

TMM 3102: Protein Structure, Function and Disease

- Integrative Structural Biology: Large Protein Complexes
(October 21st, 2021)

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(Partially adopted from former lectures by Drs. Jean-François Couture & Patrick Giguère)



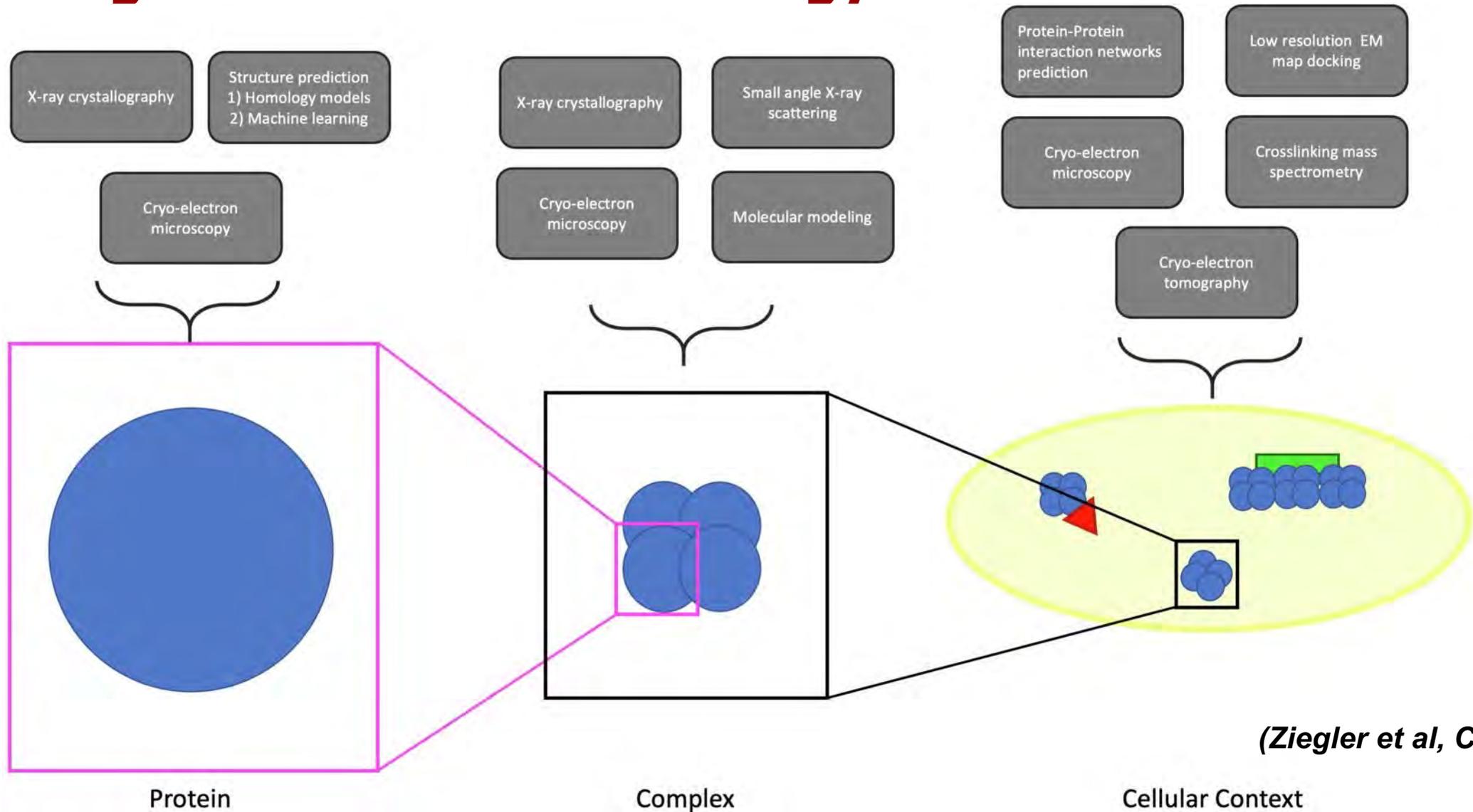
Lecture Outline

- 1. Why studying large/multi-subunit protein complexes?**
- 2. Ways to study large protein complexes**
 - **X-ray crystallography ✓**
 - **Electron microscopy ✓**
 - **Mass spectrometry ✓**
 - **Cross-linking / hydrogen-deuterium (H/D) exchange**
 - **Computer simulation, NMR, fluorescence, ...**
- 3. Case study**

Why studying large/multi-subunit protein complexes?

- **Protein structures define protein functions. Yet, a biological process is usually not performed by one single protein.**
- **Exactly how proteins send signals through their structures remains to be determined.**
- **Proteins function in ensemble with other protein partners, such signal transduction, transcription, translation, exo/endocytosis, etc.**
- **Activation/deactivation of individual protein functions requires formation of multi-protein complexes.**
- **Intracellular environment is very viscous and crowded by proteins and other biological molecules, *i.e.*, the spatial limitation of protein functions.**

Integrative Structural Biology



(Ziegler et al, CSBJ, 2021)

Integrative Structural Biology

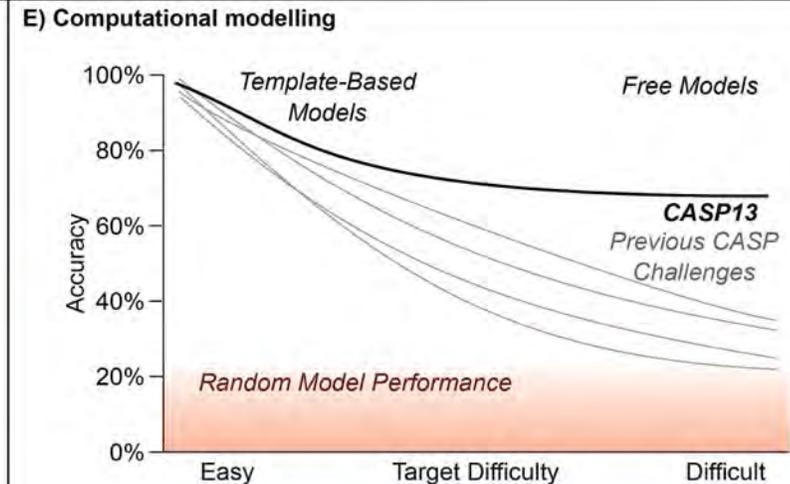
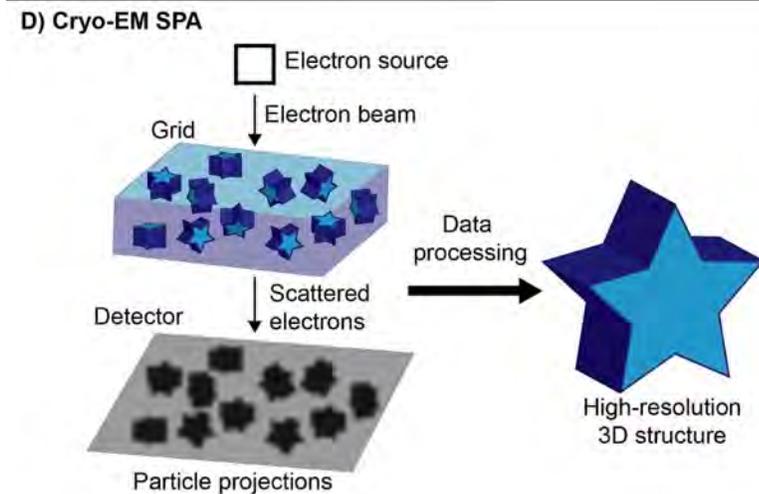
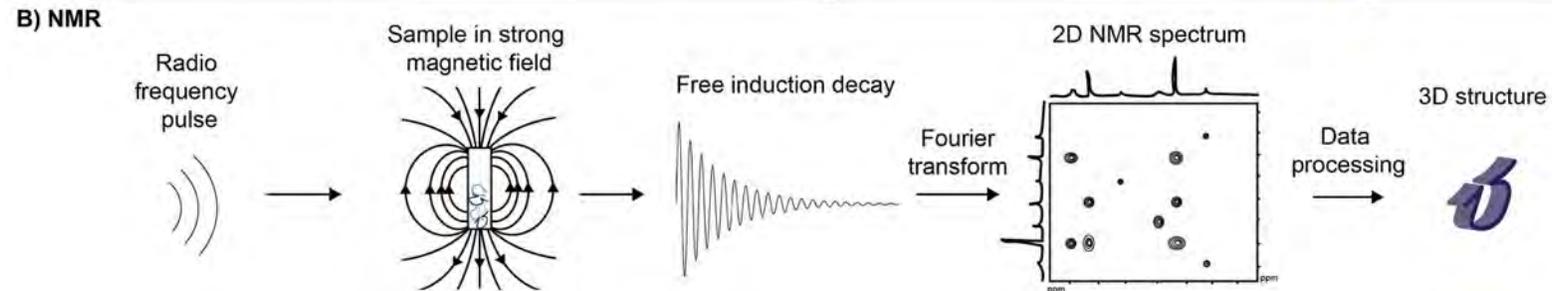
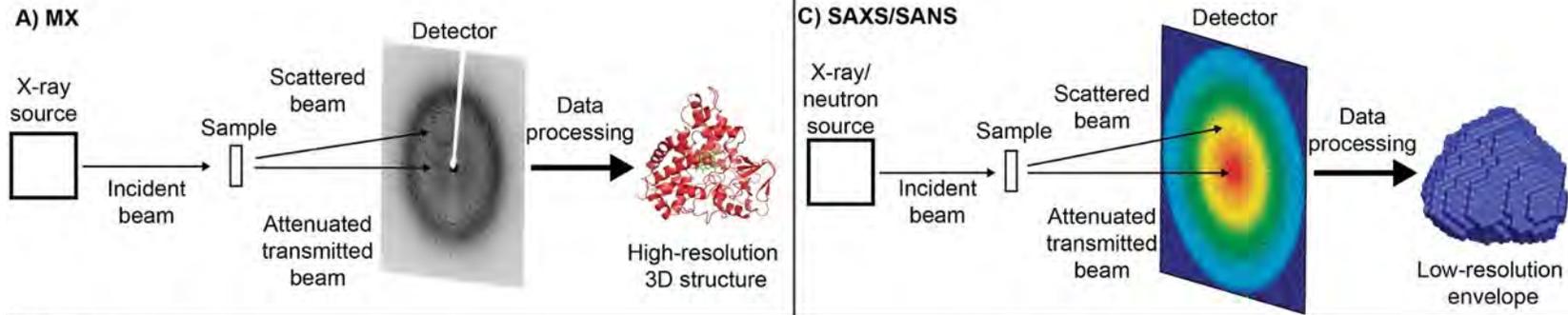
A multi-disciplinary science that collects diverse expertise from various fields, such as

**biochemistry/biophysics,
analytical chemistry,
cell biology,
protein engineering,
computer science,
...**

	Technique	Desc
<i>Structural characterization of proteins</i>		
Common technique	Macromolecular crystallography NMR	
Recent advancement	SAXS/SANS Cryo-EM SPA	
	Computational modeling	
<i>Identification and characterization of protein-protein interactions</i>		
Common technique	Co-IP FRET	
Recent advancement	XL-MS	
	Molecular docking Proximity labeling	
<i>Contextualization of protein-protein interactions</i>		
Future of ISB	Whole-cell cryo-ET Single-cell cryo-EM XL-MS and cryo-EM SPA	

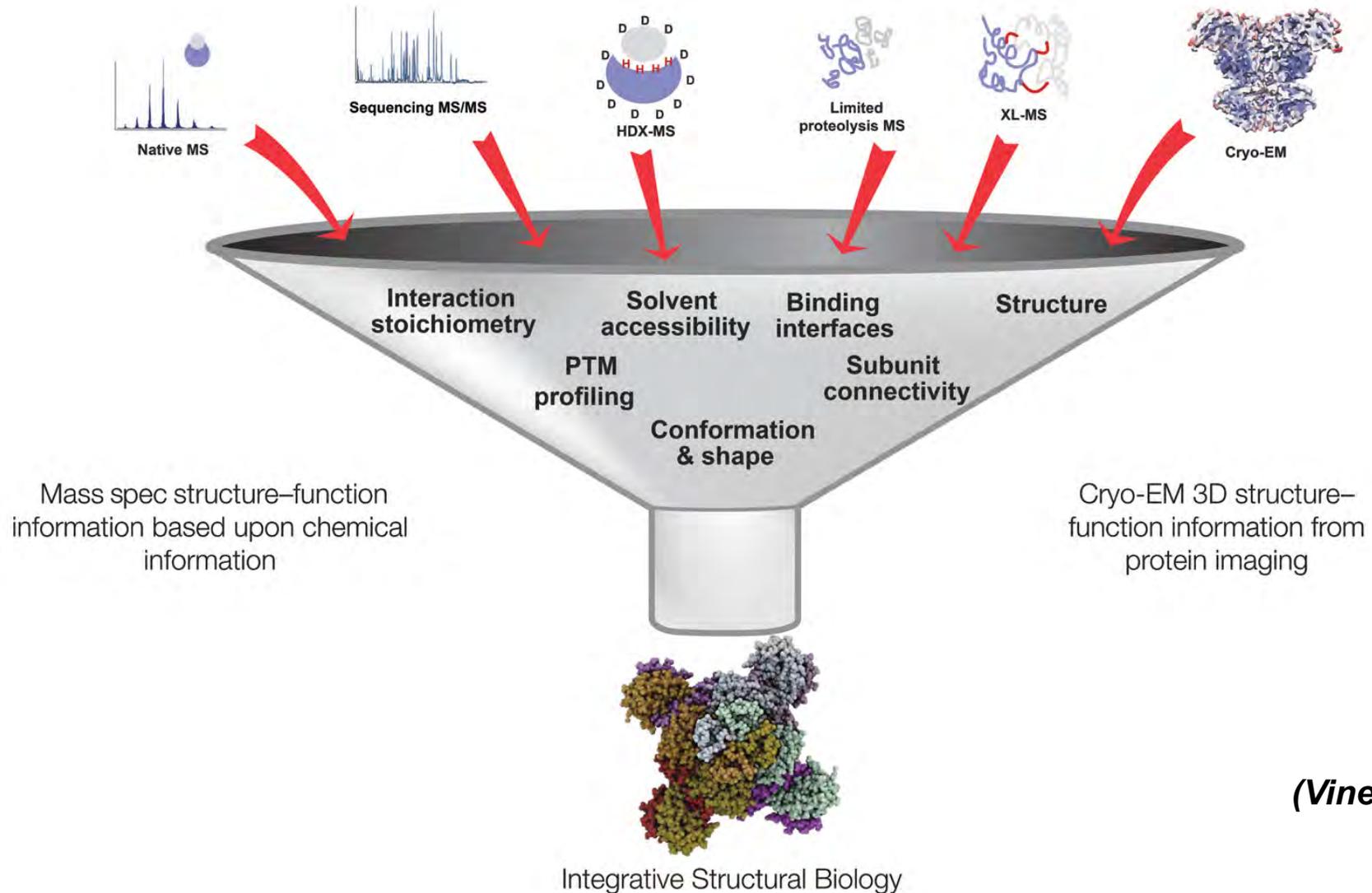
(Ziegler et al, CSBJ, 2021)

Integrative Structural Biology



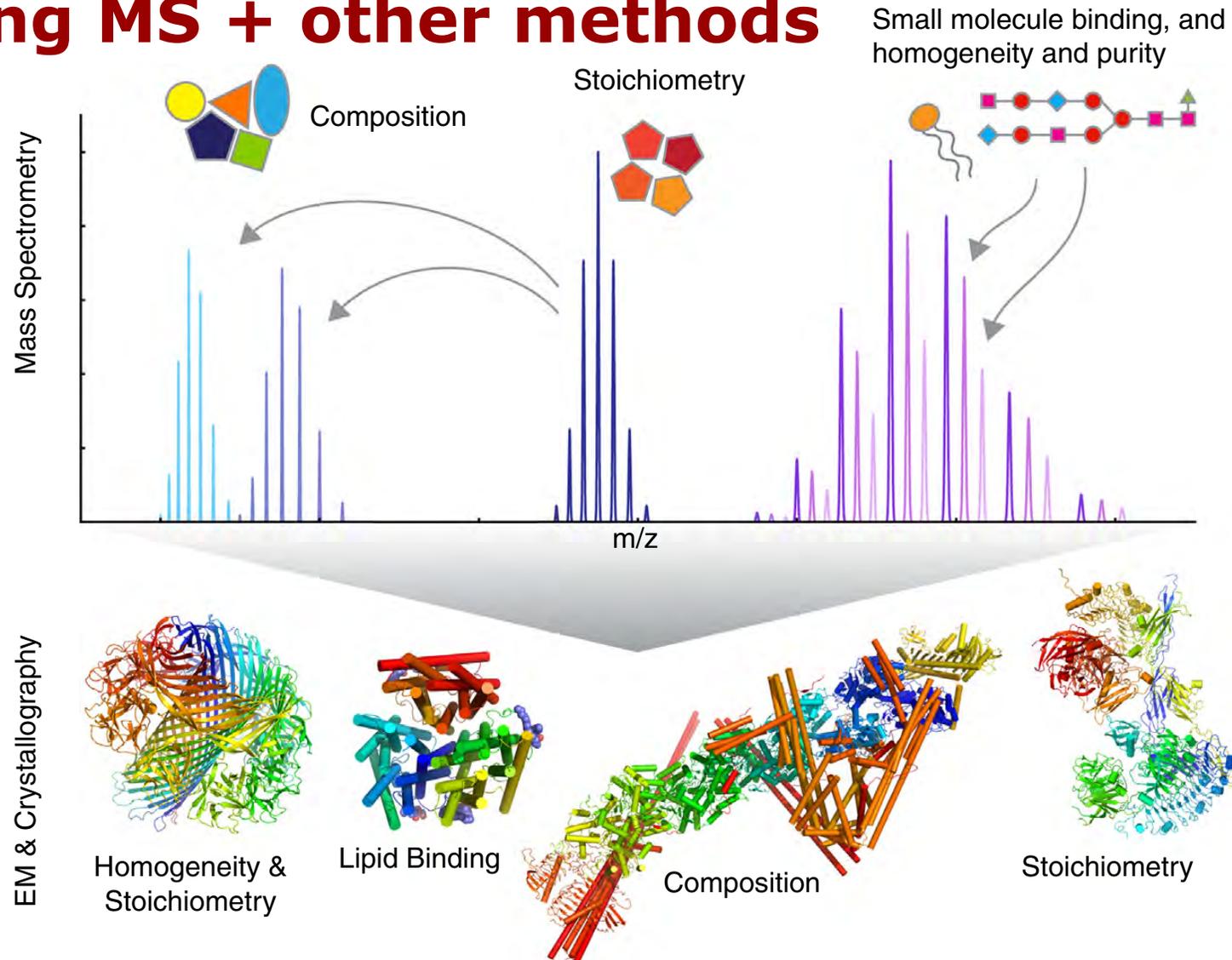
(Ziegler et al, CSBJ, 2021)

Integrative Structural Biology



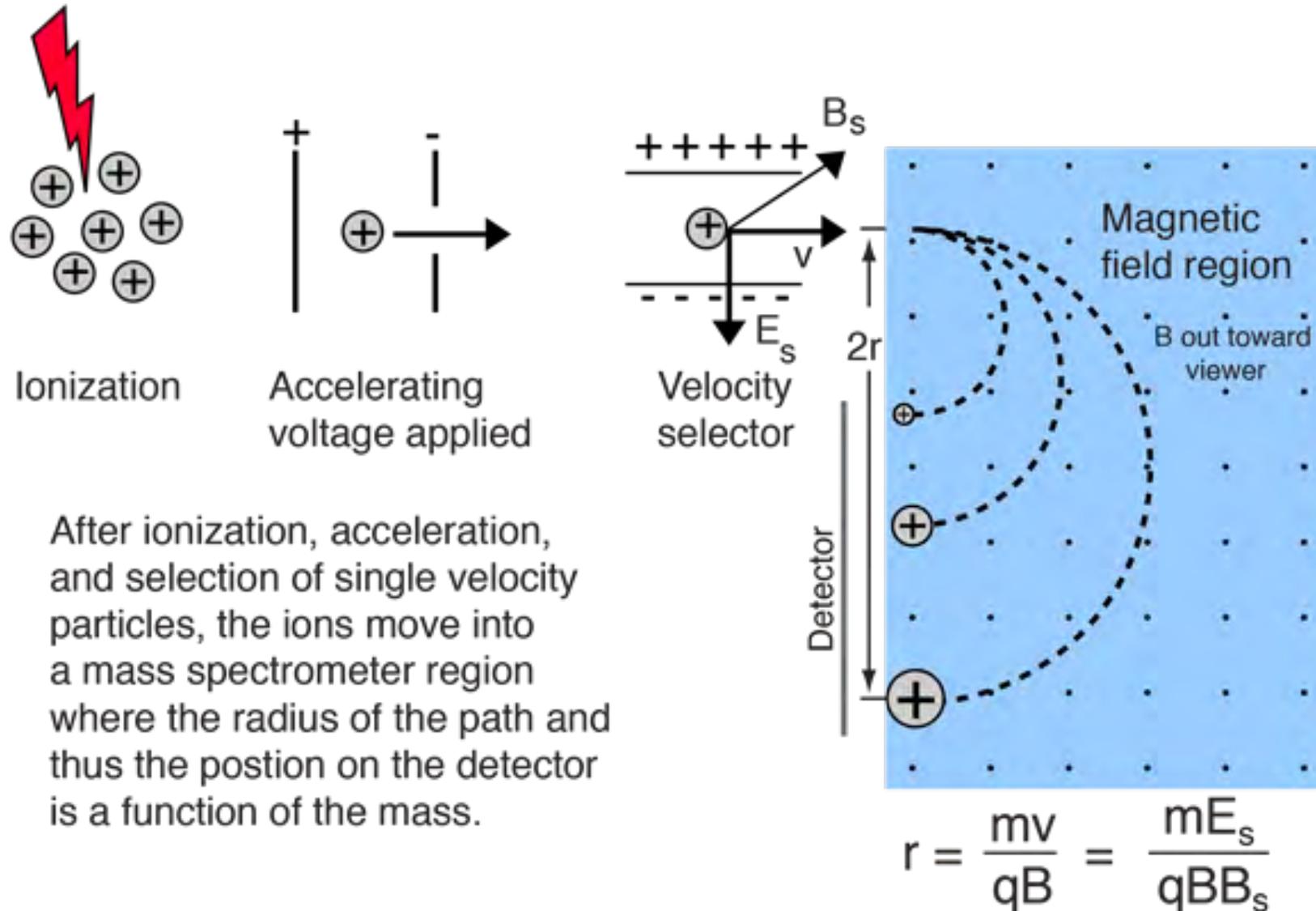
(Viner, Science, 2017)

Integrative Structural Biology using MS + other methods

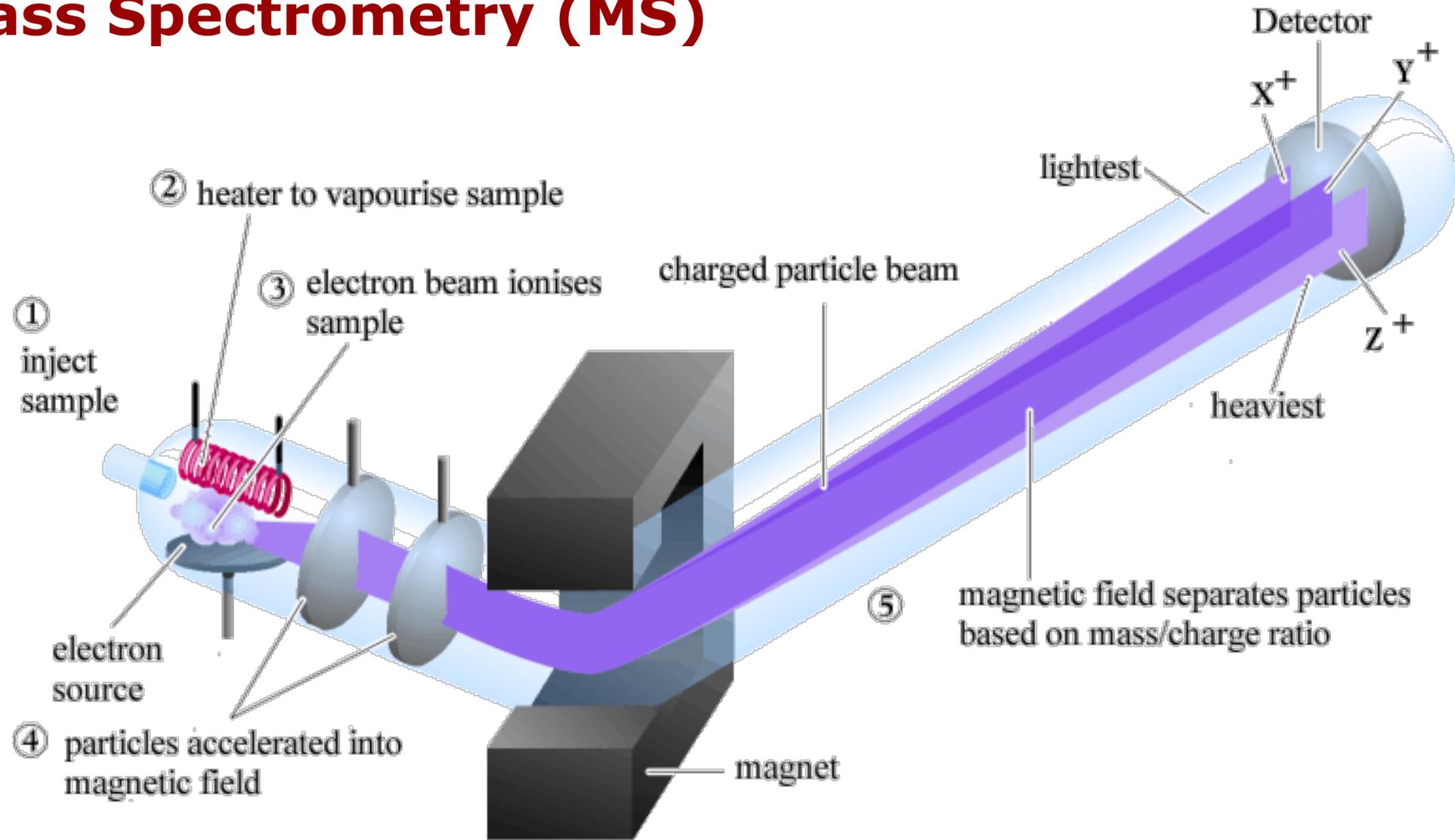


(Liko et al, *Curr Opin Struct Biol*, 2016)

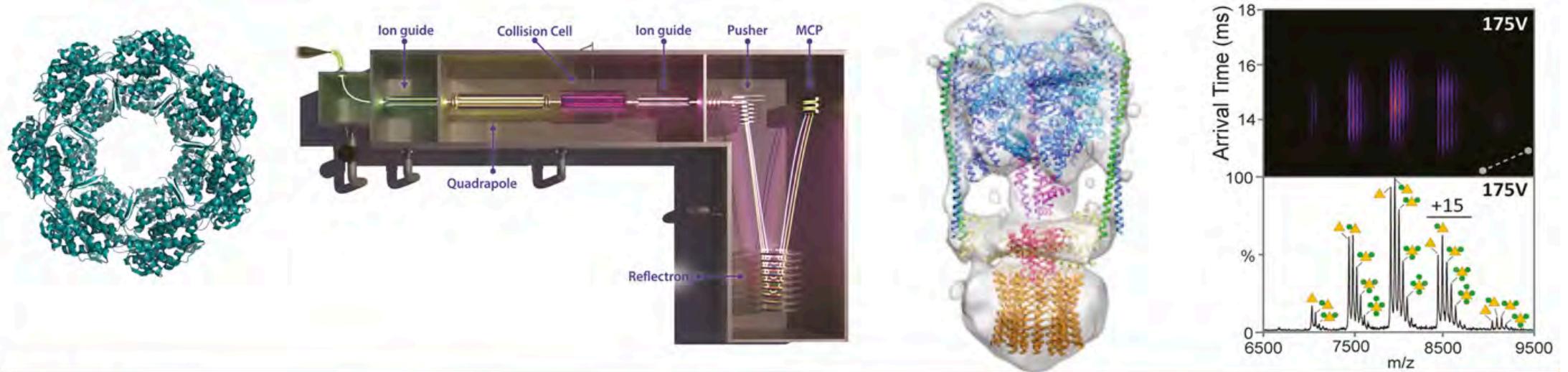
Mass Spectrometry (MS)



Mass Spectrometry (MS)

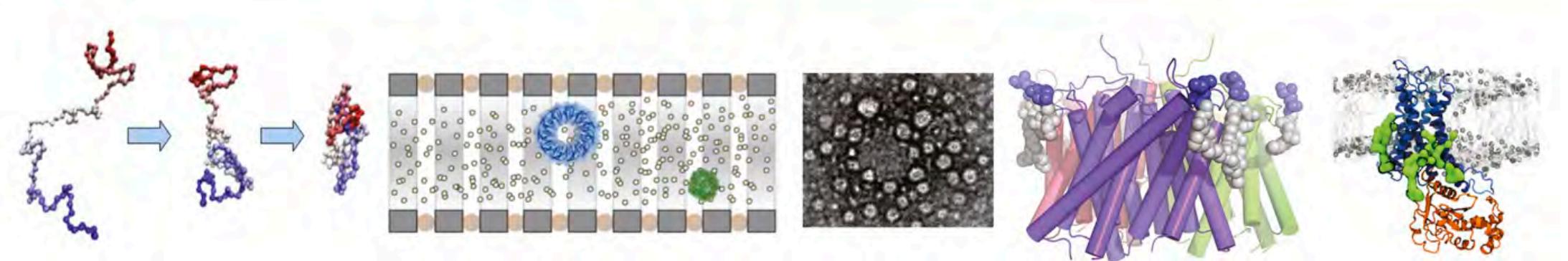


Mass Spectrometry (MS)

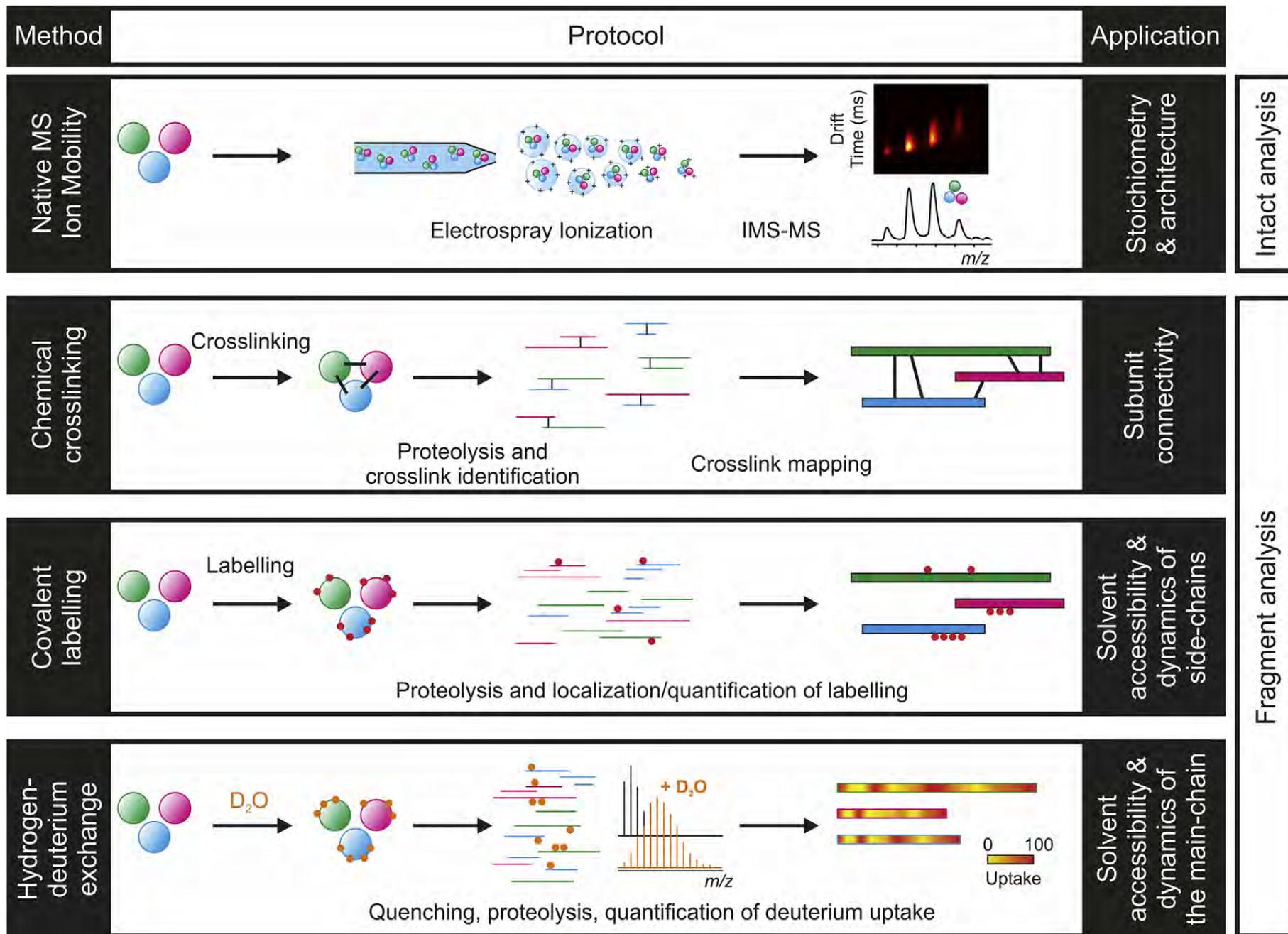


1993

2018



(Robinson, PNAS, 2017)

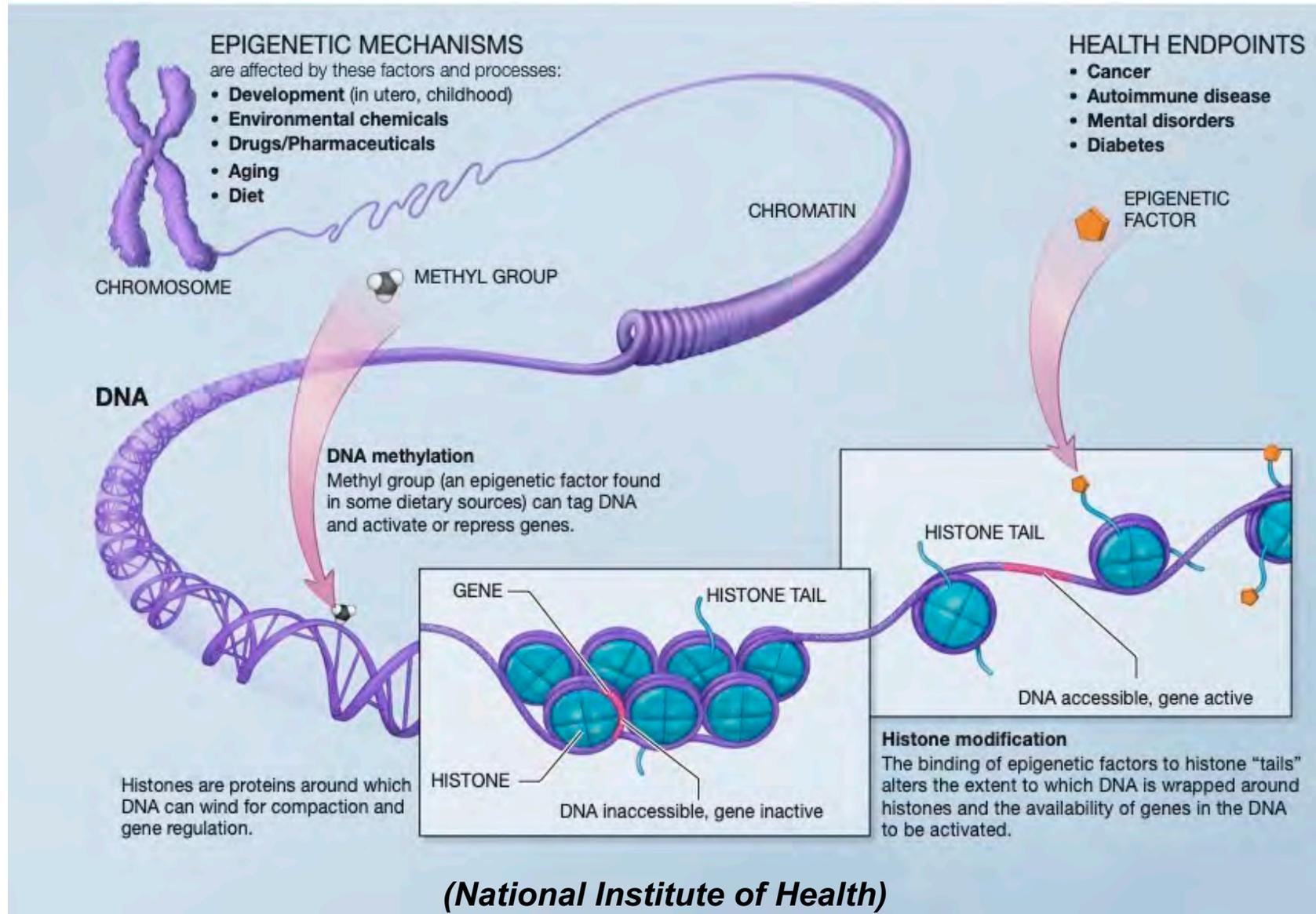


Intact analysis

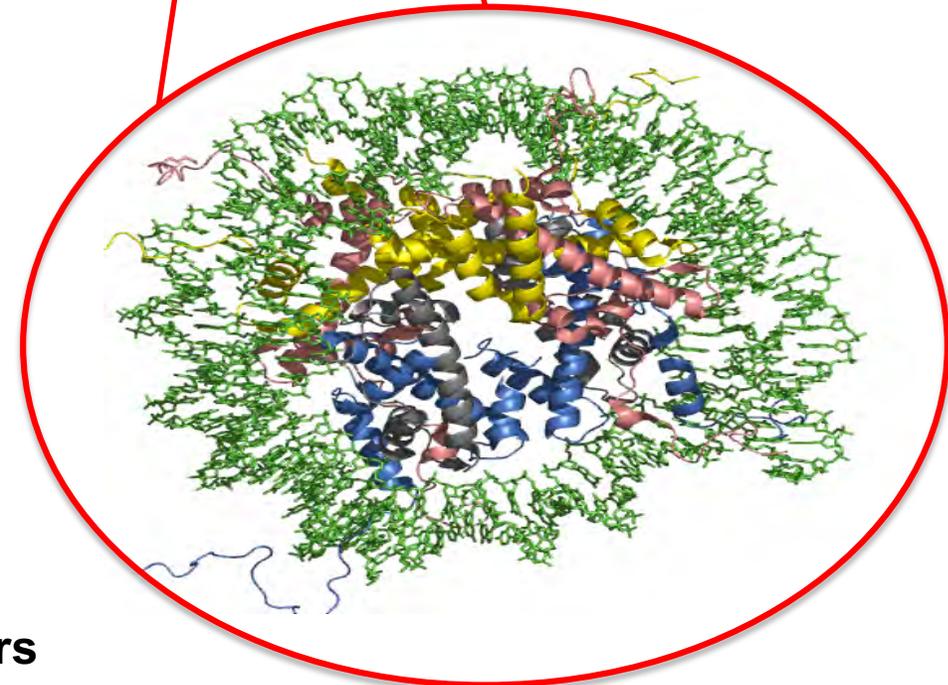
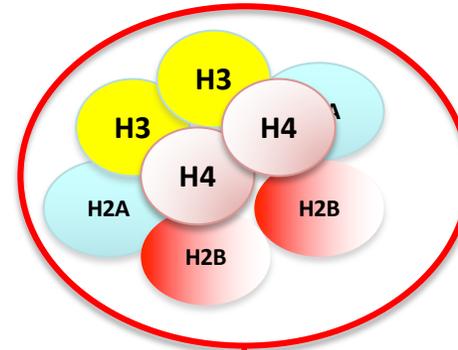
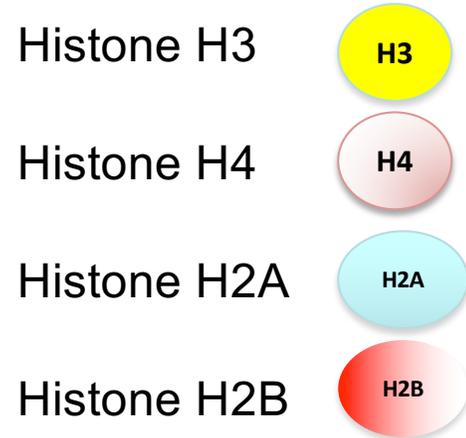
Fragment analysis

(Calabrese & Radford, *Methods*, 2018)

Case Study: Epigenetics



Case Study: Epigenetics



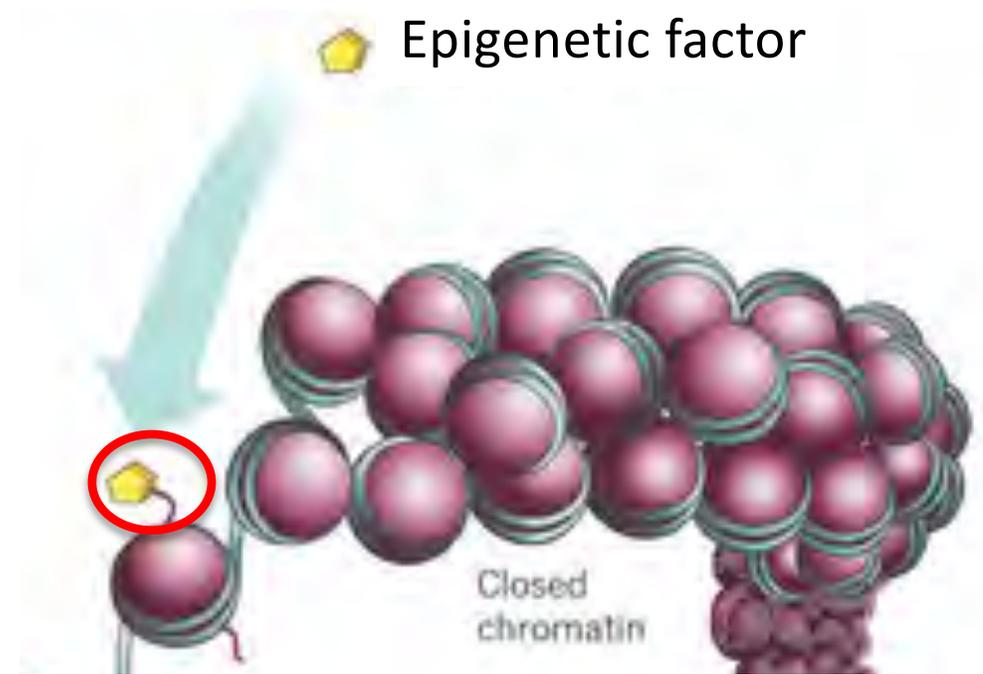
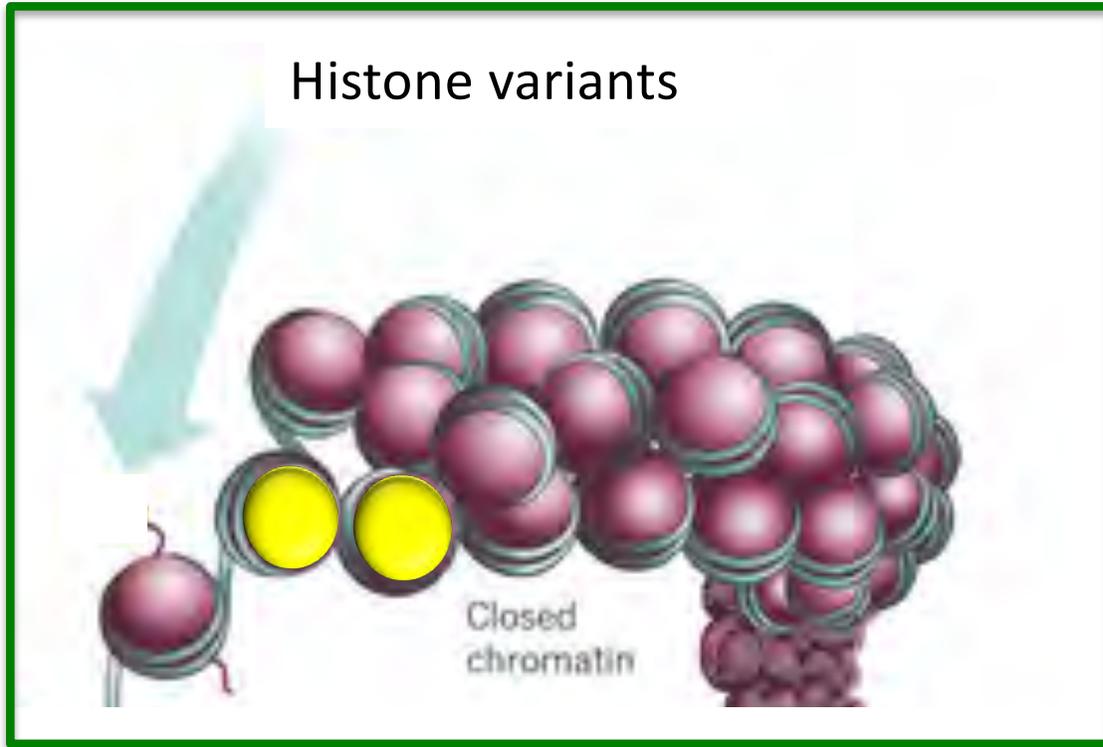
Wrap a fragment of 150bp.

Left-handed

H3 associate with H4 more readily

H3-H4 tetramer binds two H2A-H2B dimers

Case Study: Epigenetics



Case Study: Epigenetics

Post-translational modifications of histone proteins



Phosphorylation of serine and threonine residues

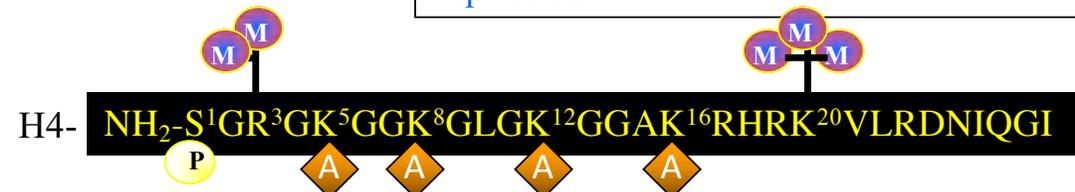
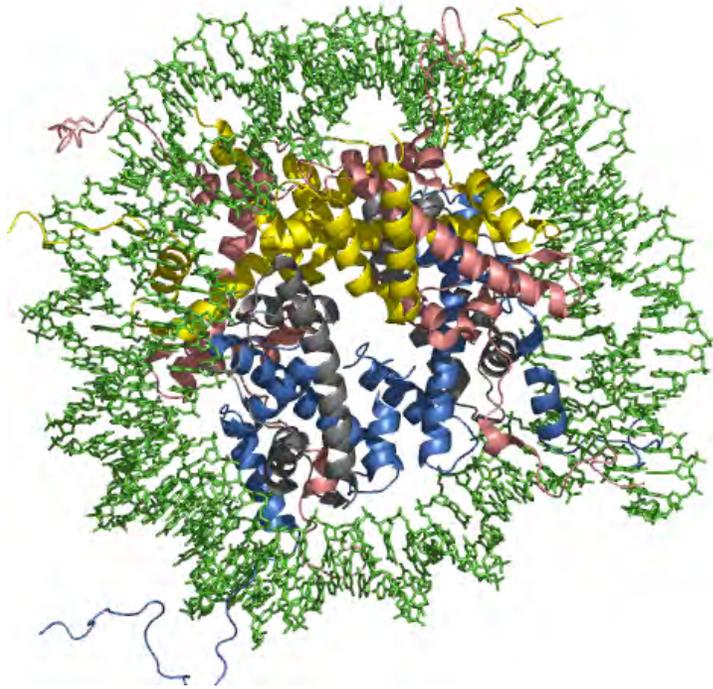
Acetylation of lysine residues

Mono-methylation of K20 is linked to gene silencing.

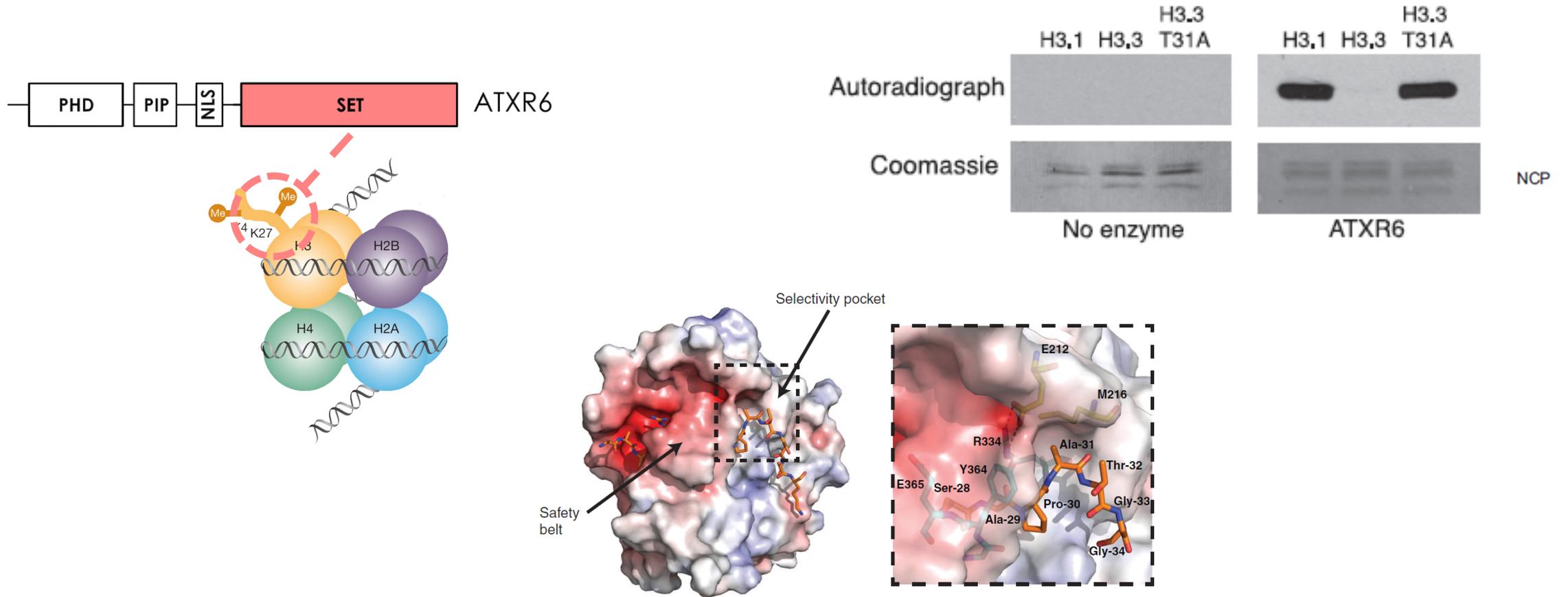
Methylation of arginine residues.

*Tri-methylation of lysine 4 is found in the promoter region of actively transcribed genes.

*Tri-methylation of lysine residues 20, 9 and 27 is linked to repression of gene expression.

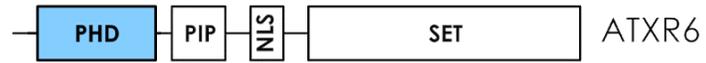


Case Study: Epigenetics by X-ray crystallography

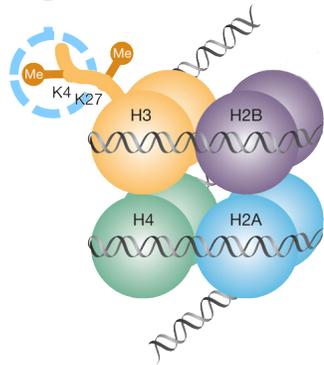


(Jacob et al, *Science*, 2014; Bergamin et al, *Nuclear Acids Res*, 2017)

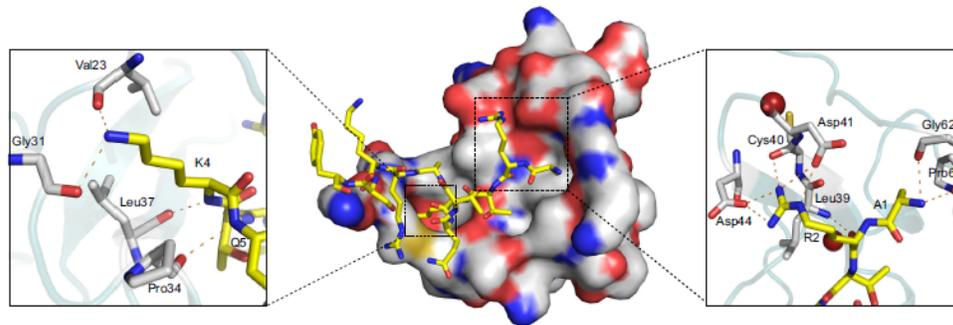
Case Study: Epigenetics by X-ray crystallography



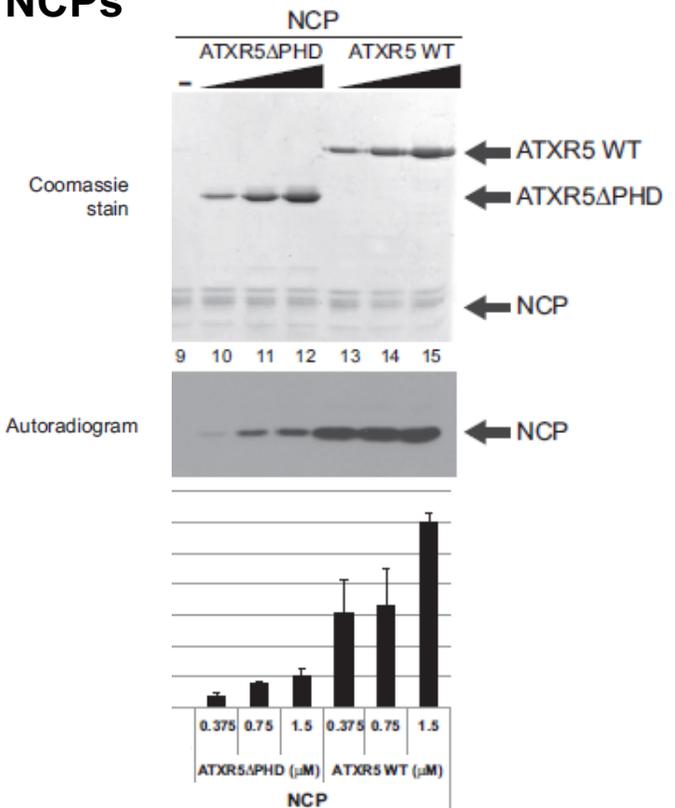
PHD binds H3 N-terminus or SET



PHD domains enhance activity of ATXR5/6 on NCPs

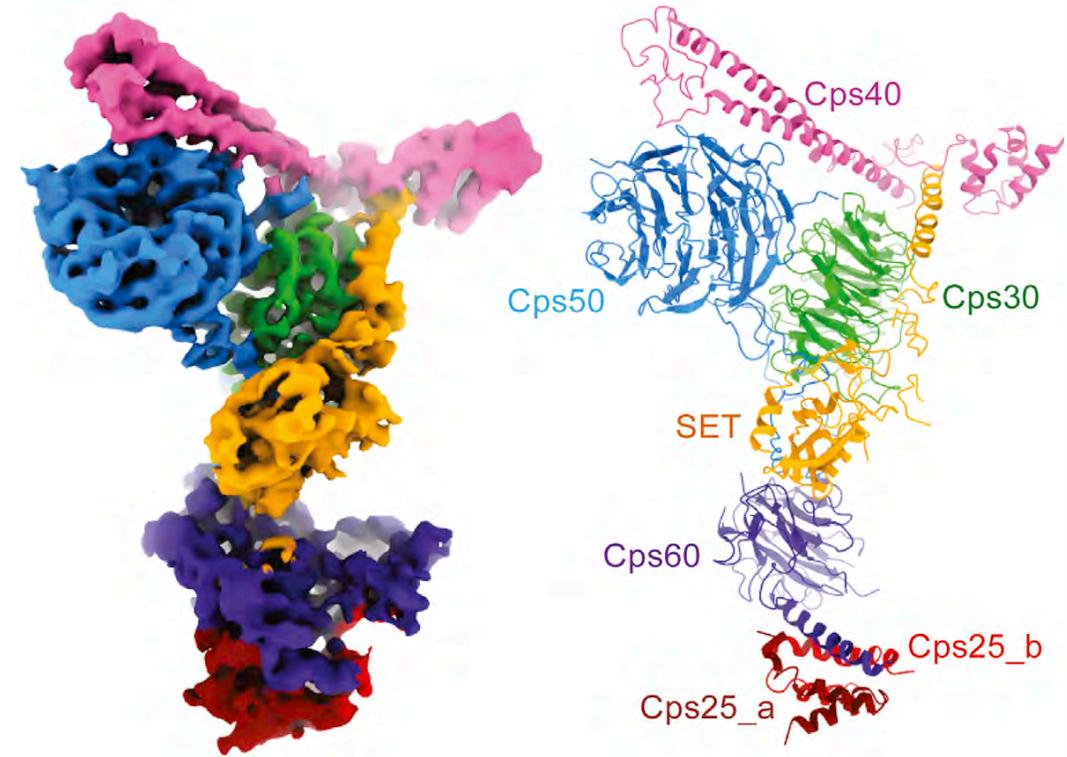
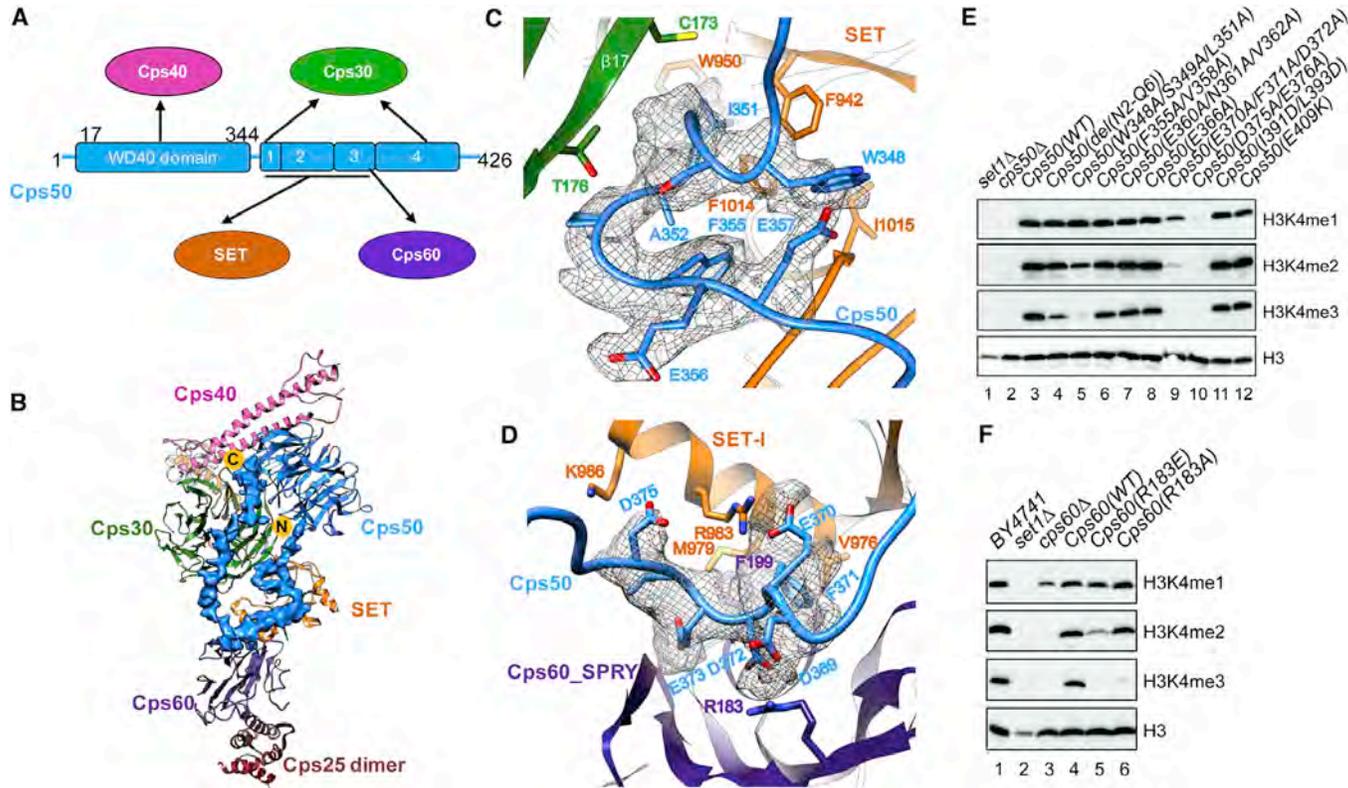


preferentially binds unmethylated H3 K4



(Bergamin et al, Nuclear Acids Res, 2017)

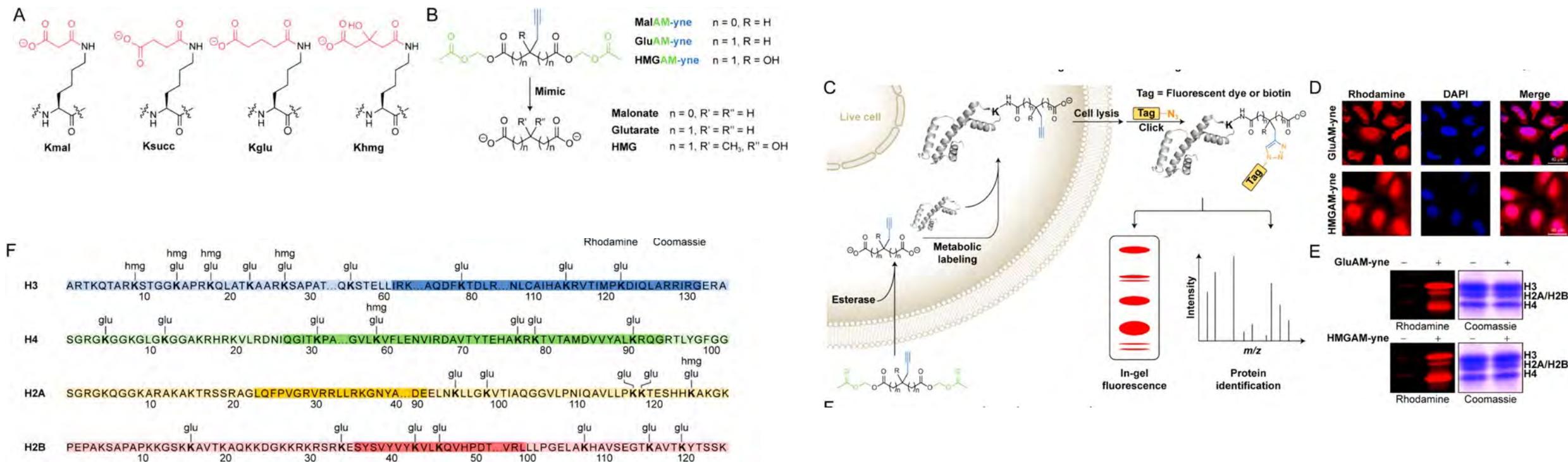
Case Study: Epigenetics by cryo-EM



Structure of a COMPASS histone H3K4 methyltransferase complex

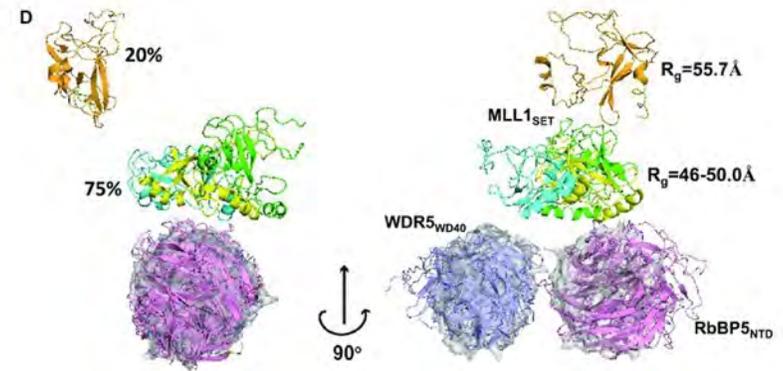
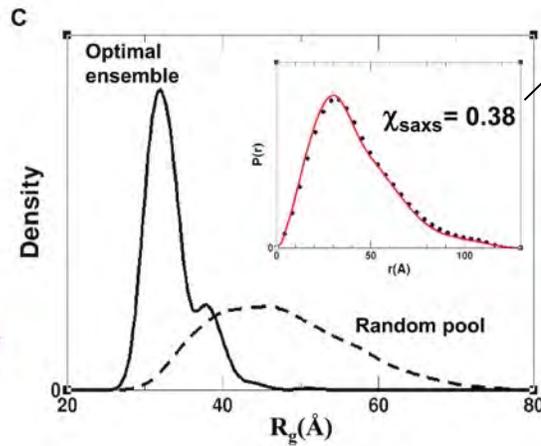
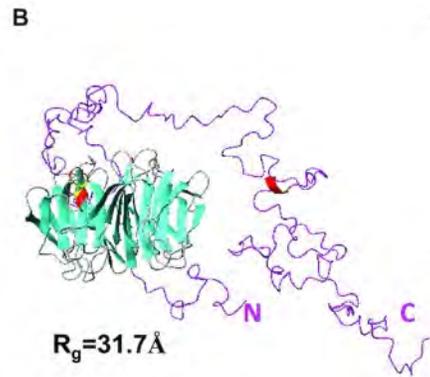
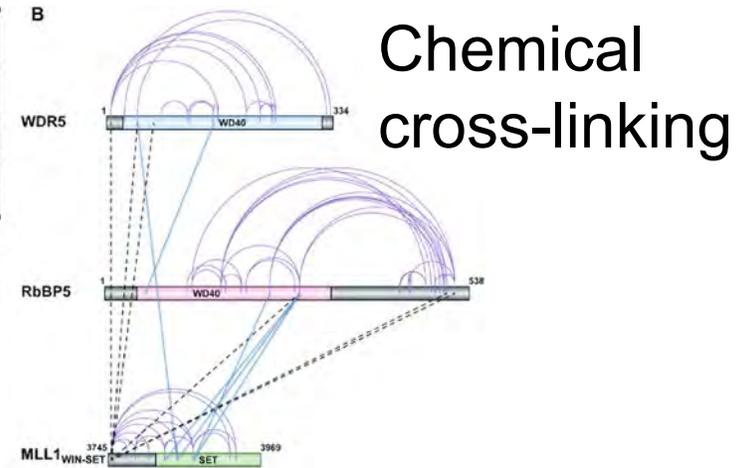
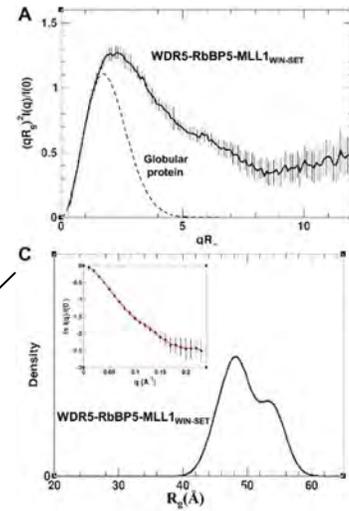
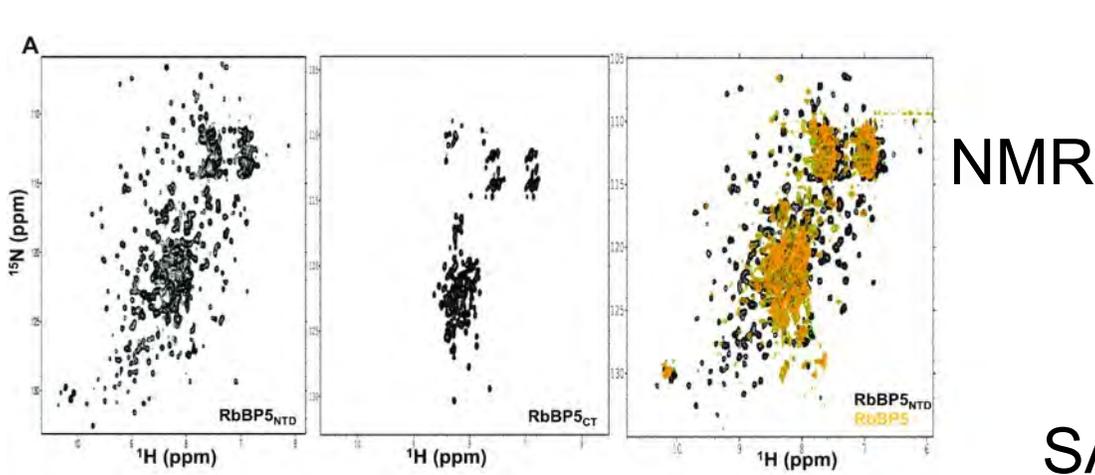
(Qu et al, Cell, 2018)

Case Study: Epigenetics by chemical reporters



(Li & Li, Acc Chem Res, 2021)

Case Study: Epigenetics by NMR, SAXS & cross-linking

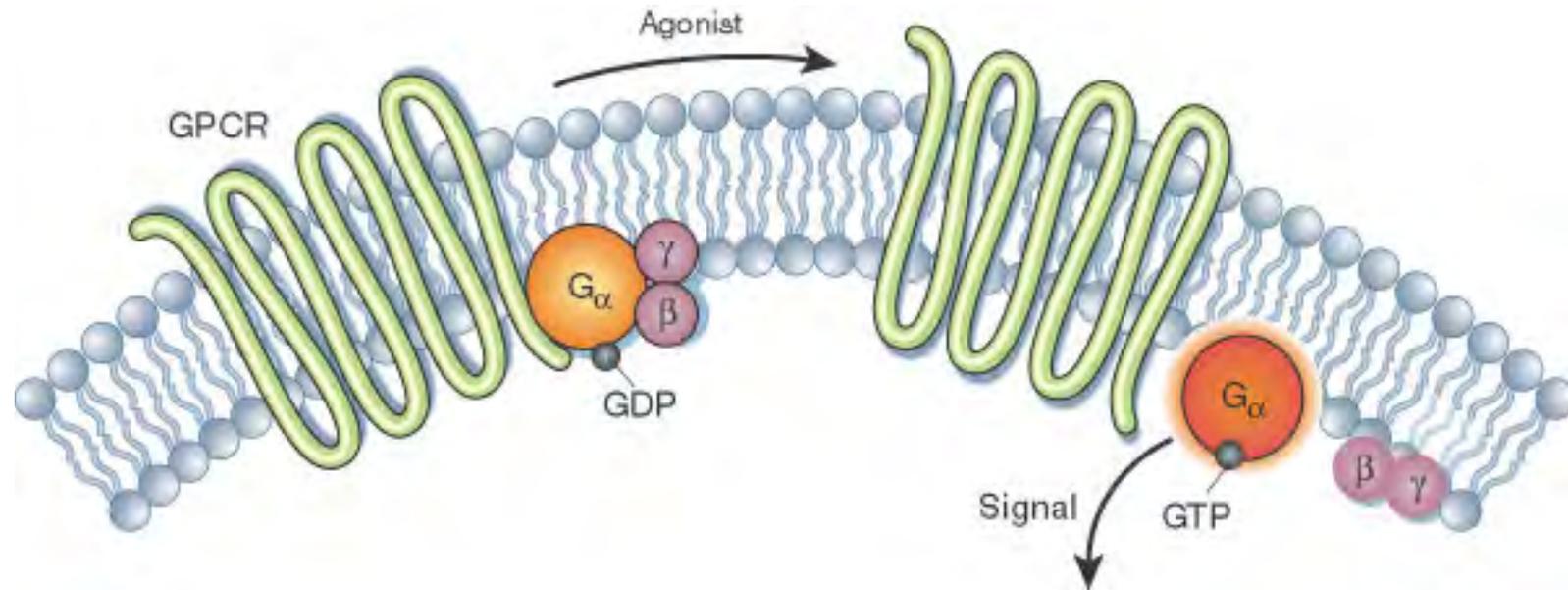


- * SAXS: small-angle X-ray scattering
- * MLL: mixed lineage leukemia

(Kaustov et al, *Nucleic Acids Res*, 2019)

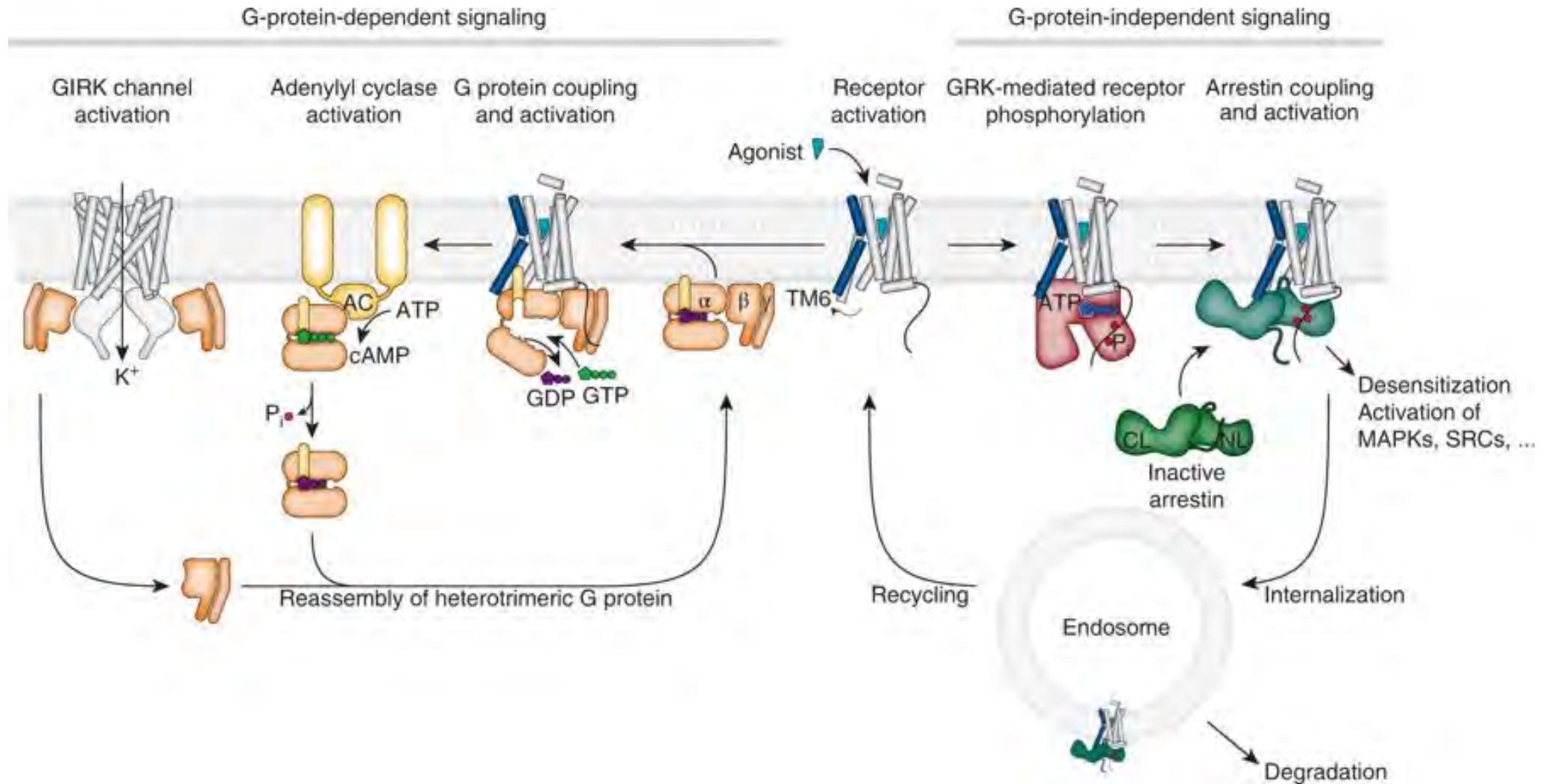
Case Study: G protein signaling

GPCR: G protein-coupled receptor

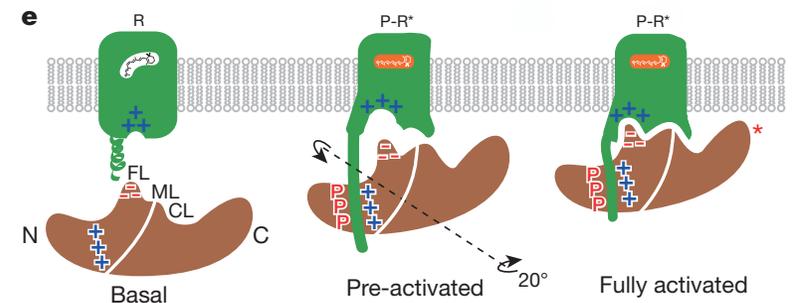
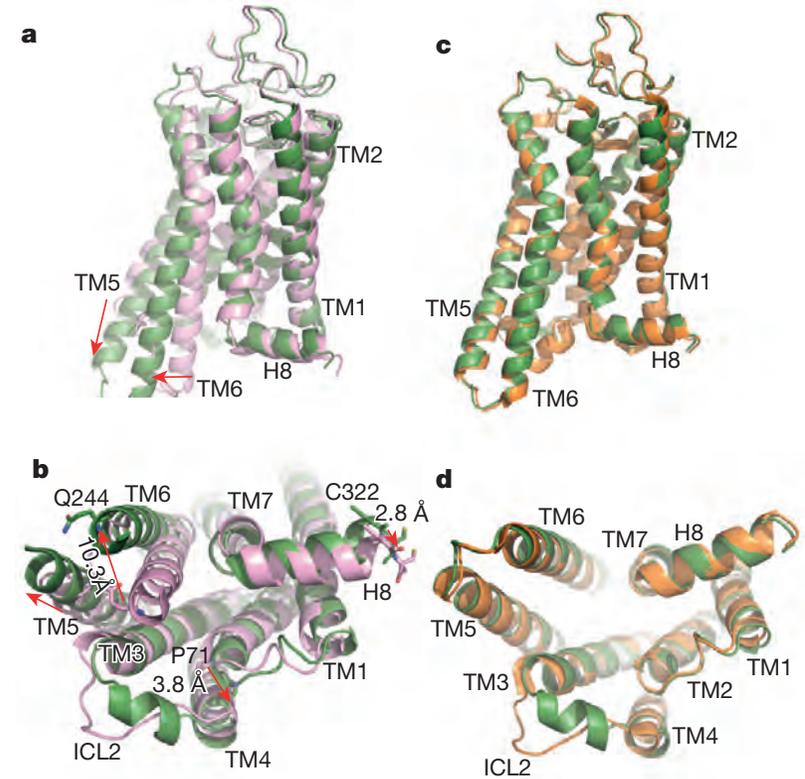
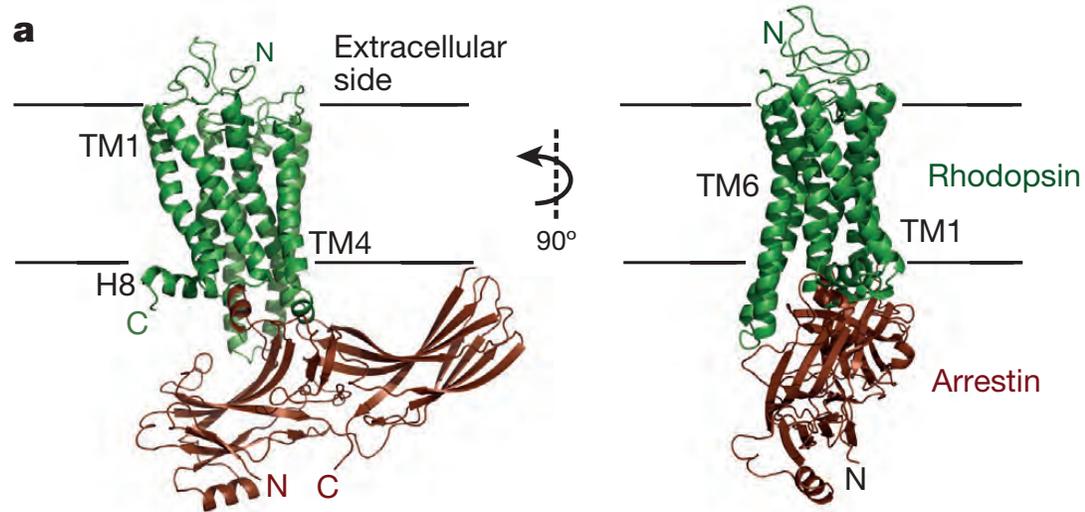


(Li et al, Nature, 2002)

Case Study: G protein signaling



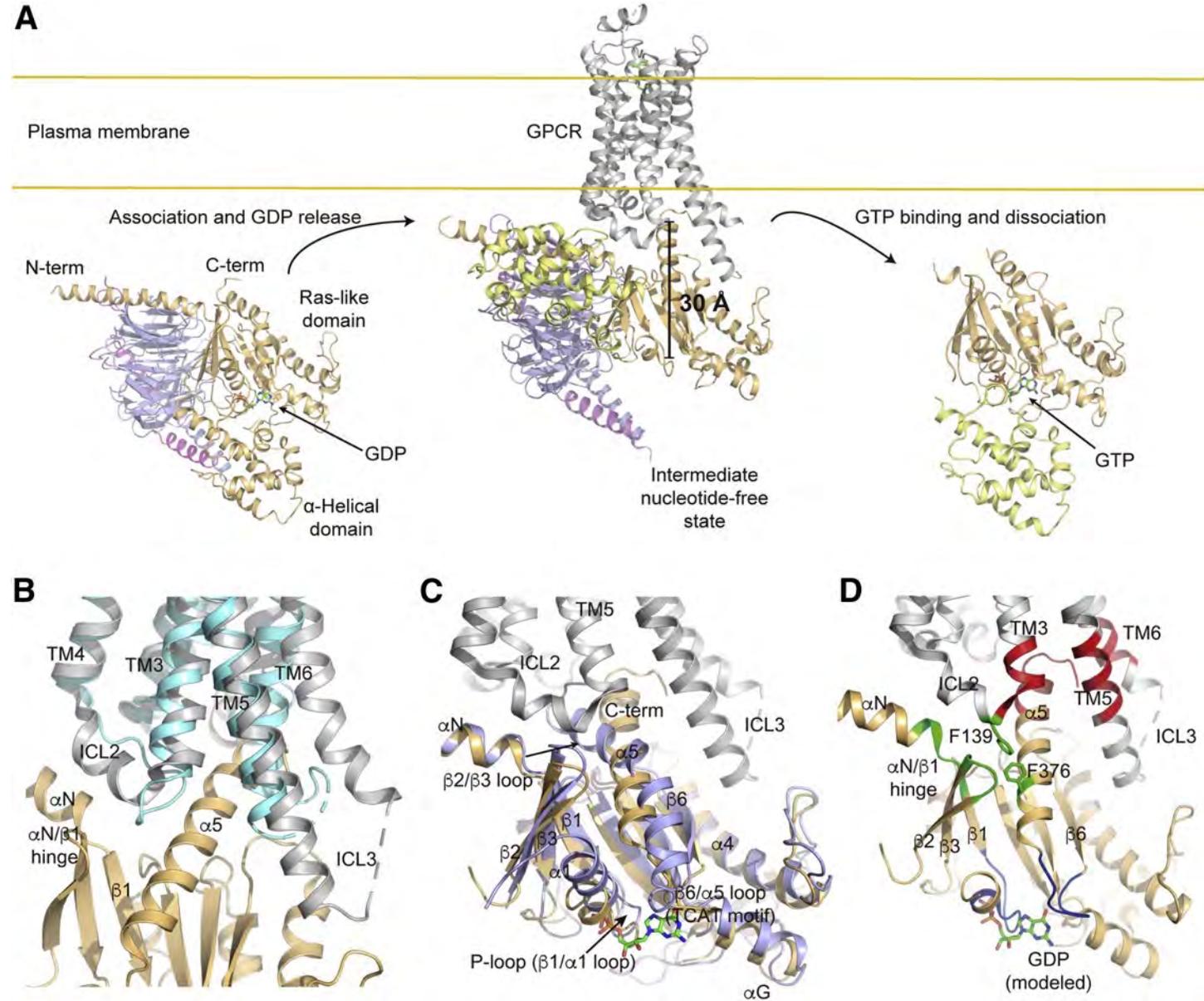
Case Study: GPCR by X-ray crystallography



(Kang et al, Nature, 2015)

Case Study: Assembly of GPCR-G protein complex

X-ray crystallography
Cryo-EM



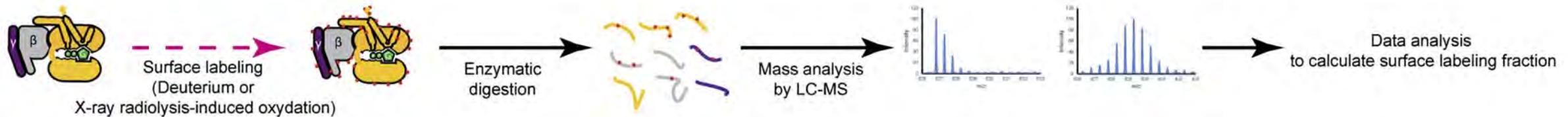
(Du et al, Cell, 2019)

Case Study: Assembly of GPCR-G protein complex

Mass spectrometry (HDX-MS & HRF-MS)

- * HDX: hydrogen/deuterium exchange
- * HRF: hydroxyl radical mediated protein footprinting

A Surface labeling followed by mass spectrometry analysis

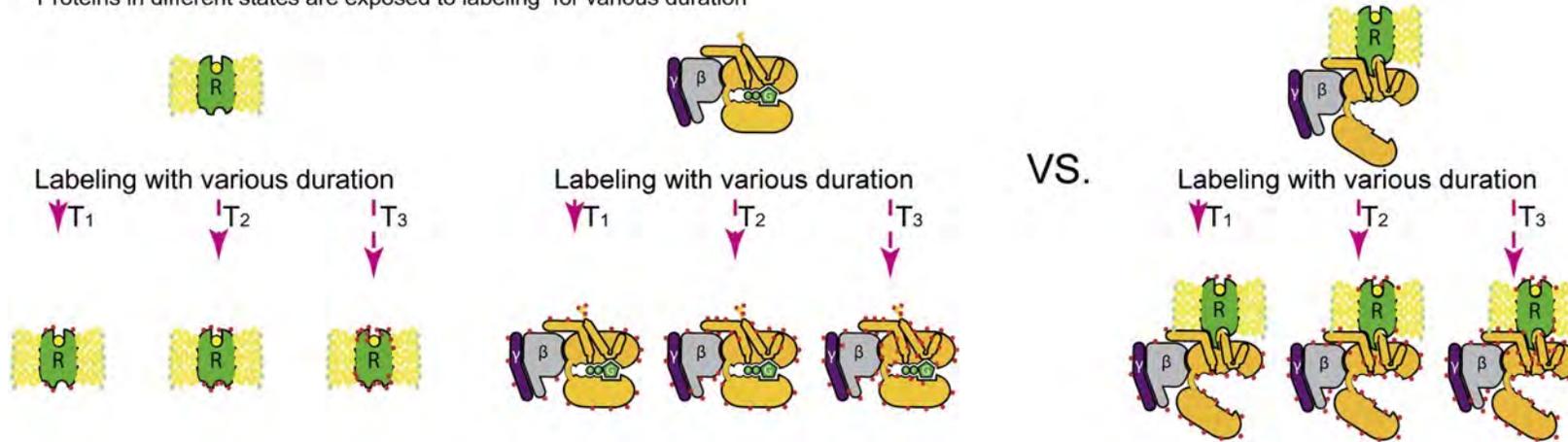


(Du et al, Cell, 2019)

Case Study: Assembly of GPCR-G protein complex

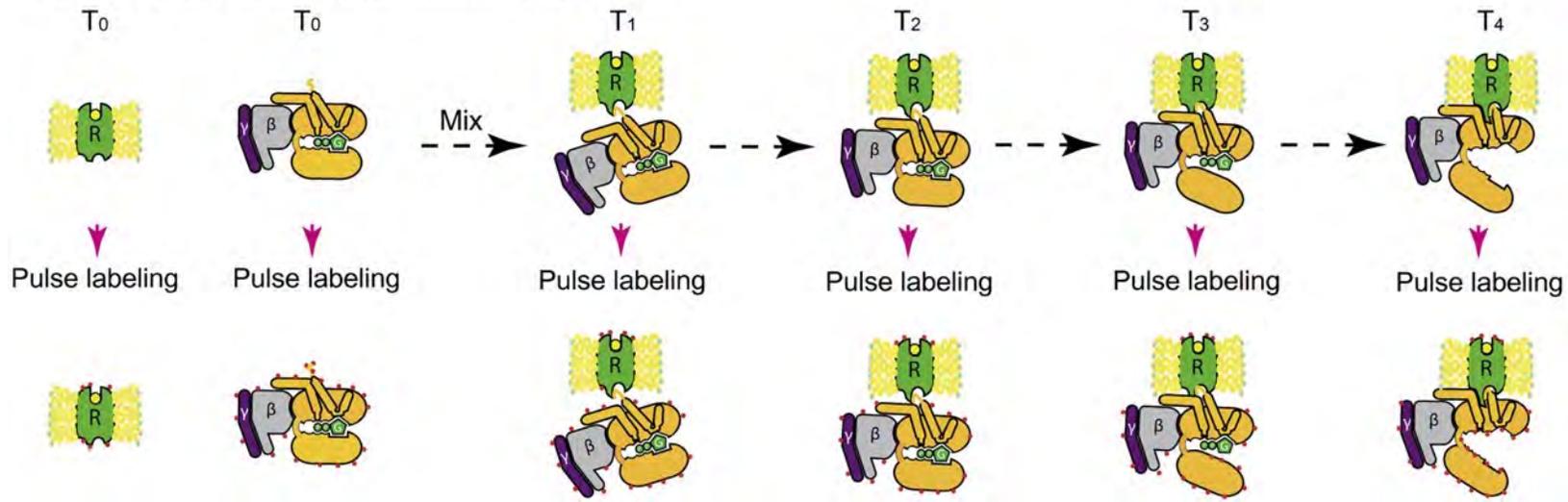
B Continuous-labeling:

Proteins in different states are exposed to labeling for various duration



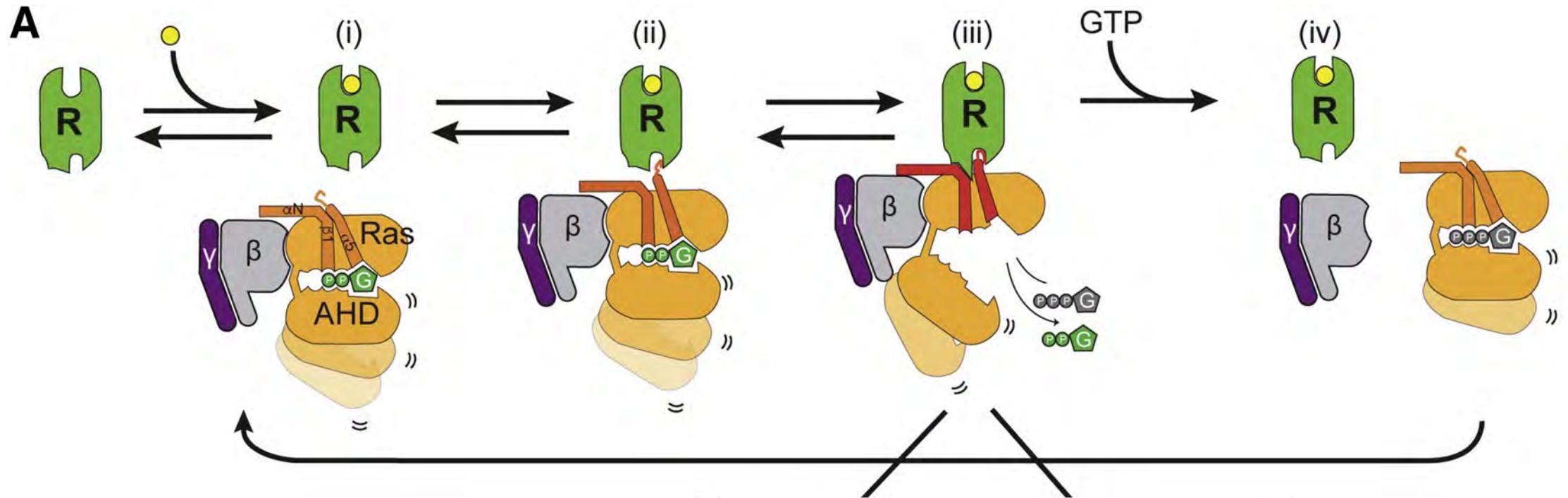
C Pulse-labeling:

A protein is pulsed with labeling during conformational change



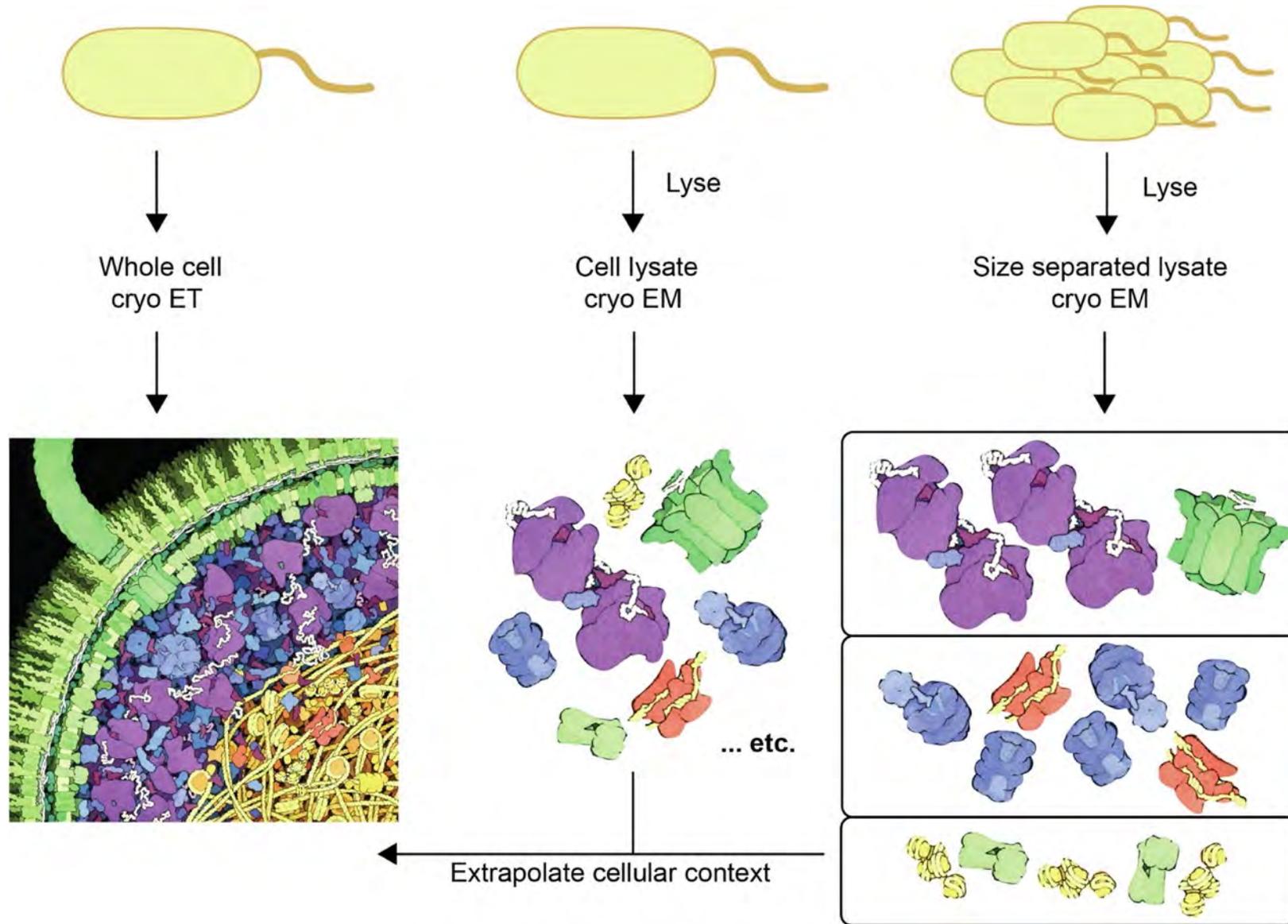
Case Study: Assembly of GPCR-G protein complex

Proposed Model



(Du et al, Cell, 2019)

Outlook: *in situ* structural characterization



(Ziegler et al, CSBJ, 2021)

Outlook: Integrative Structural Biology Strategies

