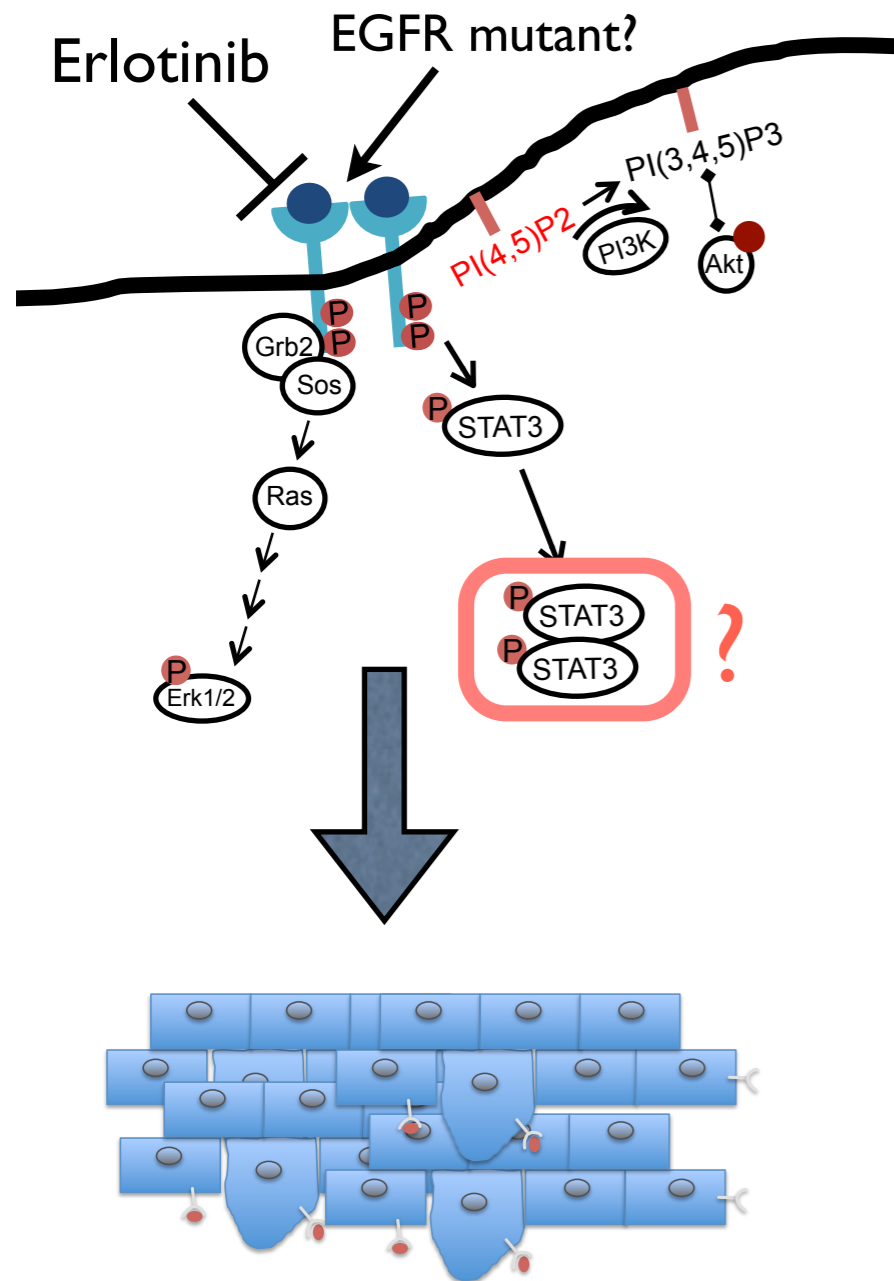


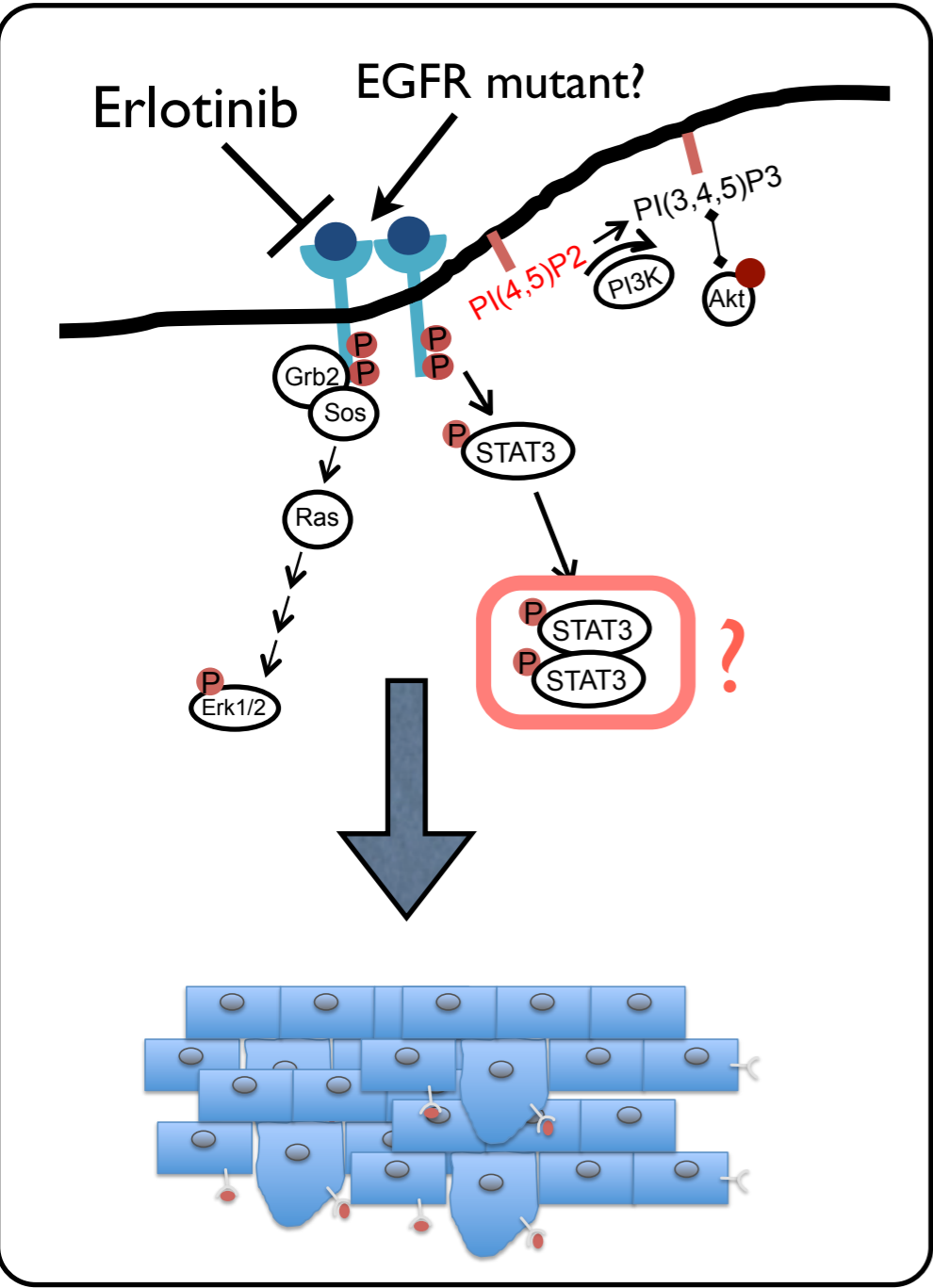
# Module 2: Systems Engineering (M2D6)

- Reminder of goals of Module 2
- Scale it up! (For real this time!)
- Densitometry -- how to analyze your WB data.
- M2D7 -- to robot or not to robot...

# Module 2 overview:



# Module 2 overview:



Mutation Analysis

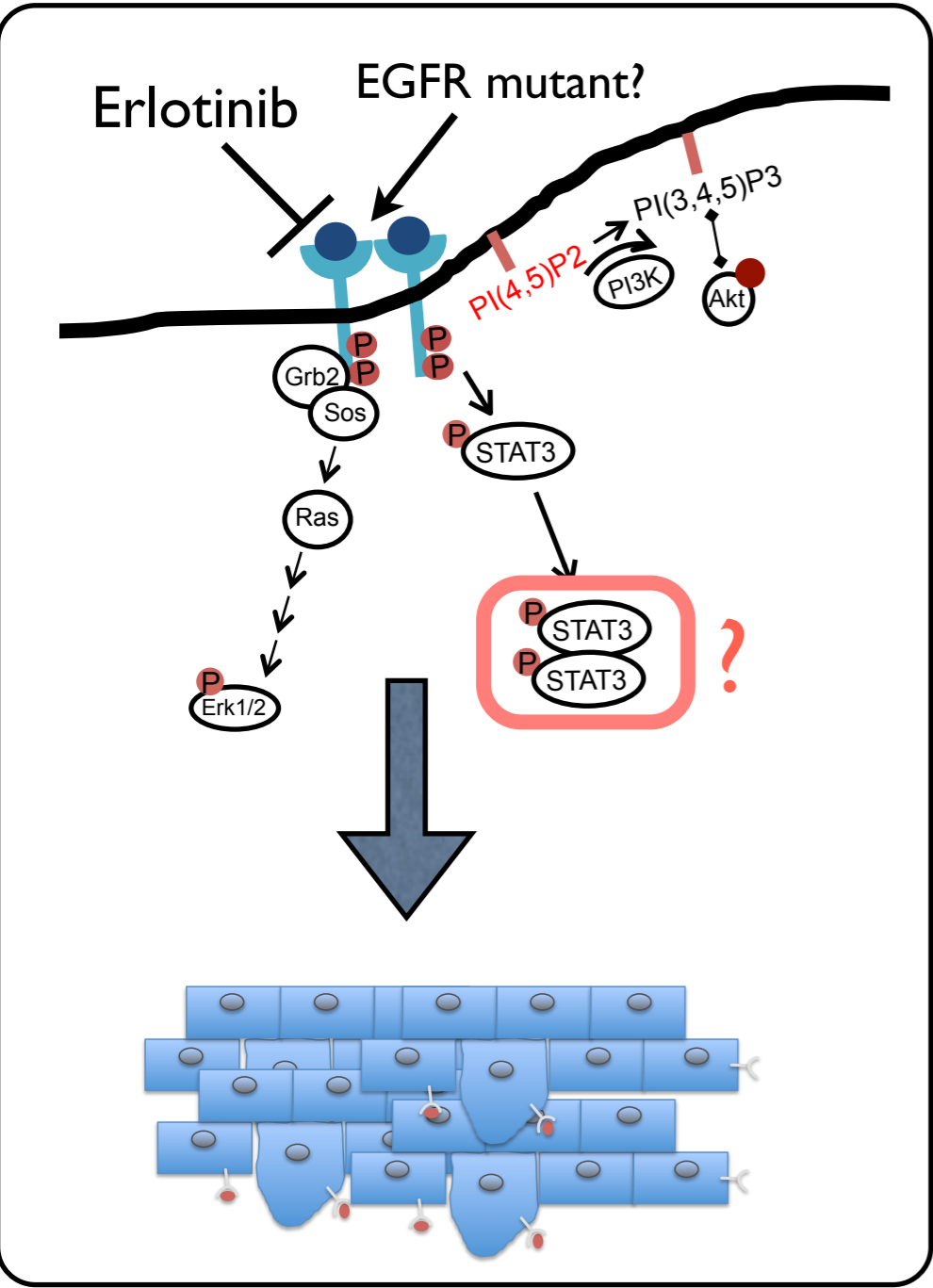


WB Analysis



Viability Analysis

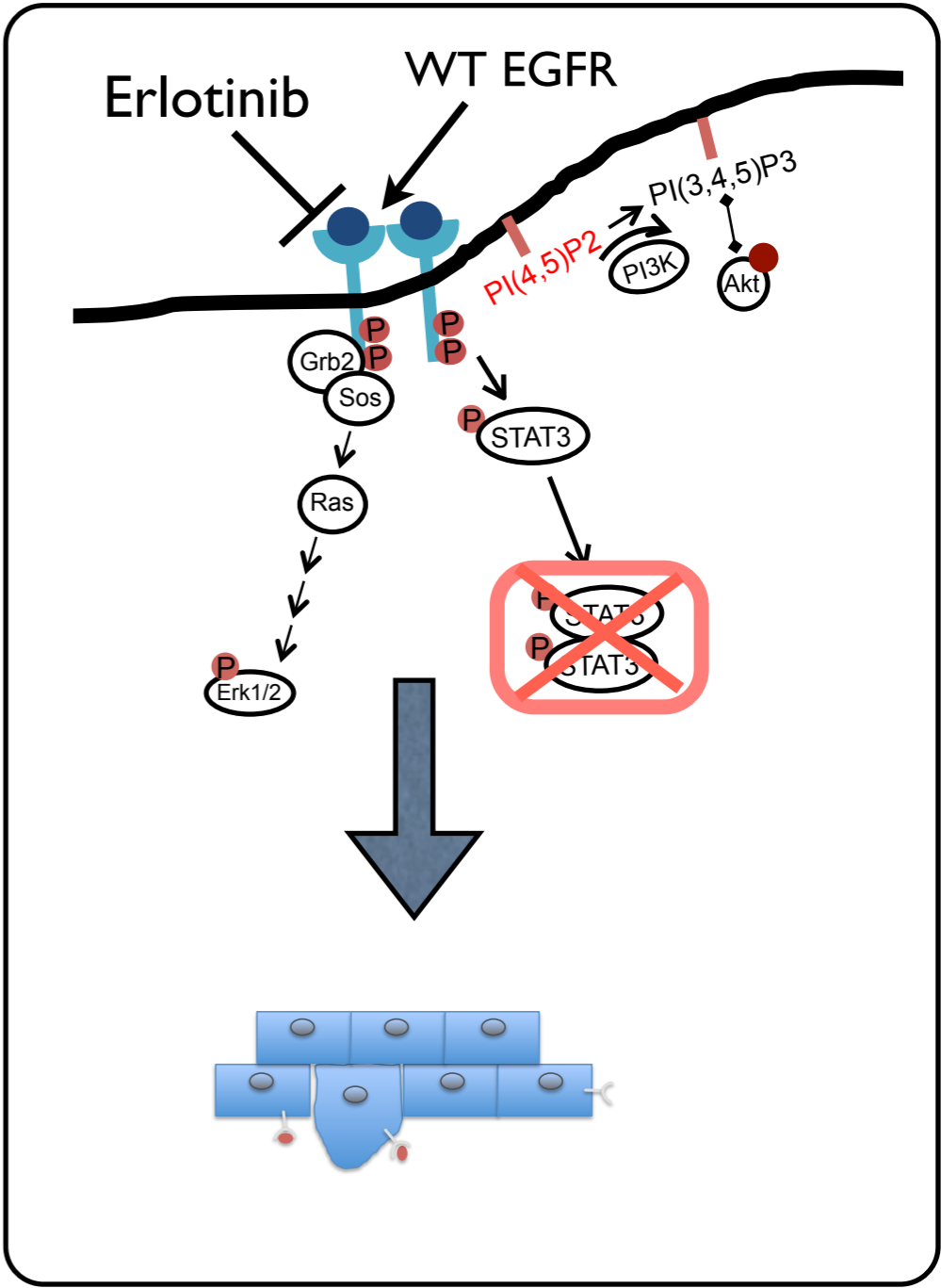
# Module 2 overview:



Mutation Analysis

WB Analysis

Viability Analysis



# Semi-quantitative analysis: Your experiment

1. All: pY1068-EGFR & Total EGFR

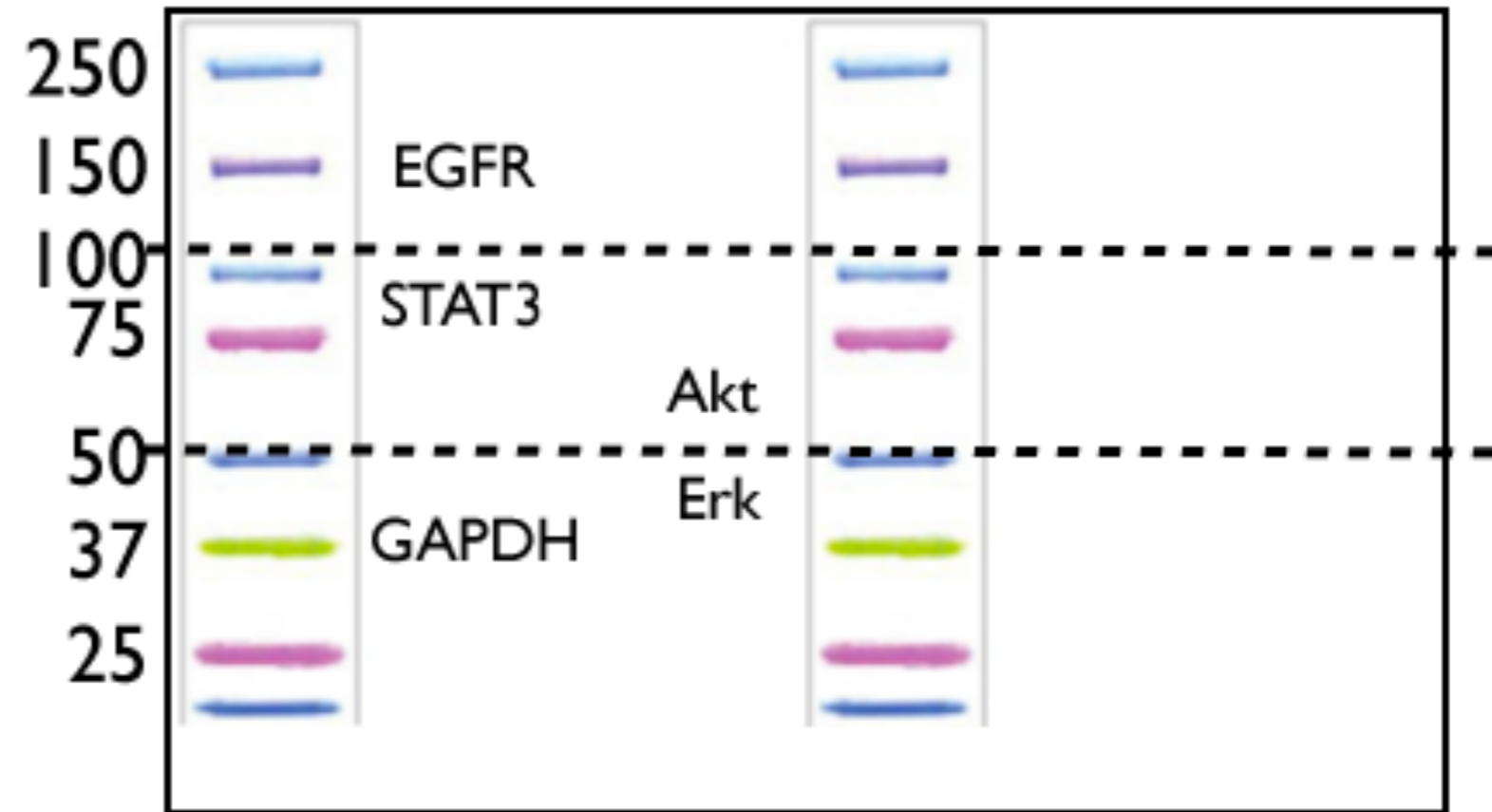
2. Pathway specific:

pERK & Total ERK

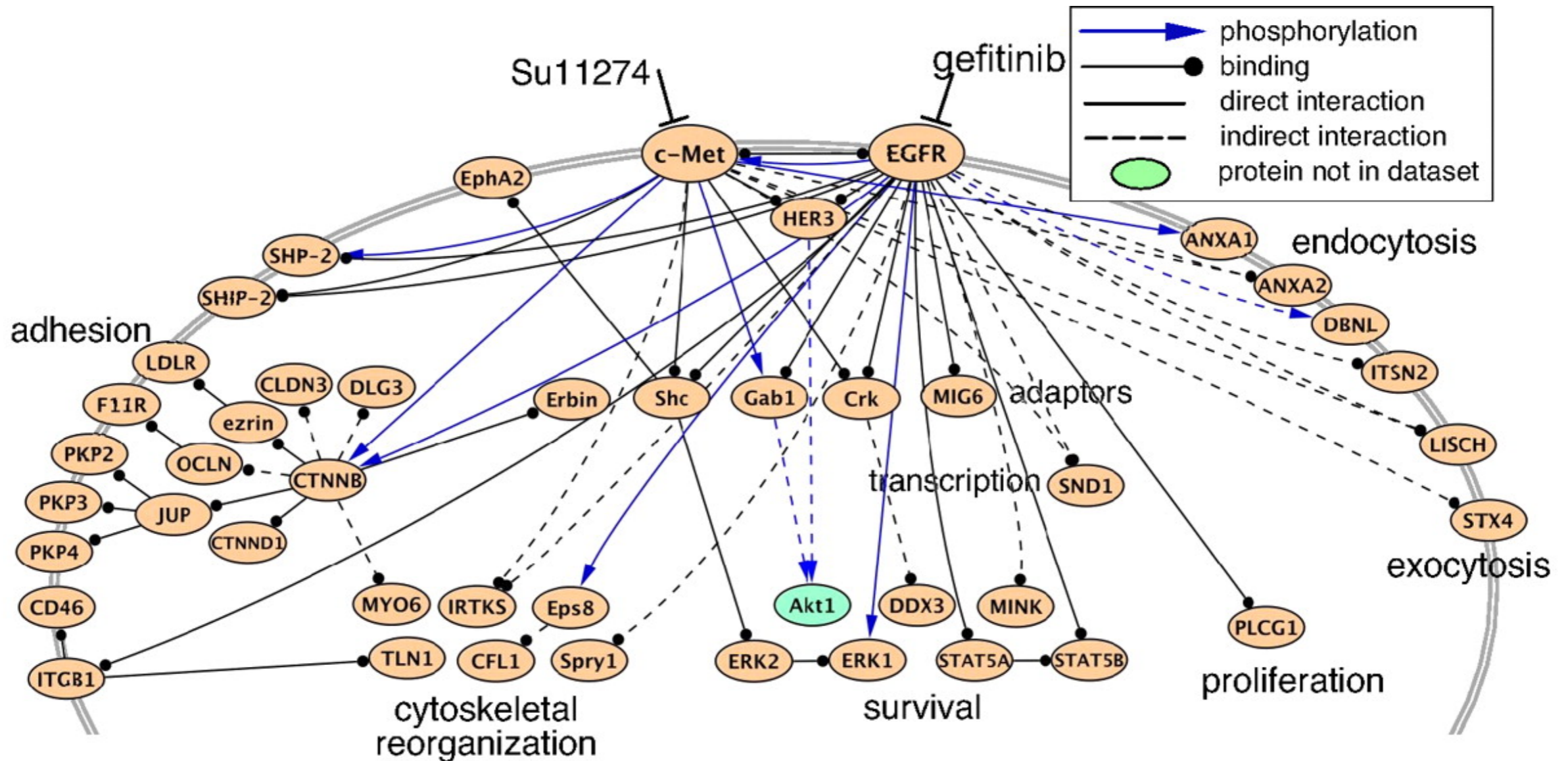
pAkt & Total Akt

pSTAT3 & Total STAT3

\*Last two + GAPDH



# What if we wanted a broader network view?



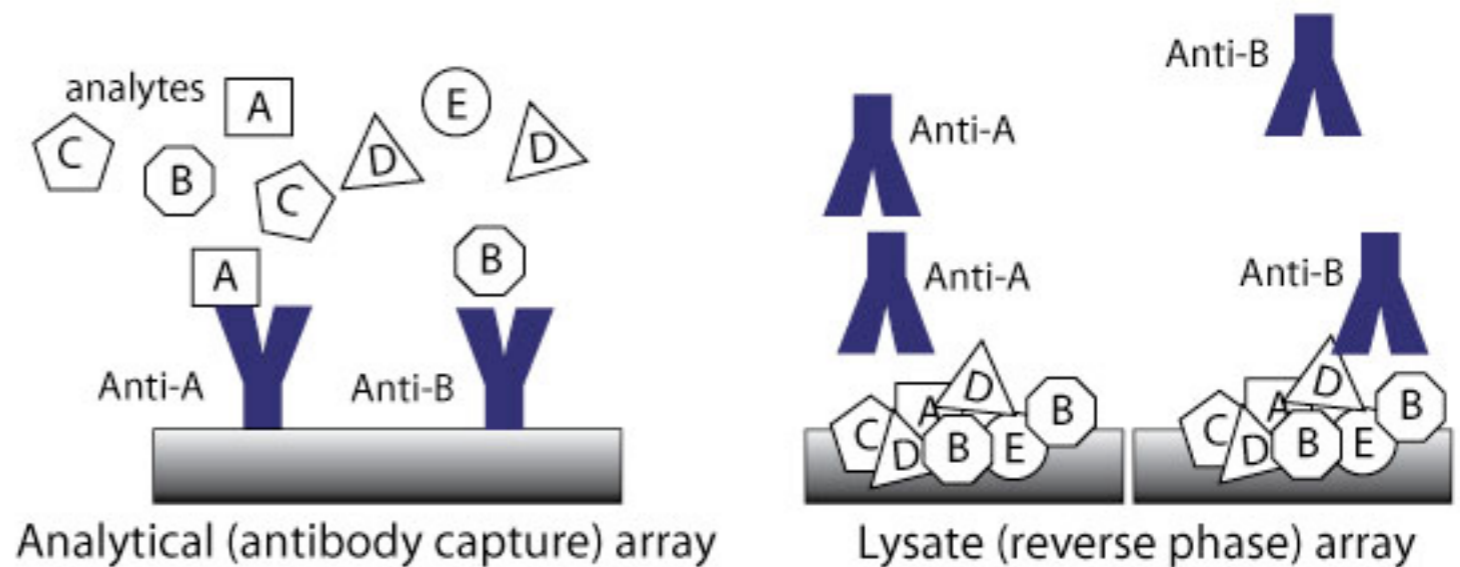
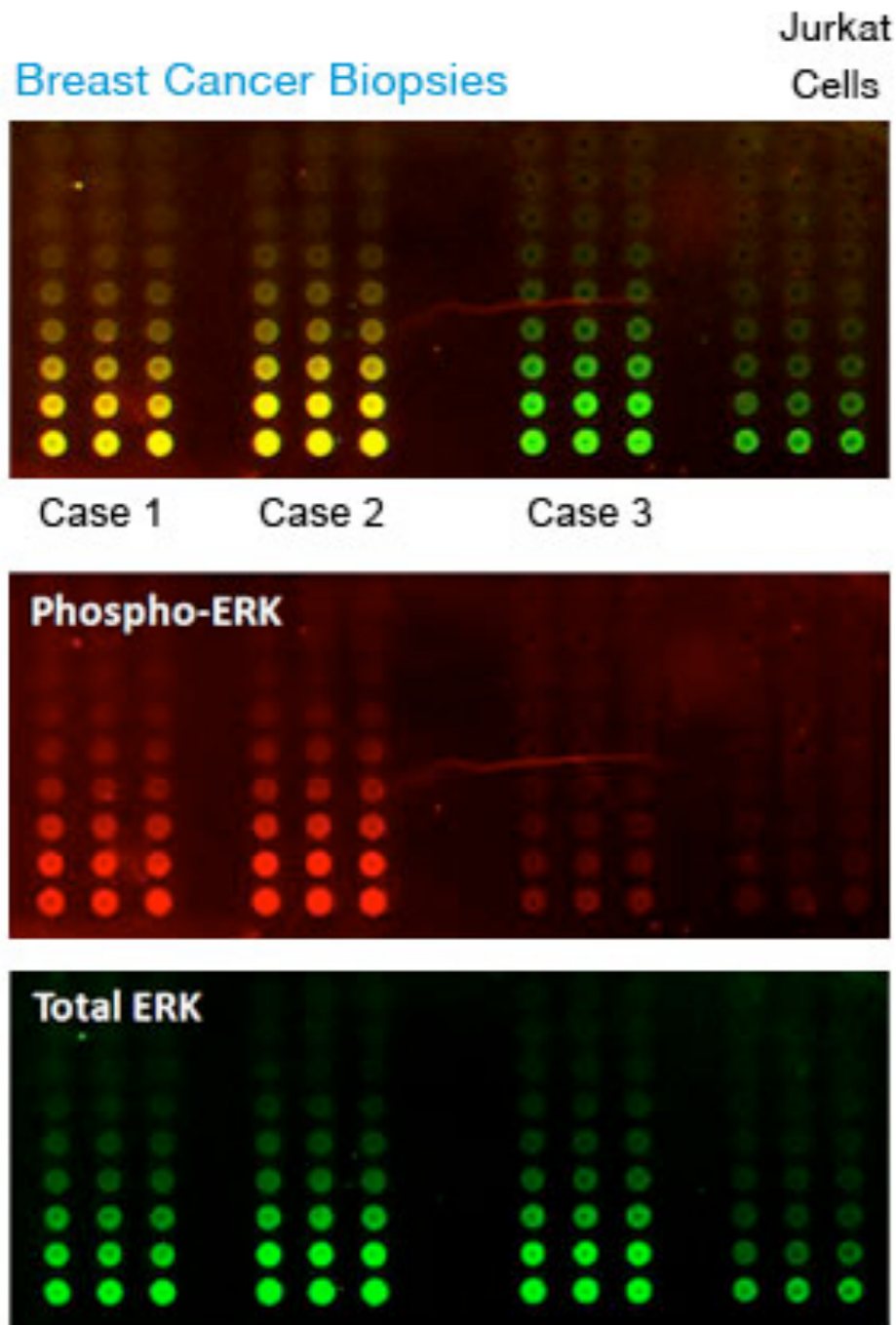
Regulatory networks sensitive to tyrosine kinase inhibitors in H3255 and MKN45 cells revealed by PhosphoScan-SILAC study.

Guo A et al. PNAS 2008;105:692-697

©2008 by National Academy of Sciences

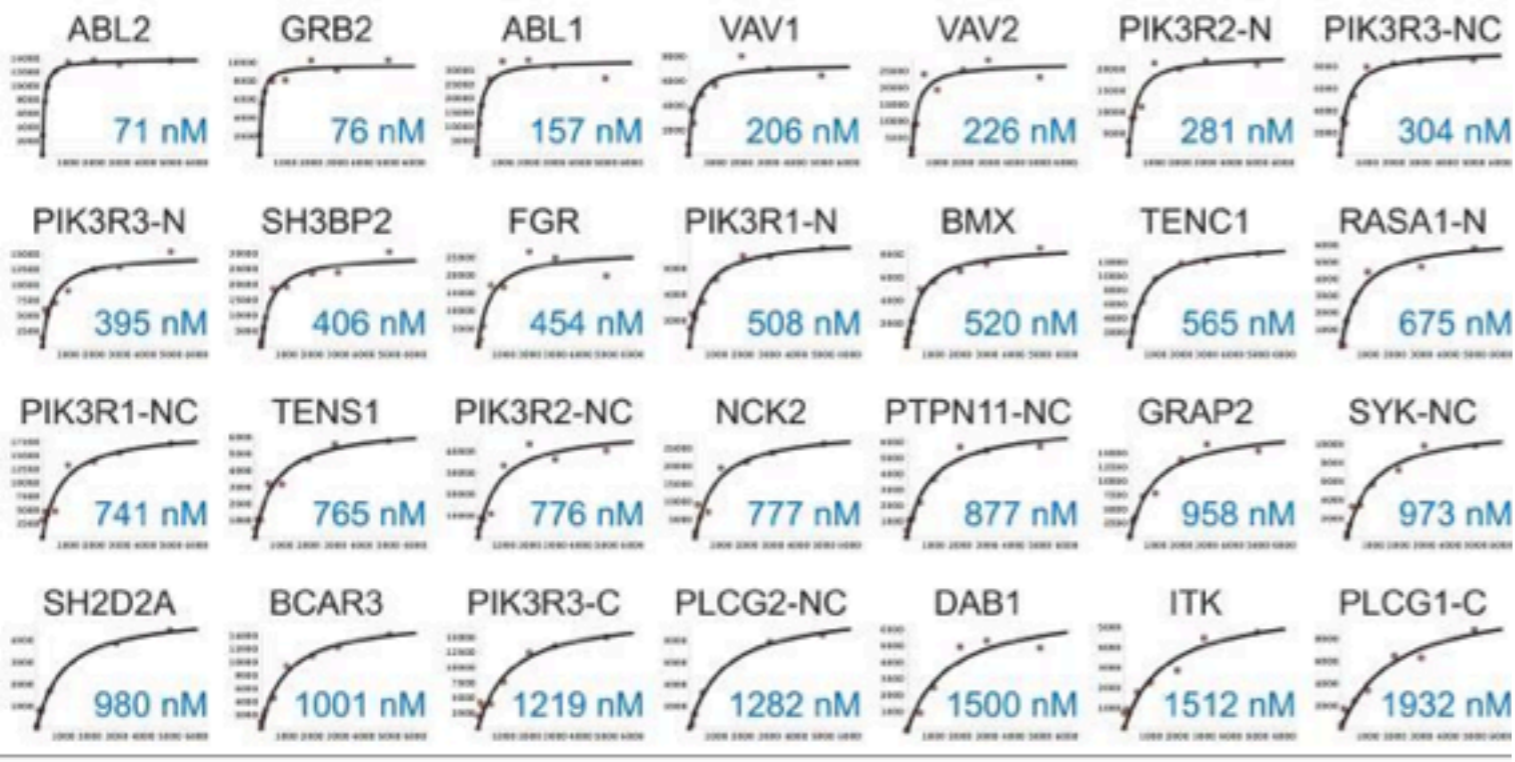
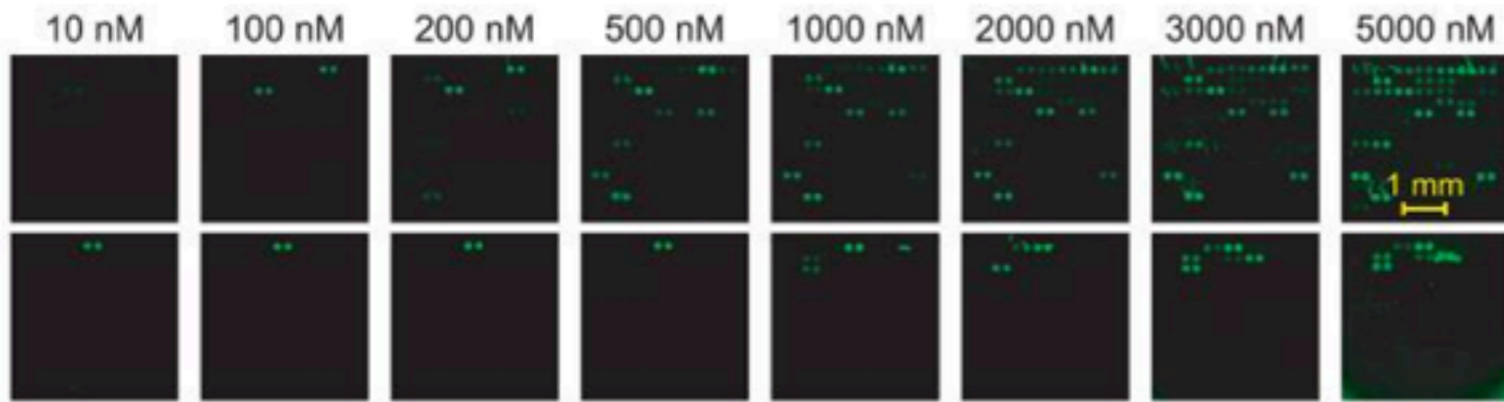
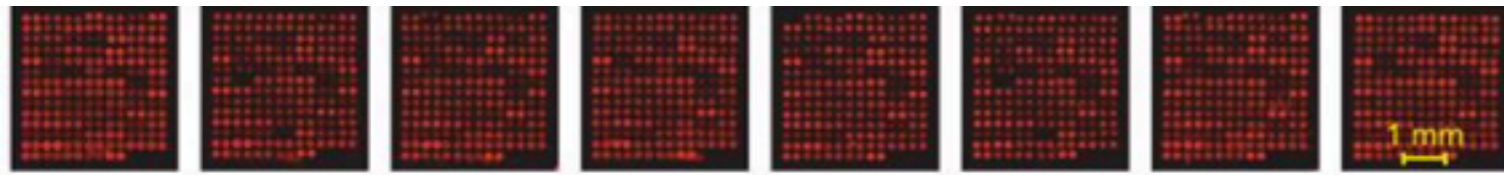
PNAS

# Semi-quantitative analysis: Protein Microarray



A few signals per spot -- up to hundreds of spots per slide.

# Semi-quantitative analysis: Protein Microarray



Strength:

Multiplex -- many conditions can be screened

Weakness:

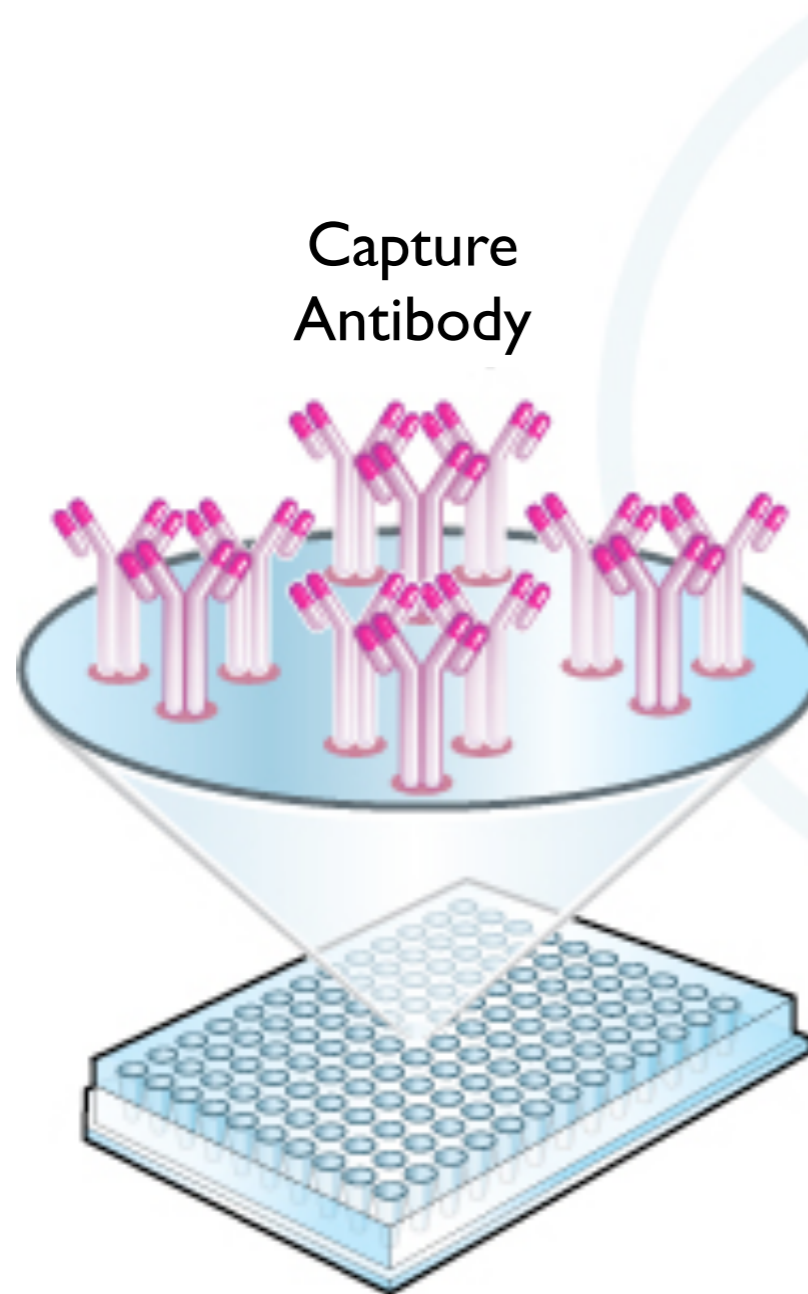
Non-specific interactions (also huge problem in WB)

Dissecting Protein Function and Signaling Using Protein Microarrays  
Curr Opin Chem Biol. 2009 October; 13(4): 398-405.

A few signals per spot -- up to hundreds of spots per slide.

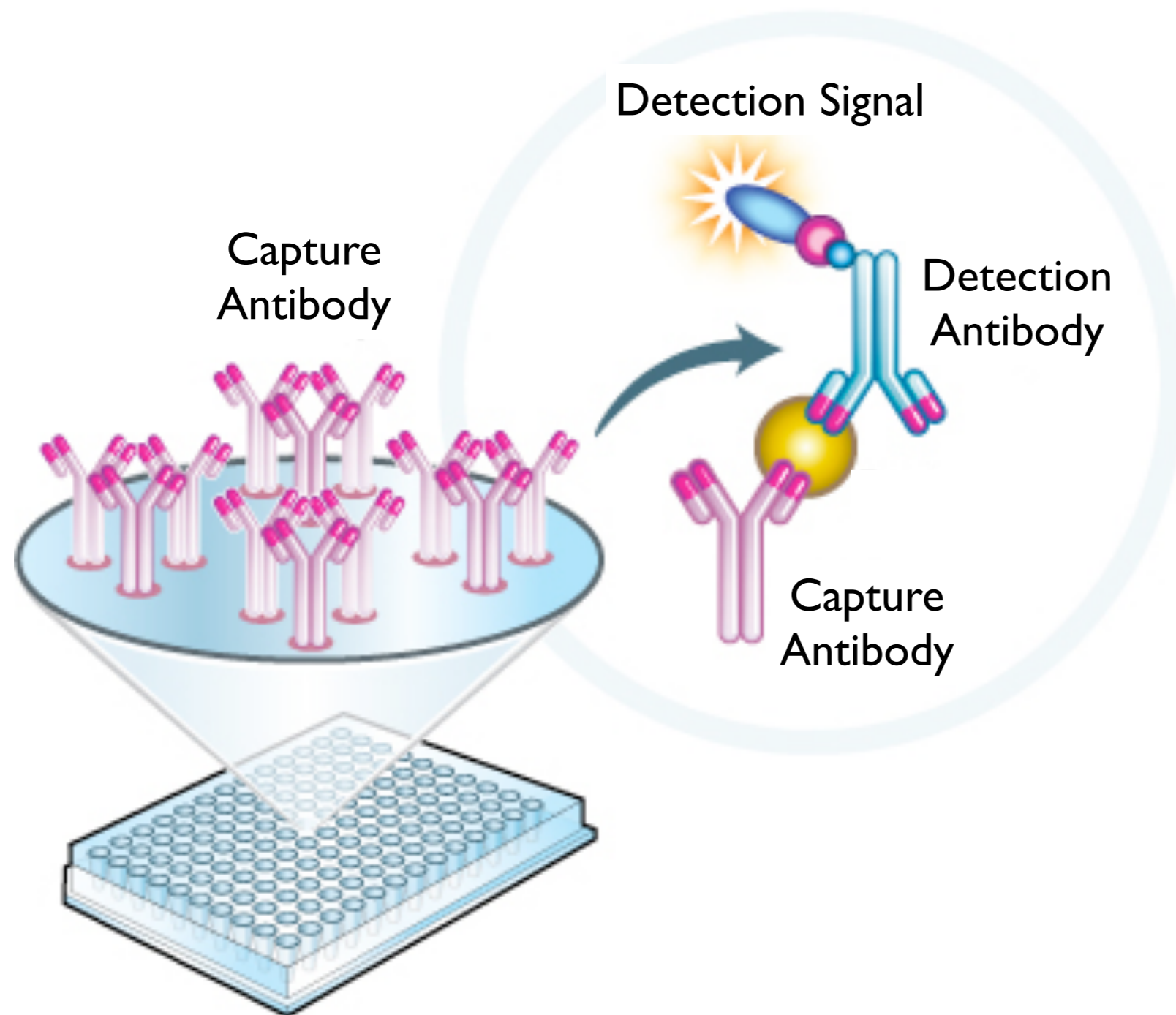


# Quantitative analysis: ELISA



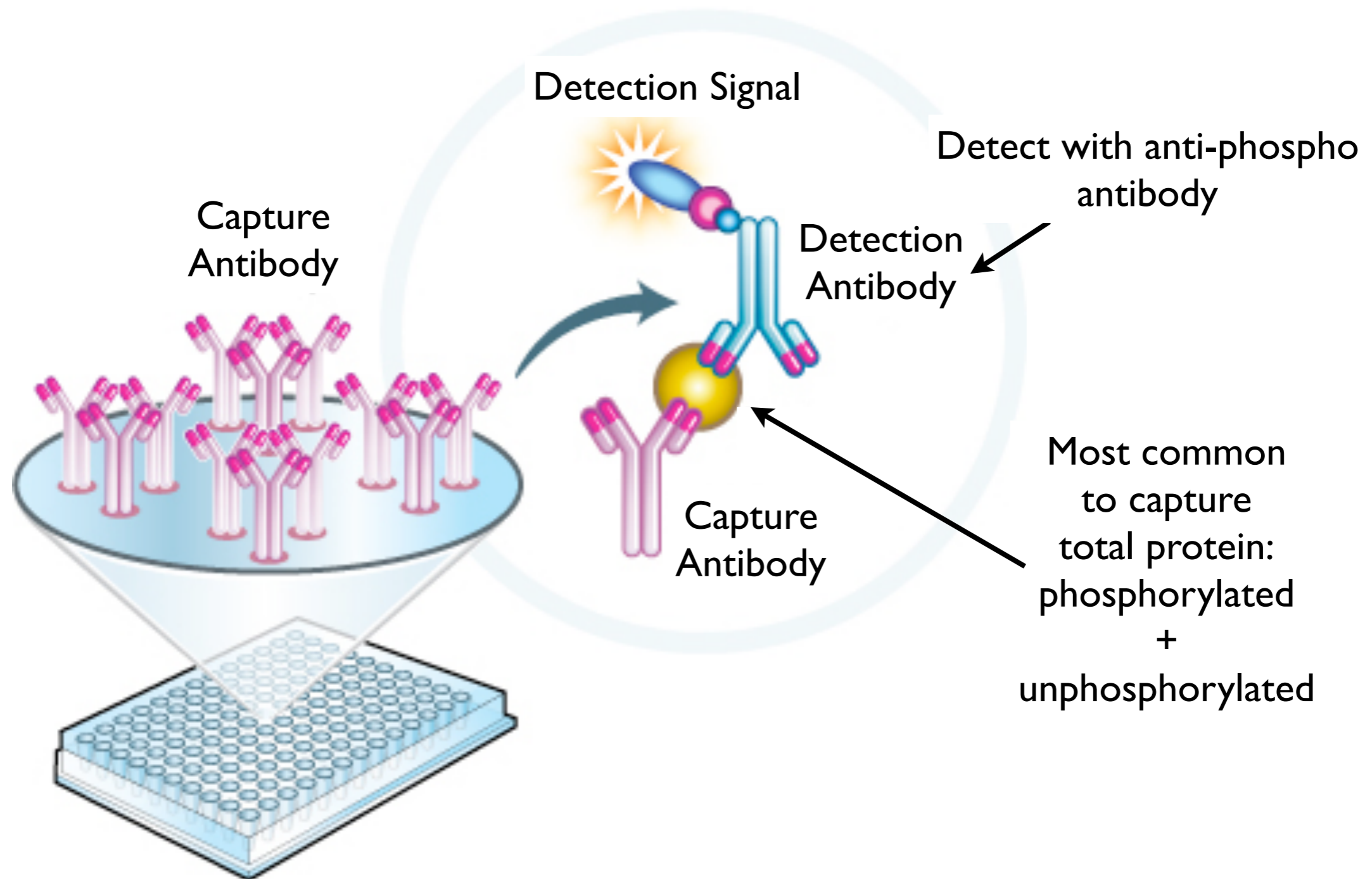
One signal per well - but up to 384 wells/experiment.

# Quantitative analysis: ELISA



One signal per well - but up to 384 wells/experiment.

# Quantitative analysis: ELISA



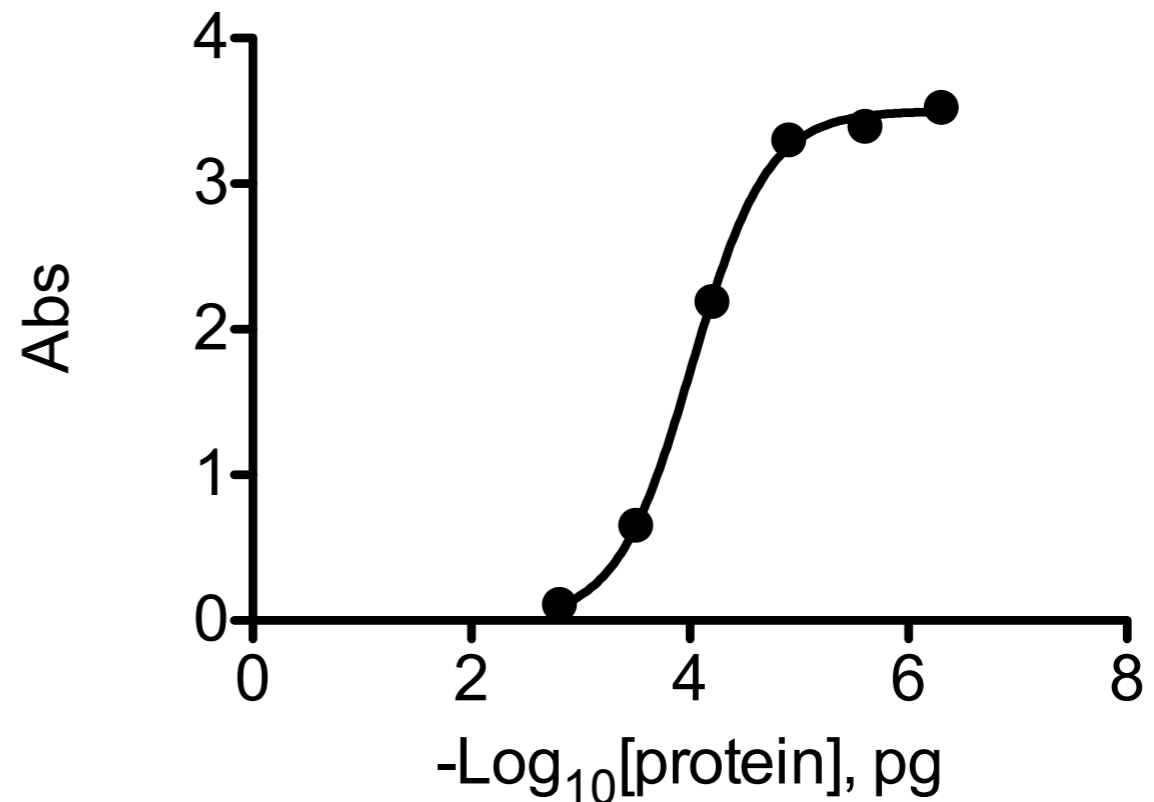
One signal per well - but up to 384 wells/experiment.

# Quantitative analysis: ELISA

How to quantify:

Very often not linear relationships

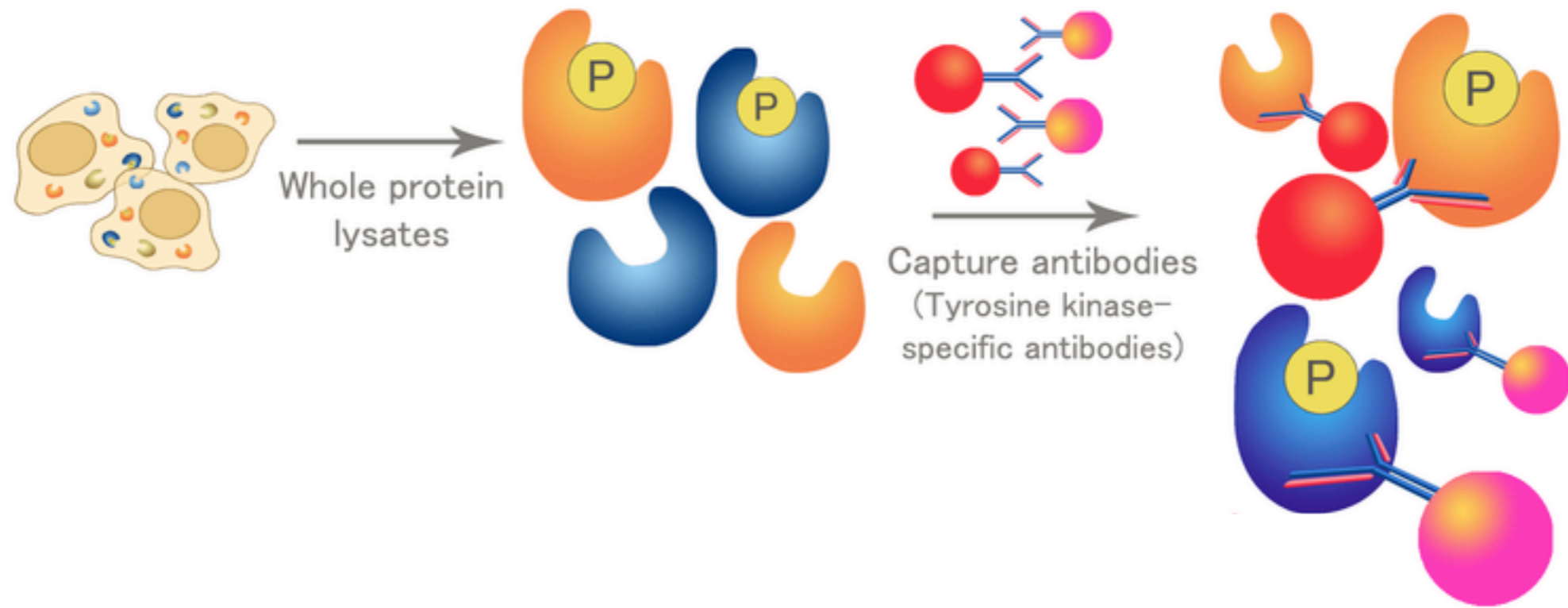
ELISA Standard Curve #2



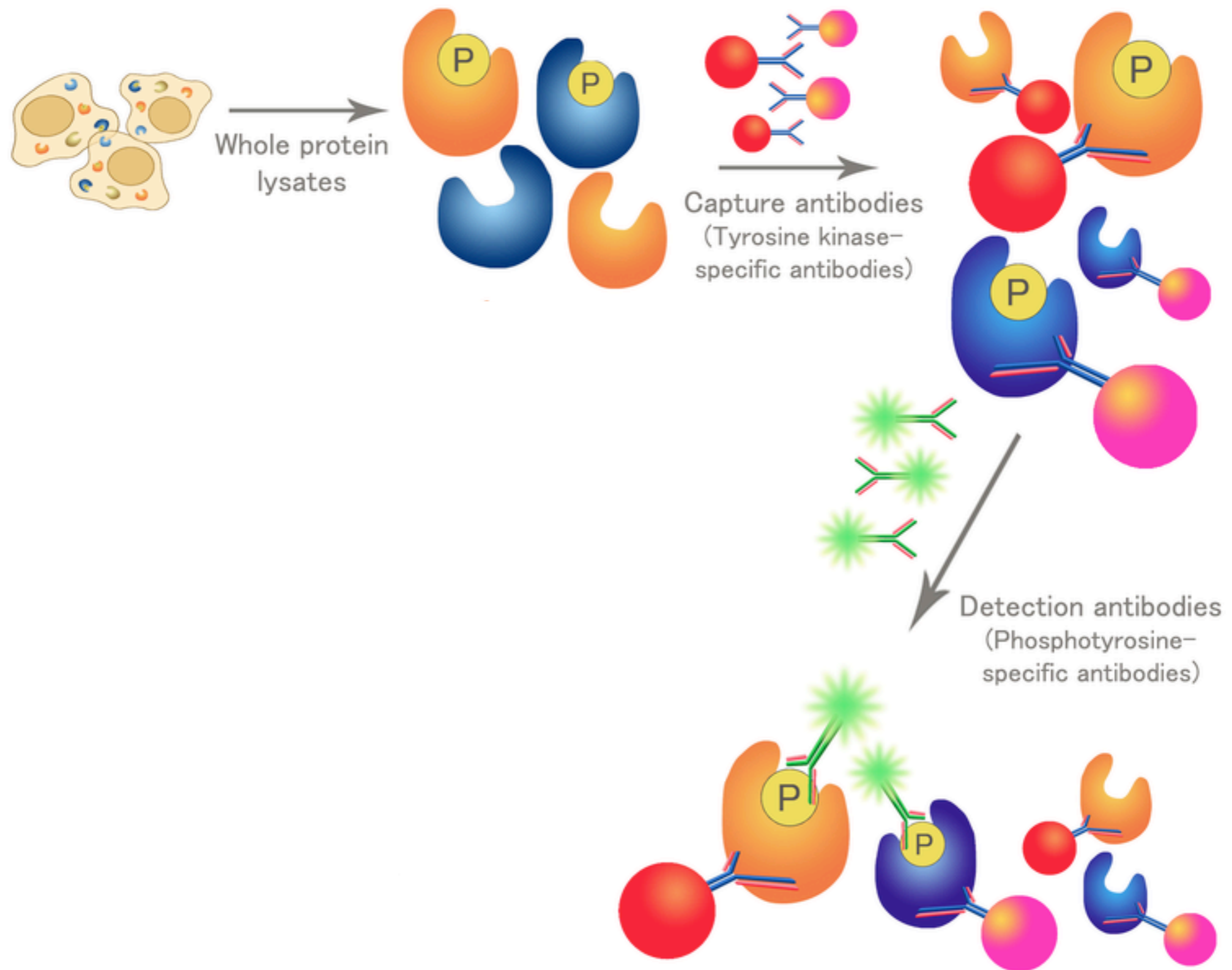
$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogEC50} - X) * \text{HillSlope})})$$

General protocol: Create a standard curve on each plate (usually supplied with kits or make your own)

# Quantitative analysis: Multiplexed bead based ELISA

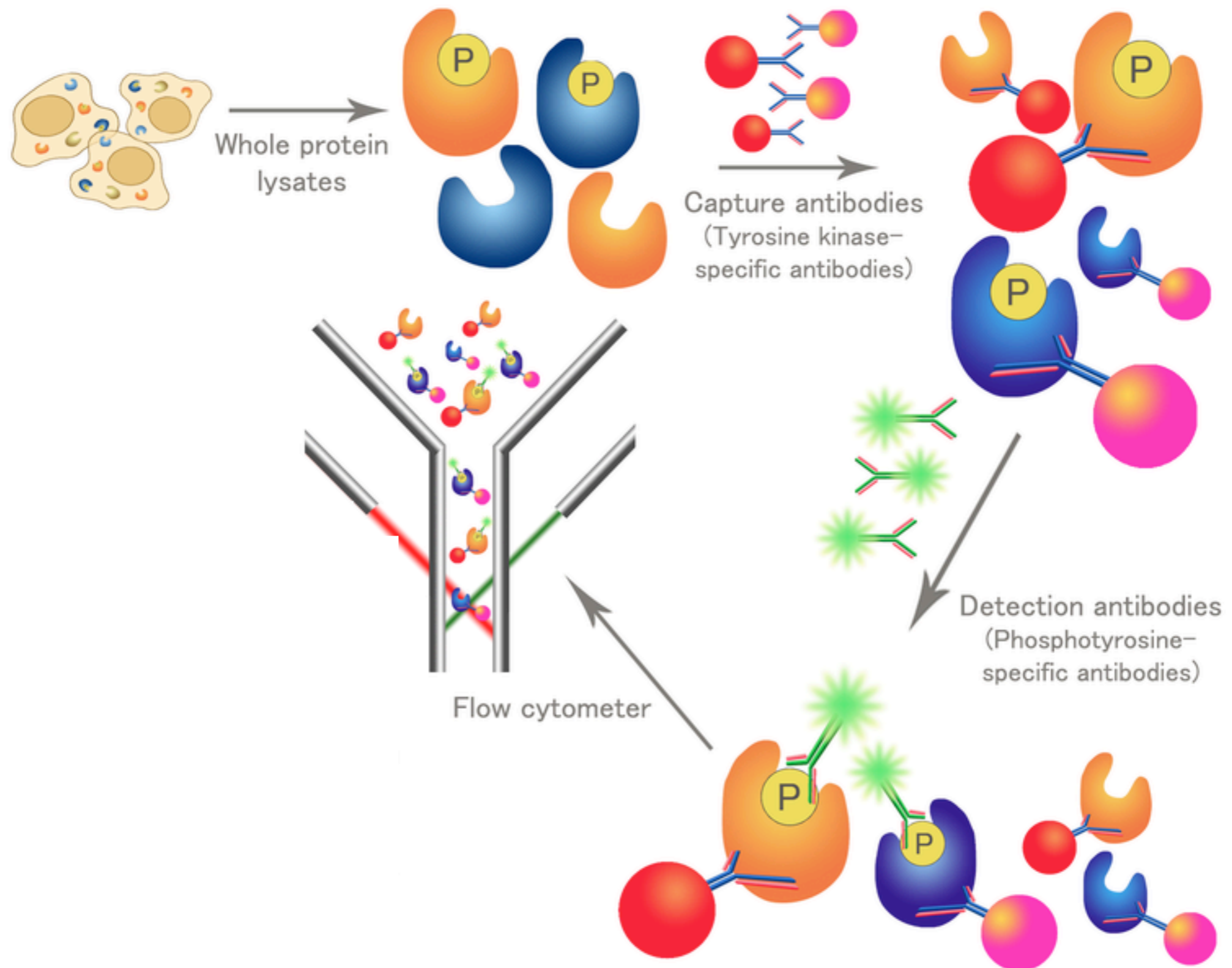


# Quantitative analysis: Multiplexed bead based ELISA



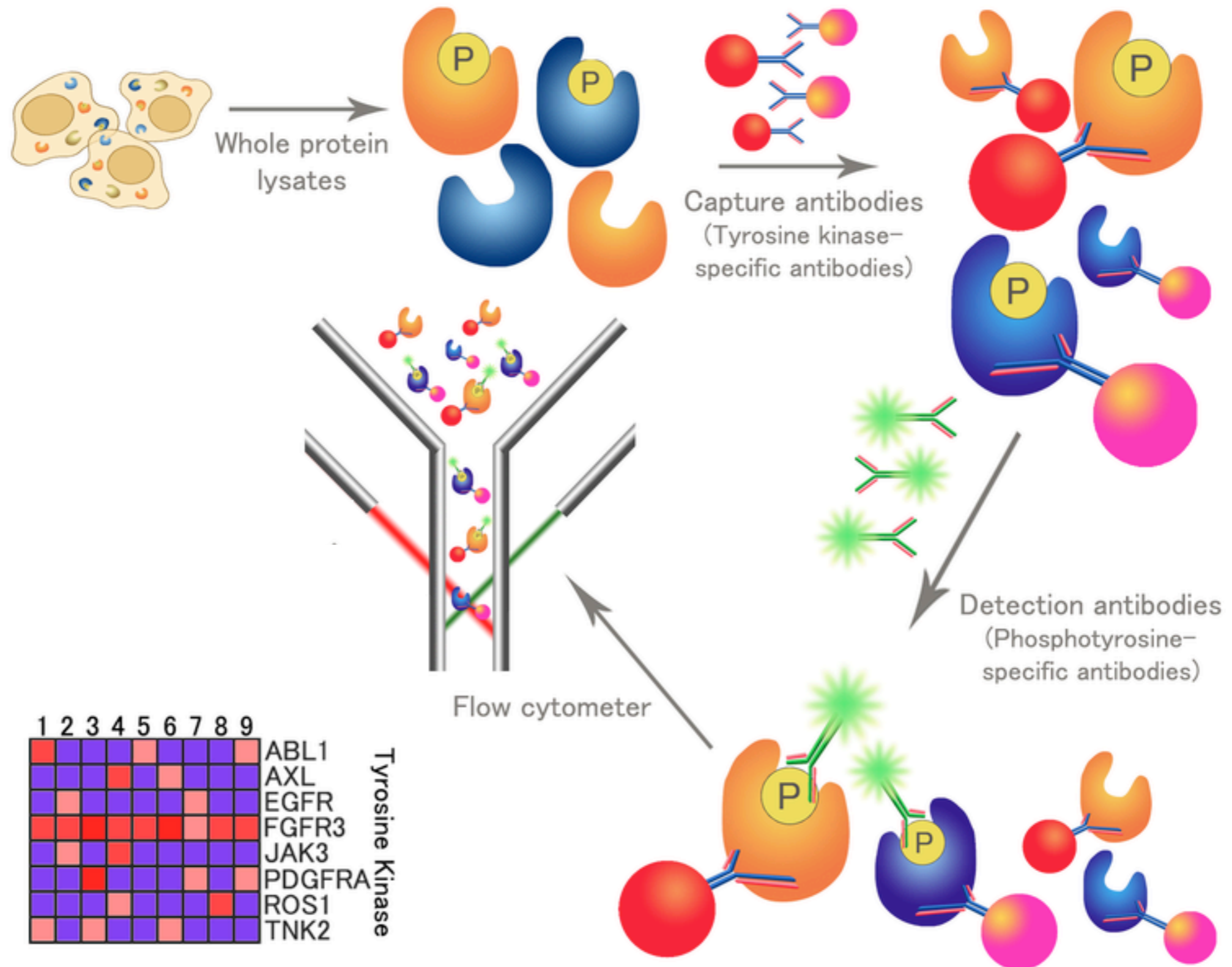
[http://commons.wikimedia.org/wiki/File:Workflow\\_IA.png](http://commons.wikimedia.org/wiki/File:Workflow_IA.png)

# Quantitative analysis: Multiplexed bead based ELISA



[http://commons.wikimedia.org/wiki/File:Workflow\\_IA.png](http://commons.wikimedia.org/wiki/File:Workflow_IA.png)

# Quantitative analysis: Multiplexed bead based ELISA



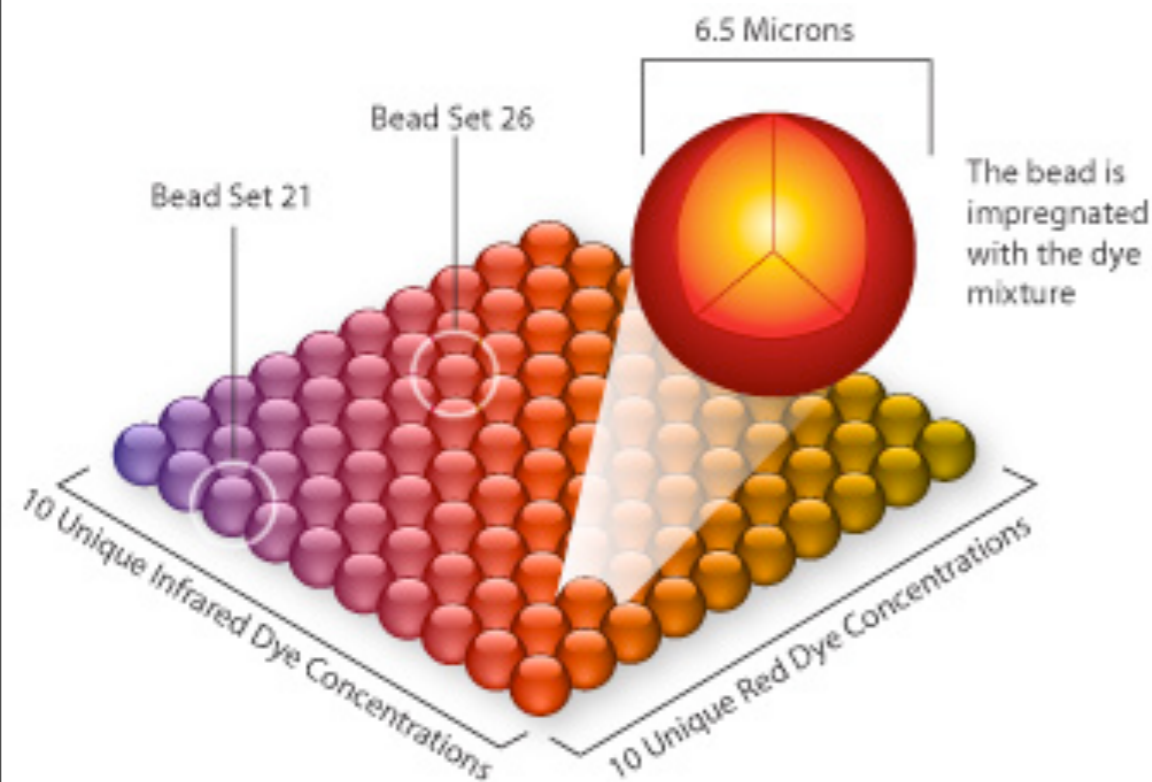
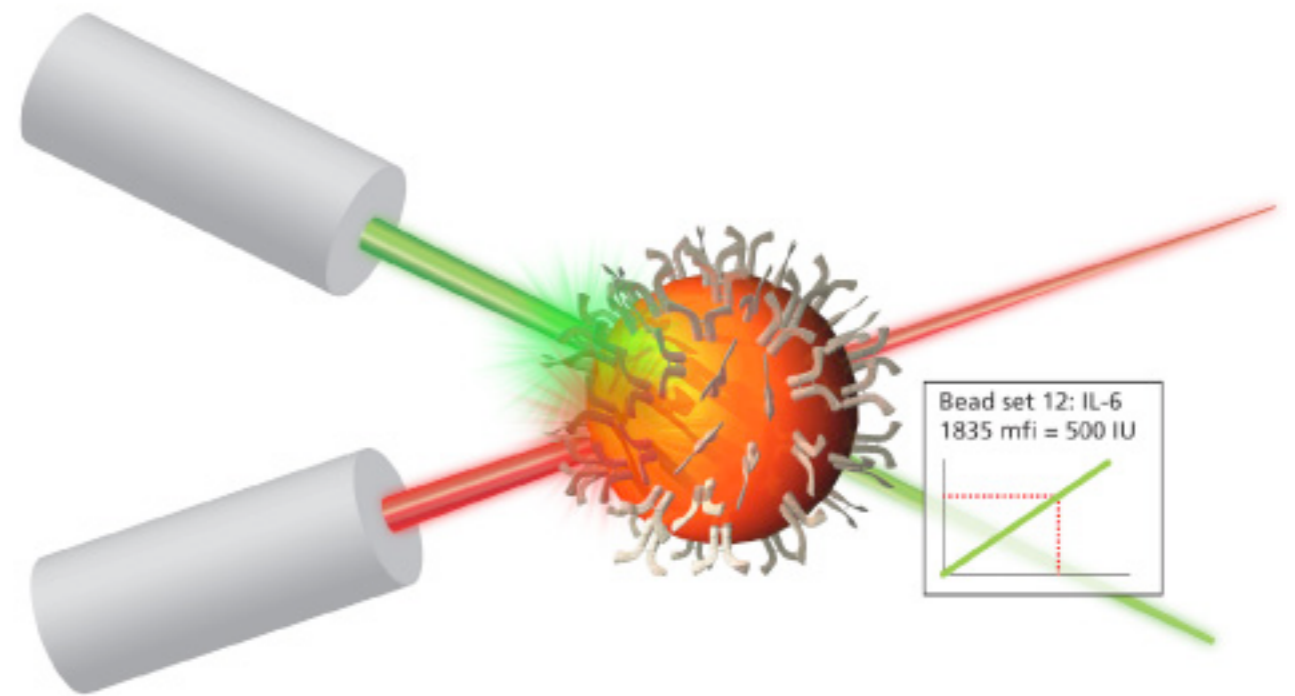
[http://commons.wikimedia.org/wiki/File:Workflow\\_IA.png](http://commons.wikimedia.org/wiki/File:Workflow_IA.png)



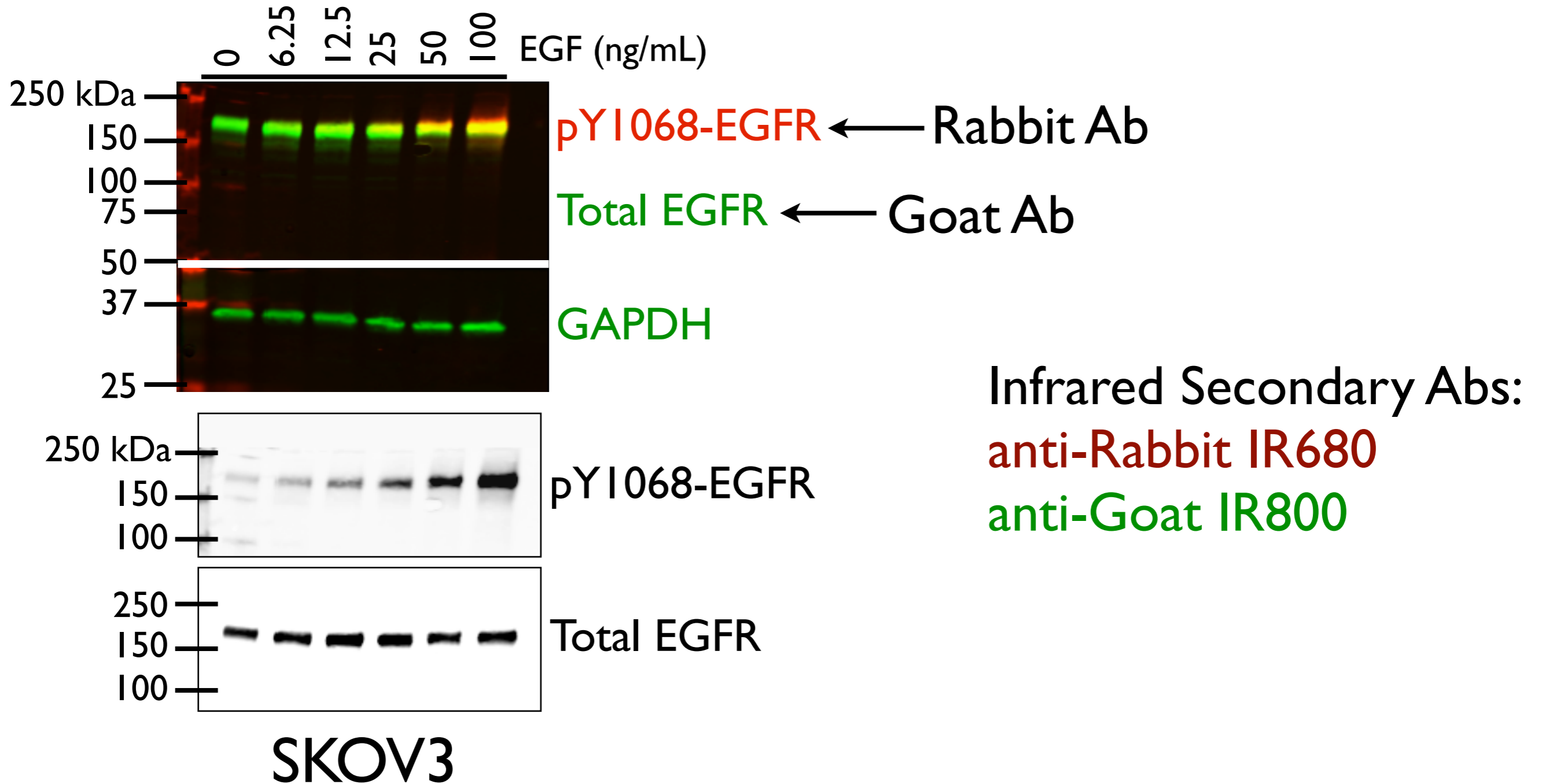
# Quantitative analysis: Multiplexed bead based ELISA

Theoretically up to hundreds of conditions per well -- 384 (sometimes >1500!) wells per experiment

In reality: 20-30 different phosphoproteins / well max



# Semi-quantitative analysis: Western blot

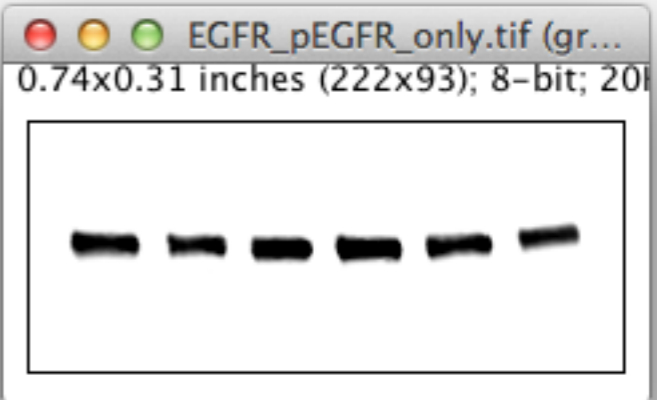
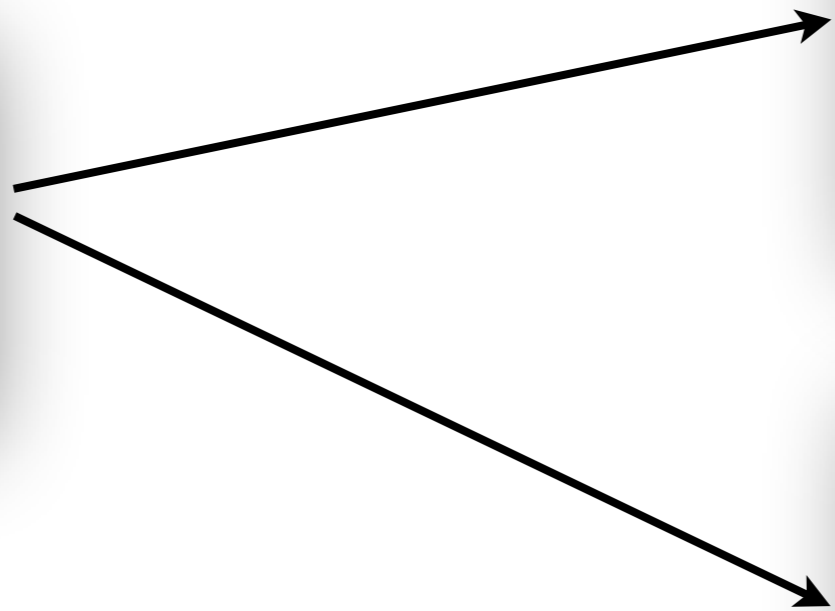


A few signals per lane -- up to ~17 lanes per mini gel.

# Densitometry:



Step I: Separate Channels & Invert

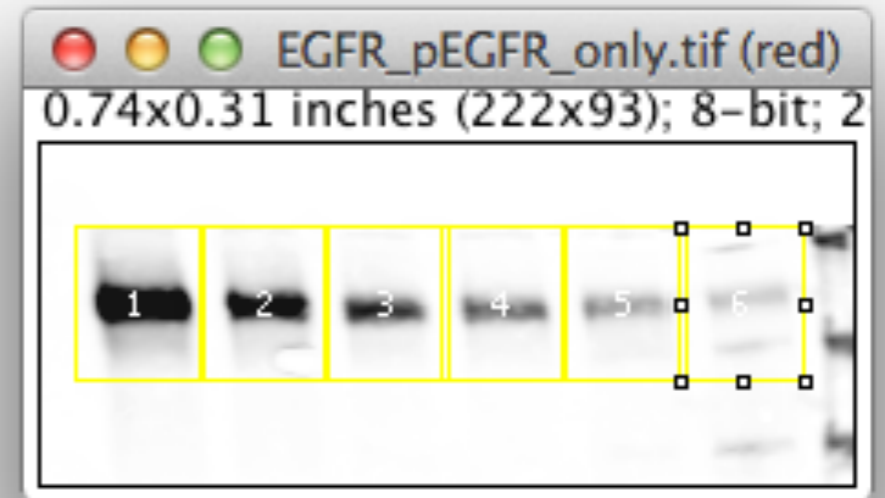


# Densitometry:

Step2: Choose regions of interest

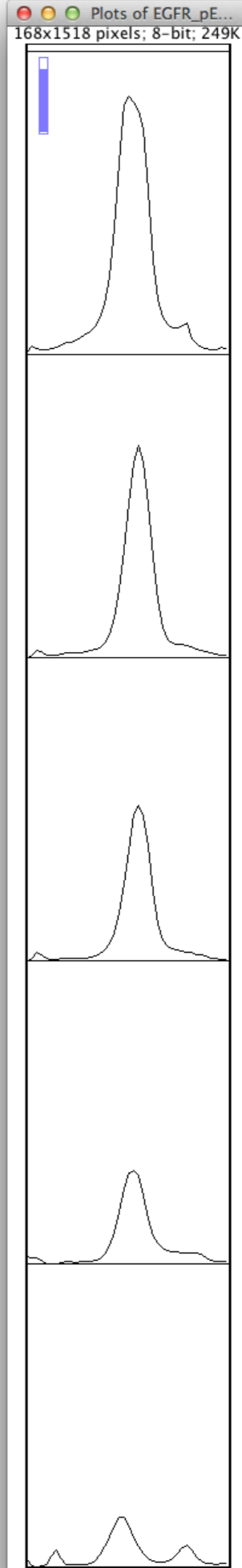
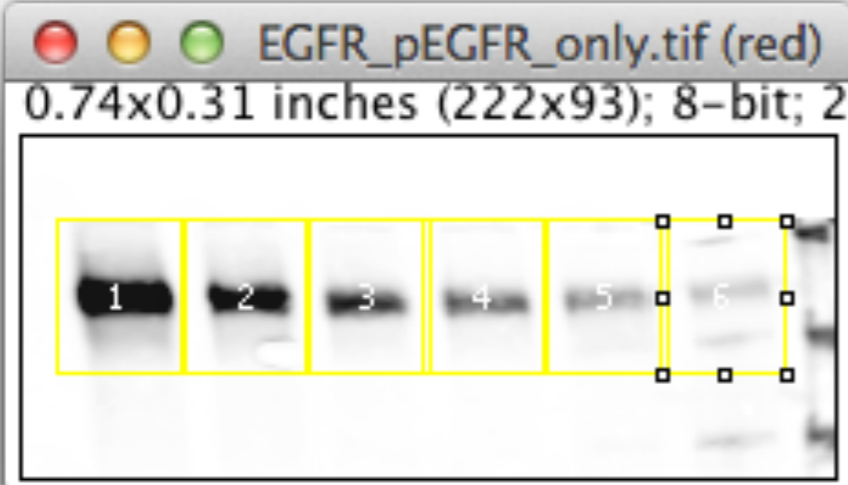


This is what it looks like in ImageJ



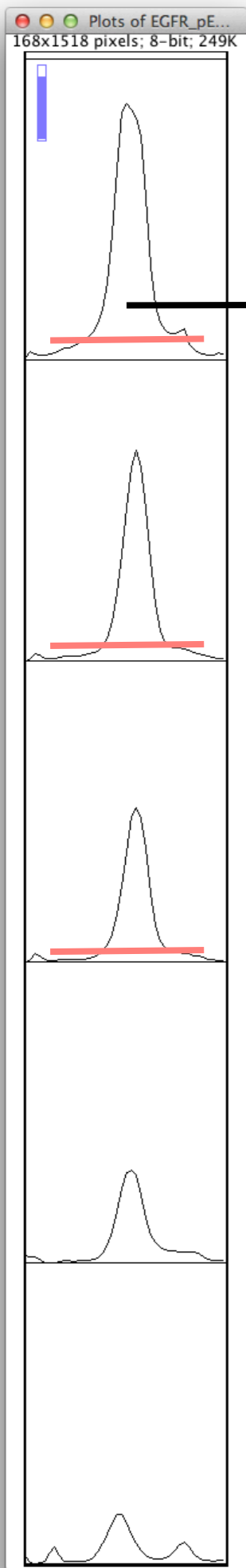
# Densitometry:

Step3: Plot pixel intensity (line drawn vertically through box)

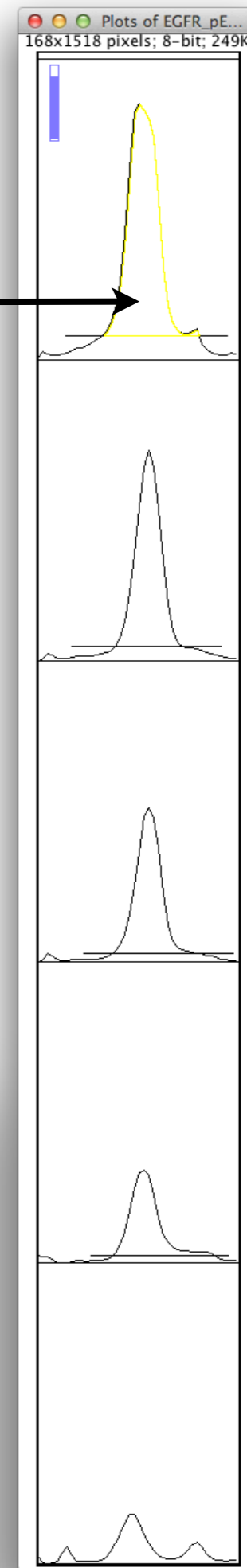


This is what it looks like in ImageJ

Step 4: Define area to analyze  
Warning: Can be very subjective!



Step 5: Use magic wand to calculate area under the curve



Step 6: Keep track of what value corresponds to each experimental condition

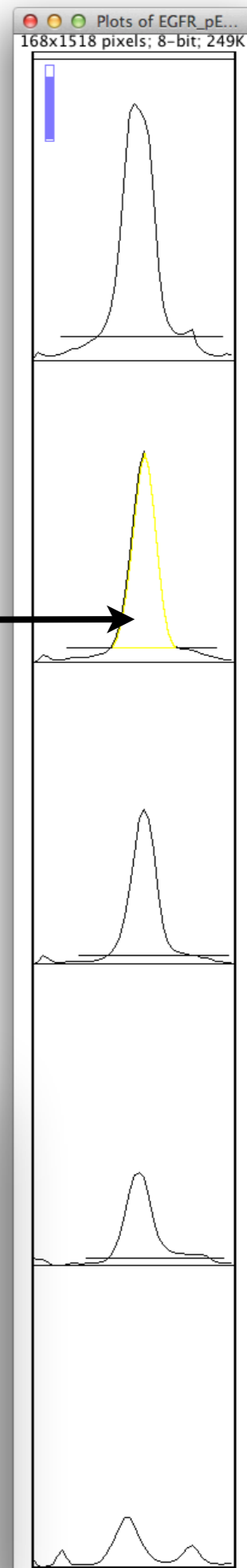
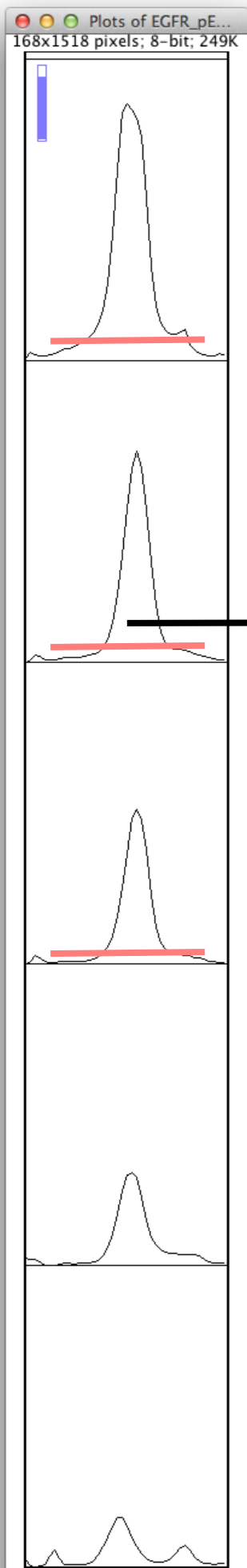
The 'Results' window displays a table with the following data:

|   | Area     |
|---|----------|
| 1 | 5779.912 |

Step 4: Define area to analyze  
Warning: Can be very subjective!

Step 5: Use magic wand to calculate area under the curve

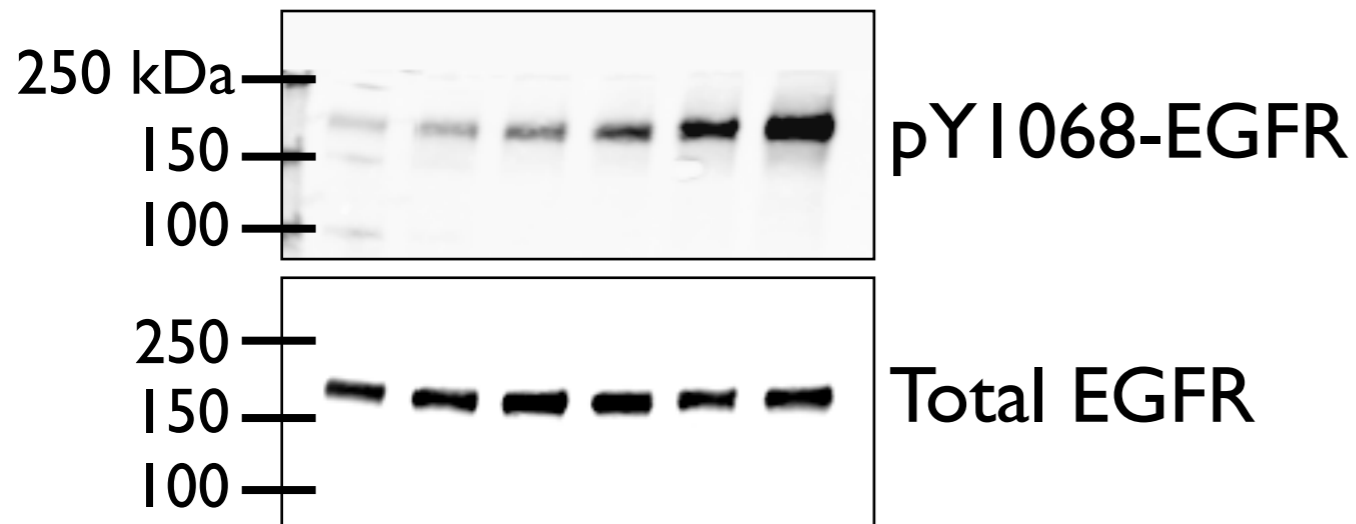
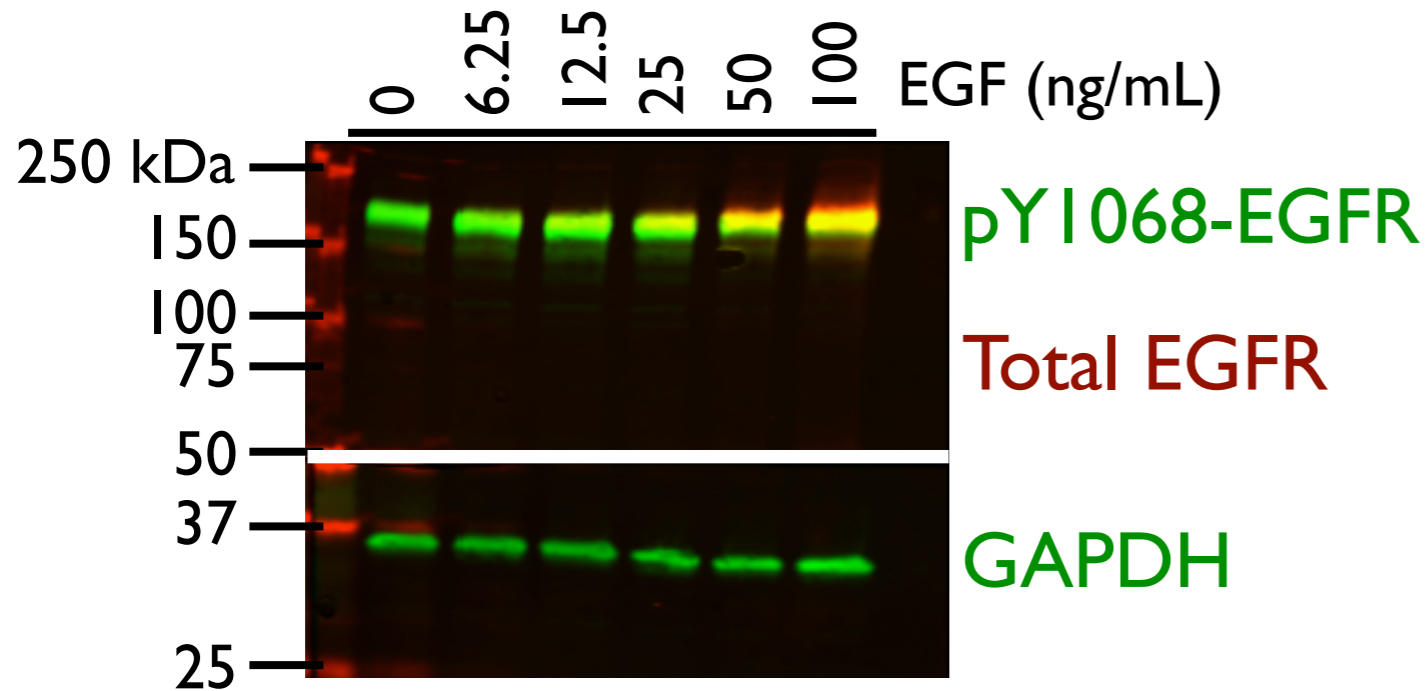
Step 6: Keep track of what value corresponds to each experimental condition



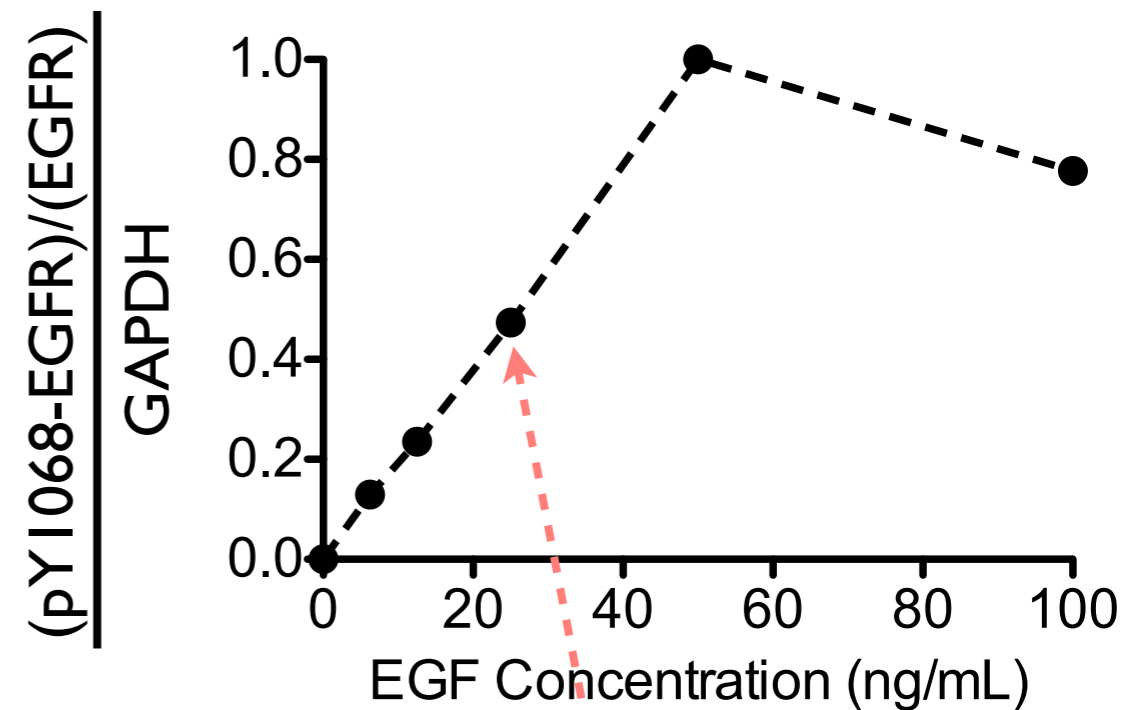
| Results |          |
|---------|----------|
|         | Area     |
| 1       | 5779.912 |
| 2       | 3851.184 |

**“Total pixel intensity”**

# Semi-quantitative analysis: Western blot



## Densitometric Analysis



**"Relative pixel intensity"**

SKOV3



# Module 2: Systems Engineering (M2D6)

- Reminder of goals of Module 2
- Scale it up! (For real this time!)
- Densitometry -- how to analyze your WB data.
- M2D7 -- to robot or not to robot...