

Click chemistry: an original approach for drug discovery, Yves Bourne,^{a*} Hartmuth C. Kolb,^b Zoran Radic,^c Barry Sharpless,^b Palmer Taylor^c and Pascale Marchot^d, ^aAFMB CNRS UMR 6098, France, ^bThe Scripps Research Institute, USA, ^cUniversity of California at San Diego, USA, and ^dIngénierie des Protéines, CNRS UMR 6560, France. E-mail: yves.bourne@afmb.cnrs-mrs.fr

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Acetylcholinesterase (AChE) inhibitors bind either to the enzyme's active site, located at the bottom of a deep gorge, or to a secondary site at the rim of the gorge. Those that bind the active site have long been used to treat Alzheimer's dementia, but the search for more potent and less toxic inhibitors continues. We have used the cycloaddition reaction between azides and alkynes to synthesize new potent AChE inhibitors. This reaction is extremely slow at room temperature and can be accelerated by heating, but adding *wt* AChE to the reaction mixture even more dramatically accelerates it. By using the enzyme's gorge as a template, one pair of building blocks represents, after cycloaddition, the most potent noncovalent inhibitors of AChE synthesized to date [1]. Our structural approach using mouse AChE explains not only why the click-chemistry inhibitors are so powerful but also why the enzyme-synthesized *syn* product is so much better than the chemically synthesized *anti* product (**Fig. 1**) [2]. Similarly, the Y337A mutant shows for the *anti* and *syn* isomers, two to three orders of magnitude greater affinities, compared to *wt* AChE, with K_i values in the 1-10 fM range.

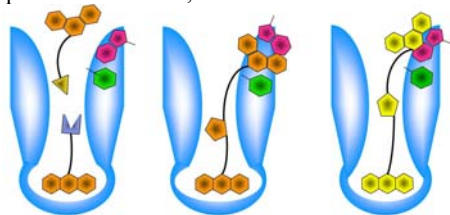


Fig. 1: Schematic representation of apo, *anti* and *syn* complexes

The potency difference between the *syn* and *anti* products is explained by their binding at the gorge's rim. A part of the *syn* product intercalates between aromatic residues near the rim, whereas the *anti* product does not. The insertion is made possible by a newly found, more-open conformation of the enzyme, in which a tryptophan residue at the gorge's rim flips out to the solvent, creating the space that makes intercalation possible. Such a conformation had not been seen in other structures of the free enzyme or its complexes with inhibitors bound at the gorge's rim [3]. In conclusion, the freeze-frame reaction offers both a strategically original approach for drug discovery and a means for kinetically controlled capture, as a high-affinity complex between the enzyme and its self-created inhibitor, of a highly reactive minor abundance conformer of a fluctuating protein template. Details of these structures solved in the 2.45-2.6 Å resolution range will be presented.

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[2] Bourne Y., Kolb H.C., Radic Z., Sharpless K.B., Taylor P. & Marchot P. (2004) *Proc Natl Acad Sci U S A.* **101**, 1449-54

[3] Bourne Y., Taylor P., Radic Z. & Marchot P. (2003) *EMBO J.* **22**, 1–12.