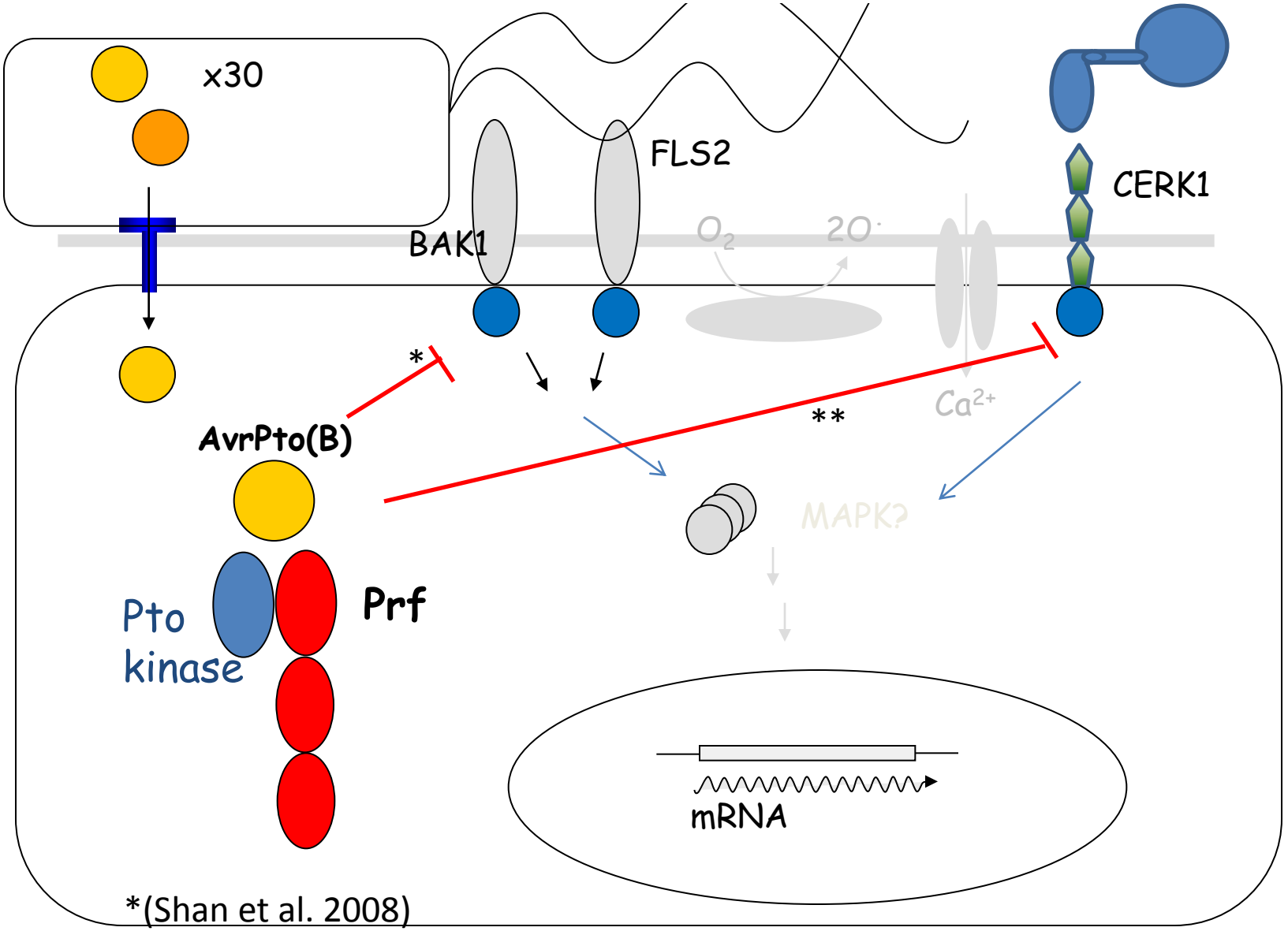
Moving from identification to quantification of phosphorylation events in plant-pathogen interactions

Effector triggered susceptibility and immunity

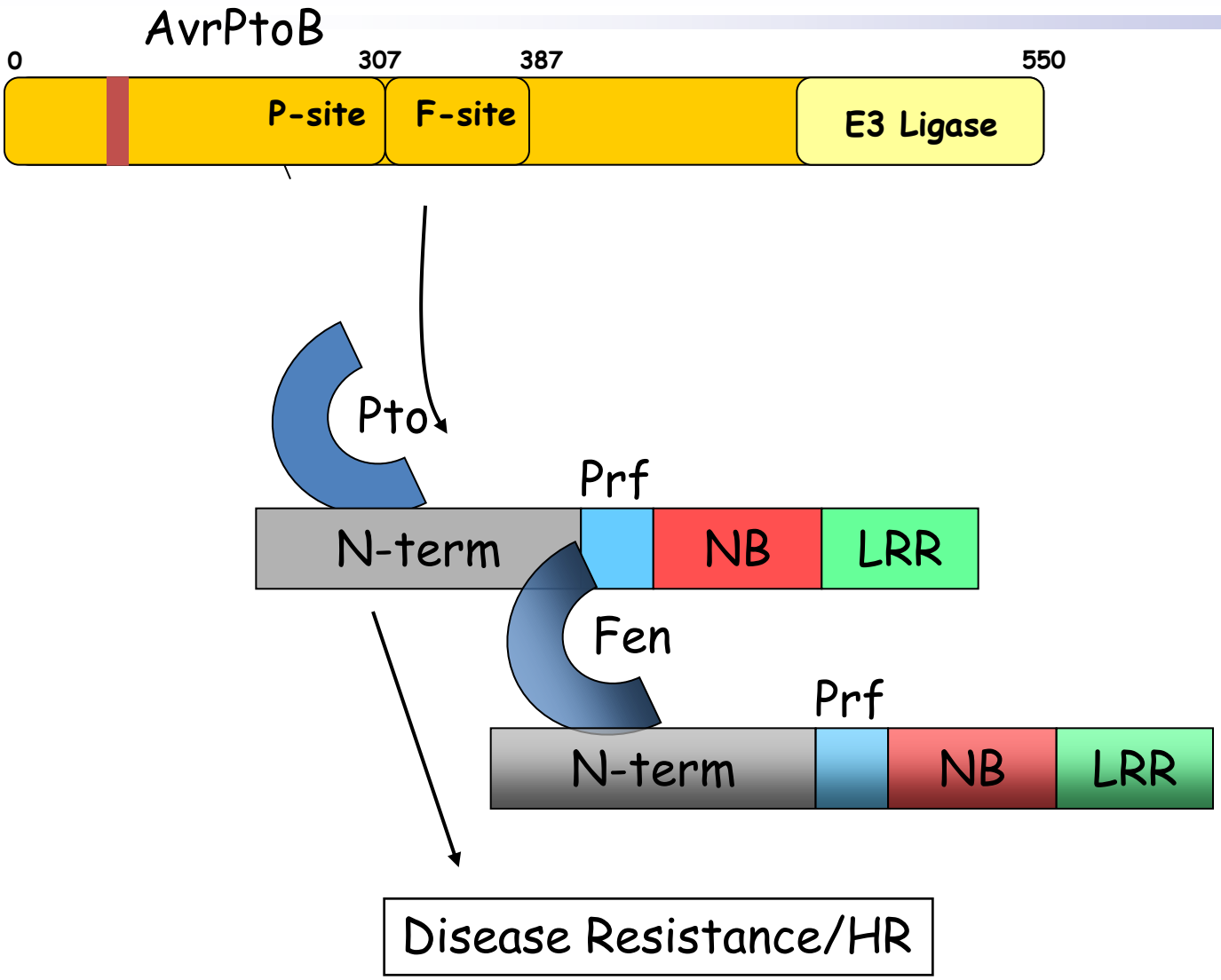


*(Shan et al. 2008)

**Gimenez-Ibanez et al Current Biol. 2009

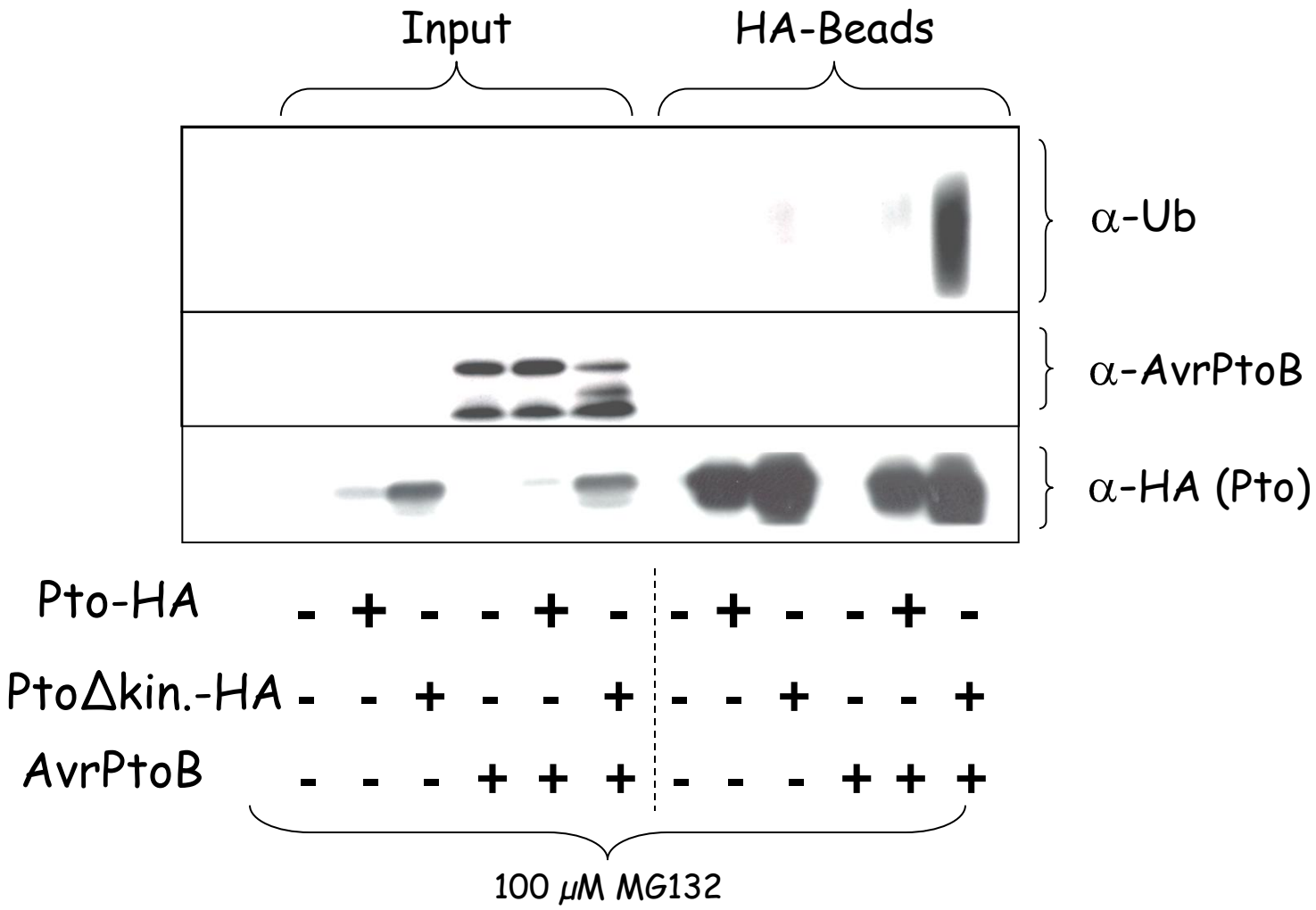
With thanks to John Rathjen for slide

Signal transduction of AvrPtoB



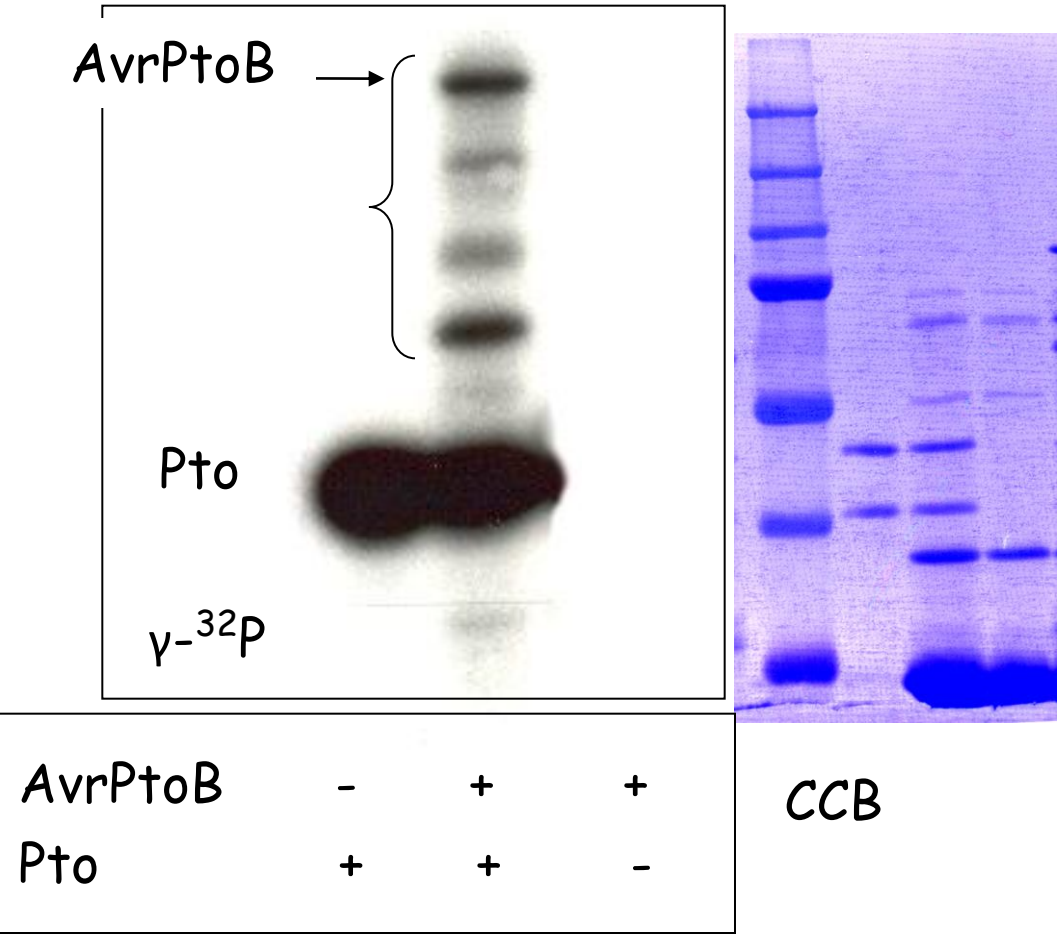
Guttierez-Pulgar Plant J. 2009

AvrPtoB ubiquitinates kinase-dead Pto

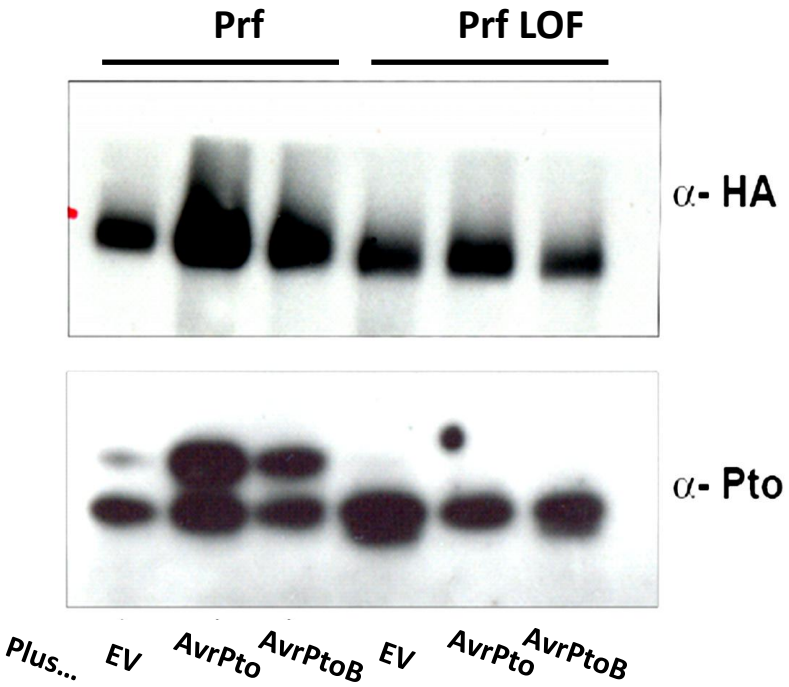


Ntoukakis et al. Science 2009

1. Pto phosphorylates AvrPtoB



2 .Pto is phosphorylated after elicitation



Ntoukakis et al. Science 2009

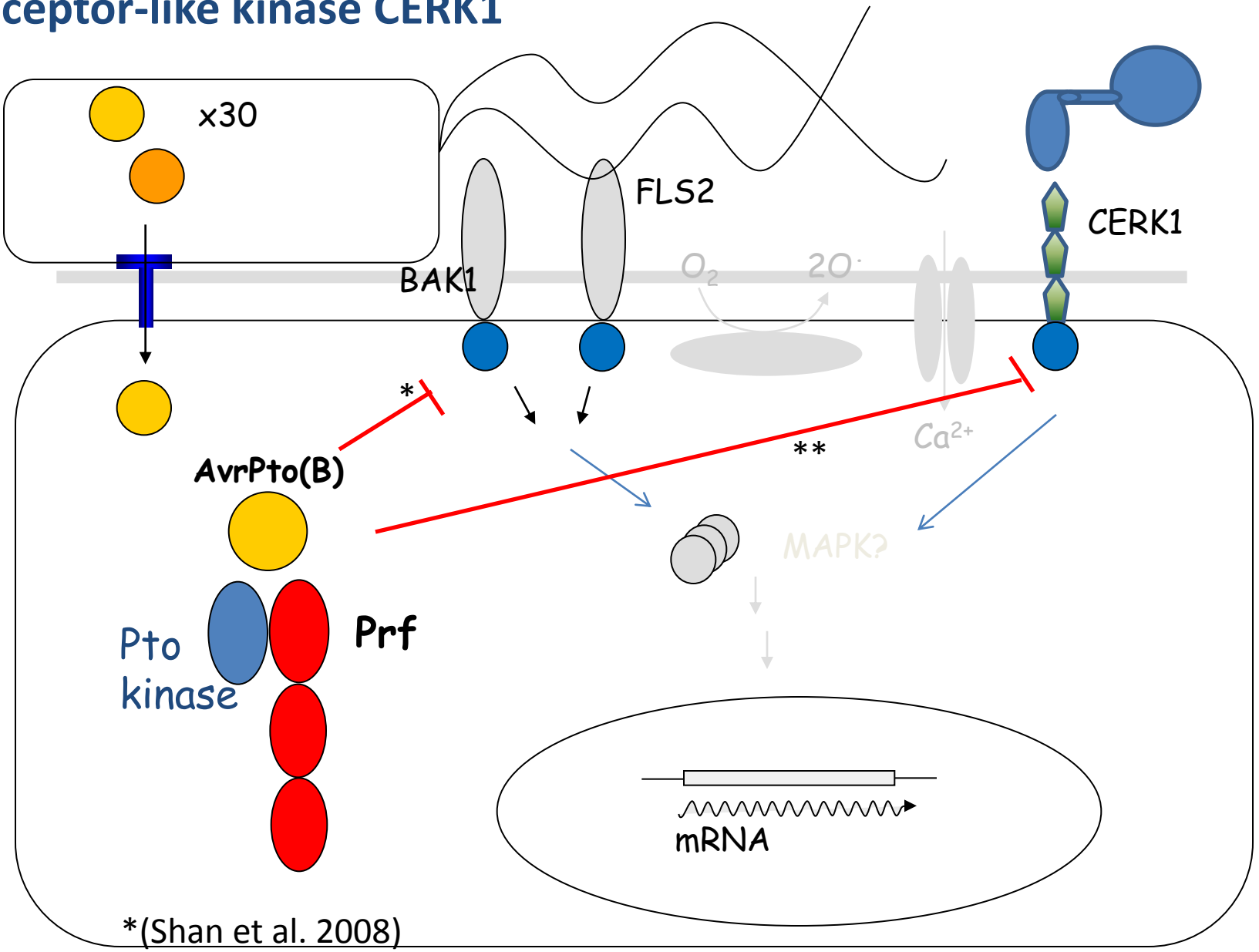
N. benthamiana

Modifications on Pto kinase

Proteins: AvrPtoB (purple) Pto pink/yellow.
Space fill: Active site red, ‘ubiquitination’ site (fen), P-loop residues blue, alternative phosphorylation sites green

Based on model 2qkw by Xing et al. Nature 2007

Receptor-like kinase CERK1

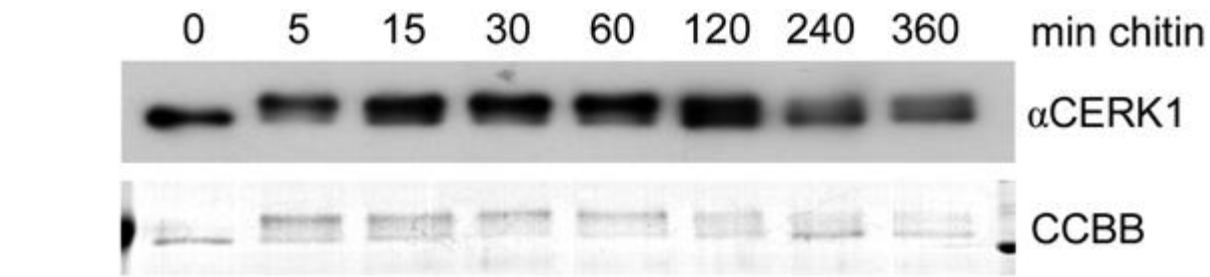


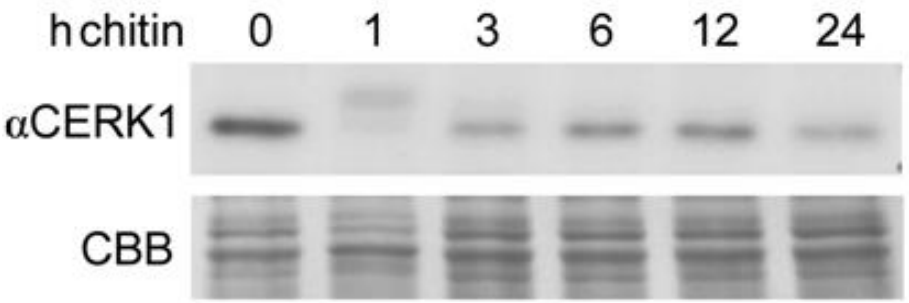
*(Shan et al. 2008)

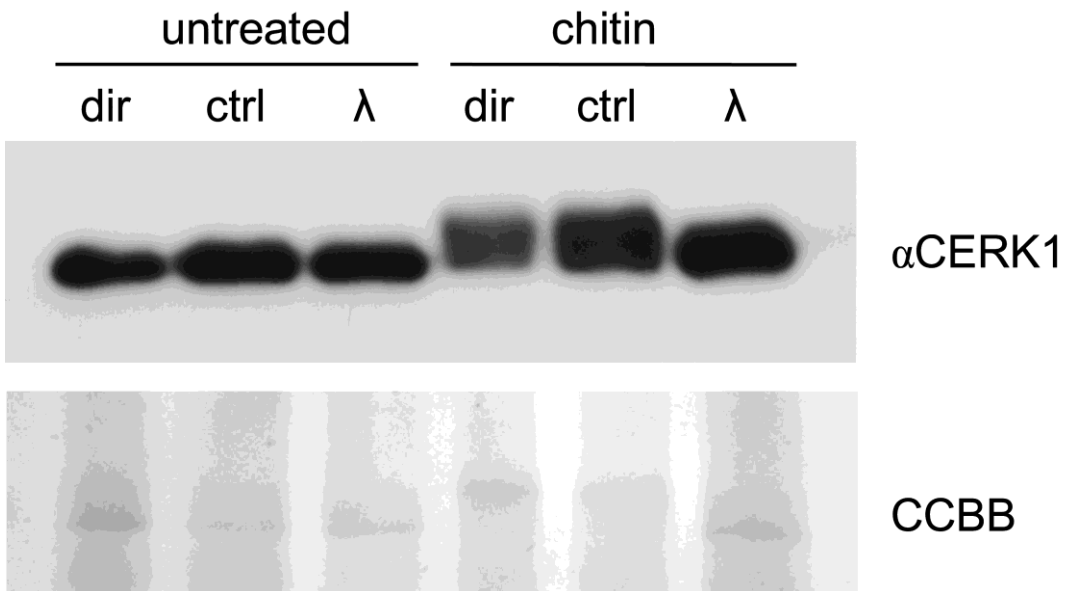
**Gimenez-Ibanez et al Current Biol. 2009

With thanks to John Rathjen for slide

Treatment with chitin induces a transient band-shift







Elena Petutschnig

Identification of constitutive and induced phosphorylation

‘Quantified‘ by spectral counts

				dephosphorylated with λ-phosphatase	
	Phosphoresidue	control	chitin	control	Chitin
SKGDSFSSSIPLSTK	S266	7	15	1	4
	S268	0	2	0	0
	S270	0	1	0	0
	S274	0	4	0	0
GAVVK[oxM]TEAVGEFR	T519	0	4	0	0

Two weak points in this analysis:

quantification

position of phosphorylation

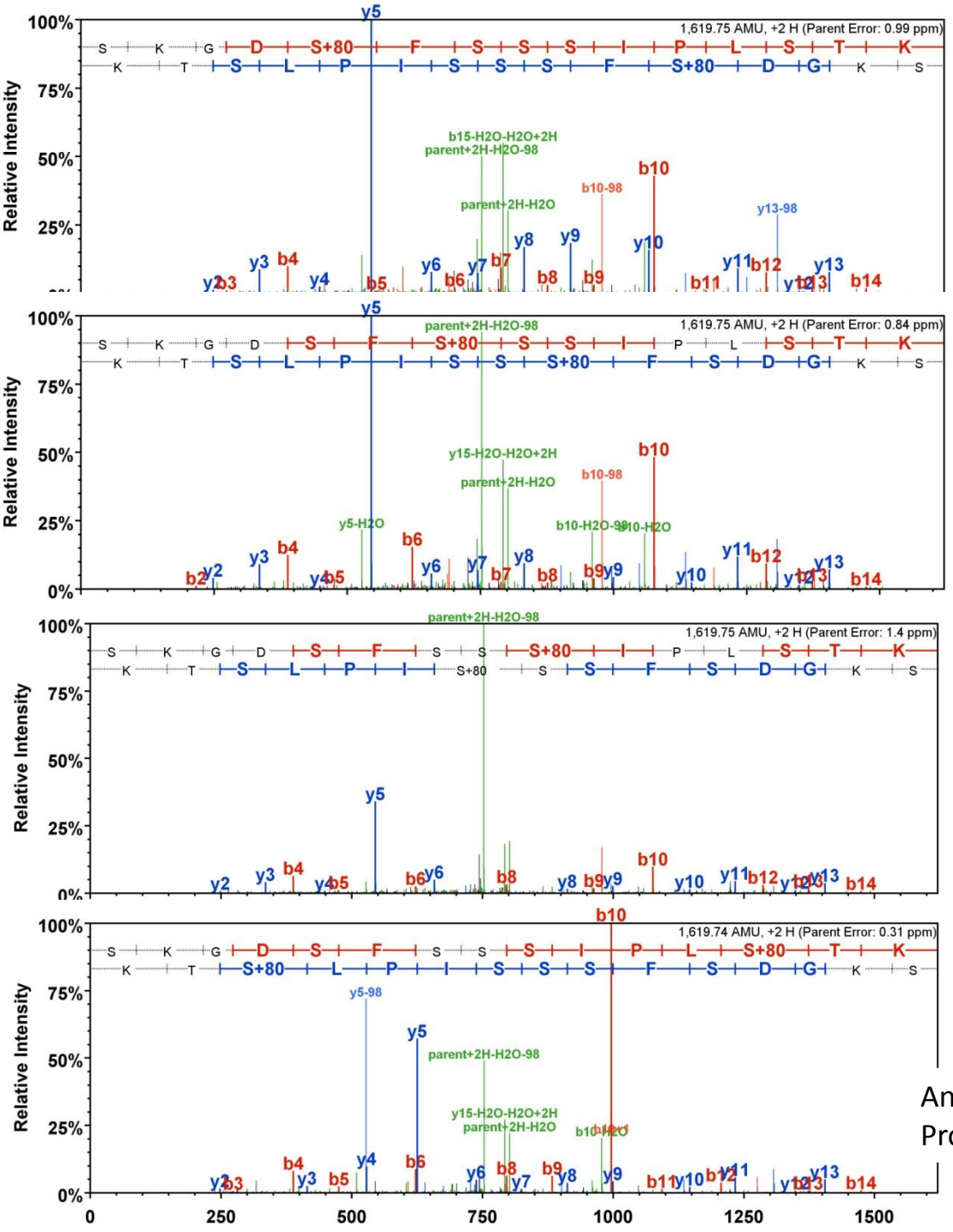
S266

S268

SKGD**S**F**S**S**S**IPL**S**TK

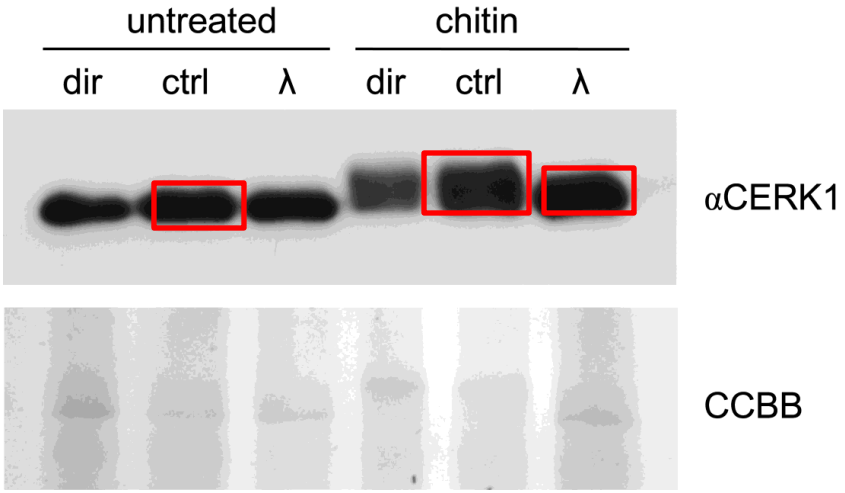
S270

S274



Annotation by Scaffold, Proteome Software

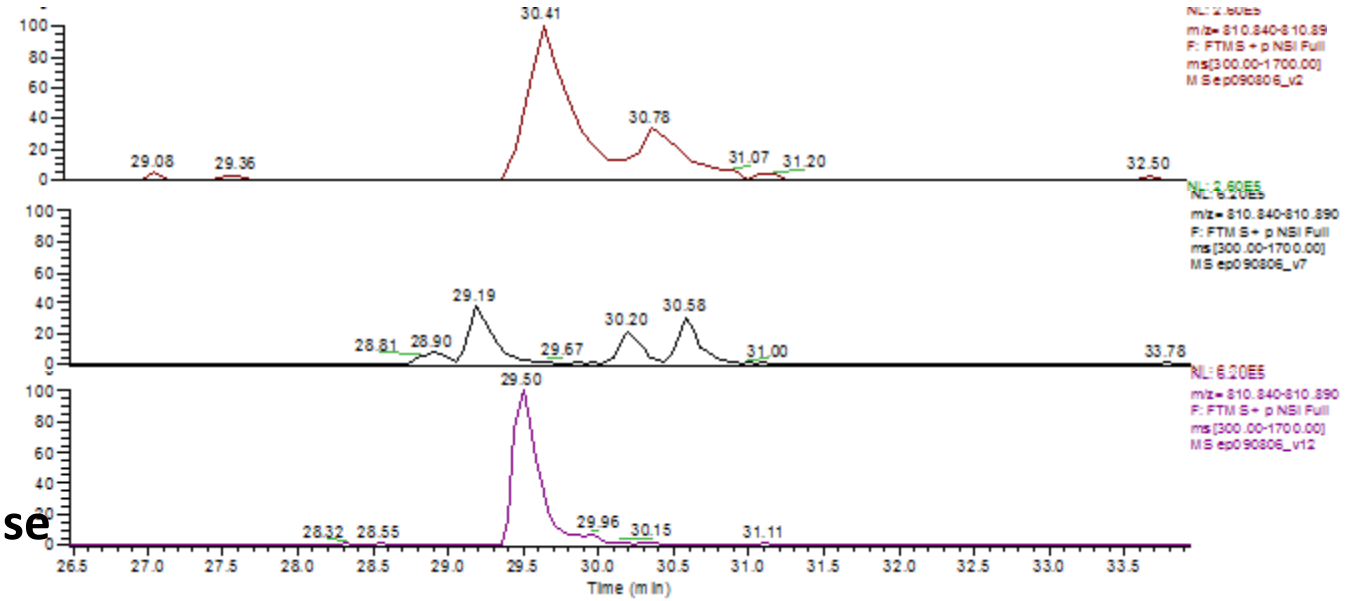
Extracted ion chromatograms of SKGDSFSSSIPLSTK



No chitin

plus chitin

plus chitin
Plus phosphatase



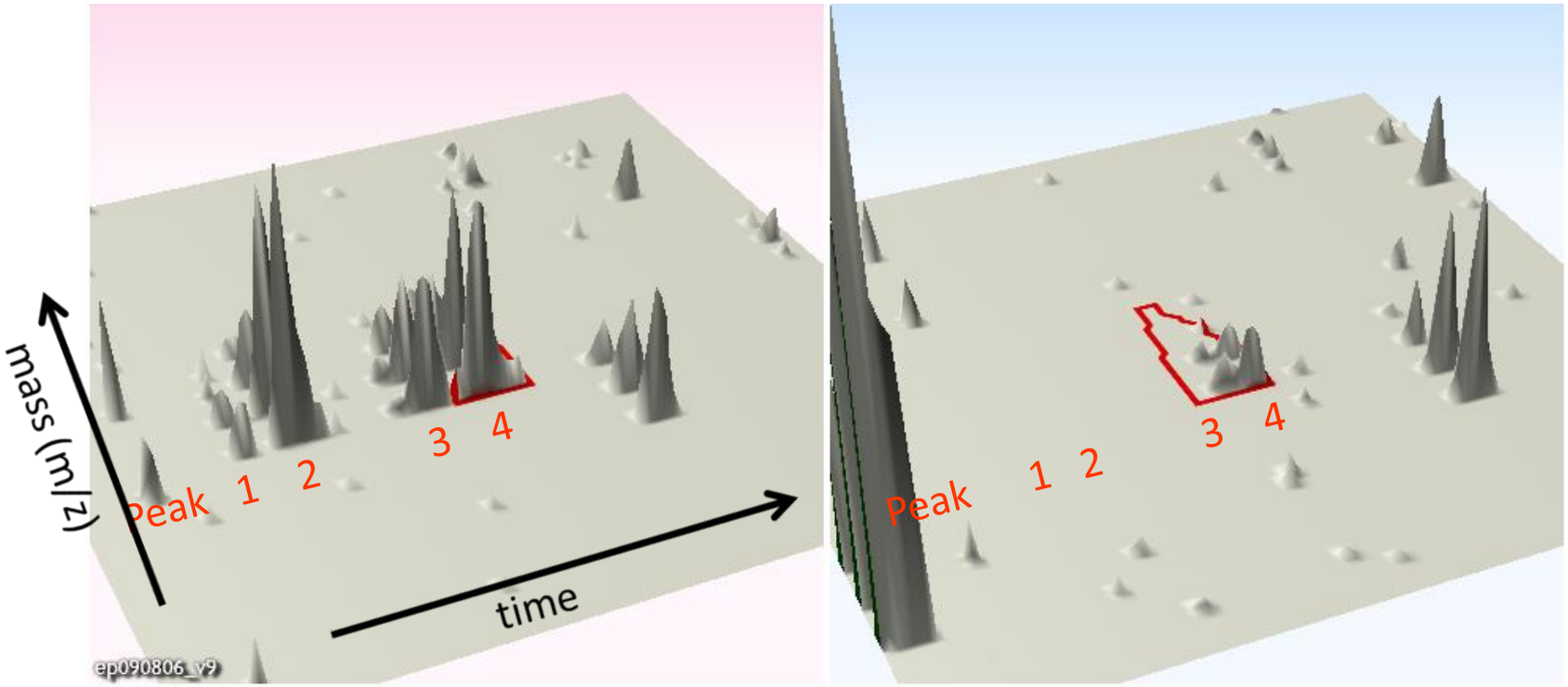
810.88 mz 2+ ion

XIC by QualBrowswer, ThermoScientific

Peak areas of SKGDSFSSSIPLSTK phosphorylated

elicited with chitin

Not elicited with chitin



XIC by Progenesis, Non-Linear Dynamics

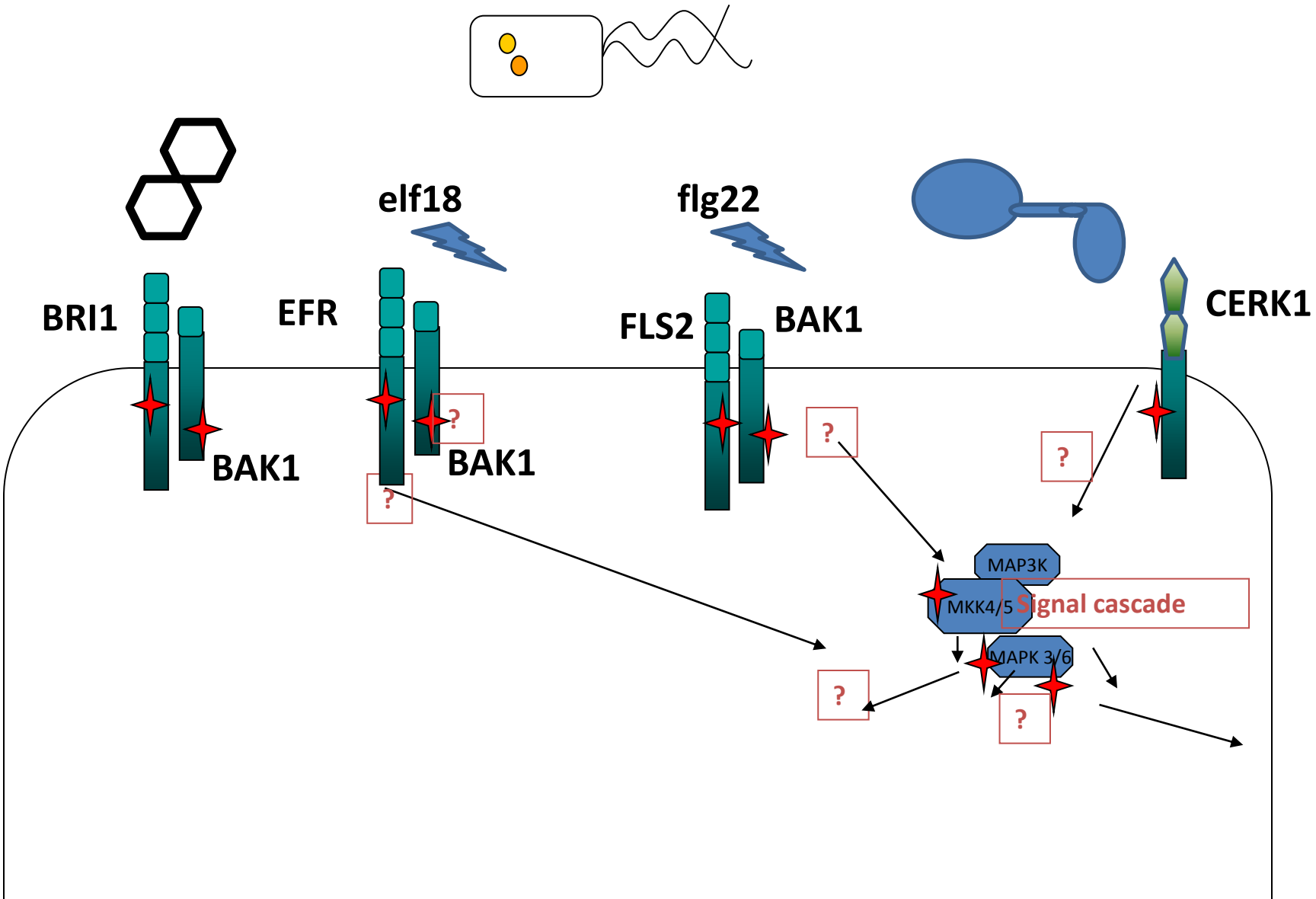
Constitutive and induced phosphorylation revisited

		percent of specific forms of peptide							
		plus chitin				no chitin			
Peptide	**modification**	**B1T1**	**B1T2**	**B2T1**	**B2T2**	**B1T1**	**B1T2**	**B2T1**	**B2T2**
GDSFSSSIPLSTK		34	36	35	32	40	30	39	47
GDSFSSSIPLSTK	Phospho (S274)	0	0	1	1	0	0	-	0
SKGDSFSSSIPLSTK 2+	Phospho (S274)	1	2	4	4	0	0	0	0
SKGDSFSSSIPLSTK 2+	Phospho (S266)	2	1	4	3	2	1	2	2
SKGDSFSSSIPLSTK 2+	Phospho (S268)	3	4	3	3	1	1	0	0
SKGDSFSSSIPLSTK 2+		35	34	37	40	32	37	39	33
SKGDSFSSSIPLSTK 3+		25	24	17	17	25	30	19	17
GAVVKMTEAVGEFR	Oxidation (M)	81	71	44	36	100	99	100	100
GAVVKMTEAVGEFR	Oxidation (M) Phospho (T519)	19	29	56	64	-	1	-	-

B= biological replicate, T= technical replicate

Petutschnig et al., Journal of Biological Chemsitry 2010

The co-receptor kinase BAK1



Current BAK1 strategy for mapping and quantifying phosphorylation sites

Expressed, tagged proteins

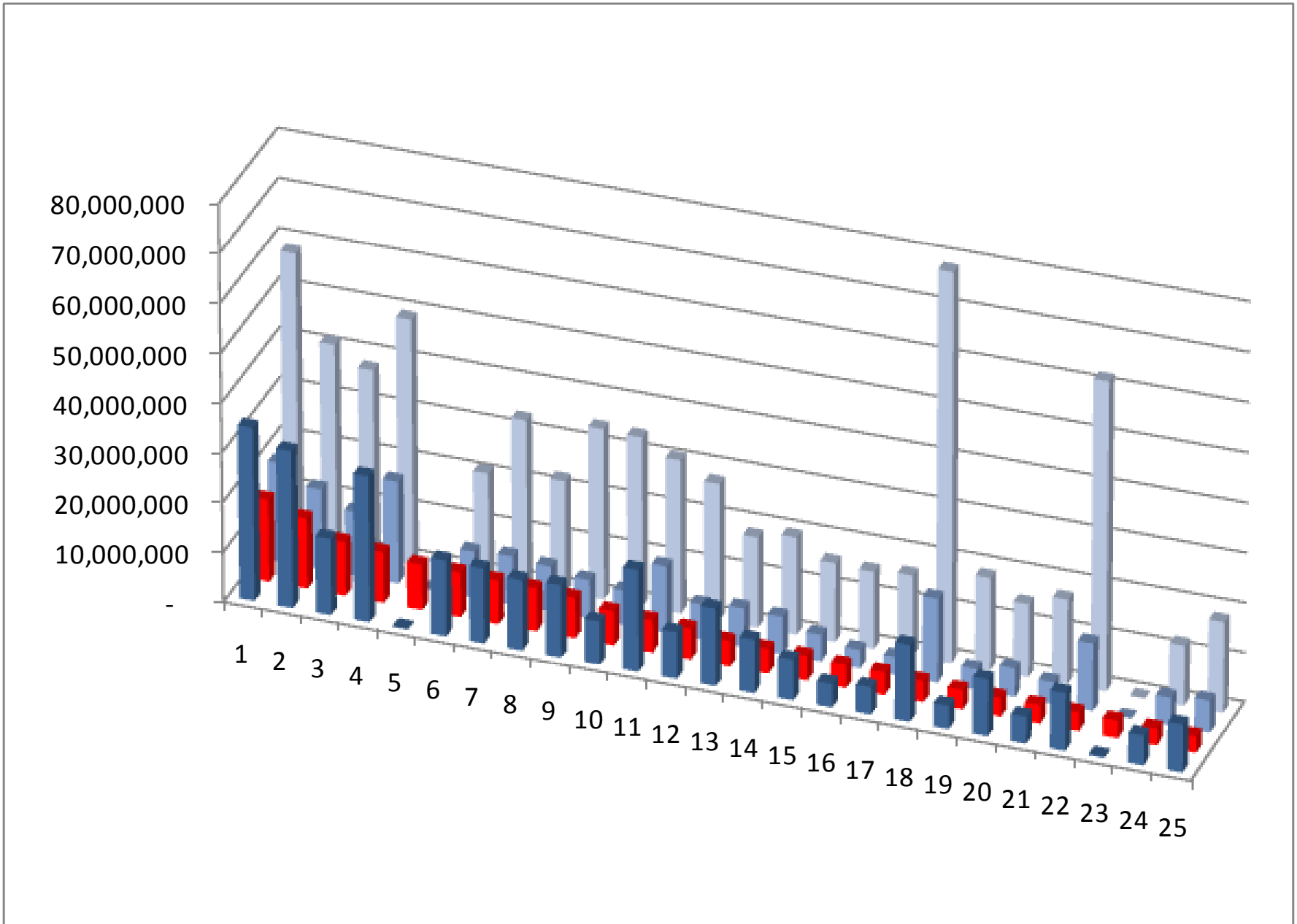
WT, hyperactive mutant, kinase dead versions

1. In vitro: 'Hot + cold' kinase assays
   Gel-LC-MS/MS mapping

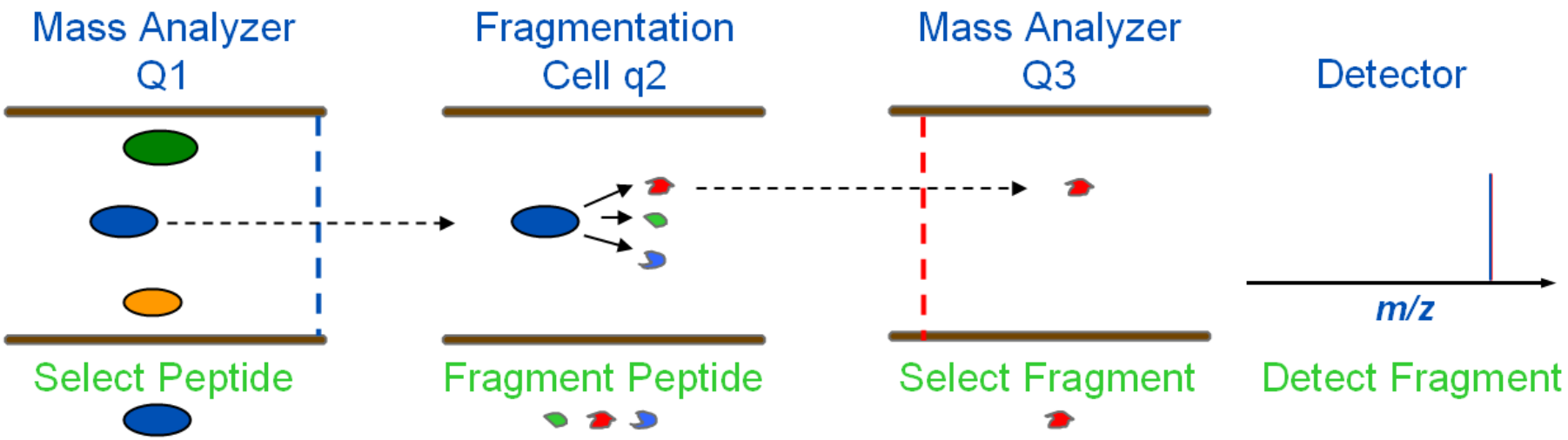
2. In vivo: IP from transient expression N. benth,
   IP stable transgenics Arabidopsis

3. In vivo: native promoters, SRM

Peak areas of top 25 BAK1 peptides



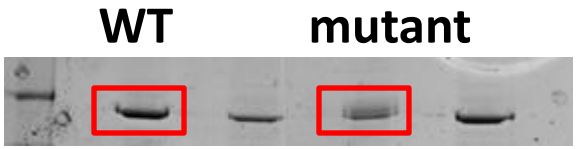
Multiple reaction monitoring



Note:
Modified forms of a peptide differ in both Q1 (intact mass) and Q3 (fragment mass)

With thanks to AB Sciex for slide

Phosphorylation events on BAK1

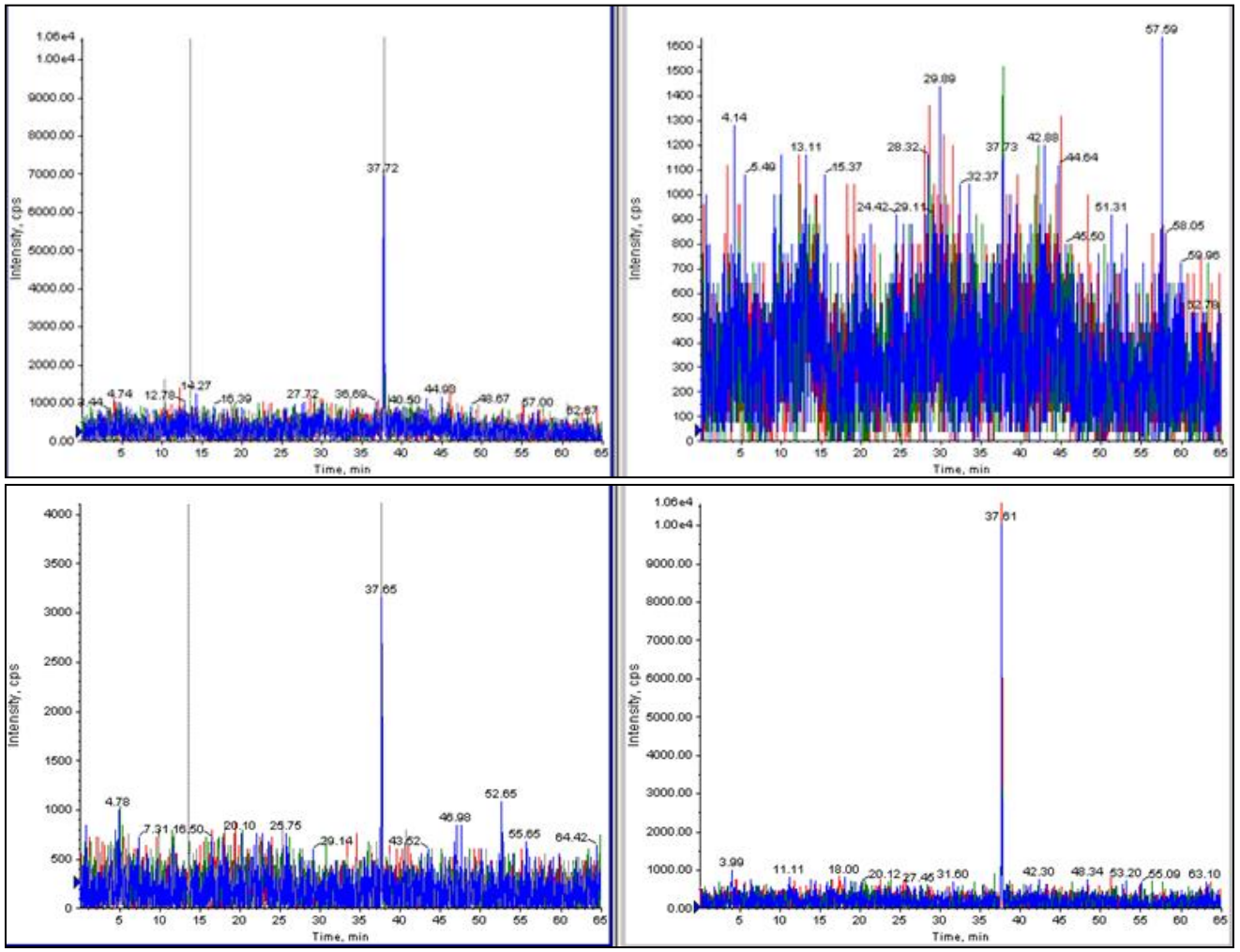


Non-phosphorylated

phosphorylated

WT

mutant



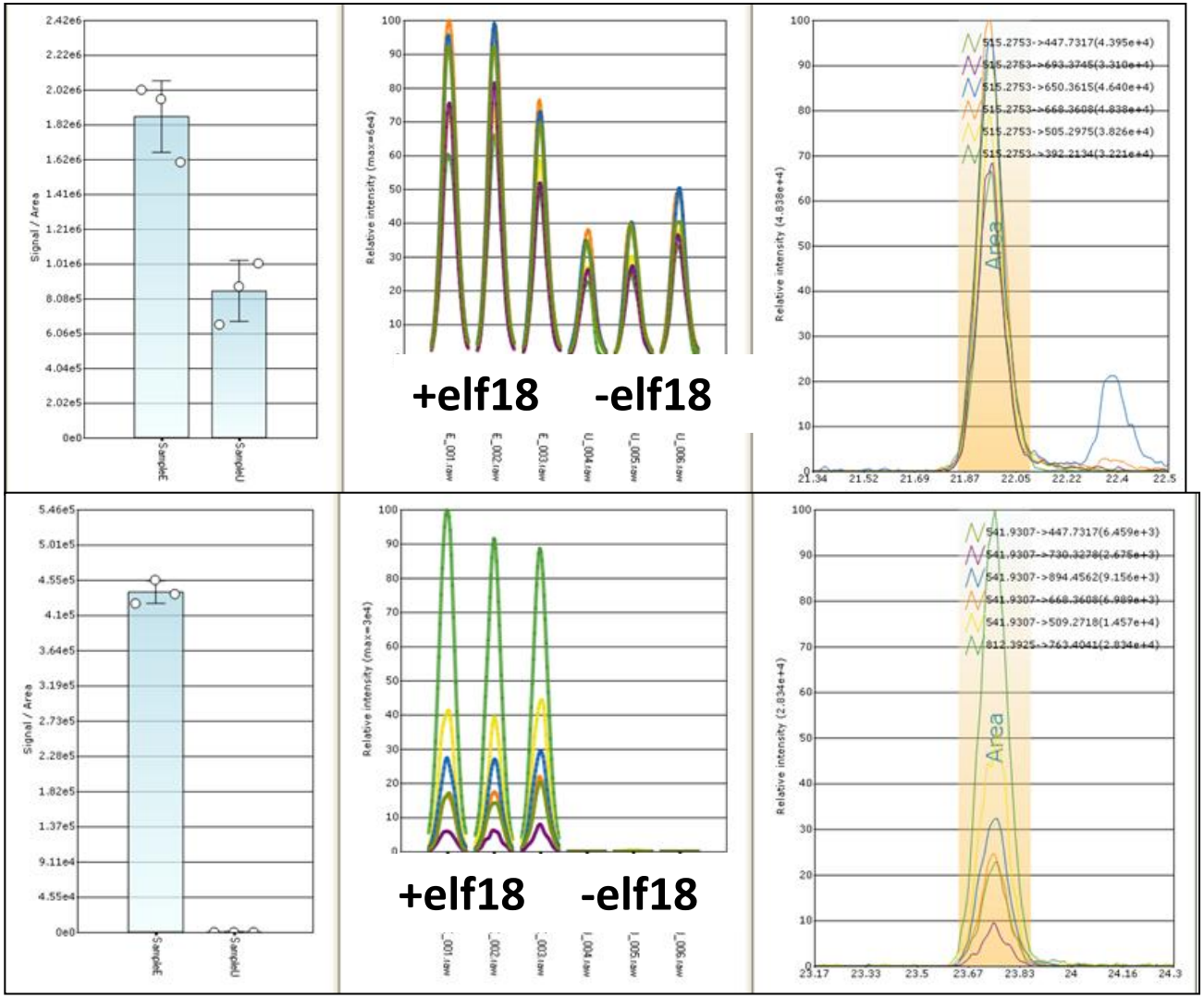
in-house Qtrap 4000

Analyst image, AB Sciex

Phosphorylation events on BAK1 with elicitors

Non-phosphorylated

phosphorylated



ThermoScientific demo data TSQ Vantage, Pinpoint software

Summary

- Plant proteomics is highly feasible
- Phosphorylation is important in plant-pathogen interactions
- Identification of sites is becoming routine

Current challenges are

assignment of correct sites*

quantification

- Future challenges

subcellular localisation,

compsition of complexes

*Note biochem noise, possible flexibility of enzymes

CERK1

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BAK1

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MS group

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