Inorganic Mass Spectrometry Analysis of Single Cell Protein Quantification; A Road Map

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Introduction

Duchenne muscular dystrophy (DMD), a genetic disorder characterized by the progressive degeneration of muscle, affects approximately 1 in 4,000 male births worldwide. DMD is caused by a variety of mutations in the gene encoding dystrophin, a critical structural component of muscle fibers. In this work, we will highlight the utilization of rare earth metal-tagged antibodies (M-Ab) to localize and quantify differentially expressed proteins of the Dystrophin-glycoprotein complex.

Objectives

- Develop novel methods for protein quantitation in biological and clinical research samples
- Exploit the low abundance of rare-earth elements in biological systems
- Combine cutting edge laser capture and laser ablation methods with ICP-MS/MS to achieve highly multiplexed single cell protein quantitation

Approach

- We are developing a novel technique to protein quantification exploiting the inherently low concentration of rare earth elements (REE) in biological systems
- By coupling REE-antibody immunolabeling with laser capture microdissection (LCM) of single cells or laser ablation of tissue sections, and measurement by ICP-QQQ, we are achieving multiplexed protein measurement in histological sections and single cells

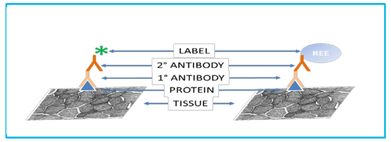


Figure 1: Traditional immunofluorescence versus heavy metal immunostaining

Experimental

Experimental Design

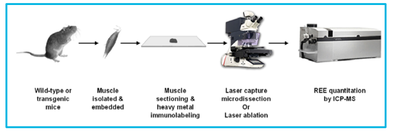


Figure 2: Workflow for single cell protein detection by LCM-ICP-MS/MS.

Detection Target	REE Label
Myosin (skeletal, fast)	Praseodymium-141
GAPDH	Ytterbium-171
IL-6	Erbium-167
DNA intercalator	Rhodium-103

Table 1: REE Labeling Reagents

Laser microdissection of single cells for ICP-MS/MS

- Immunolabeled cells are isolated by laser capture microdissection into individual wells of a 48-well microscope slide

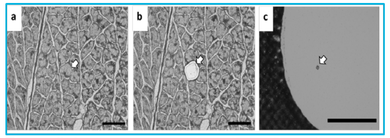


Figure 3: Laser capture microdissection of a single REE-immunolabeled muscle cell.

Black bar is 100 microns in panels a and b and 500 microns in panel c.

- Captured cells are digested with 1uL of 70% nitric acid & taken up in 9uL of deionized water.
- Ten microliter samples were either injected in the Agilent 8800 ICP-MS/MS via syringe pump or by an Agilent 1260 Cap-LC at a flow rate of 10uL/min
- The ICP-MS/MS was equipped with a low-flow nebulizer & a total consumption spray chamber.

Results and Discussion

LCM-ICP-MS/MS multiplexing across individual cells

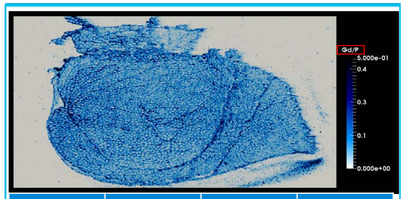
REE Label [a]	Fiber 1	Fiber 2	Fiber 3	Fiber 4	Fiber 5	Avg Count [b]
^{141}Pr-Ab-Myosin	2562	2726	2835	3198	1976	2659± 200
^{167}Er-Ab-IL6	178	273	345	299	233	266± 28
^{171}Yb-Ab-GAPDH	105	126	160	138	110	127± 10
^{103}Rh-DNA	877	712	1482	776	1132	996± 141

Table 2: ICP-QQQ data from single, REE-labeled muscle fibers.

[a] Data are represented as counts for each respective mass analyzed.

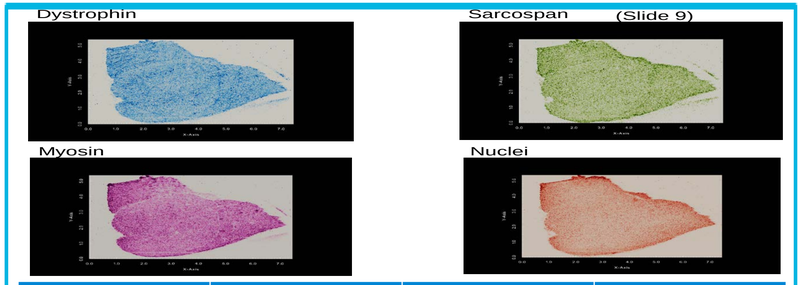
b Average counts for each REE label ± SEM.

High dimensional mass cytometry of mouse quadriceps with multiplex REE protein and DNA quantitation



Label – Single	Spot Size	Scan Speed	Integration Time
158-Gd (single)	15um	60um/s	0.250

Figure 4: WT mouse quadriceps stained for dystrophin



Label – Multiplexed	Spot Size	Scan Speed	Integration Time
158-Gd (dystrophin)	15 µm	45 µm/s	0.33
162-Dy (sarcospan)	15 µm	45 µm/s	0.33
141-Pr (myosin)	15 µm	45 µm/s	0.33
103-Rh (DNA intercalator)	15 µm	45 µm/s	0.33

Figure 5: High dimensional mass cytometry of mouse quadriceps with multiplex REE protein and DNA

Status and Conclusions

- Human muscle biopsy samples are now being collected to validate clinical research applications of these methods
- Further multiplex targets are being developed
- Trials are underway of REE immunolabeling as a replacement for traditional ELISA

Next Step

Simplifying the Workflow

While LCM-ICP-MS/MS has proved successful for single cell extraction, LCM is a tedious process. The ability to run single cell suspensions by ICP-MS/MS would remove the need for laser capture microdissection to separate cells prior to REE analysis and would increase analytical throughput.

Objective

Perform Single-Cell Analysis on an Agilent ICP-MS platform using a metabolically enriched cellular suspension for comparison against a non-enriched cellular suspension.

Experimental

Two yeast strains of saccharomyces cerevisiae (selenium enriched) and saccharomyces cerevisiae (non-enriched) were prepared in a 1x PBS and 5% glucose media. The suspensions were homogenized on an orbital shaker.

Experimental (continued)

The suspensions were allowed to propagate for 8 hours prior to sub-sampling. An analytical aliquot was then diluted twenty fold into 0.1% NaCl for introduction to the ICP-MS. Samples were delivered to the ICP-MS via syringe pump at a 10uL/min flow rate. A total consumption nebulizer and spray chamber were utilized to maximize transport efficiency. The nebulizer and spraychamber gas flows were optimized to ensure the cells stayed intact during sample transport to the plasma.

Results

- High nebulization efficiency achieved
- Multi-element scan successful for enriched and metabolic elements (Mn,Ni,Cu,Zn,Se,Fe)
- Noticeable difference between enriched and non-enriched strains for both enriched and metabolic elements

		Sample		60 Ni				63 Cu				66 Zn				78 Se				56 Fe			
Vial	R	Acq. Date-Time	Type	Sample Name	Nebulization Efficiency	Particle Conc (particles/l)	Mass Conc (ng/l)	Ionic Conc (ppb)	Nebulization Efficiency	Particle Conc (particles/l)	Mass Conc (ng/l)	Ionic Conc (ppb)	Nebulization Efficiency	Particle Conc (particles/l)	Mass Conc (ng/l)	Ionic Conc (ppb)	Nebulization Efficiency	Particle Conc (particles/l)	Mass Conc (ng/l)	Ionic Conc (ppb)	Nebulization Efficiency	Particle Conc (particles/l)	Mass Conc (ng/l)	Ionic Conc (ppb)
		10/5/2017 6:46:09 PM	Sample	Yeast SC	0.843	1.5E+6	21.2	0.7431	0.843	4.2E+9	71.7	<0.0000	0.843	5.6E+8	61.7	0.0523	0.843	5.1E+8	601.4	0.9900	0.843	1.5E+9	137.5	0.2062
		10/5/2017 6:53:31 PM	Sample	Yeast SC	0.843	9.0E+7	14.0	0.5725	0.843	4.1E+9	77.4	<0.0000	0.843	4.0E+8	66.0	0.1317	0.843	5.2E+8	540.0	1.0036	0.843	1.5E+9	133.9	0.2049
		10/5/2017 7:00:53 PM	Sample	Yeast SC	0.843	9.0E+7	12.7	0.5022	0.843	4.0E+9	53.5	<0.0000	0.843	2.0E+8	36.6	0.1665	0.843	5.5E+8	726.5	1.0950	0.843	1.5E+9	120.0	0.1932
		10/5/2017 7:09:11 PM	Sample	Yeast SC	0.843	9.1E+7	12.4	0.4234	0.843	3.9E+9	58.6	<0.0000	0.843	4.6E+7	24.3	0.0736	0.843	5.8E+8	603.9	1.1094	0.843	8.3E+8	112.6	0.2122
		10/5/2017 7:16:49 PM	Sample	Yeast SC	0.843	6.9E+7	11.3	0.3722	0.843	3.6E+9	48.7	<0.0000	0.843	3.3E+8	294.8	<0.0000	0.843	5.7E+8	743.8	1.1489	0.843	1.4E+9	161.8	0.2481
		10/5/2017 8:04:40 PM	Sample	Bread Yeast	0.843	3.8E+9	70.7	<0.0000	0.843	1.4E+9	21.8	<0.0000	0.843	3.0E+9	65.4	<0.0000	0.843	1.1E+7	4.1	<0.0000	0.843	3.2E+9	988.9	0.7586
		10/5/2017 8:12:51 PM	Sample	Bread Yeast	0.843	3.7E+9	59.6	<0.0000	0.843	1.3E+9	20.1	<0.0000	0.843	3.1E+9	90.8	<0.0000	0.843	1.2E+7	3.9	<0.0000	0.843	6.1E+7	70.1	2.5398
		10/5/2017 8:25:09 PM	Sample	Bread Yeast	0.843	3.6E+9	48.6	<0.0000	0.843	1.3E+9	33.0	<0.0000	0.843	2.2E+9	48.4	<0.0000	0.843	1.4E+7	4.5	<0.0000	0.843	6.9E+8	220.2	1.8022
		10/5/2017 8:33:54 PM	Sample	Bread Yeast	0.843	3.9E+9	52.2	<0.0000	0.843	1.4E+9	38.3	<0.0000	0.843	2.1E+9	66.4	<0.0000	0.843	1.8E+7	6.9	<0.0000	0.843	5.7E+8	186.1	2.6926
		10/5/2017 8:41:25 PM	Sample	Bread Yeast	0.843	3.7E+9	49.1	<0.0000	0.843	1.1E+9	33.5	<0.0000	0.843	1.7E+9	39.2	<0.0000	0.843	2.0E+7	6.1	<0.0000	0.843	2.5E+8	124.3	2.7643

Figure 6: Multi-Element scan of single cell homogenates of enriched and non-enriched yeast cultures.

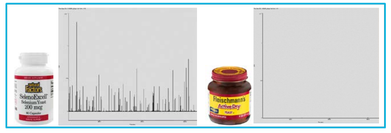


Figure 7: Time Scan of Se signal on same scale for both selenium enriched and non-enriched yeast cultures.

Future Work

The ability to analyze single cell suspensions by ICP-MS will complement other advanced analytical techniques, while also minimizing sample preparation time and providing a simplified analytical workflow.

Duchenne muscular dystrophy tissue samples immunostained with REE will be prepared utilizing collagenase to remove cells from the tissue. These samples will be analyzed for REE single-cell analysis by ICP-MS.