Separation of Cell Subpopulations by FCM for Proteome Analysis

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Clontech
Discovery Labware
Immunocytometry Systems
Pharmingen

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Overview

- Current approaches adequate for monotypic cell populations
- Cellular proteome analysis complicated by mixed populations
- Separation of subpopulations focuses analyses on desired targets
- Combine the power of cell separation techniques with mass spectrometry for global proteome analysis

Goals

- Demonstrate power of combining FACS and mass spectrometry in several model systems: lymphocytes, solid tissue, organelles
- Determine sensitivity of protein detection
- Optimize approaches for surface proteins
- Model Systems:
  - $CD4^+$ and $CD8^+$ lymphocytes
  - Hepatocyte populations
  - Mitochondria from mouse liver

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Reagents & Instruments

- Reagents
  - Anti-CD4-FITC
  - Anti-CD8-RPE
  - Anti-CD45-APC
- Cell Sorting & Analysis
  - BD FACS Vantage™ SE
  - BD FACS Calibur™
- Mass Spectrometry
  - Surveyor® HPLC
  - LCQ™ Deca Ion Trap Mass Spectrometer
  - TurboSequest™

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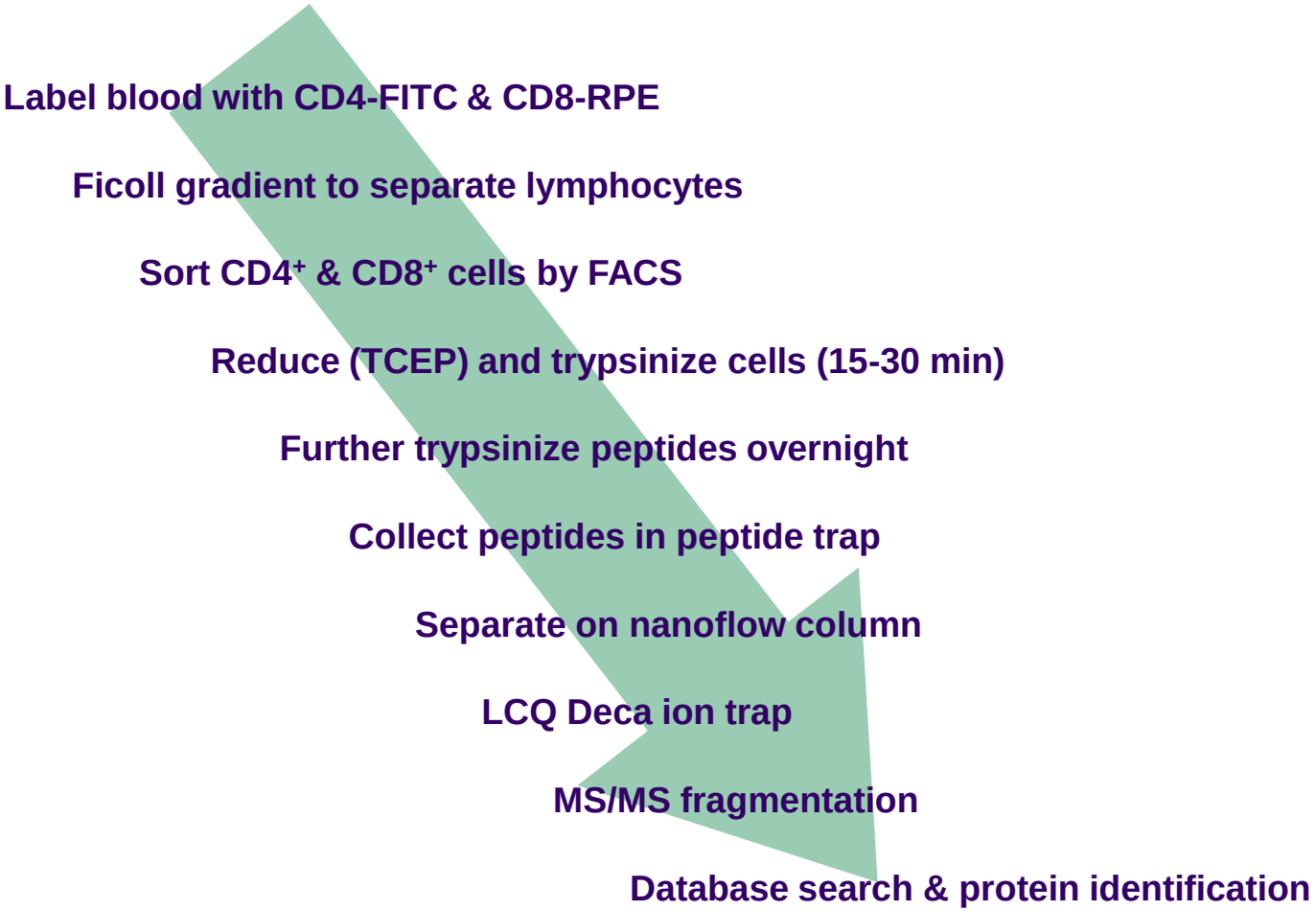
Lymphocyte Sample Preparation



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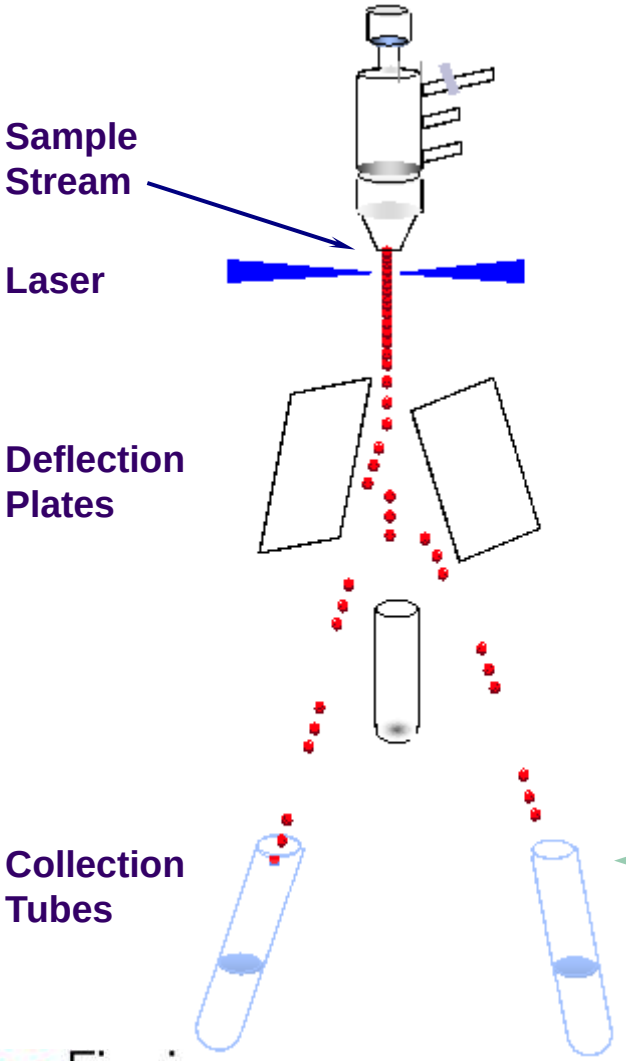
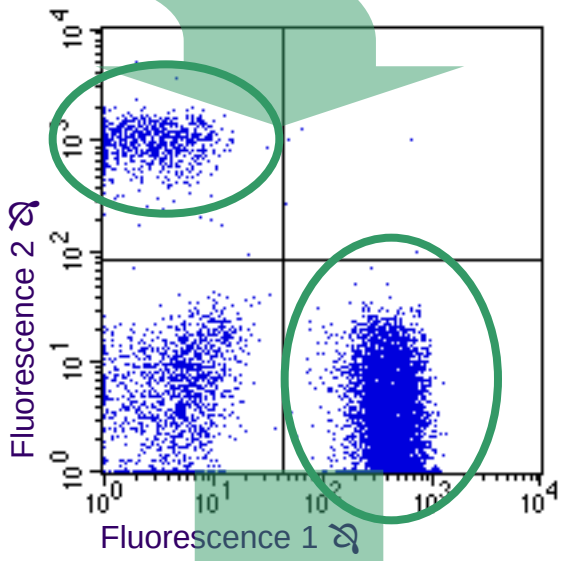
Fluorescence Activated Cell Sorting





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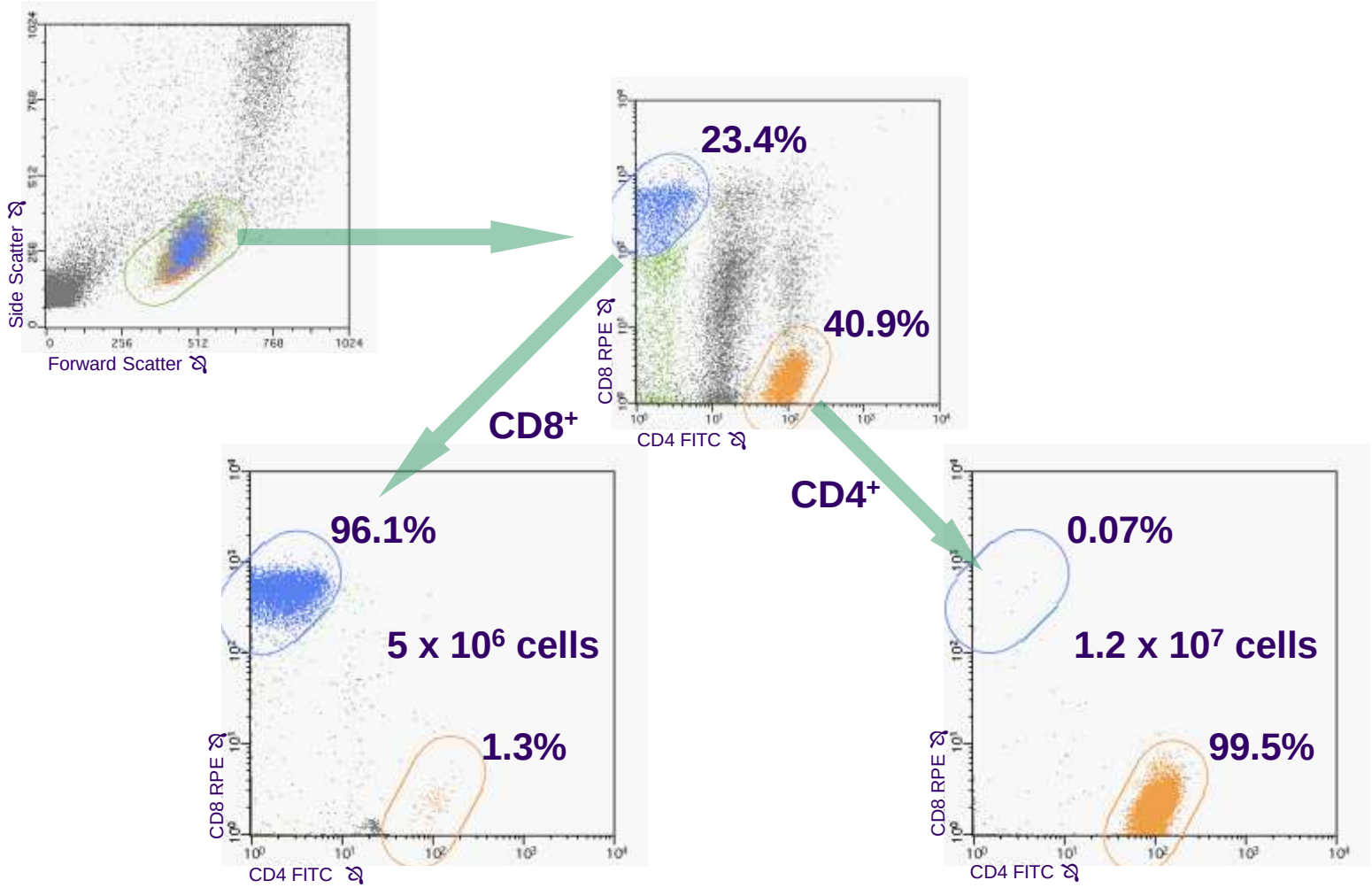
Analysis of Sorted Populations



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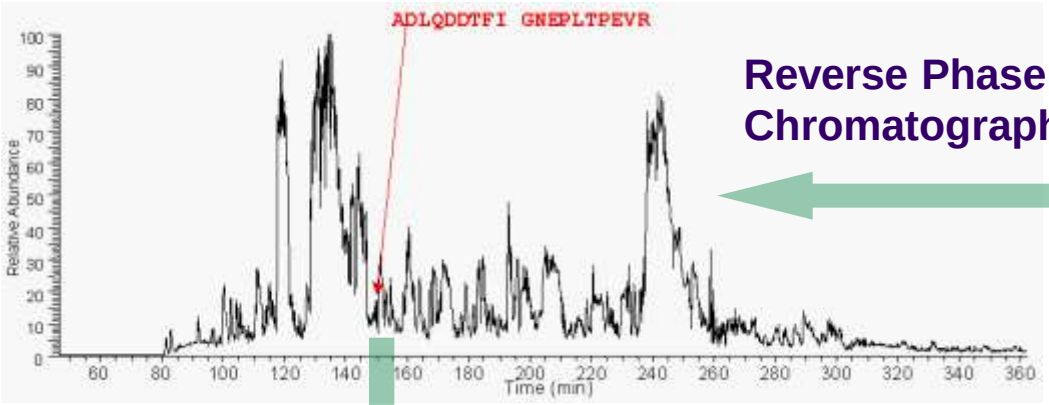
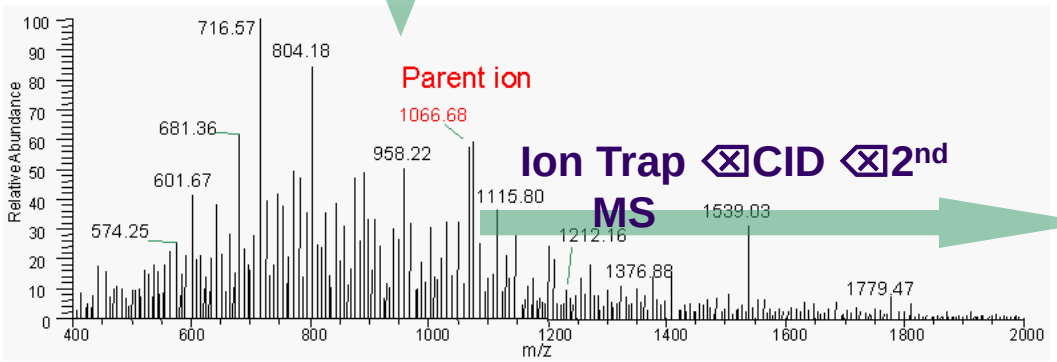
Mass Spectrometry - Peptide Sequence



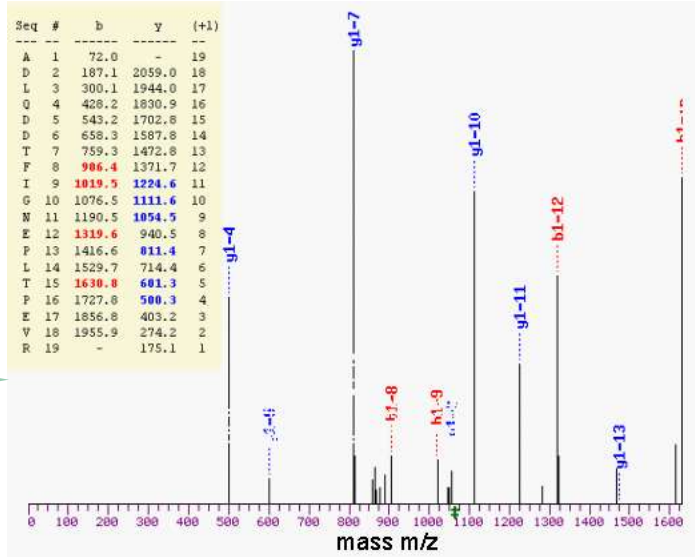
Reverse Phase Chromatography

Tryptic Peptide Mix

EIS⌧
1st MS



Ion Trap ⌧CID ⌧2nd MS



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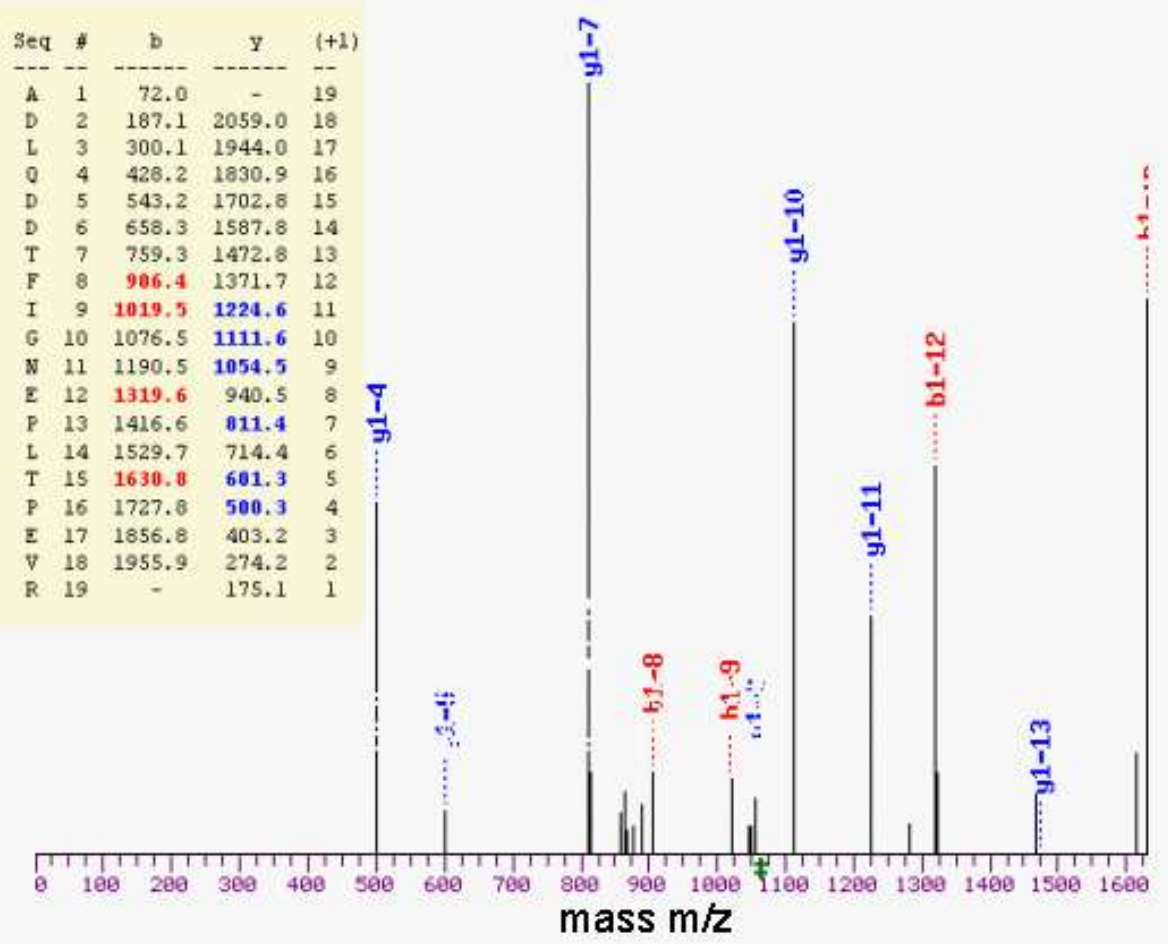
Results

Spectrum of peptide *ADLQDDTFIGNEPLTPEVR* from CD4+ cells

Matched to **CD11a**

Seq	#	b	y	(+1)
A	1	72.0	-	19
D	2	187.1	2059.0	18
L	3	300.1	1944.0	17
Q	4	428.2	1830.9	16
D	5	543.2	1702.8	15
D	6	658.3	1587.8	14
T	7	759.3	1472.8	13
F	8	906.4	1371.7	12
I	9	1019.5	1224.6	11
G	10	1076.5	1111.6	10
N	11	1190.5	1054.5	9
E	12	1319.6	940.5	8
P	13	1416.6	811.4	7
L	14	1529.7	714.4	6
T	15	1630.8	601.3	5
P	16	1727.8	500.3	4
E	17	1856.8	403.2	3
V	18	1955.9	274.2	2
R	19	-	175.1	1



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Proteins Identified

> 350 proteins matched, including 30 hypothetical protein product sequences

CD4+ Cells

- 9 CD Proteins
  - CD3, CD11a, CD16, CD35, CD99, CD117, CD121b, CD128b, CD132
- 28 Other Membrane-Related Proteins
  - Includes cell adhesion molecules, interleukin and other receptors
- Immunoglobulins

CD8+ Cells

- 14 CD Proteins
  - CD3, CD8, CD16, CD18, CD47, CD49b, CD51, CD71, CD80, CD84, CD98, CD99, CD120b, CDB9
- 20 Other Membrane-Related Proteins
  - Includes cell adhesion molecules, interleukin and other receptors
- Immunoglobulins

Challenges:

- CD4 not identified in $CD4^+$ population; CD45 not detected
- CD117 (c-kit, early development marker) observed

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CD4 Model for "Difficult" Target

Recombinant CD4 (Protein Sciences Corp.) & HPB-ALL cells ⌫ tryptic digest ⌫ mass spectrometry

- rCD4 (800 fmole)

CD4	20 scans	11 unique peptides
HSA precursor	2 scans	2 unique peptides

- rCD4 bound to anti-CD4-IMag particles (800 fmole)

CD4	5 scans	4 unique peptides
HSA precursor	10 scans	5 unique peptides

- CD4 on HPB-ALL cells (80000 copies/cell, 6 x 10^6 cells, ~ 800 fmole)

CD4	not detected

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Molecule Copy Numbers

Molecule	Per T-cell	fmoles/10^6 cells	LC-MS
CD3	8.1×10^4	130	++
CD4	5.9×10^4	98	-
CD8	1.4×10^5	230	+
CD11a	2.7×10^4	45	+
CD16	7.9×10^4	130	+
CD18	3.1×10^4	52	+
CD45	1.9×10^5	320	-

Appendix A, Cell Separation Methods and Applications. 1998. Recktenwald D and Radbruch A, eds.

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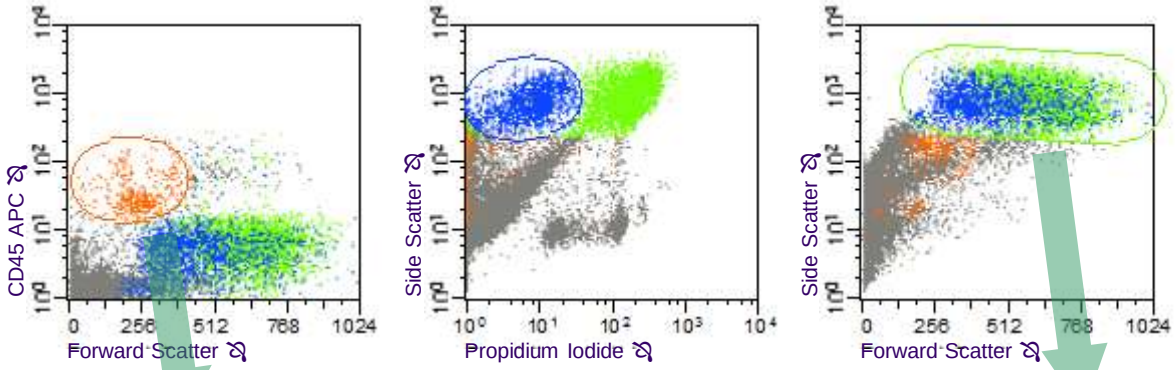
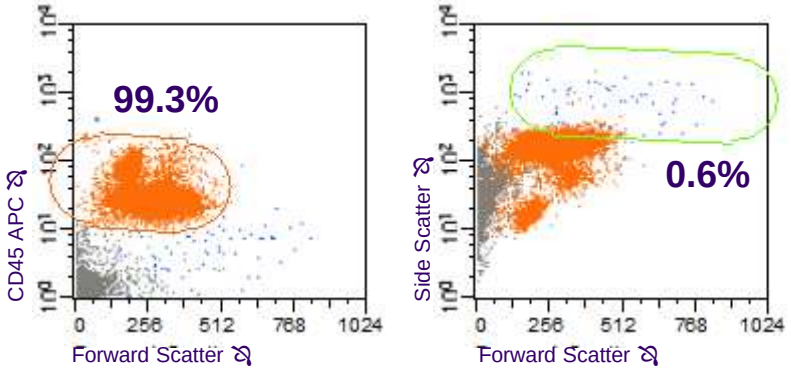
Liver Model

- Collagenase perfusate of human liver (BD Gentest, Woburn, MA)
- Anti-CD45-APC / propidium iodide ⌫ Sort (viability, CD45 expression, scatter) ⌫ LC-MS/MS analysis

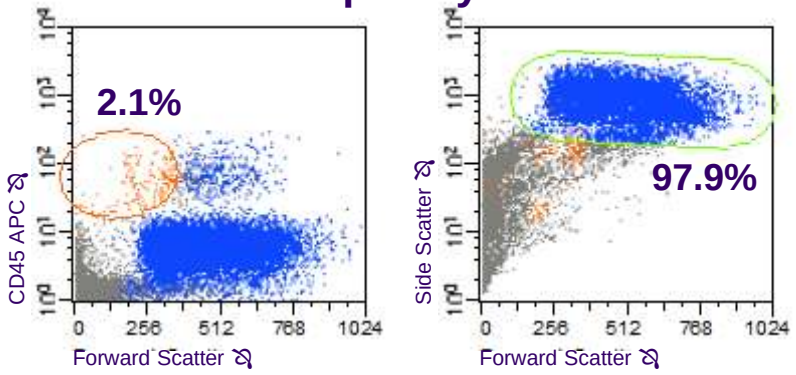
Starting cell suspension



CD45⁺



Hepatocytes



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Preliminary Results - Hepatocyte Fraction

- 35 proteins identified with high confidence from 54 unique peptides
- 8 mitochondrial-specific proteins and precursor proteins
- Hepatic arginase
- Large proportion of cytoplasmic proteins

Mitochondria

Homogenate from mouse liver cells

↓

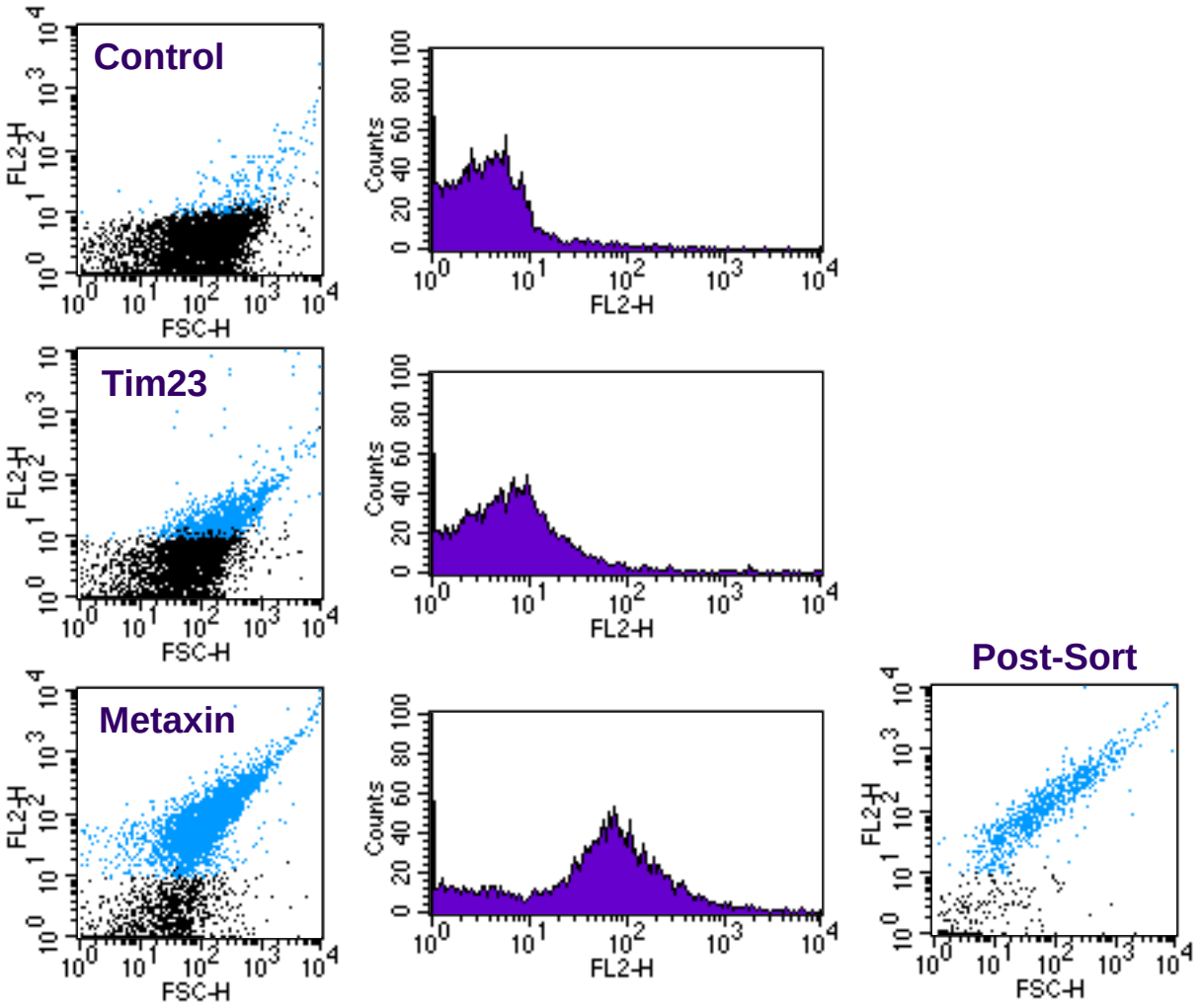
Antibodies against metaxin or Tim23 (BD Biosciences Pharmingen)

↓

Anti-Kappa-RPE

↓

Analysis & sorting



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Summary

- Cell separation focuses LC-MS/MS on a simpler target population
- Can detect large number of lineage-specific proteins
- Peptide digestion off cells is not complete and varies with target
- May be more difficult to digest the antigens used for selection
- Able to detect many proteins in complex mixtures at femtomolar levels (<100,000 copies/cell)
- Powerful tool - room for further optimization

Collaborators

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(San Jose, CA)

- Colette Rudd
- Pavel Bondarenko
- Thomas Shaler
- Sally Swedberg
- William Hancock

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- William Godfrey
- Diether Recktenwald

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