s1.m8.p6 **Structural analysis of Acetylcholine-Binding Protein from the snail *Bulinus truncatus*.** Patrick Celie[a], Remco Klaassen[b], Sari van Rossum-Fikkert[a], , René van Elk[b], Guus Smit[b] and Titia K. Sixma[a]. [a]*Division of Molecular Carcinogenesis, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.* [b]*Department of Molecular and Cellular Neurobiology, Institute of Neurosciences, Faculty of Earth and Life Sciences, Vrije Universiteit, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands. E-mail: p.celie@nki.nl*

Keywords: AChBP; ITC; Ligand binding.

Nicotinic acetylcholine receptors (nAChRs) mediate transmission of synaptic signals and contribute to learning and memory capacities as well as to nicotine addiction in tobacco smokers. Signals are transmitted upon binding of agonists to the ligand binding domain and opening of the transmembrane ion channel. nAChRs consist as pentameric proteins and the ligand binding site is located at the interfaces between the so-called principal and complementary subunits.

Acetylcholine binding proteins (AChBPs) have been identified in snails and are soluble homologs of the ligand binding domain of nAChRs. Recently, we have solved the crystal structures of AChBP from the *snail Lymnaea stagnalis* in complex with the nAChR agonists nicotine and carbamylcholine. Ligand binding is characterized by vdWaals contacts, hydrogen bonds and aromatic (cation-π) interactions. Here, we report the crystal structure of AChBP from *Bulinus truncatus* at 2.0 Å. Although the *Bulinus* AChBP sequence is only 44% identical, the structure is remarkably similar to the Lymnaea AChBP structure. We have compared the *Bulinus* and *Lymnaea* AChBPs using crystallographic, thermodynamic and spectroscopic techniques. Our biophysical and structural results provide novel information about stability and ligand recognition by AChBP and nAChRs.

s1.m8.p7 **Structure of the response regulator PleD with a novel di-guanylate cyclase domain.** Carmen Chan[a], Nicolas Amiot[b], Urs Jenal[c] and Tilman Schirmer[a], *Departments of* [a]*Structural Biology and* [c]*Molecular Biology, Biozentrum,* [b]*Department of Chemistry, University of Basel, Switzerland. E-mail: carmen.chan@unibas.ch*

Keywords: Response regulator; Multi-domain protein; Di-guanylate cyclase

Most bacteria use "two-component" systems in signal transduction which involves the transfer of a phosphoryl group from a histidine kinase to a response regulator. Response regulators typically contain two domains: a receiver domain and an output, or effector, domain. Phosphorylation of the receiver domain at a conserved aspartate activates the effector domain to elicit specific responses. The response regulator PleD is required for pole development in *Caulobacter crescentus* [1,2]. It is an unorthodox response regulator that consists of two receiver domains (D1 and D2), of which only D1 can be phosphorylated [2], and a novel effector domain (D3) with a signature GG(D/E)EF sequence motif. Despite its abundance in bacteria, the exact function and structure of the GGDEF domain have been unclear. Until recently, PleD was shown to be a di-guanylate cyclase [3], and this function was ascribed to this domain. Remote structural similarity of this domain to adenylate cyclase was predicted [4].

Here, we present the 3D crystal structure of PleD. PleD protein in complex with the product, cyclic-diGMP, gave crystals diffracting to 2.7 Å at the SLS synchrotron. The space group is $P4_22_12$ *(*$a = b = 135$ Å, $c = 169$ Å) and the same form was also obtained with Se-Met substituted protein. MAD phasing has produced interpretable electron density maps. The PleD crystal structure is a homodimer of three domains. D1 and D2 adopt the CheY $(\beta/\alpha)_5$ fold and form the dimer interface. D3 is of mixed αβ type with $(\alpha(\beta\alpha\beta)_2\alpha\beta)$ topology. A product molecule is bound at the β-hairpin where the conserved GGDEF motif is located. Structural similarity to adenylate cyclase, including the position of the active site, is apparent. Intriguingly, two additional product molecules are bound to a secondary site at the D2-D3 domain interface. These results may imply an important role for D2 in the transmission of the activation signal from D1 to the effector domain. A possible role in allosteric regulation for the PleD reaction is also suggested.

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