2024 SEA Faculty Meeting
Sunday, June 9, Morning Session
AlphaFold

ChimeraX
Download the most recent daily build to be able to run the AlphaFold2 ColabFold. Only versions released after March 2024 can now run AlphaFold2.
https://www.cgl.ucsf.edu/chimerax/download.html

AlphaFold3 Server
Sign in with Google account. Each user gets 20 runs per day
https://golgi.sandbox.google.com/

FoldSeek
https://search.foldseek.com/search

Background Slides:
https://www.dropbox.com/scl/fi/yn2vsdliwuafwkatjy8vs/20230609_AlphaFold.pdf?rlkey=zpv3atje6g6kvk3ocmjw7ud5n&dl=0

AlphaFold3 Paper: https://www.nature.com/articles/s41586-024-07487-w

EMBL-EBI AlphaFold Practical Guide:
https://www.ebi.ac.uk/training/online/courses/alphafold/inputs-and-outputs/evaluating-alphafolds-predicted-structures-using-confidence-scores/

Investigation 1: Continuation from your CAT work: Use AlphaFold3 to gather additional structural evidence to inform your functional annotations. Choose a gene from your CAT cluster that you were unsure about or could not assign a function for based on HHPRED/BLAST evidence.

a. Does AlphaFold3 yield a high confidence structure?
b. Does this structure yield any potentially informative hits on FoldSeek? (Let's say E-value <0, Probability >0.9)

Investigation 2: Phage-encoded Toxin-Antitoxin Systems: Type II toxin-antitoxin (TA) systems are abundant in bacterial and phage genomes. In these systems, the more stable toxin protein targets some essential cellular factors (many toxins are endonucleases), and the more labile antitoxin proteins bind to the toxin to neutralize toxicity. Many Type II TA proteins form stable heterotetrameric complexes (e.g., PDB 3G50). Antitoxin proteins typically can bind within the TA promoter region to repress TA expression, and this autorepression is enhanced by binding of the antitoxin to the toxin. Below are several sequences, play around and see what AlphaFold predicts for the following scenarios.

What are the predicted dimer and tetramer structures for the following predicted type II TA systems?

> Alexphander_gp46 (RelE-like toxin)
MFVVLKYGPYFWYHFGMSSYRVEIETSAAKQIQRLQRSEQKRVMVAITALADDPRPHGCTKLSGTT
DAYRIRVGNFRVVYVIDDGLHIVNVTRVGHRREVYKR
>Alexphander_gp47 (RelB-like antitoxin)
MSVLVPISKAKAKLSELVRQSEDTDVVLMNHSTPAAVLISVERYESLQEELEDLRDRLSVHERSGVT
VSVDKLMAELGLSTD
>Bunnybear_gp29 (RelE-like toxin)
MSGYDLRITTSAAKSLMKLPRVEQKRIRSAIDGLTTDPRPHGVTKLSGTTDSYRIRVGNYRIVYTIDD
GELIIVVVRIGHRKEIYR
>Bunnybear_gp28 (RelB-like antitoxin)
MSYSIREAKAKLSEVIRDAEDEPTIISNHGKPAAVVLSPERYEGLLEEIEDLRDRLAVHESRGEPTMS
FDKLVAELGLGD
>Waterfoul_gp86 (BrnT-like toxin)
MAKWTHEQVAHLRERHDLSVAEAEEALHDPSRVTIVPDPASRSGRGIRVVGYSRSAGTVLCVLIVE
HEGVMYGATAFPANATYRRIYQEGE
>Waterfoul_gp87 (BrnA-like antitoxin)
MMSKQTRDDLAATIREVATDAEVYEEVMGEHVELADVKITRGGPRTRVLQVRLDEREMQFVEDAA
AARGLPASTVAREILLSTLRRPSVDPDAKAELVGAFVRYLEGVDQKIRCDATTGPASVHETGPASM
QSAG
>LastHope_gp97 (HicB-like antitoxin)
MDSATKYTAIATPGEPGWLLVYVPEIEQYTQARGADEVAPMARDLIATWLDVPVESIEVEVTMTR
>LastHope_gp98 (HicA-like toxin)
MPNRTEVIAKIRKAARAKGLTFEFEREGGNHEIWILDGIRVPISRHKYVHDHLALKIYKQCQPKLGTG
WWR
(Or any other annotated toxin-antitoxin system in PhagesDB!)

Does AlphaFold3 predict interaction between these predicted TA complexes and the upstream DNA sequence?

- Relevant intergenic DNA sequences can be found in DNAMaster or by downloading the genome file from GenBank and viewing in free software SnapgeneViewer

Are these Cluster N HicB-like antitoxins orphan antitoxins? Or is there evidence that the adjacent gene is the cognate toxin?

- Xeno_gp31
- IdentityCrisis_gp33

TA systems are known to be somewhat promiscuous in their binding. Are these phage-encoded toxin or antitoxin proteins predicted to form complexes with host TA systems?

- To find various mycobacterial TA systems: https://mycobrowser.epfl.ch/