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