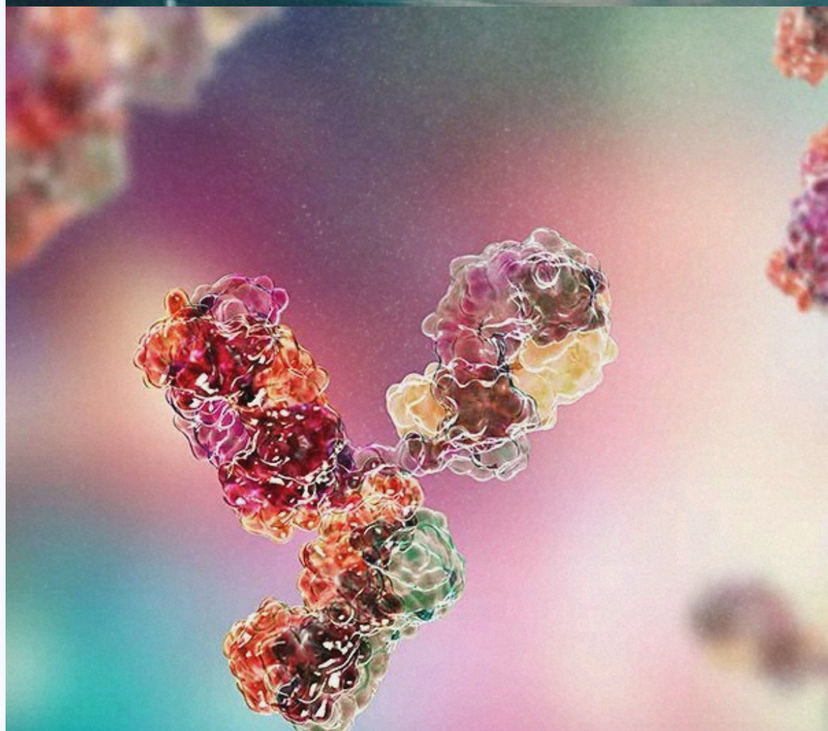
Topic 1: Recombinant Protein Production

2026 Sakura Science Exchange Program – The University of Osaka

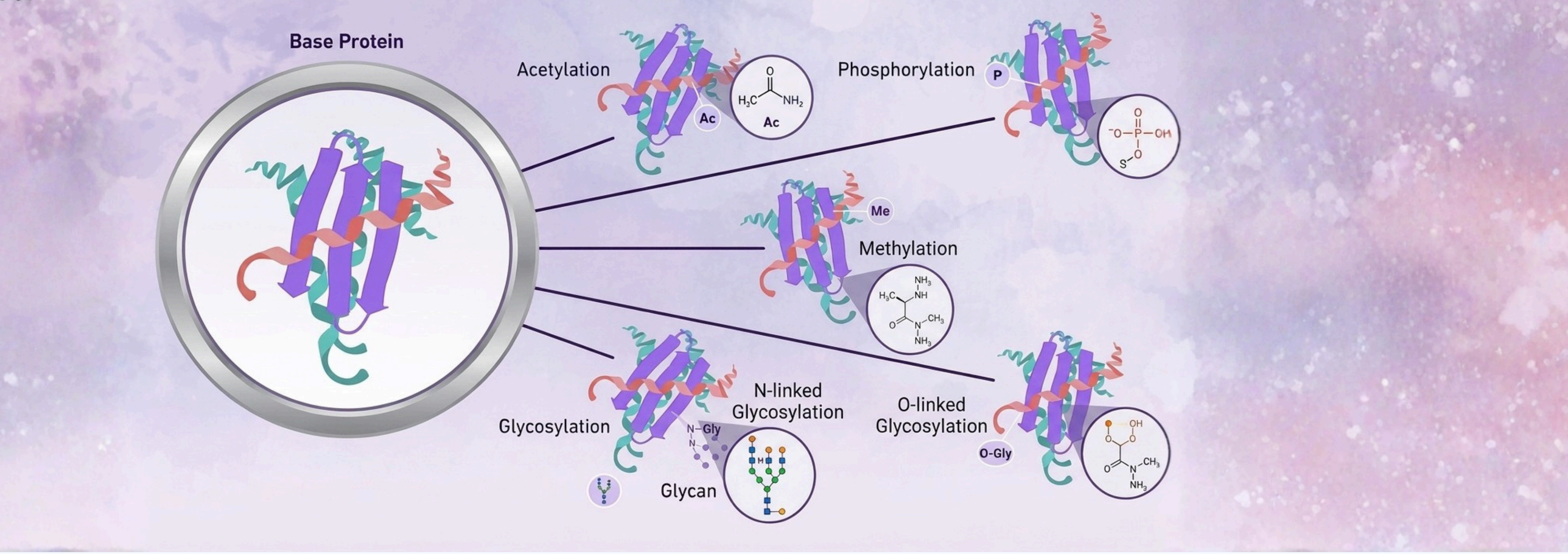
ALERTA KAYE ANN LOOT
NAZHIFAH BINTI HAJI JAPAR
SEVERINUS ADRIAN MAITRI
SATTIYASHELAN KUNAJAKARAN





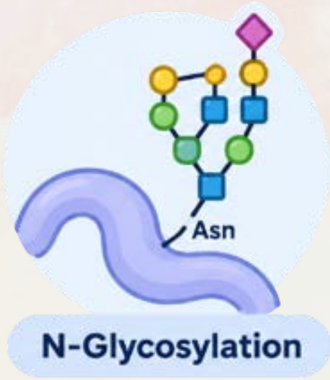
Advantages of producing recombinant proteins

Aspect			
Production costs & speed	Generally low and fast	Depending on production platform	Culture requirements & process control
Culture requirements	Simple	Depends on the system	Carefully controlled
Scalability	High ; Fermentation	Good, potentially high	OK with lots of control
Protein folding	Good for general protein, troubled with complex one	Complex Protein & some post-translational modifications	Highly suitable complex mamalian cell
Glycosylation	Does not possess the machinery	Can perform, but glycan structures differ	はい
Applications	Insulin, certain enzymes	Vaccine candidates, antigens	Monoclonal antibodies & complex therapeutic proteins



Differences in post-translational modification (PTM) mechanisms

N-Glycosylation





Microorganism

Rare; mainly occurs in specific pathogens (e.g., Campylobacter). Relies on cytoplasmic or periplasmic pathways.



Animal

Complex, hybrid, and high-mannose structures. Extensively modified in the Golgi with terminal sialic acid, galactose, and α-1,6-fucose.



Plants

Complex, hybrid, and high-mannose structures. Uniquely feature β-1,2-xylose and α-1,3-fucose linkages. Strictly no sialic acid.





O-Glycosylation





Microorganism

Attach sugars directly to Ser/Thr, primarily in structural or surface proteins.



Animal

Highly diverse, featuring mucin-type O-glycans (initiating with O-GalNAc) and regulatory O-GlcNAcylation in the cytoplasm/nucleus.



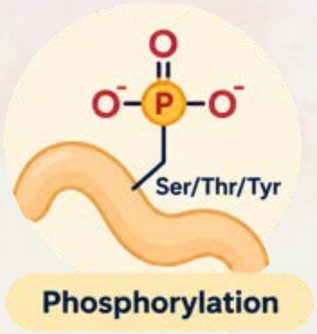
Plants

Dominated by hydroxyproline (Hyp) O-glycosylation. Attaches complex arabinogalactans or short arabinosides to cell wall proteins.





Phosphorylation





Microorganism

Primarily occurs on Histidine and Aspartate residues via two-component regulatory systems. Ser/Thr/Tyr phosphorylation exists but is less prevalent.



Animal

Predominantly occurs on Serine, Threonine, and Tyrosine (via distinct tyrosine kinases) for complex signaling cascades and oncogenic regulation.



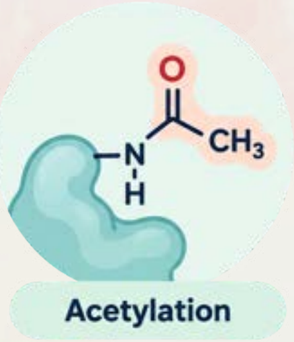
Plants

Heavily relies on Serine and Threonine phosphorylation. Tyrosine phosphorylation occurs via dual-specificity kinases, as plants lack dedicated tyrosine kinases.





Acetylation





Microorganism

Mostly functions as N^{ε}-lysine acetylation to regulate metabolic enzymes and transcription factors. Lack classical histone architecture in bacteria.



Animal

Extensive N^{α}-terminal acetylation (irreversible) and N^{ε}-lysine acetylation on histones and non-histone proteins for epigenetic control.



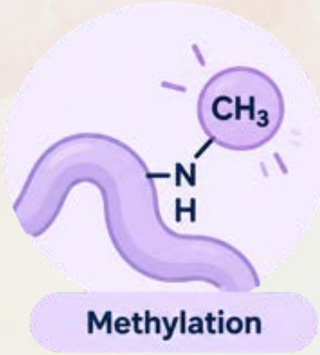
Plants

Widespread across histones, metabolic enzymes, and chloroplast proteins (e.g., Rubisco) to dynamically modulate photosynthesis and stress responses.





Methylation





Microorganism

Primarily targets DNA rather than proteins in bacteria (used for restriction-modification immunity).



Animal

Highly targeted to Lysine and Arginine residues on histones (e.g., H3K4, H3K9) for precise transcriptional activation or repression.



Plants

Crucial for epigenetic silencing, flowering time regulation, and immunity. Plant-specific enzymes methylate both histones and developmental RNA/protein complexes.



How IgGs were purified from cell culture medium

- **Ion-exchange Chromatography**
  - Separates IgG and contaminants according to their **electrical charge** at a selected pH.
- **Protein A/G magnetic beads**
  - **Beads bind IgG** while mixed with the supernatant. A magnet holds the beads during washing, followed by antibody elution.
- **Protein G affinity chromatography**
  - **Protein G captures the IgGs** through their Fc region
- **Protein A affinity chromatography**
  - Protein A captures compatible IgGs through their Fc region, **similarly to Protein G**.

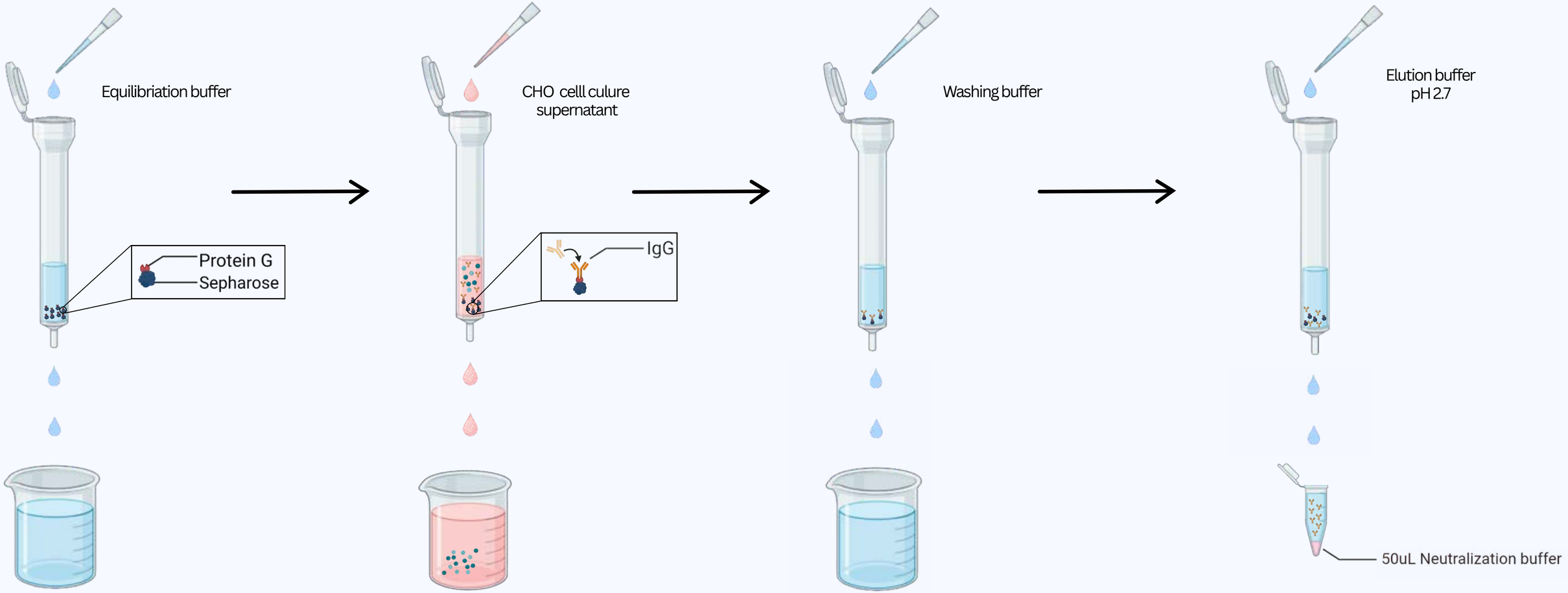
Protein G Affinity Chromatography

1 Equilibrate resin

2 Load sample

3 Wash column

4 Elute purified protein







Thank you for your attention!