



THE FRONT ROW
at Scripps Research

VISUALIZING THE INVISIBLE MACHINERY OF

LIFE

&

DEATH

Gabe Lander

 Scripps Research

Integrative Structural and Computational Biology

 @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

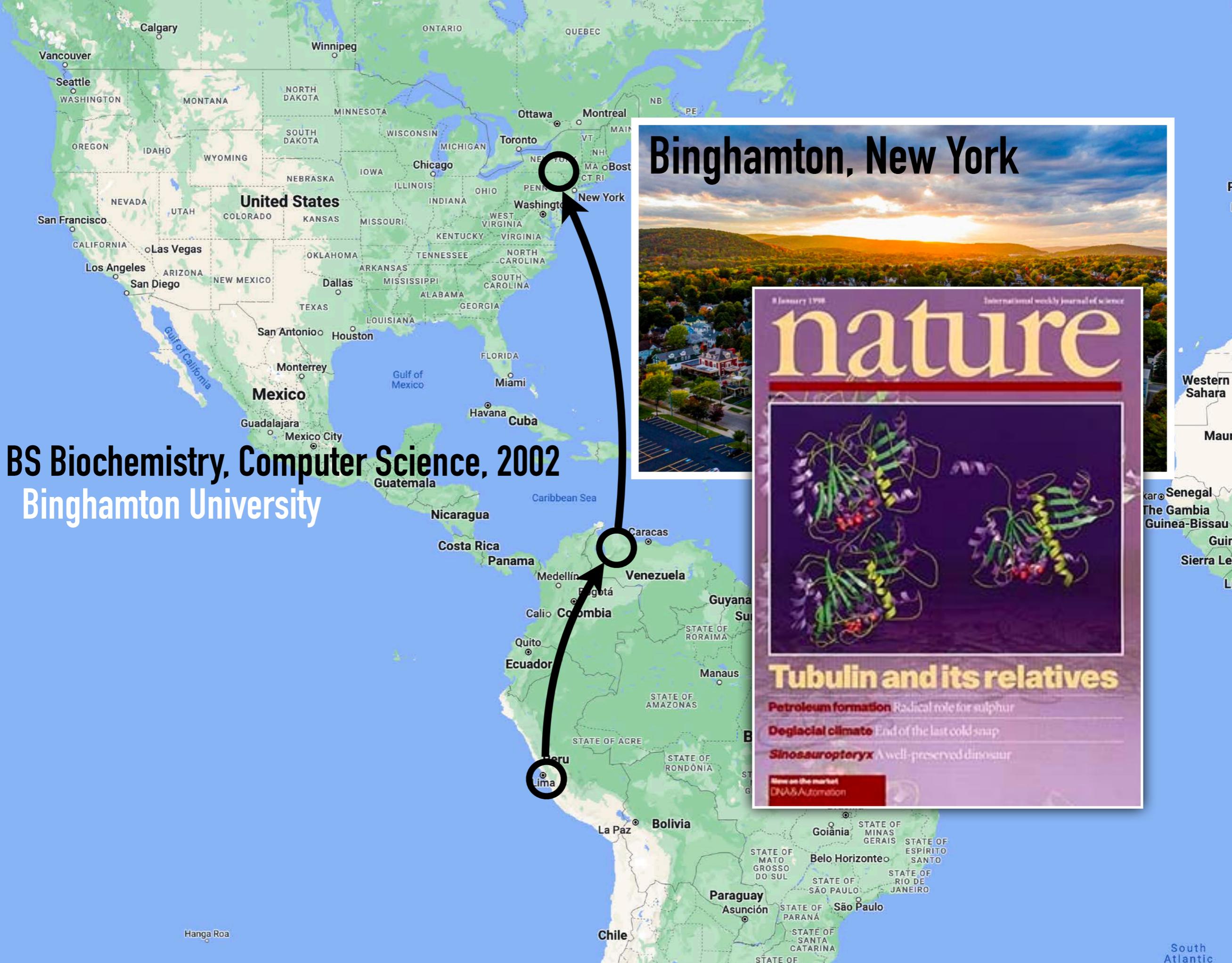
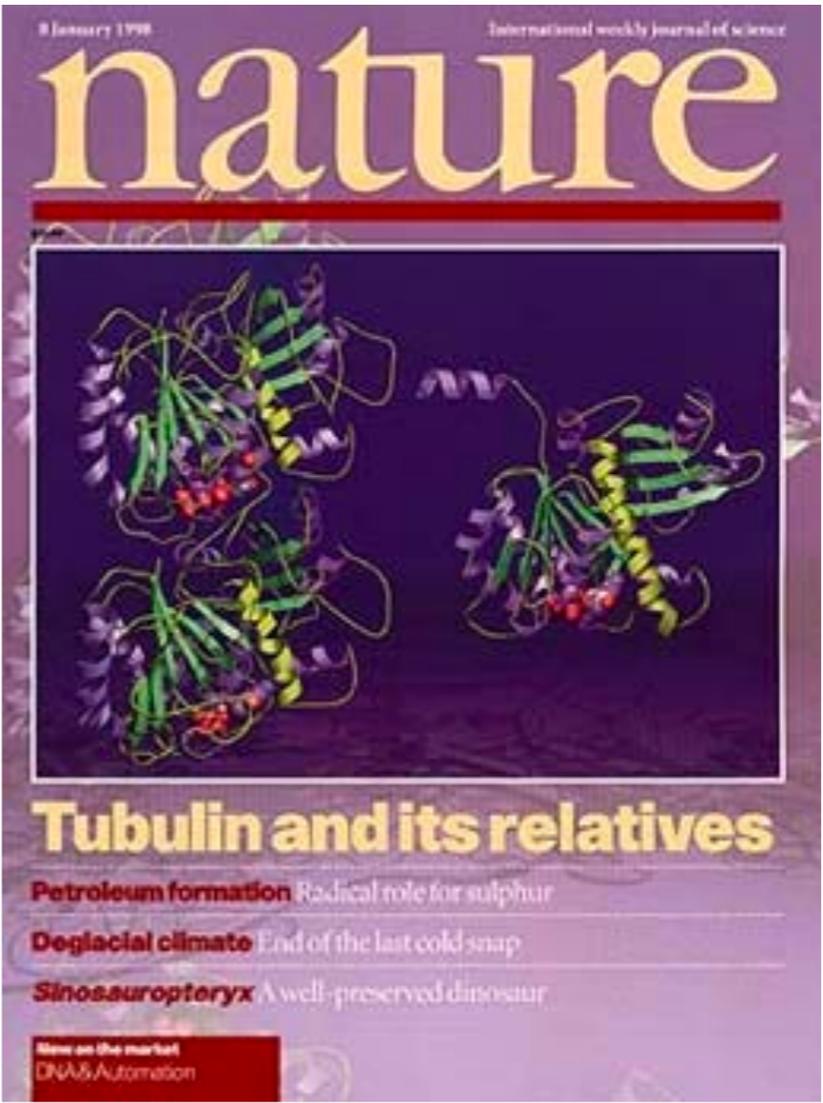


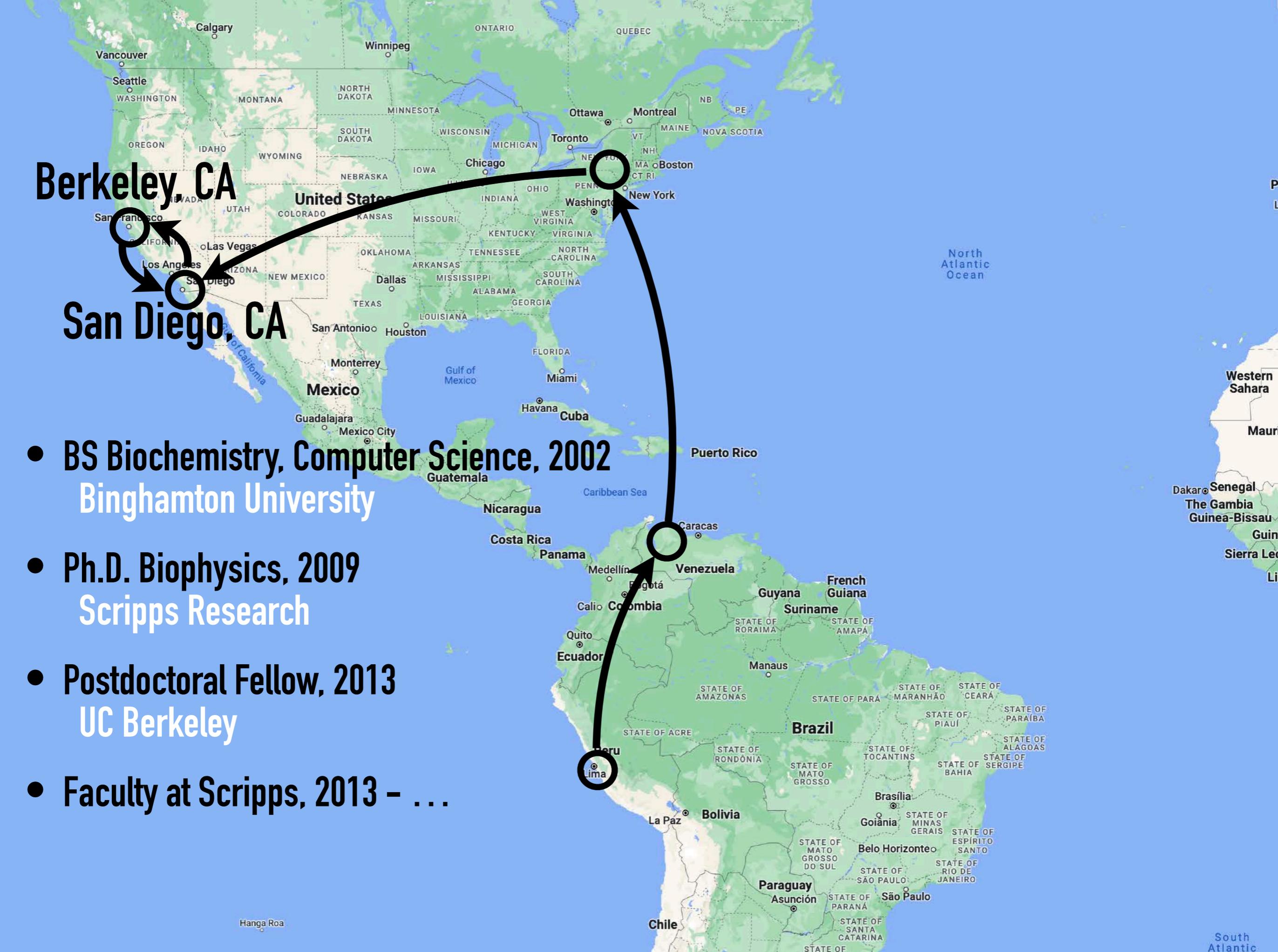
Lima, Peru



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

Binghamton, New York





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

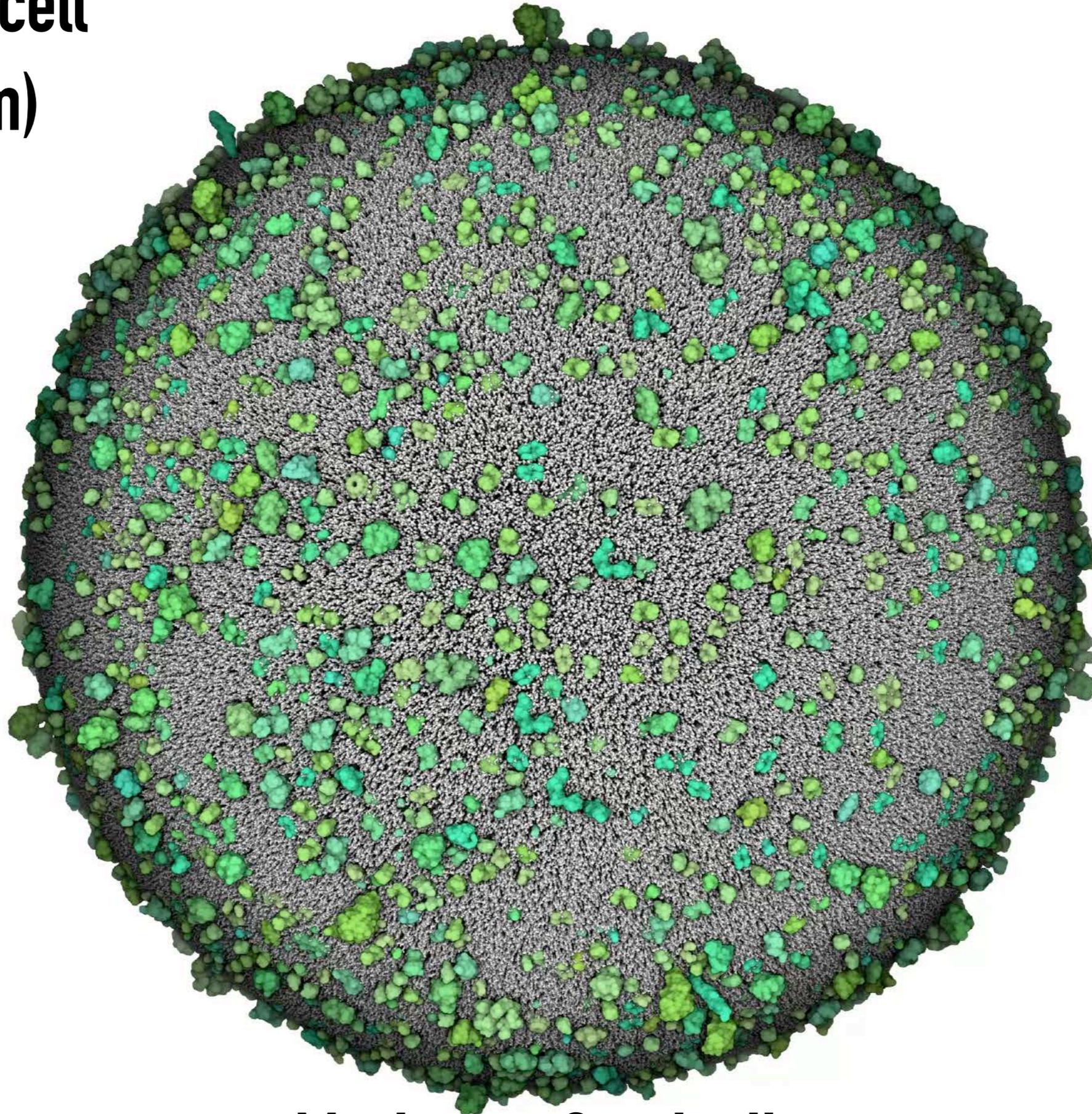
- ▶ **Part 1:** How do we see the invisible?
- ▶ **Part 2:** What are the key molecular machines involved in maintaining healthy protein levels?
- ▶ **Part 3:** How can we design better cancer therapeutics by looking at molecular machines?

HOW SMALL IS “INVISIBLE”



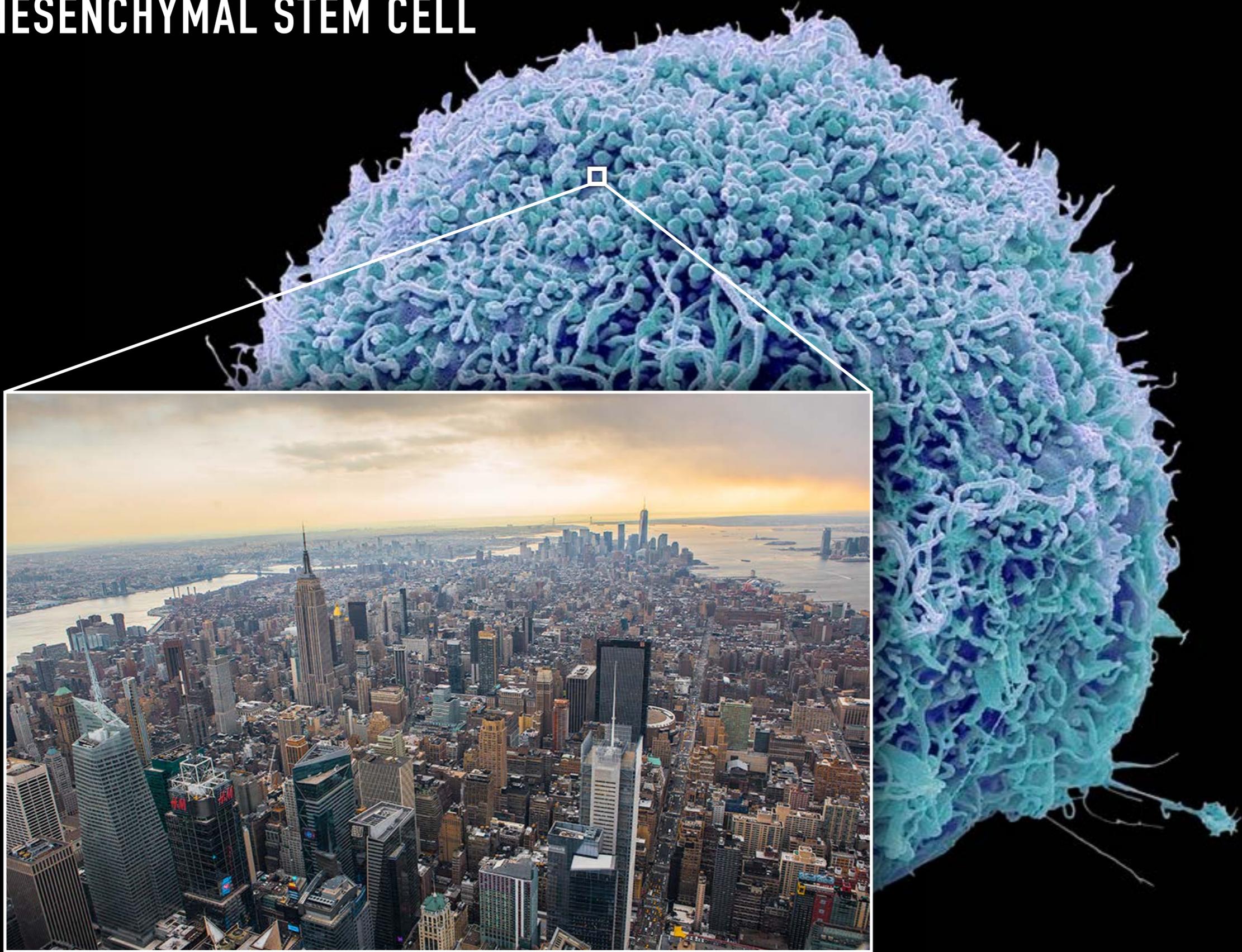
**objects 0.04 mm wide
(the width of a fine human hair)**

**Bacterial cell
(0.002 mm)**



Maritan & Goodsell

MESENCHYMAL STEM CELL



MANHATTAN, POPULATION ~1.6 MILLION

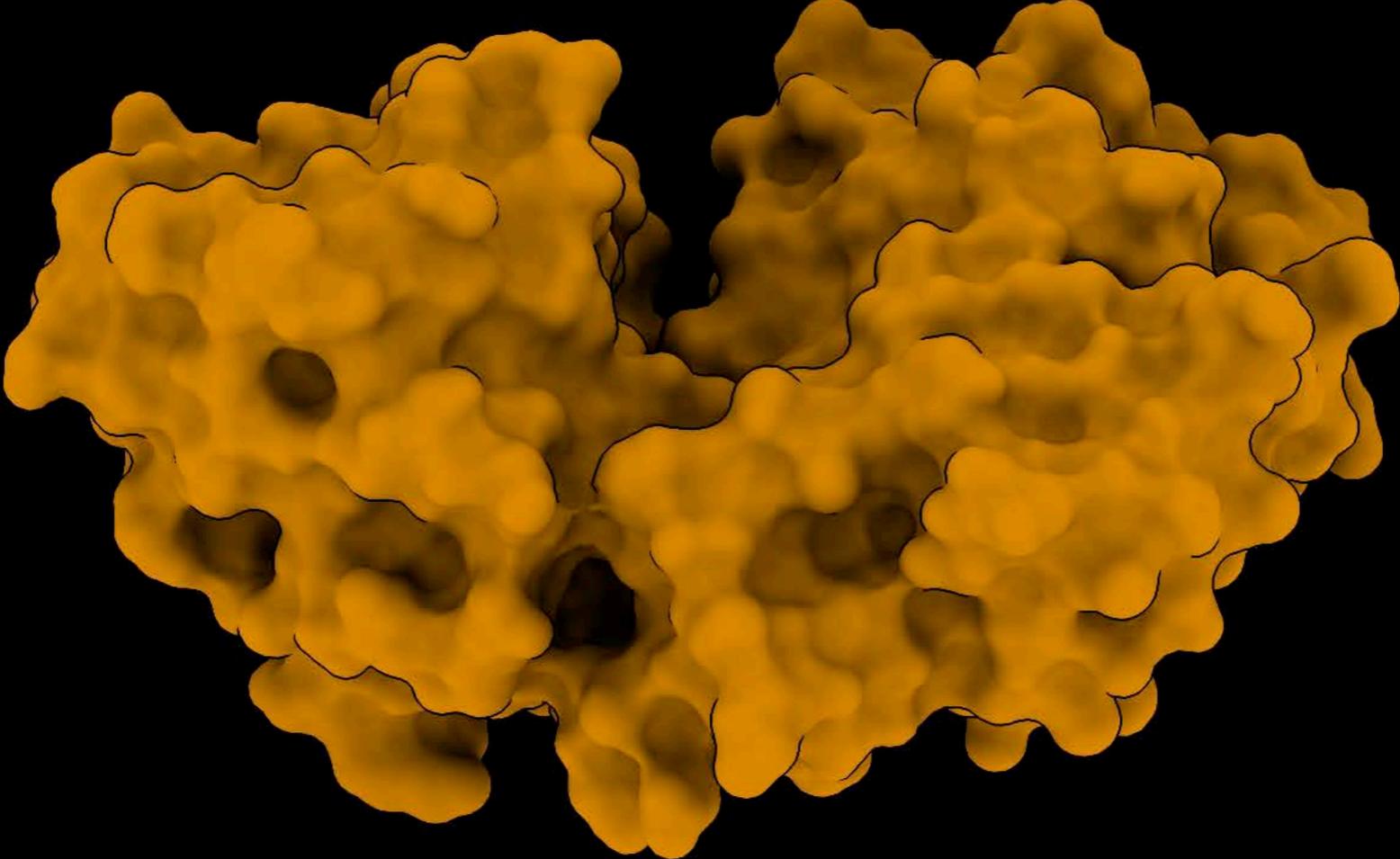


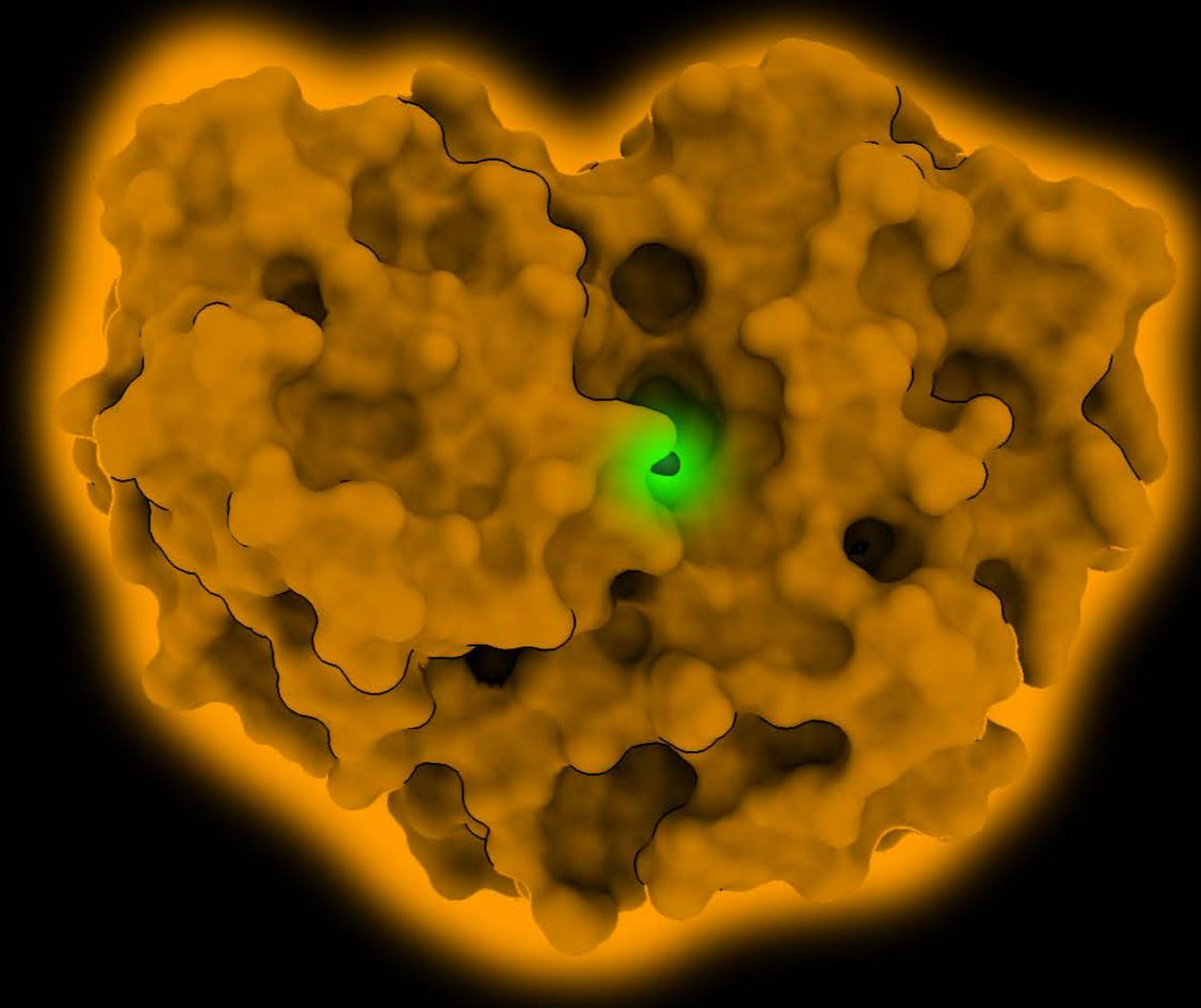
HOW DO WE LOOK AT SOMETHING
FORTY THOUSAND
TIMES SMALLER THAN A HAIR?



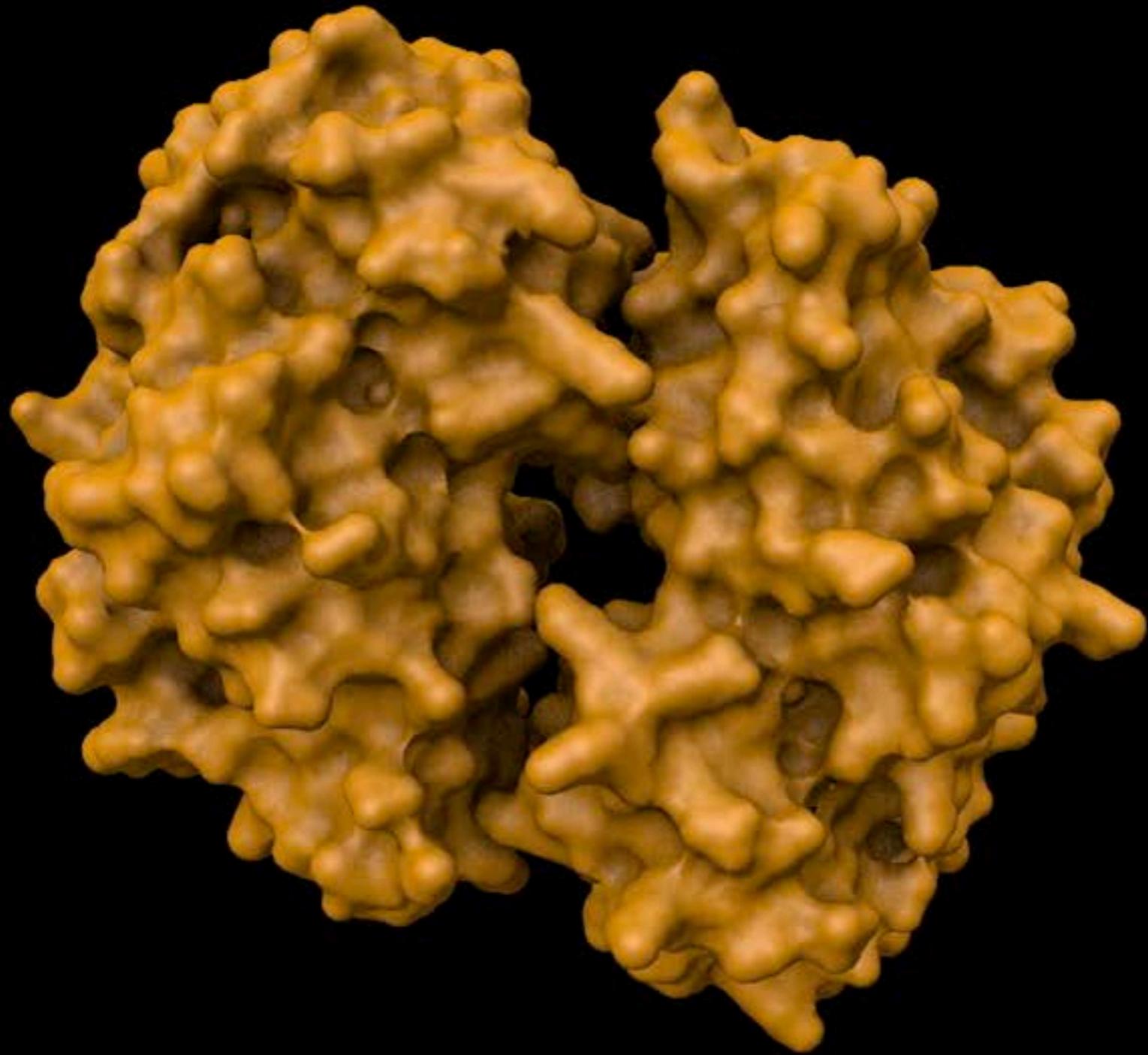
OPTION 1:

CRYSTALLOGRAPHY



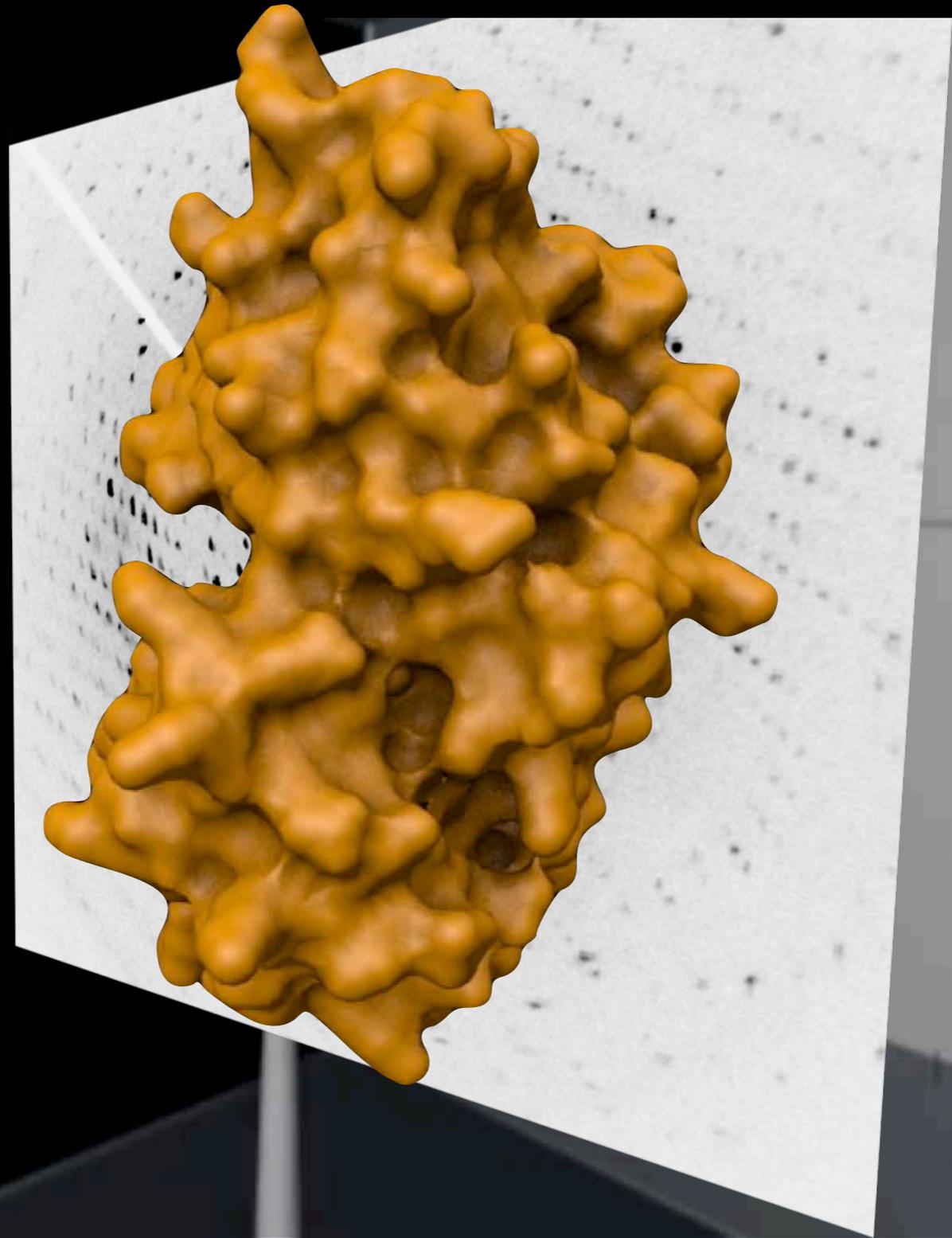








Ri

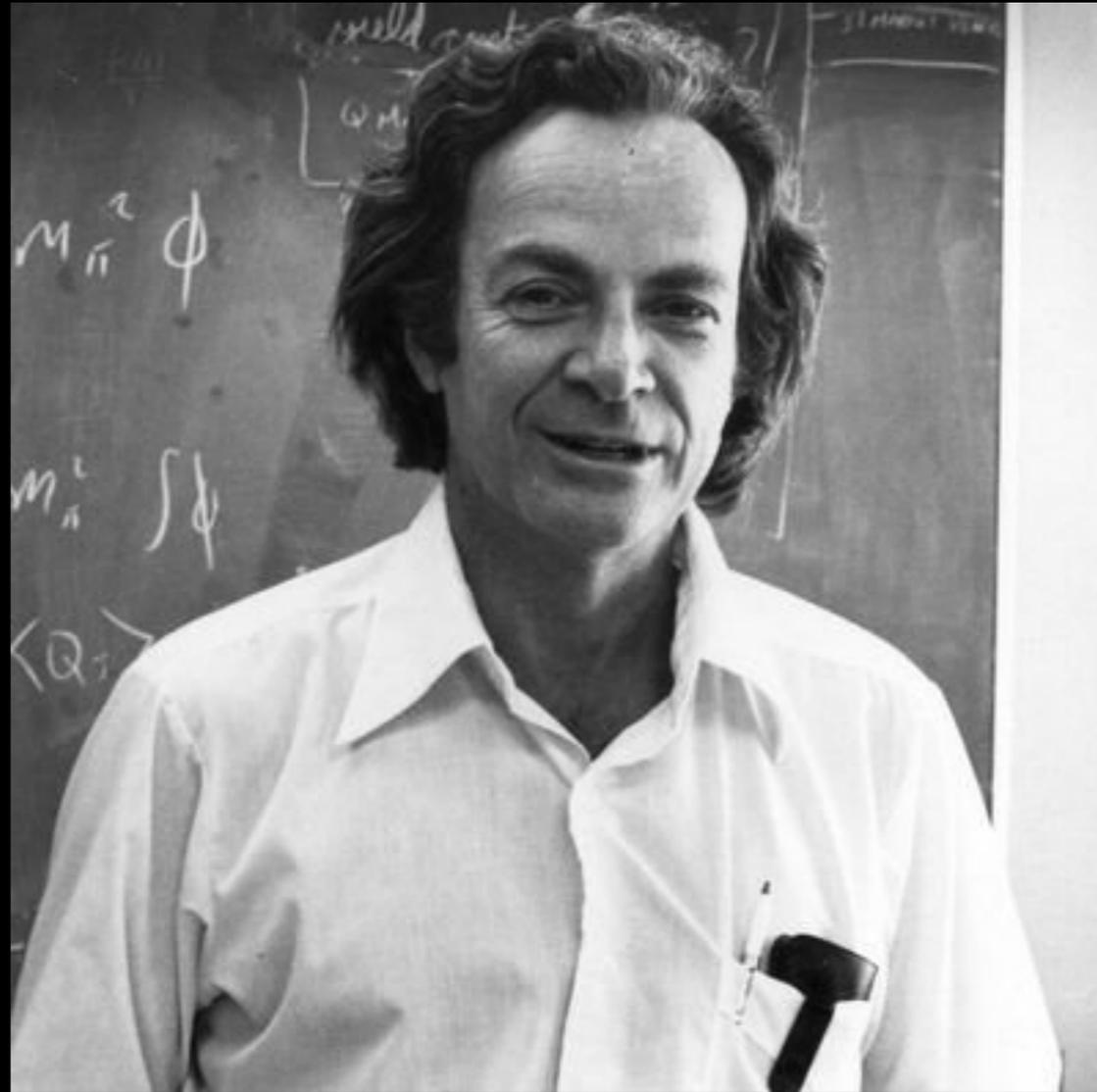




VS.



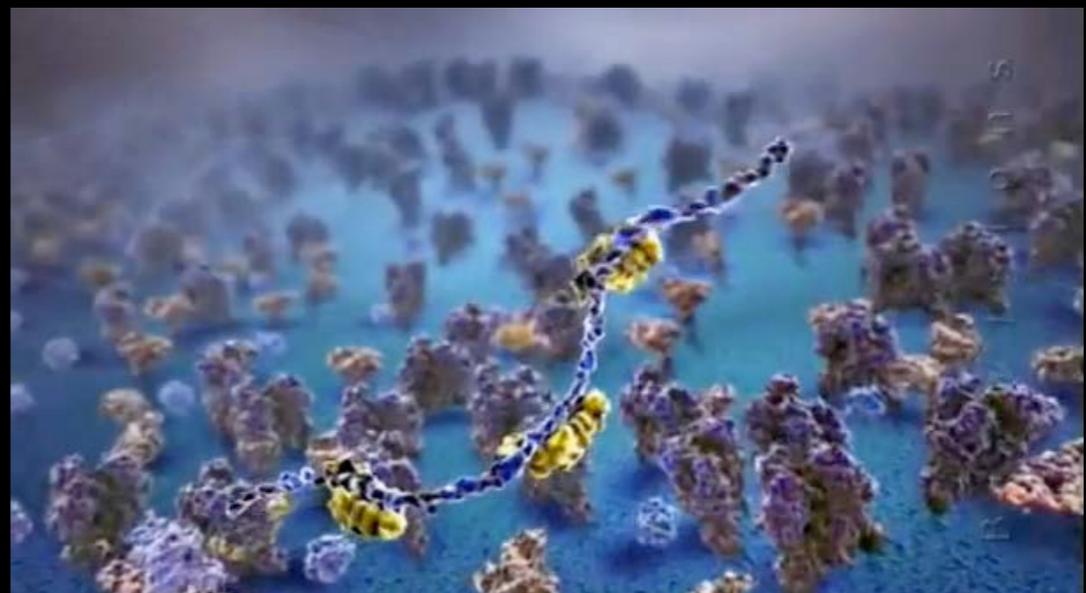
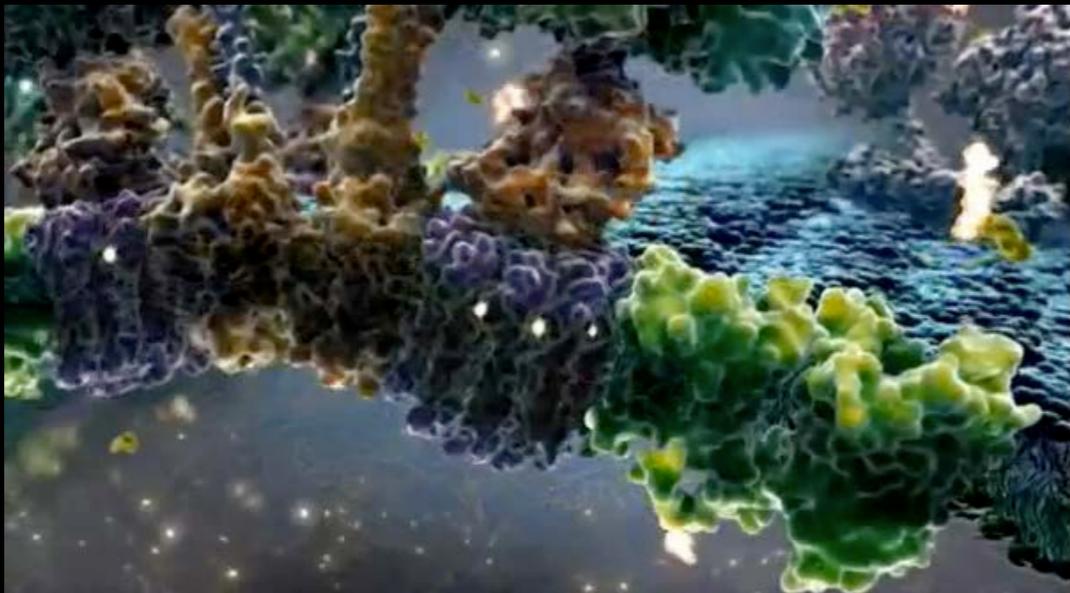
Richard P. Feynman's Solution



"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."

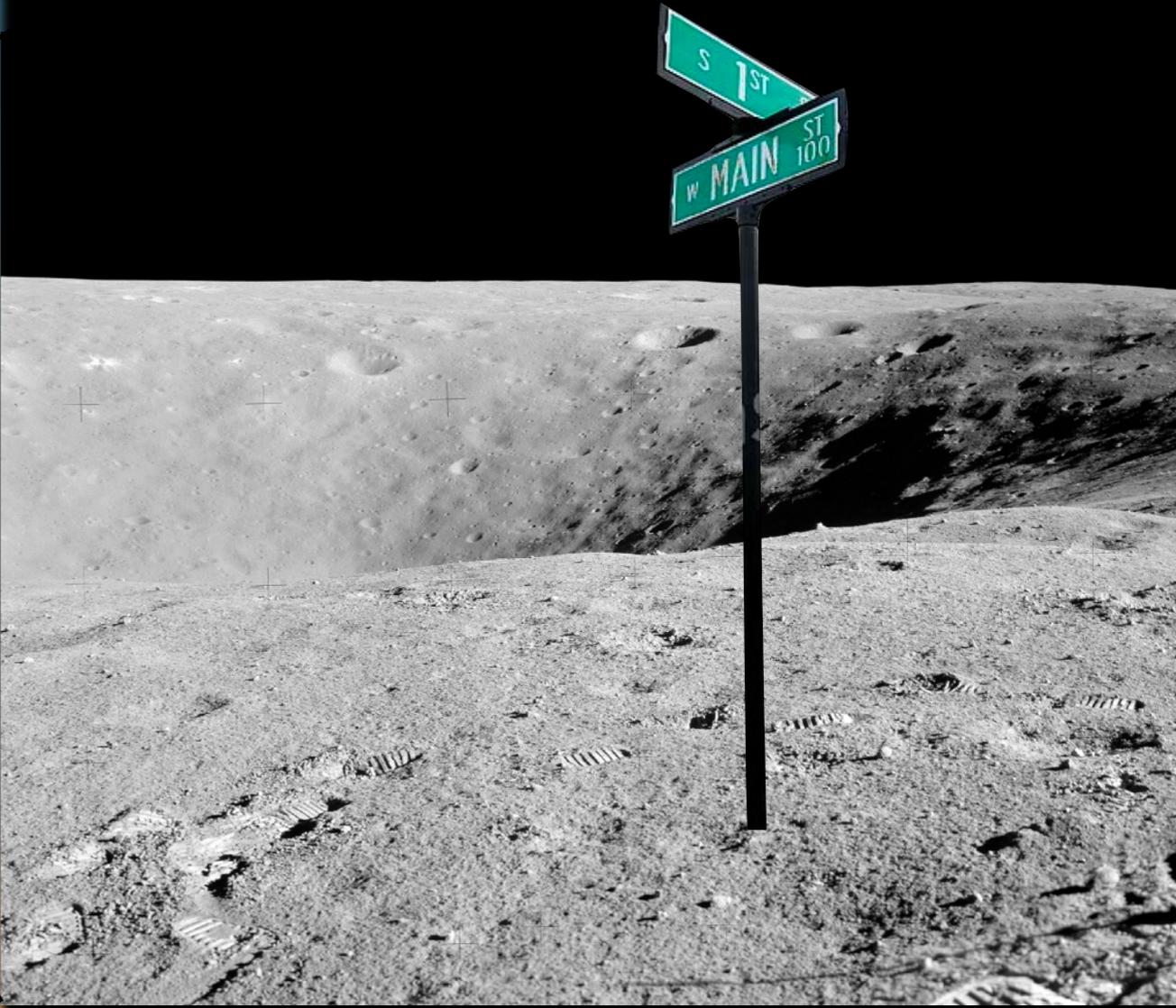
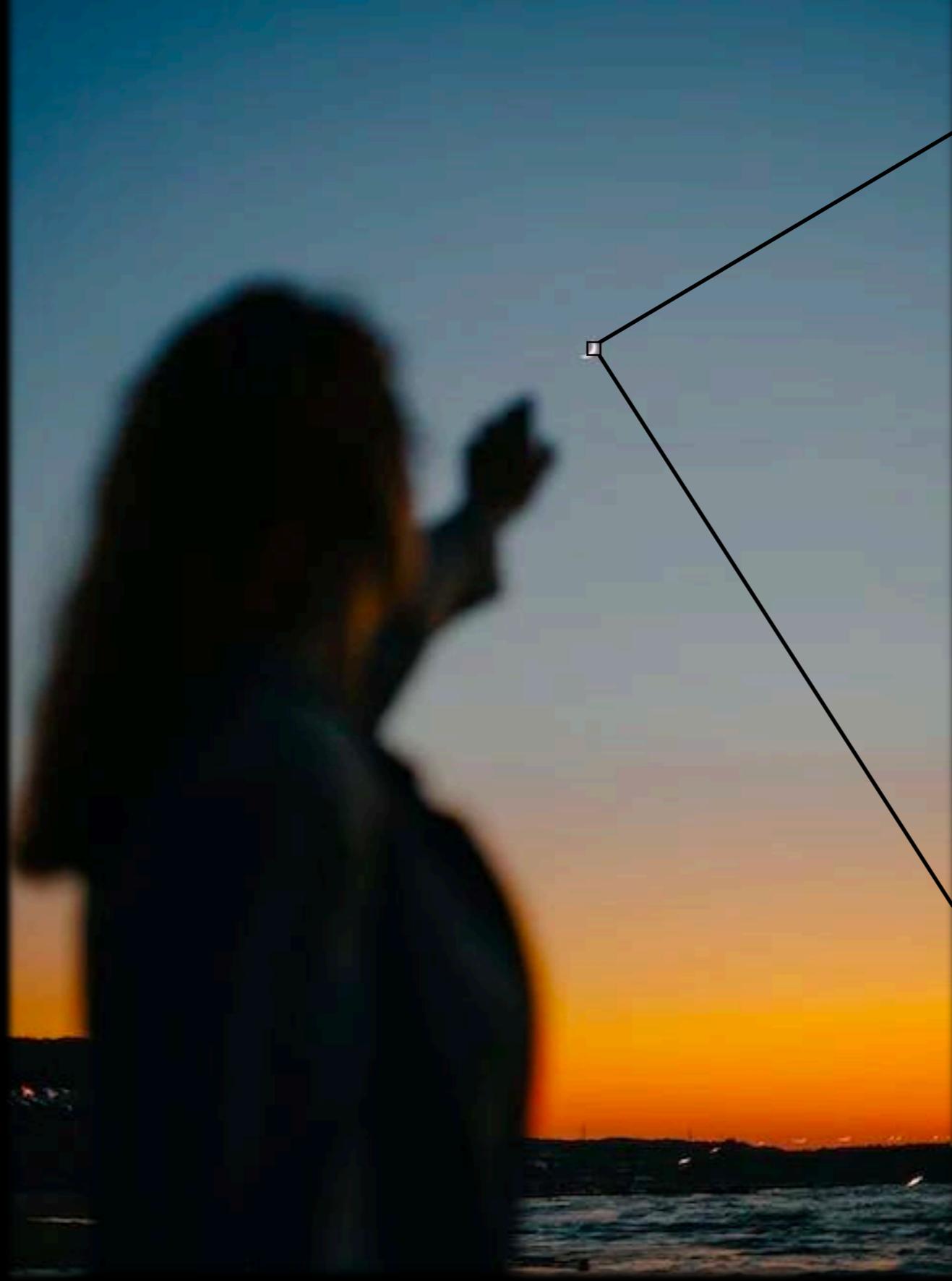
"There's Plenty of Room at the Bottom", a lecture given to the American Physical Society in 1959.

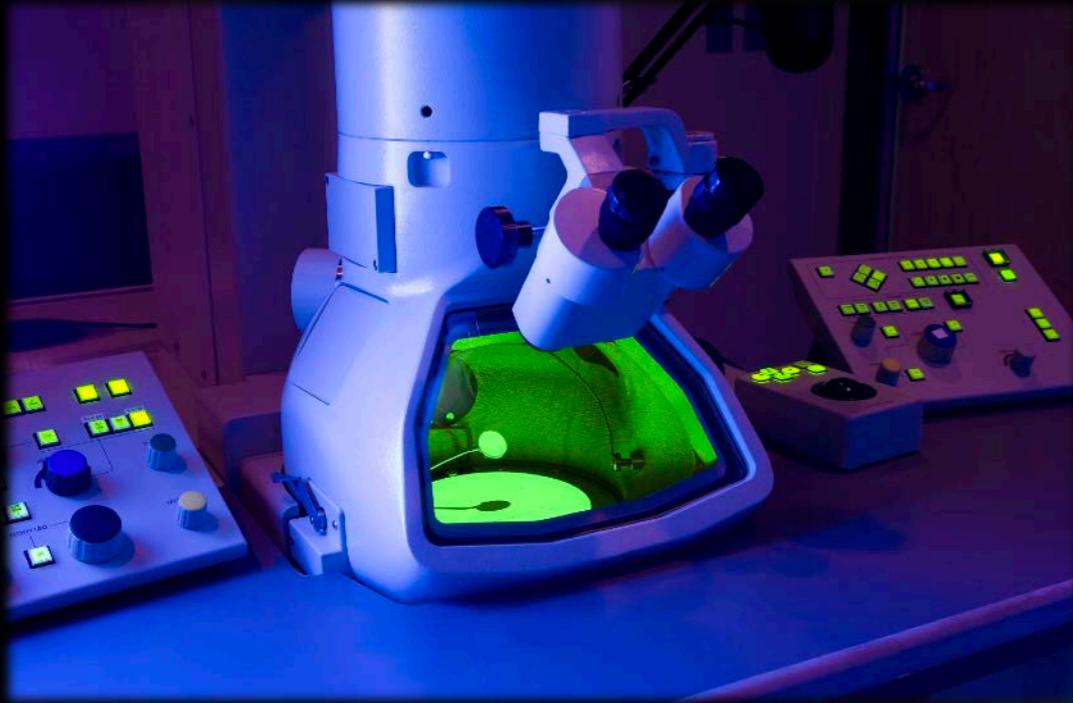
WHAT MAGNIFYING POWER DO WE NEED?



XVIVO medical animation

WHAT **MAGNIFYING** POWER DO WE NEED?

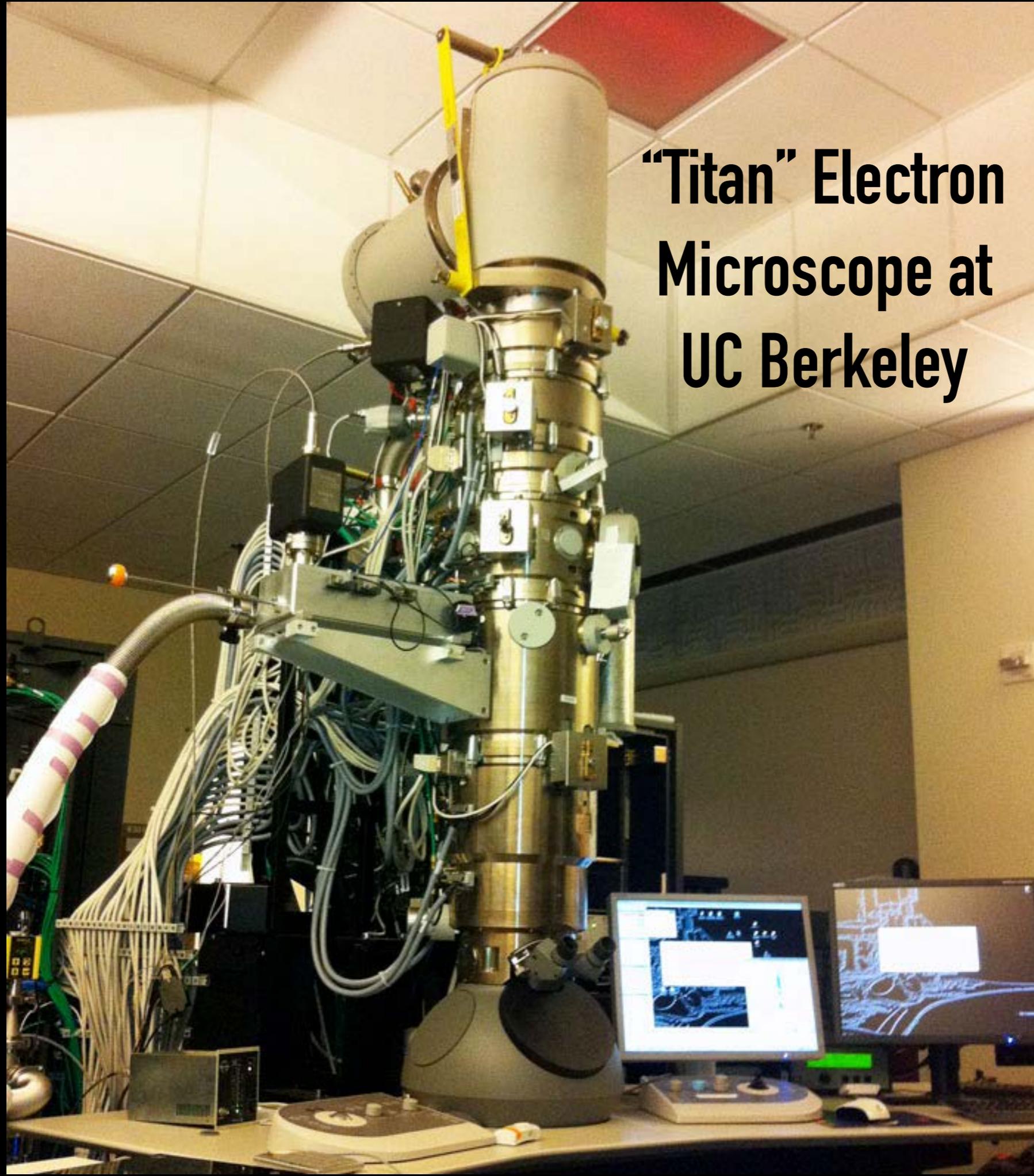




**OPTION 2:
CRYOGENIC ELECTRON
MICROSCOPY
(CRYO-EM)**



**“Titan” Electron
Microscope at
UC Berkeley**

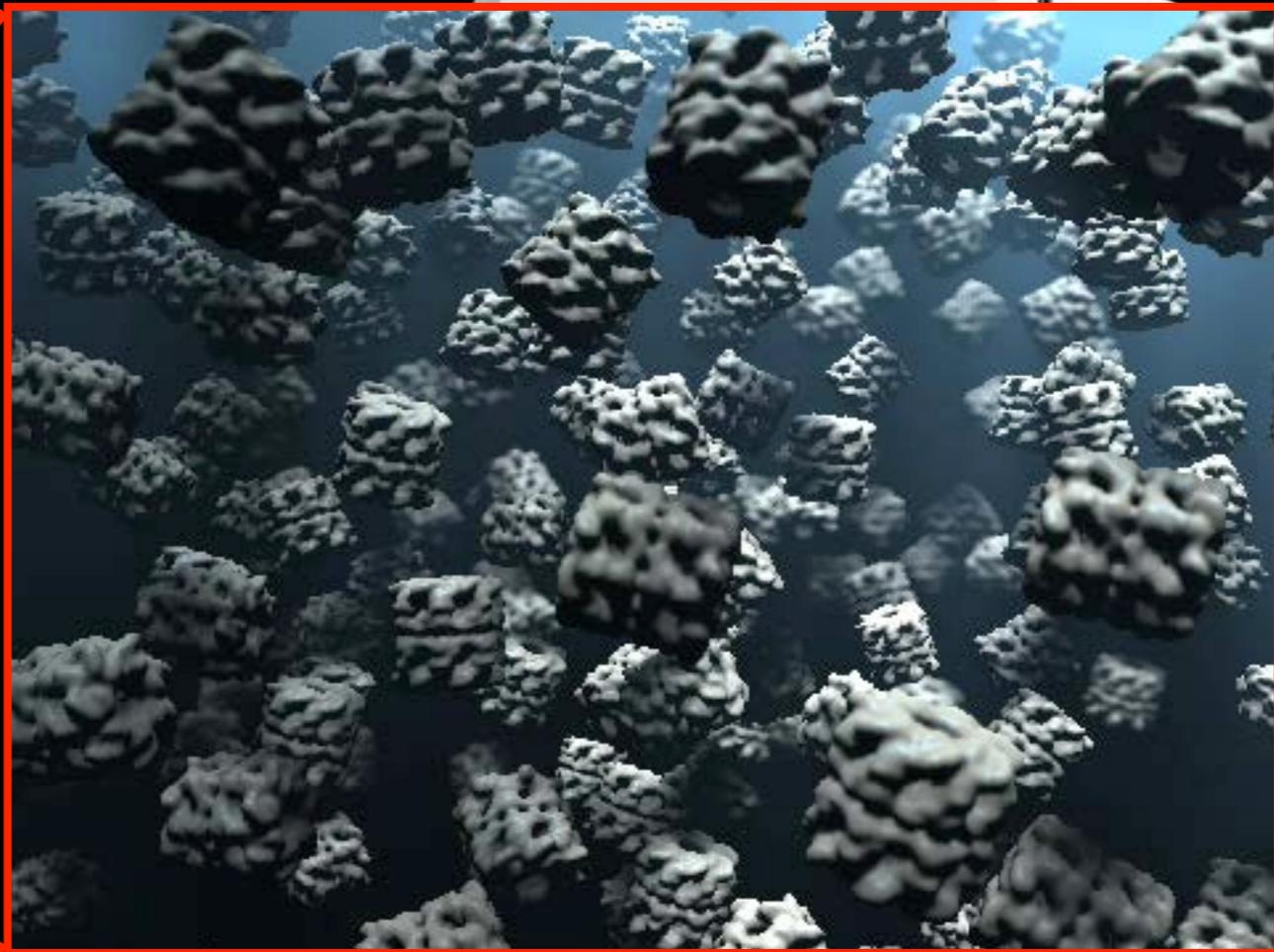


VISUALIZING MOLECULES AT THE
Scripps Research
**ELECTRON
MICROSCOPY FACILITY**

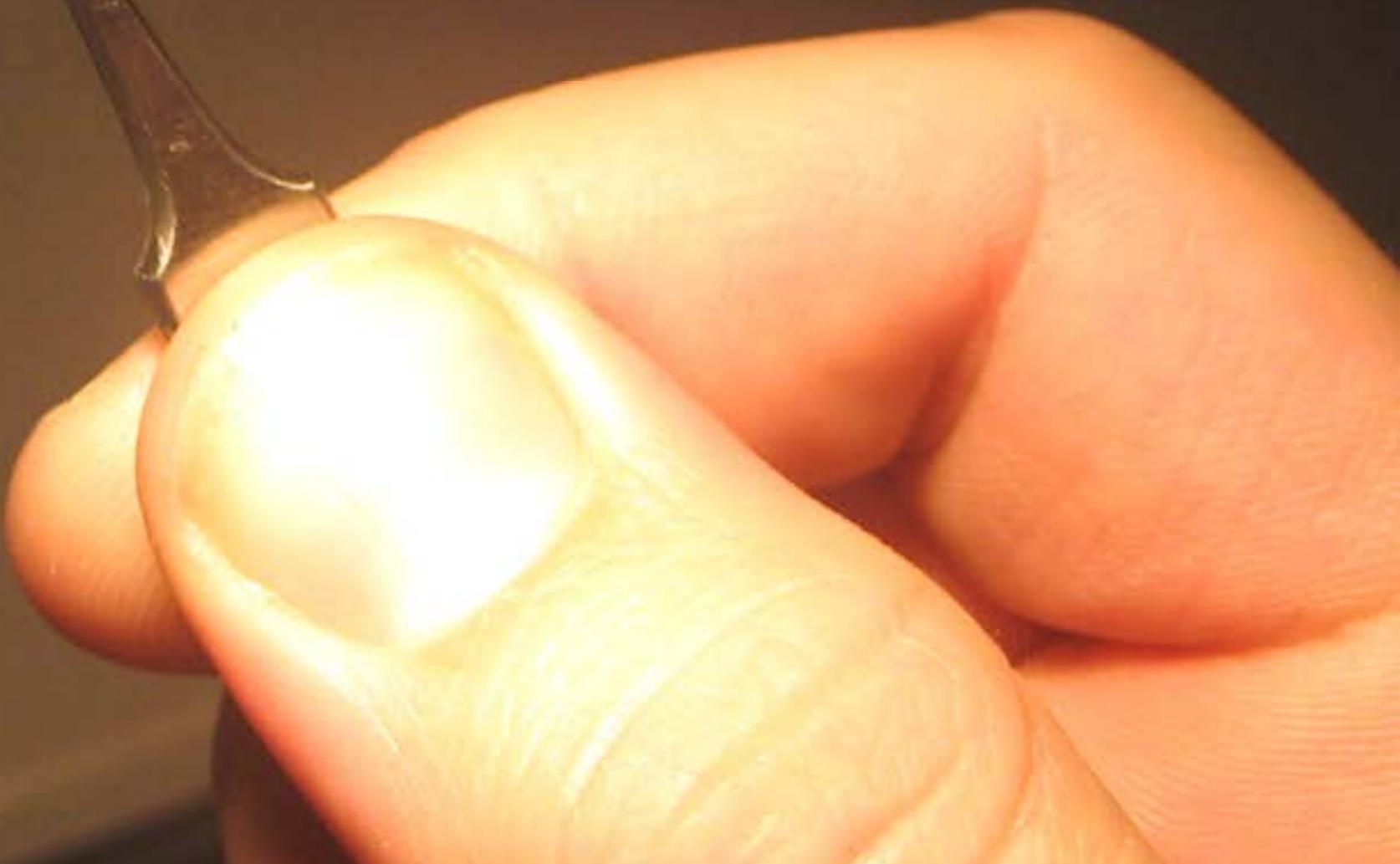




Protein sample



**3 millimeters
wide**



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



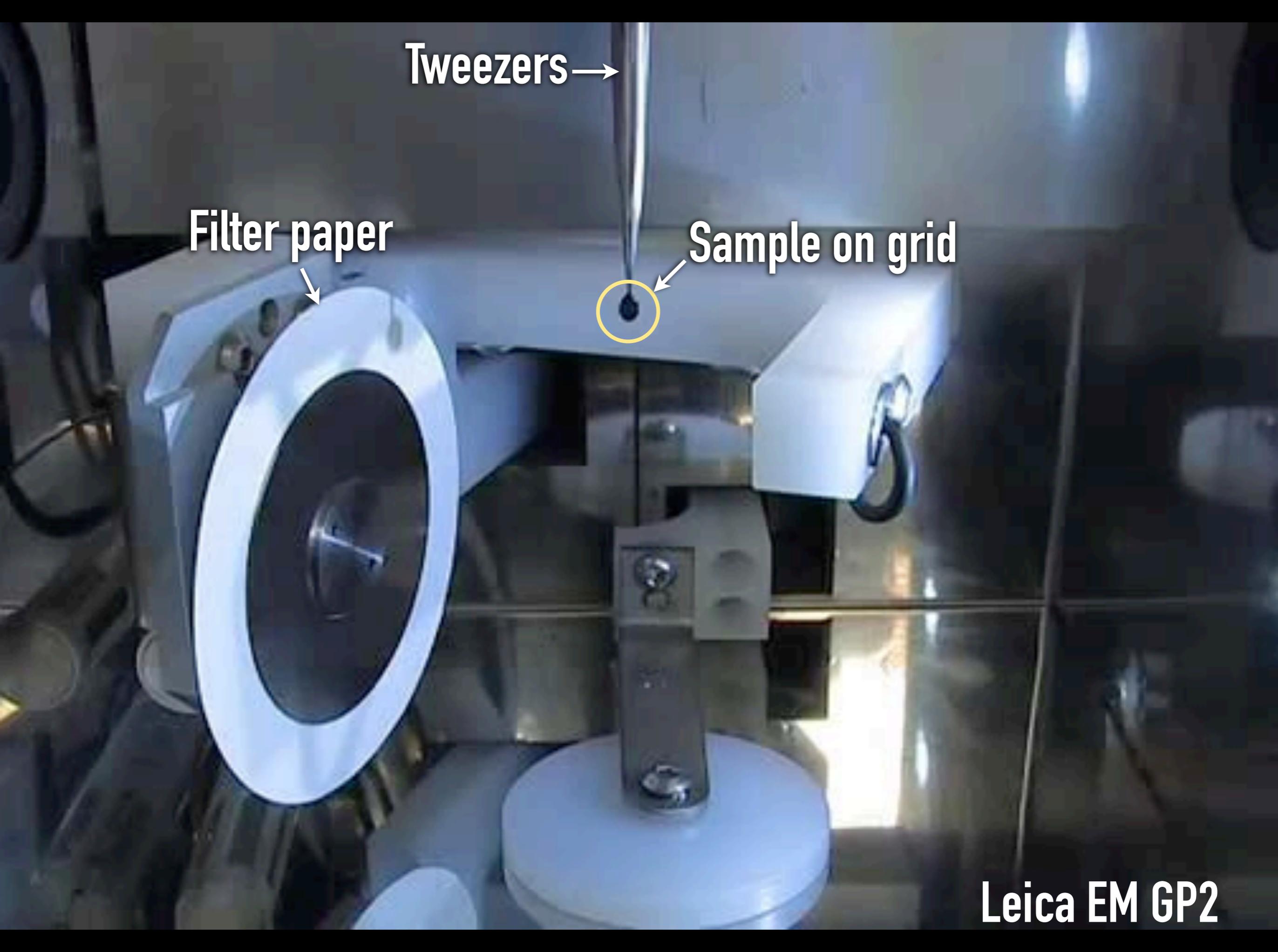
100,000 times too thick

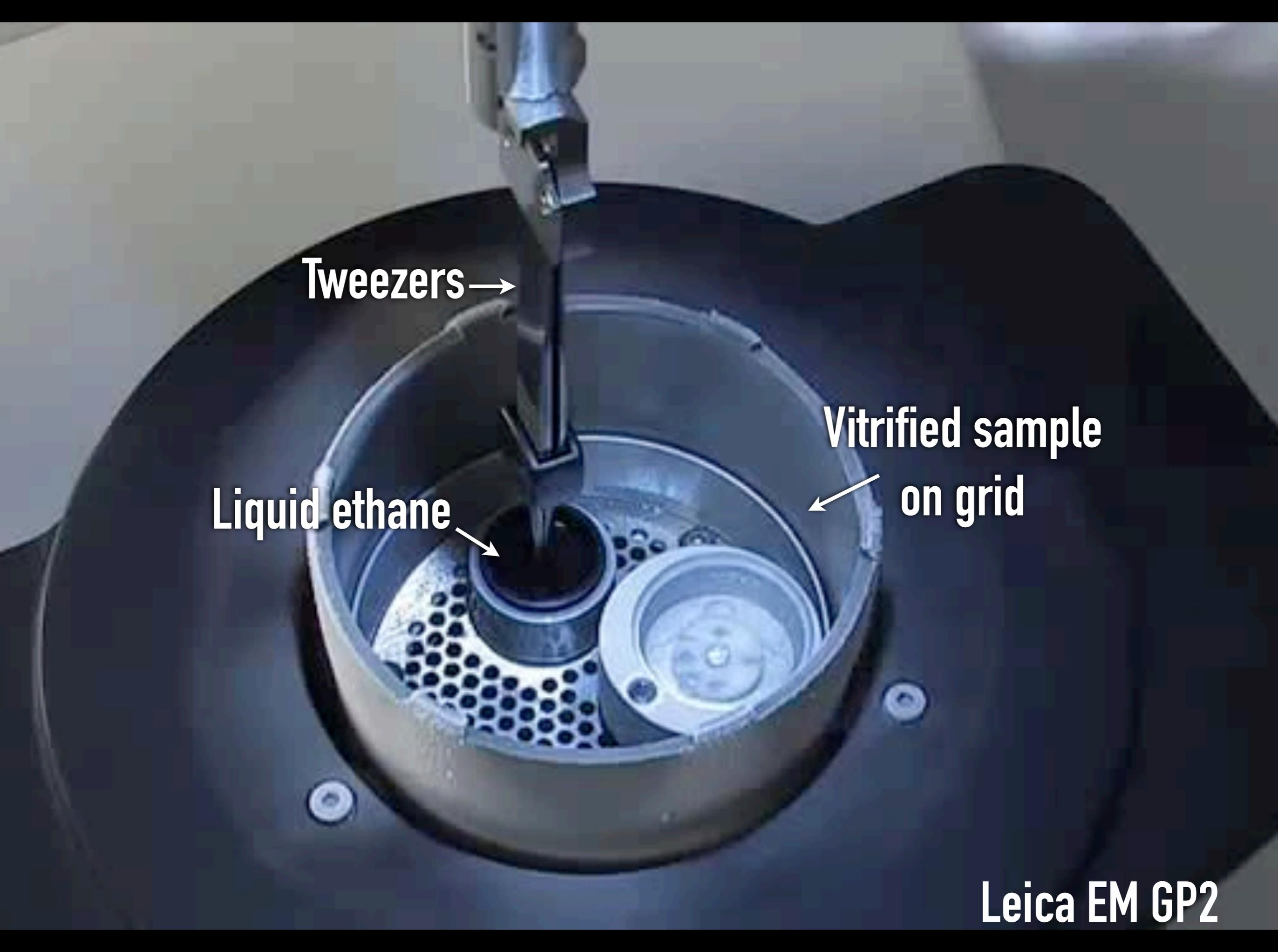
Tweezers →

Filter paper

Sample on grid

Leica EM GP2



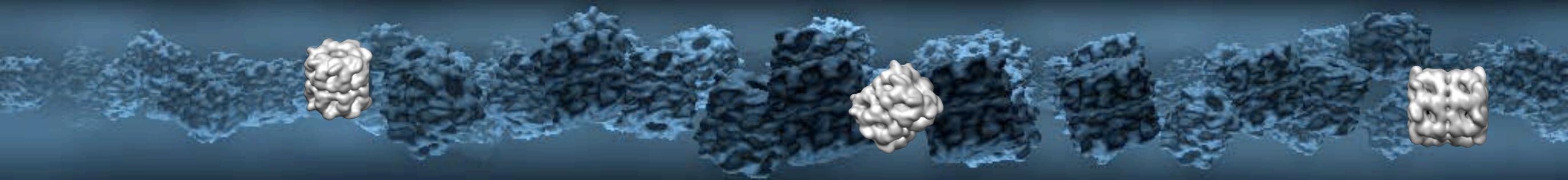
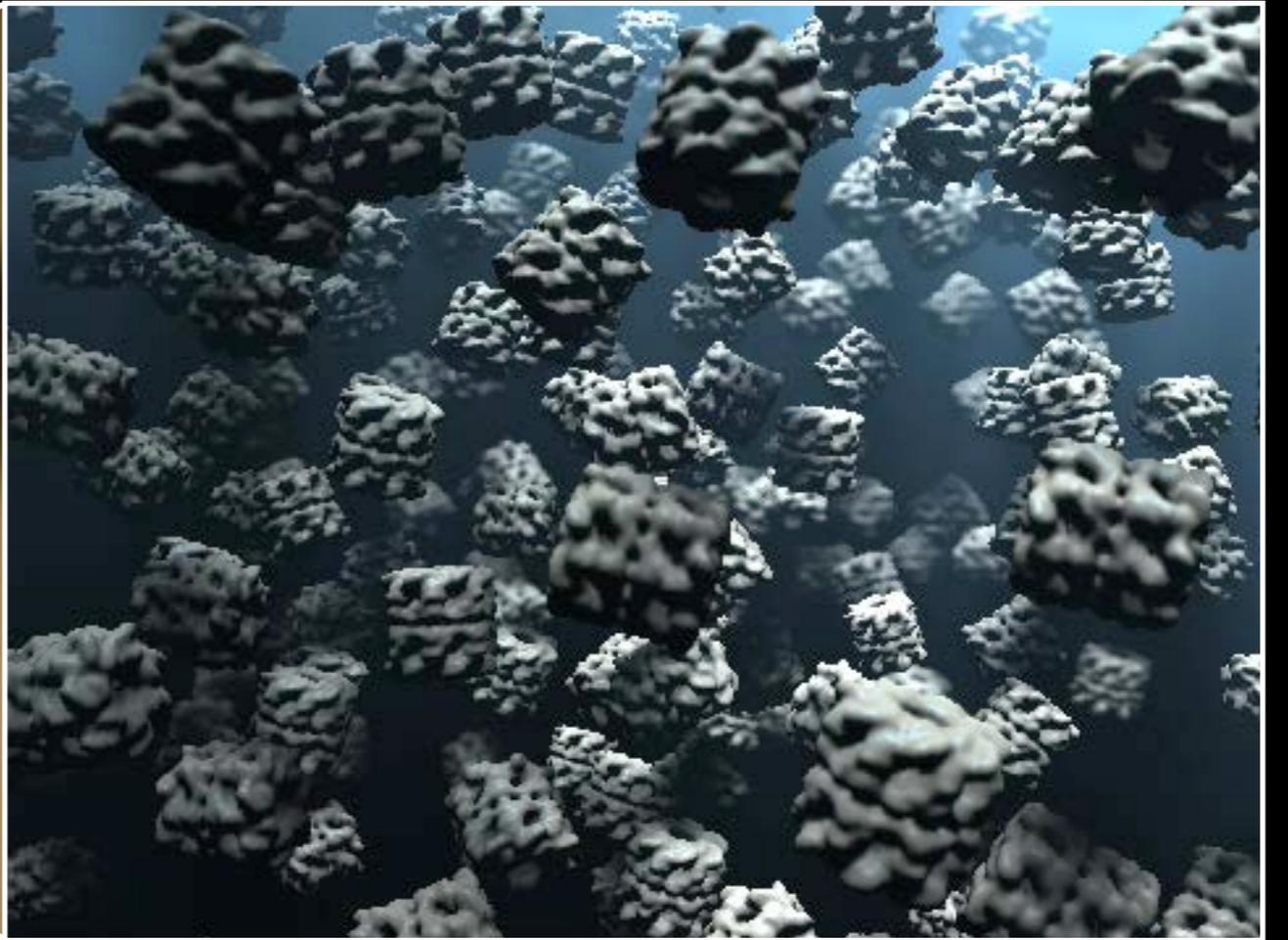
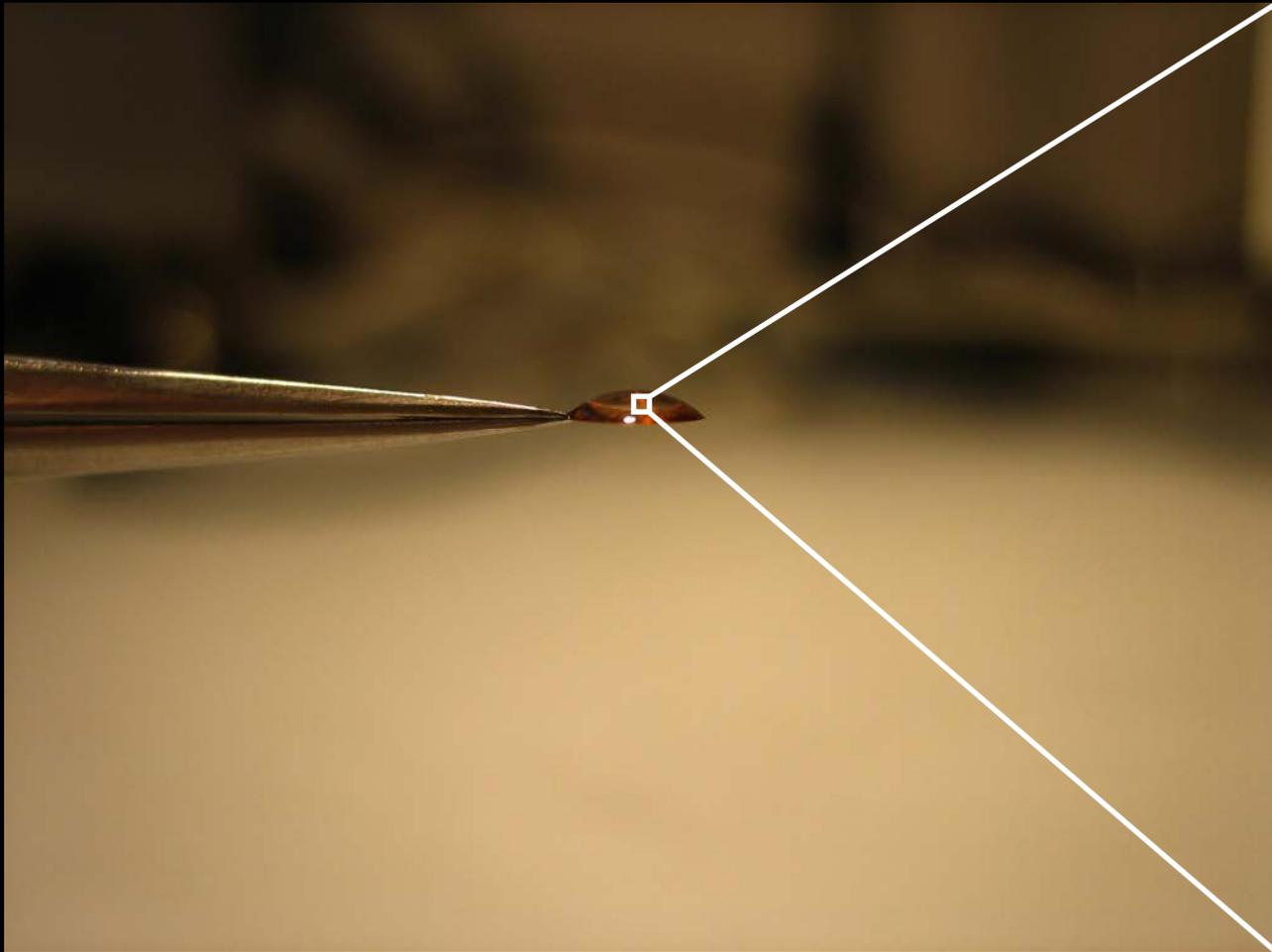


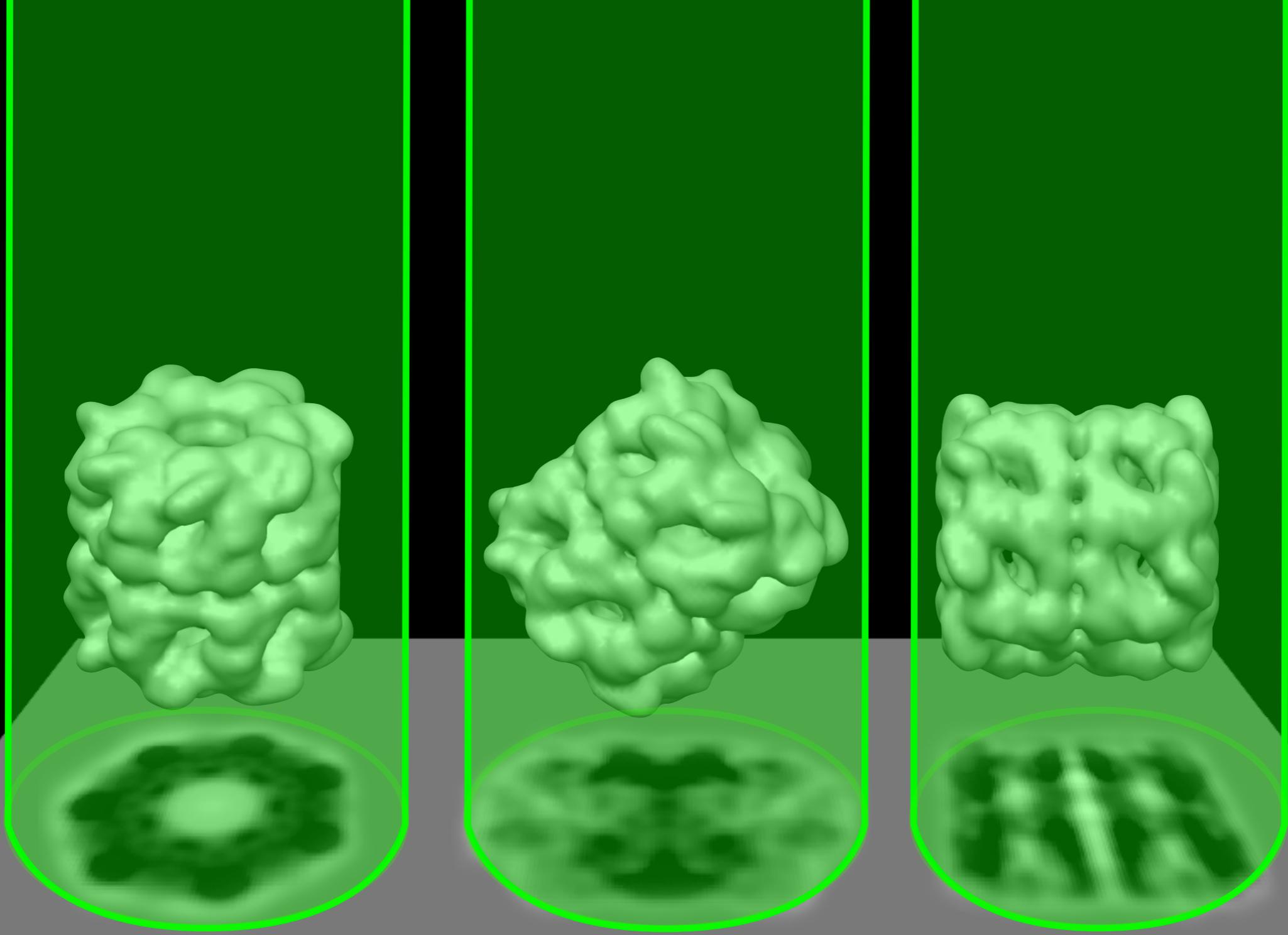
Tweezers →

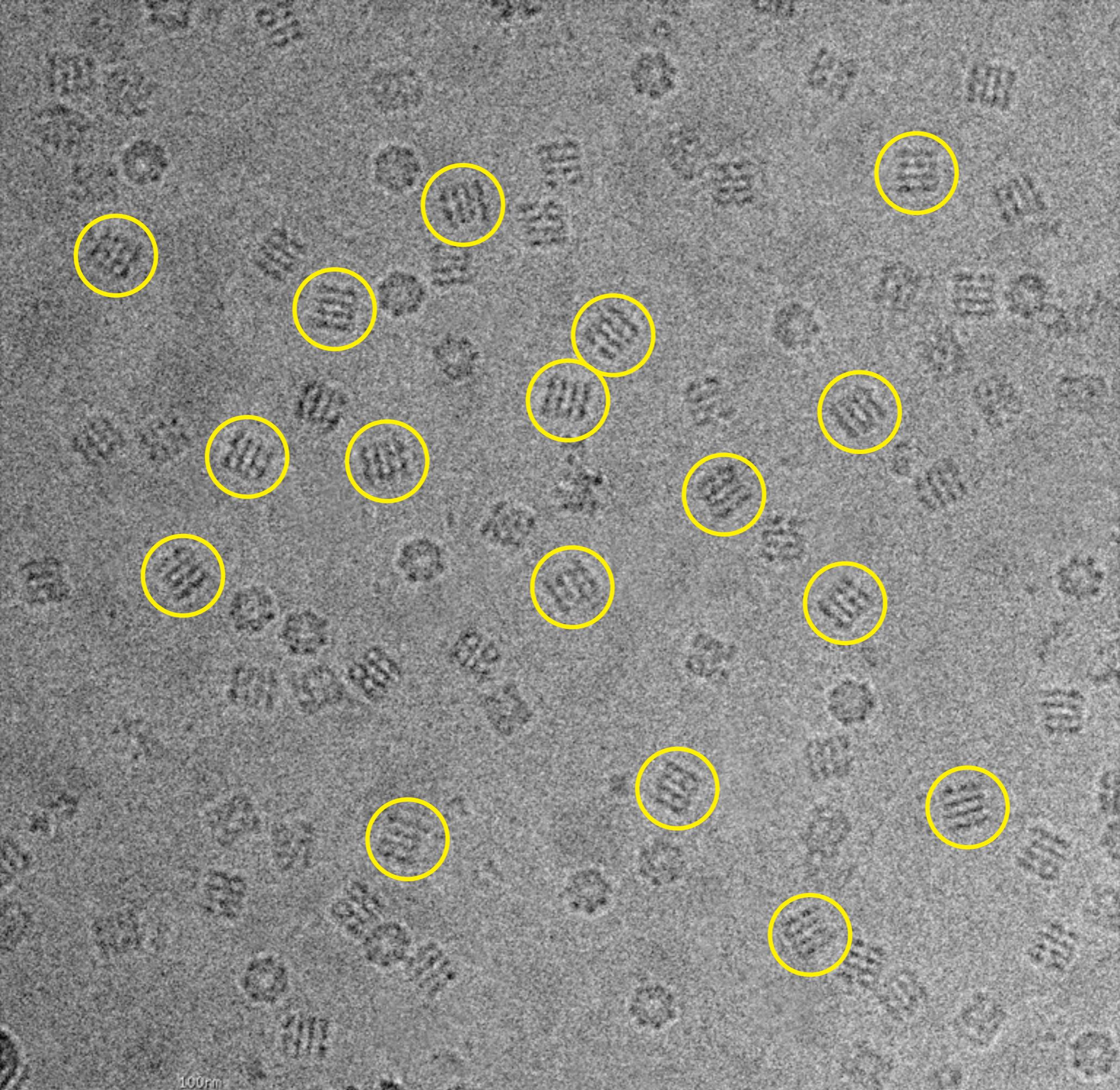
Liquid ethane ↘

**Vitrified sample
on grid** ↙

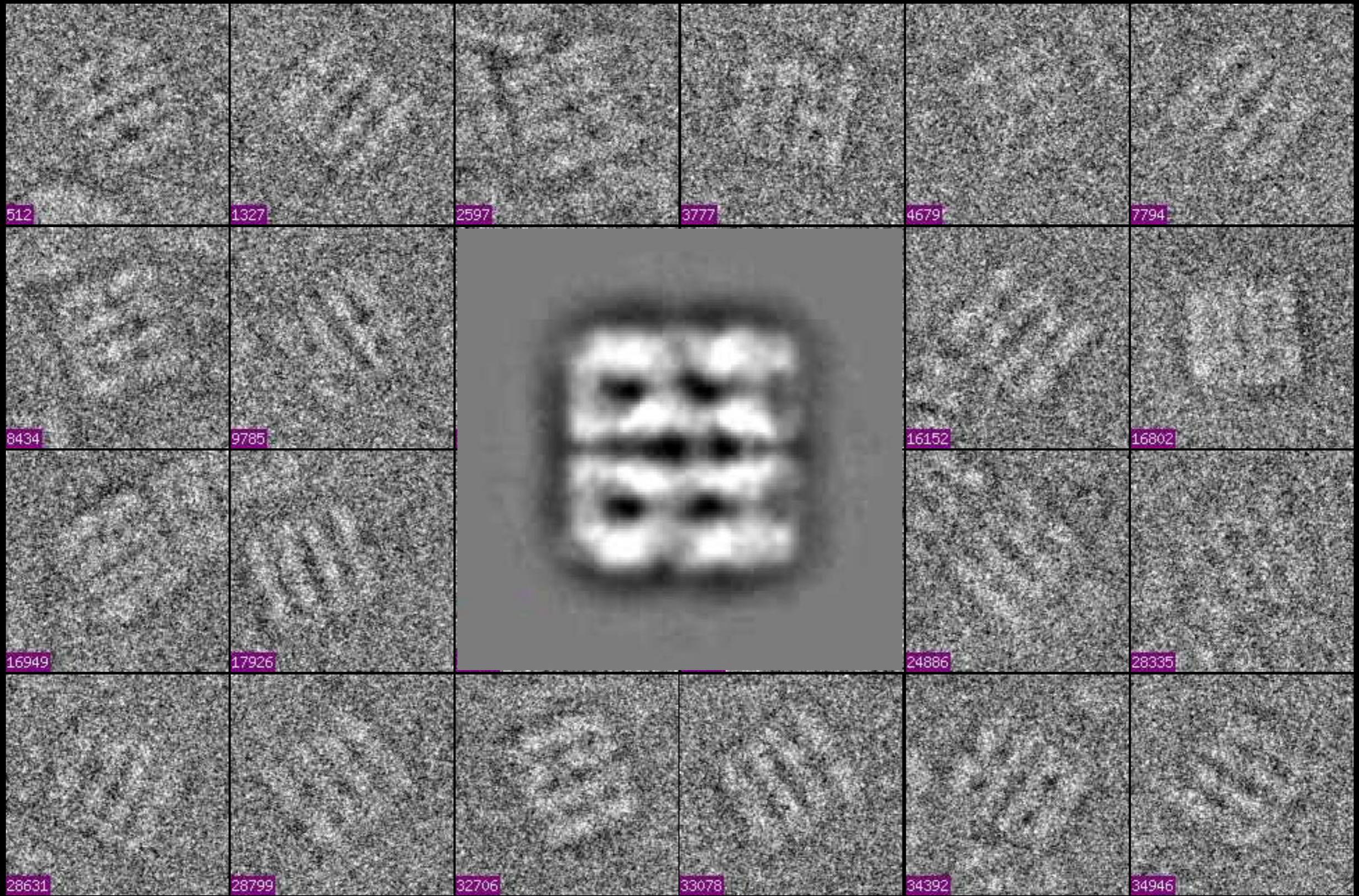
Leica EM GP2

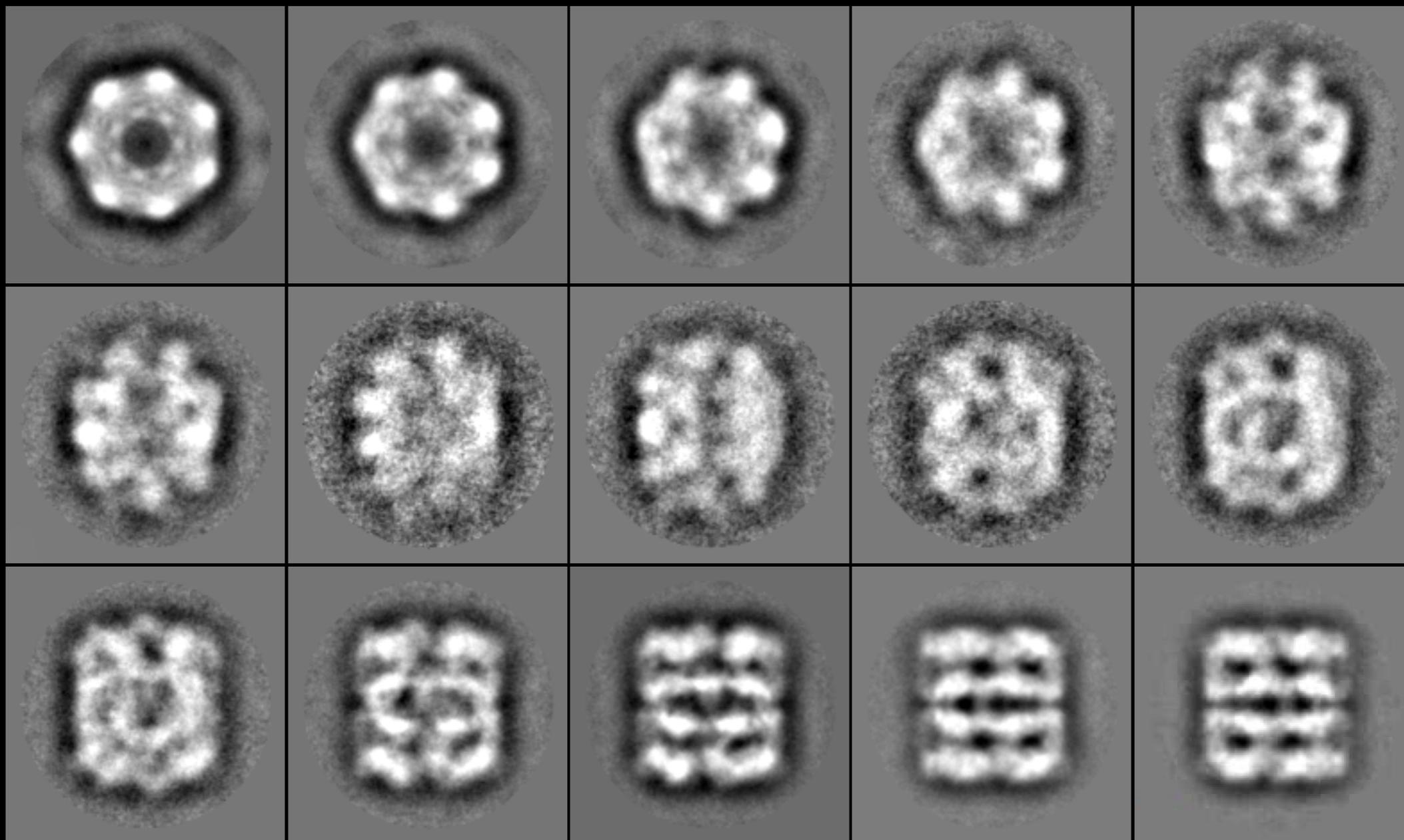


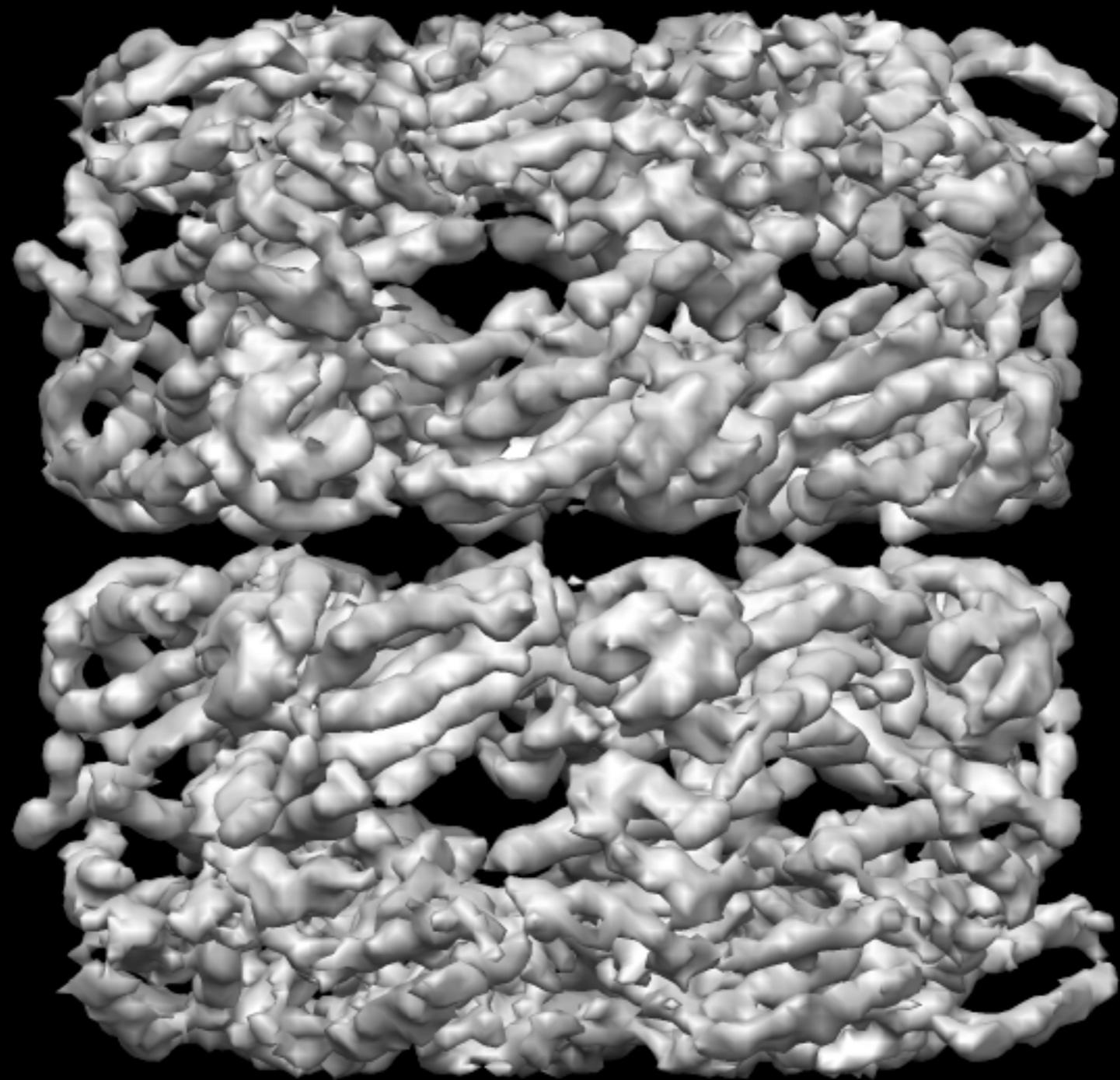




100nm







Current cryo-EM instrumentation and algorithms

Using 2009
instrumentation
& algorithms

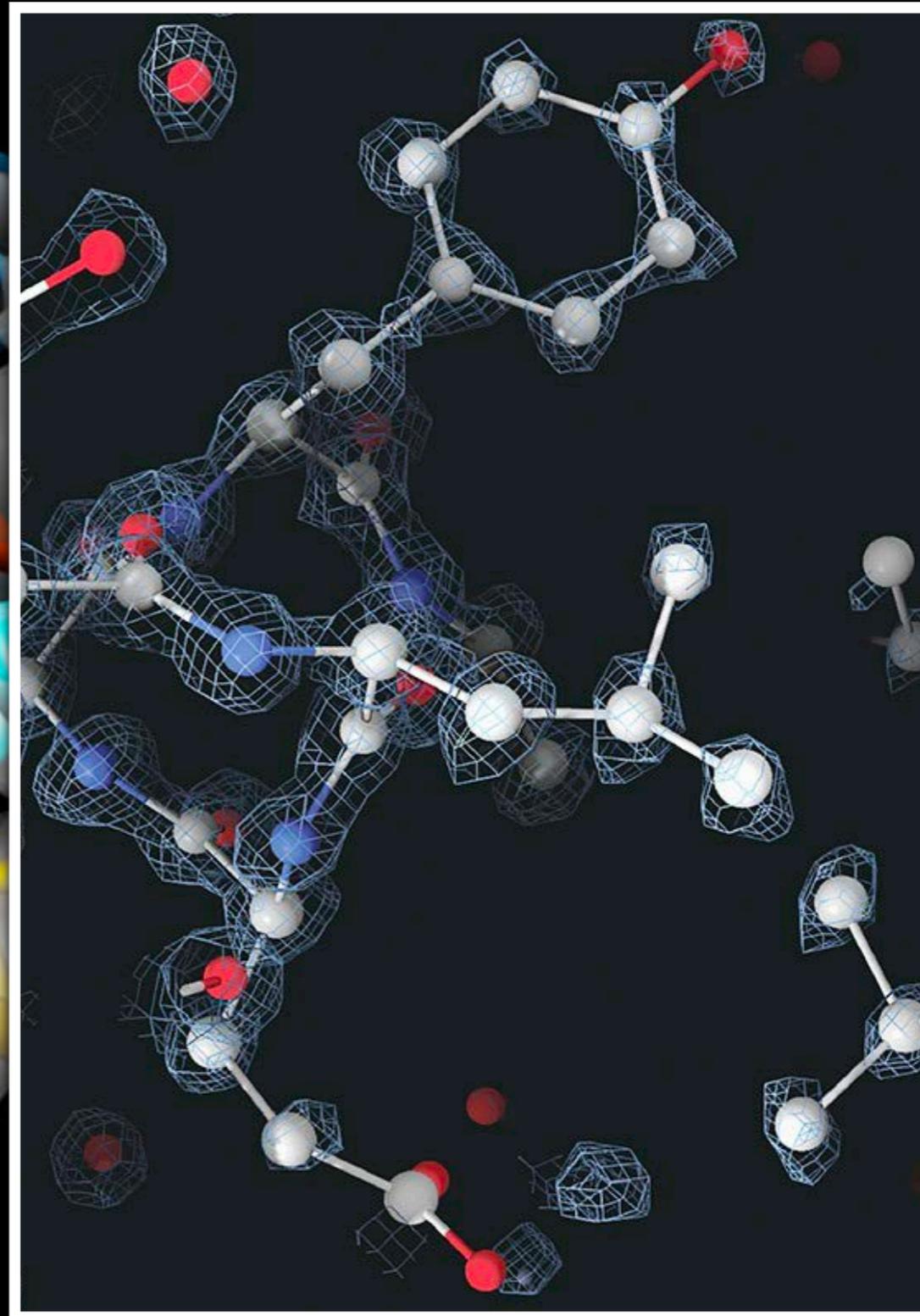
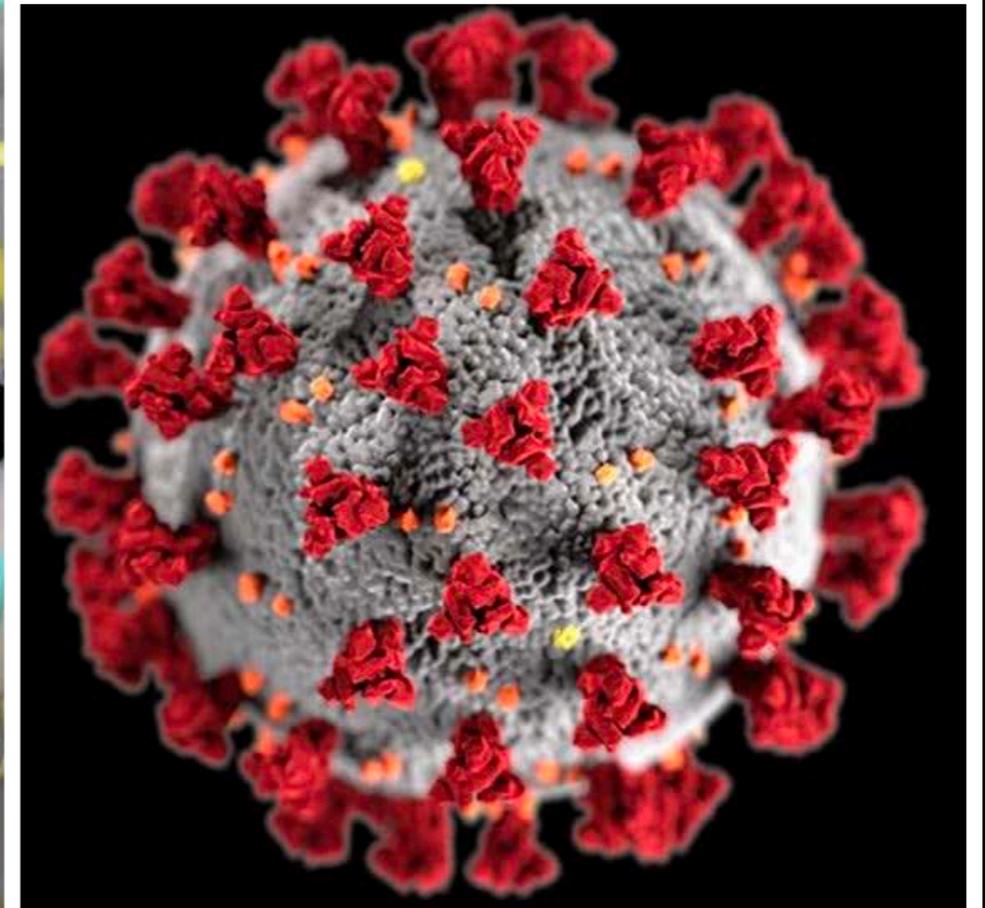
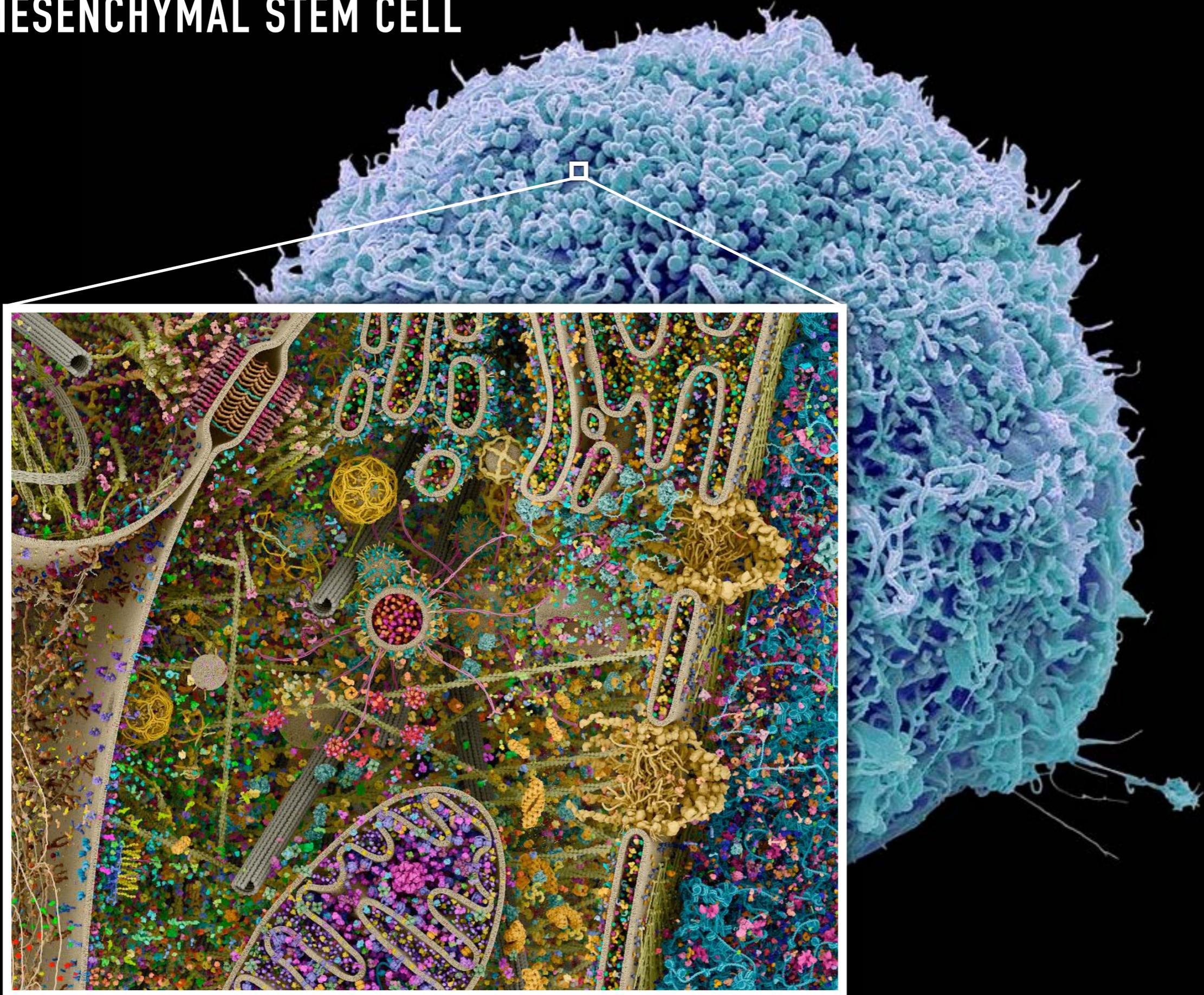


image from Herzik 2020

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

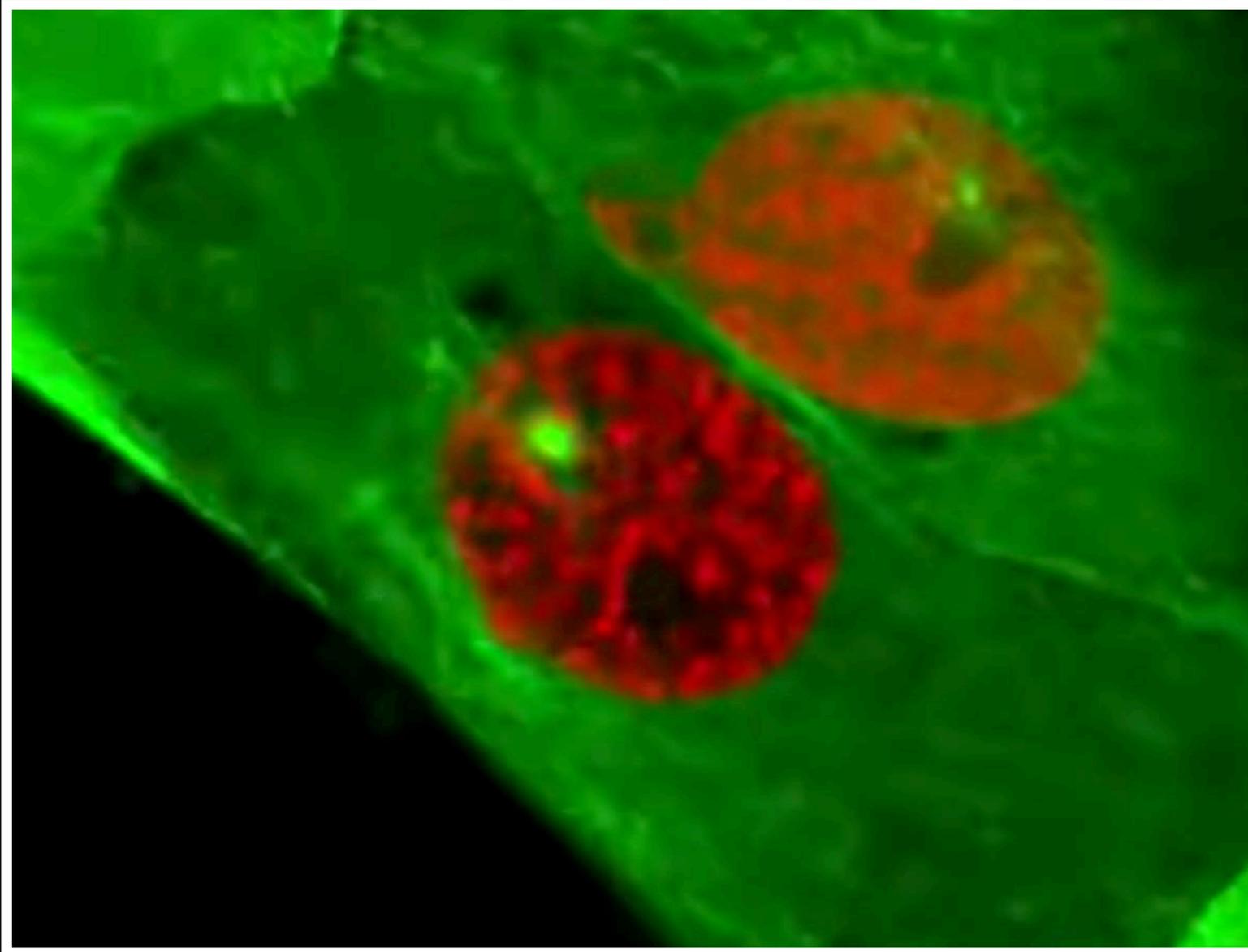
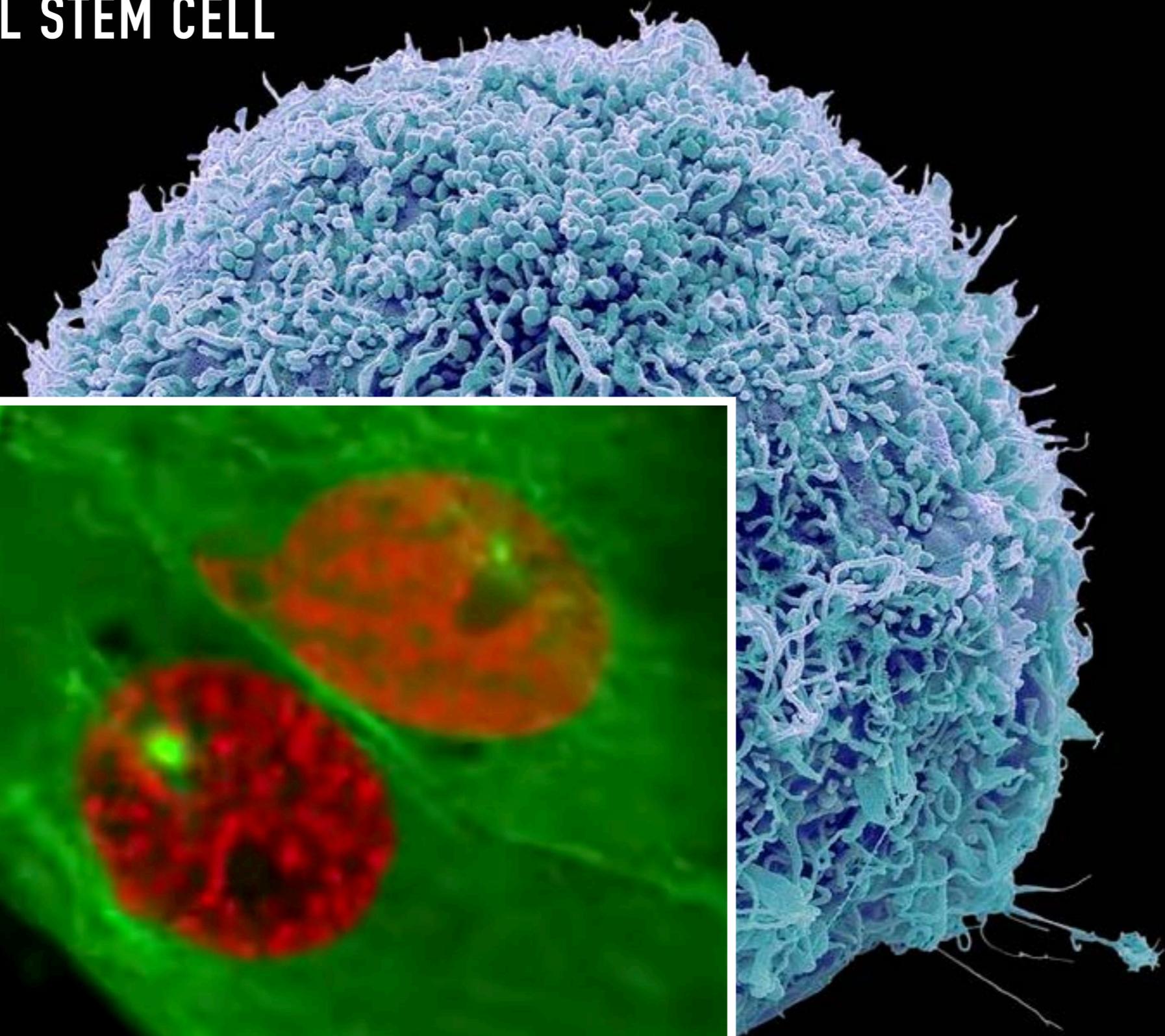


MESENCHYMAL STEM CELL

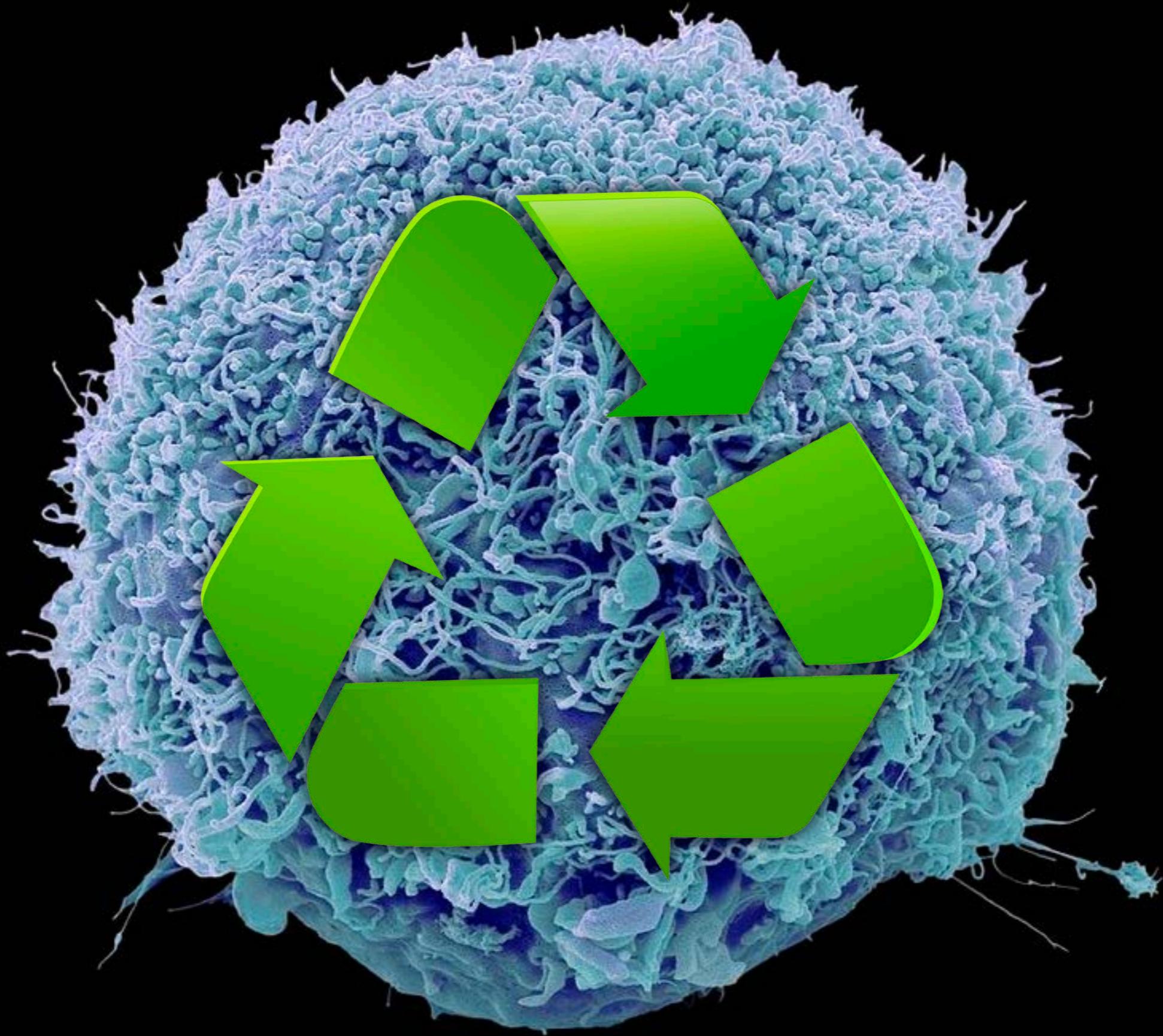


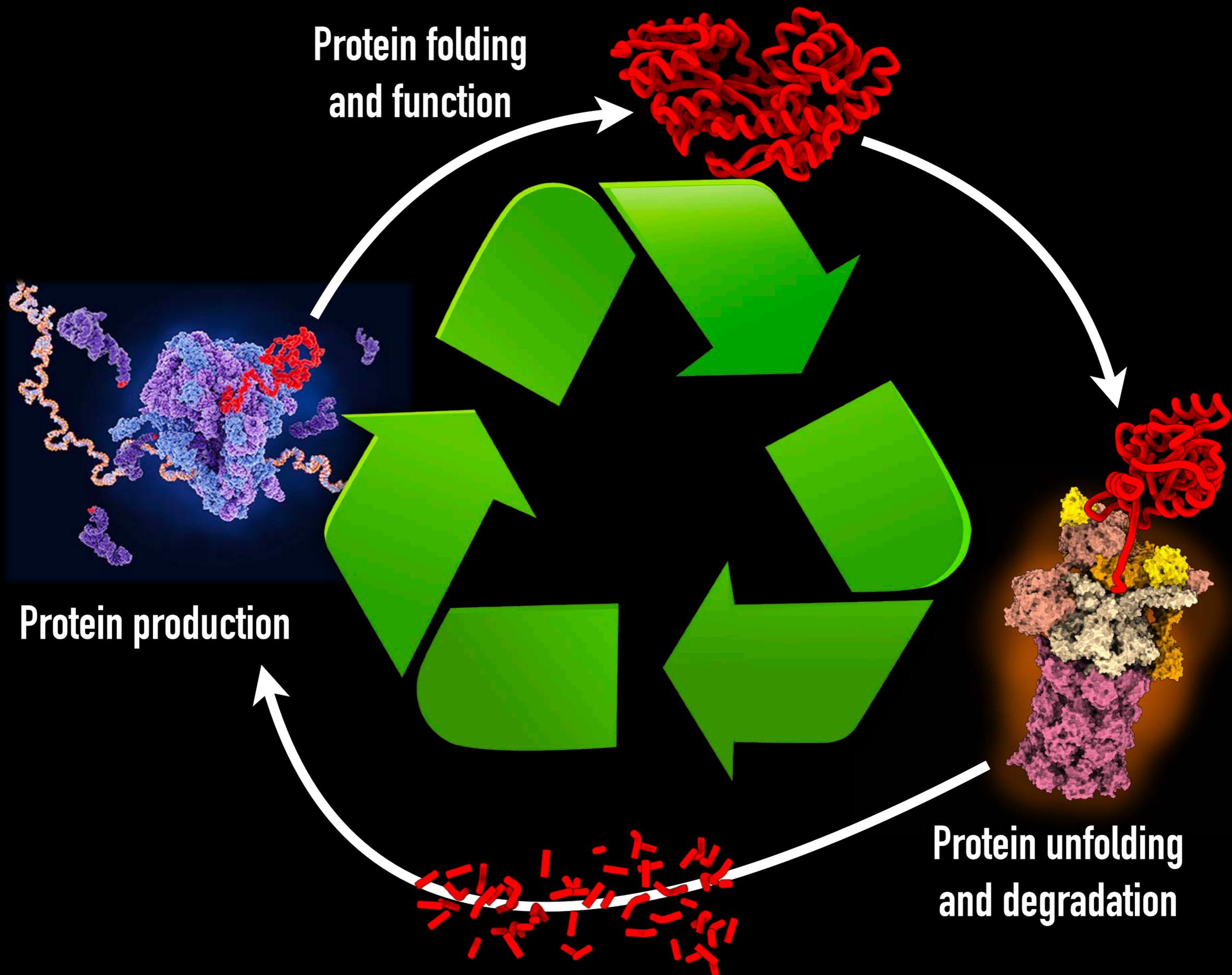
Evan Ingersoll & Gael McGill

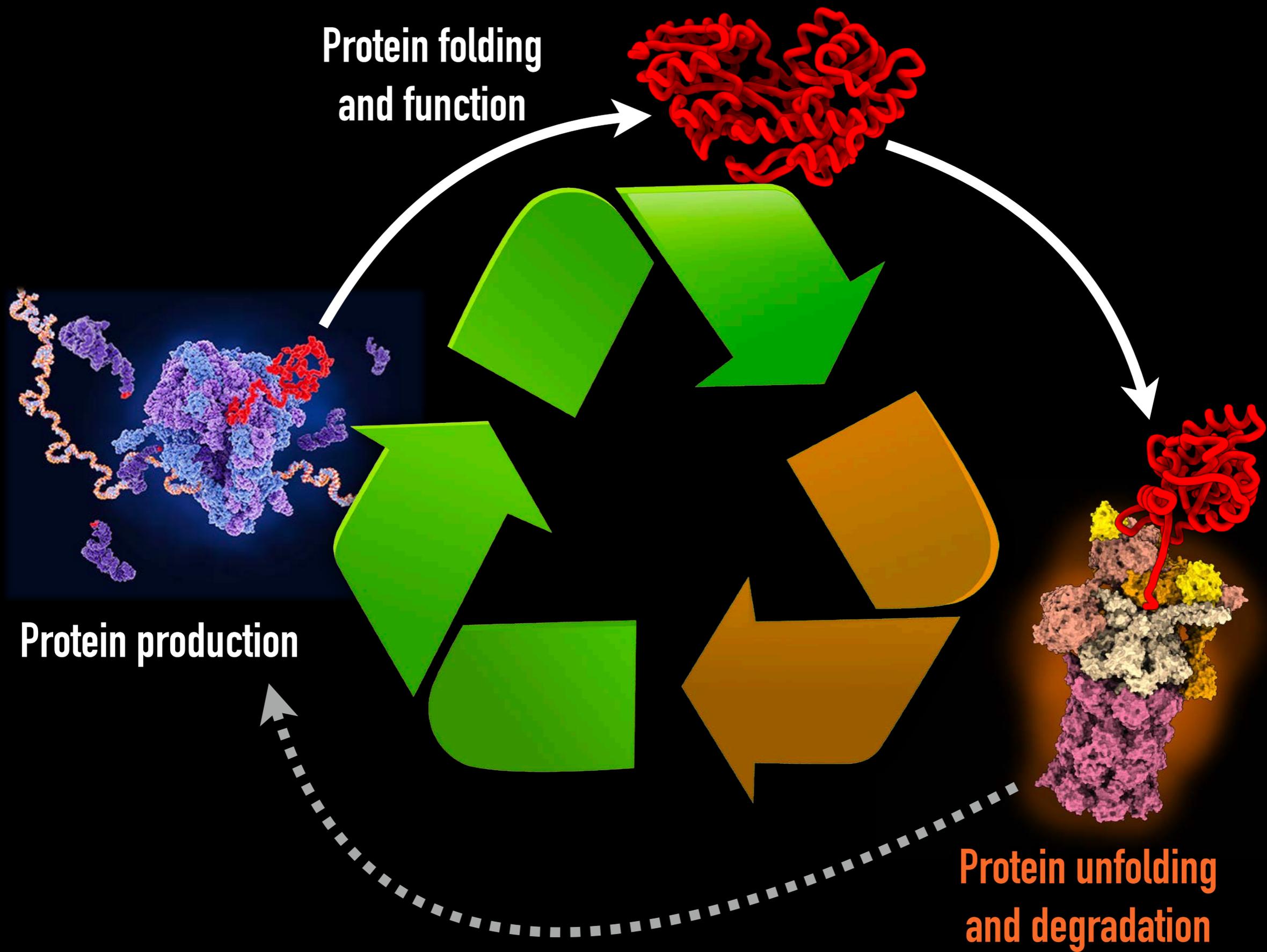
MESENCHYMAL STEM CELL

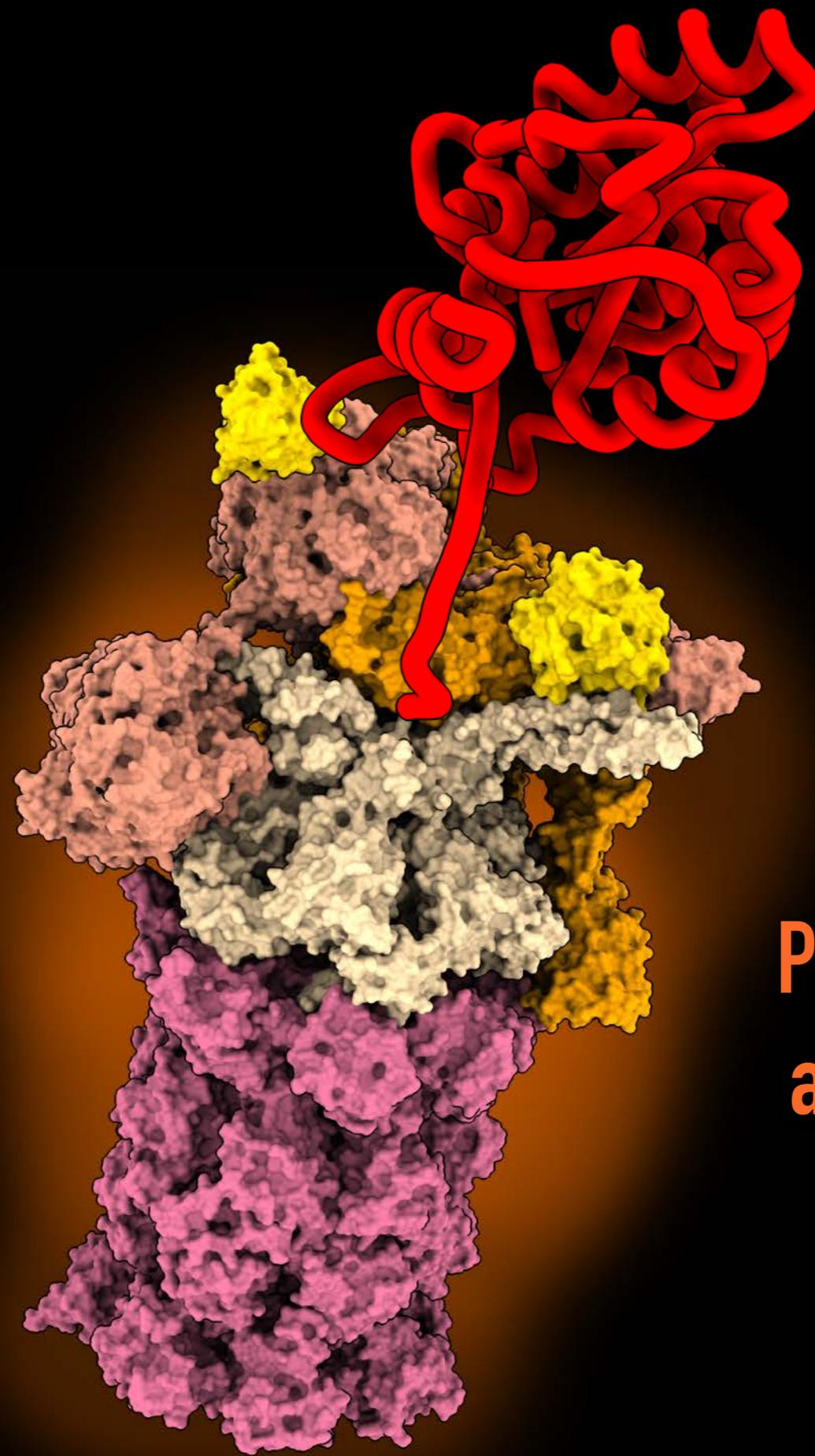


Dividing kidney cells



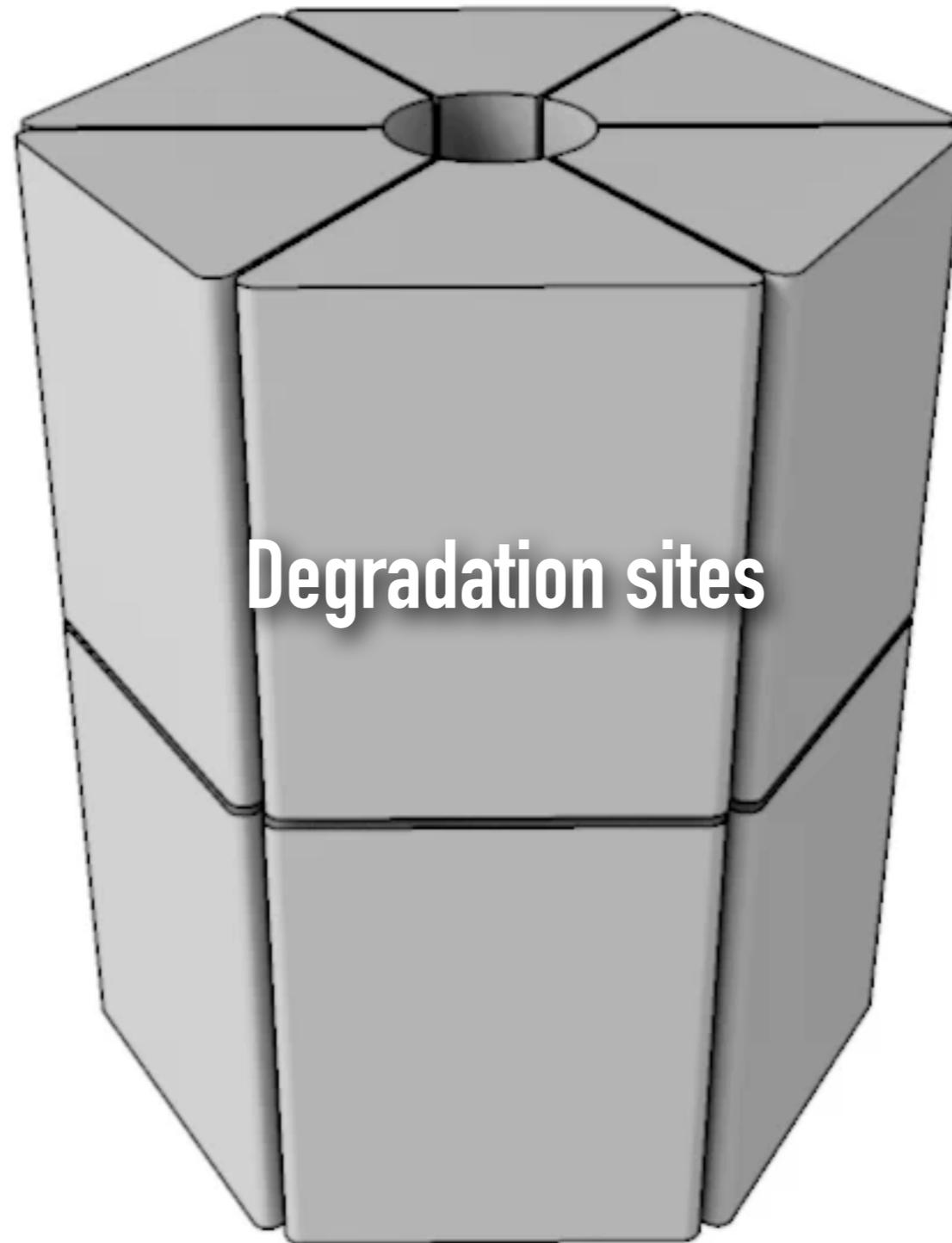






**Protein unfolding
and degradation**

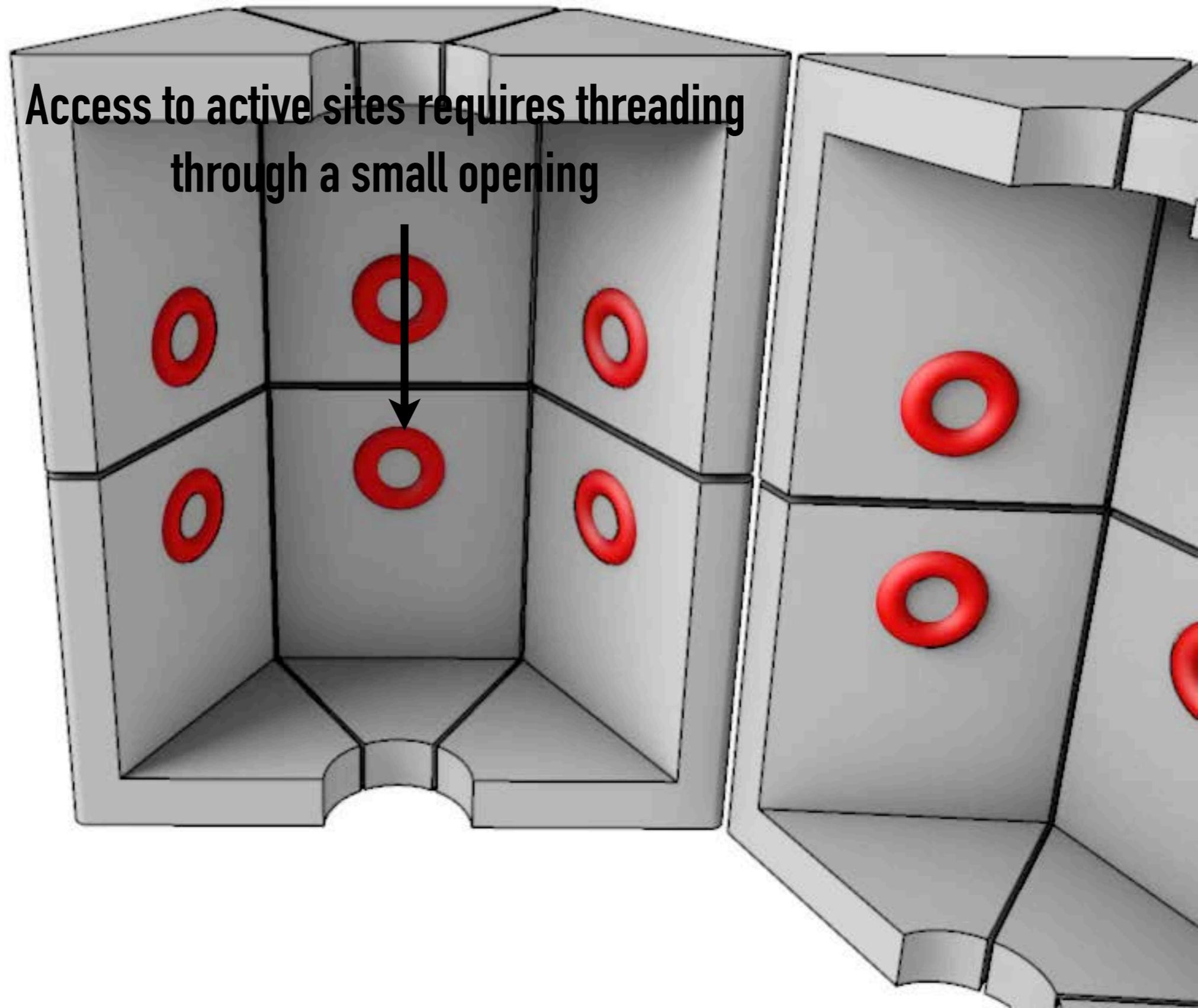
MOLECULAR UNDERTAKERS



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

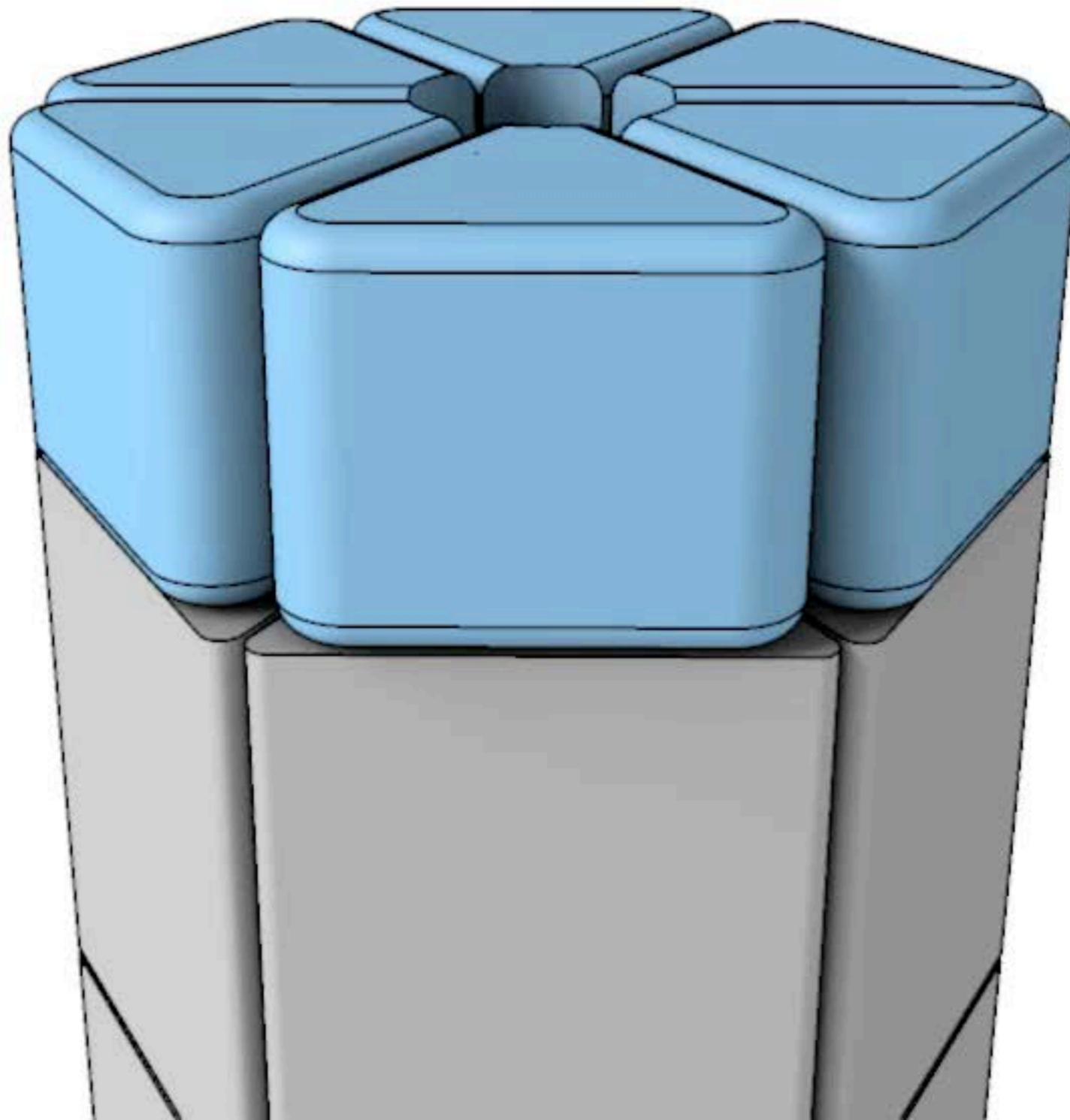
Degradation sites

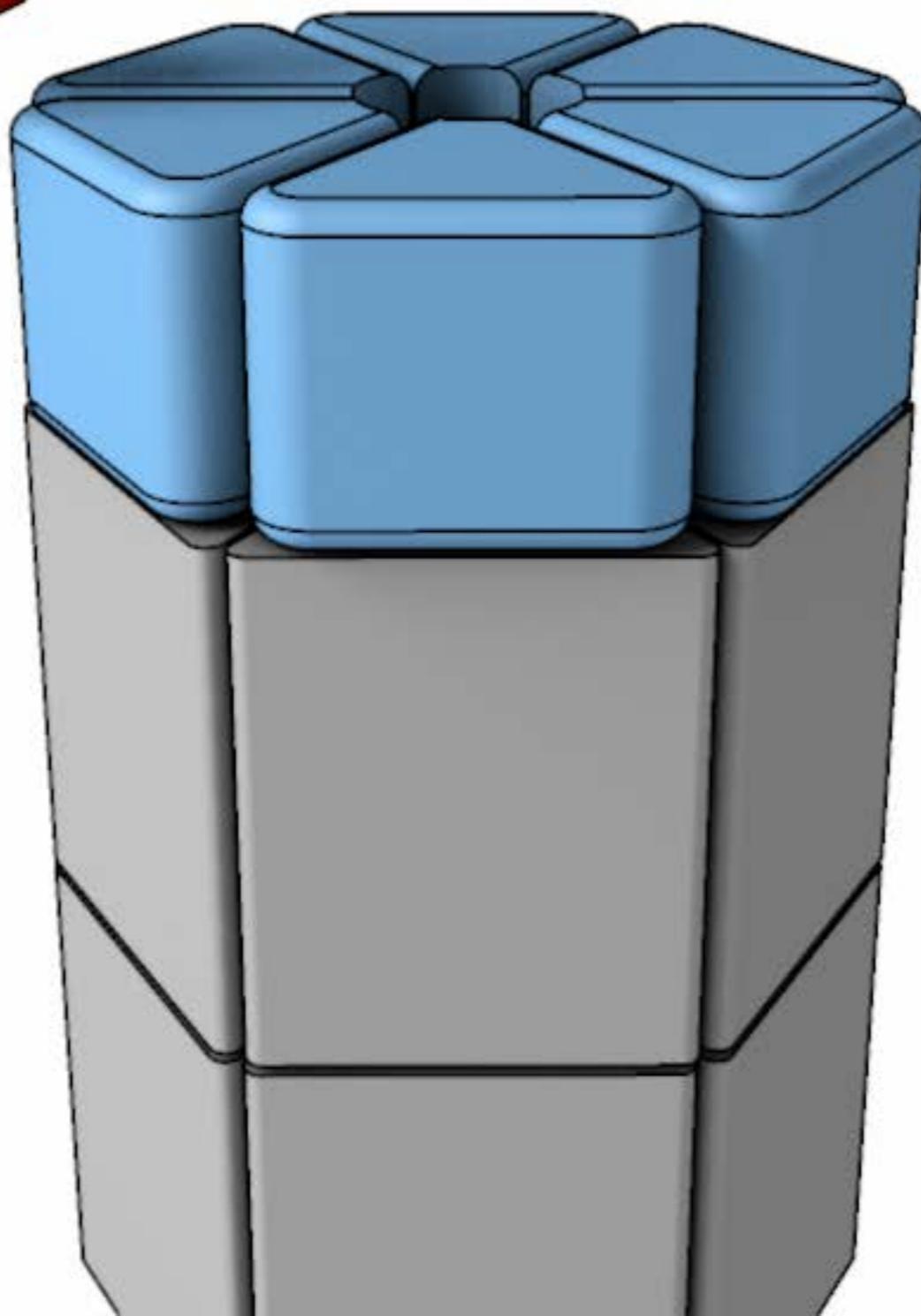
MOLECULAR UNDERTAKERS

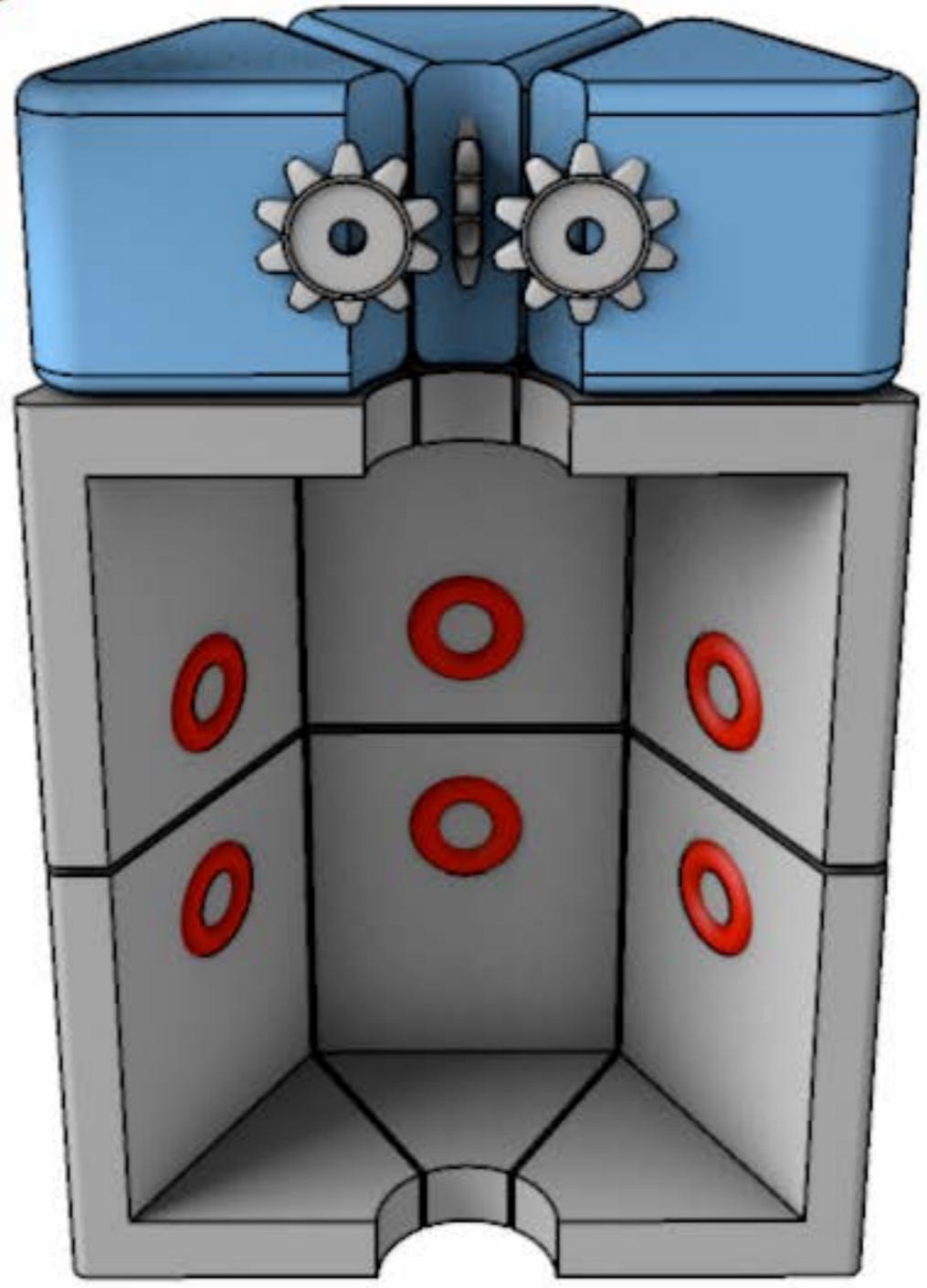
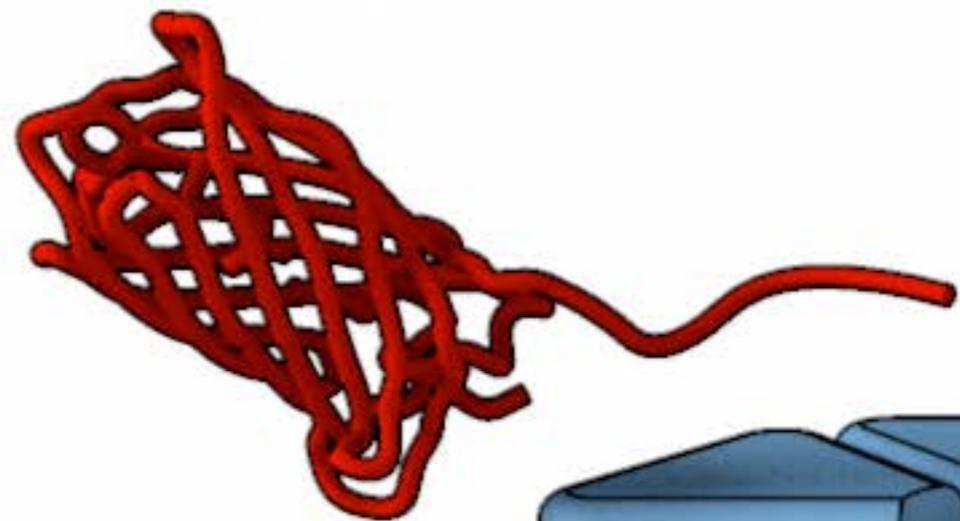


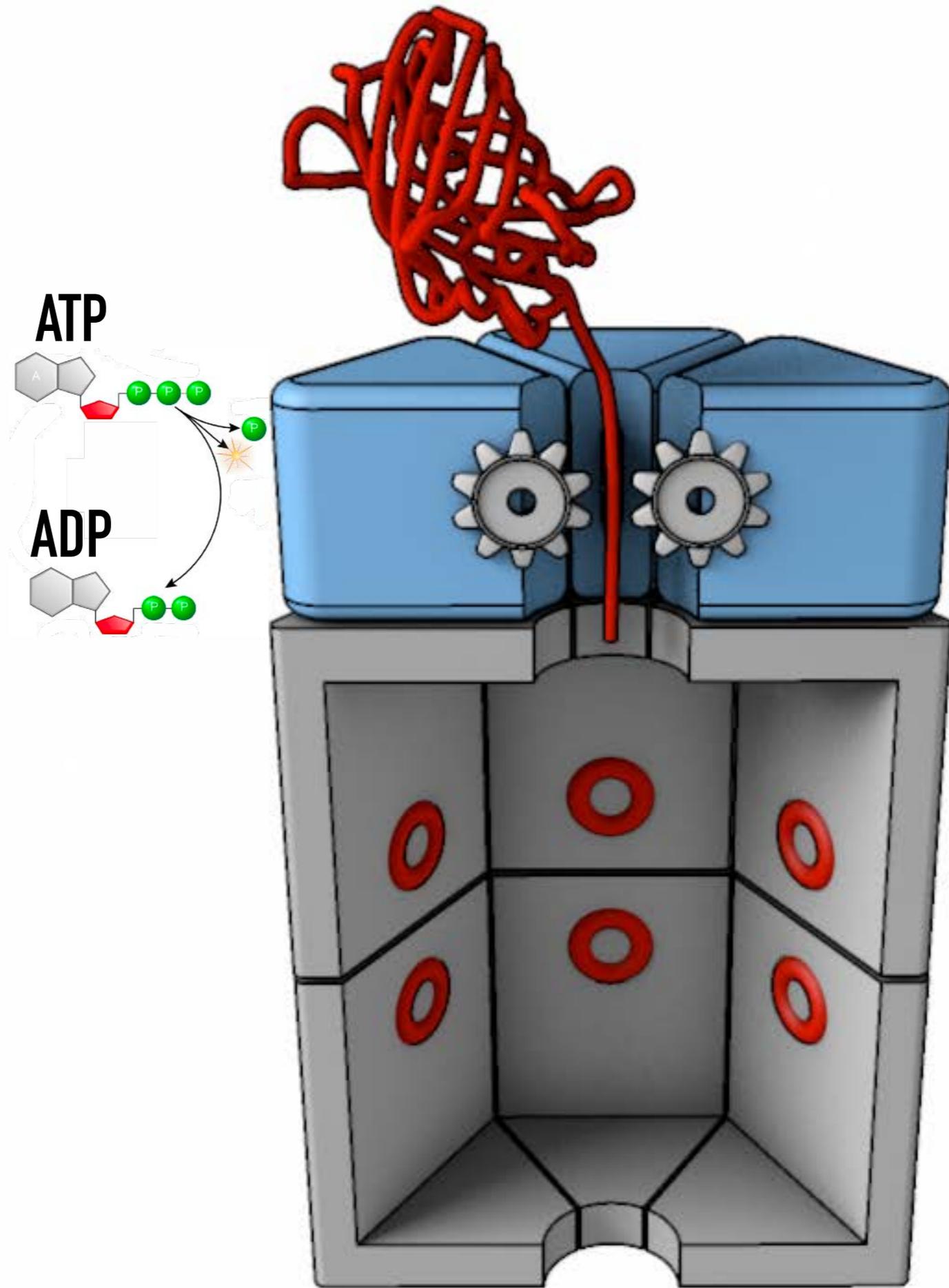
MOLECULAR UNDERTAKERS

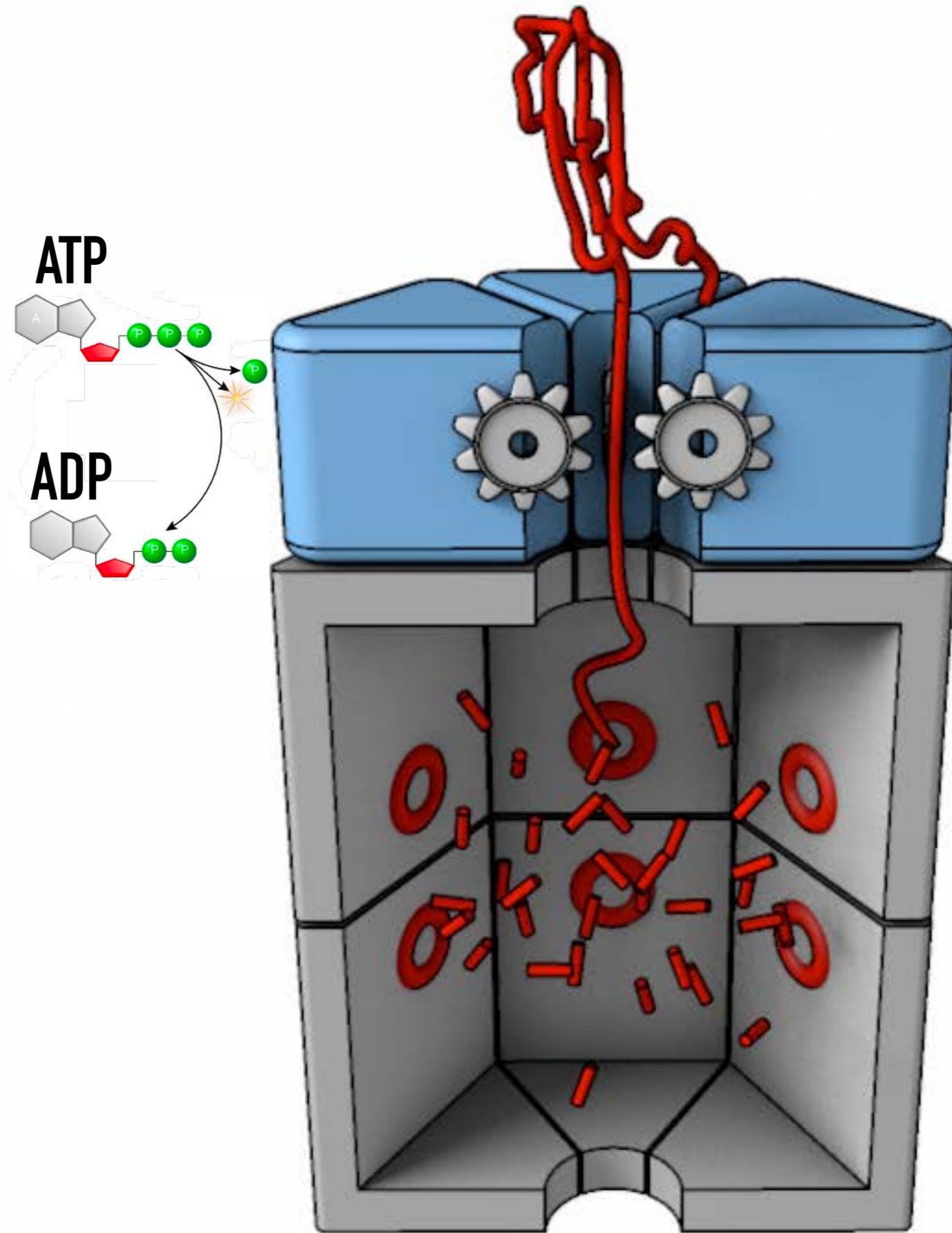
Molecular Motor



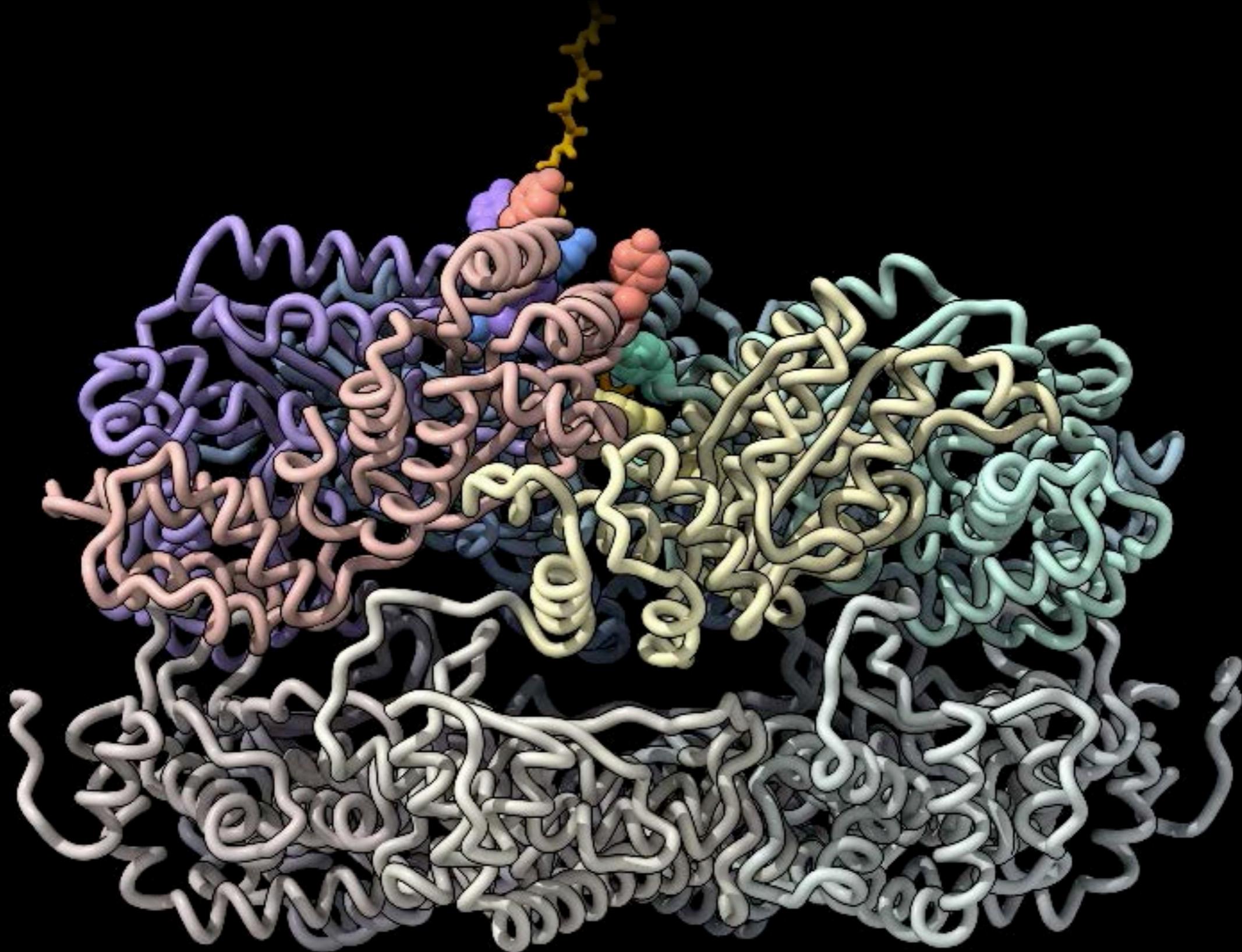






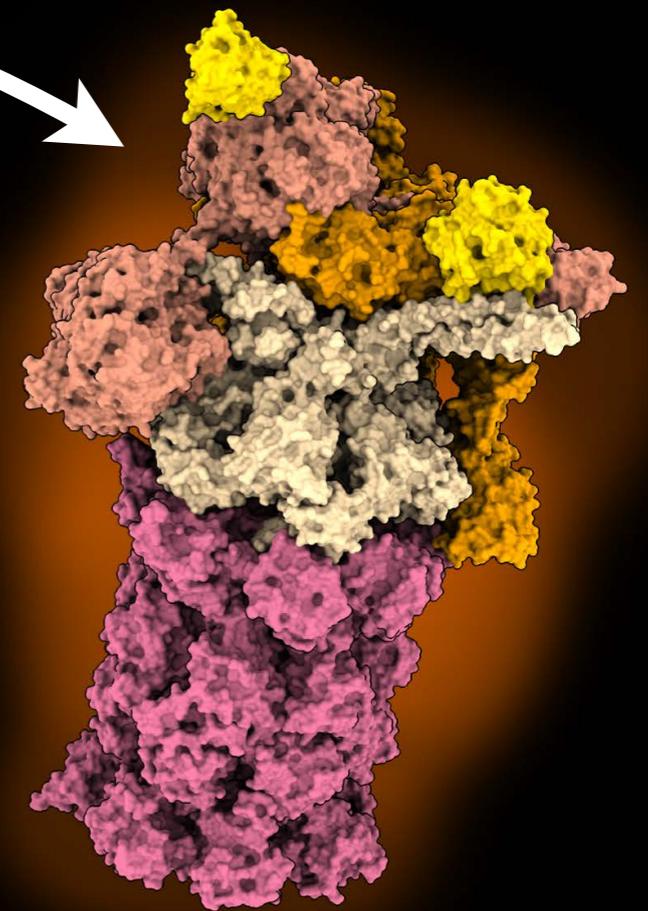


UNFOLDING AND DESTROYING PROTEINS

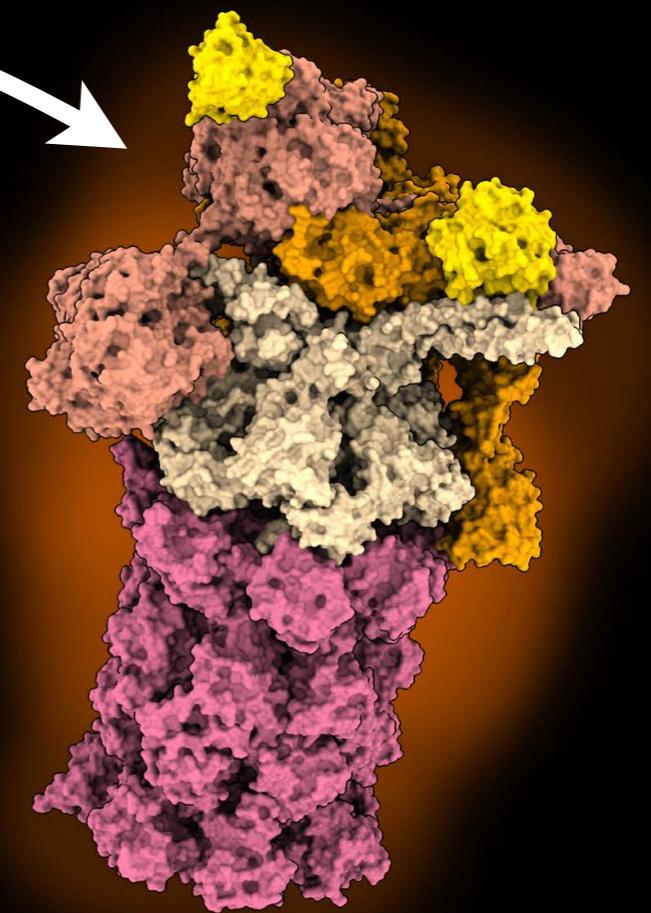


Puchades et al. 2017, 2019, 2020

HOW DO THE DEGRADERS KNOW WHAT TO TARGET?

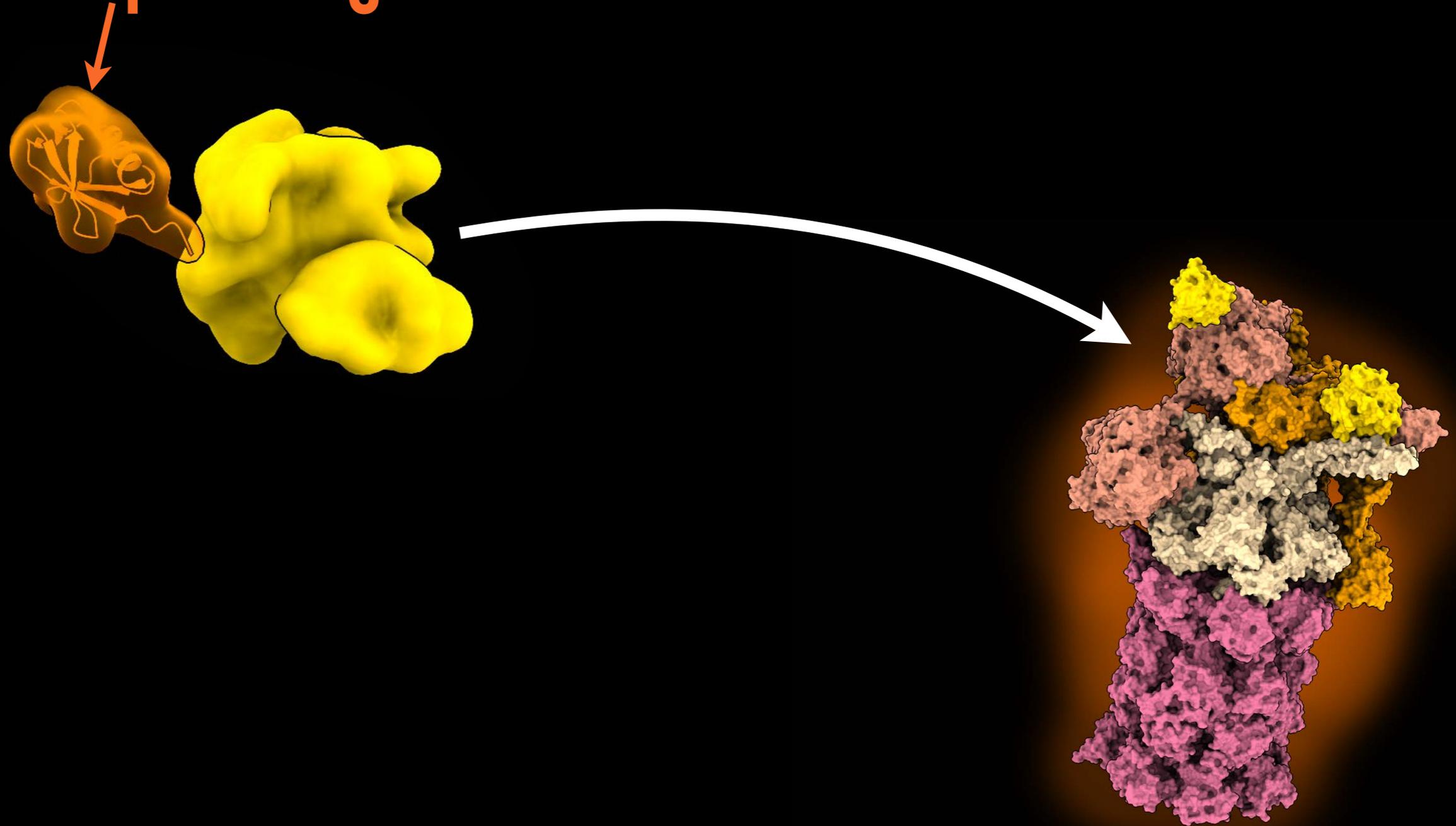


HOW DO THE DEGRADERS KNOW WHAT TO TARGET?

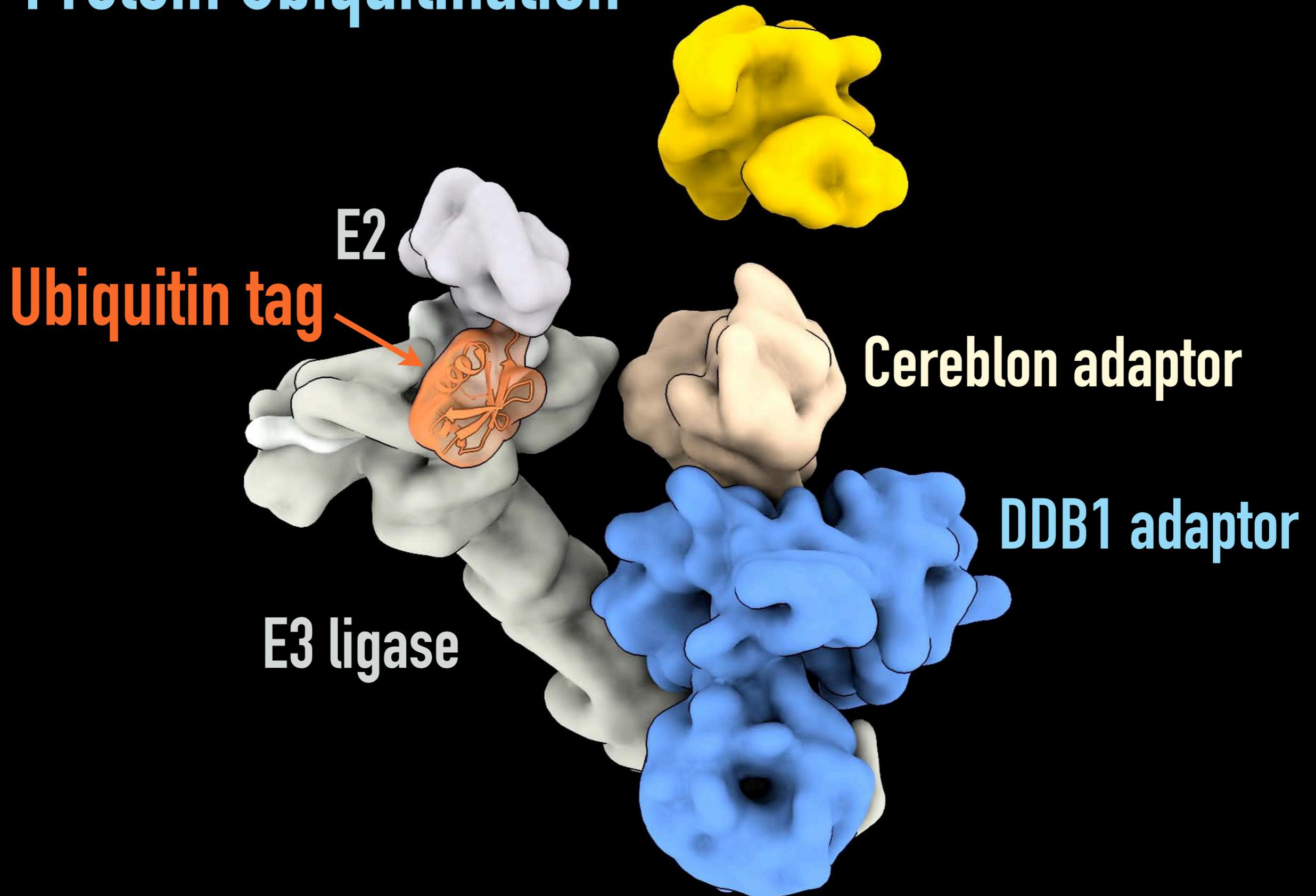


HOW DO THE DEGRADERS KNOW WHAT TO TARGET?

Ubiquitin tag

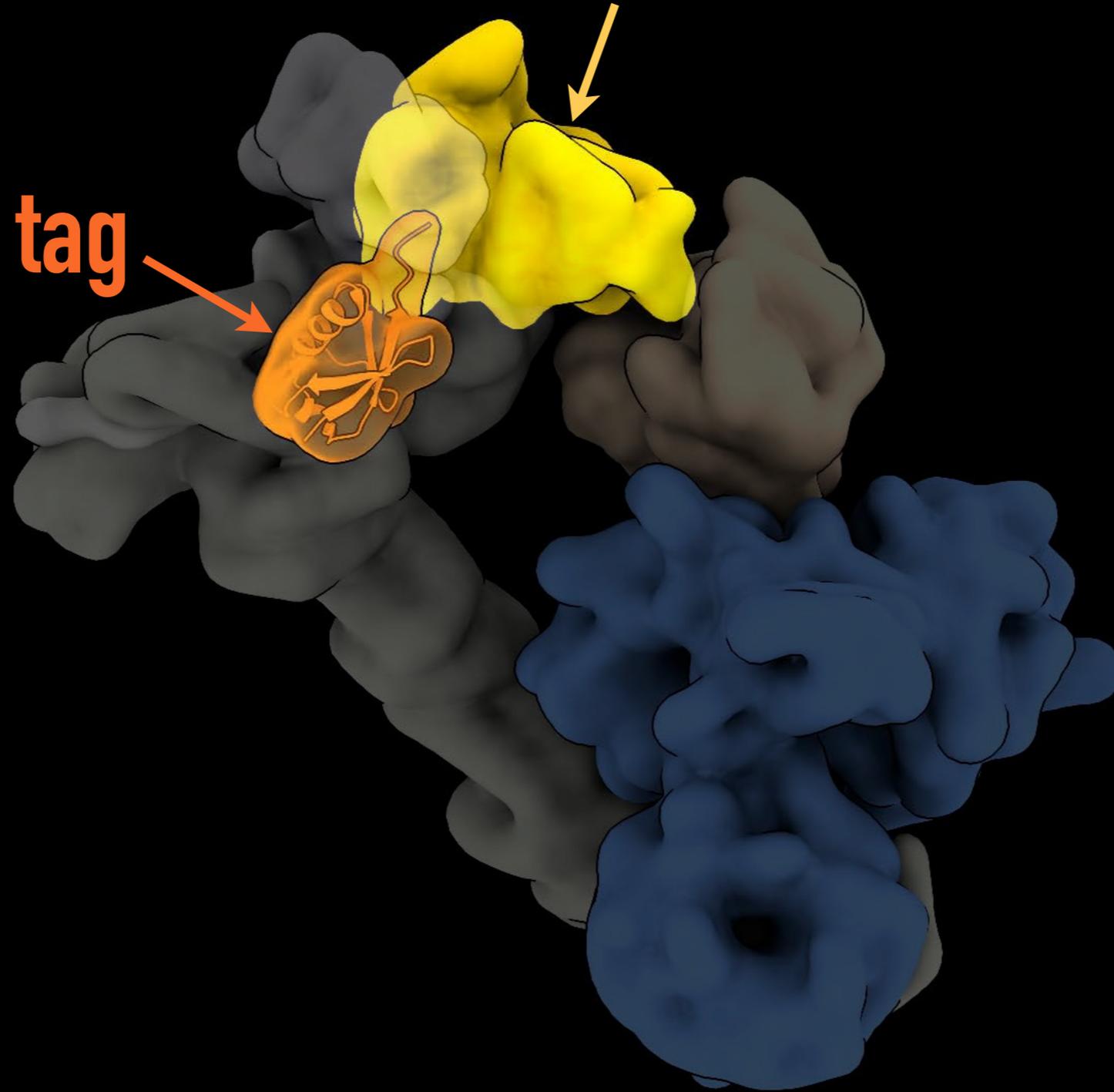


Protein Ubiquitination

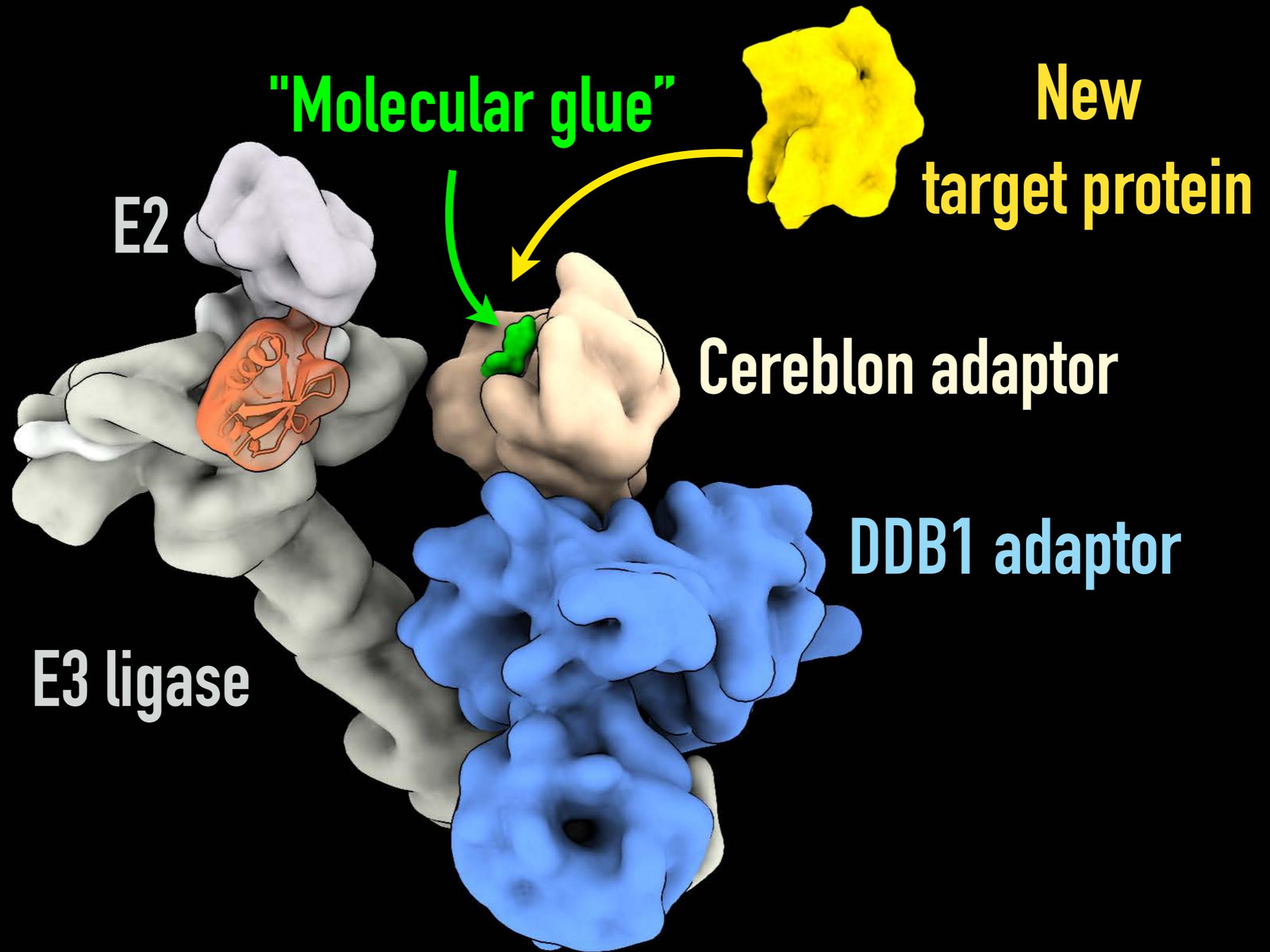


Target protein

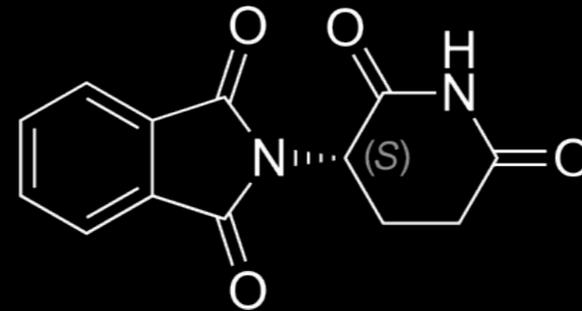
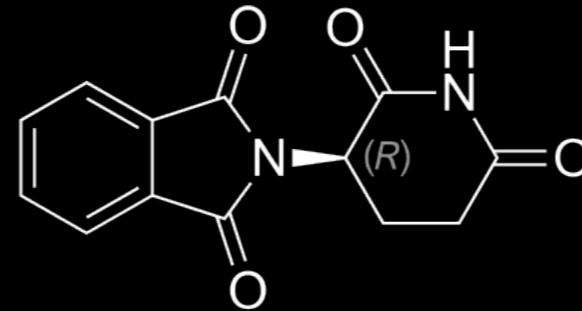
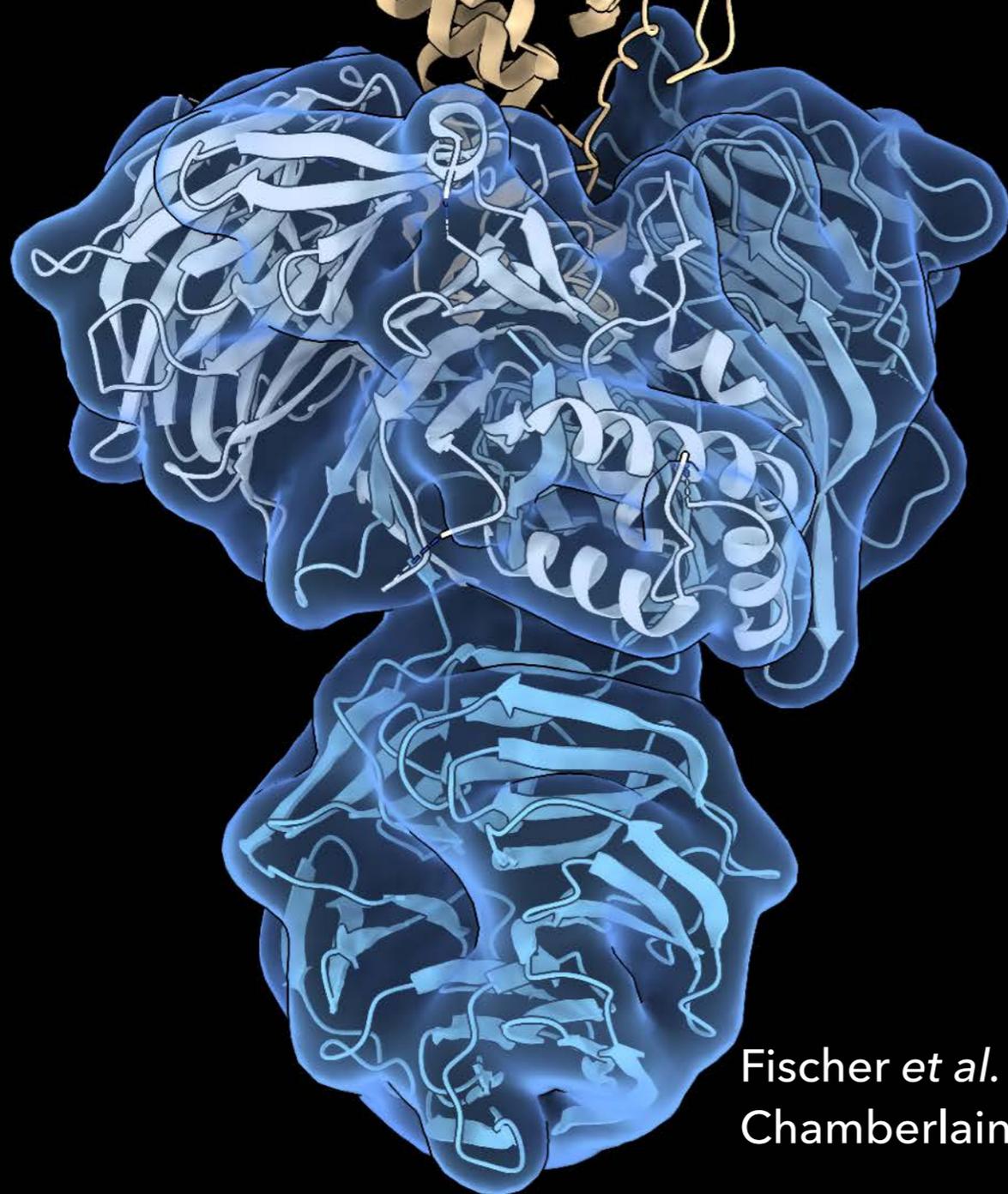
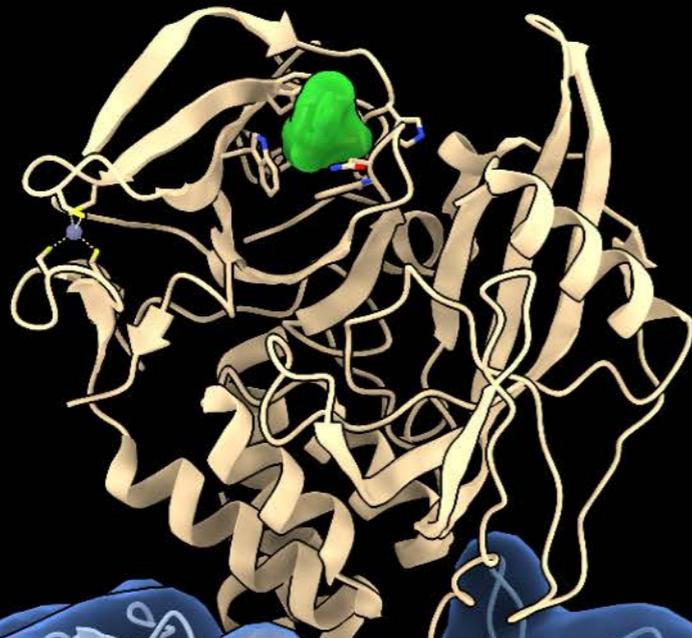
Ubiquitin tag



Targeting Disease-related proteins



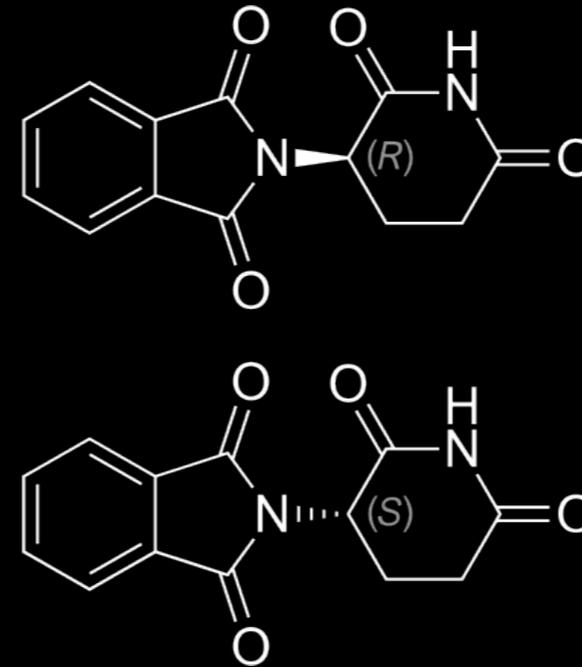
THALIDOMIDE



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

THALIDOMIDE

Cereblon

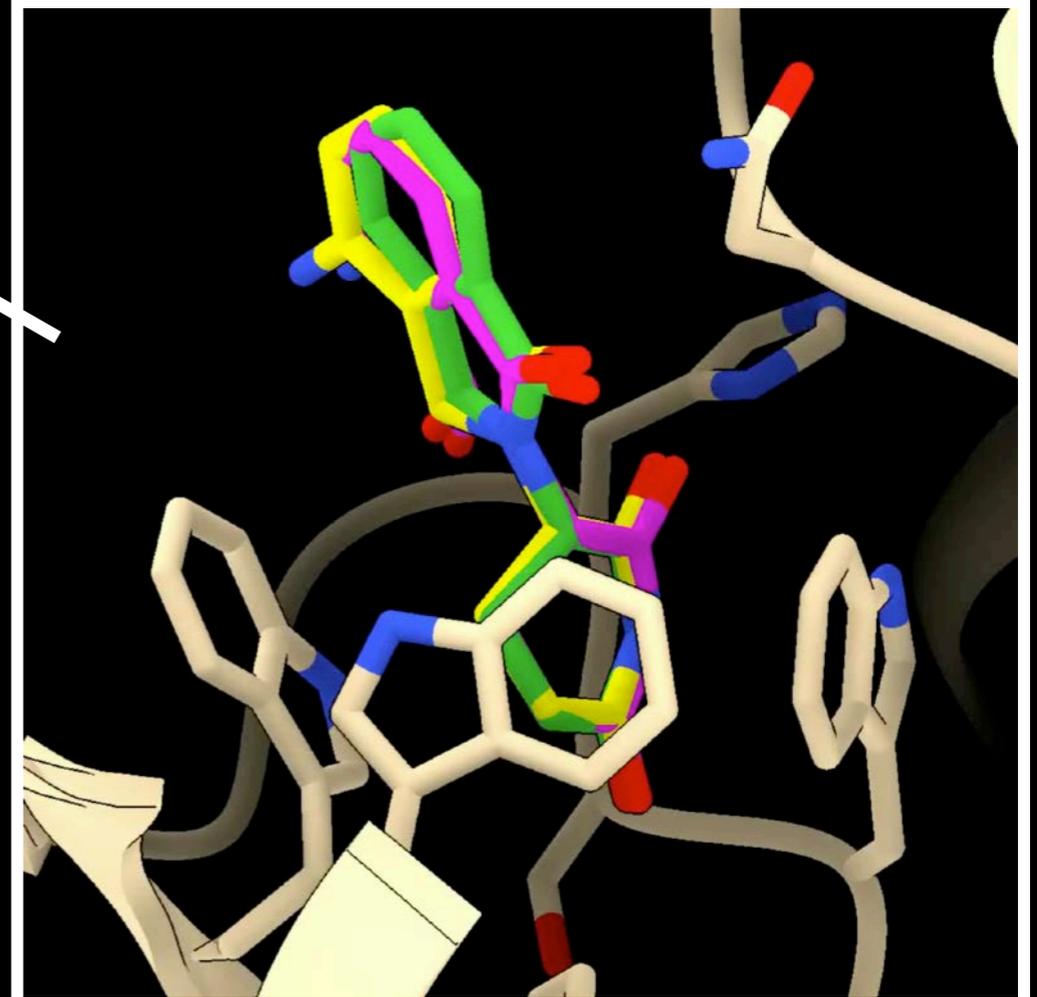


- ▶ In early 2000's approved for use in treatment of multiple myeloma, graft-vs-host disease, and leprosy

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

DRUGS BIND TO THE CEREBLON PROTEIN

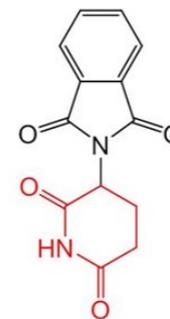
Cereblon



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

THALOMID
(thalidomide) Capsules

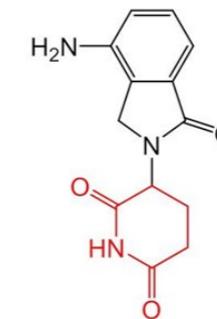
thalidomide



Glutarimide

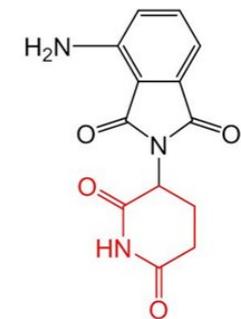
Revlimid
(lenalidomide) capsules

lenalidomide

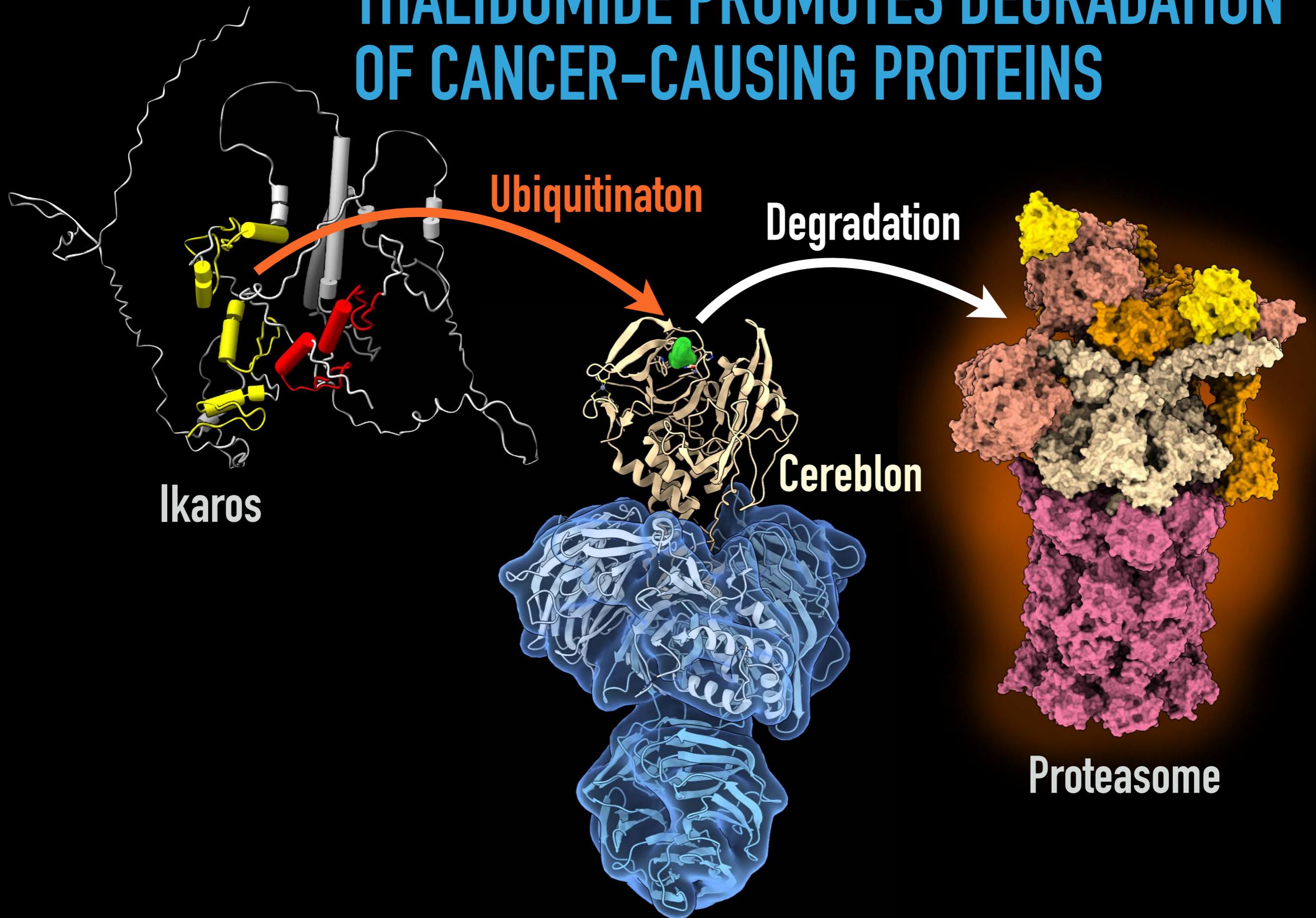


Pomalyst
(pomalidomide) capsules

pomalidomide



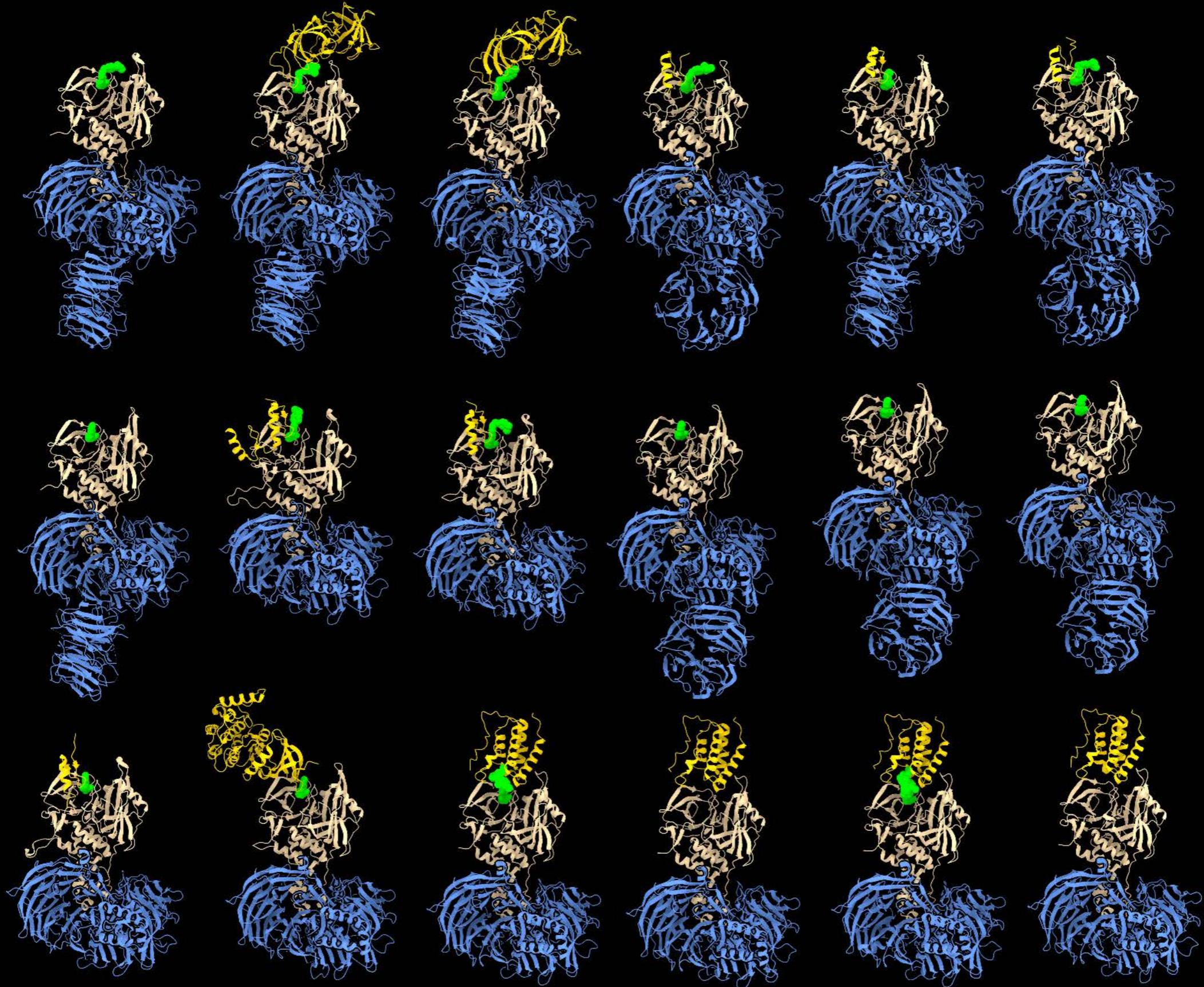
THALIDOMIDE PROMOTES DEGRADATION OF CANCER-CAUSING PROTEINS



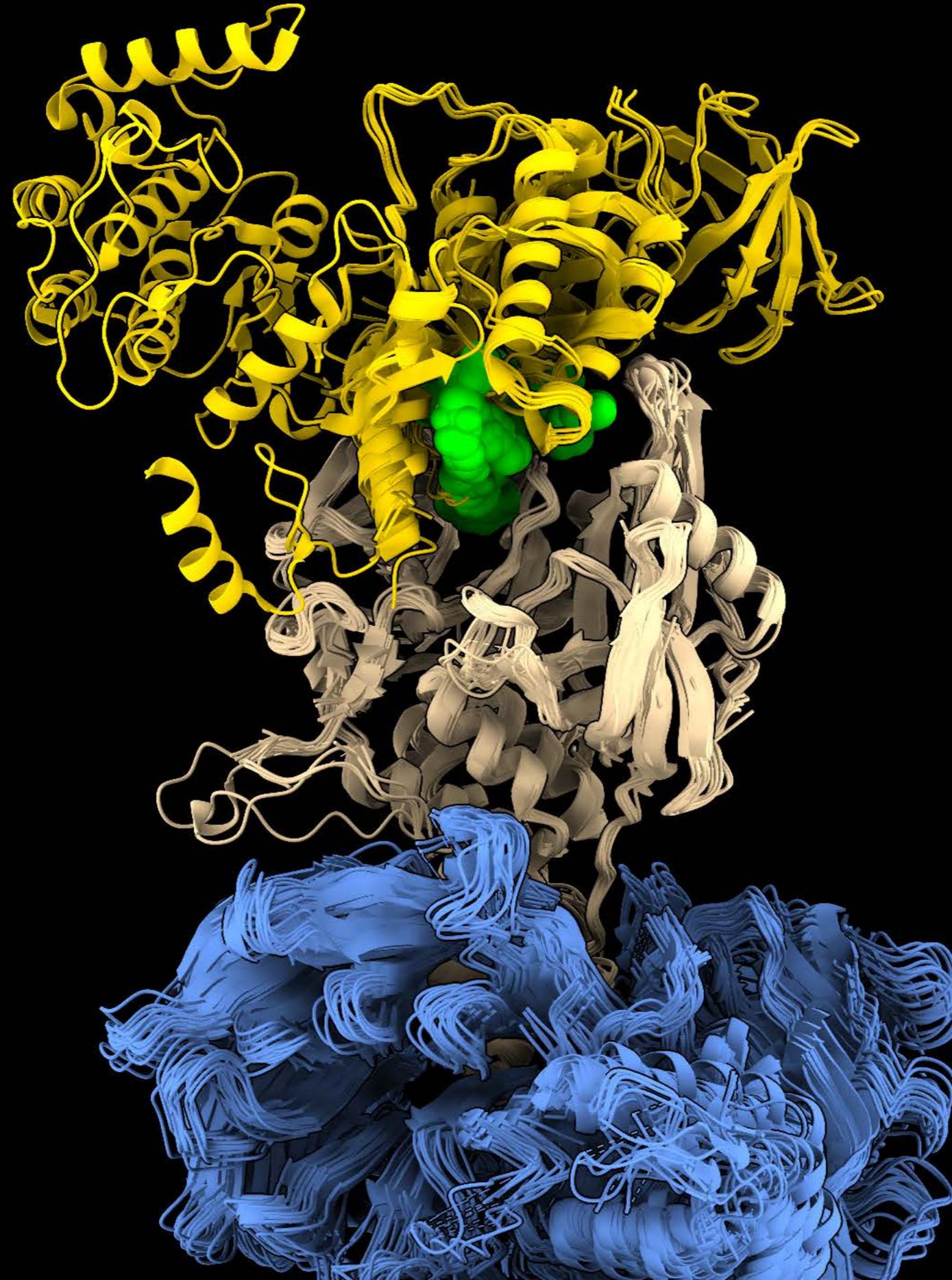
DEGRADERS IN AND APPROACHING THE CLINIC

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

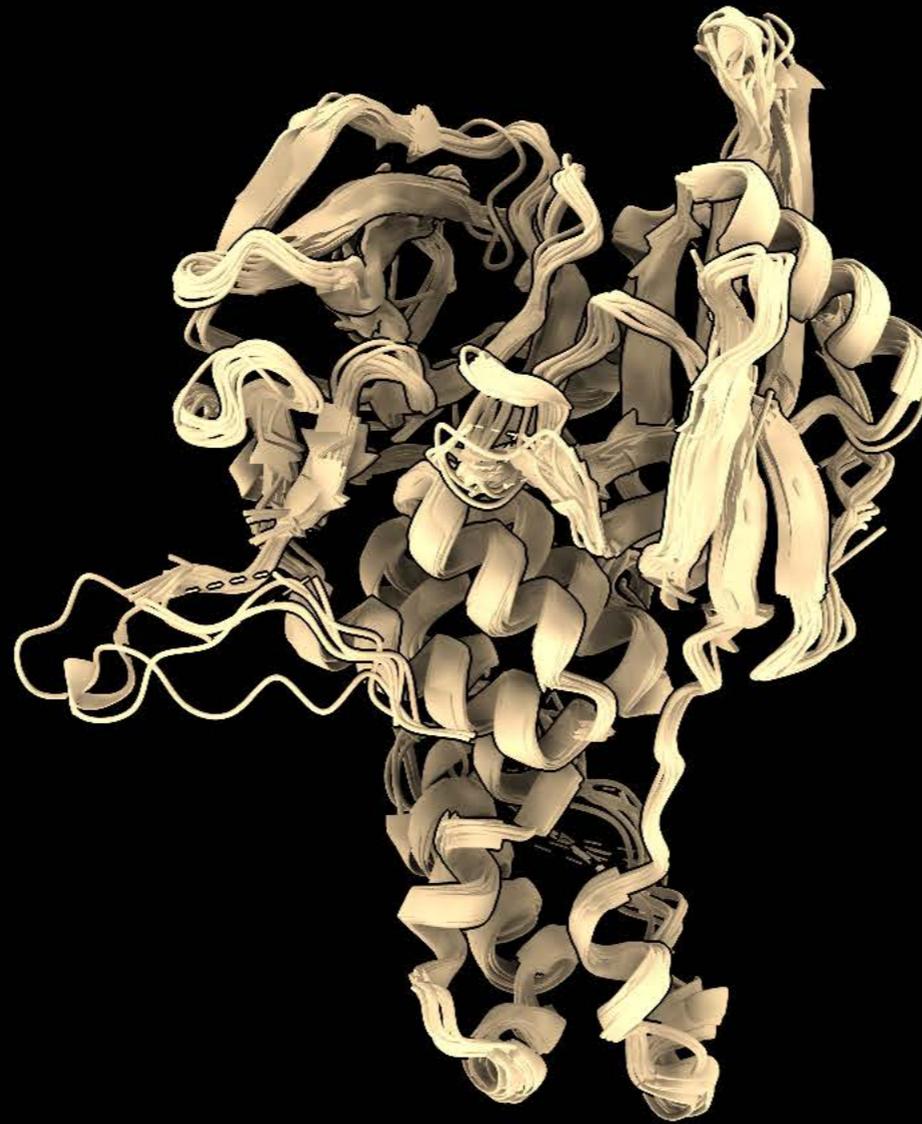
Crystal structures of the Cereblon complex



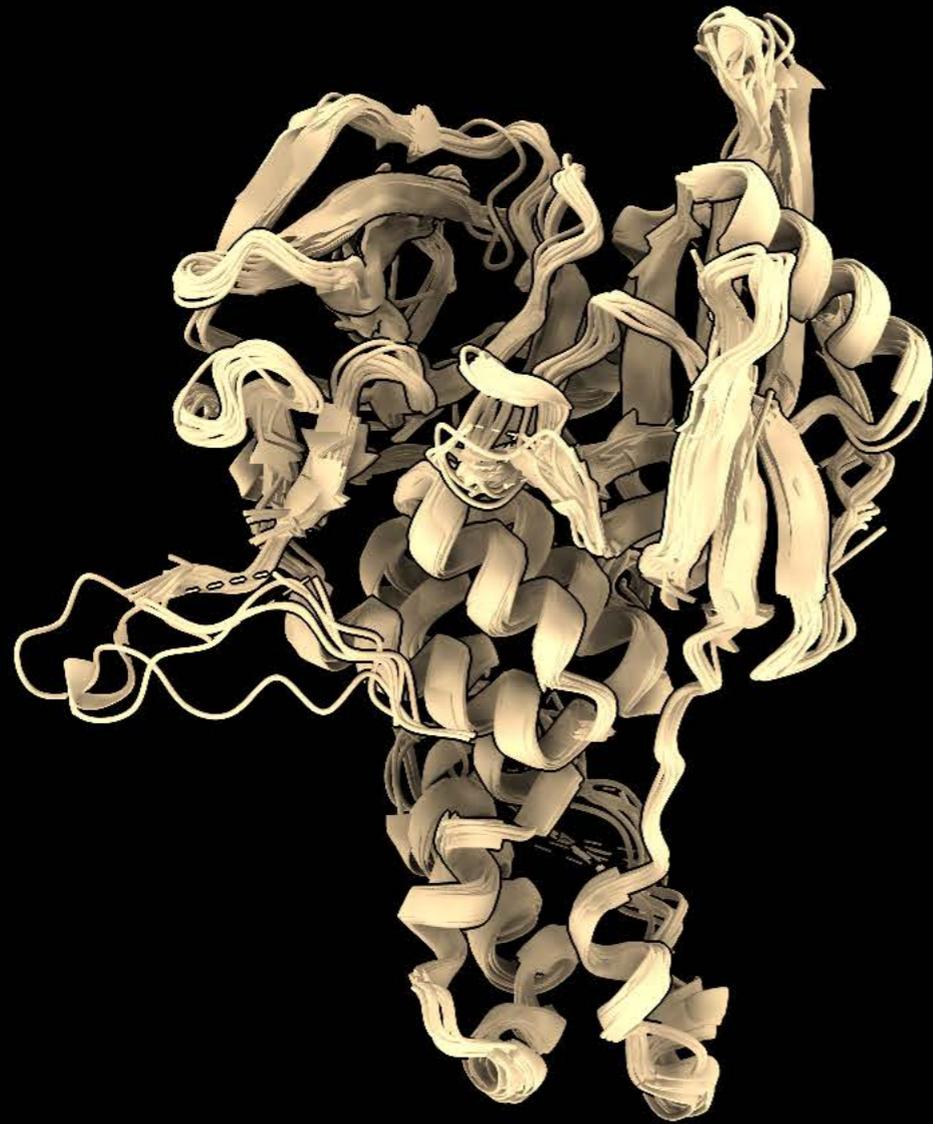
Crystal structures of the Cereblon complex



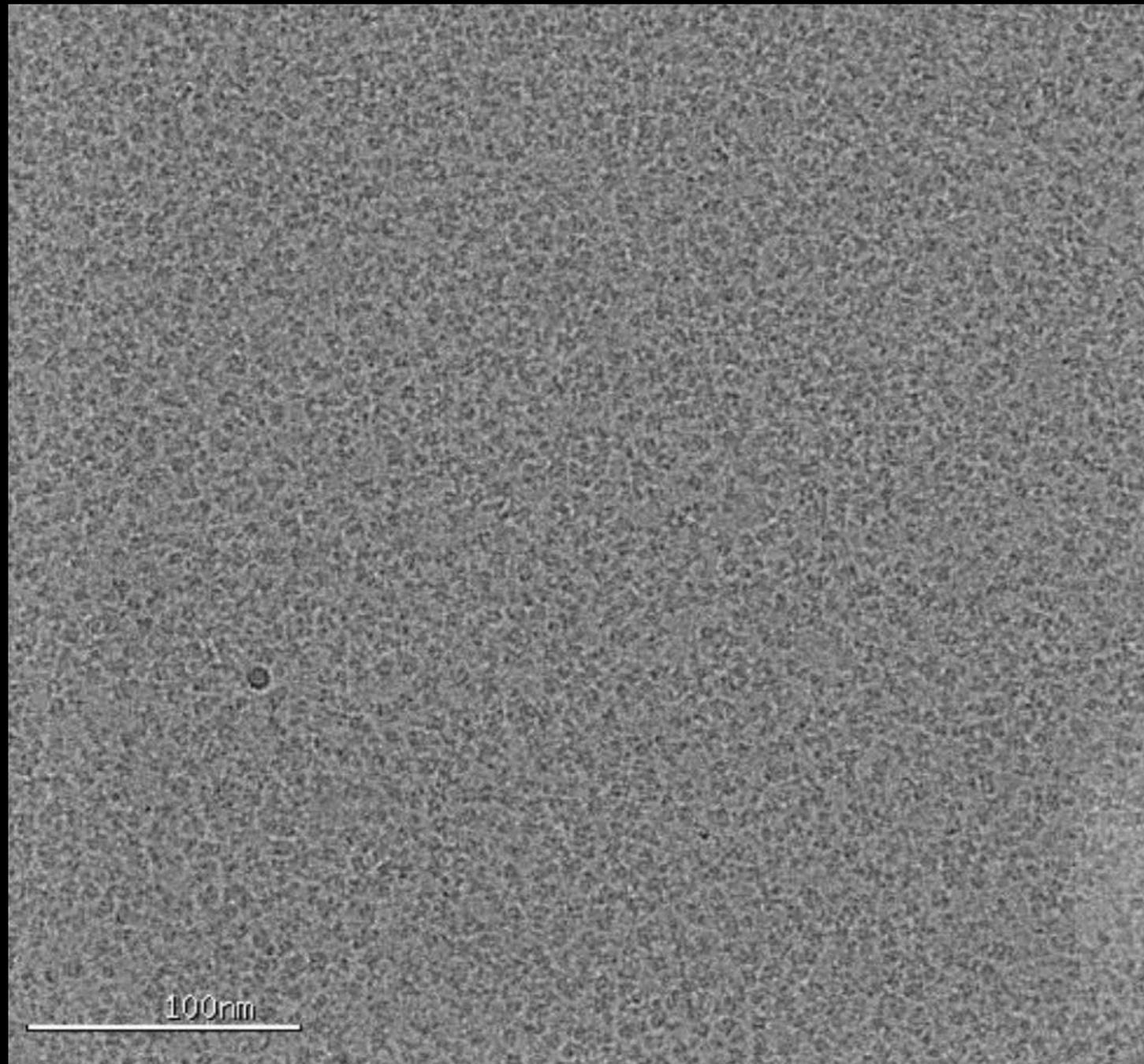
Crystal structures of Cereblon



Crystal structures of Cereblon



CRYOEM STRUCTURE OF THE CEREBLON COMPLEX



45,000 X magnification



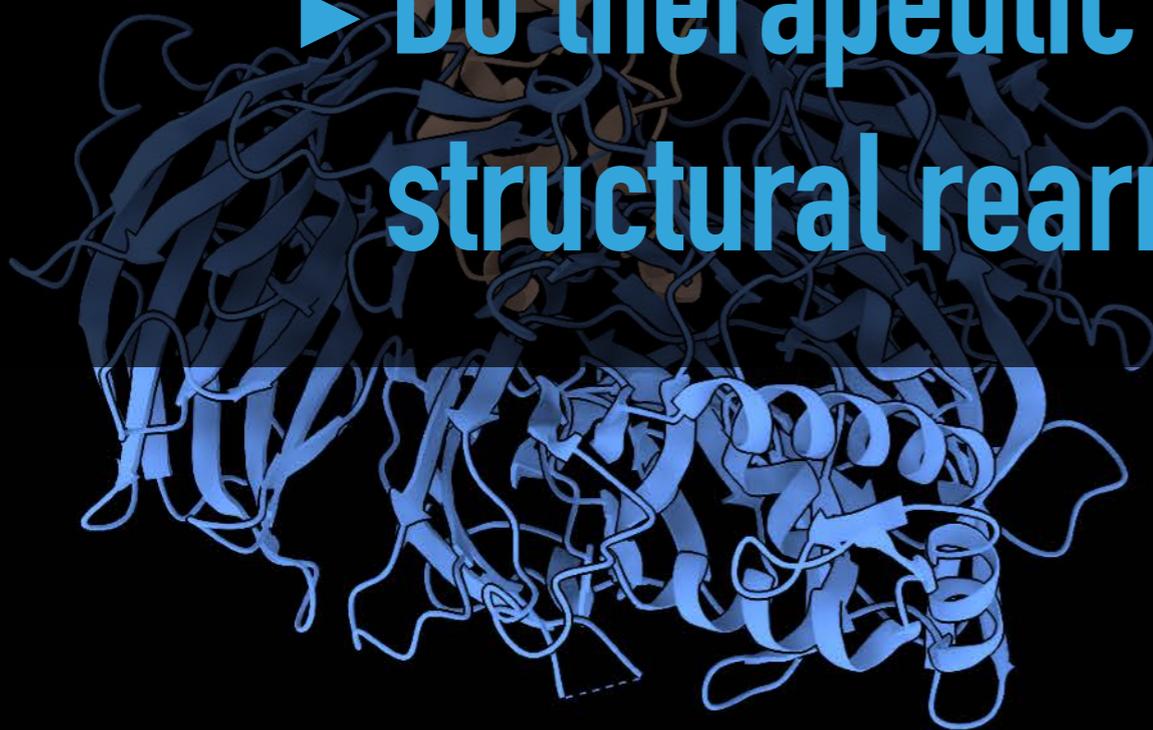
Randy Watson, PhD

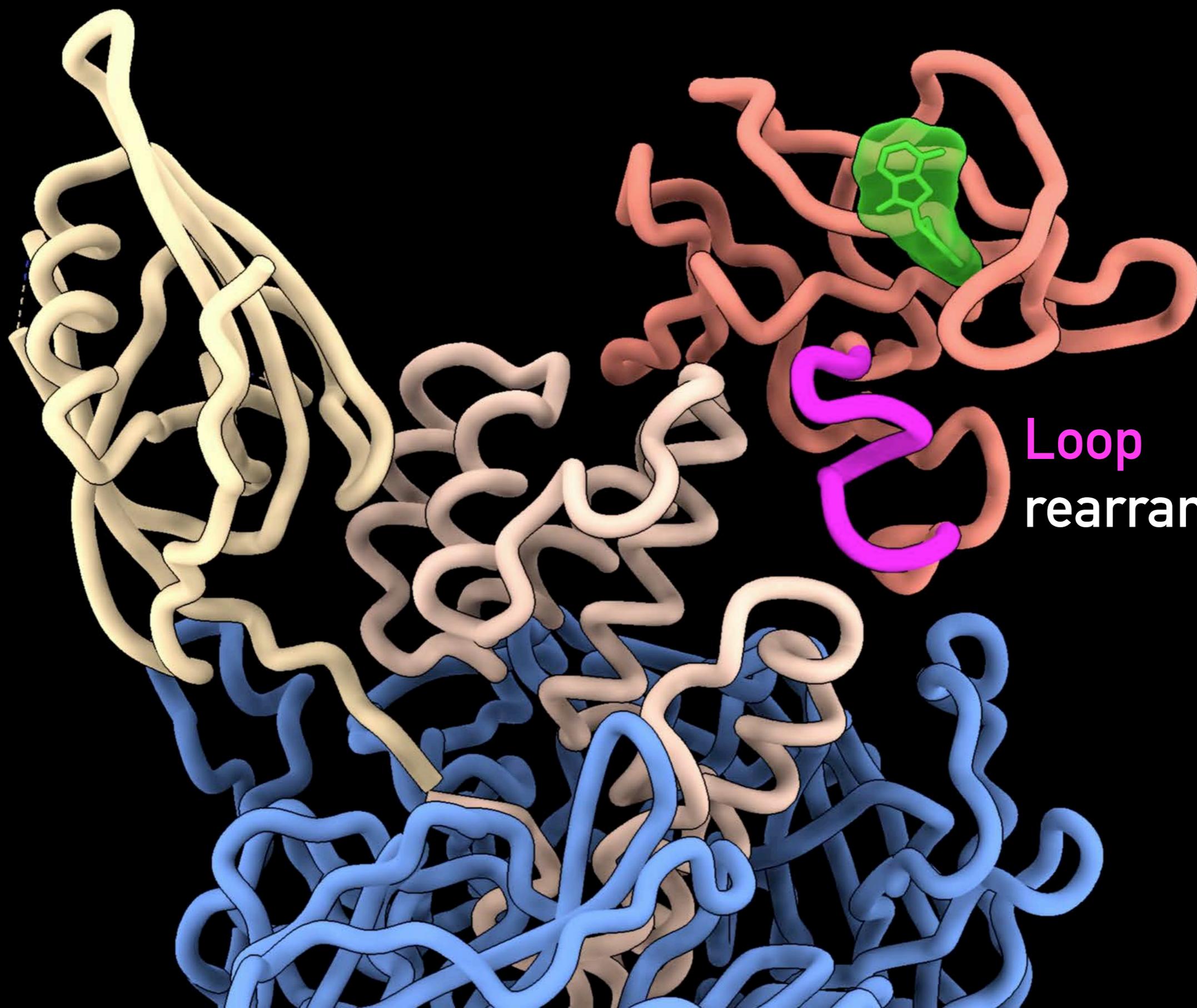
CRYSTALLOGRAPHY

CRYO-EM



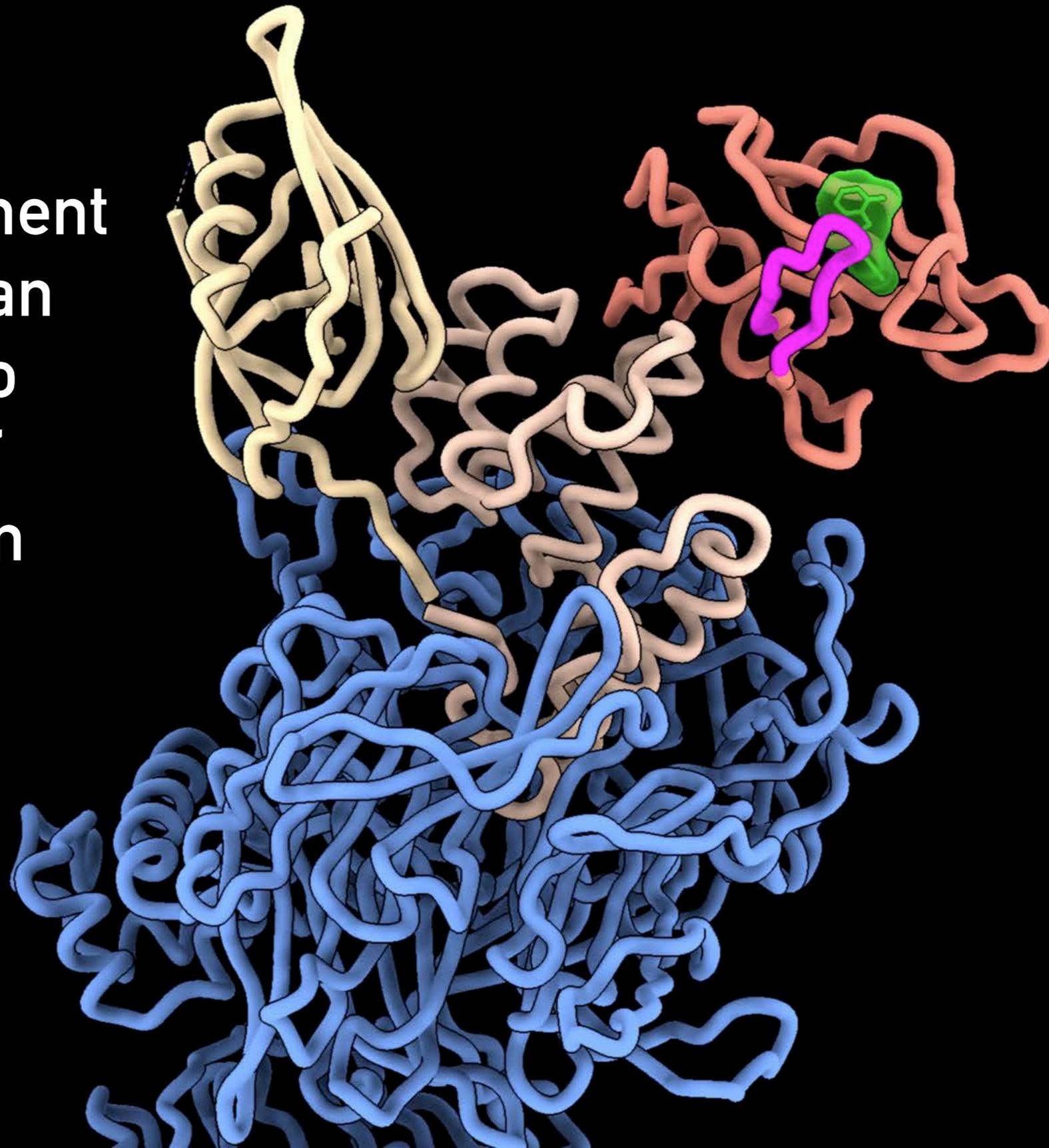
► Do therapeutic drugs impact this structural rearrangement?

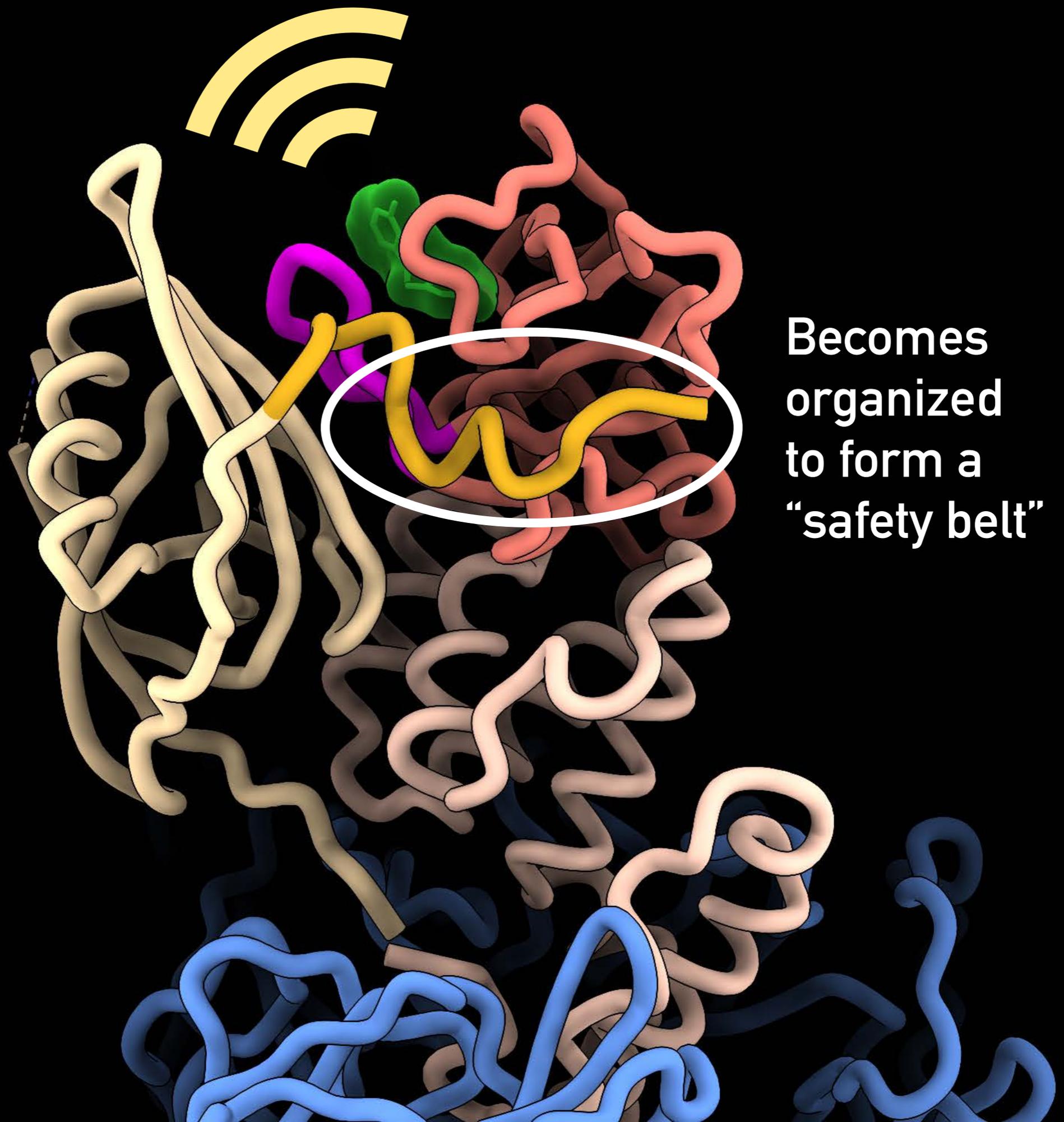




Loop
rearrangement

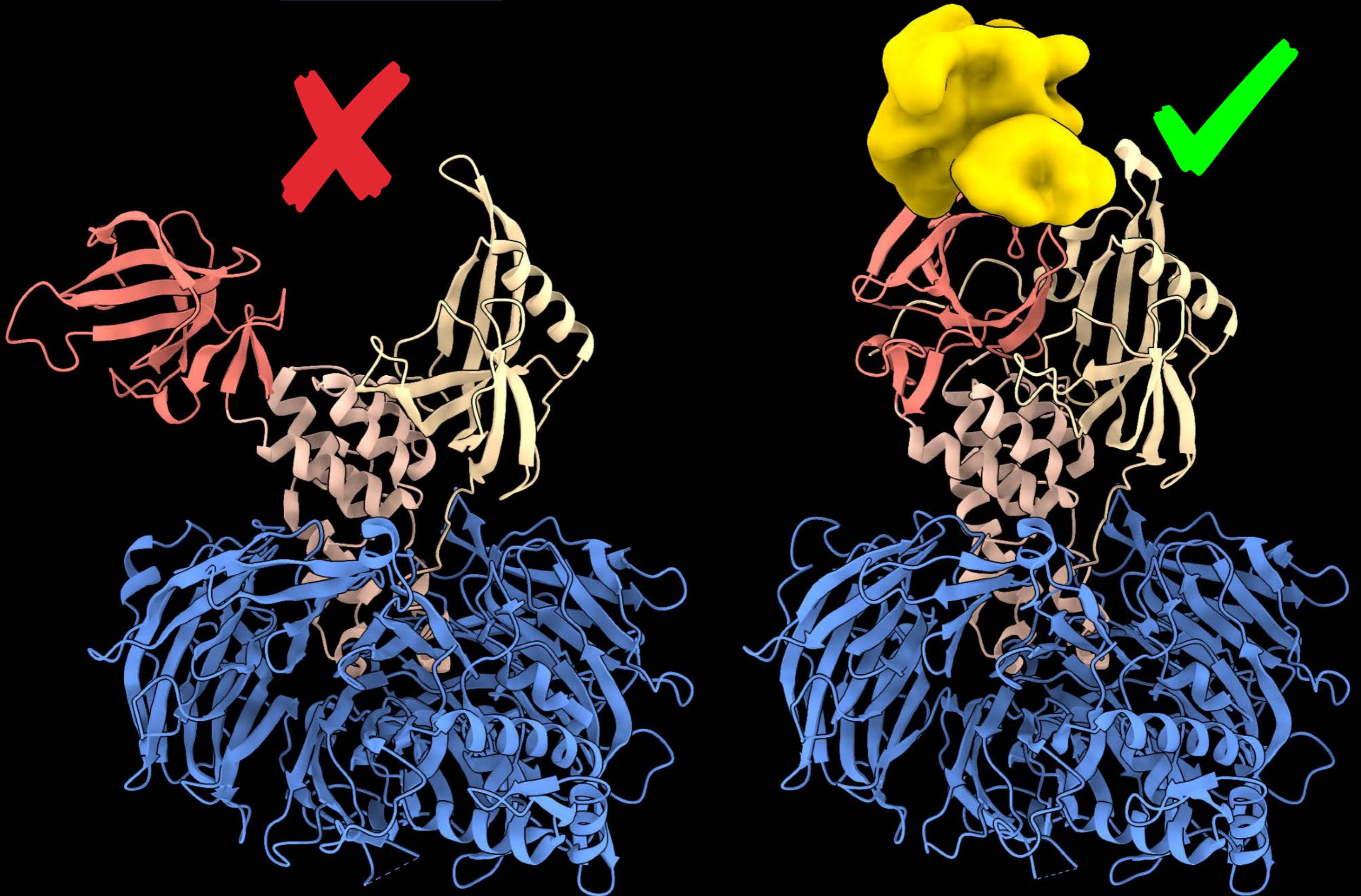
Loop
rearrangement
prompts an
“open” to
“closed”
transition





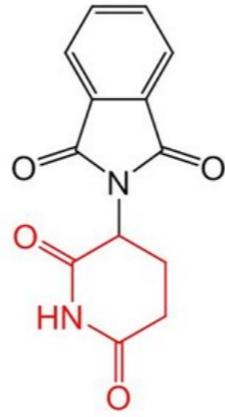
Becomes
organized
to form a
"safety belt"

ONLY THE CLOSED FORM BINDS TARGET PROTEINS



THALOMID[®]
(thalidomide) Capsules

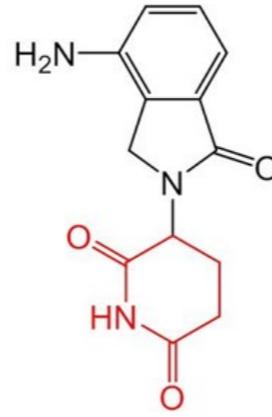
thalidomide



Glutarimide

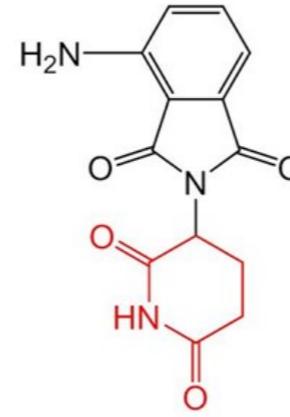
Revlimid[®]
(lenalidomide) capsules

lenalidomide



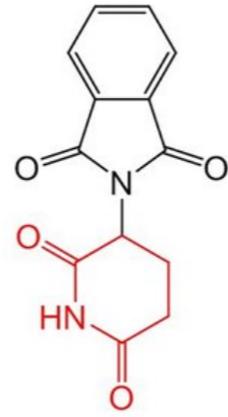
Pomalyst[®]
(pomalidomide) capsules

pomalidomide



THALOMID[®]
(thalidomide) Capsules

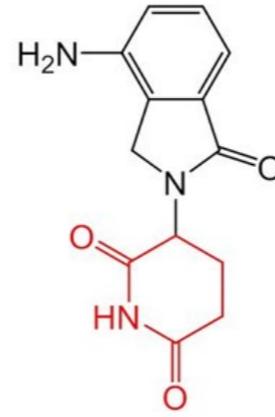
thalidomide



Glutarimide

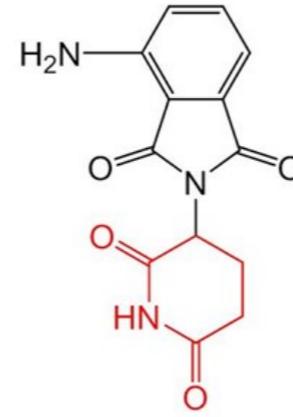
Revlimid[®]
(lenalidomide) capsules

lenalidomide



Pomalyst[®]
(pomalidomide) capsules

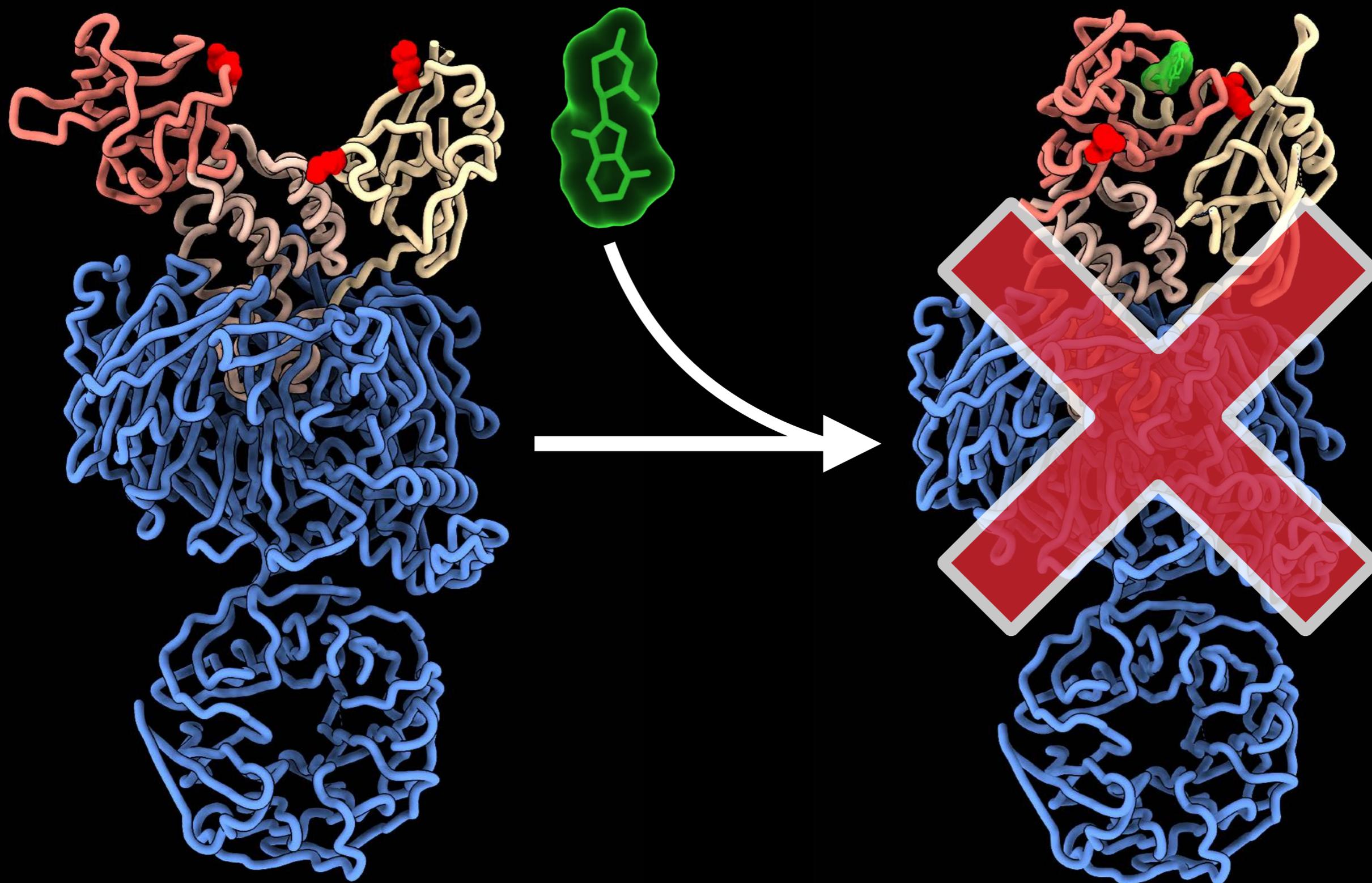
pomalidomide



NO RESPONSE



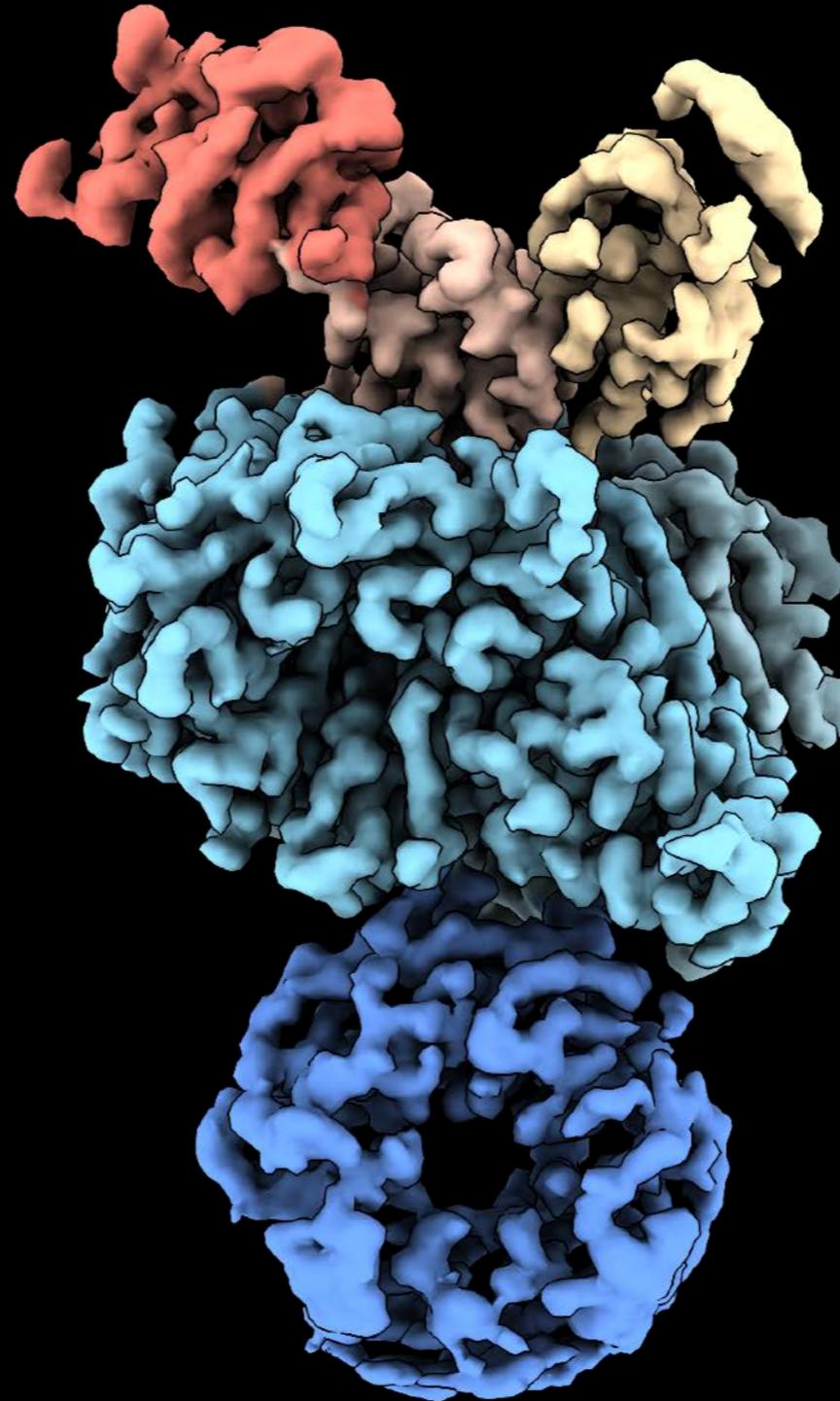
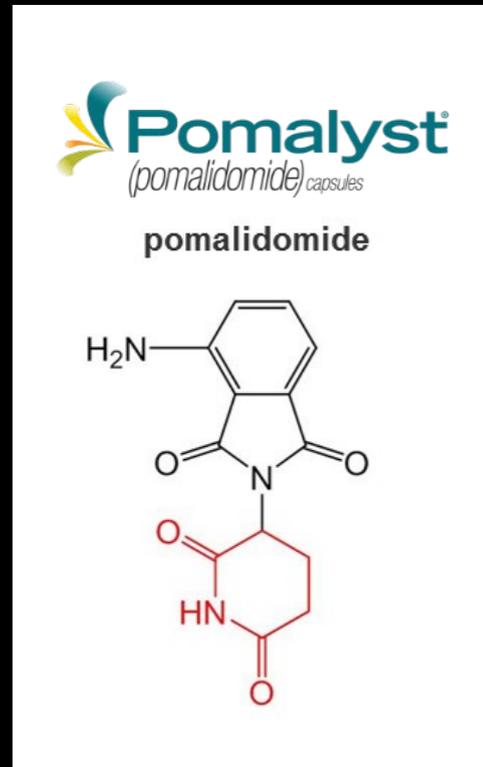
PATIENT MUTATIONS MAY PREVENT CLOSED FORM



TEAMING UP WITH BRISTOL MYERS SQUIBB



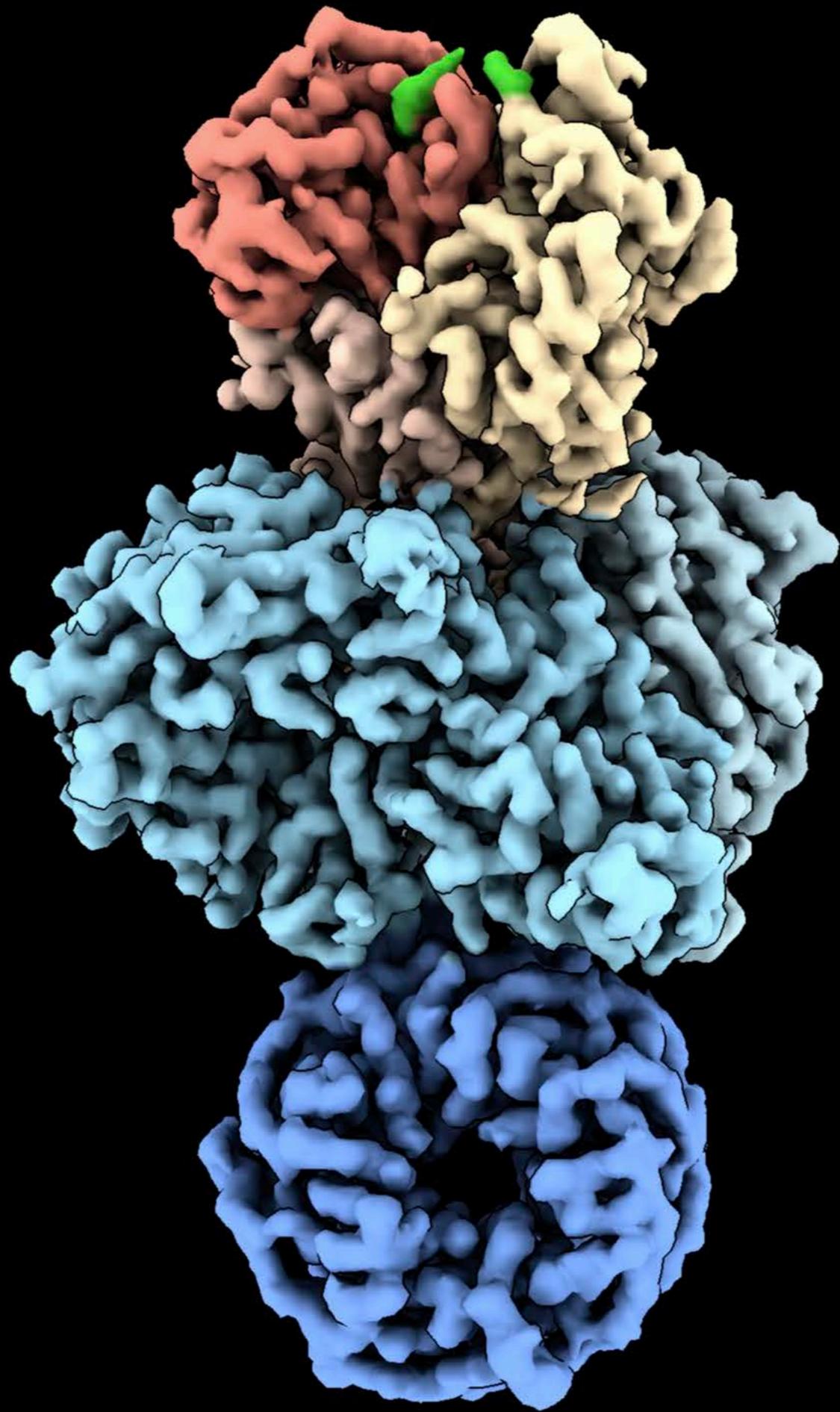
TRADITIONAL THERAPEUTIC + MUTANT CEREBLON



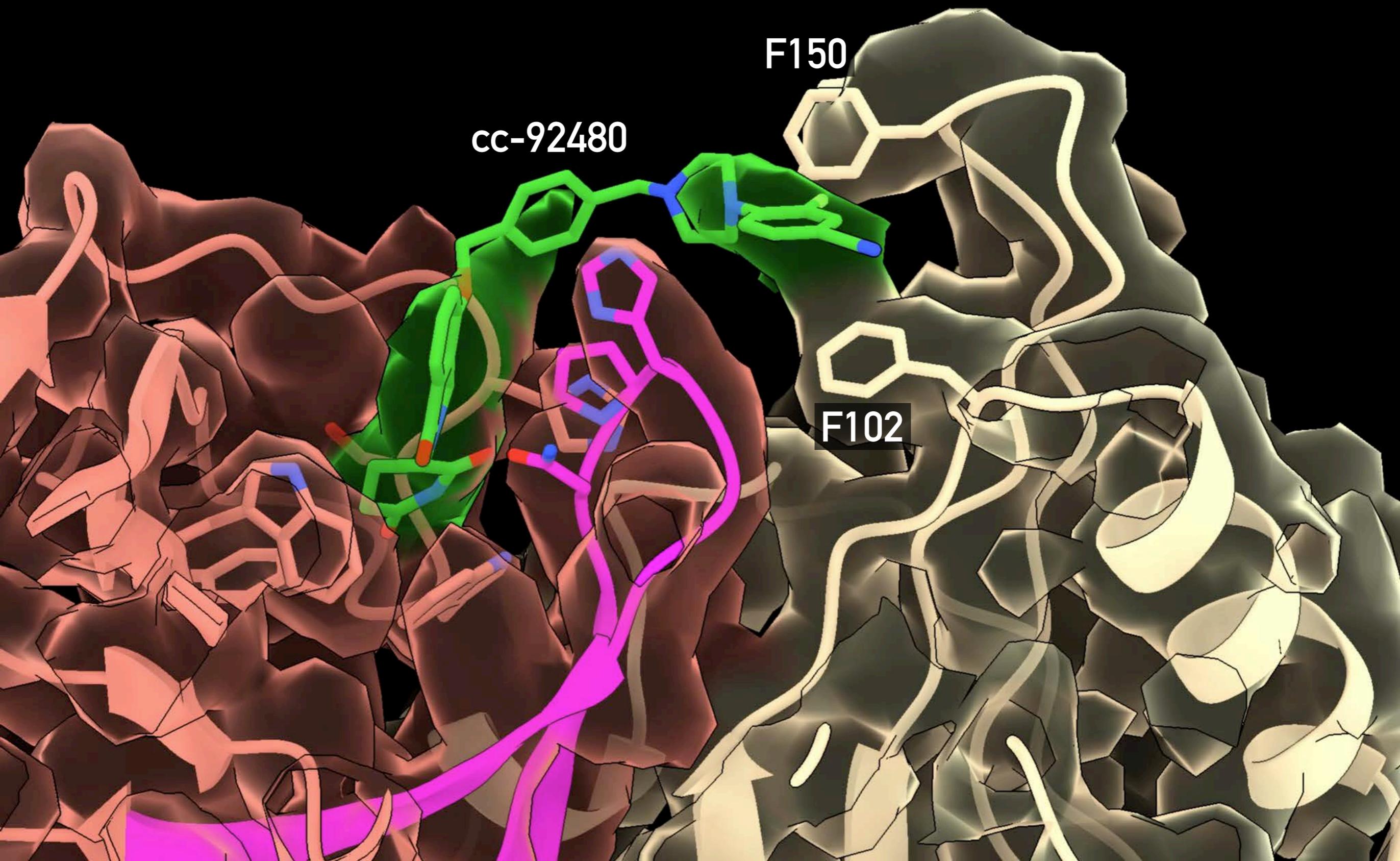
No closure
of Cereblon

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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