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Structural analysis of the *Plasmodium falciparum* cysteine protease falcipain-2

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Malaria is one of the major infectious diseases in the world. Approximately 300 - 500 million people are infected and 1 - 3 million deaths occur annually[1]. The multidrug resistance of the parasite and limitations with existing drugs (e.g., toxicity, side effects, high cost)[2] emphasize the urgent need for new drugs, ideally directed against potential new targets such as plasmodial proteases. These proteases are multifunctional: During the merozoite stage of the parasite they facilitate erythrocyte escape and subsequent reinvasion. In the trophozoite stage these enzymes are critical for the degradation of hemoglobin[3]. The falcipains are cysteine proteases belonging to the papain family (C1A). The best-studied members of this group are falcipains-1, -2 and -3. Falcipain-2 and -3 degrade hemoglobin at acidic pH. In addition, falcipain-2 degrades cytoskeletal proteins at neutral pH and thus facilitates the release of the mature merozoites. There is experimental evidence that cysteine protease inhibitors such as E-64, fluoromethyl ketones or aziridine derivatives inhibit falcipain-2 at low micromolar concentrations, and thereby block the development of the parasite[4,5]. However, a falcipain-specific inhibitor with *in-vivo* activity remains to be developed. It is expected that the elucidation of the structure of falcipain-2 will open new avenues for the development of antimalarial drugs with lowered toxicity and drug resistance. Towards this goal, we have crystallized recombinant falcipain-2 and determined its structure to ~ 3.1 Å resolution. There are two major structural differences between papain and falcipain-2. First, the presence of a ~ 20-residue N-terminal extension which is necessary for the proper folding of the mature protein[6]. Interestingly, this sequence element is functionally conserved within the *plasmodial* cysteine proteases[6]. Second, falcipain-2 contains a rather unique structural motif responsible for the binding of its natural substrate, hemoglobin[7]: a beta-hairpin located between the active-site residues His174 and Asn204 where it protrudes out of the core of the enzyme. On the basis of our results, we will discuss the interaction between falcipain and its substrate, hemoglobin. Additional experimental data on substrate binding and specificity will be presented.

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Towards a functional mapping of the BLM helicase

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The RecQ family of DNA helicases, highly conserved from bacteria to humans, plays important roles in the maintenance of genome integrity. Mutations in the human RecQ helicase BLM are responsible for Bloom Syndrome, an autosomal recessive disorder characterized by genomic instability and a predisposition to a wide variety of cancers. We adjusted a DNA heteroduplex in the BLM structure we computed with Modeller. Using this BLM-DNA model, we attempted to correlate structural possible consequences with the activities measured for five mutations observed in Bloom Syndrome patients and four directed mutations altering highly conserved residues. All the mutants have been expressed in *E. coli* and their activities compared to the wild BLM enzyme. Three groups can be distinguished: the mutations affecting DNA binding, the mutations affecting the coupling between DNA-binding and ATPase and the mutation revealing, for the fist time, a direct coupling of ATPase and unwinding activities. This work led to the identification and localization of important sites and provided valuable information towards a functional mapping of the Bloom syndrome protein. From this base, we proposed a biochemical and structural explanation of the drastic consequences of the mutations observed in Bloom Syndrome. We recently initiated the crystallization of the human BLM helicase core in presence of various DNA substrates. The crystallographic study should clarify the unwinding active site as well as all the DNA binding sites.