VACCINE AND INFECTIOUS DISEASE DIVISION

The Power of Structural Biology to Elucidate Immune System Function with Roland Strong

Mindy Miner

Dr. Roland Strong went to graduate school at Harvard University and did a postdoctoral fellowship at Caltech. He then joined the Hutchinson Center faculty in the nascent crystallography program. Since coming to Seattle, he has learned to

As of this year, over 88,000 biological molecular structures have been deposited in a federally funded, online protein data bank. A majority of these protein structures were solved using the laboratory technique x-ray crystallography. This powerful tool works just as the name suggests: scientists purify protein, which, under the right conditions, will grow into crystals. The protein crystals are then shot with an x-ray beam and the resulting x-ray diffraction pattern is translated into a protein structure.

Dr. Roland Strong, joint member of VIDD and the Basic Sciences Division, specializes in solving protein structures via x-ray crystallography and using biophysics and molecular methods for then characterizing molecule-molecule interactions. The multi-disciplinary nature of Strong's field of study facilitates cross-divisional collaborations and exemplifies the interconnected spirit of VIDD.

"I started my career in antibodies," Strong said. "And I've been very interested in virology and various aspects of the immune system, particularly receptor-ligand interactions that drive immune responses."

Strong is specifically interested in HIV immunogen-antibody binding properties, iron-scavenging antimicrobial pathways, and antiviral NK-cell signaling. Ultimately, he hopes to expand our understanding of these processes via structural molecular immunology.

scuba dive and recently had an encounter with the beloved North Pacific giant octopus. Both parties left unharmed.

An Ironic Story

Neutrophils are one type of 'first responder' innate immune cell that secrete antimicrobial proteins in response to infection. Strong's lab studies one such protein, NGAL, that is a member of a large family of proteins called lipocalins, which have a very telling "cup" structure for binding ligands. Despite detailed structural and biochemical analyses of NGAL, which has a comparatively large and obvious binding pocket, the Strong lab was unable to identify a ligand.

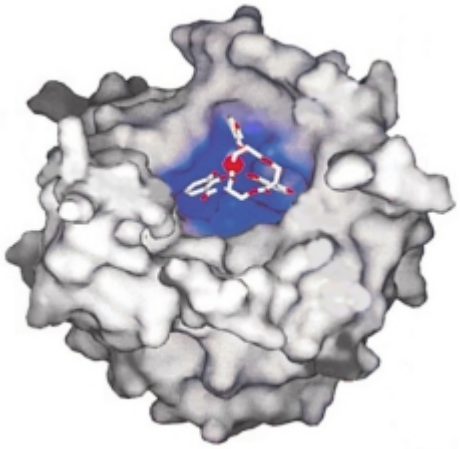
"It (NGAL) looked like a coffee mug," Strong said. "A deep cavity containing positively charged amino acids almost undoubtedly suggests a binding pocket for a negatively charged molecule."

Continuing their pursuit to identify and characterize NGAL molecular interactions, Strong's lab decided to switch from a viral expression system to a more efficient *E. coli* system for generating the large amount of protein needed for x-ray crystallography.

This protocol change resulted in serendipitously deciphering NGAL's function: the purified protein was now red, a color indicating the presence of iron. They identified the red ligand bound in the pocket: an *E. coli* siderophore-iron complex.

Almost every life form studied to date requires iron. In many environments, iron is a limited resource and therefore organisms employ adroit mechanisms to compete for the precious element. Siderophores (Greek for 'iron carrier') are exceptionally efficient in binding iron and many bacteria secrete these chemicals to bind iron and then deposit it back to the cell. Strong discovered that neutrophils secreted NGAL to prevent bacterial (in this case, *E. coli*) iron acquisition by sequestering the bacterium's own siderophore.

This was the first identification of an iron-scavenging antibacterial protein that didn't bind to iron, but actually bound the iron-siderophore complex. They renamed the protein Siderocalin.



A structural cartoon of siderocalin, which resembles a coffee mug. Siderocalin is gray; the view is looking down into the 'cup' region, which is colored blue. The siderophore-iron

complex (white with red) is bound inside the pocket.

Adapted from Goetz, et al. Mol Cell. 2002 Nov;10:1033–1043.

Moving Toward a Vaccine By Going in Reverse

Most successful vaccines have been made by following a relatively standard formula. Briefly, the disease-causing agent, such as a virus, is 1) isolated; 2) killed or attenuated (becoming an immunogen); and 3) administered to healthy individuals, who then make a strong adaptive immune response that is protective against infection. Unfortunately, despite 30 years of research, this method has heretofore been unsuccessful for HIV.

“Our goal is to develop a prophylactic humoral HIV vaccine so that when you vaccinate a healthy person, the antibody response is sufficient to prevent infection later on,” said Strong.

As opposed to the standard vaccine formula, Strong’s lab uses a novel ‘backward’ approach for HIV vaccine development called reverse vaccinology. There are a minute number of HIV-infected people who have lucked out because their bodies make anti-HIV antibodies that can control infection. His team analyzes these antibodies to determine why they work and what viral immunogens they recognize, and then designs recombinant immunogens that specifically re-elicit that antibody response.

“We don’t truly understand exactly how these antibodies really work: the specific mechanism by which they stop the virus. We have pieces of it, but we don’t have the whole story,” Strong said.

In collaboration with Seattle BioMed’s Dr. Leo Stamatatos, Strong is analyzing two antibodies, called b12 and 4E10, that are directed against certain regions of the HIV protein Env. They use detailed molecular and biophysics techniques to determine the binding kinetics and affinity, thermodynamic properties and conformational changes relating to the interactions between 4E10 and b12 with different Env immunogens. They are also studying how the parental forms of these antibodies interact with immunogens at the start of the immunization process.

In a related study, Strong and Stamatatos recently uncovered a potential reason why the recombinant form of a different Env immunogen fails to induce the broadly neutralizing capability of antibody b12. It turns out that during natural infection, Env actually affects b12 B-cell maturation, a multi-step process that ultimately leads to a person’s highly diverse antibody pool. Without this engagement, Env cannot elicit a productive b12 antibody response.

The capability to define the action/reaction dynamics of molecular interactions allows scientists to explain the big picture by understanding the small picture. Some of Strong's contributions include illuminating how in-depth antibody-immunogen interaction studies are crucial to the design of future HIV vaccines, discovering and characterizing novel innate immune strategies that regulate microbial infections via iron sequestration, and parsing out various NK- and T-cell receptor interactions with cognate ligands and target cells. Strong has developed a unique niche in VIDD where he uses structural biology and biophysics to address human health issues such as infectious disease and basic immune system function.

»**Roland Strong faculty page**

»**Strong Lab website**