DEVELOPMENT OF A HIGH-THROUGHPUT SCREENING ASSAY TO IDENTIFY INHIBITORS OF S. AUREUS ISDB BINDING TO HUMAN HEMOGLOBIN

Francesca Spyrakis [a], Omar De Bei [b], Marialaura Marchetti [a], Eleonora Gianquinto [a], Barbara Campanini [b], Luca Ronda [c], Stefano Bettati [c].

[a] Department of Drug Science and Technology, University of Turin, Italy; [b] Department of Food and Drug, University of Parma, Italy; [c] Department of Medicine and Surgery, University of Parma, Italy.

INTRODUCTION

Nowadays antimicrobial resistance is among the most serious health treats, and is expected to become increasingly threatening in the next decades. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most dangerous multi-drug resistant bacteria worldwide and it is included as "high priority" in the Global Priority Pathogens List (Global-PPL) drafted by WHO in 2017. The pathogenesis of *S. aureus* human infections has been shown to be reliant on the acquisition of iron. To overcome the human nutritional immunity, *S. aureus* has developed the Iron-regulated Surface Determinant (Isd) system aimed at iron retrieval based on hemoglobin (Hb) binding followed by heme extraction.

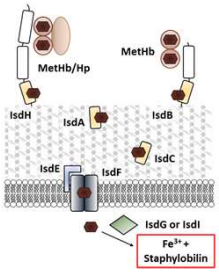
THE PROJECT

i) Identification of small molecules able to interfere with the IsdB-Hb recognition process by means of *in silico* techniques.

ii) Development of an ELISA-based assay for the detection of IsdB-Hb complex formation and affinity and for inhibitors validation.

IRON REGULATED SURFACE DETERMINANTS

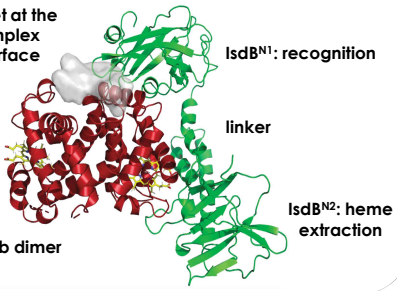
Iron-regulated Surface Determinant (Isd) system consists of eight components: four surface proteins covalently anchored to the peptidoglycan that reversibly bind Hb and heme (IsdA, IsdB, IsdC and IsdH), an ABC transporter (IsdF) with an associated lipoprotein (IsdE), and two intracellular heme-degrading enzymes (IsdG and IsdI) [1].



THE TARGET

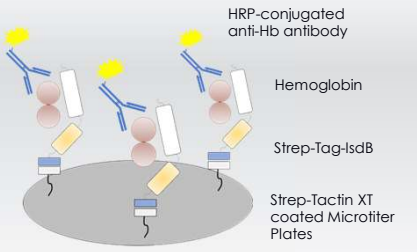
In the Isd system, IsdB and IsdH are the proteins in charge of binding Hb to extract heme. Interestingly, antibodies and vaccines directed against IsdB have been demonstrated to be active in the protection against *S. aureus* infections [2].

IsdB-Hb complex



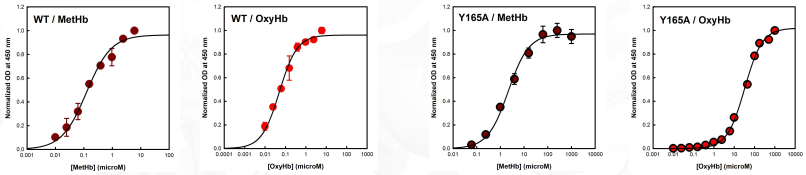
THE RESULTS

THE ASSAY

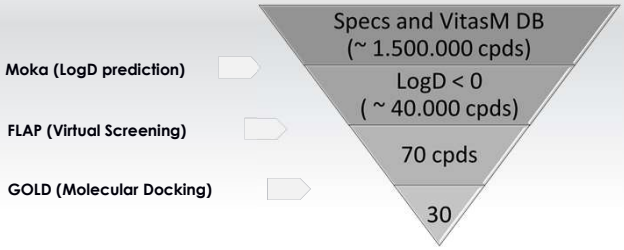


ELISA-based assay for the detection of IsdB-Hb complex, where the affinity-tag recombinantly added to IsdB ensures its binding to the well and Hb bound to IsdB is detected through an HRP-conjugated anti-Hb antibody.
The assay allows to calculate the K_D of IsdB for OxyHb (oxygenated hemoglobin) and MetHb (methemoglobin) in good agreement with published data [3]. The analyses were also performed on the Y165A IsdB mutant, having an alanine replacing tyrosine 165, a residue known to play a fundamental role in Hb binding. Indeed, the mutant was resonably found to have a lower affinity towards hemoglobin with respect to the wild type form.

		K_D (μM)	OD max
WT	MetHb	0.12 ±0.02	2.55
	OxyHb	0.05 ±0.01	0.43
Y165A	MetHb	2.14 ±0.28	1.75
	OxyHb	32.86 ±2.58	1.07



VIRTUAL SCREENING

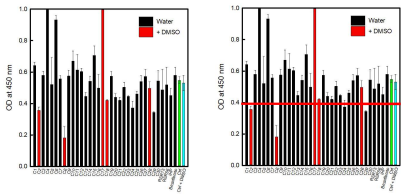


With the aim to identify small molecules able to destabilize the IsdB-Hb interaction, a virtual screening campaign was carried out with FLAP (Fingerprint for Ligand and proteins), developed by Molecular Discovery Ldt. (www.moleculardiscovery.org). The best scored compounds were submitted to molecular docking, and the most promising ones were chosen according to their docking score as well as to their pose in the binding site, their capability to establish H bonds and their chemical diversity. 30 compounds were selected and purchased for in vitro assays.

PROTEIN PURIFICATION

EXPERIMENTAL SCREENING

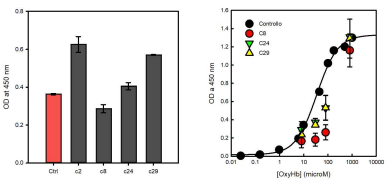
In this preliminary stage of inhibitor selection, the compound activity in terms of capability to interfere with IsdB-Hb interaction was tested towards the IsdB^{Y165A} – OxyHb complex. The relatively weak interaction of the Y165A mutant allowed to detect inhibitors with apparent binding constants in the micromolar range.



- 40 nM IsdB^{Y165A}
- 30 μM OxyHb
- 1 mM inhibitor

A cutoff equal to 0.4 OD was set to select the most promising inhibitors.

Four compounds have been selected and tested for their capability of interfering with the recognition of the IsdB ^{Y165A} – MetHb complex, having an affinity in the micromolar range



CONCLUSIONS

The *in silico* screening has allowed the identification of possible agents interfering with the stabilization of the IsdB-Hb complex.
The ELISA-based screening assays on IsdB^{Y165A}-Hb complex has identified four compounds that interfere with the complex formation. Further experiments are ongoing to calculate the potency of these compounds, and possibly ameliorate their activity and their pharmacokinetic properties

ACKNOWLEDGEMENTS

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