## Protocol S1

```
# i/o
-in:file:fasta AdiU_A.fasta
-nstruct 2000
-parser:protocol rosetta_cm.xml
-out:file:silent silent.out
-out:file:scorefile scores.sc

# relax options
-default_max_cycles 200
-relax:min_type lbfgs_armijo_nonmonotone
-relax:dualspace
-relax:jump_move true
-score:weights talaris2013.wts
-use_bicubic_interpolation
-hybridize:stage1_probability 1.0
-sog_upper_bound 15
-out:level 100
```

## Protocol S2

```
-in:file:fasta AxoU_Cterm.fasta
-in:file:frag3 AxoUCT.3mers.frags
-in:file:frag9 AxoUCT.9mers.frags
-abinitio:relax
-relax:fast


-abinitio:increase_cycles 10
-abinitio:rg_reweight 0.5
-abinitio:rsd_wt_helix 0.5
-abinitio:rsd_wt_loop 0.5


-use_filters true
-psipred_ss2 AxoUCT.psipred_ss2
-out:file:silent silent.out
-out:file:scorefile score.sc
-nstruct 50000
```

**S3. Codon optimized VpdA protein (665 aa)**

The VpdA open reading frame is identified in yellow highlight. Nucleotide sequences not highlighted are included for cloning into pEGFPC1.

Codon optimization was targeted for protein expression in human cells.

TACAAGTCCGGACTCAGATCTCGAGCTATGAAAACTAAGCAGGAAGTAAGCCAACAAGATA
AGCTCAAGGACTCAAAGTCAAGCACACCTTTGCAAACTAAAGAGCCATGGTTTATCTCAGA
CACACTTAATATCACGTTTGATAGCTACGATTTTAGCATATCAGTTACGGAACAAGCACCGA
TTCCATCACGAATCGTGTTTTCCGGAGGTGGCTCTAGGATCCTGGCTCATATCGGTGCACT
TGACGAATTGACGCGGCATGGTCTCAAATTCACCGAATTTAGCGGGAGCGCGGCCGGTGC
AATGGTTGCAGCTTTCGCGTATTTGGGATACAATTGCAGCGAGATCAAACAGATAATTTCTT
GGTTTAATGAAGACAAACTCCTGGATTCCCCACTGATATTTAACTTCAACAACATTAAACAA
ATTTTCAACAAAGGCGGATTGTCATCAGCCAAATTGATGCGCCAAGCGGCGAACTACGTCA
TACTGAAGAAAGTTATGGACATCATATCTGATGAGAAGTTTAAAACTCGCTTTGCGAAGTTT
CAGAATTTCCTTGAGGAAAACATTTATTCTTGCCCCGAGAACATTACCTTCCAGACGCTGG
CACGCATCAAGGAAATTTGTCCCGAATGCGAGCTCGGCGAGAAGCTCTTTATAACCGGAA
CCAACCTCAGCACTCAGAAACATGAGGTTTTCAGTATCGACACAACTCCTTCAATGGCGCT
GGCGGATGCTATTATAATATCAGCAAACCTGCCAATAGCATTCGAGAGGATCTGTTACCAA
GGCAACGTCTACTCCGACGGCGGTATAAGTAACAATCTCCCTGCTCATTGCTTTAGTGAGA
AGGGTCACAAGACTACGTTCCTGAAGCACAAGGATGATGTAGACTTTAGCGTCCTGGCTCT
TCAATTTGACAATGGTCTCGAGGAAAACGCCTTGTATAGCCAAAACCCGATACCTAAGTGG
AGCTGGCTCTCAAATACGTTCTACTCTTTGATAACTGGGCACCCTAATGTAACTGAAAACTG
GCACGAGGACCTCCAAATACTCCGACGACACGCGCATCAGAGTATCTTGATTAAGACGCC
CACGATCGCTCTCACCAACTTGACCATCAGTCAGGATACGAAAAAAGCCCTCGTGGAAAGT
GGCCGGACTGCTGCGAAAACCTATCTGGAGTTGCATGAATTCTACACAGATGATTACGGGA
ACATCCGGCACAACGAGTGTTTGCACGAGAAGTTCCAGAAGCCGGAGGAGCTGTTGGACT
ACTGCGTGCTTCACTCACATTTTGCTCTCCTCAAAAAAATCAAACAAGCGATTTCCTGTAGT
CAGTACCTGGAGAAGGGCTATAAGCATTATCTTTGCGAACTCTGCGACAATTTGTTGCCTC
CTCAGCTTAAATGCCCTAACGAGGGCTCTGGTACGGAACAGCCAGAAATCAAACTGGAAAA
GGACACTATTATTTGTAAAAAAAACAATAACAGTGGGCTCACTTTCTCCATGACGTTCTTTG
GAGTCCCCAGTCCGCTCGGAAAAACACTGAATCAAGATTCCCCCGAACTCAAGATAAAGTT
GTTTACAGGACTTTACCCAATATTGATTCAAAACTGGCAAAATCTTTGCCCAGTTTCTGGAA
TAAGTGGGATTCTCAATAGTATTCGGATGTCTTTCGTTGAGATTAGTAGTACCGATACGTGT
ATCAAGACACTTATTGACAAACTGAACGAAATCGAGATCGGGCACTTTCTTATATTCGTTTT
CAAAGCTGCCCTTAAGAATTACGACAAGCATGATTTTATACTTCTTCTGAAAAATCTTAAGC
ACCTCCACCACAGTATTGAGCTCATTAGAAACAAGCCCTTCCACTCAGACGATAGATTCTA
CGGCCAATGGTCCTTTGAGGGTCACGACCCTAAGCGGATTCTTGAATTCATCAAGAGTGAC
GATATTTCTGGCTTGATGACTATTCTGGAAGATAAGAAAGCACTCCCAAATAATAAGCCTAA
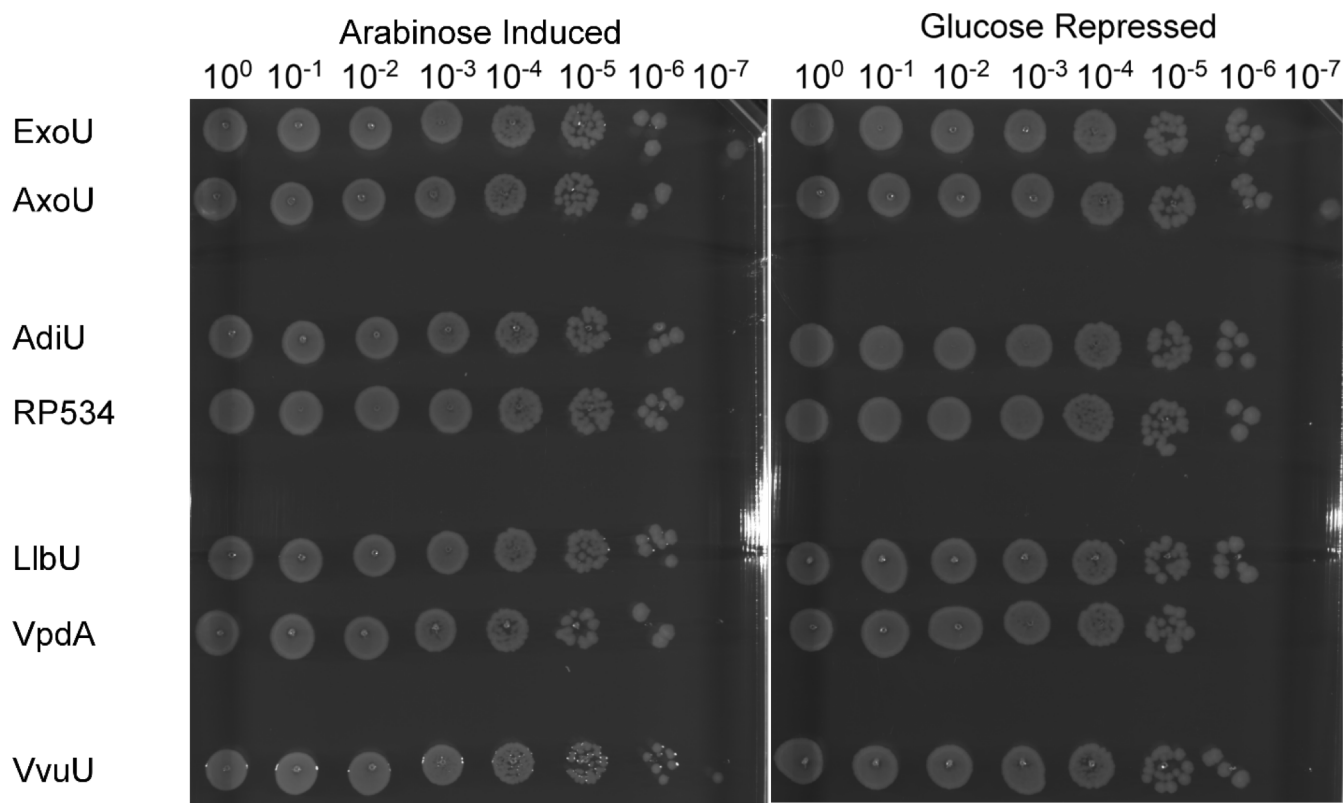TTAATAAGGATCCACCGGATCTAGATAACTG

Figure S1. Spot plate assay with BL21 (DE3) pJY2 strains containing an arabinose inducible clone of each of the parental orthologous enzymes tested in this manuscript. BL21 strains encoding ExoU or an orthologous enzyme were grown on LB plates with chloramphenicol, gentamycin and glucose. Each culture was emulsified, normalized to $OD_{600}$=3.0 and rested for 40 minutes as described in the materials and methods. Each culture was then diluted serially ($10^0 - 10^{-7}$) and spotted on control (glucose) or induction medium (arabinose).
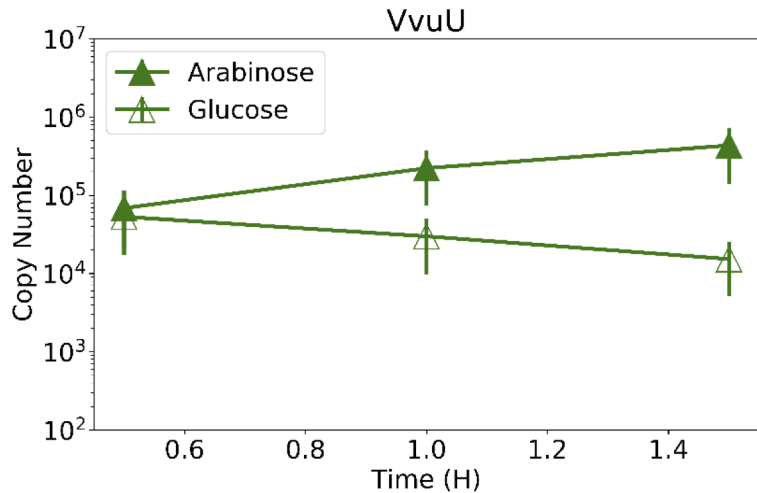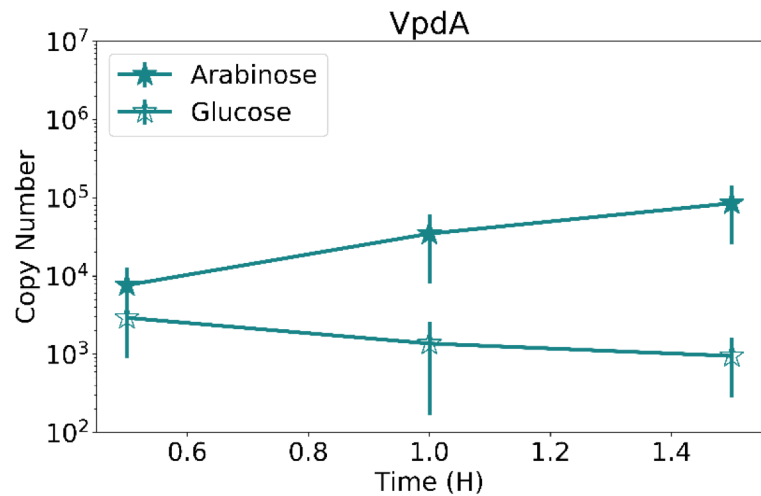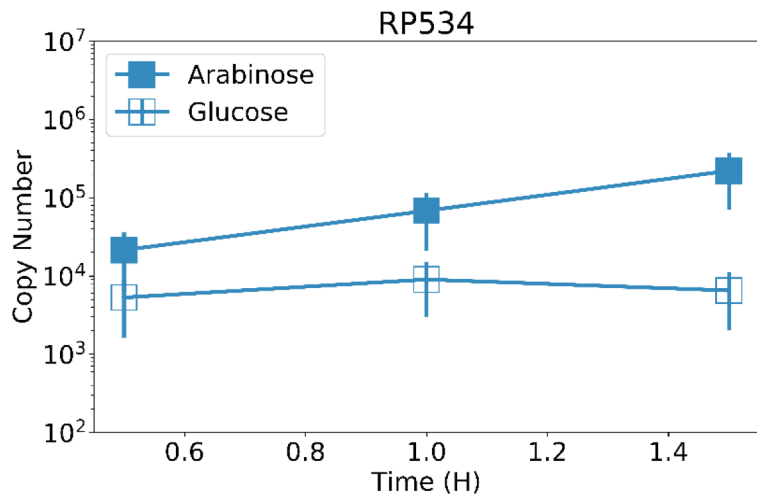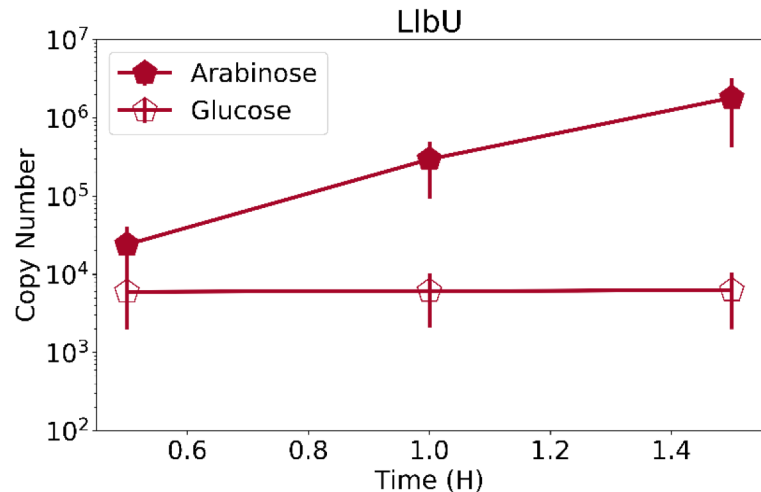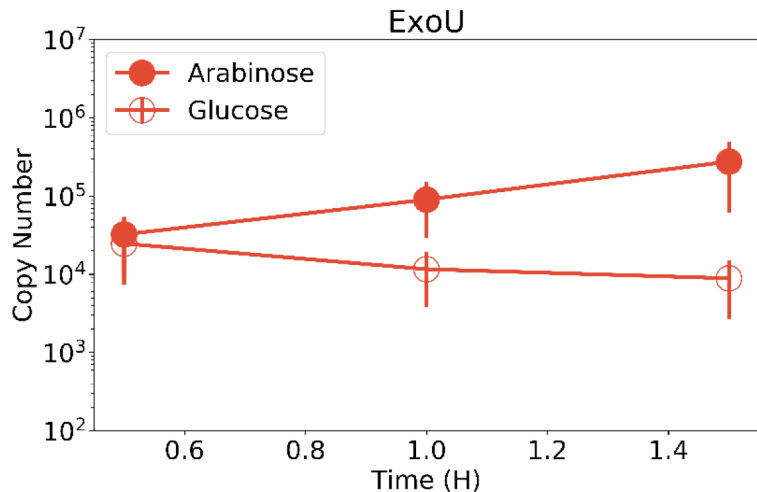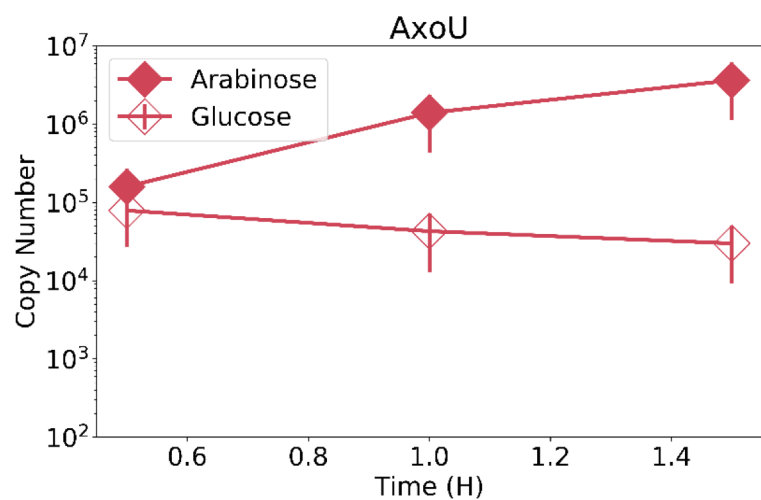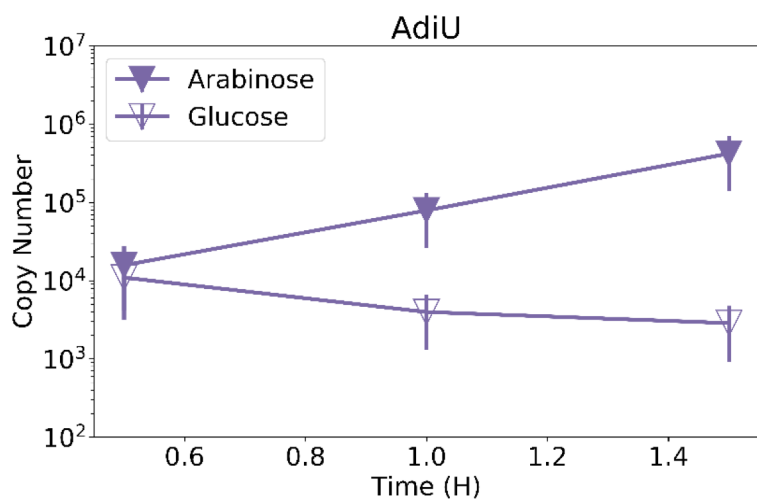
Figure S2. mRNA expression of toxin constructs in BL21 (DE3) pJY2 after induction with arabinose or repression with glucose containing medium. Copy number is plotted on the Y axis versus time post induction on the X axis. Copy number is based on a titration of plasmid DNA, with the same primers during the same RT-PCR assay performed in triplicate.
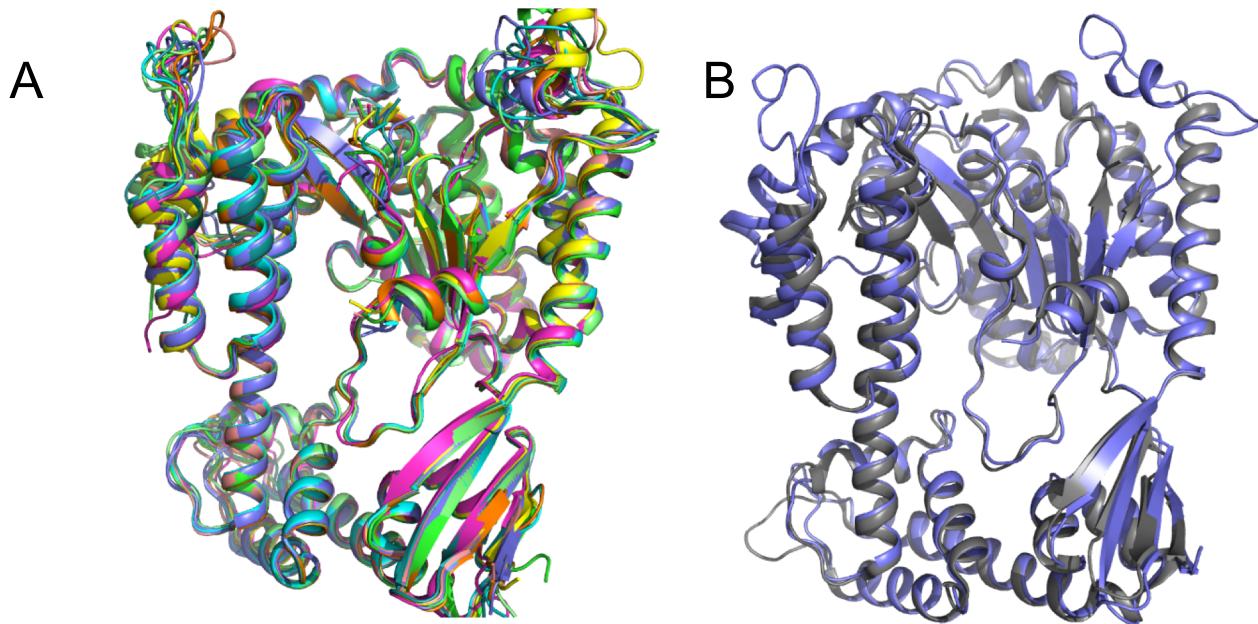
Figure S3. AdiU homology model predictions. AdiU was modeled using RosettaCM and previously published ExoU structures as templates (3TU3, 4QMK). A. The top ten scoring models align with each other with less than 2.0 Å RMSD and adopt a highly similar fold to that of both published 4QMK and 3TU3. B. The top scoring AdiU (blue) structure aligns with the published ExoU crystal structure (gray) with an RMSD less than 2.2 Å.
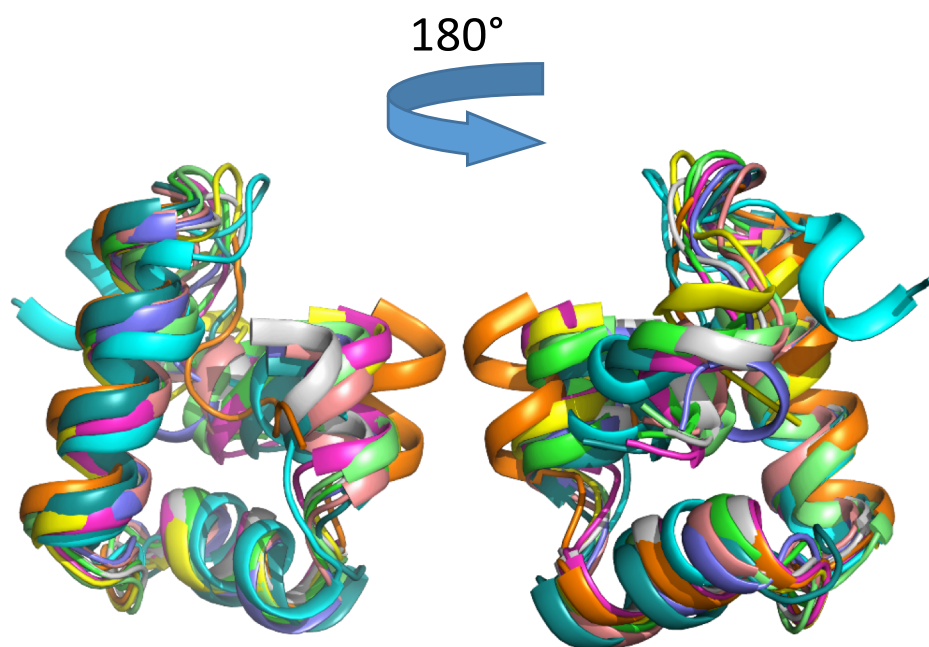
Figure S4. *Ab initio* models of AxoU C-terminus. Top ten scoring models were aligned with a similar fold suggesting convergence. The AxoU C-term model suggests a globular fold of small helices.