Acetylcholinesterase Inactivation and Reactivation: A Tale of Two Consequences

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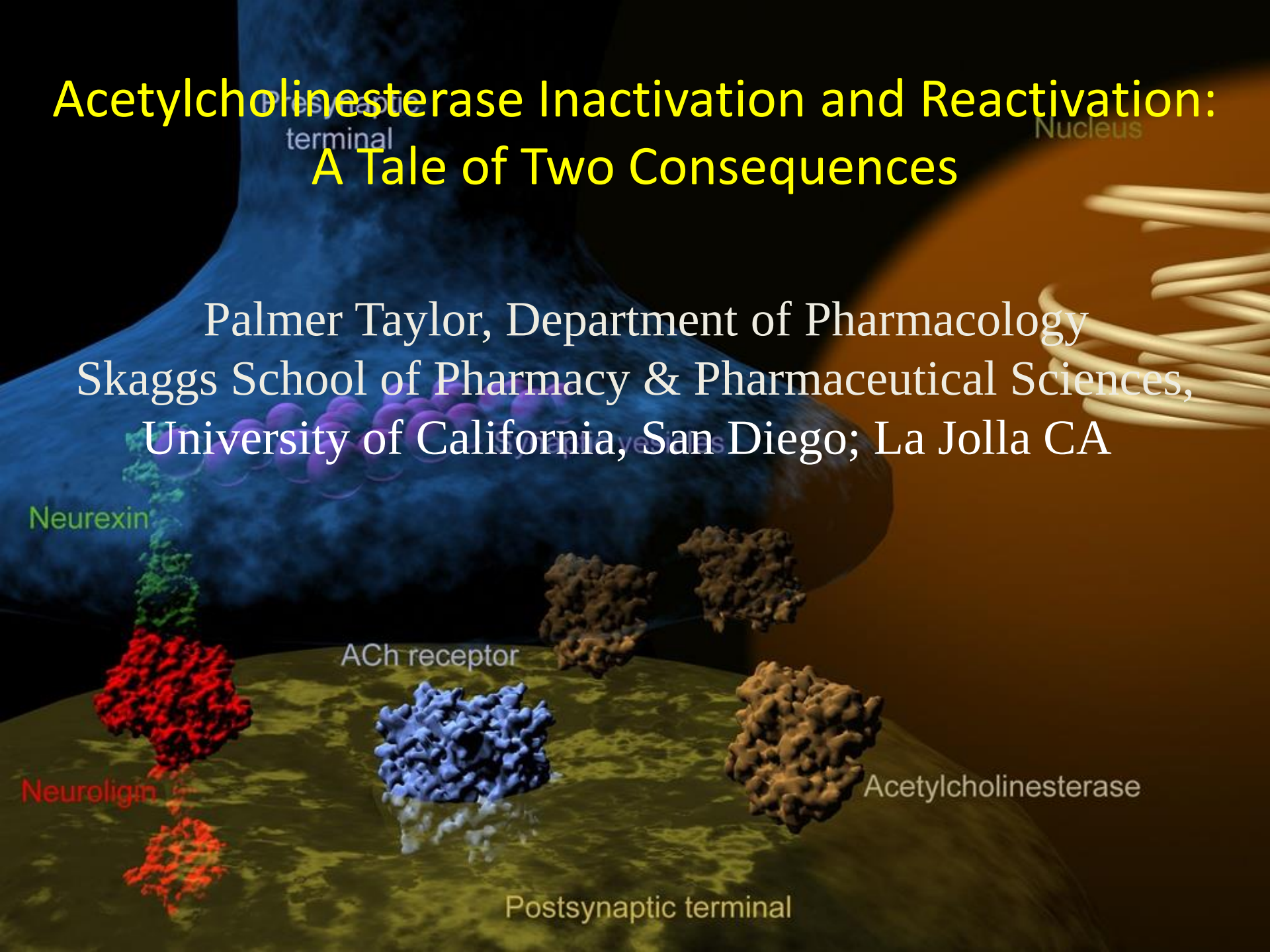
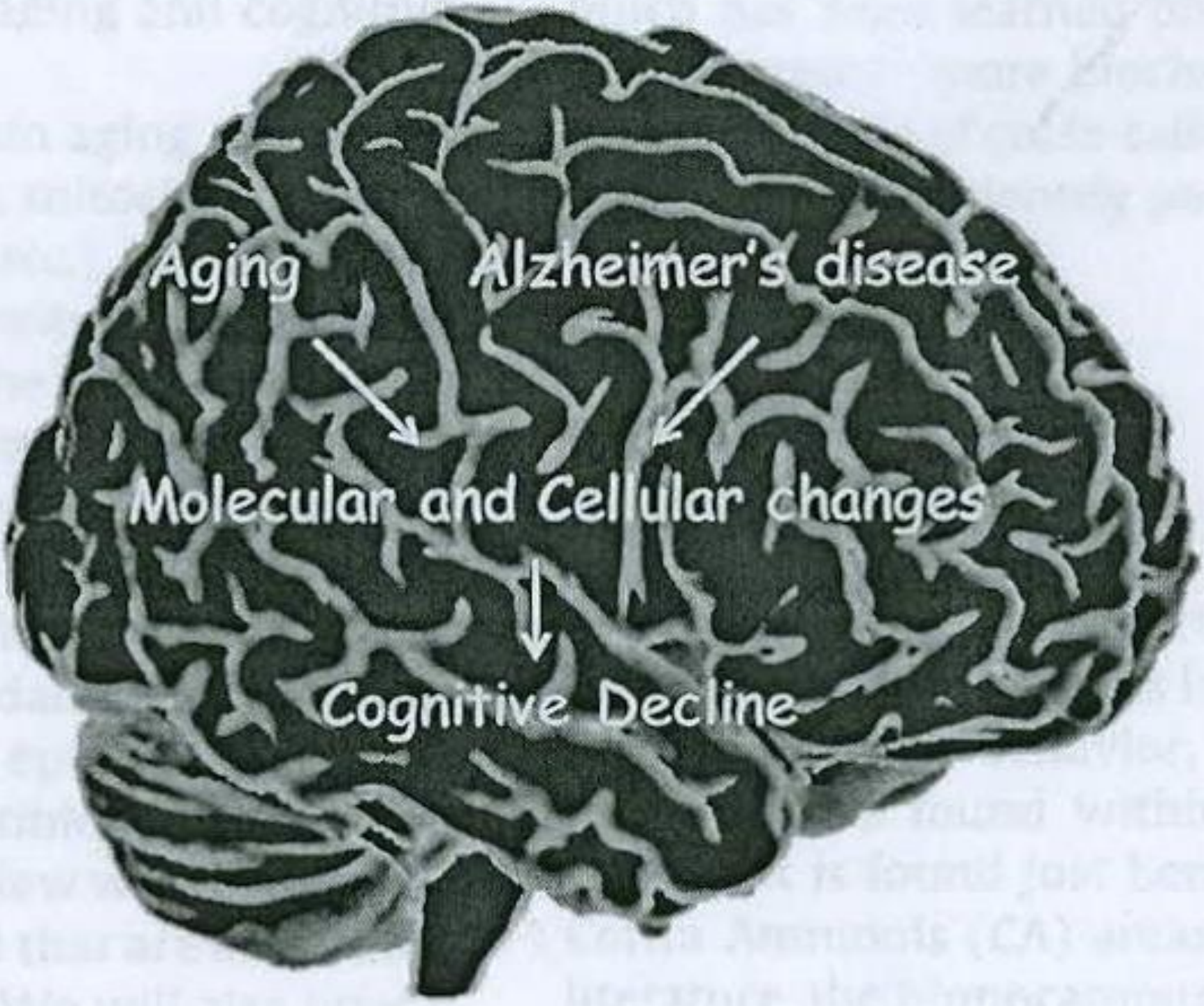
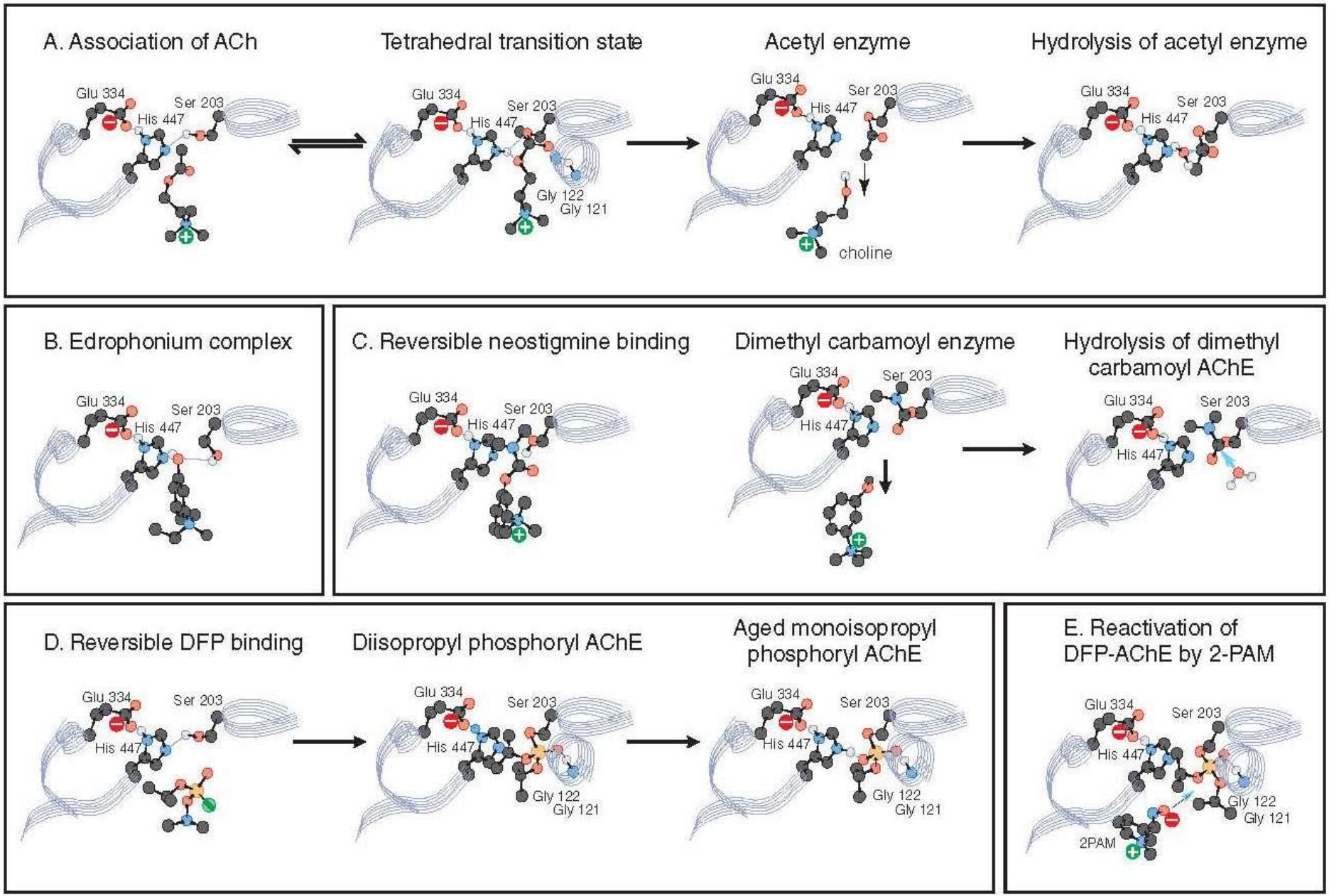
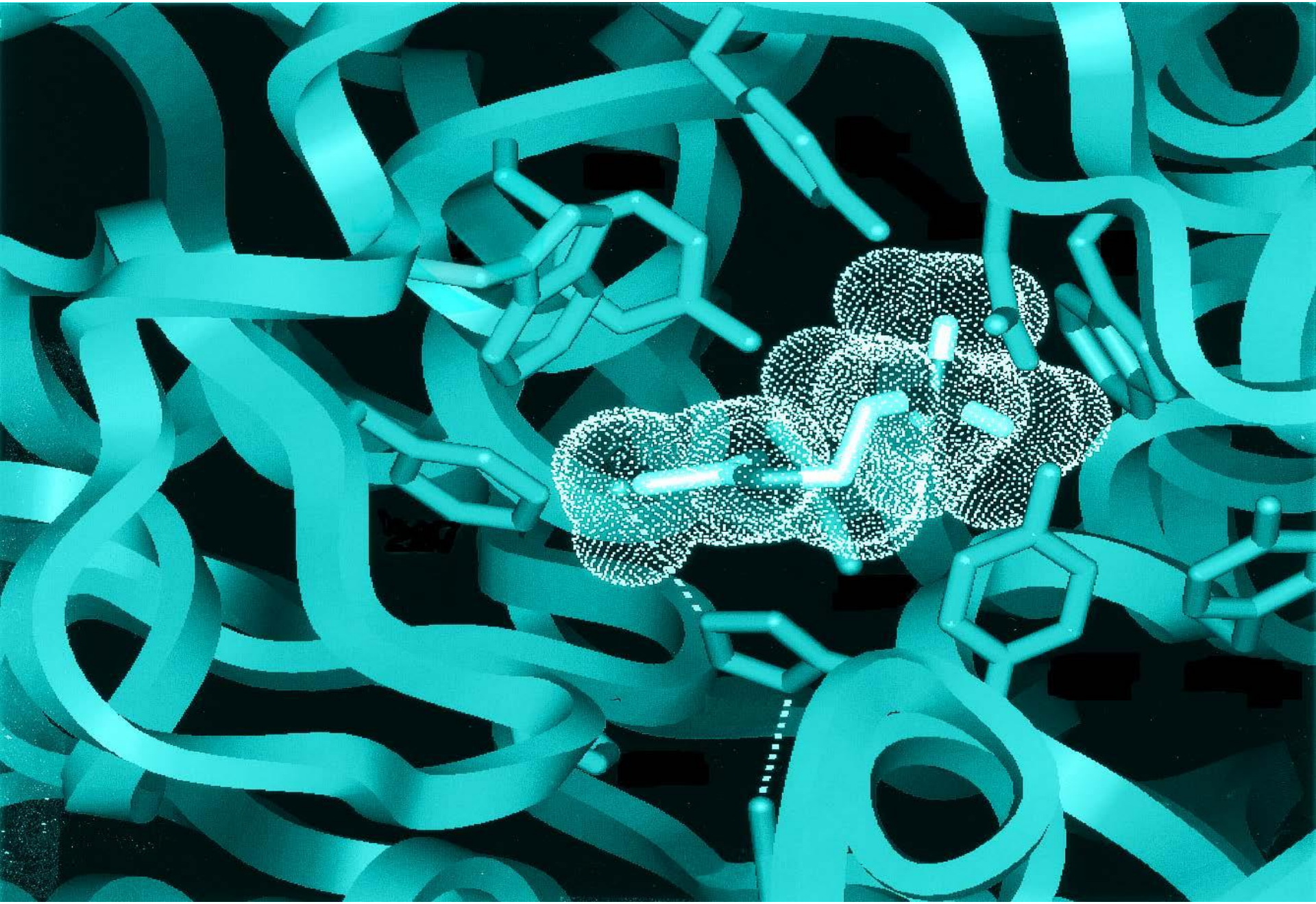



Aging
Alzheimer's disease
Molecular and Cellular changes
Cognitive Decline

Catalysis, Inhibition and Reactivation of Acetylcholinesterase



Acetylcholine at the Base of the Active Center Gorge

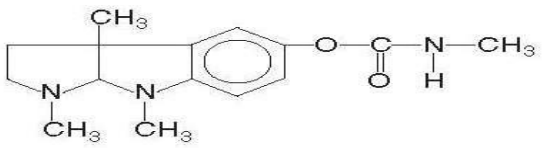


Cholinesterase Inhibitors

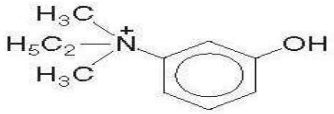
- Produce only a short lived plateau (6-12 months)
- Increasing the dose enhances side effects
- Little enhancement with paired agents (donepezil and memantine)
- Balance of benefit and risk
  a. Quality of life
  b. Caregiver relief
  c. Pharmacoeconomics-Cappell et al., CNS Drugs 24: 909-927 (2010)

Structures of Cholinesterase Inhibitors

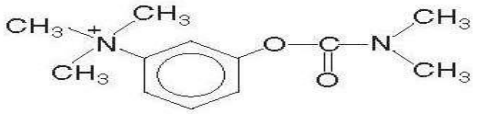
Donepezil and Galantamine are reversible inhibitors, while Rivastigmine is a carbamylating agent (slowly reversible)



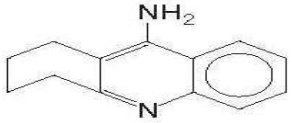
PHYSOSTIGMINE



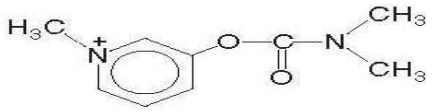
EDROPHONIUM



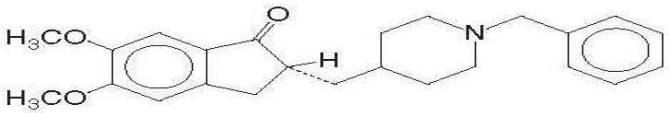
NEOSTIGMINE



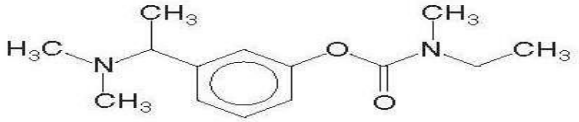
TACRINE



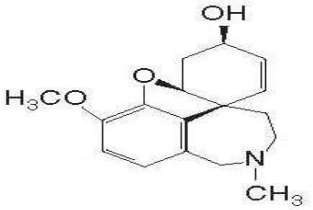
PYRIDOSTIGMINE



DONEPEZIL



RIVASTIGMINE



GALANTAMINE

Properties of an Ideal Cholinesterase Inhibitor

- CNS activity exceeds peripheral autonomic and motor activities. (partial success)
- No liver toxicity as seen with Tacrine (tetrahydroacridine) (Success)
- Devoid of peripheral side effects (Lack of Success)

Side Effects

- Gastrointestinal Disturbances: nausea, vomiting, hyper-motility
- Visual field limitations; meiosis
- Insomnia-long acting
- Muscle tremors and fasciculations
- “Cholinergic Crisis”

“Cholinergic Crisis” Results in

- Meiosis (intense pupil constriction) in the absence of a sympathetic reflex response.
- Brow pain
- Excessive gastrointestinal activity
- Muscle tremors and fasciculations
- In the extreme--convulsions

Why do we see greater efficacy in Parkinson's Disease (PD) than Alzheimer's Disease (AD)?

- Balance of dopaminergic and cholinergic stimulation in PD allows for a greater window for therapeutic endpoints.
- Multiple transmitters responsible for Alzheimer's dementias --cholinergic pathways are primarily presynaptic, releasing other transmitters
- Nigrostriatal pathway is more discrete in PD etiology.
- Neurodegeneration is more advanced in AD, before symptoms appear-need for early biomarkers. Giocomeli, Danielle, Martini, *Biochem. Pharmacol. 131*, 1-15 (2017)

Reactivation Mechanisms and Tissue Disposition Affecting Efficacy of Oximes in Averting Toxicity from Organophosphate Exposure

Sarin Experiences Japan: 1994-1995; Arum Cult

Yanagisawa *et al*.(2006) *J. Neurol Sci 249*, 76

Matsumoto -Outdoor Exposure

Tokyo Subway-Controlled Ventilation System

Sarin Experience Syria: Despotic and Inhumane Leader

Up to 1,000 deaths and 3,000 toxic events

Concepts that Require Continuing Re-evaluation

- Sarin Use (Vapor pressure and dispersion-Partial pressure latency of action distal to ground zero.
- Distinctions of Pre- and Post-exposure Antidotes versus Scavenging Agents (Applicability in *in vivo,* field situations?)
- Importance of Studies in Multiple Animal Species (double jeopardy for toxicant-antidote combinations in FDA Animal Rule)
- Value and Limitations of Molecular-based Techniques: Capture the transition state)
- Requirements for Parallel Pharmacokinetic, Toxicity and Efficacy Studies for Acute Exposures.

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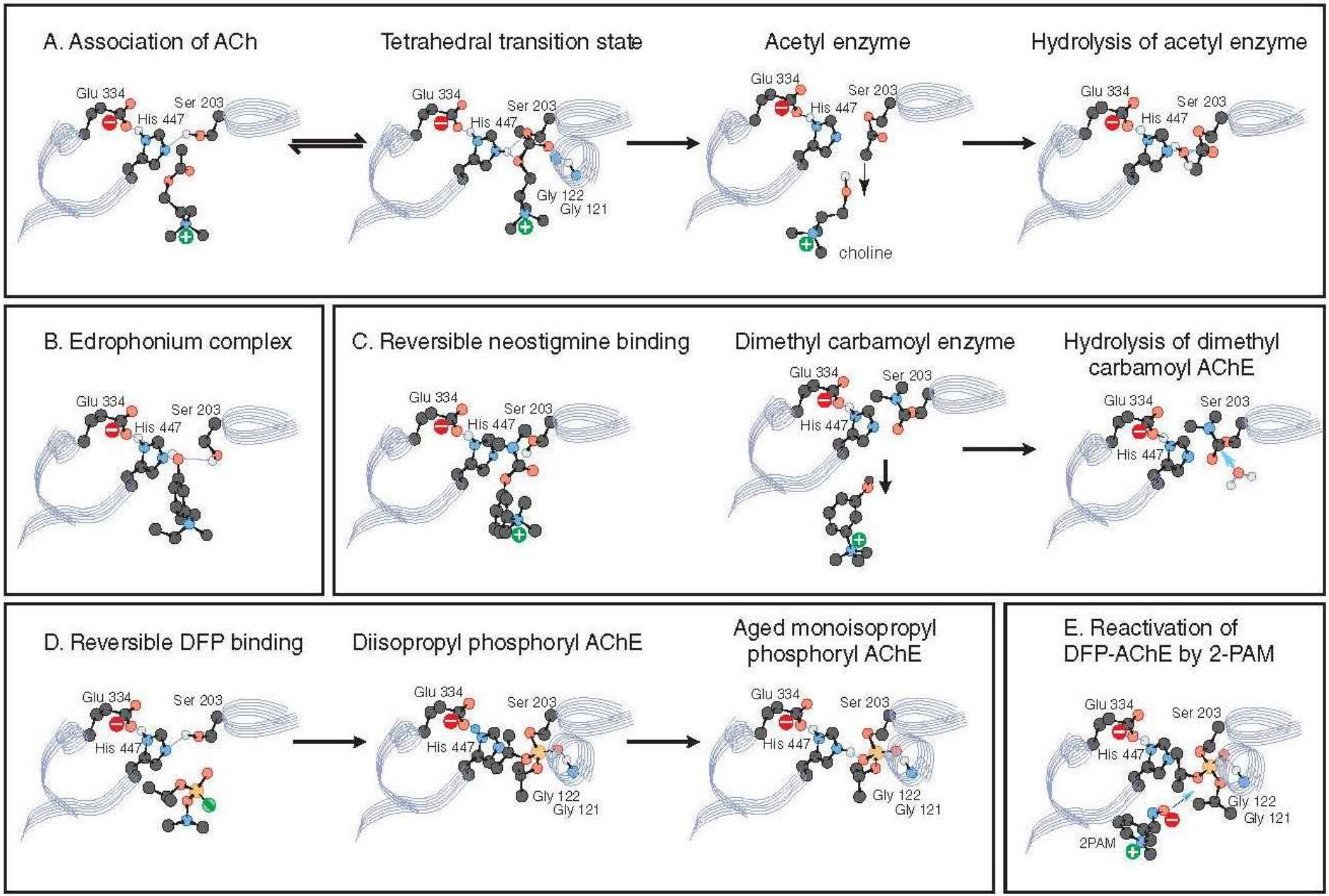
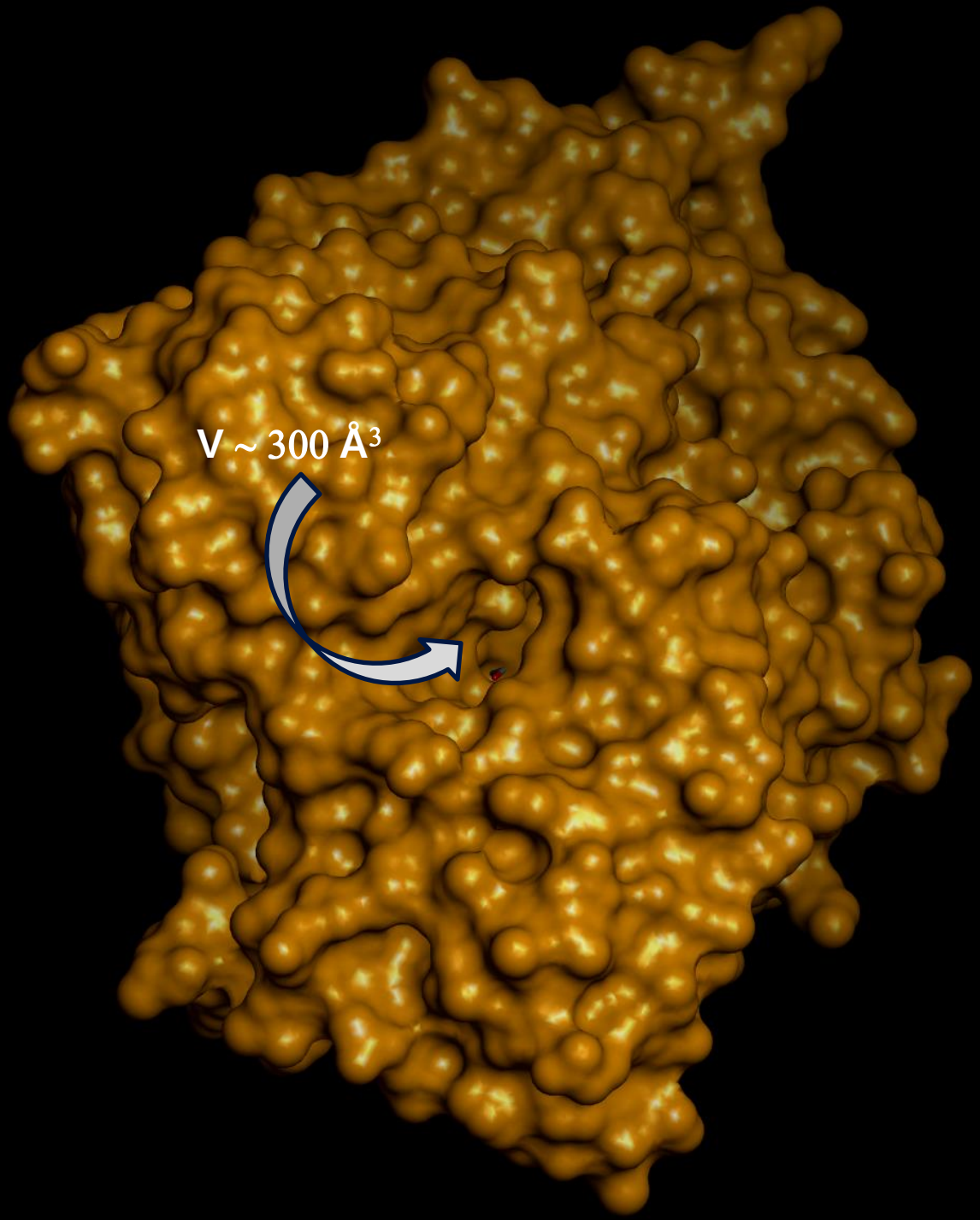
Catalysis, Inhibition and Reactivation of Acetylcholinesterase

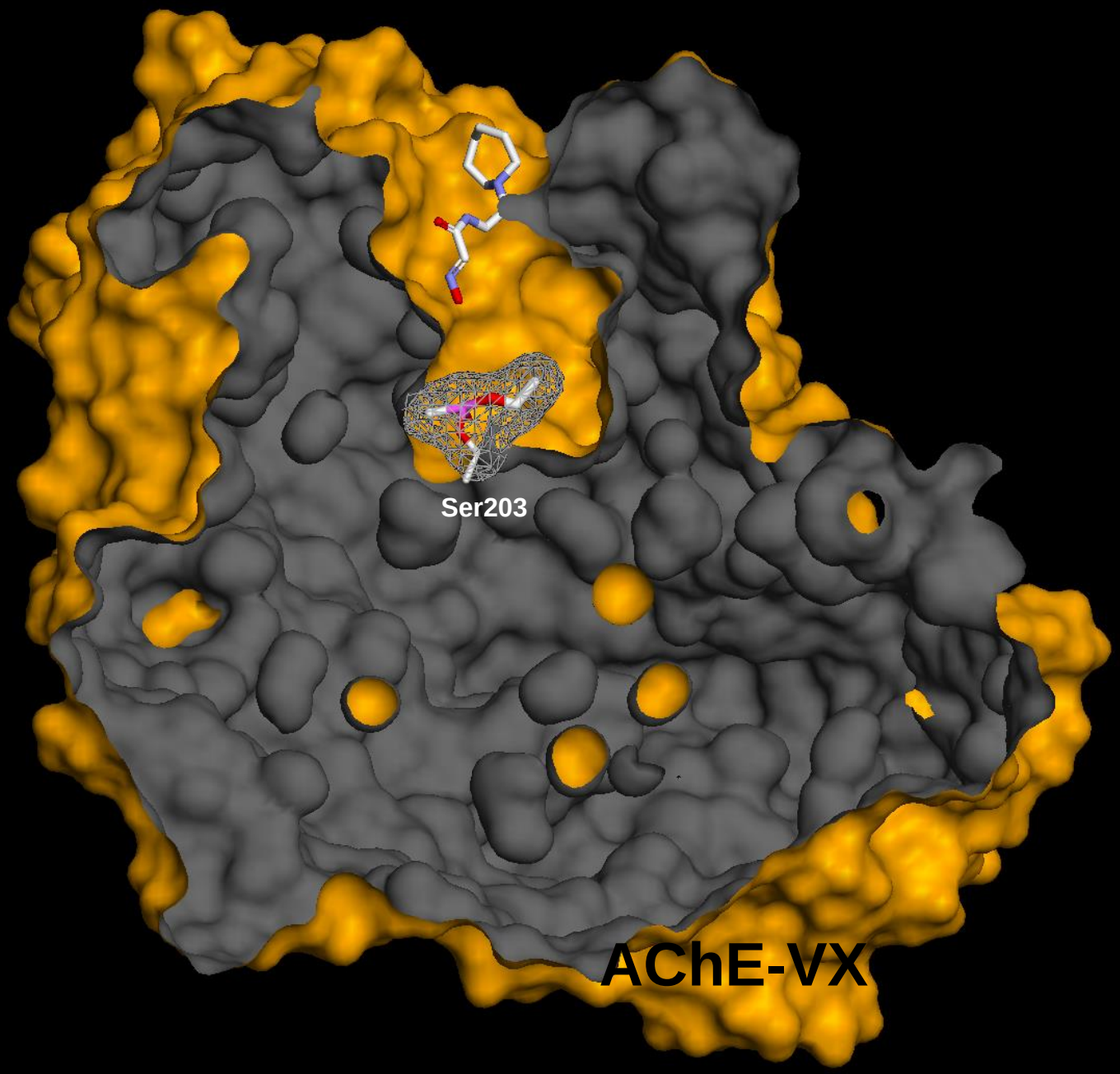




OP	V (Å^3)
cyclosarin	156
POX	121
sarin	115
VX	105
DDVP	90

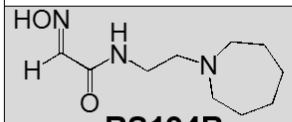
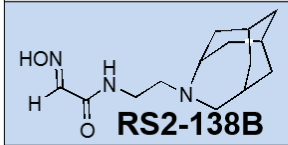
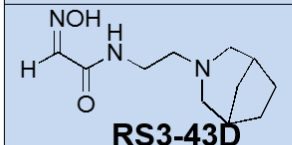
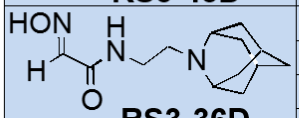
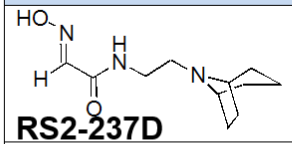
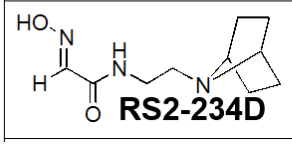
ACh	**145**

RS194B + VX inhibited AChE

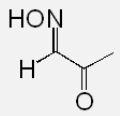
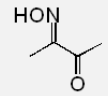
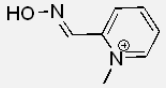


In vitro *Reactivation Constants for Recovery of AChE Activity*

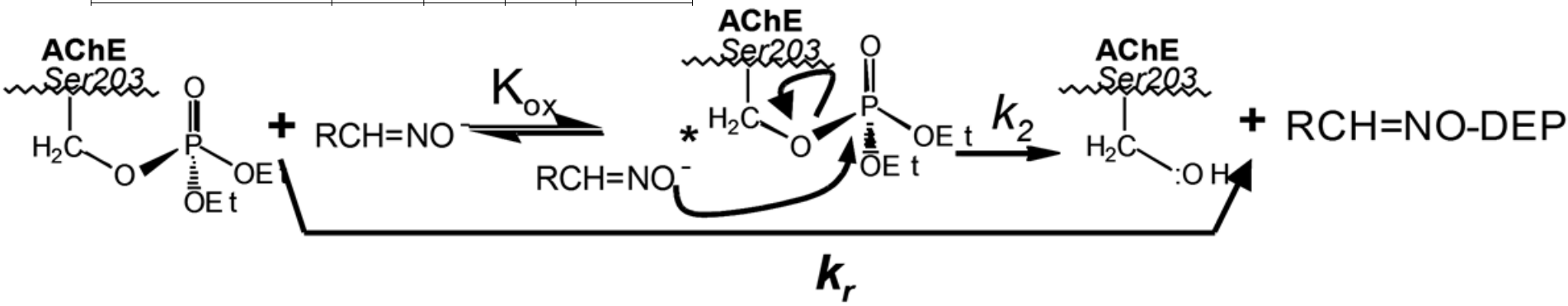
Hydroxyimino acetamide Alkyl amines

oxime	OP	k_2 (min^{-1})	K_{ox} (mM^{-1})	k_r (M^{-1}min^{-1})
 RS194B	VX	2.8	1.6	1800
	sarin	2.5	1.9	1300
	CS	0.88	3.9	230
	POX	0.38	7.4	51
 RS2-138B	VX	2.6	1.6	1600
	sarin	2.4	1.2	2000
	CS	0.83	6.8	120
	POX	0.23	2.2	100
 RS3-43D	VX	1.6	1.3	1200
	sarin	1.1	0.8	1400
	CS	0.63	3.3	190
	POX	0.22	11	19
 RS3-36D	VX	6.7	7.7	870
	sarin	>10	>10	820
	CS	0.69	5.9	120
	POX	0.71	29	25
 RS2-237D	VX	2.1	3.6	570
	sarin	2.9	4.9	590
	CS	-	-	89
	POX	0.15	5.2	29
 RS2-234D	VX	3.2	6.2	520
	sarin	1.7	1.8	940
	CS	0.79	15	51
	POX	0.14	4.6	30

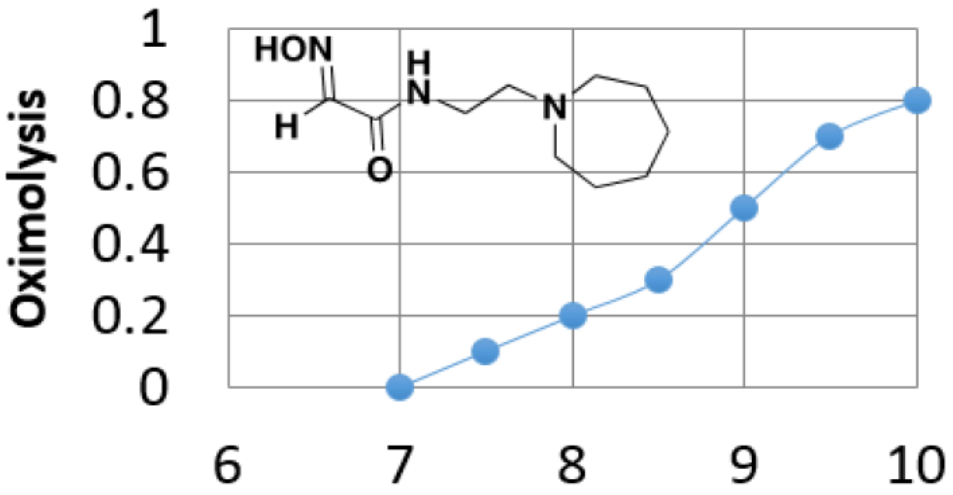
Reference Compounds

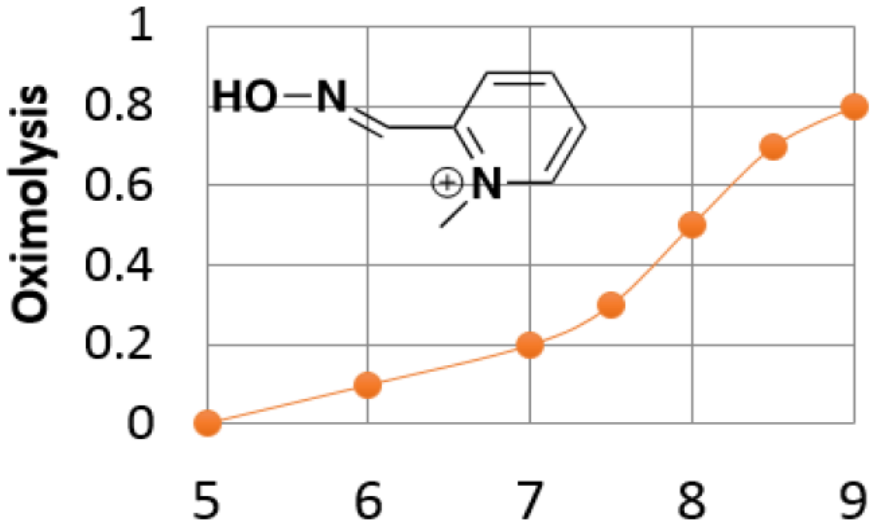
oxime	OP	k_2 (min^{-1})	K_{ox} (mM^{-1})	k_r (M^{-1}min^{-1})
 MINA	VX	>0.7	>6	110
	sarin	1.6	14	120
	CS	1.2	16	75
	POX	>0.2	>20	8.4
 DAM	VX	>0.2	>100	1.7
	sarin	0.13	88	1.5
	CS	>0.3	>100	2.5
	POX	0.027	46	0.58
 2PAM	VX	0.73	0.3	2400
	sarin	1.1	0.34	3200
	CS	0.73	6.6	110
	POX	0.27	1.8	150

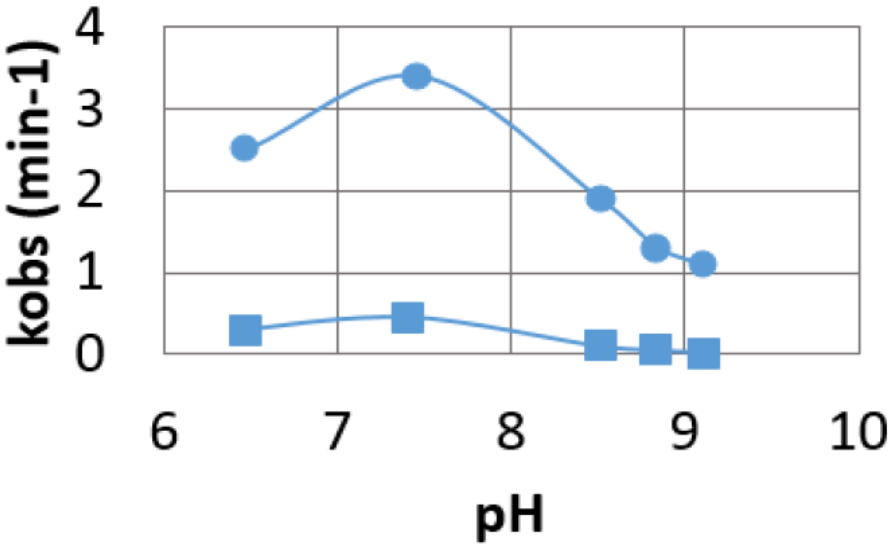
$$k_r = k_2/(1+K_{ox}/[\text{oxime}])$$

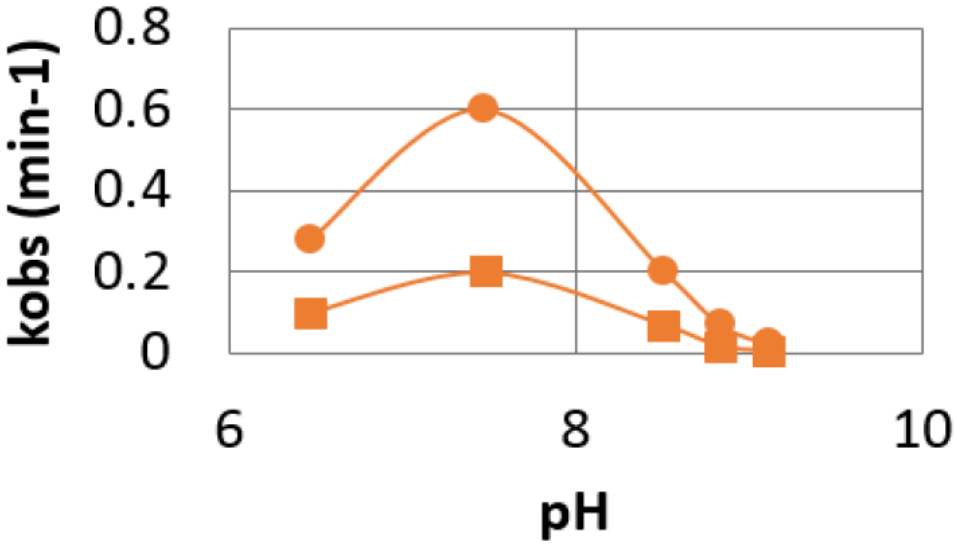


pH Dependences of Oxymolysis of VX in Buffer (Top) *versus* Oxime Catalyzed VX Conjugates with AChE Serine (Bottom)

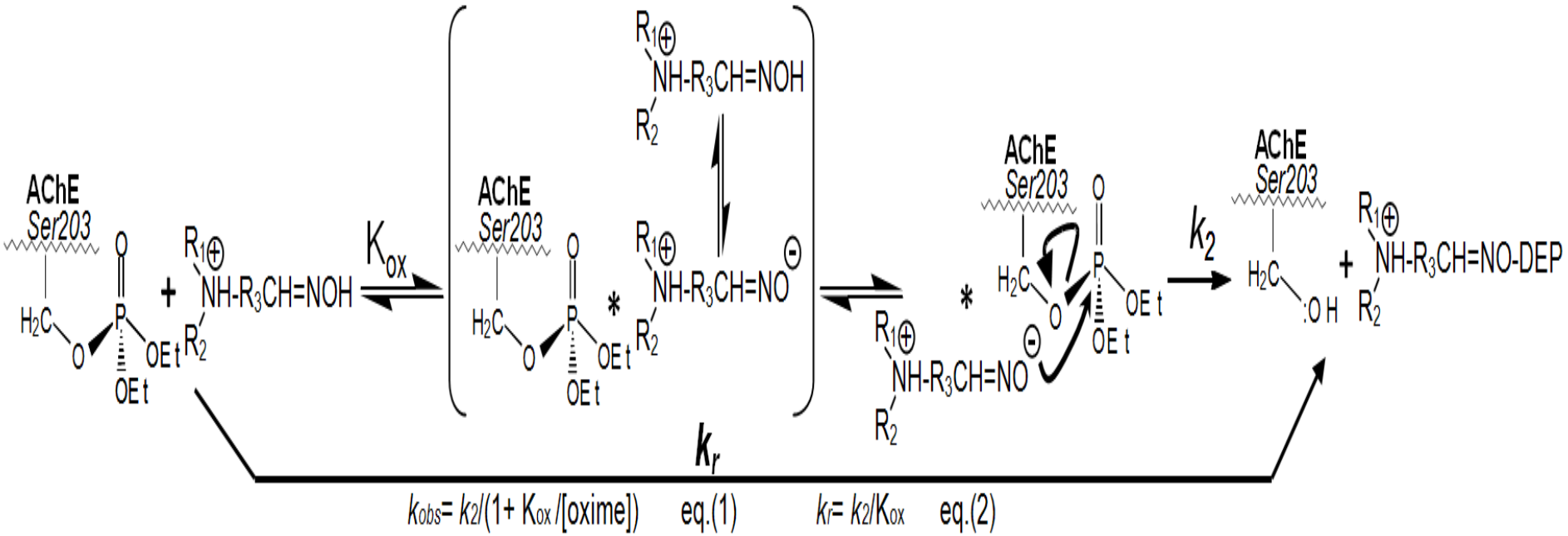








Proton Abstraction & Tunneling in Oxime Reactivation



Reaction steps in nucleophilic reactivation of OP-AChE with an oxime (RCH=NOH). K_{ox} is a Michaelis type constant for formation of sarin-AChE*oxime reversible complex that is practically identical to the equilibrium dissociation constant, since the maximal phosphylation rate constant (k_2) appears much slower in vast majority of cases than the dissociation rate constant of the complex.

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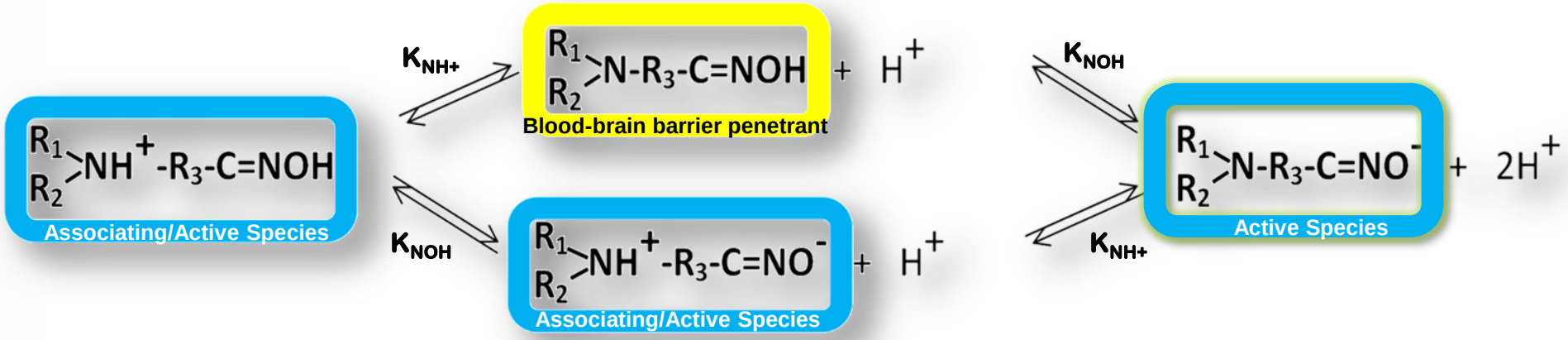
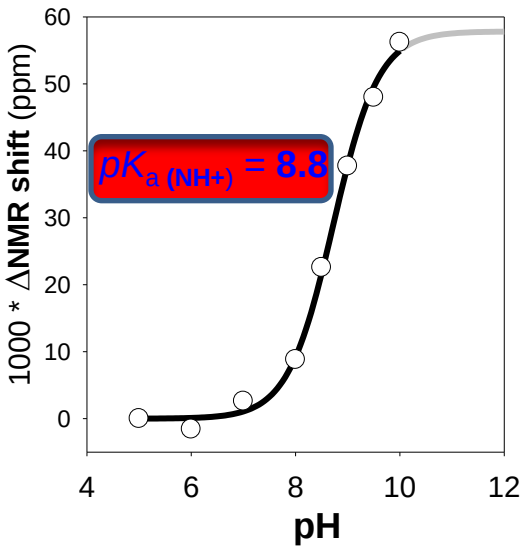
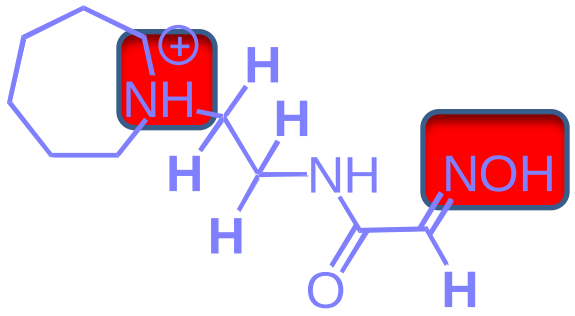
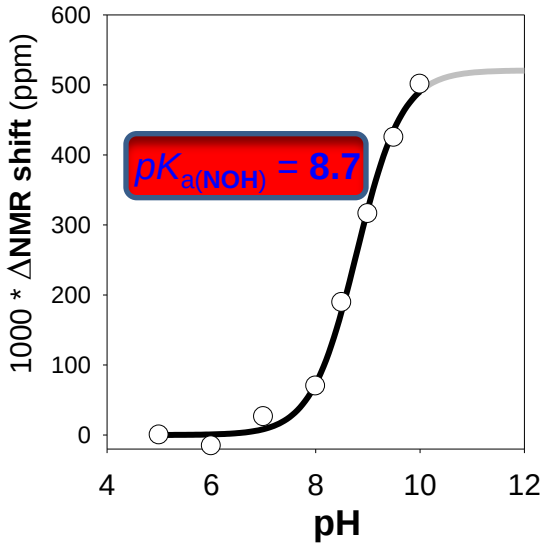
Oxime-Amine Zwitterion with Ionizing Species



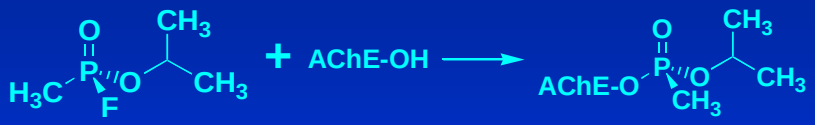




RS194B

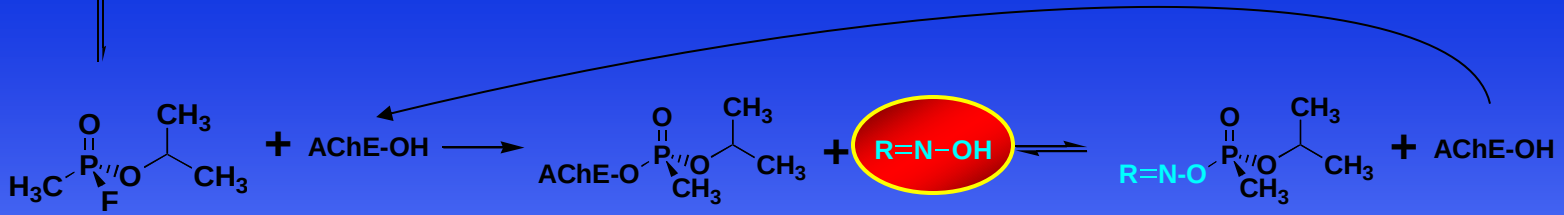


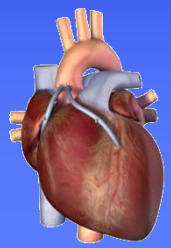
CNS



Blood-Brain Barrier

Plasma



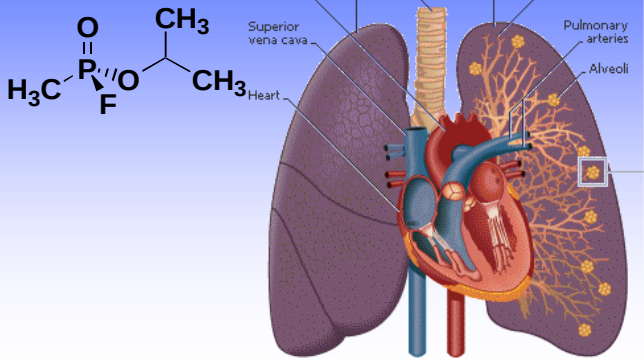


Reactivation of native peripheral AChE

Oxime-assisted Catalytic OP hydrolysis in Plasma

Alveolar Membrane

Site of Exposure

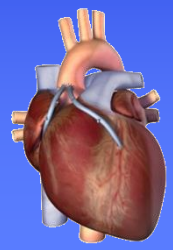


CNS

Oxime Reactivation of native AChE in CNS

Blood-Brain Barrier

Plasma



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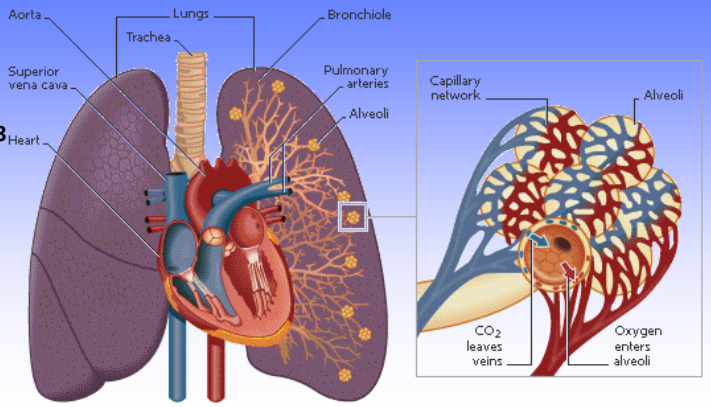
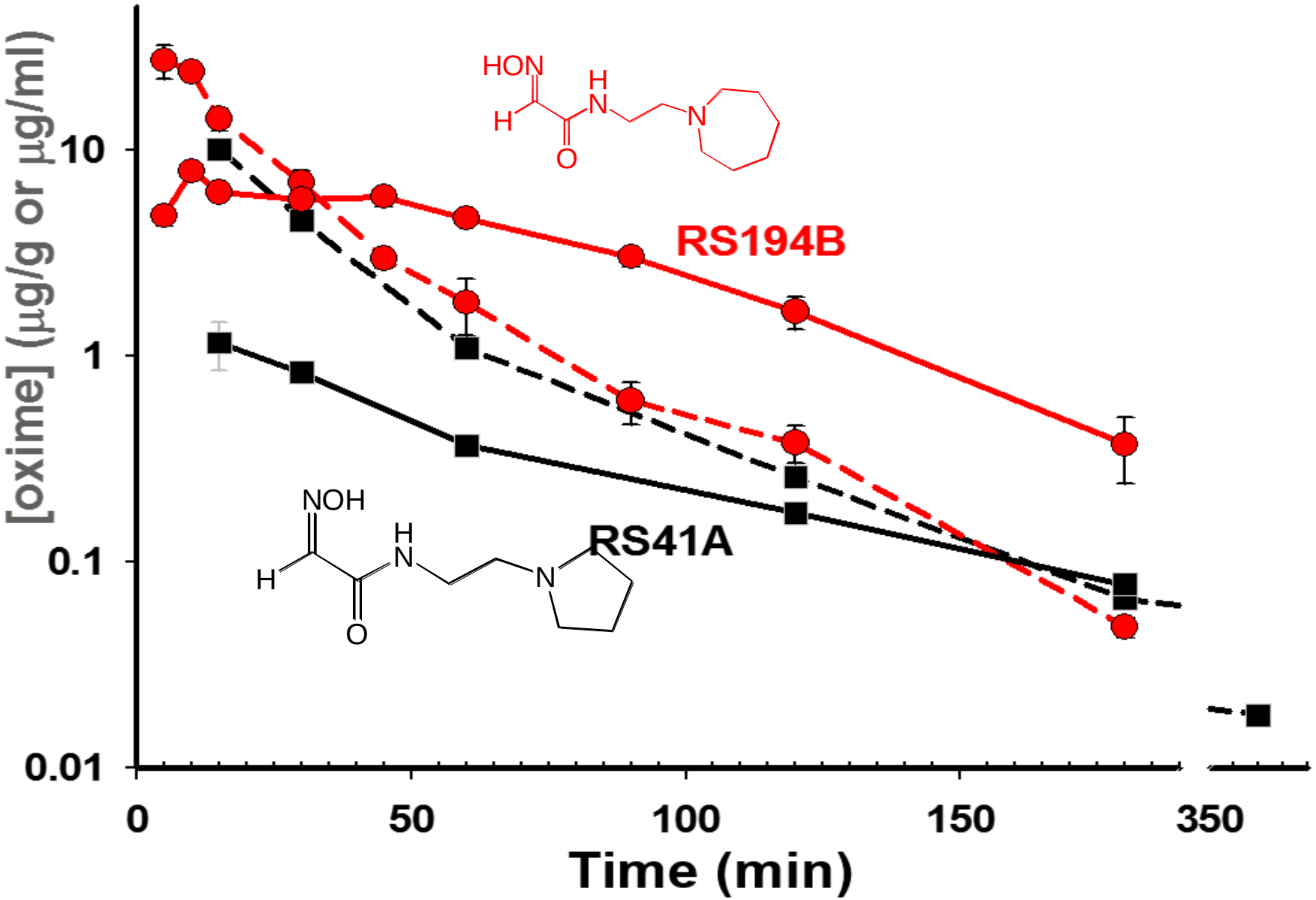
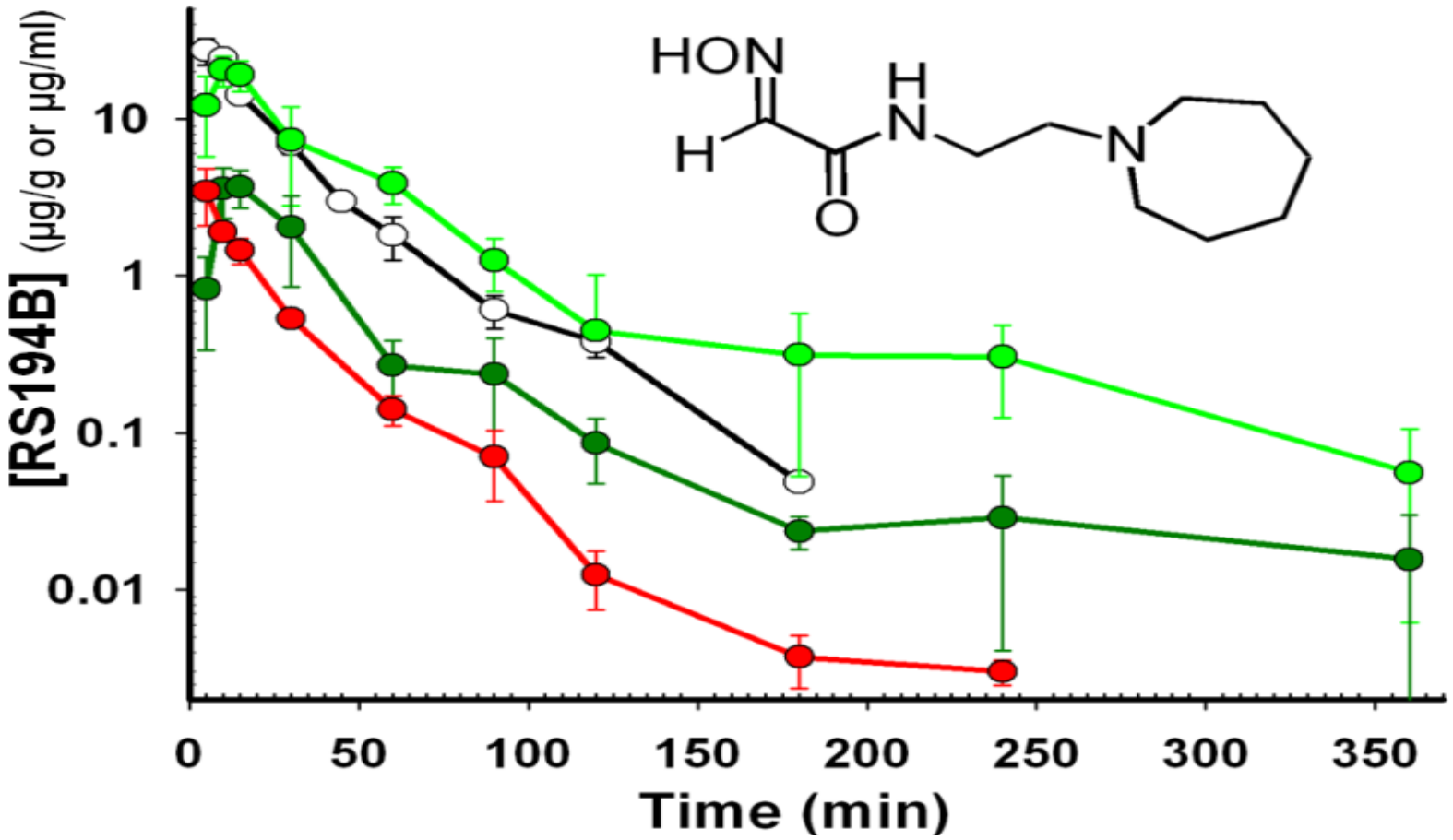


Superiority of RS 194B-Plasma and Tissue Kinetics Plasma in Mice: dashed line Brain: solid lines

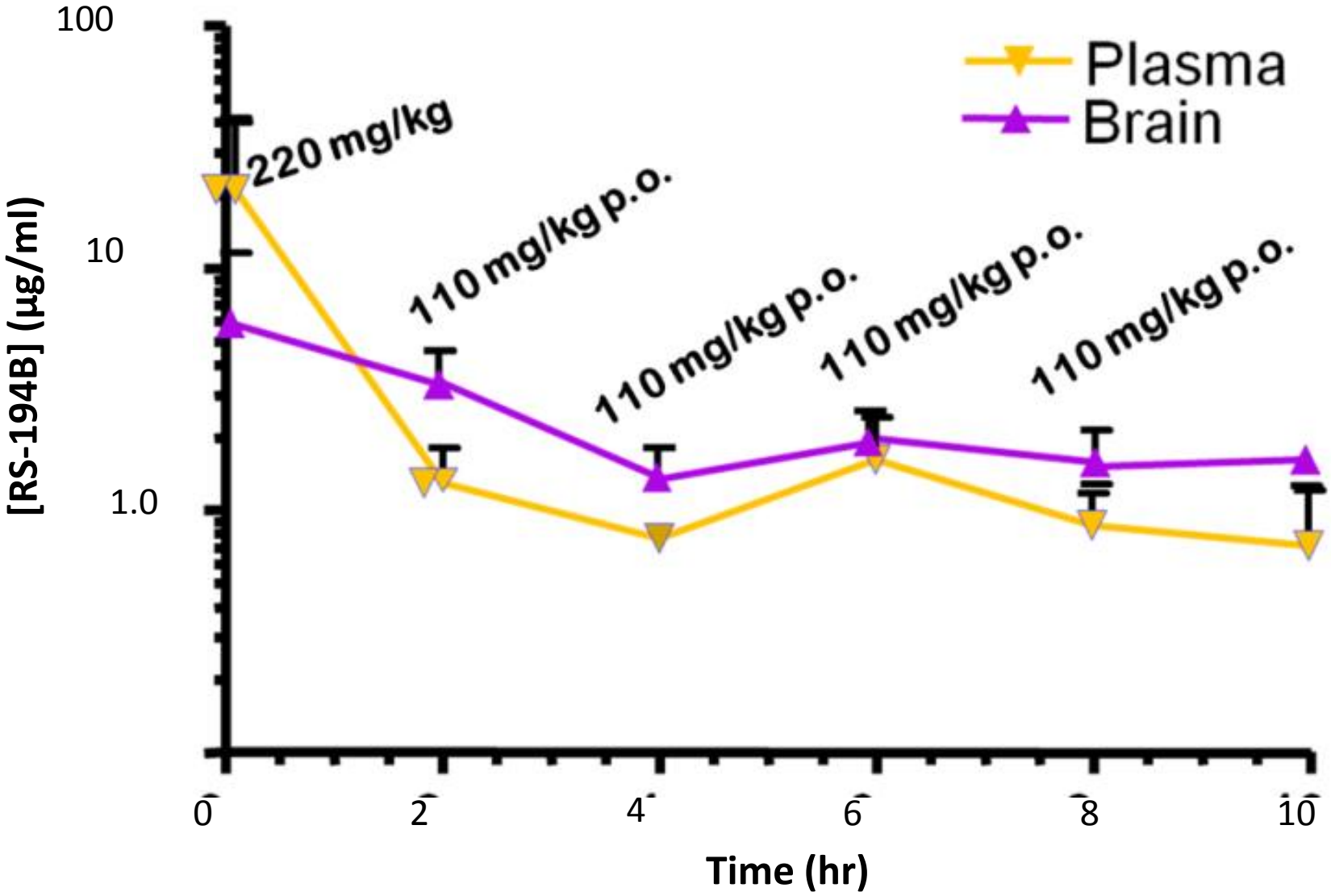


Plasma Levels after Intravenous, Intramuscular and Oral Dosing in Mice



PK profiles of RS194B in mouse plasma following various routes of administration to the mouse: **i.v.-**20 mg/kg; **p.o., 50 mg/kg; p.o. 200 mg/kg, i.m. 80 mg/kg;** The data show rapid oral absorption within 20 min and bioavailability >50% **.**

Plasma and Brain Concentrations After an i.m. Loading Dose Followed by Four Oral Maintenance Doses in Mice



RS194B and 2PAM Protective Indices upon i.m. and p.o. (gastric lavage) Administration in Mice

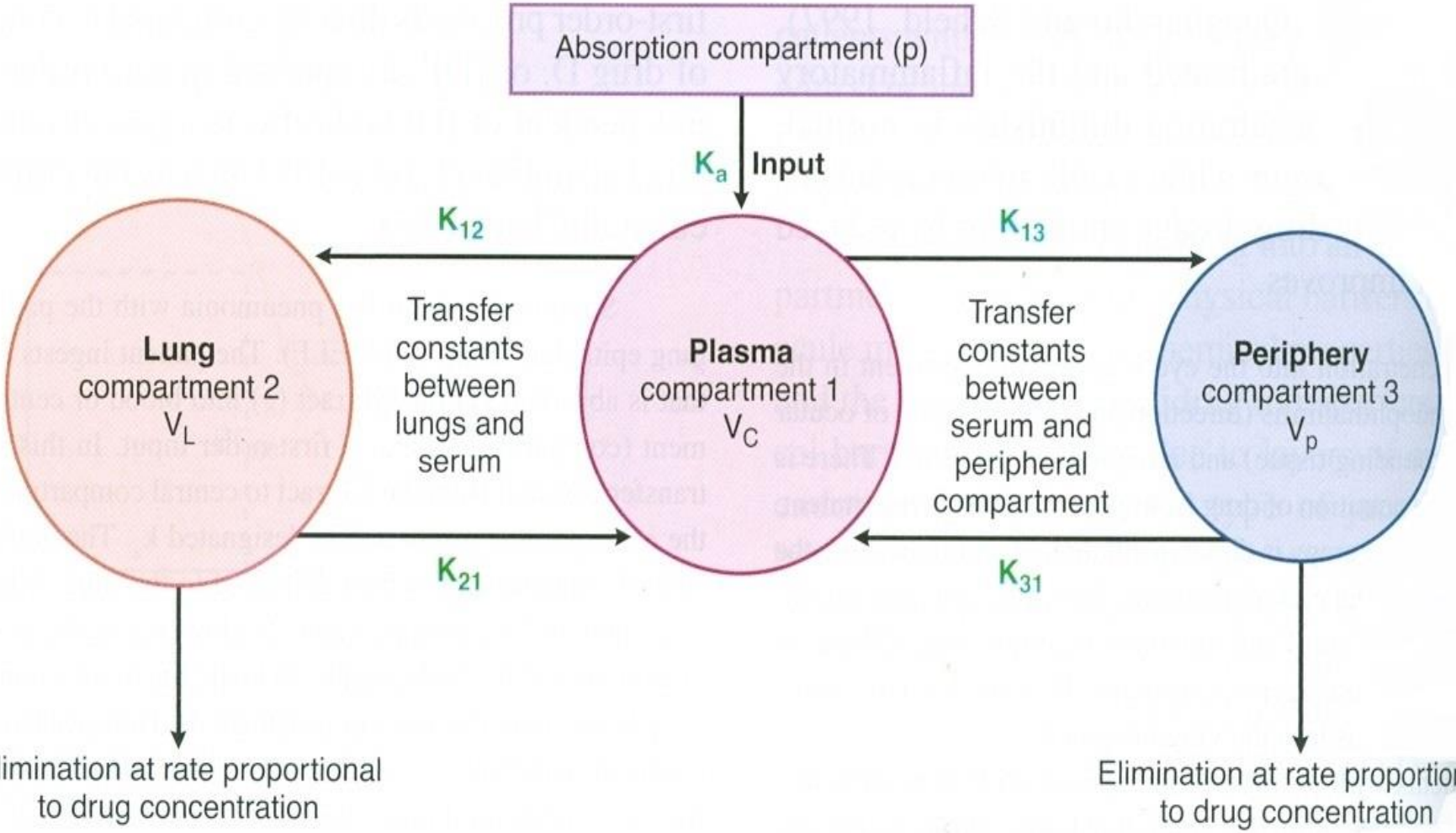
oxime	Protective Index [*(OP LD_{50} with oxime) / (OP LD_{50} without oxime)*]	
	1 min after VX i.m.	15 min before VX p.o.
RS194B		
125 mg/kg i.m.	18	-
200 mg/kg p.o.	-	40
2PAM		
26 mg/kg i.m.	9.3	-
42 mg/kg p.o.	-	1.1

Oxime doses set as 25% of individual oxime LD_{50} for i.m. administration.

FDA Animal Rule for Toxicants and Antidotes

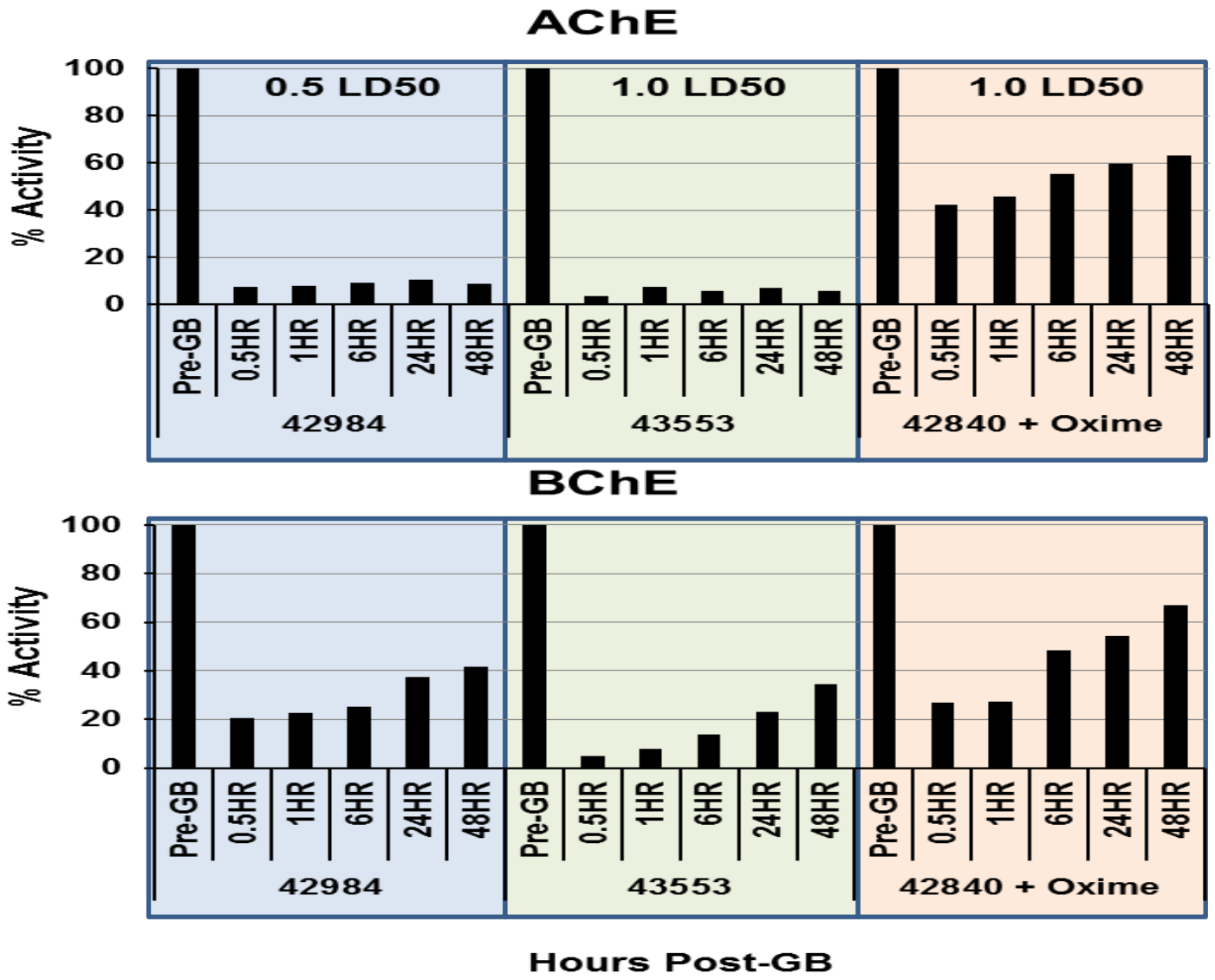
- Mode of Administration
- Pulmonary design and animal size
- Pulmonary physiology in non-human primates and rodents.
- Olfactory system dependence-turbenate structures
- Fraction of cardiac output from absorption point
- Sequelae of cholinergic symptoms

Disposition of Antidote from Absorption to the Lung

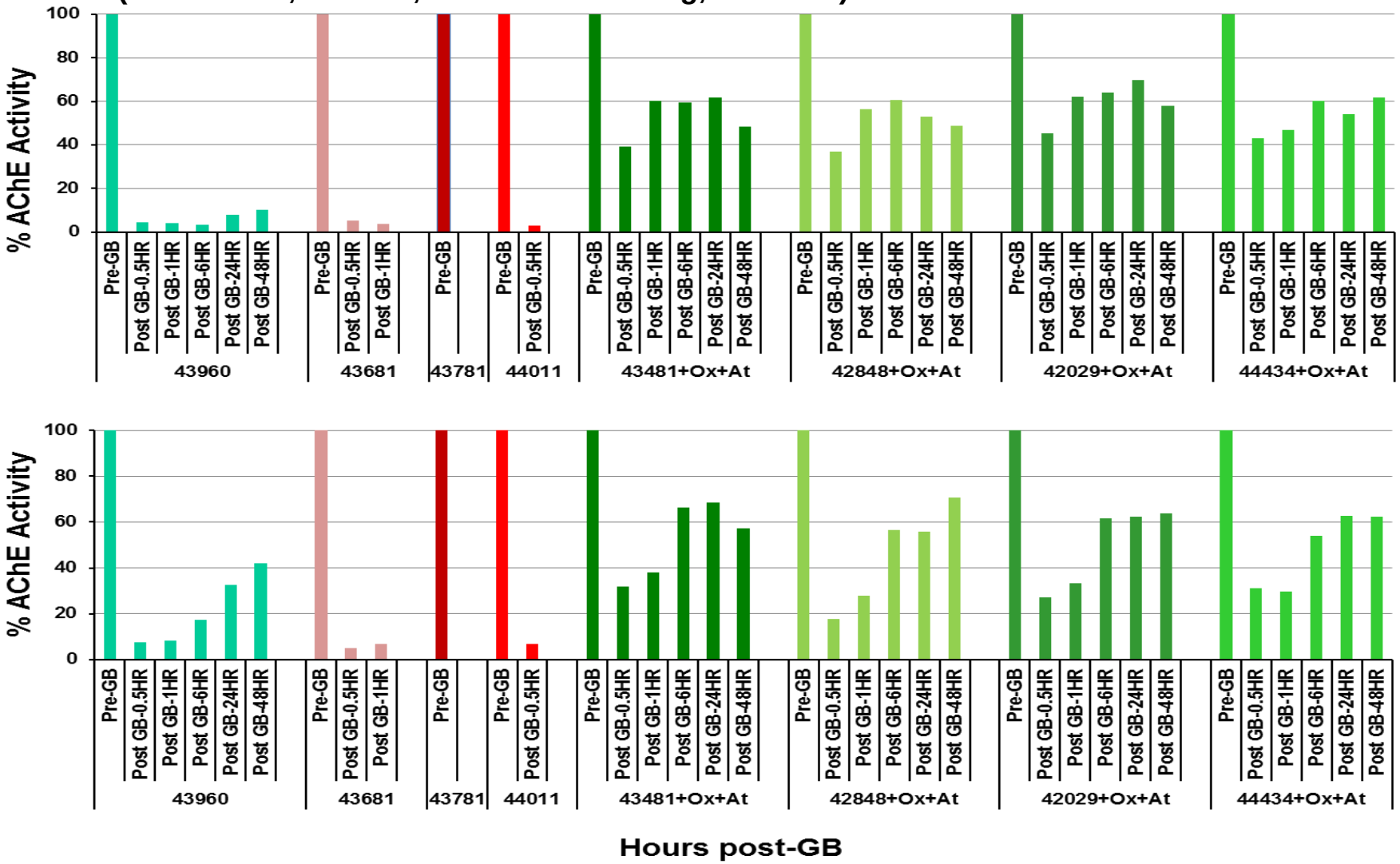


Recovery of Macaque Blood Cholinesterase Activities from Sarin

Yvonne Rosenberg, PlantVax; Tom Snider, Battelle Institute



Recovery of Macaque Blood Cholinesterase Activities after Sarin Exposure
(Top) AChE; (Bottom) BChE
(Tom Snider, Battelle; Yvonne Rosenberg, PlantVax)



Oxime (RS194B) administered at 62.5 mg/kg and Atropine 0.28 mg/kg 2.75 min after a 31 min sarin exposure. BChE pre-administered by inhalation to obtain 6.0 mg/kg deposited. Green monkeys survived, red and orange monkeys died.

Chemical Design &Synthesis

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AChE Structure-Function

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AChE Crystal Structure

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Pharmacokinetics, Toxicity & Disposition

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Gratitude and Recognition of the Many Students and Fellows from Yokohama City University Who Trained at UC San Diego

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