

Developing predictive models of enzyme function from physics and phylogeny

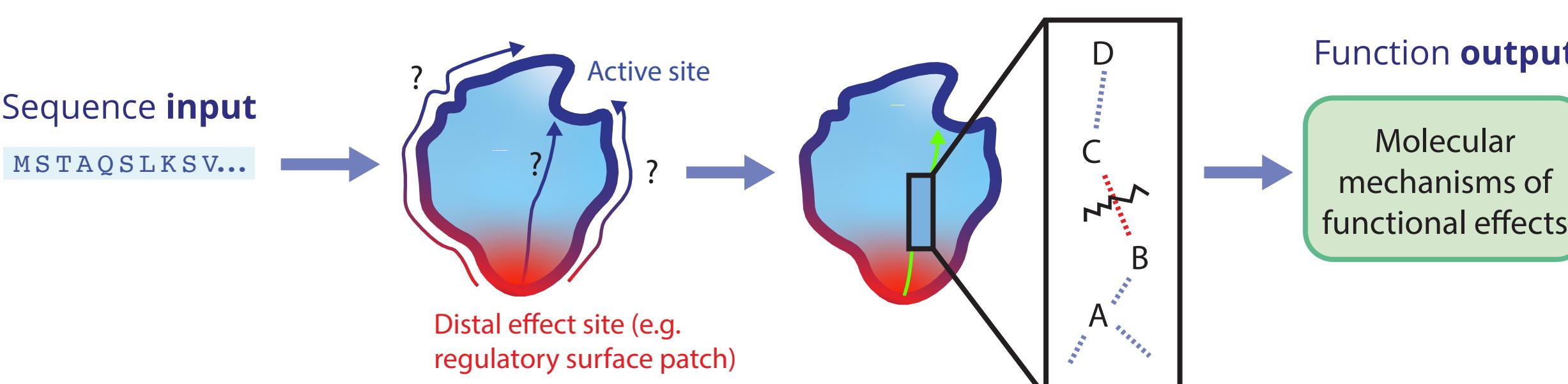
Micah Olivas¹, Craig Markin³, Dan Herschlag³, and Polly Fordyce^{1,2}

¹ Department of Genetics, Stanford University

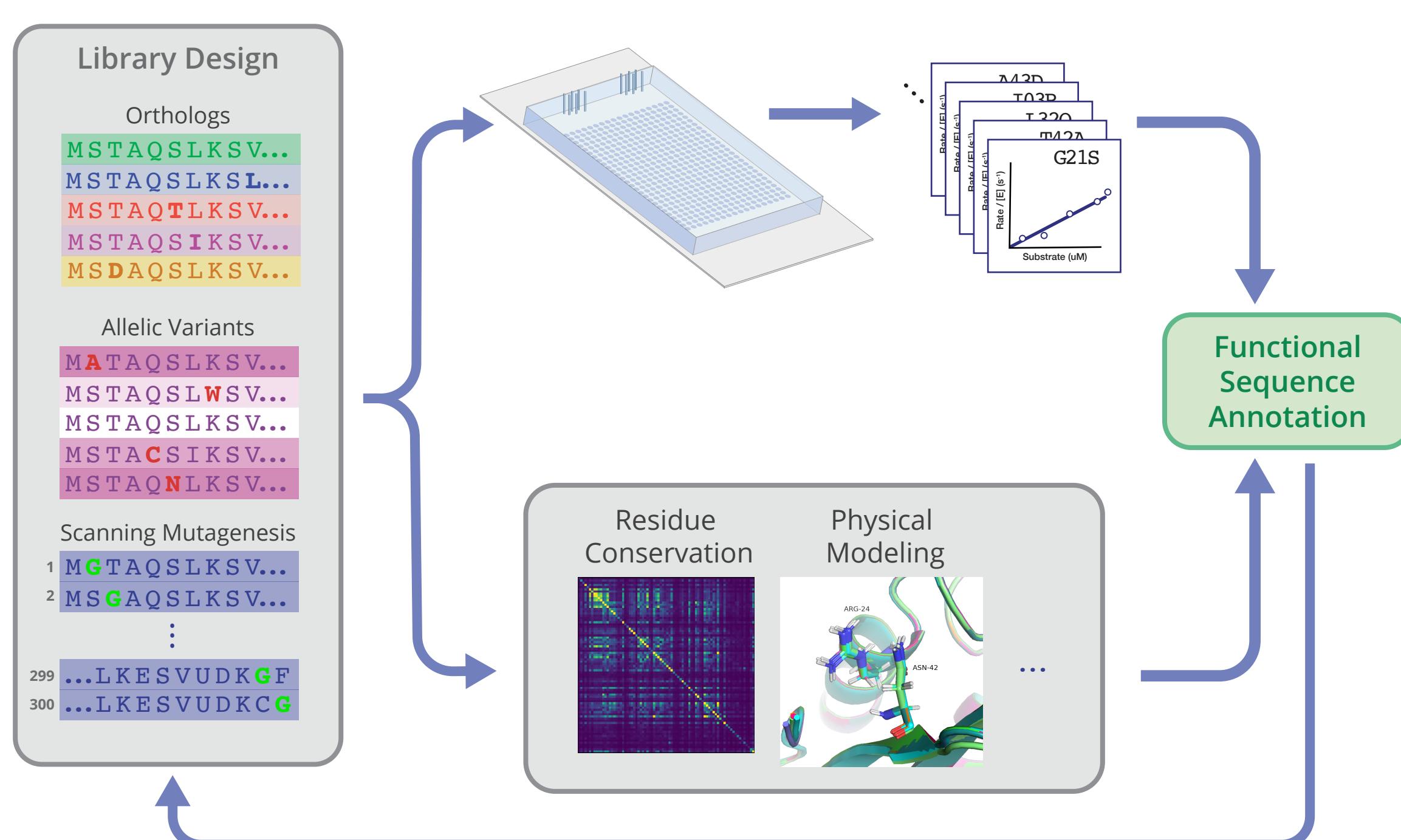
² Department of Bioengineering, Stanford University

³ Department of Biochemistry, Stanford University

Challenge: Predict protein function from amino acid sequence

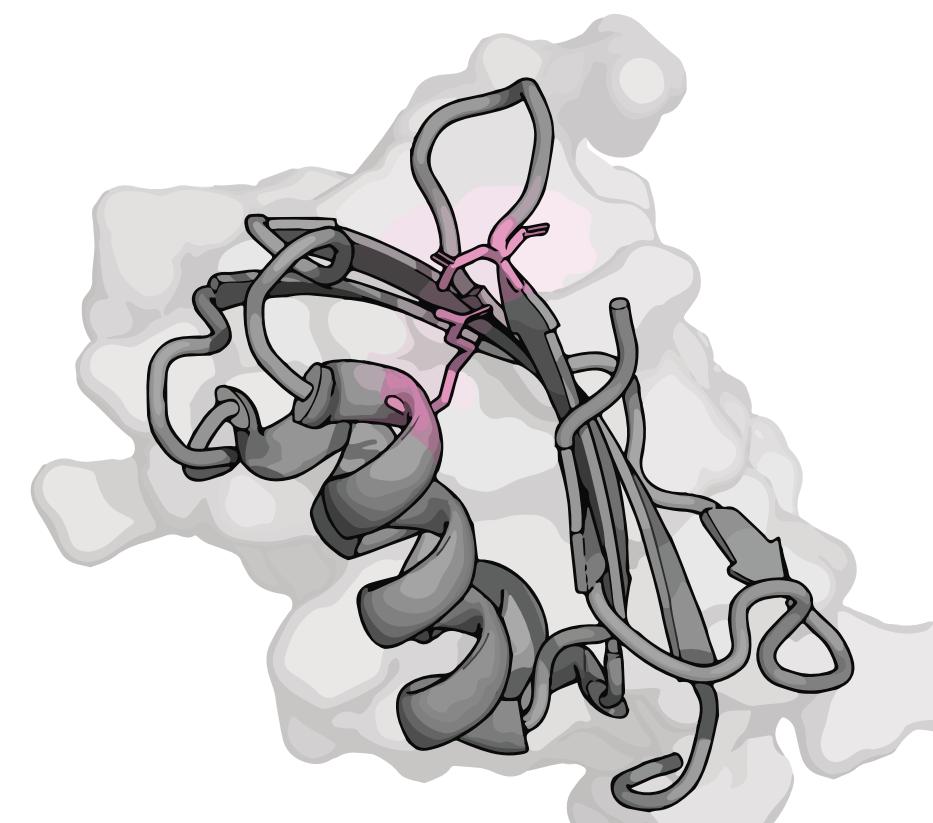


Approach: Pair mutagenesis scans and protein prediction models to infer function



Acylphosphatase 2 is a small, catalytically efficient, universally-conserved model enzyme

1 One of the smallest enzymes at 100 amino acids



PDB ID: 1URR
(*D. melanogaster*)
Acylphosphatase 2

2 Catalytic mechanism is well established

ACYP2 catalysis was accurately quantified on chip

1 Enzyme concentration-normalized rates were found to be linear across chambers of the chip

2 Kinetic proficiency of wildtype enzyme (K_{cat}/K_m) with acetyl phosphate was within 2-fold of literature rates and a separate off-chip validation

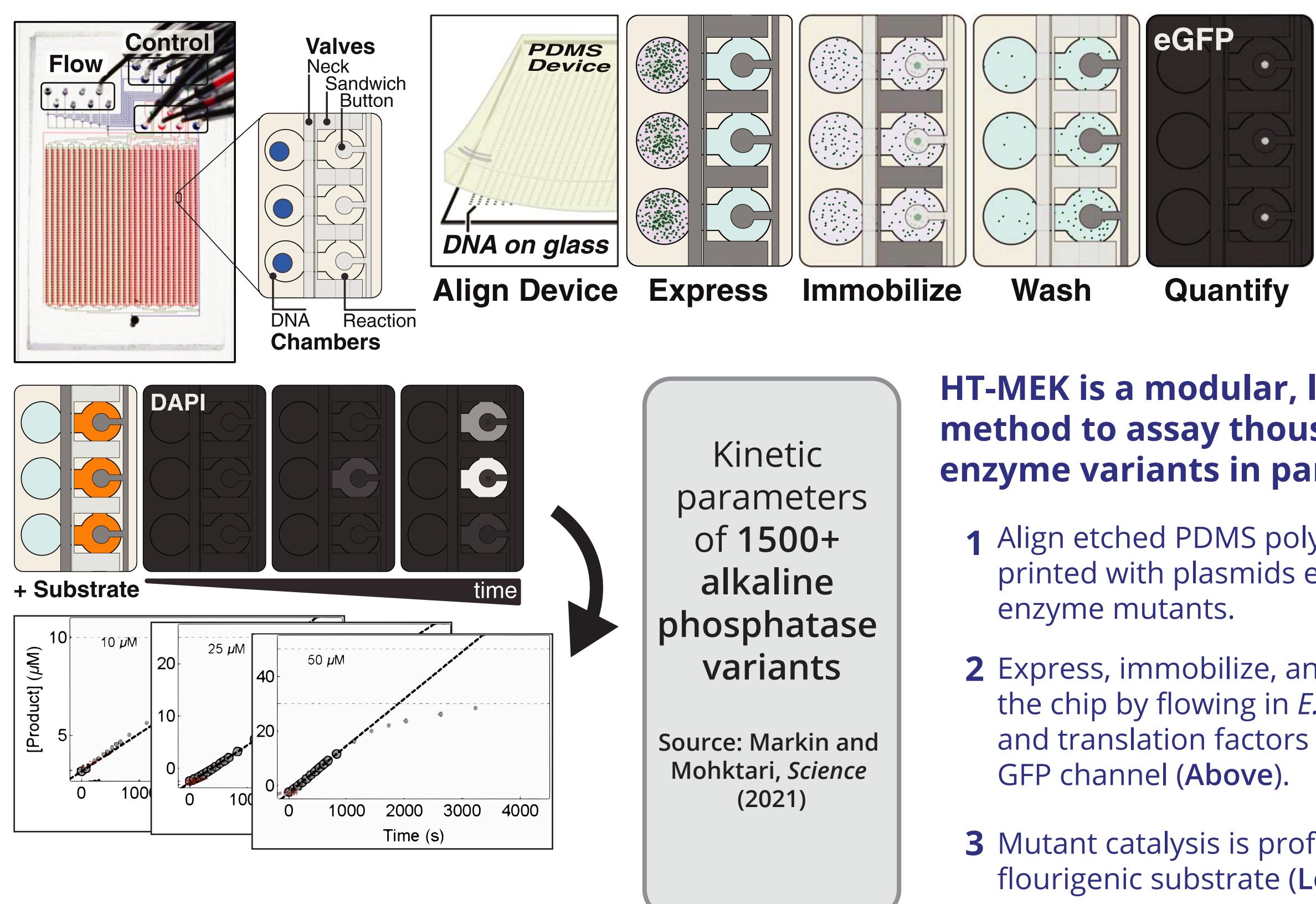
Future:

Quantify mutant catalytic parameters on chip. Small library experiment in progress (10 mutants) and full scan (900+ mutants) planned

4 Reactive with multiple classes of substrates

5 Several penetrant allelic variants

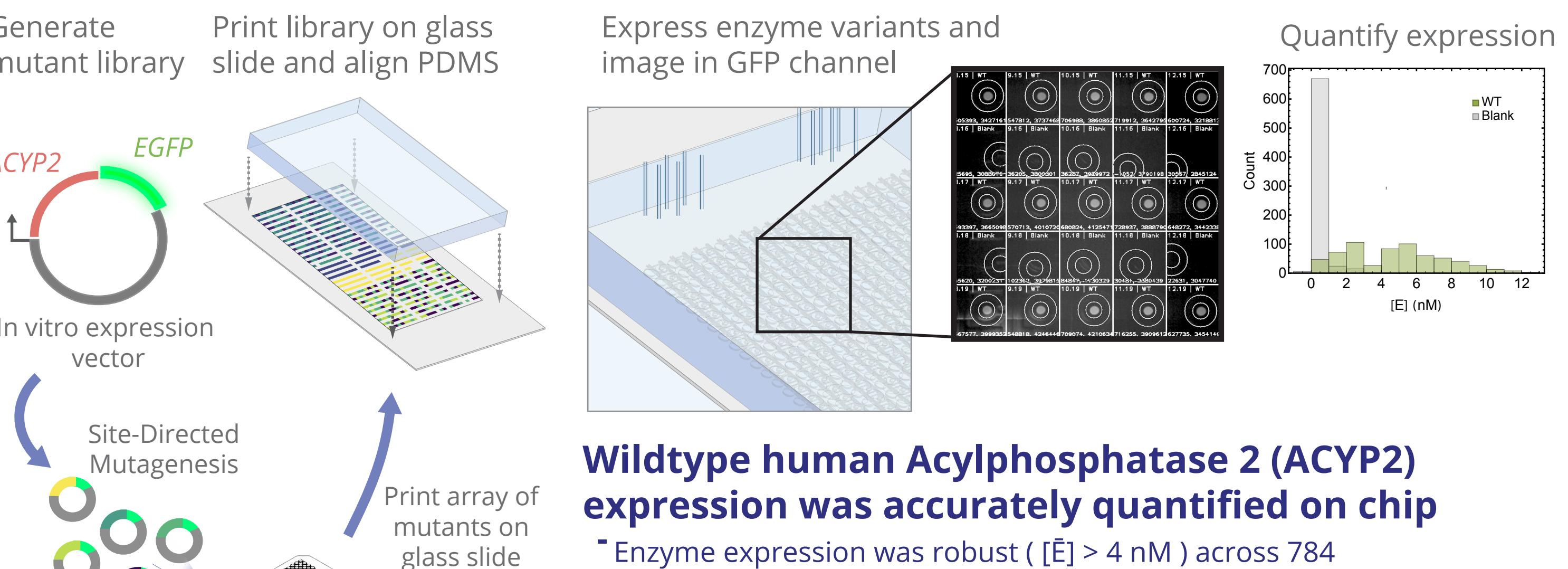
High-Throughput Microfluidic Enzyme Kinetics (HT-MEK)



HT-MEK is a modular, low-cost method to assay thousands of enzyme variants in parallel.

- 1 Align etched PDMS polymer to glass slide printed with plasmids encoding eGFP-tagged enzyme mutants.
- 2 Express, immobilize, and quantify enzyme in the chip by flowing in *E. coli* transcription and translation factors and imaging in the GFP channel (Above).
- 3 Mutant catalysis is profiled by flowing on a fluorogenic substrate (Left).

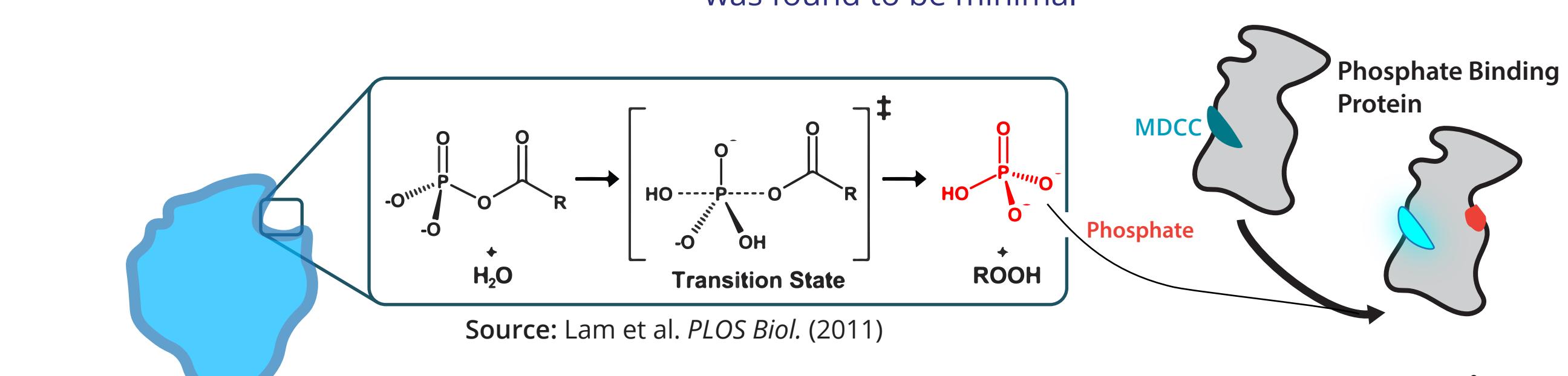
Can we accurately quantify Acylphosphatase 2 activity in high throughput?



Wildtype human Acylphosphatase 2 (ACYP2) expression was accurately quantified on chip

- Enzyme expression was robust ($[E] > 4 \text{ nM}$) across 784 replicates on device

- Crosstalk between chambers (diffusion of enzyme or product) was found to be minimal



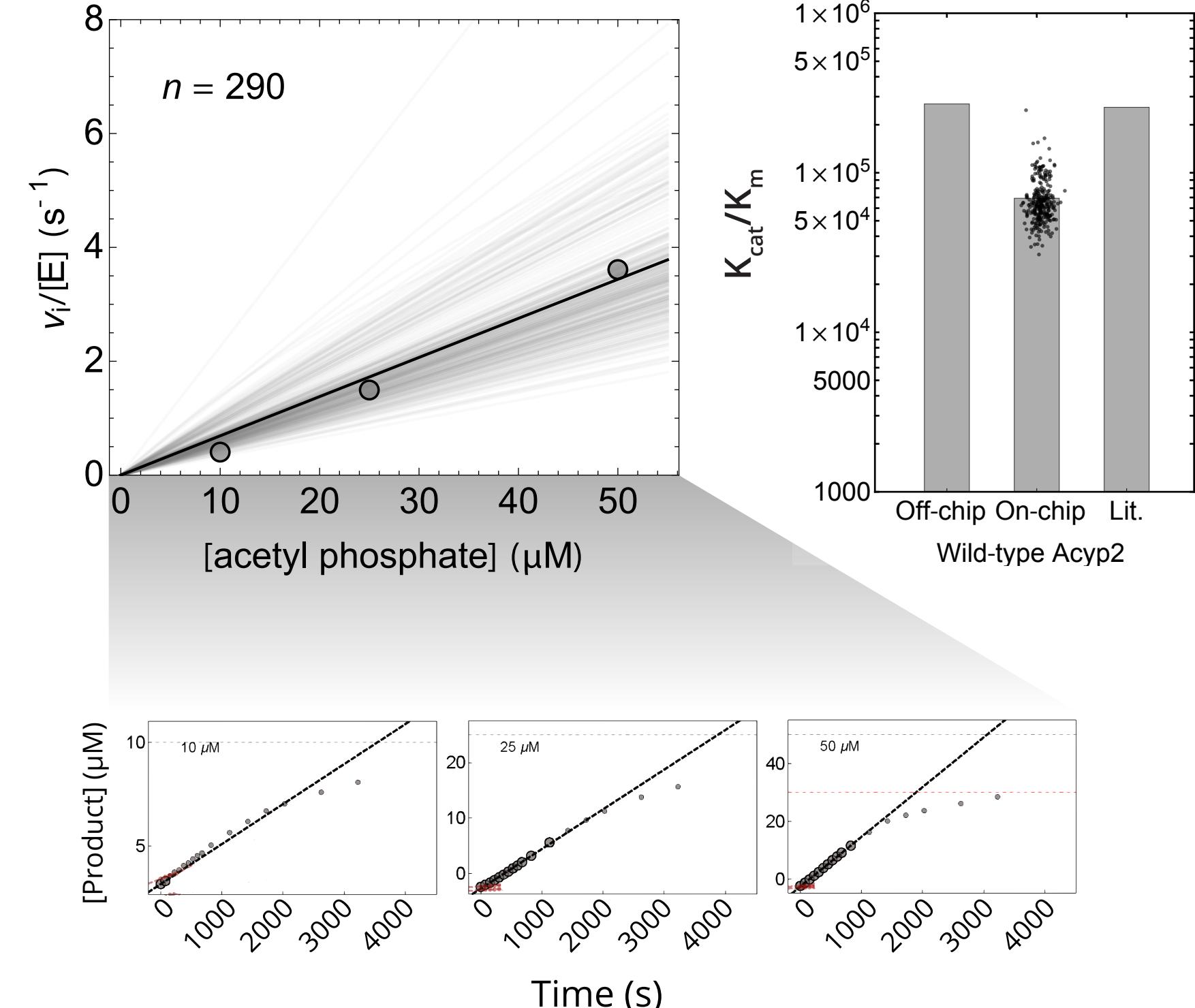
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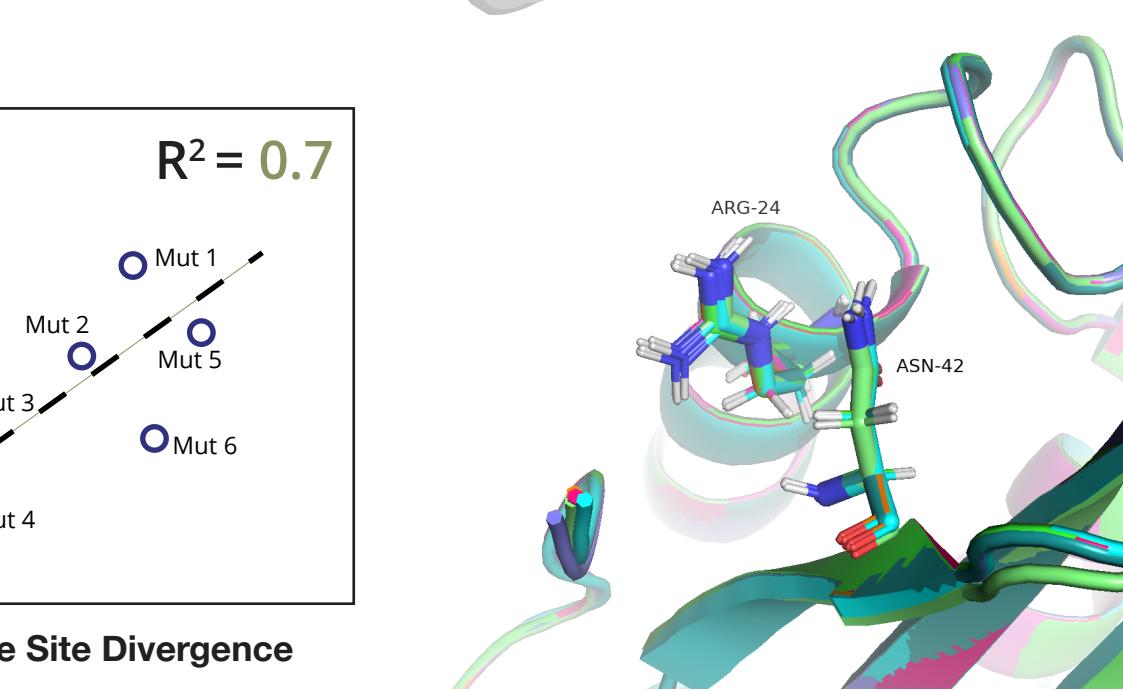
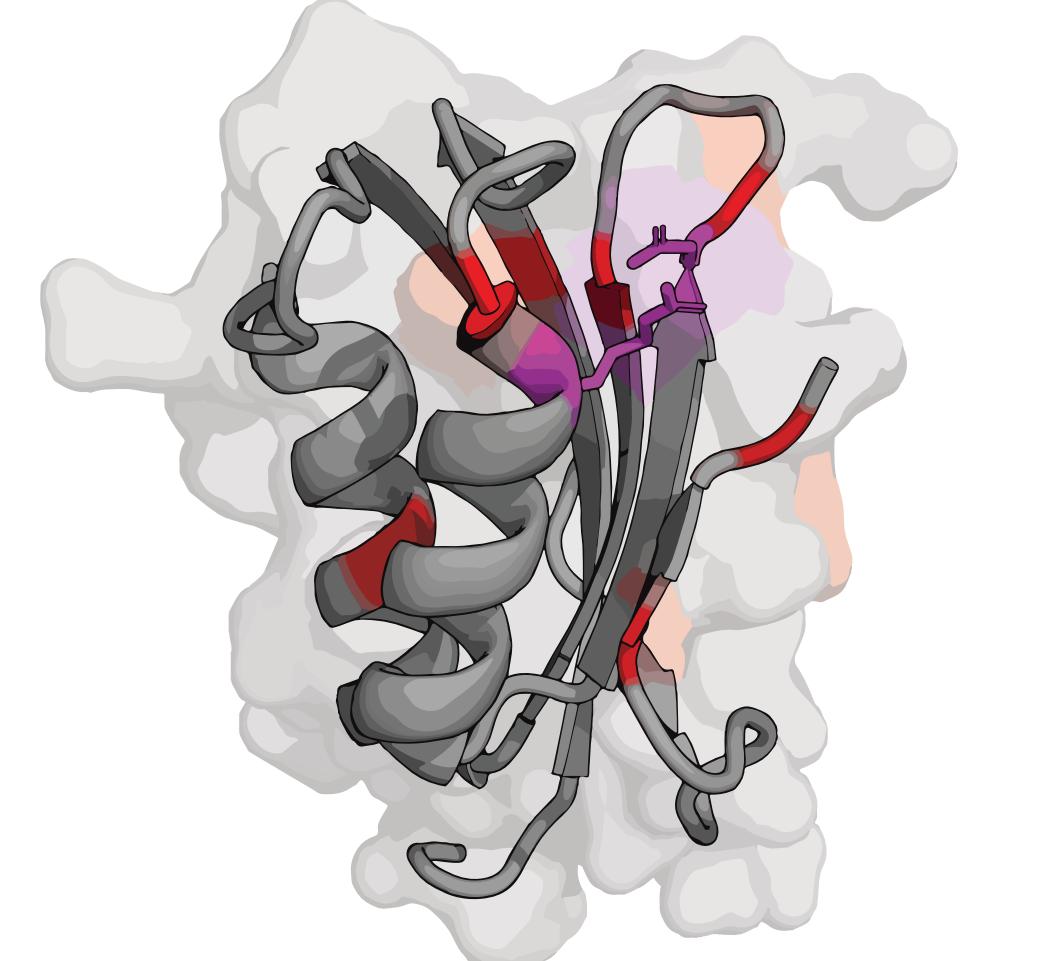


Toward sequence-to-function prediction

Pairing mutant kinetic effects and AlphaFold structure predictions

Residue	Effect	$\Delta\Delta G_{U-F}^{H_2O} (\text{kJ mol}^{-1})$
C21S	Stability	-
F94L	Stability	-18.1 ± 1.1
M61A	Stability	-16.6 ± 2.2
N41A	Active Site	-
N41Q	Active Site	-
R23A	Active Site	-
R97A	Active Site-adjacent	-
R97Q	Active Site-adjacent	-
T42A	Stability	-7.7 ± 1.7
V13A	Stability	-11.0 ± 1.4

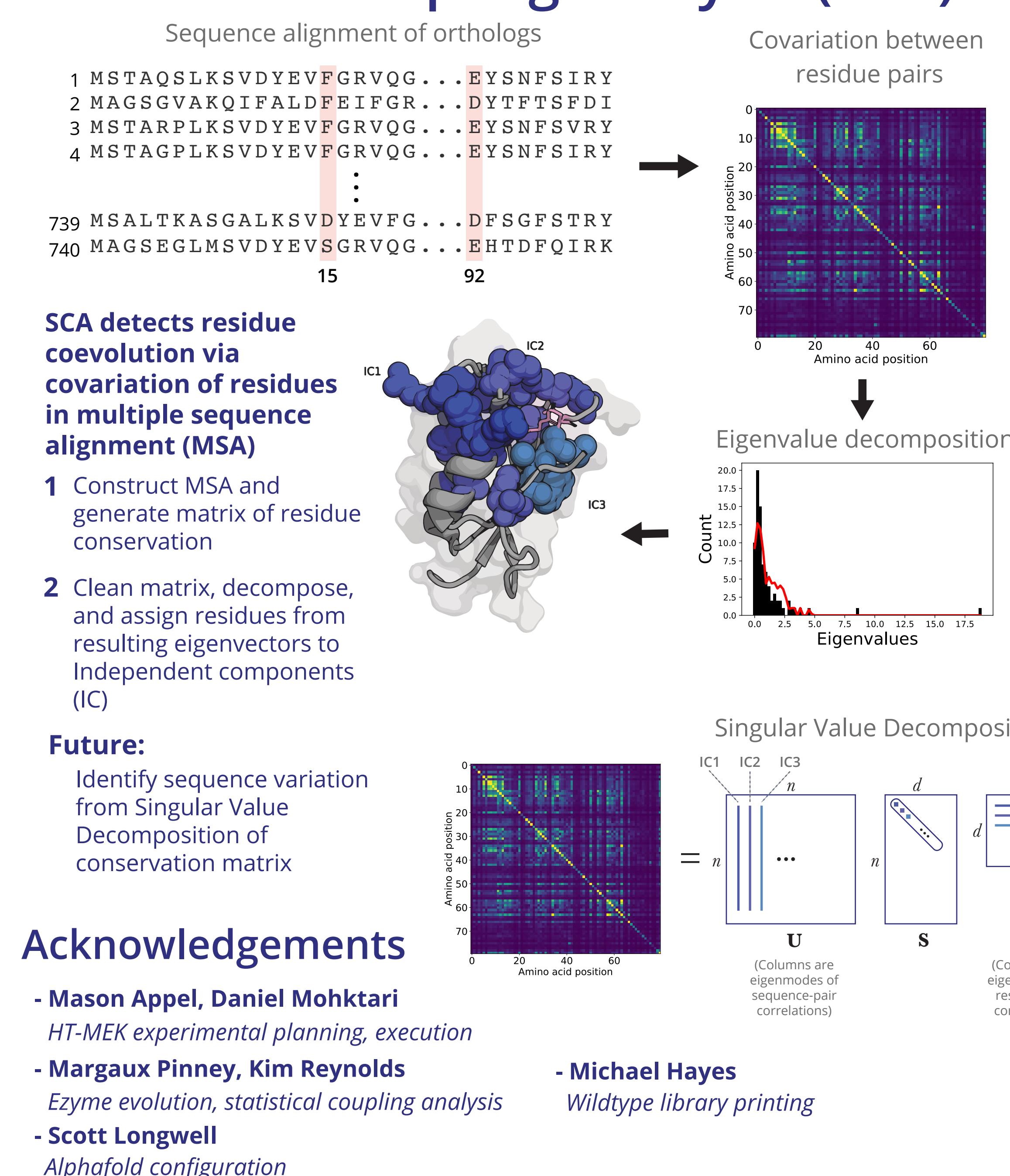
Source: Chiti et al. 1999. *Nat Struct Biol*



AlphaFold structure predictions of mutants reveal conformational change at active site

- Structural predictions of mutants at all residue positions will be compared to mutant on-chip kinetics (mutants in table above-left; aligned structures above-right) in correlation matrix (simulated data; left)

Detecting residue coevolution by Statistical Coupling Analysis (SCA)



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- Mason Appel, Daniel Mohktari
HT-MEK experimental planning, execution

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Enzyme evolution, statistical coupling analysis

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AlphaFold configuration

- Michael Hayes
Wildtype library printing

