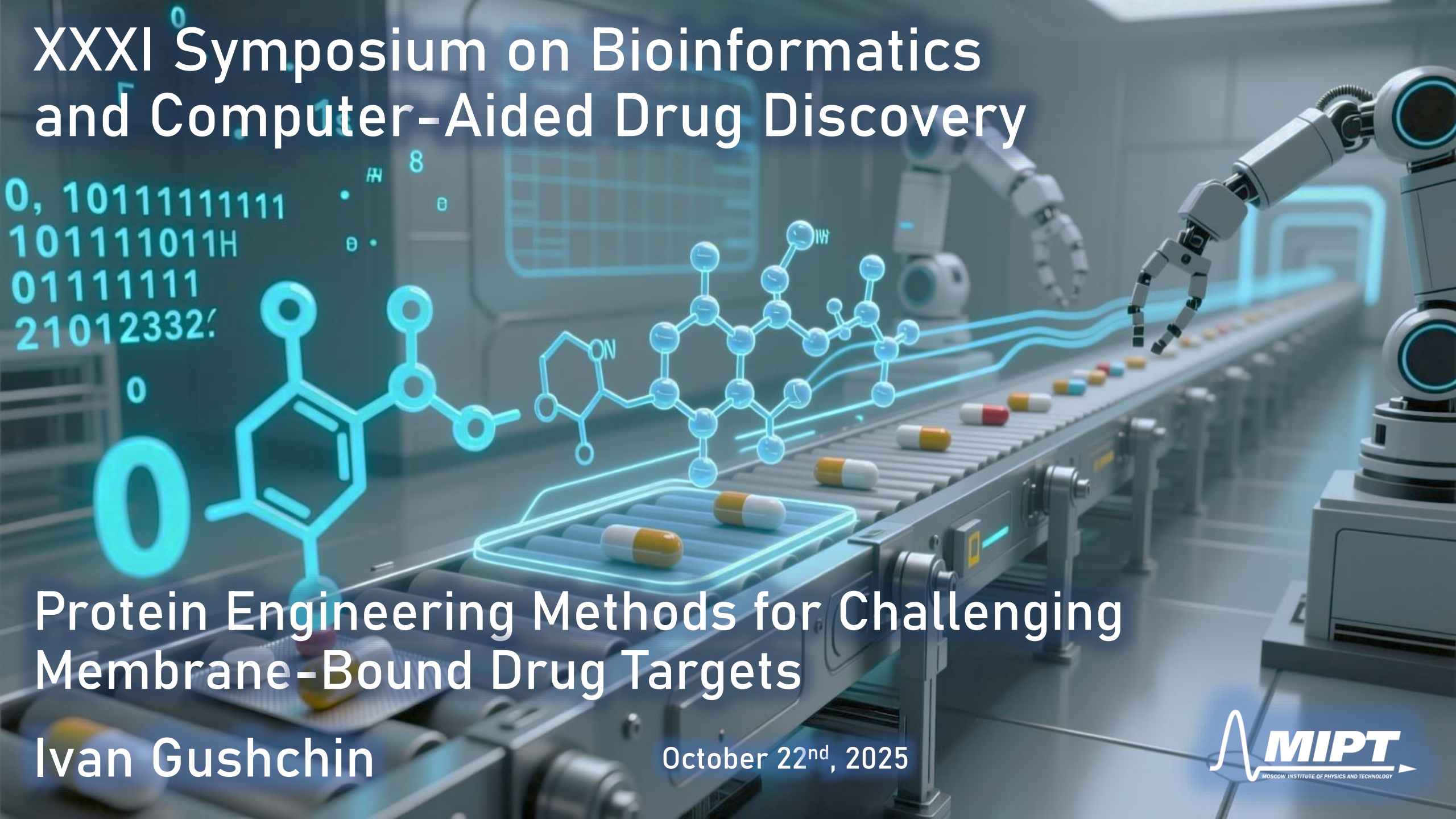
# XXXI Symposium on Bioinformatics and Computer-Aided Drug Discovery



## Protein Engineering Methods for Challenging Membrane-Bound Drug Targets

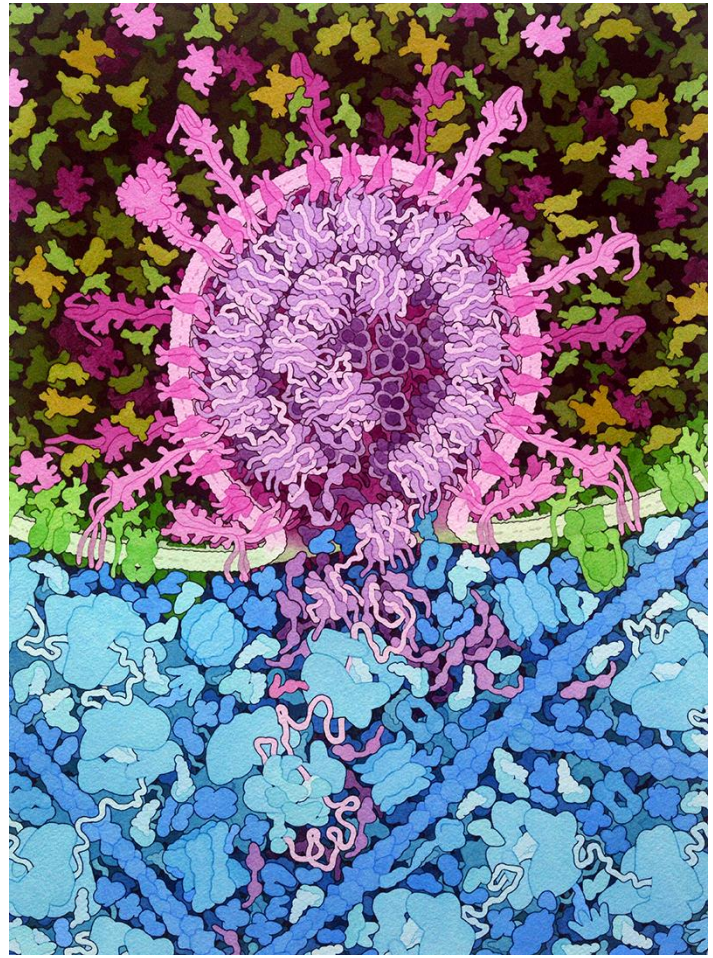
Ivan Gushchin

October 22nd, 2025

MIPT
MOSCOW INSTITUTE OF PHYSICS AND TECHNOLOGY

# Molecular interactions at the basis of disease

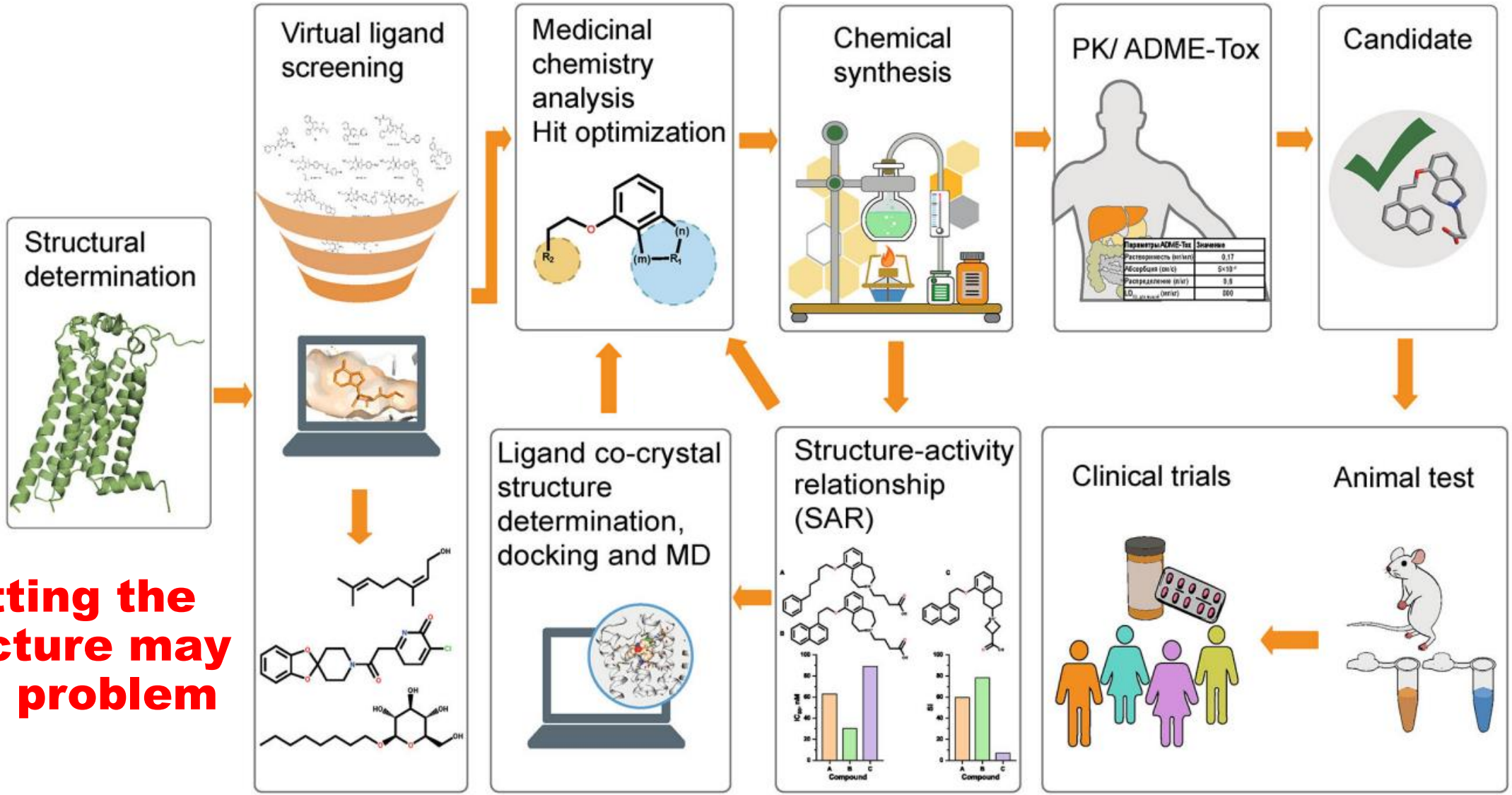
https://www.nih.gov/news-events/nih-research-matters/most-covid-19-hospitalizations-due-four-conditions



https://pdb101.rcsb.org/sci-art/goodsell-gallery/sars-cov-2-fusion

**Need to identify the target & disrupt (or enable) interactions**

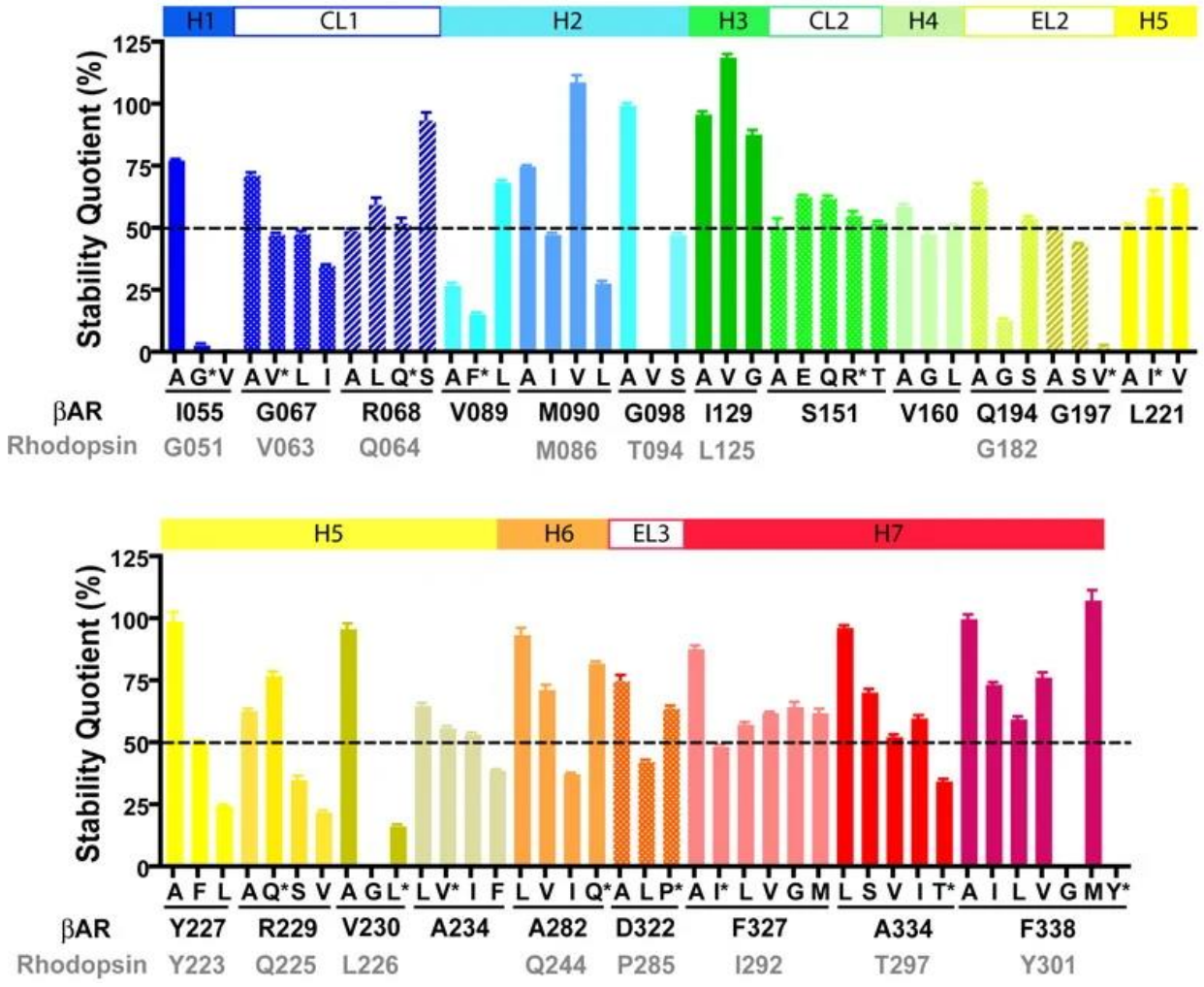
# Structure-based drug development

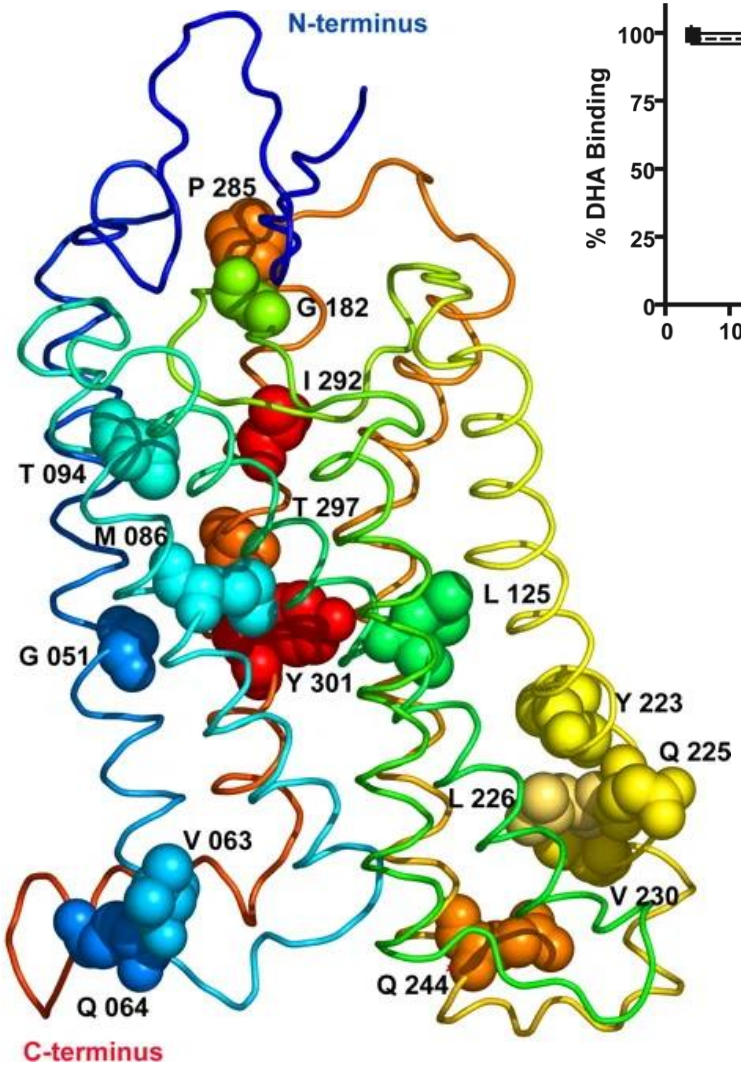


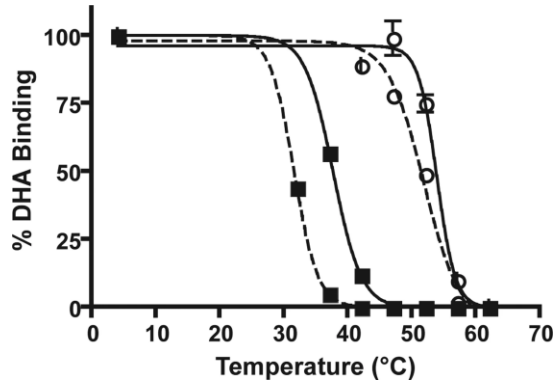

**Getting the structure may be a problem**

https://doi.org/10.1134/S0006297924040138

# Protein stabilization before AI

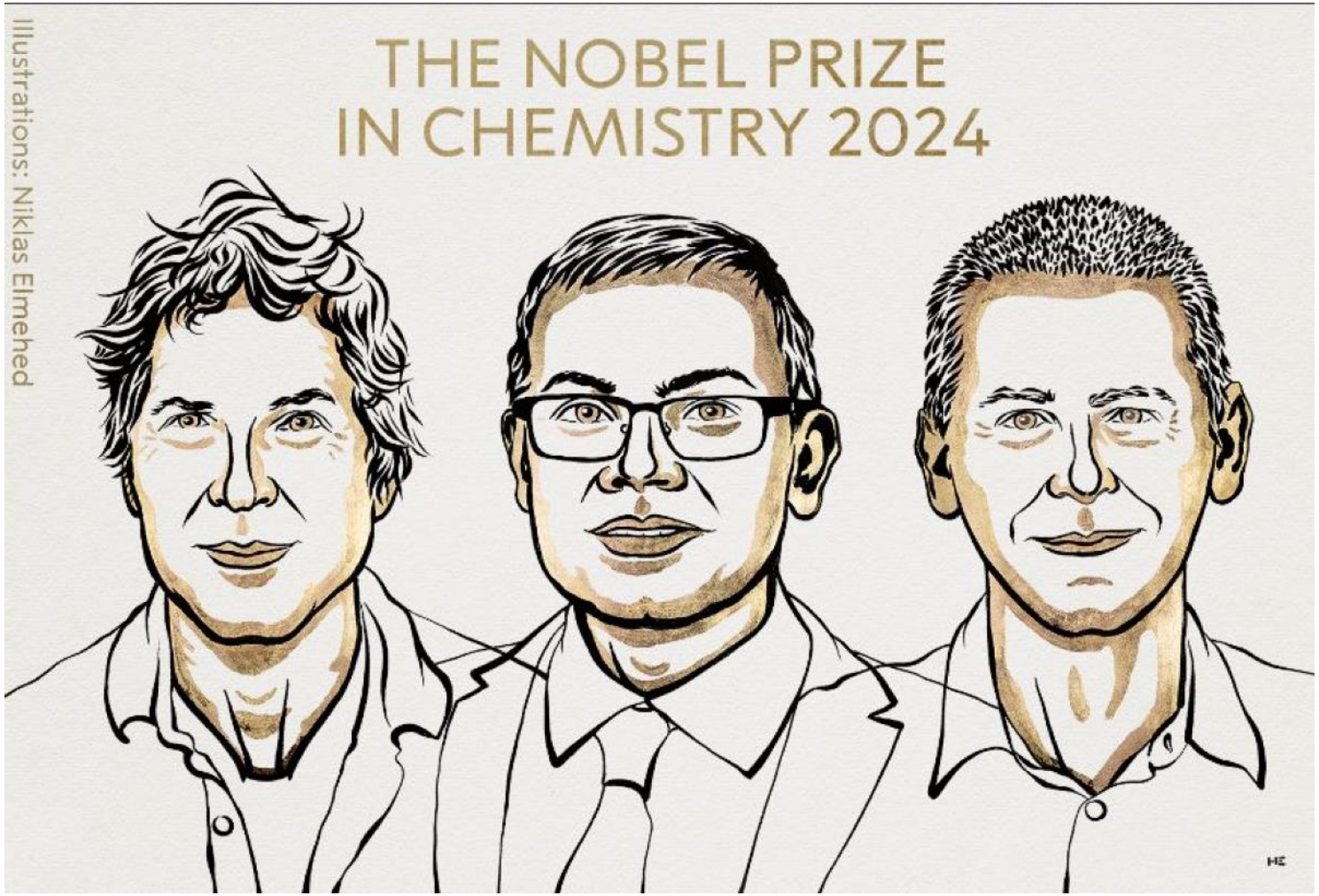



https://doi.org/10.1073/pnas.0711253105

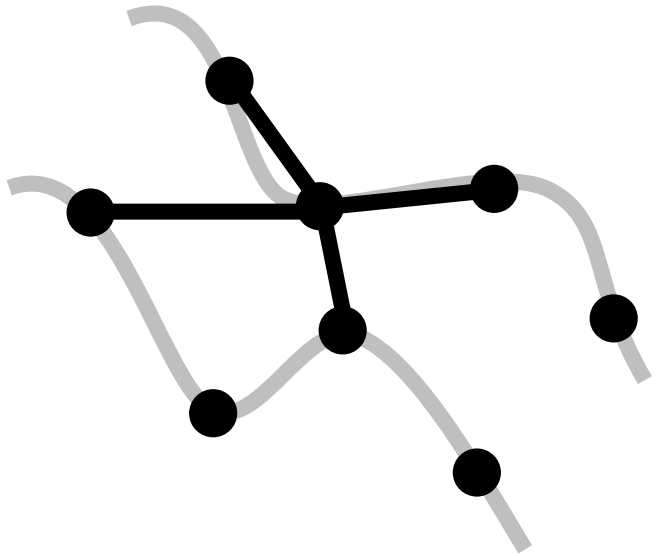
# AI triumphs in structural biology

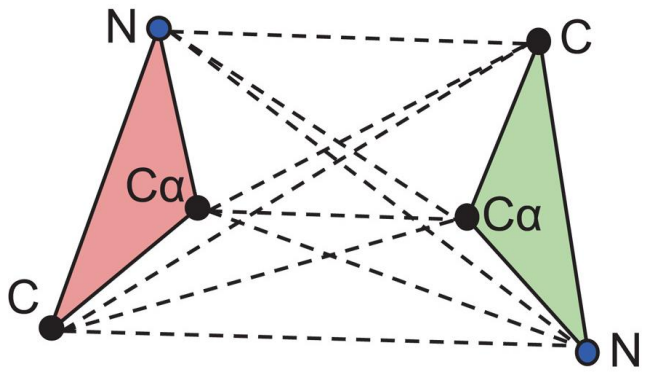


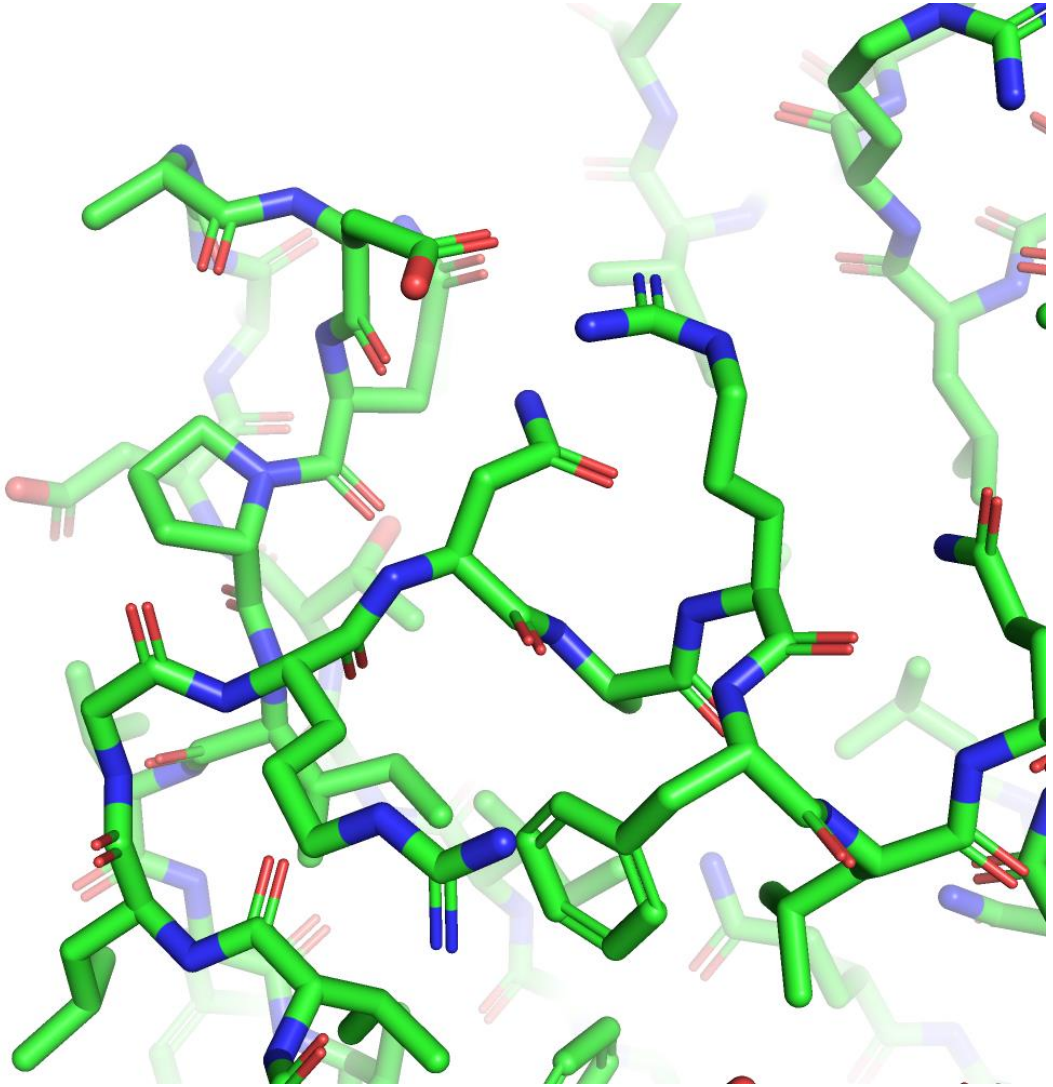

**“for protein structure prediction”**

**“for computational protein design”**

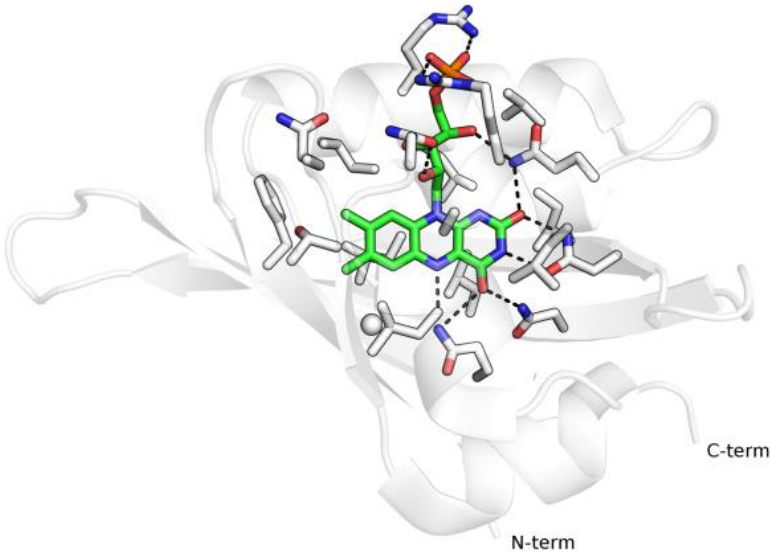
# ProteinMPNN: identification of “optimal” amino acids





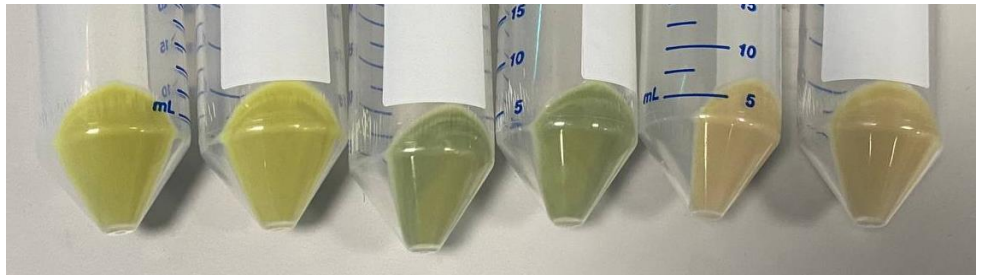



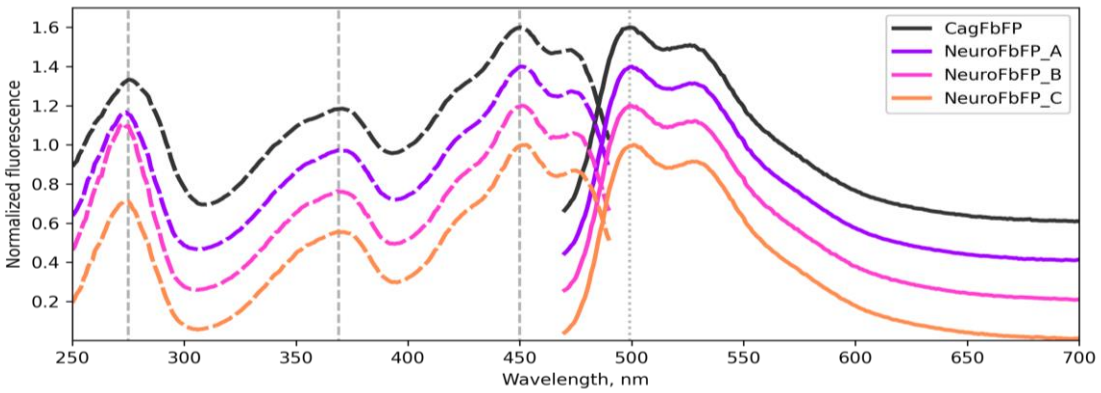
# Protein stabilization after AI

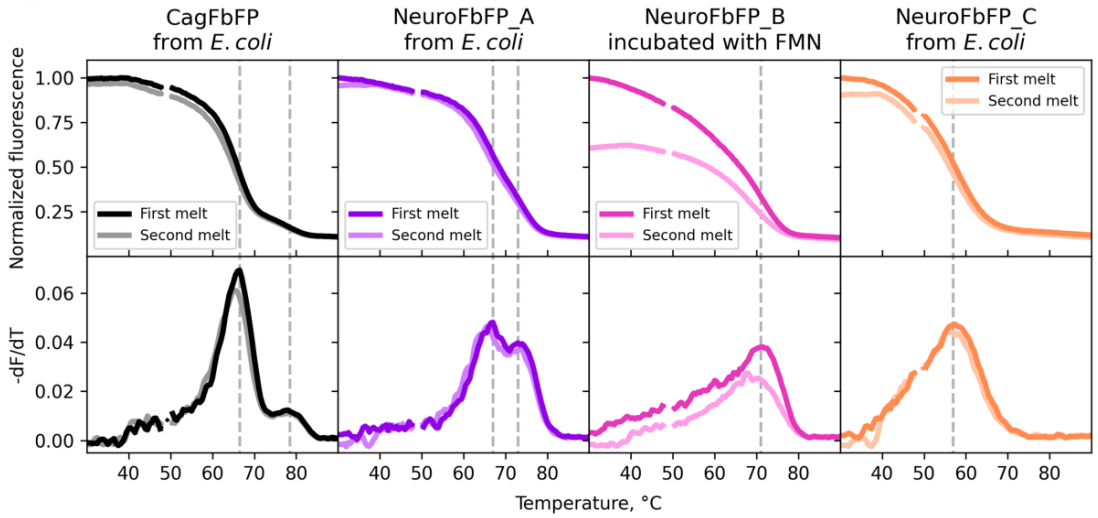



**55-66% sequence identity**


Original | Engineered





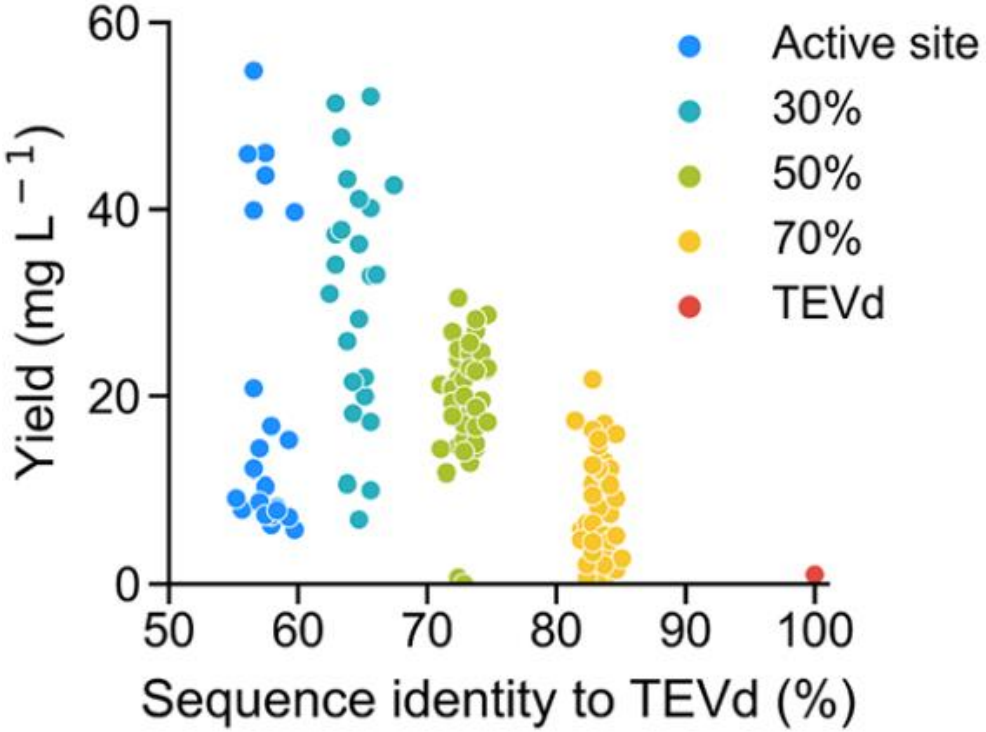

https://doi.org/10.1002/pro.4958

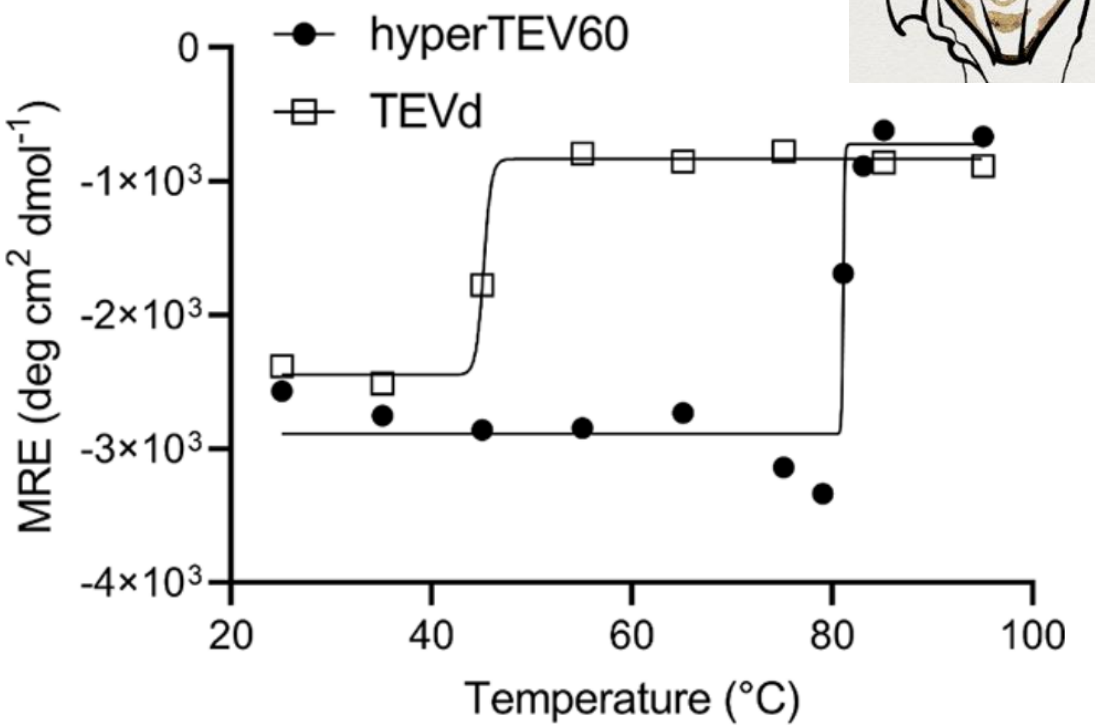
# Protein stabilization after AI




https://doi.org/10.1021/jacs.3c10941

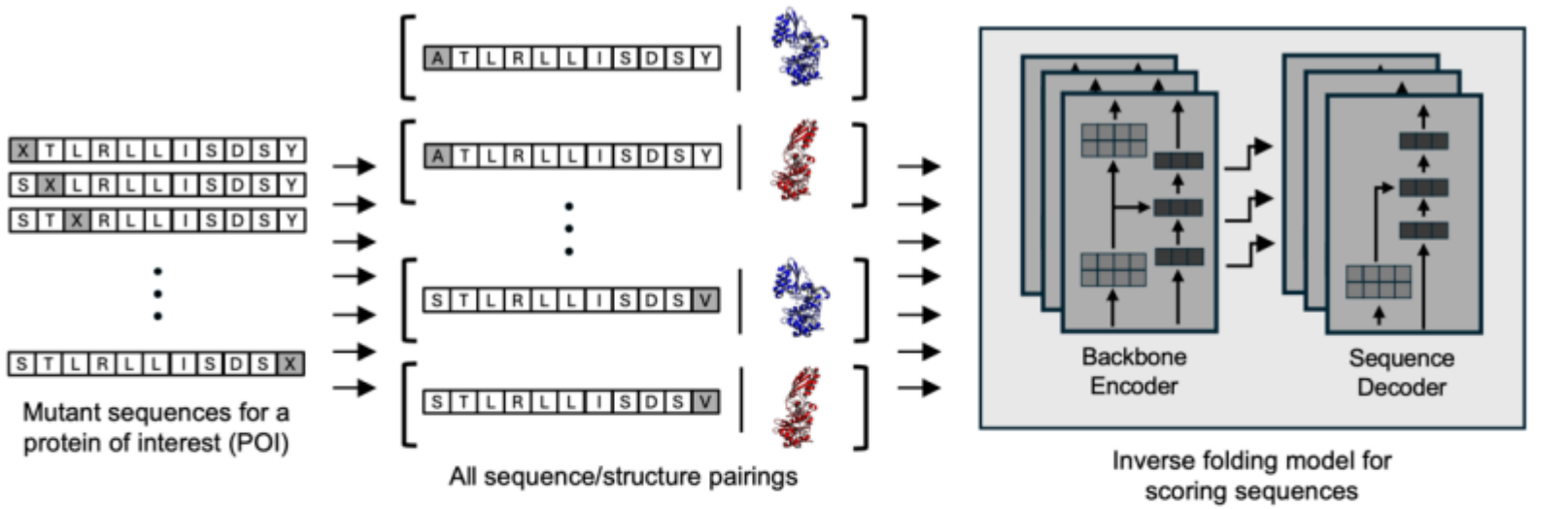
# Protein stabilization after AI

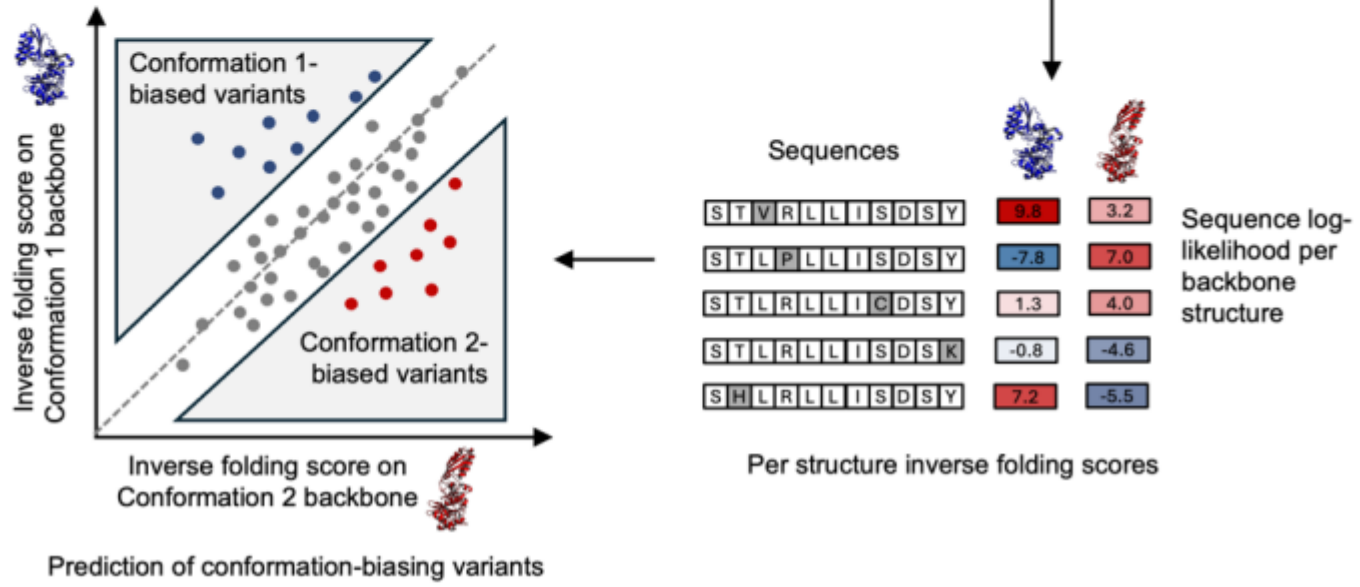


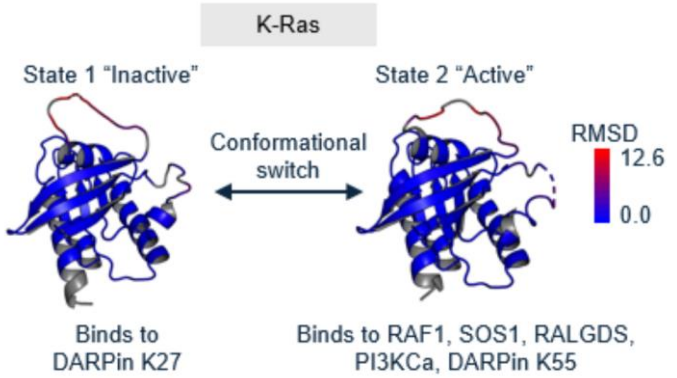



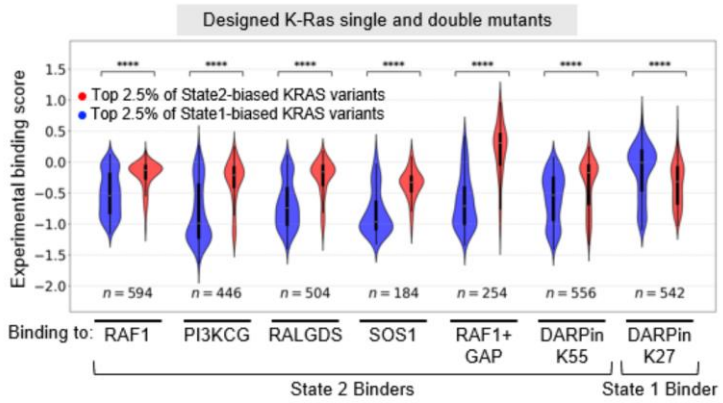

https://doi.org/10.1021/jacs.3c10941

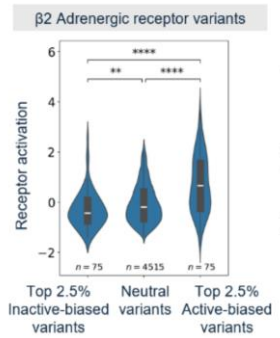
# Stabilization of a specific conformation



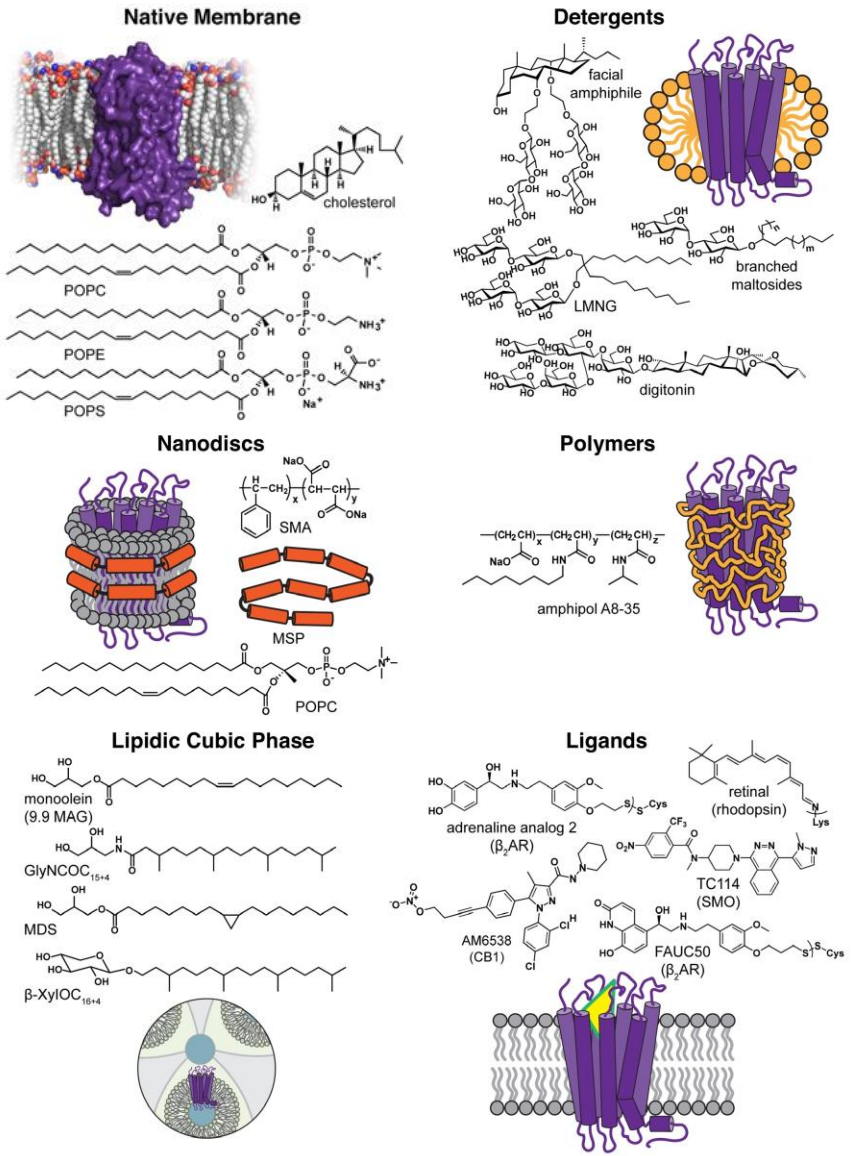




https://doi.org/10.7554/eLife.54895

Alice Ting lab, https://doi.org/10.1101/2025.05.03.652001

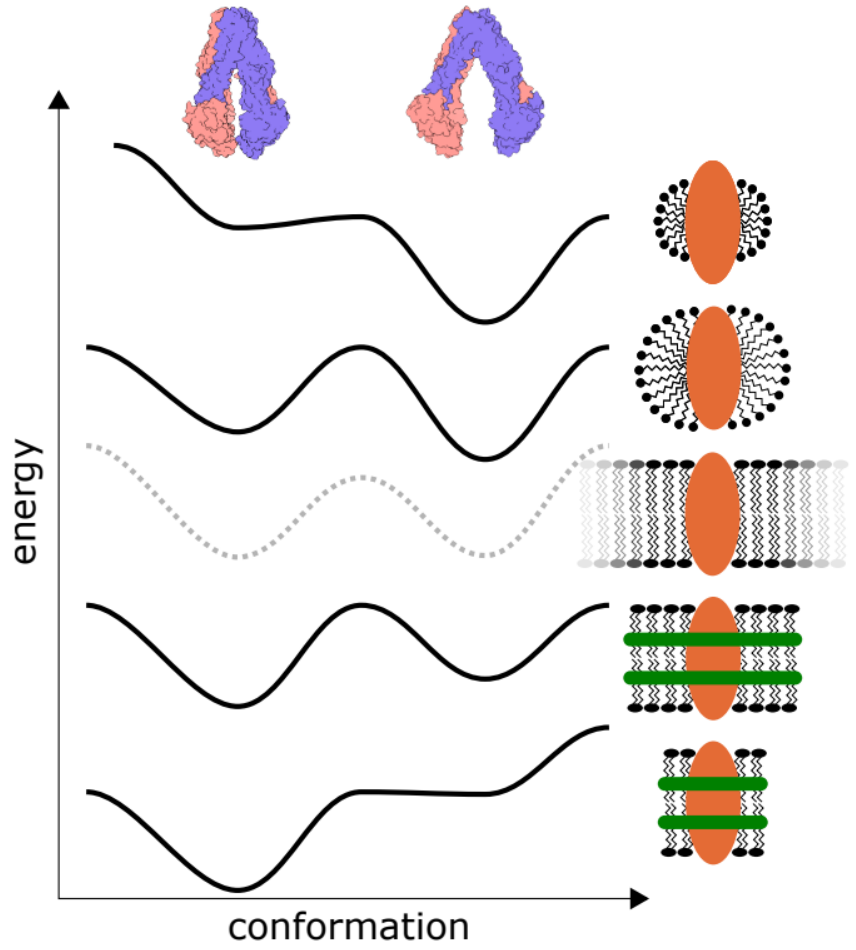
# Solubilization of membrane proteins is a challenge




Zhang & Cherezov, https://doi.org/10.1016/j.sbi.2019.06.002

**Finding a membrane mimic is laborious**

**Conformations may be affected**

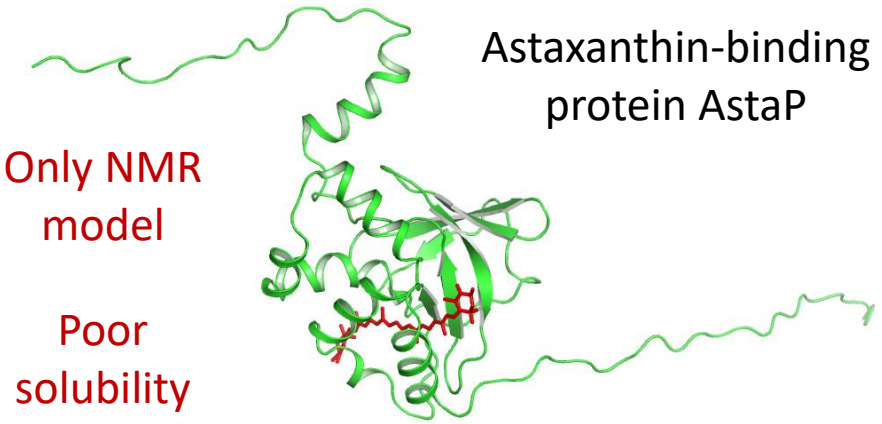



Nury & Efremov, https://doi.org/10.1016/j.str.2025.04.002

# Enhancing solubility of a membrane-associated protein

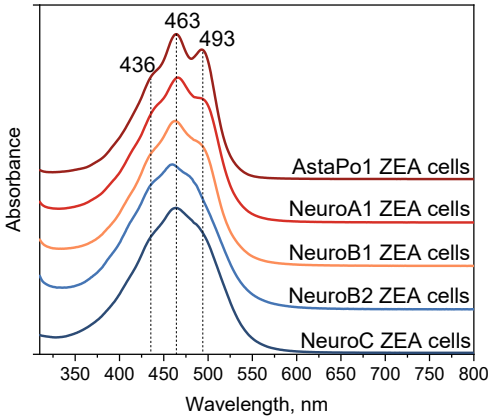
Only NMR model

Poor solubility



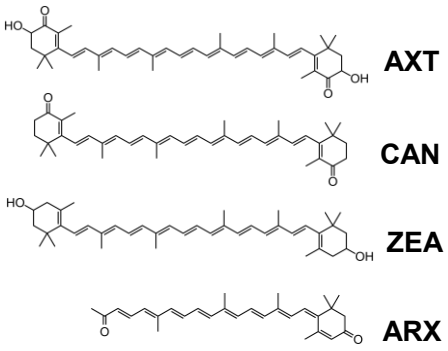
Astaxanthin-binding protein AstaP

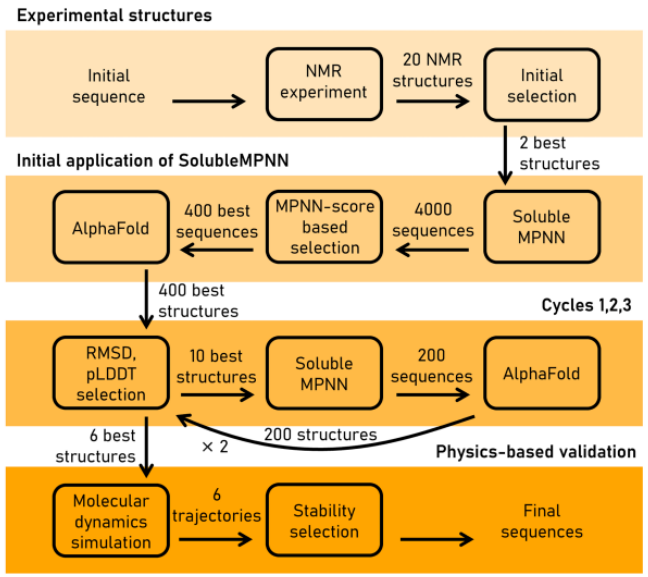
**58-62% residues exchanged**

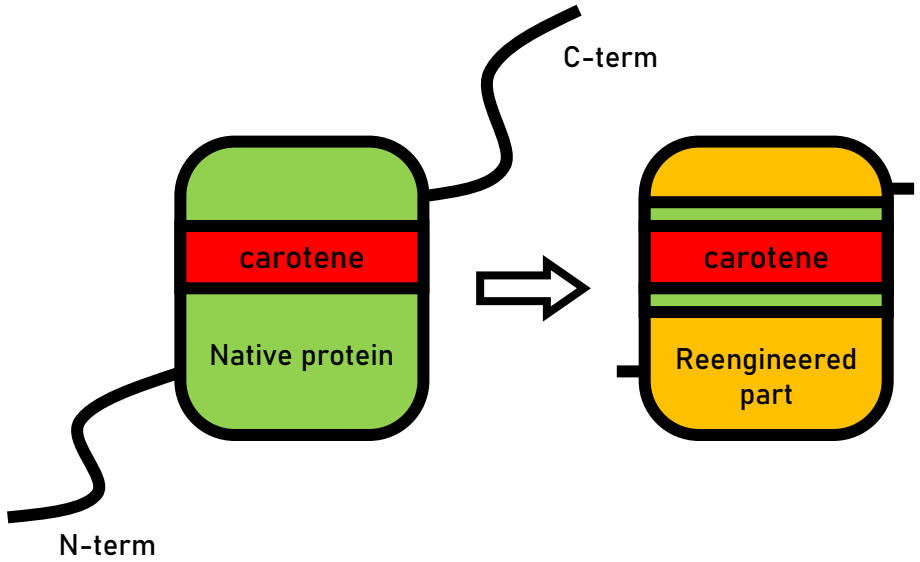






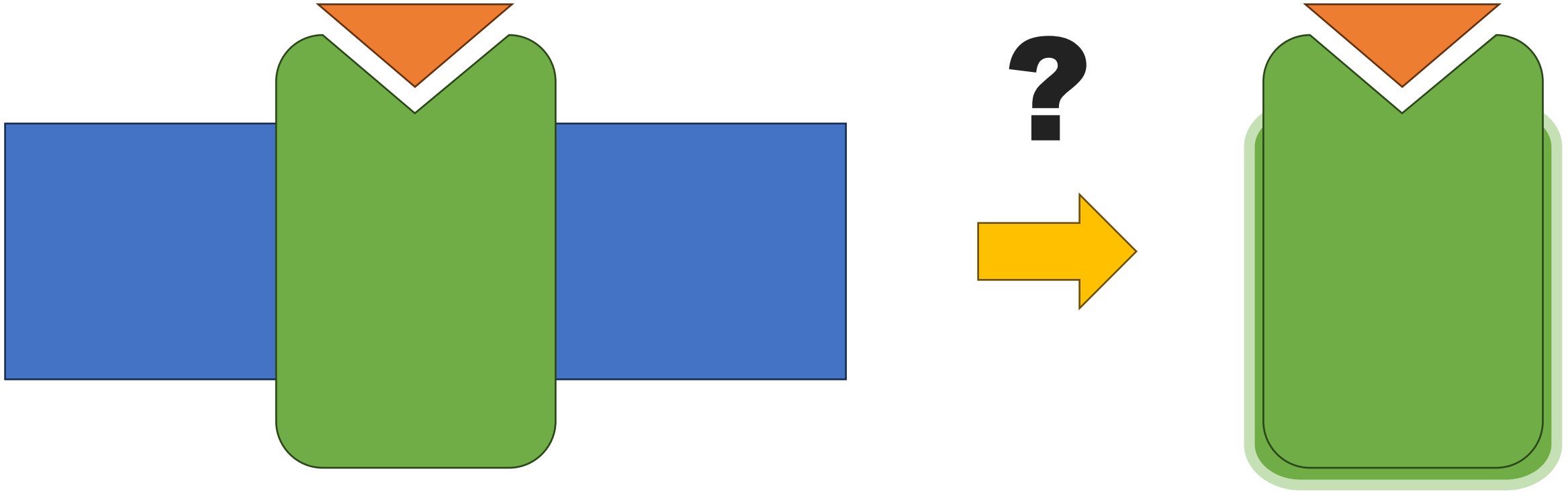



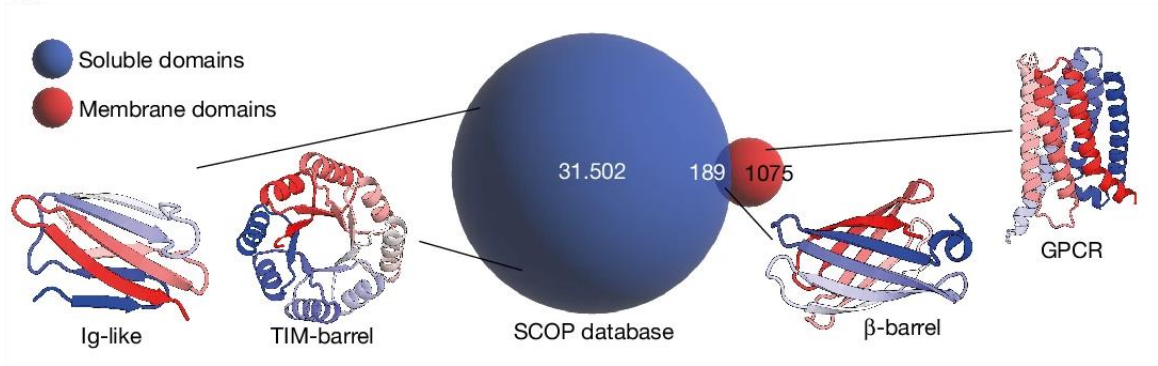



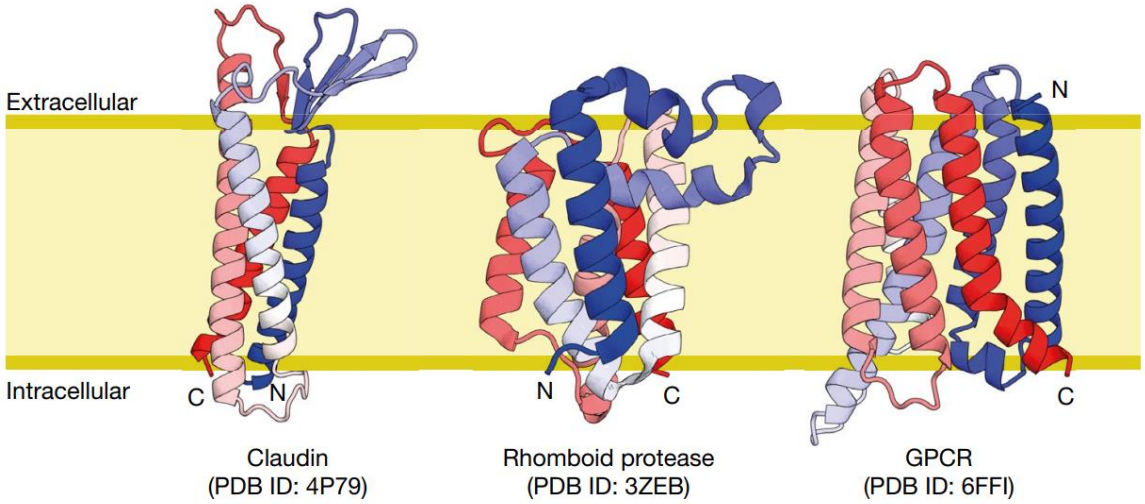

https://doi.org/10.1002/pro.5216

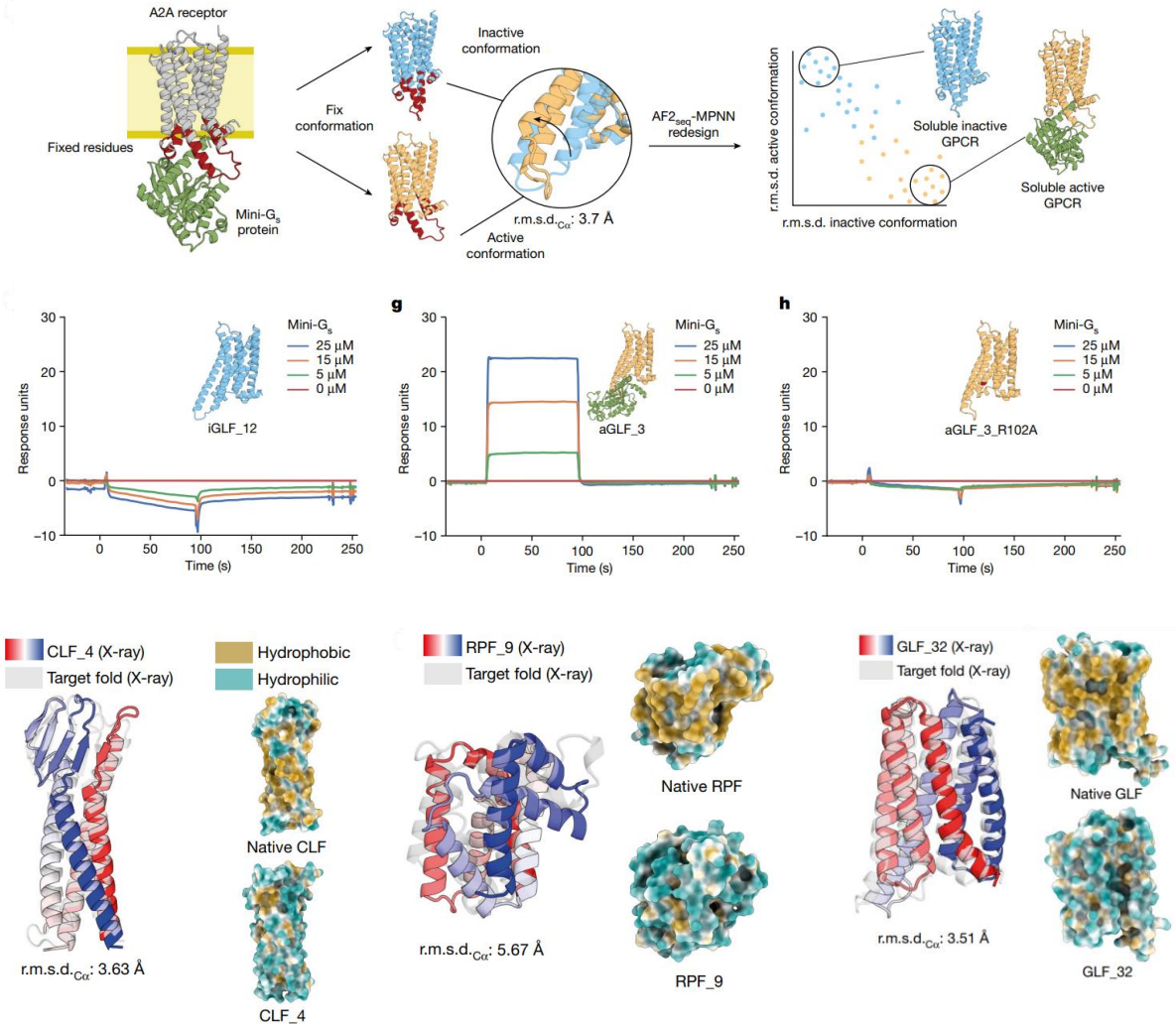
# Active site mimics for challenging targets



# Computational design of soluble and functional membrane protein analogues

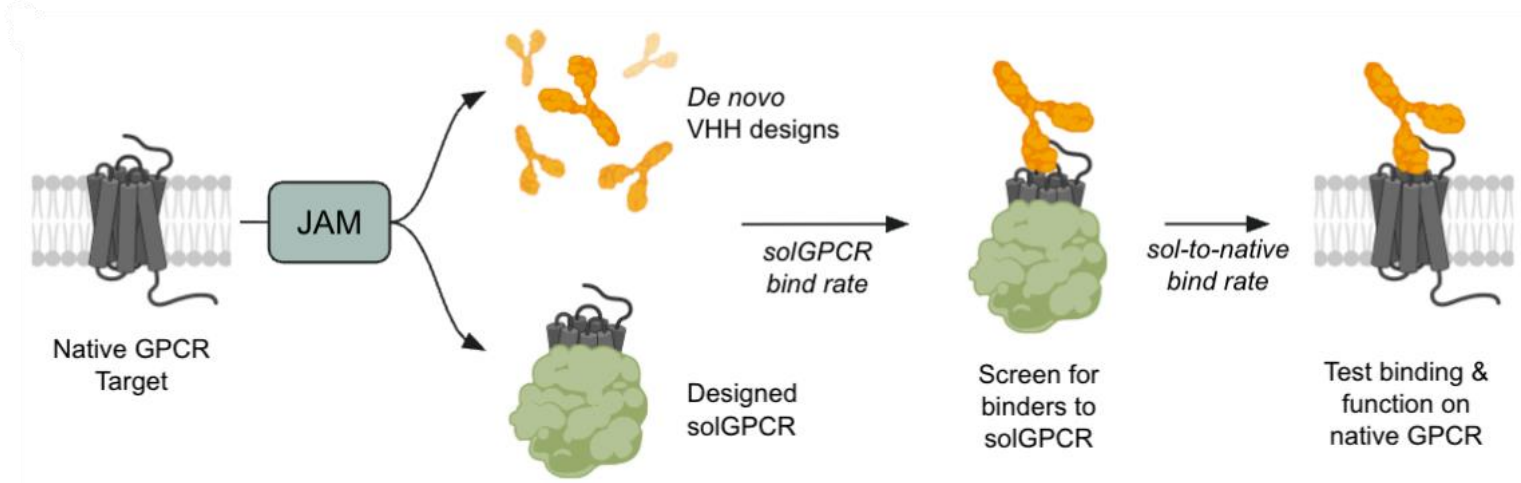


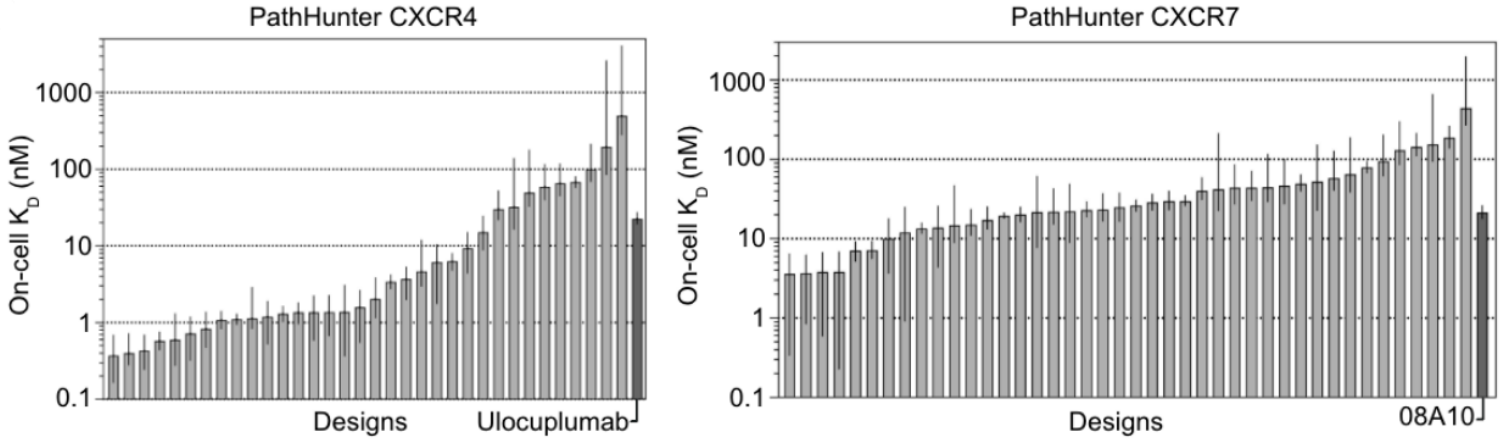



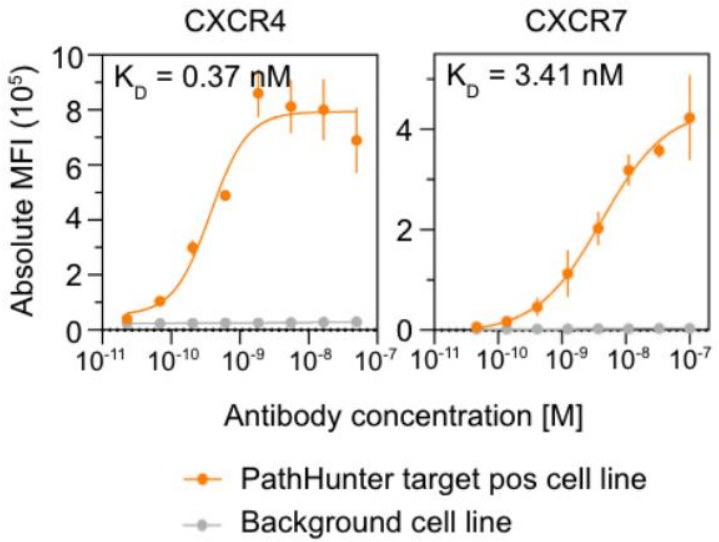

Goverde et al., Correia lab, https://doi.org/10.1038/s41586-024-07601-y

# Soluble analogs for ligand screening




Nabla Bio

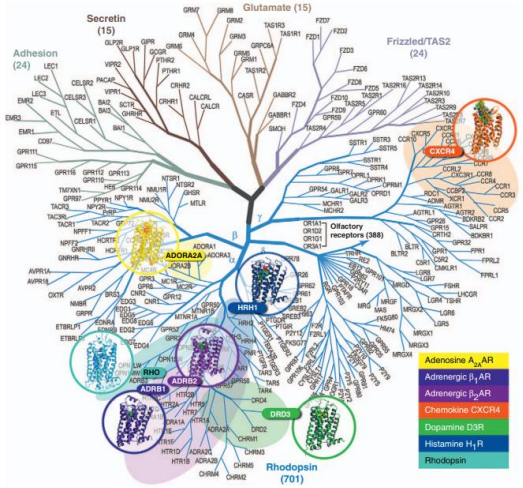


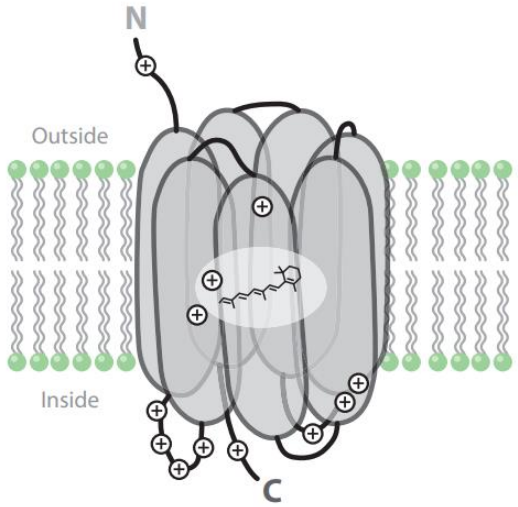

https://doi.org/10.1101/2025.01.21.633066

See also: https://doi.org/10.1002/pro.70184

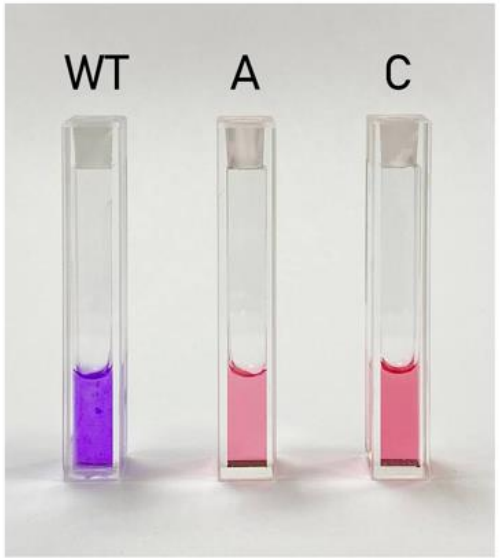
https://doi.org/10.1101/2025.08.18.670068

# Keeping intramembrane binding site during “solubilization”

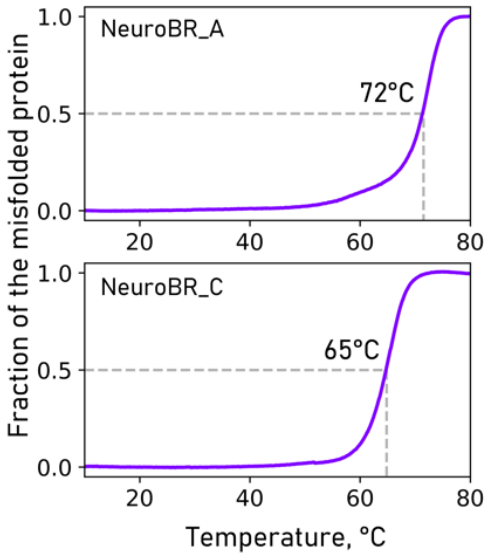


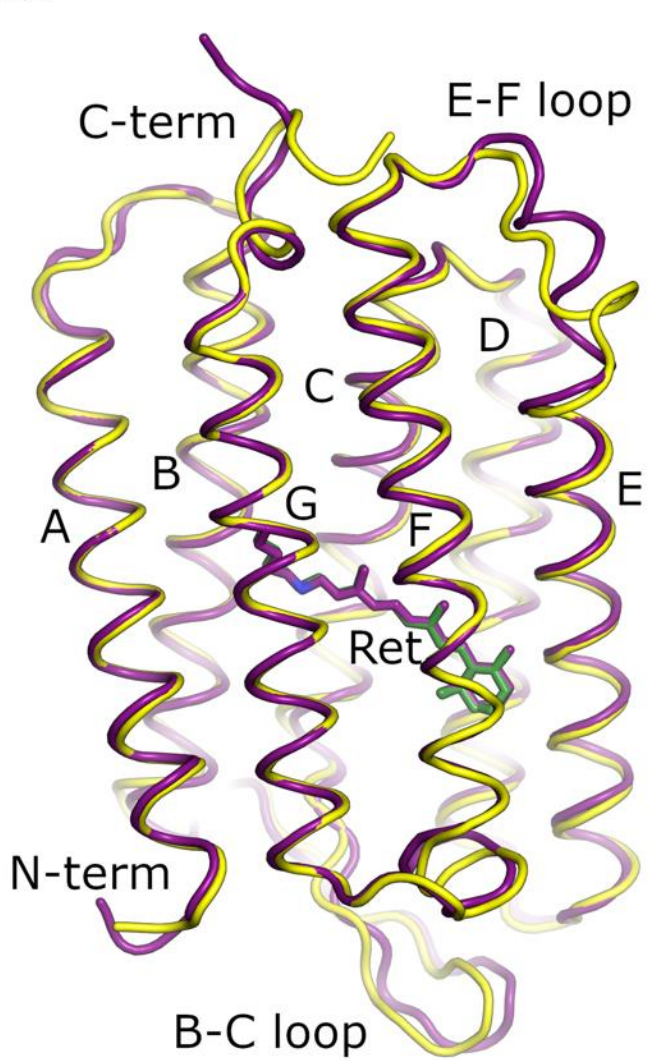



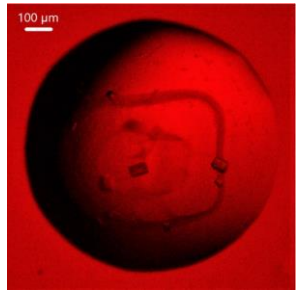

10.1146/annurev-micro-031721-020452, 10.1016/j.tips.2011.09.003



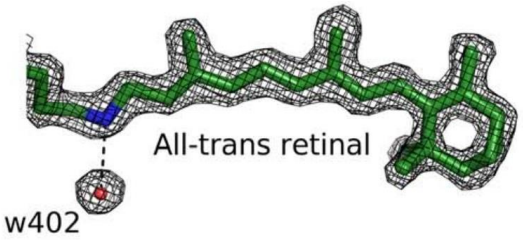

**42-45% seq. id., 2/3**

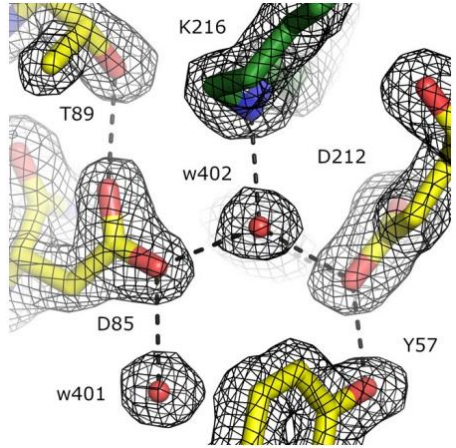


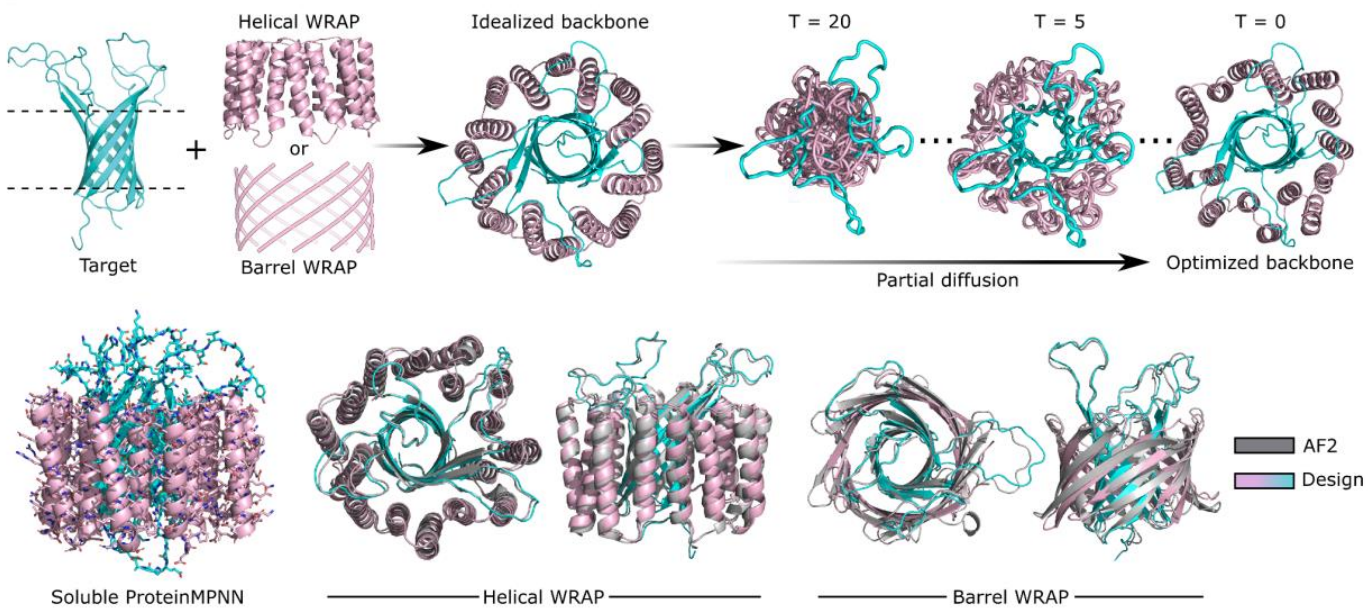


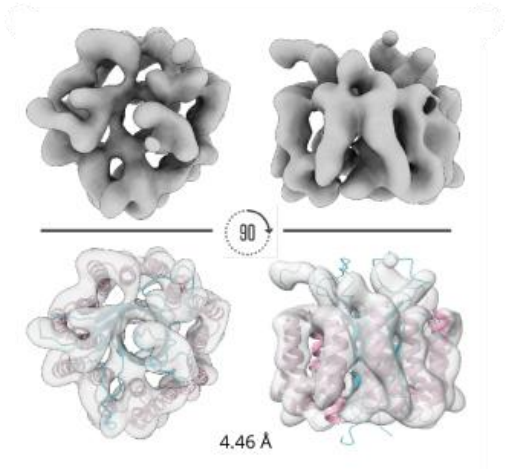


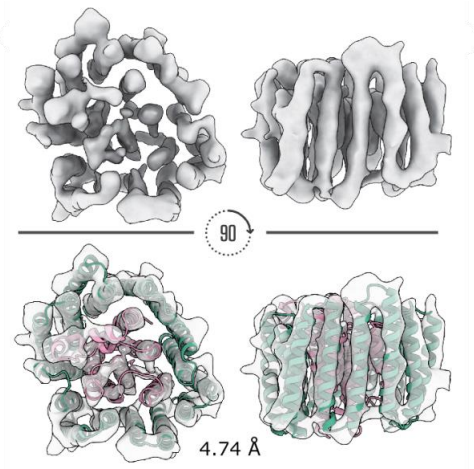




https://doi.org/10.1039/d5sc02453f

# Wrapping membrane proteins for solubility

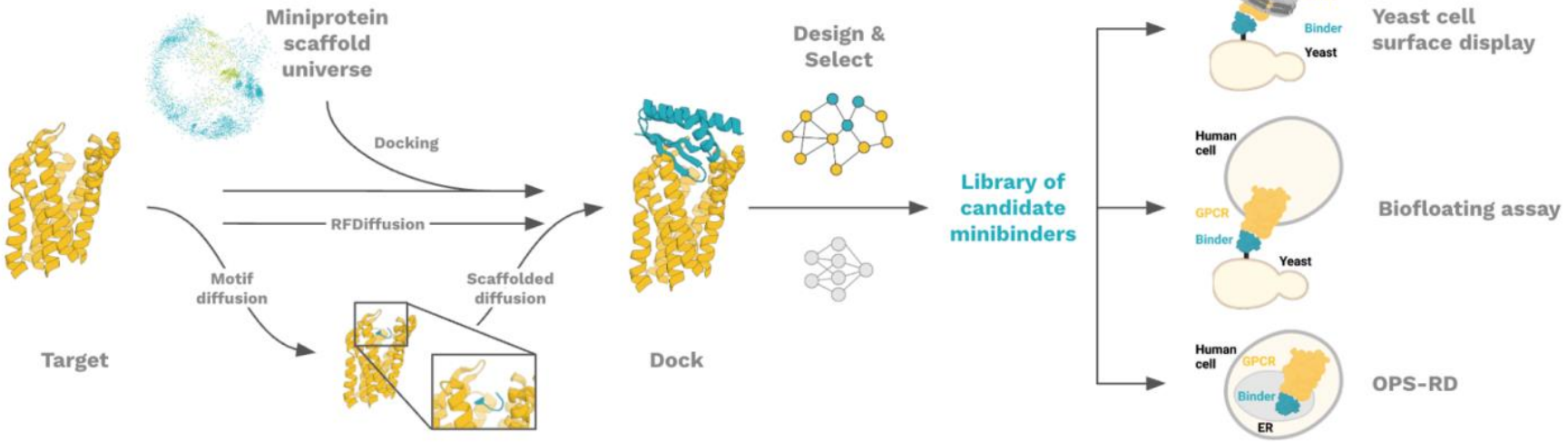


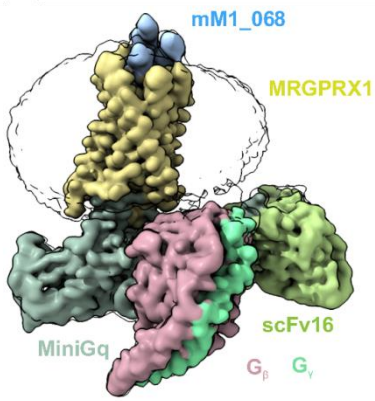



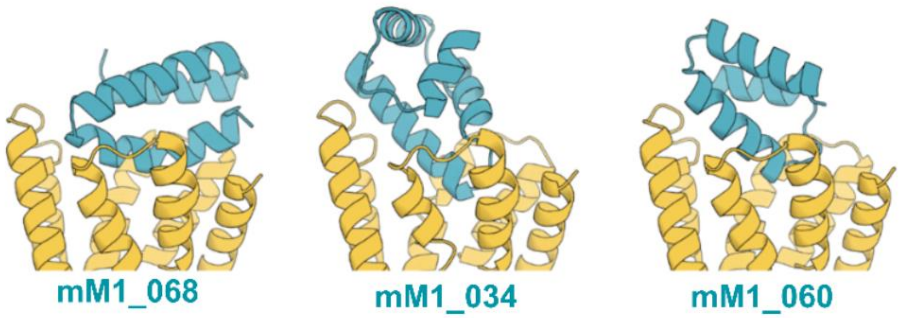



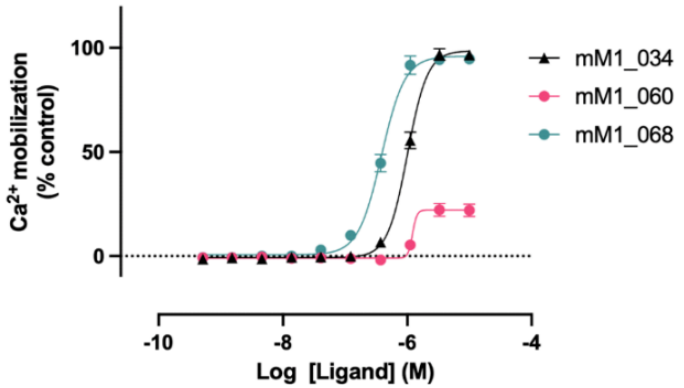

doi.org/10.1101/2025.02.04.636539

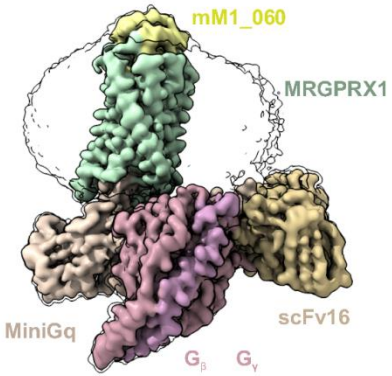
# Binder proteins: GPCR activators and inhibitors



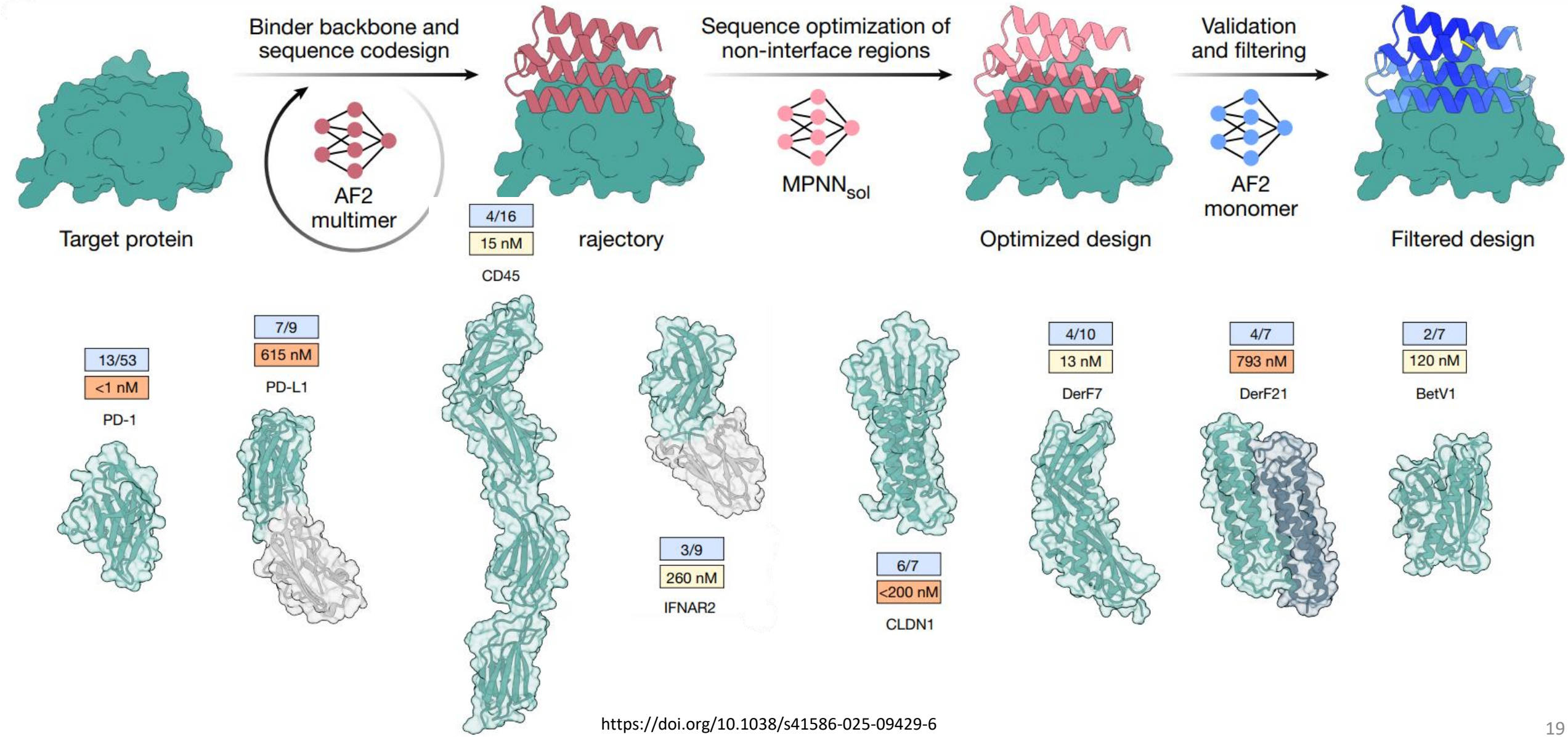




doi.org/10.1101/2025.03.23.644666

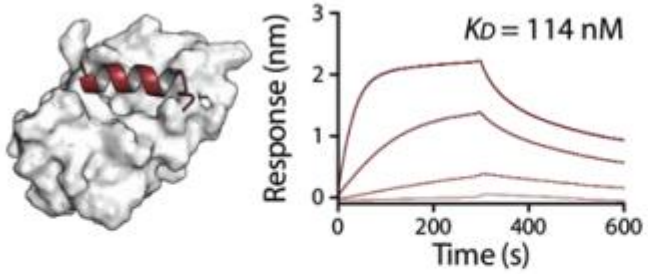
# Simplified binder design with BindCraft



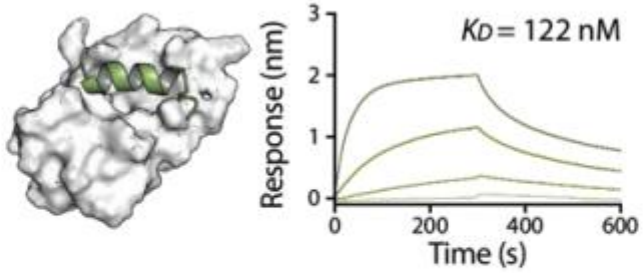

https://doi.org/10.1038/s41586-025-09429-6

# Peptide binder design with BindCraft

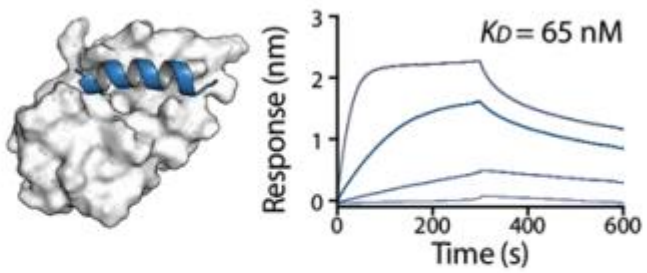
**MDM2 B3** SDLSGFGKLWEDLMNS



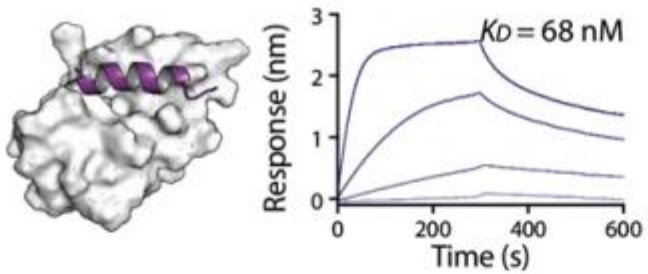

**MDM2 B4** SDLSGFGKLWQDLMNS



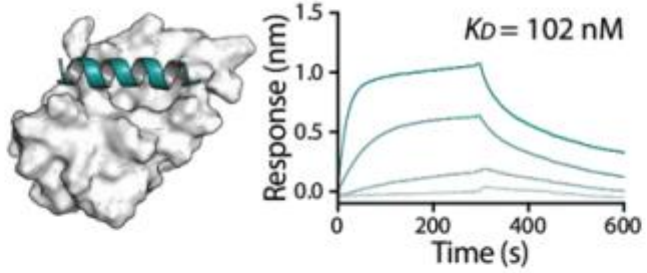

**MDM2 B5** SASDEFQKEWEDLMNF



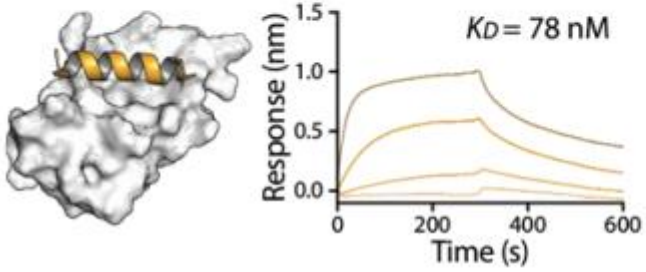

**MDM2 B6** SASDEFQKEWQDLMNF



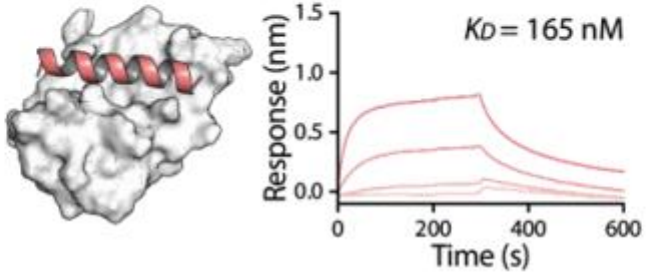

**MDM2 B16** SPSEFQKHWQDLWNDYMK



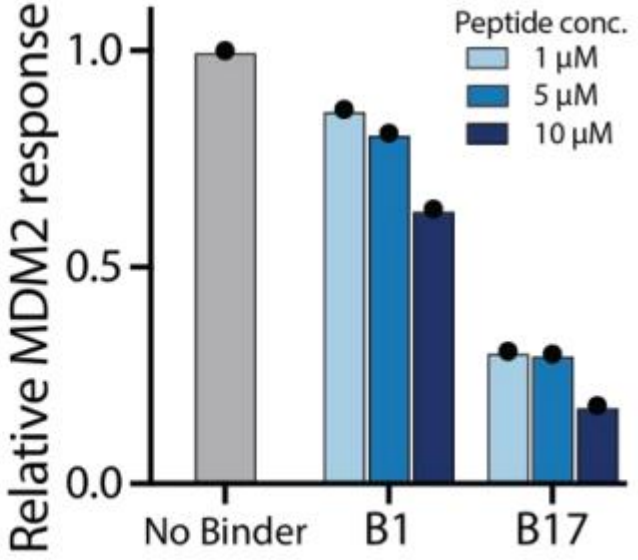

**MDM2 B17** SPSEFQKHWQDLWDDYMK




**MDM2 B20** GWEGFMKQWKEFSENLEKYM

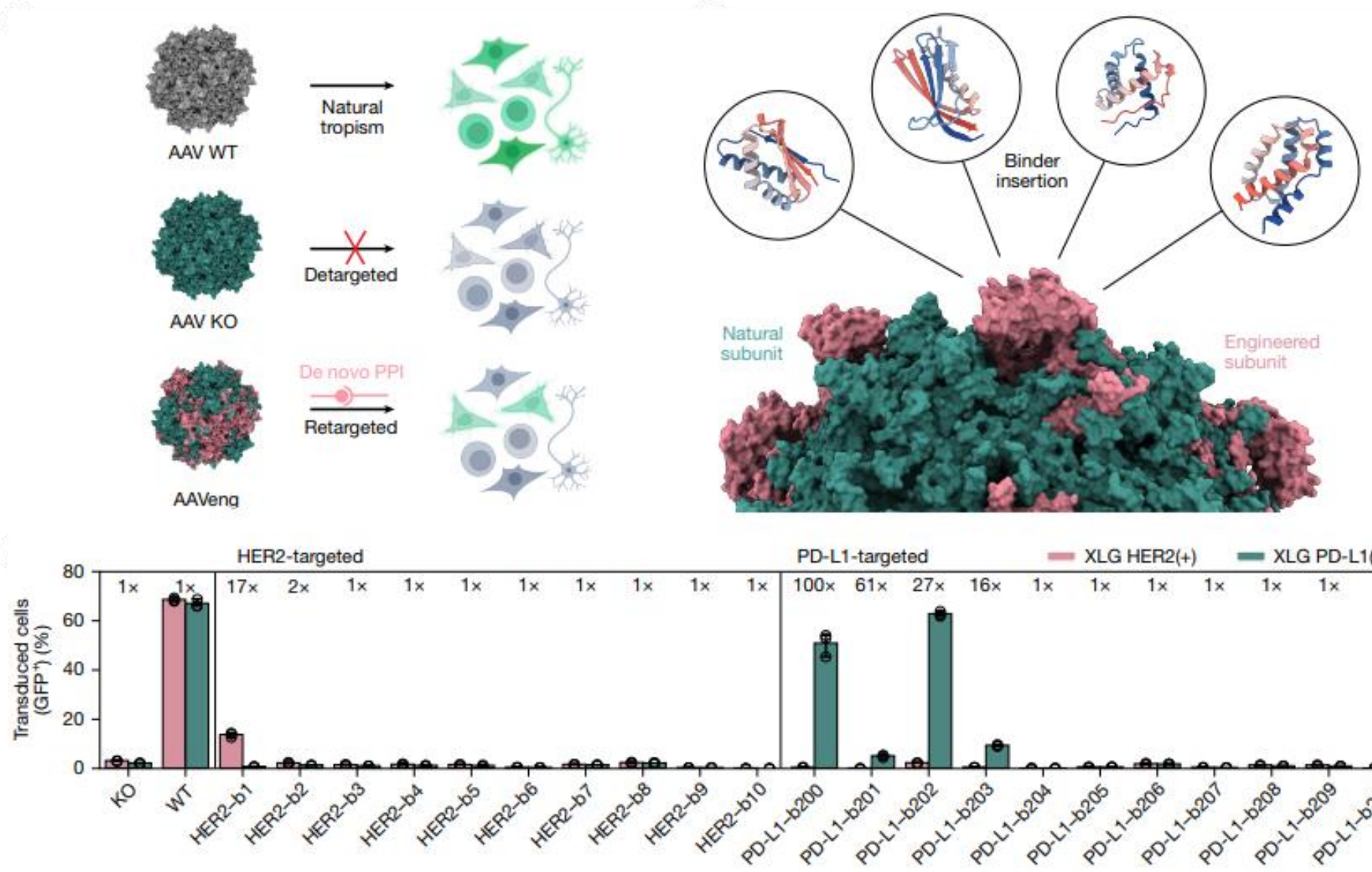



**g**




https://doi.org/10.1101/2025.07.23.666285

# AAV tropism design with BindCraft




https://doi.org/10.1038/s41586-025-09429-6

# Overall success rates

**Table 1. Metrics of AI-designed *de novo* binders to different targets - published**

| Targets | Design method | No. designs (backbones) | Exp. Hit Rate | $K_d$ (Top hits) | $T_M$ (°C) |
|---|---|---|---|---|---|
| RFdiffusion, Watson et al., *Nature* (July 2023) | | | | | |
| Influenza An H1 Hemagglutinin (HA), IL-7Rα, PD-L1, InsR, TrkA | RFdiffusion | Several thousand | 7.4–34.7% | 27nM-1.4μM (BLI) | – |
| Helical peptide binders, protein biosensors, Vázquez Torres, Leung, Venkatesh et al., *Nature* (December 2023) | | | | | |
| Bid | Hallucination | ~2000 | 8.7% | 7nM (FP) | 95 |
| Bim, Parathyroid hormone, PTH | Unconditional RFdiffusion | ~2000 | 26.0–58.3% | <500pM (FP) | 95 |
| Glucagon, GCG, Neuropeptide Y, NPY | Parametrically designed inpainted binders ($RF_{joint}$) | – | 1.0% | 231nM-3.5μM (FP) | – |
| | Partial diffusion optimization of inpainted binders | ~2000 | 20.8–26.0% | <500pM-5.3nM (FP) | – |
| GCG, NPY, PTH, Peptide YY, PYY | Fine-tuned RFdiffusion (trained on 2 chain systems from PDB) | – | – | 24.5nM-3.82μM (FP) | – |
| RFdiffusion All-Atom, non-protein ligands, Krishna, Wang, Ahern et al., *Science* (March 2024) | | | | | |
| Digoxigenin, heme, bilin | RFdiffusionAA | 25,000, ~10,000, 2,776 | 0.07–66.7%[a] | 343nM (ITC) (DIG_1) | 95 |
| TNFR agonist and antagonist binders, Glögl et al., *Science* (December 2024) | | | | | |
| TNFR1 | RFdiffusion (hotspot residues) | 30,000 | 6.7% | 29, 24.5nM, >10μM (SPR) | 95 |
| | Partial diffusion optimization | 25,000 | 29.8% | 5nM, <10pM, 20nM (SPR) | 95 |
| TNFR2, OX40, 4-1BB | Partial diffusion (RFdiffusion), using TNFR1 designs as input | 25,000 | 2.1–45.8% | 198pM-44nM (SPR) | 95 |
| Elapid snake venom binders, Vázquez Torres et al., *Nature* (January 2025) | | | | | |
| ScNtx, α-cobratoxin (P01391) | RFdiffusion (β-edge pairing) | ~2000 | 2.3–2.4% | 842nM-1.3μM (BLI) | – |
| | Partial diffusion optimization | ~2000 | 10.5–14.1% | 0.7–6.7nM (BLI, SPR) | 78- >95 |
| Consensus cytotoxin | RFdiffusion (hotspot residues) | ~2000 | 5.6% | 271nM (SPR) | 61 |
| | CTYX with an introduced disulfide bond to reduce flexibility | NA | NA | 740nM (SPR) | 70.3 |
| Macrocyclic binders, Rettie, Juergens, Adebomi et al., *Nature Chemical Biology* (June 2025) | | | | | |
| MCL1, MDM2, GABARAP, RbtA | RFpeptides (+/– hotspot residues) | 10,000–20,000 | 21.4–66.7% | 6nM-2μM (SPR) | – |
| Anti-microbial/heme-uptake transporter binders, Fox et al., *Nature Communications* (July 2025) | | | | | |
| ChuA | RFdiffusion (hotspot residues) | 25,000 | 8.3% | 64.4–155nM (BLI) | – |

**Table 2. Metrics of AI-designed *de novo* binders to different targets - preprints**

| Targets | Design method | No. designs (backbones) | Exp. Hit Rate | $K_d$ (Top hits) | $T_M$ (°C) |
|---|---|---|---|---|---|
| Binders to intrinsically disordered proteins and regions, Liu, Wu, Choi et al., *bioRxiv* (July 2024) | | | | | |
| Amylin (hIAPP), C-peptide, VP48, G3bp1, IL2RG, Prion | RFdiffusion (either with varied constraints or unconstrained) | ~10,000–50,000 | 1.0–5.1% | 14nM-16μM (BLI) | 95 |
| | Two-sided partial diffusion optimization | ~10,000–50,000 | 3.2–61.5% | 3.8nM-2μM | 95 |
| AlphaProteo (closed-source), Zambaldi, La, Chu, Patani, Danson, Kwan, Frerix, Schneider, Saxton, Thillaisundaram, Wu et al., *arXiv* (September 2024) | | | | | |
| BHRF1, SARS-CoV-2 spike RBD, IL-7Rα, PD-L1, TrkA, IL-17A, VEGF-A, TNFα | AlphaProteo (hotspot residues) | – | 0-88% | 82pM-26nM (HTRF) | 95 |
| BindCraft, Pacesa, Nickel, Schellhaas et al., *bioRxiv* (October 2024) | | | | | |
| PD-1, PD-L1, IFNAR2, CD45, BBF-14, CrSAS-6, Der f7, Der f21, Bet v1, SpCas9 | BindCraft (+/– hotspot residues) | 300–6000 | 10-100% | <1nM-5.7μM (SPR) | 90 |
| Improved binder design with beta-pairing RFdiffusion, polar targets, Sappington et al., *bioRxiv* (October 2024) | | | | | |
| KIT (SCFR, CD117), PDGFRα, ALK-2, ALK-3, FCRL5, NRP1, α-cobratoxin | RFdiffusion (β-strand pairing, target residues) | ~10,000 | – | 76pM-193nM (SPR) | 63.2- >95 |
| Binders for use in CAR-T therapies, Mergen, Abele et al., *bioRxiv* (November 2024) | | | | | |
| BCMA (CD269) | RFdiffusion (Google Colab, hotspot residues) | 1-28 designs for 18 binders | 16.7% | – | – |
| pMHC-I binders, CAR-T therapies, Johansen, Wolff, Scapolo, Fernández-Quintero et al., *bioRxiv* (December 2024) | | | | | |
| Cancer/Testis Antigen 1B NY-ESO-$1^{(157\text{-}165)}$/HLA-A*02:01, Metastatic melanoma neoantigen RVTDESILSY /HLA-A*01:01 | RFdiffusion (against non-achor peptide residues) | 2,100, 5,500 | 2.1–2.3% | – | – |
| Anti-CRISPR binders (Alcrs), Taveneau et al., *bioRxiv* (December 2024) | | | | | |
| LbuCas13a | RFdiffusion (hotspot residues) | 10,000 | 5.2–10.4% | – | 70 |
| TCR-mimic binders, Householder et al., *bioRxiv* (December 2024) | | | | | |
| NY-ESO-$1^{(157\text{-}165)}$/HLA-A*02 | RFdiffusion (hotspots residues) | 100 | 40% | 9.5nM (SPR) | – |
| GPCR agonist and antagonist binders, Muratspahić, Feldman, Kim, Qu, Bratovianu, Rivera-Sánchez et al., *bioRxiv* (March 2025) | | | | | |
| MRGPRX1, CXCR4, GLP1R, GIPR, GCGR, CGRPR, | RFdiffusion (scaffold-guided) | 26,000–100,000 | 0.008–2.1%[a] | 5.3–27nM (SPR) | 95 |
| CGRPR | Partial diffusion optimization | 3,000 | 46.2% | – | – |
| Chai-2 (closed-source), Chai Discovery Team et al., *bioRxiv* (July 2025) | | | | | |
| PDGFR, IL-7Rα, PD-L1, InsulinR, TNFα | Chai-2 | – | 22-95% | 18.3pM-11.7nM[b] (BLI) | – |

**Gene synthesis cost as low as 1-3 k$ per screening round**

https://doi.org/10.1016/j.str.2025.08.007

# Progress in protein engineering

Before 2021

Complicated software

Complicated calculations

Thousands of trials

Low success rate
(0-0.1%)

AI/ML
advances

In 2025

Easy-to-use* software

Minutes to days on
gaming PC

3-10 trials

High success rate*
(20-100%)

# Summary

**1. Protein design strongly advances drug discovery efforts and provides new avenues (stabilization, mimics, binders)**

**2. Rapid development of new methods leads to higher competition and search for new biological insights**

**3. Protein engineering gets simple: try it yourself!**

# Acknowledgements

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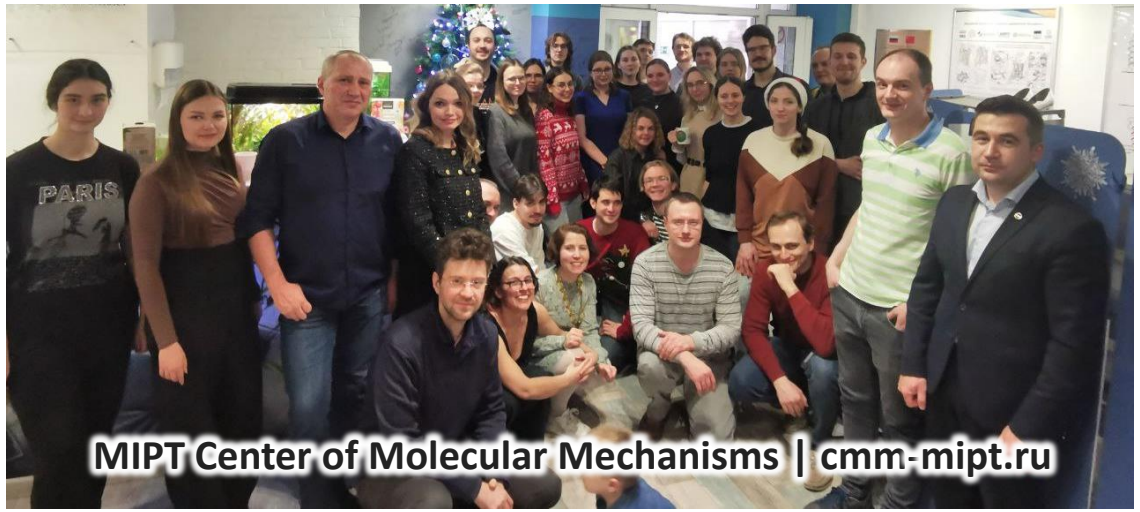

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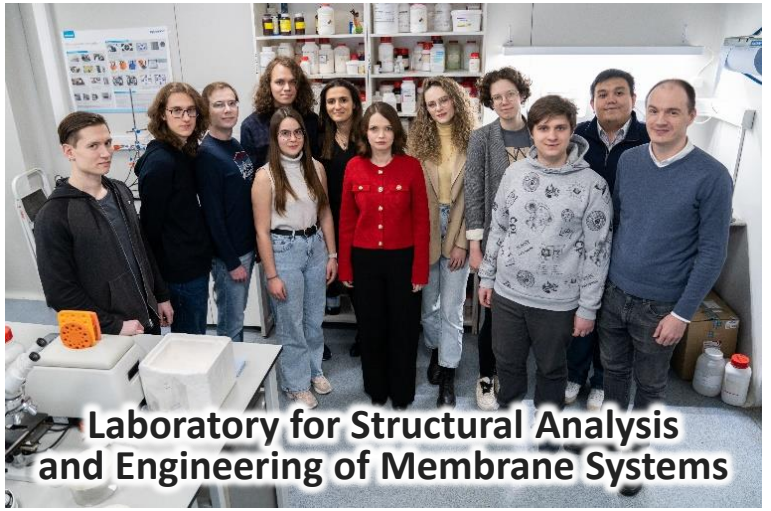

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