s1.m6.o3 **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], [a]*AFMB CNRS UMR 6098, France,* [b]*The Scripps Research Institute, USA,* [c]*University of California at San Diego, USA, and* [d]*Ingénierie des Protéines, CNRS UMR 6560, France. E-mail: yves.bourne@afmb.cnrs-mrs.fr*

Keywords: Acetylcholinesterase; Crystallography; Combinatorial Chemistry

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 acetylenes 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 synthesize 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 Ki values in the 1-10 fM range.

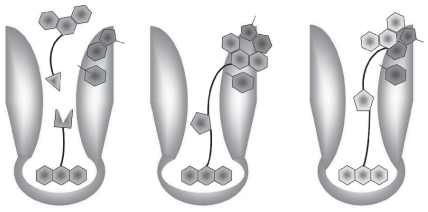

Fig. 1: Schematic representation of apo, *anti* and *syn* complexes

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 betweent he 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 HC, Radic Z, Sharpless KB, 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

s1.m6.o4 **Structure Based Drug Design of a New Class of Antibacterial Agents.** Véronique Blanc, Marc Capet, Chantal Carrez, Arielle Genevois-Borelli, Jean-Pierre Guilloteau, Jean-Paul Martin, Magali Mathieu, Sophie Vincent, Sylvie Wentzler, Vincent Mikol, *Drug Innovation & Approval, Aventis Pharma, 13 Quai Jules Guesde, BP.14, F 94403 Vitry-sur-Seine, France. vincent.mikol@aventis.com; phone 33 158933093, fax 33 158938063*

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The deformylase project is featured by the extensive use of the genomic and structural data to highlight and define the molecular requirements to generate a potent, large-spectrum new class of antibiotics. Bacterial peptide deformylase (PDF) belongs to a sub-family of metalloproteases, which catalyses the removal of the N-terminal formyl group from newly synthesized proteins. PDF is essential in prokaryotes and conserved throughout the eubacteria. It has therefore qualified as an attractive target for developing new antibacterial agents. Several structures of four bacterial PDFs, including two from the major bacterial pathogens, free or bound to actinonin, a natural occurring antibiotic, two of which belong to the Gram negative strains (*Escherichia coli* and *Pseudomonas aeruginosa*) and two to the Gram positive strains (*Staphylococcus aureus* and *Bacillus stearothermophillus*) have been determined. It is shown that the overall tertiary structure is essentially conserved but shows significant differences namely at the C-terminus which are directly related to the deformylase class (i.e. I or II) they belong to. The geometry around the catalytic metal exhibits high similarity within the different enzymes as does the binding mode of actinonin to the various PDFs. However some significant structural differences are found in the vicinity of the active site highlighting the structural and molecular requirements for the design of a PDF inhibitor active against a broad spectrum of bacterial strains. Using this information a new class of macrocyclic compound has been designed based upon the actinonin molecule and synthesized. This resulted in a new chemical series of compounds that display a significantly improved biochemical activity, an inhibitory activity against a large spectrum of bacterial strains and in vivo activity in a mouse septicemia model both by the i.v. and oral route.