

Phosphoproteomics and Cellular Signaling

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Outline

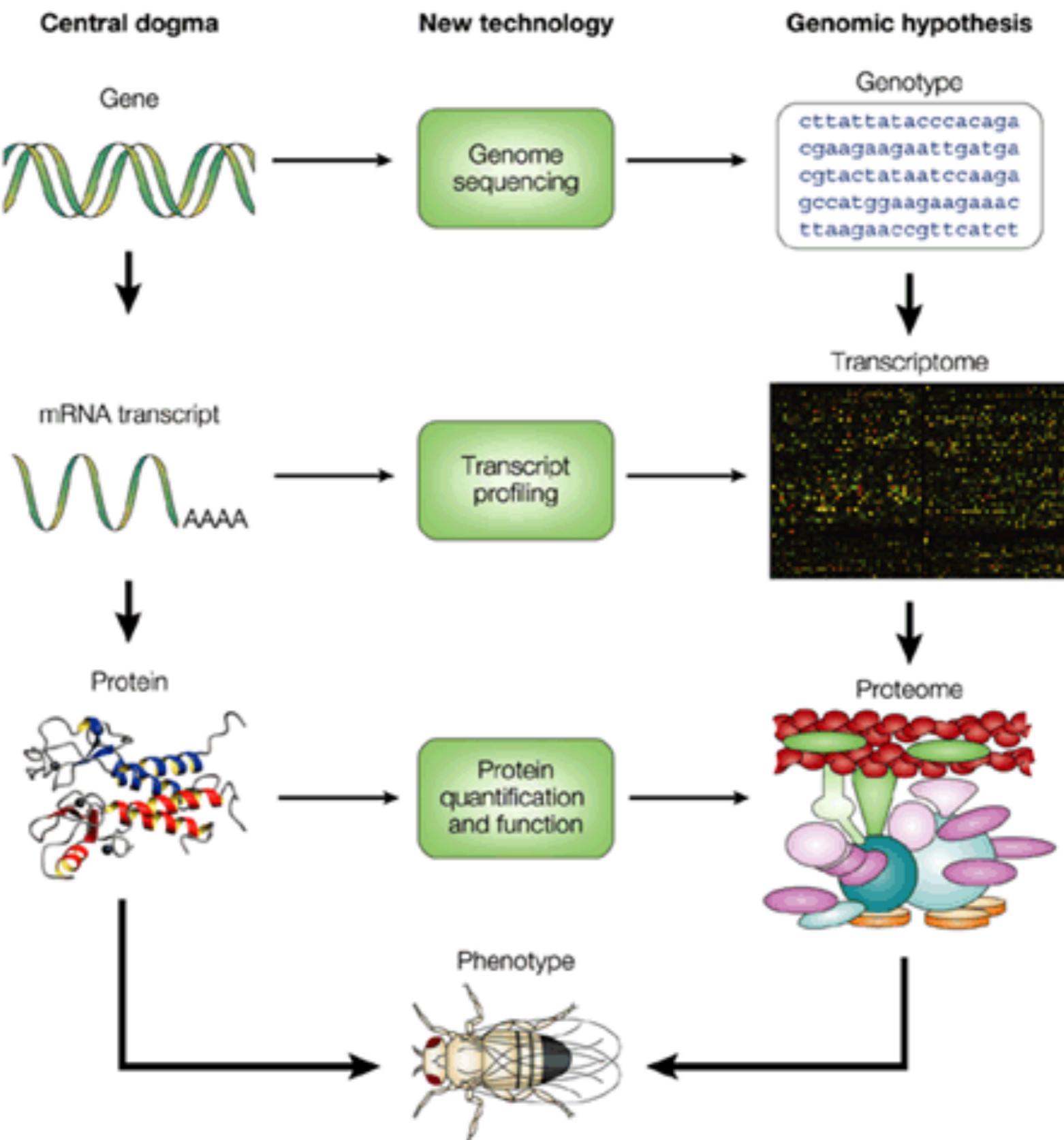
What is proteomics?

What is Mass Spectrometry(MS)?

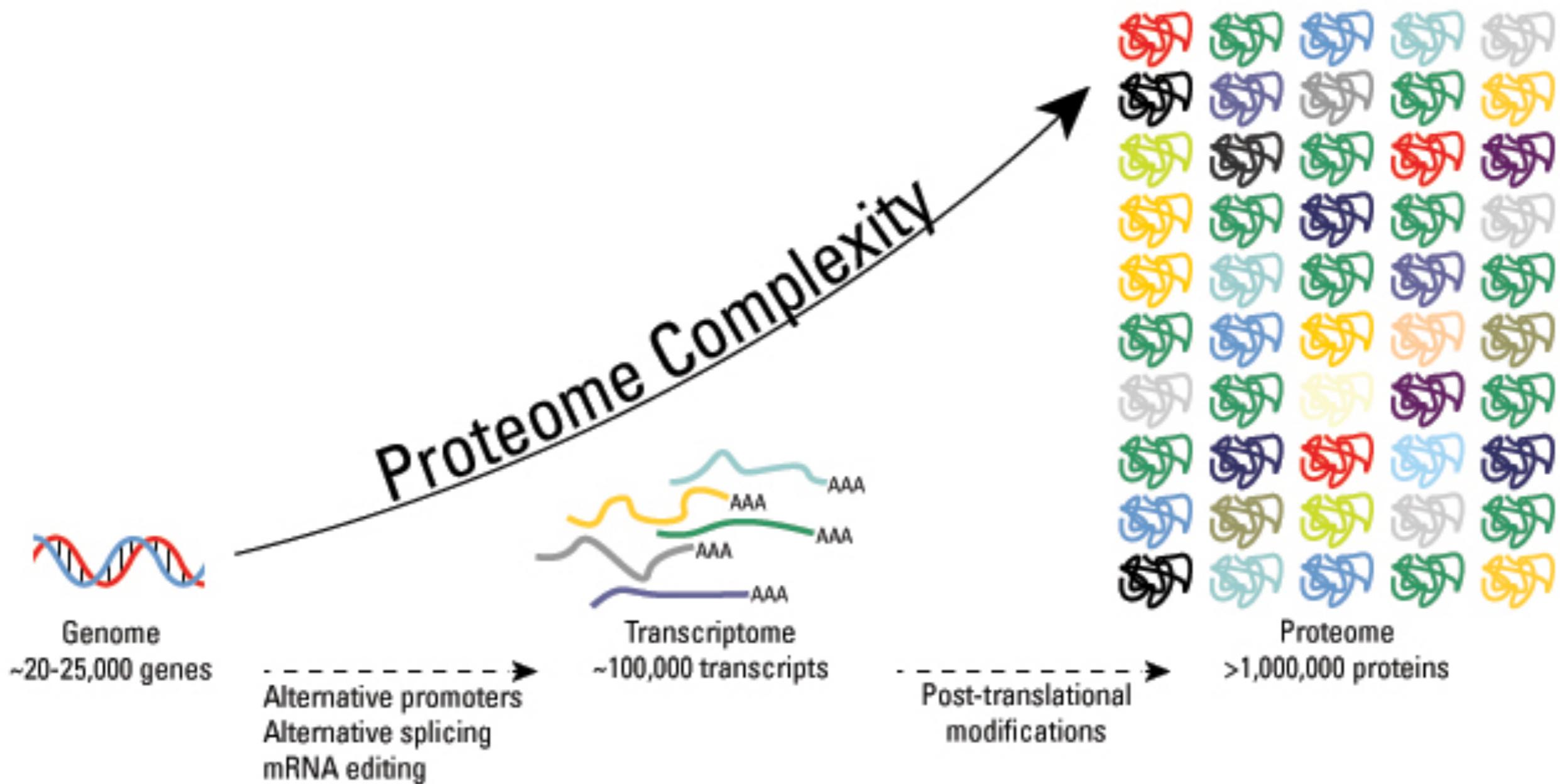
What is phosphoproteomics?

High-Throughput Phosphoproteomics

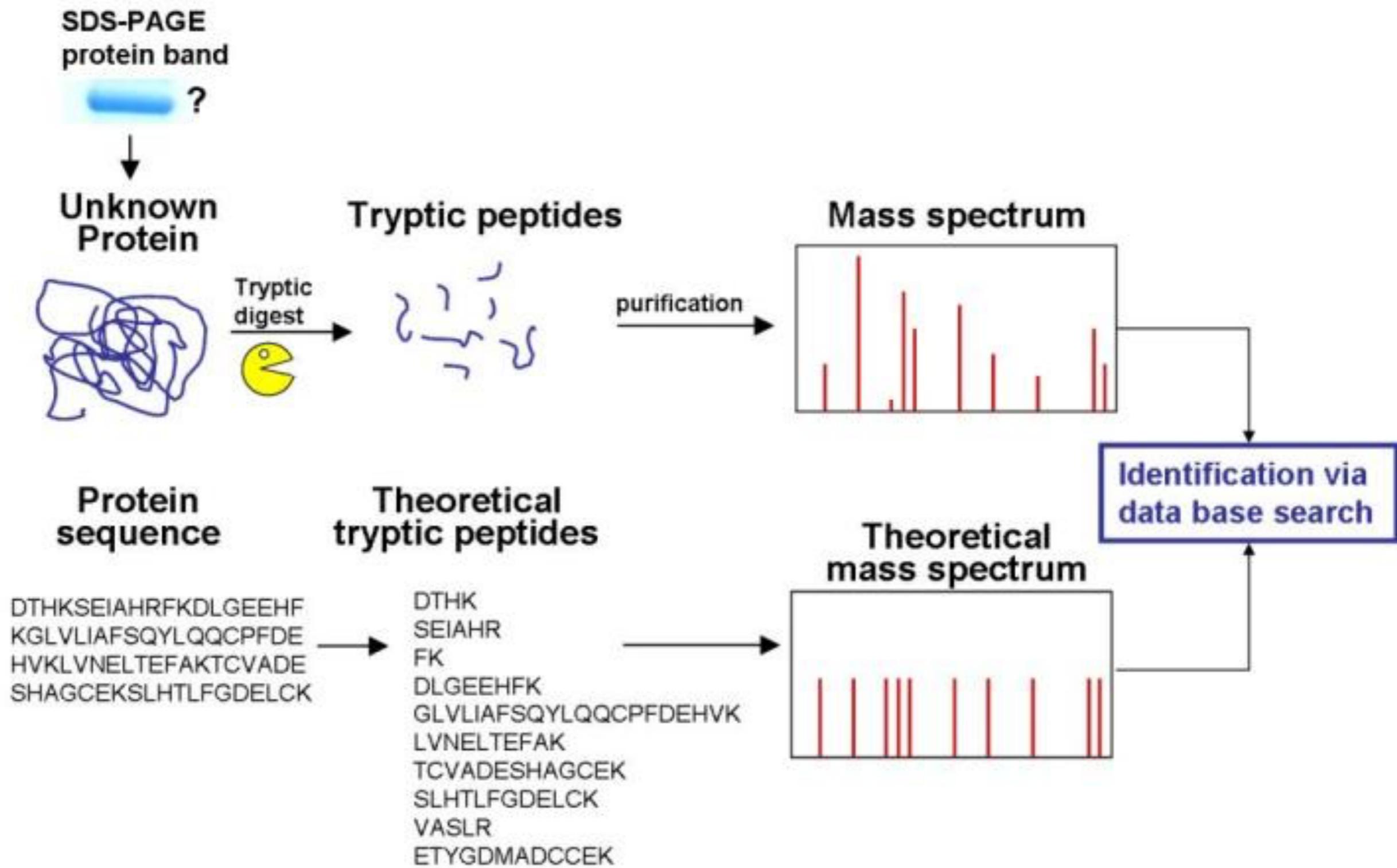
What does the central dogma suggest about the importance of studying proteins?



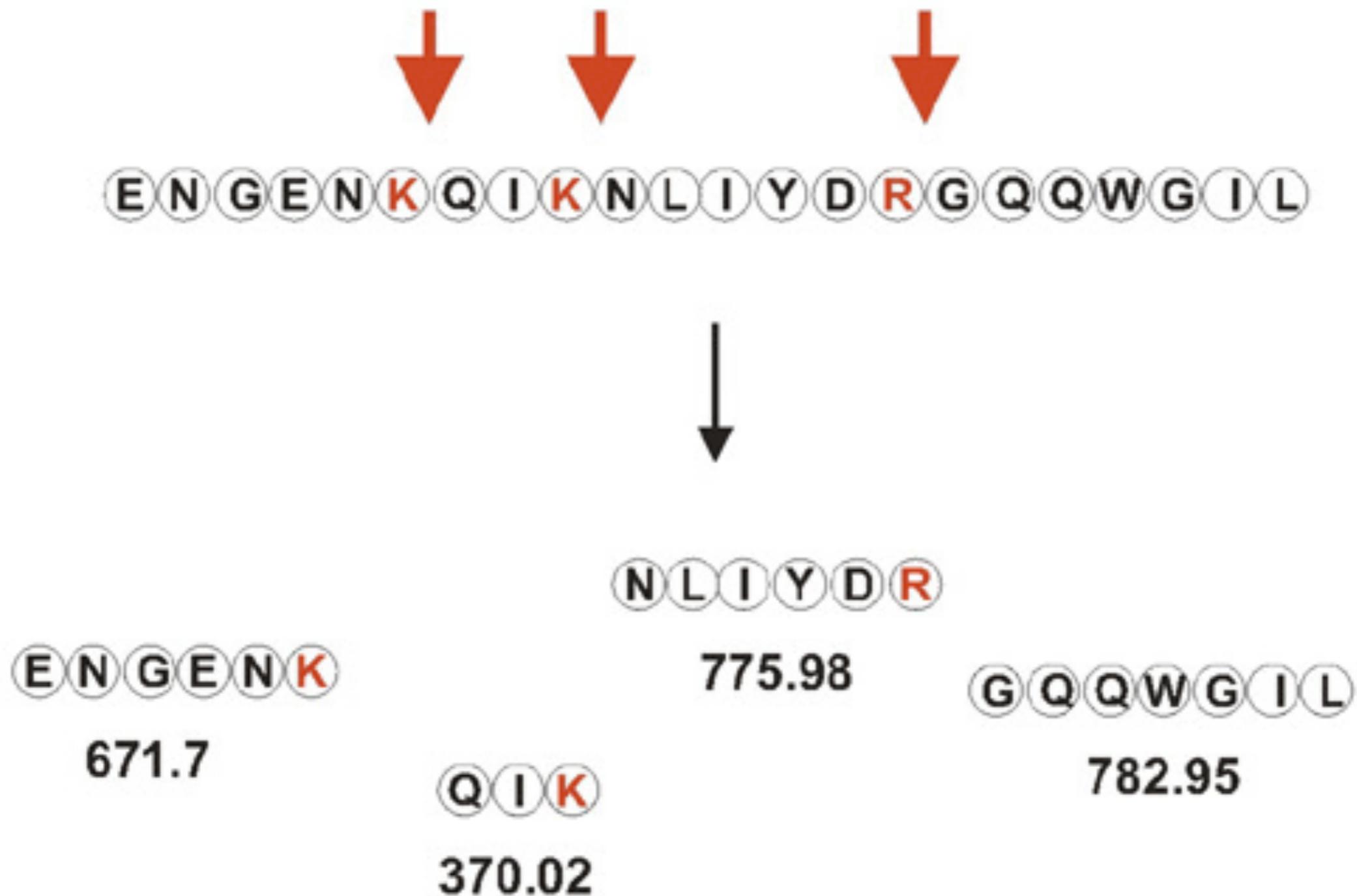
The proteome is complex



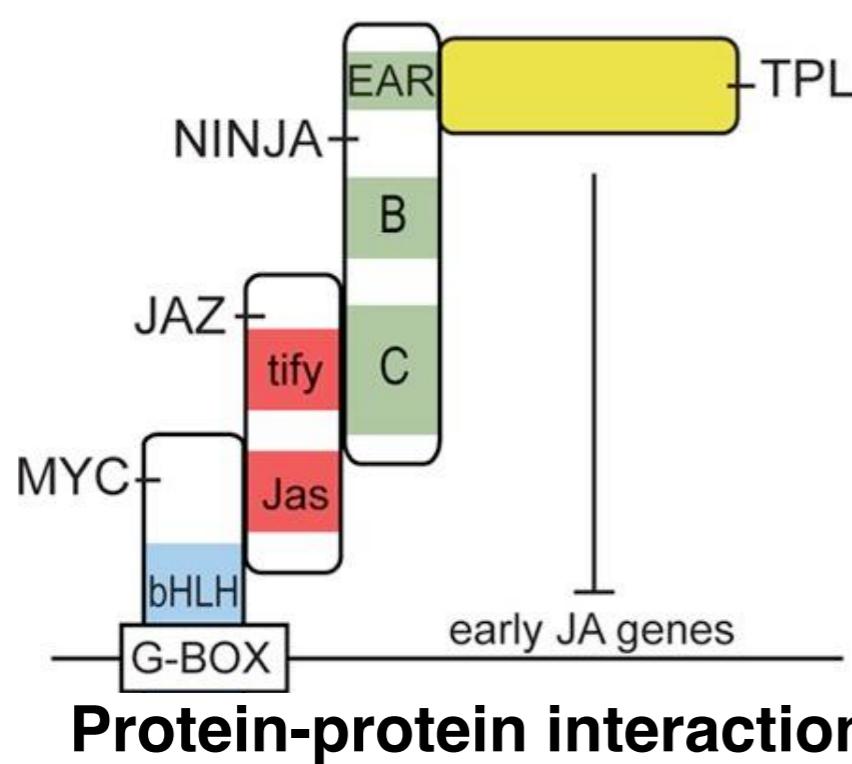
What is proteomics?



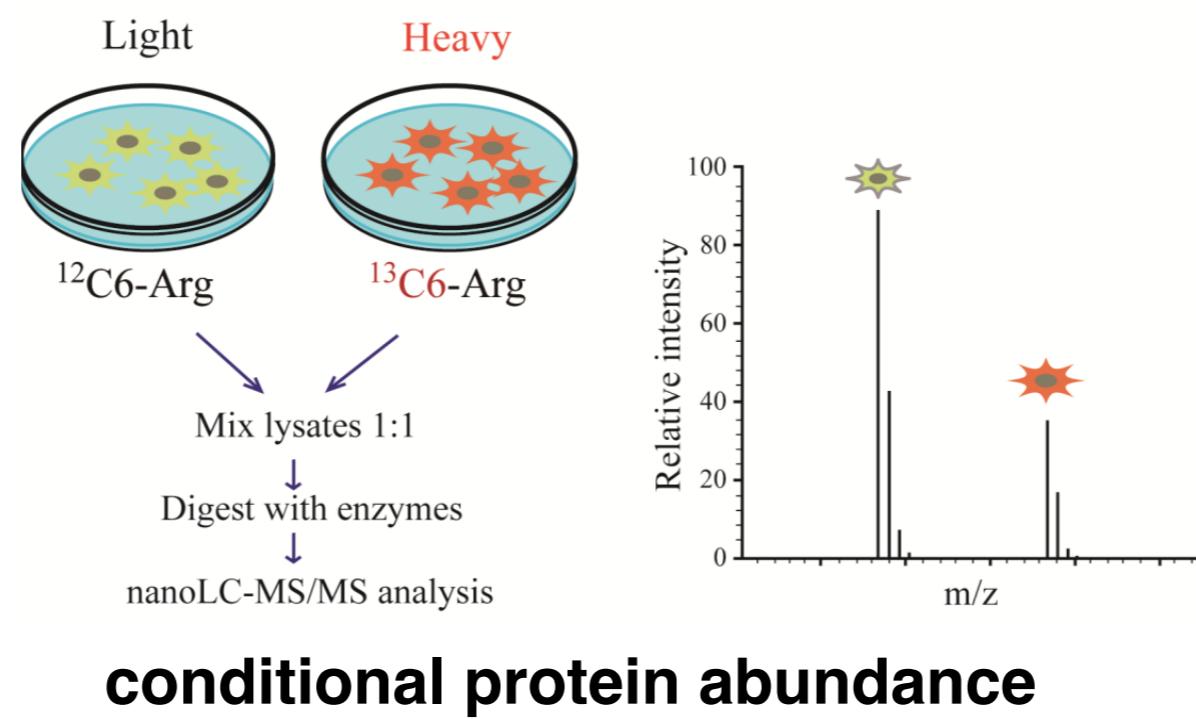
How does trypsin cut proteins?



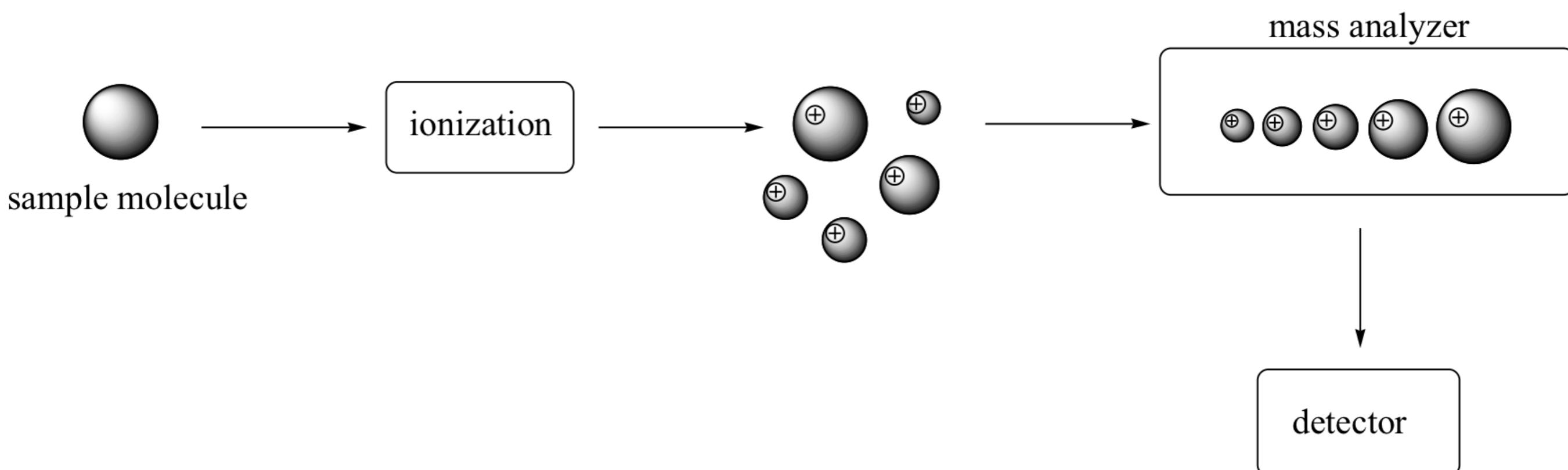
Why is proteomics useful?



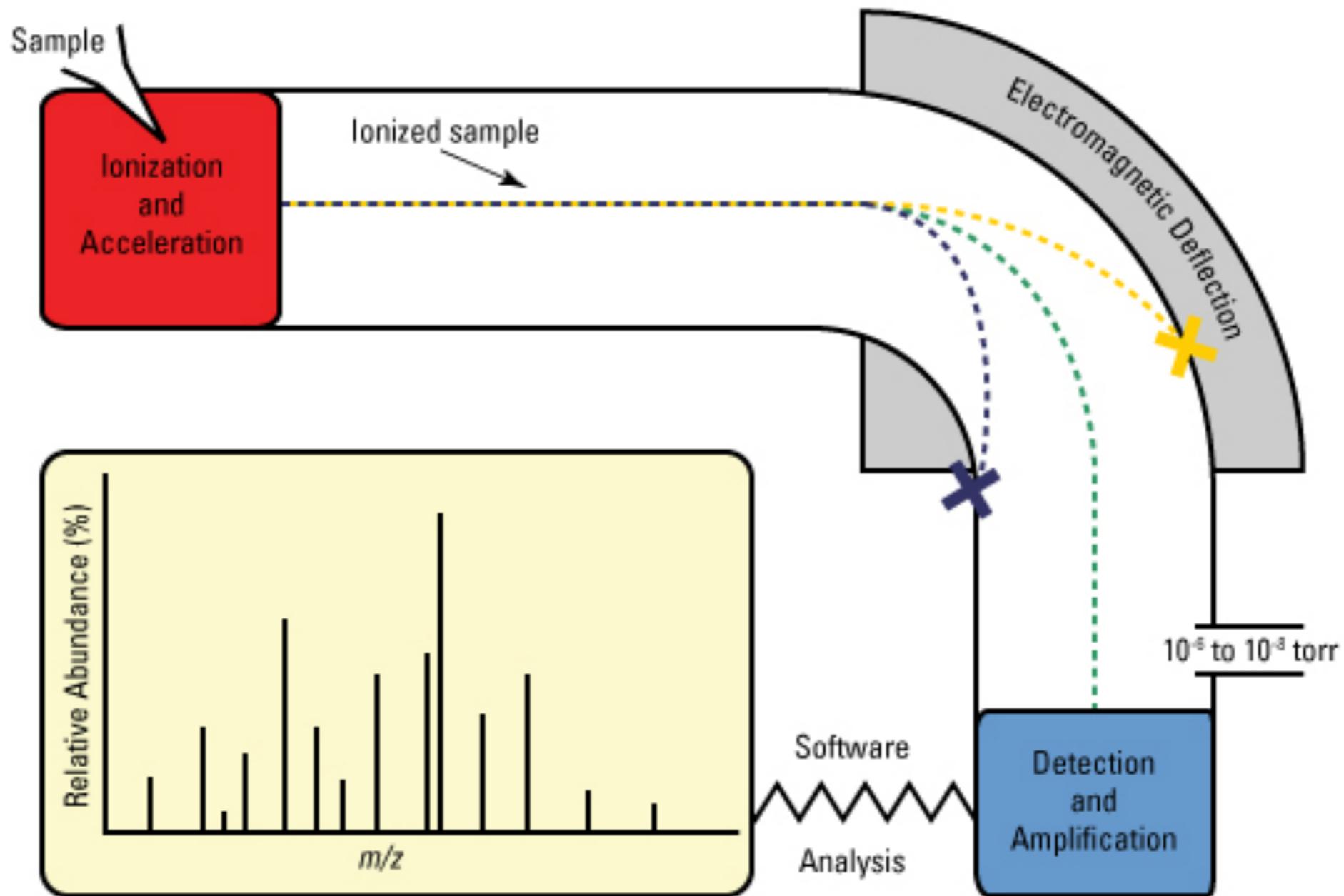
Crystal structure & a.a. sequencing



What is the basic science behind mass spectrometry?



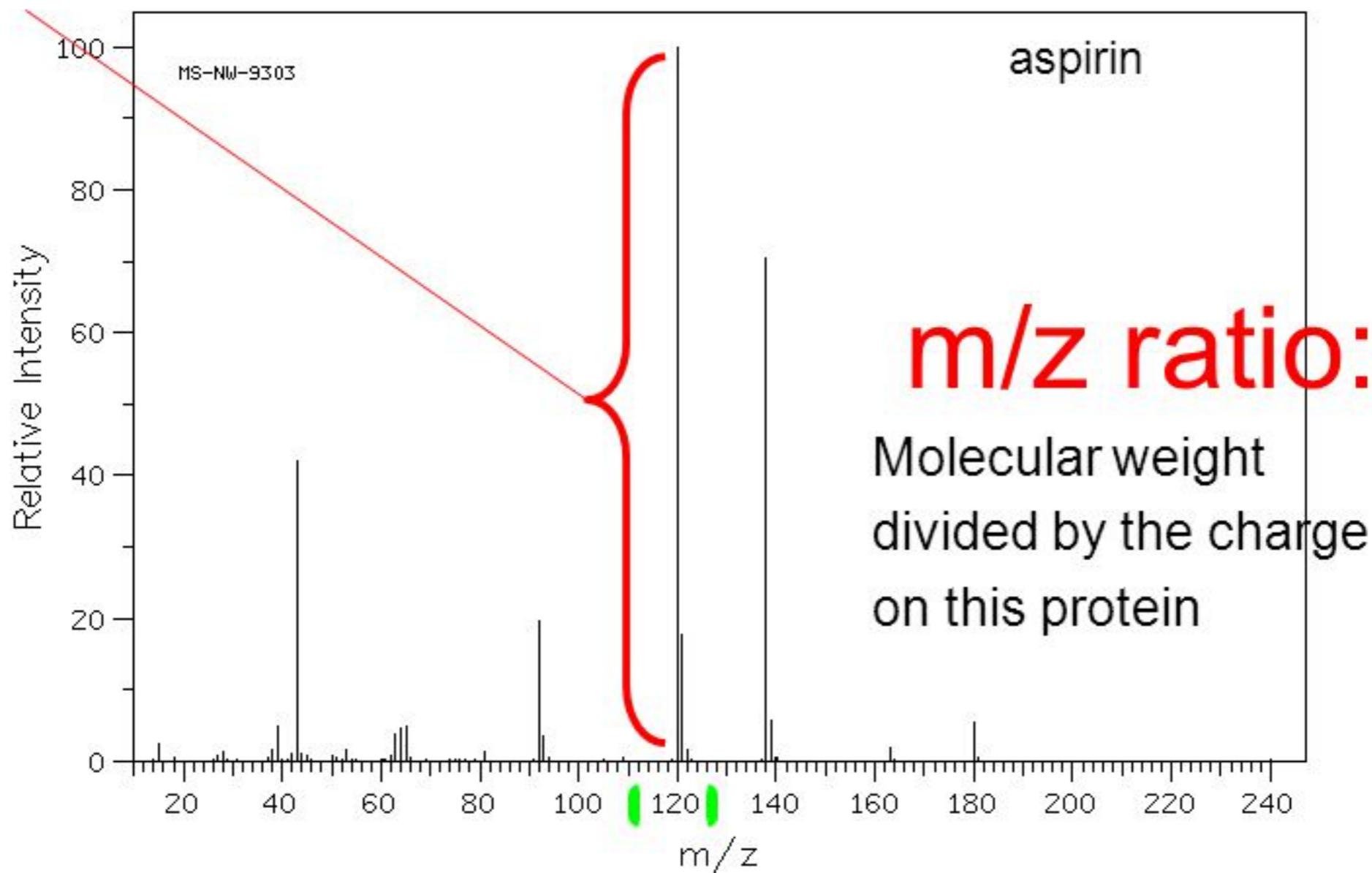
How is a peptide determined via mass spectrometry?



Mass spectrometry is the measurement of the mass to charge ratios of an ions

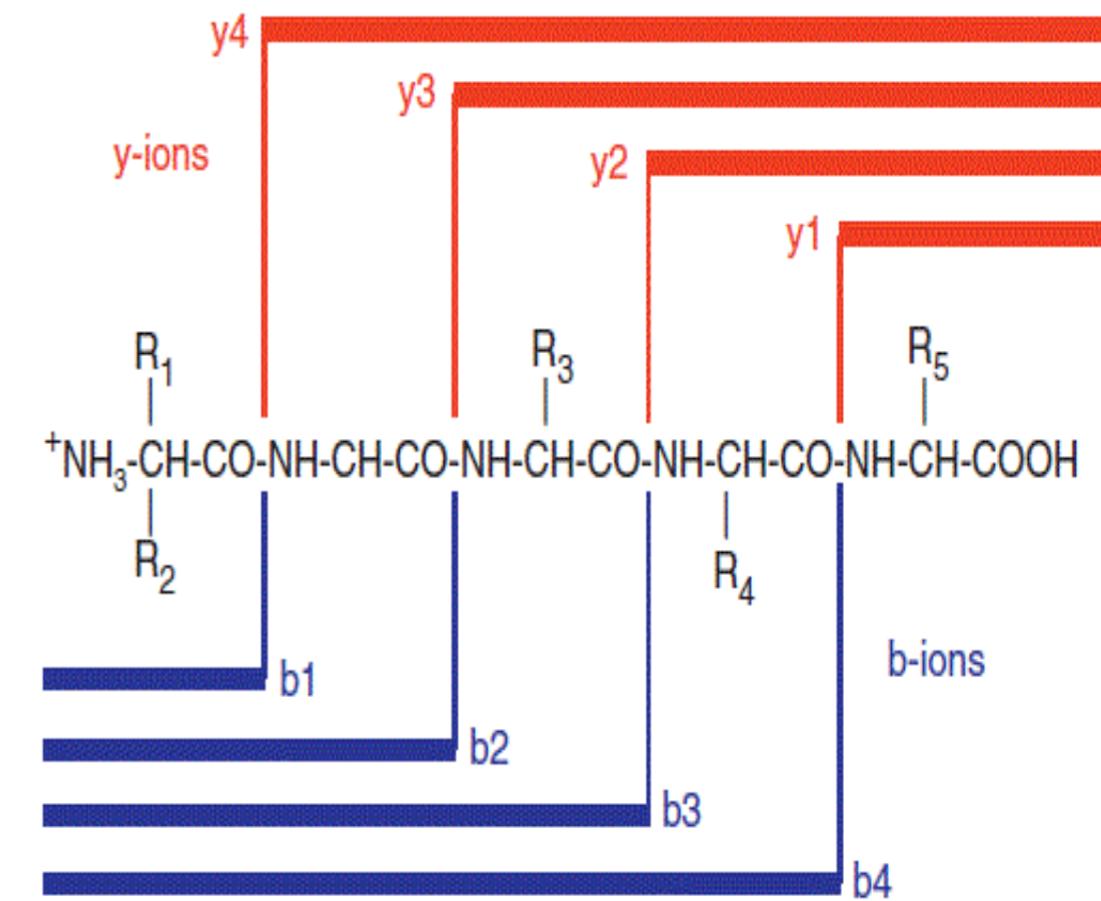
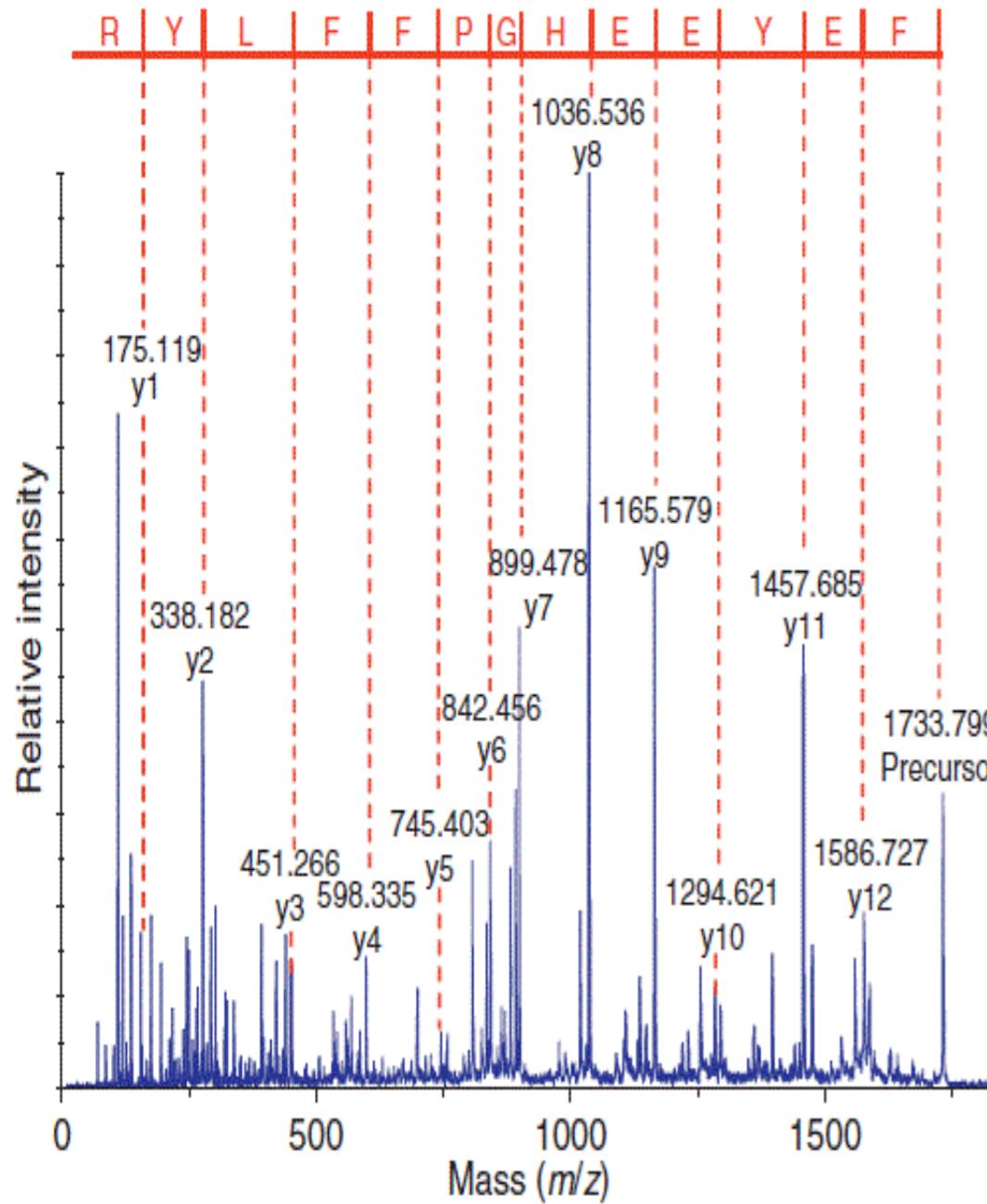
What is a m/z ratio?

Relative Abundance



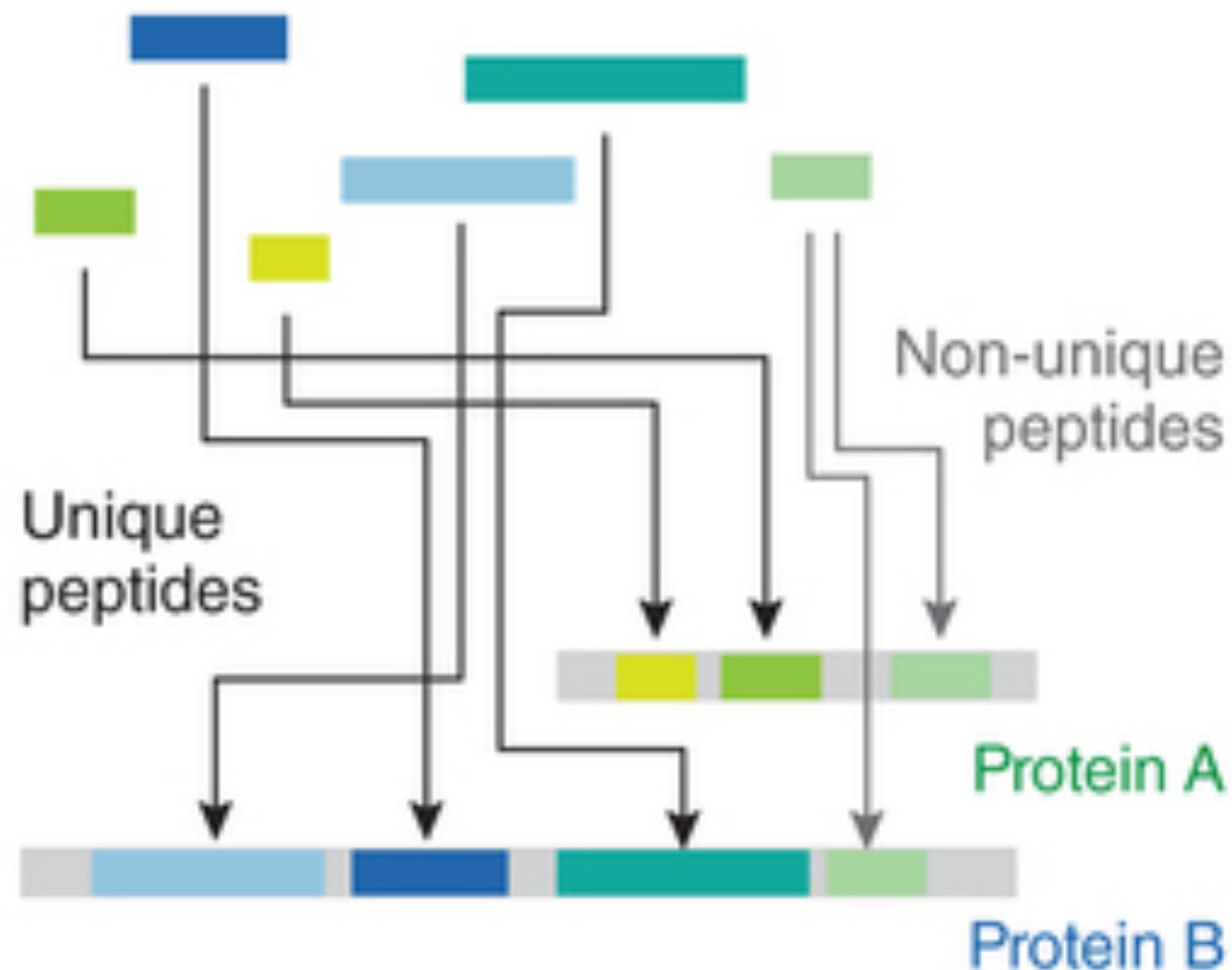
120 m/z-for singly charged ion this is the mass

How do we extract amino acid sequence from mass spec data?



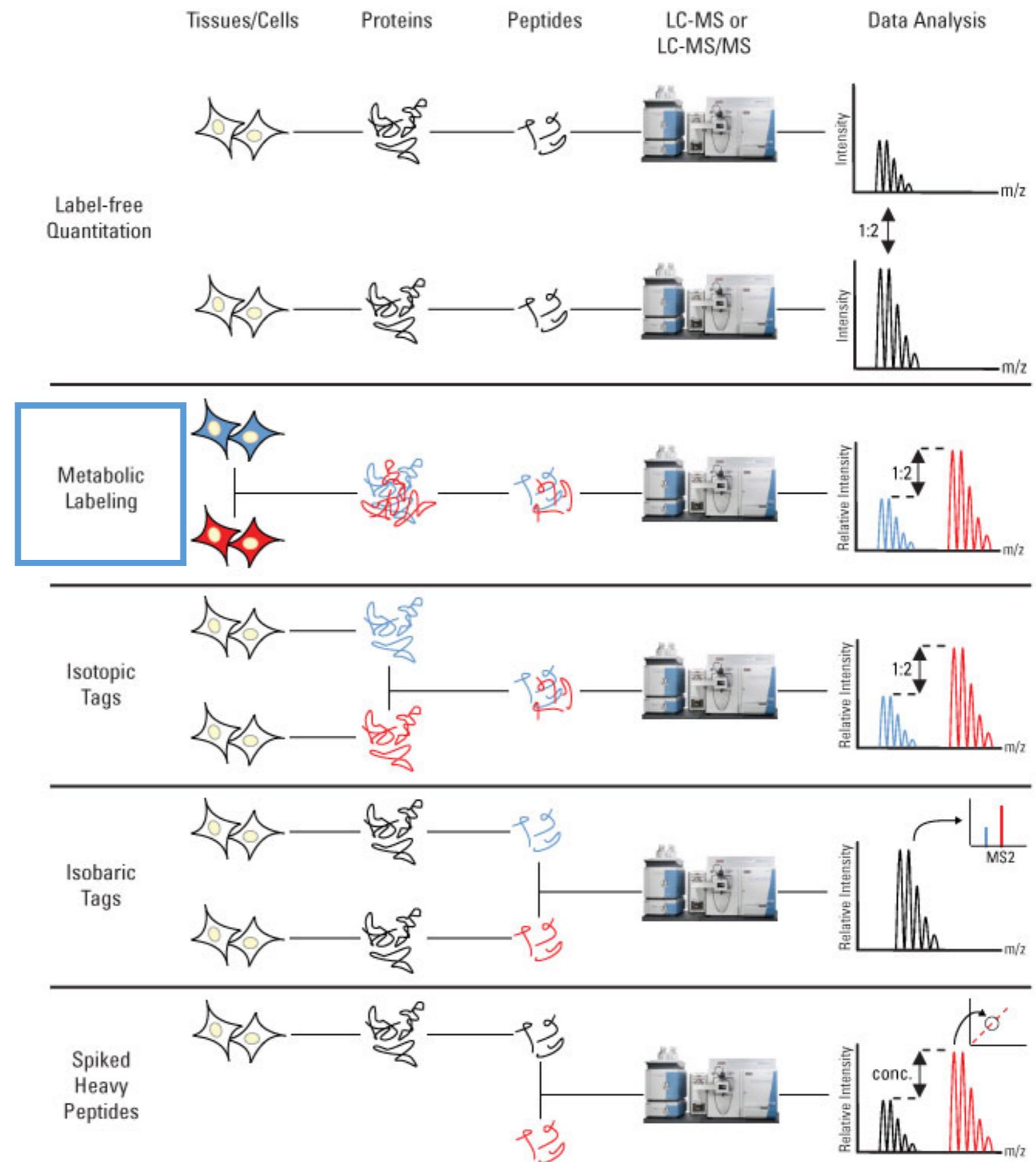
Visualization of peptide fragments

How do they determine which peptide belongs to which protein?



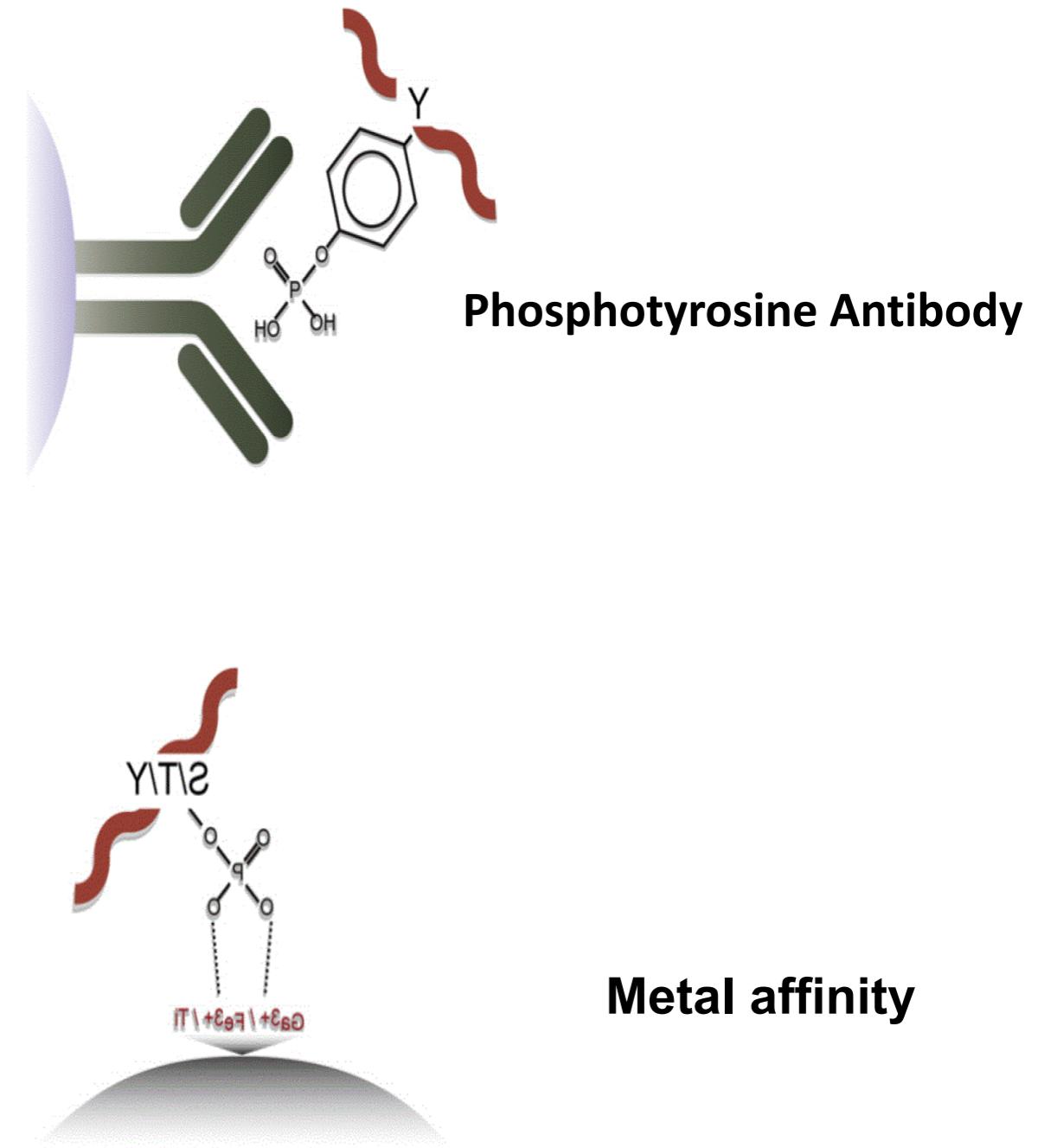
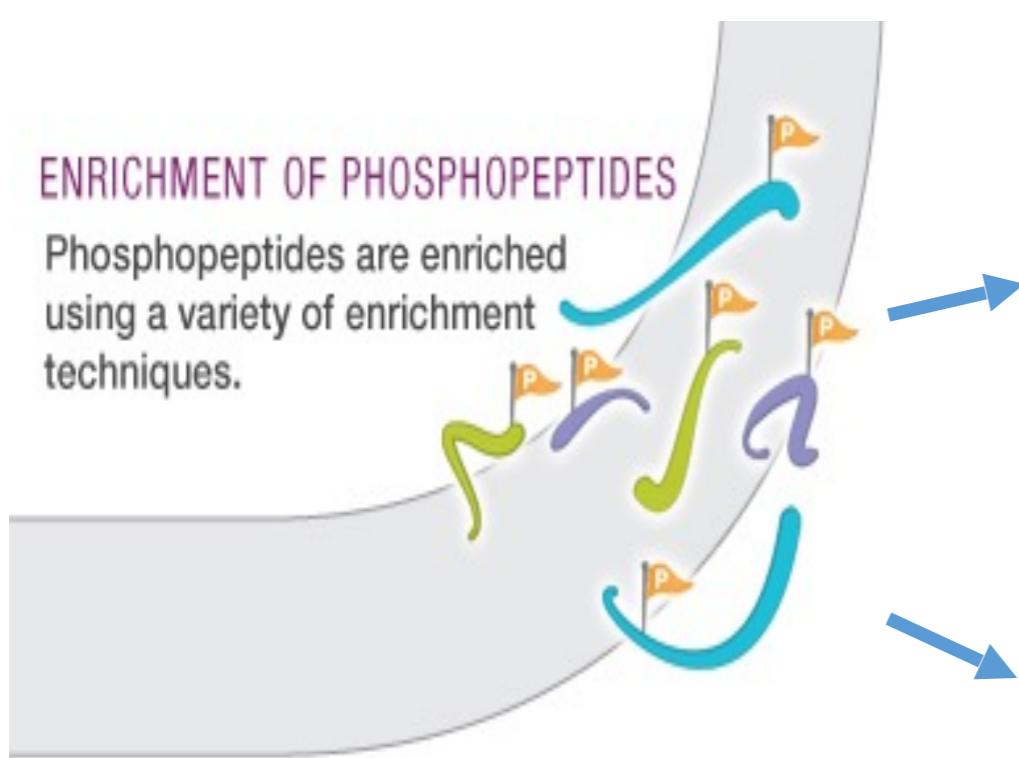
How are proteins regulated?

How can we quantify proteins?



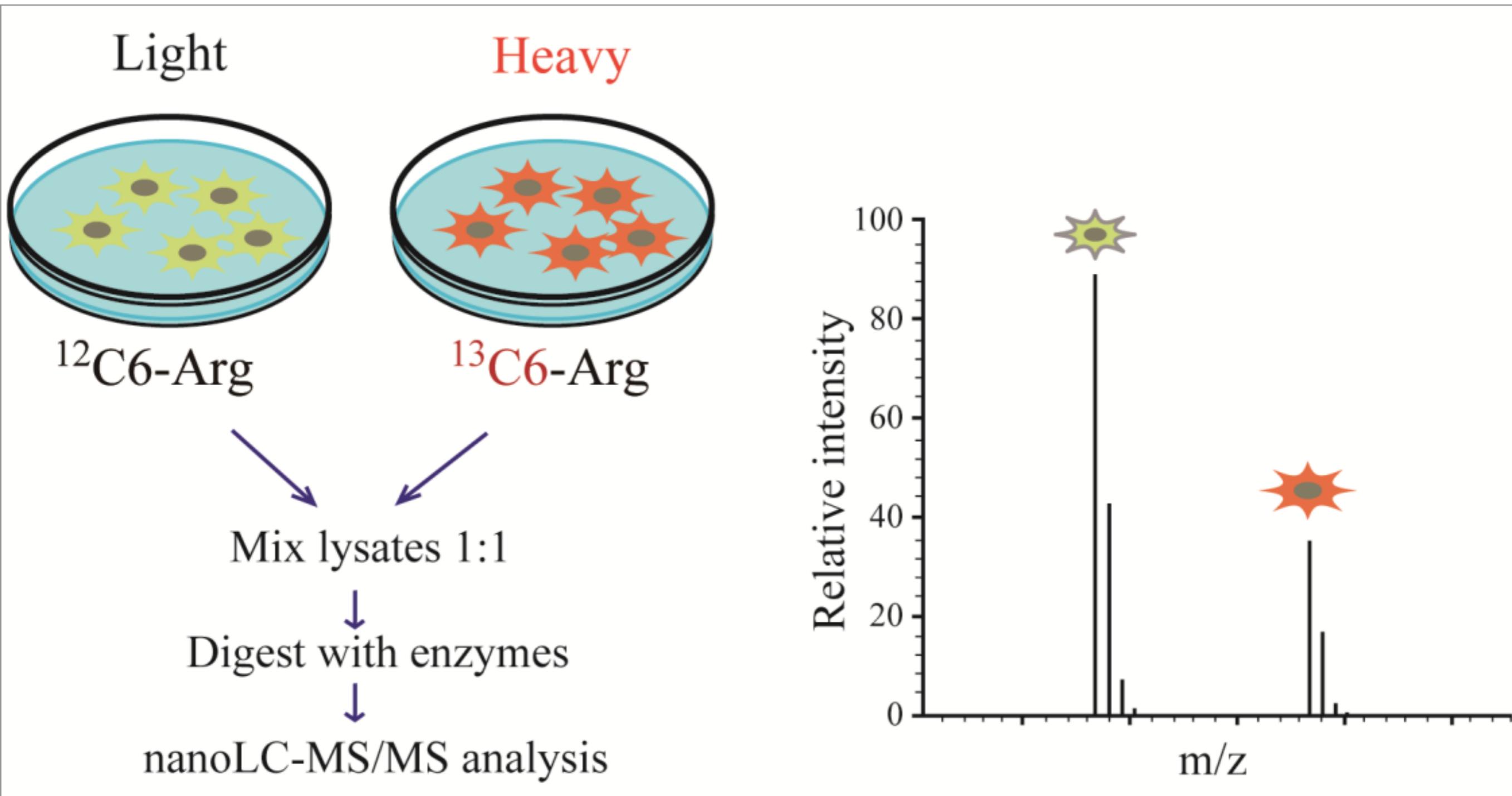
Which amino acids are phosphorylated?

How are samples prepared for phosphoproteomic analysis?



**Requires an additional step
that increase the amount of
phosphorylated peptides**

What is SILAC?



What are the advantages vs. disadvantages?

Purification step does not affect relative concentrations
Data analysis is easier
Not too expensive

Protein loss due to mass spec
Works only in cell culture
Preparation more complicated

High-throughput phosphoproteomics reveals in vivo insulin signaling dynamics

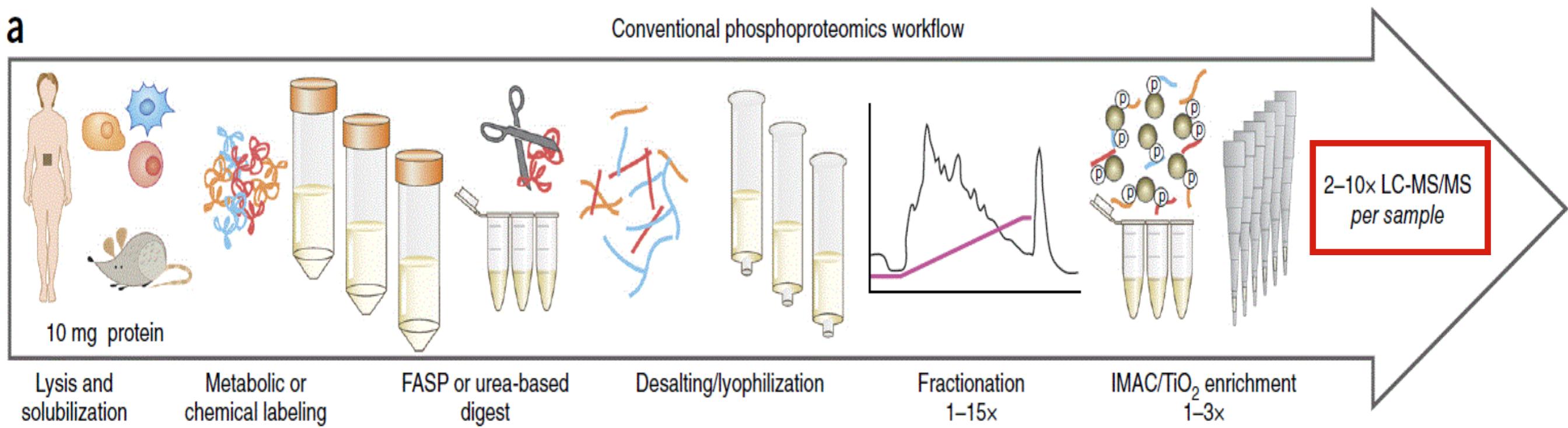
Sean J Humphrey, S Babak Azimifar & Matthias Mann
Nature Biotechnology
August 2015

What is cell signaling?

What is the purpose of insulin?

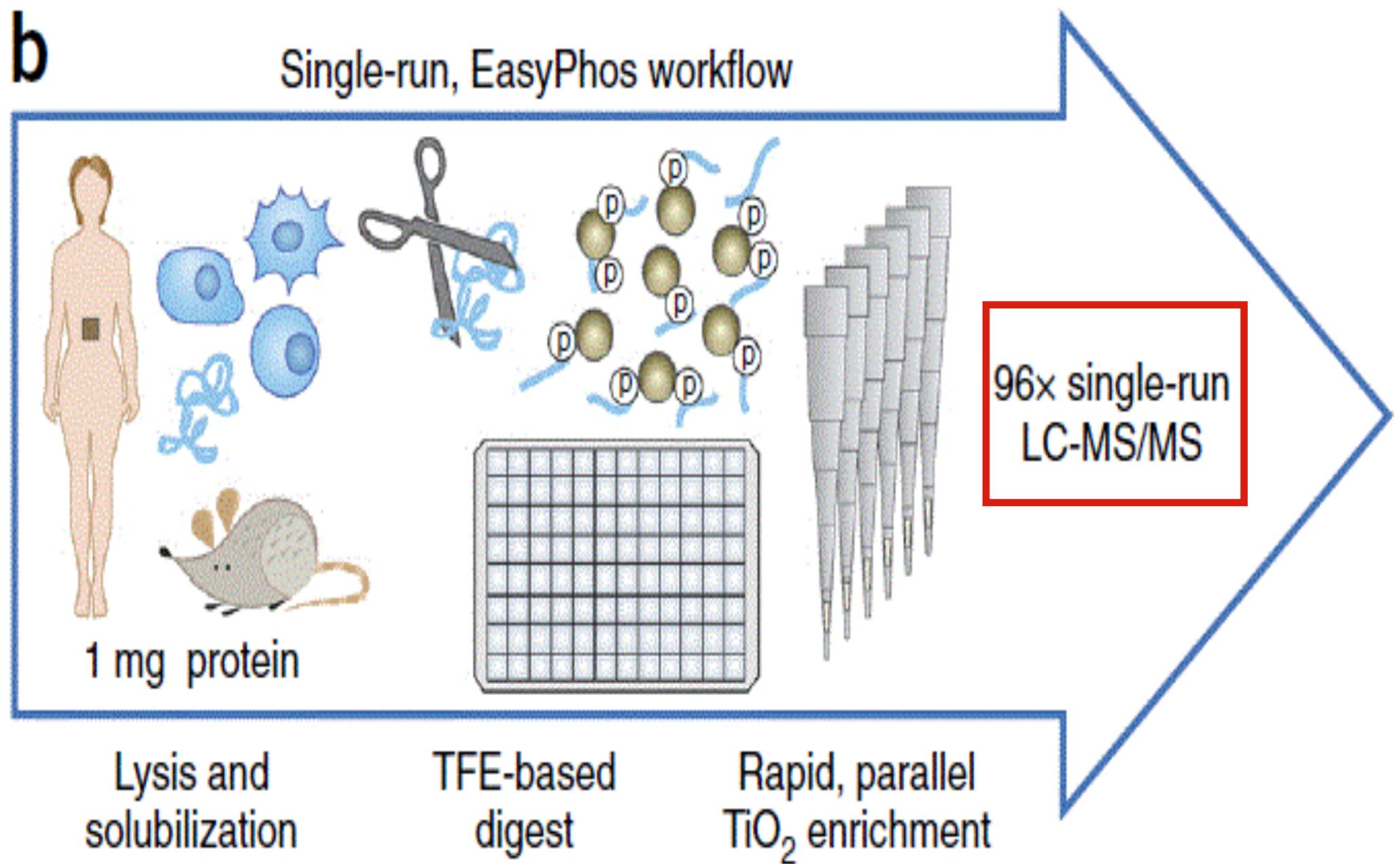
What were the steps of past phosphoproteomics?

a



**Very time consuming and meticulous
Many steps and more materials involved**

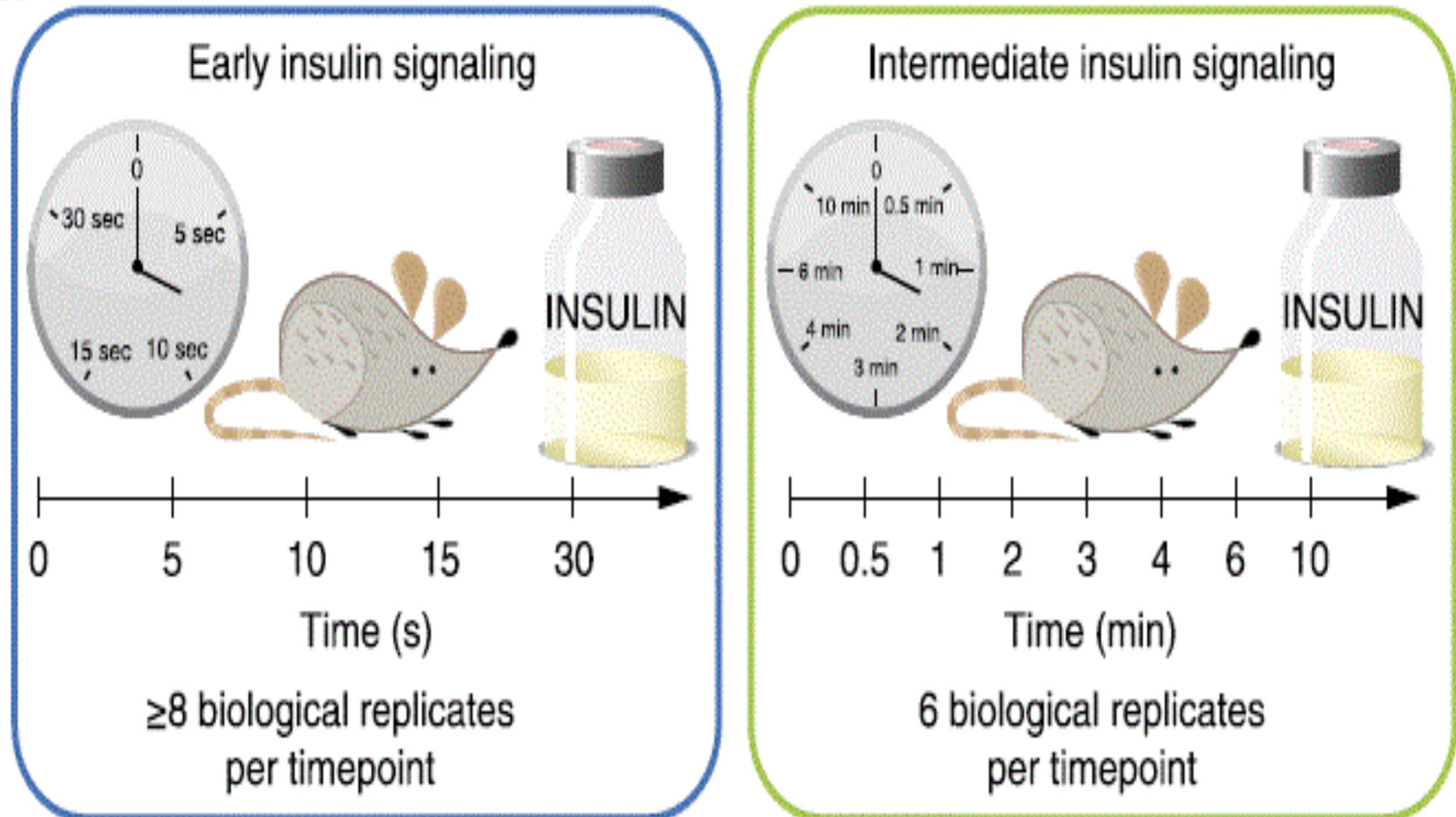
What makes EasyPhos a better technique?



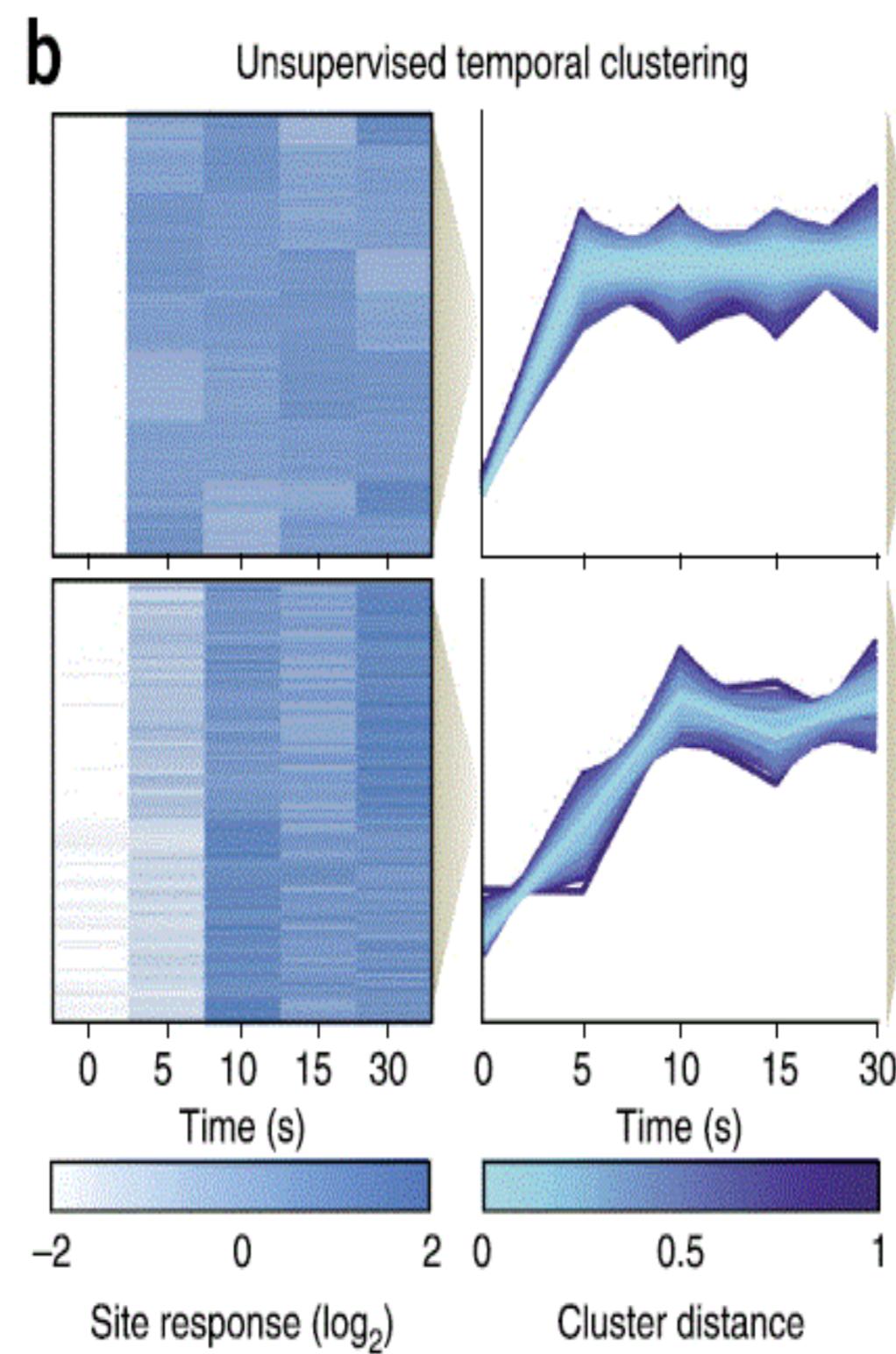
Few steps that allow for faster production

Fig. 2a: How did the response to insulin change?

a



Are the phosphorylation events linked?

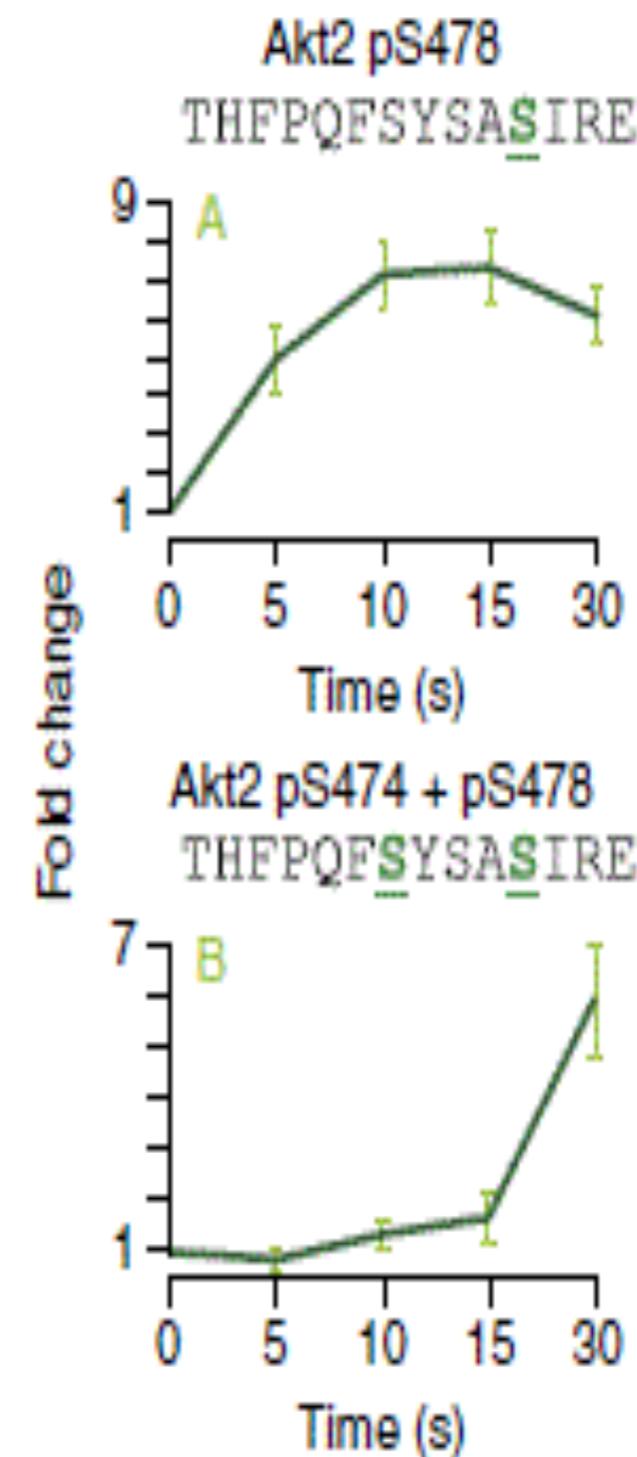
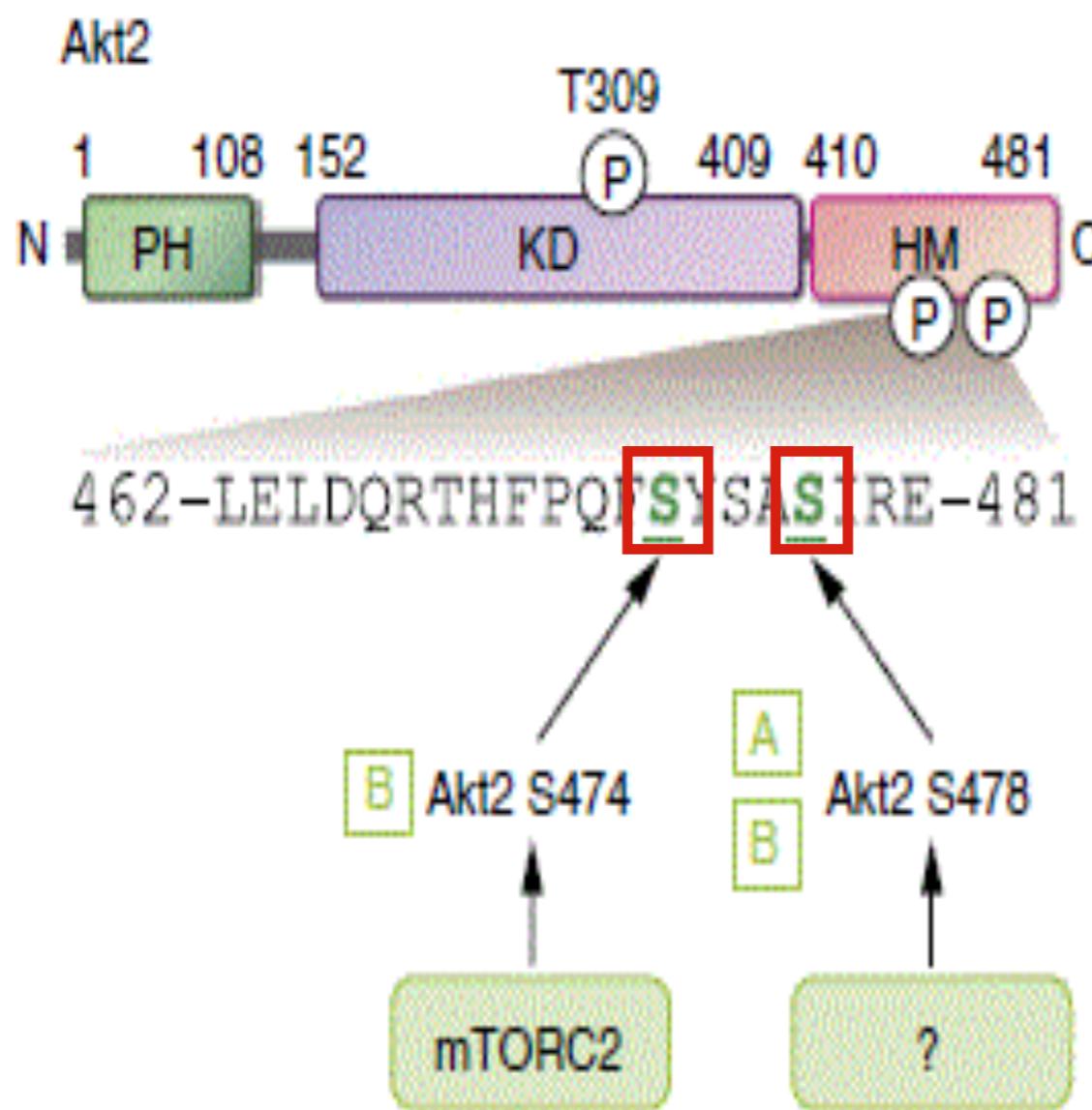




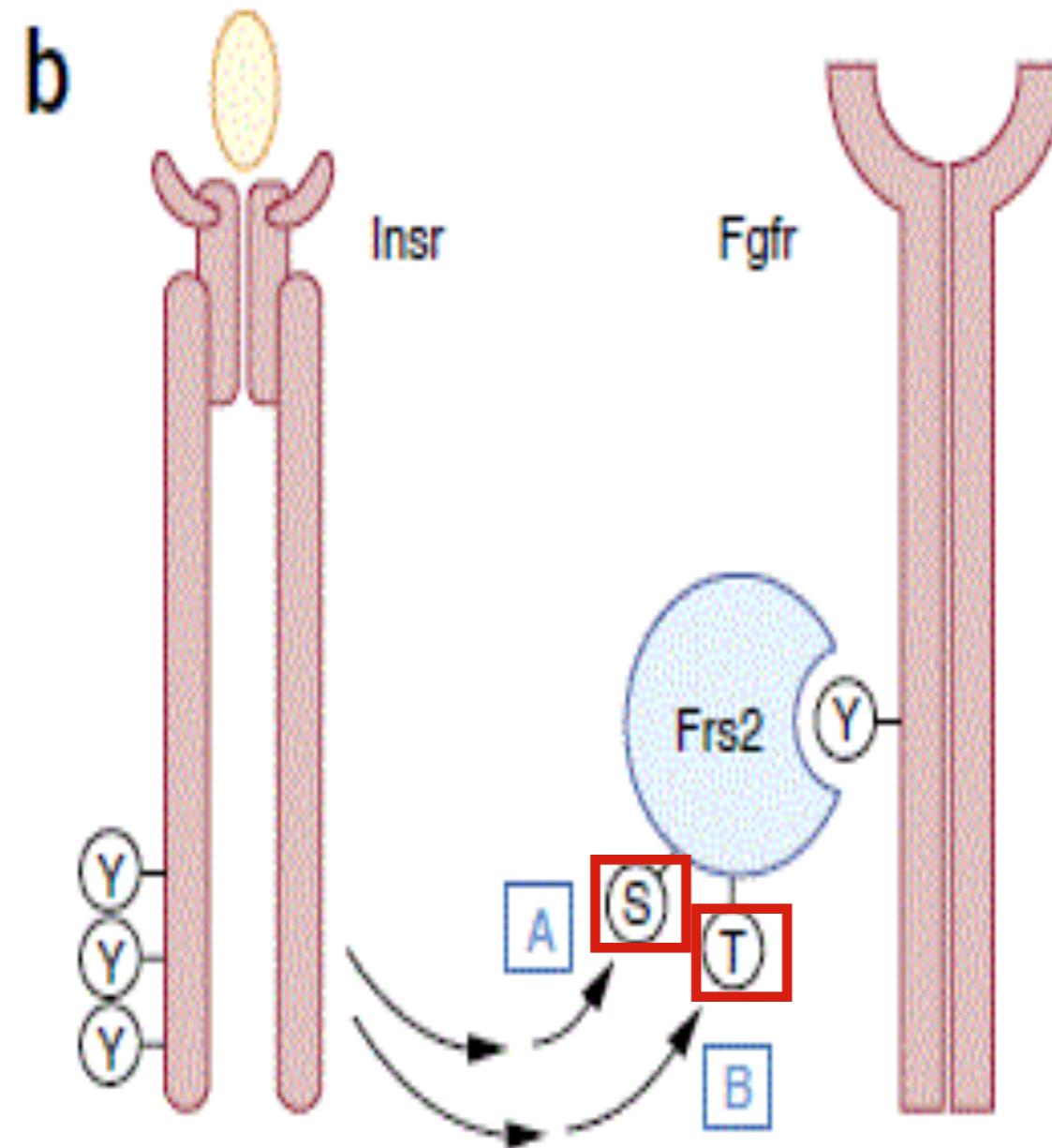
What did they
find?

How long did it take for Akt2 to respond?

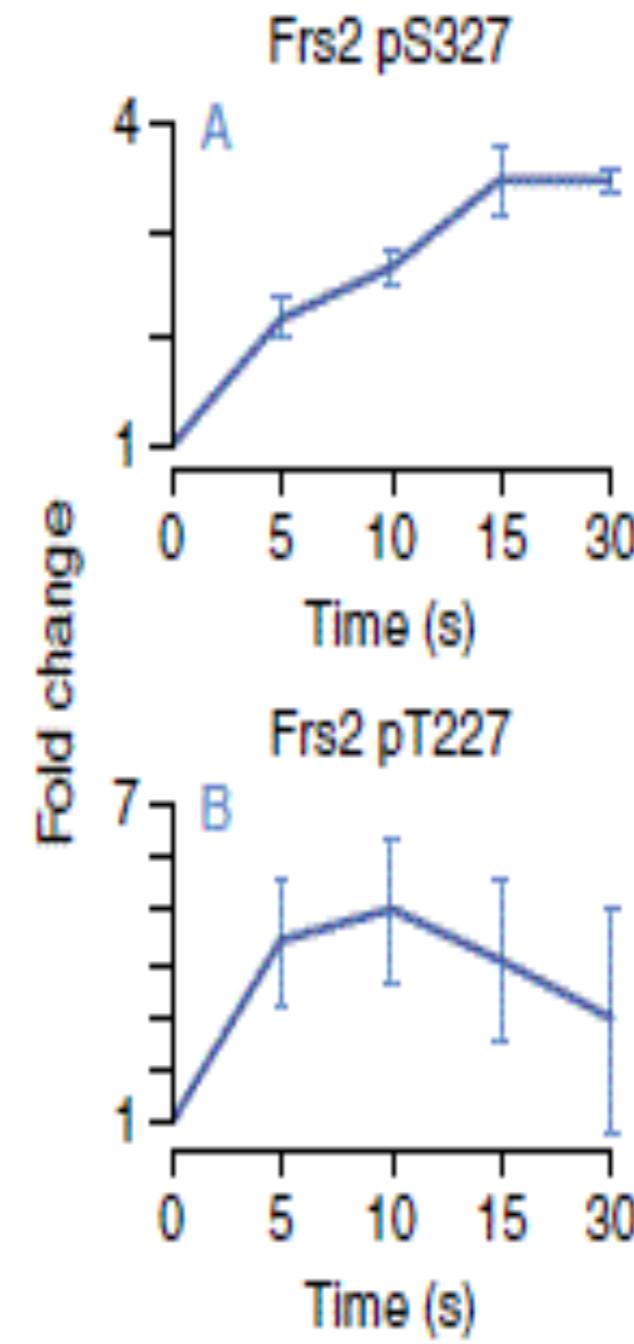
a



What is the rate of phosphorylation on Frs2?

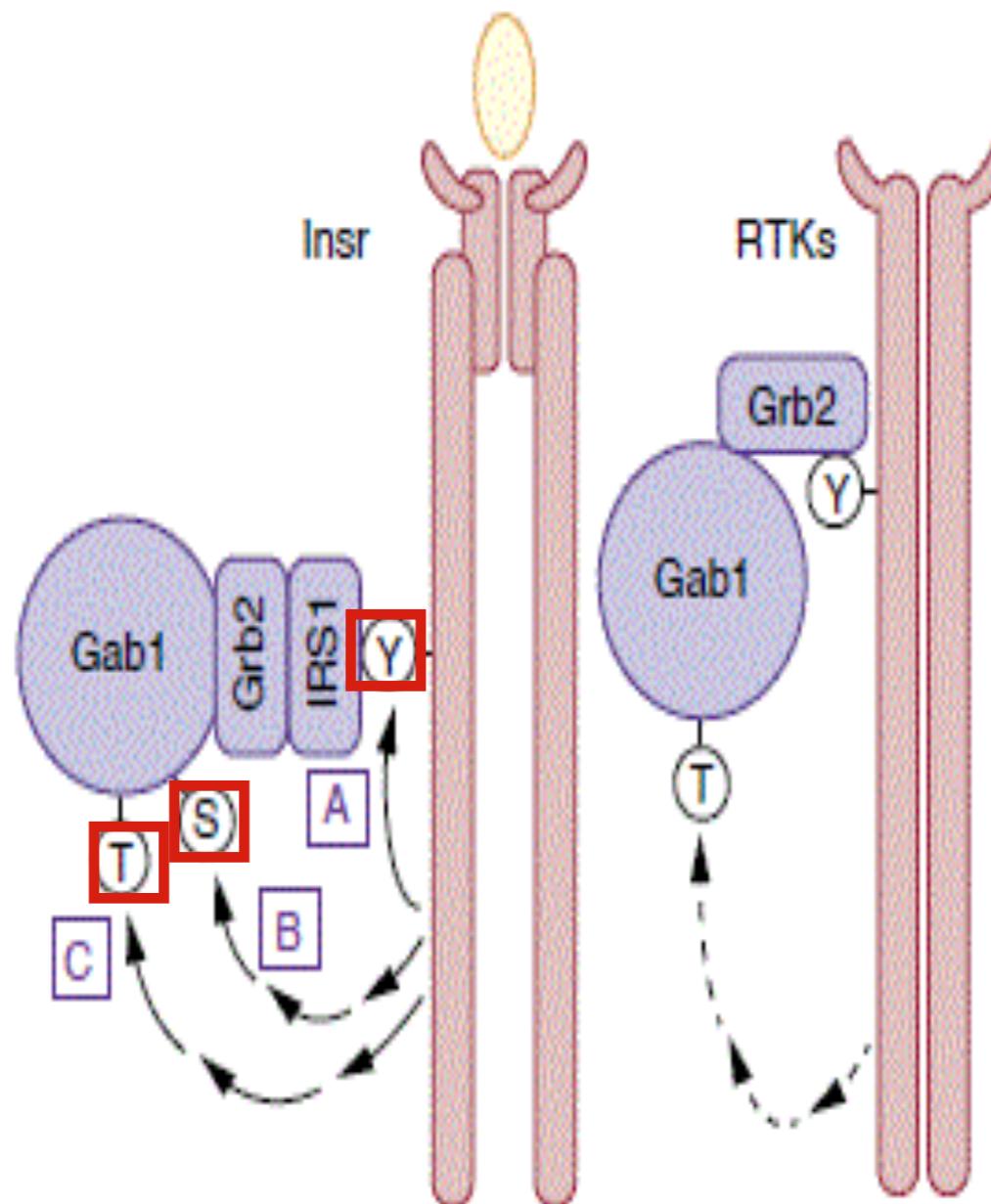


Unknown Intermediates

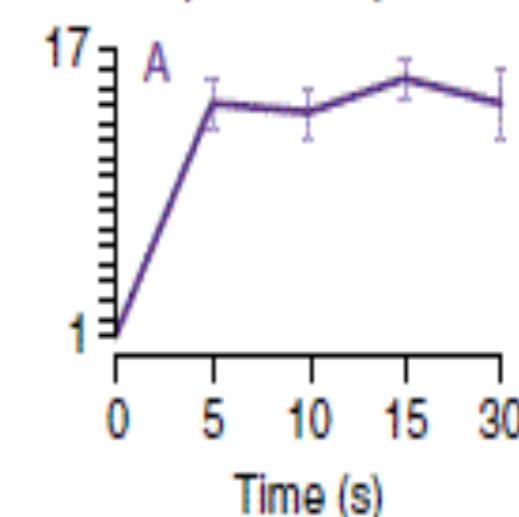


What is the rate of Gab1 Insulin-regulated phosphorylation?

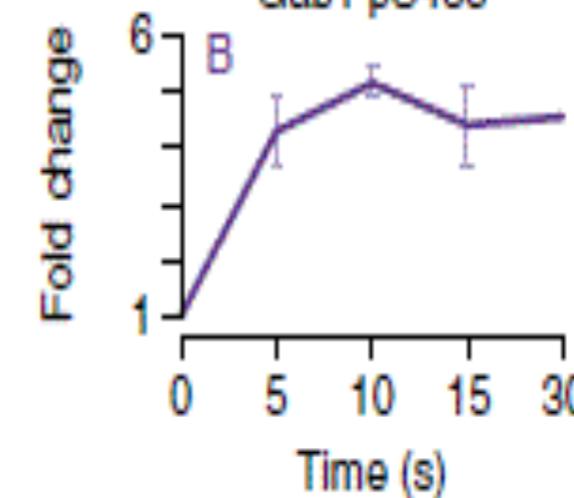
C



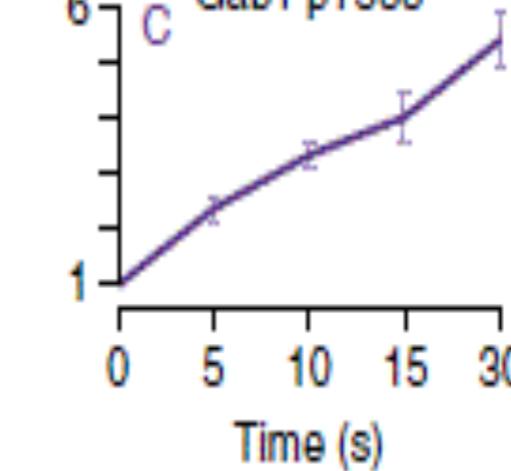
Insr pY1175 + pY1179



Gab1 pS455

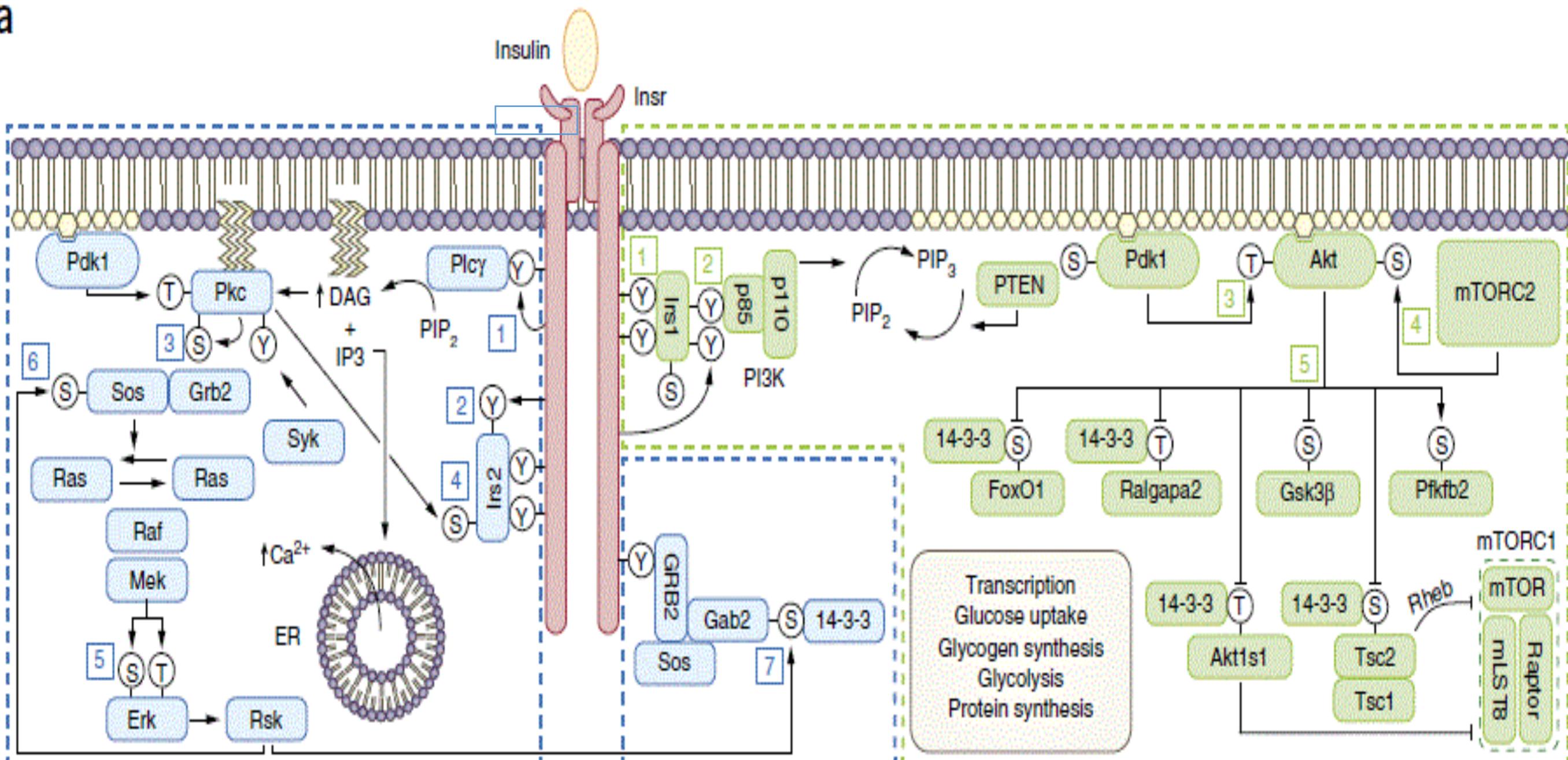


Gab1 pT388



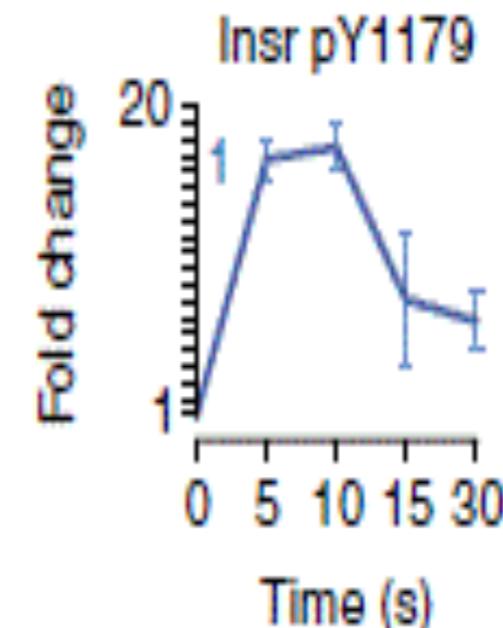
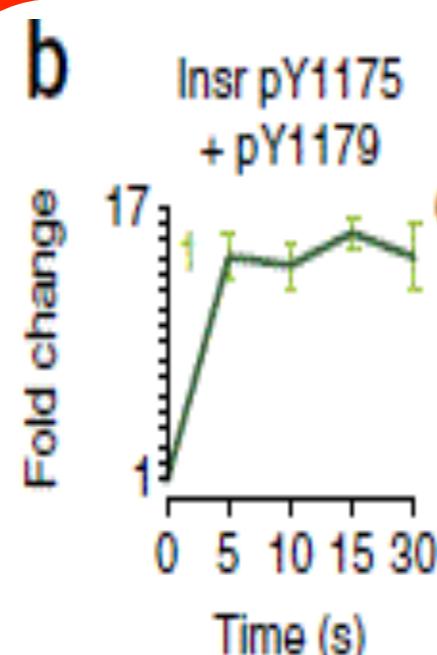
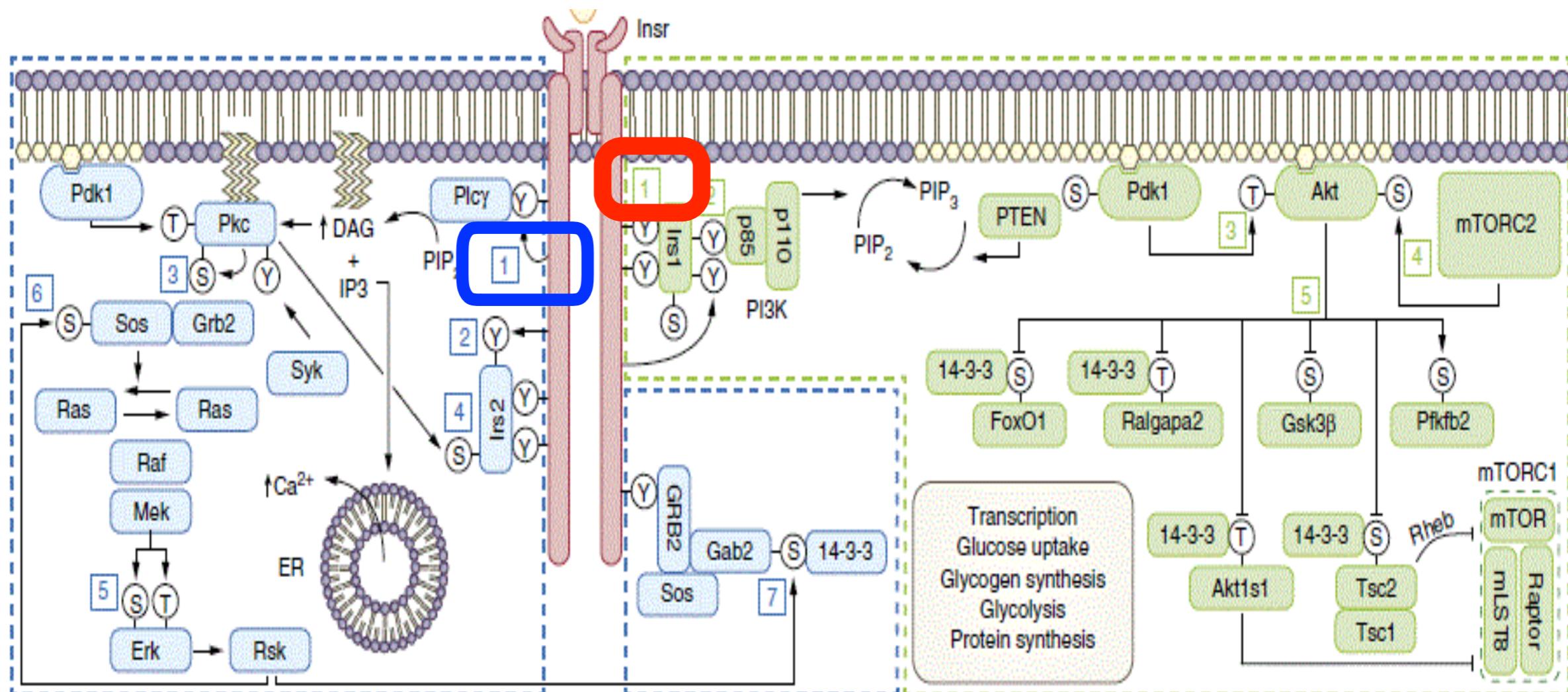
Where are the phosphorylation events once treated with insulin?

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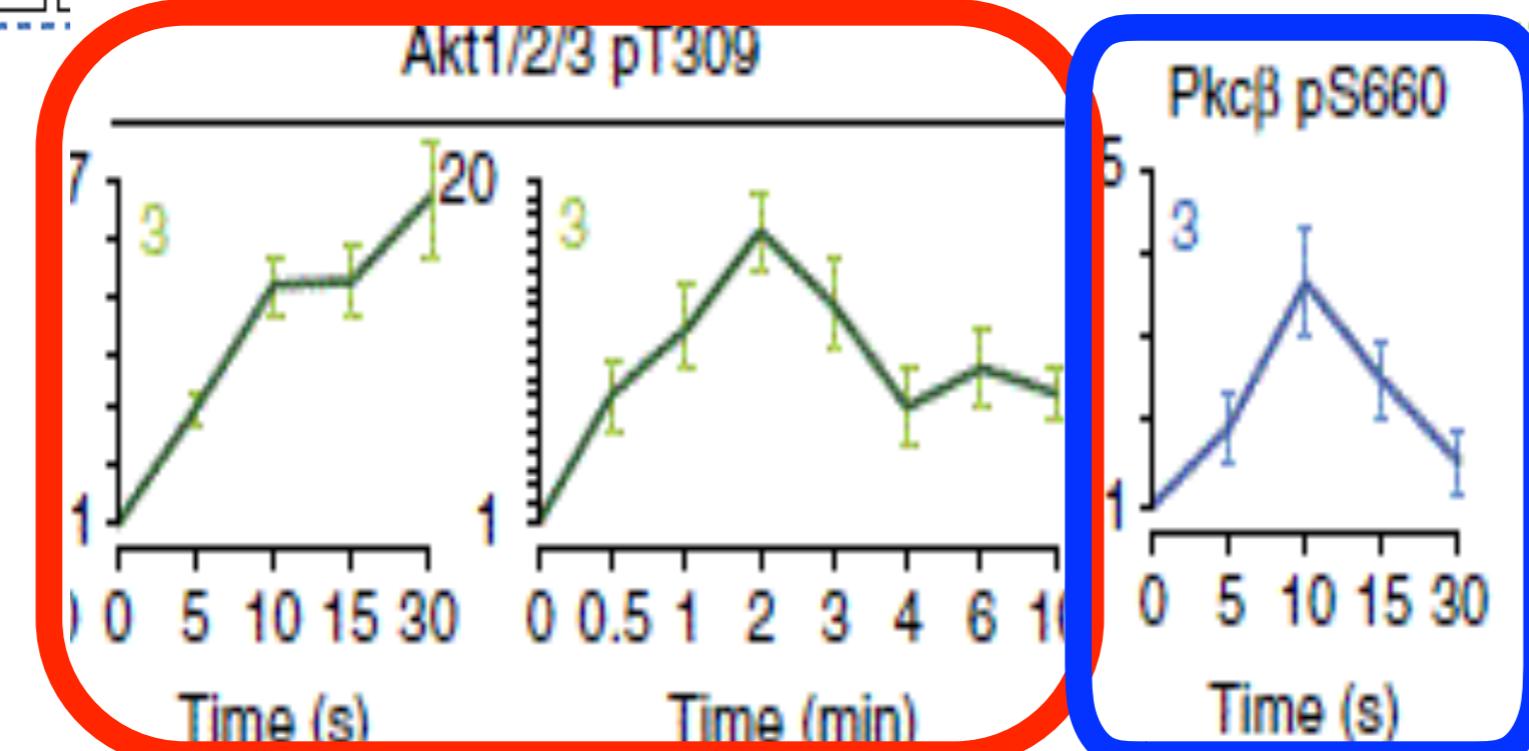
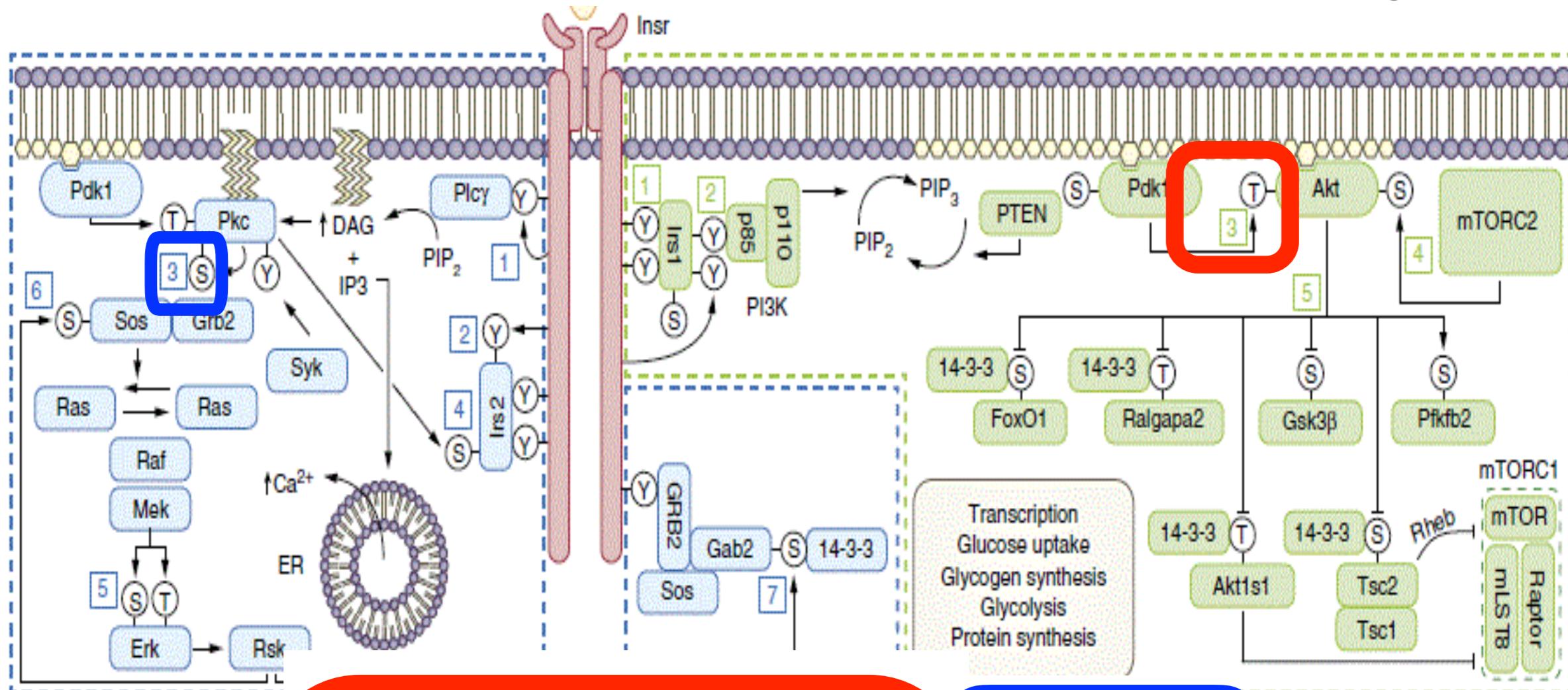
Members of the pathway that are activated once treated with insulin

How does phosphorylation compare on both sides of the Insulin Receptor?

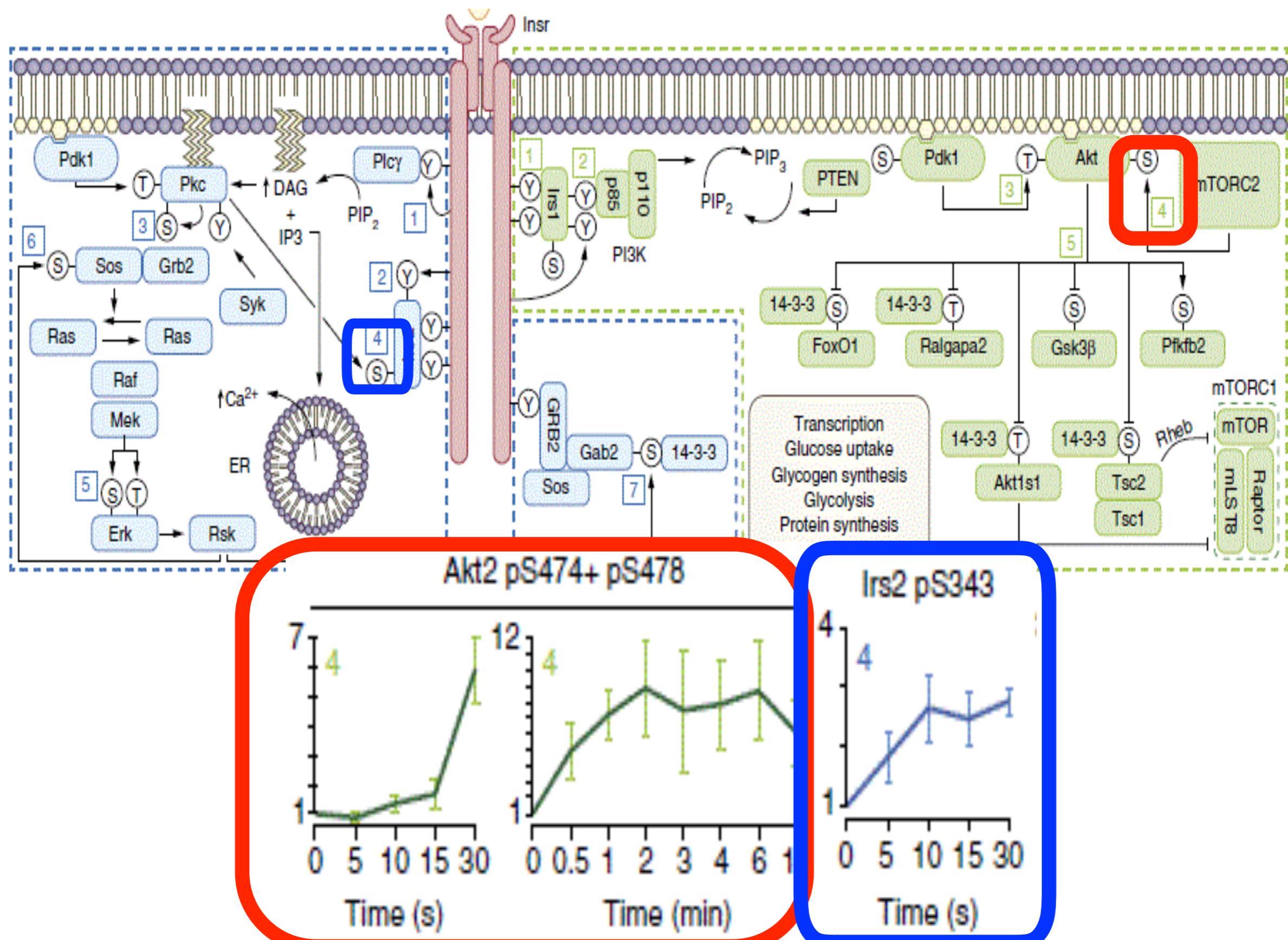


put your

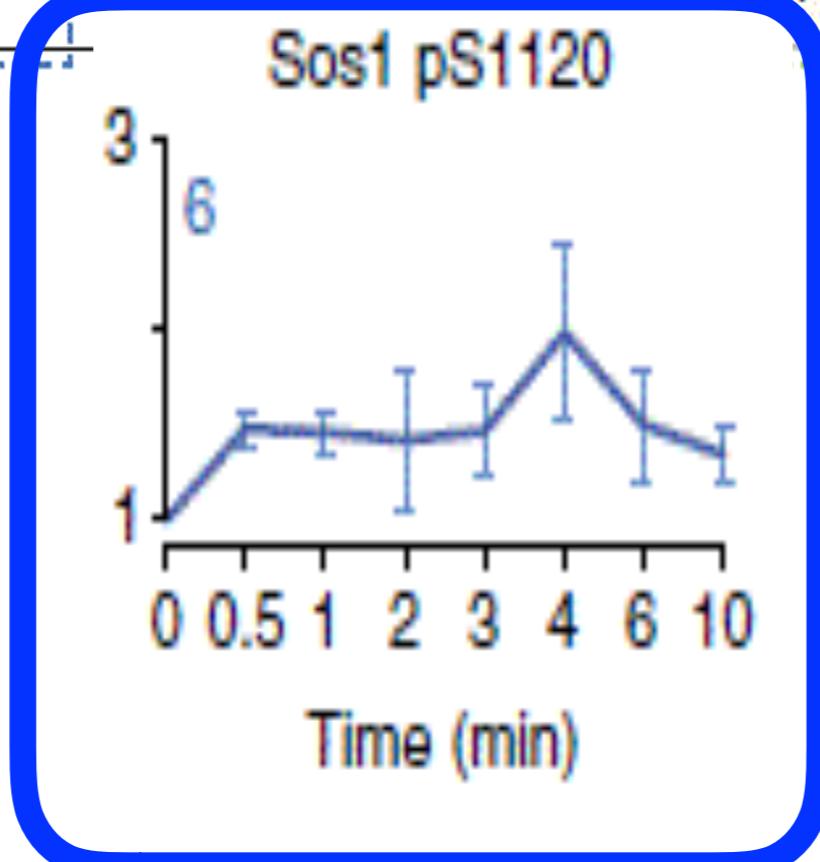
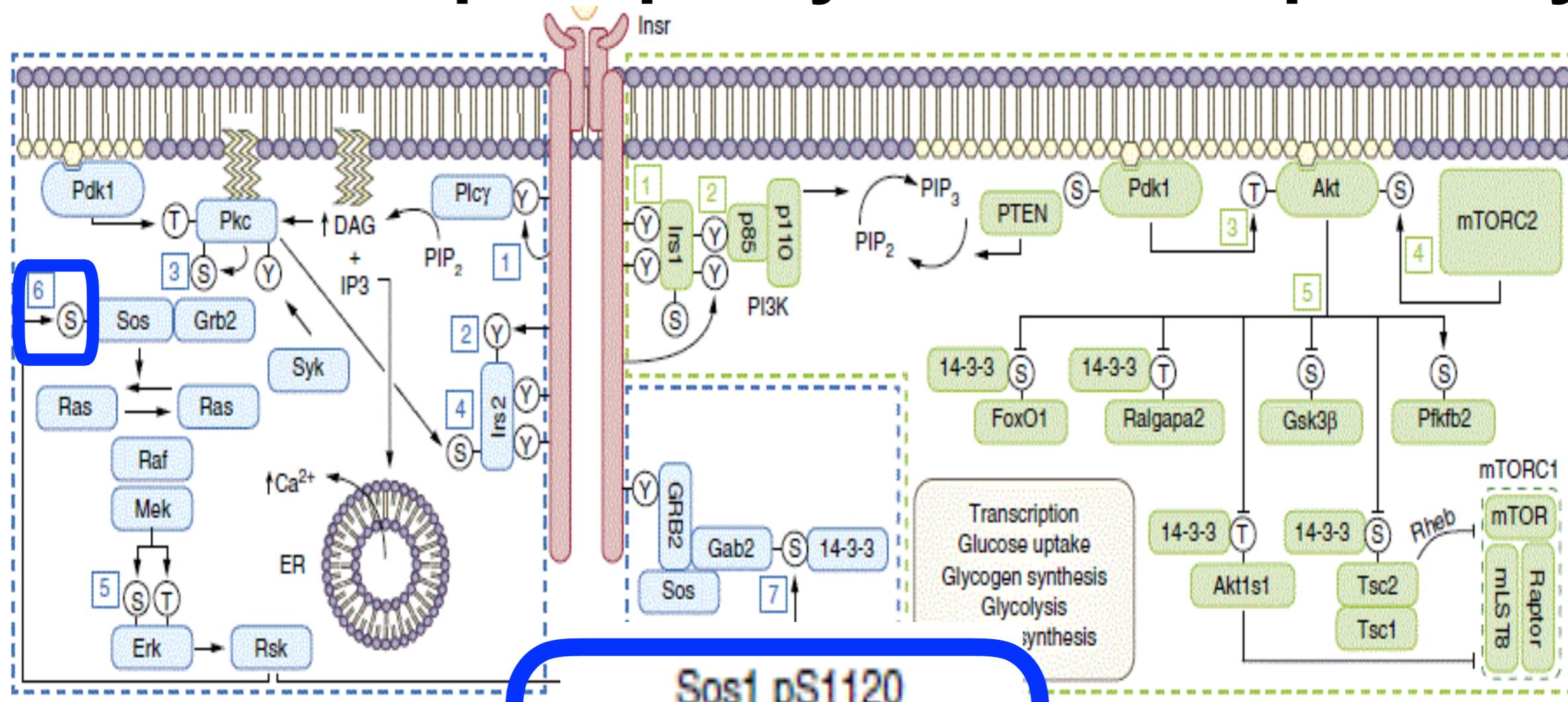
How fast are Akt1 and PkcB phosphorylated?



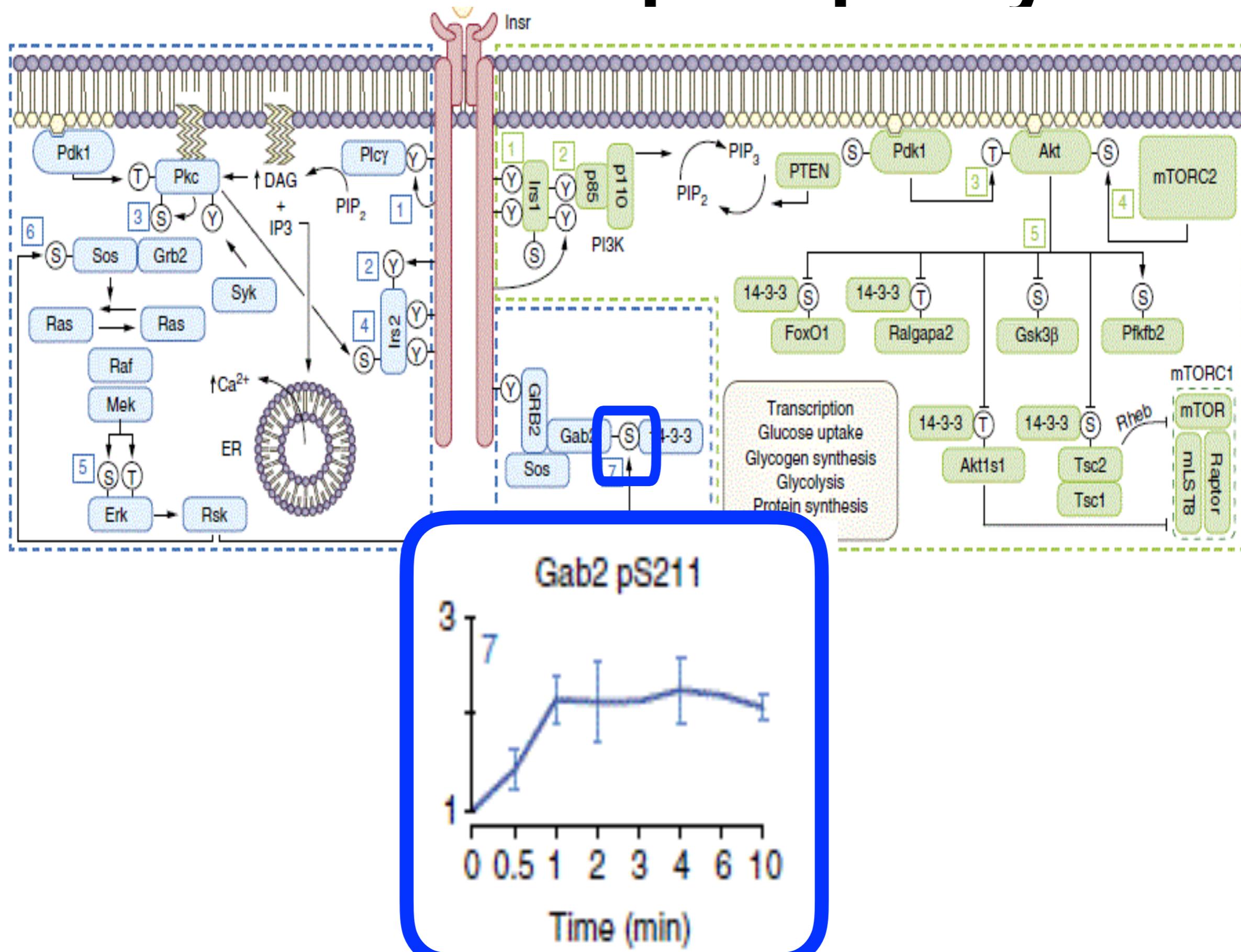
How does phosphorylation compare at multiple sites on Akt2?



When is Sos1 phosphorylated in the pathway?



What rate is Gab2 phosphorylated?



Summary

EasyPhos Technology for determining cell signaling networks

Time scale of phosphorylation events

Insulin Regulated Phosphorylation Pathway

A close-up photograph of a baby with light brown hair and blue eyes. The baby has a neutral to slightly grumpy expression, with thin eyebrows and a small frown. They are wearing a light blue t-shirt with a green collar and a small white tag or piece of fabric hanging from the neckline. The background is a soft-focus outdoor scene, possibly a beach or coastal area.

I'M DONE

GOT ANY QUESTIONS?