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UNIVERSITAS MASARYKIANA BRUNENSIS

Structural Bioinformatics and Molecular Modeling

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EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
INVESTING IN YOUR FUTURE

2007–13
OP Research and Development for Innovation

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Structural bioinformatics

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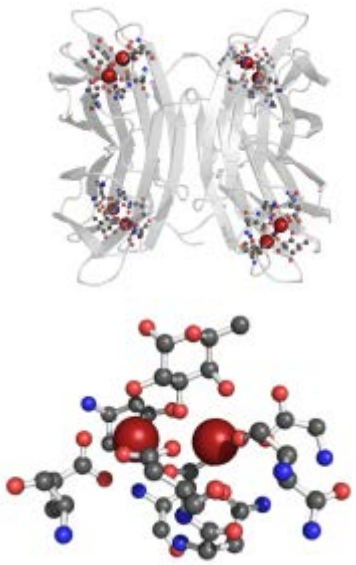
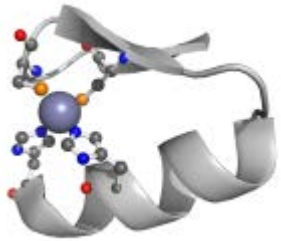
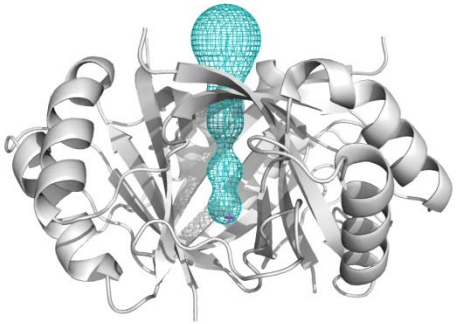
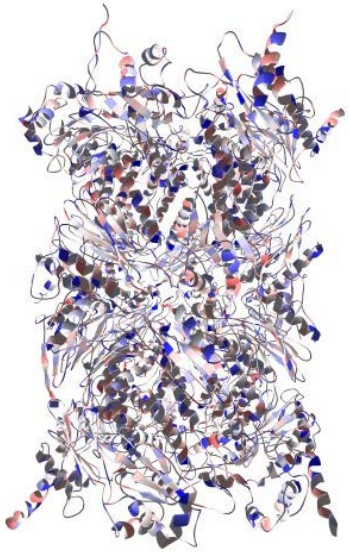
Mission

Input: **Information about biomacromolecular structure**









Outputs:

- Understanding of biomacromolecular function
- Prediction of structural change influence
- Classification of biomacromolecules
- Understanding of relations between biomacromolecules

Model of a molecule in computer

Atoms:

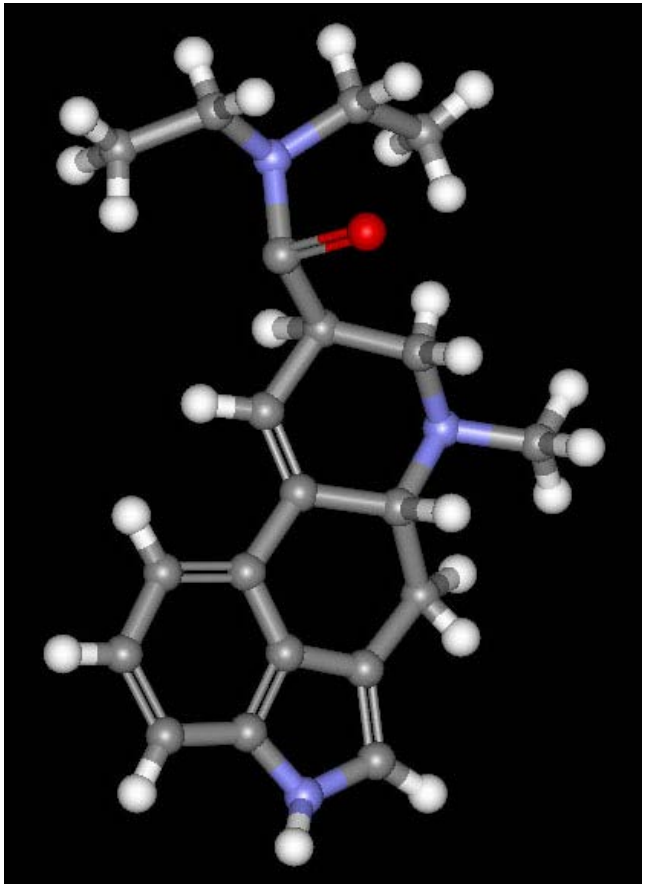
Points in a 3D space

Information about a chemical element

Bonds:

Information about two bound atoms

Bond order (single, double, triple, …)



Molecule in a computer – MOL format

number of atoms

number of bonds

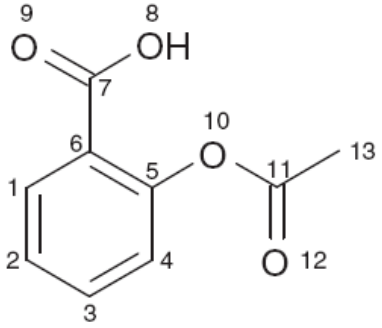
the first atom is a carbon

```
  -ISIS-  09270222202D

 13 13  0  0  0  0  0  0  0  0999 V2000
   -3.4639   -1.5375    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
   -3.4651   -2.3648    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
   -2.7503   -2.7777    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
   -2.0338   -2.3644    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
   -2.0367   -1.5338    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
   -2.7521   -1.1247    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
   -2.7545   -0.2997    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
   -2.0413    0.1149    0.0000 O   0  0  0  0  0  0  0  0  0  0  0  0
   -3.4702    0.1107    0.0000 O   0  0  0  0  0  0  0  0  0  0  0  0
   -1.3238   -1.1186    0.0000 O   0  0  0  0  0  0  0  0  0  0  0  0
   -0.6125   -1.5292    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
   -0.6167   -2.3542    0.0000 O   0  0  0  0  0  0  0  0  0  0  0  0
    0.1000   -1.1125    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
  1  2  2  0  0  0  0
  6  7  1  0  0  0  0
  3  4  2  0  0  0  0
  7  8  1  0  0  0  0
  7  9  2  0  0  0  0
  4  5  1  0  0  0  0
  5 10  1  0  0  0  0
  2  3  1  0  0  0  0
 10 11  1  0  0  0  0
  5  6  2  0  0  0  0
 11 12  2  0  0  0  0
  6  1  1  0  0  0  0
 11 13  1  0  0  0  0
M END
```

the first three numbers are the *x*, *y* and *z* coordinates of the atom

the first bond is between atoms 1 and 2 and has order 2



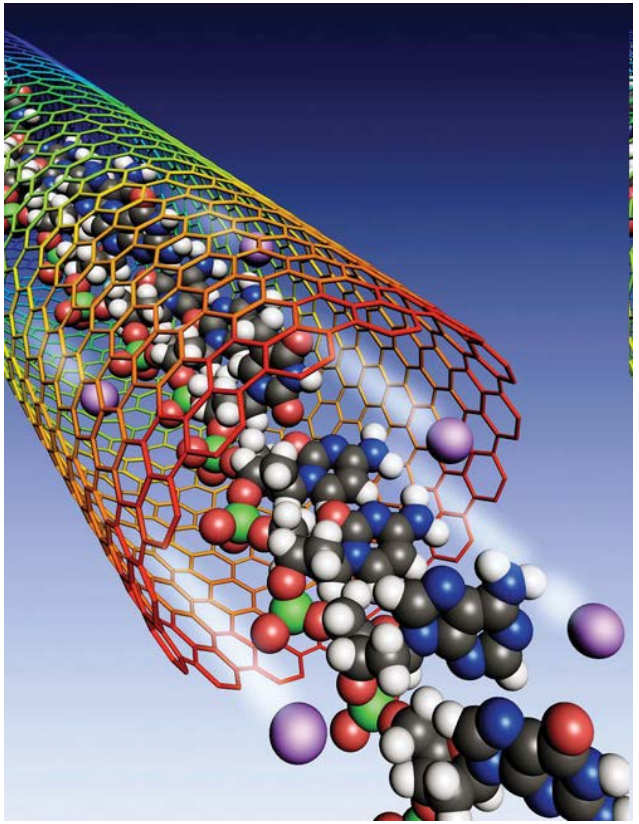
Current databases of bio(macro)molecules

„**Information boom**“ in the field of bio(macro)molecular structures

Why?: High performance techniques of structural analysis were developed.

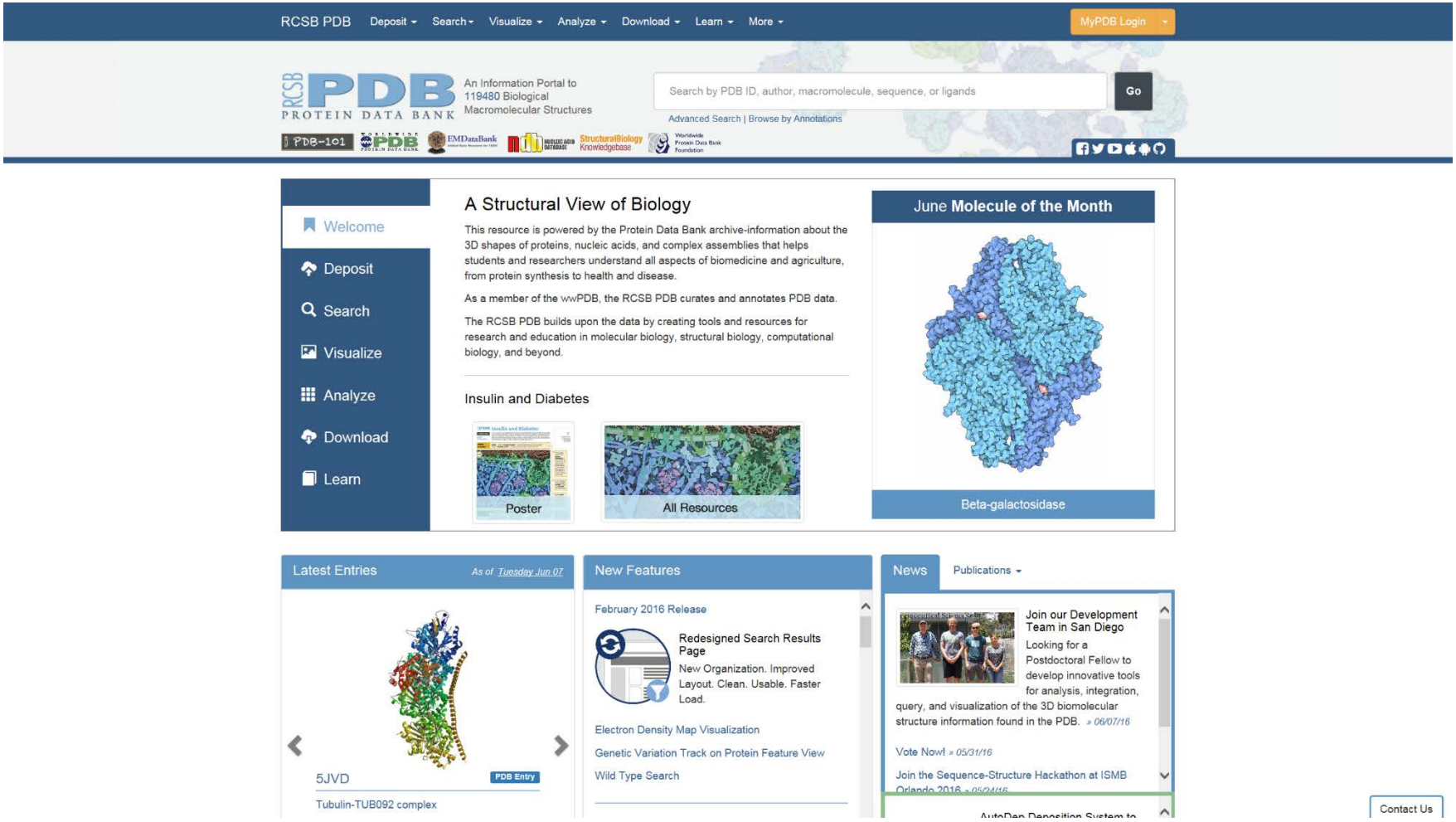
Results:

- We are able to get information about a genome of a person in a week and for low price
- More than 120 000 structures of proteins and nucleic acids are available in Protein Data Bank
- Millions of structures of small chemical compounds (drug-like molecules, ligands…) are stored in Pubchem, Zinc, Drugbank and other database

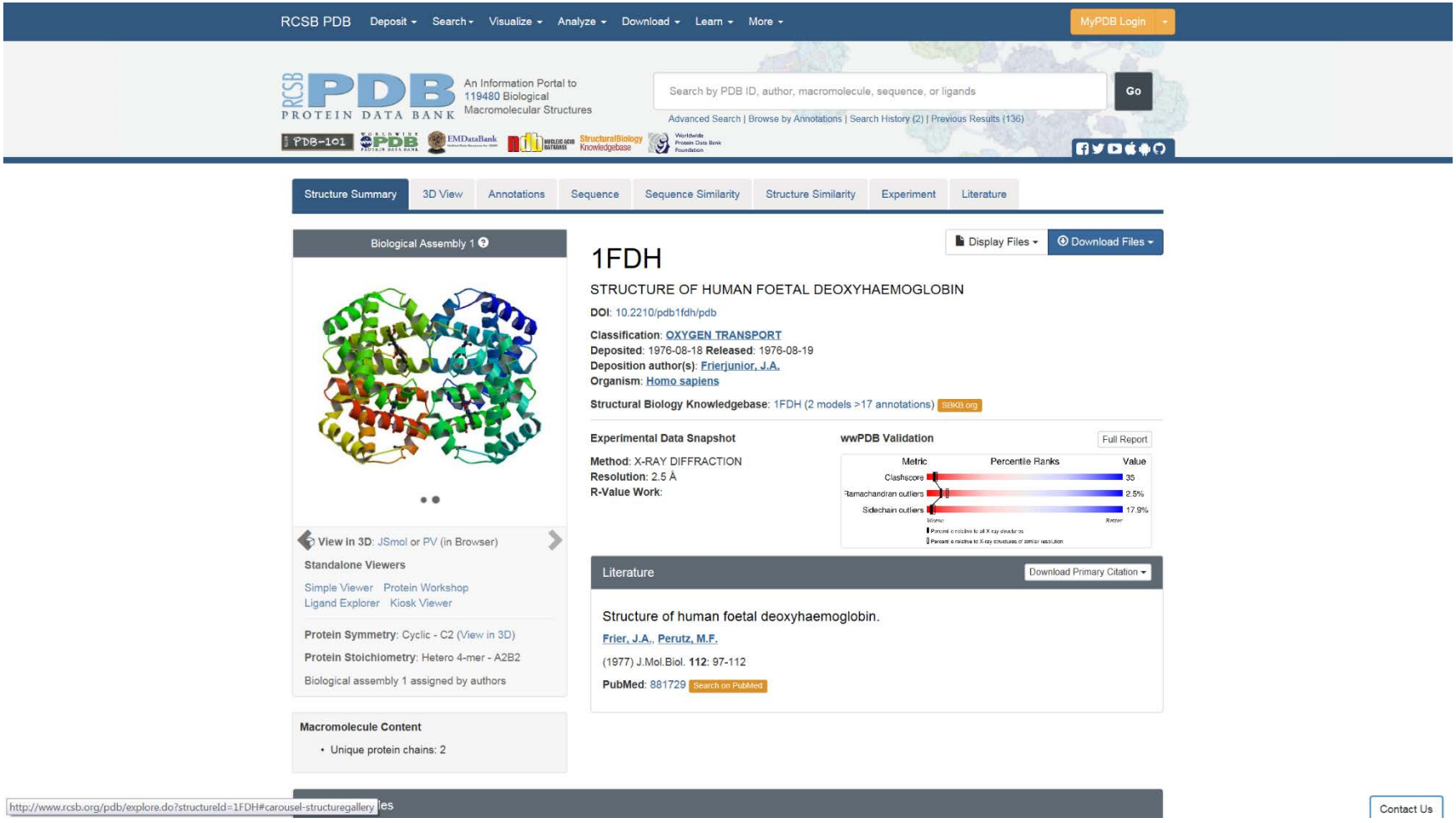


Most of this information is publicly and freely accessible:-)

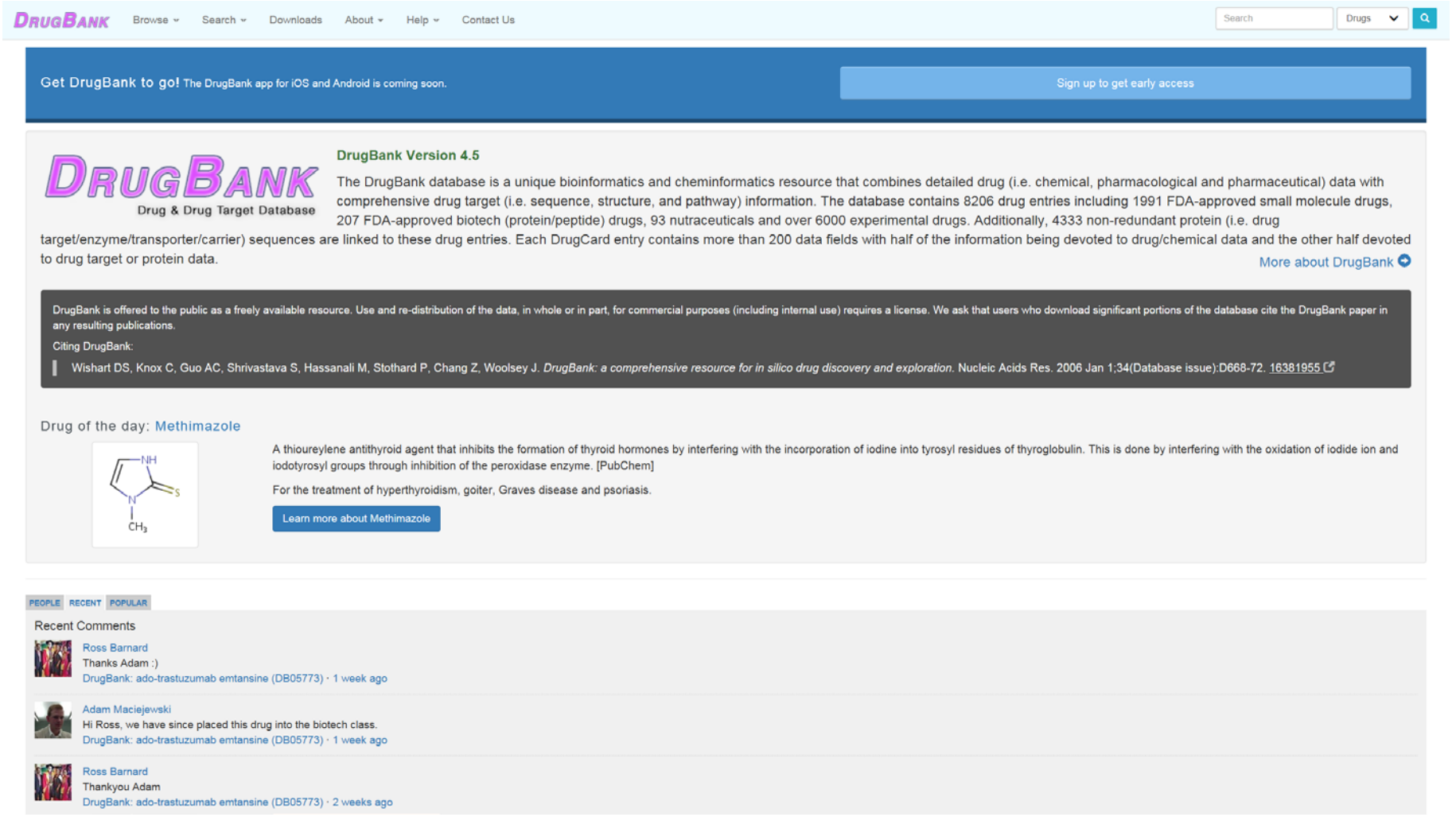
Protein Data Bank
– example of biomacromolecular structure DB



Protein Data Bank
– example of biomacromolecular structure DB II



DrugBank
– example of drug DB



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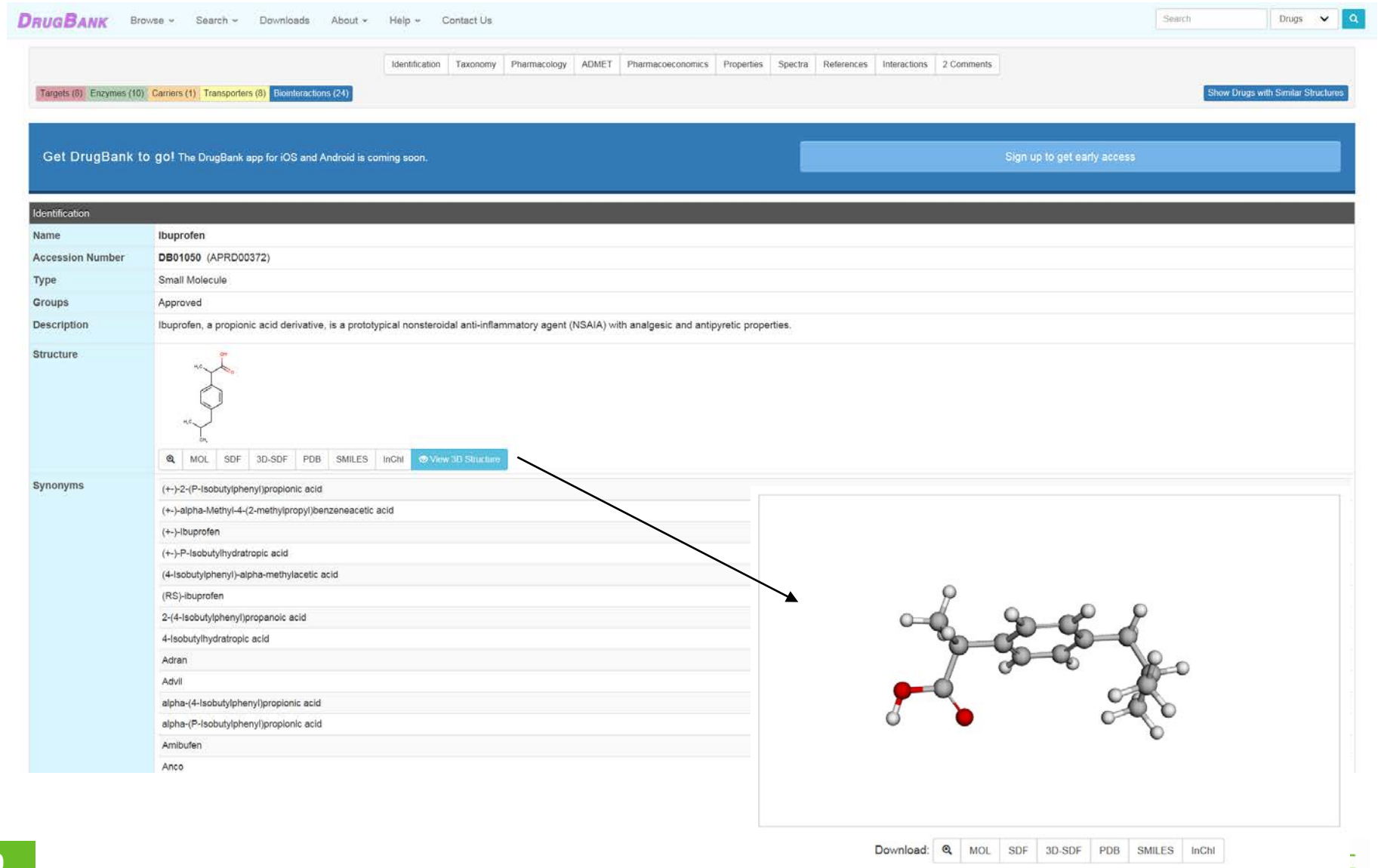
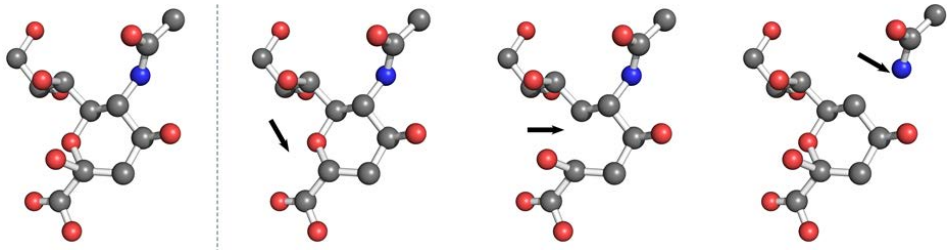
DrugBank
– example of drug DB II

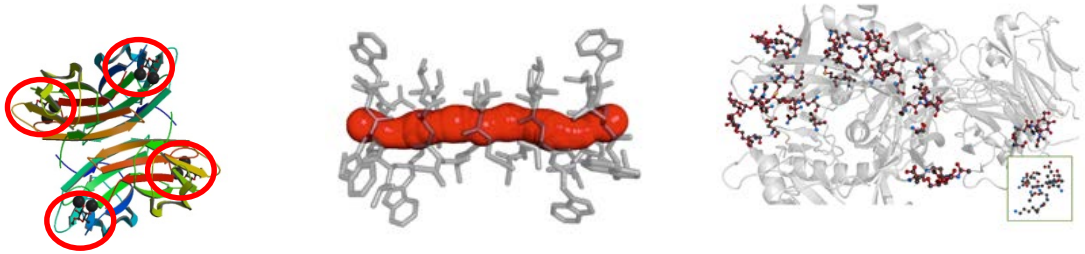


Methodologies of structural bioinformatics
- processing and analysis of the structures

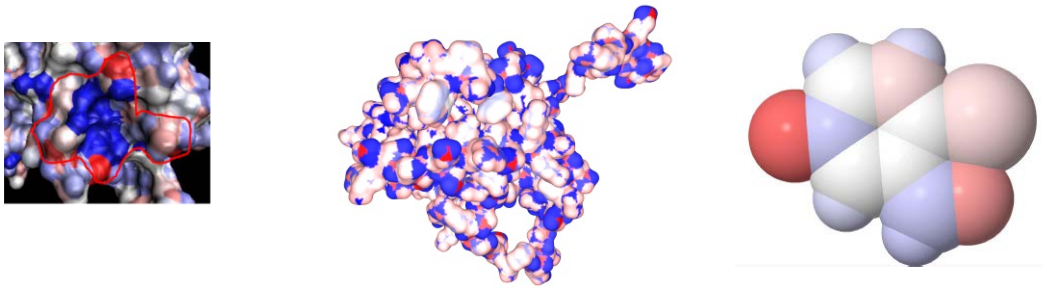
Validation



Detection of biologically important parts

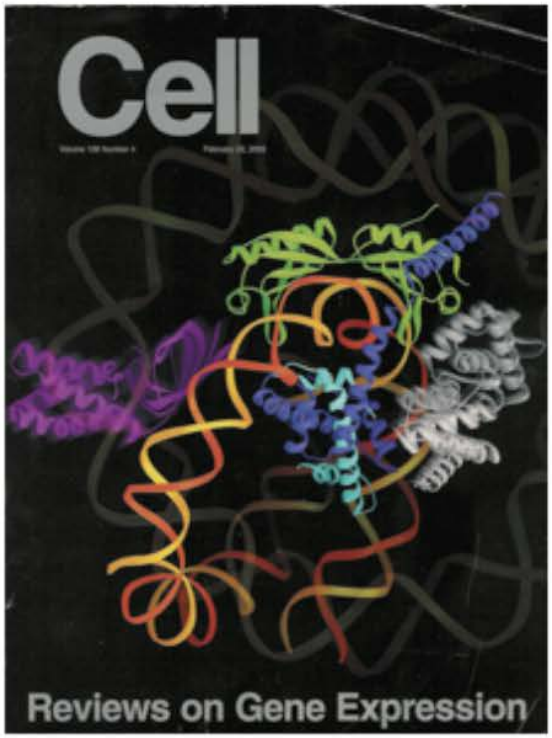


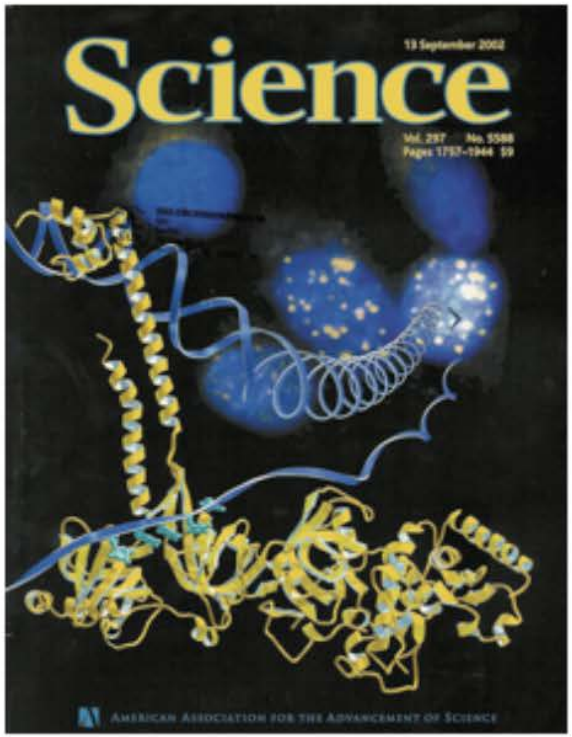
Characterization



Validation: Why to validate?

- High-throughput experimental techniques produce a large amount of data on the 3D structure of proteins and their complexes
- This allows us to produce impressive research results





Validation: Why to validate?

- But are our results correct?
- **Structural biology community found that some published structures contained serious errors**
- For this reason, validation of biomolecular structures arose as a major issue

Retraction

WE WISH TO RETRACT OUR RESEARCH ARTICLE "STRUCTURE OF MsbA from *E. coli*: A homolog of the multidrug resistance ATP binding cassette (ABC) transporters" and both of our Reports "Structure of the ABC transporter MsbA in complex with ADP·vanadate and lipopolysaccharide" and "X-ray structure of the EmrE multidrug transporter in complex with a substrate" (*1*–*3*).

The recently reported structure of Sav1866 (*4*) indicated that our MsbA structures (*1*, *2*, *5*) were incorrect in both the hand of the structure and the topology. Thus, our biological interpretations based on these inverted models for MsbA are invalid.

An in-house data reduction program introduced a change in sign for anomalous differences. This program, which was not part of a conventional data processing package, converted the anomalous pairs (I+ and I–) to (F– and F+), thereby introducing a sign change. As the diffraction data collected for each set of MsbA crystals and for the EmrE crystals were processed with the same program, the structures reported in (*1*–*3*, *5*, *6*) had the wrong hand.

The error in the topology of the original MsbA structure was a consequence of the low resolution of the data as well as breaks in the electron density for the connecting loop regions. Unfortunately, the use of the multicopy refinement procedure still allowed us to obtain reasonable refinement values for the wrong structures.

The Protein Data Bank (PDB) files 1JSQ, 1PF4, and 1Z2R for MsbA and 1S7B and 2F2M for EmrE have been moved to the archive of obsolete PDB entries. The MsbA and EmrE structures will be recalculated from the original data using the proper sign for the anomalous differences, and the new Cα coordinates and structure factors will be deposited.

We very sincerely regret the confusion that these papers have caused and, in particular, subsequent research efforts that were unproductive as a result of our original findings.

GEOFFREY CHANG, CHRISTOPHER B. ROTH, CHRISTOPHER L. REYES, OWEN PORNILLOS, YEN-JU CHEN, ANDY P. CHEN

Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037, USA.

References

1. G. Chang, C. B. Roth, *Science* **293**, 1793 (2001).
2. C. L. Reyes, G. Chang, *Science* **308**, 1028 (2005).
3. O. Pornillos, Y.-J. Chen, A. P. Chen, G. Chang, *Science* **310**, 1950 (2005).
4. R. J. Dawson, K. P. Locher, *Nature* **443**, 180 (2006).
5. G. Chang, *J. Mol. Biol.* **330**, 419 (2003).
6. C. Ma, G. Chang, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 2852 (2004).

Validation: Validation of biomacromolecules

Various tools for the validation of the protein and nucleic acid 3D structures are well established:

WHAT_CHECK, PROCHECK, MolProbity, OOPS, Mogul, Coot, PHENIX

They are focused on checking of structure and geometry properties:

- Electron density
- Atom clashes
- Bond length
- Bond angles
- Chirality and planarity

Validation: Ligand validation

Very important:

- Ligands play a key role in a function of biomacromolecules
- Ligands are the main source of errors in structures

Challenging:

- High diversity and nontriviality of their structure
- Lack of information about correct structures

Validation against tabular values of properties:

- Compares geometrical properties of molecules with tabular value
- Example of errors: Atom clashes, bong length errors,
- Tools: ValLigURL, Mogul, Coot, PHENIX

Validation against a template molecule:

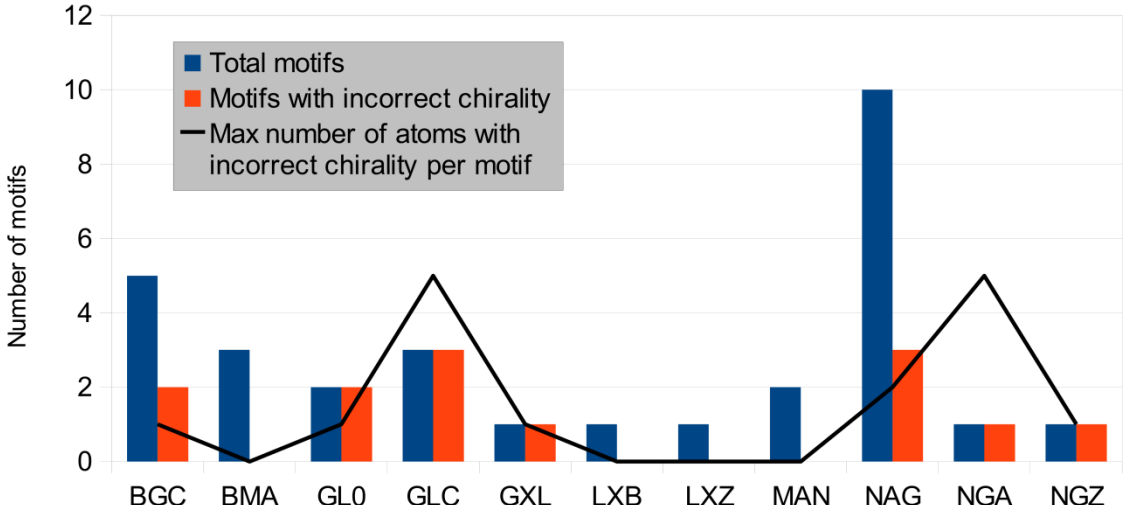
- Compares a validated molecule with a correct (template) molecule
- Example of errors: Missing atoms, wrong chirality, atom substiotutions
- Tools: PDB care, MotiveValidator, ValidatorDB, PDB validation reports

Validation: Example - PDB entry validation
Validation of 3D12

Nipah G attachment glycoprotein
Contains 30 instances of 11 different carbohydrates, each with one ring and five chiral atoms.

Results:
- 13 of these ligands have incorrect chirality
- In a few cases, all chiral atoms exhibit incorrect chirality

Not very good ☹



http://ncbr.muni.cz/MotiveValidator http://ncbr.muni.cz/ValidatorDb

Validation: Exercise

Validation of Nipah G attachment glycoprotein (3D12):

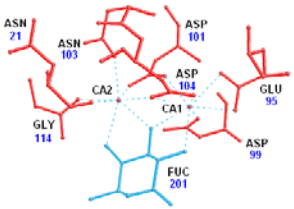
- See validation results for 3D12 in ValidatorDB:
  Open ValidatorDB (http://ncbr.muni.cz/ValidatorDB), use:
  Search -> PDB Entry -> 3D12 -> Quick Search. Browse the bookmarks "Overview", "Summary" and "Details" to get all the required information.

- See PDB validation report for 3D12:
  Go to Protein Data Bank Europe (http://www.ebi.ac.uk/pdbe/node/1), use:
  Search 3D12, Download files, Validation, Full report (PDF)

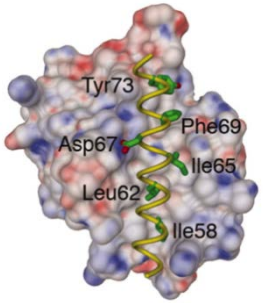
Detection: Which biomacromolecular parts can we detect?

Fragments:

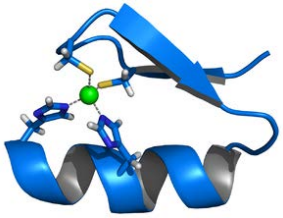
- Binding sites



- Elements of secondary structure

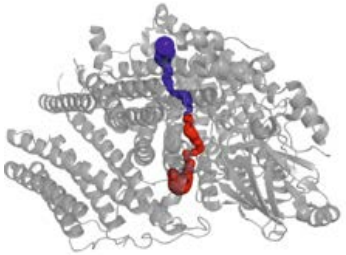


- Supersecondary motifs

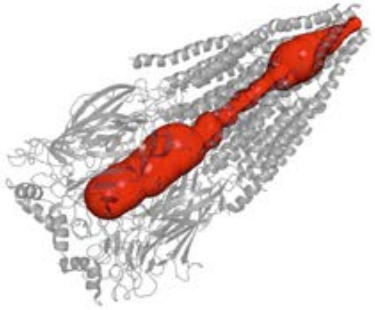


Channels:

- Pathways from surface to a binding site



- Pores



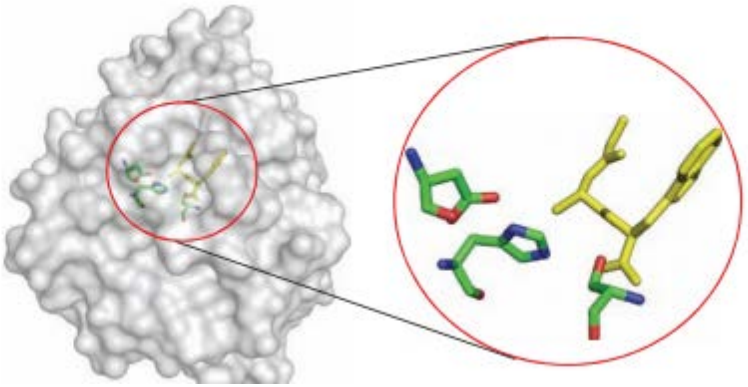
Detection: Why to detect biomacromolecular parts?

Fragments:

- Patterns for drug design
- Comparison of biomacromolecules
- Understanding / discovery of biomaromolecule function

Channels:

- Key objects for biomacromolecule function
- Influence the binding site selectivity (only some substrate can path through)



Detection: How to detect biomacromolecular fragments?

- **Methodology**:
  - Describe a fragment via a defined expression (query)
  - Find all suitable fragments
- **Tools:** PatternQuery, RASMOT-3D PRO, Promotif, Prosite, IMAAAGine, PDBeMotif, SPRITE& ASSAM, 3Dfit, SPASM, Protein segment finder

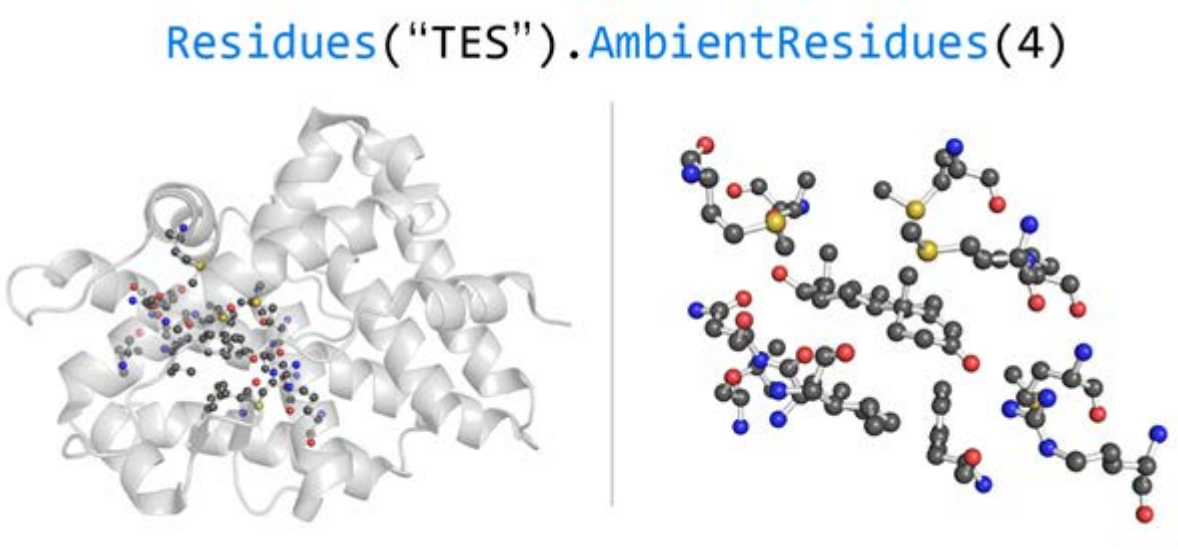
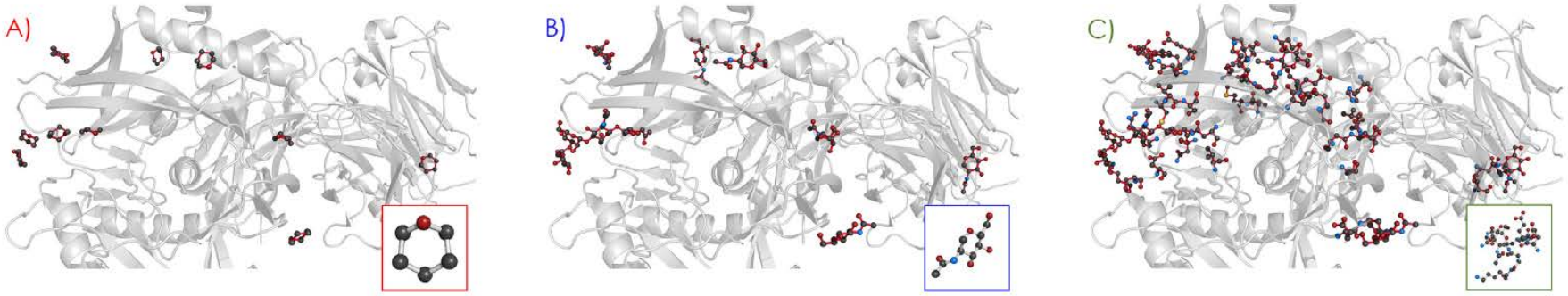


Figure: Detection of testosterone (TES) and its 4 Å large surrounding via PatternQuery: A query and a picture of the detected fragment.

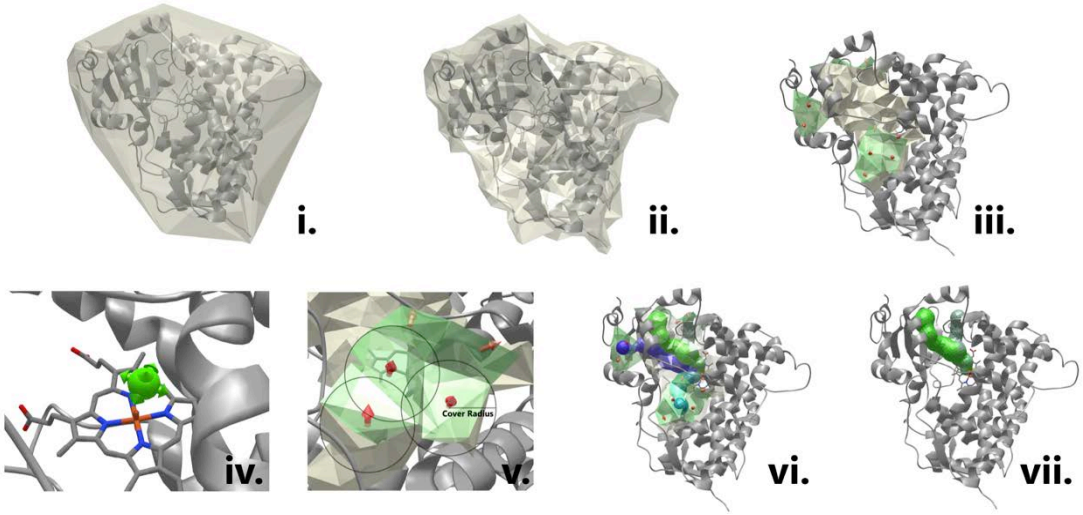
Detection: Example – binding pocket detection
Detection of fragments within 3U7Y

- **Glycoprotein gp160** from Human immunodeficiency virus 1 in complex with Homo sapiens immunoglobulins (PDB ID: 3U7Y).
- **Goal:** Detect a binding pocket of any residue containing a pyranose
- **Results** - via PatternQuery (http://ncbr.muni.cz/PatternQuery):



A) First, the query identifies a pyranose moiety (a ring composed of 5 carbons and an oxygen atom). B) Then, all residues which include this pattern in their structure are identified. C) Finally, all the residues that are at most 4Å from any of the pyranose containing residues are detected as well.

Detection: How to detect biomacromolecular channels?



- **Methodology:**
  - Delaunay triangulation/Voronoi diagram (i.)
  - Approximating the molecular surface (ii.) and identifying cavities (iii.)
  - Identifying possible start (iv.) and end (v.) points of channels
  - Computing channels via Dijkstra's algorithm (vi.)
  - Filtering of channels – removing too similar channels (vii.)
- **Tools:** MOLE, Caver, MolAxis

Detection: Example – channels detection
Detection of channels within 1TQN

- **Microsomal cytochrome P450 3A4** (PDB ID: 1TQN).
- **Goal:** Detect channels from a buried active site (in Glu 308 and Thr 309 residues, according to Catalytic Site Atlas) to a surface of the cytochrome
- **Results** - via MOLEonline (http://ncbr.muni.cz/mole) :

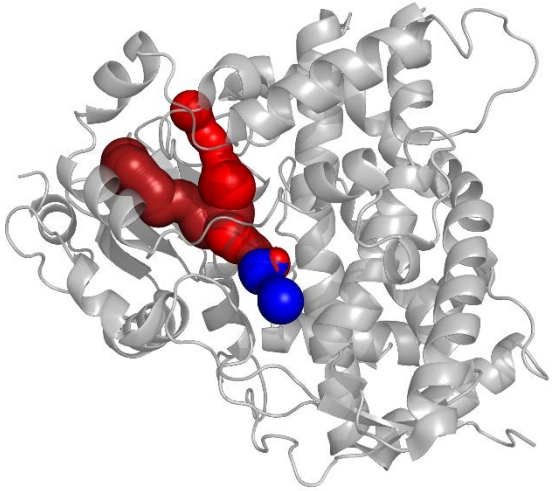


Figure: Results of channel analysis of Cytochrome P450 3A4. Three channels found from user-specified starting point (i.e., Glu 308 and Thr 309) are shown – the solvent channel (in blue), the channel 2a (in dark red) and the channel 2e (in light red).

Detection: Exercise

Exercise 1 - Fragment detection:

- Detect a binding pocket of any residue containing a pyranose within glycoprotein gp160 (3U7Y).

  Open PatternQuery (http://ncbr.muni.cz/PatternQuery), do:

  Query Protein Data Bank

  Queries:

  Unique Name ...: Test111

  Rings(5*['C']+['O']).ConnectedResidues(0).AmbientResidues(4)

  Add+

  PDB ID List: 3U7Y

  Submit

  Details: Test111 10/1

Exercise 2 – Channel detection:

- Detect channels from a buried active site (in Glu 308 and Thr 309 residues, according to Catalytic Site Atlas) to a surface of the cytochrome P450 3A4 (1TQN).

  Open MOLEonline (http://ncbr.muni.cz/mole), do:

  Quick start -> 1TQN -> Next -> Starting point: A Glu 308, A thr 309 -> Submit

Characterization: Which characteristics can we calculate?

Geometrical properties:
- Biomacromolecular surface
- Channel length, channel volume, ….
- Binding site size
- ….

Biochemical composition:
- Channel lining residues
- Binding site residues composition

Physico-chemical properties:
- **Partial atomics charges**
- Hydrophobicity
- Partition cofficients
- …

We will now focus on them, because they are very useful
+
They present an illustrative example of structure characterization

Characterization: Why are partial atomic charges useful?

- **Real numbers describing a distribution of electron density within a molecule**
- **Provide clues to the chemical behaviour of molecules**
- **Applications:**
  - **Computational chemistry and molecular modeling:**
    - molecular dynamics
    - docking
    - conformational searches
    - binding site predictions
  - **Chemoinformatics:**
    - descriptors for QSAR and QSPR modelling
    - virtual screening
    - similarity searches
  - **Structural bioinformatics:**
    - study of mechanisms and effects connected with certain chemical action, e.g.:
      - an activation of some biomacromolecule
      - a binding of some ligand
    - predict influences of structural changes, e.g.:
      - an influence of a certain point mutation

Characterization: How to calculate partial atomic charges?

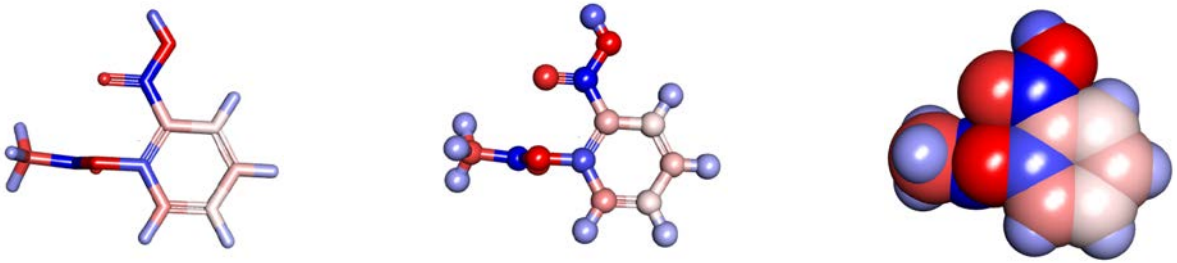
- **Methodology**:
  - Quantum mechanical (QM) charge calculations: Accurate, but very time demanding
  - Empirical charge calculations: Accuracy comparable to QM, markedly faster

- **Tools:**
  - **QM charges:** Gaussian
  - **Empirical charges:** AtomicChargeCalculator, OpenBabel, NEEMP, EEM_Solver

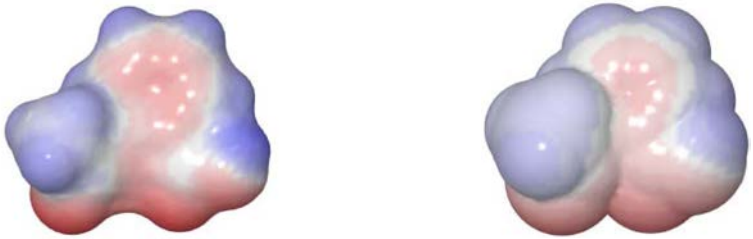
Characterization: How to visualize partial atomic charges?

Coloring of molecular structure models according charges:

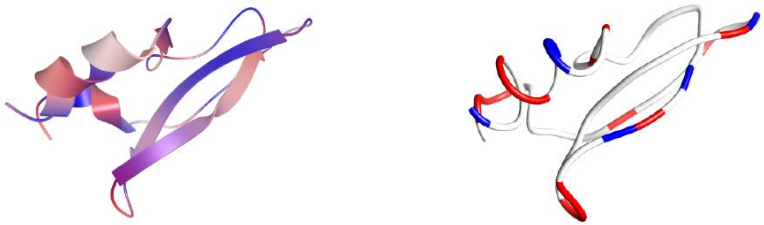
- Standard models (sticks, balls & sticks, CPK):



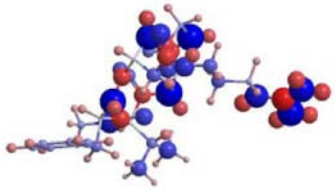
- Surface models (van der Waals surface, solvent accessible surface):



- Schematic models (cartoon, ribbon):



Size of atoms based on charges
(only for balls & stick models):



Characterization: Example – charge calculation in proteins
Calculation of charges in inactive and active BAX protein

- **Apoptotic protein BAX:** inactive BAX (PDB ID: 1F16), inactive BAX with inhibitor (PDB ID: 2LR1), active BAX with activator (PDB ID: 2K7W)
- **Goal:** Detect a binding pocket of any residue containing a pyranose
- **Results** - via AtomicChargeCalculator (http://ncbr.muni.cz/ACC):

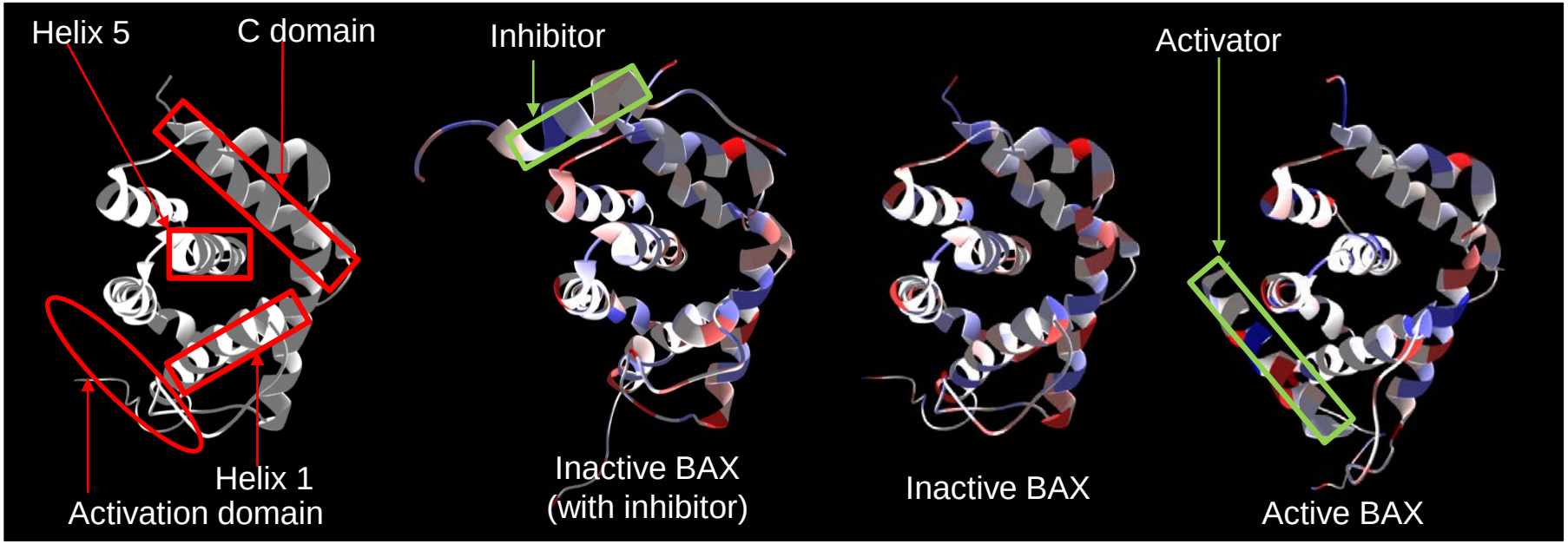


Figure:

- Inactive BAX with and without an inhibitor show very similar charge distribution
- Activation of BAX is performed via binding of a strongly charged activator
- Binding of this activator causes vanishing of charge in Helix 1, Helix 5 and C domain (they are white in the figure of activated BAX).
- It causes a release of C domain in activated BAX

Characterization: Exercise

Exercise 1 – Charge calculation in small organic molecule:

- Toluene is known to have more negative charges in the positions ortho- and para- (comparable to meta- position) and therefore it directs a follow-up substitution into these positions. Calculate charge distribution in toluene (it can be obtained from Pubchem, CID 1140) and check, if the results agree with this fact.

  Download toluene 3D structure (in SDF format) from Pubchem and save it to a file Structure3D_CID_1140.sdf.
  Open AtomicChargeCalculator (http://ncbr.muni.cz/ACC), do:
  Submit a Computation -> Select file: Structure3D_CID_1140.SDF -> Upload -> Compute -> Click on Structure3D_CID_1140 -> 3D model -> hover mouse on the atoms and you can see charge values.

Exercise 2 – Charge calculation in protein:

- Calculate charges for inactive BAX (PDB ID: 1F16), inactive BAX with inhibitor (PDB ID: 2LR1) and active BAX with activator (PDB ID: 2K7W). Study differences in charge distributions of these structures.

  Download PDB files for structures 1F16, 2LR1 and 2K7W from Protein Data Bank.
  Open AtomicChargeCalculator (http://ncbr.muni.cz/ACC) and do the same steps as in the previous exercise.

Further reading:

Koča J., Svobodová Vařeková R., Pravda L., Berka L., ...

Structural bioinformatics tools for drug design
Extraction of biologically relevant information from structural databases

Springer, should appear in the autumn of 2016

Thank you for your attention

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