Investigating and Disrupting the Interaction between Hemoglobin and MRSA Hemophores: *In Silico* Approaches to Design Novel Antimicrobials

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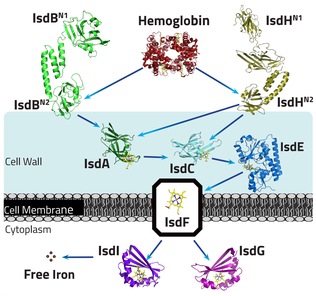
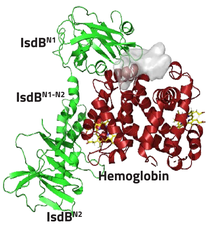
>> Background

The emergence of bacteria resistant to last resort antibiotics is responsible for more than 700,000 annual deaths and to respond to this emerging threat, new therapies are urgently needed. Methicillin-Resistant *Staphylococcus aureus* (MRSA) has been prioritized as the most threatening multidrug resistant Gram+ (1).

>> Introduction



The Isd Family. Iron is an essential nutrient for all organisms, MRSA included. Iron-regulated Surface Determinants (Isd) proteins, also called hemophores, safeguard the iron supply: the Isd-mediated pathway starts with heme extraction from human hemoglobin (Hb) until intracellular heme degradation, which will provide free iron for the bacteria.



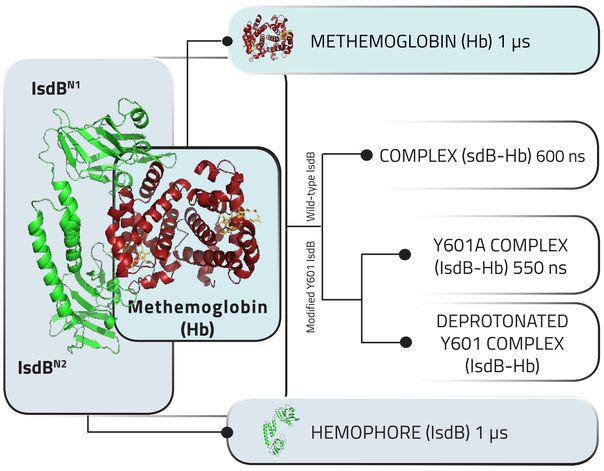
IsdB-Hb Complex. The upstream process of hemoglobin binding is performed by hemophores IsdB and IsdH: in this image, the Hb (red) - hemophore (green) complex is shown. In particular, IsdB^{N1} domain binds Hb, while in IsdB^{N2} the heme transfer occurs. Interfering with the recognition site (grey) can be an effective strategy to design a new class of antimicrobials.

>> Aims

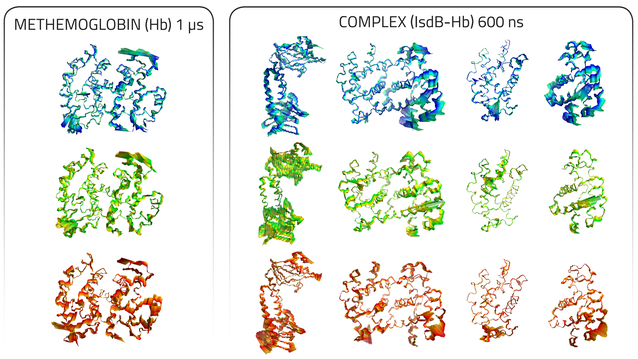
> **Give a mechanicistic insight** on the heme extraction process;
> **Design a first-in-class antimicrobial** to disrupt the hemophore-hemoglobin complex, cutting down the iron supply, on which bacterial survival relies completely.

>> Materials and methods

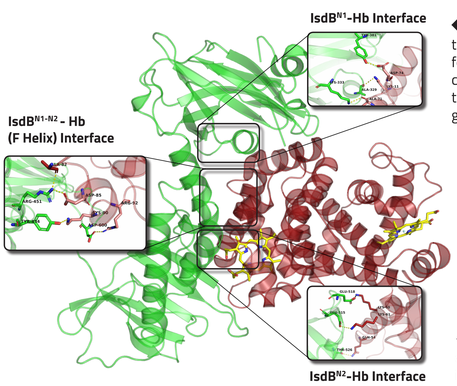
Molecular dynamics to investigate the complex. IsdB and methemoglobin structures (PDB IDs 5vmm and 3p5q, respectively) were retrieved from RCSB Protein Data Bank (www.rcsb.org). The complex was then parametrized with Amber 99SB-ILDN force field, and solvated with TIP3P model. All the simulations were performed with GROMACS 4.6.5. The following scheme summarizes several MD experiments that were carried out to test the dynamic evolution of the IsdB-Hb complex.



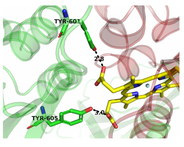
>> Results



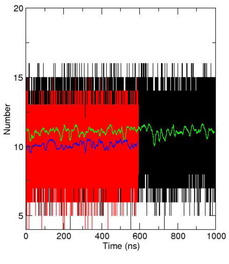
Essential dynamics. The 1 µs methemoglobin and the 600ns IsdB-Hb complex were analysed by means of Essential Dynamics (ED) with GROMACS 4.6.5 to extract the principal vectors of motion. It can be clearly seen how IsdB can affect both alpha and beta chains of methemoglobin, even if the binding of IsdB in this simulation does not involve the beta chain. The trajectories were filtered along the first, the second and the third eigenvectors (shown in blue, green and red, respectively).

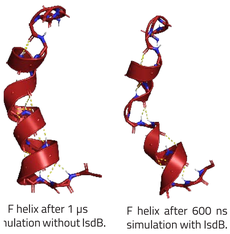


Hydrogen bonds. During the production, several H bonds formed at the IsdB-Hb interface, revealing a pattern of contacts which is crucial for hemoglobin recognition and binding.



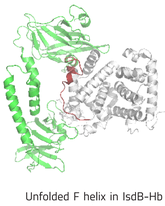
Heme extraction. Detail of Y601 and Y605 contracting polar interactions with the propionates of the heme group (measures in ångström).





F helix after 1 µs simulation without IsdB.

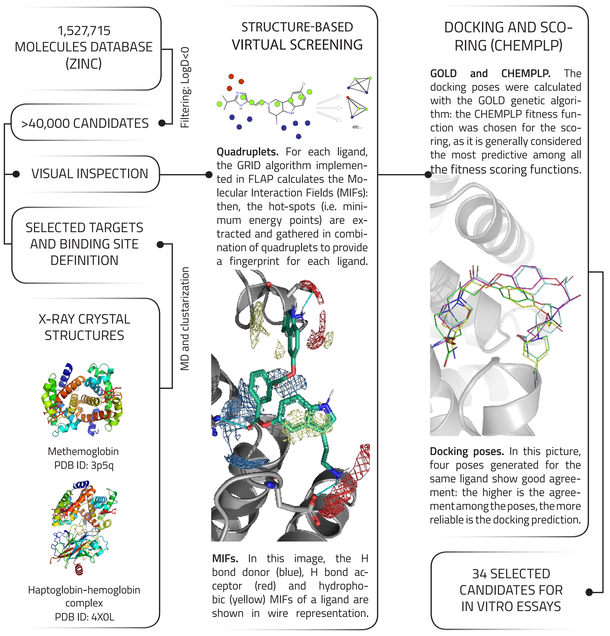
F helix after 600 ns simulation with IsdB.



Unfolded F helix in IsdB-Hb x-ray structure (PDB ID: 5vmm).

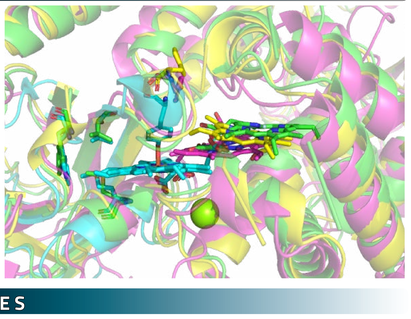
Emerging F helix disruption. As in the crystal structure of the IsdB-Hb complex the F helix of hemoglobin appears completely unfolded, the structure of this helix was monitored along the MD production. In this line plot, the number of H bonds is quantified for each frame (red line for IsdB-Hb complex 600 ns, black line for methemoglobin 1 µs). Averages clearly show how the number of H bonds intra-backbone is higher for F helix in methemoglobin 1 µs (green line) than for F helix in IsdB-Hb complex 600 ns (blue line).

Virtual screening to find inhibitors. The structures of methemoglobin (PDB ID: 3p5q) and methemoglobin-haptoglobin complex (PDB ID: 4X0L) were submitted to Molecular Dynamics for a more accurate conformational sampling (see the protocol in the previous section). The virtual screening campaign was carried out with FLAP (Fingerprint for Ligand and proteins), developed by Molecular Discovery Ldt. (www.moleculardiscovery.org). Details are provided by the following flowchart.



>> Perspectives

Dynamic docking and steered molecular dynamics. These simulations will provide detailed information about the heme path from hemoglobin to IsdB^{N2}. This image shows an alignment among the starting minimized structure for the dynamics (green), the cristal structure 5vmm (yellow), a frame of one dynamic docking replica (magenta) and finally the crystal structure of IsdB^{N2} (cyan).



>> Refercences

(1) [http://www.who.int/medicines/areas/rational_use/PPLreport_2017_09_19.pdf?ua=1]

>> Aknowledgements

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>> Partnerships & Affiliations