Addressing cancer heterogeneity via technologies for making in vitro protein diagnostics cheap

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Senior collaborators: UCLA: Paul Mischel; Caius Radu, Owen Witte, Toni Ribas
ISB: Leroy Hood

Heath group contributors: Chao Ma, Dr. Rong Fan (Yale); Dr. Ann Cheung, Young Shik Shin, Ophir Vermesh, Udi Vermesh, Habib Ahmad, Dr. Lidong Qin, Dr. Qihui Shi

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Disclaimers: Founder & Board Member of Integrated Diagnostics; Momentum Biosciences

In vitro diagnostics Challenges

Pound for pound, proteins are the most informative biomarkers

As we march towards the $1000 genome…

Price of quantitative protein diagnostics unchanged in ~30 yrs (~$50/protein)

If Proteins can be measured for ~1cent/per, the impact would be transformative

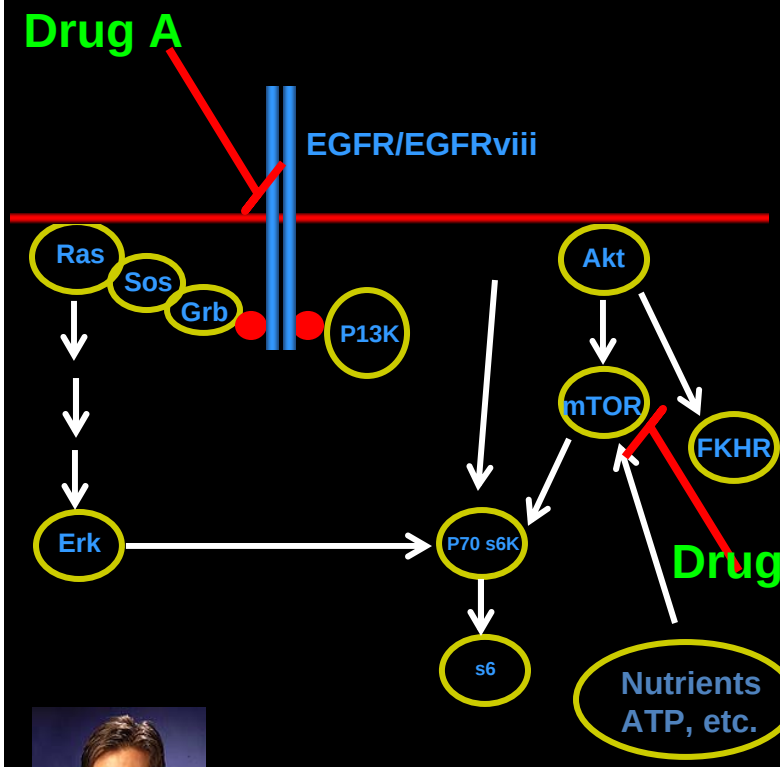
- **monitor all candidate biomarkers, not just 2 or 3 from a list of ~100**
- **time dependent (velocity) profiles consistent with the kinetics of drug action**
- **capture disease heterogeneity & function at the single cell level**

Many technology challenges

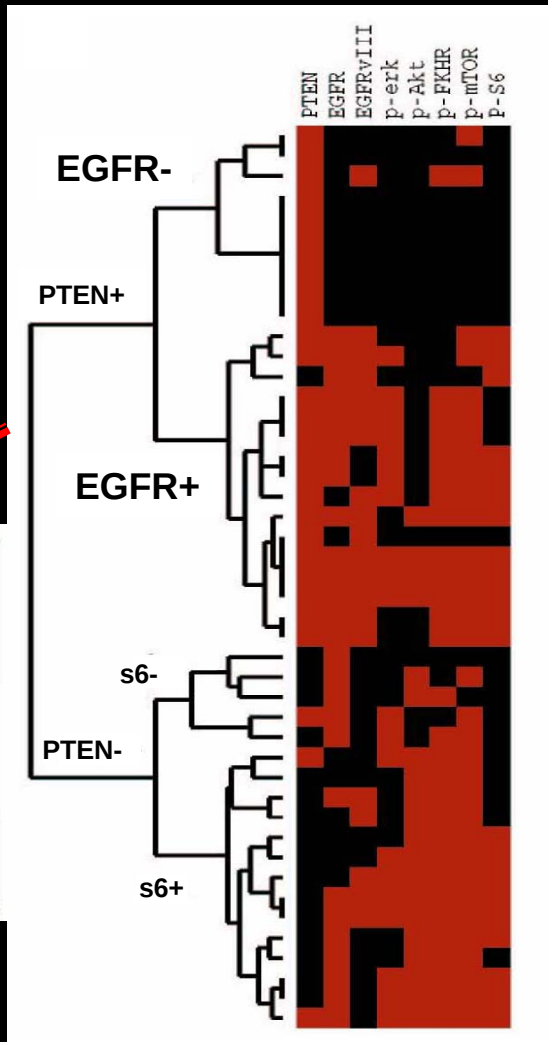
- protein capture agents (the biggest one!)
- increased multiplexing at reduced cost
- improved quantitation and sensitivity at reduced cost
- time-dependent measurements

A few examples of what is possible….

Cancer Pathways & Glioblastoma Multiforme

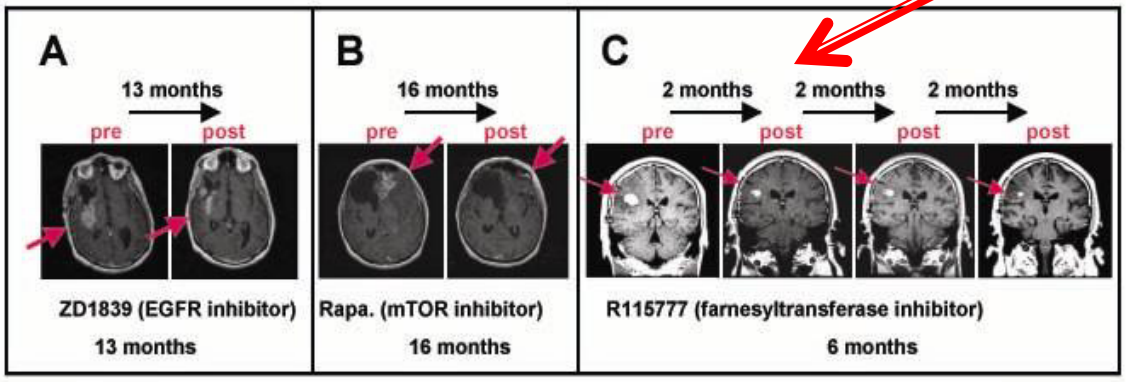


Pathology TREE to stratify patients for therapy



And to produce a positive outcome for patients

Paul Mischel



Unfortunately, this doesn't work

The tumor microenvironment represents a rich & highly heterogeneous information network

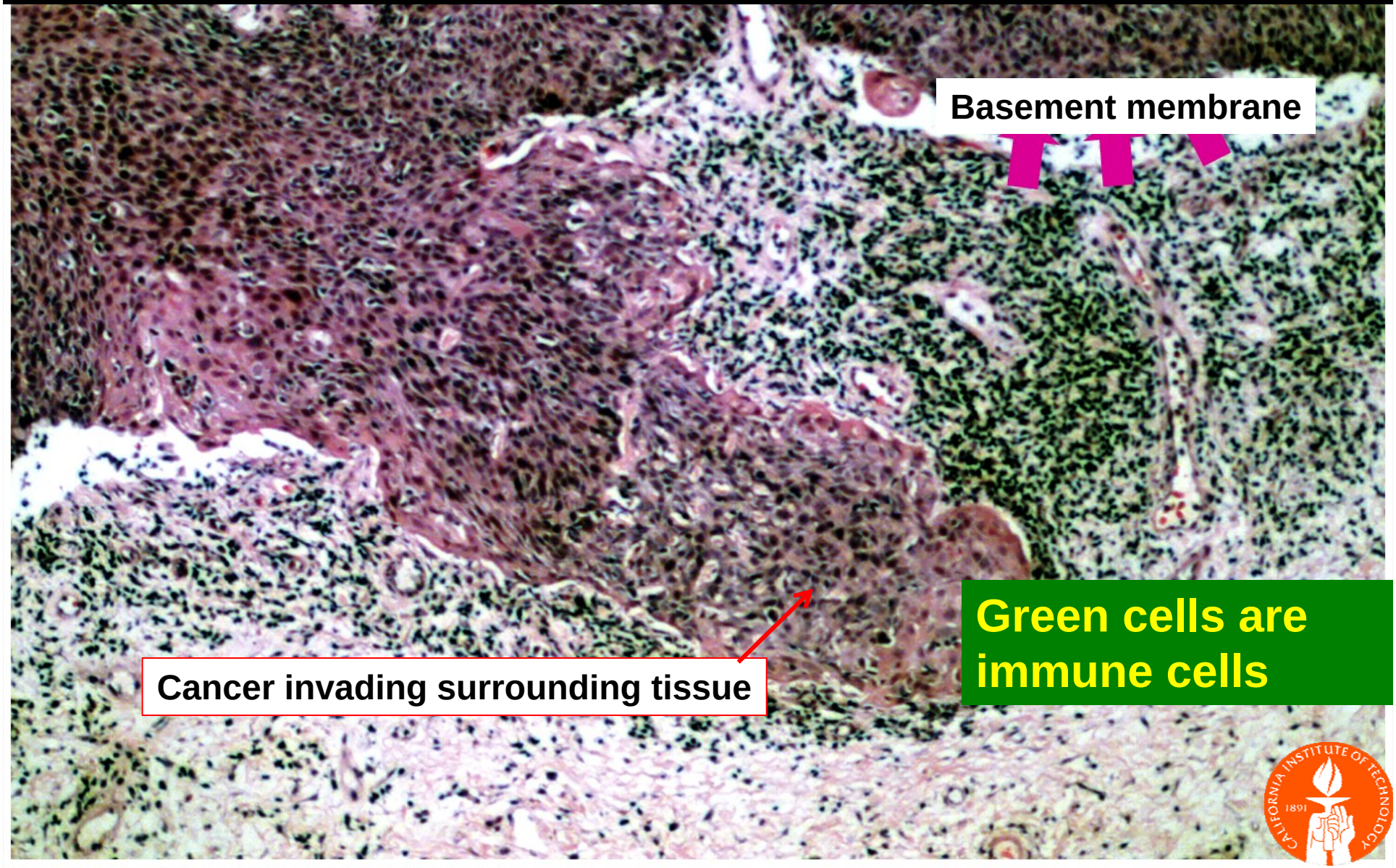


Figure 14-5c The Biology of Cancer (© Garland Science 2007)

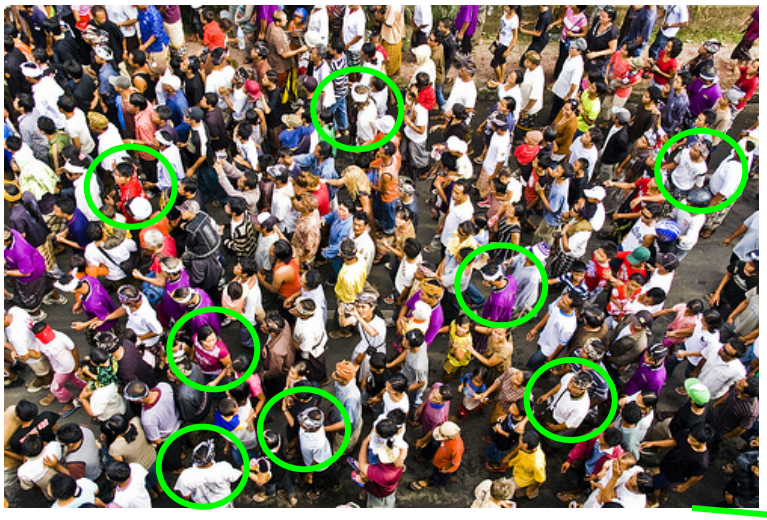
Targeted Therapeutics: Avastin is an angiogenesis inhibitor that costs ~$100k per GBM patient, ~30% responders; benefit is minimal, but better than anything else.

No biomarkers exist other than *watch and wait* (via MRI & CT imaging) for tumor to regress (~10 weeks)

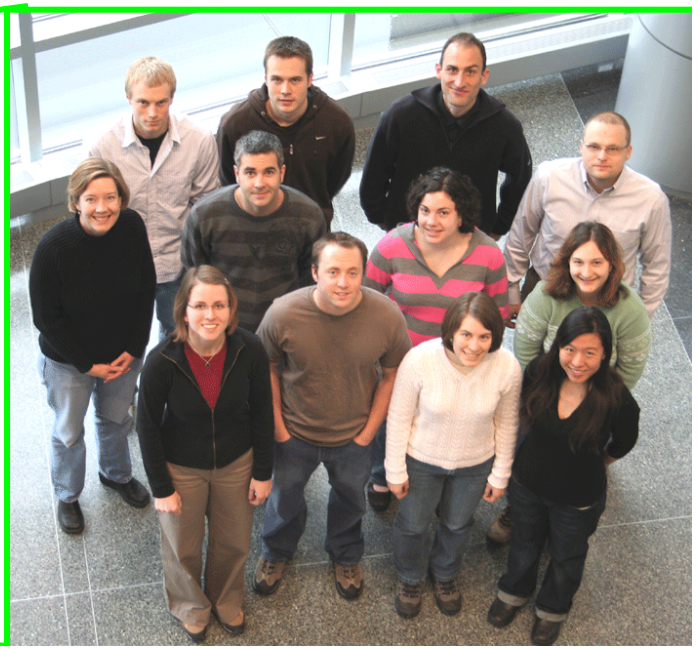
We identified ~50 proteins associated with the GBM microenvironment

500 patients x 50 proteins x $50 per protein = $1.25M

find a different way…..

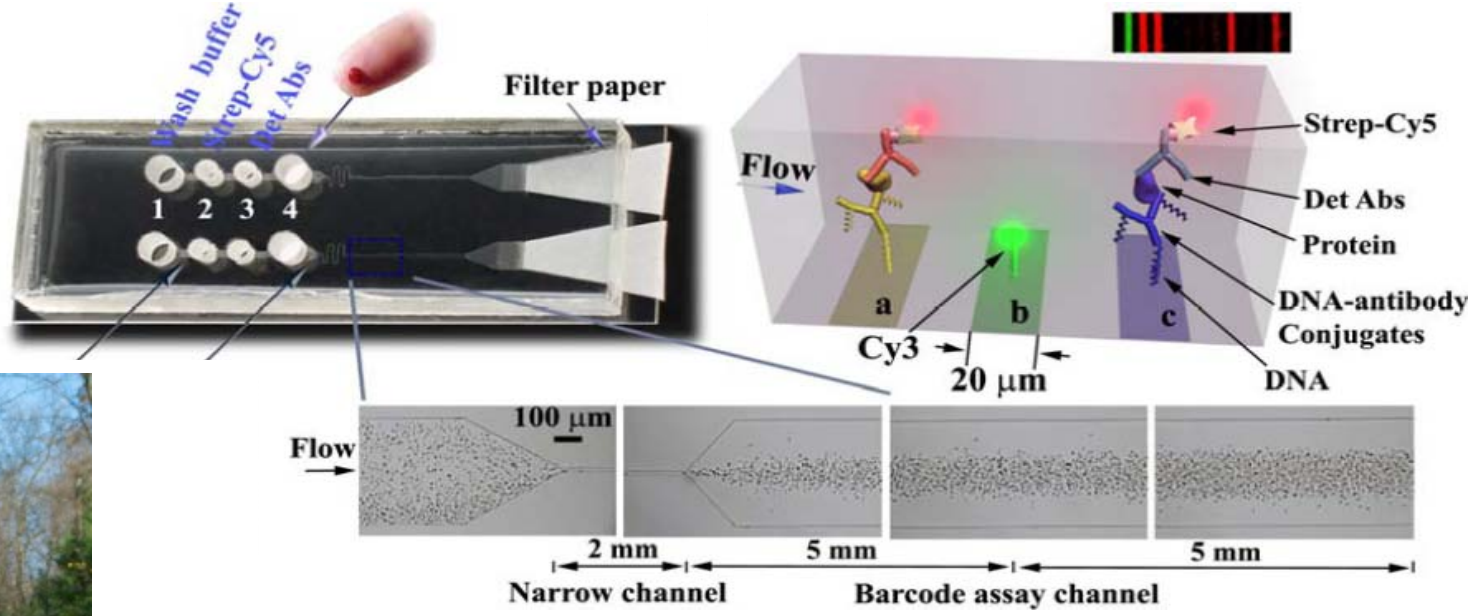


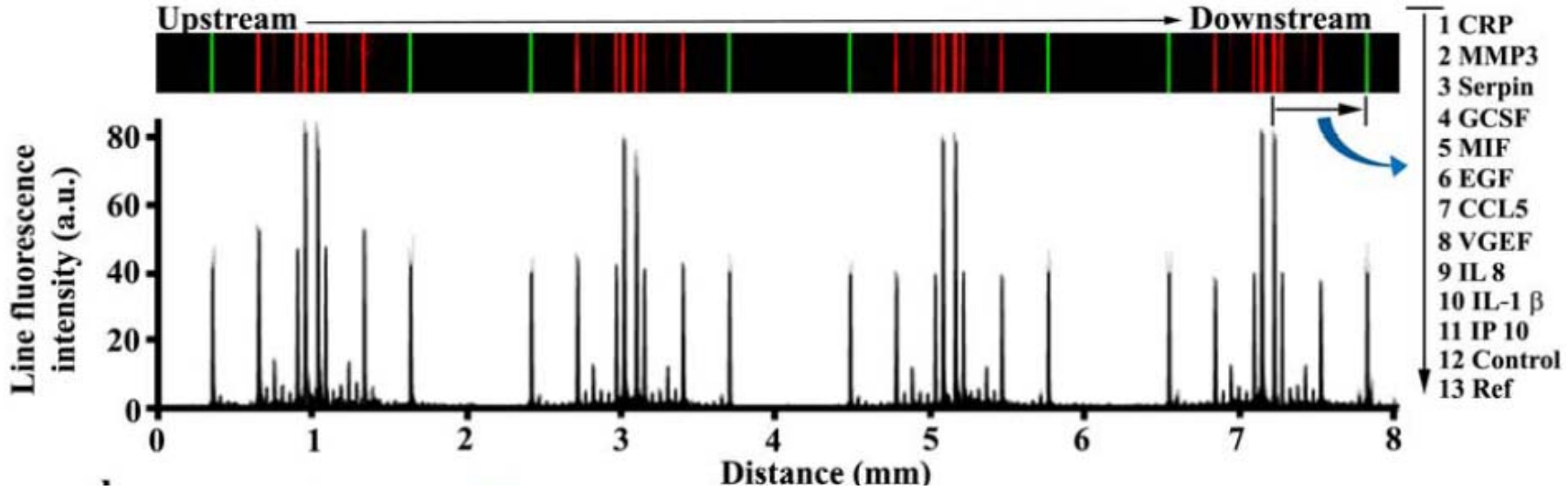
Challenge:
Identify
Responders from
Non-responders



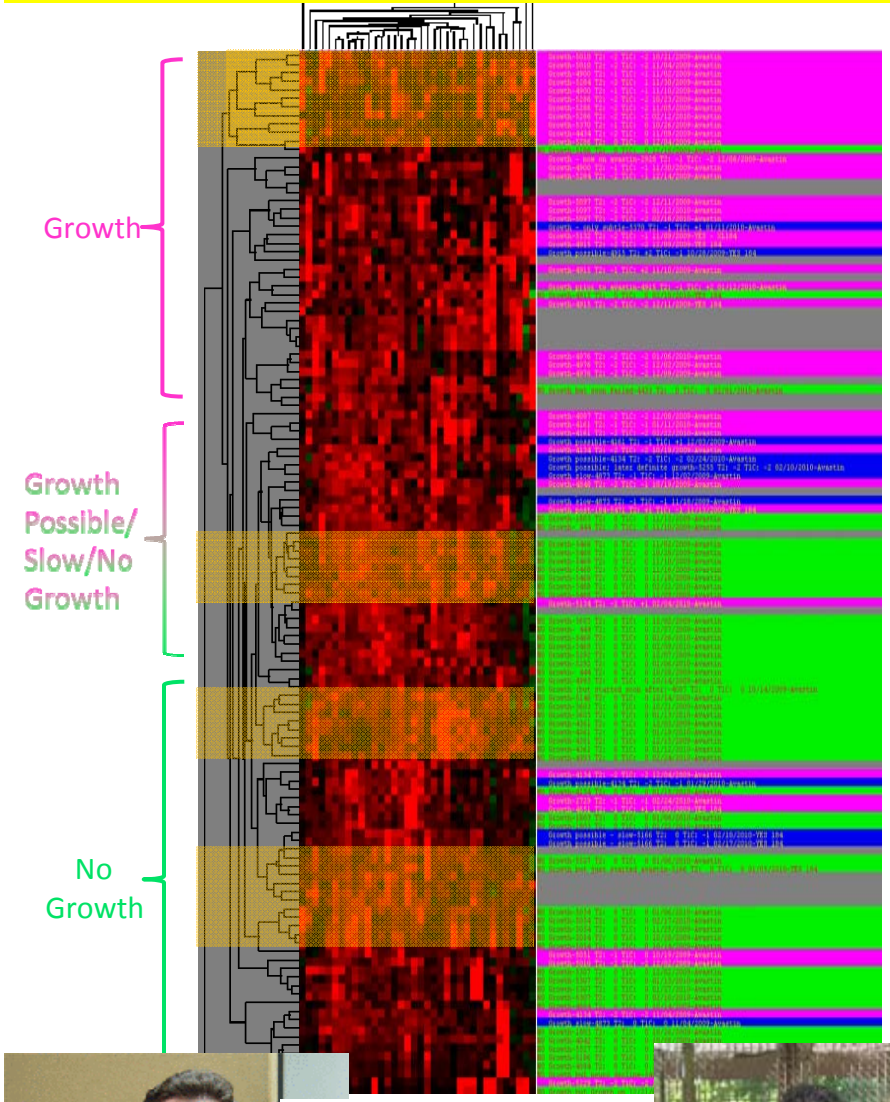
A Rapid, Quantitative, Multiplexed, Lateral Flow Immuoassay Chip for Whole Blood Analysis

Dr Jun Wang
bioengineering





Discriminating Growing versus nonGrowing GBM tumors



	Test Positive	Test Negative
Applied to All Patients		
Growth	37 TP	13 FN
No Growth	10 FP	36 TN

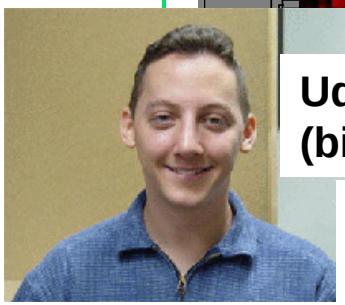
PPV = 78.7%
NPV = 73.5%

Applied Only to Patients in unambiguous Clusters		
Growth	18 TP	2 FN
No Growth	1 FP	22 TN

PPV = 94.7%
NPV = 91.7%

Applied Only to Patients in Perfectly Homogeneous "Zones"		
Growth	9 TP	1 FN
No Growth	0 FP	7 TN

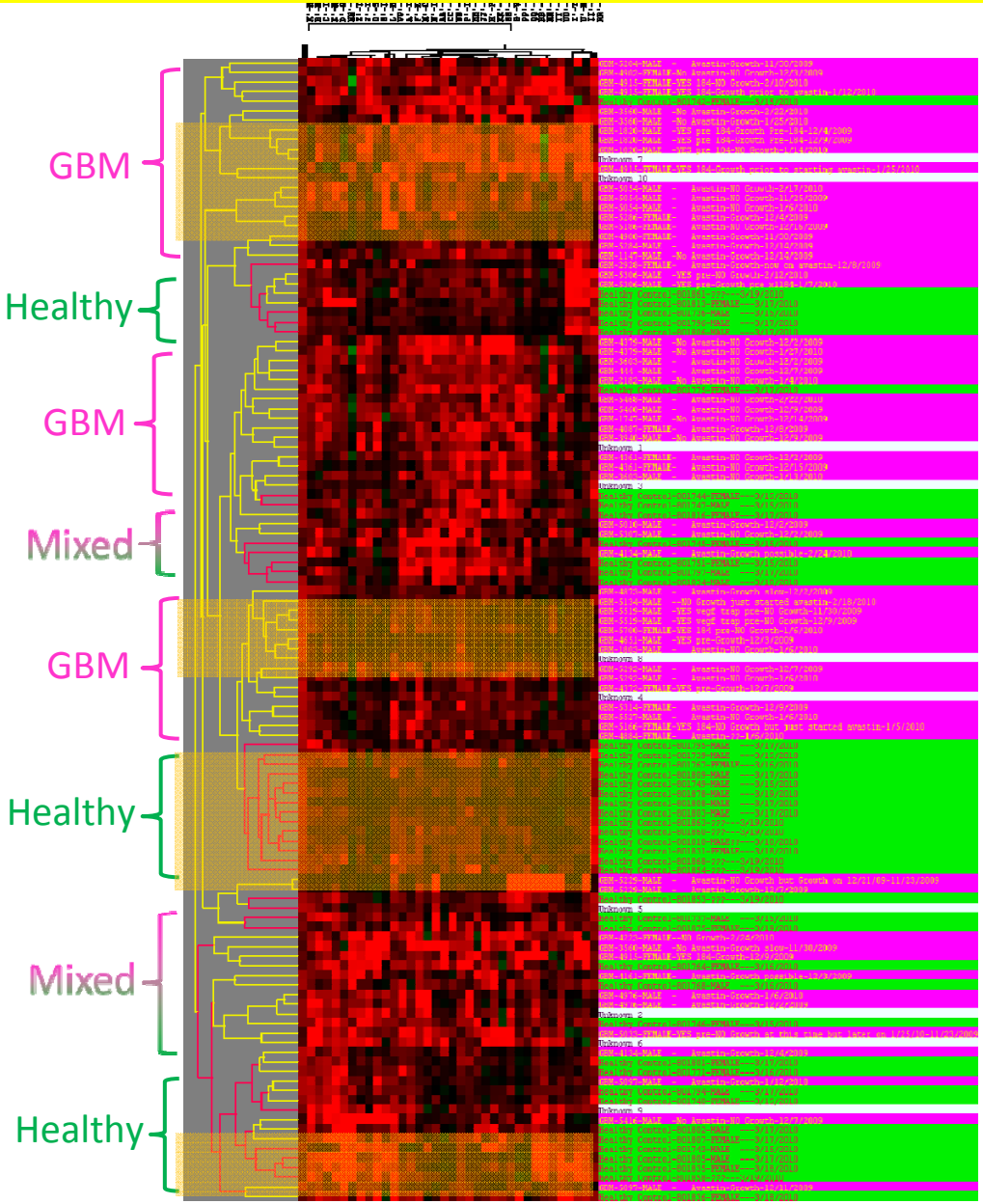
PPV = 100%
NPV = 87.5%



Udi Vermesh (bioengineering)

Ophir Vermesh MD/Ph.D.

Discriminating GBM vs Healthy



Applied to All Patients

	Test Positive	Test Negative
GBM	47 TP	9 FN
Healthy	3 FP	25 TN

PPV = 94%
NPV = 73.5%

Applied Only to Patients in Unambiguous Clusters

GBM	37 TP	4 FN
Healthy	1 FP	17 TN

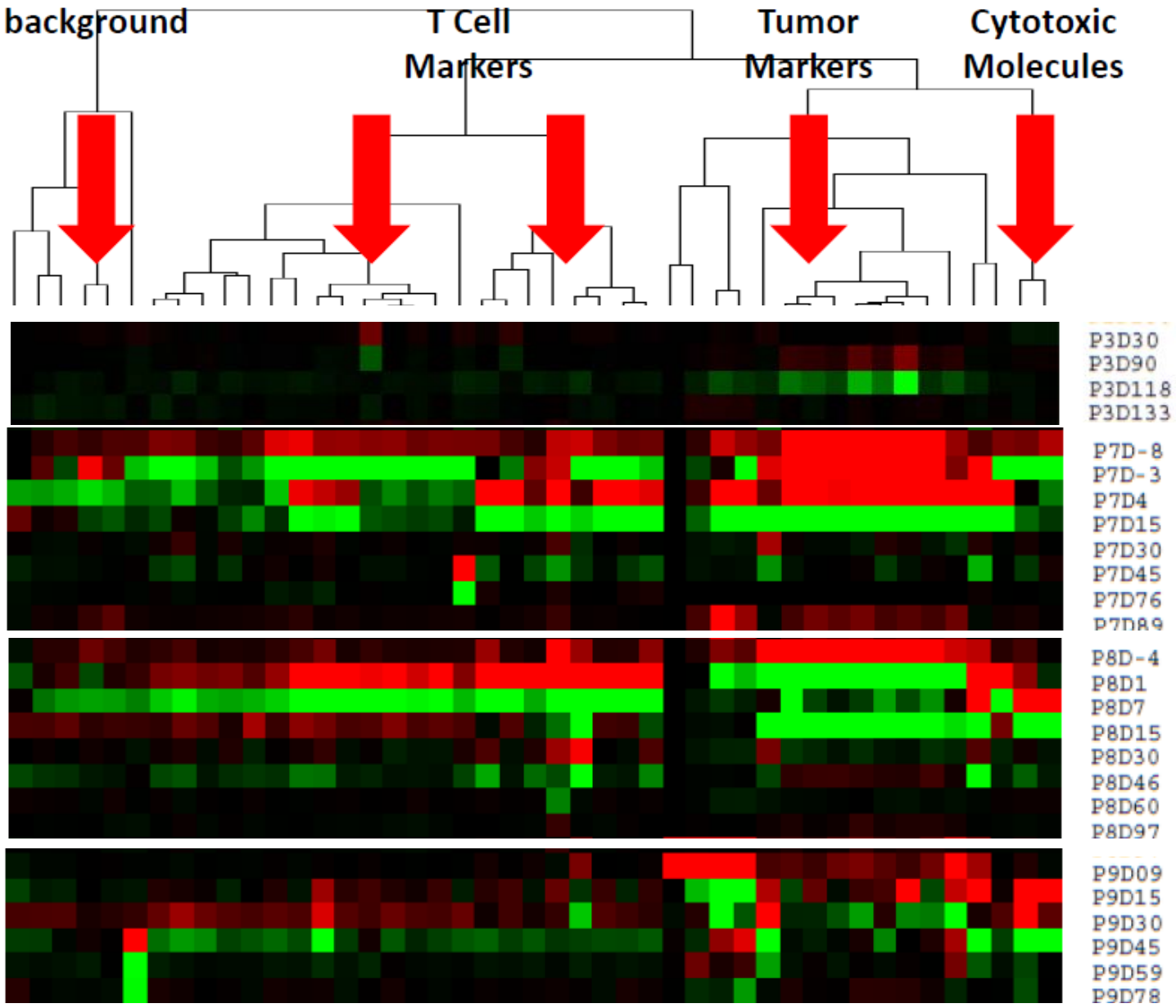
PPV = 97.4%
NPV = 81%

Applied Only to Patients in Perfectly Homogeneous "Zones"

GBM	19 TP	0 FN
Healthy	0 FP	8 TN

PPV = 100%
NPV = 100%

Velocity Profiles: Measurement period matches therapy kinetics
example: Melanoma patient on adoptive T-cell Immunotherapy Trial



Dr. Ann Cheung
(immunology)

Chao Ma
physics

Reduce the Heterogeneity of the Microenvironment to the level of a single cell

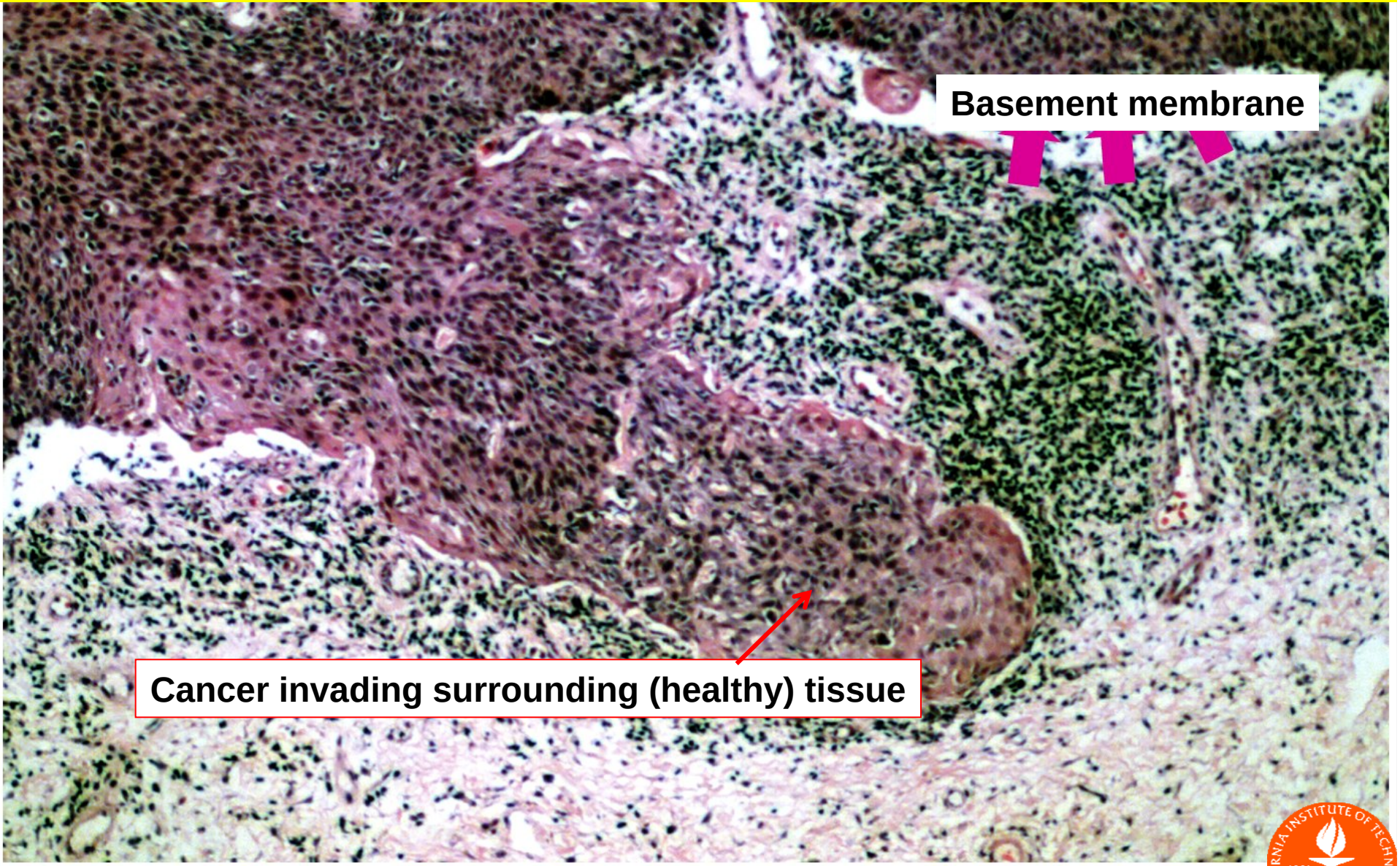


Figure 14-5c The Biology of Cancer (© Garland Science 2007)

Take a system at equilibrium

$$aA + bB \rightleftharpoons cC + dD$$

$$-\Delta G^0 = RT \ln K_{eq}$$

$$K = \frac{[C]^c [D]^d}{[A]^a [B]^b}$$

If we can count the numbers of A, B, C, D molecules, at Temp=T, we can extract the the Gibbs free energy

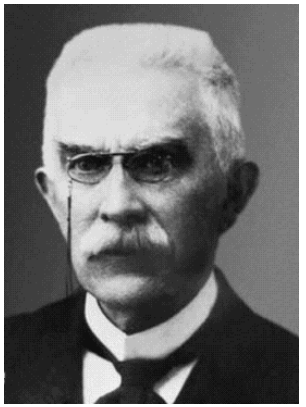
This is called capturing the fluctuations of the system

Le Chatelier's Principle (or the fluctuation/dissipation theorem)

When a system at equilibrium is perturbed, the system will adjust to relieve the stress.

- concentration changes
- temperature changes
- pressure changes
- addition of a catalyst

If we know the free energy, then we can predict how the system will response to changes



Information Theory to Extract the Signaling Networks

w/ Raphy Levine & Francoise Remacle

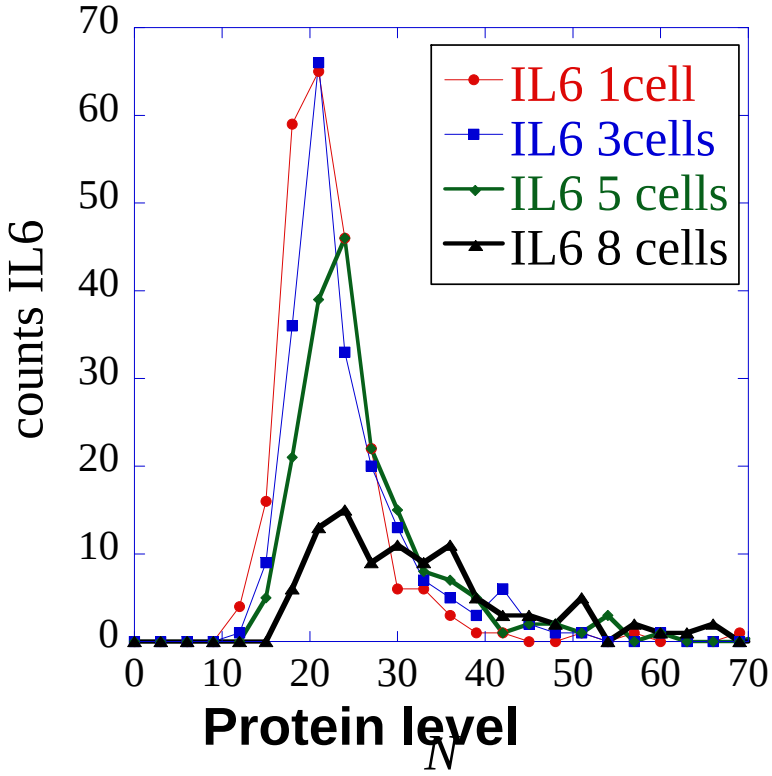
The system is a single cell

The fluctuations of this system are captured by measuring many cytoplasmic and secreted proteins from each of many single cells

Cell is perturbed

perturbation can be

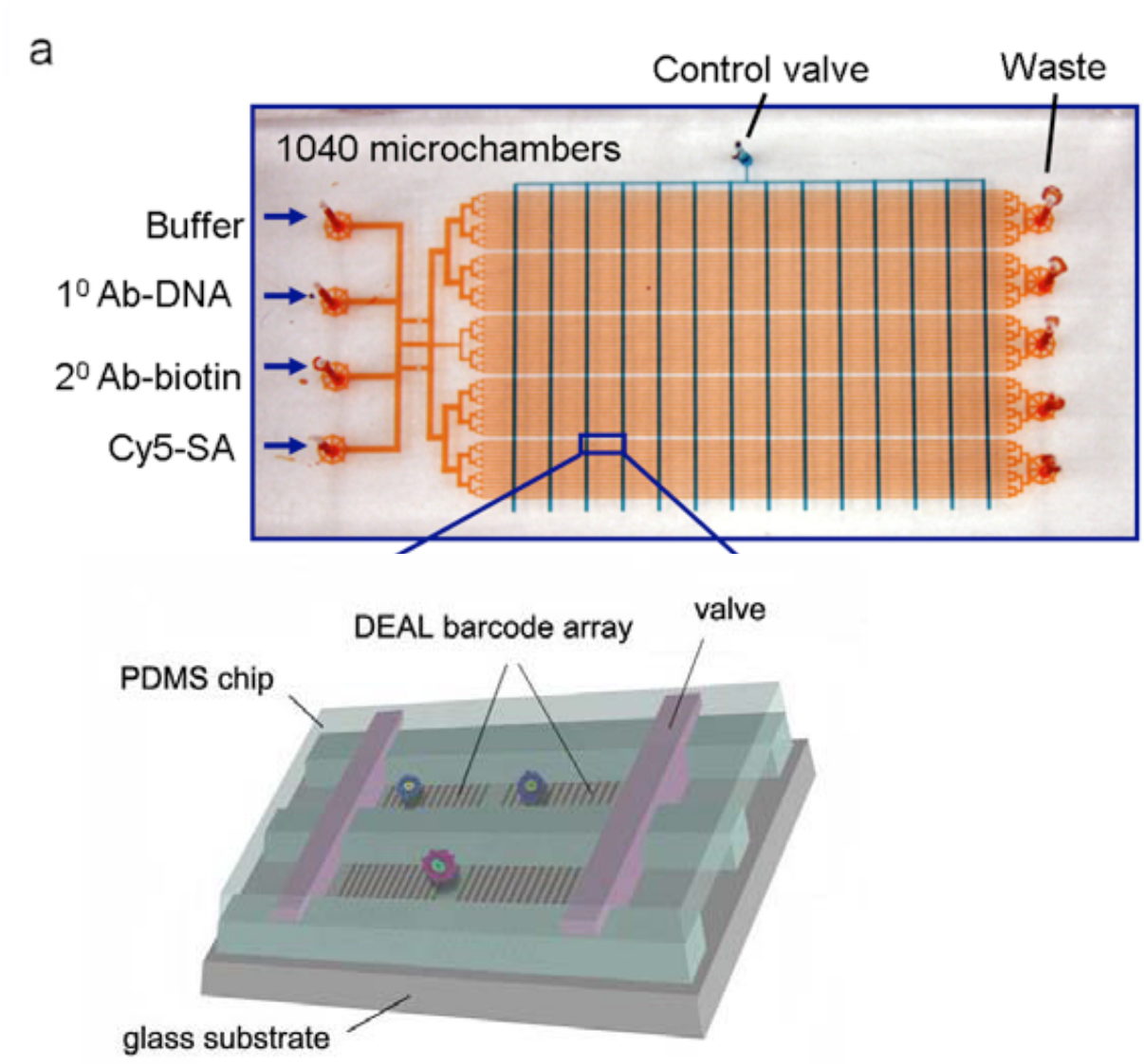
- ***another cell;***
- ***cell of a different type;***
- ***pH or O_2(Warburg effect);***
- ***temperature;***
- ***drug;***
- ***antibody, etc.***



Le Chatelier's principle is invoked to understand how the system responds to the perturbation

Capturing Single Cell Fluctuations: Secreted Proteins

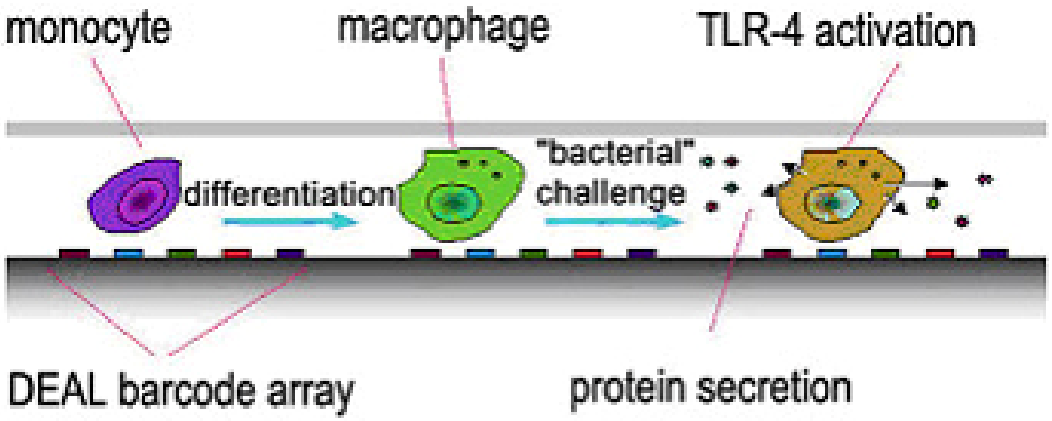
1060 parallel experiments; 12 proteins per experiment



Single cell Secretome Measurements

Validate approach via secreted protein measurements from macrophages

perturbations: increasing # of cells in colony & molecular perturbations

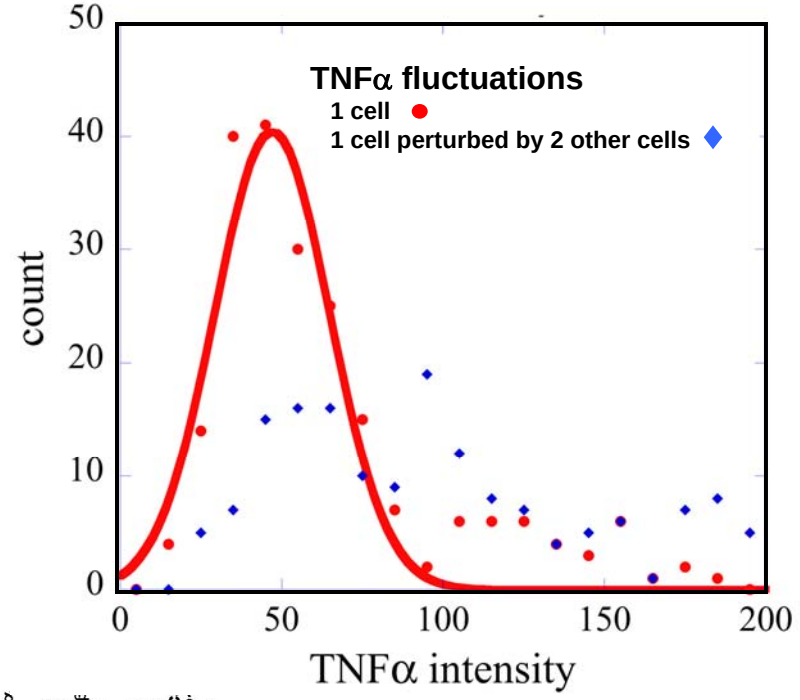


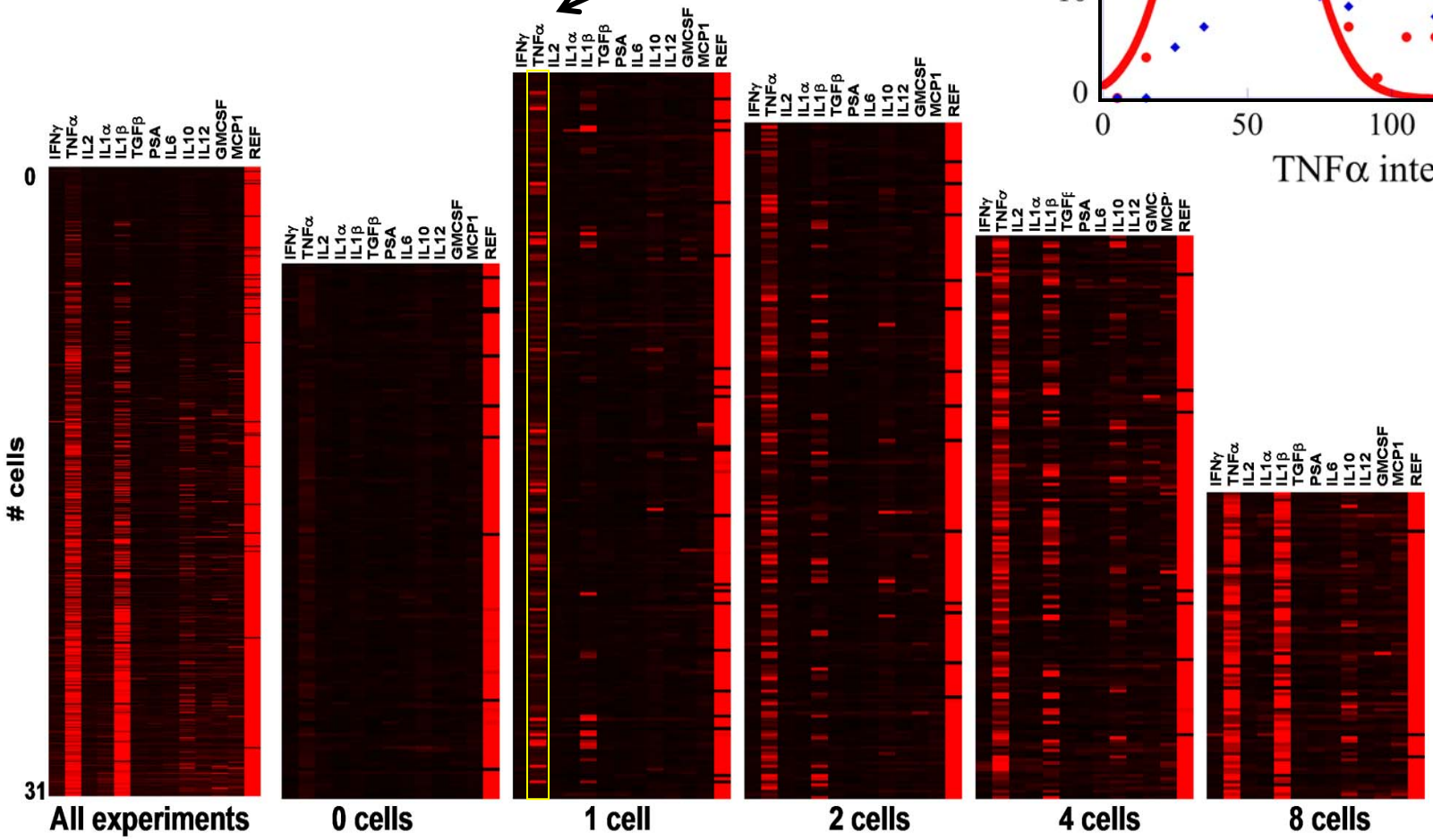
Dr. Rong Fan (materials science) now Prof at Yale

Habib Ahmad chemistry

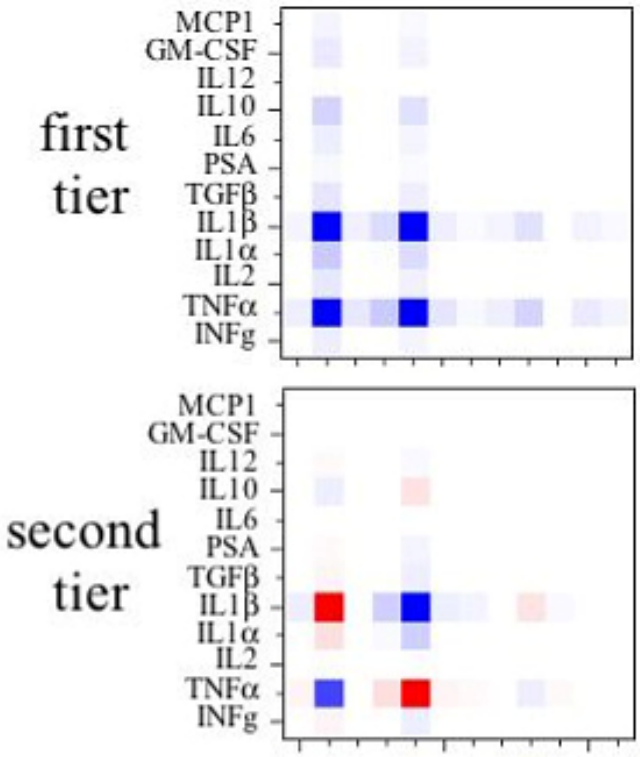
This system emulates the response of macrophages to gram negative bacteria

Raw Data sorted by #cells per experiment



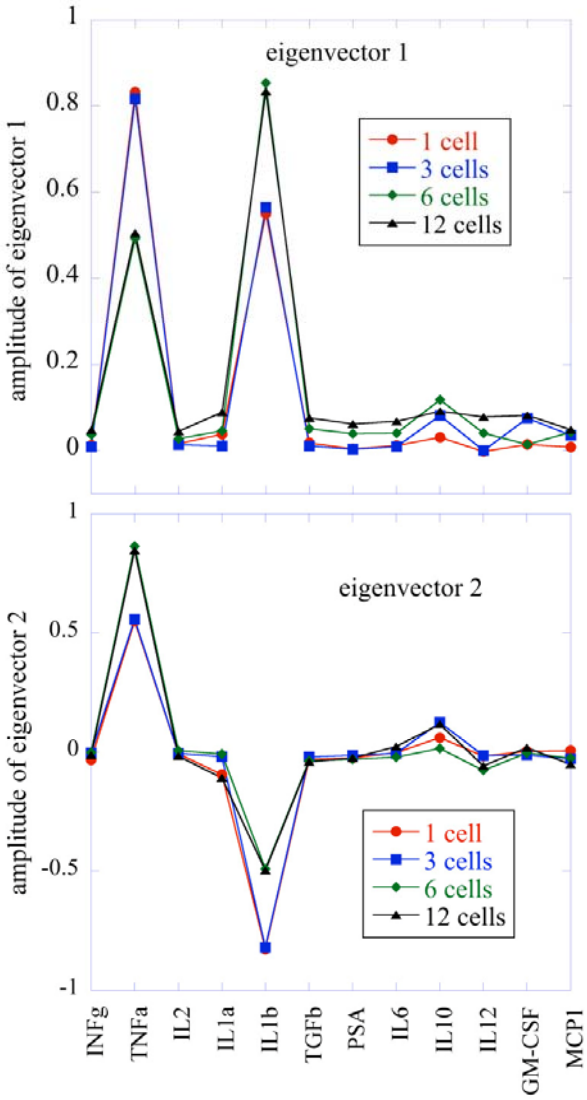


Protein-protein interactions Extracted from Single-Cell Fluctuations

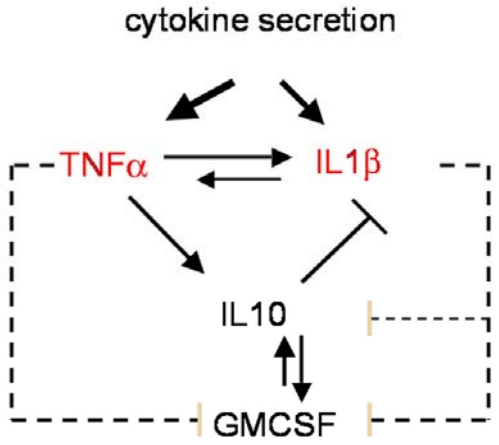


inhibitory interactions emerge at 2nd tier

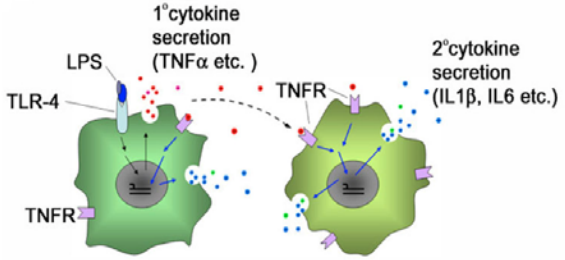
a



b

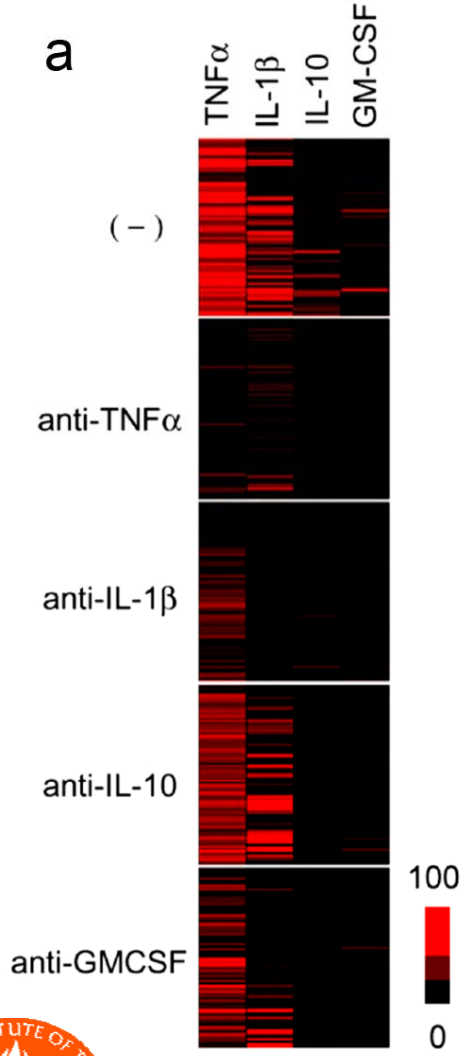


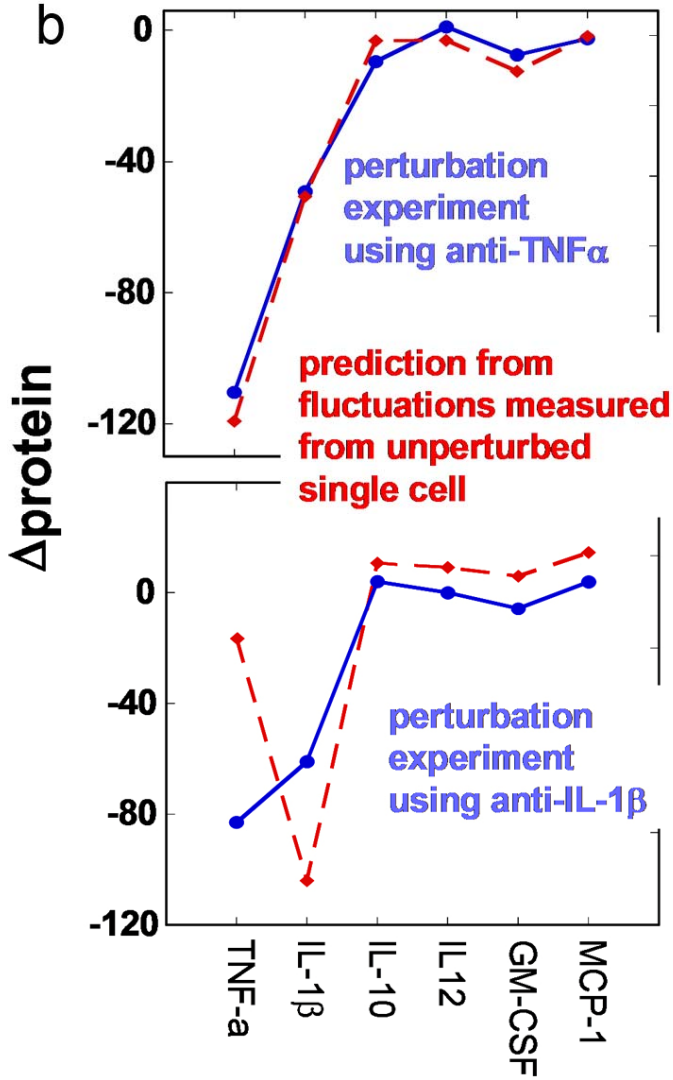
c

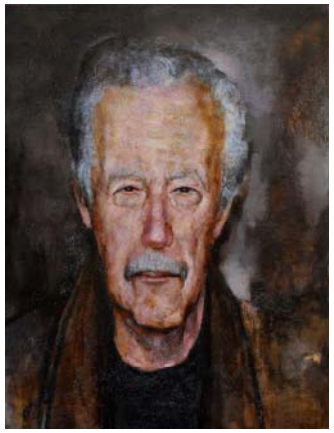


w/ Francoise Remacle & Raphael Levine

Using Le Chatelier's Theorem to Predict the Response to Antibody Perturbations







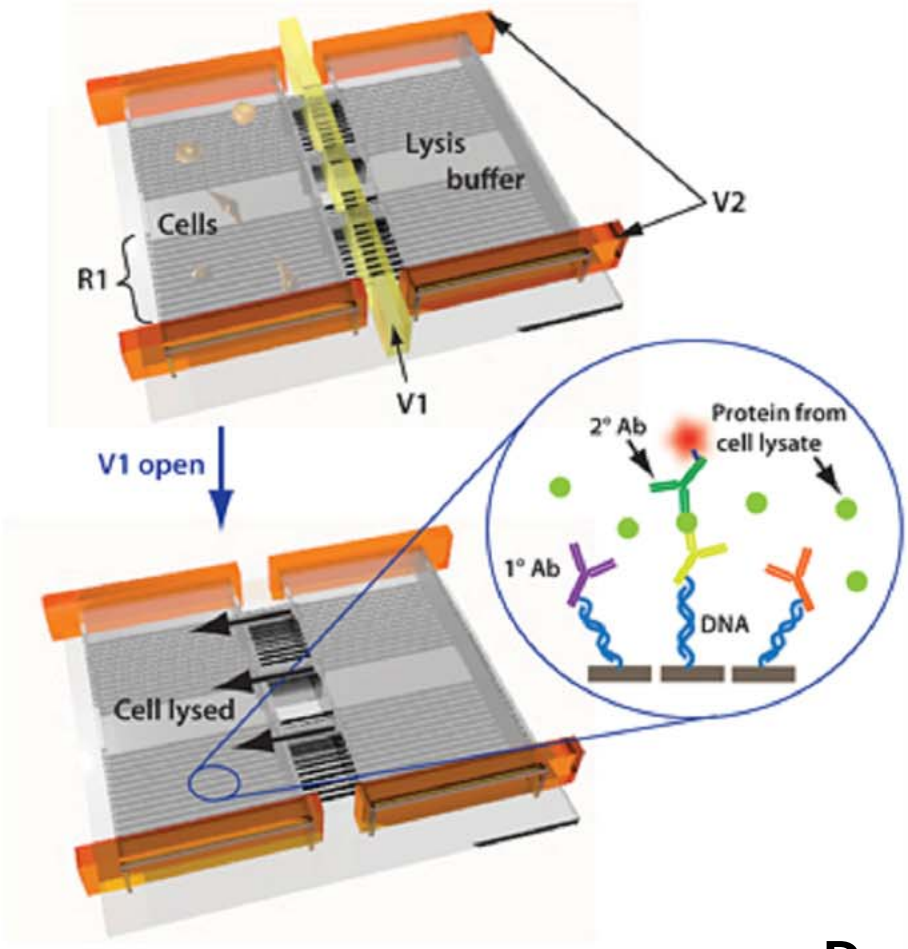
Prof Raphy Levine
(math/chemistry)
Hebrew Univ

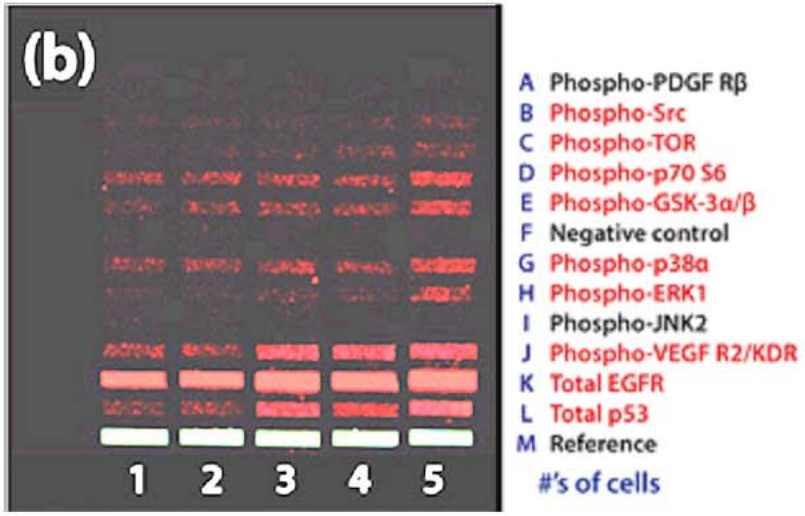
Prof Francoise Remacle
chemistry
Univ. Liege, Belgium

w/ Francoise Remacle & Raphael Levine

Applied to Cytoplasmic PI3K pathway proteins

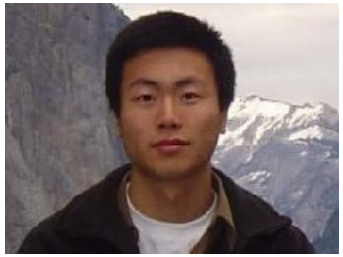
Sensitivity is Sufficient to Detect PI3K pathway proteins from single cells





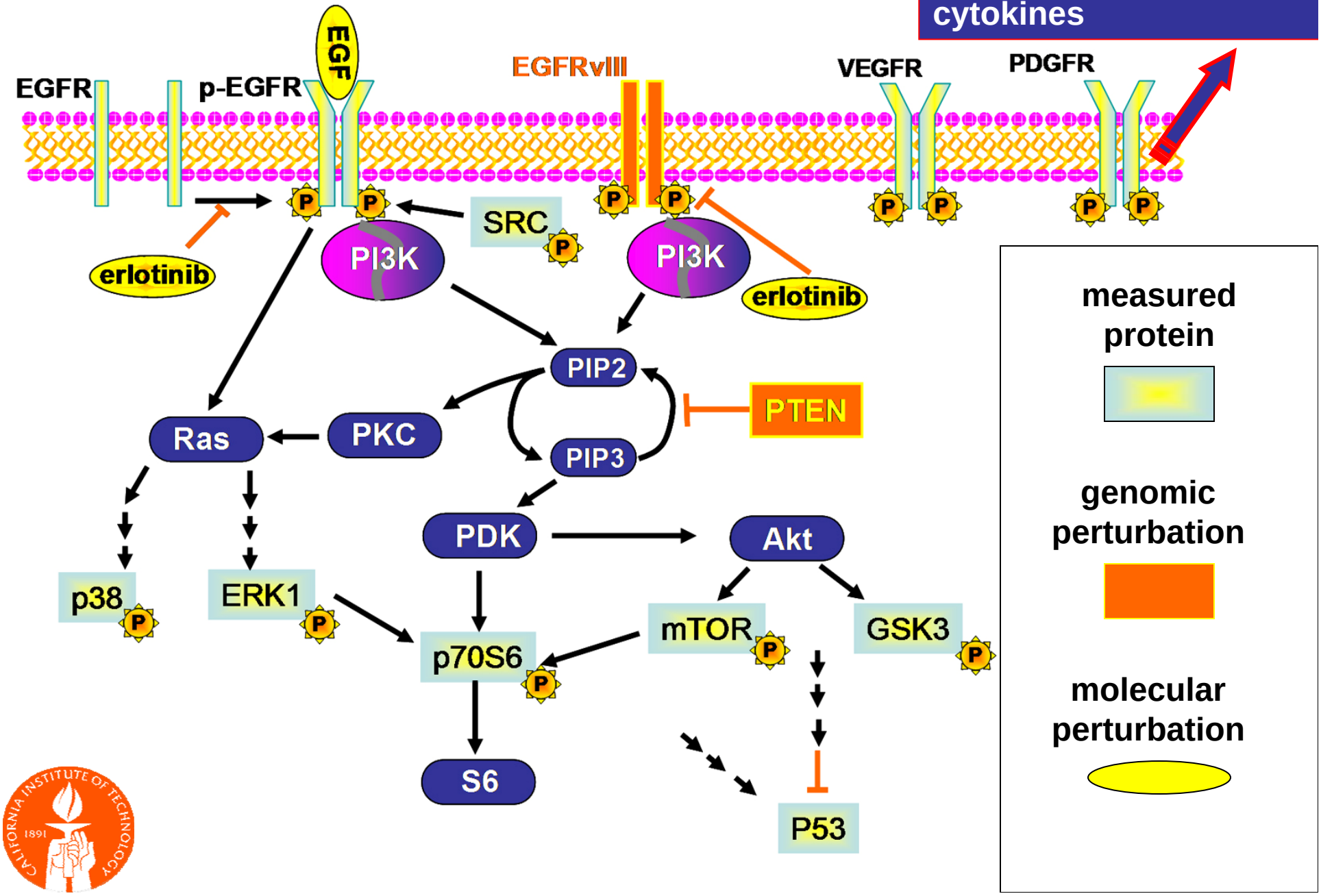
contrast enhanced to show all proteins detected

Dr. Qihui Shi
materials science

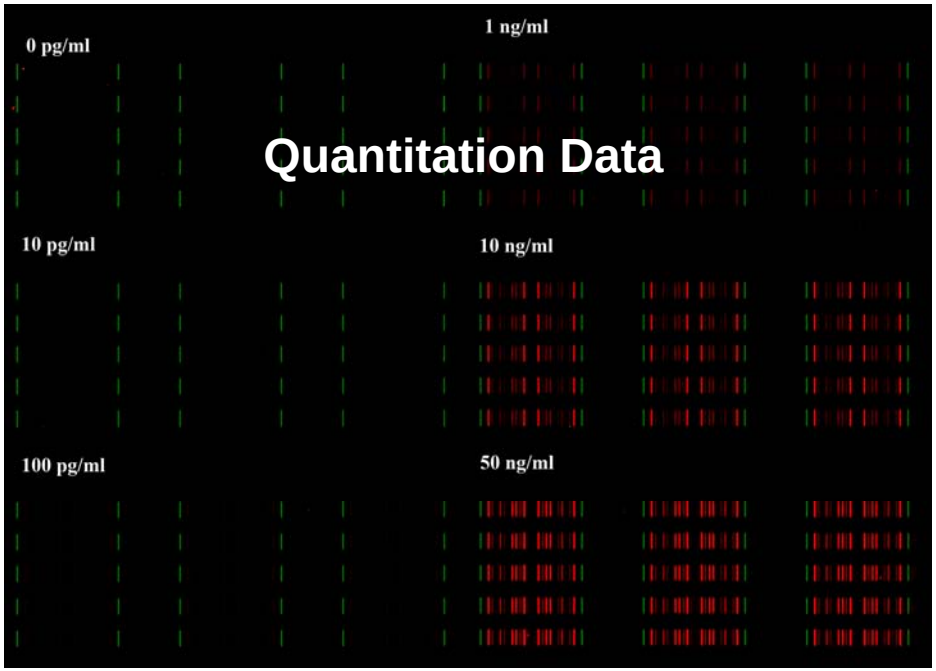


Our experiment (3 cell lines; U87; U87 EGFRvIII; U87EGFRvIII + PTEN

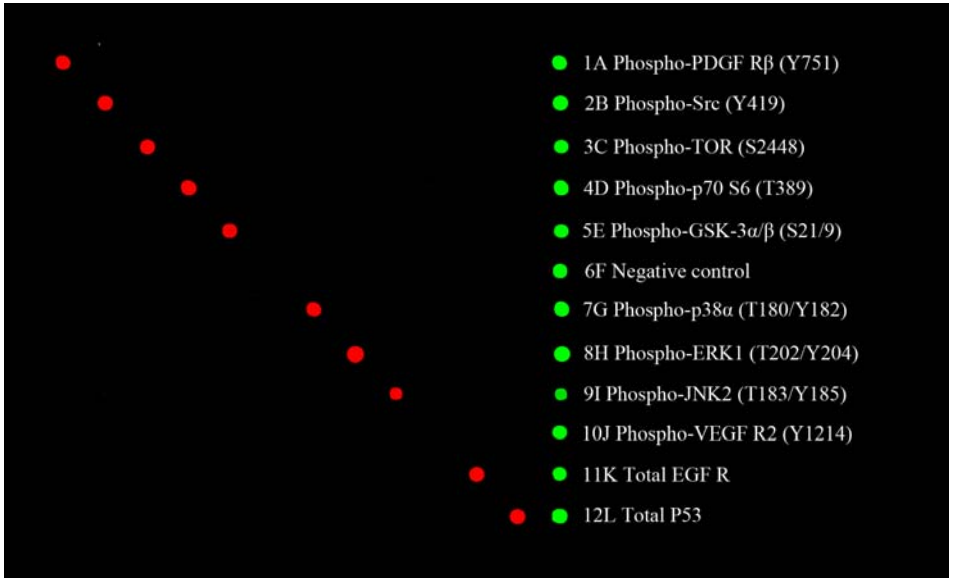
secreted growth factors
proteases
cytokines

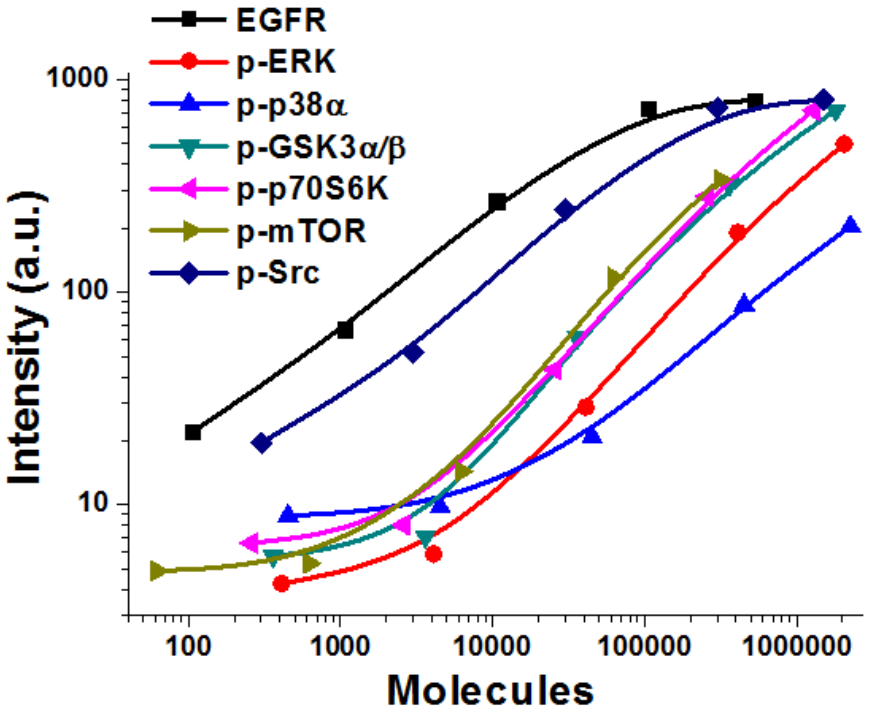


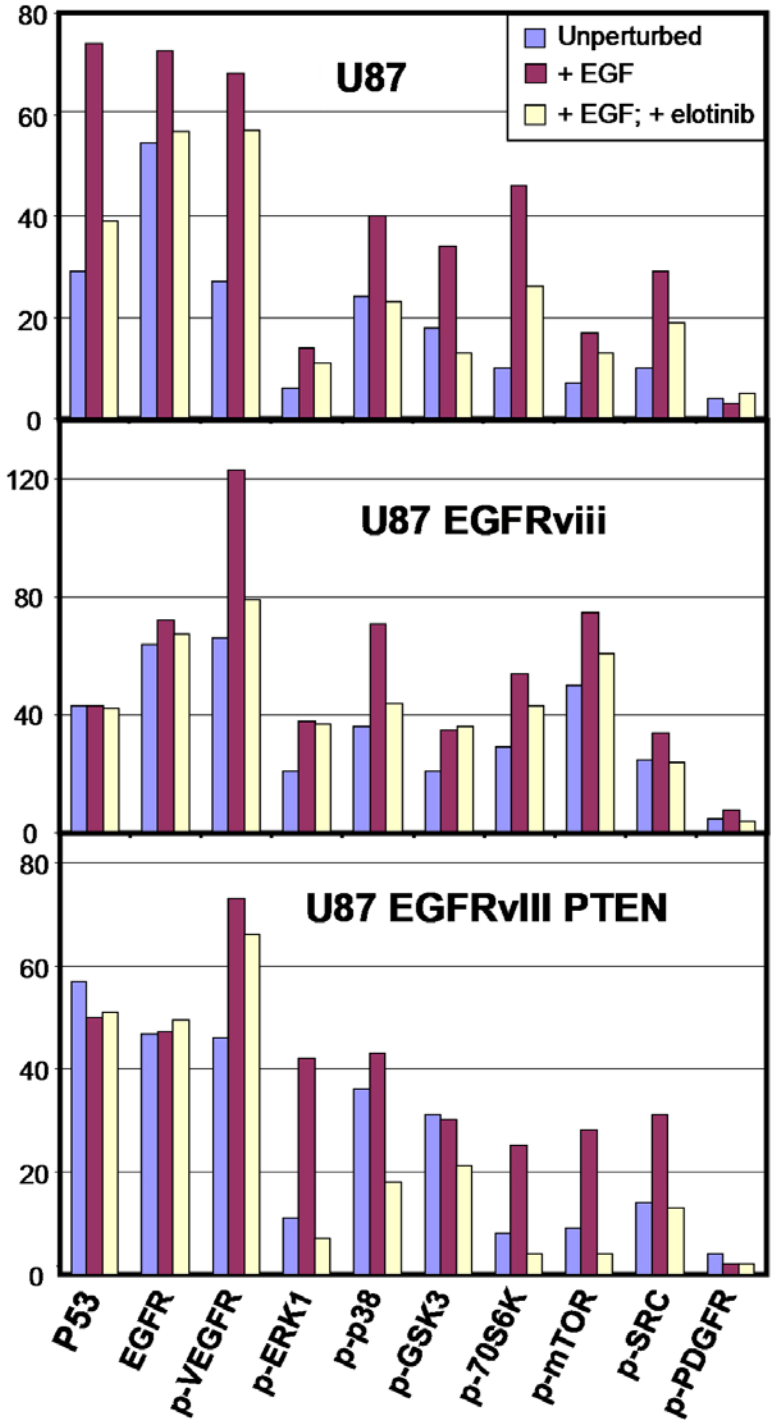
Just to be clear on why we believe our results



Cross Reactivity Data

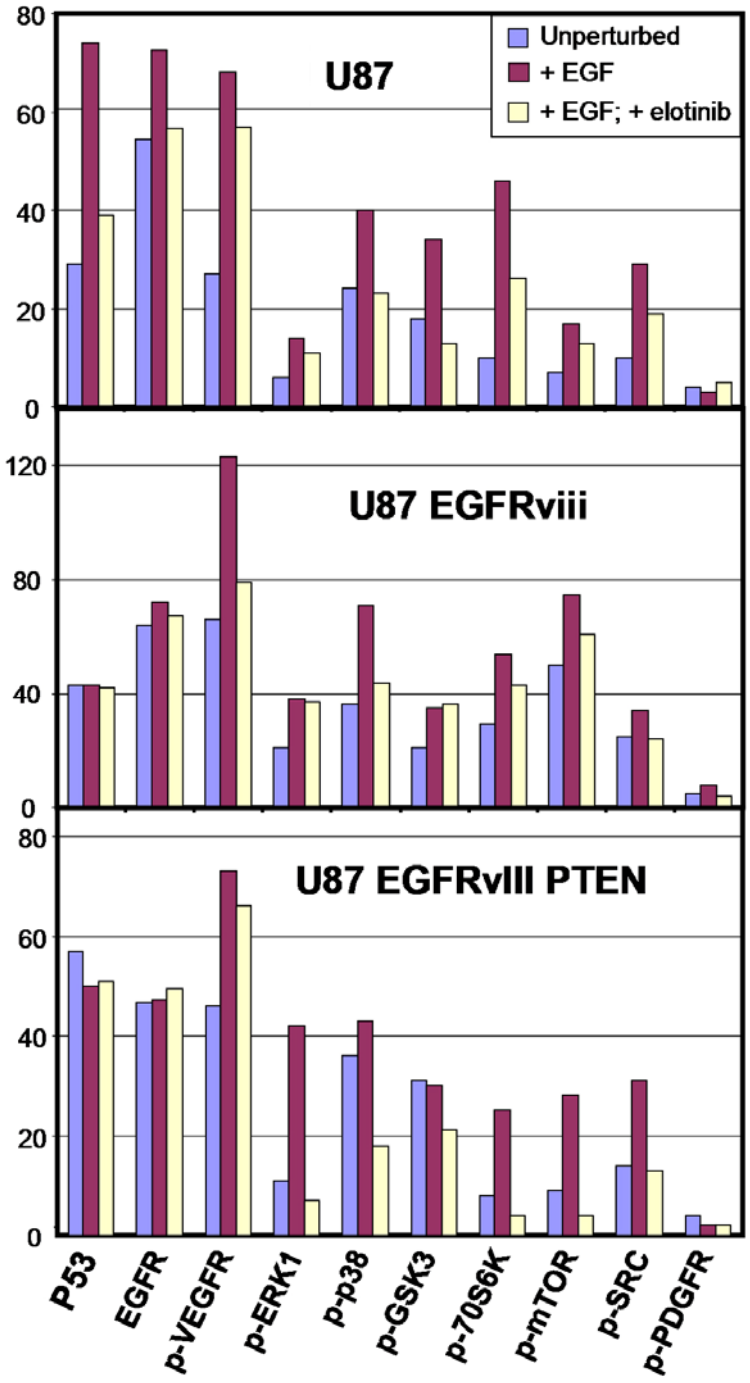




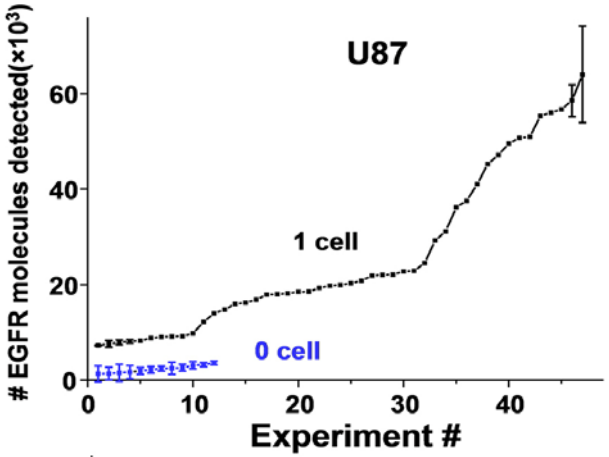


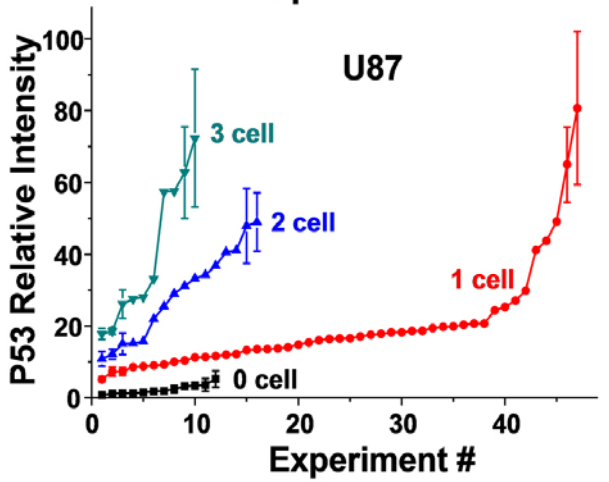
Statistical Responses Measured at the three cell level

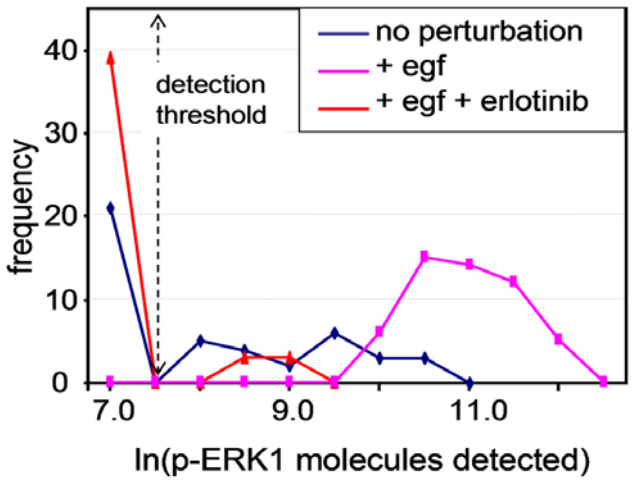
Somewhat analogous to what would be extracted from Western Blots from cell lines



Single Cell Profiles vs statistical responses

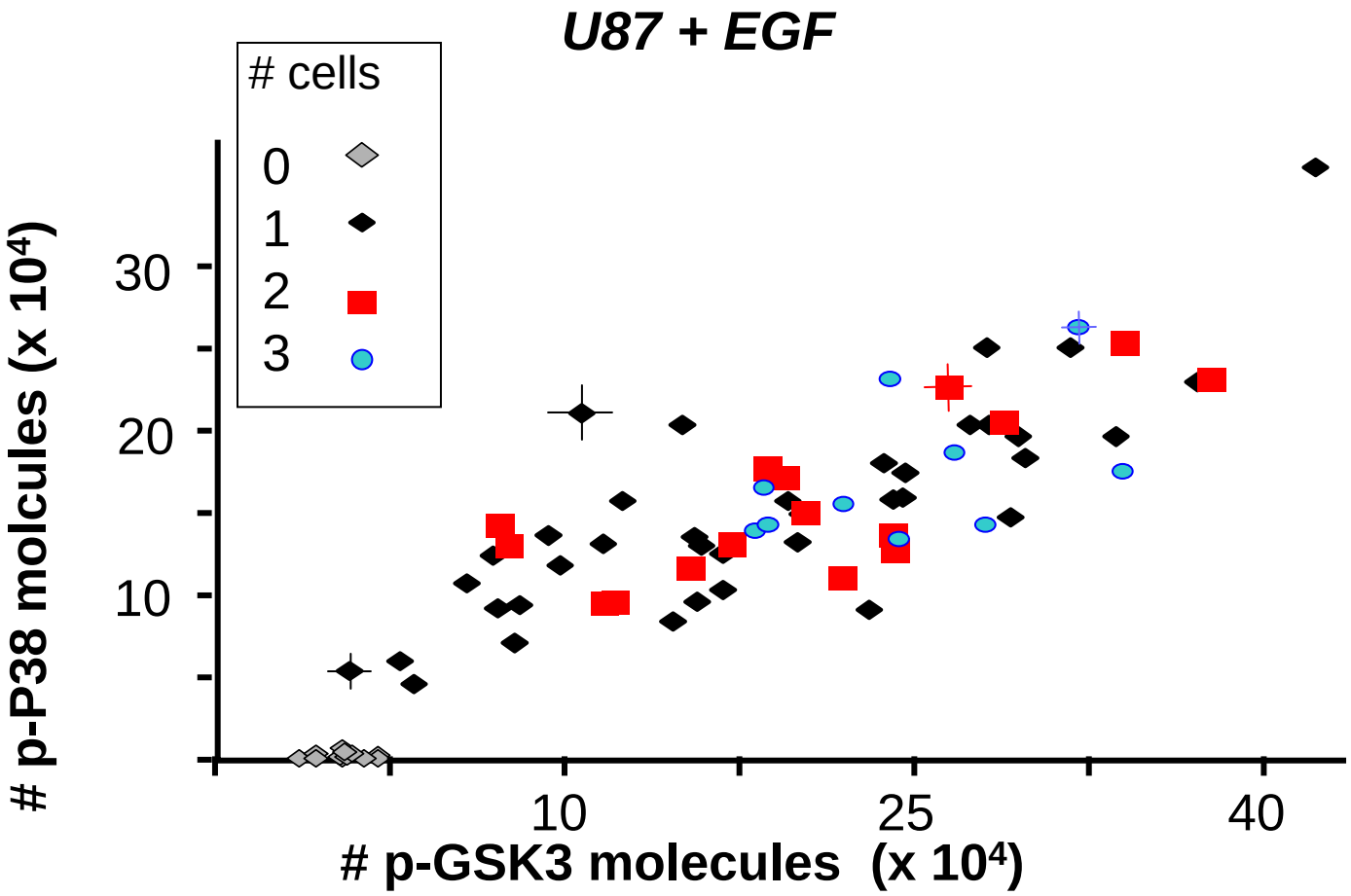




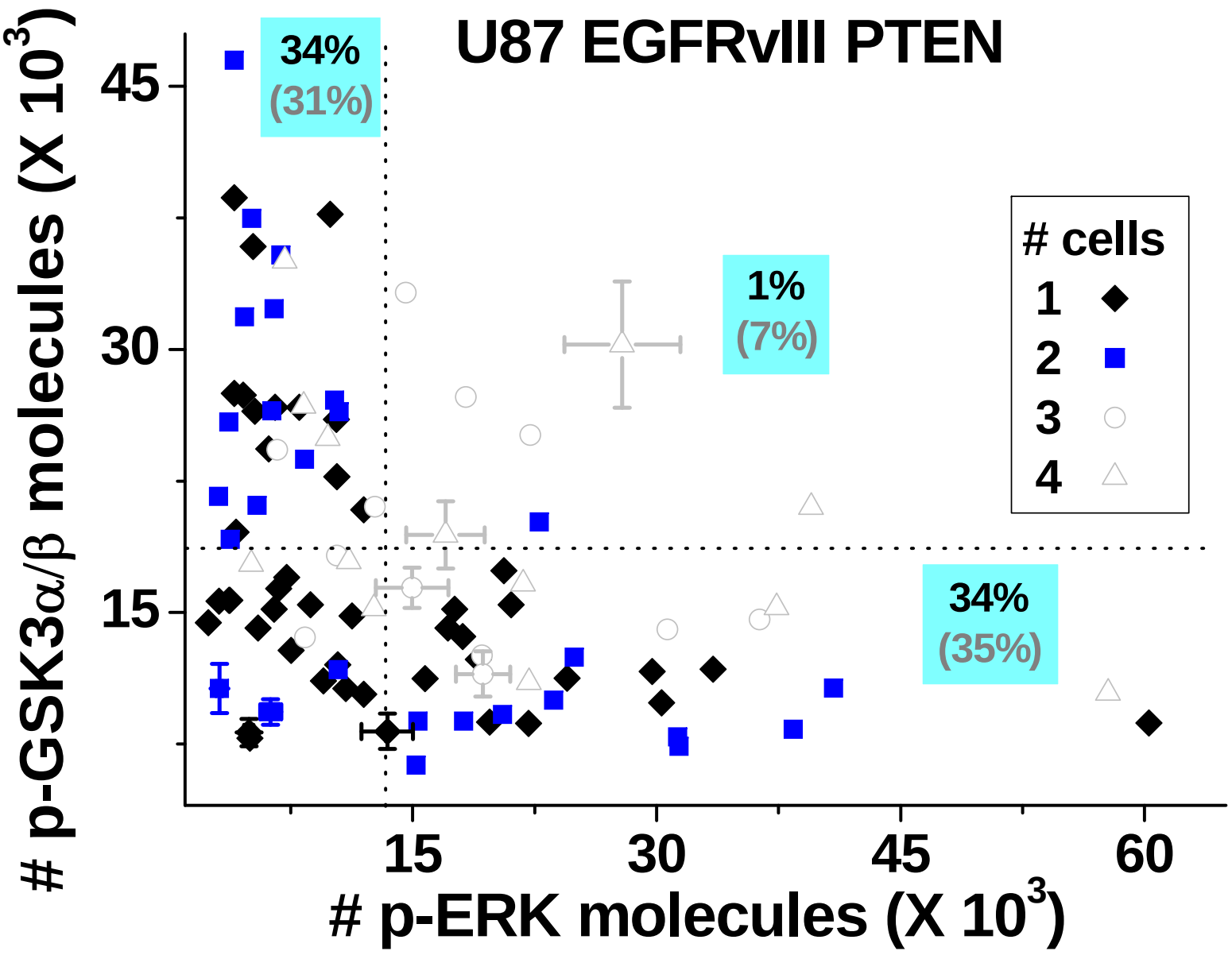


Types of Protein-Protein Interactions

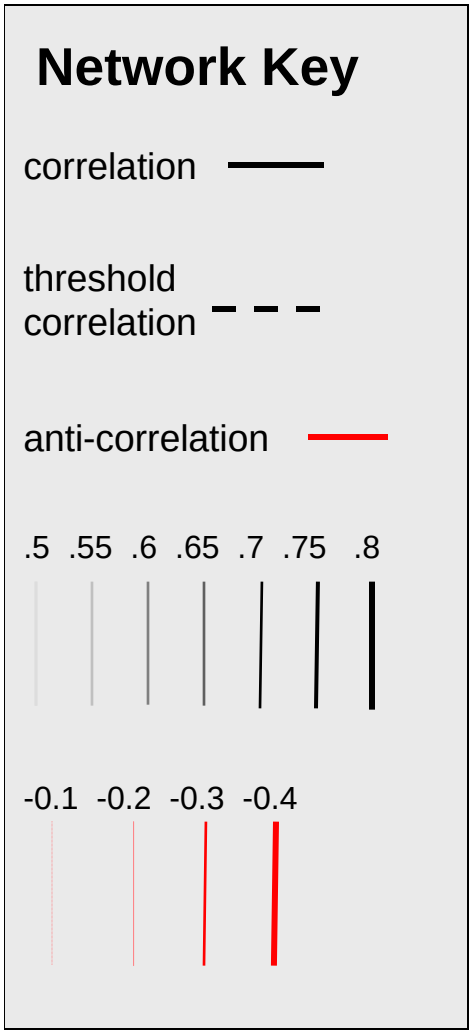
Correlated Proteins

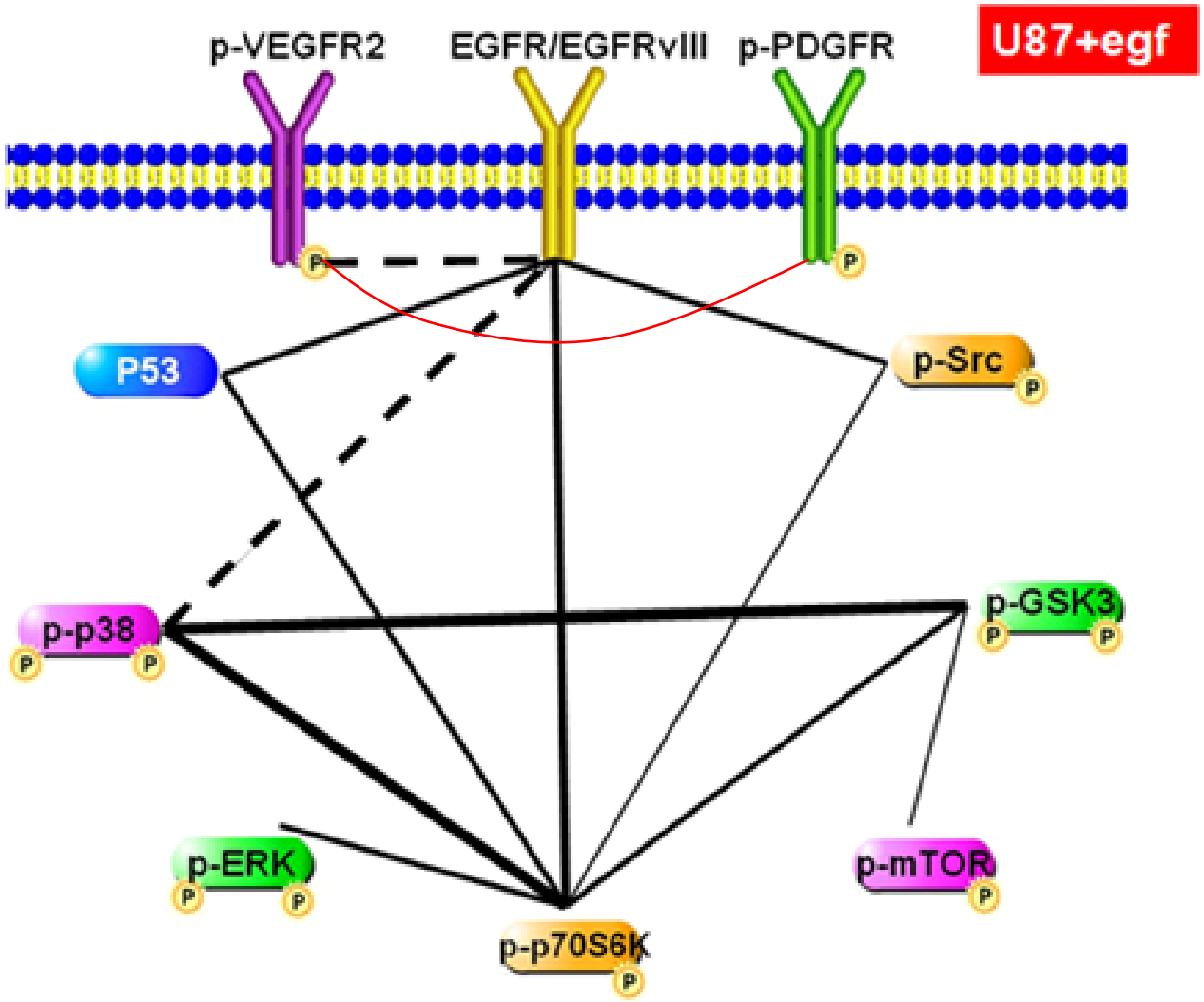


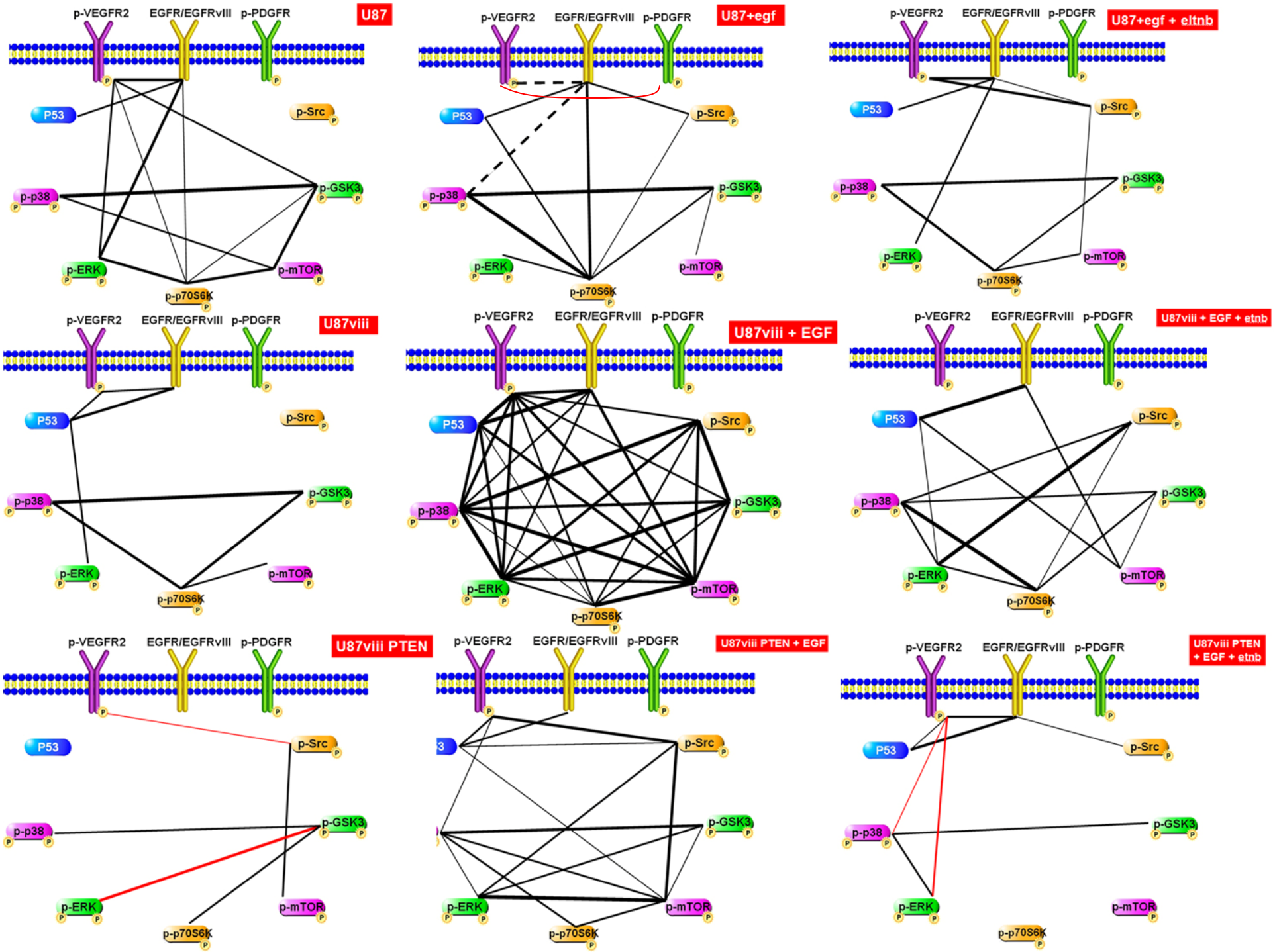
Protein-protein Interactions: Anti-Correlations



An example of a Network: egf stimulated U87 cells






U87
p-VEGFR2
EGFR/EGFRvIII
p-PDGFR
P53
p-Src
p-p38
p-GSK3
p-ERK
p-mTOR
p-p70S6K
U87+egf
U87+egf + eltnb
U87viii
U87viii + EGF
U87viii + EGF + etnb
U87viii PTEN
U87viii PTEN + EGF
U87viii PTEN
+ EGF + etnb

Some Results Identified (Expected & New)

PTEN induces a switch to ERK signaling (expected)

The mechanics of this switch are due to feedback; Pandolfi's group showed that mTOR inhibits PI3K signaling as a requlatory negative feedback. Abrogating it releases PI3K which (surprising new result) drives ERK signaling as well.

Paul Mischel

2nd expected connection: **PTEN protects p53.**

ERK and P70s6k are tightly linked (Blenis's group has shown this) - Our data suggest that PTEN prevents that link (new result).

Presence of EGFRvIII is not the same as activating EGFR!! EGFRvIII rewires virtually all pathways – explains why patients with EGFRvIII are so drug resistant.

We also detect (known) why GBM patients with EGFRvIII + PTEN respond to Erlotinib.

In vitro diagnostics Challenges

Inexpensive, quantitative protein measurements can enable a huge number of fundamental & translational opportunities

We have quite a distance to go before this is a reality

Many technology challenges, but only one central bottleneck

cheap & robust protein capture agents

solve this problem, and the problem of highly quantitating highly multiplexed protein assays goes away!

Thank you