The social network of a cell: Recent advances in interactome mapping

Sebastian Charbonnier, Oriol Gallego and Anne-Claude Gavin*
EMBL, Structural and Computational Biology Unit, Meyerhofstrasse 1, D-69117 Heidelberg, Germany

Abstract. Proteins very rarely act in isolation. Biomolecular interactions are central to all biological functions. In human, for example, interference with biomolecular networks often lead to disease. Protein–protein and protein–metabolite interactions have traditionally been studied one by one. Recently, significant progresses have been made in adapting suitable tools for the global analysis of biomolecular interactions. Here we review this suite of powerful technologies that enable an exponentially growing number of large-scale interaction datasets. These new technologies have already contributed to a more comprehensive cartography of several pathways relevant to human pathologies, offering a broader choice for therapeutic targets. Genome-wide scale analyses in model organisms reveal general organizational principles of eukaryotic proteomes. We also review the biochemical approaches that have been used in the past on a smaller scale for the quantification of the binding constant and the thermodynamics parameters governing biomolecular interaction. The adaptation of these technologies to the large-scale measurement of biomolecular interactions in (semi-)quantitative terms represents an important challenge.

Keywords: yeast-two hybrids, TAP/MS, protein complexes, affinity constant, affinity purification, pulldown, phage display, ITC, SPR, metabolites, small-molecules, interaction.

Introduction

Since the sequencing of the first eukaryotic genome, *Saccharomyces cerevisiae*, some 10 years ago [1], our understanding of the basic building blocks that make up a cell has spectacularly improved. The explosion of new analytical tools in the fields of genomics, proteomics and metabolomics contributes ever-growing molecular repertoires of a cell. However, biology does not rely on biomolecules acting in isolation. Biological function usually depends on the concerted action of molecules acting in protein complexes, metabolic or signaling pathways or networks.

Traditionally, assays were designed to study a few selected gene products and their interactions in a defined number of chosen biological contexts. These decades of one-by-one studies have contributed a wealth of knowledge on how cells sense, store and transduce information through a defined number of signaling routes or pathways [2]. In human, impaired or deregulated protein–protein or protein–metabolite interaction often leads

*Corresponding author: Tel.: +49-0-6221-387-8816.
E-mail: gavin@embl.de (A.-C. Gavin).

BIOTECHNOLOGY ANNUAL REVIEW
VOLUME 14 ISSN 1387-2656
DOI: 10.1016/S1387-2656(08)00001-X

© 2008 ELSEVIER B.V.
ALL RIGHTS RESERVED

to disease. Also, the majority of targets of current therapeutics are part of a limited number of cellular pathways [3]. A precise cartography of these pathways is believed to contribute to the identification of drug targets, and to help understanding the mechanism of action and side effects of therapeutic compounds. One of the goals of this review is to exemplify and illustrate the importance of protein–protein, but also protein–small-molecule (metabolite) interactions for human biology and pathology.

Recently, the study of protein–protein, and more broadly biomolecular interactions, has taken center stage. New strategies have been designed that allow the study of interactions more globally at the level of entire biological systems. A section of this review is dedicated to the description of the technologies adapted to chart biomolecular interactions on a systems-wide scale. Their respective advantages, shortcomings and limitations are presented. These methods have already contributed molecular maps of several pathways involved in human pathologies. More global, genome-scale protein–protein interaction screens performed in a model organism, *S. cerevisiae*, have provided a molecular network that serves as basis for the interpretation of simple genetic data such as gene essentiality. There is a greater tendency for proteins central in networks to be lethal when deleted [4].

Biomolecular interactions in human diseases

The spatial and temporal coordination of the many cellular enzyme activities through extensive and highly regulated protein–protein interaction networks bears remarkable functional relevance. The specificity of protein–protein recognition is believed to essentially rely on two distinct mechanisms. In some cases, specialized domains or binding sites accommodate smaller determinants or peptides present on the interaction partner [2]. For example, Src homology 2 (SH2) domains specifically interact with small peptides containing a phosphotyrosyl residue. PSD95-DLG-ZO1 (PDZ) domains target small (4 amino acid long) consensus binding motifs located at the C-terminus of the interaction partners. These short linear motifs are critical to many biological processes. They often show medium to low affinities (0.5–10 μM) and thus tend to be mediators in transient interactions, especially in signaling networks. The derived plasticity from these weak affinities and the often low conservations might be an advantage for fast adaptation of networks according to changing environment [5–7]. In other cases, protein–protein interaction involves much larger interfaces. This modality of recognition requires mostly folded domains and occurs with higher affinities in the low nanomolar or even picomolar range. A famous example are proteins containing a leucine zipper, in which α-helices interact tightly and fit together [2,8].

Very similar to the associations taking place between proteins, interactions involving small molecule metabolites and proteins play key biological

functions. The relationship between metabolomes and proteomes is not limited to enzyme–substrate or –product interactions. Many metabolites have well-known signaling roles, as second messengers. Others, such as succinate and α-ketoglutarate, two intermediates of the citric acid cycle, are ligands of mammalian G-coupled receptors, GPR91 and GPR99 [9]. Many metabolic enzymes and many signaling proteins are allosterically modulated by metabolites. Binding to small molecules is often mediated by a variety of more than thousand specific domains (Protein Data Bank: www.rcsb.org/pdb/).

Among the 2,000 monogenic syndromes with a known molecular basis (Online Mendelian Inheritance in Man, 2000), mutations that affect biomolecular interactions are not uncommon (Table 1). Noteworthy examples are mutations in receptors that affect their ability to interact with the cognate ligands. The Apert syndrome, characterized by skull malformation, syndactyly and often mental deficiency, is caused by mutations in the fibroblast growth factor receptor 2 (FGFR2) that selectively increase the affinity for FGF2 [10]. Enzymes are also extensively involved in protein–protein interactions. For instance, DNMT3B is a DNA methyltransferase implicated in the immunodeficiency, centromeric instability, facial anomalies (ICF) syndrome, a rare autosomal recessive disorder. Missense mutations characterized in ICF patients map not only within the catalytic site but also affect an N-terminal PWWP domain, involved in protein–protein interactions [11]. Also, mutations have been characterized that prevent the assembly of a functional multiprotein complex. A good example is a RFXANK gene mutant that fails to assemble a regulatory factor X (RFX) complex required for the expression of MHC class II genes. This leads to the bare lymphocyte syndrome [12]. Finally, a variety of abnormal or erroneous interactions between brain proteins can result in the formation of toxic aggregates of proteinacious fibrils. These ‘‘fatal attractions’’ [13] are the apparent cause of a variety of neurodegenerative disorders, such as sporadic and familial Alzheimer’s disease, Parkinson’s disease, amyotrophic lateral sclerosis and prion encephalopathies.

Similarly to protein–protein interactions, deregulation of protein–metabolite interactions leads to many pathological status in human (Table 1). For example, the Bannayan-Riley-Ruvalcaba syndrome, characterized by macrocephaly, multiple lipomas and hemangiomas [14], is caused by mutation in the phosphatase PTEN. Different mutations map in a protein kinase C conserved region 2 (C2) domain that has relatively broad specificities for phospholipids. Pleckstrin-homology domains (PH) (http://smart.embl-heidelberg.de/), the 11th most frequent domain in human, mediate the specific recruitment of key signaling proteins to the membrane pools of phosphatidylinositol phosphates (PIPs). PH domains share an extremely conserved fold, despite divergent primary sequences. X-linked a-gamma-globulinaemia (XLA), characterized by the absence of mature B-lymphocyte and all immunoglobulin

Table 1. Altered biomolecular interaction in human diseases.

Disease and syndrome	Mutated gene product	Interacting partner	References
Protein–protein interaction			
Apert syndrome	Fibroblast growth factor receptor 2: FGFR2	FGF	[10]
Familial melanoma	Tumor suppressor gene: p16(INK4)	Cyclin-dependent kinases (CDK4, CDK6)	[110,111]
CADASIL	NOTCH3	NOTCH3, Fringe	[112,113]
Bare lymphocyte syndrome	RFXANK	RFX complex	[12]
Branchio-oto-renal/ Branchio-otic syndromes	SIX1	EYA1	[114]
Adrenoleukodystrophy	ATP-binding cassette transporter: ABCD1	ABCD1	[115]
Holt-Oram syndrome	Tbx5	NKX2.5	[116]
ICF syndrome	Methytransferase gene: DNMT3B	Unknown	[117]
Giant axonal neuropathy	Gigaxonin	MAP1B-LC	[118]
Hereditary nonpolyposis colorectal cancer	Mismatch repair gene: MLH1	PMS2	[119]
Protein-metabolite interaction			
Bannayan-Riley-Ruvalcaba syndrome	PTEN, C2	Phospholipids	[14]
X-linked agammaglobulinemia	BTK, PH	PI3,4,5P	[15,16]
Pseudoxanthoma elasticum	ABCC6, NBF (nucleotide binding fold)	ATP	[120]
Vitamin D-dependent Rickets, Type II	VDR, Steroid hormon-binding	1,25-dihydroxyvitamin D3	[121]
Sporadic colon cancers, Familial Partial Lipodystrophy Type 3	PPARG, Ligand-binding	Broad variety of ligand	[122]
Human breast, colorectal and ovarian cancers	AKT1, PH	PI3,4,5P, PI3,4P	[123]

isotypes, is caused by mutations in the Bruton's protein tyrosine kinase (Btk) (Table 1) [15]. Many mutations have been reported that cluster within the tyrosine kinase domain and also in the amino terminal PH domain of Btk [15,16].

The examples listed in Table 1 do not represent a comprehensive inventory. They illustrate that the spatial and temporal orchestration of the many enzyme activities through extensive and highly regulated biomolecular interaction networks bears remarkable functional relevance. Mutational

lesions or environmental factors impairing the pathway flow or deregulating connections lead to pathology, as surely as interferences with the catalytically active sites do.

Charting biomolecular interactions

The sequencing of the full repertoire of genes in several organisms and significant breakthrough in the fields of proteomics opened the way to more holistic protein–protein interaction analyses. Mainly two types of approaches have been adapted: the yeast two-hybrid system that allows the mapping of pairwise associations and affinity purification methods coupled to mass spectrometry, MS-based protein identification, designed for the characterization of protein complexes (Table 2). Alternative strategies are emerging, such as pulldown coupled to MS or microarrays that will also be presented. These screening methods are generally limited to qualitative measurements.

Table 2. Overview of large-scale protein–protein interaction studies.

Method	Organism	Interactions	References
Yeast two-hybrid			
Y2H	*H. pylori*	~1,520	[47]
	S. cerevisiae	~4,500	[124]
	S. cerevisiae	~1,000	[48]
	C. elegans	~5,000	[51]
	D. melanogaster	~20,400	[49]
	D. melanogaster	~2,300	[50]
	H. sapiens	~2,800	[52]
	H. sapiens	~3,200	[53]
	Kaposi sarcoma-associated herpes virus	~120	[102]
	Varicella-zoster virus	~170	[102]
	Epstein-Barr virus	~40	[101]
	C. jejuni	~11,600	[125]
Affinity chromatography			
TAP/mass spectrometry	*S. cerevisiae*	~4,100	[28]
	S. cerevisiae		[29]
	S. cerevisiae		[30]
	E. coli	~5,250	[27]
	H. sapiens		[32]
	O. sativae	~220	[126]
Immuno-affinity purification/mass spectrometry	*S. cerevisiae*	~3,620	[24]
Others			
Protein microarrays	*S. cerevisiae*	~40	[77]

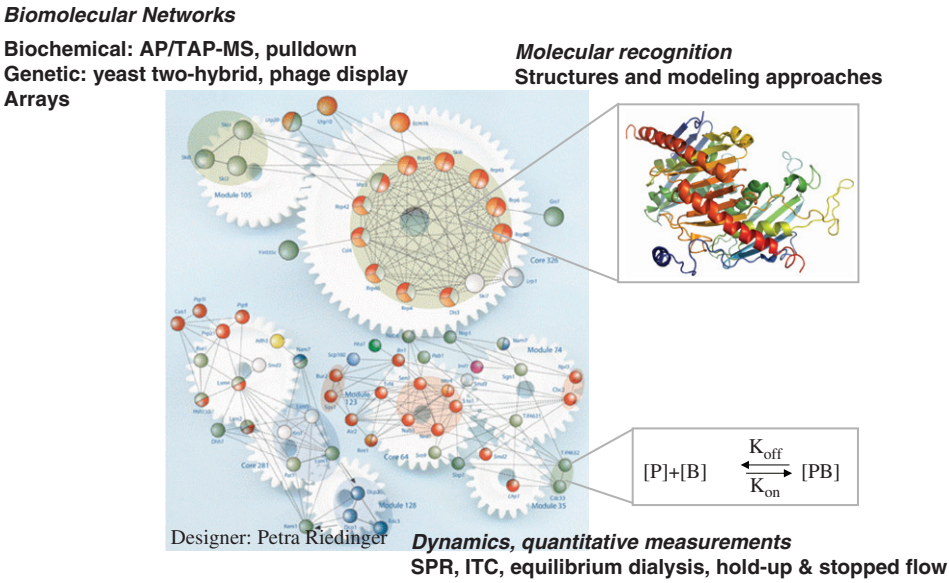


Fig. 1. Main methodologies in the study of biomolecular interactions. The approaches are grouped according to their aim. (The color version of this figure is hosted on Science Direct.)

We also review the arsenal of quantitative approaches that are currently amenable to smaller scale work (Fig. 1). Finally, a number of databases have been developed that integrate biomolecular interaction data from various origins (large-scale physical or genetic interactions, literature mining) and provide a very rich source of information (Table 3). The necessity to integrate the exponentially growing number of protein interaction datasets being generated, recently lead to the development of "The Minimum Information required for reporting a Molecular Interaction experiment (MIMIx)," a community standard for the representation of protein interaction data [17].

Large-scale approaches: Interactome mapping

Biochemical approaches

Analysis of protein complexes: Affinity purification-mass spectrometry
The emergence of sensitive and high-throughput MS methods has fuelled the development of methods employing the biochemical purification of whole cellular assemblies. Pioneering work used specific antibodies directed against epitopes present on endogenous protein complexes or recombinant specific interaction domains combined with MS to identify protein complexes. This

Table 3. Protein–protein interaction databases available on the Internet and their URL addresses. See also The Jena Center for Bioinformatics Protein–Protein Interaction Website (http://www.imb-jena.de/jcb/ppi/).

Database	Internet URL address (http://)	Species
Experimental datasets for protein–protein interactions:		
The GRID	www.thebiogrid.org	*C. elegans*, *D. melanogaster*, *S. cerevisiae*
SGD	www.yeastgenome.org/	*S. cerevisiae*
CYGD	mips.gsf.de/genre/proj/yeast/	*S. cerevisiae*
BOND	bond.unleashedinformatics.com/Action?	*A. thaliana*, *B. taurus*, *C. elegans*, *D. melanogaster*, *G. gallus*, *H. pylori*, *HIV1*, *H. sapiens*, *R. norvegicus*, *S. cerevisiae*, *M. musculus*
HPRD	www.hprd.org/	*H. sapiens*
DIP	dip.doe-mbi.ucla.edu/	*C. elegans*, *D. melanogaster*, *E. coli*, *H. pylori*, *H. sapiens*, *M. musculus*, *R. norvegicus*, *S. cerevisiae*
MINT	mint.bio.uniroma2.it/mint/Welcome.do	*B. taurus*, *C. elegans*, *D. melanogaster*, *E. coli*, *H. pylori*, *H. sapiens*, *M. musculus*, *R. norvegicus*, *S. cerevisiae*
IntAct	www.ebi.ac.uk/intact/index.jsp	*C. elegans*, *D. melanogaster*, *E. coli*, *H. sapiens*, *M. musculus*, *S. cerevisiae*
Biocarta	www.biocarta.com/	*H. sapiens*, *M. musculus*
MPPI	mips.gsf.de/proj/ppi/	*Mammals*
PDB	www.rcsb.org/pdb/	*Archae*, *Bacteria*, *Eucaryota*, *Viruses*
Experimental datasets for protein–small-molecule interactions:		
KDBI	xin.cz3.nus.edu.sg/group/kdbi/kdbi.asp	
AffinDB	www.agklebe.de/affinity	
LPDB	lpdb.chem.lsa.umich.edu	

has been successfully applied to the protein assembled around neurotransmitter receptors N-methyl-D-aspartate (NMDA) and 5-hydroxytryptamine 2c (5-HT2C) [18,19]. The main advantage is that endogenously expressed and native proteins are retrieved from cells or even tissues, which is closest to physiological conditions. Availability of specific antibodies or other affinity capturing agents remains a major limitation.

More generic approaches exploiting known high-affinity interactions have been broadly developed where the proteins of interest "baits" are fused to an affinity-tag that is eventually captured on a suitable affinity chromatography

resin. Many different genes can be fused to the same tag in parallel, expressed in the appropriate cell type and isolated using the same affinity resins. The components of the purified protein complexes are identified by MS either directly (shot-gun sequencing approaches) or after one-dimensional gel electrophoresis. The tag is often an epitope-tag. An antibody directed against the tag, instead of the bait protein itself, is used for protein complex purification. A broad variety of epitope-tags are available such as Myc, HA, Flag and KT3. Other tagging systems have also been developed that exploit enzyme–substrate interaction, for example, between glutathione-S-transferase (GST) and glutathione (GSH) or the use of strep tags [20]. A variety of tag-affinity resin pairs are currently available. Their respective performances in a variety of expression systems have been extensively reviewed [21–23]. In the past, mono-affinity approaches have been adapted to the large-scale purification of yeast protein complexes assembled around more than 700 baits involved in cell signaling and in the DNA damage response [24] (Table 2).

Further improvements aimed at a higher discrimination against unspecific protein background while retaining the essential components of the protein complex. More stringent washing steps in ''traditional'' mono-affinity purification schemes are not always compatible with the preservation of the protein complex integrity. The tandem affinity purification (TAP) protocol utilizes sequentially two epitope-tags, Protein A and calmodulin binding peptide (CBP), instead of one and addresses some of the signal-to-noise issues [25,26]. The TAP-fusion protein is expressed in cells and a protein complex can assemble under physiological conditions with the endogenous components. The tagged protein along with associated partners is retrieved by two steps of affinity purifications (Fig. 2A). First, the protein A tag is immobilized on immunoglobulin resin. The protein complex is specifically eluted by protease cleavage, using Tobacco Etch Virus (TEV) protease. The TEV protease very specifically cleaves a seven amino-acid sequence that has been introduced between the Protein A and the CBP tags. The TEV cleavage sequence is only found in a few human, mouse or yeast proteins, ensuring that the retrieved complexes are not digested. In a second affinity step, the complex is immobilized to calmodulin-coated beads via the CBP tag. This step removes the TEV and further contaminants. As the CBP–calmodulin interaction is calcium dependent, a second specific elution step is achieved through the removal of calcium with a chelating agent (EGTA).

The TAP/MS protocol has been rapidly adapted to high-throughput analyses of protein complexes in a variety of organisms, including the bacteria *Escherichia coli* [27], *S. cerevisiae* [28–30], plants [31] and human [32] (Table 2). Proteome-wide screens in yeast, including more than 2,000 baits, provided the largest repertoire of eukaryotic protein complexes so far [29,33].

The TAP–MS approach is not limited to one cell type, it is possible to monitor and quantify changes in complex compositions in different cell lines,

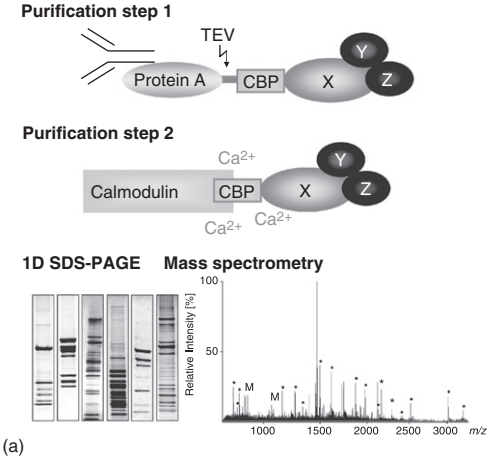
during development or following various cell stimulations [34–36]. Only one protein is cloned and tagged, all other components of the complex are native proteins and reflect the natural diversity of protein isoforms, such as alternative splicing and post-translational modifications. Because of the two steps of purification, it generally efficiently reduces the unspecific protein background and most importantly, stringent purification condition can be avoided. The protein complexes can be kept under "native" conditions throughout the purification procedure. For example, TAP-purified complexes have been successfully used for electron microscopy studies [37,38]. A diversity of different tag combinations are also now available that have been optimized for expression in mammalian or insect cells [39,40].

The affinity purification/MS methods are not generally designed to monitor very labile or transient interactions (generally $K_d \leqslant$ mid nM; unpublished data). In addition, the fusion with an epitope-tag may sometimes interfere with the biological function of the tagged protein, as it impairs its folding, its recruitment within a protein complex or its sub-cellular localization. These risks can be significantly reduced by creating and analyzing both N-terminal and C-terminal fusions in parallel. Finally, over-expression can also lead to aberrant localization, protein aggregation or toxicity. Generally, tight controls over the expression levels and the bait-protein localization should be included in any systematic screen setting [32].

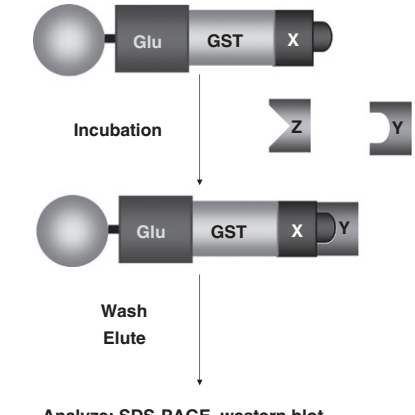
Protein or small molecule pulldown

The pulldown assay is probably one of the oldest and widest spread techniques to identify biomolecular interactions [41]. The assay monitors the ability of a ligand (bait), for example, a recombinant protein, a domain, a peptide or a metabolite bound to a matrix, to specifically capture proteins from a complex cell extract (Fig. 2B). The binding of the bait to the matrix can be achieved by chemical cross-linking. Alternatively, the bait can be expressed as a tag-fusion, for example, GST-fusion, with specificity for a particular affinity resin, for example, glutathione sepharose (GST-pulldown). Analyte proteins (preys), typically a cellular extract, are incubated with the bound bait and non-interacting proteins are washed away under mild washing conditions. Protein interactors can then be eluted by high-salt conditions, cofactors, competitors, chaotropic agents or sodium dodecyl sulfate (SDS) and are identified with specific antibodies (western blot) or by MS.

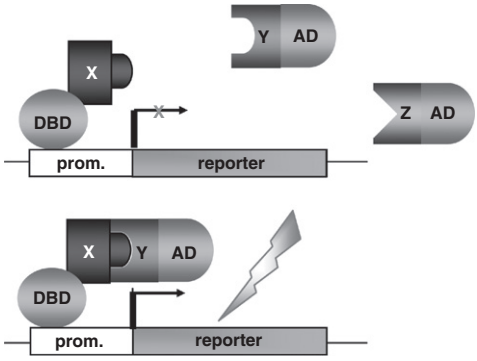
In contrast to classical affinity chromatography methods (see aforementioned), pulldown is less physiological, as the binding happens *in vitro* on a solid support. The assay, however, is generally very sensitive. With appropriate concentrations of the immobilized ligand, *i.e.*, well above the K_d of the interaction, interactions with binding constants as weak as 10^{-5} M can be detected. This high sensitivity, however, comes at the cost of a relatively low specificity and a high rate of false positives. Generally,


Purification step 1
TEV
Protein A
CBP
X
Y
Z
Purification step 2
Ca2+
Calmodulin
CBP
X
Y
Z
Ca2+
Ca2+
1D SDS-PAGE
Mass spectrometry
100
50
Relative Intensity [%]
M
M
1000
1500
2000
2500
3000
m/z

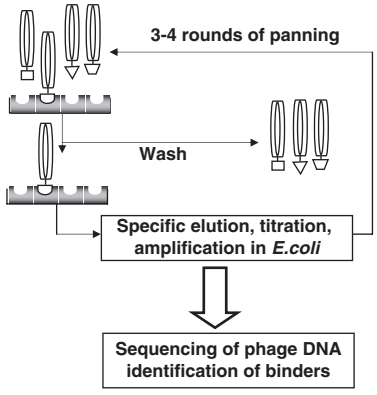
(a)


Glu
GST
X
Incubation
Z
Y
Glu
GST
X
Y
Wash
Elute
Analyze: SDS-PAGE, western blot,

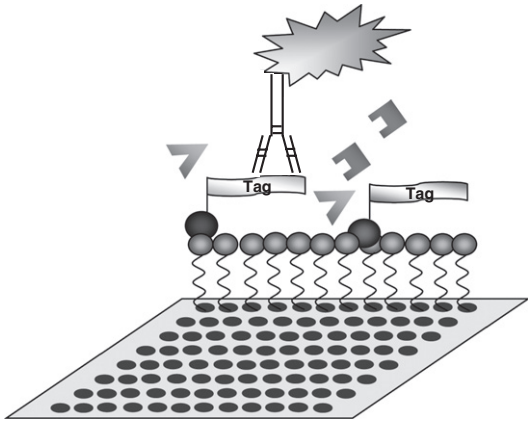
(b)


Y
AD
X
DBD
prom.
reporter
Z
AD
X
Y
AD
DBD
prom.
reporter

(c)


3-4 rounds of panning
Wash
Specific elution, titration, amplification in E.coli
Sequencing of phage DNA identification of binders

(d)


Tag
Tag

(e)

adequate controls, for example, inactive mutants or analogs, must be carefully designed to discriminate artifactual binding.

Mann's group used synthetic peptides from the four members of the ErbB-receptor family either tyrosine phosphorylated or non-phosphorylated in pulldown experiments. By quantitative MS they characterized the phosphotyrosine-dependent interactions induced by growth factors stimulation. The analysis recapitulates almost all previously known epidermal growth factor receptor substrates as well as 31 novel effectors [42].

Another interesting area of pulldown application is the monitoring of small molecule- or metabolite-binding profiles in complex proteomes. Such approaches have been broadly used to study protein–lipid interactions using, for example, biotinylated liposome of varying lipid composition or

Fig. 2. Technologies for the large-scale charting of biomolecular interactions. (A) Tandem Affinity Purification (TAP): A bait protein is fused to a TAP-tag built of a protein A coupled via a TEV protease sensitive linker to a calmodulin-binding peptide. The TAP-bait fusion is expressed at endogenous levels and can form a functional complex in native like conditions. The protein complex is isolated via two subsequent chromatographic purification steps, the first involving purification on IgG beads followed by elution via TEV cleavage and a second step on calmodulin beads followed by EGTA elution. Individual protein complex subunits can be visualized on SDS PAGE and identified by mass spectrometry. X, bait protein; Y and Z, natural complex subunits (preys); CBP, Calmodulin-binding peptide. (B) Pulldown: GST pulldown is shown here as an example for chromatographic affinity protein or small molecule pulldown. The bait protein is fused to an affinity tag (GST) which can bind an affinity chromatography resin (Glu, gutathione sepharose beads). It is incubated with prey proteins and unspecific proteins are washed away, while interacting proteins remain bound to the bait. Binders can be visualized by SDS PAGE, western blot or any other suitable method. (The color version of this figure is hosted on Science Direct.) (C) Yeast two hybrid: A bait protein is fused to a DNA-binding domain of a specific promoter, which is coupled to a reporter gene. A bank of prey proteins fused to an activation domain is cloned into the yeast cells, where only one prey protein is expressed in one yeast clone. If interaction between bait and prey takes place the reporter gene is activated and an expression phenotype can be visualized. DBD, DNA binding domain; AD, activation domain; X, bait protein; Y and Z, prey proteins. (D) Phage display: A phage library with individual phages expressing different prey proteins are incubated with a bait protein, which is immobilized on a solid surface. Non-specific phages are washed away and specific interacting phages are titrated and amplified. Specifically binding phages are amplified in 4–5 rounds of panning and the binding preys identified by sequencing of the phage DNA. (E) Protein or small molecule microarray: A bank of prey proteins or small molecules is spotted on a solid surface. The array is then incubated with labeled or tagged bait. Labels can be visualized either directly (GFP, Radioactivity) or indirectly via antibodies.

concentration [43]. More recently, pulldown approaches coupled to MS have elegantly been applied to the proteome-wide charting of cAMP/cGMP- [44] and purine-binding proteomes [45].

Genetic approaches

Monitoring binary interaction; the yeast two-hybrid system

The yeast two-hybrid system is a genetic, *ex vivo* assay that allows the charting of binary interactions. Its principle relies on the modular nature of transcription factors that contain both a site-specific DNA-binding domain (DBD) and a transcriptional activation domain (AD) that recruits the transcriptional machinery to the promoters. The interaction between a ''bait'' fusion (protein X-DBD hybrid protein) and a ''prey'' fusion (protein Y-AD hybrid protein) reconstitutes a functional transcription factor which turns on the expression of reporter genes or selection markers [46] (Fig. 2C).

The system is readily scalable and has very rapidly evolved to genome-wide strategies that have been broadly applied to the charting of protein–protein interactions in a variety of organisms (Table 2), including *Helicobacter pylori* (Rain *et al*. [47]), budding yeast [48], *Drosophila melanogaster* [49,50], *Caenorhabditis elegans* [51] and *Homo sapiens* [52,53]. The two-hybrid system is a sensitive assay suitable for the detection of weak and/or very transient interactions. Dissociation constants down to 10^{-6} M, corresponding to the range of the weakest interaction occurring in the cell, can be detected this way.

The system also has drawbacks mainly related to its *ex vivo* nature. Expressed fusion proteins are forced to the nucleus, which may not be their natural location. For example, membrane proteins are usually not compatible with such a nuclear-based assay. Ectopically expressed proteins may not undergo the appropriate post-translational modifications. Similarly, interaction often involves cooperative, allosteric events or chaperone-assisted assembly that may not occur in the nucleus. Finally, transcription factors, as well as other proteins (about 5–10% of gene products) can auto-activate transcription of the reporter genes making them unsuitable for this approach.

Several modified versions of the yeast two-hybrid system have been developed that address some of these limitations. They involve the reconstitution of modular proteins other than transcription factors that enable the analyses of proteins not amenable to the ''classical'' two-hybrid assay (essentially membrane proteins and transcription factors). It includes the SOS [54] or the Ras recruitment systems [55], the G-protein-based screening assay [56], the split-ubiquitin system [57] and the mammalian protein–protein interaction trap (MAPPIT) based on the complementation of signaling-deficient type I cytokine receptors [58]. Although some of these assays are apparently robust [59], none of them has yet been used in high-throughput proteome-wide screens.

Reverse versions of the two-hybrid system have been developed, where the disruption of a given protein–protein interaction generates a signal. These reverse approaches, initially developed in yeast [60], have matured in an arsenal of assays that allow the screening for small molecules disrupting selected protein–protein interactions [61,62]. Finally, the most recent application of the two-hybrid principle is MASPIT, a three-hybrid trap for quantitative screening of small molecule–protein interactions in mammalian cells [63]. Using MASPIT, Caligiuri *et al.* [63] could show that besides its well-known inhibitory action on the SRC kinase, the pyrido[2,3-d]pyrimidine PD173955 is also a potent inhibitor of several ephrin receptor tyrosine kinases.

Phage display

Smith, Scott and colleagues first proposed a way of displaying polypeptides on the surface of filamentous M13-derived bacteriophages [64]. Polypeptides (preys) are expressed as fusions with the phage coat protein pIII [64,65]. During the phage assembly process, the resulting fusion proteins are transported to the bacterial cell membrane and are incorporated into the phage particle along with the single-stranded DNA (ssDNA) encoding the displayed fusion protein [65]. Phage libraries displaying large diversity of preys (10^9 unique sequences) can be created, amplified and screened for the specific binding to an immobilized target protein (bait). Usually three to five rounds of panning are sufficient to enrich for phages expressing a peptide sequence interacting with the bait. The identity of the polypeptide binder is deduced by sequencing the corresponding phage DNA (Fig. 2D). When necessary, affinity maturation for individual clones can be performed through generation of secondary libraries of mutated peptides [66–68].

Phage display is suitable for the charting of interactions with affinity constants in the micromolar to nanomolar range. To fine-tune the sensitivity of the assay to a particular bait, alternative systems have been engineered. They use different viral coat proteins that are expressed and displayed at the phage surface with different stoichiometry [69]. The system has clear limitations as many proteins do not fold properly in bacterial periplasm. To circumvent this problem, new strategies use lytic bacteriophages, such as T4 [70], T7 [71] or P4 [72,73], that assemble their capsids in the cytoplasm.

Phage display has proven particularly powerful to the selection of relatively small peptides. Using phage display, Robinson *et al.* [74] screened a peptide library for the selection of nonameric sequences that specifically bind and target human papilloma viruses (HPVs) transformed cells. They identified three different consensus tumor-targeting sequences that could be employed for the selected delivery of therapeutics. Other illustrative examples are recent epitomics screens where peptide libraries were selected for the specific binding to antibodies from serum of cancer patients. The selected

peptides were used to develop a peptide array used for the diagnostic of cancer [75,76].

Protein and small molecule microarrays

It has become routine to use DNA microarrays to probe the expression of thousands of genes in parallel. Similarly, protein or small molecule microarrays have been developed. They provide a mean to the rapid and parallel screening of large numbers of proteins for biochemical activities, protein–protein, protein–lipid, protein–nucleic acid and protein–small molecule interactions (Fig. 2E). The first high-density proteome microarray consisted of 5,800 GST-HisX6 tag-fusion yeast proteins [77]. Such protein microarrays are currently commercially available. Details on the procedure have been extensively reviewed [78,79]. Protein arrays are in principle amenable to proteome-wide screens for protein–protein interactions. So far though, they have been only used on limited scale to identify new calmodulin- and phospholipid-binding proteins as well as to monitor domain–domain and antigen–antibody interactions [77,80–82].

Similarly, a variety of small molecule arrays have been developed, that involve the covalent coupling of entire chemical collections of synthetic small molecules [83,84] or natural products [85] to a solid surface. Small molecule micorarrays have been used in a broad range of applications such as the determination of protease activity profile on cell lysates [86], the study of ligand binding specificity of proteins and domains [87] and the identification of protein modulators [88]. For instance, using a small molecule microarray containing a selection of 3,800 compounds, Kuruvilla *et al.* [88] identified 8 small molecules that selectively bind the yeast transcriptional regulator Ure2.

Measurement of the dynamics of biomolecular interactions: The quantitative methods

Generally, the information contributed by large-scale studies of biomolecular interactions is static. Indeed, the methods designed to the charting of networks on a large-scale fail to capture the dynamic aspect of recognition that is central to the whole cell functioning. Many of the large-scale methods imply the expression of proteins under non-physiological conditions and in these *ex vivo* or *in vitro* systems, the regulation and the fine-tuning of the molecular interactions at cellular and physiological levels are usually lost.

To fill this gap, approaches based on the affinity purification of molecular assemblies formed inside the cell and the measurement, by quantitative MS, of changes in complex stoichiometry following various cell stimulations have emerged [34,36]. Also, *in silico* strategies imply the integration of interaction

with global expression data [89–91]. Pseudo-affinity scores have been developed that approximate the tendency of protein pairs to associate and form direct physical contact [29].

In this paragraph, we review several biochemical approaches that have been used in the past on a smaller scale to quantify the binding constants and the thermodynamic parameters governing biomolecular interactions. Some of the methods might be amenable to more global strategies (Fig. 3). Fluorescence-based assays such as fluorescence resonance energy transfer (FRET) also hold great promises. They allow the monitoring and quantification of protein–protein interactions inside a living cell. These technologies are outside the scope of this review and will not be discussed; they have been extensively reviewed elsewhere [92,93].

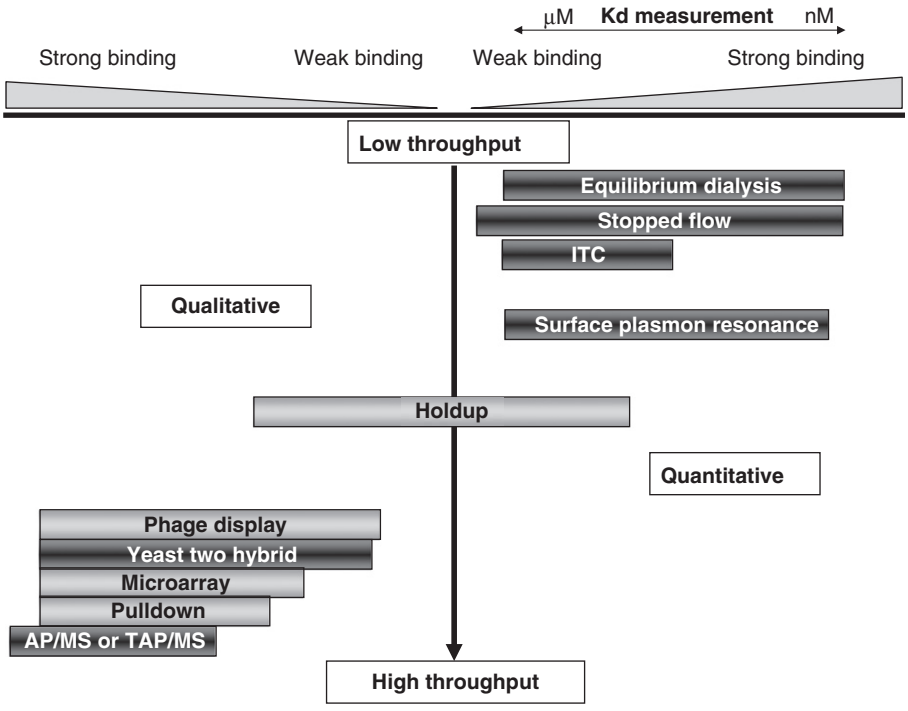


Fig. 3. Recapitulative plot of the described methods. The methods cited in the paper are plotted according to their features. Qualitative methods are depicted on the left side, quantitative on the right side. The Y-axis codes compatibility for high throughput. The X-axis codes for the capacity to detect and analyze weak or strong interactions. In case K_d can be quantitatively described, the working range is indicated as a double-headed arrow, ranging from sub-micromolar to nanomolar. Black boxes are indicative for techniques which allow working in solution; grey boxes for techniques, where one partner is bound to a solid matrix.

Surface plasmon resonance: Equilibrium and competition in solution

Almost 15 years after the development of the first biosensor relying on the principle of surface plasmon resonance (SPR) [94], SPR-based strategies have gained popularity in the field of interactomics, because of the high accuracy, reliability and sensitivity in reporting binding rates. An electromagnetic wave is measured, which propagates in a sensor surface between a dielectric and a metal (usually gold), the so-called surface plasma wave (SPW). Biacore and other SPR-based instruments use an optical method to measure the refractive index near the sensor surface (within 300 nm). A sensor surface is integrated in a flow cell (IFC) through which an aqueous solution passes under continuous flow (1–100 µl/min). The ligand (bait) is immobilized by chemical cross-linking onto the sensor surface either directly or on different carboxymethylated dextran matrices. The binding of the analyte (prey) results in an increase in local density at the surface of the sensor. This change is measured in real time and expressed in resonance units (RU). One RU represents the binding of approximately 1 pg protein/mm^2.

SPR has been broadly used to study the binding behavior of macromolecules such as recombinant proteins with their natural ligands. It generates real-time data and is well designed for the analysis of binding kinetics. An interesting extension is the possibility to design experiments, where measurements are made at different temperatures. This allows the monitoring of thermodynamic parameters (entropy, etc.). SPR performs for the measurement of interactions that cover broad ranges of K_d, from nM to the high µM range. An important limitation to kinetic analysis is due to the effect of mass transport that affects interactions with fast k_{on} values. At k_{on} faster than about 10^6–10^7 $M^{-1}s^{-1}$, the measurements significantly lose accuracy.

Stopped flow

Stopped flow experiments [95,96] are designed to measure interactions that have a very fast kinetic (rapid k_{on} and k_{off}) and that are usually not amenable to SPR or other methods. The principle relies on the very fast mixing of bait and prey solutions. The time required for mixing the two solutions, the ''dead time,'' is the shortest measurable time point in a stopped flow experiment. It ranges from 500 to 40 µs for the latest generations of stopped flow instruments. Fast reactions also require fast measurement methods. The mixing chamber is usually coupled to an external device for measuring the binding reaction, such as UV or visible spectroscopy, circular dichroism spectrometer, fluorescence spectrometer or electrical conductivity. Besides the possibility to monitor fast kinetics other advantages rely on the fact that measurement happens in solution. In consequence, there is no need of coupling the bait or the prey to a solid surface. Finally, only low amounts of biological material are needed.

Calorimetry – isothermal titration calorimetry (ITC)

Isothermal titration calorimetry (ITC) is a quantitative technique that is designed to measure the binding affinity (K_a), enthalpy changes (dH) and binding stoichiometry (n) between two or more molecules interacting in solution. An isothermal titration calorimeter is composed of two identical cells made of a highly efficient thermal conducting material surrounded by an adiabatic jacket. Sensitive thermocouple circuits are used to detect temperature differences between a buffer-filled reference cell and a sample cell containing the interacting macromolecule. During the experiment, the ligand (bait) is injected into the sample cell containing a prey. An exothermic reaction produces heat; the opposite occurs in case of an endothermic reaction. A power is applied to the reference cell and is coupled to a feedback circuit, activating a heater, located on the sample cell. The calorimeter measures the power needed over time to maintain the reference and the sample cell at an identical temperature.

ITC measures interaction directly in solution and does not require any modification or immobilization of bait or prey. ITC directly measures the heat change during the complex formation and, in addition to the binding constants, it also measures the thermodynamic parameters governing the interaction [97,98]. The main limitation remains the need of relatively high amount of sample (in the order of milligrams).

Equilibrium dialysis

Equilibrium dialysis is probably one of the simplest and nevertheless an effective assay for the study of interactions between molecules. The experimental setup is based on two chambers which are separated by a dialysis membrane. The molecular weight cut off of this membrane is chosen such that it will retain the ligand (bait) while the analyte (prey) diffuses freely. A known concentration and volume of prey is placed into one of the chambers. Its diffusion across the membrane and binding to the bait takes place until equilibrium has been reached. At equilibrium, the concentration of prey in free solution is the same in both chambers. In the bait chamber, however, the overall concentration is higher due to the presence of bait–prey complexes. The equilibrium binding of various concentrations of the prey and bait can be used to determine the K_d as well as number of binding sites on the bait.

Equilibrium dialysis also offers the ability to study low affinity interactions that are undetectable using other methods. It has been used to the detailed study of antigen–antibody interactions [99].

Hold up

The hold up assay, also called "comparative chromatography retention assay," is based, like the pulldown assay, on the reversible binding of a bait

to affinity resin. The main difference is that the hold up assay does not include washing steps and directly measures the amounts of prey remaining in solution upon exposure to the resin-bound bait. Therefore, in contrast to pulldown experiments, the hold up assay gives access to visualization of complexes at equilibrium conditions. Because it measures interaction at equilibrium, it allows detection of fast-exchanging protein complexes. Equilibrium dissociation constants (K_d) are measured that are comparable to the ones measured using SPR [100]. The hold up assay is specifically adapted to monitor weak protein interactions, where high concentrations of bait are necessary. The method is extremely simple and the general experimental setup is prone to automation. Work on this aspect is actually in progress.

Conclusions and perspectives

Nowadays, a growing choice of technologies is available to the scientists for the global charting of biomolecular interactions. These large-scale approaches have already contributed comprehensive cartographies of the proteins functionally involved in various human pathways that underlie pathologies. For instance, in human, the systematic mapping, by TAP/MS, of the protein interaction network around 32 components of the pro-inflammatory TNF-alpha/NF-kappa B signaling cascade led to the identification of 221 molecular associations. The analysis of the network and directed functional perturbation studies using RNA interference highlighted 10 new functional modulators that provided significant insight into the logic of the pathway as well as new candidate targets for pharmacological intervention [32]. Recently, global analysis of the interaction between a variety of viruses and their hosts provided new hypotheses on viral strategies for replication and persistence [101–103]. Generally, the elucidation of pathways or cellular processes important to human diseases is expected to contribute alternative therapeutic targets with better chemical tractability and also to provide a molecular frame for the interpretation of genetic links.

In the simple eukaryote, *S. cerevisiae*, more global, genome-wide screens for protein complexes raised the interesting view that protein multifunctionality may be a more general attribute than initially anticipated [28,104]. Different protein complexes very often use the same protein to exert their different biological functions. About 37% of the proteins were found to be part of more than one protein complex [105]. Proteins, similar to the globular domains they are made of, are used in a combinatorial manner and contribute to the assembly of a variety of "molecular machines." Protein modularity or multifunctionality has been proposed to support parsimonious increases in organismal complexity, *i.e.*, with a relatively constant number of genes. The understanding of protein modularity in higher eukaryotes may

not only provide a molecular frame for the explanation of genetic traits such as genetic pleiotropy, but is also expected to contribute to the selection of more specific and "safer" drug targets.

The systematic and unbiased charting of biomolecular networks in a variety of organisms contributes to our understanding of the sequences, motifs and structural folds involved in the processes of molecular recognition [5]. These recent advances opened new avenues for the identification of leads that specifically abrogate or modulate disease-relevant interactions. Promising successes include FTY720 (fingolimod; 2-amino-2[2-(4-octylphenyl) ethyl]-1,3-propanediol, Novartis) a sphingosine-one phosphate (S1P) analog that binds four of the S1P receptors [106], disruptors of the interaction between p53 and murine double minute 2 (MDM2) [107], compounds that interfere with the interaction between Bcl-2 and Bak [108] and small molecules inhibitors of SH3-mediated interactions [109].

Finally, the adaptation of existing technology to the large-scale measurement of biomolecular interactions in (semi-)quantitative terms represents an important challenge.

Acknowledgments

Our work is supported by the EMBL and the EU-grant "3D repertoire." We would like to thank Petra Riedinger for help with the figures. We are grateful to Christoph Müller, Vladimir Rybin, Gilles Travé and Yves Nominé for critical reading of the manuscript.

References

1. Goffeau A, Barrell BG, Bussey H, Davis RW, Dujon B, Feldmann H, Galibert F, Hoheisel JD, Jacq C, Johnston M, Louis EJ, Mewes HW, Murakami Y, Philippsen P, Tettelin H and Oliver SG. Life with 6000 genes. Science 1996;274:546, 563–567.
2. Pawson T, Raina M and Nash P. Interaction domains: from simple binding events to complex cellular behavior. FEBS Lett 2002;513:2–10.
3. Brown D and Superti-Furga G. Rediscovering the sweet spot in drug discovery. Drug Discov Today 2003;8:1067–1077.
4. Jeong H, Mason SP, Barabasi AL and Oltvai ZN. Lethality and centrality in protein networks. Nature 2001;411:41–42.
5. Neduva V, Linding R, Su-Angrand I, Stark A, de Masi F, Gibson TJ, Lewis J, Serrano L and Russell RB. Systematic discovery of new recognition peptides mediating protein interaction networks. PLoS Biol 2005;3:e405.
6. Neduva V and Russell RB. DILIMOT: discovery of linear motifs in proteins. Nucleic Acids Res 2006;34:W350–W355.
7. Neduva V and Russell RB. Peptides mediating interaction networks: new leads at last. Curr Opin Biotechnol 2006;17:465–471.
8. Landschulz WH, Johnson PF and McKnight SL. The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. Science 1988;240:1759–1764.

9. He W, Miao FJ, Lin DC, Schwandner RT, Wang Z, Gao J, Chen JL, Tian H and Ling L. Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. Nature 2004;429:188–193.
10. Anderson J, Burns HD, Enriquez-Harris P, Wilkie AO and Heath JK. Apert syndrome mutations in fibroblast growth factor receptor 2 exhibit increased affinity for FGF ligand. Hum Mol Genet 1998;7:1475–1483.
11. Shirohzu H, Kubota T, Kumazawa A, Sado T, Chijiwa T, Inagaki K, Suetake I, Tajima S, Wakui K, Miki Y, Hayashi M, Fukushima Y and Sasaki H. Three novel DNMT3B mutations in Japanese patients with ICF syndrome. Am J Med Genet 2002;112:31–37.
12. Wiszniewski W, Fondaneche MC, Louise-Plence P, Prochnicka-Chalufour A, Selz F, Picard C, Le Deist F, Eliaou JF, Fischer A and Lisowska-Grospierre B. Novel mutations in the RFXANK gene: RFX complex containing in-vitro-generated RFXANK mutant binds the promoter without transactivating MHC II. Immunogenetics 2003;54:747–755.
13. Trojanowski JQ and Lee VM. "Fatal attractions" of proteins: a comprehensive hypothetical mechanism underlying Alzheimer's disease and other neurodegenerative disorders. Ann N Y Acad Sci 2000;924:62–67.
14. Eng C. PTEN: one gene, many syndromes. Hum Mutat 2003;22:183–198.
15. Hagemann TL, Chen Y, Rosen FS and Kwan SP. Genomic organization of the Btk gene and exon scanning for mutations in patients with X-linked agammaglobulinemia. Hum Mol Genet 1994;3:1743–1749.
16. Hyvonen M and Saraste M. Structure of the PH domain and Btk motif from Bruton's tyrosine kinase: molecular explanations for X-linked agammaglobulinaemia. Embo J 1997;16:3396–3404.
17. Orchard S, Salwinski L, Kerrien S, Montecchi-Palazzi L, Oesterheld M, Stumpflen V, Ceol A, Chatr-Aryamontri A, Armstrong J, Woollard P, Salama JJ, Moore S, Wojcik J, Bader GD, Vidal M, Cusick ME, Gerstein M, Gavin AC, Superti-Furga G, Greenblatt J, Bader J, Uetz P, Tyers M, Legrain P, Fields S, Mulder N, Gilson M, Niepmann M, Burgoon L, Rivas Jde L, Prieto C, Perreau VM, Hogue C, Mewes HW, Apweiler R, Xenarios I, Eisenberg D, Cesareni G and Hermjakob H. The minimum information required for reporting a molecular interaction experiment (MIMIx). Nat Biotechnol 2007;25:894–898.
18. Husi H, Ward MA, Choudhary JS, Blackstock WP and Grant SG. Proteomic analysis of NMDA receptor-adhesion protein signaling complexes. Nat Neurosci 2000;3:661–669.
19. Becamel C, Alonso G, Galeotti N, Demey E, Jouin P, Ullmer C, Dumuis A, Bockaert J and Marin P. Synaptic multiprotein complexes associated with 5-HT(2C) receptors: a proteomic approach. Embo J 2002;21:2332–2342.
20. Junttila MR, Saarinen S, Schmidt T, Kast J and Westermarck J. Single-step Strep-tag purification for the isolation and identification of protein complexes from mammalian cells. Proteomics 2005;5:1199–1203.
21. Korf U, Kohl T, van der Zandt H, Zahn R, Schleeger S, Ueberle B, Wandschneider S, Bechtel S, Schnolzer M, Ottleben H, Wiemann S and Poustka A. Large-scale protein expression for proteome research. Proteomics 2005;5:3571–3580.
22. Lichty JJ, Malecki JL, Agnew HD, Michelson-Horowitz DJ and Tan S. Comparison of affinity tags for protein purification. Protein Expr Purif 2005;41:98–105.
23. Terpe K. Overview of tag protein fusions: from molecular and biochemical fundamentals to commercial systems. Appl Microbiol Biotechnol 2003;60:523–533.
24. Ho Y, Gruhler A, Heilbut A, Bader GD, Moore L, Adams SL, Millar A, Taylor P, Bennett K, Boutilier K, Yang L, Wolting C, Donaldson I, Schandorff S, Shewnarane J, Vo M, Taggart J, Goudreault M, Muskat B, Alfarano C, Dewar D, Lin Z, Michalickova K,

Willems H, Sassi H, Nielsen PA, Rasmussen KJ, Andersen JR, Johansen LE, Hansen LH, Jespersen H, Podtelejnikov A, Nielsen E, Crawford J, Poulsen V, Sorensen BD, Matthiesen J, Hendrickson RC, Gleeson F, Pawson T, Moran MF, Durocher D, Mann M, Hogue CW, Figeys D and Tyers M. Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry. Nature 2002;415:180–183.

25. Rigaut G, Shevchenko A, Rutz B, Wilm M, Mann M and Seraphin B. A generic protein purification method for protein complex characterization and proteome exploration. Nat Biotechnol 1999;17:1030–1032.
26. Puig O, Caspary F, Rigaut G, Rutz B, Bouveret E, Bragado-Nilsson E, Wilm M and Seraphin B. The tandem affinity purification (TAP) method: a general procedure of protein complex purification. Methods 2001;24:218–229.
27. Butland G, Peregrin-Alvarez JM, Li J, Yang W, Yang X, Canadien V, Starostine A, Richards D, Beattie B, Krogan N, Davey M, Parkinson J, Greenblatt J and Emili A. Interaction network containing conserved and essential protein complexes in *Escherichia coli*. Nature 2005;433:531–537.
28. Gavin AC, Bosche M, Krause R, Grandi P, Marzioch M, Bauer A, Schultz J, Rick JM, Michon AM, Cruciat CM, Remor M, Hofert C, Schelder M, Brajenovic M, Ruffner H, Merino A, Klein K, Hudak M, Dickson D, Rudi T, Gnau V, Bauch A, Bastuck S, Huhse B, Leutwein C, Heurtier MA, Copley RR, Edelmann A, Querfurth E, Rybin V, Drewes G, Raida M, Bouwmeester T, Bork P, Seraphin B, Kuster B, Neubauer G and Superti-Furga G. Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature 2002;415:141–147.
29. Gavin AC, Aloy P, Grandi P, Krause R, Boesche M, Marzioch M, Rau C, Jensen LJ, Bastuck S, Dumpelfeld B, Edelmann A, Heurtier MA, Hoffman V, Hoefert C, Klein K, Hudak M, Michon AM, Schelder M, Schirle M, Remor M, Rudi T, Hooper S, Bauer A, Bouwmeester T, Casari G, Drewes G, Neubauer G, Rick JM, Kuster B, Bork P, Russell RB and Superti-Furga G. Proteome survey reveals modularity of the yeast cell machinery. Nature 2006;
30. Krogan NJ, Cagney G, Yu H, Zhong G, Guo X, Ignatchenko A, Li J, Pu S, Datta N, Tikuisis AP, Punna T, Peregrin-Alvarez JM, Shales M, Zhang X, Davey M, Robinson MD, Paccanaro A, Bray JE, Sheung A, Beattie B, Richards DP, Canadien V, Lalev A, Mena F, Wong P, Starostine A, Canete MM, Vlasblom J, Wu S, Orsi C, Collins SR, Chandran S, Haw R, Rilstone JJ, Gandi K, Thompson NJ, Musso G, St Onge P, Ghanny S, Lam MH, Butland G, Altaf-Ul AM, Kanaya S, Shilatifard A, O'Shea E, Weissman JS, Ingles CJ, Hughes TR, Parkinson J, Gerstein M, Wodak SJ, Emili A and Greenblatt JF. Global landscape of protein complexes in the yeast S*accharomyces cerevisiae*. Nature 2006;440:637–643.
31. Rohila JS, Chen M, Chen S, Chen J, Cerny R, Dardick C, Canlas P, Xu X, Gribskov M, Kanrar S, Zhu JK, Ronald P and Fromm ME. Protein–protein interactions of tandem affinity purification-tagged protein kinases in rice. Plant J 2006;46:1–13.
32. Bouwmeester T, Bauch A, Ruffner H, Angrand PO, Bergamini G, Croughton K, Cruciat C, Eberhard D, Gagneur J, Ghidelli S, Hopf C, Huhse B, Mangano R, Michon AM, Schirle M, Schlegl J, Schwab M, Stein MA, Bauer A, Casari G, Drewes G, Gavin AC, Jackson DB, Joberty G, Neubauer G, Rick J, Kuster B and Superti-Furga G. A physical and functional map of the human TNF-alpha/NF-kappa B signal transduction pathway. Nat Cell Biol 2004;6:97–105.
33. Krogan NJ, Peng WT, Cagney G, Robinson MD, Haw R, Zhong G, Guo X, Zhang X, Canadien V, Richards DP, Beattie BK, Lalev A, Zhang W, Davierwala AP, Mnaimneh S, Starostine A, Tikuisis AP, Grigull J, Datta N, Bray JE, Hughes TR, Emili A and

Greenblatt JF. High-definition macromolecular composition of yeast RNA-processing complexes. Mol Cell 2004;13:225–239.
34. Ranish JA, Yi EC, Leslie DM, Purvine SO, Goodlett DR, Eng J and Aebersold R. The study of macromolecular complexes by quantitative proteomics. Nat Genet 2003;33:349–355.
35. Gingras AC, Aebersold R and Raught B. Advances in protein complex analysis using mass spectrometry. J Physiol 2005;563:11–21.
36. Gingras AC, Gstaiger M, Raught B and Aebersold R. Analysis of protein complexes using mass spectrometry. Nat Rev Mol Cell Biol 2007;8:645–654.
37. Aloy P, Ciccarelli FD, Leutwein C, Gavin AC, Superti-Furga G, Bork P, Bottcher B and Russell RB. A complex prediction: three-dimensional model of the yeast exosome. EMBO Rep 2002;3:628–635.
38. Aloy P, Bottcher B, Ceulemans H, Leutwein C, Mellwig C, Fischer S, Gavin AC, Bork P, Superti-Furga G, Serrano L and Russell RB. Structure-based assembly of protein complexes in yeast. Science 2004;303:2026–2029.
39. Burckstummer T, Bennett KL, Preradovic A, Schutze G, Hantschel O, Superti-Furga G and Bauch A. An efficient tandem affinity purification procedure for interaction proteomics in mammalian cells. Nat Methods 2006;3:1013–1019.
40. Yang P, Sampson HM and Krause HM. A modified tandem affinity purification strategy identifies cofactors of the Drosophila nuclear receptor dHNF4. Proteomics 2006;6:927–935.
41. Ratner D. The interaction bacterial and phage proteins with immobilized *Escherichia coli* RNA polymerase. J Mol Biol 1974;88:373–383.
42. Schulze WX, Deng L and Mann M. Phosphotyrosine interactome of the ErbB-receptor kinase family, Mol Syst Biol 2005;1:2005.0008.
43. Davidson WS, Ghering AB, Beish L, Tubb MR, Hui DY and Pearson K. The biotincapture lipid affinity assay: a rapid method for determining lipid binding parameters for apolipoproteins. J Lipid Res 2006;47:440–449.
44. Scholten A, Poh MK, van Veen TA, van Breukelen B, Vos MA and Heck AJ. Analysis of the cGMP/cAMP interactome using a chemical proteomics approach in mammalian heart tissue validates sphingosine kinase type 1-interacting protein as a genuine and highly abundant AKAP. J Proteome Res 2006;5:1435–1447.
45. Bantscheff M, Eberhard D, Abraham Y, Bastuck S, Boesche M, Hobson S, Mathieson T, Perrin J, Raida M, Rau C, Reader V, Sweetman G, Bauer A, Bouwmeester T, Hopf C, Kruse U, Neubauer G, Ramsden N, Rick J, Kuster B and Drewes G. Quantitative chemical proteomics reveals mechanisms of action of clinical ABL kinase inhibitors. Nat Biotechnol 2007;25:1035–1044.
46. Fields S and Song O. A novel genetic system to detect protein–protein interactions. Nature 1989;340:245–246.
47. Rain JC, Selig L, De Reuse H, Battaglia V, Reverdy C, Simon S, Lenzen G, Petel F, Wojcik J, Schachter V, Chemama Y, Labigne A and Legrain P. The protein–protein interaction map of Helicobacter pylori. Nature 2001;409:211–215.
48. Uetz P, Giot L, Cagney G, Mansfield TA, Judson RS, Knight JR, Lockshon D, Narayan V, Srinivasan M, Pochart P, Qureshi-Emili A, Li Y, Godwin B, Conover D, Kalbfleisch T, Vijayadamodar G, Yang M, Johnston M, Fields S and Rothberg JM. A comprehensive analysis of protein–protein interactions in *Saccharomyces cerevisiae*. Nature 2000;403:623–627.
49. Giot L, Bader JS, Brouwer C, Chaudhuri A, Kuang B, Li Y, Hao YL, Ooi CE, Godwin B, Vitols E, Vijayadamodar G, Pochart P, Machineni H, Welsh M, Kong Y, Zerhusen B, Malcolm R, Varrone Z, Collis A, Minto M, Burgess S, McDaniel L, Stimpson E,

Spriggs F, Williams J, Neurath K, Ioime N, Agee M, Voss E, Furtak K, Renzulli R, Aanensen N, Carrolla S, Bickelhaupt E, Lazovatsky Y, DaSilva A, Zhong J, Stanyon CA, Finley RL, Jr., White KP, Braverman M, Jarvie T, Gold S, Leach M, Knight J, Shimkets RA, McKenna MP, Chant J and Rothberg JM. A protein interaction map of *Drosophila melanogaster*. Science 2003;302:1727–1736.

50. Formstecher E, Aresta S, Collura V, Hamburger A, Meil A, Trehin A, Reverdy C, Betin V, Maire S, Brun C, Jacq B, Arpin M, Bellaiche Y, Bellusci S, Benaroch P, Bornens M, Chanet R, Chavrier P, Delattre O, Doye V, Fehon R, Faye G, Galli T, Girault JA, Goud B, de Gunzburg J, Johannes L, Junier MP, Mirouse V, Mukherjee A, Papadopoulo D, Perez F, Plessis A, Rosse C, Saule S, Stoppa-Lyonnet D, Vincent A, White M, Legrain P, Wojcik J, Camonis J and Daviet L. Protein interaction mapping: a Drosophila case study. Genome Res 2005;15:376–384.
51. Li S, Armstrong CM, Bertin N, Ge H, Milstein S, Boxem M, Vidalain PO, Han JD, Chesneau A, Hao T, Goldberg DS, Li N, Martinez M, Rual JF, Lamesch P, Xu L, Tewari M, Wong SL, Zhang LV, Berriz GF, Jacotot L, Vaglio P, Reboul J, Hirozane-Kishikawa T, Li Q, Gabel HW, Elewa A, Baumgartner B, Rose DJ, Yu H, Bosak S, Sequerra R, Fraser A, Mango SE, Saxton WM, Strome S, Van Den Heuvel S, Piano F, Vandenhaute J, Sardet C, Gerstein M, Doucette-Stamm L, Gunsalus KC, Harper JW, Cusick ME, Roth FP, Hill DE and Vidal M. A map of the interactome network of the metazoan *C. elegans*. Science 2004;303:540–543.
52. Rual JF, Venkatesan K, Hao T, Hirozane-Kishikawa T, Dricot A, Li N, Berriz GF, Gibbons FD, Dreze M, Ayivi-Guedehoussou N, Klitgord N, Simon C, Boxem M, Milstein S, Rosenberg J, Goldberg DS, Zhang LV, Wong SL, Franklin G, Li S, Albala JS, Lim J, Fraughton C, Llamosas E, Cevik S, Bex C, Lamesch P, Sikorski RS, Vandenhaute J, Zoghbi HY, Smolyar A, Bosak S, Sequerra R, Doucette-Stamm L, Cusick ME, Hill DE, Roth FP and Vidal M. Towards a proteome-scale map of the human protein–protein interaction network. Nature 2005;
53. Stelzl U, Worm U, Lalowski M, Haenig C, Brembeck FH, Goehler H, Stroedicke M, Zenkner M, Schoenherr A, Koeppen S, Timm J, Mintzlaff S, Abraham C, Bock N, Kietzmann S, Goedde A, Toksoz E, Droege A, Krobitsch S, Korn B, Birchmeier W, Lehrach H and Wanker EE. A human protein–protein interaction network: a resource for annotating the proteome. Cell 2005;122:957–968.
54. Aronheim A, Zandi E, Hennemann H, Elledge SJ and Karin M. Isolation of an AP-1 repressor by a novel method for detecting protein–protein interactions. Mol Cell Biol 1997;17:3094–3102.
55. Broder YC, Katz S and Aronheim A. The ras recruitment system, a novel approach to the study of protein–protein interactions. Curr Biol 1998;8:1121–1124.
56. Ehrhard KN, Jacoby JJ, Fu XY, Jahn R and Dohlman HG. Use of G-protein fusions to monitor integral membrane protein–protein interactions in yeast. Nat Biotechnol 2000;18:1075–1079.
57. Stagljar I, Korostensky C, Johnsson N and te Heesen S. A genetic system based on split-ubiquitin for the analysis of interactions between membrane proteins in vivo. Proc Natl Acad Sci USA 1998;95:5187–5192.
58. Eyckerman S, Verhee A, der Heyden JV, Lemmens I, Ostade XV, Vandekerckhove J and Tavernier J. Design and application of a cytokine-receptor-based interaction trap. Nat Cell Biol 2001;3:1114–1119.
59. Iyer K, Burkle L, Auerbach D, Thaminy S, Dinkel M, Engels K and Stagljar I. Utilizing the split-ubiquitin membrane yeast two-hybrid system to identify protein–protein interactions of integral membrane proteins. Sci STKE 2005;2005:pl3.

60. Vidal M, Brachmann RK, Fattaey A, Harlow E and Boeke JD. Reverse two-hybrid and one-hybrid systems to detect dissociation of protein–protein and DNA-protein interactions. Proc Natl Acad Sci USA 1996;93:10315–10320.
61. Kato-Stankiewicz J, Hakimi I, Zhi G, Zhang J, Serebriiskii I, Guo L, Edamatsu H, Koide H, Menon S, Eckl R, Sakamuri S, Lu Y, Chen QZ, Agarwal S, Baumbach WR, Golemis EA, Tamanoi F and Khazak V. Inhibitors of Ras/Raf-1 interaction identified by two-hybrid screening revert Ras-dependent transformation phenotypes in human cancer cells. Proc Natl Acad Sci USA 2002;99:14398–14403.
62. Eyckerman S, Lemmens I, Catteeuw D, Verhee A, Vandekerckhove J, Lievens S and Tavernier J. Reverse MAPPIT: screening for protein-protein interaction modifiers in mammalian cells. Nat Methods 2005;2:427–433.
63. Caligiuri M, Molz L, Liu Q, Kaplan F, Xu JP, Majeti JZ, Ramos-Kelsey R, Murthi K, Lievens S, Tavernier J and Kley N. MASPIT: Three-hybrid trap for quantitative proteome fingerprinting of small molecule–protein interactions in mammalian cells. Chem Biol 2006;13:711–722.
64. Smith GP. Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. Science 1985;228:1315–1317.
65. Kehoe JW and Kay BK. Filamentous phage display in the new millennium. Chem Rev 2005;105:4056–4072.
66. Irving MB, Pan O and Scott JK. Random-peptide libraries and antigen-fragment libraries for epitope mapping and the development of vaccines and diagnostics. Curr Opin Chem Biol 2001;5:314–324.
67. Groves M, Lane S, Douthwaite J, Lowne D, Rees DG, Edwards B and Jackson RH. Affinity maturation of phage display antibody populations using ribosome display. J Immunol Methods 2006;313:129–139.
68. Ho M, Nagata S and Pastan I. Isolation of anti-CD22 Fv with high affinity by Fv display on human cells. Proc Natl Acad Sci USA 2006;103:9637–9642.
69. Sergeeva A, Kolonin MG, Molldrem JJ, Pasqualini R and Arap W. Display technologies: application for the discovery of drug and gene delivery agents. Adv Drug Deliv Rev 2006;58:1622–1654.
70. Jiang J, Abu-Shilbayeh L and Rao VB. Display of a PorA peptide from Neisseria meningitidis on the bacteriophage T4 capsid surface. Infect Immun 1997;65:4770–4777.
71. Danner S and Belasco JG. T7 phage display: a novel genetic selection system for cloning RNA-binding proteins from cDNA libraries. Proc Natl Acad Sci USA 2001;98:12954–12959.
72. Castagnoli L, Zucconi A, Quondam M, Rossi M, Vaccaro P, Panni S, Paoluzi S, Santonico E, Dente L and Cesareni G. Alternative bacteriophage display systems. Comb Chem High Throughput Screen 2001;4:121–133.
73. Malys N, Chang DY, Baumann RG, Xie D and Black LW. A bipartite bacteriophage T4 SOC and HOC randomized peptide display library: detection and analysis of phage T4 terminase (gp17) and late sigma factor (gp55) interaction. J Mol Biol 2002;319: 289–304.
74. Robinson P, Stuber D, Deryckere F, Tedbury P, Lagrange M and Orfanoudakis G. Identification using phage display of peptides promoting targeting and internalization into HPV-transformed cell lines. J Mol Recognit 2005;18:175–182.
75. Wang X, Yu J, Sreekumar A, Varambally S, Shen R, Giacherio D, Mehra R, Montie JE, Pienta KJ, Sanda MG, Kantoff PW, Rubin MA, Wei JT, Ghosh D and Chinnaiyan AM. Autoantibody signatures in prostate cancer. N Engl J Med 2005;353:1224–1235.

76. Draghici S, Chatterjee M and Tainsky MA. Epitomics: serum screening for the early detection of cancer on microarrays using complex panels of tumor antigens. Expert Rev Mol Diagn 2005;5:735–743.
77. Zhu H, Bilgin M, Bangham R, Hall D, Casamayor A, Bertone P, Lan N, Jansen R, Bidlingmaier S, Houfek T, Mitchell T, Miller P, Dean RA, Gerstein M and Snyder M. Global analysis of protein activities using proteome chips. Science 2001;293:2101–2105.
78. Schweitzer B, Predki P and Snyder M. Microarrays to characterize protein interactions on a whole-proteome scale. Proteomics 2003;3:2190–2199.
79. Poetz O, Schwenk JM, Kramer S, Stoll D, Templin MF and Joos TO. Protein microarrays: catching the proteome. Mech Ageing Dev 2005;126:161–170.
80. Poetz O, Ostendorp R, Brocks B, Schwenk JM, Stoll D, Joos TO and Templin MF. Protein microarrays for antibody profiling: specificity and affinity determination on a chip. Proteomics 2005;5:2402–2411.
81. Espejo A, Cote J, Bednarek A, Richard S and Bedford MT. A protein-domain microarray identifies novel protein–protein interactions. Biochem J 2002;367:697–702.
82. Hiller R, Laffer S, Harwanegg C, Huber M, Schmidt WM, Twardosz A, Barletta B, Becker WM, Blaser K, Breiteneder H, Chapman M, Crameri R, Duchene M, Ferreira F, Fiebig H, Hoffmann-Sommergruber K, King TP, Kleber-Janke T, Kurup VP, Lehrer SB, Lidholm J, Muller U, Pini C, Reese G, Scheiner O, Scheynius A, Shen HD, Spitzauer S, Suck R, Swoboda I, Thomas W, Tinghino R, Van Hage-Hamsten M, Virtanen T, Kraft D, Muller MW and Valenta R. Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. Faseb J 2002;16:414–416.
83. Uttamchandani M, Walsh DP, Yao SQ and Chang YT. Small molecule microarrays: recent advances and applications. Curr Opin Chem Biol 2005;9:4–13.
84. MacBeath G, Koehler AN and Schreiber SL. Printing small molecules as microarrays and detecting protein–ligand interactions en masse. J Am Chem Soc 1999;121:7967–7968.
85. Schmitz K, Haggarty SJ, McPherson OM, Clardy J and Koehler AN. Detecting binding interactions using microarrays of natural product extracts. J Am Chem Soc 2007;129:11346–11347.
86. Winssinger N, Ficarro S, Schultz PG and Harris JL. Profiling protein function with small molecule microarrays. Proc Natl Acad Sci USA 2002;99:11139–11144.
87. Blixt O, Head S, Mondala T, Scanlan C, Huflejt ME, Alvarez R, Bryan MC, Fazio F, Calarese D, Stevens J, Razi N, Stevens DJ, Skehel JJ, van Die I, Burton DR, Wilson IA, Cummings R, Bovin N, Wong CH and Paulson JC. Printed covalent glycan array for ligand profiling of diverse glycan binding proteins. Proc Natl Acad Sci USA 2004;101:17033–17038.
88. Kuruvilla FG, Shamji AF, Sternson SM, Hergenrother PJ and Schreiber SL. Dissecting glucose signalling with diversity-oriented synthesis and small-molecule microarrays. Nature 2002;416:653–657.
89. Luscombe NM, Babu MM, Yu H, Snyder M, Teichmann SA and Gerstein M. Genomic analysis of regulatory network dynamics reveals large topological changes. Nature 2004;431:308–312.
90. Han JD, Bertin N, Hao T, Goldberg DS, Berriz GF, Zhang LV, Dupuy D, Walhout AJ, Cusick ME, Roth FP and Vidal M. Evidence for dynamically organized modularity in the yeast protein–protein interaction network. Nature 2004;430:88–93.
91. de Lichtenberg U, Jensen LJ, Brunak S and Bork P. Dynamic complex formation during the yeast cell cycle. Science 2005;307:724–727.

92. Presley JF. Imaging the secretory pathway: the past and future impact of live cell optical techniques. Biochim Biophys Acta 2005;1744:259–272.
93. Wallrabe H and Periasamy A. Imaging protein molecules using FRET and FLIM microscopy. Curr Opin Biotechnol 2005;16:19–27.
94. Jonsson U, Fagerstam L, Ivarsson B, Johnsson B, Karlsson R, Lundh K, Lofas S, Persson B, Roos H, Ronnberg I, et al. Real-time biospecific interaction analysis using surface plasmon resonance and a sensor chip technology. Biotechniques 1991;11: 620–627.
95. Gibson QH and Milnes L. Apparatus for rapid and sensitive spectrophotometry. Biochem J 1964;91:161–171.
96. Chance B. The kinetics of the enzyme-substrate compound of peroxidase. 1943. Adv Enzymol Relat Areas Mol Biol 1999;73:3–23.
97. Weber PC and Salemme FR. Applications of calorimetric methods to drug discovery and the study of protein interactions. Curr Opin Struct Biol 2003;13:115–121.
98. Perozzo R, Folkers G and Scapozza L. Thermodynamics of protein–ligand interactions: history, presence, and future aspects. J Recept Signal Transduct Res 2004; 24:1–52.
99. Ji QC, Rodila R, Morgan SJ, Humerickhouse RA and El-Shourbagy TA. Investigation of the immunogenicity of a protein drug using equilibrium dialysis and liquid chromatography tandem mass spectrometry detection. Anal Chem 2005;77:5529–5533.
100. Charbonnier S, Zanier K, Masson M and Trave G. Capturing protein–protein complexes at equilibrium: the holdup comparative chromatographic retention assay. Protein Expr Purif 2006;50:89–101.
101. Calderwood MA, Venkatesan K, Xing L, Chase MR, Vazquez A, Holthaus AM, Ewence AE, Li N, Hirozane-Kishikawa T, Hill DE, Vidal M, Kieff E and Johannsen E. Epstein-Barr virus and virus human protein interaction maps. Proc Natl Acad Sci USA 2007;104:7606–7611.
102. Uetz P, Dong YA, Zeretzke C, Atzler C, Baiker A, Berger B, Rajagopala SV, Roupelieva M, Rose D, Fossum E and Haas J. Herpesviral protein networks and their interaction with the human proteome. Science 2006;311:239–242.
103. Brizard JP, Carapito C, Delalande F, Van Dorsselaer A and Brugidou C. Proteome analysis of plant-virus interactome: comprehensive data for virus multiplication inside their hosts. Mol Cell Proteomics 2006;5:2279–2297.
104. Gavin AC and Superti-Furga G. Protein complexes and proteome organization from yeast to man. Curr Opin Chem Biol 2003;7:21–27.
105. Krause R, von Mering C, Bork P and Dandekar T. Shared components of protein complexes-versatile building blocks or biochemical artefacts? Bioessays 2004;26: 1333–1343.
106. Sonoda Y, Yamamoto D, Sakurai S, Hasegawa M, Aizu-Yokota E, Momoi T and Kasahara T. FTY720, a novel immunosuppressive agent, induces apoptosis in human glioma cells. Biochem Biophys Res Commun 2001;281:282–288.
107. Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, Kong N, Kammlott U, Lukacs C, Klein C, Fotouhi N and Liu EA. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. Science 2004;303:844–848.
108. Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, Bruncko M, Deckwerth TL, Dinges J, Hajduk PJ, Joseph MK, Kitada S, Korsmeyer SJ, Kunzer AR, Letai A, Li C, Mitten MJ, Nettesheim DG, Ng S, Nimmer PM, O'Connor JM, Oleksijew A, Petros AM, Reed JC, Shen W, Tahir SK, Thompson CB, Tomaselli KJ, Wang B, Wendt MD, Zhang H, Fesik SW and Rosenberg SH. An

inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature 2005;435:677–681.
109. Inglis SR, Stojkoski C, Branson KM, Cawthray JF, Fritz D, Wiadrowski E, Pyke SM and Booker GW. Identification and specificity studies of small-molecule ligands for SH3 protein domains. J Med Chem 2004;47:5405–5417.
110. Yarbrough WG, Buckmire RA, Bessho M and Liu ET. Biologic and biochemical analyses of p16(INK4a) mutations from primary tumors. J Natl Cancer Inst 1999;91:1569–1574.
111. Cammett TJ, Luo L and Peng ZY. Design and characterization of a hyperstable p16INK4a that restores Cdk4 binding activity when combined with oncogenic mutations. J Mol Biol 2003;327:285–297.
112. Zweifel ME, Leahy DJ, Hughson FM and Barrick D. Structure and stability of the ankyrin domain of the Drosophila Notch receptor. Protein Sci 2003;12:2622–2632.
113. Arboleda-Velasquez JF, Rampal R, Fung E, Darland DC, Liu M, Martinez MC, Donahue CP, Navarro-Gonzalez MF, Libby P, D'Amore PA, Aikawa M, Haltiwanger RS and Kosik KS. CADASIL mutations impair Notch3 glycosylation by Fringe. Hum Mol Genet 2005;14:1631–1639.
114. Ruf RG, Xu PX, Silvius D, Otto EA, Beekmann F, Muerb UT, Kumar S, Neuhaus TJ, Kemper MJ, Raymond RM, Jr., Brophy PD, Berkman J, Gattas M, Hyland V, Ruf EM, Schwartz C, Chang EH, Smith RJ, Stratakis CA, Weil D, Petit C and Hildebrandt F. SIX1 mutations cause branchio-oto-renal syndrome by disruption of EYA1-SIX1-DNA complexes. Proc Natl Acad Sci USA 2004;101:8090–8095.
115. Zhou HX. Improving the understanding of human genetic diseases through predictions of protein structures and protein–protein interaction sites. Curr Med Chem 2004;11:539–549.
116. Fan C, Liu M and Wang Q. Functional analysis of TBX5 missense mutations associated with Holt-Oram syndrome. J Biol Chem 2003;278:8780–8785.
117. Chen T, Tsujimoto N and Li E. The PWWP domain of Dnmt3a and Dnmt3b is required for directing DNA methylation to the major satellite repeats at pericentric heterochromatin. Mol Cell Biol 2004;24:9048–9058.
118. Ding J, Liu JJ, Kowal AS, Nardine T, Bhattacharya P, Lee A and Yang Y. Microtubule-associated protein 1B: a neuronal binding partner for gigaxonin. J Cell Biol 2002;158:427–433.
119. Guerrette S, Acharya S and Fishel R. The interaction of the human MutL homologues in hereditary nonpolyposis colon cancer. J Biol Chem 1999;274:6336–6341.
120. Hu X, Plomp A, Wijnholds J, Ten Brink J, van Soest S, van den Born LI, Leys A, Peek R, de Jong PT and Bergen AA. ABCC6/MRP6 mutations: further insight into the molecular pathology of pseudoxanthoma elasticum. Eur J Hum Genet 2003;11:215–224.
121. Ritchie HH, Hughes MR, Thompson ET, Malloy PJ, Hochberg Z, Feldman D, Pike JW and O'Malley BW. An ochre mutation in the vitamin D receptor gene causes hereditary 1,25-dihydroxyvitamin D3-resistant rickets in three families. Proc Natl Acad Sci USA 1989;86:9783–9787.
122. Barroso I, Gurnell M, Crowley VE, Agostini M, Schwabe JW, Soos MA, Maslen GL, Williams TD, Lewis H, Schafer AJ, Chatterjee VK and O'Rahilly S. Dominant negative mutations in human PPARgamma associated with severe insulin resistance, diabetes mellitus and hypertension. Nature 1999;402:880–883.
123. Carpten JD, Faber AL, Horn C, Donoho GP, Briggs SL, Robbins CM, Hostetter G, Boguslawski S, Moses TY, Savage S, Uhlik M, Lin A, Du J, Qian YW, Zeckner DJ, Tucker-Kellogg G, Touchman J, Patel K, Mousses S, Bittner M, Schevitz R, Lai MH,

Blanchard JE and Thomas JE. A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature 2007;448:439–444.
124. Ito T, Chiba T, Ozawa R, Yoshida M, Hattori M and Sakaki Y. A comprehensive two-hybrid analysis to explore the yeast protein interactome. Proc Natl Acad Sci USA 2001;98:4569–4574.
125. Parrish JR, Yu J, Liu G, Hines JA, Chan JE, Mangiola BA, Zhang H, Pacifico S, Fotouhi F, Dirita VJ, Ideker T, Andrews P and Finley RL, Jr. A proteome-wide protein interaction map for *Campylobacter jejuni*. Genome Biol 2007;8:R130.
126. Rohila JS, Chen M, Cerny R and Fromm ME. Improved tandem affinity purification tag and methods for isolation of protein heterocomplexes from plants. Plant J 2004;38: 172–181.