

# Towards a Pseudo-Catalytic Bioscavenger: *in vitro* Reactivation of Organophosphorus-Inhibited and Resurrection of Organophosphorus-Aged Butyrylcholinesterase by Quinone Methide Precursors

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## INTRODUCTION

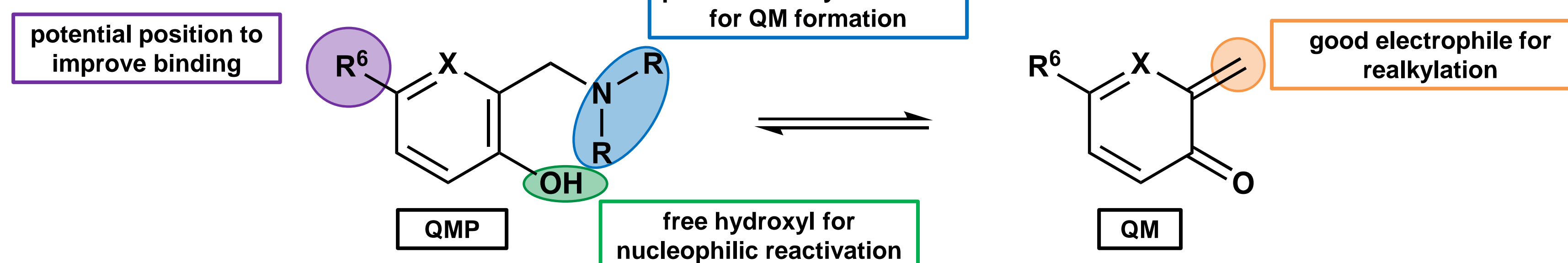
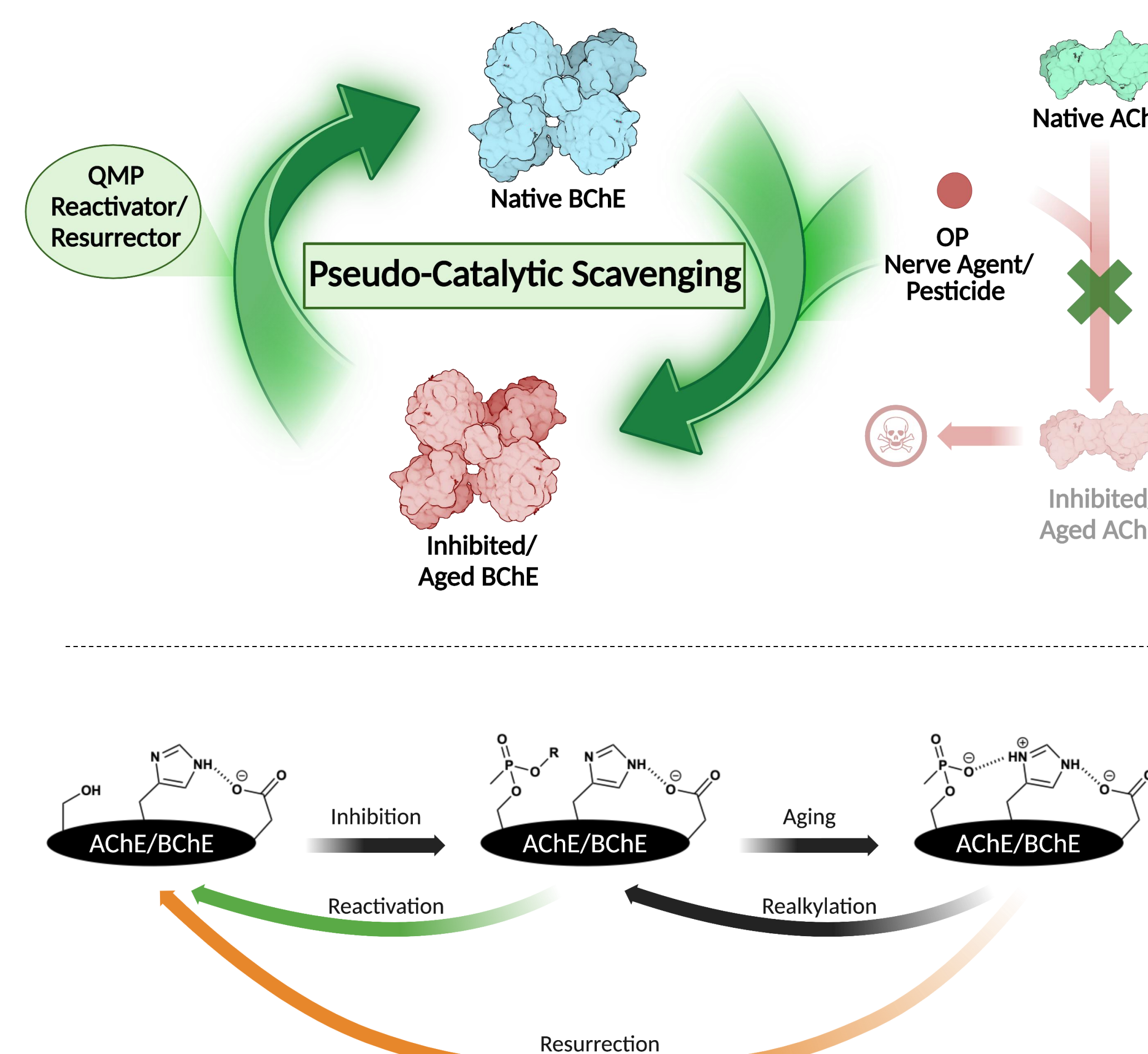
Cholinesterases are covalently inhibited by **organophosphorus (OP) compounds** used as pesticides and chemical warfare agents.

Inhibition of acetylcholinesterase (AChE) can lead to cholinergic crisis and death.

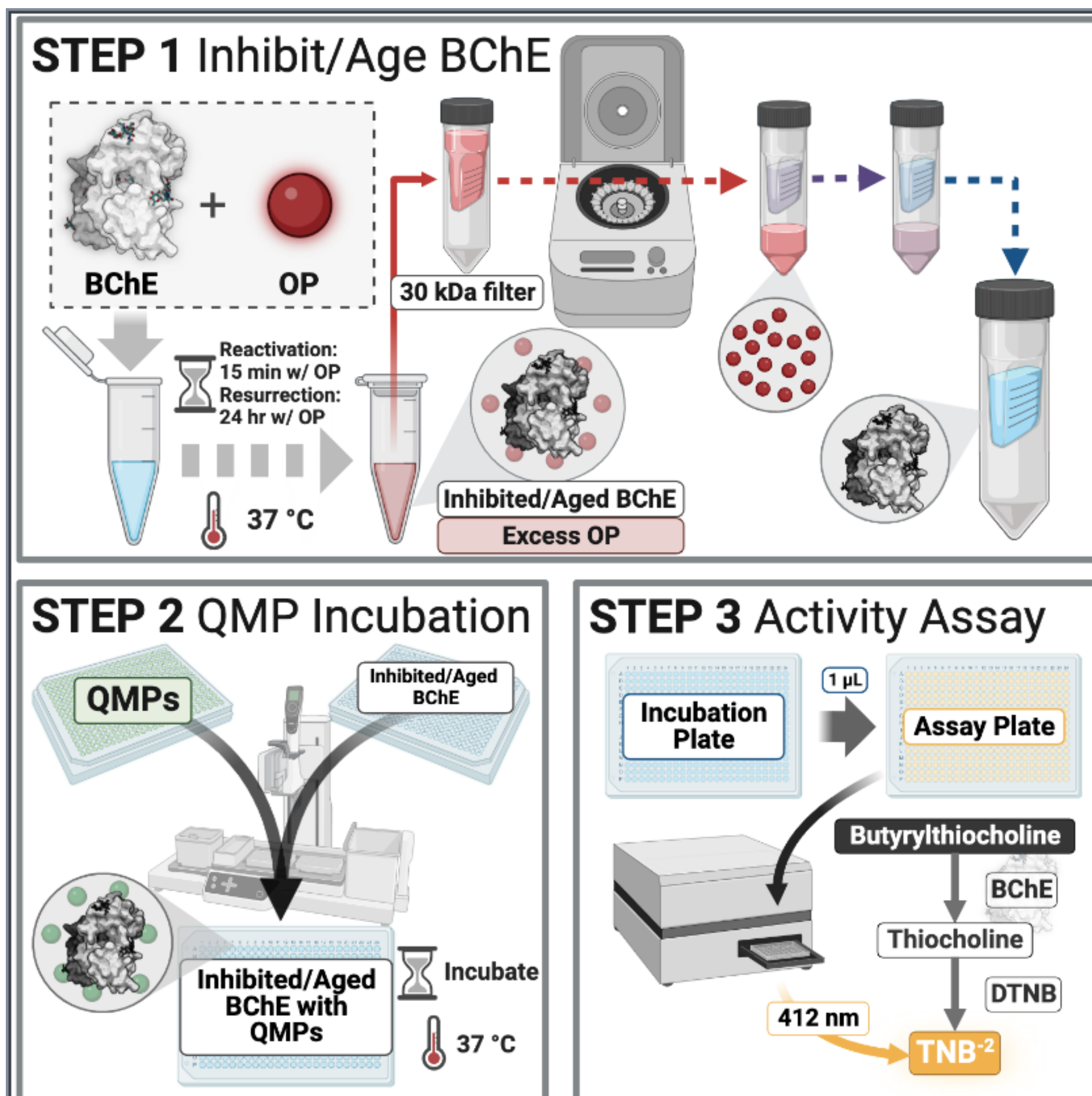
**Butyrylcholinesterase (BChE)** is a non-essential cholinesterase naturally found in the body capable of **stoichiometrically** scavenging OP compounds.

Upon AChE/BChE inhibition, a second **spontaneous O-dealkylation** event known as **aging** occurs that is **recalcitrant** to reactivation by pyridinium oximes.

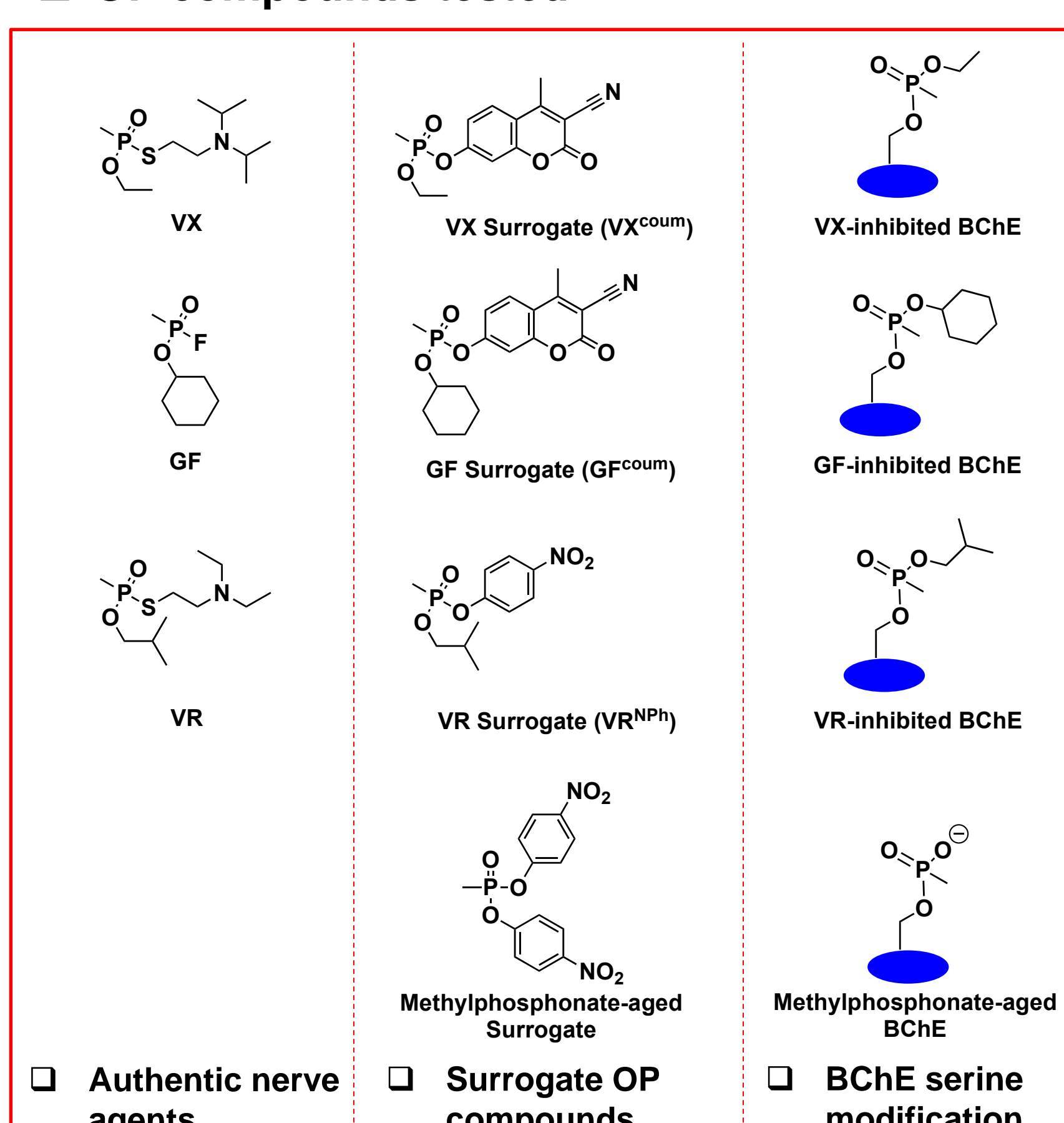
**Quinone methide precursors (QMPs)** have previously been shown to reactivate OP-inhibited and OP-aged AChE, and here are being tested against BChE.<sup>1</sup>



## METHODS



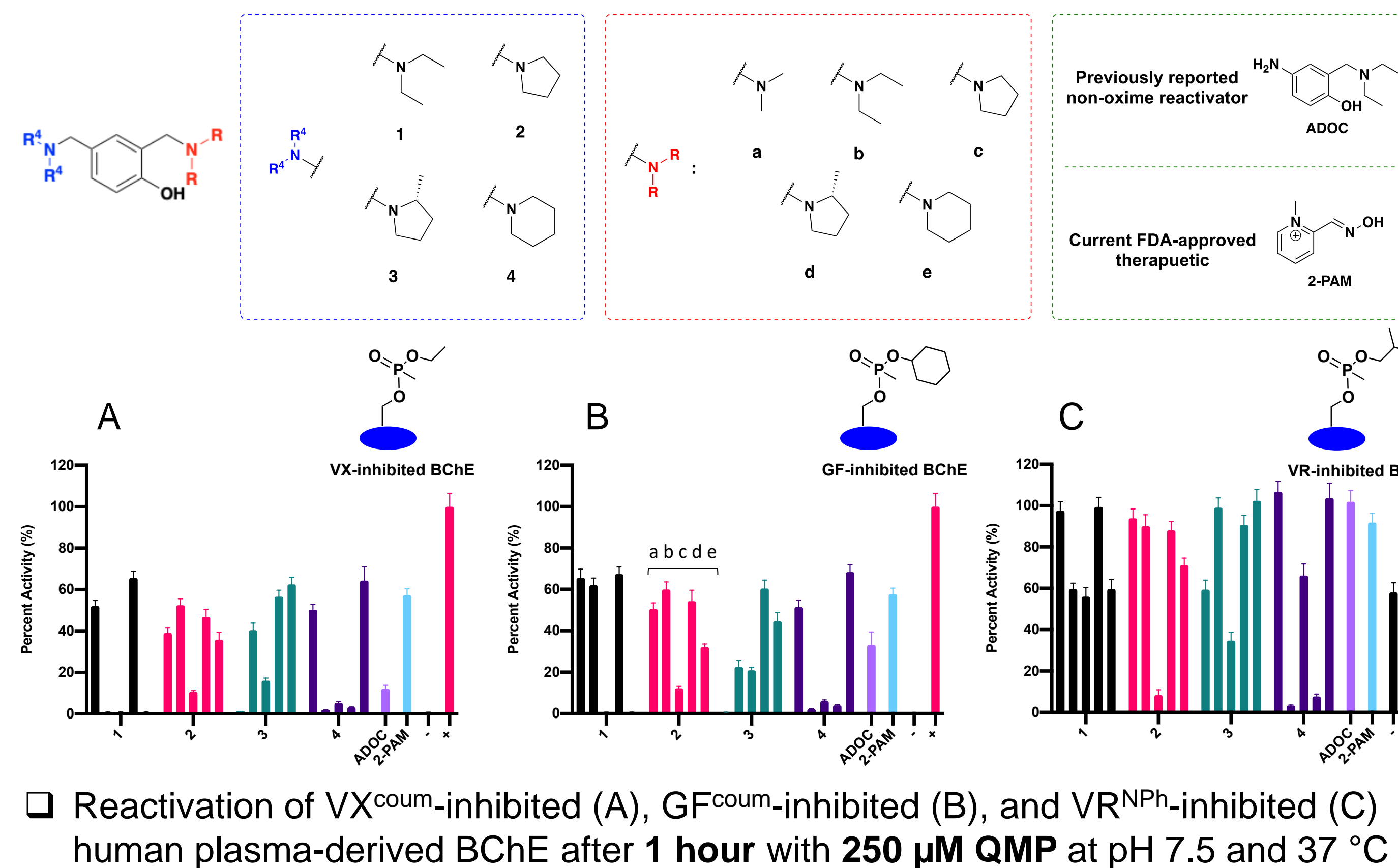
### OP compounds tested



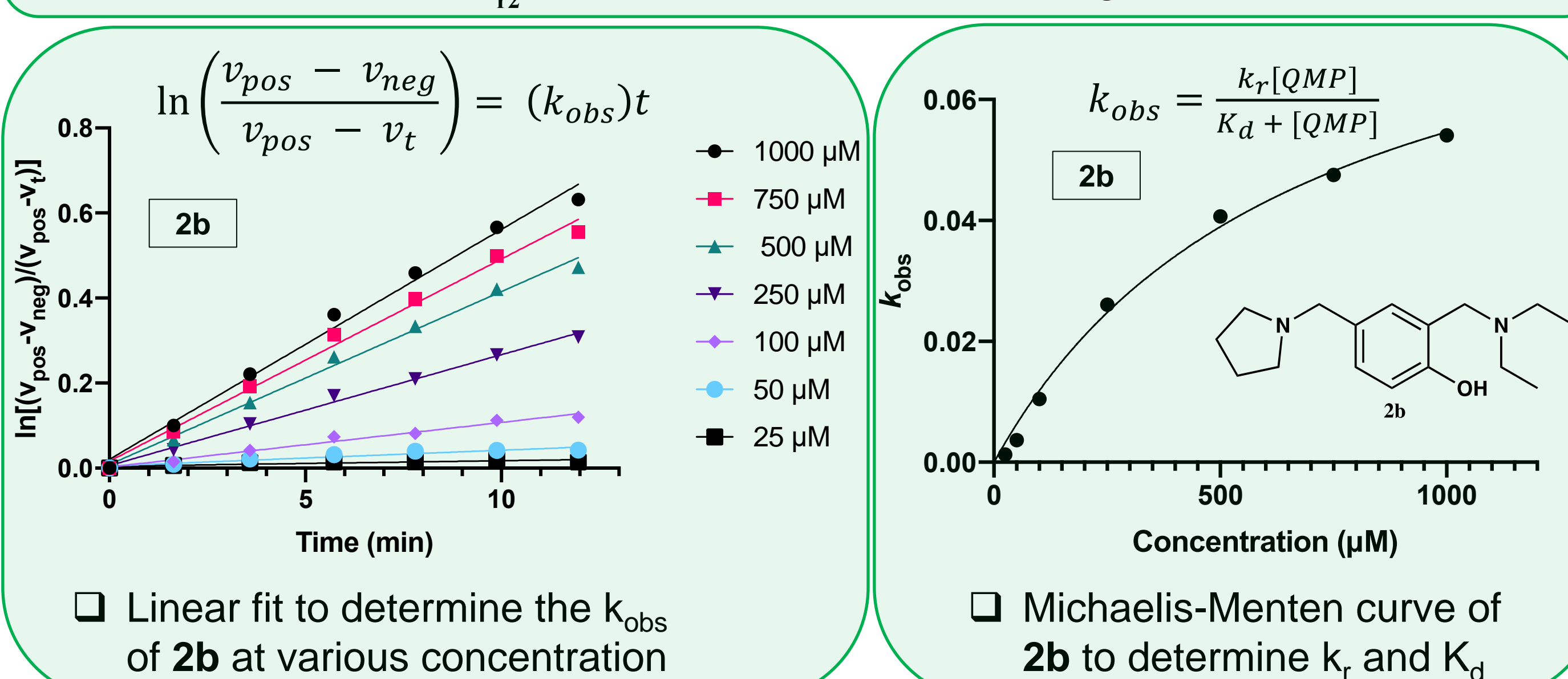
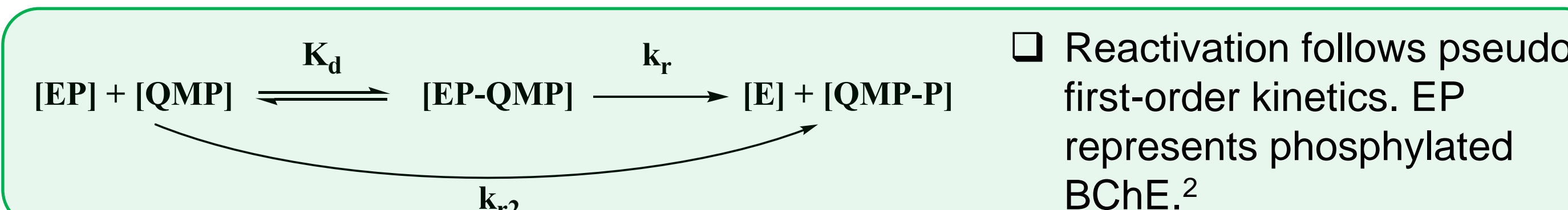
## RESULTS

### Reactivation of OP-Inhibited BChE

#### QMPs screened for reactivation



Reactivation of VX<sup>coum</sup>-inhibited (A), GF<sup>coum</sup>-inhibited (B), and VR<sup>NPh</sup>-inhibited (C) human plasma-derived BChE after 1 hour with 250  $\mu$ M QMP at pH 7.5 and 37 °C



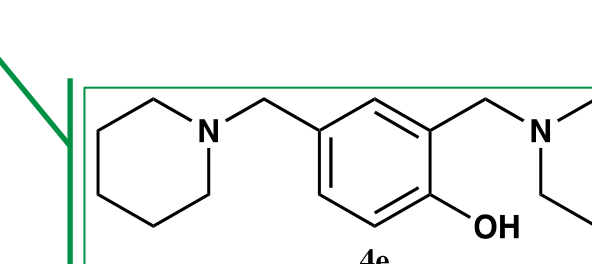
Linear fit to determine the  $k_{obs}$  of **2b** at various concentration

Michaelis-Menten curve of **2b** to determine  $k_r$  and  $K_d$

OP	$k_r$ (min <sup>-1</sup> )			$K_d$ ( $\mu$ M)			$k_{r2}$ (min <sup>-1</sup> mM <sup>-1</sup> )		
	VX <sup>coum</sup>	GF <sup>coum</sup>	VR <sup>NPh</sup>	VX <sup>coum</sup>	GF <sup>coum</sup>	VR <sup>NPh</sup>	VX <sup>coum</sup>	GF <sup>coum</sup>	VR <sup>NPh</sup>
1a	0.061	- <sup>a</sup>	0.340	2394	>10,000	323	0.025	- <sup>a</sup>	1.04
1d	0.055	0.169	0.677	540	2901	212	0.102	0.058	3.20
2a	0.027	0.107	0.561	1289	4456	858	0.021	0.024	0.653
2b	0.038	0.100	0.370	363	847	115	0.106	0.118	3.22
2d	0.020	0.033	0.239	497	853	137	0.039	0.038	1.74
3a	0.038	0.096	0.583	1053	3107	291	0.036	0.031	2.00
3e	0.065	- <sup>a</sup>	0.584	1011	>10,000	86	0.065	- <sup>a</sup>	6.77
4a	0.011	0.060	0.516	746	5163	676	0.014	0.011	0.763
4e	0.082	0.070	0.663	832	3385	79	0.098	0.021	8.35
ADOC	0.002	- <sup>a</sup>	0.199	- <sup>b</sup>	- <sup>a</sup>	1798	- <sup>b</sup>	- <sup>a</sup>	0.111
2-PAM	0.036	0.051	0.088	522	2451	1084	0.068	0.021	0.081

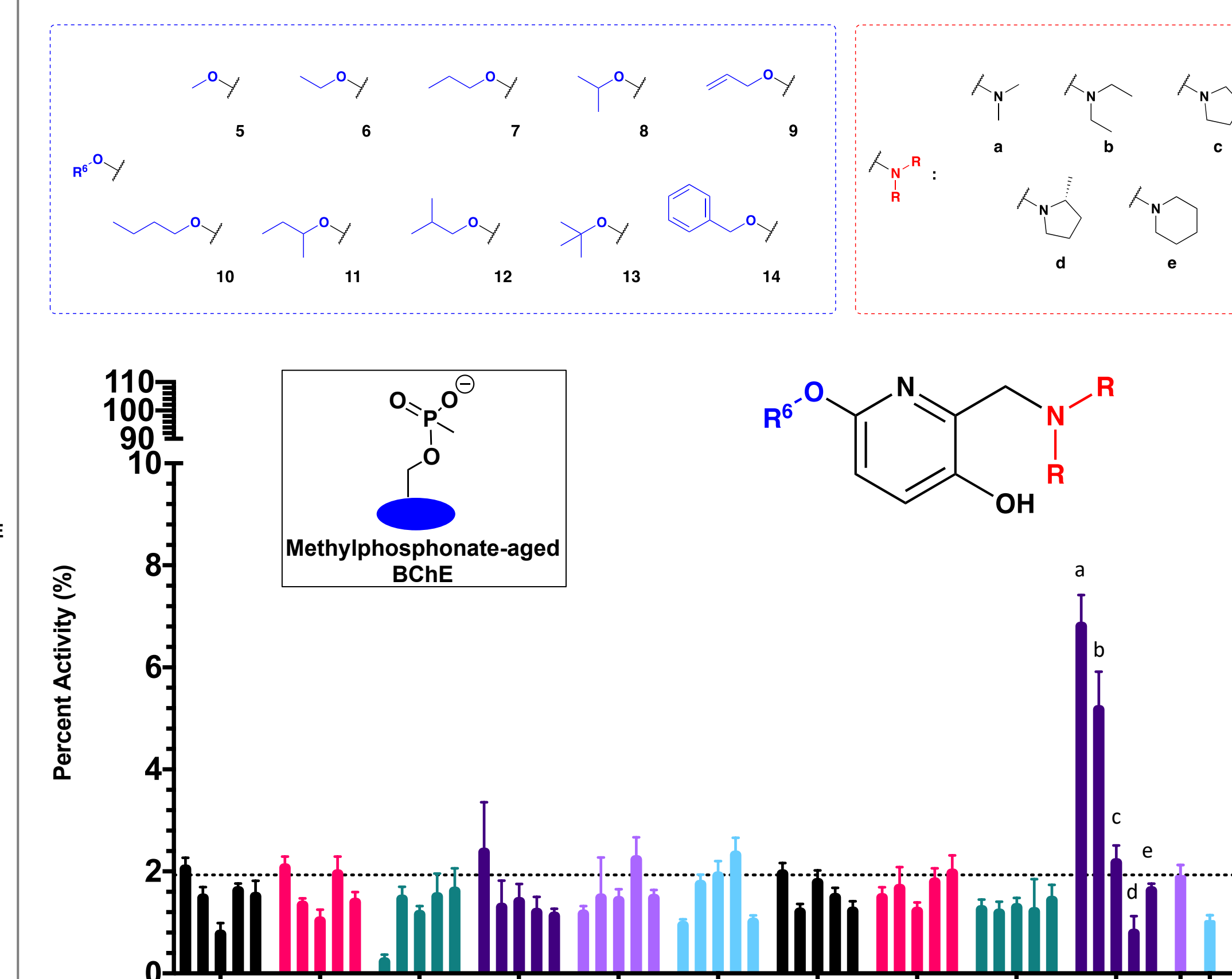
<sup>a</sup>: Could not be determined due to poor curve fitting  
<sup>b</sup>: Could not be determined due to minimal reactivation

Kinetic constants for top performing QMPs  
 $k_{r2}$  is equal to  $k_r$  divided by the  $K_d$   
All values are averages of 2 replicates.

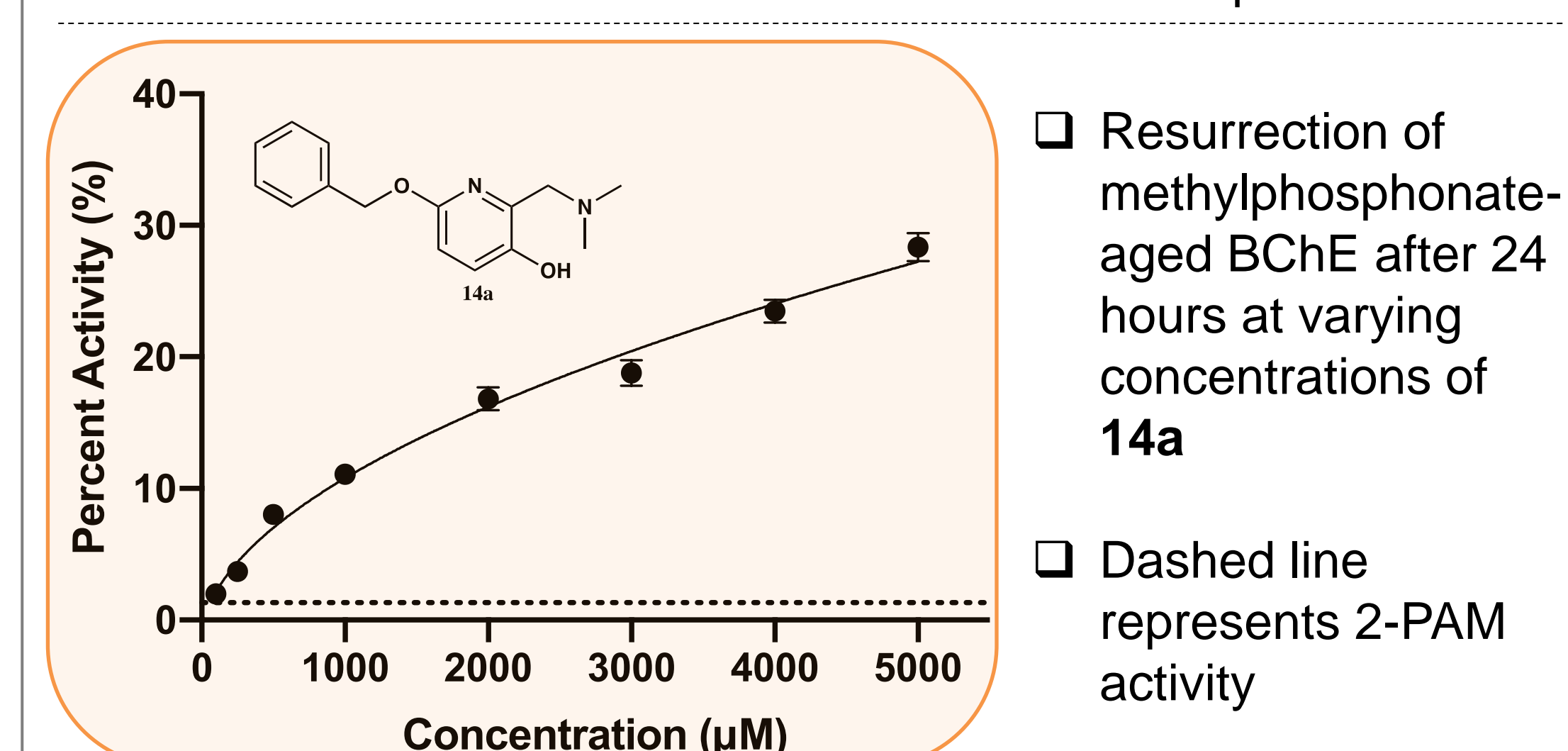


### Resurrection of OP-Aged BChE

#### QMPs screened for resurrection



Resurrection of methylphosphonate-aged human plasma-derived BChE after 24 hours with 1 mM QMP at pH 7.5



Resurrection of methylphosphonate-aged BChE after 24 hours at varying concentrations of **14a**  
Dashed line represents 2-PAM activity

## Conclusions

### Reactivation

- Multiple QMPs show more efficient reactivation of OP-inhibited BChE than current therapeutics (such as, 2-PAM).
- Notably, **2b** shows broad activity against all three OP compounds and **4e** is very effective against the VR surrogate.
- The extended and larger amine at the 4-position may improve reactivation compared to ADOC due to the larger active site of BChE.

### Resurrection

- Best resurrection is observed with smaller NMe<sub>2</sub> amines.
- Resurrection only observed with large 6-alkoxy substituent.

## Bibliography

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(2) Horn, G.; Wille, T.; Musilek, K.; Kuca, K.; Thiermann, H.; Worek, F. Reactivation Kinetics of 31 Structurally Different Bispyridinium Oximes with Organophosphate-Inhibited Human Butyrylcholinesterase. *Arch. Toxicol.* **2015**, *89* (3), 405–414. <https://doi.org/10.1007/s00204-014-1288-5>

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