

MEETING ABSTRACTS

RECENT BREAKTHROUGHS IN THE STRUCTURE/FUNCTION STUDIES OF ACETYLCHOLINESTERASE

J.L. Sussman¹ & I. Silman²

¹ Departments of Structural Biology, Weizmann Institute of Science, Rehovot 76100, Israel,
Joel.Sussman@weizmann.ac.il

² Neurobiology Department, Weizmann Institute of Science, Rehovot 76100, Israel

The synaptic enzyme acetylcholinesterase (AChE) terminates transmission at cholinergic synapses by rapidly hydrolysing acetylcholine. Examination of the 3D structure of AChE¹ shows that the active site is located at the bottom of a deep and narrow gorge, lined largely by aromatic residues, with its peripheral anionic site located at the top, near the entrance to of the gorge. 3D structures of AChE have been determined for the *Torpedo*, *Electrophorus*, mouse, *Drosophila* and human enzymes. Overall, more than a hundred crystal structures of AChEs, and of covalent conjugates and reversible complexes with various inhibitors and substrate analogues have been determined. Although the 3D structure of the enzyme itself, and of its molecular dimer, are highly conserved, subtle structural differences are seen to occur upon the binding of certain inhibitors. These changes are well correlated with molecular dynamics data, and appear to be of functional significance.

Unfortunately, upon heterologous overexpression, many proteins misfold or aggregate, thus resulting in low functional yields. Human AChE is a typical case of a human protein that necessitates mammalian systems to obtain functional expression. Using a novel computational strategy, we designed an AChE variant bearing 51 mutations that improved core packing, surface polarity, and backbone rigidity. This variant expressed at ~2,000-fold higher levels in *E. coli* compared to wild-type hAChE, and exhibited 20°C higher thermostability with no change in enzymatic properties or in the active-site configuration as determined by crystallography^{2,3}.

Keywords: *acetylcholinesterase; improved stability; improved expression; Proteopedia; 3D structure*

References

1. Sussman, J. L., Harel, M., Frolow, F., Oefner, C., Goldman, A., Toker, L. & Silman, I. Atomic structure of acetylcholinesterase from *Torpedo californica*: a prototypic acetylcholine-binding protein. *Science* **253**, 872-879 (1991).
2. Goldenzweig, A., Goldsmith, M., Hill, S. E., Gertman, O., Laurino, P., Ashani, Y., Dym, O., Unger, T., Albeck, S., Prilusky, J., Lieberman, R. L., Aharoni, A., Silman, I., Sussman, J. L., Tawfik, D. S. & Fleishman, S. J. Automated structure- and sequence-based design of proteins for high bacterial expression and stability. *Mol. Cell* **63**, 337–346 (2016).
3. *Proteopedia: Automated computational design of human enzymes for high bacterial expression and stability*, http://www.proteopedia.org/w/Journal:Molecular_Cell:1 (2016).