Inference of Predictive Phospho-regulatory Networks from LC-MS/MS Phosphoproteomics Data

Sebastian Vlaic[1], Robert Altwasser[1], Peter Kupfer[1], Carol L. Nilsson[2], Mark Emmett[3], Anke Meyer-Baese[3] and Reinhard Guthke[1]

[1]*Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, Beutenbergstr. 11a,D-07745 Jena, Germany*

[2]*Department of Pharmacology & Toxicology, The University of Texas Medical Branch, Galveston, Texas 77555-1074, U.S.A.*

[3]*Department of Scientific Computing, Florida State University, Tallahassee, Florida 32310-4120, U.S.A.*

Keywords: Phospho-regulatory networks, Network Inference, Glioblastoma Cancer Stem Cells.

Abstract: In the field of transcriptomics data the automated inference of predictive gene regulatory networks from high-throughput data is a common approach for the identification of novel genes with potential therapeutic value. Sophisticated methods have been developed that extensively make use of diverse sources of prior-knowledge to obtain biologically relevant hypotheses. Transferring such concepts to the field of phosphoproteomics data has the potential to reveal new insights into phosphorylation-related signaling mechanisms. In this study we conceptually adapt the TILAR network inference algorithm for the inference of a phospho-regulatory network. Therefore, we use published phosphoproteomics data of WP1193 treated and IL6-stimulated glioblastoma stem cells under normoxic and hypoxic condition. Peptides corresponding to 21 differentially phosphorylated proteins were used for network inference. Topological analysis of the phospho-regulatory network suggests lamin B2 (LMNB2) and spectrin, beta, non-erythrocytic 1 (SPTBN1) as potential hub-proteins associated with the alteration of phosphorylation under the observed conditions. Altogether, our results show that inference of phospho-regulatory networks can aid in the understanding of complex molecular mechanisms and cellular processes of biological systems.

1 INTRODUCTION

Network inference is a well established tool to uncover the complex underlying regulatory mechanisms of biological systems. The immediate reaction of a cell towards changing conditions or external perturbations is often mediated by alteration of already existing proteins via post-translational modifications such as phosphorylation, methylation, acetylation, sumoylation or ubiquitination (Seet et al., 2006).

Changes in phosphorylation were found to have an impact on almost all aspects of cell biology (Jørgensen and Linding, 2008). Therefore, the analysis of causal relations between changes in phosphorylation state of proteins is essential for understanding regulatory mechanisms of the biological system studied. For the inference of gene regulatory networks (GRNs) a variety of diverse approaches have been proposed, which can be used to identify causal relations in the expression of genes (Hecker et al., 2009b). The adaptation and application of concepts developed for GRNs to data from other layers of regulation has therefore the potential to provide new insights into regulatory relations of cellular processes.

With analytical methods such as high resolution mass spectrometry, the quality and quantity of phosphoproteomics data is continuously increasing. This offers new possibilities but also challenges regarding interpretation and computational analysis of large scale data sets. (Terfve and Saez-Rodriguez, 2012) divided the approaches that have been applied to high-throughput phosphoproteomics data into two categories. On the one hand, there are descriptive methods such as mapping of measured data to pathways, enrichment analysis, and supervised as well as unsupervised learning methods. These approaches provide an overview of the data and often serve as a starting point for a more detailed investigation. On the other hand, methods such as difference and differential equations, Bayesian, boolean and rule-based

Vlaic, S., Altwasser, R., Kupfer, P., Nilsson, C., Emmett, M., Meyer-Baese, A. and Guthke, R.
Inference of Predictive Phospho-regulatory Networks from LC-MS/MS Phosphoproteomics Data.
DOI: 10.5220/0005743000850091
In *Proceedings of the 9th International Joint Conference on Biomedical Engineering Systems and Technologies (BIOSTEC 2016) - Volume 3: BIOINFORMATICS*, pages 85-91
ISBN: 978-989-758-170-0

networks are considered to be predictive since they are able to process measured data and produce interpretable models that predict the behavior of biological systems.

In this study, we apply an inference algorithm that combines these two concepts by using knowledge of descriptive approaches to produce predictive models, based on a system of linear equations. To this end, we apply the previously published transcription factor binding site integrating LARS (TILAR) algorithm (Hecker et al., 2009a), which uses a linear model to infer GRNs from expression data based on least angle regression (LARS) (Efron et al., 2004). One feature of TILAR is its ability to make use of several sources of prior-knowledge. Initially, a network structure template is created, which is iteratively refined to explain the measured data in the best possible way, while integrating as much prior-knowledge as possible. The concept of modeling is based on the idea that the expression of genes is regulated by transcription factors (TFs). This introduces the possibility of using known transcription factor binding sites (TFBSs) as knowledge base to group genes that are regulated by the same TFs.

Analogous to the TFBS-based concept of TILAR, we will show how prior-knowledge about phosphorylation regulating phosphatases and kinases (PKs) can be used to group different phosphorylation sites creating a network template for the inference of phosphoregulatory networks (PRNs). We will exemplify this idea by applying the algorithm to published phosphoproteomics data of glioblastoma stem cells (GSC11) perturbed with the JAK2/STAT3 phosphorylation inhibitor WP1193, IL-6 stimulation and hypoxia.

2 METHODS

2.1 Data Pre-processing

Raw data from (Nilsson et al., 2010) was processed using R 2.14 (R Core Team, 2014). Duplicated peptide measurements were mean averaged and log10 transformed. Peptides containing no tyrosine, serine or threonine amino acid were removed from the list of measured peptides, since only those can be phosphorylated.

2.2 TILAR Concept of Modeling for the Inference of GRNs

The basic idea of the TILAR algorithm as published by (Hecker et al., 2009a) is to include knowledge about the expression regulation of the modeled genes by TFs. Accordingly, the resulting GRNs are composed of relations between the regulating TFs and their target genes (*TF-to-gene* relations) as well as relations between the modeled genes and the regulating TFs (*gene-to-TF* relations). The *TF-to-gene* relations can be, e.g., derived from literature, TFBS-databases or TFBS prediction tools and serve as a network structure template for the inference of the *gene-to-TF* relations. The prior-knowledge structure template can thus effectively assist the algorithm in the identification of biologically meaningful networks. Using TILAR, the predicted expression value of a gene i ($\hat{x}_i$) (with $i = 1 \ldots N$) is the sum of the weighted expression values ($w_{kj}x_j$) of all other genes j via the TF k (with $k = 1 \ldots F$) if (1) the regulated gene i has a TFBS for the TF k and (2) the gene j does not have a TFBS for the TF k (equation 1).

$$\hat{x}_i = \sum_{k=1}^{F} \sum_{j=1}^{N} (1 - b_{kj}) w_{kj} x_j b_{ki}$$
$$b_{kj} = \begin{cases} 1, & \text{if gene } j \text{ possesses a binding site for TF } k \\ 0, & \text{else} \end{cases} \quad (1)$$

The result is a system of linear equations for each gene that has a TFBS for at least one TF. The interaction weights w_{kj} corresponding to the *gene-to-TF* relations can then be estimated by LARS regression (see (Hecker et al., 2009a) for details).

2.3 Network Inference

Network inference of PRNs is performed similar to the inference of GRNs using TILAR (see (Hecker et al., 2009a) for details). The conceptually adapted TILAR algorithm uses the PK binding knowledge as a structure template. Variable selection and estimation is performed using LARS. Initially, the model that minimizes Mallows Cp statistic (Mallows, 1973) is selected and the achieved residual sums of squares (RSS) serves as the initial error. Subsequently, stepwise backward elimination is performed to iteratively remove *PK-to-peptide* edges from the template for which inference with LARS results in a model with a RSS lower than the one measured in the previous iteration. The iterative algorithm stops when the removal of a *PK-to-peptide* knowledge edge does not decrease the RSS of the model. The final model is then selected based on the drop of the RSS compared to the amount of model parameters. The model parameters are then estimated using linear regression.

3 RESULTS

3.1 Data Pre-processing

Peptides with an absolute log10 fold-change greater 0.3 between treatment and control were selected as differentially phosphorylated peptides (DPPs). This was checked for the WP1193 treated, IL6 stimulated cells with respect to their corresponding WP1193 treated control under normoxic (NO) (IL6-NO) and under hypoxic (HO) (IL6-HO) conditions separately. 11 DPPs were identified for the IL6-NO condition and 13 DPPs for the IL6-HO condition, each corresponding to a unique protein (Table 1). The results show that only 2 peptides overlap between these two sets of DPPs, corresponding to septin 2 (SEPT2) and spectrin repeat containing, nuclear envelope 2 (SYNE2). Furthermore, we identified 28 DPPs between the two stimuli, i.e., IL6-NO and IL6-HO respectively (HO-NO), to include the effect of the condition under which the cells where cultured.

Table 1: Proteins (Name) corresponding to the DPPs identified in the different comparisons.

Comparison	Name
IL6 - NO	EIF3C, GTF2F1, LMNA, MSN, NES, NUMA1, SEPT2, SLTM, SPTBN1, SRSF1, SYNE2
IL6 - HO	EIF4G3, FSCN1, HSP90B1, ILF3, KRT1, LMNB2, MARCKS, NOLC1, RBM8A, SEPT2, SYNE2, TUBB3, YBX1
HO - NO	CLASP1, EIF3C, EIF4G3, G3BP1, GTF2F1, ILF3, KRT1, LMNB2, MAP1B, MARCKS, MCM3, MSN, MYH9, NES, NOLC1, NUMA1, PI4KB, PLCB3, SEPT2, SEPT7, SLTM, SRSF1, SRSF11, SYNE2, TUBB3, VIM, YBX1

DAVID (Huang et al., 2009) was applied to identify significantly enriched Gene Ontology (GO)-terms using the union of all sets of DPPs. For a Benjamini-Hochberg (BH)-corrected p-value < 0.01 the results show mainly categories related to the cell cycle or structure building processes (Table 2).

3.2 Network Inference

3.2.1 Application of the TILAR Concept to Phosphoproteomic Data

The TILAR algorithm is primarily applied for the inference of GRNs using gene expression data (see methods). The basic idea of the adapted algorithm is that a change in the phosphorylation state of a peptide alters the activity of the corresponding protein. As a result, this protein potentially changes the activity of PKs and therefore, the phosphorylation state of another peptide. Given our data the identified DPPs all correspond to a unique protein and thus, the adaption is straight forward and can be formulated as outlined in equation 1 in the methods section. The predicted change in phosphorylation state of the peptide i (x_i , with $i = 1 \dots N$) is the sum of the weighted (w_{kj}) change in activity of the protein j ($w_{kj}x_j$) via the PK k (with $k = 1 \dots F$) if (1) the change in the phosphorylation state of peptide i is mediated by the PK k , and (2) the phosphorylation state of any peptide of protein j is not.

Table 2: List of significantly enriched GO-terms identified with DAVID. Shown are the GO-terms (GO-term), the corresponding id (GO-term ID), the number of DPPs associated with the term (Count) and the BH-corrected p-value (Pvalue).

GO-term ID	GO-term	Count	Pvalue
GO:0005198	structural molecule activity	12	1.16e-5
GO:0008092	cytoskeletal protein binding	9	8.47e-4
GO:0000279	M phase	8	1.56e-3
GO:0003779	actin binding	7	3.35e-3
GO:0022403	cell cycle phase	8	3.51e-3
GO:0003723	RNA binding	9	5.15e-3
GO:0007049	cell cycle	9	7.77e-3
GO:0048285	organelle fission	6	8.23e-3
GO:0022402	cell cycle process	8	8.46e-3
GO:0000087	M phase of mitotic cell cycle	6	9.26e-3

3.3 Extraction of Prior-knowledge

According to the adapted concept there are two types of prior-knowledge that can be integrated into the inference process, i.e., knowledge about PKs that are known to alter the phosphorylation of any of the DPPs (*PK-to-peptide*) and knowledge about relations between the corresponding proteins and the regulating PKs (*protein-to-PK*). Accordingly, PhosphoSitePlus (Hornbeck et al., 2012) was used to find PKs known to alter the phosphorylation state of the DPPs. Using this approach, regulators for five DPPs were extracted corresponding to the proteins phospholipase C, beta 3, phosphatidylinositol-specific (PLCB3), minichromosome maintenance complex component 3 (MCM3), myosin (MSN), heavy chain 9, non-muscle (MYH9), SEPT2 and eukaryotic translation initiation factor 4 gamma, 3 (EIF4G3). Additionally, NetworKIN (Linding et al., 2008) as well as GPS 2.0 (Xue et al., 2008) were used to predict binding PKs for the re-

maining DPPs. The obtained prior-knowledge network was used as a template for the network inference with TILAR. We also used PathwayStudio 9 (Nikitin et al., 2003) to find known regulatory interactions between the proteins of the corresponding peptides and the identified PKs (*protein-to-PK*) as well as known regulatory interactions between the proteins themselves. However, we did not find any supporting known relation.

3.4 Network Inference

DPPs with corresponding proteins associated to the GO-terms 'cell cycle' (9 DPPs) and 'structural molecule activity' (12 DPPs) were selected to investigate the effect of IL6 stimulation in WP1193 treated GSC11 cells with respect to the different oxidation environments. Four DPPs were found to overlap between both groups leading to a total of 17 DPPs. While 'structural molecule activity' is the term with the smallest p-value we selected 'cell cycle' as a second category since it is the most general term among the other cell cycle related GO-terms. Moreover, all DPPs annotated with one of the cell-cycle associated GO-terms are contained in GO-term 'cell cycle'. Additionally, we included myristoylated alanine-rich protein kinase C substrate (MARCKS), GTPase activating protein SH3 domain Binding Protein 1 (G3BP1), general transcription factor IIF, polypeptide 1, 74kDa (GTF2F1) and Y box binding protein 1 (YBX1), as the corresponding peptides share common PK regulators with the 17 selected peptides. Altogether, 21 DPPs were selected for the inference. After refinement of the initial structure template and LARS regression (see methods) the final model was manually selected. Figure 1 shows that an increase in the number of parameters (green line) in the model beyond the selected cutoff of 41 (black dotted line) does not drastically decrease the RSS (red line).

The final PRN (Figure 2) is composed of 18 inferred edges, 36 prior-knowledge edges, 19 PK-nodes and

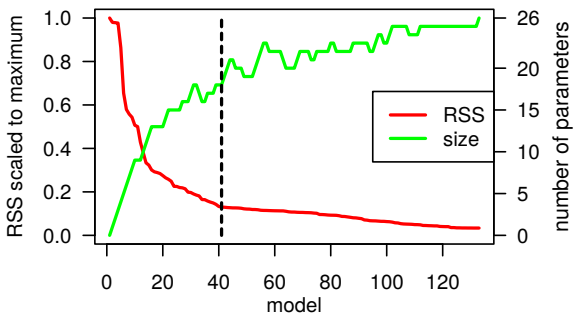

Figure 1: Drop of the RSS with increasing model size.

18 proteins, representing the corresponding DPPs. The proteins keratin 1, type II (KRT1), MSN and YBX1 are not included in the network since no *protein-to-PK* edge was inferred connecting the corresponding DPPs to any PK in the network. Lamin B2 (LMNB2) and spectrin, beta, non-erythrocytic 1 (SPTBN1) are the two nodes with the highest out-degree indicating a signal distributing function in the PRN. Stimulating the cells with IL6, the peptide that denotes for LMNB2 is differentially phosphorylated under HO condition and not affected under NO condition, whereas SPTBN1 is dephosphorylated under NO conditions and not affected under HO conditions. Notably, the PRN itself shows a clustering of proteins associated with either of the two GO terms. Interleukin enhancer binding factor 3, 90kDa (ILF3), nucleolar and coiled-body phosphoprotein 1 (NOLC1), septin 7 (SEPT7), SEPT2, MARCKS and CLASP1 form a cluster that is mainly regulated by LMNB2 and tubulin, beta 3 class III (TUBB3). These proteins are mostly associated with the mitotic phase of the cell cycle. Interestingly, all DPPs beside SEPT2 are affected by IL6 treatment in HO, but not NO. In contrast, GTF2F1, lamin A/C (LMNA), nestin (NES) and nuclear mitotic apparatus protein 1 (NUMA1) are mainly regulated by SPTBN1. All of the associated DPPs are affected by IL6 treatment in NO but not HO.

4 DISCUSSION

In our data set we identified only DPPs corresponding to unique proteins. However, it is well known that multiple phosphorylation sites can belong to the same protein and can be phosphorylated independently producing many phosphoisoforms leading to a potentially distinct activity of the protein (Yang, 2005). In cases of multiple DPPs for one protein, these biological properties are important and will have to be considered in future studies. Our results highlight two structurally connected groups of proteins in the inferred PRN, which are either mostly differentially phosphorylated in HO (ILF3, TUBB3, NOLC1, SEPT2, CLASP1, LMNB2) or in NO (SPTBN1, NES, LMNA, GTF2F1, NUMA1). Within the PRN the de-phosphorylation of ILF3 and NOLC1 is mainly due to the negative relation to the phosphorylated MARCKS. This is in agreement with literature as ILF3 and NOLC1 are known to be phosphorylated during the mitotic phase of the cell cycle (Smith and Miskimins, 2011; Pai et al., 1995). Moreover, Rombouts et al. hypothesized based on their data that phosphorylation of MARCKS could inhibit the cell cycle (Rombouts et al., 2012). In our

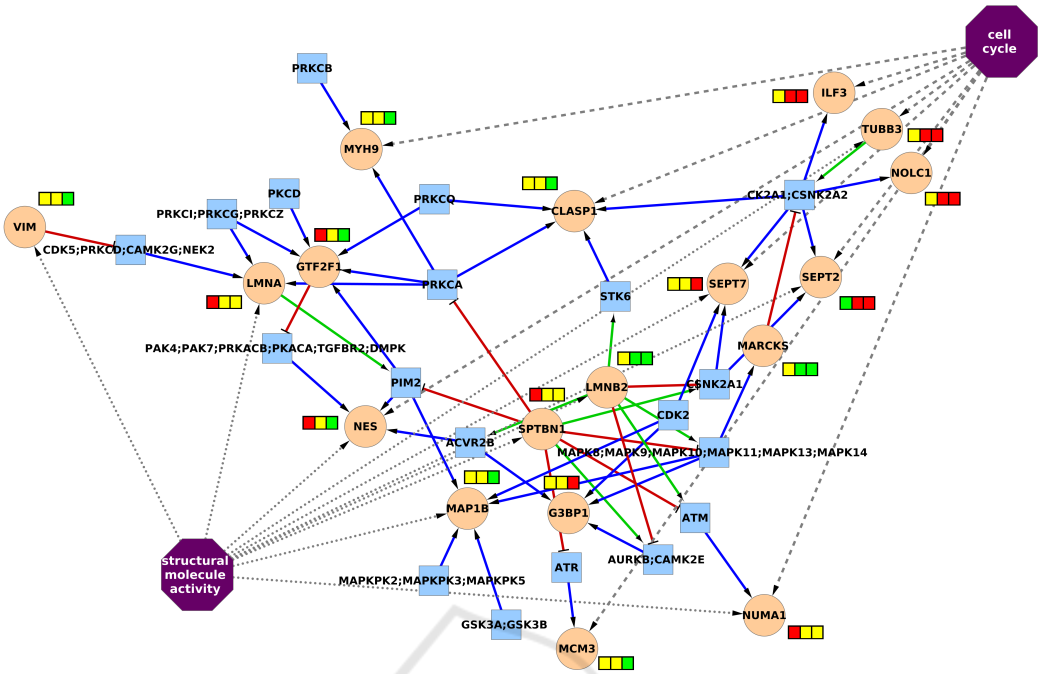

Figure 2: The inferred PRN. Rectangles denote for PKs and circles represent Proteins of the corresponding DPPs. GO-terms are outlined as octagons and associated proteins are connected with dotted lines. Blue edges correspond to the extracted *PK-to-peptide* prior-knowledge while red and green edges represent the inferred inhibiting and activating edges, respectively. Colored annotation boxes denote for direction of regulation (green: up; red: down; yellow: none) for the comparisons (left:) IL6-NO; (middle:) IL6-HO; (right:) HO-NO.

PRN, the increase in phosphorylated MARCKS follows an increase in LMNB2 phosphorylation. Lamins are known to be phosphorylated during mitosis to mediate matrix disassembly (Dechat et al., 2008). This indicates that the mitotic phase might be initiated under the HO condition, but not the NO condition, with MARCKS preventing the phosphorylation of mitosis important proteins such as ILF3 and NOLC1 as well as SEPT2 and SEPT7. Both proteins are associated with the coordination of cytokinesis (Spiliotis et al., 2005). According to the inferred PRN the second hub-protein is SPTBN1. In erythrocytes, the phosphorylation of this protein was found to be linked to mechanical stability of the membrane. Manno et al. demonstrated that increased phosphorylation of SPTBN1 destabilizes the membrane while reduced phosphorylation has the adverse effect (Manno et al., 1995). In the PRN, SPTBN1-phosphorylation is positively associated with SEPT7 and SEPT2 phosphorylation and negatively related to MARCKS phosphorylation. This indicates that destabilization of the membrane by SPTBN1 could be directly linked to mitosis. The data shows that SPTBN1 phosphorylation decreases upon IL6 stimulation in NO while no effect is seen in HO. Other proteins connected to SPTBN1 are NUMA1, NES, GTF2F1 and LMNA, all of which are negatively related to SPTBN1 phosphorylation. NUMA1 was reported an important mitotic component by contributing to the initiation and maintenance of the focused spindle poles (Sparks et al., 1995). Interestingly, Sparks et al. observed multiple mitotic phosphorylation events occuring in similar timing of lamin B phosphorylation (Sparks et al., 1995). Thus, our results show that phosphorylation of SPTBN1 might negatively control the phosphorylation of NUMA1 acting in an antagonistic fashion to the effect of LMNB2 phosphorylation. Similar to NUMA1 the phosphorylation of NES is also positively related to the phosphorylation of LMNB2 and negatively related to the phosphorylation of SPTBN1. NES phosphorylation was shown to be an important regulator for its organization and dynamics during mitosis in a neuronal progenitor cell line (Sahlgren et al., 2001). Similar to NES the data reports LMNA phosphorylation to be lower in IL6 treated cells in NO, but not in HO. Like LMNB2, LMNA phosphorylation is associated with matrix disassembly in mitosis. Finally, phosphorylation of GTF2F1 is associated with the stimulation of transcription (Solow et al., 2001). The observed de-phosphorylation in IL6 treated cells in NO therefore might correspond to a decreased transcription rate.

5 CONCLUSION

In this study, we used phosphoproteomics data derived from LC-MS/MS to illustrate the inference of phospho-regulatory networks based on the modeling concept of the published TILAR algorithm. The advantage of this approach is that knowledge about regulating phosphatases and kinases serves as a knowledge base to create a network structure template that guides the inference. This way, prior-knowledge can be automatically integrated to create biologically meaningful, intuitive network models representing the experimentally measured data. For the inference of the PRN we used published data measuring the changes in phosphorylation of proteins from JAK2/STAT3 phosphorylation inhibitor WP1193 perturbed GSC11 cells treated with IL6 under NO and HO conditions. In total, 21 DPPs were selected for the inference with most of them related to the GO-terms 'structural molecule activity' and 'cell cycle'. Our results suggest that the oxygen concentration has an impact on IL6 induced changes in protein phosphorylation. While phosphorylation of most of the selected DPPs does not change upon IL6 treatment in NO, there is an increased phosphorylation in mitosis-associated proteins such as LMNB2 and MARCKS in HO. This exemplifies how PRNs can aid in the interpretation of phosphoproteomics data. However, due to the shortage of experimental data the derived hypotheses will have to be verified using additional experimental data.

ACKNOWLEDGEMENTS

This work was supported by the BMBF (Virtual Liver Network, FKZ: 0315736) and the excellence graduate school Jena School for Microbial Communication (JSMC).

REFERENCES

Dechat, T., Pfleghaar, K., Sengupta, K., Shimi, T., Shumaker, D. K., Solimando, L., and Goldman, R. D. (2008). Nuclear lamins: major factors in the structural organization and function of the nucleus and chromatin. *Genes Dev*, 22(7):832–853.

Efron, B., Hastie, T., Johnstone, I., Tibshirani, R., et al. (2004). Least angle regression. *The Annals of statistics*, 32(2):407–499.

Hecker, M., Goertsches, R. H., Engelmann, R., Thiesen, H.-J., and Guthke, R. (2009a). Integrative modeling of transcriptional regulation in response to antirheumatic therapy. *BMC Bioinformatics*, 10:262.

Hecker, M., Lambeck, S., Toepfer, S., van Someren, E., and Guthke, R. (2009b). Gene regulatory network inference: data integration in dynamic models-a review. *Biosystems*, 96(1):86–103.

Hornbeck, P. V., Kornhauser, J. M., Tkachev, S., Zhang, B., Skrzypek, E., Murray, B., Latham, V., and Sullivan, M. (2012). Phosphositeplus: a comprehensive resource for investigating the structure and function of experimentally determined post-translational modifications in man and mouse. *Nucleic Acids Res*, 40(Database issue):D261–D270.

Huang, D. W., Sherman, B. T., and Lempicki, R. A. (2009). Systematic and integrative analysis of large gene lists using david bioinformatics resources. *Nat Protoc*, 4(1):44–57.

Jørgensen, C. and Linding, R. (2008). Directional and quantitative phosphorylation networks. *Brief Funct Genomic Proteomic*, 7(1):17–26.

Linding, R., Jensen, L. J., Pasculescu, A., Olhovsky, M., Colwill, K., Bork, P., Yaffe, M. B., and Pawson, T. (2008). Networkin: a resource for exploring cellular phosphorylation networks. *Nucleic Acids Res*, 36(Database issue):D695–D699.

Mallows, C. L. (1973). Some comments on c p. *Technometrics*, 15(4):661–675.

Manno, S., Takakuwa, Y., Nagao, K., and Mohandas, N. (1995). Modulation of erythrocyte membrane mechanical function by beta-spectrin phosphorylation and dephosphorylation. *J Biol Chem*, 270(10):5659–5665.

Nikitin, A., Egorov, S., Daraselia, N., and Mazo, I. (2003). Pathway studiothe analysis and navigation of molecular networks. *Bioinformatics*, 19(16):2155–2157.

Nilsson, C. L., Dillon, R., Devakumar, A., Shi, S. D.-H., Greig, M., Rogers, J. C., Krastins, B., Rosenblatt, M., Kilmer, G., Major, M., Kaboord, B. J., Sarracino, D., Rezai, T., Prakash, A., Lopez, M., Ji, Y., Priebe, W., Lang, F. F., Colman, H., and Conrad, C. A. (2010). Quantitative phosphoproteomic analysis of the stat3/il-6/hif1alpha signaling network: an initial study in gsc11 glioblastoma stem cells. *J Proteome Res*, 9(1):430–443.

Pai, C. Y., Chen, H. K., Sheu, H. L., and Yeh, N. H. (1995). Cell-cycle-dependent alterations of a highly phosphorylated nucleolar protein p130 are associated with nucleologenesis. *J Cell Sci*, 108 (Pt 5):1911–1920.

R Core Team (2014). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rombouts, K., Mello, T., Liotta, F., Galli, A., Caligiuri, A., Annunziato, F., and Pinzani, M. (2012). Marcks actin-binding capacity mediates actin filament assembly during mitosis in human hepatic stellate cells. *Am J Physiol Cell Physiol*, 303(4):C357–C367.

Sahlgren, C. M., Mikhailov, A., Hellman, J., Chou, Y. H., Lendahl, U., Goldman, R. D., and Eriksson, J. E. (2001). Mitotic reorganization of the intermediate filament protein nestin involves phosphorylation by cdc2 kinase. *J Biol Chem*, 276(19):16456–16463.

Seet, B. T., Dikic, I., Zhou, M.-M., and Pawson, T. (2006). Reading protein modifications with interaction domains. *Nat Rev Mol Cell Biol*, 7(7):473–483.

Smith, N. L. and Miskimins, W. K. (2011). Phosphorylation at serine 482 affects stability of nf90 and its functional role in mitosis. *Cell Prolif*, 44(2):147–155.

Solow, S., Salunek, M., Ryan, R., and Lieberman, P. M. (2001). Taf(ii) 250 phosphorylates human transcription factor iia on serine residues important for tbp binding and transcription activity. *J Biol Chem*, 276(19):15886–15892.

Sparks, C. A., Fey, E. G., Vidair, C. A., and Doxsey, S. J. (1995). Phosphorylation of numa occurs during nuclear breakdown and not mitotic spindle assembly. *J Cell Sci*, 108 (Pt 11):3389–3396.

Spiliotis, E. T., Kinoshita, M., and Nelson, W. J. (2005). A mitotic septin scaffold required for mammalian chromosome congression and segregation. *Science*, 307(5716):1781–1785.

Terfve, C. and Saez-Rodriguez, J. (2012). Modeling signaling networks using high-throughput phosphoproteomics. *Adv Exp Med Biol*, 736:19–57.

Xue, Y., Ren, J., Gao, X., Jin, C., Wen, L., and Yao, X. (2008). Gps 2.0, a tool to predict kinase-specific phosphorylation sites in hierarchy. *Mol Cell Proteomics*, 7(9):1598–1608.

Yang, X.-J. (2005). Multisite protein modification and intramolecular signaling. *Oncogene*, 24(10):1653–1662.