



THE FRONT ROW
at Scripps Research

VISUALIZING THE INVISIBLE MACHINERY OF LIFE

Gabe Lander



Scripps Research

Integrative Structural and Computational Biology

&

DEATH



@LanderLab

ANTOINE DE SAINT-EXUPÉRY

Le Petit Prince

Avec des aquarelles de l'auteur

L'essentiel est
invisible pour les
yeux.
- Le Petit Prince



folio



Lima, Peru





Barquisimeto, Venezuela



- **BS Biochemistry, Computer Science, 2002**
Binghamton University

Binghamton, New York



Tubulin and its relatives

Petroleum formation Radical role for sulphur

Deglacial climate End of the last cold snap

Sinosauropteryx A well-preserved dinosaur

Now on the market
DNAS Automation

A map of North and South America showing travel routes. A thick black line with circular markers at each stop connects Lima, Peru; Caracas, Venezuela; Washington, D.C.; and San Diego, CA. From San Diego, a curved arrow points to Berkeley, CA. The map includes labels for major cities, states, and countries.

Berkeley, CA

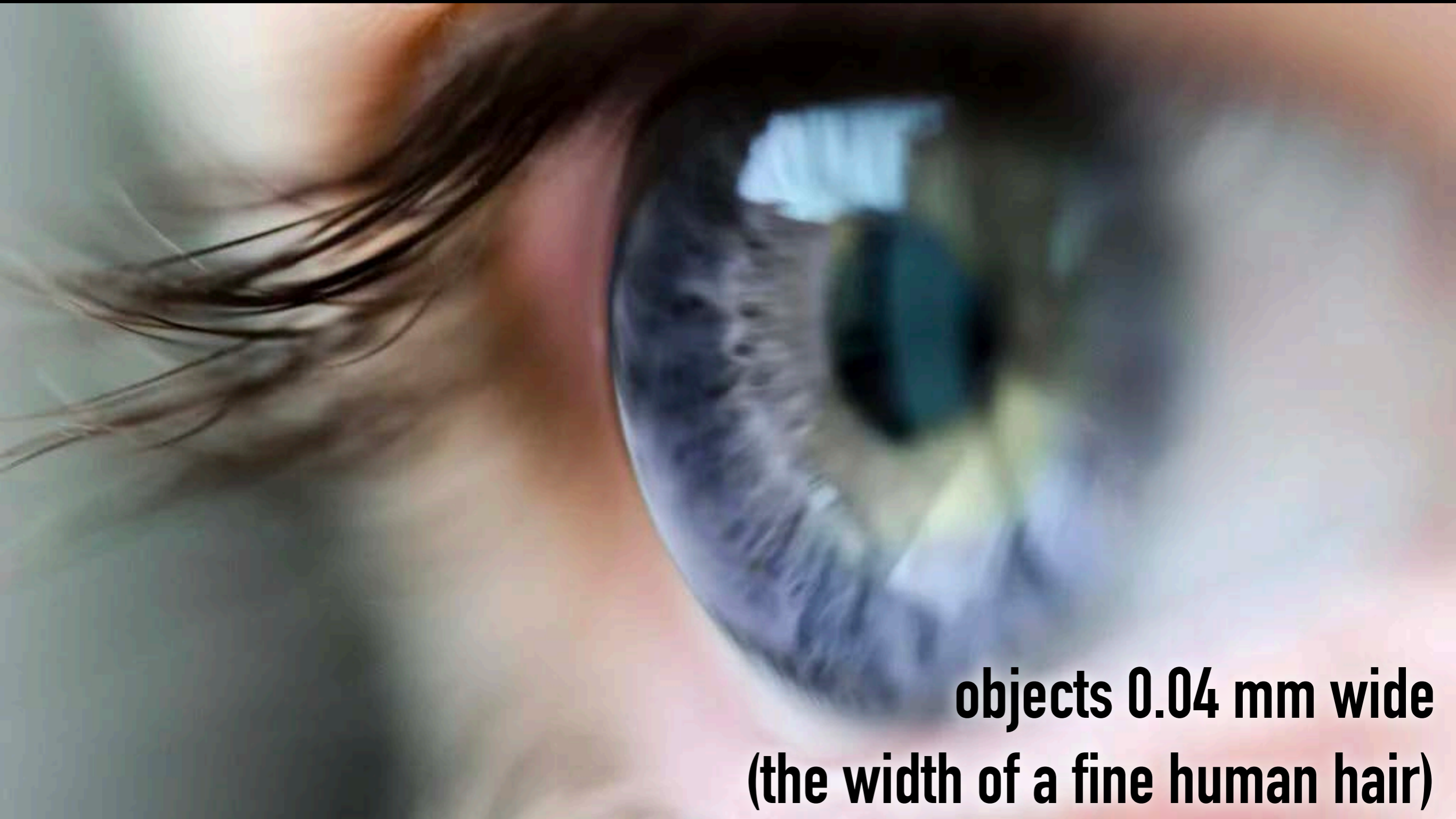
San Diego, CA

- **BS Biochemistry, Computer Science, 2002**
Binghamton University
- **Ph.D. Biophysics, 2009**
Scripps Research
- **Postdoctoral Fellow, 2013**
UC Berkeley
- **Faculty at Scripps, 2013 – ...**

OUTLINE

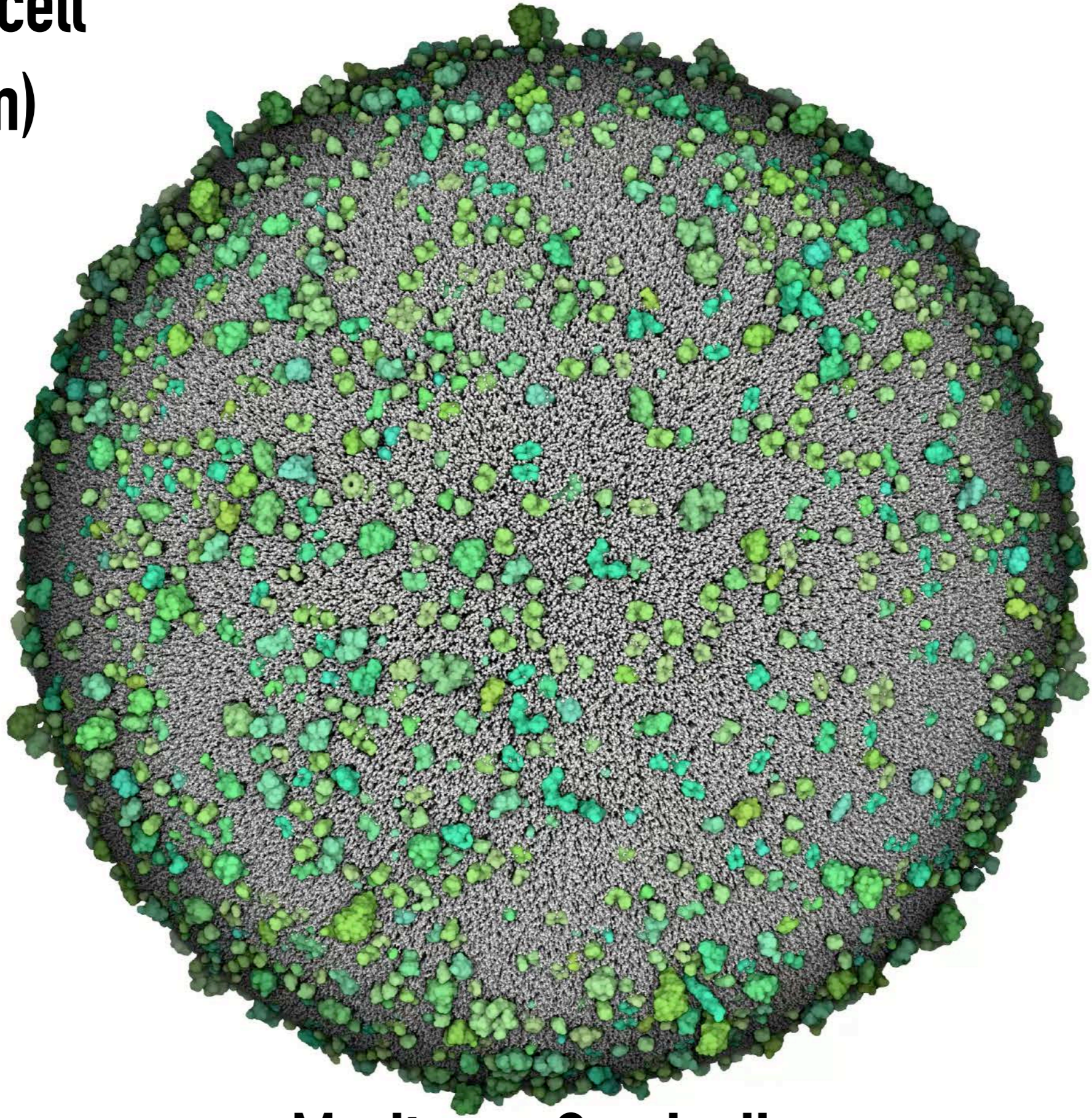
- ▶ **Part 1:** How do we see the invisible?
- ▶ **Part 2:** What are the key molecular machines involved in maintaining healthy protein levels?
- ▶ **Part 3:** How can we design better cancer therapeutics by looking at molecular machines?

HOW SMALL IS “INVISIBLE”



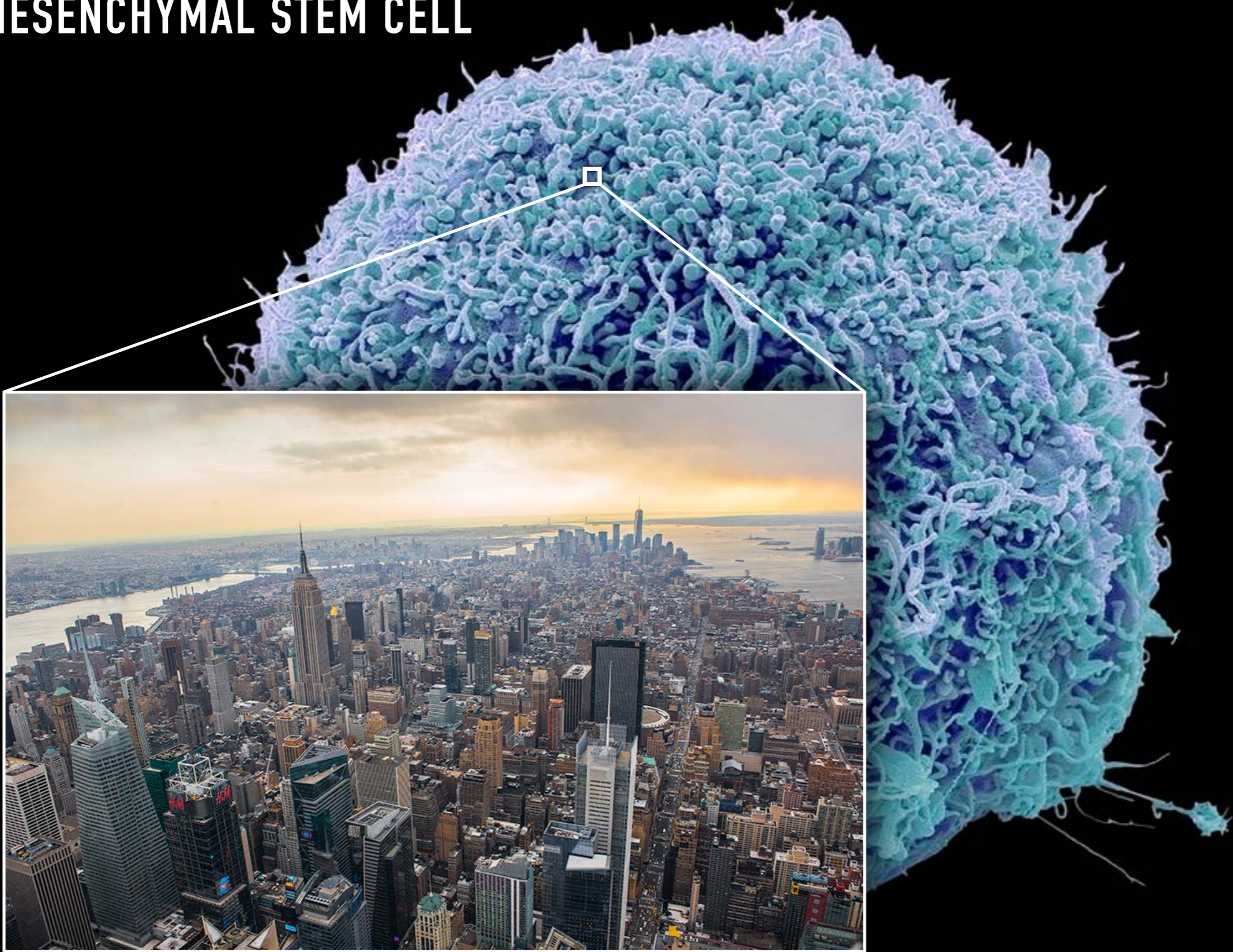
**objects 0.04 mm wide
(the width of a fine human hair)**

**Bacterial cell
(0.002 mm)**



Maritan & Goodsell

MESENCHYMAL STEM CELL



MANHATTAN, POPULATION ~1.6 MILLION

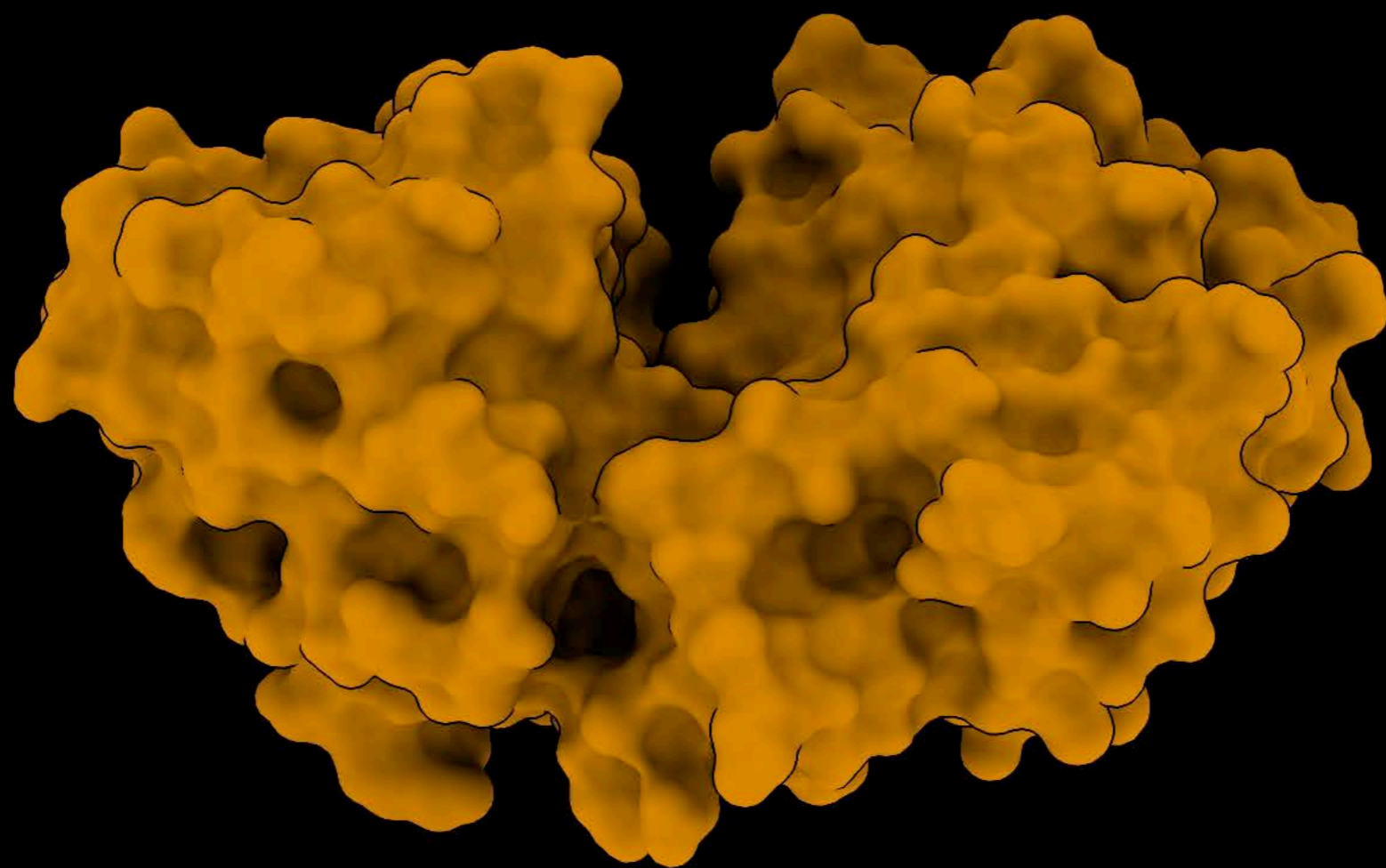


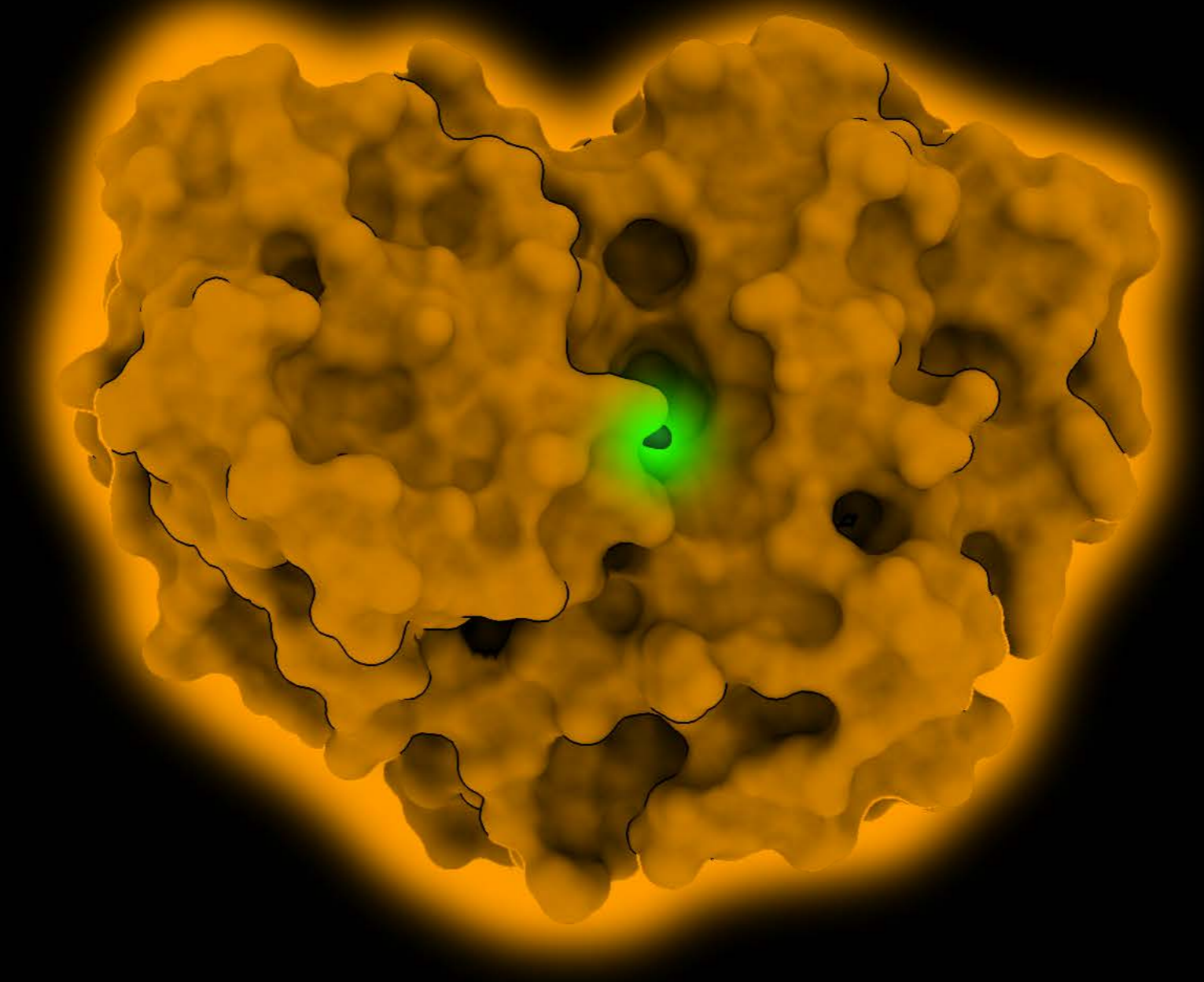
HOW DO WE LOOK AT SOMETHING
FORTY THOUSAND
TIMES SMALLER THAN A HAIR?

OPTION 1:

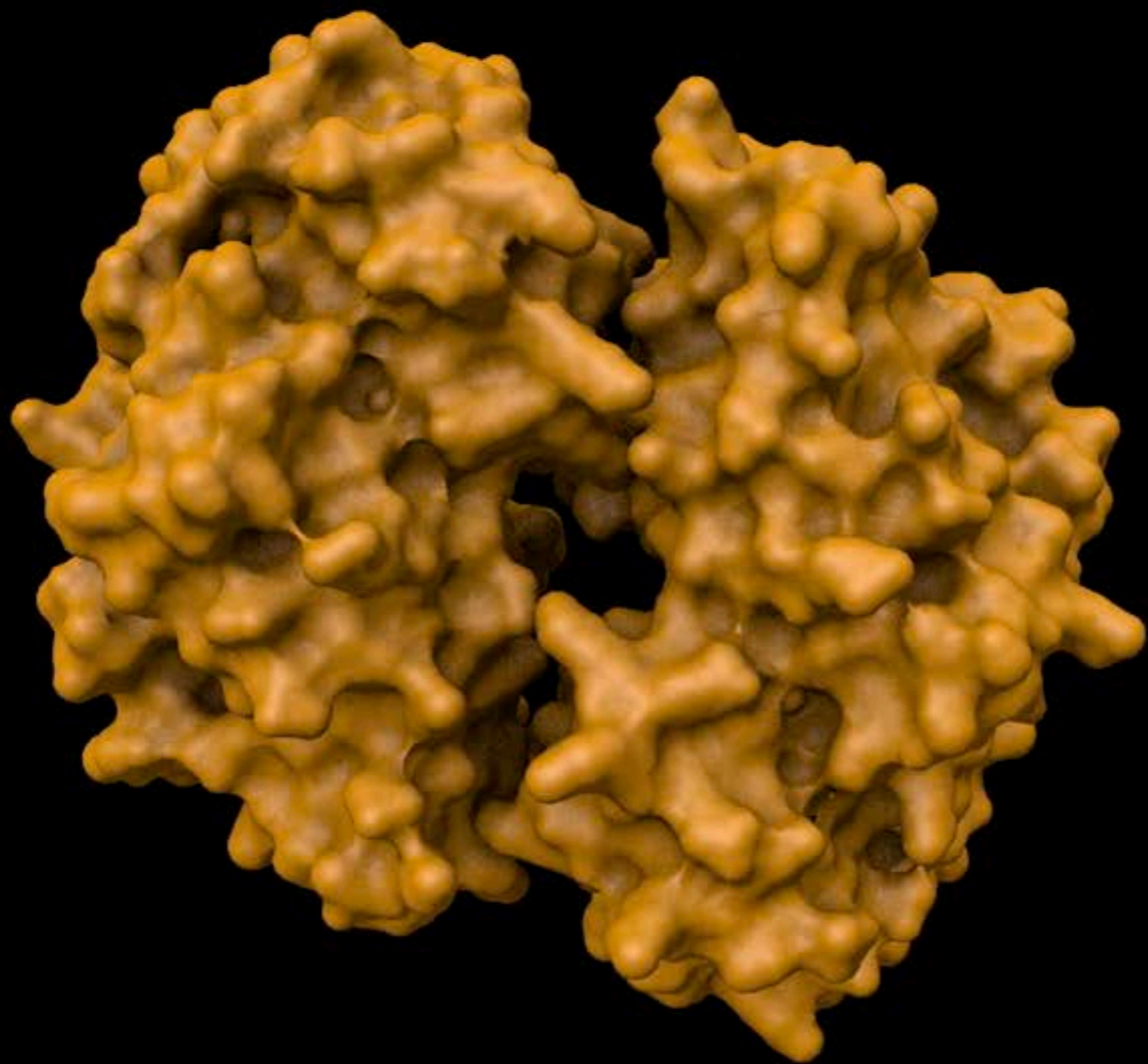
CRYSTALLOGRAPHY



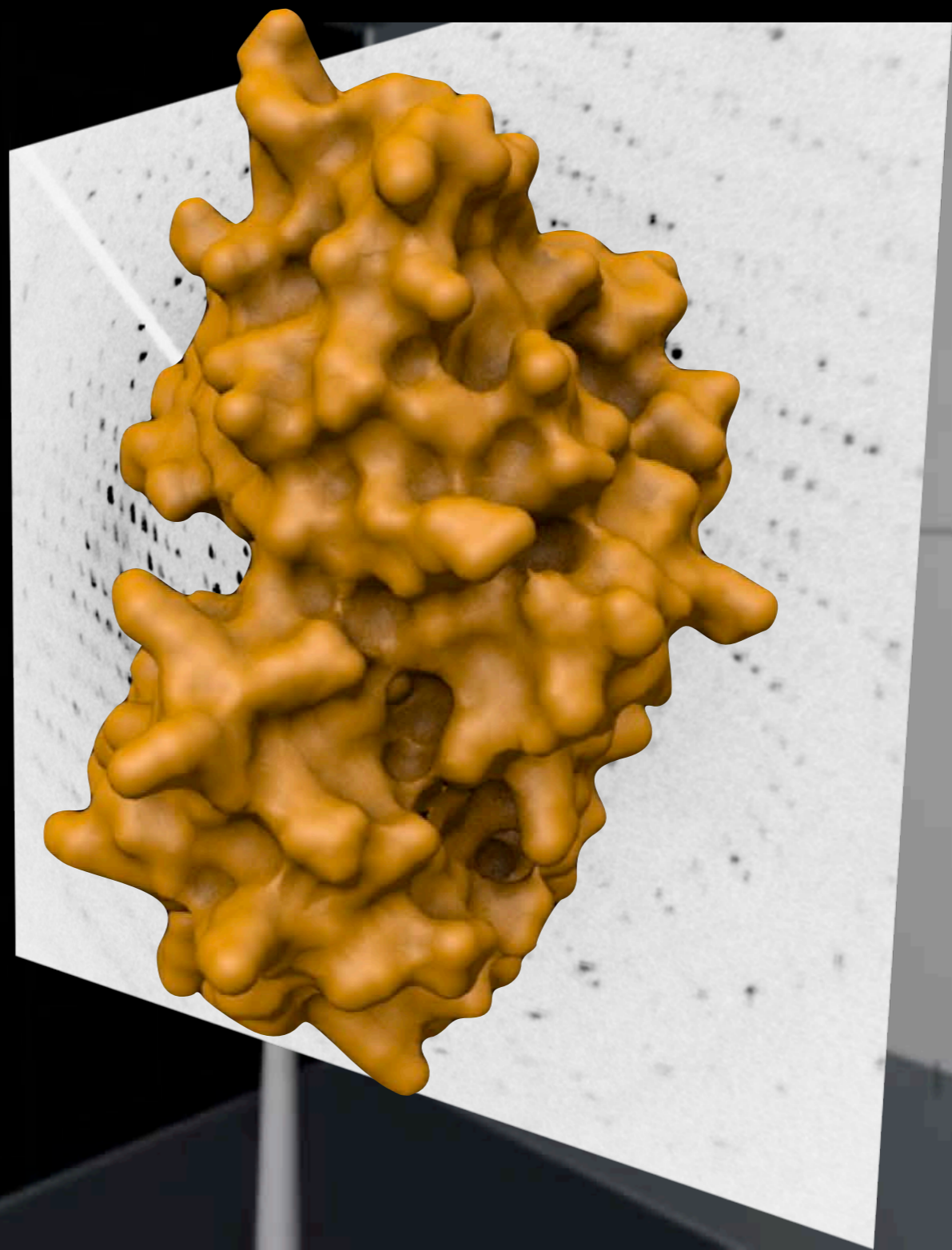










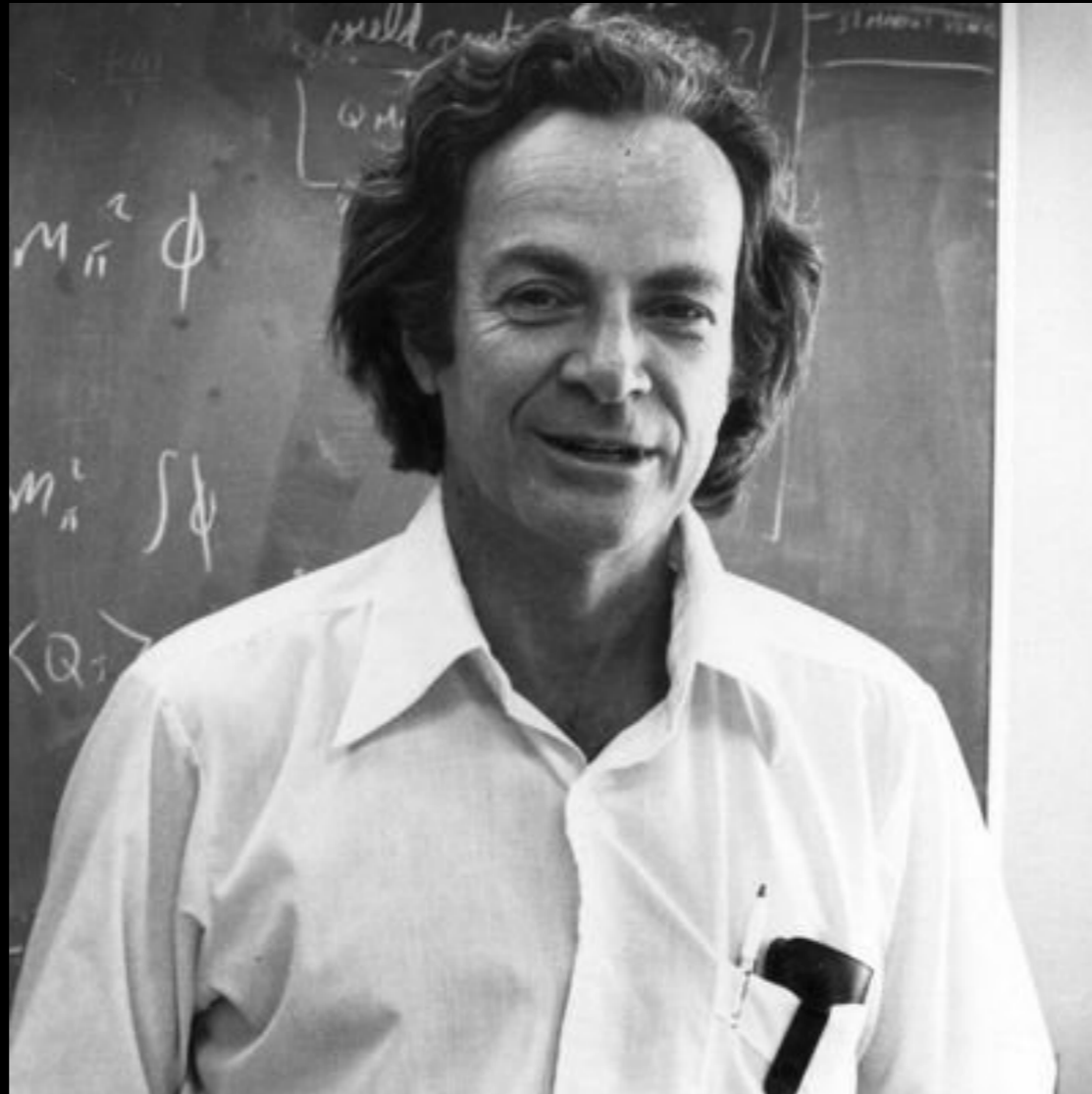




VS.



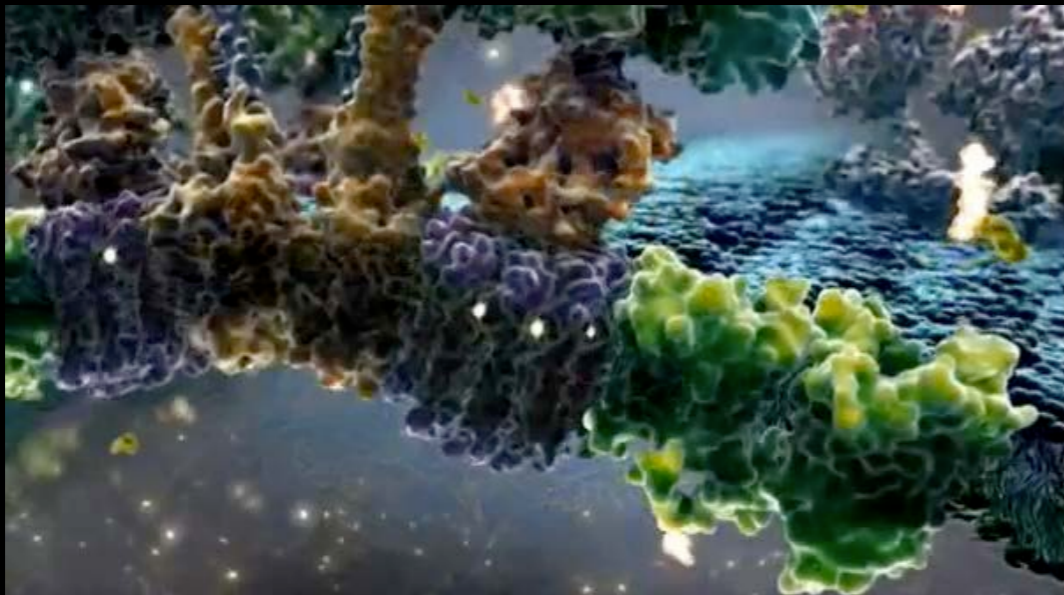
Richard P. Feynman's Solution



"It is very easy to answer many of these fundamental biological questions; you just look at the thing!
Make the microscope one hundred times more powerful,
and many problems of biology would be made very much easier."

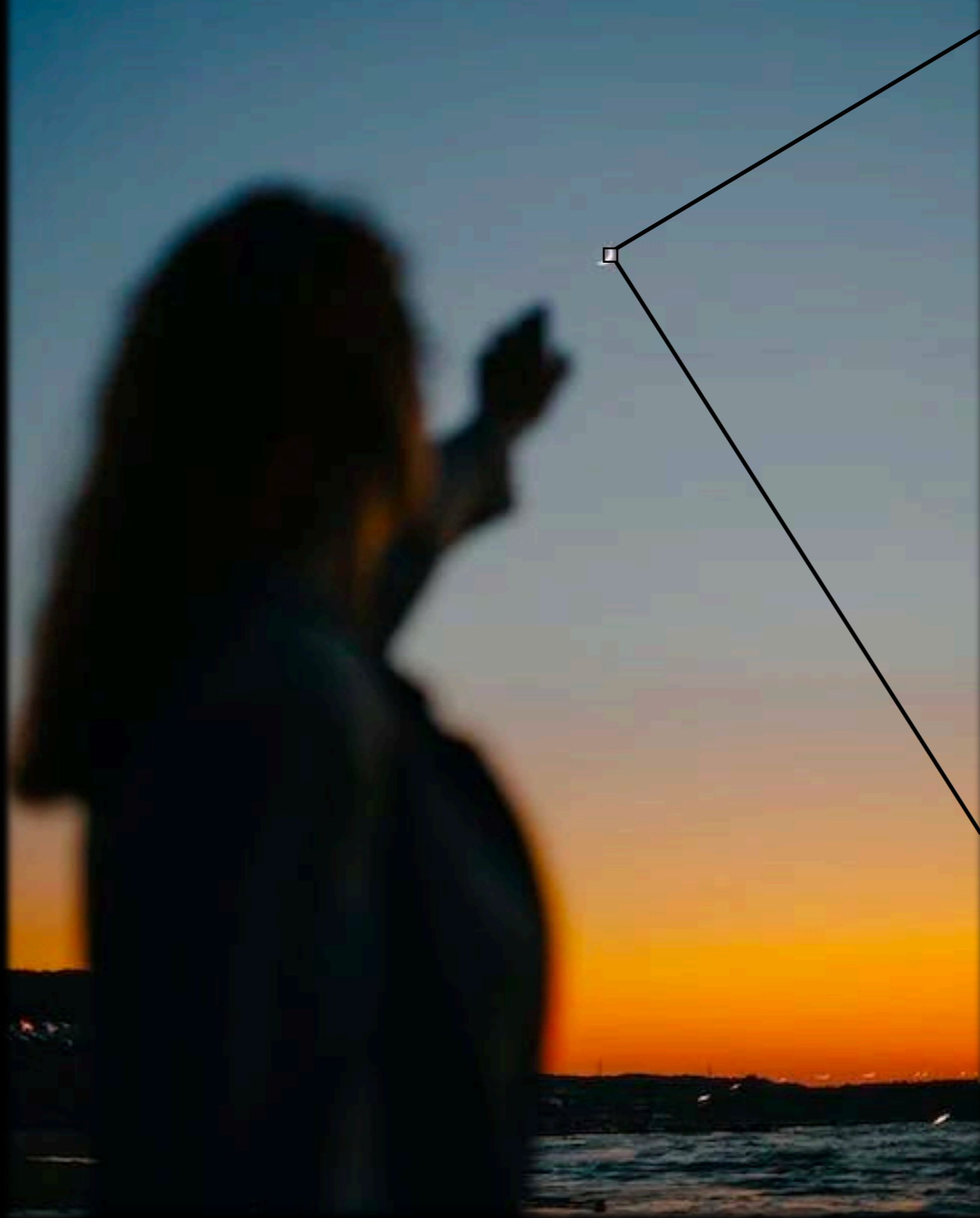
"There's Plenty of Room at the Bottom", a lecture given to the American Physical Society in 1959.

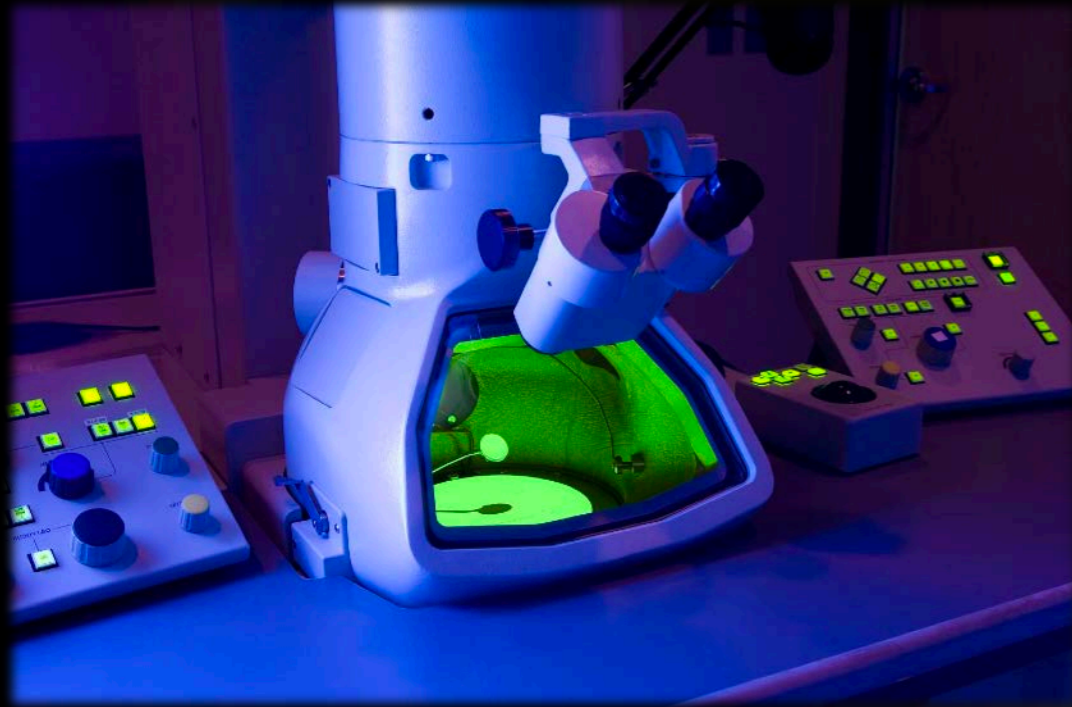
WHAT MAGNIFYING POWER DO WE NEED?



XVIVO medical animation

WHAT **MAGNIFYING** POWER DO WE NEED?

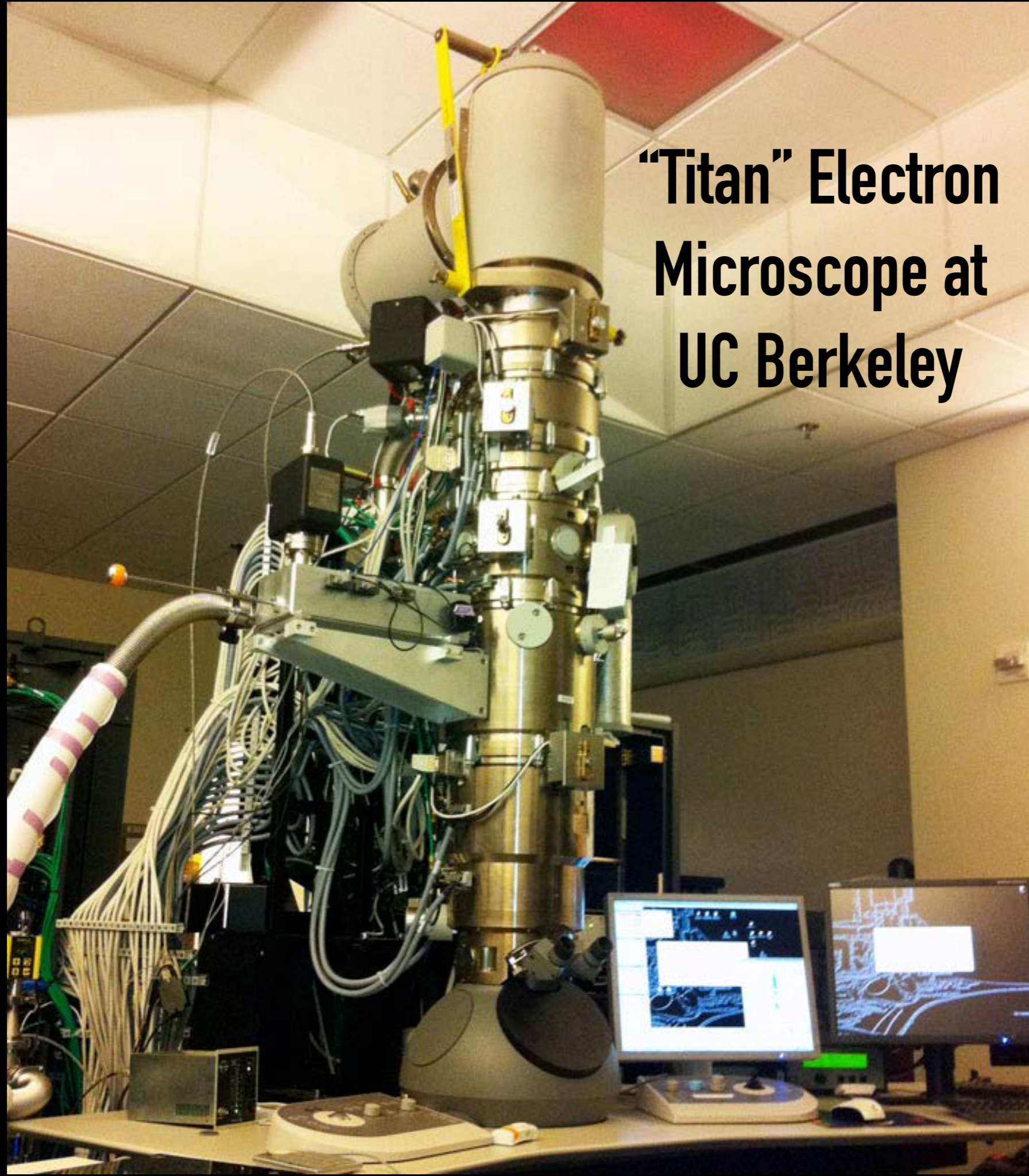




OPTION 2: CRYOGENIC ELECTRON MICROSCOPY (CRYO-EM)



“Titan” Electron Microscope at UC Berkeley

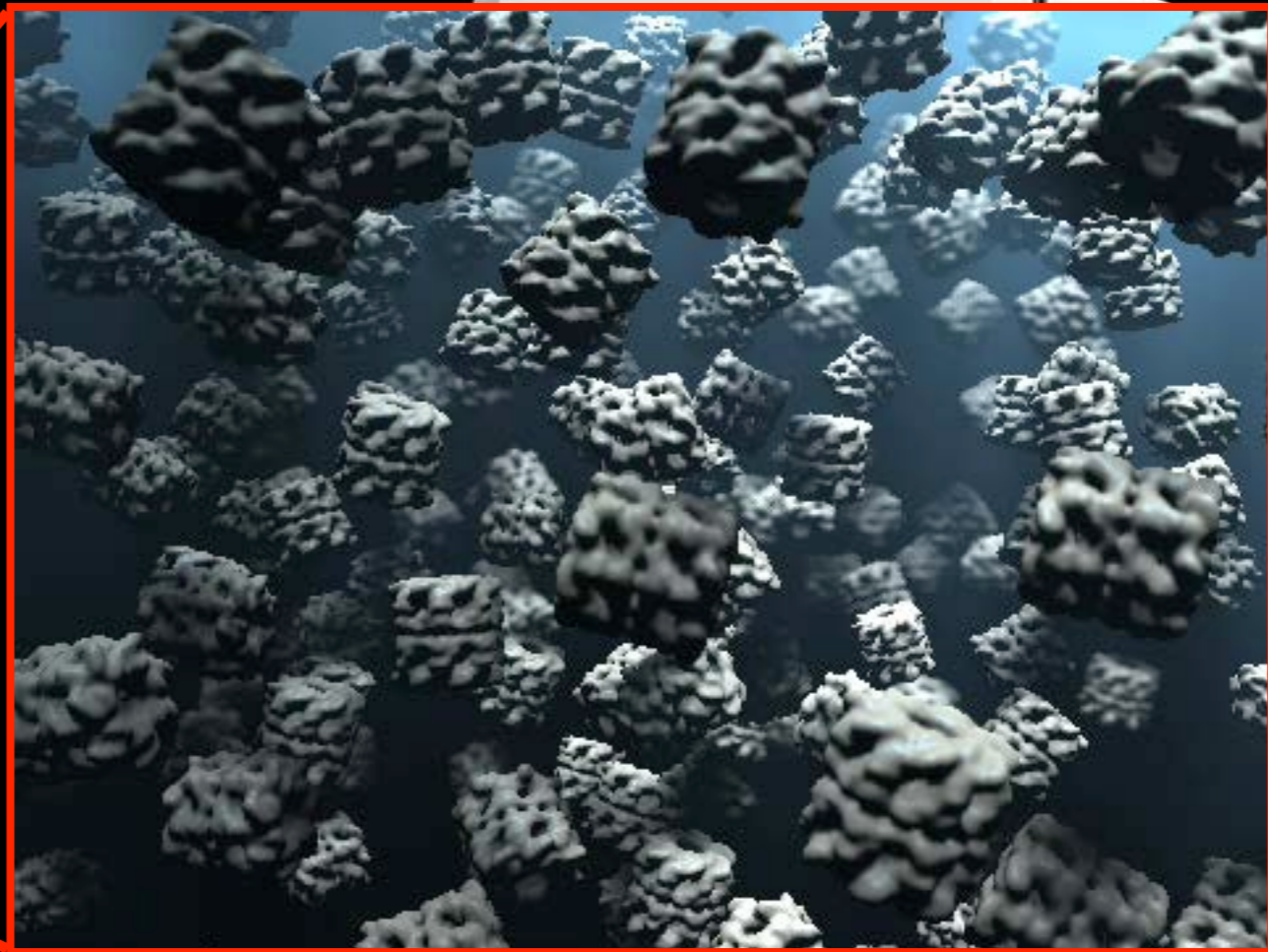


VISUALIZING MOLECULES AT THE
Scripps Research
ELECTRON
MICROSCOPY FACILITY





Protein sample



**3 millimeters
wide**



**3 microliters of sample
(0.000101442 fluid oz)**



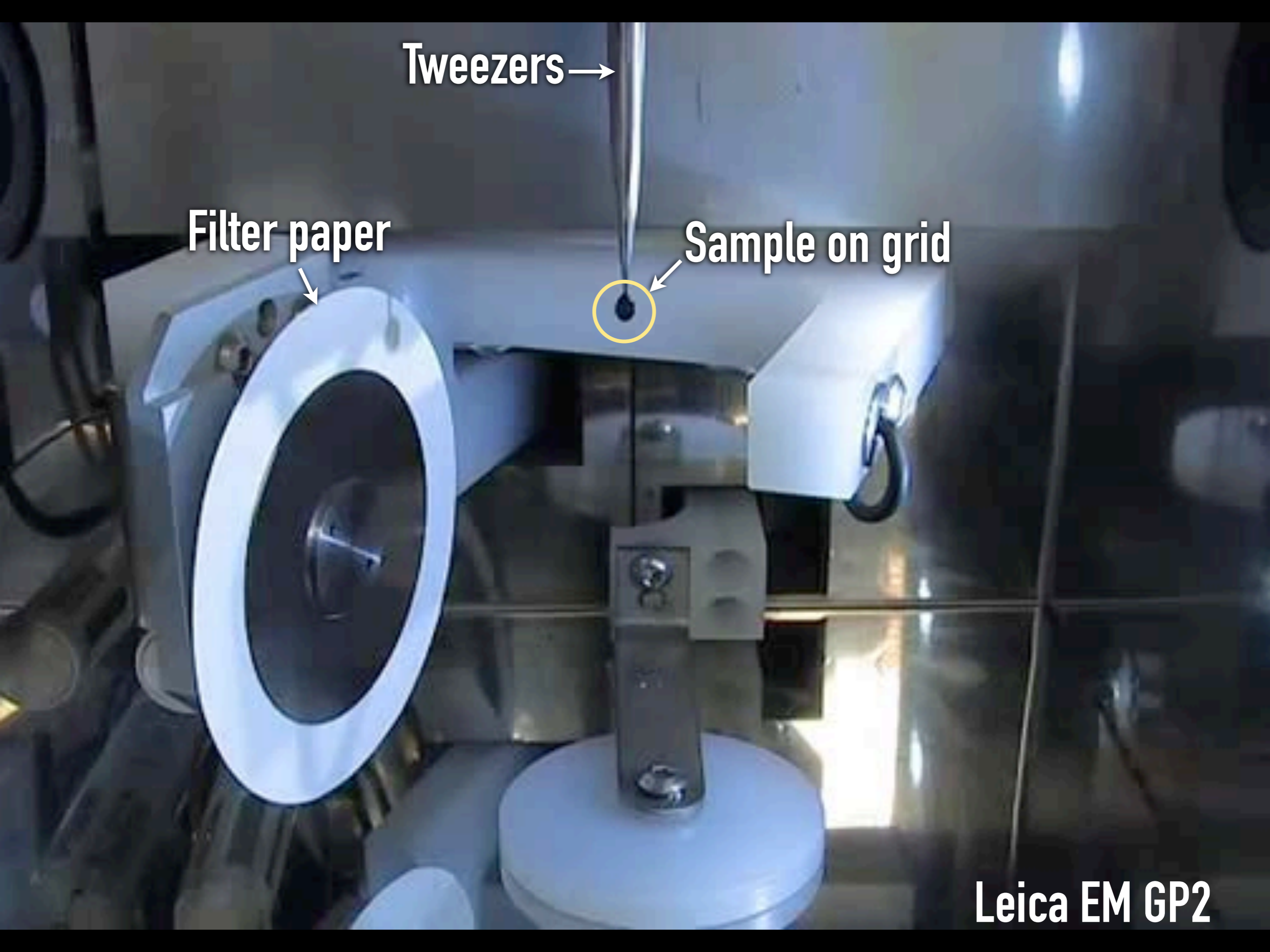
100,000 times too thick

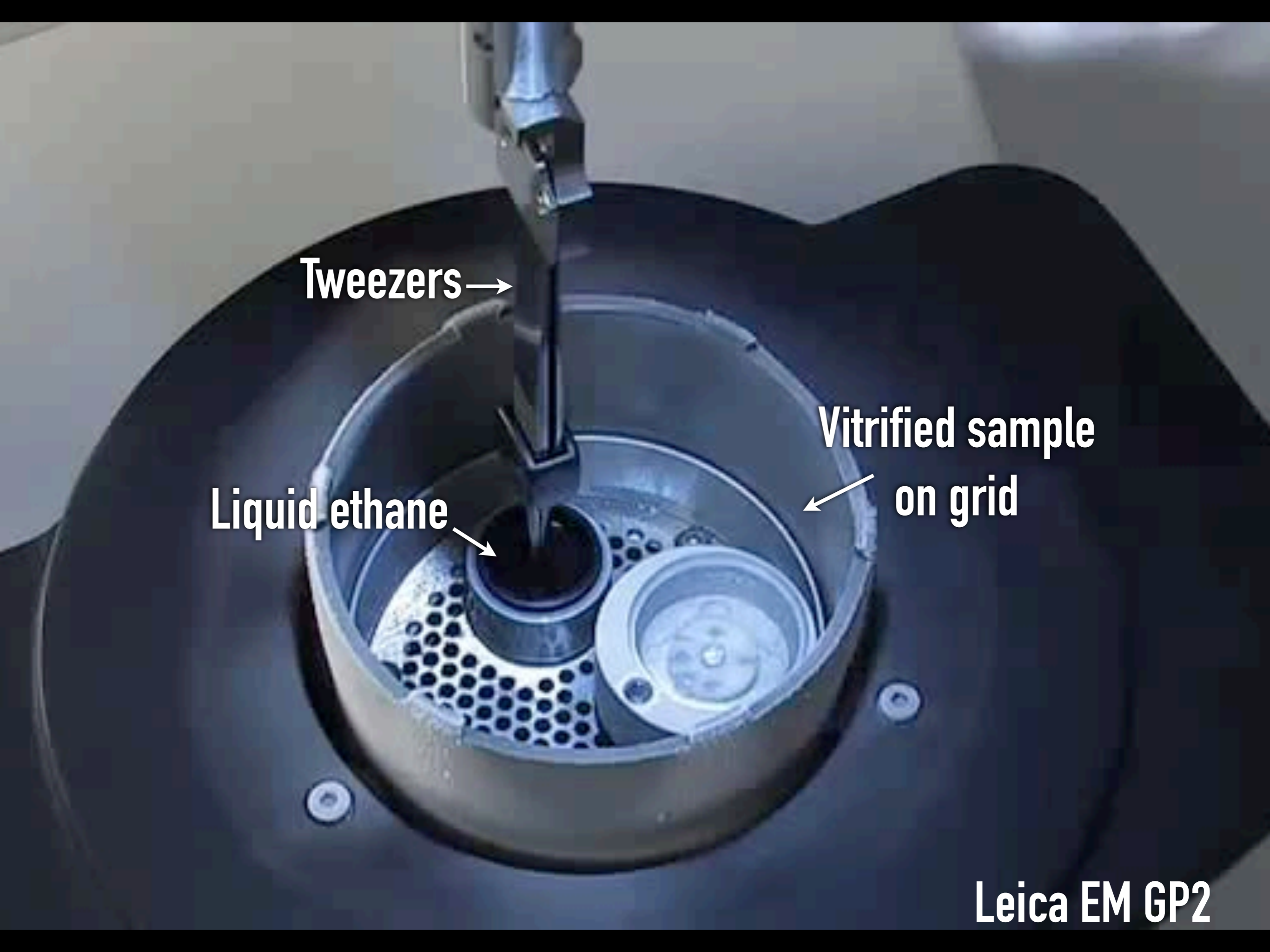
Tweezers →

Filter paper

Sample on grid

Leica EM GP2



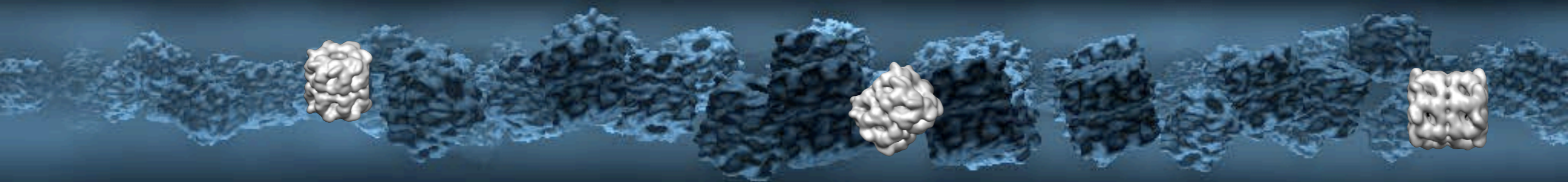
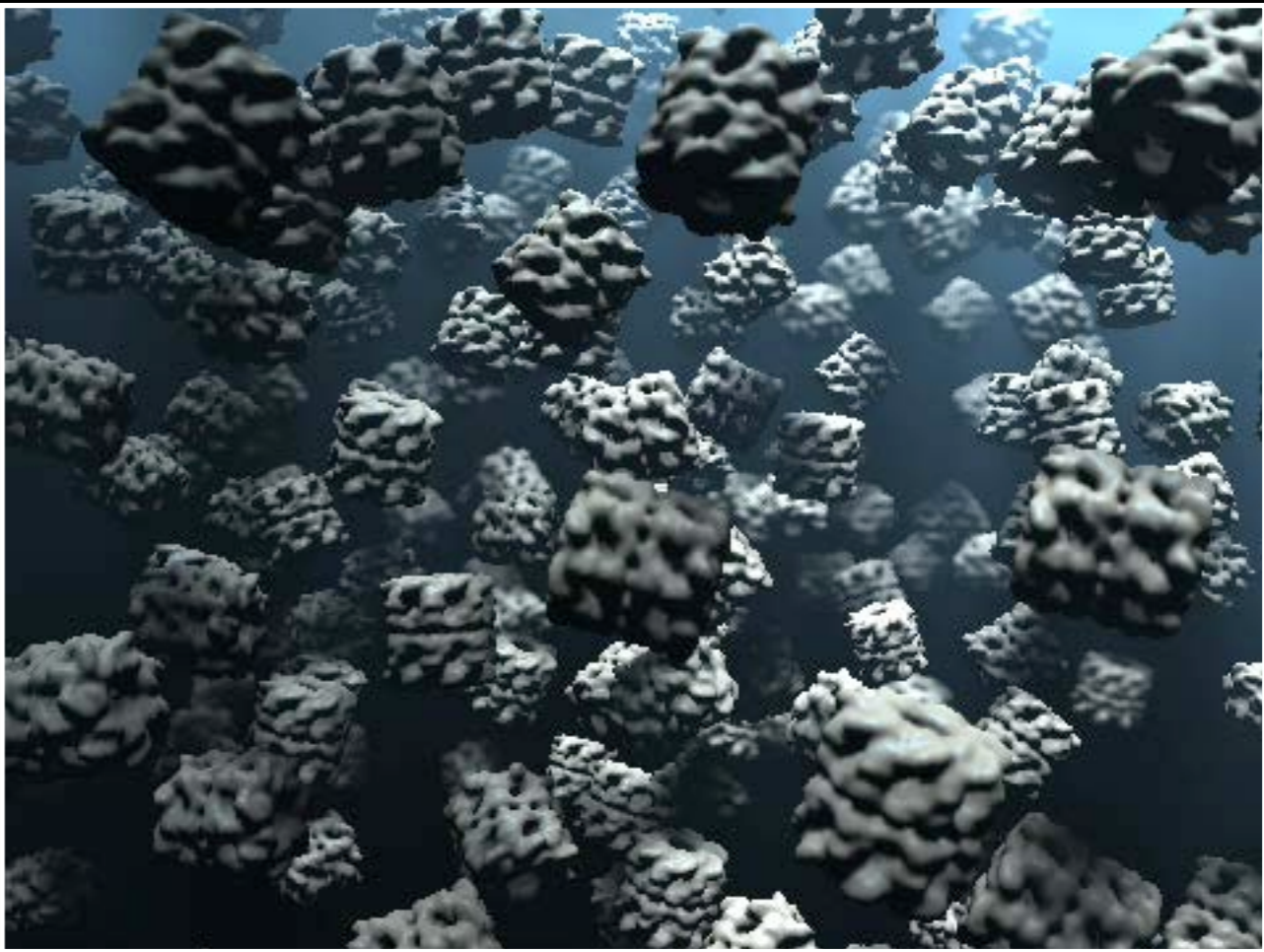
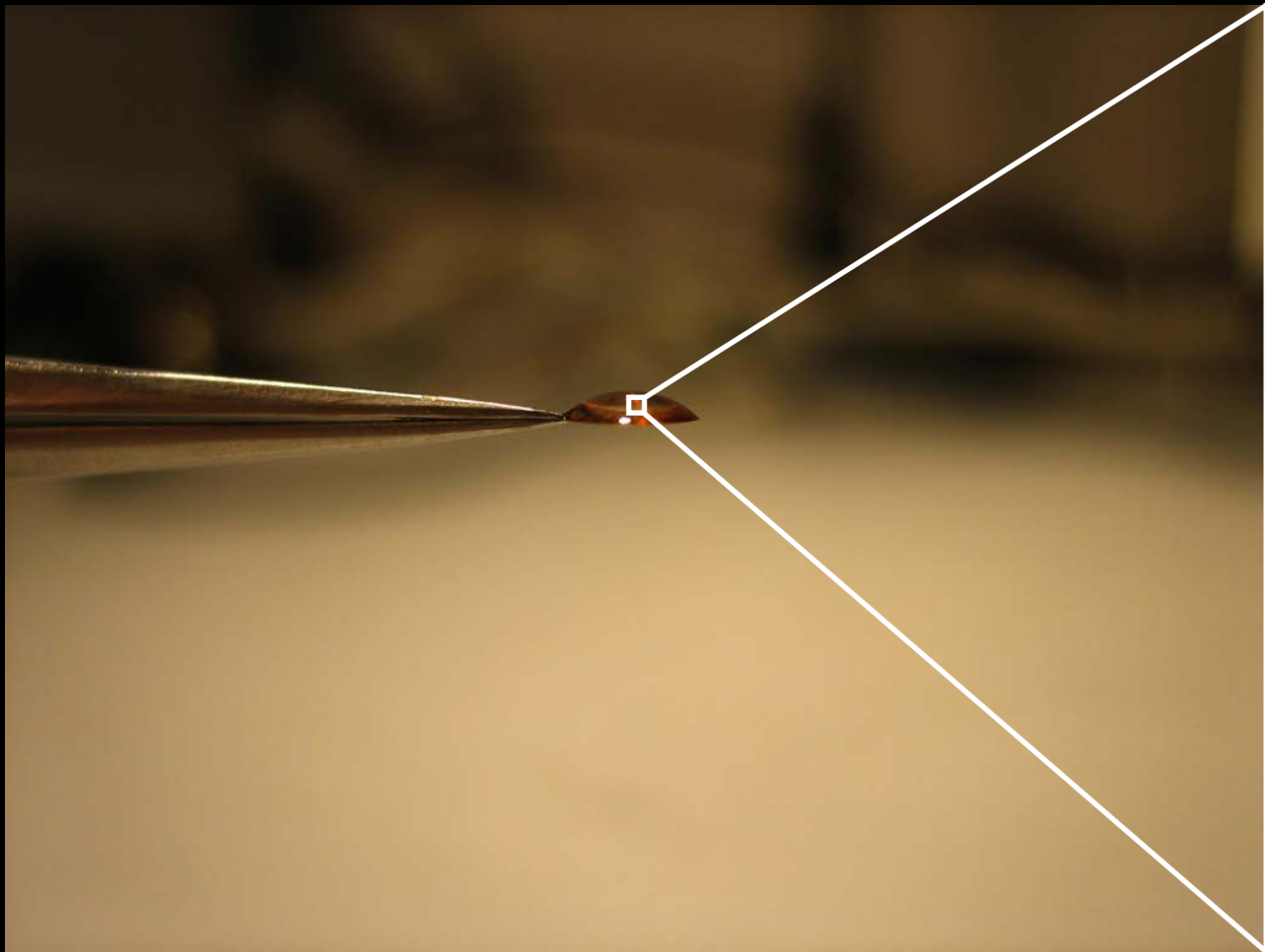


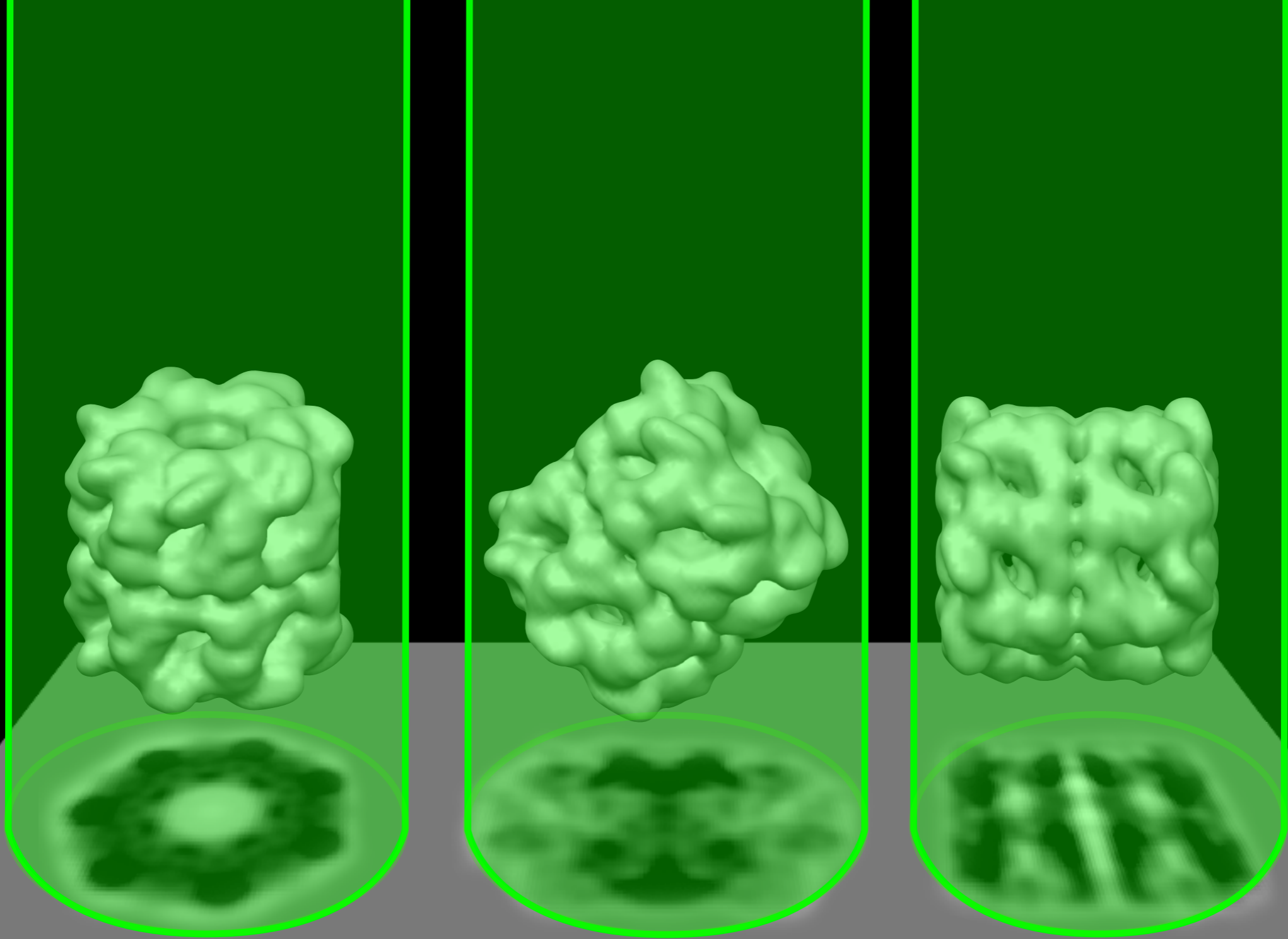
Tweezers →

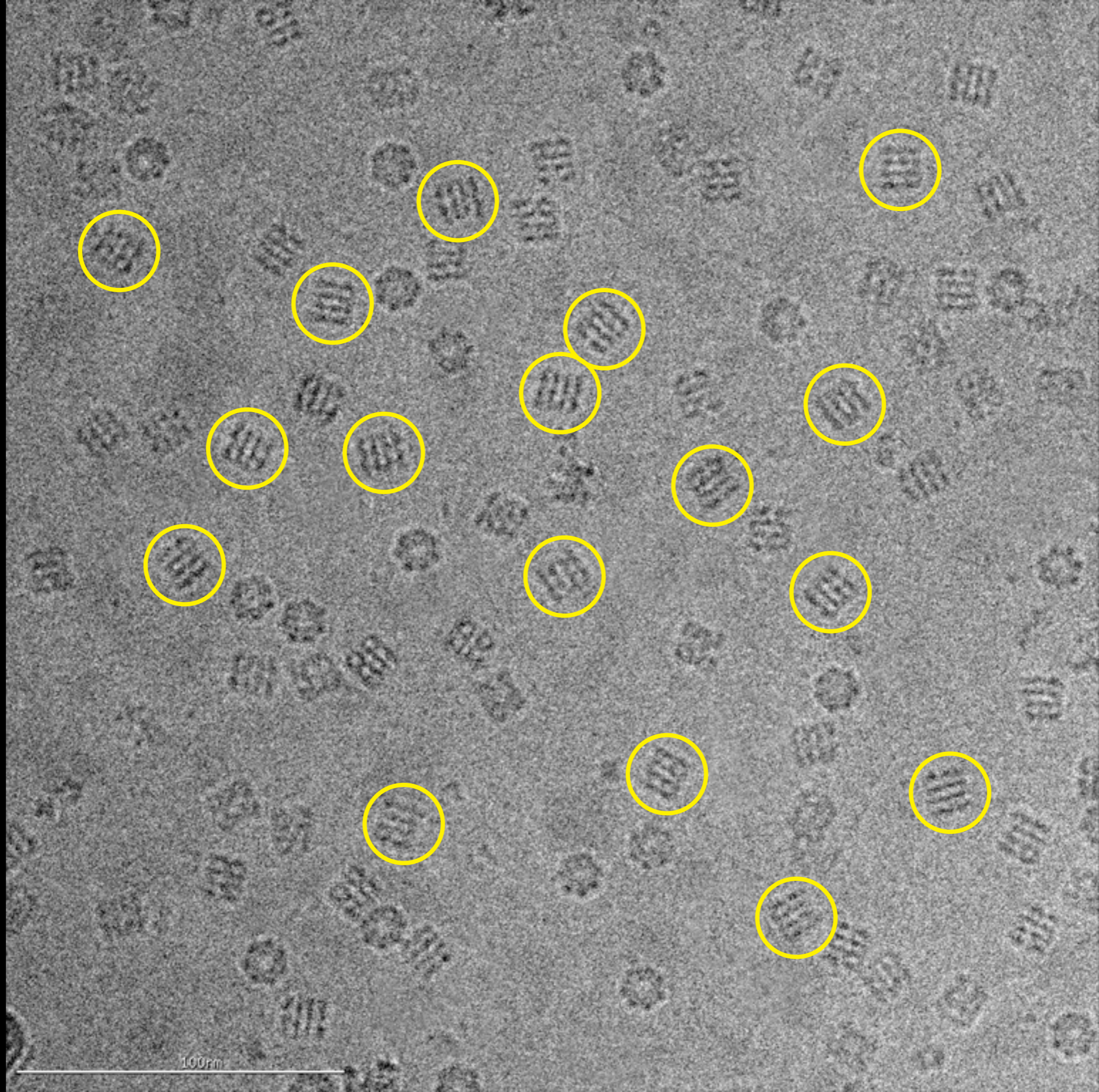
Liquid ethane →

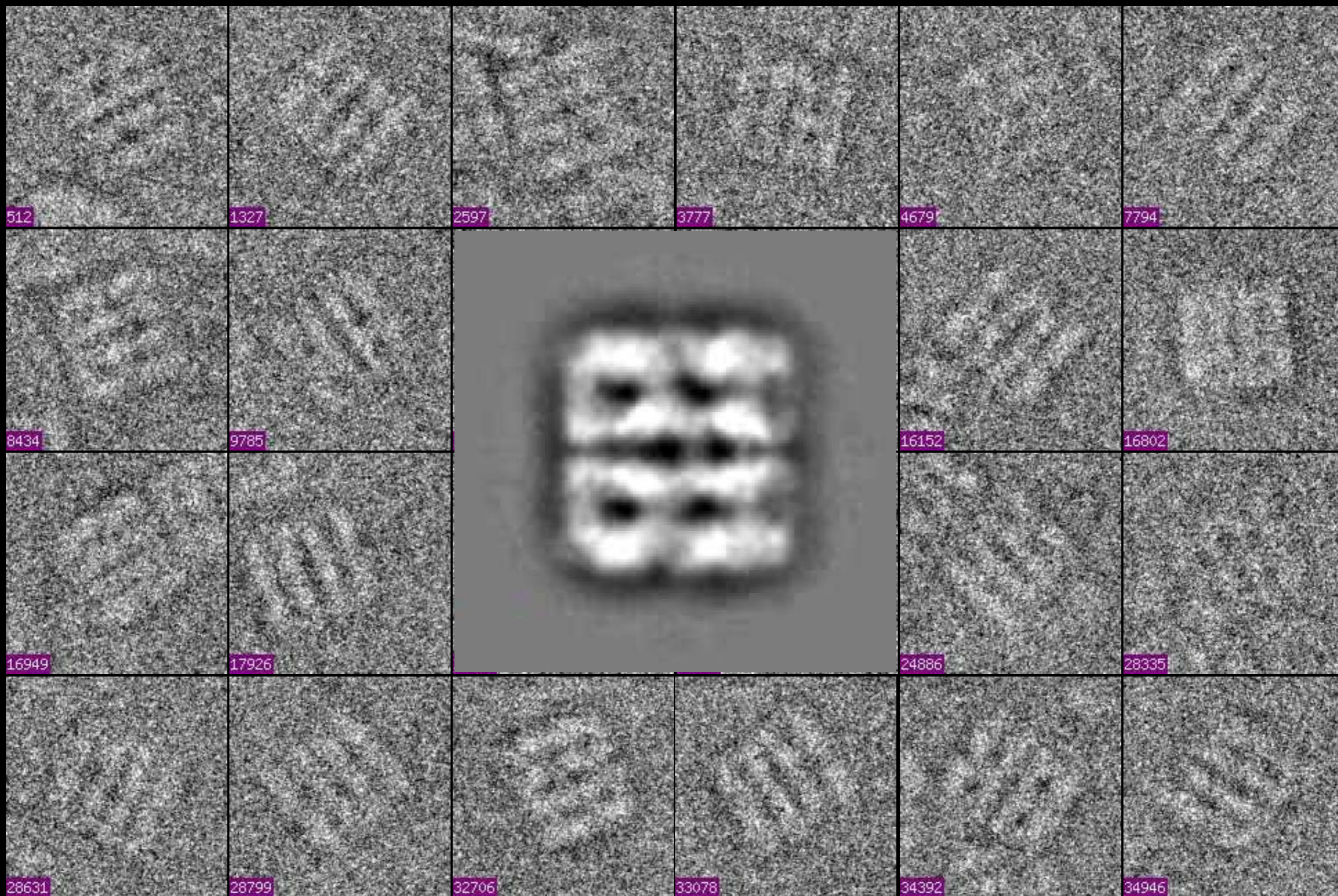
**Vitrified sample
on grid** ←

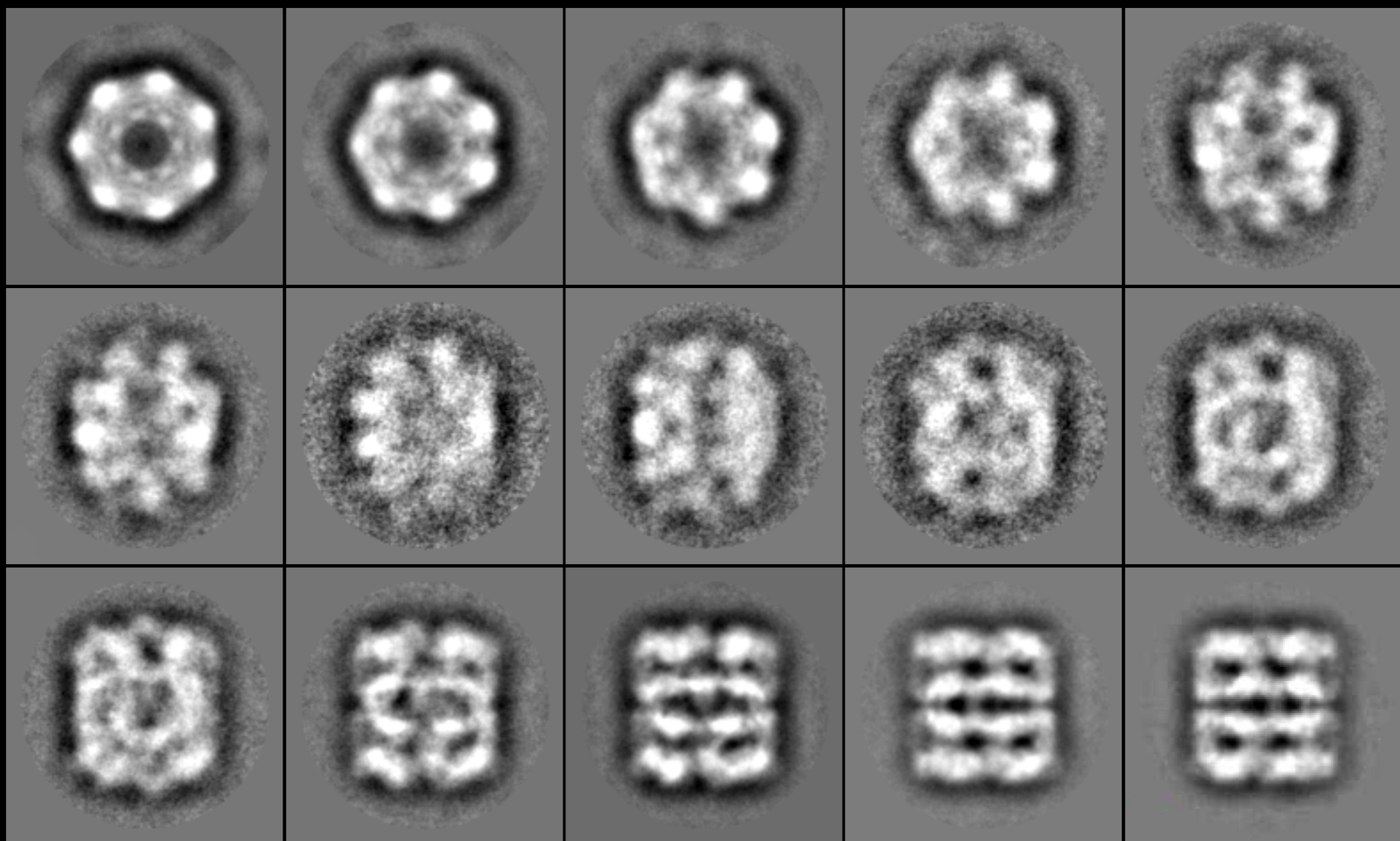
Leica EM GP2

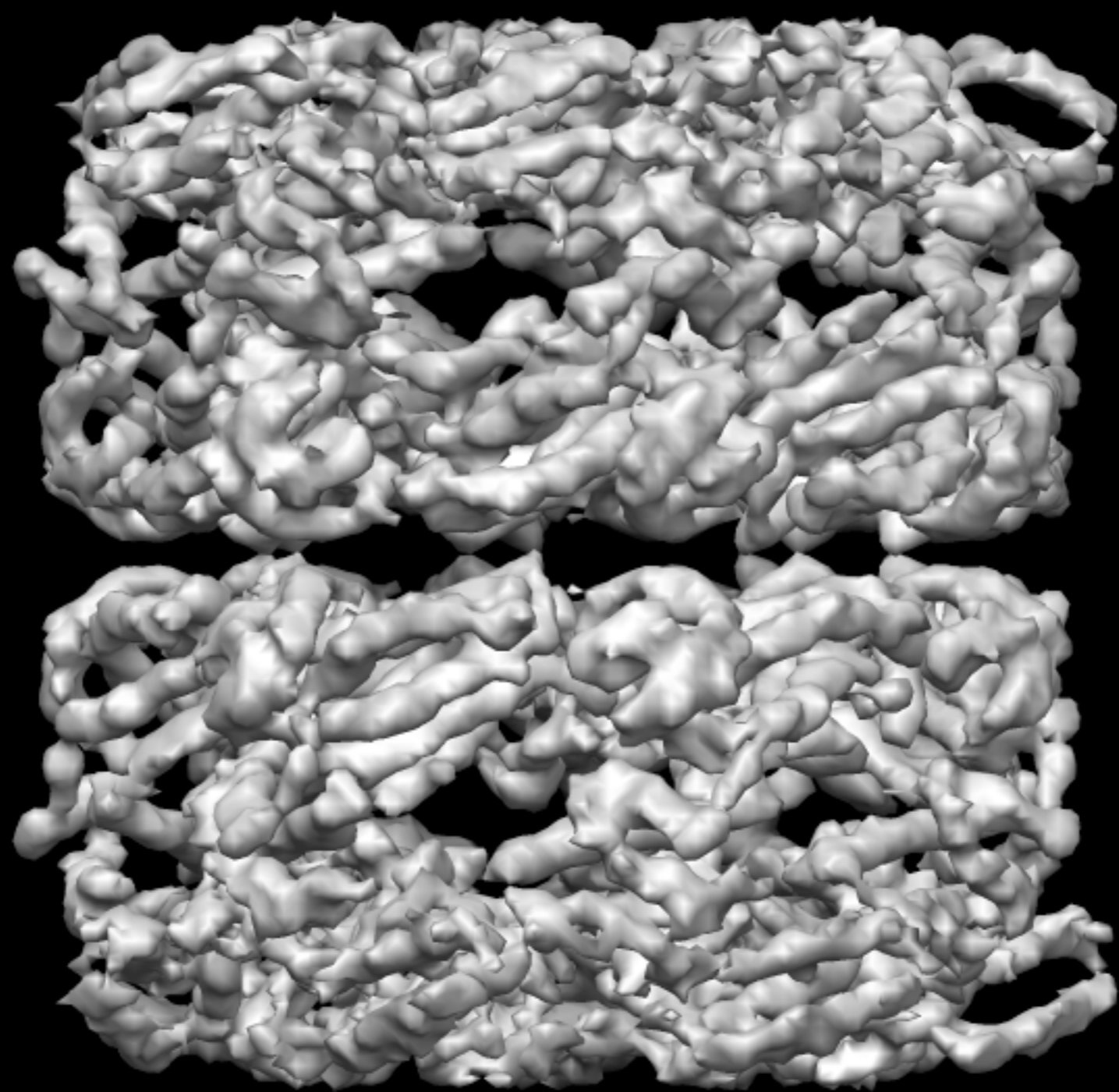












Current cryo-EM instrumentation and algorithms

Using 2009
instrumentation
& algorithms

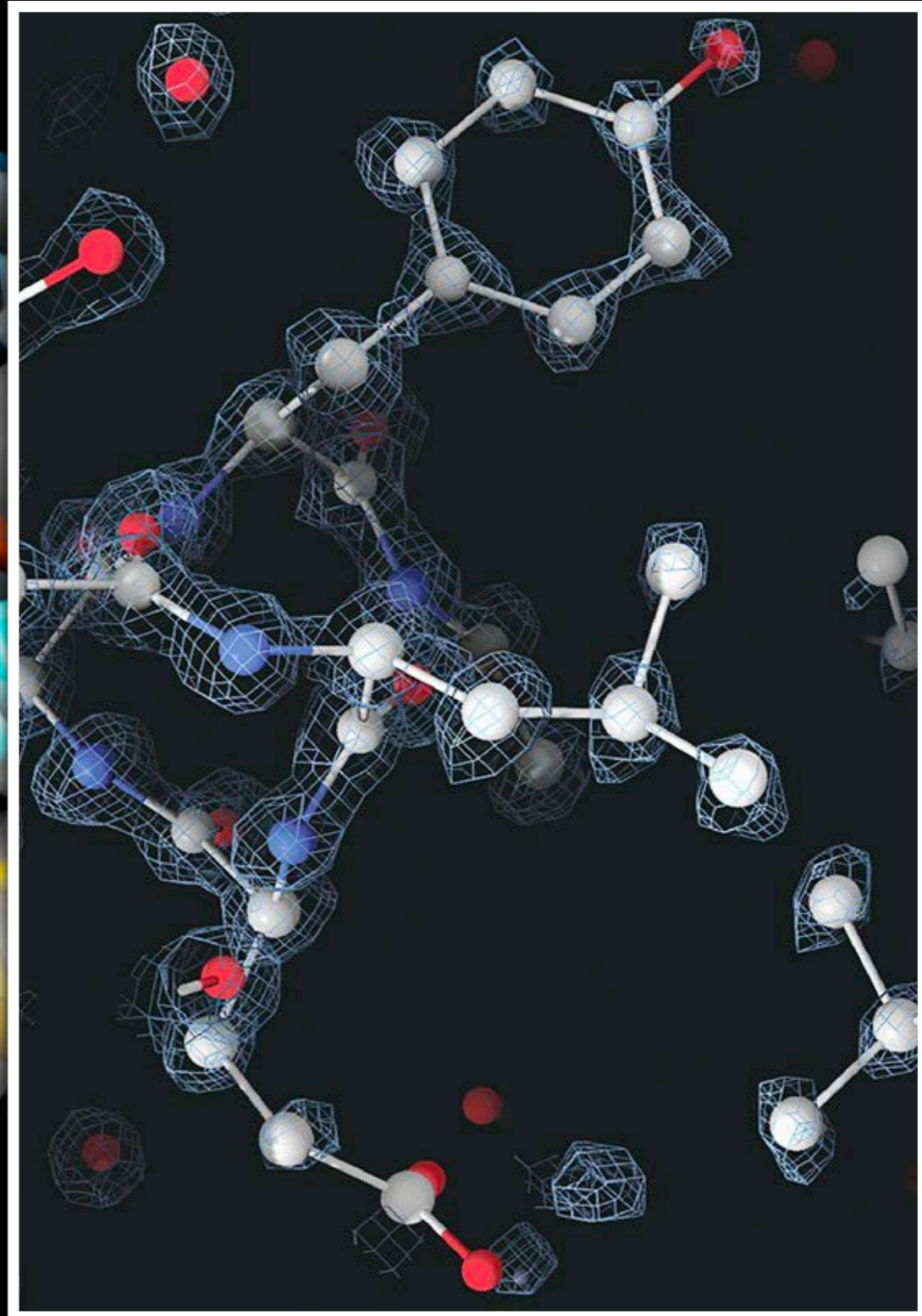
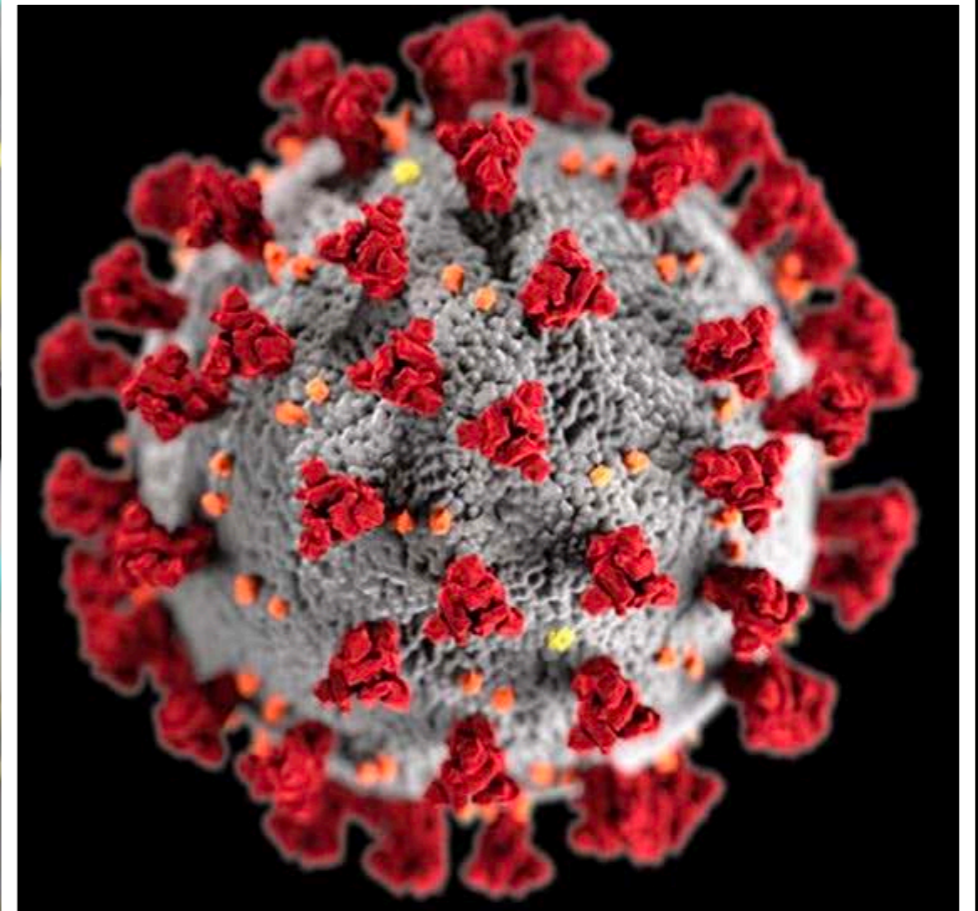


image from Herzik 2020

Current cryo-EM instrumentation and algorithms used to fight COVID-19

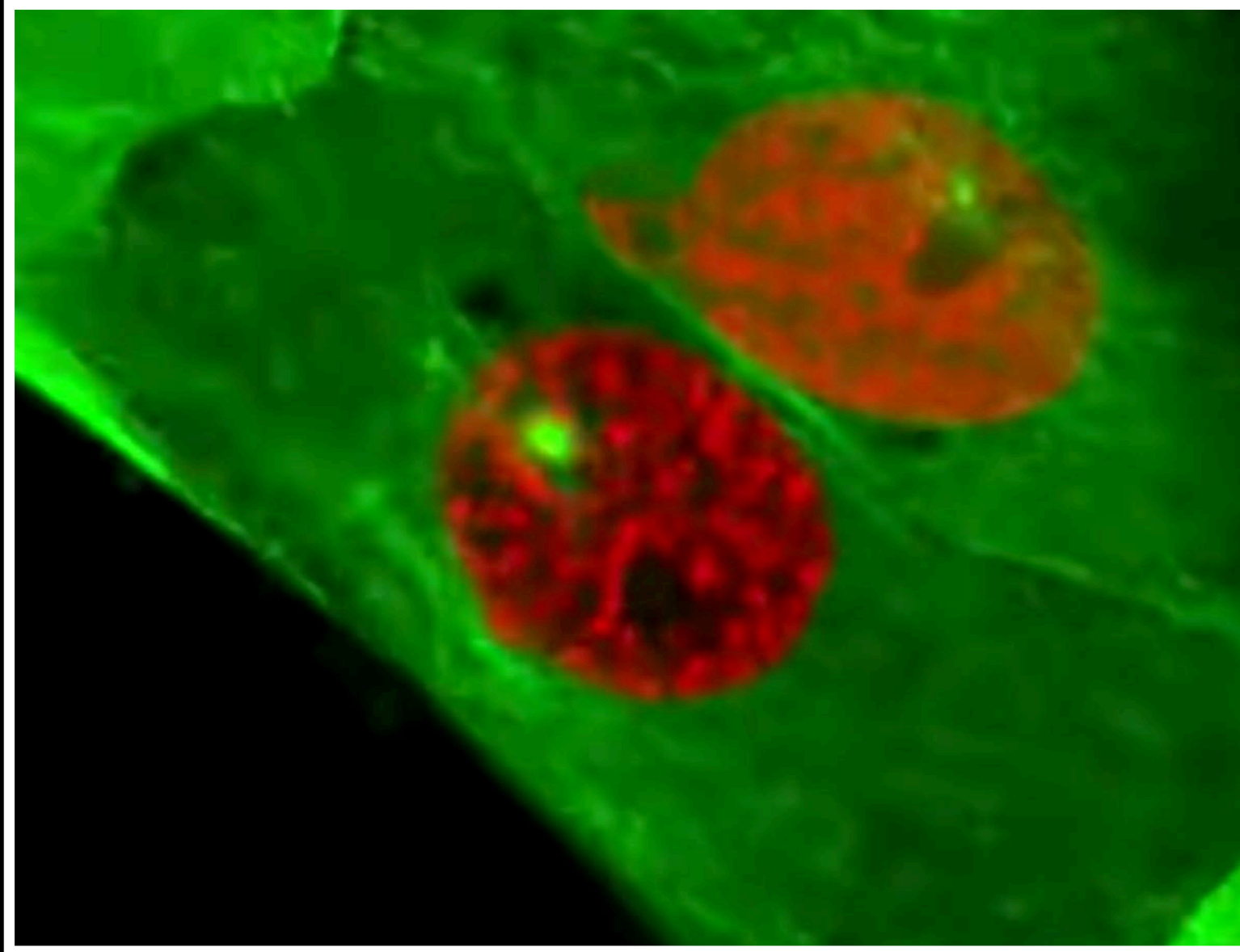


MESENCHYMAL STEM CELL

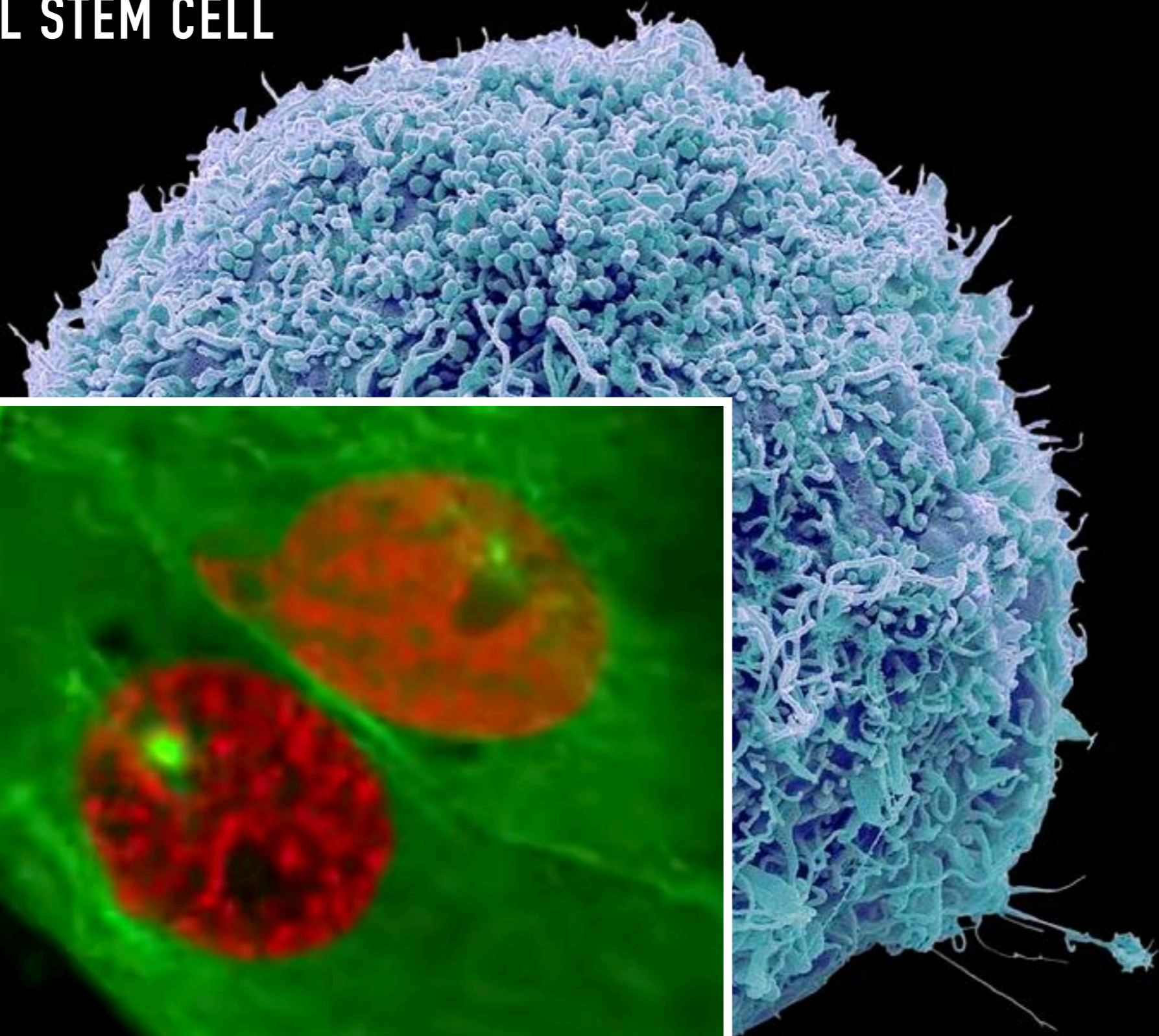


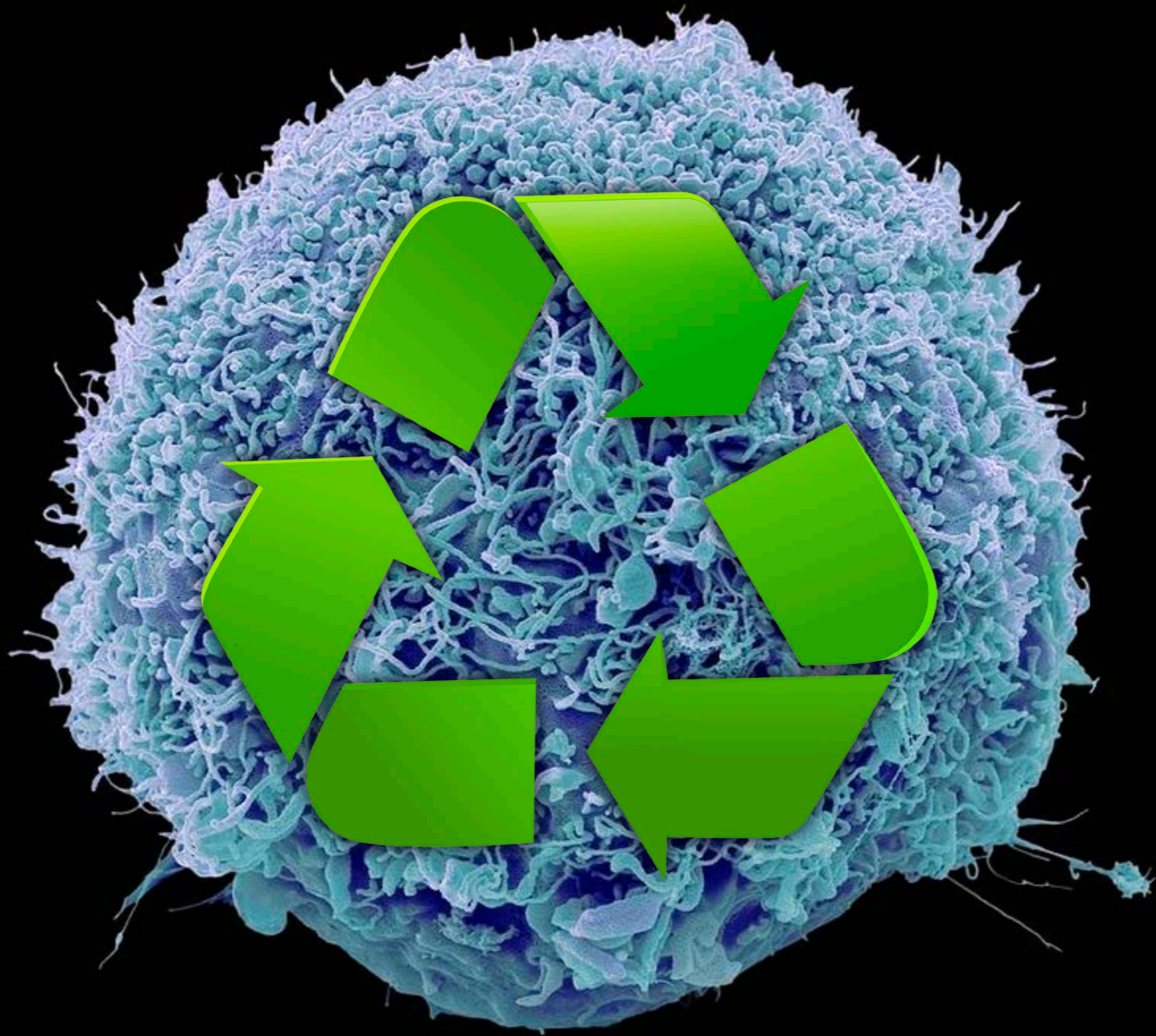
Evan Ingersoll & Gael McGill

MESENCHYMAL STEM CELL

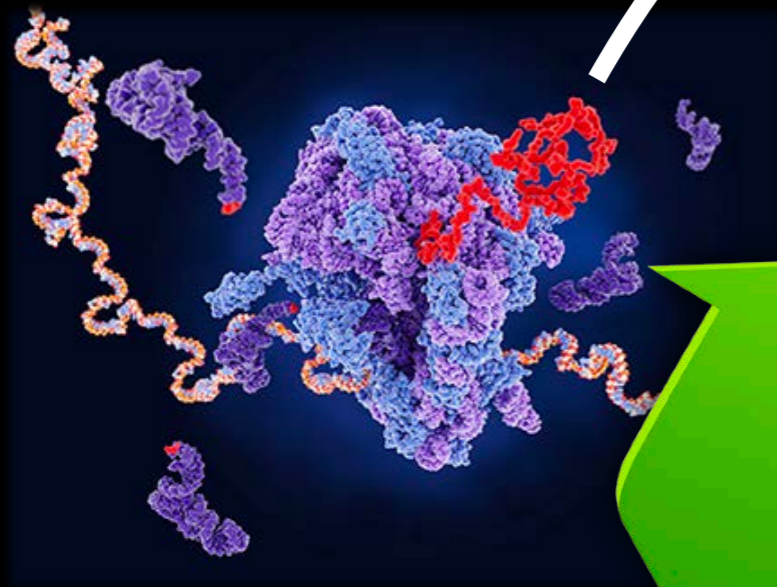
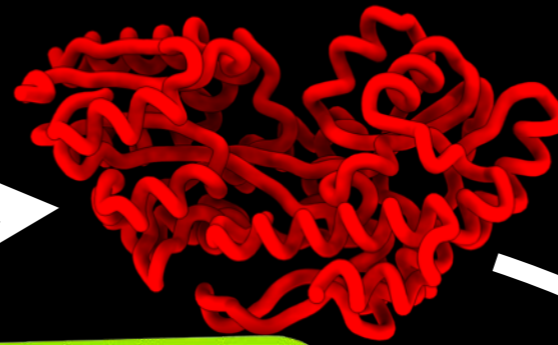


Dividing kidney cells

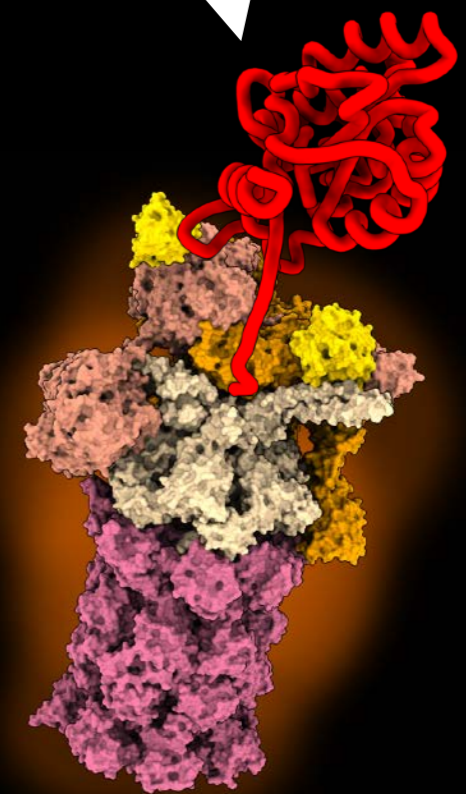




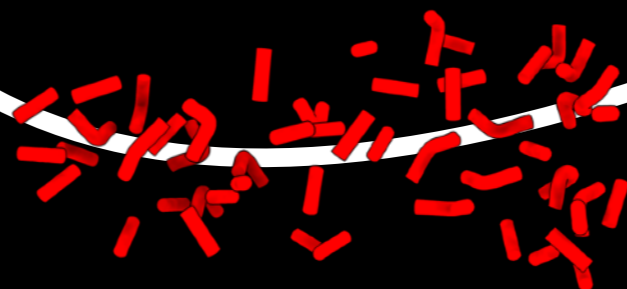
**Protein folding
and function**

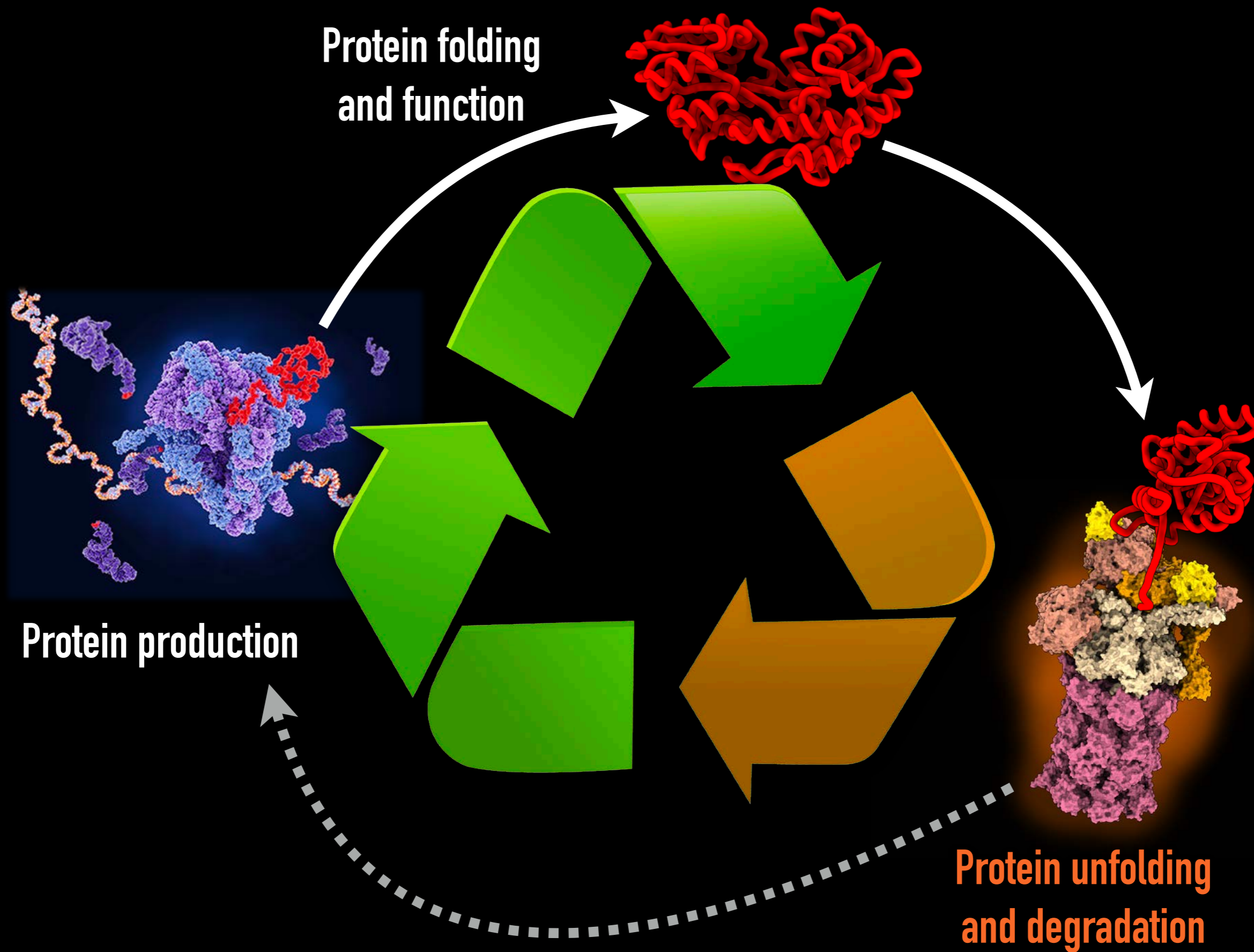


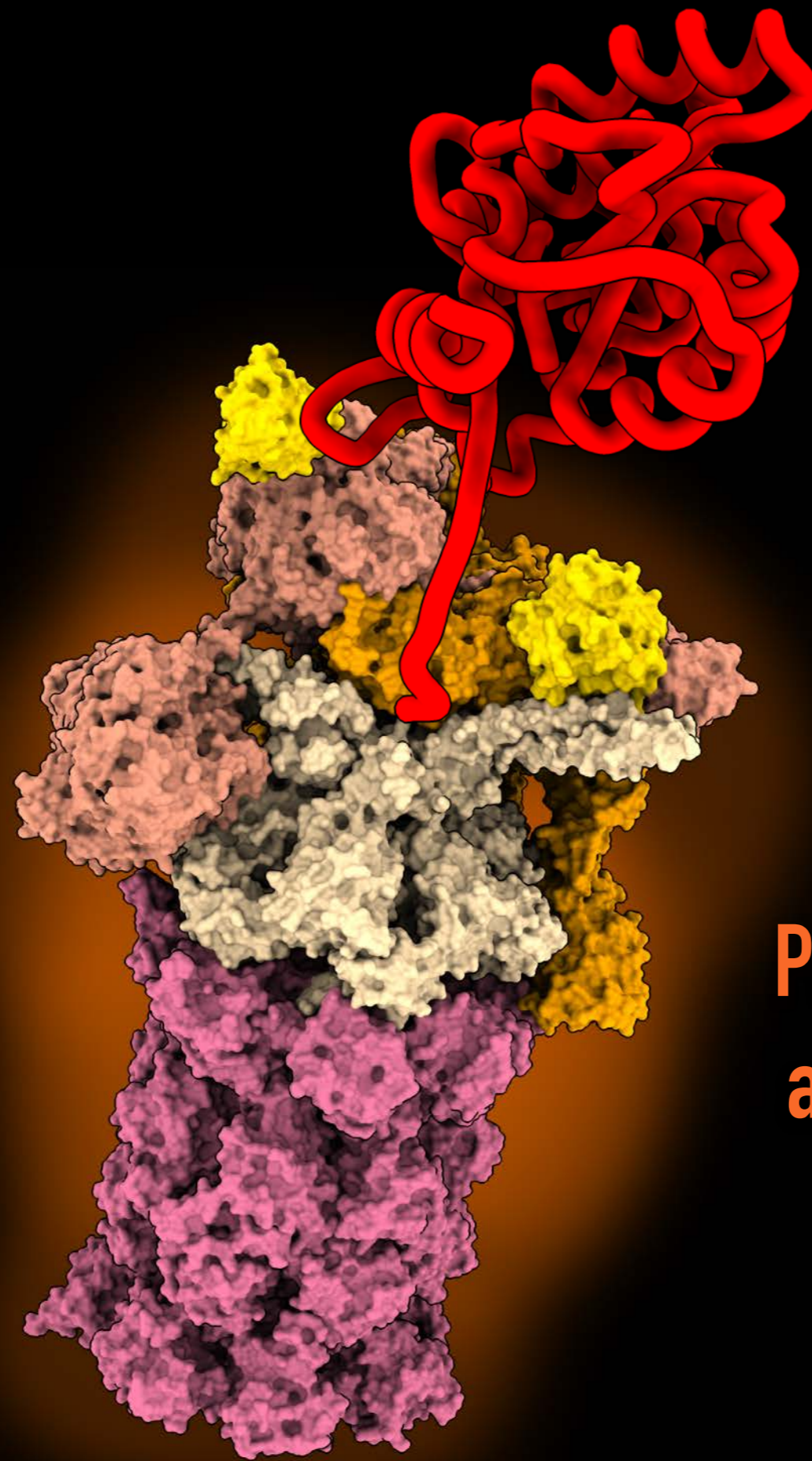
Protein production



**Protein unfolding
and degradation**

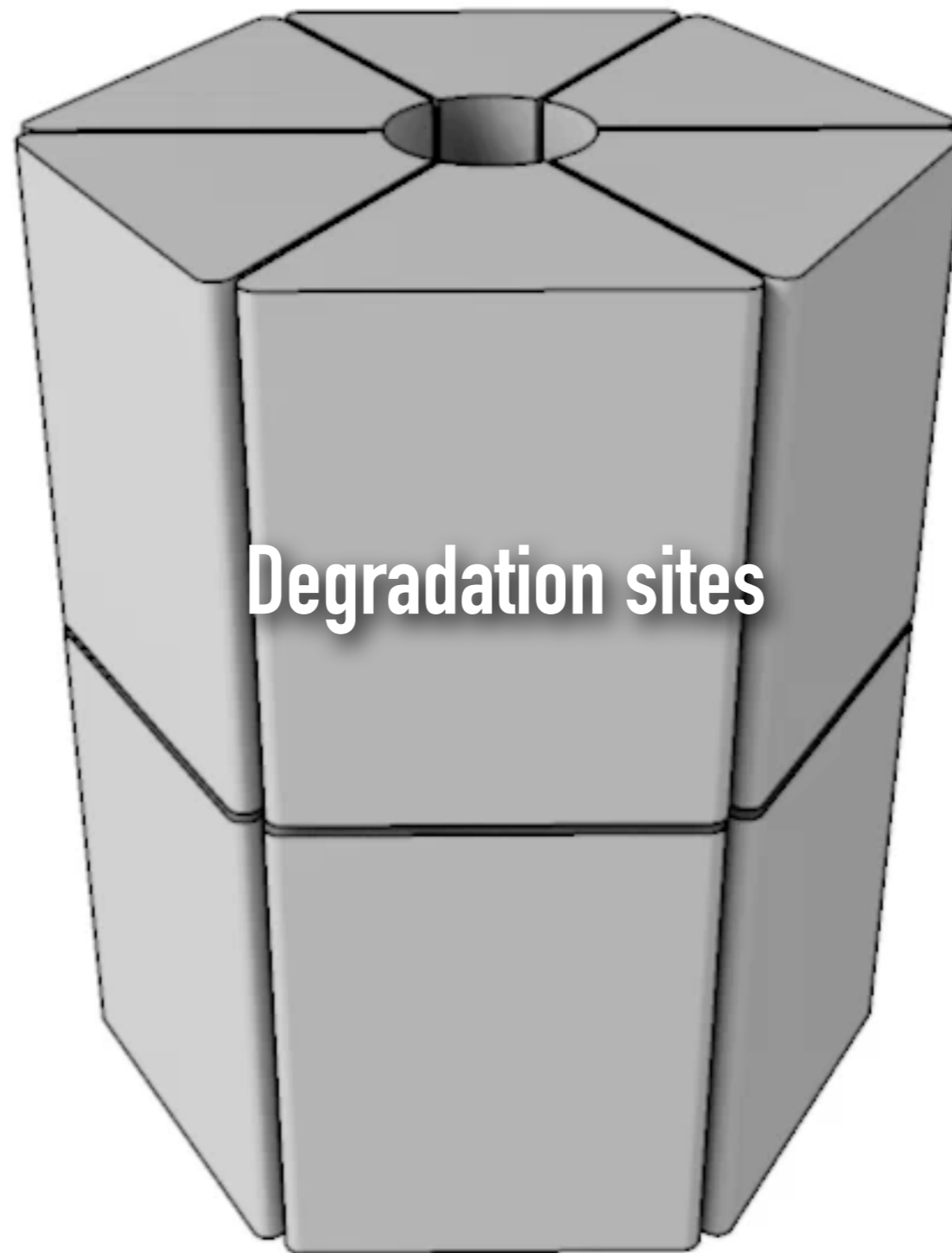






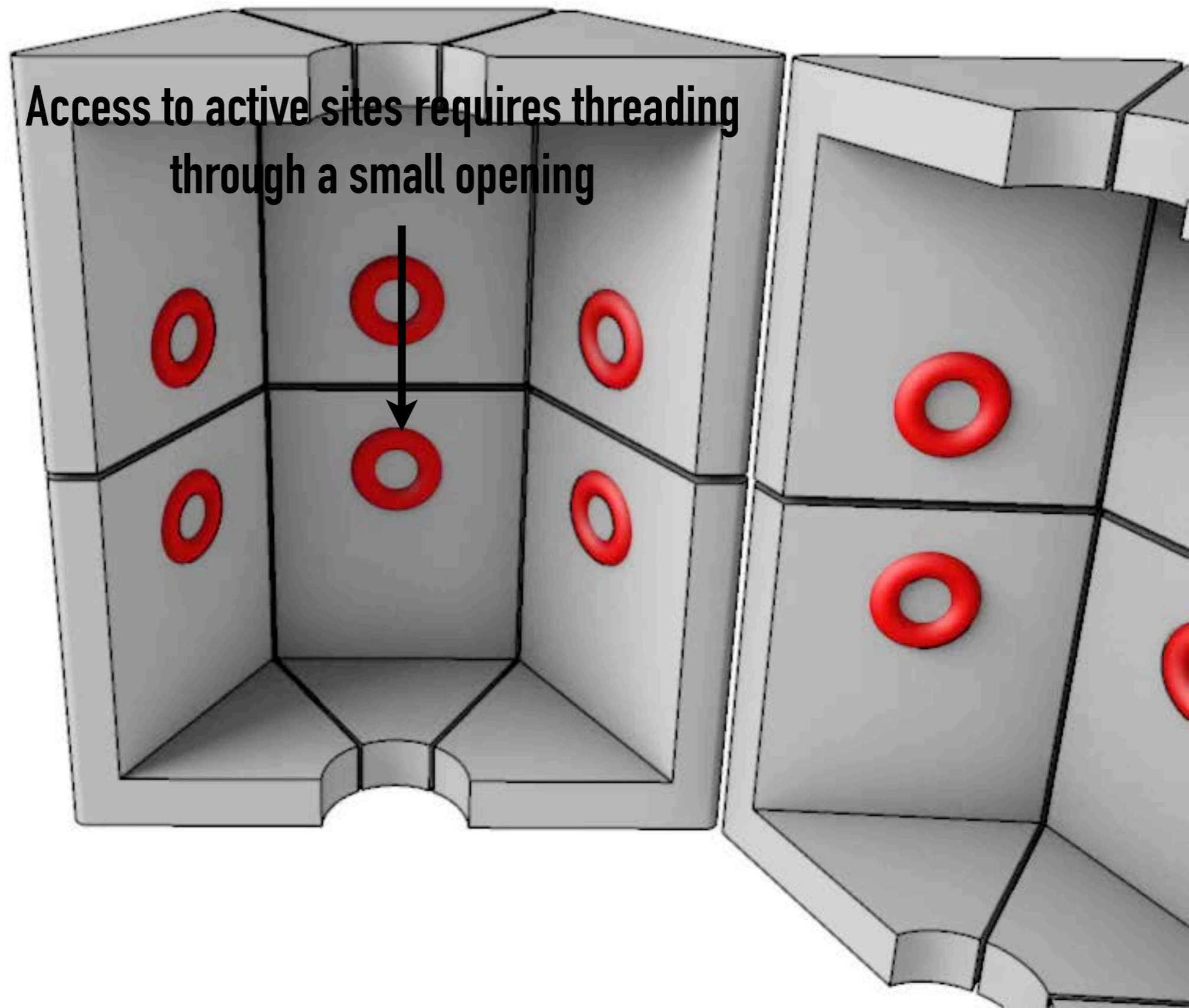
**Protein unfolding
and degradation**

MOLECULAR UNDERTAKERS



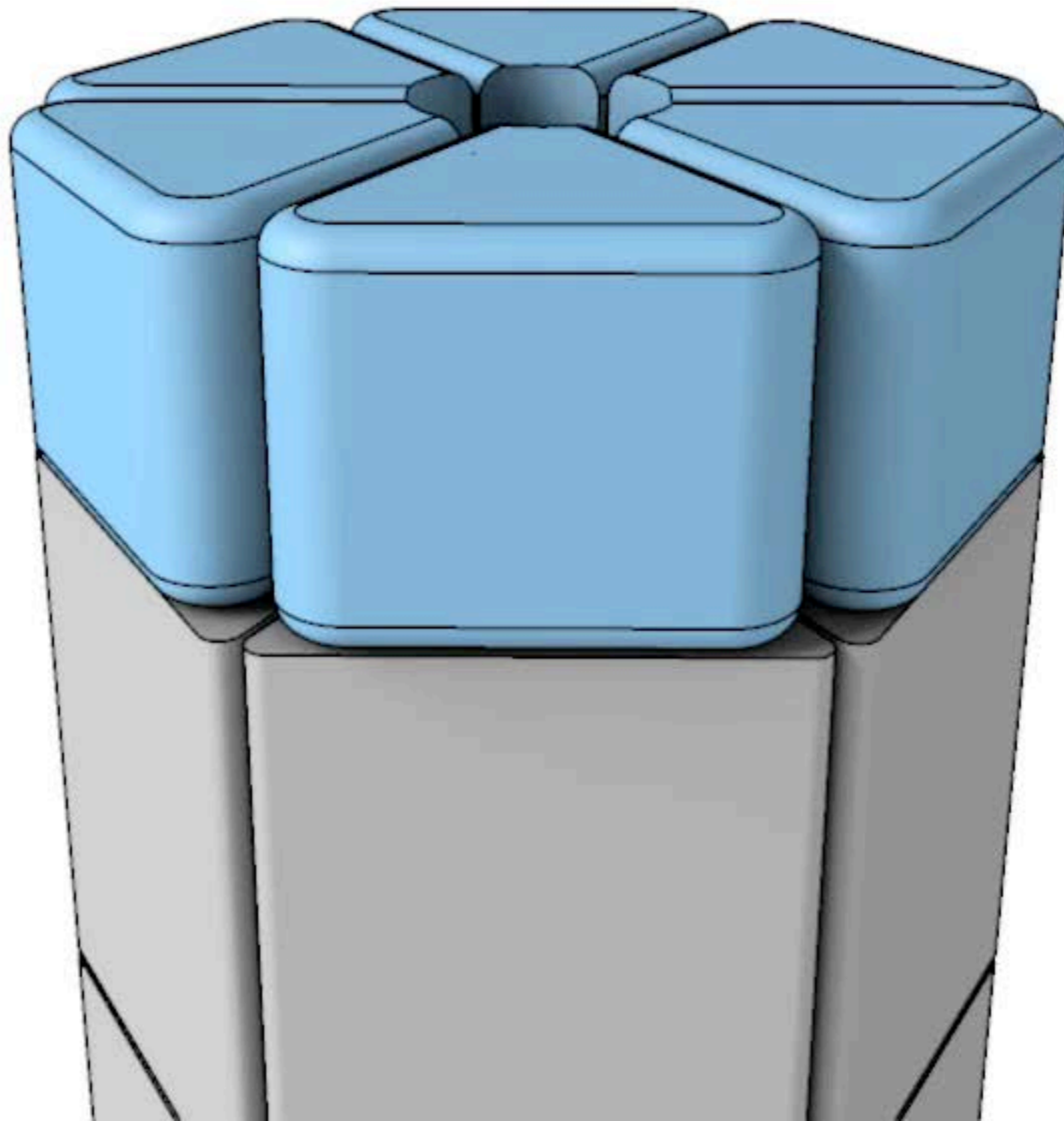
**Degradation sites
sequestered within
barrel-shaped
structure**

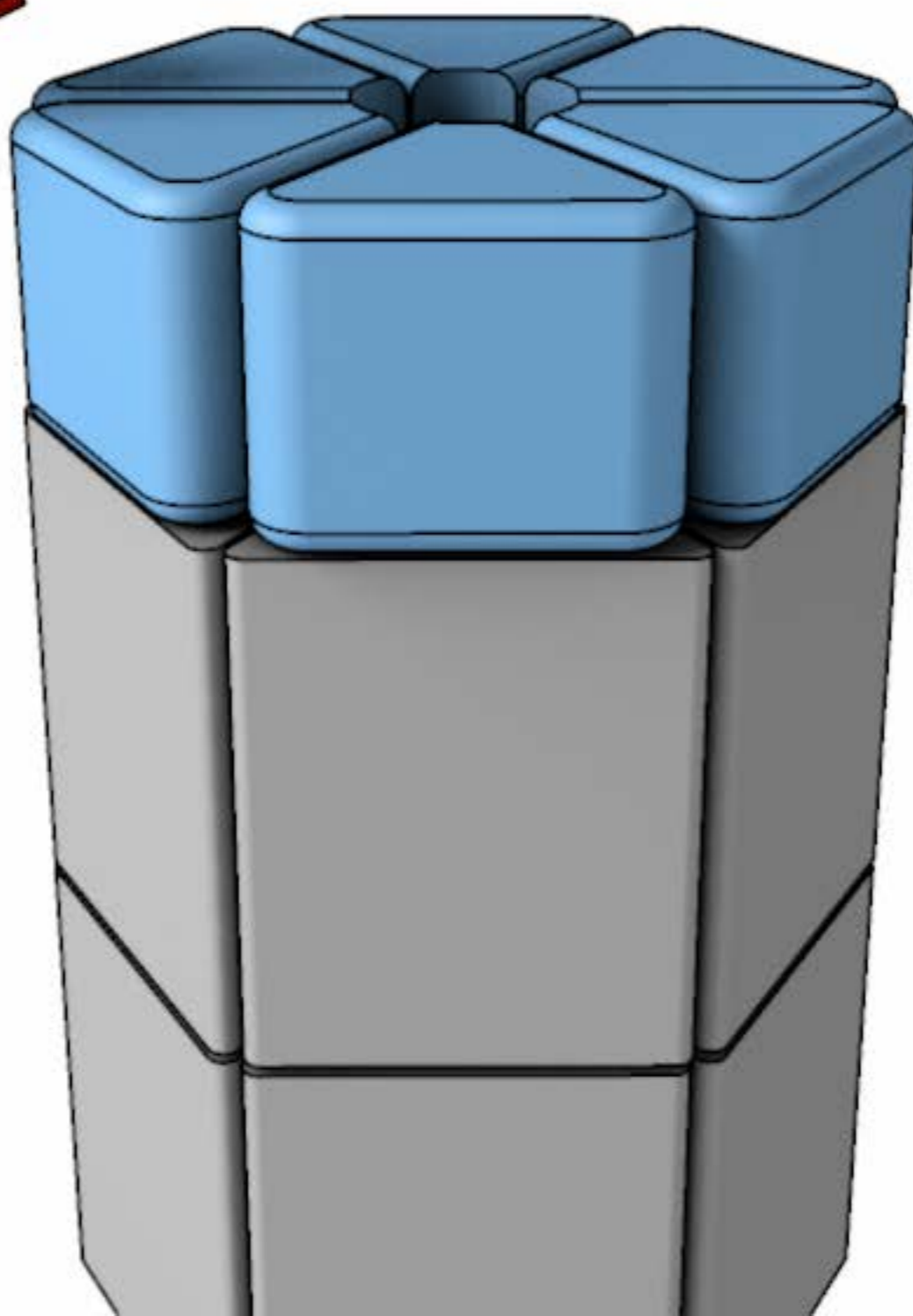
MOLECULAR UNDERTAKERS

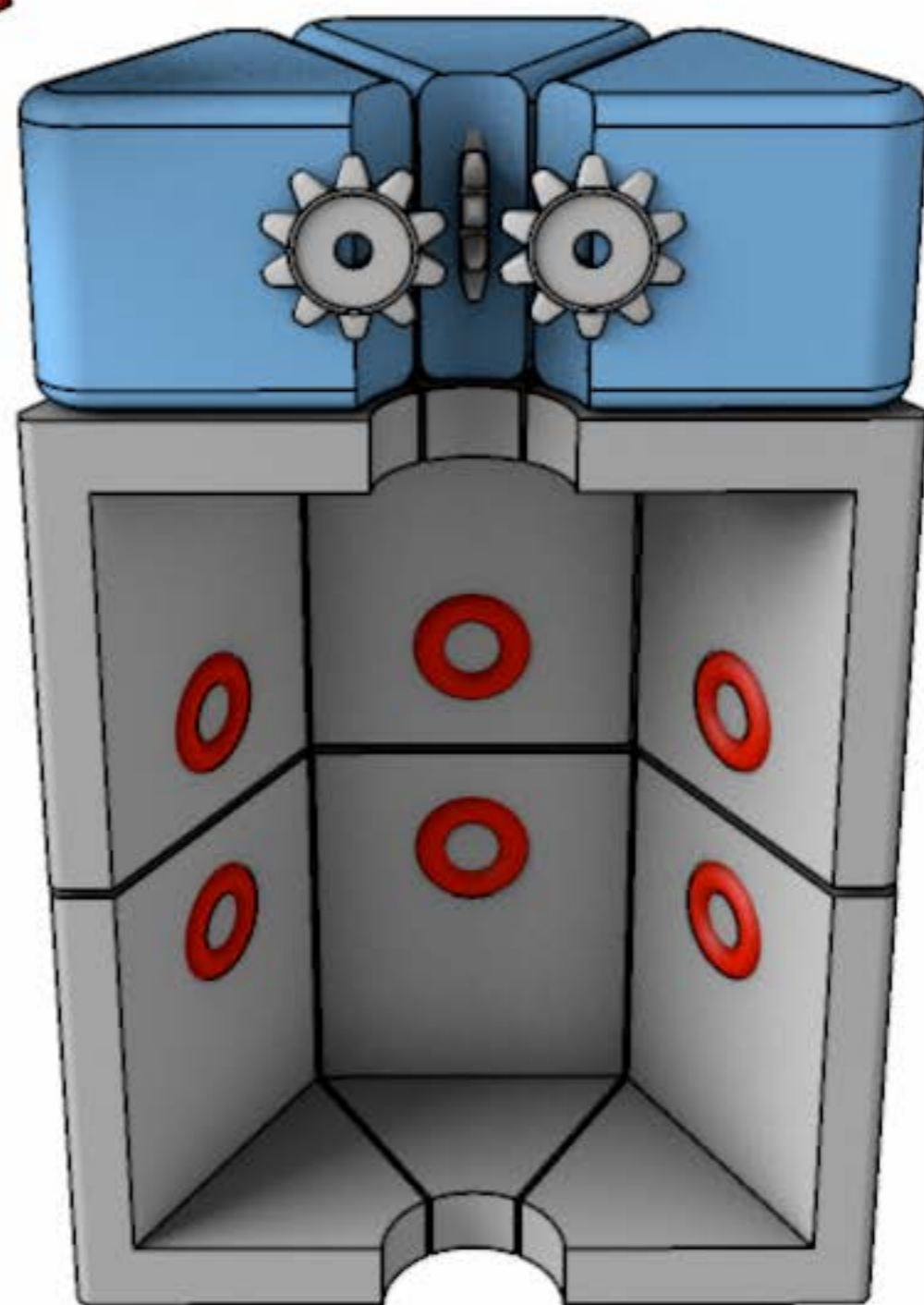


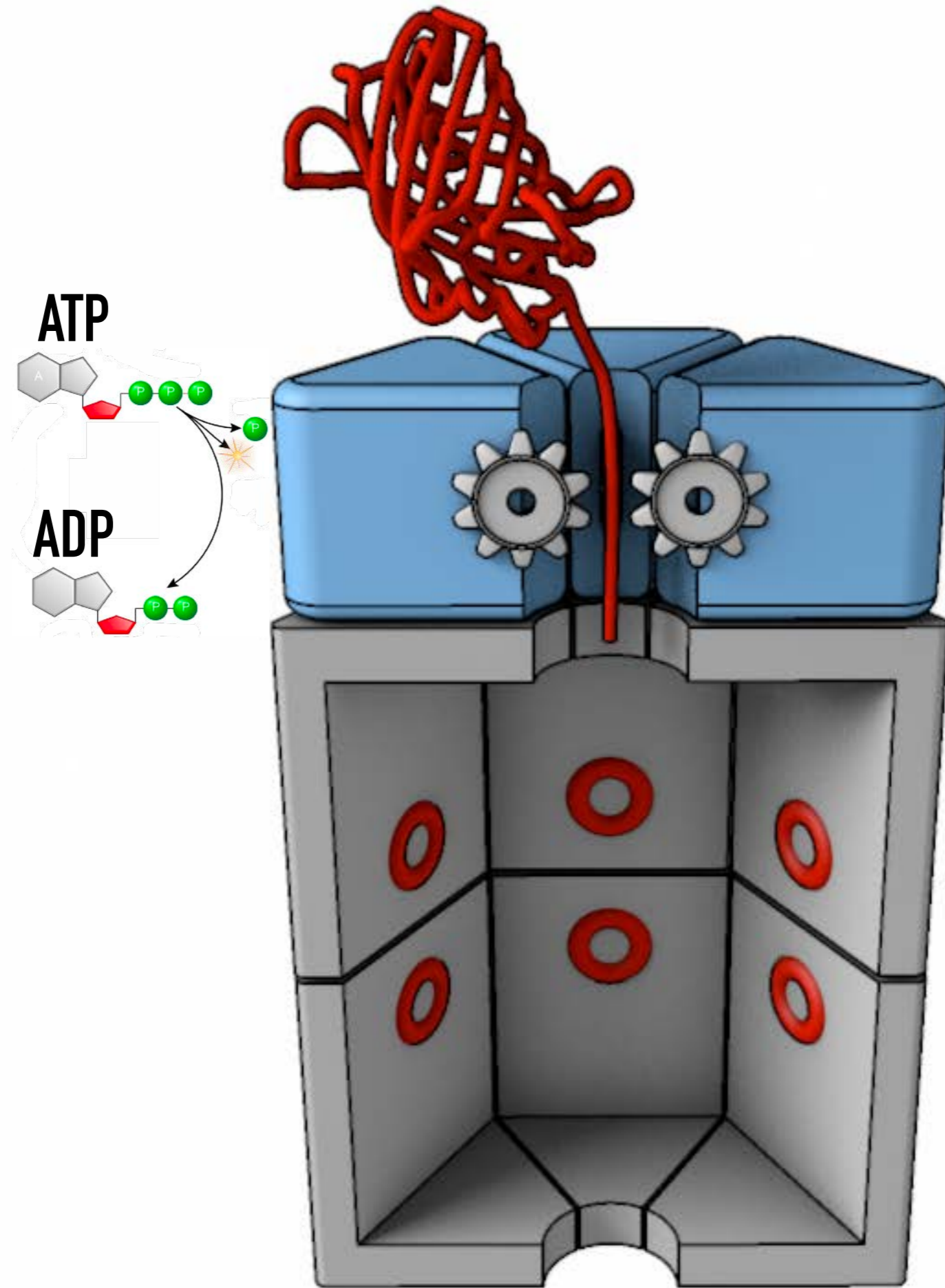
MOLECULAR UNDERTAKERS

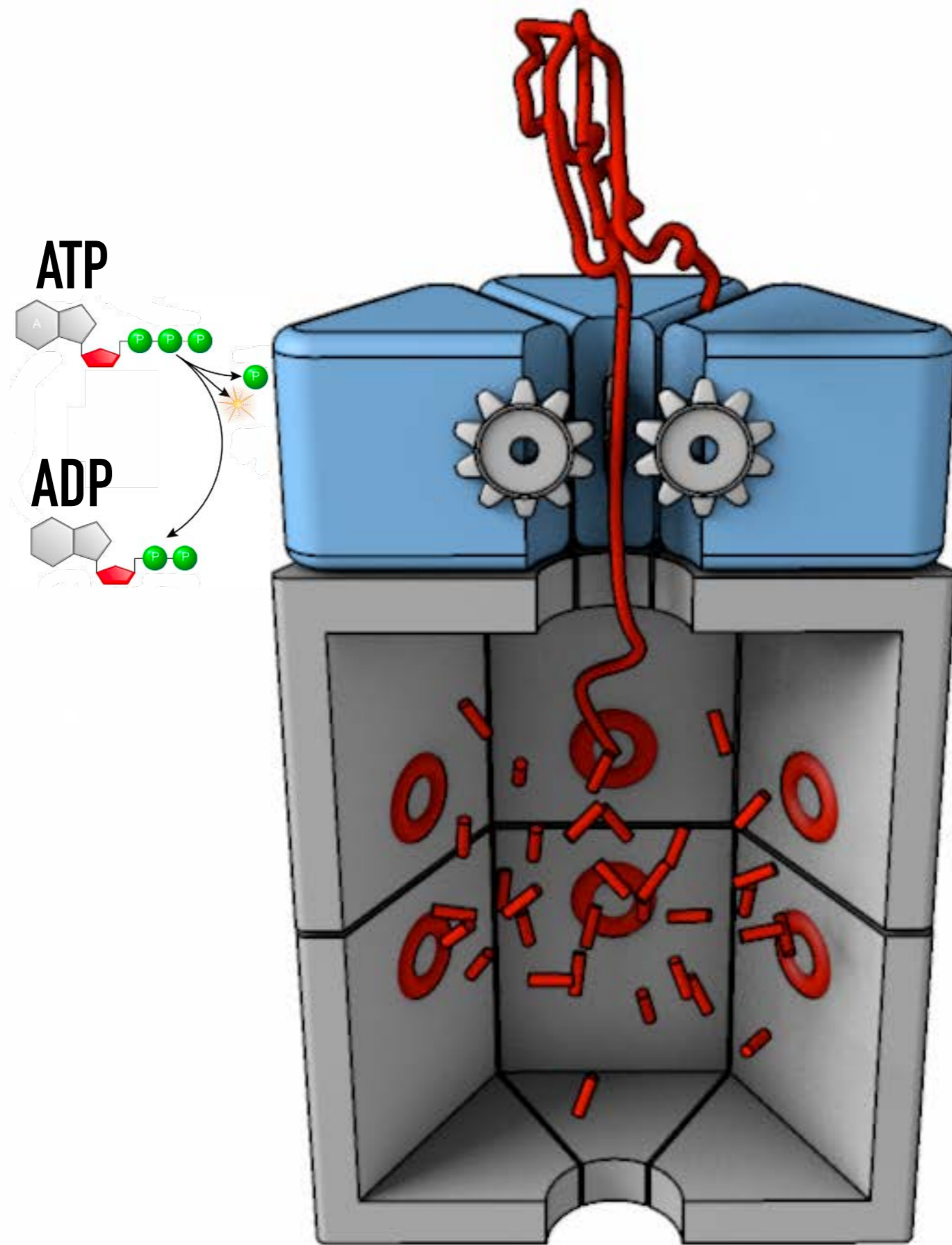
Molecular Motor



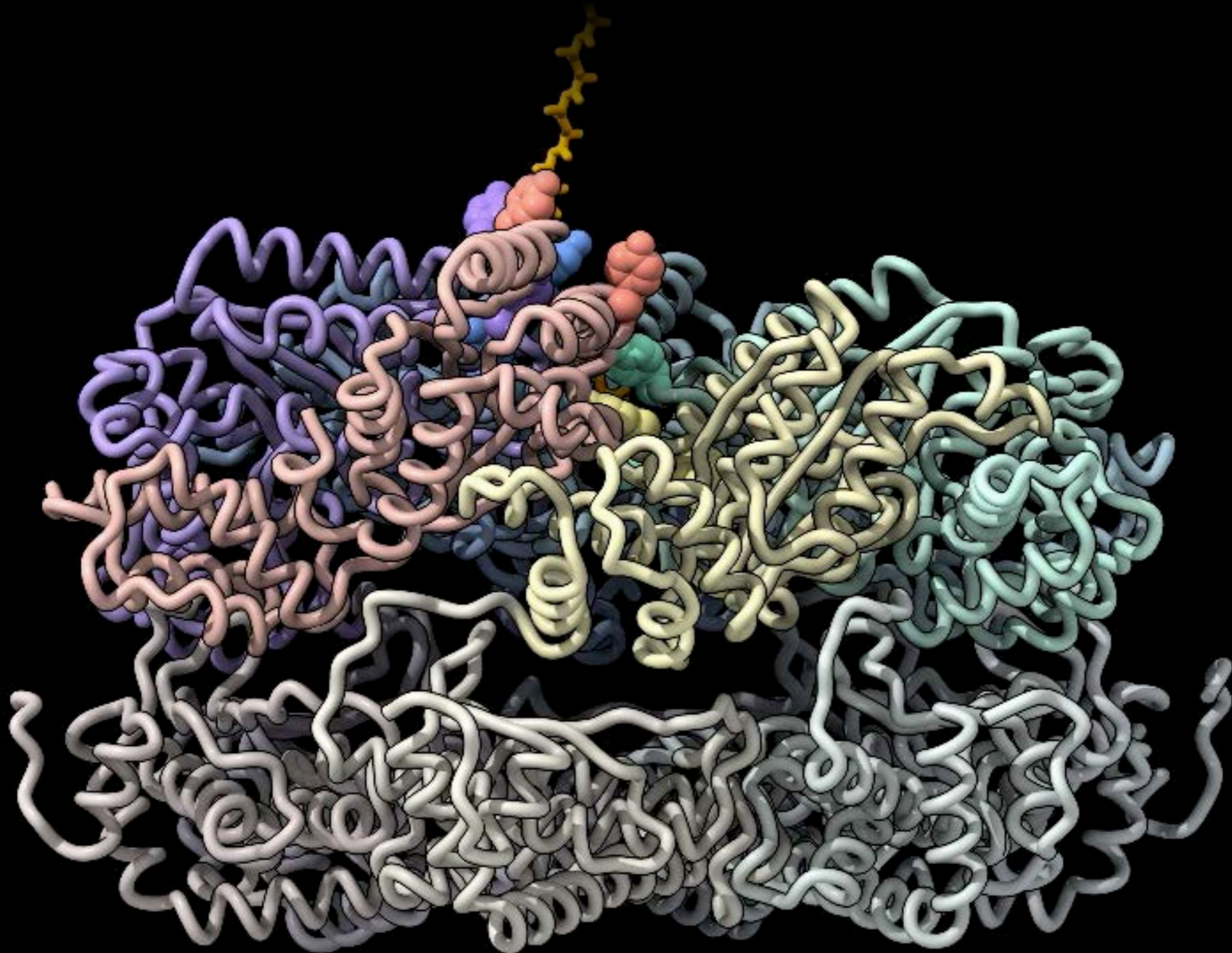






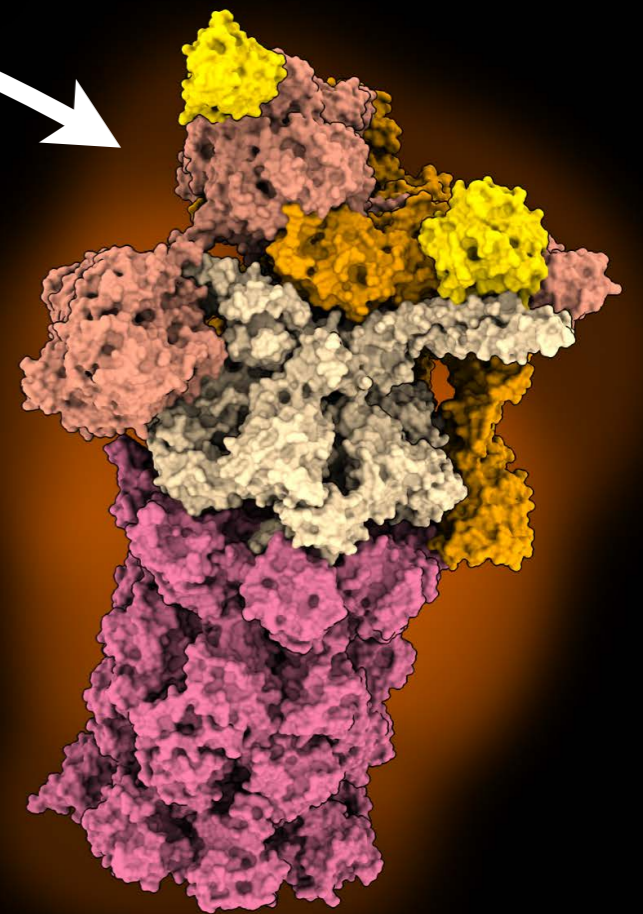


UNFOLDING AND DESTROYING PROTEINS

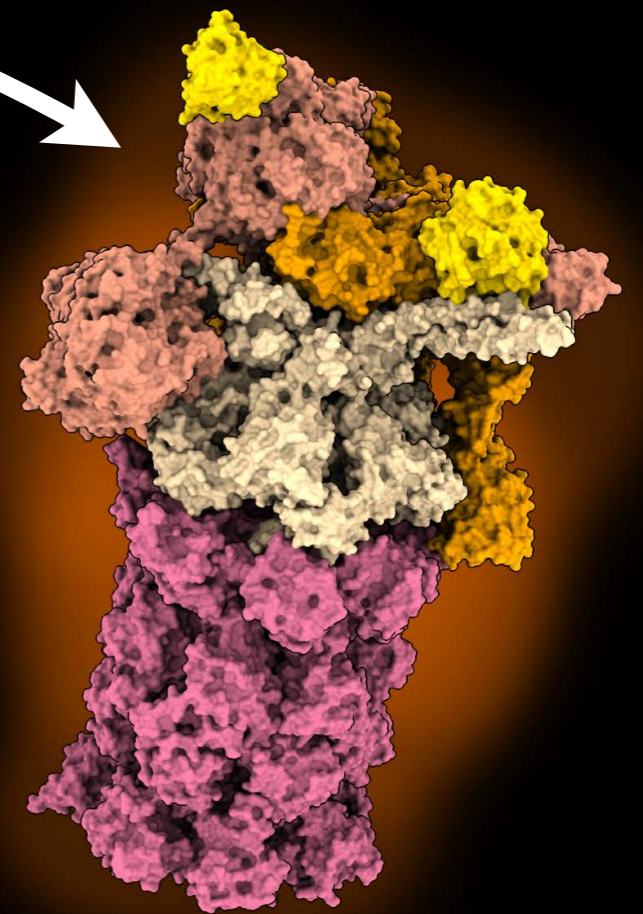


Puchades et al. 2017, 2019, 2020

HOW DO THE DEGRADERS KNOW WHAT TO TARGET?

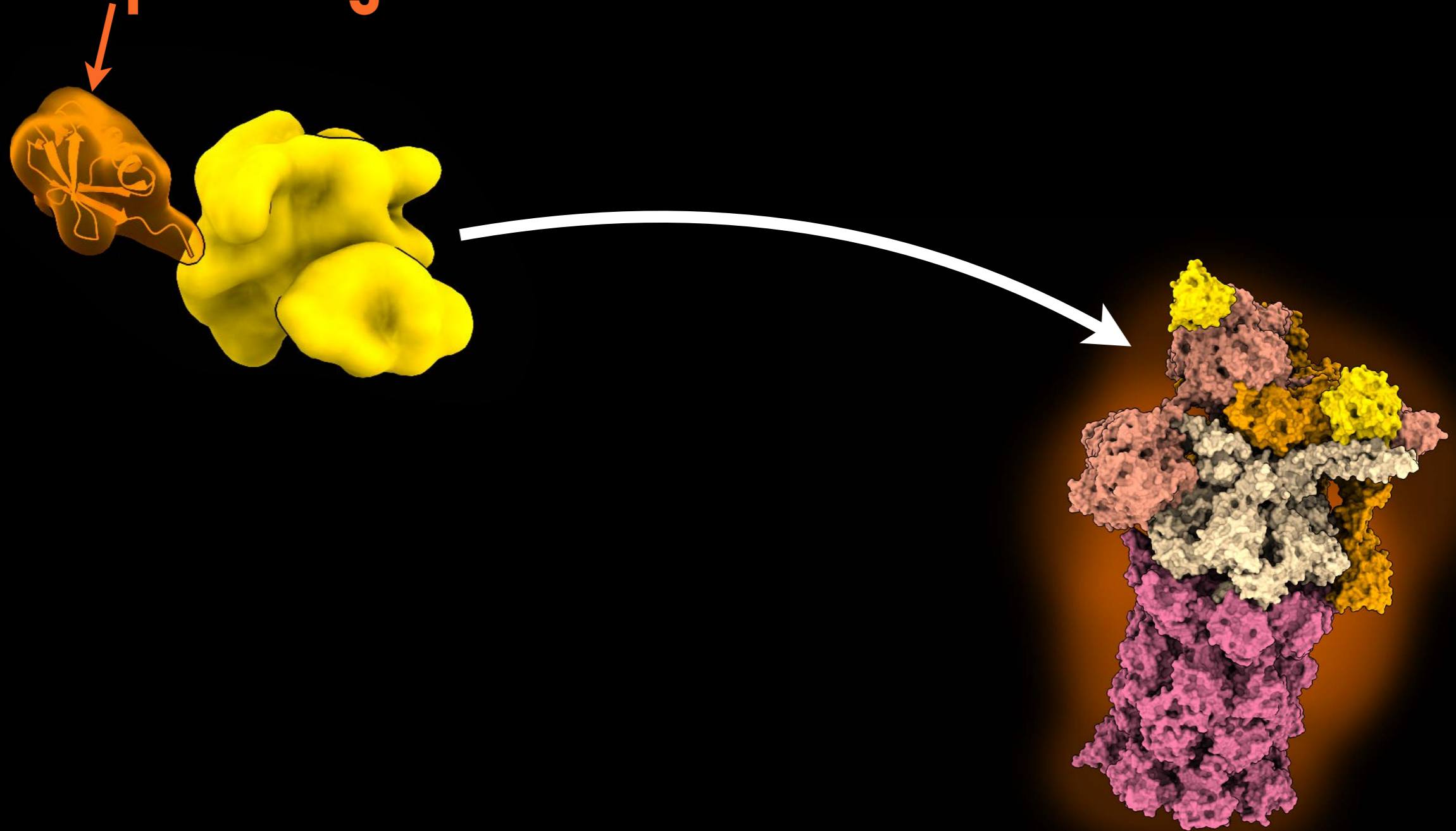


HOW DO THE DEGRADERS KNOW WHAT TO TARGET?

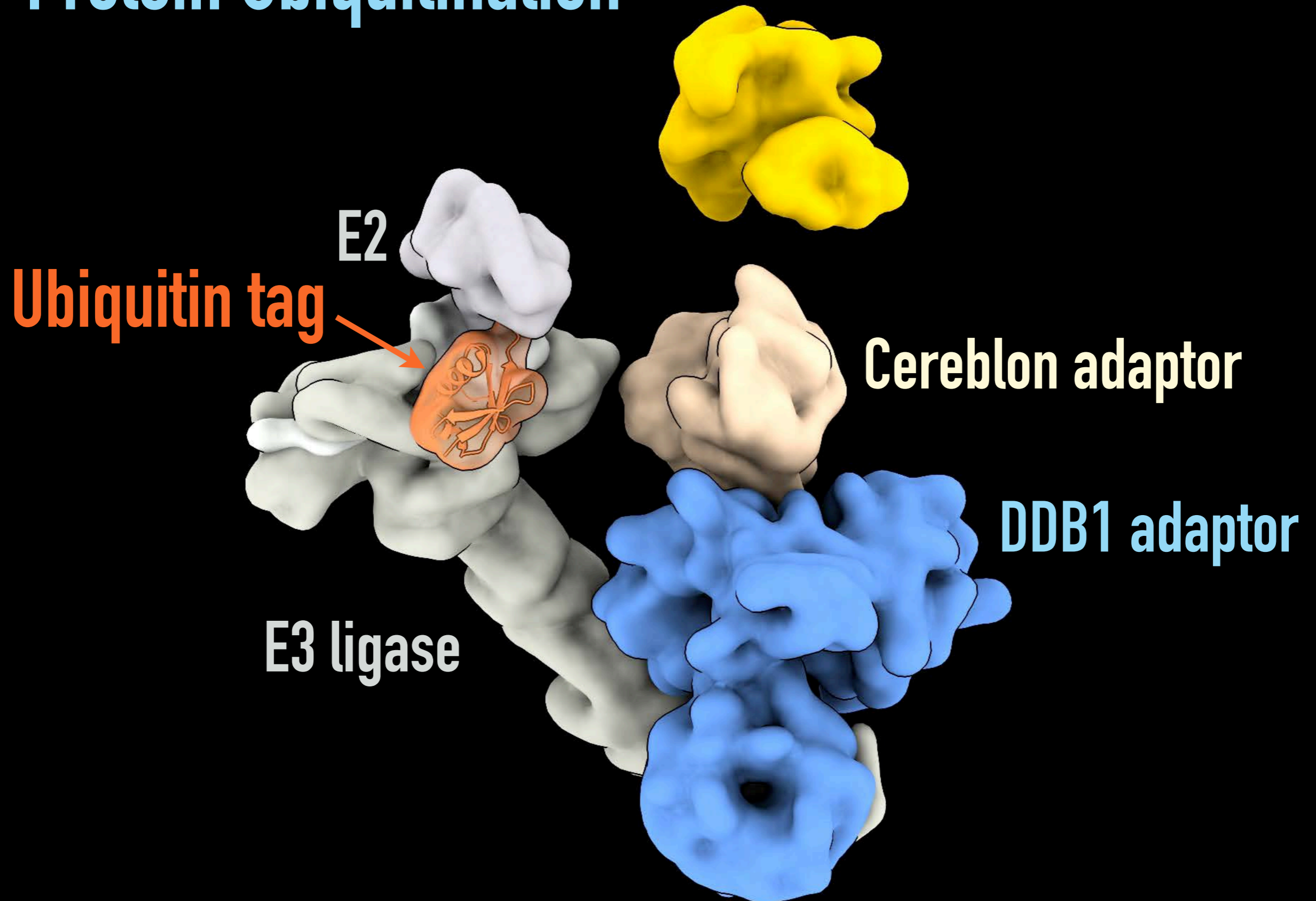


HOW DO THE DEGRADERS KNOW WHAT TO TARGET?

Ubiquitin tag

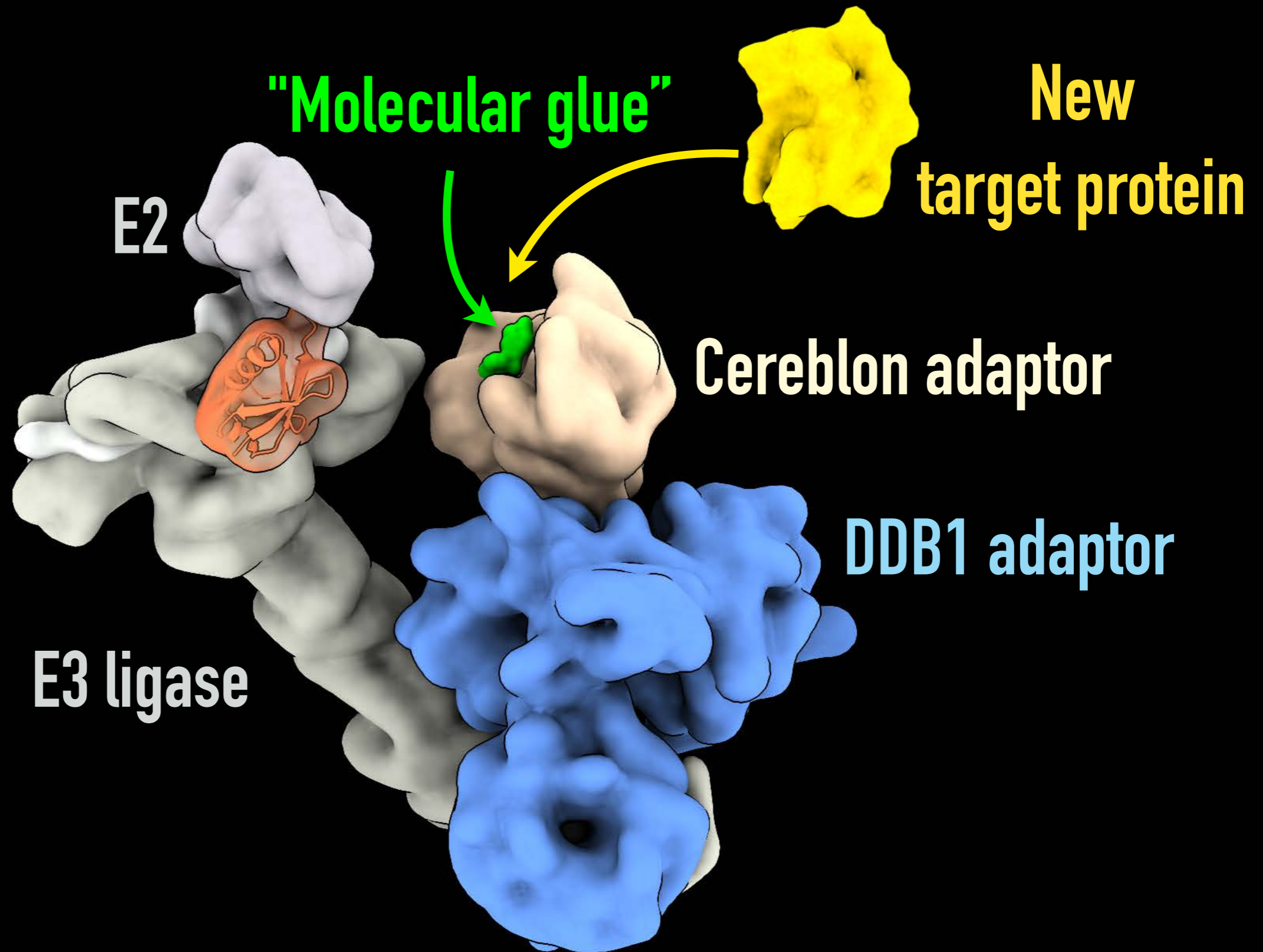


Protein Ubiquitination

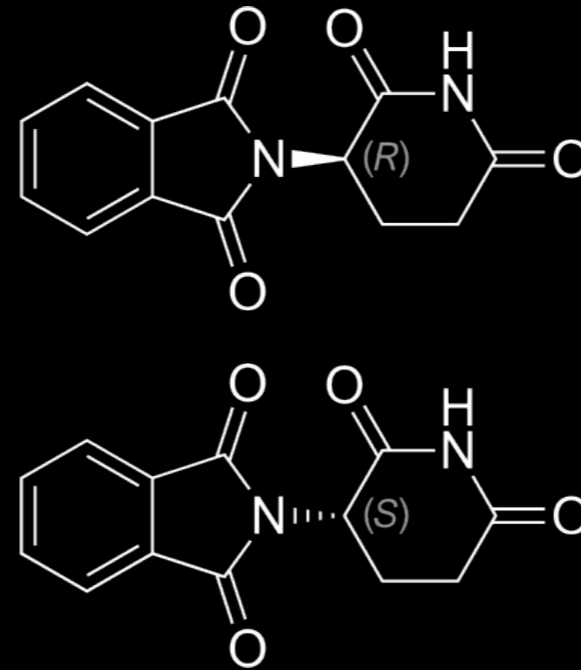




Targeting Disease-related proteins

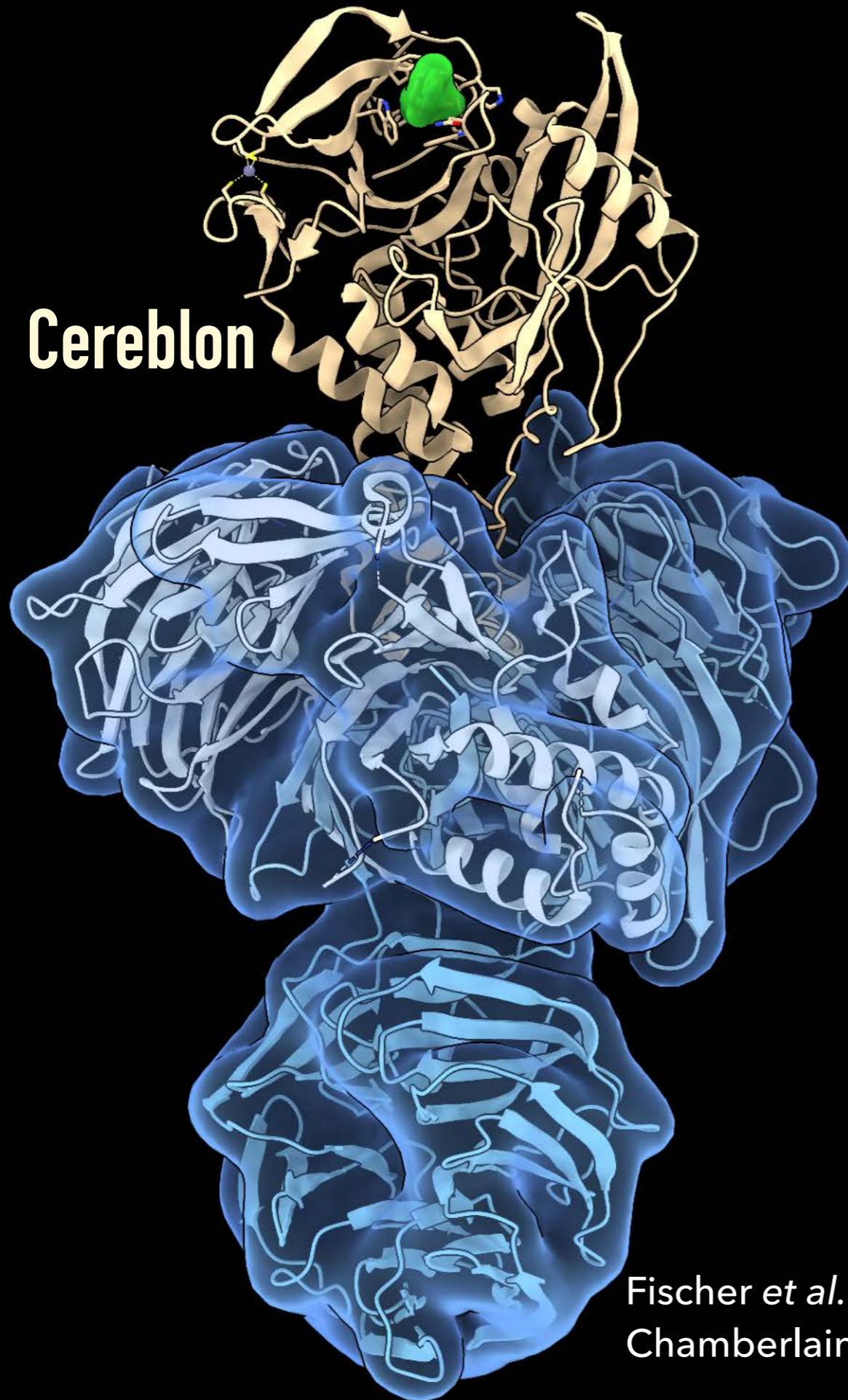


THALIDOMIDE

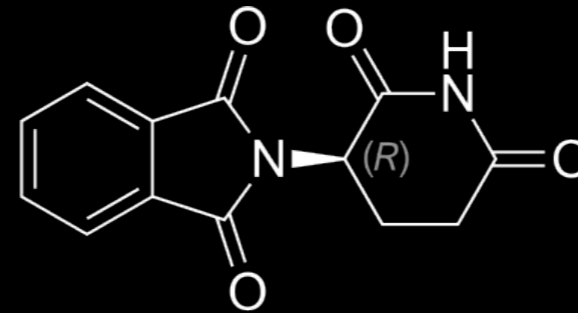


Fischer *et al.* 2014
Chamberlain *et al.* 2014

Cereblon



THALIDOMIDE

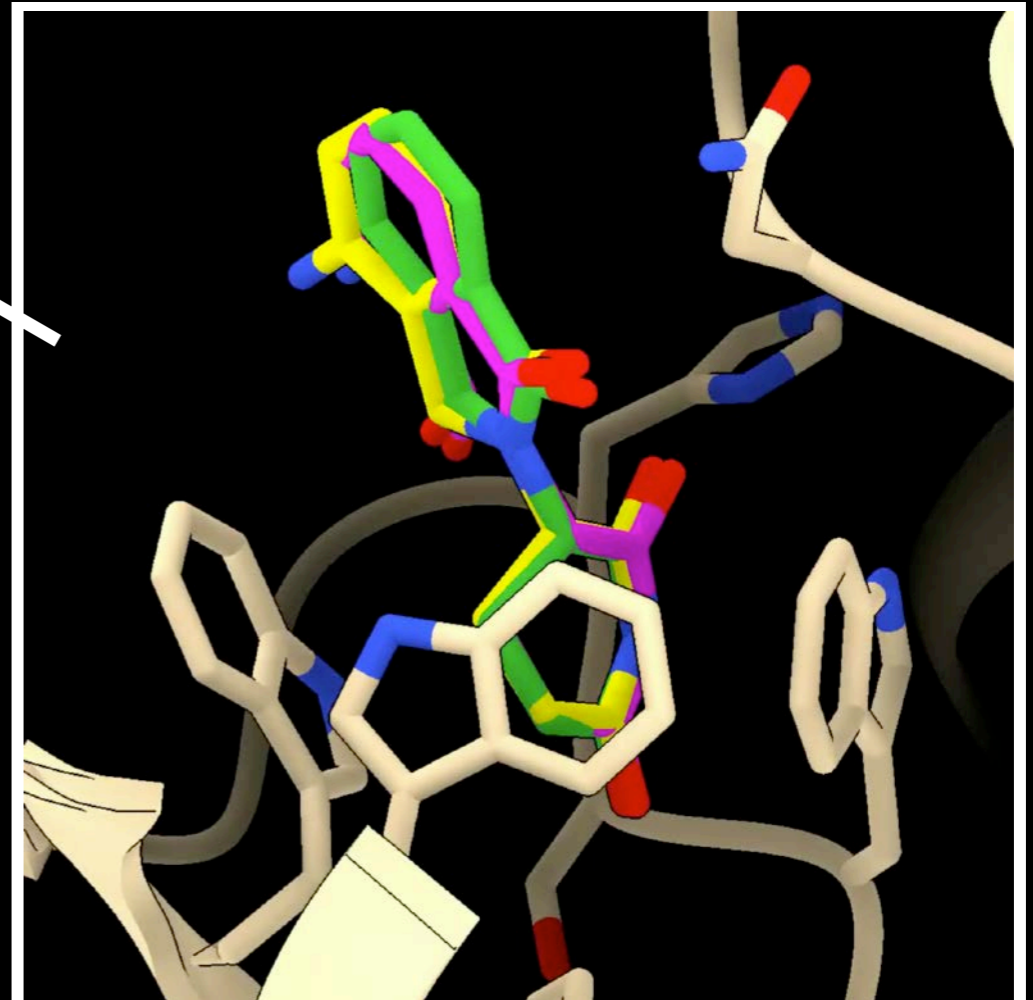
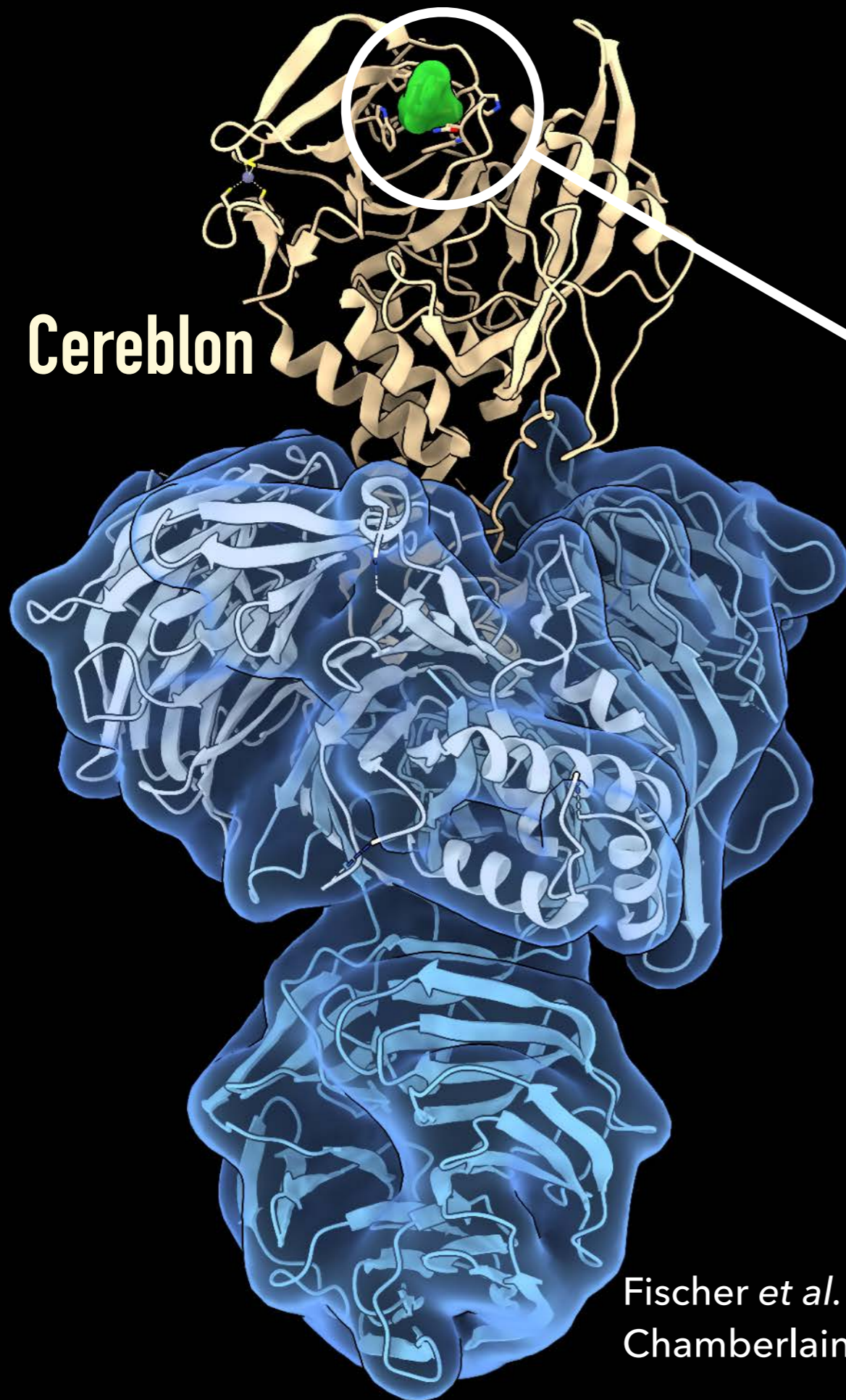


- ▶ In early 2000's approved for use in treatment of multiple myeloma, graft-vs-host disease, and leprosy

Fischer *et al.* 2014
Chamberlain *et al.* 2014

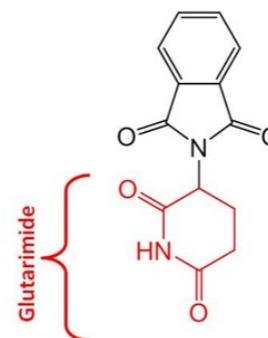
DRUGS BIND TO THE CEREBLON PROTEIN

Cereblon



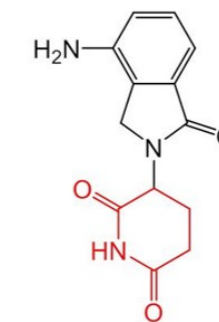
THALOMID
(thalidomide) Capsules

thalidomide



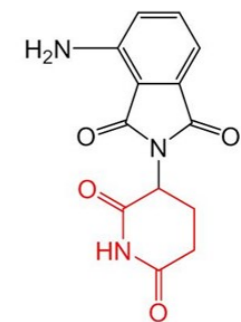
Revlimid
(lenalidomide) capsules

lenalidomide



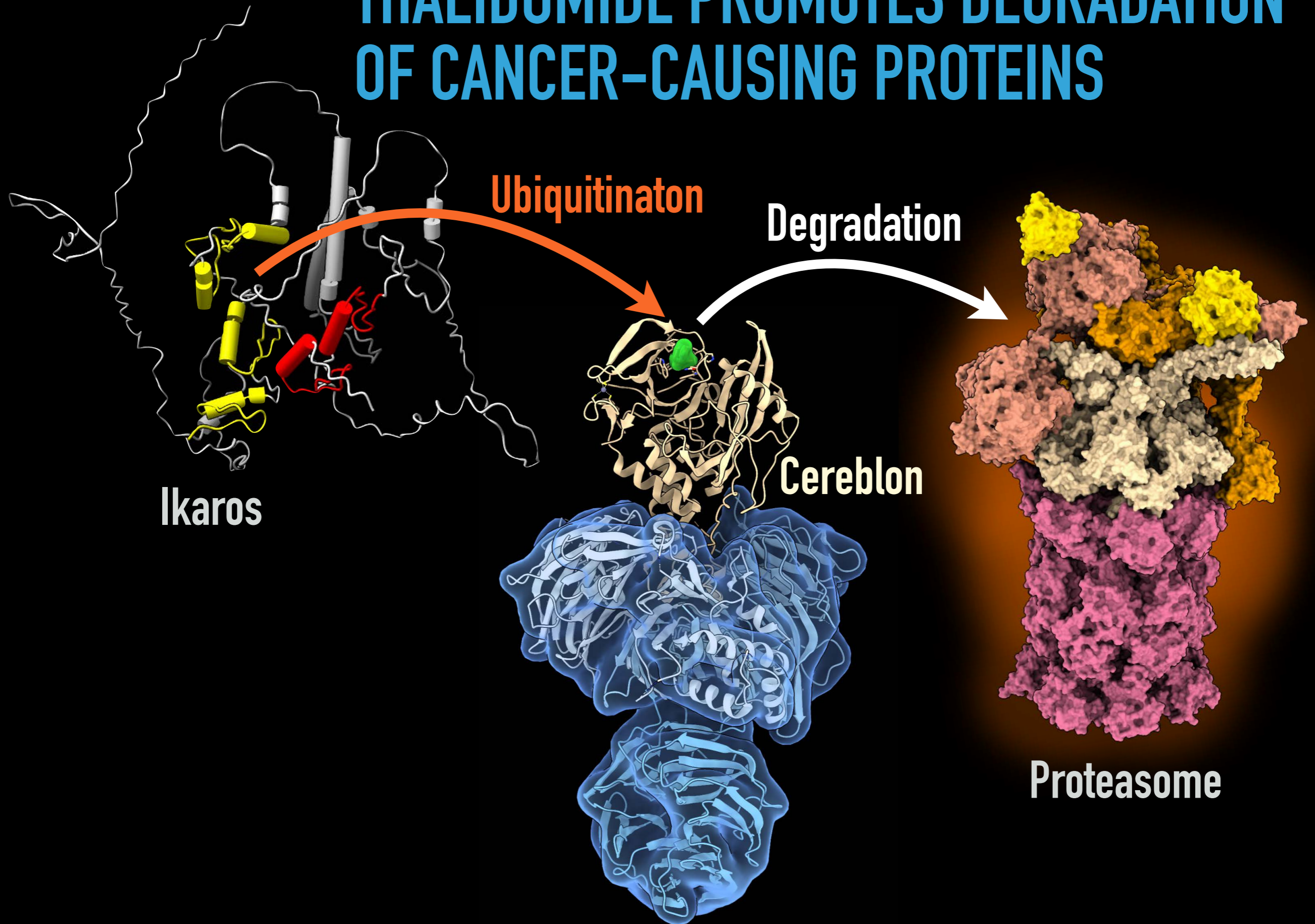
Pomalyst
(pomalidomide) capsules

pomalidomide



Fischer *et al.* 2014
Chamberlain *et al.* 2014

THALIDOMIDE PROMOTES DEGRADATION OF CANCER-CAUSING PROTEINS

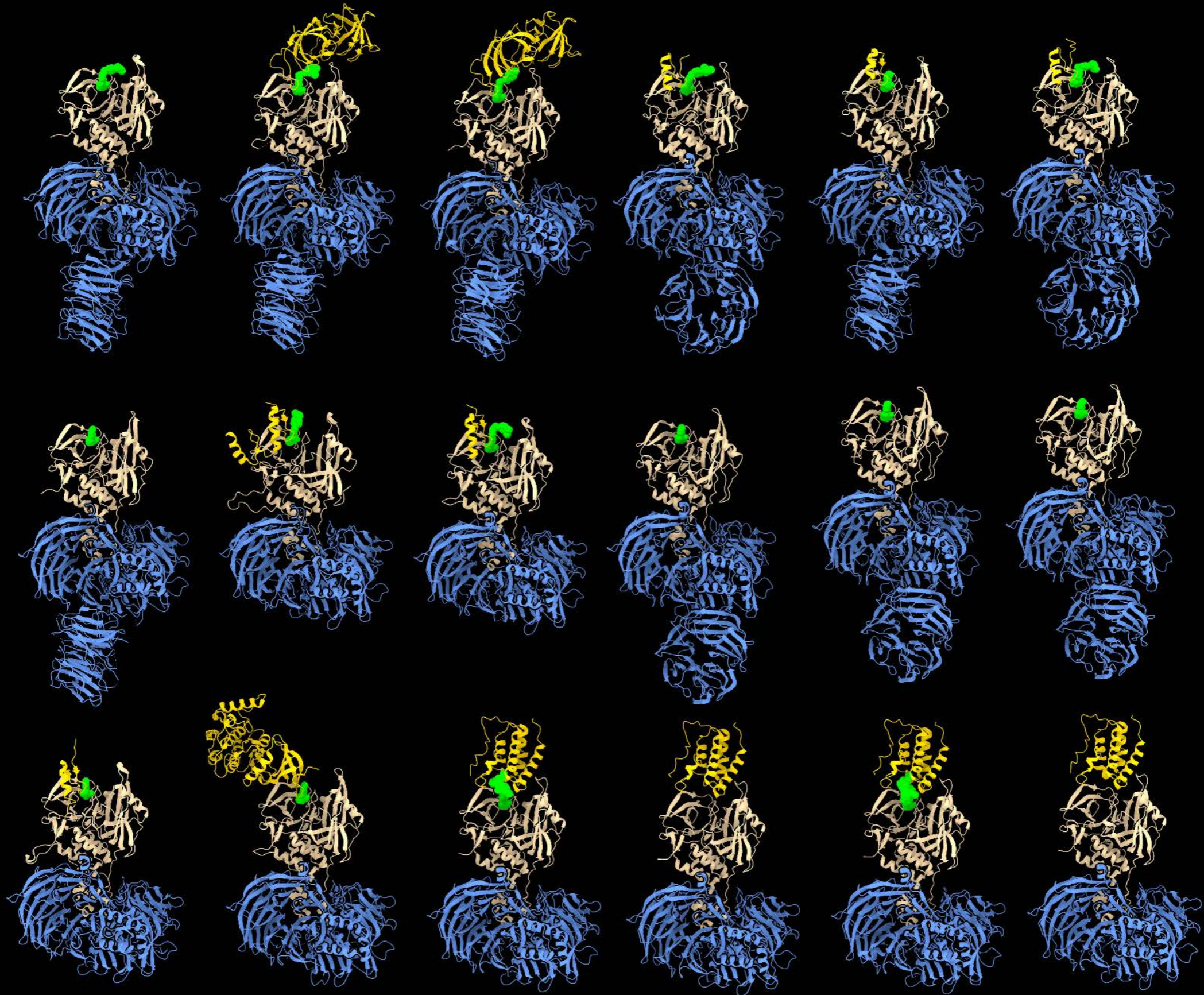


DEGRADERS IN AND APPROACHING THE CLINIC

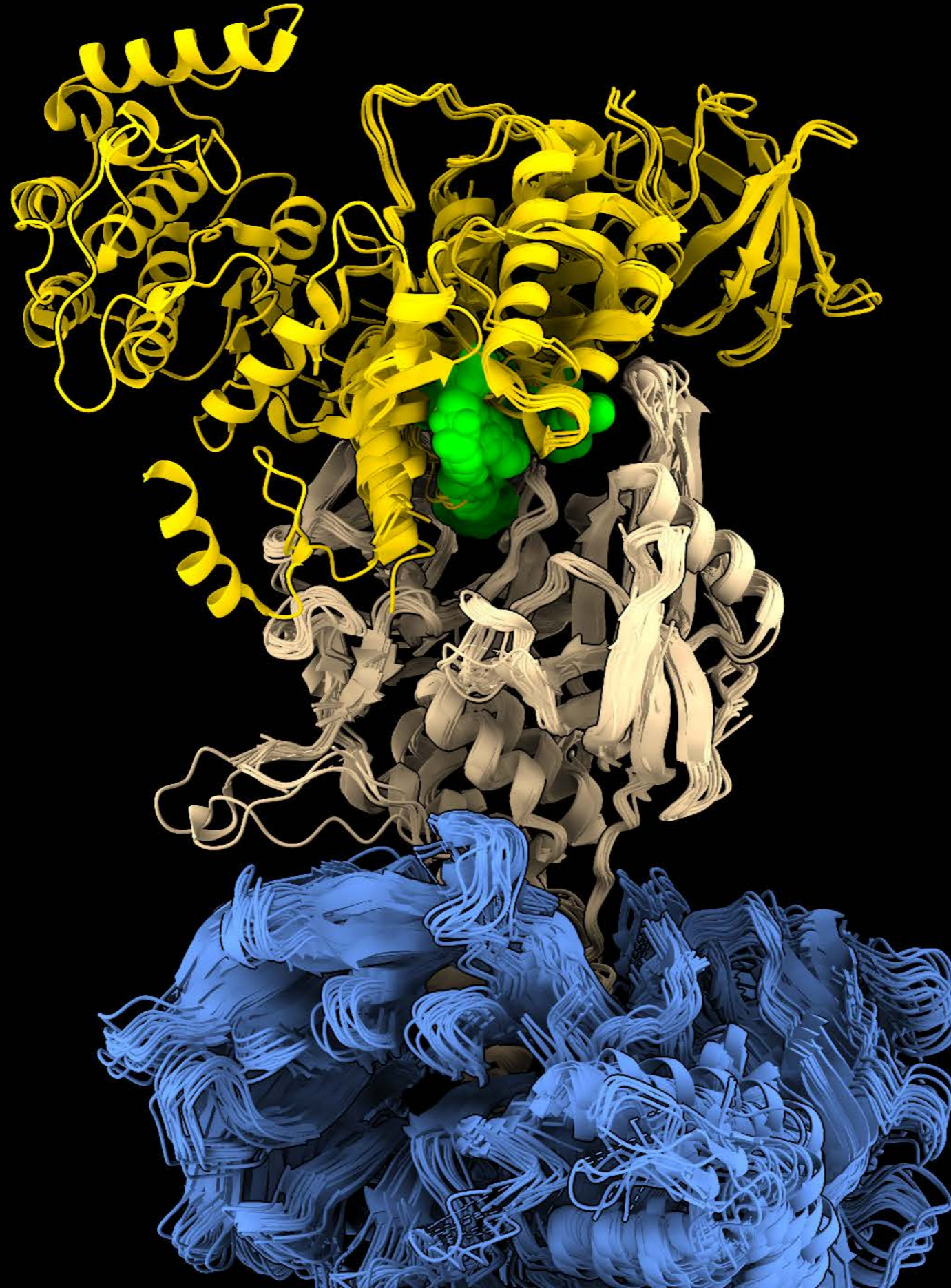
Drug	Sponsor	Properties	Lead indication	Status
<i>Heterobifunctional degraders (PROTACs, BiDACs, etc.)</i>				
ARV-110	Arvinas	Androgen receptor degrader	Prostate cancer	Phase II
ARV-471	Arvinas	Oestrogen receptor degrader	Breast cancer	Phase II
ARV-766	Arvinas	Androgen receptor degrader	Prostate cancer	Phase I in 2021
AR-LDD	Bristol Myers Squibb	Androgen receptor degrader	Prostate cancer	Phase I
DT2216	Dialectic	BCL-XL degrader	Liquid and solid cancers	Phase I
KT-474	Kymera/Sanofi	IRAK4 degrader	Autoimmune including AD, HS and RA	Phase I
KT-413	Kymera	IRAK4 degrader with IMiD activity	MYD88-mutant DLBCL	Phase I in 2H2021
KT-333	Kymera	STAT3 degrader	Liquid and solid tumours	Phase I in 2H2021
NX-2127	Nurix	BTK degrader with IMiD activity	B cell malignancies	Phase I
NX-5948	Nurix	BTK degrader	B cell malignancies and autoimmune	Phase I in 2H2021
CG001419	Cullgen	TRK degrader	Cancer and other diseases	IND in 2021
CFT8634	C4 Therapeutics	BRD9 degrader	Synovial sarcoma	IND in 2H2021
FHD-609	Foghorn	BRD9 degrader	Synovial sarcoma	IND in 1H2021
<i>Molecular glue degrader (CELMoDs, MonoDACs, etc.)</i>				
DKY709	Novartis	Helios (IKZF2) degrader	Solid cancers	Phase I
CC-90009	Bristol Myers Squibb	GSPT1 degrader	Acute myeloid leukaemia	Phase I
CC-92480	Bristol Myers Squibb	Ikaros/Aiolos (IKZF1/3) degrader	Multiple myeloma	Phase I
CC-99282	Bristol Myers Squibb	Ikaros/Aiolos (IKZF1/3) degrader	Lymphoma	Phase I
CFT7455	C4 Therapeutics	Ikaros/Aiolos (IKZF1/3) degrader	Multiple myeloma and lymphoma	Phase I in 1H2021

Mullard 2021

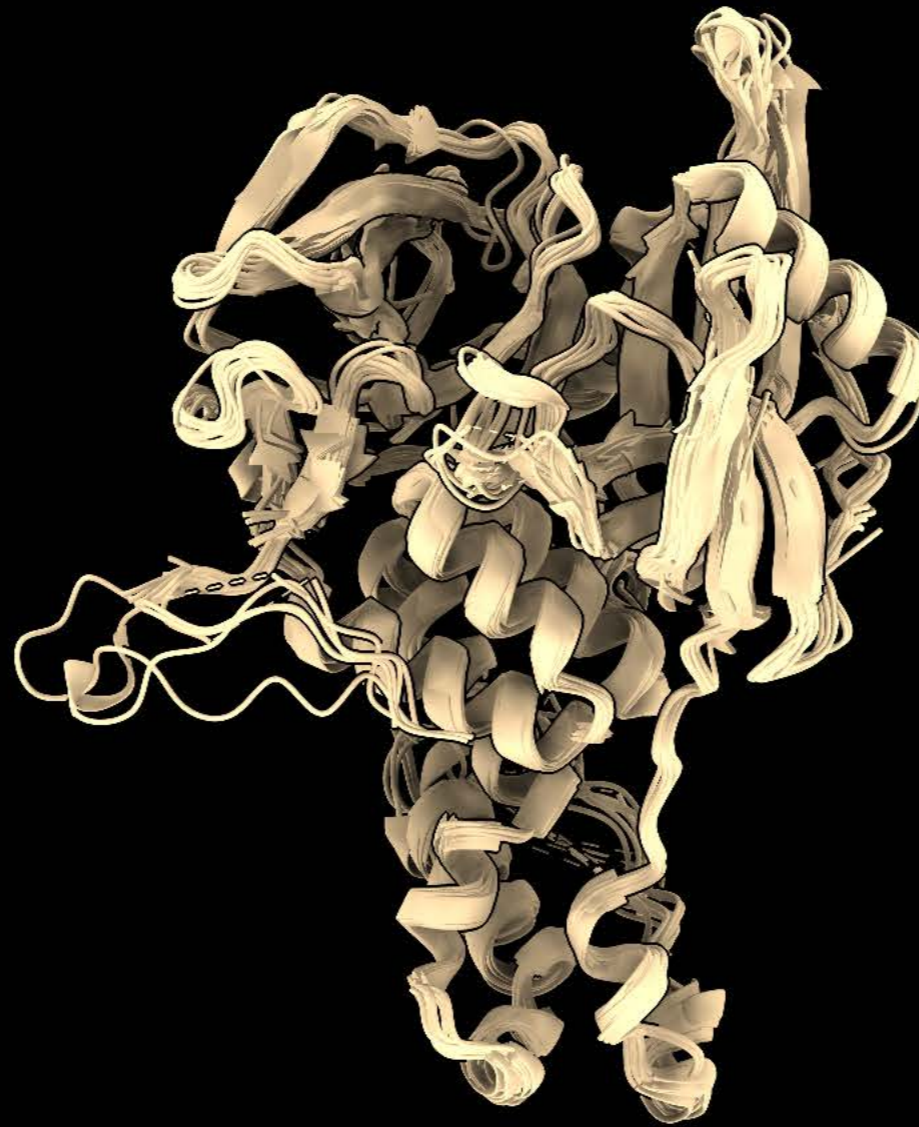
Crystal structures of the Cereblon complex



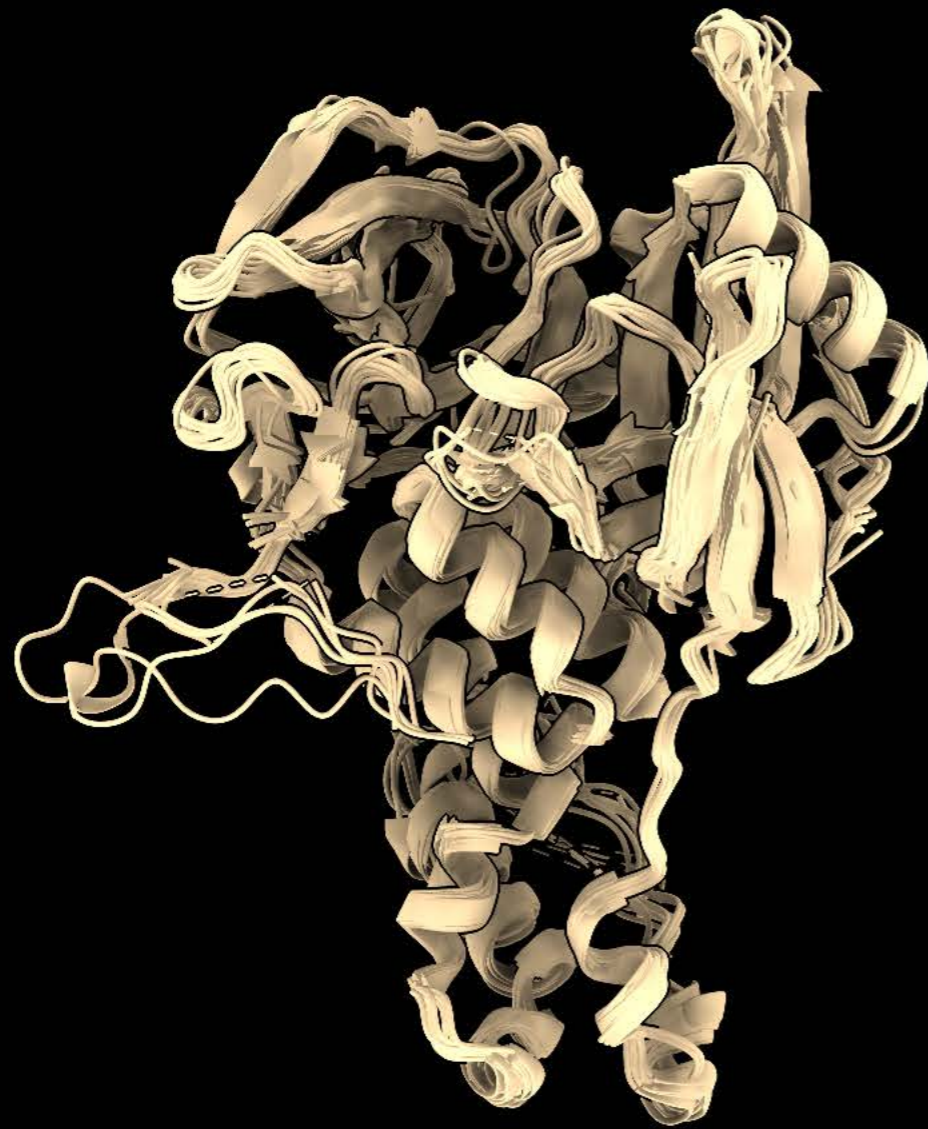
Crystal structures of the Cereblon complex



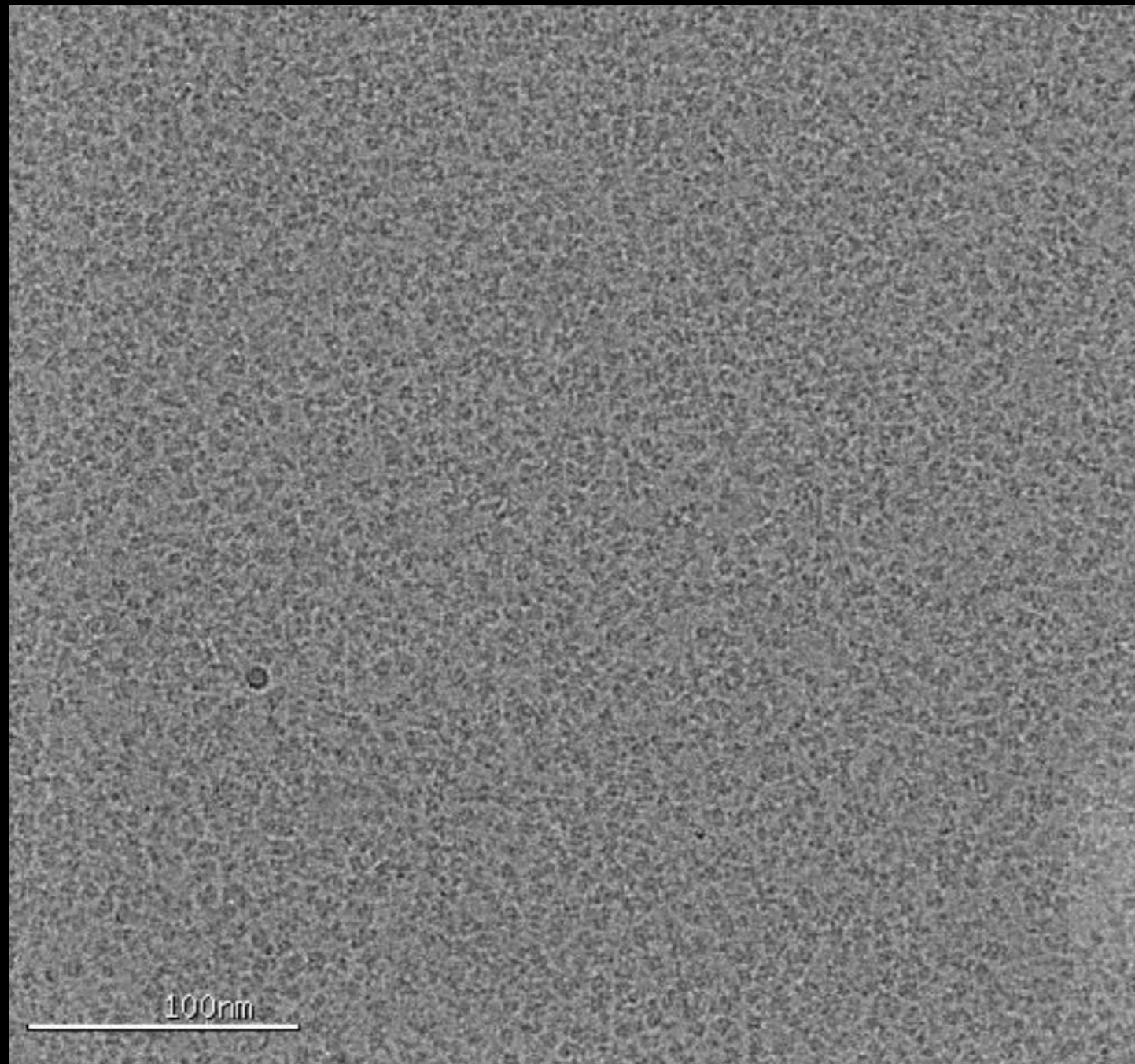
Crystal structures of Cereblon



Crystal structures of Cereblon



CRYOEM STRUCTURE OF THE CEREBLON COMPLEX



45,000 X magnification

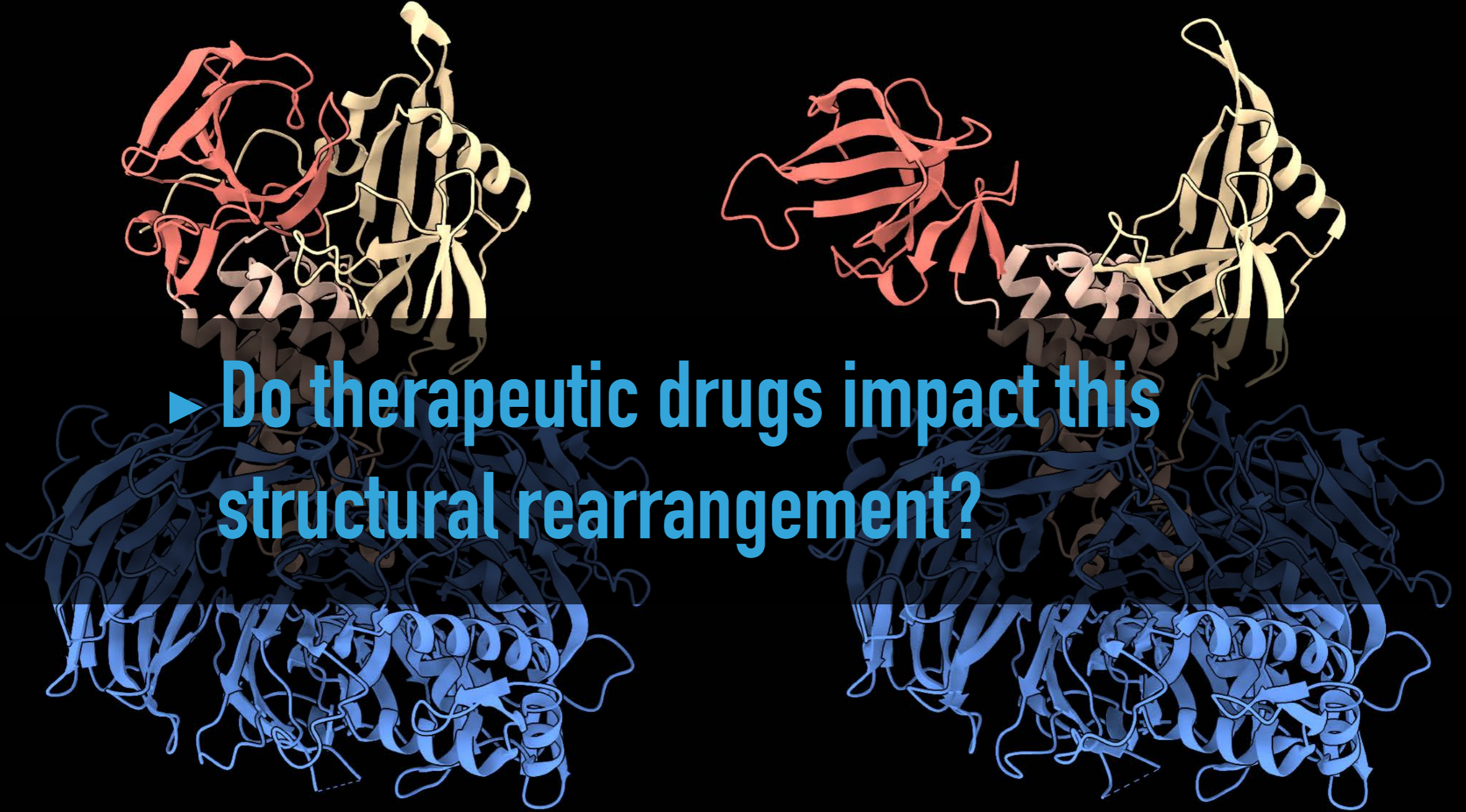


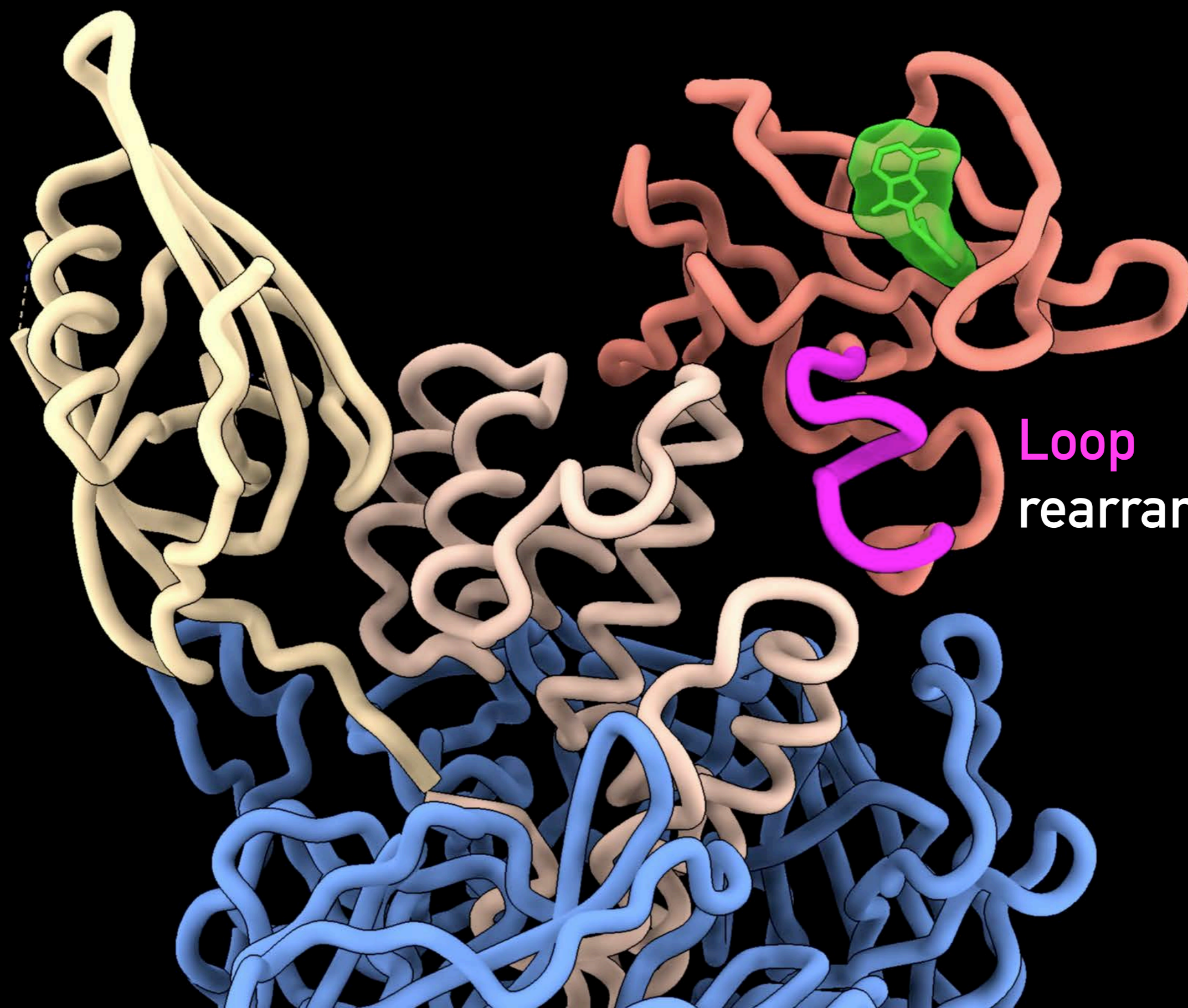
Randy Watson, PhD

CRYSTALLOGRAPHY

CRYO-EM

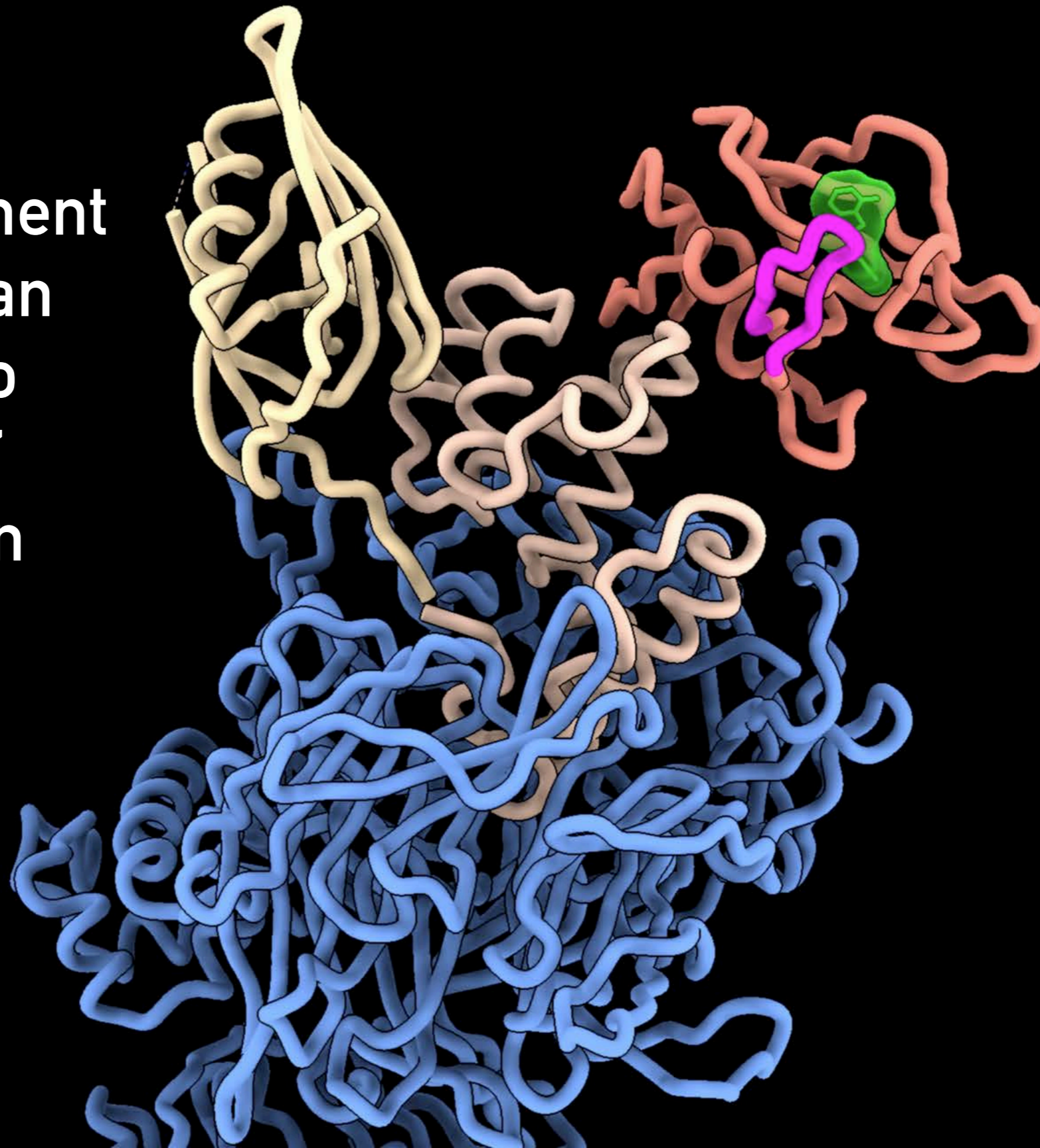
► Do therapeutic drugs impact this structural rearrangement?

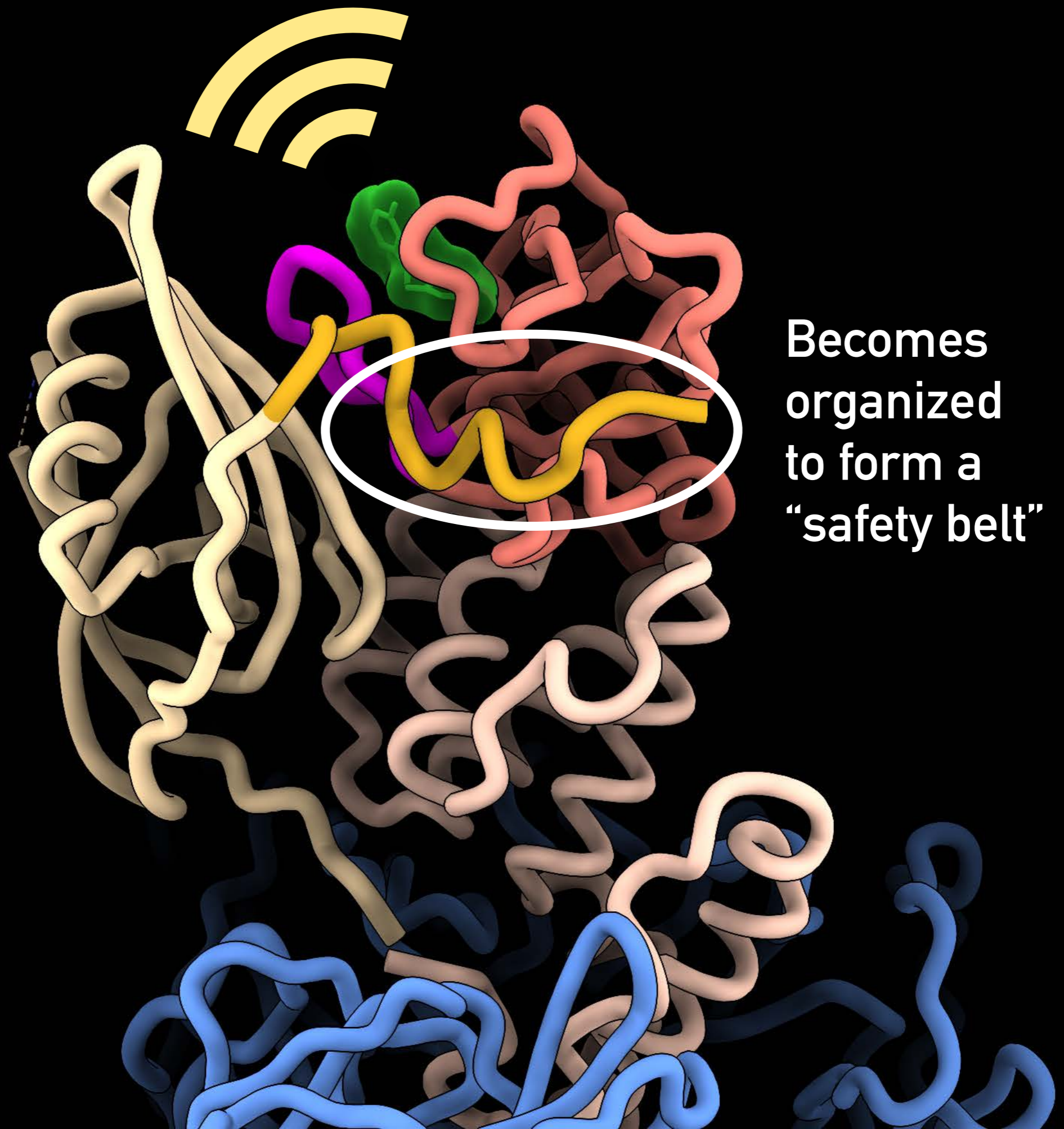




Loop
rearrangement

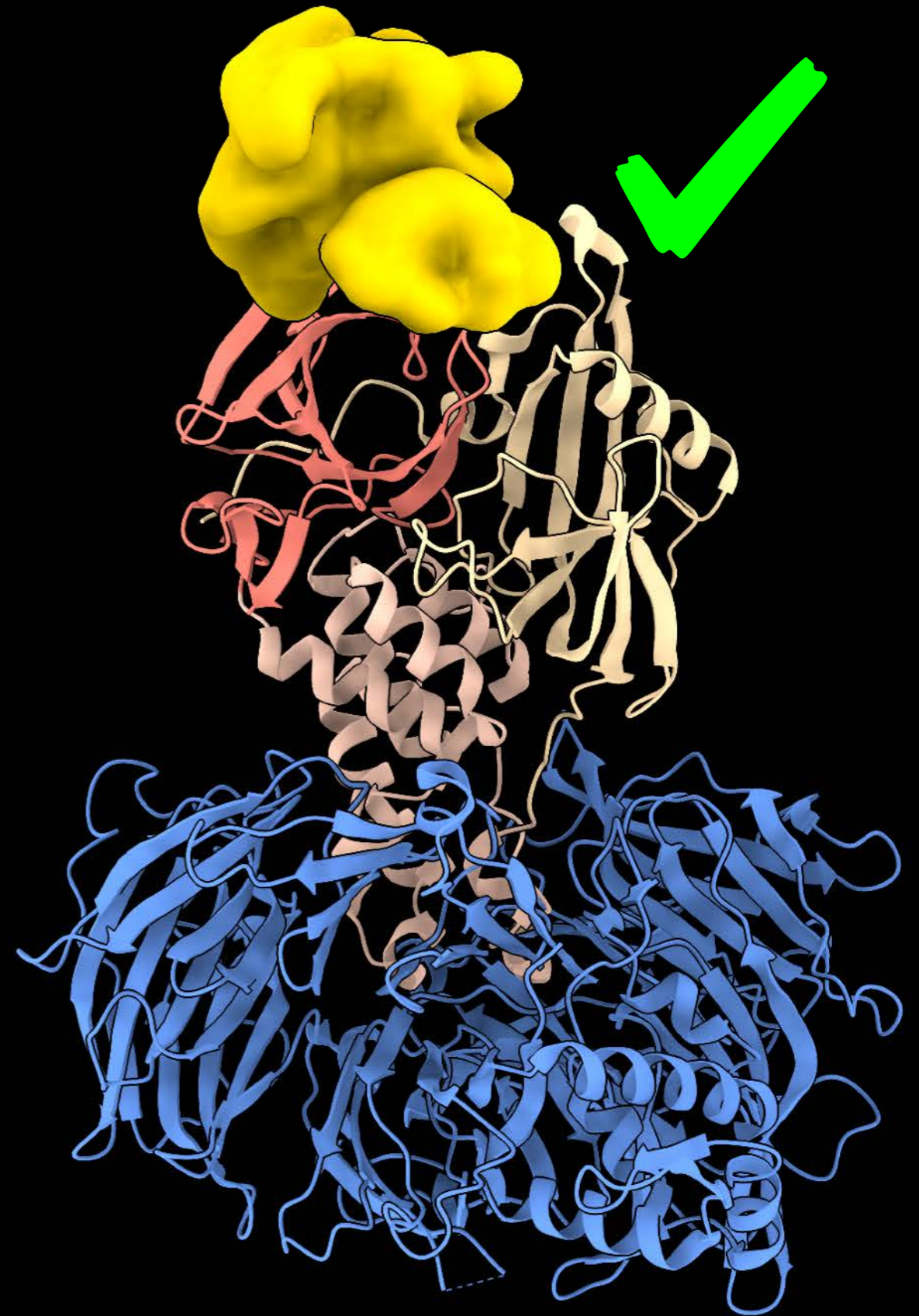
Loop
rearrangement
prompts an
“open” to
“closed”
transition





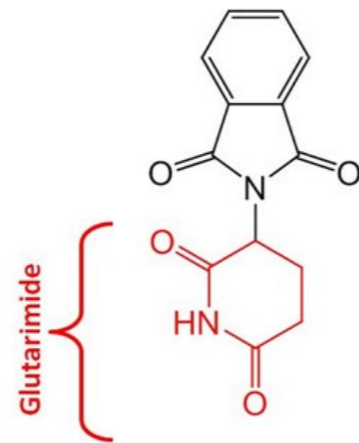
Becomes
organized
to form a
“safety belt”

ONLY THE CLOSED FORM BINDS TARGET PROTEINS



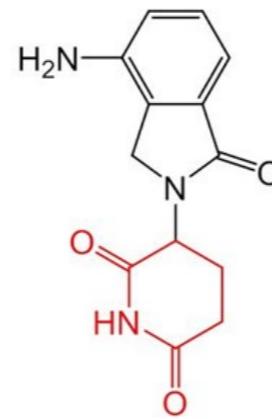
THALOMID[®]
(thalidomide) Capsules

thalidomide



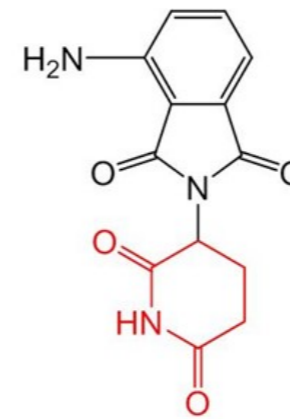
Revlimid[®]
(lenalidomide) capsules

lenalidomide



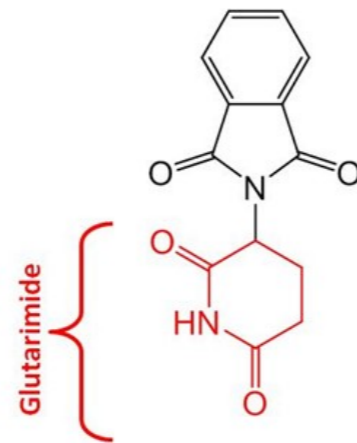
Pomalyst[®]
(pomalidomide) capsules

pomalidomide



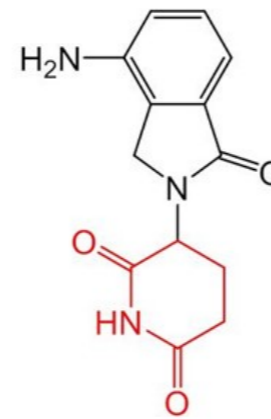
THALOMID[®]
(thalidomide) Capsules

thalidomide



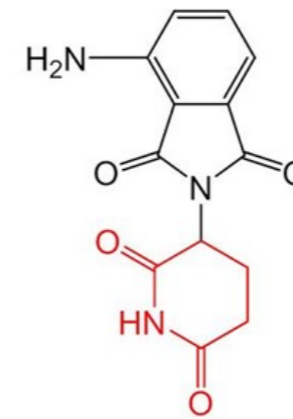
Revlimid[®]
(lenalidomide) capsules

lenalidomide

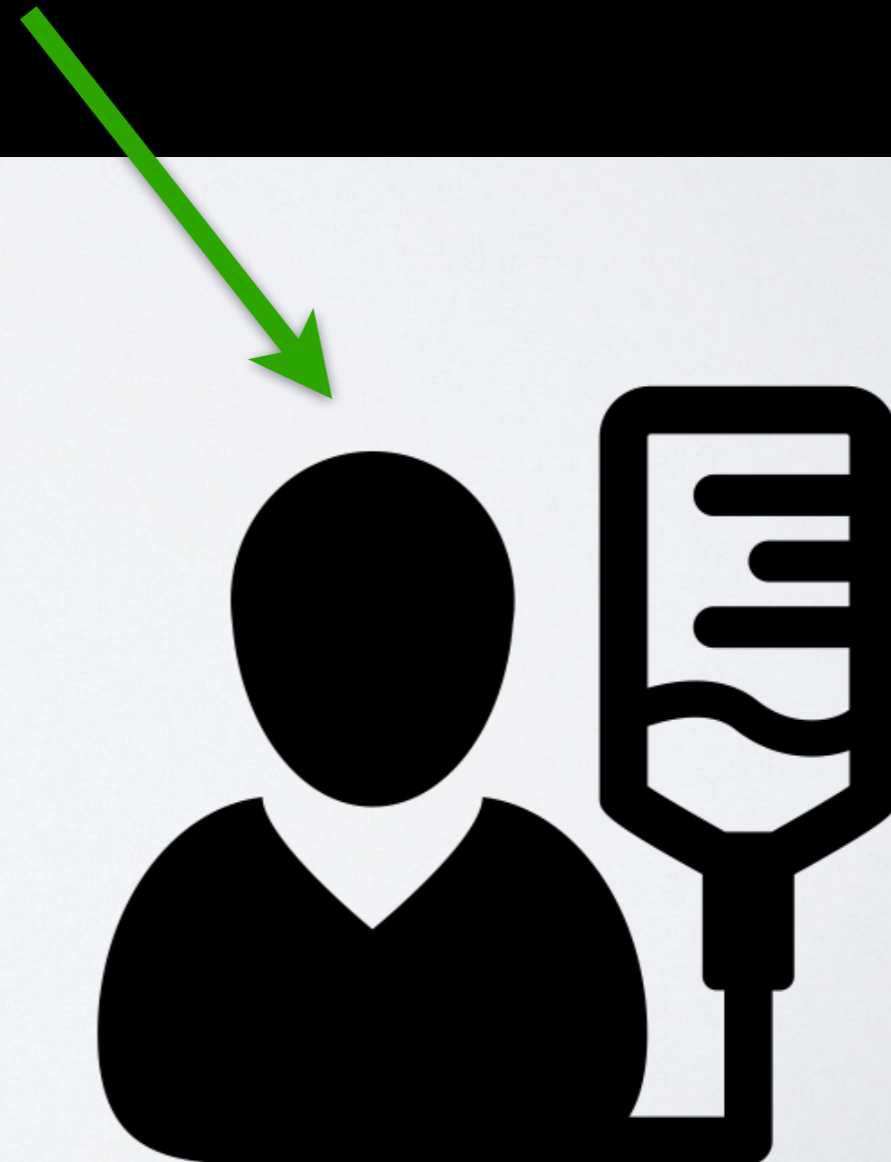


Pomalyst[®]
(pomalidomide) capsules

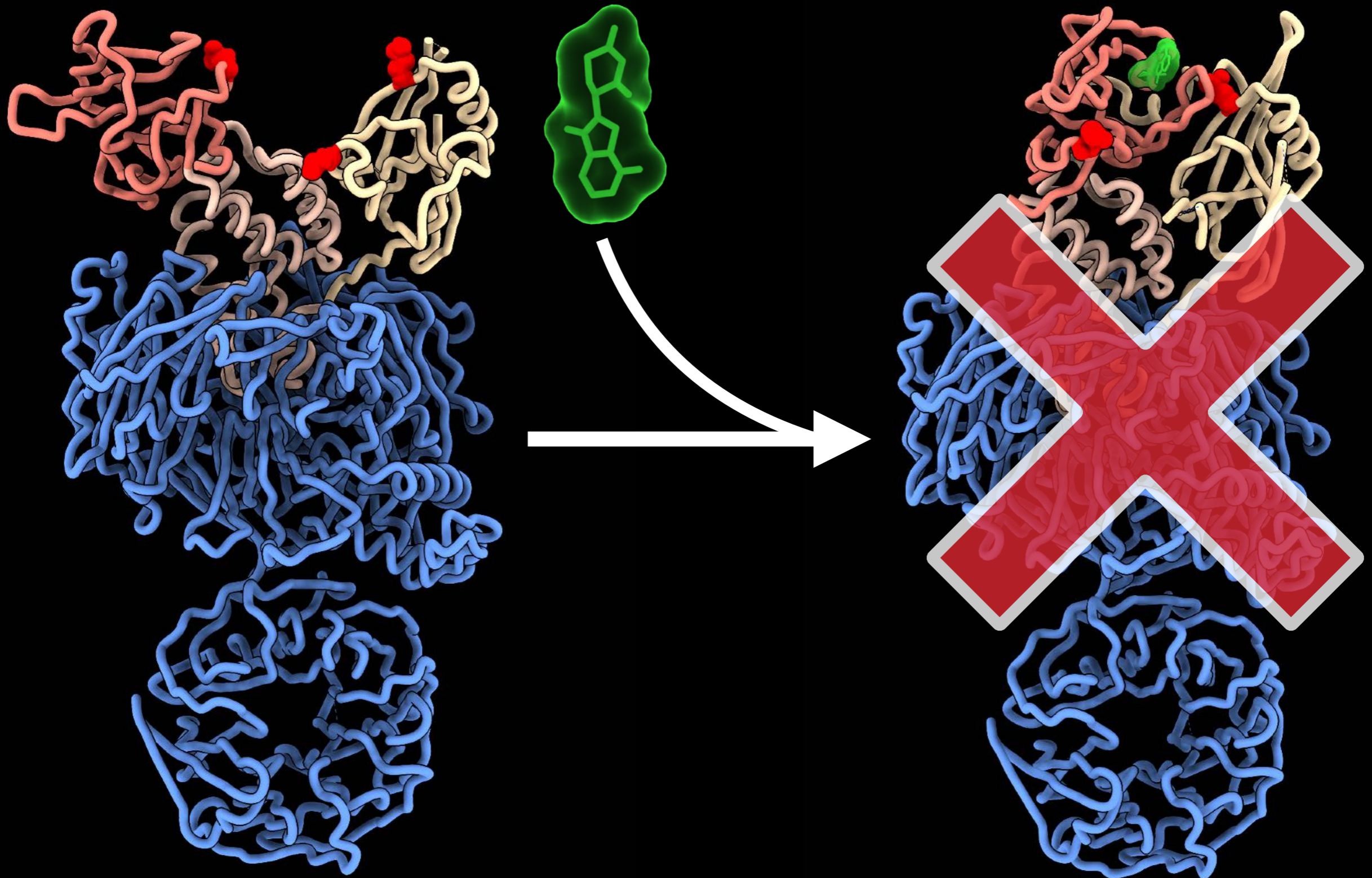
pomalidomide



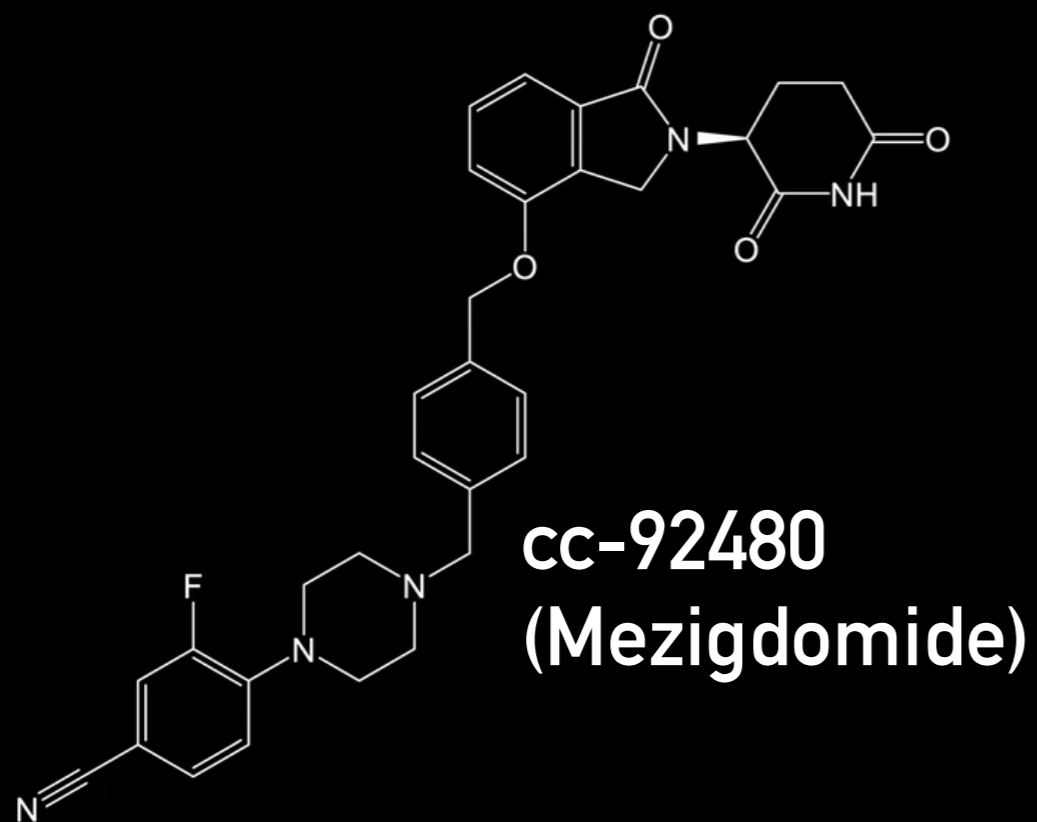
NO RESPONSE



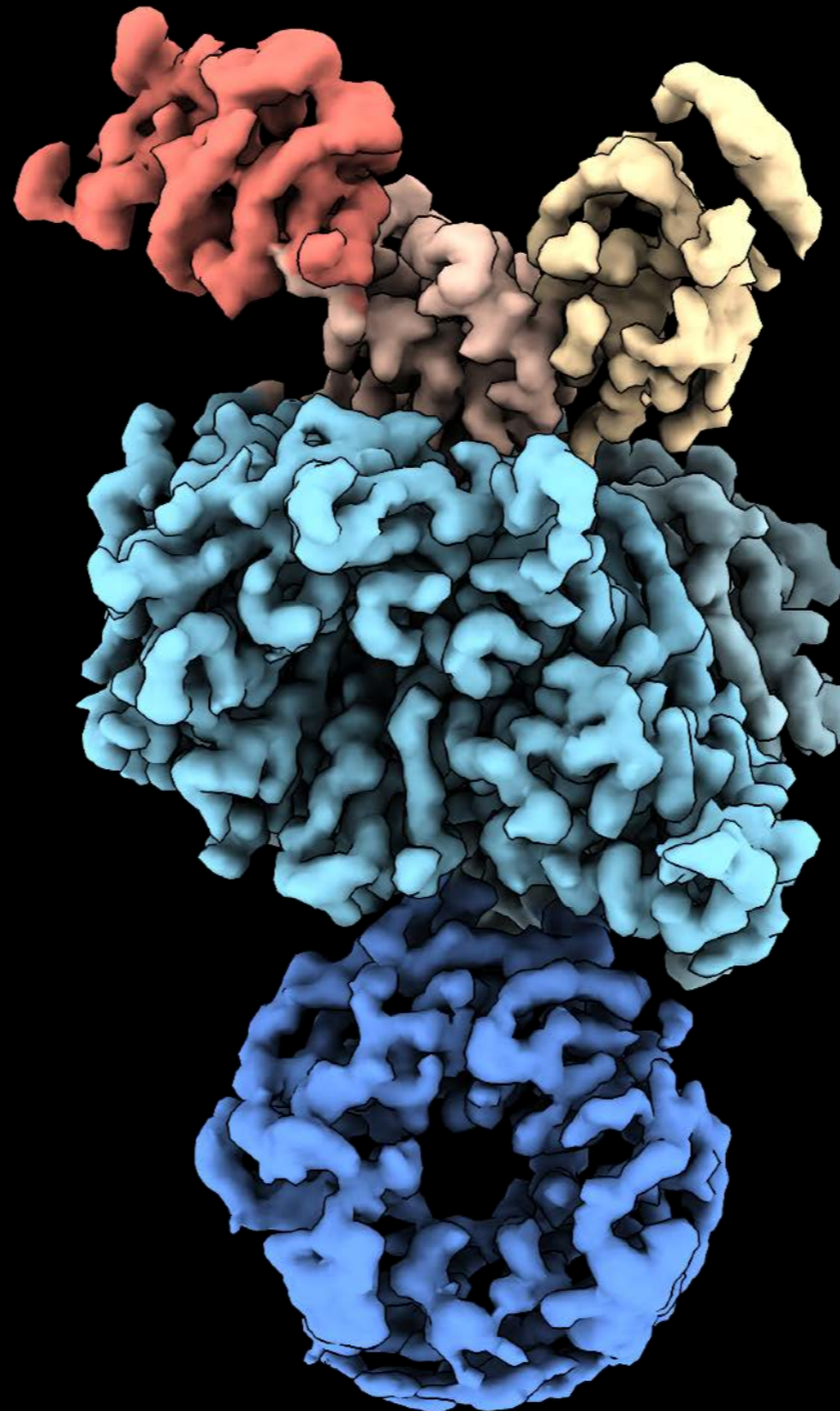
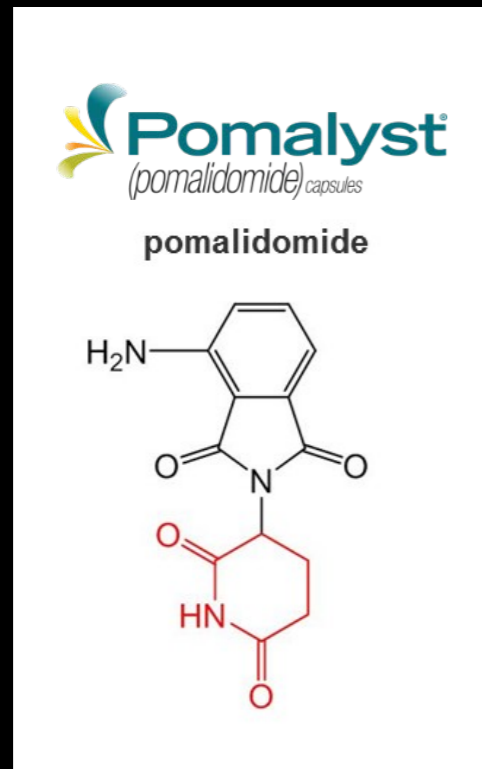
PATIENT **MUTATIONS** MAY PREVENT CLOSED FORM



TEAMING UP WITH BRISTOL MYERS SQUIBB

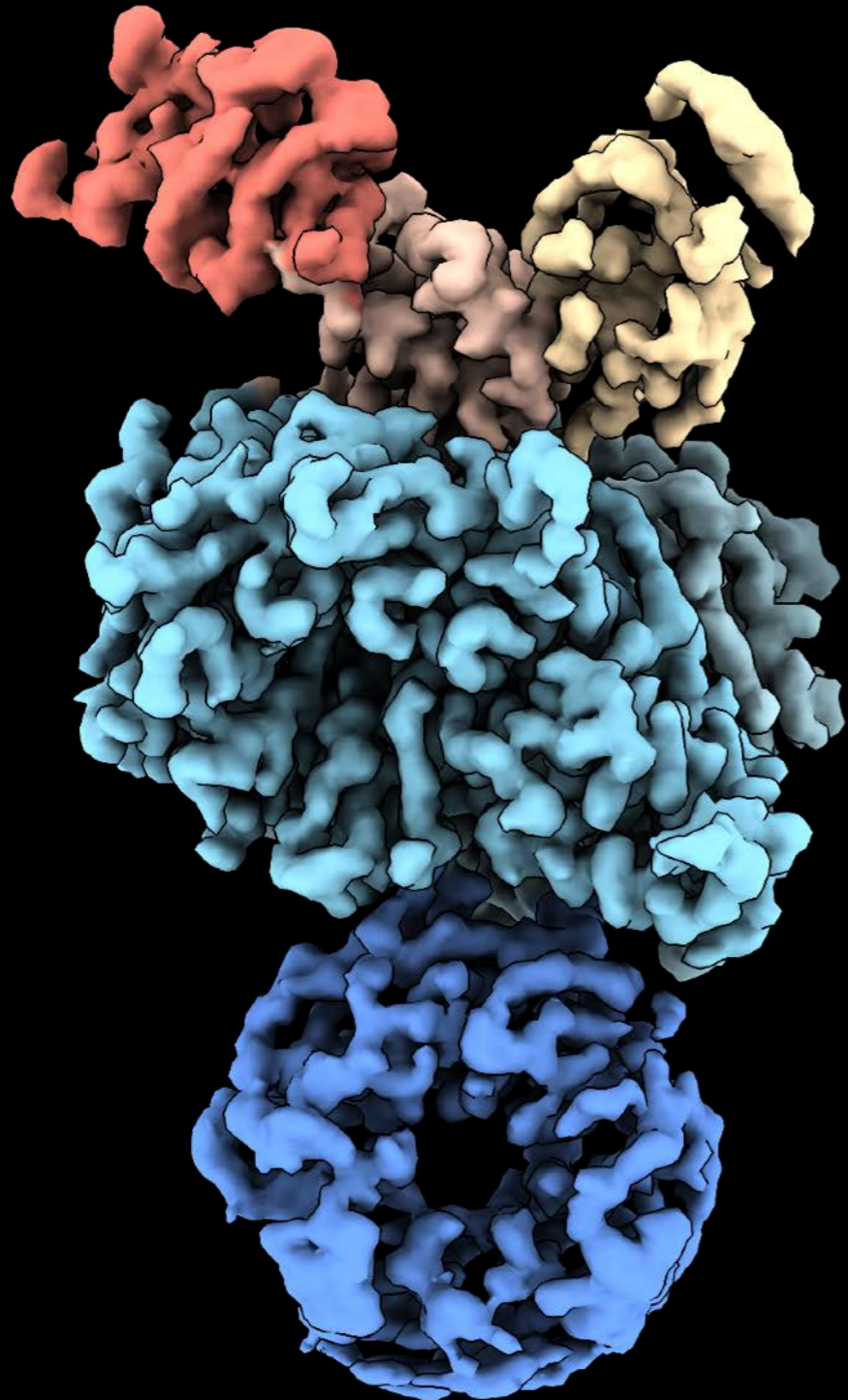


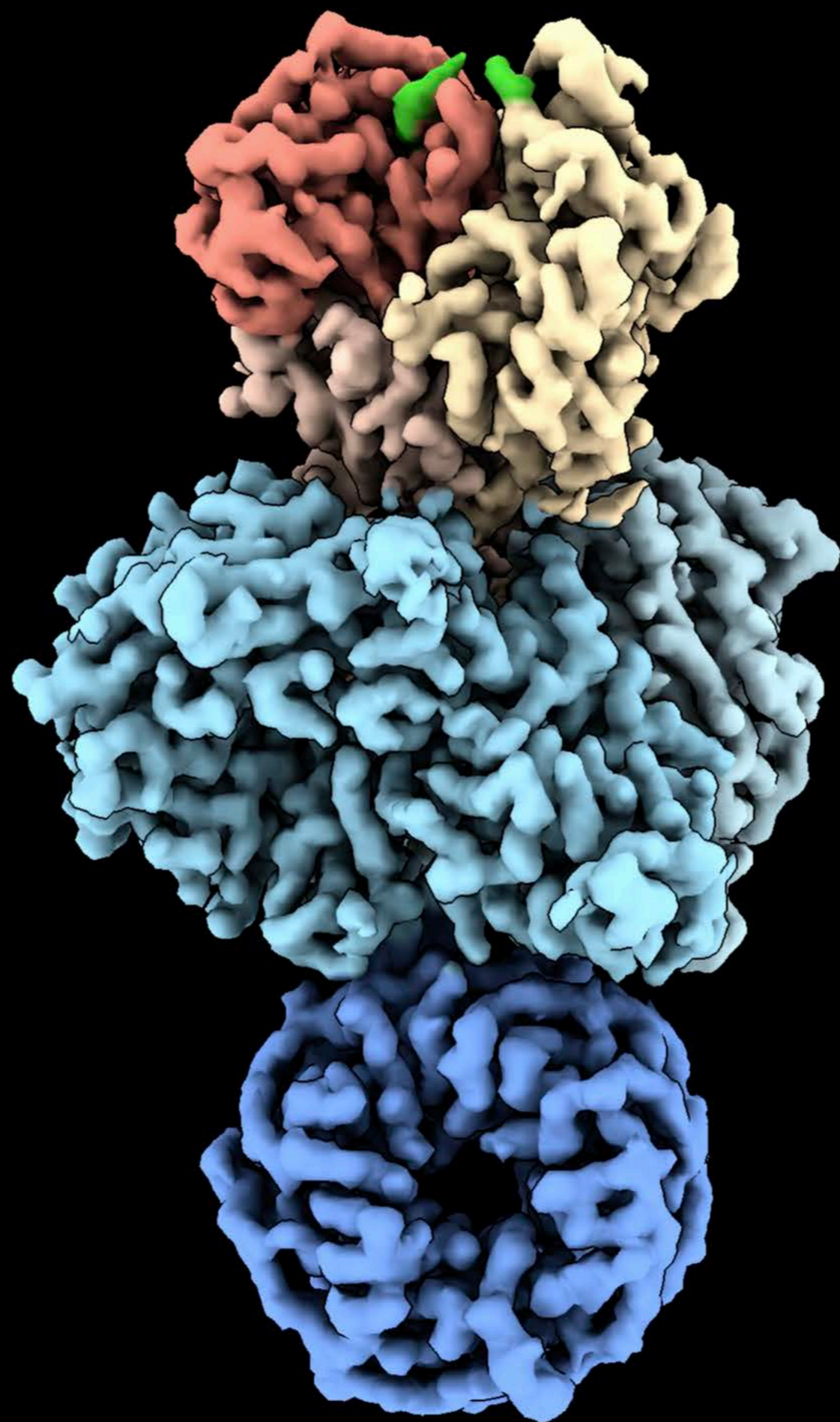
TRADITIONAL THERAPEUTIC + MUTANT CEREBLON



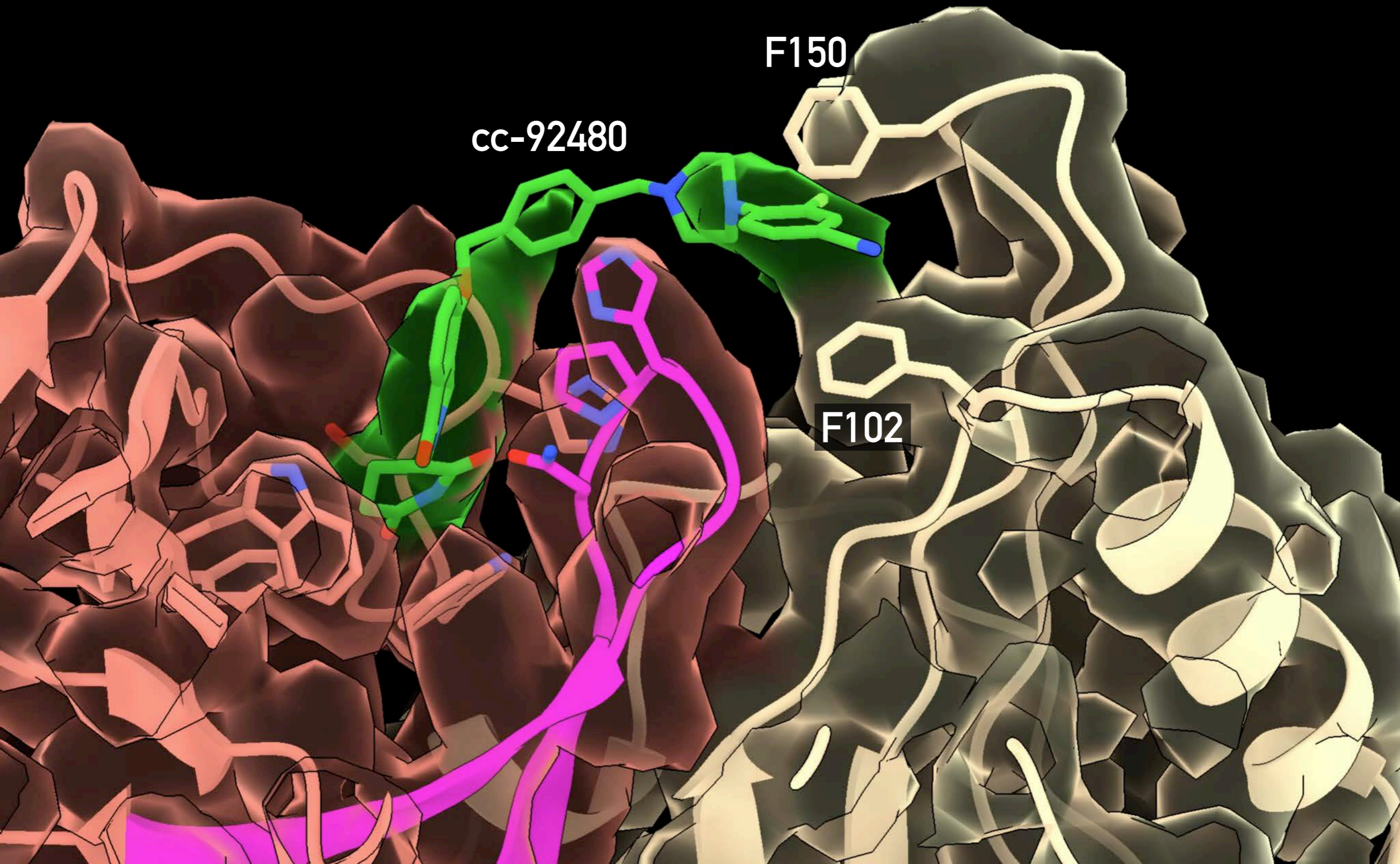
No closure
of Cereblon

TRADITIONAL THERAPEUTIC + **MUTANT** CEREBLON





MEZIGDOMIDE IS A MOLECULAR “STAPLE”



IF THIS IS YOUR TARGET...

**IT IS IMPORTANT TO
UNDERSTAND THIS**



SUMMARY

- ▶ **Part 1:** We can see the invisible!
- ▶ **Part 2:** There are a variety of fascinating molecular machines involved in maintaining protein homeostasis.
- ▶ **Part 3:** Visualizing molecular structures and their motions is critical to designing more effective therapeutics.

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