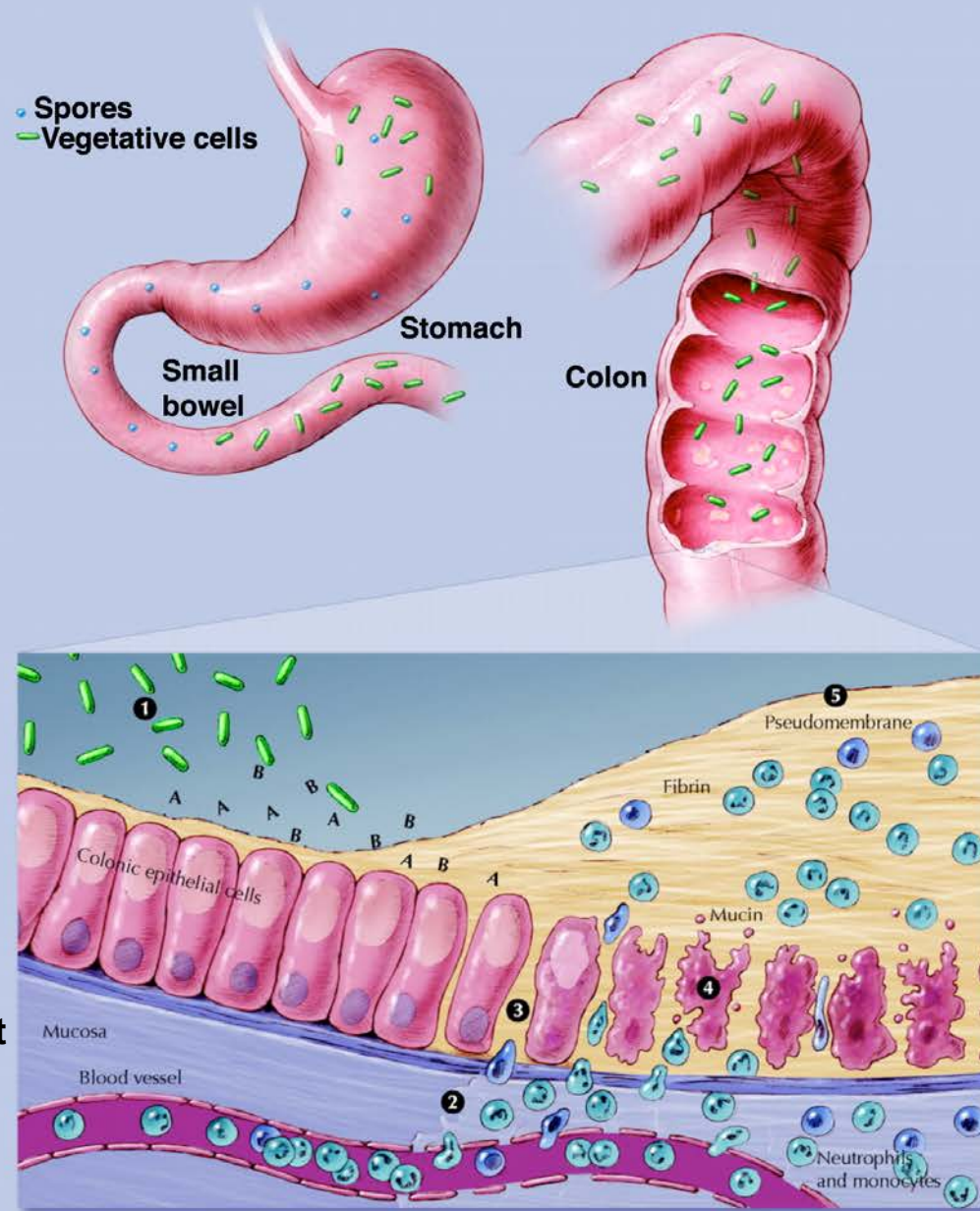


Structures and functions of *Clostridium difficile* TcdA and TcdB

D. Borden Lacy, Ph.D.
Vanderbilt University School of Medicine
Nashville, TN USA

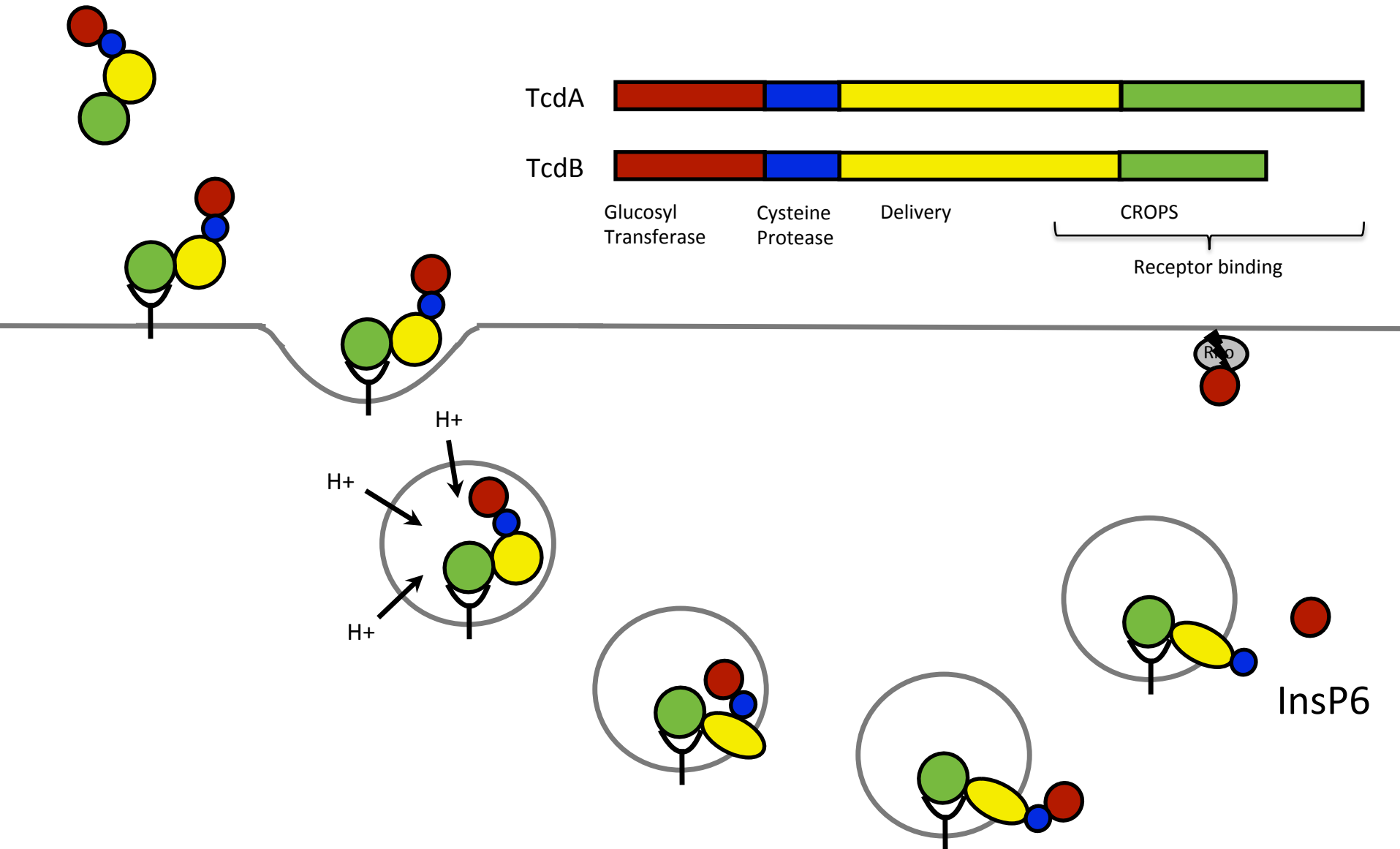
Pathogenesis of *C. difficile*-associated disease



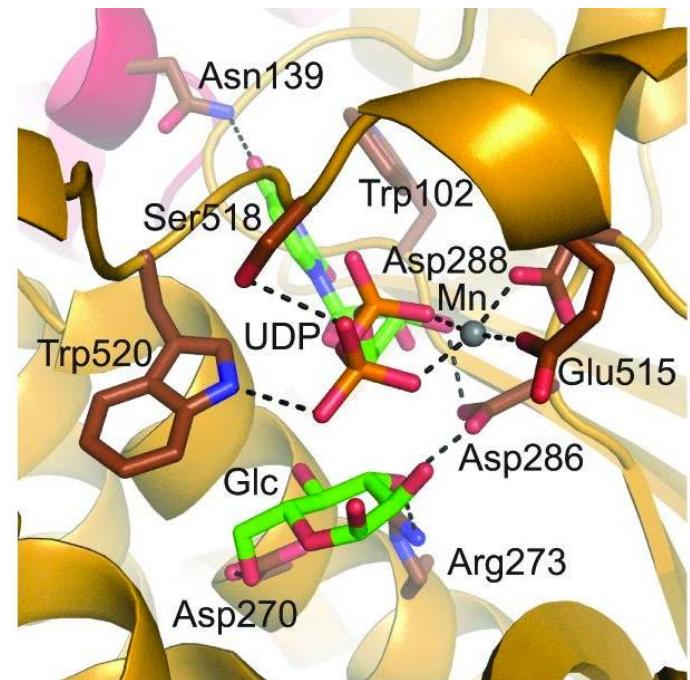
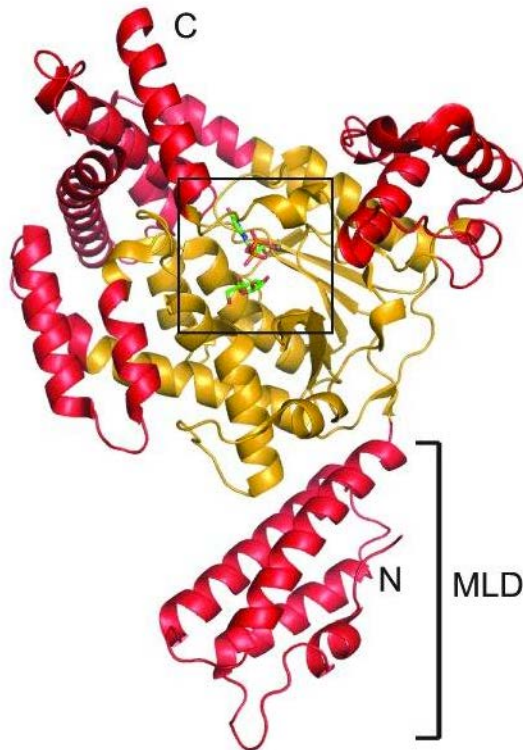
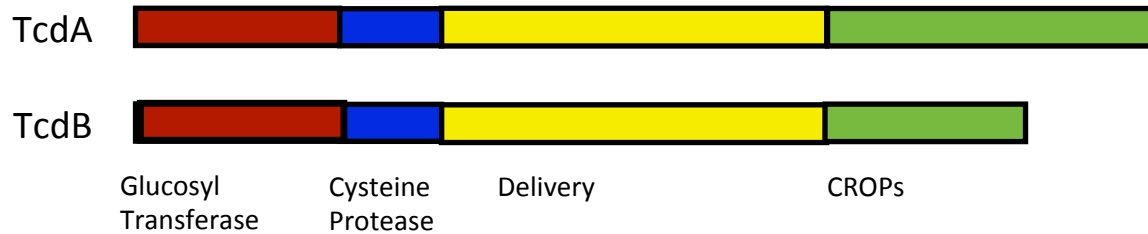
1. Toxin production.
2. TNF α , IL-8, vascular leakage, monocyte & neutrophil recruitment

3. Disrupt tight junctions
4. Cell death
5. Pseudomembrane formation

Mechanism of Host Cell Entry

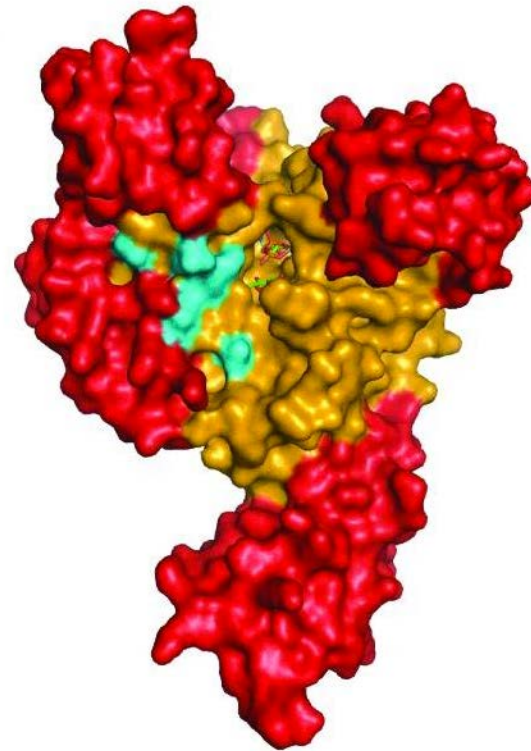
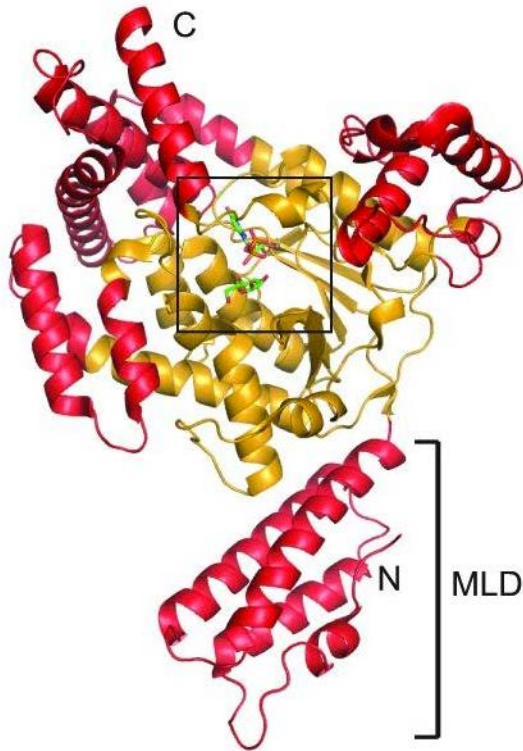


X-ray crystal structures of individual functional domains

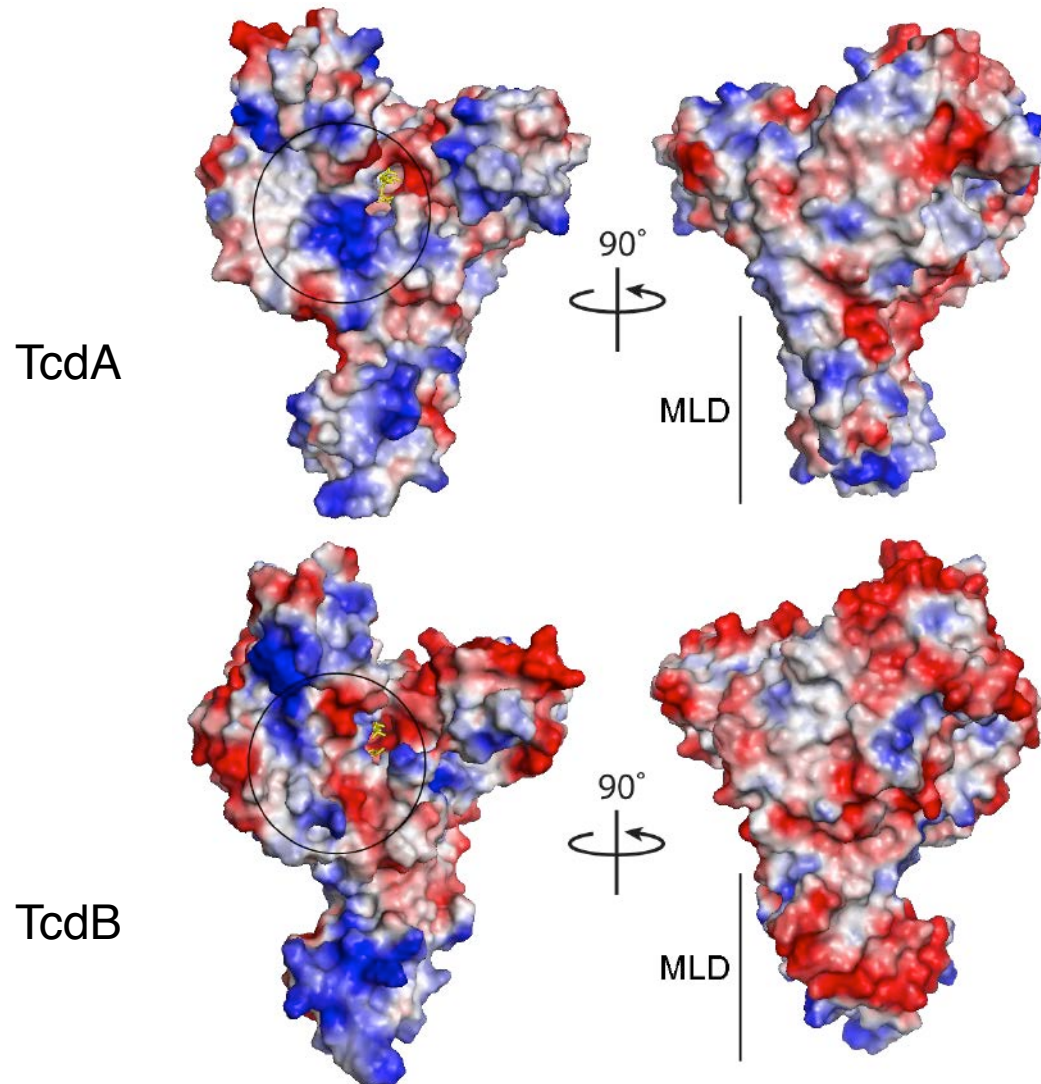


Reinert DJ, Jank T, Aktories K, Schulz GE.
J Mol Biol. 2005 Sep 2;351(5):973-81.

Defining the GTPase binding surface of the TcdB glucosyltransferase domain



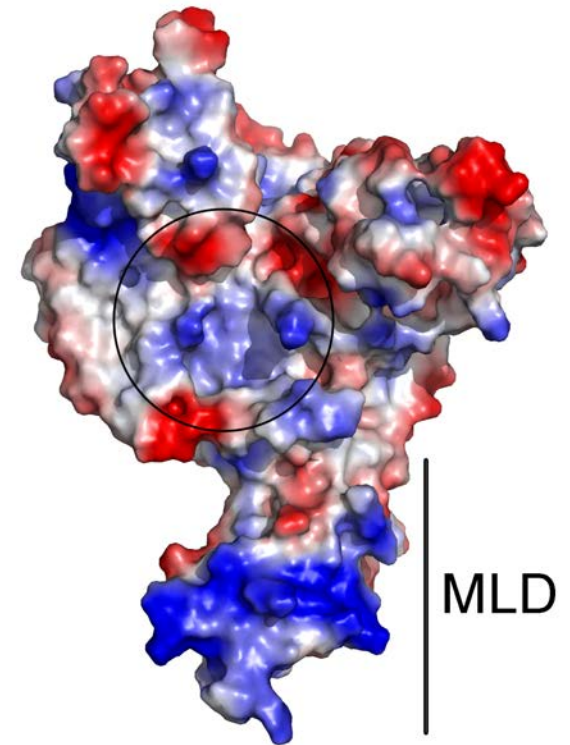
The proposed GTPase binding surface differs in TcdA



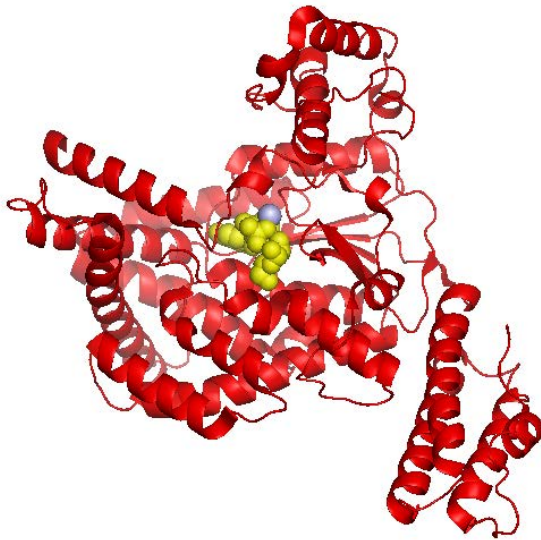
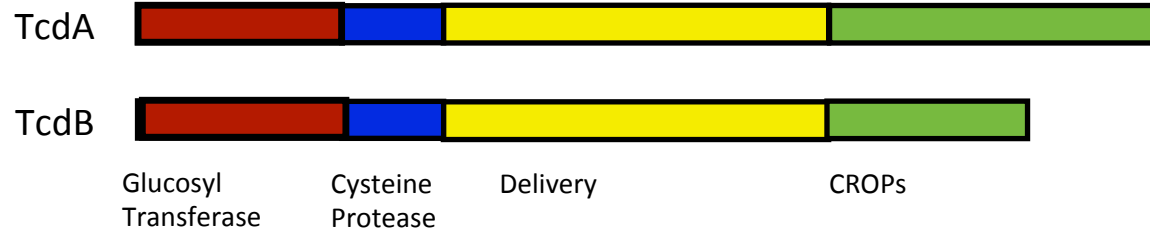
Variant TcdB GTDs

- Several *C. difficile* strains have mutations in the TcdB GTD that allow them to modify Ras family substrates
 - TcdB (630) – RhoA, Rac1, Cdc42
 - TcdB (8864) – Rac1, Cdc42, **Ral**, **Rap**, **Ras**
 - TcdB (1470) – Rac1, Cdc42, **Ral**, **Rap**, **Ras**

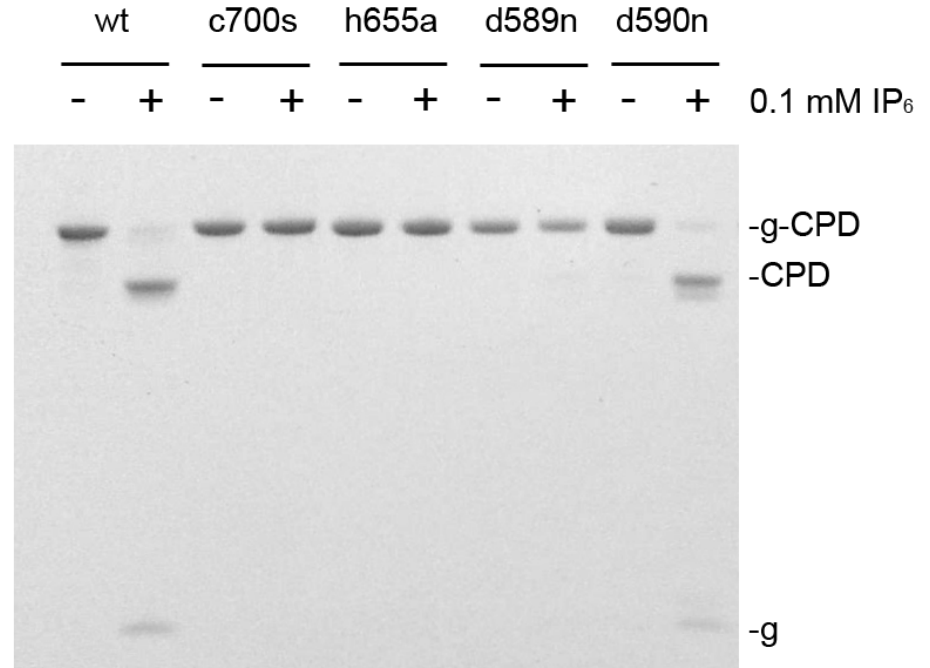
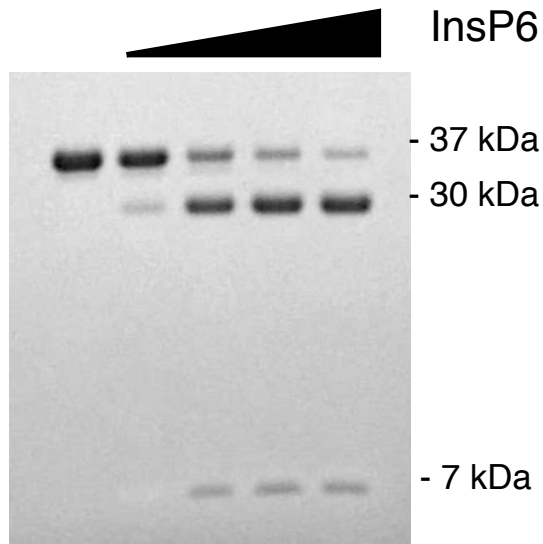
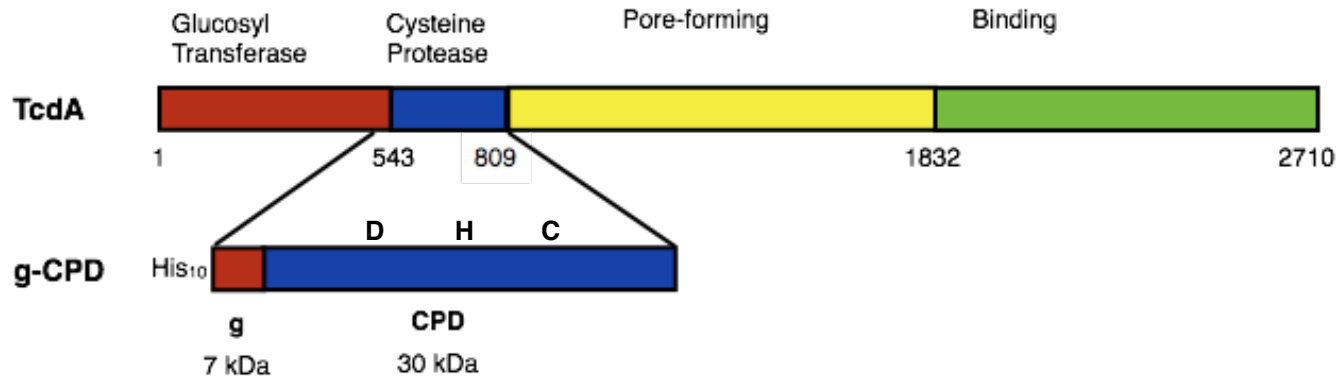
Homology model of
TcdB from strain 1470



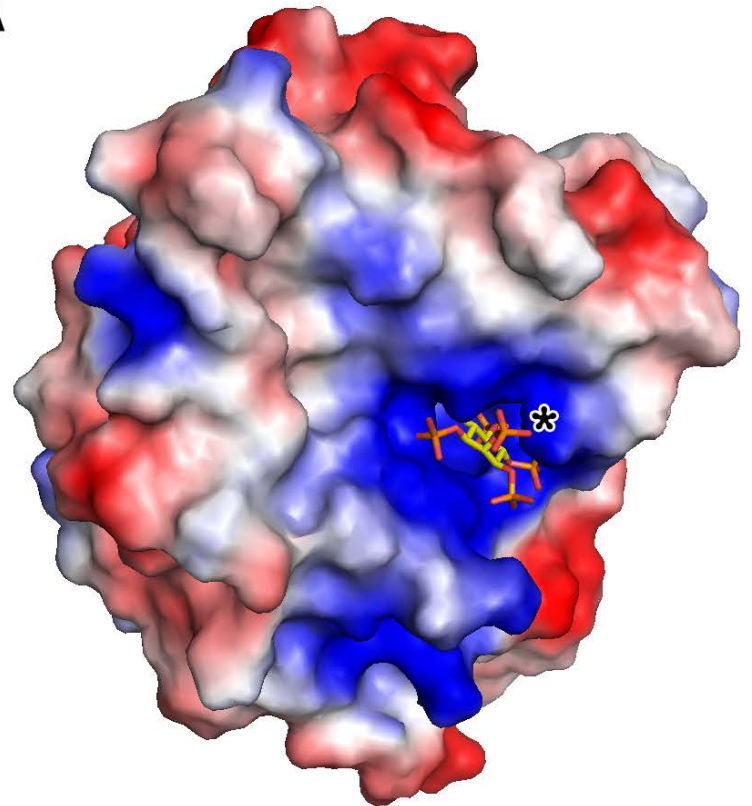
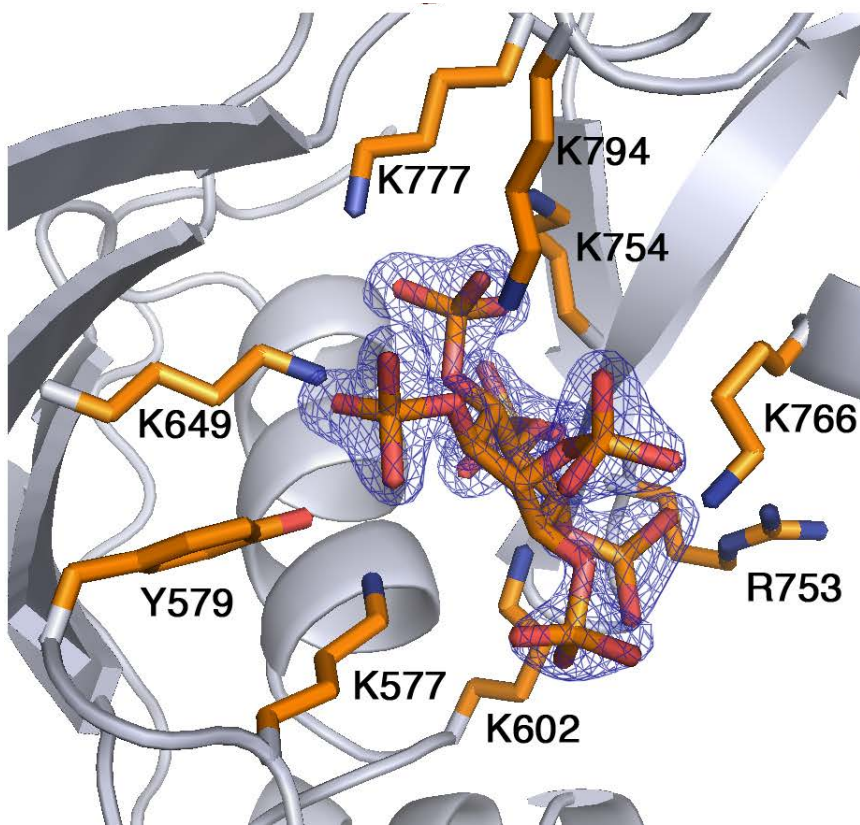
X-ray crystal structures of individual functional domains



The cysteine protease domain of TcdA

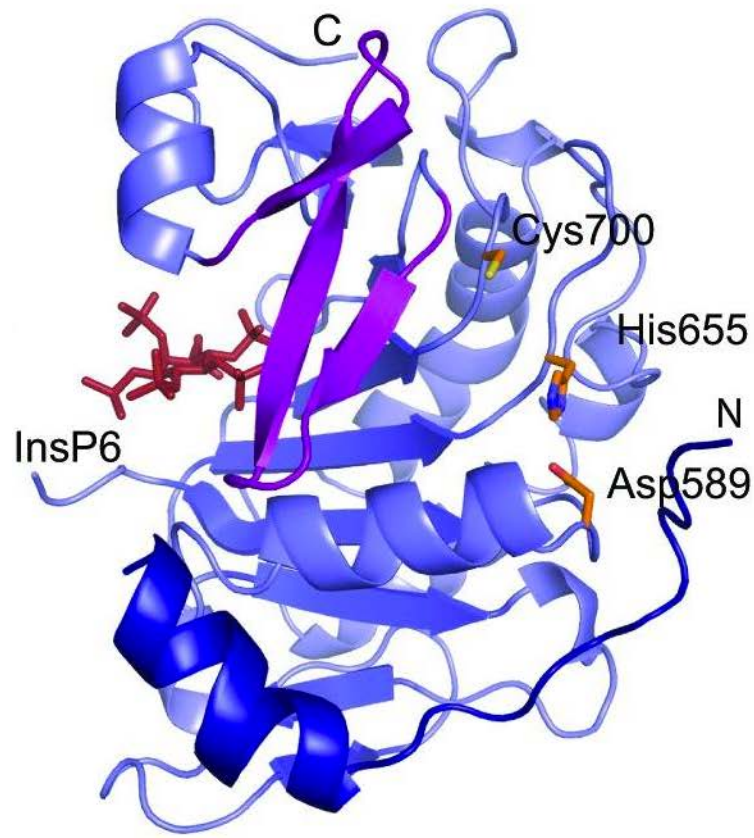


InsP6 binds a positively charged pocket



Structural homology between the cysteine protease domains of TcdA and TcdB

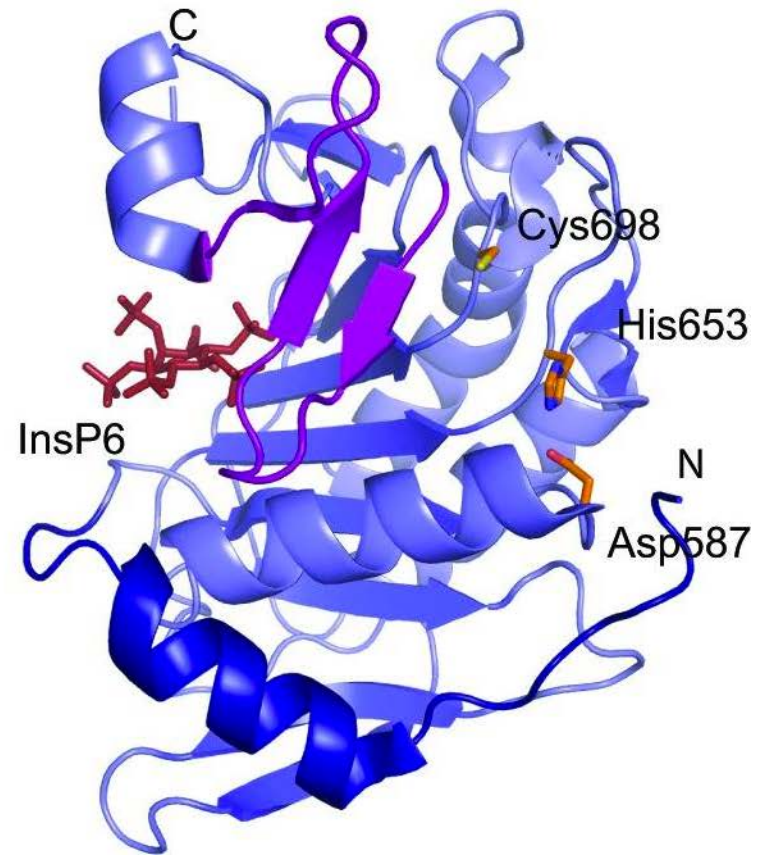
TcdA CPD



Pruitt *et. al.*

(2009) J.Biol.Chem. 284: 21934-21940

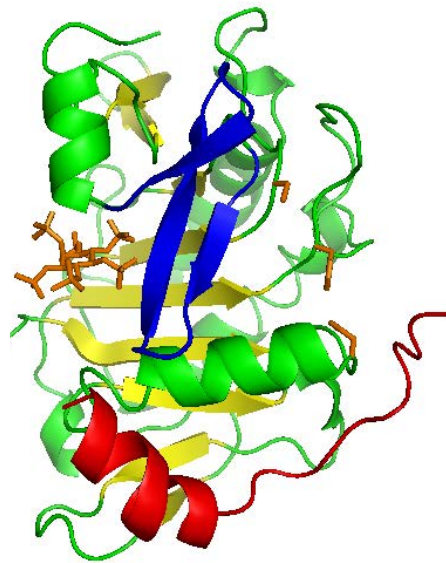
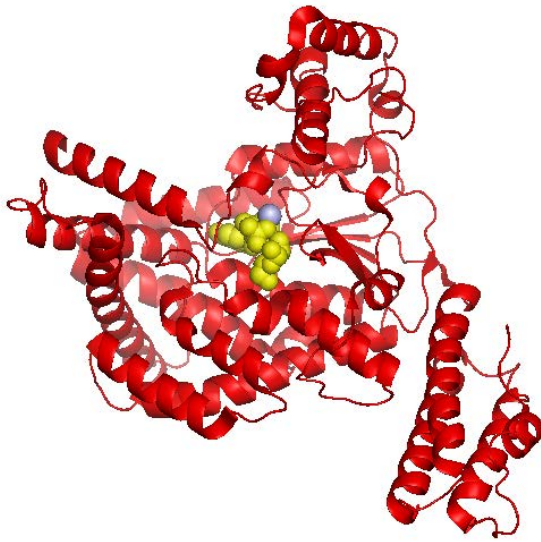
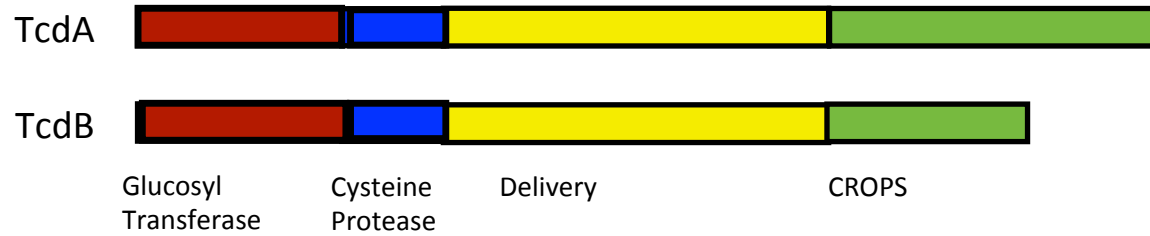
TcdB CPD



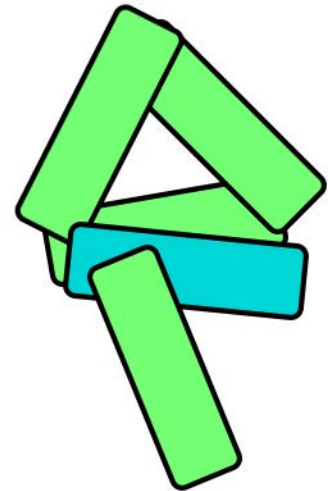
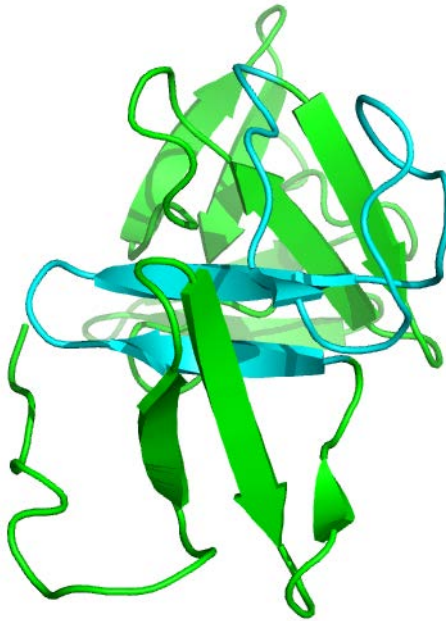
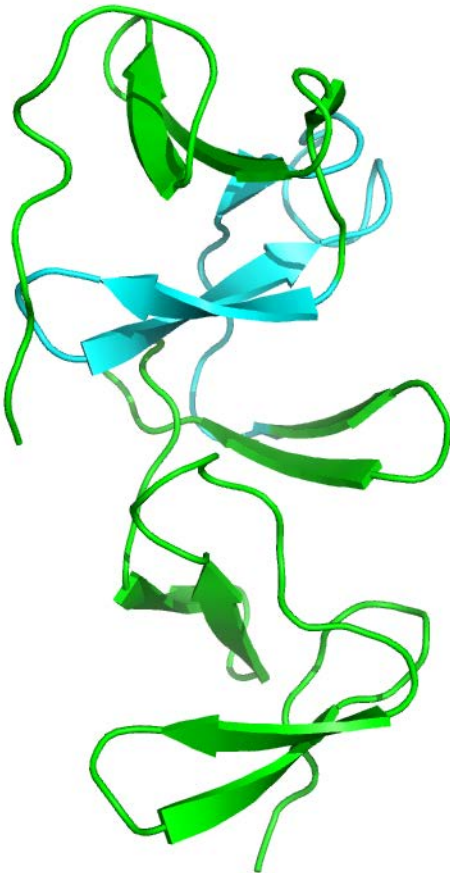
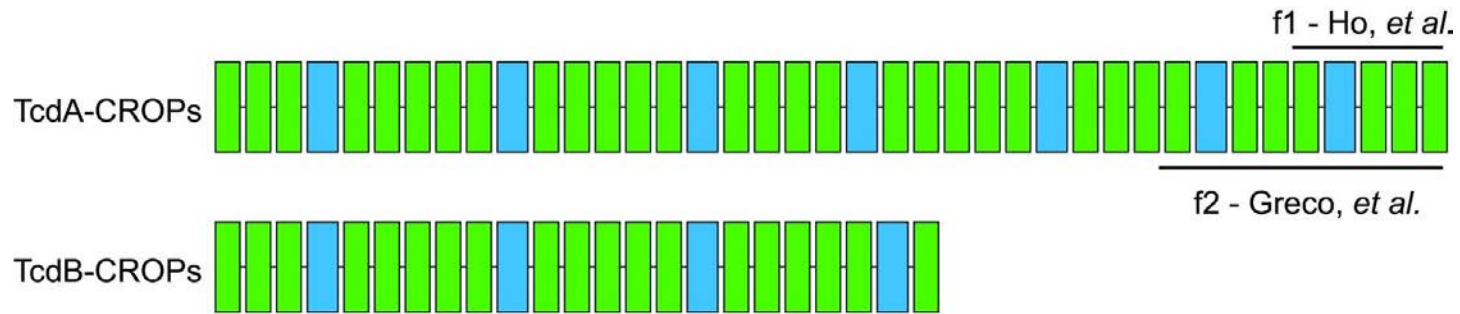
Puri *et. al.*

(2010) Chem.Biol. 17: 1201-1211

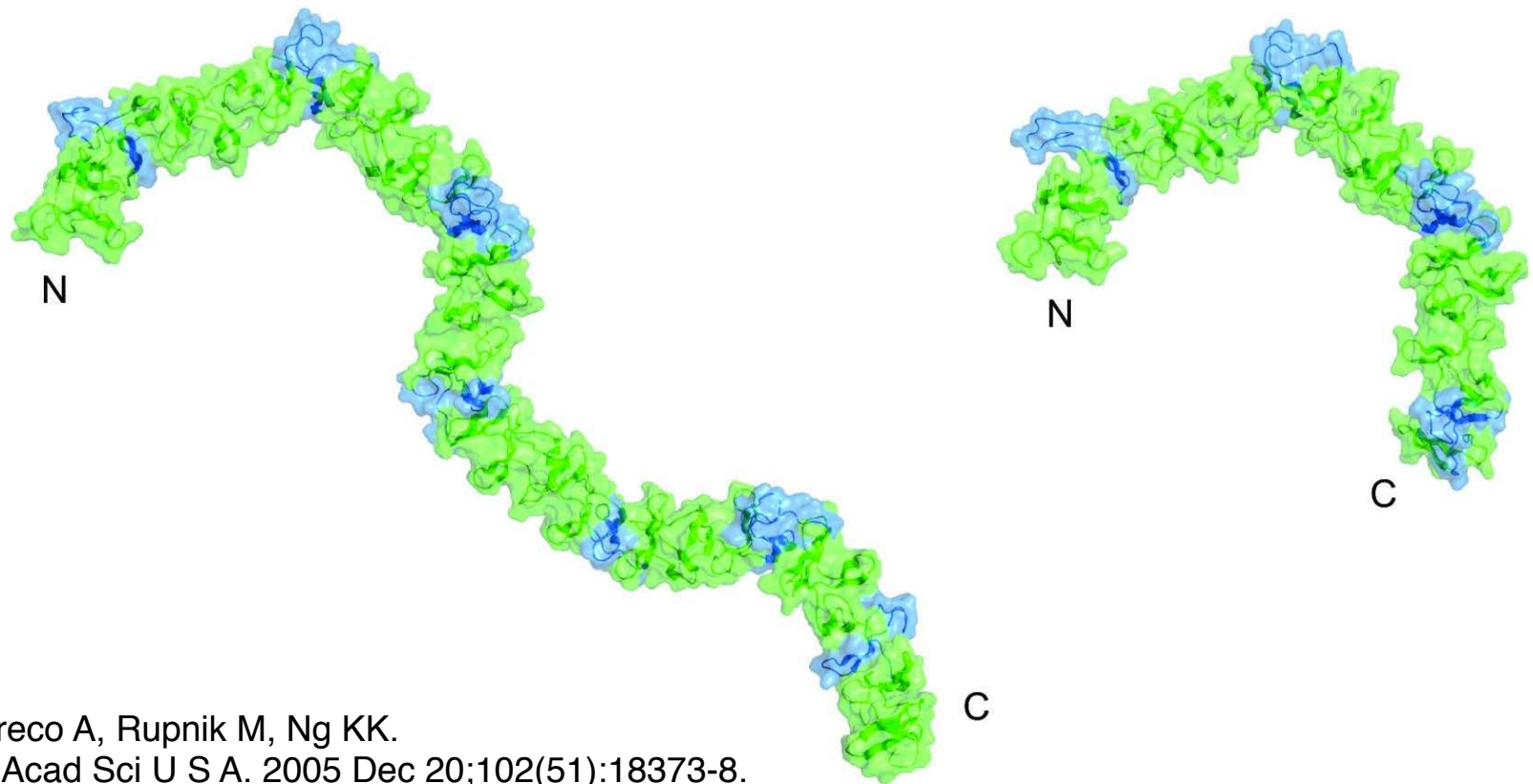
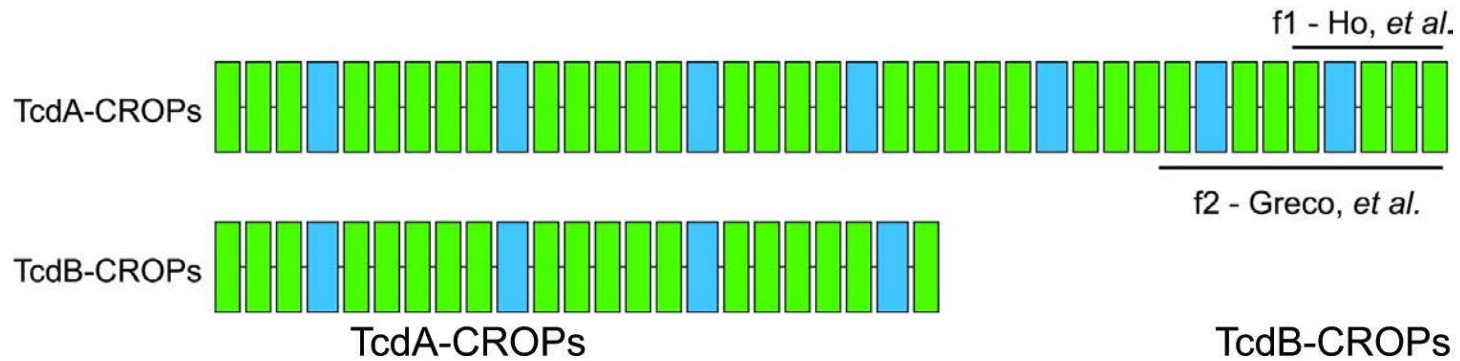
X-ray crystal structures of individual functional domains



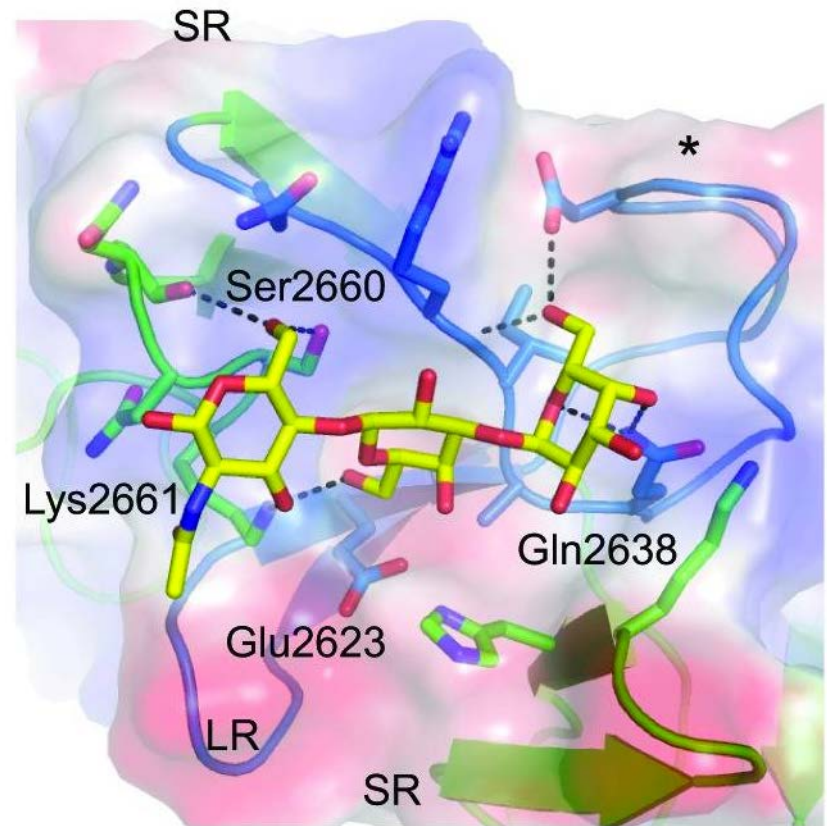
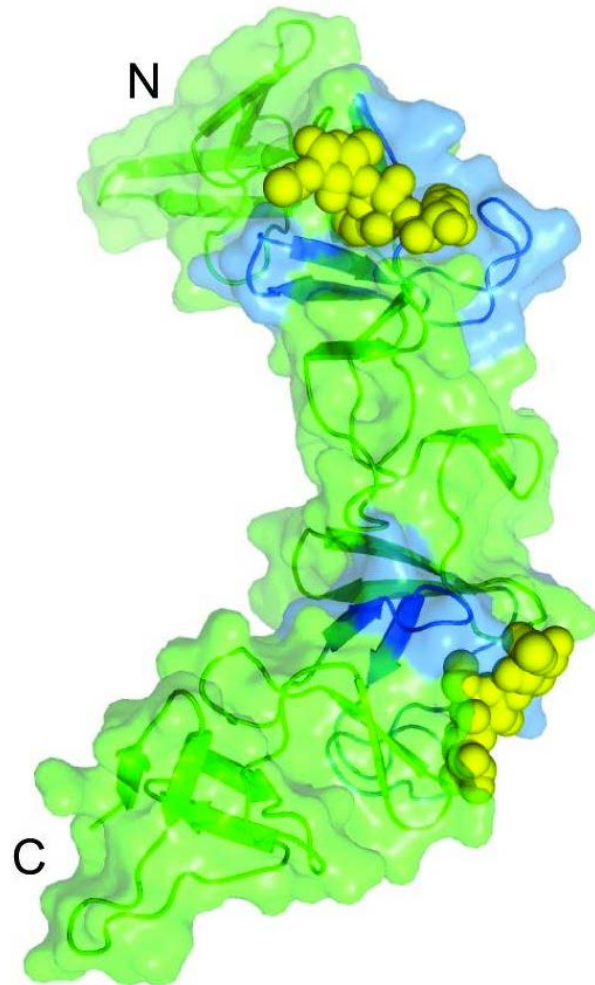
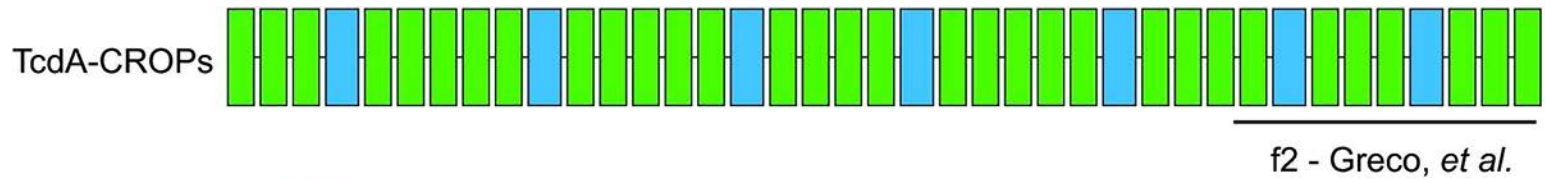
CROPs: Combined repetitive oligopeptides



CROPs: Combined repetitive oligopeptides

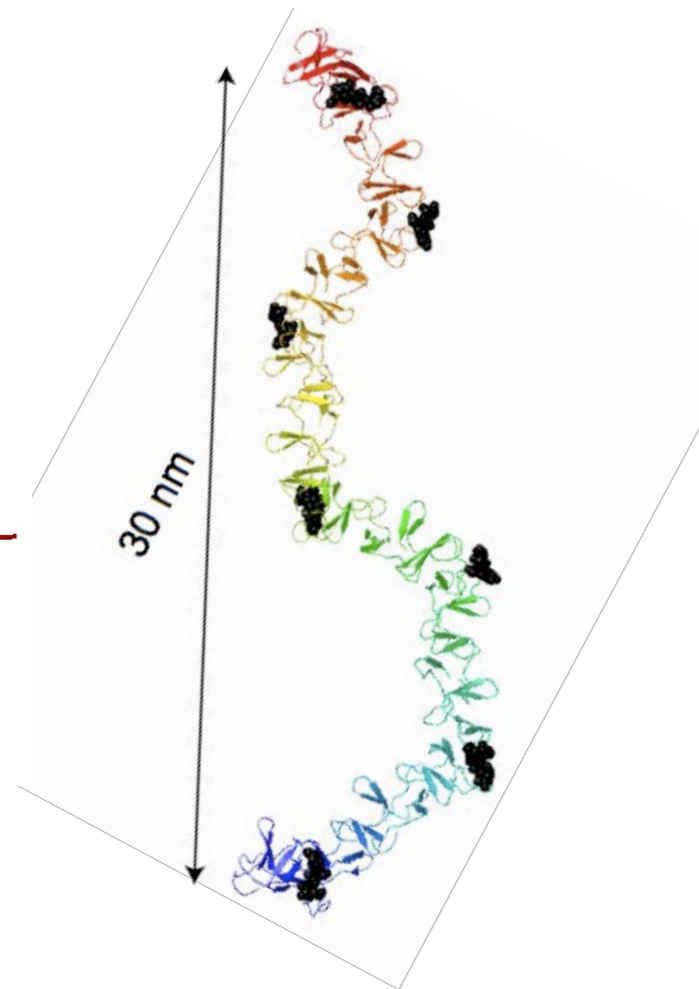
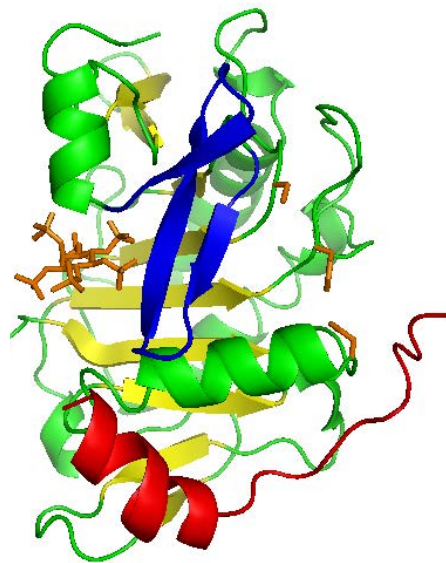
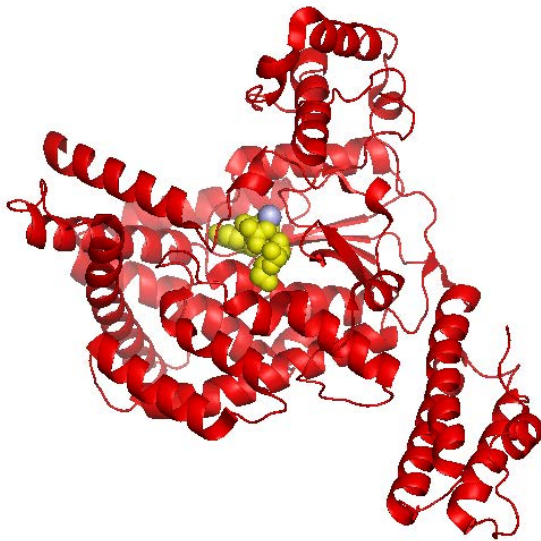
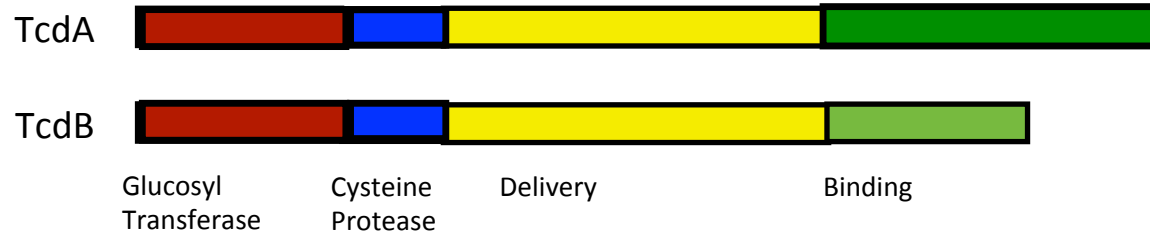


The TcdA CROPs bind carbohydrates



Greco A, Ho JG, Lin SJ, Palcic MM, Rupnik M, Ng KK.
Nat Struct Mol Biol. 2006 May;13(5):460-1.

How do the domains assemble to form the holotoxin?



Purification of TcdA and TcdB

Spin down dialysis sac cultures



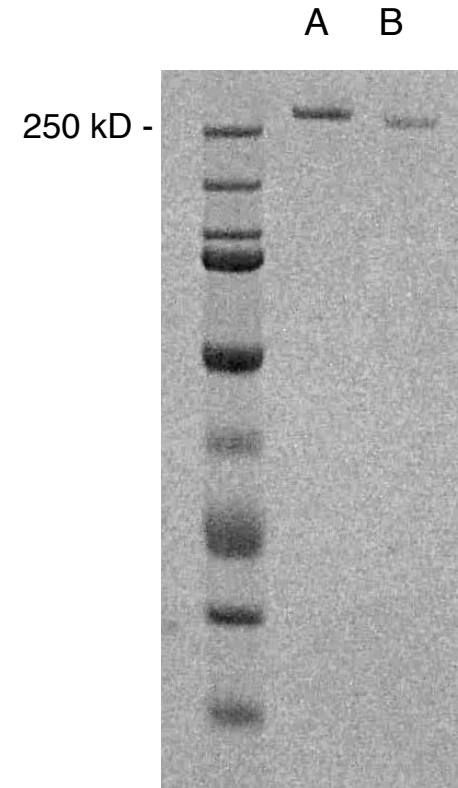
Filter supernatant



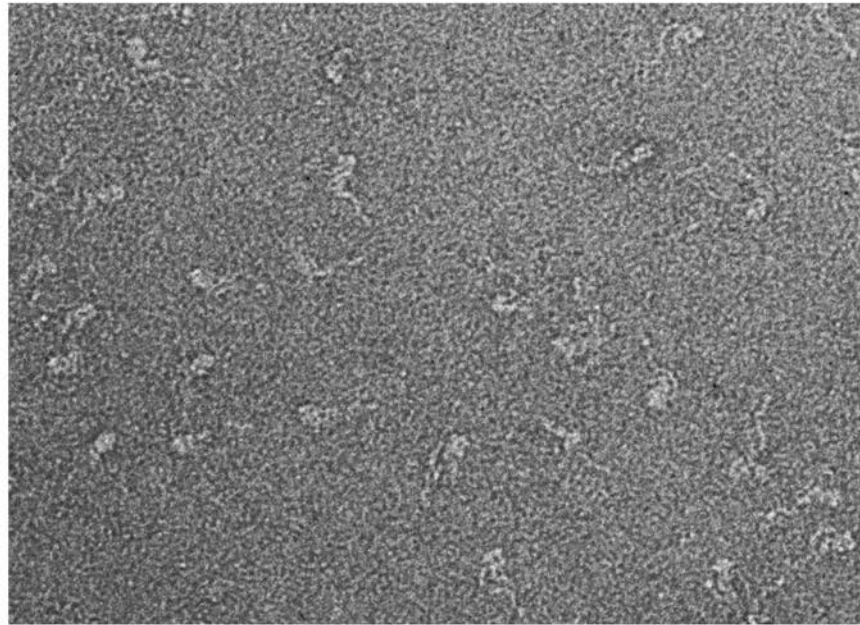
Anion exchange columns



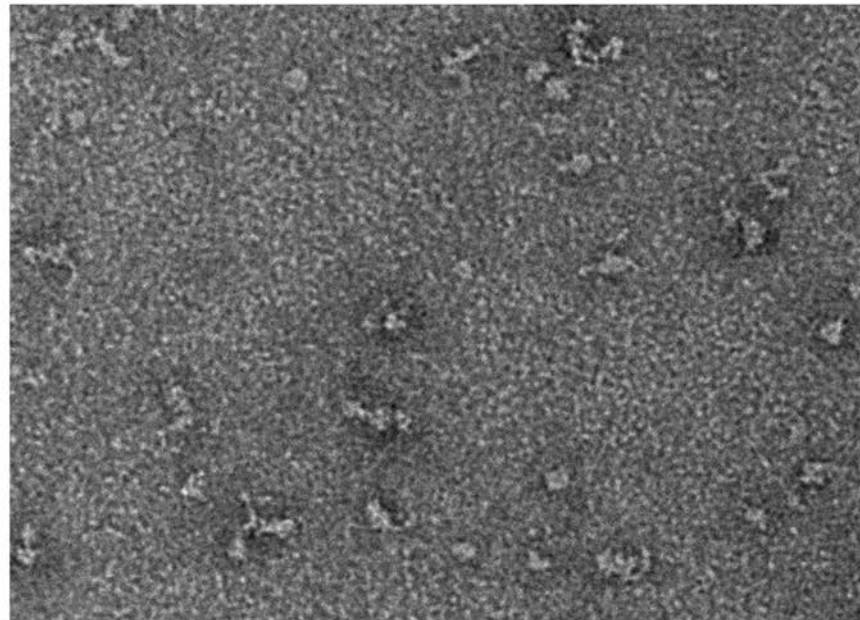
Gel filtration column



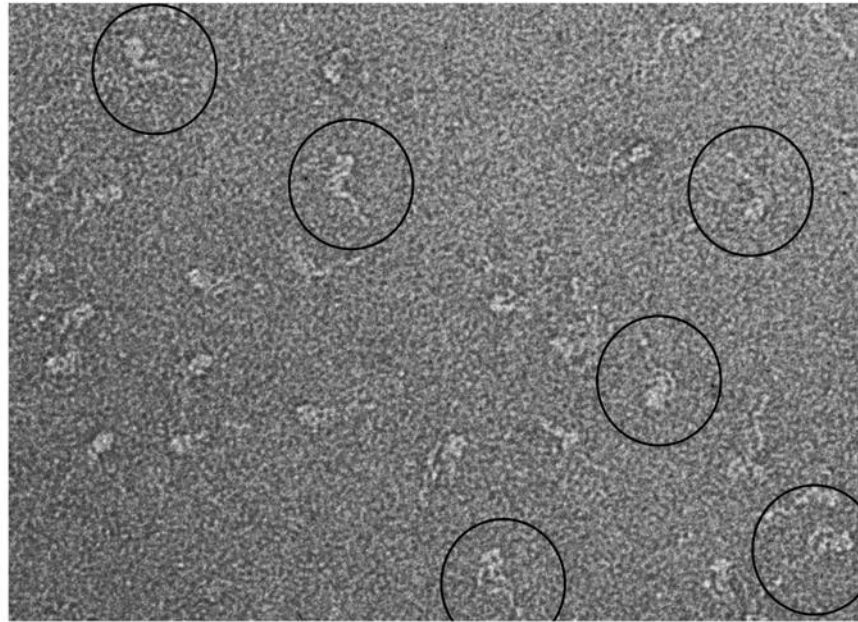
TcdA



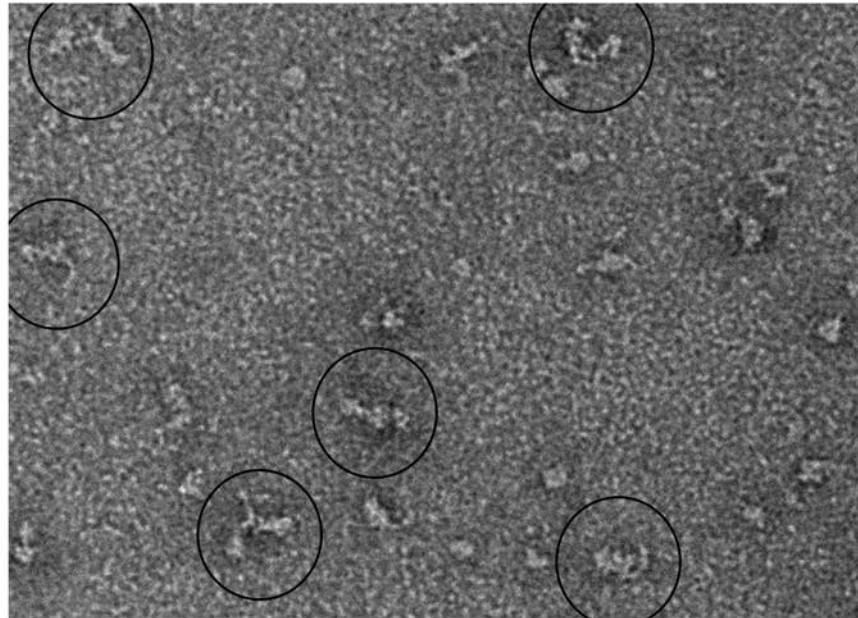
TcdB



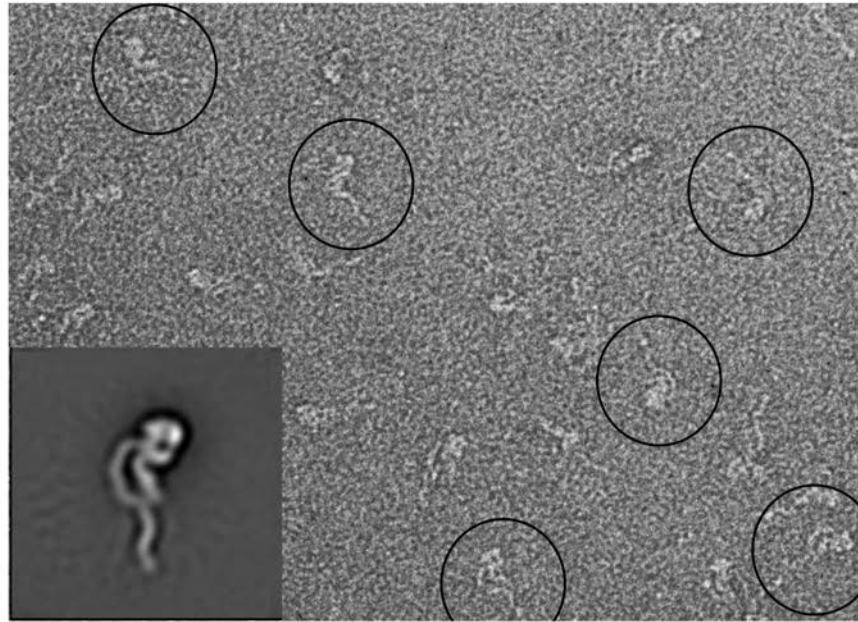
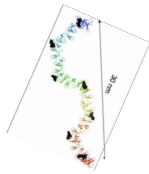
TcdA



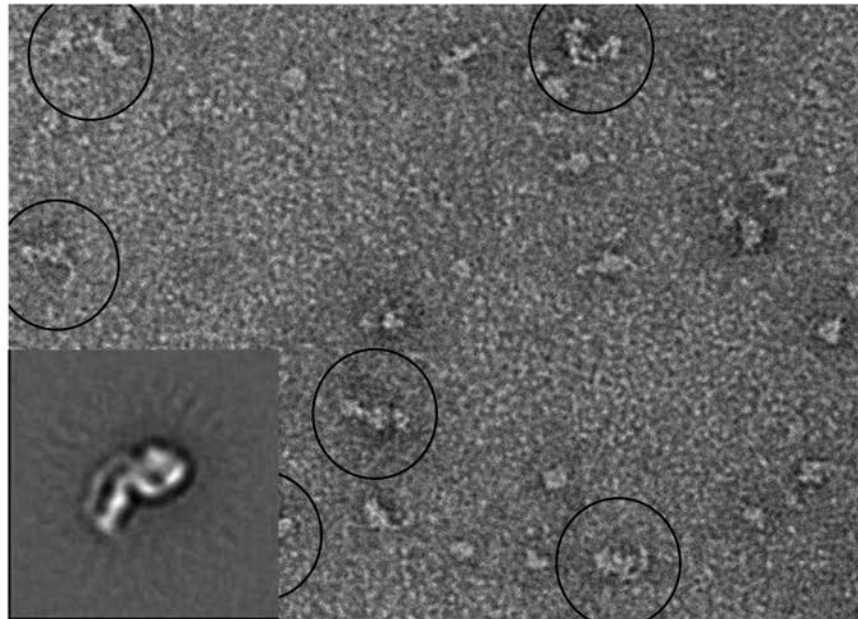
TcdB



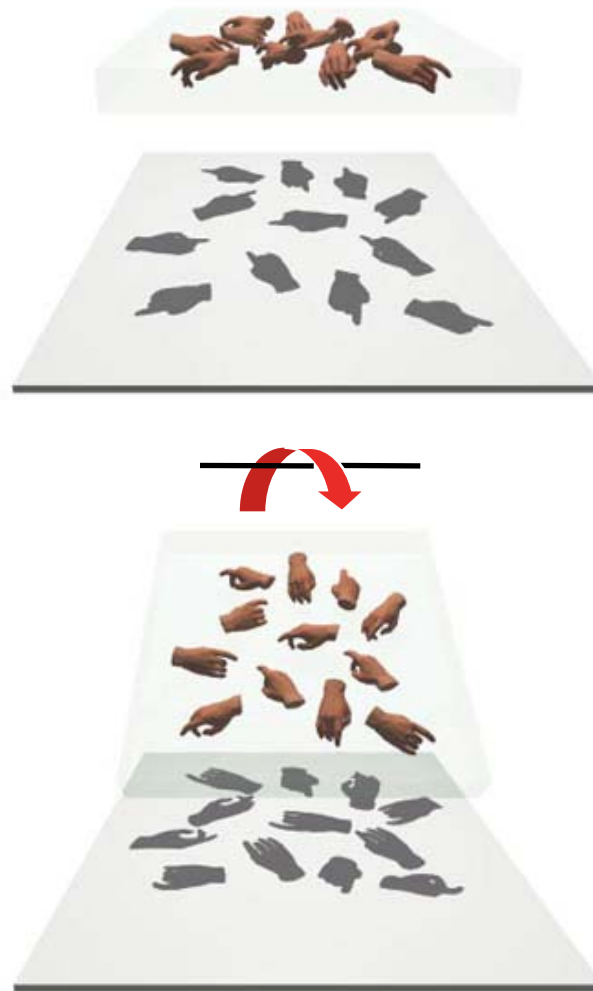
TcdA



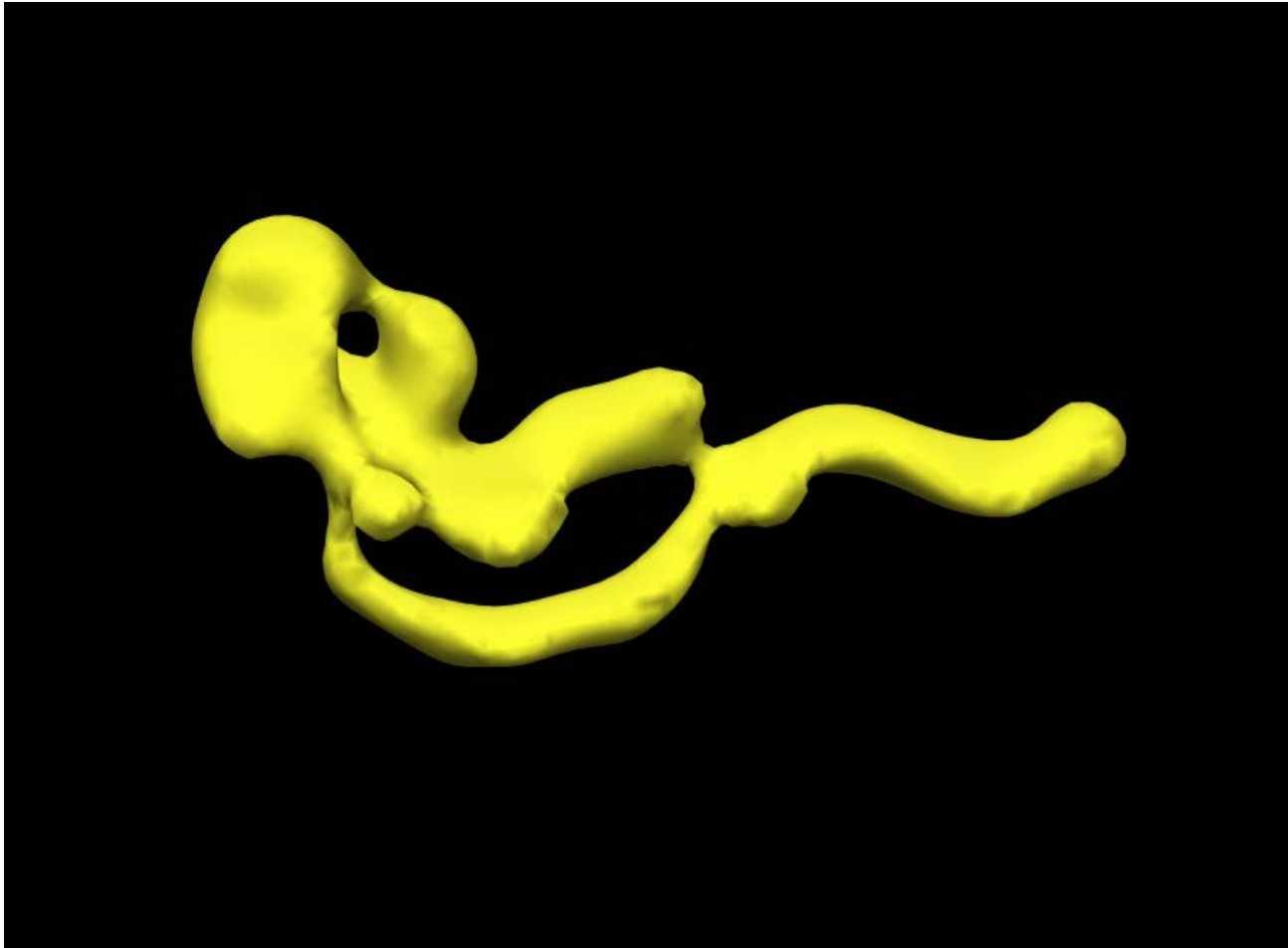
TcdB



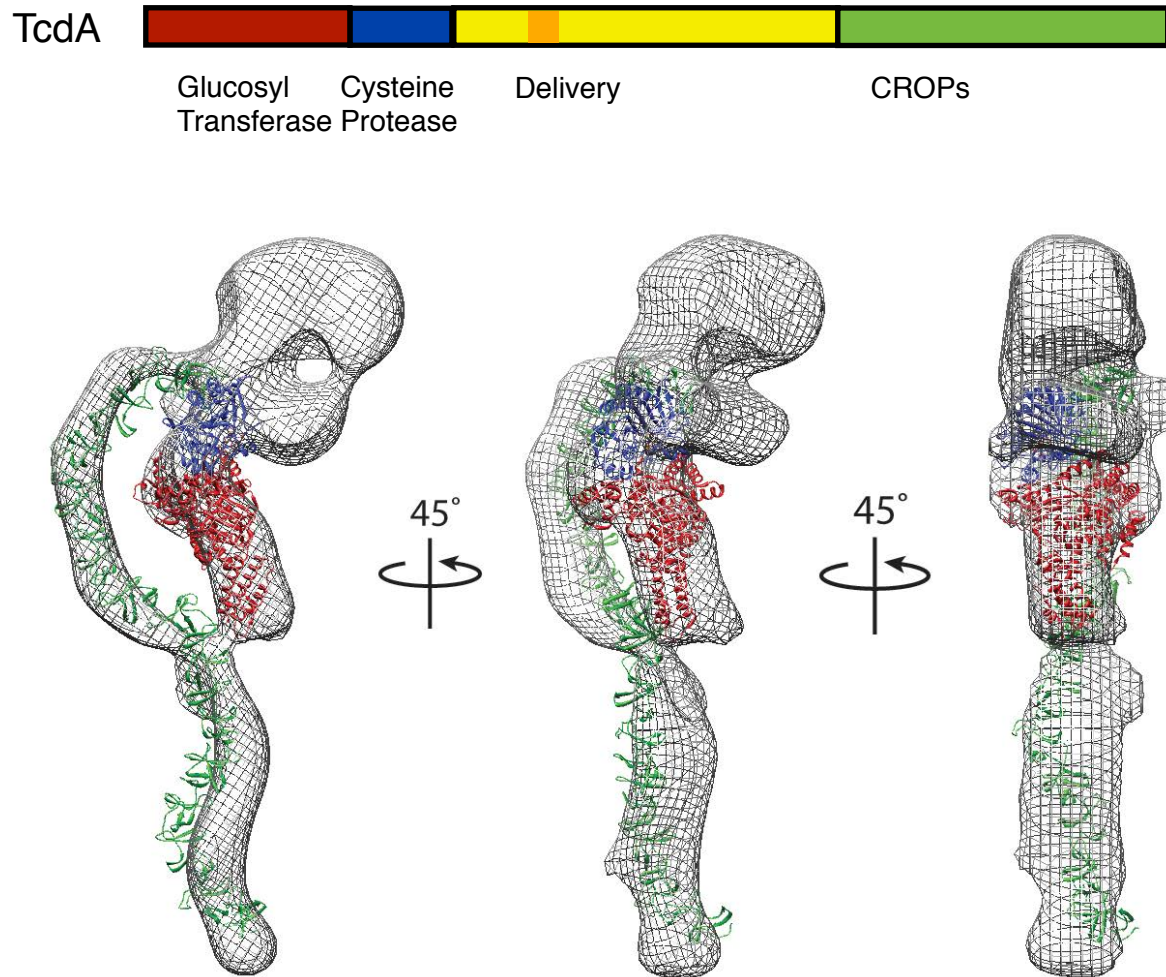
3D structure using Random Conical Tilt



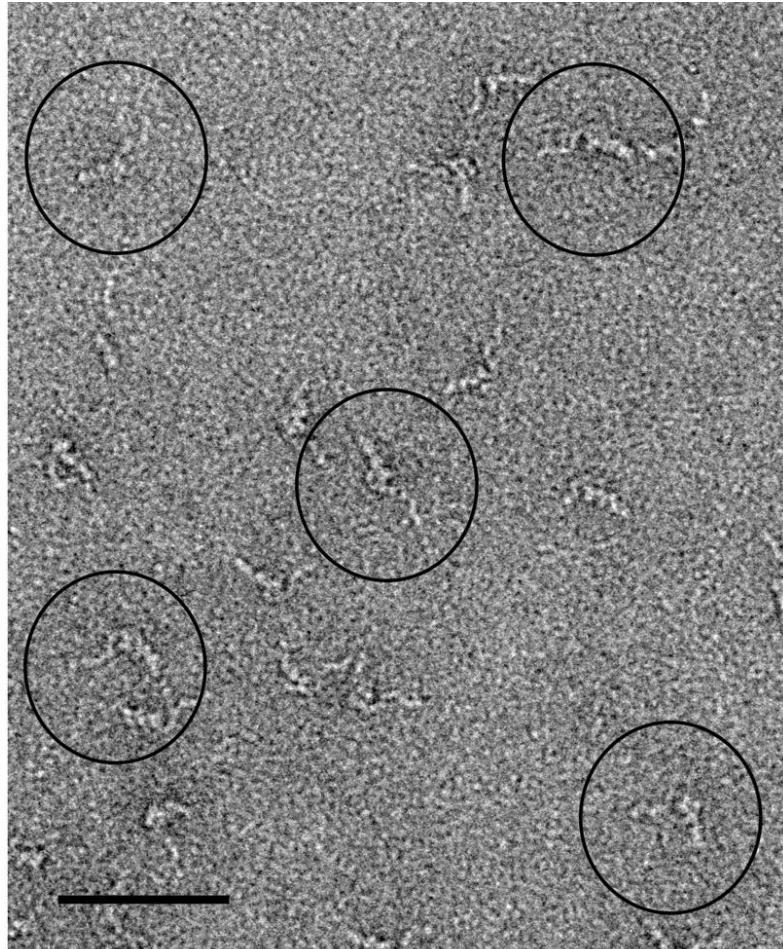
3-D reconstruction of TcdA



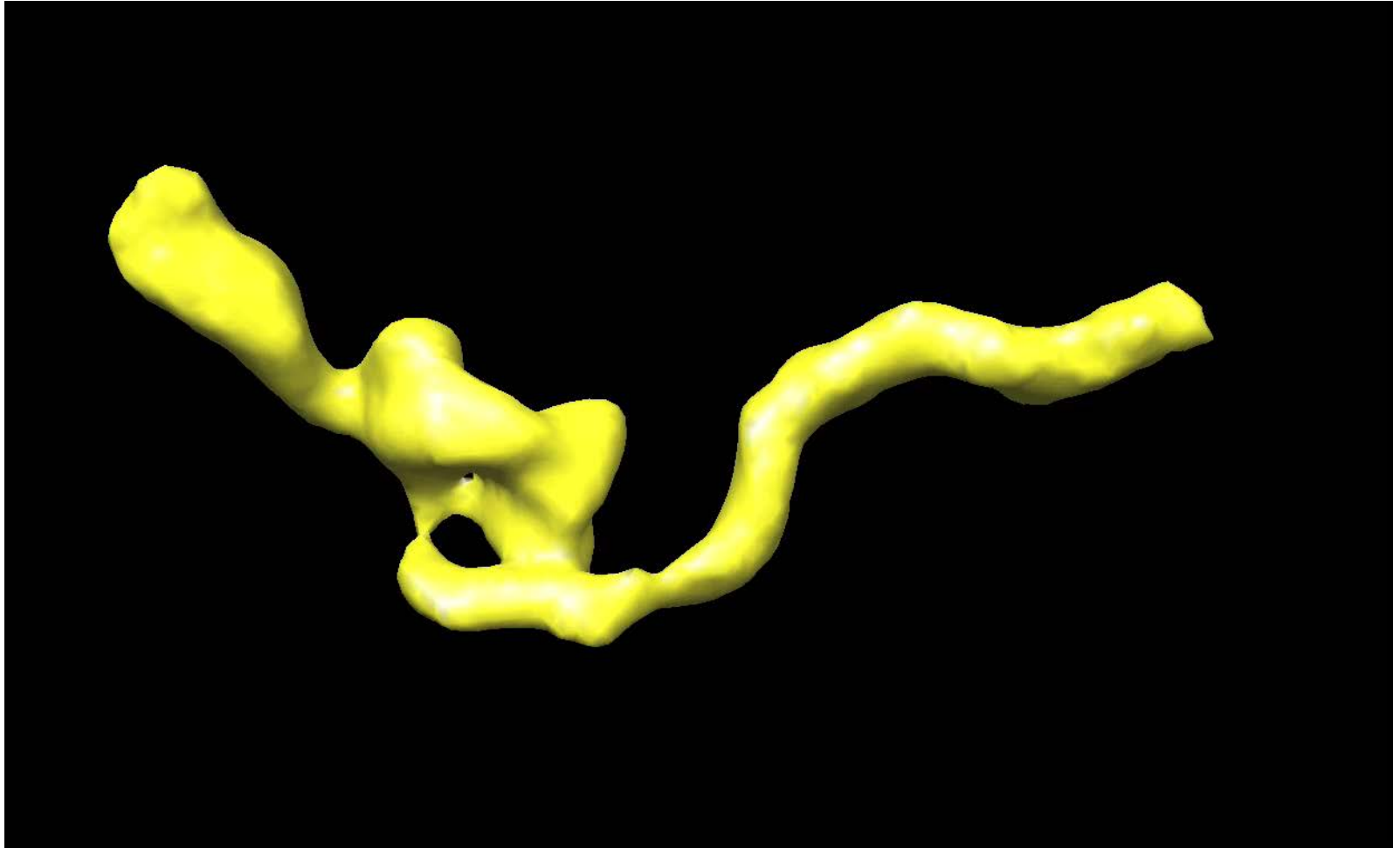
Proposed Model of TcdA Domain Organization



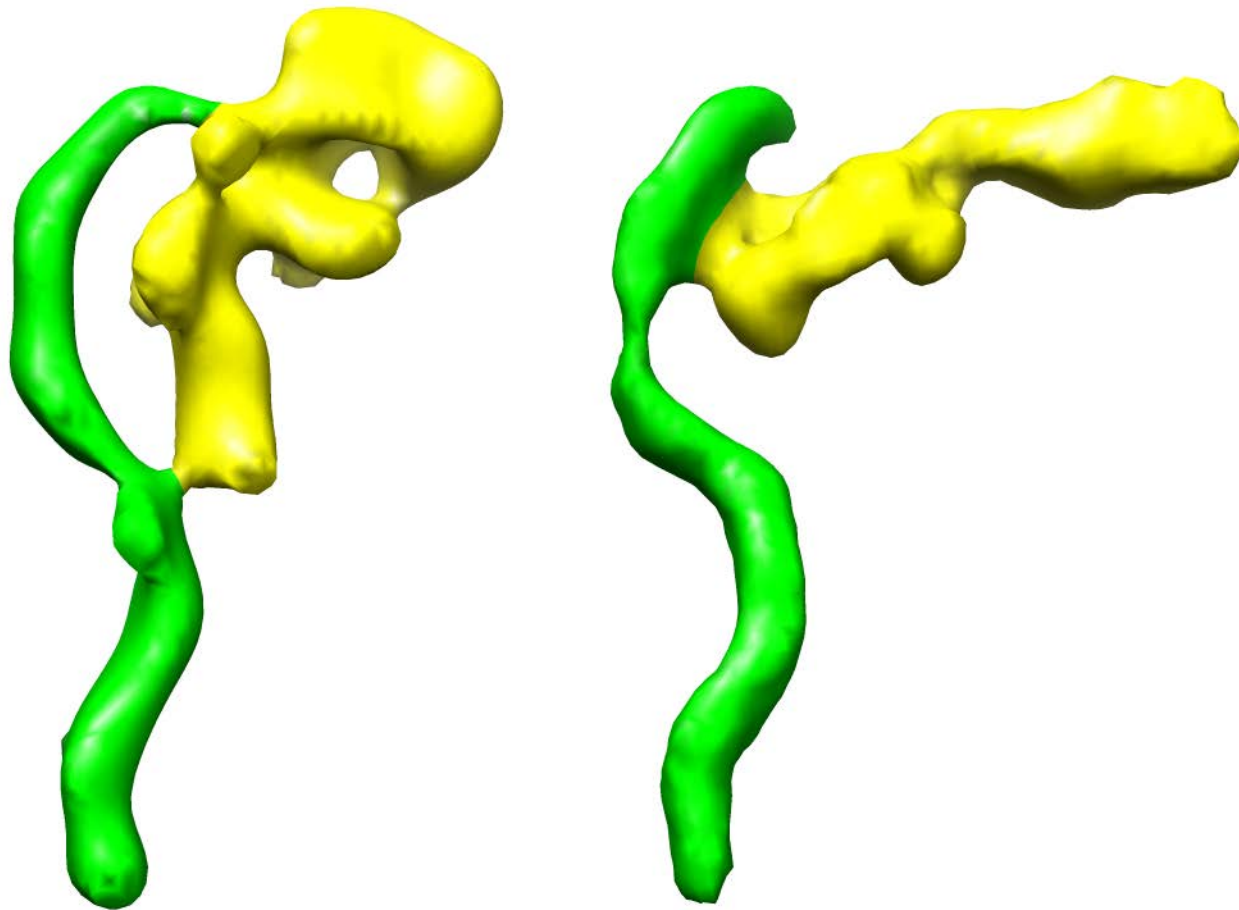
TcdA at Acidic pH

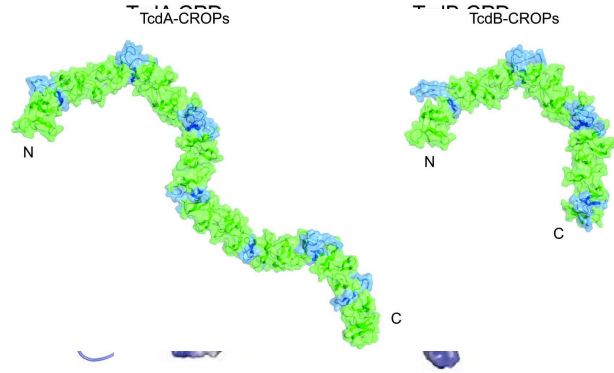


3-D reconstruction of TcdA at low pH

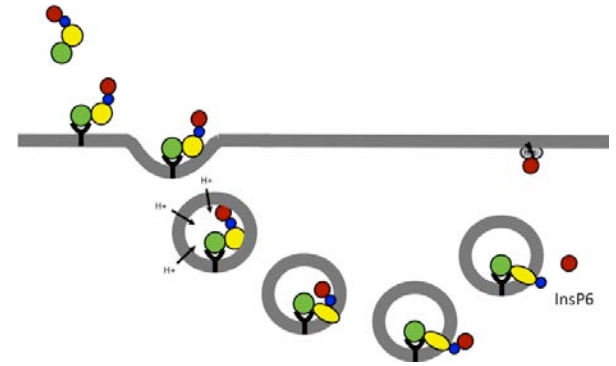


EM structures of TcdA at neutral and low pH





Summary



- Crystal structures of GTDs from several LCTs reveal differences in the GTPase binding surfaces. *Are differences in GTPase specificity important for pathogenesis?*
- Crystal structures of the CPDs bound to InsP6 suggest that an allosteric mechanism of activation. *What is the structure in the absence of InsP6?*
- Structures from the CROPs domain of TcdA are consistent with a multivalent model of low affinity carbohydrate interactions. *How does TcdB interact with cells?*
- EM imaging suggests TcdA and TcdB are structural homologs and that TcdA undergoes a significant rearrangement in response to low pH. *How does the delivery domain facilitate the entry of the GTD?*

Do any of these structural states represent good targets for the design and discovery of novel therapeutics?

Acknowledgments

Joe Alvin
Desirée Benefield
Ramya
Chandrasekaran
Nicole Chumbler
Ryan Craven

Heather Kroh
Melissa Farrow
Mitch LaFrance
Darren Mushrush
Stacey Seeback



Rory Pruitt

Electron Microscopy

Melissa Chambers
Melanie Ohi

X-ray Crystallography

Ben Spiller
Argonne National Lab

Funding

Burroughs Wellcome Foundation
Digestive Disease Research Center Pilot Project
National Institutes of Health NIAID

