Filling the Biomarker Pipeline: Overview and Historical Context

Leigh Anderson, Ph.D.
Plasma Proteome Institute

ASMS Asilomar Meeting, Oct 14-18 2005

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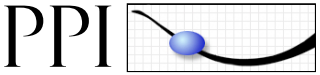
Good biomarkers have been found before, and with better tools it should be easy, right?



Many Important Existing Clinical Tests Measure Proteins in Plasma

Since when?	Decades
How many tests?	> 10 million/yr
What instruments?	50-100,000 machines in hospitals etc
How accurate?	CV~5-10% worldwide at 100pg/ml

Cardiac damage	TnI, CK-MB, Mb, MPO, BNP
Cancer	PSA, CA-125, Her-2
Inflammation	CRP, SAA, cytokines, RF
Liver Damage	ALT, ALP, AST, GGT (enzyme assays)
Coagulation	AT-III, proteins C&S, fibrinogen, VWF
Allergy	IgE against various antigens
Infectious disease	HIV-1, Hepatitis BsAg

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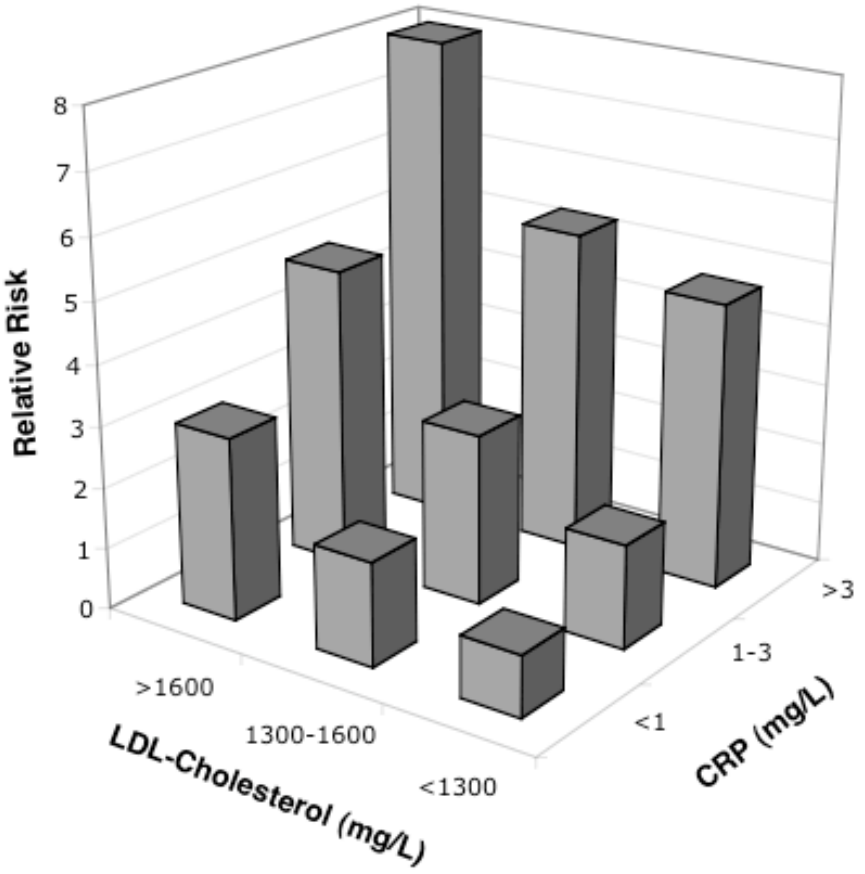
Often One Marker Is Not Enough: Two or More Together Are Better



Data replotted from Rifai, N. & Ridker, P. M. (2003). *Clin Chem* 49, 666-669.

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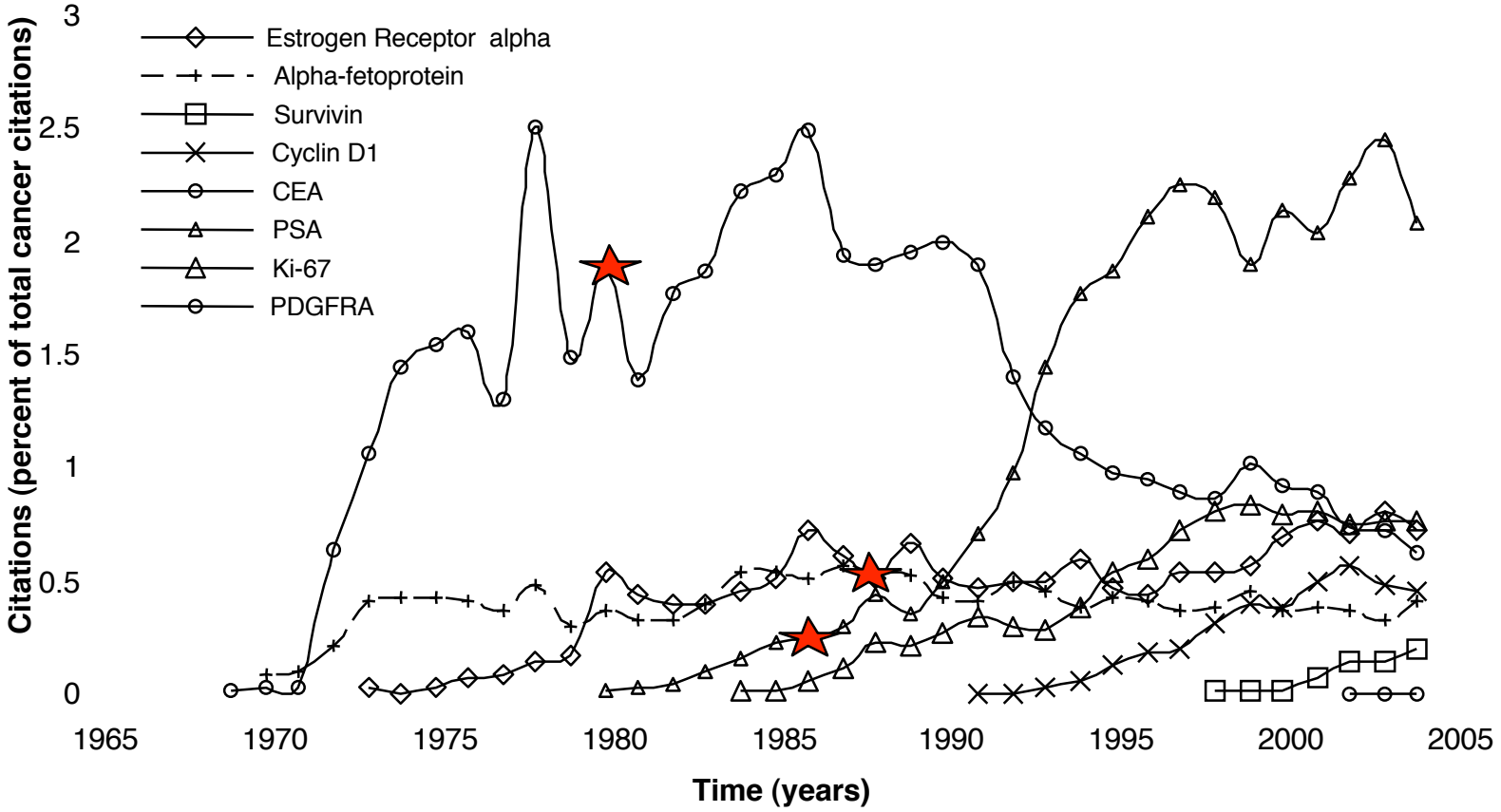
Timescale for Diagnostic Marker Development Has Been Long: Cancer Markers



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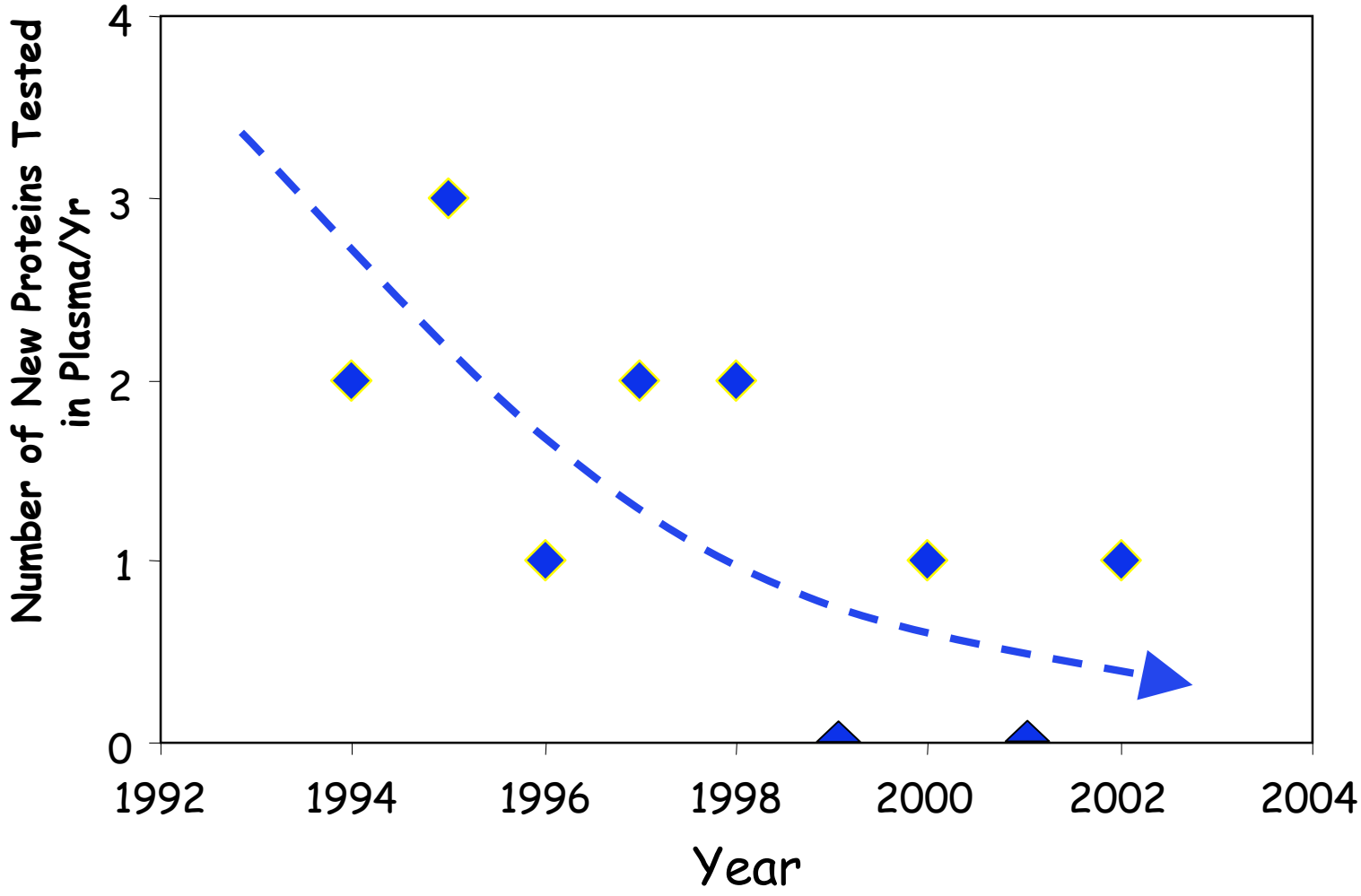
Introduction of New Clinical Biomarkers is Slowing

New FDA-Approved (CLIA) Diagnostic Protein Tests in Serum/Plasma Declined for the Last Decade



From: The human plasma proteome: history, character, and diagnostic prospects. Anderson, N. L. Anderson, N. G., Mol Cell Proteomics (2002) 1:845-67.

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"The appealing notion that research advances travel from bench to bedside is laudable, but conceptually flawed. Even though the U.S. Congress fully anticipates that funding to the National Institutes of Health (NIH) will result in advances in clinical medicine and that other forces, presumably non-governmental, will translate the latest in exciting science into health technologies, under the system of healthcare we have today, this advancement is not likely to happen."

Why are we focused on plasma?

The Plasma Proteome Is Unique

Picograms to Megagrams

- Samples the imports and exports of nearly all cells in the body
- Large dilution volume:
  - ~2.5L plasma,
  - ~12L extracellular fluid
- 22+ million liters are fractionated annually to make human therapeutic proteins
- >1 million patients receive plasma protein products
- Largest products:
  - human serum albumin (50,000 kg/yr in US)
  - intravenous immunoglobulin (25,000 kg/yr in US
- Temporally dynamic:
  - rapid clearance of proteins <60kd
  - albumin halflife of 21 days
- Collected routinely for diagnosis

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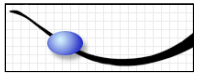
The plasma proteome is a hard one…



Plasma is the largest, and deepest, version of the human proteome

- Largest = Most proteins
- Deepest = Widest dynamic range

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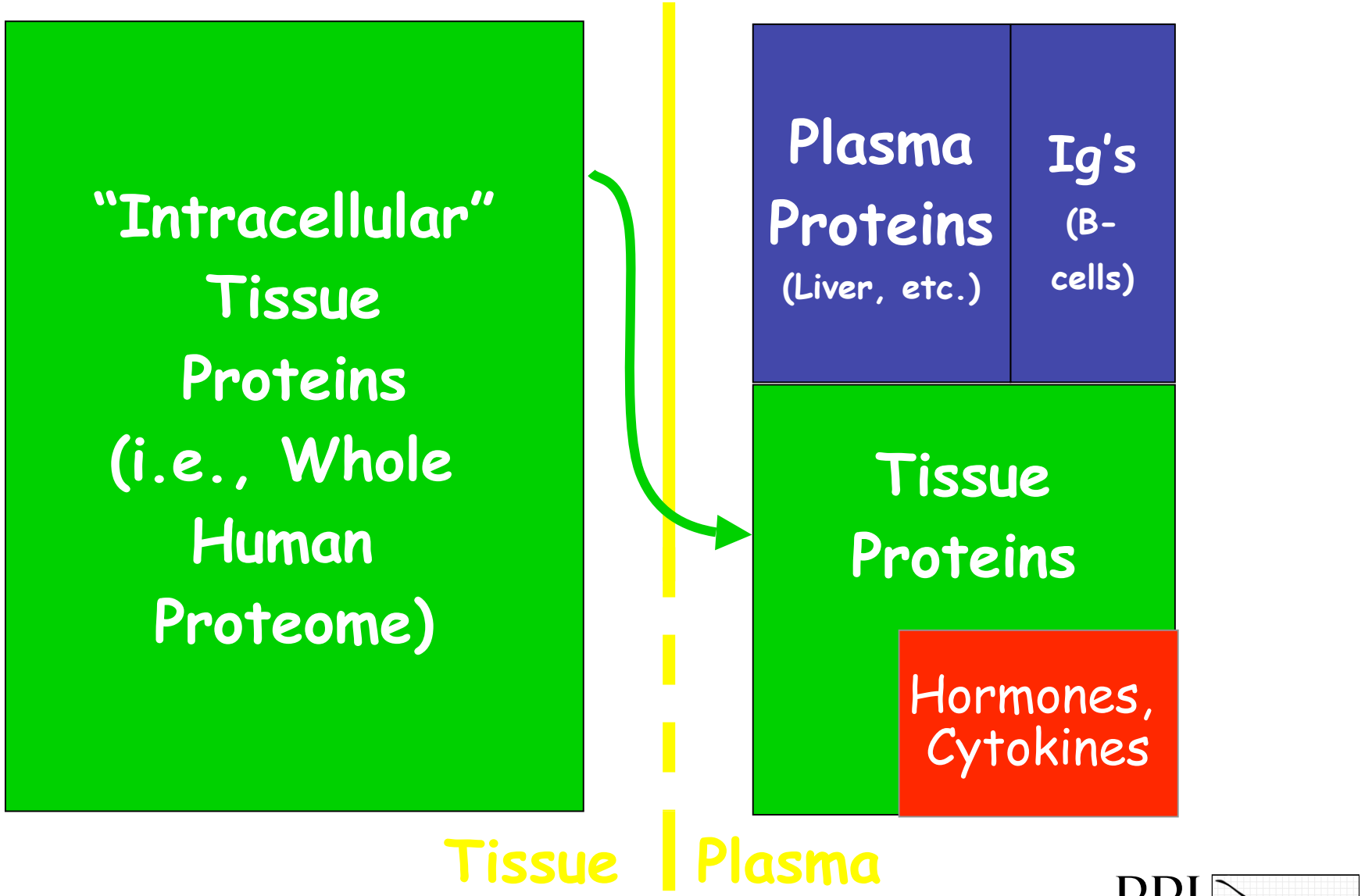
Tissues Leak Proteins: Any Human Protein May Be Detectable in Plasma at Some Level



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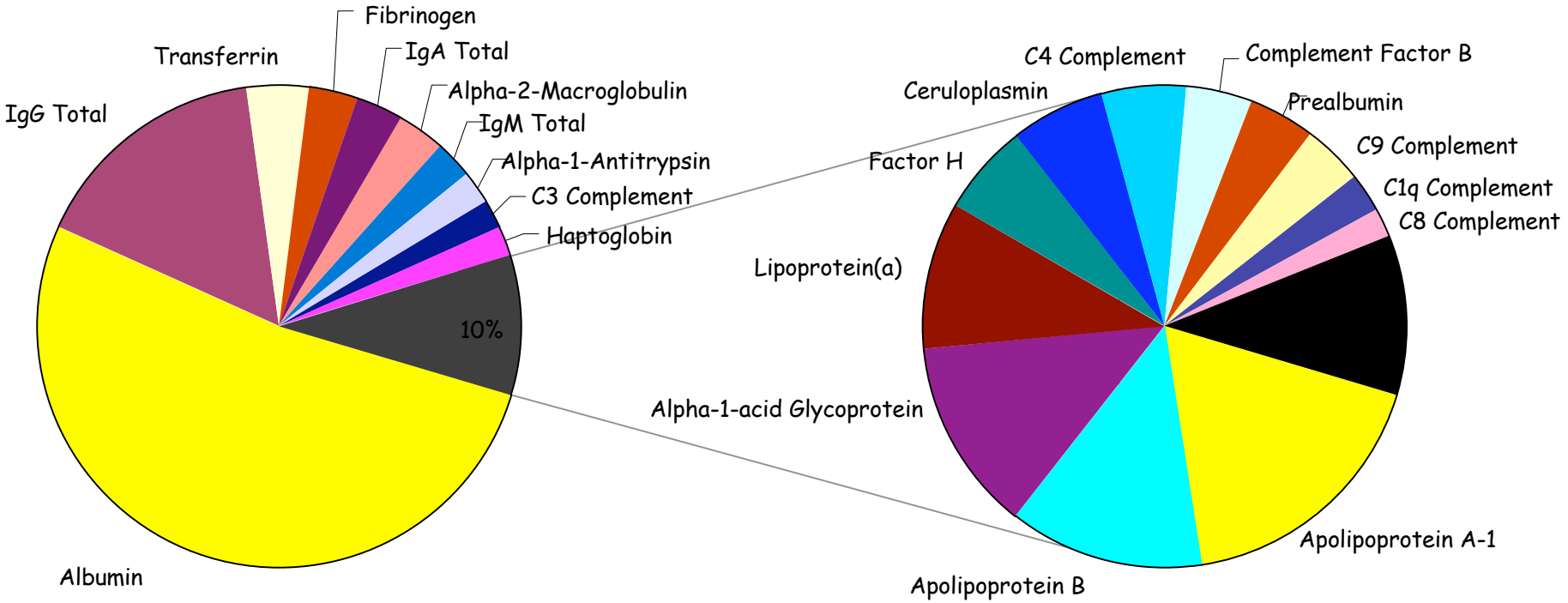
A Small Number of Proteins Make Up the Top 99% of Plasma by Mass



0 - 90%

(10 proteins)

90 - 99%

(12 proteins)

The human plasma proteome: History, character, and diagnostic prospects. Anderson, N.L. and Anderson, N.G., Molecular and Cellular Proteomics, 1.11, 845-867 (2002)

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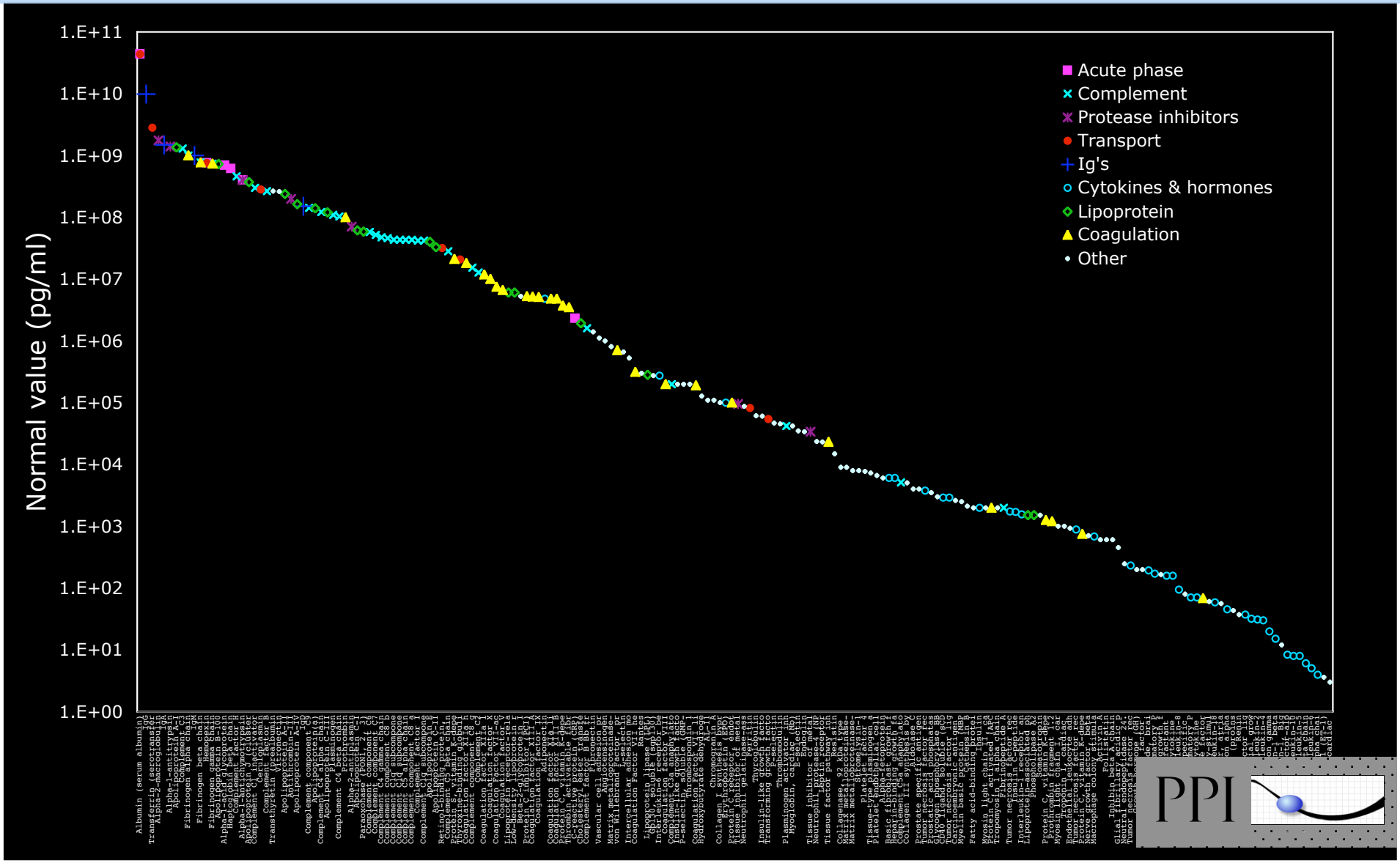
Proteins Measured Clinically in Plasma Span > 10 Orders of Magnitude in Abundance

(199 proteins, literature values)



Detection Limits in Clinical Diagnostics

for a 50 kdal protein analyte

Biomarker concentration	pg/ml	amol/ml	Molecules/ml
50 mg/ml	50,000,000,000	1,000,000,000	6.02E+17
10 mg/ml	10,000,000,000	200,000,000	1.20E+17
1 mg/ml	1,000,000,000	20,000,000	1.20E+16
100 ug/ml	100,000,000	2,000,000	1.20E+15
10 ug/ml	10,000,000	200,000	1.20E+14
1 ug/ml	1,000,000	20,000	1.20E+13
100 ng/ml	100,000	2,000	1.20E+12
10 ng/ml	10,000	200	1.20E+11
1 ng/ml	1,000	20	1.20E+10
100 pg/ml	100	2	1.20E+09
10 pg/ml	10	0.2	1.20E+08
1 pg/ml	1	0.02	1.20E+07

Immuno assays

QqQMS given 1000-fold enrichment

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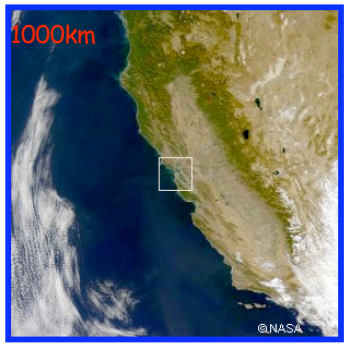
10^{10} Really Is Wide Dynamic Range

(Here on a linear scale)

10



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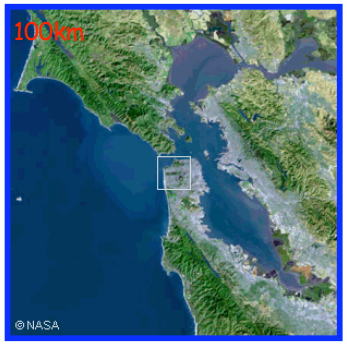

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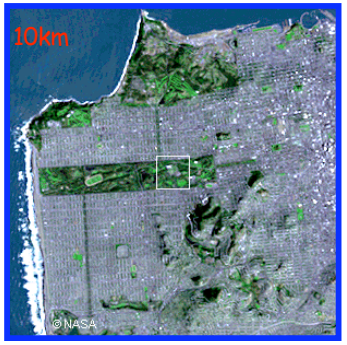

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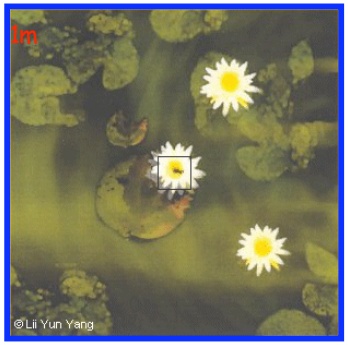

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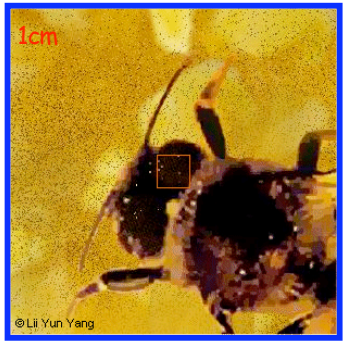

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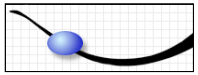
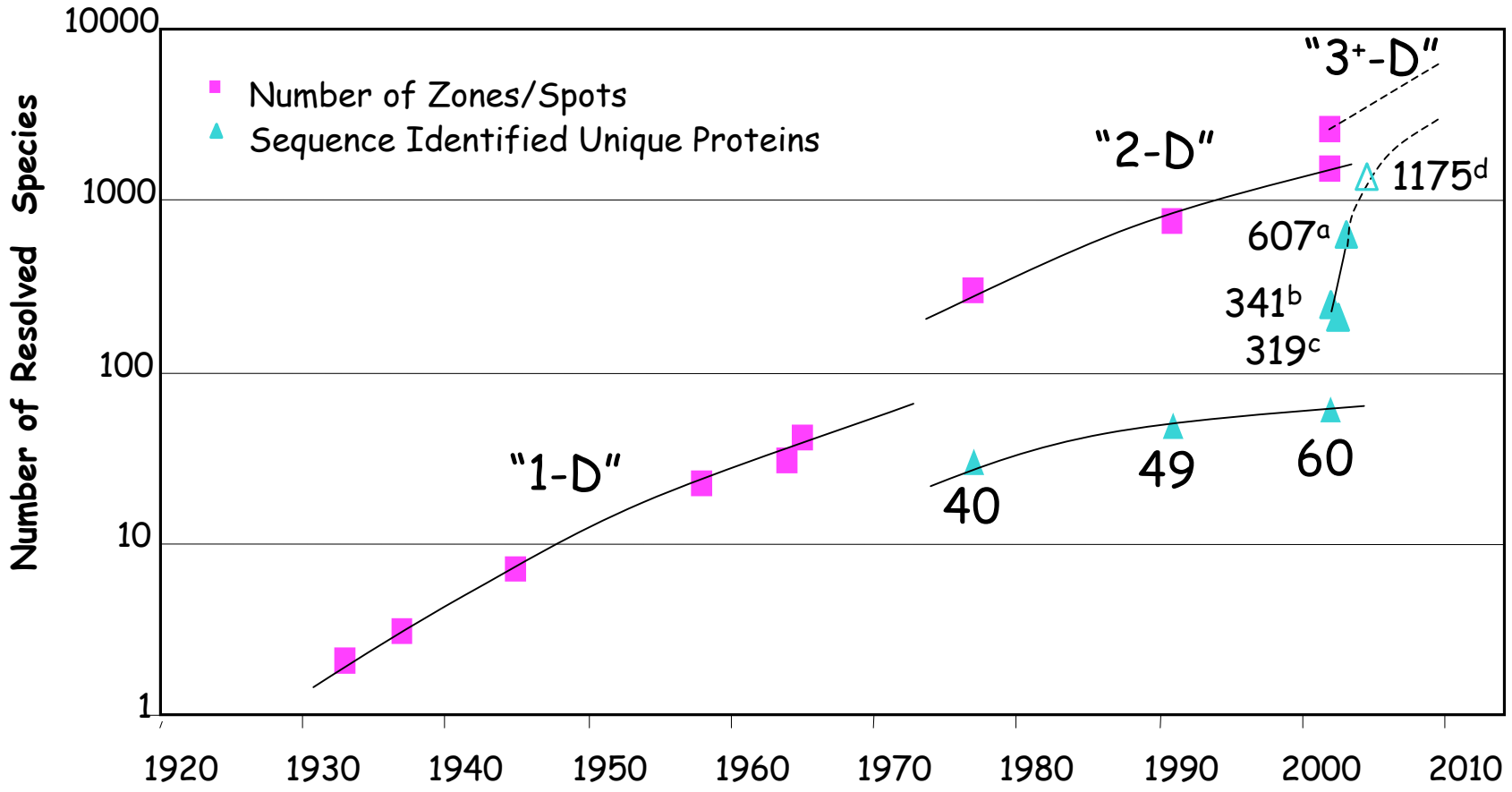
Slide courtesy Bruno Domon, ETH Zurich

How does proteomics technology stack up against this problem?



History of Plasma Proteomics



- a. J. N. Adkins, et al, *Mol Cell Proteomics* **1**, 947-55.
- b. R. S. Tirumalai, et al, *Mol Cell Proteomics* **2**, 1096-103.
- c. R. Pieper, et al, *Proteomics* **3**, 422-32.
- d. H_Plasma_NR-v2

The human plasma proteome: History, character, and diagnostic prospects. Anderson, N.L. and Anderson, N.G., Molecular and Cellular Proteomics, 1.11, 845-867 (2002)

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The State of the Art in Plasma Proteomics c. 1976: 2-D Gels

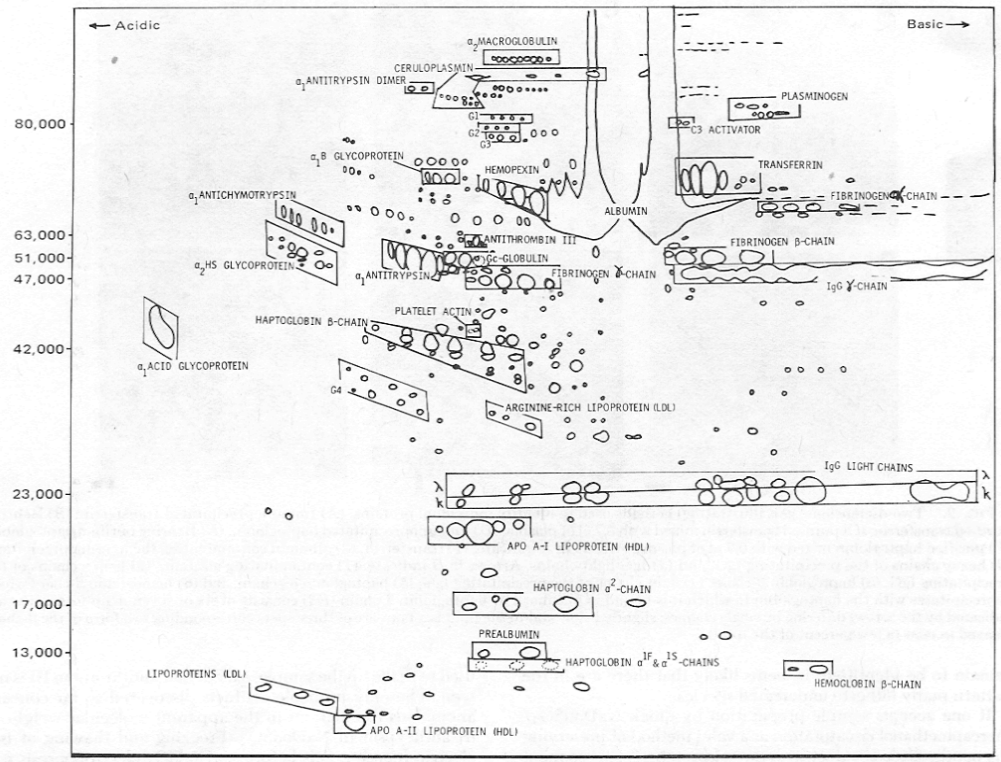

FIG. 3. Diagram drawn from the gel shown in Fig. 1, and labeled to indicate positions of known plasma proteins. Hemopexin and the C3-activator are somewhat obscured by albumin overloading. Ceruloplasmin appears to be present in two major and two minor forms (all between 80,000 and 90,000 daltons), each present as a row of four or more dots due to sialic acid heterogeneity. The highest molecular weight form interacts strongly with the albumin precipitate, while the others do not. Plasminogen exists in two forms: the Glu-form (upper horizontal row of dots) and the Lys-form (lower row, more basic) (19). Gc-globulin can be present as three spots; the left-hand pair appears to correspond to type 1, and the right-hand spot to the type 2 allele. The immunoglobulin light chains (κ and λ) are partially resolved (20) and show similar isoelectric distributions. Identification of the lipoproteins is based on the presence of spots in certain of the low (LDL) and high (HDL) density lipoprotein fractions, as well as similarity to isolated materials for the arginine-rich and apo A-I lipoproteins. Platelet actin, Gc-globulin spot 3, and the haptoglobin α^{1F} and α^{1S} chains are shown although they were not present in the sample run in Fig. 1. As yet unrecognized glycoproteins G1, 2, 3, and 4 are labeled for use in the *text*. The hemoglobin α-chain is too basic to appear in a separation with these ampholytes.

Anderson,L., Anderson,N. G. High resolution two-dimensional electrophoresis of human plasma proteins. (1977) PNAS 74, 5421-5

2-D Electrophoresis

300+ resolved spots

40 identified proteins

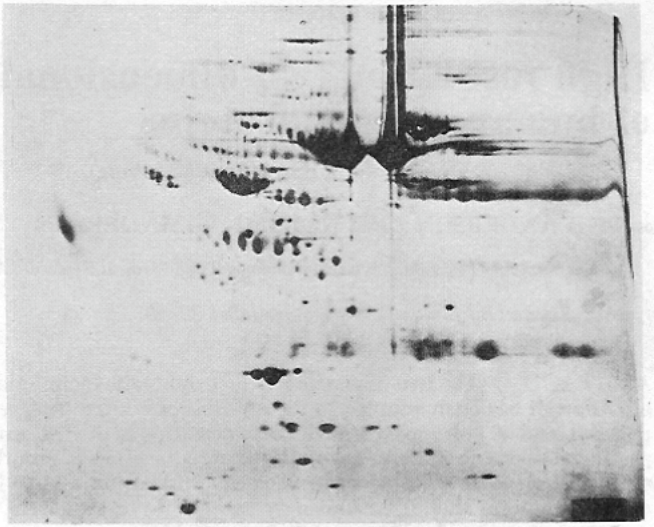

FIG. 1. Two-dimensional gel of human plasma proteins. The sample was 10 μl of fresh heparinized plasma denatured in Na-DodSO$_4$/mercaptoethanol.

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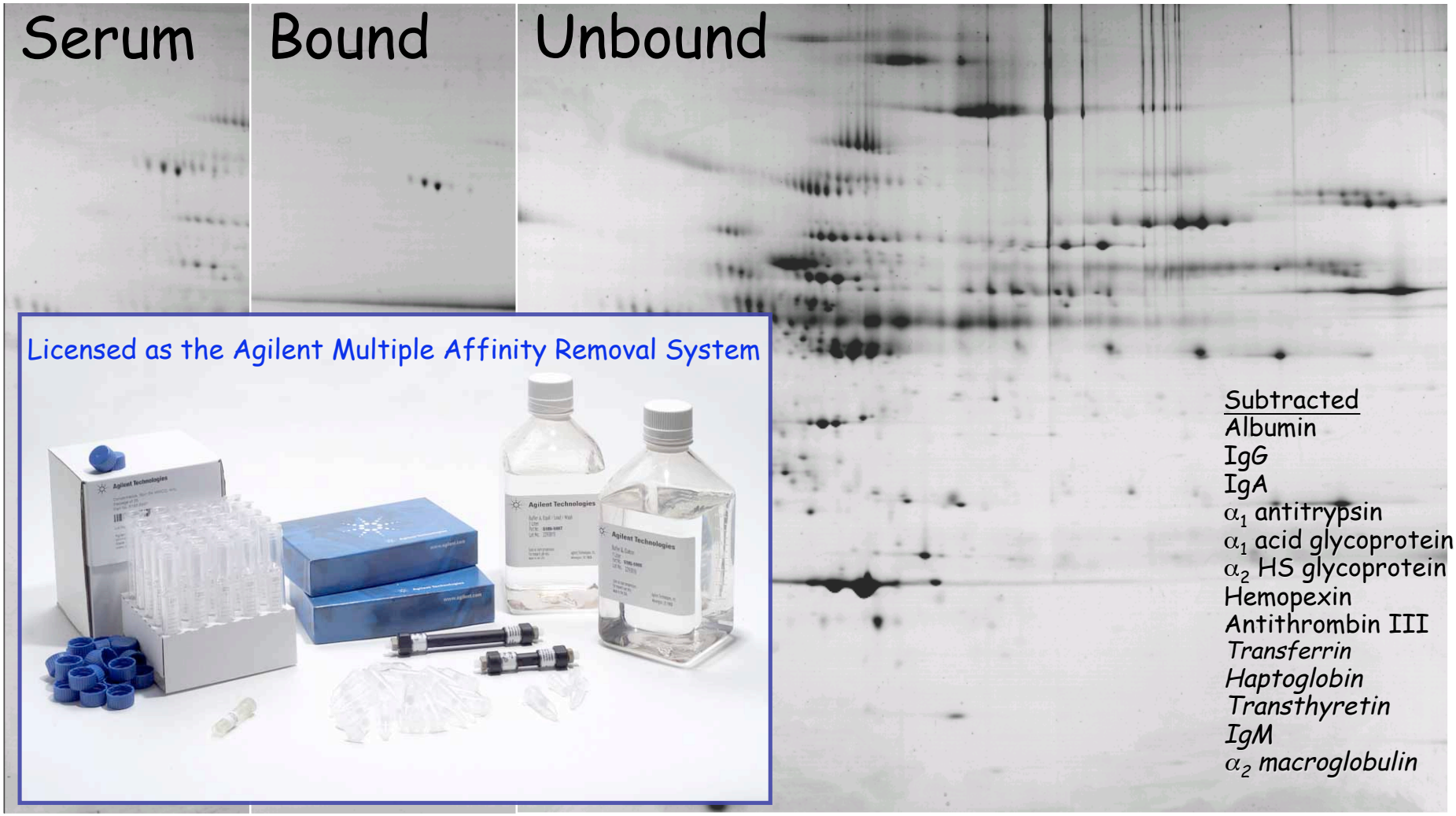
Antibody Affinity Subtraction as a Starting Point for Serum Fractionation



Multi-Component Immunoaffinity Subtraction Chromatography, An Innovative Step Towards A Comprehensive Survey Of The Human Plasma Proteome,
Rembert Pieper, et al, Proteomics, (2003) *Proteomics* **3,** 422-32.

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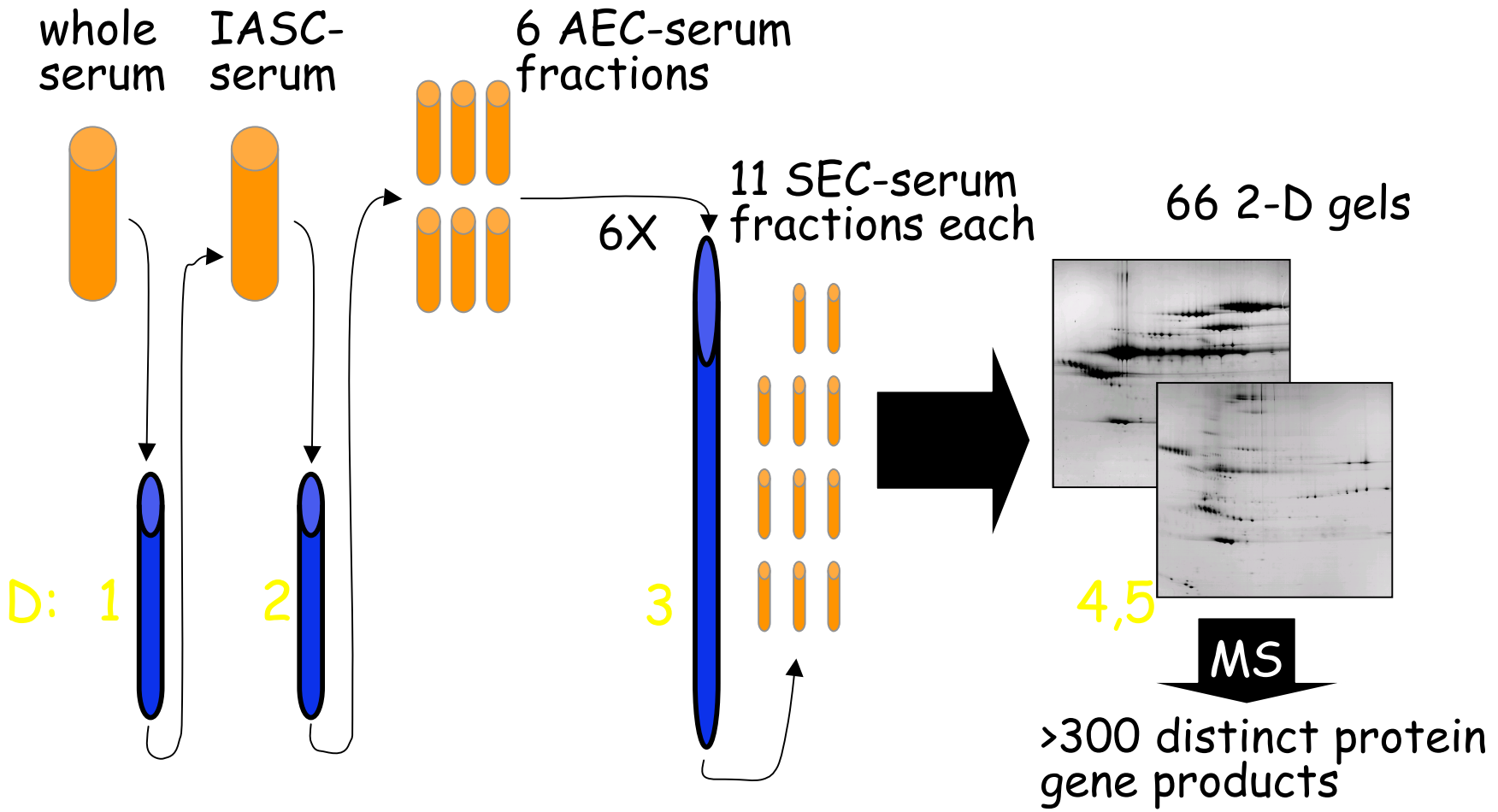
Multi-Dimensional 2DE-Based Plasma Biomarker Discovery Proteomics

(e.g., 3-D Chromatography + 2-DE + LC/MS)



The human serum proteome: Display of nearly 3700 chromatographically separated protein spots on two-dimensional electrophoresis gels and identification of 325 distinct proteins. Pieper, R., et al Proteomics 3(7): 1345-64. (2003).

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Different Technologies Provide Different Views of the Human Plasma Proteome

- Four datasets compared:
  - Base list of ~450 proteins reported in "non-proteomics" literature as measured/detected in plasma or serum
  - Three sets of 300-600 proteins each from proteomics surveys (2-D gels + MS/MS; LC/LC-MS/MS)
- Made non-redundant using methods of genomics
  - Redundancy definition: >95% homology over >=15 amino acid subsequence
- Questions:
  - What is the overlap between different methods?
  - Can low-abundance proteins be detected in surveys?
  - Can a useful plasma database be collected?

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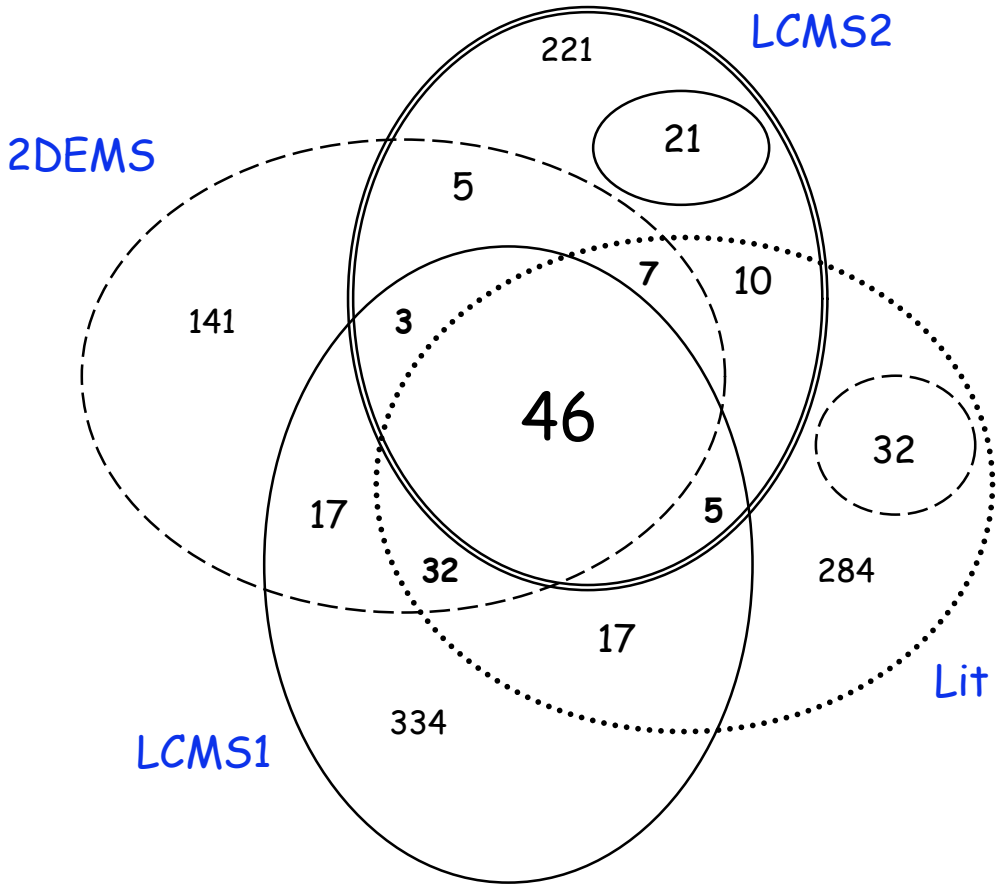
Overlap of Four Plasma Proteome Datasets

(Number of NR proteins)



- 46 proteins in all four lists
- 195 proteins in 2 or more lists
- 1175 NR proteins total

From: The Human Plasma Proteome: A Non-Redundant List Developed by Combination of Four Separate Sources, N. L. Anderson et al, Molec. Cell Proteomics, 3: 311-326 (2004).

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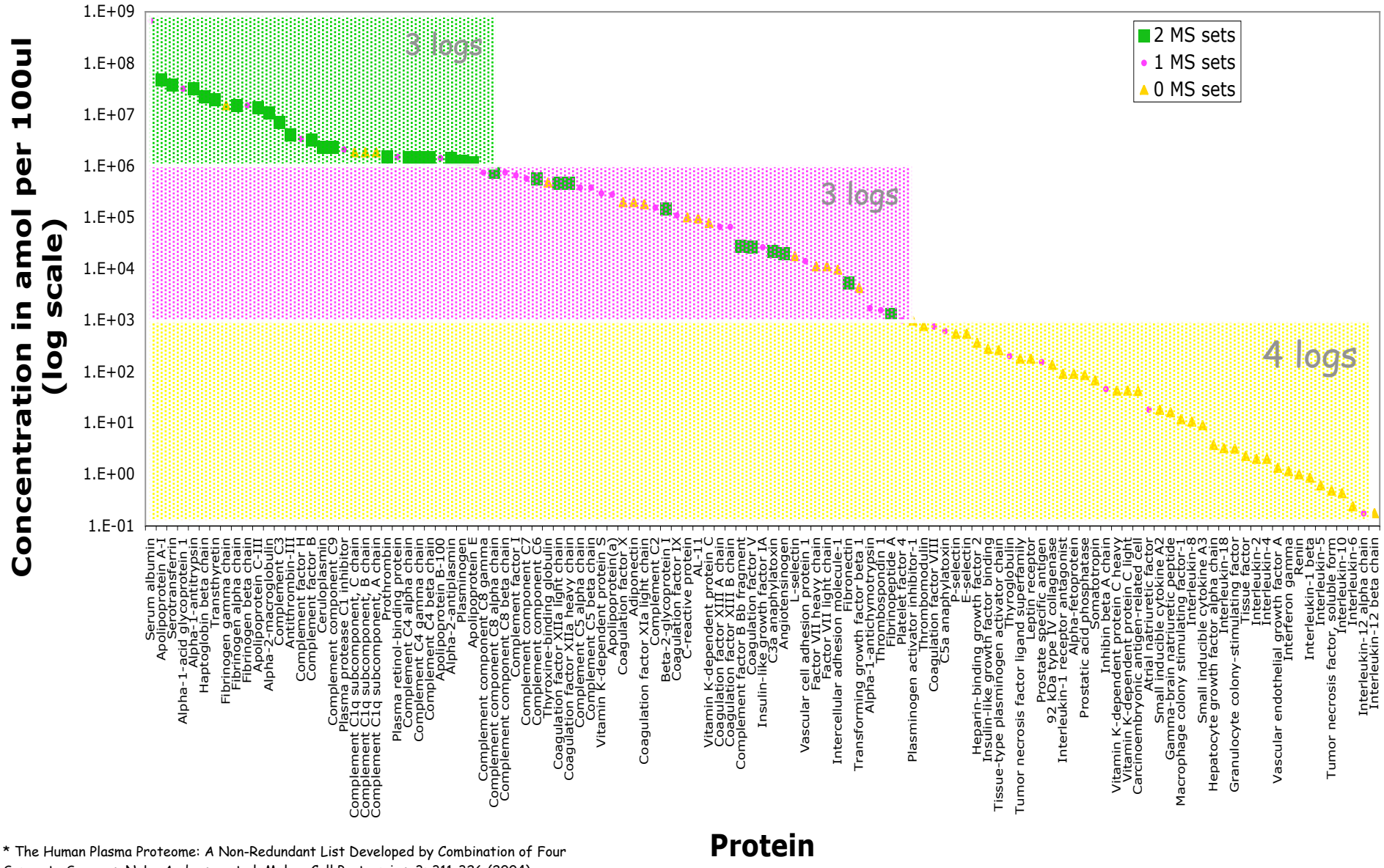
Plasma Proteome Surveys Detect Primarily Higher Abundance Proteins



* The Human Plasma Proteome: A Non-Redundant List Developed by Combination of Four Separate Sources, N. L. Anderson et al, Molec. Cell Proteomics, 3: 311-326 (2004).

Challenges and Limitations of Plasma Discovery Proteomics

- So far, there is no comprehensive exploratory proteomics platform for plasma
- Multi-dimensional fractionation is crucial to penetrate deeper into the abundance distribution
  - However this multiples analytical effort, increasing cost and decreasing sample numbers
- Adapting/confirming candidates between platforms is challenging
- Use of multiple platforms significantly enhances chance of finding new markers
- Where possible, it may make more sense to discover the markers in tissues or non-plasma fluids where they occur at higher concentrations… but we ultimately need to measure them in plasma

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What kind of pipeline could translate the results of disparate plasma biomarker discovery efforts into In Vitro Diagnostics (IVD)?

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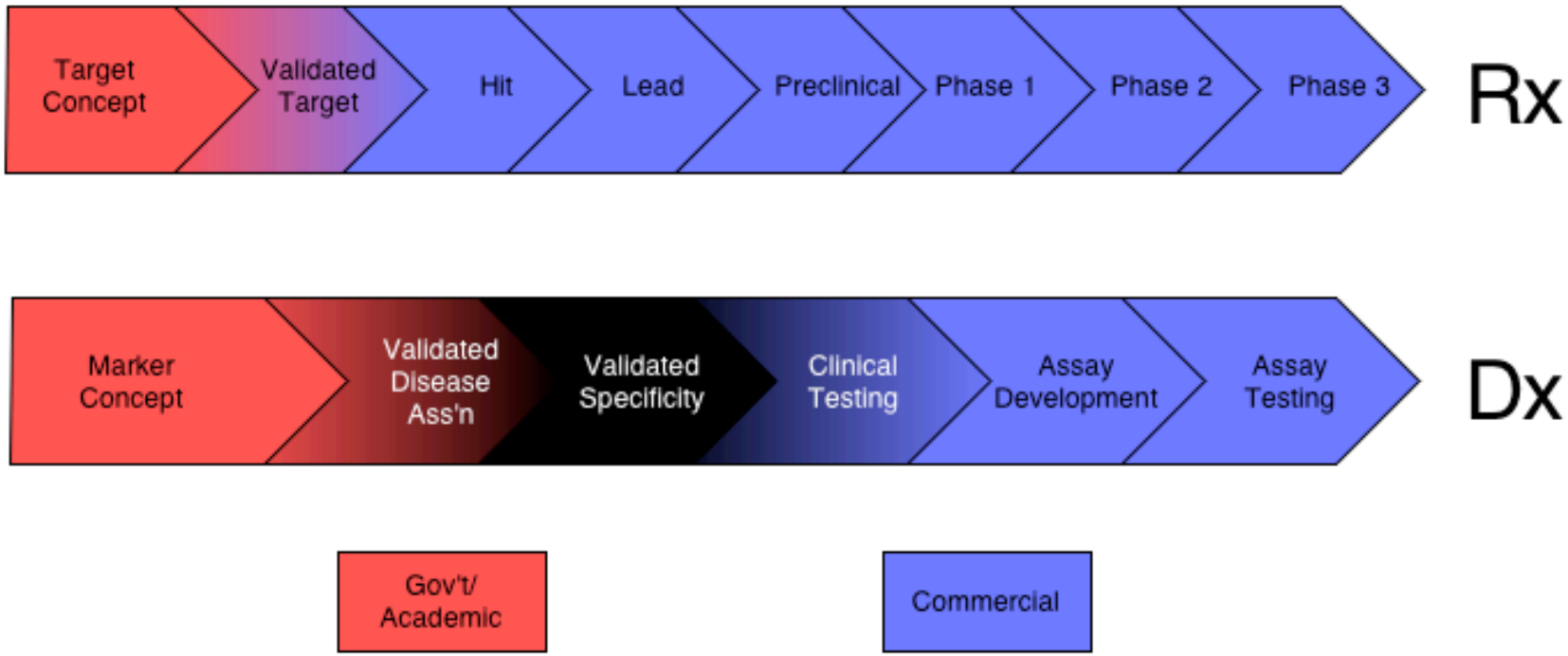
At Present We Don't Really Have a Pipeline



- Dx industry does not cover discovery or validation as Rx does (Dx scale = Rx * 0.05)
- NIH has not funded marker validation
- A major resource gap has developed in the Dx pipeline at the point of marker validation

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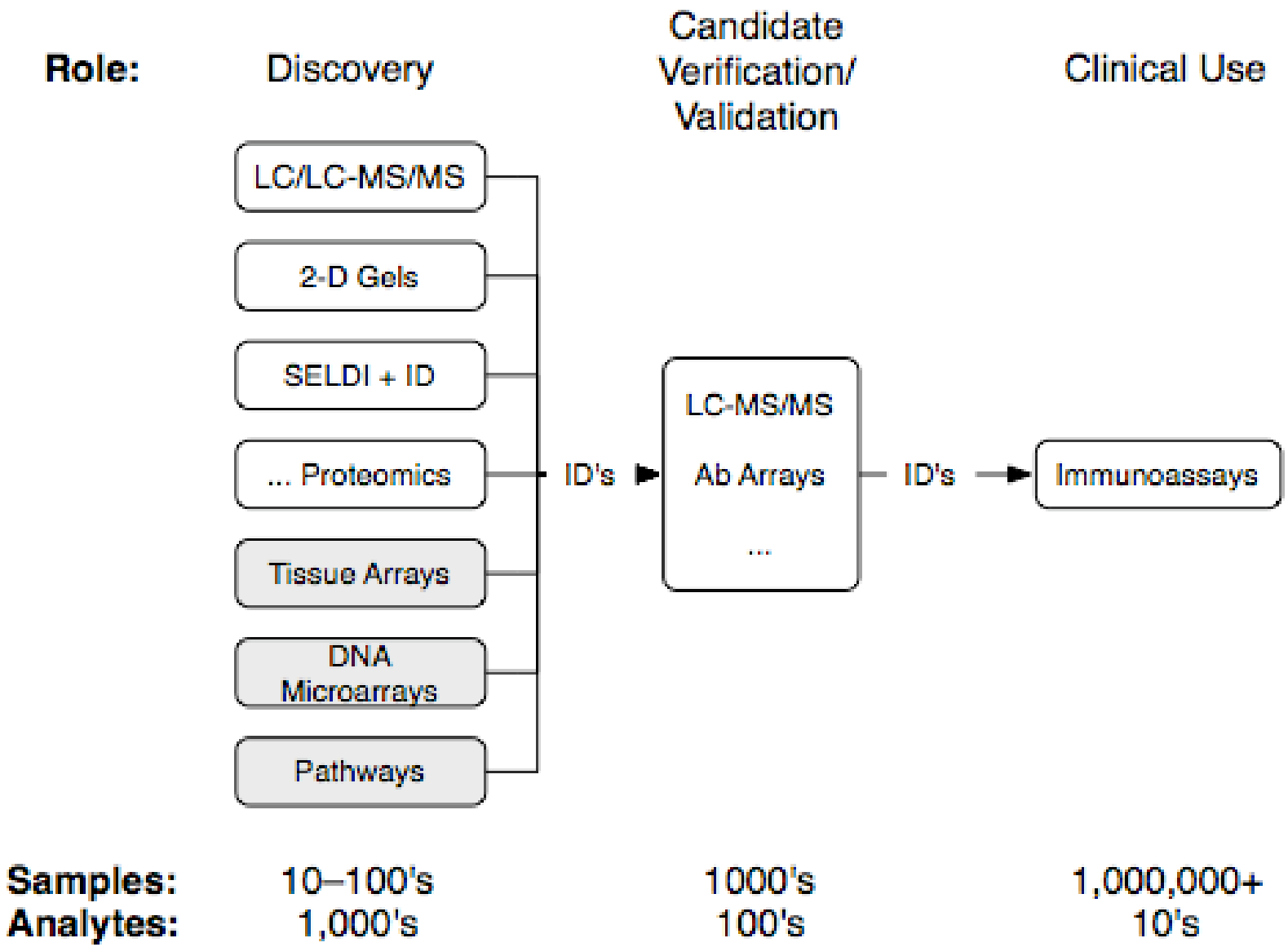
Proposed Three-Stage Dx Pipeline



Biomarker validation is where the rubber meets the road

- Is your marker really associated with the target disease in real-world samples?

- Is the signal strong enough relative to underlying biological variation (including all the other diseases)?

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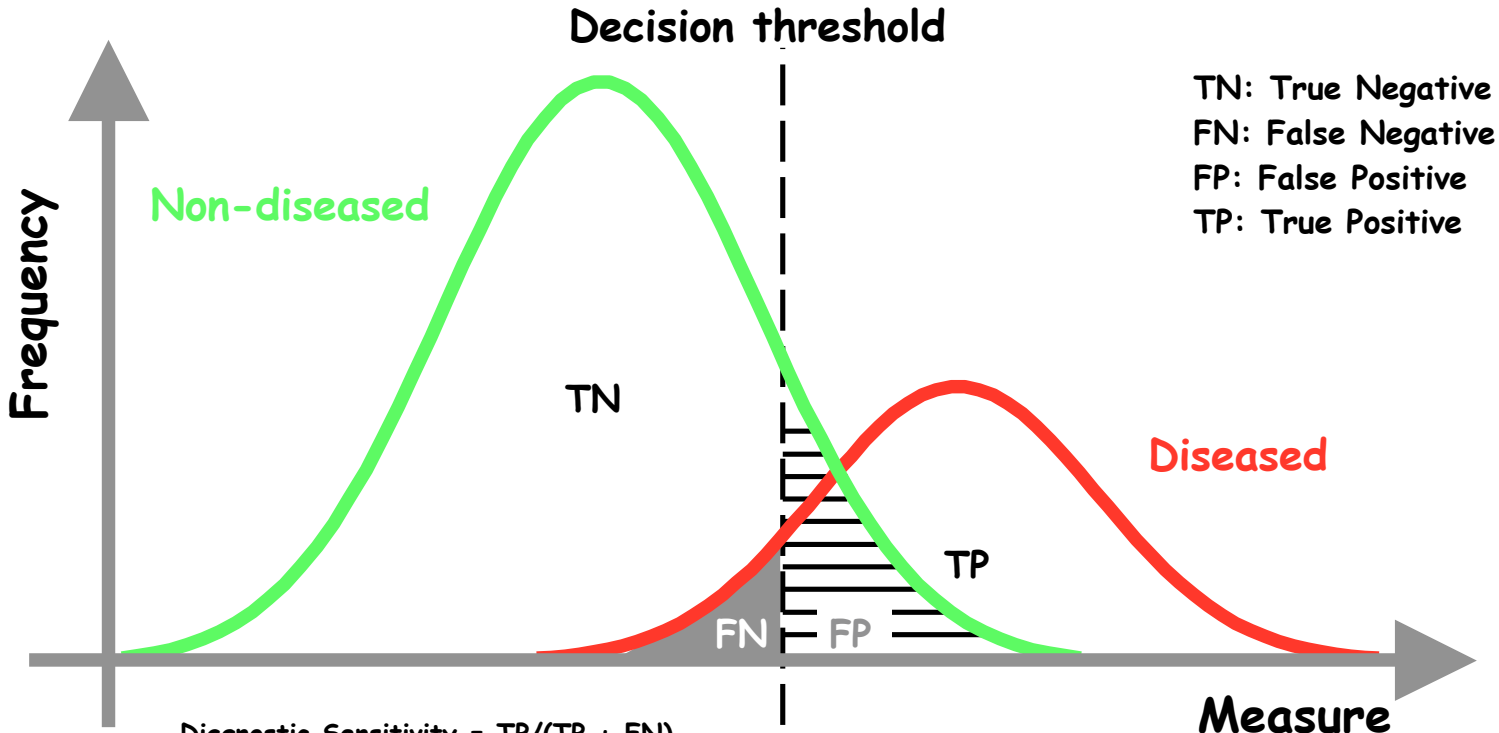
Parameters of Diagnostic Tests



Diagnostic Sensitivity = TP/(TP + FN)
Diagnostic Specificity = TN/(TN + FP)

Positive Predictive Value (PPV) = TP/(TP + FP)
Negative Predictive Value (NPV) = TN/(TN + FN)

Disease frequency ($AUC_{diseased}/AUC_{non\text{-}diseased}$) affects PPV and NPV

Proteomics: From Basic Research to Diagnostic Application. A Review of Requirements & Needs
Vitzthum, Behrens, Anderson, and Shaw, 2005, J Proteome Res, in press

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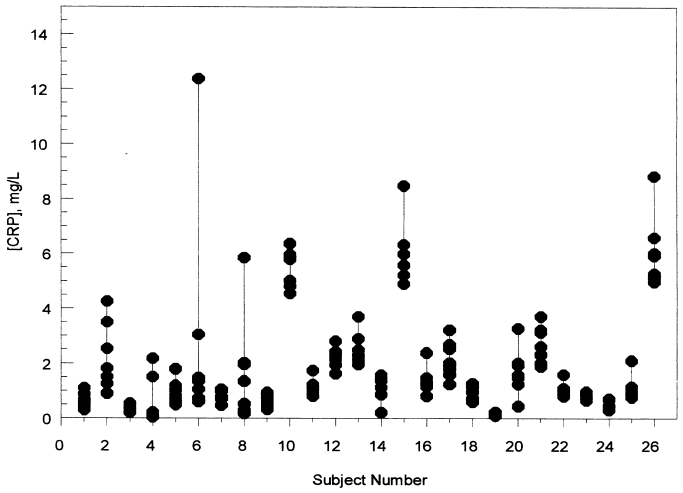
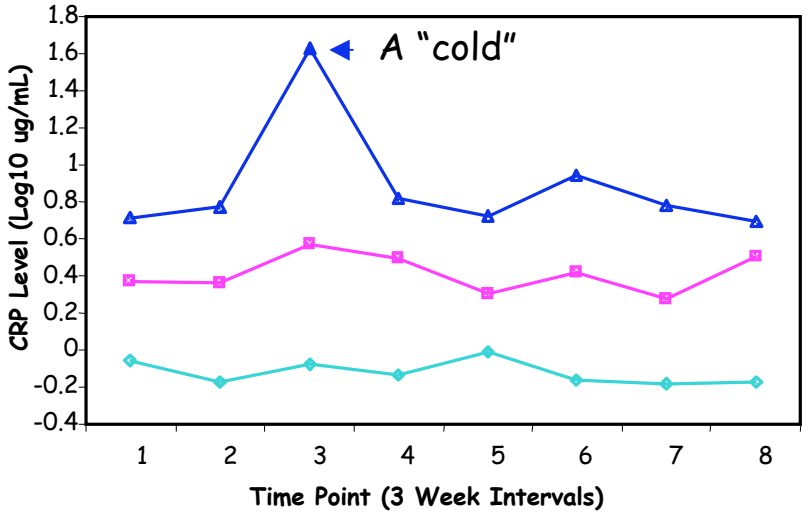
Biovariability: Critical Parameters of Candidate Biomarkers Can Only Be Measured in Populations

- Many proteins have very stable level in an individual, even when the population shows a wide abundance distribution
- A disease relationship may be detected in an individual over time that is not apparent in comparing one sample with a population





Macy, E. M., Hayes, T. E. and Tracy, R. P., Variability in the measurement of C-reactive protein in healthy subjects implications for reference intervals and epidemiological applications. Clin. Chem. 43, 52-8 (1997)

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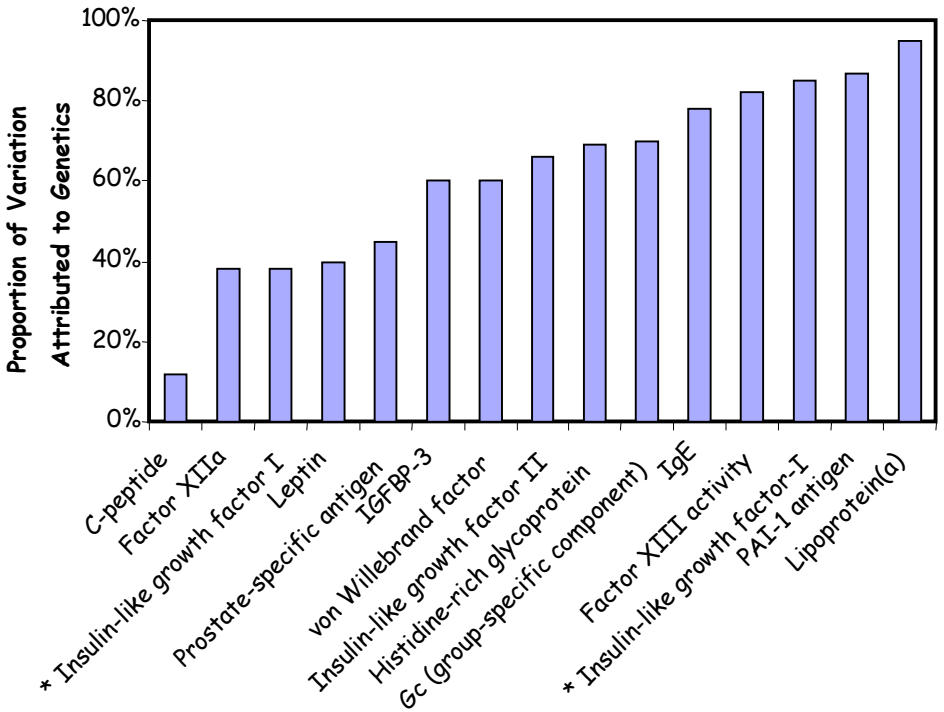
Biovariability: Critical Parameters of Candidate Biomarkers Can Only Be Measured in Populations

- Measured heritability of plasma concentrations range from 5% to 95% (average 50%)
- Highly heritable markers provide essentially a genetic measurement
- Low heritability markers provide essentially a phenotype measurement
- The difference between genetic and disease phenotype measurements may be critical in evaluating biomarker value



The human plasma proteome: History, character, and diagnostic prospects. Anderson, N.L. and Anderson, N.G., Molecular and Cellular Proteomics, 1.11, 845-867 (2002)

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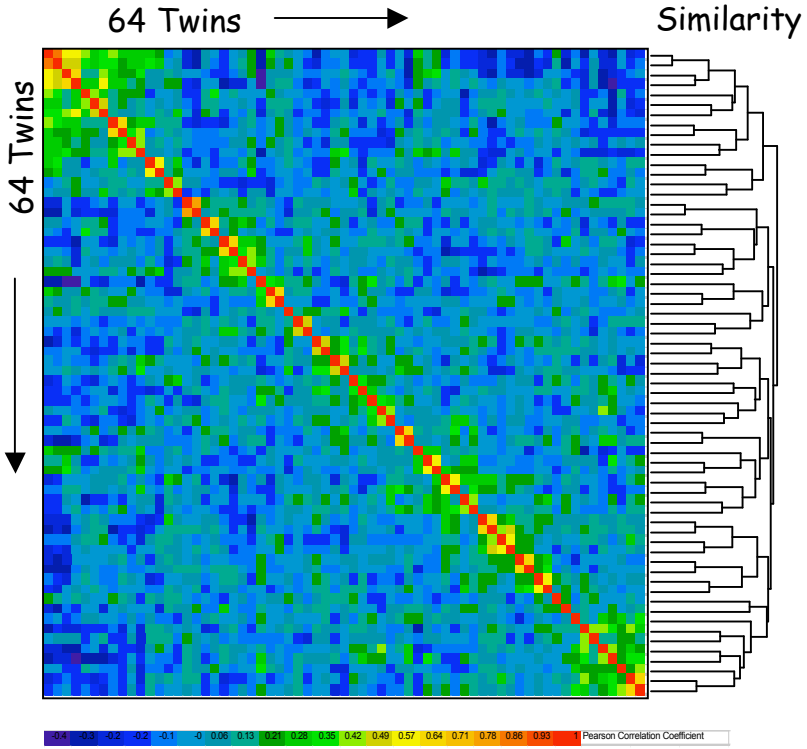
Biovariability: Critical Parameters of Candidate Biomarkers Can Only Be Measured in Populations

- Using only abundances of major plasma proteins, 90% of pairs of MZ twins can be correctly clustered together

- Genotype matters…..



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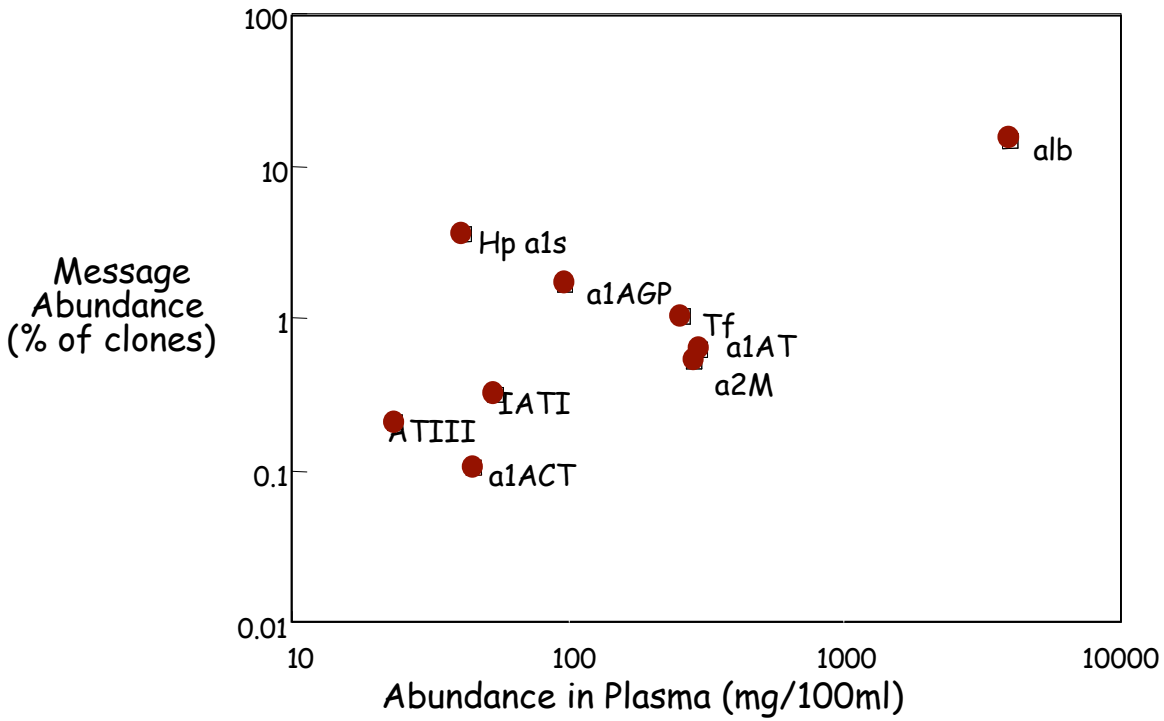
Gene Expression vs Protein Abundance in Plasma

- Almost no correlation with liver mRNA levels



Data reploted from: Kawamoto, S., Matsumoto, Y., Mizuno, K., Okubo, K. and Matsubara, K., *Gene* 1996, 174, 151-158.

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Biomarker validation, in the context of a Dx pipeline, requires pools of candidates and sensitive, high-throughput specific assays

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There Are More Existing Candidate Disease Markers Than You Might Think: 177 Candidate Cardiovascular Disease Marker Proteins

	Name	Accession	Normal Concentration (pg/ml)	Source for concentration	Reason
1	activin A	P08476	6.0E+02	(Eldar-Geva *et al.*, 2001)	Released by heparin from vascular endothelium (Phillips *et al.*, 2000)
2	adiponectin (ADPN)	Q15848	4.8E+06	(Mallamaci *et al.*, 2002)	Higher levels in essential hypertensives (Mallamaci *et al.*, 2002)
3	albumin	P02768	4.1E+10	(Laboratories, 2001)	Negative acute phase reactant, lower levels associated with increased risk of cardiovascular mortality (Shaper *et al.*, 2004)
4	aldolase C	P09972	4.0E+03	(Asaka *et al.*, 1990)	A more specific and sensitive marker of cerebrovascular diseases than aldolase A (Asaka *et al.*, 1990)
5	alpha 2 antiplasmin (alpha 2 AP)	P08697	7.0E+07	Progen test insert	An important regulator of the fibrinolytic system
6	alpha 2 macroglobulin (alpha 2 M)	P01023	1.8E+09	(Laboratories, 2001)	Major plasma protease inhibitor
7	alpha(1)-antichymotrypsin (ACT)	P01011	4.2E+07	(Putnam, 1975)	Major plasma protease inhibitor
8	alpha1 acid-glycoprotein (AAG)	P02763	6.9E+08	(Laboratories, 2001)	Acute phase reactant
9	alpha1-antitrypsin (AAT)	P01009	1.4E+09	(Laboratories, 2001)	Major plasma protease inhibitor
10	angiotensin-converting enzyme (ACE)	P12821			Lower in stroke patients than controls (Catto *et al.*, 1996)
11	angiotensinogen	P01019	1.5E+06	(Bloem *et al.*, 1995)	Precursor of major blood pressure control peptide
12	antithrombin III (AT III)	P01008	2.0E+08	(Kalafatis *et al.*, 1997)	Major inhibitor of thrombin
13	apolipoprotein A-I	P02647	1.4E+09	(Glowinska *et al.*, 2003)	Low level associated with mortality and myocardial infarction five years after CABG(Skinner *et al.*, 1999)
14	apolipoprotein A-II	P02652	2.4E+08	(Luo & Liu, 1994)	Lipoprotein
15	apolipoprotein A-IV	P06727	1.6E+08	(Kondo *et al.*, 1989)	A relatively independent risk factor for CHD(Warner *et al.*, 2001)
16	apolipoprotein B	P04114	7.3E+08	(Glowinska *et al.*, 2003)	Major component of LDL
17	apolipoprotein C-I	P02654	6.1E+07	(Riesen & Sturzenegger, 1986)	Lipoprotein
18	apolipoprotein C-II	P02655	3.3E+07	(Bury *et al.*, 1986)	Lipoprotein
19	apolipoprotein CIII	P02656	1.2E+08	(Onat *et al.*, 2003)	marker of CHD independent of cholesterol(Onat *et al.*, 2003)
20	apolipoprotein D	P05090			Lipoprotein
21	apolipoprotein E	P02649	4.0E+07		presence of epsilon4 allele a strong independent predictor of adverse events (Brscic *et al.*, 2000)
22	apolipoprotein L1	O14791			Lipoprotein
23	aspartate aminotransferase, mitochondrial (m-type)	P00505			diagnostic for early detection of myocardial infarction (Yoneda *et al.*, 1992)
24	basic fibroblast growth factor (bFGF)	P09038	6.0E+03	(Song *et al.*, 2002)	sICAM-1level increases in acute cerebral infarction (Song *et al.*, 2002)
25	beta(2)-glycoprotein I, nicked	P02749			may control extrinsic fibrinolysis via a negative feedback pathway loop (Yasuda *et al.*, 2004)
26	B-type neurotrophic growth factor (BNGF)	P01138	7.0E+02	(Reynolds *et al.*, 2003)	Candidate stroke marker (Reynolds *et al.*, 2003)

From: Candidate-Based Proteomics in the Search for Biomarkers of Cardiovascular Disease, Leigh Anderson, J. Physiol., in press 2004

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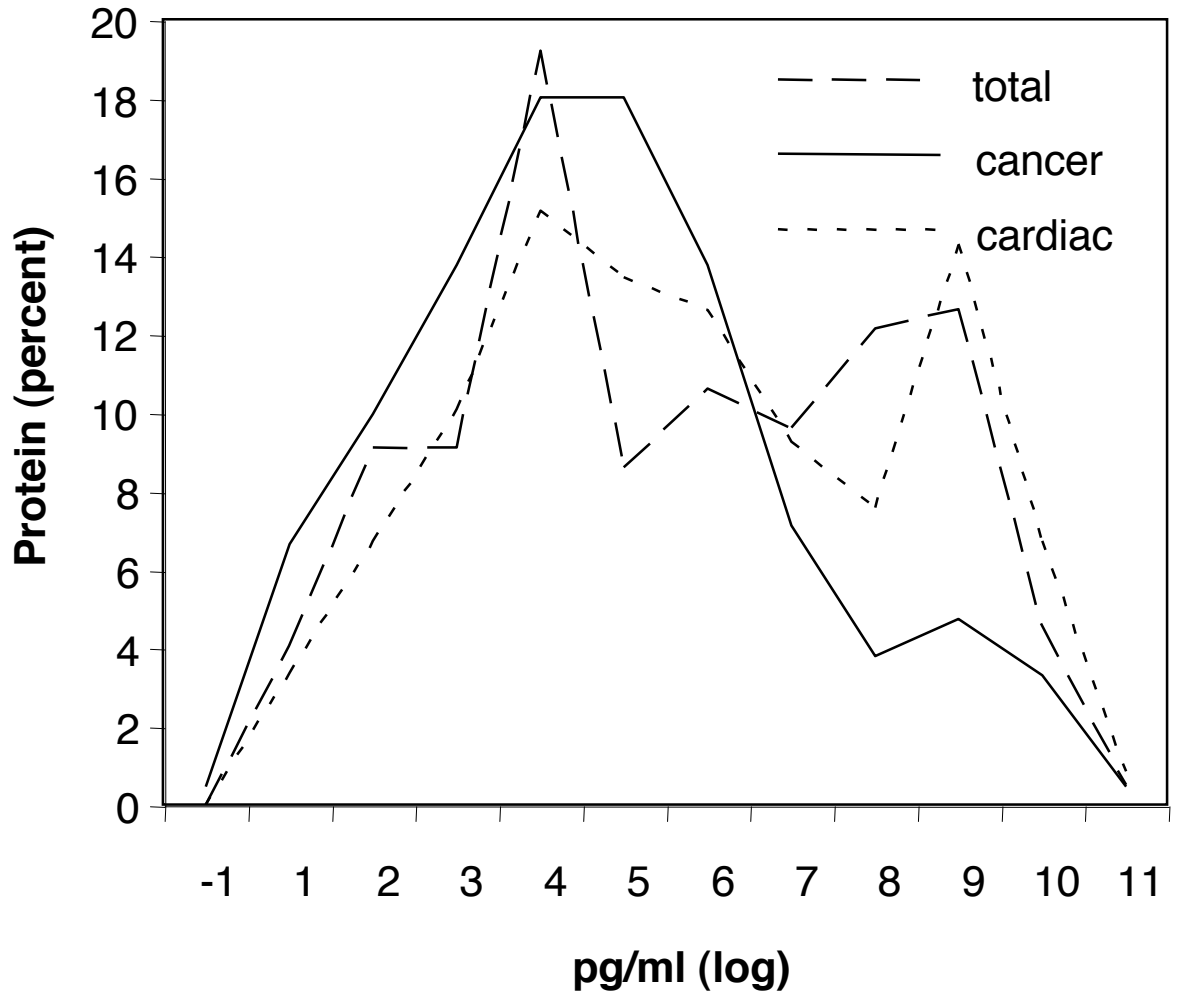
Candidate Cancer Markers Appear Distributed at Lower Plasma Concentrations Than Unselected Proteins or Cardiac Markers



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Technology Alternatives for Candidate-Based Proteomics

- Immunoassays (likely clinical test implementation)
  - Very sensitive
  - Expensive: IVD-quality assays cost $2-4 million
  - Specificity issues with less well-developed assays
  - Multiplexing limits in a single assay volume

- Hybrid MS-based assays
  - Peptide MS for quantitation and identification
  - Specific enrichment for sensitivity
  - Absolute analyte specificity
  - Multiplex 25-200 assays/analysis

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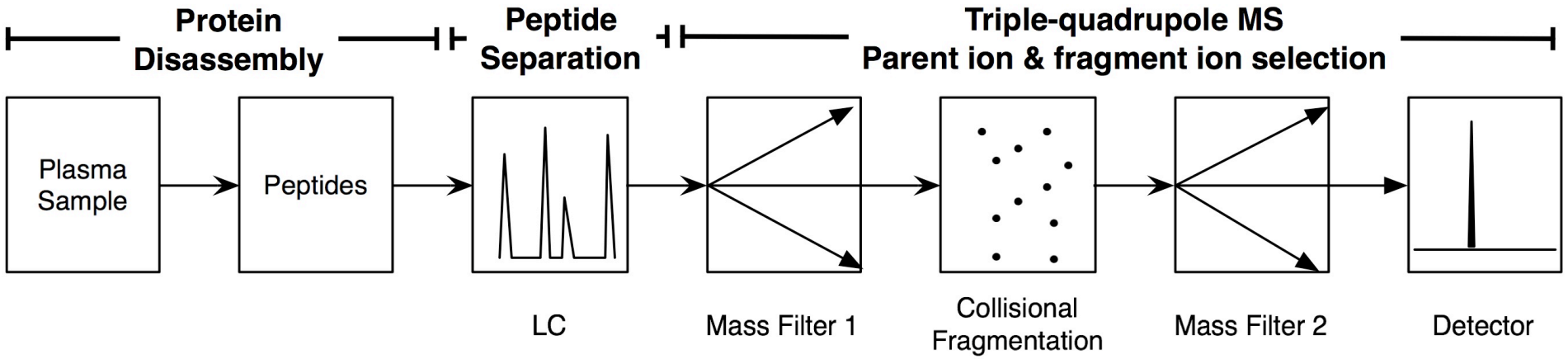
A General Approach to Protein Measurement via MS/MS

- Objective: direct design of protein assays from sequence, using peptides as measurement surrogates:
  - Variation of protein physical properties make them hard to address comprehensively
  - Postulate: within every protein (good,bad) there is ≥1 good tryptic peptide
  - Absolute quantitation using isotope dilution (stable isotope labeled peptide internal standards)



  - The triple-quadrupole MS (LC-MS/MS) platform is very widely used for small molecular assays in plasma (drug metabolites, inborn errors, pesticides)
  - Multiplex 100-200 assays per run

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Alpha-1-acid glycoprotein 1 peptide EQLGEFYEALDBLR 1742.8

Detected ions passing MS1 and MS2 (871.9/1186.6) over course of 180 minute LC peptide separation: single sequence-specific peak

MS1: 871.9 ± 1 amu
Whole peptide, +2 charge

↓

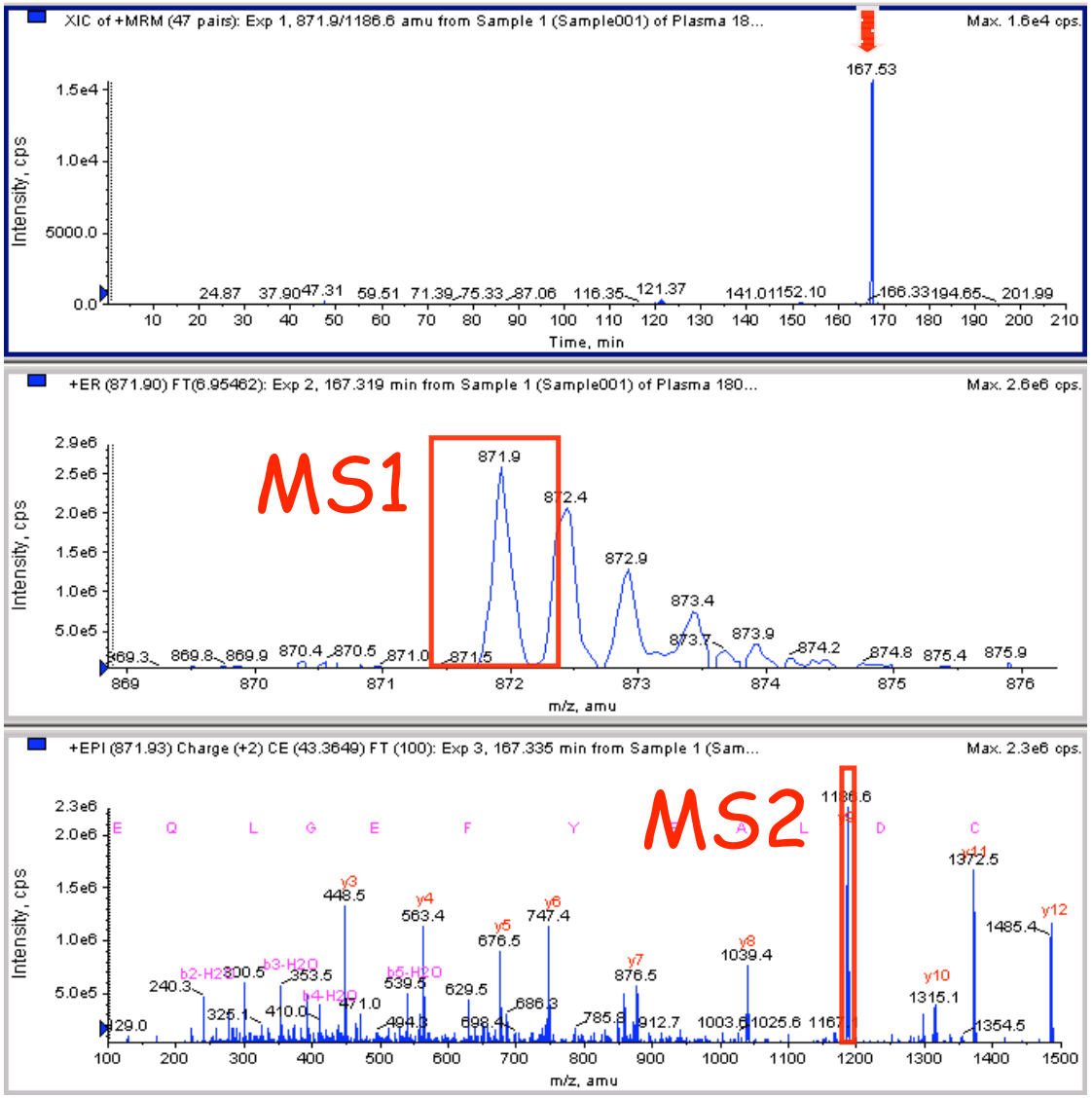
Peptide fragmented

↓

MS2: 1186.6 ± 1 amu
Fragment ion, +1 charge

↓

Detector



This defines an MRM assay, referring to "multiple reaction monitoring"

Collaboration with Christie Hunter (ABI) using 4000 Q TRAP MS

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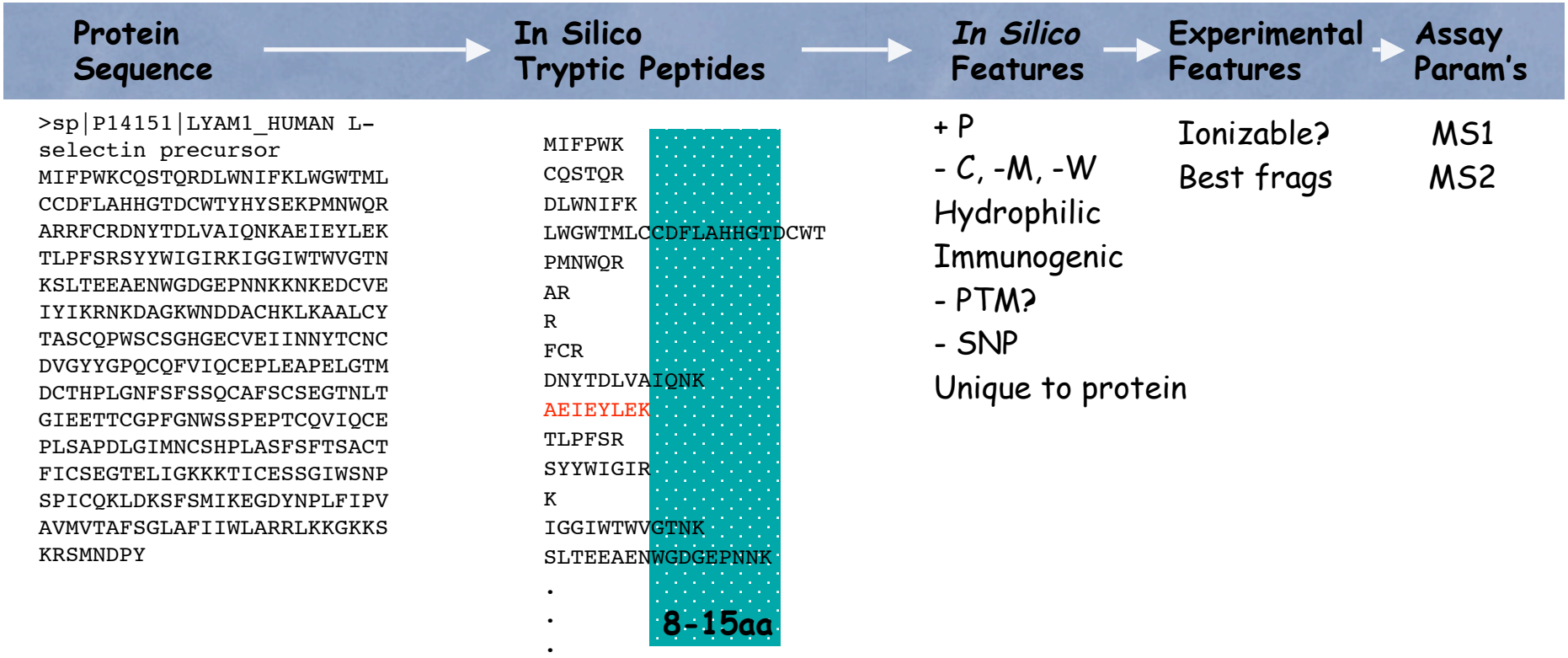
In Silico MS Assay Design via Bioinformatics



- Pure in silico yields a low proportion of good peptides
- Addition of experimental data yields >90% success

- Improvements needed in prediction of ionization and fragmentation

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Efficient Production of Stable Isotope-labeled Standards (SIS) as polySIS*

Apolipoprotein A-I	ATEHLSTLSEK
Alpha-1-acid glycoprotein 1	NWGLSVYADKPETTK
Haptoglobin beta chain	ILGGHLDAK
Fibrinogen gamma chain	DTVQIHDITGK
Fibrinogen alpha chain	TVIGPDGHK
Fibrinogen beta chain	QGFGNVATNTDGK
Alpha-1-antichymotrypsin	EIGELYLPK
Complement C3	TGLQEVEVK
Antithrombin-III	DDLYVSDAFHK
Ceruloplasmin	IYHSHIDAPK
Prothrombin	ETAASLLQAGYK
Complement C4 gamma chain	ITQVLHFTK
Apolipoprotein B-100	FPEVDVLTK
Alpha-2-antiplasmin	LGNQEPGGQTALK
Plasminogen	LSSPAVITDK
Apolipoprotein E	QWAGLVEK
Coagulation factor XIIa heavy chain	IPPWEAPK
Apolipoprotein(a)	LFLEPTQADIALLK
Coagulation factor X	SHAPEVITSSPLK
Adiponectin	IFYNQQNHYDGSTGK
Beta-2-glycoprotein I	EHSSLAFWK
Coagulation factor IX	VSVSQTSK
C-reactive protein	ESDTSYVSLK
Vitamin K-dependent protein C	WELDLDIK
Coagulation factor XIII A chain	STVLTIPEIIIK
Coagulation factor XIII B chain	LIENGYFHPVK
Cholesteryl ester transfer protein	ASYPDITGEK
Coagulation factor V	DPPSDLLLLK
Angiotensinogen	ALQDQLVLVAAK
L-selectin	AEIEYLEK

KATEHLSTLSEKNWGLSVYADKPETTKILG
GHLDAKDTVQIHDITGKTVIGPDGHKQGF
GNVATNTDGKEIGELYLPKTGLQEVEVKD
DLYVSDAFHKIYHSHIDAPKETAASLLQAG
YKITQVLHFTKFPEVDVLTKLGNQEPGGQT
ALKLSSPAVITDKQWAGLVEKIPPWEAPKL
FLEPTQADIALLKSHAPEVITSSPLKIFYNQ
QNHYDGSTGKEHSSLAFWKVSVSQTSKE
SDTSYVSLKWELDLDIKSTVLTIPEIIIKLIE
NGYFHPVKASYPDITGEKDPPSDLLLLKAL
QDQLVLVAAKAEIEYLEK

Synthesize gene CVD_1a (codon optimized)

Clone into pIVEX2.4d expression vector

Express in Roche RTS E coli-based cell-free system (^{15}N,^{13}C-Lys)

polySIS protein standard

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* Patent Pending

Absolute Protein Quantitation in Relation to polySIS* Peptide Standards

polySIS CVD_1 (lys-labeled standard)

ATEHLSTLSEKNWGLSVYADKPETTKILGGHLDAKD
TVQIHDITGKTVIGPDGHK**QGFGNVATNTDGK**EI
GELYLPKTGLQEVEVKDDLYVSDAFHKIYHSHIDAP
KETAASLLQAGYKITQVLHFTKFPEVDVLTKLGNQEP
GGQTALKLSSPAVITDKQWAGLVEKIPPWEAPKLFL
EPTQADIALLKSHAPEVITSSPLKIFYNQQNHYDGST
GKEHSSLAFWKVSVSQTSKESDTSYVSLKWELDLD
IKSTVLTIPEIIIKLIENGYFHPVKASYPDITGEKDPPS
DLLLLKALQDQLVLVAAKAEIEYLEK

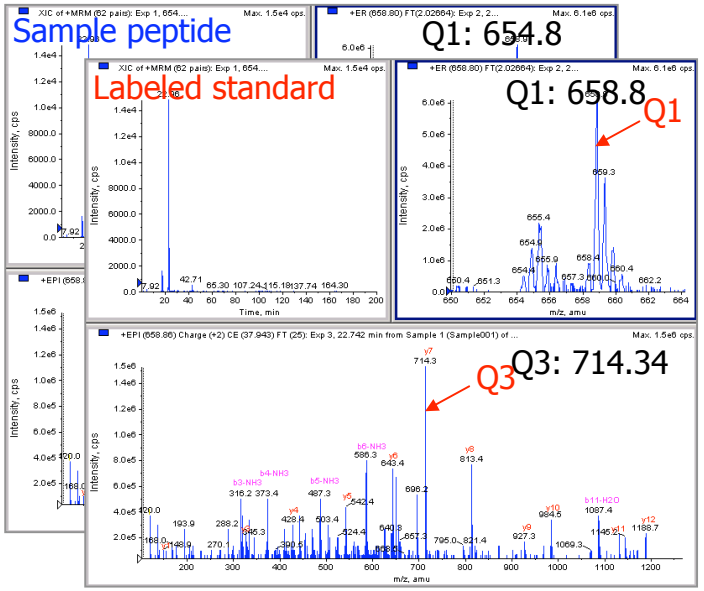
Fibrinogen β chain (in sample)

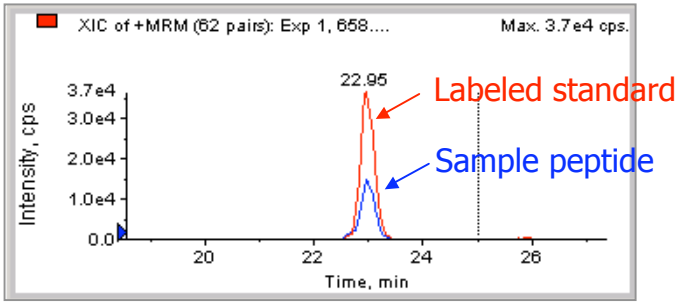
MKRMVSWSFHKLKTMKHLLLLLLCVFLVKSQGVND
NEEGFFSARGHRPLDKKREEAPSLRPAPPPISGGGY
RARPAKAAATQKKVERKAPDAGGCLHADPDLGVLC
PTGCQLQEALLQQERPIRNSVDELNNNVEAVSQTSS
SSFQYMYLLKDLWQKRQKQVKDNENVVNEYSSELE
KHQLYIDETVNSNIPTNLRVLRSILENLRSKIQKLESD
VSAQMEYCRTPCTVSCNIPVVSGKECEEIIRKGGETS
EMYLIQPDSSVKPYRVYCDMNTENGGWTVIQNRQD
GSVDFGRKWDPYK**QGFGNVATNTDGK**NYCGLPG
EYWLGNDKISQLTRMGPTELLIEMEDWKGDKVKAH
YGGFTVQNEANKYQISVNKYRGTAGNALMDGASQL
MGENRTMTIHNGMFFSTYDRDNDGWLTSDPRKQC
SKEDGGGWWYNRCHAANPNGRYYWGGQYTWDM
AKHGTDDGVVWMNWKGSWYSMRKMSMKIRPFFP
QQ

* Patent Pending

Digest

***LC-TQMS of* QGFGNVATNTDGK**





MRM Chromatogram

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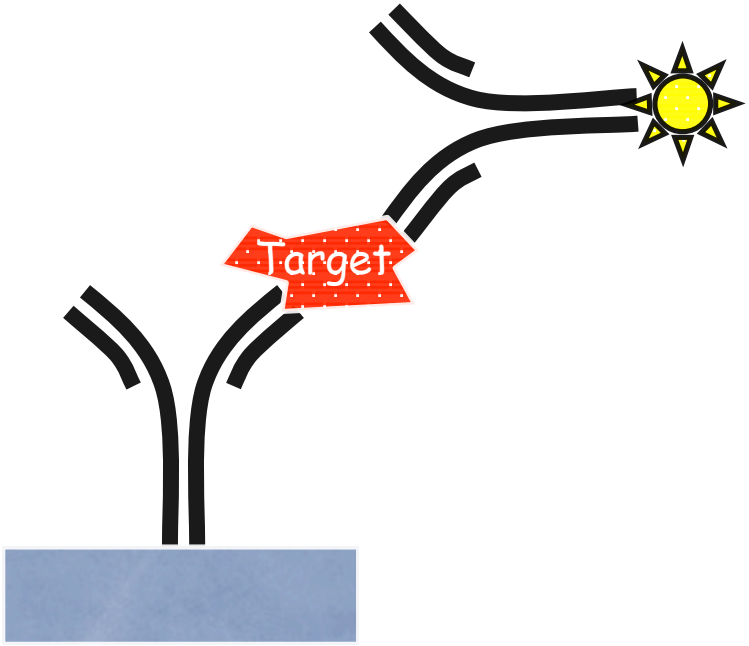
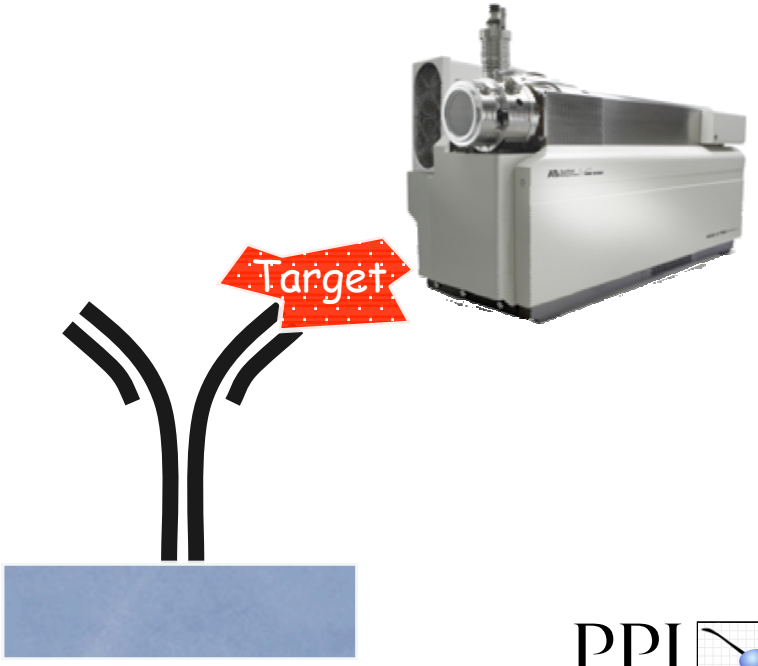
Hybrid Ab-MS Assays: MS as a Universal Second Antibody

	Antibody	Mass Spectrometer
Sensitivity	+++	++
Specificity	++	++++
Shared across targets	No	Yes





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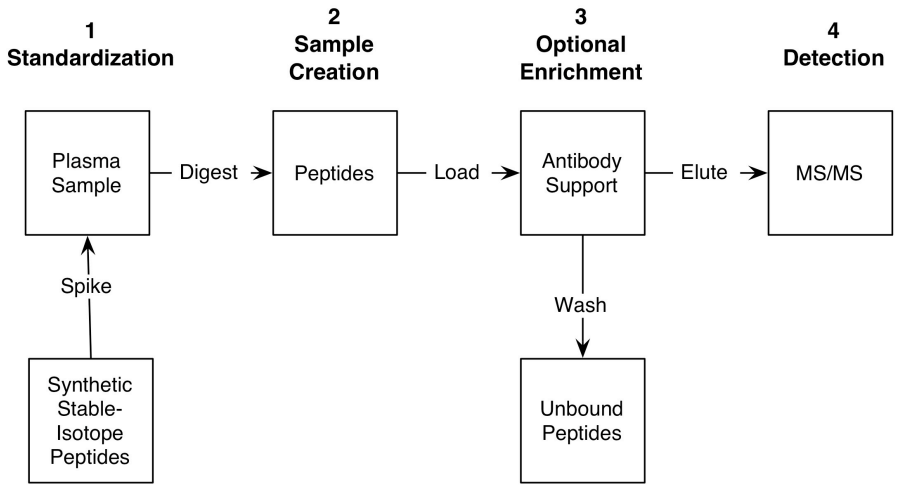
Sensitivity Enhancement via Specific Capture

- Employ MS/MS assay as "2nd antibody" with absolute specificity
- Add specific capture step, e.g., using anti-peptide antibody
  - Demonstrated 10^2-fold sensitivity improvement with polyclonals
  - Up to 10^5-fold enhancement expected with monoclonal Ab's
- Forgiving of Ab performance
  - Complete specificity not required (MS does this)
  - Ab does not quantitate (stable isotope standards + MS do this)

SISCAPA is an immunoassay in which the second (detection) antibody is replaced by a mass spectrometer. This provides:

- Increased assay specificity (absolute structural specificity of MS/MS)
- High sensitivity (low fmol- high amol at peptide level)
- Lower cost (one Ab instead of two)
- Lower analyte-analyte interference (and greater sample stability) by digesting proteins to peptides



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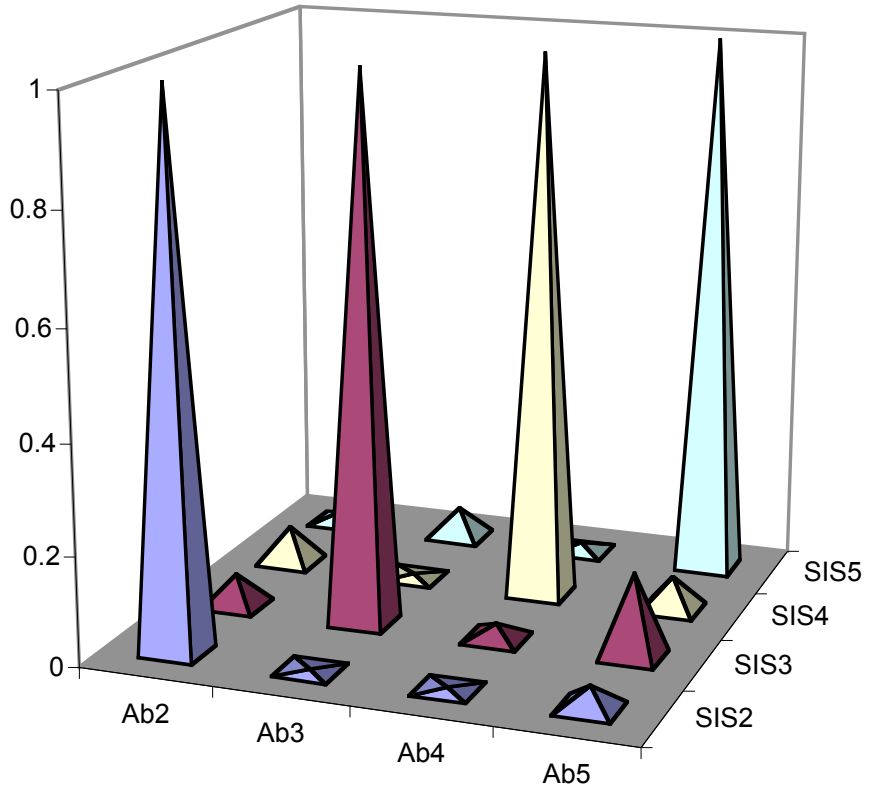
Relative Quantities of Four SIS Peptides Bound by Four Anti-Peptide Antibodies, Using Two-stage MS Selection (SRM)
Average Peptide Enrichment by Ab > 100-fold



The signals (vertical axis) for each antibody are normalized to the largest signal for that antibody

Mass Spectrometric Quantitation of Peptides and Proteins Using Stable Isotope Standards and Capture by Anti-Peptide Antibodies (SISCAPA).
Anderson, N.L., et al, Journal of Proteome Research, 3: 235-44 (2004).

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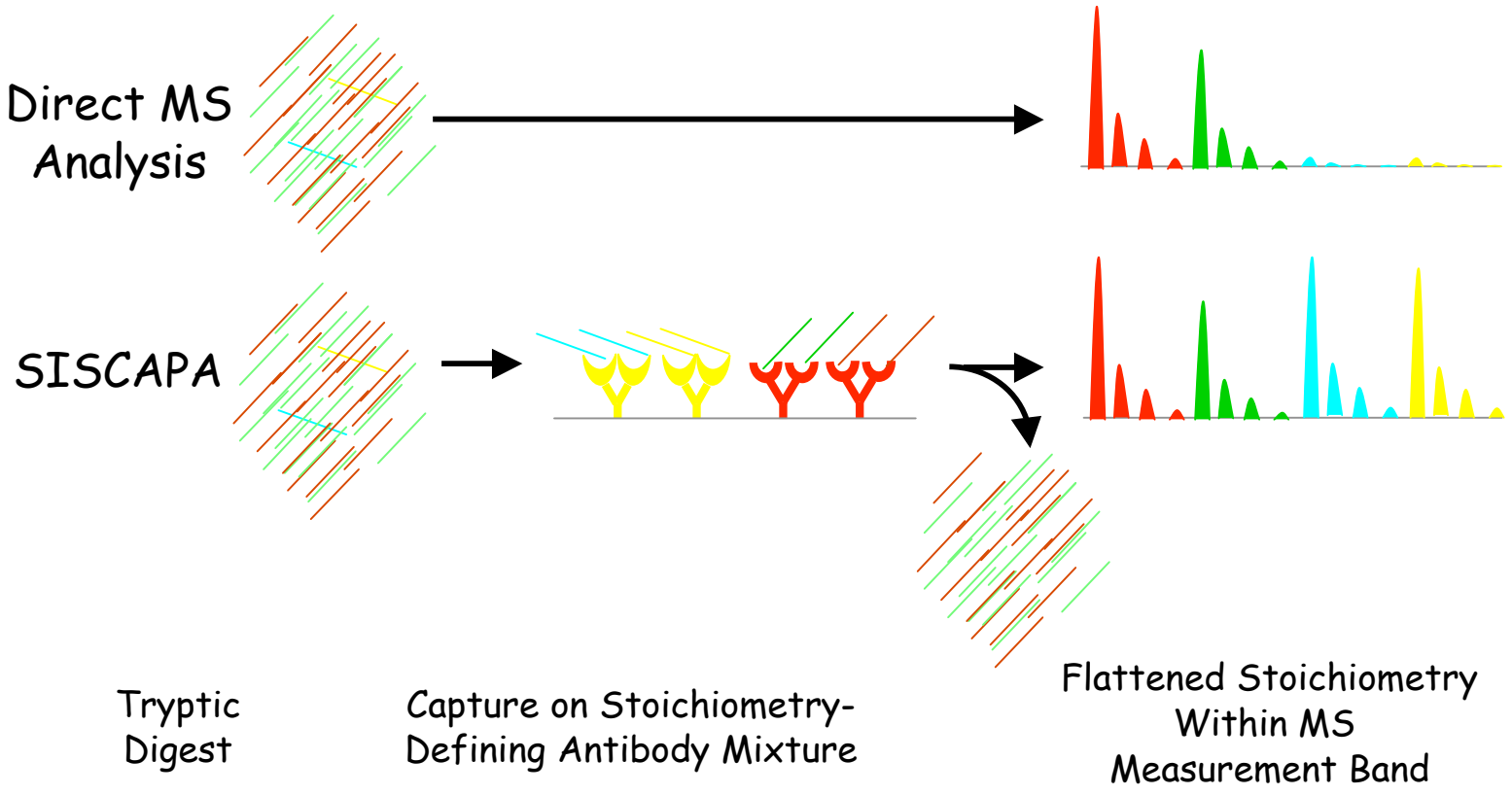
SISCAPA Captures Similar Amounts of Peptides, Flattening the Abundance Distribution to Accommodate MS Dynamic Range



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Some Definitions of Success for Biomarker Proteomics

Growing funding for biomarker discovery	Yes
Candidates in at least one commercial pipeline	Yes?
Consensus on best experimental approach to biomarker discovery	No
Generally-accessible pipeline for translating candidates to tests	No
Clinical tests in general use	No

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The Heroic Age of Mass Spectrometry

A bank of 32 Calutrons (of 1152 built) for preparative MS of isotopes at Oak Ridge built circa 1945

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Acknowledgements

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  - Bob Olafson, Derryl Hardy, UVIC-Genome B.C. Proteomics Centre
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- Plasma Proteome Database
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- polySIS labeled peptide standards
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- MRM Assay Development
  - Christie Hunter, Applied Biosystems, Foster City
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www.plasmaproteome.org

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Recent Relevant Papers

- **The human plasma proteome: History, character, and diagnostic prospects**. Anderson, N.L. and Anderson, N.G., Molecular and Cellular Proteomics, 1.11, 845-867 (2002)
- **The human serum proteome: Display of nearly 3700 chromatographically separated protein spots on two-dimensional electrophoresis gels and identification of 325 distinct proteins**. Pieper, R., et al Proteomics 3(7): 1345-64. (2003).
- **Multi-component immunoaffinity subtraction chromatography: An innovative step towards a comprehensive survey of the human plasma proteome.** Pieper, R., Su, Q., Gatlin, C. L., Huang, S. T., Anderson, N. L., Steiner, S. Proteomics 3(4): 422-32 (2003).
- **Therapeutic potential of the plasma proteome**. Lathrop, J.T., Anderson, N.L., Anderson, N.G., and Hammond, D.J. Current Opinion in Mol. Therapeutics 5:250-257 (2003).
- **Mass Spectrometric Quantitation of Peptides and Proteins Using Stable Isotope Standards and Capture by Anti-Peptide Antibodies (SISCAPA).** Anderson, N.L., Anderson, N.G., Haines, L.R., Hardie, D.B., Olafson. R.W., and Pearson, T.W. Journal of Proteome Research, 3: 235-44 (2004).
- **NHLBI Clinical Proteomics Working Group Report.** Granger, C.B., Van Eyk, J.E., Mockrin, S.C., and Anderson, N.L., on behalf of the Working Group Members. Circulation 109: 1697-703 (2004).
- **Candidate-Based Proteomics in the Search for Biomarkers of Cardiovascular Disease,** Leigh Anderson, J. Physiol., 563.1:23-60 (2005).
- **The Roles of Multiple Proteomics Platforms in a Pipeline for New Diagnostics**, N. Leigh Anderson, Mol Cell Proteomics, in press 2005

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