six lectures on systems biology

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lecture 2
31 march 2011

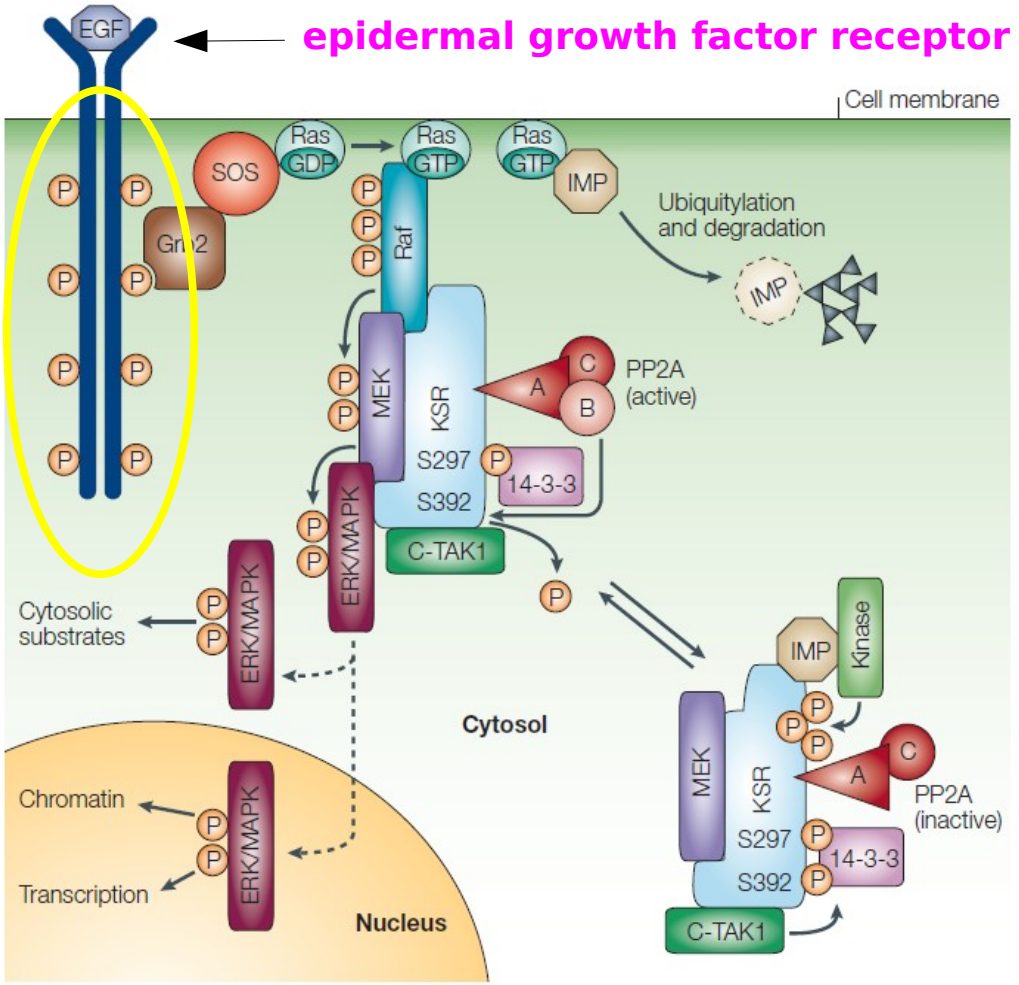
part 2 seminar room, department of genetics

a rather provisional syllabus

0. why mathematical models?
1. post-translational modification of proteins
2. microscopic cybernetics
3. development and evolution

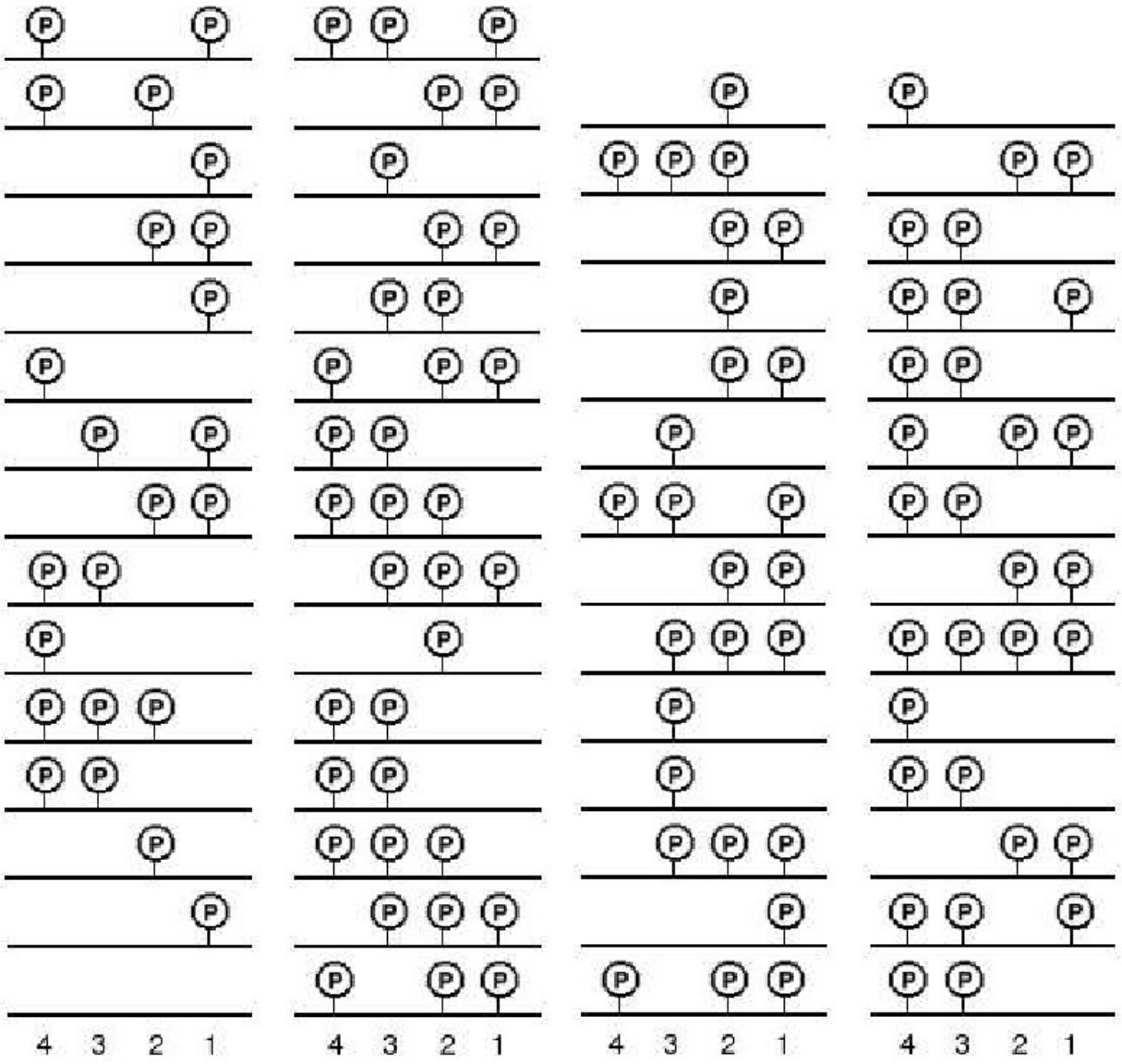
1. post-translational modification (PTM)

the cartoon view of PTMs



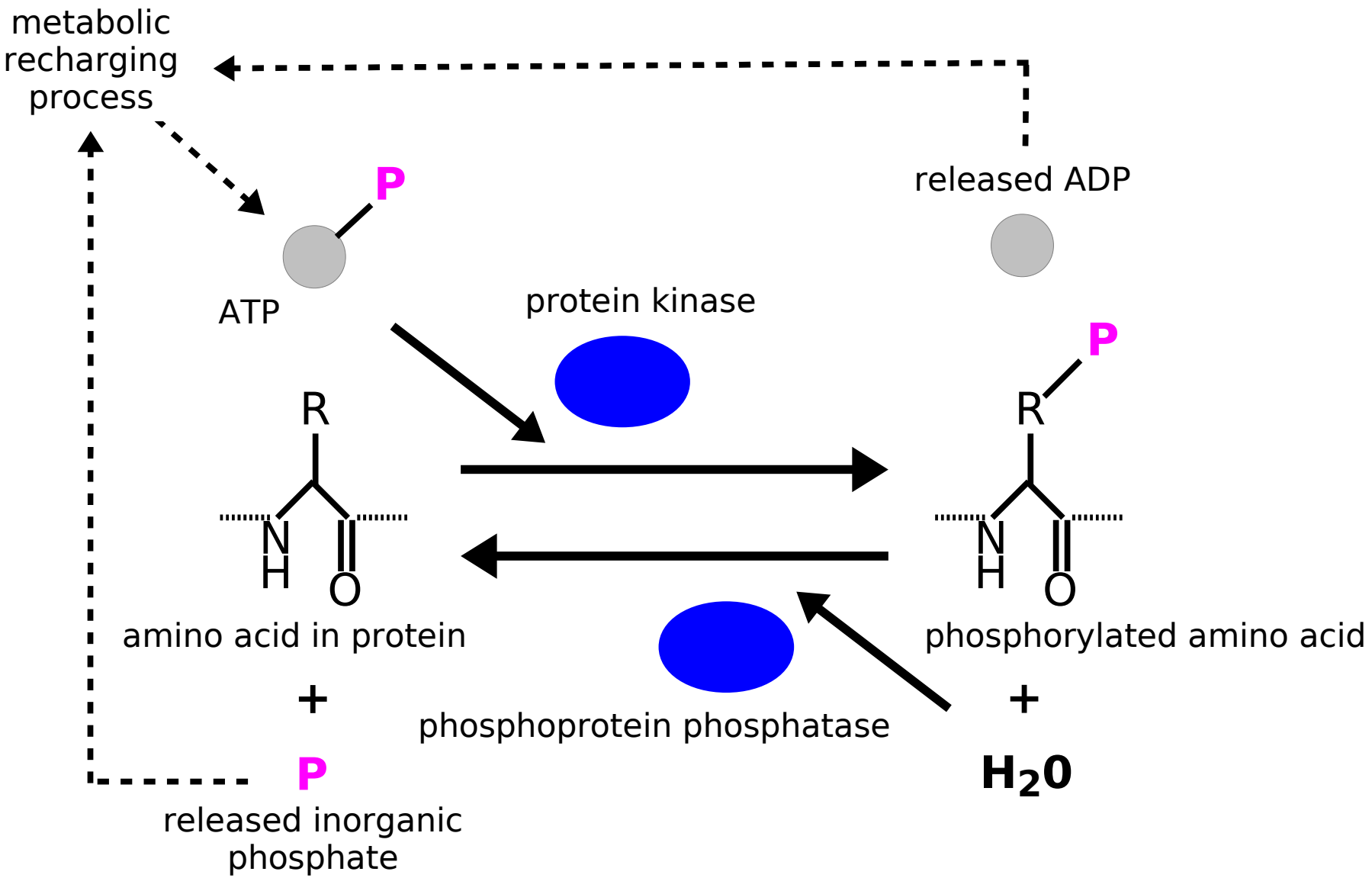
Walter Kolch, *"Coordinating Erk/MAPK signalling through scaffolds and inhibitors"*, Nature Rev Mol Cell Biol **6**:827-37 2005

but under the hood



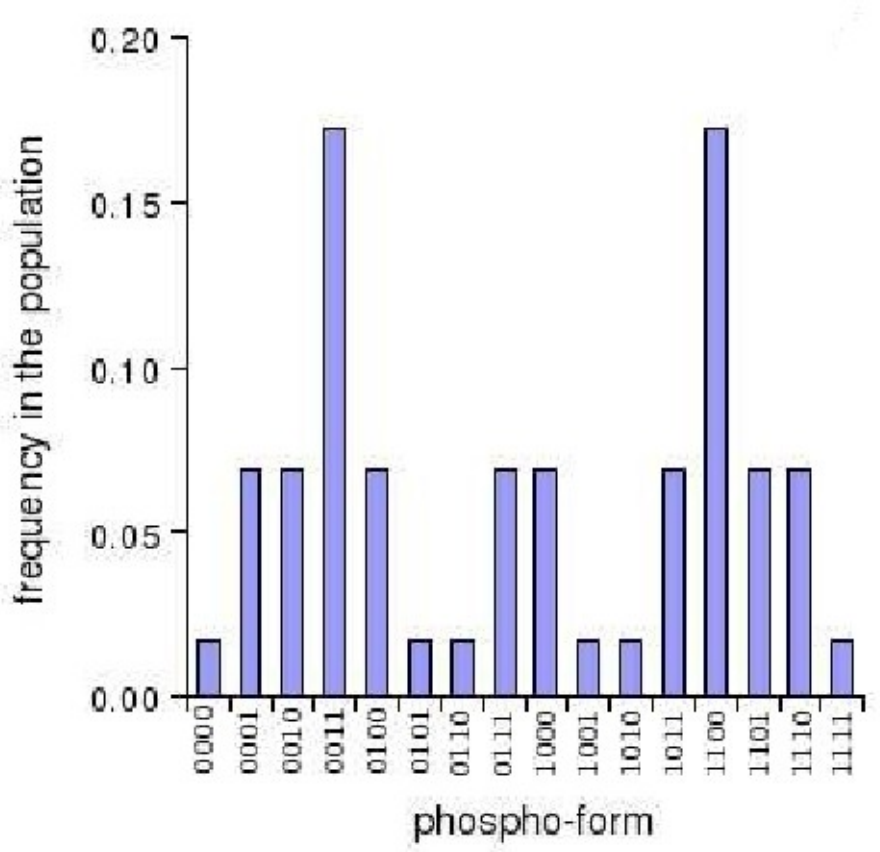
many potential phospho-forms

phosphorylation is reversible and dynamic



phospho-form distribution

relative proportion of each phospho-form in the molecular population



many kinds of reversible PTMs

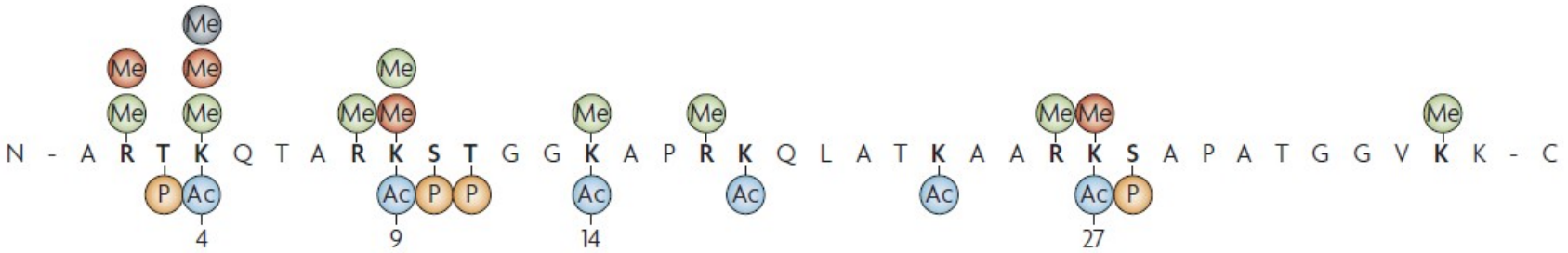
phosphorylation	PO_3^{2-}	**ATP**	S, T, Y H, D
sulfation	SO_3^-	**PAPS**	Y† (extracellular)
methylation	CH_3	**SAM**	E, K(1-3), R(1-2)†
acetylation	CH_3CO	**AcCoA**	K
GlcNAcylation	$C_8H_{15}NO_6$	**UDP-GlcNAc**	S, T
ubiquitin-like	Ub, SUMO, Nedd		K (linear/branched polymers)

† = reverse enzymes not known

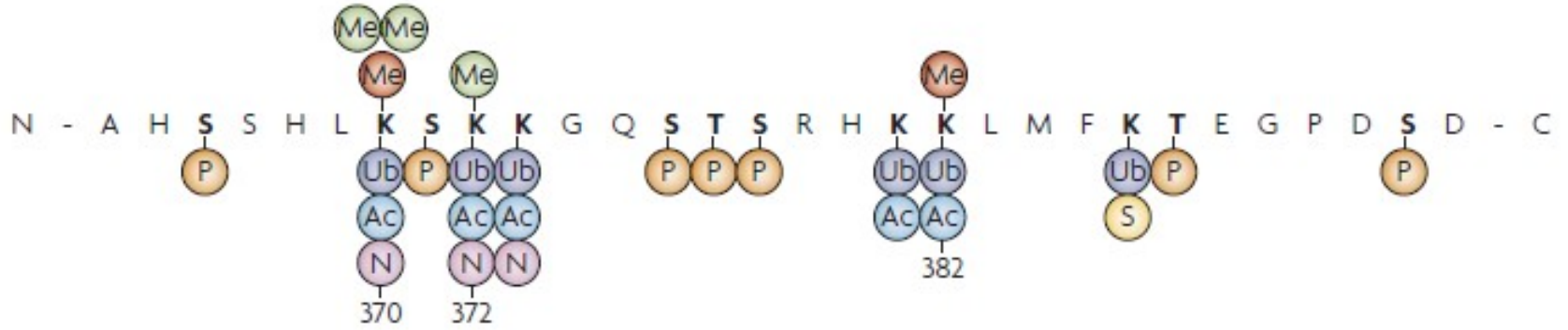
Walsh, **Posttranslational Modification of Proteins: Expanding Nature's Inventory**, Roberts & Co 2006

which interact with each other

histone H3 N-terminal tail



C-terminal domain of p53

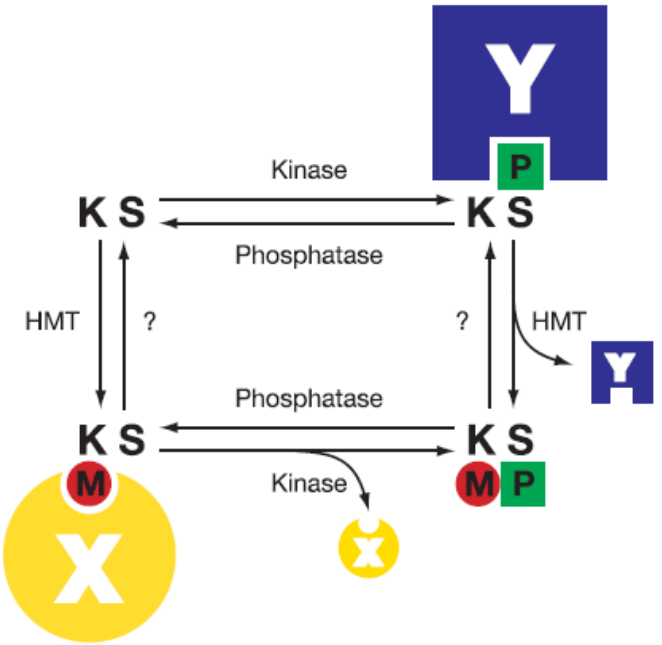


R J Sims & D Reinberg, *"Is there a code embedded in proteins that is based on post-translational modification"*, Nature Rev Mol Cell Biol **9**:815-20 2008

in many different ways

Binary switches and modification cassettes in histone biology and beyond

Fischle, Wang, Allis, Nature **452**:475-9 2003

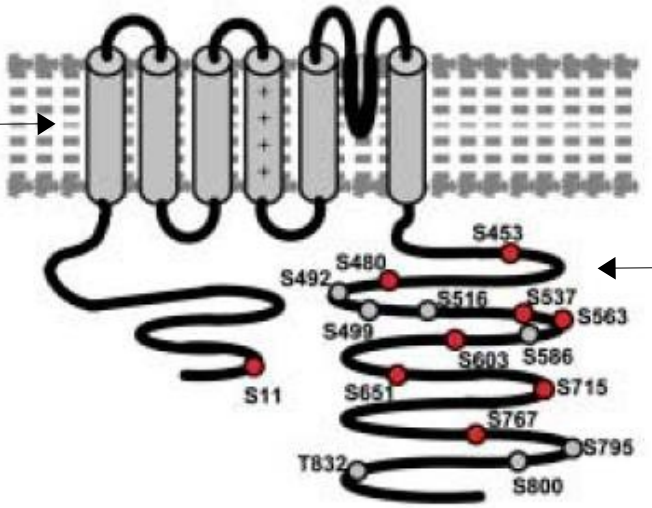


The Age of Crosstalk: Phosphorylation, Ubiquitination, and Beyond

Tony Hunter, Mol Cell **28**:730-8 2007

distributions are biologically relevant – example 1

Kv2.1 voltage-gated pottasium channel



16 S/T phosphorylation sites

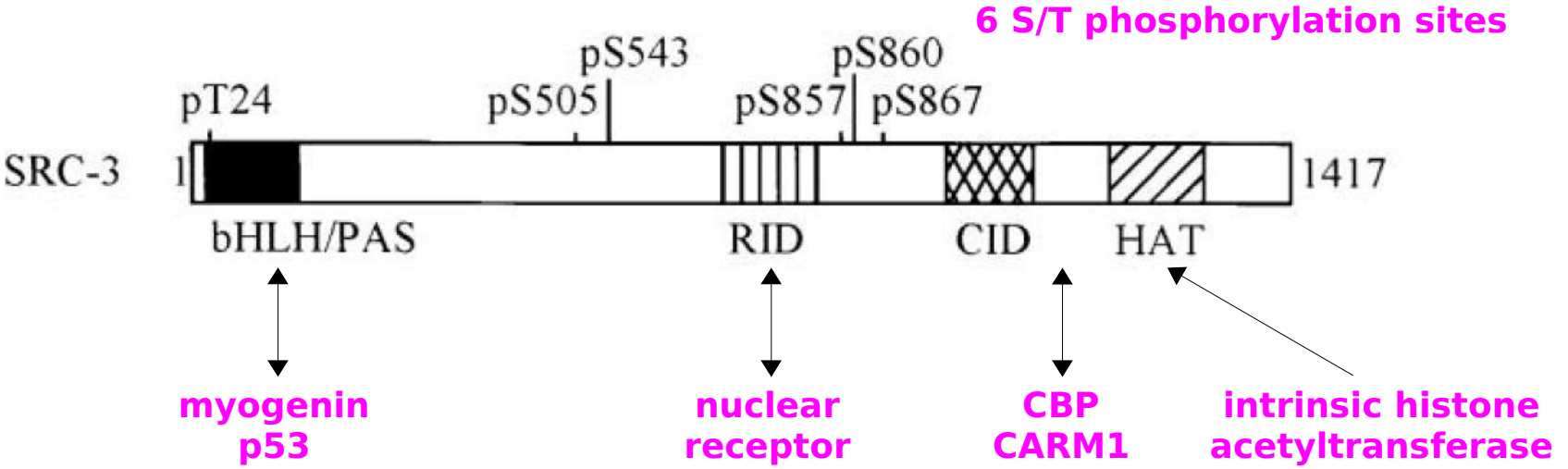
7 are dephosphorylated by calcineurin in response to elevation of intracellular Ca^{2+}

Muta-genesis studies reveal that each of the seven calcineurin-modulated sites imparts a unique and incremental change in voltage-dependent gating.

Park, Mohapatra, Misonou, Trimmer, *"Graded regulation of the Kv2.1 potassium channel by variable phosphorylation"*, Science **313**:976-9 2006

distributions are biologically relevant – example 2

steroid receptor co-activator 3 (SRC-3)

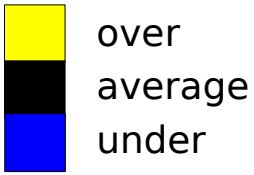


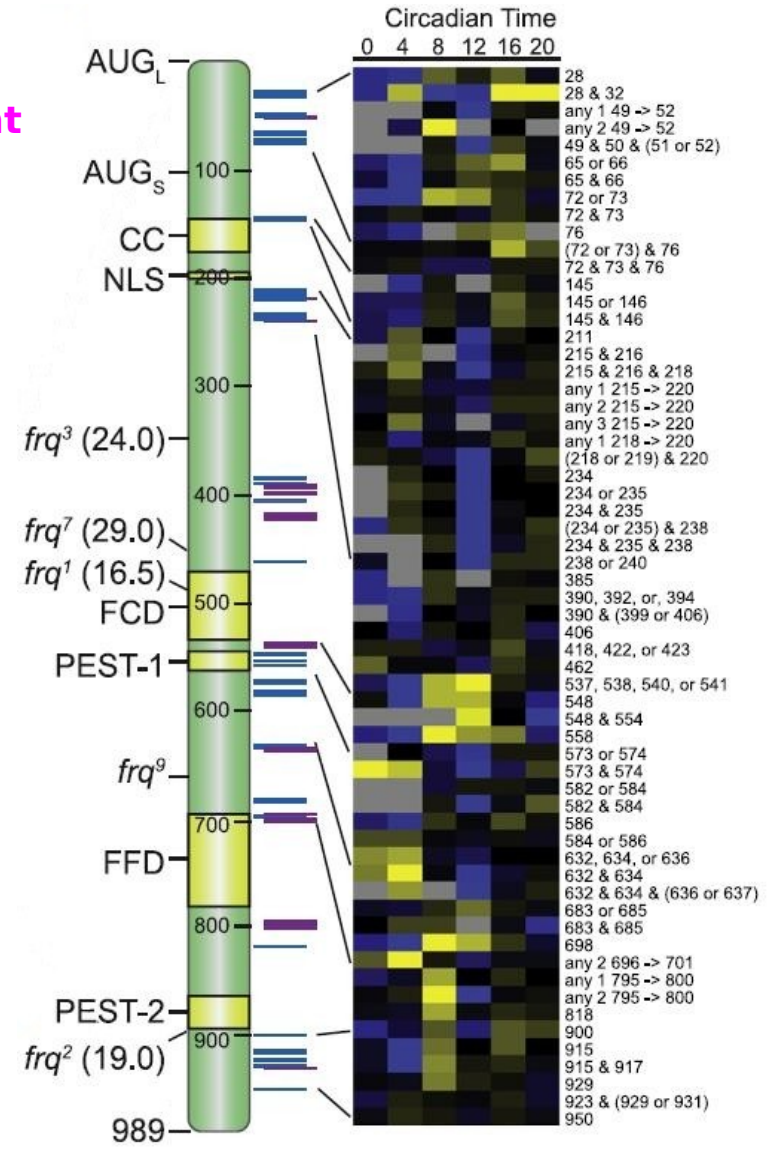
We showed that distinct combinations of phosphorylation sites were responsible for the function of different transcription factors and identified multiple cellular kinsases involved in the site-specific phosphorylations. Finally, we showed that different combinations of phosphorylation sites were required for different physiological functions.

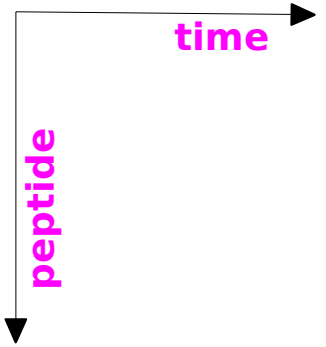
Wu, Qin, Yi, Wong, Tsai, Tsai, O'Malley , *"Selective phosphorylations of the SRC-3/AIB1 coactivator integrate genomic responses to multiple cellular signaling pathways"*, Mol Cell **15**:937-49 2004

distributions are biologically relevant – example 3

FRQ
circadian clock component
from *Neurospora crassa*



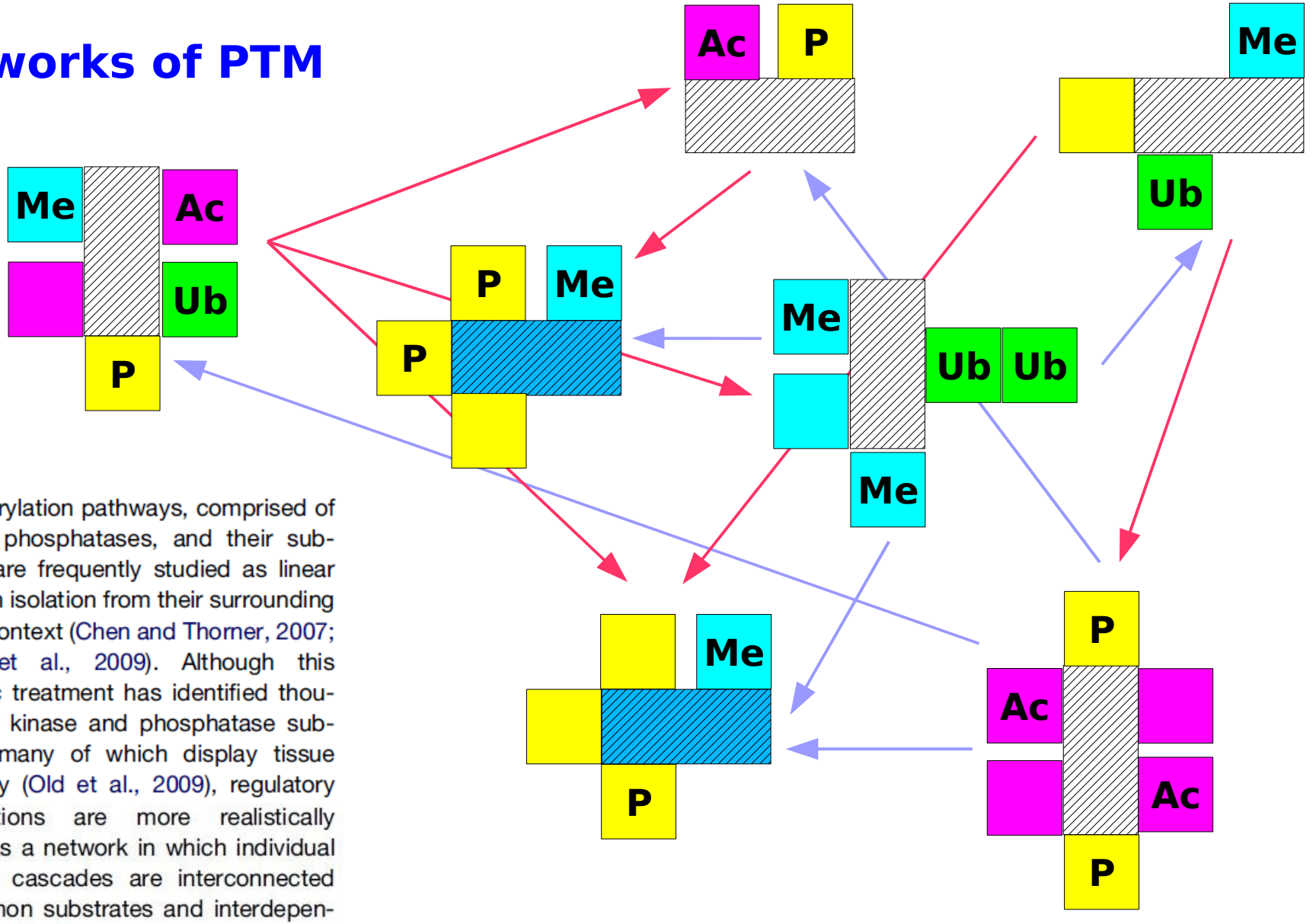




75 S/T phosphorylation sites on 63 tryptic peptides

Baker, Kettenbach, Loros, Gerber, Dunlap, *"Quantitative proteomics reveals a dynamic interactome and phase-specific phosphorylation in the Neurospora circadian clock"*, Mol Cell **34**:354-63 2009

networks of PTM



Phosphorylation pathways, comprised of kinases, phosphatases, and their substrates, are frequently studied as linear entities in isolation from their surrounding cellular context (Chen and Thorner, 2007; Fiedler et al., 2009). Although this simplistic treatment has identified thousands of kinase and phosphatase substrates, many of which display tissue specificity (Old et al., 2009), regulatory modifications are more realistically viewed as a network in which individual signaling cascades are interconnected by common substrates and interdependent regulation.

Klein, Dioum, Cobb, *"Exposing contingency plans for kinase networks"*, Cell **143**:867-9 2010

another enchanting loom?

“Swiftly the head mass becomes an enchanted loom where millions of flashing shuttles weave a dissolving pattern, always a meaningful pattern though never an abiding one; a shifting harmony of subpatterns.”

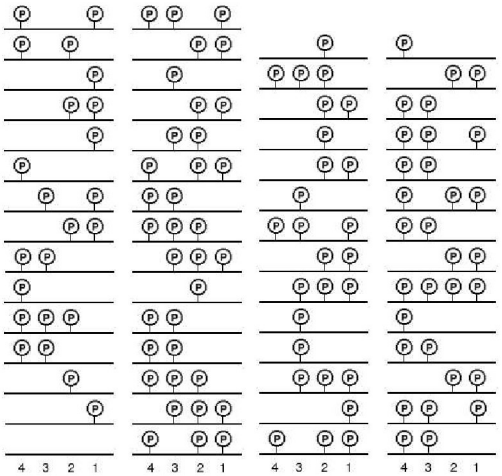
Charles Sherrington, **Man on his Nature**, CUP 1942

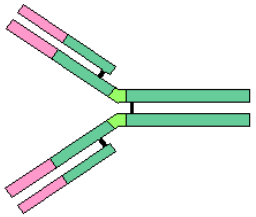
1. how can we measure mod-form distributions?

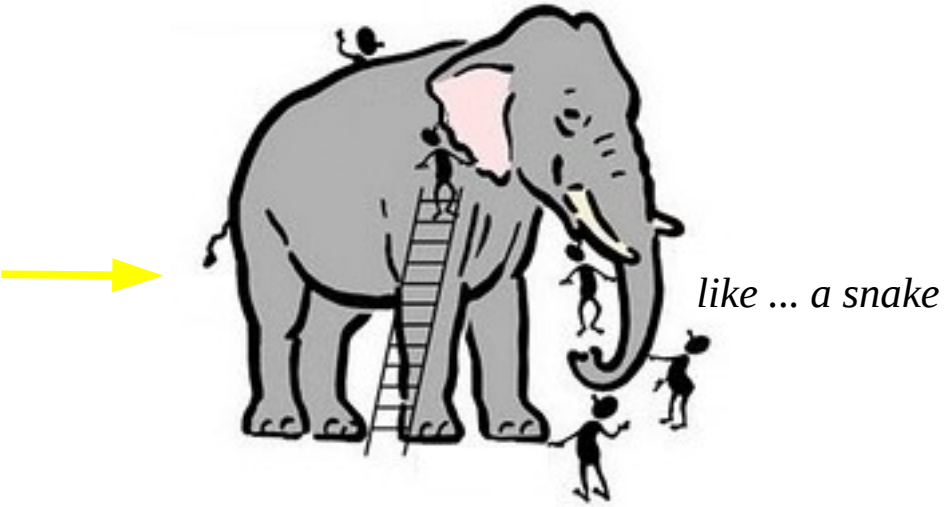
2. how do PTM networks regulate the distributions?

measuring phospho-form distributions

1. phospho-specific antibodies – site-specific information







2. mass spectrometry – excellent for small proteins like histone tails

Phanstiel, Brumbaugh, Berggren, Conrad, Feng, Levenstein, McAlister, Thomson, Coon, *"Mass spectrometry identifies and quantifies 74 unique histone H4 isoforms in differentiating human embryonic stem cells"*, PNAS **105**:4093-8 2008

Pesavento, Mizzen, Kelleher, *"Quantitative analysis of modified proteins and their positional isomers by tandem mass-spectrometry: human histone H4 "*, Anal Chem **78**:4271-80 2006

measuring phospho-form distributions

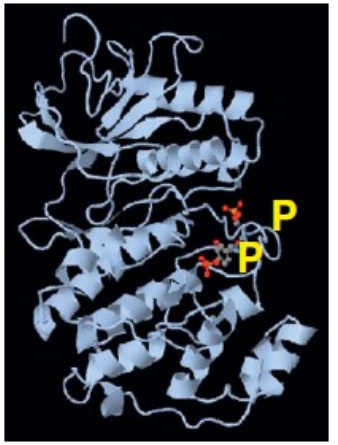
there is no oracle to tell us what is “really” there, so we take a comparative approach –

1. mass spectrometry (MS)
2. nuclear magnetic resonance spectroscopy (NMR)
3. phospho-specific antibodies

using differentially phosphorylated samples of Erk2

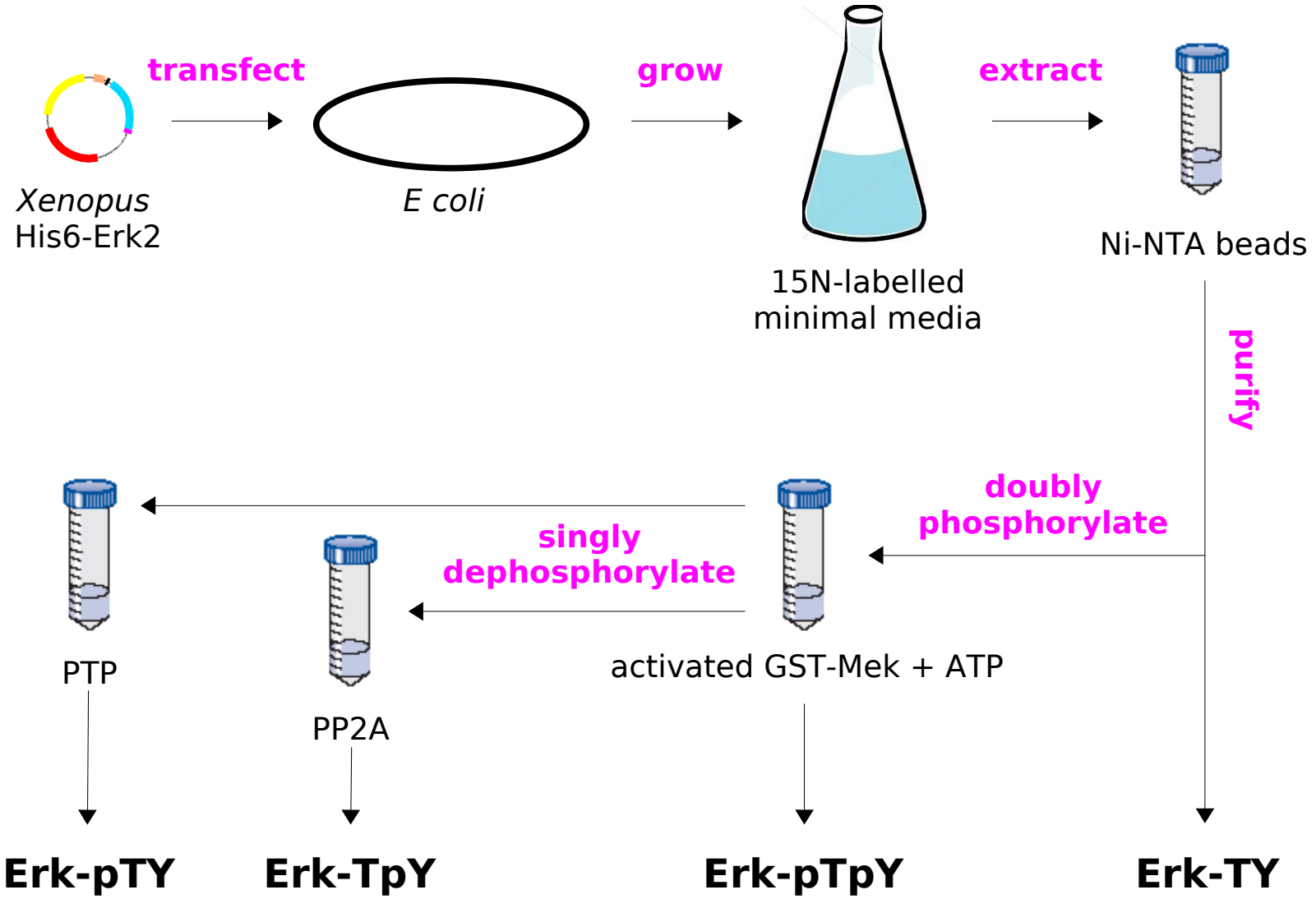
42kD MAP kinase, doubly phosphorylatable on **T**E**Y**

4 phospho-forms – *TY*, *pTY*, *TpY*, *pTpY*



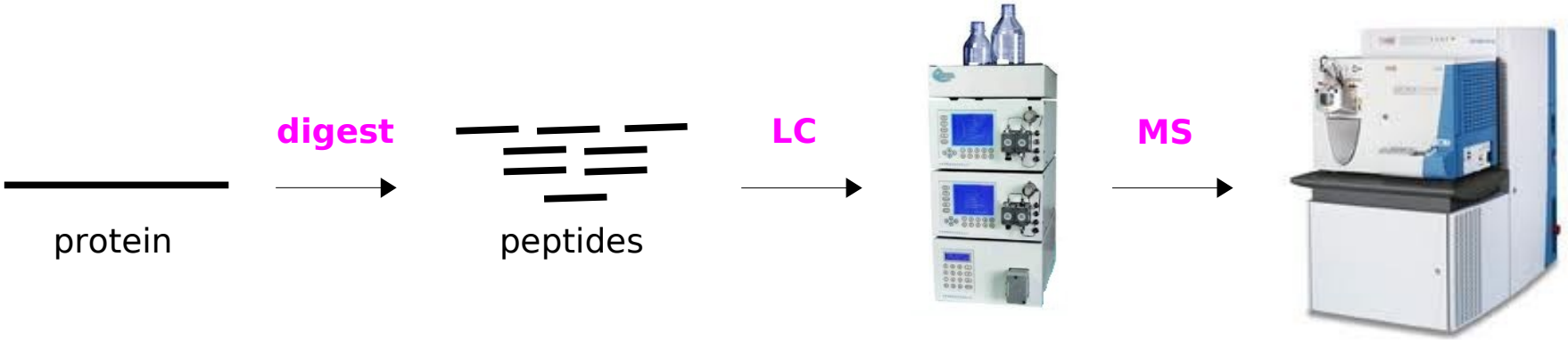
Prabhakaran, Everley, Landrieu, Wieruszeski, Lippens, Steen, Gunawardena, *“Comparative analysis of Erk phosphorylation suggests a mixed strategy for measuring phospho-form distributions”*, Mol Sys Biol, to appear, 2011

four samples

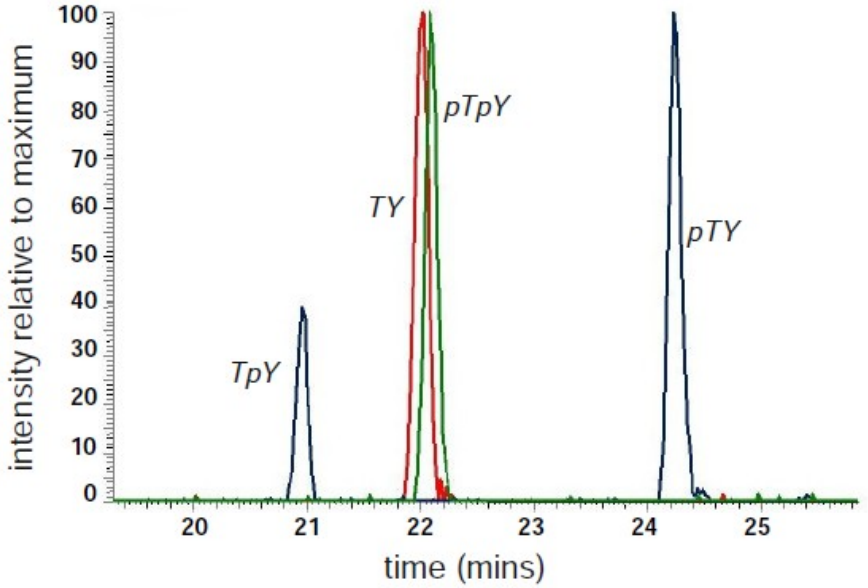


mass spectrometry with peptides

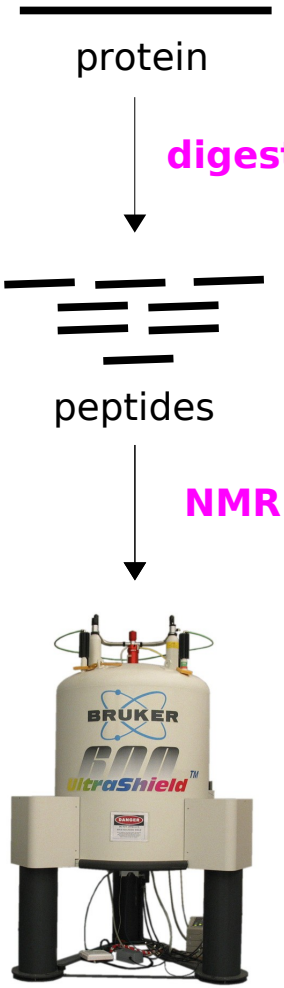
peptide-based LC/MS (pepMS) with internal standards



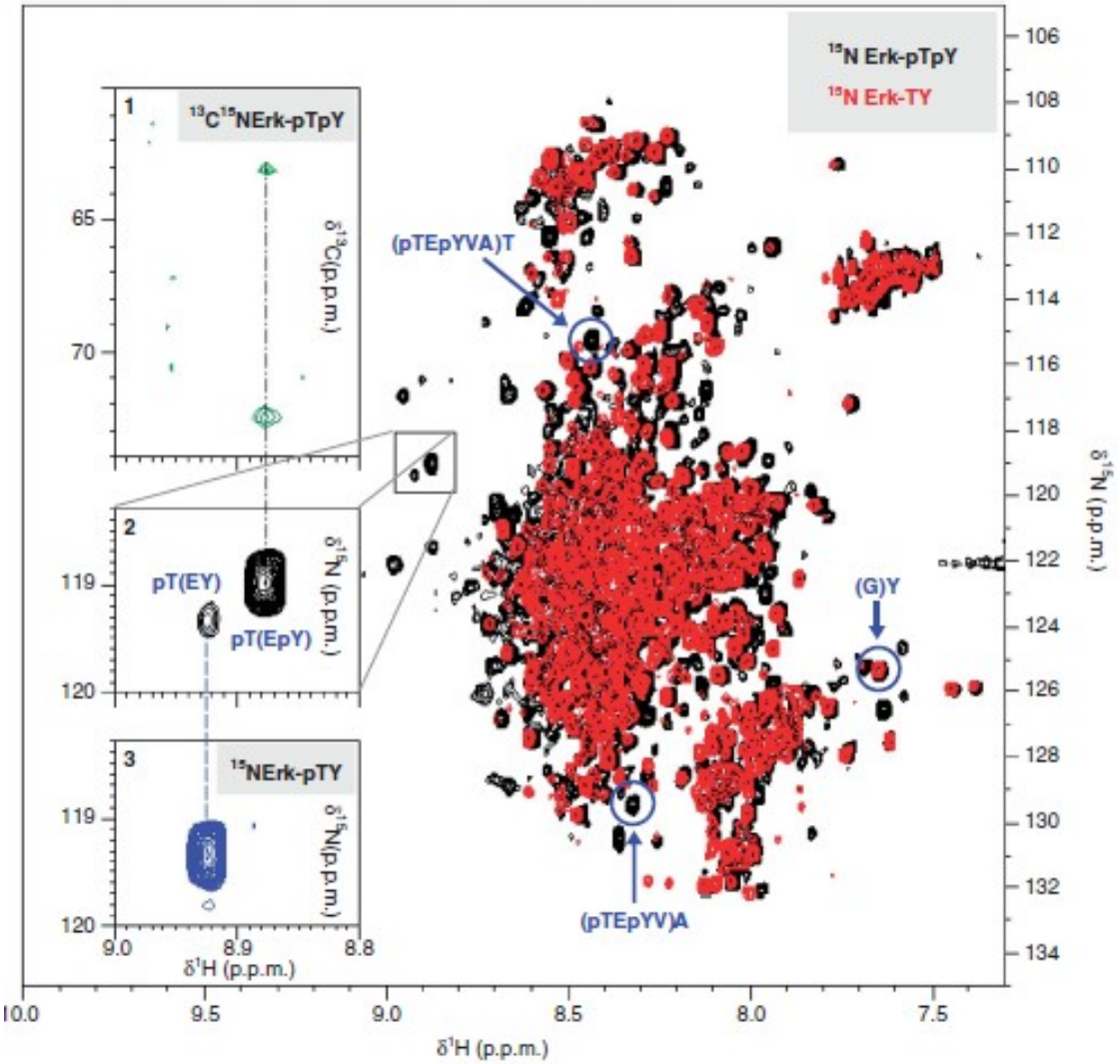
LTQ - orbitrap

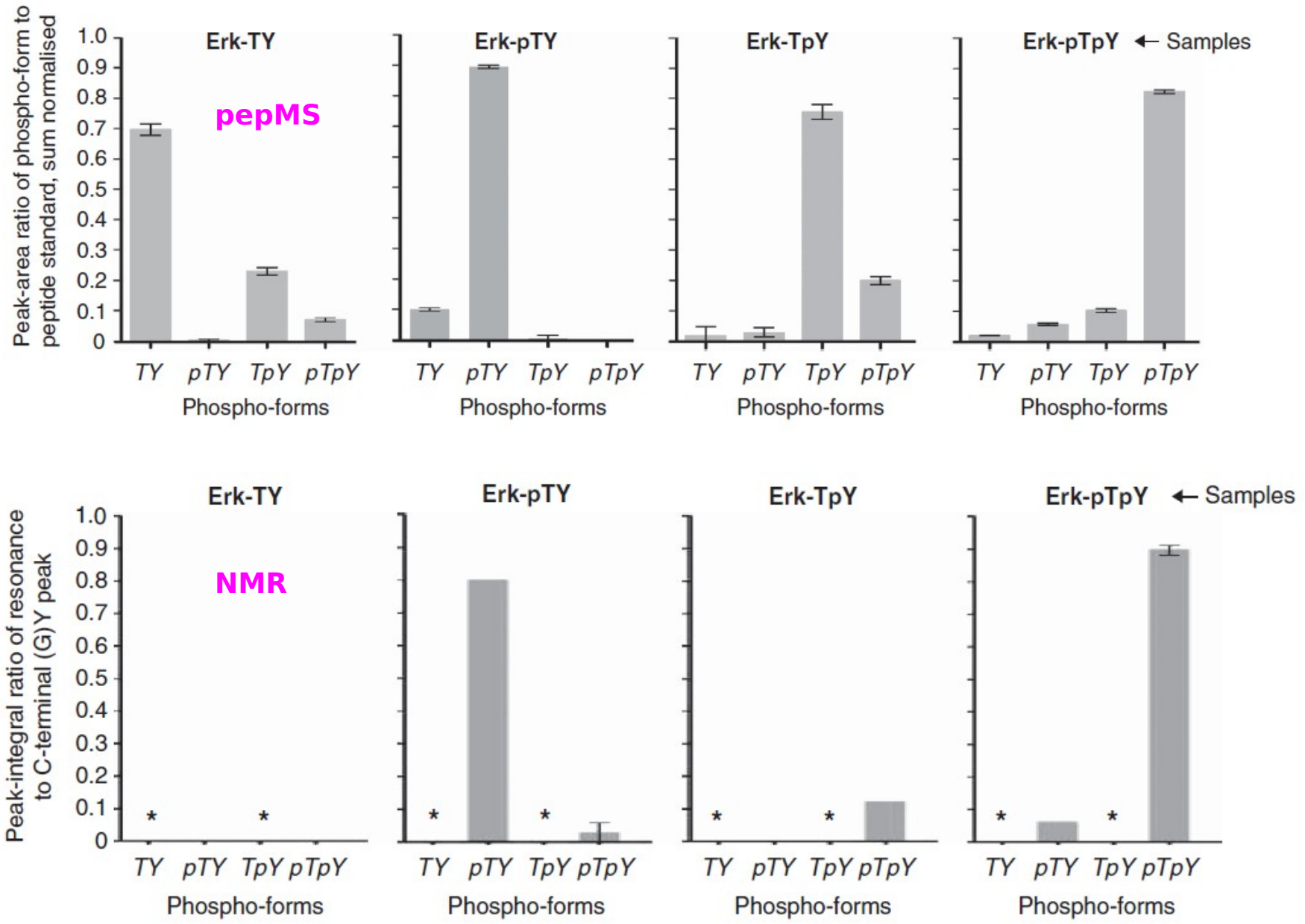


nuclear magnetic resonance

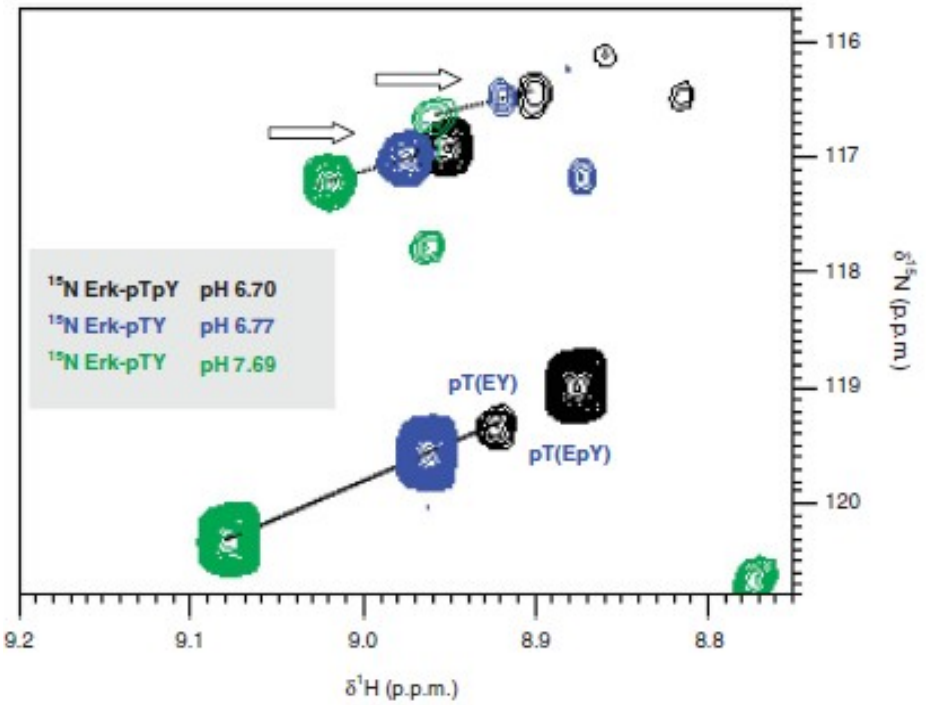


Bruker Avance
600MHz




Peak-area ratio of phospho-form to peptide standard, sum normalised
Erk-TY
Erk-pTY
Erk-TpY
Erk-pTpY
← Samples
pepMS
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
TY pTY TpY pTpY
Phospho-forms
Peak-integral ratio of resonance to C-terminal (G)Y peak
NMR
*

but this elephant has a tail ...



at least two additional S/T phosphorylations on the **Erk-pTpY** sample

mass spectrometry with proteins

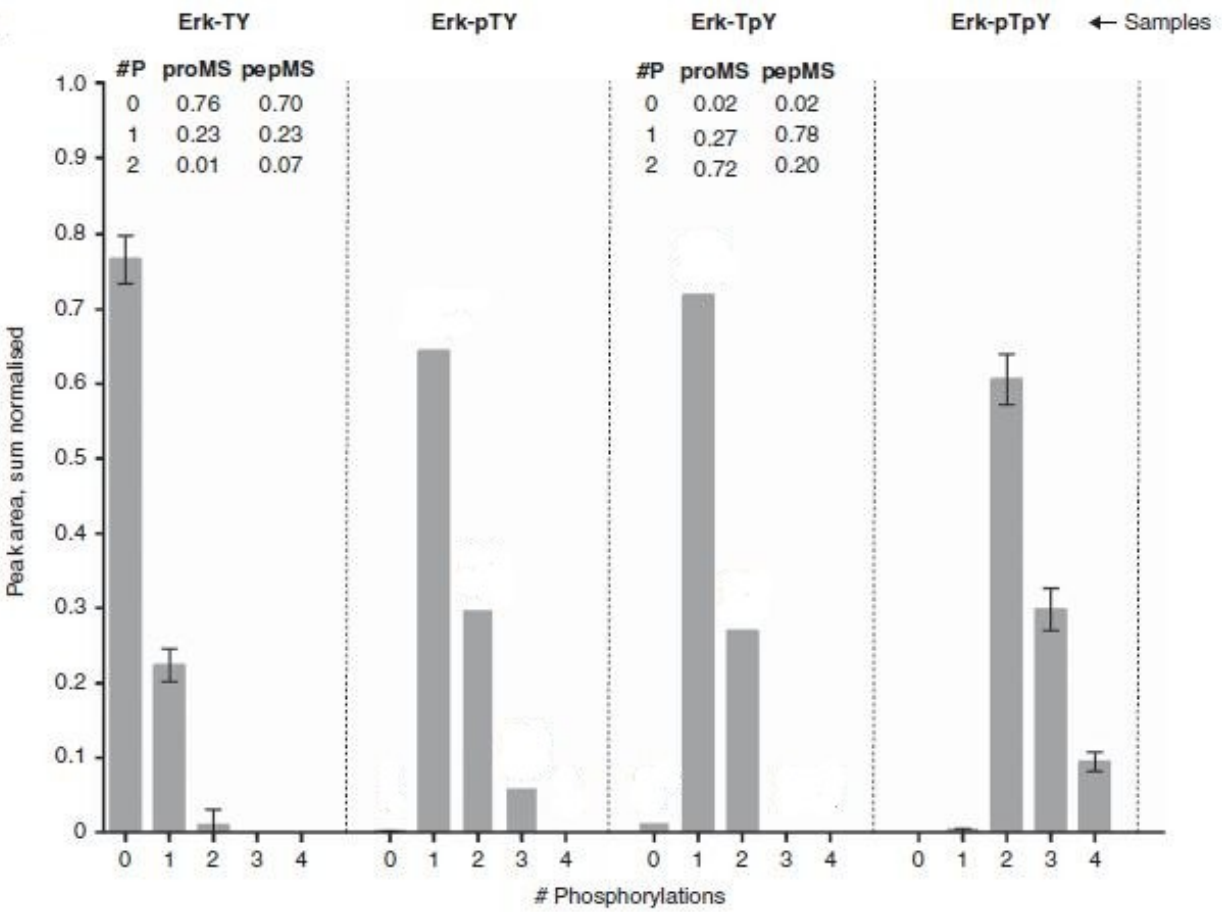
protein-based LC/MS (proMS)

protein

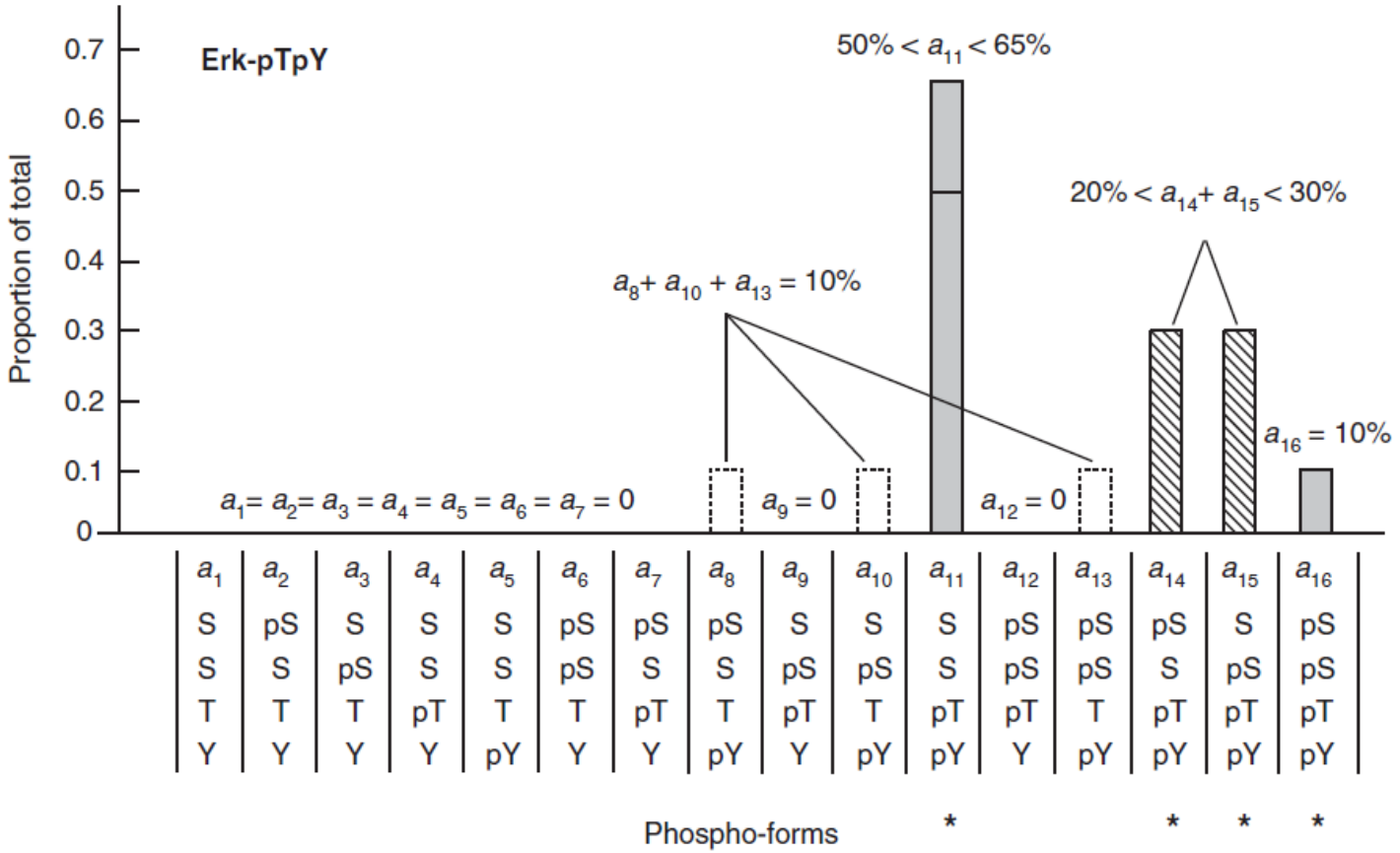
LC

MS

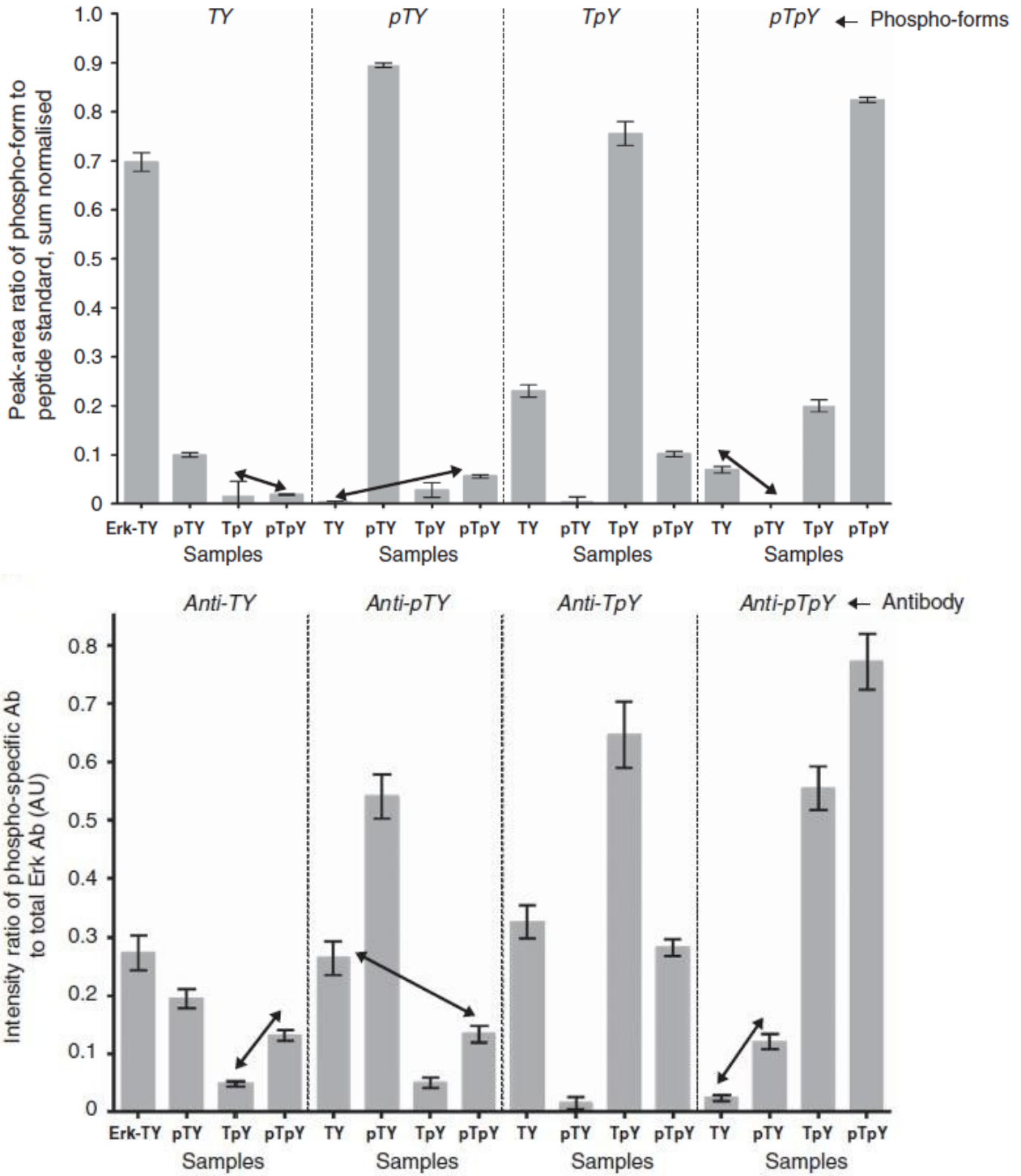
QSTAR



4-site phospho-form distribution




```
  1  MAHHHHHHAS AAAAASSNPG GGPEMVRGQA FDVGPRYTNL SYIGEGAYGM
 51  VCSAHCNINK VRVAIKKISP FEHQTYCQRT LREIKILLRF KHENIIGIND
101  IIRAPTIEQM KDVYIVQDLM ETDLYKLLKT QHLSNDHICY FLYQILRGLK
151  YIHSANVLHR DLKPSNLLLN TTCDLKICDF GLARVADPDH DHTGFLTEYV
201  ATRWYRAPEI MLNSKGYTKS IDIWSVGCIL AEMLSNRPIF PGKHYLDQLN
251  HILGILGSPS QEDLNCIINL KARNYLLSLP HKNKVPWNRL FPNADPKALD
301  LLDKMLTFNP HKRIEVEAAL AHPYLEQYYD PSDEPVAEAP FKFEMELDDL
351  PKETLKELIF EETARFQPGY
```


TY
pTY
TpY
pTpY
Phospho-forms
Peak-area ratio of phospho-form to peptide standard, sum normalised
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Erk-TY
pTY
TpY
pTpY
Samples
Anti-TY
Anti-pTY
Anti-TpY
Anti-pTpY
Antibody
Intensity ratio of phospho-specific Ab to total Erk Ab (AU)

summing up

1. *mod-form distributions carry the most information about PTM state*

2. *downstream biological function depends on the distribution*

3. *biophysical measurements (pepMS, proMS, NMR) agree to within 10%*

4. *a hybrid strategy of pepMS + proMS can uncover phospho-form distributions for small numbers of sites (n < 10?)*

5. *phospho-specific antibodies are a biological readout (see point 2) – beware!*