Supplemental Material to:
Numan Oezguen, Trevor D. Power, Petri Urvil, Hanping Feng, Charalabos Pothoulakis, Jonathan S. Stamler, Werner Braun and Tor C. Savidge. Clostridial toxins: Sensing a target in a hostile gut environment. Gut Microbes 2012; 3(1):
http://dx.doi.org/10.4161/gmic.3.1.19250
http://www.landesbioscience.com/journals/gutmicrobes/article/19250/

AMBER force field parameters for $InsP_6$ were generated in-house using the standard procedure described in the AMBER manual for determining parameters for new compounds. By geometrically optimizing the parameters for hydrogen atoms only, a gas-phase partial geometry optimization was performed on $InsP_6$ at the Hartee-Fock level in basis set 6-31G(d) with GAUSSIAN03[1] as a 1) neutral species, 2) minus 6 anion and 3) minus 12 anion. Since the heavy atoms of $InsP_6$ were supplied in the crystal structure, these atoms remained frozen in the crystal structure geometry during the optimization to default convergence criteria. Charges were approximated with RESP using the highest degree of symmetry for $InsP_6$ at all 3 ionization states. The input for RESP was generated in a three-cycle procedure utilizing the Merz–Singh–Kollman[2,3] electrostatic potential charge fitting analysis from anab initio gas-phase single-point calculation in GAUSSIAN03 at the Hartree–Fock level in basis set 6-31G(d).
Table S1: Calculated RESP partial charges for $InsP_6$

	$InsP_6$	**$[InsP_6]^{6-}$**	**$[InsP_6]^{12-}$**
C1	0.134540	-0.074038	-0.227779
H1	0.085726	0.177874	0.228840
O1	-0.435376	-0.263488	-0.049786
P1	1.164856	1.045046	1.026972
O2	-0.743376	-0.814017	-0.992749
O3	-0.583007	-0.646954	N/A
H2	0.479822	0.389592	N/A

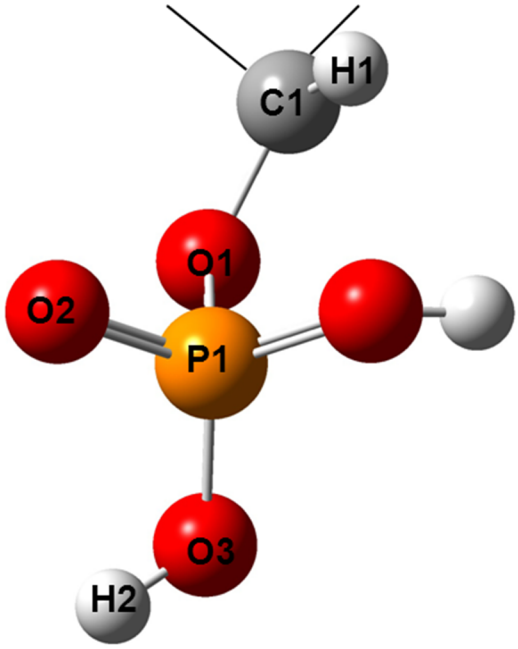
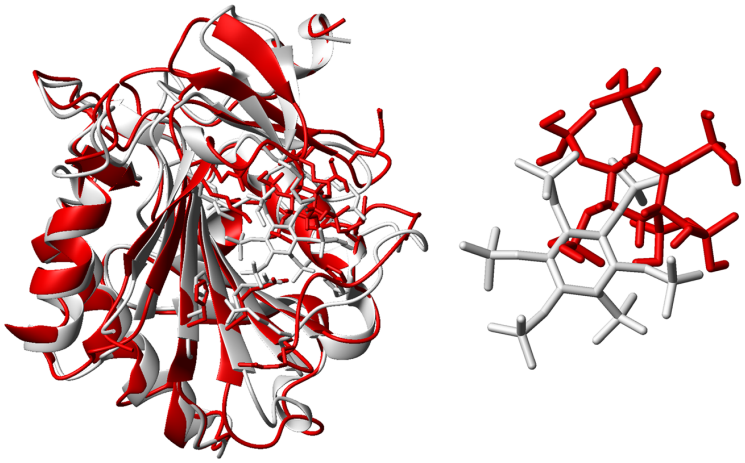
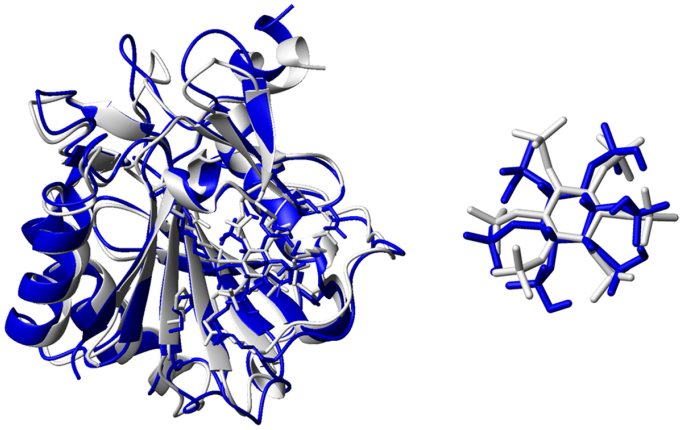


Figure S1: Definition of the atom names used in Table S1. To test which of the three $InsP_6$ forms (neutral, 6^- or 12^- total charge) should be used in the MD-simulations, we performed MD-simulations of the RTX_{VC} crystal structure 3FZY including bound $InsP_6$. The form that carries a total charge of 12^- reproduced the crystal structure with least deviation (Figure S2c).

a: Neutral $InsP_6$



b: $[InsP_6]^{6-}$



c: $[InsP_6]^{12-}$

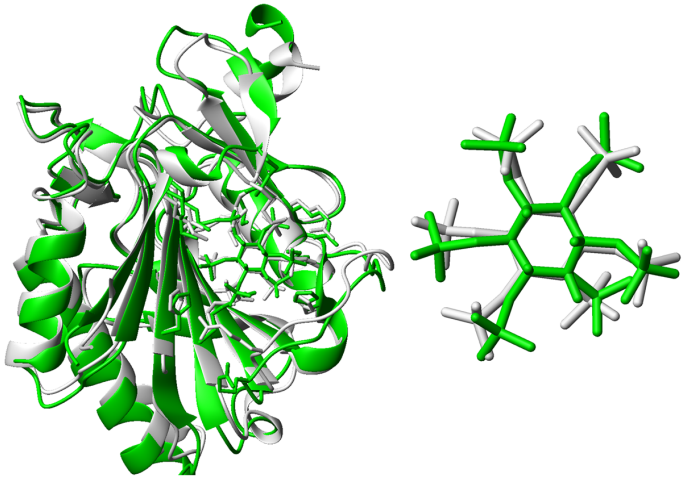


Figure S2: Alignment of the RTX_{VC} crystal structure 3FZY (in grey) with conformations of the same after 5ns MD-simulation (in red, blue and green). Panel a, b and c show the result of the MD-simulation with different parameter sets for $InsP_6$ (neutral, -6 and -12 total charge). On the right are views to the alignment in the $InsP_6$ region.

Movie legends

Legends for movies 1 and 3: MD-simulation of the TcdA crystal structure (3HO6; video 1) and RTXvc (3EEB; video 3) without the $InsP_6$ bound. The color of the ribbon indicates the simulation time (red at the beginning and blue at the end after 10 ns simulation). Please note that the movie starts with the end at 10 ns and progresses back towards the beginning of the simulation (towards the crystal structure). Highlighted residues from the right to the left are the catalytic triad Asp (red), His (blue), Cys (yellow), activity regulating Glu (red) and Trp (grey) on the β-flap (green). Green dotted lines are H-bonds.

Legend for movies 2 and 4: MD-simulation of the long model of TcdB (based on the TcdB crystal structure 3PA8 and RTX_{VC} crystal structure 3FZY; movie 2) and RTXvc (3FYZ; movie 4) without the $InsP_6$ bound. The color of the ribbon indicates the simulation time (red at the beginning and blue at the end after 5 ns simulation). Please note that the movie starts with the end at 5 ns and progresses back towards the beginning of the simulation (towards the crystal structure). Highlighted residues from the right to the left are the catalytic triad Asp (red), His (blue), Cys (yellow), activity regulating Glu (red) and Trp (grey) on the β-flap (green). Green dotted lines are H-bonds.

Supplement References

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