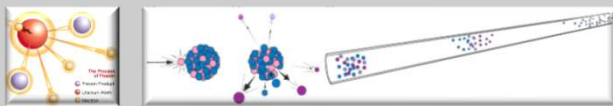


Accelerating structure-based design of rapid uncharged reactivators of organophosphate-inhibited human acetylcholinesterase by joint X-ray/neutron mechanistic studies

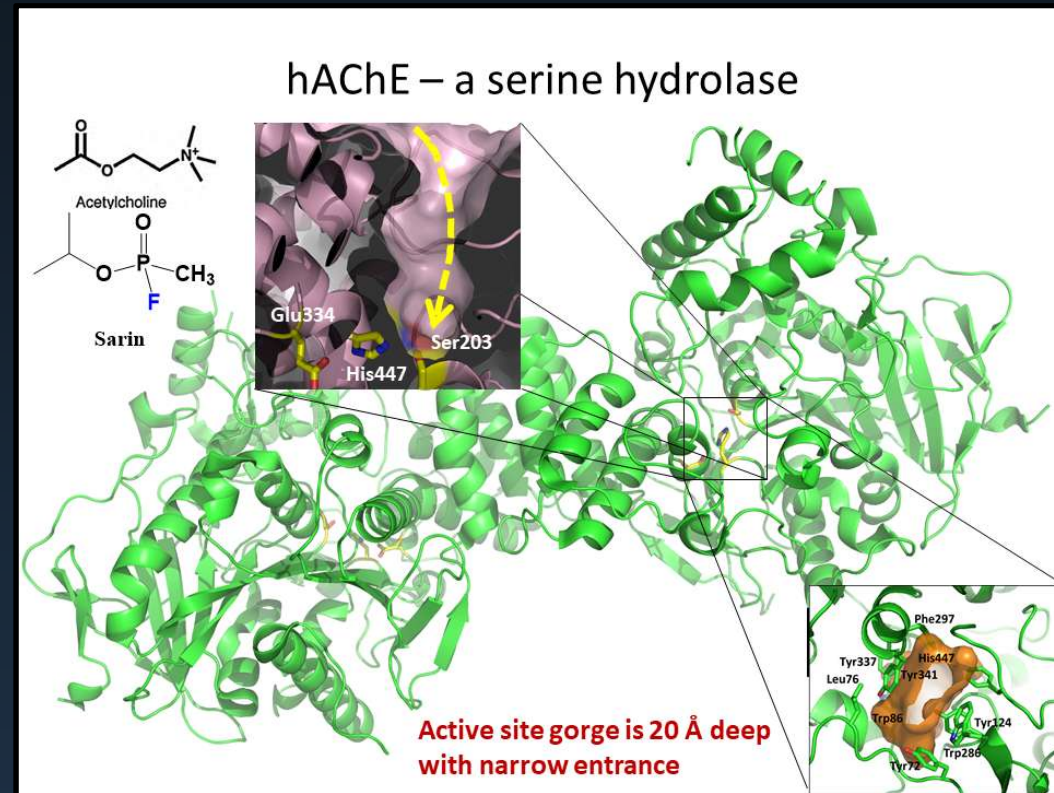
Andrey Kovalevsky
Neutron Scattering Division
Oak Ridge National Laboratory
Oak Ridge, TN

ORNL is managed by UT-Battelle, LLC for the US Department of Energy

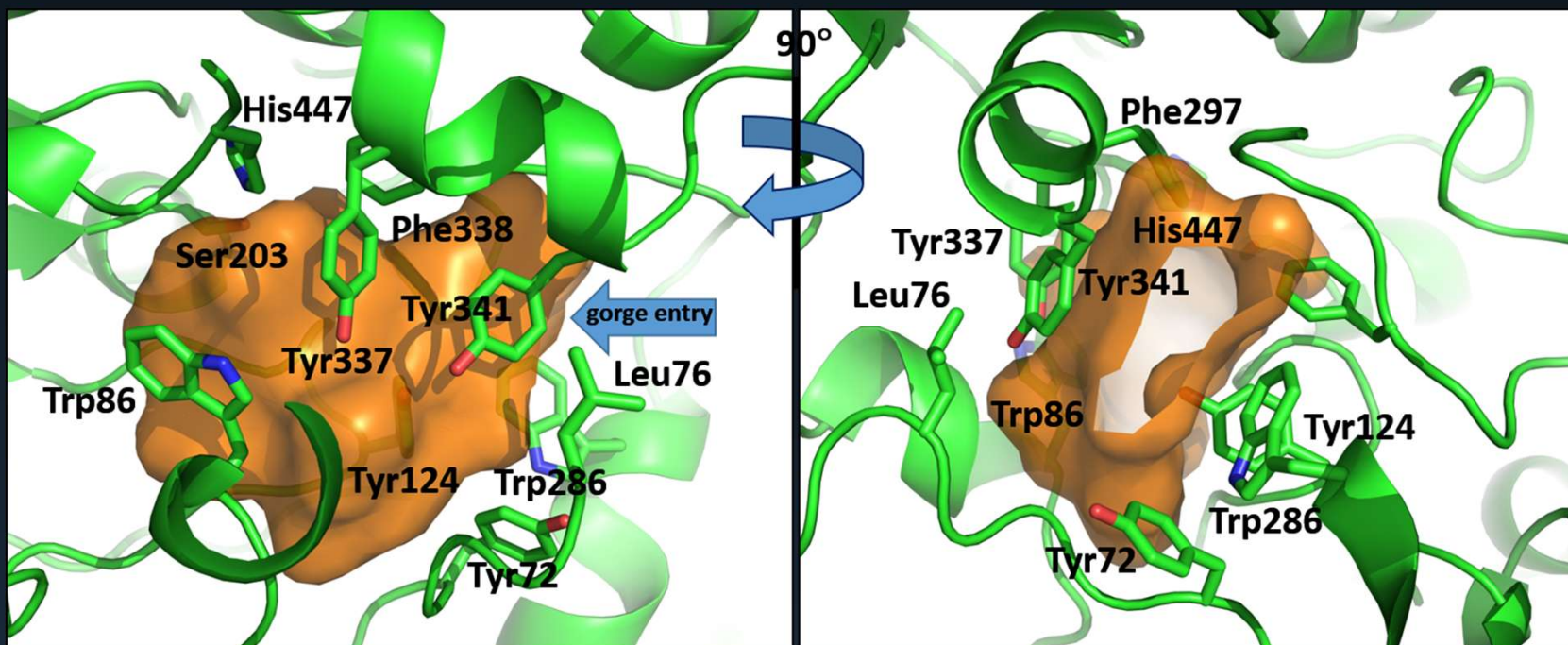


Talk outline

- ❑ New crystal form of hAChE amenable for growing neutron-diffraction quality crystals
- ❑ Low- and room-temperature X-ray crystallography of RS-170B complexes
- ❑ Low-temperature structures of RS194B complexes
- ❑ Neutron vibrational spectroscopy of ligand-free and paraoxon-conjugated hAChE
- ❑ Design of uncharged bis-oximes



Limitations of oxime therapy



Many oximes have low penetration levels across the blood-brain barrier reactivators

Slow reactivation rates (a few reactions per minute)

Reactivation efficiency depends on structures of OPs and reactivators

HI-6 is an efficient reactivator of AChE conjugated to sarin but not to tabun

RS2-170B is more effective than HI-6 for soman conjugate

Aged conjugates are resistant to current oxime

Half-lives of aging for different conjugates:

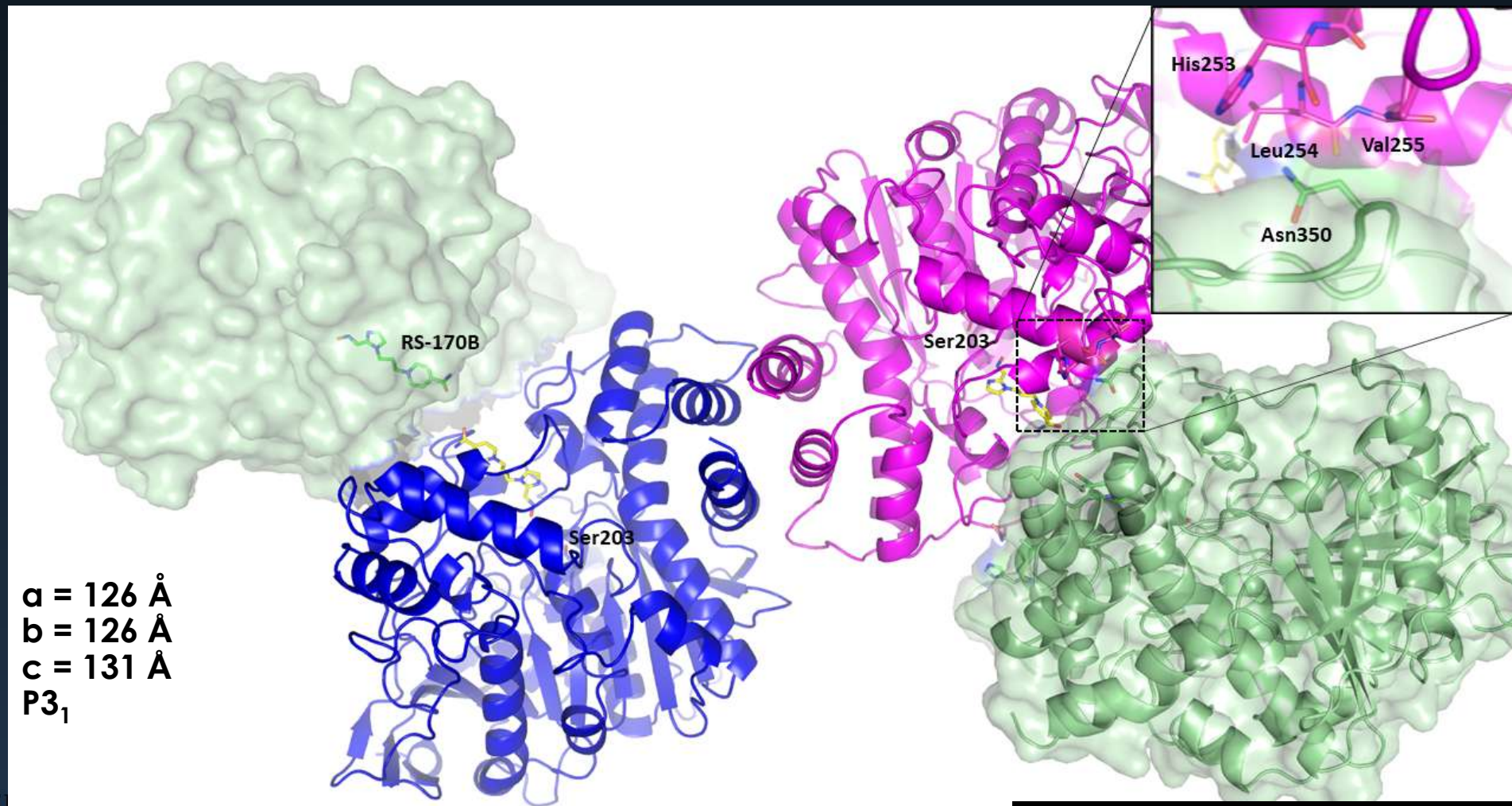
For soman-hAChE ~ 2 min

For sarin-hAChE several hours

For VX-hAChE over 30 hours

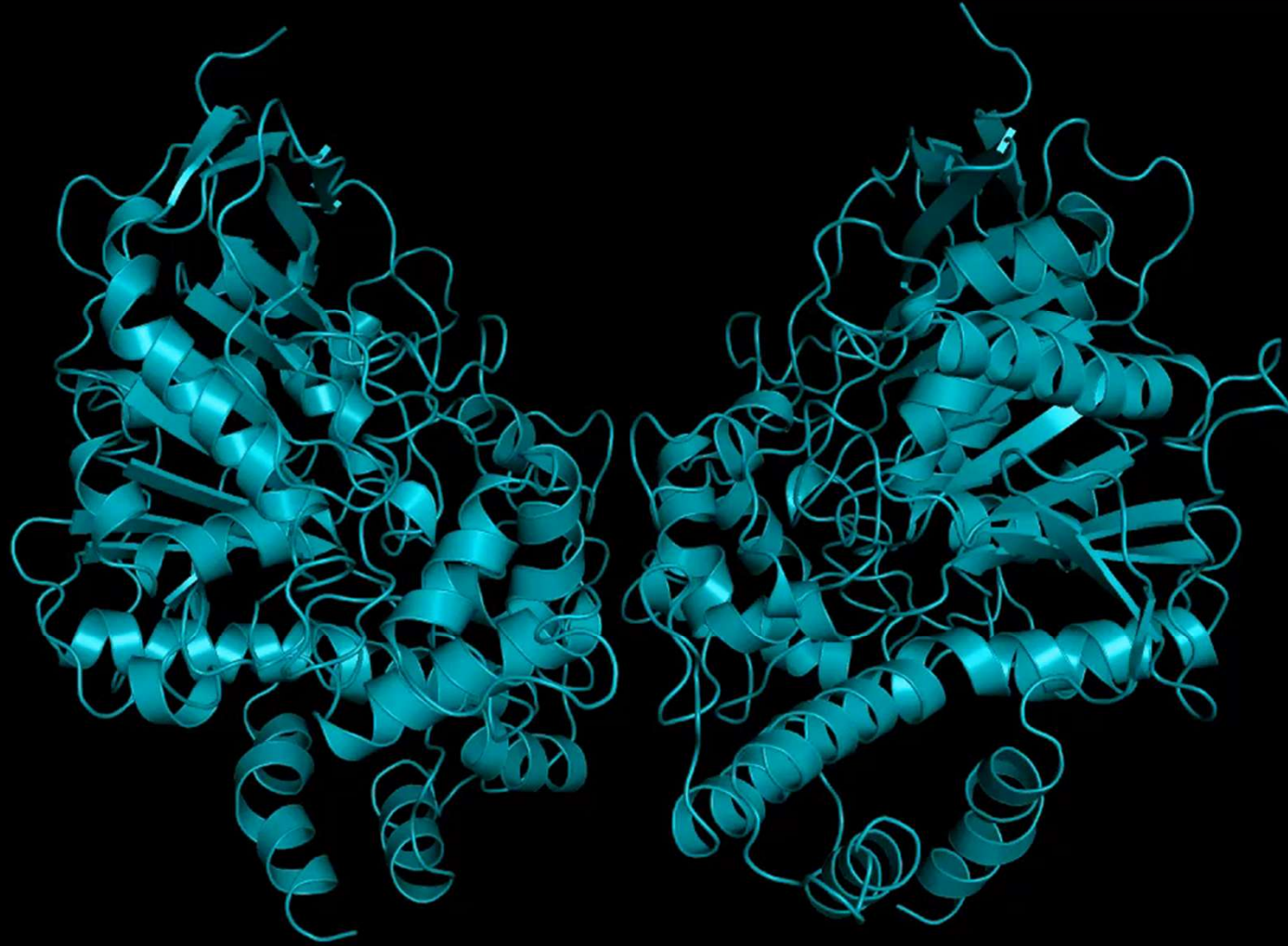
The new crystal form of hAChE

lack of glycosylation on Asn350 allows a different type of crystal packing

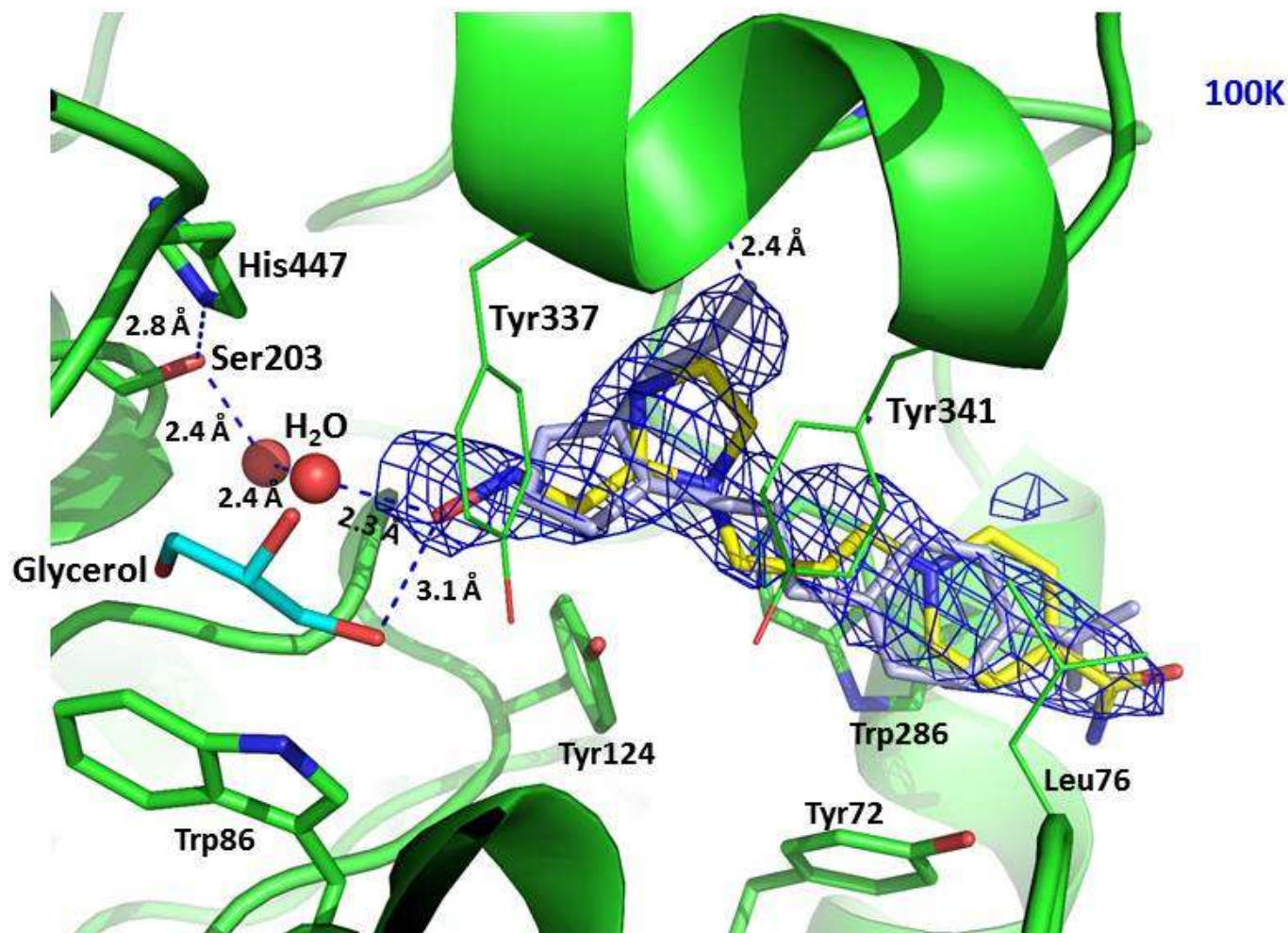


Gerlits et al. 2019 *Chem. Biol. Interact.* **309**, 108698

The new crystal form of hAChE
face-to-face and 4-helix bundle dimers are formed



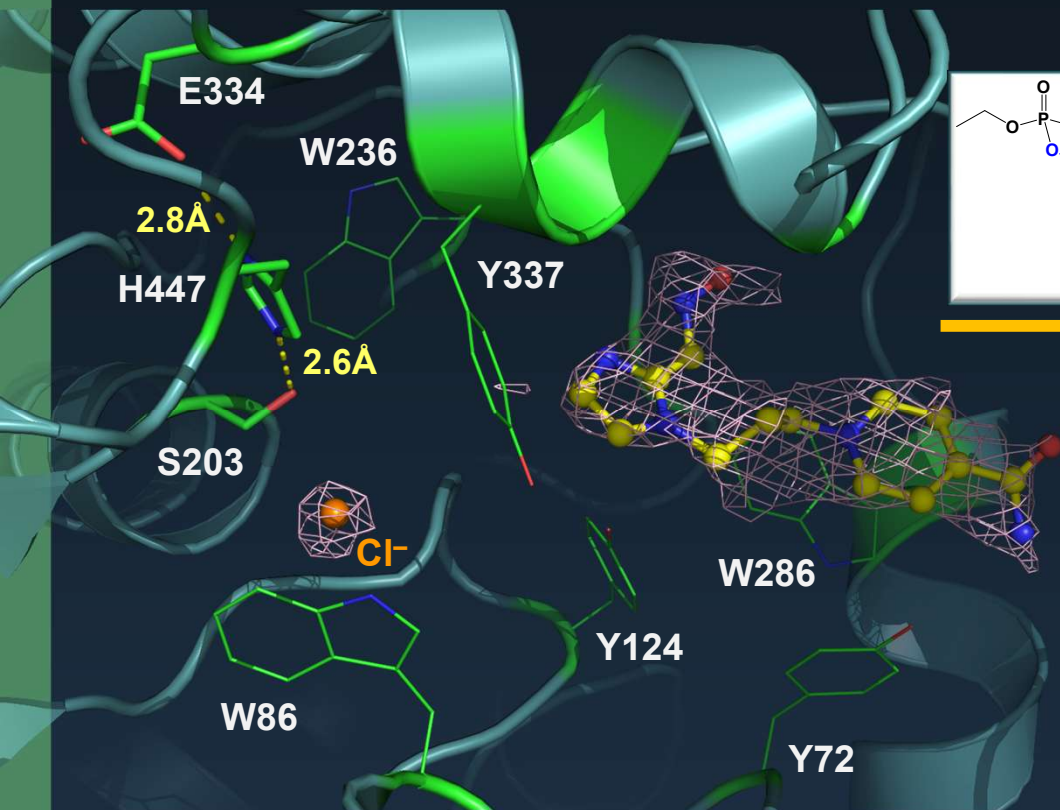
RS-170B oxime binds in two conformations to non-modified active site; one conformation extends to Ser203



Gerlits *et al.* 2019 *J. Biol. Chem.* 294, 10607-10618

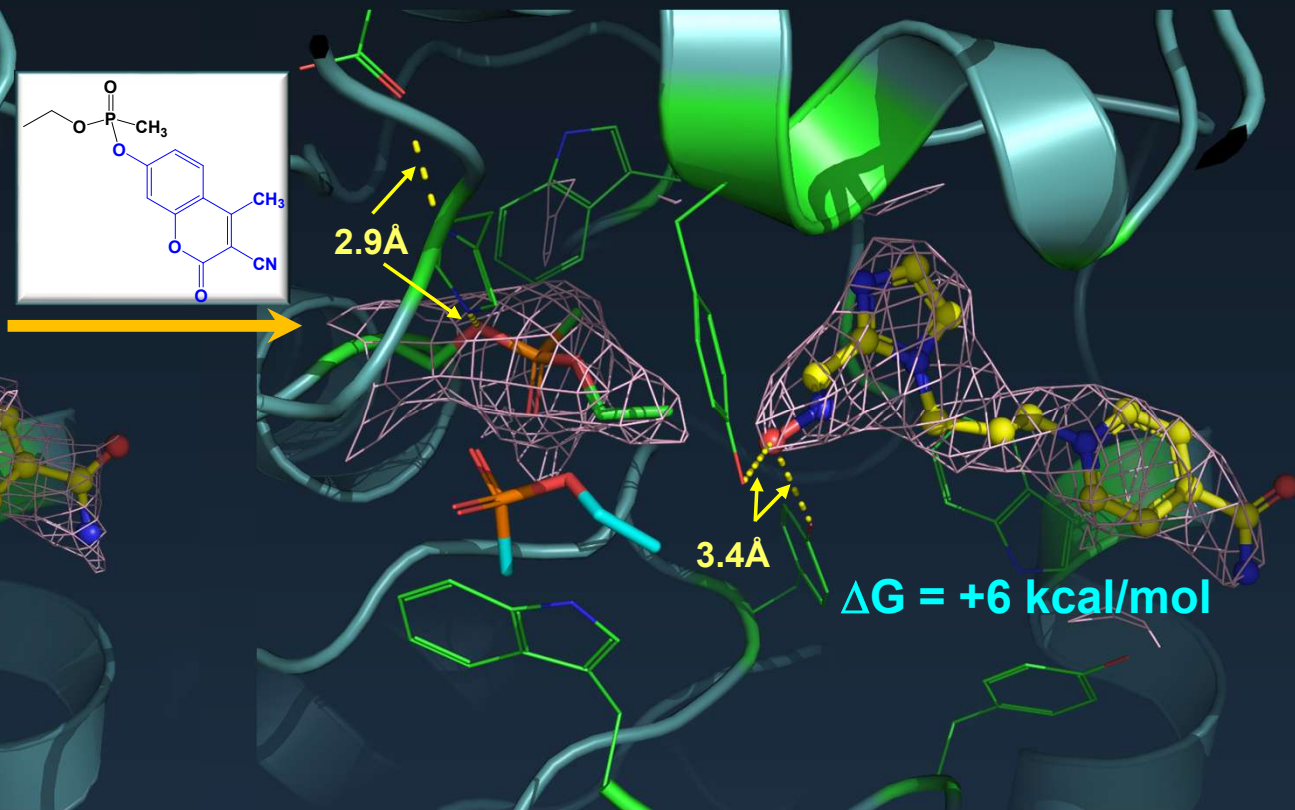
Room-temperature structures

hAChE:RS-170B

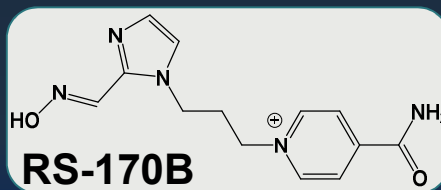


2.8 Å resolution

VX-hAChE:RS-170B

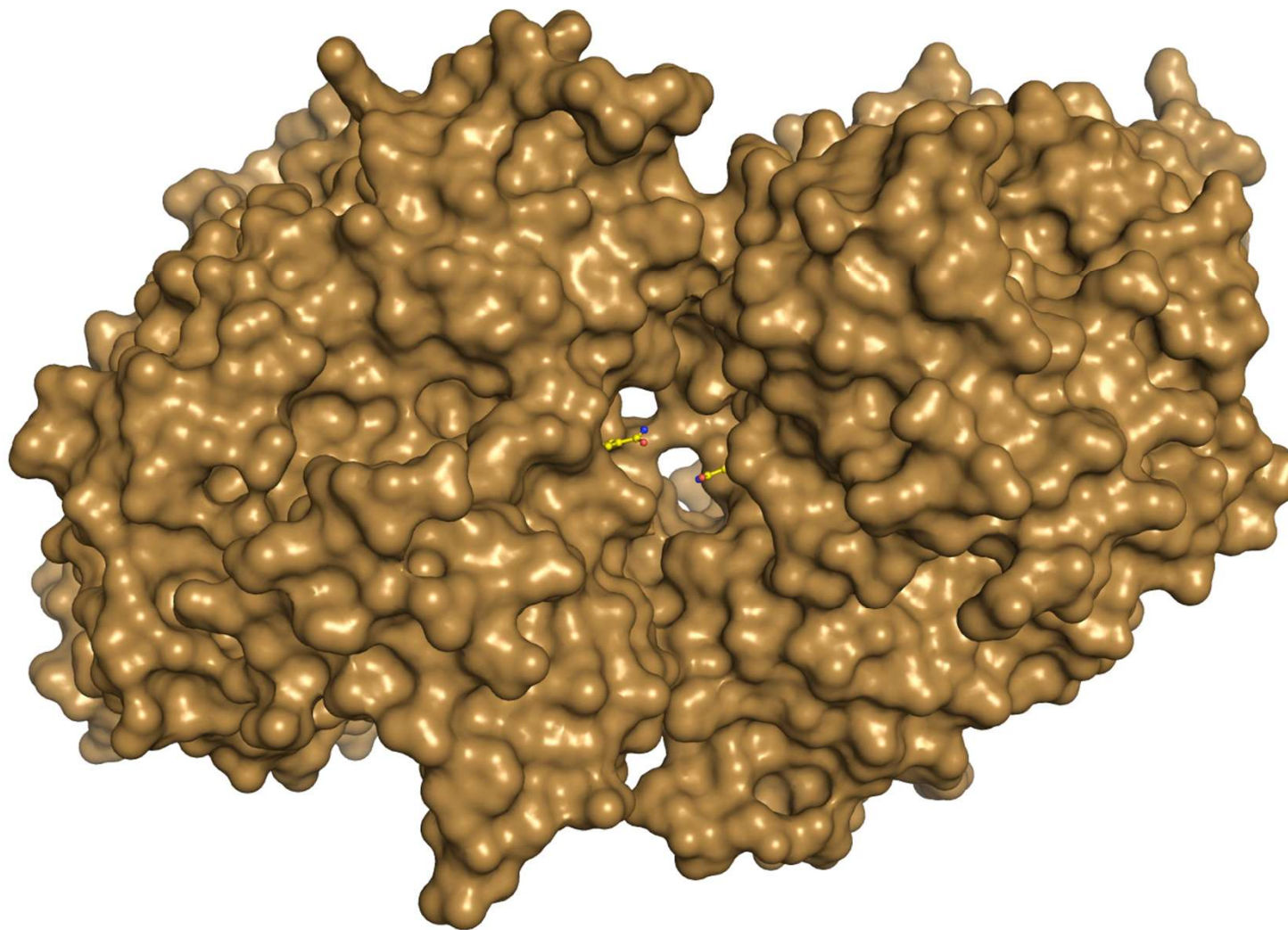


2.8 Å resolution



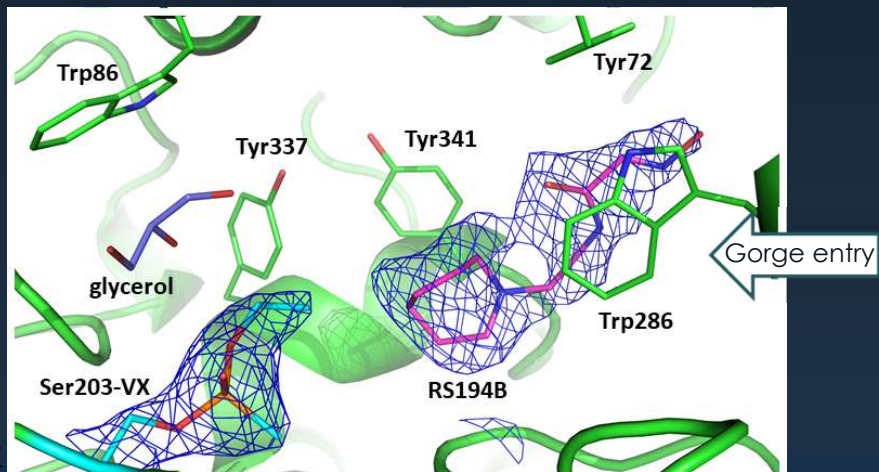
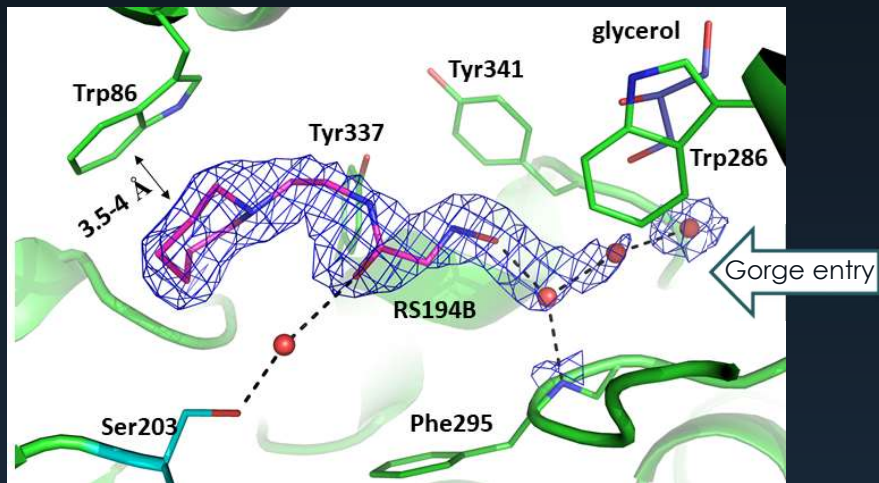
Gerlits et al. 2019 *J. Biol. Chem.* 294, 10607-10618

Room-temperature structures

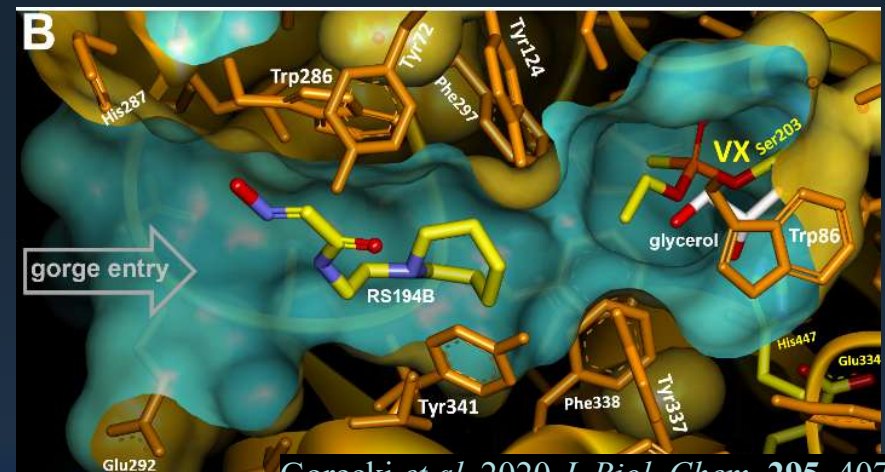
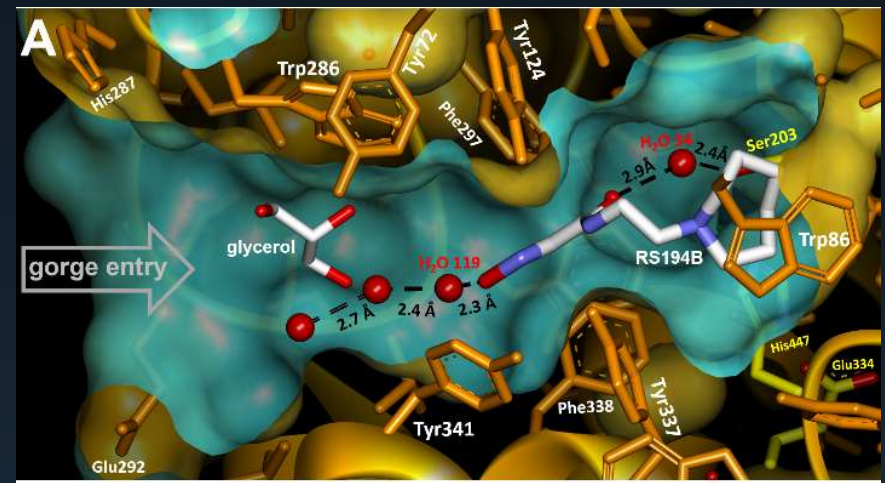


Low-temperature structures

hAChE:RS194B

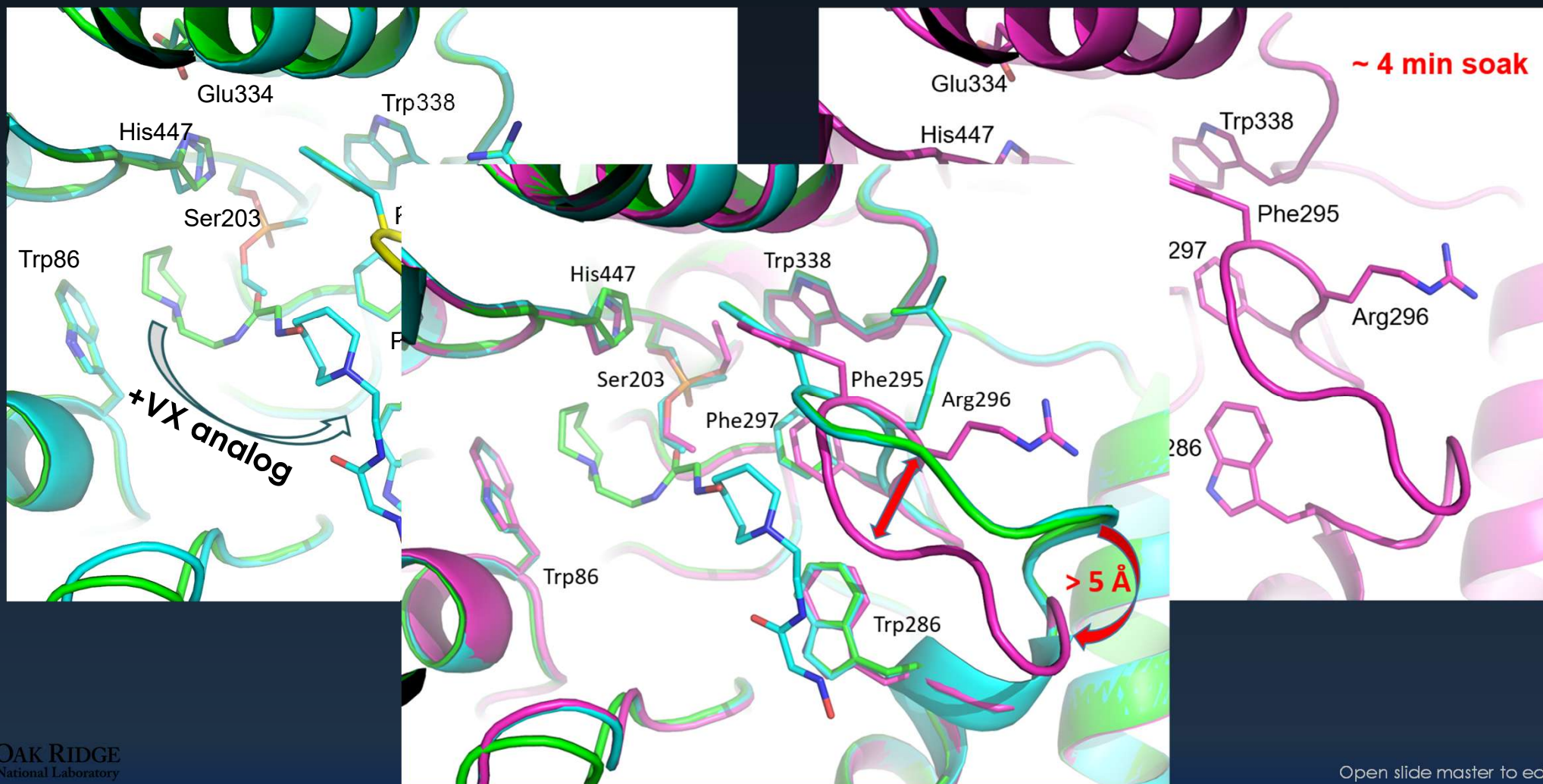


VX-hAChE:RS194B

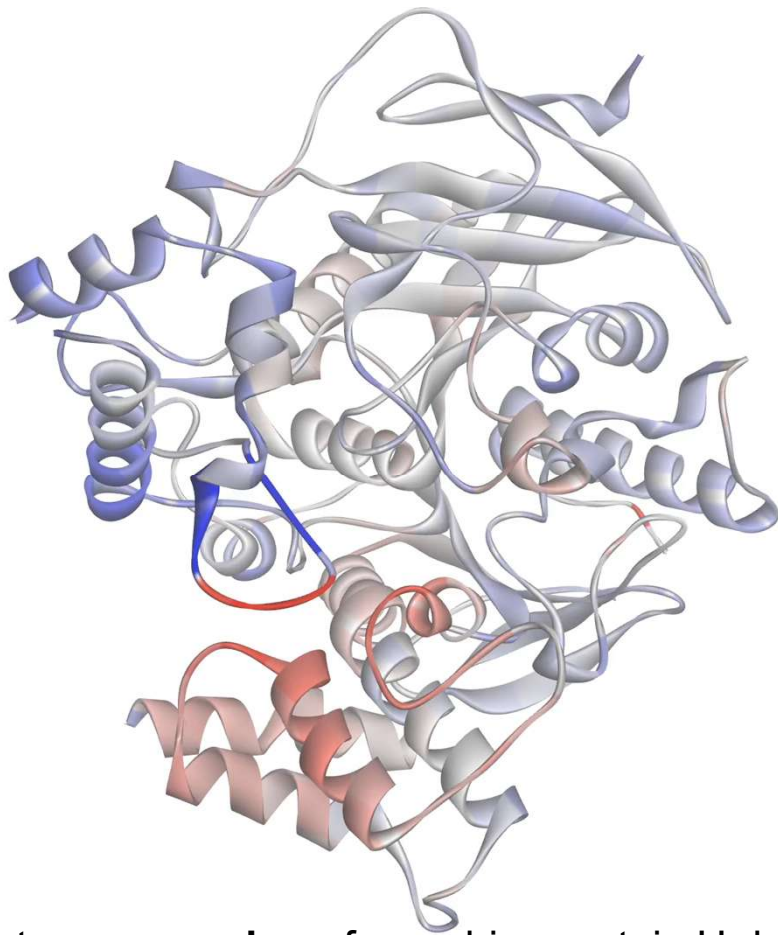


Gorecki et al. 2020 *J. Biol. Chem.* 295, 4079-4092

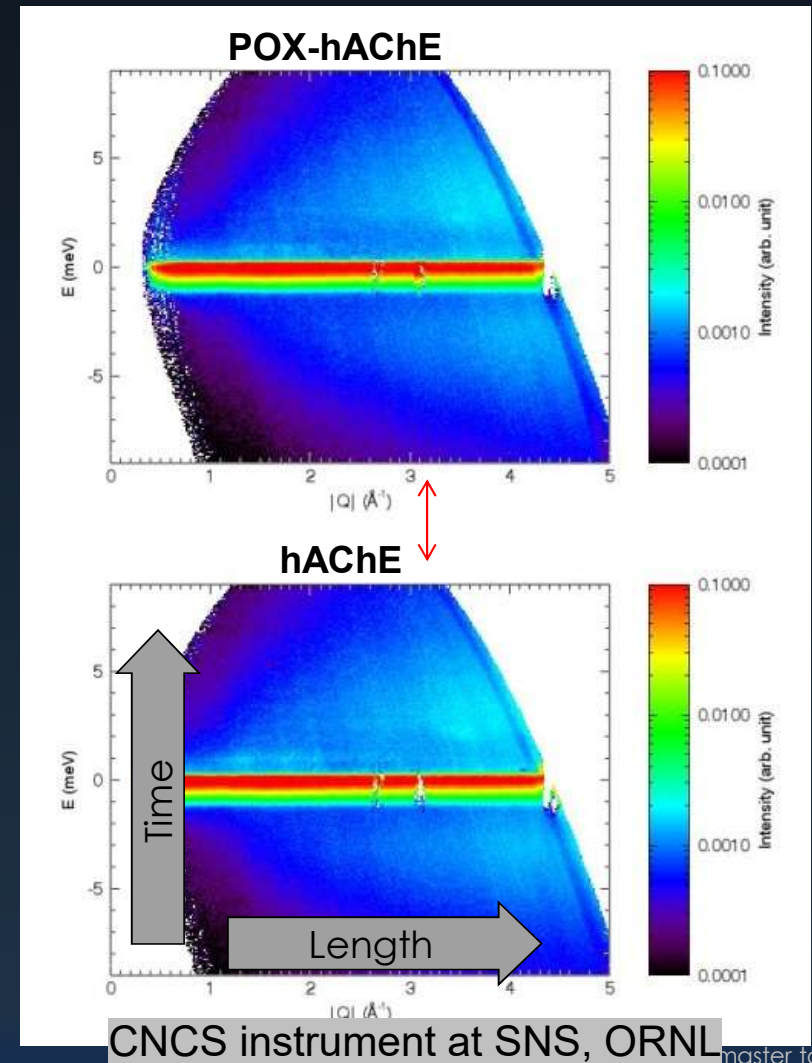
POX conjugation alters acyl pocket loop conformation



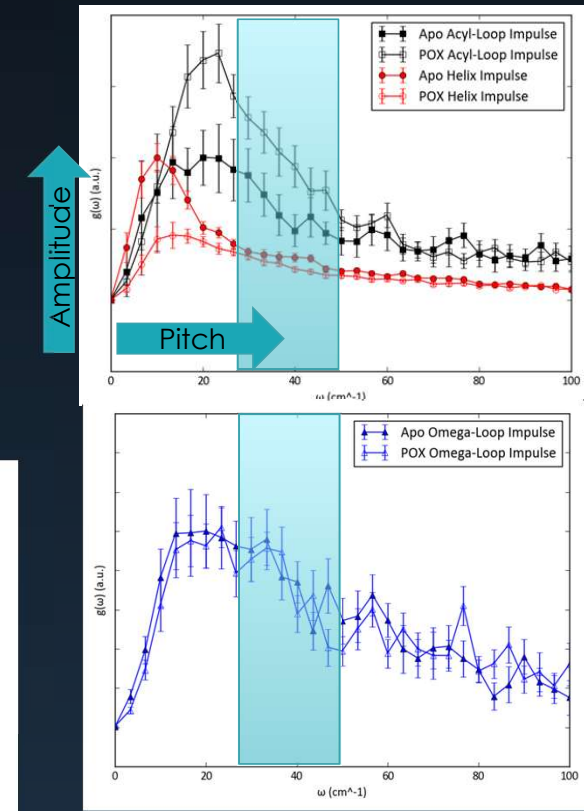
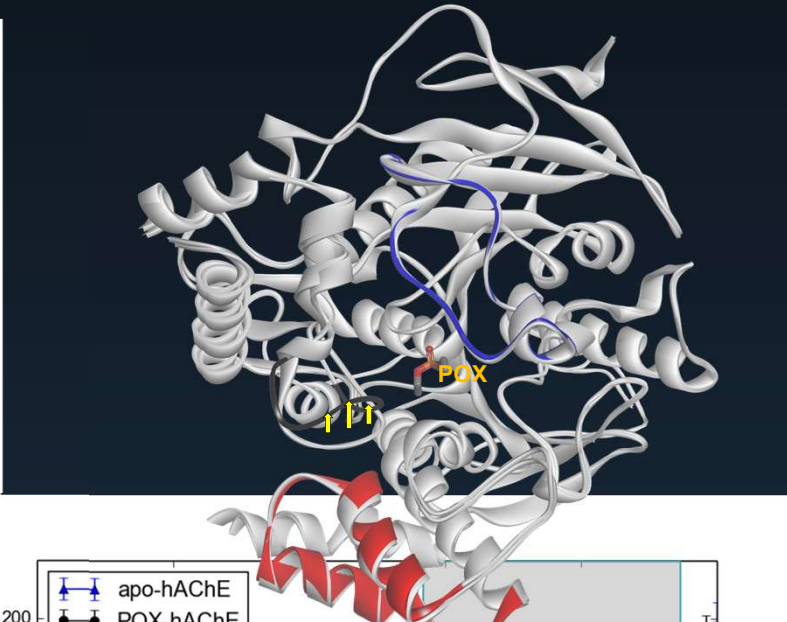
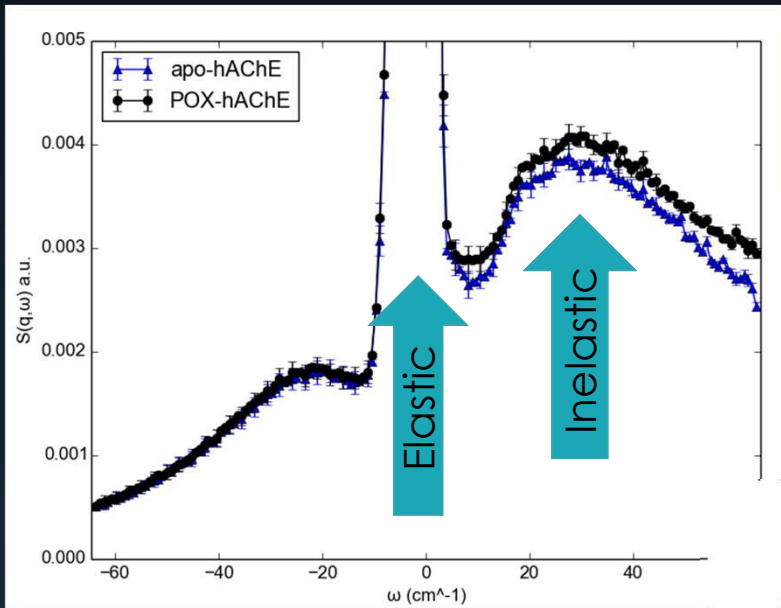
Protein vibrational dynamics on the picosecond timescale



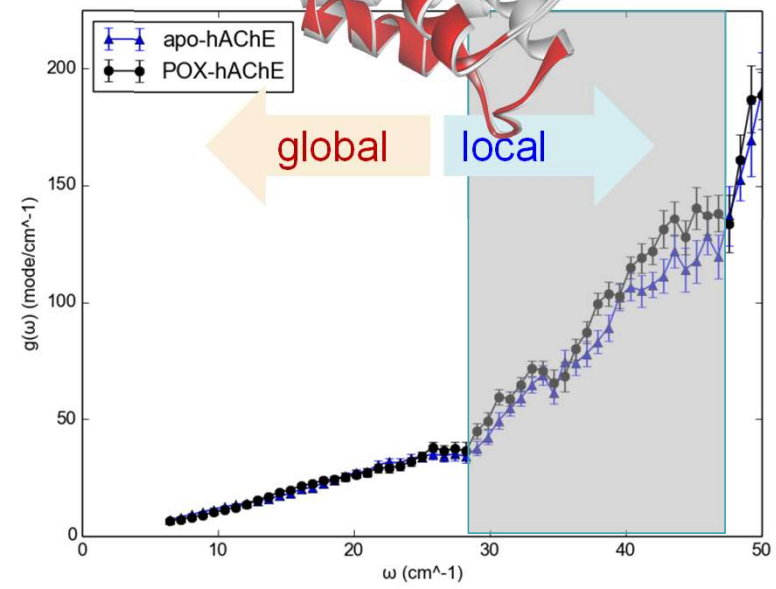
Neutrons are **unique** for probing protein H dynamics –
both spatial and temporal resolution.



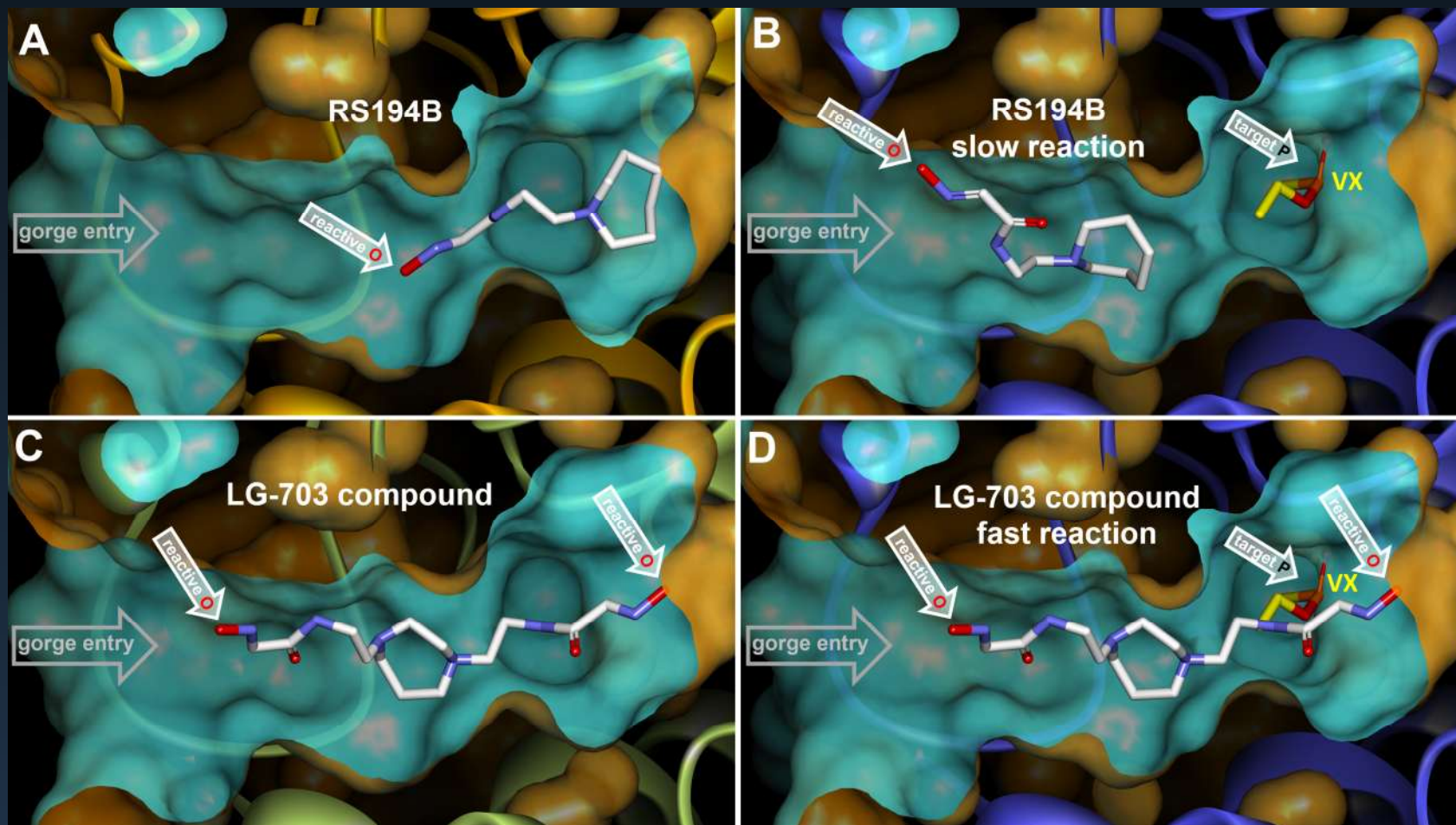
Protein vibrational dynamics on the picosecond timescale



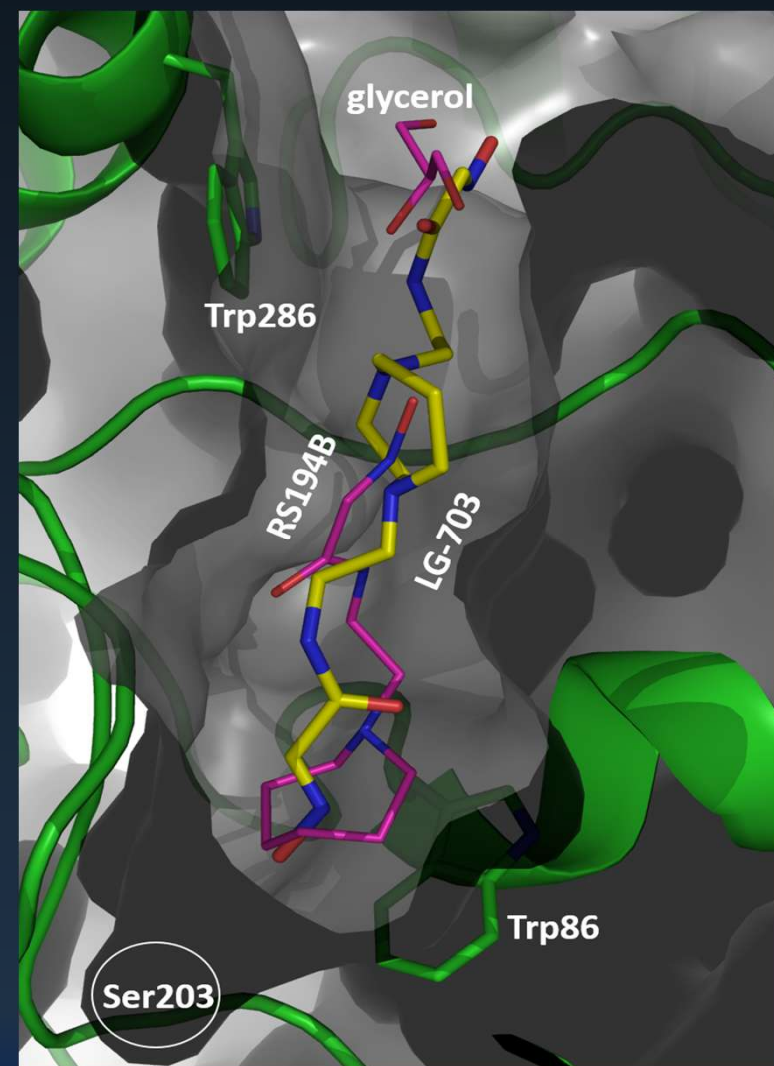
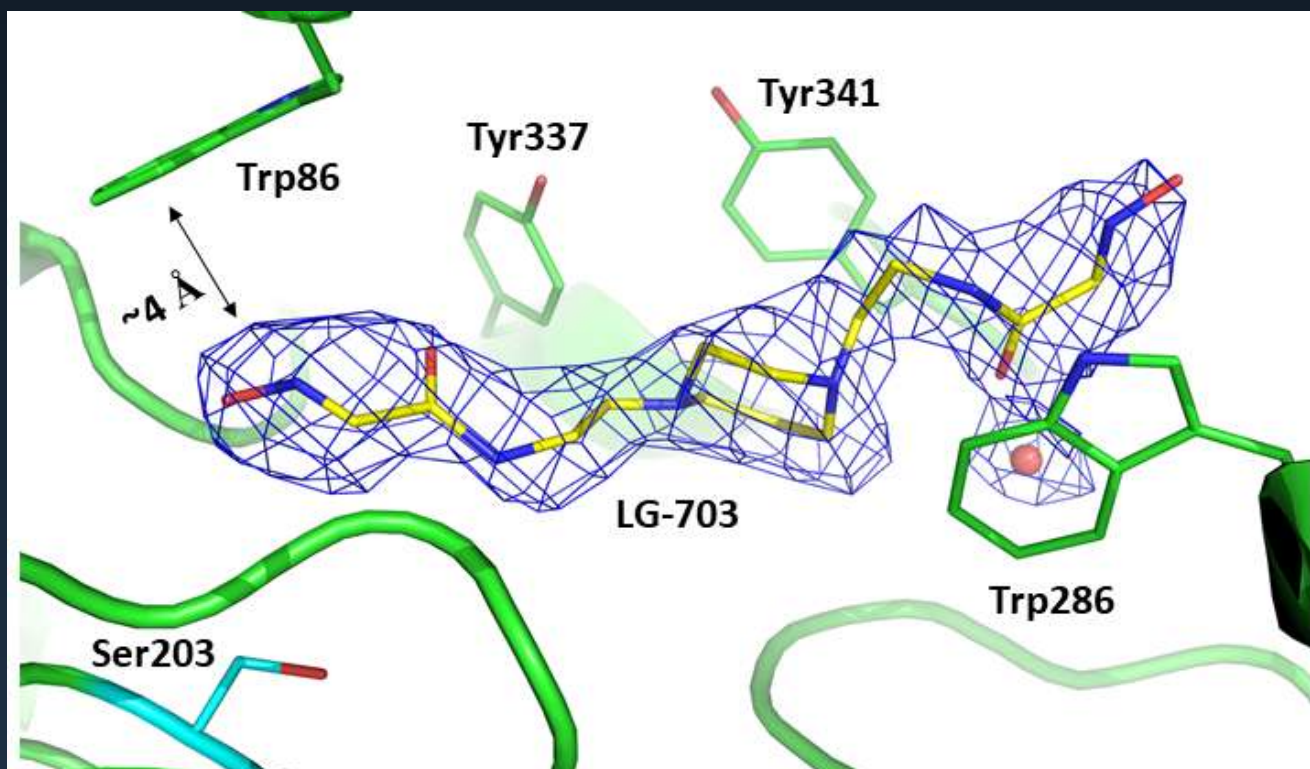
Global, collective motions remain unchanged with POX-conjugation
POX-conjugation promotes some regional motions (e.g., vibrational dynamics of loops)



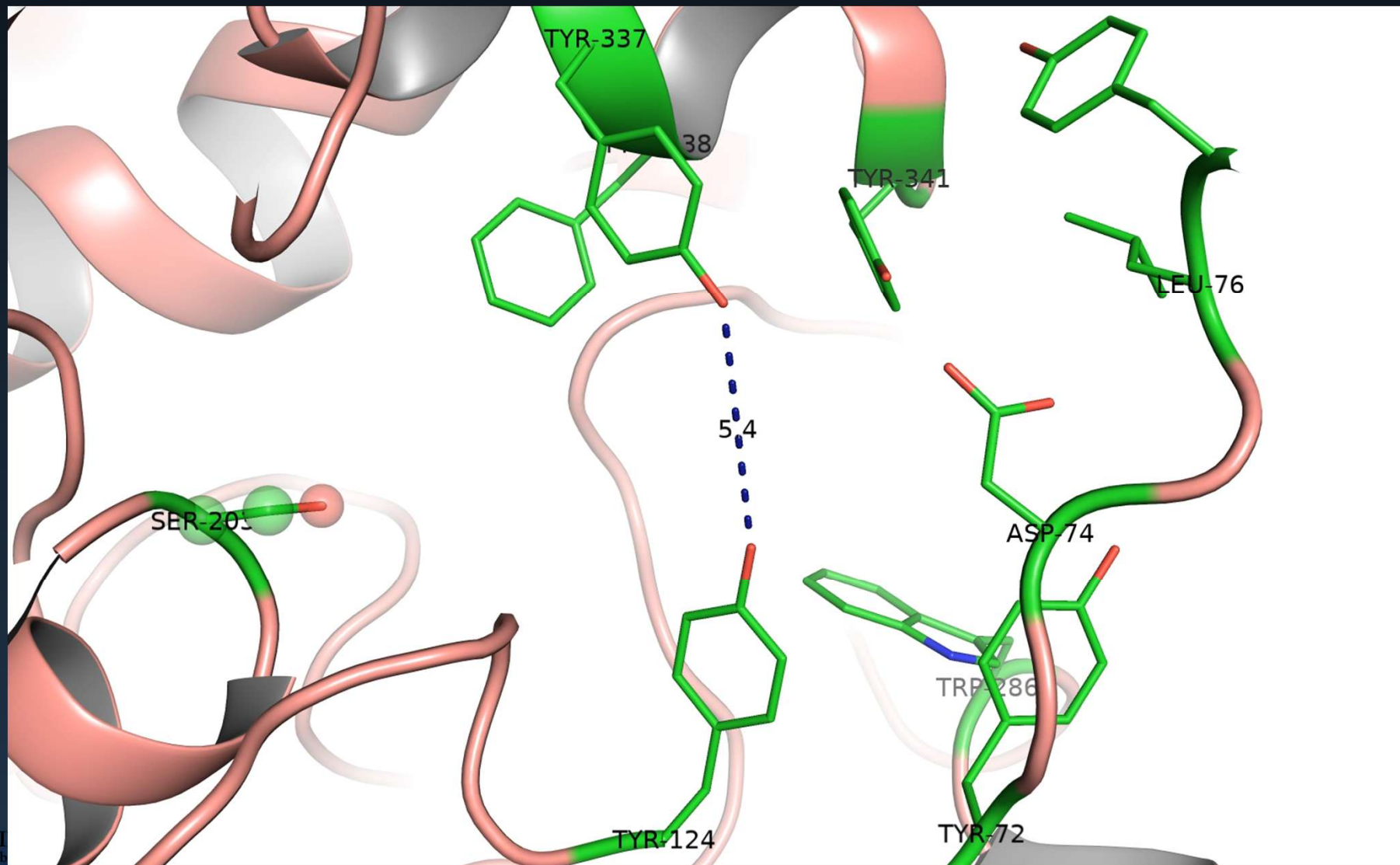
Design of uncharged bis-oximes



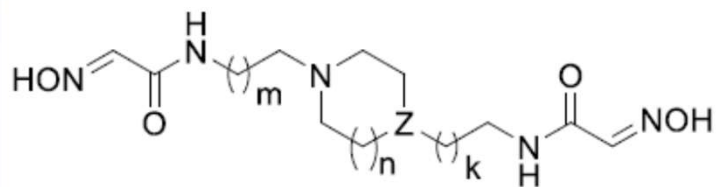
Low-temperature structure of hAChE:LG-703 complex



Gorge reshaping due to LG-703 binding



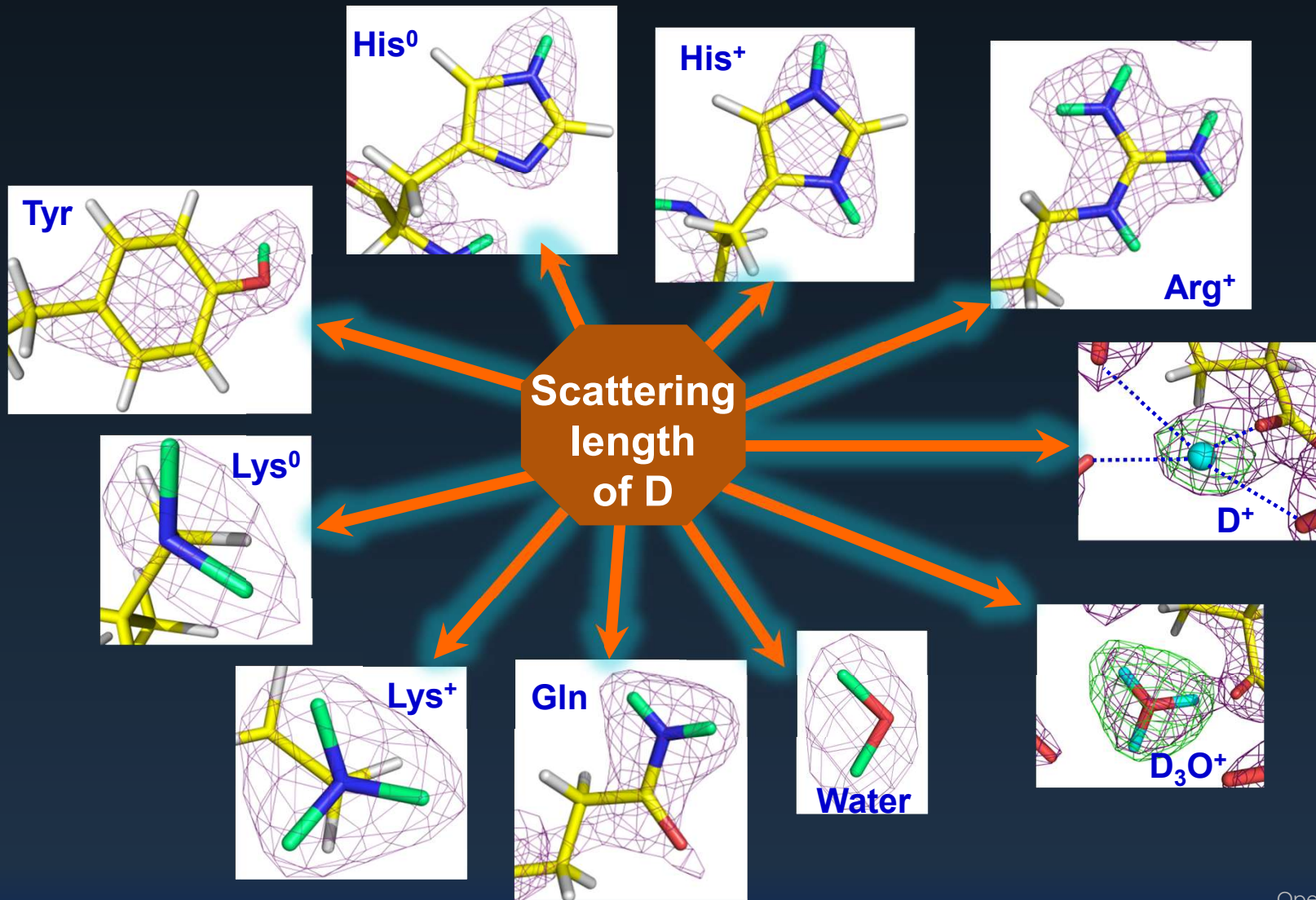
Design of uncharged bis-oximes



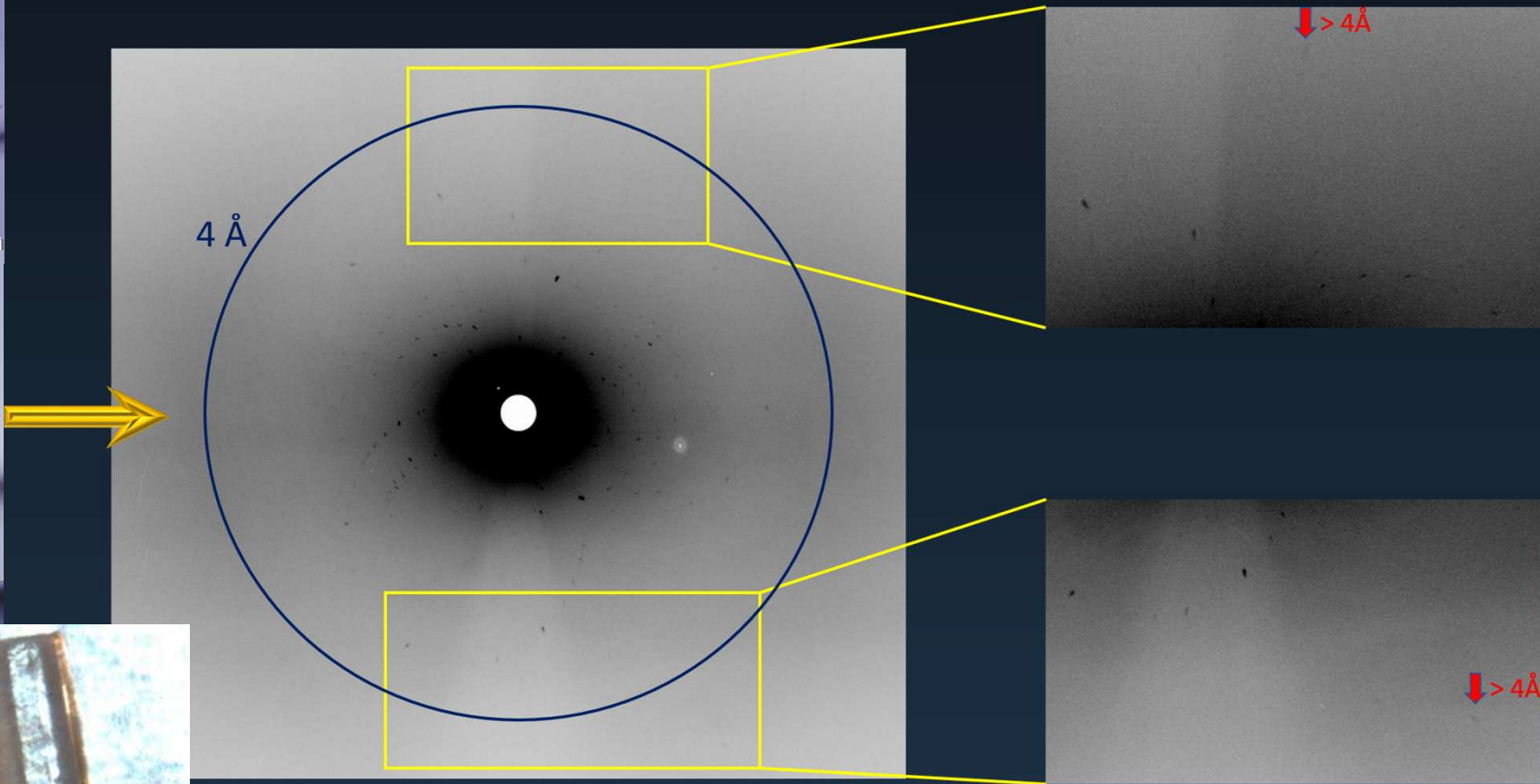
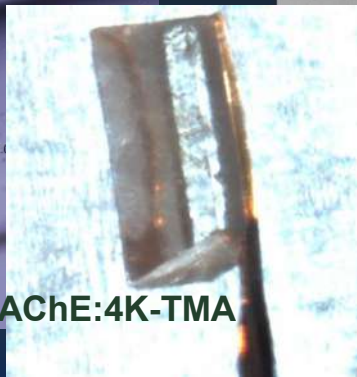
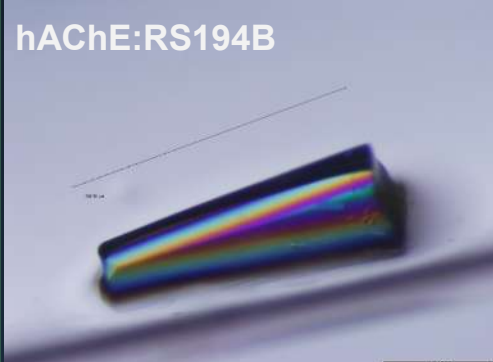
LG-700 Z = N; n = 1; k = 1; m = 1
LG-703 Z = N; n = 2; k = 1; m = 1
LG-750 Z = N; n = 1; k = 1; m = 2
LG-747 Z = N; n = 1; k = 2; m = 2
LG-804 Z = N; n = 2; k = 2; m = 2
LG-823 Z
LG-829 Z

Oxime	Paraoxon			Sarin			Cyclosarin			VX		
	k_2	K_{ox}	k_r	k_2	K_{ox}	k_r	k_2	K_{ox}	k_r	k_2	K_{ox}	k_r
LG-703	0.14	2.0	69	0.80	0.90	890	0.50	2.9	170	1.3	1.2	1100
LG-804	0.14	1.7	86	0.73	0.45	1700	0.57	4.5	130	1.1	0.63	1800
LG-700	0.012	0.065	180	0.15	0.16	910	0.046	0.25	190	0.14	0.16	880
LG-750	0.081	1.1	71	0.91	1.9	480	0.32	1.4	220	0.59	0.43	1400
LG-747	0.053	0.45	120	0.33	0.25	1300	0.055	0.51	110	0.44	0.11	3900
LG-823	0.10	1.4	72	0.55	0.35	1600	0.26	0.89	300	0.65	0.45	1500
LG-829	0.17	1.9	87	0.78	0.37	2100	>0.5	>2.0	100	0.98	0.43	2300
RS194B	0.080	0.97	83	0.60	1.0	590	0.17	1.3	140	0.6	0.53	1100
2PAM	0.27	1.8	150	1.1	0.34	3200	0.73	6.6	110	0.65	0.25	2600

Neutrons reveal atomic details



Crystal growth of hAChE for neutron diffraction



Neutron diffraction image was collected on LADI instrument at the Institut Laue Langevin (Grenoble, France) for 16 hours.

Open slide master to edit

In search for better diffracting crystals – towards micro gravity growth



A. Pre-flight

All hardware transferred to a +12°C refrigerator following hardware integration.

The flight hardware turned over to Cold Storage on May 30, 2017 and loaded into the DCB on the same day.

PCG-6 SpX-11 mission



Acknowledgements

ORNL:

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Dr. Kevin Weiss (CSMB)



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CNCS:

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Stephanie Luedtke



Yunshen Li



Celine Bojo

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