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X-ray Structure for an RNase H Inhibitor Bound to HIV-1 Reverse Transcriptase

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We have determined a 3.0 Å resolution X-ray crystal structure of HIV-1 reverse transcriptase (RT) complexed with DHBNH, an RNase H inhibitor (RNHI). HIV-1 RT uses two enzymatic activites, a polymerase and an RNase H, to convert the viral genomic single-stranded RNA into double-stranded DNA suitable for integration into the host genome [1]. RNase H is essential for virus replication; however, very few small molecule inhibitors targeting this function have been reported, and there are no crystal structures of HIV RT in a complex with an RNase H inhibitor. DHBNH is an N-acyl hydrazone derivative that inhibits RNase H with an IC_{50} of 0.5 μM but does not inhibit the RT polymerase (IC_{50}>20 μM). Despite this specificity, the inhibitor binds more than 40 Å away from the RNase H active site, at a novel binding site in the palm of the p66 subunit, between the primer grip and the polymerase active site. The inhibitor partially overlaps the non-nucleoside RT inhibitor (NNRTI) binding pocket. The inhibitor appears to interact with the conserved residues Asp186 and Trp229, as well as with Tyr188, Lys223, Asp224, Pro226, Phe227, and Leu228. Certain substitutions on DHBNH can enhance interactions in the NNRTI binding pocket, resulting in "dual inhibitors" that inhibit both the polymerase and RNase H activities of HIV-1 RT. Our results are consistent with the view that binding of DHBNH alters the trajectory of the nucleic acid substrate, affecting the RNase H activity. Knowledge gained from this study provides new opportunities for structure-based drug design.

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Keywords: HIV-1 reverse transcriptase, RNase H inhibitor, rational inhibitor design

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Crystal Structure of the NAD Kinase from *Listeria monocytogenes*

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The NAD kinase is involved in the essential step for the biosynthesis of NADP the dinucleotidic cofactor for numerous enzymes. The NAD kinases was previously proposed to share the ATP-binding site of phosphofructokinases despite important sequence divergence [1]. The first crystal structure of a NAD kinases was recently solved [2]. We present, here, the structure of one of the two NAD kinases from *Listeria monocytogenes*, a food-borne human pathogen. The crystal structure, refined at 2.4 A resolution, reveals the conserved tetrameric structure of NAD kinases in agreeement with its oligomeric state in solution. Co-crystallization as well as complementary biophysical characterizations (SAXS, ...) in presence of the ligands are currently undertaken in order to analyse putative conformation changes. Meanwhile, the structure allows virtual screening in order to identify potential inhibitors.

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Keywords: protein crystallography, rational drug design, comparative modelling

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Identical Sets of Residues Produce Two Strikingly Different Dimers in the NF-κB Family of Proteins

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The proteins of the Nuclear Factor-kappaB (NF- κB) family proteins are important transcription factors that regulate the expression of genes involved in immune and inflammatory response and apoptosis. There are five known NF-κB proteins, p50(NF-κB1), p52(NF-κB2), p65(RelA), c-Rel and RelB, that exist as homo- and heterodimers. Unlike other family members that form all possible functional combinatorial dimers, RelB forms heterodimers with only p100/p52 and p105/p50. The X-ray crystal structure of the RelB dimerization domain (DD) alone, and in complex with p52 DD have been determined. This reveals that RelB/p52 DD heterodimer forms a "regular" dimer similar to other NF-κB dimers, unlike RelB DD which forms an intertwined homodimer. We have shown that RelB forms an intertwined homodimer in solution as well. The residues that are critical in NF-κB dimer formation are invariant in RelB, however, a solvent exposed hydrophobic patch destabilizes the RelB domain fold, a feature that is also essential for its association with p52. We propose that the intertwined unstable RelB homodimer may serves as an intermediate to before converting into highly stable heterodimers with p105/p50 or p100/ p52.

Keywords: transcription factor structures, NF-kB proteins, residues

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Crystal structure of the *Actinomadura* R39 DD-peptidase (PBP4)

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Actinomadura sp. R39 produces an exocellular DD-peptidase penicillin-binding protein (PBP) whose primary structure is similar to *Escherichia coli* PBP4. It is characterized by a high beta-lactam-binding activity (second order rate constant for the acylation of the active site serine by benzylpenicillin: k_2/K = 300 mM^{-1} s^{-1}). The crystal structure of the DD-peptidase from *Actinomadura* R39 was solved at a resolution of 1.8 Å by single anomalous dispersion at the cobalt resonance wavelength. The structure is composed of three domains: a penicillin-binding domain similar to the penicillin-binding domain of *E. coli* PBP5 and two domains of unknown function. In most multimodular PBPs, additional domains are generally located at the C- or N-termini of the penicillin-binding domain. In R39, the other two domains are inserted in the penicillin-binding domain, between the SXXK and SXN motifs, in the way of "Matryoshka dolls". One of these domains is composed of a five-stranded beta-sheet with two helices on one side and the other domain is a double three-stranded beta-sheet inserted in the previous domain. Additionally, the 2.4 Å structure of the acyl-enzyme complex of R39 with nitrocefin reveals the absence of active site conformational change upon binding the beta-lactams.

Keywords: peptidoglycan biosynthesis, carboxypeptidase, penicillin-binding protein

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Sulfur SAD Structure of Heparin-Binding CRISP from *Naja atra* Reveals Protease and Ion Channel Blocking Domains

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Various cysteine-rich secretory proteins (CRISP) have been identified in diverse organisms with conserved sequences, including