

Stanford University Mass Spectrometry

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The Vincent Coates Foundation Mass Spectrometry Laboratory

is named in honor of a generous gift from Vincent and Stella Coates, given to support the mass spec & proteomics facility as a shared core resource.

Overviews of three ongoing projects are presented at right.

Services

Consultation & experimental design

Proteomics:

- protein identification
- peptide mapping
- protein modifications – PTMs & synthetic
- capillary & multi-dimensional LC-MS (MudPIT); long column chromatography
- *de novo* peptide sequencing

Quantitative applications & assays:

- small molecule quantitation in biological matrices
- metabolic, PK studies
- protein/peptide quantitation
- biomarker verification

General analysis:

- mass determination
- LC-MS
- MS/MS & MSn
- High-resolution MS

Open access lab:

- ESI-MS
- LC-MS
- GC-MS
- Data analysis workstations

Custom projects: method development, protein folding, non-covalent interactions, etc.

Identification of proteins associated with actomyosin-independent cytokinesis. Dr. Masayuki Onishi, Meng Wang, Dr. John Pringle; Dept. of Genetics, Stanford School of Medicine

Cytokinesis — division of the cytoplasm in a cell — occurs as a terminal event in the cell cycle. Despite a common model in which the actomyosin ring at the division site pulls the membrane invaginating into the cytosol, several recent reports suggest that the actomyosin ring is not essential for cytokinesis in some cell types, including our model organism, *S. cerevisiae*.¹

By genetic and cytological approaches, we have identified two key proteins involved in the “actomyosin-independent cytokinesis,” Cyk3p and Hof1p. Our preliminary data suggest that Cyk3p and Hof1p form a transient complex as cells undergo cytokinesis, yet they play distinct roles. The aim of this project is to identify Cyk3p- and Hof1p-binding proteins, in order to increase understanding of the molecular events conducted by these proteins.

Few Cyk3p- and Hof1p-binding proteins have been identified by the large-scale genome-wide TAP-purification/MS analysis project, likely due to the poor solubility of these proteins. In the current project, Western blotting analysis has confirmed most of the candidate proteins identified at SUMS to be true interactors, including EF1-alpha, RPL4, and Ynl152wp.

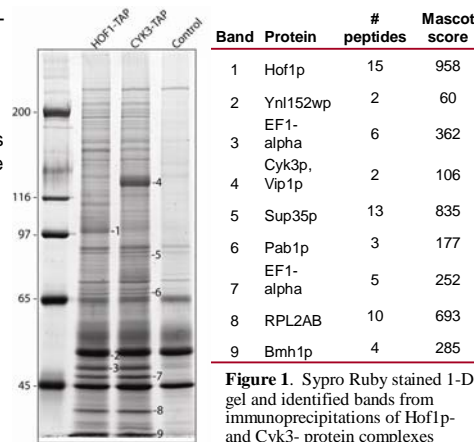


Figure 1. Sypro Ruby stained 1-D gel and identified bands from immunoprecipitations of Hof1p- and Cyk3- protein complexes

Ubiquitination activity of a human protein involved in DNA repair. Dr. Brian Scottoline, Dr. Beverly Mitchell; Dept. of Medicine, Div. of Oncology, Stanford School of Medicine

DNA repair is essential to the preservation of genomic integrity, and deficiency in this process is associated with a wide variety of genetic diseases including those with predisposition to malignancy and immunodeficiency, as well as sporadic cancer. Although there continues to be much progress in identifying the proteins involved in DNA repair, both genetic as well as biochemical evidence indicates that there are yet to be defined proteins in repair processes.

hPso4 is the human homologue of *S. cerevisiae* Pso4/Prp19, which has been identified in yeast as a being involved in DNA repair, most likely with DNA double strand break (DSB) repair.² hPso4 contains a U-box domain which is associated with E3 ubiquitin ligase activity; this activity is being studied as part of our investigation of the role of hPso4 in DNA repair processes.

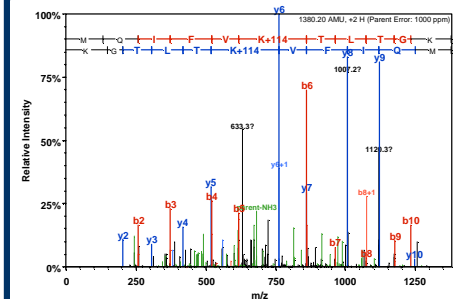
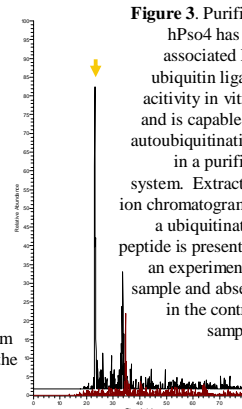


Figure 2. MS/MS pinpoints ubiquitination site. Following trypsin digestion of a ubiquitinated protein, a Gly-Gly tag from the ubiquitin sequence is left behind on the lysine residue of the modified peptide. In the example above, the modified lysine (K+114 Da) is confirmed by both the b- and y- ion series.

Figure 3. Purified hPso4 has an associated E3 ubiquitin ligase activity in vitro, and is capable of autoubiquitination in a purified system. Extracted ion chromatograms: a ubiquitinated peptide is present in an experimental sample and absent in the control sample.



Neurosteroid Quantitation: Role of placental factors in neonatal brain development. Dr. Florian Ermini, Dr. Anna Penn; Dept. of Pediatrics, Stanford School of Medicine

During pregnancy, steroid hormone levels increase 100-fold in maternal and fetal plasma, but abruptly drop to baseline levels at birth. Both estrogen and progesterone have been shown to stimulate brain development and have neuroprotective effects. Prematurely born infants suffer from an early withdrawal of these important neurodevelopmental factors, and this may contribute to the prevalence of later cognitive impairment.

To investigate the effect of this early steroid withdrawal on the development of the mouse brain, LC-MS/MS is used to quantify steroids extracted from plasma and brain tissue.

The method developed enables the concurrent detection and quantitation of five steroids at picomolar levels from extremely small samples.

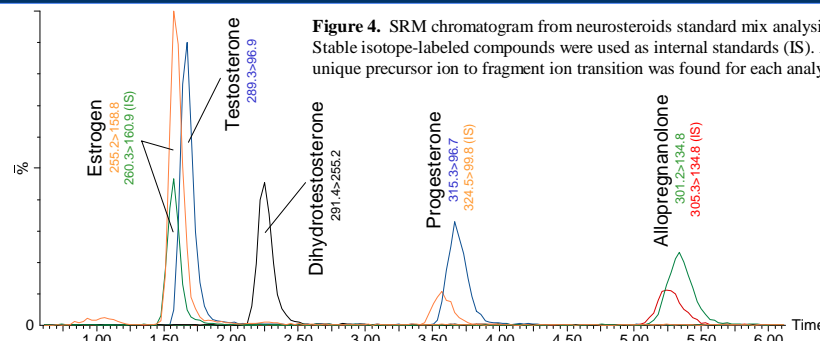


Figure 4. SRM chromatogram from neurosteroids standard mix analysis. Stable isotope-labeled compounds were used as internal standards (IS). A unique precursor ion to fragment ion transition was found for each analyte.

Operations

SUMS is staffed by 4 scientists and operates 6 mass spectrometers and associated instrumentation in an 1800 ft² facility on the Stanford campus.

Instrumentation

- 6890/5973 GC-MS
- LCQ Deca XP+ ion trap LC-MS
- LCQ "Classic" ion trap LC-MS
- Q-ToF hybrid quadrupole-time of flight LC-MS
- Quattro Premier triple quadrupole LC-MS
- ZQ single quadrupole LC-MS
- NanoMate nano-electrospray robots
- NanoLC-2D capillary HPLC

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- Stanford Bio-X
- Stanford Cancer Center

References

- 1 Ko N, Nishihama R, Tully GH, Ostapenko D, Solomon MJ, Morgan DO, Pringle JR "Identification of Yeast IQGAP (Iqg1p) as an Anaphase-Promoting-Complex Substrate and Its Role in Actomyosin-Ring-Independent Cytokinesis." *Mol Biol Cell* 2007; 18: 12: 5139-53.
- 2 Mahajan KN, Mitchell BS "Role of human Pso4 in mammalian DNA repair and association with terminal deoxynucleotidyl transferase." *Proc Natl Acad Sci USA* 2003; 100: 19: 10746-51.

Acknowledgements

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This poster may be downloaded from <http://mass-spec.stanford.edu/Publications.html>

