Protein Expression and Purification 72 (2010) 149–156

Contents lists available at ScienceDirect

Protein Expression and Purification

journal homepage: www.elsevier.com/locate/yprep

Review

The tandem affinity purification method: An efficient system for protein complex purification and protein interaction identification

Xiaoli Xu, Yuan Song, Yuhua Li, Jianfeng Chang, Hua zhang *, Lizhe An **

Key Laboratory of Arid and Grassland Agroecology of Ministry of Education, School of Life Sciences, Lanzhou University, Lanzhou 730000, China

ARTICLE INFO

Article history:
Received 17 February 2010
and in revised form 8 April 2010
Available online 23 April 2010

Keywords:
Tandem affinity purification
Protein–protein interaction
Protein complex
TAP tag

ABSTRACT

Isolation and identification of protein partners in multi-protein complexes are important in gaining further insights into the cellular roles of proteins and determining the possible mechanisms by which proteins have an effect in the molecular environment. The tandem affinity purification (TAP) method was originally developed in yeast for the purification of protein complexes and identification of protein–protein interactions. With modifications to this method and many variations in the original tag made over the past few years, the TAP system could be applied in mammalian, plant, bacteria and other systems for protein complex analysis. In this review, we describe the application of the TAP method in various organisms, the modification in the tag, the disadvantages, the developments and the future prospects of the TAP method.

Introduction

Recently, a large number of studies have focused on proteins because it was realized that the intact genome sequence information was not enough to explain and predict cellular mechanisms. Proteins carry out and regulate the majority of cellular activities and generally interact with neighboring proteins and form multi-protein complexes in a time- and space-dependent manner [1], or in response to intra- and intercellular signals. Within a protein complex, each individual protein has a significant role within the whole function of the complex and this function may rely on association with other proteins. This combination of proteins may provide regulation of protein activity through conformational transformation or post-translational modification. It is increasingly clear that functional research of single proteins in a complex organization may yield a better understanding of their functions [2].

Genome-wide yeast two-hybrid screens [3–5] and protein chip-based methods [6] allow broader insight into the interaction networks. However, the yeast two-hybrid system only produces binary interactions and has the potential for false–positive and false–negative results. As for protein chips, the task of purifying and spotting proteins is time consuming and labor intensive. These defects may limit their application in large scale protein complex purification.

A generic protein complex purification strategy, named tandem affinity purification (TAP)[1] [7,8], in combination with mass spectrometry allows identification of interaction partners and purification of protein complexes. This strategy was originally developed in yeast and has been tested in many cells and organisms.

Overview of the TAP tag and the TAP method

The TAP method requires fusing a TAP tag to the target protein. The TAP tag is composed of two IgG-binding units of protein A of *Staphylococcus aureus* (ProtA) and a calmodulin-binding domain (CBP), with a cleavage site for the tobacco etch virus (TEV) protease inserted between them [7]. In addition to the C-terminal TAP tag, an N-terminal TAP tag [8], which is a reverse orientation of the C-terminal TAP tag, was also generated (Fig. 1A).

The TAP method involves the fusion of the TAP tag to proteins of interest, either at the C- or N-terminus, and the transformation of the construct into appropriate host organisms. Protein complexes containing the TAP-tagged protein are purified from cell extracts by two specific affinity purification steps (Fig. 2).

The TAP method has many advantages for researching protein complexes and interactions. First, the TAP system enables rapid

* Corresponding author.
** Corresponding author. Fax: +86 931 8625576.
E-mail addresses: zhanghua@lzu.edu.cn (H. zhang), lizhean@lzu.edu.cn (L. An).

[1] *Abbreviations used:* TAP, tandem affinity purification; ProtA, IgG-binding units of protein Aof *Staphylococcus aureus*; CBP, calmodulin-binding domain; TEV, tobacco etch virus; ADAP, adhesion and degranulation promoting adaptor protein; MCM, minichromosome maintenance; EBNA1, Epstein–Barr nuclear antigen-1; Dm, *Drosophila melanogaster*; RNAi, RNA interference;RNP, ribonucleoprotein; ProtC, protein C epitope; HRV 3C, human rhinovirus 3C protease cleavage site; ProtG, IgG binding units of protein G from *Streptococcus* sp.; SBP, streptavidin-binding peptide; HA, hemagglutinin.

1046-5928/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pep.2010.04.009

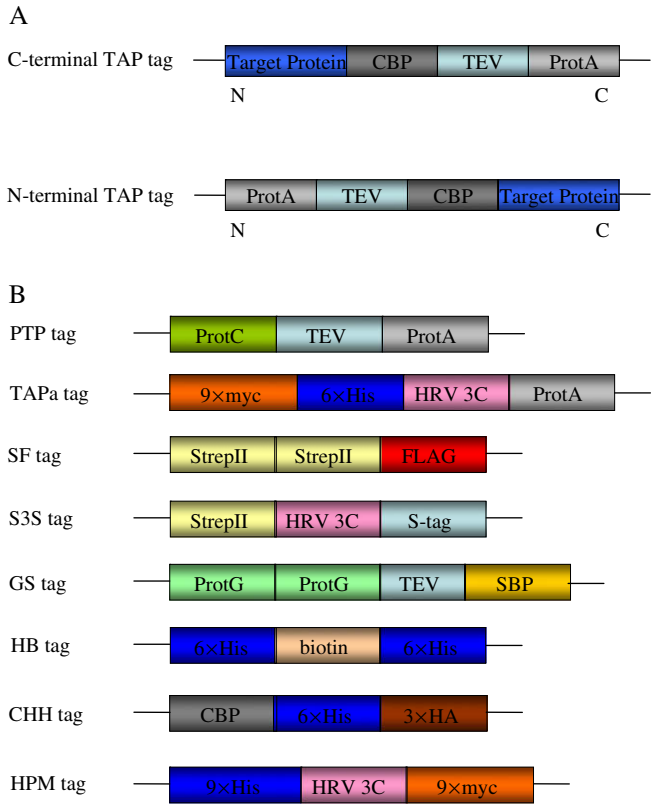

Fig. 1. Diagrammatic sketch of the TAP tag. (A) The original C- and N-terminal TAP tag. (B) Diversity of TAP tags developed over the past few years.

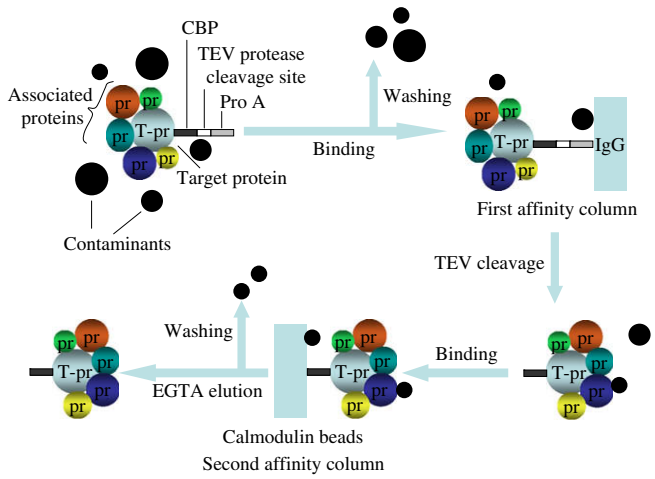

Fig. 2. Schematic of the original TAP method. In the first step, the protein complex, which contains the tagged target protein, combines with an IgG matrix by the ProtA fraction. The protein complex is then eluted using TEV protease under native conditions. In the second step, the elution fraction of the first purification step is incubated with beads coated by calmodulin in the presence of calcium. Subsequently, contaminants and the remainder of TEV protease used in the first step are eliminated through washing. Ultimately, the target protein complex is obtained by elution using EGTA.

purification of protein complexes without knowing their function or structure. Second, the TAP method allows for protein complex purification under native conditions [7,8]. Third, the tandem purification steps are highly specific and eliminate the high background caused by contaminants. Finally, all protein complex purification can be processed under the same conditions, thus the results are reproducible and comparable, which is meaningful in a large-scale systematic proteome analysis. Due to these advantages, the TAP method has been successfully applied in the research of protein–protein interactions in prokaryotic and eukaryotic cells.

Application of the TAP method

With the development of the TAP approach over the past decade, this method has been employed in the analysis of protein–protein interactions and protein complexes in many different organisms, including yeast, mammals, plants, *Drosophila* and bacteria (Table 1) [9].

TAP in yeast

The TAP method was originally developed for analysis of protein complexes in yeast at near-physiological conditions. Gavin et al. [10] utilized TAP in the large-scale analysis of multi-protein complexes in *Saccharomyces cerevisiae*, in which 589 tagged proteins were successfully purified and the associated proteins were isolated and identified. Later, the same research group performed the first genome-wide screen for protein complexes in budding yeast to investigate the organization of the eukaryotic cellular machinery [11]. At the same time, a different group also utilized TAP in a large-scale study involving yeast [12]. They successfully purified 2357 proteins from 4562 different tagged proteins, and revealed 7123 protein–protein interactions for 2708 proteins. These studies yielded results that were able to provide a deep insight into the functional and organizational network of proteins in cells.

In the case of a given protein, the TAP procedure could also be used to investigate protein interactions. For example, Krogan et al. [13] used the TAP strategy to systematically describe the interactions of 25 putative yeast transcription elongation factors. In addition to known or suspected interactions, the results revealed novel protein–protein interactions and new polypeptides involved in transcriptional elongation in *S. cerevisiae*. However, it is not known whether all these identified transcription elongation factors can combine into a transcription complex simultaneously and whether a certain factor can associate with the transcription complex all the time. These issues cannot be explained by the TAP strategy. The results from the TAP system only demonstrated the total interaction network rather than time-dependent interactions. In studies attempting to decipher the 26S proteasome interaction networks *in vivo* from budding yeast [14], a total of 64 putative interactions were identified. In another study, TAP analysis uncovered 102 previously known and 179 possible interacting proteins for 21 tagged proteins involved in transcription and progression during mitosis [15]. Among these interactions, a new interacting protein of Swi2p/Snf2p was revealed. Honey et al. [16] used a tagged protein as bait to purify an active Clb2-Cdc28 kinase complex from yeast cell lysate by TAP. Following identification by mass spectrometry, four proteins were found to be associated with this complex.

It is possible to use the TAP protocol not only in *S. cerevisiae*, but also in *Schizosaccharomyces pombe*. Several known associated partners of the cyclin-dependent kinase were eluted with the bait protein by the TAP process [17]. Furthermore, Arp2p was used to isolate the Arp2/3 complex using the TAP strategy [18]. This was the first time the Arp2/3 complex was biochemically identified in *S. pombe*. Cipak et al. [19] purified proteins which associated with two evolutionarily conserved proteins, Swi5 and Sfr1, from *S. pombe* and revealed two different Swi5-containing protein complexes for the first time. Furthermore, the TAP strategy could be used to isolate protein complexes in *Candida albicans* [20].

Table 1
Representative applications of the TAP method.

Protein complex	Bait protein	Tandem tag	Organism	Proteins identified	Functional pathway	Reference
RNA polymerase II elongation factors	15 proteins	Protein A, CBP	Yeast	25	Transcriptional elongation	[13]
Cyclin-dependent kinase complex	Cdc2p	Protein A, CBP	Yeast	3	Orchestrating cell cycle	[17]
Arp2/3 complex	Arp2p	Protein A, CBP	Yeast	6	Nucleation	[18]
Swi 5 containing complex	Swi 5	Protein A, CBP	Yeast	4	Mating-type switching and homologous recombination regulation	[19]
Spetin complex	CaCDC11	His, FLAG	Yeast	4	Cytokinesis, virulence	[20]
DNA-dependent protein kinase	Ku70, Ku80	Protein G, SBP	Human cells	Many	Multiple	[22]
Human cytomegalovirus protein kinase	UL97	Protein A, Protein C	Human cell	1	Human cytomegalovirus replication	[24]
ADAP	ADAP	S-tag, Strep II	Human cells	Many	Integrin adhesion regulation	[25]
Many	Spartin	Protein A, CBP	Human cells	94	Multiple	[26]
Parkin-associated complex	Parkin	streptavidin-binding peptide, CBP	Human cells	14	Regulation mitochondrial activity	[27]
Many	32 proteins	Protein A, CBP	Human cells	Many	mRNA formation	[28]
MCM complex	MCM-BP	Protein A, CBP	Human cells	5	Initiation of DNA replication	[29]
Resistance protein N containing complex	Resistance protein N	ProteinA, Myc, His	*Nicotiana benthamiana*	1	Plant defense	[35]
Many	6 proteins	Protein A, CBP	*Arabidopsis*	42	Cell division signaling	[41]
Notch signaling complex	4 proteins	Protein A, CBP	*Drosophila*	400	Signaling pathway	[44]
Thioredoxin associated complex	Thioredoxin	Protein A, CBP	*Escherichia coli*	80	Multiple	[47]
RecQ complex	RecQ	Protein A, CBP	*Escherichia coli*	3	DNA unwinding	[48]
RNP	SrmB	Protein A, CBP	*Escherichia coli*	2	Ribosome assembly	[49]
Microtubule-associated protein	DdEB1, DdCP224	Protein A, CBP	*Dictyostelium*	Many	Multiple	[52]
Arp2/3 complex	pArc-34	Protein A, CBP	*Dictyostelium*	6	Nucleation	[53]
RNA polymerase I	RPB6z	Protein A, Protein C	*Trypanosome brucei*	3	Transcription	[57]
Translation elongation factor	PfEF-1β	Protein A, Protein C	*Plasmodium falciparum*	4	Translation elongation	[58]

TAP in mammalian systems

Recent studies have shown that the TAP strategy is useful in mammalian protein complex analysis. For instance, human SMAD3 was fused to a TAP tag at the N-terminus and stably expressed in mammalian cells at appropriate levels [21]. Active SMAD3 protein complex was purified from cell lysates, and HSP70 was identified as a novel combination partner of SMAD3. However, the TAP system used in this research employed a FLAG tag for the second purification step, the elution conditions of which was incompatible with the liquid chromatography-MS/MS sequence application. An additional purification step may be needed to eliminate this problem, which may lead to increased labor time and greater sample loss. Ku70 and Ku80 [22], two cofactors of the DNA-dependent protein kinase, were expressed with TAP tags to uncover the partners of the DNA-dependent protein kinase complex in human HEK293 cells. Besides the identification of new combining proteins, the two bait proteins, Ku70 and Ku80, were shown to form a complex at a ratio of 1:1. This result is consistent with previous findings [23] and demonstrates the advantage of TAP in simple quantity analysis. In human foreskin fibroblasts, Kamil et al. [24] showed that pp65, the virion tegument phosphoprotein, was a major associated subunit of cytomegalovirus protein kinase UL97. Taking the tagged adhesion and degranulation promoting adaptor protein (ADAP) as bait [25], several presumed associated partners including the cytoskeletal proteins, DOCK2 and the guanine nucleotide exchange factor, GEF-H1were isolated from T cells. In HEK293T cells, Milewska et al. [26] suggested that spartin may have a role in protein folding both in mitochondria and the endoplasmic reticulum by using the TAP process. Davison et al. [27] employed TAP to isolate and identify components of Parkin complexes from stably transfected HEK293 cells. As a result, 14 proteins were demonstrated to combine with tagged Parkin, nine of which took part in mitochondrial energy metabolism and glycolysis. This would suggest that Parkin may act in regulating mitochondrial activity. To characterize soluble human protein complexes containing key components of the transcription and RNA processing machineries [28], 32 tagged proteins including subunits of RNA polymerase II and basal transcription factors were purified with their associated partners. These interactions yielded a high-density protein interaction network for the mammalian transcription machinery and revealed multiple factors regulating the transcription machinery. It is noteworthy that a large number of purified associated partners were also tagged and subjected to the same procedure. The advantage of this is that the reverse TAP can confirm the confidence of interactions and enrich the interaction data set. In another study using the TAP system in human cells, a new form of the minichromosome maintenance (MCM) complex was identified [29]. With the aim of learning more about the Epstein-Barr nuclear antigen-1 (EBNA1) protein, Holowaty et al. [30] expressed EBNA1 in fusion with a TAP tag at the C-terminus in human 293T cells. Several specific cellular protein interactions and some important regulating proteins were discovered. TAP also allows for purification of protein complexes from mouse fibroblast cells growing in monolayer cultures [31].

TAP in plants

Rohila et al. [32] were the first research group that successfully applied the TAP protocol to isolate and purify protein complexes from plants. By using a TAP-tagged hybrid transcription factor as bait, HSP70 and HSP60 were co-purified. This result was consistent with former reports [33,34]. In another study, Hsp90 was shown to

associate with the plant resistance protein N through the TAP strategy [35]. This result suggests that Hsp90 plays an important role in plant defense [36,37]. Rivas et al. [38] employed TAP to investigate Cf-9 protein function in initiating defense signaling. The TAP studies described above were carried out in a transient expression system of *Nicotiana benthamiana*.

In 2005, the TAP system was utilized to purify a protein complex from stably transformed *Arabidopsis thaliana* for the first time [39]. The components of the CSN complex were all co-purified with CSN3-TAP. The TAP strategy utilized a constitutive promoter, which allowed for over-expression of TAP fusion proteins. The strength of this method is that over-expression increases incorporation of the tagged protein into a protein complex, when the tagged protein is the essential component of a complex or a mutant and suppressed expression for the target protein is not available. In a different study, TAP tagged fatty acid synthase components were utilized to investigate protein interactions *in vivo* from stably transfected *A. thaliana* [40]. In addition to the application of TAP to a whole, strategies have also been developed for *A. thaliana* cell suspension cultures to investigate a protein–protein interaction map surrounding six core cell cycle proteins [41].

Purification of protein complexes by TAP was demonstrated to be effective in rice [42], in which 95% of TAP-tagged rice protein kinases were recovered from transgenic rice plants. This result suggested that the TAP method could be utilized in cereal crops.

TAP in Drosophila

In 2003, Forler et al. [43] successfully expressed TAP-tagged human proteins and purified their *Drosophila melanogaster* (Dm) binding partners in Dm Schneider cells. The critical advantage in this system is the combination of the TAP approach and double-stranded RNA interference (RNAi). The introduction of RNAi can suppress the expression of the corresponding endogenous proteins, thereby avoiding competition from them during protein complex assembly. But the complexes purified through this system consisted of human bait protein and Dm binding partners, two different source proteins, therefore validation of the interaction needed support from previously published reports. In *Drosophila* cultured cells and embryos, several components of the Notch signaling pathway were tagged with a TAP tag to reveal many novel interactions [44]. Throughout the TAP progress, Hsc70 and Hsp83 were validated as cofactors of the *Drosophila* nuclear receptor protein for the first time [45].

TAP in bacteria

In recent years, with the development of the TAP procedure, the application of TAP was extended to purification of protein complexes from bacteria. Gully et al. [46] first used the TAP protocol in *Escherichia coli* to isolate native protein complexes. Kumar et al. [47] have identified 80 proteins associated with thioredoxin in *E. coli* suggesting that thioredoxin is involved in at least 26 different cellular processes including transcription regulation, cell division, energy transduction, and some biosynthetic pathways. RecQ DNA helicases are involved in DNA replication, recombination and repair machinery in all eukaryotes and bacteria. Shereda et al. [48] employed the TAP approach to purify the RecQ complexes. As a result, three heterologous proteins were co-purified with TAP-RecQ: single-stranded DNA-binding proteins, exonuclease I, and RecJ exonuclease. According to the amount of these three binding proteins, the authors also classed these interactions as direct or indirect. This may be a new application aspect of TAP in interaction identification. SrmB, one of the five *E. coli* DEAD-box proteins was discovered to form a specific ribonucleoprotein (RNP) complex with r-proteins L4, L24 and the 5′ region of 23S rRNA using the TAP procedure [49]. Similar to the application of the TAP method in global protein complexes analysis in yeast, a large-scale analysis of protein complexes, which revealed a novel protein interaction network in *E. coli*, was reported [50]. Besides the application of the TAP method in *E. coli*, TAP was also carried out in *Thiocapsa roseopersicina* [51].

TAP in other organisms

The efficiency of the TAP method in purification of protein complexes and identification of interactions was also validated in other organisms, such as *Dictyostelium* [52,53], *Trypanosoma brucei* [54–57] and *Plasmodium falciparum* [58].

Diversity of TAP tags

Although the TAP system was originally developed in yeast, it has been proven to successfully work in a broad range of organisms. The classic ProtA-TEV-CBP tag is unable to provide high efficiency for all given protein complexes. Thus several variations of the TAP tag based on other affinity tags have been developed that offer advantages in specific cases (Fig. 1B). The properties of these basic affinity tags [59–62] are summarized in Table 2 to highlight the advantages and disadvantages of corresponding recombinant tags.

One type of variation is the replacement of the CBP tag that cannot always efficiently recover protein complexes. For example, when purifying protein complexes from mammalian cells growing in monolayer cultures, a biotinylation tag is used as the second affinity tag, taking advantage of the high biotin–avidin binding affinity and resulting in an increased yield of the fusion protein [31]. Another example where the CBP tag has been replaced with a protein C epitope (ProtC) resulting in a new TAP tag, designated PTP [63]. The advantage of this is that ProtC eliminates the inefficiency of the CBP tag and allows tagged proteins to be eluted either by EGTA or by the ProtC peptide in cases where EGTA may irreversibly interfere with the metal-binding protein function. With respect to isolation of active metal-binding proteins, another replacement of the CBP moiety has been a 9×myc with a 6×His sequence [39]. This tag is known as a TAPa a tag and also contains a human rhinovirus 3C protease cleavage site (HRV 3C) instead of the original TEV site for elution from IgG beads. In contrast to TEV protease, 3C protease still has enzymatic activity at 4 °C. These modifications are thought to help to stabilize protein complex structures and activities.

Another type of variation is a series TAP tags with smaller size. The original TAP tag is large, at approximately 21 kDa, and this size might impair the function of the tagged protein or interfere with protein complex formation. Because of this, many affinity tags, which range in size from 5 to 51 amino acids, can be used to replace of CBP or ProtA moiety [60]. One example of a smaller TAP tag is the sequential peptide affinity tag made by substituting 3×FLAG for ProtA [64]. Replacement of the CBP with a spacer and a single FLAG sequence is another example of a smaller tag for TAP [21]. These FLAG-containing combination tags may reduce the tag length and result in higher purity fusion proteins, but the system of purification comes at a relatively high cost. Recently, use of another alternative tandem affinity tag, composed of a Strep-tag II and a FLAG tag (SF), has been published [65]. This SF tag reduced the size of the TAP tag to 4.6 kDa. This smaller size is less likely to disturb protein activity and structure. Because both tags can be eluted under native conditions, the SF-TAP strategy allows purification of protein complexes in less than 2.5 h. Another similar tandem combination of FLAG-tag-Strep-tag II has been developed to purify protein complexes efficiently from *Thiocapsa*

Table 2
Properties of some commonly used affinity tags.

Affinity tag	Length	Binding matrix	Elution condition	Comments
Staphylococcus protein A (ProtA)	58 aa	IgG	TEV cleavage	Protein A binds to IgG with high affinity. Elution needs low pH, so cleavage is recommended for elution. The large size may impair the function and structure of tagged protein
Calmodulin-binding domain (CBP)	26 aa	Calmodulin	EGTA or EGTA with 1 M NaCl	Tight binding of CBP to calmodulin permits stringent washing to remove almost all non-specific binding proteins. CBP tag may be unsuitable for purification in mammalian cells, because calmodulin interacts with many endogenous proteins. Elution with chelating agents may disturb metal-binding protein activity
Biotinylation tag	15 aa	Avidin	Biotin	The biotinylation tag has high-affinity binding to biotin. But this strategy requires co-transfection of *BirA* gene in the same host cell
Protein C epitope (ProtC)	12 aa	Anti-protein C affinity matrix	EGTA or ProtC peptide	When EGTA may interfere with protein function, ProtC peptide can be chose for elution
c-myc tag	10 aa	Anti-myc monoclonal antibody	Low pH	c-myc tag is rarely expected to hamper protein activity and structure. It is widely used in monitoring expression of recombinant protein but is seldom applied for purification
His tag	6 or 9 aa	Ni^{2+}–NTA	Imidazole	His tag is widely employed for purification with immobilized metal-affinity chromatography. Relatively small size rarely affects protein function and folding. His tag can provide high purification yield, but can not eliminate all contaminant. Proteins containing EDTA and EGTA may not be purified using it
FLAG (or 3 × FLAG)	8 (or 22) aa	Anti-FLAG monoclonal antibody	FLAG peptide or low pH	FLAG tag can provide high purity to protein purification and can be removed by enterokinase. Small size tag gives little opportunity to impair tagged protein activity and structure. The monoclonal antibody binding matrix is not very stable and is expensive
Strep-tag II	8 aa	Strep-Tactin	Desthiobiotin	Strep-tag II can bring high yield and purity to protein purification. The purification conditions are highly variable. Strep II tag is less likely interferes with target protein property, but it was shown to interfere with the crystallization of one particular enzyme
S-tag	15 aa	S protein	Guanidine thiocyanate, citrate, magnesium chloride	S-tag exhibits high-affinity binding to S protein and also can be used for detecting expression level of fusion proteins. Elution conditions are extremely harsh, e.g. low pH, so it is recommended to elute with protease cleavage
Streptococcus protein G (ProtG)	60 aa	IgG	TEV cleavage	ProtG shows a slightly higher affinity for a broader rang of immunoglobulins than Protein A and also with a large size
Streptavidin-binding peptide (SBP)	38 aa	Streptavidin	Biotin	SBP tag has a strong binding to streptavidin and enables proteins purified with high yield and purity
Hemagglutinin (HA)	9 aa	Anti-HA monoclonal antibody	HA peptide or low pH	HA tag is often used for detecting expression level of fusion protein, rarely used for purification

roseopersicina [51]. Lehmann et al. [25] developed a novel S3S tag comprising a S-tag, a HRV 3C and a Strep-tag II. The S3S tag with a size of 4.2 kDa fulfils the requirements of specificity, high yield and no adverse effects on protein function. Nevertheless, it is doubtful as to whether large tags actually disturb the function of tagged proteins. It would appear that the majority of proteins tagged with the original protA-TEV-CBP tag remain functional, and even small proteins such as acyl-carrier protein (<10 kDa) [46] and thioredoxin (~12 kDa) [47] can be used as bait to purify protein complexes.

In addition to those described above, there are a variety of other TAP tags that are largely different from the classic TAP tag. Bürckstümmer et al. [22] designed a new TAP tag, designated as GS tag, to enable a 10-fold increase in protein-complex yield. This tag comprised two copies of IgG-binding units of protein G from *Streptococcus* sp. (ProtG) and a streptavidin-binding peptide (SBP). The GS tag was able to purify recombinant proteins with high efficiency and purity. The disadvantage was the size of the GS tag, at approximately 19 kDa, which is comparable to the original TAP tag. In a recently published paper, a new tandem affinity tag, the HB tag [14], consisting of two 6×His motifs and a biotinylation signal peptide has been developed. The advantage of the HB tag is that it is compatible with *in vivo* cross-linking to purify protein complexes under fully denaturing conditions. *In vivo* cross-linking is beneficial to detect transient and weak protein–protein interactions. A useful derivative of the HB tag is the HTB tag, which includes a TEV cleavage site allowing for protease-driven elution from streptavidin resins [66]. Honey et al. [16] have designed a CHH tag consisting of a CBP, 6×His residues and three copies of the hemagglutinin (3×HA). Theoretically, this tag permits two or three purification steps resulting in very high purity. However, the 3×HA peptide is usually used to detect the expression levels of tagged proteins with its commercially available antibody. In practice, the elution buffer for the calmodulin resin is incompatible with binding to the Ni^{2+} resin. Although buffer exchange may solve this problem, it results in a significant loss of yield. The combination of CBP and His tags is generally not recommended. As for purification of associated proteins from *Drosophila* tissues, Yang et al. [45] have shown that the 3×FLAG–6×His tag provided yields in the 10–20% range, while the original ProtA-TEV-CBP tag only provided yields of around 1%. At the same time, a similar combination of His and FLAG epitope was constructed to isolate protein complexes from pathogenic fungus [20]. The HPM tag, another bipartite affinity tag, consisting of 9×His, 9×myc epitope and two copies of HRV 3C between them was successfully applied in yeast [15].

Problems and future prospects

The TAP method has been successfully used for purification and identification of protein complexes and complex components both in prokaryotic and eukaryotic organisms. However, in a practical situation, some inherent shortcomings of the method have been uncovered. In a systematic analysis of the yeast proteome, Gavin et al. [10] found that they were unable to isolate and identify interacting proteins in 22% of purified tagged proteins and were unable to purify all of the tagged proteins. They ascribed this failure to the intrinsic quality of the TAP tag. The classic TAP tag added to a target protein may interfere with protein function, location and

complex formation. In this situation, a simple alternative solution is to add the tag at the other terminal of the protein or to replace the original tag with another novel TAP tag. A relatively low efficiency of purification for proteins at the CBP affinity step for some protein complexes proved to be problematic because many endogenous proteins of mammalian cells interact with calmodulin in a calcium-dependent manner [67,68]. This can be resolved by replacing the CBP tag with other affinity tags, such as the FLAG sequence [21,65], ProtC [63] and biotinylation tag [31]. The main challenge facing the TAP strategy comes from the competition of endogenous proteins with the tagged protein in protein complex assembly. Therefore, the use of RNAi to reduce the expression level of endogenous proteins has been shown to be a great aid in insect cell systems [43]. However, when the target protein is essential and a mutant of it might be harmful and lethal, the over-expression strategy is a perfect strategy to obtain a protein complex containing the tagged target protein [39,42,69].

It is thought that the TAP approach is not a powerful tool to detect transient interactions. Therefore, an *in vivo* cross-linking step is added to freeze both weak and transient interactions taking place in intact cells before lysis [14,32]. The cross-linking method has been widely used in the investigation of protein-DNA and protein–protein interactions [70–77].

Although the two sequential purification steps of the TAP method largely reduce the background resulting from non-specific protein binding compared to a single purification step, these contaminants cannot be removed completely. Collins et al. [78] have compared the results from the two large-scale studies of protein complexes in yeast [11,12] and found the two datasets shared very low degrees of overlap. The major difference between the two datasets was mainly caused by non-specific interactions. The problem of non-specifically interacting proteins can be overcome by comparing several interaction datasets [79], using stable-isotope labeling by amino acids in cell culture [80,81] or isotope-coded affinity tag [82], thereby completely eliminating false-positive interactions.

The TAP system is considered to be inefficient in identifying interactions occurring only in special physiological states or those which occur for a short period. Whether the TAP tag impairs protein function and complex assembly also remains largely unknown and speculative. These disadvantages may affect its application in such instances.

Understanding protein function is a major goal in biology. Although the TAP method has some inherent shortcomings, it is undoubtedly a reasonable system for use in purification of protein complexes and identification of protein–protein interactions. In addition to identifying interactions between proteins, the TAP method could be used to characterize and verify interactions between protein and DNA or between protein and RNA [83]. At the same time, the TAP method can also be used to analyze the effect of mutants on protein interaction and association, possibly resulting in the discovery of binding sites. Protein purification under near-physiological conditions through the TAP strategy is compatible with functional studies and this advantage allows for mapping of large-scale functional interaction networks. As the procedures and conditions used during the TAP process do not vary greatly among different proteins, the results that are generated by this method should be compiled in a database in order to provide comparable and detailed information on the potential and confirmed functions of proteins, as well as the composition of protein complexes and even the structure and activity of protein complexes.

Acknowledgments

This work has been supported by the National Outstanding Youth Foundation of China (30625008); the major project of cultivating new varieties of Transgenic organisms (2009ZX08009-029B); the National High Technology Research and Development Program (863 Program) (2007AA021401); the National Basic Research Program of China (973 Program) (2007CB108902).

References

[1] B. Alberts, The cell as a collection of protein machines: preparing the next generation of molecular biologists, Cell 92 (1998) 291–294.
[2] M.E. Cusick, N. Klitgord, M. Vidal, D.E. Hill, Interactome: gateway into systems biology, Hum. Mol. Genet. 14 (2005) 171–181.
[3] M. Fromont-Racine, J.C. Rain, P. Legrain, Toward a functional analysis of the yeast genome through exhaustive two-hybrid screens, Nat. Genet. 16 (1997) 277–282.
[4] P. Uetz, L. Giot, G. Cagney, T.A. Mansfield, R.S. Judson, J.R. Knight, D. Lockshon, V. Narayan, M. Srinivasan, P. Pochart, et al., A comprehensive analysis of protein–protein interactions in *Saccharomyces cerevisiae*, Nature 403 (2000) 623–627.
[5] T. Ito, T. Chiba, R. Ozawa, M. Yoshida, M. Hattori, Y. Sakaki, A comprehensive two-hybrid analysis to explore the yeast protein interactome, Proc. Natl. Acad. Sci. USA 98 (2001) 4569–4574.
[6] H. Zhu, M. Bilgin, R. Bangham, D. Hall, A. Casamayor, P. Bertone, N. Lan, R. Jansen, S. Bidlingmaier, T. Houfek, et al., Global analysis of protein activities using proteome chips, Science 293 (2001) 2101–2105.
[7] G. Rigaut, A. Shevchenko, B. Rutz, M. Wilm, M. Mann, B. Seraphin, A generic protein purification method for protein complex characterization and proteome exploration, Nat. Biotechnol. 17 (1999) 1030–1032.
[8] O. Puig, F. Caspary, G. Rigaut, B. Rutz, E. Bouveret, E. Bragado-Nilsson, M. Wilm, B. Seraphin, The tandem affinity purification (TAP) method: a general procedure of protein complex purification, Methods 24 (2001) 218–229.
[9] I.F. Chang, Mass spectrometry-based proteomic analysis of the epitope-tag affinity purified protein complexes in eukaryotes, Proteomics 6 (2006) 6158–6166.
[10] A.C. Gavin, M. Bosche, R. Krause, P. Grandi, M. Marzioch, A. Bauer, J. Schultz, J.M. Rick, A.M. Michon, C.M. Cruciat, et al., Functional organization of the yeast proteome by systematic analysis of protein complexes, Nature 415 (2002) 141–147.
[11] A.C. Gavin, P. Aloy, P. Grandi, R. Krause, M. Boesche, M. Marzioch, C. Rau, L.J. Jensen, S. Bastuck, B. Dumpelfeld, et al., Proteome survey reveals modularity of the yeast cell machinery, Nature 440 (2006) 631–636.
[12] N.J. Krogan, G. Cagney, H. Yu, G. Zhong, X. Guo, A. Ignatchenko, J. Li, S. Pu, N. Datta, A.P. Tikuisis, et al., Global landscape of protein complexes in the yeast *Saccharomyces cerevisiae*, Nature 440 (2006) 637–643.
[13] N.J. Krogan, M. Kim, S.H. Ahn, G. Zhong, M.S. Kobor, G. Cagney, A. Emili, A. Shilatifard, S. Buratowski, J.F. Greenblatt, RNA polymerase II elongation factors of *Saccharomyces cerevisiae*: a targeted proteomics approach, Mol. Cell. Biol. 22 (2002) 6979–6992.
[14] C. Guerrero, C. Tagwerker, P. Kaiser, L. Huang, An integrated mass spectrometry-based proteomic approach: quantitative analysis of tandem affinity-purified *in vivo* cross-linked protein complexes (QTAX) to decipher the 26 S proteasome-interacting network, Mol. Cell. Proteomics 5 (2006) 366–378.
[15] J. Graumann, L.A. Dunipace, J.H. Seol, W.H. McDonald, J.R. Yates 3rd, B.J. Wold, R.J. Deshaies, Applicability of tandem affinity purification MudPIT to pathway proteomics in yeast, Mol. Cell. Proteomics 3 (2004) 226–237.
[16] S. Honey, B.L. Schneider, D.M. Schieltz, J.R. Yates, B. Futcher, A novel multiple affinity purification tag and its use in identification of proteins associated with a cyclin–CDK complex, Nucleic Acids Res. 29 (2001) 24–32.
[17] J.J. Tasto, R.H. Carnahan, W.H. McDonald, K.L. Gould, Vectors and gene targeting modules for tandem affinity purification in *Schizosaccharomyces pombe*, Yeast 18 (2001) 657–662.
[18] K.L. Gould, L. Ren, A.S. Feoktistova, J.L. Jennings, A.J. Link, Tandem affinity purification and identification of protein complex components, Methods 33 (2004) 239–244.
[19] L. Cipak, M. Spirek, M. Novatchkova, Z. Chen, C. Rumpf, W. Lugmayr, K. Mechtler, G. Ammerer, E. Csaszar, J. Gregan, An improved strategy for tandem affinity purification-tagging of *Schizosaccharomyces pombe* genes, Proteomics 9 (2009) 4825–4828.
[20] A. Kaneko, T. Umeyama, N. Hanaoka, B.C. Monk, Y. Uehara, M. Niimi, Tandem affinity purification of the *Candida albicans* septin protein complex, Yeast 21 (2004) 1025–1033.
[21] M. Knuesel, Y. Wan, Z. Xiao, E. Holinger, N. Lowe, W. Wang, X. Liu, Identification of novel protein–protein interactions using a versatile mammalian tandem affinity purification expression system, Mol. Cell. Proteomics 2 (2003) 1225–1233.
[22] T. Bürckstümmer, K.L. Bennett, A. Preradovic, G. Schutze, O. Hantschel, G. Superti-Furga, A. Bauch, An efficient tandem affinity purification procedure for interaction proteomics in mammalian cells, Nat. Methods 3 (2006) 1013–1019.
[23] T.M. Gottlieb, S.P. Jackson, The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen, Cell 72 (1993) 131–142.
[24] J.P. Kamil, D.M. Coen, Human cytomegalovirus protein kinase UL97 forms a complex with the tegument phosphoprotein pp65, J. Virol. 81 (2007) 10659–10668.

[25] R. Lehmann, J. Meyer, M. Schuemann, E. Krause, C. Freund, A novel S3S-TAP-tag for the isolation of T-cell interaction partners of adhesion and degranulation promoting adaptor protein, Proteomics 9 (2009) 5288–5295.
[26] M. Milewska, J. McRedmond, P.C. Byrne, Identification of novel spartin-interactors shows spartin is a multifunctional protein, J. Neurochem. 111 (2009) 1022–1030.
[27] E.J. Davison, K. Pennington, C.C. Hung, J. Peng, R. Rafiq, A. Ostareck-Lederer, D.H. Ostareck, H.C. Ardley, R.E. Banks, P.A. Robinson, Proteomic analysis of increased Parkin expression and its interactants provides evidence for a role in modulation of mitochondrial function, Proteomics 9 (2009) 4284–4297.
[28] C. Jeronimo, D. Forget, A. Bouchard, Q. Li, G. Chua, C. Poitras, C. Therien, D. Bergeron, S. Bourassa, J. Greenblatt, et al., Systematic analysis of the protein interaction network for the human transcription machinery reveals the identity of the 7SK capping enzyme, Mol. Cell 27 (2007) 262–274.
[29] A.M. Sakwe, T. Nguyen, V. Athanasopoulos, K. Shire, L. Frappier, Identification and characterization of a novel component of the human minichromosome maintenance complex, Mol. Cell. Biol. 27 (2007) 3044–3055.
[30] M.N. Holowaty, M. Zeghouf, H. Wu, J. Tellam, V. Athanasopoulos, J. Greenblatt, L. Frappier, Protein profiling with Epstein–Barr nuclear antigen-1 reveals an interaction with the herpesvirus-associated ubiquitin-specific protease HAUSP/USP7, J. Biol. Chem. 278 (2003) 29987–29994.
[31] R. Drakas, M. Prisco, R. Baserga, A modified tandem affinity purification tag technique for the purification of protein complexes in mammalian cells, Proteomics 5 (2005) 132–137.
[32] J.S. Rohila, M. Chen, R. Cerny, M.E. Fromm, Improved tandem affinity purification tag and methods for isolation of protein heterocomplexes from plants, Plant J. 38 (2004) 172–181.
[33] K.D. Dittmar, D.R. Demady, L.F. Stancato, P. Krishna, W.B. Pratt, Folding of the glucocorticoid receptor by the heat shock protein (hsp) 90-based chaperone machinery. The role of p23 is to stabilize receptor.hsp90 heterocomplexes formed by hsp90.p60.hsp70, J. Biol. Chem. 272 (1997) 21213–21220.
[34] L.F. Stancato, K.A. Hutchison, P. Krishna, W.B. Pratt, Animal and plant cell lysates share a conserved chaperone system that assembles the glucocorticoid receptor into a functional heterocomplex with hsp90, Biochemistry 35 (1996) 554–561.
[35] Y. Liu, T. Burch-Smith, M. Schiff, S. Feng, S.P. Dinesh-Kumar, Molecular chaperone Hsp90 associates with resistance protein N and its signaling proteins SGT1 and Rar1 to modulate an innate immune response in plants, J. Biol. Chem. 279 (2004) 2101–2108.
[36] A. Takahashi, C. Casais, K. Ichimura, K. Shirasu, HSP90 interacts with RAR1 and SGT1 and is essential for RPS2-mediated disease resistance in Arabidopsis, Proc. Natl. Acad. Sci. USA 100 (2003) 11777–11782.
[37] H. Kanzaki, H. Saitoh, A. Ito, S. Fyjisawa, S. Kamoun, S. Katou, H. Yoshioka, R. Terauchi, Mol. Plant Pathol. 4 (2003) 383–391.
[38] S. Rivas, T. Romeis, J.D. Jones, The Cf-9 disease resistance protein is present in an approximately 420-kilodalton heteromultimeric membrane-associated complex at one molecule per complex, Plant Cell 14 (2002) 689–702.
[39] V. Rubio, Y. Shen, Y. Saijo, Y. Liu, G. Gusmaroli, S.P. Dinesh-Kumar, X.W. Deng, An alternative tandem affinity purification strategy applied to *Arabidopsis* protein complex isolation, Plant J. 41 (2005) 767–778.
[40] A.P. Brown, V. Affleck, T. Fawcett, A.R. Slabas, Tandem affinity purification tagging of fatty acid biosynthetic enzymes in *Synechocystis* sp. PCC6803 and *Arabidopsis thaliana*, J. Exp. Bot. 57 (2006) 1563–1571.
[41] J. Van Leene, H. Stals, D. Eeckhout, G. Persiau, E. Van De Slijke, G. Van Isterdael, A. De Clercq, E. Bonnet, K. Laukens, N. Remmerie, et al., A tandem affinity purification-based technology platform to study the cell cycle interactome in *Arabidopsis thaliana*, Mol. Cell. Proteomics 6 (2007) 1226–1238.
[42] J.S. Rohila, M. Chen, S. Chen, J. Chen, R. Cerny, C. Dardick, P. Canlas, X. Xu, M. Gribskov, S. Kanrar, et al., Protein–protein interactions of tandem affinity purification-tagged protein kinases in rice, Plant J. 46 (2006) 1–13.
[43] D. Forler, T. Kocher, M. Rode, M. Gentzel, E. Izaurralde, M. Wilm, An efficient protein complex purification method for functional proteomics in higher eukaryotes, Nat. Biotechnol. 21 (2003) 89–92.
[44] A. Veraksa, A. Bauer, S. Artavanis-Tsakonas, Analyzing protein complexes in *Drosophila* with tandem affinity purification-mass spectrometry, Dev. Dyn. 232 (2005) 827–834.
[45] P. Yang, H.M. Sampson, H.M. Krause, A modified tandem affinity purification strategy identifies cofactors of the *Drosophila* nuclear receptor dHNF4, Proteomics 6 (2006) 927–935.
[46] D. Gully, D. Moinier, L. Loiseau, E. Bouveret, New partners of acyl carrier protein detected in Escherichia coli by tandem affinity purification, FEBS Lett. 548 (2003) 90–96.
[47] J.K. Kumar, S. Tabor, C.C. Richardson, Proteomic analysis of thioredoxin-targeted proteins in *Escherichia coli*, Proc. Natl. Acad. Sci. USA 101 (2004) 3759–3764.
[48] R.D. Shereda, D.A. Bernstein, J.L. Keck, A central role for SSB in *Escherichia coli* RecQ DNA helicase function, J. Biol. Chem. 282 (2007) 19247–19258.
[49] D. Trubetskoy, F. Proux, F. Allemand, M. Dreyfus, I. Iost, SrmB, a DEAD-box helicase involved in *Escherichia coli* ribosome assembly, is specifically targeted to 23S rRNA *in vivo*, Nucleic Acids Res. 37 (2009) 6540–6549.
[50] G. Butland, J.M. Peregrin-Alvarez, J. Li, W. Yang, X. Yang, V. Canadien, A. Starostine, D. Richards, B. Beattie, N. Krogan, et al., Interaction network containing conserved and essential protein complexes in *Escherichia coli*, Nature 433 (2005) 531–537.
[51] B.D. Fodor, A.T. Kovacs, R. Csaki, E. Hunyadi-Gulyas, E. Klement, G. Maroti, L.S. Meszaros, K.F. Medzihradszky, G. Rakhely, K.L. Kovacs, Modular broad-host-range expression vectors for single-protein and protein complex purification, Appl. Environ. Microbiol. 70 (2004) 712–721.
[52] K.V. Koch, Y. Reinders, T.H. Ho, A. Sickmann, R. Graf, Identification and isolation of Dictyostelium microtubule-associated protein interactors by tandem affinity purification, Eur. J. Cell Biol. 85 (2006) 1079–1090.
[53] M.E. Meima, K.E. Weening, P. Schaap, Vectors for expression of proteins with single or combinatorial fluorescent protein and tandem affinity purification tags in Dictyostelium, Protein Expr. Purif. 53 (2007) 283–288.
[54] D. Walgraffe, S. Devaux, L. Lecordier, J.F. Dierick, M. Dieu, J. Van den Abbeele, E. Pays, L. Vanhamme, Characterization of subunits of the RNA polymerase I complex in *Trypanosoma brucei*, Mol. Biochem. Parasitol. 139 (2005) 249–260.
[55] Z. Palfi, B. Schimanski, A. Gunzl, S. Lucke, A. Bindereif, U1 small nuclear RNP from *Trypanosoma brucei*: a minimal U1 snRNA with unusual protein components, Nucleic Acids Res. 33 (2005) 2493–2503.
[56] B. Schimanski, T.N. Nguyen, A. Gunzl, Characterization of a multisubunit transcription factor complex essential for spliced-leader RNA gene transcription in *Trypanosoma brucei*, Mol. Cell. Biol. 25 (2005) 7303–7313.
[57] T.N. Nguyen, B. Schimanski, A. Gunzl, Active RNA polymerase I of *Trypanosoma brucei* harbors a novel subunit essential for transcription, Mol. Cell Biol. 27 (2007) 6254–6263.
[58] S. Takebe, W.H. Witola, B. Schimanski, A. Gunzl, C. Ben Mamoun, Purification of components of the translation elongation factor complex of Plasmodium falciparum by tandem affinity purification, Eukaryot. Cell 6 (2007) 584–591.
[59] R.C. Stevens, Design of high-throughput methods of protein production for structural biology, Structure 8 (2000) 177–185.
[60] K. Terpe, Overview of tag protein fusions: from molecular and biochemical fundamentals to commercial systems, Appl. Microbiol. Biotechnol. 60 (2003) 523–533.
[61] J.J. Lichty, J.L. Malecki, H.D. Agnew, D.J. Michelson-Horowitz, S. Tan, Comparison of affinity tags for protein purification, Protein Expr. Purif. 41 (2005) 98–105.
[62] Y.F. Li, Commonly used tag combinations for tandem affinity purification, Biotechnol. Appl. Biochem. 55 (2010) 73–83.
[63] B. Schimanski, T.N. Nguyen, A. Gunzl, Highly efficient tandem affinity purification of trypanosome protein complexes based on a novel epitope combination, Eukaryot. Cell 4 (2005) 1942–1950.
[64] M. Zeghouf, J. Li, G. Butland, A. Borkowska, V. Canadien, D. Richards, B. Beattie, A. Emili, J.F. Greenblatt, Sequential Peptide Affinity (SPA) system for the identification of mammalian and bacterial protein complexes, J. Proteome Res. 3 (2004) 463–468.
[65] C.J. Gloeckner, K. Boldt, A. Schumacher, R. Roepman, M. Ueffing, A novel tandem affinity purification strategy for the efficient isolation and characterisation of native protein complexes, Proteomics 7 (2007) 4228–4234.
[66] C. Tagwerker, H. Zhang, X. Wang, L.S. Larsen, R.H. Lathrop, G.W. Hatfield, B. Auer, L. Huang, P. Kaiser, HB tag modules for PCR-based gene tagging and tandem affinity purification in Saccharomyces cerevisiae, Yeast 23 (2006) 623–632.
[67] N. Agell, O. Bachs, N. Rocamora, P. Villalonga, Modulation of the Ras/Raf/MEK/ERK pathway by Ca(2+), and calmodulin, Cell. Signal. 14 (2002) 649–654.
[68] J.F. Head, A better grip on calmodulin, Curr. Biol. 2 (1992) 609–611.
[69] Y. Ho, A. Gruhler, A. Heilbut, G.D. Bader, L. Moore, S.L. Adams, A. Millar, P. Taylor, K. Bennett, K. Boutilier, et al., Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry, Nature 415 (2002) 180–183.
[70] V. Jackson, Formaldehyde cross-linking for studying nucleosomal dynamics, Methods 17 (1999) 125–139.
[71] M.H. Kuo, C.D. Allis, *In vivo* cross-linking and immunoprecipitation for studying dynamic protein:DNA associations in a chromatin environment, Methods 19 (1999) 425–433.
[72] G. Schmitt-Ulms, G. Legname, M.A. Baldwin, H.L. Ball, N. Bradon, P.J. Bosque, K.L. Crossin, G.M. Edelman, S.J. DeArmond, F.E. Cohen, Et al., Binding of neural cell adhesion molecules (N-CAMs) to the cellular prion protein, J. Mol. Biol. 314 (2001) 1209–1225.
[73] G. Schmitt-Ulms, K. Hansen, J. Liu, C. Cowdrey, J. Yang, S.J. DeArmond, F.E. Cohen, S.B. Prusiner, M.A. Baldwin, Time-controlled transcardiac perfusion cross-linking for the study of protein interactions in complex tissues, Nat. Biotechnol. 22 (2004) 724–731.
[74] V. Orlando, H. Strutt, R. Paro, Analysis of chromatin structure by *in vivo* formaldehyde cross-linking, Methods 11 (1997) 205–214.
[75] J. Vasilescu, X. Guo, J. Kast, Identification of protein–protein interactions using *in vivo* cross-linking and mass spectrometry, Proteomics 4 (2004) 3845–3854.
[76] M. Otsu, F. Omura, T. Yoshimori, M. Kikuchi, Protein disulfide isomerase associates with misfolded human lysozyme *in vivo*, J. Biol. Chem. 269 (1994) 6874–6877.
[77] D.B. Hall, K. Struhl, The VP16 activation domain interacts with multiple transcriptional components as determined by protein–protein cross-linking *in vivo*, J. Biol. Chem. 277 (2002) 46043–46050.
[78] S.R. Collins, P. Kemmeren, X.C. Zhao, J.F. Greenblatt, F. Spencer, F.C. Holstege, J.S. Weissman, N.J. Krogan, Toward a comprehensive atlas of the physical interactome of *Saccharomyces cerevisiae*, Mol. Cell. Proteomics 6 (2007) 439–450.
[79] R.M. Ewing, P. Chu, F. Elisma, H. Li, P. Taylor, S. Climie, L. McBroom-Cerajewski, M.D. Robinson, L. O'Connor, M. Li, Et al., Large-scale mapping of human protein–protein interactions by mass spectrometry, Mol. Syst. Biol. 3 (2007) 89–105.

[80] B. Blagoev, I. Kratchmarova, S.E. Ong, M. Nielsen, L.J. Foster, M. Mann, A proteomics strategy to elucidate functional protein–protein interactions applied to EGF signaling, Nat. Biotechnol. 21 (2003) 315–318.
[81] M. Mann, Functional and quantitative proteomics using SILAC, Nat. Rev. Mol. Cell Biol. 7 (2006) 952–958.
[82] J.A. Ranish, E.C. Yi, D.M. Leslie, S.O. Purvine, D.R. Goodlett, J. Eng, R. Aebersold, The study of macromolecular complexes by quantitative proteomics, Nat. Genet. 33 (2003) 349–355.
[83] J.R. Hogg, K. Collins, RNA-based affinity purification reveals 7SK RNPs with distinct composition and regulation, RNA 13 (2007) 868–880.