Research Statement
Understanding the molecular basis of bacterial pathogenesis with an emphasis on characterizing the role of bacterial toxins in the disease process.

Bacteria are a highly successful group of single-cell organisms that have evolved to colonize and grow in many natural environments once considered too extreme for any form of life. Humans are invariably exposed to and colonized by bacterial organisms living within the biosphere, and these interactions can be viewed as beneficial, harmful or benign with regard to human life. Many of the bacteria that live on the external surfaces of humans perform beneficial and sometimes essential functions required for our wellbeing.

Bacterial pathogens are those organisms which can cause disease of humans. In addition to those bacteria which are obligate pathogens of humans, bacteria such as *Staphylococcus aureus* may exist as a commensal organism on the skin and nasal tissues, initiating disease only when normal barriers have been breached. Bacteria are able to produce disease because they possess certain structural, biochemical, or genetic traits (referred to as virulence determinants) that render them pathogenic. The outcome of infection by a bacterial pathogen is dynamic, depending of many factors, such as the route of entry, the virulence of the bacteria, the number of bacteria encountered during the initial exposure, and the immune status of the host.

**Botulinum Neurotoxins**

Botulism is a rare and distinctive disease – the hallmark of which is a descending flaccid paralysis with a long duration of illness; and results from exposure to botulinum neurotoxins (BoNTs). BoNTs are the most potent bacterial toxins known and share classical A-B structure-function properties. BoNT potency derives from the remarkable selectivity of the toxins for peripheral a-motor neurons and the efficient cleavage of one or more neuronal SNARE proteins (SNAP-25, Syntaxin 1A and Synaptobrevin-2). Cleavage of neuronal SNARE proteins prevents neurotransmitter release through inhibition of synaptic vesicle exocytosis. BoNTs are produced as single-chain molecules that undergo post-translational proteolytic cleavage to form a di-chain molecule composed of a catalytic light-chain (LC, ~50,000 kDa) and a heavy chain (HC, ~100 kDa) component linked through a single disulfide bond. The HC component contains two functional domains: an N-terminal translocation domain (HCT, ~50 kDa) and a C-terminal receptor binding domain (HCR, ~50 kDa) (Figure 1). BoNTs are divided into seven distinct serotypes (A, B, C, D, E, F, and G) based on their antigenic specificity. While experimental evidence suggests humans are sensitive to all serotypes, natural intoxications are associated with serotypes A, B, E, and F. In addition is widely used as a therapeutic agent for the treatment of a
range of neurological disorders in humans including dystonias, hyperhydrosis, cerebral palsy and pain management.

**Neurotransmission**

Synaptic transmission is initiated when an action potential triggers neurotransmitter release from a presynaptic nerve terminal. An action potential induces the opening of Ca2+ channels present on the presynaptic membrane, and the resulting Ca2+ transient stimulates synaptic vesicle (SV) exocytosis and neurotransmitter release. Most neurons form >500 presynaptic nerve terminals that are often separated by large distances from the neuronal cell body. Action potentials, initiated in the neuronal cell body, travel to the nerve terminals where they are transformed into synaptic secretory signals. Synaptic vesicles (SVs), harboring the neurotransmitter cargoes, undergo a unique trafficking cycle within the nerve terminal that can be divided into sequential steps (Figure 2): (1) SVs are loaded with neurotransmitters and cluster in front of the active zone, (2) SVs are docked to the presynaptic membrane and primed for exocytosis, (3) Ca2+-triggered fusion-pore opening results in neurotransmitter release, SV endocytosis and subsequent reloading of neurotransmitter for a second round of release.

![Fig. 2](image)

**Botulinum Neurotoxin Mode of Action**

BoNTs act at femtomolar concentrations and are specific for the presynaptic membranes of α-motor neurons. However, mechanisms of BoNT neuronal binding and subsequent trafficking remain poorly defined. To explain these observations, Montecucco developed the longstanding “dual receptor” model (Figure 2). BoNTs initially associates with the presynaptic membrane of
α-motor neurons through interaction with a protein- or lipid-linked oligosaccharide, concentrating the toxin at the site of synaptic vesicle exocytosis. (4) Fusion of SVs to the membrane exposes a second protein co-receptor to which the toxin can now bind. As the SV-toxin complex undergoes endocytosis (5), the SV lumen is acidified (6) resulting in a conformational change in the toxin driving the transcytosis of the light chain (LC) subunit into the host cytosol (7). The LC is then able to cleave its SNARE protein substrate (8) resulting in inhibition of SV exocytosis and disruption of neurotransmission (9).

Projects
At present there are two projects within the laboratory:

1. **Characterize the interaction of botulinum neurotoxins with neuronal receptors.** The exceptional potency and specificity of the clostridial neurotoxins raises unique questions about the nature of their receptor(s) and the mode of their membrane binding. Recently, it has been shown that certain BoNT serotypes associate with α-motor neurons via interaction with two co-receptor molecules: namely polysialogangliosides and synaptic vesicle proteins. We wish to understand at the molecular level how this family of toxins recognizes both co-receptor molecules. This will be achieved using a wide range of approaches including protein chemistry, molecular biology, advanced microscopy, and structural biology.

2. **Understand the mechanisms of botulinum neurotoxin binding, entry and transcytosis across epithelial and endothelial cells.** BoNTs can enter the body by several different routes, but the most common of these is the gastrointestinal system. Thus, most cases of intoxication are due to ingestion of food contaminated with preformed toxin or ingestion of food contaminated with bacteria that can produce toxin in the gut. In either case, orally ingested BoNTs must pass through several biological barriers to reach peripheral cholinergic nerve endings, which are the target cells for toxin action. It has been shown that the first event is toxin absorption in the stomach and upper small intestine. If BoNT were to enter and remain within cells of the gastrointestinal system, this would prevent it from reaching peripheral cholinergic nerve endings. Thus, the series of steps that underlie toxin movement at the level of individual gut cells cannot be identical to the steps that underlie toxin movement within neurons. After absorption from the digestive tract, BoNTs enter the lymphatic system, from where they are transported to the bloodstream and disseminated throughout the organism. Finally, the toxin exits the microvasculature via unknown mechanisms to reach the peripheral cholinergic nerve endings. We wish to understand these differences at the molecular level by identifying the receptor molecules present on epithelial and endothelial cells as well as determine the host cell components involved in toxin trafficking.