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

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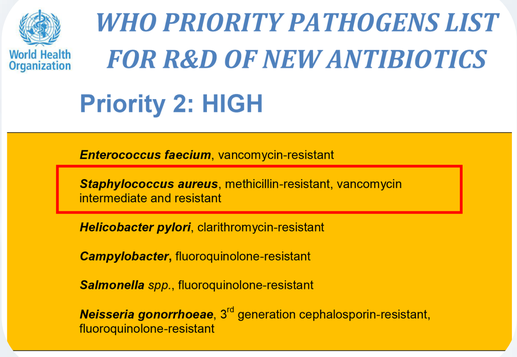
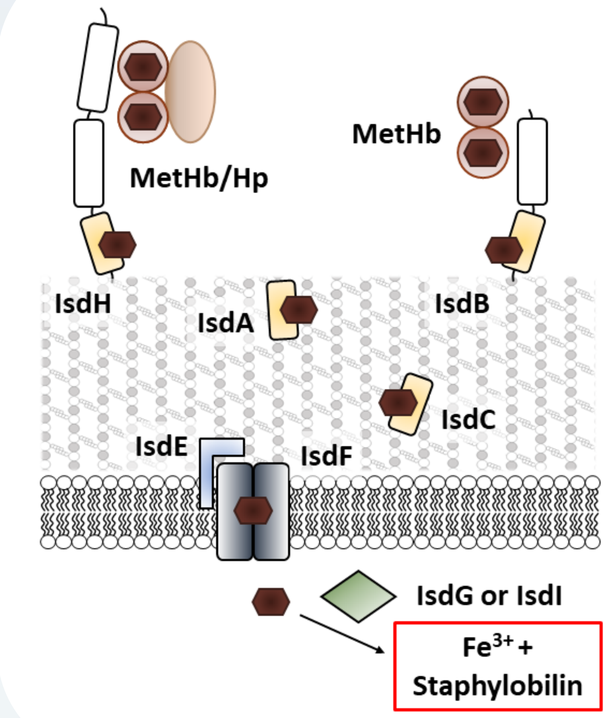


Nowadays and in the coming decades antimicrobial resistance (AMR) will be among the most serious health threats. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most dangerous multi-drug resistant bacteria worldwide and it is included as "high priority" in the Global Priority Pathogens List (Global-PPL) drafted by WHO in 2017.

The *Staphylococcus aureus* pathogenesis of 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 Hb binding. In detail, Iron-regulated Surface Determinant (Isd) system consists of nine components: four surface proteins covalently anchored to the peptidoglycan that reversibly bind hemoglobin (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]. 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].

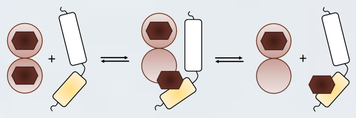


[1] E. P. Skaar and O. Schneewind, "Iron-regulated surface determinants (Isd) of Staphylococcus aureus: Stealing iron from heme," *Microbes Infect.*, vol. 6, no. 4, pp. 390–397, 2004.
[2] N. a Kuklin *et al.*, "A Novel Staphylococcus aureus Vaccine: Iron Surface Determinant B Induces Rapid Antibody Responses in Rhesus Macaques and Specific Increased Survival in a Murin S. aureus Sepsis Model," *Society*, vol. 74, no. 4, pp. 2215–2223, 2006.

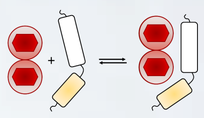
IsdB STRUCTURE

→ The NEAr Transporter (NEAT) domain

IsdB - MetHb Complex



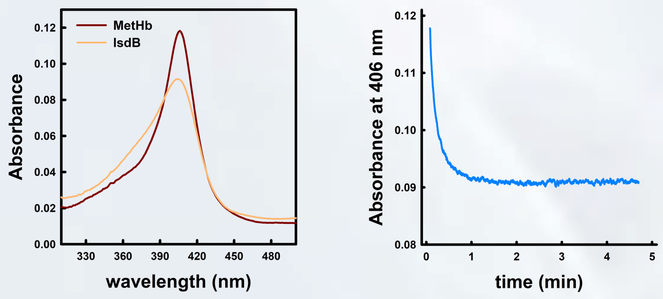
IsdB - OxyHb Complex



NEAT domains are found exclusively in cell surface proteins in Gram-positive bacteria. IsdB presents two NEAT domains: the first allows the receptor to bind hemoglobin, the second is implicated in heme extraction. IsdB is able to scavenge only Fe^{3+}-heme (from MetHb).

UV-VIS SPECTROSCOPY

→ ASSESSMENT OF HEME EXTRACTION

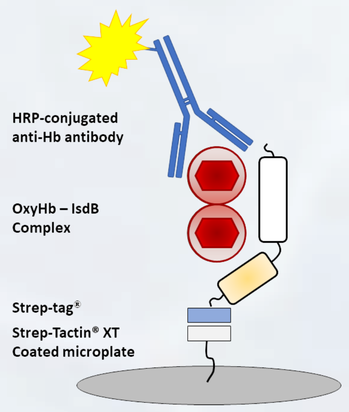


Soret absorption peak changes when heme is transferred from MetHb to IsdB.

Extraction kinetics can be followed at 406 nm.

ELISA ASSAY

→ DETECTION OF COMPLEX FORMATION



The Strept-tag®* recombinantly added to IsdB allows stable binding of the protein to the wells of a plate functionalized with Strep-Tactin® XT*.
The amount of OxyHb bound to IsdB is determined by a HRP-conjugated anti-Hb antibody

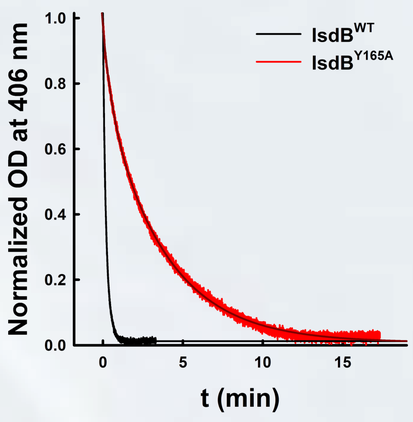
* IBA GmbH, Germany

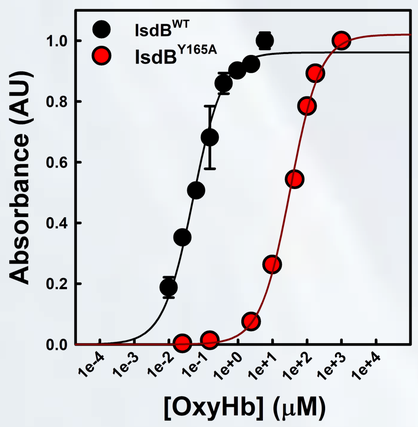
wt IsdB AND Y165A IsdB MUTANT

→ PURIFICATION AND CHARACTERIZATION

$IsdB^{WT}$ and $IsdB^{Y165A}$ mutant have been recombinantly expressed purified in *E. coli*. In the mutant, an Ala replaces Tyr 165, the residue that plays a fundamental role in Hb binding [3]. $IsdB^{Y165A}$ was exploited to increase the sensitivity of the ELISA screening assay.

[3] G. Pishchany et al., "IsdB-dependent hemoglobin binding is required for acquisition of heme by Staphylococcus aureus," J. Infect. Dis., vol. 209, no. 11, pp. 1764–1772, 2014.





	Extraction rate 1/2* (s^{-1})	K_D (μM)
wt	9.4 ± 0.4 / 4.3 ± 0.1	0.05 ± 0.01
Y165A	1.9 ± 0.1 / 0.30 ± 0.01	32.9 ± 2.6

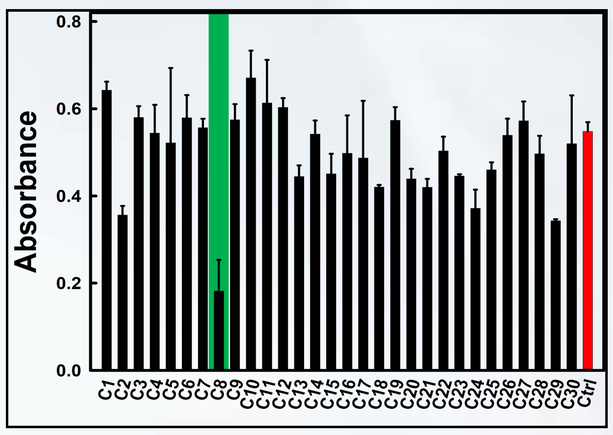
*Double Exponential Decay

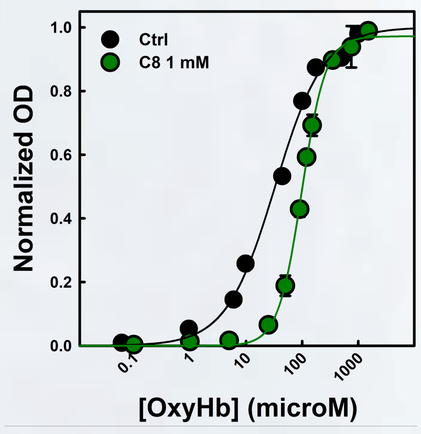
SCREENING OF POTENTIAL INHIBITORS

→ ELISA ASSAY ON $ISDB^{Y165A}$

ELISA assay was used for a self-confident identification of inhibitors of IsdB-Hb complex formation and SAR development. Thirty molecules, identified by structure-based virtual screening and molecular docking (by Prof. Francesca Spyrakis, Università di Torino) were purchased from commercial suppliers (Specs and VITAS)* and tested.

* Specs, The Netherlands andVitas M Laboratory, Ltd., The Nederlands





Although the method allows easy and fast detection of PPI inhibitors, the calculation of K_I for the selected compound will require the development of a dedicated assay.