For most pathogens, the ability to feed on iron within their host is critical for survival and virulence. Although iron is plentiful in the human body (~5 g of the stuff), it is guarded in ways that make it inconvenient for sharing, with the bulk of it locked up in hemoglobin, mineralized in ferritin cages, bound in transferrin, or otherwise lodged within enzyme active sites. Elucidating the chemical principles underlying the multiple strategies that microbes employ to confiscate iron from these kinetically intransient sources is important for understanding microbial-host interactions and combating infectious diseases. In this issue of ACS Central Science, Endicott and co-workers reveal new mechanistic insights into ferric iron uptake facilitated by membrane-anchored siderophore binding proteins (SBP) in Staphylococcus aureus, a Gram-positive human pathogen.

Many aspects of siderophore-mediated iron uptake have been elucidated, but an overriding perplexing question has persisted: how exactly do siderophores facilitate iron uptake from host iron sources? Even high-affinity siderophores with a thermodynamic advantage do not extract Fe(III) from human transferrin at pH 7.4 on any kind of time scale pertinent to microbial growth. By using a fluorescent siderophore probe to measure rates of iron exchange between biological ligands, Wencewicz and his team discovered a previously unrecognized function for siderophore-binding proteins that expands their role as siderophore receptor to include iron-transfer catalyst. Experiments with site-directed SBP mutants led to identification of key tyrosine and tryptophan residues that do not affect substrate binding but significantly accelerate Fe(III) transfer, for example, from human iron-loaded holo-transferrin to the linear siderophore desferrioxamine B. In essence, the siderophore serves dual roles for a dual-function enzyme: substrate for SBP as receptor, and cofactor for SBP as catalyst. Dynamic conformational flexibility of SBPs is likely to stabilize transition states of siderophore substrates in the binding pocket to facilitate

![Figure 1. A revised paradigm for siderophore-mediated ferric iron uptake assigns catalytic ferrichelatase function to siderophore binding proteins, providing a conceptually satisfying explanation for how bacteria acquire ferric iron from kinetically intransient host proteins, including human transferrin. Membrane-anchored SBP binds with apo-siderophore to form a cofactor complex (a) that interacts with transferrin via a putative Fe-coordinated intermediate (b). The resulting Fe-siderophore-SBP complex adopts a closed conformation (c) and associates with a membrane permease (d) to transfer the cargo across the membrane (e, f).](https://dx.doi.org/10.1021/acscentsci.0c00179)
ferric iron transfer via ligand-exchange reactions. With a nod to the ferrochelatases, which mediate ferrous iron insertion for heme biosynthesis, the authors propose to name this class of catalytic SBPs siderophore-dependent ferrochelatases, to reflect their ferric iron transfer activity.4

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The key new insight based on evidence of ferrochelatase activity expands the paradigm of cell-surface ligand exchange mechanisms for nonreductive iron uptake first proposed by Raymond and co-workers.5 In that model, iron shuttles from holo-siderophore to a receptor-bound apo-siderophore.6 Shown schematically in Figure 1, the newly revised paradigm provides a chemically satisfying explanation for how SBPs impart a kinetic advantage to extract iron from host sources. The iron-free apo-siderophore binds to SBP in its resting state to form an enzyme−cofactor complex (Figure 1a). The plasticity of the cofactor and SBP enables interaction with host iron sources, in this case forming a putative quininy species consisting of SBP, siderophore, transferrin, iron, and carbonate, the synergistic anion that fulfills the iron coordination sphere in holo-transferrin (Figure 1b). Exactly what takes the first “bite of the apple” remains to be determined, but possible culprits include one of several conserved tyrosines on SBP or the tail hydroxamate of the linear siderophore, either of which could act as the first sticky finger to reach into iron’s inner coordination sphere and initiate ligand exchange. Regardless of these details, which constitute another curious knowledge gap to be elucidated in future research, the authors suggest that SBP adopts a closed conformation after it strips iron from transferrin to form the holo-siderophore-SBP complex (Figure 1c), which is then primed to associate with the membrane permease (Figure 1d). The remaining steps involve ATP-driven translocation across the membrane (Figure 1e−f) and ultimately release of iron from the siderophore complex by one of several mechanisms that have been well characterized, including reduction to Fe(II), protonation, and siderophore hydrolysis.1,3

The chemical principles that underlie this newly recognized function for a siderophore receptor protein were presaged by earlier work in the field. Detailed kinetic investigations by Crumbliss and co-workers, for example, emphasized the requirement of an additional driving force for iron transfer between siderophores and iron-binding proteins to occur in a biological time frame.1,7−9 Notably, they proposed stepwise mechanisms in which competing chelators (siderophores) displace a gate-keeping synergistic anion to form a relatively stable ternary intermediate, an idea invoked in Figure 1b.9 Additionally, they emphasized the importance of molecular recognition in the first and second coordination shells of siderophore complexes, thus predicting that hydrogen bonding interactions between ferrioxamine B and specific iron-binding residues of membrane receptors could explain the relatively fast cellular uptake of iron compared to other ferrioxamine siderophores.7

By building on the Raymond membrane transport paradigm and invoking concepts of synergistic anion displacement, cofactor plasticity and molecular recognition predicted by Crumbliss, the authors of this recent work present a satisfying model for linear hydroxamate siderophores in Gram-positive bacteria. But what of other organisms? And what of other siderophores? A wide diversity exists among siderophore structures and other small molecule metallophores, which of course prompts additional questions about their potential cofactor plasticity, molecular recognition, and aspects of their roles in mediating host−pathogen metallobiology.

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