

A Response to Reimportation

In this issue of *Structure*, [Holmes et al. \(2005\)](#) describe elements of an innate defense mechanism that provides mammals a means to restrict bacterial growth. The host protein siderocalin scavenges structurally dissimilar bacterial siderophores and prevents the uptake of Fe^{3+} already earmarked for bacterial import.

Despite the low solubility of ferric iron, bacteria are able to acquire the quantity necessary to support growth with a specific transport system that involves the reimportation of secreted siderophores that forage Fe^{3+} from the surrounding milieu (for review, see [Faraldo-Gomez and Sansom, 2003](#)). While the chemical structures of the siderophores synthesized by diverse bacteria may differ substantially, the overall design of the uptake system is the same; the bacteria manufacture and secrete high affinity Fe^{3+} chelators (MW ~500–1000) and specific outer membrane proteins such as FhuA and FepA, membrane-spanning 22-stranded antiparallel β -barrels with plugs, allow the reimportation of the scavengers. Thus, despite low concentrations of ferric iron, bacteria are able to salvage the requisite amount to support growth.

Defense against bacterial reimportation of the ferric-siderophore is afforded the host by a member of the lipocalin superfamily: small, secretory proteins (~170 amino acids) that adopt eight-stranded antiparallel β -barrel structures and bind labile or hydrophobic ligands within the barrel. The lobster lipocalin crustacyanin binds the carotenoid astaxanthin and upon denaturation releases the bound pigment to take on a bright red color. The lipocalin bilin binding protein is involved in insect coloration, while the superfamily member known as nitrophorin, found in blood sucking insects releases NO into the tissue of the insect's prey to induce vasodilation. In mammals, lipocalins mediate specific transport systems; for example, the serum retinol binding protein is involved in transport of vitamin A, the precursor of the morphogen retinoic acid, and odorant

binding proteins transport odorants in the nasal mucosa. However, the function of the lipocalin formerly known as neutrophil-gelatinase associated lipocalin (NGAL), lipocalin-2, uterocalin, or 24p3 remained elusive until recently. In 2002, Strong and colleagues showed that the protein binds the siderophore enterochelin produced by *Escherichia coli* ([Goetz et al., 2002](#)). This observation raised the intriguing possibility that the protein, long recognized as a marker for bacterial infection when it is observed at elevated levels, is a natural antibiotic. Renamed siderocalin to reflect its newly revealed function as a lipocalin that binds siderophores, the protein is the host's scavenger of the siderophores produced by invading bacteria. The first hint of its activity came from overproduction of the protein in a heterologous *E. coli* expression system, as the bacteria failed to grow under Fe limiting conditions, and the protein copurified with a bright red chromophore. Subsequently, experiments with siderocalin knockout mice ([Flo et al., 2004](#)) supported an antibiotic function for the lipocalin, as mice deficient in the protein are more susceptible to infection by bacteria that are dependent upon iron uptake by the siderophore enterochelin. These results led the authors to suggest that siderocalin is a component of the innate immune system and the acute phase response to infection.

In the current paper, the authors have extended their work and demonstrate that in addition to siderophores from enterobacter, siderocalin is able to bind those produced by mycobacteria as well. While this makes biological sense in that the specificity is for compounds of similar function, it is not obvious from a chemical perspective how this is achieved, given the dissimilar structures of the siderophores. The enterobacter siderophores that siderocalin binds might be roughly described as tripartite structures with a central ring of varying size, three identical spokes radiate from the central ring. Carboxymycobactins, the mycobacteria siderophores, are tripartite as well, but each of their three regions is unique; they lack both the 3-fold repeating motif and the central ring ([Figure 1](#)). The recessed binding site of siderocalin is able to accommodate the tripod-like structures of enterochelins with subsites for each of the identical feet ([Figure 2](#)). These

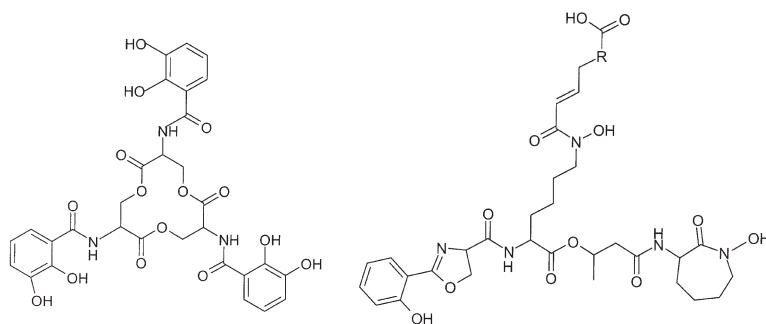


Figure 1. The Siderophores Enterochelin (Left) and Carboxymycobactin-S (Right)

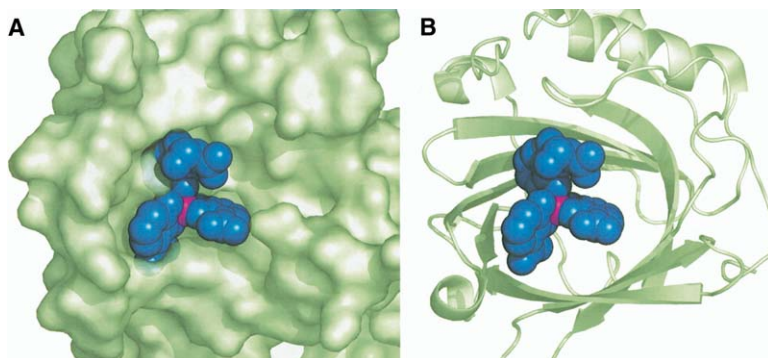


Figure 2. The Tripartite Binding Cavity of Siderocalin with Ferric Enterochelin Hydrolysis Products (Blue)

The Fe is in pink and protein (1L6M) is in white (A, surface rendering; B, backbone illustration).

same three subsites host specific parts of the asymmetric carboxymycobactins.

Perhaps even more remarkable than this broad specificity is the fact that it is not a consequence of a malleable binding site that adjusts itself to accommodate different ligands; only two amino acids (of the ~25 that line the cavity) are observed with different rotamer conformations in the various siderocalin-siderophore structures described. This is in stark contrast to the structural basis for the “directed promiscuity” of the pregnane X receptor (PXR) (Watkins et al., 2001, 2003), the binding site of which can expand or “breathe” to adapt to different ligands. Thus PXR is able to upregulate the expression of drug-metabolizing enzymes in response to the binding of structurally diverse xenobiotics. In the PXR isolated ligand binding domain, a single ligand can be observed in three distinct orientations. In contrast, although the siderophores are not tightly fixed in the siderocalin binding site (as judged from the appearance of the electron density), they are trapped in a single orientation primarily by electrostatic and cation- π interactions.

Given the large number of sequences that are associated with the lipocalin superfamily, and the fact that for many of these proteins a function remains to be ascribed, the work of Holmes and colleagues (2005) sug-

gests new avenues to be considered for function, and is another remarkable illustration of just how different the lives of members of the same superfamily can be. At the same time, it is a sobering reminder of the complexity of the task of inferring biological function from structure.

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Selected Reading

- Holmes, M.A., Paulsene, W., Jide, X., Rattledge, C., and Strong, R.K. (2005). *Structure* 13, this issue, 29–41.
- Faraldo-Gomez, J.D., and Sansom, M.S. (2003). *Nat. Rev. Mol. Cell Biol.* 4, 105–116.
- Flo, T.H., Smith, K.D., Sato, S., Rodriguez, D.J., Holmes, M.A., Strong, R.K., Akira, S., and Aderem, A. (2004). *Nature* 432, 917–921.
- Goetz, D.H., Holmes, M.A., Borregaard, N., Bluhm, M.E., Raymond, K.N., and Strong, R.K. (2002). *Mol. Cell* 10, 1033–1043.
- Watkins, R.E., Maglich, J.M., Moore, L.B., Wisely, G.B., Noble, S.M., Davis-Searles, P.R., Lambert, M.H., Kliewer, S.A., and Redinbo, M.R. (2003). *Biochemistry* 42, 1430–1438.
- Watkins, R.E., Wisely, G.B., Moore, L.B., Collins, J.L., Lambert, M.H., Williams, S.P., Willson, T.M., Kliewer, S.A., and Redinbo, M.R. (2001). *Science* 292, 2329–2333.