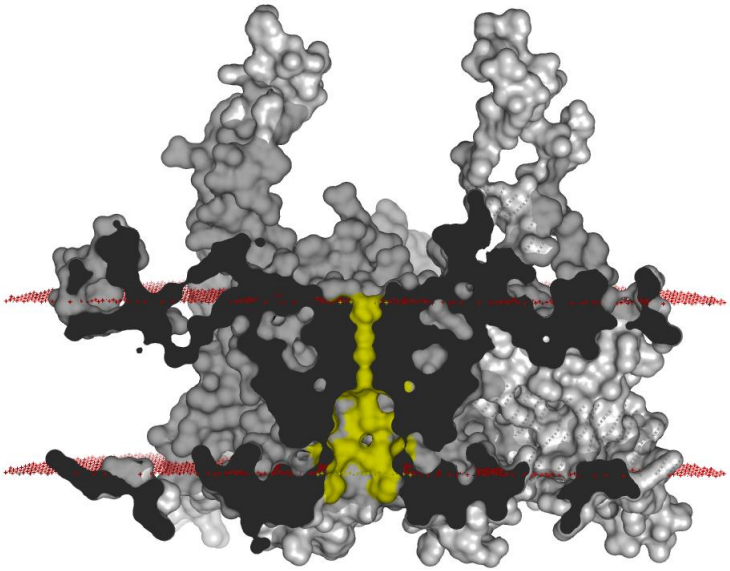
Ligand-transport pathways

Analogies with ion-channels

Soluble/globular proteins



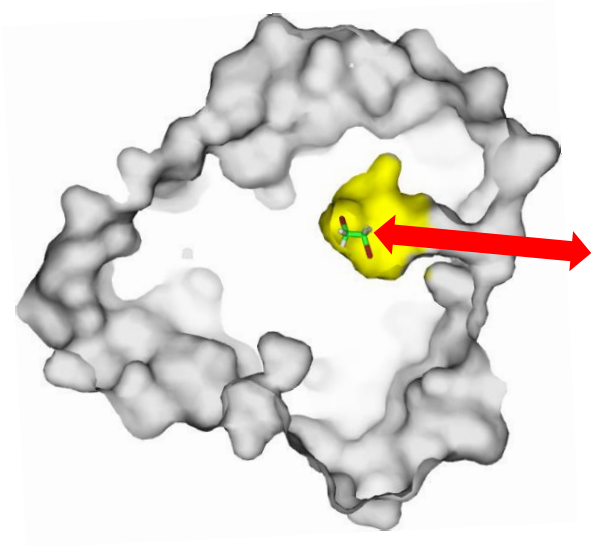
Transmembrane proteins



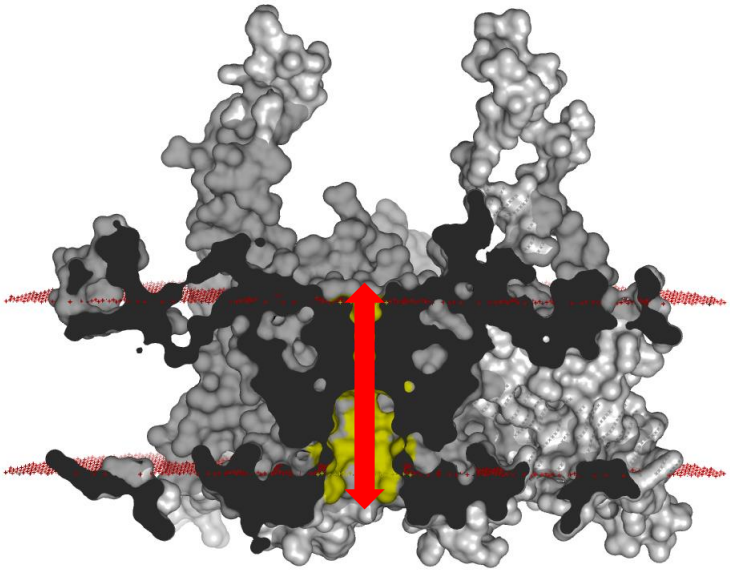
Ligand-transport pathways

Analogies with ion-channels

Soluble/globular proteins



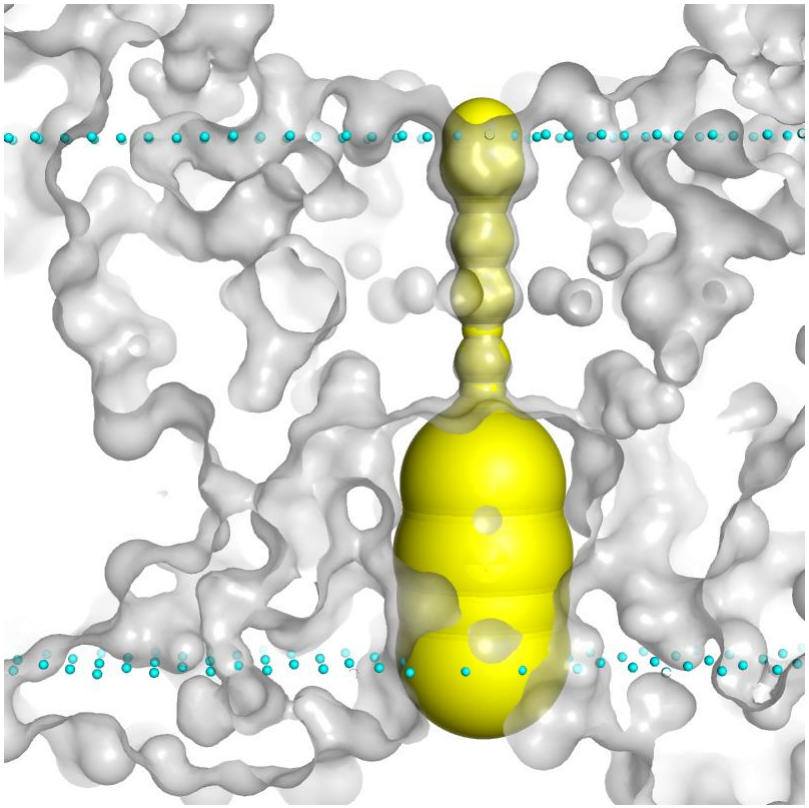
Transmembrane proteins



Ligand-transport pathways

Gain-of-function mutations in potential pathways

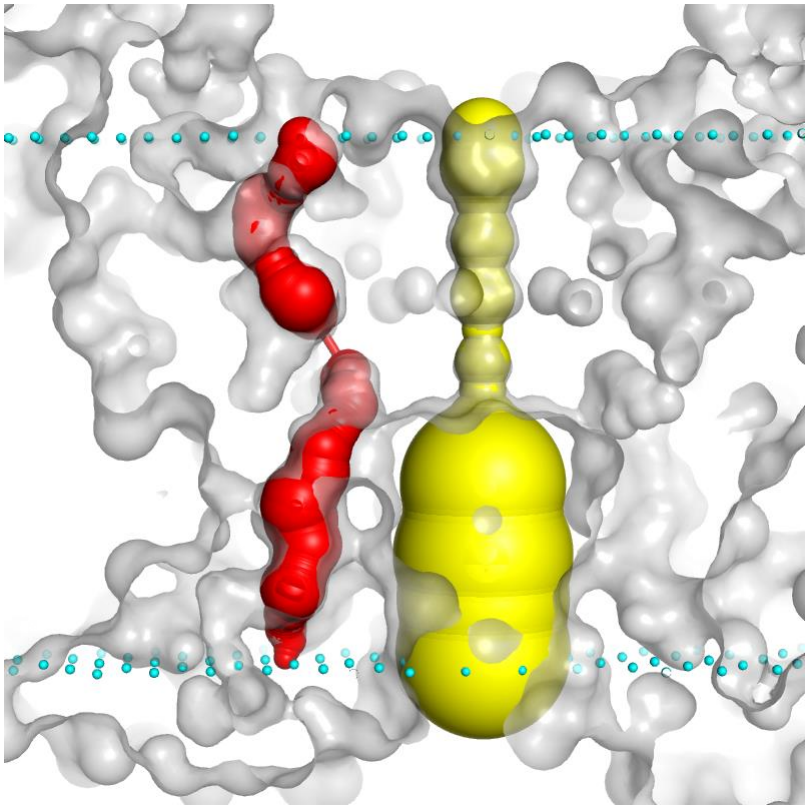
- **Potential pathways – transmembrane channels**
  - leading through voids of proteins with only sub-Å dimension



Prutting et al. 2011, J. Biol. Chem. 286: 20031-20042.

Gain-of-function mutations in potential pathways

- **Potential pathways – transmembrane channels**
  - leading through voids of proteins with only sub-Å dimension



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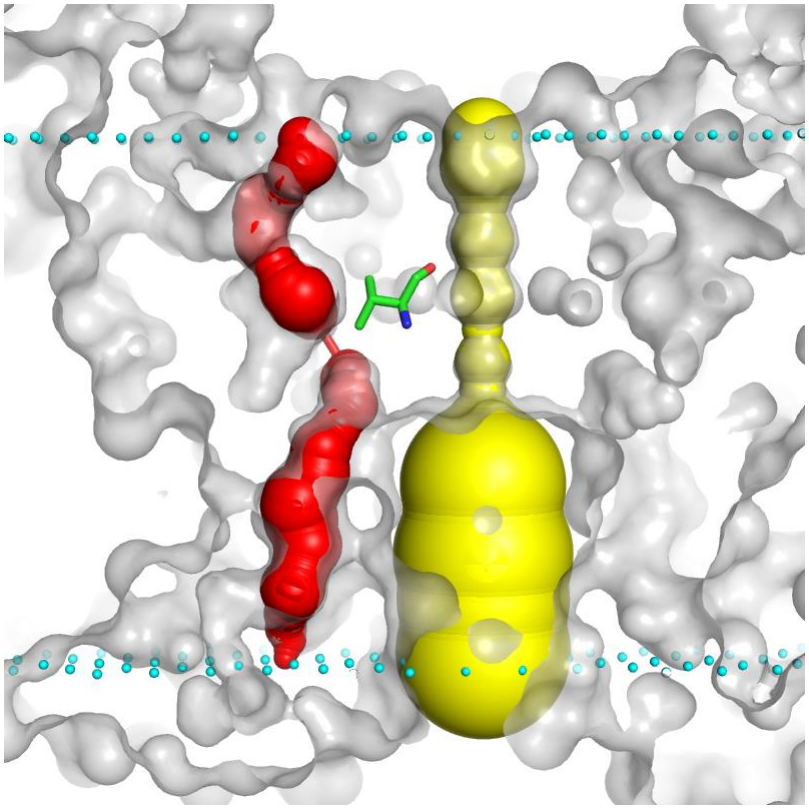

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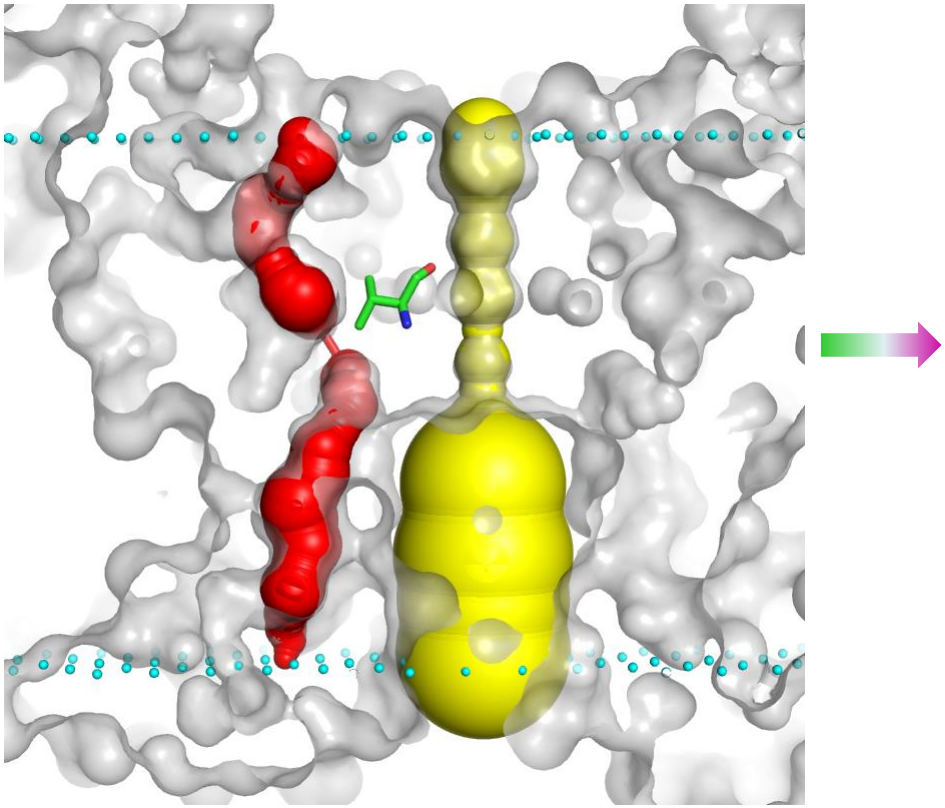

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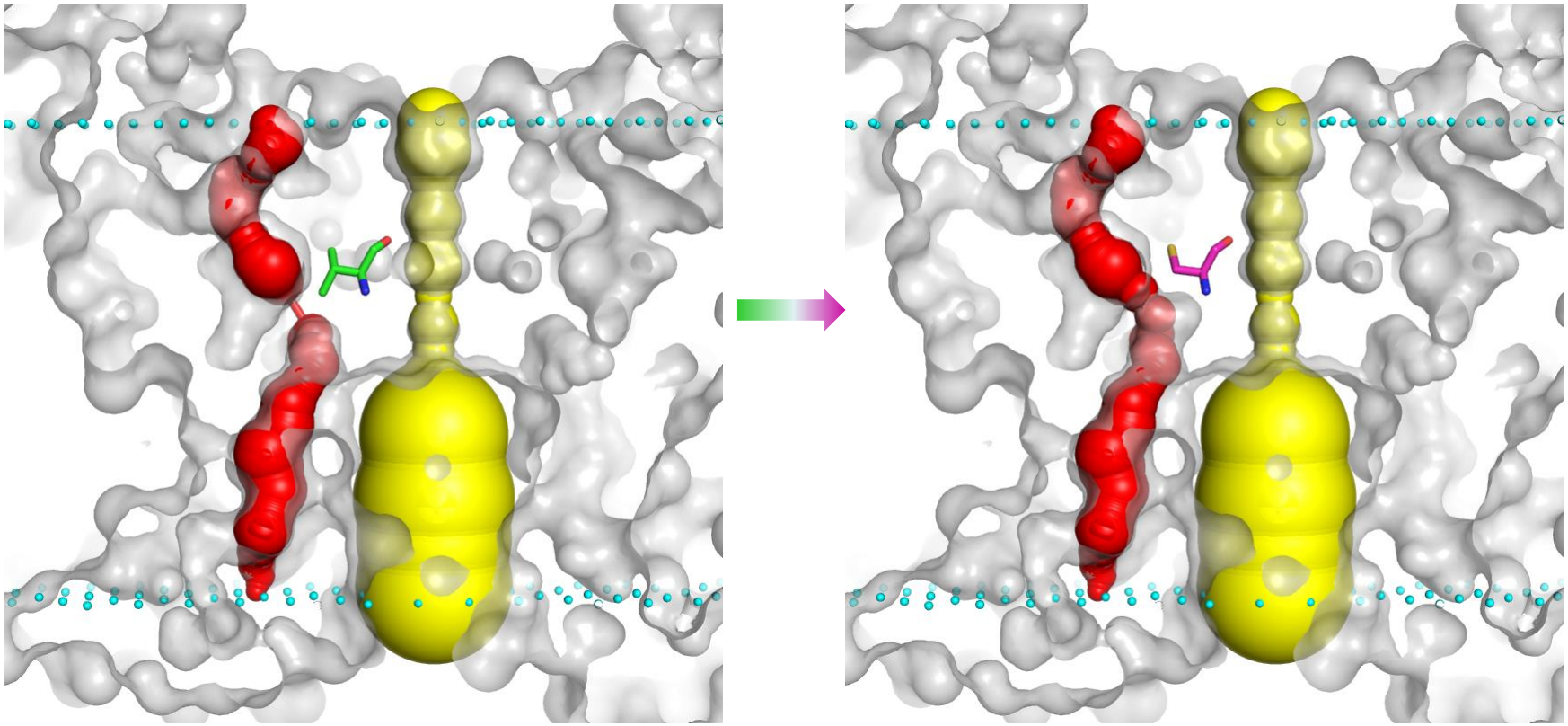
- **Potential pathways – transmembrane channels**
  - viable upon gain-of-function mutations



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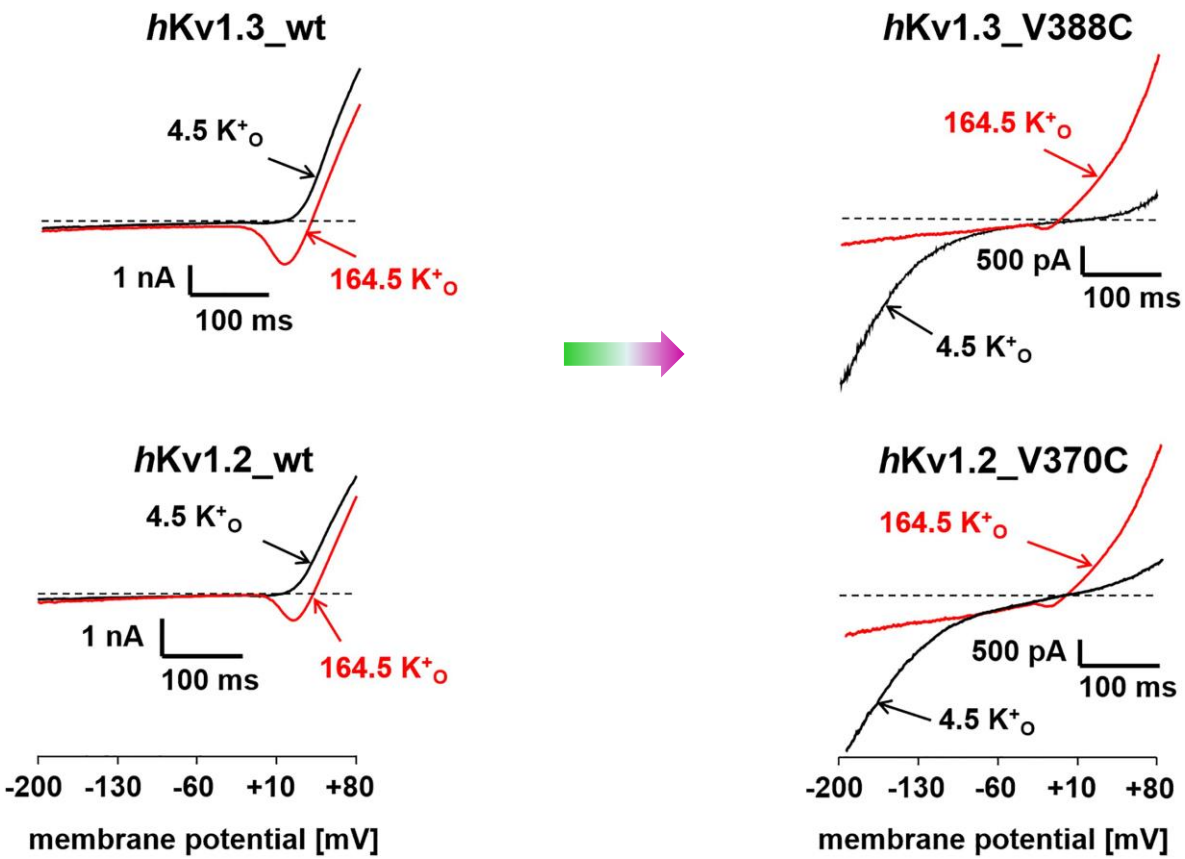
Gain-of-function mutations in potential pathways

Potential pathways – transmembrane channels

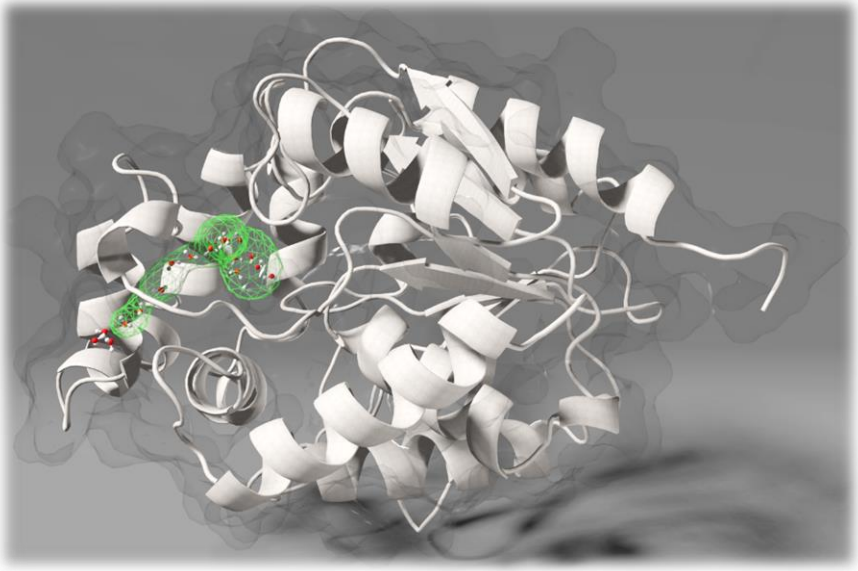
- notable functional consequences



Prutting et al. 2011, J. Biol. Chem. 286: 20031-20042.

Gain-of-function mutations in water pathways

- hydrolytic enzymes require water for their catalytic action
- water migrate via internal tunnel network
- despite its vital role, water transport has been largely neglected due to difficulties in tracking those rare phenomena

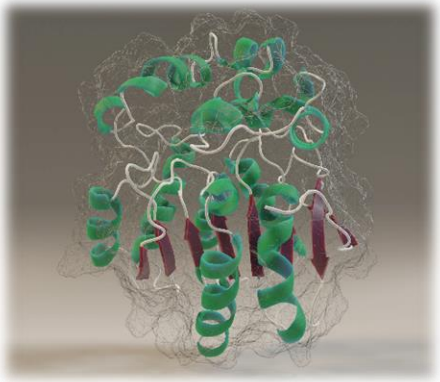


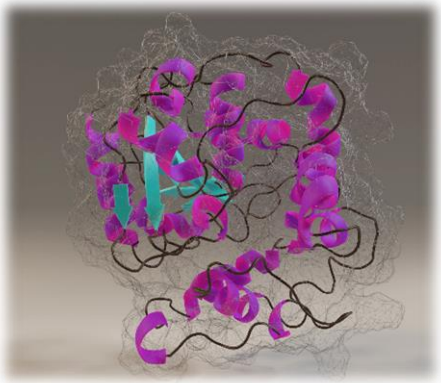
Sequeiros-Borja et al., in preparation

Gain-of-function mutations in water pathways

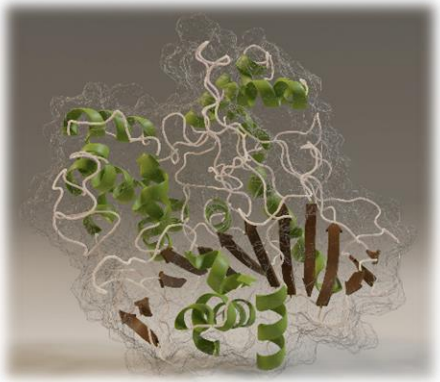
- extensive MD simulations (5 µs) of three members of the hydrolase superfamily
  - haloalkane dehalogenase from *Rhodococcus rhodochrous* (**Dha**)
  - potato epoxide hydrolase 1 (**Epx**)
  - lipase from *Candida rugose* (**Lip**)

Dha





Epx

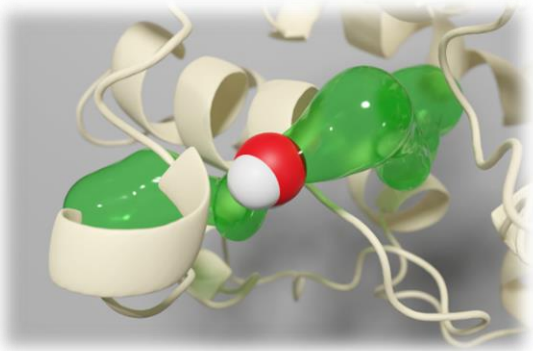


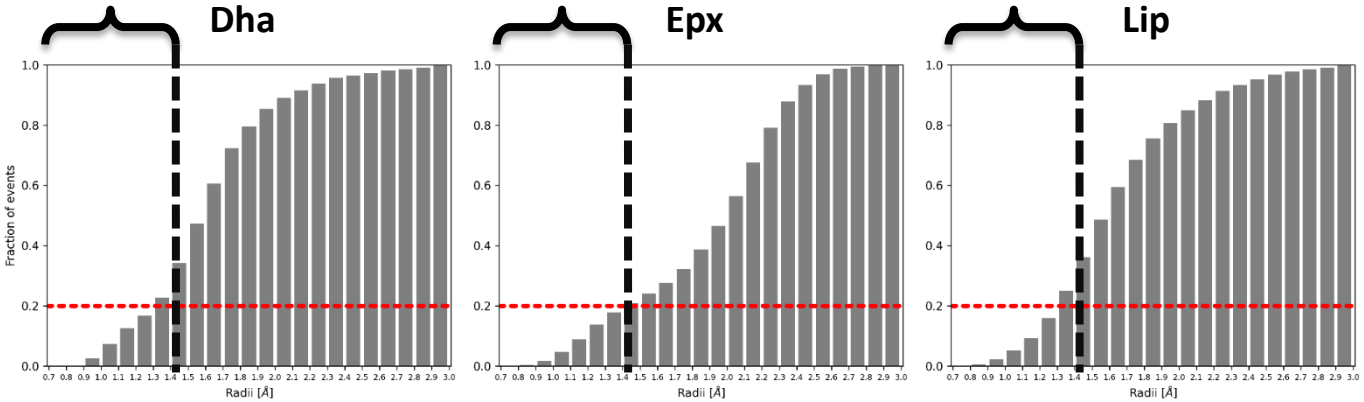
Lip

Sequeiros-Borja et al., in preparation

Gain-of-function mutations in water pathways

- ~20% of water travels through unexpectedly narrow tunnels (bottleneck radius <1.4 Å)

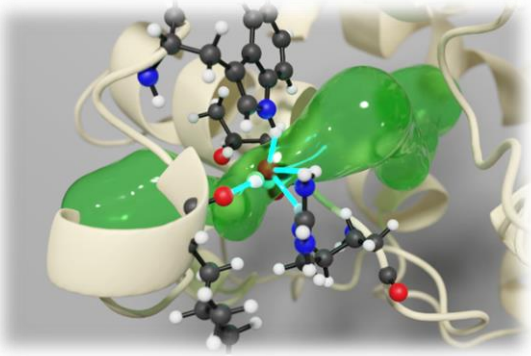


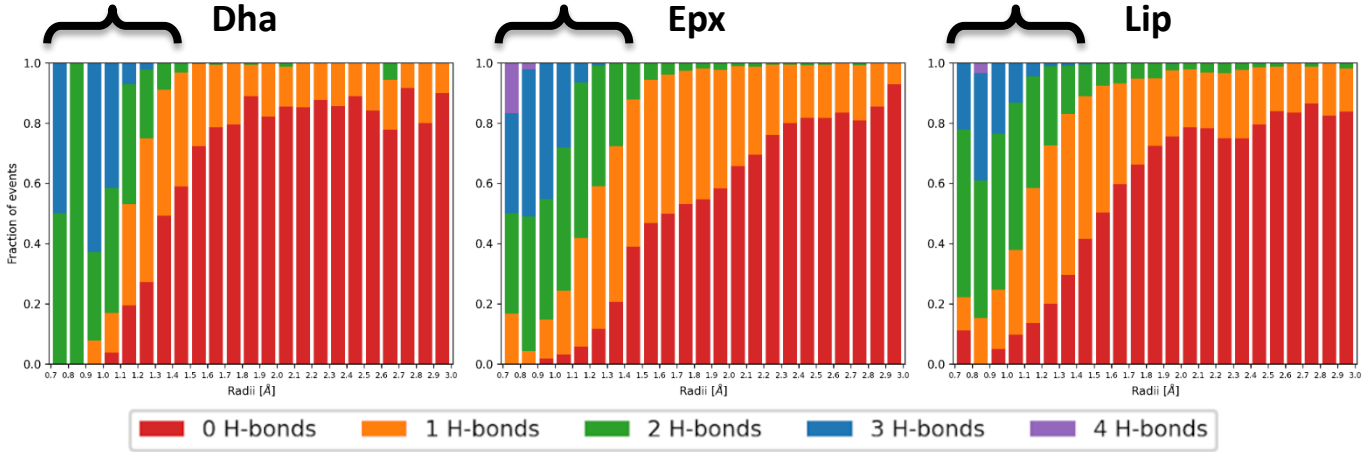


Sequeiros-Borja et al., in preparation

Gain-of-function mutations in water pathways

- multiple H-bonds stabilize water transport via such narrow tunnels



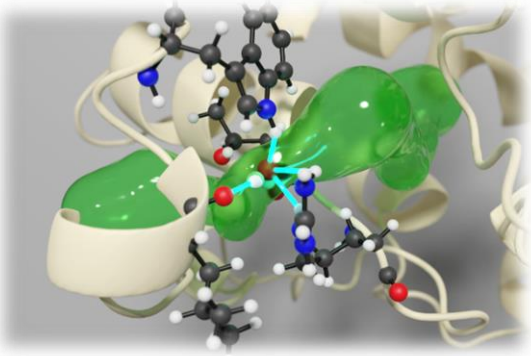


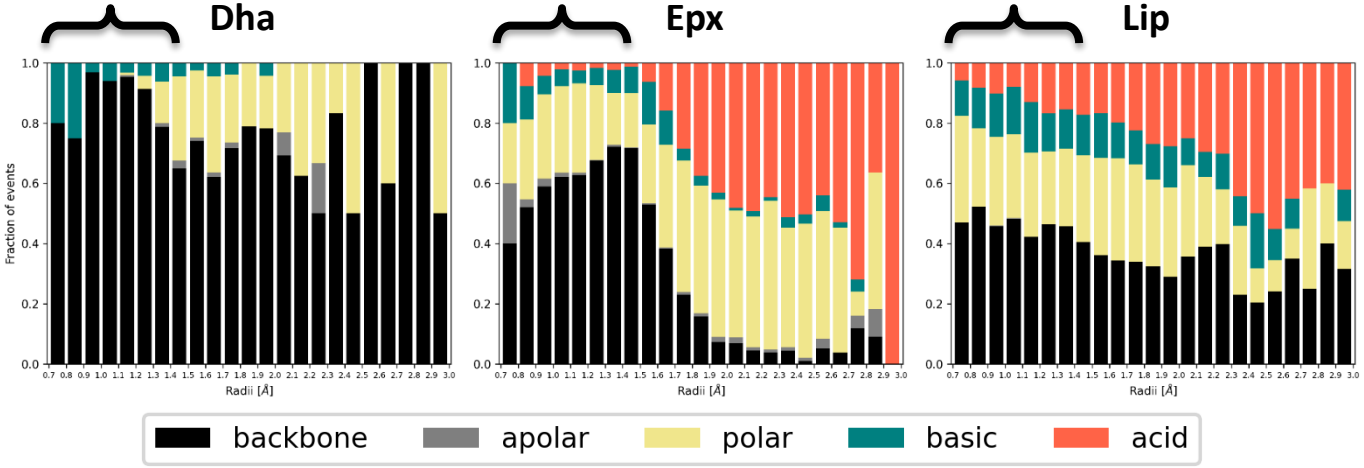
=> New functionally relevant sites

Sequeiros-Borja et al., in preparation

Gain-of-function mutations in water pathways

- these H-bond are mainly formed with backbone atoms



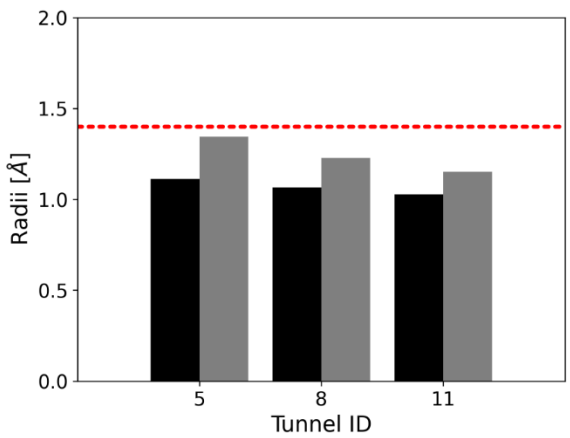


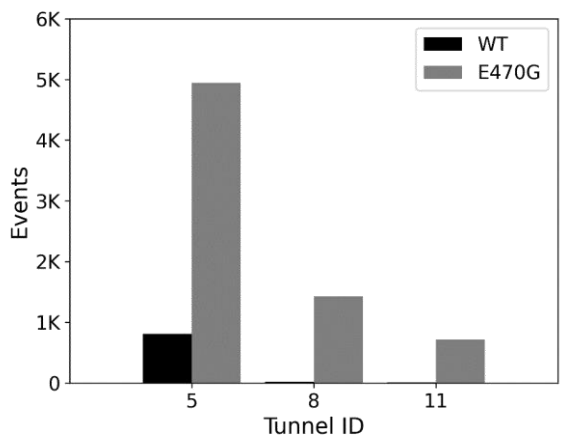
=> Conservation of paths within a protein fold

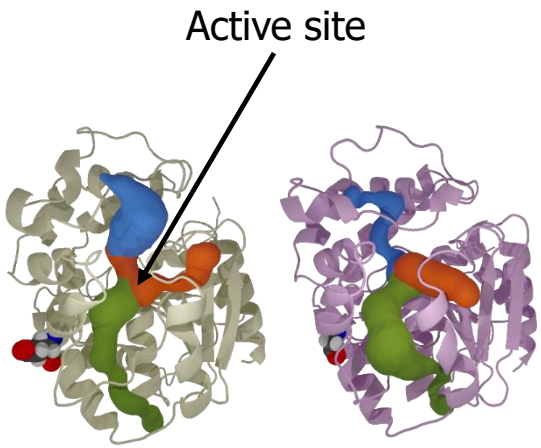
Sequeiros-Borja et al., in preparation

Gain-of-function mutations in water pathways

- **human epoxide hydrolase pathology:** mutation E470G remote from active site => increased catalytic activity; associated with higher stroke incidence
- simulations showed visible differences in the three narrow tunnels
- facilitates water transport => significantly increasing availability of water co-substrates for catalytic action of the enzyme







=> Novel indicator of mutation hot-spots

Sequeiros-Borja et al., in preparation

Ligand-transport pathways – pathology

- **Only rarely considered when interpreting molecular bases of diseases**
  - tunnels in soluble proteins have been accepted as functionally important just recently
  - hindered by the lack of information on the presence of transient tunnels and potential ones with high propensity for opening

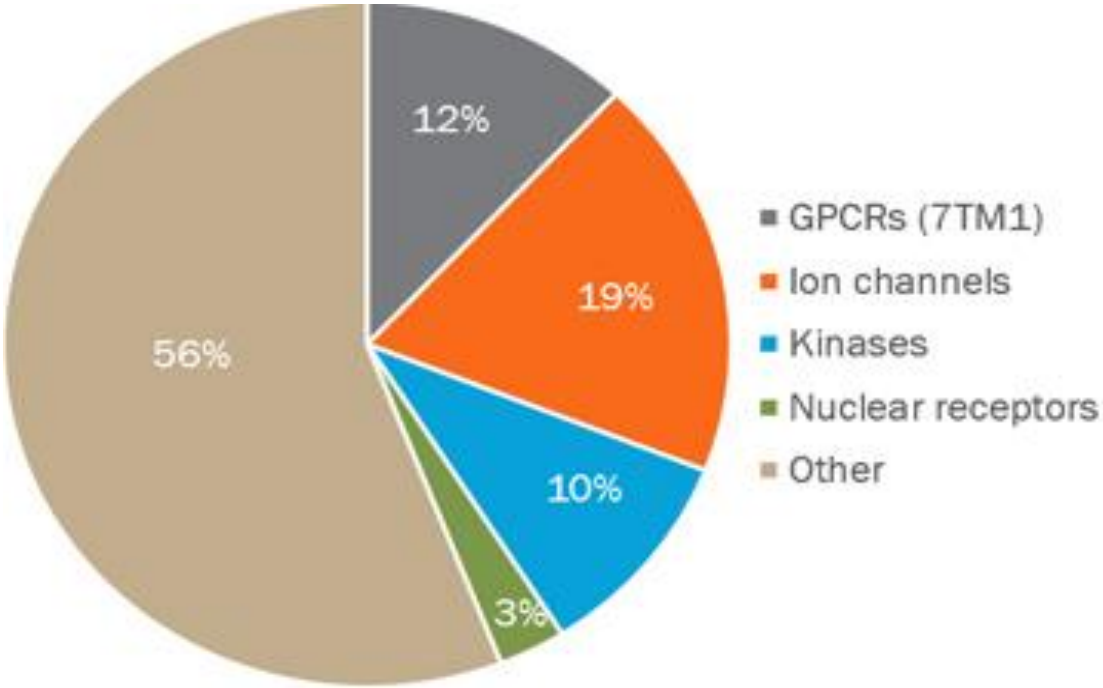
Marques et al. 2016, Med. Res. Rev. 37: 1095-1139

Pathologies linked to ligand-transport pathways

Protein	Disease/pathology
Dihydroorotate dehydrogenase	autoimmune and parasitic diseases, immunosuppression, cancer, inflammation
Nitric oxide synthase	neurological diseases, inflammation, rheumatoid arthritis, immune-type diabetes, stroke, cancer, thrombosis, infection susceptibilities
Glycogen phosphorylase	diabetes
Leukotriene-A4 hydrolase	inflammatory diseases
Neurolysin	nervous and endocrine systems disorders
Plasma cholesteryl ester transfer protein CETP	atherosclerosis
β-hydroxyacyl-acyl carrier protein dehydratase FabZ	gastric diseases
voltage-gated Na, K, Ca channels	periodic paralyses, mixed arrhythmias, dilated cardiomyopathy, neuronal hyperexcitability, …

Ligand-transport pathways – drug discovery

- **Tunnels promising targets in drug discovery**
  - new functional locations to target
  - selective drugs due to relatively lower evolutionary conservation



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Santos et al. 2017, Nat. Rev. Drug Discov. 16: 19-34

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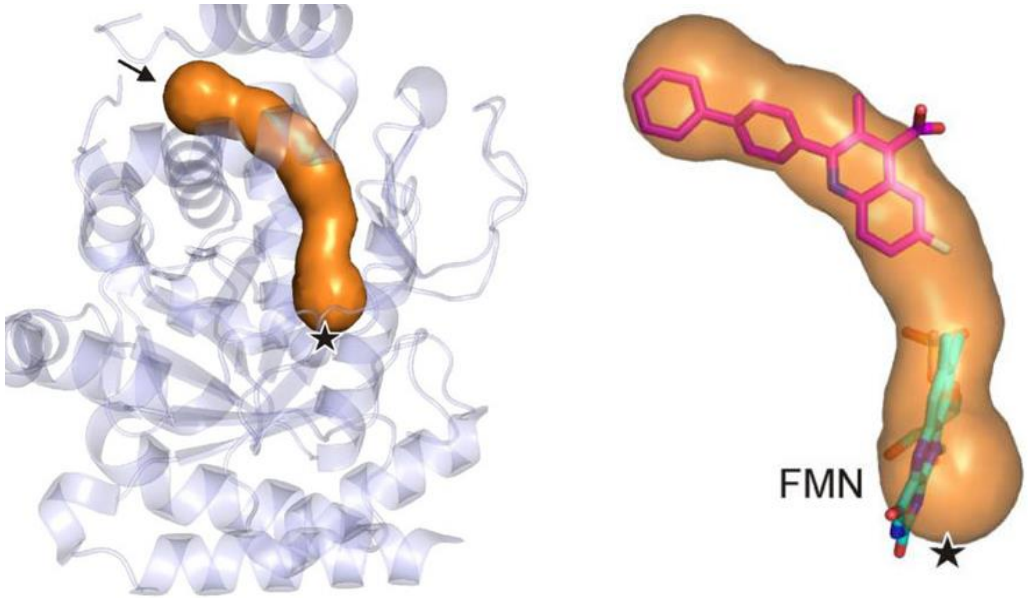

Dihydroorotate dehydrogenase inhibited by *brequinar* analog. While the active sites of the these enzymes in human and pathogenic species (e.g. *Plasmodium falciparum* or *Helicobacter pylori*) closely resemble one another, their access tunnels differ markedly.

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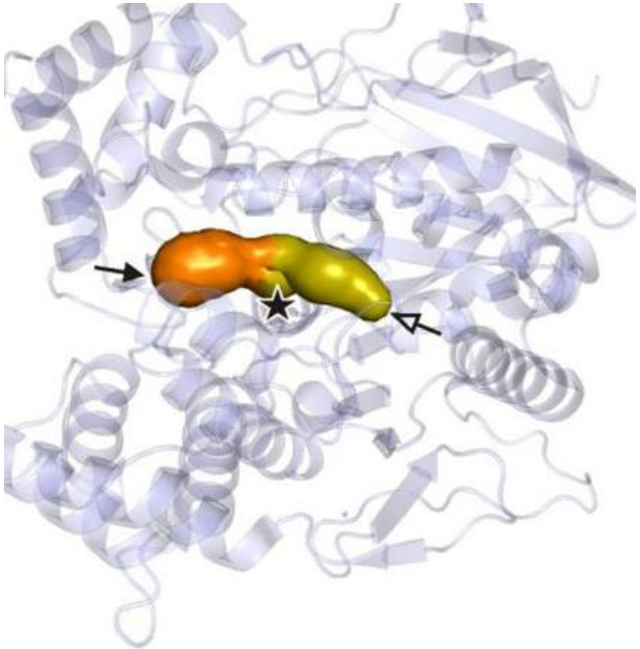

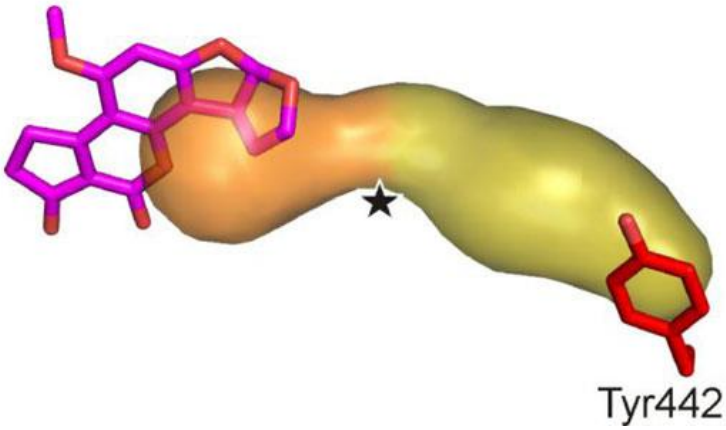

Aflatoxin blocking the main tunnel of Acetylcholinesterase, the secondary transient tunnel gated by Tyr442 still enables residual activity.

Marques et al. 2016, Med. Res. Rev. 37: 1095-1139

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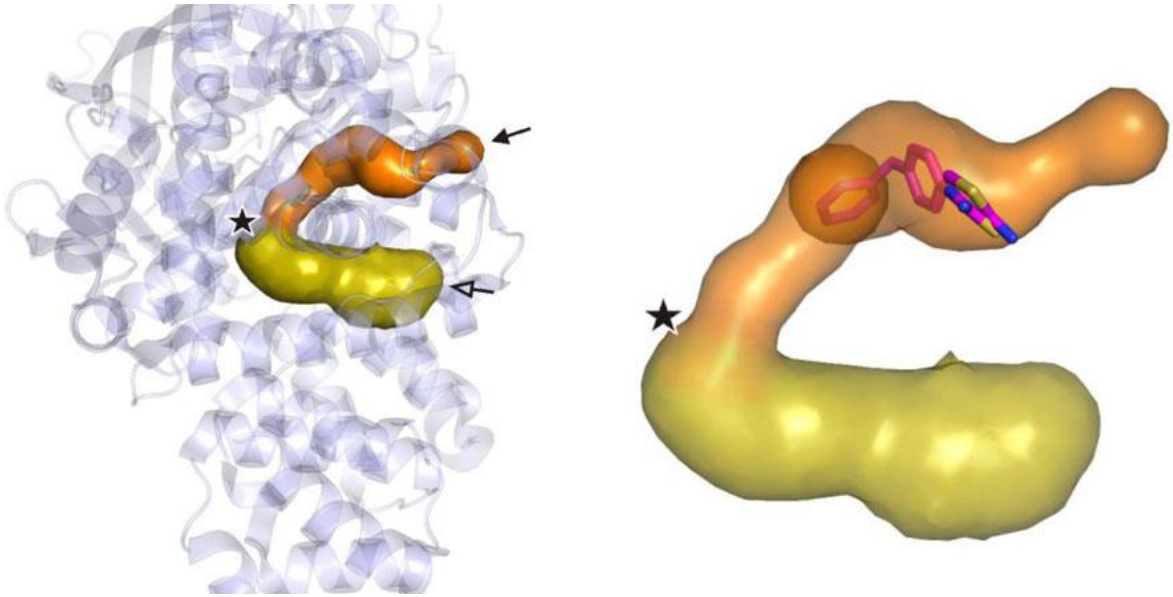
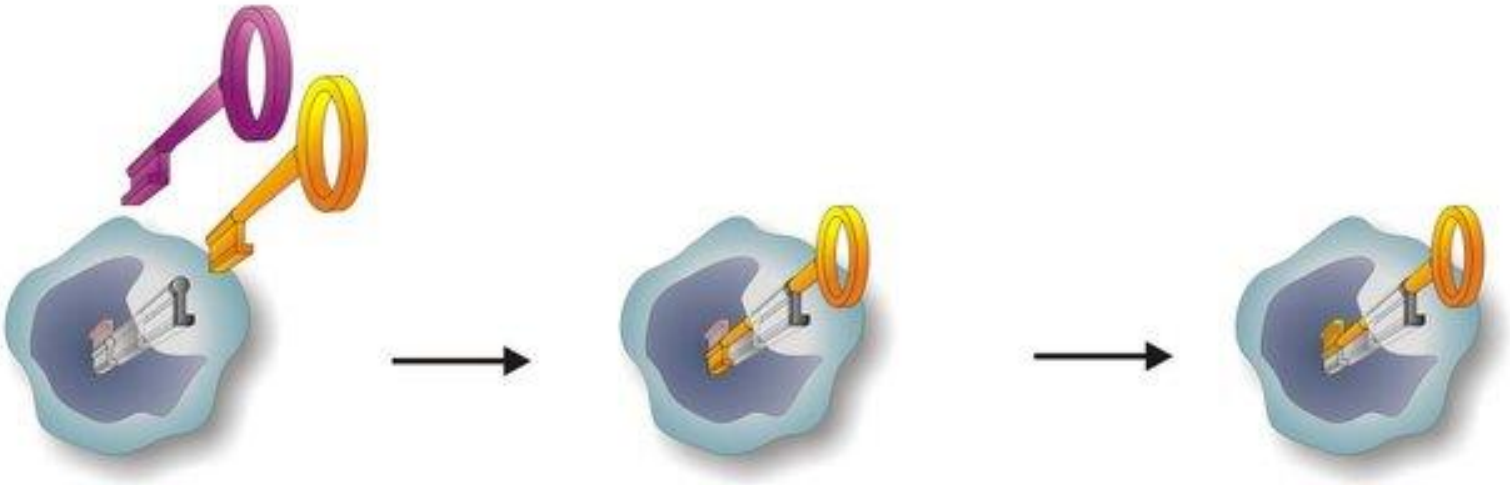


ARM1 inhibitor blocking the tunnel used for the access of LTA4 substrate to Leukotriene A4 hydrolase/aminopeptidase, while leaving the transport path for PGP unaffected.

Marques et al. 2016, Med. Res. Rev. 37: 1095-1139

Conclusions

- **Ligand-transport pathways are of functional importance not only in transmembrane proteins but also in a wide range of soluble proteins**



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- **Pathways represents interesting targets for drug discovery**