

# Mass spectrometric compatibility of Deep Purple and SYPRO Ruby total protein stains for high-throughput proteomics using large-format two-dimensional gel electrophoresis

Christina M. Nock<sup>1†</sup>, Malcolm S. Ball<sup>2†</sup>, Ian R. White<sup>1</sup>, J. Mark Skehel<sup>1</sup>, Louisa Bill<sup>1</sup> and Peter Karuso<sup>2,3\*</sup>

<sup>1</sup>GlaxoSmithKline Pharmaceuticals, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY, UK

<sup>2</sup>FLUOROTECH Pty Ltd., Macquarie University, Sydney NSW 2109, Australia

<sup>3</sup>Department of Chemistry & Biomolecular Sciences, Macquarie University, Sydney NSW 2109, Australia

Received 25 November 2007; Revised 31 January 2008; Accepted 3 February 2008

**In order to identify putative biomarkers from two-dimensional (2D) gel electrophoresis it is necessary to use a visualization technique that is sensitive, has a large dynamic range and does not interfere with the identification of the protein. As mass spectrometry increases in sensitivity more pressure is placed on visualization techniques that facilitate proteomic workflows but do not interfere with downstream processing. Two stains reported to meet these requirements are SYPRO Ruby (Invitrogen) and Deep Purple (GE Healthcare). This study examined the compatibility of these stains with protein identification by selecting spots from replicate 2D gels of human plasma and subjecting these to protein identification using liquid chromatography/tandem mass spectrometry (LC/MS/MS). Using a test of two populations of proportions it was found that proteins were statistically more likely to be identified from gels stained with Deep Purple. Additionally, the identifications from Deep Purple stained gels are of higher quality because they are based on multiple peptides. Copyright © 2008 John Wiley & Sons, Ltd.**

Two-dimensional polyacrylamide gel electrophoresis (2DE) is one of the most widely used techniques in proteomics today. The methodology is well understood, provides highly visual, analog data with equipment available for automated running, staining and analysis of multiple gels. Accordingly, it is well suited to relatively high-throughput analysis of samples and identification of putative biomarkers in research and clinical settings. For example, 2DE has been applied successfully for identification of tumor markers<sup>1</sup> and identification of individuals susceptible to diabetes.<sup>2</sup> The challenge in biomarker discovery is that complex biological samples, such as plasma, may contain proteins in a wide range of concentrations exceeding 10 orders of magnitude<sup>3</sup> and the desired biomarker may be present in low abundance. For this reason protein stains must be sensitive and have sufficient dynamic range to allow determination of concentration changes of potential biomarkers. In practice this can best be achieved with radioisotope or fluorescence techniques, because colorimetric stains, such as Coomassie or silver, are either not sensitive enough for modern proteomics or have too small a dynamic range.<sup>4–6</sup> The safety concerns

with use of radioisotopes have resulted in replacement of this technique with fluorescence in recent years.

While a 2DE gel generates a large amount of high-resolution analog data in a single experiment, proteins with expression differences contributing to spot intensity changes must be identified using techniques such as peptide mass fingerprinting (PMF), peptide mapping (high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS)), Edman sequencing,<sup>7</sup> or immunochemical staining. Mass spectrometric techniques are particularly useful for protein identification from 2DE gels as they lend themselves to automation and high-throughput methodologies.<sup>8,9</sup> The sensitivity of mass spectrometers has enabled proteins to be detected at femtomole to attomole levels from pure samples which means that, in theory, any protein visualized on a 2D gel should be detectable by mass spectrometry.<sup>10</sup> However, in practice, interference from the gel matrix, sample handling and stain interference conspire with protein sequence specific issues to reduce realistic detection limits to the mid-to-high femtomolar level.<sup>11</sup>

The ability of 2D gels to be used as a diagnostic tool therefore relies on three characteristics: (1) that the detection method is sensitive enough to detect proteins present in the sample with sufficient dynamic range to allow quantification of proteins over a broad range of concentrations; (2) that the variability of the detection method is less than any real change in expression levels of the protein of interest; and

\*Correspondence to: P. Karuso, Department of Chemistry & Biomolecular Sciences, Macquarie University, Sydney NSW 2109, Australia.

E-mail: peter.karuso@mq.edu.au

<sup>†</sup>These authors contributed equally to this work.

(3) that the spots of interest can be identified. Silver staining is still one of the most sensitive methods for visualization but because it is not an end-point method, timings are critical and variability is high. In addition, silver staining has been widely reported to interfere with protein identification by mass spectrometry.<sup>11,12</sup> In contrast, fluorescent dyes offer similar sensitivity to silver whilst having a larger linear dynamic range and lower susceptibility to interference with down-stream processes such as mass spectrometry. SYPRO Ruby, a stain based on ruthenium tris-bathophenanthroline,<sup>13</sup> has dominated this field because it is almost as sensitive as silver but has a linear dynamic range of three to four orders of magnitude.<sup>14</sup> However, as mass spectrometric techniques have improved, it has recently been reported to interfere with subsequent analysis by mass spectrometry.<sup>15,16</sup>

Deep Purple is a fluorescent stain based on the natural product epicocconone<sup>17,18</sup> that has a unique reversible binding mechanism.<sup>19</sup> The stain has been shown to be sensitive in the femtomole range for proteins in gel electrophoresis and to exhibit linearity over four orders of magnitude.<sup>20</sup> The reversible nature of the stain suggests that it should be removed under conditions used for tryptic digestion of proteins prior to mass spectrometry by PMF or LC/MS/MS.

In this study we have investigated the reproducibility and compatibility with mass spectrometry of SYPRO Ruby and Deep Purple gel stains using human plasma depleted of the six most abundant proteins resulting in a better understanding of the optimal staining methodology for high-throughput large-format gels with Deep Purple. Furthermore, following *in situ* trypsin digestion, mass spectrometric analysis of a representative selection of protein spots suggests that staining with Deep Purple has advantages over SYPRO Ruby when downstream protein identification is necessary.

## EXPERIMENTAL

### Materials

Boric acid, citric acid, and sodium hydroxide (50% (w/v) solution) were purchased from Sigma Aldrich (St. Louis, MO, USA). Pre-mixed acrylamide solutions and MES buffer were purchased from Invitrogen (Paisley, UK). TEMED and ammonium persulfate were purchased from BioRad (Hemel Hempstead, UK). Human plasma was obtained from healthy human volunteers. Multiple affinity removal system partitioning columns were purchased from Agilent (Stockport, UK). Linear immobilized pH gradient strips (24 cm, pH 4–7) were purchased from GE Healthcare (Amersham, UK).

### Gel electrophoresis

Human EDTA-plasma was treated with Agilent Hu-6 depletion columns to remove albumin, IgG, IgA, transferrin, antitrypsin, and haptoglobin. Total protein (140 µg) was loaded onto 24 cm pH 4–7 IEF strips by rehydration. The strips were focused for 60 000 Vh using Multiphor electrophoresis units (GE Healthcare, Amersham, UK) and stored at –80°C until needed. Strips were reduced and alkylated in sodium dodecyl sulfate (SDS) equilibration buffer as

described by Görg and coworkers<sup>21</sup> and overlaid onto large-format (20 × 24 cm) 11% (w/v) acrylamide gels, poured using the Invitrogen bis-tris buffer system (Paisley, UK). The strips were embedded in 0.5% (w/v) agarose and the gels run on Ettan 12 gel electrophoresis apparatus (GE Healthcare) at 15 mA constant current for approximately 26 h. A total of 48 developed gels were randomized and placed into four linked Dodeca gel-staining units (BioRad) for staining. Half the gels were fixed in 10% (v/v) ethanol, 7% (v/v) acetic acid (10 L; 3 × 60 min) and the other half fixed in 15% (v/v) ethanol, 1% (w/v) citric acid (10 L; overnight).

### Staining

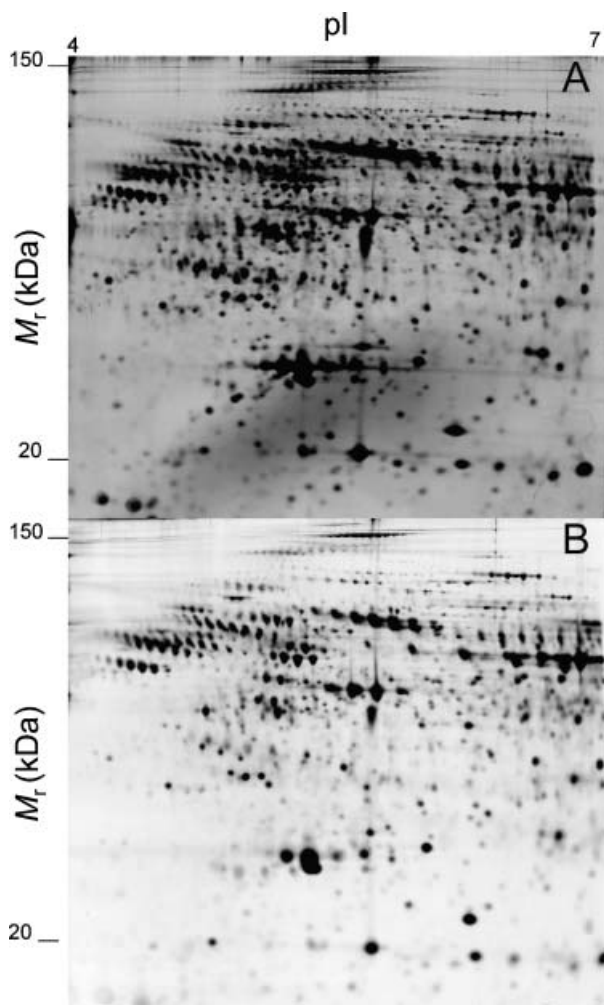
The ethanol/acetic acid fixed gels were stained overnight, 8 gels per unit, in 3 × Dodeca gel stainers using 1 × SYPRO Ruby then de-stained with 10% (v/v) ethanol, 7% (v/v) acetic acid (10 L; 3 × 60 min). Finally, the gels were washed in water (10 min) to remove excess de-staining solution. The gels fixed in ethanol/citric acid were stained similarly in 3 × Dodeca staining units with a 1:200 dilution of Deep Purple dissolved in 100 mM sodium borate (pH 11.0; 90 min.). They were then washed in 15% (v/v) ethanol (60 min) and 15% (v/v) ethanol, 1% (w/v) citric acid (60 min), and finally water (10 min).

Gels were imaged on an FLA-5000 imager (Raytek, Sheffield, UK) using a 473 nm laser in conjunction with a 575 nm Long Pass Green filter for SYPRO Ruby stained gels and a 532 nm laser with the same 575 nm emission filter for gels stained with Deep Purple (Fig. 1). Protein spots were detected using Progenesis software (Nonlinear Dynamics, Newcastle-upon-Tyne, UK) and matched across all gels of both stains.

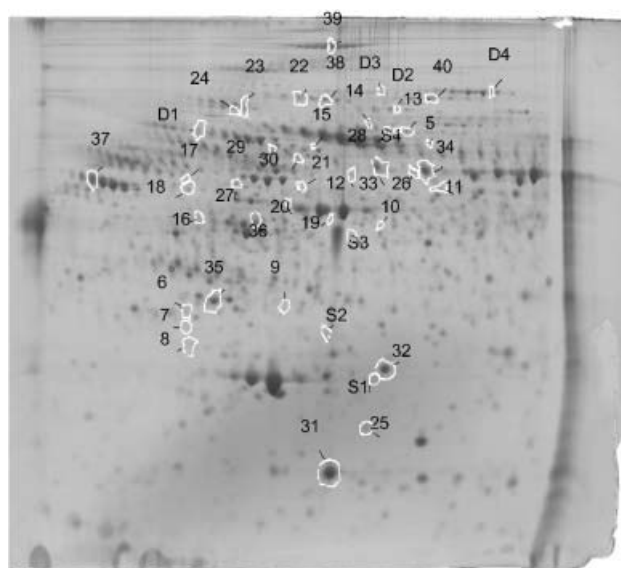
### Mass spectrometric analysis

Spots matched across all 48 gels were ranked in order by their average volume. Spots were assigned to four intensity groups: extra large, large, medium and small spots, each group containing 25% of all spots. From group 1 containing the most intense (extra large) spots, 10 spots were chosen, from group 2 (large spots) 11 spots, from group 3 (medium sized spots) 9 spots and from group 4 (small spots) 6 spots. A fifth group of eight spots were chosen, four of which were uniquely stained with Deep Purple and four with SYPRO Ruby (Fig. 2, D1–4 and S1–4; see Supplementary Material).

Protein spots were excised from gels using a Kcore automated spot cutter (Kbiosystems, Basildon, UK), placed in 96-well plates, de-stained with 50% (v/v) acetonitrile and 50 mM ammonium bicarbonate, reduced with 10 mM dithiothreitol (DTT), and alkylated with 55 mM iodoacetamide. After alkylation, proteins in gel pieces were digested *in situ* with 6 ng/µL trypsin overnight at 37°C on a MassPrep automated liquid-handling system (Waters, Manchester, UK) as previously described.<sup>14</sup> The resulting peptides were extracted in 1% (v/v) formic acid, 2% (v/v) acetonitrile and analyzed by nano-scale capillary LC/MS/MS (Waters nanoAcquity UPLC) to deliver a flow of approximately 300 nL/min. A Waters µ-Pre-column, C18 Symmetry 5 µm, 180 µm × 20 mm (Waters, Manchester, UK) guard column trapped the peptides prior to separation on a C18 BEH130 1.7 µm, 75 µm × 100 mm nanoAcquity UPLC column. Pep-



**Figure 1.** Representative gels stained with either SYPRO Ruby (A) or Deep Purple (B).



**Figure 2.** Average gel map (Progenesis) depicting spots selected for analysis by LC/MS/MS. S1–S4 proteins found only with SYPRO Ruby, D1–D4 proteins found only with Deep Purple; 5–40 are the 36 spots that are spread across the four intensity classes; small, medium, large and extra large.

tides were flushed from the guard column onto the analytical column at 300 nL/min and eluted with a gradient of acetonitrile. The column outlet was directly coupled to the manufacturer's standard emitter. Mass spectrometric information was obtained using an orthogonal acceleration quadrupole time-of-flight (TOF) mass spectrometer in data-dependent acquisition mode (Q-ToF ULTIMA, Waters, Manchester, UK), equipped with a Z-spray source for nanoflow analysis. Data-dependent analysis was carried out where automatic MS/MS data were acquired on the eight most intense, multiply charged precursor ions in the  $m/z$  range 400–1500. MS/MS data were acquired over the  $m/z$  range 50–1975. LC/MS/MS data were then searched against a non-redundant protein database using the Mascot search engine (Matrix Science, London, UK).<sup>22</sup> All LC/MS/MS data were searched against an in-house non-redundant protein database. Taxonomy was left open to all species, trypsin was specified as the enzyme and up to one missed cleavage was allowed. Variable modifications allowed were: carbamidomethylation (C), deamidation (NQ), Phospho (S/T), Phospho (Y) and propionamide (C). The peptide precursor tolerance was  $\pm 0.25$  Da and the MS/MS fragment ion tolerance set to  $\pm 0.1$  Da. Individual fragment ion spectra were manually assessed for quality and extent of  $y$  or  $b$  ion series. Characteristic features, associated with the presence of a proline, in the spectra were taken into account.

### Statistical analysis

The number of proteins identified by LC/MS/MS for both stains was determined as a percentage of the total spots tested, and the success rates compared using a test of two populations of proportions for differences. The process was repeated for each of the spot groups 1–4. Group 5 was not tested because they were composed of unique spots to each stain. The test was repeated using only those proteins identified with two peptides or more. Groups 3–4 did not contain enough data for statistical analysis (see Supplementary Material).

## RESULTS

### Gel staining

In general, both stains produced high-quality images that were consistent across all 24 gels in each stain group (Fig. 1). Spots were selected for *in situ* trypsin digestion and mass spectrometric analysis representing a broad pI/size and intensity range. SYPRO Ruby stained gels were fixed and de-stained in 10% ethanol/7% acetic acid rather than 50% methanol/7% acetic acid as recommended in the Invitrogen protocol. This was done for two reasons; firstly the large volumes of buffers used for the study meant that the amounts of methanol required were considered hazardous; and secondly our previous work has shown that the method used improved image quality by reducing speckling. It is possible that the reduced alcohol concentration in the fix may have negatively impacted on protein identification; however, this is unlikely as it has been reported that the fixation system used is fully compatible with protein identification by mass spectrometry.<sup>13</sup>

**Table 1.** Summary of the analysis of gel spots by LC/MS/MS when stained with Deep Purple (DP) or SYPRO Ruby (SR)

MS results	Group 5		Group 4		Group 3		Group 2		Group 1		Total for all spots	
	DP	SR	DP	SR	DP	SR	DP	SR	DP	SR	DP	SR
No. Spots identified	4/4	1/4	3/6	2/6	6/8	5/8	11/12	3/12	10/10	4/10	33/40	15/40
% spots with proteins identified	100.0	25.0	50.0	33.0	75.0	62.5	91.7	25.0	100.0	40.0	82.5	37.5
Number of spots with multiple proteins identified	3	0	0	1	1	0	4	0	4	1	12	2
Total # of proteins identified	8	1	3	4	7	5	15	3	16	7	49	20
No. proteins > 1 peptide (%)	2 (50)	1 (25)	0 (0)	1 (17)	6 (63)	1 (13)	10 (58)	3 (25)	12 (100)	4 (30)	30 (68)	10 (23)
Average Mowse score	69.3	223*	80.7	116.7	111.0	77.6	160.7	63.3	230.5	96.0	130.4	88.4
Statistical significance	Not Tested		$p > 0.05$		$p > 0.05$		$p < 0.01$		$p < 0.01$		$p < 0.0001$	

\*Only one protein was identified in SYPRO group 5 and was assigned from multiple peptides.

## Protein identification

Protein identifications are summarized in Table 1. Overall more proteins were identified from gels stained with Deep Purple as compared to SYPRO Ruby (49 proteins were identified from 33 of the spots using Deep Purple compared to 20 proteins from 15 spots using SYPRO Ruby). Using a two populations of proportions test this difference was found to be significant with a confidence level of  $p < 0.0001$ . Surprisingly, even in the darkest stained proteins (group 1), less than half the proteins from the SYPRO Ruby stained gels were positively identified while all of the proteins stained with Deep Purple in this group were identified (significance of  $p < 0.01$  in a test of proportions). Similarly, Deep Purple identified significantly more proteins in groups 2 ( $p \leq 0.01$ ), 3 and 4, though the small number of matches in groups 3 and 4 precluded statistical analysis ( $n < 5$ ). Of the proteins identified, Deep Purple also gave a better average MOWSE score across all gels (130 vs. 88 for SYPRO).

Current HUPO reporting requirements for protein identification suggest more than one peptide should be identified or additional *de novo* data reported to substantiate the identification.<sup>23</sup> As these processes (e.g. Western blotting or MS sequencing) are labor intensive compared to automated LC/MS/MS, it is worth noting that protein identifications from spots stained with Deep Purple were more often identified with more than one peptide (30/49 for Deep Purple compared to 10/20 for SYPRO Ruby). Therefore, proteins were not only more likely to be identified using Deep Purple as a stain, they were also less likely to require additional sequence confirmation in studies requiring positive identifications.

## DISCUSSION

### Gel staining

SYPRO Ruby and Deep Purple are the two oldest and best-accepted post-electrophoresis fluorescent proteomics stains on the market. Both stains produced high-quality images suitable for image analysis and spot selection for protein identification. Despite some qualitative differences in staining patterns and intensity, both stains were found to be suitable for high-throughput analysis using the Dodeca staining units. Deep Purple does however exhibit some

protocol advantages over SYPRO Ruby when high-throughput analysis is considered. Firstly, SYPRO Ruby is supplied as a 1× solution. While this is convenient for researchers staining low numbers of gels at a time, and removes any potential errors caused by diluting a concentrate, storage space for 80 L of stain per 100 large-format gels is required for SYPRO Ruby. Deep Purple, being supplied as a 200× concentrate, required storage for only 0.4 L. Secondly, SYPRO Ruby staining typically uses high concentrations of methanol and acetic acid that must be disposed of as hazardous waste (MSDS information); however, Deep Purple is non-toxic and readily biodegradable<sup>24</sup> and the ethanol and citric acid concentrations used for staining are able to be disposed of without special requirements (MSDS information). Finally, the methanol and acetic acid used in SYPRO Ruby fixing solutions produce fumes when large volumes are required for continuous running of multiple Dodeca staining units. The protocol used at GSK replaces the high percentage of methanol with lower percentages of ethanol to minimize any health risks.

### Protein identification

Being able to produce a sharp image with a large number of spots on a 2D gel is a necessary prerequisite for biomarker discovery. However, protein identification is heavily reliant on mass spectrometry, which can be viewed as the last but critical step in the long trail of sample preparation, separation, visualization and identification. Moreover, mass spectrometry in proteomics requires significant expert input if quality data is to be obtained. For these reasons, the highest possible yield is required for this final phase.<sup>25</sup> In particular, the time involved in LC/MS/MS techniques makes identification of all of the proteins present in a sample as complex as plasma extremely daunting. This study was designed to directly compare the MS compatibility of the two stains, and as such the majority of the spots selected for analysis were taken from the region of the gels where both stains performed well (Fig. 2). This was done to limit the influence of gel staining variability on the results. Selecting spots in this way resulted in the majority of proteins being selected from the mid-range regions (both Mr and pI) of the gel, as differences in staining efficiency were most noticeable at the extreme corners of the gels.

As it has been previously reported that SYPRO Ruby staining can impact negatively on protein identification,<sup>15,16</sup> we decided to examine the relationship between protein characteristics such as size, pI, and concentration and successful protein identification. Thus protein spots, widely distributed throughout the gels, were selected for analysis and included isoforms from post-translationally modified protein trains as well as individual, well-resolved spots (Fig. 2). Overall it was found that protein spots stained with Deep Purple were more than twice as likely to be positively identified than proteins from gels stained with SYPRO Ruby (Table 1). This trend was reflected in all the groups, though only groups 1 and 2 were found to be statistically significant ( $p < 0.01$ ). Of the identical spots selected for comparison (groups 1–4), Deep Purple stained spots were identified over a broad molecular weight and charge range. Whilst some proteins were identified exclusively in spots stained with either Deep Purple or SYPRO Ruby, only two proteins (in one spot) were identified in the SYPRO Ruby stained gels that was not identified by Deep Purple. In contrast, 29 proteins, originating from 17 spots, were identified in the Deep Purple stained gels only. Whilst it is generally accepted that SYPRO Ruby staining has better MS compatibility than silver,<sup>14,26</sup> reports differ when compared to other stains, with success rates of 50–75%. These estimates<sup>15,16,27–29</sup> are almost exclusively based on matrix-assisted laser desorption/ionization (MALDI)-TOF PMF and often on small sample sizes that may not be statistically significant. The generally poorer performance of SYPRO Ruby in this study compared to published data may be based on the analytical method (LC/MS/MS), staining method (optimized for image quality), or that human plasma was used as a sample. Regardless of success rates reported in the literature, it is commonly reported that sequence coverage and peptide recoveries are reduced with SYPRO Ruby compared to other stains.<sup>13,15,16,27,30</sup> It has been suggested that the reason SYPRO Ruby interferes with protein identification by MALDI-TOF PMF is due to a chemical modification of cysteine-containing peptides.<sup>15</sup> Due to the generally low number of peptides identified in high-throughput LC/MS/MS techniques it is difficult to draw conclusions on the few peptides positively identified. There was no discernible correlation with molecular weight for example. However, all of the proteins identified from Deep Purple gels that were not identified from SYPRO Ruby gels originated in the most basic half of the gel and exhibited staining patterns suggestive of significant post-translational modification. A comparison of the compositions of proteins that were identified using Deep Purple that were not identified using SYPRO Ruby indicated no strong trends, though proteins unsuccessfully identified from gels stained with SYPRO Ruby tended to have higher amounts of serine and threonine, two amino acids associated with O-linked glycosylation. In contrast, the proteins that were identified by SYPRO Ruby were either apparently unmodified or lightly post-translationally modified. In general proteins identified from Deep Purple stained gels had a higher number of peptides recovered (see Table S-1, Supplementary Material). In the case of complement factor H (spot 39), 17 peptides were recovered from the Deep Purple

stained spot, and no identification was made from the SYPRO Ruby stained spot. Theoretical digestion of this protein (Table S-4, Supplementary Material) indicated that 75% of the peptides (allowing for one missed cleavage) would contain at least one cysteine residue. None of the spots from isoform trains were identified correctly from SYPRO Ruby stained gels even though the majority of these spots were in the top 50% of protein intensity (groups 1 and 2). In contrast, Deep Purple identified all of these proteins.

In general, protein spot intensity did not seem to be related to identification efficiency. In fact, the difference in the two stains was most marked in the most abundant proteins (groups 1 and 2) where Deep Purple stained gels allowed the identification of proteins from 20/21 excised spots compared to only 7/21 for SYPRO Ruby (Table 1). Neither stain performed particularly well with the least intense spots (group 4) suggesting that the amounts of protein in the gel were close to the limit of detection of the LC/MS/MS method used.

New reporting standards for proteomic analysis of human samples<sup>31</sup> require two or more peptides for an identification to be considered valid. Following implementation of these standards Deep Purple stained gels yielded proteins from over 75% of the excised spots compared to 25% for SYPRO Ruby stained gels suggesting that SYPRO Ruby does impact peptide identification by LC/MS/MS.

Similarly, the MASCOT Mowse algorithm score, a quantitative indication of the confidence of each protein identification, was better overall for Deep Purple (Table 1) and markedly better for groups 1–3. In the faintest spots (group 4) SYPRO Ruby had a better score although this result could not be tested for statistical significance.

Of the spots unique to each stain (group 5), Deep Purple was able to identify proteins within all of the excised spots, albeit not always with two or more peptides. SYPRO Ruby was able to identify only one protein from this group, apolipoprotein AIV, assigning four peptides resulting in a high Mowse score. This protein is not glycosylated and notably contains no cysteine residues.

## CONCLUSIONS

SYPRO Ruby and Deep Purple are two fluorescent stains that have been shown to be easy to use and meet the image quality requirements of biomarker discovery in terms of sensitivity. This study, focusing on the mass spectrometric compatibility of two post-electrophoresis stains, has shown that protein spots excised from gels stained with Deep Purple are more likely to result in protein identification by LC/MS/MS than those taken from gels stained with SYPRO Ruby. Furthermore, identifications originating from gels stained with Deep Purple are also more likely to be of higher quality, resulting from assignment of multiple peptides. These results, coupled with significant environmental and safety benefits associated with the use of Deep Purple, have implications in the efficiency and cost of large-scale, high-throughput proteomics for biomarker discovery.

## SUPPLEMENTARY MATERIAL

The supplementary electronic material for this paper is available in Wiley InterScience at: <http://www.interscience.wiley.com/jpages/0951-4198/suppmat/>.

## Acknowledgements

The authors are indebted to Dr Neil Hopkins (University of Western Sydney) for assistance with the statistical analyses. All experimental work was conducted at GlaxoSmithKline, Stevenage, UK. Statistical analysis of the data and image analyses were conducted by FLUORotechnics. Professor Karuso and Dr Ball are employees of FLUORotechnics and as such have a commercial interest in Deep Purple.

## REFERENCES

- Verma M, Srivastava S. *Recent Results Cancer Res.* 2003; **163**: 72.
- Herber S, Grus FH, Sabuncuo P, Augustin AJ. *Electrophoresis* 2001; **22**: 1838.
- Anderson NL, Anderson NG. *Mol. Cell. Proteomics* 2002; **1**: 845.
- Jin LT, Hwang SY, Yoo GS, Choi JK. *Electrophoresis* 2004; **25**: 2494.
- Jin LT, Hwang SY, Yoo GS, Choi JK. *Proteomics* 2006; **6**: 2334.
- Switzer RC, 3rd, Merril CR, Shifrin S. *Anal. Biochem.* 1979; **98**: 231.
- Chen WQ, Kang SU, Lubec G. *Nature Protocols* 2006; **1**: 1446.
- Li X, Gong Y, Wang Y, Wu S, Cai Y, He P, Lu Z, Ying W, Zhang Y, Jiao L, He H, Zhang Z, He F, Zhao X, Qian X. *Proteomics* 2005; **5**: 3423.
- Sommerer N, Centeno D, Rossignol M. *Methods Mol. Biol.* 2007; **355**: 219.
- Wei H, Nolkranz K, Powell DH, Woods JH, Ko MC, Kennedy RT. *Rapid Commun. Mass Spectrom.* 2004; **18**: 1193.
- Patton WF. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2002; **771**: 3.
- Mortz E, Krogh TN, Vorum H, Gorg A. *Proteomics* 2001; **1**: 1359.
- Berggren KN, Schulenberg B, Lopez MF, Steinberg TH, Bogdanova A, Smejkal G, Wang A, Patton WF. *Proteomics* 2002; **2**: 486.
- White IR, Pickford R, Wood J, Skehel JM, Gangadharan B, Cutler P. *Electrophoresis* 2004; **25**: 3048.
- Lanne B, Panfilov O. *J. Proteome Res.* 2005; **4**: 175.
- Tannu NS, Sanchez-Brambila G, Kirby P, Andacht TM. *Electrophoresis* 2006; **27**: 3136.
- Bell PJ, Karuso P. *J. Am. Chem. Soc.* 2003; **125**: 9304.
- Mackintosh JA, Veal DA, Karuso P. *Proteomics* 2005; **5**: 4673.
- Coghlan DR, Mackintosh JA, Karuso P. *Org. Lett.* 2005; **7**: 2401.
- Mackintosh JA, Choi HY, Bae SH, Veal DA, Bell PJ, Ferrari BC, Van Dyk DD, Verrills NM, Paik YK, Karuso P. *Proteomics* 2003; **3**: 2273.
- Gorg A, Weiss W, Dunn MJ. *Proteomics* 2004; **4**: 3665.
- Available: [www.matrixscience.com](http://www.matrixscience.com).
- Taylor GK, Goodlett DR. *Rapid Commun. Mass Spectrom.* 2005; **19**: 3420.
- Choi HY, Veal DA, Karuso P. *J. Fluorescence* 2006; **16**: 475.
- Haebel S, Albrecht T, Sparbier K, Walden P, Korner R, Steup M. *Electrophoresis* 1998; **19**: 679.
- Lauber WM, Carroll JA, Dufield DR, Kiesel JR, Radabaugh MR, Malone JP. *Electrophoresis* 2001; **22**: 906.
- Ball MS, Karuso P. *J. Proteome Res.* 2007; **6**: 4313.
- Berggren K, Steinberg TH, Lauber WM, Carroll JA, Lopez MF, Chernokalskaya E, Zieske L, Diwu Z, Haugland RP, Patton WF. *Anal. Biochem.* 1999; **276**: 129.
- Chevalier F, Rofidal V, Vanova P, Bergoin A, Rossignol M. *Phytochemistry* 2004; **65**: 1499.
- Chevalier F, Centeno D, Rofidal V, Tauzin M, Martin O, Sommerer N, Rossignol M. *J. Proteome Res.* 2006; **5**: 512.
- Taylor CF. *Proteomics* 2006; **6**(Suppl 2): 39.