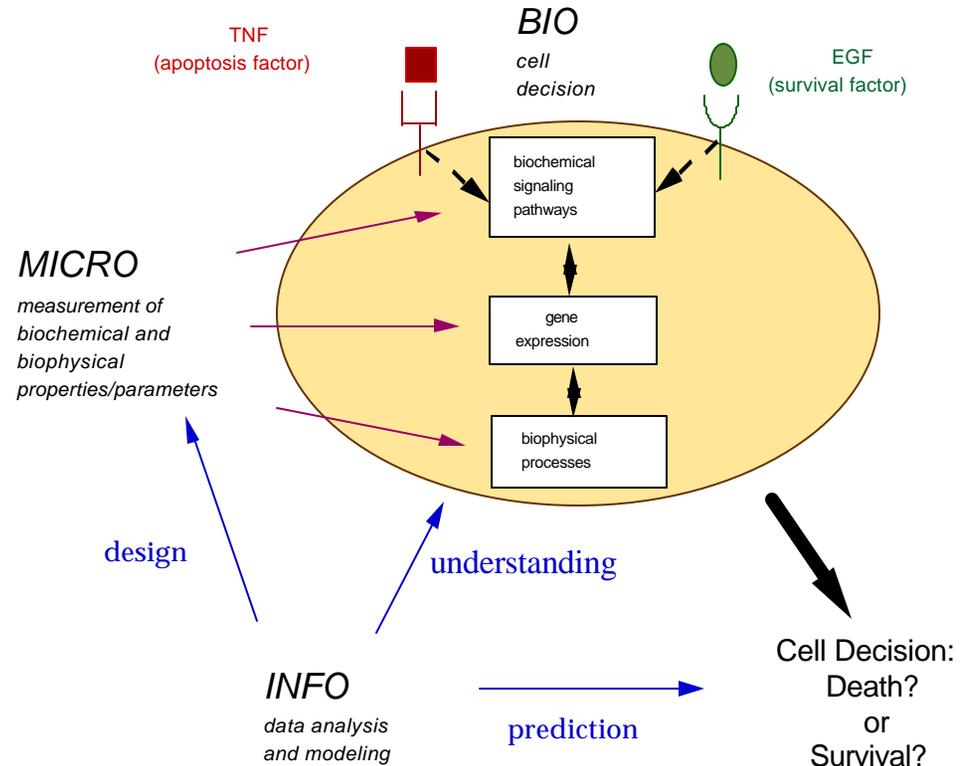


Cell Decision Processes

MIT

Aims:

- * **Create integrated-systems approach for understanding how cells make behavioral ‘decisions’ in response to stimuli**
- * **Elucidate new ‘biological signal processing’ paradigms for non-biological systems**



Project Objectives

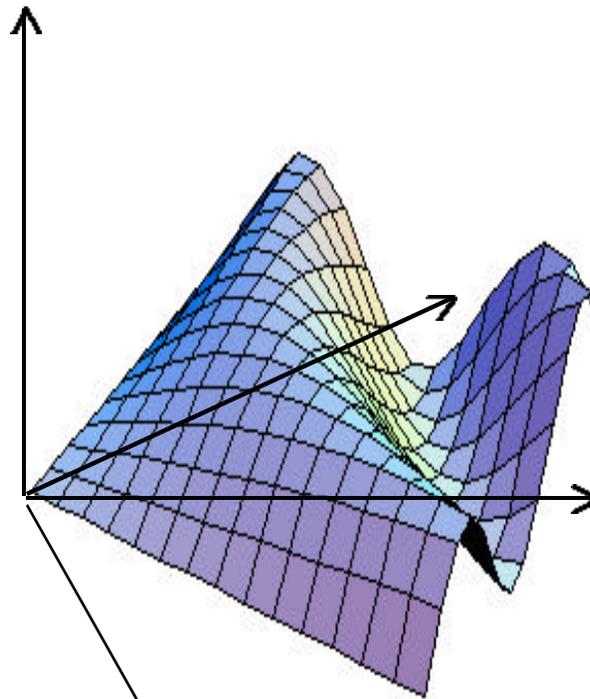
Create a 'bioengineering science' base for how cells function -- for scientific understanding, technological manipulation, and paradigm elucidation

Generate advances in micro-instrumentation for biological measurement methods, informatics for biological data handling, and computational modeling frameworks for biological cell decision processes

Discover new concepts in decision-making strategies for non-biological systems

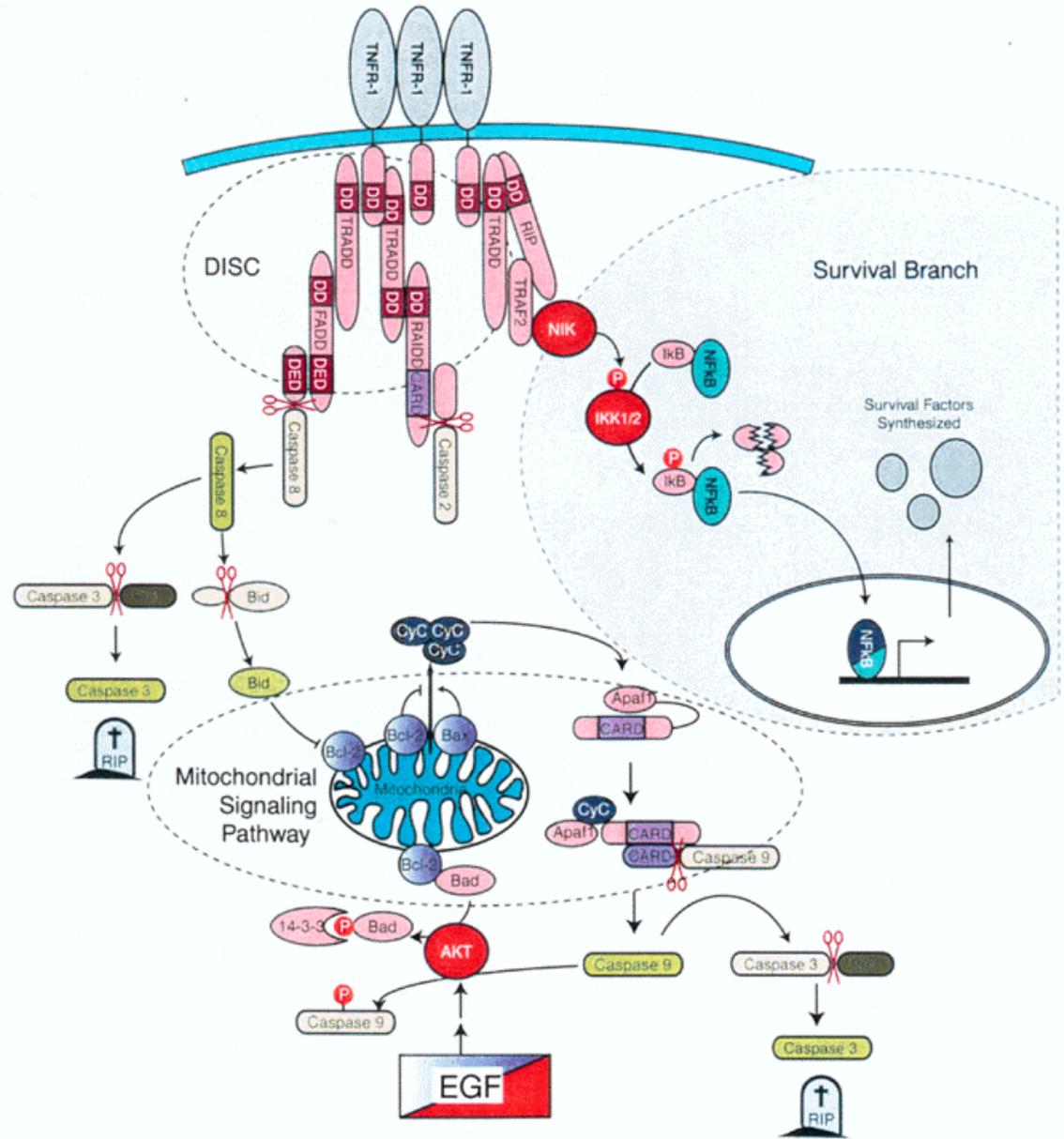
Educate students/postdocs (and faculty!) in cross-disciplinary research at the biology/engineering interface

Cellular
system
function:
apoptosis-vs-
survival
decision



System
component
parameters:
Protein state/location/
interactions, gene expression)

TNF/EGF Apoptosis/Survival Signaling Networks



- Regulation by Protein-Protein Association
- Regulation by Transcriptional Activation
- Regulation by Cleavage
- Regulation by Phosphorylation
- Regulation by Translocation

*Measurement & Modeling Needed
for Integrated Systems Analysis of
Molecular Networks
Governing Cell Decisions*

“Primary” Networks -- protein signaling pathways

- * Protein state (e.g., cleavage, phosphorylation)
- * Protein location (e.g., cytosol, mitochondria, plasma membrane-associated)
- * Protein-protein coupling

“Secondary Networks” -- gene expression responses

- * mRNA levels
- * Protein levels

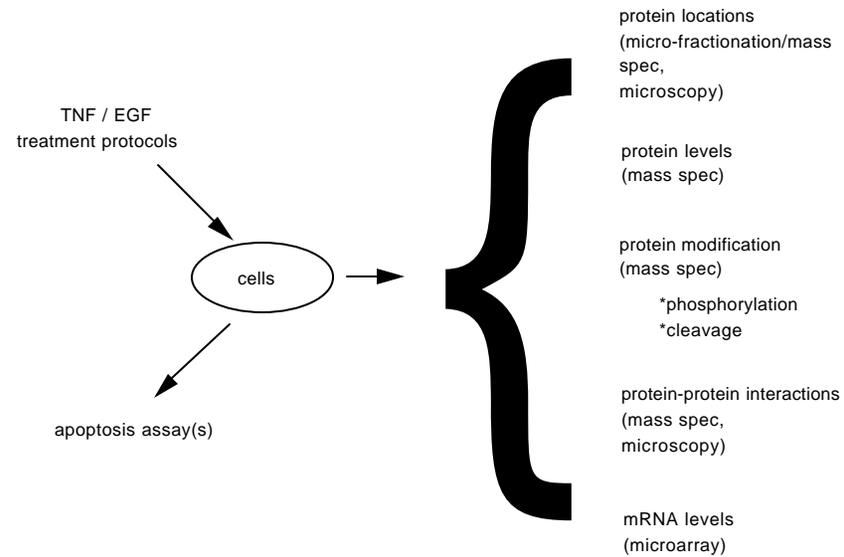
Protein/Gene/Protein network feedback

Bio-Info-Micro Driving Forces

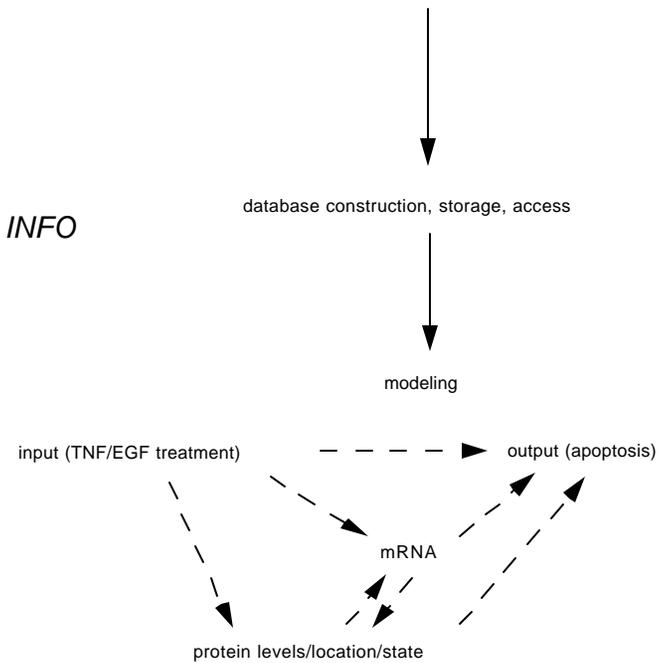
- Bio -- how can we:
 - Go from cartoons to predictive models despite incomplete data
 - Emphasize quantitative, dynamic molecular/cell data
 - Elucidate cell “design principles”
- Micro -- how can we:
 - Determine intracellular molecular locations and interactions
 - Quantify molecular and organellar properties
- Info -- how can we:
 - Model molecular networks in face of incomplete data
 - Physico-chemical
 - Relational
 - Engineering analogies
 - Handle heterogeneous expt/modeling interface
 - Identify new bio-inspired paradigms for non-biological systems

Program Outline

BIO / MICRO



INFO



Project Areas & Investigators

Area 1: Biology / Genomics: cell responses to TNF/EGF

- * ***P Sorger (Biol)***
- * M Cardone (Biol)
- * J Yuan (Biol - HMS)
- * D Lauffenburger (ChE / BEH)
- * L Griffith (ChE / BEH)

Area 2: Biology / Proteomics: signaling protein levels/states/locations

- * ***S Tannenbaum (BEH)***
- * P Wishnok (BEH)
- * M Cardone (Biol)
- * K Jensen (ChE)

Area 3: Bio-microanalytics: signaling protein locations

- * ***K Jensen (ChE)***
- * M Schmidt (EECS)
- * I Hunter (ME / BEH)
- * F Dewey (ME / BEH)
- * P Sorger (Biol)

Areas 4/5/6: Bio-informatics: data analysis, network modeling, and 'BSP'

- * ***F Dewey (ME / BEH)***
- * ***D Gifford (EECS)***
- * T Jaakkola (EECS)
- * ***A Oppenheim (EECS)***
- * D Lauffenburger (ChE / BEH)
- * P Sorger (Biol)

Students/Postdocs/Staff

- ◆ **Patrick Anquetil (Mech Eng)**
- ◆ Jim Bear (Biol)
- ◆ Yuan Cheng (Mech Eng)
- ◆ Ngon Dao (Mech Eng)
- ◆ Robert David (Mech Eng)
- ◆ Ben Fu (Elect Eng / Comp Sci)
- ◆ Suzanne Gaudet (Biol)
- ◆ Rebecca Jackman (Chem Eng)
- ◆ **Ji-Eun Kim (Bioeng / Env Hlth)**
- ◆ **Hang Lu (Chem Eng)**
- ◆ **John Mehl (Bioeng / Env Hlth)**
- ◆ Ulrik Nielsen (Biol)
- ◆ Can Ozbal (Bioeng / Env Hlth)
- ◆ **Karen Sachs Bioeng / Env Hlth)**
- ◆ **Maya Said (Elect Eng / Comp Sci)**
- ◆ **Stas Shvartsman (Chem Eng)**
- ◆ Jamie Tuttle (Biol)
- ◆ **Shixin Zhang (Mech Eng)**

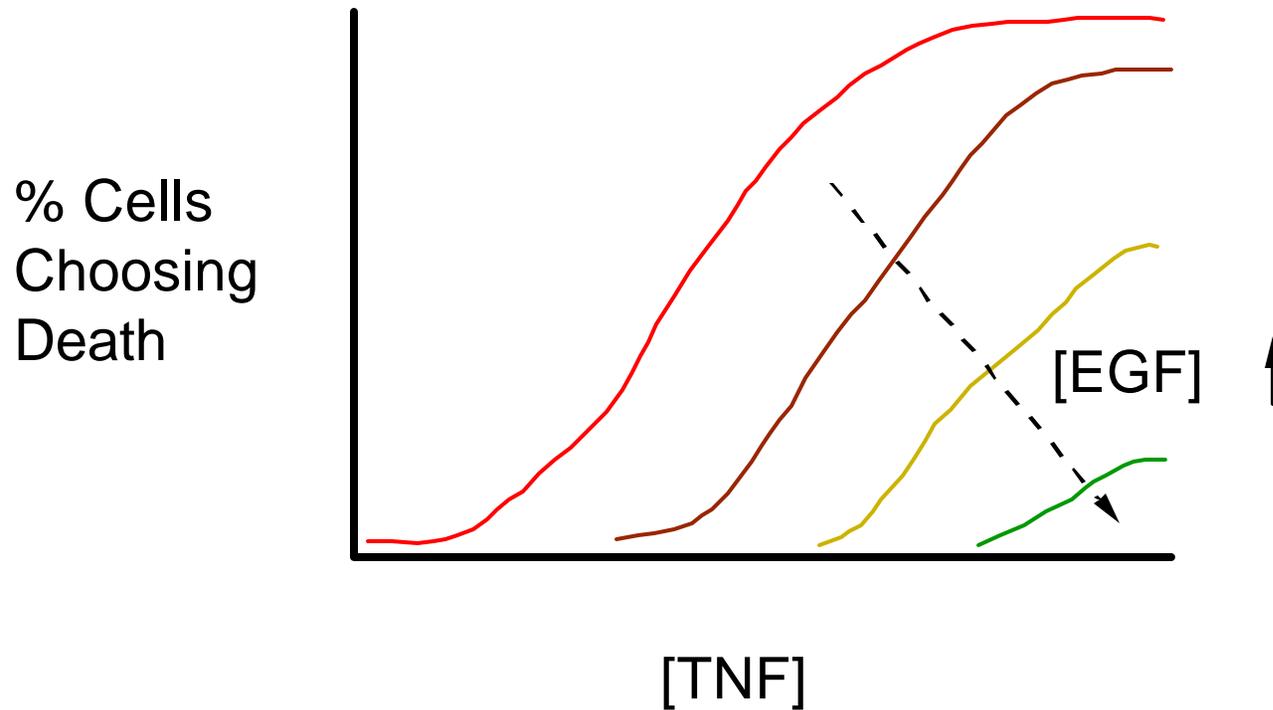
Program Operation

- Monthly MIT BIM mtgs
- Special Fall 2000 seminar series (joint w/ Whitehead Inst for Biomed Res & BEH Div)
 - ‘Proteomics for Cell Signaling Processes’
 - Matthias Mann (Denmark)
 - Technical interchanges
 - Social interactions
- Journal club? (student/postdoc-driven)

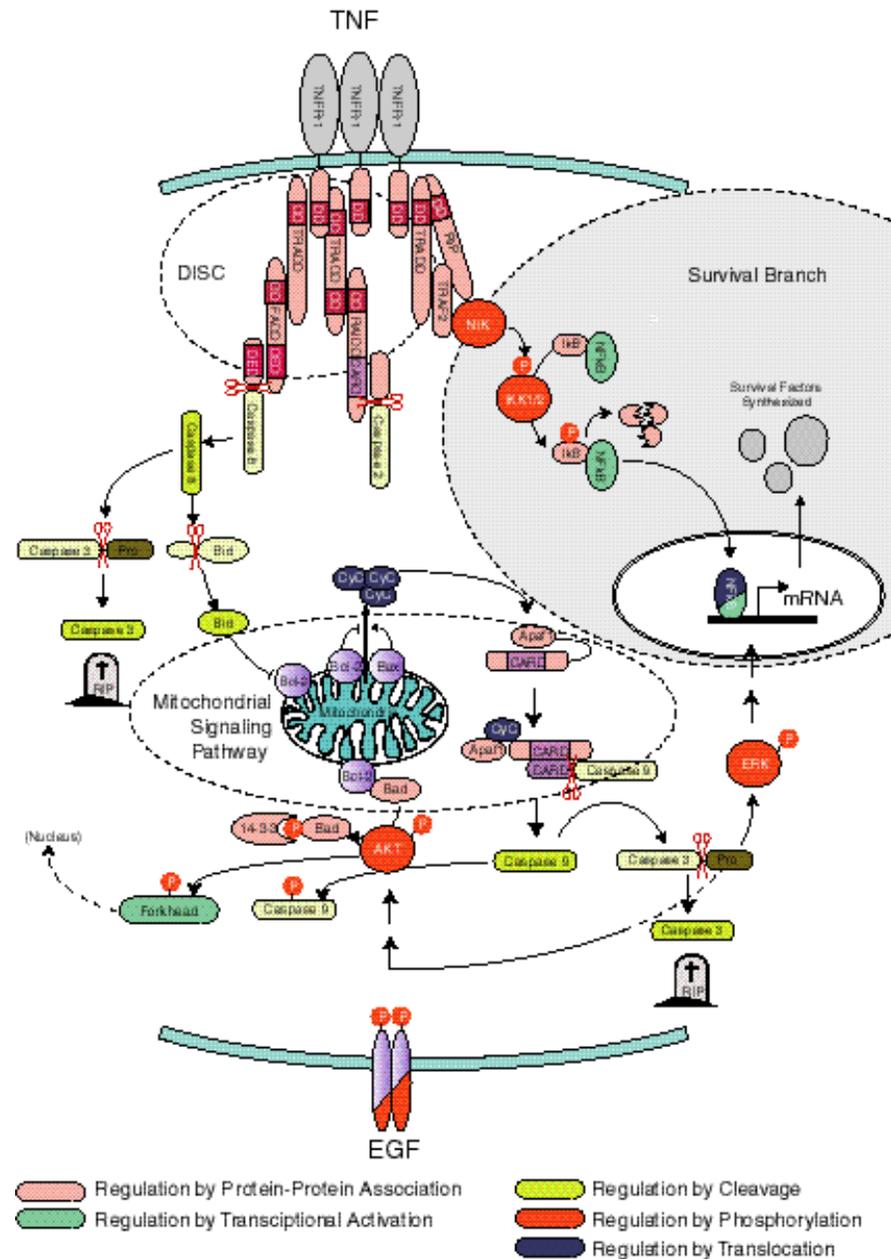
*Area 1: Biology / Genomics -- cell
responses to TNF/EGF*

- * ***P Sorger (Biol)***
- * M Cardone (Biol)
- * J Yuan (Biol - HMS)
- * D Lauffenburger (Chem Eng / BEH)
- * L Griffith (Chem Eng / BEH)

*Cell Biological Decision: Response to
Death-promoting versus Survival-promoting
Inputs*



TNF/EGF Apoptosis/Survival Signaling Networks



Measurement & Modeling Targets

- T1. TNFR – TNF binding [Area 1]**
- T2. IKK – phosphorylation state & level [Area 2]**
- T3. NFkB – location (nucleus vs cytosol) [Area 3]**
- T4. Caspase 8 – interactions (DISC) [Area 3]
- T5. Caspase 8 – cleavage state (loss of DED) [Areas 1,2]
- T6. CIAP2 – level [Area 1]
- T7. Bid – cleavage state [Areas 1,2]
- T8. CytC – location (mitochondria vs cytosol) [Area 3]**
- T9. CytC – interactions (Apaf/CARD/Casp9) [Areas 2,3]
- T10. Bax, Bad, Bid, Bcl2 – location (mitochondria vs cytosol) [Area 3]

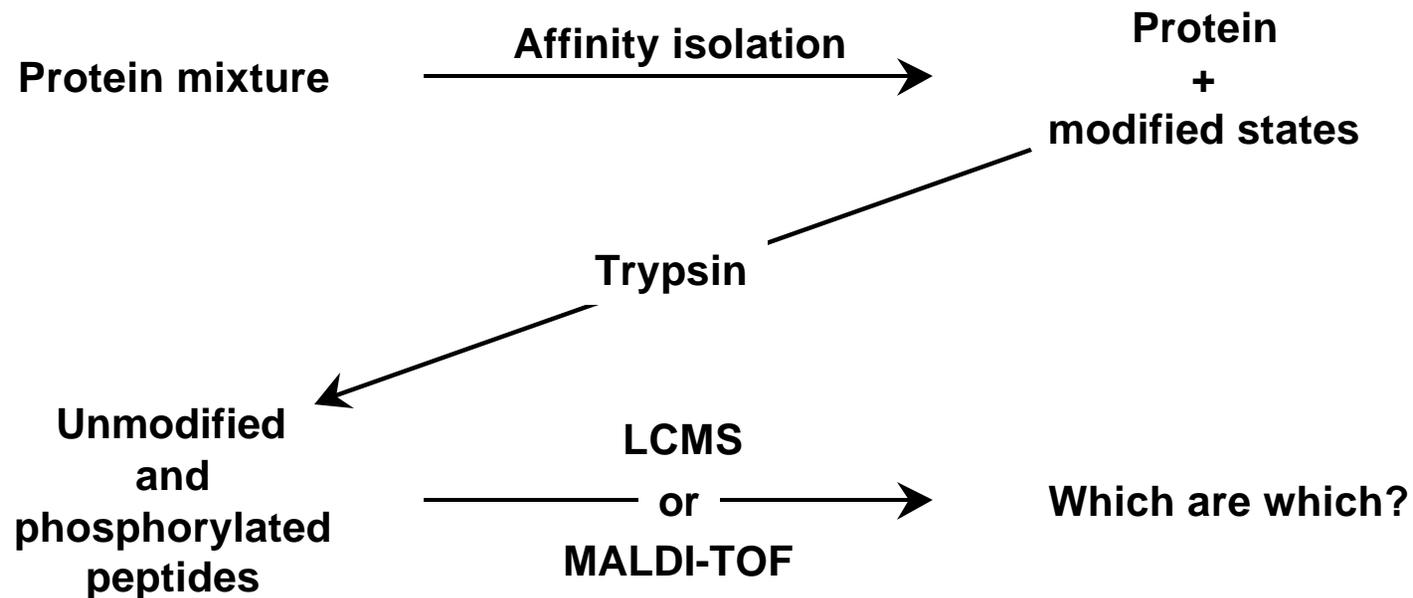
- E1. EGFR – EGF binding, phosphorylation state & level [Area 1]**
- E2. Akt – phosphorylation state [Area 2]**
- E3. Bad – phosphorylation state [Area 2]
- E4. Casp9 – phosphorylation state [Area 2]**
- E5. Forkhead – phosphorylation state [Area 2]
- E6. ERK – phosphorylation state & location (cytosol vs nucleus) [Areas 1,2,3]**

- C1. apoptosis [Area 1]**
- C2. gene expression [Area 1]**

*Area 2: Biology / Proteomics --
signaling protein levels/states/locations*

- * ***S Tannenbaum (BEH)***
- * P Wishnok (BEH)
- * M Cardone (Biol)
- * K Jensen (Chem Eng)

Protein Isolation & Analysis Strategy



Protein Capture

- Affinity chromatography on chip

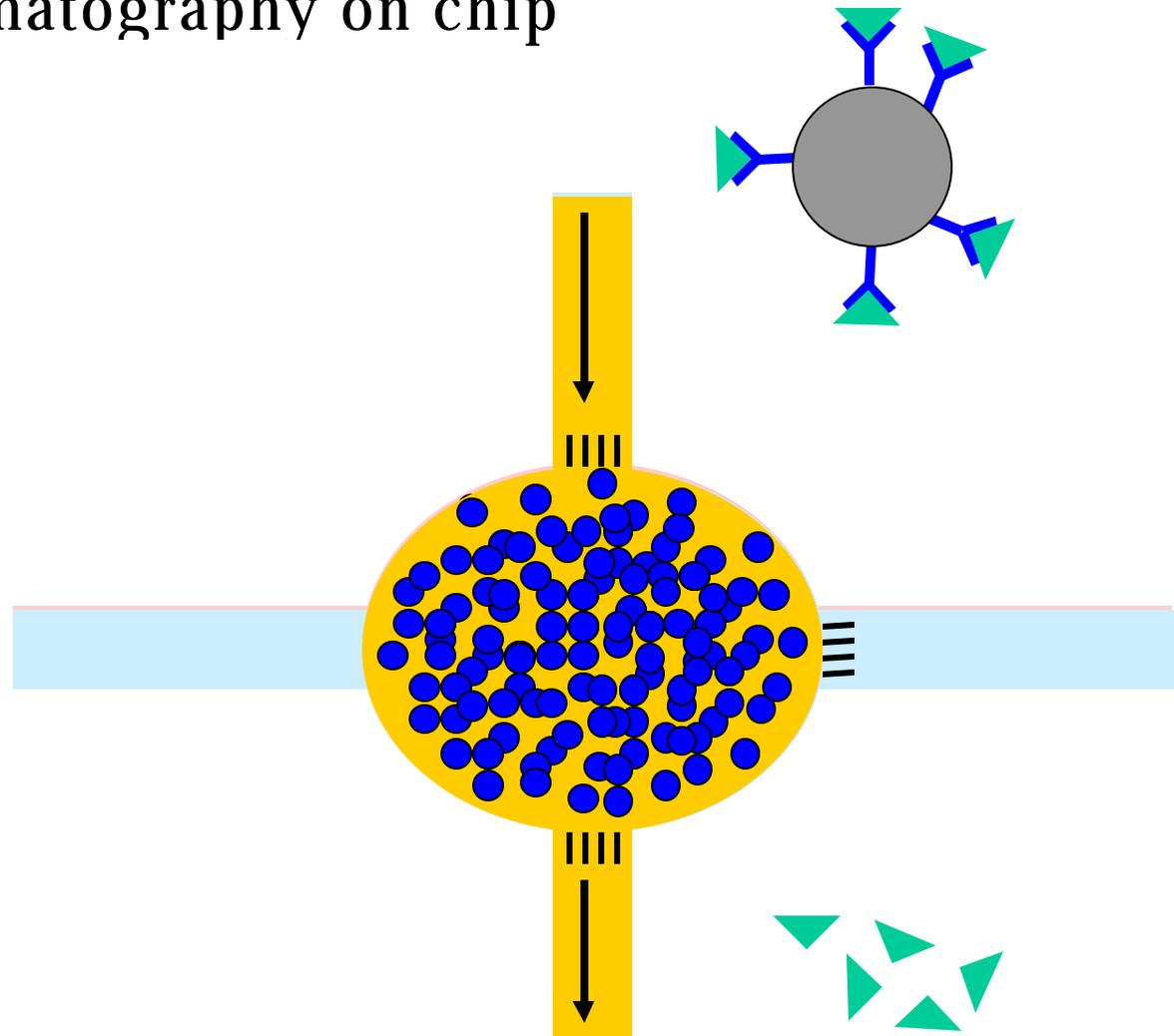
- bind (off chip)

- load

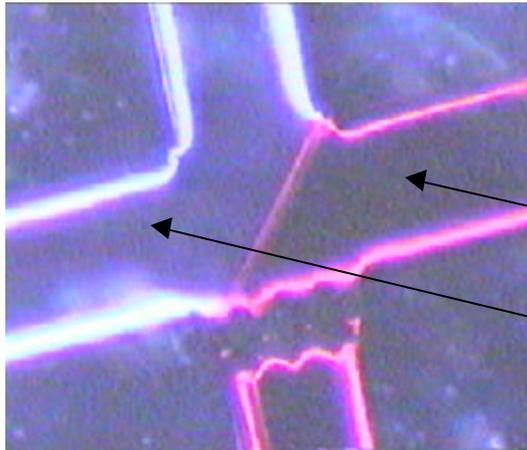
- wash

- elute

- unload



Protein Capture Microfilter Device

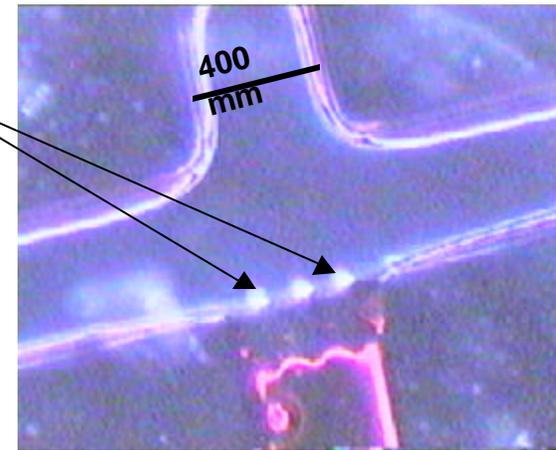


Fluid streams laminate

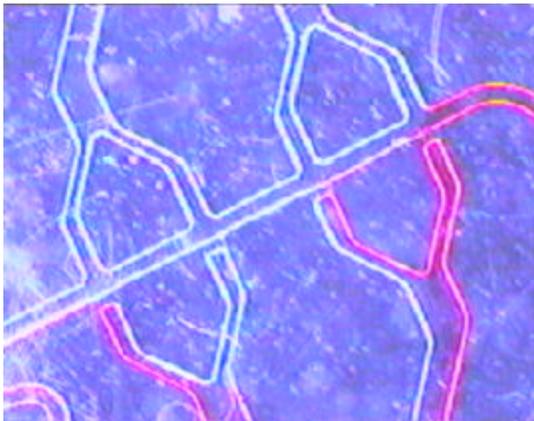
polystyrene
beads

phenol red
solution

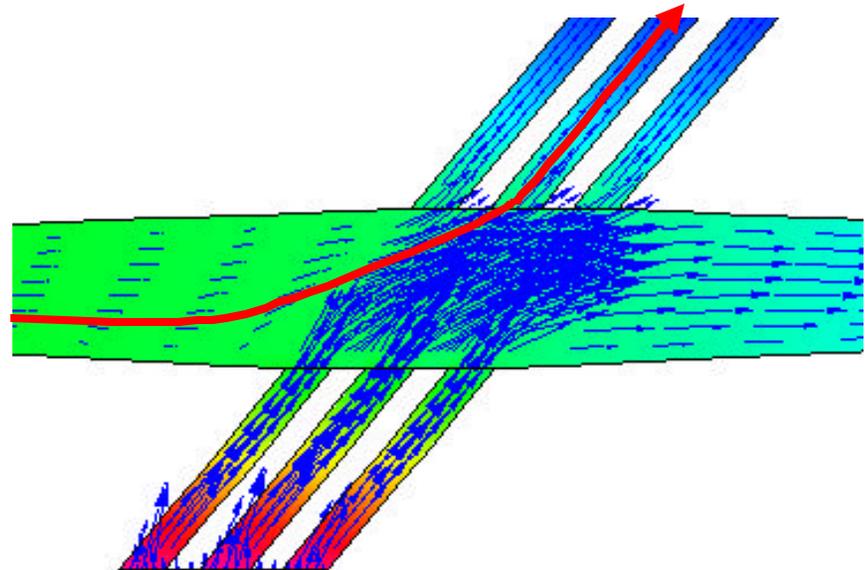
water



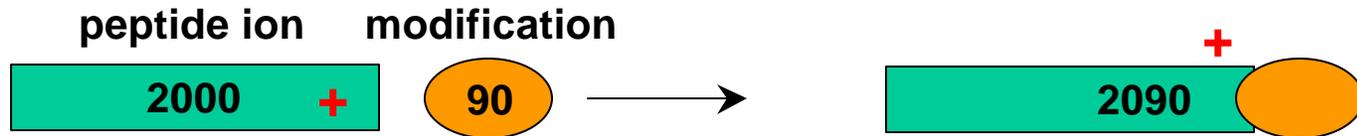
Microfilters strain beads



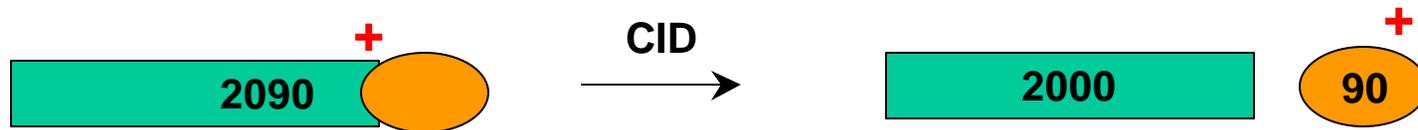
**Microfilter device fabricated
in silicon using DRIE**



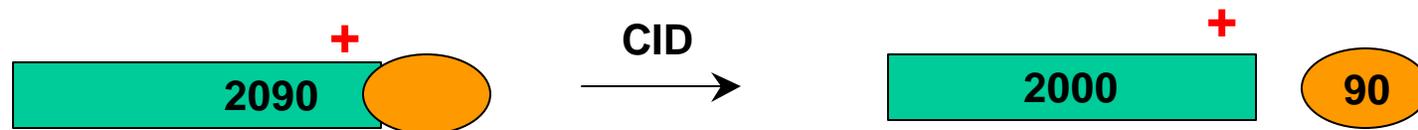
Ferretting out modified peptides by mass spectrometry



1. Look for ions 90 Da higher than those from tryptic peptides.
2. Collision-induced dissociation:



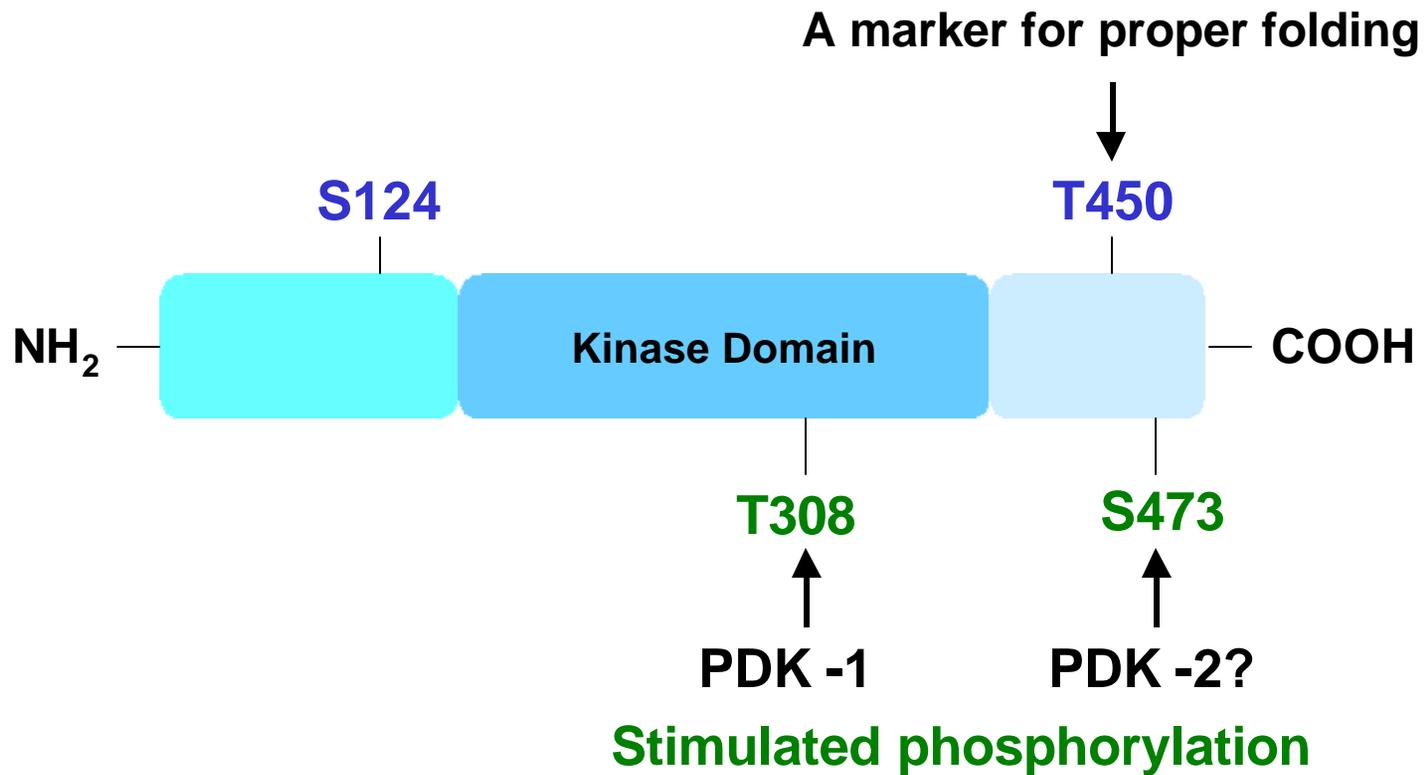
- a. Look for characteristic modification ion, e.g. m/z 90.



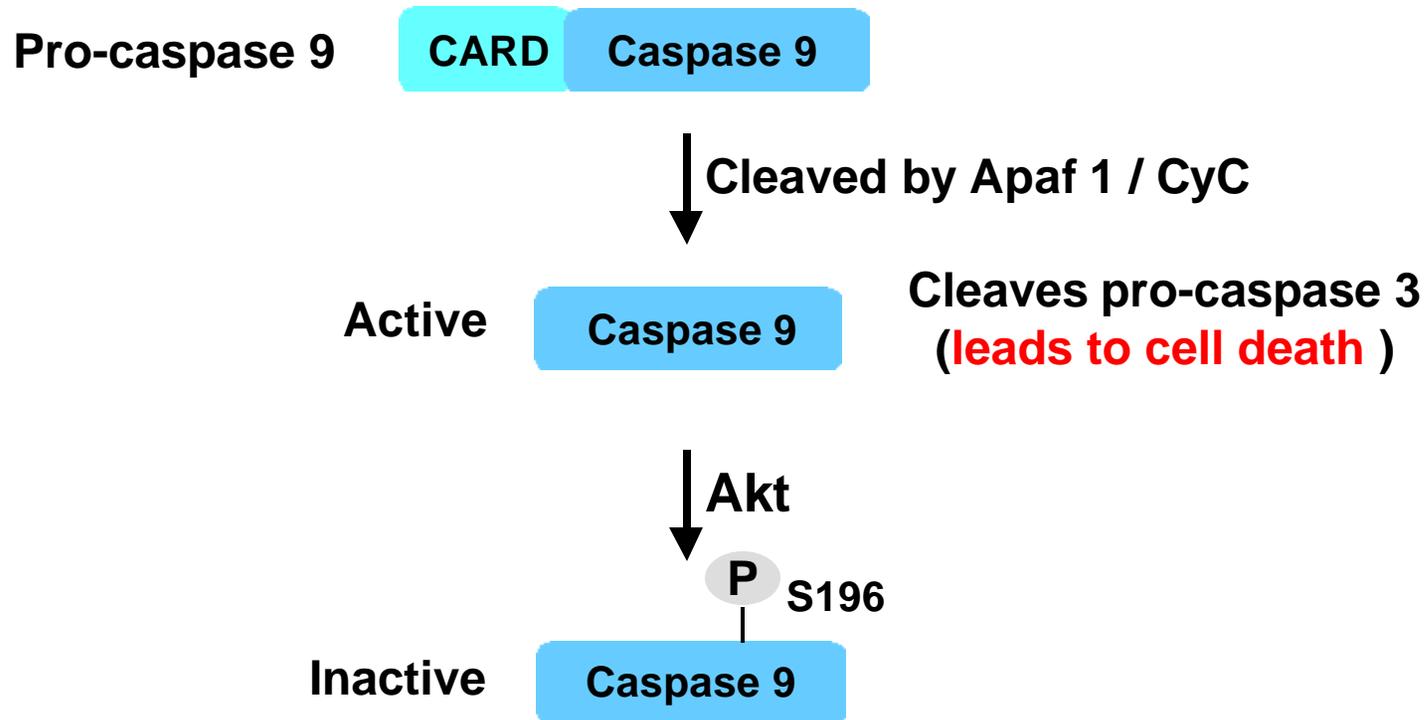
- b. Look for loss of characteristic neutral fragment, e.g. m/z 90.

Domain Structure of Akt/PKB

Constitutive phosphorylation



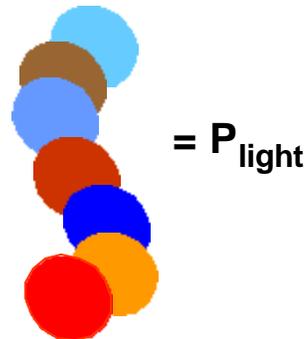
Caspase 9 Cleavage & Phosphorylation



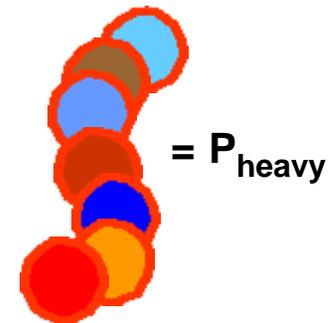
CARD: Caspase Activation Recruitment Domain

Quantitation with isotopomeric internal standards

1. Select peptide for analysis:



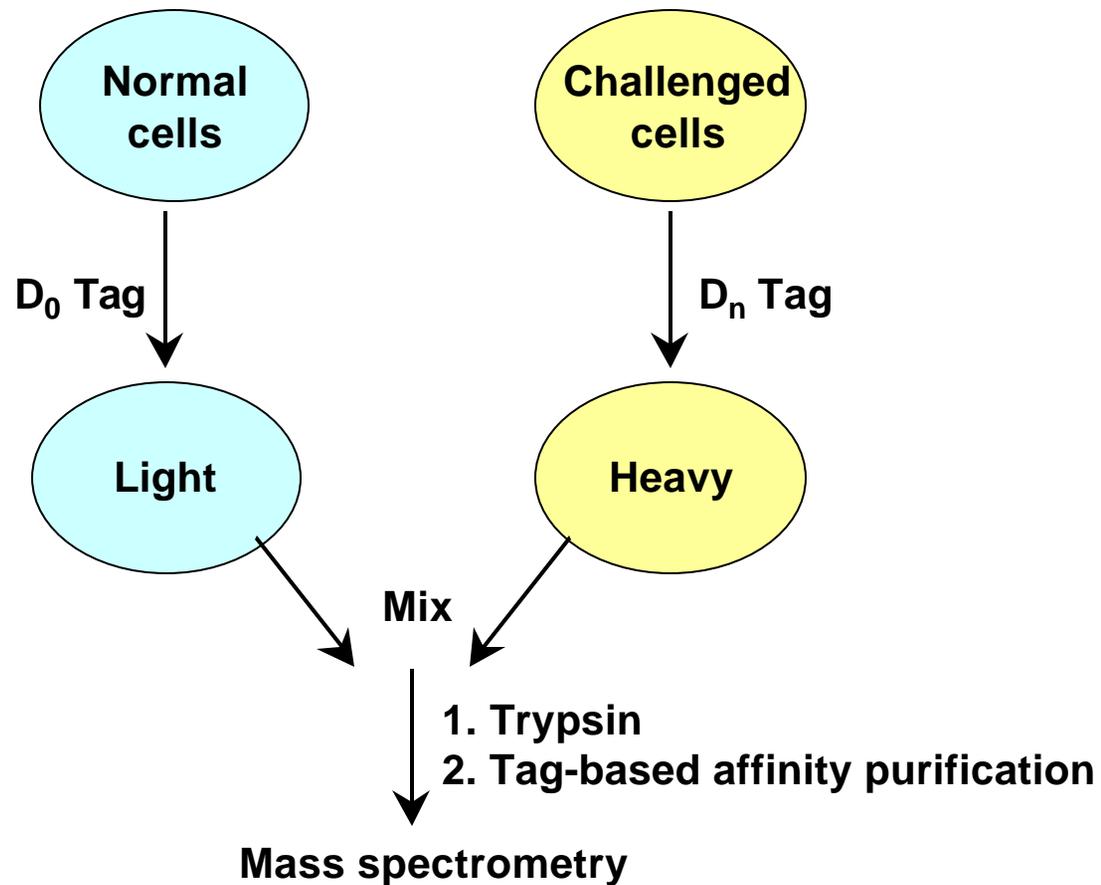
2. Synthesize same sequence with labeled amino acids:



3. Add known amount of labeled peptide to digest.

4. Concentration of analyte = $[P_{\text{heavy}}] * P_{\text{light}} / P_{\text{heavy}}$

Isotope-Coded Affinity Tags



Labeled peptides detected as doublets separated by n Daltons

Mass Spectrometers

PE Biosystems Voyager Elite DE MALDI-TOF

Rapid analysis of tryptic digests and intact proteins

Finnigan TSQ 7000 Tandem Quadrupole

Quantitation with high sensitivity in selective modes

MS/MS

Standard and capillary HPLC, naanospray

Agilent 1100 Ion-Trap LCMS

Qualitative analysis with high sensitivity in full scan mode

MSⁿ

Standard and capillary HPLC, nanospray

*Area 3: Bio-microanalytics -- signaling
protein locations*

- * ***K Jensen (Chem Eng)***
- * M Schmidt (Elect Eng & Comp Sci)
- * I Hunter (Mech Eng / BEH)
- * F Dewey (Mech Eng / BEH)
- * P Sorger (Biol)

Motivations

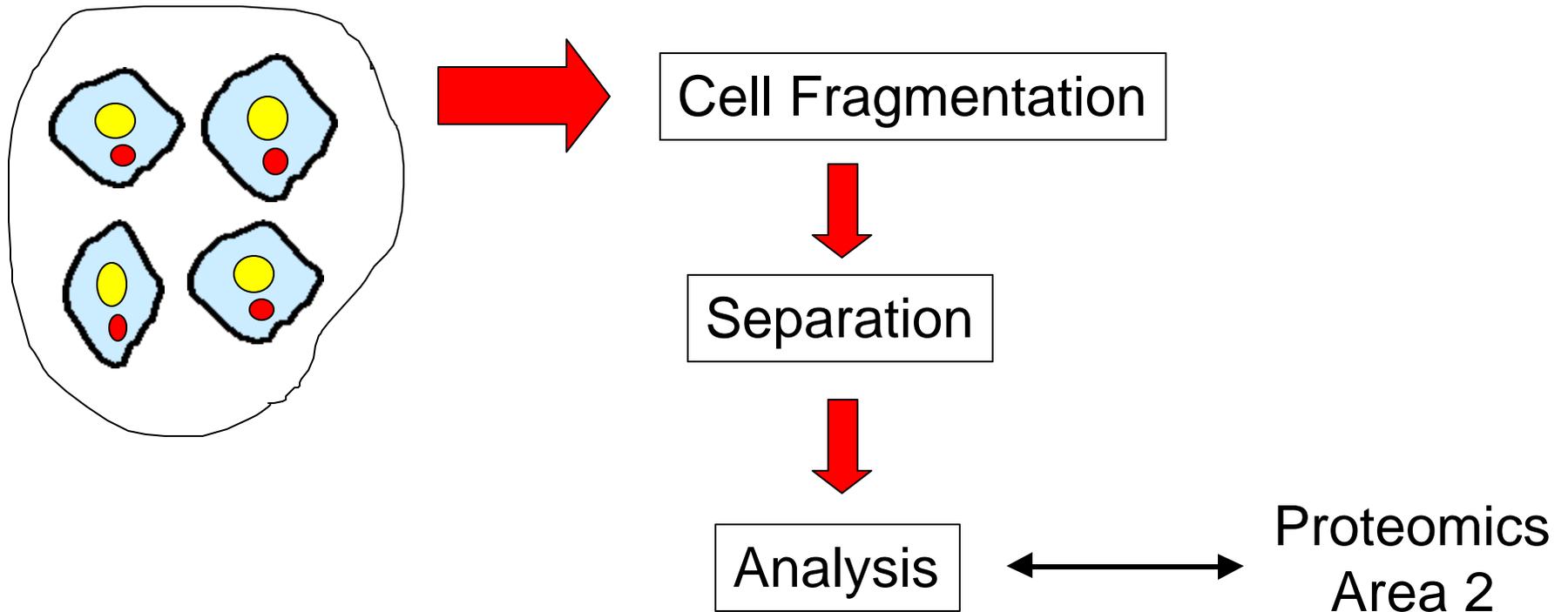
- Need to know locations of proteins with cell!
- Increase appropriateness of data by studying intracellular functional units
- Current, macroscopic techniques (e.g., cell grinding, lysis) - are highly problematic (slow, prone to loss of resolution)
- Fluorescence labeling capacity is limited
- Handle small volumes in integrated fashion
- Parallel investigations

Strategy

Build on advances in BioMEMS and μ TAS to develop techniques for:

fracturing cells and separating organelles - specifically mitochondria and nuclei -- for molecular studies

handling small cell numbers and individual cells



Multiple Cells

Cell membrane “fracture” to obtain intracellular contents

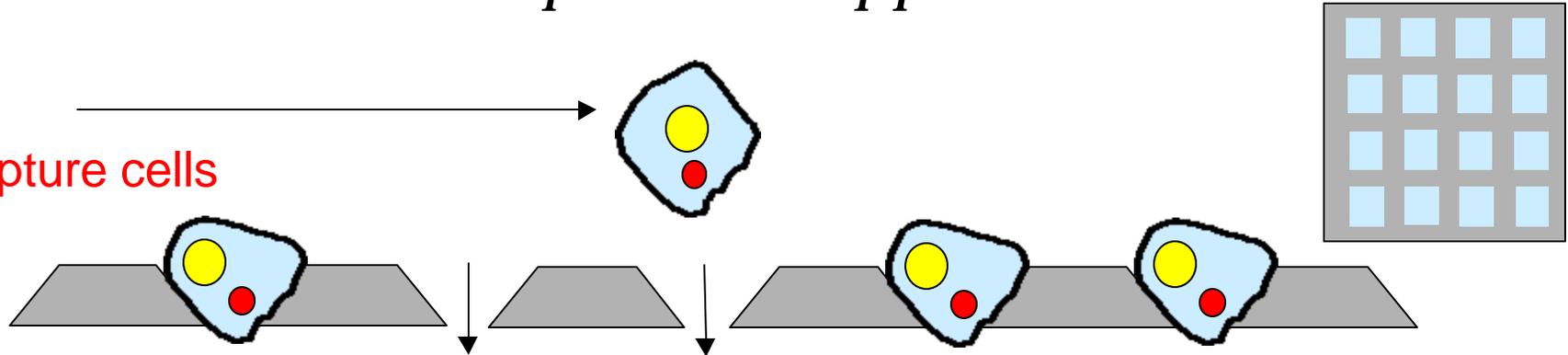
- * targeted use of lysing agents (detergents) to minimize contamination
- * mechanical approaches - controlled puncturing

Sorting

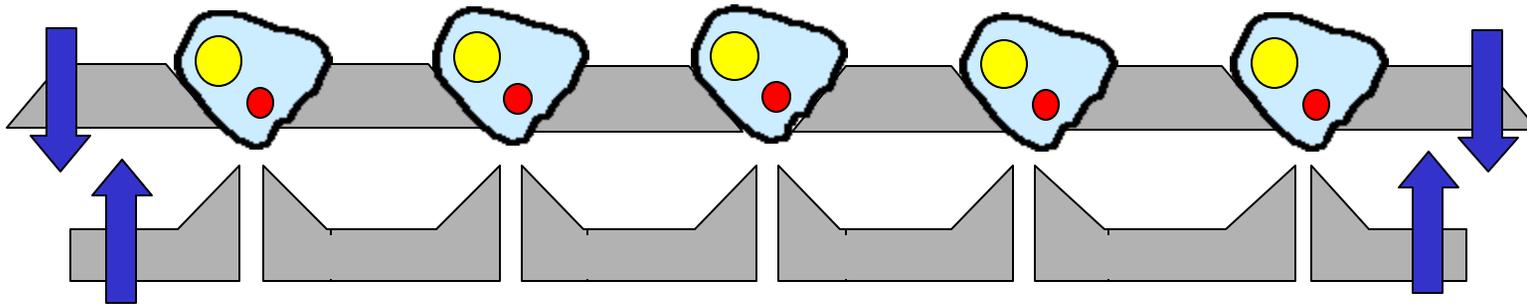
- * by size
 - microfilters
- * by size, charge, and dielectric characteristics
 - dielectrophoresis
 - free-flow electrophoresis

Multiple Cell Approach

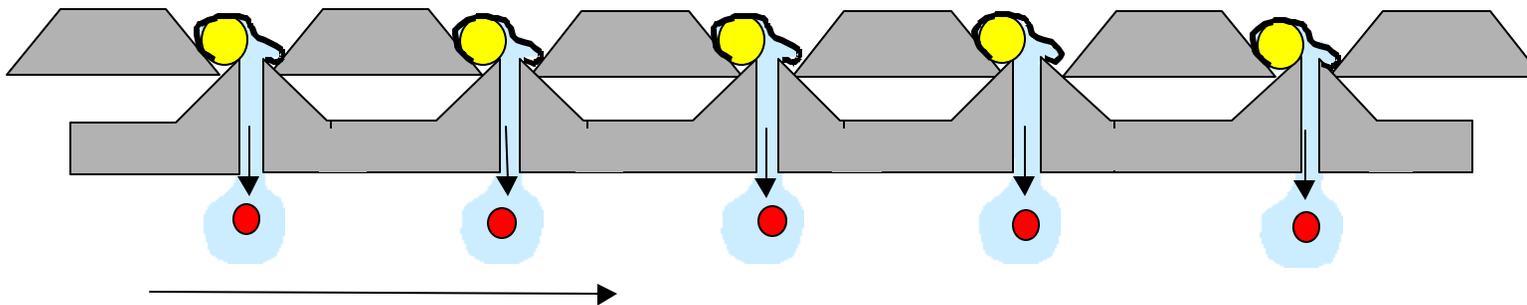
Capture cells



Fracture cell membrane

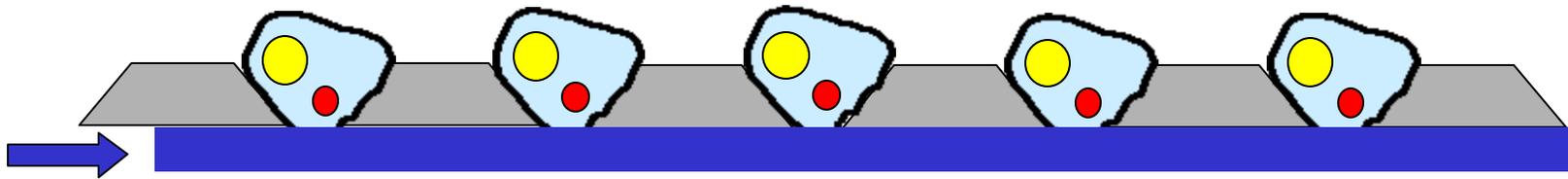


Extract cell contents



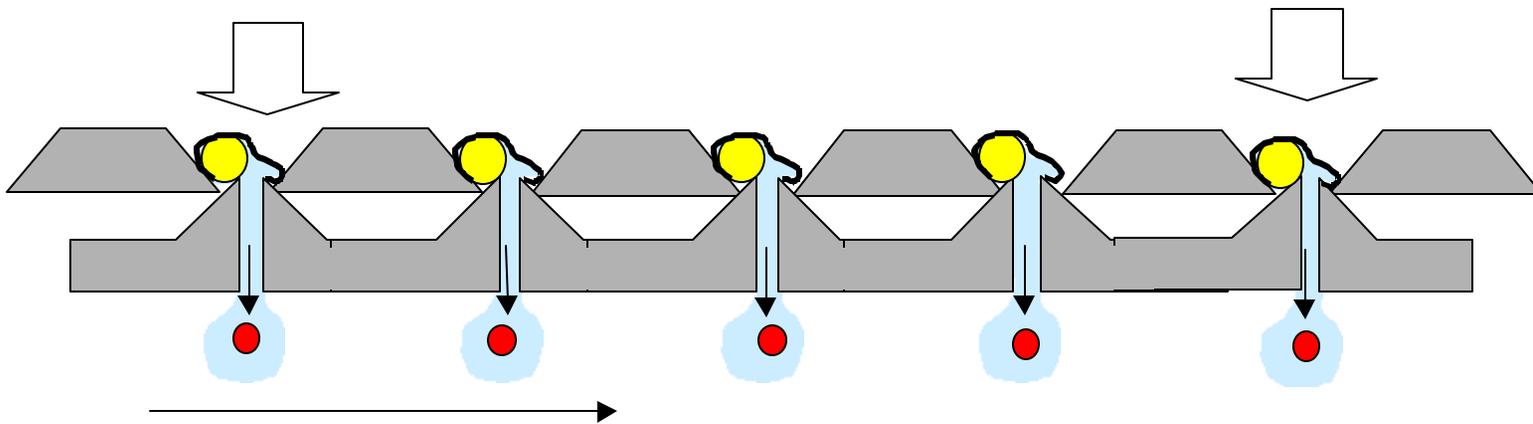
Alternative Strategy

Use laminar flow characteristics to open exposed membrane segments by exposure to a detergent

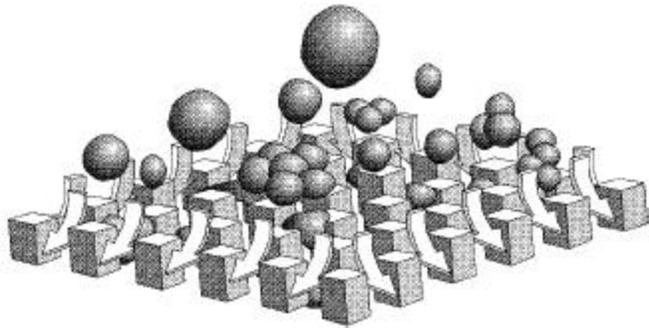


detergent

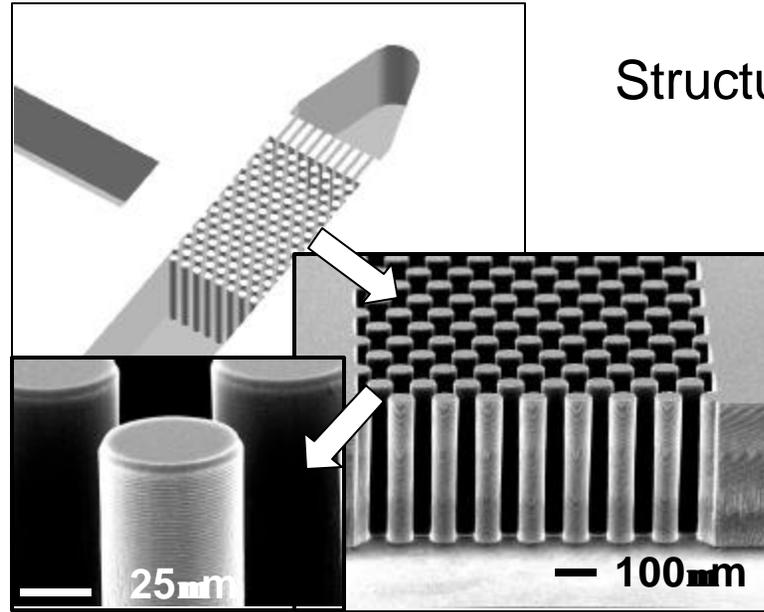
Push out cell contents



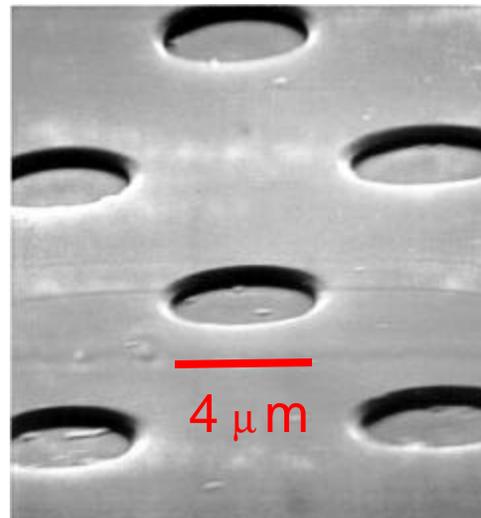
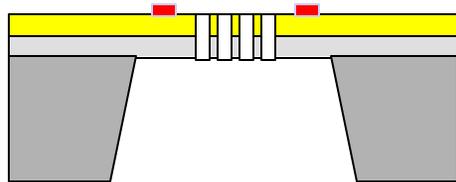
Separation of Cell Components by Mechanical Filtering



Non-clogging filter
Fred Regnier*
Analytical Chemistry
1999, 71, 1464-1468



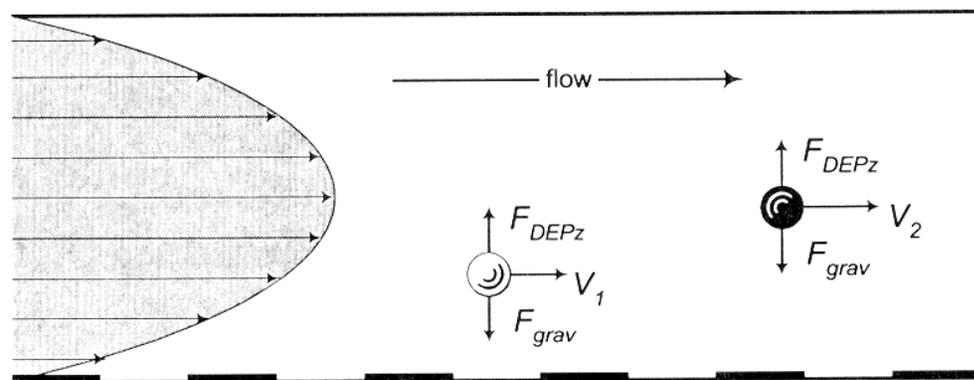
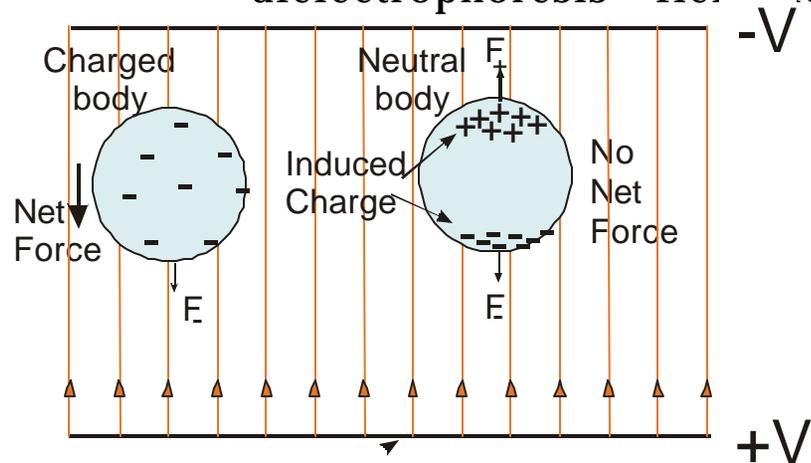
Structured packaging



Membrane with μm sized holes

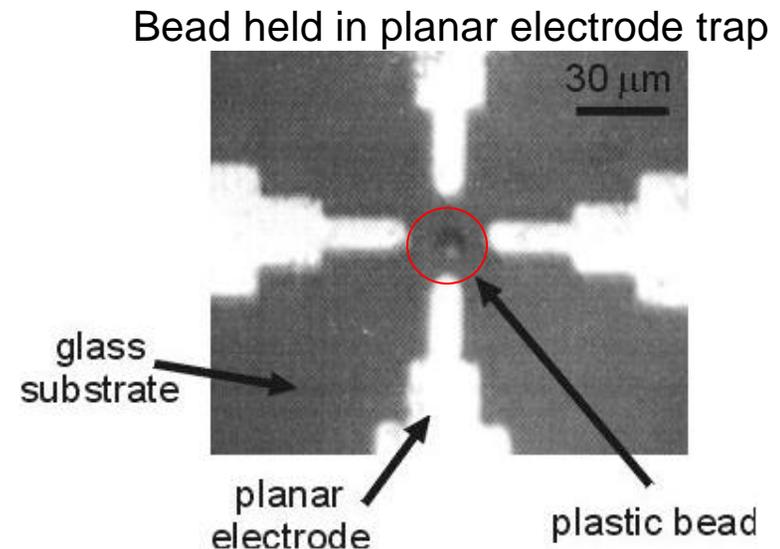
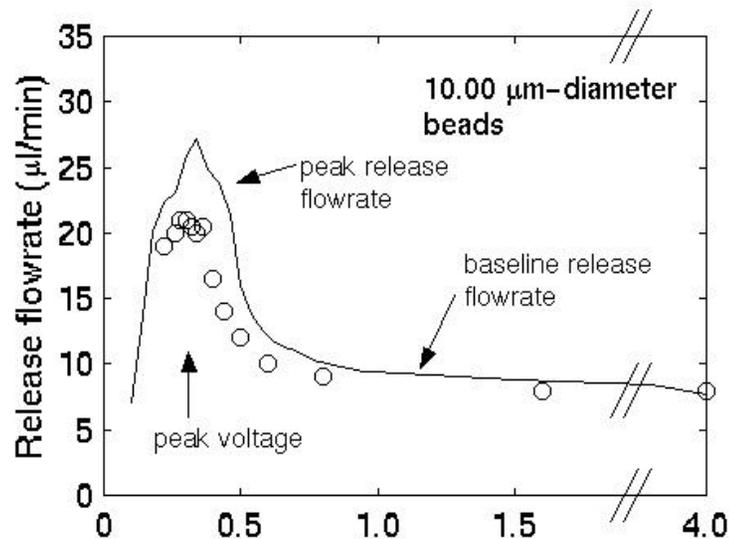
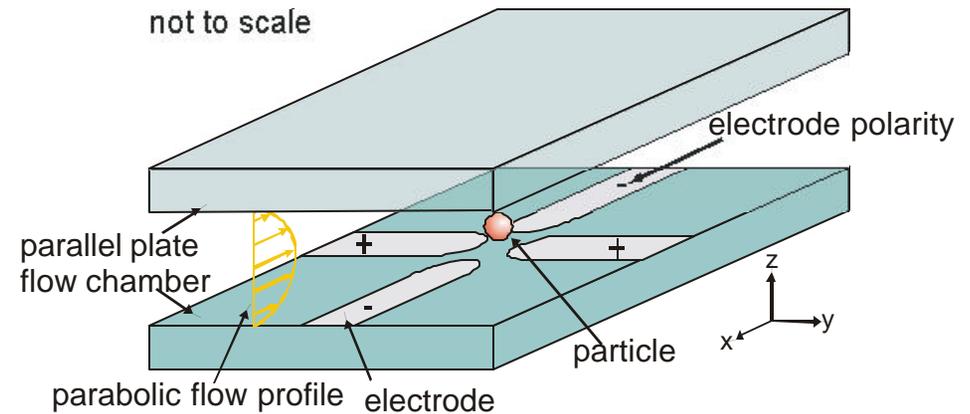
Separation of Organelles and Cell Fragments

- Electrophoresis separation by charge characteristics
- Separation by size and dielectric properties
 - dielectrophoresis - field-flow fractionation



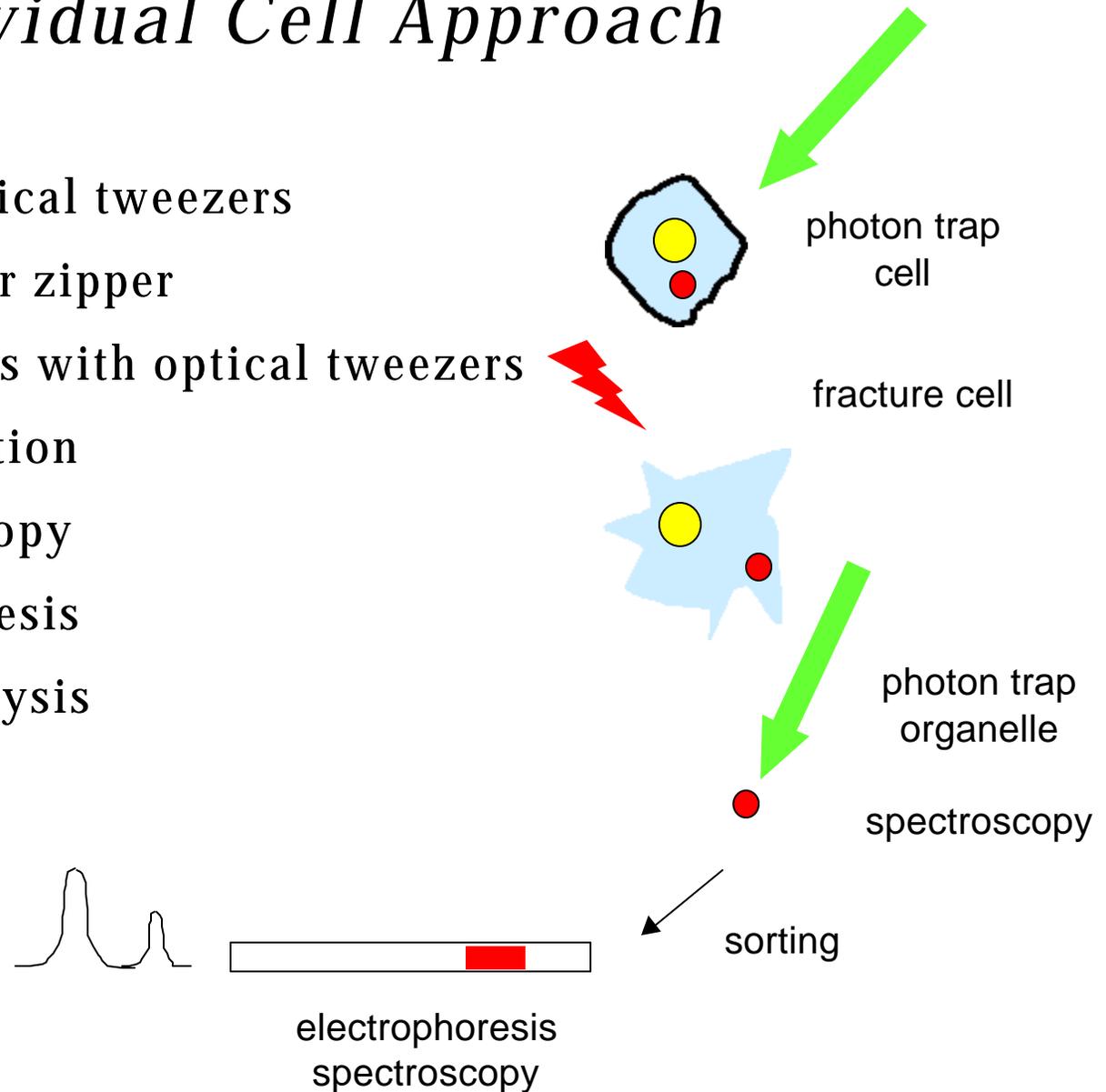
Dielectrophoresis Traps

- Can hold particles against liquid flows
- Can predict holding of
 - different particles (cells, beads)
 - in arbitrary traps
 - under a wide variety of experimental conditions



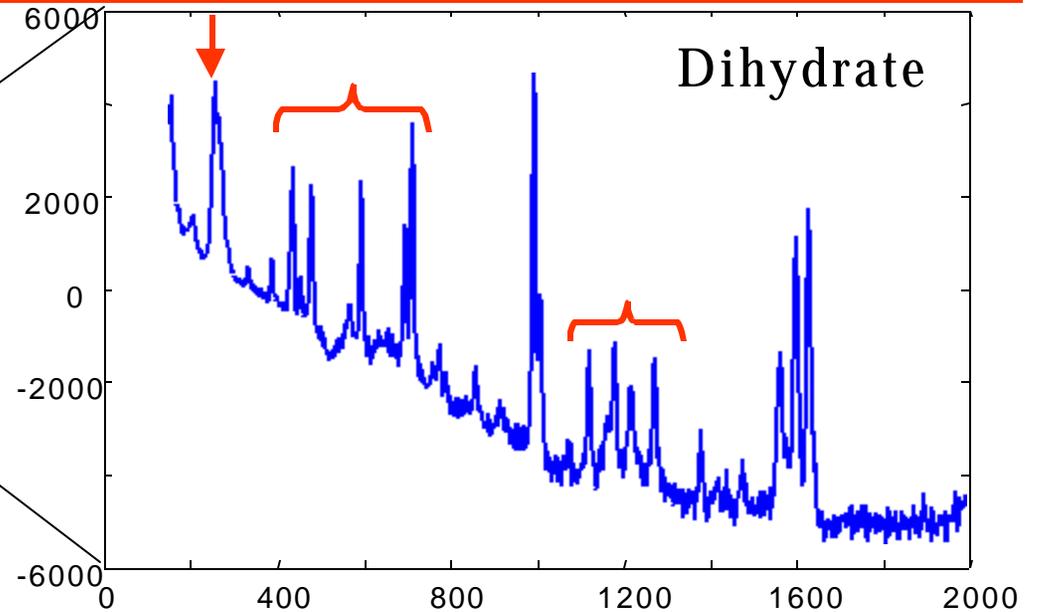
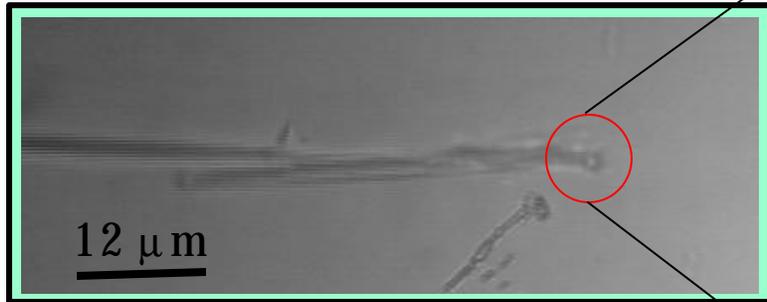
Individual Cell Approach

- trap cell with optical tweezers
- fracture cell, laser zipper
- capture organelles with optical tweezers
- optical identification
- *in situ* spectroscopy
- nano-electrophoresis
- fluorescence analysis

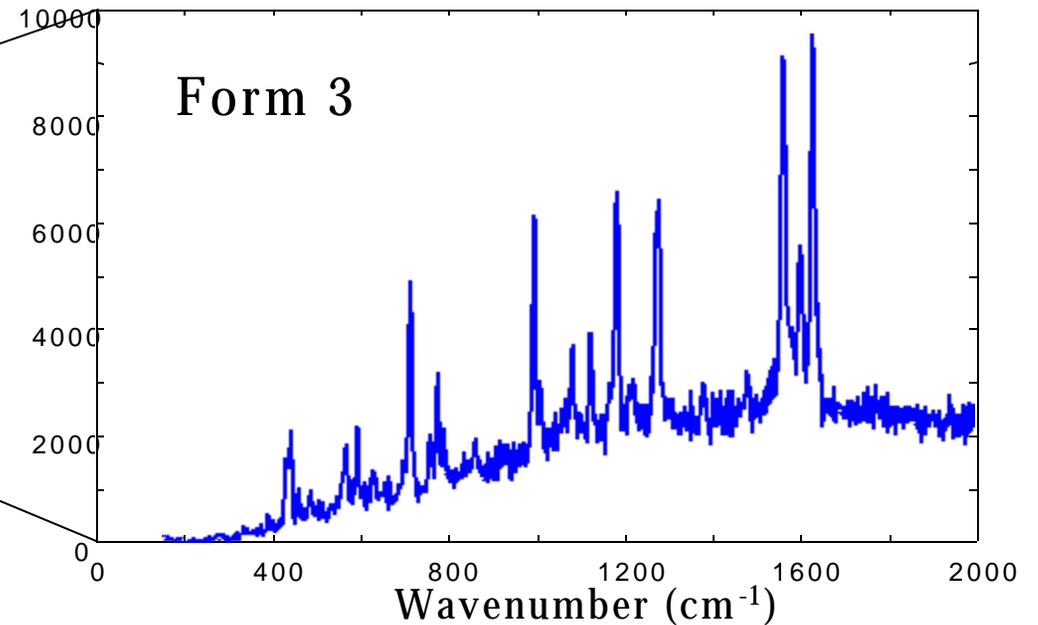
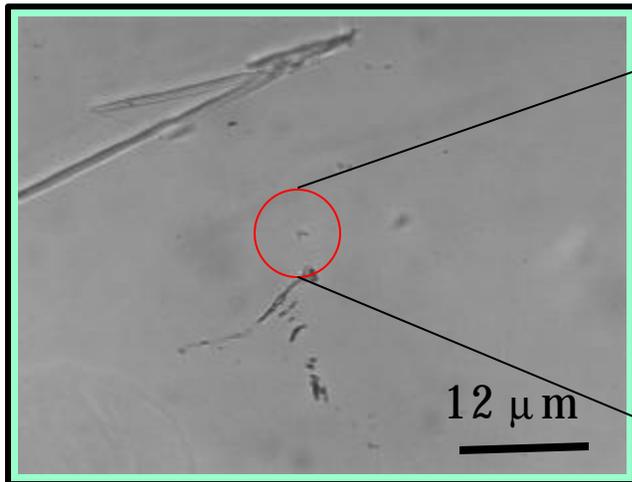


Raman Spectral Characterization of Organelles

Carbamazepin – Dihydrate Form



Carbamazepin – Form 3 (Prism)



*Area 4: Bio-informatics -- heterogeneous
extpl/model interfaces*

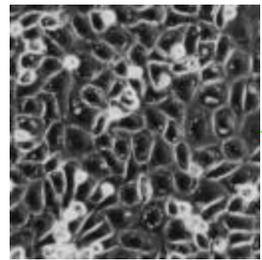
* ***F Dewey (Mech Eng / BEH)***

* D Gifford (Elect Eng & Comp Sci)

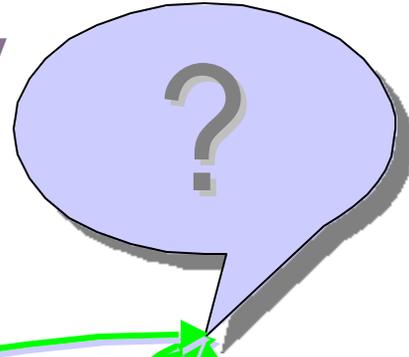
* P Sorger (Biol)

Goal

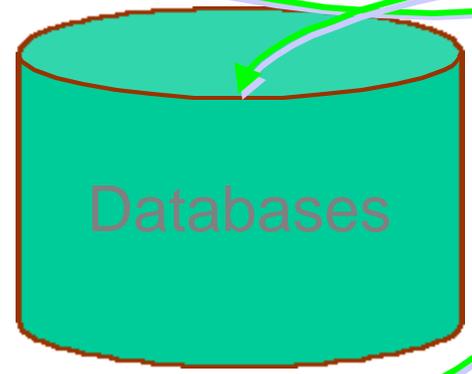
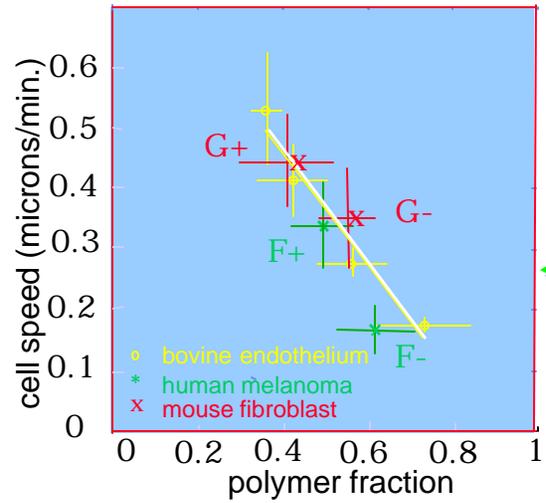
Experiments



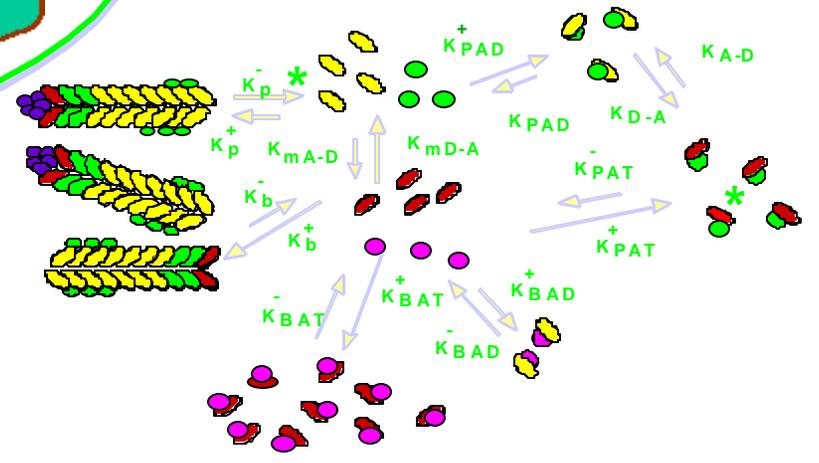
Query



Interpretation



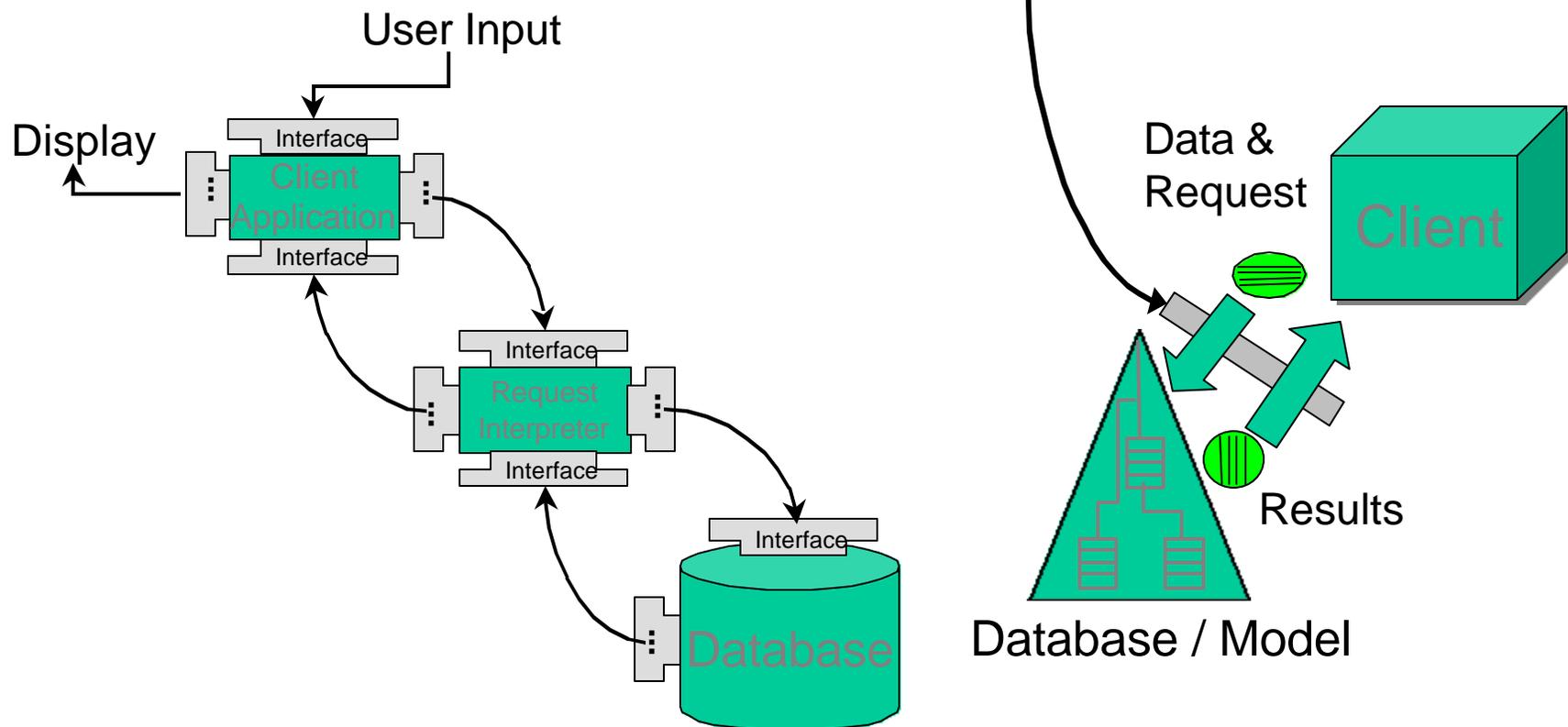
Models



Mediated data exchange and connection between entities

The Interface Implementation:

- “Class Mapper” produces object view of database
- XML wrapper for multimedia data
- CORBA used for transport and exchange of objects



Area 5: Modeling of Cell Regulatory Pathways

- * D Gifford (Elect Eng & Comp Sci)
- * T Jaakkola (Elect Eng & Comp Sci)
- * D Lauffenburger (Chem Eng / BEH)

Goal and principles

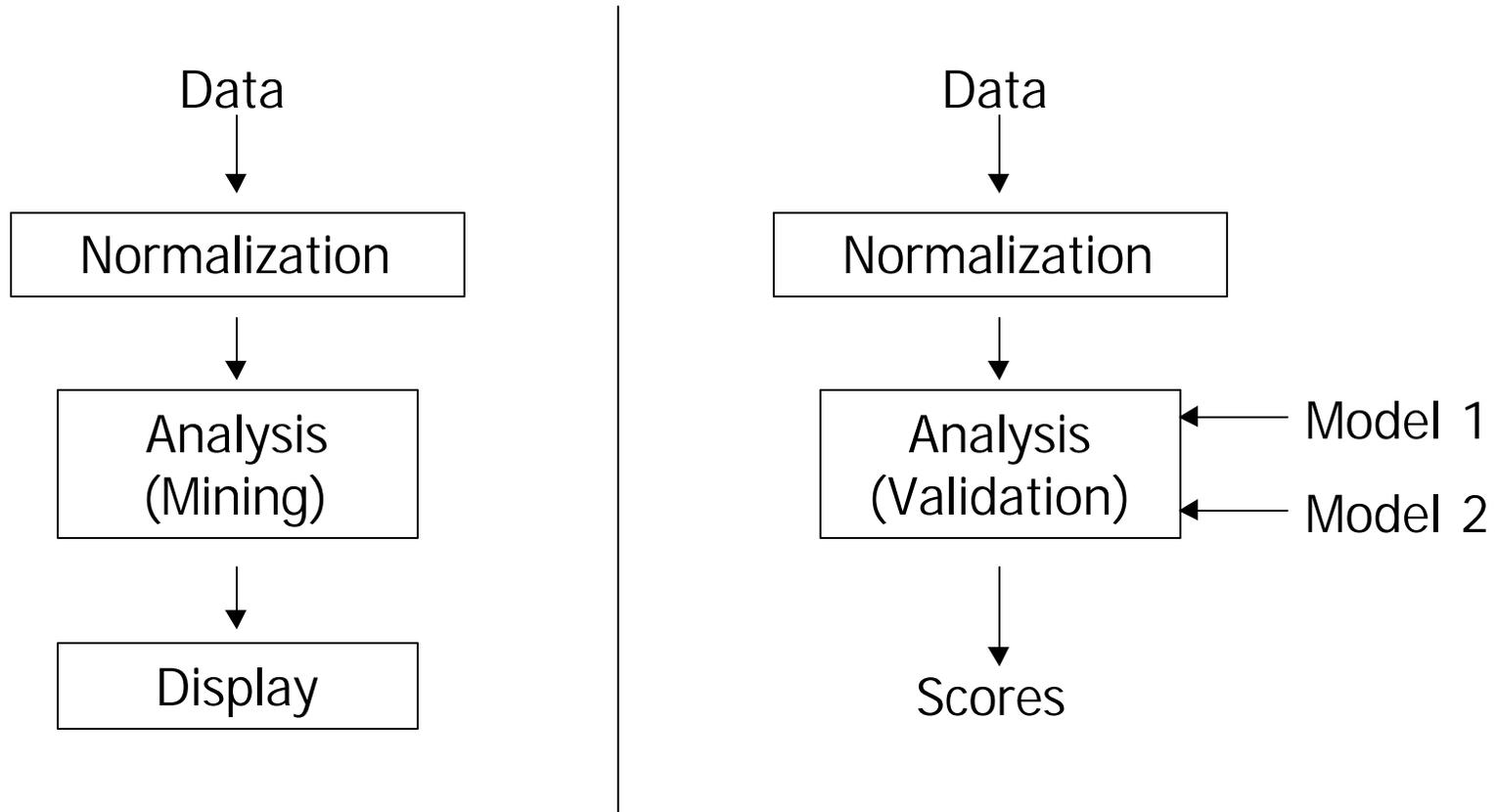
Goals

- * Develop robust models for molecular networks governing cell responses to stimuli, and their predictive capability for cell death-vs-survival decisions
- * Address both protein signaling networks and gene regulatory networks

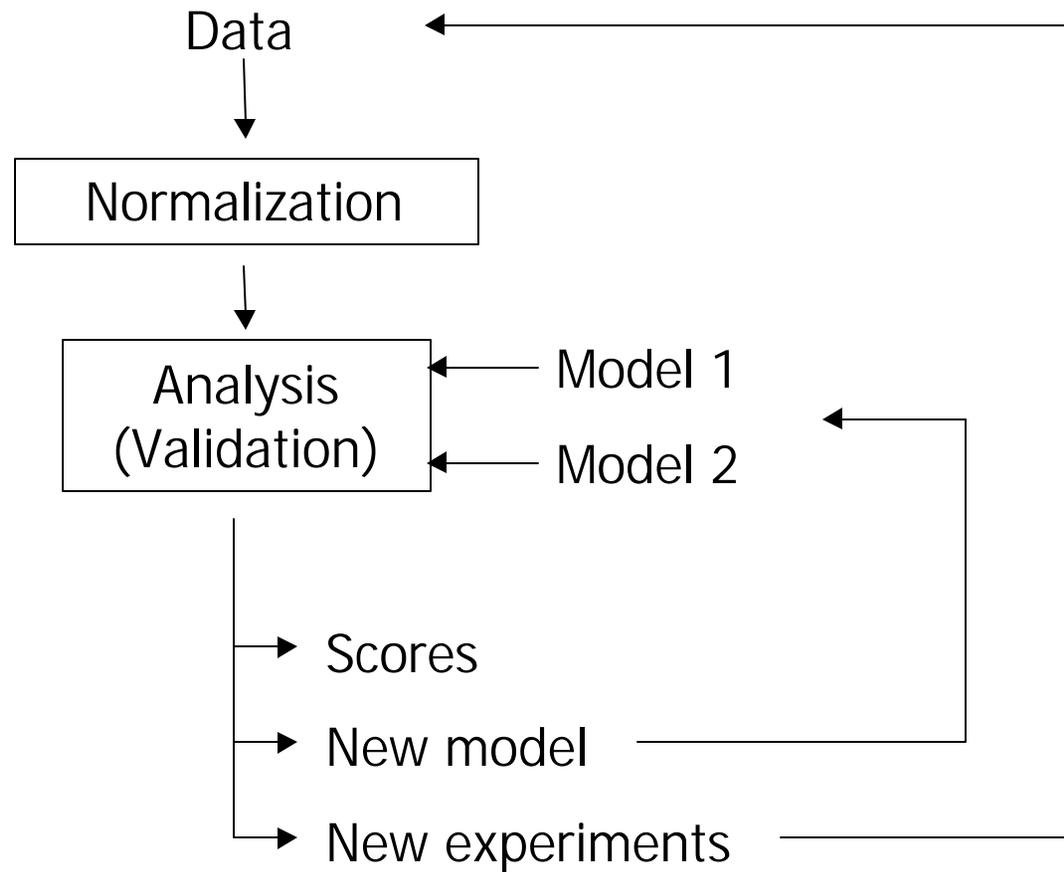
Principles

- * Handle imperfect data and imperfect theory robustly
- * Employ models that are biologically interpretable
- * Work from high level to low level models
- * Produce results with statistical significance

Models for Enhancement of Data Analysis



Computationally-represented Models for Project Integration



Probabilistic Representations

Modeling complex molecular regulatory networks requires probabilistic representations because:

- * data is inherently noisy
- * knowledge of networks is incomplete
- * networks are in some cases inherently stochastic

We can validate probabilistic models of networks using rigorous statistical metrics.

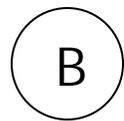
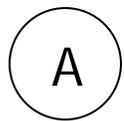
Graph Models of Regulatory Networks

Graphical models employ graphs to encode dependence relationships between variables

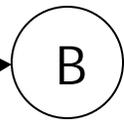
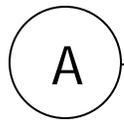
- * graph vertices represent variables
- * graph edges represent dependencies
- * dependencies describe probabilistic relationships between variables

We use Bayesian networks (directed graphs)

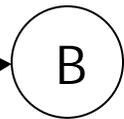
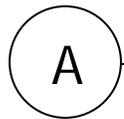
*Graphical models can represent
knowledge at varying levels of refinement
in a single model*



A and B are independent

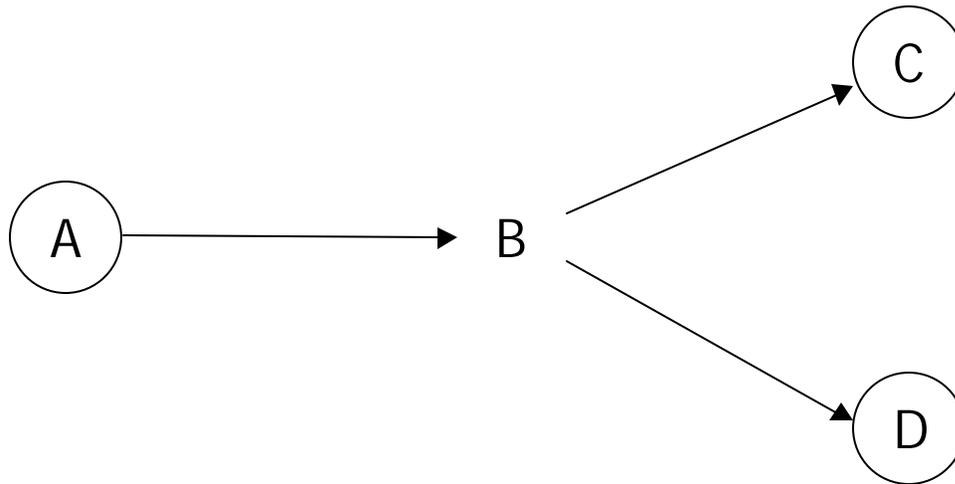


A has an influence on B



A has a positive influence on B

*Graphical models can include
latent variables*



Here, B can be unobserved, unobservable, or unknown

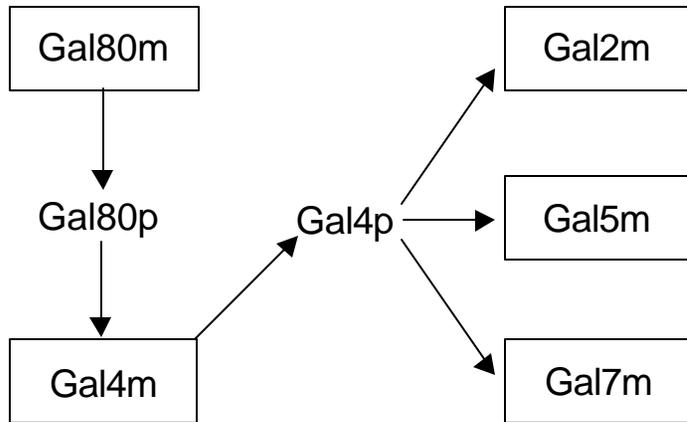
Bayesian scoring metric allows us to compare models with statistical rigor

Bayesian approach: we score model structure as ensemble with a distribution over possible parameters

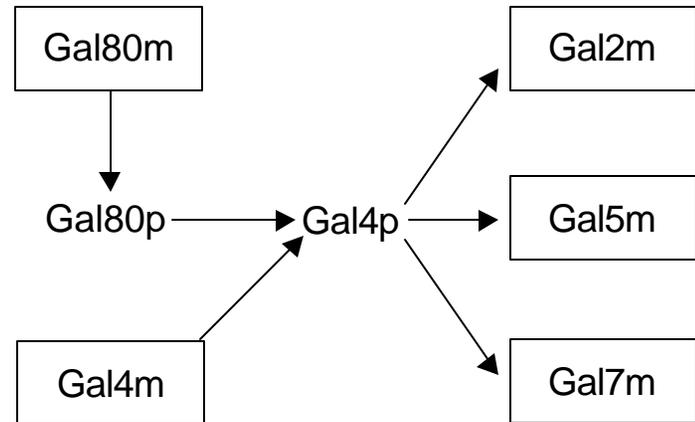
Score is proportional to average likelihood of observing data over all possible parameter settings:

$$P(D | S) = \int P(D | \theta) P(\theta) d\theta$$

Example Graphical Models

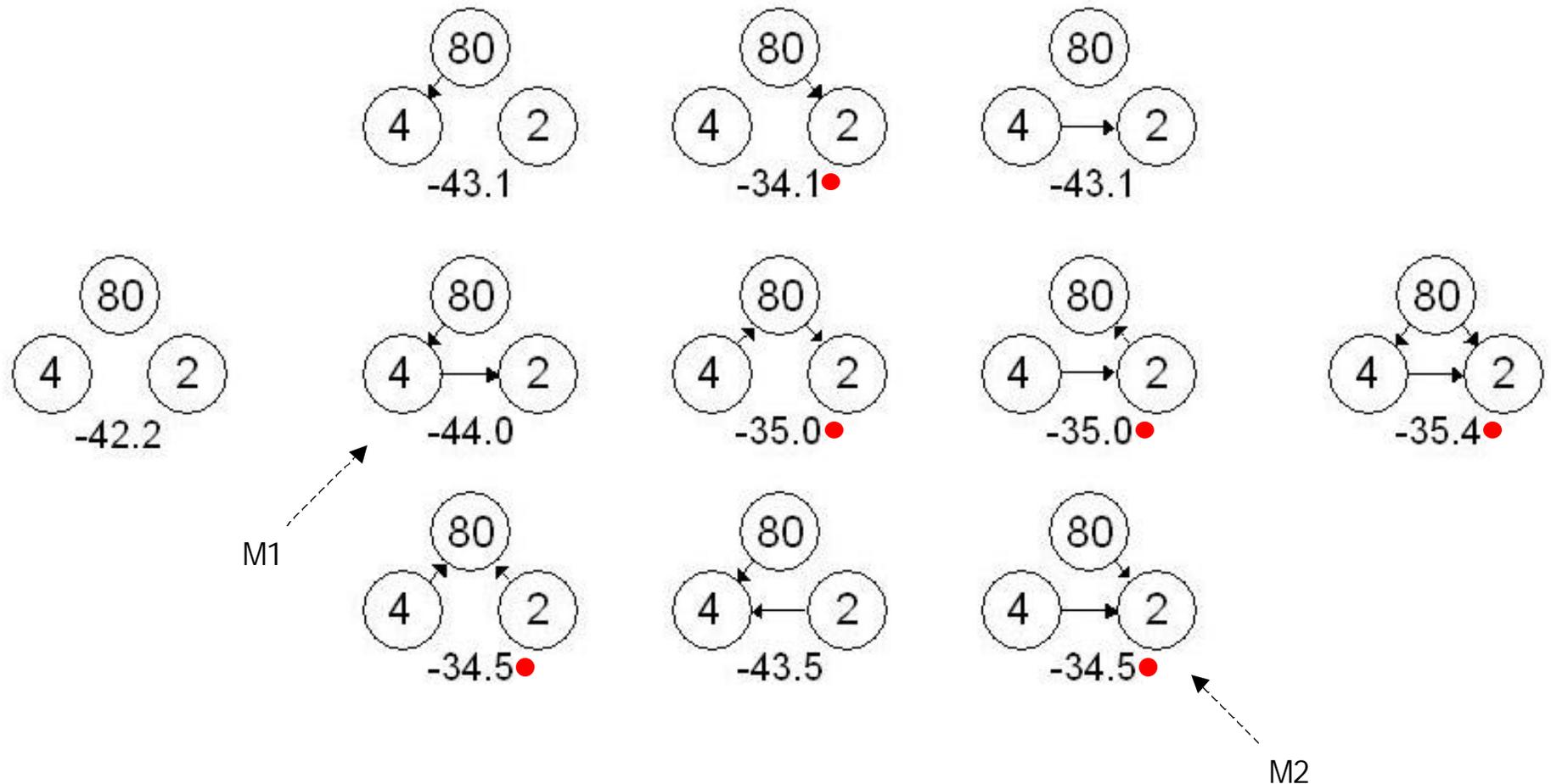


M1

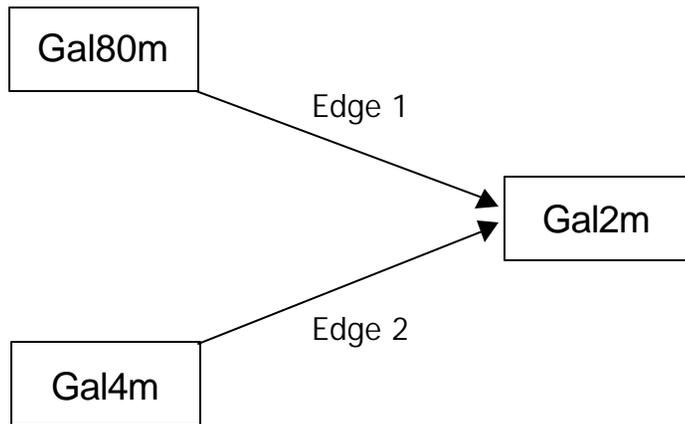


M2

*Scoring all possible models relating
Gal80m, Gal4m, and Gal2m*



Edges can be annotated to add constraints and the resulting models scored



		Edge 2			
		-	Uncons.	+/-	+
Edge 1	-	-48.89	-49.27	-47.27	-46.68
	Uncons.	-36.06	-34.46	-35.76	-35.53
	+/-	-35.53	-34.31	-35.44	-35.36
	+	-34.83	-33.61	-34.75	-34.66

Limitations of this modeling approach

Connection to physico-chemical mechanisms?
(complementary approach)

Cannot assess the validity of a single model in isolation, must compare alternative models --
opportunity for biology exploration

Cannot compare complex models without sufficient data -- driving Areas 1,2,3,4

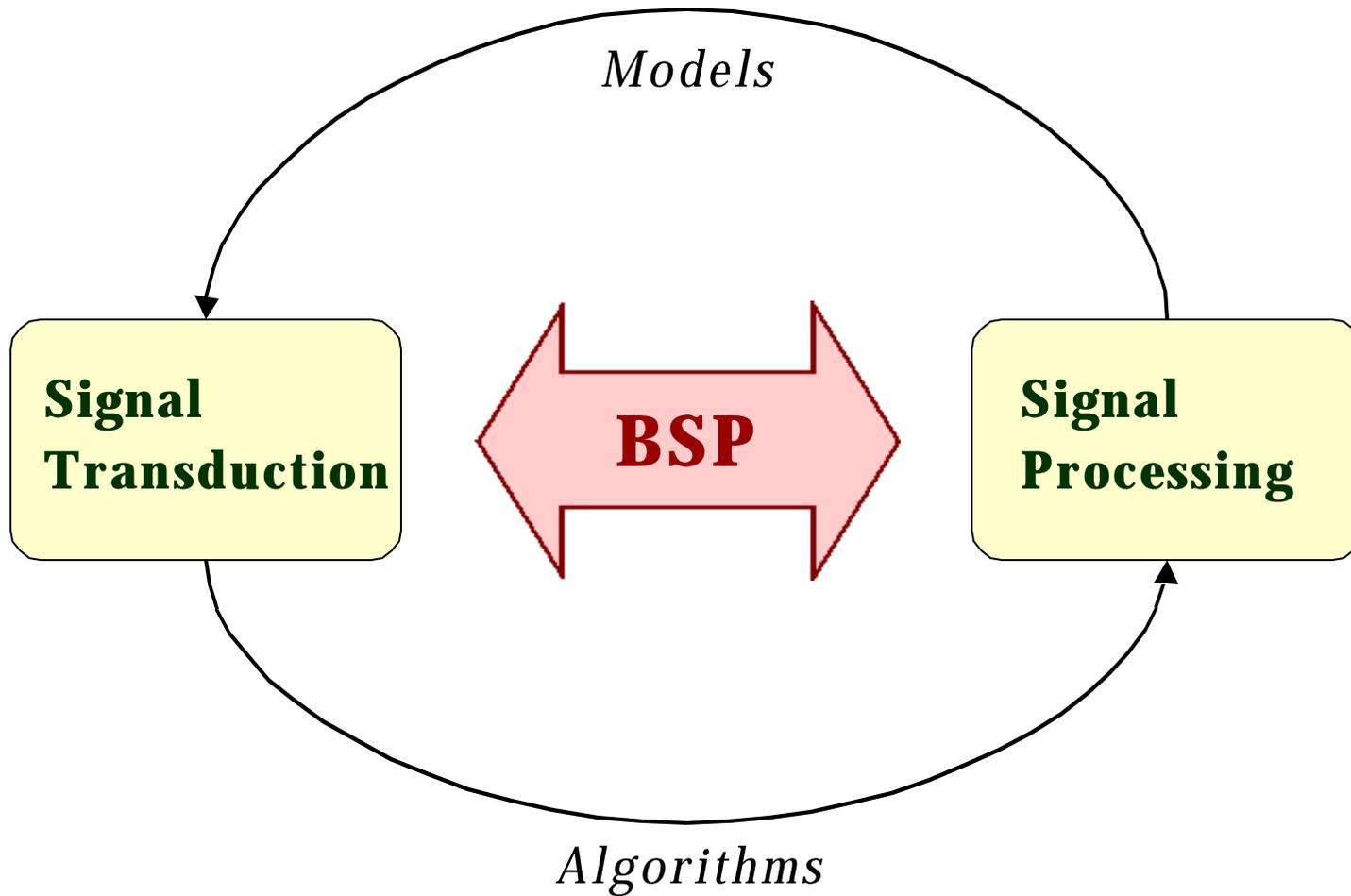
Area 6: Biological Signal Processing

- * A Oppenheim (Elect Eng & Comp Sci)
- * D Lauffenburger (Chem Eng / BEH)

Modeling Approach

- Define layers of **Abstraction**
- Apply Engineering **Analogies**
- Explore **Input/Output** Relationships

Establish a Dialogue



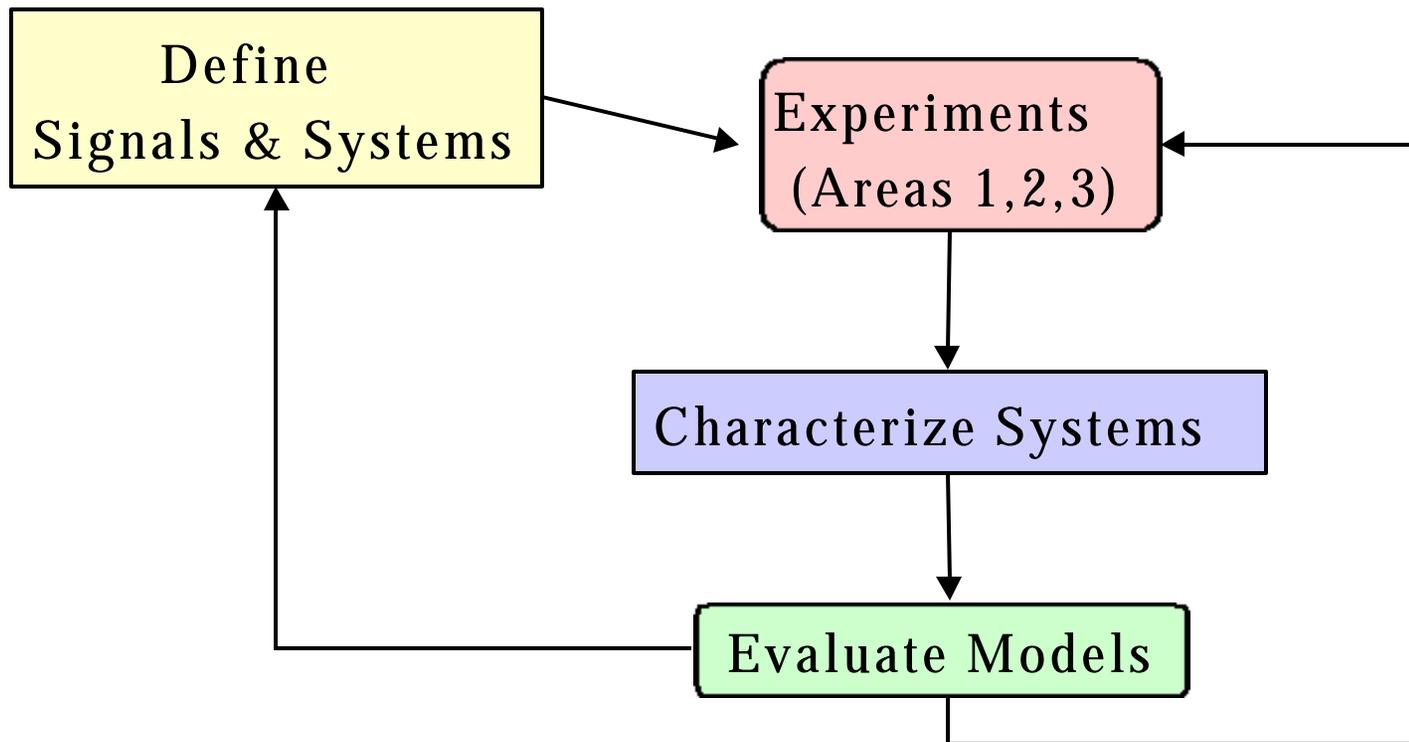
BSP Fundamentals

- **‘Arithmetic’ Operations**
 - phosphorylation (*I κ B* , *Akt*)
 - cleavage (*caspases*)
- **Hardware Assembly**
 - protein-protein associations (*TNFR*)
 - transcriptional activation (*NF κ B*)
- **Signal Transmission**
 - mitochondrial membrane (*cyt c*)
 - nuclear membrane (*NF κ B*)

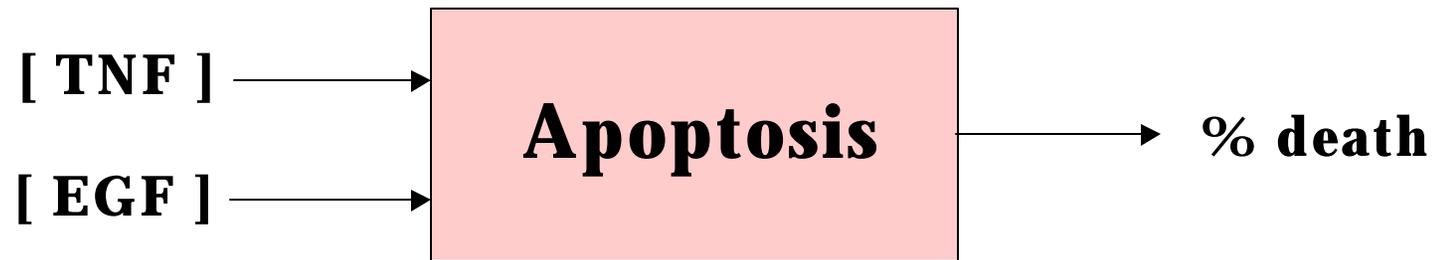
“Algorithms”

- **Fault Tolerance**
 - Bax & Bid ‘dual switch’
- **Distributed Signal Processing**
 - ‘mitochondrial checkpoint’ (types 1 and 2 cells)
- **Coding/Control**
 - Apaf-1 and Smac/DIABLO

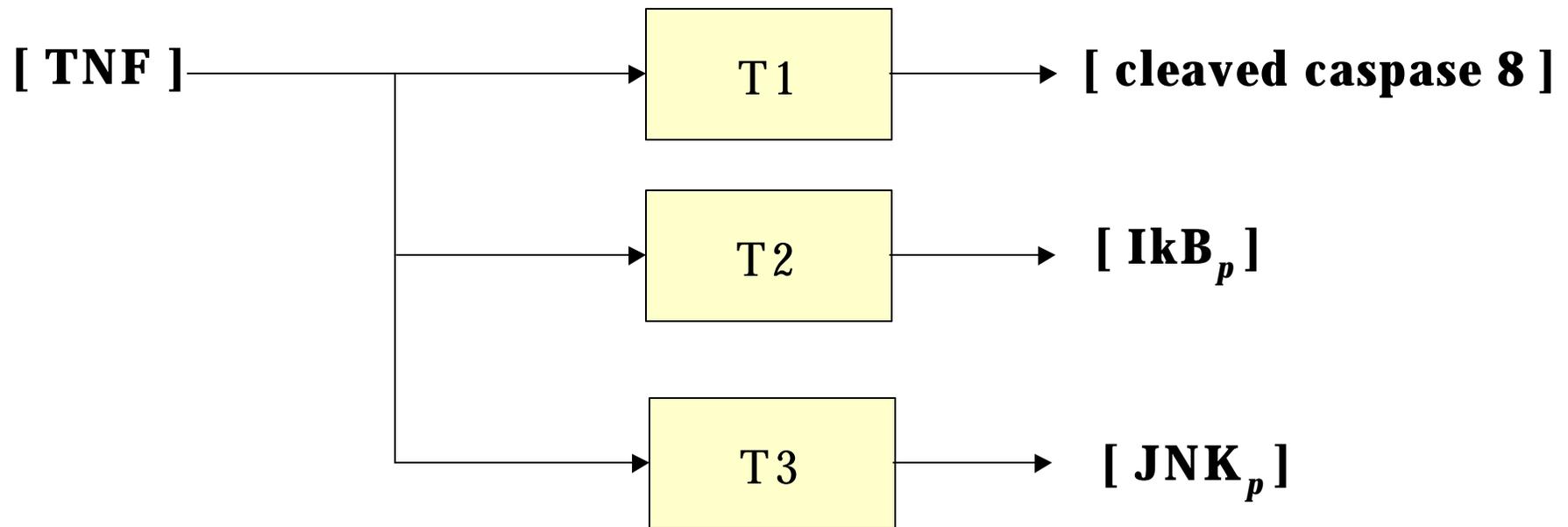
Approach



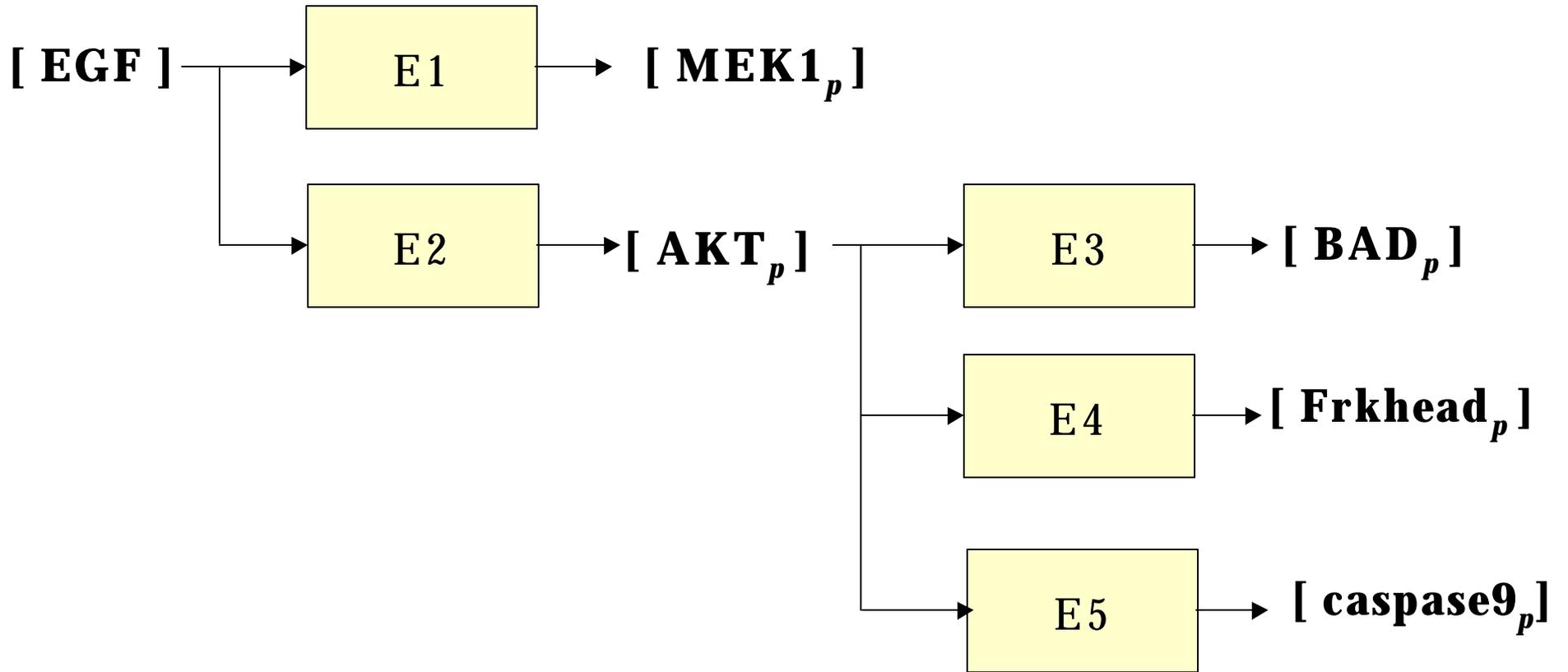
*The **BIG** Picture*



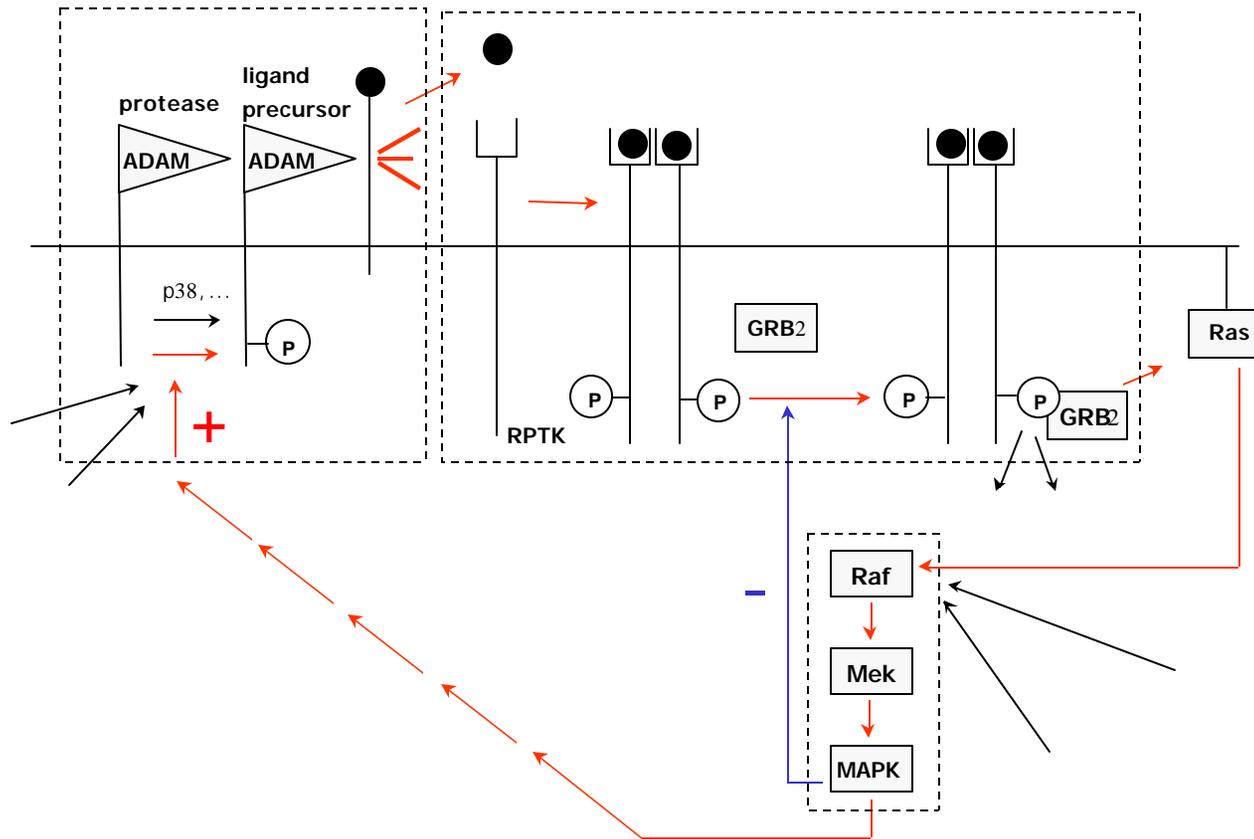
TNF 'subsystem'



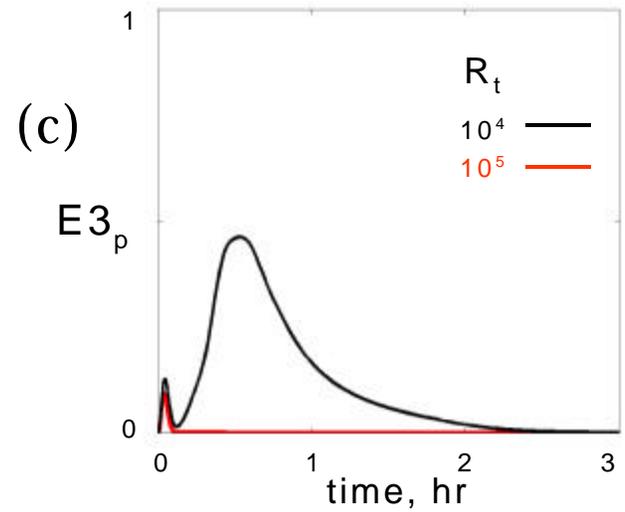
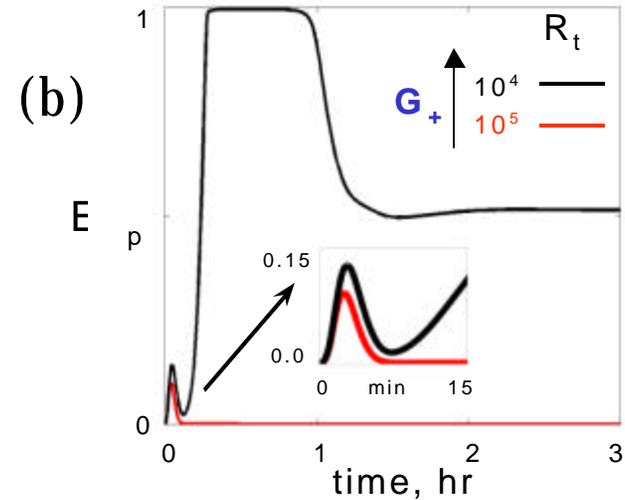
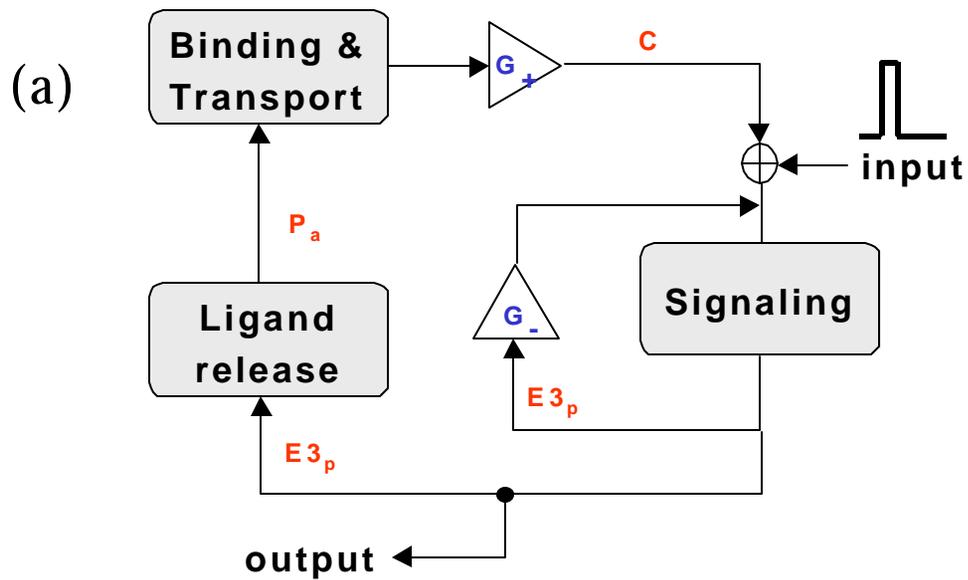
EGF 'subsystem'



Example: EGF Receptor Autocrine Signaling Feedback Loop

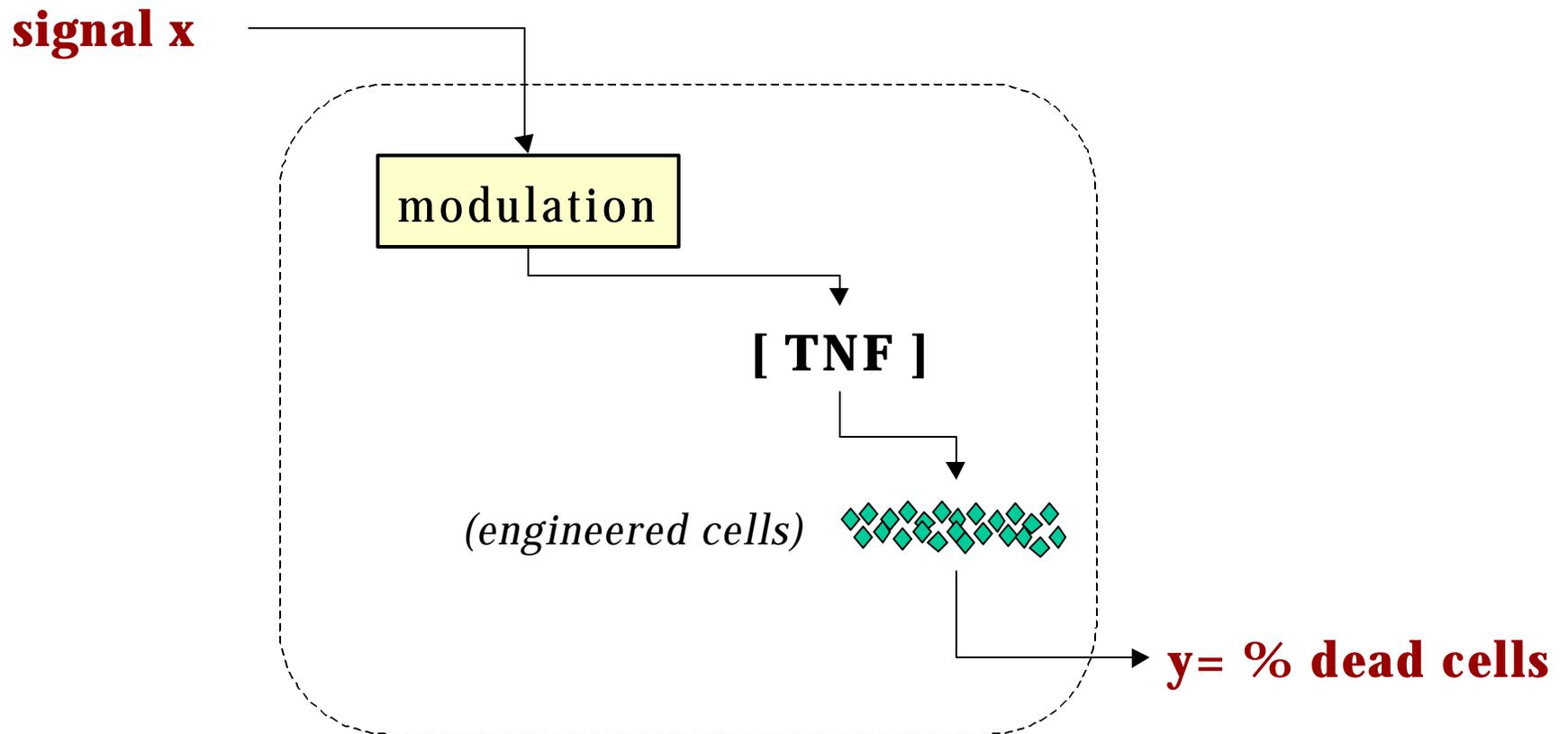


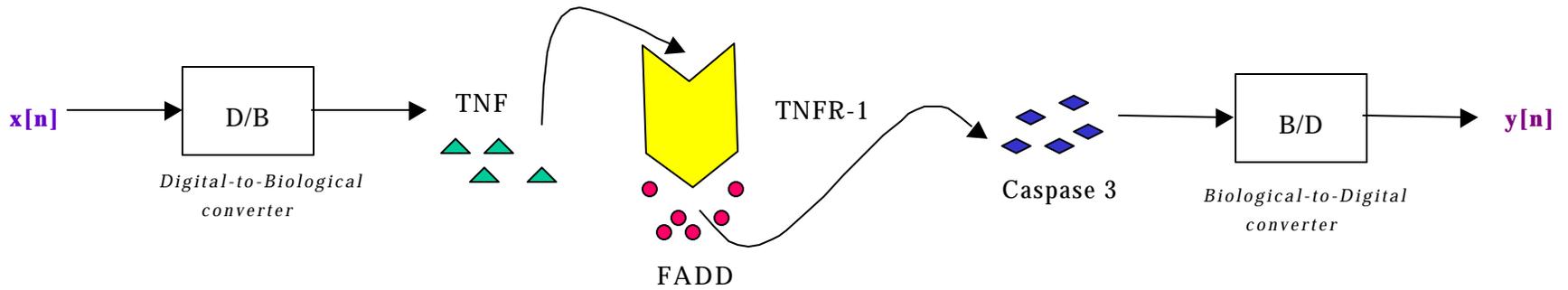
EGFR Autocrine Signaling Loop: Bi-Directional Communication



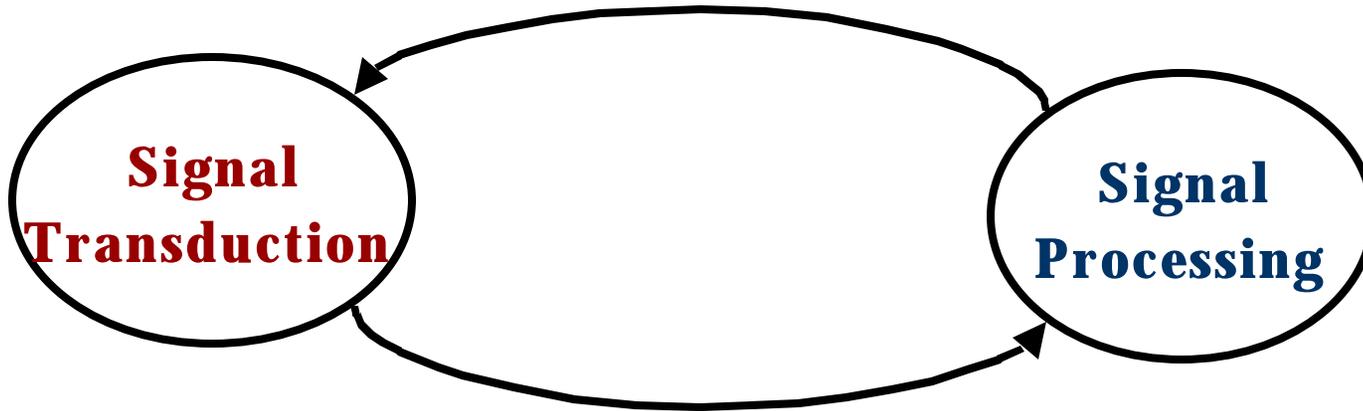
G_-

BSP: Biological Sensors





Biological Signal Processing



Signal Transduction Modeling

