Response Record for March 5, 2020

Directed evolution of a genetic circuit
Yokobayashi Y, Weiss R, Arnold FH.
Proc Natl Acad Sci U S A. 2002 Dec 24;99(26):16587-91.

Presented by SystemSally and DeviceDude

Main conclusion and my assessment
The authors of this paper used directed evolution *in vivo* to optimize the performance of a genetic circuit. They started with a circuit in which the output of an IMPLIES gate was not matched to the input requirements of the subsequent NOT gate. They mutated the *cI* gene and its RBS in order to get the protein output of the IMPLIES gate to fall within the useable protein concentration range of the downstream NOT gate. They identified mutations in the RBS, mutants with altered start sites, and mutant proteins that were truncated due to premature stop codon introduction – none of which were especially interesting results. The mutants that they tout are the full-length λ repressor cI proteins with no change in the regulatory sequence – all of which affected the dimerization and cooperativity of cI. They admit that the mutations in the RBS are predictable (in fact, they were predicted by Weiss), but they also make the somewhat dubious claim that the mutations in the oligomerization domain would have been difficult to predict. Given the "rich structural data" available for this protein, it's strange to think that one couldn't have predicted mutations to this domain – especially since they were able to post-experimentally simulate the effect that altering the dimerization constant had on the circuit performance. I agree with the comments about this paper that were made in class during the discussion: that it was an interesting demonstration of a technique (directed evolution) to a problem (genetic circuit design), but that the authors should have extended the technique to develop new mutants in a system that was not as well understood as λ repressor cI.

Justification for inclusion in 20.902 and connection to synthetic biology field
This work falls into the "artificial network construction" part of the field of synthetic biology. The authors attempt to address an important problem in this field – that biological circuits are buggy. Synthetic biologists have found it quite difficult to precisely predict the behavior of simple circuits. This problem must be addressed before the field can hope to move to the more complicated circuits that will be needed to create solutions to real-world problems. In addition to adding another tool to the synthetic biologist's repertoire, the authors created a set of new genetic devices with a range of behaviors.

For the presenters
Sally and Dude gave a well-organized presentation. I liked how they pulled some of the modeling results out of supplementary information. Sally is clearly comfortable speaking in front of groups and her talk was easy to follow. Dude seemed a bit nervous and did not make much eye contact. I think he knows a lot more than he appears to know – a bit more confidence would make his audience trust him more.