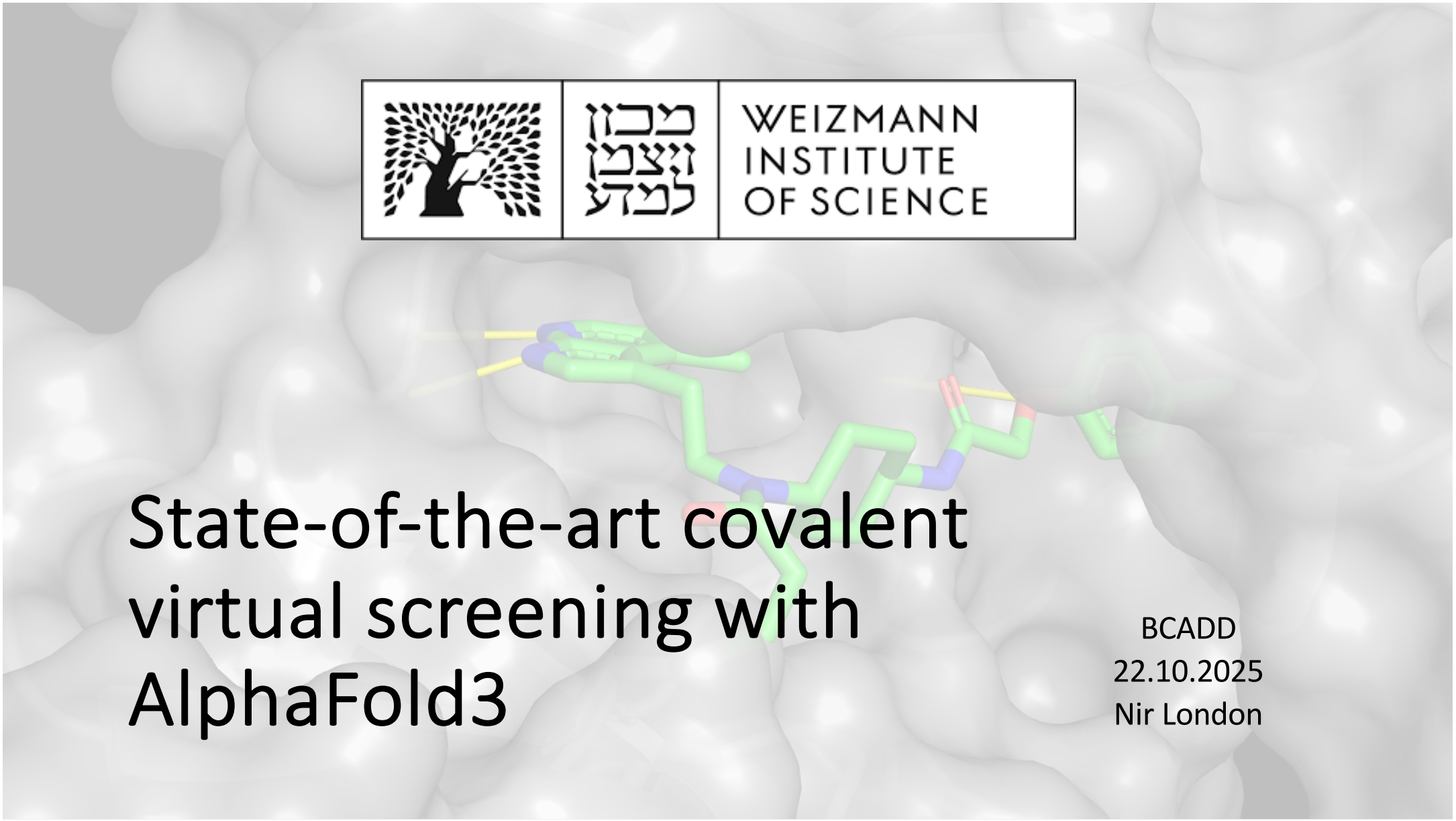
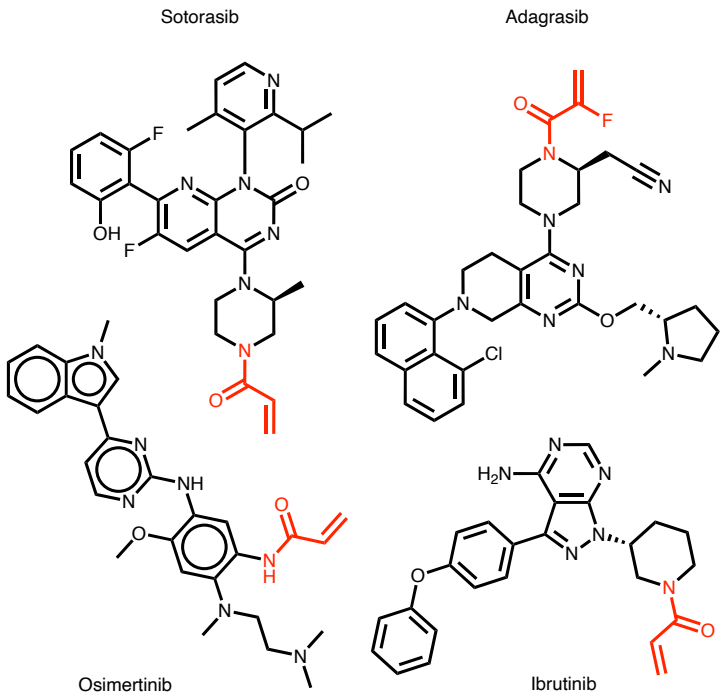


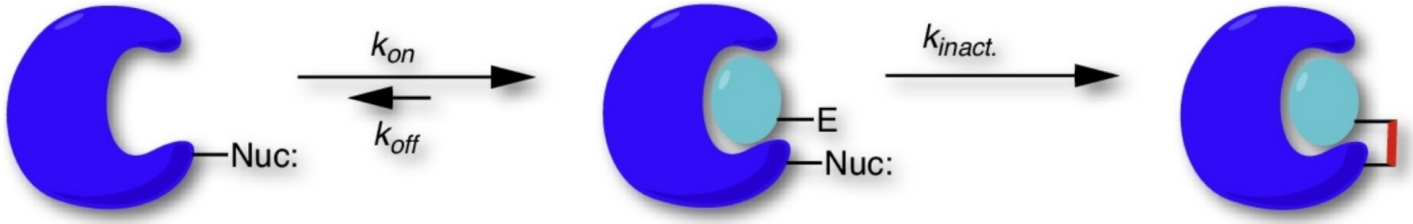
State-of-the-art covalent virtual screening with AlphaFold3

BCADD
22.10.2025
Nir London

Covalent inhibitors

- Molecules that are able to form a covalent bond with their target show:
  - Increased biochemical efficiency
  - Longer duration of action
  - Can be very selective
- Several successful drugs including clinical candidates for challenging targets



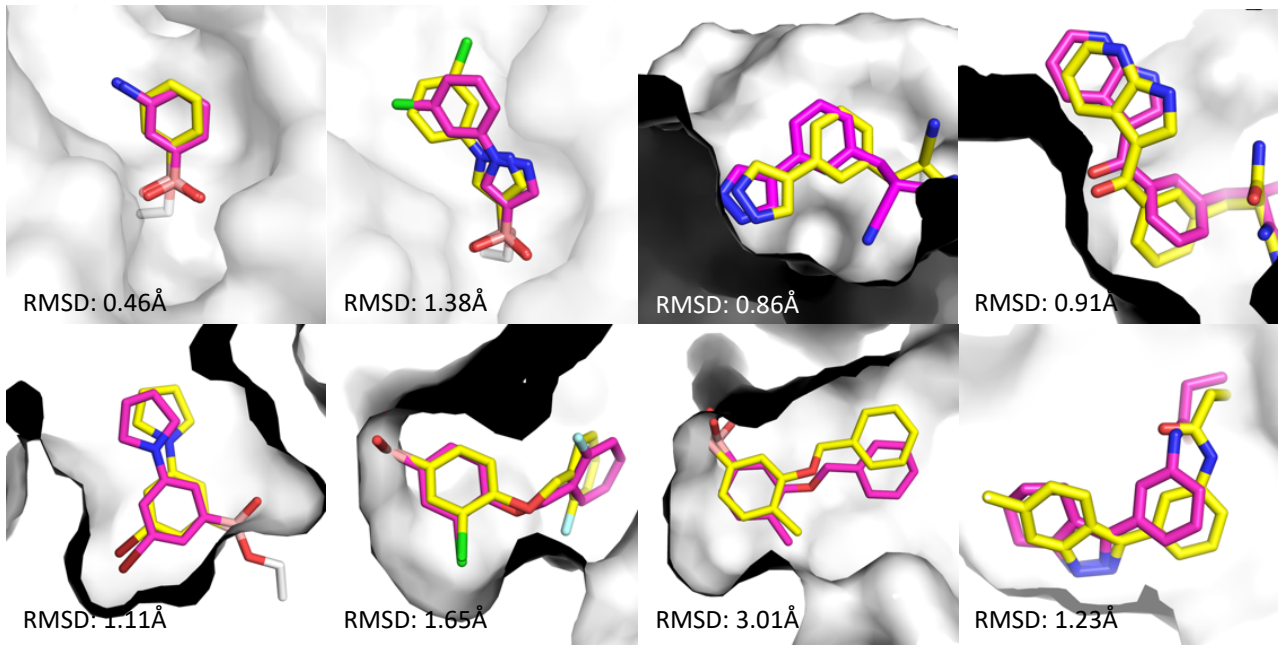


DOCKovalent

nM inhibitors directly from primary screen

Target	Hit Rate	Best 'activity'
AmpC	5/6	K_i=10 nM
a7-CBE	5/5	K_i=250 pM
RSK2	5/8	IC_{50}=42 nM
JAK3	9/15	IC_{50}=49 nM
MKK7	4/10	IC_{50}=11 nM
KRas^{G12C}	5/31	labeling
SETD8	0/21	n/a

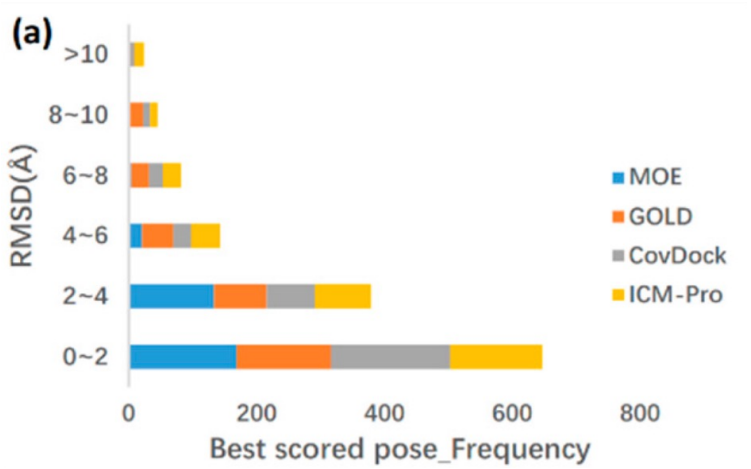
Atomic accuracy of prospective docking predictions

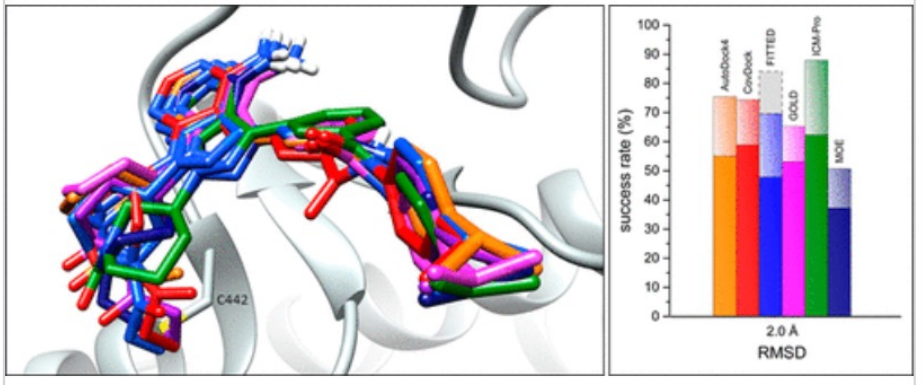


London et al. Nat. Chem. Biol, 2014
Correy et al. PNAS, 2019
Shraga et al. Cell Chem Biol, 2019

A benchmark set for covalent virtual screening

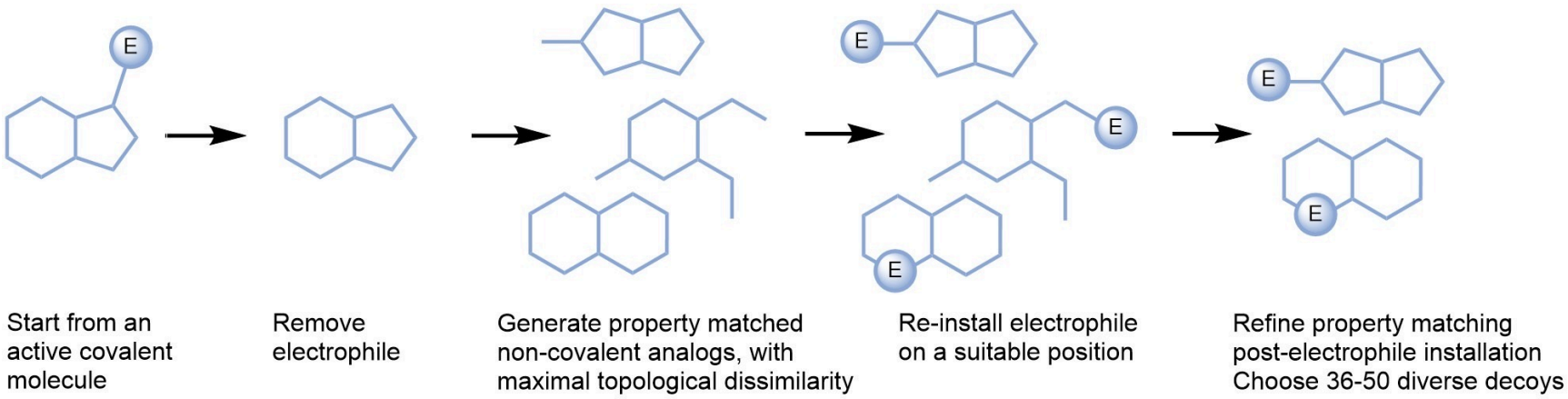
- With the proliferation of covalent docking methods, several covalent benchmarks were published, but all were focused on “Modelling” i.e. accuracy of pose recapitulation





Scarpino et al. JCIM, 2018
Wen et al. Molecules, 2019

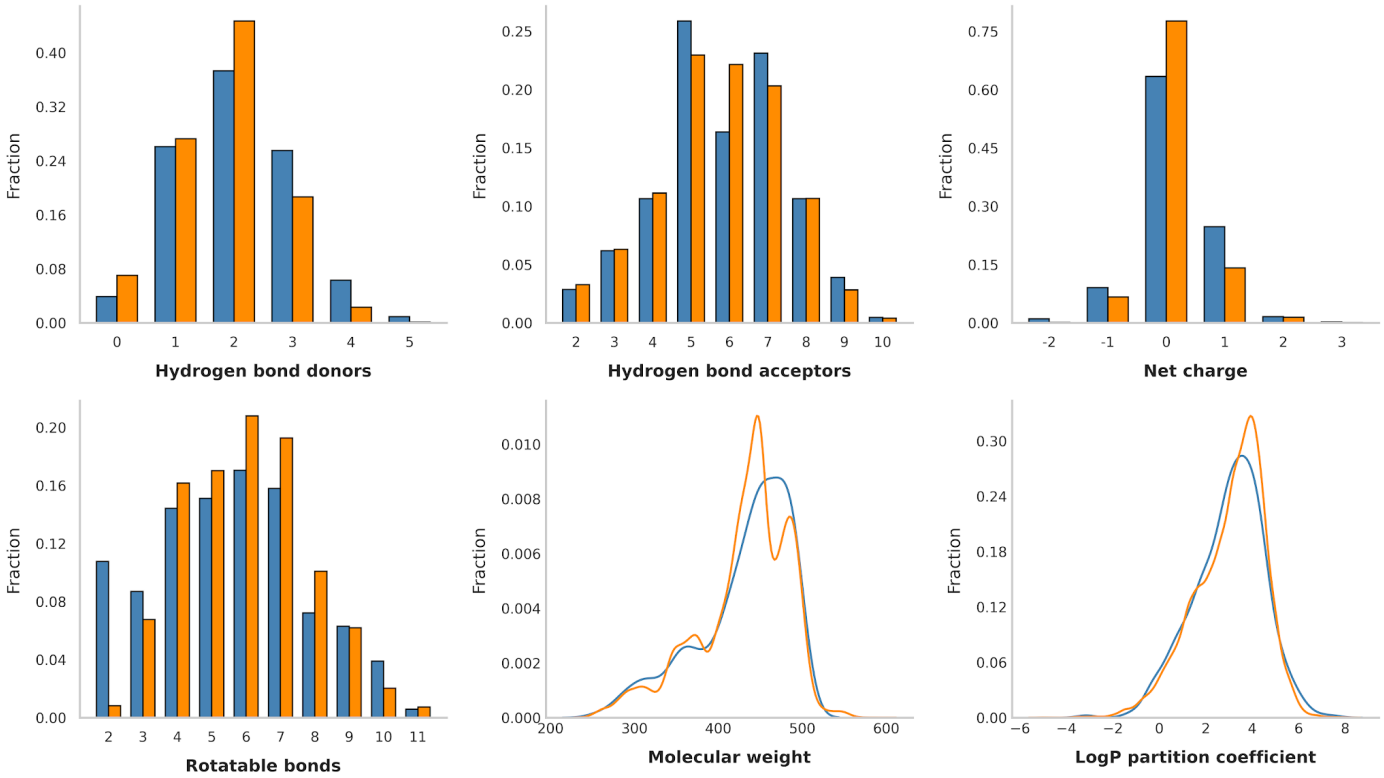
Introducing COValid – a Covalent VS benchmark



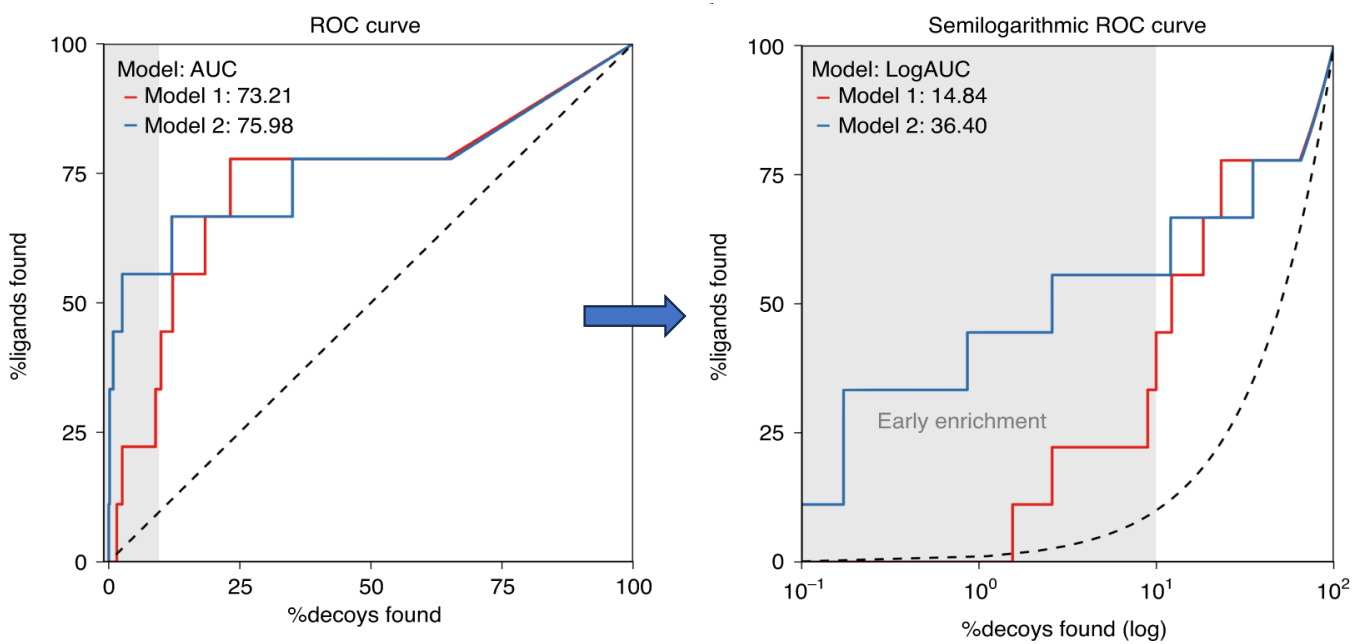
Mysinger et al. J. Med. Chem 2012

Introducing COValid – a Covalent VS benchmark

Nucleophilic attachment site	Active protomers	Decoy protomers
BMX C496	36	1714
BTK C481	182	8724
EGFR C797	155	5678
FGFR1 C488	53	2581
FGFR4 C477	21	1009
FGFR4 C552	44	1916
ITK C442	75	3420
JAK3 C909	197	9041
KRAS G12C C12	93	3178
MAP3K7 C174	18	658
Total	**874**	**37919**



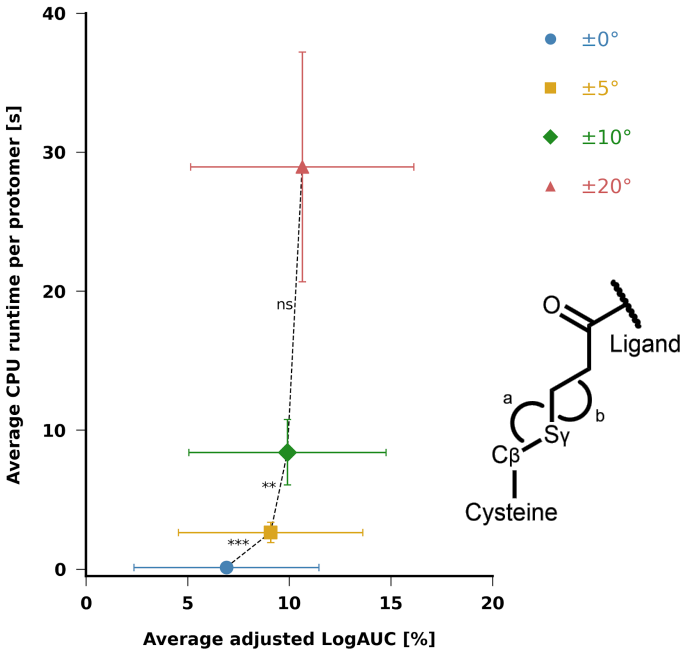
The enrichment metric: Adjusted LogAUC

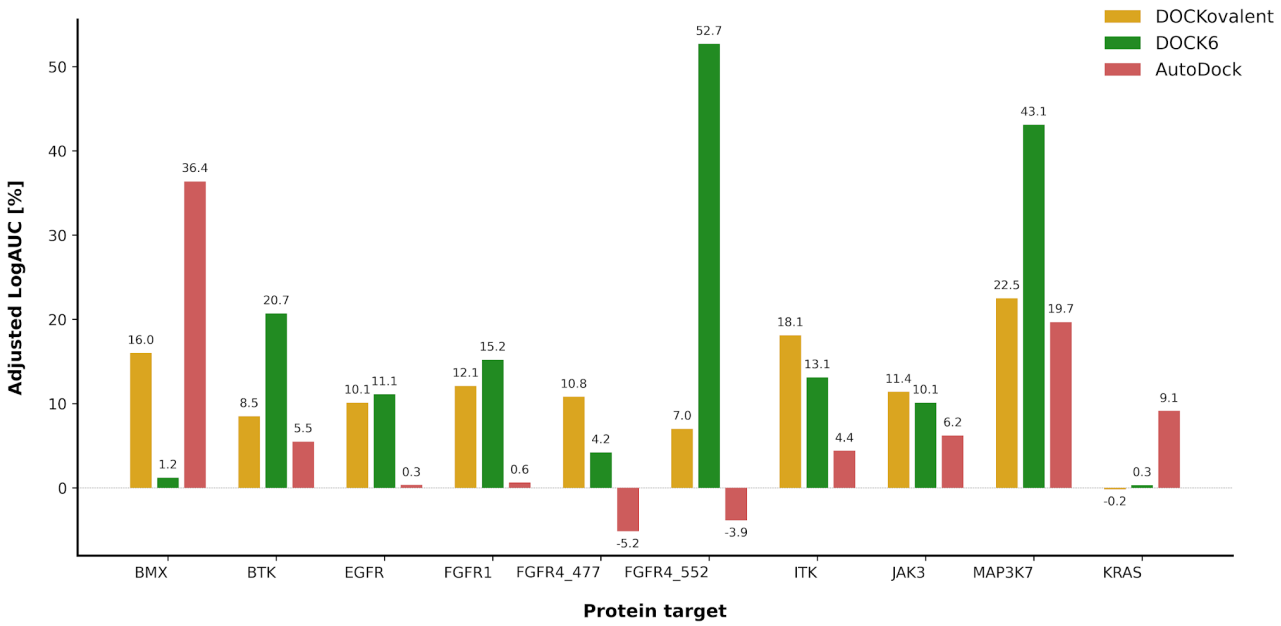


- Adjusted LogAUC –
  - 0% = random enrichment
  - 85.5% = perfect ranking

Mysinger et al. JCIM, 2010

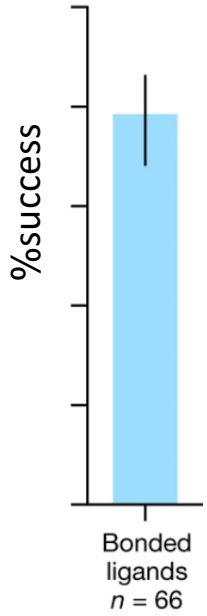
Classical covalent docking perfrom well

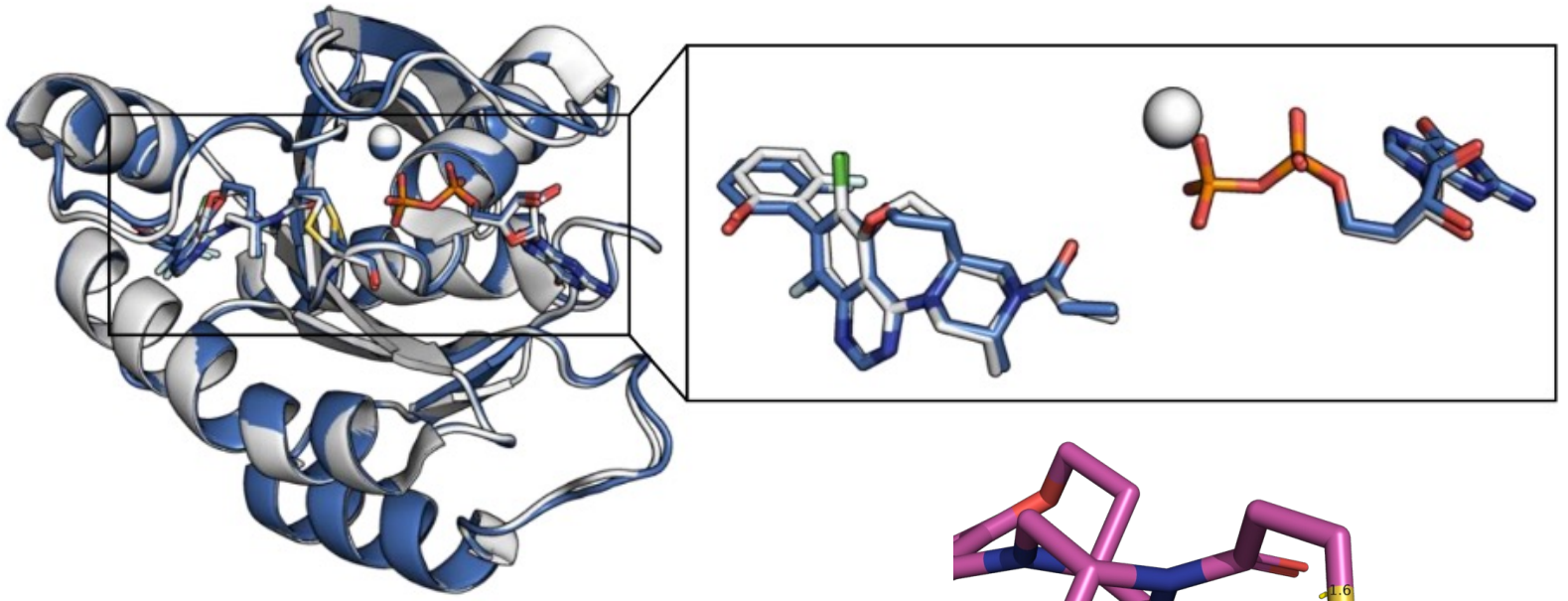


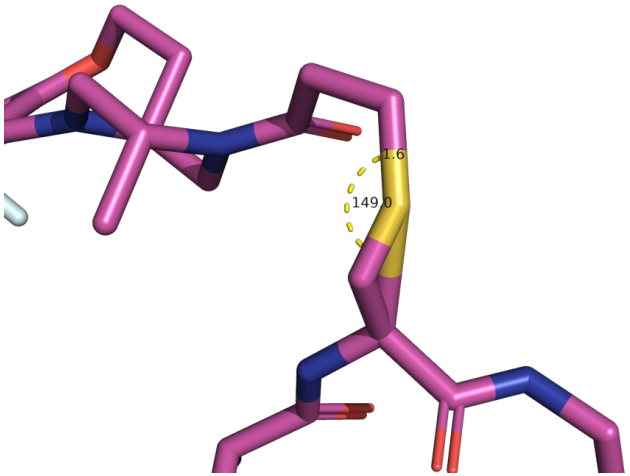


London et al. Nat. Chem. Biol. 2014
Bianco et al. Prot. Sci, 2015
Tan et al. JCIM, 2025

AlphaFold3 can model covalent ligands

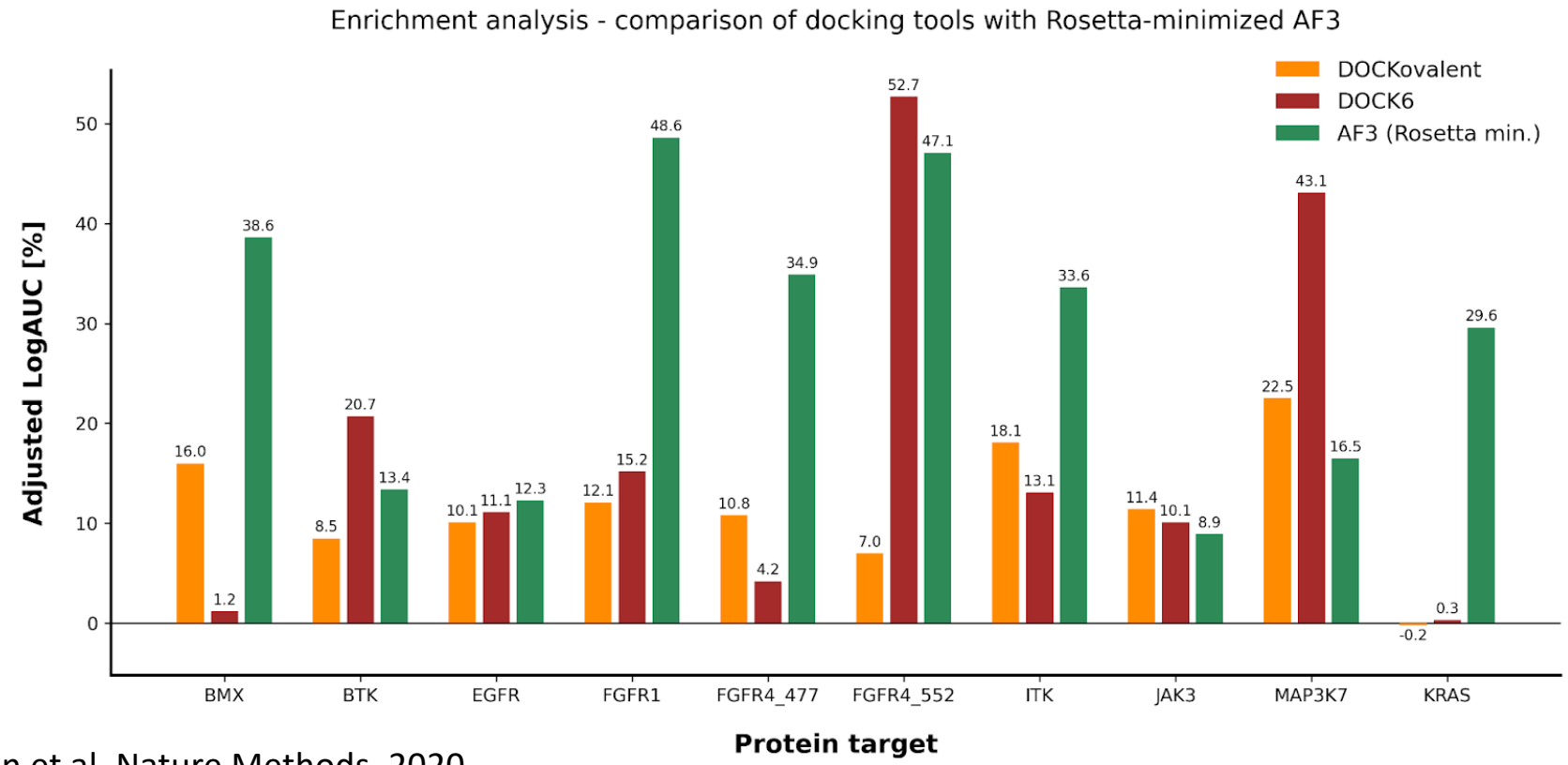






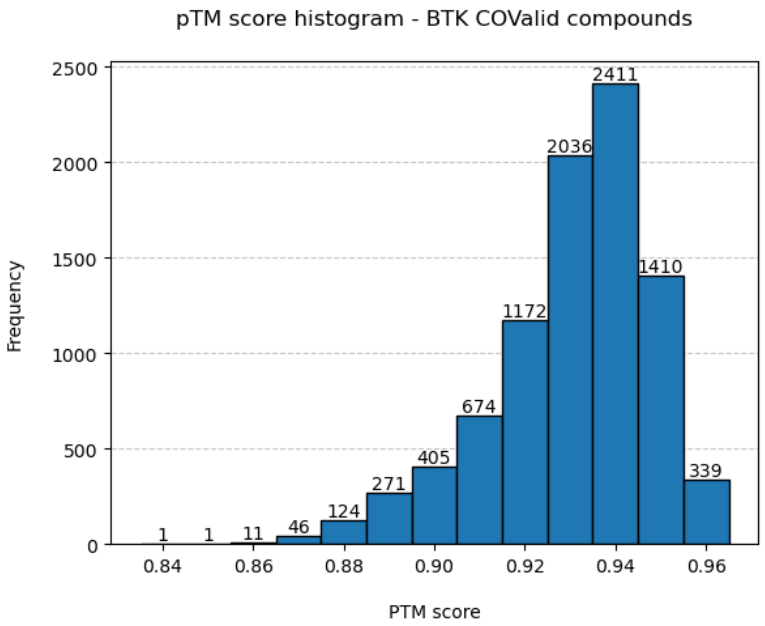
Abramson et al. Nature, 2024

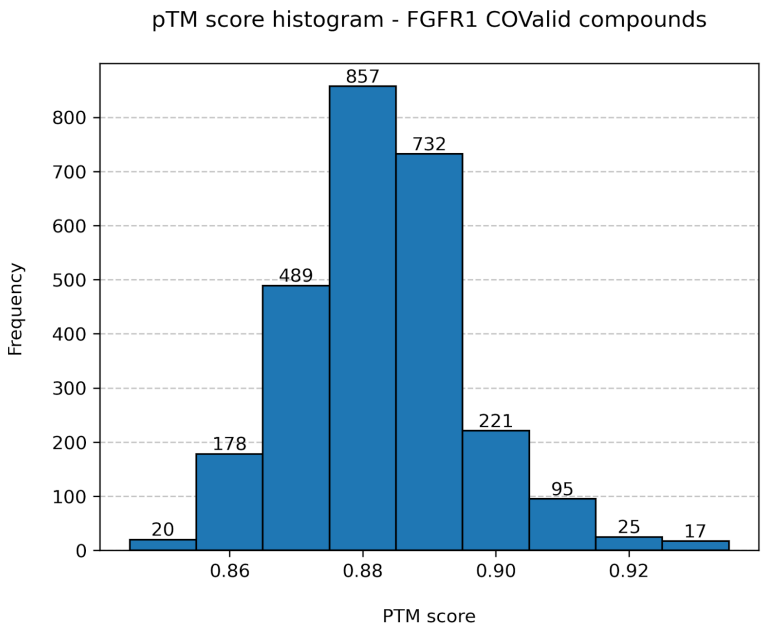
AF3, rescored by Rosetta, outperforms docking



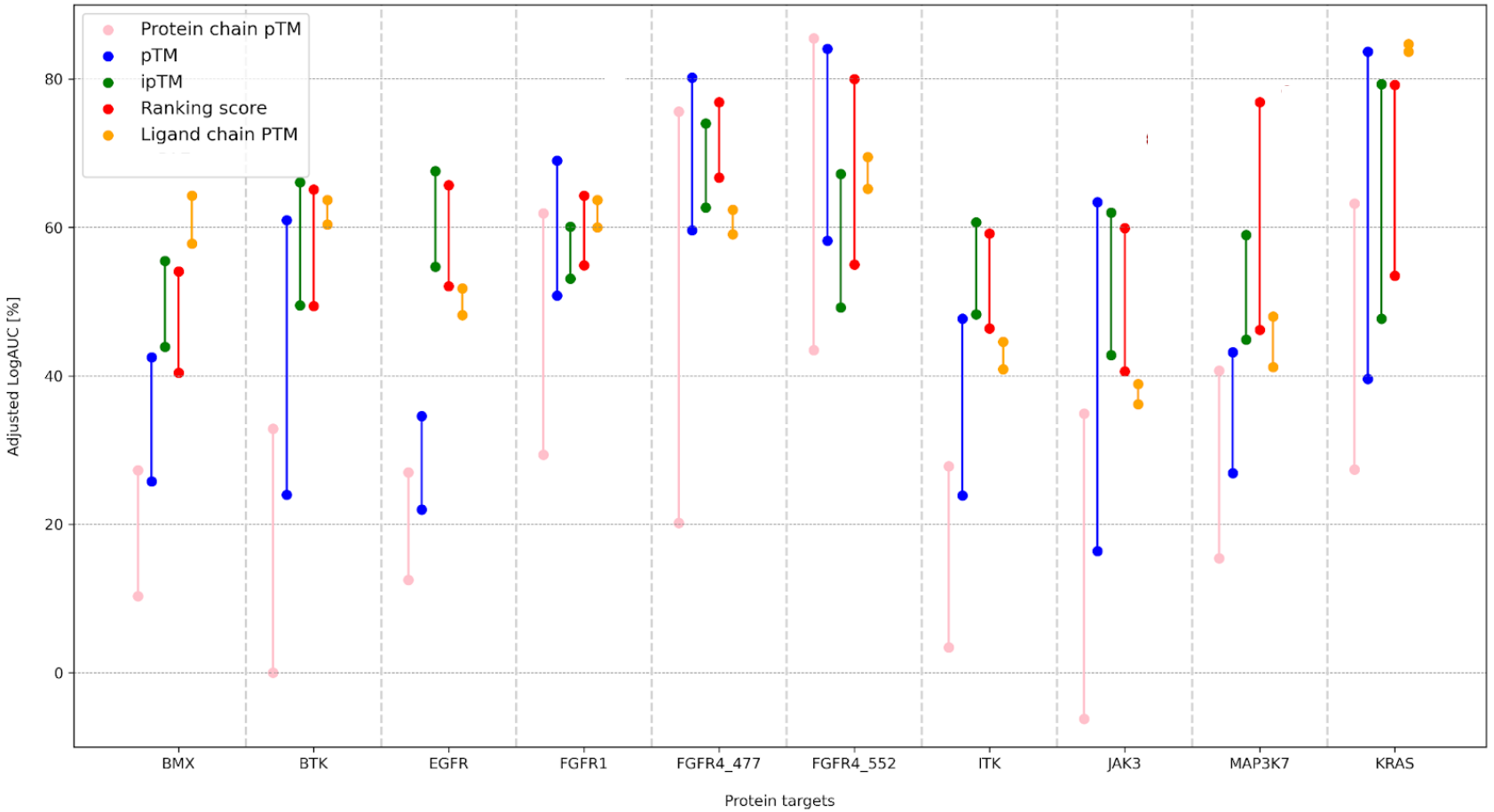
Leman et al. Nature Methods, 2020

Most metrics lack the resolution to rank

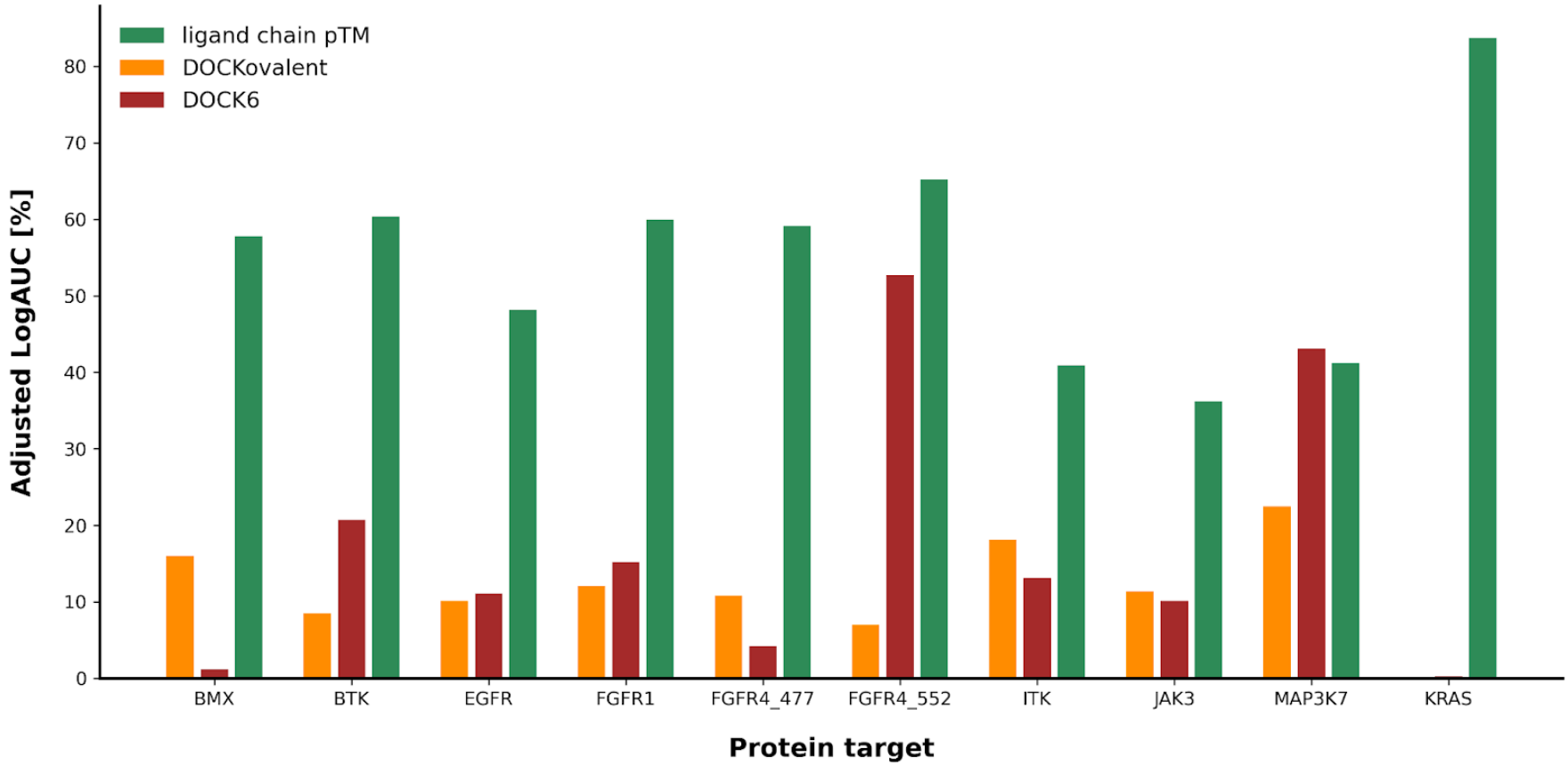




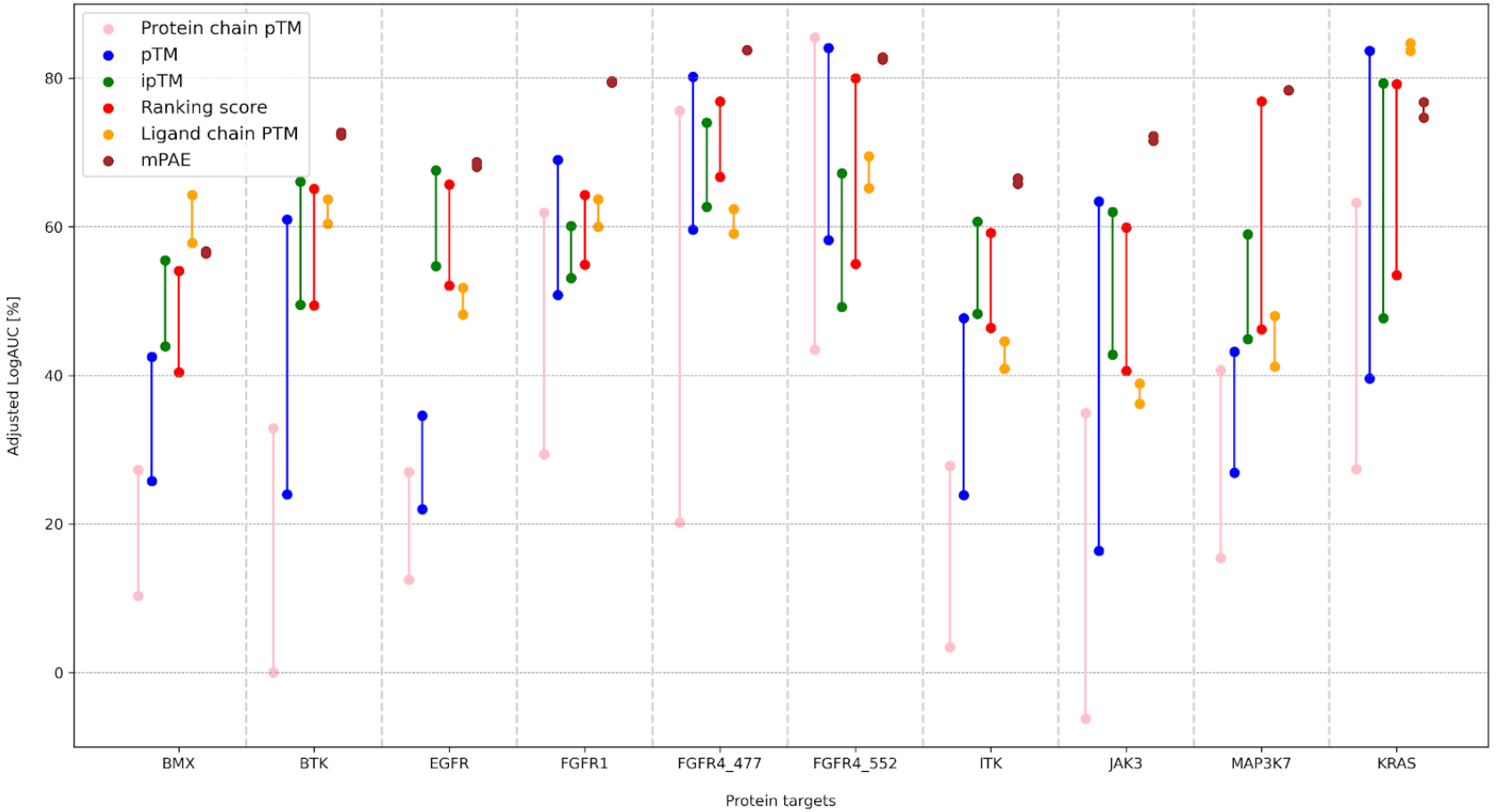
Most metrics lack the resolution to rank



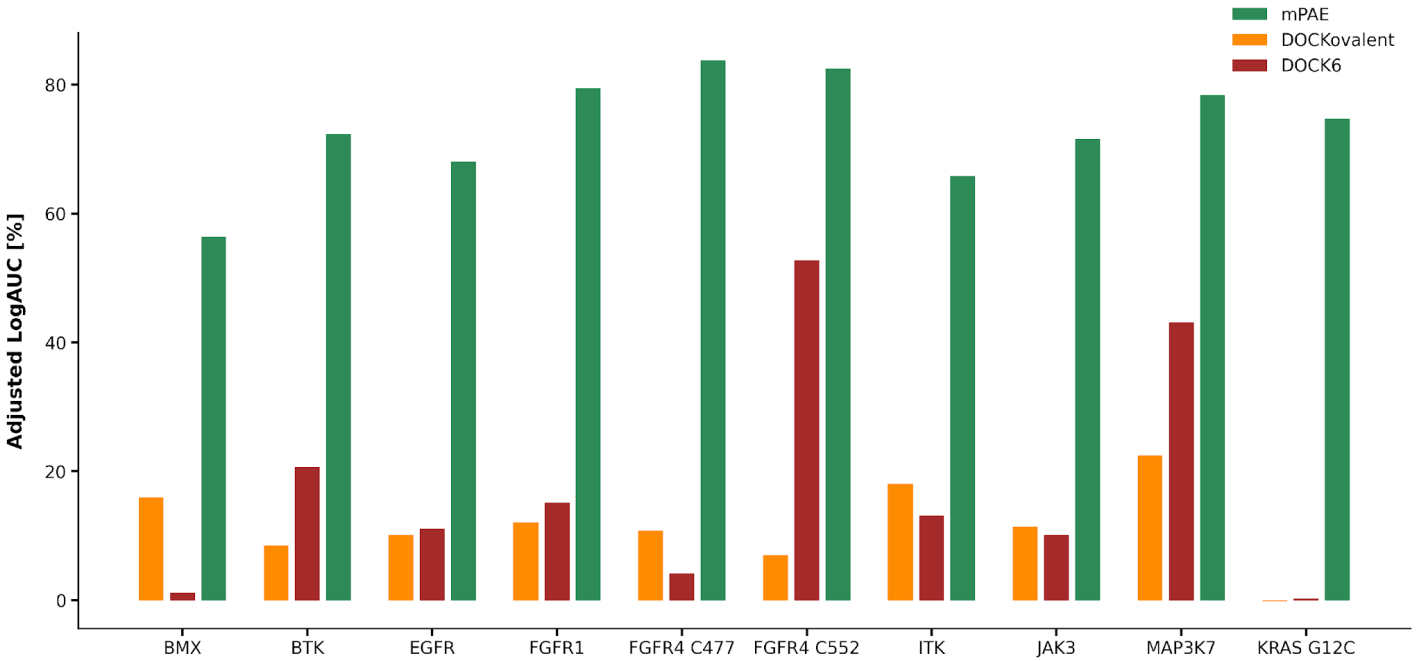
Ligand chain pTM outperforms docking



mPAE outperforms all metrics

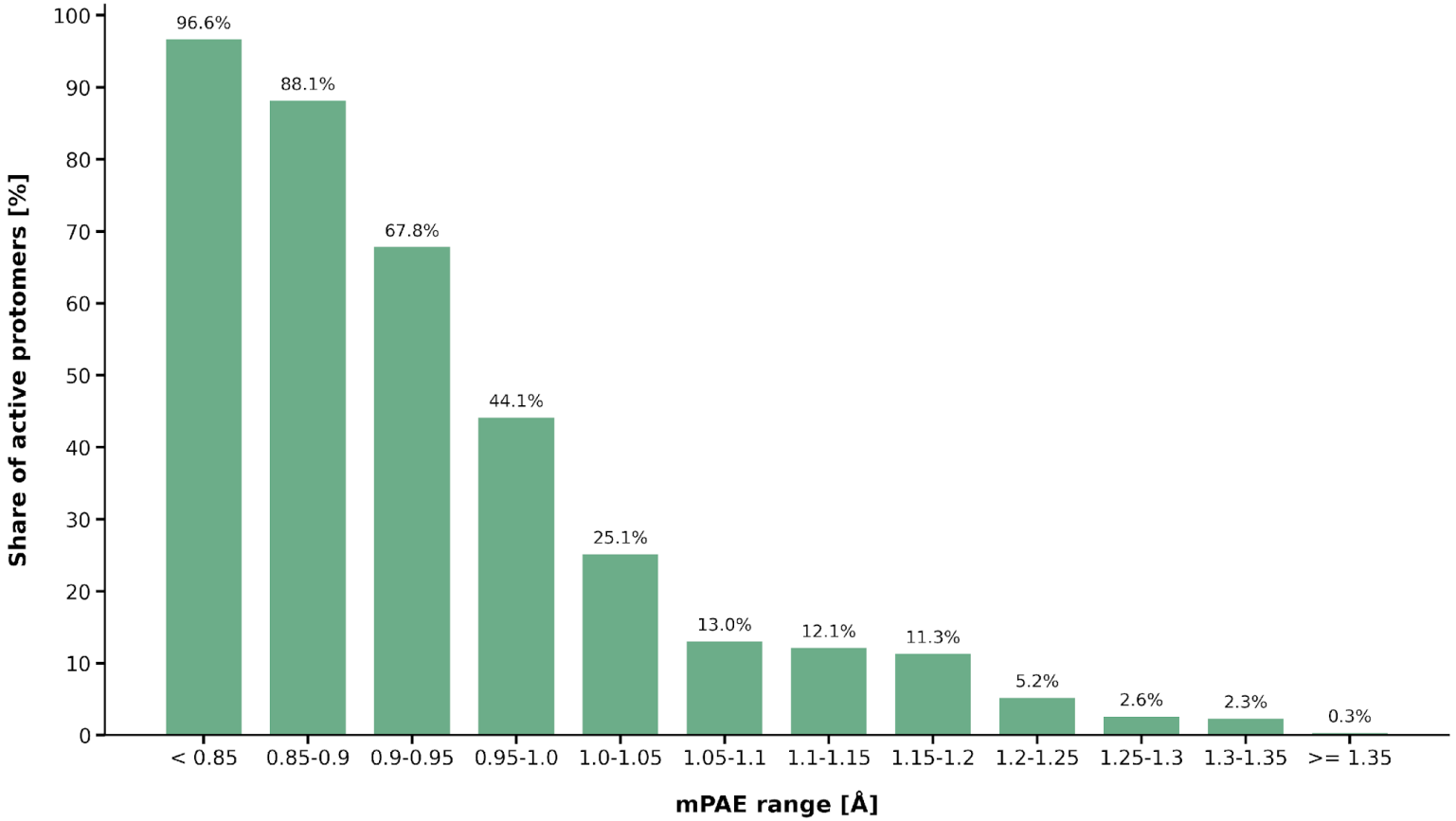


mPAE outperforms all methods

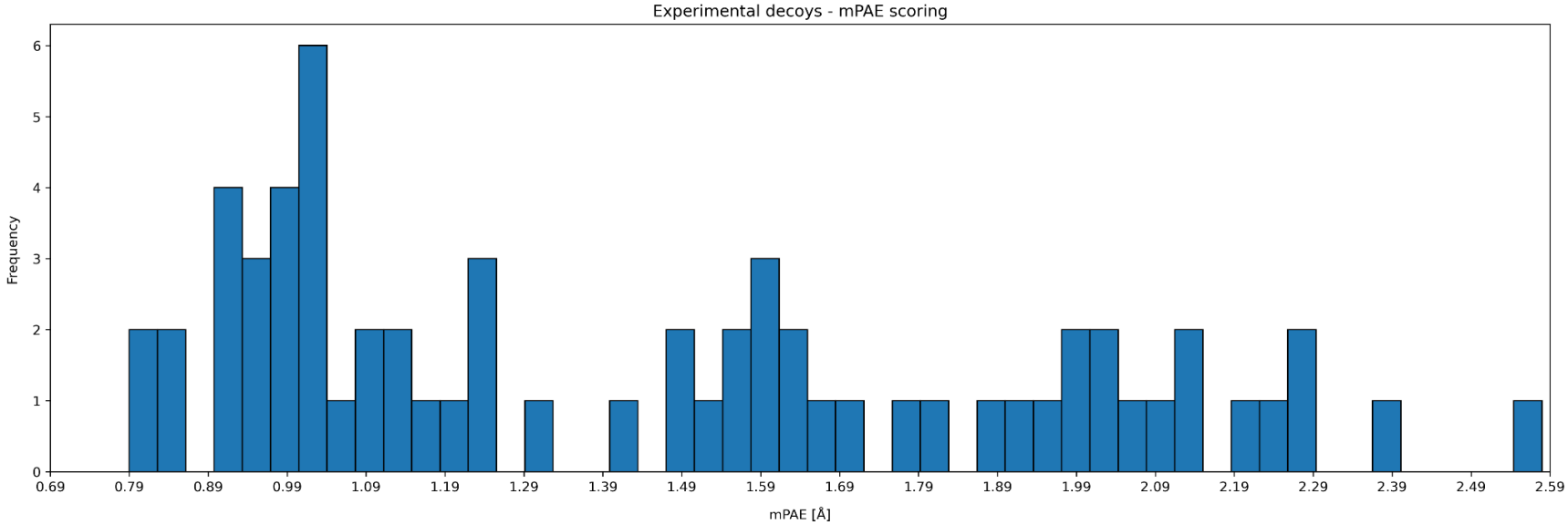


	DOCKovalent[a]	DOCK6	AutoDock	AF3 (Rosetta)	AF3 (mPAE)
Average Adj. LocAUC (%)	9.9±4.8	12.1±9.9	5.7±7.9	28.5±16.1	71.8±5.9
Average run-time per ligand (Sec.)	8.4±2.4	7.0±2.0	6.7±0.4	266±114	

Probablity of ‘binding’ can be extrapolated (and is target independent)

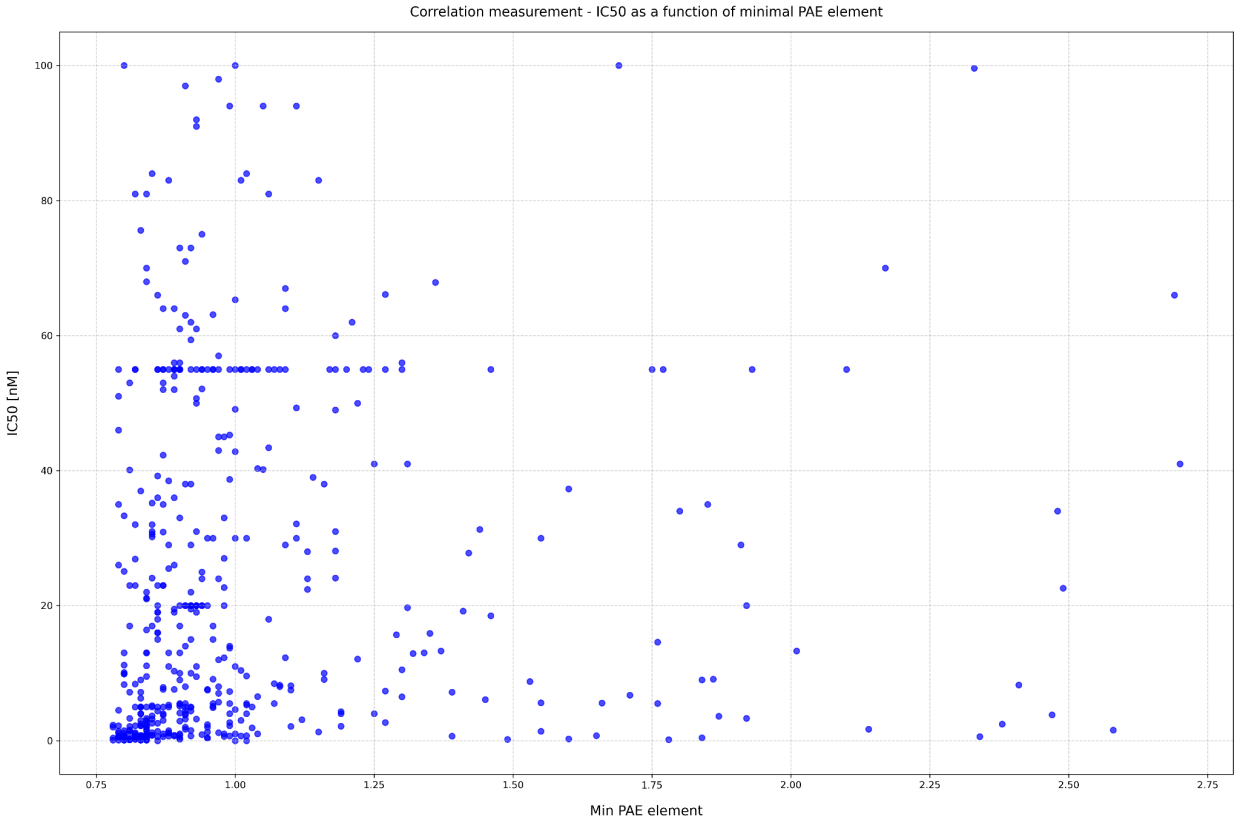


mPAE can identify experimental decoys

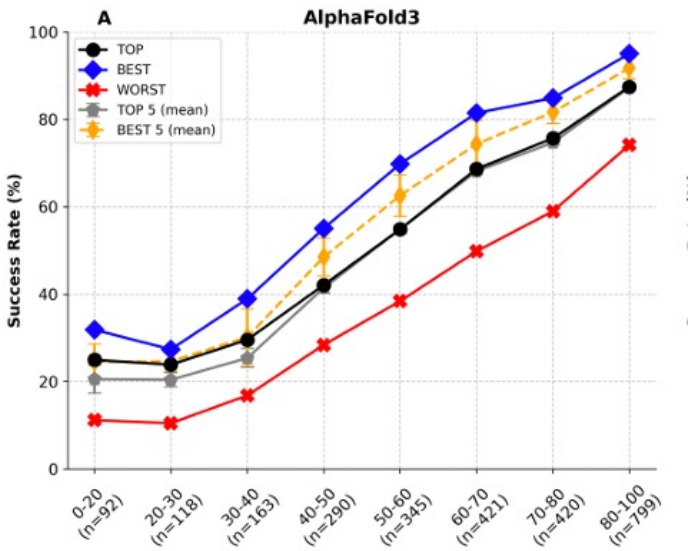


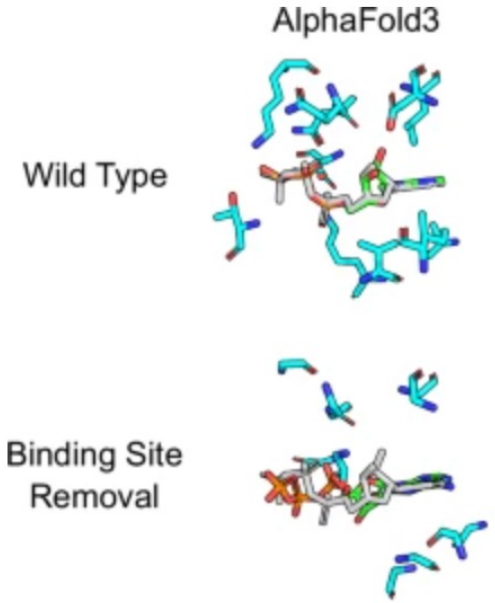
64 ligands with annotated >10 µM activity ; The average mPAE is 1.5±0.5Å.

mPAE cannot predict affinity



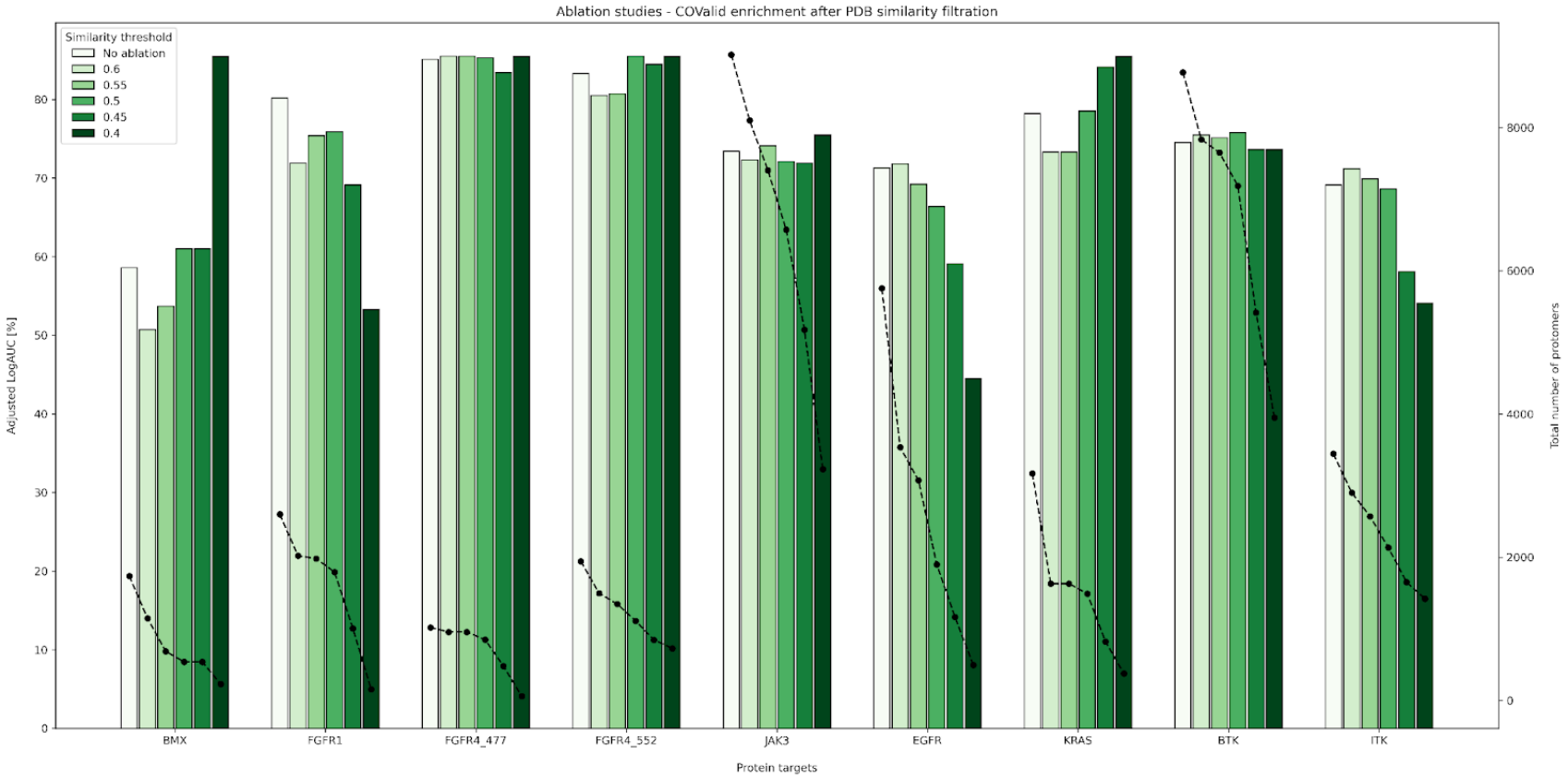
Is this “Memorization” or “Generalization”?



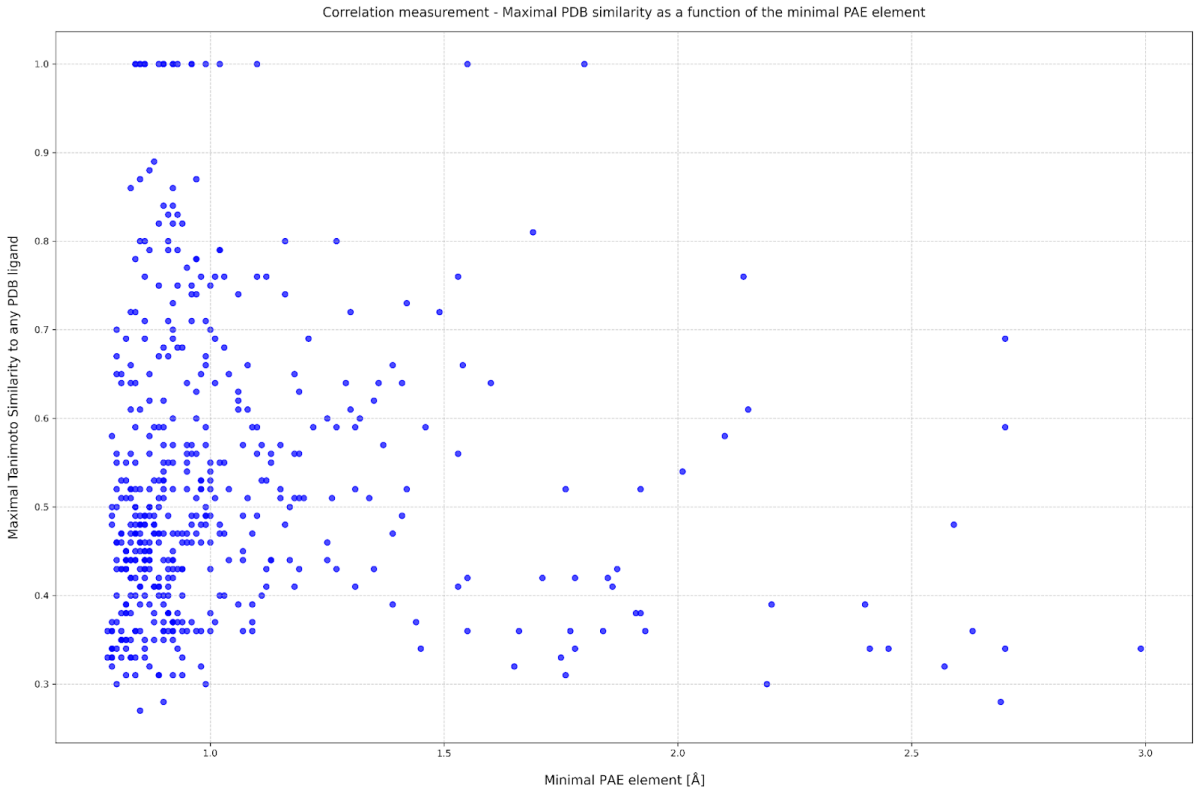


Masters et al. Nature Comms, 2025
Škrinjar et al. bioRxiv, 2025

Performance does not deterioirate when restricting to ‘distant’ compounds

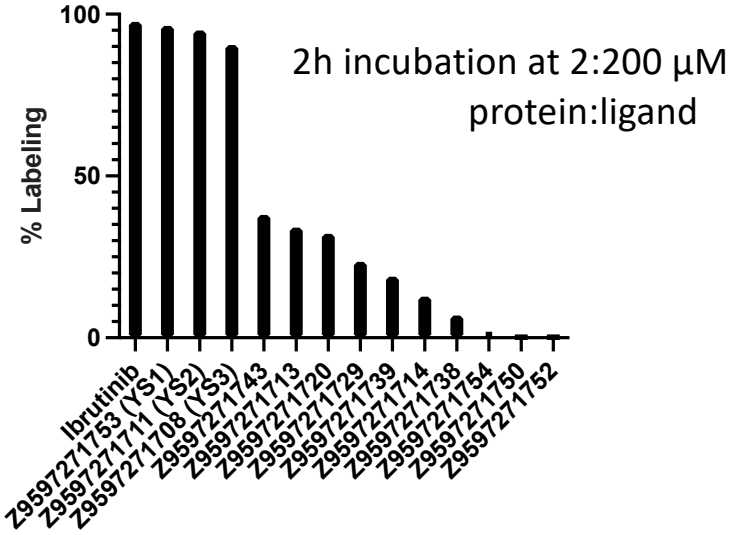


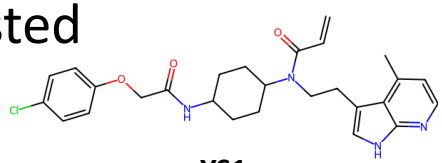
No correlation between mPAE and the maximal Tanimoto similarity to PDB ligands

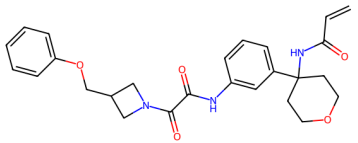


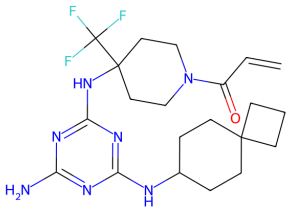
The ultimate test is prospective screening

- Generated a library of 900K acrylamide compounds
  - 250 Da < MW < 500 Da
  - Diverse subset of free amines sampled from Enamine REAL
- Screened by AF3 against BTK as a model kinase
  - Filtered by mPAE ($\leq 0.9[\text{Å}]$)
    - 440 compounds remain
  - Filtered by maximal similarity to any ChEMBL compound (T_c<0.35)
    - 390 compound remain
- After clustering and manual inspection, synthesized and tested 13 compounds

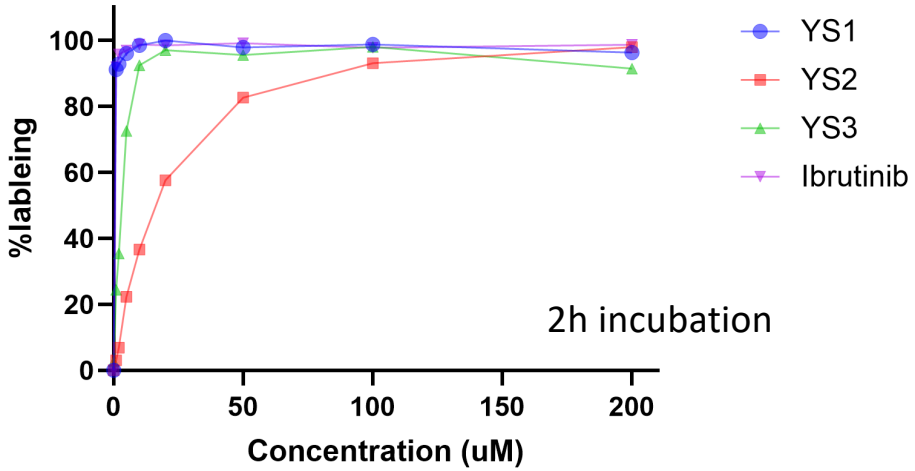


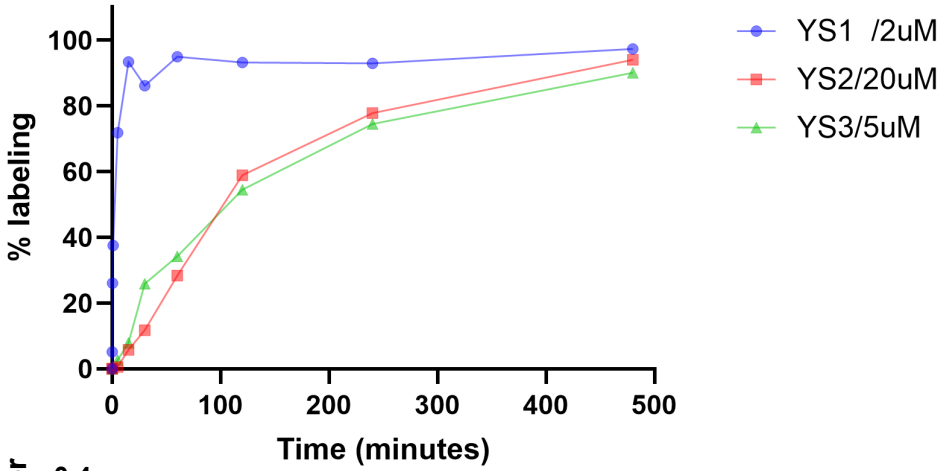

YS1

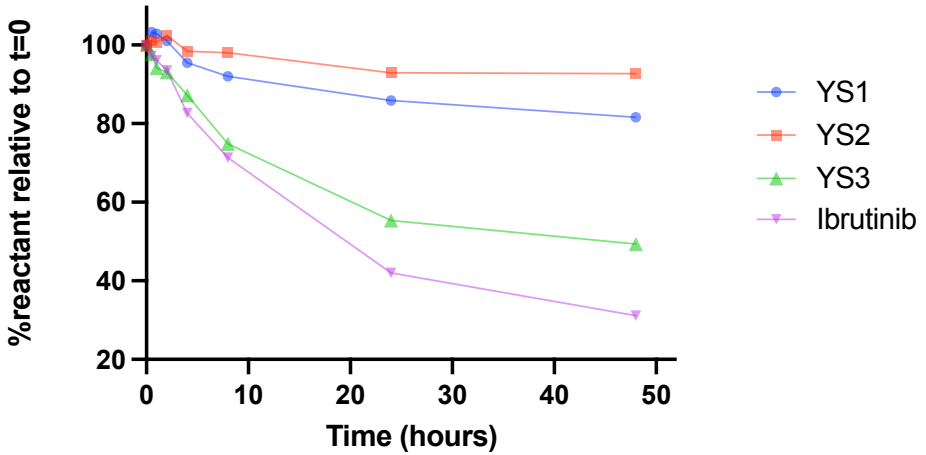

YS2

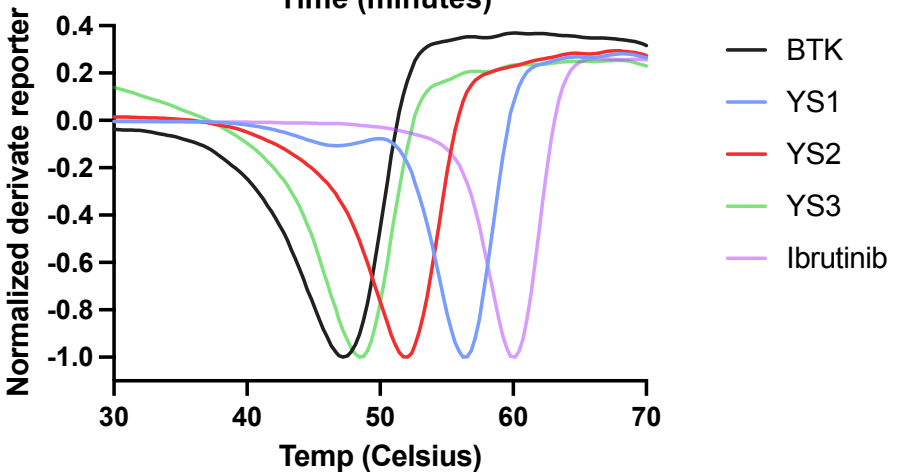

YS3

Experimental validation of AF3 hits

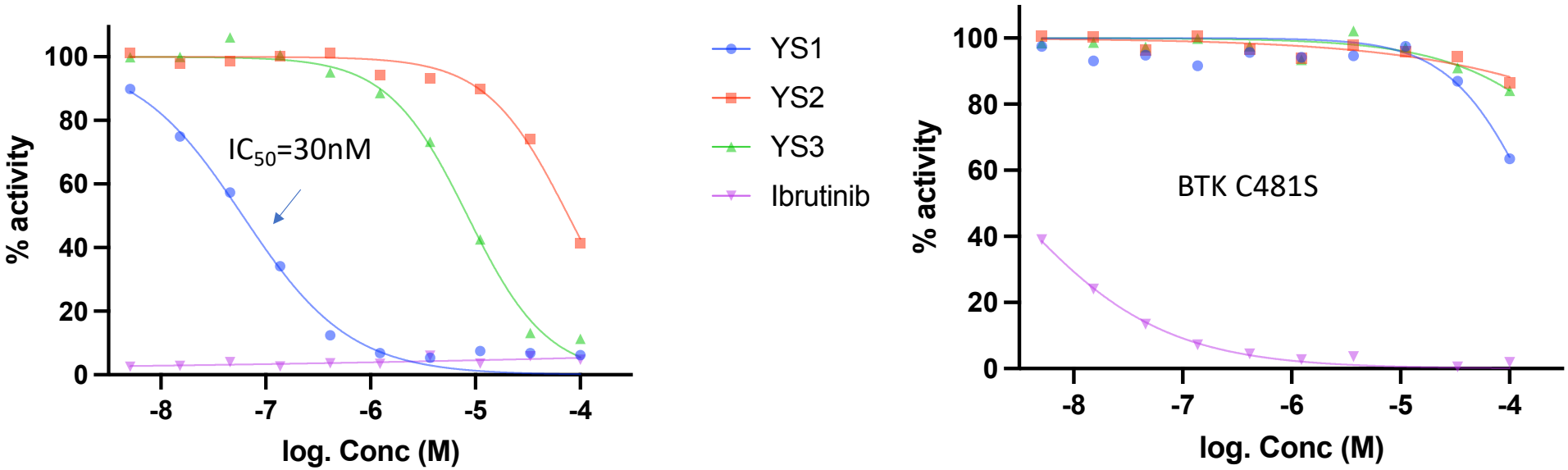




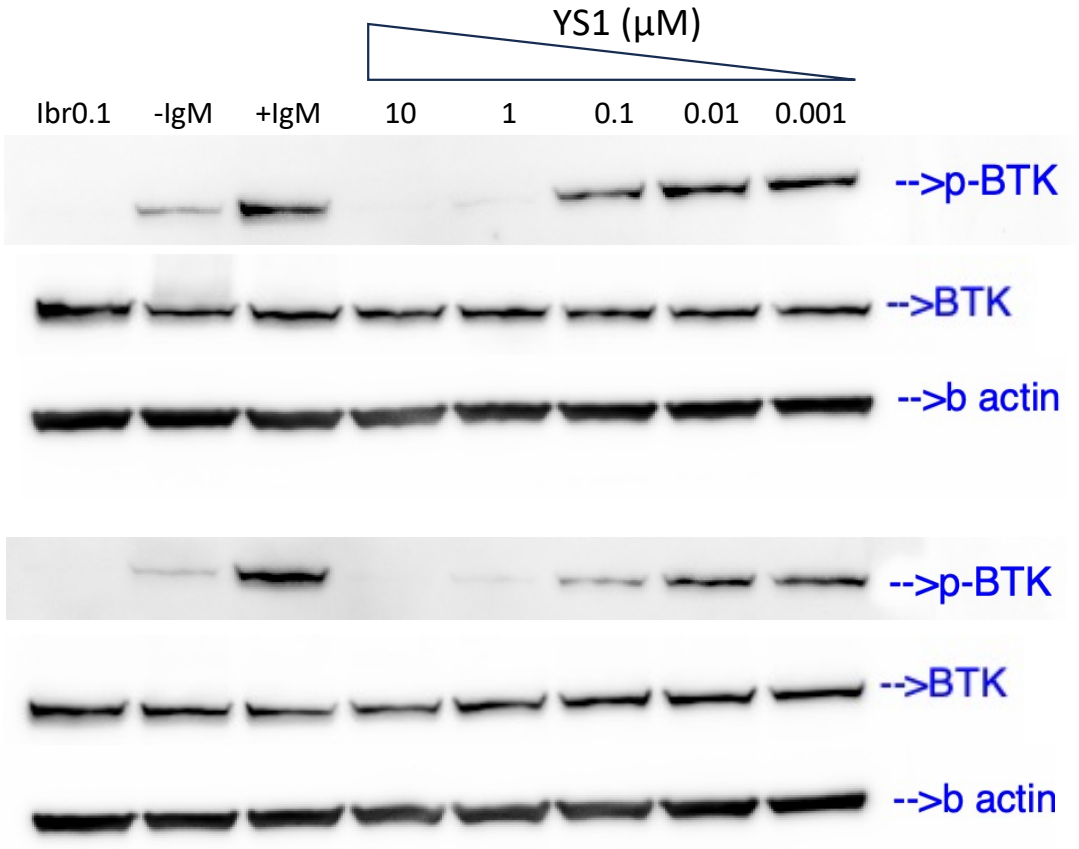




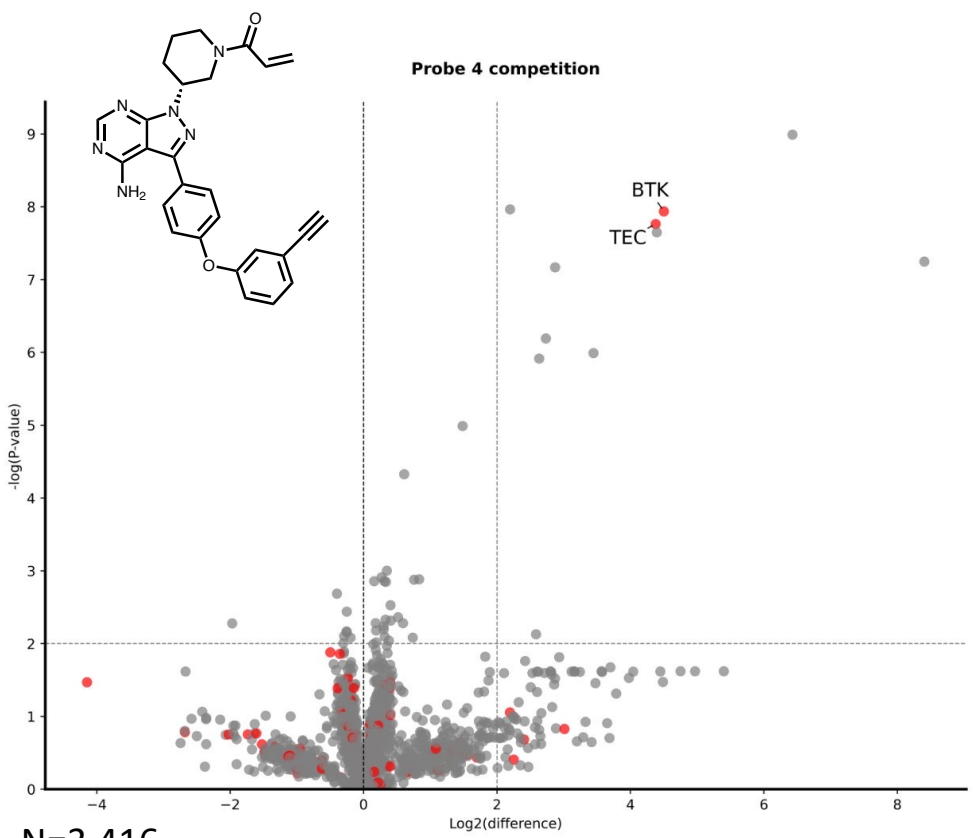
YS1 is a potent (covalent) BTK inhibitor



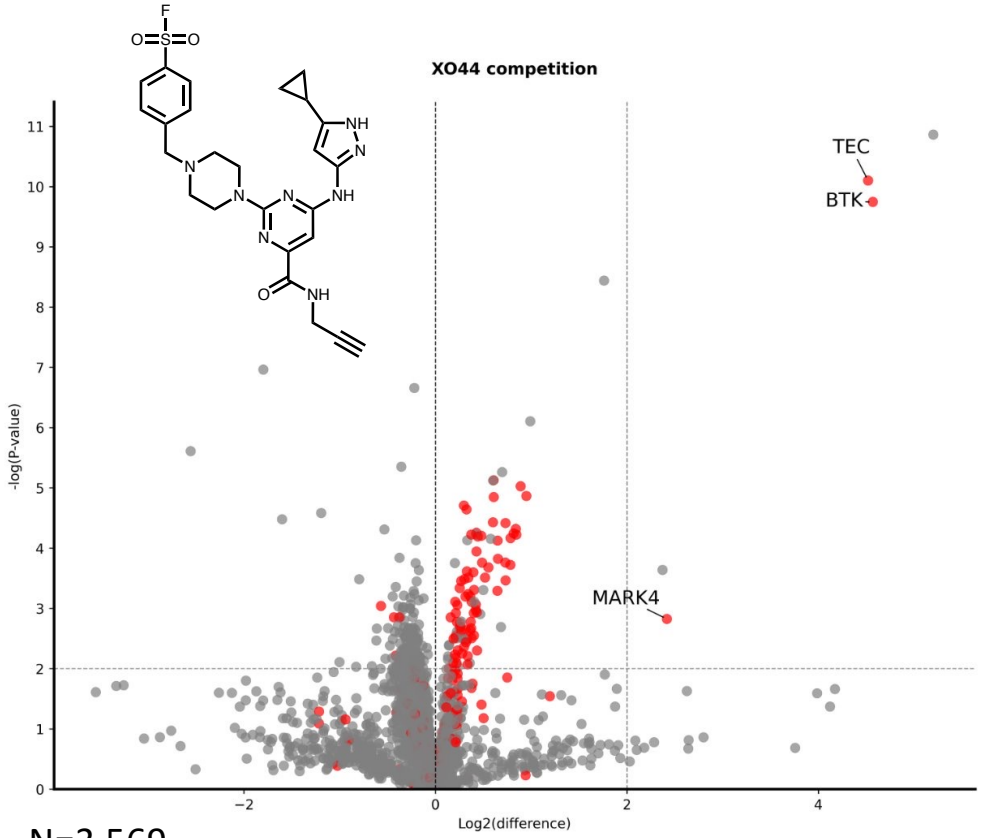
YS1 is <100nM potent in cells



YS1 is extremely selective for BTK in cells

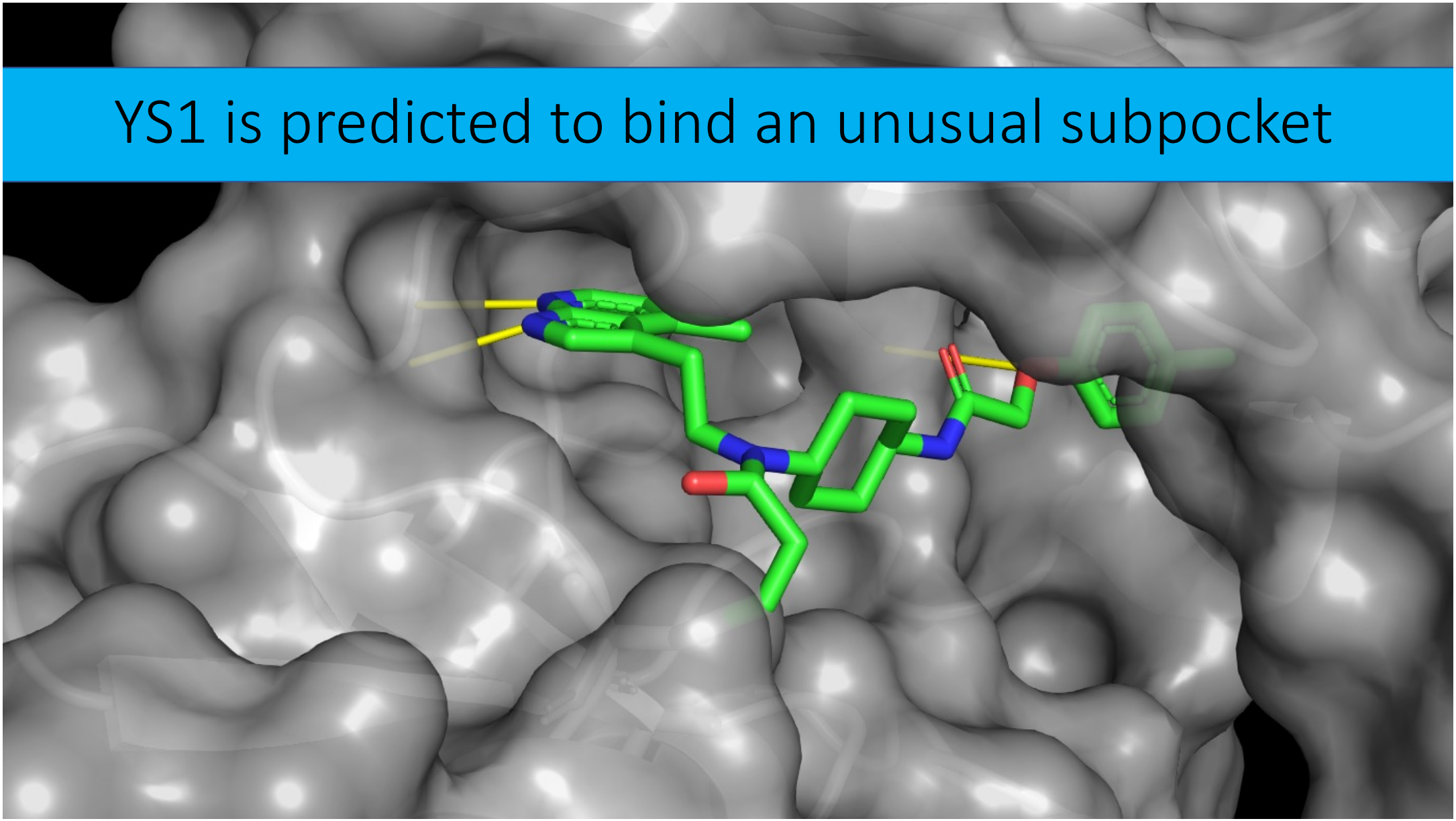


N=3,416



N=3,569

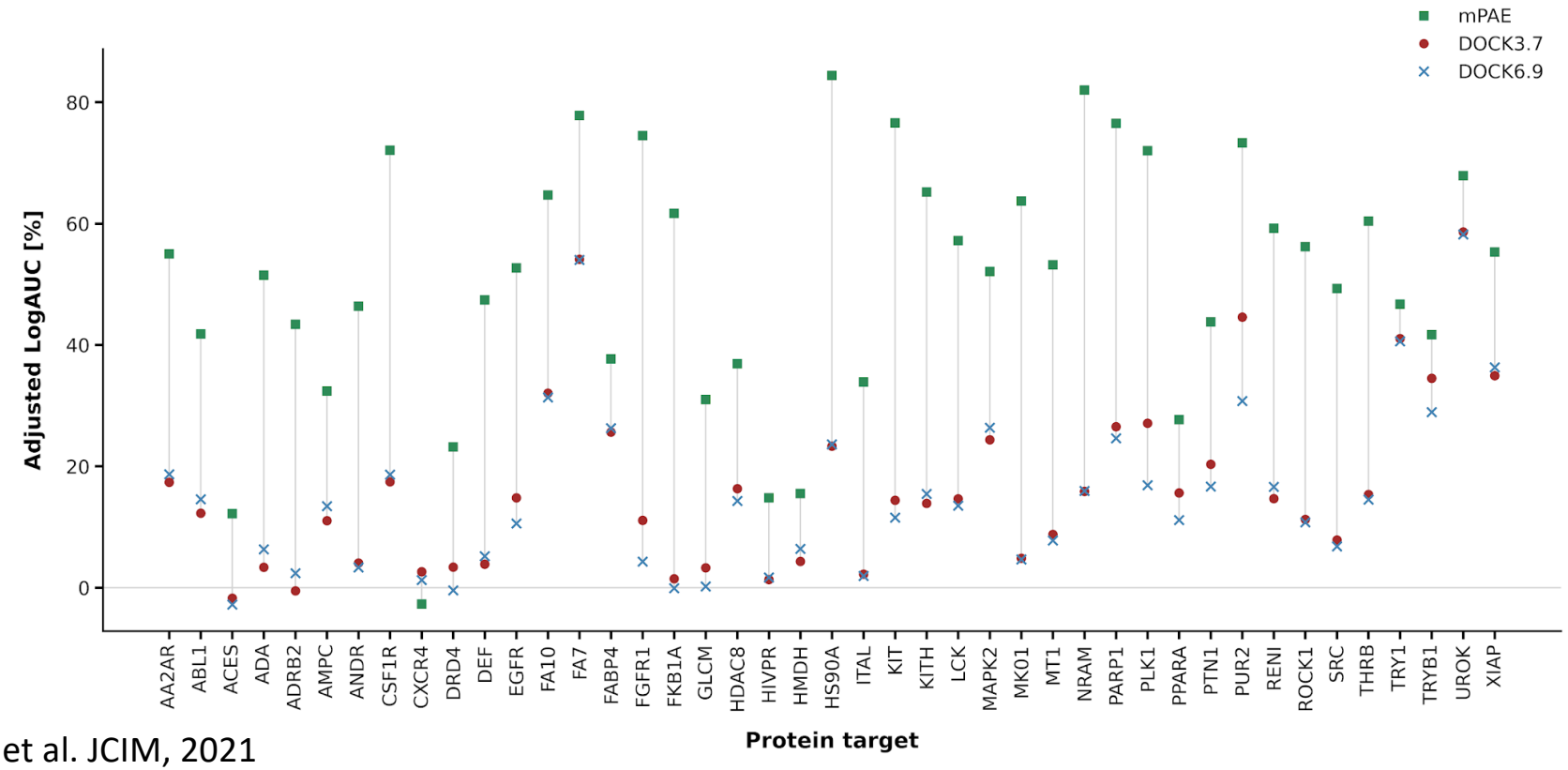
YS1 is predicted to bind an unusual subpocket



Summary and future directions

- AF3 looks like a promising direction for covalent virtual screening despite being x40 slower.
- mPEA dramatically outpeforms any other method and metric and leads to near perfect performance in the benchmark.
- Prospective screening identified a novel ligand, refuting concerns of ‘memorization’.
- What are we dooing now:
  - Crystallographic validation of pose
  - Improvement of screening libraries
  - Two teir screening
  - Application for non-covalent screening

Preliminary application suggests AF3 also useful for non-covalent VS



Stein et al. JCIM, 2021

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  - Dr. David Lin

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- Niv Koriat
- Almog Nadir

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