

Supplemental Material

Structures of distant diphtheria toxin homologs reveal functional determinants of an evolutionarily conserved toxin scaffold

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DT      G-ADDVVSSKSFVMENFSSYHGTGKPGYVDSIQKGIQK--PKSG-----TQGNYYDDWKGEFYSTDNKYDAAGYSVDNENPLSGKAGGVVKVYTPGLTKVLALKVDNAETI----- 102
DT-like (S.a) MTTAVENIQVAEITPSTRIVYRGVSPAEFIY-LEGNKFSRAQSP-----TQGNDDPQWKALYTGSDANVSSRN---I---TDNPGGVVKIEYPSDWKVLITSTTPSQKWHNDMGEA 105
DT-like (S.p) MPNNESIV-VEIVKLNHFHYRGVSPIGVKDYQVGQKI--VKSTSTDEHGNPVLGNYDPHWQGLYAAEHLHHAASYAVDNN---SGVPGGLFKIKLPEDVRVRYENKDAQAII-TP-GRL 114

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DT      -----KKEL-GLSLTEPLME-QVGTEEFIKRFGDGASRVVLSLP-----F-AEGSSSVFYNNWEQAK-ALSVEL-----EINFETRGRK----- 172
DT-like (S.a) WPVWRVAVKKAASNQVDLPDVT-ASNIDDYLLDELGKKIILKKP-----IGEDDVSSHFEIIPWKMAE-TVAQNKIDSTSPPAKFFTPDDLDSTTKQ-----P 199
DT-like (S.p) YRALR-EEGLI-KLTAKELNETHFNSNQFLTNELGKEKILIIITDEFESFTDINGMKIPRLFEIIPWNIATEQVQVSE-----EVKVWYKGRDFSSSLNAKERLELMKLRGP 221

      furin site
      * * * * *
DT      RGQDAMYEYM-A---QA-CA---GNRVRRSVGSSSLSCINLDWDVIRDKTKTKIESLKEHGPPIKNKMSSESPNKTVSEEKAKQYLEEFHOTA-LEHPELSELKTVTGTNPVFAGANYAAWAVNV 285
DT-like (S.a) KDQAAVRRIILKKW-DAYSCKGGA-SATFG---VASLCCGINVAAYKADIEKLIKDVYEDPNFSD-LKN-RTGGPQ--KDDTLKGYIERLKPKIVETLRPLKA--GVSSAVGAAGAIWAIGV 309
DT-like (S.p) YENDL-TSYEAKFKDLIICR--S-ASYYS---SGSSCLDWEKIKITESQRIVKQIIEHPPELQ---S-HSKNAV--TDKEKLQKIYNDYAPKIDKLSLKE--GVSRAATTALNIASWAAGL 326

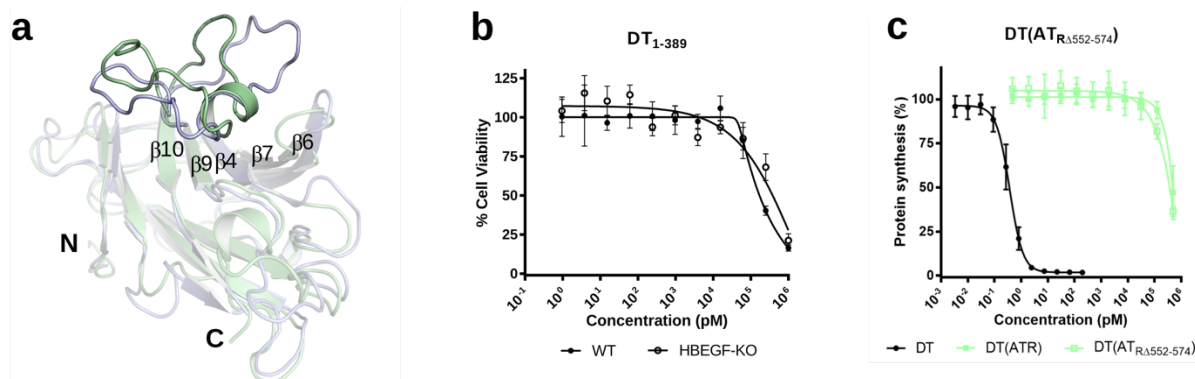
      * * * * *
DT      AQVIDSETADNLEKTTAALSILPGIGSVMGIADGAVHHNTEEIVASIALSSLMVAQAIPLVG---ELVDIGFAAYNFVESIINLFQVVHNSYNRPAYSPGHKTPQPELHDGYAVSWNT-VED 403
DT-like (S.a) ADAFTSENVSSFDKAAAVTAIVPGLGECVGIANAIIDKRDPEGLIINTISMAALMASAAVPIVLPIGVALDAGLAAAGVATVLEYLEI---GQPARTPLPVSSPKTHKGVTAAWVG-SER 425
DT-like (S.p) AETFSNKNADGLDKAAAVTAIIPGLQAVGIANGIEKHDGEAIIANSIALSALVVAQAIPIVG---EIAADVVGAGLILAGGLAQLIQSVS--PDTPPHVEPPHFYPQTSNHNVTVGWLNQKID 443

      * * * * *
DT      SIIRT--GFQ-GESGHDIKITAE-----NTPLPIAGVLL--PTIPGKLDVNKSKTHISVNGRKIRMRCRAIDG---DVTFCRPKSPVYVNGVGHANLHVAFHRSSEKIHSNE-----IS 505
DT-like (S.a) IIAHR--PR-PGMRQHIFSVSIDSSKPEY-TAPLIEVAG--VRADGKLDPSPEWIRIRQNHYPPIPRFEKLSGDSPYAFRCVLLRPTTIITRTEPVYVTFAYMTSDMTCRTGESDPNKACSPN 541
DT-like (S.p) EMIHAWYPH-EGYRSHHFVIKIANAPENTTMPITEIMAKLGSQTKQLDLVPERVWYQNNVITCTKQTVSLKTDRAVIRPLFTMLTKSRPIVVRMAYITGENSCITD---ANPTCFPE 561

      * * * * *
DT      SDSIGVLGYQKTVDHDK-----VNSKLSLFFEIKS- 535
DT-like (S.a) NPAIAVRV-FGLVKNEDERSVLAVTWPGPSIRPETNWIKLPYSIHYP 587
DT-like (S.p) NPAIAVRV-TPLPSNNECDWHTPLHPSYQNGDKADFVRLGYRIGV- 606

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Supplemental Figure 1. Sequence alignment.



Supplemental Figure 2. R domain. (a) Structural differences in the lid structure present in AT and PT. In PT_R the 'lid' is more splayed, and the apex of the loop does not possess as much helical character while in AT_R, it adopts a full helical-turn. Sequence conservation between the two proteins in this region is low (11% identical, 22% similar) relative to the full domain (28% identical, 42% similar). (b) Dose titration of DT₁₋₃₈₉ on WT and HBEGF_{KO} cells (mean $\pm$ SD, n=3). Sensitivity to DT₁₋₃₈₉ was unaffected by HBEGF expression (EC₅₀'s: WT-207 nM, HBEGF_{KO}-498 nM). (c) The 'lid' sequence in AT_R (residues 552-574) was replaced with two Gly residues in the DT(AT_R) chimeric construct. Dose titration of DT, DT(AT_R), and DT(AT_R Δ 552-574) on Vero cells (mean $\pm$ SD, n=3).

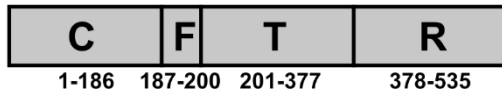
Diphtheria Toxin (DT)

Albireti Toxin (AT)

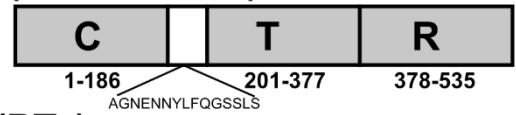
Peptono Toxin (PT)

Catalytic (C) Furin-site (F) Translocation (T) Receptor-binding (R)

DT



DT (uncleavable)



DT(AT_C)



DT(PT_C)



DT(AT_F)



DT(PT_F)



DT(AT_T)



DT(PT_T)



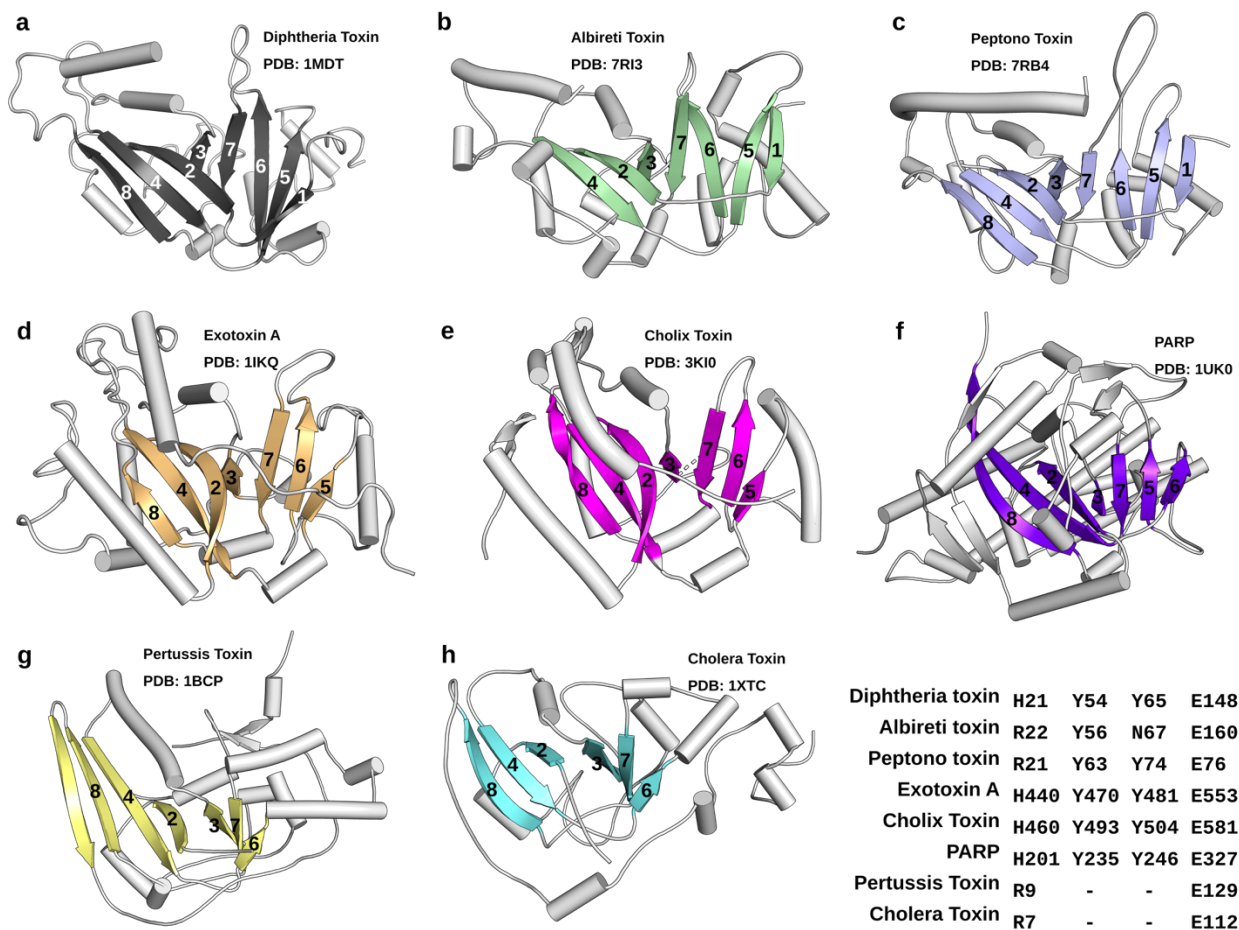
DT(AT_R)



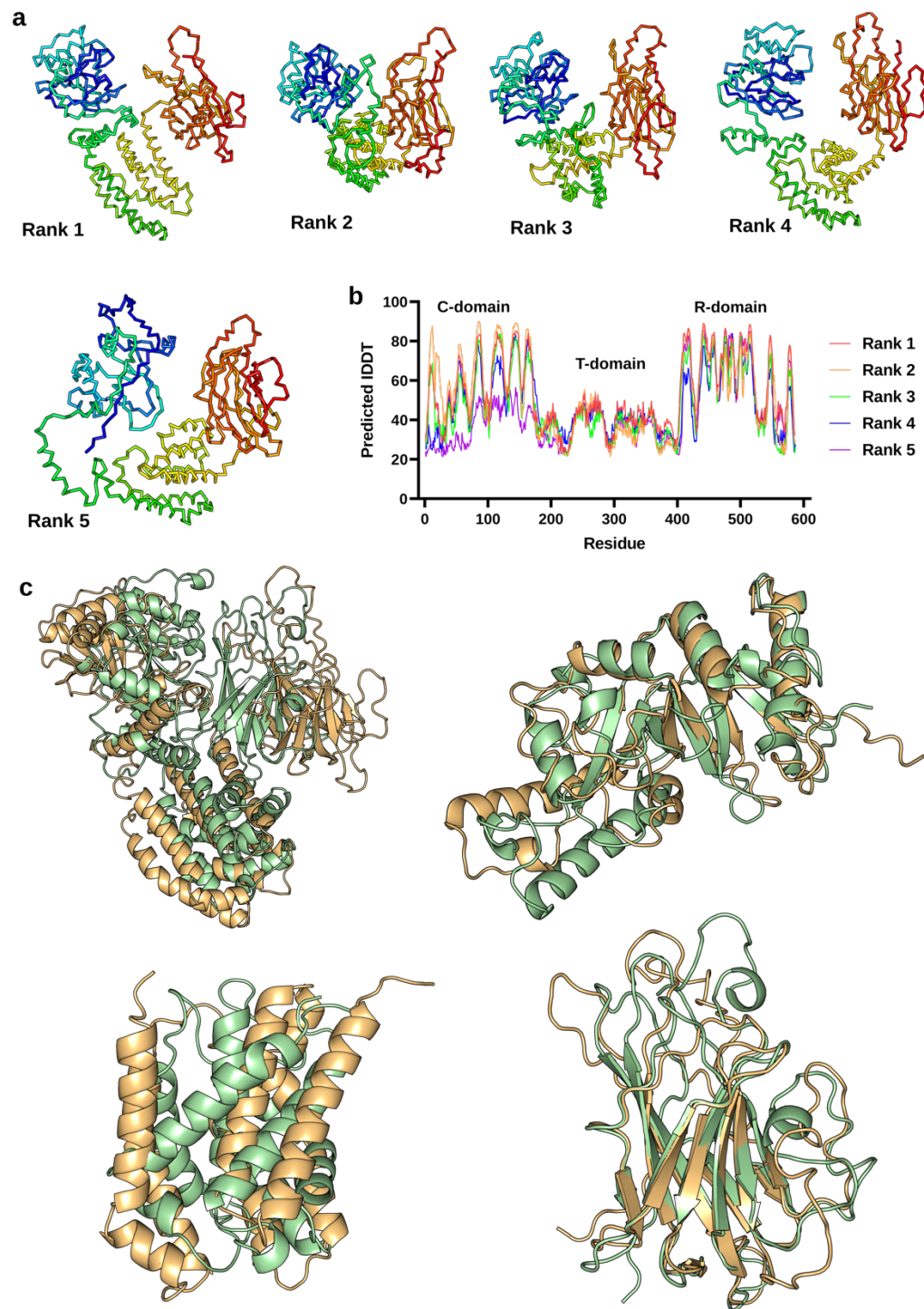
DT(PT_R)



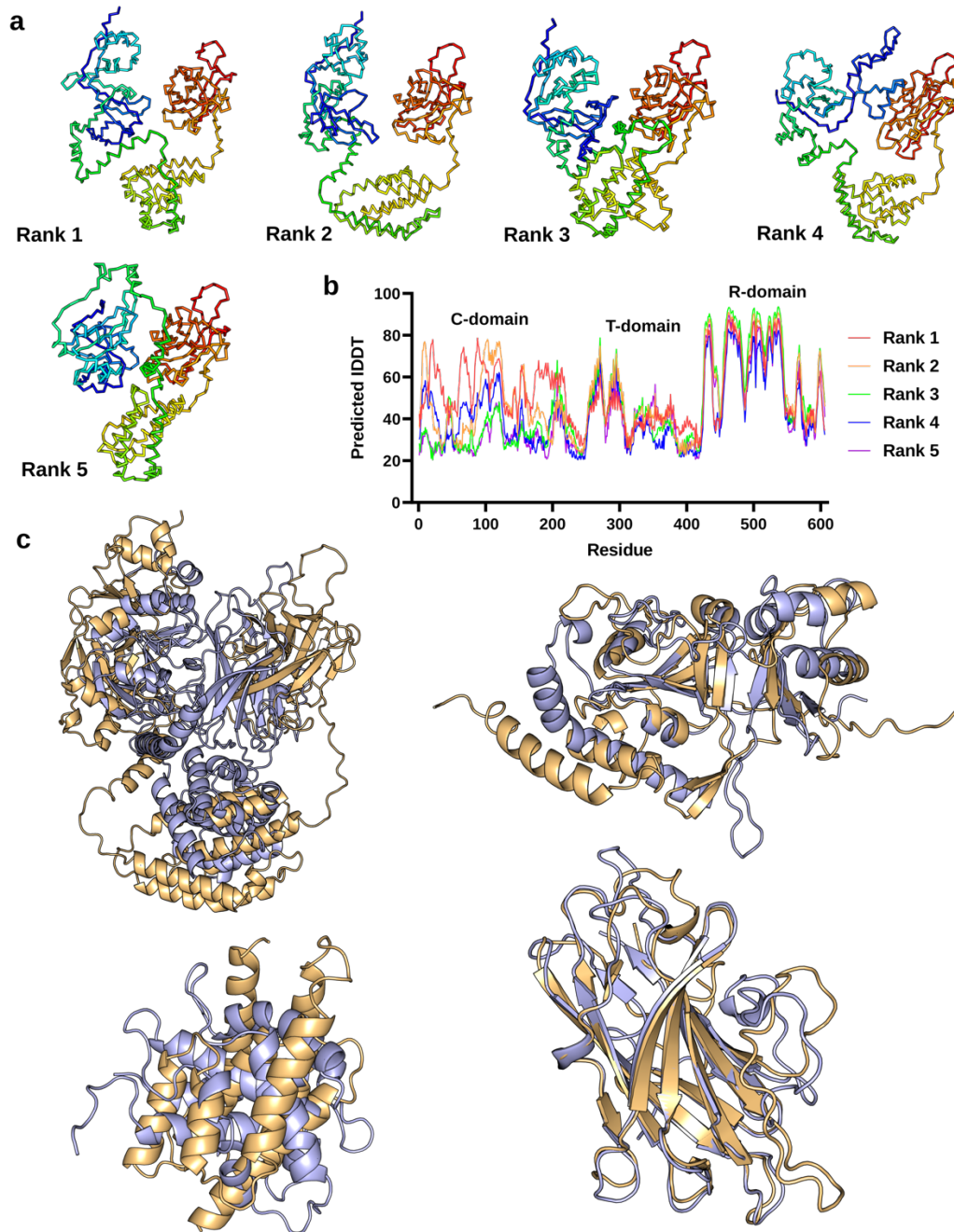
Supplemental Figure 3. DT chimeras. Architecture and residue numbering of DT domain swapped chimeras.



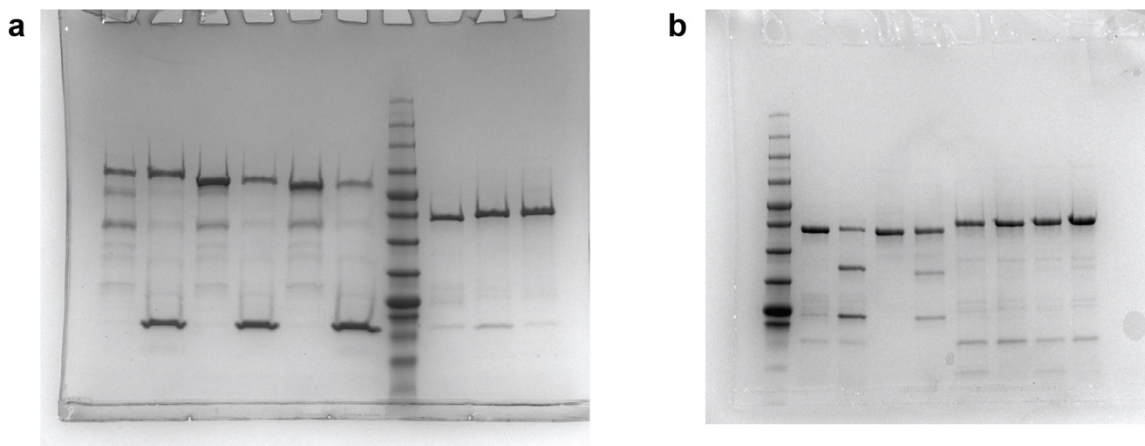
Supplemental Figure 4. ADP ribosylating enzyme folds. Conservation of the split β -sheet amongst ART family catalytic domains.



Supplemental Figure 5. AT Prediction – (a) 5 models of AT generated by the AlphaFold network using ColabFold coloured N→C (blue to red). (b) Predicted IDDT scores by residue number for models ranked 1 to 5. (c) Superpositions of top ranked AlphaFold prediction with the crystal structure of AT: holotoxin (top-left), C-domain (top-right), T-domain (bottom-left), R-domain (bottom-right).



Supplemental Figure 6. PT Prediction – (a) 5 models of PT generated by the AlphaFold network using ColabFold coloured N→C (blue to red). (b) Predicted IDDT scores by residue number for models ranked 1 to 5. (c) Superpositions of top ranked AlphaFold prediction with the crystal structure of PT: holotoxin (top-left), C-domain (top-right), T-domain (bottom-left), R-domain (bottom-right).



Supplemental Figure 7. Uncropped gels used in this work. (a) uncropped gel from which gel presented in Figure 1f was derived. Only Lanes 7-10 were used in figure. Lane 7 corresponds to molecular weight markers. Lane 8 is purified DT; Lane 9 is purified AT; Lane 10 is purified PT (b) uncropped gel from which gel presented in Figure 5c was derived. Only Lanes 1-3 and 6-9 were used were used in figure. Lane 1 corresponds to molecular weight markers. Lane 2 is DT – lysate; Lane 3 is DT + lysate; Lane 6 is AT -lysate; Lane 7 is AT + lysate; Lane 8 is PT – lysate; Lane 9 is PT + Lysate.