nature microbiology

SUPPLEMENTARY INFORMATION

ARTICLE NUMBER: 15002 | DOI: 10.1038/NMICROBIOL.2015.2

Crystal structure of *Clostridium difficile* Toxin A

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Supplementary information Table 1: Data collection and refinement statistics.

	Native	$HgCl_2$ (90 min)	$HgCl_2$ (3 days)	S1329C $HgCl_2$ (3 days)
Data collection				
Wavelength (Å)	1.28149	1.00273	1.00275	1.00798
Cell dimensions				
a, *b*, *c* (Å)	303.61, 124.54, 75.98	300.34, 123.80, 75.83	302.0, 123.99, 75.76	300.87, 124.34, 76.34
α, *β*, *γ* (°)	90, 97.5, 90	90, 98.0, 90	90, 97.73, 90	90, 97.5, 90
Resolution (Å)	50.0-3.25 (3.31-3.25)*	50.0-3.6 (3.66-3.6)	50.0-3.8 (3.87-3.8)	50.0-4.15 (4.22-4.15)
R_{merge}	6.2 (63.4)	6.9 (50.7)	8.0 (68.2)	8.7 (57.1)
$I/\sigma I$	21.0 (2.5)	9.6 (1.8)	10.3 (1.9)	10.4 (2.3)
Completeness (%)	100 (99.9)	99.3 (99.3)	99.8 (99.8)	99.7 (99.5)
Redundancy	6.0 (6.0)	2.7 (2.7)	3.8 (3.8)	5.1 (5.1)

	K_2PtCl_2	$HAuCl_4$
Wavelength (Å)	1.06578	1.03501
Cell dimensions		
a, *b*, *c* (Å)	315.88, 123.41, 75.43	297.66, 123.92, 75.52
α, *β*, *γ* (°)	90, 97, 90	90, 98.2, 90
Resolution (Å)	50.0-4.1 (4.17-4.1)	50.0-3.5 (3.56-3.5)
R_{merge}	5.4 (51.9)	6.1 (44.1)
$I/\sigma I$	15.9 (2.2)	16.0 (2.5)
Completeness (%)	99.9 (100.0)	99.4 (95.8)
Redundancy	4.0 (4.0)	5.2 (4.5)

Refinement	
Resolution (Å)	50-3.26
No. reflections	43,813
R_{work}/ R_{free}† (%)	18.21/23.74
No. atoms	14412
Protein	14411
Zinc	1
Water	0
B-factors (Å^2)	
Wilson B	106.15
Protein	123.95
Zinc	146.46
R.m.s deviations	
Bond lengths (Å)	0.018
Bond angles (°)	1.975

*Highest resolution shell is shown in parenthesis.

† Rfree calculated with 4.57% of the reflections (2002) that were omitted from the refinement process.

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Supplementary information Table 2: Alignment of enzymatic domains to isolated domain structures.

Toxin	Ligands	PDB ID	Rmsd Å^2	No of Cα atoms	Reference
TcdA	Apo	4DMV	0.65	428	46
TcdA	Apo	3SS1	0.78	484	24
TcdA	UDP, Mn	4DMW	0.7	430	46
TcdA	UDP-glucose, Mn	3SRZ	0.92	455	24
TcdB	UDP, glucose, Mn	2BVL	1.22	469	47
TcsL	UDP-glucose, Mn	2VKD	0.96	461	48
Tcna	Apo	2VK9	1.99	420	48

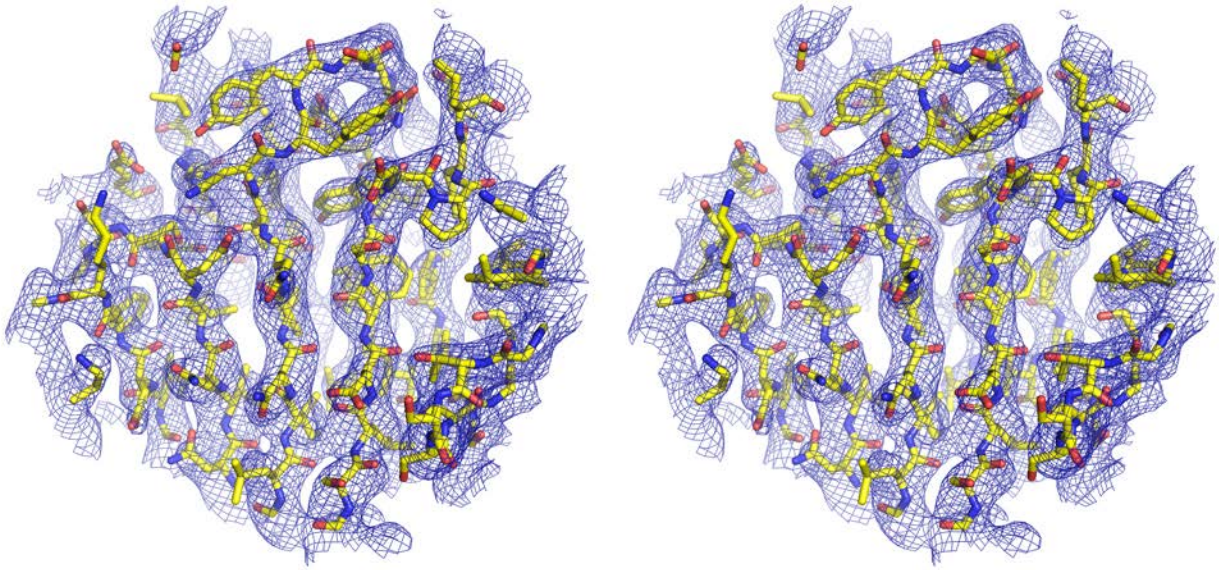
Supplementary information Table 3: Zinc to protein ratios determined by ICP-MS

Sample*	Zinc/Protein			Sample	Zinc/Protein		
$TcdA_{1795}$	0.8	0.83	0.72	TcdB	0.74	0.68	0.6
$TcdA_{1795}$ C700A	0.08			TcdB C698A	0.09		
$TcdA_{1795}$ H655A	0.06			TcdB H653A	0.04		
$TcdA_{1795}$ H759A	0.3			TcdB H757A	0.12	0.31	0.5
$TcdA_{1795}$ + InsP6	0.57	0.51		TcdB + InsP6	0.45	0.55	
$TcdA_{1795}$ + TPEN	0.04			TcdB + TPEN	0.05		
$TcdA_{1795}$ (apo) + $ZnCl_2$	0.48			TcdB (apo) + $ZnCl_2$	0.38		

* Each entry represents a measurement from a unique protein preparation.

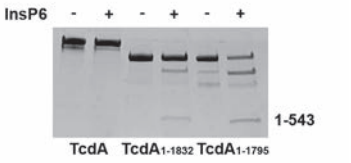
Supplementary information Table 4: Primers used to generate the plasmids for this study.

Protein	Plasmid	Primers
$TcdA_{1832}$ S1329C	pBL578	5' -CTTACTCTTTATTATTATGTTCATATCCAATATCAACG-3' 5'-CGTTGATATTGGATATGAACATAATAATAAAGAGTAAG-3'
$TcdA_{1795}$	pBL656	5'-GATATTAAAAAACTATCATTAGGATATATAATGAGTGGAGGGCATGCCGGC-3' 5'-GCCGGCATGCCCTCCACTCATTATATATCCTAATGATAGTTTTTTAATATC-3'
$TcdA_{1795}$ C700A	pBL712	5'-GTAGAAGTAAACTTACTTGGAGCTAATATGTTTAGTTATGATTTTAATG-3' 5'-CATTAAAATCATAACTAAACATATTAGCTCCAAGTAAGTTTACTTCTAC-3'
$TcdA_{1795}$ H655A	pBL713	5'-GTAAAAGTAACCTTTATTGGAGCGGGTAAAGATGAATTCAAC -3' 5'-GTTGAATTCATCTTTACCCGCTCCAATAAAGGTTACTTTTAC-3'
$TcdA_{1795}$ H759A	pBL714	5'-GAAAAGAACTTCTGGCTGCGTCAGGTAAATGGATAAATAAAG-3' 5'-CTTTATTTATCCATTTACCTGACGCAGCCAGAAGTTCTTTTC-3'
TcdB H757A	pBL689	5'-GGAAGAAGAGAATTATTGGATGCGTCTGGTGAATGGATAAATAAAG-3' 5'-CTTTATTTATCCATTCACCAGACGCATCCAATAATTCTCTTCTTCC-3'
TcdASAS V1109S, N1110A, N1111S	pBL675	5'- GCAGGAATACCTTCATTATCTGCTTCTGAATTAATATTGCATGATAAGGC-3' '5'-GCCTTATCATGCAATATTAATTCAGAAGCAGATAATGAAGGTATTCCTGC-3'



Supplementary information Figure 1: Wall-eyed stereo view of representative electron density.

Weighted 2Fo-Fc map contoured at 1 sigma.



Supplementary information Figure 2: A truncated TcdA construct ($TcdA_{1795}$) undergoes autoprocessing more efficiently than TcdA and $TcdA_{1832}$. The three proteins were incubated with or without 10 mM InsP6 for 2 hours in a buffer containing 20 mM HEPES pH 6.9, 50 mM NaCl and then subjected to SDS-PAGE. Autoprocessing was most efficient in the $TcdA_{1795}$ sample. Gel is representative of three independent experiments.

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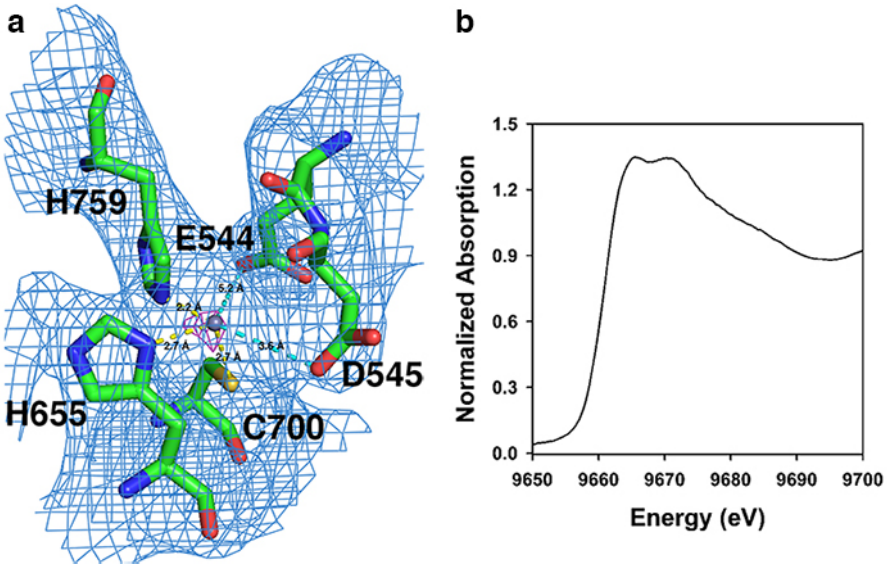
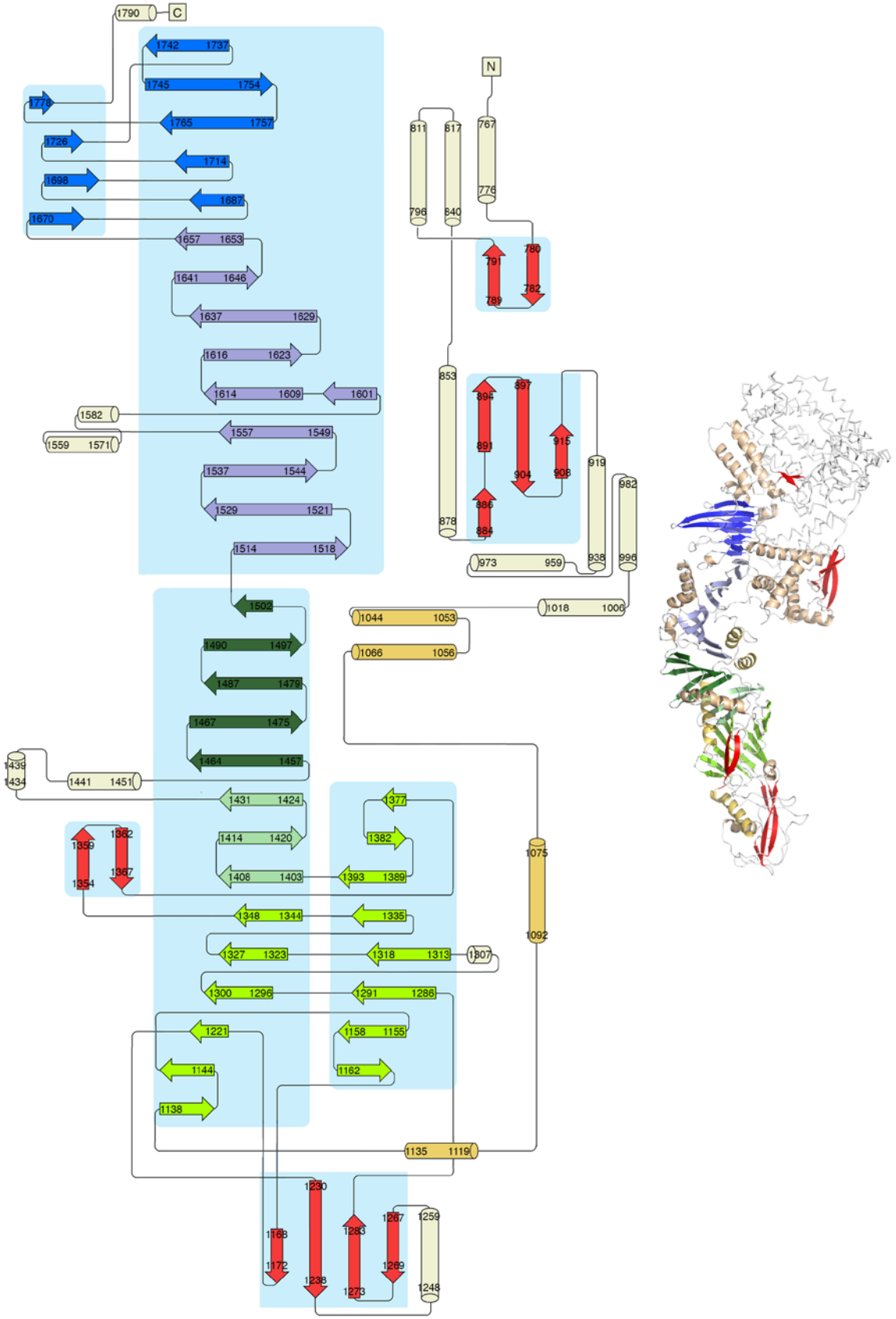


Supplementary information Figure 3: Zinc is present in the APD active sites of TcdA. **a.** Analysis of the anomalous zinc signal, reveals a single peak, shown here in purple at 7 sigma, located in the APD active site. The zinc is coordinated by H655 (2.7 Å), C700 (2.7 Å), and H759 (2.2 Å) and may have indirect contacts with E544 or D545. The blue mesh depicts a weighted 2Fo-Fc map contoured around the 5 active site amino acids at 1 sigma. **b.** X-ray absorption near-edge spectrum of TcdA in solution.



Supplementary information Figure 4: Topology of the TcdA delivery domain. Cylinders indicate α-helices, arrows represent β-strands.

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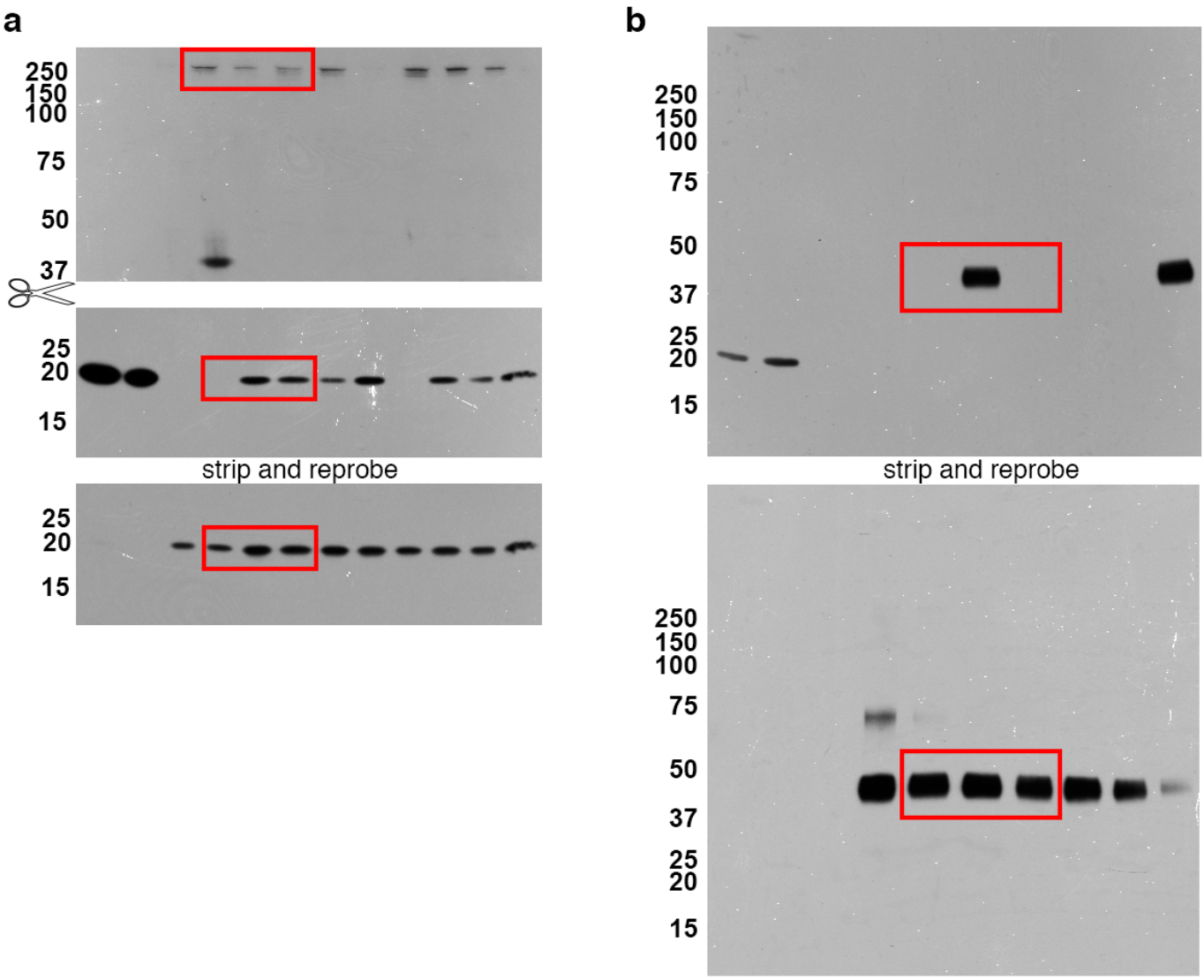

Supplementary information Figure 5: Full Western blots associated with Figures 3c and 3d. a. Proteins were separated by SDS-PAGE and transferred to PVDF membrane. The membrane was cut between the 37 kDa and 25 kDa molecular weight markers so that the top blot could be probed with an antibody against the TcdA CROPS domain and the middle blot could be probed for unglucosylated Rac1. After exposure to film, the middle blot was stripped and reprobed with an antibody against total Rac1. b. The membrane was initially probed with an antibody that recognizes unglucosylated Rac1. After exposure to film, the blot was stripped and reprobed with an antibody against total Rac1.

References

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