# Polyvalent ligand trounces Shiga toxin

A multivalent carbohydrate ligand fashioned by researchers in Canada and the U.K. neutralizes Shiga toxin in vitro up to 10 million times more potently than univalent ligands do [*Nature,* **403,** 669 (2000)]. David R. Bundle, a professor of chemistry at the University of Alberta, led the eight-member team that synthesized the inhibitor.

Shiga toxin, produced by the bacterium *Shigella dysenteriae*, belongs to a family of toxins, including cholera, that are made up of two subunits. The so-called B subunit binds to susceptible mammalian cells, allowing the A subunit to gain entry and do the toxin's dirty work. A strain of *Escherichia coli* has picked up the gene for Shiga toxin, Bundle notes, expressing it as the "hamburger toxin" that's proven deadly in undercooked meat.

Shiga toxin's doughnut-shaped B subunit is made up of five identical monomers, each of which has three binding sites with varying affinities for cell-surface carbohydrates. The pentamer locks onto cells by gripping five or more carbohydrate ligands simultaneously. Bundle and his colleagues sought an inhibitor that would mimic this binding approach and latch tenaciously onto the toxin. In designing their molecule, they exploited the crystal structure of the *E. coli* toxin's B subunit complexed to an analog of its carbohydrate receptor.

Initially, Bundle's team designed a ligand they anticipated might have a high affinity for binding sites 11 Å apart on the subunit's monomers. That ligand consisted of two trisaccharide units connected by an 11-Å-long tether. But the results were disappointing—"almost useless actually," Bundle says. "Then my postdoc, Pavel I. Kitov, came up with the idea of attaching the trisaccharide dimer on each of five spokes on a core molecule [glucose] in the hopes of 'hitting' 10 binding sites on the five monomers." Because of its configuration, the new ligand was dubbed Starfish.

Starfish sticks like Velcro to each of the five B subunit monomers—but not as expected. Instead of binding to two sites per monomer, it binds only to one. Only five of Starfish's trisaccharide arms are engaged, leaving the other five free to bind another B subunit. The upshot is that Starfish is sandwiched between two subunits. "That mode of action differs from the one envisioned by rational design," Bundle notes.

Nonetheless, "the generation of a high-affinity inhibitor for Shiga toxin by rational design is a landmark achievement," says James Paulson, a molecular biologist at Scripps Research Institute, La Jolla, Calif. "Like Shiga toxin, microbial and eukaryotic carbohydrate-binding proteins (lectins) in general have low intrinsic affinities for their ligands, and many laboratories have pursued the design of multivalent ligands to increase molar affinities with notable lack of success."

George M. Whitesides, a professor of chemistry at Harvard University, calls the work "a spectacular example of polyvalency. It is also another signpost pointing to new classes of medicinal agents that act with targets on cell surfaces (and with oligomeric proteins)" via polyvalent interactions.

"It's a beautiful study," comments Laura L. Kiessling, a professor of chemistry at the University of Wisconsin, Madison. "The structural studies reveal that these potent ligands function by two mechanisms: They act by the chelate effect, and they cluster two receptor complexes. These results are highly significant."

More work is on Bundle's agenda. Antidotes to treat Shiga toxin in the gut are in clinical trials, but no compounds are on the horizon for treating the toxin after it enters the bloodstream, an event that can trigger kidney toxicity and death. "The Starfish molecule offers the potential for the design of a potent [injectable] drug" that can prevent such toxicity, Paulson suggests. Bundle's group is gearing up to produce Starfish in sufficient quantities to explore that possibility in animal models for "hamburger disease."

*Mairin Brennan*



**Starfish inhibitor**