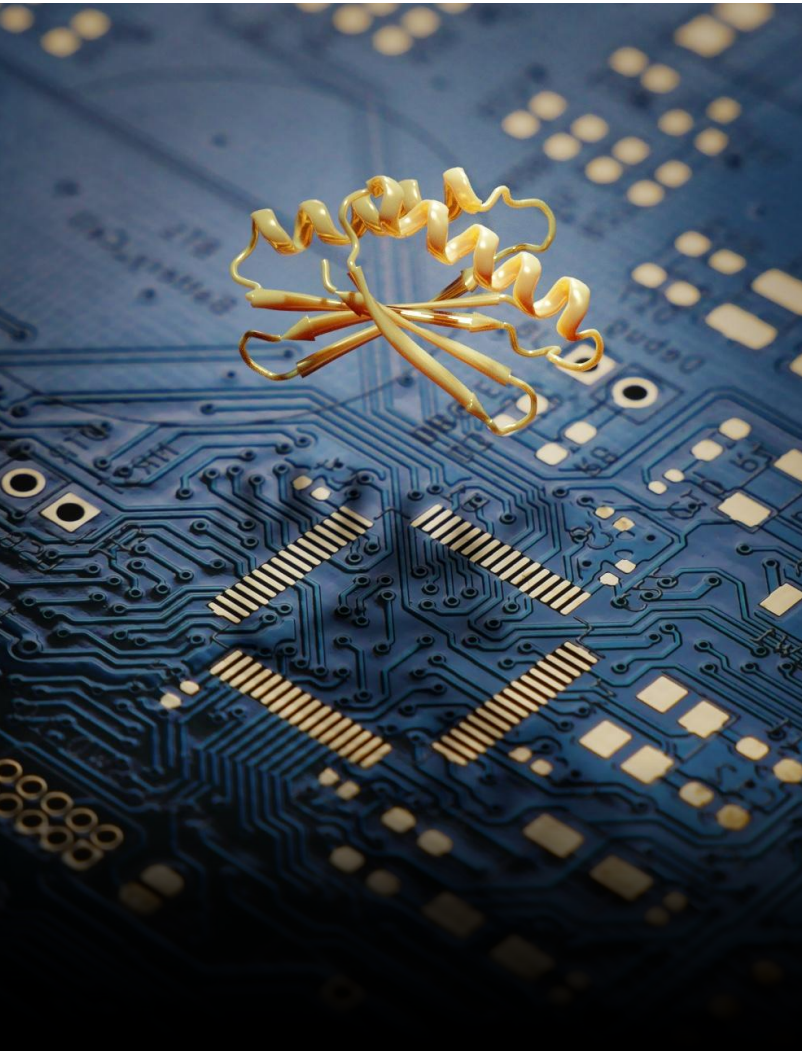
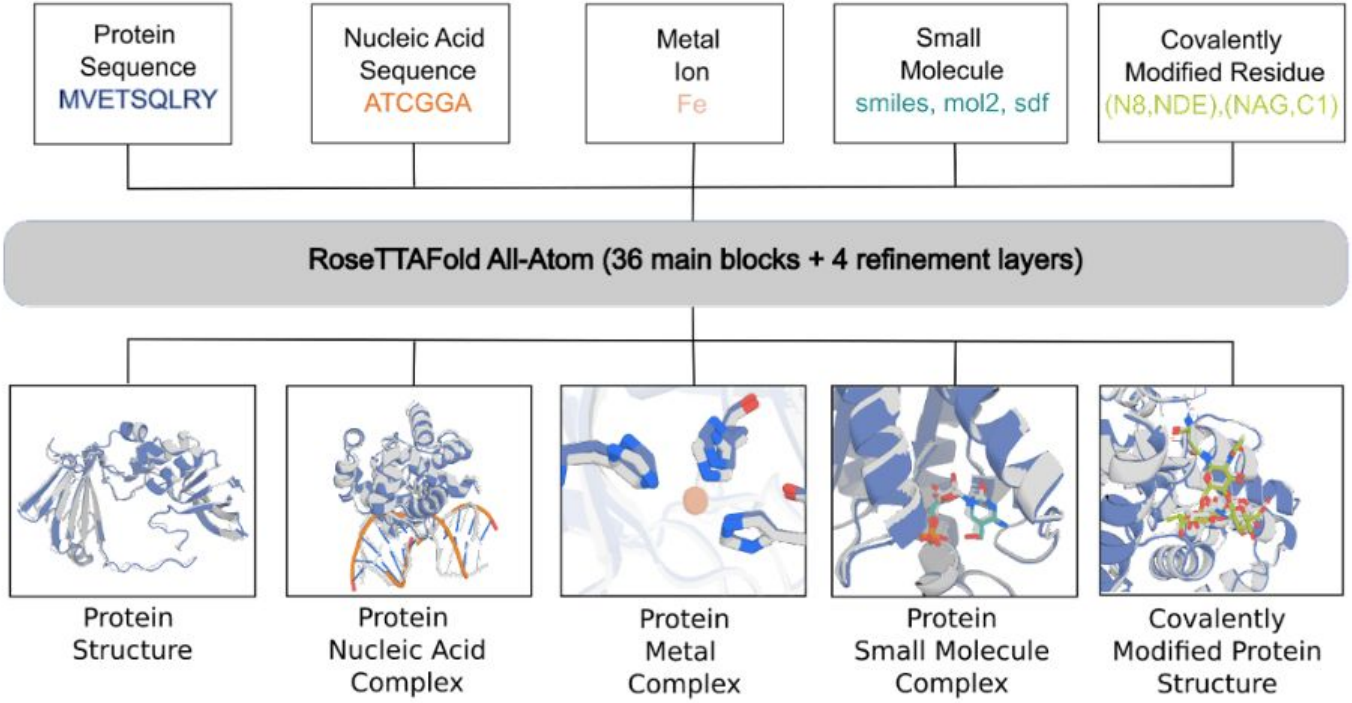


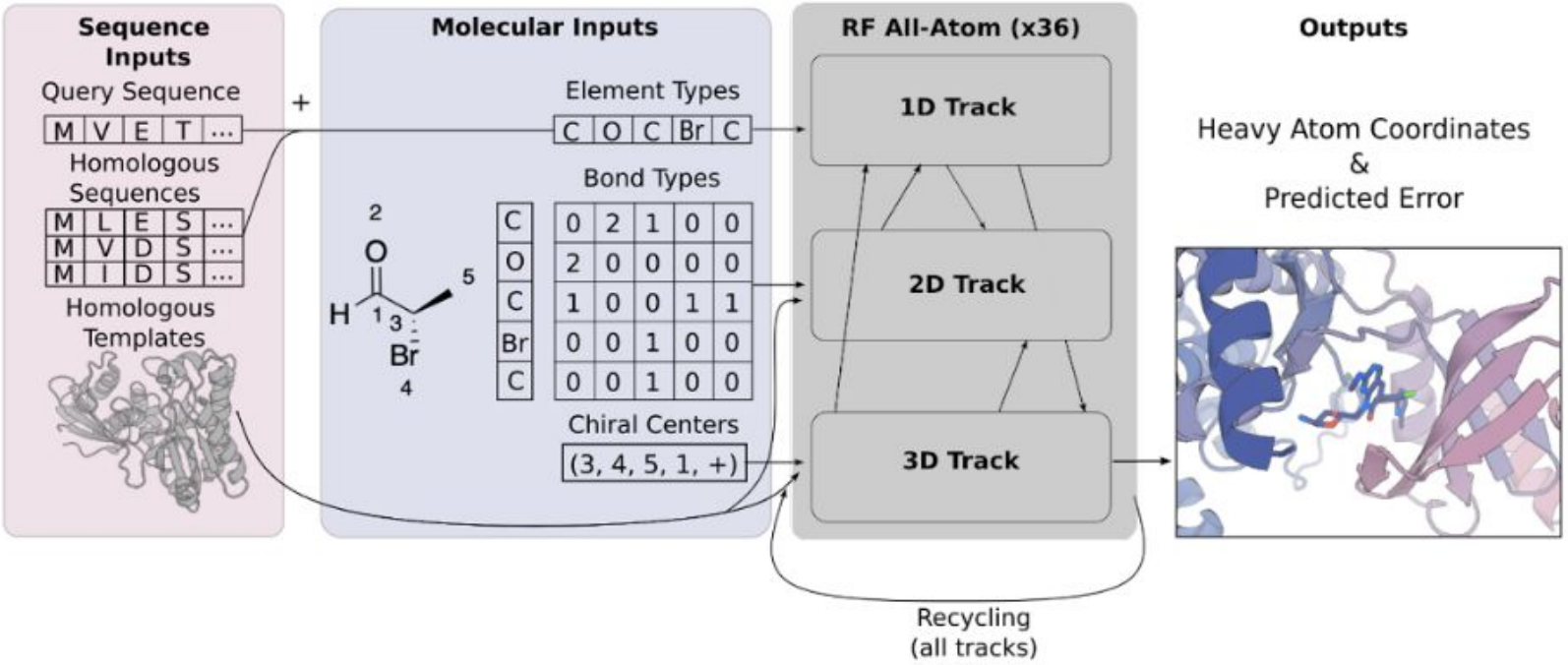
De Novo Protein Design

We are transitioning from physically based models (Rosetta) to deep learning methods (RFdiffusion, ProteinMPNN)

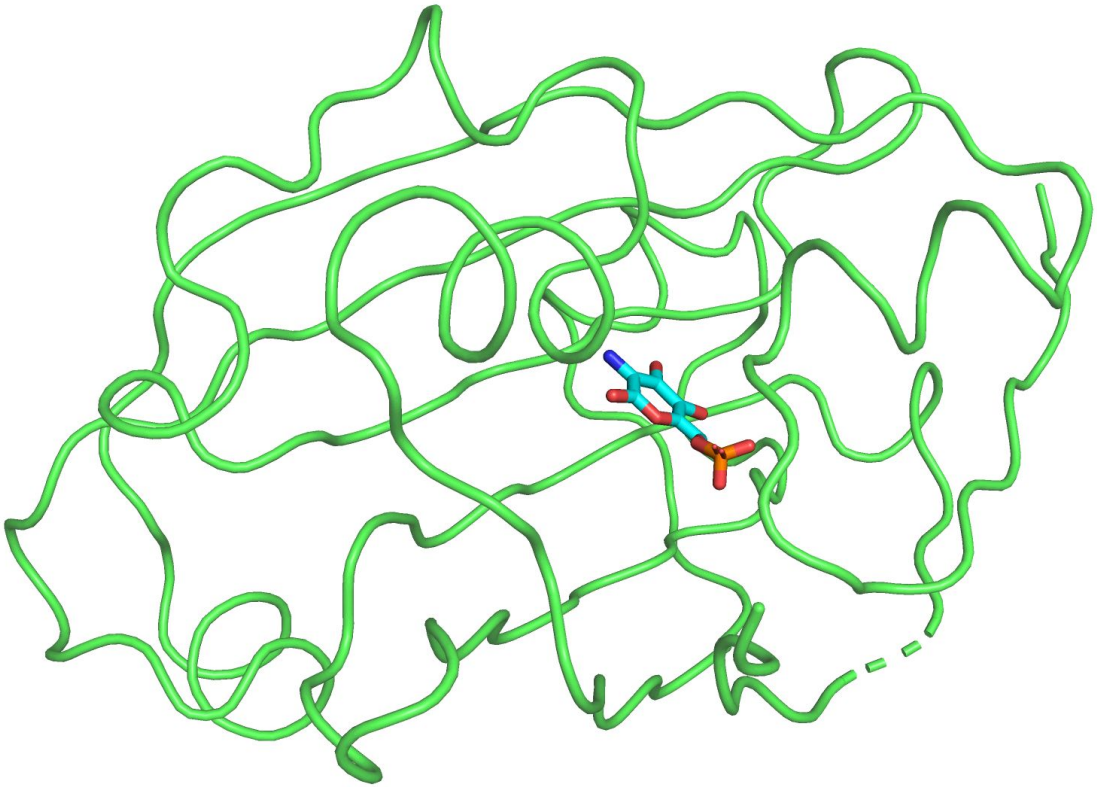
1. Overview of New Methods
2. The Design Frontier

Can we design a neural architecture that models all molecules in the PDB?

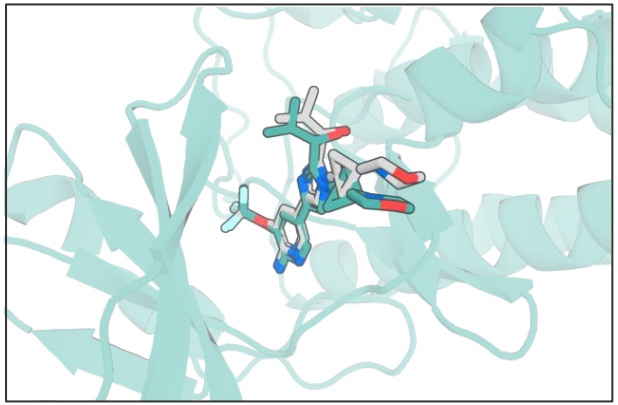


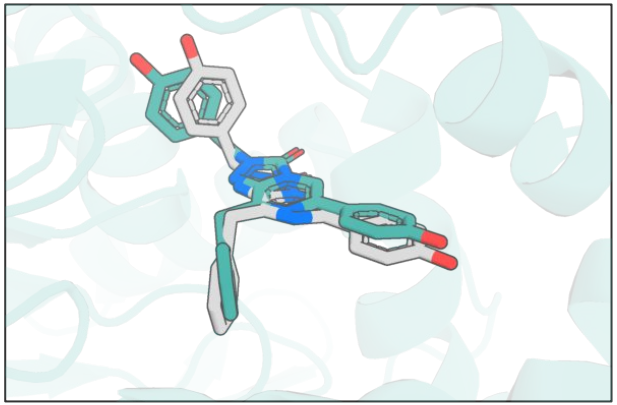

Sequence Inputs
Query Sequence
M V E T ...
Homologous Sequences
M L E S ...
M V D S ...
M I D S ...
Homologous Templates
+
Molecular Inputs
Element Types
C O C Br C
Bond Types
C 0 2 1 0 0
O 2 0 0 0 0
C 1 0 0 1 1
Br 0 0 1 0 0
C 0 0 1 0 0
Chiral Centers
(3, 4, 5, 1, +)
RF All-Atom (x36)
1D Track
2D Track
3D Track
Recycling
(all tracks)
Outputs
Heavy Atom Coordinates
&
Predicted Error

Results



Protein Small Molecule Complex Prediction





Complexes that are dissimilar to training dataset

Our deep-learning toolkit for protein design

1 **RFdiffusion**

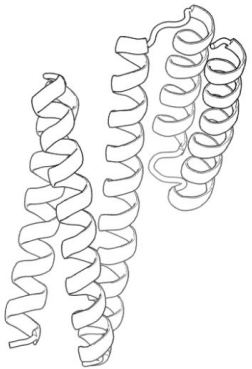
Generates new protein structures

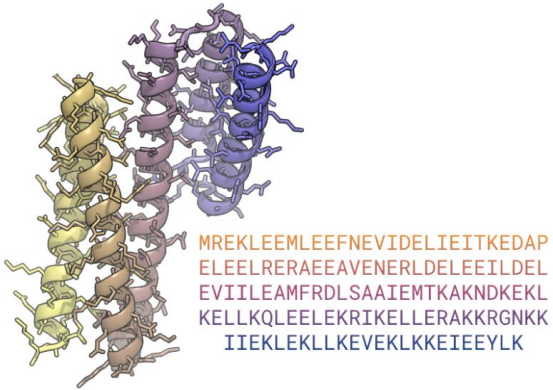
2 **ProteinMPNN**

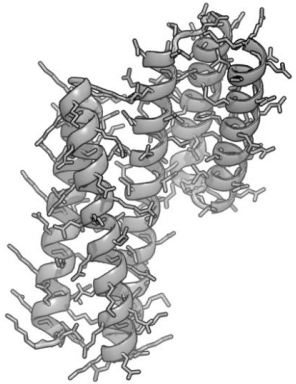
Assigns amino acids to protein structures

3 **RoseTTAFold**

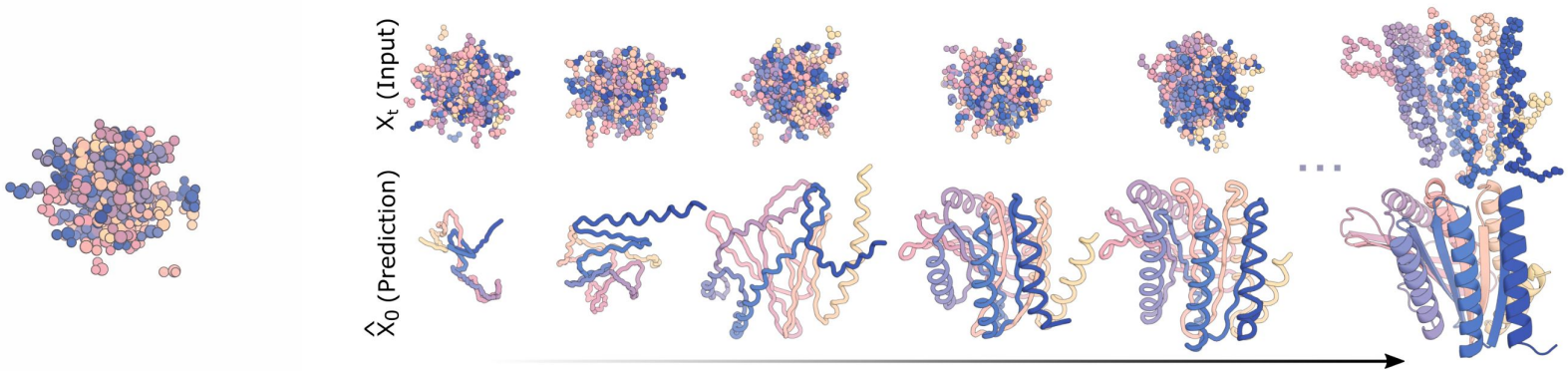
Predicts structures from sequence







De novo protein design using RFdiffusion



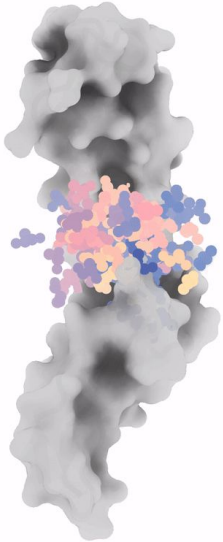
Watson et al, 2023. PMID: 37433327Z

Inspired by deep-learning methods for generating synthetic images.
e.g. DALL-E

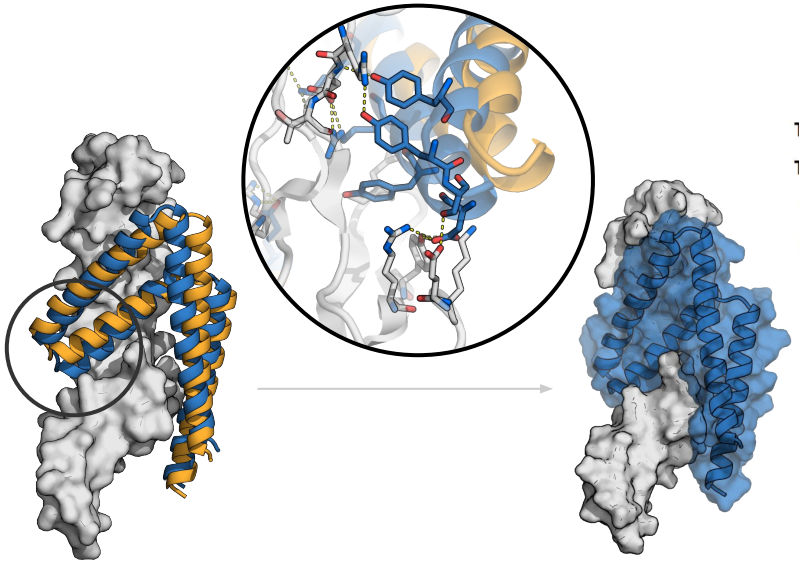
Synthetic image trajectory from NVIDIA

Shape matching binders to TNF superfamily receptors

RFdiffusion generates shape matched binders to TNFR1



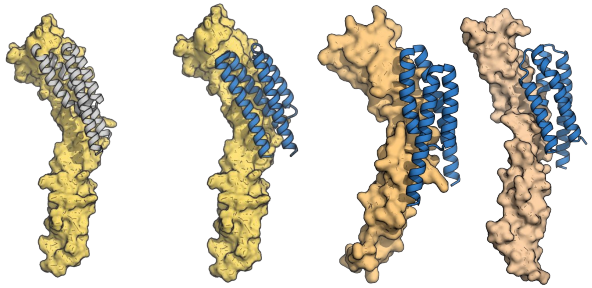
Partial diffusion improves shape complementarity and binding affinity



Initial binder: K_D = 16 nM
Partially diffused binder: K_D = 9 pM

Binders can be retargeted to other family members by partial diffusion

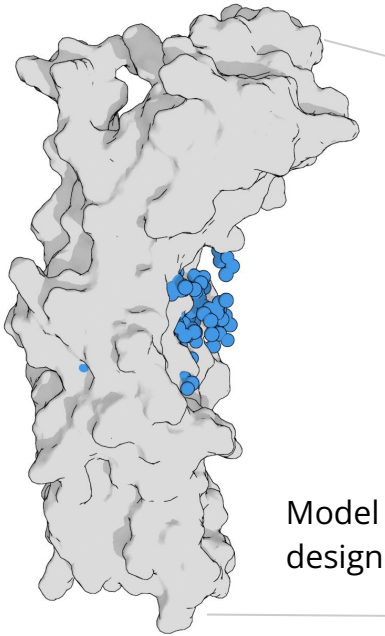
	Original	Retargeted		
	TNFR1 binder	TNFR2 binder	OX40 binder	4-1BB binder
TNFR1	16	n.b.	n.b.	n.b.
TNFR2	n.b.	0.058	n.b.	n.b.
OX40	n.b.	n.b.	24	n.b.
4-1BB	n.b.	n.b.	n.b.	64



TNFR1 TNFR2 OX40 41-BB

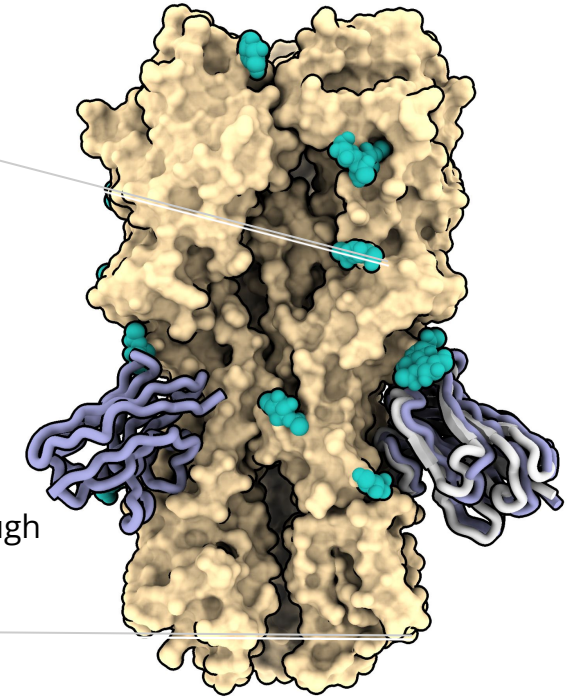
RFdiffusion for antibody design

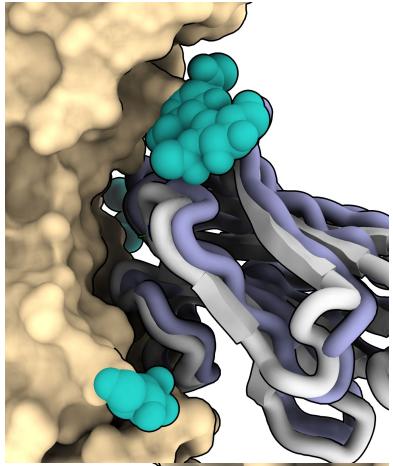
Start from random noise placed around chosen target epitope

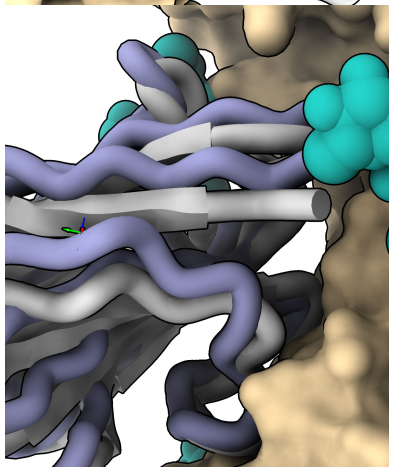


Model binds to hotspots through designed CDR loops

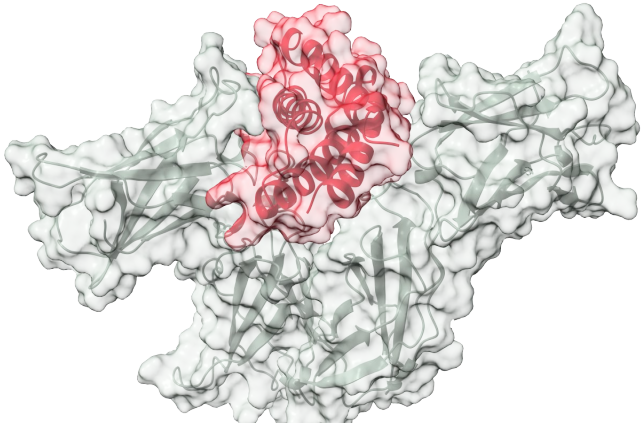
Cryo-EM confirms accuracy of diffused anti-HA antibody





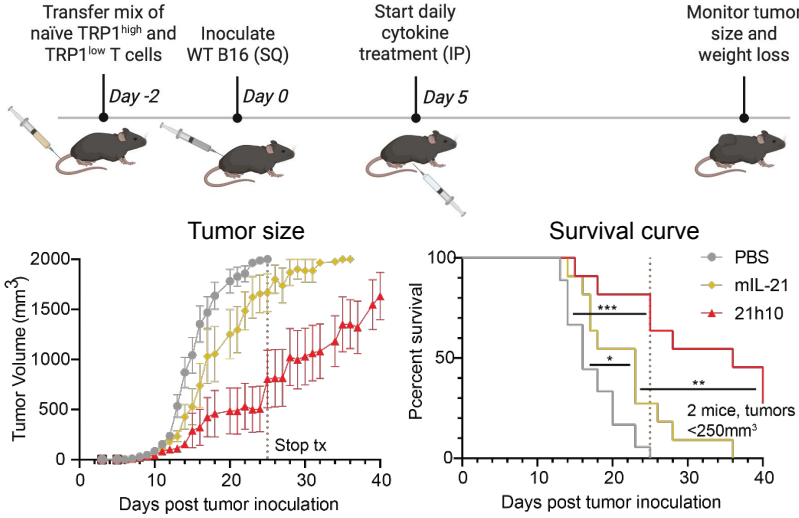


Potent anti-tumor immunomodulators

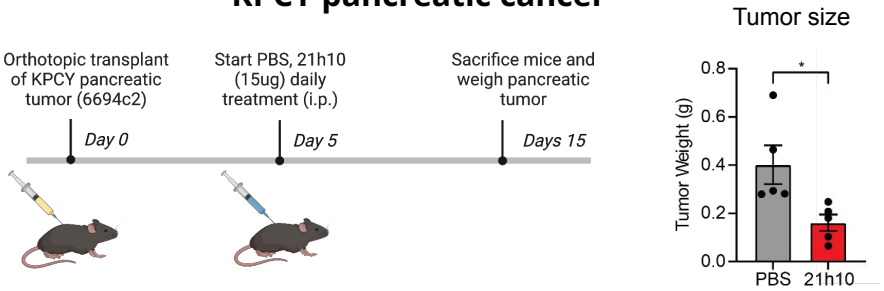


IL-21 mimic (21h10)

B16F10 melanoma



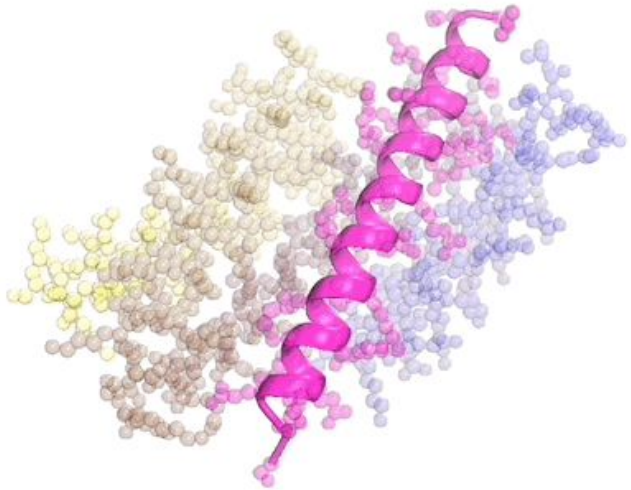
KPCY pancreatic cancer

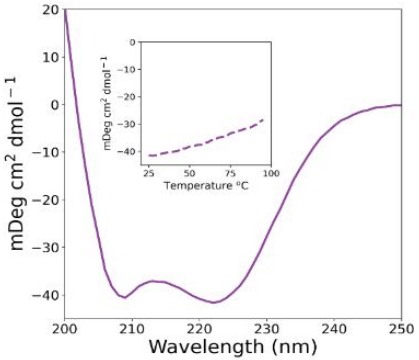


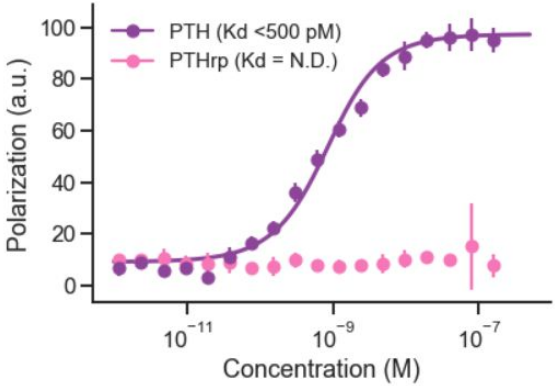
with Dougan Lab (Dana Farber)

Design of peptide/disordered protein binding

Design of peptide-binding proteins via RFdiffusion

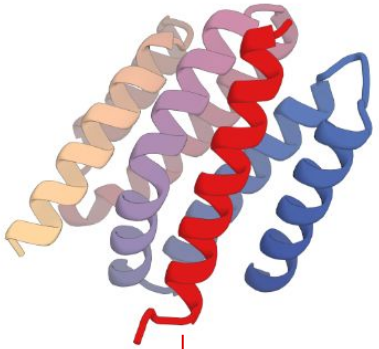




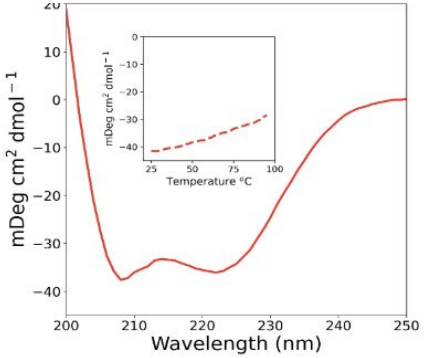


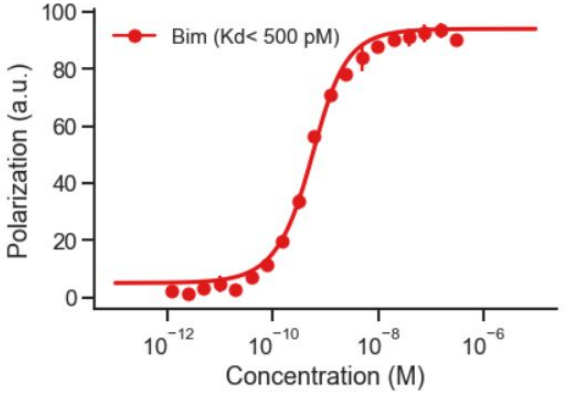
Preetham Venkatesh, Susana Vazquez Torres, Phil Leung, Joe Watson

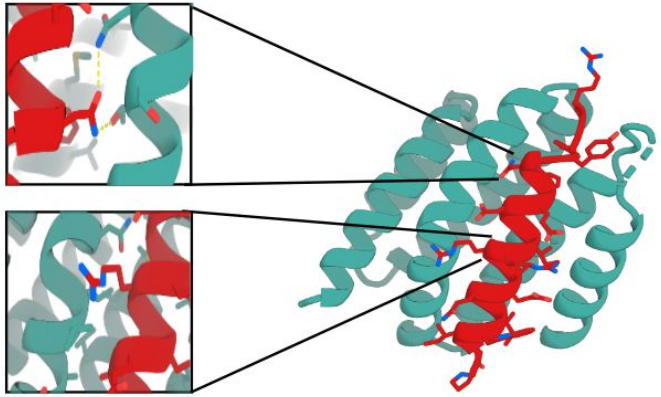
Diffused peptide binders have picomolar affinites



Bim peptide



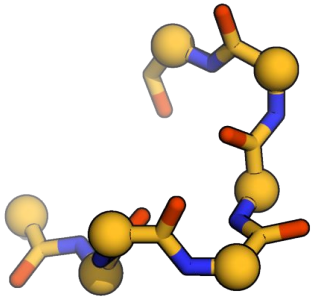




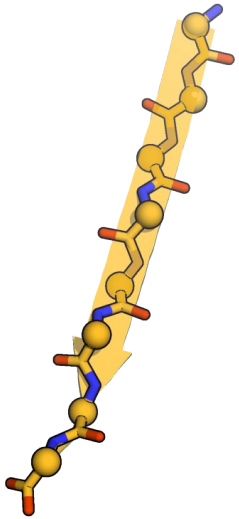
Crystal structure

Preetham Venkatesh, Susana Vazquez Torres, Phil Leung, Joe Watson

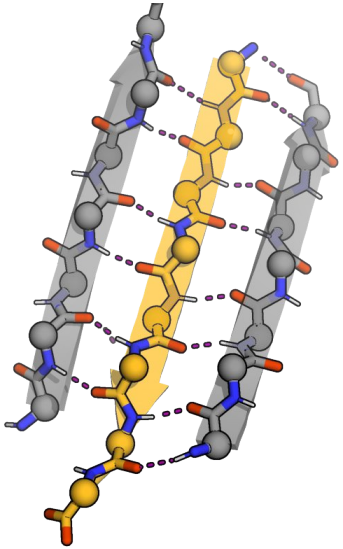
Design strategy for binding amyloid forming peptides



Peptide may have many conformations, making them hard to bind.

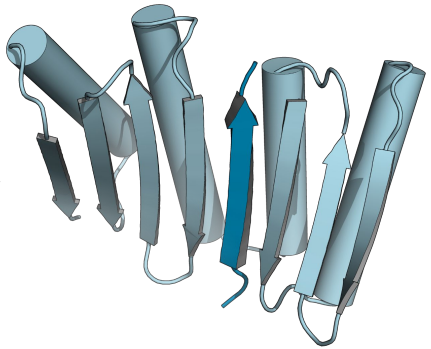


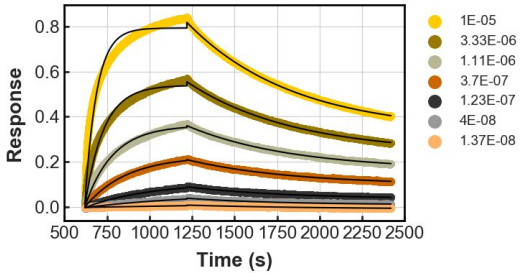
We use knowledge of protein structure to guide binding peptide.



We include design features that bind peptides in beta conformation.

C104

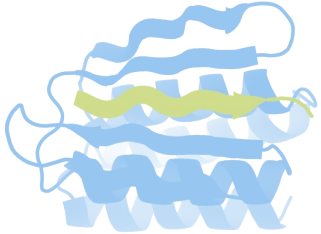


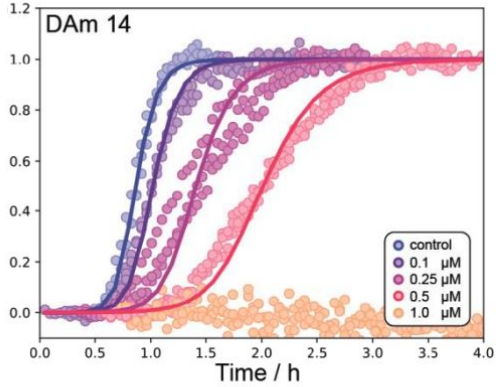


Danny Sahtoe, Hannah Han

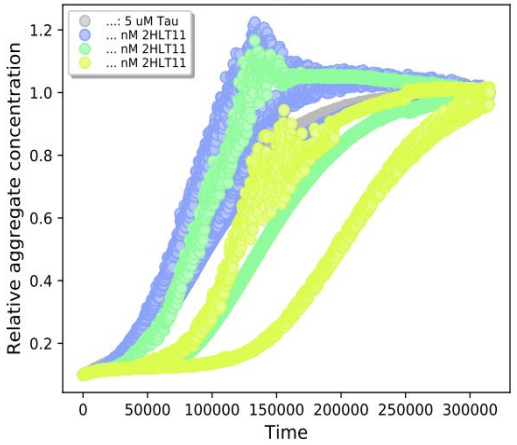
Blocking amyloid formation

Target: **Amyloid Beta** | KLVFFAEDV
Design: DAm_014



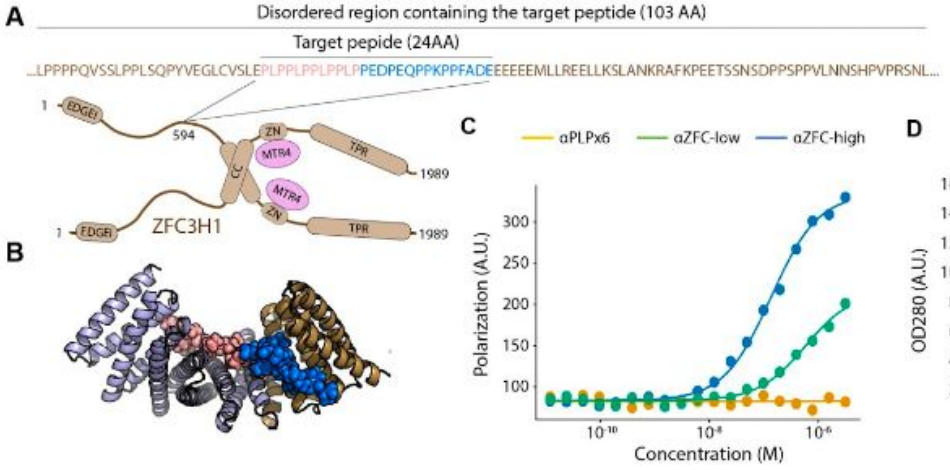


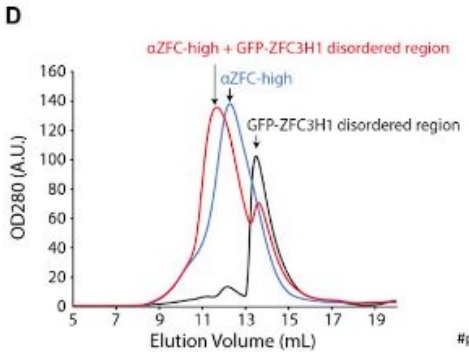
Target: **Tau** | GGSVQIVYKP
Design: 2HLTAU_011

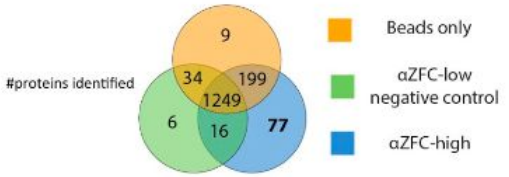


Designed binders for native disordered proteins function in cells

General approach for targeting disordered proteins







Protein	Description	Ctrl	αZFC-low	αZFC-high
ZFC3H1	PAXT complex	0	0	**27 (19%)**
MTR4	PAXT complex	8 (11%)	5 (5.8%)	**35 (37%)**
BUB3	Mitotic checkpoint	3 (13%)	3 (13%)	**23 (84%)**
ZN207	Mitotic checkpoint	2 (2.7%)	3 (5.4%)	**13 (14%)**
RBM12	RNA processing	4 (3.8%)	6 (6.3%)	**43 (43%)**
RBM26	RNA processing	3 (3.6%)	2 (3.7%)	**47 (42%)**

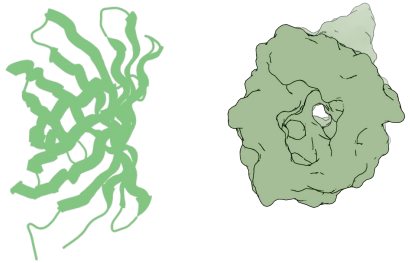
number indicates exclusive unique peptide count

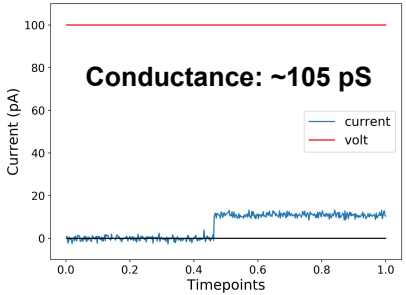
Design: Kejia Wu
Cell assays/Mass spec the Emmanuel Derivery lab (UK)

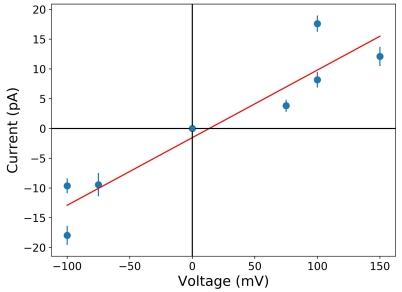
Design of transmembrane nanopore sensors

Design of transmembrane beta barrel nanopores

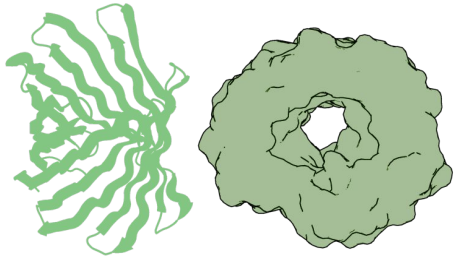
10 Strands

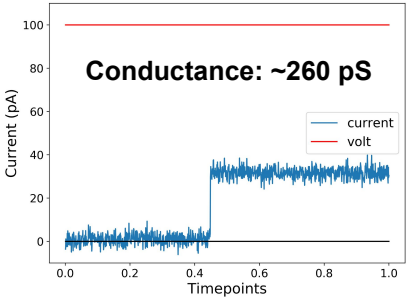


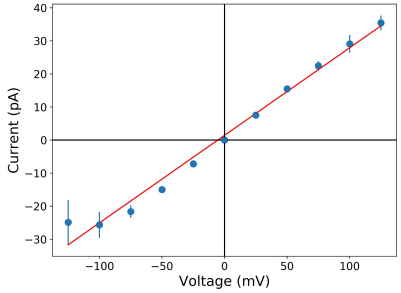




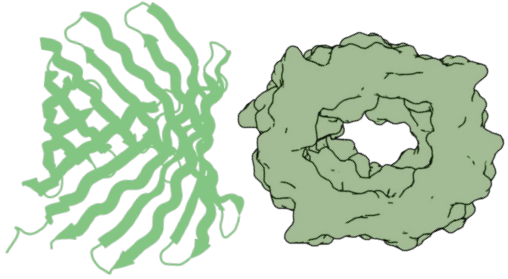
12 Strands

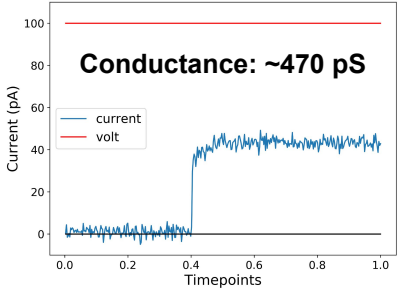


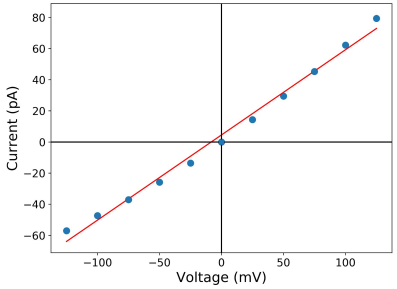




14 Strands





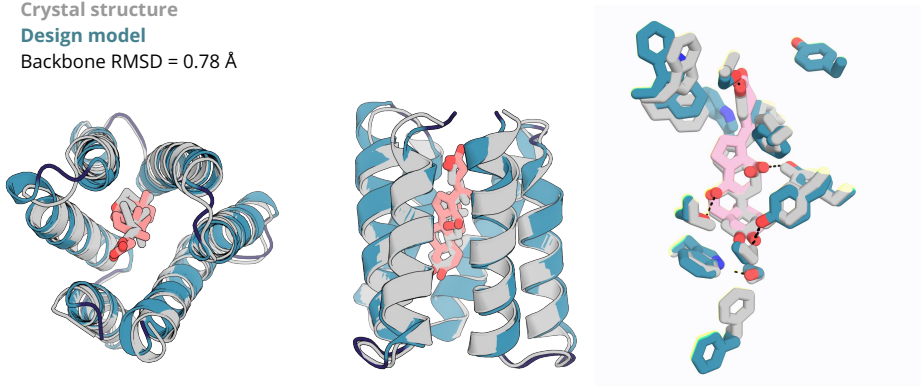


Samuel Lemma, Sagardip Majumder,
Carolin Berner, Anastassia Vorobieva

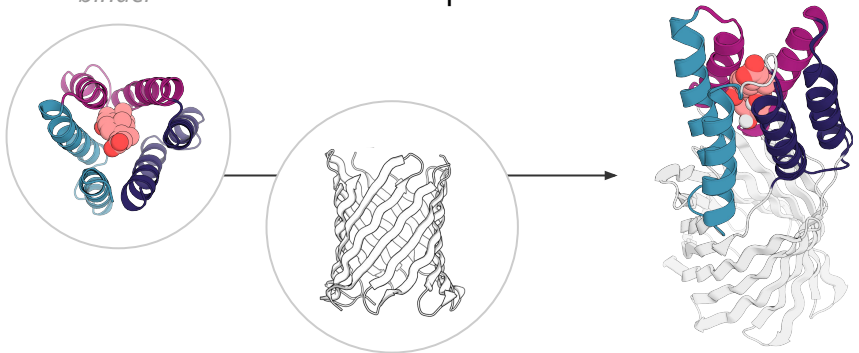
Ligand gated nanopores

Designed cholic acid binder

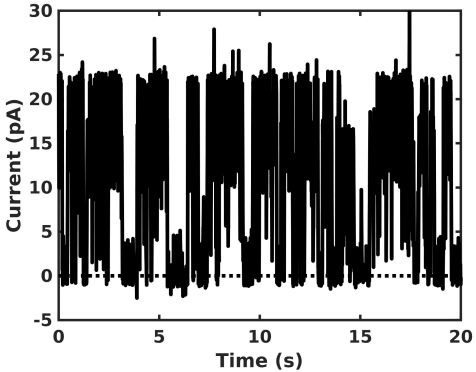
Crystal structure
Design model
Backbone RMSD = 0.78 Å



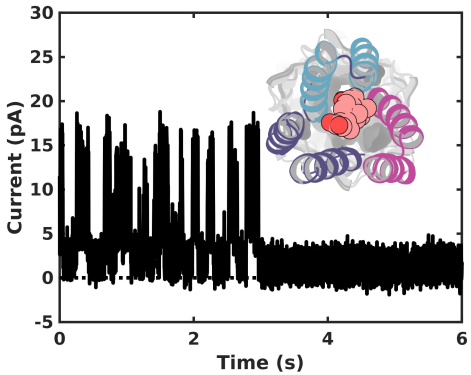
Fuse to pore



Gated nanopore without cholic acid



Gated nanopore with cholic acid



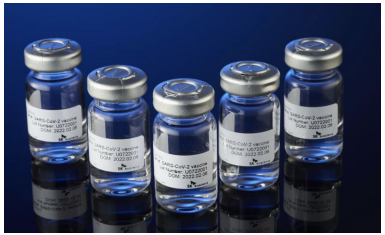
Design of protein nanomaterials

First approved de novo designed medicine!

- **June 29: South Korea approved SKYCovione for use in adults!**
- Completed a multinational Phase 3 trial with 4,037 adults
- SKYCovione™ generated **~3x more neutralizing antibodies**
- Antibody conversion rate: **98%** for SKYCovione (v.s. 87%)
    - Among subject ≥65 years of age: **95%** for SKYCovione (v.s. 79%)
- Comparable levels of T-cell activation
- No serious adverse reactions
- Heterologous booster trials now underway

SKYCovione™ employs IPD's self-assembling protein nanoparticle technology and GSK's pandemic adjuvant

SK bioscience

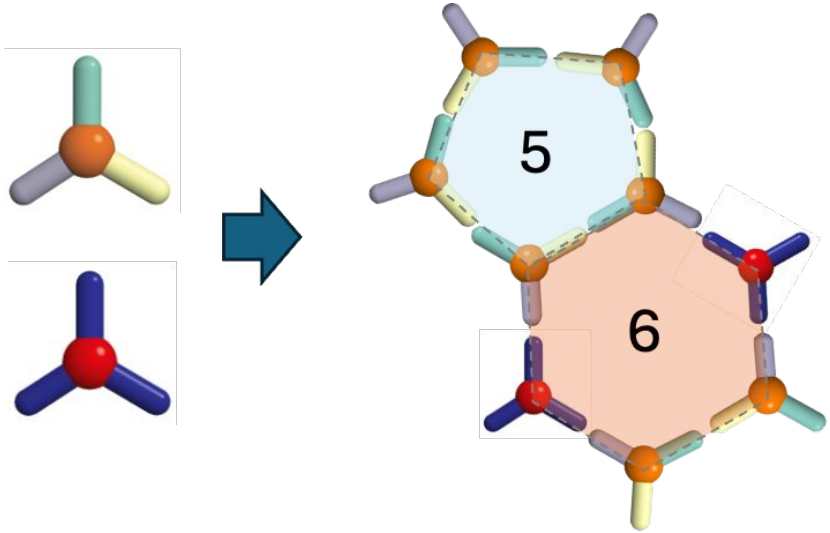


How to break symmetry

1. Pseudo-symmetry
(Programmable assembly)

Multi components

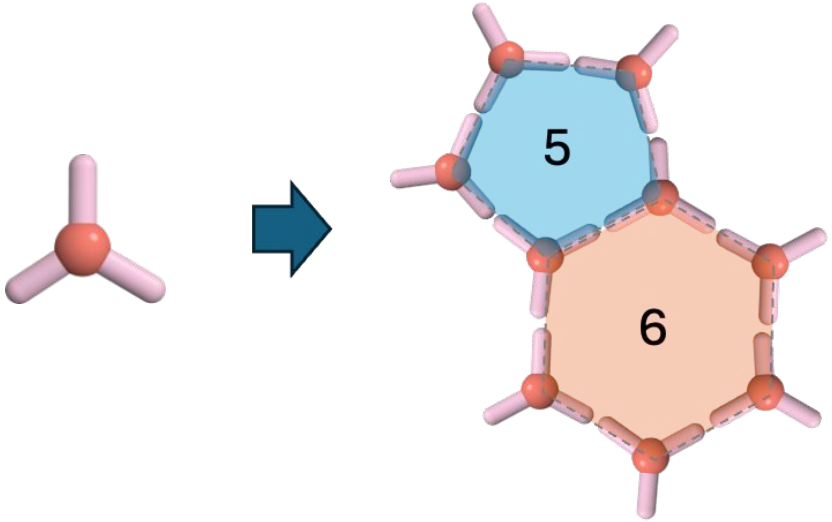
Interface programming



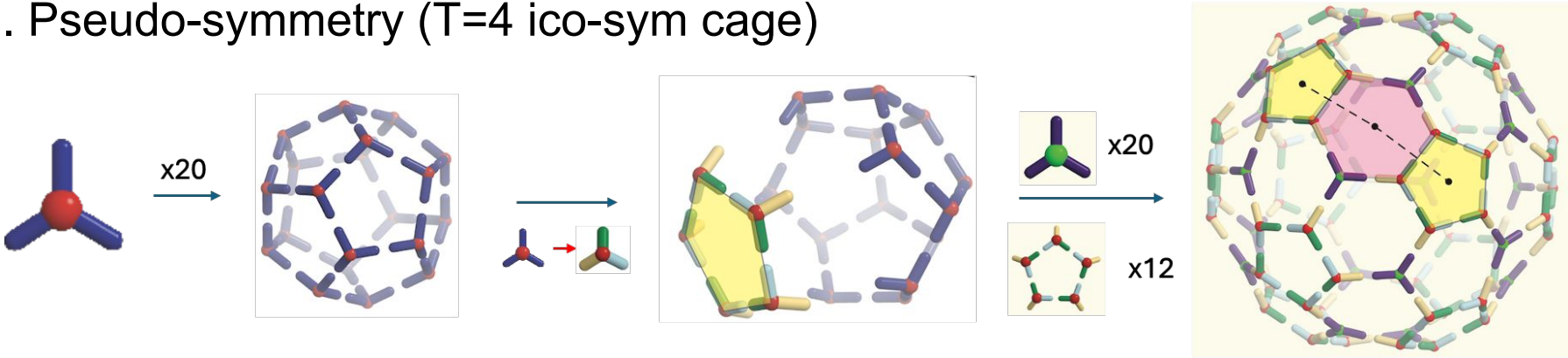
2. Quasi-symmetry
(Multi-state assembly)

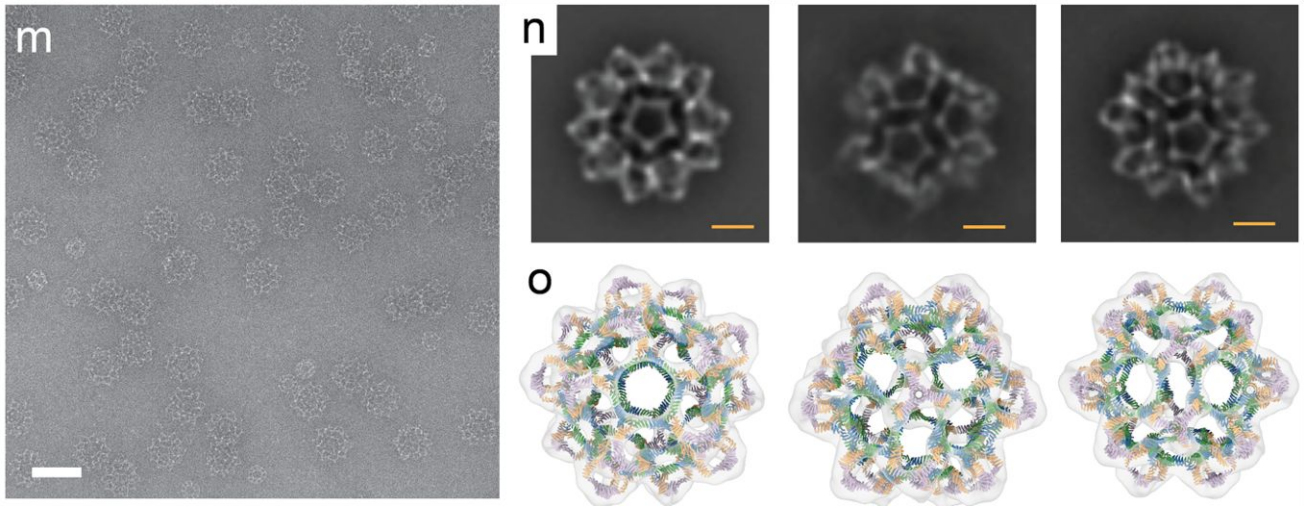
Single component

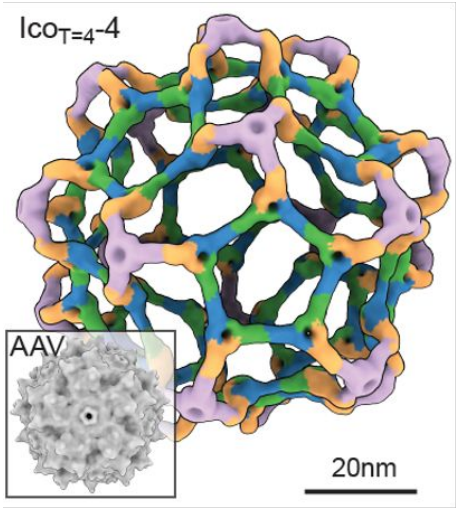
Multiple types of local motifs



1. Pseudo-symmetry (T=4 ico-sym cage)





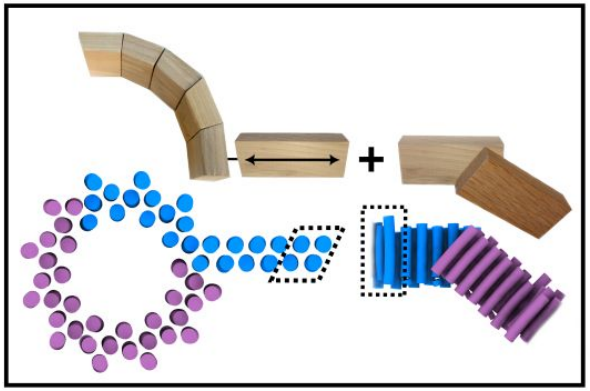


S. Lee* & R. Kibler et al., *bioRxiv* 2023 (**currently Assistant Prof. @ POSTECH, South Korea)*

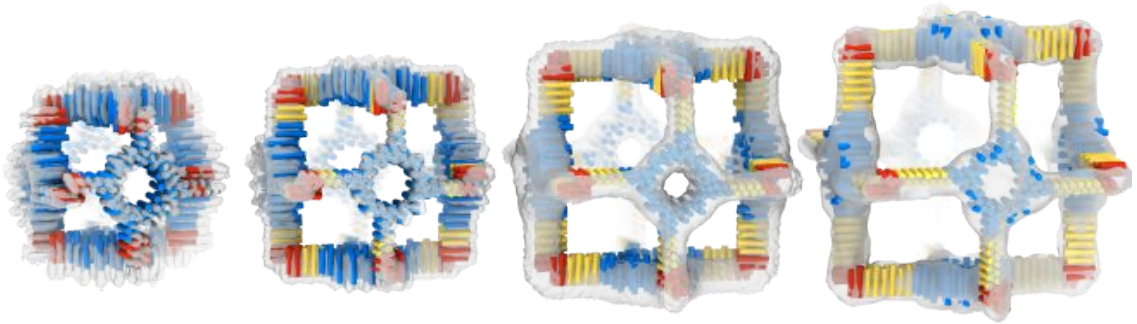
Resolution: ~10A

Expandable nanomaterials

Building blocks:



EM structures:



Tim Huddy, Yang Hsia, Ryan Kibler

Create proteins that solve modern challenges in medicine, technology & sustainability.

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Cancer Immunotherapy	Protein-Silicon Devices	Carbon Sequestration
Drug Delivery Systems	Bio-Based Computers	Artificial Photosynthesis

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NSERC!!!