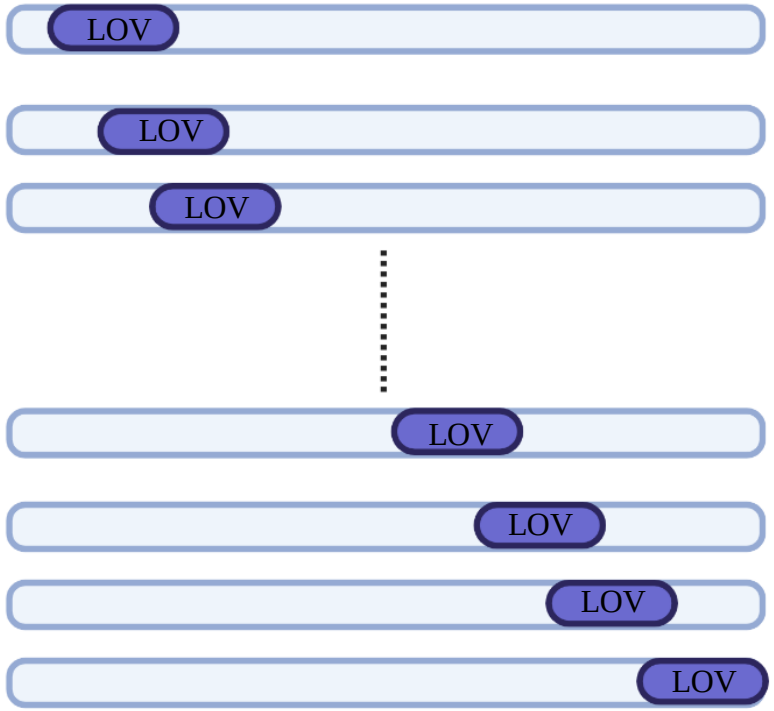
Engineering a photo-controlled Rep helicase

Sonisilpa Mohapatra [a], Tunc Kayikcioglu [a] , Chang-Ting Lin [a], Taekjip Ha [a] *

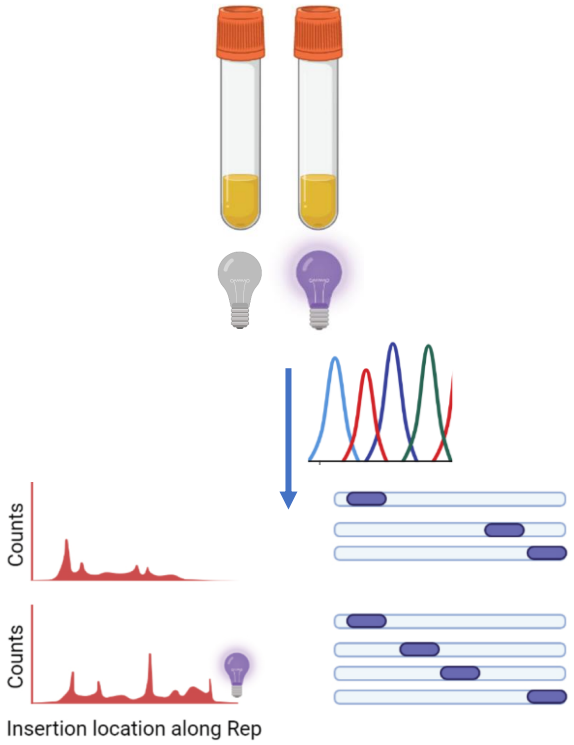
[a] Department of Biophysics and Biophysical Chemistry, School of Medicine, Johns Hopkins University

*tjha@jhu.edu

Generation of Rep-LOV library

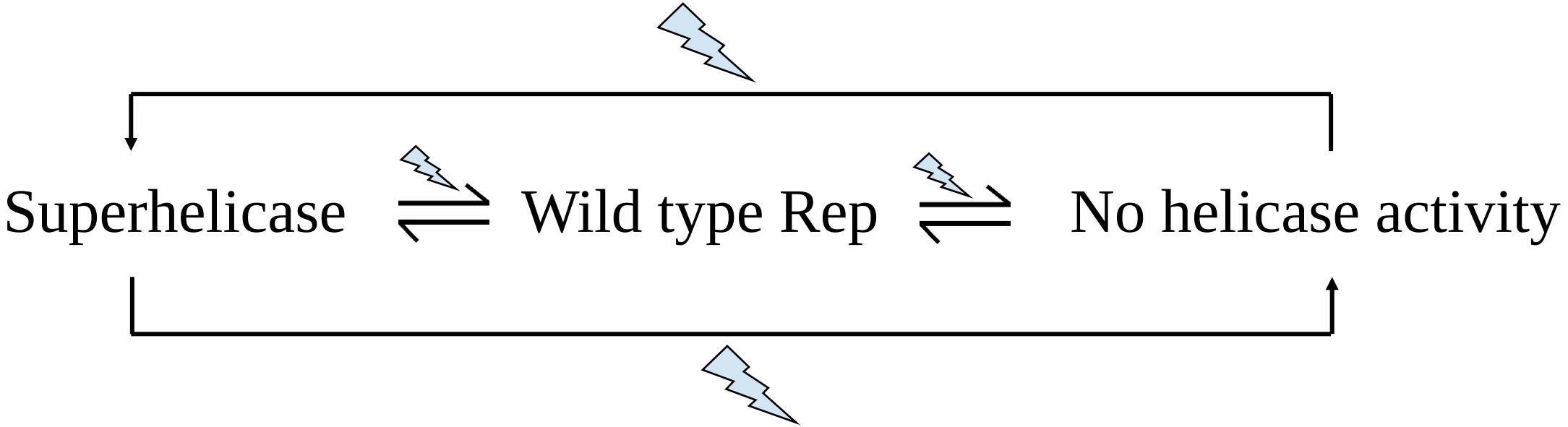


High-throughput screening of Rep

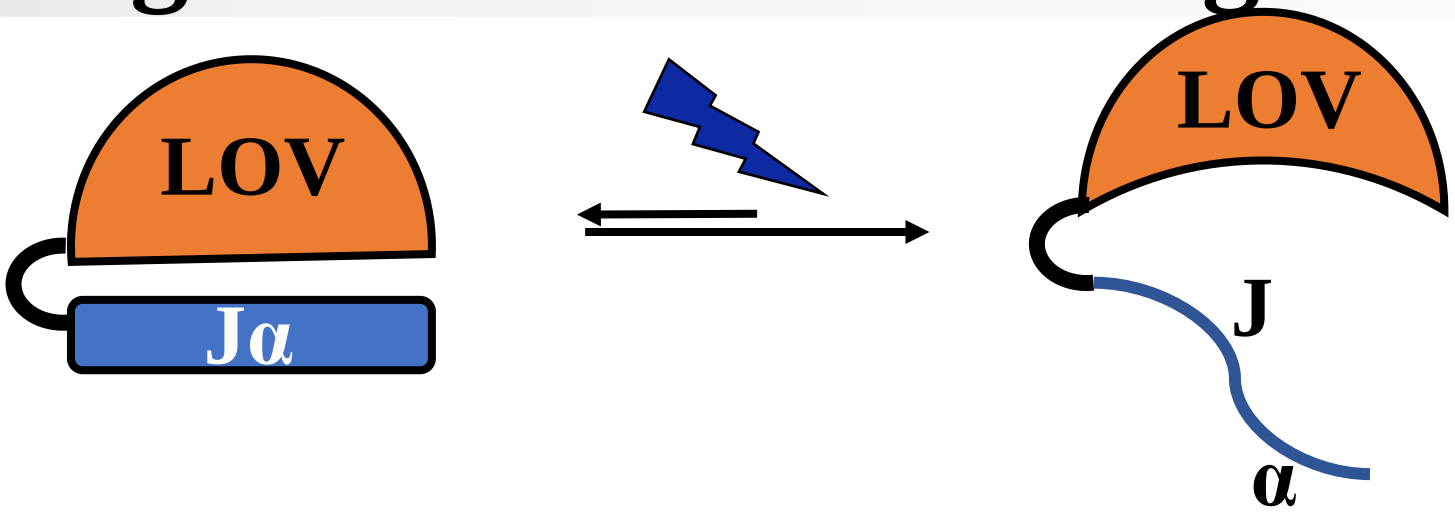


Identify survivors

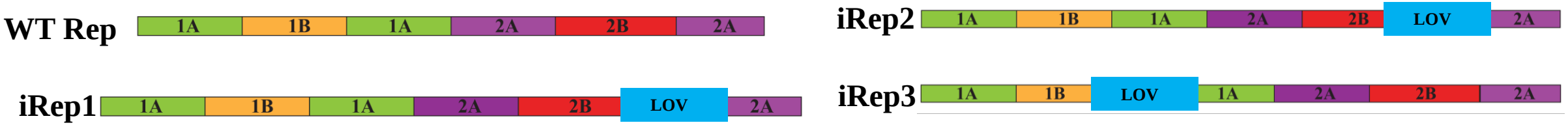
1. Photo-controlled Rep helicases

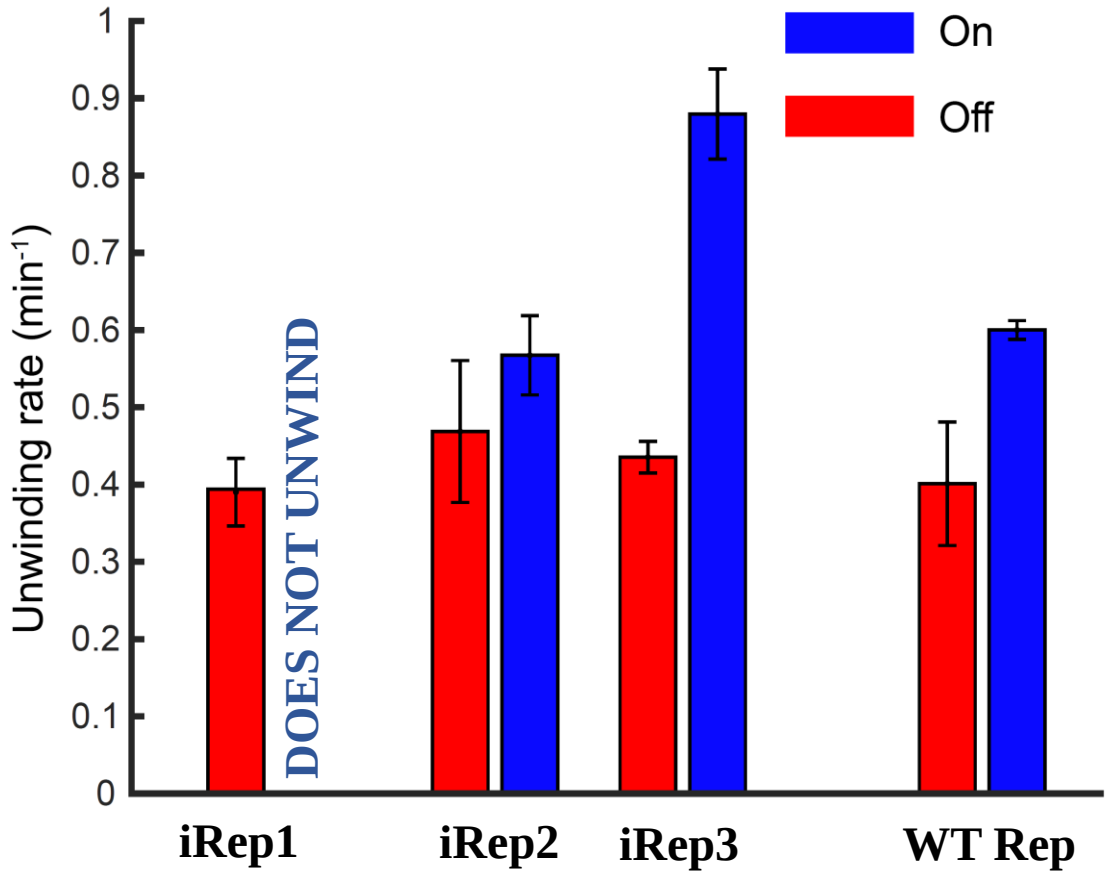


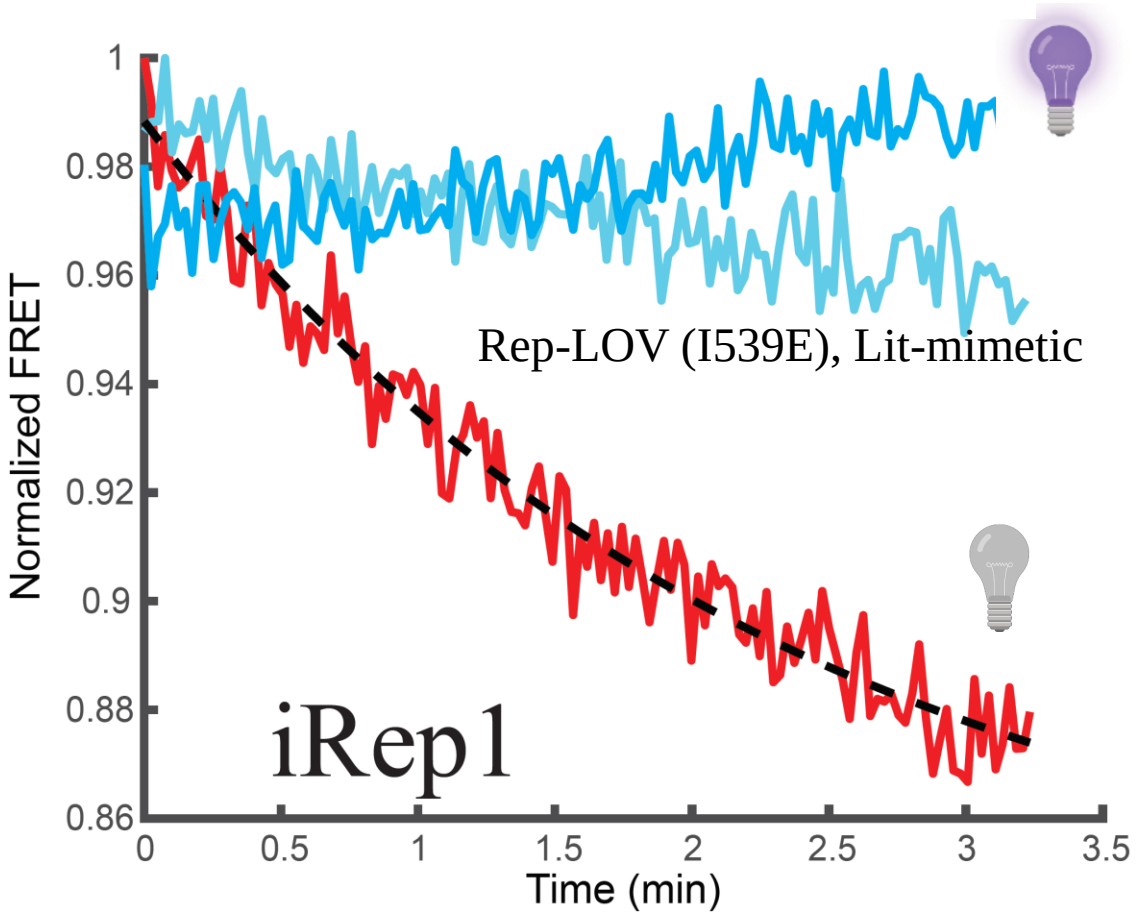
2. Using LOV domain for engineering Rep



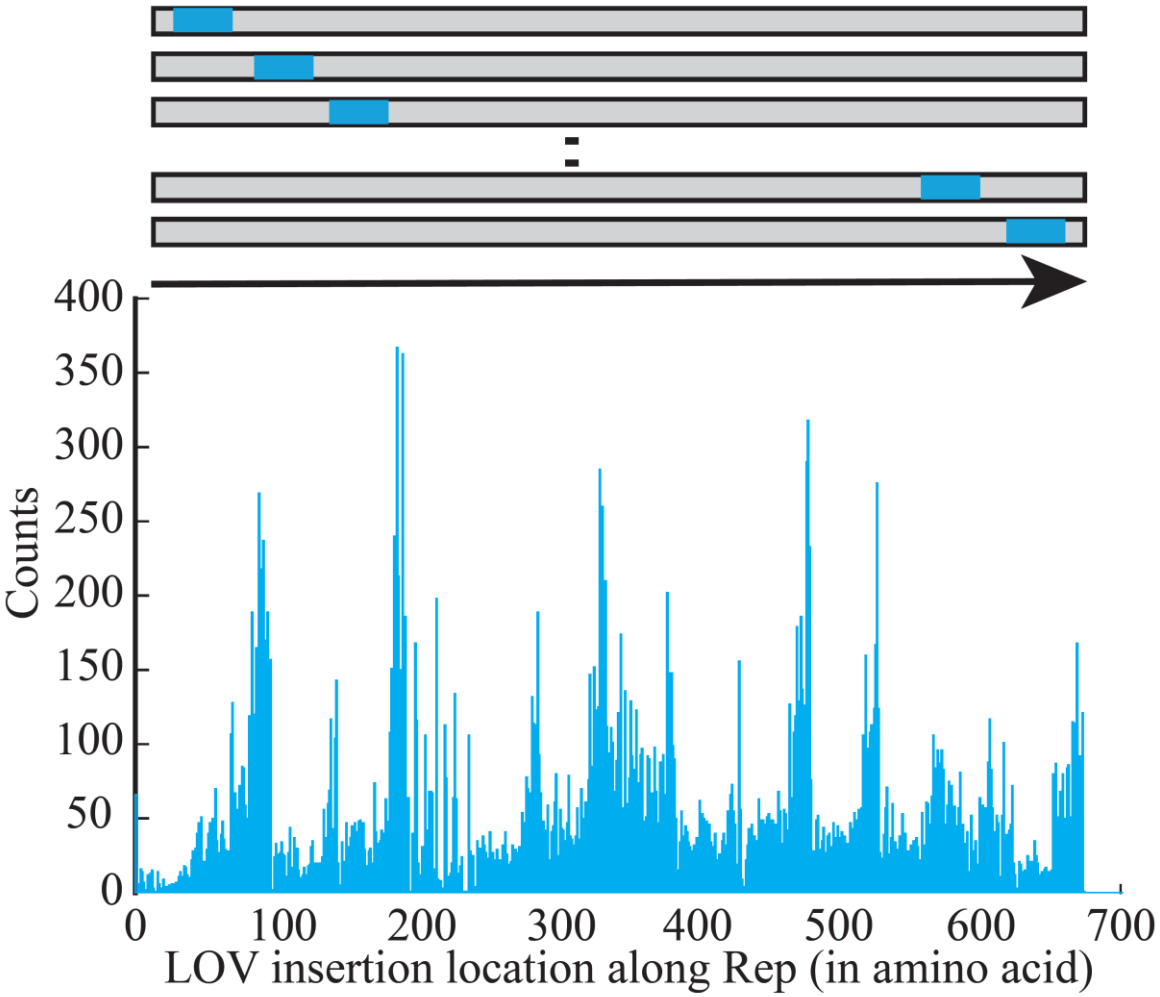
3. Unwinding activity of different Rep-LOV variants





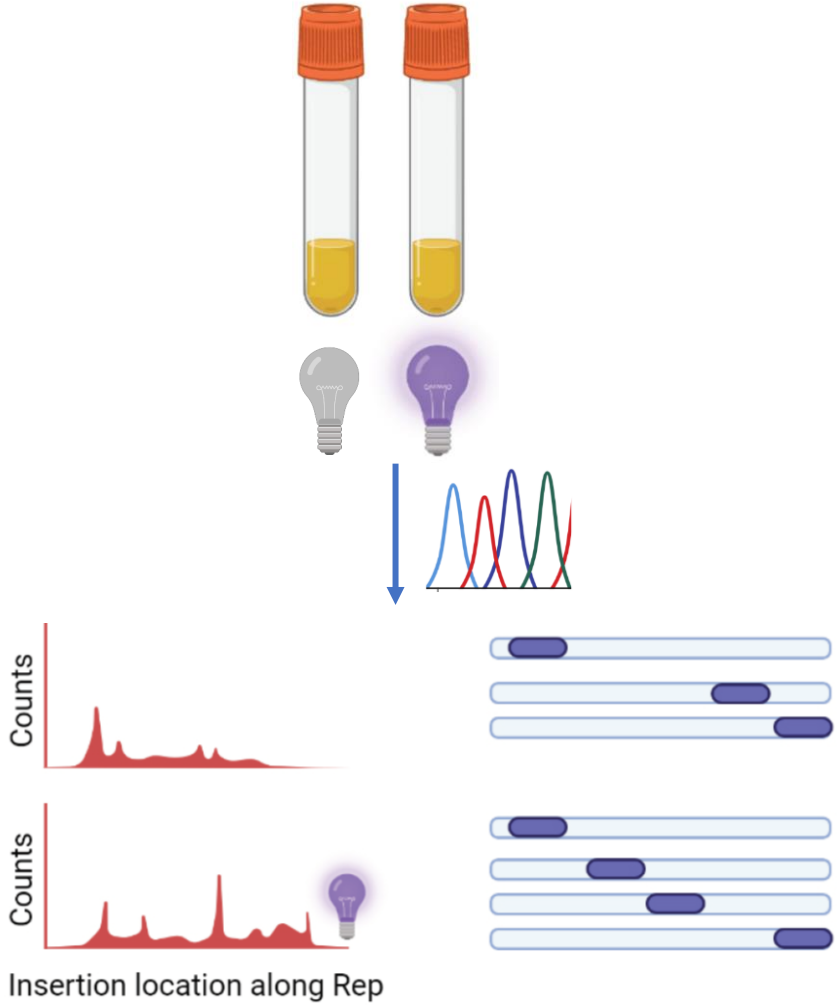


4. Generation of Rep-LOV library



Coyote - Maestas *et al*, Nucleic Acids Research (2019)

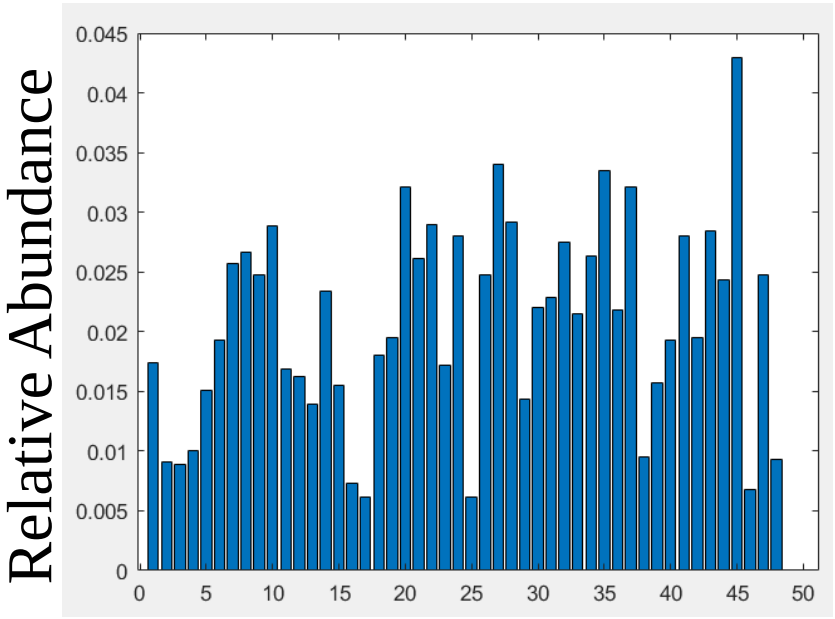
5. High-throughput screening of photo-controlled Rep

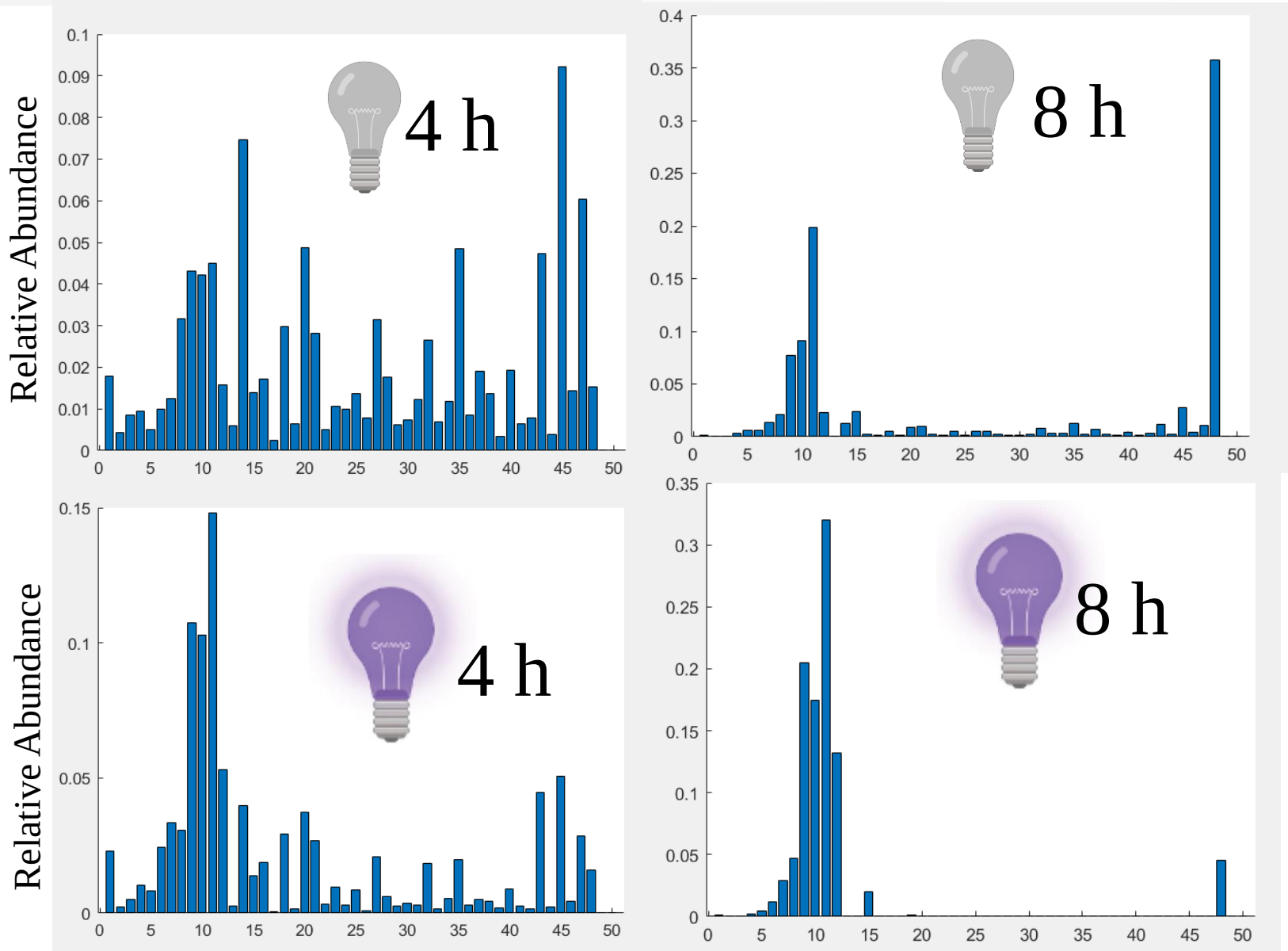


Identify survivors

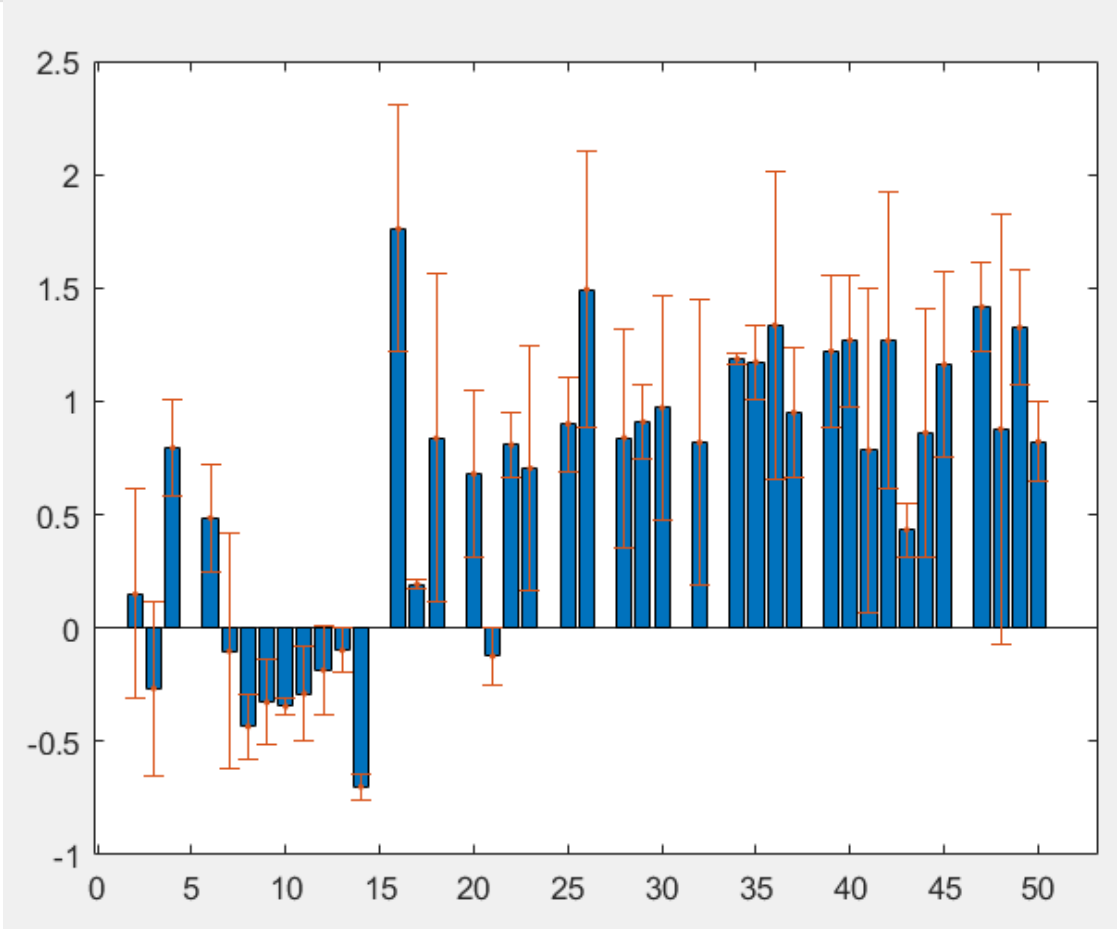
6. High-throughput screening of a small prototype Rep – LOV library

Starting library





7. Identification of photo-controlled Rep variants



8. Conclusions

- Engineered a photo-activatable and a photo-deactivatable Rep helicase
- Generated a ~ 700 membered library of Rep-LOV variants
- Developed high throughput screening assay for Rep-LOV library

9. Acknowledgments

Howard Hughes Medical Institute

Prof. Mark Dillingham
Prof. Mark Leake
Prof. Peter Mcglynn