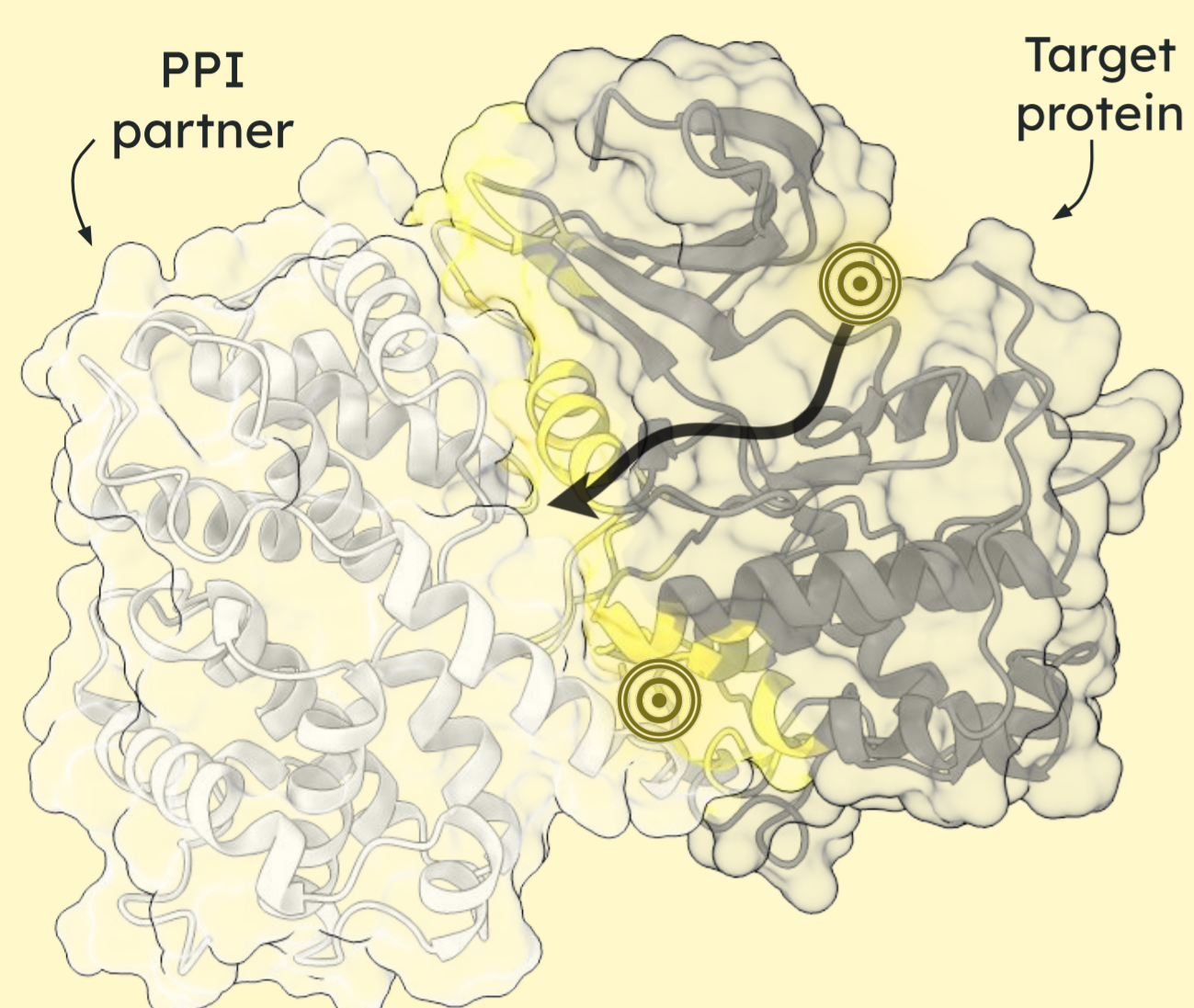


PPI hotspot mapping for the rapid discovery of novel therapeutics

Mireia Seuma[†], Sol Adaglio[†], Pablo Baeza[†], Grzegorz Chojnowski, Júlia Domingo[†], André J Faure[†], Lukas Lim[†], Cristina Lorca, Youssef Mansour[†], Raphaëlle Palau[†], Andrei Papkou[†]

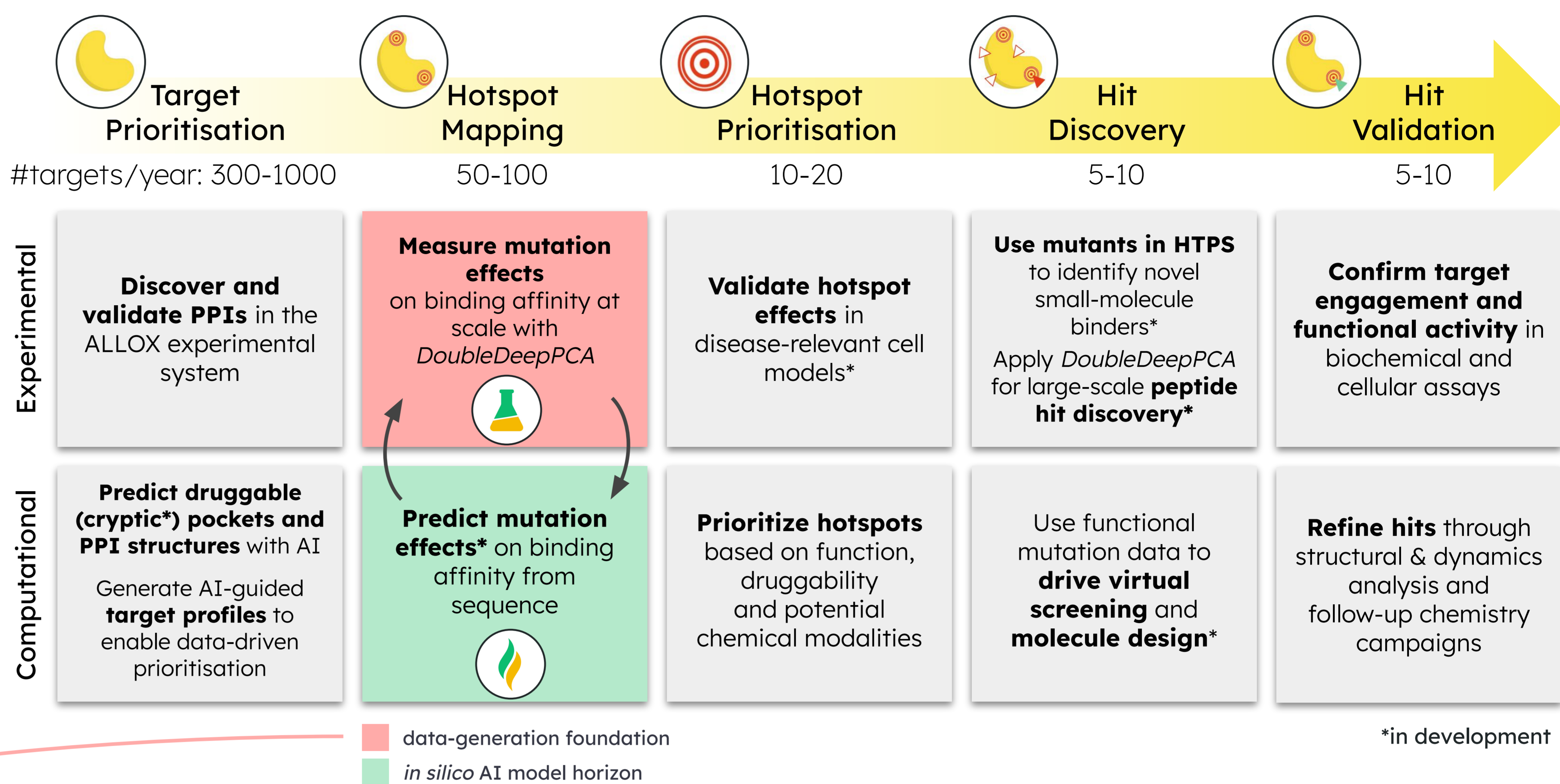
ALLOX's mission

Challenges of targeting PPIs	Solutions
▶ Large & featureless	▶ Allosteric hotspot
▶ Highly conserved	▶ Orthosteric hotspot



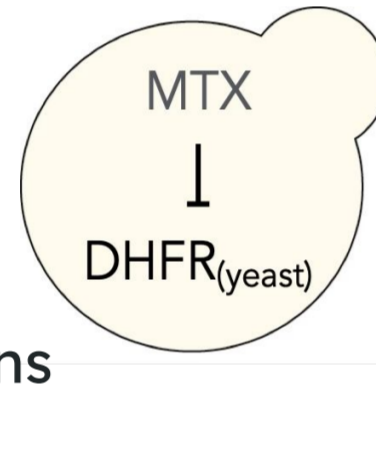
Need to systematically map therapeutic hotspots

Integrated Drug Discovery Platform

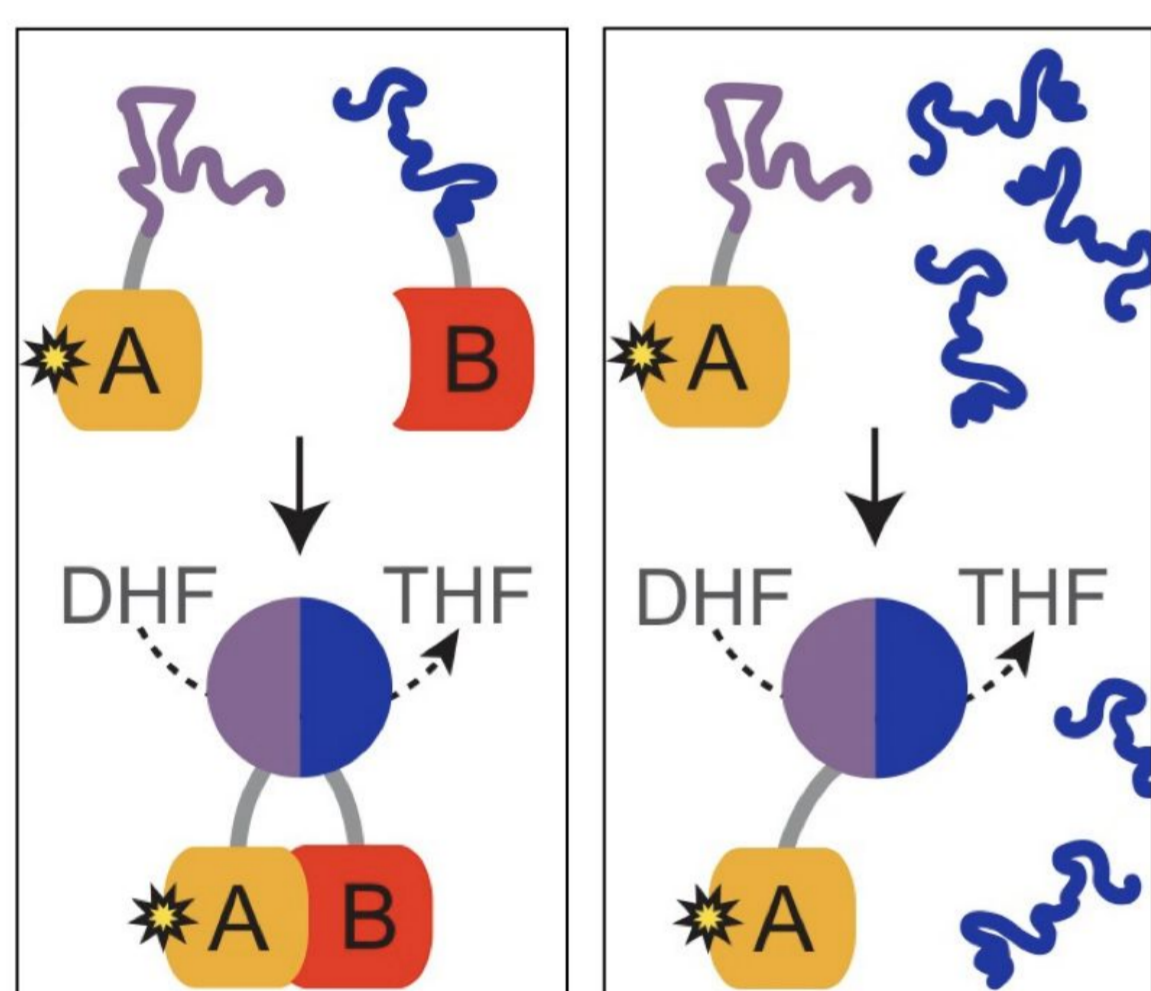


DoubleDeepPCA is a DMS-based protein complementation assay (PCA) approach to infer changes in binding affinities via the combined measurement of mutation effects on binding & abundance.

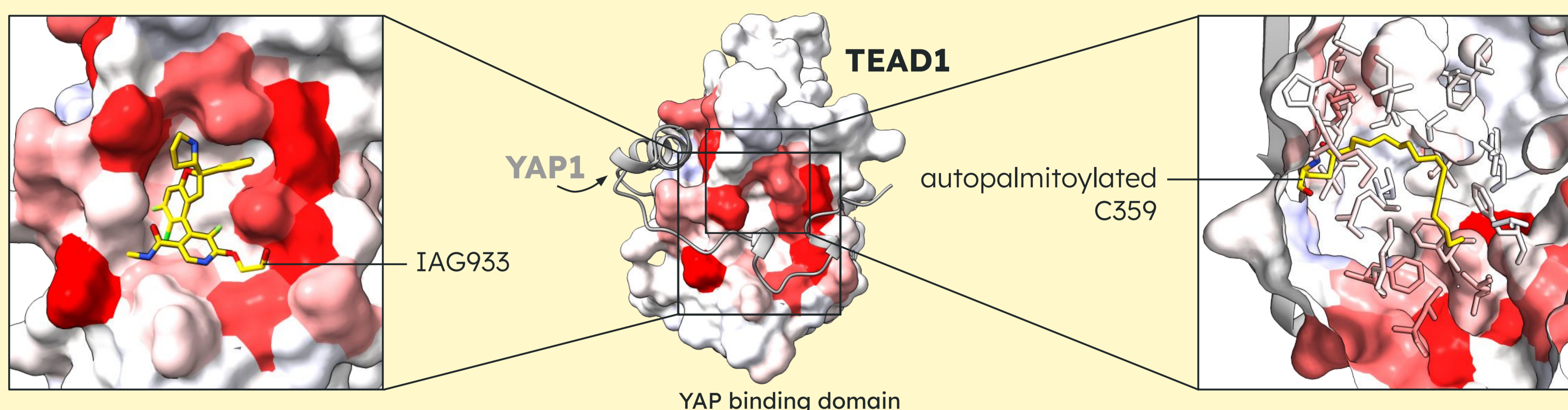
Complementation of exogenous split DHFR enzyme rescues growth defect in selective conditions (MTX, Methotrexate)



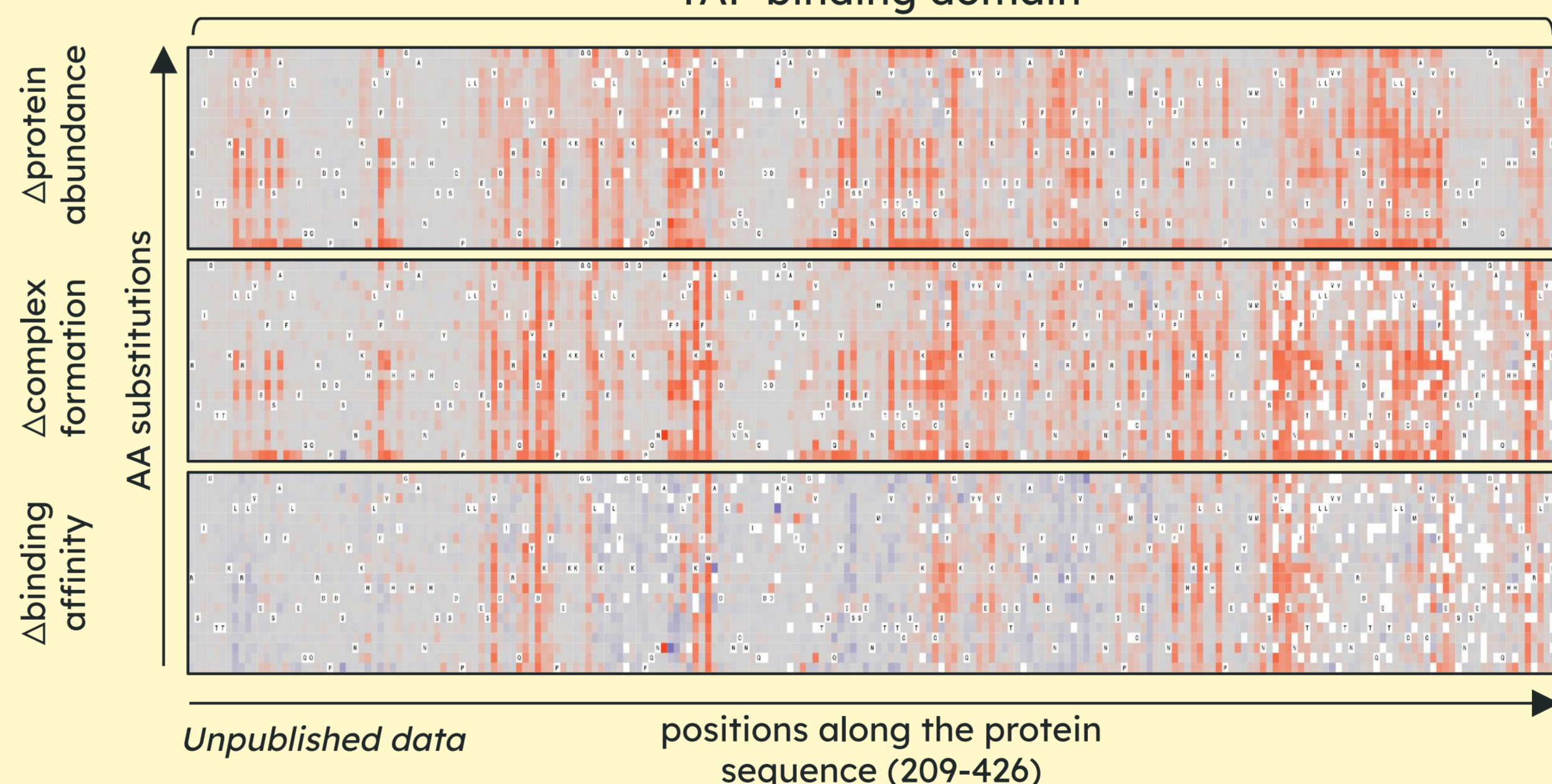
bindingPCA abundancePCA



Case study: Validation of TEAD1 therapeutic hotspots



Identification of binding interface hotspots
6/7 top residues are within 5Å of the orthosteric PPI inhibitor IAG933*



Identification of allosteric lipid pocket residues
Binding site of known allosteric drugs**

Hotspot mapping status

Pathways	Status	
GTPases	MAPK pathway, growth signaling	●
CDK	Cell cycle progression	●
CDK regulators	Cell cycle progression	●
Translation factors	Translation initiation	●
Bcl-2 family	Apoptosis	●
Ubiquitin ligase complex	Ubiquitination	●
Phosphatases	Growth & cytokine signaling	●
Transcription factors	Transcriptional regulation, Hippo signaling pathway	●
Transcriptional cofactors	Transcriptional regulation, mRNA deadenylation, miRNA silencing	●
Transmembrane receptor intracellular domains	NF-κB pathway, MAPK pathway, growth signaling	●
F-box	Ubiquitination, cell cycle regulation	●
Scaffolding	DNA damage repair, ubiquitination, transcriptional regulation	●
Undisclosed	p53 signaling, undisclosed	●

ALLOX has generated **30**

proprietary maps of oncology targets as well as targets in the immunology & inflammation space

Unleashing the power of a true platform

	PLATFORM + DATA	PIPELINE	PARTNERSHIPS
VALUE PROPOSITION	Unprecedented speed and data quality for training AI models	Novel discoveries to drug challenging targets that feed differentiated pipelines	Massive optionality across the PPI disease universe
THREE YEAR GOALS	<ul style="list-style-type: none"> Develop internal hit discovery & optimisation capabilities Generate a massive training dataset of 300 maps Build an AI model to predict hotspots from sequence 	<ul style="list-style-type: none"> Complete 20+ early Hit ID campaigns Expand pipeline with 3 new high-potential pre-clinical programs Deliver 1 program to pre-candidate nomination 	<ul style="list-style-type: none"> Secure 1 strategic multi-target and/or in-licence pharma partnerships Sign 1 AI software based deal for in silico hotspot discovery

>160,000 total number of biophysical parameters measured today

300+ total maps

>2M total number of biophysical parameters measured

Faure, Domingo, Schmiedel, et al., *Nature* 2020
 *Chapeau, E.A., et al., *Nature Cancer* 2024
 **Hagenbeek, T.J., et al., *Nature Cancer* 2023
[†]Affiliated with ALLOX. Authors are listed in alphabetical order.