

Technical Aspects of a European Multicentre SELDI Study in Pharmaceutical Toxicology



Ben C. Collins^{1,2}, Alexandra Walijew³, Sonja Vorderwülbecke⁴, Phillip Hewitt³, Jean-Charles Gautier⁵, Stephen R. Pennington² and William M. Gallagher¹.

UCD School of Biomolecular and Biomedical Science¹ and Proteome Research Centre², UCD Conway Institute, University College Dublin, Ireland; Institute for Toxicology³, Merck KGaA, Darmstadt, Germany; Bio-Rad Laboratories GmbH⁴, Munich, Germany; Drug Safety Evaluation⁵, Sanofi Aventis, Alfortville, France.



Introduction

Toxicity and safety issues remain a significant problem for drug development efforts by pharmaceutical and biotechnology companies. Specifically, current early biomarkers of toxicity are insufficient and this is demonstrated by the high failure rate of candidate therapeutics due to toxicity problems in both preclinical and clinical stages of development. PredTox (Predictive Toxicology) is a collaborative project partly funded by the EU involving a consortium of 15 industrial (13 large pharma, 1 technology provider and 1 SME) and 3 academic partners. The primary aim of this consortium is to assess the value of combining data generated from 'omics technologies (proteomics, transcriptomics, metabolomics) with the results from more conventional toxicology methods to facilitate more informed decision making in preclinical safety evaluation (see figure 1). SELDI-TOF-MS (surface enhanced laser desorption/ionisation – time of flight – mass spectrometry) is a widely used technique in biomarker discovery and forms one component of the proteomic strategy for this collaborative programme (2D-PAGE and 2D-DIGE studies are also included). Plasma and tissue samples from male Wistar rats, treated with both proprietary and reference hepato- and nephrotoxicants at varying dose levels and timepoints, were divided across three sites for SELDI analysis. Here, we present findings regarding technical issues arising from multi-site SELDI experiments including harmonisation of protocols, albumin/IgG depletion of plasma samples, and use of a cross-site reference sample amongst other factors. These preliminary experiments indicate that the SELDI proteomics platform, when used with suitable attention to the factors that influence reproducibility^{1,2}, can provide a robust and high-throughput technique for biomarker discovery in the context of pharmaceutical toxicology³.

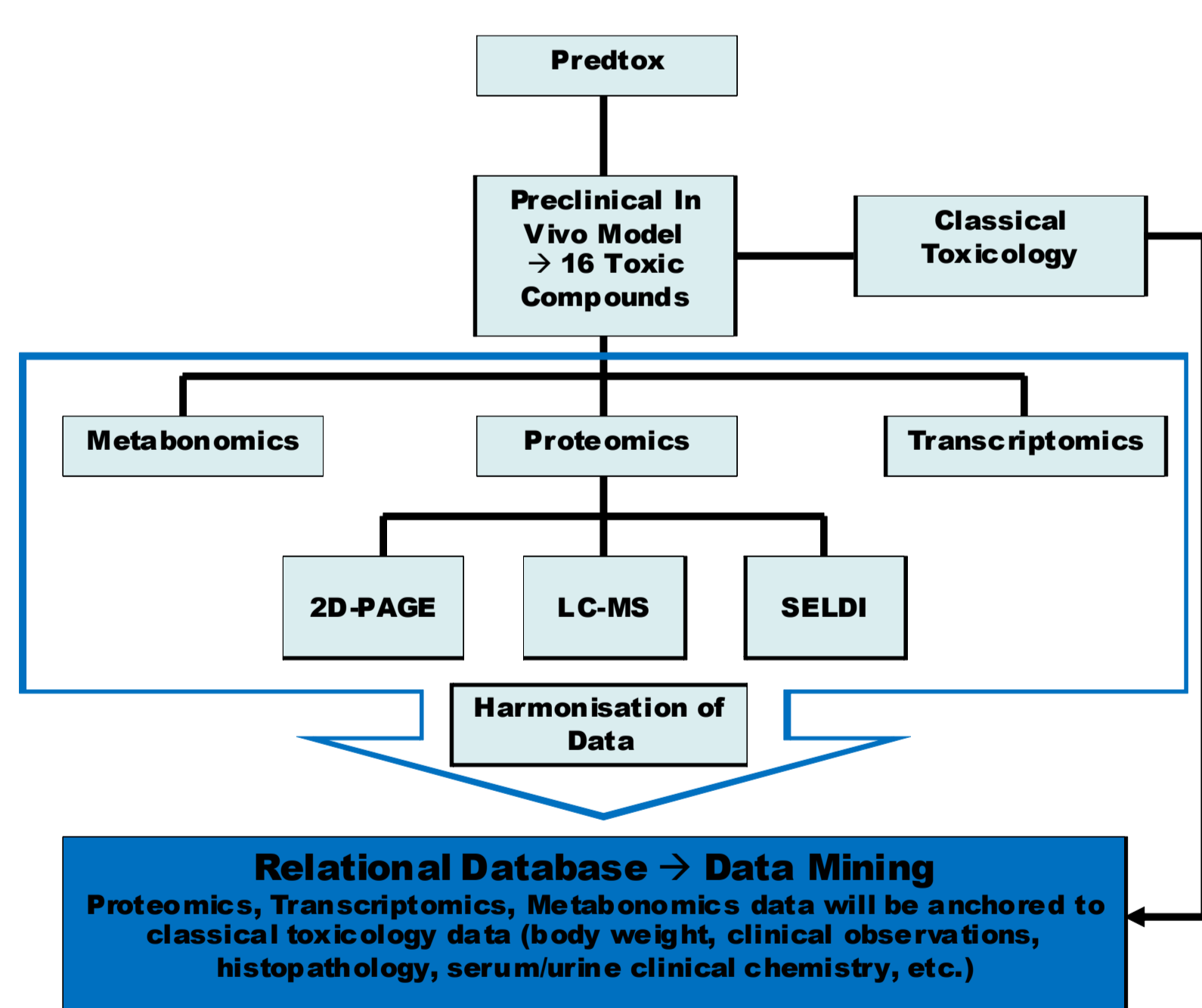


Figure 1 - Schematic diagram of the PredTox consortium. 'Omics-based molecular profiling is combined with classical toxicology endpoints to elucidate biomarker signatures of specific mechanisms of toxicity.

The FP6 PredTox Consortium

(www.innomed-predtox.com)

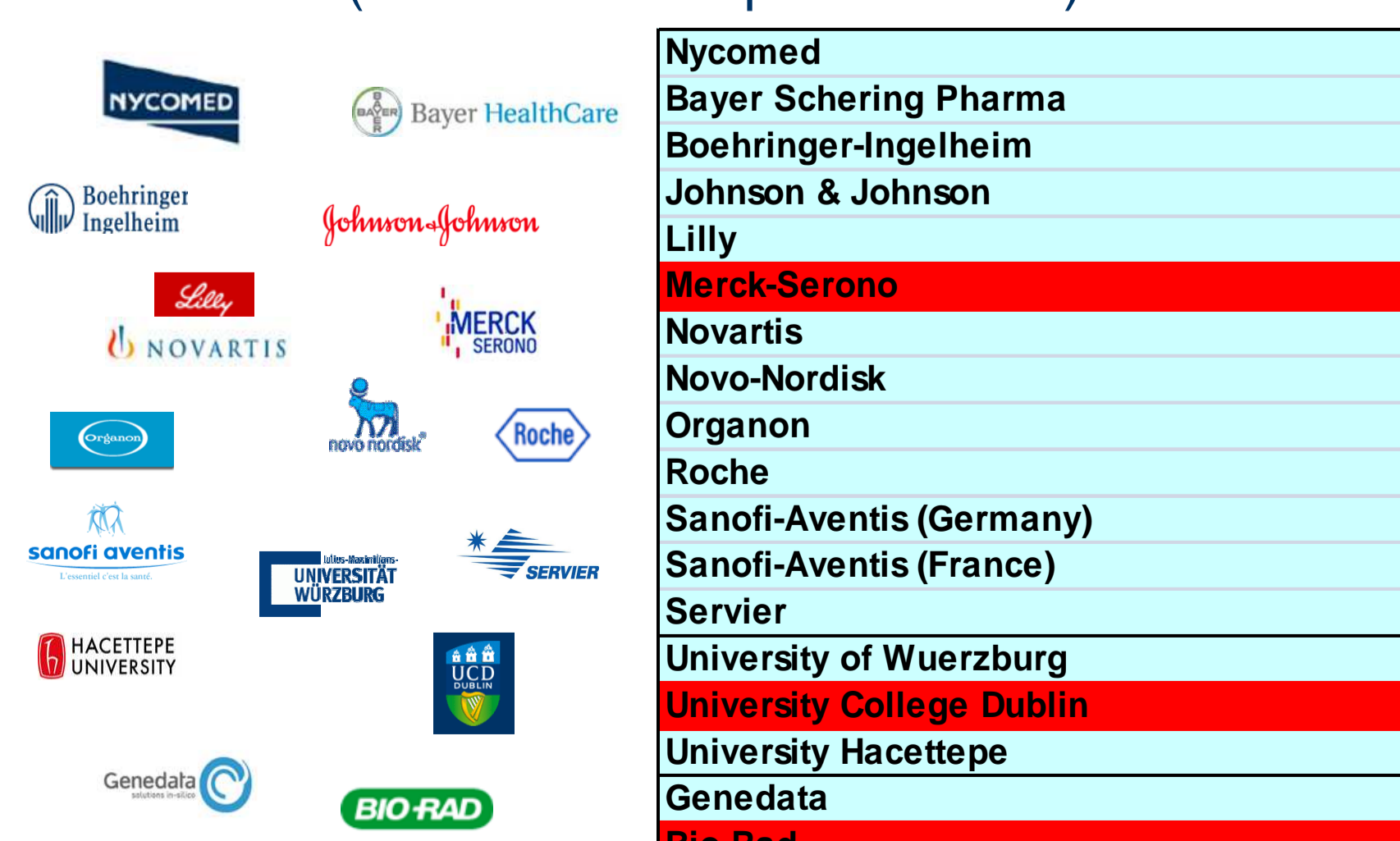


Figure 2 – The PredTox consortium partners. The participants in the SELDI component of the study are highlighted in red.

Primary Technical Considerations

1. Depletion of plasma

- EDTA plasma was depleted of albumin and IgG using Proteoextract™ (Calbiochem) single use gravity flow columns.
- Depleted plasma was concentrated by acetone precipitation with re-suspension in a denaturing buffer (9M urea, 2% CHAPS, 50 mM Tris-HCl pH 9.0).

2. Reference sample

- A reference sample consisting of a commercial rat EDTA plasma sample (Taconic) was distributed to the 3 sites.
- This sample was included on every array in the study to serve as measure of variation within and between participating sites

3. Randomisation

- Spectra were acquired using 4 technical replicates per sample. 2 batches per study compound were required to accommodate all of the samples. Technical replicates were divided equally across the 2 batches and randomised to average any batch bias.

4. Chromatographic surfaces

- Surface conditions were selected to maximise the number of detectable features (data not shown)
- CM10 pH 4.0 (weak cation exchange)
- Q10 pH 9.0 (strong anion exchange)

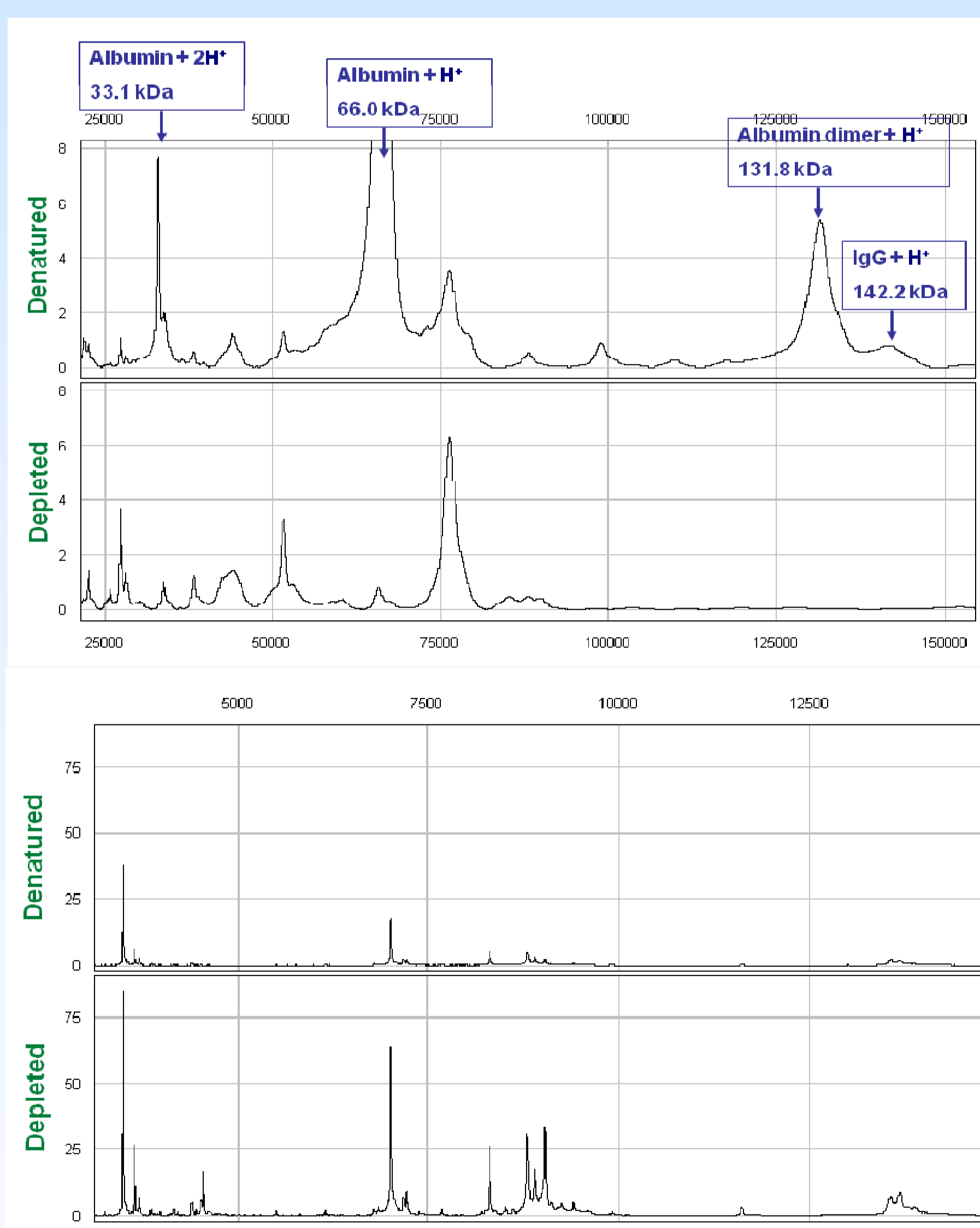


Figure 3 – SELDI spectra (CM10 pH 4.0 surface) from equal protein loadings of denatured plasma and albumin/IgG depleted plasma using high mass (upper panel) and low mass (lower panel) acquisition protocols

	Signal/Noise	Minimum Peak Threshold	Second Pass S/N	Add Estimated Peaks	No. of Peaks Detected	Percentage Increase in Peaks Detected
Denatured Plasma	10	80	-	N	14	-
	5	50	3	Y	22	-
	3.5	50	3	Y	33	-
	3.5	20	3	Y	46	-
Depleted Plasma	10	80	-	N	25	+ 79 %
	5	50	3	Y	40	+ 82 %
	3.5	50	3	Y	51	+ 56 %
	3.5	20	3	Y	69	+ 50 %

Table 1 – The number of peaks detected at differing levels of stringency was compared for denatured plasma versus albumin/IgG depleted plasma

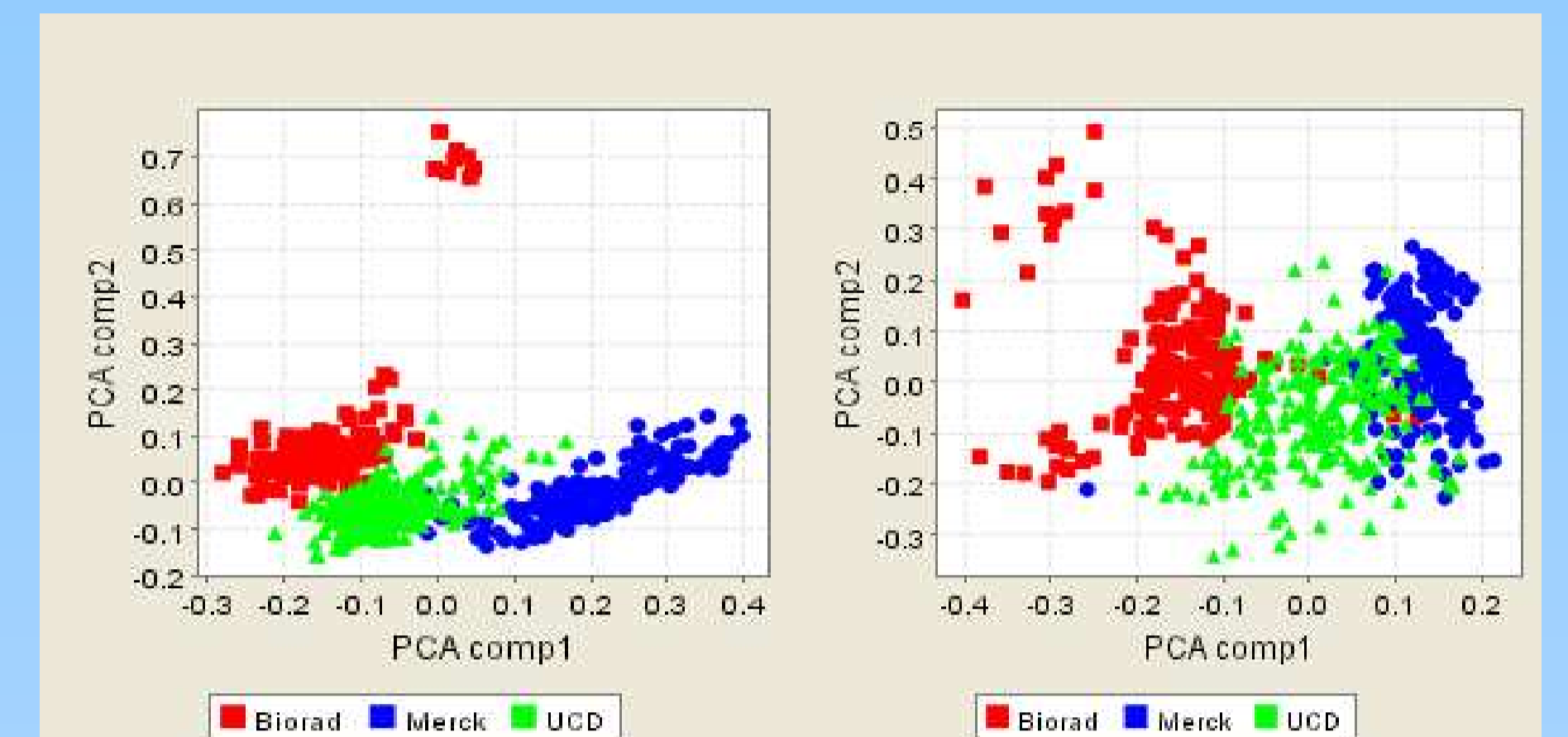


Figure 4 – Principal component analysis of reference spectra generated at all three sites – CM10 low mass (left) and Q10 low mass (right)

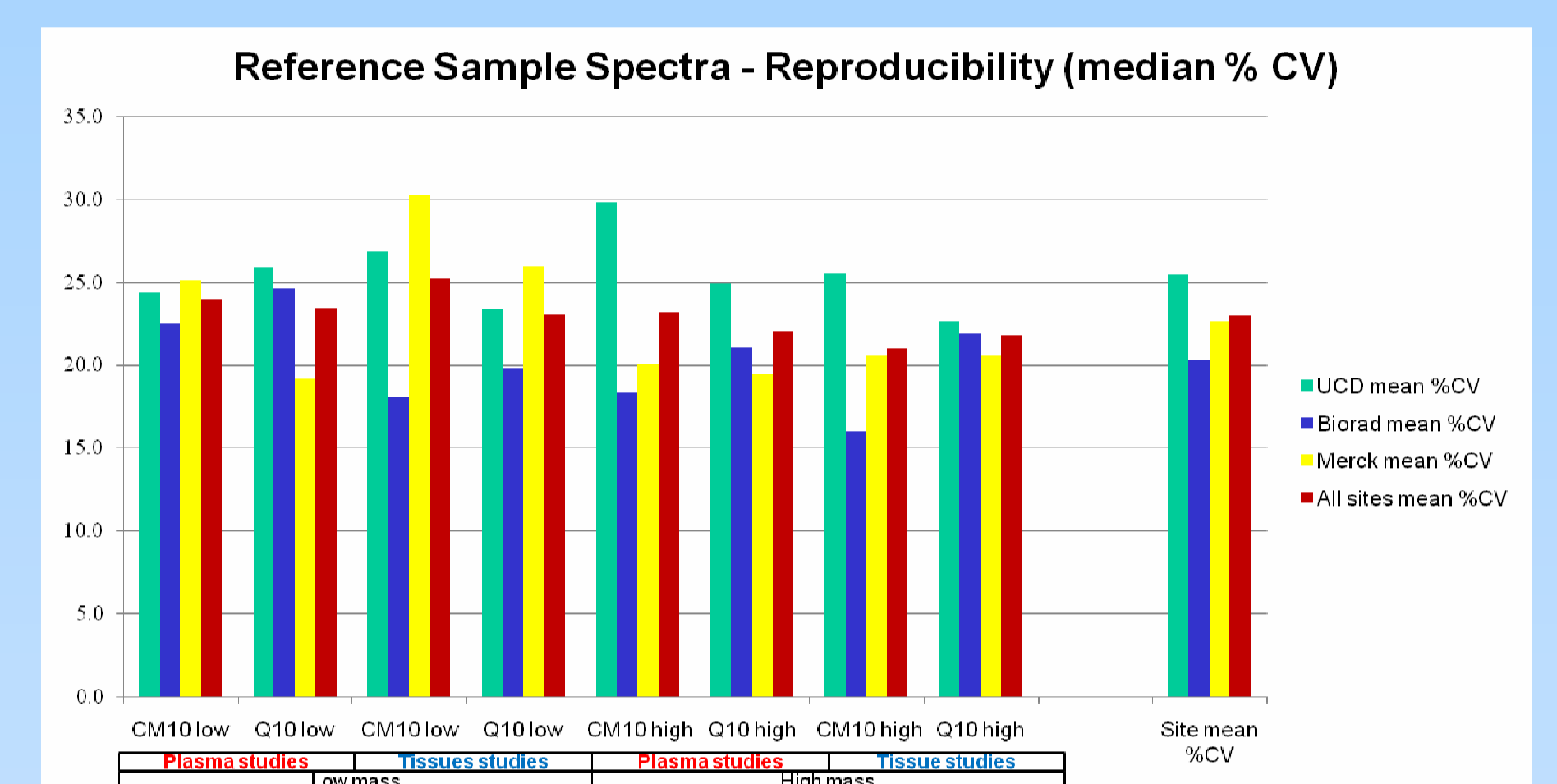


Figure 5 – Reproducibility of reference spectra as measured by median % CV

Results

Depletion of plasma

- Depletion of albumin and IgG from plasma samples resulted in an increase in feature detection of 50%-79% (depending on peak detection stringency) – see table 1
- No significant increase in the reproducibility of peak intensity was apparent across 4 technical replicates

Condition	Peak Intensity % CV (median)
Denatured Plasma	15.3
Depleted Plasma	15.9

Reproducibility and Inter-site Variation

- Per study reproducibility was measured by median % coefficient of variation (% CV). The global average % CV for these studies was determined to be 23.0 % (see figure 5).
- Some inter-site variation was observed as determined by principal component analysis (see figure 4).

References

1. Banks RE, Stanley AJ, Cairns DA et al.: Influences of blood sample processing on low-molecular-weight proteome identified by surface-enhanced laser desorption/ionization mass spectrometry. *Clin Chem* (2005) 51(9):1637-1649.
2. Rai AJ, Stemmer PM, Zhang Z et al.: Analysis of Human Proteome Organization Plasma Proteome Project (HUPO PPP) reference specimens using surface enhanced laser desorption/ionization-time of flight (SELDI-TOF) mass spectrometry: multi-institution correlation of spectra and identification of biomarkers. *Proteomics* (2005) 5(13):3467-3474.
3. Collins BC, Clarke A, Kitteringham NR, Gallagher WG, Pennington SR.: Use of Proteomics for the Discovery of Early Markers of Drug Toxicity. *Expert Opin Drug Metab Toxicol* (2007) in press.

Acknowledgements

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