The Clinical Potential of the Human Plasma Proteome

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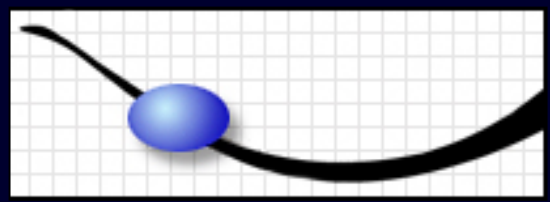

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Plasma is the largest, and deepest, version of the human proteome

- Largest = Most proteins
- Deepest = Widest dynamic range

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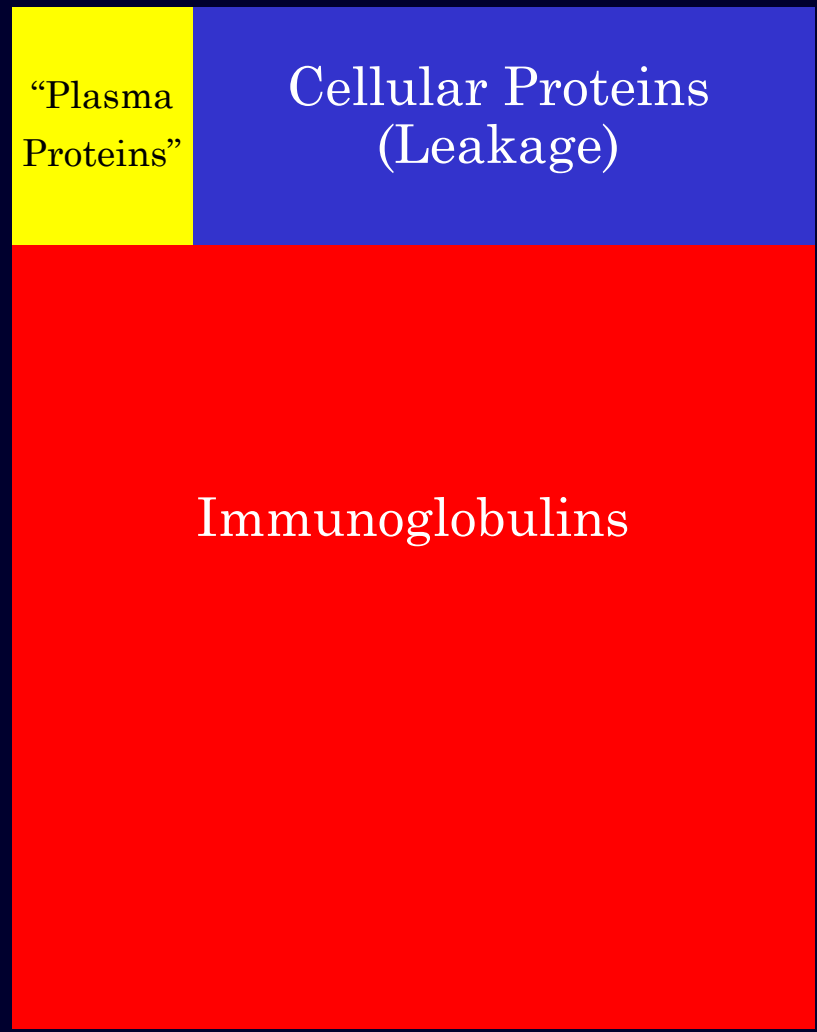
Major Components of the Plasma Proteome

- ~40,000 forms of proteins secreted to function in plasma, most glycoproteins
  - Assume 500 gene products x 2 splice variants x 20 glycoforms x 2 clip forms
- ~500,000 forms of tissue proteins
  - Essentially all tissue proteins x splice and PTM variants
- ~10,000,000 clonal forms of immunoglobulin



Total: the largest version of the human proteome

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A New Functional Classification of Proteins in Plasma

1. Secreted proteins that act in plasma (e.g., albumin, fibrinogen)
2. Immunoglobulins (Ig's A,M,G,D,E)
3. Tissue leakage products (e.g., cardiac Mb)
4. "Distant" receptor ligands (e.g.,insulin)
5. "Local" receptor ligands (e.g., IL-8)
6. Aberrant secretions (e.g., PSA in cancer)
7. Temporary passengers (e.g., lysosomal enz.)
8. Foreign proteins (e.g., virus)

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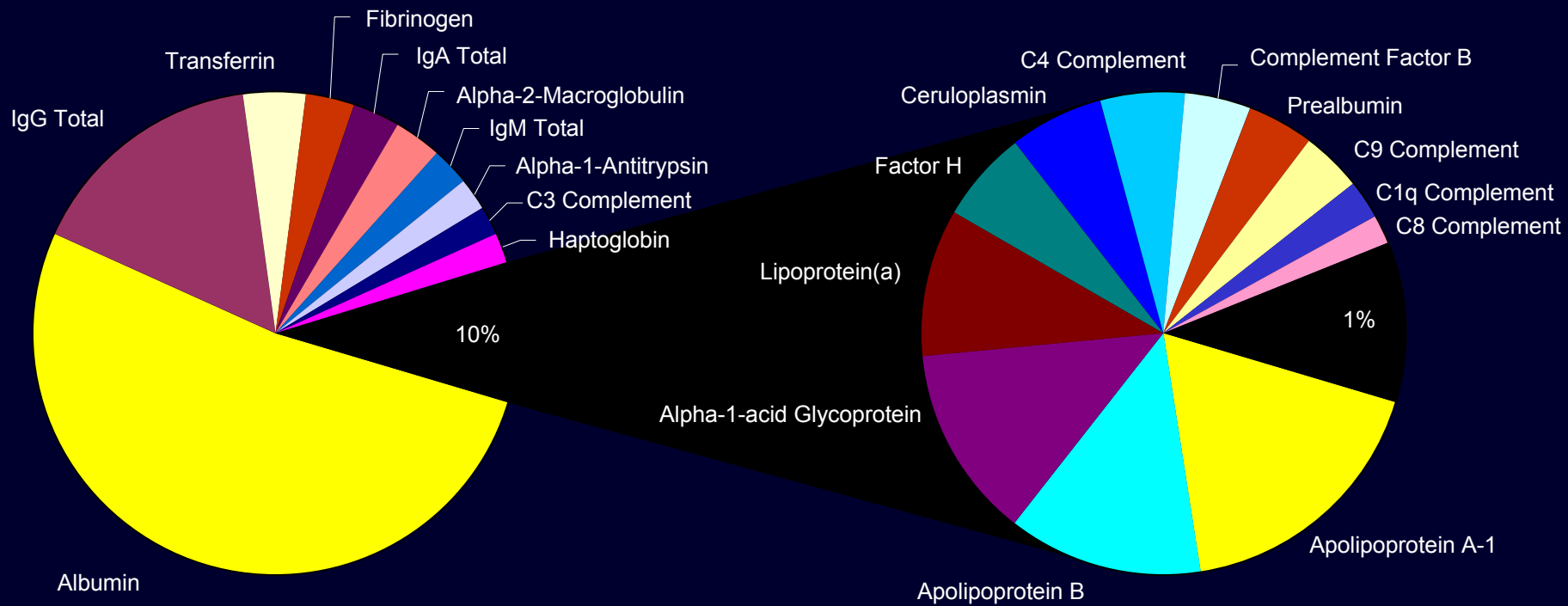
Major Plasma Proteins

99% of plasma protein mass



0 - 90%

90 - 99%

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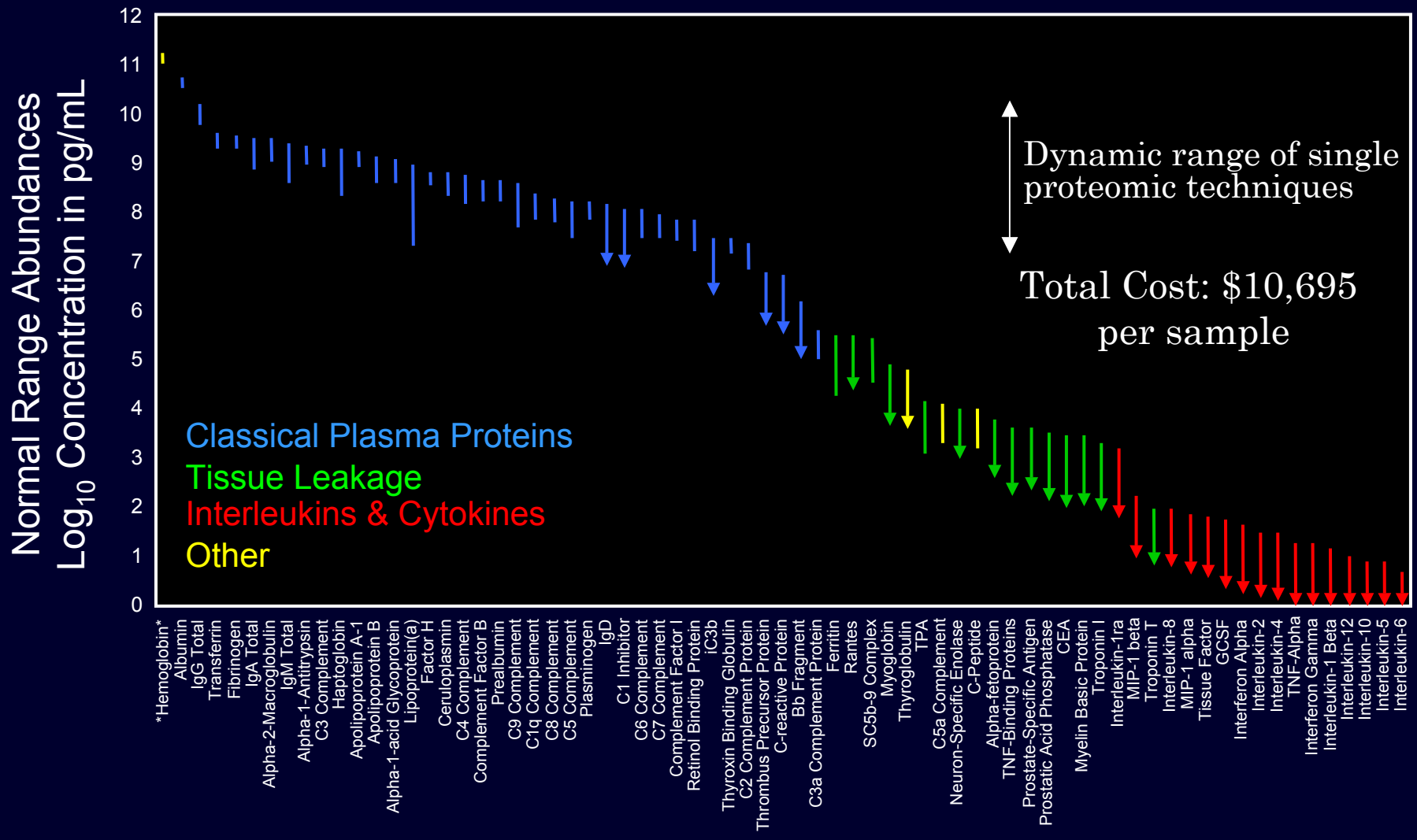
Proteins Measured Clinically in Plasma Span > 10 Orders of Magnitude in Abundance



(Human male 50yr - from Specialty Laboratories Books)

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Plasma "Proteomics" Began With 2-D Gels (c. 1976)

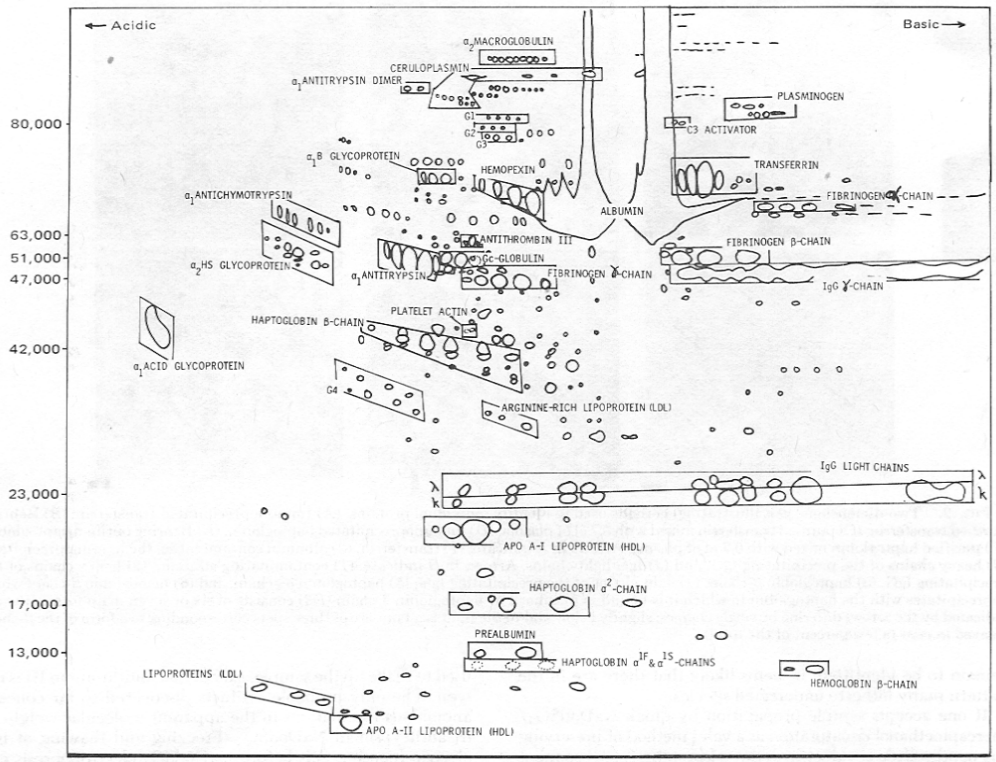

FIG. 3. Diagram drawn from the gel shown in Fig. 1, and labeled to indicate positions of known plasma proteins. Hemopexin and the C3-activator are somewhat obscured by albumin overloading. Ceruloplasmin appears to be present in two major and two minor forms (all between 80,000 and 90,000 daltons), each 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.

2-D Electrophoresis

300+ resolved spots

40 identified proteins

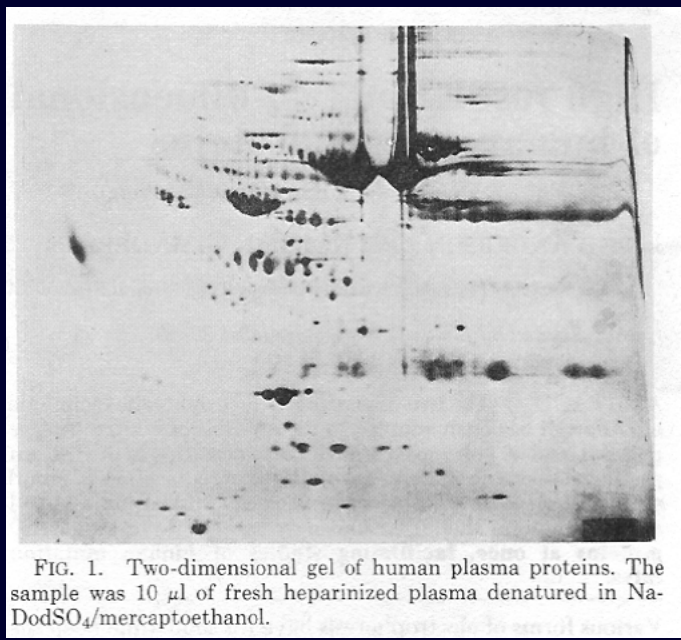

FIG. 1. Two-dimensional gel of human plasma proteins. The sample was 10 μl of fresh heparinized plasma denatured in Na-DodSO$_4$/mercaptoethanol.

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

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Short History

Most protein technologies have been applied to plasma rapidly

Year	Event
1933	Von Mutzenbacher uses Svedberg's analytical ultracentrifuge to resolve serum albumin and globulin fractions by molecular weight: demonstrates proteins are not heterogeneous colloids but rather specific structures
1937	Tiselius uses his electrophoresis to resolve serum proteins into α, β, and γ globulins, establishing a naming convention that still persists
1939	Tiselius and Kabat demonstrate that antibodies are components of the γ globulin fraction
1940's	Svensson and Longsworth further resolve serum globulins into α_1, α_2, β_1, β_2, γ_1 and γ_2
1950	Gofman finds lipoproteins float in ultracentrifuge and can be measured clinically
1950's	Paper electrophoresis of serum proteins is widely introduced into clinical chemistry
1958	Smithies and Poulik resolve 22 zones of serum proteins using starch gel electrophoresis
1960	Grabar and Burtin describe immunoelectrophoresis
1964	Ornstein and Davis introduce acrylamide 'disc' gel electrophoresis with resolution even higher than starch
1965	Laurell introduces crossed immunoelectrophoresis, resolving more than 40 different serum proteins
1966	Laurell introduces quantitative "rocket" electrophoresis
1977	Anderson and Anderson use 2-D electrophoresis to resolve hundreds of serum protein forms (40 identifications via immunoprecipitation)
1991	Published 2-D plasma protein database with 49 identified proteins and 727 spots
2002	Current SWISS-2D PAGE database with 60 identified proteins and est. 1500 spots
2002	Immunosubtraction/chromatography/2-DE with >250 identified proteins and est. 1000-1500 spots (Pieper, et al, manuscript in preparation)

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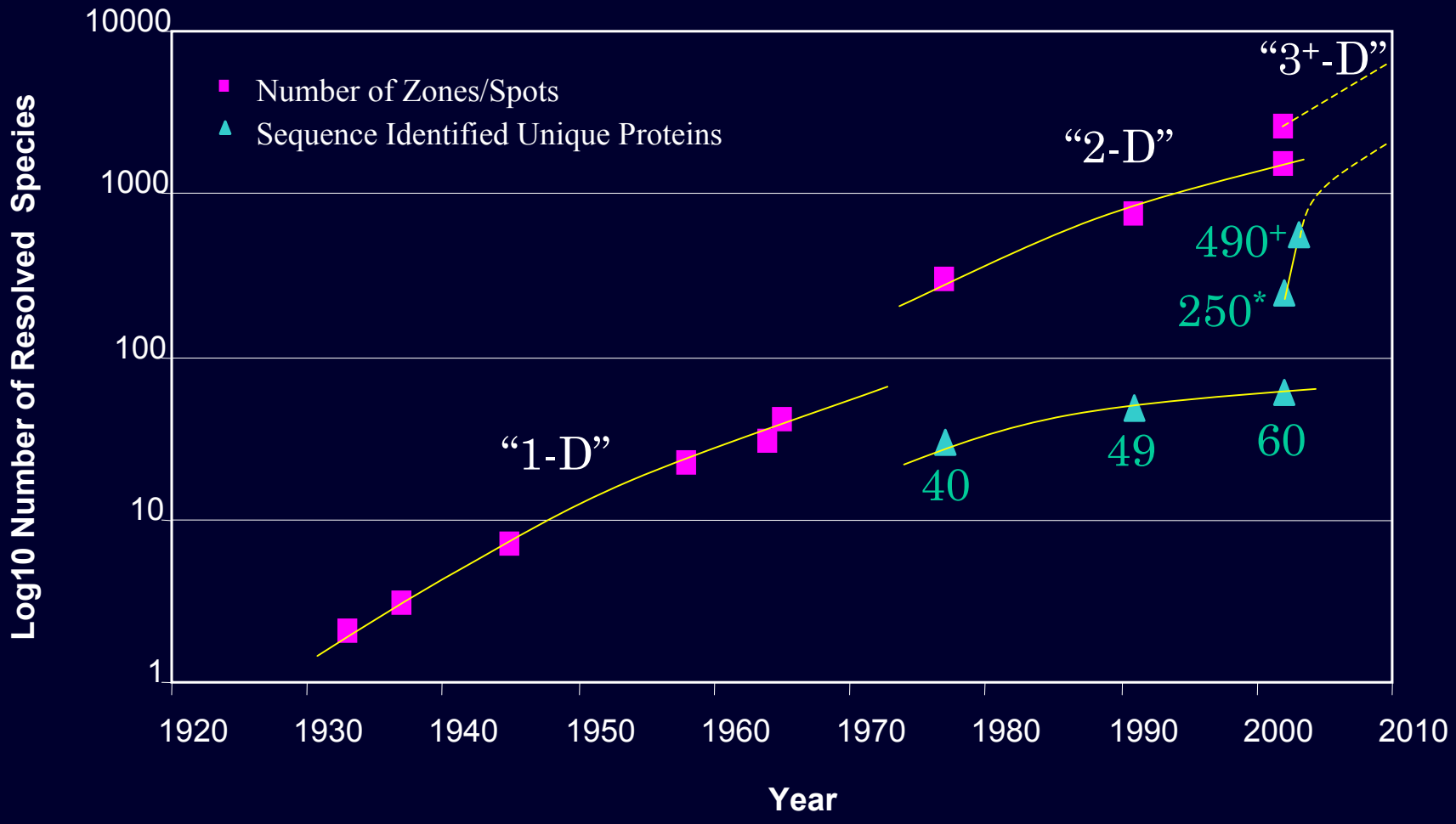
Growth in the Number of Protein Species Observed in Plasma Over Time



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* R. Pieper et al, manuscript in preparation
+ J.N. Adkins, in press

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A Provisional Plasma Proteome:

289 Proteins Observed in Plasma (Scientific Literature pre-2002)

Almost none were discovered by proteomics

Very little published on prediction of secreted proteins from human genome

5'-Nucleotidase
Acid labile subunit of IGFBP
Acid phophatase, tartrate-resistant
Acid phosphatase, prostatic
Actin beta (from platelets)
Actin gamma (from platelets)
Adenosine Deaminase
Adiponectin
Alanine aminotransferase (ALT)
Albumin
Aldolase (muscle type)
Alkaline Phosphatase (bone)
Alpha1,3-fucosyltransferase (FUT6)
Alpha-1-acid Glycoprotein
Alpha-1-Antichymotrypsin
Alpha-1-Antitrypsin
Alpha-1-B Glycoprotein
Alpha-1-Microglobulin
Alpha-2-Antiplasmin
Alpha-2-HS Glycoprotein
Alpha-2-Macroglobulin
Alpha-fetoprotein
Amylase (pancreatic)
Angiostatin
Angiotensin converting enzyme (ACE)
Angiotensinogen
Antithrombin III (AT3)
Apolipoprotein A-I
Apolipoprotein A-II
Apolipoprotein A-IV
Apolipoprotein B-100
Apolipoprotein B-48
Apolipoprotein C-I
Apolipoprotein C-II
Apolipoprotein C-III
Apolipoprotein C-IV
Apolipoprotein D
Apolipoprotein E
Apolipoprotein F
Apolipoprotein H
Apolipoprotein J (Clusterin)
Apolipoprotein(a)
Aspartate aminotransferase (AST)
Beta Thromboglobulin
Beta-2-microglobulin
CA 125
CA 19-9
CA 72-4
CA27.29/15-3 (MUC1 mucin antigens)
Calreticulin
Carboxypeptidase N , regulatory
Carboxypeptidase N, catalytic
Carcinoembryonic Antigen
Cathepsin D
CD5 antigen-like protein
Ceruloplasmin
Cholinesterase Plasma
Chorionic Gonadotropin Beta (hCG)
Chromogranin A
Chromogranin B (secretogranin I)
Coagulation Factor II (Prothrombin)
Coagulation Factor IX
Coagulation Factor V
Coagulation Factor VII , H
Coagulation Factor VII, L
Coagulation Factor VIII
Coagulation Factor X
Coagulation Factor XI
Coagulation Factor XII
Coagulation Factor XIII A
Coagulation Factor XIII B
Collagen I c-terminal propeptide
Collagen I c-terminal telopeptide (ICTP)
Collagen I n-terminal propeptide
Collagen I n-terminal telopeptide (NTx)
Collagen III c-terminal propeptide
Collagen III n-terminal propeptide
Collagen IV 7S n-terminal propeptide
Complement C1 Inhibitor
Complement C1q, A
Complement C1q, B
Complement C1q, C
Complement C1r
Complement C1s
Complement C2
Complement C3A anaphylotoxin
Complement C3B, alpha'
Complement C3B, beta
Complement C4 anaphylotoxin
Complement C4, alpha
Complement C4, beta
Complement C4, gamma
Complement C4-binding protein, alpha
Complement C4-binding protein, beta
Complement C5A anaphylotoxin
Complement C5B, alpha'
Complement C5B, beta
Complement C6
Complement C7
Complement C8, alpha
Complement C8, beta
Complement C8, gamma
Complement C9
Complement Factor B
Complement Factor B - Bb Fragment
Complement Factor D
Complement Factor H
Complement Factor I
Connective Tissue Activating Peptide III
Corticotropin Releasing Hormone (CRH)
C-reactive Protein
Creatine Kinase, B
Creatine Kinase, M
CRHBP
Cystatin C
Elastase (neutrophil)
Eosinophil granule major basic protein
E-selectin, soluble
Ferritin, H
Ferritin, L
Fibrin fragment D-dimer
Fibrinogen extended gamma chain
Fibrinogen, alpha
Fibrinogen, beta
Fibrinogen, gamma
Fibronectin
Fibulin-1
Ficolin 1
Ficolin 2
Ficolin 3
Follicle stimulating hormone
G-6-PD
Galactoglycoprotein (Leukosialin)
Gamma-glutamyl transferase alpha
Gc-globulin
GCSF
Gelsolin
GHRH
Glutamate carboxypeptidase II
Glutathione Peroxidase
Glutathione S-transferase
Glycoprotein hormones alpha chain
GMCSF
Growth Hormone
Growth Hormone Binding Protein
Haptoglobin alpha-1
Haptoglobin alpha-2-chain
Haptoglobin beta chain
Haptoglobin beta chain, cleaved
Haptoglobin-related gene product
Hemoglobin, alpha
Hemoglobin, beta
Hemopexin (Beta-1B-glycoprotein)
Histidine-rich Alpha-2-glycoprotein
ICAM-1, soluble
Ig Kappa light chain
Ig Lambda light chain
IgA1
IgA2
IgD
IgE
IGFBP-3
IgG1
IgG2
IgG3
IgG4
IgJ-chain
IgM
Inhibin (activin), beta A
Inhibin (activin), beta B
Inhibin (activin), beta C
Inhibin (activin), beta E
Inhibin, alpha
Insulin C-Peptide
Insulin, A chain
Insulin, B chain
Insulin-like growth factor IA
Insulin-like growth factor II
Inter-alpha trypsin inhibitor, H1
Inter-alpha trypsin inhibitor, H2
Inter-alpha trypsin inhibitor, H4
Inter-alpha-trypsin inhibitorL
Interferon Alpha
Interferon Beta
Interferon Gamma
Interleukin-1 Beta
Interleukin-10
Interleukin-12, alpha
Interleukin-12, beta
Interleukin-1receptor antagonist
Interleukin-2
Interleukin-4
Interleukin-5
Interleukin-6
Interleukin-8
IP-10, Small inducible cytokine B10
Isocitrate dehydrogenase
Kininogen
Ksp37
Laminin, alpha
Laminin, beta
Laminin, gamma
LDH (heart)
Lecithin-cholesterol acyltransferase
Leucine-rich Alpha-2-glycoprotein
LHRH
Lipase
Luteinizing hormone (LH), beta
Mannose-binding Protein
Matrix metalloproteinase-2
M-CSF
Melastatin
MIP-1 alpha
MIP-1 beta
MSE55
Myelin Basic Protein
Myoglobin
N-Acetyl-B-D-Glucosaminidase, alpha
N-Acetyl-B-D-Glucosaminidase, beta
N-Acetylmuramyl-L-alanine amidase
Neuron-specific Enolase
Neutrophil-activating peptide 2
Osteocalcin
Osteonectin
Pancreatic zymogen granule membrane protein GP-2
Paraoxonase Parathyroid Hormone
Parathyroid Hormone-Related Protein
PASP
Pepsinogen A
Plasma hyaluronan binding protein
Plasma kallikrein
Plasma serine protease inhibitor
Plasminogen
Plasminogen
Platelet Factor 4
Pre-alpha trypsin inhibitor, H3
Pregnancy-associated plasma protein-A
Pregnancy-associated plasma protein-A2
Pregnancy-specific beta-1-glycoprotein 3
Prolactin
Prolyl hydroxylase, alpha
Prolyl hydroxylase, beta
Prostaglandin-H2 D-isomerase
Prostate Specific Antigen
Protein C, H
Protein C, L
Protein S
Protein Z
P-selectin, soluble
Rantes
Renin
Retinol Binding Protein
S100 protein
Secretogranin V
Serum Amyloid A
Serum Amyloid P
Sex Hormone Binding Globulin
Tetranectin
Thyroglobulin
Thyroid Stimulating Hormone
Thyrotropin-releasing hormone
Thyroxin Binding Globulin
Tissue Factor
Tissue inhibitor of metalloproteinases-1
Tissue inhibitor of metalloproteinases-2
Tissue Plasminogen Activator
Tissue Plasminogen Activator Inhibitor
TNF-Alpha
TNF-Binding Protein 1
TNF-Binding Protein 2
Transcobalamin
Transcortin
Transferrin
Transferrin (asialo-, tau-, beta-2-)
Transferrin Receptor (Soluble)
Transthyretin
Triacylglycerol lipase (pancreatic)
Troponin I (cardiac)
Troponin I, (skeletal)
Troponin T (cardiac)
Tryptase, beta-2
Tyrosine hydroxylase
Urokinase (High MW kidney type)A
Urokinase (High MW kidney type)B
VCAM-1, soluble
Vitronectin
Von Willebrand F
Zn-Alpha-2-glyco

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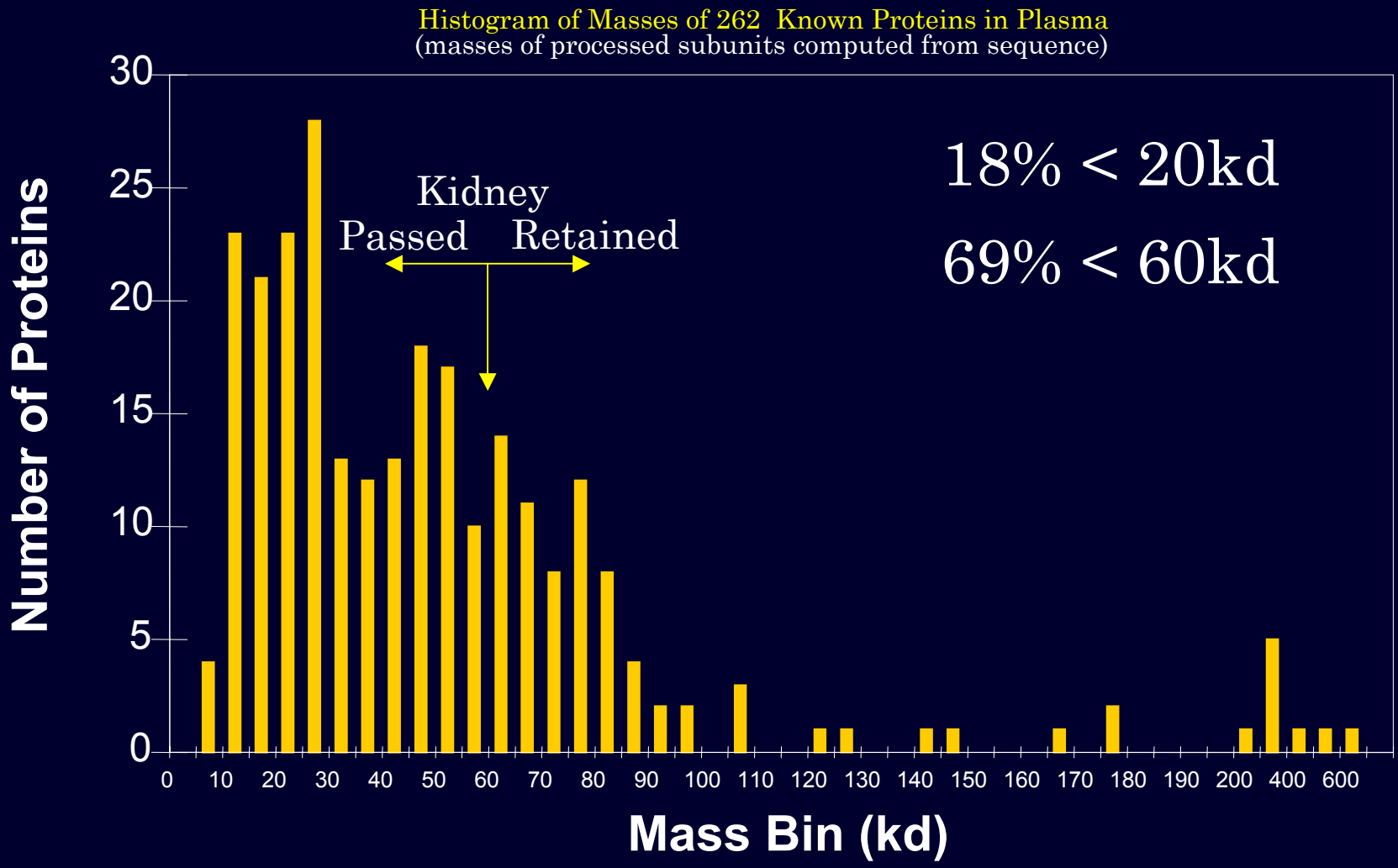
>70% of Proteins in Plasma Are Likely To Be In Complexes



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Protein Diagnostics

- How does it compare to the discovery trend in proteomics?
- What are the figures on protein tests approved by FDA (CLIA)?

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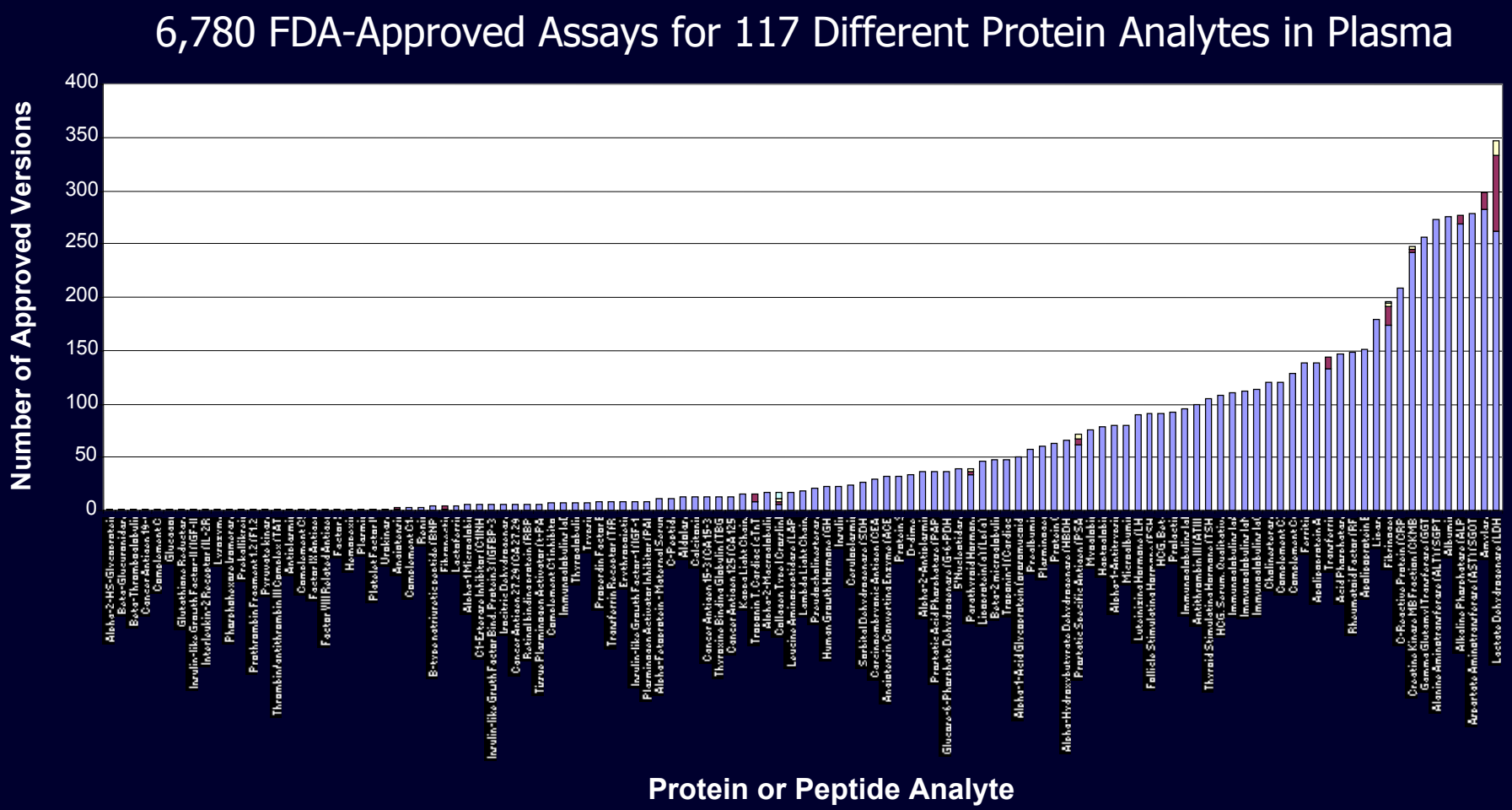
The Many Diagnostics Available Test For A Small Number of Protein Analytes



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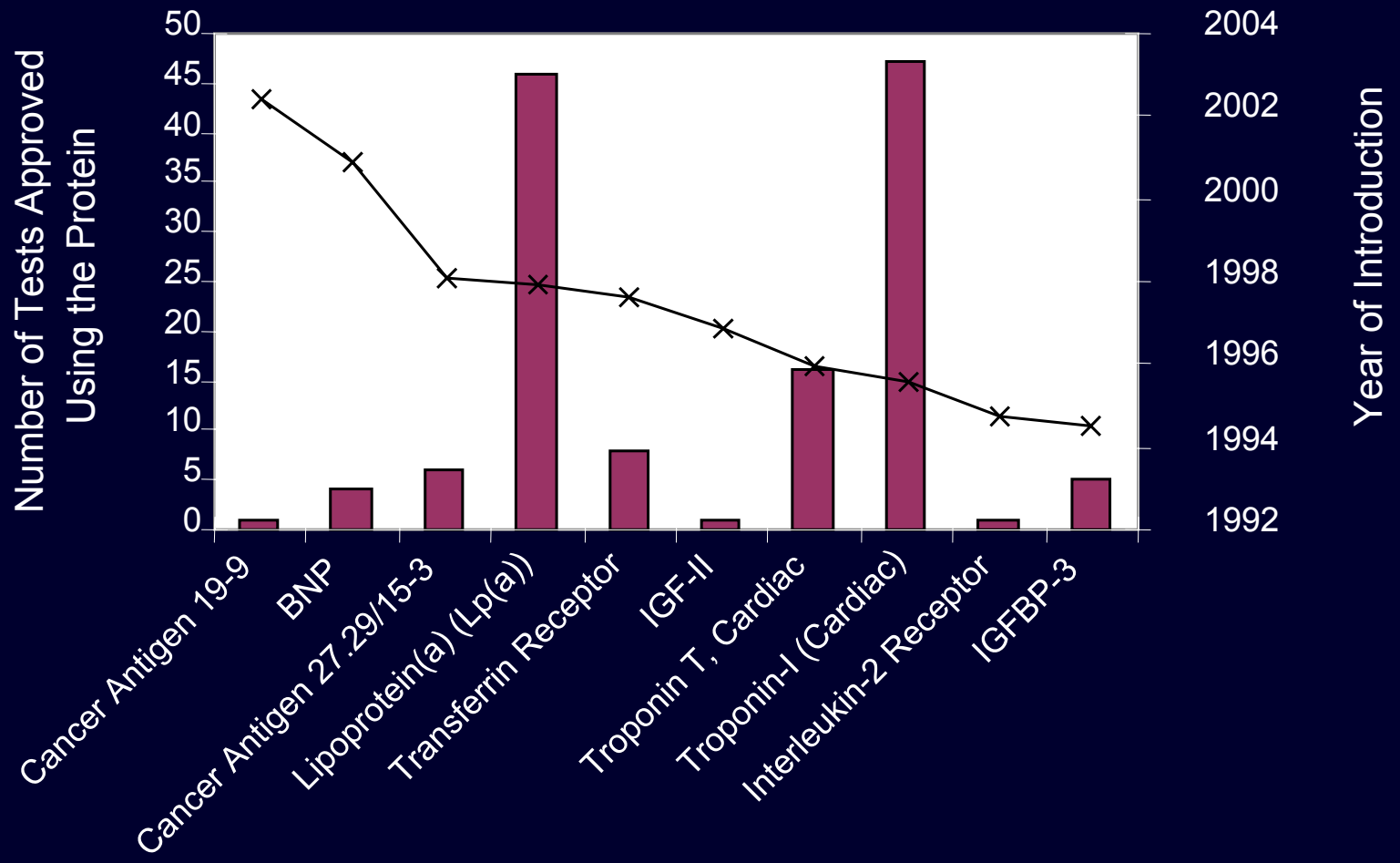
Assays for Only 10 New Proteins in Plasma Have Been Approved by FDA Since 1993



Based on the CLIA (Clinical Laboratory Improvement Amendments) Database maintained by the US Food and Drug Administration.

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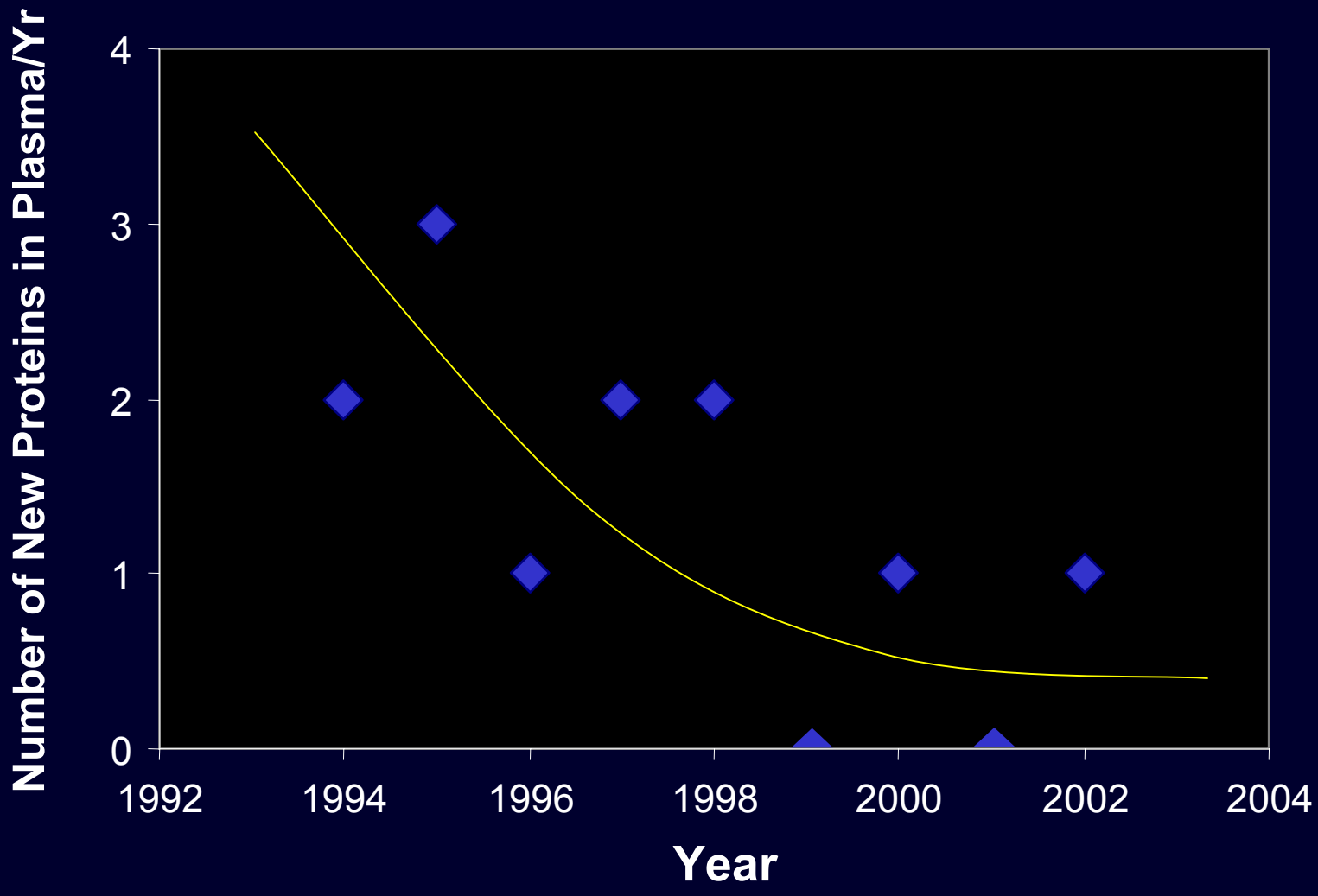
The Rate of Introduction of New FDA-Approved (CLIA) Diagnostic Protein Analytes Has Decreased to ~Zero



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Some Useful Protein Analytes Were Not Even "Identified" By the Standards of Proteomics: CA-125 Ovarian Cancer Marker

- Discovered in 1984
- Defined by monoclonal Ab(s)
- 2000+ publications on clinical use
- Identified with reference to protein sequence only in late 2001:
  - Tethered mainly-extracellular glycoprotein of 1,269,525 Daltons

∴ Proteomics ID standards not necessarily critical for applied use

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Expanding the Diagnostic Proteome

- A declining rate of introduction of new protein analytes contradicts the widespread expectation that genomics and proteomics are rapidly advancing non-genetic diagnostics
- Suggests a major problem in translation of basic research into commercial diagnostics
- PPI seeks to identify and overcome these barriers

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Some Barriers Impeding a Major Advance in Protein Diagnostics

Multivariate marker concept	Not accepted
Individual baseline concept	Not accepted
Cost per protein analyte (for multi-protein markers)	100x too high

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Multivariate Markers

- Available data indicates that multivariate (multi-protein) markers characterize disease states and drug effects better than single markers
- Examples
  - Acute phase proteins in RA, in CV risk, in bacterial *vs* viral infections
  - CK-MB, Mb, TnI(T) in MI
  - Rodent tox studies of compound classes
- Despite examples and theory, not enough weight of evidence to convince wide audience

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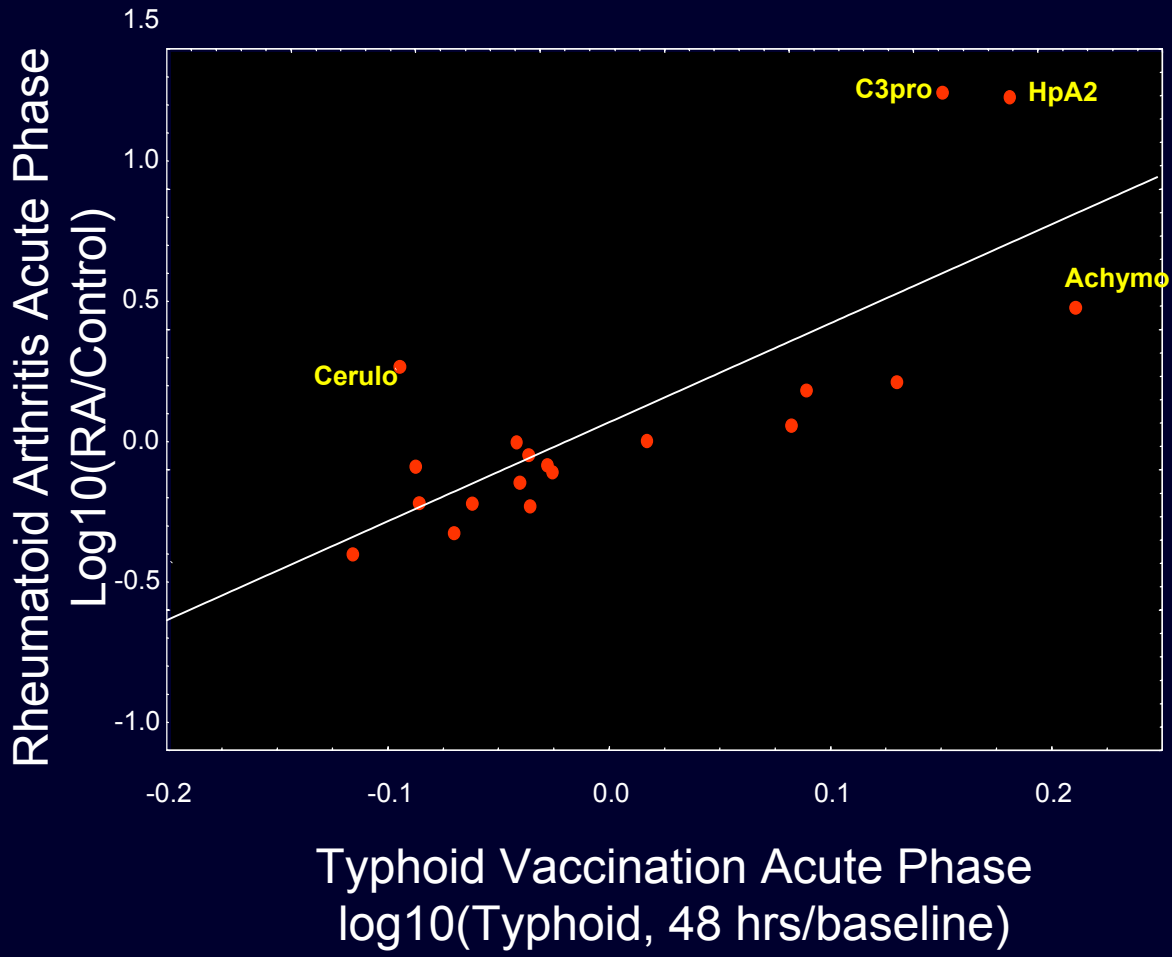
A Co-Varying Set of Protein Markers Yields a Disease Index More Sensitive and Robust Than a Single Protein Assay

Relationship Between RA and Typhoid Vaccination
Effects on Human Serum Protein Abundances



Analysis of Changes in Acute Phase Plasma Proteins in an Acute inflammatory Response and in Rheumatoid Arthritis using 2D-gel electrophoresis. NS Doherty, BH Littman, K Reilly, AC Swindell, Jane M Buss, NL Anderson, Electrophoresis.

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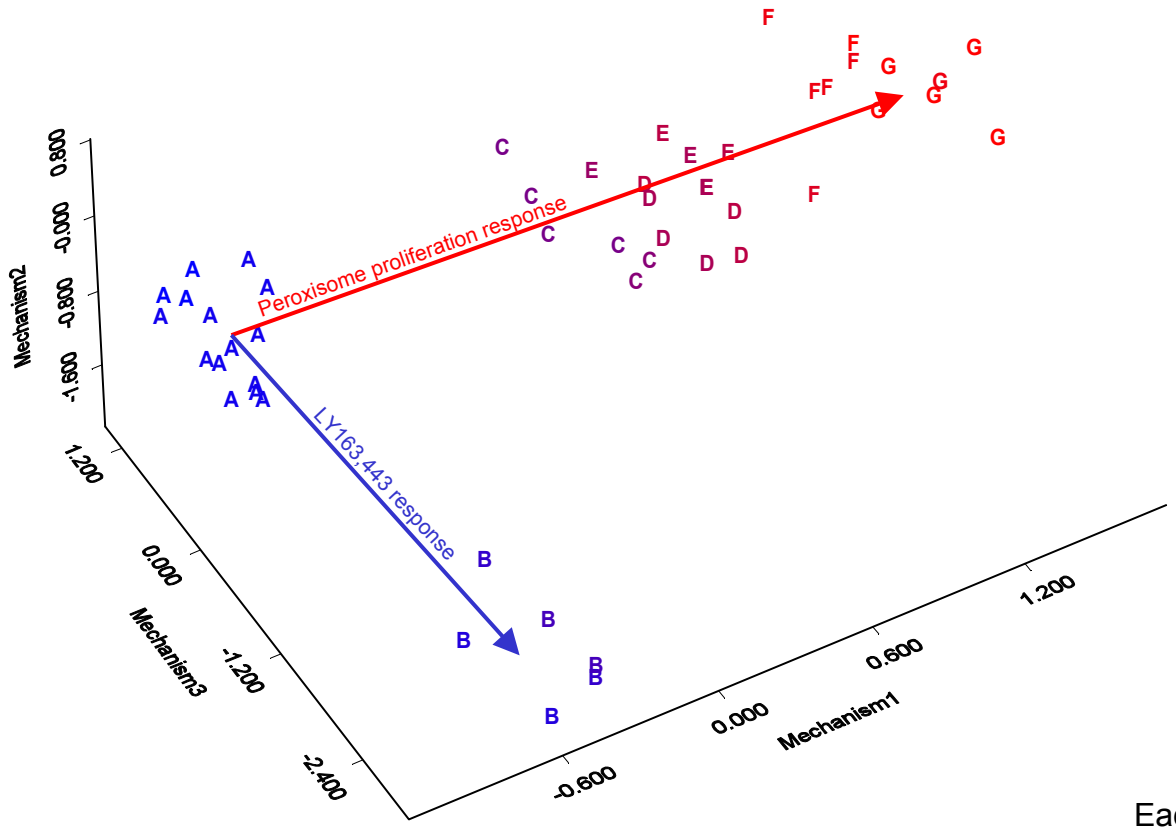
Multivariate Protein Markers Resolve Drug Mechanisms As They Do Disease States

Data from Quantitative 2-D Gel Studies in Mouse Liver
With Test Panels Involving > 100 Proteins



Peroxisome Proliferator Treatments

A= Control
B= LY163,443
C= LY171,883
D= DEHP
E= Clofibric acid
F= WY14,643
G= Nafenopin

Each symbol represents the liver protein pattern for 100+ proteins in the liver of an individual mouse

Peroxisome Prolferators: 6 Compounds Compared Over 107 Selected Protein Spots
The effects of peroxisome proliferators on protein abundances in mouse liver.
Anderson, N.L., Esquer-Blasco, R., Richardson, F., Foxworthy, P. and Eacho, P.
Toxicology and Applied Pharmacology, 137, 75-89, 1996.

Plasma Markers: Monitoring Genetic Risk or Current Health Status?

- Published data for different markers shows a wide distribution from almost total genetic control (unvarying levels) to none
  - Ratio of intra-individual to inter-individual CV's (epidemiology studies)
  - % variation due to genetics (MZ twin studies)
- Both methods show Lp(a) marker level is genetically determined: i.e., appropriate as a risk factor measure (assay needed one time)
- Average proportion genetic is ~50%
- Genetic component >20% suggests patient is best control for marker changes
- Genetic component >80% suggests patient value will not change

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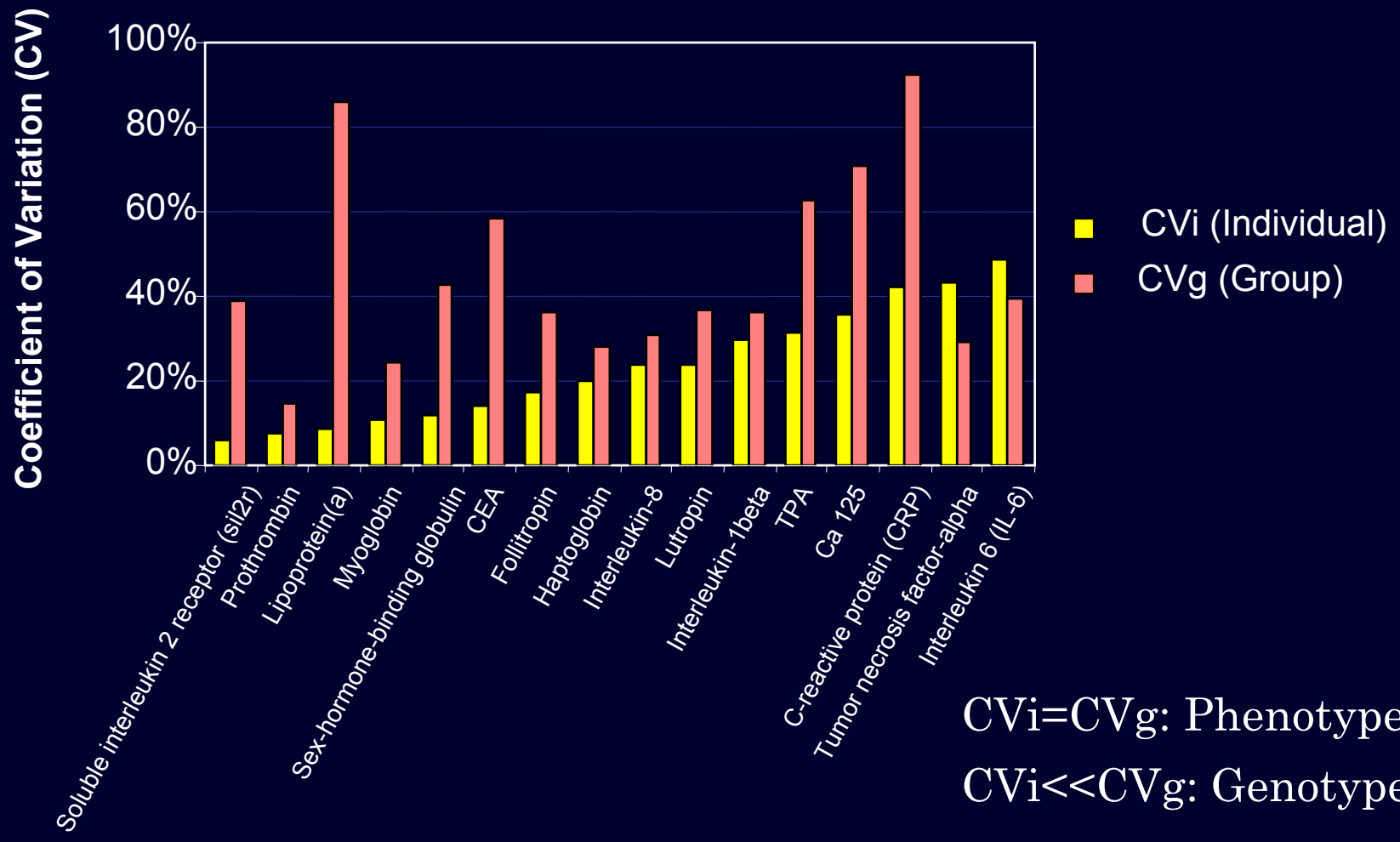
Intra-individual vs Inter-individual Coefficients of Variation for 16 Proteins in Plasma



CVi=CVg: Phenotype

CVi<<CVg: Genotype

(Published values from many sources)

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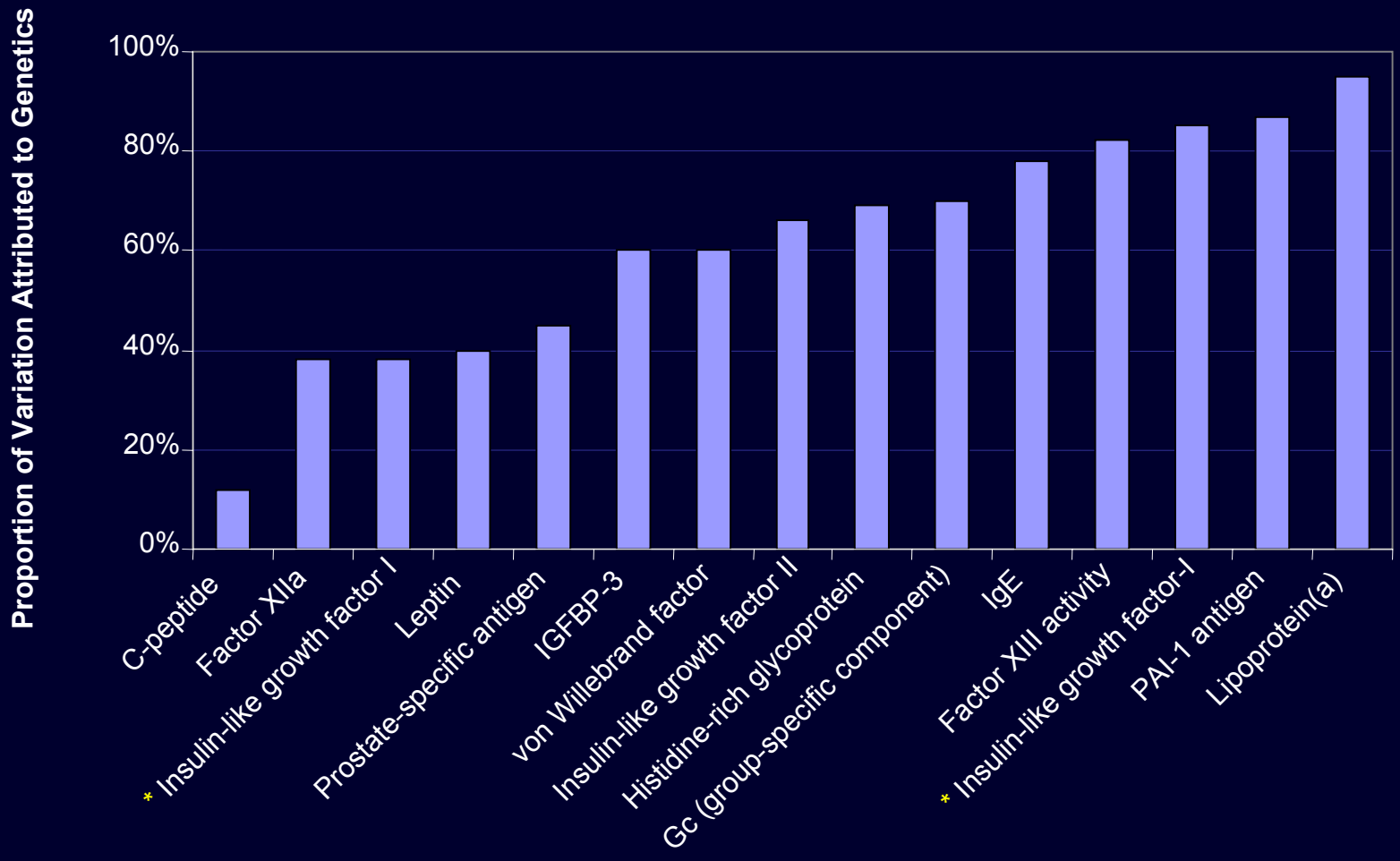
Genetic Component of Variation in Abundance of 15 Proteins in Plasma



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(Published values from many sources
*= two discordant studies)

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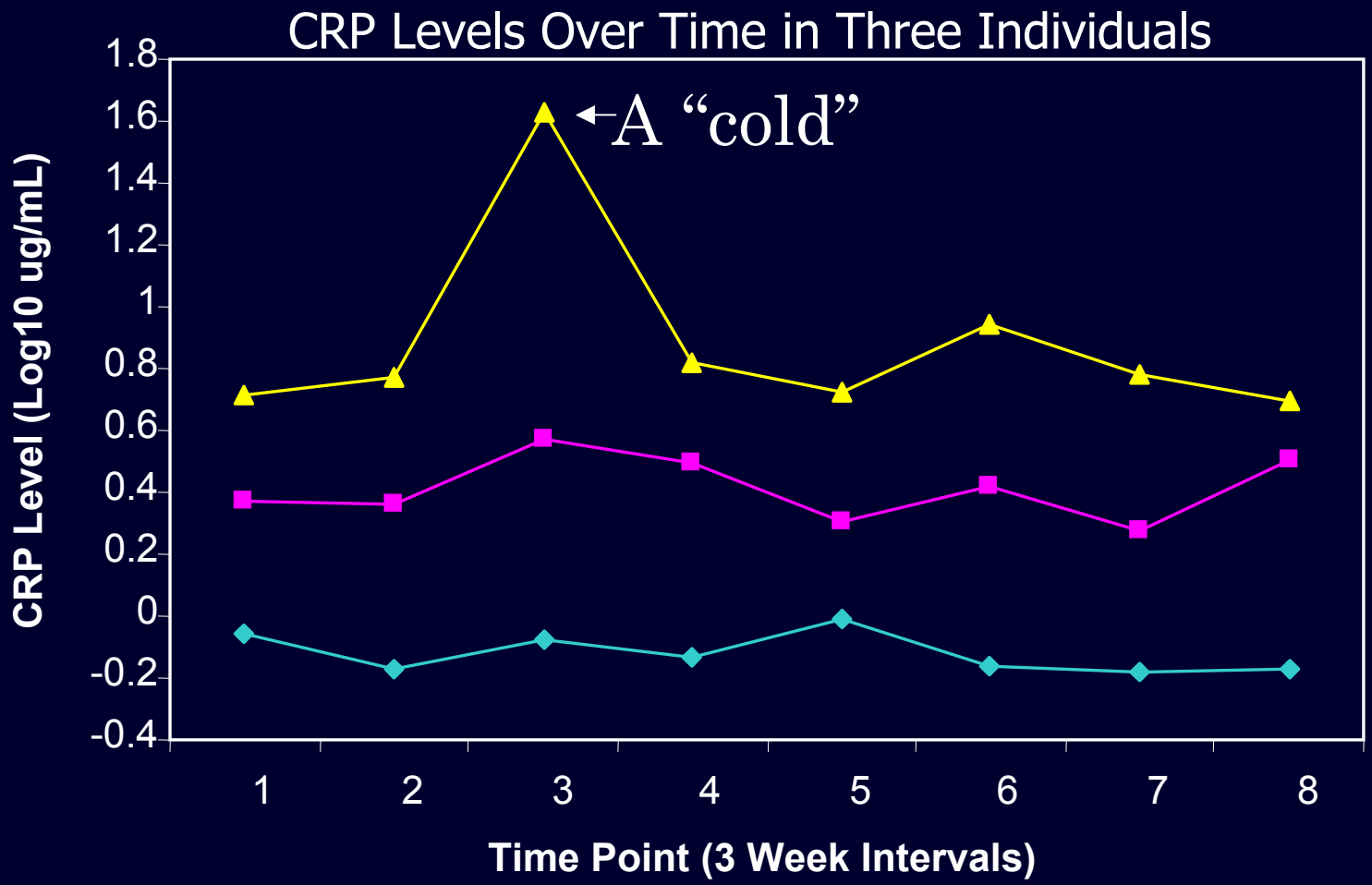
Individual Variation in CRP Over Time:
Stability of Non-Disease Levels Allows Finer Characterization of Disease



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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Conclusions

- Diagnostic value of plasma proteome measurements substantially enhanced by
  - Use of more protein markers
  - Use of multiprotein panels
  - Use of patient as self-control
- Current data is convincing but sparse
- A series of demonstration studies is needed affect change in consensus view
- Regulatory issues need debate
- Integration of the above is timely

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- Purpose

  Expand the range of protein analytes and indications through application of rapid proteomics quantitation systems to sets of well-characterized clinical samples

- Aims

  Promote multivariate protein tests

  Promote repeatedly sampling of individuals for detection of trends

  Advance technologies for routine plasma proteome measurement

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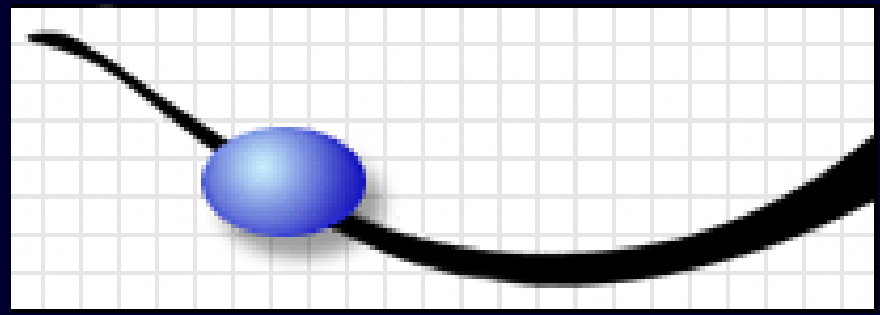

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