UNIVERSITY *of* WASHINGTON

# Massively-Parallel Measurement of Protein-Protein Interactions

Alexandr Baryshev, Benjamin Groves, Sergii Pochekailov, Peter Matulich, Georg Seelig

## INTRODUCTION

Quantitatively characterizing protein-protein interactions (PPI) is essential for understanding the function and evolution of proteins inside cells, the disruption of protein interaction networks in disease, or the de novo design of protein assemblies.

## OBJECTIVE

Our project aims to develop an easy-to-use high-throughput method for measuring protein-protein interactions by translating it into a next generation sequencing experiment. With our method we aim to provide a simple and robust method for measuring:
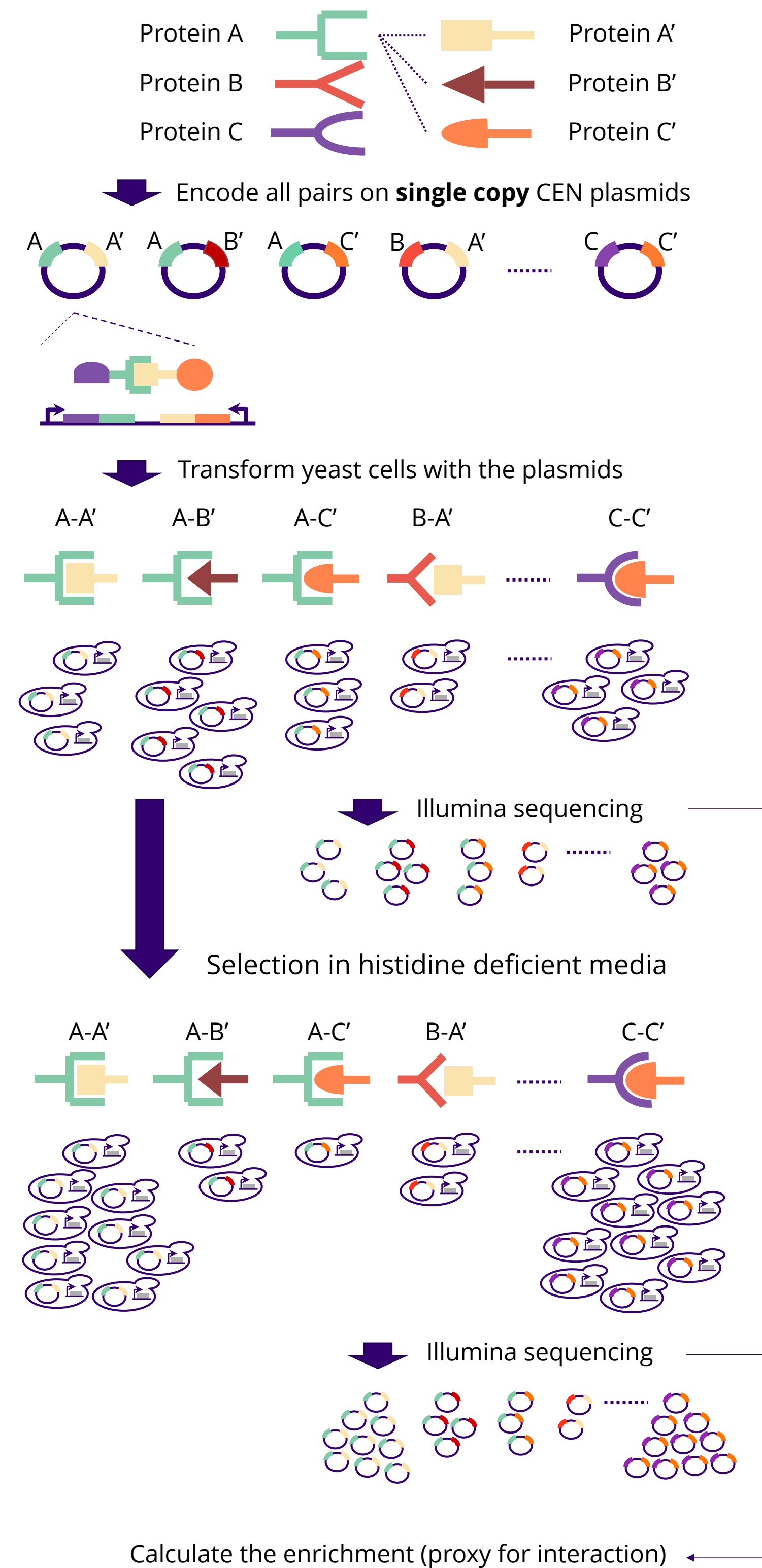
- De novo protein assembly
- Determining the functional domains of proteins
- Uncovering the human interactome

## OVERVIEW

Our approach is based on the classic yeast two-hybrid (Y2H) system [1], in which the growth rate of yeast is proportional to the strength of a protein-protein interaction.
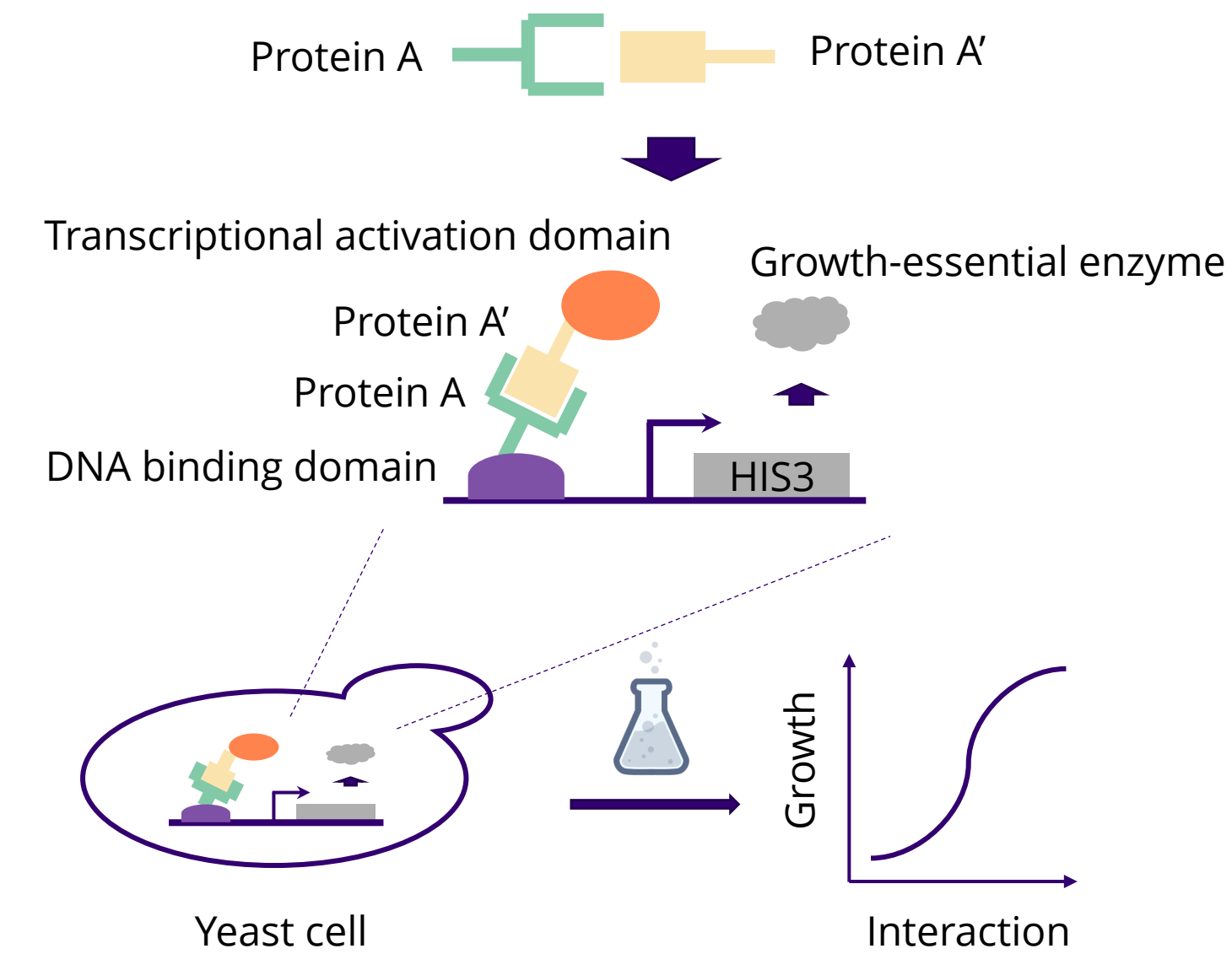


## SCALING UP



## PROOF-OF-CONCEPT

In a proof-of-concept experiment, we have simultaneously screened all possible interactions between 6 homologous proteins from BCL2 family proteins and 9 de novo designed inhibitors of those proteins using the Illumina NextSeq platform. These interactions have been previously thoroughly characterized by a biolayer interferometry [2] and AlphaSeq [3] methods with the $R^2$=0.89 correlation between the two methods.

In figure below, we compare log values of the enrichment scores we obtained using our high-throughput yeast two-hybrid approach (left) and negative log values of dissociation constants KD obtained for the same pairs using a biolayer interferometry method in [2] (center). We are getting $R^2$=0.62 correlation between the methods.

In the next steps, we plan to further optimize our method to achieve higher correlation numbers.
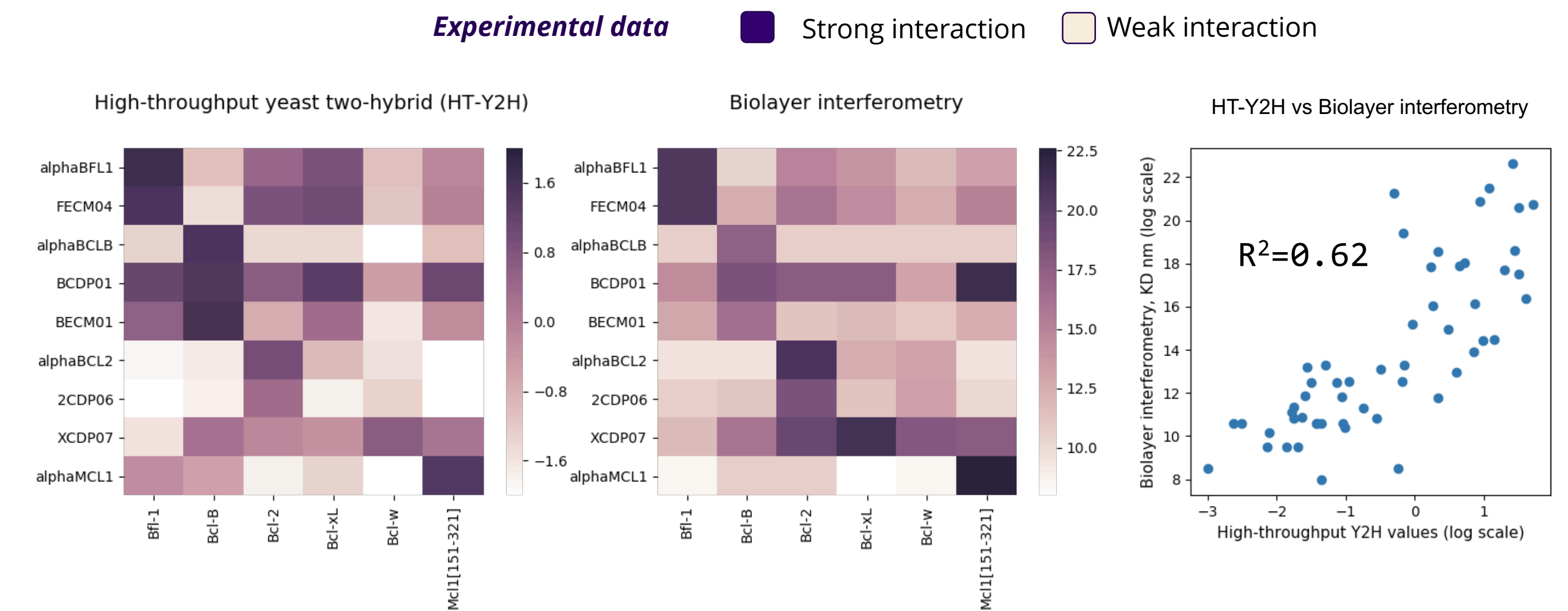


## CONCLUSIONS

- We developed a novel method for high-throughput protein-protein interaction measurements.
- Our method has good correlation with other methods on previously thoroughly studied protein-protein interaction sets.
- In future work, we will increase library size to study thousands of proteins with millions of possible interactions.
- We will apply our method to study interactions in libraries of designed as well as naturally occurring proteins.

## REFERENCES

1. Fields, S., & Song, O. (1989). A novel genetic system to detect protein–protein interactions. *Nature*, 340(6230), 245-246. doi:10.1038/340245a0
2. Berger, S., . . . Baker, D. (2016). Computationally designed high specificity inhibitors delineate the roles of BCL2 family proteins in cancer. *Elife*, 2016 Nov 2;5. pii: e20352. doi: 10.7554/eLife.20352.
3. Younger, D., Berger, S., Baker, D., Klavins, E. (2017) Proc Natl Acad Sci U S A. Nov 14;114(46):12166-12171. doi: 10.1073/pnas.1705867114.

## CONTACTS

Alexandr Baryshev (baryshev@uw.edu)
Sergii Pochekailov (sergii@uw.edu)
Benjamin Groves (benjamingroves@gmail.com)
Georg Seelig (gseelig@uw.edu)