>> Partnerships & Affiliations

# Investigating the Interaction between Human Hemoglobin and *S. aureus* IsdB to Design Novel Antimicrobials


[1]Francesca Spyrakis, [1]Eleonora Gianquinto, [2]Santiago Di Lella, [1]Loretta Lazzarato, [1]Stefano Guglielmo, [1]Roberta Fruttero, [3]Omar De Bei, [1]Marialaura Marchetti, [3]Barbara Campanini, [4]Luca Ronda, [4]Stefano Bettati.

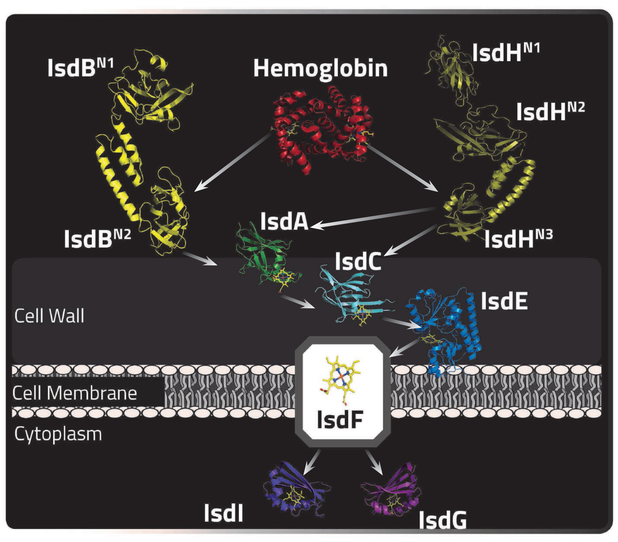
[1]Dep. of Drug Science and Technology, University of Turin; [2] Dep. of Biological Chemistry IQUIBICEN-CONICET, University of Buenos Aires; [3]Dep. of Food and Drug, University of Parma; [4]Dep. of Medicine and Surgery, University of Parma.

*francesca.spyrakis@unito.it*


## >> Introduction

**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).

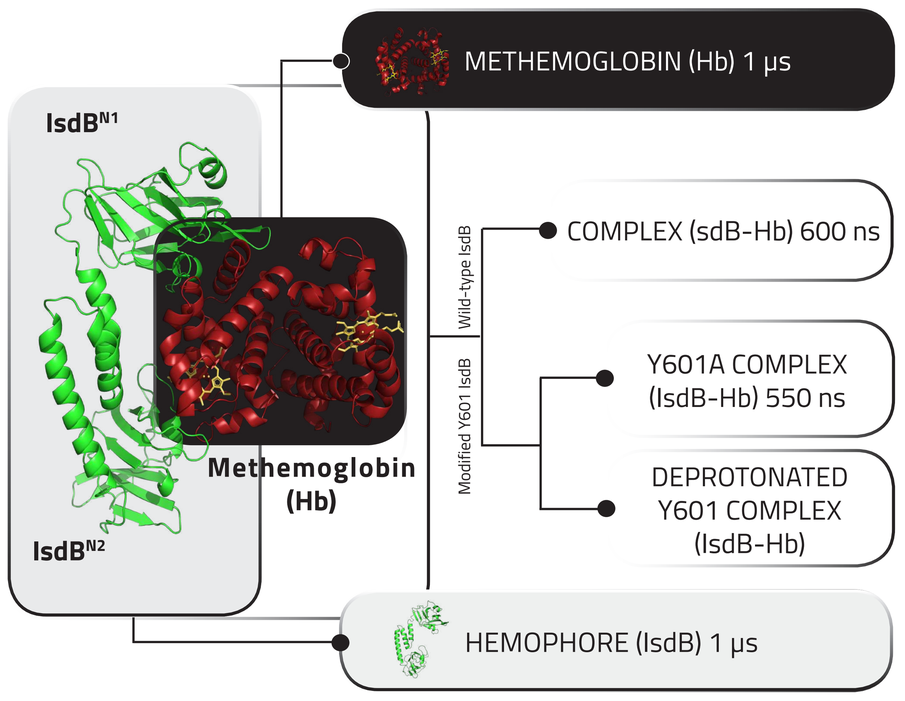
**The Isd Family.** MRSA expresses Iron-regulated Surface Determinants (Isd) proteins to bind human hemoglobin (Hb), extract the heme and degrade it to seize iron, which is essential for bacterial growth and virulence. Among Isd proteins, IsdB and IsdH are responsible for hemoglobin capturing and heme mining. To date, however, the mechanism of heme extraction has not been completely understood.



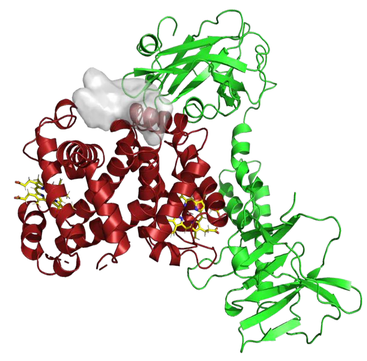

## >> Materials and Methods

**Molecular dynamics.** 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, while the TIP3P model was used for solvating the system.

**Investigating the complex.** This scheme summarizes several MD experiments that were carried out to test the dynamic evolution of the Isdb-Hb complex. All the simulations were performed with GROMACS 4.6.5 (www.gromacs.org). The system was firstly minimized and then equilibrated through seven steps, from 0 to 300 K. The simulations were performed in the NVT ensemble.

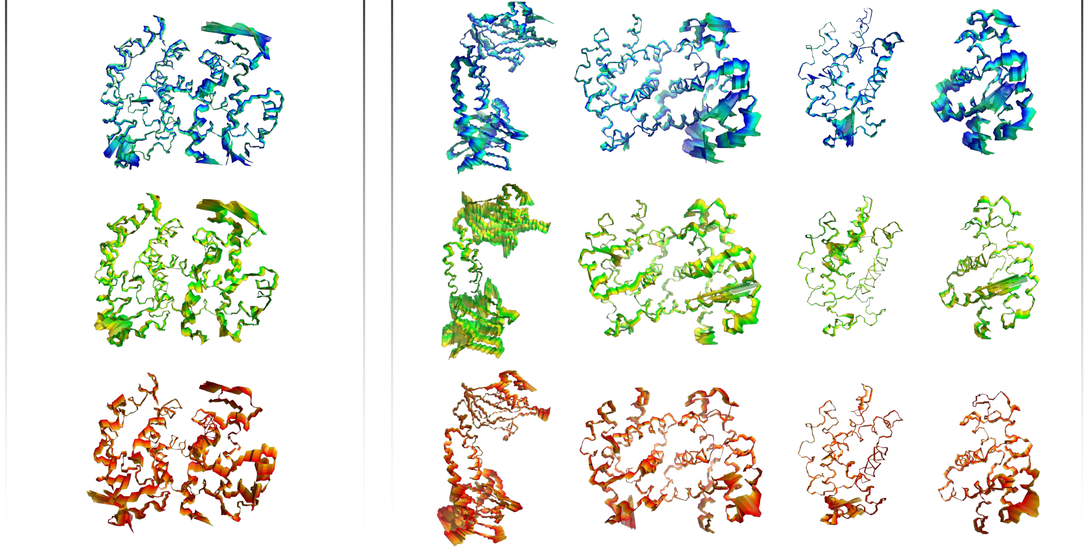



**Virtual screening.** The structures of methemoglobin (PDB ID: 3p5q) and methemoglobin-haptoglobin complex (PDB ID: 4X0L) were submitted to Molecular Dynamics for a more accurate conformational sampling (see the protocol in the previous section). The virtual screening campaign was carried out with FLAP (Fingerprint for Ligand and proteins), developed by Molecular Discovery Ldt. (www.moleculardiscovery.org). The best scored compounds were submitted to molecular docking, and the most promising ones were chosen according to their docking score as well as for their pose, their capability to establish H bonds and their chemical diversity. Details are provided by the following flowchart.

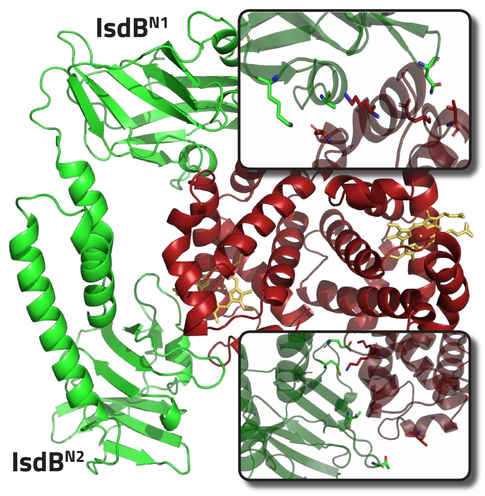


**Binding site selection.** The hemophore (green) and the methemoglobin (red) are shown in cartoon representation. The pocket depicted in grey was chosen as the binding site for the VS: this surface is indeed essential for the hemophore to recognize and bind hemoglobin.

## >> Results

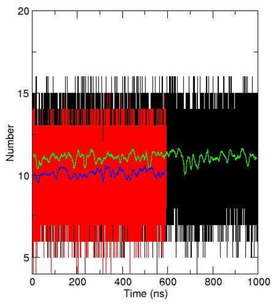


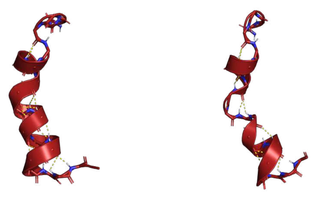

**Essential dynamics.** The 1 µs methemoglobin and the 600ns IsdB-Hb complex were analysed by means of Essential Dynamics (ED) with GROMACS 4.6.5 to extract the principal vectors of motion. It can be clearly seen how IsdB can affect both alpha and beta chains of methemoglobin, even if the binding of 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.

**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 shows 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).

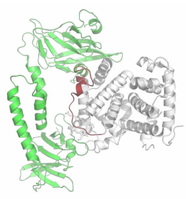


F helix after 1 µs simulation without IsdB.

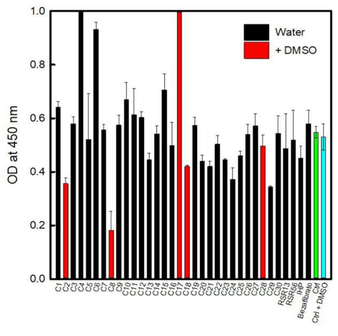


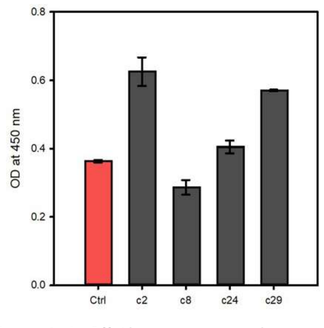
F helix after 600 ns simulation with IsdB.



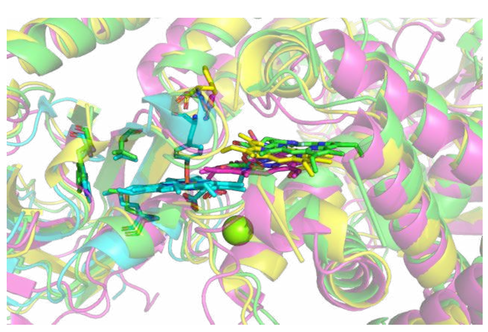
Unfolded F helix in IsdB-Hb x-ray structure (PDB ID: 5vmm).

## >> Perspectives




***In vitro* inhibition assays and experimental.** All the compounds selected by the virtual screening procedure were submitted to an ELISA assay: in this test, the activity threshold was fixed at or below 0.4. These panels show the results of the inhibition assays on Oxy-Hb-Y165A IsdB (left) and on MetHb-Y165A IsdB (right). In particular, compound C8 showed the best activity: the synthesis and the activity validation of compound 8 are currently ongoing. Meanwhile, SAXS and WAXS experiments are being carried out for further clarifying the structure and the stoichiometry of the complex.



**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).


## >> Aknowledgements

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## >> Refercences


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