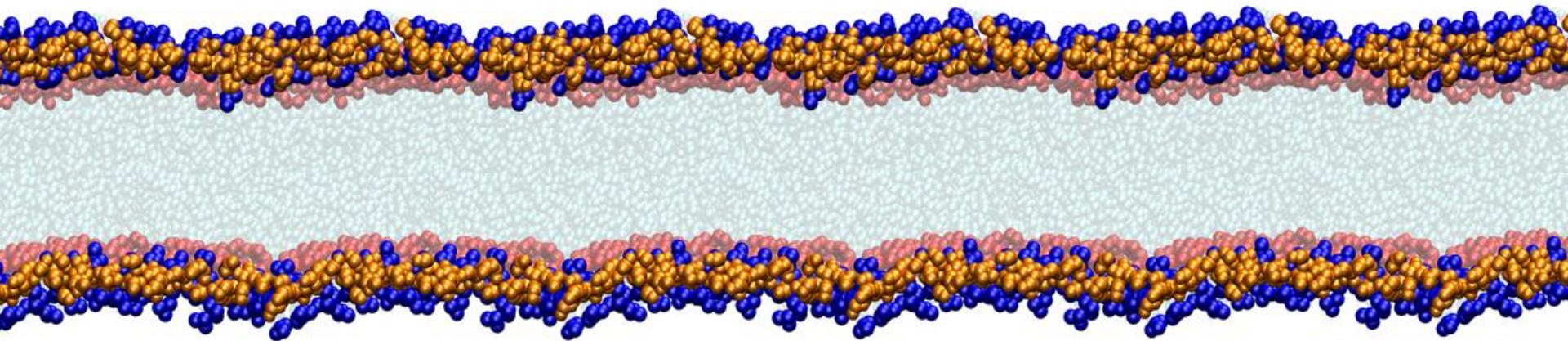


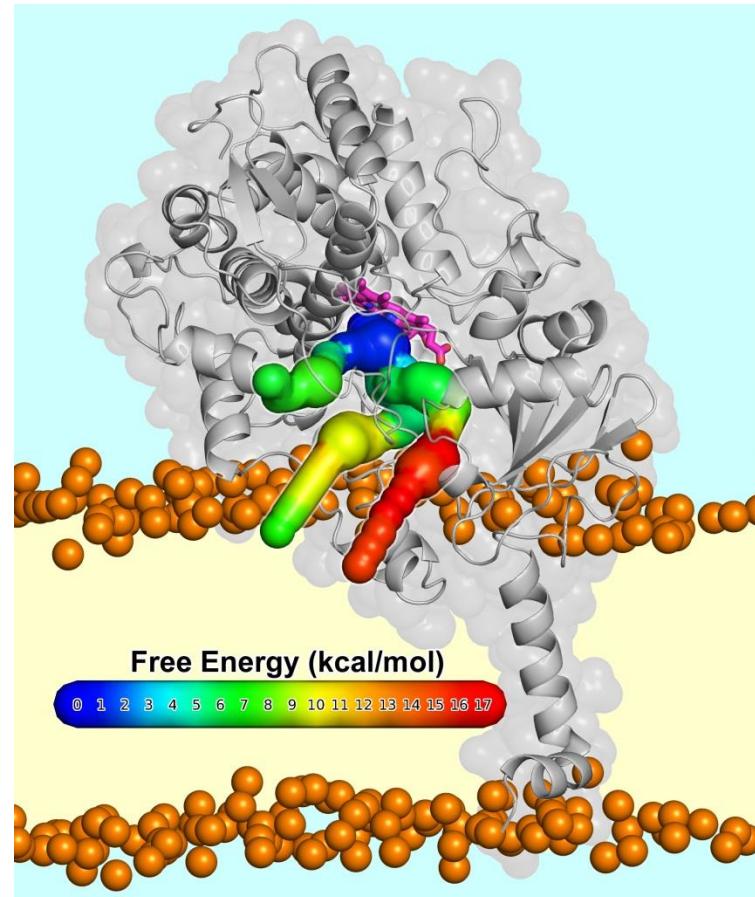
Role of Enzyme Flexibility in Ligand Access and Egress to Active Site



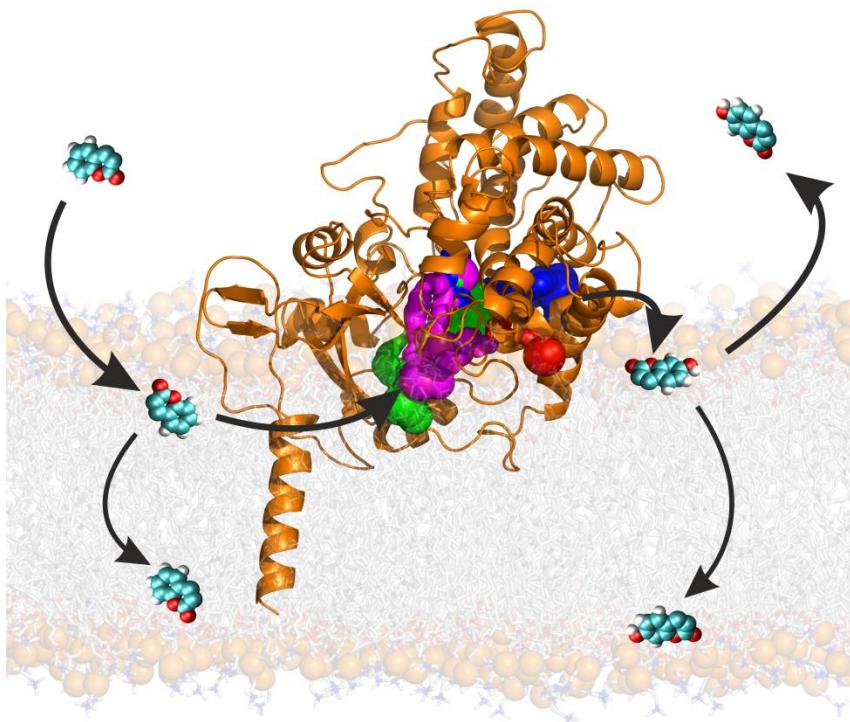
Markéta Paloncýová
Regional Center of Advanced Technologies and
Materials
Department of Physical Chemistry
Palacký University, Olomouc

Outline

- Cytochrome P450
- DMSDDrug, Spath
- Results
 - CYP3A4
+1,3,7-trimethyluric acid
 - CYP11A1 + cholesterol
 - CYP1A1
+dioxin-like compounds
- Issues of flexible enzymes



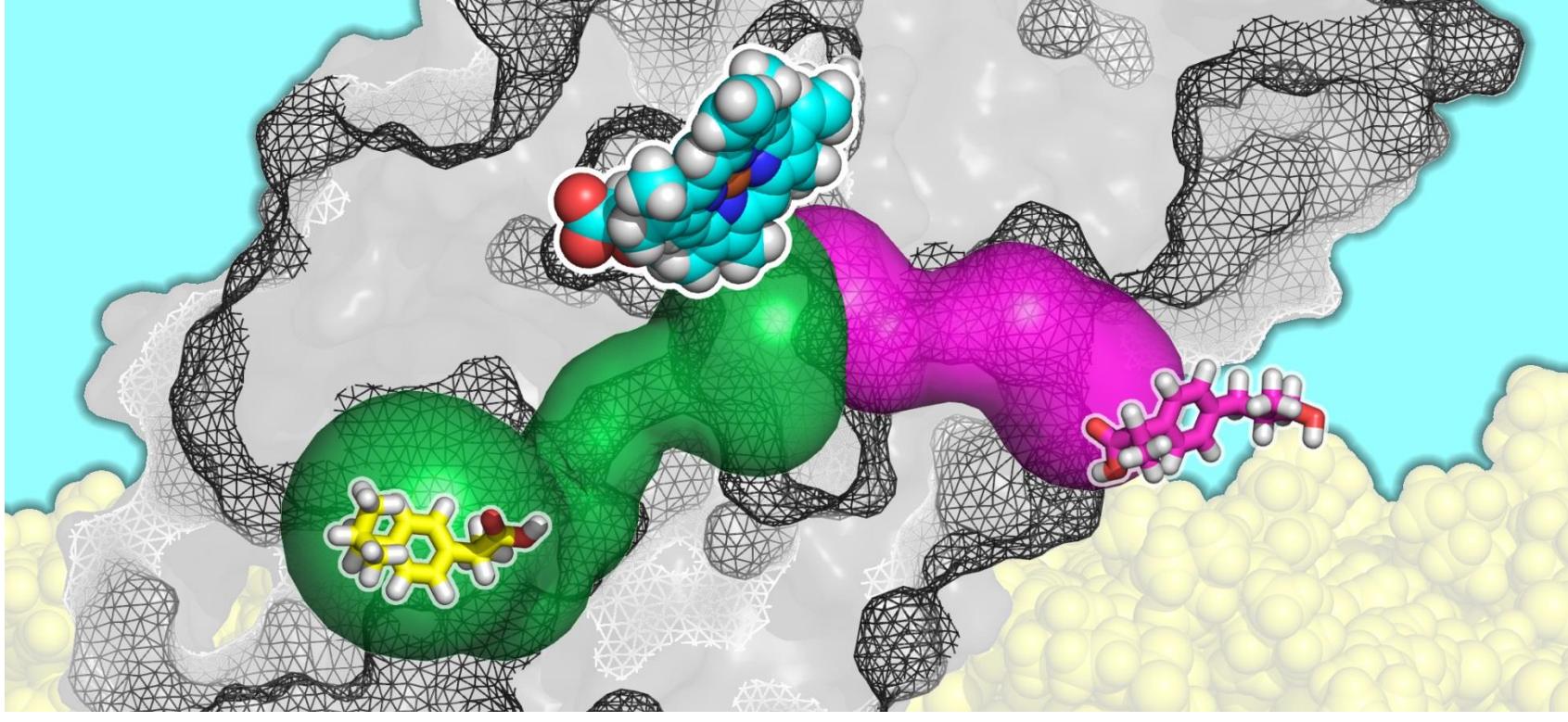
Cytochromes P450



- Drug metabolizing enzymes
 - 75 % commonly used drugs
- Embedded in the membrane
- Burried active site - HEME

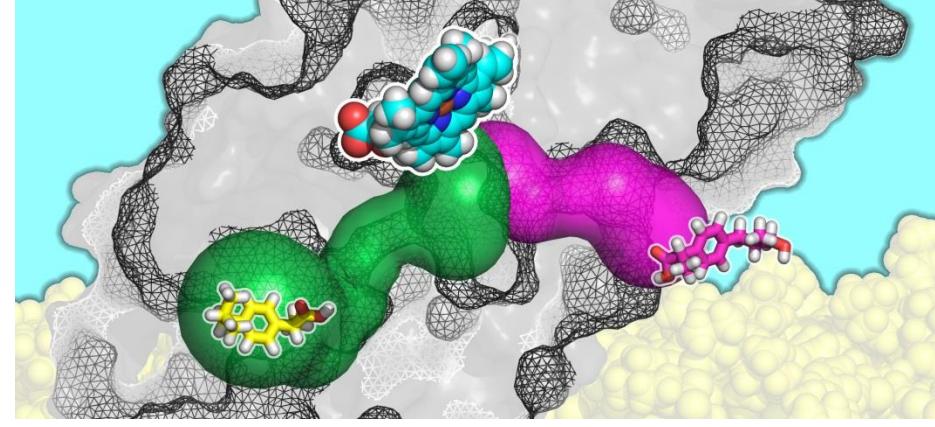
Cytochrome P450 Channels

- Active site
 - Connected with the surface by channels
- Channels
 - Uptake and release of substrates&metabolites



BE-META preparation

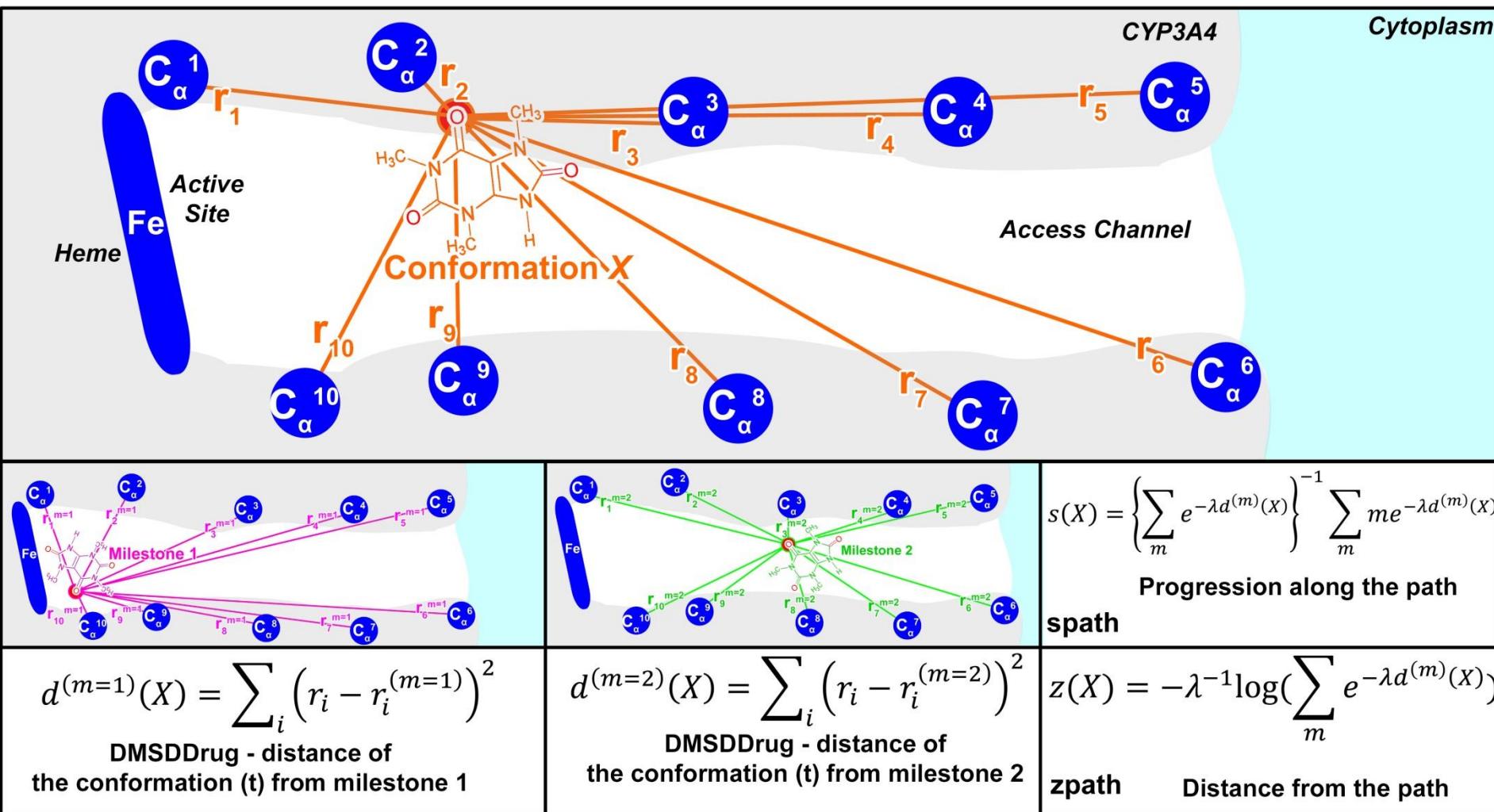
- Free simulation CYP3A4 on DOPC
- Channel analysis
 - MOLE 2.0
- Docking into channels
- Short free simulations
- Path choice



Berka, K.; Hanák, O.; Sehnal, D.; Banás, P.; Navrátilová, V.; Jaiswal, D.; Ionescu, C.-M.; Svobodová Vařeková, R.; Koca, J.; Otyepka, M. *Nucleic acids research* **2012**, *40*, W222–7

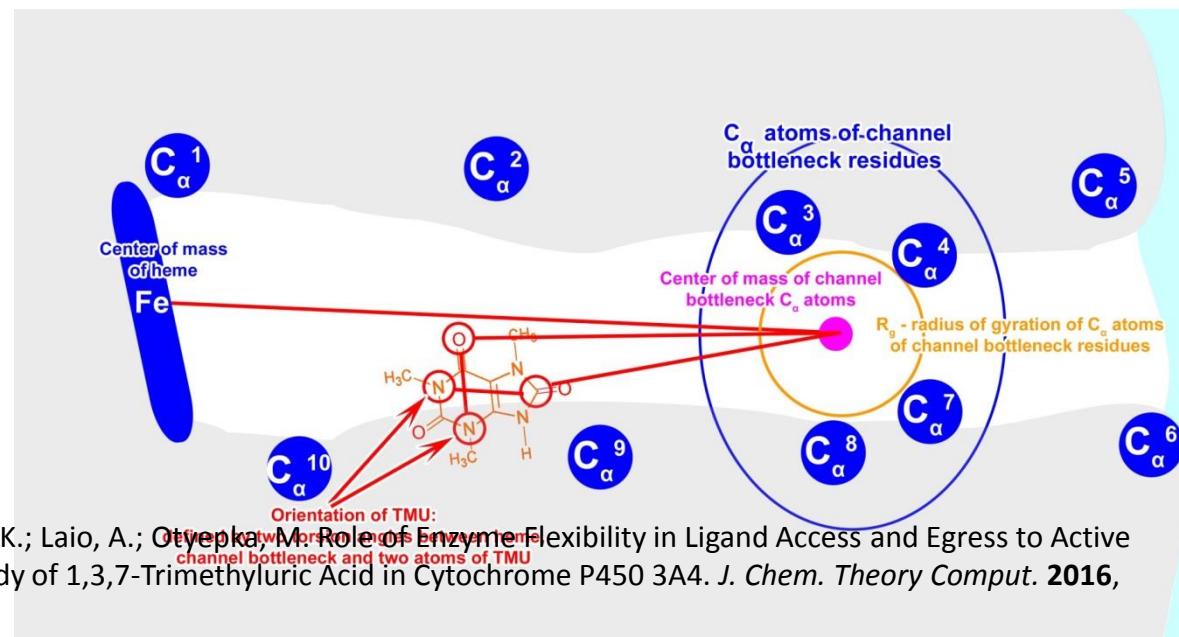
Sehnal, D.; Svobodová Vařeková, R.; Berka, K.; Pravda, L.; Navrátilová, V.; Banáš, P.; Ionescu, C.-M.; Otyepka, M.; Koča, J. *J. J. Cheminform.* **2013**, *5*, 39.

DMSDDrug



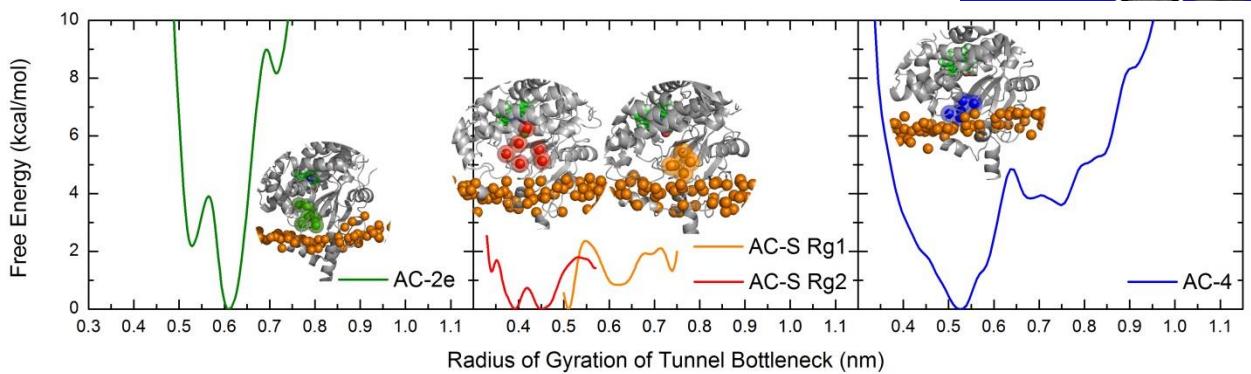
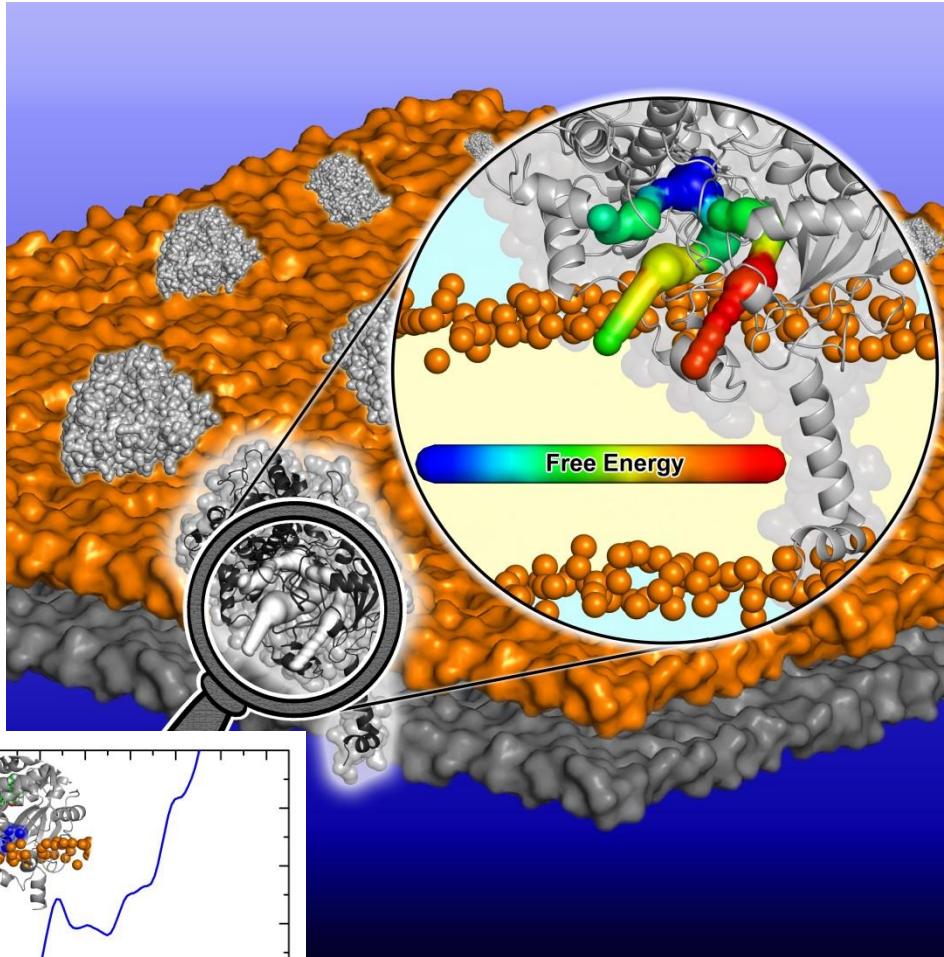
BE-META Setup

- Spath, Zpath
- Drug
 - Orientation
 - Distance from the heme
 - Contacts with channel residues
- Gyration radius of channel bottleneck
- Gromacs 4.6
 - DOPC – Slipids
 - Amber99sb



Results

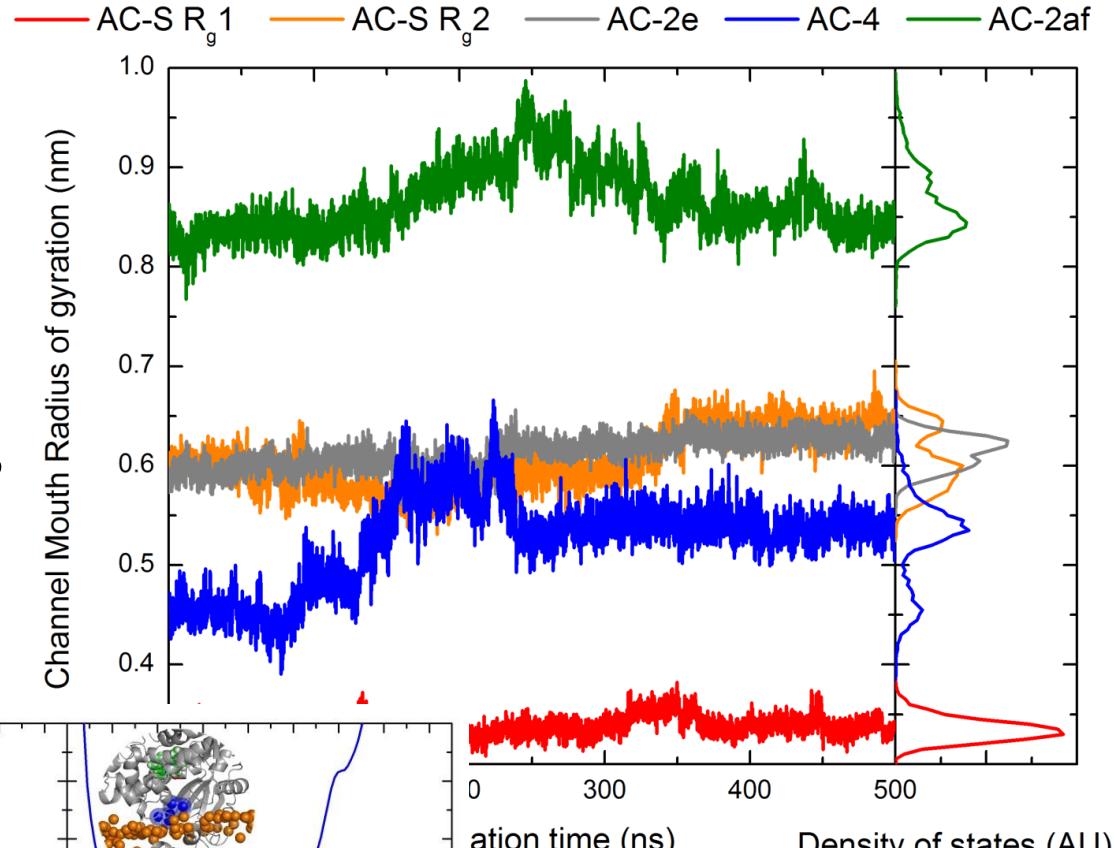
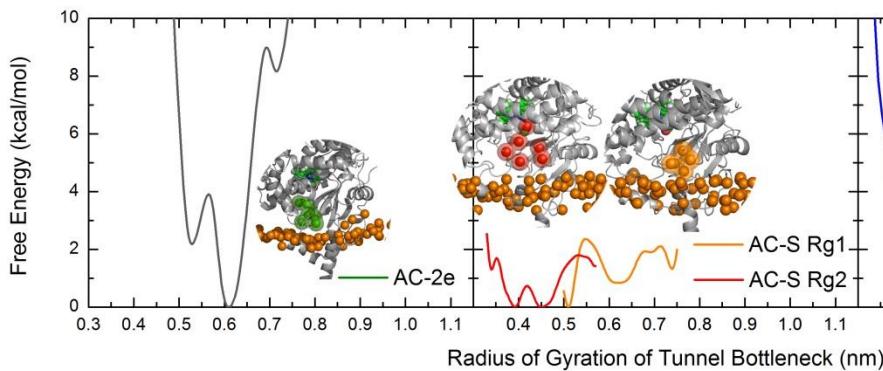
- Solvent Channel
 - Most flexible
 - Most favorable
- Transition States
 - Close to enzyme surface



Palonciová, M.; Navrátilová, V.; Berka, K.; Lai, A.; Otyepka, M. *J. Chem. Theory Comput.* 2016, 12, 2101–2109.

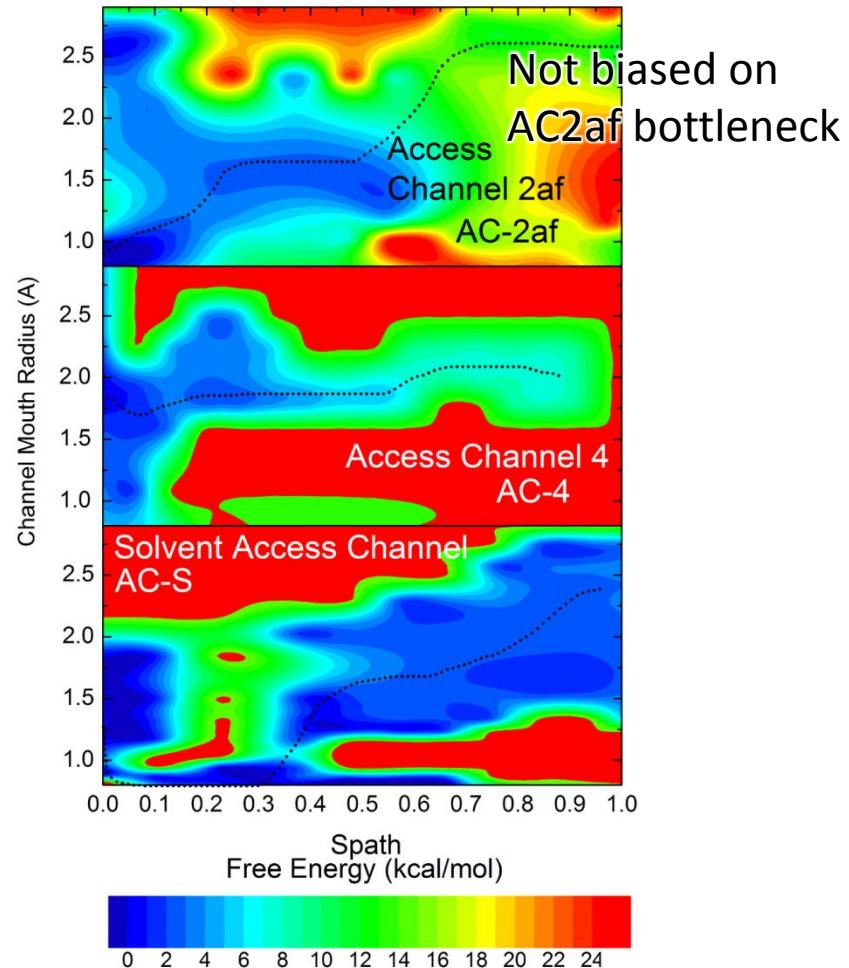
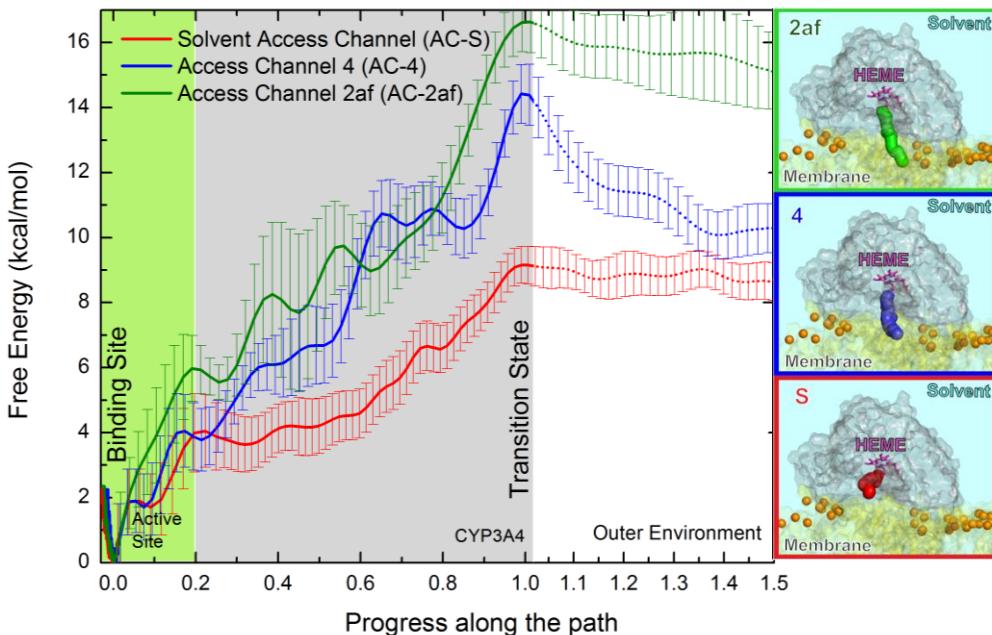
Spontaneous Channel Opening

- Free Simulation
- Breathing motion
- !Gyration radii of channel bottlenecks

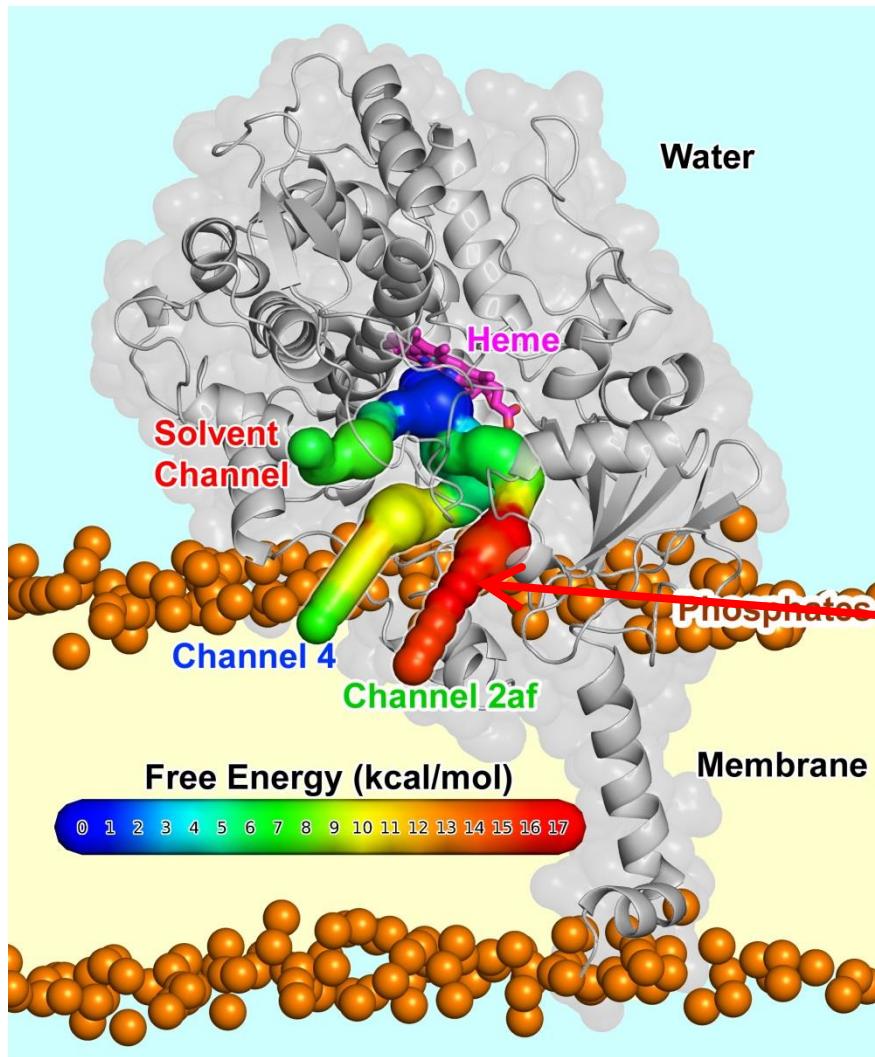


Free Energy Profiles

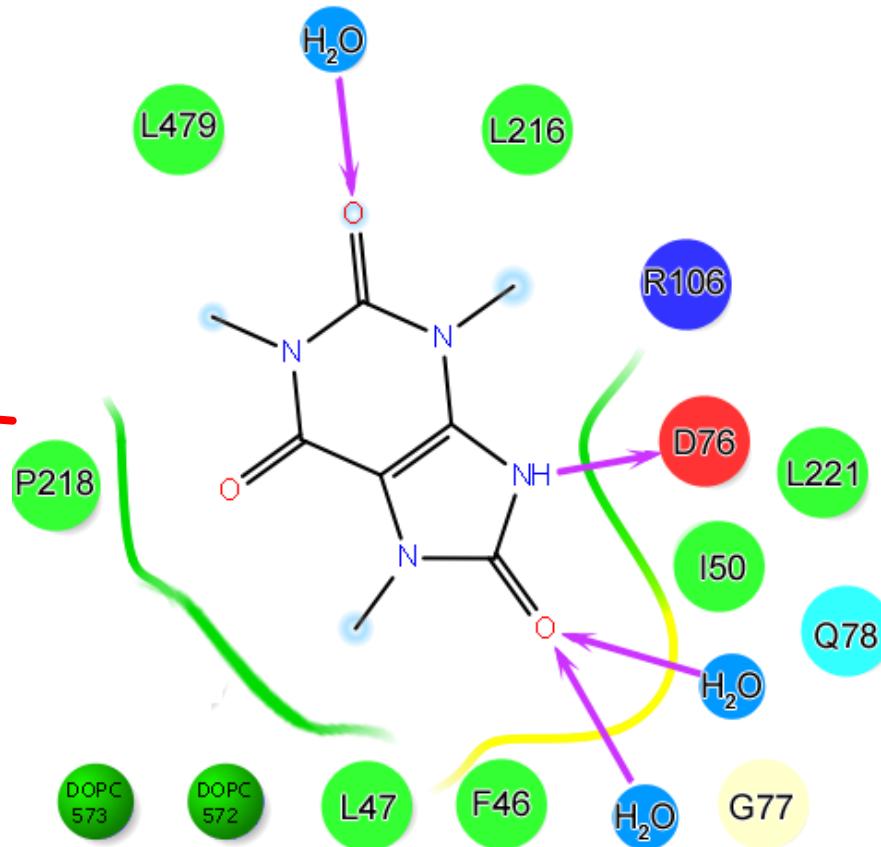
- Channel permeation
 - Channel opening



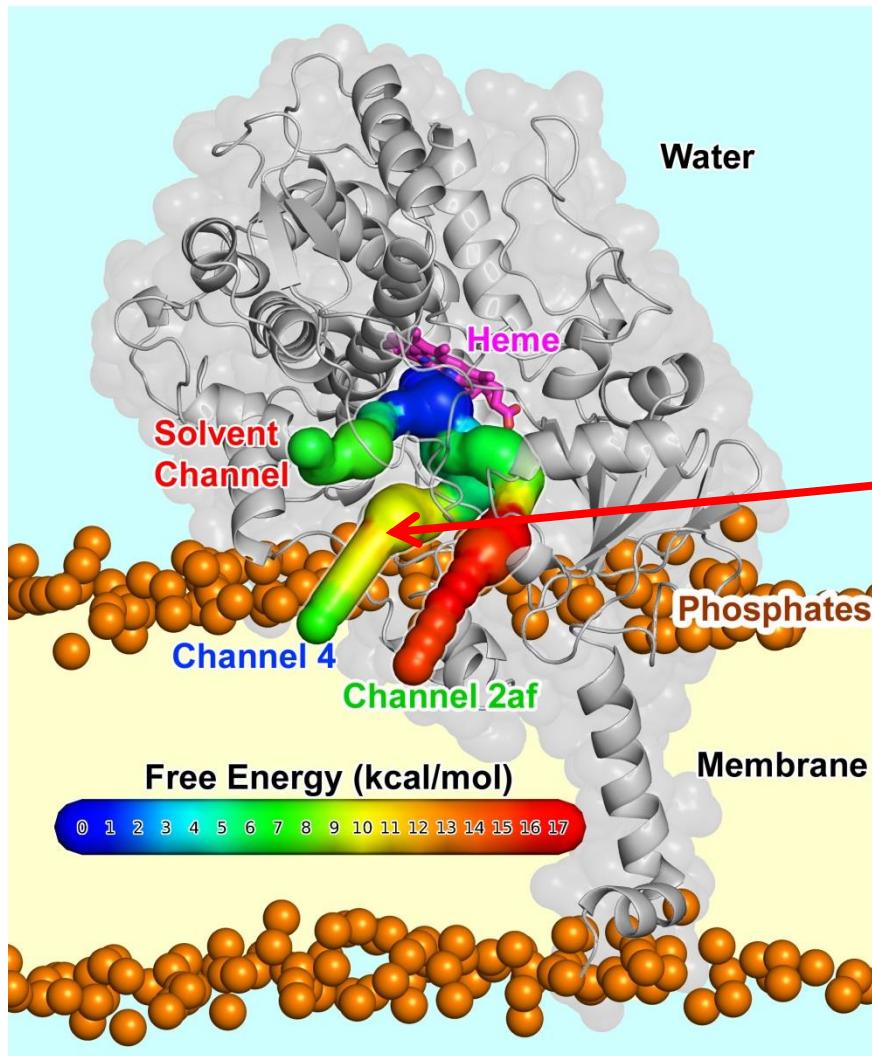
Transition States



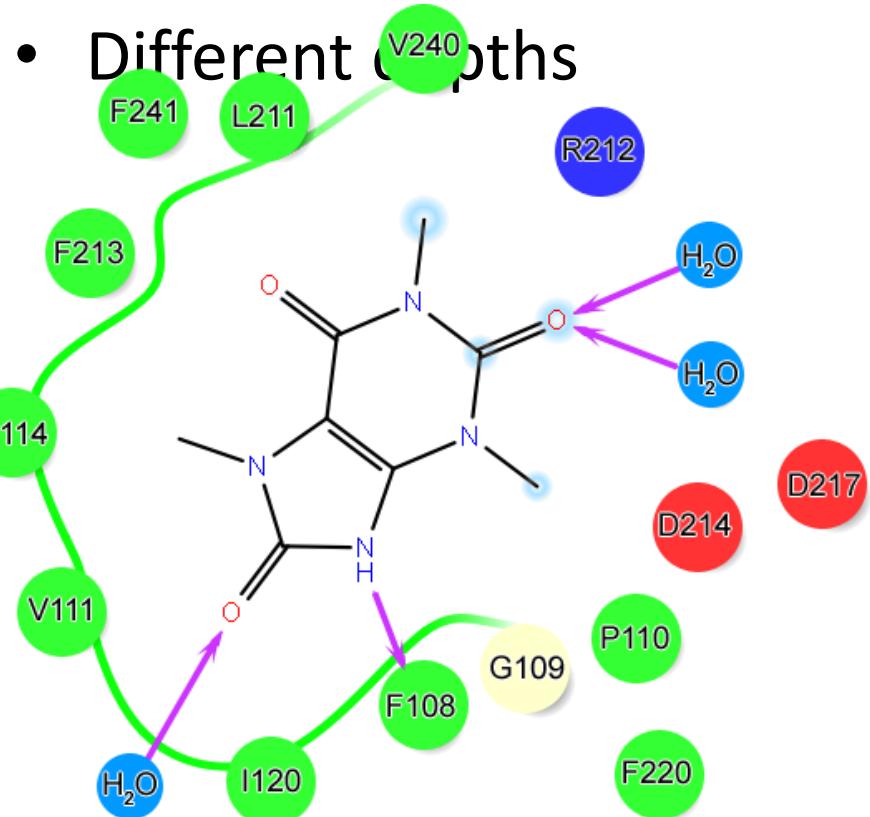
- On CYP3A4 surface
- Different depths



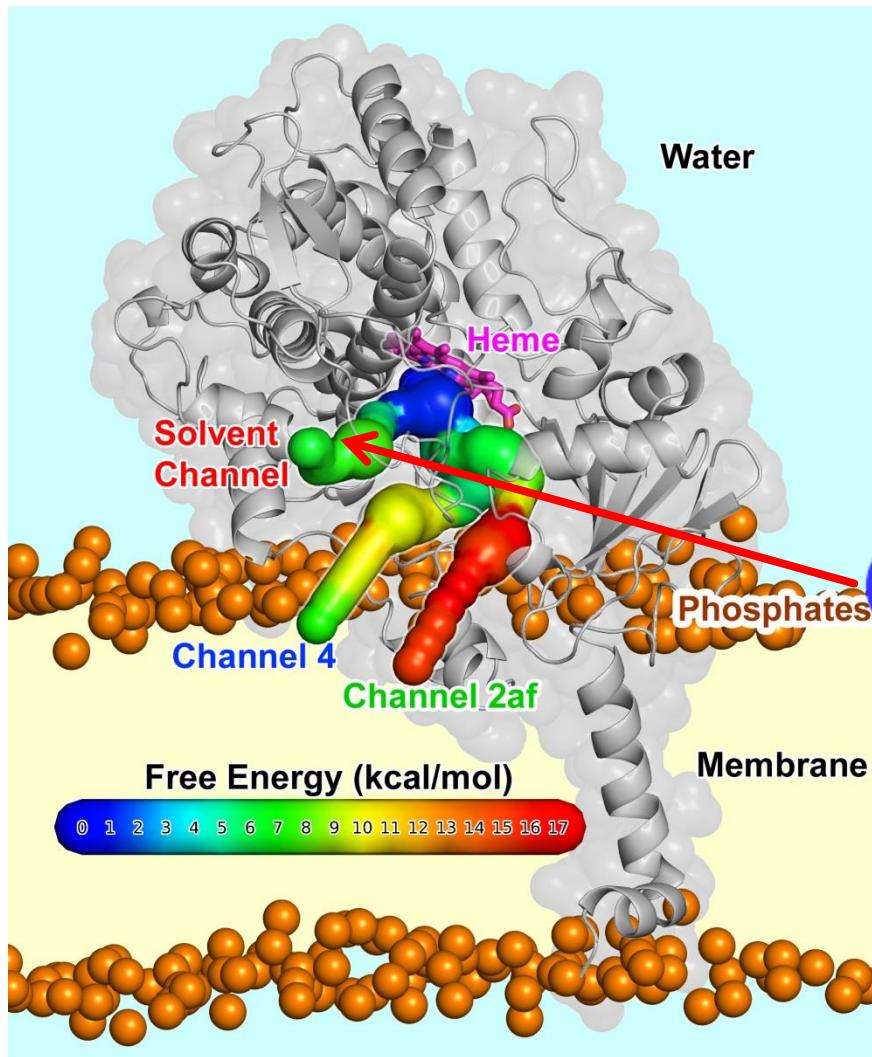
Transition States



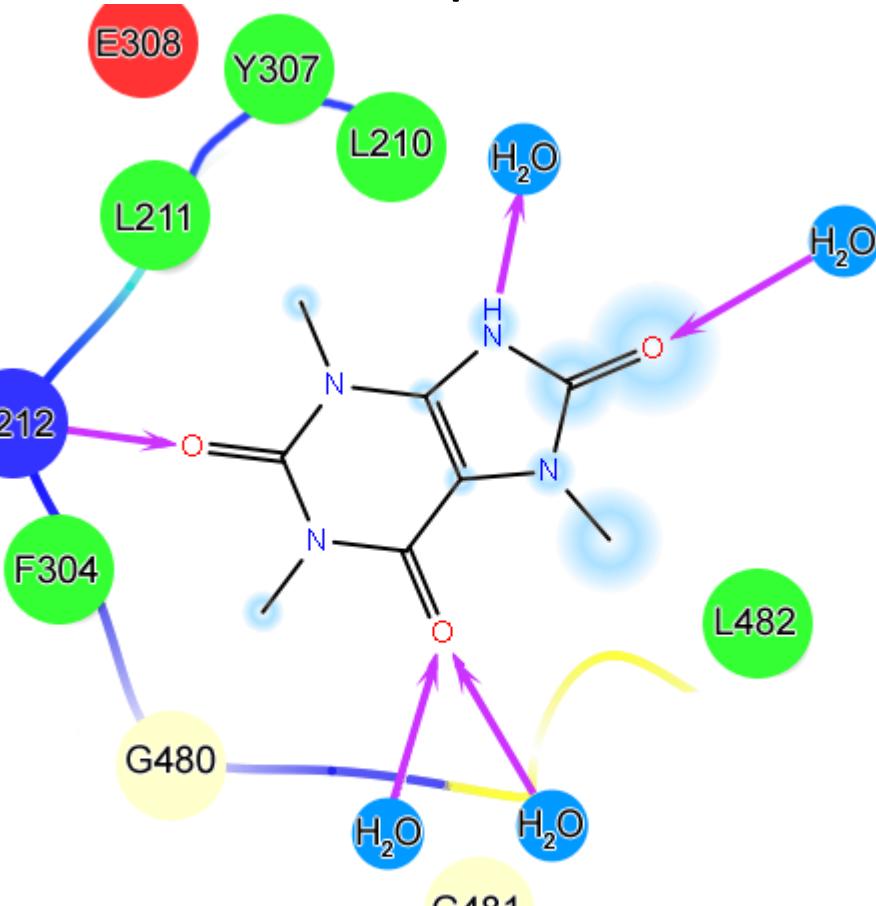
- On CYP3A4 surface
- Different Depths



Transition States

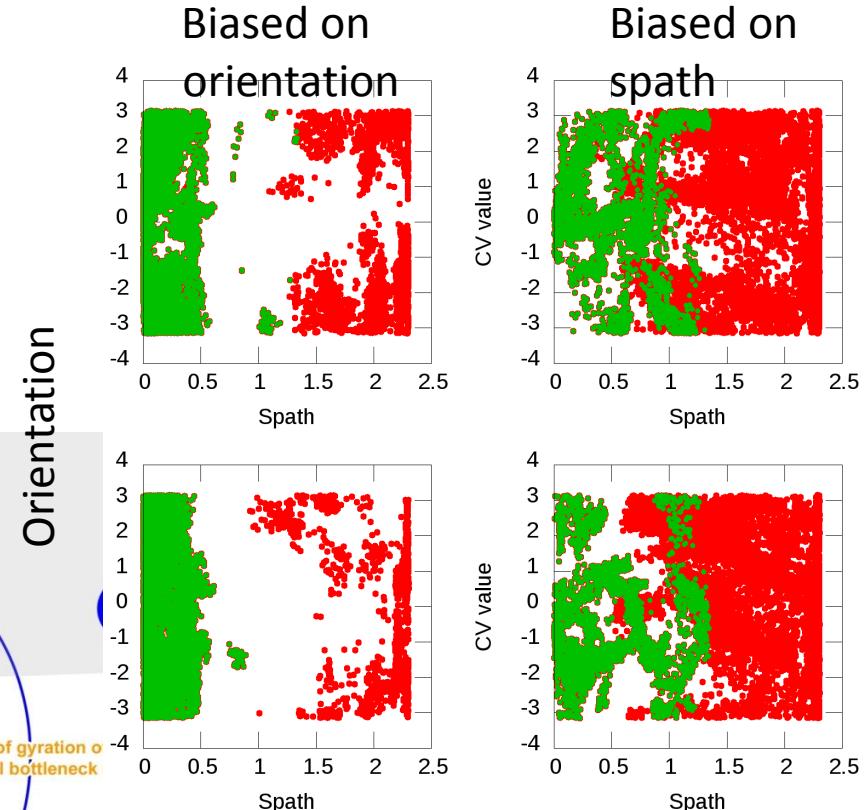
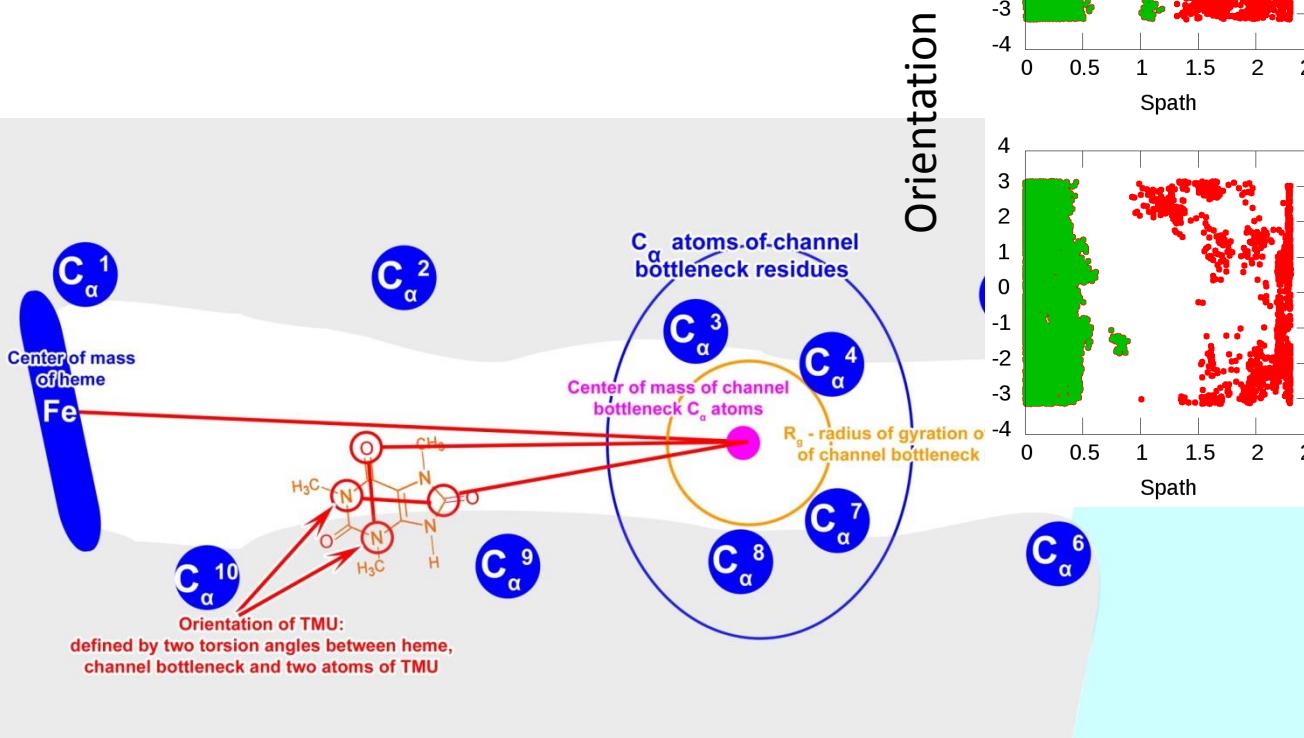


- On CYP3A4 surface
- Different depths



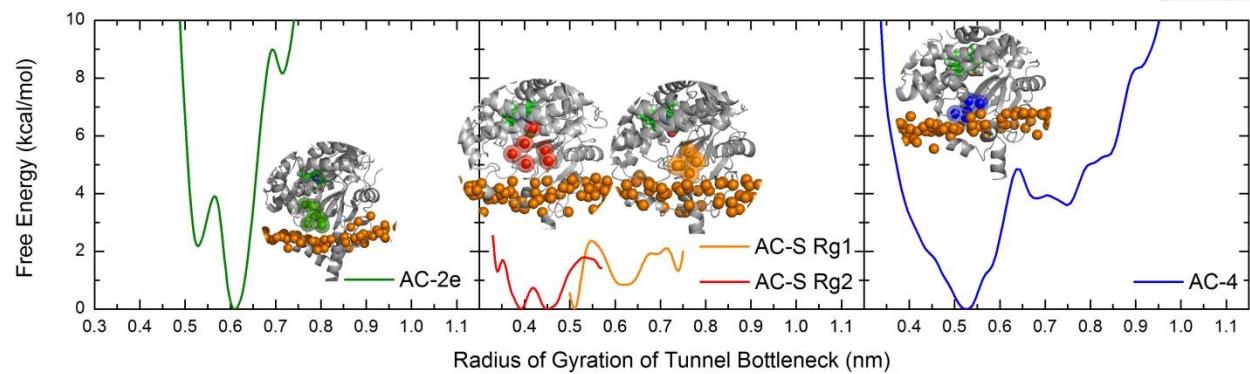
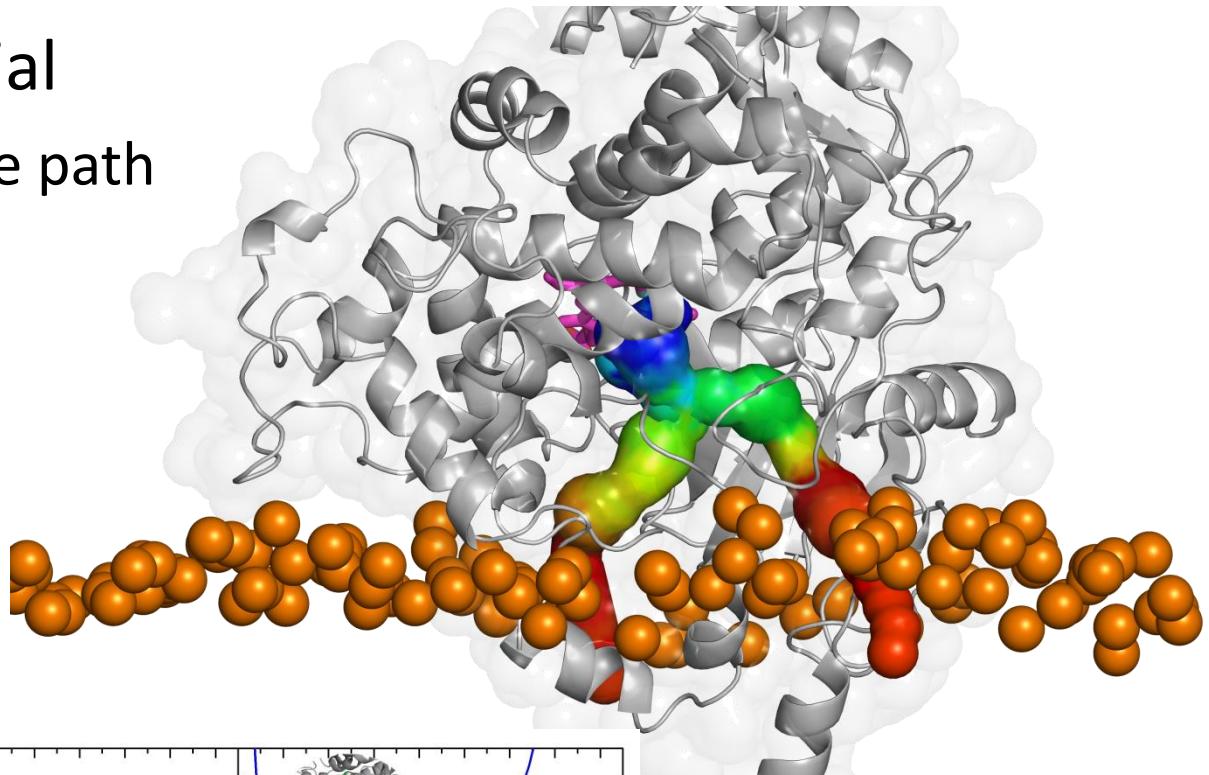
Active Site

- A long time spent in active site



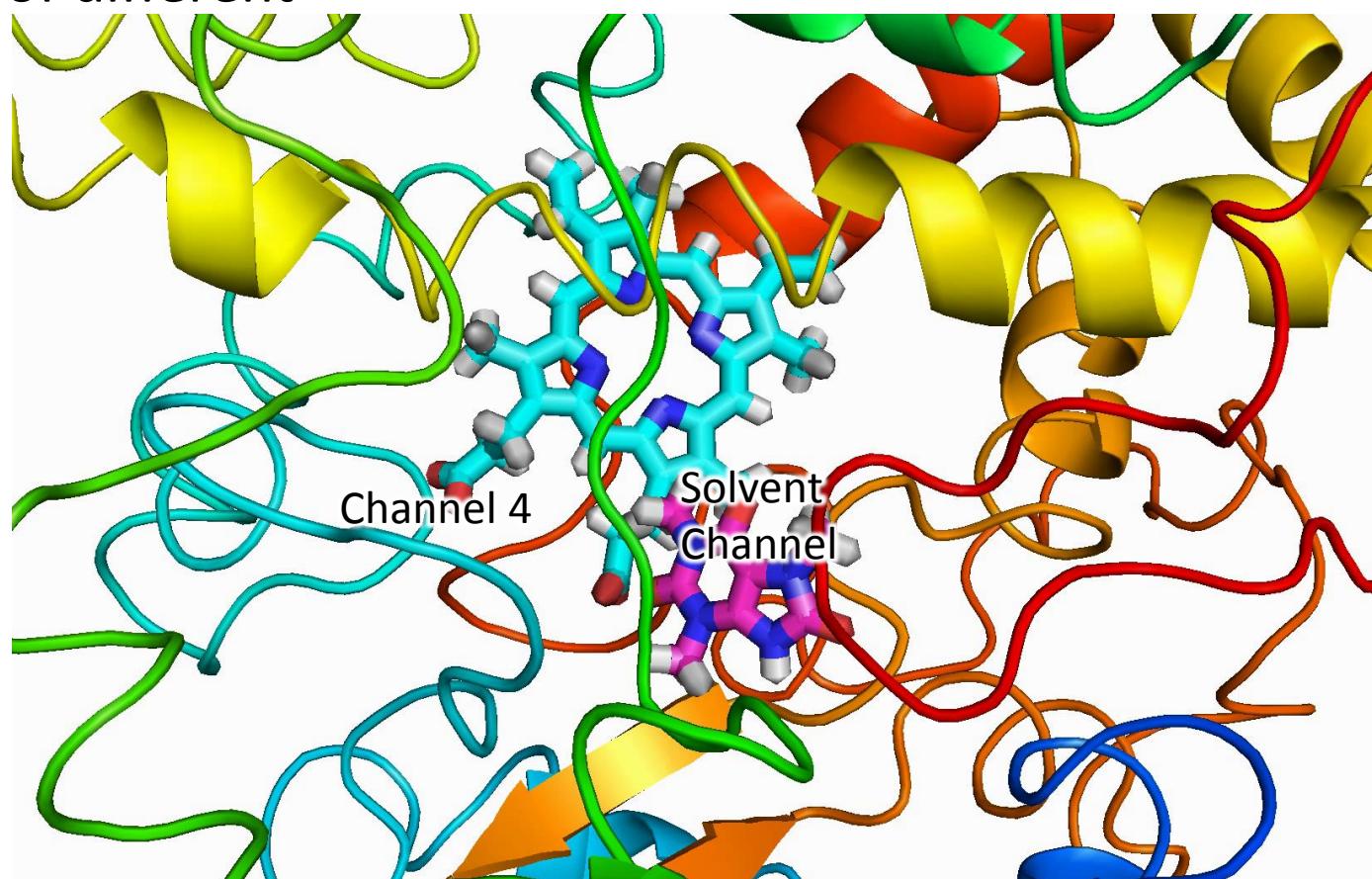
III path

- Can be beneficial
 - More favorable path
 - Rigid channel



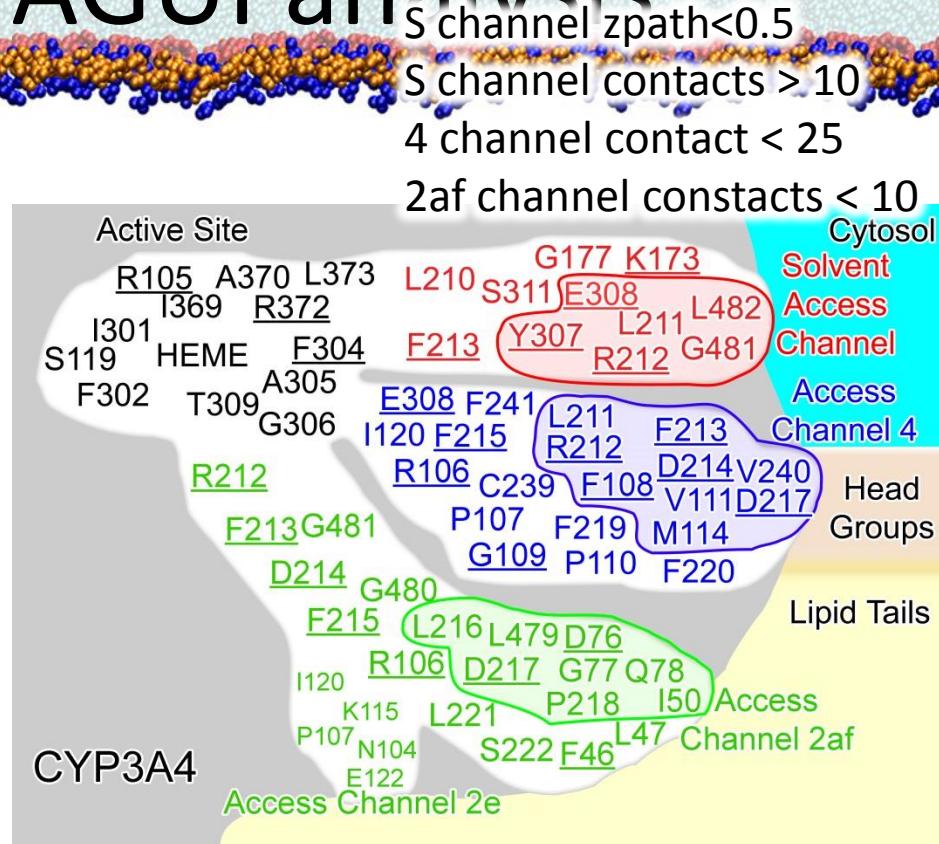
III path

- Takes simulation time
 - Sampling of different states!



III Path – METAGUI analysis

- Rerun all simulations with CVs of other channels
- Define CVs combination for each of the channel
- Add 'variable' [1,0] for each channel



```
#! FIELDS time cv1 cv2 cv3 cv4 cv5 cv6 cv7 cv8 cv9 cv10 cv11 cv12 cv13 cv14 cv15 cv16 cv17
cv18 cv18 vbias vwall
#! ACTIVE 1 1 A
84760.004026 5.900330 6.312607 0.556896 0.534900 2.154190 0.350205 1.560944 0.225318
2.952815 0.705062 3.509343 0.551059 0.092045 4.904541 0 0 0 1 0 0.000000
84770.004026 1.000708 5.367663 0.432814 0.702810 2.020260 0.901817 0.395557 0.266395
1.215017 0.191348 1.256036 0.175368 5.282368 1.442028 1 1 0 1 0 0.000000
84780.004027 1.000917 5.240266 0.454415 0.731346 1.509274 0.561382 0.453839 0.265061
1.203397 0.176278 1.243171 0.170602 4.163817 1.762012 1 1 0 1 0 0.530433
84790.004027 1.001113 3.953920 0.449510 0.736065 1.282513 0.391532 0.521209 0.233419
1.203145 0.163627 1.230279 0.163090 4.029465 1.786654 1 1 0 1 0 1.061140
84800.004028 1.000800 4.247048 0.443140 0.702162 1.829936 0.757202 0.460737 0.287428
```

III Path – METAGUI analysis

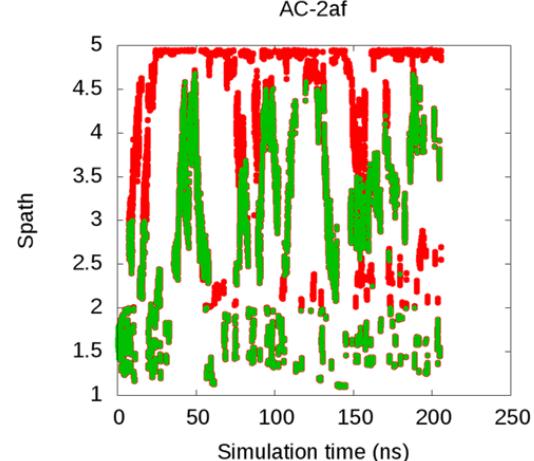
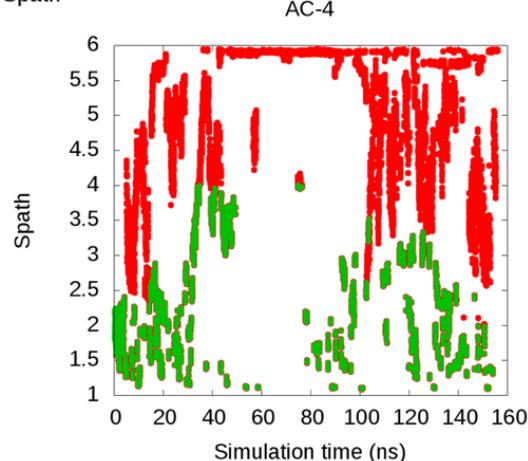
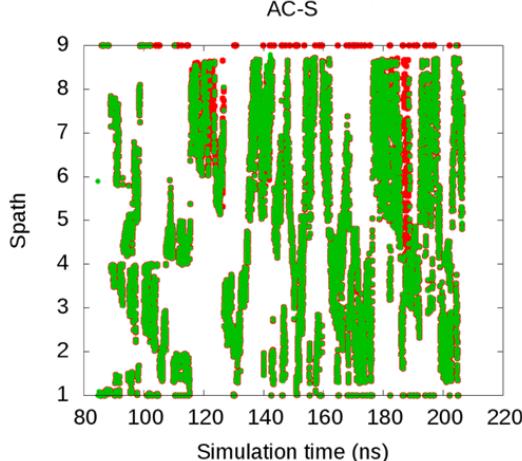
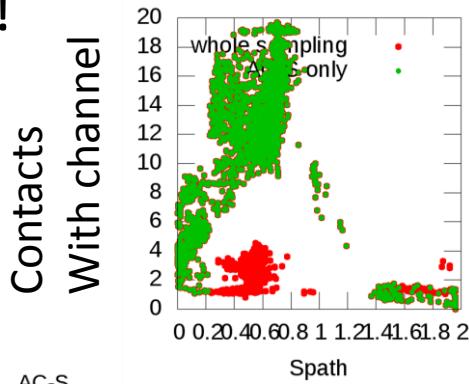
- Rerun all simulations with CVs of other channels
- Define CVs combination for each of the channel
- Add 'variable' [1,0] for each channel

```
#! FIELDS time cv1 cv2 cv3 cv4 cv5 cv6 cv7 cv8  
cv18 cv18 vbias vwall  
#! ACTIVE 1 1 A  
84760.004026 5.900330 6.312607 0.556896 0.53490  
2.952815 0.705062 3.509343 0.551059 0.092045 4.  
84770.004026 1.000708 5.367663 0.432814 0.70281  
1.215017 0.191348 1.256036 0.175368 5.282368 1.  
84780.004027 1.000917 5.240266 0.454415 0.73134  
1.203397 0.176278 1.243171 0.170602 4.163817 1.762012 1 1 0 1 0 0.530433  
84790.004027 1.001113 3.953920 0.449510 0.736065 1.282513 0.391532 0.521209 0.233419  
1.203145 0.163627 1.230279 0.163090 4.029465 1.786654 1 1 0 1 0 1.061140  
84800.004028 1.000800 4.247048 0.443140 0.702162 1.829936 0.757202 0.460737 0.287428
```

Collective Variables:						
(-)	CV type	min	max	grid	lperulse	
CV1: dmsd.s	1.5	8	15	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
CV2: coord	1.0	13	15	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
CV3: gyr1	0.32	0.6	15	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
CV4: gyr2	0.55	0.73	15	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
CV5: torsion1	-3.14	3.14	15	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
CV6: torsion2	-3.14	3.14	15	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
CV7: distance	0.3	3.2	15	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
CV8: dmsd.z	0	0.5	15	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
CV9: 2espath	1.173566	4.951145	15	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
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CV11: 4spath	1.152120	5.950023	15	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
CV12: 4zpath	0.159630	4.833244	15	<input type="checkbox"/>	<input type="checkbox"/>	
CV13: 2ecoord	0.000000	36.36923	15	<input type="checkbox"/>	<input type="checkbox"/>	
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CV15: 2f	0	1	15	<input type="checkbox"/>	<input type="checkbox"/>	
CV16: 4	0	1	15	<input type="checkbox"/>	<input type="checkbox"/>	
CV17: 1	0.5	1.5	15	<input type="checkbox"/>	<input type="checkbox"/>	
CV18: solvent	0	1	15	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
CV19: all	0.5	1.5	15	<input type="checkbox"/>	<input type="checkbox"/>	

III path

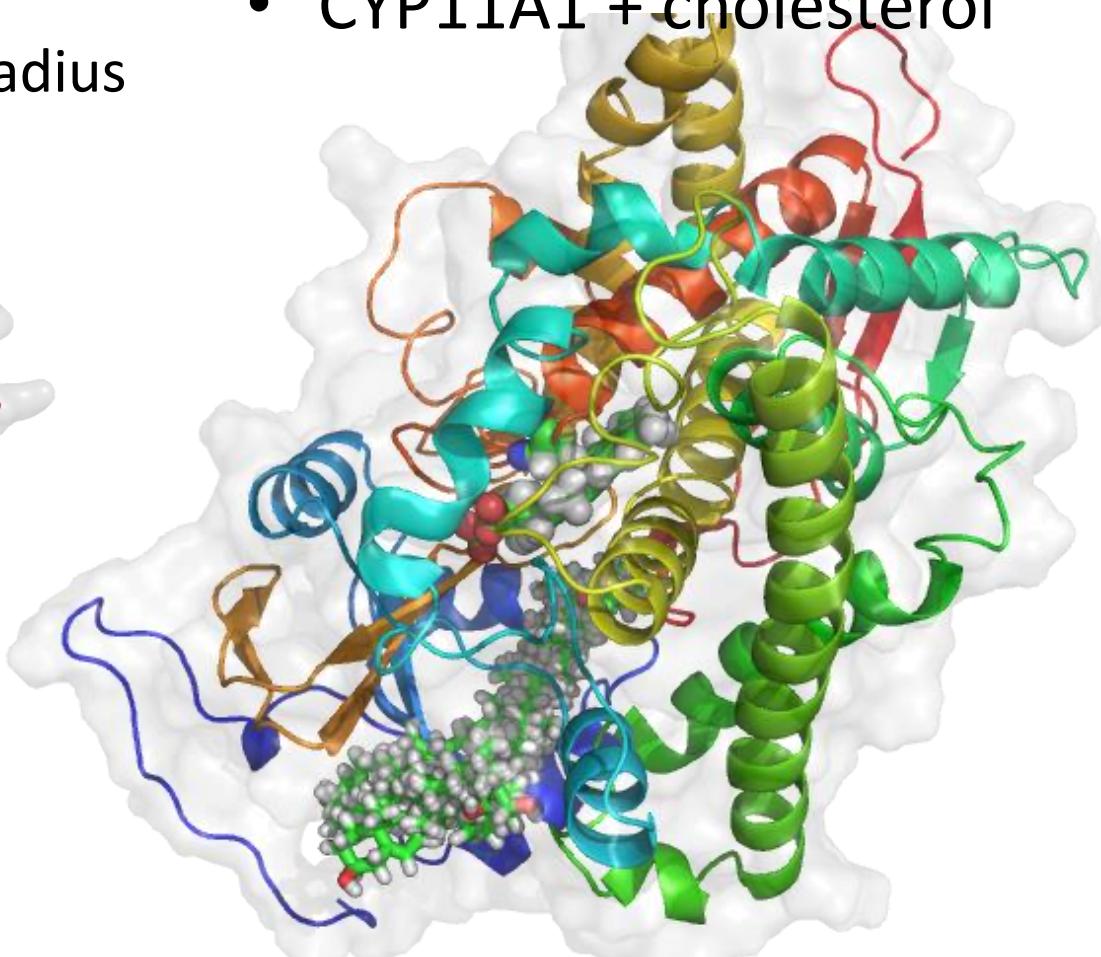
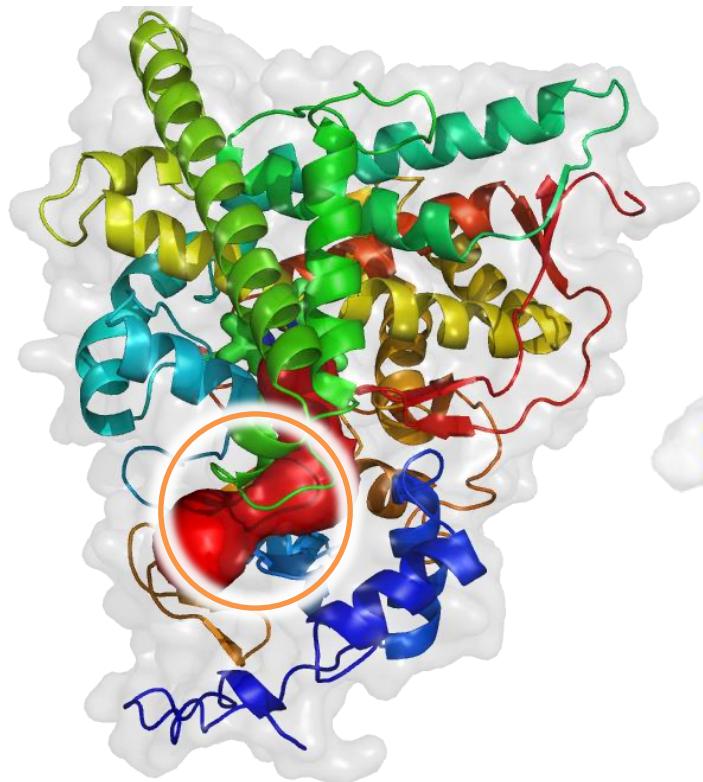
- Takes simulation time
 - Sampling of different states!
- Zpath, wall
 - Reduction of simulation speed



- Supportive CVs
 - Contacts ...

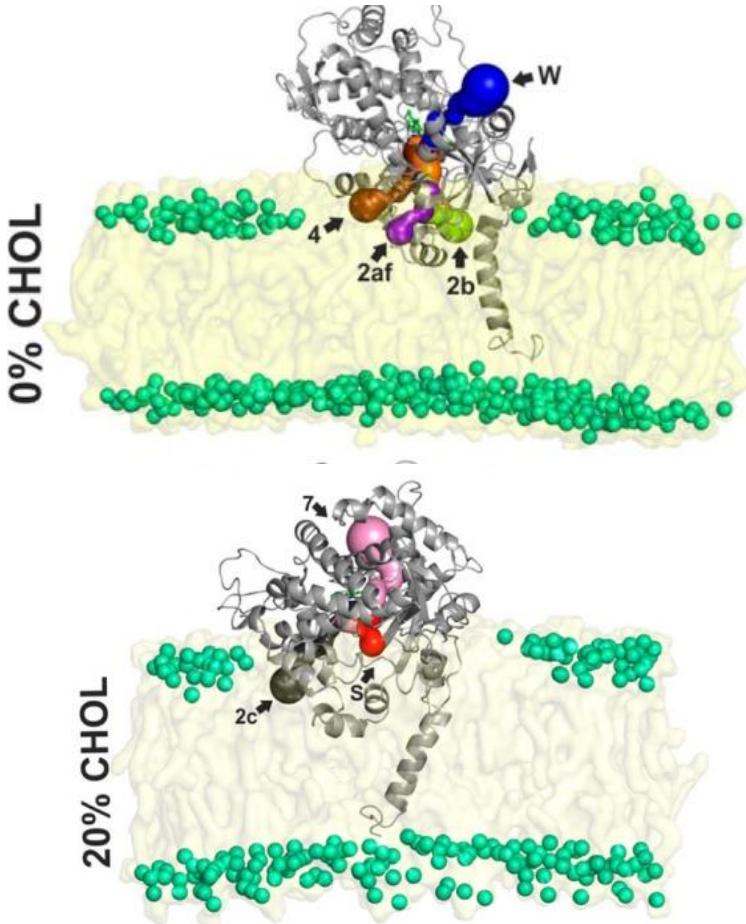
Initial Frames

- Breathing motion
 - Change in channel radius
- CYP11A1 + cholesterol



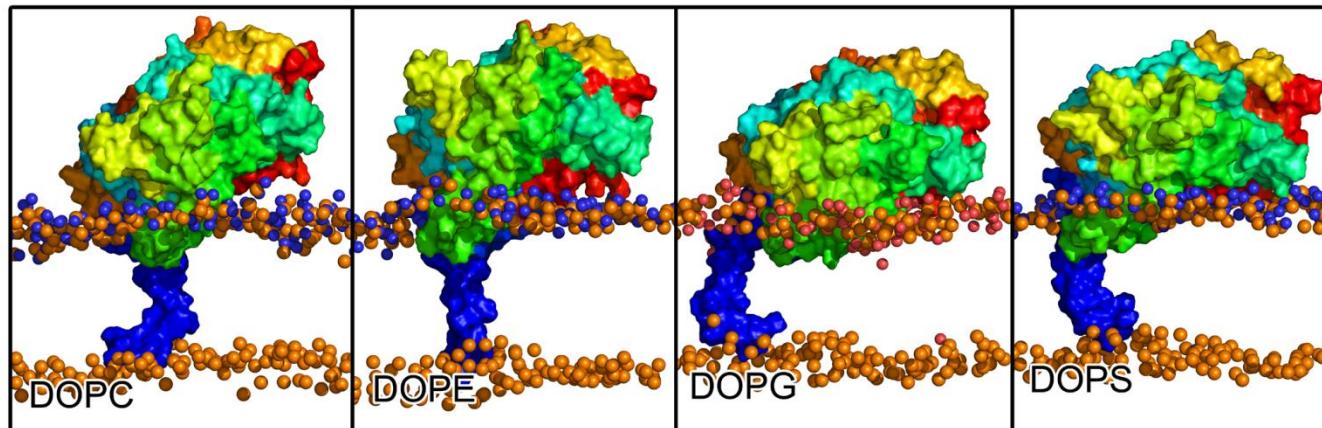
Membrane Model - Cholesterol

- DOPC with Cholesterol
 - 0, 3, 6, 20, 50 weight %
- Orientation
- Heme move deeper
- Conformation changes



Membrane Model - Headgroups

- DOPC, DOPE
 - Zwitterionic
- DOPG, DOPS
 - Anionic
- Different orientation
- Mild conformational changes
- AS

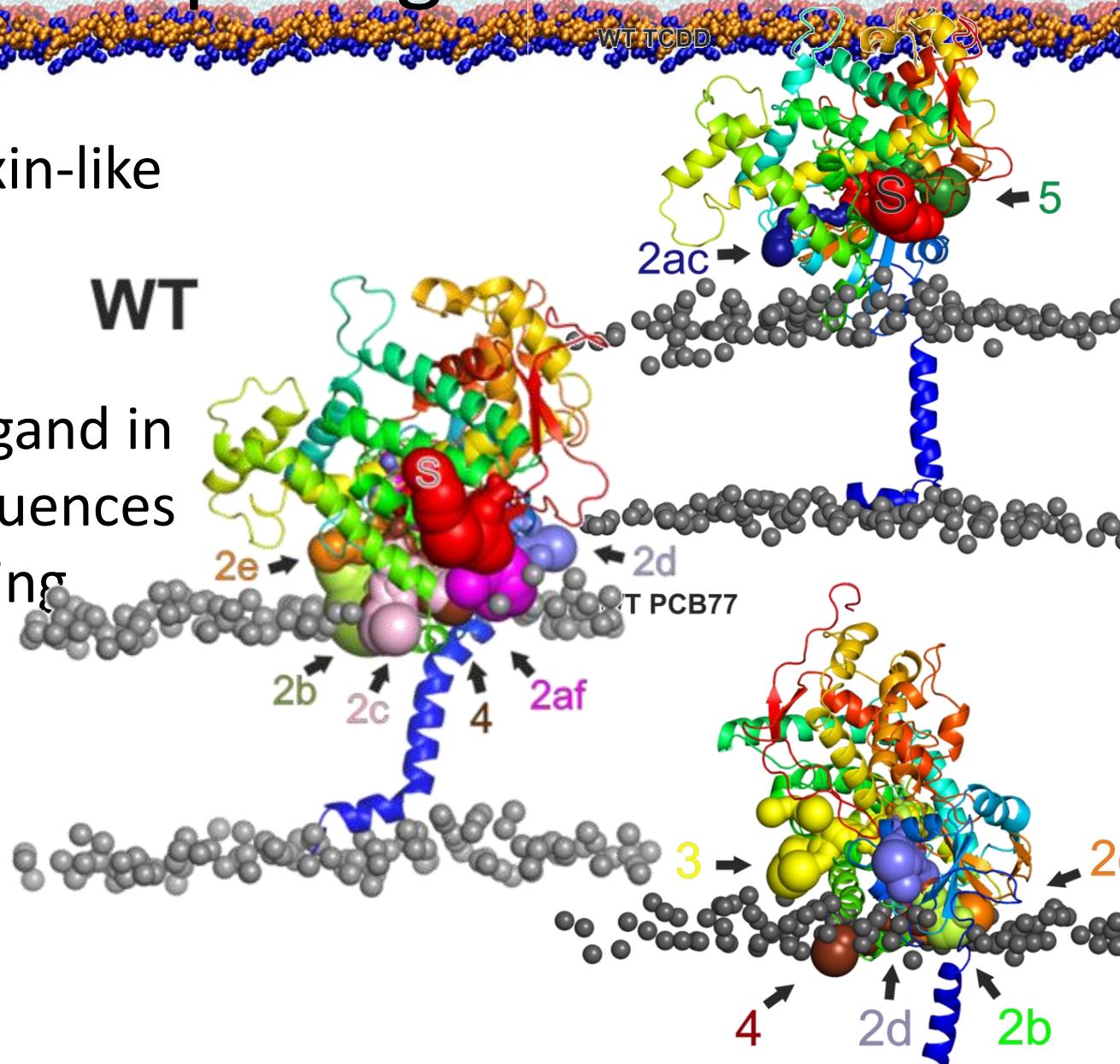


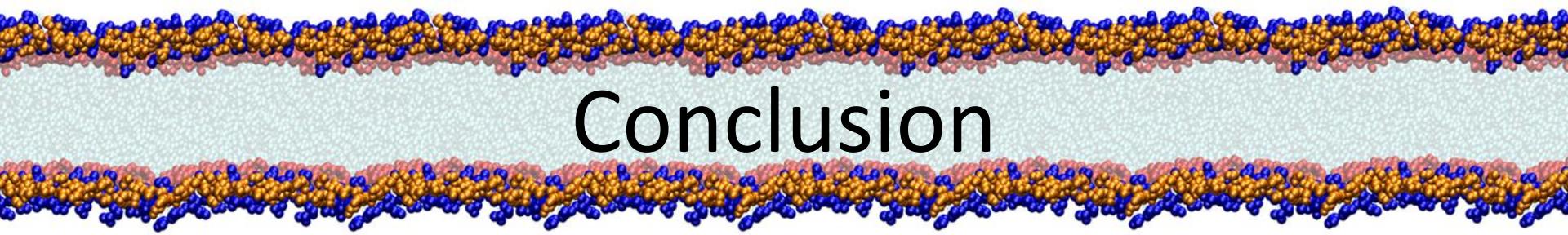
Multiple Ligands

- CYP1A1 + dioxin-like compounds

WT

- Presence of ligand in active site influences channel opening





Conclusion

- Use of BE-META for CYP3A4 channel permeation
 - Identified most favorable channel
 - Flexibility
 - Transition states
 - Close to enzyme surface
- Active Site Cavity
 - Separate sampling of AS and narrow channel
- Ill paths
 - Use whenever possible
 - Control/Check
- Channels affected by
 - Enzyme flexibility
 - Membrane
 - Ligand
- Take time to define everything

Acknowledgement

- Michal Otyepka
- Karel Berka
- Veronika Navrátilová
- Martin Šrejber
- Eliška Ryšavá



- Alessandro Laio
- Ina Bisha
- Alex Rodriguez



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Thank you for your attention