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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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Keywords: response regulator, cyclic dinucleotide, allosteric control

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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

P.04.13.3

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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

P4

forms between the S_γ atom of the catalytic residue Cys-145 of the enzyme and one of the epoxide carbon atoms of the peptide, thereby blocking the active site of the enzyme. With an appropriate sequence, the peptide also has its side chains nicely fitted into in the specificity pockets of the enzyme. These results form the structural basis for our suggestion that the aza-peptide epoxide is a potential inhibitor of SARS-CoV M^{pro} worthy of further evaluation as in the development of leads for anti-SARS agents.

Keywords: SARS, viral proteins, protease inhibitors

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EED, a Cellular Partner of the Viral Proteins MA, IN and Nef from HIV-1

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The human protein EED (Embryonic Ectoderm Development) seems to be important during HIV-1 replication cycle, interacting with the viral proteins MA, IN [1] and Nef [2].

In vitro, data from mutagenesis studies, pull-down assays, and phage biopanning suggest that the interaction between EED and IN requires the integrity of the two C-Terminal WD-40 motifs of EED. Besides, EED shows an apparent positive effect on IN-mediated DNA integration reaction *in vitro*, in a dose-dependent manner. *In situ* analysis by immunoelectron microscopy (IEM) shows that IN and EED colocalise in the nucleus and near nuclear pores [3].

EED displays along its amino-acid sequence 7 repeated WD-40 motifs and should be folded as a β-propeller homolog to the G-protein β [4]. The structure of this β subunit has been used as a template in order to obtain a model of EED. Antigenic domains localised on loops due to interact with viral partners have been confirmed by phage-display.

Crystallisation trials are under way in order to determine crystallographic structures of EED and/or in complex with its viral partners and in particularly with Nef.

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Keywords: HIV, crystal structure, human protein

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Kinetic and Crystallographic Analyses of SARS Coronavirus 3CLpro Inhibitors

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Severe Acute Respiratory Syndrome (SARS) is a life-threatening, acute, atypical pneumonia caused by the SARS coronavirus (SARS-CoV). The genome of SARS-CoV is composed of a single RNA strand with positive polarity and encodes a polyprotein that must be cleaved by two virally encoded proteases, PLpro and 3CLpro, for viral replication. We have initiated structure-based drug design studies on SARS 3CLpro using the rhinovirus 3C-protease inhibitor, AG7088, as a starting template. The SARS 3CLpro enzyme was over-expressed, purified, and crystallized without the use of affinity-tags. A high throughput, FRET-based fluorescence assay was developed to measure the kinetic parameters of the wild-type and two mutant enzymes. Ten compounds were synthesized and tested as inhibitors of SARS 3CLpro in vitro. Two of the compounds that inhibit SARS 3CLpro activity also show antiviral activity against SARS-CoV infected cells with EC50s <100 μM, and one was more effective at reducing viral titer than the protease inhibitor E64-D. The crystal structures of wild type and mutant SARS 3CLpro enzymes in complex with these inhibitors and others have been determined to between 1.9 and 2.1 Å resolution. These structures should serve as important drug-design templates for the development of anti SARS-CoV therapeutics.

Keywords: SARS, proteases, viral proteins

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Crystallization of Recombinant HIV-1 Nef

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Negative factor (Nef) is a 27 kDA myristoylated protein of the primate immunodeficiency viruses (HIV and SIV). The protein is crucial in the pathogenesis of HIV and therefore an attractive target for basic research but also for drug discovery and vaccine development.

Nef has a two-domain structure consisting of a folded core domain and of a more flexible anchor domain. Nef has not been crystallized before as whole protein: only the core domain (alone or with Fyn SH-3 kinase) has been crystallized successfully[1,2]. The structure of the anchor-domain has been determined only by NMR-method.

In this work, we have expressed HIV-1 Nef as non-myristoylated GST-tagged fusionprotein in *E. coli.* Protein was purified by glutathione-sepharose column chromatography and GST-tag was cleaved by overnight treatment with bovine thrombin. Resulting Nef-concentrate was diafiltrated to water and concentrated to concentration of 10-18 mg/ml. Crystallization screens with polyethylene glycols and isopropanol in RT produced small crystals. The first experiments to identify the crystals by SDS-PAGE gave promising results of these being the first crystallized full-length Nef.

The crystallization parameters are still further optimized in order to produce crystals suitable for x-ray crystallography.

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Keywords: HIV, protein crystallization, protein structure

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Structural and Immunological Characterization of the Fusion Core of the SARS-coronavirus Spike Protein

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Severe Acute Respiratory Syndrome (SARS) has been one of the most epidemic diseases threatening the lives of human beings in the 21st century. The SARS-CoV spike (S) protein, a glycoprotein essential for viral entry, is a primary target for vaccine and drug development.

The polyclonal antibodies produced by recombinant S2 protein were tested for the antigenicity of the two heptad repeats. Two peptides denoted HR-N(SN50) and HR-C(SC40), corresponding to the Leu/Ile/Val-rich heptad-repeat regions from the N-terminal and C-terminal segments of the SARS-CoV spike S2 sequence, respectively, were synthesized and predicted to form trimeric assembly of hairpin-like structures. The crystallographic study of the SARS spike HR-N/HR-C complex presents the crystal belongs to the triclinic space group P1 and the data-set collected to 2.98 Å resolution showed noncrystallographic pseudo-222 and 3-fold symmetries. Based on these data, comparative modeling of the SARS-CoV fusion core was performed. Structural and biophysical studies of SARS-CoV spike fusion core with inhibitor are in progress. The immunological and structural information presented herein may provide a more detailed understanding of the viral fusion mechanism as well as the development of effective therapy against SARS-CoV infection.

Keywords: SARS, spike, X-ray crystallography