SYSTEMS BIOLOGY CENTRE WITH PLYMOUTH UNIVERSITY

DISCOVER WITH PLYMOUTH UNIVERSITY

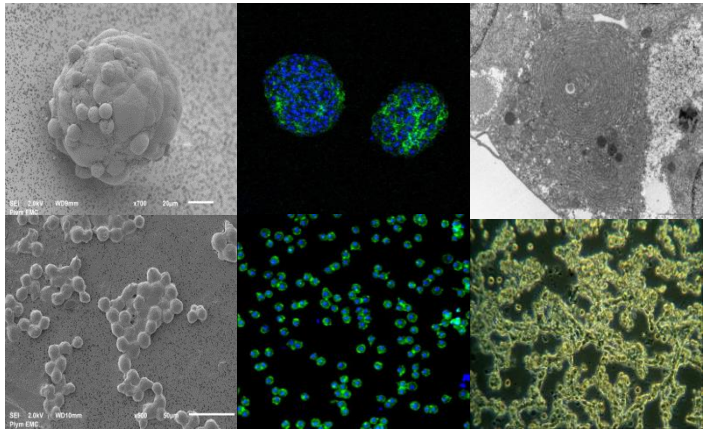
Applications of Proteomics in Fish Liver Studies

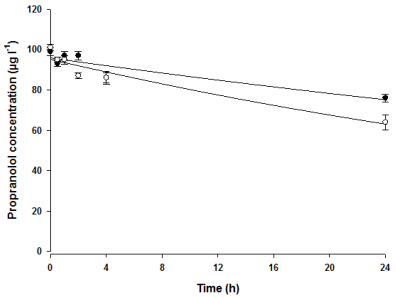
Vikram Sharma, PhD
Systems Biology Centre, Plymouth University

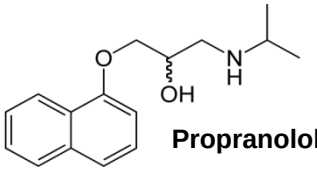
AstraZeneca

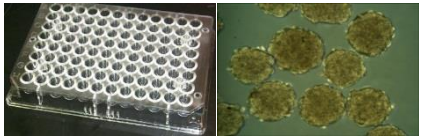
NERC SCIENCE OF THE ENVIRONMENT

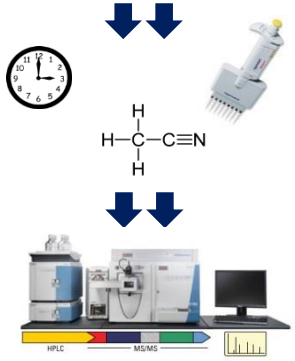
BBSRC bioscience for the future





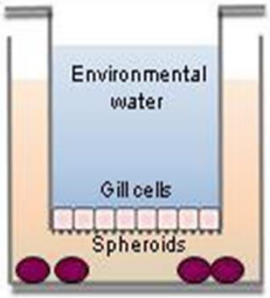


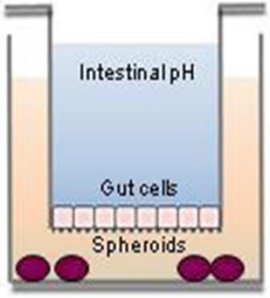




Metabolism

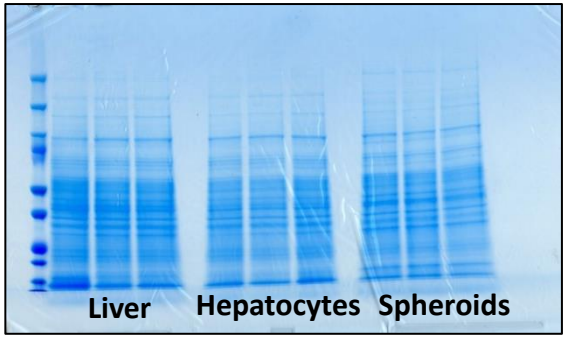
Physiology

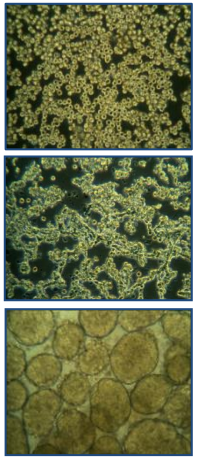


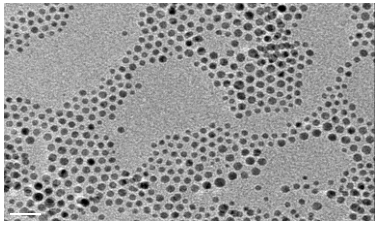


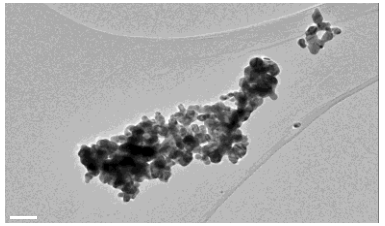
Proteomics

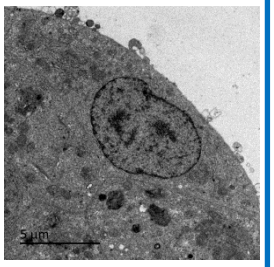
Toxicology

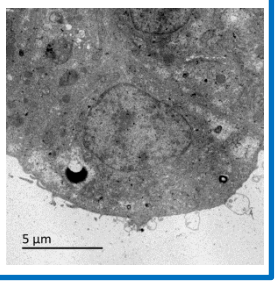




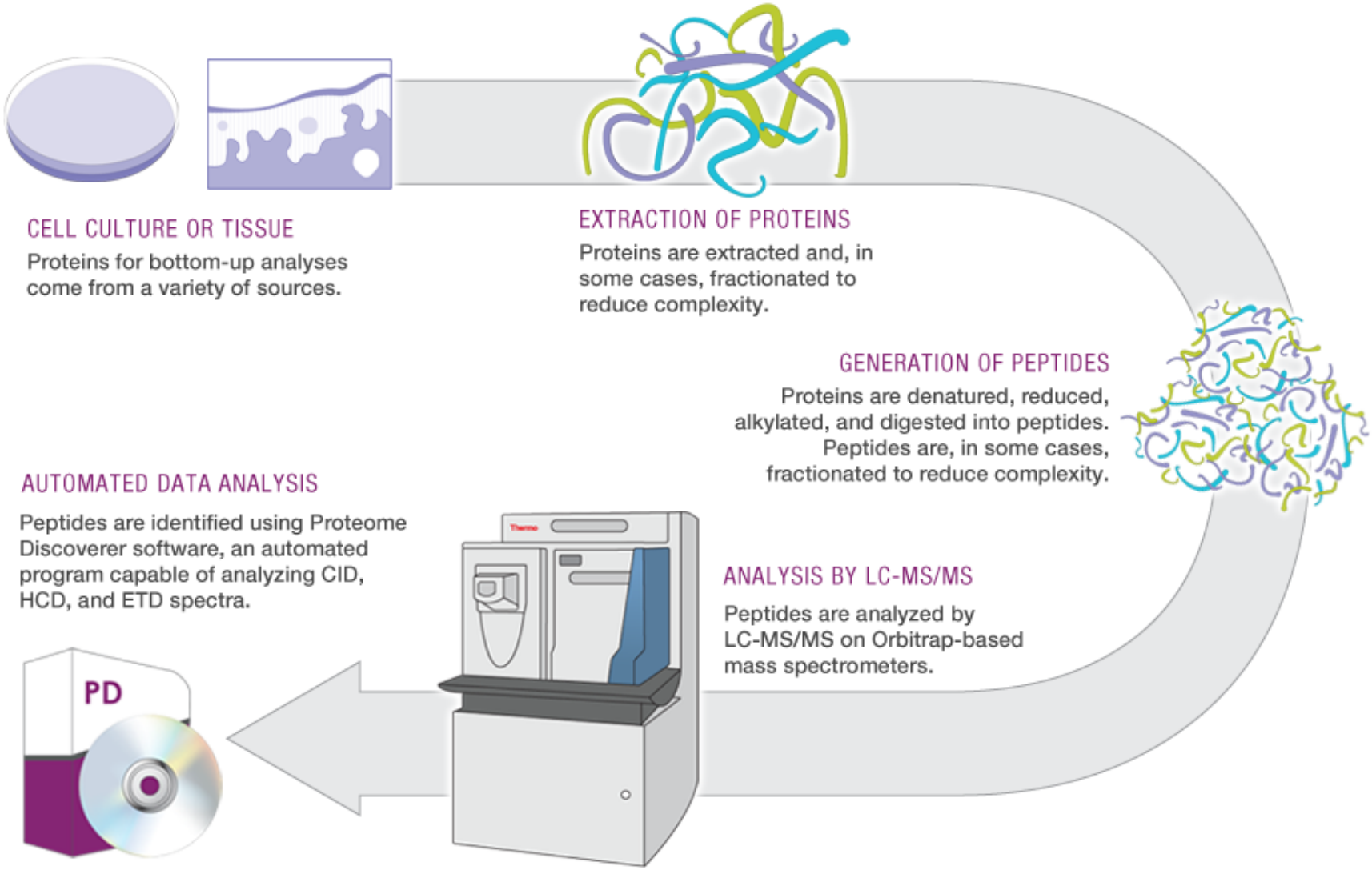








Workflow: Proteomics with Plymouth University

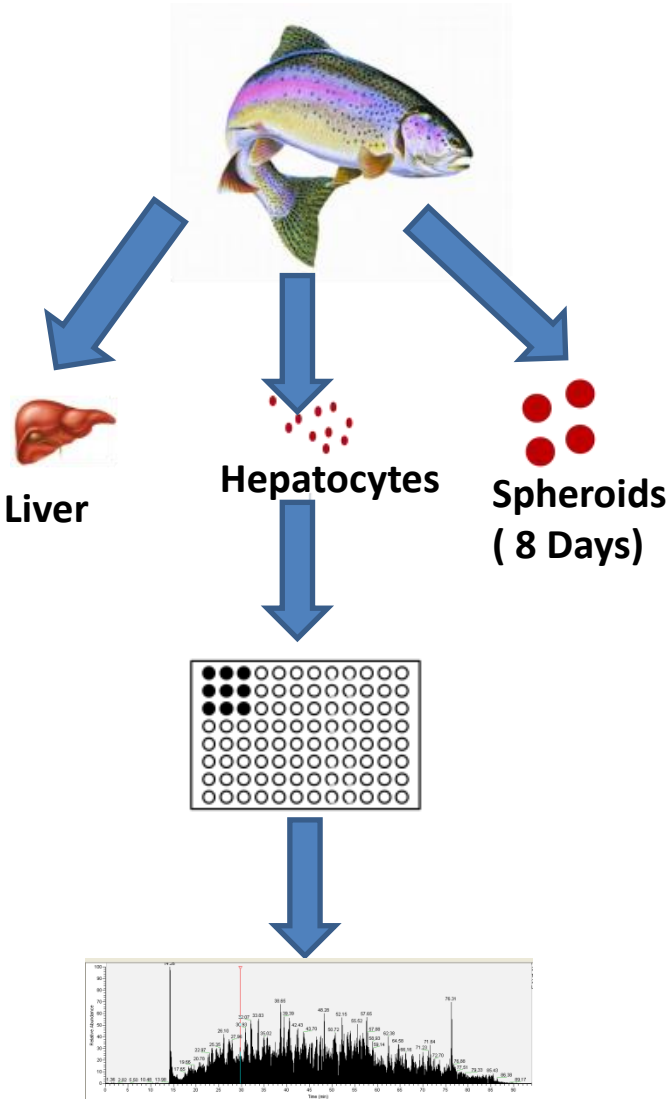


Source: www.planetorbitrap.com

Proteomic Characterisation of *in vivo* and *in vitro* liver models

(Liver Tissue, Primary Cells & Spheroids)

Experimental Design



3 Biological Replicates
(Number of Samples:9)

Author's personal copy

Ecotoxicology (2012) 21:2419–2429
DOI 10.1007/s10646-012-0965-5

TECHNICAL NOTE

Towards a more representative in vitro method for fish ecotoxicology: morphological and biochemical characterisation of three-dimensional spheroidal hepatocytes

Matthew G. Baron · Wendy M. Purcell · Simon K. Jackson · Stewart F. Owen · Awadhesh N. Jha

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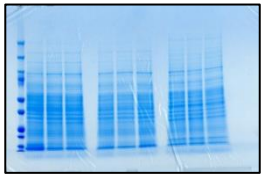
Abstract The use of fish primary cells and cell lines offer an in vitro alternative for assessment of chemical toxicity and the evaluation of environmental samples in ecotoxicology. However, their uses are not without limitations such as short culture periods and loss of functionality, particularly with primary tissue. While three-dimensional (spheroid) technology is now established for in vitro mammalian toxicity studies, to date it has not been considered for environmental applications in a model aquatic species. In this study we report development of a reproducible six-well plate, gyratory-mediated method for rainbow trout (*Oncorhynchus mykiss*) hepatocyte spheroid culture and compare their lower than 2D cultures ($P < 0.01$). It is therefore suggested that mature spheroids can maintain a high degree of functional, biochemical and morphological status over-time in culture that is superior to conventional 2D models and can provide realistic organotypic responses in vitro. Trout spheroids that take ~6–8 days to reach maturity would be suitable for use in acute toxicological tests and since it is possible to culture individual spheroids for over a month, there is potential for this work to lead towards in vitro bioaccumulation alternatives and to conduct high throughput screens of chronic exposure. This is an important step forward for developing alternative in vitro tools in future fish

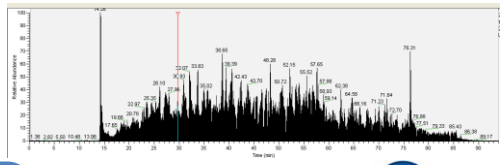
Baron et al, 2012

Workflow: Rainbow Trout Proteomics

Protein Extraction , Estimation and QC



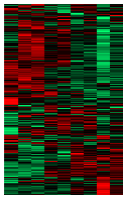
Sample Processing for Mass Spectrometry

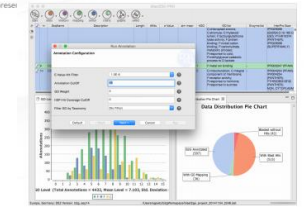


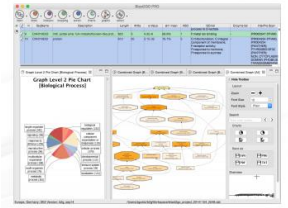
Analysis of Samples by LCMS

Database Search (www. uniprot.org)

Bioinformatic Analysis of Dataset





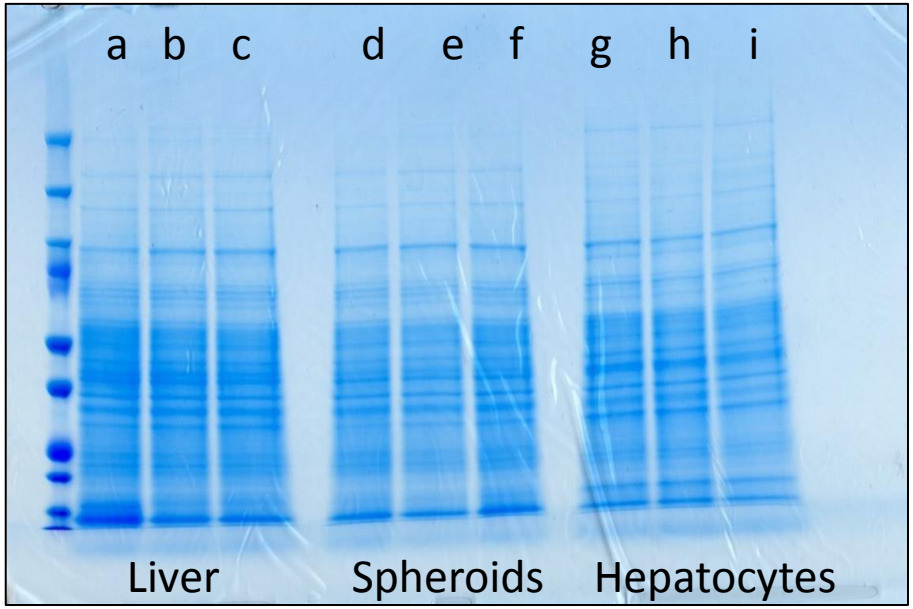


Under Progress

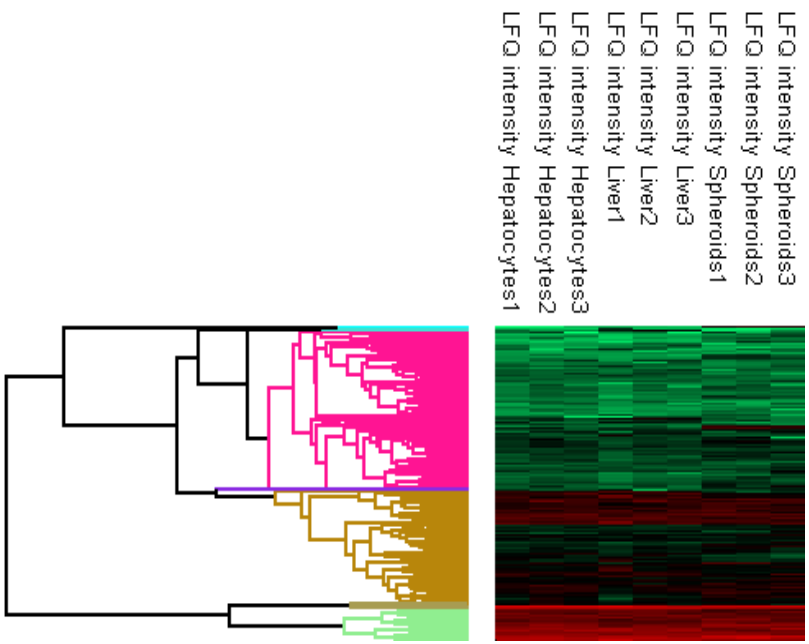
Conclusions

Preliminary Data

SDS GEL & MS Analysis



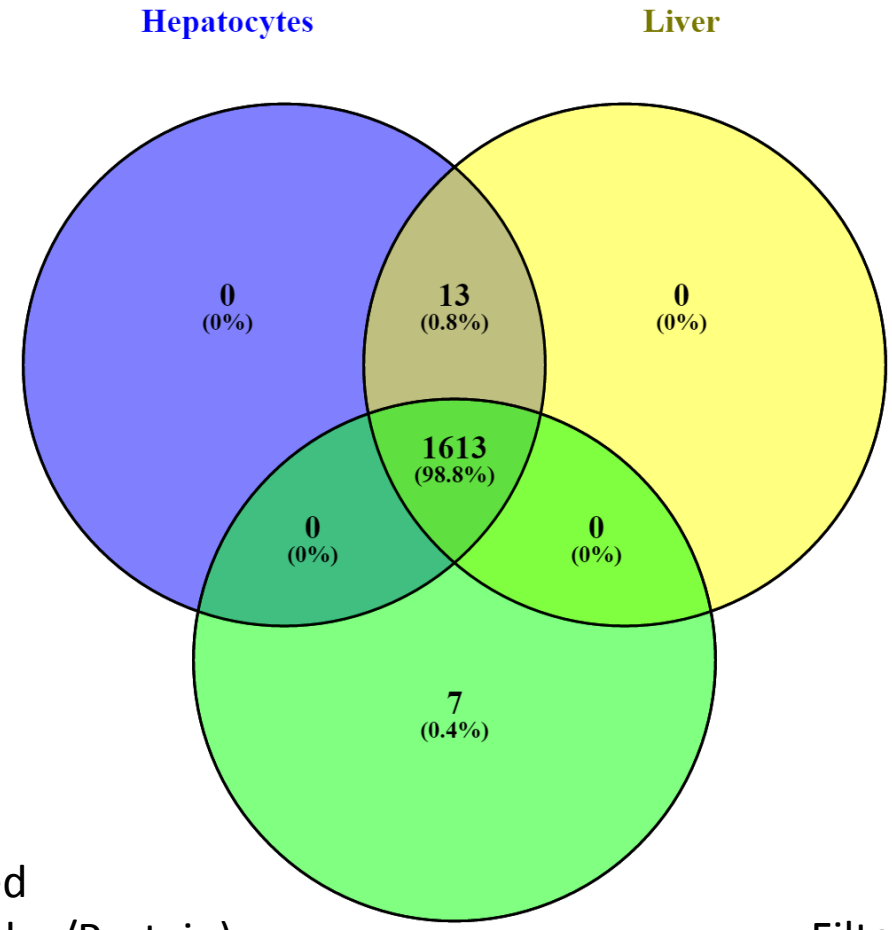
Quality Check on 4-12% Gradient Gel



Hierarchical Clustering on LFQs

LFQ: Label Free Quantification

Identified Proteins among models

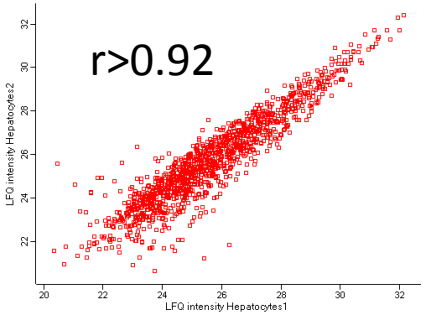


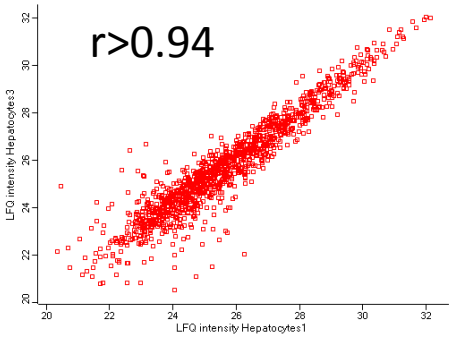
~2300 Proteins Identified
1633 Proteins (<2 peptides/Protein)

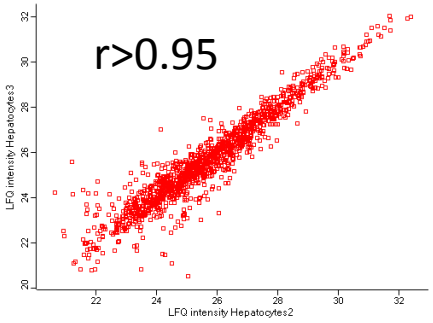
Filtering Criteria: 2 Unique Peptides/Protein

Correlation in Replicates

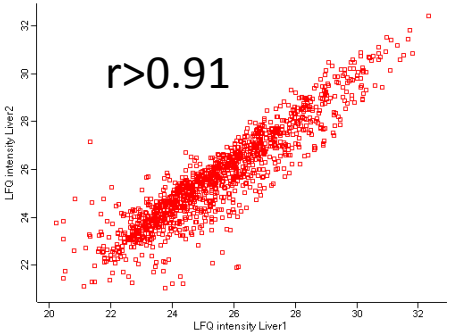
Hepatocytes

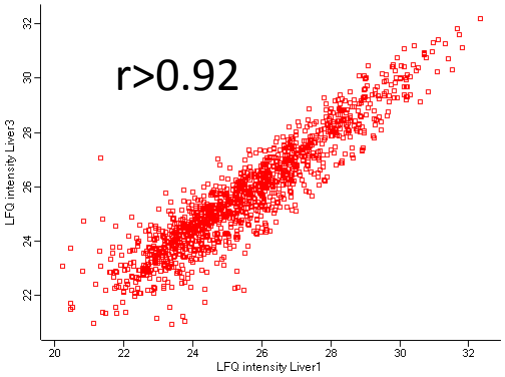


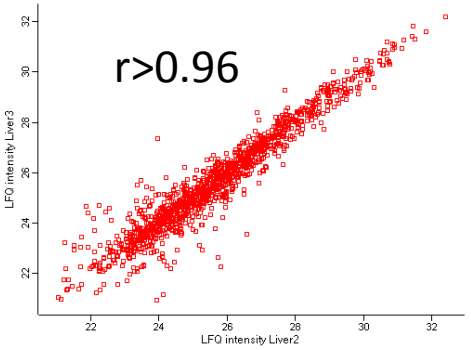




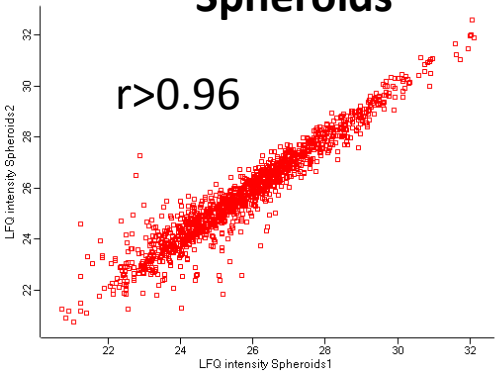
Liver

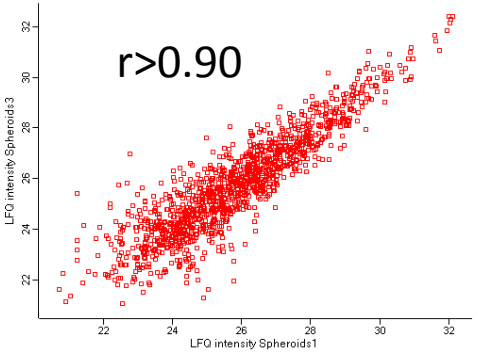


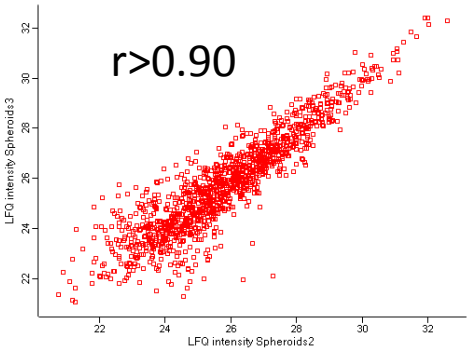




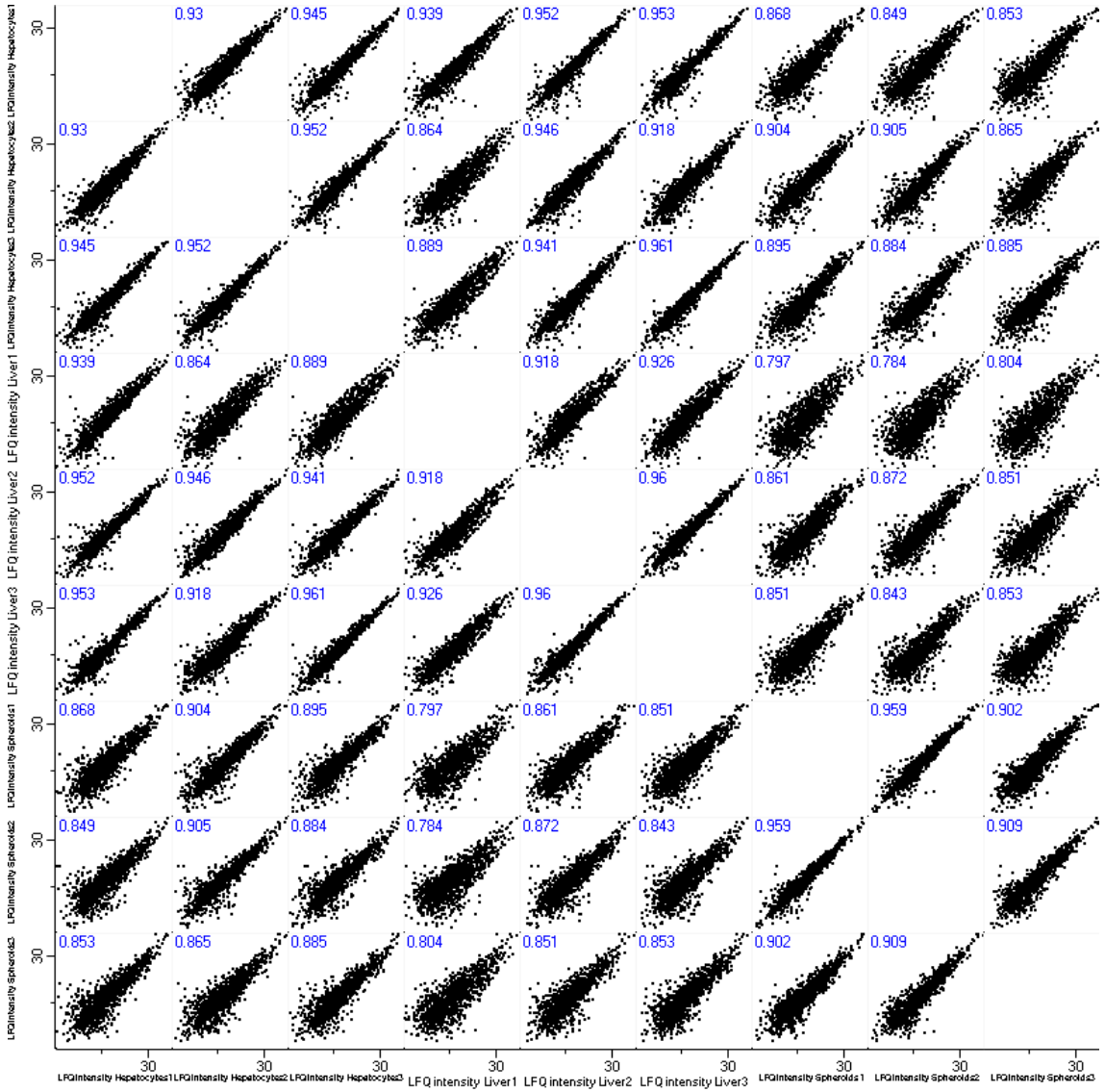
Spheroids





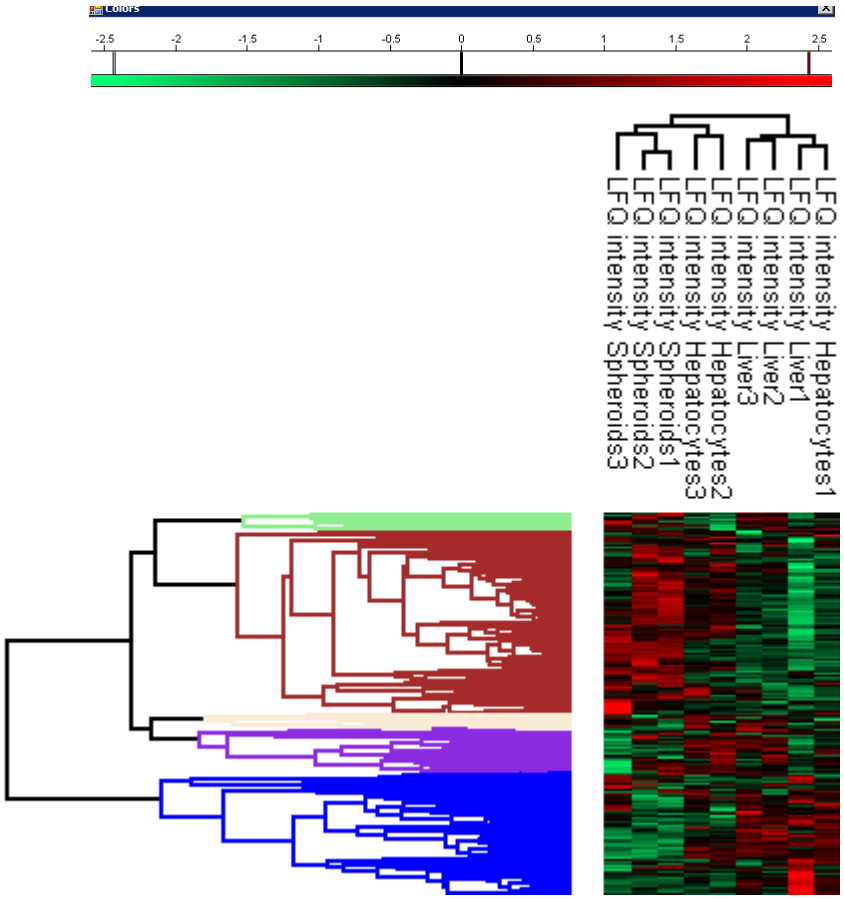


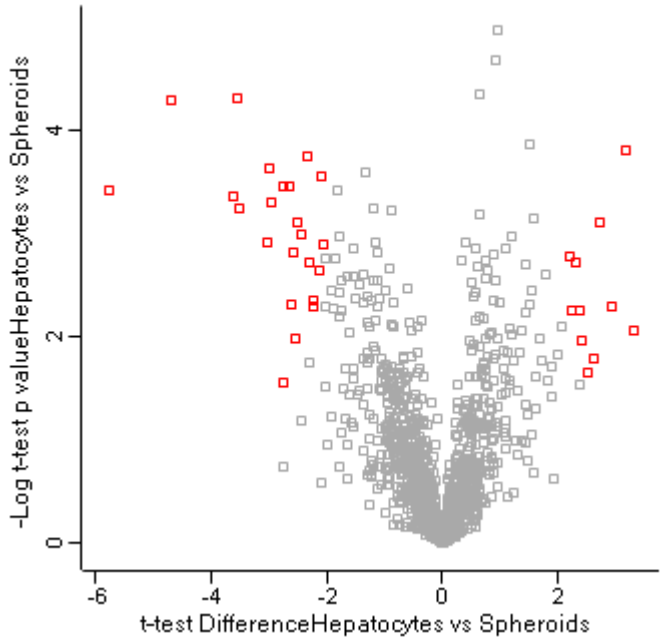
Correlation: Experimental Groups



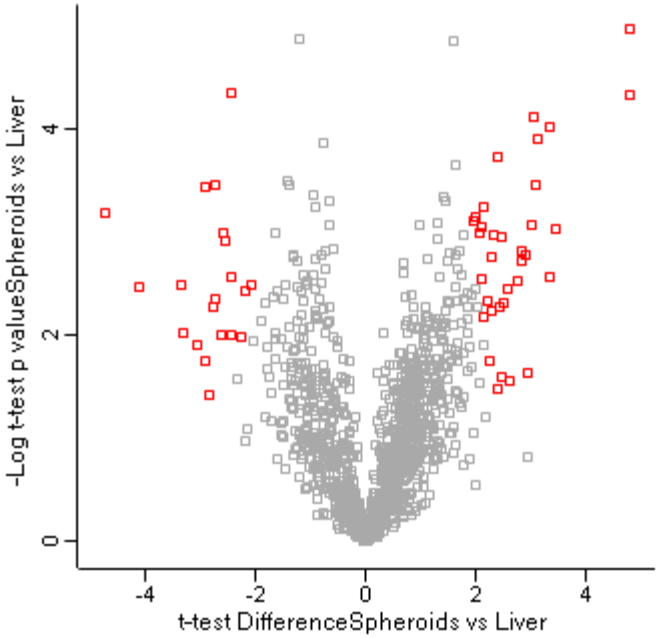
r=0.78 to 0.96

Hierarchical Clustering Analysis (z normalised LFQs)



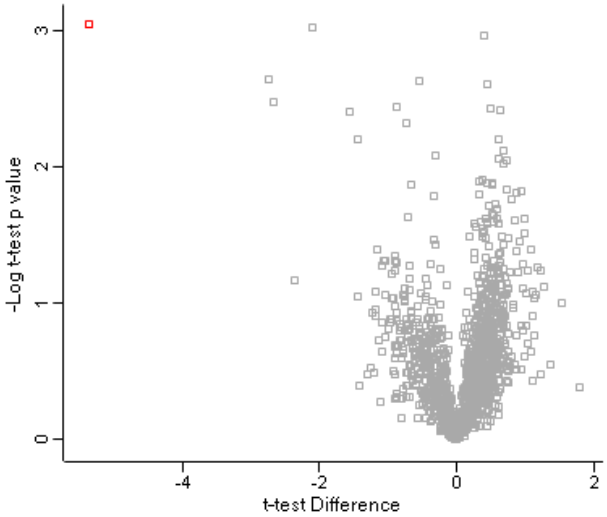


Spheroids vs Hepatocytes



Spheroids vs Liver

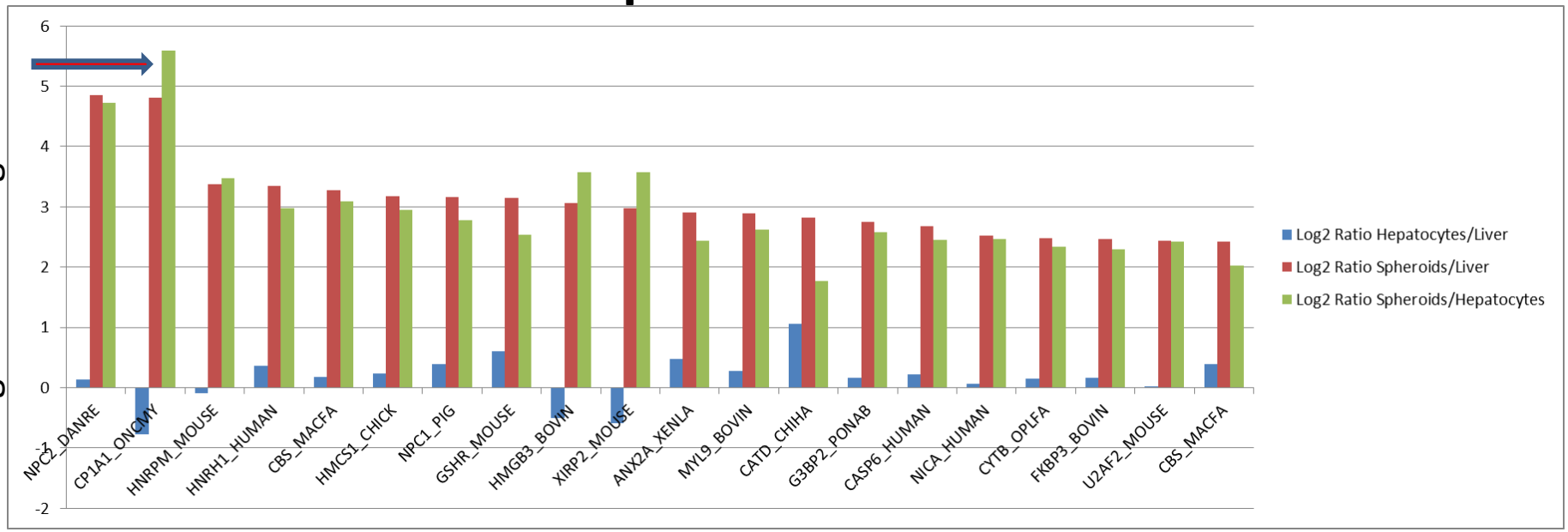
p<0.01)

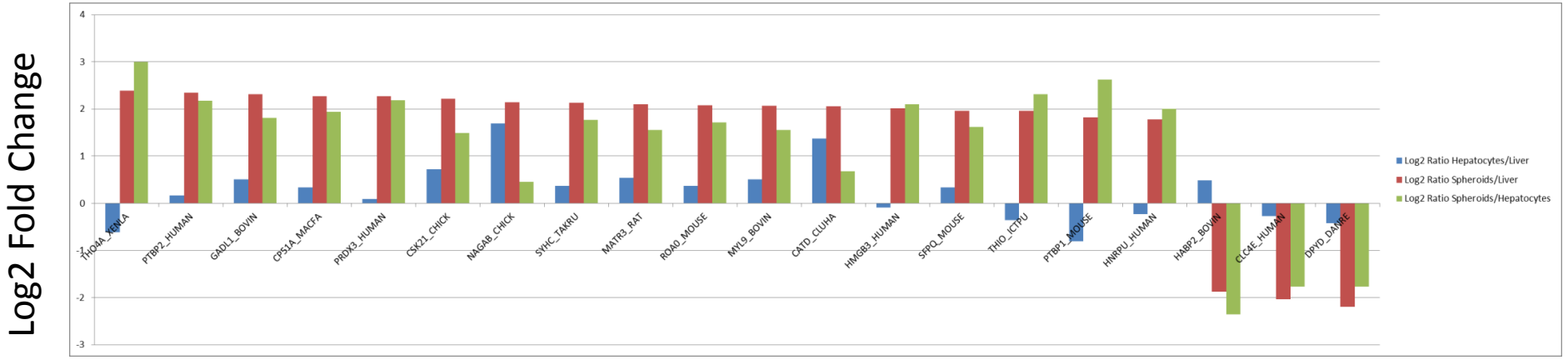


Liver vs Hepatocytes

Annotation Extraction by Blast2go

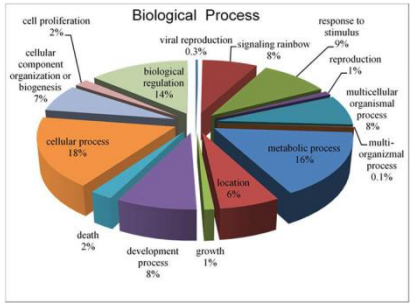
Proteomic Profile: Significantly altered proteins

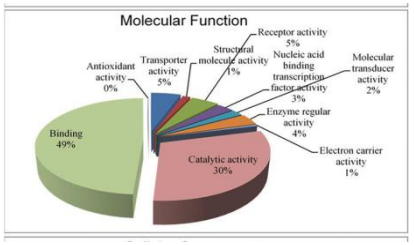


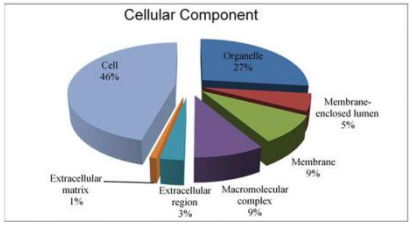


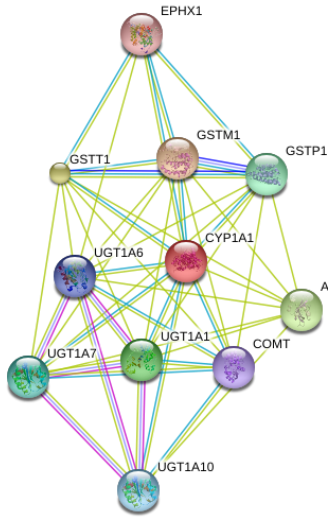
Ongoing Analysis

- Functional Significance of Altered Proteins
- Annotation Enrichment Analysis
- Comparative Proteomics







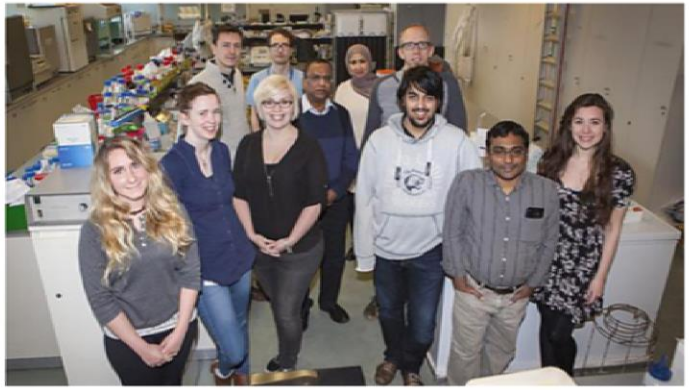


PLOS ONE | DOI:10.1371/journal.pone.0121778 March 20, 2015

STRING DB- CYP1A1

SYSTEMS BIOLOGY CENTRE WITH PLYMOUTH UNIVERSITY

RESEARCH WITH PLYMOUTH UNIVERSITY



Our current team

> The Genetic Toxicology & Ecotoxicology Research Group has a varied resource of researchers.

Meet our team

AstraZeneca

NERC SCIENCE OF THE ENVIRONMENT

BBSRC bioscience for the future