

MERCK

INVENTING FOR LIFE



Beyond the Microscope: Cryo-EM's Impact on Accelerating Drug Discovery

Sandra Gabelli

NASEM 2023

November 16, 2023

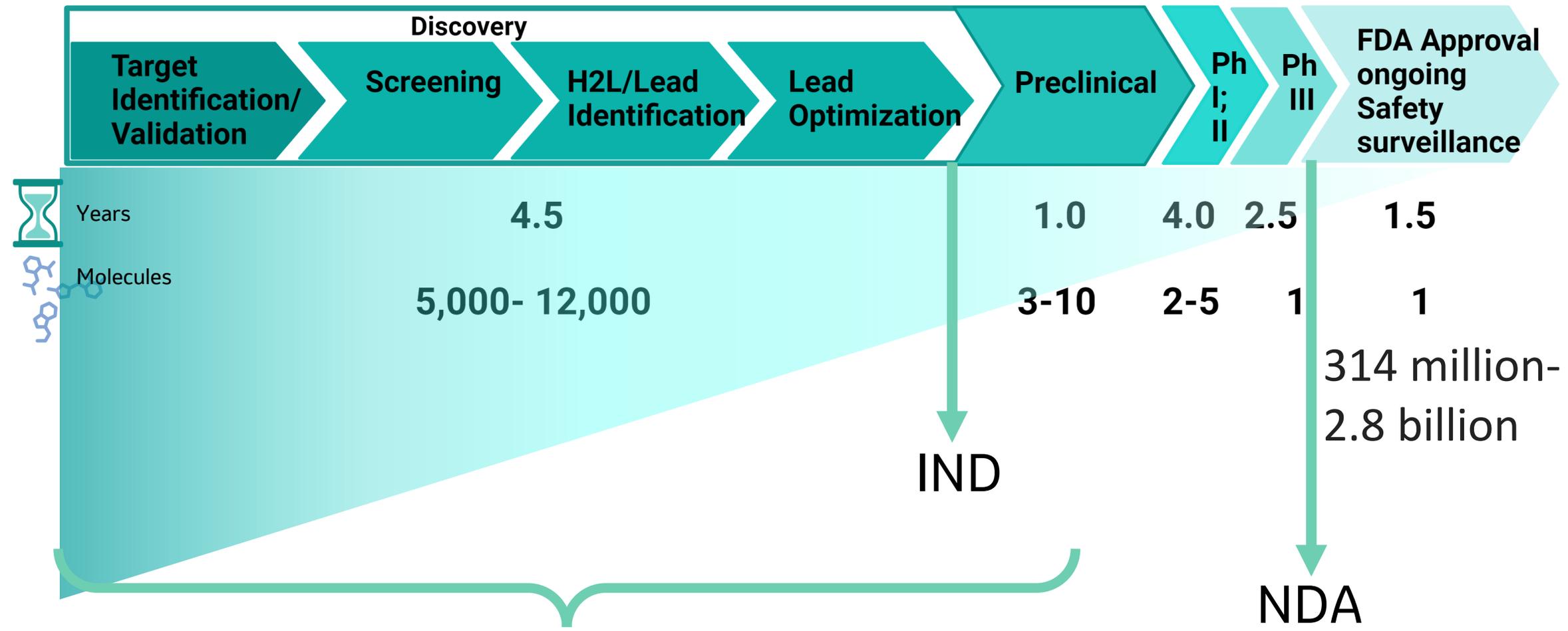
Beyond the microscope: Merck



We are a networked team, impacting drug discovery



Beyond the microscope: from hit to market ~15 years

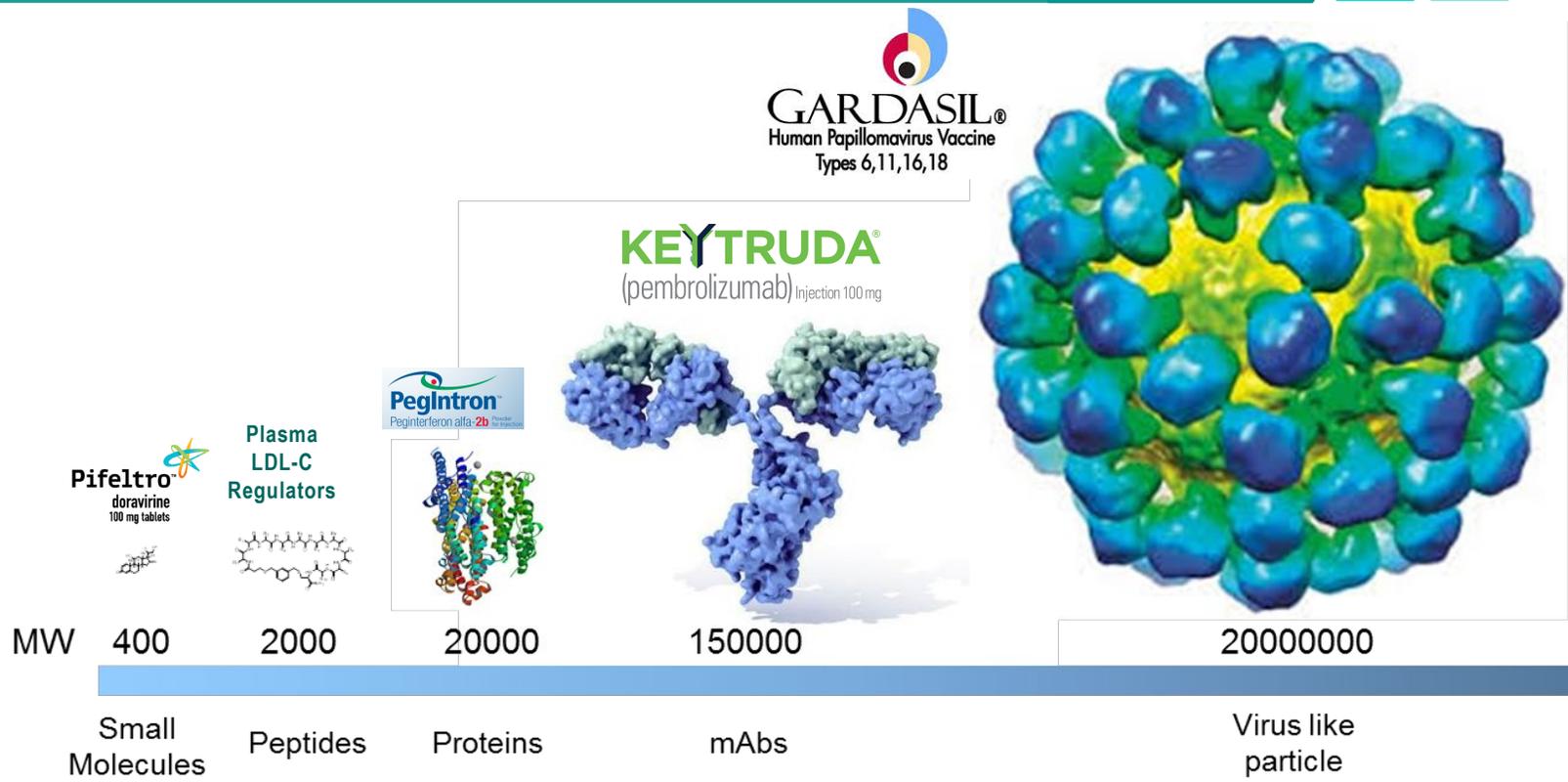


Wouters, Kee &Luyten, JAMA 2020

Attrition

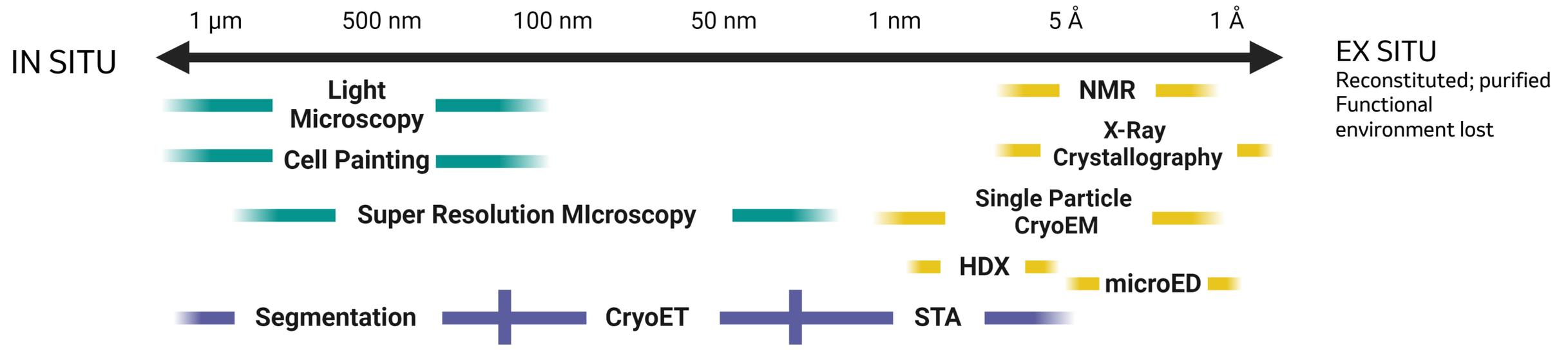
Mullard, 2023 Nature Review Drug Discovery
Fojo 2023 JAMA journey to failure
Zurdo, J April 2013 [Pharmaceutical Bioprocessing](#) 1(1):29-50

Beyond de microscope: structural biology impacts all modalities



Beyond the microscope: ex situ structural biology techniques

FROM RECONSTITUTED TO IN-CELL VISUALIZATION



Visualization of the effects of a therapeutic in the cell and understanding how cells work

Beyond the microscope: cryo-EM timeline

Xray generator
Home sources



Insulin Receptor

Orexin2

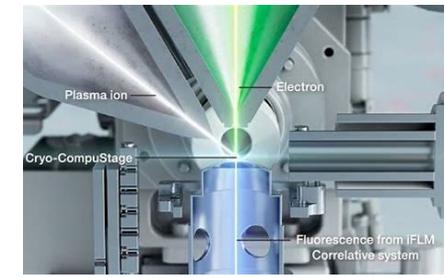
Arginase

RSV rPOL

mAB hMPV

VHH aSARS

KV3.1 VGK

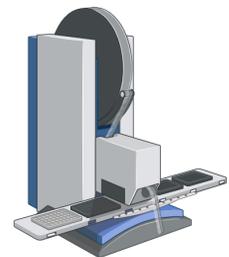


Beyond the microscope: X-ray vs CryoEM

X-RAY Crystallography

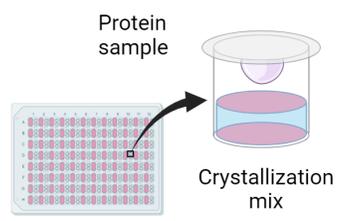
Crystal plate setup

Protein and crystallization mix are added to 96-well plate



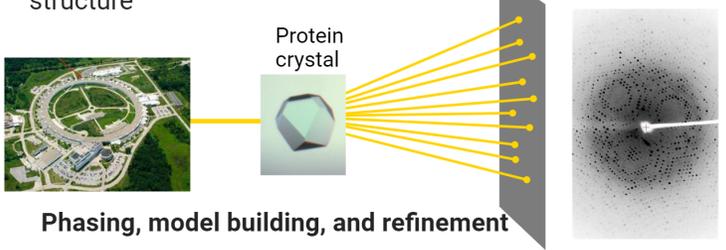
Crystallization

Protein crystallizes under unique conditions



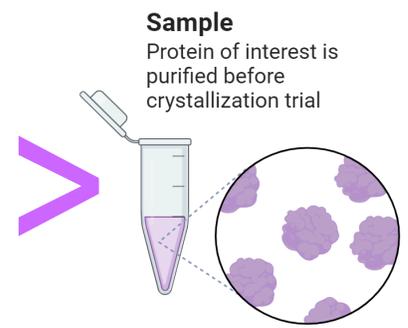
Macromolecular Crystallography Data collection

Protein crystals are shot with X-rays to produce a diffraction pattern unique to the protein structure



Phasing, model building, and refinement

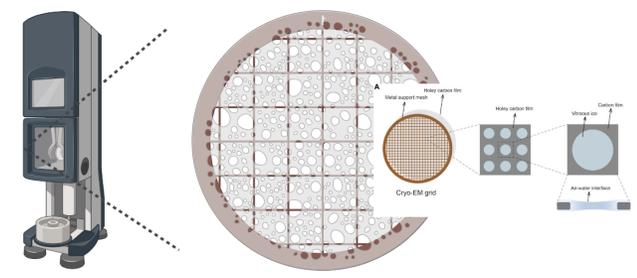
Using the diffraction pattern and specialized software, scientists are able to determine the protein's 3D structure; calculation ED map by using Fast Fourier.



CryoEM Single Particle Analysis

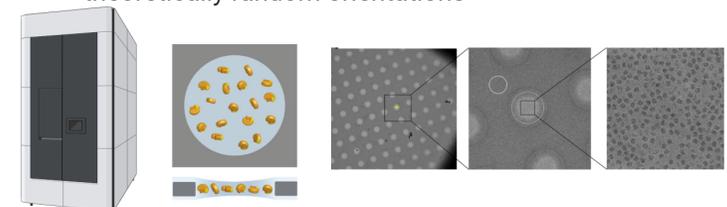
Plunge Freezing

Protein Sample is vitrified in grid by plunge freezing

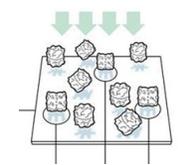


Cryo Electron Microscopy Data collection

Well dispersed proteins on grid are imaged with an electron beam to produce 2D projections of the protein in theoretically random orientations



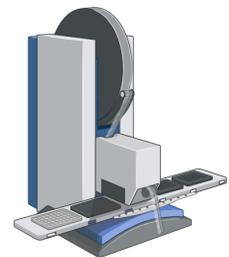
Following data collection, individual protein particles are computationally picked and classified in 2D based on their orientations. Back projections of these particles based on their relative angular orientations gives an initial 3D reconstruction that can then be refined to give a high resolution electron density map.



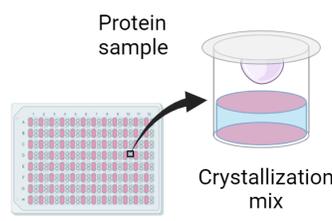
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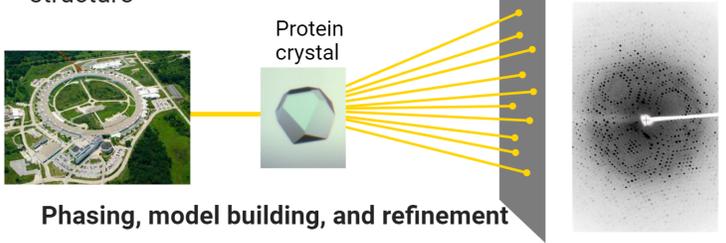


Crystallization
Protein crystallizes under unique conditions



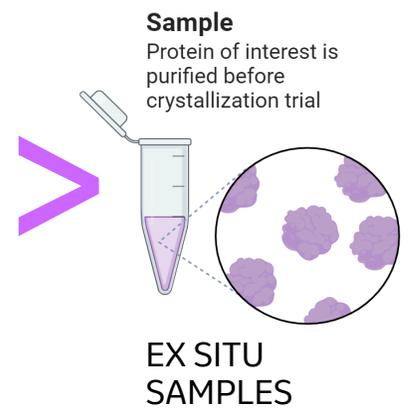
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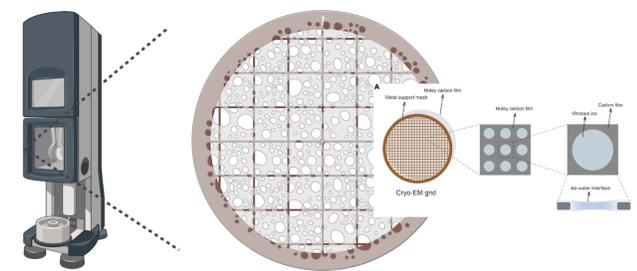
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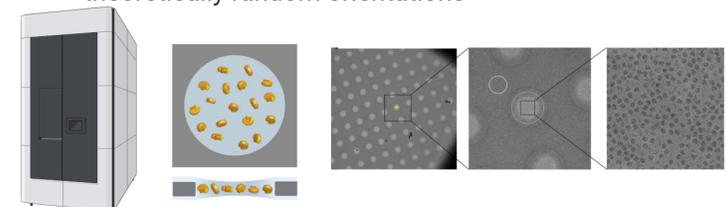
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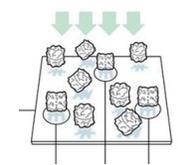


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Beyond the microscope: Xray vs Cryo-EM

X-RAY Crystallography

- High resolution (0.6-3.5 Å)
- Broad MW 12 kDa- 200 kDa
- Crystal requirement
- No need vitrification
- Only amplitudes are recorded; Phase problem
- Ice rings affect data completeness
- Higher amounts of protein required crystal screening
- Heterogeneous samples often fail to crystallize
- Excellent for ion characterization with ligands

CryoEM Single Particle Analysis

- Medium resolution (2.0 -10.0 Å)
- > 80-90 kDa (been done with 40 kDa)
- No need of crystals
- Vitrification, thin ice
- Amplitudes and phases are recorded
- Resolution is inversely proportional to ice thickness
- Smaller amount of protein (technique better suited for complexes with yield ~ 2µg)
- Allows analysis of heterogeneous samples
- Not particularly good for ion characterization

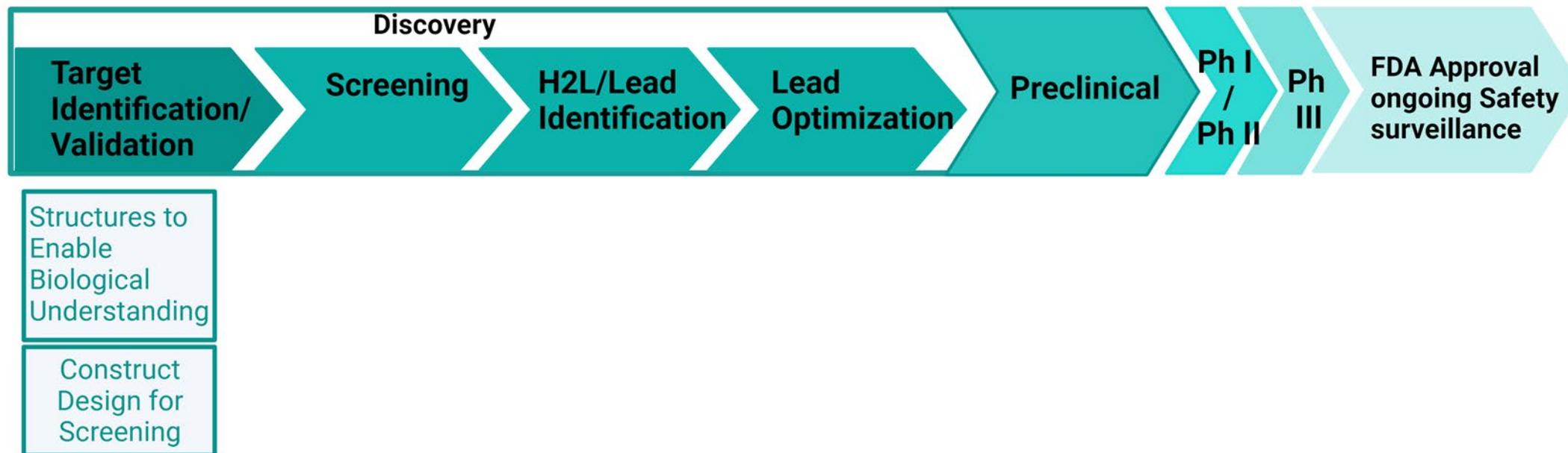
Beyond the microscope: impact of cryo-EM on pipeline



Structural Biology

Beyond the microscope: enable biological understanding

Insulin Receptor



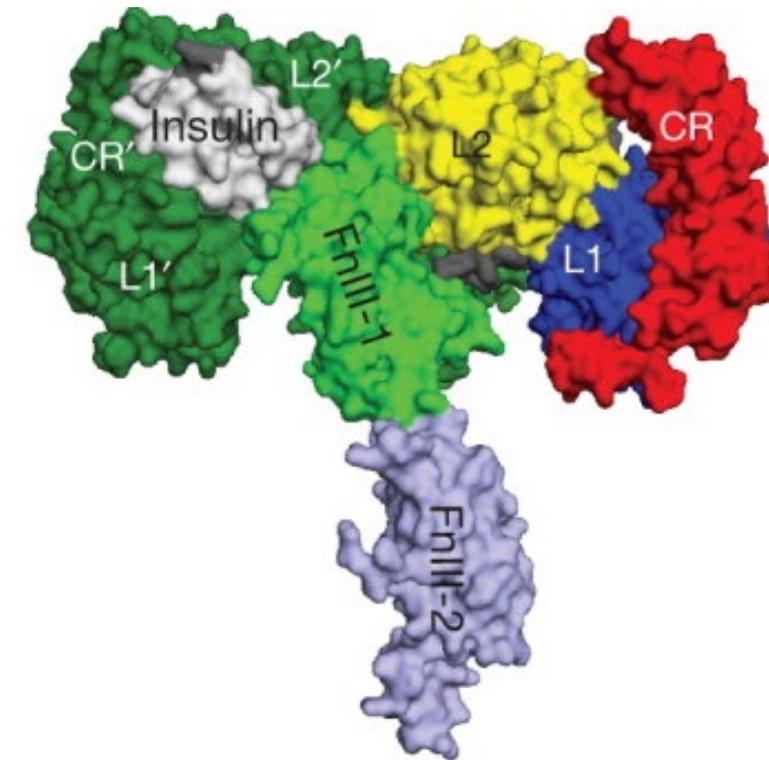
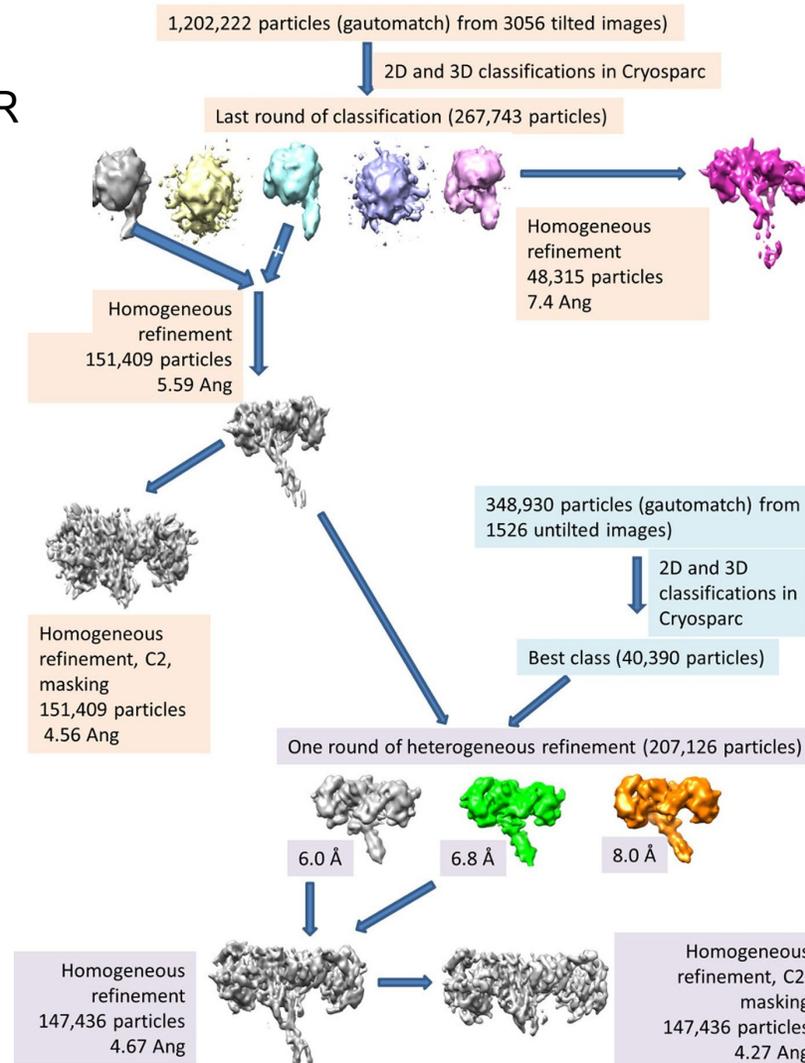
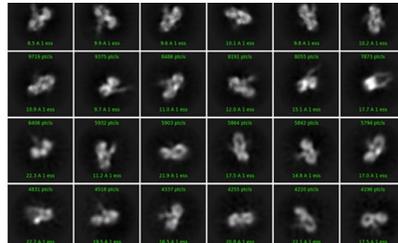
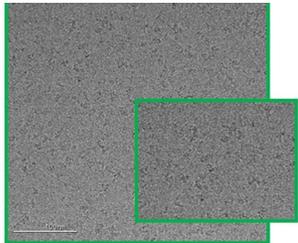
Help to understand
druggability and MOA
of tool molecules

**Structures to Enable
Biological Understanding**

Beyond the microscope: enable biological understanding

Insulin receptor

Indication/Relevance: role in controlling glucose homeostasis, regulating lipid, protein, and carbohydrase metabolism. IR dysfunction has been associated with diabetes, cancer and Alzheimer.



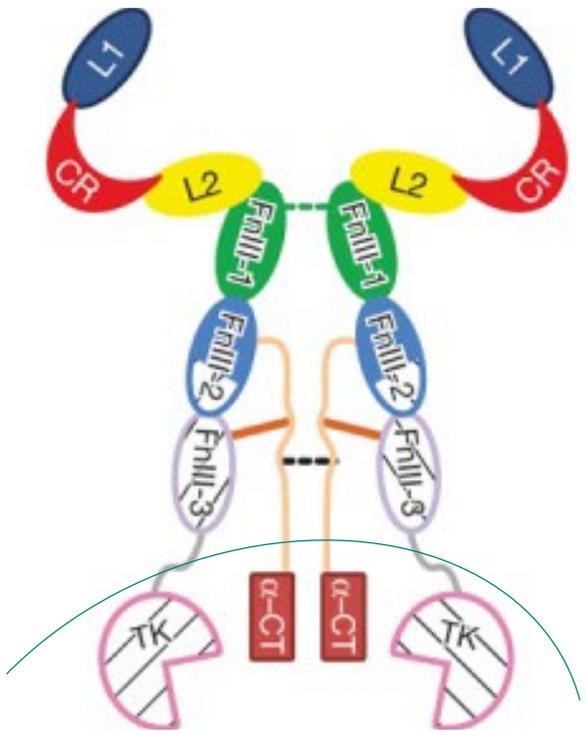
Beyond the microscope: enable biological understanding

Insulin receptor

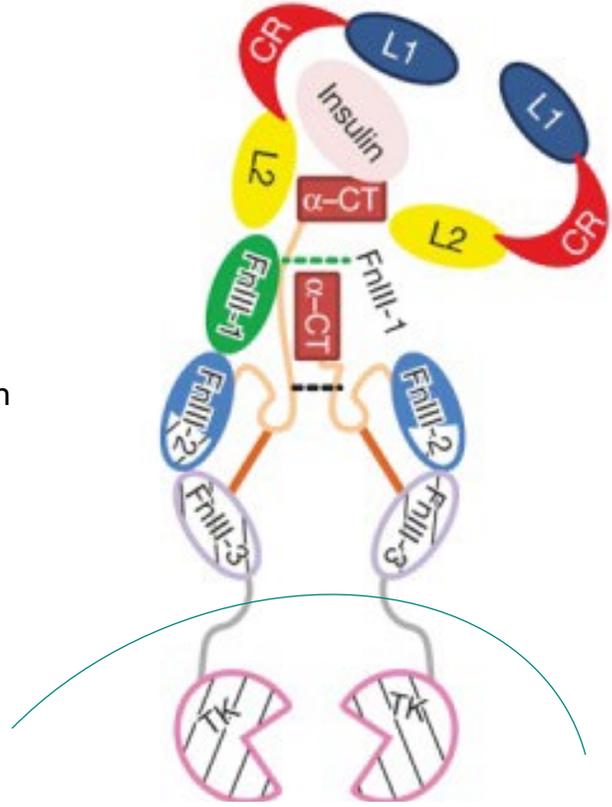
Indication/Relevance: role in controlling glucose homeostasis, regulating lipid, protein, and carbohydrate metabolism. IR dysfunction has been associated with diabetes, cancer and Alzheimer.

IMPACT: Structural and mechanistic knowledge of complex formation and activation. Insulin binding induces a conformational change that closes top L1-CR-L2 with respect to FnIII-1 domain

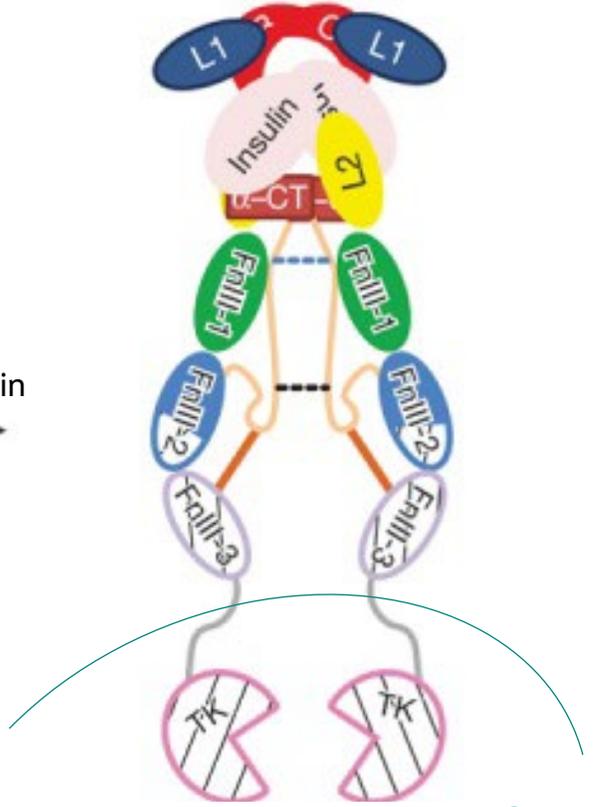
CHALLENGES OVERCOME: Sample heterogeneity; preferred orientation addressed by use of Spotiton and tilting



+ Insulin
→



+ Insulin
→



Beyond the microscope: exploring SAR

GPCRs: the orexin Receptor



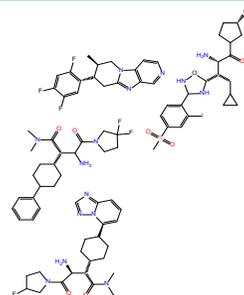
Structures to Enable Biological Understanding

Construct Design for Screening

Help to understand druggability and MOA of tool molecules

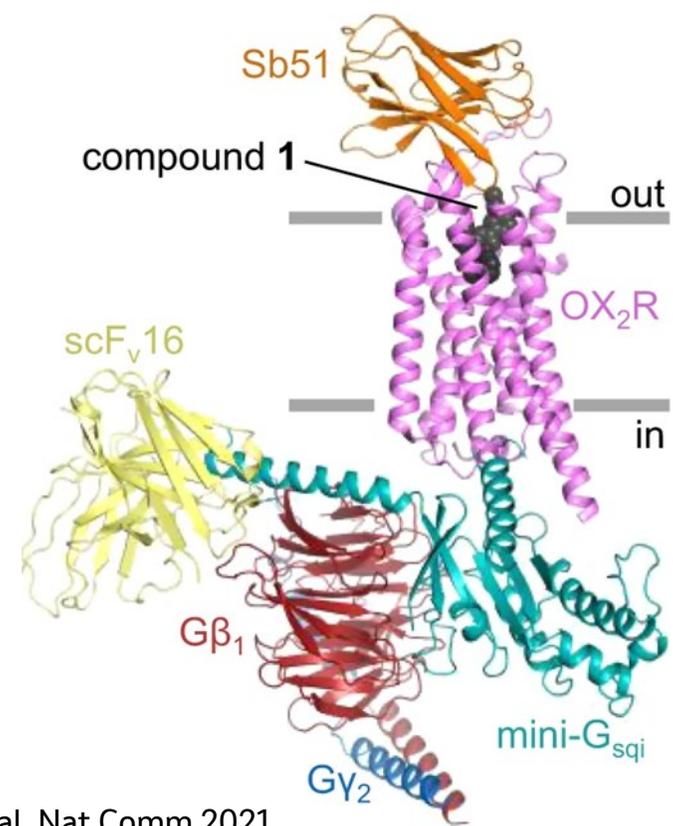
Structures to Enable Biological Understanding

Structure of Screening hits to explore SAR



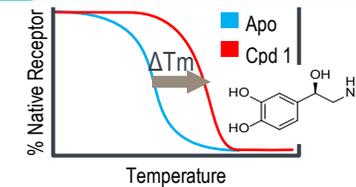
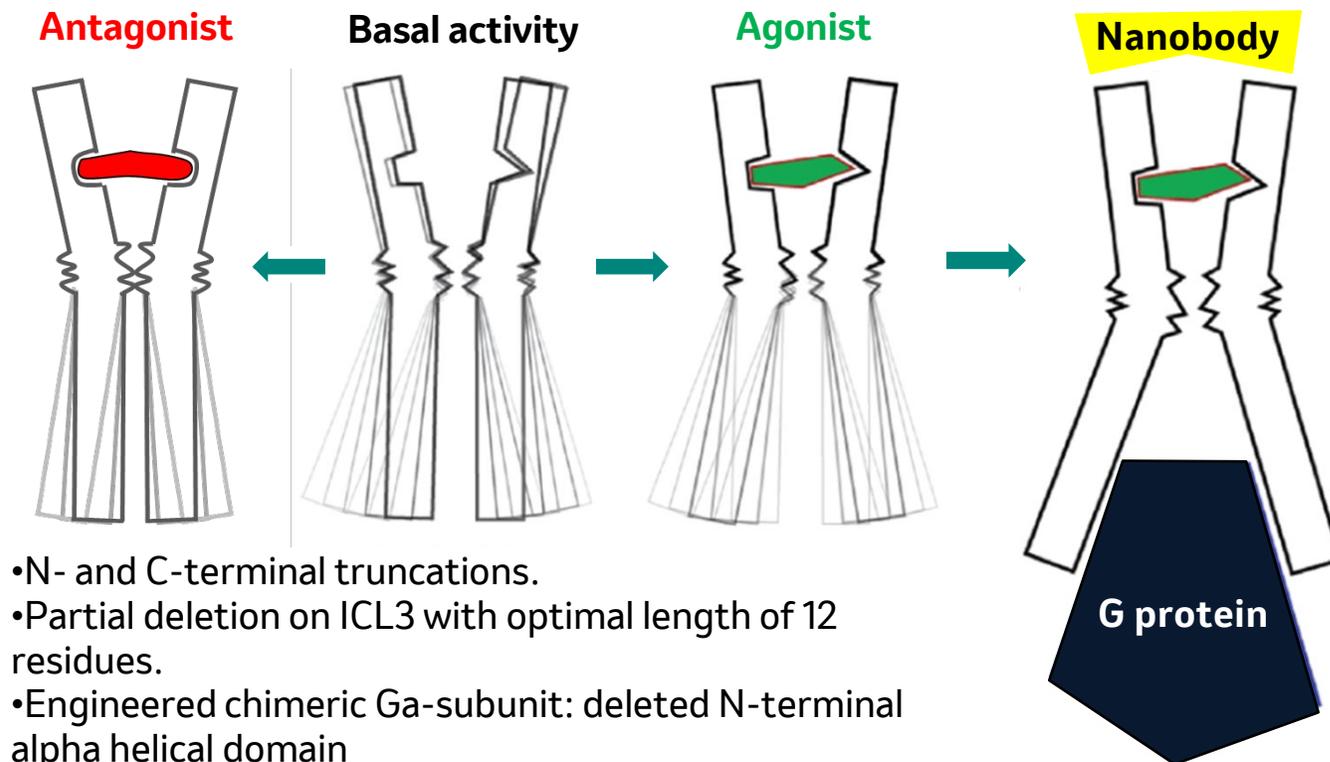
Structures of Screening hits to explore SAR

Iterative structures to improve SAR and remove liabilities



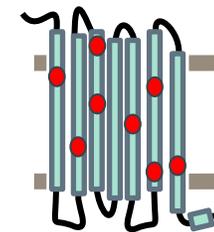
Beyond the microscope: exploring SAR

GPCRs: what makes GPCRs challenging for structural determination?



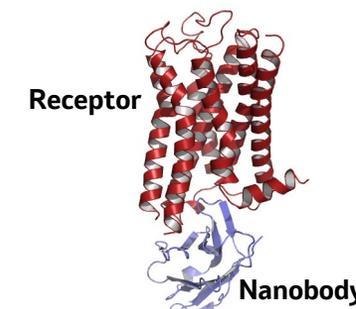
Ligands

1



Mutations

2



Structural chaperones

3

Beyond the microscope: impact on SAR

Orexin 2

Indication/Relevance: To design an agonist of the orexin receptor 2 (OX2R) since they have promise as novel therapeutics that directly target the pathophysiology of narcolepsy. Narcolepsy type 1 (NT1) is a chronic neurological disorder that impairs the brain's ability to control sleep-wake cycles



Excessive Daytime Sleepiness



Loss of Muscle tone (Cataplexy)



Disrupted Nighttime Sleep

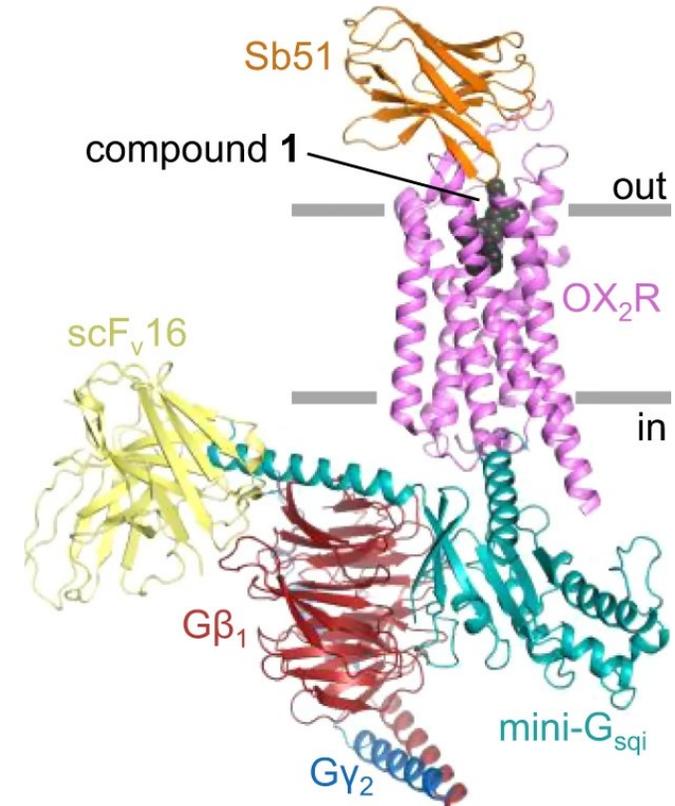
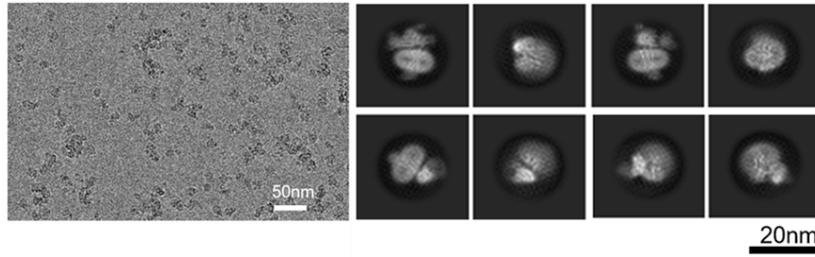


Hypnagogic / Hypnopompic Hallucinations



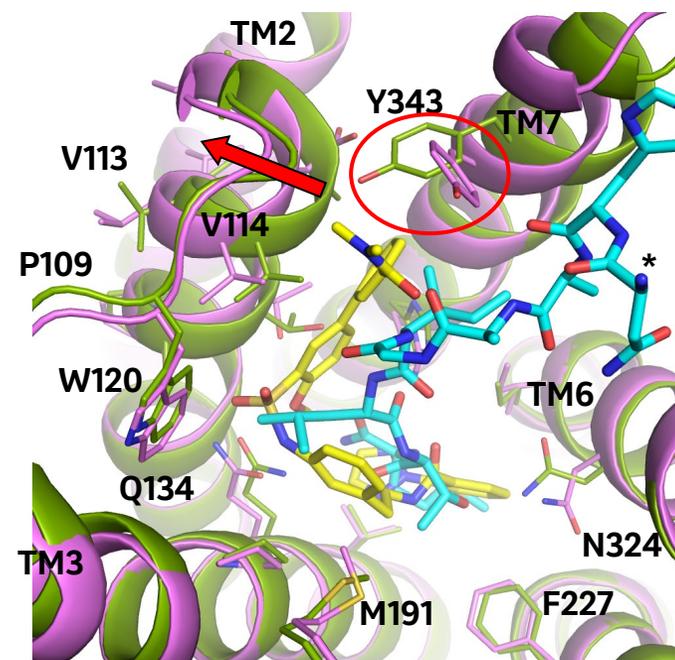
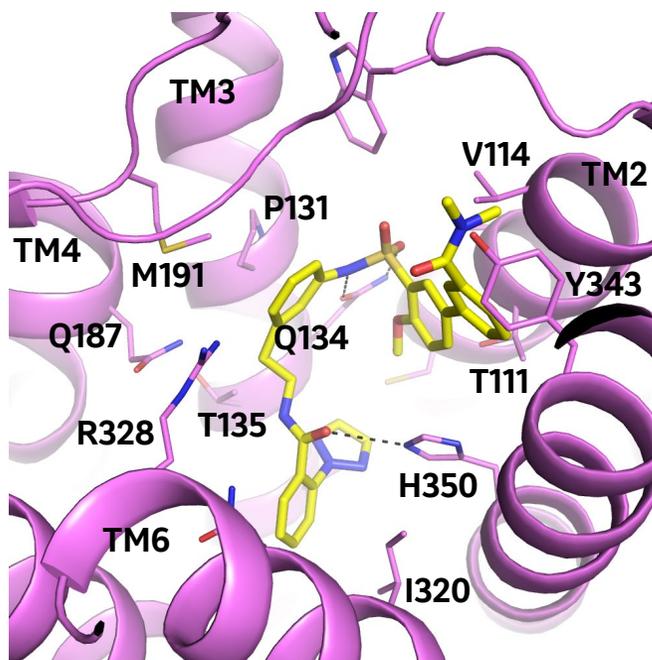
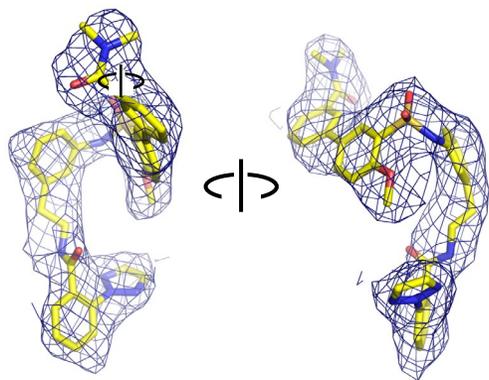
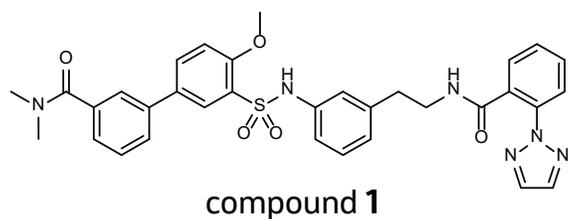
Sleep Paralysis

- CHALLENGES OVERCOME: stabilization of complex with nanobody and G protein.



Beyond the microscope: impacting SAR

Small-molecule agonist recognition



- Sybody 51 improves resolution of complex with small-molecule agonist overall, and in particular in the extracellular half of OX₂R where the agonist binding pocket is located.
- Cpd 1 sits deep down in the pocket overlapping with the binding site of the last few residues of OxB.
- Local backbone and sidechain rearrangements in TM2 and TM7 accommodate distal portion of Cpd 1.

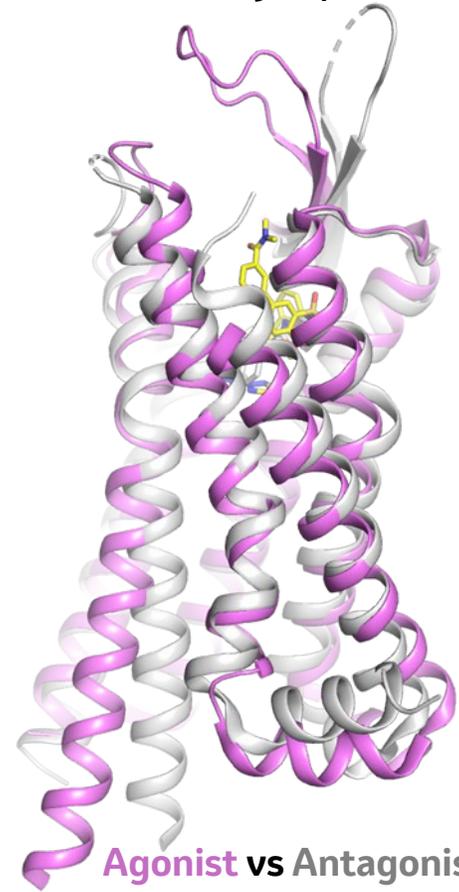
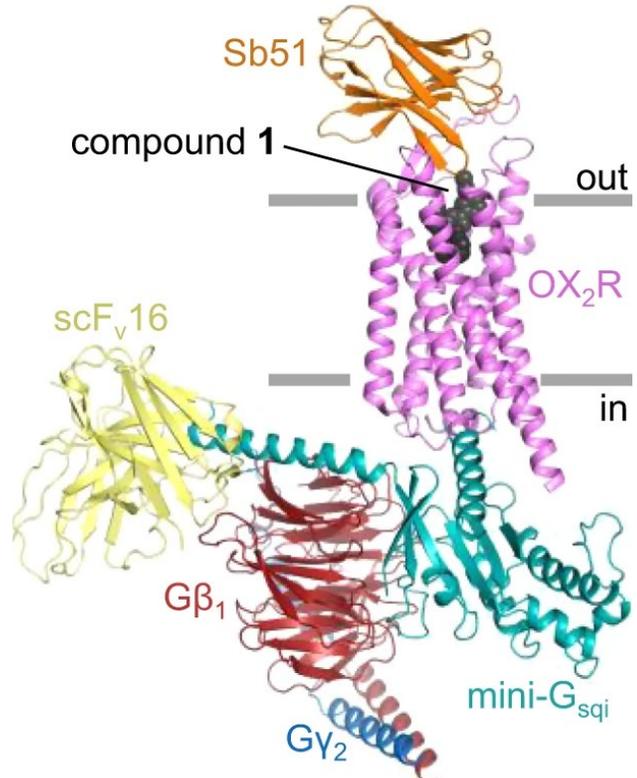
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IMPACT: with inactive-state OX2R suggests mechanism of receptor activation Q134 is a switch that senses agonist bound vs antagonist (toward cytoplasm)

CHALLENGES OVERCOME: stabilization of complex with nanobody and G protein.

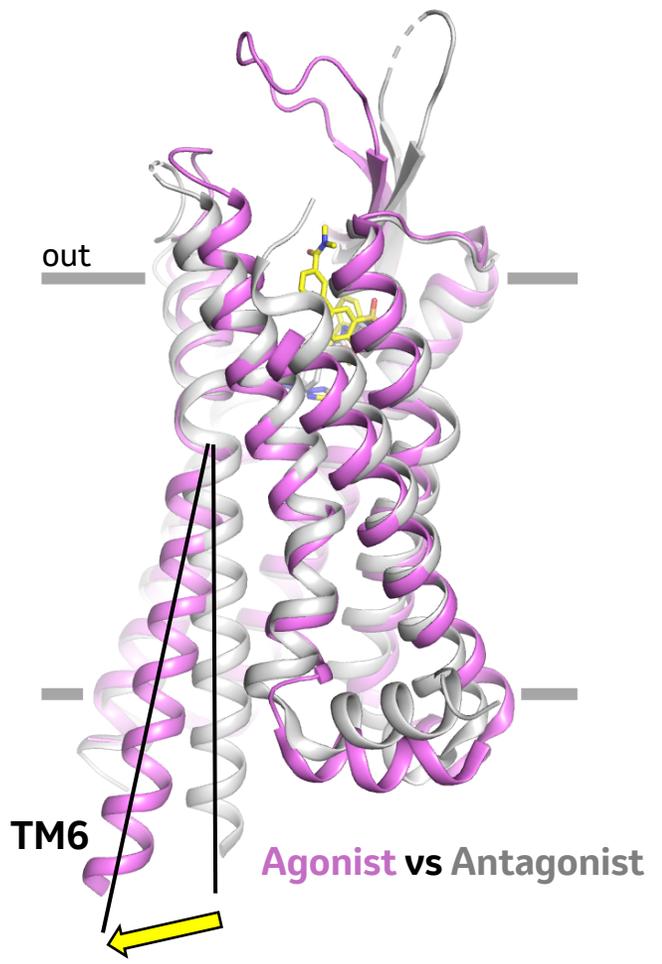


Agonist vs Antagonist

Hong, Hollenstein et al. Nat Comm 2021

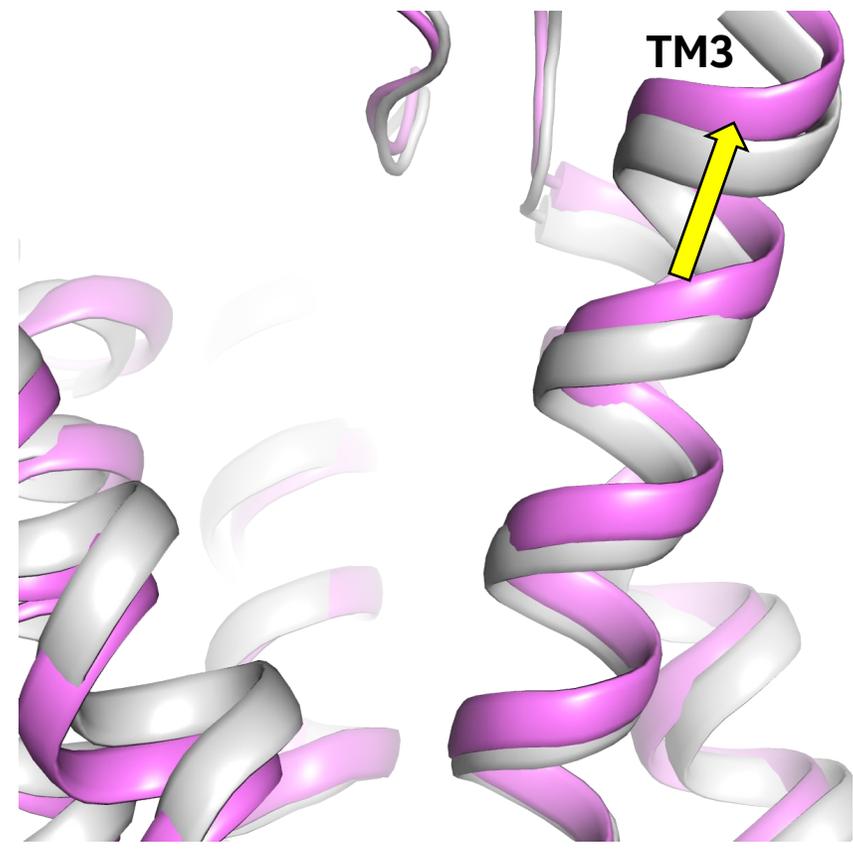
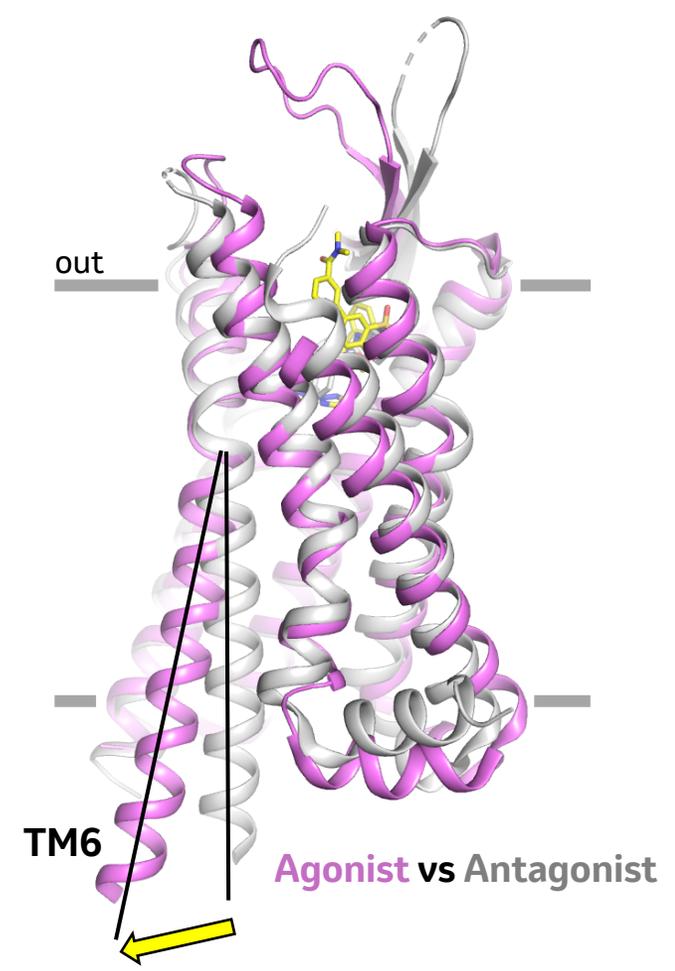
Beyond the microscope: Exploring SAR

Orexin: Comparison inactive -state OX2R suggests mechanism of receptor activation



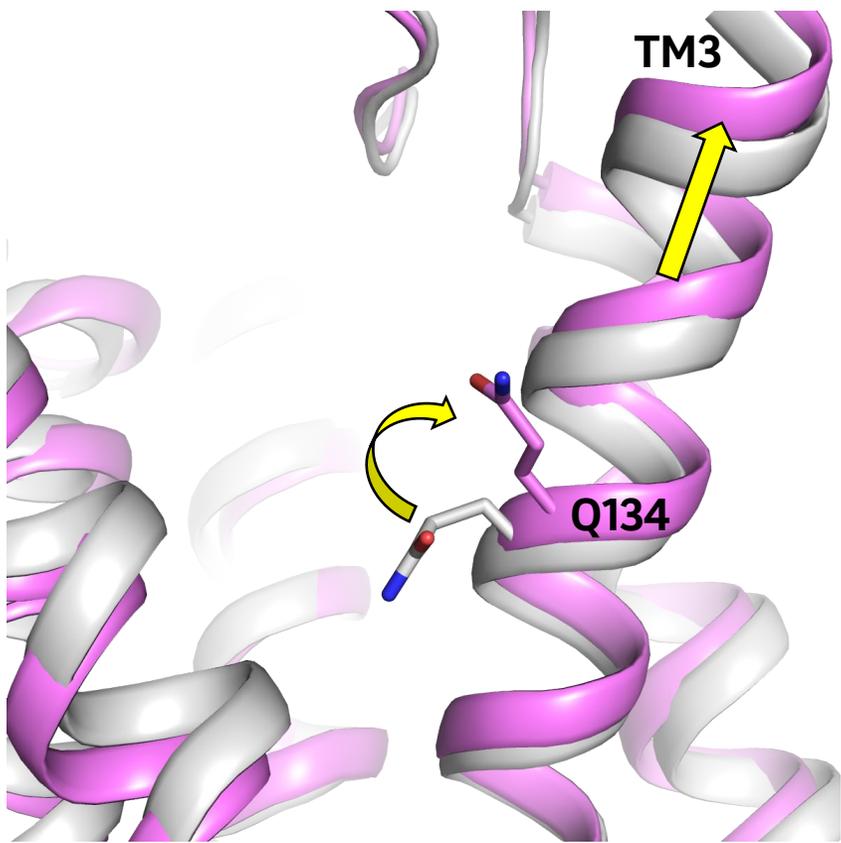
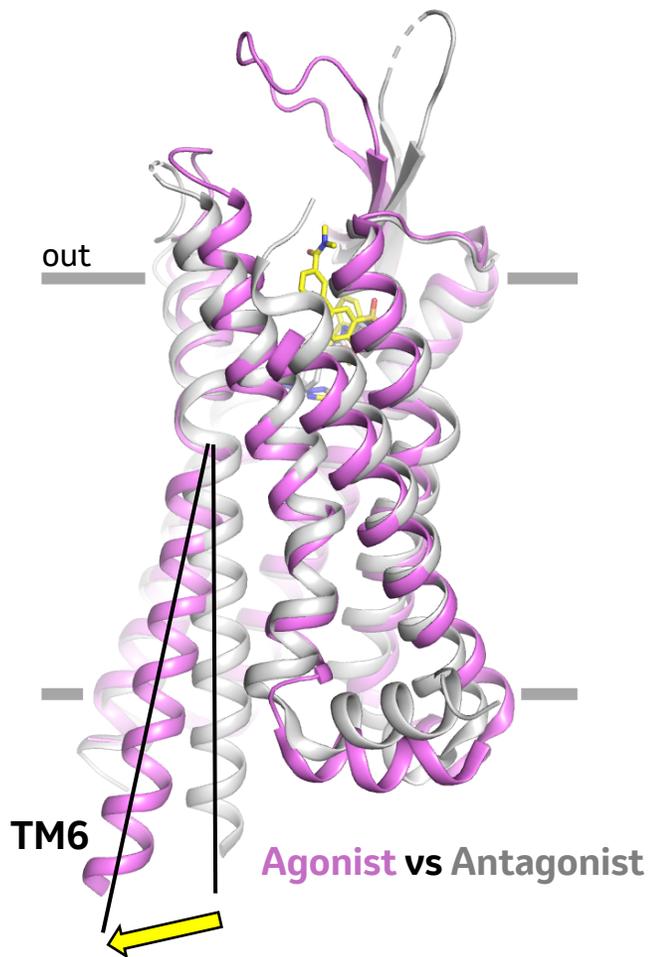
Beyond the microscope: exploring SAR

Orexin: comparison with inactive state OX2R suggests mechanism of receptor inactivation



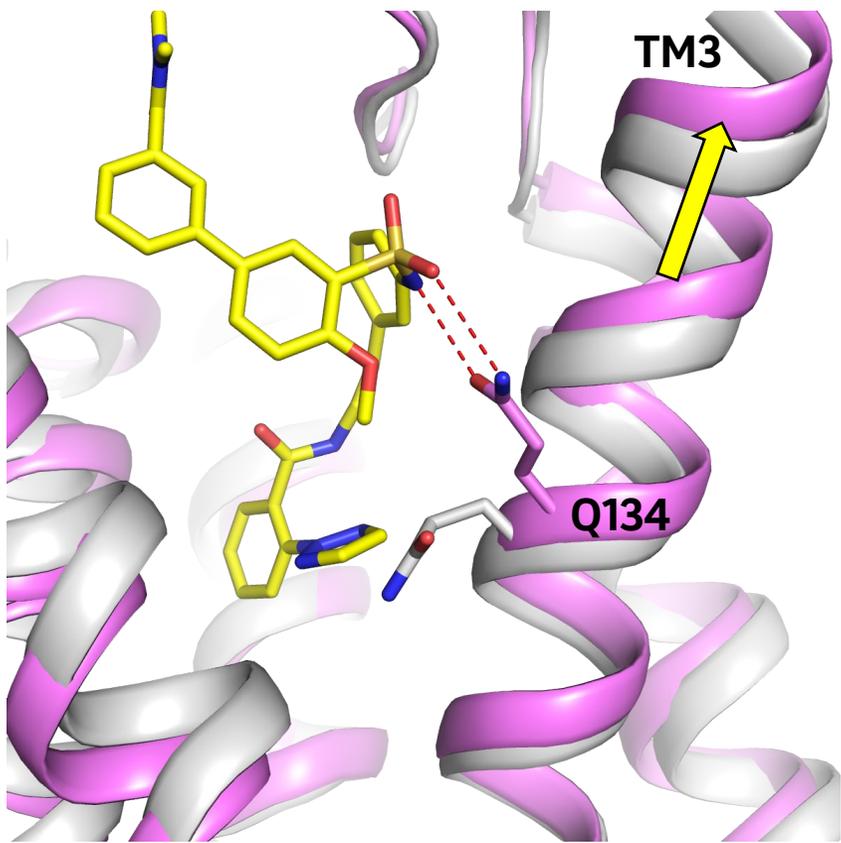
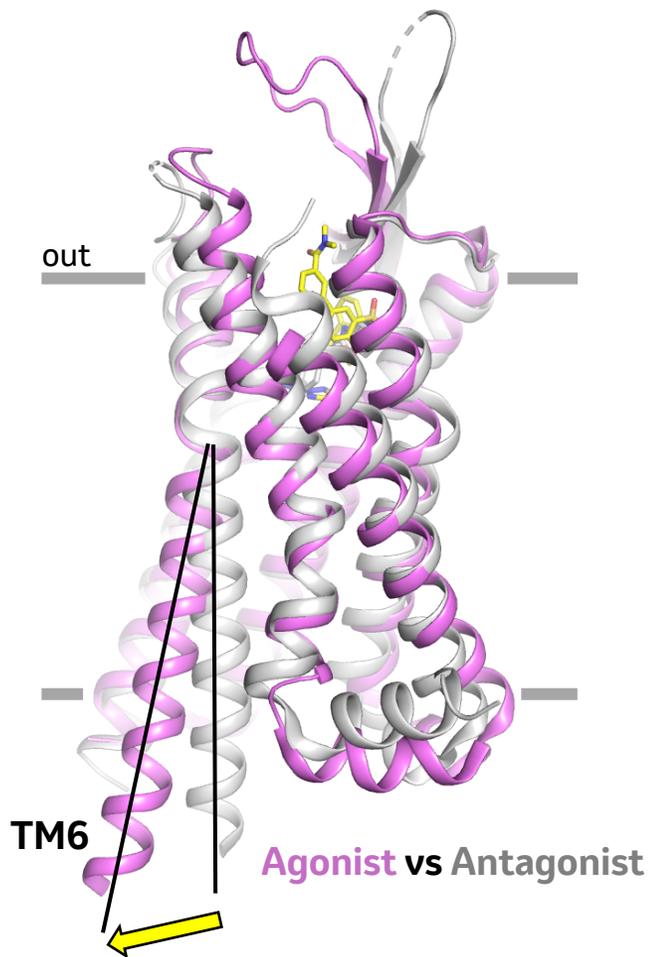
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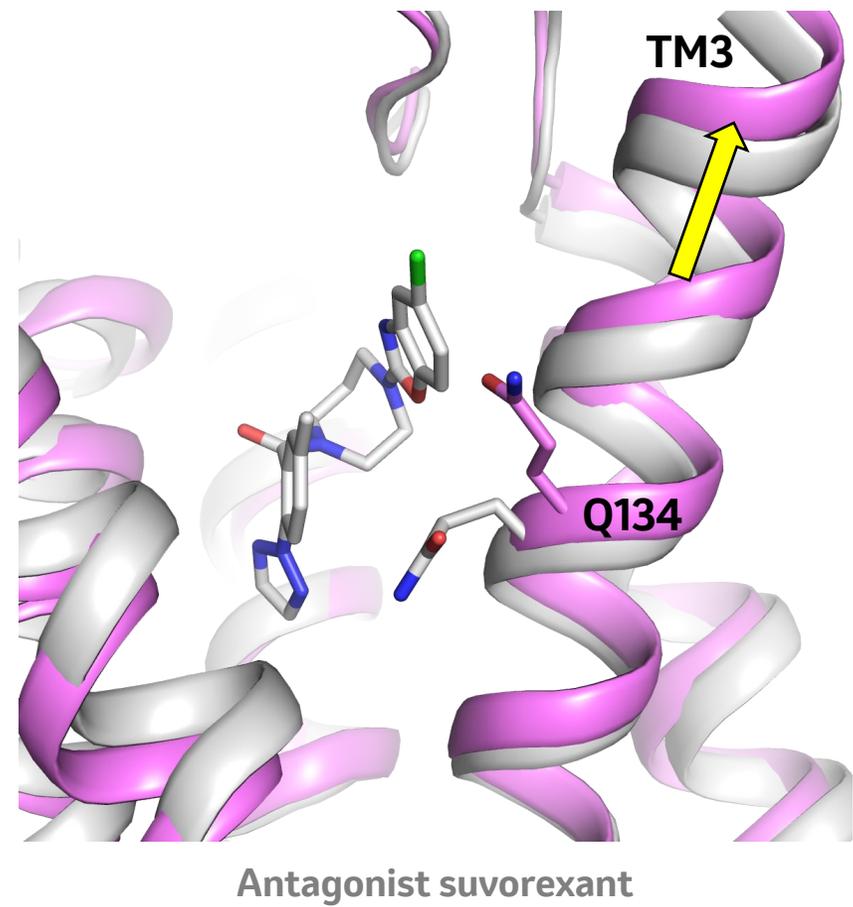
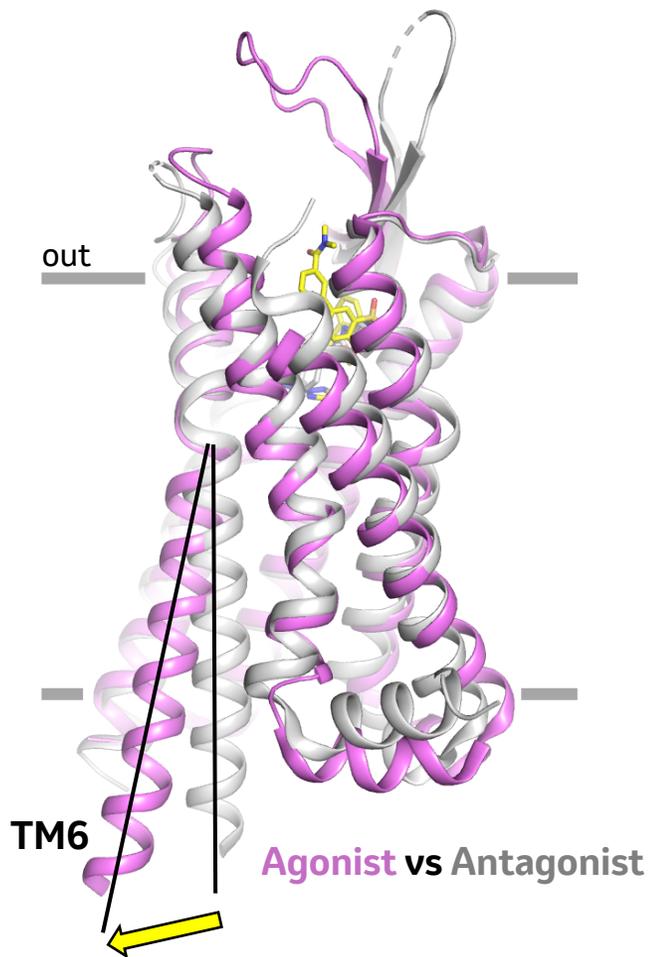
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Small-molecule agonist Cpd 1

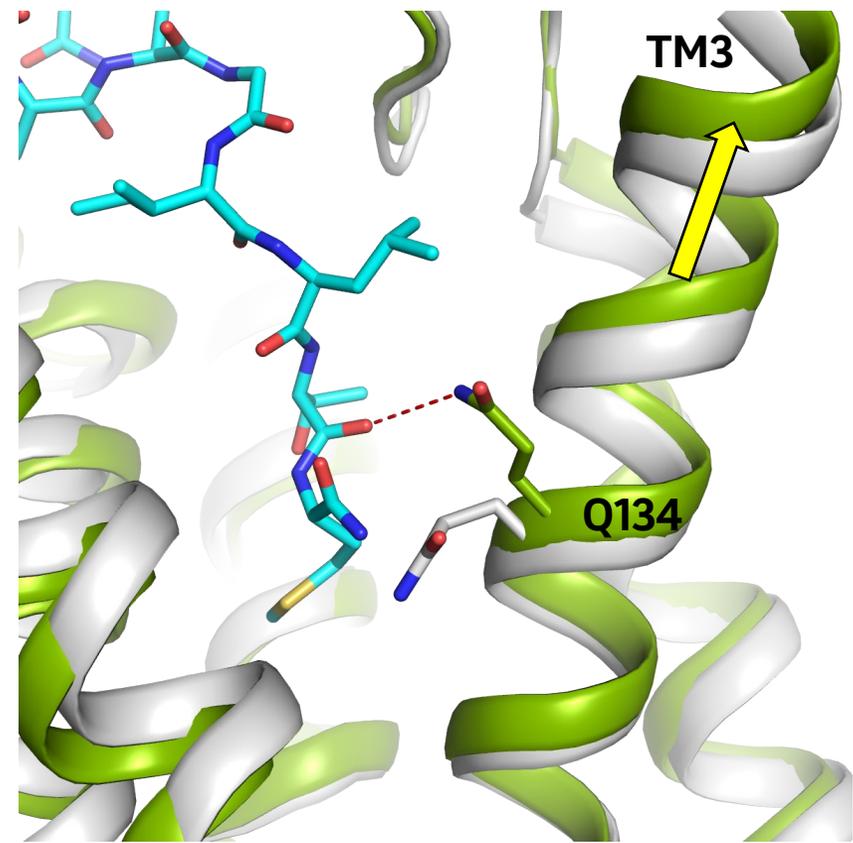
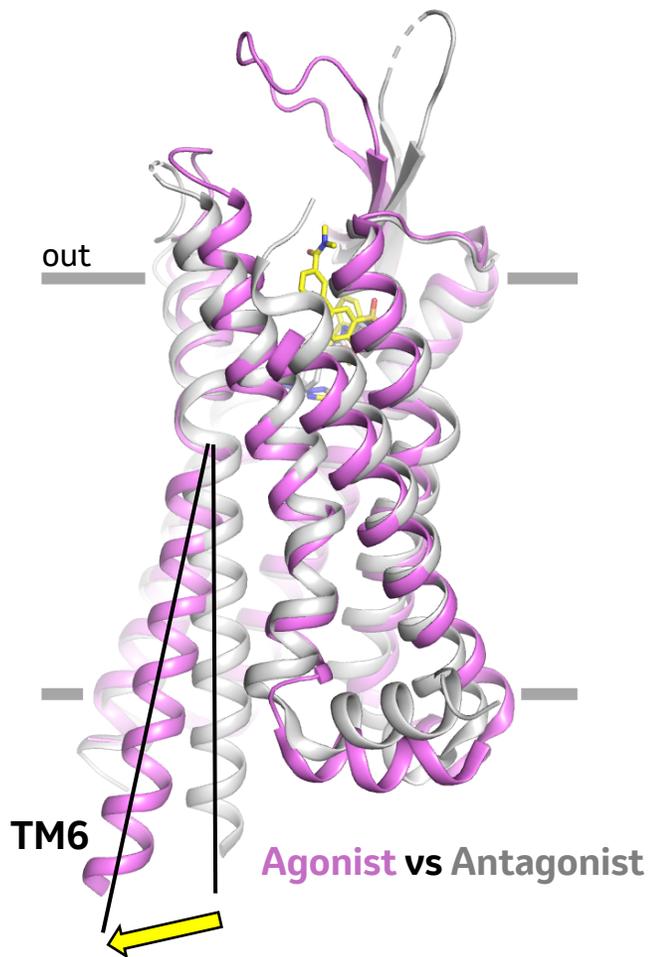
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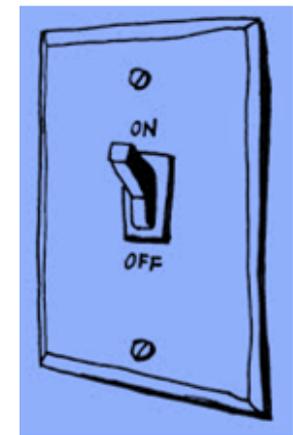


Beyond the microscope: exploring SAR

Orexin: comparison with inactive state OX2R suggests mechanism of receptor inactivation



Natural peptide agonist OxB

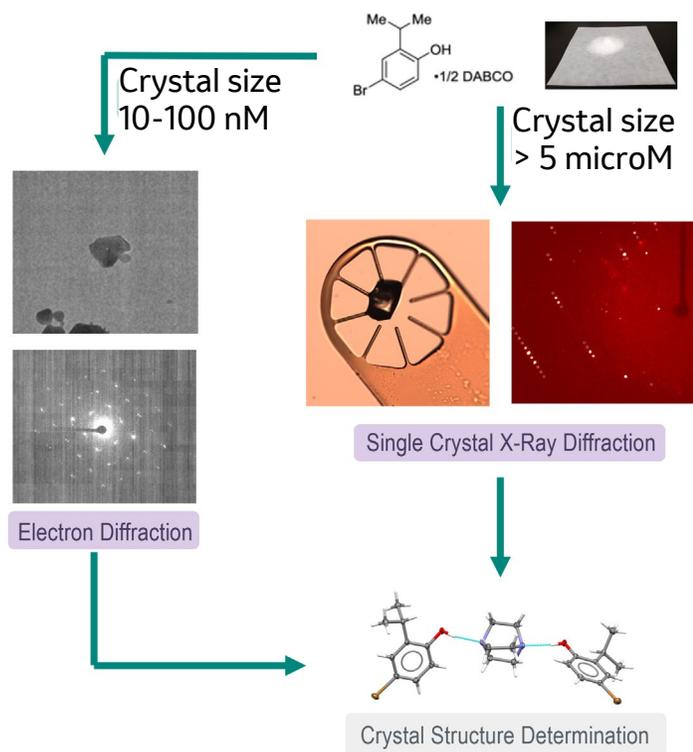


Beyond the microscope: Micro-electron diffraction (μ ED) to evaluate formulations of the final drug



MicroED/
Crystallization to
evaluate formulation

Beyond the microscope: Micro-electron diffraction (μ ED) to evaluate formulations of the final drug



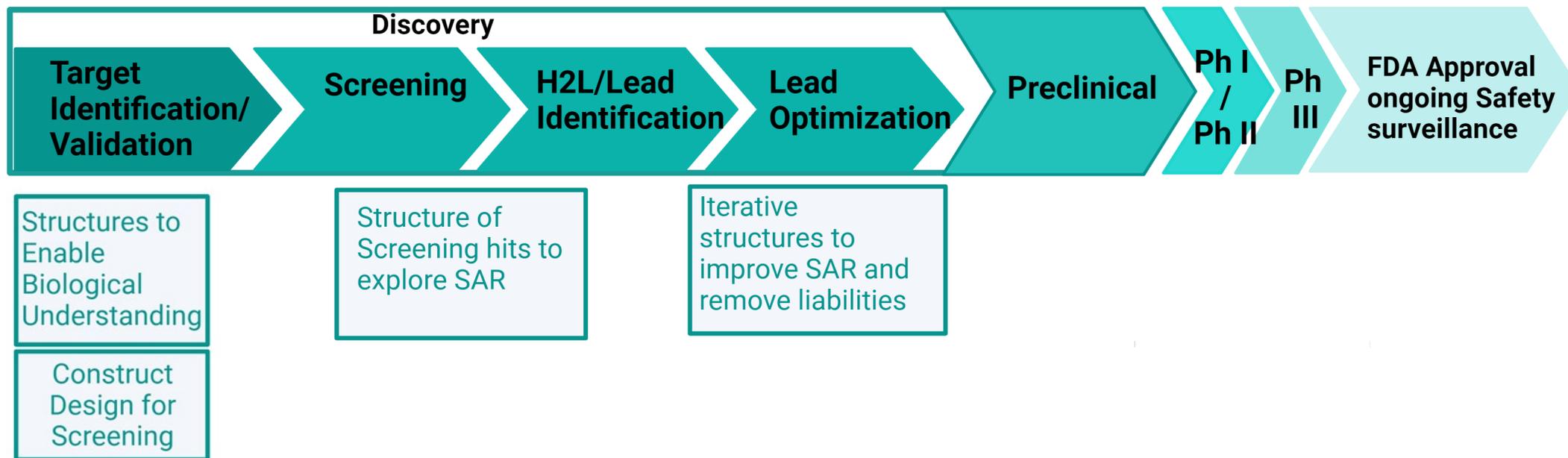
MicroED/
Crystallization to
evaluate formulation

μ ED allows the study of:

- Structure and key interactions of the molecules in the crystal.
- Absolute configuration determination.
- Solubility optimization.
- Salt vs. Co-crystal, solvent stoichiometry.
- Molecular conformation.

Beyond the microscope: exploring biologics SAR

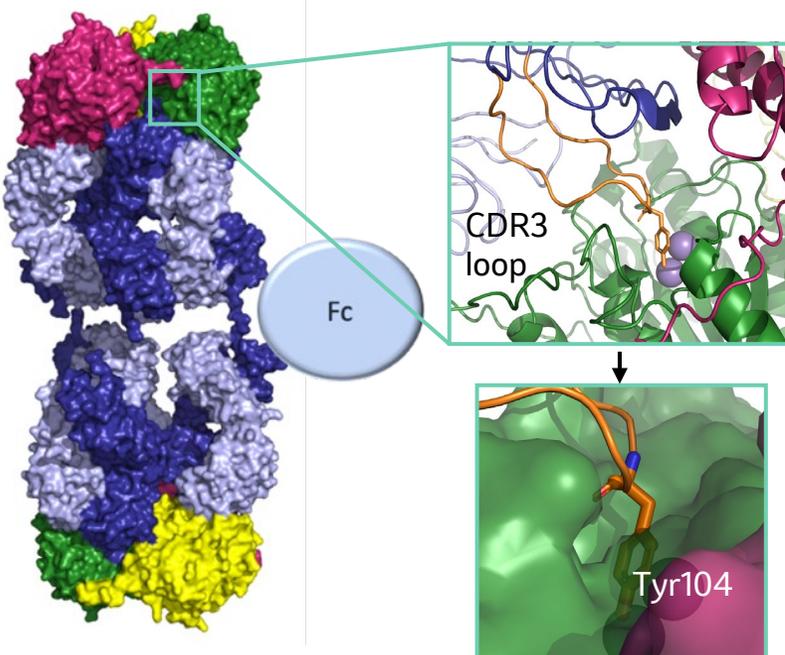
Arginase



Beyond the microscope: exploring biologics SAR

Arginase

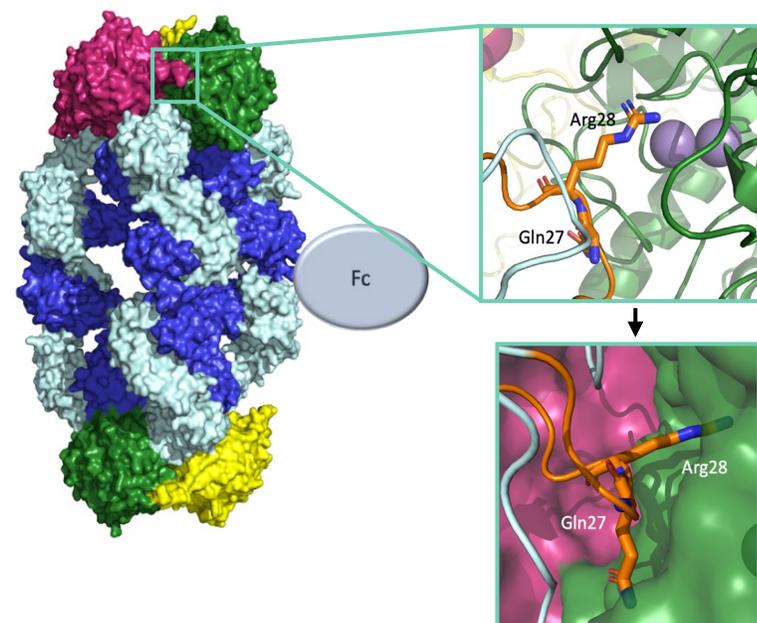
Indication/Relevance: Activation of lymphocytes has resulted in unprecedented rates of durable response among cancer patients. Since human Arginase 1 (hArg1) modulates T-cell-mediated immune response we sought to design mABs that can illuminate mechanism of inhibition.



mAB1: Mechanism of inhibition by occluding the orthosteric site with Tyr104 of the CDR3 Loop

IMPACT: Cryo-EM studies reveal large macromolecular complexes of competitive, inhibitory antibodies; inform on new means of inhibition to inspire new designs. MOA of mAB1-4 function through orthosteric inhibition by 'insertion/binding' of an amino acid side chains in Arginase active site.

CHALLENGES OVERCOME: highly variable resolution across the complex with best definition at the hARG1-fab interface

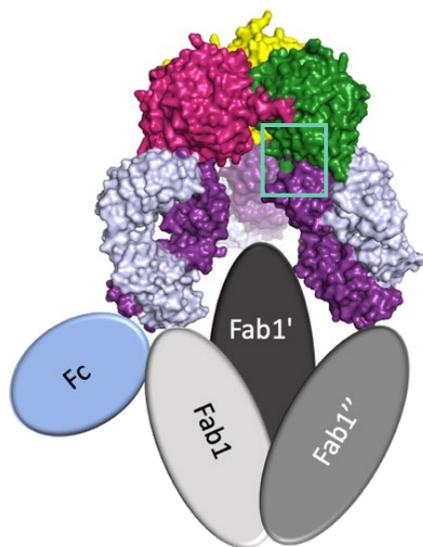


mAB4: Mechanism of inhibition by occluding the orthosteric site with Arg28

Beyond the microscope: exploring biologics SAR

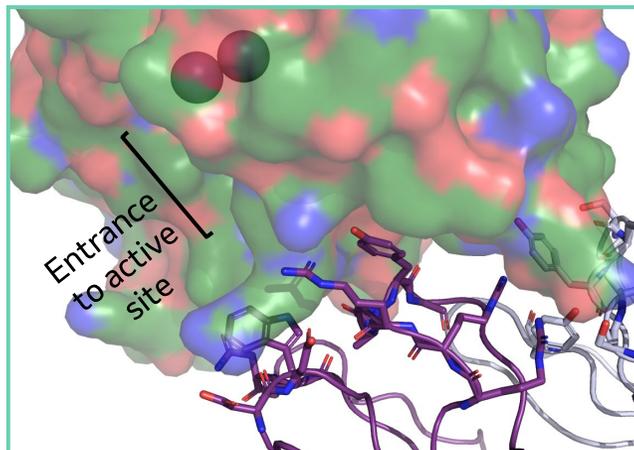
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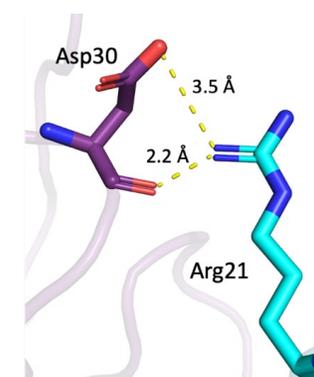


mAb5 forms 1:3 Arginase trimer: mABs (smaller) complex and does not cover active site entrance

IMPACT: Cryo-EM studies reveal large macromolecular complexes of competitive, inhibitory antibodies; inform on new means of inhibition to inspire new designs. MOA of mAB1-4 function through orthosteric inhibition by 'insertion/binding' of an amino acid side chains in Arginase active site; mAB5 by blocking interaction of Arg21 which orients substrate



CHALLENGES OVERCOME: highly variable resolution across the complex with best definition at the hARG1-fab interface



hArg1's Arg21 is at salt bridge distance Asp30 of mAb5. Arg21 is essential for orienting substrate and product. Prevention of Arg21's interaction leads to inhibition.

Beyond the microscope: from ex-situ to in-situ



Structures to Enable Biological Understanding

Structure of Screening hits to explore SAR

Construct Design for Screening

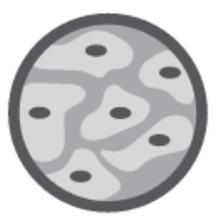
MicroED/ Crystallization to evaluate formulation

IN SITU

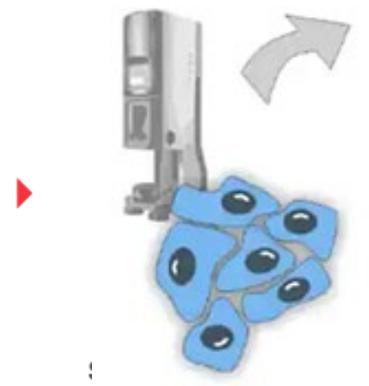


Beyond the microscope: from ex-situ to in-situ

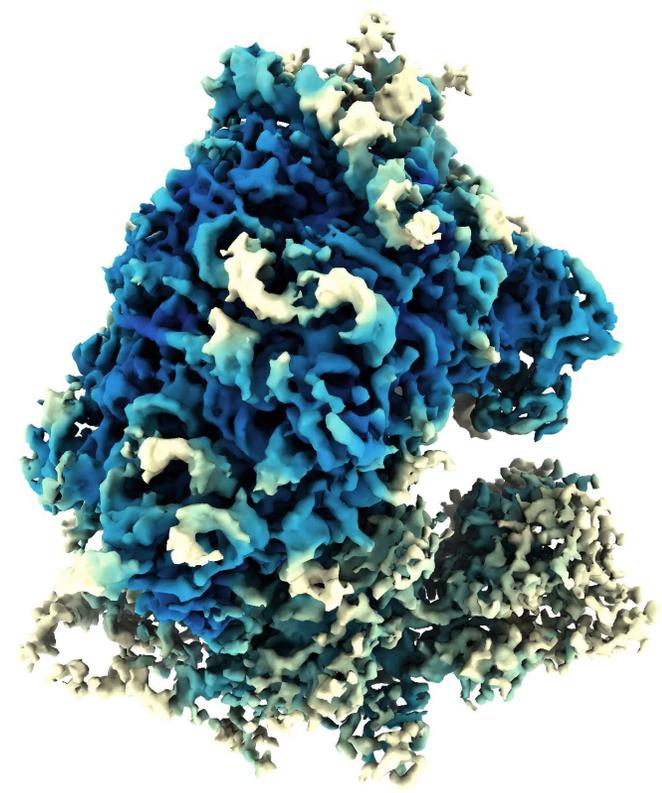
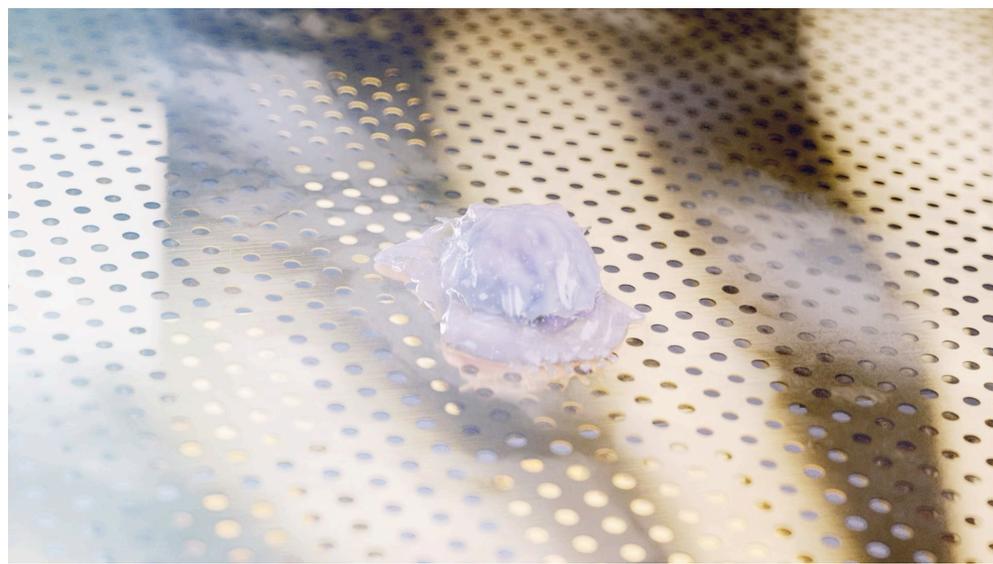
Cryo ET fib lamellae



Cell culture



Cells grown on grids
or placed on grids
Or iPSCs



Visualization of the effects of a therapeutic in the cell and understanding how cells work

Integration of data across scale and techniques will change our understanding of the chemistry and biology

Acknowledgments: Protein and structural Chemistry





Thank you