Capturing Critical Sites in Disorder-To-Order Complexation to Manipulate Protein-Protein Interfaces

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Our team has a longstanding interest in tightly interacting toxin-antitoxin pairs, which typically have dissociation constants in the picomolar range and have co-evolved to impart high specificity of pairings. These bacterial toxin proteins possess useful activities in controlling bacterial cell growth, and we and others hypothesize they could have a role in new infection control approaches. The key to any such application, however, is the manipulation of this extensive high affinity protein-protein interface of toxin with its cognate antitoxin.

Our focus is on the family of toxins named ParE that are found in many pathogenic gram- negative bacteria. ParE toxins are potent inhibitors of DNA gyrase, and phenotypic outcomes are similar to gyrase-inhibiting fluoroquinolones antibiotics. These toxins interact with an antitoxin, named ParD, that keeps ParE from inhibiting gyrase. We are keenly interested in understanding the determinants of specificity of their interaction and probing how such interactions can be interrupted.

Using in vitro and structural approaches, we have determined that despite the large interface only a few C-terminal contacts are absolutely required for interaction of antitoxin with toxin. These map to a conformationally dynamic helix that is induced to form this secondary structure when interactions with ParE toxin are satisfied. Our recent studies have identified the minimal C-terminal peptide derived from the antitoxin that can successfully interact with toxin. Our on-going studies are validating if ParE toxin bound to a minimal antitoxin peptide is able to inhibit gyrase, and to assess what levels of competition would be required for these minimal peptides to block interaction with wild type antitoxin inside bacterial cells. Overall, we are providing new knowledge on disorder-to-order structural transitions resulting in highly specific and strong interactions, and excitingly, we have identified a simple means to potently interrupt this binding process that appears applicable throughout this TA system family of gyrase-targeting toxins.