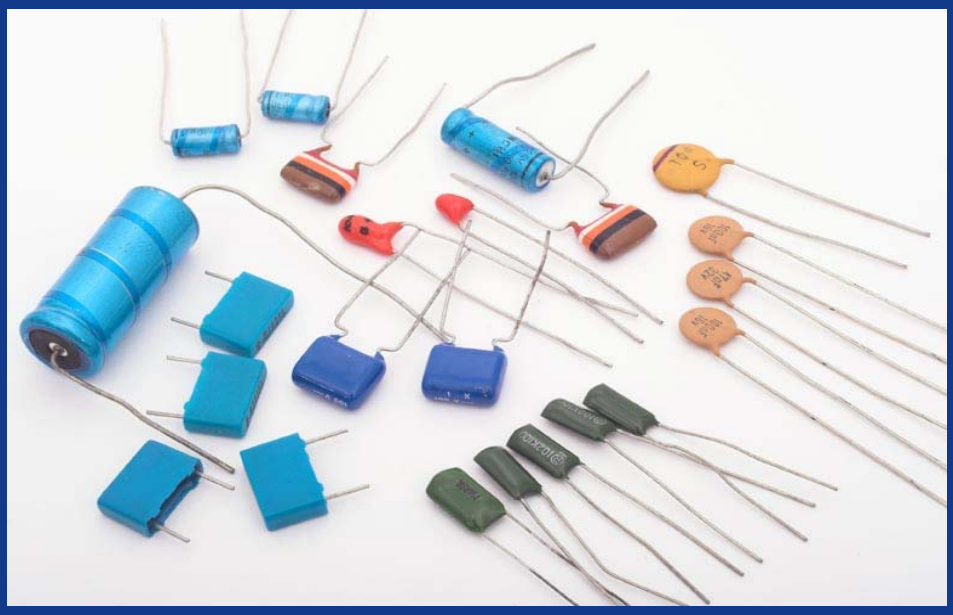
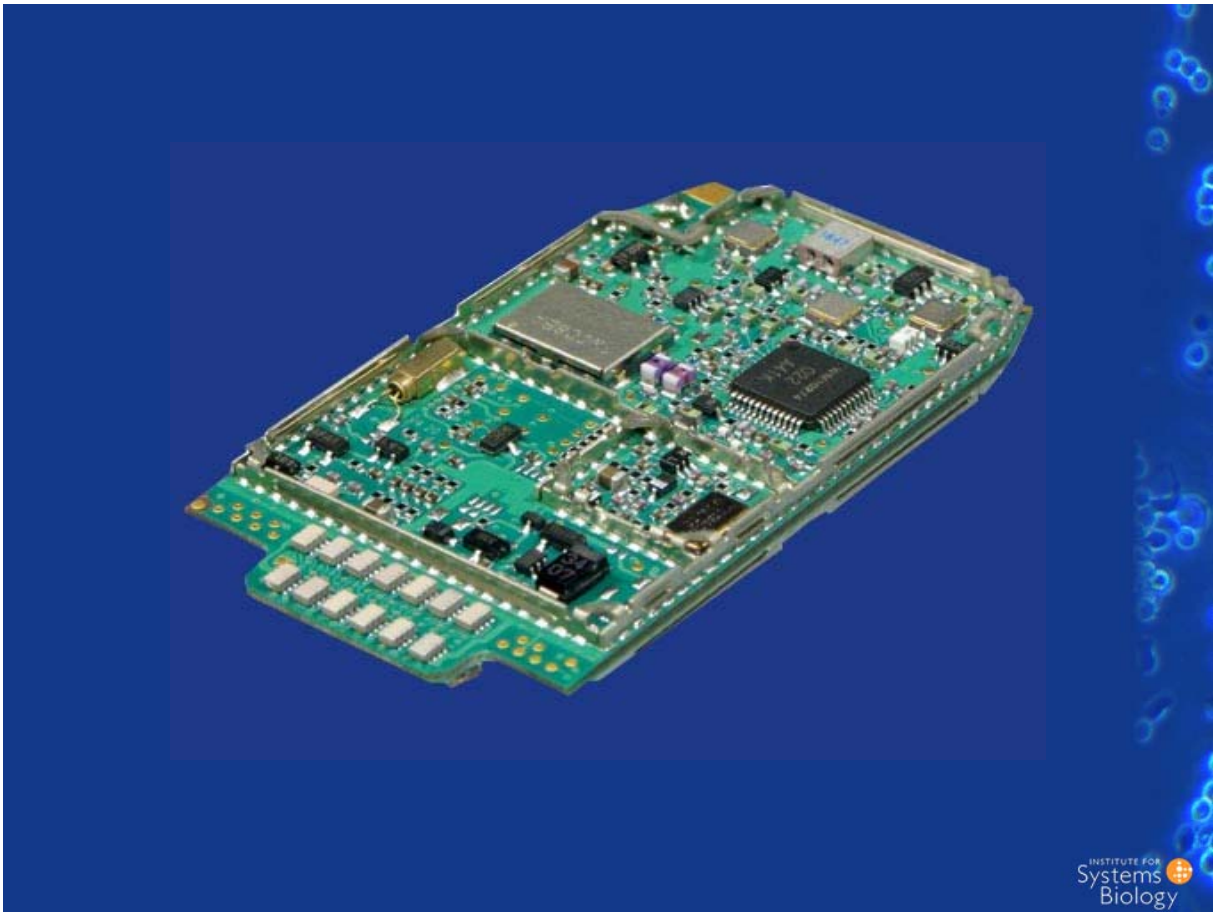
Systems Biology and Systems Medicine: Technology, Measurement and Validation

Lee Hood
Institute for Systems Biology, Seattle

How Might One Think About Systems Biology?

Radio Waves →  → Sound Waves





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Development
Physiology

Immune Response

Intra- and inter-
cellular networks

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Contemporary Systems Biology is Predicated on Viewing Biology is an Informational Science

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There are two types of Biological Information

- The digital information of the genome

- The environmental information that impinges upon and modifies the digital information.

```
CCAGAAAGGC CGAGGCTCTG CAGCGGGAGG
GCAGGGCACA GGGACAGCCC CCCTCCACAG
CCAGGAGGTT GCTTCTTCCA GGAGGCTTTT
GCTCCCAGCT GCTGTGAGTG CTGCACATTC
CACTTCTGGT GCCCACTGTG GCCACAGCAA
GCCTCCTGGG GAGCTGCTGA CCCTAGGCAG
CACCCCAGTG TTTGCCAGTG TTTGCCCGTG
TTTGCTCGCC AGTGTTCGCC ACTTGTCCCT
GAAGTTGCAG GTCCCTCCAG GACAGTTGGC
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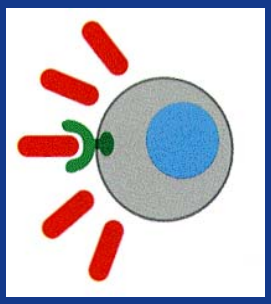

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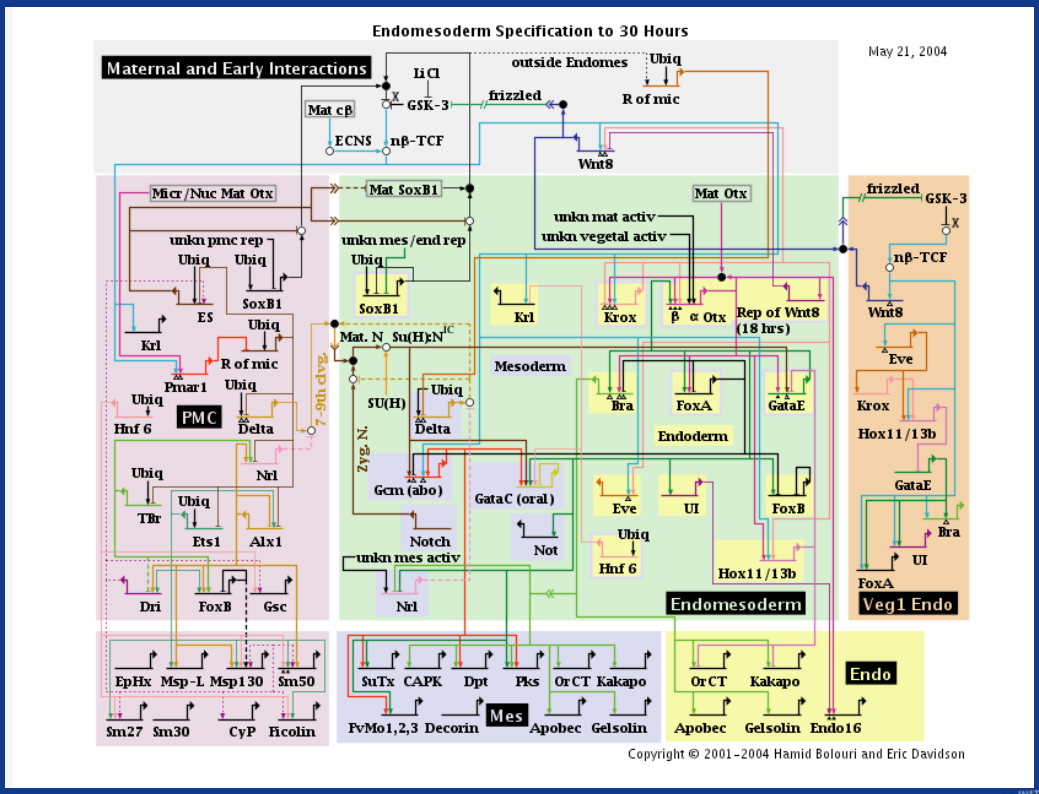
Biological Structures that Handle Information

- Biological networks capture, transmit, process and pass on information
  - Protein networks
  - Gene regulatory networks
  - MicroRNA networks
  - Genetic networks
- Simple and complex molecular machines-- execute biological functions

Most Sophisticated Integrated Biological Network Defined to Date



Hierarchical or Multiscalar Levels of Biological Information

Top Down and Bottom Up

Level of System Analysis

Connect to Digital Core

Integration of Different Levels

DNA

mRNA

Protein

Protein interactions and biomodules

Protein and gene networks

Cells

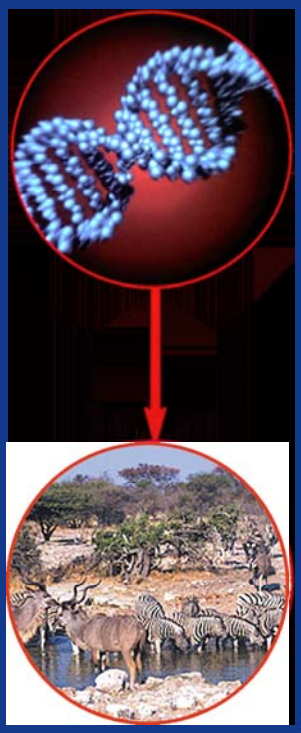
Phenotypes

Organs

Individuals

Populations

Ecologies



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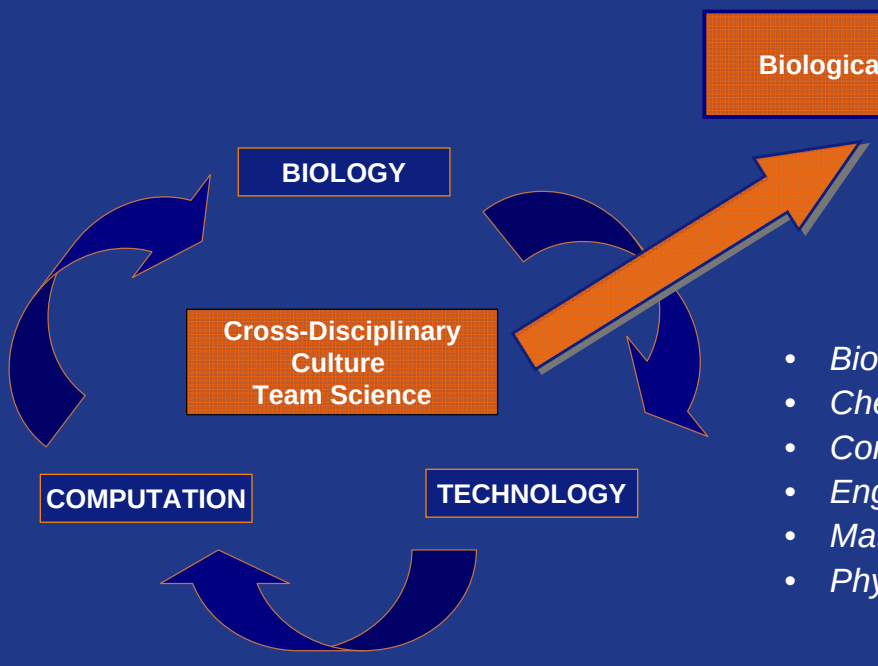
Agenda: Use biology to drive technology and computation.
Need to create a cross-disciplinary culture.



- *Biology*
- *Chemistry*
- *Computer Science*
- *Engineering*
- *Mathematics*
- *Physics*

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What Distinguishes Contemporary Systems Biology?

Six essential features of contemporary systems biology

***Systems biology cycle**: model--hypotheses--perturbation--measurement--model--hypothesis--perturbation--etc. Utilization of carefully formulated **systems perturbations**. Complexity requires simulations.*

***Global measurements**--measure dynamic changes in all genes, mRNAs, proteins, etc, across state changes.*

*Computational and mathematically **integrate different data types**--DNA, RNA, Protein, Interactions, etc.--to capture distinct types of environmental information.*

***Dynamic measurements**--across developmental, physiological, disease, or environmental exposure transitions.*

*Integration of **discovery-driven** and **hypothesis-driven** (global or focused) measurements .*

***Quantitative measurements** for all types of biological information.*

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Systems Medicine

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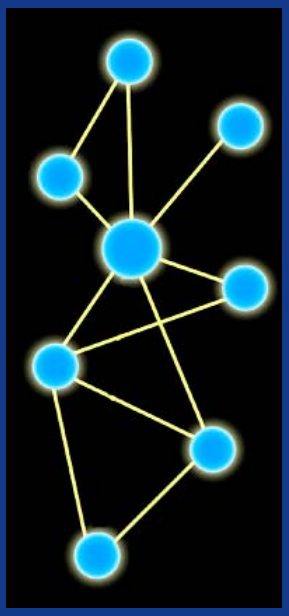
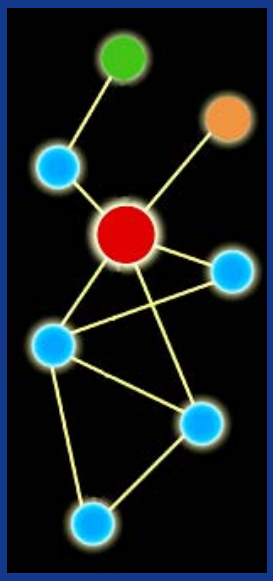
A Systems View of Disease Postulates that Disease Arises from Disease-Perturbed Networks



Non-Diseased

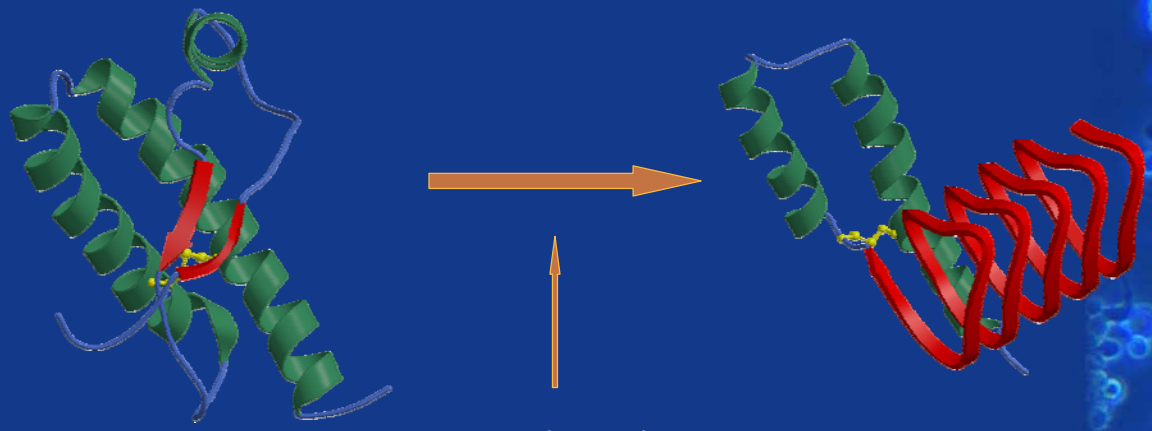


Diseased

- dynamics of pathophysiology
- diagnosis
- therapy
- prevention

A Systems Approach to Prion Disease in Mice

Prion disease example:
Prion Protein Exists in Two Forms



Cellular **PrP^{C}**

PrP Genetic Mutations
PrPSc Infections
Spontaneous conversion

Infectious **PrP^{Sc}**

Multiple groups:five inbred strains, two transgenic strains and one knockout strain

Group	Mouse	*Prnp* Genotype	Prion Strain	Incubation Time (d)
1	C57BL/6J	*a/a*	RML	~150
2	B6.I-1	*b/b*	301V	~120
3	FVB/NCr	*a/a*	RML	~150
4	B6.I-1	*b/b*	RML	~350
5	C57BL/6J	*a/a*	301V	~260
6	(FVB x FVB.129-*Prnp*^{tm1Zrch})	*a/0*	RML	~400
7	Tg(MoPrP-A)B4053	*30 x a*	RML	~60
8	FVB.129-*Prnp*^{tm1Zrch}	*0/0*	RML	No illness

Differentially Expressed Genes--DEGs--7400 to 333

Differentially Expressed Genes shared by eight mouse strain combinations: *Encode major disease responses*

333 genes shared by eight mouse strain combinations are highly likely to be involved in prion replication and neuropathogenesis.

Use protein interaction and gene regulatory databases to build hypothetical protein interaction networks for major disease features:

- Microglial activation
- Astrocytic hypertrophy
- Presynaptic bouton degeneration
- Dendritic atrophy
- Prion replication and accumulation
- Nerve cell death

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DEGs Encoding Known and Novel Prion Disease Phenotypes

- 137/333 DEGs encode known disease pathogenic pathways
- 196/333 DEGs encode novel pathogenic pathways--the dark genes of prion disease

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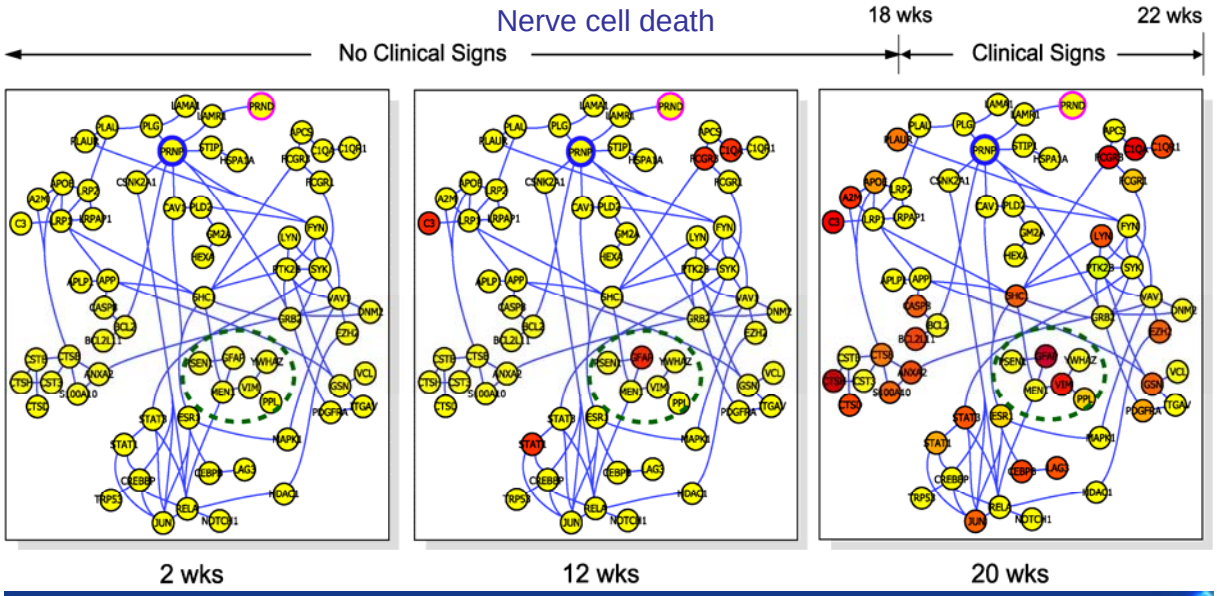
Systems Approach to Blood Diagnostics



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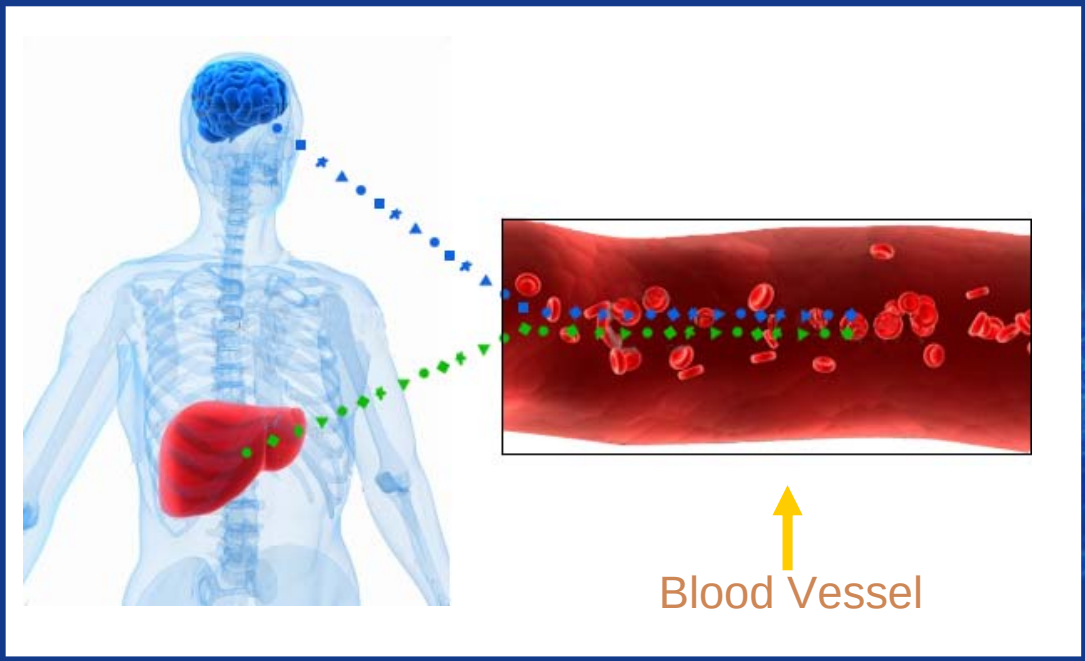
Organ-Specific Blood Fingerprints

Making Blood A Window Distinguishing Health and Disease



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Organ-Specific Blood Proteins Will Make the Blood a Window into Health and Disease

- Blood baths all organs. All organs secrete proteins into the blood. The blood is easily accessible for diagnostics.
- Perhaps 50 major organs or cell types--each secreting protein blood molecular fingerprint.
- The levels of each protein in a particular blood fingerprint will report the status of that organ and thus distinguish health from disease--and if disease, which disease. Probably need perhaps 50 organ-specific proteins per organ.
- Will need to quantify 2500 blood proteins from a droplet of blood. Discovery and validation (targeted MRM mass spectrometry) vs. typing (microfluidics/nanotechnology).
- Key point: changes in the levels of organ-specific markers can assess virtually **all** diseases challenges for a particular organ

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Uses of Organ-Specific Blood Biomarkers

- Disease diagnostics--early, stratification, progression
- Assessing the use of drugs in individuals--toxicity, response, dose
- Wellness assessment--longitudinal data gathering – patient is their own control

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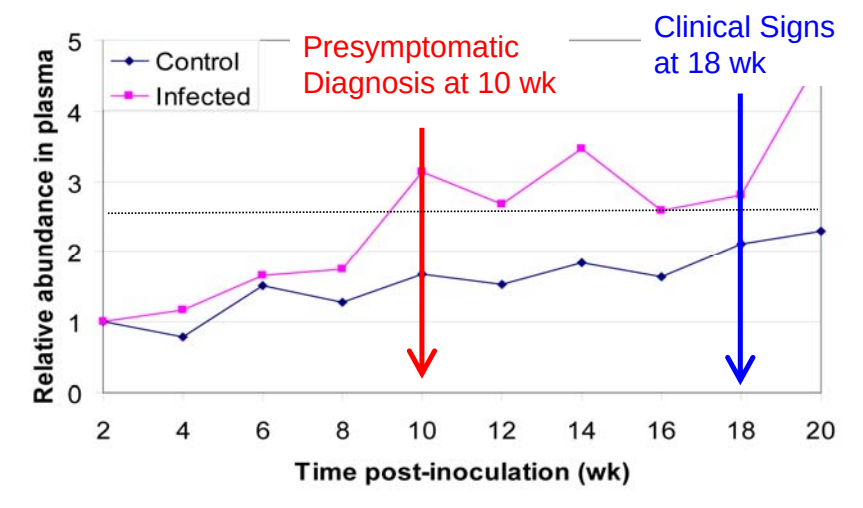
Presymptomatic Diagnosis of Murine Prion Disease with Organ-Specific Protein in Blood Samples

Dynamic change in relative abundance of "Protein A"



Systems Biology

Enable Integrated Blood and Tissue Diagnostics

- DNA
- mRNA
- miRNA
- Protein
- Metabolites
- Networks
- Single-cell analyses (DNA, RNA, protein, networks)

Systems Biology

General Comments on Measurements

Measurement and Visualization Challenges for Systems Biology

- DNA
- RNA
- Proteins
- Interactions
- Integrated biological networks
- Cells
- Phenotypes
- Organs
- Individuals
- Populations
- Ecologies

Identification
Quantification
Processing
Modification
Localization
Half-life
3-D structure
3-D dynamics
Structure-function

- Data validation, analysis, integration and modeling

Data Required to Delineate Biological Networks

- Dynamically changing mRNA and miRNA levels
- Dynamically changing protein levels
- Dynamically changing protein/protein interactions
- Dynamically changing DNA/protein interactions
- Dynamically changing mRNA, miRNA and protein modifications
- Dynamically changing protein localizations
- Validate, standardize, integrate and model various types of biological data

- Key points: global vs selected analyses
  quantitative analyses

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What are the technologies that will transform systems or P4 medicine?

- High throughput DNA sequencing for individual human genome sequencing
- Targeted MRM mass spectrometry for discovery and validation of blood organ-specific fingerprints
- Microfluidic protein chip to measure blood organ-specific protein fingerprints and type millions of individuals
- Single-cell analyses--deciphering the interplay of the digital genome and the environment
- In vivo and in vitro molecular imaging to assess disease distribution and follow therapy

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Next Generation DNA Sequencing: Microfluidic and Nanotechnology Approaches

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Key Technical Issues in Next Generation DNA Sequencing

- Throughput--parallization
- Accuracy--validation--standardization
- Read length--sequence assembly
- Ease of sample preparation--single-stranded DNA sequencing
- Ease of acquiring and translating data into assembled digital genome sequence delineating both the maternal and paternal haplotypes

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Proteomics

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Monitor Reaction Measurement (MRM) Mass Spectrometry-- Directed Target Identification

- Pioneered for proteins by Ruedi Aebersold at ISB and ETH
- Perhaps1500 fragments measured per hour at the low femptomole level
- For blood need automatable and reproducible front-end separation procedure(s) to reduce protein complexity (glycocapture)
- For discovery of biomarkers and their validation

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MRM and GlycoCapture

	Glycocapture and MRM	Antibody-Based Assays
Multiplex Limit	250 proteins	100 proteins (Luminex)
Sensitivity	pg/ml	pg/ml
Time to Create Assay (10 proteins)	6 weeks	104 weeks
Cost to Create Assay (10 proteins)	$20 000	$2 000 000
Precision	5% - 15%	5% - 10%

Key Points:

• Rapid validation by glycocapture and MRM overcomes an industry-wide barrier to diagnostics development by reducing development time 95% and reducing cost 100 fold.

• Our MRM is highly multiplexed enabling the simultaneous quantification of hundreds of proteins in a single analysis.

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Microfluidic and Nanotechnology Platforms for Protein Measurements--Large Scale Typing of Individuals

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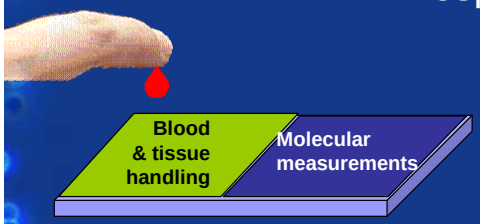
In vitro blood protein diagnostics

Quantitate 2500 organ-specific proteins to:

identify disease;
stratify disease;
progression of disease;
response of disease to therapy etc.



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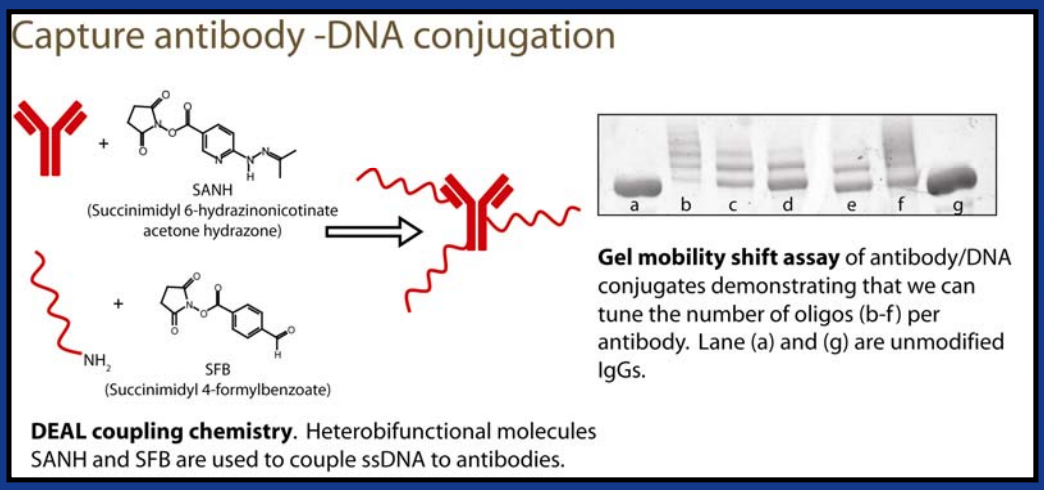
DNA Encoded Antibody Library (DEAL)



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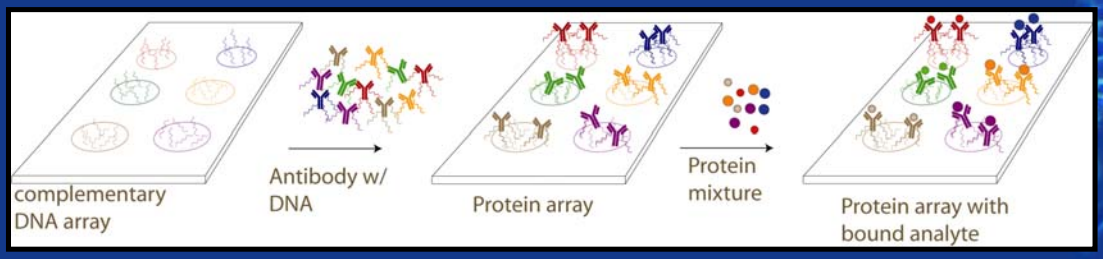
Protein Measurement by DEAL

- DNA strands are spotted on arrays.
- Antibodies are localized to specific DNA spots.



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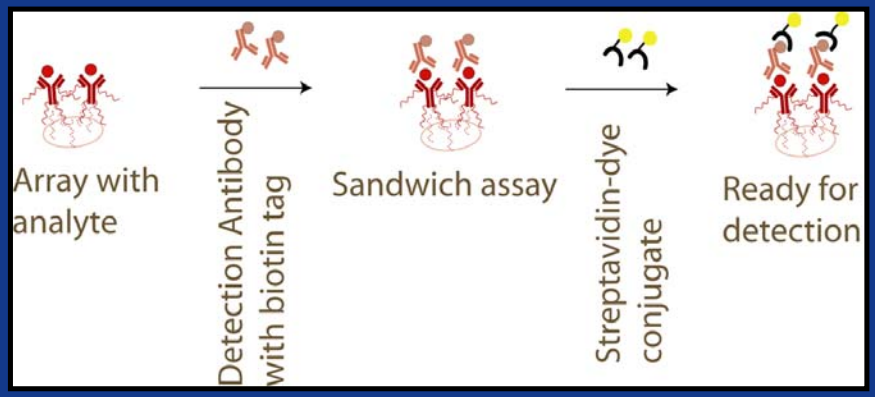
Protein Measurement by DEAL

- Sandwich assays are performed with the capture antibody and a detection antibody.
- Detection antibody has a biotin tag.
- Streptavidin dye conjugates allow visualization.



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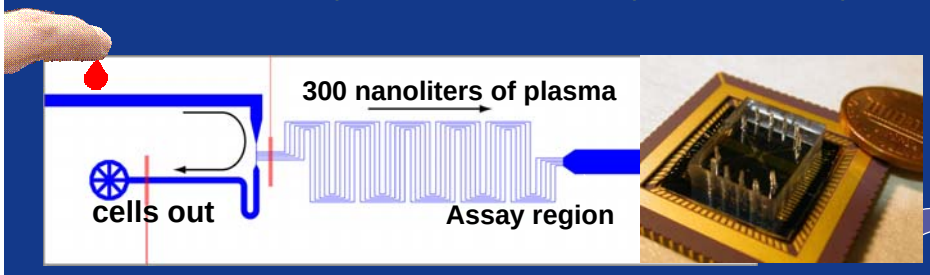
DEAL for *In vitro* molecular diagnostics:

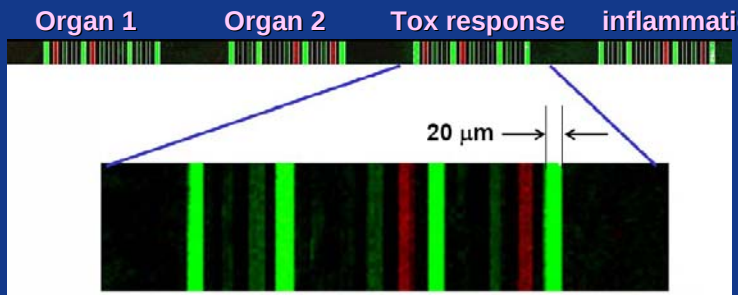
Integrated nanotech/microfluidics platform

Separate plasma & rapidly quantitate protein biomarker panels

- Profile health status of individual organs
- Select appropriate therapies or combination therapies
- Profile positive & adverse responses to therapies



Panel of protein biomarkers measured in a single microfluidics channel (15 min assay time)



Dynamic range--10^8

Sensitivity--high atmole

5 minute measurement

Jim Heath, et al

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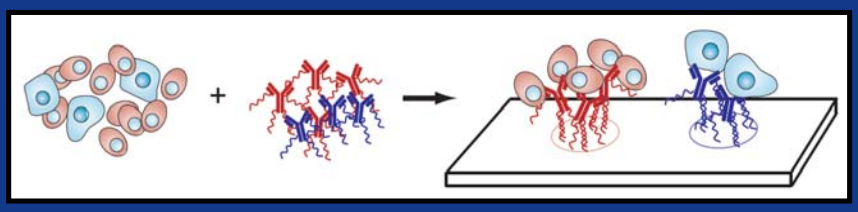
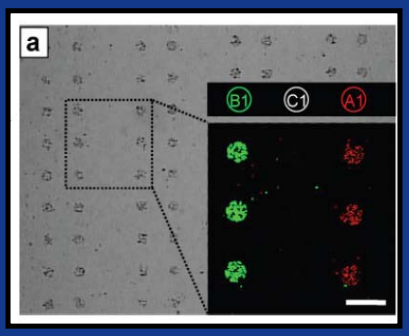
Surface Marker Specific Cell Separation: A DEAL Application



A1: mRP-expressing T cells

B1: EGFP-expressing B cells

C1: no cell capture agent



Source: Gabe Kwong

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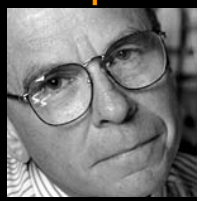
Click Chemistry: a New Approach to Protein Capture Agents--Key to All Antibody-Based Assays

Our approach: Multi-ligand Protein Capture agents

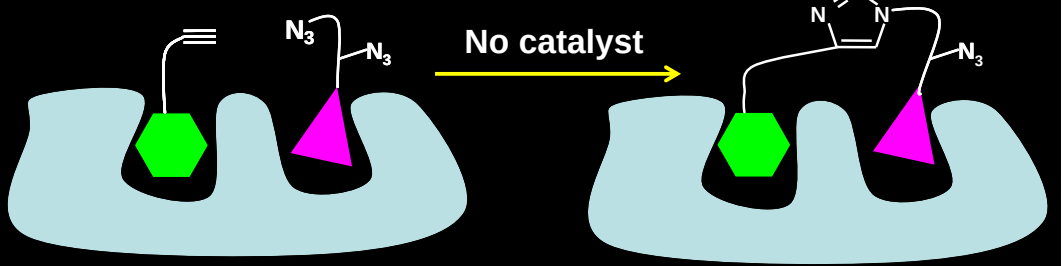
Very old chemistry (Huisgen 1,3-dipolar cycloaddition)

R. Huisgen, G. Szeimies, L. Möbius, *Chem. Ber.* **1967**, *100*, 2494–2507.

K. Barry Sharpless



Newer *in situ* click chemistry

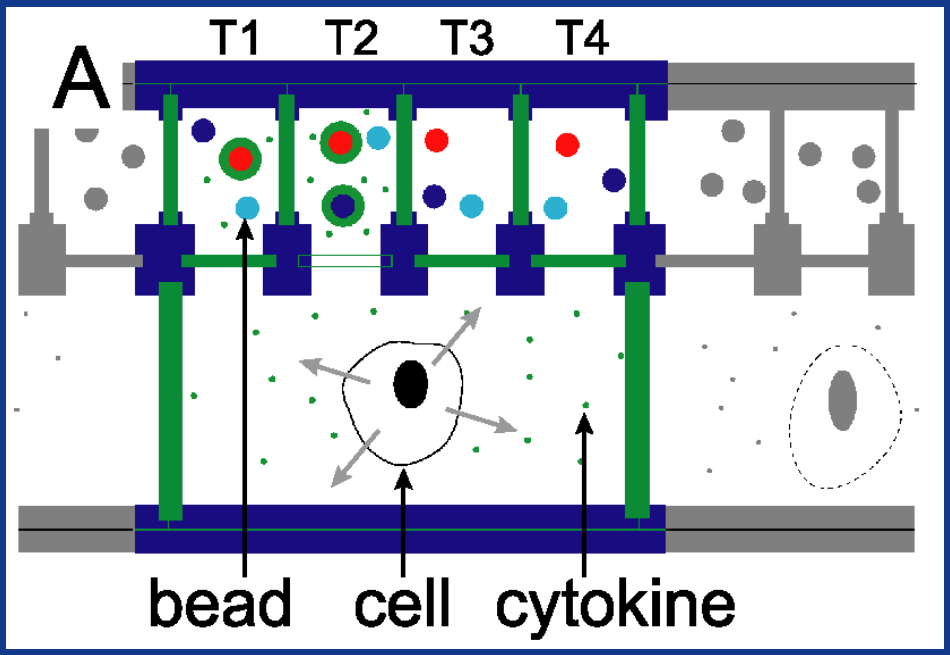


H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021.

Single-cell Analyses

Single Cell Analyses

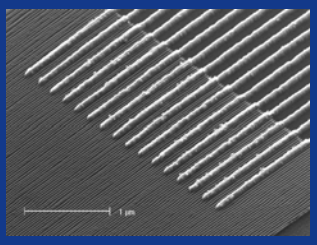
Multiplexed ELISA for secreted proteins



Adrian Ozinsky

Predictive, Personalized, Preventive and Participatory (P4) Medicine

- Driven by systems approaches to disease, new measurement (nanotechnology) and visualization technologies and powerful new computational tools, P4 medicine will emerge over the next 10-20 years



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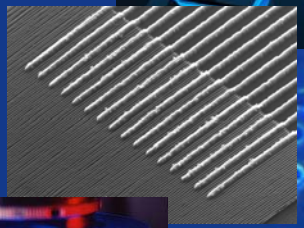
P4 Medicine

- Predictive:
  - Probabilistic health history--DNA sequence
  - Biannual multi-parameter blood protein measurements
  - In vivo molecular imaging
  - Systems genetics



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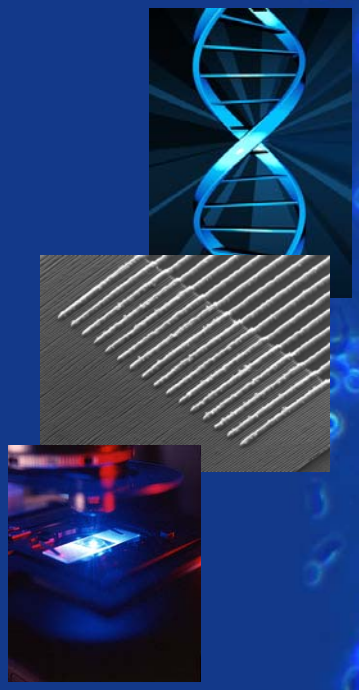
P4 Medicine

- Personalized:
  - Unique individual human genetic variation mandates individual treatment
  - Patient is his or her own control
  - Billions of data points on each individual



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P4 Medicine

- Preventive:
  - Design of therapeutic and preventive drugs via systems approaches
  - Systems approaches to vaccines will transform prevention of infectious diseases
  - Move to wellness assessment

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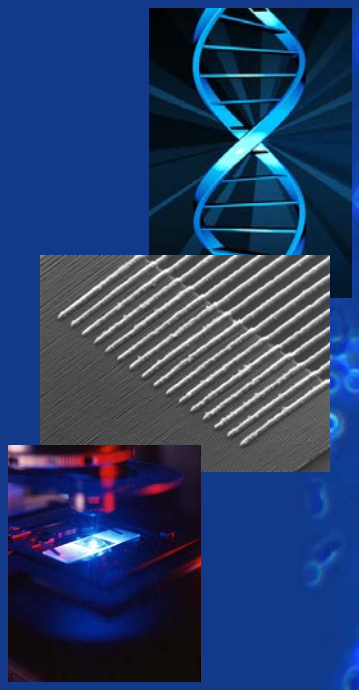
P4 Medicine

- Participatory:
  - Patient understands and participates in medical choices
  - Physicians will become the integrators of medical information--and must learn to educate patients effectively.



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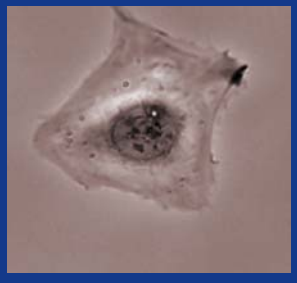
Digitalization of Biology and Medicine Will Transform Medicine

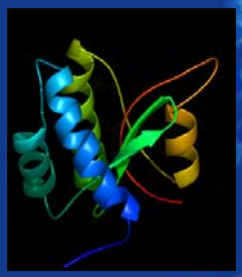
- Analysis of single molecules, single cells and single individuals
- A revolution that will transform medicine even more than digitalization transformed information technologies and communications--in 10 years
- Billions of data points on each patient
- Digitization of medicine will lead to dramatically lower healthcare measurement costs

Single individual

Single cell



Single molecule



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P4 Medicine Will Catalyze a Reduction in Healthcare Costs

- New diagnostics will stratify patients and disease making treatment more efficient
- New therapeutics will be far less expensive and more rapidly generated--preventive drugs will emerge
- The focus will shift from treating disease to promoting wellness
- Digitalization of medicine--cheaper measurements and more global data gathering

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Systems Medicine Will Transform Productivity

- P4 medicine, advances in understanding aging, stem cell therapies, powerful new approaches to developing vaccines and new approaches to degenerative brain disease will over the next 20 or so years allow individuals to remain mentally alert and physically active into their 90's.

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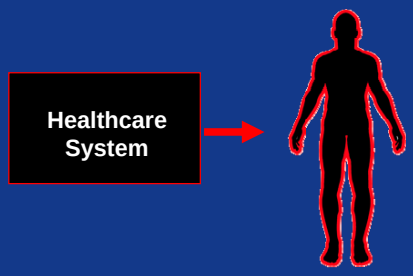
P4 Medicine Will Transform the Health Care Industry

Will impact the health care system significantly:

- Pharmaceuticals
- Biotechnology
- Diagnostics
- IT for healthcare
- Healthcare industry
- Health insurance
- Medicine--diagnostics, therapy, prevention, wellness
- Nutrition
- Assessments of environmental toxicities
- Academia and medical schools



Fundamentally new ideas need
New organizational structures

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Inventing the Future

- The foundations of P4 medicine
  - Systems analysis of biological and medical problems
  - Technology development
  - New computational tools
- Strategic partnerships
  - Solve hard problems with partners
  - Extend our reach & impact
- Bringing the benefits of advances to society
  - Education (K-12, graduate, medical)
  - Commercialization of technology
  - Bringing P4 medicine to reality

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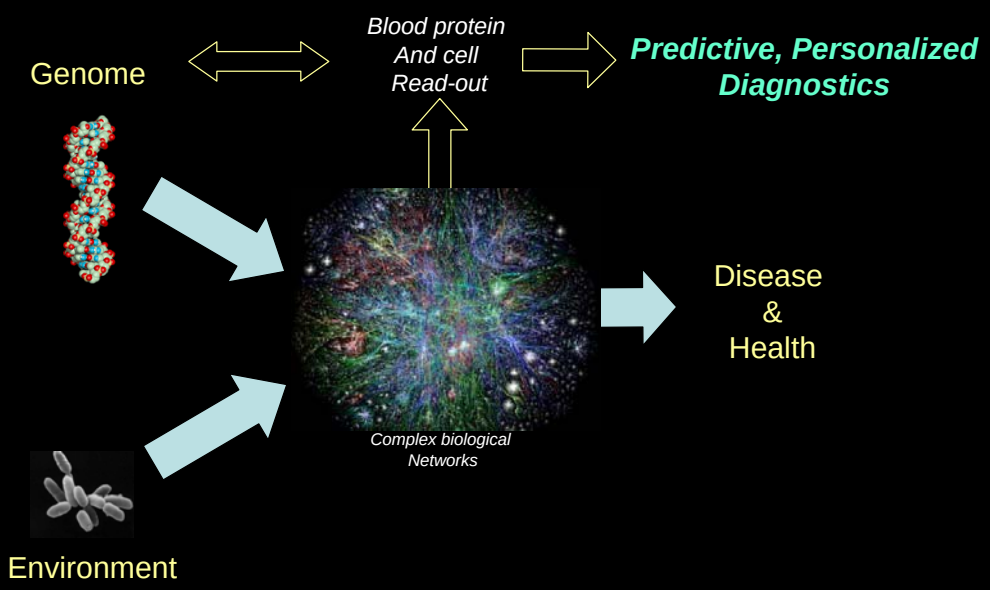
ISB Strategic Partnerships for P4 Medicine

- P4 medical institute
- Bring systems medicine to a US medical school
- Bring systems biology and P4 medicine to Luxembourg

ISB's Approach to P4 Medicine: Genetics and Environment

integration is key to future medicine



$ 100 million over 5 years

Integrated Diagnostics--a Platform Company for Personalized Medicine

Disease diagnosis--preclinical diagnosis, stratification and progression

Drug monitoring-- monitoring organ-specific toxicity/effectiveness in clinical trials and individual personalized medicine

Wellness monitoring--assess all major organ systems

Strategy: a systems approach to diagnostics
--organ-specific blood protein fingerprints

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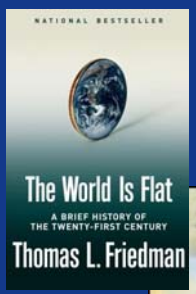
The Flattening of Many Worlds: Strategic Partnerships and the Globalization of Science

The worlds of science, technology, health are flattening.

Tremendous opportunities for national and international strategic partnerships in science, technology and education.



- Network of interacting complementary, institutions
  - Training in systems biology and recruiting the best world talent
  - Transferring and collaborating on new technologies and computational tools
  - Strategic partnerships on systems approaches to biology and P4 medicine
  - New patient populations
  - New fundraising and commercialization opportunities

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Final Comments

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Cooperation and Balance Between Cross Disciplinary and Small Biology is Essential

Cross Disciplinary Systems Biology

Small Biology

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The Disease Proteome: A Proposed Strategic Partnership with NIST and Others Using Glioblastoma as a Pilot Project

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Disease Proteome: Pilot Project

- Registry with 100s of samples per year--excellent patient records and sample preservation. Integrate molecular and phenotypic data.
- DNA sequence of tumors (sequence exons, SNPs, gain loss mutations, epigenetic markers)
- Transcriptomes of tumors and cell lines (primary, tumor stem cell, and stromal)--interactomes.
- Proteomics--tumors, cell lines, secretomes
- Blood brain-specific markers for longitudinal analyses--early diagnosis, stratification, progression.
- Single-cell analysis of cell lines and stem cells
- Follow known drug perturbations of cell lines.
- Developing computational techniques to identify brain
- Perturb relevant networks in cell lines with RNAi. disease-perturbed networks from the brain-specific fingerprints--dynamical
- Determine how to re-engineer disease-perturbed networks
- Identify drugs to re-engineer networks.
- Use glioblastoma cell lines to test new drugs
- Integration and modeling of all data types.

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Glioblastoma: Initial Objectives

- Brain-specific blood protein fingerprints for early diagnosis, disease stratification and following progression
- Single-cell and population analyses of stem cells and primary tumor cell lines for identifying new drug targets and exploring new drugs

NIST's Possible Roles

- Validation and standardization organ-specific blood molecular fingerprints
- Validation and standardization of blood single-cell analyses for disease and immunological status
- Technology development
  - Microfluidic/nanotechnology protein chips for blood protein measurements
  - Develop new types of protein capture-agents (beyond antibodies)--standardization and validation
  - Develop and standardize and validation measurements for single cells

Measurement and Standardization Bio Challenges in the 21st Century

- Complexity in multiparameter individual data types--genomes, RNAs, proteins, networks, cells, phenotypes, etc.
- Computational and mathematical integration and modeling of complex data types to create predictive models that will distinguish health from disease

Realize Individual Must be their Own Control

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Prion Disease

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