Charge-based analysis of antibodies with engineered cysteines
From multiple peaks to a single main peak

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Abbreviations: CEX, cation-exchange chromatography; cIEF, capillary isoelectric focusing; mAb, monoclonal antibody; ADC, antibody-drug conjugate; Cys, cysteine; Ctn, cystine; LC/MS, liquid chromatography/mass spectrometry

THIOMABs are antibodies with an engineered unpaired cysteine residue on each heavy chain that can be used as intermediates to generate antibody-drug conjugates. Multiple charge variant peaks were observed during cation-exchange chromatography (CEX) and imaged capillary isoelectric focusing (cIEF) analysis of several different THIOMABs. This charge heterogeneity was due to cysteinylation and/or glutathionylation at the engineered and unpaired cysteines through disulfide bonds formed during the cell culture process. Cysteine treatment followed by analysis using CEX, LC/MS and electrophoresis demonstrates that cysteine is a mild reductant that can remove glutathione and cysteine bound to the engineered cysteines without disrupting the inter- or intra-chain disulfide bonds of antibodies. We further demonstrated that using a cysteine/cystine redox pair (rather than cysteine alone) can not only effectively remove glutathione at the engineered cysteines, but also generate homogeneously cysteinylated species, which resulted in one main peak in both CEX-HPLC and imaged cIEF assays for antibodies with engineered and unpaired cysteines.

Introduction

Recombinant monoclonal antibodies (mAbs) represent one of the fastest growing protein therapeutic areas.1,2 Within this product class, antibody-drug conjugates (ADCs) are attracting significant recent attention.3-13 ADCs may improve the therapeutic index of cytotoxic drugs by using the interaction of the antibody with tumor-specific antigens to deliver chemotherapy directly to cancer cells, thereby reducing toxicity to normal tissue. THIOMABs are antibodies with an engineered and unpaired cysteine residue on each heavy chain in a non-CDR region.13 These can be used as intermediates to generate ADCs. Unlike conventional antibody conjugation strategies, where drug attachment is either through exposed lysine residues5,6 or through inter-chain disulfides7-9 forming a heterogeneous mixture of ADCs with different drug-to-antibody ratios (DARs), the THIOMAB platform provides one specific site on each heavy chain (Fab region) for drug attachment. Therefore, conjugation of a THIOMAB generates a more homogeneous ADC product with a primary DAR close to two. The resulting homogeneity of these ADCs leads to a simplified drug profile and improved therapeutic index.13

The presence of unpaired cysteine residues in a protein, however, may lead to heterogeneity due to their reactivity.14-19 THIOMABs produced in Chinese hamster ovary (CHO) cells have shown some intrinsic complexity where the thiol of the engineered cysteine (Fig. 1) was found to be blocked through a disulfide bond by a cysteine (Cys), a glutathione (GSH) or an extra mAb light chain introduced during the cell culture process. While the purification process can remove the light chain-adduct successfully, the cysteinylated and glutathionylated molecules still exist as the predominant species in the isolated THIOMAB. Although these species are not a concern for the final ADC, as de-cysteinylation and de-glutathionylation occur during the first step of the drug conjugation process,13 they present a significant challenge in the development of charge-based assays, such as CEX-HPLC and imaged cIEF for analysis of the antibody intermediate. The cysteinylation and glutathionylation alter the surface charge distribution of THIOMABs and change the typically observed charge heterogeneity profile of an antibody from one main peak to multiple peaks. To observe the true charge variants that are associated with the antibody polypeptide chain, and to simplify the charge heterogeneity profile for batch-to-batch purity comparison and stability studies, we needed to develop a method that either completely removes the modifications from the engineered cysteines or homogeneously modifies them prior to charge-based analysis.
In this report, we show that cysteine is a mild reductant that can remove GSH or Cys on the engineered cysteine while keeping the inter- and intra-chain disulfides intact. We further demonstrate that using a cysteine/cystine redox pair (rather than Cys alone) cannot only remove glutathione at the engineered cysteines, but also effectively generate homogeneously cysteinylated species, which significantly simplifies the charge distribution profile of THIOMABs.

Results and Discussion

Analysis of the purified bulk drug substance of the THIOMAB by CEX-HPLC resulted in three main peaks compared to one, seen in the conventional mAb analyzed under identical conditions (Fig. 2A). The disparity between these two chromatograms is not expected to be due to differences in pI values since the theoretical pI values of the THIOMAB and the conventional mAb are identical (pI = 9.1) based on amino acid sequence. Additionally, the relative intensities of the three main THIOMAB peaks on CEX-HPLC can vary from lot to lot. Therefore a mass spectrometry study (data not shown) was conducted and results from this study indicated that the multiple peaks observed by CEX-HPLC were due to cysteinylation and/or glutathionylation at the engineered cysteine in each heavy chain, shown as +2GSH, +1GSH+1Cys, +2Cys species. Since GSH is more acidic than Cys, the glutathionylated species elute earlier than the cysteinylated species (Fig. 2A), as if these were the acidic variants of the cysteinylated species. These main peaks obscure the true charge variants due to the side chain modifications, commonly observed as acidic and basic regions in a conventional mAb (the side chain modifications, commonly observed as acidic and basic regions in a conventional mAb). These main peaks obscure the true charge variants due to the antibody poly-peptides. Therefore, cysteine, a mild reductant, was evaluated for eliminating the chemical heterogeneity at the engineered and unpaired cysteine.

Time course study of Cys treatment and peak identification. An initial study suggested that glutathione removal could be achieved using 5 mM Cys treatment at 37°C. As shown in Figure 3A, after Cys treatment for 24 hours, the first two main peaks, +2GSH and +1Cys+1GSH in CEX-HPLC of the THIOMAB disappear, while a cluster of peaks (labeled p1, p2 and p3 in all figures) appear. A more detailed time course study as described in the Materials and Methods Section revealed changes to the relative intensities of the peaks in the cluster: peak p1 increased while peaks p2 and p3 decreased with time. Peak p1, eluting with a retention time equivalent to that of the +2Cys form in the untreated THIOMAB, became the dominant peak after 24 hours (Fig. 3A). Banks et al. also observed a similar CEX-HPLC chromatogram shift from multiple peaks to a more basic homogenous peak after cysteine treatment of a mAb that contained unpaired cysteine residues (MAB007). To identify the peaks in the CEX-HPLC profile of Cys-treated THIOMAB, fractions were collected, concentrated, deglycosylated and analyzed using LC/MS. As seen in Figure 4, the deconvoluted mass spectra of the collected peak fractions (p1-p3) indicate that peaks in the cluster are isoforms of cysteinylated species: peak p1 is fully cysteinylated (+2Cys), peak p2 is half cysteinylated (+1Cys, although there is significant amount of the +2Cys), and peak p3 contains predominantly the non-cysteinylated, +0Cys form. LC/MS analysis of the fractions eluted immediately before and after the isoforms of cysteinylated species are consistent with what is typically observed with recombinant mAbs, shown as acidic peaks and basic peaks (containing C-terminal lysine) in cysteinylated forms (data not shown). Due to the excellent resolution of this CEX-HPLC method, we observed that during Cys treatment, THIOMAB first underwent reductive de-glutathionylation and de-cysteinylation, resulting in a non-cysteinylated (+0Cys) species. With increased time, this was followed by an oxidative cysteinylation reaction yielding cysteinylated (+1Cys and +2Cys) species.

Imaged cIEF analysis (Fig. 3B) of the cysteine treatment time course samples also gave rise to a cluster of peaks (labeled p1, p2 and p3) changing intensity with the same pattern observed by CEX-HPLC (Fig. 3A). Imaged cIEF analysis of the fractions
Figure 2. Charge heterogeneity profile overlay of conventional mAb (without unpaired cysteine residues, line a in each panel) vs. THIOMAB (with engineered and unpaired cysteine residues, line b in each panel). (A) CEX-HPLC chromatograms. The insert is the mass spectrum of THIOMAB. (B) Imaged cIEF electropherograms.

Figure 3. Overlay of charge heterogeneity profiles of the THIOMAB treated with 5 mM Cys at 37°C for different durations, and the untreated sample. Peaks labeled as p1, p2 and p3 are fully cysteinylated (+2Cys), half cysteinylated (+1Cys), and non-cysteinylated (+0Cys) THIOMAB, respectively. (A) CEX-HPLC chromatograms. (B) Imaged cIEF electropherograms.
the untreated THIOMAB. Finally, the integrity of intra-chain disulfide bonds in the THIOMAB was confirmed by analyzing the mass spectra of Fab fragments generated by papain digestion in the presence of 5 mM Cys at 37°C. As shown in Figure 6A, the THIOMAB lot used for the time course study was initially highly glutathionylated, but GSH is removed in less than 2 hours of Cys treatment (Fig. 6B). During Cys treatment of up to 24 hours (Fig. 6B and C), only two masses, 48,313 Da and 48,432 Da are observed, corresponding to Fab+0Cys (theoretical mass: 48,313) and Fab+1Cys (theoretical mass: 48,432). This demonstrated the integrity of the Fab structure during Cys treatment, and further indicated that Cys treatment is a mild approach to reducing chemical heterogeneity at the engineered and unpaired cysteine of the THIOMAB, while keeping the integrity of inter- and intra-chain disulfide bonds.

Sample treatment with Cys/Ctn redox pair. As mentioned previously, when the THIOMAB was treated with 5 mM Cys at 37°C, GSH was effectively removed at the engineered cysteine in less than two hours, as identified by Fab-LC/MS analysis (Fig. 5). As a reducing agent, Cys treatment may affect the integrity of inter-chain disulfide bonds of mAb, leading to the dissociation of light chain and heavy chain under denaturing conditions. A series of experiments was conducted to evaluate the impact of Cys treatment on the THIOMAB. First, the conventional mAb was treated with 20 mM Cys at 37°C for 24 hours. The CEX-HPLC profiles are identical pre- and post-treatment (data not shown). This suggested that Cys treatment was mild, at least with no obvious impact on CEX-HPLC profile of the conventional mAb. Second, Bioanalyzer analysis of the time course samples indicated that there was no significant impact on inter-chain disulfide bonds induced by Cys treatment. As a control, the THIOMAB was also treated without Cys at 37°C for 24 hours. As displayed in Figure 5, the profile overlay of the size standards and the time course samples shows no appreciable light chain, heavy chain, or half antibody from the THIOMAB treated with 5 mM Cys at 37°C for up to 24 hours, and the time course samples have no significant change in the amount of fragments (>15 kDa) or aggregates, as compared to the control and the untreated THIOMAB.
Figure 5. Overlay of microchip electropherograms of the THIOMAB treated with 5 mM Cys at 37°C for different durations and the untreated THIOMAB.

Figure 6. Deconvoluted mass spectra of the THIOMAB treated with papain and 5 mM Cys at 37°C for different durations. (A) Starting material-intact THIOMAB. (B) 2 hours. (C) 24 hours.
(Fig. 6B and C), as well as by the presence of a significant amount of Fab+0Cys species even after 24 hours of Cys treatment (Fig. 6C). The low rate of cysteinylolation was likely due to the lack of a sufficient level of oxidant in the reaction mixture to increase the redox potential to favor cysteinylolation. To achieve homogenously cysteinylolated species in a short time, sample treatment with a Cys/Ctn redox pair was evaluated.

The ratio and concentration of Cys/Ctn redox pair was optimized and the final reaction condition is described in Section Materials and Methods. Figure 7 shows the ion-exchange profile overlaying the THIOMAB treated with different ratios of Cys:Ctn at 37°C for two hours. At a 1:4 ratio of Cys:Ctn (1 mM cysteine: 4 mM cystine), the various isoforms were efficiently converted to the fully-cysteinylolated species (+2Cys). This is shown as a single main peak in CEX-HPLC profile, similar to that of the conventional mAb (Fig. 8). A small shoulder on the tailing side of the main peak was observed that could be related to a low content of the +1Cys species. Since cysteinylolation involves disulfide formation through a reversible equilibrium (see the reaction scheme below), the reaction may not reach 100% of the +2Cys form with this redox reagent.

\[
\text{THIOMAB-S-S-Cys + Cys-SH} \rightleftharpoons k_1 k_2 \text{THIOMAB-SH + Cys-S-S-Cys}
\]

In imaged cIEF, a method with higher resolution than CEX-HPLC, the half-cysteinylolated (+1Cys) species was resolved as a small peak (~6%) (Fig. 9), and its identity was confirmed by a co-mix experiment. As shown in Figure 9, the profiles of the Cys-treated, Cys/Ctn-treated and a co-mix of the two are overlaid. In imaged cIEF, a method with higher resolution than CEX-HPLC, the half-cysteinylolated (+1Cys) species was resolved as a small peak (~6%) (Fig. 9), and its identity was confirmed by a co-mix experiment. As shown in Figure 9, the profiles of the Cys-treated, Cys/Ctn-treated and a co-mix of the two are overlaid. Based on imaged cIEF peak identification as described previously, the small peak immediately after the highest peak of the Cys/Ctn-treated in imaged cIEF was considered to be the half-cysteinylolated species (+1Cys). As a result, those two peaks were integrated as the main peak in imaged cIEF. Also owing to the high resolution of imaged cIEF, a subtle front-shoulder of the main peak in CEX-HPLC (Fig. 8) was resolved better in imaged cIEF (Fig. 9), contributing partially to the higher acidic peak percentage obtained by imaged cIEF as compared to CEX-HPLC (Table 1). Due to its higher resolution and shorter run time, the imaged cIEF method is the preferred method for analysis of THIOMAB.

Conclusions

Our characterization work indicates that cysteine can be used as a mild reductant to decrease the heterogeneity at the engineered cysteines of monoclonal antibodies without disrupting their inter- and intra-chain disulfide bonds. We further demonstrated that using a cysteine/cystine redox pair (rather than cysteine alone) can not only effectively remove glutathionylation at the engineered cysteines, but also generate homogeneously cysteinylolated species. We have thus developed an effective sample preparation method using a cysteine/cystine redox pair to obtain homogenously cysteinylolated antibodies with unpaired cysteines. By obtaining one main peak in both cation-exchange chromatography and imaged cIEF assays, these methods can be used to...
Figure 8. CEX-HPLC chromatograms of Cys/Ctn-treated (1 mM Cys:4 mM Ctn, 37°C, 2 hours) THIOMAB and untreated conventional mAb.

Figure 9. Identification of half-cysteinylated peak in imaged cIEF electropherogram of THIOMAB treated with Cys/Ctn redox pair. (A) Cys-treated (5 mM Cys, 37°C, 5 hours), (B) a mixture of (A and C) at 1:1 (v/v), (C) Cys/Ctn-treated (1 mM Cys:4 mM Ctn, 37°C, 2 hours).
The mAb to Cys in the solution was approximately 1 to 600–700. For (final concentration) at 37°C for 2 hours. The final molar ratio of treatment, 1–1.25 mg/mL mAb in 20 mM HEPES buffer (pH 8.0). The reagents were mixed thoroughly and incubated in a water bath at 37°C. At various time points, aliquots were withdrawn from the mixture and kept refrigerated for characterization using CEX-HPLC, Bioanalyzer, and imaged cIEF. After 6 hours, 6 mL of the mixture was withdrawn for CEX-HPLC fraction collection. The fractions were concentrated using Ultrafree centrifugal 10k filter (Millipore, Billerica, MA, USA) and analyzed using LC/MS. A second time course study was performed by adding 10 µL of 0.1 mg/mL papain to 250 µL of the above reaction mixture at time zero, followed by incubation at 37°C. At various time points during the papain digestion, aliquots were withdrawn for Fab-LC/MS analysis.

### Materials and Methods

**Materials.** THIOMABs, i.e., recombinant IgG1 monoclonal antibodies with engineered cysteine residues, or the conventional mAb were expressed in CHO cells and purified using protein A and ion-exchange chromatography. L-cysteine (Cys) and L-cystine (Ctn), 4-[(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and sodium chloride (NaCl) were obtained from J.T. Baker (Phillipsburg, NJ, USA). 50% sodium hydroxide (NaOH) and 85% phosphoric acid (H3PO4) were from Mallinckrodt (Phillipsburg, NJ, USA). Tris (2-carboxyethyl) phosphine hydrochloride (TCEP), dithiothreitol (DTT), and trifluoroacetic acid (TFA) were obtained from Pierce Biological (Rockford, IL, USA). Acetonitrile (ACN) and HPLC-grade water were obtained from Burdick & Jackson (Honeywell International Inc., Muskegon, MI, USA). Pharmalyte® were from GE Healthcare (Piscataway, NJ, USA). 1% methylcellulose and pH markers were from Convergent Bioscience (Toronto, ON, Canada). N-glycosidase (PNGase F; P0704L, 500 units/µL) was from New England Biolabs (Ipswich, MA, USA). Carboxypeptidase B (CpB) (DFP treated), and papain were obtained from Roche (Indianapolis, IN, USA). Iodoacetamide (IAM), formic acid (FA), and sodium dodecyl sulfate (SDS) were obtained from Fluka (Sigma-Aldrich, Steinheim, Germany).

**mAb treatment with Cys or Cys/Ctn redox pair.** For Cys treatment, 1–1.25 mg/mL mAb in 20 mM HEPES buffer (pH 8.0, adjusted using 50% NaOH) was incubated with 5 mM Cys (final concentration) at 37°C for 2 hours. The final molar ratio of mAb to Cys in the solution was approximately 1 to 600–700. For Cys/Ctn redox pair treatment, 900 µL of 1.25 mg/mL mAb in 40 mM HEPES buffer (pH 8.0) was mixed with 100 µL 10 mM Cys:40 mM Ctn stock solution (final concentration: Cys:Ctn = 1 mM:4 mM) and then incubated at 37°C for 2 hours. The treated mAb solutions were stored at 2–8°C to stop the reaction prior to analysis. Note the condition was optimized for the specific THIOMAB described in this report; reaction conditions for other proteins with unpaired cysteine residues may require further study depending on the stability of the protein and the accessibility of the unpaired cysteine residues. For convenience, 50 mM Cys and 50 mM Ctn stock solution were prepared, aliquoted and stored at -20°C. Prior to use, 50 mM Cys and 50 mM Ctn were thawed and mixed at a ratio of 1 to 4 (v/v) to achieve 10 mM Cys:40 mM Ctn stock solution. Since Ctn is sparingly soluble in water, a minimal amount of 50% NaOH was added to prepare 50 mM Ctn stock solution. C-terminal lysine residues of each mAb were removed by incubating 11 µL of 1 mg/mL CpB with 900 µL of 1.25 mg/mL mAb at 37°C for 20 minutes. This reaction was allowed to proceed prior to Cys or Cys/Ctn treatment.

**Time course study of Cys treatment.** Eighty microliters of 0.5 M Cys were added to 8 mL of THIOMAB at 1 mg/mL in 20 mM HEPES, pH 8.0. The reagents were mixed thoroughly and incubated in a water bath at 37°C. At various time points, aliquots were withdrawn from the mixture and kept refrigerated for characterization using CEX-HPLC, Bioanalyzer, and imaged cIEF. After 6 hours, 6 mL of the mixture was withdrawn for CEX-HPLC fraction collection. The fractions were concentrated using Ultrafree centrifugal 10k filter (Millipore, Billerica, MA, USA) and analyzed using LC/MS. A second time course study was performed by adding 10 µL of 0.1 mg/mL papain to 250 µL of the above reaction mixture at time zero, followed by incubation at 37°C. At various time points during the papain digestion, aliquots were withdrawn for Fab-LC/MS analysis.

### Table 1. Peak area percentage of Cys/Ctn-treated (1 mM/4 mM) THIOMAB analyzed by CEX-HPLC and imaged cIEF

| No CpB | Acidic | Main | Basic | Acidic | Main | Basic |
|--------|--------|------|-------|--------|------|-------|
| **CEX-HPLC** | | | | | | |
| 21     | 71     | 8    | 22    | 75     | 3    | |
| **Imaged cIEF** | | | | | | |
| 32     | 61     | 7    | 36    | 61     | 2    | |

assess charge-based changes to the engineered antibody product, such as during manufacturing or storage.
Fab-LC/MS. The Fab fragment was generated from the time course study of papain digestion. RP-HPLC was carried out on an Agilent 1100 binary pump LC system equipped with a Poroshell 300SB C18, column, 1 x 75 mm, 5 μm, 300 Å (Agilent Inc., Santa Clara, CA, USA). Five microliters of each sample were injected onto the column equilibrated in 82% buffer A (0.1% formic acid, 0.025% TFA in water) and 18% buffer B (0.1% formic acid, 0.025% TFA in ACN) and held for 10 minutes at 18% buffer B. Sample was eluted by a linear gradient to 50% buffer B over a 16-minute period with detection at 214 nm. The column temperature and flow rate were maintained at 75°C and 0.2 mL/min. The mass spectrometric analysis was carried as described previously, with the exception that the declustering and focusing potentials were set at 45 and 300, respectively.

Microchip electrophoresis on bioanalyzer. Analysis of low molecular weight species was accomplished by CE-SDS analysis using an Agilent 2100 Bioanalyzer (Agilent Inc., Santa Clara, CA, USA). Twenty four microliters of IAM-SDS solution (50 mM IAM, 0.5% SDS) and 2 μL of sample buffer (Agilent Protein 230 Kit) were added to 4 μL of each sample from the time course study followed by incubation at 70°C for 5 minutes. Then, 60 μL of water was added to each sample and the sample was loaded on a protein chip of Agilent 2100 Bioanalyzer (Agilent, Waldbronn, Germany) for analysis. The results were analyzed using Agilent 2100 Expert software.

Imaged cIEF. Imaged cIEF was carried out on an iCE280 Analyzer (Convergent Bioscience, Toronto, Canada) equipped with a PrinCE Microinjector autosampler (Prince Technologies, the Netherlands). The ampholyte solution consisted of a 15%/85%/ (v/v) mixture of Pharmalyte ™ solution pH 3–10 and pH 8–10.5, respectively, with 0.2% (v/v) of each of the pH markers 7.4 and 9.77. The ampholyte solution also contained 0.35% (v/v) methylcellulose (MC). Samples at 1.25 mg/mL were mixed with the ampholyte solution at a 1:4 (v/v) ratio. Separation was carried out on a fluorocarbon-coated fused-silica capillary (5 cm long, 100 μm I.D.) cartridge (Convergent Bioscience, Toronto, Canada). The catholyte was 100 mM NaOH in 0.1% methylcellulose and the anolyte was 80 mM H3PO4 in 0.1% methylcellulose. Samples were introduced from the autosampler (set at 8°C) and transferred to the cartridge for about 150 seconds by pressure. Period I pre-focusing was conducted at 1,500 V for 1 minute followed by period II focusing at 3,000 V for 10 minutes. The focused image at 280 nm was captured by a charge-coupled device (CCD) camera. Data were converted by iCE280 Analyzer and analyzed using EZChrom software version 6.8 (Scientific Software International Inc., Lincolnwood, IL, USA).

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References

1. Reichert JM, Rosenzweig CJ, Faden LB, Dewitz MC. Monoclonal antibody successes in the clinic. Nat Biotechnol 2005; 23:1073-8.

2. Clark M. Antibody humanization: a case of the "Emperor's new clothes"? Immunol Today 2000; 21:397-402.

3. Hsieh FY, Tenggardner E, Li LY, Huang YN, Milton MN, Silverman L, et al. Toxicological Protein Biomarker Analysis—An Investigative One-week Single Dose Intravenous Inflation Toxicity and Toxicokinetic Study in Cynomolgus Monkeys using an Antibody-cytotoxic Conjugate against Ovarian Cancer. Pharm Res 2007; 25:1309-17.

4. Senter PD. Potent antibody drug conjugates for cancer therapy. Curr Opin Chem Biol 2009; 13:1-10.

5. Dotson SO, Toki BE, Torgov MY, Mendelssohn BA, Cerveny CG, Chace DF, et al. Development of potent monoclonal antibody auristatin conjugates for cancer therapy. Nat Biotechnol 2003; 21:778-84.

6. Hamblett KJ, Senter PD, Chace DF, Sun MM, Lenox J, Cerveny CG, et al. Effects of drug loading on the antitumor activity of a monoclonal antibody drug conjugate. Clin Cancer Res 2004; 10:7063-70.

7. Lu SX, Takach EJ, Solomon M, Zhu Q, Law SJ, Hsieh FY. Mass spectral analyses of labile DOTA-NHS and glutathione/glutaredoxin reductase system. Biochem Biophys Res Commun 2004; 321:108-14.

8. Landino LM, Mozyniak KL, Todd JV, Kessler KL, Modulation of the redox state of tubulin by the glutathione/glutaredoxin reductase system. Biochem Biophys Res Commun 2004; 314:555-60.

9. Dormann PB, Borchers T, Koff U, Hojrup P, Roepstorff P, Spener F. Amino acid exchange and covalent modification by cysteine and glutathione explain isoforms of fatty acid-binding protein occurring in bovine liver. J Biol Chem 1993; 268:16286-92.

10. Landino LM, Mozyniak KL, Todd JV, Kessler KL, Modulation of the redox state of tubulin by the glutathione/glutaredoxin reductase system. Biochem Biophys Res Commun 2004; 314:555-60.