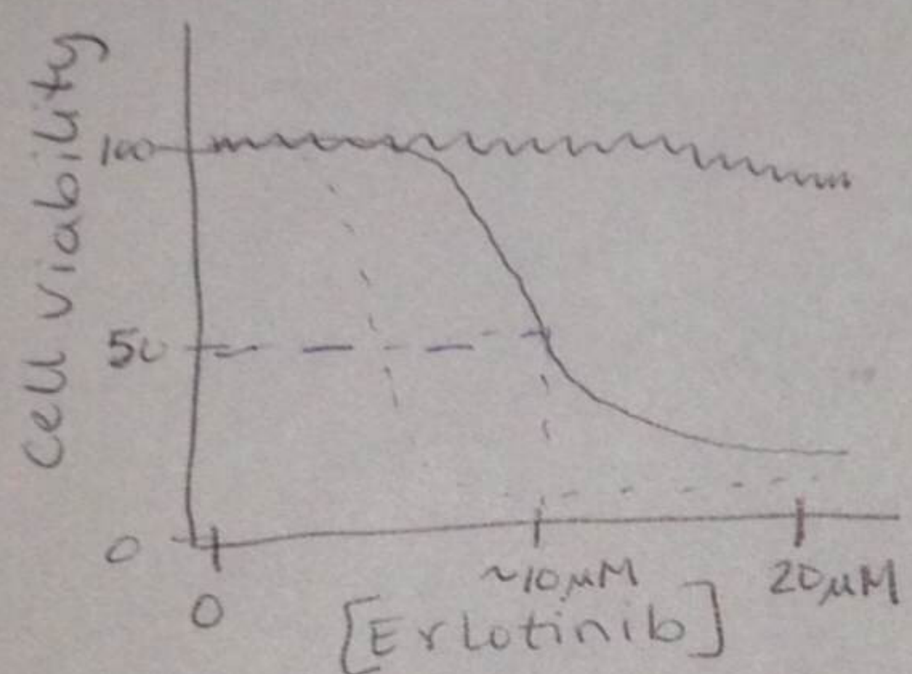
① System → SKOV3 ovarian cancer

- Why? 60% o.e EGFR

- EGFR inhibitors are unsuccessful in the clinic alone

- The mutation rate in ovarian cancer is low < 5% ⇐ "easy" to check

② Biological Question / Design challenge



- - - - "sensitive" cells

——— "normal" cells

mmmmm SKOV3

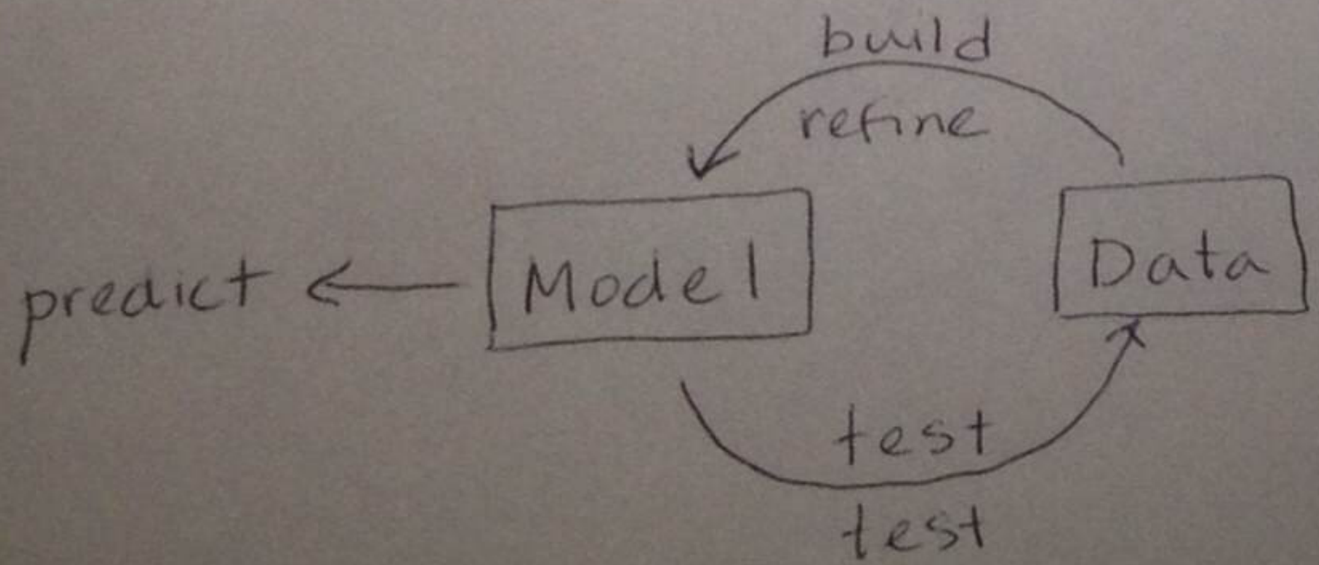
@ 50% { IC50 (normal) ≈ 10μM
*could be lower

EC50

③ How can we use a "systems biology" approach to increase effectiveness of EGFR inhibitors?

What is "systems biology"?

- Network view vs. favorite protein/gene
- quantification / analysis ⇒ modeling
- lots of data needed



④ We use a "simple" ODE model to make a prediction ⇒ we will test our prediction on M2D7

⑤ In the ~~meat~~ meantime: Let's arm ourselves with more data?

What can facilitate resistance?

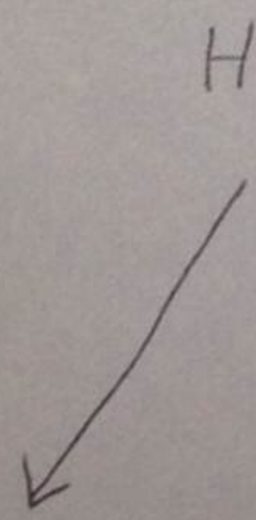
① Mutation ✓ (we tested for highest probability)

↳ conclusion → no mutation in EGFR

② Alternative signaling pathways

EGFR: ~260K
HER2: 1.4M
HER3: ~14K

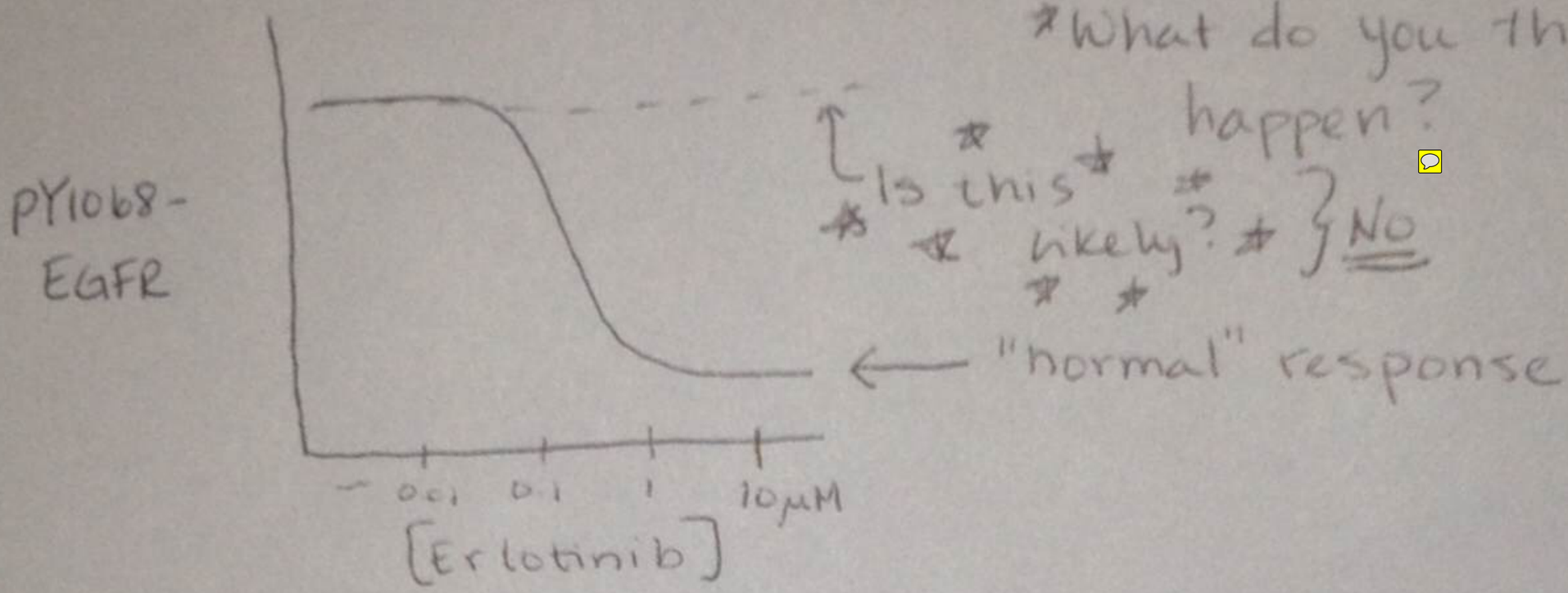
} What do we know about this?



Look at pAKt, pErK & pSTAT3 after Erlotinib treatment

★go to slide

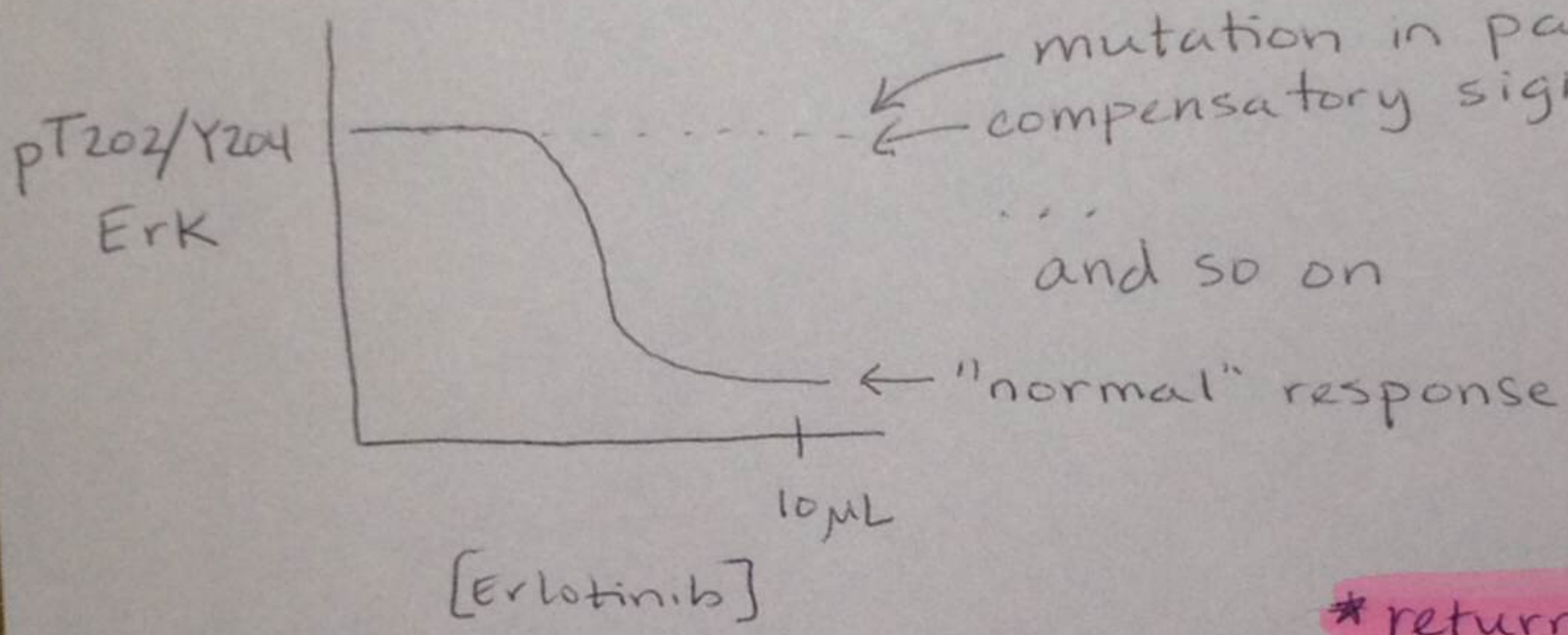
(6) Can we make predictions?



*What do you think will happen?

Is this likely? } No

← "normal" response



*return to lecture here

---

(7) What if they are all the same? ← Next time

Module 2: Systems Engineering (M2D5)

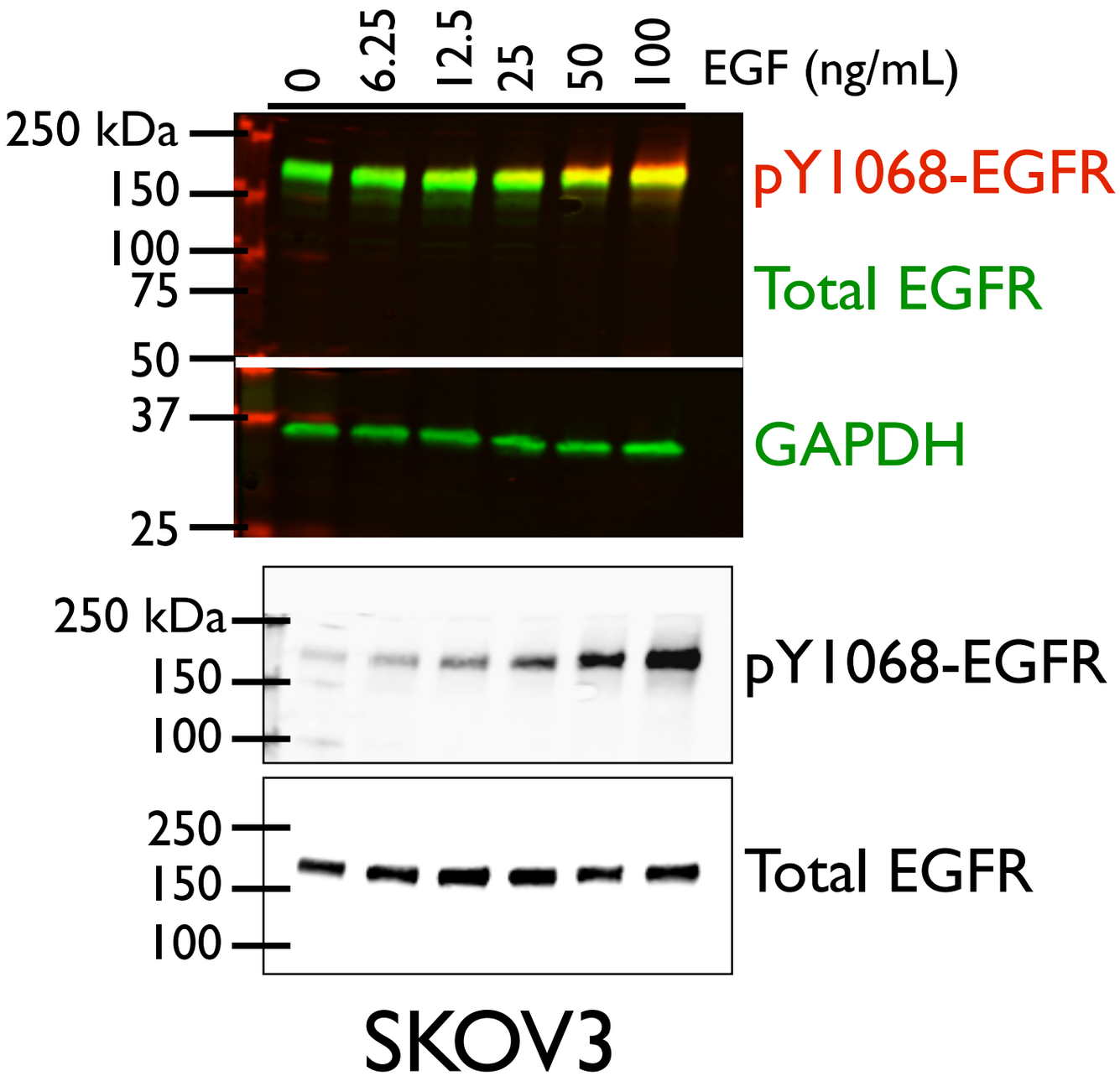
- In-depth about goals of Module 2 -- how does it all fit together?
- Scale it up!
- Talk about next time in lab: wiki pages done Saturday.
- Journal club!

Module 2 overview:

Module 2 overview:

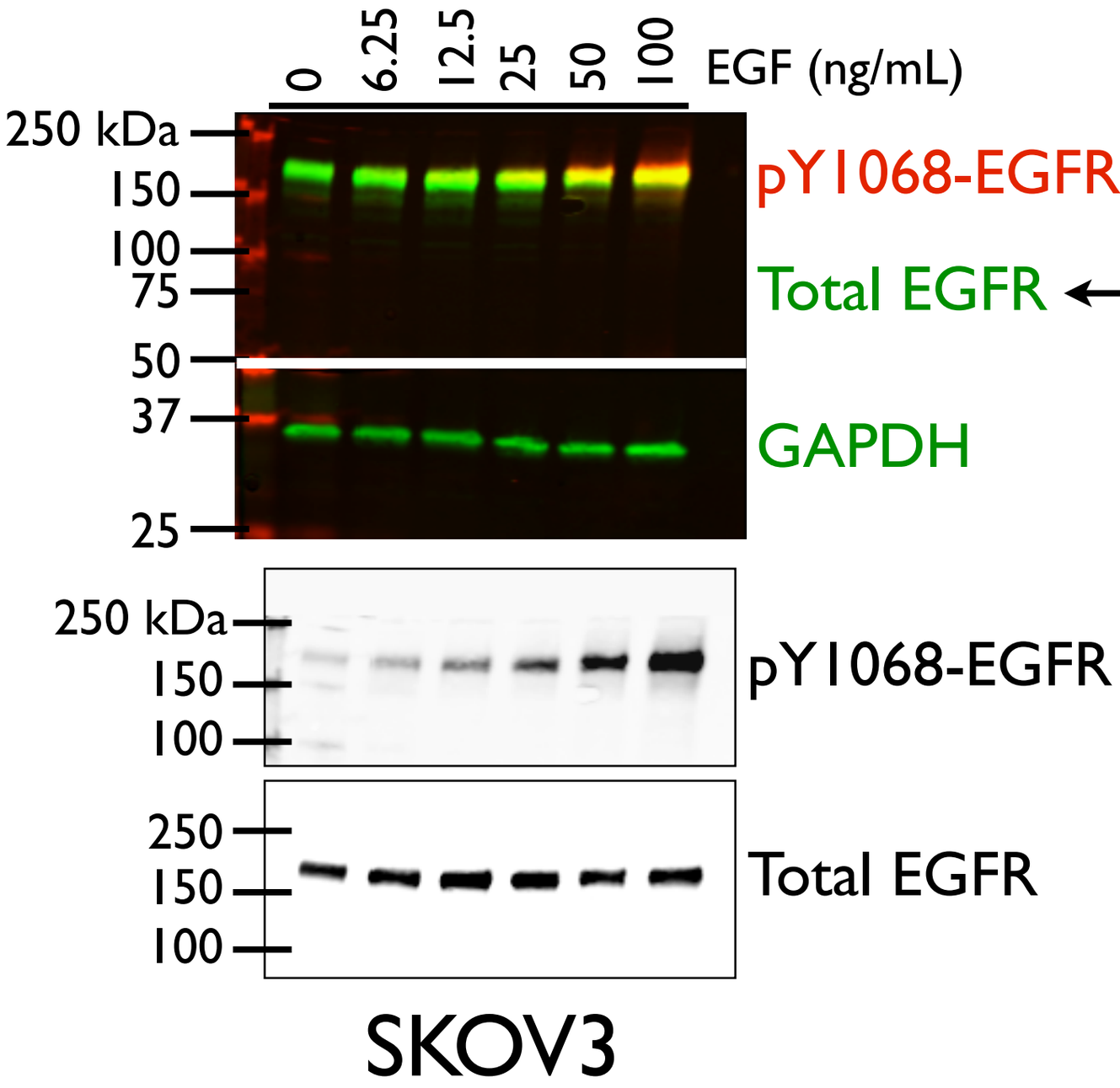
Module 2 overview:

Semi-quantitative analysis: Western blot



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

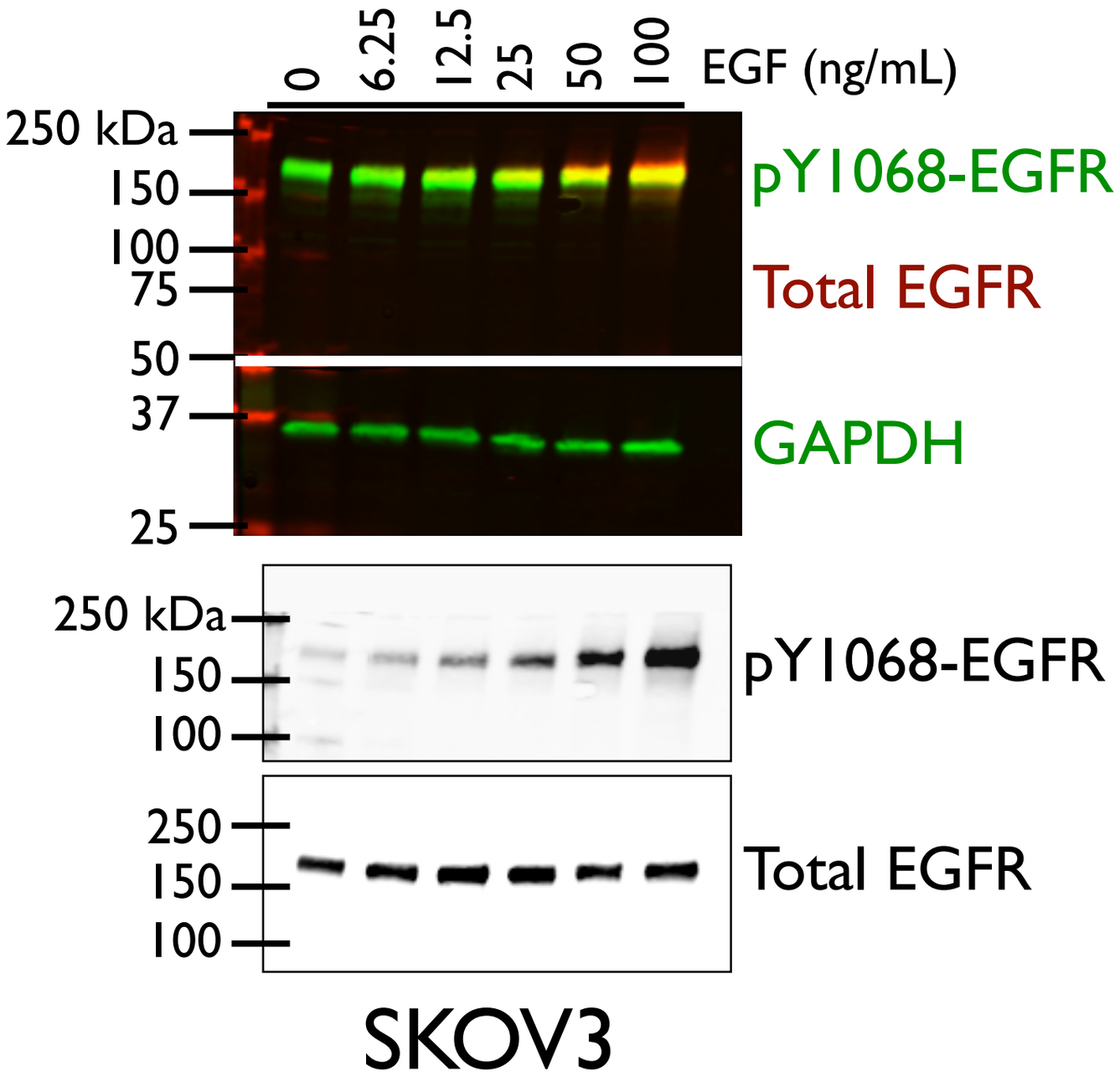
Semi-quantitative analysis: Western blot



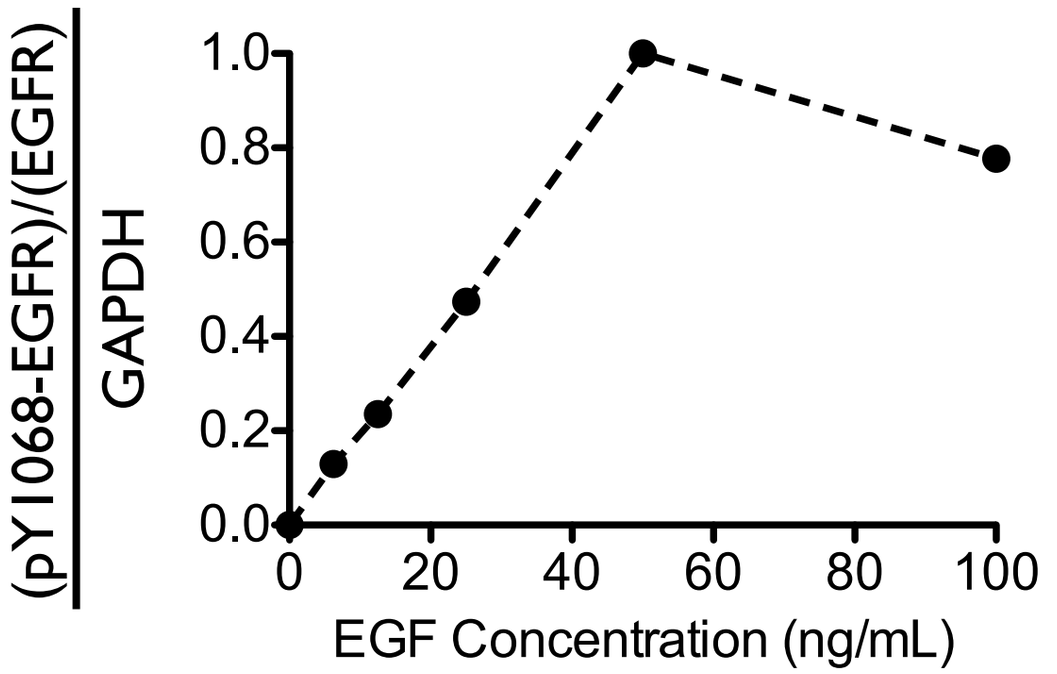
Infrared Secondary Abs:
anti-Rabbit IR680
anti-Goat IR800

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

Semi-quantitative analysis: Western blot



Densitometric Analysis



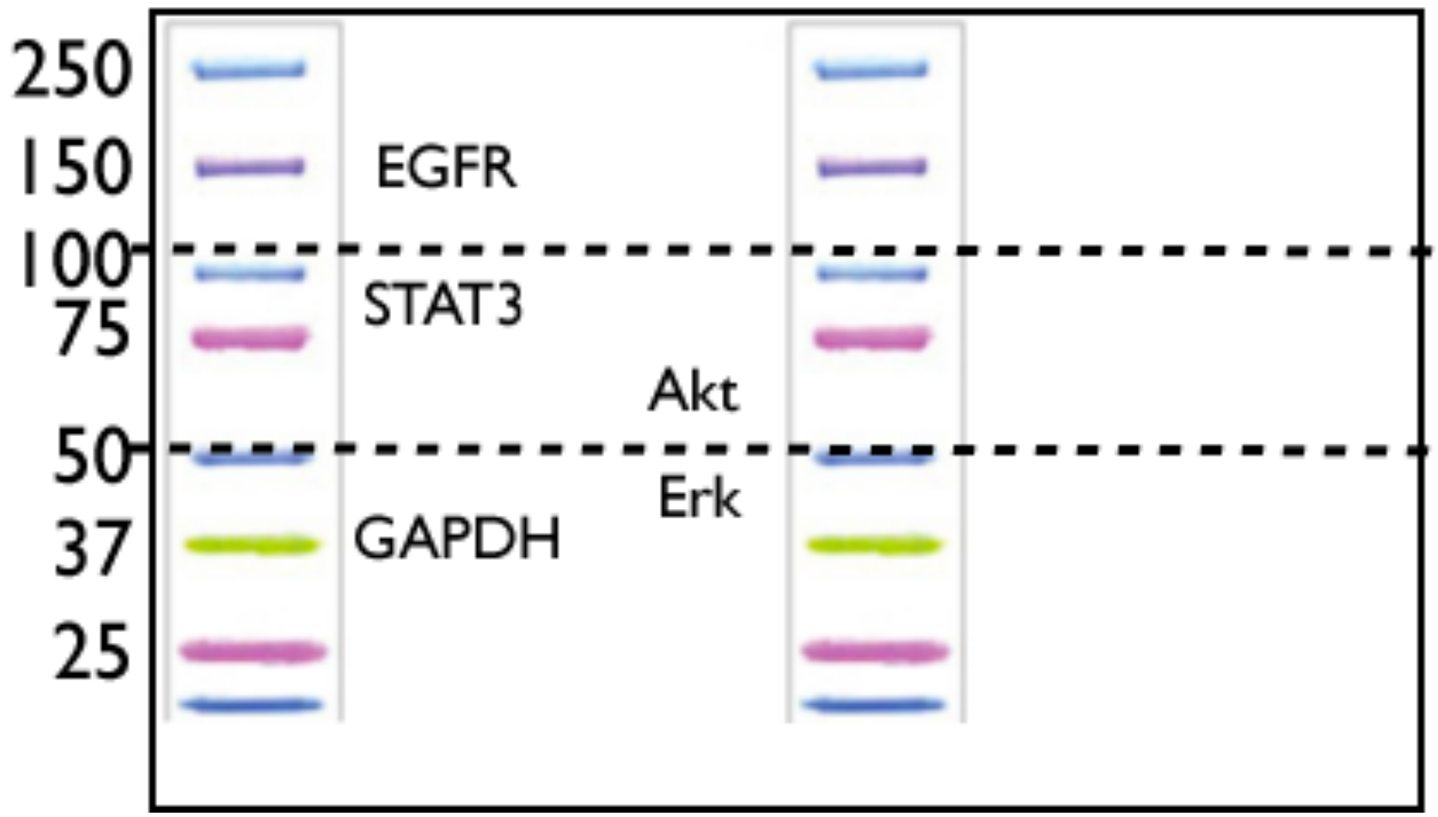
A few signals per lane -- up to ~17 lanes per mini gel.

Semi-quantitative analysis: Your experiment

1. All: pY1068-EGFR & Total EGFR
2. Pathway specific:

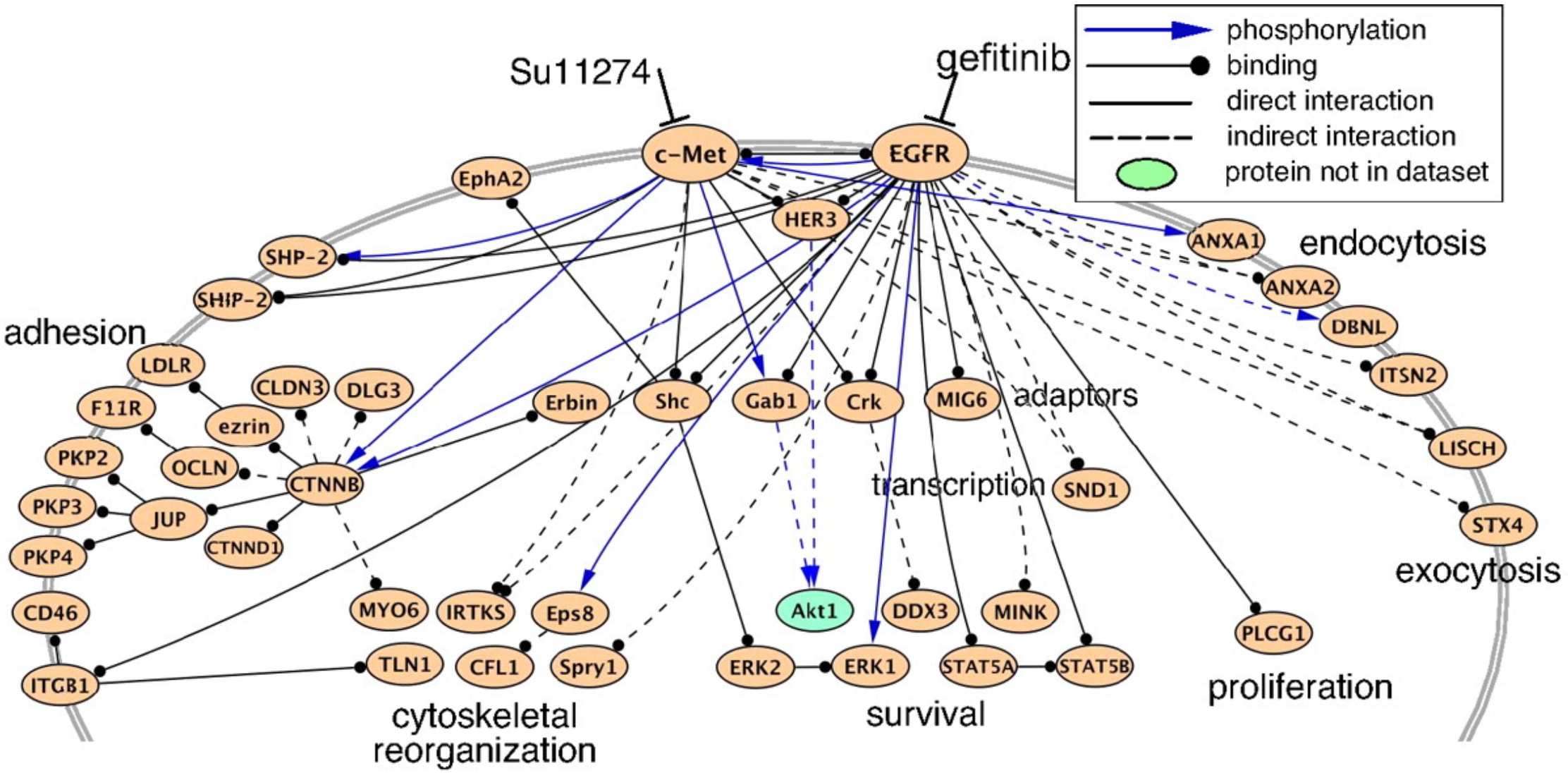
pERK & Total ERK
pAkt & Total Akt
pSTAT3 & Total STAT3

*Last two + GAPDH



Semi-quantitative analysis: Your experiment

What if we wanted a broader network view?

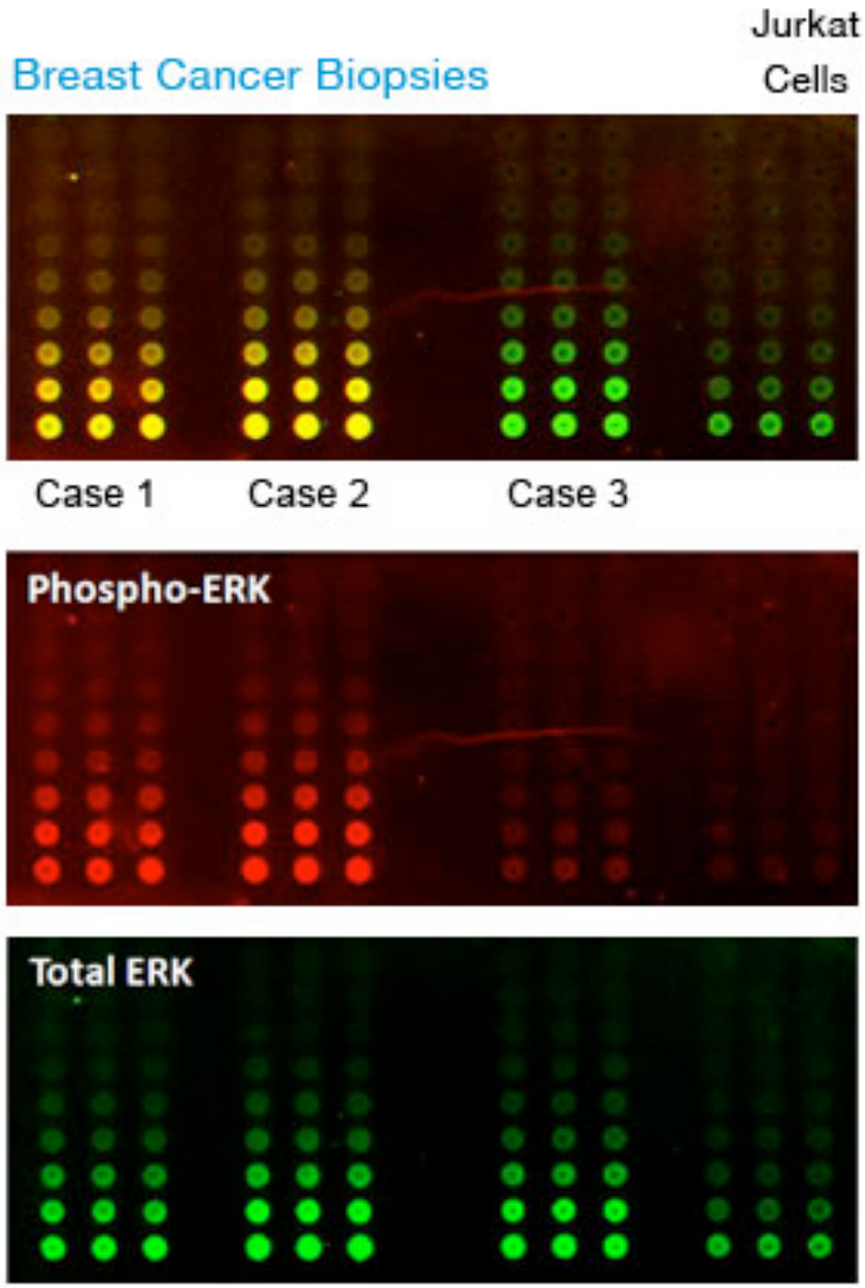


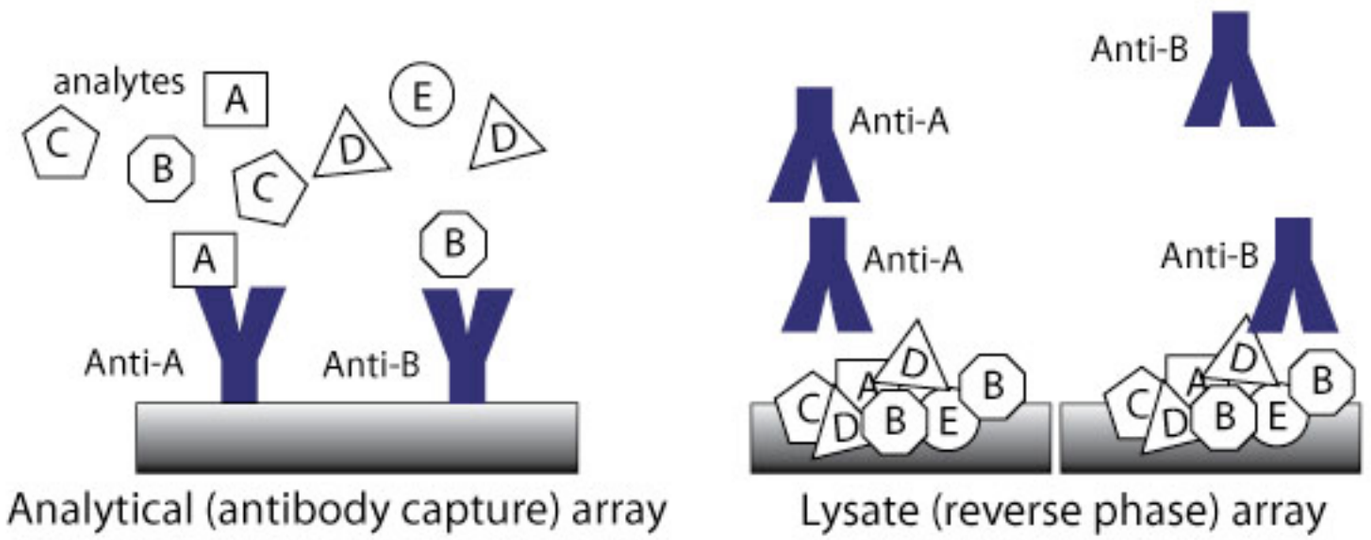
Regulatory networks sensitive to tyrosine kinase inhibitors in H3255 and MKN45 cells revealed by PhosphpScan-SILAC study.

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

PNAS

Semi-quantitative analysis: Protein Microarray

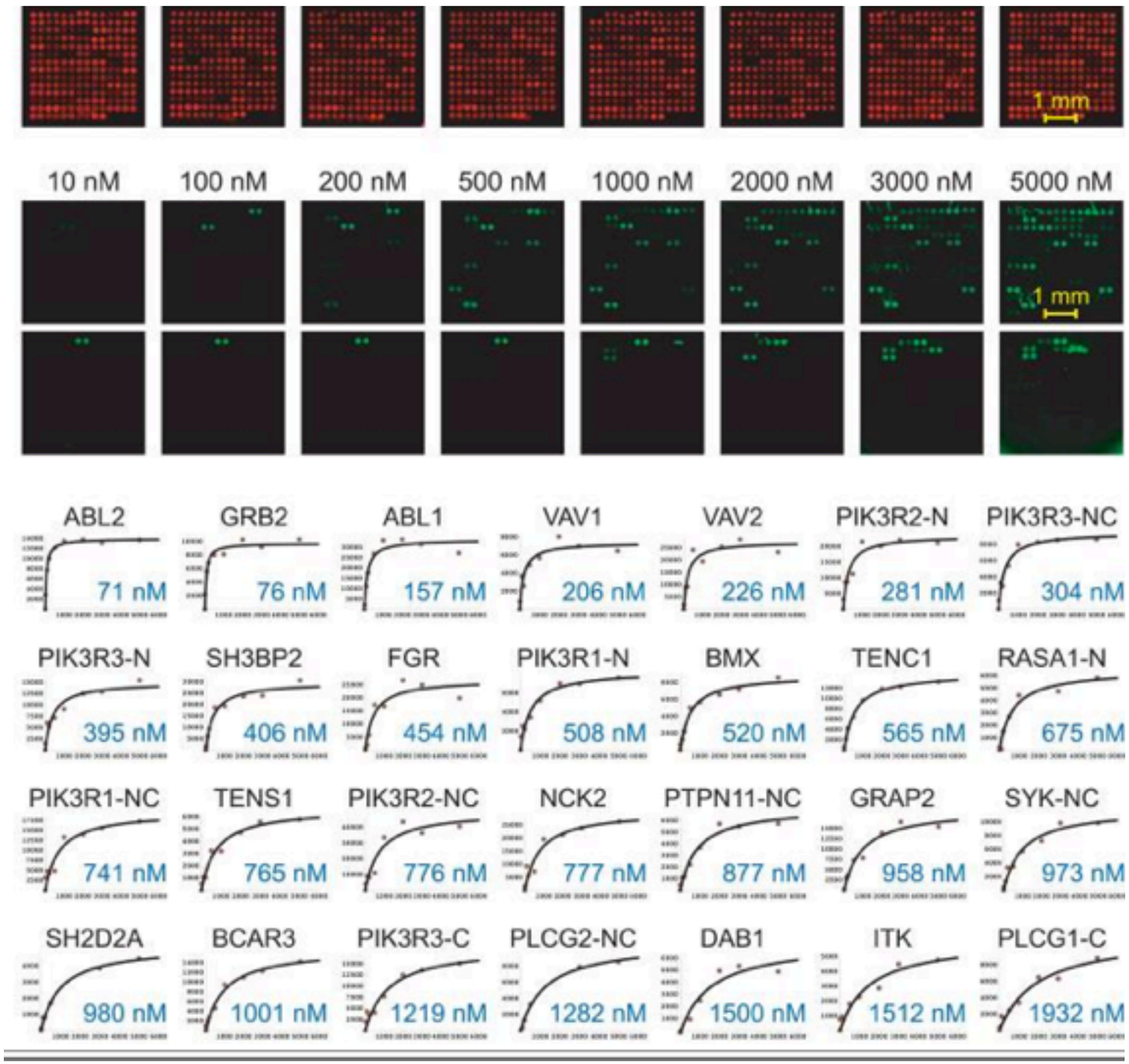




A few signals per spot -- up to hundreds of spots per slide.

http://www.licor.com/bio/applications/odyssey_applications/rpa.jsp

Semi-quantitative analysis: Protein Microarray



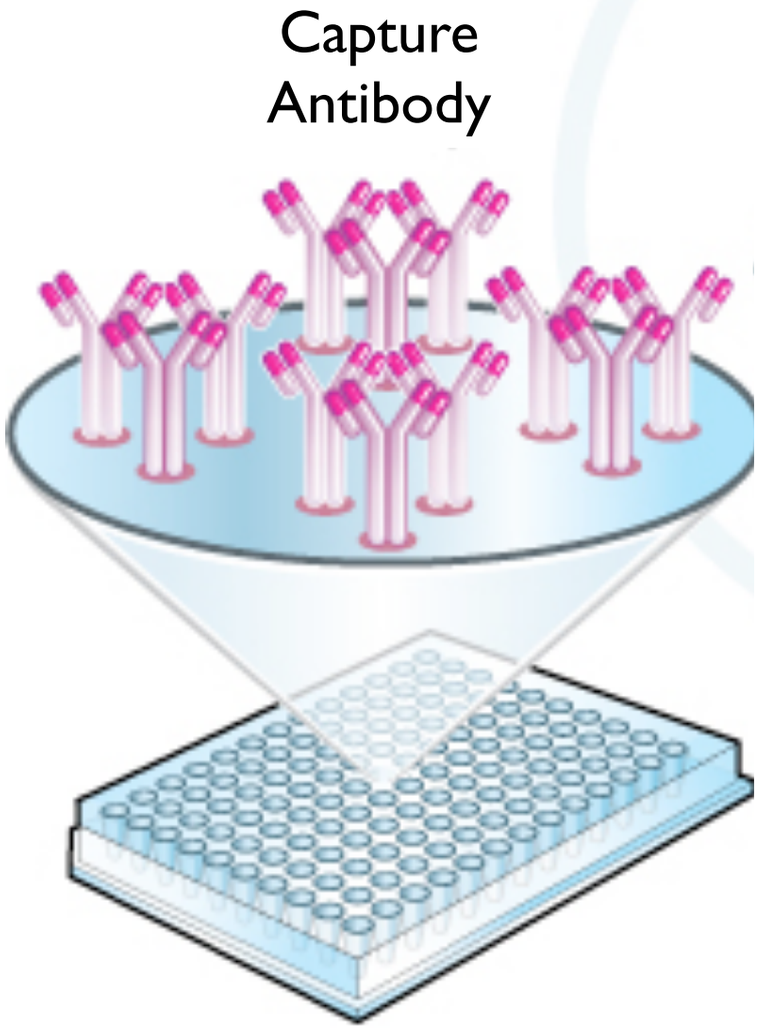
Strength:
Multiplex -- many conditions can be screened

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.

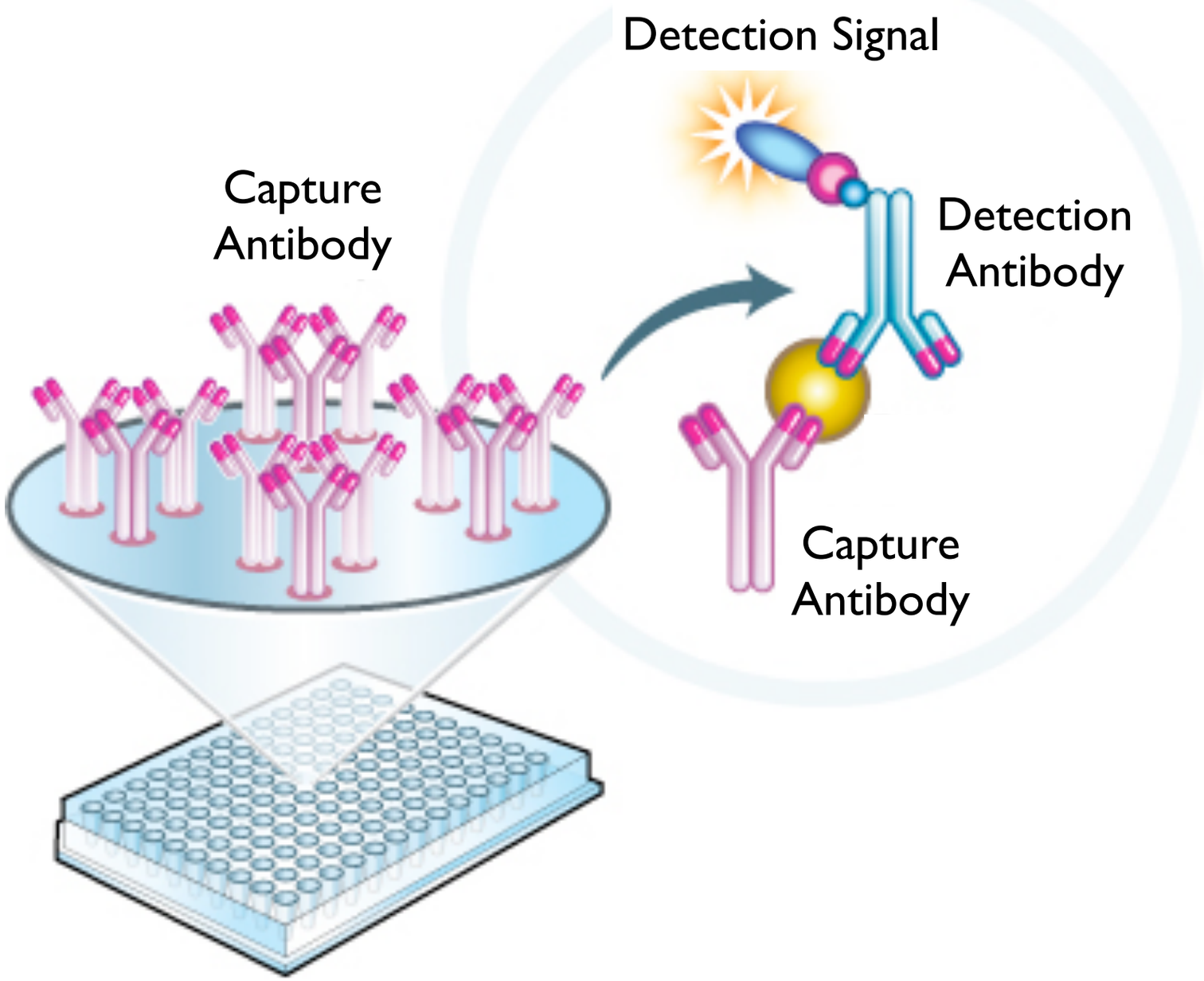
A few signals per spot -- up to hundreds of spots per slide.

Quantitative analysis: ELISA



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

Quantitative analysis: ELISA



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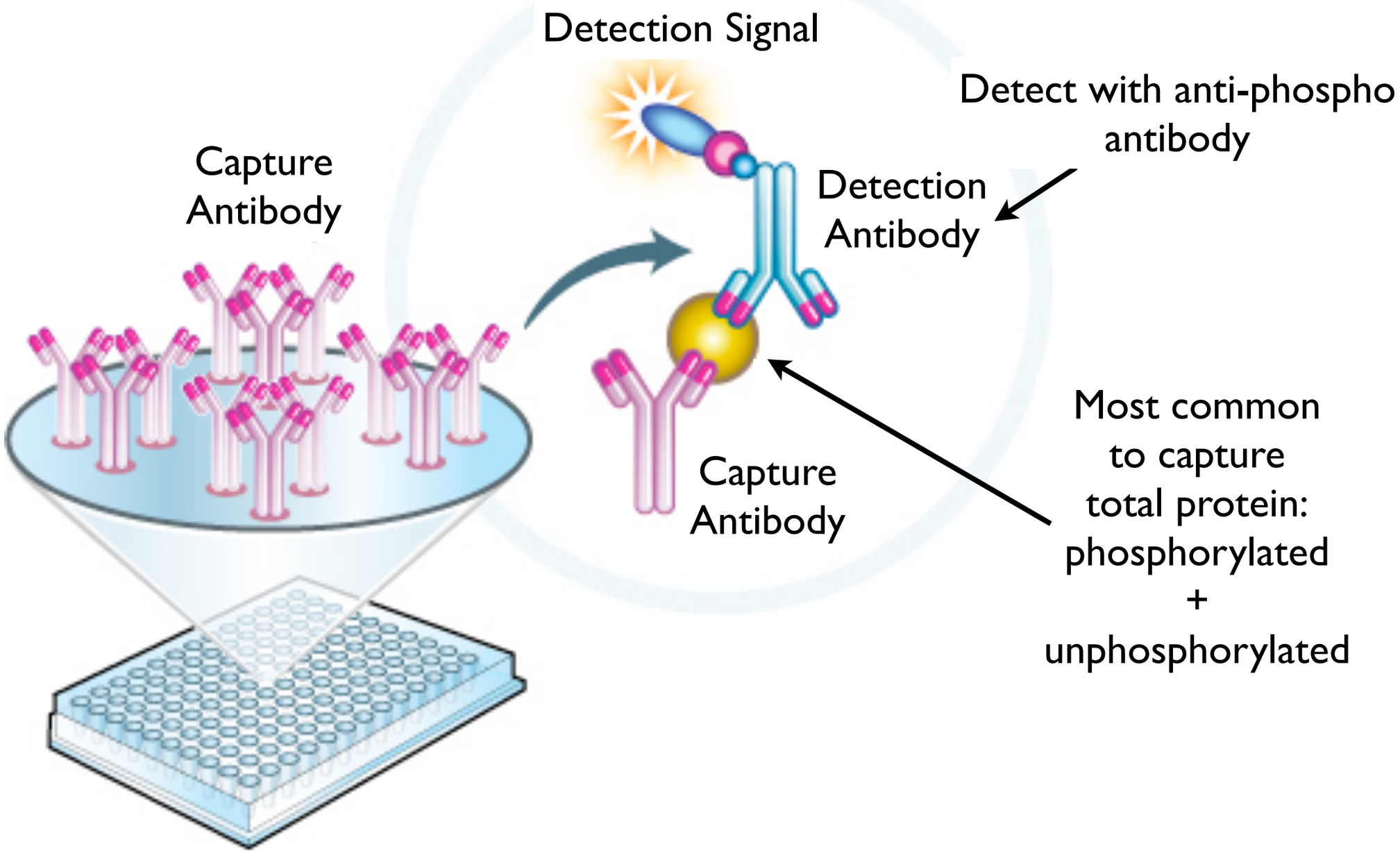
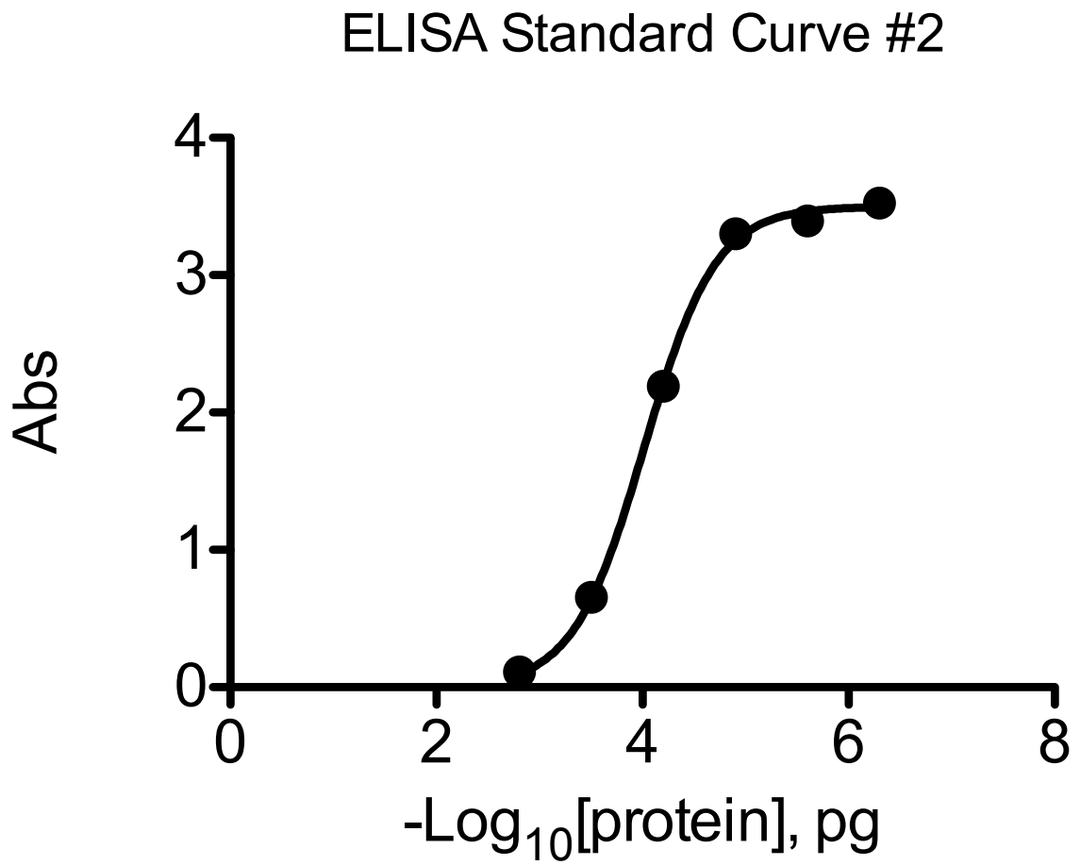


One signal per well - but up to 384 wells/experiment.

Quantitative analysis: ELISA

How to quantify:

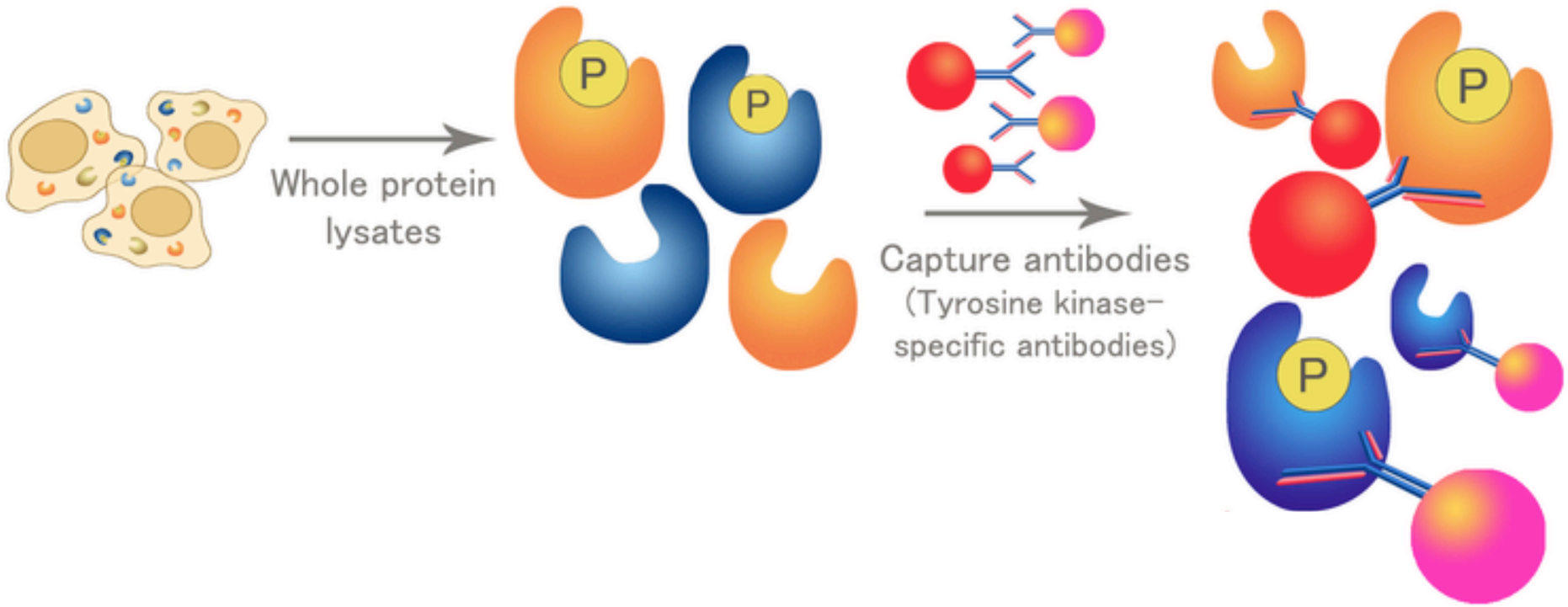
Very often not linear relationships




```
Y=Bottom + (Top-Bottom)/(1+10^((LogEC50-X)*HillSlope))
```

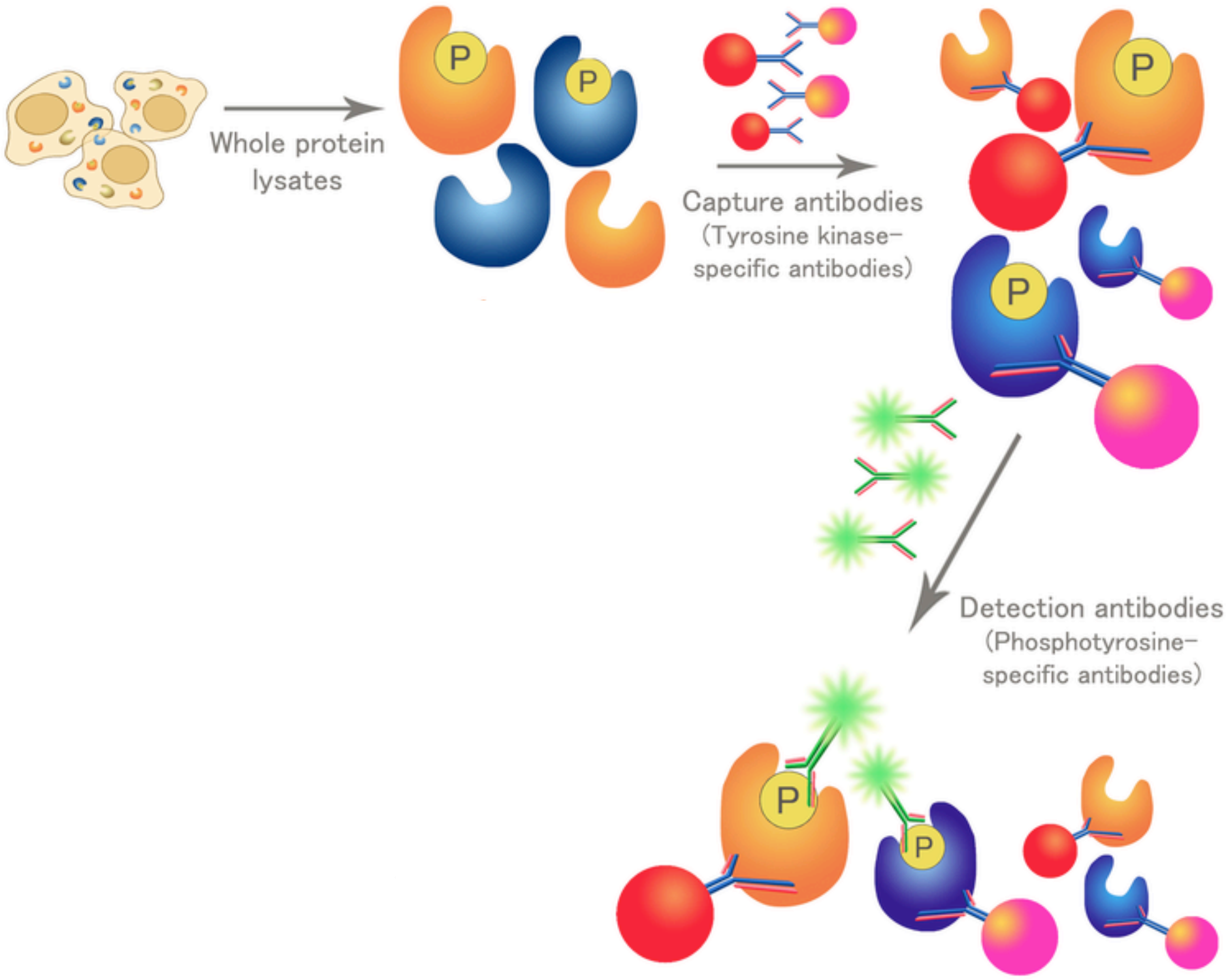
General protocol: Create a standard curve on each plate (usually supplied with kits or make your own)

Quantitative analysis: Multiplexed bead based ELISA



http://commons.wikimedia.org/wiki/File:Workflow_IA.png

Quantitative analysis: Multiplexed bead based ELISA



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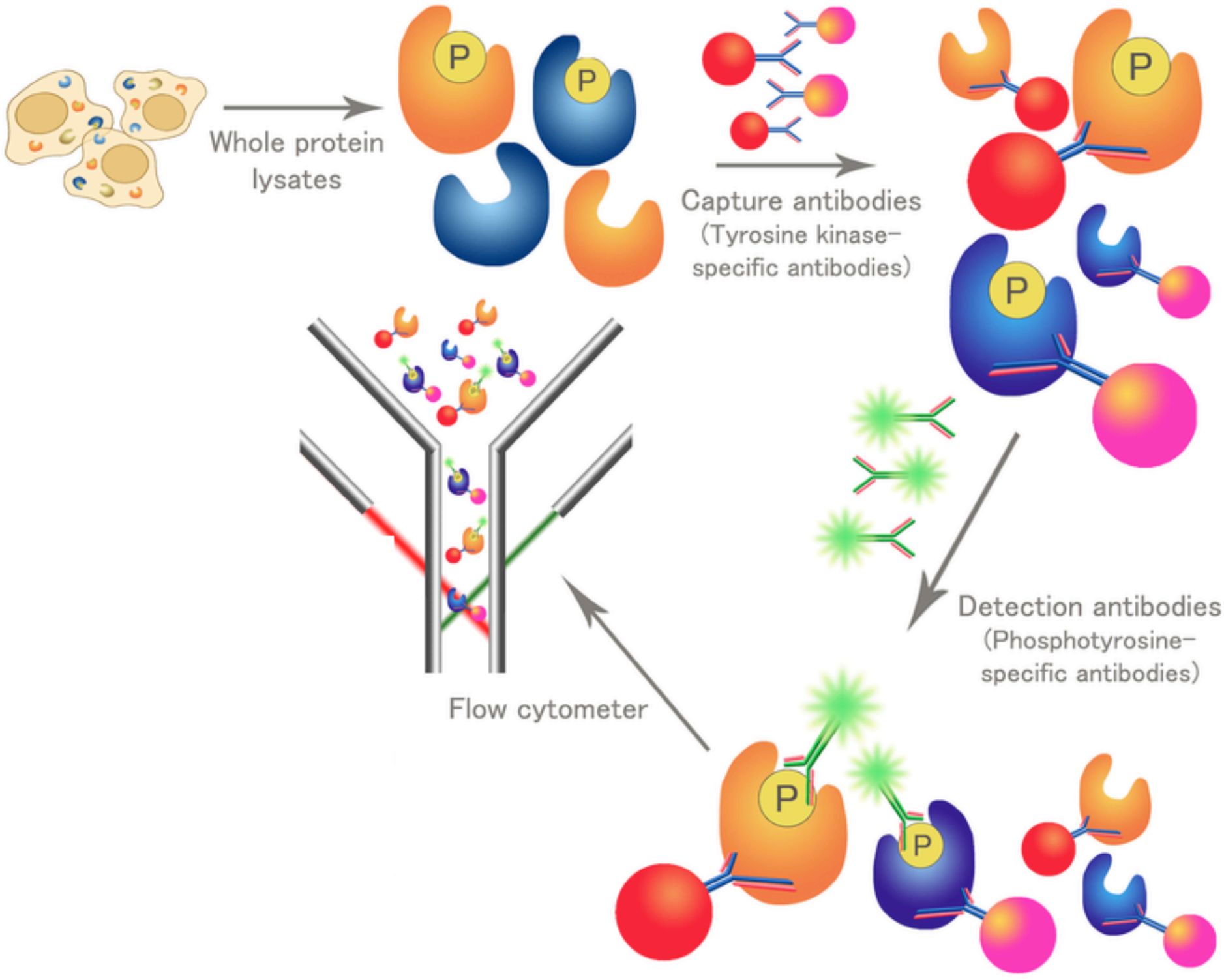
Quantitative analysis: Multiplexed bead based ELISA



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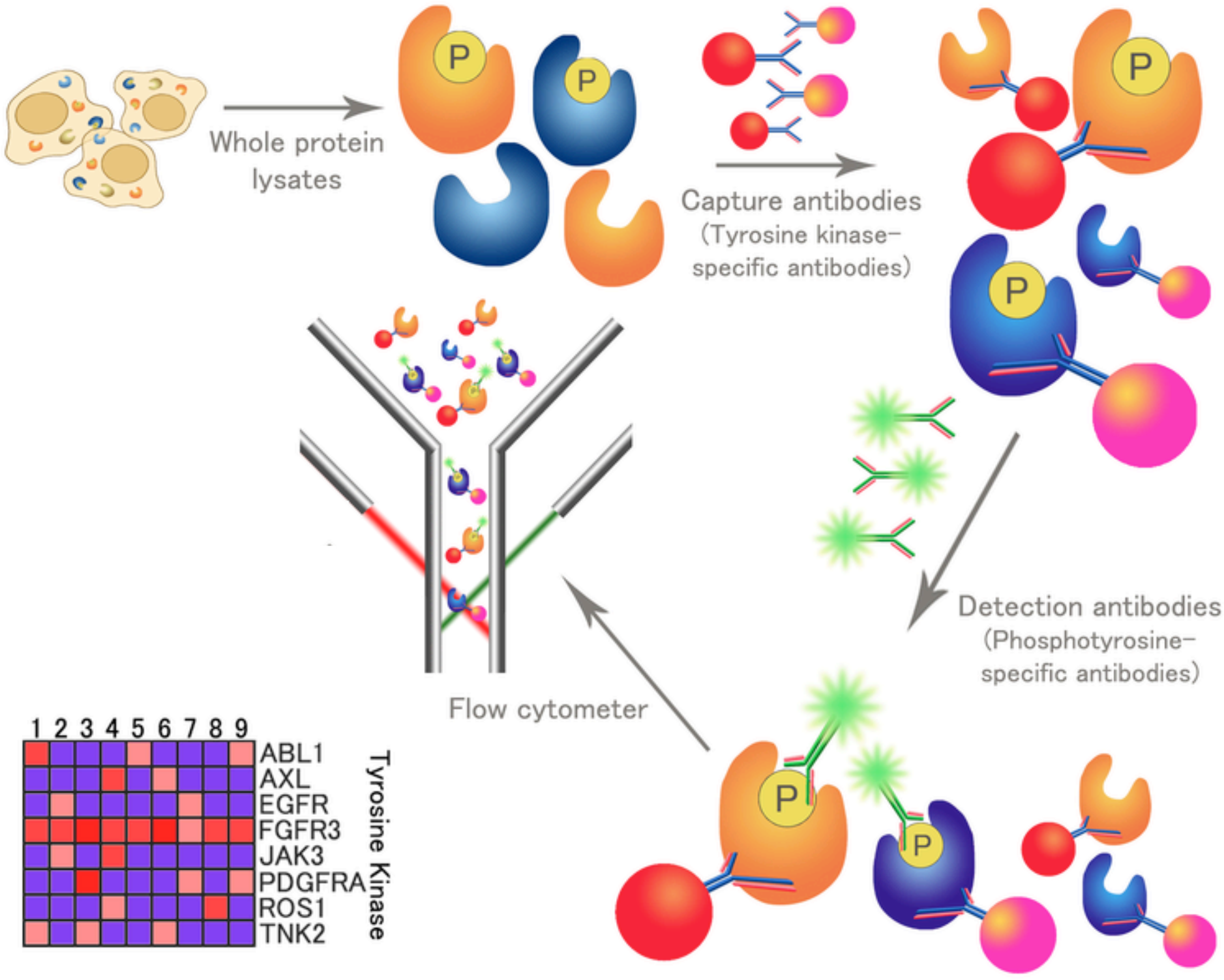
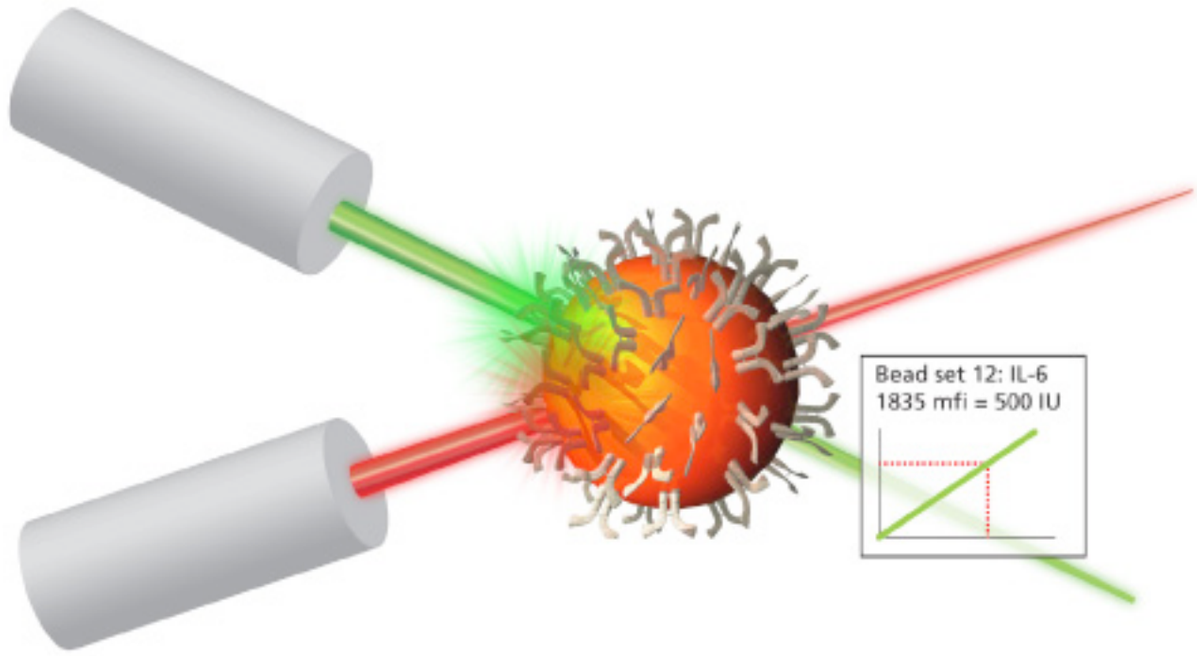


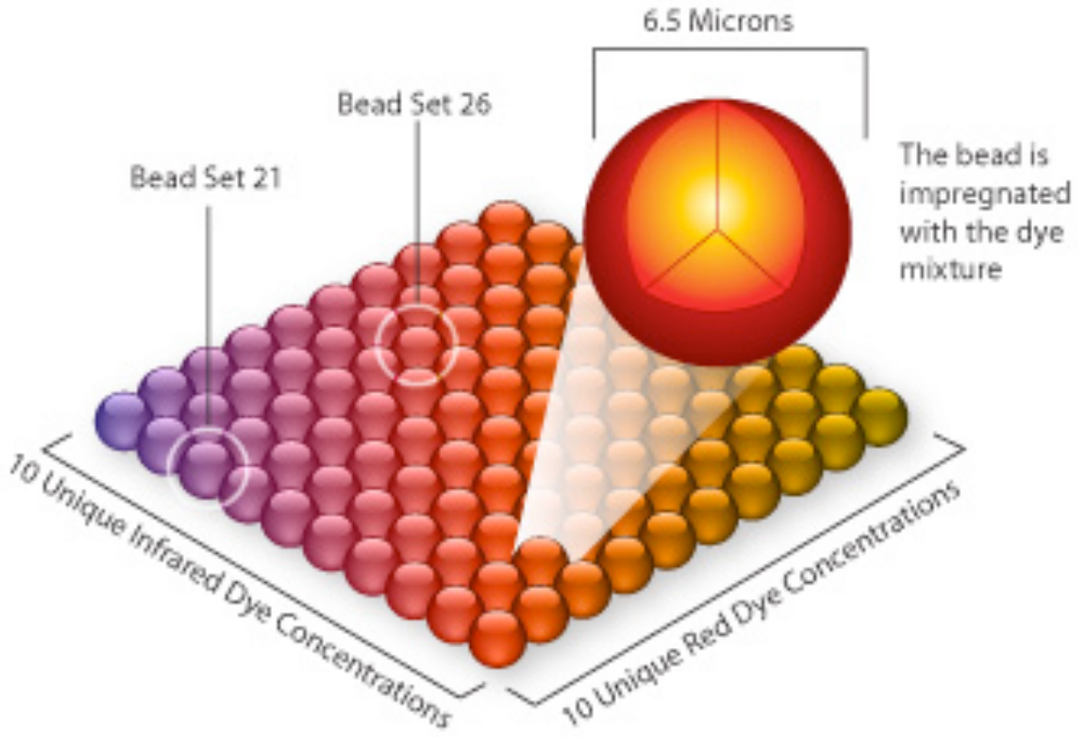
http://commons.wikimedia.org/wiki/File:Workflow_IA.png

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





http://www.ebioscience.com/knowledge-center/product-line/procartaplex/technology.htm

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