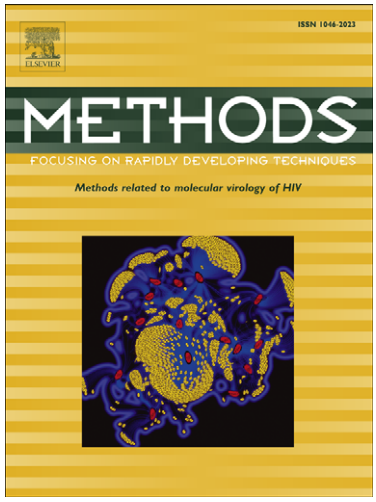

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Method for suppressing non-specific protein interactions observed with affinity resins.

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Abstract

Previous high throughput data analysis from several different approaches to affinity purification of protein complexes have revealed catalogues of contaminating proteins that persistently co-purify. Some of these contaminating proteins appear to be specific to one particular affinity matrix used or even to the artificial affinity tags introduced into endogenous proteins for the purposed of purification.

A recent approach to minimising non-specific protein interactions in high throughput screens utilises pre-equilibration of affinity surfaces with thiocyanate anions to reduce non-specific binding of proteins. This approach not only reduces the effect of contaminating proteins but also promotes the enrichment of the specific binding partners. Here, we have taken this method and adapted it in an attempt to reduce the abundance of common contaminants in affinity purification experiments. We found the effect varied depending on the bait used, most likely due to its endogenous abundance.

Keywords. Affinity purification, non-specific, contaminants, thiocyanate, Mass Spectrometry

1. Introduction

The characterisation of native protein interactions is essential for our understanding of the processes which underlie biological functions. In order to gain a comprehensive knowledge of multi-component protein complexes it has been necessary to develop and utilise high throughput methods which allow the identification of genuine interaction partners. This has lead to the inception of the field of interactomics, a rapidly growing field with numerous different approaches developed to allow the characterisation of proteins within functional complexes. Many of these approaches involve the affinity capture of a bait protein, either by its interaction with a specific antibody or by interaction of an engineered component such as a short protein epitope tag or full length fusion protein. After affinity capture, identification of the bait and its interacting partners are generally achieved by mass spectrometry. Approaches such as the tandem affinity purification method (TAP) allow high-throughput screening of interactomes in multicellular organisms [1]. Here, bait proteins are tagged with two affinity tags and purification of the tagged bait and its interacting partners is then carried out using the affinity properties of each tag sequentially. Another recent technique, iPAC (interactomes by parallel affinity capture), favours parallel purifications of a multiply tagged protein to increase yields of purified complexes as tandem approaches often result in very low recoveries of protein complex components after multiple sequential application and elutions from affinity matrices [2]. In all these approaches, conditions are utilised to minimise the sampling of contaminants such as stringent washing of affinity matrices before specific elution of the bait and its binding partners and occasionally the implementation of exclusion lists of ions associated with common contaminating proteins during mass spectrometric analysis. Despite these precautions, contaminants that have high affinity to single or multiple resins continue to be a problem in blocking available binding sites for the tagged protein(s) thus resulting in low recovery yields of genuine interacting partners. Moreover, these proteins can dominate mass spectrometric analyses, usually in the form of peptides generated upon proteolytic digestion of eluted complex components prior to analysis. Without appropriate experimental designs it can be challenging to differentiate between genuine interacting partners and contaminants. One method which aids differentiation involves the use of quantitative approaches where a negative control such as a system without a tagged bait is applied to the same affinity

matrix as the tagged version and the abundance of eluted proteins compared and the enrichment of genuine partners is established [3,4]. Trinkle-Mulchay and co-workers used this approach to quantify proteins that non-specifically bound to a GFP-Trap resin used for isolating and purifying GFP-tagged proteins and their binding partners from complex mixtures [5,6].

A complementary approach is to minimise binding of contaminants prior to purifying proteins of interest. High throughput chip arrays often use a blocking system that enable genuine binders, that have higher affinity, to preferentially bind to specific binding sites. This approach is especially useful for isolating proteins of low abundance from complex mixtures from whole cell lysates. Recently, Richens and co-workers used thiocyanate, a member of the Hofmeister series [7,8] in order to reduce the non specific binding of abundant proteins such as albumin on label free protein arrays. Thiocyanate is a relatively large anion which has a very high entropy of hydration. It is thought to disrupt non-specific interactions of proteins by modulating the structure of water in surrounding interacting regions of macromolecules and thus disrupts selectively the non-polar effects that facilitate non-specific binding events [8,9]. Richens and colleagues demonstrated enrichment of low abundance proteins, often 10 orders of magnitude lower than that of albumin, the predominant component of serum, when binding assays were carried out in the presence of thiocyanate anions [9].

Here we apply the approach of pre-treating affinity matrices designed for the purification of tagged protein baits and their interacting partners with thiocyanate containing buffers and demonstrate a reduction in the co-purification of some of the common contaminants regularly described in the literature. In taking this approach we facilitate the maintenance of transient or short lived interactions. We demonstrate that thiocyanate pre-treatment of affinity binding matrices and, more importantly, inclusion in the binding step is efficient at increasing bait peptide identification as well as reducing non-specific binding events within the iPAC protocol.

The objective of the study presented here is to assess the effect of inclusion of thiocyanate ions in affinity purifications using multiple affinity resins and to reduce the numbers of non-specific contaminants allowing surveying of lower abundance proteins in complex mixtures.

2. Methods

2.1 Tagged lines

A number of affinity tagged *Drosophila melanogaster* lines from the FlyProt collection (Kyoto stock centre, www.flyprot.org) were randomly selected for affinity purifications to analyse both genuine and non-specific binding proteins. These comprised a tandem triple tag of FLAG-Strep-YFP-Strep with the former used for affinity purifications and the YFP for visual assessment (Figure 1A). We also used non-tagged w118 control flies to determine non-specific binding proteins to identify proteins that bind to the resin material and a positive control Vha55-YFP-Strep to test the effect of the treatment.

2.2 Assessing suitability of thiocyantate anions

All affinity purifications of triple tagged proteins from *D. melanogaster* embryo lysates were performed as described in Rees *et al* [2] with the following additions and modifications. To determine the optimal buffering conditions and chaotropic anion concentration a pilot study was performed with a well characterised, high abundance, bait and a non-tagged control. Lysates were prepared in Veraksa buffer [10] including protease inhibitor cocktail (Roche) with or without 50-500 mM NaSCN (thiocyanate) and 2.85 mM PBS and incubated with either ANTI-FLAG M2 MAb (Sigma) or *Strep*-Tactin (IBA) sepharose resins that were pre-equilibrated in 50-500 mM thiocyanate and 2.85 mM PBS. After binding for one hour resins were washed three times for 10 min in Veraksa buffer to remove non specific binders. *Bona fida* bound native protein complexes were eluted twice each with either 50 l (100 g/ml) FLAG peptide (Sigma) or 50 l of 10 mM Biotin (Sigma) respectively in Veraksa buffer containing protease inhibitor cocktail for 30 min at 4ºC on a rotary mixer.
.Pooled eluates and non-binding fractions were firstly analysed by immunoblot to detect recovery of the bait and actin abundance. The workflow is summarised in Figure 1B.

Suggested location for Figure 1 here.

2.3 Protein identification by mass spectrometry.

Total eluates were partially resolved by SDS-PAGE, stained with Coomassie, excised, reduced in 2 mM DTT for 1 hour at RT and alkylated in 10 mM iodoacetamide for 30

min at RT. Proteins were digested with 2 g sequencing grade trypsin (Promega) for 1 hour at 37ºC, then a further 2 g for overnight digestion to maximise complete digestion of complex mixtures.

Digests were prepared in 0.1% Formic acid and analysed in a single run by Mass Spectrometry (MS) to identify all proteins eluted. MS was performed as described in Rees *et al* [2] but without actin containing exclusion lists, in order to measure the abundance of actin with and without treatment. The Orbitrap was operated in data dependent mode, acquiring an MS scan and two subsequent MS/MS measurements with a precursor dynamic exclusion of 0.3 Da.

Peak lists were generated using Bioworks Browser version 3.3.1 (2007). Resulting fragment masses (MS/MS) were searched using the Mascot version 2.2 (Matrix Science) search engine against an in house database comprising the FlyBase *D. melanogaster* genome (version 5.9) totalling 21064 proteins, plus the FASTA sequence for YFP as a secondary confirmation of the presence of the tagged protein. Parameters included a precursor mass tolerance of 1.0 Da and fragment ion mass tolerance of 0.8 Da, 2 missed cleavages and methionine oxidation as variable and carbamidomethylated cysteine as fixed modifications. The decoy database option, comprising a scrambled *D. melanogaster* database *in silico* digested that generates a similar number of the same sized peptides, was selected to automatically calculate the protein false discovery rate (FDR). Stringent parameters were used to ensure accuracy in the datasets. For example, proteins with single peptide hits were eliminated. MS samples were run once or twice if the bait was of particularly low abundance.

Resulting proteins lists were exported and compared using the ProteinCenter software (Thermo).

2.4 Interaction validation.

To determine if the protein interaction partners we observed are genuine, we used FlyMine search queries (www.flymine.org) to mine the public interaction databases, such as IntAct. Binary search queries within *Drosophila melanogaster* interaction datasets identify proteins within our list that have had reported interactions and binary search queries in orthologous interaction datasets allow us to potentially highlight

observed interactions in orthologous species, such as yeast, worm, human, that have not yet been detected in flies.

3. Results

3.1 Parallel affinity purifications.

Previous purifications of triple tagged *Drosophila melanogaster* proteins have used parallel methods where soluble extracts are split and half purified using Strep-Tactin resins and the other half FLAG monoclonal antibody (MAb) resins, all eluates tryptic digested and analysed by Mass Spectrometry (MS) and the resulting protein lists compared *in silico.* Almost 250 proteins' interacting partners have been characterised by this method and in almost all, actin was a non-specific interactor [2].

3.2 Pre-treatment of affinity resins.

Equilibration of affinity resins is a pre-requisite for efficient binding of target proteins. Pre-binding Strep-Tactin and FLAG MAb resins with thiocyanate should enable selective competition of binding of genuine tagged proteins so we tried pre-equilibrating, post washing, with concentrations of 50-500 mM NaSCN. MS analysis of the resulting eluates showed little difference in the non-specific binding to the resins according to protein lists generated from MS analysis (data not shown).

3.3 Thiocyanate ions improve the specific binding of proteins to affinity resins.

The next approach was to include sodium thiocyanate in the binding mixture. To determine if the effect of the addition of thiocyanate ions in affinity purification of protein complexes is beneficial, we first used non-tagged control fly embryo lysates to identify all proteins that bind non-specifically to the FLAG affinity resins. Several concentrations of the sodium thiocyanate were utilised ranging 50-500 mM. The most efficient concentration of the thiocyanate anions utilised seemed to be relatively broad as over a wide concentration tested, all gave the same protein identification lists therefore 100mM thiocyanate was used in further experimentation.

This proof of principle experiment that used control lines, where proteins should not bind resin, demonstrated that the addition of 100 mM thiocyanate reduces the number of non-specific proteins eluted from FLAG resins by a specific FLAG peptide compared to purifications without thiocyanate, from 37 to 32 in one test and from 30 to 24 in a biological replicate (Supplementary Table 1).

We also used a well characterised protein, Vha55, a subunit of the mitochondrial VATPase complex to demonstrate enrichment of known and predicted binding partners in the presence of thiocyanate. Of the 175 and 85 *D. melanogaster* proteins detected with or without thiocyanate respectively, 73 and only 24 (42% and 28%) were remaining after removal of the corresponding W- negative controls. Importantly FlyMine search queries revealed 22 proteins were identified as known or predicted interacting partners for thiocyantate treated compared to only 2 for the non treated sample (Supplementary Figure 1) demonstrating that thiocyanate enriches for genuine interacting partners. A further 35 proteins from thiocyanate-treated eluates were known or predicted but were also found in negative control samples with the majority enriched for in the positive compared to negative based on peptide numbers, sequence coverage and spectral counts (Supplementary Table 2).

Based on these observations we then sampled 8 different triple- FLAG-Strep-YFP-Strep-tagged protein lysates to determine if the inclusion of thiocyanate would improve the binding and numbers of specific binders and reduce the number of non-specific binders, both to the resin and the bait. Immunoblots showed, in many cases, an increase in the yield of bait, as detected by anti GFP antibody (Supplementary Figure 2A).

Mass spectrometry analysis showed increased numbers of bait peptides identified in the presence of thiocyanate for 9 of the 10 tagged proteins tested with a range of a 16->100% increase, the average being 43.5% (Table 1). This was similar for the YFP peptides also generated from the bait protein. This trend was also observed in the % sequence coverage of the bait protein. Whilst proteins purified using Strep resin had higher numbers of peptides, the effect of thiocyanate was more dramatic for FLAG purified proteins and in general the addition of thiocyanate was beneficial for increasing the binding of bait to both FLAG and Strep resins. Mascot or emPAI scores were more ambiguous with respect to the effect of thiocyanate ions.

Suggested location for Table 1 here.

The protein lists generated by MASCOT were compared *in silico* to analyse proteins eluted in the presence or absence of thiocyanate and Venn diagrams were used to show the overlap after removal of negative control proteins (Figure 2, upper panel) and 'non-specific' binders (identified in >20% of all interaction lists irrespective of

the bait, as shown in Figure 3A and Supplementary Table 3A&B) (Figure 2, lower panel). In all cases there were different proteins identified by each unique experiment.

Suggested location for Figure 2 here.

Protein lists for thiocyanate treated lysates had reduced abundance of actin, and fewer proteins were categorised as non-specific, particularly in FLAG pulldowns.. The remaining proteins contained uncharacterised proteins and a proportion of proteins similar to those found to be non-specific binders such as heat shock proteins and tubulins that were not frequent enough to be included in the 'non-specific' lists. After identifying the recurring non-specific members, lists from non-treated lysates also contained a high proportion of other ribosomal proteins, metabolic proteins and uncharacterised proteins that are unlikely to be genuine interactors of the bait. Annotated interaction lists are shown in Supplementary Table 4A.

When analysing the interacting proteins, very few of the proteins studied had any published interaction data in *Drosophila* (Supplementary Table 4B). Using FlyMine we did observe novel interactors in eluates both from inclusion and exclusion of thiocyanate. For example, the bait Pop2 (CPTI 2818) interacts with proteins Not1 and twin in yeast. These were both found in FLAG pulldowns but in the less effective Strep pulldown, were only found with the inclusion of thiocyanate (Supplementary Table 4A). In addition, three known contaminants were found only in untreated samples. Comparing our datasets with public datasets using FlyMine, many of our interactors were seen in affinity purification studies in other species and some did indeed complement Y2H studies (Supplementary Table 4B). It appears that thiocyanate is useful in recovering some binding partners *in vivo* in some baits.

For analysing non-specific binders in more detail all of the protein lists from the 8 different baits and controls, comprising 25 different experiments, were combined *in silico* using ProteinCenter software to determine frequently occurring proteins. The most frequently occurring proteins were indeed identified in previous studies and are illustrated in Figure 3A and Supplementary Tables 3 A&B. Several proteins were eliminated in the presence of thiocyanate; CG9436, Tm1 (2 isoforms), RpL23A, Tm2, and Ald. A further 7 proteins had between 30-50% reductions in occurrence of appearances.

Suggested location for Figure 3 here.

A semi-quantitative analysis was performed to see the overall effect of the presence of thiocyanate by plotting the average number of peptides observed in the 20 most common contaminants from negative controls and tagged proteins both in the presence and absence of thiocyanate ions, determined in Figure 3A, by globally analysing all peptides from these 20 proteins generated in all 25 experiments (Figure 3B). The more commonly occurring proteins and peptides were also in negative control samples suggesting that these are resin specific contaminants irrespective of the bait protein used. Fewer peptides were observed for some structural scaffold proteins such as actins and tubulins. From the top 20 contaminants' list 8 high abundance proteins generated fewer peptides on average, ranging from a 33-2% decrease, although 9 proteins had increased peptides whilst three showed no change (Figure 3B). In addition, of the 72 proteins seen more than five times (≥20% frequency) in the 25 pooled experiments, 35 proteins had reduced numbers of observed peptides eluted from FLAG resins whilst only 29 had increased peptides in the presence of thiocyanate (n=16) (Supplementary Tables 3A&B). For Strep resins 27 proteins had increased peptides compared to 31 that had lower numbers in the presence of thiocyanate (n=9). We also looked at the changes in the average percentage sequence coverage for all proteins occurring more than once in the 25 experiments. The heat map (Supplementary Table 3B) clearly shows that the changes in protein sequence coverage mimic the changes in peptides and protein frequencies, confirming that analysing changes in peptide numbers is a good tool for assessing the effectiveness of thiocyanate. Mascot scores were not as reliable, as can be seen from the heat map of the bait proteins (Supplementary Table 3B) as these varied widely amongst biological and technical replicates.

Therefore we have not reduced all contaminant proteins but nevertheless, we have improved the coverage of the bait and identified novel interacting partners for some baits that are not present in controls or known contaminant lists.

4. Discussion.

A potential improvement to affinity purifications was performed and analysed with a view of minimalising non-specific interactions pre-MS analysis. Thiocyanate ions have been known to reduce binding of non-specific plasma proteins to protein chips

and we found that the presence of thiocyanate in the binding step in our experiments enriched for known binding partners in our positive control but did not necessarily reduce the level of known contaminants such as actin and yolk proteins, although these contaminants did not dominate in this sample. When testing poorly characterised and/or lower abundance baits, in most examples we observed enrichment of the bait and in one example we saw enrichment for known binding partners unique to thiocyanate treatment. However many novel proteins identified were unique to thiocyanate treated samples and will need further testing. In terms of reducing non specific binding contaminants in some samples we observed varying levels of reduction of one of the most abundant proteins, actin, but it varied depending on the bait.

We tried the recommended concentration discussed by Richens and found it to be effective in some of our experiments for minimalising non-specific binding of structural proteins. However, by reducing these, we did observe increased binding of other non-specific proteins such as the yolk proteins, probably because of the increased sampling of lower abundance contaminants, but the reduction of actin and other scaffold proteins outweighed the marginal increase in other contaminants. We think this is a reasonable trade-off especially as we are aware of these common contaminants in previous studies [2].

The effect of thiocyanate is clearly bait abundance specific and may be more pronounced with lower abundance baits if MS data dependant exclusion lists for other non-specific binders were used in parallel and moreover, our method has assisted in identifying proteins which are the most desirable to exclude such as the yolk proteins [2]. It is important in mass spectrometry experiments to be very careful when excluding masses for analysis, as a too large an exclusion list will result in under sampling of the proteome. Utilisation of this thiocyanate method to show which proteins persistently co-fractionate, assists in choosing the most effective exclusion parameters.

Future work would include investigating other members of Hofmeister series around position of thiocyanate and see how they fare.

Figure legends.

Figure 1. A. Construct used to generate tagged endogenous proteins. Pl= Plasmid region, PB *PiggyBac* Inverted repeats, P= P-ends, SA= Splice Acceptor, FLAG= FLAG tag, S= *Strep*II tags, SD= Splice Donor. **B.** Workflow for the purification of *Drosophila melanogaster* tagged proteins with and without the inclusion of thiocyanate at various steps indicated. S= Strep tag, w= w118 control line, x and y are tagged test lines. R= resin and E= eluate containing purified protein.

Figure 2. The effect of thiocyanate in reducing non specific protein binding to FLAG and Strep affinity resins. **A-C.** Venn diagrams to show control proteins (Wf+&-) and eluted proteins from pulldowns in the presence (+) or absence (-) of thiocyanate. Subset Venn in **A**' is the merged negative control data. **D-F.** Venn diagrams showing each bait with its respective negative control data subtracted and the proportion of the data that is present more than 20% in all samples analysed 'non-specific' (list of proteins in Supplementary Table 3A).

Figure 3. A. The numbers, and some identifications of proteins identified in 25 experiments using 8 protein baits. Single hit proteins have been excluded. Shaded blocks indicate proteins occurring in >20%-100% frequently occurring interaction lists from all pulldown experiments (including negative controls) thus defined as 'contaminants'. These proteins are listed and detailed in Supplementary Table 3A. Dashed line shows the threshold we define our cut-off for contaminants. Proteins in bold were not frequently occurring in negative control samples so are likely to be bait specific and not resin specific (from Supplementary Table 1). **B.** An analysis of the numbers of peptides generated from the 20 most abundant proteins (and their frequencies in all interaction lists) observed in Figure 3A in the presence (grey bars) or absence (black bars) of thiocyanate. The most notable differences are in the scaffold proteins, actins and tubulins. The % average peptide count decreases, compared with no treatment, are displayed above the bars.

Table 1. Mascot data from Mass Spectrometry analysis of 8 tagged (bait) proteins purified in the presence (+) or absence (-) of thiocyanate. All FDRs are below 5%. C=cytoplasmic, m=membrane, n=nuclear and unk=unknown. PG/PC are distinct protein isoforms G and C respectively.

Supplementary Figure 1. Venn diagram to show proteins identified in the Vha55 positive and W118 negative controls with (+) and without (-) thiocyanate treatment. Subsets highlight the number of known and predicted Vha55 interactors.

Supplementary Figure 2. A. Western blot to show the yields of bait recovered from pulldowns in the presence (+) or absence (-) of Thiocyanate. Wf = w118 control line pulled down with Flag (f), Heph (CPTI2584) and Cat (CPTI2786) are two FLAG-Strep-GFP tagged proteins with their CPTI identifiers. s= soluble extract, e= eluate. Black arrows indicate enrichment of bait. **B.** The number of peptides identified from a single Mass Spectrometry analysis of the bait and different actin proteins (range of peptides) in eluates from the inclusion (+) or absence (-) of thiocyanate.

Supplementary Table 1. Mass Spectrometry data from non-tagged controls to determine proteins being eluted non-specifically from FLAG and Strep resins and analysis of the peptides generated. Wf1+; W=w118 control line, F=Flag purification, 1/2= replicates and +/- = presence or absence of thiocyanate.

Supplementary Table 2. A snapshot of some of the proteins identified, numbers of contributing peptides and % protein sequence coverage in Vha55 positive control and corresponding W118 negative control experiments with (+) or without (-) thiocyanate. Proteins highlighted in orange are either direct or indirect interactors of the bait (green) and proteins highlighted in yellow are putative direct interactors based on known interactions of orthologous proteins from other species. Proteins highlighted in red are known contaminants based on previous experiments but those asterisked are putative binding partners from yeast predictions. The remaining proteins (148) that have no published interaction data have been excluded from the list. Green values indicate increase of peptides or % sequence coverage and red highlights a decrease (or increase where the 20 known contaminants are being measured).

Supplementary Table 3. A. Mass Spectrometry data from eight baits and corresponding non-tagged controls to determine proteins being eluted non-specifically from FLAG and Strep resins. **B.** Heat Map showing Mass Spectrometry data averaged from all eight baits and corresponding non-tagged controls to demonstrate changes in the average numbers of peptides and % sequence coverage after thiocyanate

treatment. Also shown are the Mascot scores for the baits to demonstrate that this is not a good measure to show the effect of thiocyanate treatment.

Supplementary Table 4. Interaction lists and validation. **A.** Interaction lists for the 8 baits used to show proteins identified in FLAG and STREP affinity purifications with or without the presence of thiocyanate ranked in decreasing order of numbers of unique peptides, then % sequence coverage. * indicates the bait protein. Respective *w118* negative control proteins found in FLAG and Strep pulldowns have been removed but common 'known' contaminants have not. Contaminants identified in pooled analysis are in italics. Grey highlighting indicates protein was detected with and without thiocyanate. Grey boxes with white text identify proteins that have interactions with the bait from orthologous species. Black highlighted boxes with white text indicate interacting proteins unique to the inclusion or exclusion of thiocyanate. **B.** Numbers of published and predicted interactors from DroID and the numbers in our lists that were published using the FlyMine interaction queries.

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Role of the funding source.

The funding source, the Wellcome Trust, had no involvement in the design, collection, analysis, interpretation of the data, in the writing of the report, or the decision to submit the paper for publication

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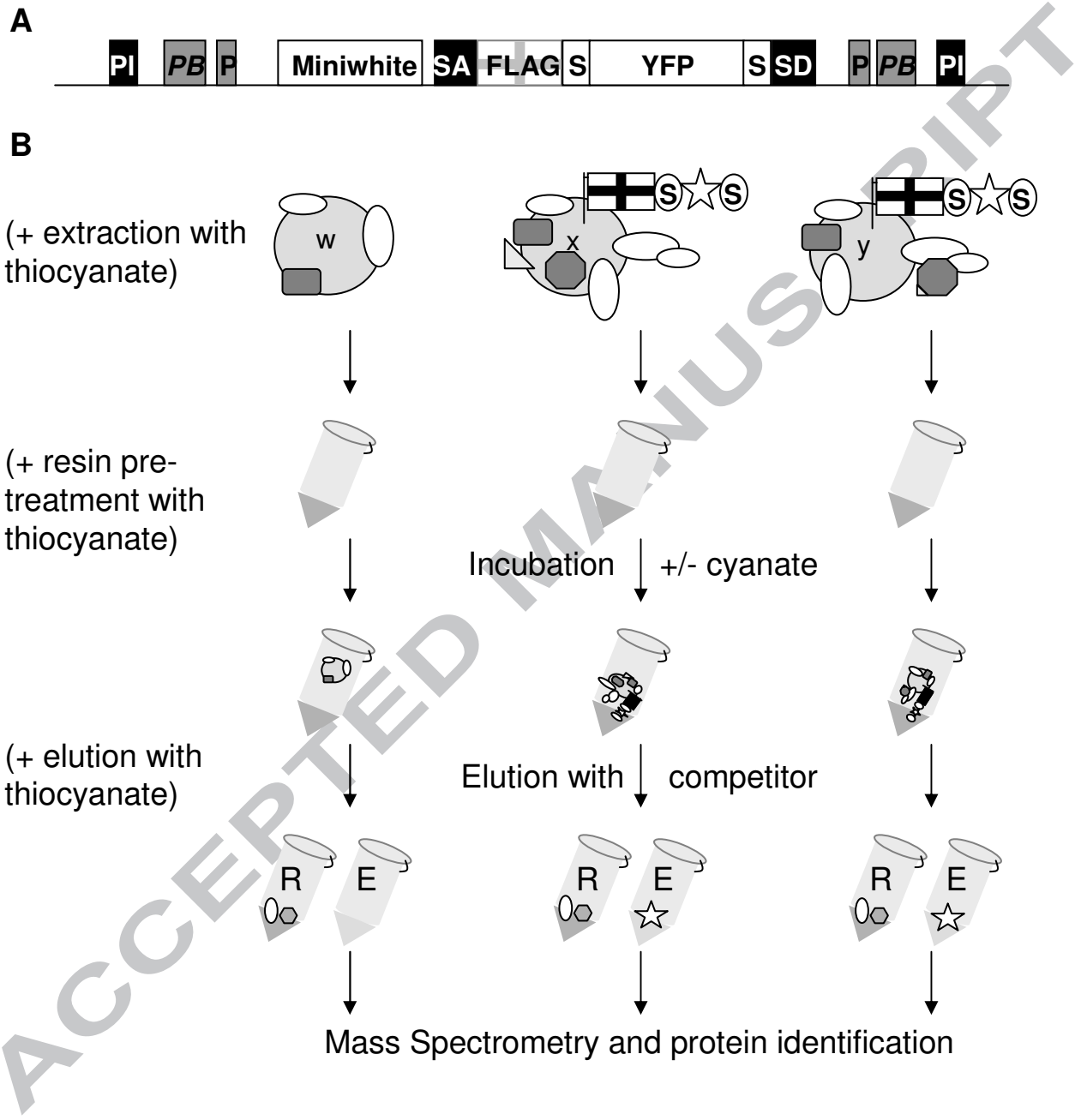

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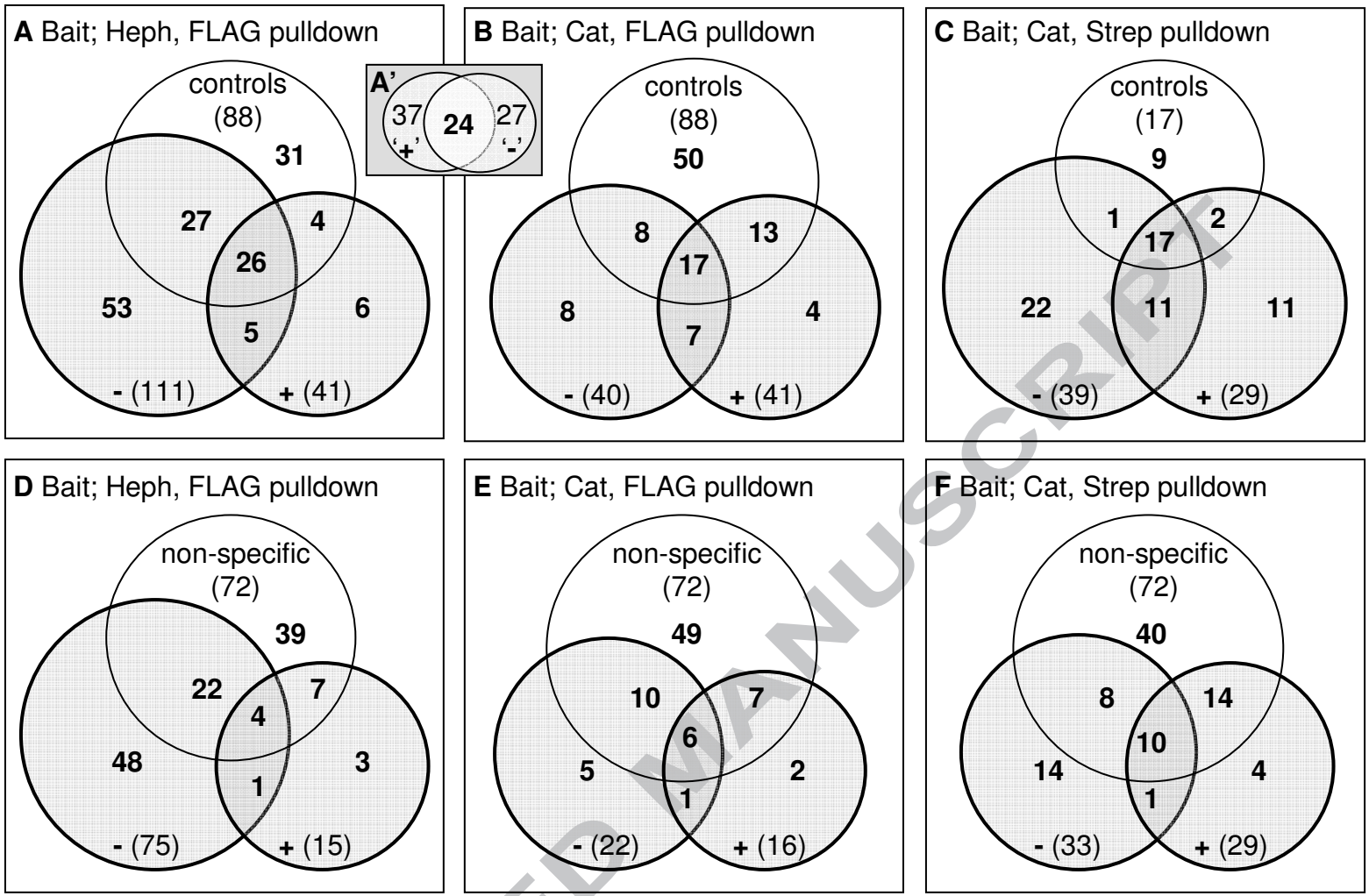

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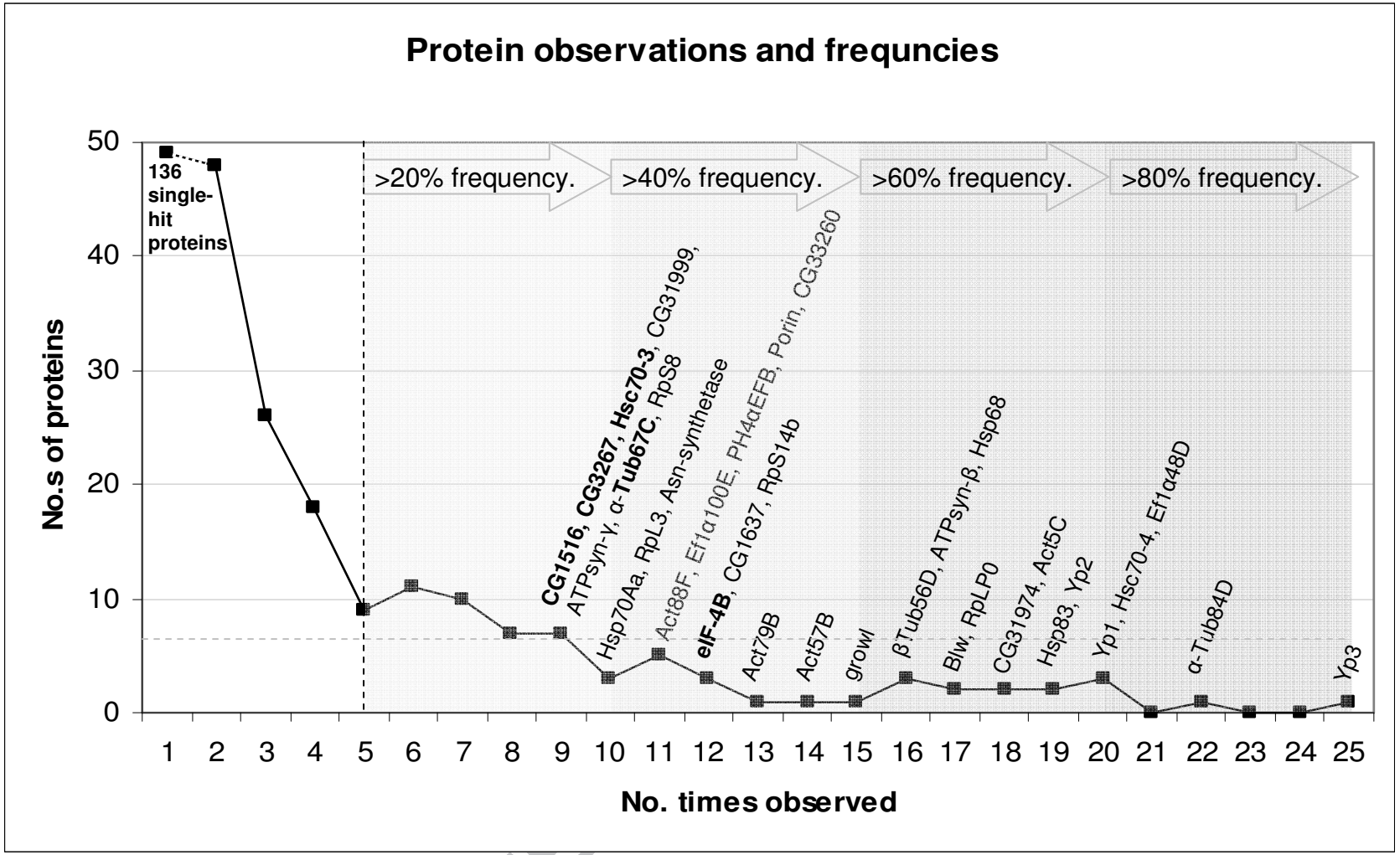

Figure 3A. The numbers, and some identifications of proteins identified in 25 experiments using 8 protein baits. Single hit proteins have been excluded. Shaded blocks indicate proteins occurring in >20%-100% frequently occurring interaction lists from all pulldown experiments (including negative controls) thus defined as 'contaminants'. These proteins are listed and detailed in Supplementary Table 3B. Dashed line shows the threshold we define our cut-off for contaminants. Proteins in bold were not frequently occurring in negative control samples so are likely to be bait specific and not resin specific (from Supplementary Table 1).

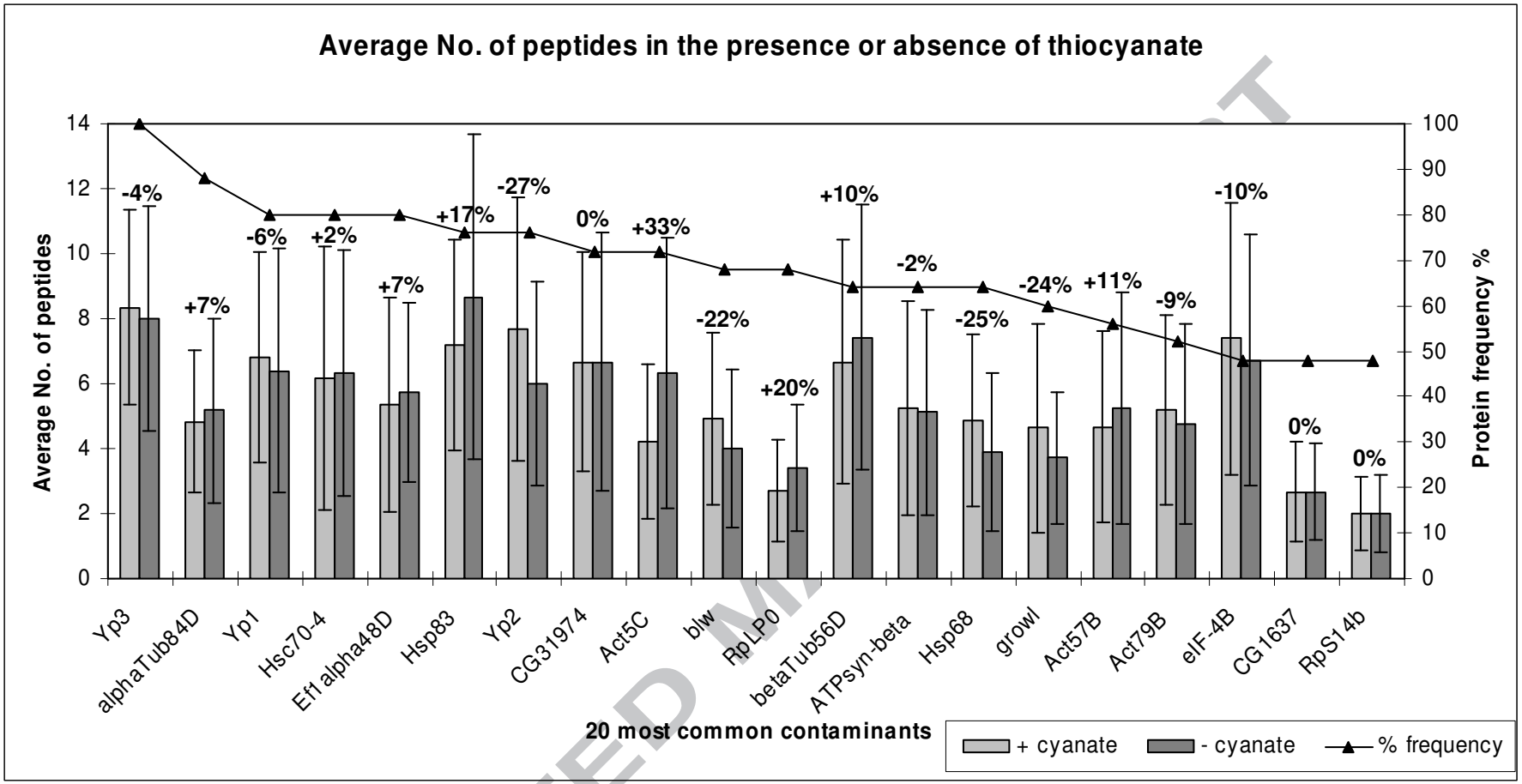

Figure 3B. An analysis of the numbers of peptides generated from the 20 most abundant proteins (with % frequencies in all interaction lists) observed in Figure 3A in the presence (light grey bars) or absence (dark grey bars) of thiocyanate with error bars. The most notable differences are in the scaffold proteins, actins and tubulins. The % average peptide count changes, compared with no treatment, are displayed above the bars.

Bait ID	Name/ FlyBase ID	size (KDa)	type	resin	bait peptides '+'	bait peptides '-'	bait seq. coverage (%) '+'	bait seq. coverage (%) '-'	YFP peptides '+'	YFP peptides '-'	YFP seq. coverage (%) '+'	YFP seq. coverage (%) '-'	Mascot score '+'	Mascot score '-'	% increase in bait peptides	% increase in bait coverage
2584	heph -PG	66.3	c	FLAG	7	6	21	18	12	12	30	41	390	106	16.67	16.67
	heph -PC	62.6	c	FLAG	6	0	17	0	12	12	30	41	375	0	>100	16.67
2785	CG11030	37.3	unk	FLAG	5	3	23	6	7	7	13	27	130	63	66.67	283.33
2786	Cat	57.1	m	FLAG	27	32	38	42	10	4	36	15	1066	1585	-15.63	-9.52
2796	shep	40-60	c	FLAG	3	2	8	11	7	8	20	25	57	75	50.00	-27.27
2818	Pop2	33.5	n&c	FLAG	6	3	28	10	7	6	28	18	89	80	100.00	180.00
3424	Aats-His	57/62	c	FLAG	9	6	19	14	4	3	10	13	366	407	50.00	35.71
2267	CG1440	55.2	c	Strep	47	39	62	48	4	6	21	42	982	853	20.51	29.17
2728	CG6084	36/40	c	Strep	68	42	66	43	36	36	38	33	898	1549	61.90	53.49
2786	Cat	57.1	m	Strep	34	24	43	39	10	5	31	21	1576	1124	41.67	10.26
2818	Pop2	33.5	n&c	Strep	0	0	0	0	5	2	34	6	0	0	0.00	0.00

Table 1. Mascot data from Mass Spec analysis of 8 tagged (bait) proteins purified in the presence (+) or absence (-) of thiocyanate. All FDRs are below 5%. C=cytoplasmic, m=membrane, n=nuclear and unk=unknown. PG/PC are distinct protein isoforms G and C respectively.

Large scale affinity purification studies reveal co-purifying contaminants.

Sodium thiocyanate (NaSCN) ions can help minimize persistent contaminants.

Importantly NaSCN also enriches for desired proteins and specific binding partners.