



Modelling cellular signal communication mediated by phosphorylation dependent interaction with 14-3-3 proteins



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ABSTRACT

The 14-3-3 proteins are important effectors of Ser/Thr phosphorylation in eukaryotic cells. Using mathematical modelling we investigated the roles of these proteins as effectors in signalling pathways that involve multi-phosphorylation events. We defined optimal conditions for positive and negative cross-talk. Particularly, synergistic signal interaction was evident at very different sets of binding affinities and phosphorylation kinetics. We identified three classes of 14-3-3 targets that all have two binding sites, but displayed synergistic interaction between converging signalling pathways for different ranges of parameter values. Consequently, these protein targets will respond differently to interventions that affect 14-3-3 binding affinities or phosphorylation kinetics.

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1. Introduction

Cellular adaption and decision-making rely on the communication between multiple signalling pathways. Post-translational protein modification on multiple sites is one important mechanism for signal cross communication. To execute a response specific to such multisite modifications, it often has to be “read” by specialized domains in the receiving signal transduction protein. Among the many specialized cellular signal transduction molecules that have been discovered during recent decades, the family of 14-3-3 proteins occupies a remarkably ubiquitous role as downstream effectors of phosphorylation events. Essentially being soluble dimers of single phospho-Ser/Thr binding domains, the 14-3-3 proteins are reported to bind hundreds of different cellular

proteins, although there are also examples of binding to non-phosphorylated target proteins (for review see [1–3]). Upon binding to their phospho-recognition site, they are reported to affect the target protein (TP) by modulating its activity [4], interaction with other molecules [5], intracellular localization [6] or stability [7].

Dimeric 14-3-3s are required for binding to many targets, and several TPs like the PKC ϵ , Cdc25B, c-Raf and Foxo4 require two sites to be phosphorylated for high affinity binding of 14-3-3 [6,8–10] (see [11] for more examples). The term “gatekeeper site” refers to the primary role of one phospho-site in determining 14-3-3 binding. A secondary phosphorylation site, which in some cases can be more divergent from the consensus sequence, can further contribute to increased affinity binding or induction of structural changes in the TP. The latter is referred to as the “molecular anvil” hypothesis [12]. Multi-phosphorylation events have also been reported to negatively regulate binding of 14-3-3 proteins, by phosphorylation of TPs at a site close to the 14-3-3 interaction site, thereby preventing complex-formation [10,13,14].

Context dependent signalling mechanisms can act at the level of signalling pathways or at their downstream targets. Examples exist for the action of 14-3-3 proteins at both levels [4,13]. We wanted to investigate in more detail how 14-3-3 proteins may influence signal transduction, particularly in multi-pathway communication executed through phosphorylation at multiple sites. A mathematical modelling approach was chosen for generality, but the

Abbreviations: AANAT, aralkylamine N-acetyltransferase; Bad, Bcl-2 antagonist of cell death; Cdc25B, cell division cycle 25B; Foxo4, forkhead box protein O4; MAPKAP-K1, mitogen activated protein kinase activated protein kinase 1; p27Kip1, cyclin-dependent kinase inhibitor 1B/p27; PAK1, p21 protein (Cdc42/Rac)-activated kinase 1; c-Raf/Raf-1, Raf proto-oncogene ser/thr protein kinase; Rap1GAP2, Rap1 GTPase activating protein 2; RGS18, regulator of G-protein signalling 18; r_n , phosphorylation rate constant ratio site n ; r_s , synergy ratio; S1, signal 1; S2, signal 2; TP, target protein; pSn-TP, TP phosphorylated on site n

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modelling was based on previously reported mechanisms of interaction between 14-3-3 and TPs and experimentally derived affinity constants. We first investigated 14-3-3 binding to TP downstream of one signalling pathway and its regulation by phosphorylation kinetics and binding affinities. Next, we modelled inhibitory signal communication for 14-3-3–TP interactions where the TPs in addition had a phosphorylation site that inhibited 14-3-3 binding, a mechanism reported for several TPs. The capability of dual phosphorylation site recognition of 14-3-3 proteins has been suggested to make them functional logic AND-gates or coincidence detectors [11]. Based on the nature of their signal output, we defined three functional classes of dual site 14-3-3 TPs. The influence of phosphorylation rate kinetics of TPs and the binding affinities of 14-3-3 to different phosphorylated states on the signalling response of the different classes was then investigated. In particular, we report on conditions that gave optimal synergistic cross-talk between the two signalling pathways, mediated by 14-3-3 interactions. These conditions varied substantially between the three classes of 14-3-3 TPs. Our findings provide new insights into the regulation of 14-3-3 target proteins and open up for new strategies for therapeutic modification of 14-3-3 regulated processes.

2. Materials and methods

2.1. Modelling of protein phosphorylation and 14-3-3 binding

We refer to the [Supplemental text](#) for details on the modelling approach. The reactions were implemented in the simulation software Copasi (v4.8) [15]. We used the LSODA algorithm for numerical integration using an absolute tolerance of 10^{-12} .

3. Results and discussion

3.1. Signalling interaction through disrupted 14-3-3 binding

Several hundred binding partners have been reported for the 14-3-3 proteins. Most of the available 14-3-3 proteins in a cell can therefore be expected to be bound to different target proteins. To tackle this in our models we classified the bulk cellular 14-3-3 TPs as high and low affinity binders, and used a buffered protein interaction modelling approach for all the calculations ([Supplemental text, Fig. S1](#)).

Starting with the simple case where only one signalling pathway targets the TP 14-3-3 binding site, we show how different signalling strengths (kinase/phosphatase rate ratios, r_R) and K_d -values of 14-3-3 binding can modulate outputs such as complex formation and TP phosphorylation ([Figs. S2 and S3](#)). This simple model would apply to many 14-3-3 TPs. Multiple signalling inputs occur when several kinases or phosphatases target the same phosphorylation site. As such, additional input signals will change the r_R -value, leading to altered response ([Fig. S3](#)).

Alternatively, signalling pathways may modulate the affinity of 14-3-3 binding, e.g., by expression of different 14-3-3 isoforms, modification of 14-3-3s [16,17] or by additional modification of their TPs [18]. The latter mechanism has been shown for the proteins Cdc25B, RGS18, Rap1GAP2 and Bad, where phosphorylation-mediated inhibition of 14-3-3 binding by direct phosphorylation of the TP close to the 14-3-3 binding site has been reported [10,13,14,19]. This provides a mechanism for inhibitory signalling communication, and we investigated this mechanism more closely where one signalling pathway downstream of signal 1 (S1) controls phosphorylation of site 1 on TP, necessary for 14-3-3 binding ([Fig. 1A, Fig. S4A](#)). We modelled the situation where a strong inhibitory phosphorylation was included downstream of signal 2 (S2), that targeted a site 2 on TP and rendered it incapable

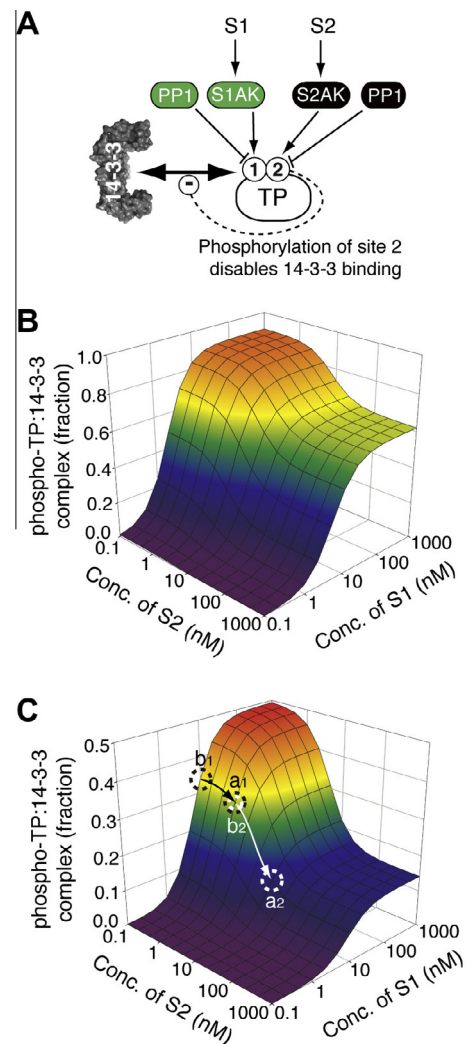


Fig. 1. Inhibitory signal communication. We modelled a TP with two phosphorylation sites (see [Fig. S4A](#) for details on the model) where site 1 was phosphorylated downstream of signal 1 (S1) and site 2 downstream of signal 2 (S2) (A). Phosphorylation of site 1 (pS1-TP, $r_1 = k_{K1}/k_{P1} = 0.1$) initiated binding of 14-3-3 with high affinity, which was necessary for the regulation of TP. Phosphorylation of TP on site 2 (pS2-TP and pS1pS2-TP, $r_2 = k_{K2}/k_{P2} = 5$) inhibited any binding to 14-3-3. Phosphorylation and dephosphorylation of the two sites were independent and did not occur on pS1-TP bound to 14-3-3 ([Fig. S4A](#)). In (B), the amount of TP (phosphorylated only on site 1) in complex with 14-3-3 (pS1-TP:14-3-3 complex, fraction of total TP) is shown for varying signalling strengths of S1 and S2. A K_d of 1 nM was used for the binding of 14-3-3 to pS1-TP. The results in panel (C) were obtained using the same model as in (B), but the K_d of 14-3-3 binding was changed to 10 nM. The dotted circles illustrate changes in TP:14-3-3 complex expected going from basal states of 0.1 or 1.0 nM S2, b1 or b2, respectively, to activated states a1 and a2. The signal strength from S1 is kept constant.

of binding 14-3-3 ([Fig. 1A, Fig. S4A](#)). As the proteins reported to be regulated by this mechanism have the inhibitory phosphorylation site (site 2) placed adjacent to the 14-3-3 binding site (site 1), we assumed that site 2 was unavailable for phosphorylation in the complex between 14-3-3 and pS1-TP.

Expectedly, for high 14-3-3 binding affinities ($K_d = 1$ nM) only modest inhibition was obtained ([Fig. 1B](#)) even at phosphorylation conditions favouring high phosphorylation stoichiometry on the inhibitory site (rate constant ratio of kinase/phosphatase of site 2, $r_2 = 5$). This low inhibition occurred as most of the TP resided in a complex with 14-3-3 unavailable for inhibitory input. This is shown in [Fig. S4B](#), where increasing S1 generated more pS1-TP:14-3-3 complex that was unresponsive to S2 signal input. A lower affinity ($K_d = 10$ nM) opened for more sensitive pathway communication

(Fig. 1C). In comparison, the influence of 14-3-3 proteins on site 2 phosphorylation was reciprocal for the two situations (Fig. S4B and C). For both TPs, the strength of signalling communication depended on the basal signalling conditions of the inhibitory signal and the signalling strength on site 1. Thus, a cell exposed to basal stimuli of 1 nM S2 (point b2 in Fig. 1C) is much more sensitive to a 10-fold increase in inhibitory signal than one experiencing a basal S2 level of 0.1 nM (point b1 in Fig. 1C). We suggest that for high affinity 14-3-3 binding TPs, this mechanism for negative regulation would only be efficient if both pathways could be modified in conjunction. This may occur automatically as the two phosphorylation sites in general are adjacent to each other. Hence, it is expected that phosphorylation of one site would also affect the phosphorylation or dephosphorylation of the other site. However, to our knowledge the possible interdependence has not been investigated for any of the reported TPs that are regulated by this mechanism.

3.2. Synergistic signal communication with 14-3-3 protein interactions

We wanted to investigate the ability of 14-3-3 proteins to mediate positive signalling cross-talk through their dual phospho-site binding capability. Several reported 14-3-3 binding proteins such as PKC ϵ , c-Raf, AANAT, Foxo4 and Bad have reported gatekeeper sites, that contribute the most to 14-3-3 binding, with secondary sites that further increase the affinity of the complex [4,8–10,20]. We modelled a TP where the binding of 14-3-3 was facilitated by phosphorylation on two sites (Fig. 2). Due to the symmetry of this model, we considered site 1 to be a gatekeeper site ($K_{d1} \leq K_{d2}$), and binding to TP phosphorylated on both site 1 and 2 (pS1pS2-TP) was at least as strong as that for site 1 ($K_{d1,2} \leq K_{d1}$). Similar to the case with single phospho-site TPs (Fig. S2), we considered different output types for phospho-TP:14-3-3 complexes (Fig. 2): Class 1, the type of phospho-TP:14-3-3 complexes where binding to any of the two sites was sufficient to mediate the biological effect (see [11]); class 2, where the functional output of 14-3-3 association

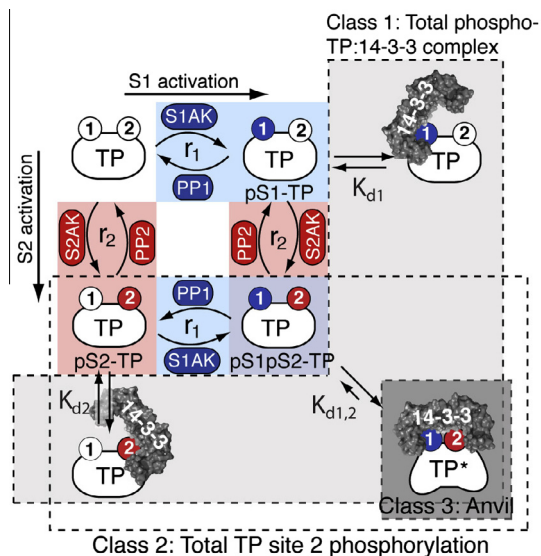


Fig. 2. Target proteins with two-site 14-3-3 binding. The model describes a TP phosphorylated on two different phosphorylation sites by signalling pathways modelled as described (Fig. 1 and Fig. S4). However, for this model both site 1 and 2 facilitated binding to 14-3-3 proteins. The figure shows the phosphorylation, dephosphorylation, and 14-3-3 binding reactions for TP upon activation of S1-pathway (blue) or S2-pathway (red). The binding of 14-3-3 to single-phosphorylated TP is quantified by K_{d1} or K_{d2} , for binding to pS1-TP or pS2-TP, respectively. Binding of 14-3-3 to double-phosphorylated TP (pS1pS2-TP) is given by $K_{d1,2}$. Three functionally different output types were defined: Class 1, total phospho-TP:14-3-3 complex, class 2, total TP phosphorylated on site 2 and class 3, 14-3-3 complex with doubly phosphorylated TP (pS1pS2-TP:14-3-3).

was increased phosphorylation of one of the sites (here shown for site 2) [20]. Finally, we defined class 3 as the type of TP where 14-3-3 binding to doubly phosphorylated TP provided the alterations in bioactivity, similar to the anvil type of complex reported for AANAT and Cdc25B [4,21].

We modelled first the case with modest affinity for 14-3-3 binding to TP when only one site was phosphorylated, whereas high affinity binding was included for the double-phosphorylated TP ($K_{d1} = 100$ nM, $K_{d2} = 1.0$ μ M, $K_{d1,2} = 1$ nM). We also used relatively low kinase activity relative to phosphatase (ratio for site n , $r_n = k_{Kn}/k_{Pn}$ (Supplemental Eqs. S1 and S2), $r_1 = r_2 = 0.1$). This was expected to provide little output when only one signalling pathway was activated, but much higher output values when both signalling pathways were activated (Fig. 3). The steady state outputs of the three different classes of 14-3-3 TP were then calculated for values of signal 1 (S1) and signal 2 (S2) varying between 0.1 nM (basal) and 1 μ M (high) (half maximal activation of kinase at 10 nM, Eq. S3). For both class 1 and 2, a low response was found ($\sim 10\%$) when only one signal was activated, whereas much higher activation was found when both signalling pathways were stimulated (Fig. 3A,C). As expected, the formed pS1pS2-TP:14-3-3 complex (class 3) had extremely low response to single-pathway stimulations (Fig. 3E), whereas in general the three values of the output types were quite similar (shown as fraction of total TP).

From the surface plots it was possible to calculate the synergy obtained by dual pathway stimulation compared to that obtained by the two pathways alone (Fig. 3B, D and F). We chose to calculate the ratio of the output for when both S1 and S2 are stimulated to that of the sum of outputs obtained with single pathway stimulation. We refer to this as the synergy ratio (r_s), and r_s will be > 1 if higher activation is obtained than could be expected from the combined effect of both pathways. The r_s for total phospho-TP:14-3-3 complex (Fig. 3B) and total TP site 2 phosphorylation (Fig. 3D) was very similar (about 5, Fig. 3D), whereas that for the anvil type was extremely high (> 200 ; Fig. 3F) due to very low levels of 14-3-3 complex with dual-phosphorylated TP when only one signalling pathway was activated.

3.3. Effect of binding affinity and phosphorylation kinetics on signalling pathway synergy

The similar behaviour of the three output classes led us to investigate how changes in the value of K_{d1} and r_2 (phosphorylation rate ratio on site 2) affected their signalling response (Fig. 4). First, lowering the K_{d1} -value to 10 nM increased 14-3-3 binding in response to S1 as expected, whereas the AND-response (S1 and S2) was not enhanced accordingly (Fig. 4A). This led to a strong decrease in the r_s of total phospho-TP:14-3-3 (Fig. 4E), whereas the change in TP site-2 phosphorylation was much lower (Fig. 4B and F).

We next investigated the effect of decreasing the activity constant of the protein phosphatase acting on site 2. This gave a more robust increase in total phospho-TP:14-3-3 complex and of TP site 2 phosphorylation (Fig. 4C and D) upon stimulation with both S1 and S2. However, only the synergy for total phospho-TP:14-3-3 complex response was increased (Fig. 4E), as TP site 2 phosphorylation in response to S2 alone also increased considerably (Fig. 4D). The two outputs therefore varied in their response to changes in different parameters. The synergy ratio for the class 3 output type varied little between the mentioned parameters (data not shown).

3.4. A comprehensive investigation of parameter variation reveals large differences between the classes of functional outputs

The results above show that both phosphorylation kinetics and binding affinities affect the response strength and synergy

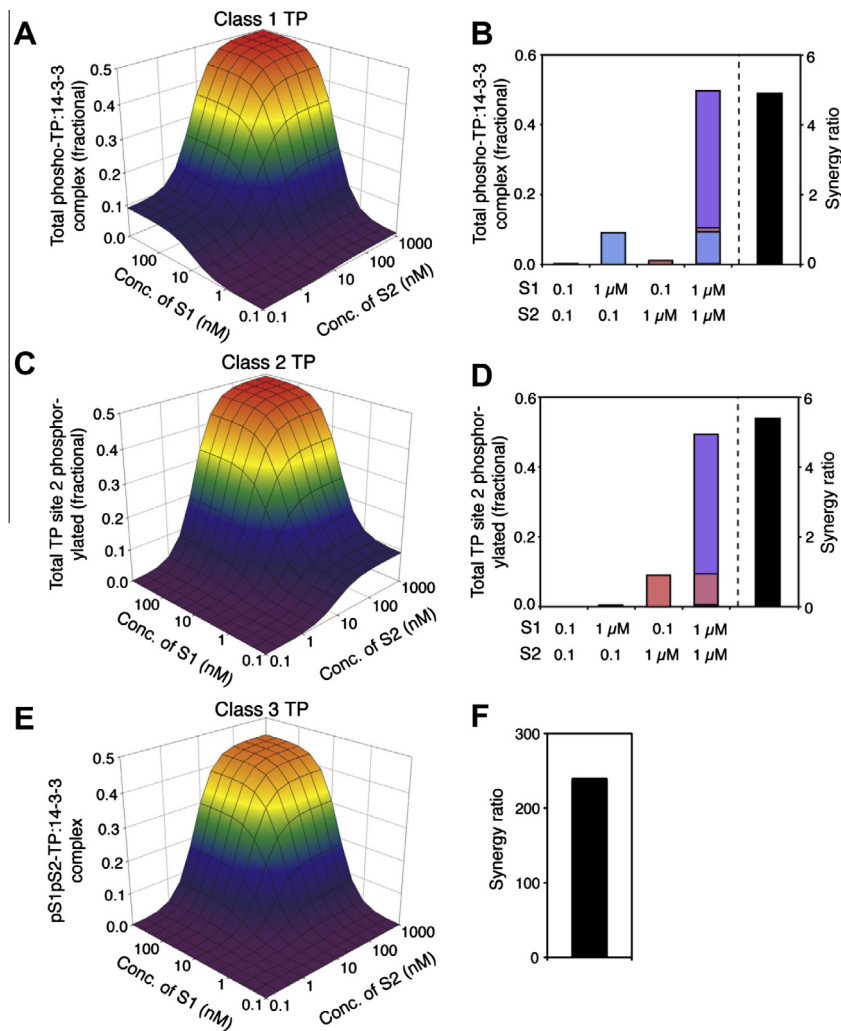


Fig. 3. Responses to multiple signalling inputs. A two-site TP (Fig. 2) was modelled using rate constant ratios ($r_n = k_{kn}/k_{pn}$, rate constants for kinase and phosphatase acting on site n) for site 1 and 2, r_1 and r_2 , set to 0.1. Moderate to weak affinity was used for singly phosphorylated TP ($K_{d1} = 100$ nM, $K_{d2} = 1.0$ μM) and high affinity binding for double phosphorylated TP ($K_{d1,2} = 1.0$ nM). Panel (A) shows the fraction of total TP bound to 14-3-3 proteins (class 1 TP) for values of S1 and S2 ranging from 0.1 nM (basal stimuli) to 1.0 μM (high stimuli). The response values at S1 and S2 at basal or high stimuli were used to calculate the synergy ratio, r_s , defined as the ratio of signal, above basal, observed when both S1 and S2 were high to the sum of that obtained for single stimuli of S1 and S2. (B) Shows the observed signal output of the class 1 type at basal and high S1 or S2 signal strengths. The corresponding r_s -value is also shown. Panel (C) and (D) shows the response for TP class 2 where 14-3-3 increases site 2 phosphorylation, analogous to that for (A) and (B), respectively. The signalling response for a class 3 TP for the same parameters is also shown (E) with the corresponding synergy ratio (F).

between the signalling pathways. They also suggest that there are differences between the three types of functional classes of 14-3-3 effector mechanisms. We therefore performed a comprehensive analysis of these three output classes and how their response changed with different values of r_1 and r_2 (0.01–100) and K_{d1} , K_{d2} , $K_{d1,2}$ (1 nM–1 μM, $K_{d1,2} \leq K_{d1} \leq K_{d2}$), which is within the typical range of K_d -values reported for 14-3-3 complexes (Table S2). We compared the responses to basal and high levels of S1 and S2 and calculated the synergy ratio (r_s) for each parameter sets (Figs. S5–S11), as this would be an important factor when considering communication between signalling pathways.

We found that for TPs where any of the three phospho-TP:14-3-3 complexes provide functional outputs (class 1), the synergetic signalling response was critically dependent on lower affinity binding to monophosphorylated TP compared to that for dual-phosphorylated TP. Increased affinity of 14-3-3 binding to pS2-TP greatly decreased the opportunity for pathway synergies, as complex formation of 14-3-3 to singly phosphorylated TP competed more strongly with that of binding to pS1pS2-TP.

It was interesting to note the narrow range of values for r_1 and r_2 that allowed potent pathway synergies and how these ranges

shift upon changes in affinities. In particular, we noted that the synergies for total phospho-TP:14-3-3 complex (class 1) and TP site 2 amplification complex type (class 2) had maxima at quite different ranges of r_1 and r_2 , for K_d -values where they both showed synergies (Fig. 5). Thus, whereas pS2-TP phosphorylation in general showed maximal synergy at low r_2 values (0.01) and medium r_1 -values (≈ 1), the total phospho-TP:14-3-3 complex had maximal synergy at medium to low r_1 values (< 1) and medium r_2 values (≈ 1) (Fig. 5A and B). The synergy of class 2 TPs was also much more robust against values of K_{d1} and K_{d2} approaching that of $K_{d1,2}$. In fact, decreasing both K_{d1} and K_{d2} retained much of the synergy and led to a peculiar r_2 -shift where maximal synergies were now observed at high r_2 -values (Fig. 5B and D). Going from a flat non-synergistic response curve at high K_{d2} and $K_{d1} = K_{d1,2}$, this shift occurred as K_{d2} decreased. At high site 2 phosphorylating conditions (high r_2) 14-3-3 complex formation could then be shifted away from pS1-TP, which does not contribute to the functional output of the class 2 TPs (Fig. S9).

As observed above, the synergy of pS1pS2-TP:14-3-3 formation (class 3 TP) was high for all sets of K_d -values, but was higher at lower values of r_1 and r_2 (Figs. S5, S10 and S11). Increasing the

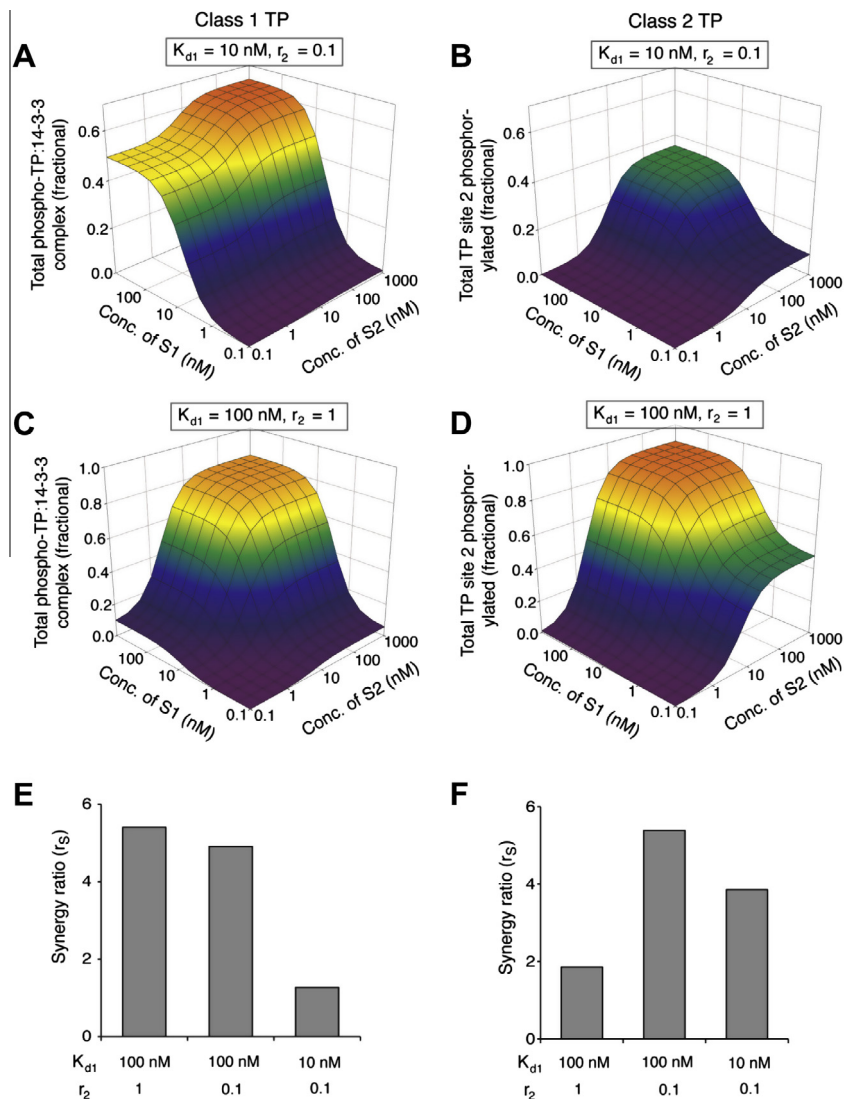


Fig. 4. Different effects of site 1 affinity and site 2 phosphorylation kinetics on signalling responses. The model from Fig. 3 was used, except that K_{d1} was decreased to 10 nM (A and B) or the phosphorylation rate ratio for site 2 (r_2) was increased to 1 (C and D). The response to different inputs of S1 or S2 was then calculated for output class 1 (total phospho-TP:14-3-3 complex (fractional) (A and C) or for total TP site 2 phosphorylation (fractional) (B and D). The corresponding synergy ratios were then calculated for total phospho-TP:14-3-3 complex (E) and total TP site 2 phosphorylation stoichiometry (F) for the three parameter sets, including that used in Fig. 3.

affinity for pS1-TP led to higher dependency of low r_1 -values, whereas decreasing both K_{d1} and K_{d2} led to a narrowing of high synergy peak giving a saddle along the line of $r_1 = r_2$. It should be noted that the extreme synergy values observed for very low r_1 -values are probably less relevant biologically as very low phosphorylation levels of low abundance proteins will be subject to stochastic variation, in particular in compartments confined to smaller volumes. Thus, the less spectacular, but still very strong synergy levels (<100) are probably more biologically feasible.

3.5. Implications of parameter sensitivities for regulation of cell signalling processes

Synergistic interactions between signalling pathways provide opportunity for context-dependent responses, but also for filtering noise. Although several known TPs have increased binding affinity for 14-3-3 upon dual phosphorylation, this increase was inadequate to guarantee signalling synergism (Figs. S5–S11). In particular, to promote synergistic output of class 1 14-3-3 TPs, binding to singly phosphorylated TP should occur with relatively

low affinity, particularly for the secondary 14-3-3 binding site (site 2). In addition, the gatekeeper site should operate at low kinase/phosphatase ratios.

The proapoptotic protein Bad is as an example of a class 1 TP. The protein complex with 14-3-3 provides a functional output by localising Bad in the cytosol, away from the outer mitochondrial membrane. Phosphorylation at Ser136 and Ser112 (murine Bad numbering) cooperate to regulate its interaction with 14-3-3 proteins [20]. Synergistic signalling would be of interest to inhibit Bad sequestration in cancer or to increase its 14-3-3 binding for neuro- or cardioprotection. For Bad the K_d -values for binding to Ser136 and Ser112, although not quantified in isolation, seem to fulfil the criteria for synergistic interaction to occur. Thus, Ser136 seems to play a primary role in controlling 14-3-3 binding, whereas Ser112 alone shows weaker binding and functions to further increase the affinity for 14-3-3 binding [20,22,23]. Additional requirement for synergy between the two pathways is low r_1 (the AKT or p70S6 kinase site) and $r_2 \sim 1$ (Ser112, analogous to site 2, phosphorylated by MAPKAP-K1 and PAK1). Furthermore, as discussed above (Section 3.1), the strength of inhibitory cross-talk

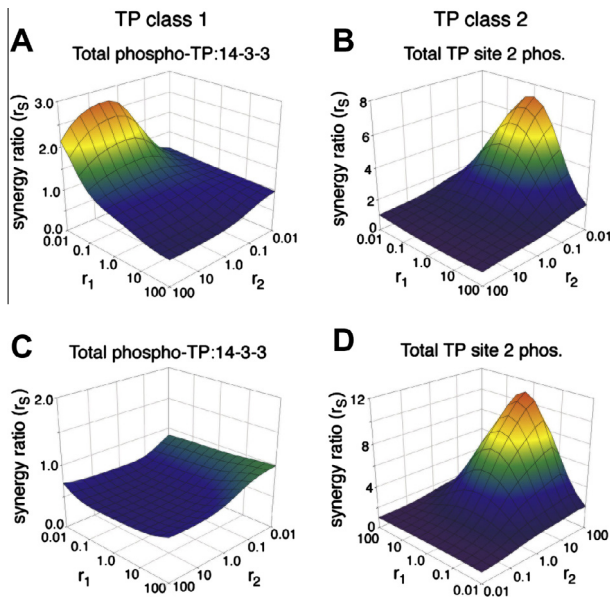


Fig. 5. Synergistic signalling is observed for different parameters for the three classes of TPs. Synergy ratios > 1 were observed for all choices of K_d -values for the anvil type of interaction (class 3). For all panels $K_{d1,2}$ was set to 1 nM, whereas K_{d1} was 10 nM (A and B) or 1 nM (C and D) and K_{d2} was 1.0 μ M (A and B) or 10 nM (C and D). The synergy ratio (r_s) was then calculated for all sets of r_1 and r_2 for TPs where the level of total phospho-TP:14-3-3 (TP of class 1) provides the functional output (A and C) or where total level of TP site 2 phosphorylation (TP of class 2) was important (B and D). See Figs. S5–S11 for other conditions.

between pathways depended on the affinity of 14-3-3 binding. Thus, the inhibitory JNK site reported in Bad (Ser128) [19] is predicted to differentially modulate the interaction with 14-3-3 depending on the affinity, i.e., if Bad is phosphorylated on Ser112 and/or Ser136. These additional requirements may explain some of the conflicting observations regarding these sites [20,24,25]. Still, considerate sequence difference between human and murine Bad, multiple isoforms, additional phosphorylation sites and other types of modifications are all complicating factors for understanding the interaction of Bad with 14-3-3s.

Numerous compounds are now available for pharmacological intervention of 14-3-3 protein interactions that can stabilise or destabilise complex-formation [26,27]. For more simple interactions involving one pathway TPs, the binding of 14-3-3 increases the robustness for perturbations of the upstream signalling pathway (r_R , Figs. S2 and S3). Efficient signal attenuation therefore necessitates both decreased affinity for 14-3-3 and inhibition of kinase/signalling input. For inhibitory cross talk, it may readily be amplified or attenuated by compounds that stabilise or destabilise the pS1-TP:14-3-3 complex, respectively. For synergistic signal communication the differences in parameter robustness and optimality could be used for selectivity among the TP classes or to compensate for off-target effects. Class 3 type of TP showed highly robust signalling output to all parameters, but can be inhibited by inhibition of both binding affinity and signalling input. For TPs of class 1 and 2 targeted by the same signalling pathways, a convenient strategy could be to decrease K_{d2} and r_1 to inhibit class 1 or to decrease K_{d1} and increase K_{d2} for class 2 TP.

4. Concluding remarks

We have investigated the quantitative requirements of signalling processes involving 14-3-3 proteins. For inhibitory signalling by phosphorylation of TPs, efficient communication depends both

on basal signalling status, as well as the 14-3-3 binding affinity. Similarly, robust signalling synergy was only observed for a limited range of affinities and phosphorylation kinetics, suggesting that it may only be observed for moderate signal strengths. Different classes of 14-3-3 TPs also responded synergistically to dual signalling inputs within very different ranges of parameters.

The kinetic properties of various 14-3-3 TPs and their phosphorylation are still largely uncharacterized, especially in cells. However, for some well-studied TPs like Bad, measurements suggest that synergetic signalling interaction is facilitated *in vivo*. Our findings also suggest novel strategies for intervention. Thus, selective modulation of different classes of 14-3-3 binding proteins can be achieved by combining modulation of kinase activity with compounds that directly target the binding of 14-3-3 proteins to their TPs [26,28].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.febslet.2013.11.012>.

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