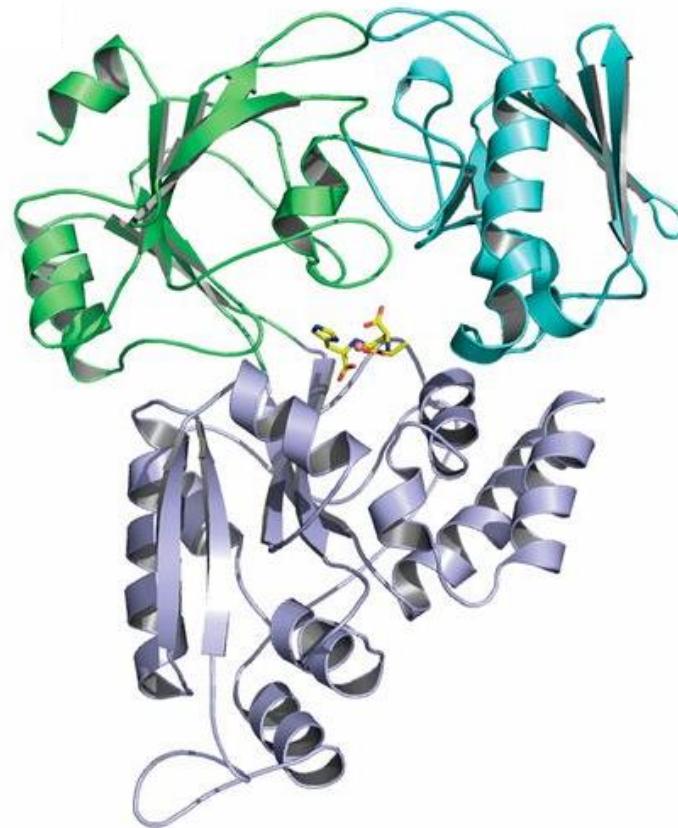


Siderophores and Metallophores



Hiroaki Itoh

Jan. 5, 2019 | Literature Seminar

The structure of the CntA-staphylopine-Co(II) complex (PDB ID: 5YHE):

Song, L.; Zhang, Y.; Chen, W.; Gu, T.; Zhang, S.-Y.; Ji, Q. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 3942.

Transition Metal Ions for Organisms

All organisms must acquire transition metals
(More than 30% of all proteins contain a transition metal cofactor)¹⁾

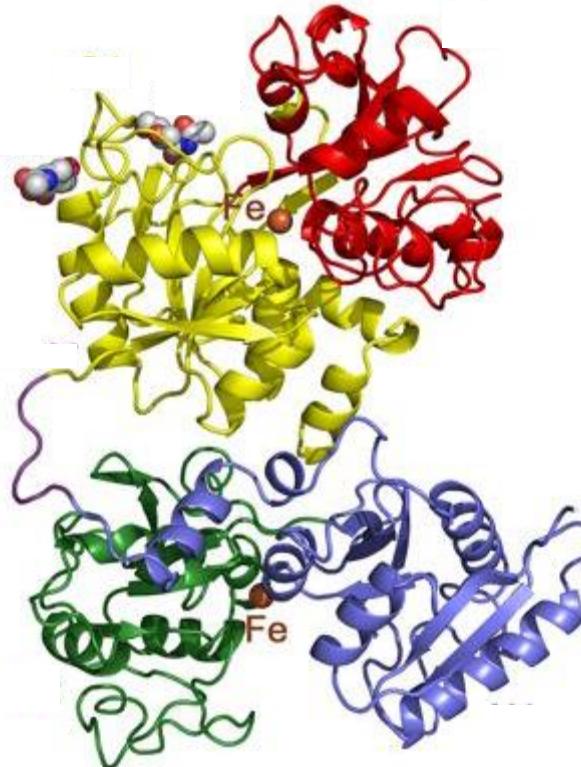


Mechanisms for transition metal acquisition are required for each cell and the whole organisms

e.g. iron utilized as a cofactor of heme proteins (heme): hemoglobin, cytochrome C

Fe(III) as free aqueous ion at pH 7.4:
 10^{-18} M

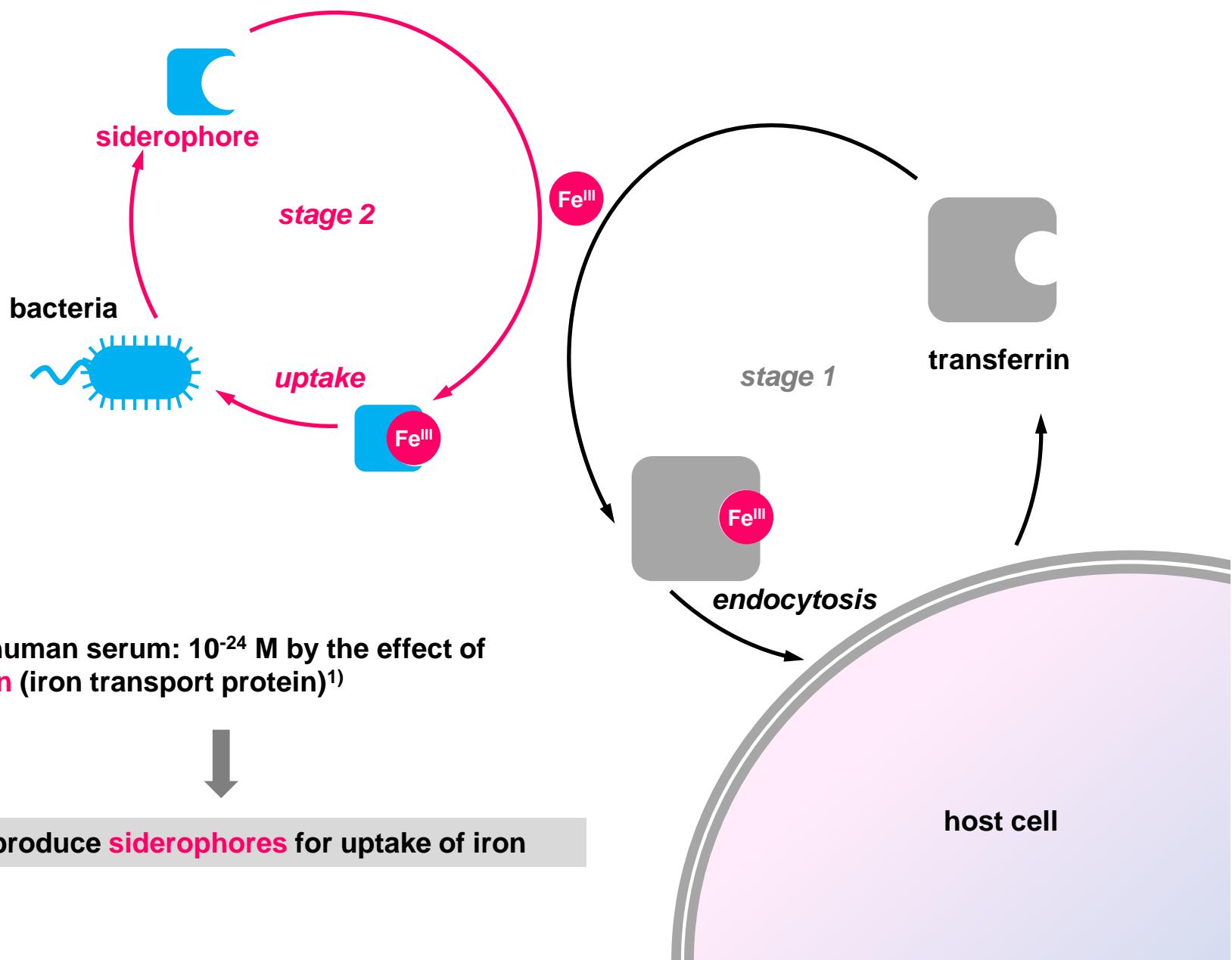
Fe(III) in human serum:
 10^{-24} M by the effect of **transferrin**
(iron transport protein)²⁾



diferric human serum transferrin and metal binding sites (PDB ID 3QYT)³⁾

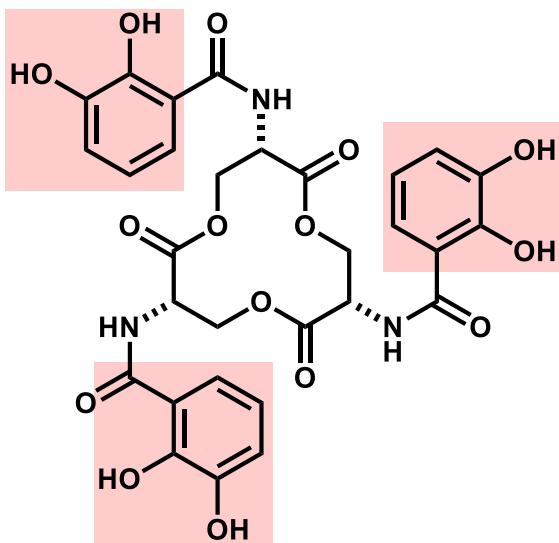
1) Waldron, K. J.; Rutherford, J. C.; Ford, D.; Robinson, N. J. *Nature* **2009**, *460*, 823. 2) Raymond, K. N.; Dertz, E. A.; Kim, S. S. *Proc. Natl. Acad. Sci.* **2003**, *100*, 3584. 3) Yang, N.; Zhang, H.; Wang, M.; Hao, Q.; Sun, H. *Sci. Rep.* **2012**, *2*, 999.

Siderophores



Classification of Siderophores by Structures

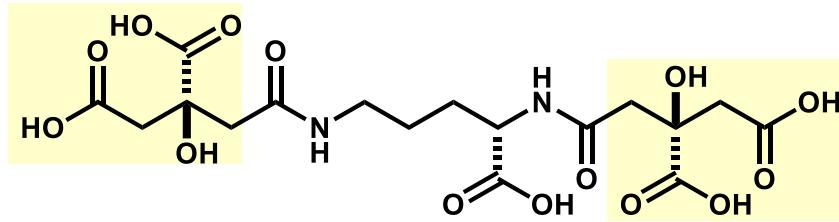
catecholate



enterobactin

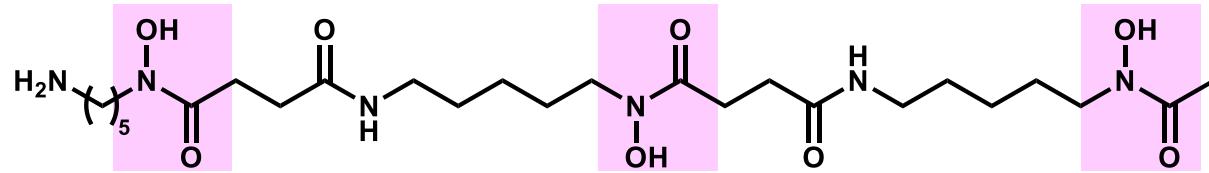
(*P. aeruginosa*, *E. coli*, *K. pneumoniae*)

carboxylate



staphyloferrin A
(*S. aureus*)

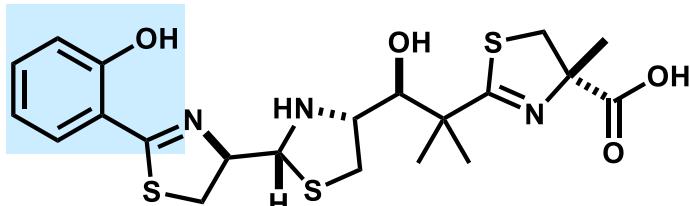
hydroxamate



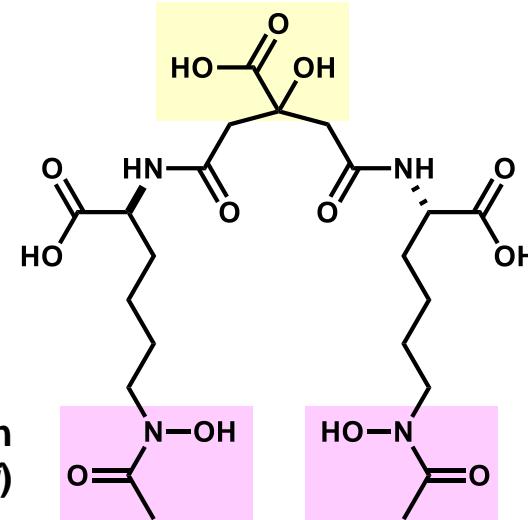
desferrioxamine B (*Streptomyces* sp.)

hybrid (hydroxamate and carboxylate)

hybrid (phenolate and thiazoline)

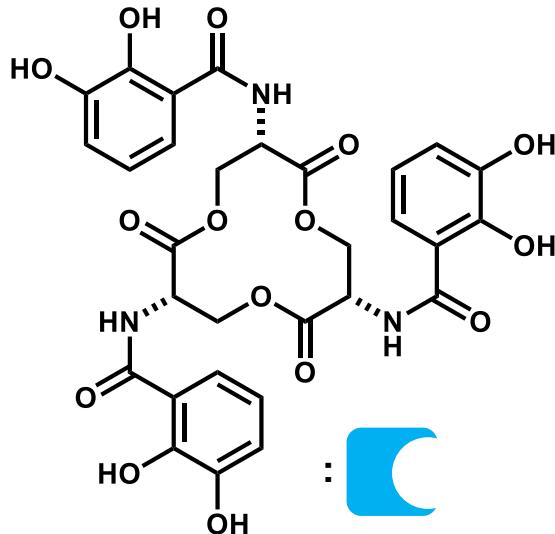


yersiniabactin (*K. pneumoniae*)



aerobactin
(*K. pneumoniae*, *E. coli*)

Biological Function of Siderophores



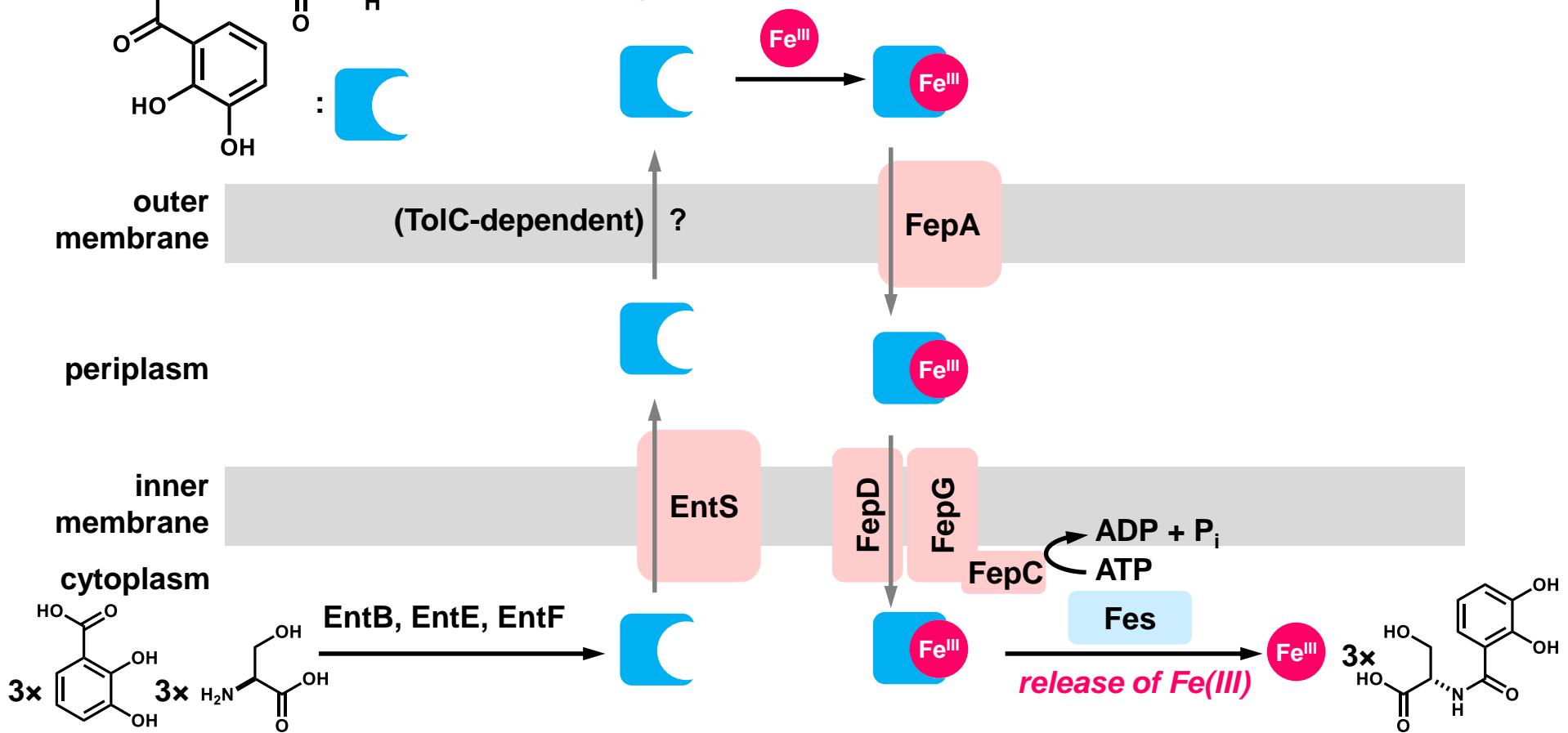
enterobactin:

produced by many Gram-negative bacteria

(*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella enterica* etc.)

biosynthesis, transport, and process of enterobactin:^{1,2)}

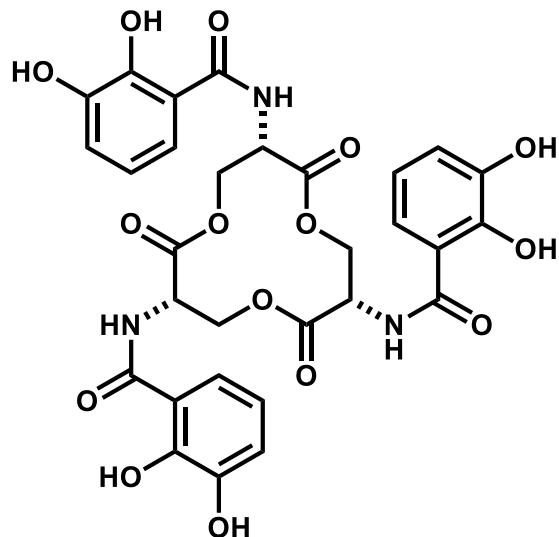
24 kb gene cluster



1) Fischbach, M. A.; Lin, H.; Liu, D. R.; Walsh, C. T. *Nat. Chem. Biol.* **2006**, 2, 132.

2) Lin, H.; Fischbach, M. A.; Liu, D. R.; Walsh, C. T. *J. Am. Chem. Soc.* **2005**, 127, 11075.

Enterobactin is not Optimal Compounds in Hosts



enterobactin: hydrophobic molecule

partitioning¹⁾

lipid bilayer

binding²⁾

serum albumin

	¹⁾
iron acquisition rate constant ($\text{mM}^{-1}\text{s}^{-1}$)	0.041
membrane partitioning coefficient	15000
relative iron acquisition rate with 10 mM lipid ^a	0.27

^aThe iron acquisition rate in the absence of lipid (SUVs consisting of DMPC) are defined as 1.

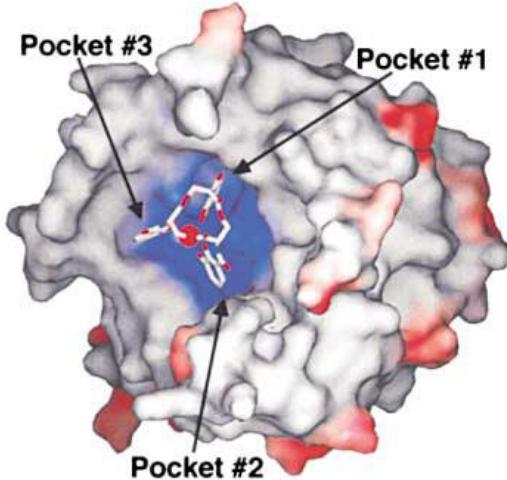
Enterobactins are captured by lipid bilayer and serum albumins in hosts due to hydrophobic catechol moieties

1) Luo, M.; Lin, H.; Fischbach, M. A.; Liu, D. R.; Walsh, C. T.; Groves, J. T. *ACS Chem. Biol.* **2006**, 1, 29.

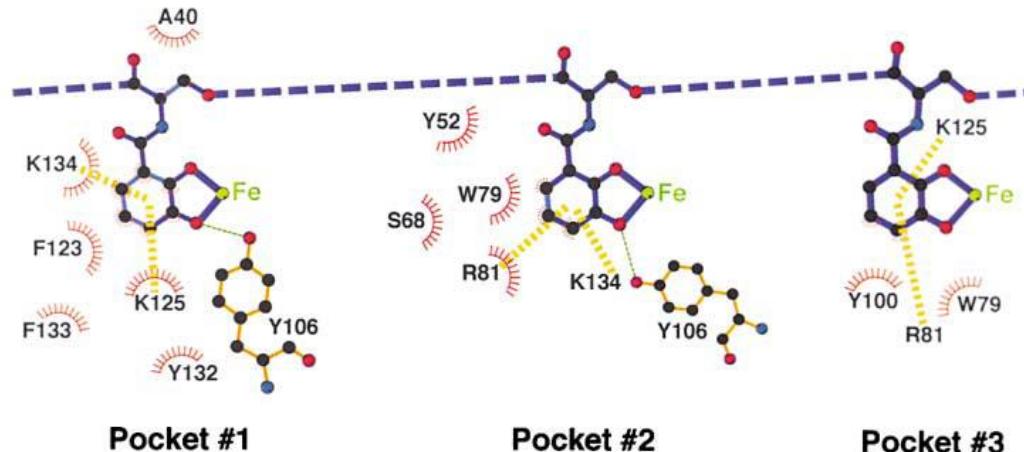
2) Konopka, K.; Neilands, J. B. *Biochemistry* **1984**, 23, 2122.

Host Defense System by Iron-binding Proteins

Sequestration of Fe(III)-enterobactin complex

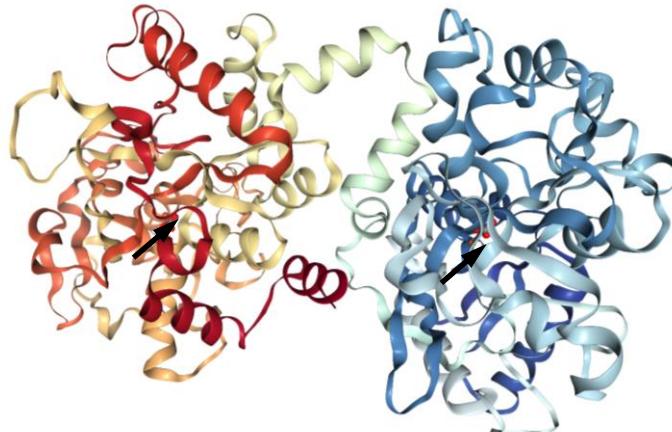


neutrophil gelatinase-associated lipocalin
(NGAL)-Fe(III)-enterobactin complex¹⁾



electrostatic potential (blue, positive; red, negative)

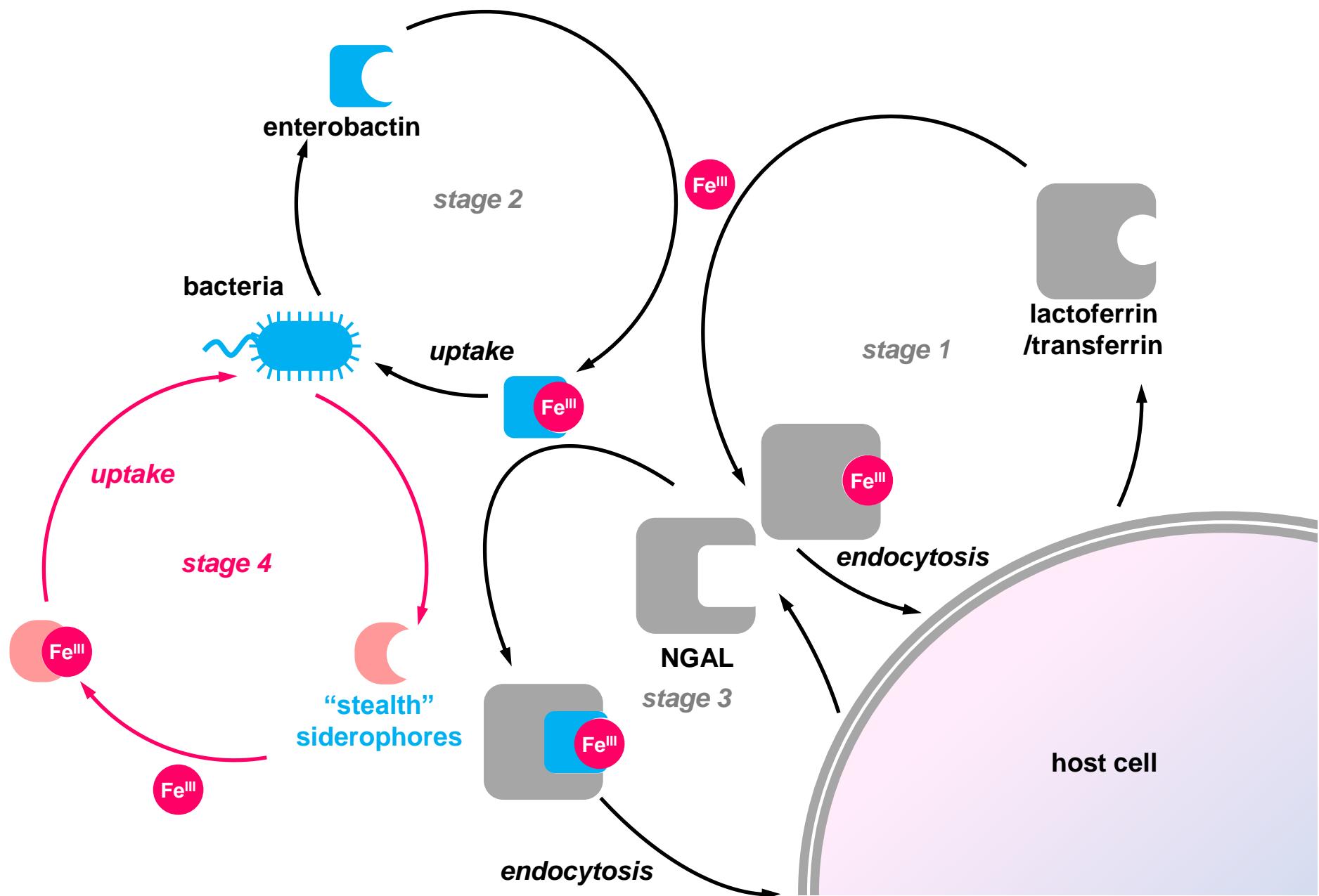
Sequestration of Fe(III)



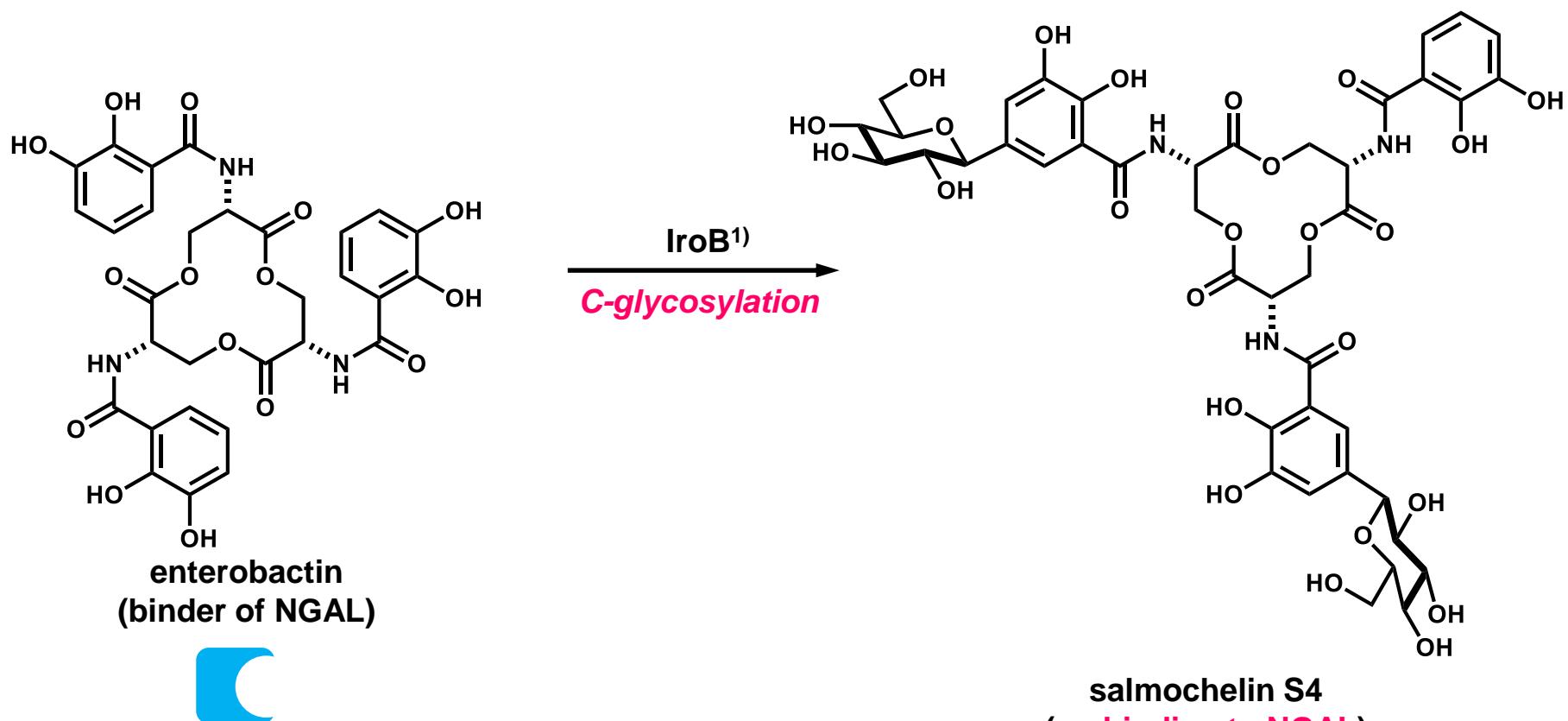
lactoferrin (PDB ID 1B0L)²⁾

- 1) Goetz, D. H.; Holmes, M. A.; Borregaard, N.; Bluhm, M. E.; Raymond, K. N.; Strong, R. K. *Mol. Cell* **2002**, *10*, 1033. 2) Sun, X. L.; Baker, H. M.; Shewry, S. C.; Jameson, G. B.; Baker, E. N. *Acta Crystallogr. D Biol. Crystallogr.* **1999**, *55*, 403.

Production of “Stealth” Siderophores



Enzymatic Modification of Enterobactin

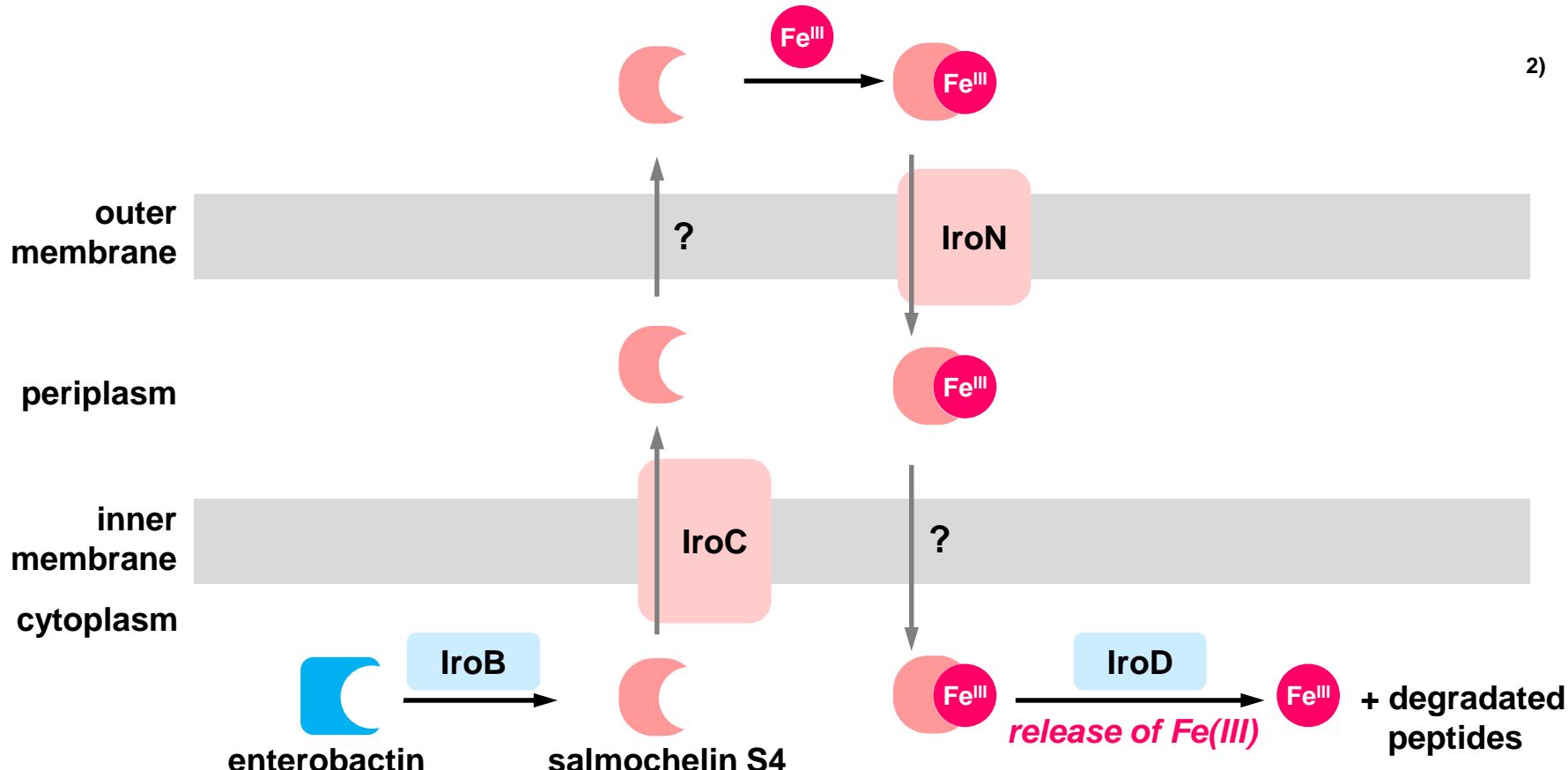


1) Bister, B.; Bischoff, D.; Nicholson, G. J.; Valdebenito, M.; Schneider, K.; Winkelmann, G.; Süßmuth, R. D. *BioMetals* **2004**, 17, 471.

Iron Uptake by Salmochelins

	enterobactin	salmochelin S4	1)
iron acquisition rate constant ($\text{mM}^{-1}\text{s}^{-1}$)	0.041	0.045	
membrane partitioning coefficient	15000	3100	
relative iron acquisition rate with 10 mM lipid ^a	0.27	0.64	

^aThe iron acquisition rate in the absence of lipid (SUVs consisting of DMPC) are defined as 1.

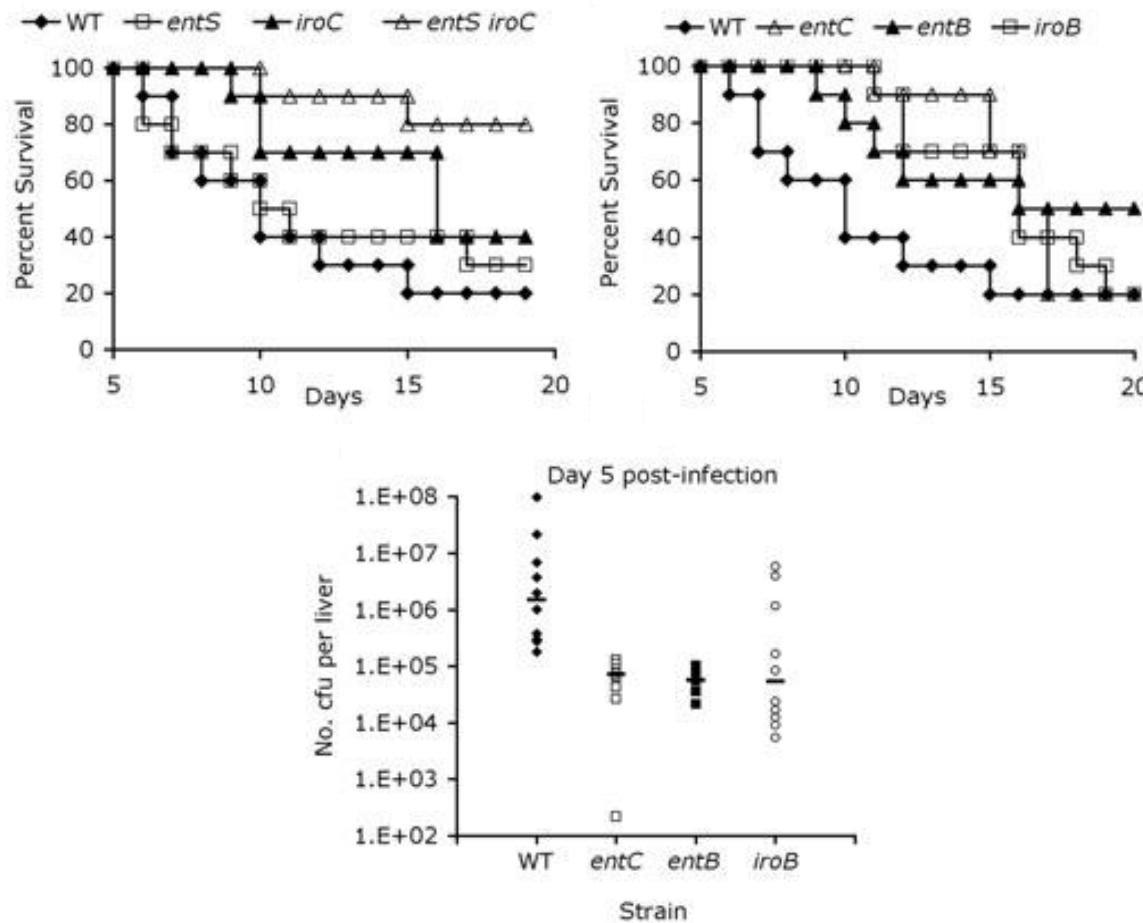


1) Luo, M.; Lin, H.; Fischbach, M. A.; Liu, D. R.; Walsh, C. T.; Groves, J. T. *ACS Chem. Biol.* **2006**, 1, 29.

2) Fischbach, M. A.; Lin, H.; Liu, D. R.; Walsh, C. T. *Nat. Chem. Biol.* **2006**, 2, 132.

Salmochelin is Essential for Virulence

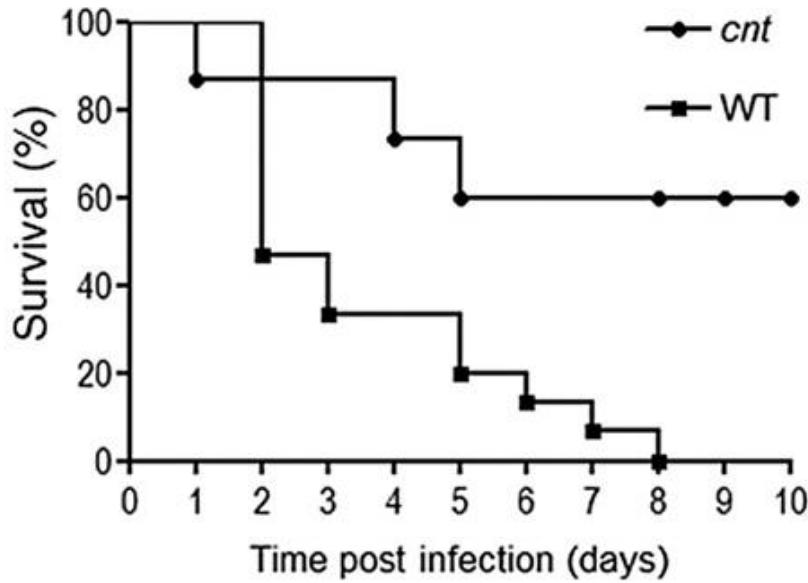
requirement for siderophore synthesis and secretion during systemic *Salmonella* infection of mice



The biosynthesis and secretion of salmochelin S4 was found to play a role in the virulence¹⁾

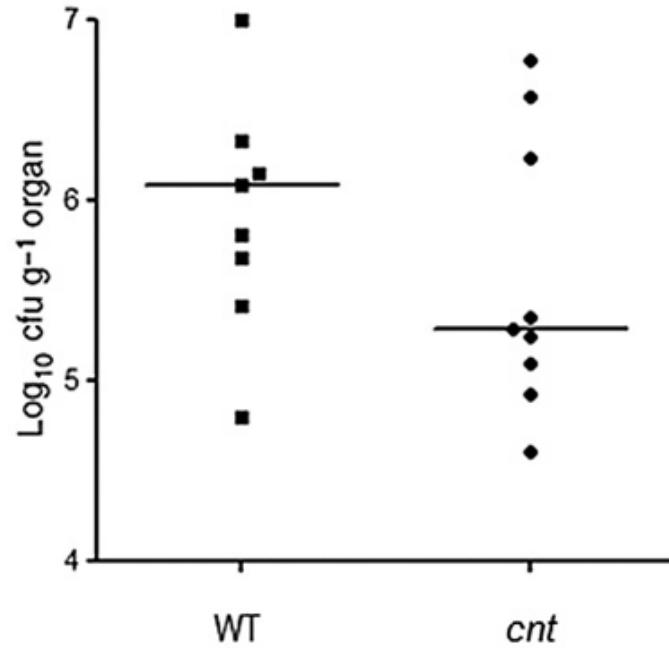
Metallophores and Virulence

A nickel/cobalt uptake system (CntA, CntB, CntC, CntD, and CntF) in *S. aureus* was found to play a role in the virulence in animal models of infection¹⁾



survival of mice infected with *S. aureus*

square: wild type (RN6390), circles: *cntABCDF* mutant



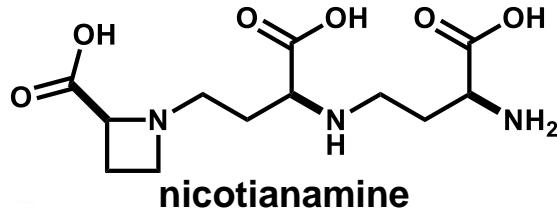
bacterial counts within kidneys of mice 24 h after intravenous infection

A carrier of these heavy metals had been unknown

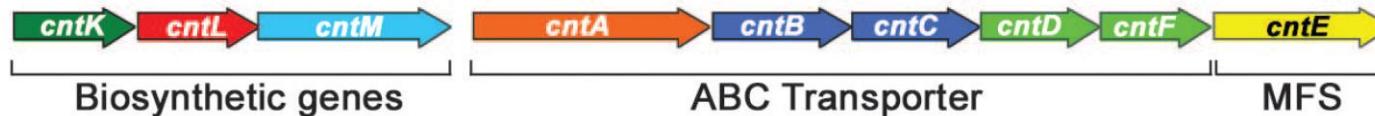
1) Remy, L.; Carrière, M.; Derré-Bobillot, A.; Martini, C.; Sanguinetti, M.; Borezée-Durant, E. *Mol. Microbiol.* 2013, 87, 730.

Nicotianamine-Like Molecule in Bacteria

nicotianamine: a metal chelator (copper, nickel, zinc, and iron) found in plants biosynthesized by nicotianamine synthase



A gene coding for nicotianamine synthase-like enzyme was found in **bacterial genome**, including that of *S. aureus*¹⁾



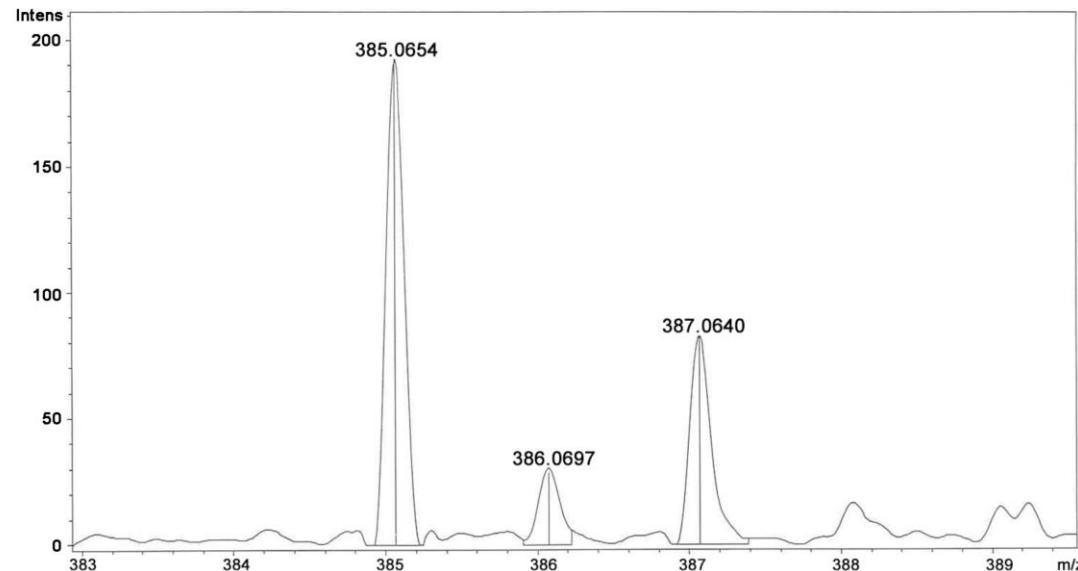
ABC transporter: ATP-binding cassette transporters, MFS: major facilitator superfamily

1. cloning, expression and purification of CntA from *E. coli*

2. incubation in supernatant of *S. aureus* *cntA* mutant with Ni(II)

3. repurification of CntA

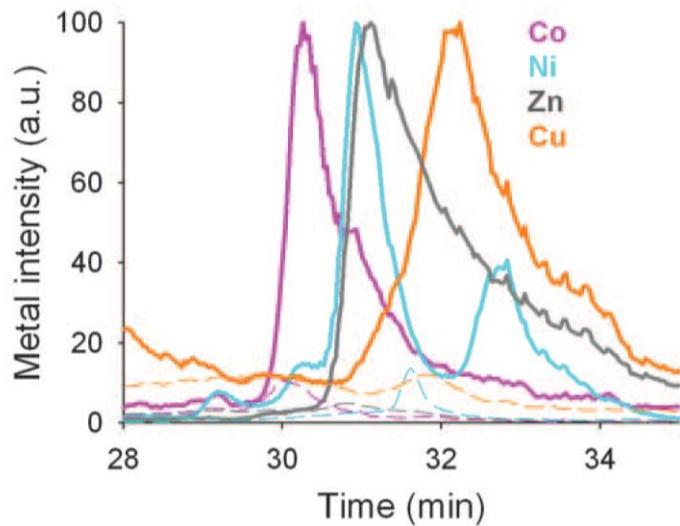
4. buffer exchange and MS analysis



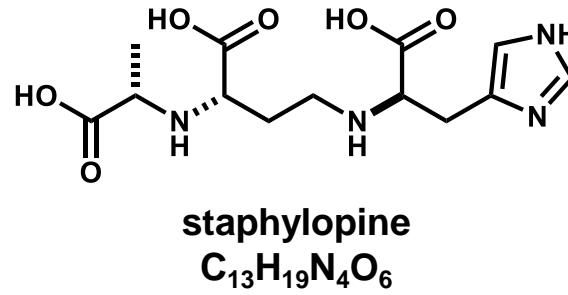
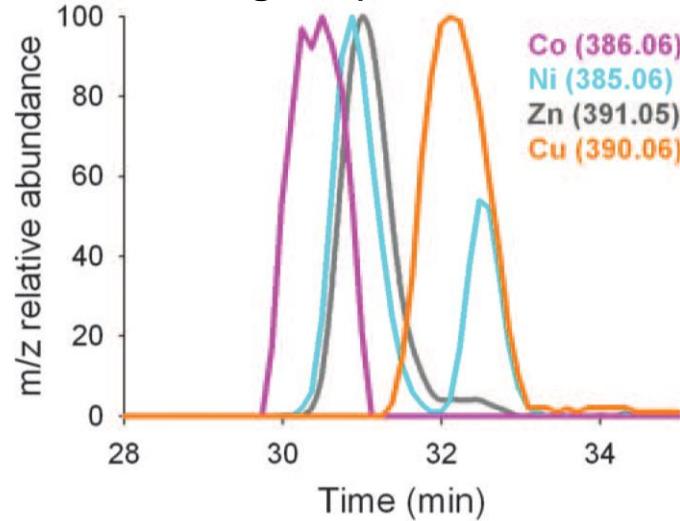
1) Ghssein, G.; Brutesco, C.; Ouerdane, L.; Fojcik, C.; Izaute, A.; Wang, S.; Hajjar, C.; Lobinski, R.; Lemaire, D.; Richaud, P.; Voulhoux, R.; Espaillat, A.; Cava, F.; Pignol, D.; Borezée-Durant, E.; Arnoux, P. *Science* **2016**, 352, 1105.

Discovery of Staphylopine

HILIC/ICP-MS chromatograms

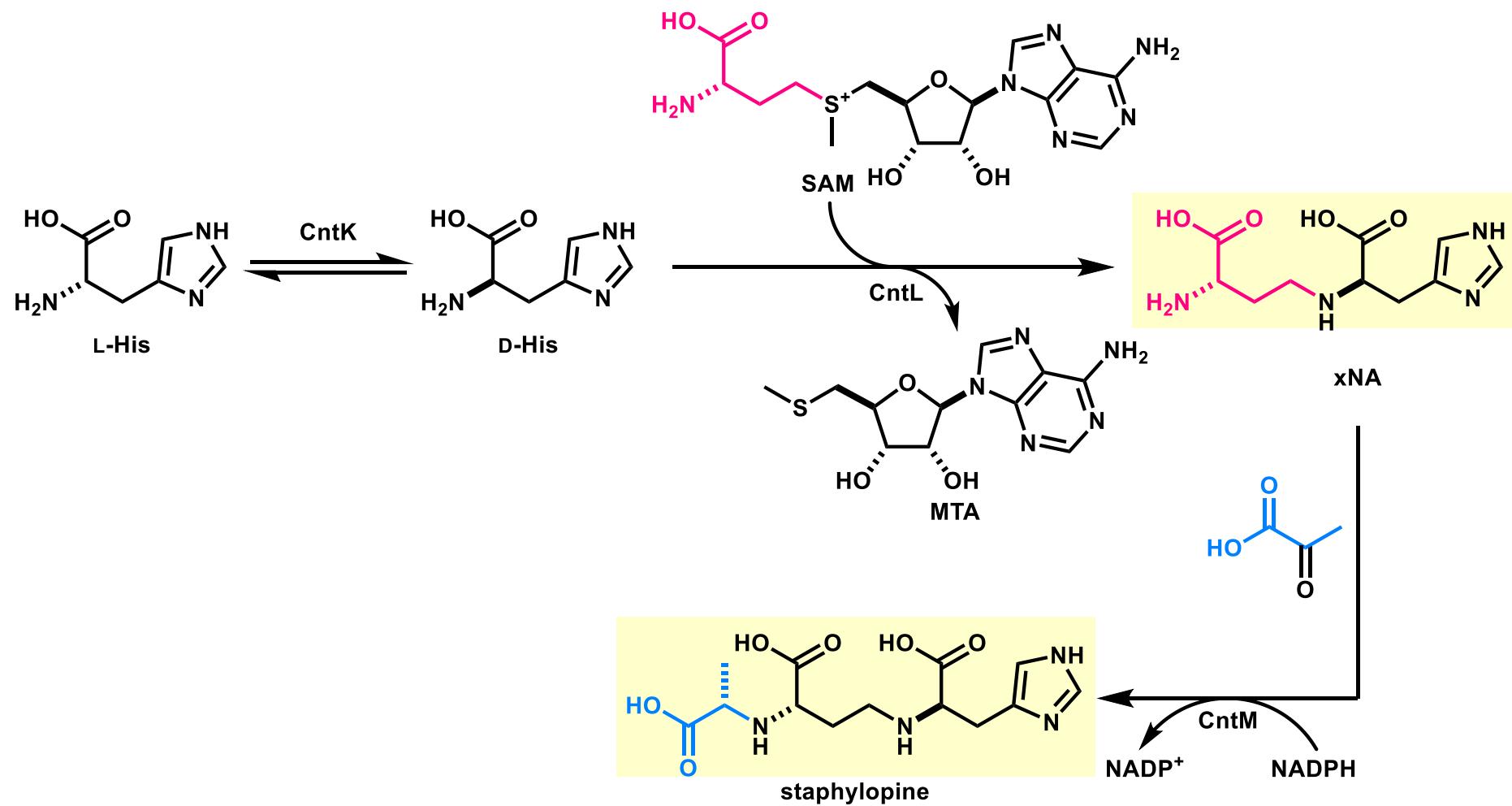


HILIC/ESI-MS chromatogram (extracted ion chromatograms)



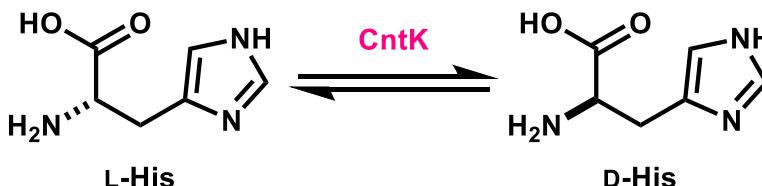
- 1) Ghssein, G.; Brutesco, C.; Ouerdane, L.; Fojcik, C.; Izaute, A.; Wang, S.; Hajjar, C.; Lobinski, R.; Lemaire, D.; Richaud, P.; Voulhoux, R.; Espaillat, A.; Cava, F.; Pignol, D.; Borezée-Durant, E.; Arnoux, P. *Science* **2016**, 352, 1105.

Biosynthesis of Staphylopine

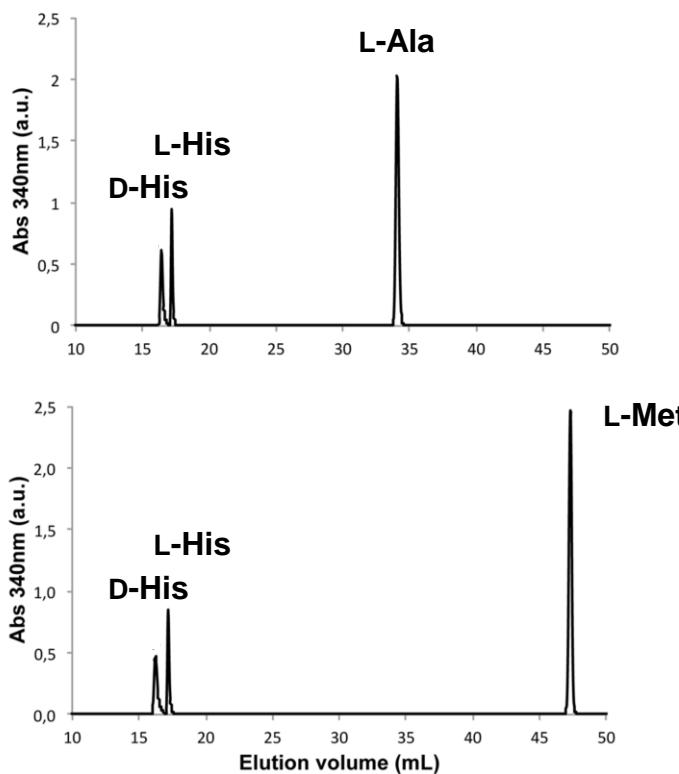
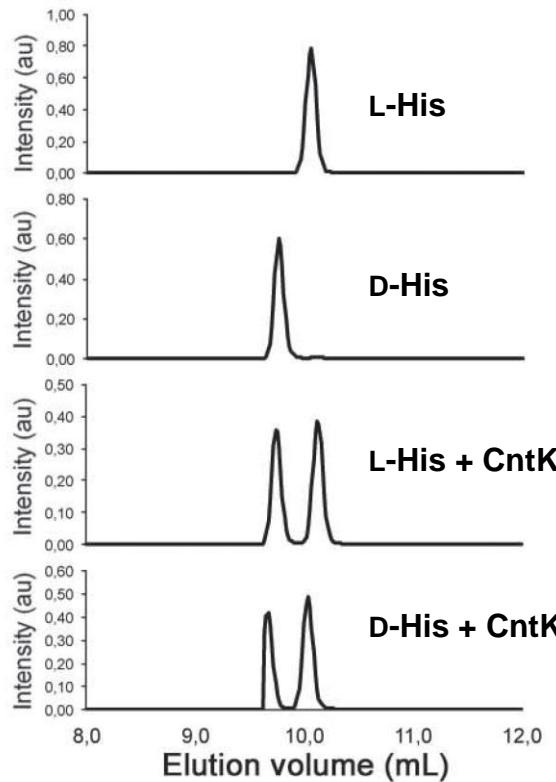


- 1) Ghssein, G.; Brutesco, C.; Ouerdane, L.; Fojcik, C.; Izaute, A.; Wang, S.; Hajjar, C.; Lobinski, R.; Lemaire, D.; Richaud, P.; Voulhoux, R.; Espaillat, A.; Cava, F.; Pignol, D.; Borezée-Durant, E.; Arnoux, P. *Science* **2016**, 352, 1105.

CntK: Histidine-Specific Racemase



Marfey's analysis (derivatization with FDAA)

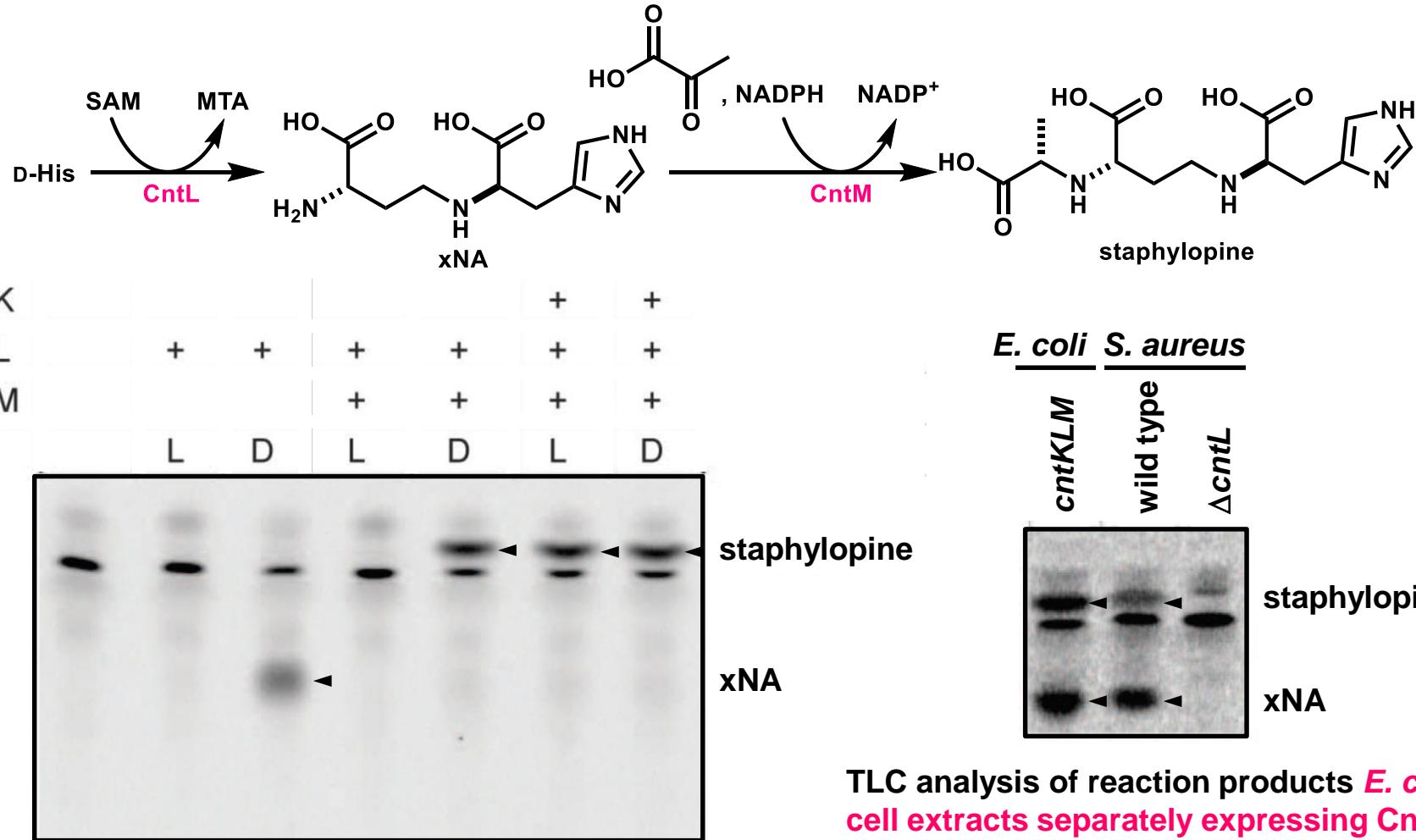


Aeris peptide column 3.6 μ m
4.6 x 250 mm
triethylamine phosphate buffer
/acetonitrile
2 mL/min

CntK was found to be histidine-specific racemase

- 1) Ghssein, G.; Brutesco, C.; Ouerdane, L.; Fojcik, C.; Izaute, A.; Wang, S.; Hajjar, C.; Lobinski, R.; Lemaire, D.; Richaud, P.; Voulhoux, R.; Espaillat, A.; Cava, F.; Pignol, D.; Borezée-Durant, E.; Arnoux, P. *Science* **2016**, 352, 1105.

Functions of CntL and CntM



TLC analysis of reaction products *E. coli* cell extracts separately expressing CntK, CntL, and CntM with ¹⁴C-labelled SAM

TLC analysis of reaction products incubating ¹⁴C-labelled SAM with purified enzymes (CntK, CntL, and CntM), D-or L-histidine, pyruvate, and NADPH

The results were consistent with the above biosynthesis.

- 1) Ghssein, G.; Brutesco, C.; Ouerdane, L.; Fojcik, C.; Izaute, A.; Wang, S.; Hajjar, C.; Lobinski, R.; Lemaire, D.; Richaud, P.; Voulhoux, R.; Espaillat, A.; Cava, F.; Pignol, D.; Borezée-Durant, E.; Arnoux, P. *Science* **2016**, 352, 1105.

Metal Selectivity of Staphylopine

pK_d values for staphylopine complexes estimated by metal competition assay with nicotianamine by ESI-MS

	pK _d (-log ₁₀ K _d) at pH 7	
	staphylopine	nicotianamine
Cu(II)	19.0	18.6
Ni(II)	16.4	16.1
Co(II)	15.1	14.8
Zn(II)	15.0	14.7
Fe(II)	12.3	12.1
Mn(II)	9.1	8.8

staphylopine has the similar K_d and affinity order:

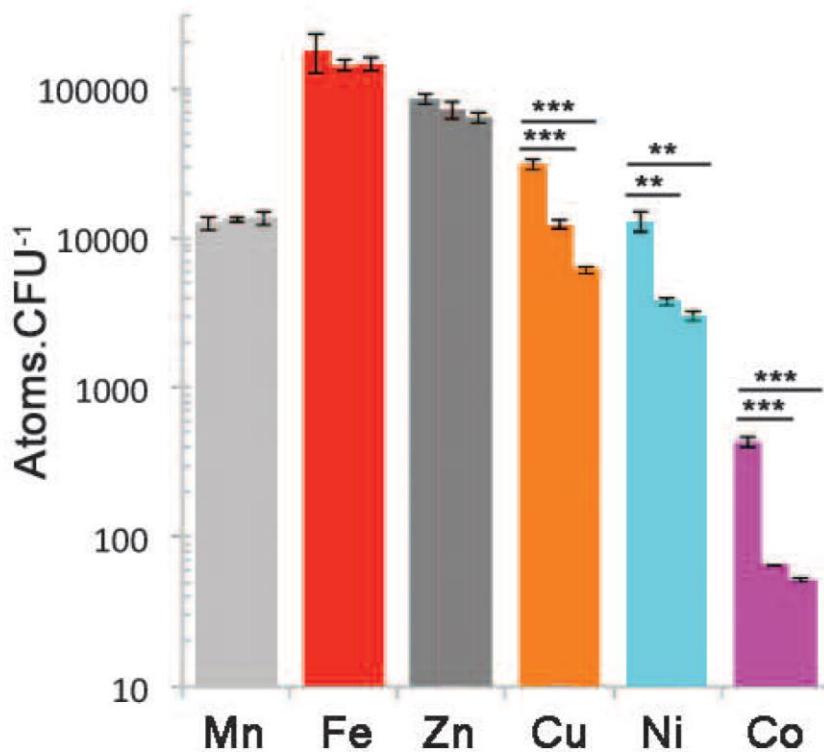


(Fe(III) has a weak affinity for staphylopine at neutral pH)

staphylopine: a broad-spectrum metallophore, which is similar to nicotianamine

- 1) Ghssein, G.; Brutesco, C.; Ouerdane, L.; Fojcik, C.; Izaute, A.; Wang, S.; Hajjar, C.; Lobinski, R.; Lemaire, D.; Richaud, P.; Voulhoux, R.; Espaillat, A.; Cava, F.; Pignol, D.; Borezée-Durant, E.; Arnoux, P. *Science* **2016**, 352, 1105.
- 2) For metal selectivity of a related molecule, aspergillomarasamine A, see: Zhang, J.; Wang, S.; Bai, Y.; Guo, Q.; Zhou, J.; Lei, X. *J. Org. Chem.* **2017**, 82, 13643.

Cellular Uptake of Metals



Intracellular metal content of the *S. aureus* was determined by ICP-MS

left: wild type
middle: *cntL* mutant
right : *cntABCDF* mutant

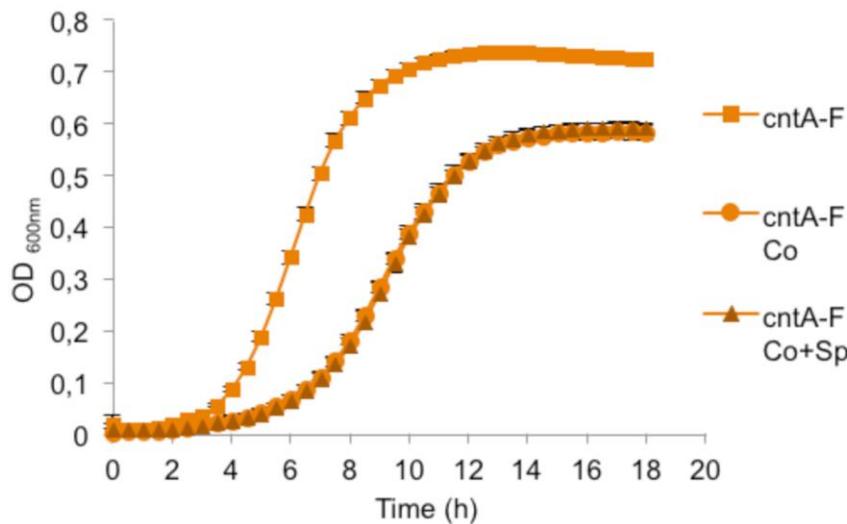
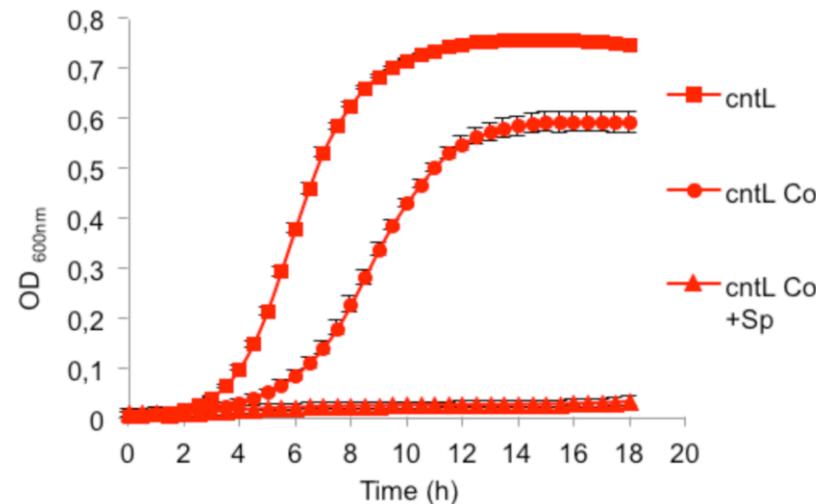
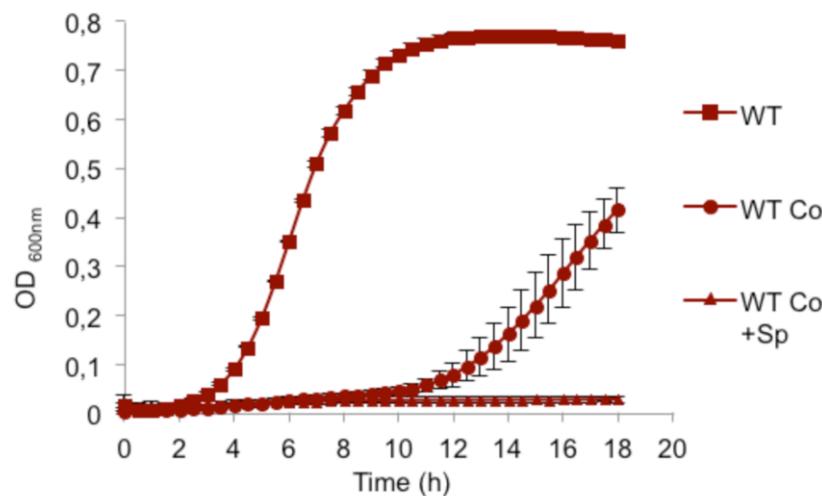
These strains were grown in chemically defined medium (CDM)

** $P < 0.01$, *** $P < 0.001$

- Both *cntL* and *cntA-F* mutants caused decrease of intracellular accumulation of copper, nickel, and cobalt
- A *cntABCDF* gene is involved with cellular uptake of metal or metal-staphylopine complexes

1) Ghssein, G.; Brutesco, C.; Ouerdane, L.; Fojcik, C.; Izaute, A.; Wang, S.; Hajjar, C.; Lobinski, R.; Lemaire, D.; Richaud, P.; Voulhoux, R.; Espaillat, A.; Cava, F.; Pignol, D.; Borezée-Durant, E.; Arnoux, P. *Science* **2016**, 352, 1105.

Transportation of Metal-Staphylopine Complexes



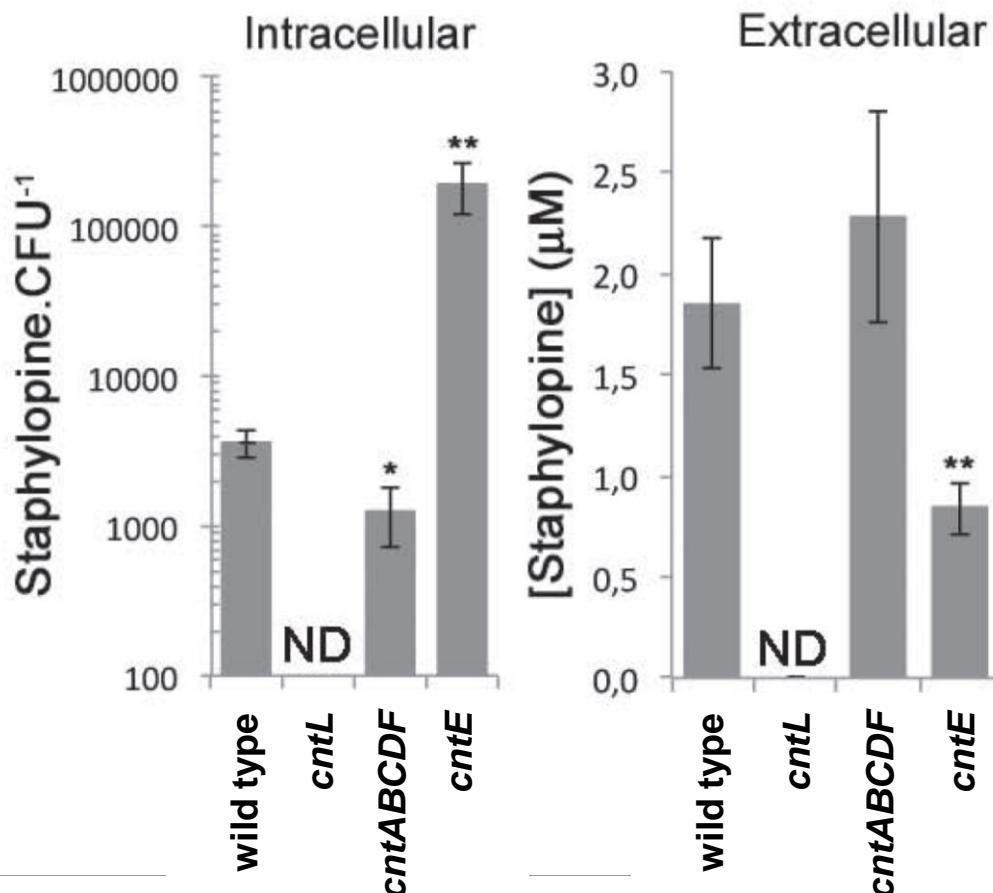
chemically defined medium (CDM) supplemented or not with 2 mM cobalt (Co), 10 μ M of staphylopine (Sp), or both (Co+Sp).

The addition of staphylopine restored the toxicity of cobalt in *cntL* mutant but not in *cntABCDF*



A gene *cntABCDF* is likely involved with a transportation of metal-staphylopine complexes

Intracellular and Extracellular Staphylospines



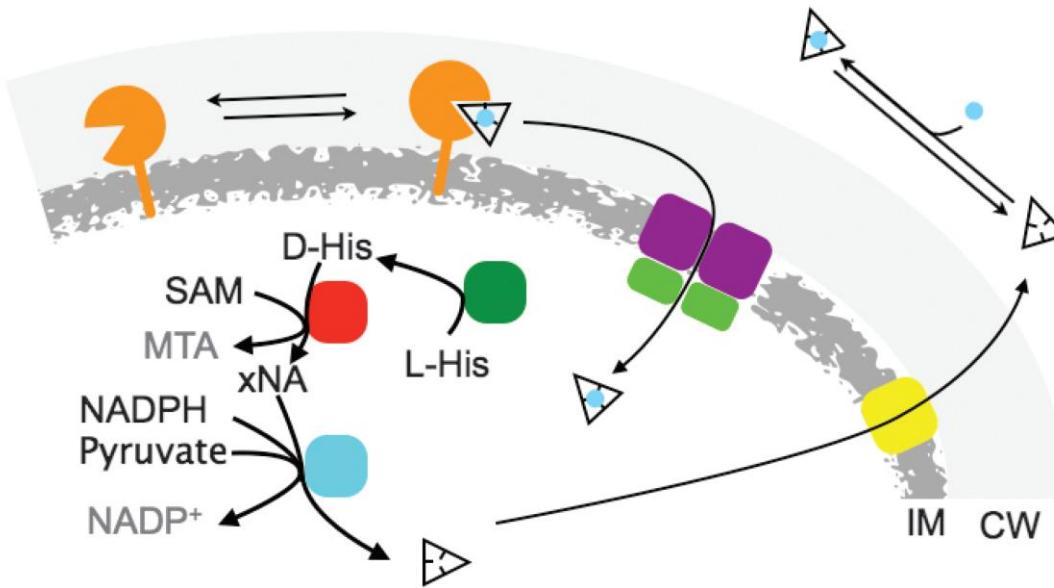
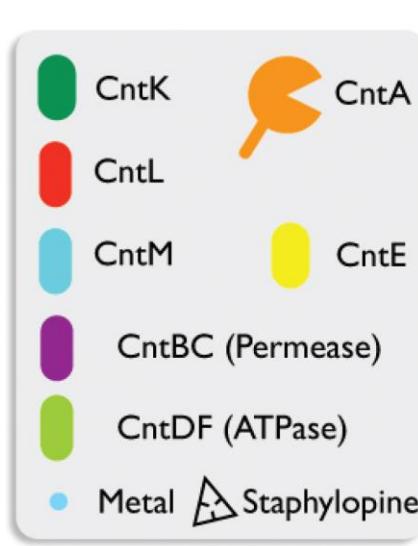
staphylospine level of the intracellular (left) and supernatant (right) fractions of the wild type, *cntABCF*, *cntL*, and *cntE* mutant strains

* $P < 0.05$, ** $P < 0.01$; ND: not detected

These results supported that the functions of *cntABCF* and *cntE* are involved with the import of staphylospine-metal complexes and the export of staphylospine, respectively

- 1) Ghssein, G.; Brutesco, C.; Ouerdane, L.; Fojcik, C.; Izaute, A.; Wang, S.; Hajjar, C.; Lobinski, R.; Lemaire, D.; Richaud, P.; Voulhoux, R.; Espaillat, A.; Cava, F.; Pignol, D.; Borezée-Durant, E.; Arnoux, P. *Science* **2016**, *352*, 1105.

Biosynthesis and Function of Staphylopine



Homologous biosynthetic genes are encoded by other microbial pathogens, including *Pseudomonas aeruginosa* and *Yersinia pestis*



Further study on metallophores would reveal the influence of them in bacterial pathogenicity

- 1) Ghssein, G.; Brutesco, C.; Ouerdane, L.; Fojcik, C.; Izaute, A.; Wang, S.; Hajjar, C.; Lobinski, R.; Lemaire, D.; Richaud, P.; Voulhoux, R.; Espaillat, A.; Cava, F.; Pignol, D.; Borezée-Durant, E.; Arnoux, P. *Science* **2016**, 352, 1105.