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Keywords: neurotrophin, protein refolding, biophysical biochemical characterization

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Structural Basis for the Activity and Allosteric Control of Diguanylate Cyclase

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Recent studies suggest that a novel second messenger, cyclic d-GMP (c-diGMP), is extensively used by bacteria to control multicellular behaviour. This cyclic dinucleotide is synthesised by the diguanylate cyclase (DGC) domain in a reaction that converts two GTP into one c-diGMP and two pyrophosphates. The DGC domain contains a highly conserved GG(D/E)EF sequence motif, and occurs in various combinations with sensory / regulatory domains in bacteria.

We have identified the response regulator, PleD, from *Caulobacter crescentus* as a diguanylate cyclase [1] and have solved its crystal structure in complex with c-diGMP to 2.7 Å [2]. PleD consists of a receiver domain D1 with a phosphorylation site, a receiver-like domain D2, and an effector domain DGC. In the structure, PleD forms a homodimer mediated by D1-D2 interactions. The DGC domain has a similar fold as the catalytic domain of adenylate cyclase but has an active site that reveals different nucleotide binding. The guanine base of c-diGMP is hydrogen bonded to Asn335 and Asp344, while the ribosyl and α-phosphate groups extend over the β2-β3 hairpin that carries the sequence motif. Interestingly, the c-diGMP molecule crosslinks two symmetrically arranged DGC domains from adjacent dimers. We propose that activation of PleD through phosphorylation leads to dimerisation, which allows the two DGC domains of a dimer to align symmetrically for c-diGMP synthesis.

Two intercalated c-diGMP molecules are bound to the domain interface between D2 and DGC. This allosteric binding site explains the observed non-competitive product inhibition. We propose that PleD inhibition is effected by DGC domain immmobilisation to the D1-D2 stem.

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Structural Differences between B and F Subtypes of HIV PR

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One of the major problems facing the development of successful therapies against AIDS is the emergence of viral variants that exhibit drug resistance, as well as viral subtypes naturally more liable to development of therapeutic failure. In this work we solved by molecular replacement the crystal structures of four HIV-1 proteases complexed with the inhibitor TL-3: of the subtype B wild type (*Bwt*) at 2.1Å resolution, of the subtype F wild type (*Fwt*) at 2.1Å, and a mutant of each subtype (*Bmut* and *Fmut*) at 1.75Å and 2.80Å, respectively. All crystals were in space group $P6_1$

The mutation V82A in the proteases *Bmut* and *Fmut* causes repacking of the S1' pocket, which rearranges the inhibitor's side chain at the P1' subsite. Our analysis further indicates that some polymorphic substitutions between subtypes B and F could lead to stabilization of naturally flexible regions of subtype F proteases, resulting in an intrinsically less active and drug resistant enzyme. On subtype F proteases the polymorphic substitution M36I leads to the displacement of the loop between residues 35-41, which would cause loss of the flexibility of the flaps and of the loop 76-83 in the active site. Our comparisons further indicate that the polymorphic substitution L89M on non-B subtypes could be equivalent to the L90M resistance mutation on subtype B proteases.

Keywords: HIV retroviral proteases, structural and biological function, biological macromolecules

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X-ray Crystallographic Structure of Virus like Particle from Hyperthermophilic Archaea *Pyrococcus furiosus*

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Hyperthermophilic archaea is grown in an ultimate environment.Interesting protein was discovered from *Pyrococcus furiosus* of them.The proteins were assembled 180 copies subunits into a virus like particle (PfV: *Pyrococcus furiosus* virus), and it has virus like icosahedral symmetry. The atomic structure of PfV was determined at 3.6Å resolution by X-ray crystallography.

Crystallization was performed by hanging-drop vapor diffusion against16-20% MPD. The crystal belongs to the space group of *P*41212 with cell dimensions of *a*=*b*=631.5Å, *c*=351.3Å. Diffraction data was collected at wavelength of 0.9Å crystals at 100K using an imaging plate DIP6040 on beamline 44XU of the SPring-8. A diffraction data set at 3.6Å resolution with 96.8% completeness with an *R*merge of 0.132 was obtained. Phase was determined by the SIRAS (Single Isomorphous Replacement with Anomalous Scattering) method using Tungsten cluster derivative. Phase improvement and extension was performed using the symmetry of a virus and electron density was calculated. The structure model was built based on this electron density map. The CNS program was used refinement against 20-3.6Å intensity data. The crystallographic *R*-factor and free *R*-factor were 0.267, respectively.

PfV subunit was a mixed alpha/beta structure. Three dimensional structure of PfV and bacteriophage HK97 capsid protein were very similar. This suggests that PfV and HK97 have a common ancestor.

Keywords: *Pyrococcus furiosus*, virus like particle, HK97

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Structures of SARS Coronavirus Main Protease Bound by an Aza-peptide Epoxide

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Soon after the global outbreak of severe acute respiratory syndrome (SARS) in the spring of 2003, a novel coronavirus (CoV) was identified to be the etiological agent of this highly infectious and fatal disease. The main protease (M^{pro}) of this virus is essential for viral replication, and therefore is one of the major targets for the development of anti-SARS agents. We have determined the crystal structures of SARS-CoV M^{pro} unbound in the space group C2, and bound by an aza-peptide epoxide in the space groups C2 and $P2_12_12_1$. These structures show that the peptide binds, like a true substrate, to the substrate-binding and active site of the enzyme, without inducing any significant change in the structure of the enzyme. A covalent bond