Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Structural Biology

Studying post-translational modifications with protein interaction networks

Jonathan Woodsmith and Ulrich Stelzl

At least 46 interactome studies, broad at proteome scale or biologically more focused, have together mapped about 75,000 human protein–protein interactions (PPIs). Many of the studies addressed local interactome data paucity analyzing specific homeostatic and regulatory systems, with recent focus demonstrating the involvement of post-translational protein modification (PTM) enzyme families in a wide range of cellular functions. These datasets provided insight into binding mechanisms, the dynamic modularity of complexes or delineated combinatorial enzymatic cascades. Furthermore, the combined study of PPI and PTM dynamics has begun to reveal conditional rewiring of molecular networks through PTM-mediated recognition events. Taken together these studies highlight the utility of local and global interaction networks to functionally prioritize the many changing PTMs mapped in human cells.

Addresses
Max Planck Institute for Molecular Genetics (MPIMG), Otto-Warburg Laboratory, Ihnestraße 63-73, D-14195 Berlin, Germany

Corresponding author: Stelzl, Ulrich (stelzl@molgen.mpg.de)

Current Opinion in Structural Biology 2014, **24**:34–44

This review comes from a themed issue on **Folding and binding**

Edited by **James CA Bardwell** and **Gideon Schreiber**

For a complete overview see the Issue and the Editorial

Available online 21st December 2013

0959-440X/$ – see front matter,

http://dx.doi.org/10.1016/j.sbi.2013.11.009

Introduction

Most cellular functions are in large part driven by the coordinated action of multiple macro-molecular assemblies of interacting protein subunits. Defining the molecular architecture of how these individual protein building blocks interact is a major task fundamental to a better understanding of cellular processes in health and disease [1,2]. Both broad and focused protein–protein interaction (PPI) studies have recently dented interactome data paucity and provided novel insight into a diverse array of cellular systems. Yet, the mapping of conditional interactions, that is, interactions that are strengthened or loosened under specific conditions and thus change with changing conditions, has just started [3]. Here, we collect recent systematically generated human interaction data: focused studies that lead to functional or mechanistic insights as well as broad, proteome wide PPI data resources. We further point out that interaction approaches are particularly useful in understanding of post-translational modification (PTM)-mediated signaling, both in defining modifying enzyme relationships and in delineating PTM-dependent, conditional interactions. Finally, we highlight how collated interactome data can be used in conjugation with PTM data to extract biological signals from PTM collections and drive insight into PTM signaling.

Recent progress in systematic human PPI mapping

Generating datasets broad in scope is fundamental to interactome mapping, providing an increasingly better framework for further analysis. Much of the work to improve data quality focused on determining and improving the specificity of large scale PPI approaches [4–6]. Given high specificity, it is relatively low coverage large unbiased data sets suffer from. Comprehensive interactome mapping requires both search space and interaction coverage: that is, methods that scale well with the number of protein pairs/complexes to be assayed and high sensitivity of the methods to actually detect the interactions in the search space. As such methods have substantially improved. For example Y2H approaches have seen second generation sequencing techniques utilized to reduce work load and increase sensitivity in large data set generation [7•,8•]. Two mass spectrometry (MS) based approaches extensively fractionated human culture cells to infer interaction relationships from the obtained co-fractionation patterns [9•,10•]. Affinity purification of cell cycle related proteins [11] and protein complexes immunoprecipitated using ~1800 antibodies targeted against more than 1000 transcription co-regulatory proteins [12] lead to additional substantial human interactome data sets (Table 1). Also, luminescence based co-IP methods, originally coined LUMIER, have been further exploited as a stringent pair wise PPI screening system [13•,14,15••]. Ultimately, high coverage data will result from combining interactomes generated with different approaches. For example in yeast two-hybrid (Y2H) analyses, weaker, more transient interactions can be better sampled [16] because interactions are measured out of a binding equilibrium via transcriptional reporter activity, and not the physical interacting proteins as such. Therefore Y2H data contain, for example, a larger fraction of signaling interactions while more cooperative associations in processes like transcription and protein homeostasis are generally better covered in complex based approaches.

Table 1

Large human protein–protein interaction data sets. Table summarizes data sets generated in a systematic, well controlled way. Studies are described by listing first author + year, ENTREZ pub med identifier, the topic of the study, number of baits, number of preys(in binary approaches) or the search space, the number of PPIs, main PPI screening method and the reference. Single protein data sets have not been included even though they report many, sometimes hundreds, of interactions. Further, significant data sets have been published describing interactions from organisms such as *E. coli*, yeast, *C. elegans, Drosophila* or Arabidopsis which are not considered here.

Study	PMID	Topic	Baits	Preys/space	PPIs[b]	Methods	Refs
Malovannaya, 2011	21620140	'Co-Regulation'; transcriptional and signaling proteins	1088	HeLa S3 cells	14443 (top 5% of [74])	IP of endogenous prots with 1796 Abs::MS	[12,74]
Havugimana, 2012	22939629	Cofractionation of a human cell line	HeLa S3 and HEK293	HeLa S3 and HEK293 cells	14168	Cofractionation::MS	[9•]
Kristensen, 2012	22863883	Size exclusion chromatography of a human cell line	HeLa (+/−EGF)	HeLa S3 cells (+/−EGF)	7173 (=291 complexes)	Cofractionation (PCP-SILAC)::MS	[10•]
Ewing, 2007	17353931	Selected annotated proteins	338	HEK293 cells	5807	FLAG-tag::MS; transient transfection	[75]
Wang, 2011	21988832	Liver PPIs	4788/1428	4740/liver cDNA library	3484	Y2H	[76]
Bell, 2009	19293945	Aging processes	175	cDNA libraries[a]	3229	Y2H	[73]
Stelzl, 2005	16169070	1st systematic human Y2H matrix screen	4456	5632	3184	Y2H	[77]
Rual, 2005	16189514	1st systematic human Y2H matrix screen	7263	7263	2754	Y2H	[78]
Mukherji, 2007	17001007	Human cell-cycle regulators	459	cDNA libraries[a]	2631	Y2H with RNAi	[79]
Vinayagam, 2011	21900206	Directed signaling network (EGF-signaling)	473	~7800	2623	Y2H	[39]
Bandyopadhyay, 2010	20936779	MAPK singaling	81	cDNA libraries[a]	2268	Y2H	[38]
Hutchins, 2010	20360068	Mitosis	239	HeLa S3 cells	1923	GFP-tag::MS; expression from BAC clones	[11]
Yu, 2011	21516116	Stitch-seq approach	~6000	~6000	1166	Y2H (stitch-seq)	[8•]
Sowa, 2009	19615732	DUBs	75	HEK293T cells	1048	GFP-tag::MS; from stable cell lines	[51]
Woods, 2012	22990118	BRCT tandem domains	7	cDNA library/HEK293FT cells	965 (350(Y2H)/ 619(TAP))	Y2H/TAP-tag::MS	[28]
Miller, 2009	19888210	Wnt signaling	640	11	812	Lumier with RNAi, cDNA overexpression	[14]
Jeronimo, 2007	17643375	Transcription and RNA Processing	32	EcR-293 cells	781	TAP-tag::MS; from stable cell lines	[80]
Lim, 2006	16713569	Ataxias	54	7263 + brain cDNA library	770	Y2H	[81]
Ravasi, 2010	20211142	Transcription factor — transcription factor, (M.m. & H.s.)	1222	1222	762	Mammalian-2H	[82]
Behrends, 2010	20562859	Autophagy	65	HEK293T cells	709	FLAG-HA-tag::MS; retroviral expression lines	[83]
Colland, 2004	15231748	Smad pathway	44	Placental cDNA library	706	Y2H	[84]
Hegele, 2012	22365833	Spliceosome	237	237	632	Y2H with Lumier	[26•]
Markson, 2009	19549727	E2–E3 RING	39	153	568	Y2H (matrix + library)	[43]
Weimann, 2013	23455924	Protein methyltransferases and demethylases	22	~13000	522	Y2H (Y2H-seq), with Lumier	[7•]
Varjosalo, 2013	23602568	CMGC group kinases	57	HEK293 TREX cells	511	SH-tag::MS; from stable cell lines	[35•]
Sardiu, 2008	18218781	TIP49 centered complexes	27	HEK293 cells	485 (probabilistic network)	FLAG-tag::MS; from stable cell lines	[85]

Table 1 (*Continued*)

Study	PMID	Topic	Baits	Preys/space	PPIs[b]	Methods	Refs
Varjosalo, 2013	23455922	Selected kinases of different kinase families	32	HEK293 TREX cells	485	SH-tag::MS; from stable cell lines	[6]
Bennett, 2010	21145461	Cullins	11	HEK293T cells	460	FLAG-HA-tag::MS; from stable cell lines	[49]
Taipale, 2012	22939624	HSP90 client interactions: kinases, E3s, TFs	1414	2	393	Lumier	[15••]
Joshi, 2013	23752268	HDACs	11	CEM T-cell lines	387	EGFP-FLAG-tag::MS; from stable cell lines	[40]
Perez-Hernandez, 2013	23463506	Tetraspanins	8	Lysates or exome extracts of prim. Lymphoblasts	359 (240 + 172)	Pulldowns with intra cellular peptides::MS from T cells or exome preparations	[27]
van Wijk, 2009	19690564	E2-E3(RING)	35	250	304	Y2H	[44]
Albers, 2005	15604093	NR interacting proteins Y2H	38	cDNA libraries	290	Y2H	[86]
Goudreault, 2009	18782753	PP2A complex	21	HEK293 cells	268	TAP or FLAG-tag::MS; from stable cell lines	[37]
Venkatesan, 2009	19060904	Empirical quality assessment of HTP-PPI data	1744	1796	239	Y2H	[4]
Woodsmith, 2012	22493164	E3(RING)-E3(RING)	119	123	228	Y2H	[47]
Bouwmeester, 2004	14743216	TNF-alpha/NF-kappa B signaling	32	HEK293 cells	200	TAP-tag::MS; from stable cell lines	[87]
Soler-Lopez, 2011	21163940	Alzheimer's disease	18	74	200	Y2H (matrix + library)	[88]
Wang, 2008	18624398	Ras-MAPK/PI3K pathways	42	cDNA library	200	Y2H	[89]
Camargo, 2007	17043677	DISC1 (Disrupted in Schizophrenia) interactome	9	cDNA library	188	Y2H	[90]
Glatter, 2009	19156129	PP2A complex	11	HEK293 cells	188	SH-tag::MS; from stable cell lines	[36]
Ellis, 2012	22749401	Splice variant specific PPIs	173	46	172	Lumier	[13•]
Gao, 2012	22325352	PRC1 Family Complexes	15	HEK293 cells	170	FLAG-HA-tag::MS; from stable cell lines with CHIP-seq	[48]
Goehler, 2004	15383276	Huntington's Disease	51	cDNA library	164	Y2H (matrix + library)	[91]
Brehme, 2009	19380743	BCR-ABL complex	10	K562 CML cell line	157 (8 core network)	TAP-tag::MS; retroviral expression lines (BCR-ABL IP with Abs)	[32]
Wallach, 2013	23555304	Circadian clock	46	46	150	Y2H with RNAi	[92]

[a] Using results from the Prolexys human protein interaction network; 345,000 individual yeast two-hybrid library screens that resulted a filtered core network with 70,358 unique binary interactions between protein fragments representing 10,425 unique genes curated as NCBI RefSeq entries [73].
[b] PPI numbers are obtained from processing the original data according to author's filtering recommendations. The PPI data are then collapsed onto NCBI Entrez GeneID and can be obtained as Supplemental file 1.

PPI data sets must carefully be quality controlled either empirically [4,17], through benchmarking [18] or, for the folded part of the proteome, assessed through structural modeling [19,20]. Computational scoring of PPIs, for example, using network properties [21,22], can be very useful for high quality network construction, however the method of choice strongly depends on the biological questions asked. PPI detection methods, PPI quality measures, scoring and PPI databases have been subject of comprehensive recent reviews including Refs. [17,18,20]. Please refer to Table 1 for a summary of recent large-scale human interactome mapping studies.

These interaction datasets, along with other recent well controlled high throughput (HTP) studies, are invaluable for their unbiased approach. They collectively fuel advanced integrative approaches, 'big data' modeling and clinical data interpretation [23,24]. On the cellular systems level, they are fundamental to the interpretation of genetic and protein variation and can lead to mechanistic insight (Figure 1a). For example, Lee *et al.* recently derived the likely mis-location of >150 proteins in glioma from a combination of PPIs, expression data and biochemical protein features [25]. This analysis derived how ectopic network structure, driven by alterations in gene expression profiles, can lead to alteration of protein localization, and by proxy altered protein function in disease. In isolation however, precisely this global interactome approach can predicate detailed mechanistic understanding of specific systems, as global mapping more or less randomly samples a subset of the interactions from large search spaces at relatively low coverage. It is difficult to answer specific mechanistic questions with sparse and somewhat technically biased data, rather the value is the high quality data resource as such and biological insight is given in form of novel global analyses, statistical trends, testable hypotheses and unexpected leads. Biologically focused screens sample a smaller number of potential interactions much more thoroughly, providing high coverage data to enable a better dynamic and mechanistic view of specific cellular processes or protein assemblies. High coverage interaction mapping among the components of relatively well defined molecular systems, for example, the spliceosome [26•], can shed new light on the potential subunit interplay and here led to unforeseen mechanistic details with regards to the dynamics of sub-complex rearrangement in the course of spliceosome assembly and intron excision. Conversely, the potential functional repertoire of less well characterized groups of proteins, such as tetraspanins [27] or tandem-BRCT domain proteins [28], are more consistently defined through focused interactome mapping approaches.

Characterizing the PTM modifying enzyme interaction space

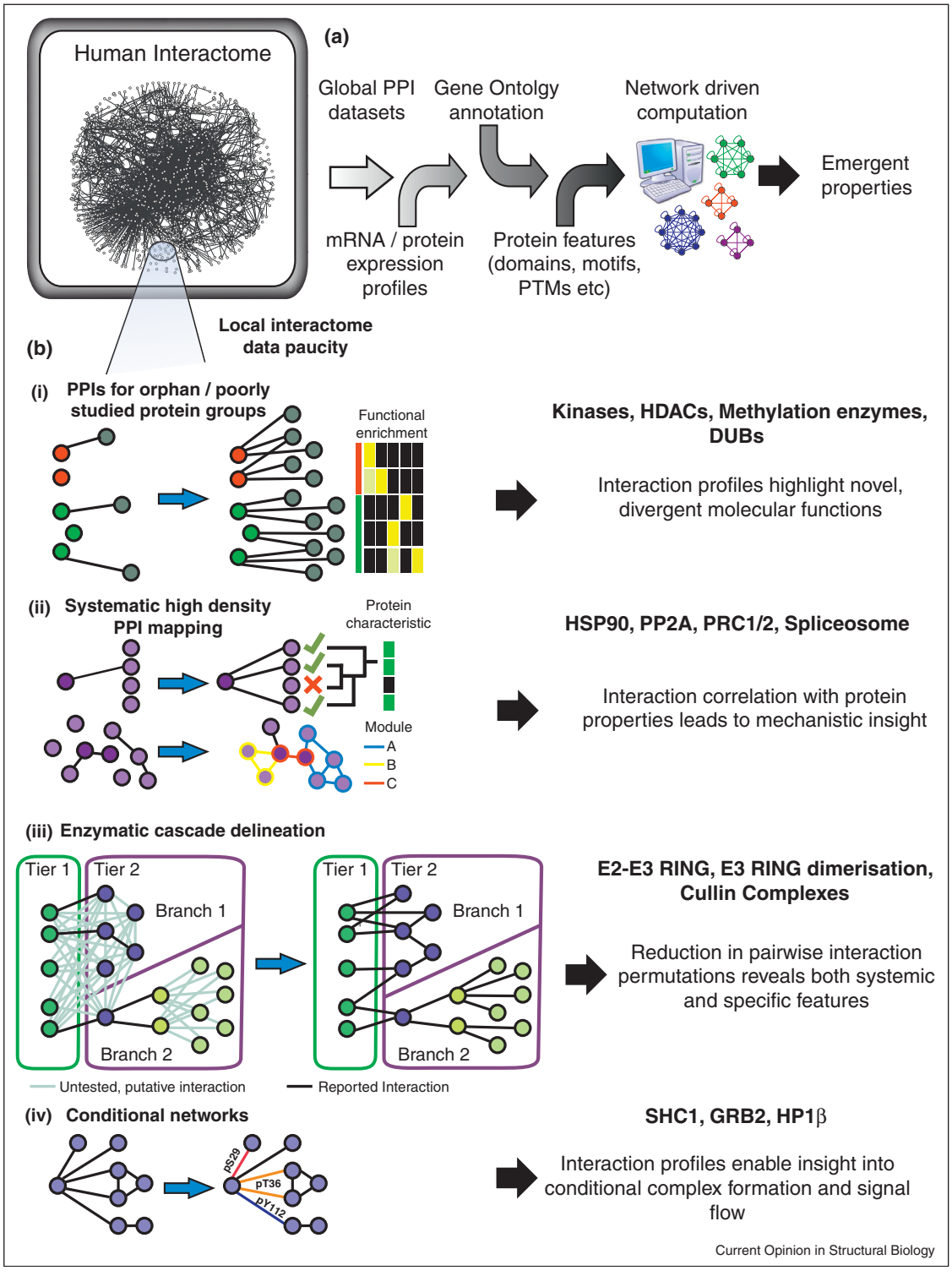
Many of the recent PPI sets focused on modifying enzymes, that is, kinases, phosphatases, methyltransferases, deacetylases, and E2/E3 ubiquitin ligases (Table 1). PTM systems, as defined through writer/reader/eraser/substrate components [29], are requisite for cellular functioning. Ectopic expression or activity can cause a wide variety of human diseases, reflected in the number of pharmaceuticals targeted at PTM components currently in clinical trials [30]. Despite this, the vast majority of research has been restricted to just a small fraction of PTM components such as the 'hottest' 50 kinases [31], highlighting the need for more systematic approaches. Also, more than 100,000 PTMs contrast the relatively small number of enzymes that mediate modification and leave the substrate–enzyme relationships largely elusive. In addition, interactions involving PTM-enzymes are thought to be relatively weak and transient thus suffer from poor coverage in the current global interactome data. Several recent interactome studies focusing on PTM-enzymes have been conducted using distinct methodological approaches and provided context for orphan or poorly annotated nodes in PTM networks, highlighting hitherto unknown functions controlled by PTM systems (Figure 1B(i)).

Many studies have tackled interactomes of disease relevant single kinases such as BCR-ABL [32,33] or LRRK2 [34]. Providing a more global context for kinase function, two recent studies by Varjosalo *et al.* presented proteomic analysis of complexes of a large number of human CMGC kinases [35•] and of selected kinases from other major families [6]. 481 and 345 co-complex members were identified for 57 and 32 kinases (512 and 488 PPIs) via LC–MS/MS, in the two studies respectively. Not surprisingly, interaction partners of the different kinases point towards their involvement in many different cellular processes. Only the most closely related kinases show significant overlapping interaction pattern and no kinase family trends are apparent, suggesting largely non-redundant functions for the enzymes. Together with global phospho-site information these data provide a basis for the inference of kinase-substrate networks as suggested for SRPK1 and SRPK2 and their spliceosomal substrates [35•].

AP-MS approaches were further utilized to assess the modular composition of the human phosphatase PP2A complex through affinity purification of the individual subunits [36,37]. Covering the complexes in extensive reciprocal purifications, the two studies observe pronounced modularity of the holo-PP2A complexes including, for example, a Striatin, STRIP1/2, CTTNBP2NL, MST4 containing module.

Two large Y2H studies [38,39] included mitogen activated protein kinases (MAPKs) and MAPK signaling proteins providing quite complementary analyses of the large signaling PPI data sets obtained (including 716 and 584 kinase PPIs, respectively). Bandyopadhyay *et al.* [38] probed MAPK-mediated signaling through

Figure 1



Interactome mapping strategies. **(a)** Collated PPI data as a framework for integrative network biology. Integration of proteome wide PPI datasets with independent protein/gene annotations uncovers emergent properties of cellular systems. **(b)** Strategies for addressing local PPI data paucity. (i) Increasing PPI coverage of poorly studied proteins. (ii) High density PPI mapping within defined protein–protein interaction types or known molecular complexes. (iii) Hierarchical cascade delineation through high density PPI mapping of specific interaction sub-types. (iv) Elucidation of interactions which are dependent on, or mediated by, specific PTMs or residues in response to a signal.

RNAi knockdowns of 45 MAPK-interacting proteins. Vinayagam *et al.* [39] used overexpression perturbation analysis of 50 proteins in the network to assess the directionality of signal flow through the large network. Though all these kinase/phosphatase interactome studies elaborate on very different aspects in their data analyses, they commonly broaden our view of phosphorylation function in the cell (Figure 1B(i)).

Similarly, less intensively studied PTM systems have also received recent attention. Affinity purifications from cultured T-cells [40] provided binding profiles for all 11 cytoplasmic and nuclear 'histone' deacetylase proteins (HDACs). Protein methylation related enzymes were investigated in an analogous approach using a novel Y2H methodology to define sets of putative methylation targets and regulators for 22 protein methyltransferases and demethylases [7•]. Both of these studies highlighted the underappreciated functions for these PTMs outside of their canonical role in epigenetic regulation of transcription, with many interactors [7•] or the enzymes themselves [40] residing outside of the nucleus. These and other studies (Table 1) provide direct systematic evidence for alternate, cytoplasmic PTM functions through observed interaction profiles, significantly extending anecdotal evidence collected from a handful of small scale studies.

Many kinases are known to require the chaperone HSP90, a quality control system of protein folding, for activity. Through study of HSP90 interactions with 314 kinases, using a modified LUMIER system, Taipale *et al.* [15••] could begin to unravel global principles of protein client recognition (Figure 1B(ii)). Interestingly they could rule out simple explanations of single amino acid determinants of binding, instead providing evidence that HSP90 could distinguish clients from none clients based on intrinsic protein stability. This applied kinome wide and could discriminate between highly homologous kinases such as ARAF and BRAF.

PTM systems such as phosphorylation operate using a direct enzyme–substrate mechanism, however the ubiquitin and ubiquitin-like families of enzymes represent a key challenge in network biology as they operate in a combinatorial, hierarchical enzymatic cascade [41,42]. The pairwise permutations of ~40 E2 conjugating enzymes with >300 E3 ligases, in combination with specific partner preferences within E3 ligase multimers, together control the target and architecture of ubiquitin modification, and are thus crucial to understanding regulation in ubiquitin biology (Figure 1B(iii)). Three distinct Y2H studies tackled the ~12,000 putative E3 RING ligase interactions with their cognate E2 conjugation enzymes [43–45], highlighting the utility in using multiple Y2H experimental set-ups to address interaction space coverage. These papers revealed similar patterns of E2 binding with several E2s (UBE2Ds/Es/W and N) appearing to operate as ubiquitination work horses, engaging in many interactions with a wide variety of E3 RINGs. Conversely, some E3s are highly selective, interacting with one or few E2 enzymes (e.g. ARIH1-UBE2L3/6, MARCH10-UBE2N/K). These and literature studies now present a biophysical framework of approximately 1000 E2–E3 RING interactions, allowing both systematic trends and reductionist hypotheses to be tested across the entire E3 RING family. In this branch of the ubiquitin cascade further regulatory potential is achieved through E3 RING homo-dimerisation and hetero-dimerisation, highlighted through structural analysis of the BARD1-BRCA1 disease related heterodimer [46]. A systematic Y2H analysis revealed these interactions highly prevalent, especially through the large TRIM subfamily and PCGF proteins [47]. Concomitantly, AP-MS and CHIP-seq experiments revealed alternate PCGF-RNF2 heterodimerisations recruit RNF2 to different genomic loci in distinct protein complexes [48], as such suggesting RING dimerisation could extend the canonical linear cascade to facilitate ubiquitination of alternate substrate proteins in *trans* [47].

A second large branch of E3 ligases is represented by the multi-subunit cullin complexes. Bennett *et al.* [49] used systematic AP-MS to highlight individual cullin complex composition in human cultured cells, suggesting a substrate adaptor model for cullin E3 ligase regulation. Furthermore, interaction partners defined a plethora of roles for well-studied de-ubiquitinases (DUBs) in processes such as the COP9 signalsome [50] and the proteosome, together with initial insight into a wide range of previously poorly characterized DUB enzymes [51].

Conditional/PTMa-dependent protein interactions

As addressed above, mapping of interaction profiles for enzymes and regulators involved in PTM signaling has accelerated in recent years, revealing multiple novel aspects of PTM regulated biology. One functional PTM paradigm provided by small scale studies is their ability to dynamically alter interaction partner preferences in response to stimuli. These conditional interactions can either be mediated through single modification events, or through multiple modifications in short sequence space.

In general, recognition of PTMs is often mediated through specialized domains in dedicated families of modular proteins [29], with most interaction knowledge stemming from assaying these domains with short modified peptides. Recently, Src homology 2 domains (SH2), known to bind phospho-tyrosine residues, were tested for interactions on peptide arrays containing phospho-peptides that resemble known *in vivo* phospho-sites [52,53].

In the context of a neuronal network prediction framework these data provided a wealth of new potential conditional interactions [52]. Furthermore, remarkable interaction specificity was seen in SILAC based quantitative pull-downs that captured cellular binding partners with methylated histone-derived peptides [54,55]. Finally, general principles of multi-site PTM recognition could be experimentally observed between structurally characterized BROMO domains and multiply-modified histone tail peptides [56].

However, studies turn more towards models/approaches which better resemble the context of the interactions in a cell using full length proteins. For example, SRM-based interaction quantification from affinity purifications of the adapter protein GRB2 was used in combination with domain mutations and short interfering RNA knockdown probes to interrogate the conditional GRB2 interactome. Bisson *et al.* [57] separated direct from indirect, and SH3-domain from SH2-domain mediated (i.e. phosphorylation-dependent), interactions within a group of 90 GRB2 complex members in response to distinct stimuli. Another approach used the Polo-box domain of polo-like Kinase 1, a domain that preferentially binds phosphoproteins, to purify 622 proteins from cell cycle arrested osteosarcoma U2OS cell lysates which were co-enriched for the optimal PLK phospho-binding and PLK1 phosphorylation-site motifs [58].

These affinity purification studies reveal mostly correlative information about *PTM-dependent* complex formation, with either the modification-site or the recognition site known and both direct or indirect relationships have explanatory potential (Table 2). A deeper mechanistic understanding implies knowledge about *PTM-mediated* interactions, where both the modified site and the corresponding recognition site are known. Recent medium scale studies have begun to address these complexities using selective reaction monitoring (SRM) based MS techniques to investigate PTM-mediated interactome alterations (Figure 1B(iv)). In an integrated approach, using a combination of peptide array, bioinformatic analysis and SRM based MS analysis, Liu *et al.* [59••] identified 40 lysine methylation sites across 28 proteins that associated with the chromo domain containing heterochromatin binding protein HP1β. They then highlighted the functional relevance of DNA-PKcs methylation mediated HP1β interaction in the DNA damage response [59••]. Elucidating both PTM-dependent and PTM-mediated complex formation, Zheng *et al.* [60••] recently tracked the relative abundance of a subset of 41 SHC1 interacting proteins during EGF stimulation and correlated these with both early pY and later pS/T modifications. A phospho-Y313-mediated, proliferative Grb2-dependent cluster of proteins bound 1–3 min after EGF treatment. At 3–9 min, the SHC1-S29 Akt-phosphosite resulting from this activation starts the signal termination, that is, recruitment of a distinct set of proteins including Ptpn12, a tyrosine phosphatase displacing SHC1 from the receptor. During the late response SHC1 somehow recruits proteins involved in cytoskeletal dynamics that cluster with phosphorylation of SHC1-T147 and S335. Although the late phospho-dependent signal flow is not understood these phospho-sites are crucial for restricting proliferation. Formation of this signaling complex depends on the PTMs, therefore the interaction isolation through IP provided the necessary enrichment to correlate the dynamics of multiple interacting species simultaneously with the dynamics of SHC1 phosphorylation in EGF-signaling [60••].

The literature provides excellent examples of studies starting to elucidate phosphorylation-dependent signaling networks in detail and first network approaches

Table 2

PTM-dependent and PTM-mediated interactions. Examples illustrating potential mechanisms for interactions/co-complex formation influenced by PTMs.

	Principle of indirect PTM effect	Example mechanism	Specific interaction	Ref
PTM dependent	Local binding partner availability/ concentration	pS/pT of NLS leads to nuclear export	HDAC3 increased binding to HDAC5 upon nuclear retention	[93]
	Allosteric conformational change	Phosphorylation regulates protein conformation	Phosphorylation of S518 in NF2 (merlin) regulates its conformation; DCAF1 binds to the (S518A) closed form	[94]
	Co-complex formation	Signal, that is, kinase-dependent complex recruitment	Upon IGF treatment, GRB2 binds PIK3 subunits in an IRS4 dependent manner	[57]
	PTM mediates recognition event directly	**Example recognition domain**	**Specific interaction**	**Ref**
PTM mediated	Single phospho-Y residue	SH2	GRB2 binding to activated EGFR	[57, 60••]
	Multiple methyl-R residues	TUDOR	SMN1-Sm D3(meR^4)	[95]
	Polyubiquitin chain	UBAN-ZF	NEMO-RIP1(UB^{Met1})	[96]

addressing the recognition of other PTMs, such as K-acetylation or K/R-methylation are also under way. However, systematic studies that assess the potential of Ub and Ub-like dependent interactions in signaling are elusive. 20 families of Ub binding proteins with the potential to recognize non-degradation triggering, alternatively conjugated Ub chains and the various Ub-like protein modifications suggest these interactions are prevalent yet poorly sampled [41,42]. Furthermore Ub/Ub-like PTMs are proteins in their own right, therefore the storage of thousands of covalent Ub modification events in interaction databases makes systematically dissecting genuine Ub-modification dependent interactions difficult.

Conclusions

In general, systematic investigation linking specific PTMs to large scale alterations in network structure have lagged behind due to the technical challenges inherent in connecting two large scale measurements, that is, protein interaction data and protein modification data. Combining recent MS studies and literature datasets, over 100,000 modifications across more than 12,000 unique proteins have been identified in human cells. PTM data sets are difficult to normalize and interpret because of extensive enrichment protocols, incomplete sampling, miscalls, large differences in protein abundance [61], different PTM-occupancy levels [62] and relatively low PTM-site conservation [63,64••,65••]. In addition most PTMs may not function alone [64••,65••,66•] yet experimental strategies to tackle these dependencies are just being developed [67]. However, upon changing conditions, PTMs do show massive dynamic responses with hundreds to thousand dynamic PTM-site changes [62,68,69], providing a strong argument for their functional importance. Predicting putative enzyme-site relationships, for example, using an integrative Bayesian approach [70], clustering dynamic changes throughout the cell cycle [62] or in stem cell development [69], or examining evolutionary conservation and structural relationships [64••,65••] can all be informative in identifying PTM functionality. However, as the MS studies described above highlight [59••,60••], an additional appropriate data filter for prioritizing the functionally most relevant PTM-signals is interaction network context. The first large MS study measuring lysine-acetylation reported that acetylation accumulates over protein complexes in human cultured cells [71] and high phosphorylation levels were observed on specific subunits of mitotic complexes [72]. Supporting and extending this idea, a first computational analysis of global acetylation, phosphorylation and ubiquitylation data revealed that modifications cluster within interaction networks [66•]. Furthermore PTMi spots, short disordered amino acid stretches with exceptional high PTM density, were found in more than 400 human proteins and may be important sites for PTM-mediated recognition events [66•]. These studies suggest that analysis of PTMs coupled to protein interaction information will promote a better understanding of enzyme–substrate relationships, the dynamics of PTM-mediated signal flow and the consequences of PTM-mediated recognition events, that is, the rewiring of molecular networks as a signaling response. Function clusters within protein interaction networks. Just as the interpretation of the large number of genetic variation between genomes is greatly aided by network information, evidence is piling up that protein–protein interaction networks will be successfully exploited in the analyses of the more than 100,000 cellular PTMs.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.sbi.2013.11.009.

1. Stelzl U, Wanker EE: **The value of high quality protein–protein interaction networks for systems biology**. *Curr Opin Chem Biol* 2006, **10**:551-558.
2. Vidal M, Cusick ME, Barabasi AL: **Interactome networks and human disease**. *Cell* 2011, **144**:986-998.
3. Ideker T, Krogan NJ: **Differential network biology**. *Mol Syst Biol* 2012, **8**:565.
4. Venkatesan K, Rual JF, Vazquez A, Stelzl U, Lemmens I, Hirozane-Kishikawa T, Hao T, Zenkner M, Xin X, Goh KI *et al.*: **An empirical framework for binary interactome mapping**. *Nat Methods* 2009, **6**:83-90.
5. Choi H, Larsen B, Lin ZY, Breitkreutz A, Mellacheruvu D, Fermin D, Qin ZS, Tyers M, Gingras AC, Nesvizhskii AI: **SAINT: probabilistic scoring of affinity purification-mass spectrometry data**. *Nat Methods* 2011, **8**:70-73.
6. Varjosalo M, Sacco R, Stukalov A, van Drogen A, Planyavsky M, Hauri S, Aebersold R, Bennett KL, Colinge J, Gstaiger M *et al.*: **Interlaboratory reproducibility of large-scale human protein-complex analysis by standardized AP-MS**. *Nat Methods* 2013, **10**:307-314.
7. • Weimann M, Grossmann A, Woodsmith J, Ozkan Z, Birth P, Meierhofer D, Benlasfer N, Valovka T, Timmermann B, Wanker EE *et al.*: **A Y2H-seq approach defines the human protein methyltransferase interactome**. *Nat Methods* 2013, **10**:339-342.

Though methodologically distinct, the two studies report substantially improved Y2H approaches involving second generation sequencing for high quality interactome mapping.

8. • Yu H, Tardivo L, Tam S, Weiner E, Gebreab F, Fan C, Svrzikapa N, Hirozane-Kishikawa T, Rietman E, Yang X *et al.*: **Next-generation sequencing to generate interactome datasets**. *Nat Methods* 2011, **8**:478-480.

See annotation of Ref. [7•].

9. • Havugimana PC, Hart GT, Nepusz T, Yang H, Turinsky AL, Li Z, Wang PI, Boutz DR, Fong V, Phanse S *et al.*: **A census of human soluble protein complexes**. *Cell* 2012, **150**:1068-1081.

These two studies extensively fractionate human cells and identify co-fractionating proteins via MS. The co-fractionation patterns provide a wealth of novel co-complex data.

10. • Kristensen AR, Gsponer J, Foster LJ: **A high-throughput approach for measuring temporal changes in the interactome**. *Nat Methods* 2012, **9**:907-909.
See annotation of Ref. [9•].

11. Hutchins JR, Toyoda Y, Hegemann B, Poser I, Heriche JK, Sykora MM, Augsburg M, Hudecz O, Buschhorn BA, Bulkescher J *et al.*: **Systematic analysis of human protein complexes identifies chromosome segregation proteins**. *Science* 2010, **328**:593-599.

12. Malovannaya A, Lanz RB, Jung SY, Bulynko Y, Le NT, Chan DW, Ding C, Shi Y, Yucer N, Krenciute G *et al.*: **Analysis of the human endogenous coregulator complexome**. *Cell* 2011, **145**:787-799.

13. • Ellis JD, Barrios-Rodiles M, Colak R, Irimia M, Kim T, Calarco JA, Wang X, Pan Q, O'Hanlon D, Kim PM *et al.*: **Tissue-specific alternative splicing remodels protein–protein interaction networks**. *Mol Cell* 2012, **46**:884-892.
This paper uses LUMIER to measure isoform specific binary interactions. It is very suggestive as it provides a first glimpse on the extent to how different isoform specific interaction networks might be.

14. Miller BW, Lau G, Grouios C, Mollica E, Barrios-Rodiles M, Liu Y, Datti A, Morris Q, Wrana JL, Attisano L: **Application of an integrated physical and functional screening approach to identify inhibitors of the Wnt pathway**. *Mol Syst Biol* 2009, **5**:315.

15. •• Taipale M, Krykbaeva I, Koeva M, Kayatekin C, Westover KD, Karras GI, Lindquist S: **Quantitative analysis of HSP90–client interactions reveals principles of substrate recognition**. *Cell* 2012, **150**:987-1001.
Well controlled, systematic LUMIER study assaying HSP90/CDC37 client interactions with kinases, E3 ligases and transcription factors. It demonstrated that a property of the client proteins, conformational flexibility, rather than specific interface residues, is the determinant for HSP90 recognition.

16. Vinayagam A, Stelzl U, Wanker EE: **Repeated two-hybrid screening detects transient protein–protein interactions**. *Theor Chem Acc* 2010, **125**:613-619.

17. Braun P: **Interactome mapping for analysis of complex phenotypes: insights from benchmarking binary interaction assays**. *Proteomics* 2012, **12**:1499-1518.

18. Wodak SJ, Vlasblom J, Turinsky AL, Pu S: **Protein–protein interaction networks: the puzzling riches**. *Curr Opin Struct Biol* 2013 http://dx.doi.org/10.1016/j.sbi.2013.08.002.

19. Hosur R, Peng J, Vinayagam A, Stelzl U, Xu J, Perrimon N, Bienkowska J, Berger B: **Coev2Net: a computational framework for boosting confidence in high-throughput protein–protein interaction datasets**. *Genome Biol* 2012, **13**:R76.

20. Mosca R, Pons T, Ceol A, Valencia A, Aloy P: **Towards a detailed atlas of protein–protein interactions**. *Curr Opin Struct Biol* 2013 http://dx.doi.org/10.1016/j.sbi.2013.07.005.

21. Royer L, Reimann M, Stewart AF, Schroeder M: **Network compression as a quality measure for protein interaction networks**. *PLoS One* 2012, **7**:e35729.

22. Kamburov A, Grossmann A, Herwig R, Stelzl U: **Cluster-based assessment of protein–protein interaction confidence**. *BMC Bioinformatics* 2012, **13**:262.

23. Califano A, Butte AJ, Friend S, Ideker T, Schadt E: **Leveraging models of cell regulation and GWAS data in integrative network-based association studies**. *Nat Genet* 2012, **44**:841-847.

24. Schadt EE, Bjorkegren JL: **NEW: network-enabled wisdom in biology, medicine, and health care**. *Sci Transl Med* 2012, **4**:115rv111.

25. Lee K, Byun K, Hong W, Chuang HY, Pack CG, Bayarsaikhan E, Paek SH, Kim H, Shin HY, Ideker T *et al.*: **Proteome-wide discovery of mislocated proteins in cancer**. *Genome Res* 2013, **23**:1283-1294.

26. • Hegele A, Kamburov A, Grossmann A, Sourlis C, Wowro S, Weimann M, Will CL, Pena V, Luhrmann R, Stelzl U: **Dynamic protein–protein interaction wiring of the human spliceosome**. *Mol Cell* 2012, **45**:567-580.
This interaction study provides a high coverage, binary interaction map for the >200 human spliceosomal proteins. Integrating a series of co-purification data, dynamic interaction patterns are revealed leading to mechanistic hypotheses for important steps in the spliceosomal reaction cycle.

27. Perez-Hernandez D, Gutierrez-Vazquez C, Jorge I, Lopez-Martin S, Ursa A, Sanchez-Madrid F, Vazquez J, Yanez-Mo M: **The intracellular interactome of tetraspanin-enriched microdomains reveals their function as sorting machineries toward exosomes**. *J Biol Chem* 2013, **288**:11649-11661.

28. Woods NT, Mesquita RD, Sweet M, Carvalho MA, Li X, Liu Y, Nguyen H, Thomas CE, Iversen ES Jr, Marsillac S *et al.*: **Charting the landscape of tandem BRCT domain-mediated protein interactions**. *Sci Signal* 2012, **5**:rs6.

29. Seet BT, Dikic I, Zhou MM, Pawson T: **Reading protein modifications with interaction domains**. *Nat Rev Mol Cell Biol* 2006, **7**:473-483.

30. Cohen P, Tcherpakov M: **Will the ubiquitin system furnish as many drug targets as protein kinases?** *Cell* 2010, **143**:686-693.

31. Edwards AM, Isserlin R, Bader GD, Frye SV, Willson TM, Yu FH: **Too many roads not taken**. *Nature* 2011, **470**:163-165.

32. Brehme M, Hantschel O, Colinge J, Kaupe I, Planyavsky M, Kocher T, Mechtler K, Bennett KL, Superti-Furga G: **Charting the molecular network of the drug target Bcr-Abl**. *Proc Natl Acad Sci U S A* 2009, **106**:7414-7419.

33. Titz B, Low T, Komisopoulou E, Chen SS, Rubbi L, Graeber TG: **The proximal signaling network of the BCR-ABL1 oncogene shows a modular organization**. *Oncogene* 2010, **29**:5895-5910.

34. Meixner A, Boldt K, Van Troys M, Askenazi M, Gloeckner CJ, Bauer M, Marto JA, Ampe C, Kinkl N, Ueffing M: **A QUICK screen for Lrrk2 interaction partners — leucine-rich repeat kinase 2 is involved in actin cytoskeleton dynamics**. *Mol Cell Proteomics* 2011, **10** M110 001172.

35. • Varjosalo M, Keskitalo S, Van Drogen A, Nurkkala H, Vichalkovski A, Aebersold R, Gstaiger M: **The protein interaction landscape of the human CMGC kinase group**. *Cell Rep* 2013, **3**:1306-1320.
An AP-MS map for a large group of kinases that defines interaction partners for many poorly characterized family members. These data will also be important for inferring kinase-substrate relationships.

36. Glatter T, Wepf A, Aebersold R, Gstaiger M: **An integrated workflow for charting the human interaction proteome: insights into the PP2A system**. *Mol Syst Biol* 2009, **5**:237.

37. Goudreault M, D'Ambrosio LM, Kean MJ, Mullin MJ, Larsen BG, Sanchez A, Chaudhry S, Chen GI, Sicheri F, Nesvizhskii AI *et al.*: **A PP2A phosphatase high density interaction network identifies a novel striatin-interacting phosphatase and kinase complex linked to the cerebral cavernous malformation 3 (CCM3) protein**. *Mol Cell Proteomics* 2009, **8**:157-171.

38. Bandyopadhyay S, Chiang CY, Srivastava J, Gersten M, White S, Bell R, Kurschner C, Martin CH, Smoot M, Sahasrabudhe S *et al.*: **A human MAP kinase interactome**. *Nat Methods* 2010, **7**:801-805.

39. Vinayagam A, Stelzl U, Foulle R, Plassmann S, Zenkner M, Timm J, Assmus HE, Andrade-Navarro MA, Wanker EE: **A directed protein interaction network for investigating intracellular signal transduction**. *Sci Signal* 2011, **4**:rs8.

40. Joshi P, Greco TM, Guise AJ, Luo Y, Yu F, Nesvizhskii AI, Cristea IM: **The functional interactome landscape of the human histone deacetylase family**. *Mol Syst Biol* 2013, **9**:672.

41. Komander D, Rape M: **The ubiquitin code**. *Annu Rev Biochem* 2012, **81**:203-229.

42. Husnjak K, Dikic I: **Ubiquitin-binding proteins: decoders of ubiquitin-mediated cellular functions**. *Annu Rev Biochem* 2012, **81**:291-322.

43. Markson G, Kiel C, Hyde R, Brown S, Charalabous P, Bremm A, Semple J, Woodsmith J, Duley S, Salehi-Ashtiani K *et al.*: **Analysis of the human E2 ubiquitin conjugating enzyme protein interaction network**. *Genome Res* 2009, **19**:1905-1911.

44. van Wijk SJ, de Vries SJ, Kemmeren P, Huang A, Boelens R, Bonvin AM, Timmers HT: **A comprehensive framework of E2-**

RING E3 interactions of the human ubiquitin–proteasome system. *Mol Syst Biol* 2009, **5**:295.

45. Napolitano LM, Jaffray EG, Hay RT, Meroni G: **Functional interactions between ubiquitin E2 enzymes and TRIM proteins**. *Biochem J* 2011, **434**:309-319.

46. Christensen DE, Brzovic PS, Klevit RE: **E2-BRCA1 RING interactions dictate synthesis of mono- or specific polyubiquitin chain linkages**. *Nat Struct Mol Biol* 2007, **14**: 941-948.

47. Woodsmith J, Jenn RC, Sanderson CM: **Systematic analysis of dimeric E3-RING interactions reveals increased combinatorial complexity in human ubiquitination networks**. *Mol Cell Proteomics* 2012, **11** M111 016162.

48. Gao Z, Zhang J, Bonasio R, Strino F, Sawai A, Parisi F, Kluger Y, Reinberg D: **PCGF homologs, CBX proteins, and RYBP define functionally distinct PRC1 family complexes**. *Mol Cell* 2012, **45**:344-356.

49. Bennett EJ, Rush J, Gygi SP, Harper JW: **Dynamics of cullin-RING ubiquitin ligase network revealed by systematic quantitative proteomics**. *Cell* 2010, **143**:951-965.

50. Olma MH, Roy M, Le Bihan T, Sumara I, Maerki S, Larsen B, Quadroni M, Peter M, Tyers M, Pintard L: **An interaction network of the mammalian COP9 signalosome identifies Dda1 as a core subunit of multiple Cul4-based E3 ligases**. *J Cell Sci* 2009, **122**:1035-1044.

51. Sowa ME, Bennett EJ, Gygi SP, Harper JW: **Defining the human deubiquitinating enzyme interaction landscape**. *Cell* 2009, **138**:389-403.

52. Tinti M, Kiemer L, Costa S, Miller ML, Sacco F, Olsen JV, Carducci M, Paoluzi S, Langone F, Workman CT *et al.*: **The SH2 domain interaction landscape**. *Cell Rep* 2013, **3**:1293-1305.

53. Koytiger G, Kaushansky A, Gordus A, Rush J, Sorger PK, MacBeath G: **Phosphotyrosine signaling proteins that drive oncogenesis tend to be highly interconnected**. *Mol Cell Proteomics* 2013, **12**:1204-1213.

54. Vermeulen M, Eberl HC, Matarese F, Marks H, Denissov S, Butter F, Lee KK, Olsen JV, Hyman AA, Stunnenberg HG *et al.*: **Quantitative interaction proteomics and genome-wide profiling of epigenetic histone marks and their readers**. *Cell* 2010, **142**:967-980.

55. Nikolov M, Stutzer A, Mosch K, Krasauskas A, Soeroes S, Stark H, Urlaub H, Fischle W: **Chromatin affinity purification and quantitative mass spectrometry defining the interactome of histone modification patterns**. *Mol Cell Proteomics* 2011, **10** M110 005371.

56. Filippakopoulos P, Picaud S, Mangos M, Keates T, Lambert JP, Barsyte-Lovejoy D, Felletar I, Volkmer R, Muller S, Pawson T *et al.*: **Histone recognition and large-scale structural analysis of the human bromodomain family**. *Cell* 2012, **149**:214-231.

57. Bisson N, James DA, Ivosev G, Tate SA, Bonner R, Taylor L, Pawson T: **Selected reaction monitoring mass spectrometry reveals the dynamics of signaling through the GRB2 adaptor**. *Nat Biotechnol* 2011, **29**:653-658.

58. Lowery DM, Clauser KR, Hjerrild M, Lim D, Alexander J, Kishi K, Ong SE, Gammeltoft S, Carr SA, Yaffe MB: **Proteomic screen defines the Polo-box domain interactome and identifies Rock2 as a Plk1 substrate**. *EMBO J* 2007, **26**:2262-2273.

59. •• Liu H, Galka M, Mori E, Liu X, Lin YF, Wei R, Pittock P, Voss C, Dhami G, Li X *et al.*: **A method for systematic mapping of protein lysine methylation identifies functions for HP1beta in DNA damage response**. *Mol Cell* 2013, **50**:723-735.
The two studies show that PTM-mediated signaling/regulatory events can be successfully studied when concomitantly assaying protein–protein interactions.

60. •• Zheng Y, Zhang C, Croucher DR, Soliman MA, St-Denis N, Pasculescu A, Taylor L, Tate SA, Hardy WR, Colwill K *et al.*: **Temporal regulation of EGF signalling networks by the scaffold protein Shc1**. *Nature* 2013, **499**:166-171.
See annotation of Ref. [59••].

61. Levy ED, Michnick SW, Landry CR: **Protein abundance is key to distinguish promiscuous from functional phosphorylation based on evolutionary information**. *Philos Trans R Soc Lond B Biol Sci* 2012, **367**:2594-2606.

62. Olsen JV, Vermeulen M, Santamaria A, Kumar C, Miller ML, Jensen LJ, Gnad F, Cox J, Jensen TS, Nigg EA *et al.*: **Quantitative phosphoproteomics reveals widespread full phosphorylation site occupancy during mitosis**. *Sci Signal* 2010, **3**:ra3.

63. Landry CR, Levy ED, Michnick SW: **Weak functional constraints on phosphoproteomes**. *Trends Genet* 2009, **25**:193-197.

64. •• Beltrao P, Albanese V, Kenner LR, Swaney DL, Burlingame A, Villen J, Lim WA, Fraser JS, Frydman J, Krogan NJ: **Systematic functional prioritization of protein posttranslational modifications**. *Cell* 2012, **150**:413-425.
Analyses of different globally mapped PTMs reveals patterns of PTM co-evolution and provides hints for functional prioritization of PTMs.

65. •• Minguez P, Parca L, Diella F, Mende DR, Kumar R, Helmer-Citterich M, Gavin AC, van Noort V, Bork P: **Deciphering a global network of functionally associated post-translational modifications**. *Mol Syst Biol* 2012, **8**:599.
See annotation of Ref. [64••].

66. • Woodsmith J, Kamburov A, Stelzl U: **Dual coordination of post translational modifications in human protein networks**. *PLoS Comput Biol* 2013, **9**:e1002933.
The global analyses of four different PTMs reveals coordination in human protein networks and PTMi spots, that is, preferentially disordered protein regions of exceptional high PTM density.

67. Mertins P, Qiao JW, Patel J, Udeshi ND, Clauser KR, Mani DR, Burgess MW, Gillette MA, Jaffe JD, Carr SA: **Integrated proteomic analysis of post-translational modifications by serial enrichment**. *Nat Methods* 2013, **10**:634-637.

68. Olsen JV, Blagoev B, Gnad F, Macek B, Kumar C, Mortensen P, Mann M: **Global, in vivo, and site-specific phosphorylation dynamics in signaling networks**. *Cell* 2006, **127**: 635-648.

69. Rigbolt KT, Prokhorova TA, Akimov V, Henningsen J, Johansen PT, Kratchmarova I, Kassem M, Mann M, Olsen JV, Blagoev B: **System-wide temporal characterization of the proteome and phosphoproteome of human embryonic stem cell differentiation**. *Sci Signal* 2011, **4**:rs3.

70. Newman RH, Hu J, Rho HS, Xie Z, Woodard C, Neiswinger J, Cooper C, Shirley M, Clark HM, Hu S *et al.*: **Construction of human activity-based phosphorylation networks**. *Mol Syst Biol* 2013, **9**:655.

71. Choudhary C, Kumar C, Gnad F, Nielsen ML, Rehman M, Walther TC, Olsen JV, Mann M: **Lysine acetylation targets protein complexes and co-regulates major cellular functions**. *Science* 2009, **325**:834-840.

72. Hegemann B, Hutchins JR, Hudecz O, Novatchkova M, Rameseder J, Sykora MM, Liu S, Mazanek M, Lenart P, Heriche JK *et al.*: **Systematic phosphorylation analysis of human mitotic protein complexes**. *Sci Signal* 2011, **4**:rs12.

73. Bell R, Hubbard A, Chettier R, Chen D, Miller JP, Kapahi P, Tarnopolsky M, Sahasrabuhde S, Melov S, Hughes RE: **A human protein interaction network shows conservation of aging processes between human and invertebrate species**. *PLoS Genet* 2009, **5**:e1000414.

74. Mazloom AR, Dannenfelser R, Clark NR, Grigoryan AV, Linder KM, Cardozo TJ, Bond JC, Boran AD, Iyengar R, Malovannaya A *et al.*: **Recovering protein–protein and domain–domain interactions from aggregation of IP-MS proteomics of coregulator complexes**. *PLoS Comput Biol* 2011, **7**:e1002319.

75. Ewing RM, Chu P, Elisma F, Li H, Taylor P, Climie S, McBroom-Cerajewski L, Robinson MD, O'Connor L, Li M *et al.*: **Large-scale mapping of human protein–protein interactions by mass spectrometry**. *Mol Syst Biol* 2007, **3**:89.

76. Wang J, Huo K, Ma L, Tang L, Li D, Huang X, Yuan Y, Li C, Wang W, Guan W *et al.*: **Toward an understanding of the protein interaction network of the human liver**. *Mol Syst Biol* 2011, **7**:536.

77. Stelzl U, Worm U, Lalowski M, Haenig C, Brembeck FH, Goehler H, Stroedicke M, Zenkner M, Schoenherr A, Koeppen S *et al.*: **A human protein–protein interaction network: a resource for annotating the proteome**. *Cell* 2005, **122**:957-968.

78. Rual JF, Venkatesan K, Hao T, Hirozane-Kishikawa T, Dricot A, Li N, Berriz GF, Gibbons FD, Dreze M, Ayivi-Guedehoussou N *et al.*: **Towards a proteome-scale map of the human protein–protein interaction network**. *Nature* 2005, **437**:1173-1178.

79. Mukherji M, Bell R, Supekova L, Wang Y, Orth AP, Batalov S, Miraglia L, Huesken D, Lange J, Martin C *et al.*: **Genome-wide functional analysis of human cell-cycle regulators**. *Proc Natl Acad Sci U S A* 2006, **103**:14819-14824.

80. Jeronimo C, Forget D, Bouchard A, Li Q, Chua G, Poitras C, Therien C, Bergeron D, Bourassa S, Greenblatt J *et al.*: **Systematic analysis of the protein interaction network for the human transcription machinery reveals the identity of the 7SK capping enzyme**. *Mol Cell* 2007, **27**:262-274.

81. Lim J, Hao T, Shaw C, Patel AJ, Szabo G, Rual JF, Fisk CJ, Li N, Smolyar A, Hill DE *et al.*: **A protein–protein interaction network for human inherited ataxias and disorders of Purkinje cell degeneration**. *Cell* 2006, **125**:801-814.

82. Ravasi T, Suzuki H, Cannistraci CV, Katayama S, Bajic VB, Tan K, Akalin A, Schmeier S, Kanamori-Katayama M, Bertin N *et al.*: **An atlas of combinatorial transcriptional regulation in mouse and man**. *Cell* 2010, **140**:744-752.

83. Behrends C, Sowa ME, Gygi SP, Harper JW: **Network organization of the human autophagy system**. *Nature* 2010, **466**:68-76.

84. Colland F, Jacq X, Trouplin V, Mougin C, Groizeleau C, Hamburger A, Meil A, Wojcik J, Legrain P, Gauthier JM: **Functional proteomics mapping of a human signaling pathway**. *Genome Res* 2004, **14**:1324-1332.

85. Sardiu ME, Cai Y, Jin J, Swanson SK, Conaway RC, Conaway JW, Florens L, Washburn MP: **Probabilistic assembly of human protein interaction networks from label-free quantitative proteomics**. *Proc Natl Acad Sci U S A* 2008, **105**:1454-1459.

86. Albers M, Kranz H, Kober I, Kaiser C, Klink M, Suckow J, Kern R, Koegl M: **Automated yeast two-hybrid screening for nuclear receptor-interacting proteins**. *Mol Cell Proteomics* 2005, **4**: 205-213.

87. Bouwmeester T, Bauch A, Ruffner H, Angrand PO, Bergamini G, Croughton K, Cruciat C, Eberhard D, Gagneur J, Ghidelli S *et al.*: **A physical and functional map of the human TNF-alpha/NF-kappa B signal transduction pathway**. *Nat Cell Biol* 2004, **6**:97-105.

88. Soler-Lopez M, Zanzoni A, Lluis R, Stelzl U, Aloy P: **Interactome mapping suggests new mechanistic details underlying Alzheimer's disease**. *Genome Res* 2011, **21**:364-376.

89. Wang J, Yuan Y, Zhou Y, Guo L, Zhang L, Kuai X, Deng B, Pan Z, Li D, He F: **Protein interaction data set highlighted with human Ras-MAPK/PI3K signaling pathways**. *J Proteome Res* 2008, **7**:3879-3889.

90. Camargo LM, Collura V, Rain JC, Mizuguchi K, Hermjakob H, Kerrien S, Bonnert TP, Whiting PJ, Brandon NJ: **Disrupted in Schizophrenia 1 Interactome: evidence for the close connectivity of risk genes and a potential synaptic basis for schizophrenia**. *Mol Psychiatry* 2007, **12**:74-86.

91. Goehler H, Lalowski M, Stelzl U, Waelter S, Stroedicke M, Worm U, Droege A, Lindenberg KS, Knoblich M, Haenig C *et al.*: **A protein interaction network links GIT1, an enhancer of huntingtin aggregation, to Huntington's disease**. *Mol Cell* 2004, **15**:853-865.

92. Wallach T, Schellenberg K, Maier B, Kalathur RK, Porras P, Wanker EE, Futschik ME, Kramer A: **Dynamic circadian protein–protein interaction networks predict temporal organization of cellular functions**. *PLoS Genet* 2013, **9**:e1003398.

93. Greco TM, Yu F, Guise AJ, Cristea IM: **Nuclear import of histone deacetylase 5 by requisite nuclear localization signal phosphorylation**. *Mol Cell Proteomics* 2011, **10** M110 004317.

94. Li W, You L, Cooper J, Schiavon G, Pepe-Caprio A, Zhou L, Ishii R, Giovannini M, Hanemann CO, Long SB *et al.*: **Merlin/NF2 suppresses tumorigenesis by inhibiting the E3 ubiquitin ligase CRL4(DCAF1) in the nucleus**. *Cell* 2010, **140**:477-490.

95. Tripsianes K, Madl T, Machyna M, Fessas D, Englbrecht C, Fischer U, Neugebauer KM, Sattler M: **Structural basis for dimethylarginine recognition by the Tudor domains of human SMN and SPF30 proteins**. *Nat Struct Mol Biol* 2011, **18**:1414-1420.

96. Rahighi S, Ikeda F, Kawasaki M, Akutsu M, Suzuki N, Kato R, Kensche T, Uejima T, Bloor S, Komander D *et al.*: **Specific recognition of linear ubiquitin chains by NEMO is important for NF-kappaB activation**. *Cell* 2009, **136**:1098-1109.