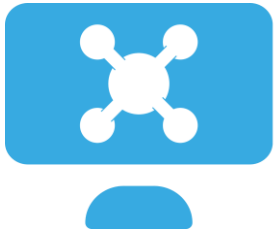
Computational insights into the biocatalytic activity of C-type halohydrin dehalogenase HheC

Višnja Stepanić, Zlatko Brkljača,
and Maja Majerić Elenkov

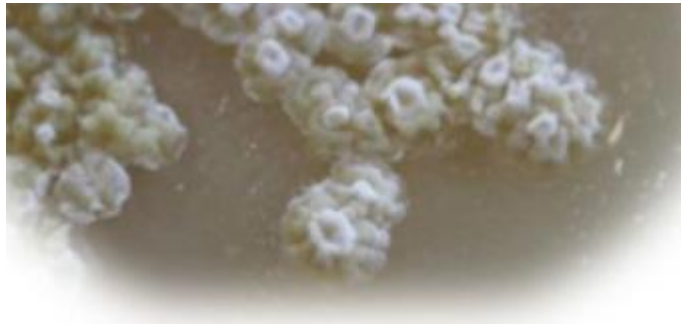


COMPUTATIONAL CHEMISTRY DAY 2023

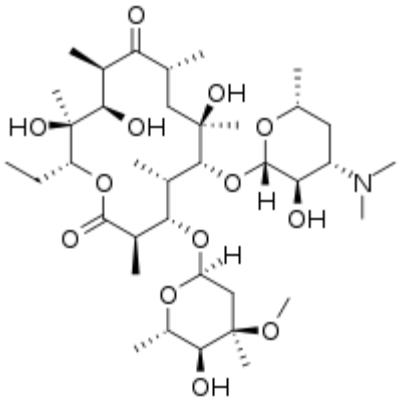
Zagreb, Hrvatska
16/9/2023

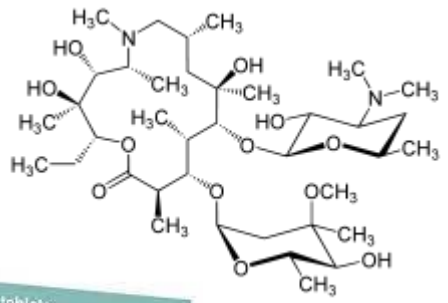
Biocatalysis

use of living biological systems or their parts to catalyse chemical reactions

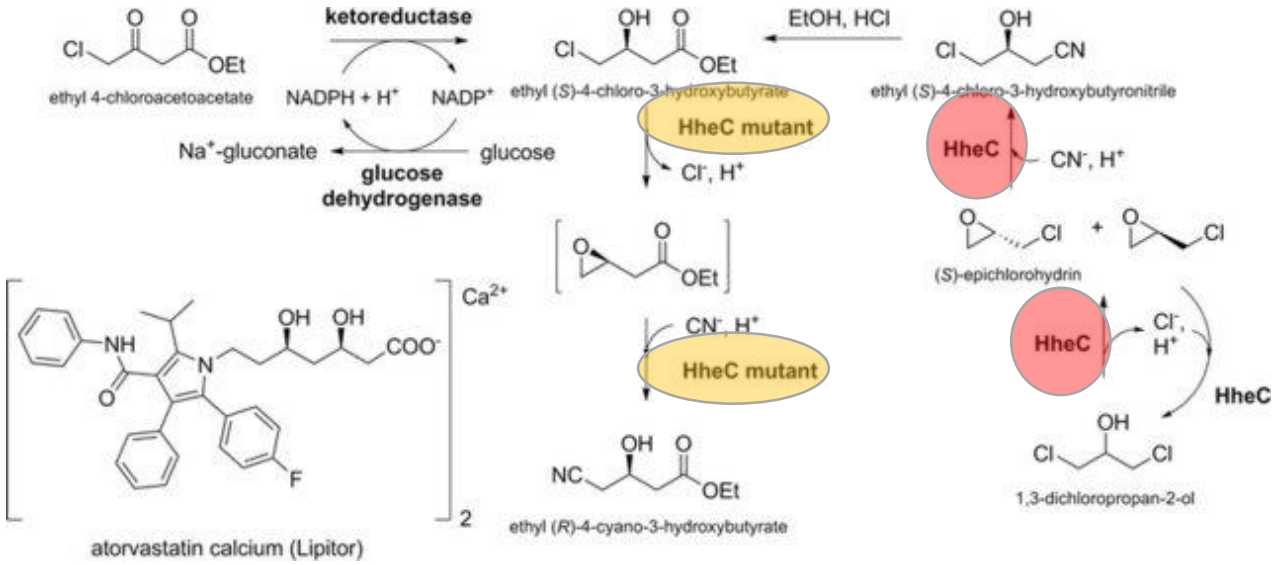


Saccharopolyspora erythraea





From 1980s



green synthesis

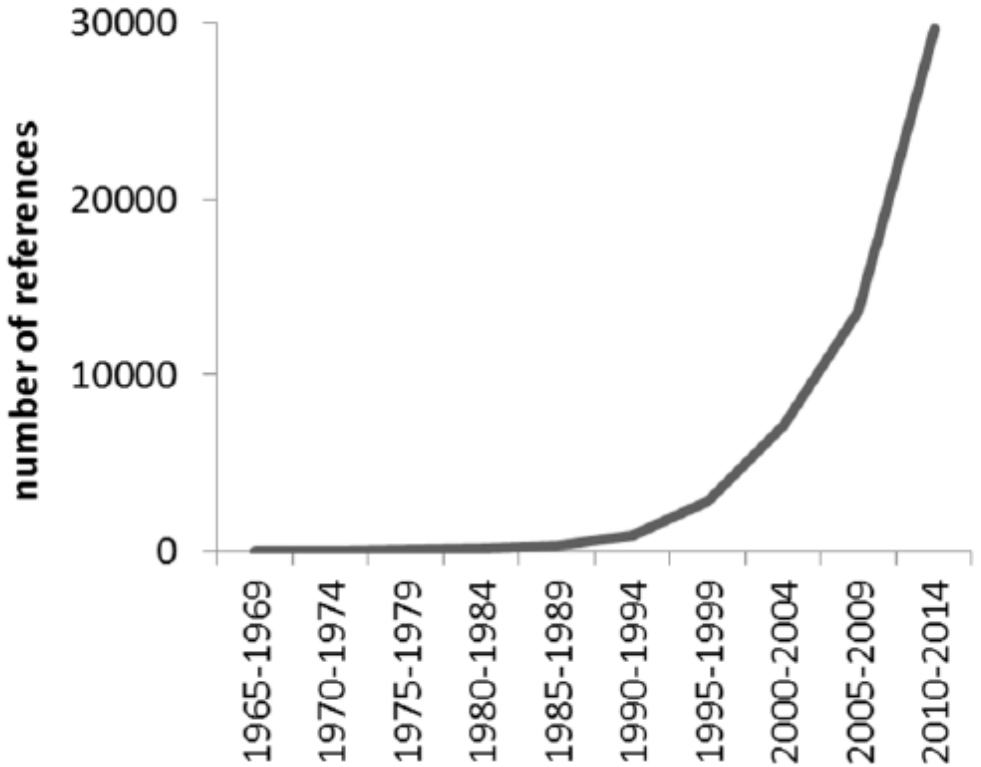


Figure 3. Number of publications and patents discussing "pharmaceutical biocatalysis" for each 5 year period of the last 50 years. Metrics from Google Scholar.

Truppo MD. Biocatalysis in the Pharmaceutical Industry: The Need for Speed. ACS Med Chem Lett. 2017;8(5):476-480

Halogenhydrin-dehalogenases (HHDHs)

Podjela u grupe: **A**, **B**, **C**, **D**, **E**, **F**, **G**

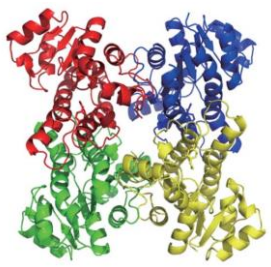
HheA (*Corynebacterium* sp.)
HheA2 (*Arthrobacter* sp.)
HheB (*Corynebacterium* sp.)
HheB2 (*Mycobacterium* sp.)
HheC (*Agrobacterium radiobacter*)
HheD (*Dechloromonas aromatica*)
HheD2 (*Gammaproteobacterium*)
HheE (*Acaryochloris*)
HheF (Uncultured bacterium)
HheG (*Ilumatobacter coccineus*)
HheG2 (*I. Nonamiensis*)

Up to 2001.

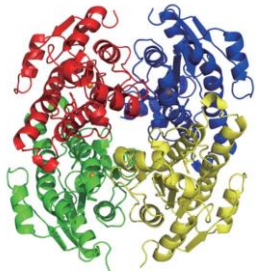
from 2014.

>40 known enzymes

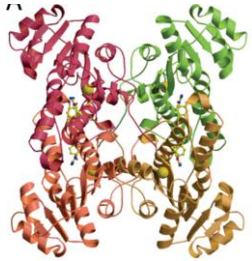
Crystal structures



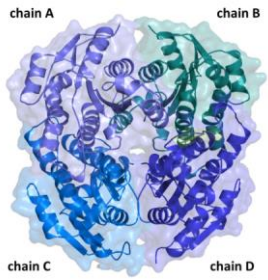
HheA/A2



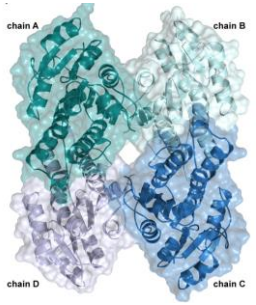
HheB



HheC



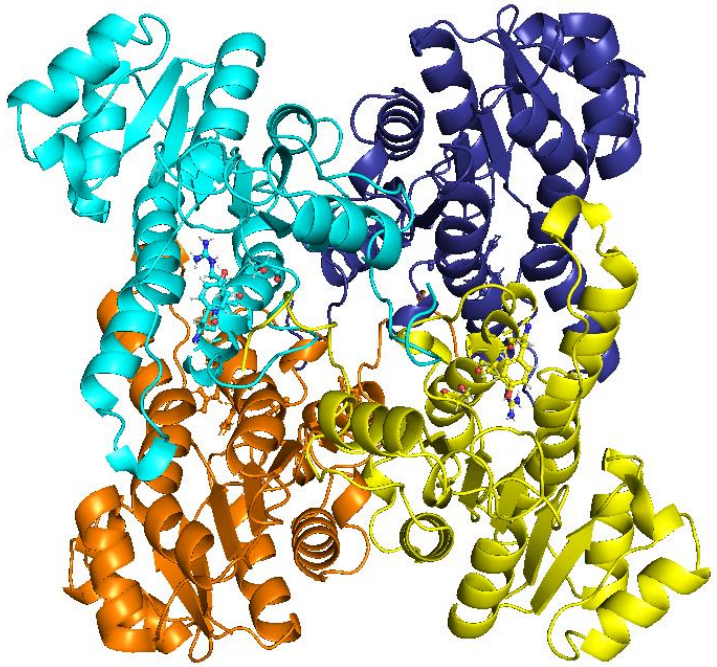
HheD2

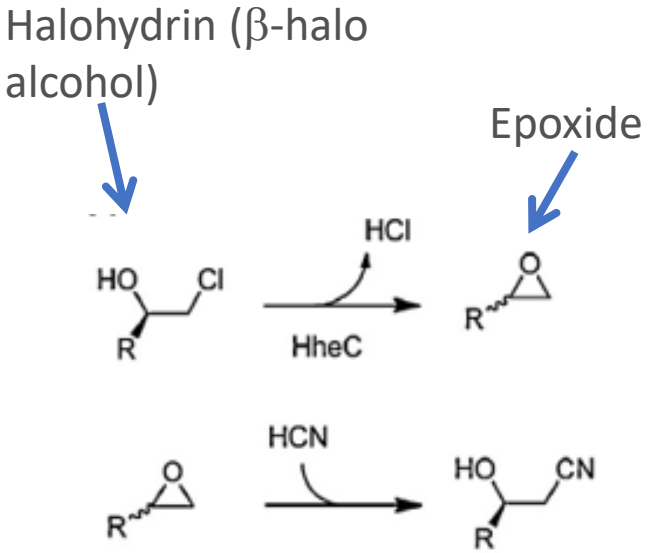


HheG

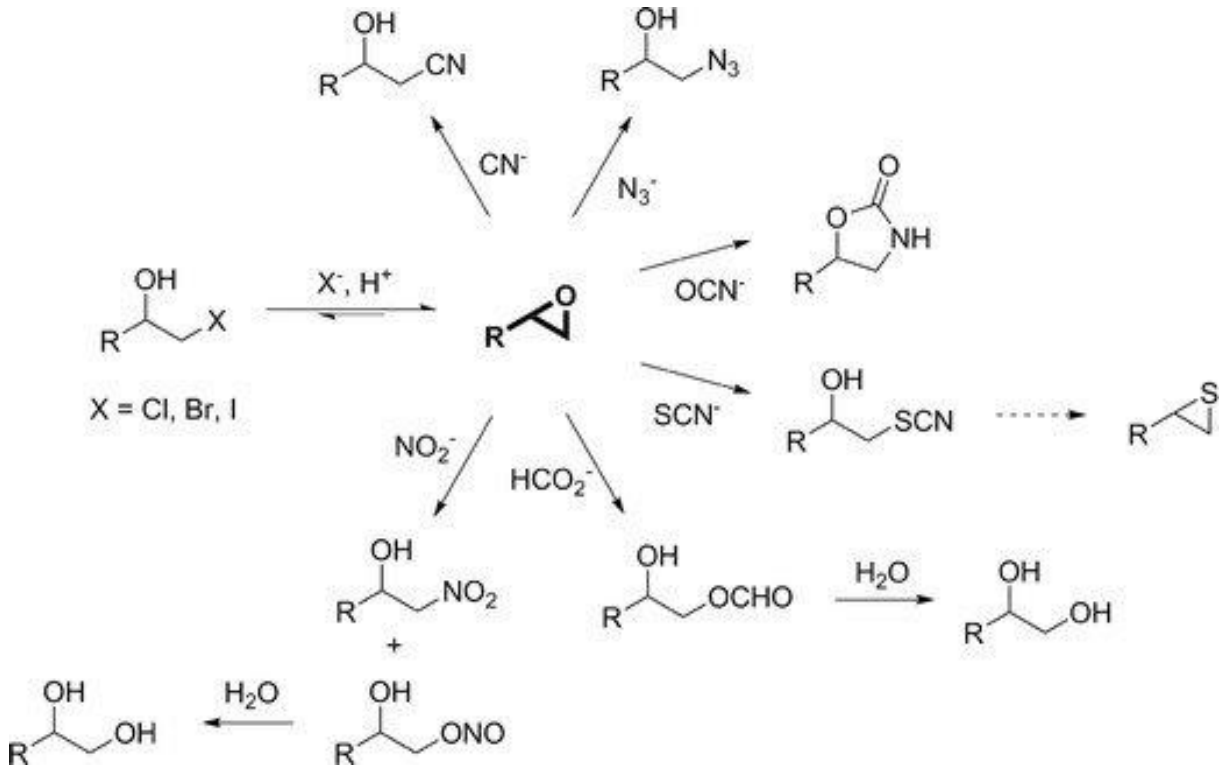
Slide made by
M. Majerić
Elenkov

HheC - HHDH from *Agrobacterium radiobacter* AD1





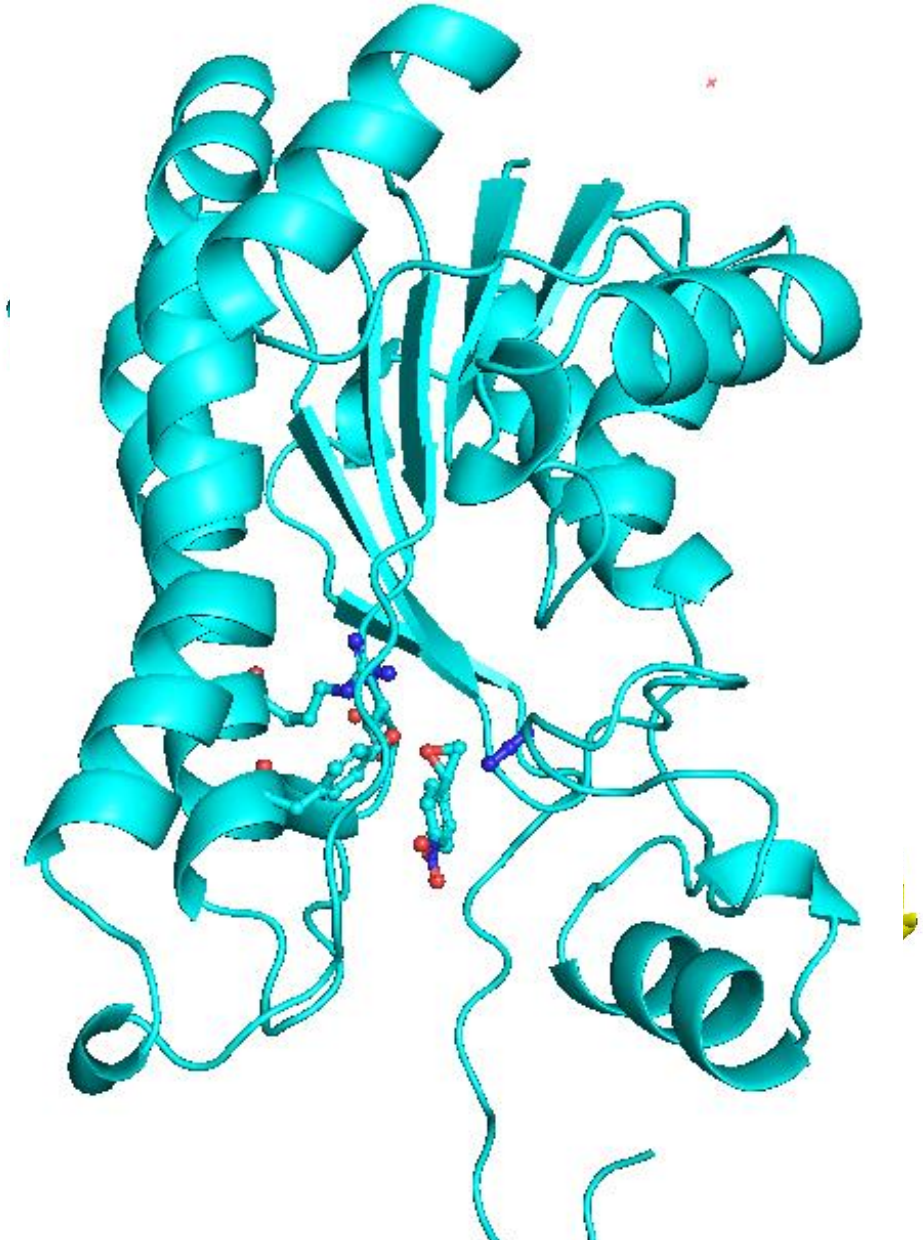
HheC -the most frequently used HHDH as biocatalyst

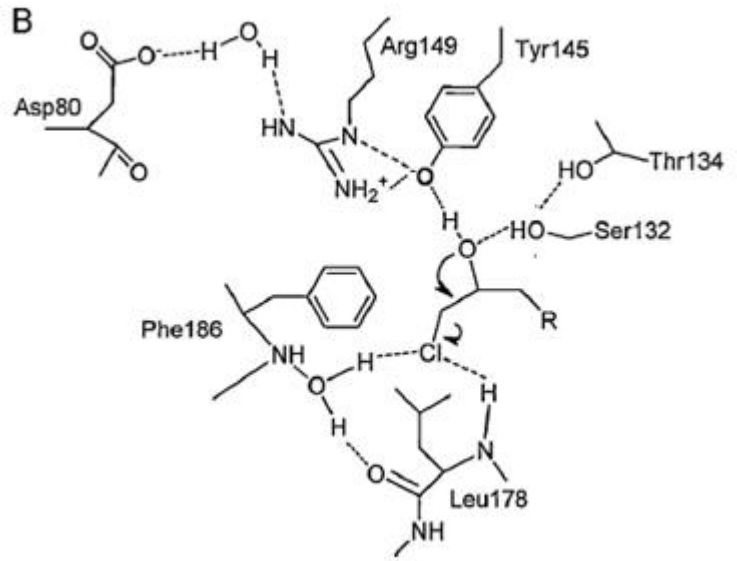


Industrially relevant enzyme

- Broad substrate specificity
- Regioselectivity
- Enantioselectivity
  - For styrene oxides (R-benzene) E > 200, *R*-enantioselectivity

HheC catalytic site





M. Schallmey et al. / Enzyme and Microbial Technology 70 (2015) 50–57

The hrzz project EnzyFluor

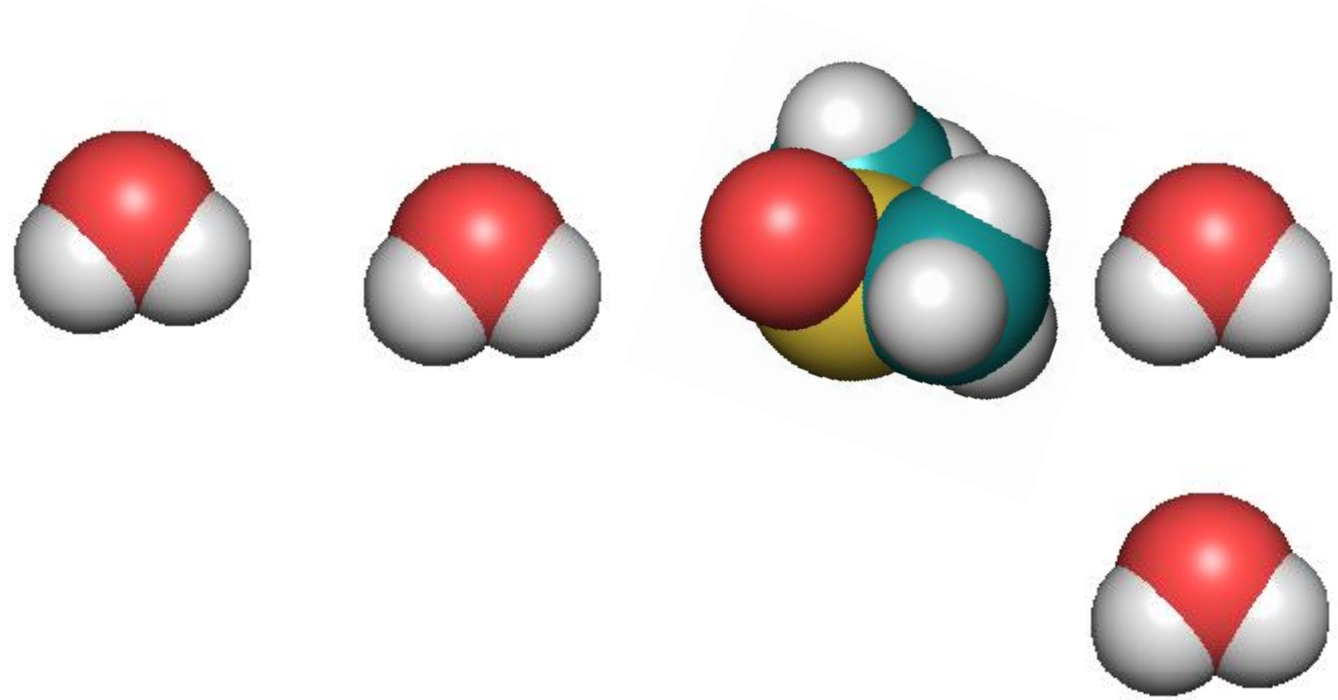
EnzyFluor – Enzymatic Synthesis of Fluorinated Chiral Building Blocks, funded Croatian Science Foundation (IP-2018-01-4493) (2018-2023, PI Maja Majerić Elenkov)

Aim: Further extending the potential for the synthetic application of HheC

Mixing buffered aqueous solution with various solvents

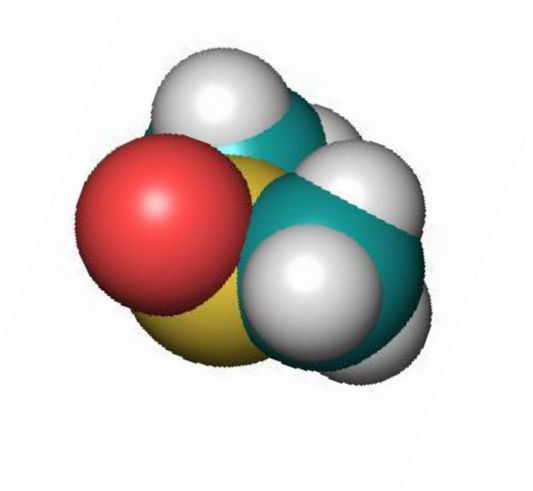
Rationally selected/designed mutants of HheC

Steric Organofluorinated Molecules



DMSO effects

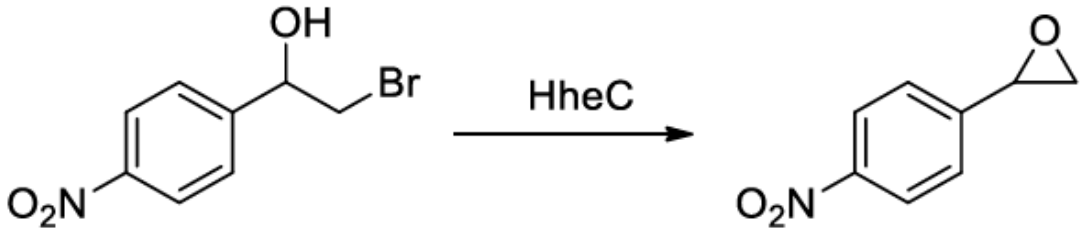
DMSO
Dimethyl sulphoxide



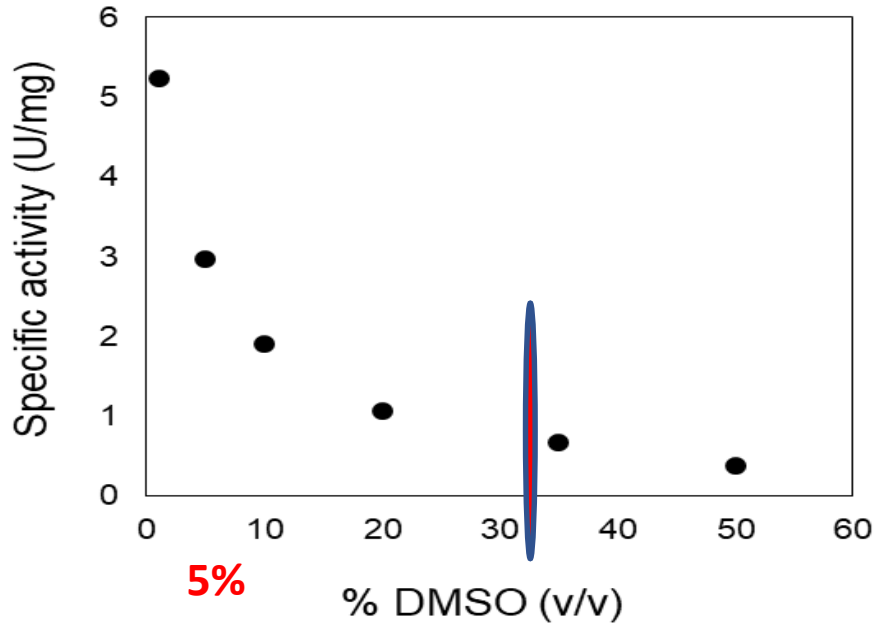
- Polar solvent miscible with water (ε = 46.7)
- Aprotic but form H-bonds as acceptor
- Has amphipathic nature -> dissolving poorly soluble polar and non-polar molecules
- High boiling temperature (190^{o}C), stable at high temperatures
- Chemically inert
- May not biochemically/biologically inert

Ring-closure reactions of PNSHH to PNSO

Kinetics



DMSO v/v (%)	k_d (min^{-1})	$t_{1/2}$ (h)	Residual activity after 24 h (%)
0	$2.22 \cdot 10^{-5} \pm 1.44 \cdot 10^{-6}$	520	97
30	$2.39 \cdot 10^{-5} \pm 5.21 \cdot 10^{-6}$	484	96.5
40	$2.81 \cdot 10^{-4} \pm 9.31 \cdot 10^{-6}$	41	67
50	$1.67 \cdot 10^{-2} \pm 2.53 \cdot 10^{-3}$	0.7	0



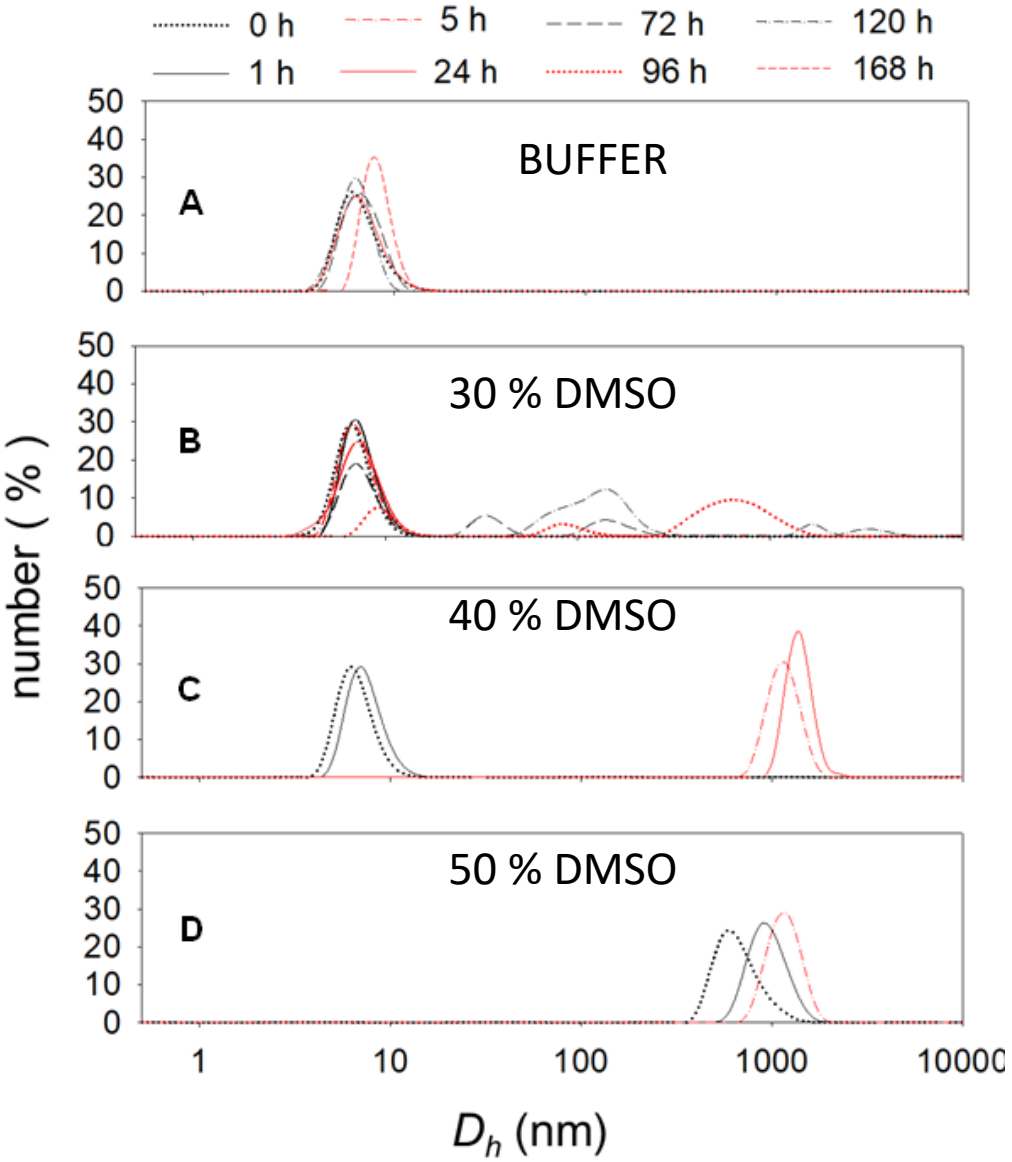
$$\frac{dS.A.}{dt} = -k_d \cdot t \qquad t_{1/2} = \frac{\ln 2}{k_d}$$

Mechanistic explanation

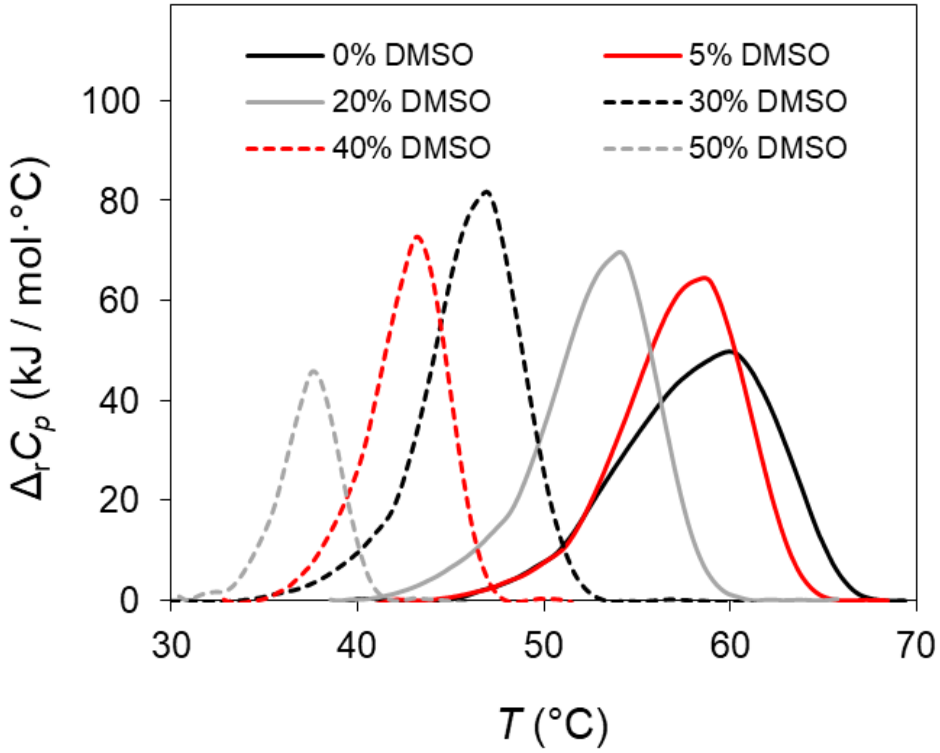
- By using **physical methods**

  - Dynamic Light Scattering (DLS) – particle size distribution
  - Differential Scanning Calorimetry (DSC) - thermal stability measurements
  - Molecular Dynamics (MD) simulations - MoA

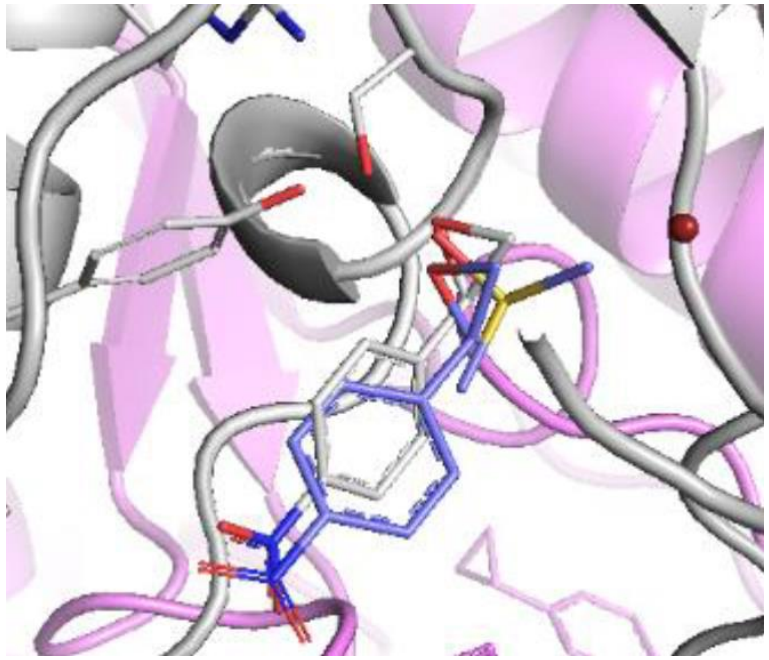
DLS – number size distibution



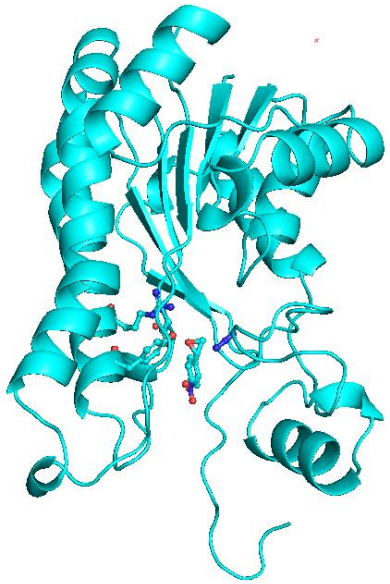
DSC thermograms



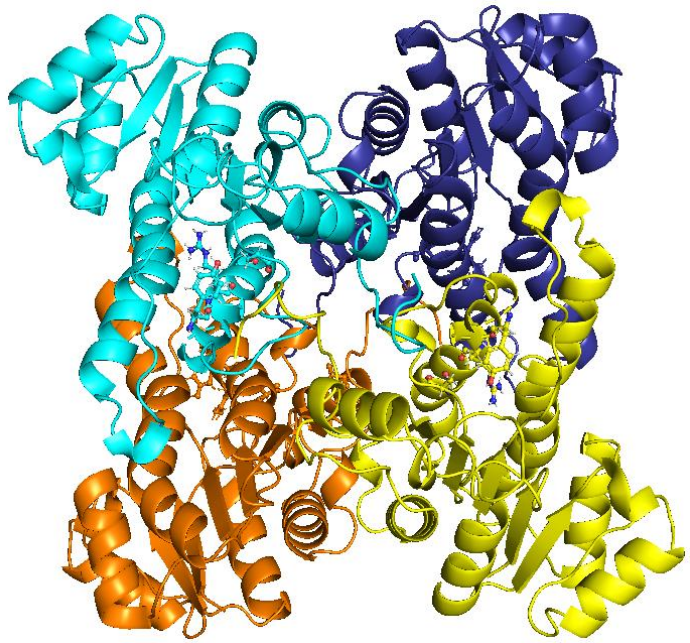
From monomer to tetramer MD simulations



Molecular docking



MD simulations on monomer



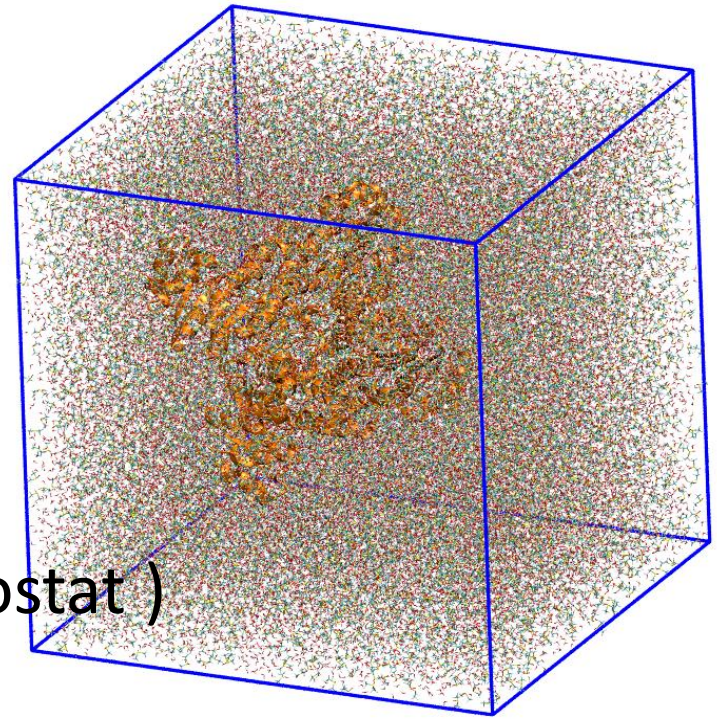
MD simulations on tetramer

FOCUS: DMSO EFFECTS ON TETRAMER STRUCTURE

All-atom MD calculations

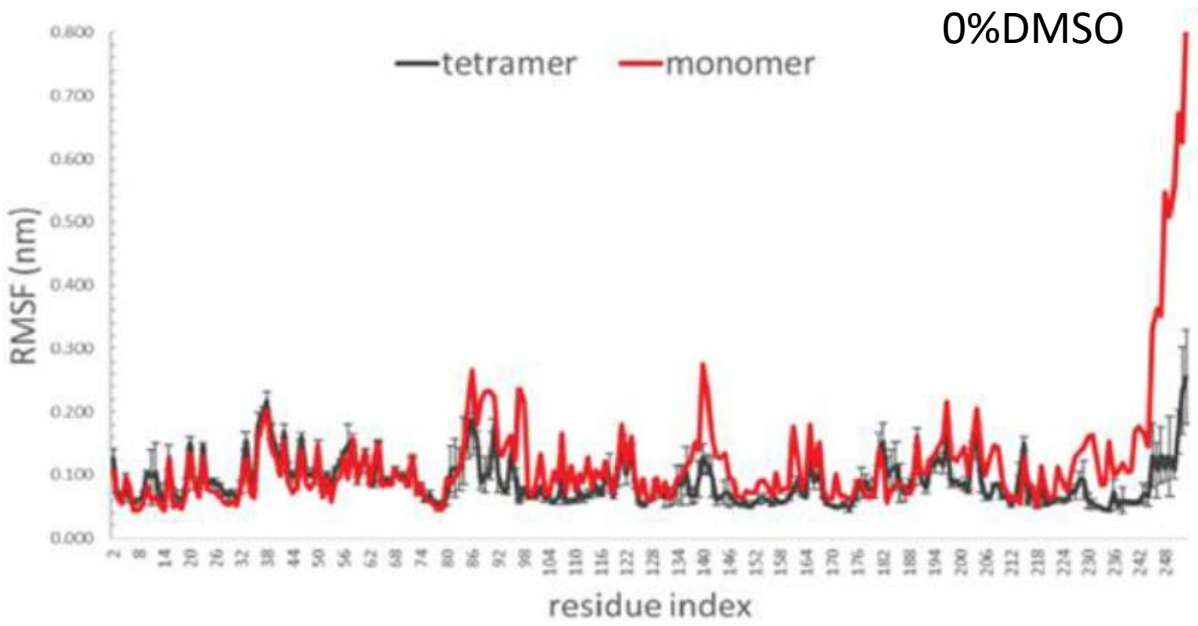
- PDB:1ZMT wild type HheC
- hypothetical monomer and tetramer
- In 0% DMSO , 20% v/v DMSO and 50% v/v DMSO
- Simulation times: 200-500 ns
- **Analyses: the last 100 ns of simulations**
- Water TIP3P, DMSO Amber
- NPT ensemble , at T = 298 K (Nose-Hoover thermostat)

& 1 bar (Parrinello-Rahman barostat)



0% v/v DMSO 80000 H_2O
50% v/v DMSO: 40000 H_2O/9200 DMSO

Monomer vs tetramer



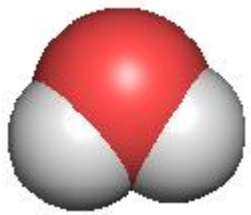
RMSF values of Cα atoms of amino acid residues

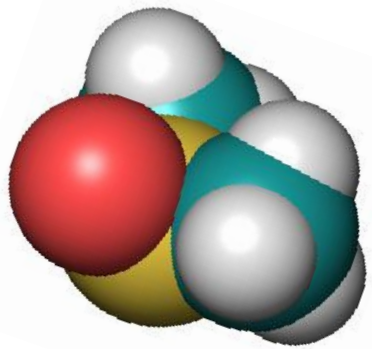
Table S1. Buried surface area for the tetramers calculated as the difference between four times SASA (solvent accessible surface area) of the hypothetical monomer and SASA of its respective tetramer.

DMSO v/v (%)	SASA / Å^2		Buried surface area / Å^2
	Hypothetical monomer	Tetramer	
0	13113	37158	**15294**
20	13301	37640	**15564**
50	13535	35977	**18163**

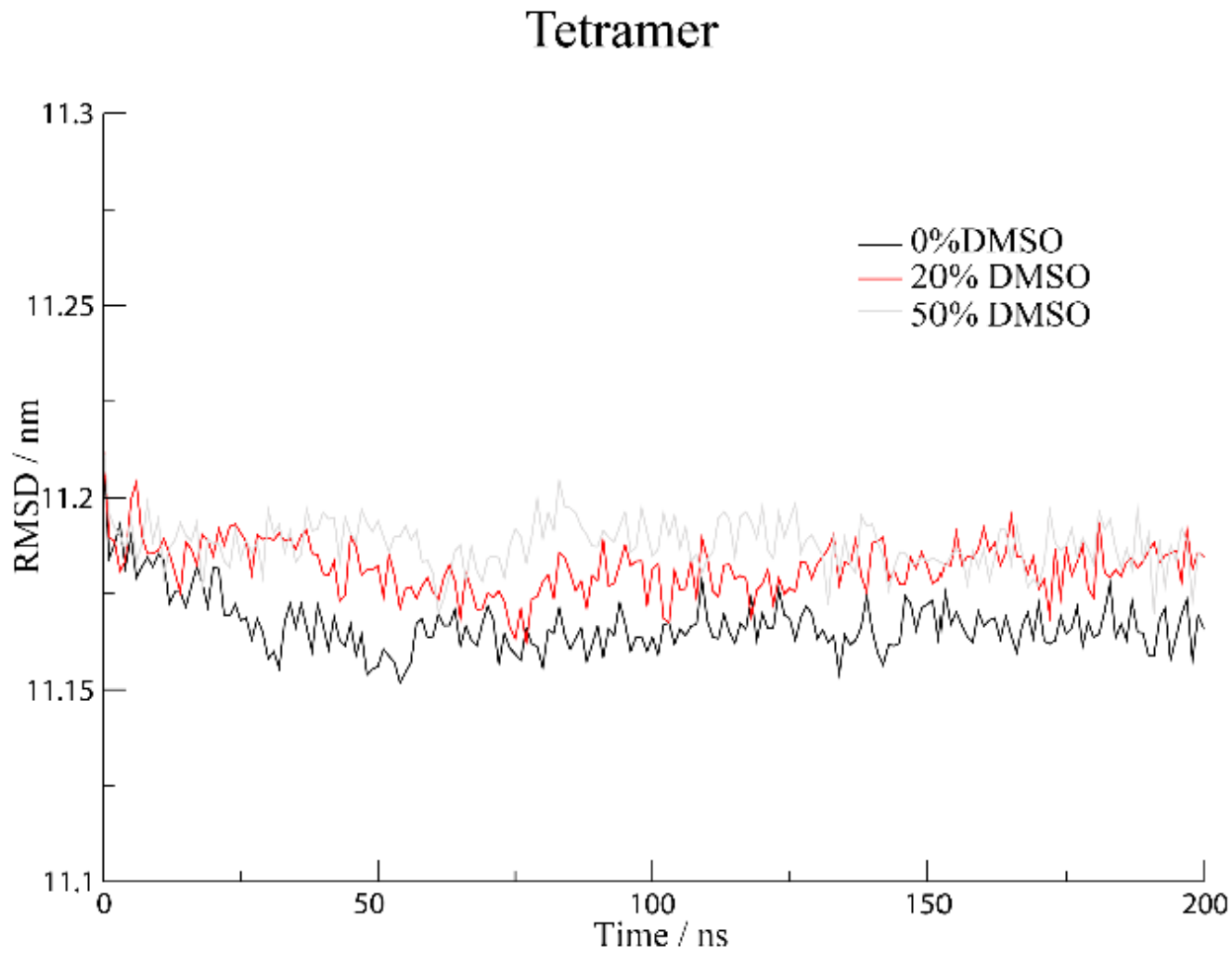
At tetramer surface

¶ DMSO·v/v·(%)¤	¶ SASA[a]·(Å²)·¤	Average·Number·of·Solvent·Molecules·in·the·First·Solvent·Shell[b]¤		Number·of·HBs[c]¤	
		H_2O¤	DMSO¤	H_2O¤	DMSO¤
0¤	37157.8·±·377.5¤	1846.9·±·29.5¤	/¤	1711.6·±·26.6¤	/¤
20¤	37639.6·±·310.4¤	1584.0·±·25.3¤	144.5·±·9.6¤	1592.5·±·21.6¤	36.8·±·5.8¤
50¤	35977.1·±·244.9¤	1243.1·±·21.3¤	281.0·±·11.8¤	1335.8·±·21.2¤	73.0·±·7.0¤



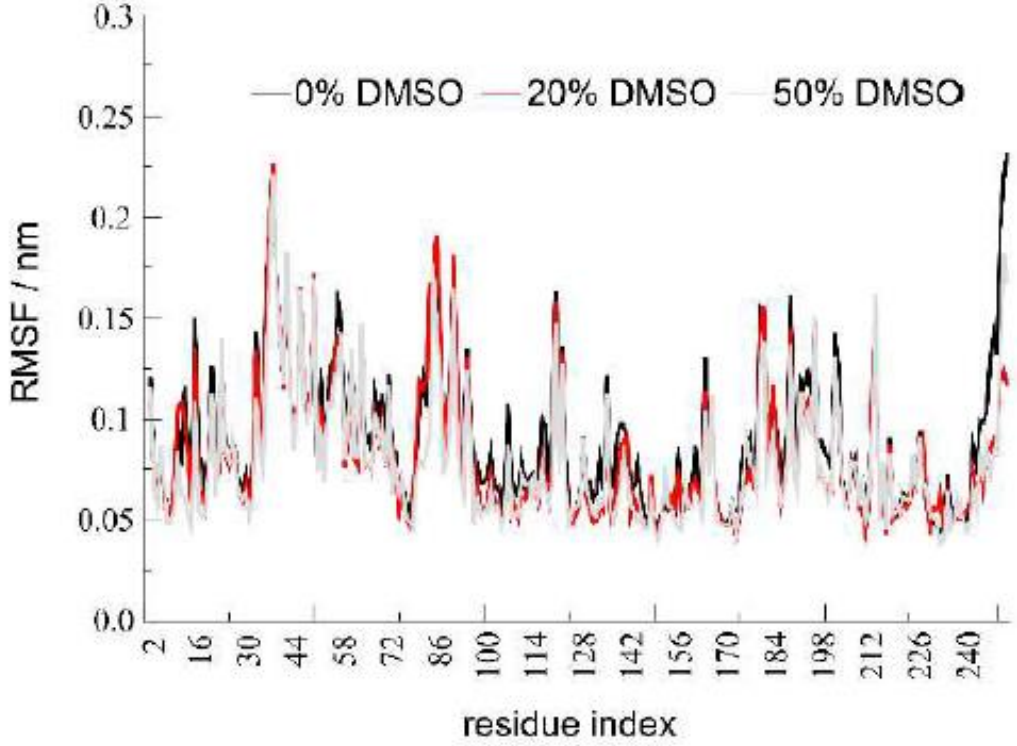


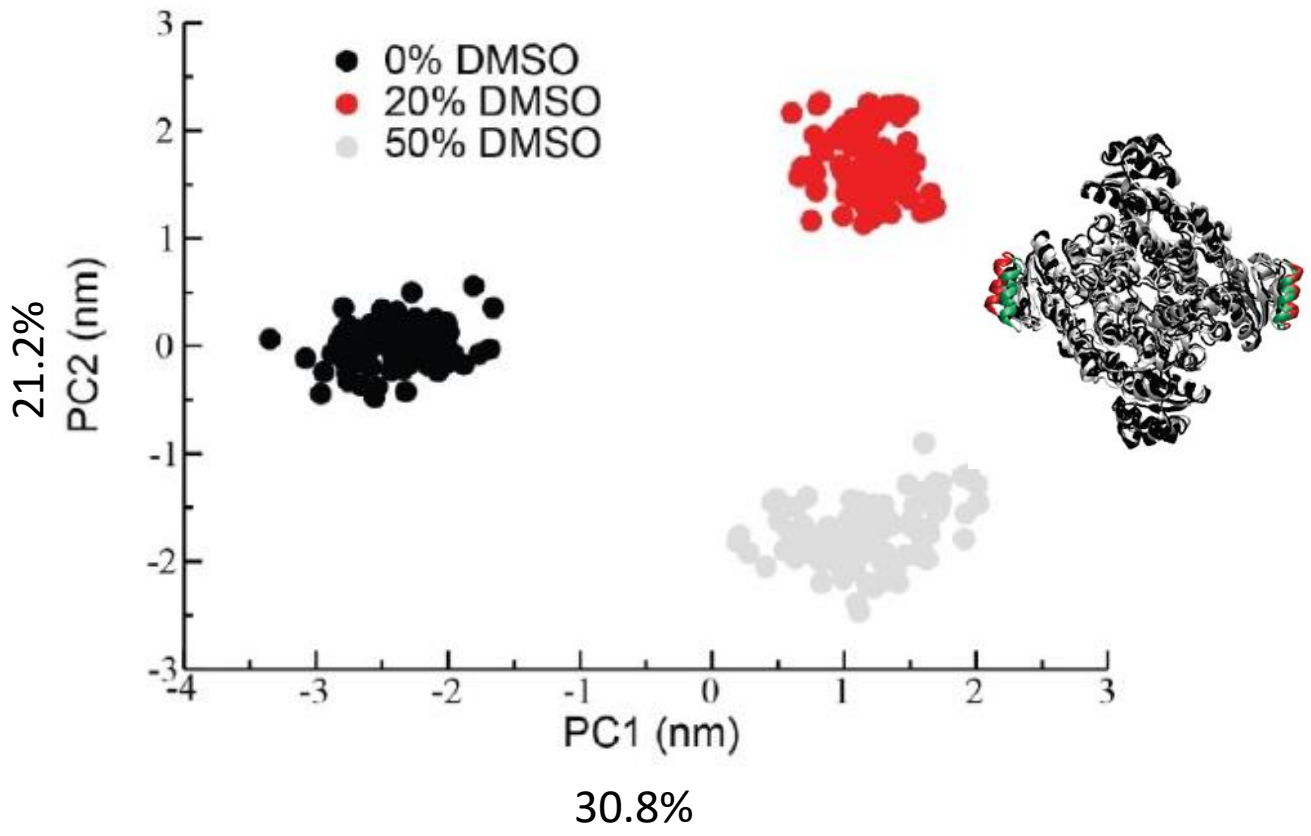
HheC stays stable during 250 ns and longer of simulations

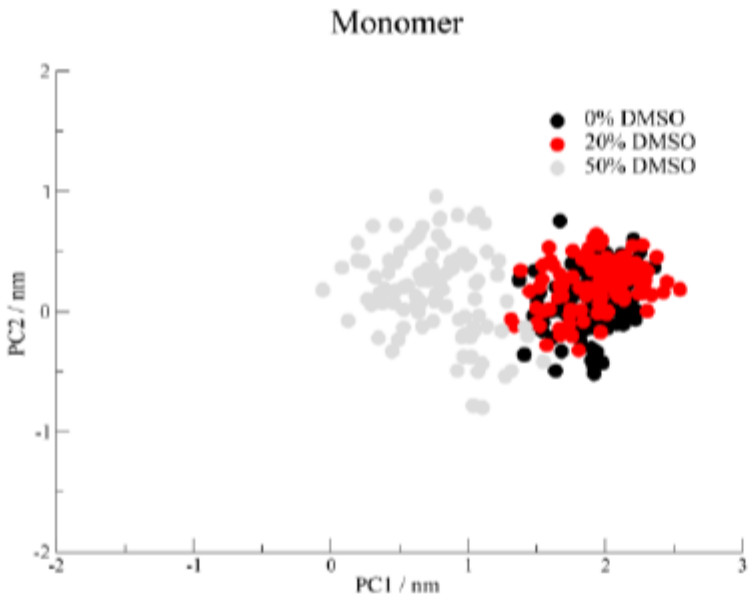


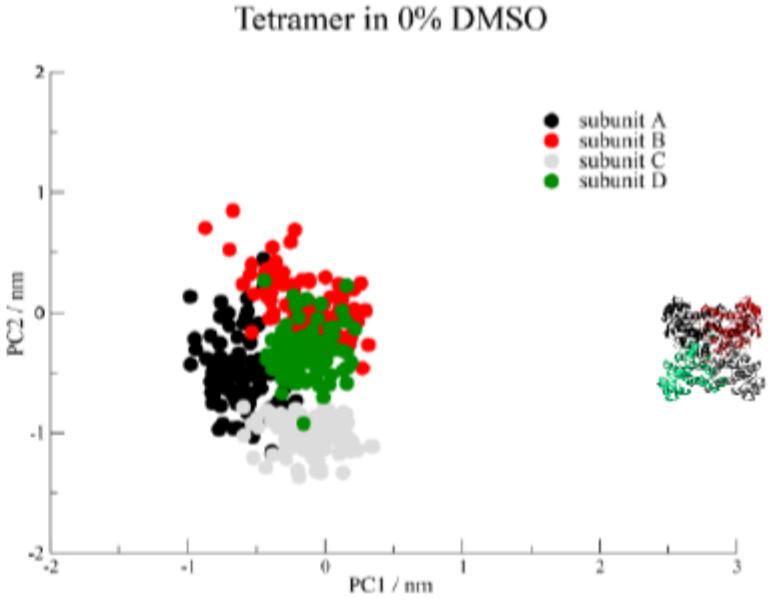
HheC

All Cα atoms from 4 subunits from 3 simulations

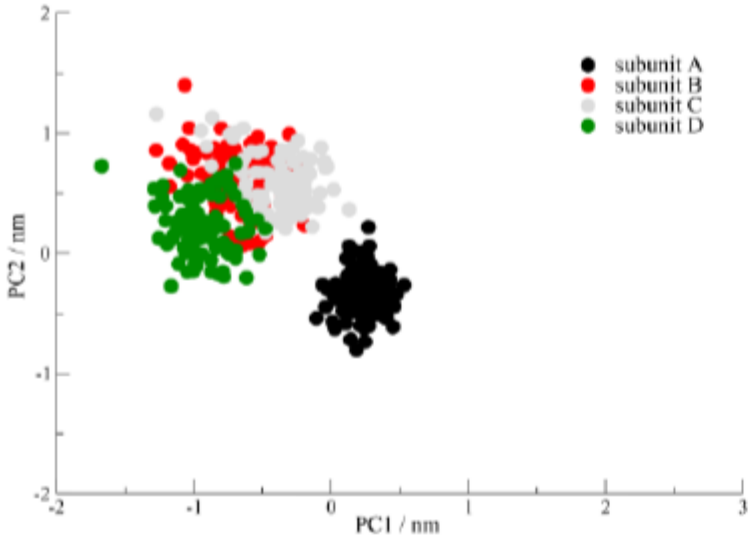


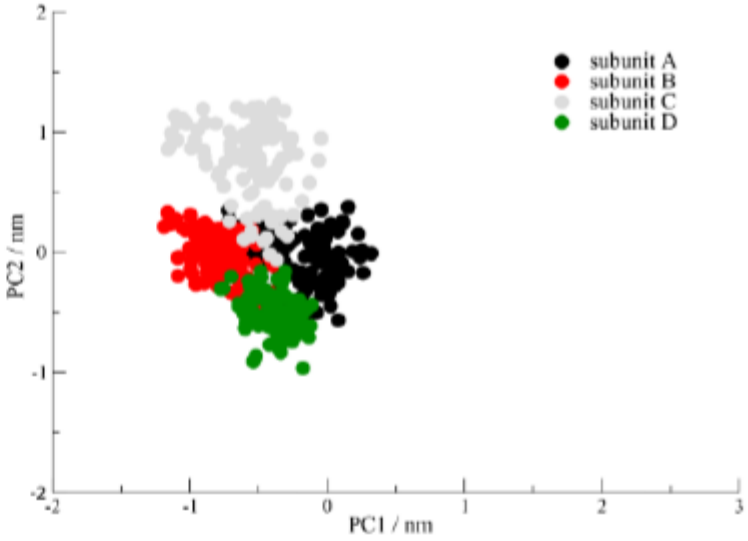






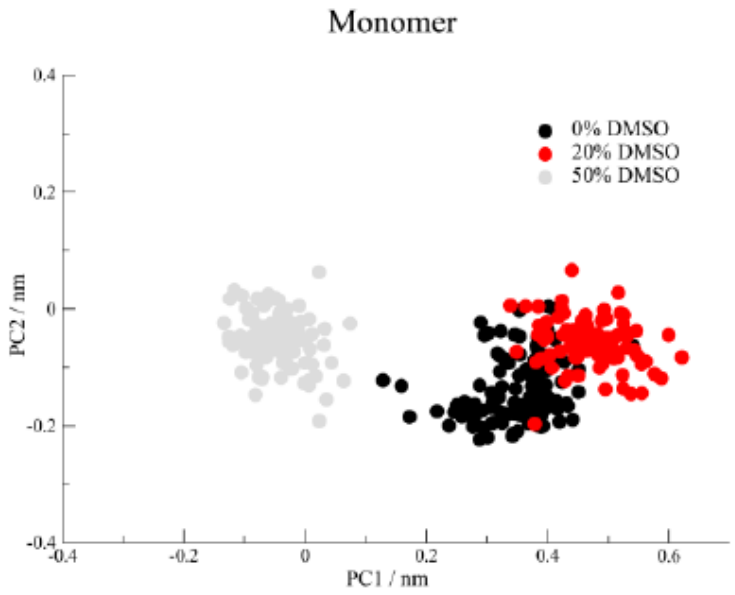
Principal component analysis (PCA) of the simulated systems without C-terminal tail

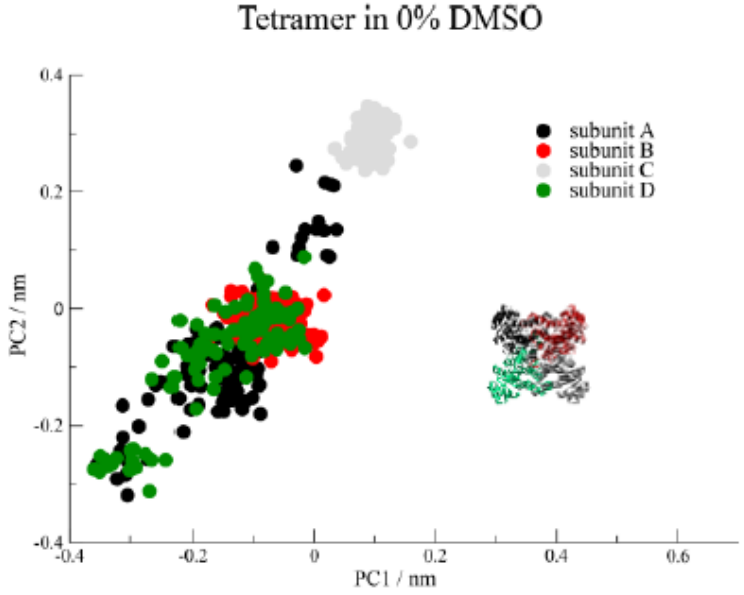


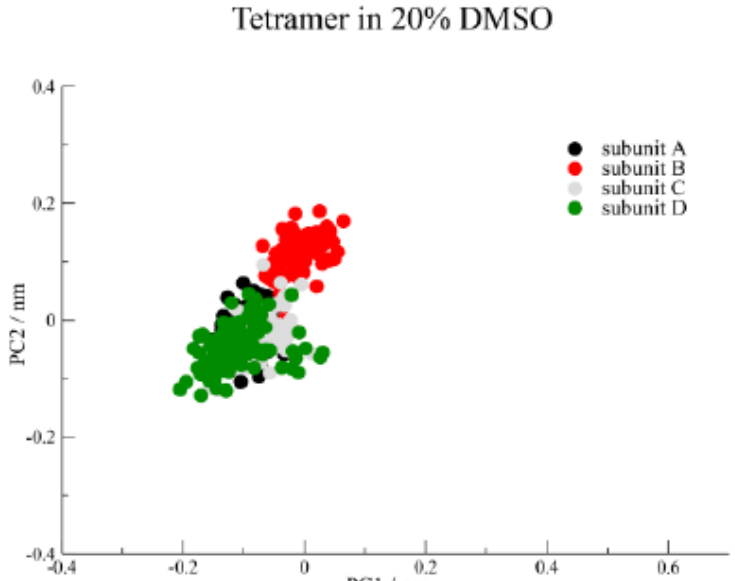


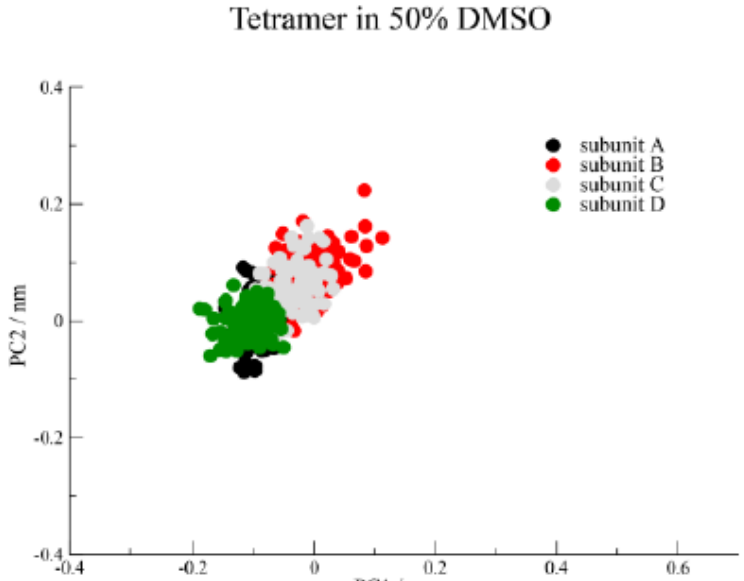
Conformational space of hypothetical monomer ≠ conformational space of tetramer subunits, particularly in DMSO/water mixtures.

In the active site



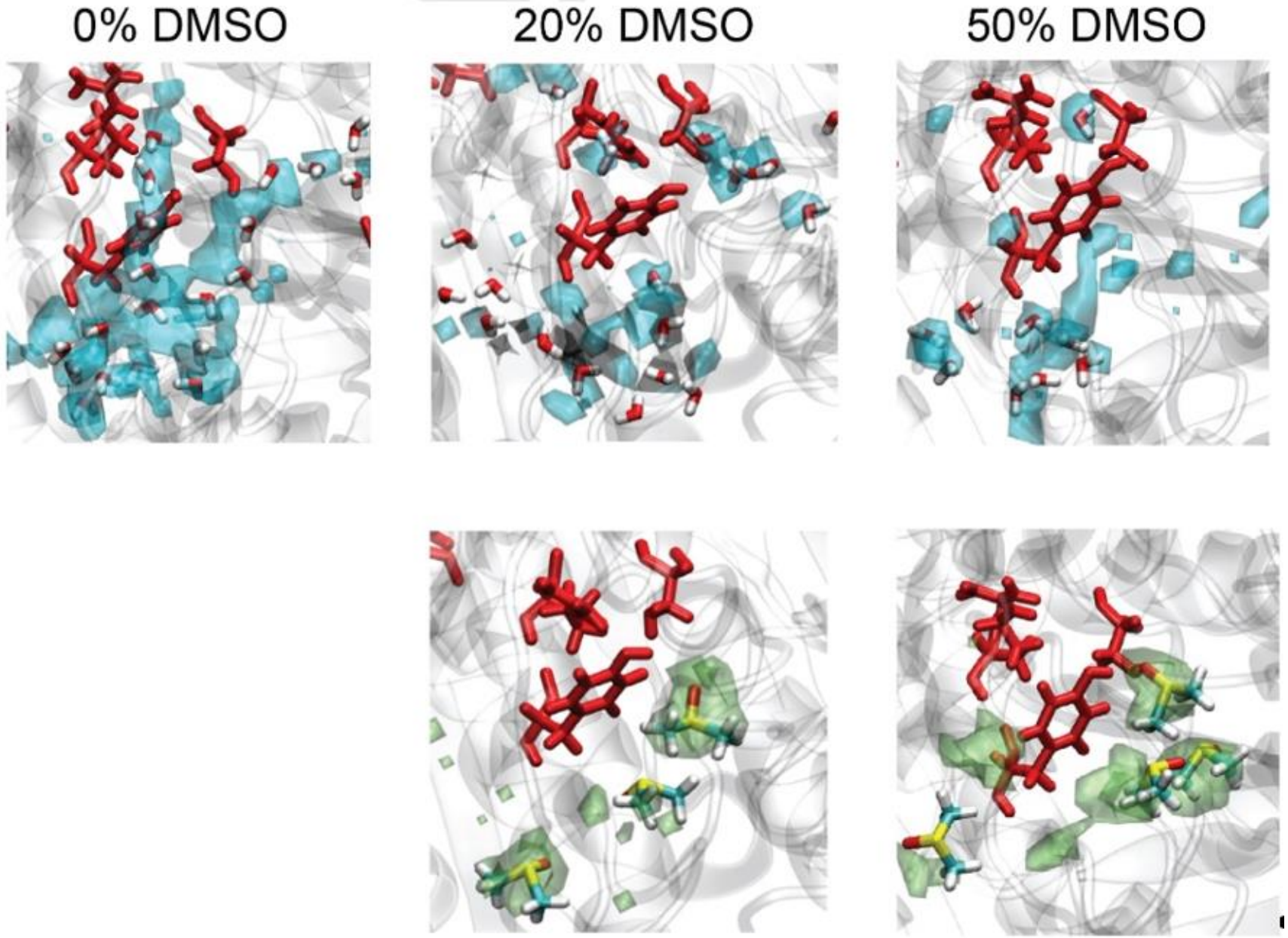






PCA by taking into account Cα atoms of the residues 132 - 149, constituting active site and the region surrounding it, of conformations of all monomers, stemming from the hypothetical monomer simulations and subunits constituting the tetramer

In the active site



Volumetric maps (isovalue = 0.5) of water (blue) and DMSO (green) inside the substrate binding site of HheC (4 Å from Ser132)

Volumetric maps of water and DMSO inside the substrate binding sites

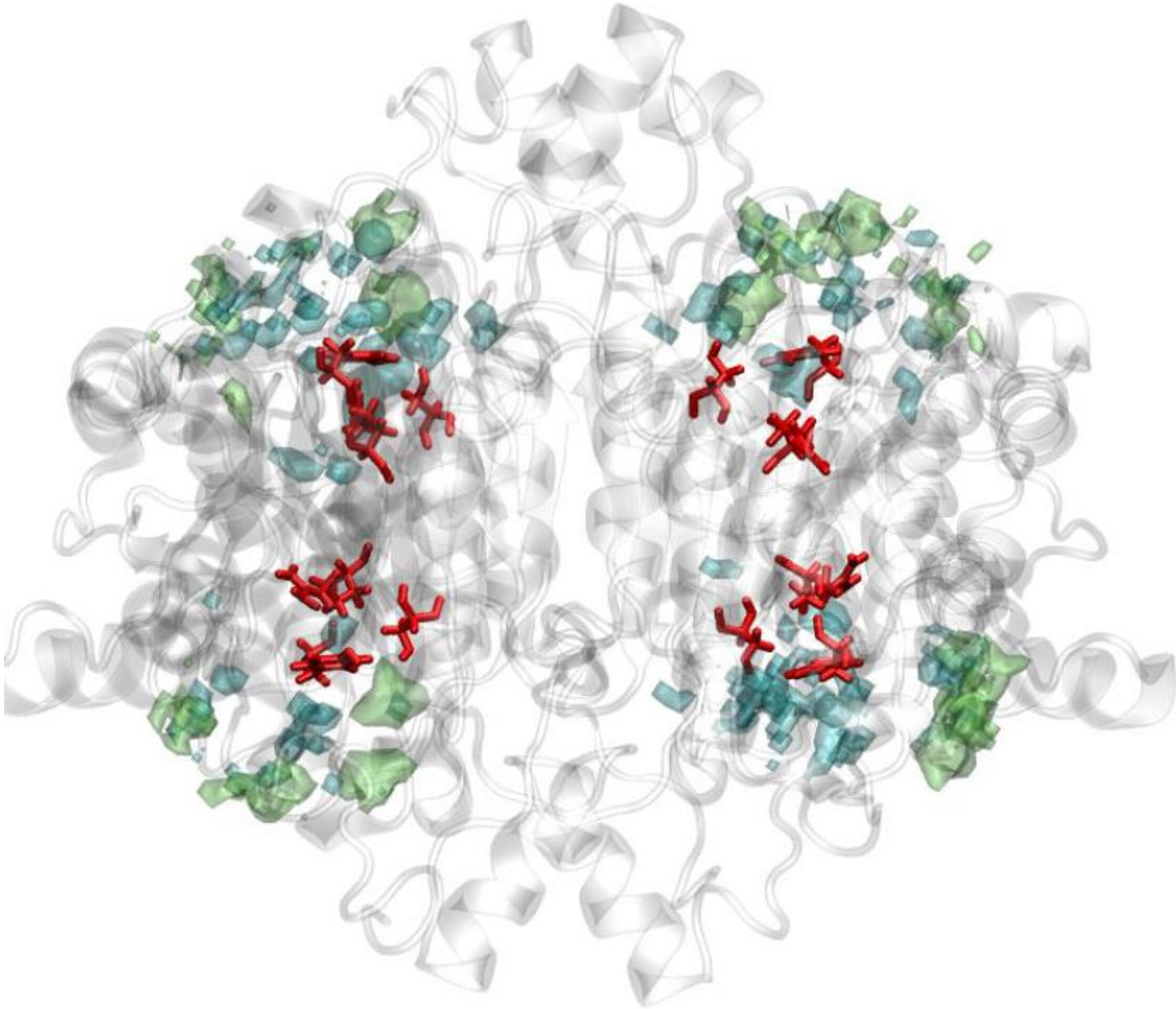


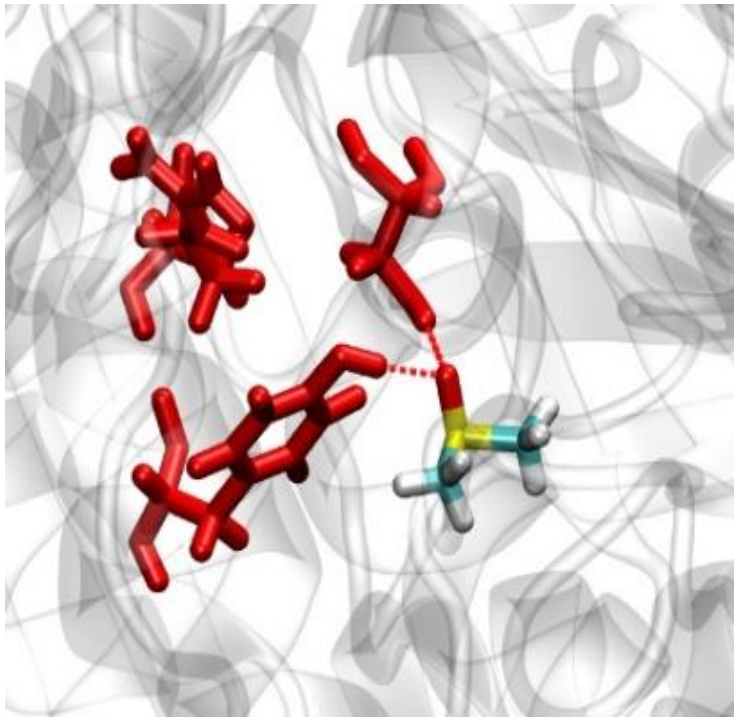
Table 4. Average numbers of solvent molecules (DMSO or water) and their HBs with the residues Ser132 or Tyr145 in the active site for HheC tetramer

DMSO v/v (%)	Average Number of Solvent Molecules				Number of HBs[c] (Ser132/Tyr145)	
	Substrate binding site[a]		Halide binding site[b]			
	H_2O	DMSO	H_2O	DMSO	H_2O	DMSO
0	19.9 ± 2.0	/	1.7 ± 0.4	/	2.1 ± 0.5	/
20	15.5 ± 1.8	2.6 ± 0.5	1.7 ± 0.3	0.3 ± 0.2	1.5 ± 0.5	0.3 ± 0.3
50	11.1 ± 1.5	2.8 ± 0.5	1.3 ± 0.3	0.2 ± 0.2	2.1 ± 0.5	0.8 ± 0.3

[a] Average number of solvent molecules inside the ligand binding region defined as the region inside a sphere of radius of 8 Å centred around the centre of mass of Tyr187. [b] Defined as the intersection of the two conditions; all water molecules found at or below 5 Å from Cα atom of His179 AND all water molecules found at or below 8 Å from Cα atom of Tyr187. [c] Average number of HBs between solvent molecules and Ser132 OR Tyr145. Only HBs with distance below 3.5 Å and angle below 30°, are counted.

Section Break (Continuous)

Alternate H-bonds of DMSO with catalytic Ser132 and Tyr145



The mean residence time in the vicinity of OH-groups monitored within radius of 4 Å from OH-group of Ser132 is for

- Water – 1 - 2.8 ns for the studied systems
- DMSO – 8.5 ns in 20% v/v DMSO
- DMSO – 23 ns in 50% v/v DMSO

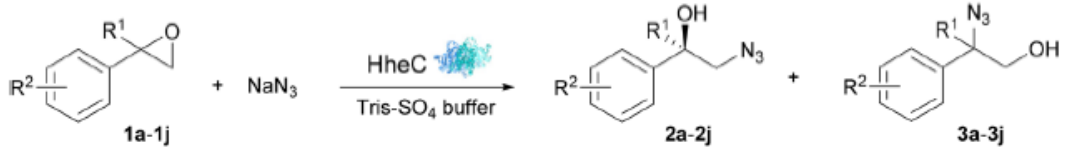
Inhibitory Effect of DMSO on Halohydrin Dehalogenase: Experimental and Computational Insights into the Influence of an Organic Co-solvent on the Structural and Catalytic …
N Milčić, V Stepanić, I Crnolatac, Z Findrik Blažević, Z Brkljača, ...
Chemistry–A European Journal 28 (56), e202201923

gmx hbond

Explanation for new types of substrates and inversion of stereospecificity for HheC – M4

Panel of fluorinated aromatic epoxides /styrene oxides

WT HheC



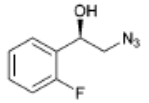
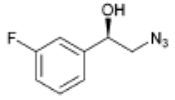
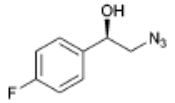
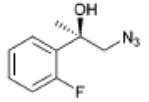
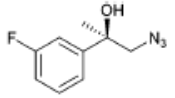
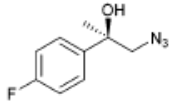
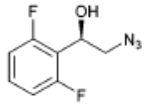
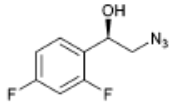
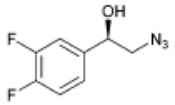
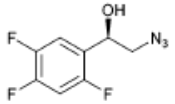
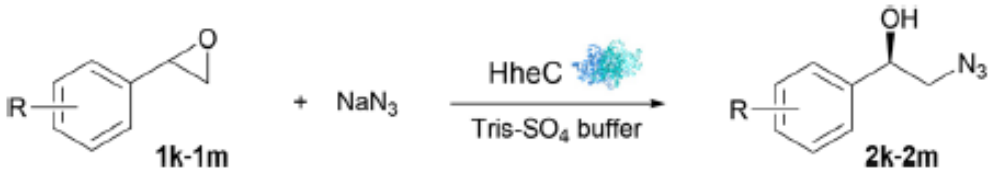
Entry	Substrate	t (h)	Conv. (%)	ee 1[b] (%)	Product 2	ee 2[b] (%)	Ratio 2 : 3[c]	E value
1	1a	3	30	35 (*S*)		83 (*R*)	99 : 1	15
2	1b	3	43	68 (*S*)		91 (*R*)	96 : 4	43
3	1c	2	43	73 (*S*)		98 (*R*)	94 : 6	>200
4	1d	3	nd[d]	nd[d]		nd[d]	nd[d]	nd[d]
5	1e	3	36	56 (*S*)		98.5 (*R*)	100 : 0	>200
6	1f	2	46	84 (*S*)		>99 (*R*)	100 : 0	>200
7	1g	3	nd[d]	nd[d]		nd[d]	nd[d]	nd[d]
8	1h	3	42	59 (*S*)		81 (*R*)	100 : 0	17
9	1i	3	47	88 (*S*)		98.5 (*R*)	100 : 0	>200
10	1j	3	36	52 (*S*)		91 (*R*)	99 : 1	36

Table 3 HheC-catalysed azidolysis of CF_3-substituted styrene oxides (**1k–1m**)[a]



Entry	Epoxide	R	HheC	t (h)	Conv.[b] (%)	ee 1[c] (%)	ee 2[d] (%)	E value
1	1k	*o*-CF_3	WT	3	na[e]	—	—	—
2	1k	*o*-CF_3	T134A	3	na[e]	—	—	—
3	1k	*o*-CF_3	N176A	3	nd[f]	nd[f]	nd[f]	nd[f]
4	1k	*o*-CF_3	M4 (P84V/F86P/T134A/N176A)	1	50	84	82 (*S*)	27
5	1l	*m*-CF_3	WT	2	46	82	95 (*R*)	100
6	1m	*p*-CF_3	WT	2	46	85	>99 (*R*)	>200

[a] Reaction conditions: epoxide (2 mM), NaN_3 (3 mM), 250 μL HheC, Tris-SO_4 buffer (2 mL, 0.5 M, pH 7.0), 5% DMSO, total volume of 2.5 mL. [b] Catalysed by the enzyme. [c] Determined by GC (for more details, see Table S1†). [d] Determined by HPLC (for more details, see Table S2†). [e] na = no activity. [f] nd = not determined due to very low enzyme activity.

Reversed activity and inversed stereoselectivity

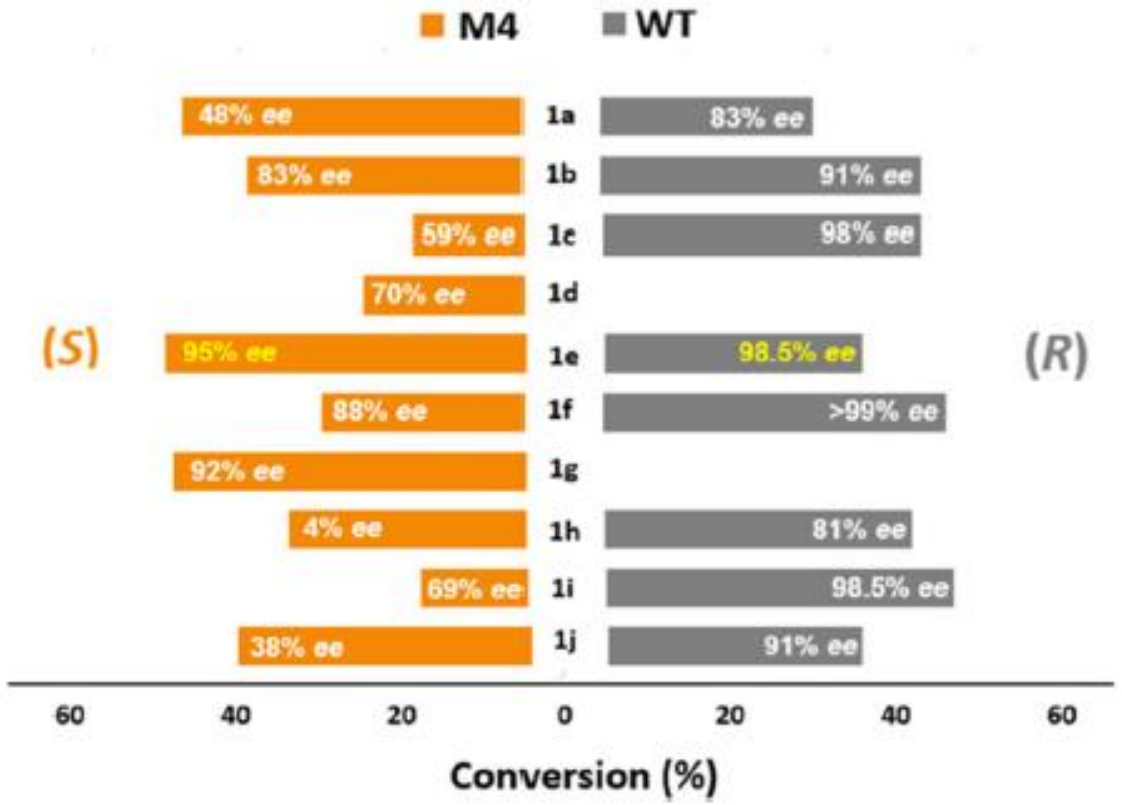


Fig. 3 HheC-catalysed azidolysis of epoxides **1a–1j**. Results for WT and M4 are given after 3 h and 1 h of the reaction time, respectively.

- Homology model (SWISS MODEL) and molecular docking – insufficient explanation

All-atom MD calculations

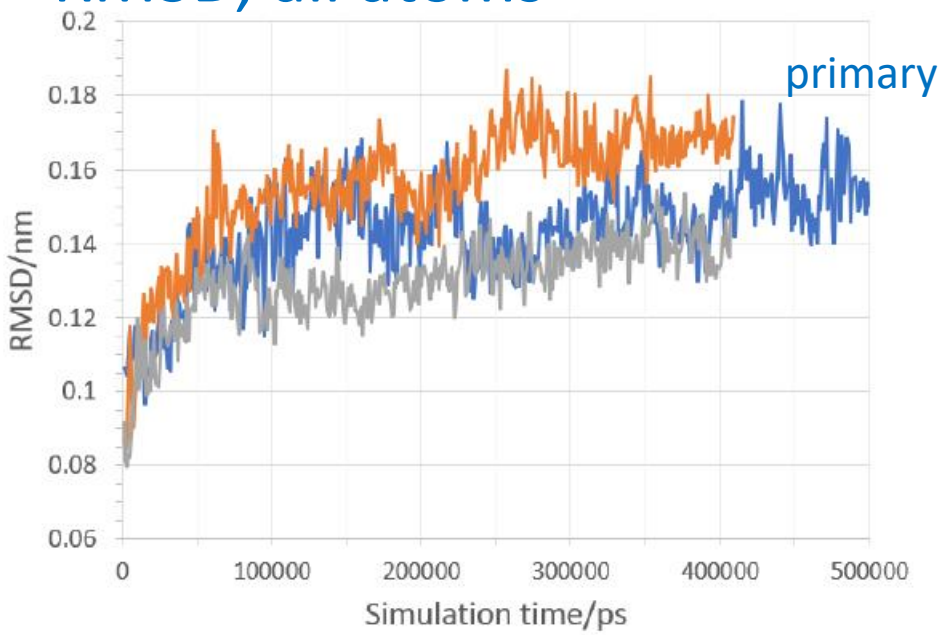
- PDB:1ZMT wild type HheC, with the mutations P84V/F86P/T134A/N176A
- Large solvent box: 80000 H2O, Na^+, Cl^- TIP3P water model and via parameters developed by Cheatham III et al. , resp.
- AMBER force field
- NPT ensemble , at T = 298 K (Nose-Hoover thermostat)

& 1 bar (Parrinello-Rahman barostat)

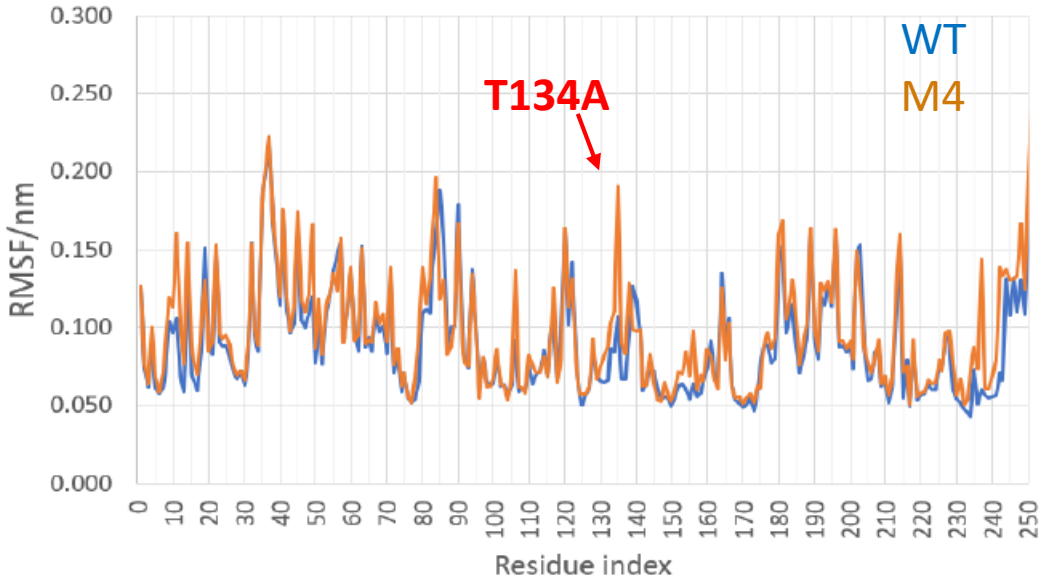
- Simulation times: 500 ns, 400 ns, 400 ns
- Analyses: without first 100 ns of simulations

HheC – M4

RMSD, all atoms

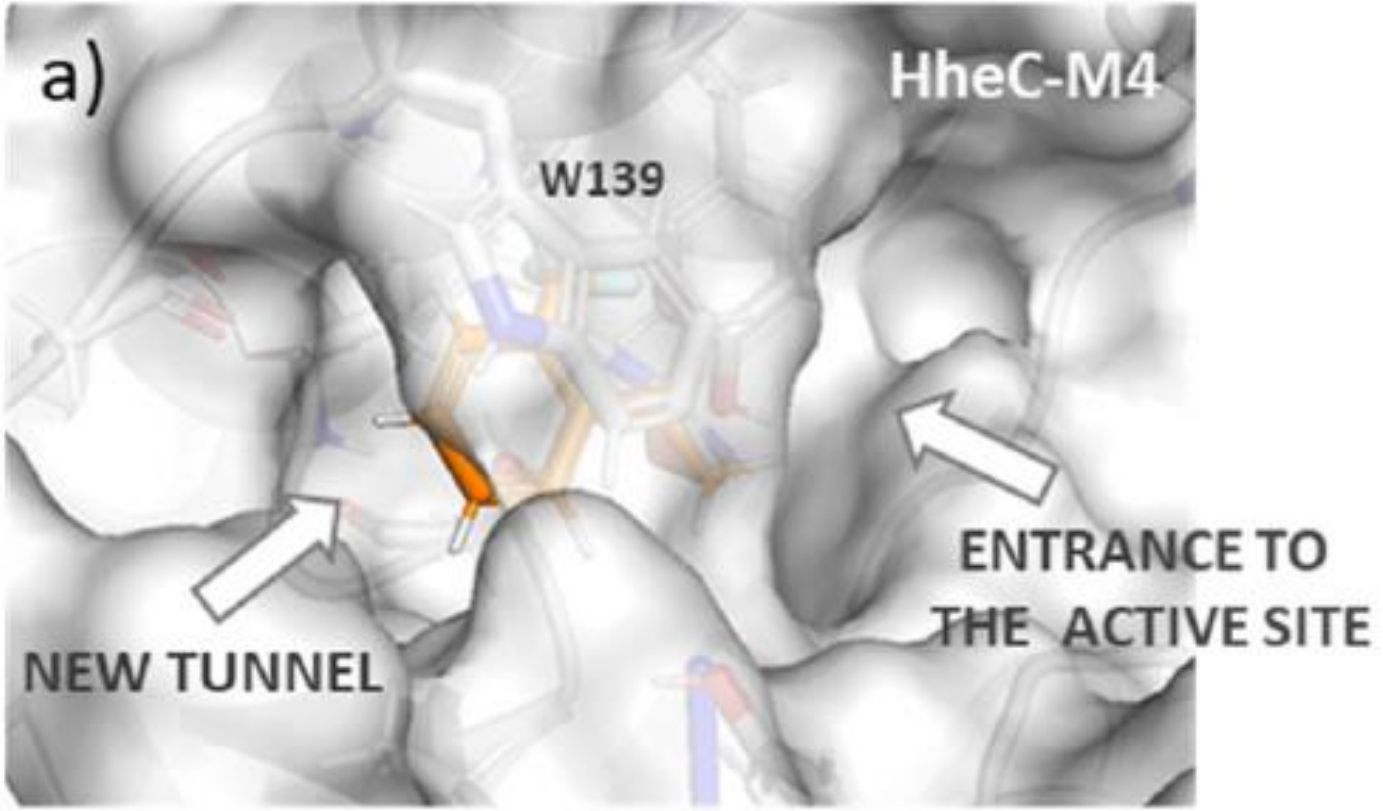


RMSF, all atoms from 12 subunits (4x3)



P84V/F86P/**T134A**/N176A

Molecular docking by GOLD



“Conformational selection” hypothesis

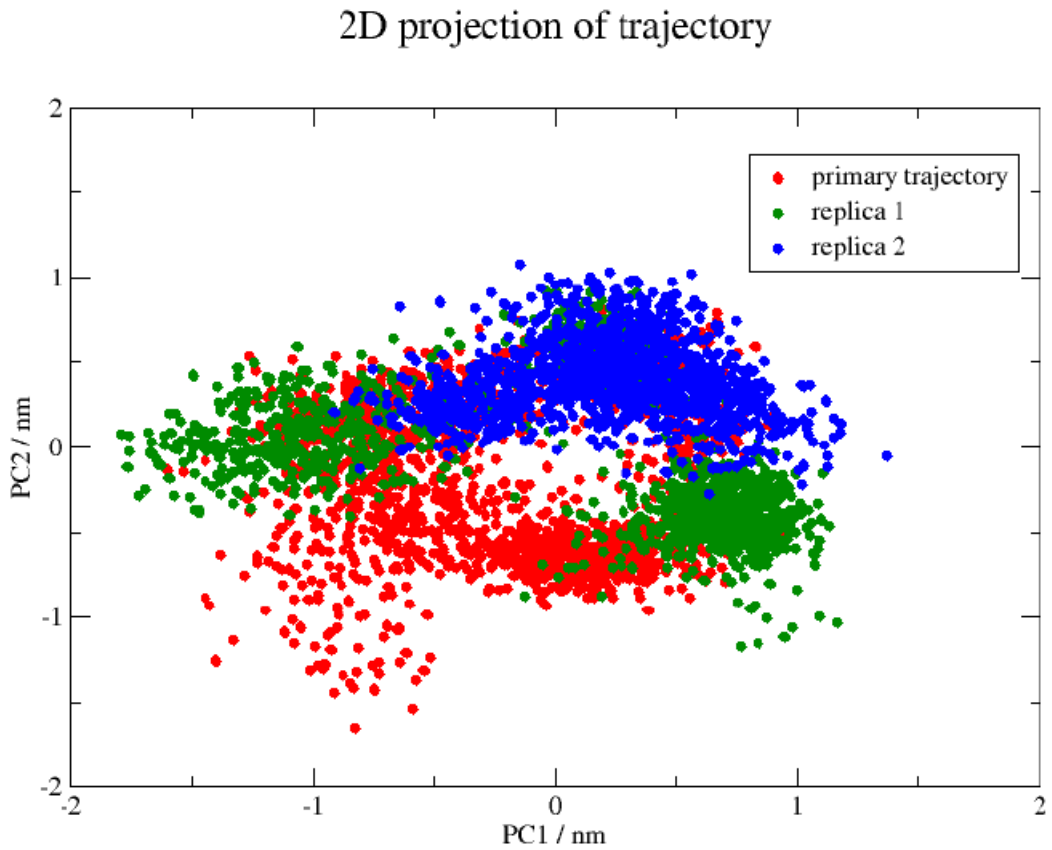
For selection of conformations for molecular docking

Jarvis-Patrick clustering on all atoms encapsulated with 5 Å sphere around the 1ZMT substrate placed near the catalytic residues Ser132 and Tyr 145

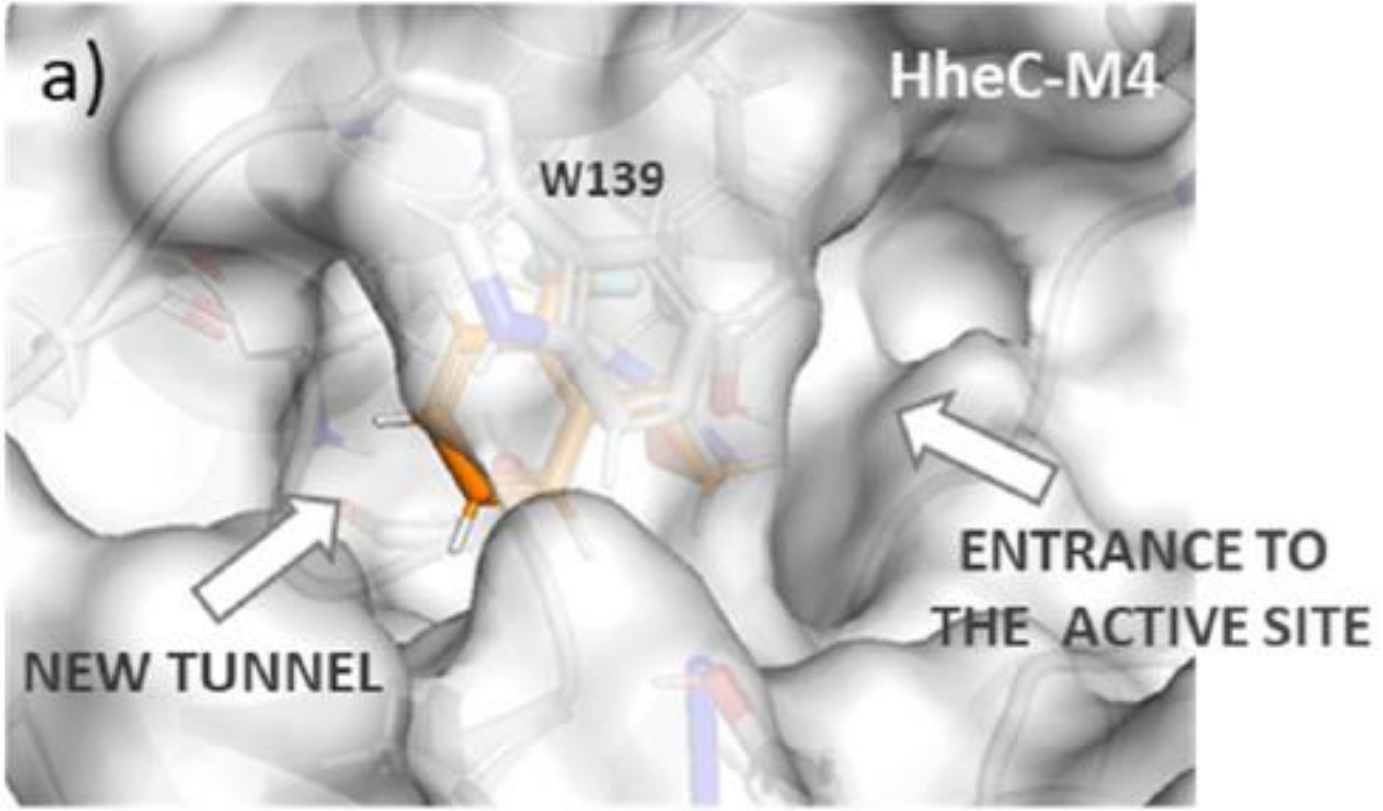
Representative conformations (7/58) were selected from clusters populated with more than 5% of all conformations (in all 80.4%) and having the mutual position of the catalytic residues Ser132 and Tyr145 close to those in the HheC 1ZMT structure.

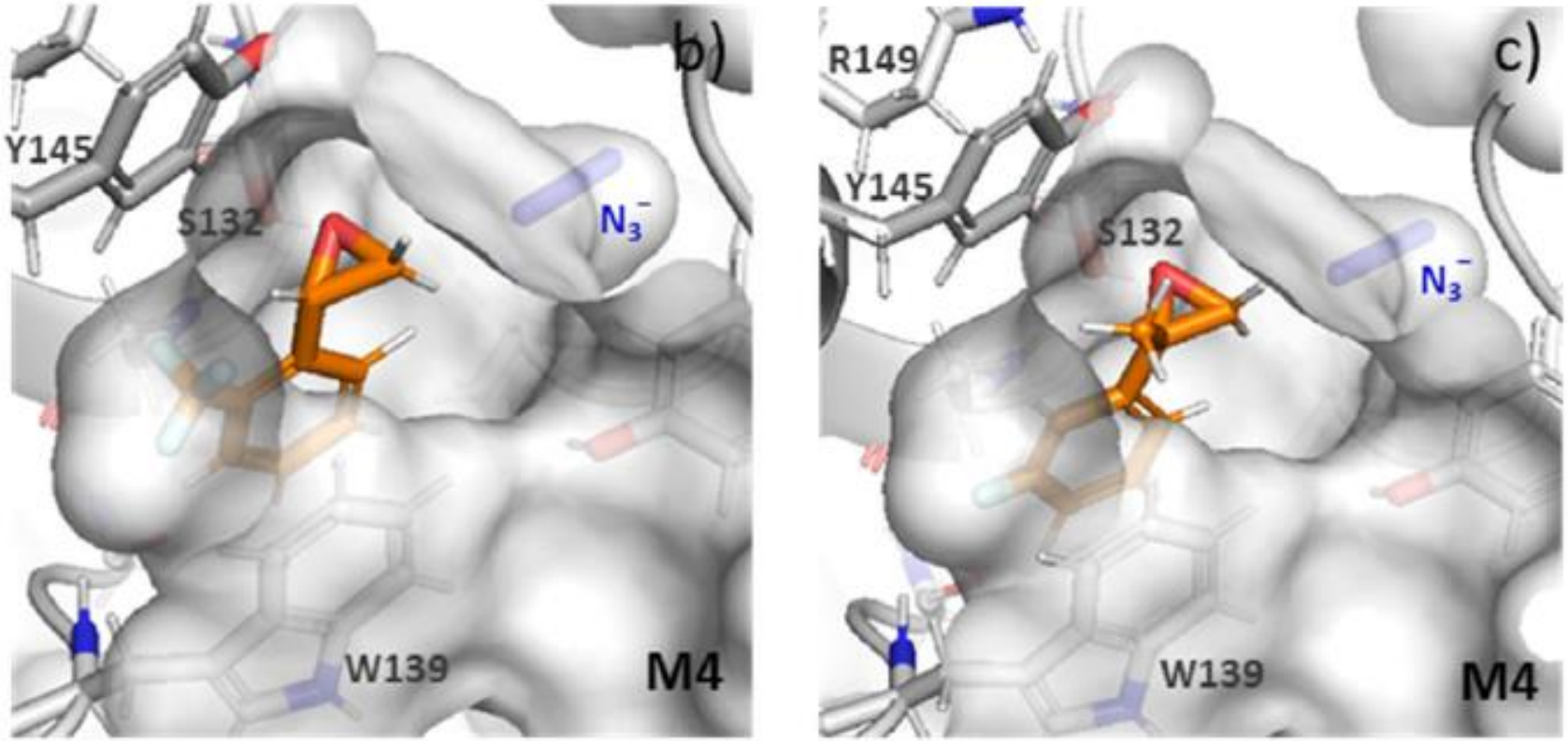
The most populated Jarvis-Patrick cluster (31.3% of the conformation space) for interpretation

4000 conformations of all subunits extracted each 10 ns, thus accounting for 1µs simulation time



Molecular docking by GOLD





Biocatalytic approach to chiral fluoroaromatic scaffolds

I Dokli, Z Brkljača, P Švaco, L Tang, V Stepanić, MM Elenkov

Organic & Biomolecular Chemistry 20 (48), 9734-9741

Conclusions

MD simulations provide mechanistic interpretation for the wet experimental results

- For negative influence of DMSO on catalytic activity HheC
  - Tetramer simulations
  - DMSO acts as a mixed-type inhibitor
  - Competitive inhibitor till 30%v/v DMSO
  - DMSO shows tendency to form small patches close to the protein surface what may cause aggregation in the presence of 50% v/v DMSO

- For favopurable extension of substrate space and inversion of stereoselectivity of quadrupole mutant HheC P84V/F86P/T134A/N176A
  - Formation of new binding channel by T134A/N176A and losing favourable interactions with F86P

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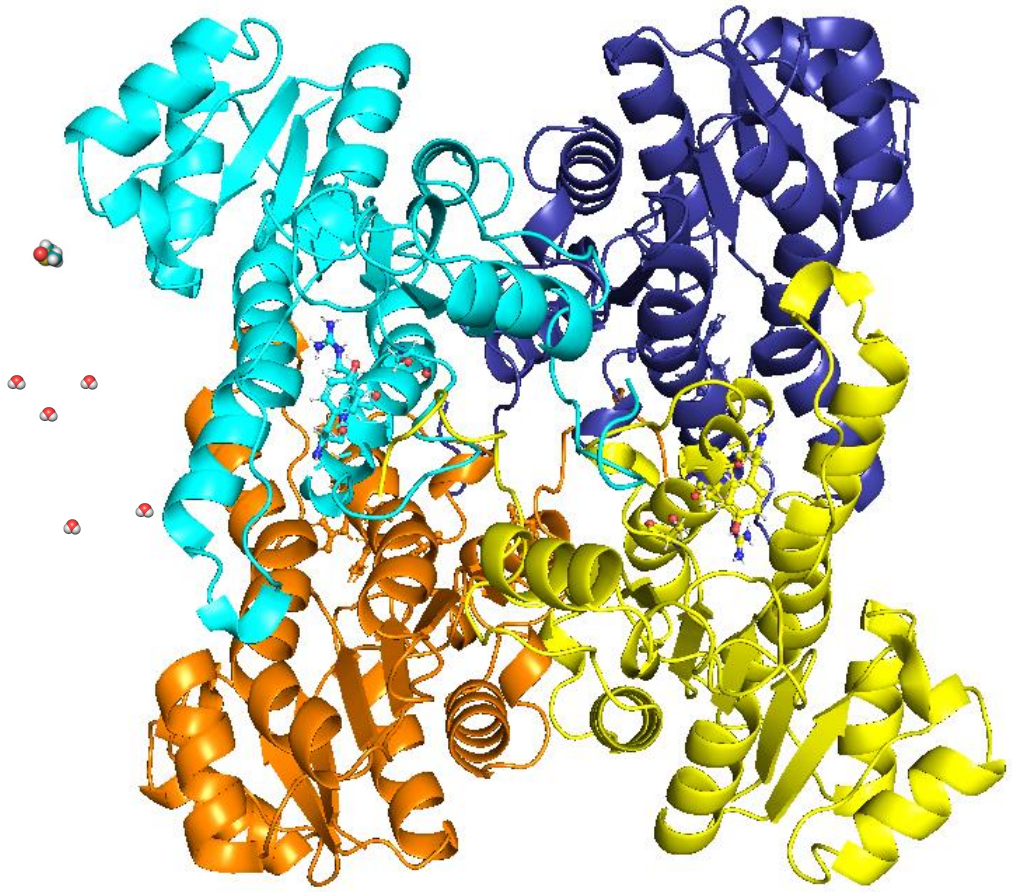

THANK YOU FOR YOUR ATTENTION