Murphy Lab - Principal Investigator

Michael Murphy
Professor

Email: Michael.Murphy [at] ubc.ca
Office Telephone: 604-822-8022
Office: LSC 4553

Address:
Dept. of Microbiology and Immunology
2350 Health Sciences Mall
Life Sciences Centre
Vancouver, BC V6T 1Z3
Canada

Research Interests

Many essential biological processes rely on the special catalytic properties of iron and thus this element is a required mineral nutrient for most organisms from bacteria to mammals. In key environments, growth of microorganisms is limited by the availability of iron. For example, humans have several systems that sequester iron to limit its availability to invading pathogens that are referred to as nutritional immunity. We are investigating systems used by bacterial pathogens such *Staphylococcus aureus* to overcome iron restriction and cause disease.

Many regions of the open ocean are iron limited receiving periodic inputs from terrestrial dust or deep water upwelling. A central focus my research is the investigation of the mechanism of iron uptake and storage systems in bacteria, yeast and diatoms employed to succeed in iron poor environments. We are combining structure determination by x-ray crystallography with biochemical and microbial approaches to define the molecular basis of iron uptake and storage.
(a) Iron uptake by the human pathogen *Staphylococcus aureus*

*S. aureus* is a Gram-positive bacterium that causes a wide range of debilitating and life-threatening infections. Furthermore, it is one of the most commonly acquired bacterial infectious agents in hospitals. The spread of multi-drug resistance in *S. aureus* (exemplified by MRSA strains, or the methicillin resistant “superbugs?”) has highlighted the imperative need for new therapeutics. *S. aureus* iron uptake systems employ two distinct mechanistic strategies. The first involves the use of receptors specific for host iron sources such as hemoglobin and transferrin. We are investigating the iron surface determinant (Isd) system to understand how heme is extracted from hemoglobin and iron is released for bacterial use. The second strategy is the secretion of small molecules termed siderophores that are high affinity iron chelators after which the iron laden siderophores are reabsorbed. Our aims are to understand the mechanisms of biosynthesis of the two siderophores produced by *S. aureus* as well as to define the recognition determinant of the siderophore-iron uptake receptors. Both the Isd and siderophore based systems are required for full virulence of the pathogen and may be exploited therapeutically.

The overall structure of the *Staphylococcus aureus* IsdA NEAT domain haem complex.
The overall structure of the *Staphylococcus aureus* IsdE-heme complex. A schematic representation of IsdE illustrates the bi-lobed architecture of the protein. Propionate stabilizing helix-1 is represented in orange.
The overall structure of the HtsA-staphyloferrin A complex. The open structure of HtsA with Staphyloferrin A shown in the binding pocket as sticks with carbon, nitrogen, oxygen and iron shown in grey, blue, red, and orange, respectively.
The structure of *Staphylococcus aureus* IsdG-hemin. Superposition of apo-IsdG (cyan) and IsdG-hemin (yellow). The loop of IsdG-hemin structure that is disordered in the apo-IsdG structure is colored red.

(b) Iron uptake by the human pathogen Campylobacter jejuni

C. jejuni is a commensal bacterium in poultry and is a leading cause of food borne illness in the developed world. C. jejuni requires low oxygen concentrations for growth as is found in the site of infection, the human gut. The iron uptake systems in C. jejuni differ from those found in E. coli and some have been shown to be required for colonization in a chick model. We have
determined crystals structures and demonstrated iron binding by three of these iron uptake proteins found in the periplasm. C. jejuni growth studies of inactivation mutants are being used to show the role of each protein in iron uptake under iron limited conditions. We aim to understand how these proteins work together to obtain the iron necessary to support bacterial colonization of intestinal epithelium.

The crystal structure of haem-bound ChaN from *Campylobacter jejuni*. (a) Monomeric and (b) dimeric holo-ChaN enclosing two cofacial heme molecules. (c) 2Fo?Fc electron density of the ChaN Tyr148?heme interaction, contoured at 0.8?.

(c) Iron storage by ferritin in the diatom Pseudonichia multiseries

Inside a typical cell, surplus iron is stored by ferritin, a protein that oxidizes Fe(II) to form a Fe(III) mineral inside a nanocage constructed from 24 subunits. The presence of ferritin is associated with the dominance of pennate diatoms such as Pseudonichia multiseries, over centric diatoms that lack ferritin in iron fertilized oceanic blooms. We have determined the crystal structure of P. multiseries ferritin (PmFTN) and have shown that this ferritin is phylogenetically distinct from those found in all other branches of life. Interest in this phenomena stems from the fact that diatoms are unicellular photosynthetic organisms that play a major role in global primary production and carbon sequestration into the deep ocean. Indeed as detailed below the ferroxidase and mineralization sites of PmFTN are unique implying a novel mechanism of iron storage.
Tertiary crystal structure and ferroxidase site of Pseudo-nitzschia multiseries ferritin. a, b, Crystal structure of the recombinant P. multiseries ferritin: a, multimer (24mer); b, monomer. Iron atom (pink) positions are according to the crystal structure. c, Ferroxidase site A is occupied by a water molecule (grey sphere). One iron atom (pink sphere) occupies site B at full occupancy. A difference anomalous dispersion map around the iron atoms is contoured at 3Å.

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