

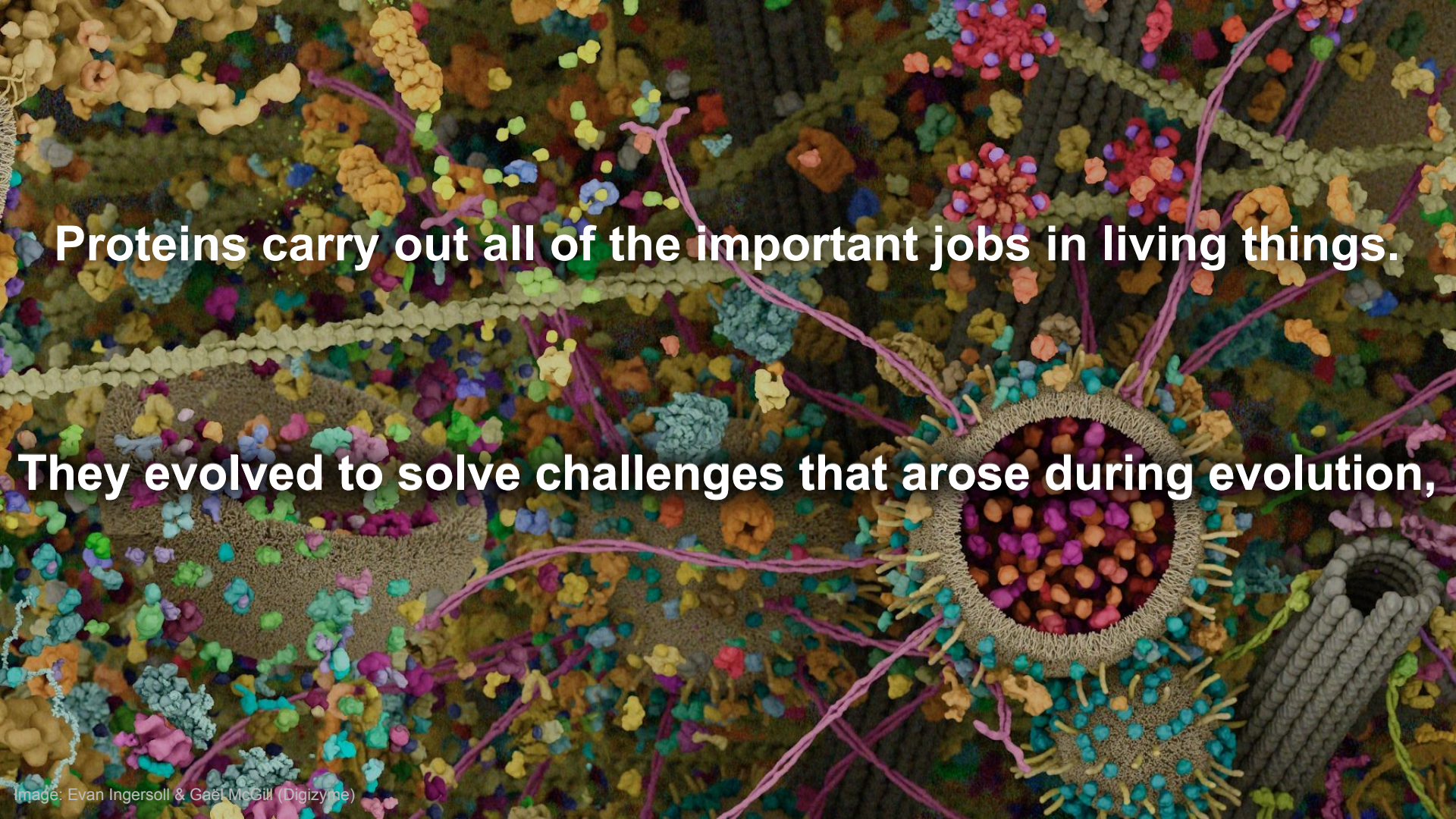


# DE NOVO PROTEIN DESIGN

Image: Evan Ingersoll & Gaël McGill (Digizyme)



**Proteins carry out all of the important jobs in living things.**



**Proteins carry out all of the important jobs in living things.**

**They evolved to solve challenges that arose during evolution,**



**Proteins carry out all of the important jobs in living things.**

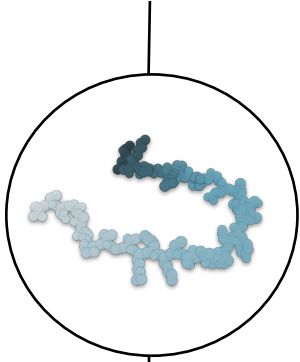
**They evolved to solve challenges that arose during evolution,  
but we face new challenges today.**

# Biology

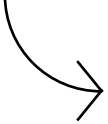


CAATGTTGC...

Gene  
Sequence



Amino Acid  
Sequence

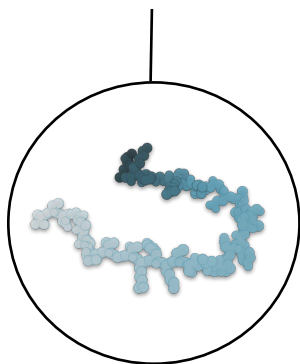


Biological  
Function

## Biology



CAATGTTGC...



Gene  
Sequence

Amino Acid  
Sequence

Biological  
Function



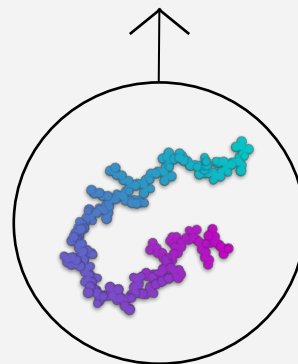
## Design

*Make protein and test*

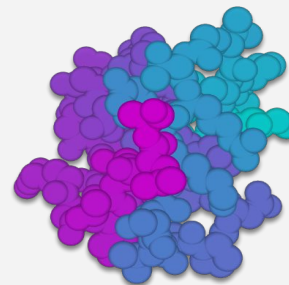
**New**  
Gene  
Sequence

TTCATGGCT...

**New**  
Amino Acid  
Sequence



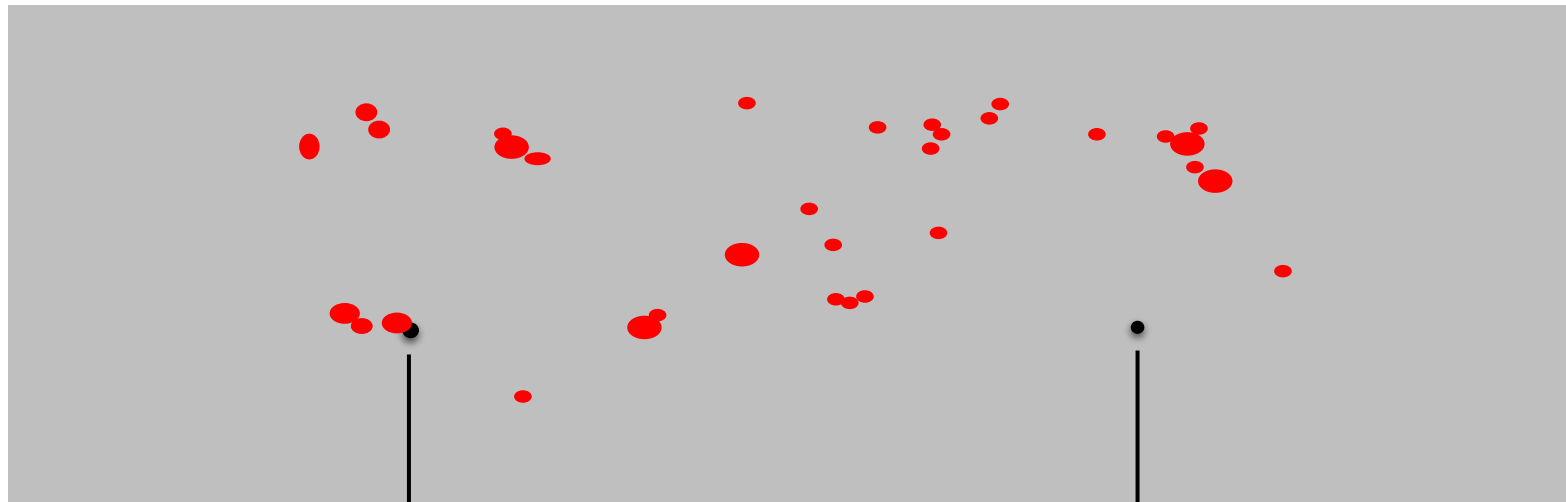
**Desired**  
Function



# A vast universe of possible proteins

● Proteins in Nature

○ Possible Proteins



Traditional protein engineering

De novo protein design

Number of 100-residue amino acid sequences:  $20^{100} = 10^{130}$

Number of naturally occurring proteins:  $\sim 10^{15}$

# A brief history of protein design

---

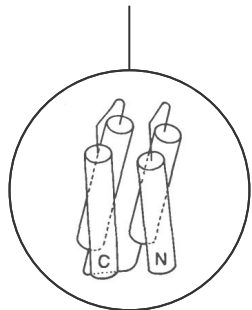
1988

now

# A brief history of protein design

## NEW STRUCTURES

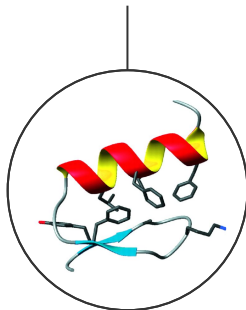
1988



**Bill DeGrado**

University of California,  
San Francisco

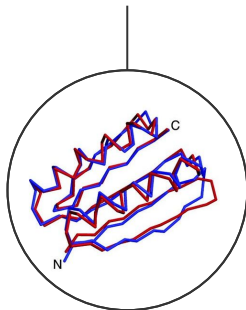
1997



**Steve Mayo**

California Institute  
of Technology

2003



**Brian Kuhlman**

University of North  
Carolina at Chapel Hill

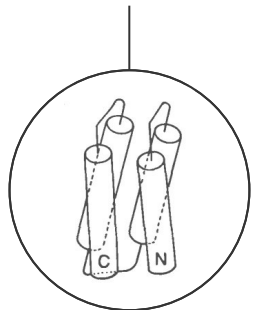
now

# A brief history of protein design

## NEW STRUCTURES

## NEW FUNCTIONS

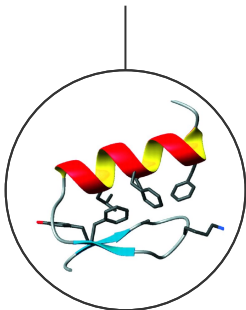
1988



**Bill DeGrado**

University of California,  
San Francisco

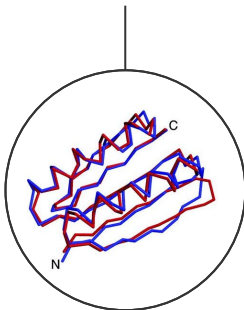
1997



**Steve Mayo**

California Institute  
of Technology

2003



**Brian Kuhlman**

University of North  
Carolina at Chapel Hill

2005



**Rosetta@Home**  
Volunteer Computing

2008



**Foldit**  
Online Game

now

**1,000s of  
Projects &  
Researchers**



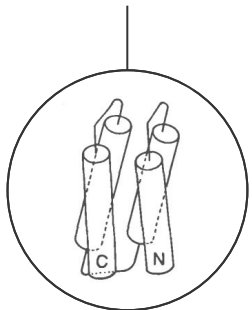
# A brief history of protein design

## NEW STRUCTURES

## NEW FUNCTIONS

## NEW ERA

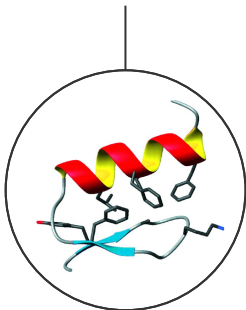
1988



**Bill DeGrado**

University of California,  
San Francisco

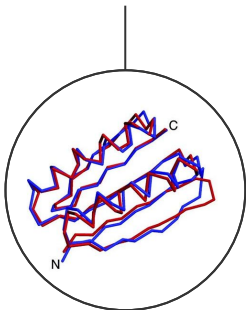
1997



**Steve Mayo**

California Institute  
of Technology

2003



**Brian Kuhlman**

University of North  
Carolina at Chapel Hill

2005



**Rosetta@Home**  
Volunteer Computing

2008



**Foldit**  
Online Game

**1,000s of  
Projects &  
Researchers**

2021–now



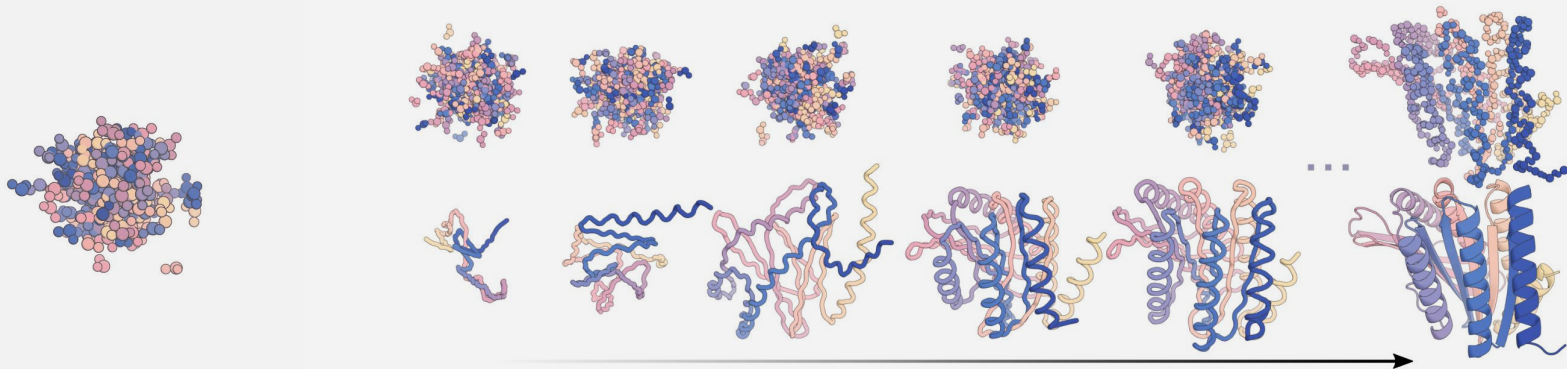
# Designing proteins with RFdiffusion

Inspired by deep-learning methods for generating synthetic images.

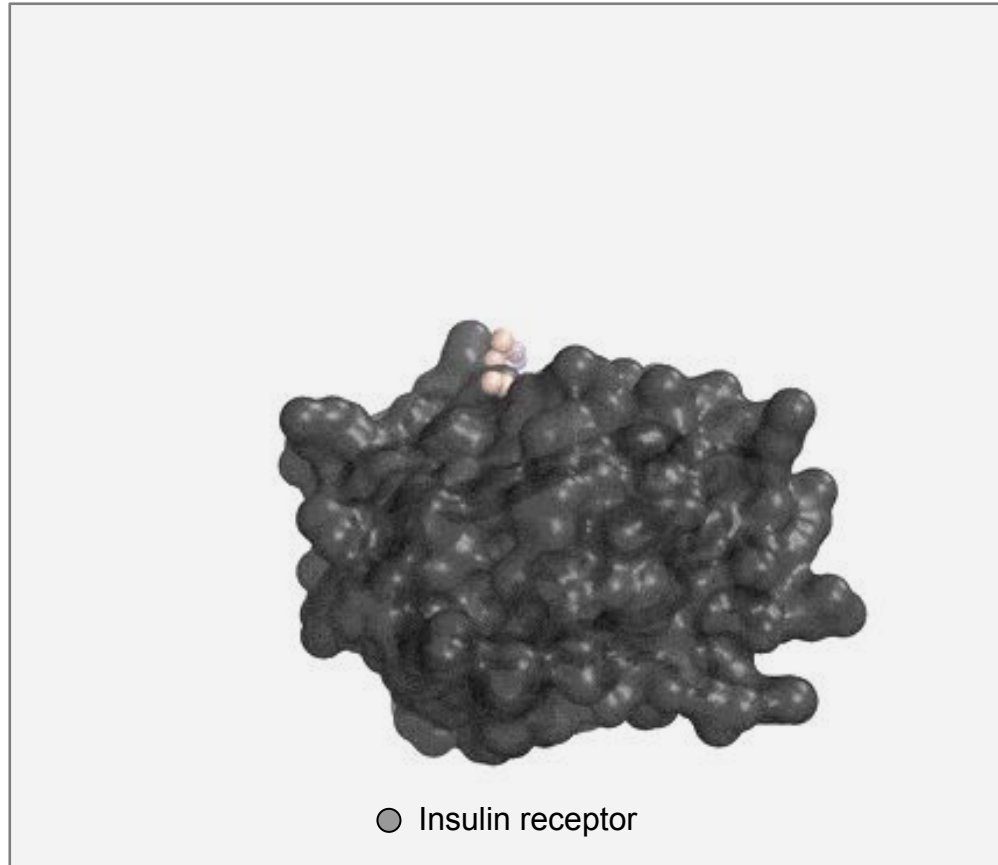
e.g. Stable Diffusion, DALL-E



Image: Arash Vahdat and Karsten Kreis (NVIDIA)



# Diffusion of an insulin mimic



# Medicine



# Technology



# Sustainability



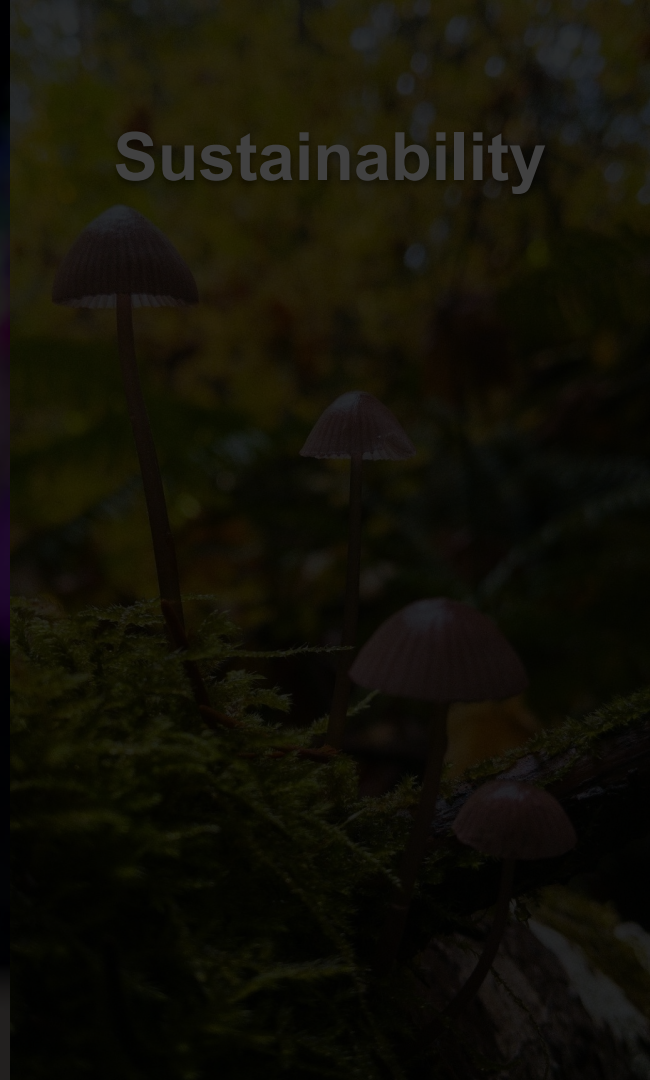
# Medicine



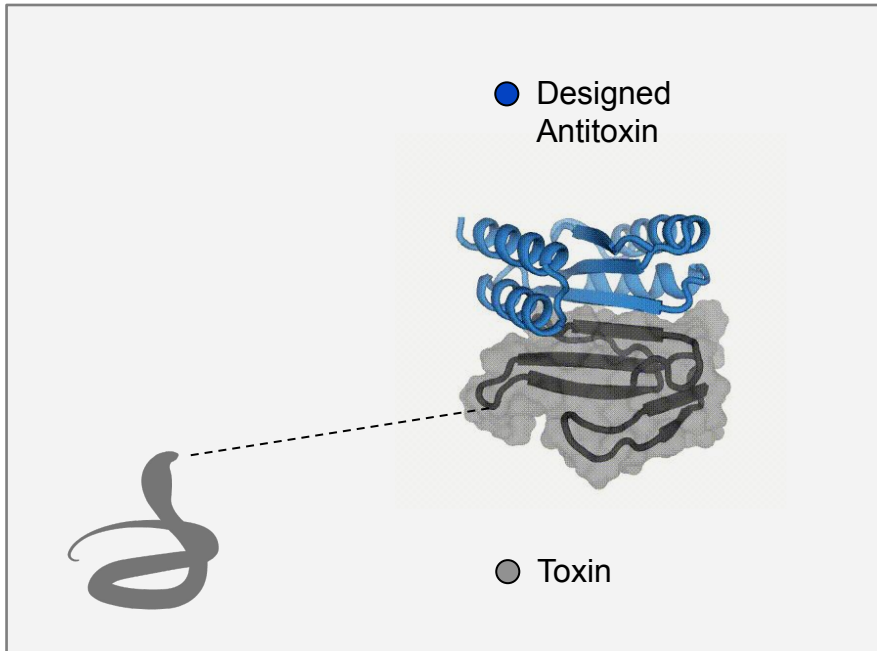
# Technology



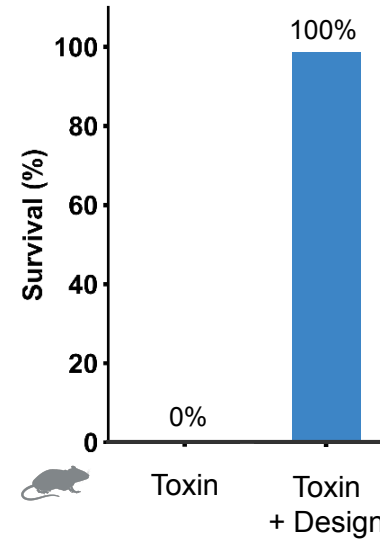
# Sustainability



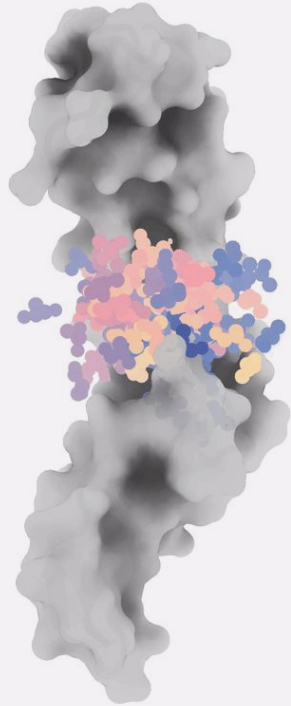
# Neutralizing snake toxin



Protects animals from lethal doses of cobratoxin.

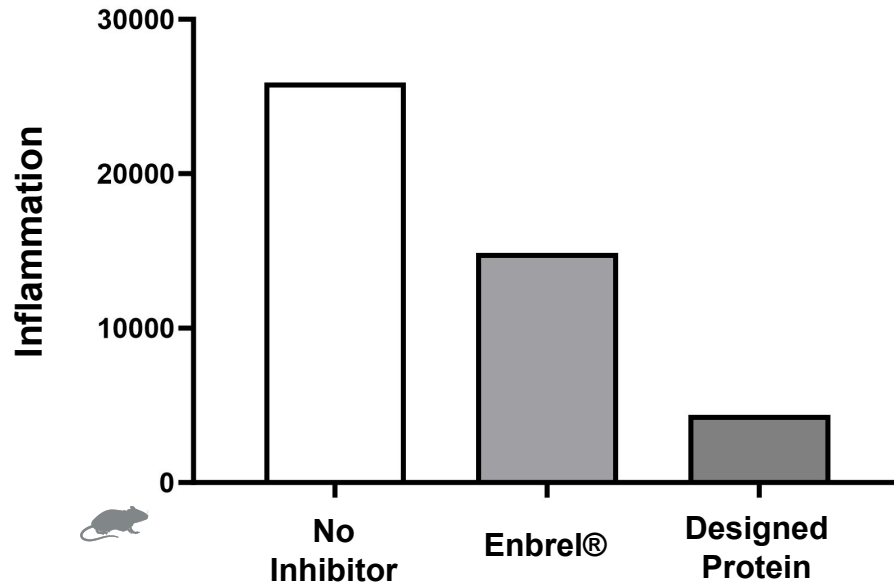


# Suppressing inflammation

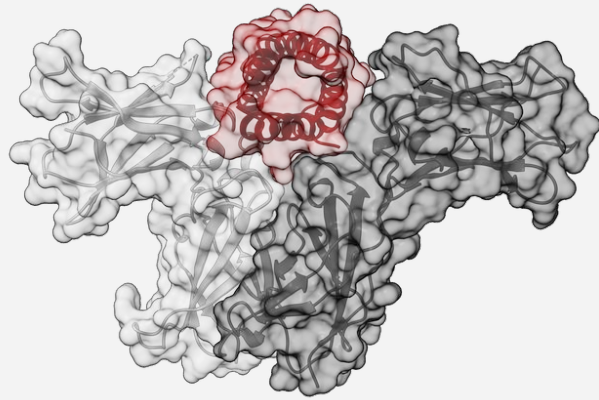


○ TNF Receptor (target)

More potent than current medicine (Enbrel®)  
at inhibiting inflammation.

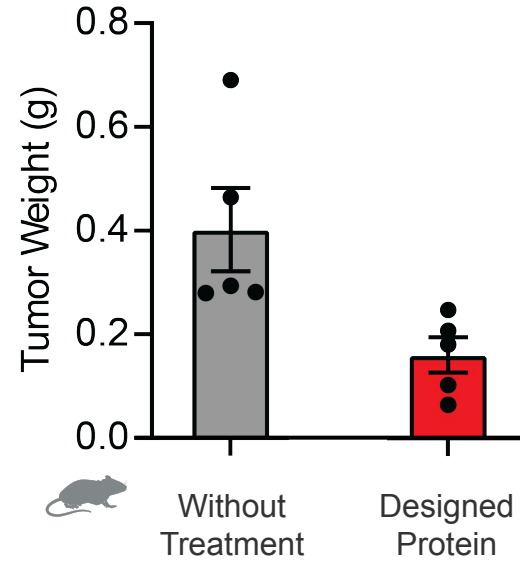


# Shrinking tumors



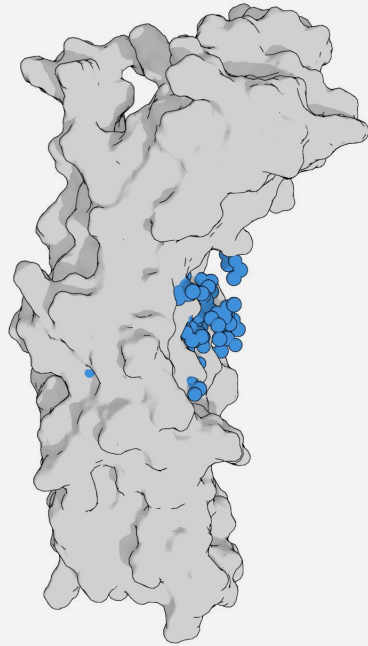
- Receptor 1 (IL-21R)
- Receptor 2 (Common  $\gamma$ )

## Pancreatic Cancer

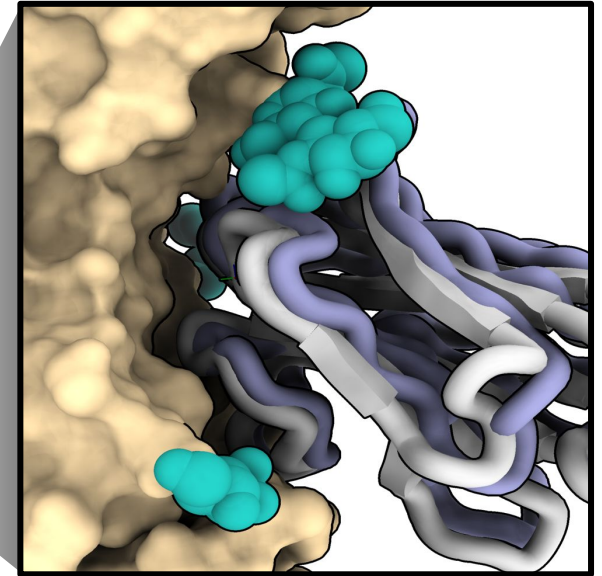
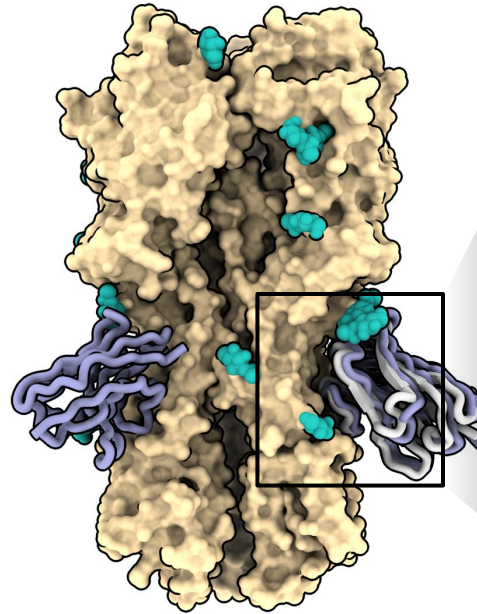


# Targeting influenza with de novo antibodies

Cryo-EM Structure

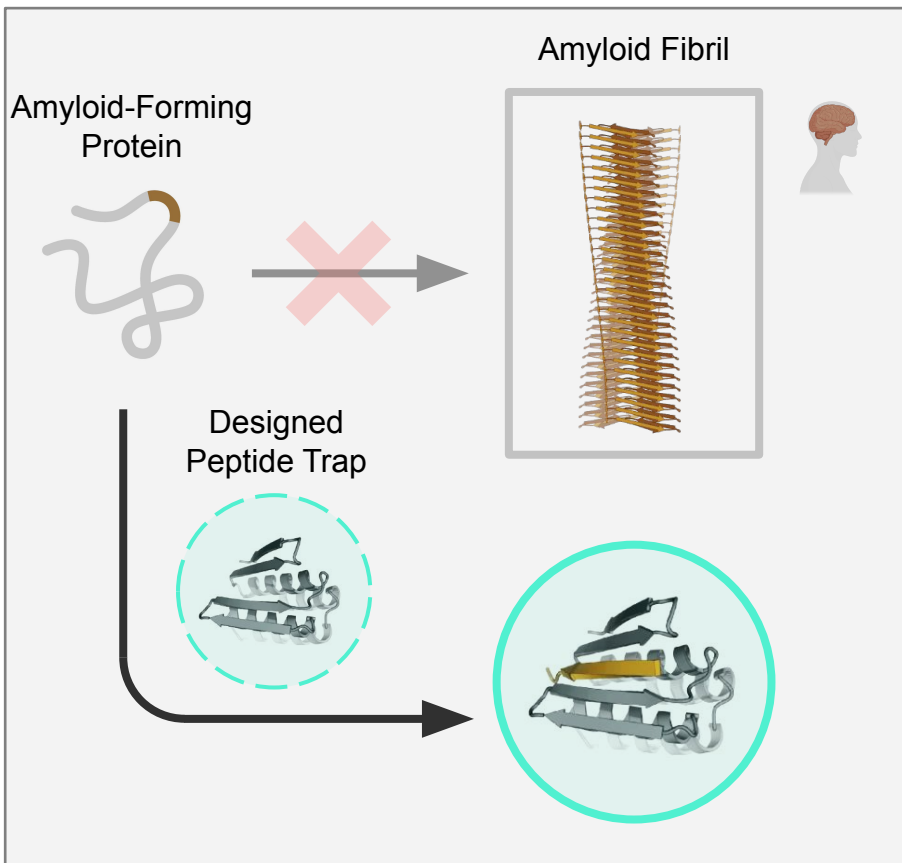


○ Influenza HA (target)

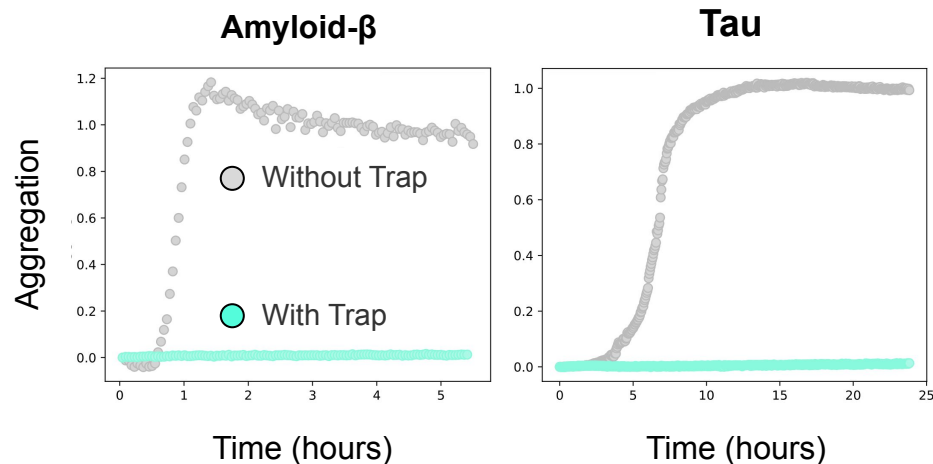


○ Intended      ● Measured

# Blocking Alzheimer's-associated amyloid fibrils



Prevents fibril formation in the lab.



## Medicine



## Technology

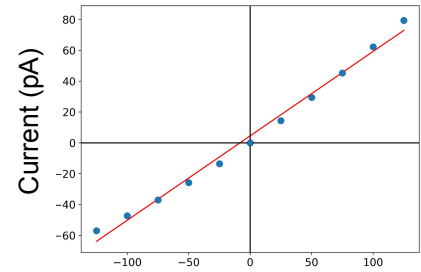
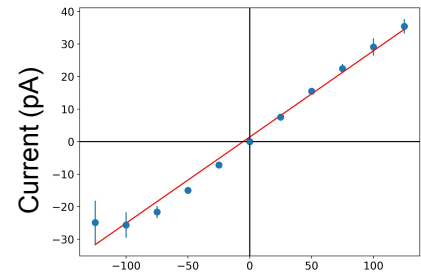
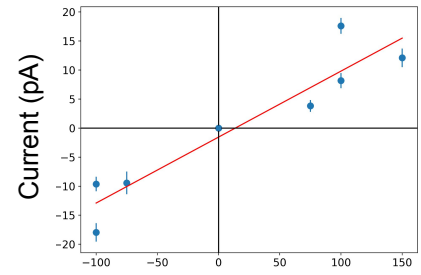
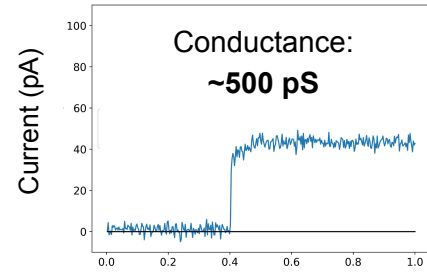
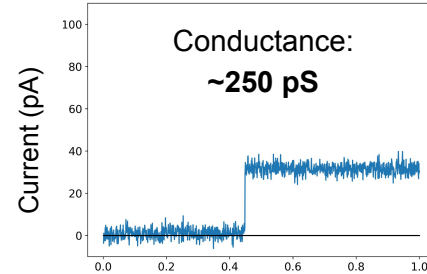
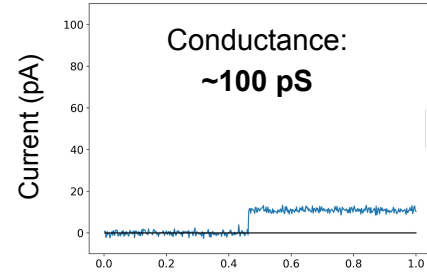
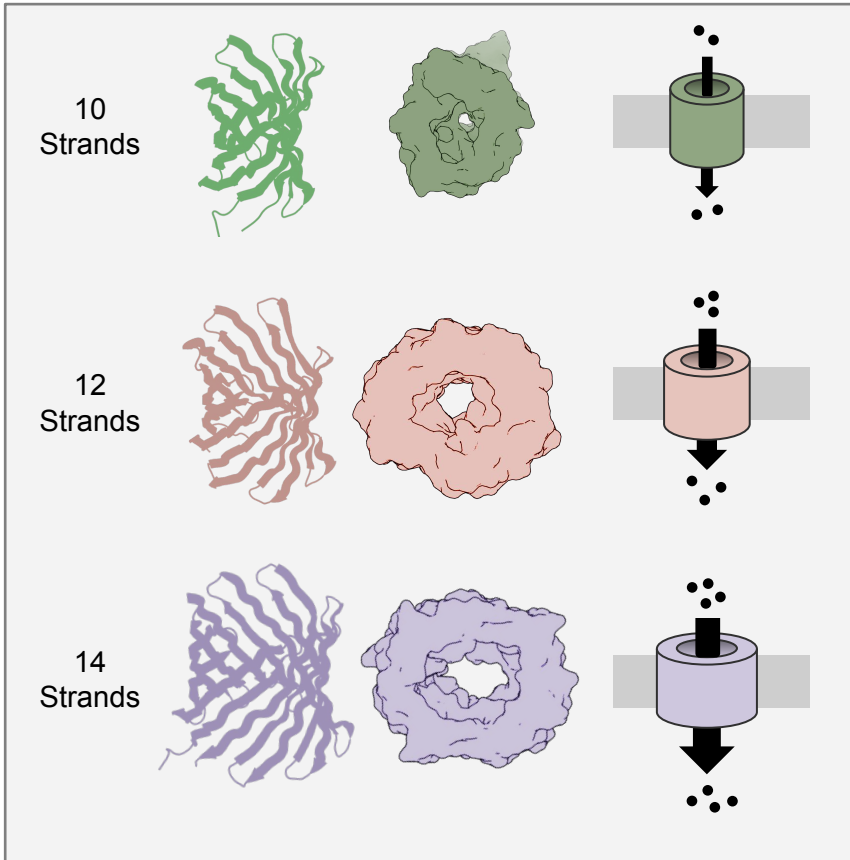


## Sustainability

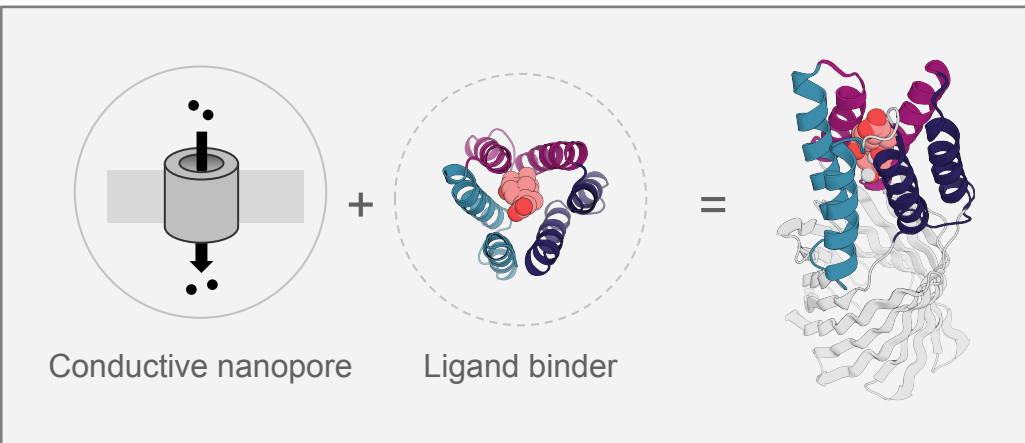


# Design of transmembrane nanopore sensors

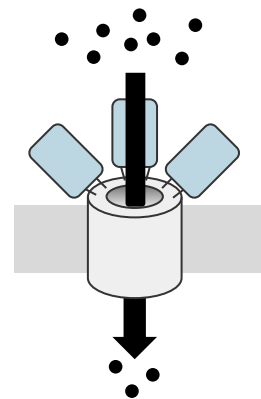
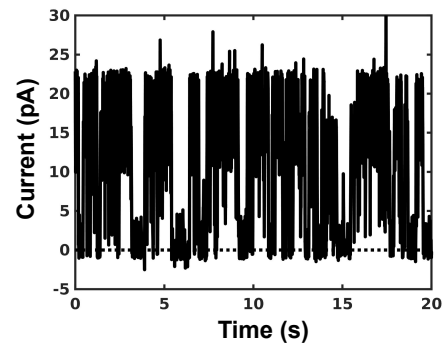
# Conductive nanopores



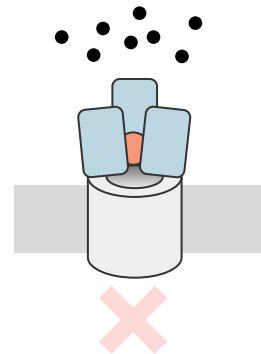
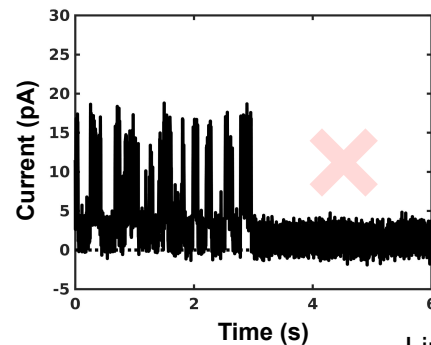
# Sensing chemicals



Without Cholic Acid



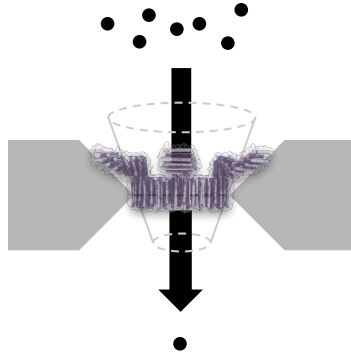
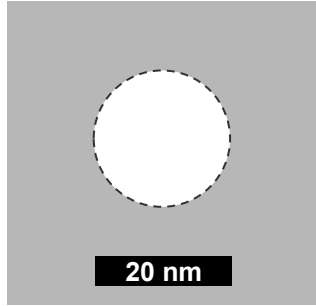
With Cholic Acid



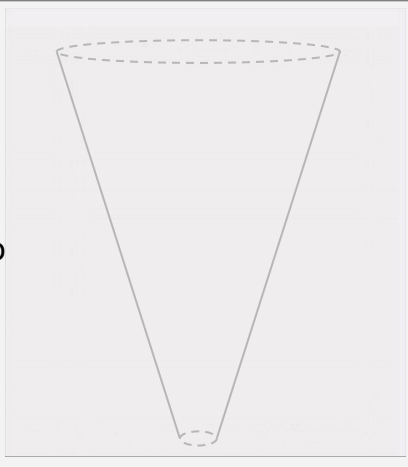


# Combining proteins and digital technology

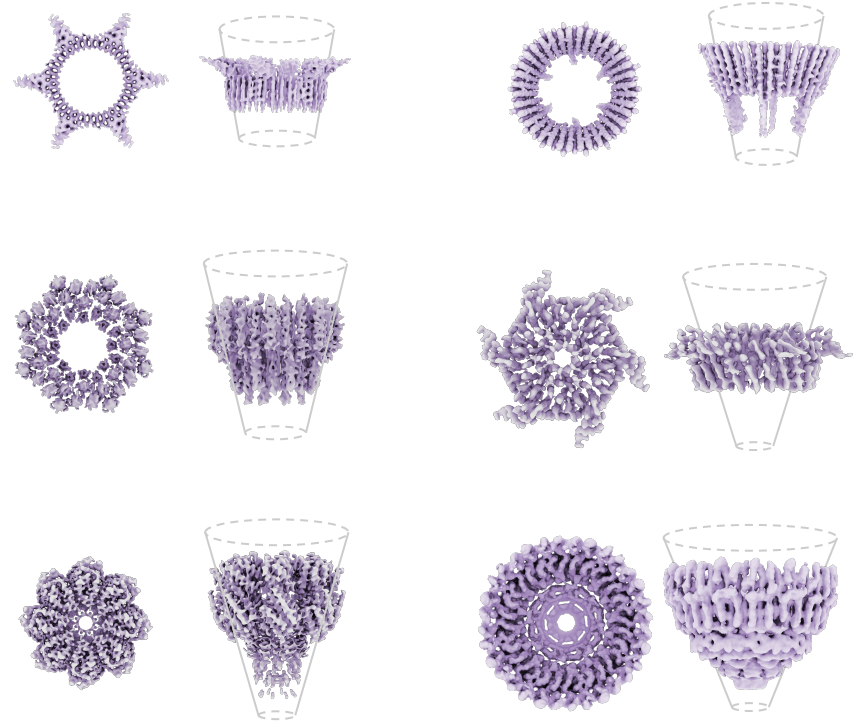
Silicon Nitride Chip



Diffusing pores to  
insert into silicon



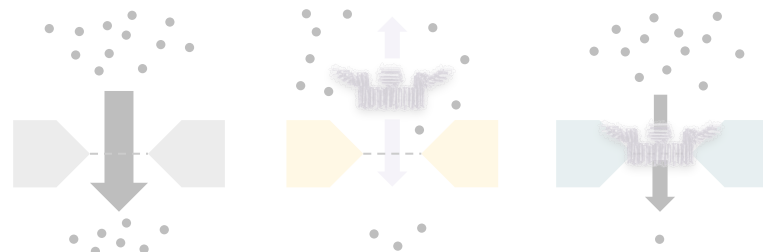
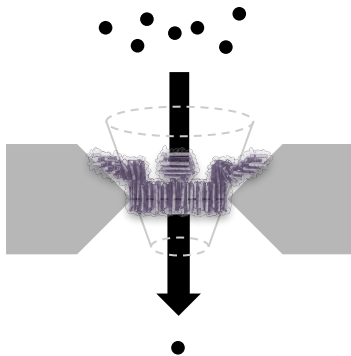
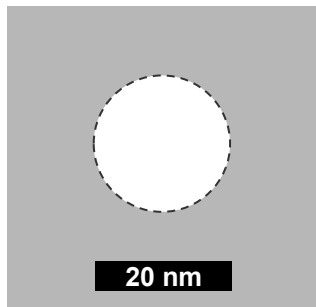
Cryo-EM Structures





# Combining proteins and digital technology

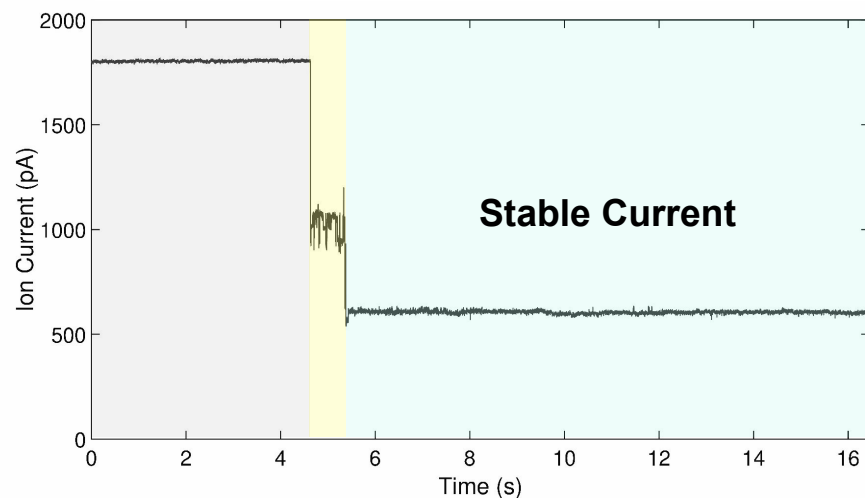
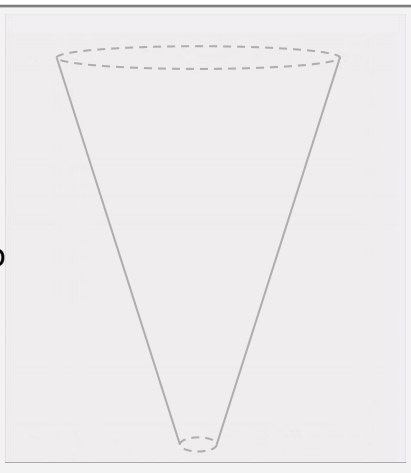
Silicon Nitride Chip



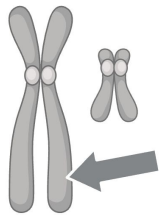
Chip Only

Integrated Protein Pore

Diffusing pores to  
insert into silicon

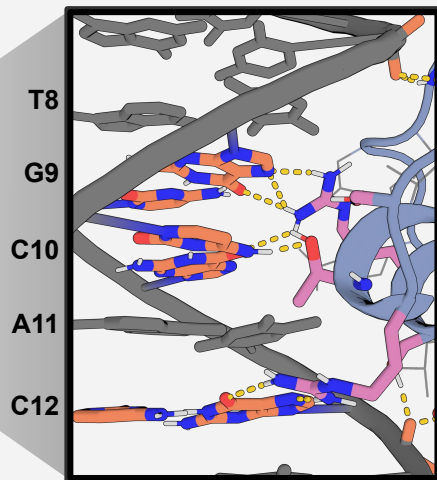


# Design of DNA-binding proteins



# Targeting DNA

## Binding Specificity

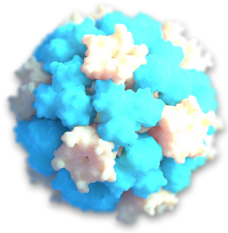


	T	G	C	A
T6	T			
C7			C	
T8	T			
→ G9		G		
→ C10			C	
A11				A
→ C12			C	
A13				A
T14	T			

# Design of protein nanomaterials

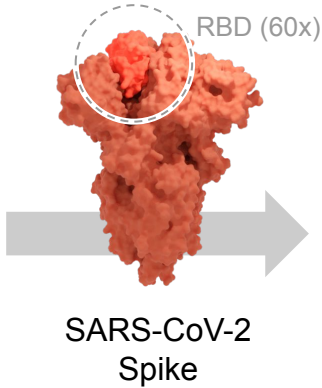
# First computationally designed protein medicine

2016



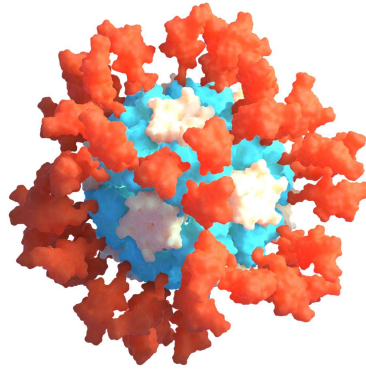
**Neil King**

UW Medicine



SARS-CoV-2  
Spike

2020



2022

SKYCovione™ COVID-19 vaccine  
approved in U.K. & South Korea



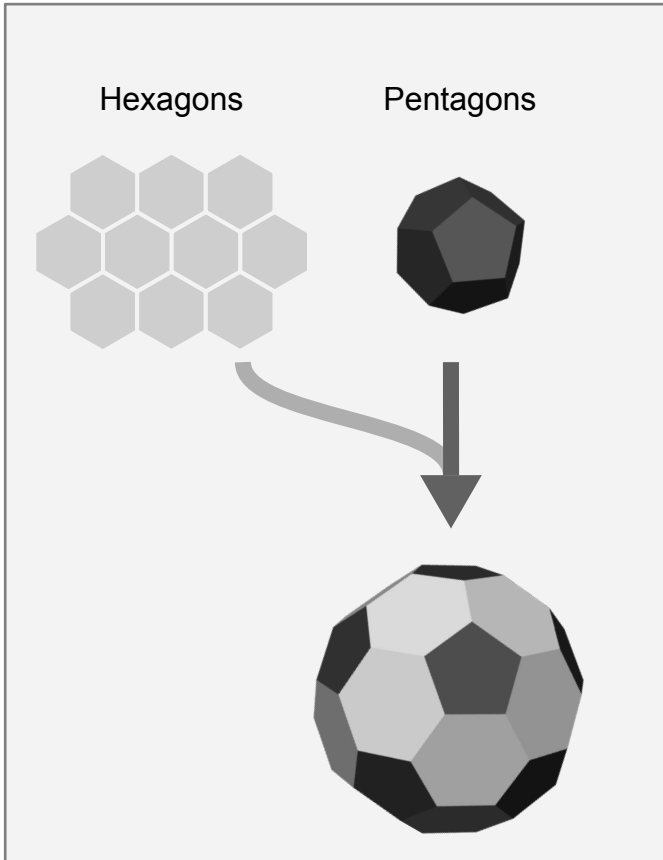
image: SK Bioscience

Alexandra Walls, Brooke Fiala, et al. Cell, 2020

Alexandra Walls, et al. Cell, 2021

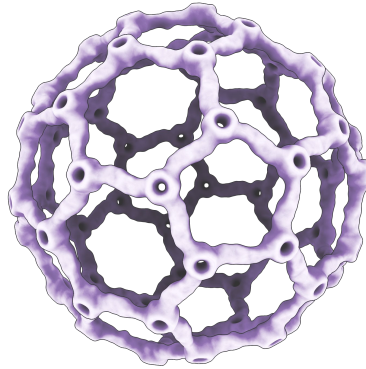
designed by King and Veesler Labs (UW Medicine)

# Larger containers through symmetry breaking



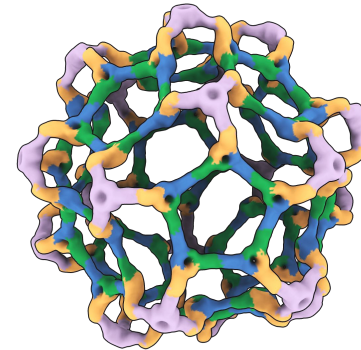
## Cryo-EM Structures

Quasisymmetry



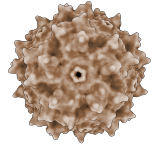
1 unique protein,  
180 chains

Pseudosymmetry



4 unique proteins,  
240 chains

AAV  
For Scale



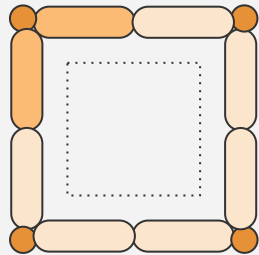
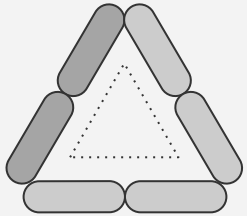
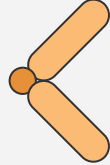
20 nm

Design of proteins with multiple low energy states

# Molecular switches

State A

State B



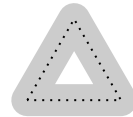
Triangle

Square

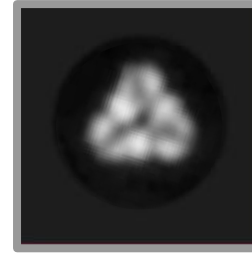
Electron Micrograph

State A

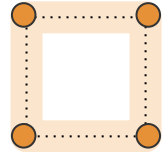
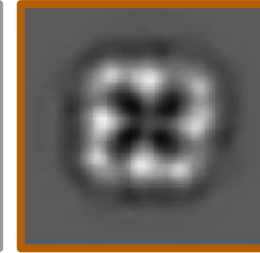
State B



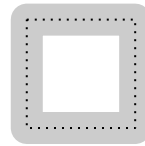
Triangle



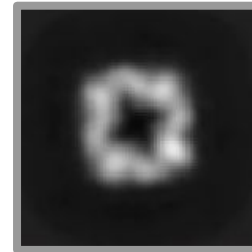
10 nm



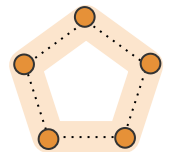
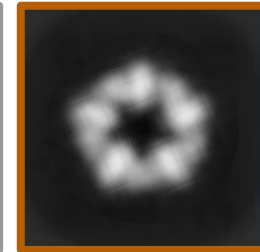
Square



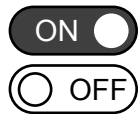
Square



10 nm



Pentagon



# Controllable immunotherapies

**Begin Treatment**  
*Add Medicine*

**End Treatment**  
*Add Effector*

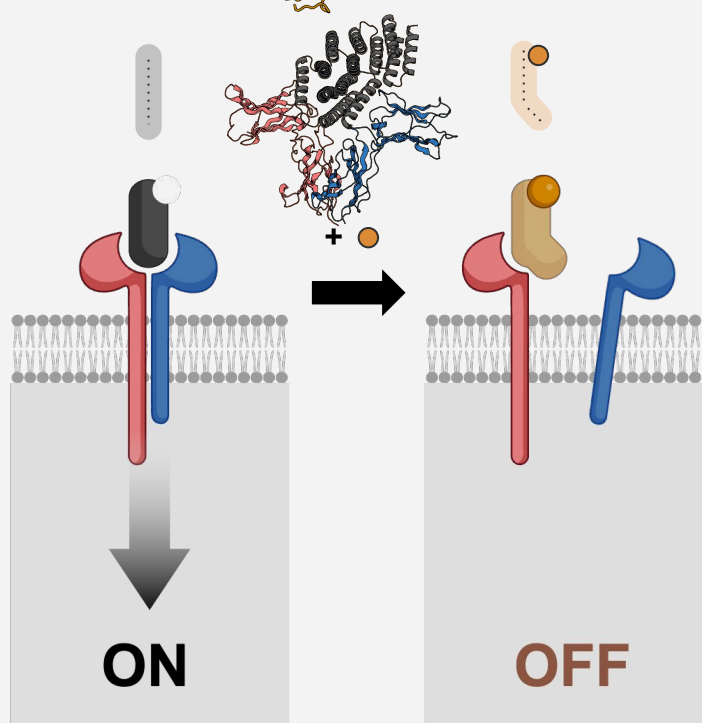
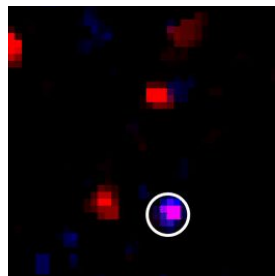
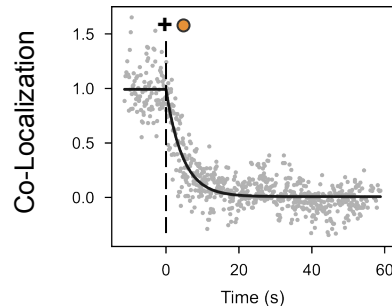


image created with BioRender.com

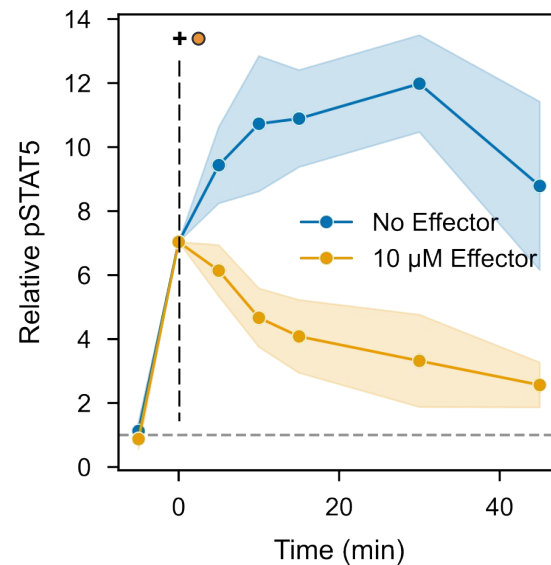
## Observed Co-Localization



- Receptor 1 (IL-2R $\beta$ )
- Receptor 2 (Common  $\gamma$ )



## Cell Signaling



## Medicine



## Technology



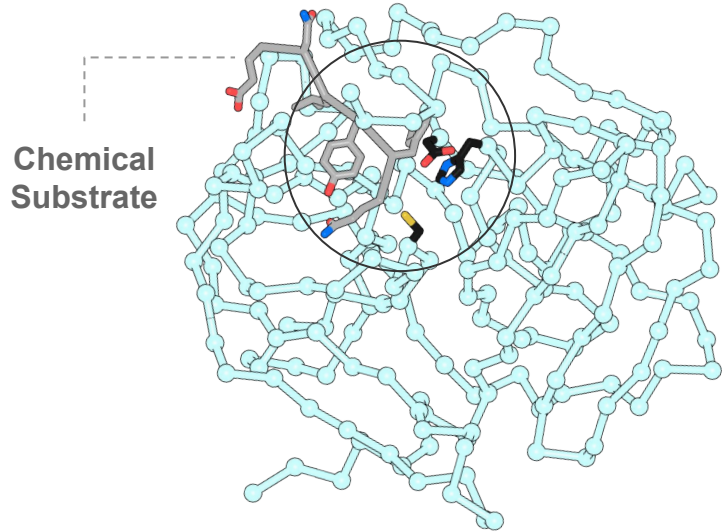
## Sustainability



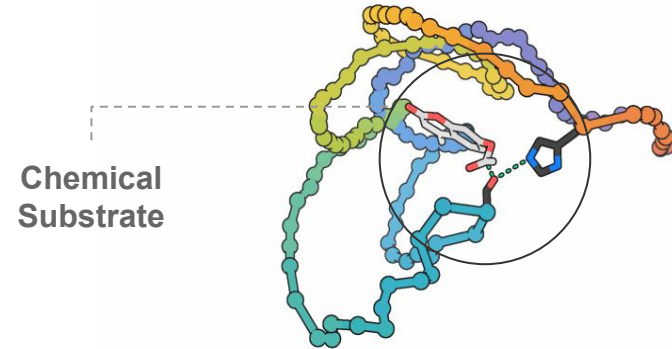
# Enzyme design

# Custom catalysts

**Natural Enzyme**



**Designed Enzyme**

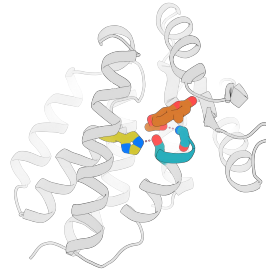
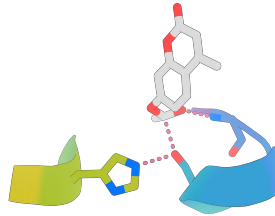


# Building up new enzymes

1

## Ser-His dyad

Oxyanion contacts: 1

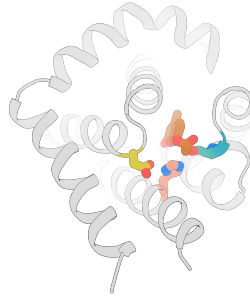
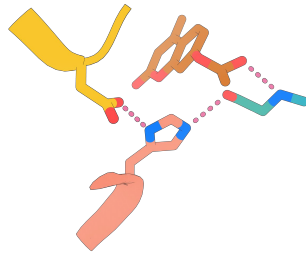


Inactive

2

## Ser-His-Asp triad

Oxyanion contacts: 1

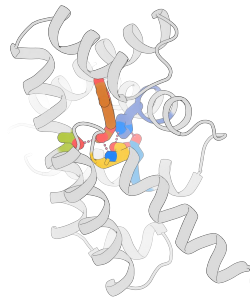
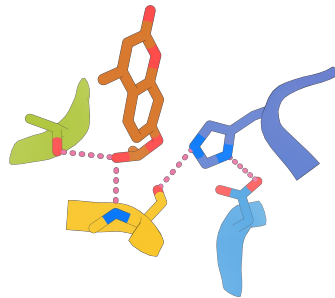


Works once... then stuck

3

## Ser-His-Asp triad

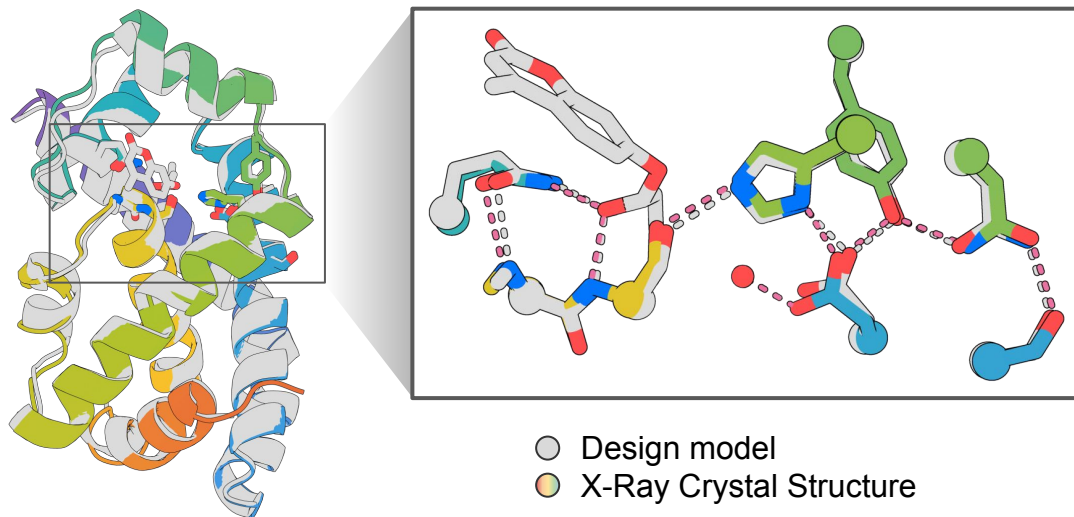
Oxyanion contacts: 2



Active!

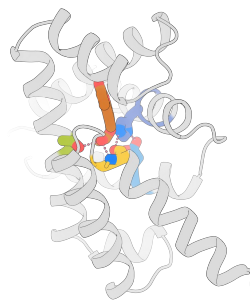
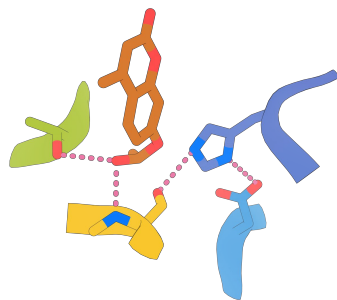
$$\frac{k_{\text{cat}}}{K_{\text{M}}} \\ 3,800 \text{ M}^{-1} \text{ s}^{-1}$$

# Building up new enzymes



3

**Ser-His-Asp triad**  
Oxyanion contacts: 2

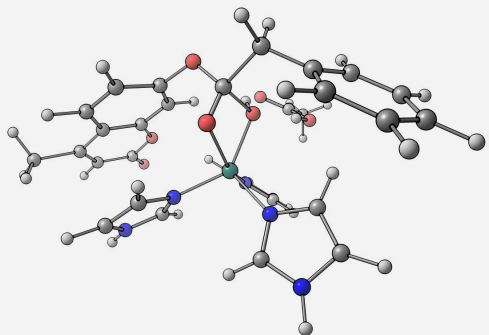


**Active!**

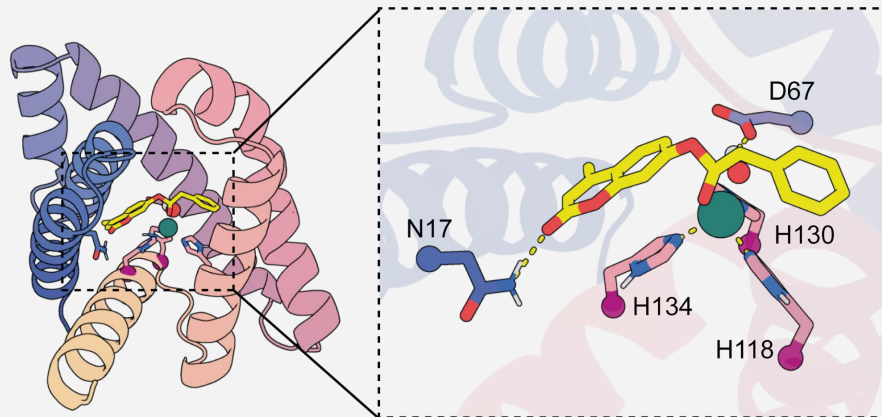
$$\frac{k_{\text{cat}}}{K_{\text{M}}} \\ 3,800 \text{ M}^{-1} \text{ s}^{-1}$$



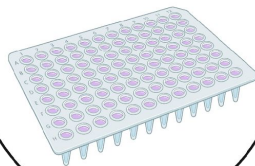
# Using metal to break chemical bonds



Start from quantum chemistry calculation



96

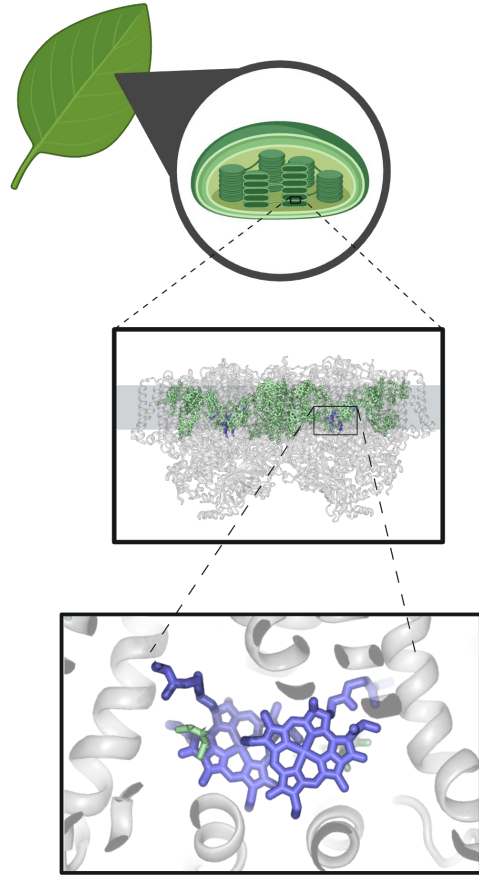


**$23,000 \text{ M}^{-1} \text{ s}^{-1}$**

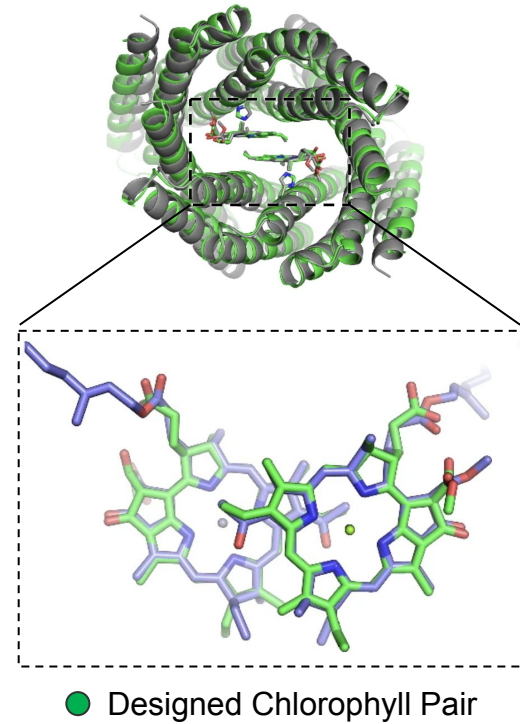
$k_{\text{cat}}/K_{\text{M}}$

# Artificial photosynthesis

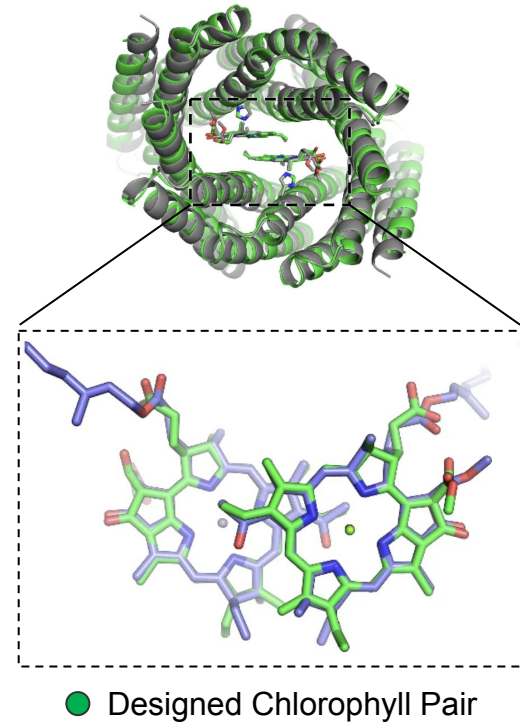
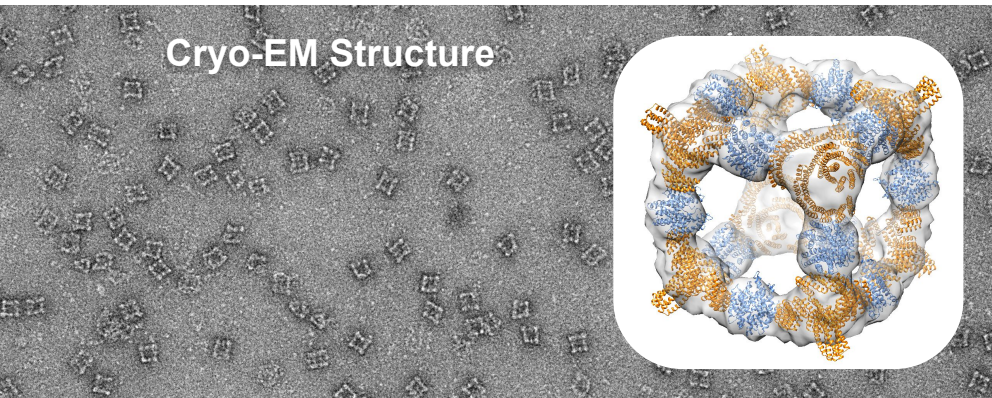
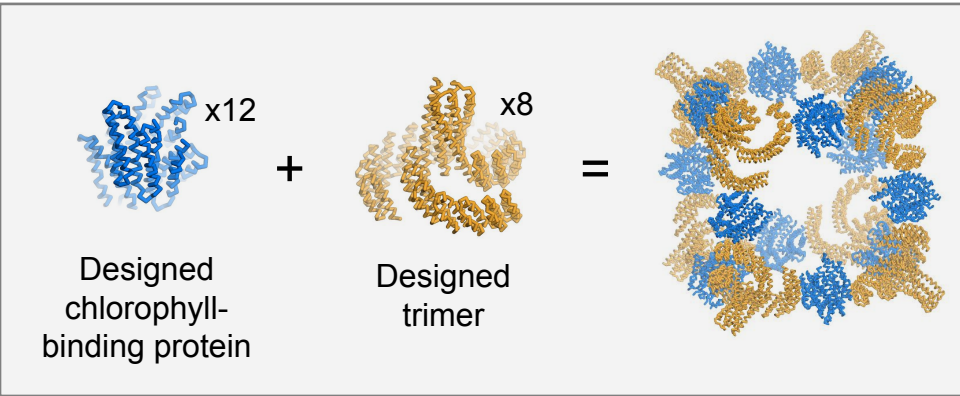
# Harvesting sunlight



● Natural Chlorophyll Pair



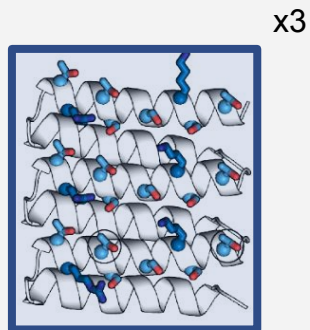
# Harvesting sunlight



# Hybrid materials

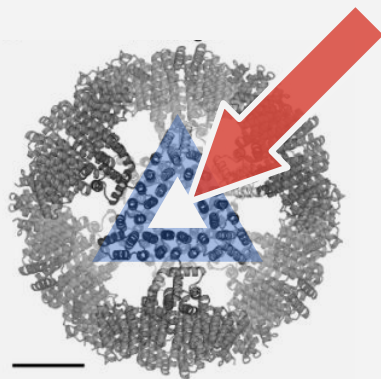


# Growing semiconductors



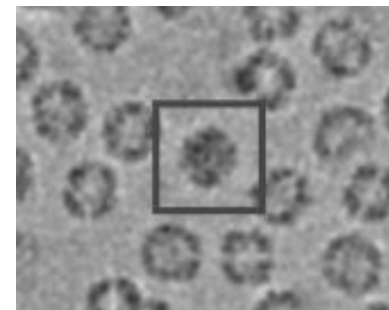
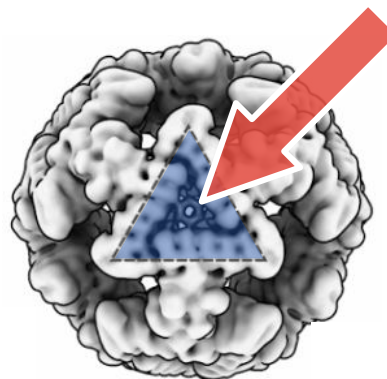
x3

Zinc Oxide  
Template



4 nm

**Cryo-EM Structure**  
*with Zinc Oxide*



# A whole new world of designed proteins

## Medicine

Pandemic Preparedness

Cancer & Immunotherapy

Neurodegeneration

## Technology

Chemical Sensors

Bio-Electronic Devices

Drug Delivery

## Sustainability

Breaking Down Plastic

Artificial Photosynthesis

Nanoscale Manufacturing

