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Systems Biology and Systems Medicine: Predictive, Personalized, Preventive and Participatory (P4)

Lee Hood

Institute for Systems Biology, Seattle

NIST Opportunities in Personalized Medicine

- Measurement--validation, standardization and databases
  - Blood protein biomarkers
- Technology development
  - Nanotechnology/microfluidics
    - Blood protein measurements
    - Single-cell analyses
  - Imaging and visualization--in vitro and in vivo
- Computation/mathematical algorithms and platforms
  - Healthcare information technology
- Outside strategic partnerships are critical for NIST--for domain expertise

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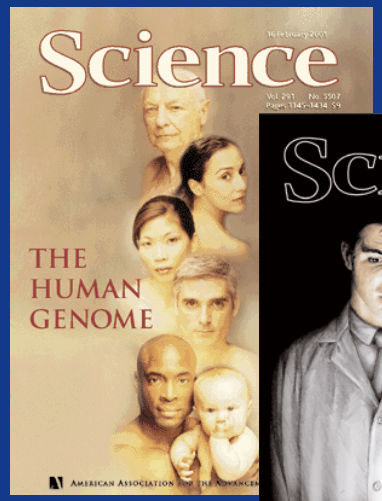
A Grand Convergence

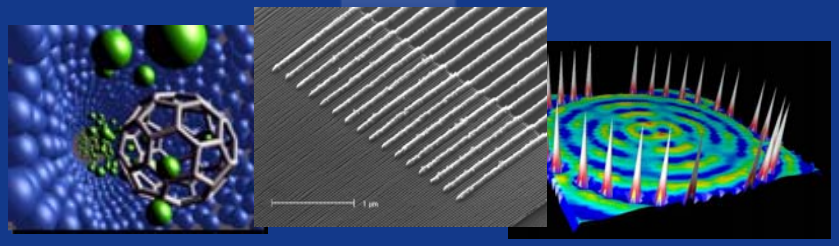


Complexity: Biology is an Information Science

Genetics, genomics

Predictive
Personalized
Preventive
Participatory
Medicine

Systems biology



Nanotechnology

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Biology as an Informational Science

- Two types of biological information--digital genome and environmental
- Biological networks capture, transmit, integrate, dispense and execute biological information.
- Biological information is hierarchical and multiscalar--DNA, RNA, protein, interactions, networks, cells, organs, individuals, ecologies.

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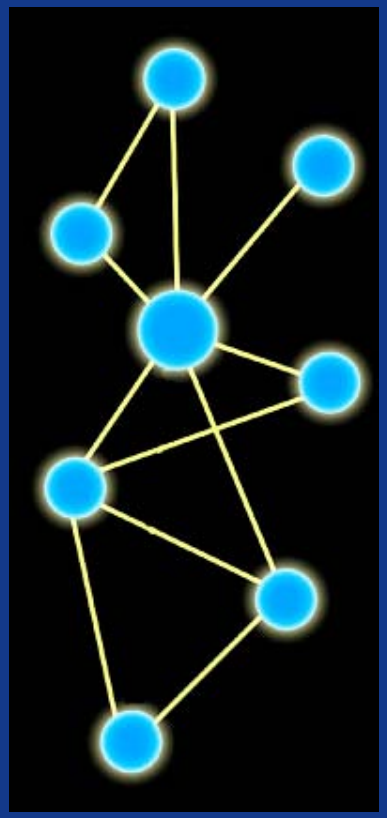
Systems Biology

Dynamic Networks

- ***Elements (genes,proteins) – "nodes"--measurements!***
- ***Interactions*** between the elements – ***"edges"--dynamic***
- Elements and their interactions are affected by the ***Context of other systems within--cells and organisms***
- Interactions between/among elements give rise to the system's ***Emergent properties***



Technology Has Transformed Contemporary Systems Biology

Four essential features of contemporary systems biology

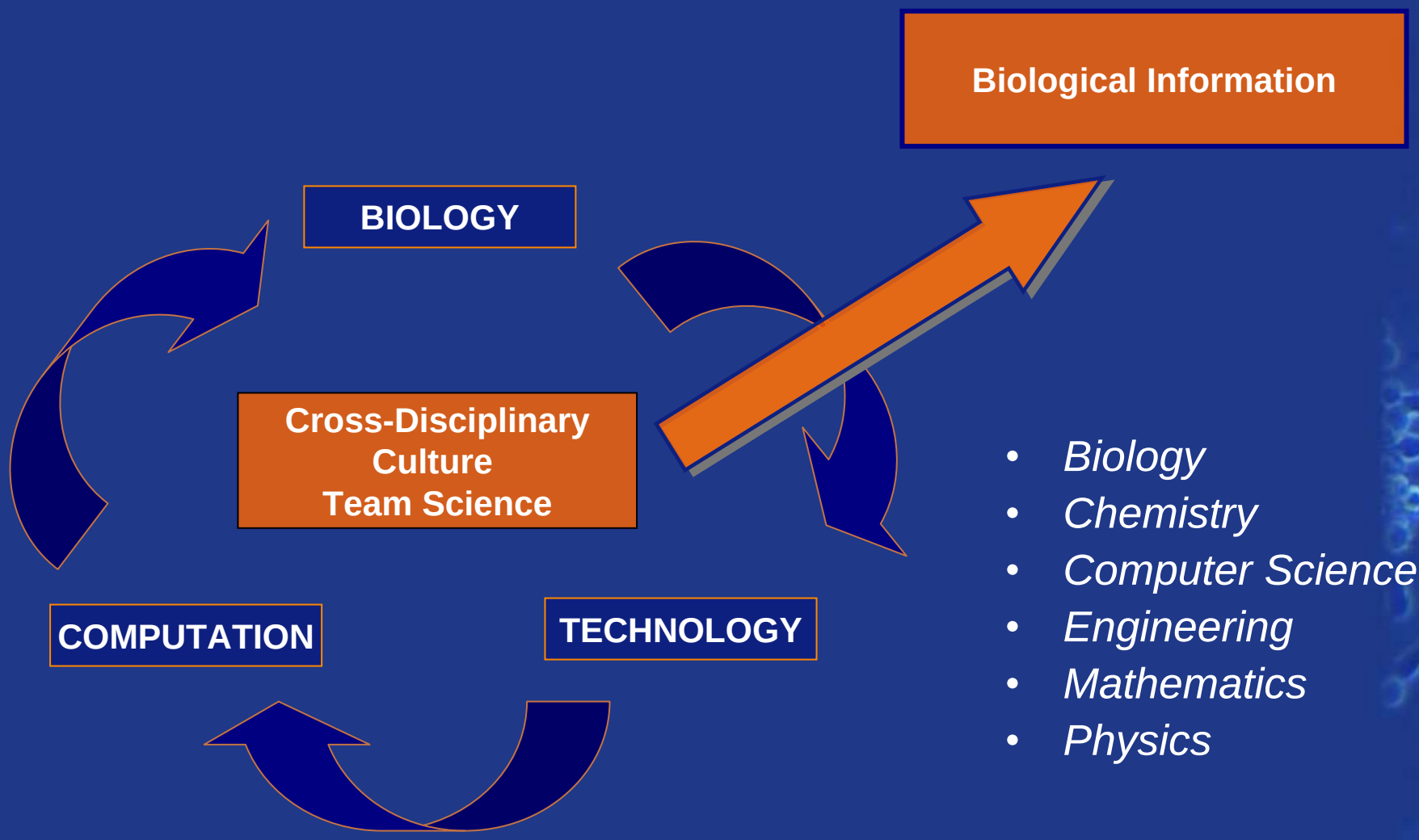
Quantitative measurements *for all types of biological information.*

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.*

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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Institute Overview

- Founded: Non-Profit Research Institute January 2000 in Seattle, WA
- Co-founders: Dr. Leroy Hood, President
  Dr. Alan Aderem, Director
  Dr. Ruedi Aebersold
- Staff: More than 200 staff members, including thirteen cross disciplinary faculty members and laboratory groups.
- Funding: More than $300 million awarded to date.
- Facilities: Highly automated biological equipment and platforms. Strong computational infrastructure.
- Partnerships: More than 10 industrial and academic partners and almost 50 research collaborators.
- Integrations: Biology/Technology/Computation/Medicine
- Transfer of Knowledge: Spin offs/Accelerator; K-12 Education; Third World Medicine; Informational Medicine and Society
  Global Partnerships: Short courses

A New Organizational Institution for Systems Biology

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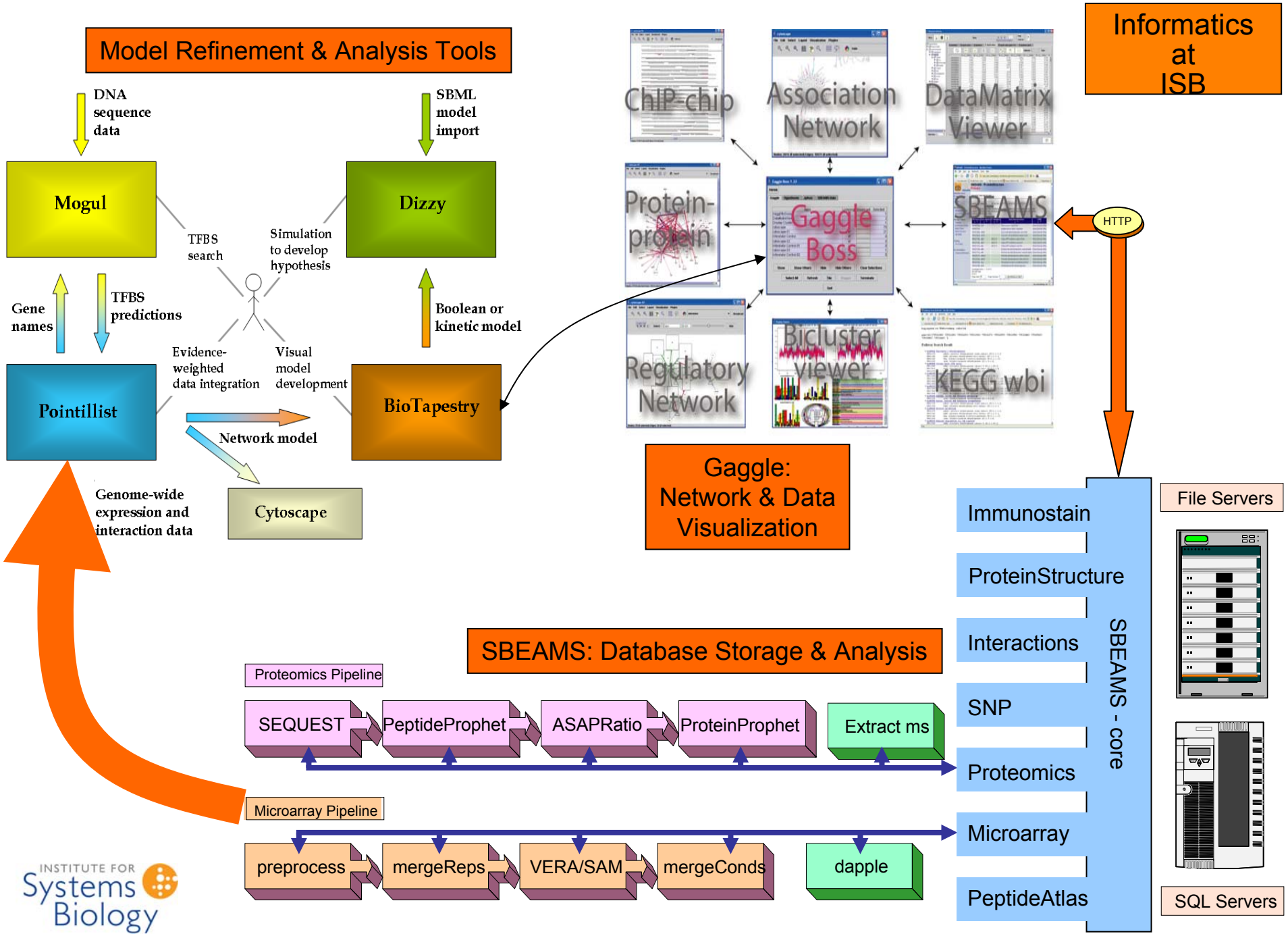
ISB Technology Development

- Genomics--Hood
  - Single strand DNA sequencing--Helicos
  - Protein folding and annotation--Hood/Baliga
  - Gene finding--FEAST
  - Personalized genome analysis--George Church--Harvard
- Proteomics-
  - Analyzing molecular machines--Ranish
  - Glycocapture of blood proteins--Aebersold/Hood
  - Serum databases--reanalysis of primary data--Atlas--Aebersold
  - Computational software programs for validation--Aebersold
  - Toward global proteomics--Hood/Aebersold
  - Measuring 2000 blood proteins--nanotechnology--Hood/Heath
  - Surface plasmon resonance--Hood, Lausted
  - Nanotechnology and microfluidic measurements of protein properties
- Single cell analyses--Ozinsky/Galitski/Aitchison/Hood/Heath
  - Microfluidics
  - Nanotechnology
- Imaging and visualization---strategic partnerships

Techniques for searching new realms of data space


Informatics at ISB
Model Refinement & Analysis Tools
DNA sequence data
SBML model import
Mogul
Dizzy
TFBS search
Simulation to develop hypothesis
Gene names
TFBS predictions
Boolean or kinetic model
Evidence-weighted data integration
Visual model development
Pointillist
BioTapestry
Network model
Cytoscape
Genome-wide expression and interaction data
ChIP-chip
Association Network
DataMatrix Viewer
Protein-protein
Gaggle Boss
SBEAMS
HTTP
Regulatory Network
Bicluster viewer
KEGG wbi
Gaggle: Network & Data Visualization
SBEAMS: Database Storage & Analysis
Proteomics Pipeline
SEQUEST
PeptideProphet
ASAPRatio
ProteinProphet
Extract ms
Microarray Pipeline
preprocess
mergeReps
VERA/SAM
mergeConds
dapple
Immunostain
ProteinStructure
Interactions
SNP
Proteomics
Microarray
PeptideAtlas
SBEAMS - core
File Servers
SQL Servers
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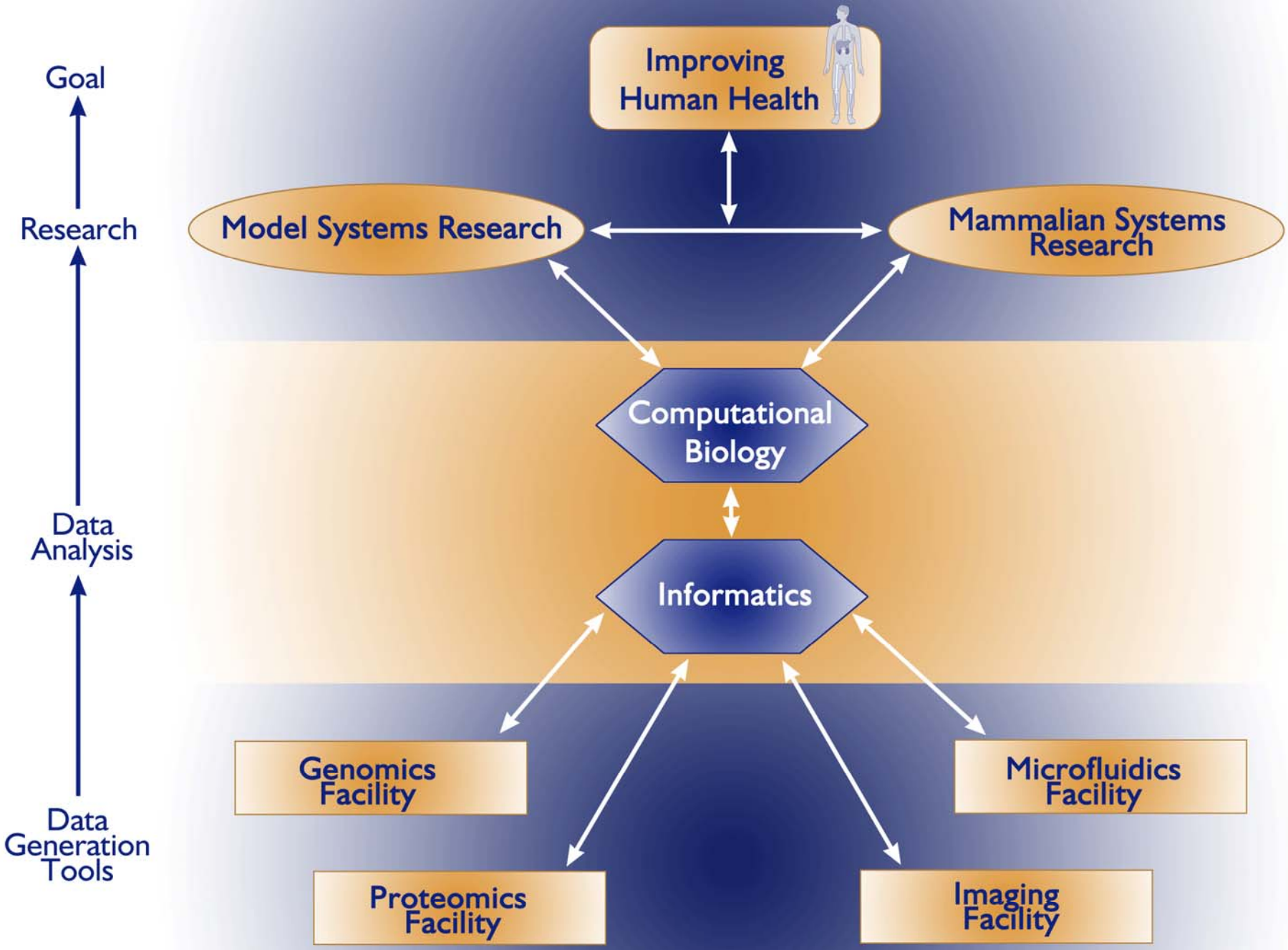
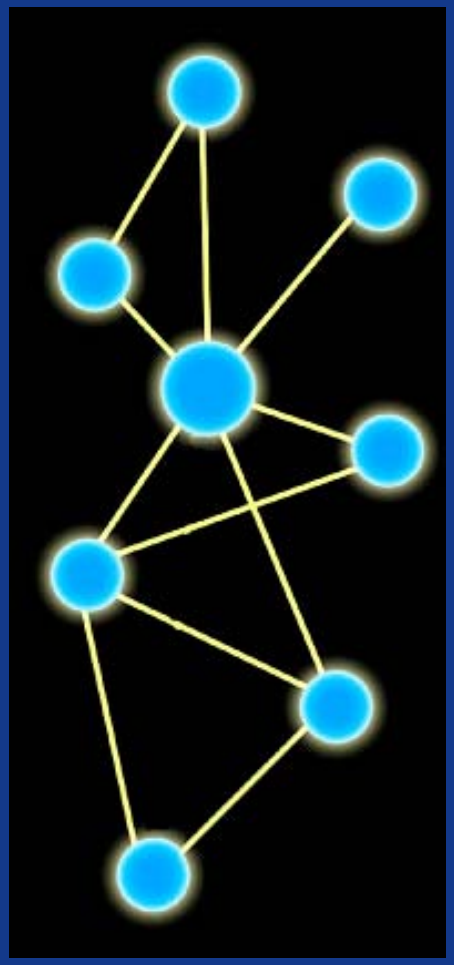
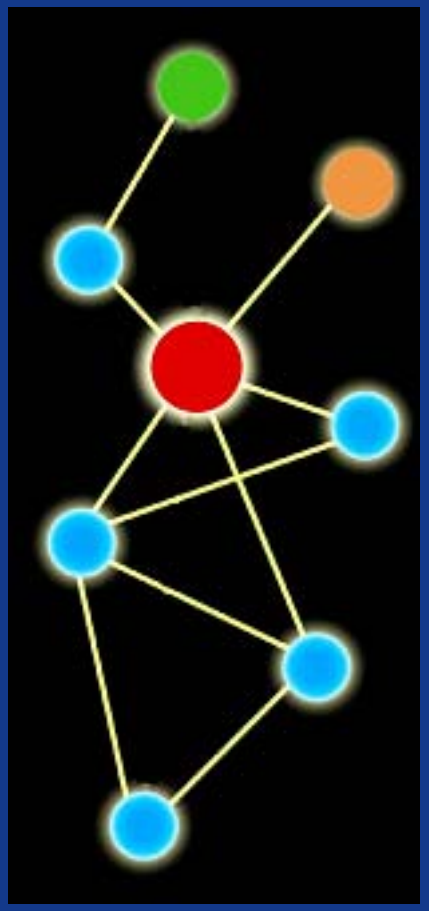

Goal
Research
Data Analysis
Data Generation Tools
Improving Human Health
Model Systems Research
Mammalian Systems Research
Computational Biology
Informatics
Genomics Facility
Microfluidics Facility
Proteomics Facility
Imaging Facility

A systems approach to disease

Disease Arises from Disease Perturbed Networks



Non-Diseased



Diseased

dynamics of pathophysiology

diagnosis

therapy

prevention

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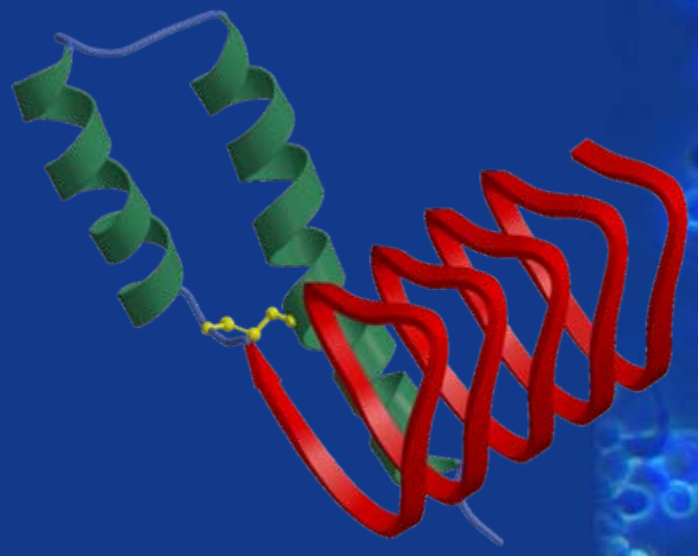
Prion Protein Exists in Two Forms

Cellular **PrP^{C}**

PrP Genetic Mutations

PrPSc Infections

Spontaneous conversion



Infectious **PrP^{Sc}**

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Differentially Expressed Genes shared by five prion-mouse combinations: Do they encode major disease responses

924 genes shared by five mouse-prion combinations are highly likely to be directly/indirectly involved in prion replication and neuropathogenesis.

Use protein interaction and gene regulatory databases to build hypothetical protein interaction networks for major disease features (uses 253/924 core prion genes--the rest do other pathology!):

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

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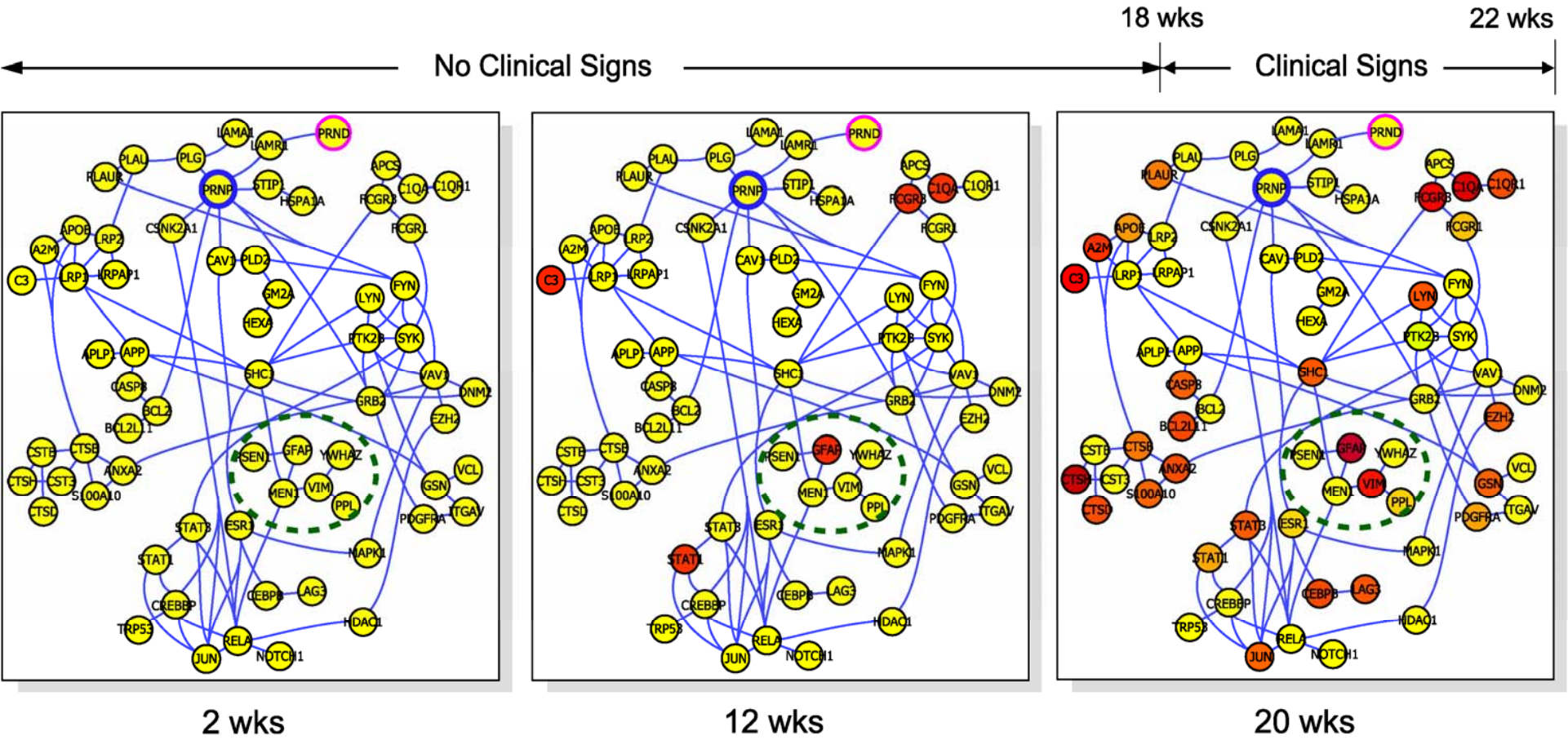
Dynamics of a Prion Perturbed Network in Mice



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Blood Protein Diagnostics

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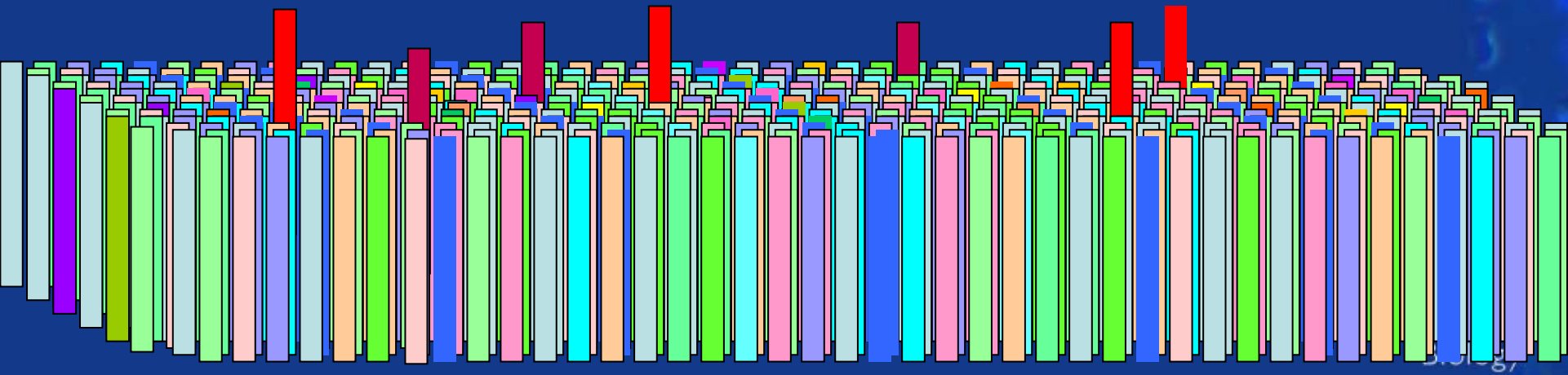
Finding diagnostic markers:
The 'needle in the haystack' problem

Hundreds of thousands proteins are present (cells, tissue, serum, etc.)

Which one(s) in the serum is/are indicator(s) for a specific disease?

Organ-specific blood markers

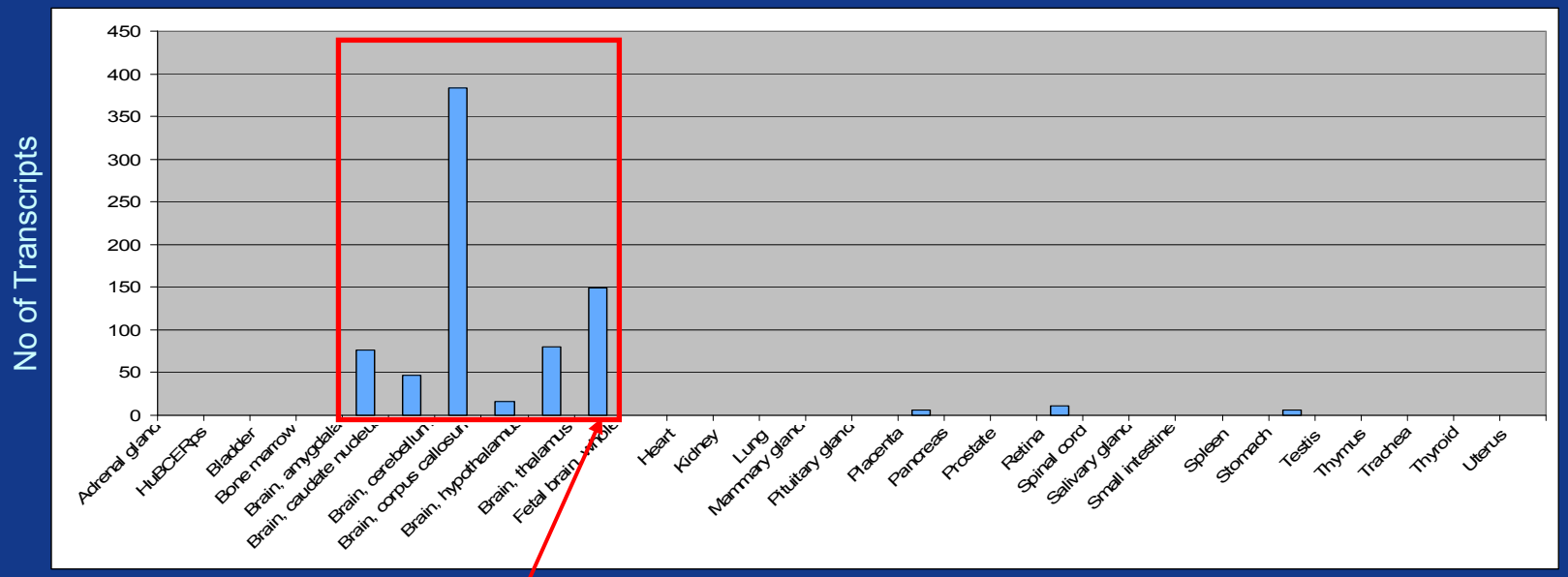


Fingerprinting Prion Disease through the Brain-Specific Blood Proteins

- Out of 900 common DEGs, 100 transcripts are predicted as CNS-specific transcripts by computational searches against transcriptome libraries of MPSS (Massively Parallel Signature Sequencing) on 40 mouse tissues (female).

- 49 CNS-specific gene products are predicted as secreted proteins.

- Some of those marker candidates are being tested by immunoassay methods on serum samples.



Brain
Sub-regions

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Blood Protein Measurements

- Proteomics--mass spectrometry
- Surface plasmon resonance
- Microfluidics/nanotechnology

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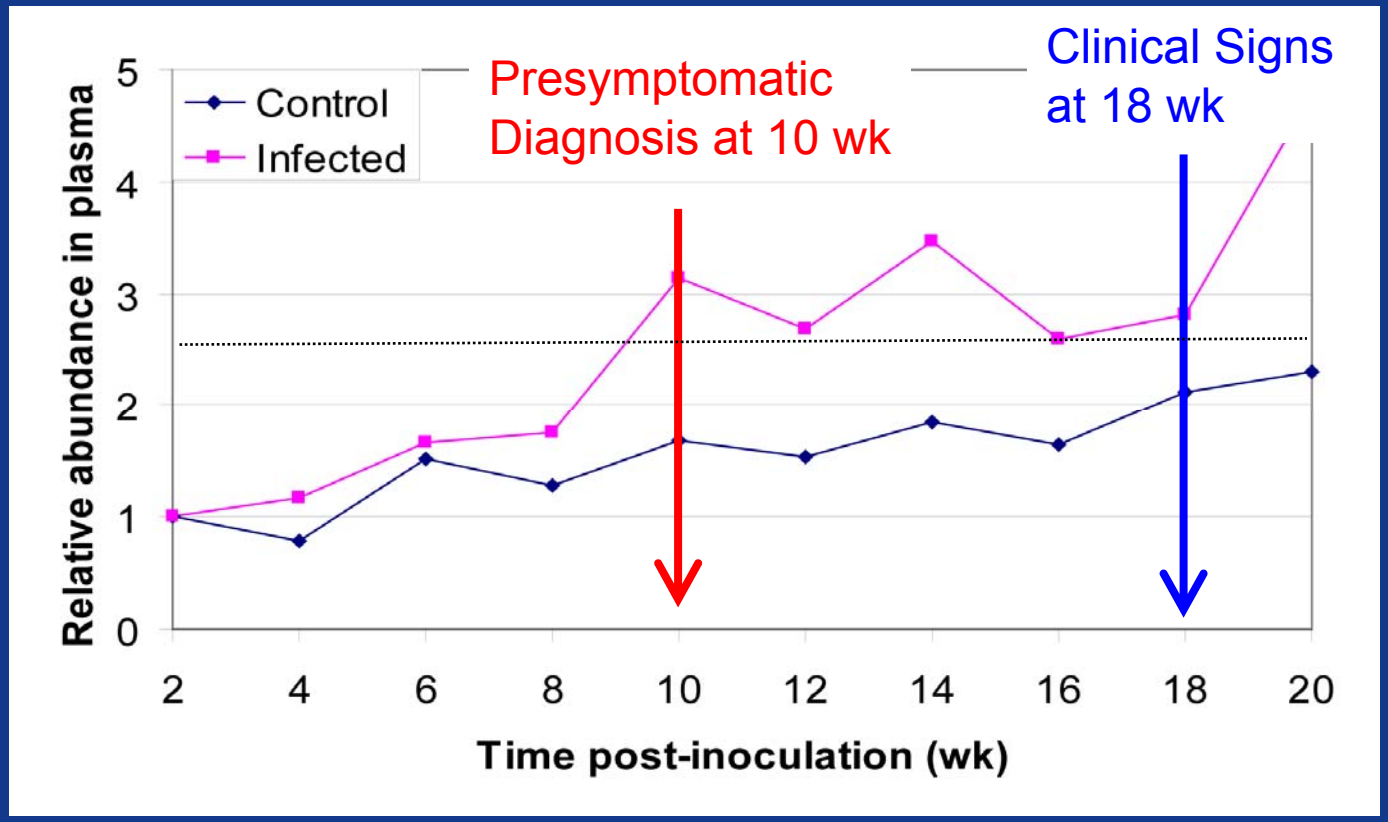
Increasing abundance of Protein A for prion-infected blood samples

Dynamic change in relative abundance of "Protein A"



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Mouse Brain-Specific Blood Proteins

- 4500 mouse blood proteins detected by mass spectrometry
- 2200 blood proteins encoded by transcripts identified by brain MPSS analyses
- 98 of these proteins are encoded by brain-specific blood transcripts
- 96/98 blood proteins have human orthologs

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Mouse liver toxicity

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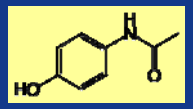

Acetaminophen Liver Toxicity

- Acetominophen (APAP) overdose causes hepatocyte death through its toxic metabolite.

- C57 BL6/J mice, IP injection of 50% lethal dose of APAP, collection of liver & blood at 0, 24h, 48h, 72h.

- Big organ--secrets lots of proteins into blood--lots of antibodies to blood liver-specific proteins

Zhiyuan Hu
Chris Lausted

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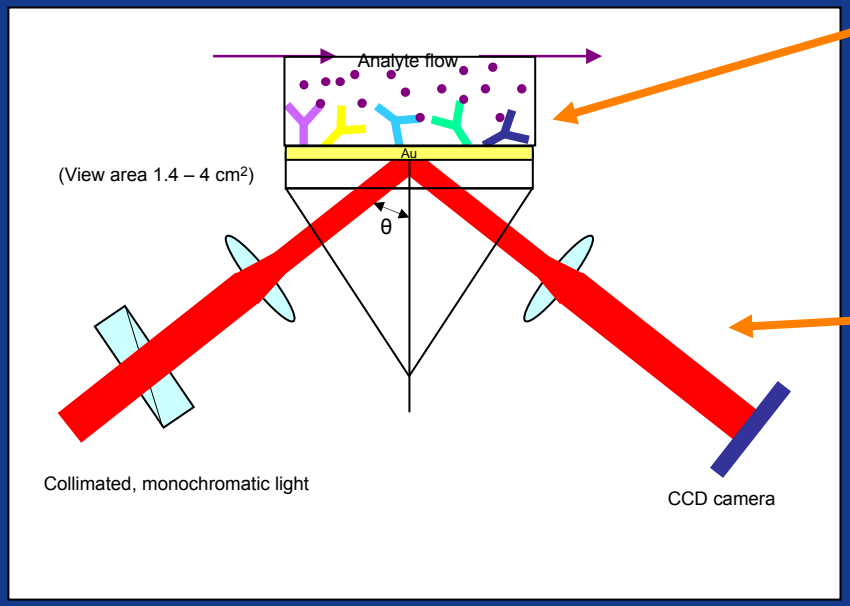
Antibody microarray with SPR

- Antibodies are printed on gold-coated glass slides .
- Array is placed at the bottom of a ~50ul flow chamber.
- Reflectance of each spot is monitored in real-time.
- Protein binding to the antibody increases refractive index near the array surface, increasing reflectance.

Surface Plasmon Resonance (SPR)



Lumera Nanocapture Gold™

Lumera ProteomicProcessor™

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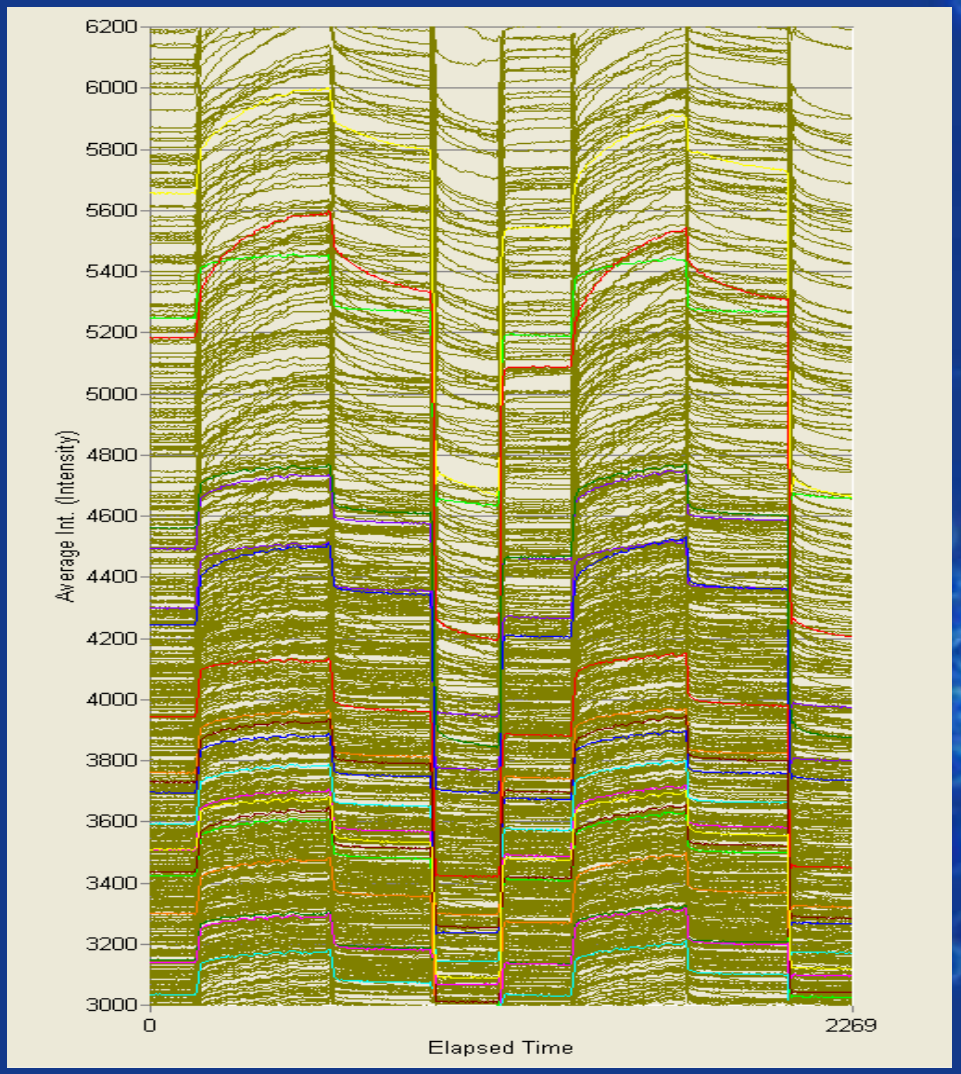
Raw image of 800 spots array
(400 antibodies in duplicates)

Raw SPR sensorgrams from 800-feature array



Advantages in biomarker discovery

- Label-free---**simple**
- 800 spots---**high throughput**
- 10 minutes for each serum sample---**fast**
- Antibody chip can be regenerated---**low cost**
- 30 samples by same chip---**low variation**

Cross reactivities--multipara-meter analyses

Platform taking few lambda blood samples coming

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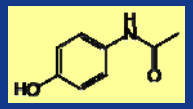
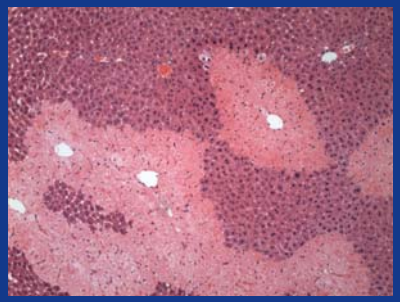


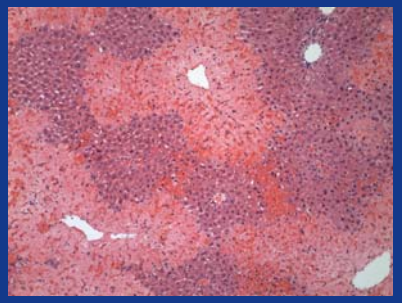
Acetaminophen Liver Toxicity

Histological confirmation of liver injury and recovery post i.p. APAP

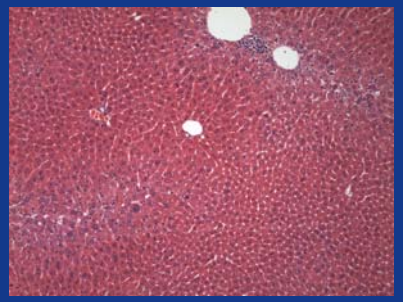
0h



24h



48h



72h

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Blood is a window into health and disease

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

- 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. Probably need perhaps 50 organ-specific proteins per organ.
- Will need to quantify 2500 blood proteins from a droplet of blood--use microfluidic/nanotechnology approaches.
- Key point: changes in the levels of organ-specific markers can assess virtually **all** diseases challenges for a particular organ

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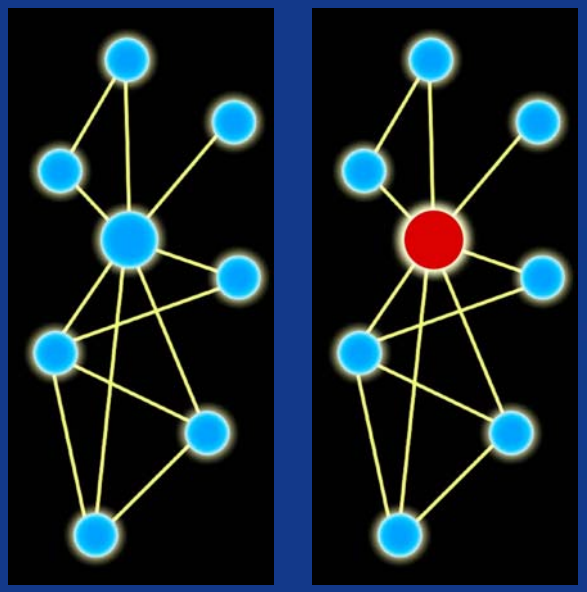
Blood Is a Window into Health and Disease

- Each human organ has an organ-specific blood molecular fingerprint composed of secreted proteins that reports the state of the organ (normal vs disease).

- The blood molecular fingerprints can be read today (discovery--10s proteins on a few controls) with antibodies and proteomics. Tomorrow they (typing--1000s proteins on millions of patients) will be read with microfluidics and nanotechnology devices.



Non-Diseased Diseased

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Microfluidic and nanotechnology approaches to blood protein measurements

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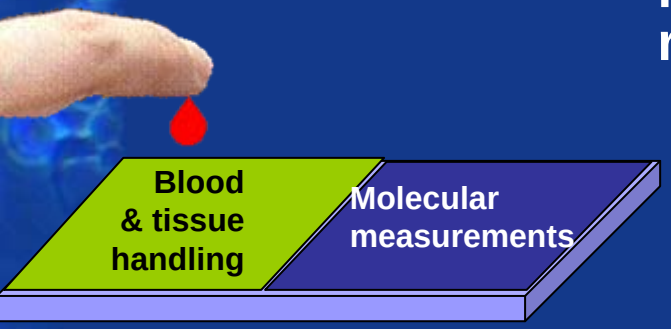
In vitro diagnostics

Quantitate 2500 organ-specific proteins to:

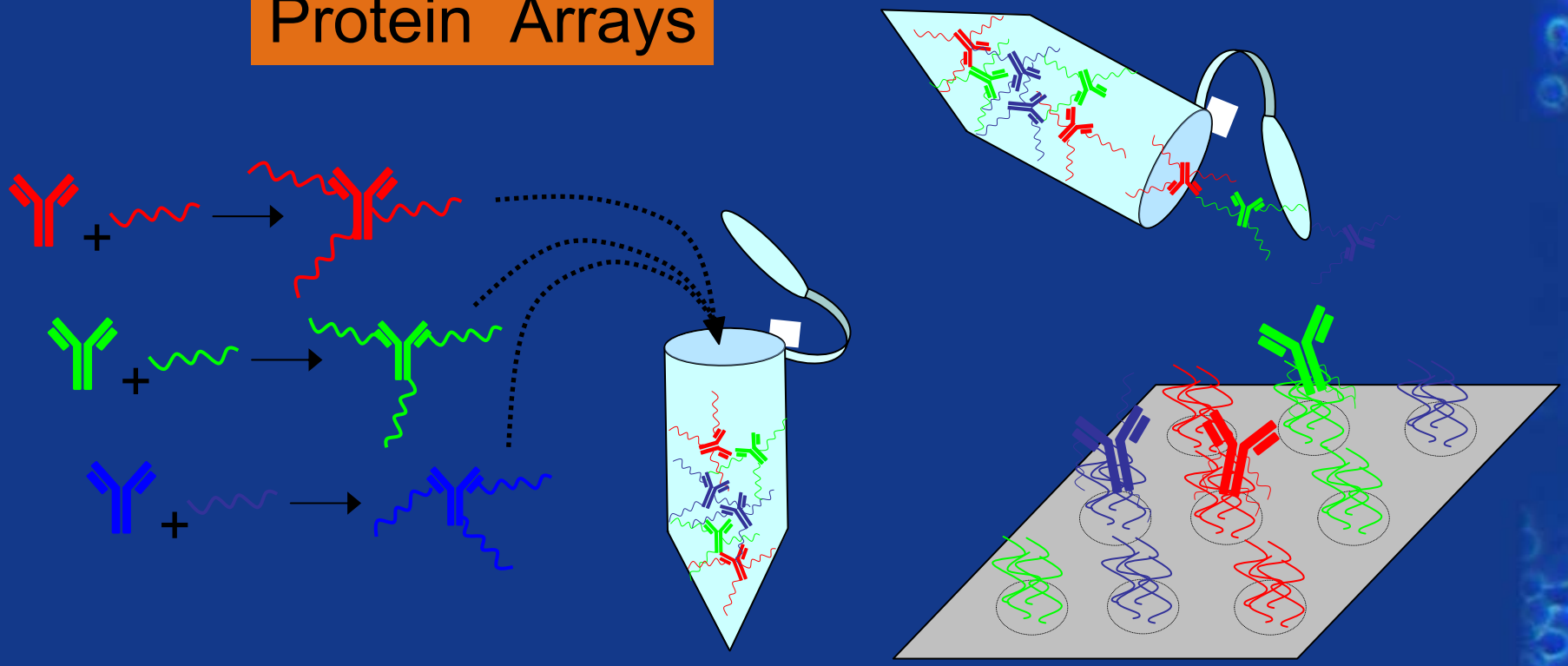
identify disease;
stratify disease;
progression of disease;
response of disease to therapy etc.



Fundamental Materials/Chemical Issues

- Scalable & Simple Detection Technologies
- Multiple Functions Integrated onto Microfluidics Chips
- Protein Capture Agents
- Manufacturability

Protein Arrays



DNA-Encoded Antibody Library scheme. Femptomole sensitivity.

Ryan Bailey and Jim Heath

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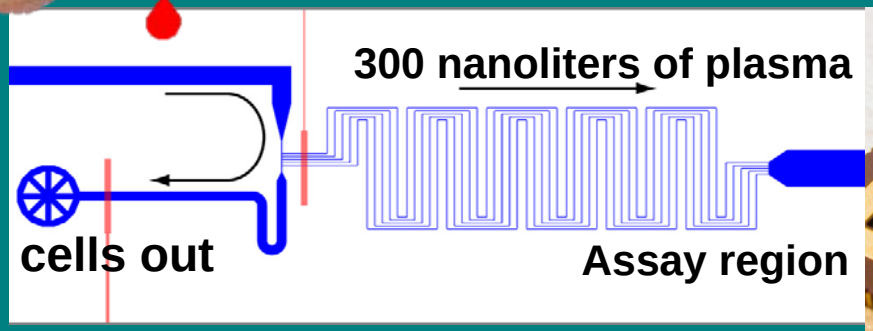
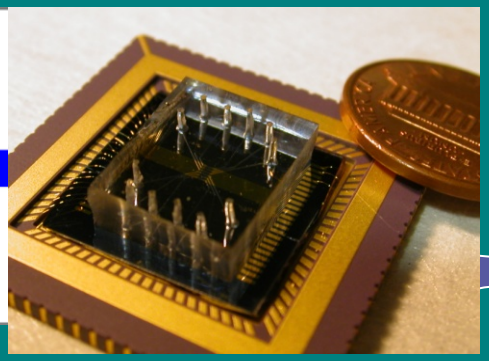
DEAL for *In vitro* molecular diagnostics:
Integrated biology/chemistry/nanotech/microfluidics platforms

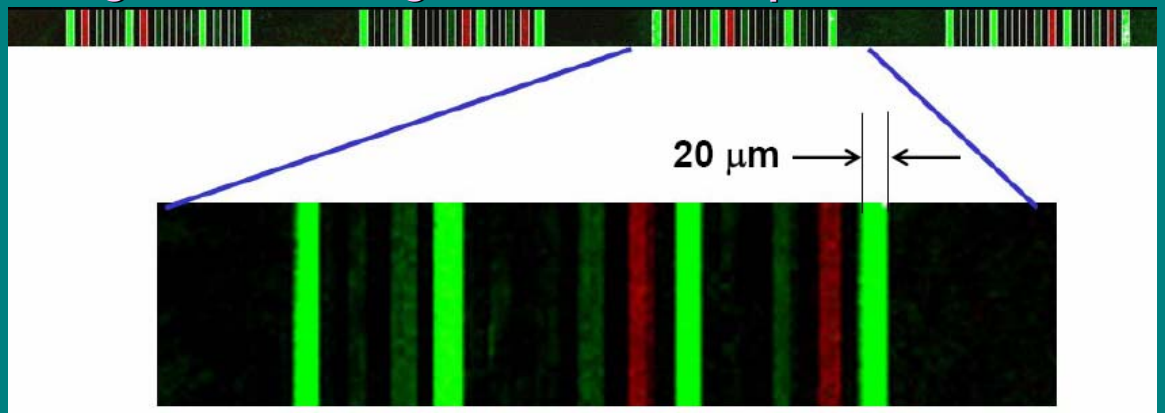
Separate plasma & rapidly quantitate protein biomarker panels to:

- **Profile health status of individual organs**
- **Detect disease prior to clinical symptoms**
- **Select appropriate therapies or combination therapies**
- **Profile positive & adverse responses to therapies**





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

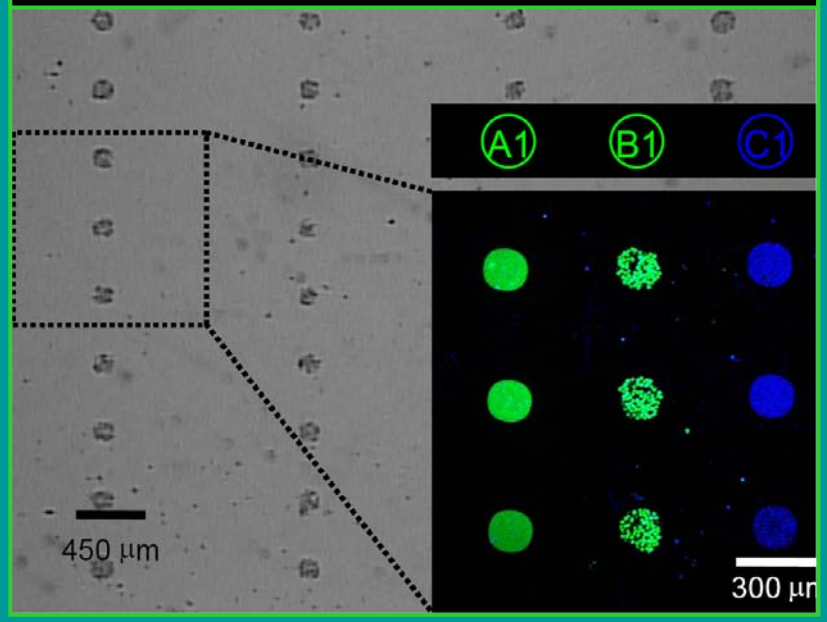


Jim Heath, et al

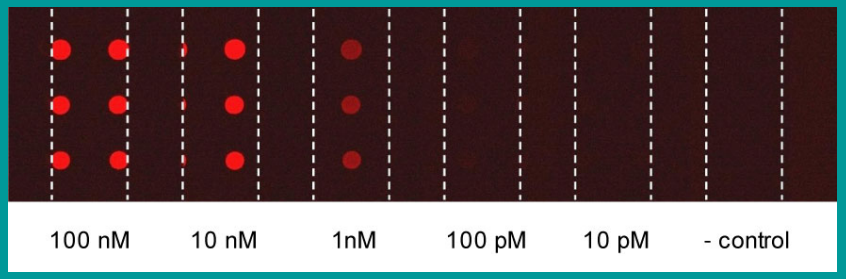
DEAL: DNA-Encoded Antibody Libraries

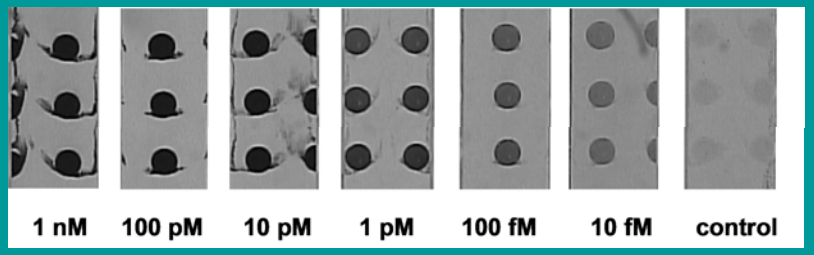
A single, non-biofouling surface chemistry for gene & protein detection & high efficiency multiplexed cell sorting. Chemistry is thermally stable towards microfluidics fabrication steps (80°C; few hours) & stable to dehydration

genes & Proteins & Cells Detected

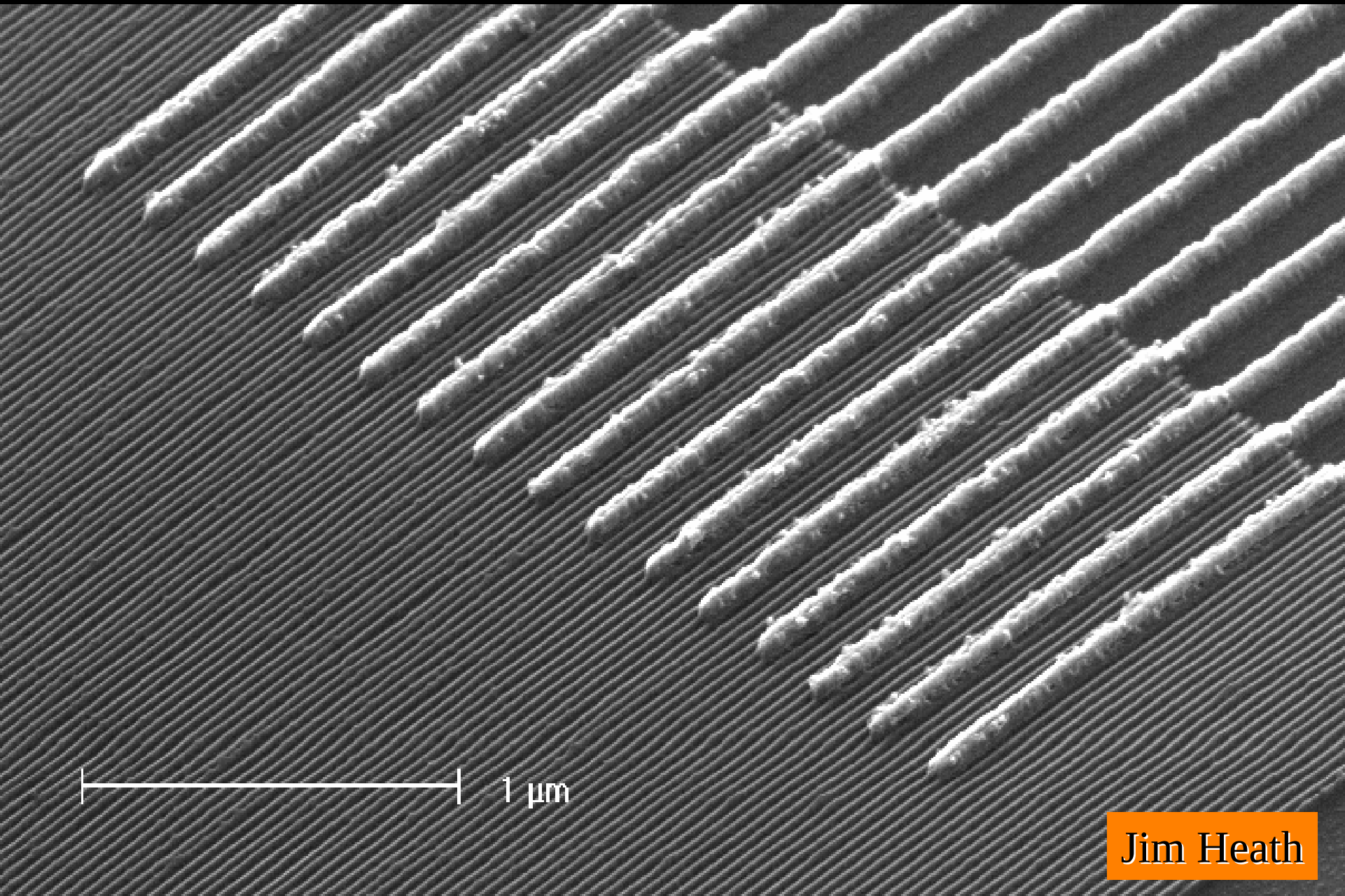


Dynamic range of 10^{-7} – 10^{-15}M





This Entire Circuit is Much Smaller than a Single Cell:
Systems Biology at the Single Cell Level: ~2000 measurements



Blood Is a Window into Health and Disease

- Read the organ-specific secreted molecular fingerprints
  - Correlation with cellular state change
  - Decipher underlying network changes

- The blood fingerprints will permit:
  - Early diagnosis
  - Disease stratification
  - Follow disease progression
  - Follow response to therapy
  - Early detection of adverse drug reactions--e.g. liver toxicity
  - Check animal disease models for validity vs humans
  - Stratify patients with regard to drug responsiveness
  - Aid in titrating drug doses
  - Monitor drug side reactions
  - Environmental toxin assessment
  - Development
  - Aging

Single Cell Analyses and Single Molecule

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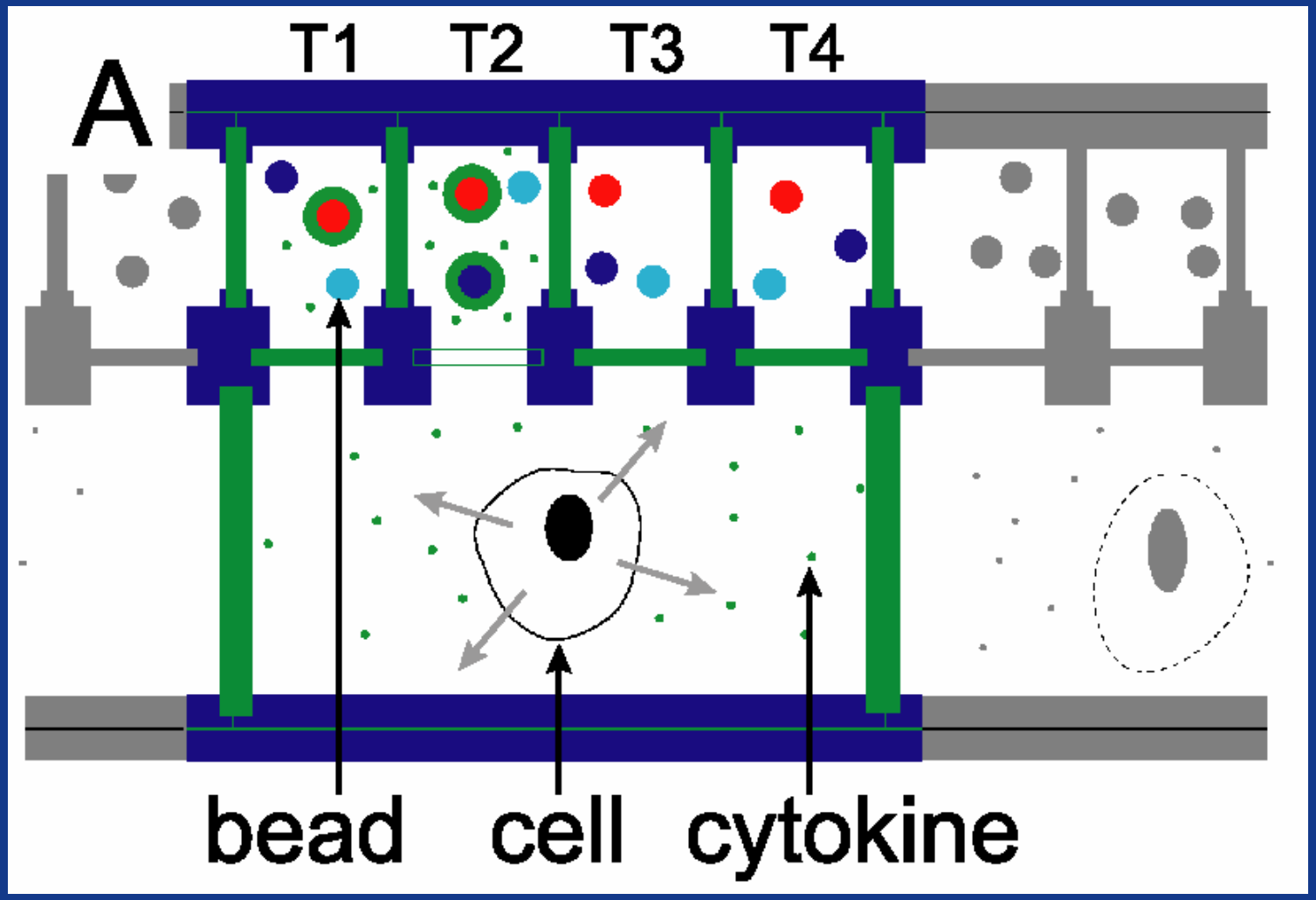
Single Cell Analyses

Multiplexed ELISA for secreted proteins



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Blood Cell Diagnosis

- Separate cells into 10 types
- Single-cell analyses
- Past and present infectious histories of exposure
- Perturbation studies looking at genetic defects

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A Nano-Device to Sequence Single DNA Molecules

- Sequence one billion DNA molecules simultaneously from one individual for 100 DNA letters

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Personalized Genome Sequences

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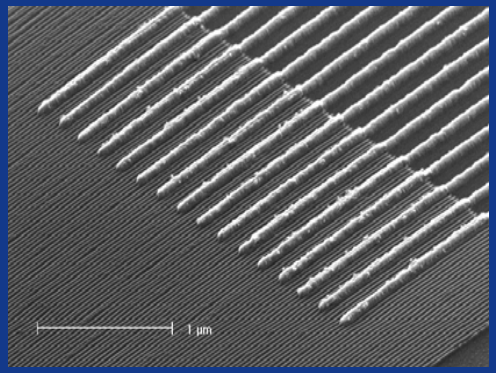
Predictive, Personalized, Preventive and Participatory (P4) Medicine

- 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



Predictive, Personalized, Preventive and Participatory Medicine (P4 Medicine)

- Predictive:
  - Probabilistic health history--DNA sequence
  - Biannual multi-parameter blood protein measurements
  - Biannual blood cell measurements--dynamic
  - In vivo and single cell molecular imaging
- Personalized:
  - Unique individual human genetic variation mandates individual treatment
  - Patient is his or her own control
  - Perturb blood cells for dynamic measurements
  - Go directly to patient and skip doctor--patient will have all medical information
- Preventive:
  - Strategies for re-engineering the behavior of disease-perturbed networks with drugs

    Vaccines

    Focus on wellness
- Participatory:
  - Patient understands and participates in medical choices

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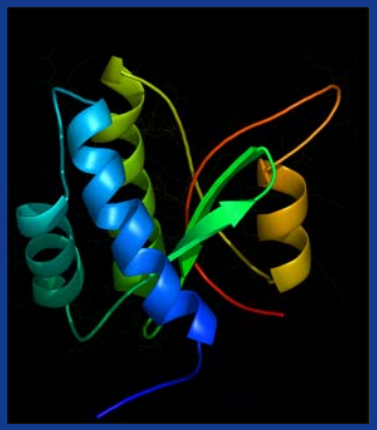
Digitalization of Biology and Medicine Will Transform Medicine

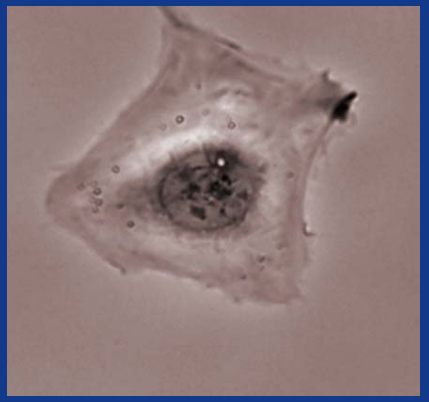
- Analysis of single molecules, single cells and single individuals--*in vitro* and *in vivo*
- A revolution that will transform medicine even more than digitalization transformed information technologies and communications

Single individual

Single molecule



Single cell



Technology and New Strategies Will Drive Down Healthcare Costs

The Elements

- Diagnostics
- Therapy
- Wellness
- Technologies

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Why P4 Medicine Will Reduce Healthcare Costs

- Diagnostic (*Impact of rapid new methods)*
  - Presymptomatic diagnosis
  - Stratification of disease—match therapy to disease
  - Follow disease progression—is therapy working?
  - Identify adverse reactions early
- Therapy
- Wellness
- Technologies

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Why P4 Medicine Will Reduce Healthcare Costs

- Diagnostics
- Therapy *(More precise and more effective)*
  - Drugs to make disease-perturbed networks more normal
  - More effective use of animal models in development
  - Use organ-specific fingerprints get drug dose right
  - Organ-specific fingerprints to assess drug toxicities
- Wellness
- Technologies

Why P4 Medicine Will Reduce Healthcare Costs

- Diagnostics
- Therapy
- Wellness
  - Assess future health history predictions
  - Drugs that will prevent diseases predicted by your genome
  - Follow blood fingerprints to look for pre-symptomatic signs of disease
  - Prevention is always less expensive than therapy
- Technologies

Why P4 Medicine Will Reduce Healthcare Costs

- Diagnostics
- Therapy
- Wellness
- Technologies
  - Inexpensive DNA sequencing for personal genomes
  - Low cost blood fingerprints—nanotechnology is essential
  - Molecular visualizations of the human body (in vivo)
  - New methods for discrimination of subtle patterns

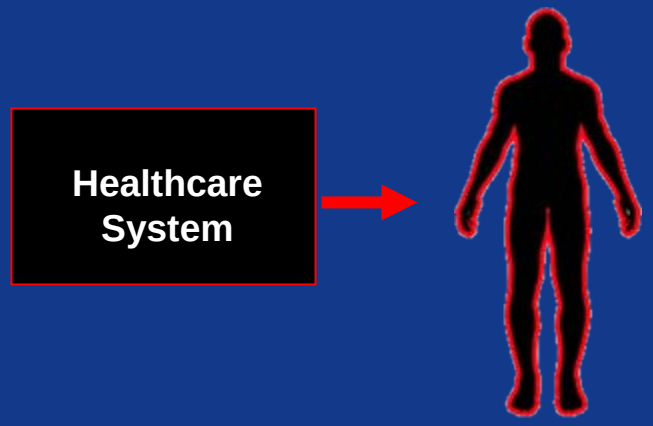
Digitalization biology medicine--bigger revolution that the digitalization of IT and communications

Strategic partnerships and their logic

P4 Medicine Will Transform the Health Care Industry

Will impact the health care system significantly:

- Pharmaceuticals
- Biotechnology
- 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

Ten Major Technical Challenges to P4 Medicine

- Methods for individualized genomics – personalized genome sequencing – new methods and approaches.
- Organ-specific proteins in the blood can be transformed into useful biomarkers. Validation of the blood protein molecular fingerprint for each organ.
- Correlate these fingerprints with health and disease states.
- Develop/invent the blood protein measurement technologies
- Imaging molecular disease indications. *In vivo* molecular imaging to follow disease, drug response, drug effectiveness, drug dosage determinations etc.
- Develop new mathematical and computational methods for extracting maximum information from blood fingerprints of individuals, the comparative analyses of genomes from individuals and their molecular images.
- Drug perturbations of networks to be understood in a predictive sense.
- Therapeutic perturbations of biological networks. Re-engineering of networks in higher organisms with drugs (diseased back to normal).
- Handling the enormous personalized data sets—policies, security, mining, reporting, modeling, etc.
- Education of patients, payers, regulators and physicians about P4 medicine.

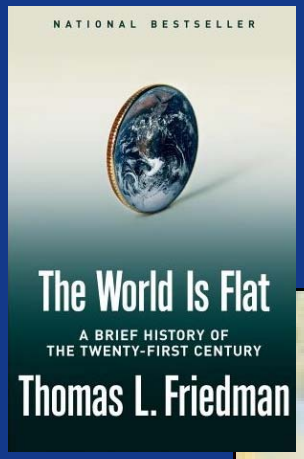
P4 Medical Consortium
Concept

- A partnership with PriceWaterhouseCoopers to accelerate and guide the emergence of P4 medicine in the healthcare arena
- Initiate with a drug company, an IT healthcare company, a payer and a provider--to help cast the business plan of the consortium
- Ultimately bring in at least one company from every sector of the health care industry
- ISB-company projects--achieve one or more milestones
- At least one medical school partner willing to embrace systems medicine--and develop and test new approaches
- International ISB-like institutes to bring new resources and support
- Integration of all advances will leverage enormously gains for each consortium member
- Begin critical analysis useful of economic, business, policy and social considerations
- Consortium will raise money to:
  - support key science efforts to attack technical barriers,
  - understand policy, societal, business and economic barriers
  - how much, how fast?

The Flattening of Many Worlds: Globalization of Science

The worlds of science, technology, health are flattening.

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



- 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 predictive, prevention, personalized and participatory medicine

INSTITUTE FOR Systems Biology

NIST Opportunities in P4 Medicine

- Diagnostic will be the key to P4 medicine for the immediate future
- Measurement--validation, standardization and databases
  - Blood organ-specific protein biomarkers
  - Blood cell diagnostic markers
  - Discovery and large scale typing
- Technology development
  - Nanotechnology/microfluidics
    - Blood protein measurement
    - Single-cell analyses
  - Imaging and visualization--in vitro and in vivo
- Computation/mathematical algorithms and platforms
  - Healthcare information technology
- Outside strategic partnerships are critical for NIST--for domain expertise

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Charge for Breakout Groups

- A personalized medicine world
- Discovery technologies
- Healthcare informatics
- Impact of personalized (P4) medicine on society
- Changing research approaches and infrastructure

INSTITUTE FOR
Systems
Biology

DEGs Encoding Known and Novel Prion Disease Phenotypes

- 7400 Differentially Expressed Genes (DEGs) in 5 inbred strains upon prion perturbation.
- Biological filters reduce to 924 core DEGs for prion disease
- 253/924 DEGs encode known disease phenotypes
- 671/924 DEGs encode novel disease phenotypes

INSTITUTE FOR Systems Biology

Biology Will be the Dominant Science in the 21st Century

- Because of biological complexity and it relevance to humans
  - We can deal with biological complexity through biology as an informational science and through systems approaches and understand life
  - Evolution has had 3 billion years of evolution to come up with potential sophisticated solutions to healthcare, energy, global warming, integrative computing, new materials, new chemistries, etc. Biology helps other scientific disciplines solve difficult problems as well as potentially solving some of society's most challenging problems .