RFdiffusion

Ana C. Chang-Gonzalez
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Article

De novo design of protein structure and function with RFdiffusion

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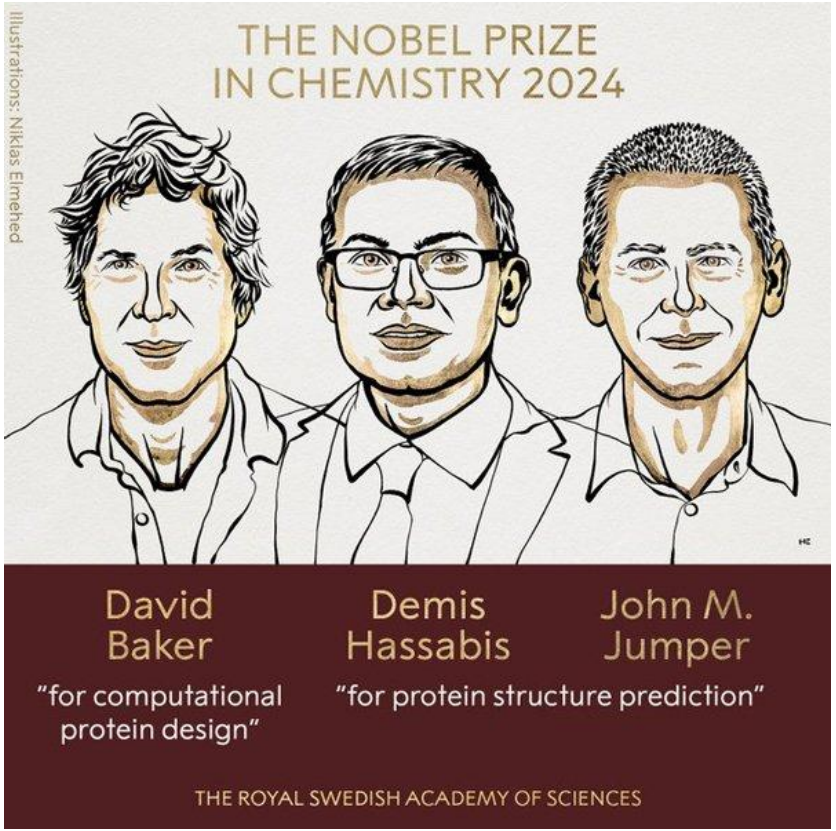
Joseph L. Watson[1,2,15], David Juergens[1,2,3,15], Nathaniel R. Bennett[1,2,3,15], Brian L. Trippe[2,4,5,15], Jason Yim[2,6,15], Helen E. Eisenach[1,2,15], Woody Ahern[1,2,7,15], Andrew J. Borst[1,2], Robert J. Ragotte[1,2], Lukas F. Milles[1,2], Basile I. M. Wicky[1,2], Nikita Hanikel[1,2], Samuel J. Pellock[1,2], Alexis Courbet[1,2,8], William Sheffler[1,2], Jue Wang[1,2], Preetham Venkatesh[1,2,9], Isaac Sappington[1,2,9], Susana Vázquez Torres[1,2,9], Anna Lauko[1,2,9], Valentin De Bortoli[8], Emile Mathieu[10], Sergey Ovchinnikov[11,12], Regina Barzilay[6], Tommi S. Jaakkola[6], Frank DiMaio[1,2], Minkyung Baek[13] & David Baker[1,2,14] ✉

There has been considerable recent progress in designing new proteins using deep-learning methods[1–9]. Despite this progress, a general deep-learning framework for protein design that enables solution of a wide range of design challenges, including de novo binder design and design of higher-order symmetric architectures, has yet to be described. Diffusion models[10,11] have had considerable success in image and language generative modelling but limited success when applied to protein modelling, probably due to the complexity of protein backbone geometry and sequence–structure relationships. Here we show that by fine-tuning the RoseTTAFold structure prediction network on protein structure denoising tasks, we obtain a generative model of protein backbones that achieves outstanding performance on unconditional and topology-constrained protein monomer design, protein binder design, symmetric oligomer design, enzyme active site scaffolding and symmetric motif scaffolding for therapeutic

Why should we care about de novo protein design?

- Enable new functions
- Tune biochemical parameters
- Control protein function
- Create composite molecular machines, assemblies



Backbone generation is a bottleneck in the protein design workflow

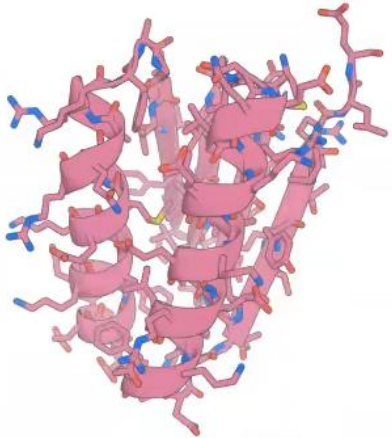
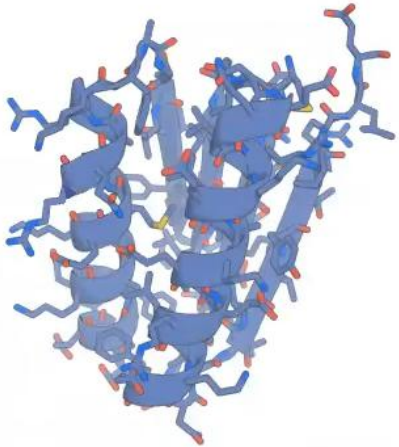
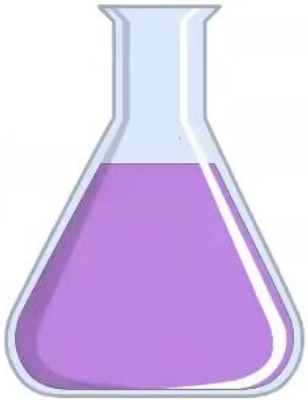
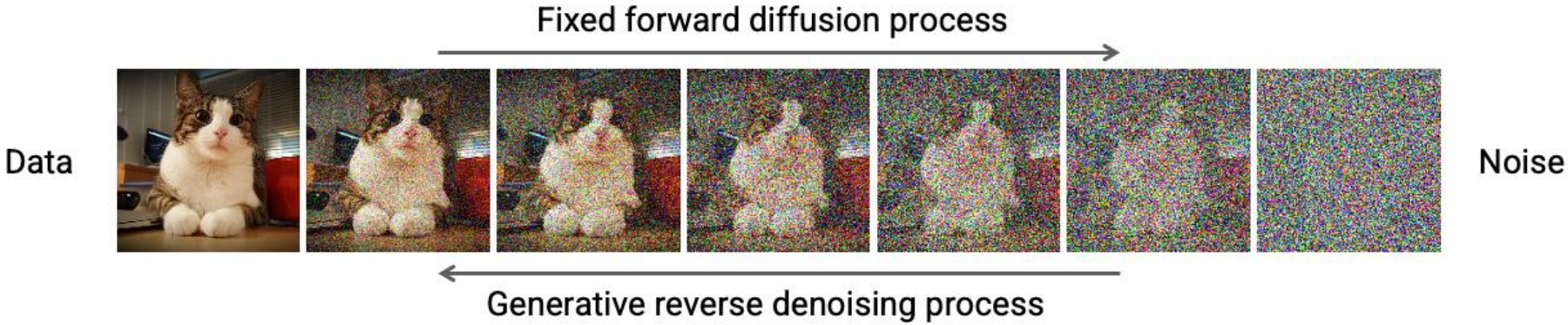
Backbone Generation	Sequence Design	Computational Filtering	Experimental Characterisation
			
RFdiffusion	ProteinMPNN	AlphaFold2 RoseTTAFold Chai-1 ESMFold OmegaFold Boltz-2	~ 1 week

Figure from video "RFdiffusion" by SBGrid Consortium.

Outline

1. What is diffusion?
2. Introduction to RFdiffusion
3. How to use RFdiffusion

Diffusion models introduce random noise iteratively and learn to reverse the process during training

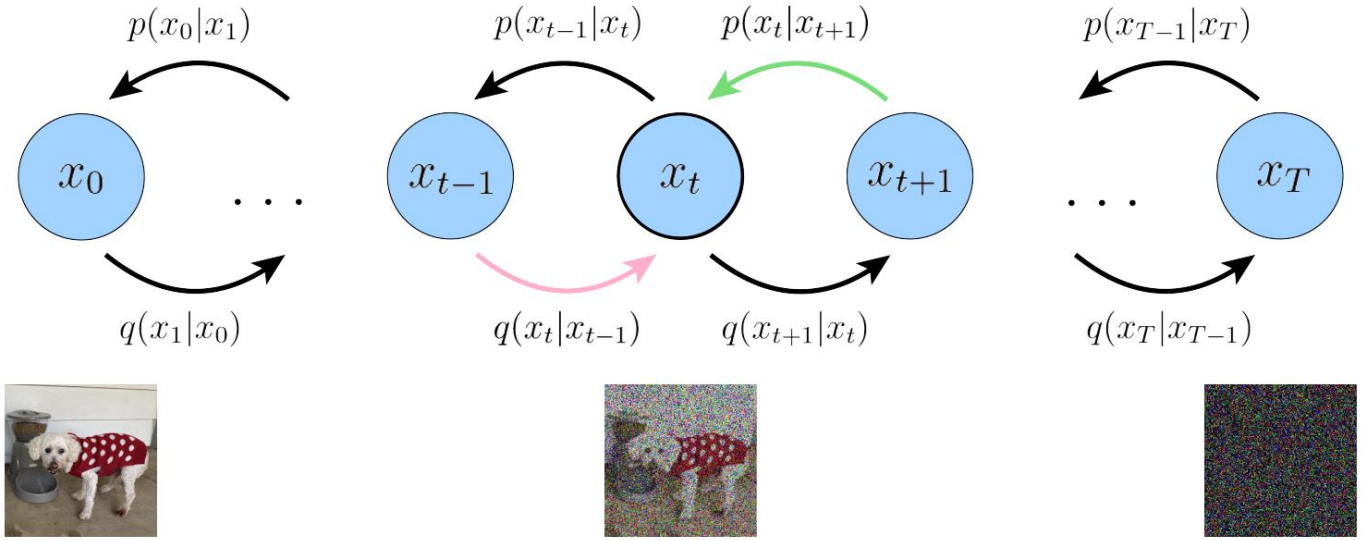


https://cvpr2022-tutorial-diffusion-models.github.io/

"Generate an image of a banana eating a hamburger while tap dancing."

DALL·E

Denoising diffusion probabilistic models



C. Luo. arXiv. 2022

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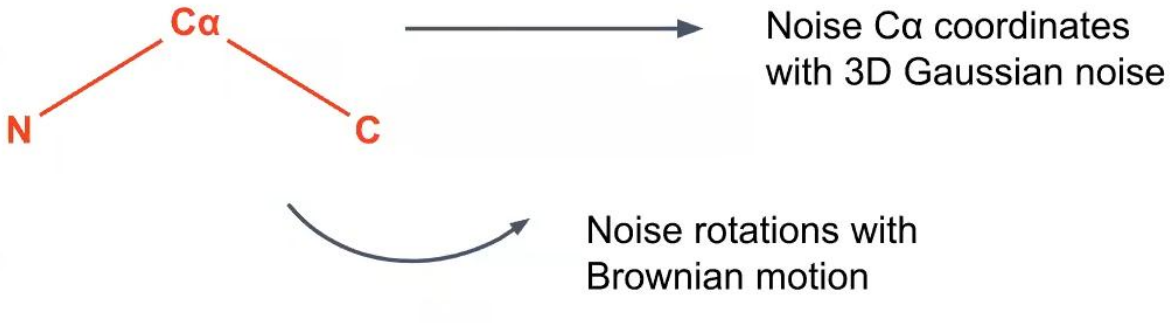
Diffusion occurs across translations and rotations



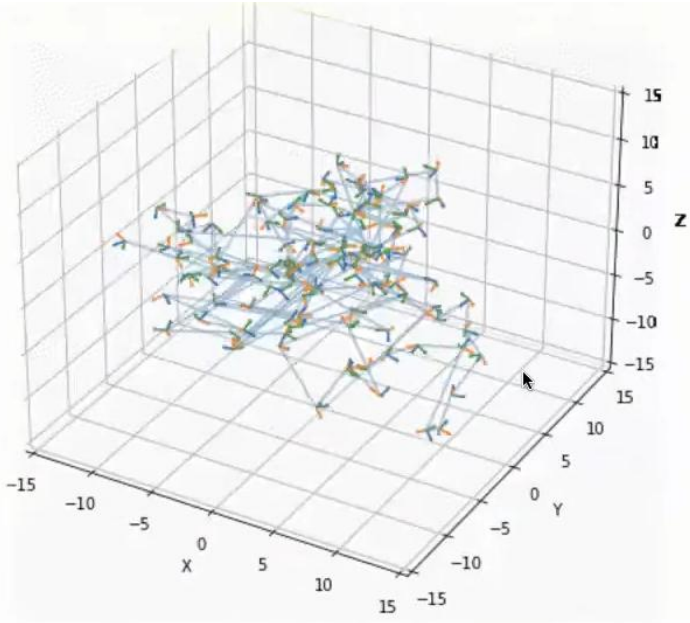
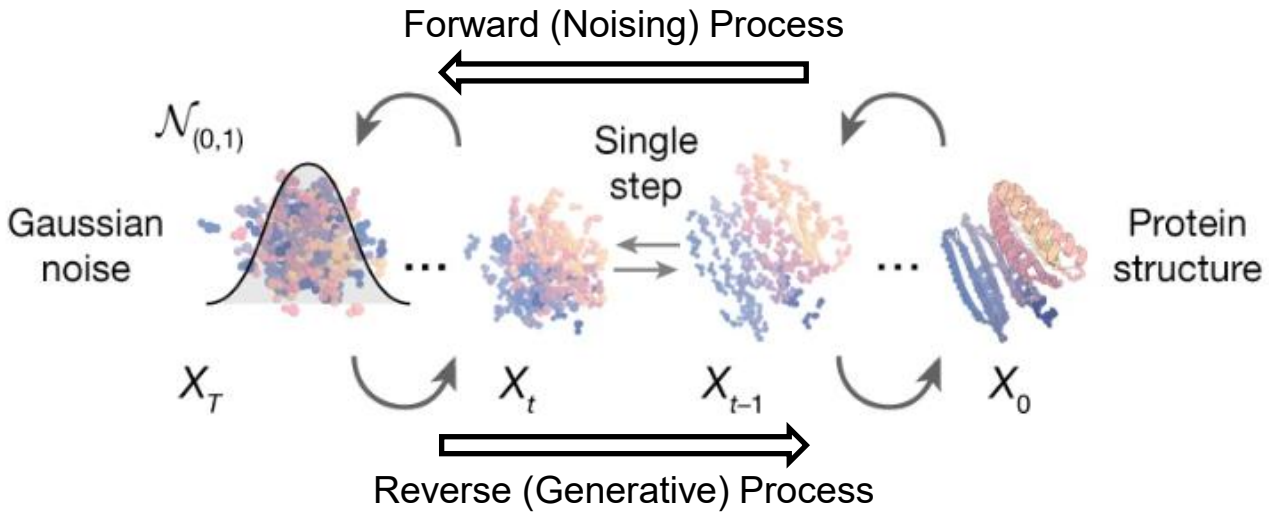
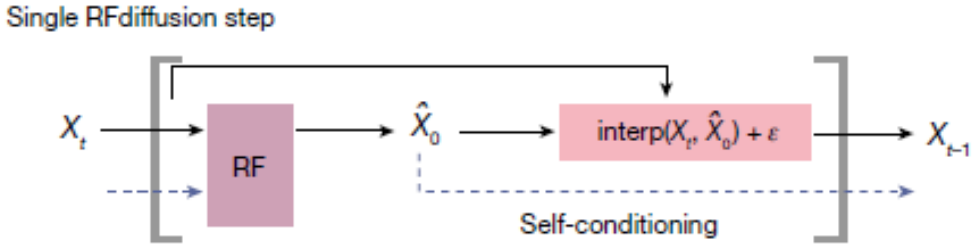


Figure from "RFDiffusion: Accurate protein design using structure prediction and diffusion generative models" video.

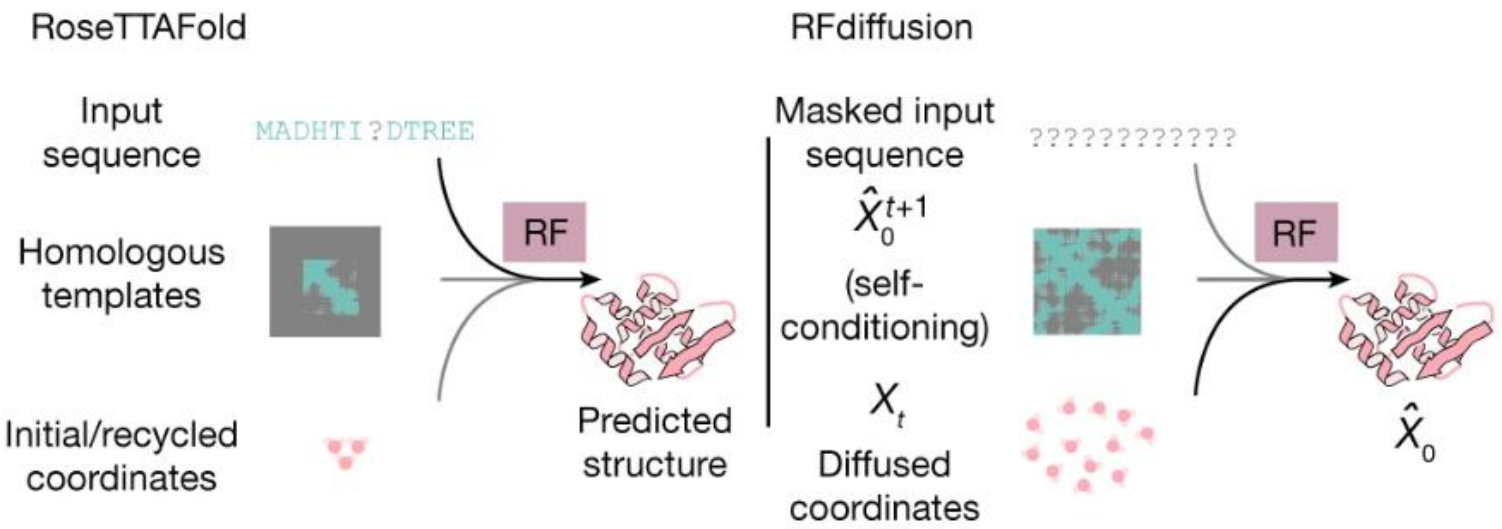
Utilizing diffusion models for use in protein design





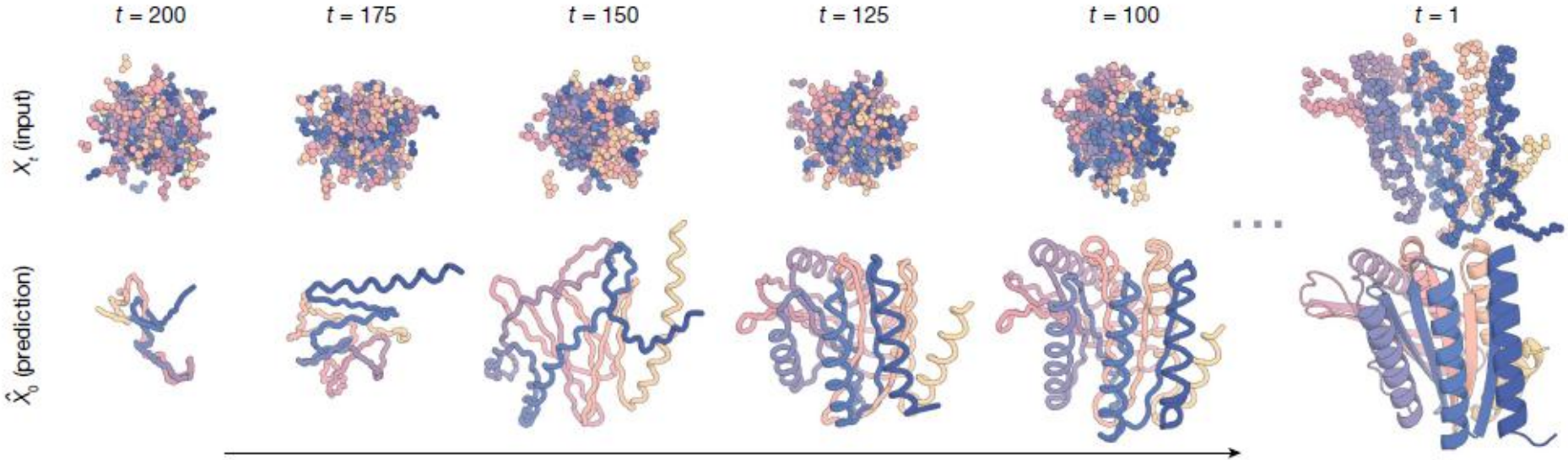
Watson et al. Nature. 2023.

RoseTTAFold is used as the denoising framework



Watson et al. Nature. 2023.

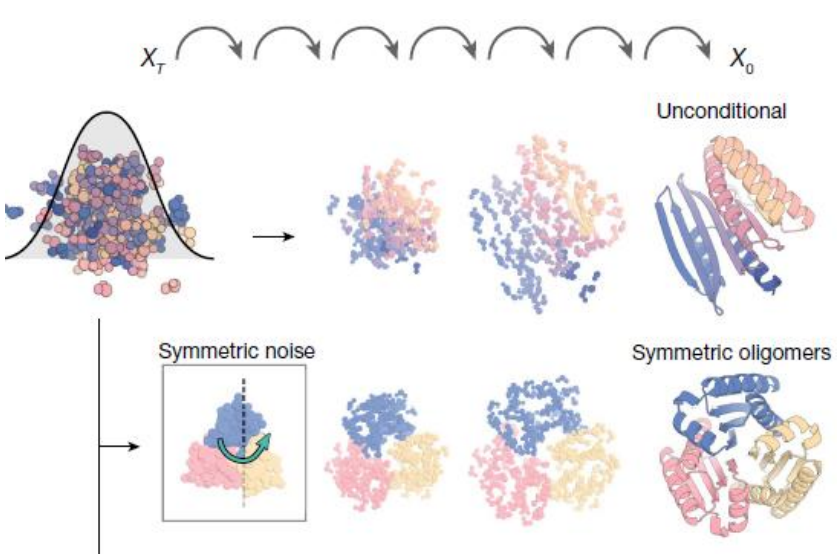
Unconditional design trajectory for a 300-residue chain

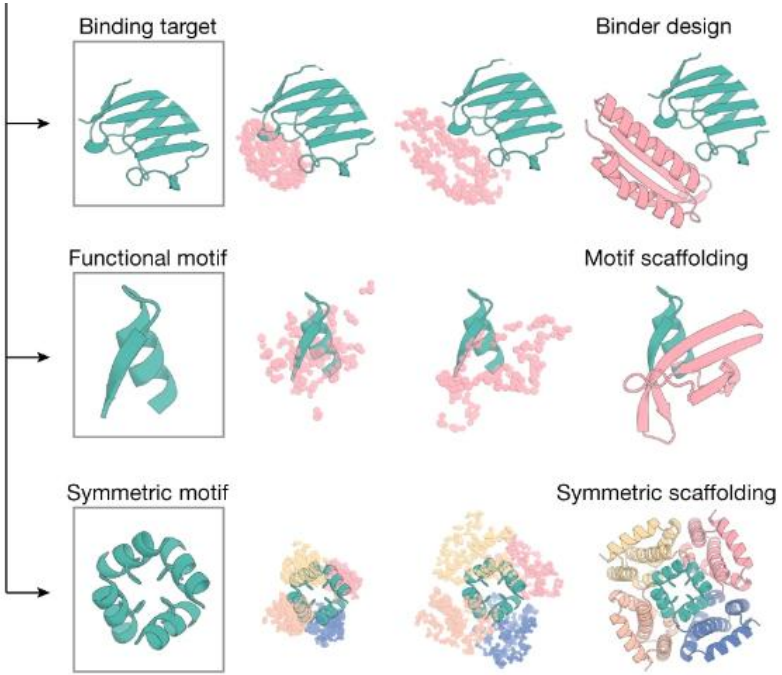


Watson et al. Nature. 2023.

Broad applications of RFdiffusion

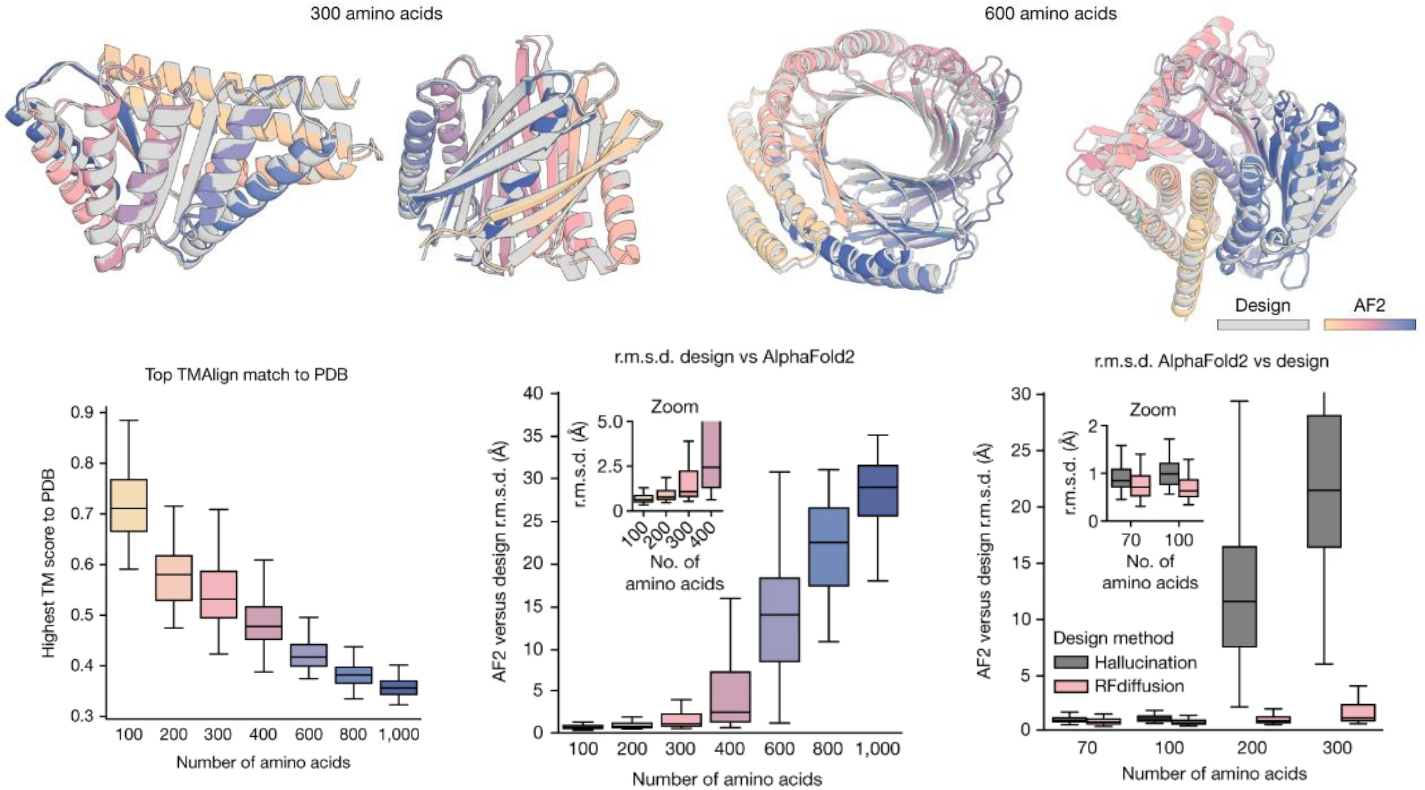
“Generate a protein that has 300 amino acids.”
“Generate a protein that can bind to this target.”





Watson et al. Nature. 2023.

RFdiffusion produces diverse and new-to-nature proteins

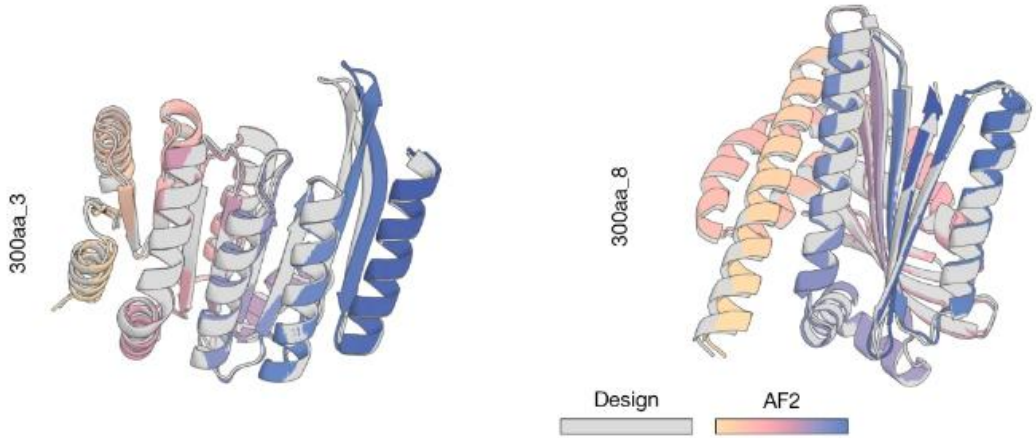


Watson et al. Nature. 2023.

Unconditional designs expressed as soluble monomers

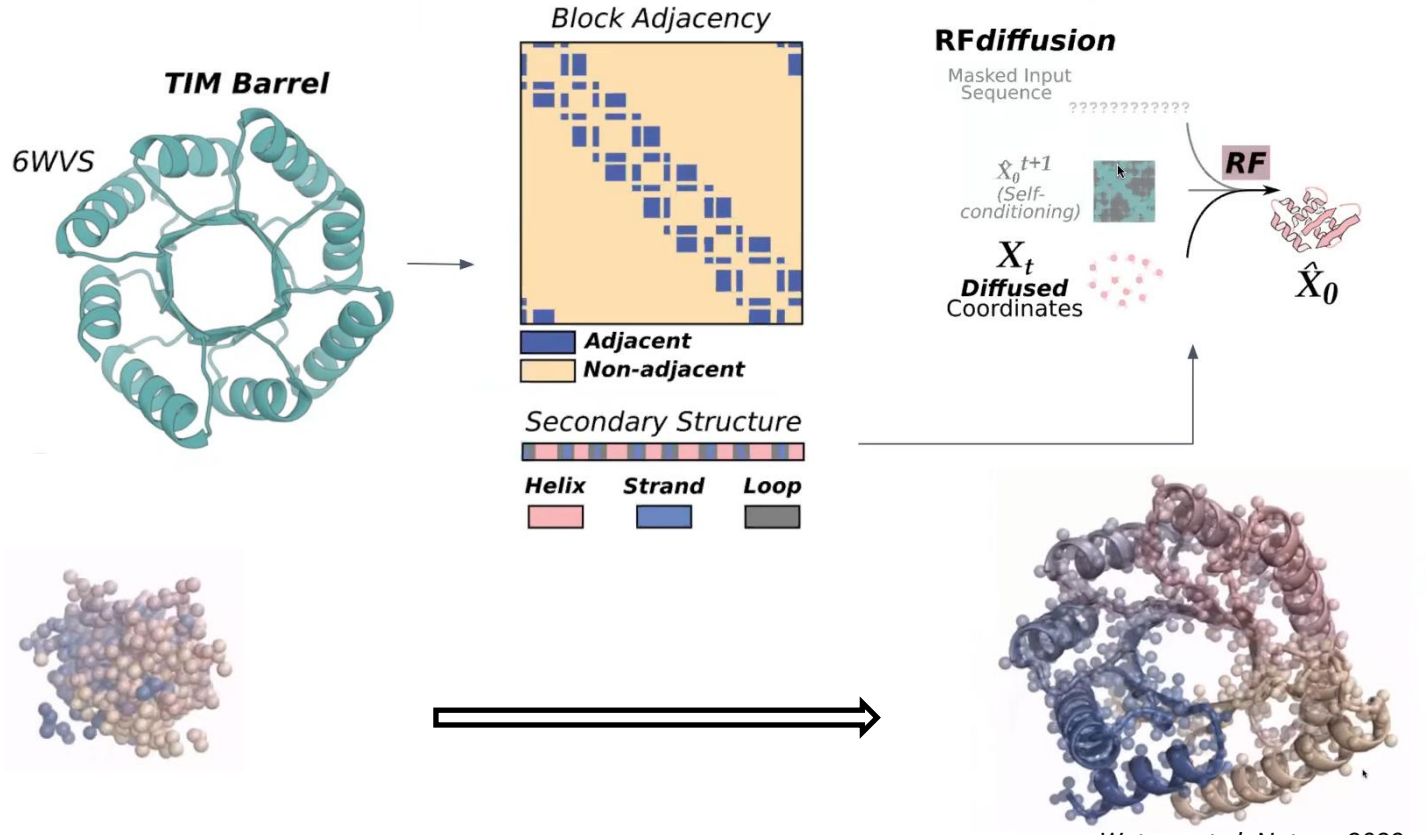
Two sample 300 aa designs that expressed as soluble monomoers

- Use ProteinMPNN to deign sequences for backbone designs
- AF2 recapitulates pose
- Designs are thermostable
- Circular dichroism spectra consistent with mixed α-β topologies



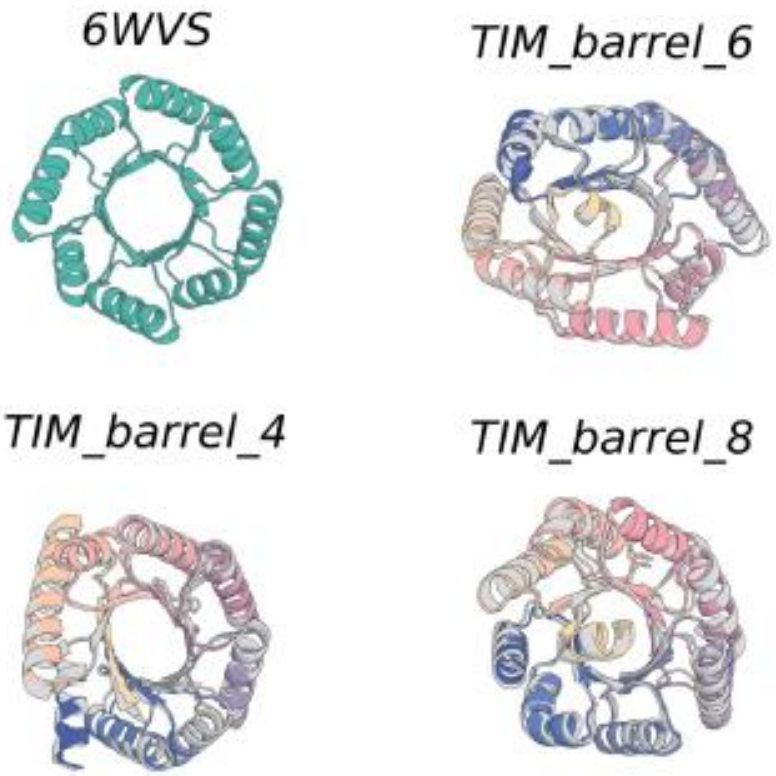
Watson et al. Nature. 2023.

Condition designs to get a specific type of fold



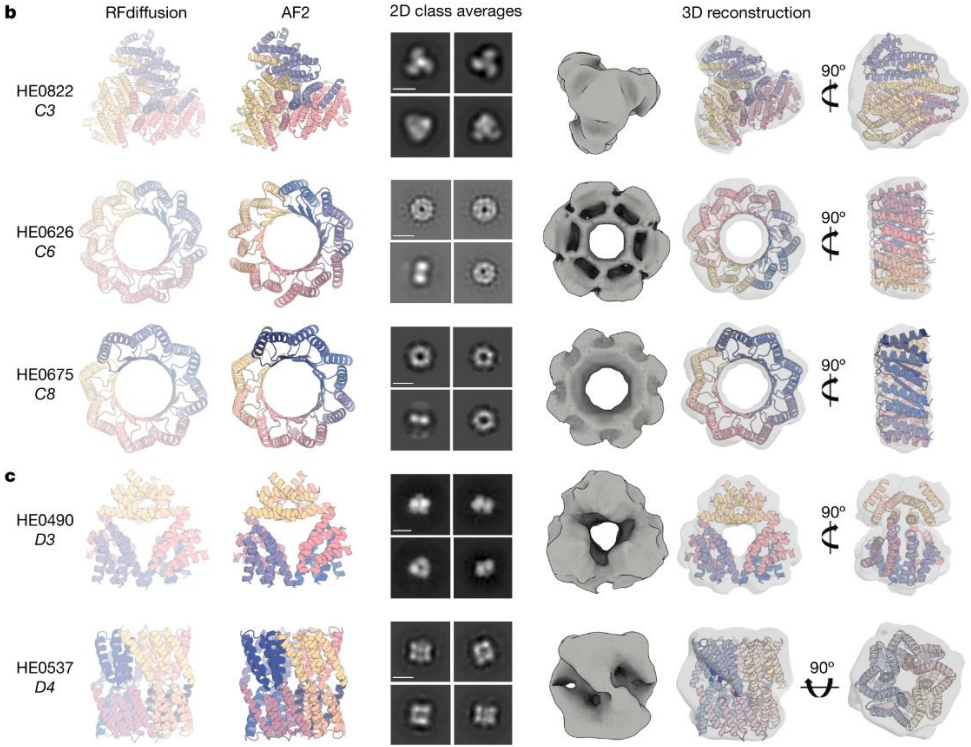
Watson et al. Nature. 2023.

Example TIM barrel-conditioned designs capture fold and are diverse

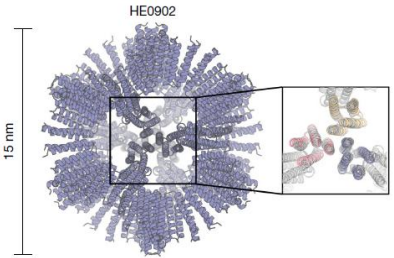


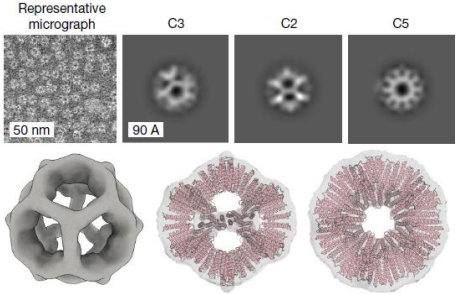
Watson et al. Nature. 2023.

Symmetric oligomer design



Design of icosahedral particle:

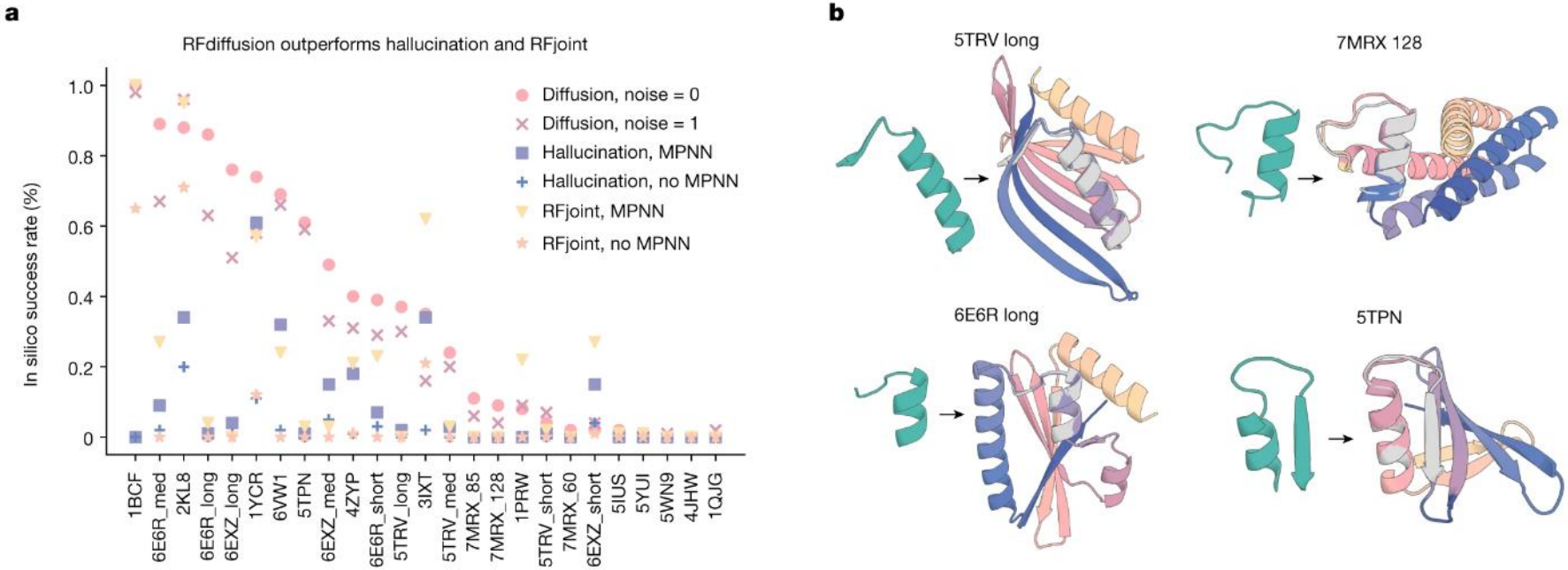




Watson et al. Nature. 2023.

Generate proteins based on functional motifs

Given a functional motif, generate a protein which captures the structure of the input functional motif.



https://www.bakerlab.org/2023/07/11/diffusion-model-for-protein-design/

Designed binders show higher affinity for target than native binder

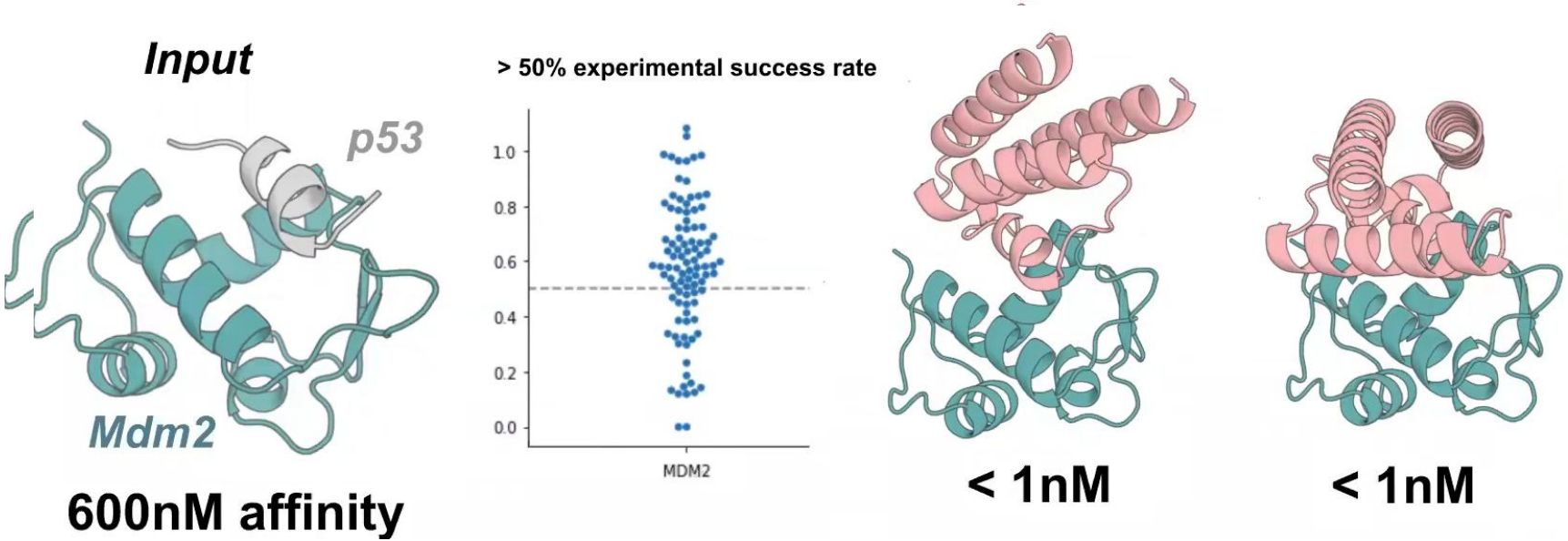
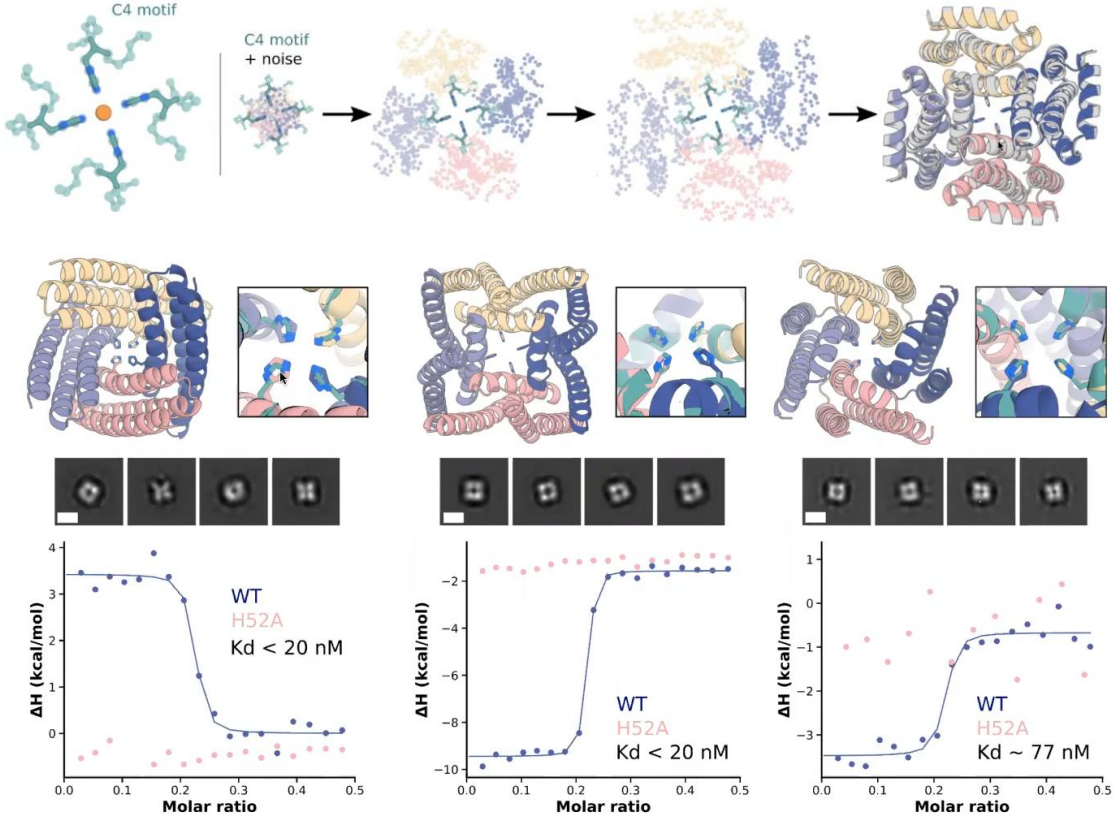
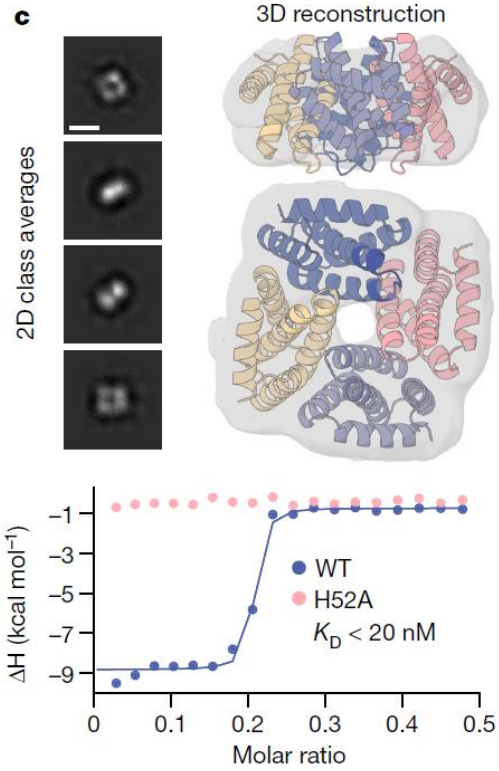


Figure from "RFDiffusion: Accurate protein design using structure prediction and diffusion generative models" video.

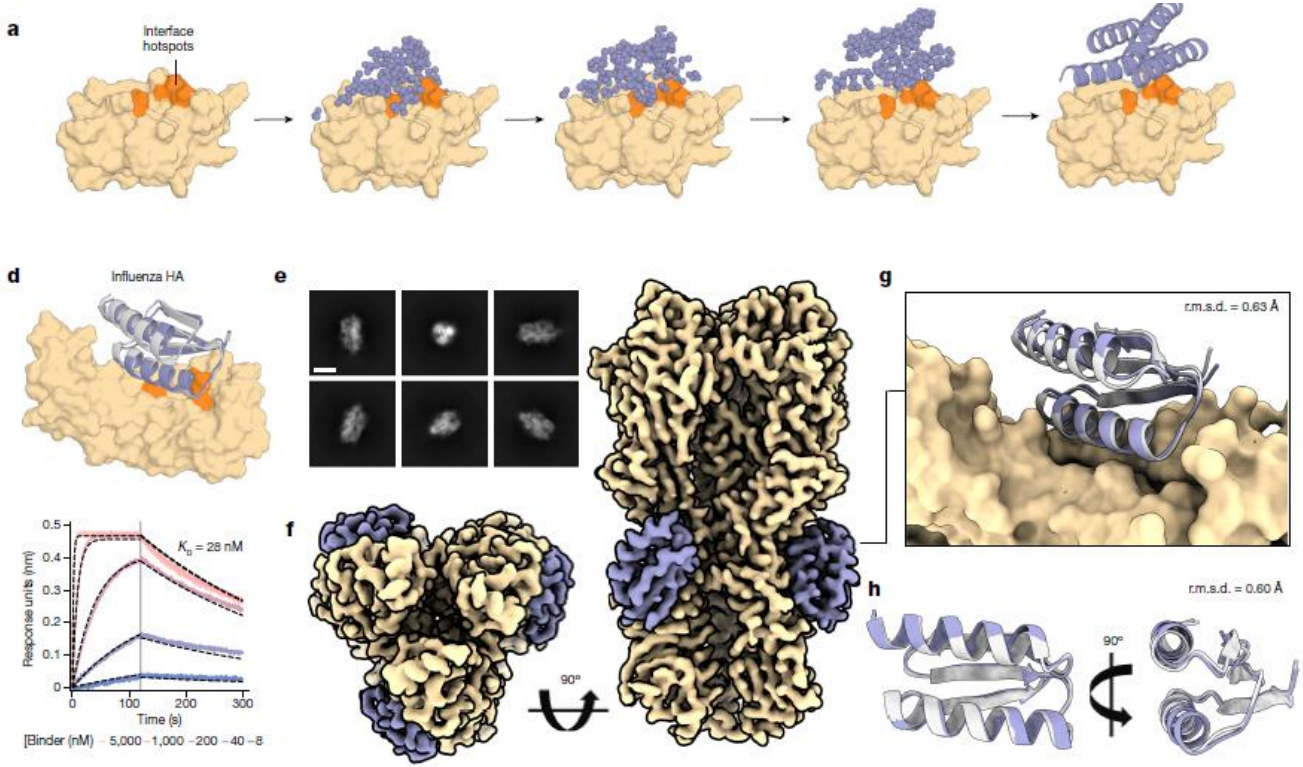
Symmetric metal-binding oligomers





Watson et al. Nature. 2023.

De novo design of protein-binding proteins



Watson et al. Nature. 2023.

Outline

1. What is diffusion?
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How to use RFdiffusion?

Activate your conda environment! `conda activate SE3nv`

- Example for unconditional monomer generation:

```
../RFdiffusion/scripts/run_inference.py \
'contigmap.contigs=[150-150]' \
inference.output_prefix=./output_monomer/monomer \
inference.num_designs=5
```

Call the code: **~/rosetta_workshop/RFdiffusion/scripts/run_inference.py**

- Specify length of the protein with **contigmap.contigs**
  Example: ‘c**ontigmap.contigs = [150-150]’**
- Specify the output
  Example: **inference.output_prefix=path_to_output**
- Set the number of backbones you want to generate
  Example: **inference.num_designs = 5**

Output of unconditional monomer generation

Output files are in **output_monomer** folder.

All outputs are poly-Glycine sequence.

- **PDB file** contains generated backbone
- **.trb file**, contains all metadata with the run, including input options
- **traj folder**, contains trajectory files (pdb)

Use PyMOL for visualization

```
pymol monomer_0.pdb
```

Motif scaffolding

- Example for generation conditioned on input motif:

```
../RFdiffusion/scripts/run_inference.py \
'contigmap.contigs=[20-30/E422-440/40-50]' \
inference.input_pdb=6UYG.pdb \
inference.output_prefix=./output_scaffold/scaffold \
inference.num_designs=1
```

- Specify motifs using **‘contigmap.contigs’**
  Example: **‘contigmap.contigs=[20-30/E422-440/40-50]’**
  → “Build a backbone with 20-30 residues at the N-terminal, then use residues 422-440 from chain E of the input as scaffold, followed by 40-50 residues for C-terminal
  - Motif is prefixed by letter followed by residue specification (**E422-440**)
- Specify input PDB with **‘inference.input_pdb=path’**

Motif scaffolding with additional chains to avoid clashes

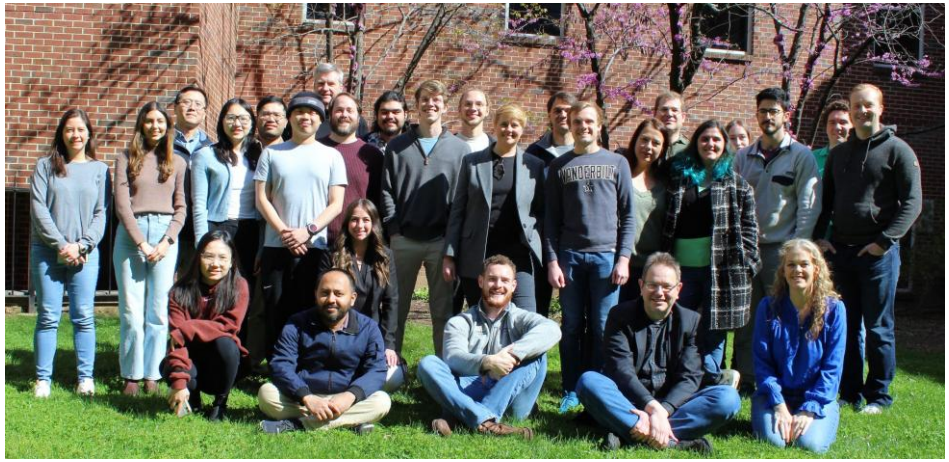
- Example where we can input structure from additional chains to avoid clashes
- Use **/0** followed by a space to symbolize a chain break

```
cd ../../
../RFdiffusion/scripts/run_inference.py \
'contigmap.contigs=[10-20/E422-440/40-50/0 H4-22/H29-71/H77-113/0
L2-29/L31-95/L97-109]' \
inference.input_pdb=6UYG.pdb \
inference.output_prefix=./output_scaffold/scaffold_Ab \
inference.num_designs=1
```

Heavy chain info

Light chain info

Thank You!



Dr. Ana C. Chang-Gonzalez
Postdoctoral Scholar

ana.chang-gonzalez@vanderbilt.edu
www.meilerlab.org

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