Phospho-form distributions of proteins – quantitation, mathematical analysis and biological significance.

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Short Abstract — **Evidence from many biological contexts—transcription factors, signalling proteins, ion channels, clock components—shows that the functional impact of multisite protein phosphorylation can depend upon both the numbers and the positions of phosphorylated sites. Such modifications on multiple sites, leads to a potentially enormous number of internal molecular states. A central problem across biology, therefore, is to quantify the relative amount of each of the 2^n phospho-forms of a protein with n sites, which we call the phospho-form distribution. Mathematically by using algebraic geometry we have shown that we can predict the distributions of phosphoforms of proteins at steady-state. To verify these predictions we have employed four potential methods: quantitative Western blots with phospho-specific antibodies, peptide-based LC/MS (pepMS), protein-based LC/MS (proMS) and NMR to quantify phosphoform distribution of our model protein Erk.**

Keywords **— multisite phopshoryaltion, phosphor-forms, quantitative MS, NMR, Erk and algebraic geometry.**

I. Introduction

Phosphorylation on serine (S), threonine (T) and tyrosine (Y) residues is the most widely-studied post-translational modification (PTM) on eukaryotic proteins [1]. Many phosphoproteins are multiply phosphorylated and the necessity for so many molecular states remains a fascinating problem [2]. Cartoon diagrams give the doubly-misleading impression that a multiply phosphorylated protein has only a single global pattern of phosphorylation, or "phospho-form", and that this pattern is static. The aim of this work is to bring this conceptual view point across to a broader audience and suggest mathematical and experimental strategies to unravel this problem.

Mathematically, we have shown that at steady state the relative concentration of each modified form is a rational function of one parameter, E/F, the ratio of free kinase, to free phosphatase [2].

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Experimentally, we optimized and employed 4 detection methods on Erk2. We assessed that a combined strategy of pepMS and proMS is best suitable to address this problem [3].

II. Method

A. *Measuring Erk phosphoforms*

Erk2 is doubly phosphorylated by the kinase Mek and dephosphorylated by the phosphatase MKP3, on a two site T-Y motif on its activation loop. The four resulting phosphoforms – TY, pTY, TpY, pTpY – can be quantified by LC/MS, where the LC stage is able to separate the two singly phosphorylated forms, which would otherwise have the same m/z ratio. This MS protocol was used to follow the phosphorylation of recombinantally purified Erk2 and the dephosphorylation of partially phosphorylated Erk2. Now steady-states can be constructed with both the enzymes present and phosphoforms measured.

B. *Steady-state prediction of Erk phosphoforms*

Multisite modification with two sites satisfies the quadratic invariant. Invariants can be calculated using Grobner bases and other techniques from computational algebraic geometry. This invariant can be visualized as a linear equation

$$m1y1 + m2y2 + m3y3 = m4$$

where the variables $y^1 = (S_{01})^2 / S_{00}S_{11}$, $y^2 = S_{01}S_{10} / S_{00}S_{11}$,$y^3 = (S_{10})^2 / S_{00}S_{11}$ [4].

III. Conclusion

Confirming such steady-state predictions needs novel experiments and new kinds of experimental strategies. We need to quantify the modform/phospho-form distribution, as shown for doubly phosphorylated Erk we need to do systems biochemistry, in which kinases and phosphatases are treated collectively, rather than separately.

References

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