

Phosphorylation profiling

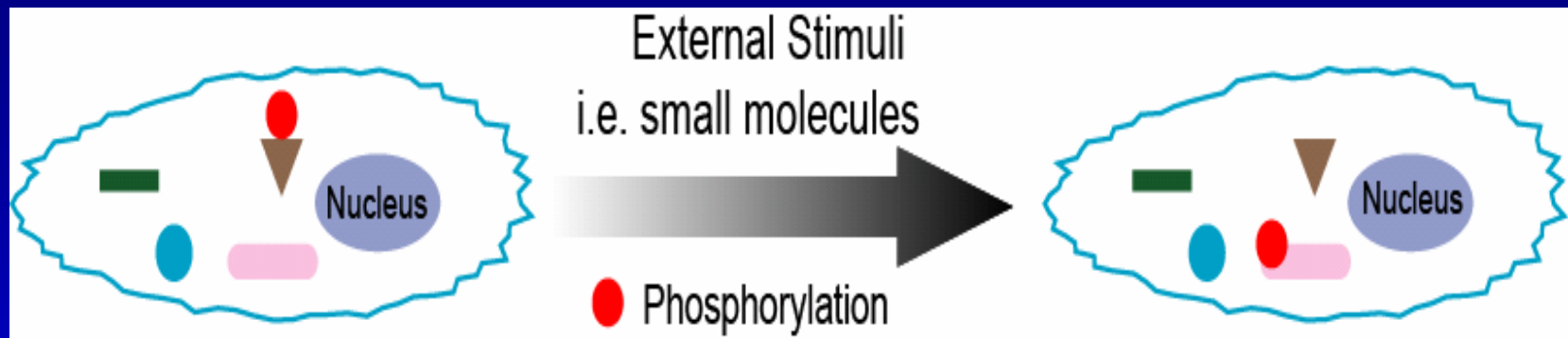
- High throughput label-free detection of protein-protein interactions with the Epic™ system

Meng Wu , Ph. D.

Department of Neuroscience and
High Throughput Biology Center (ChemCore),
School of Medicine,
Johns Hopkins University

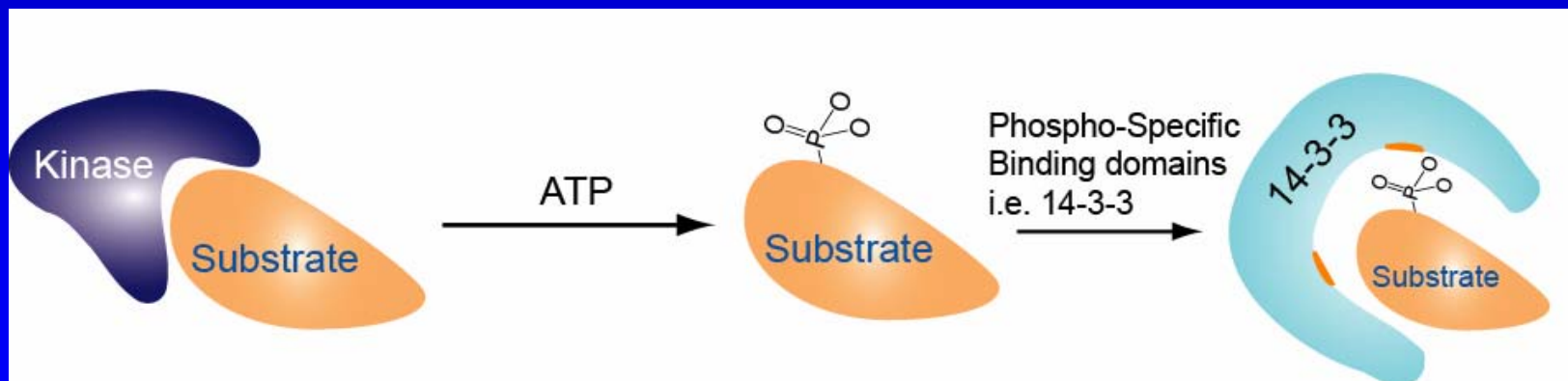
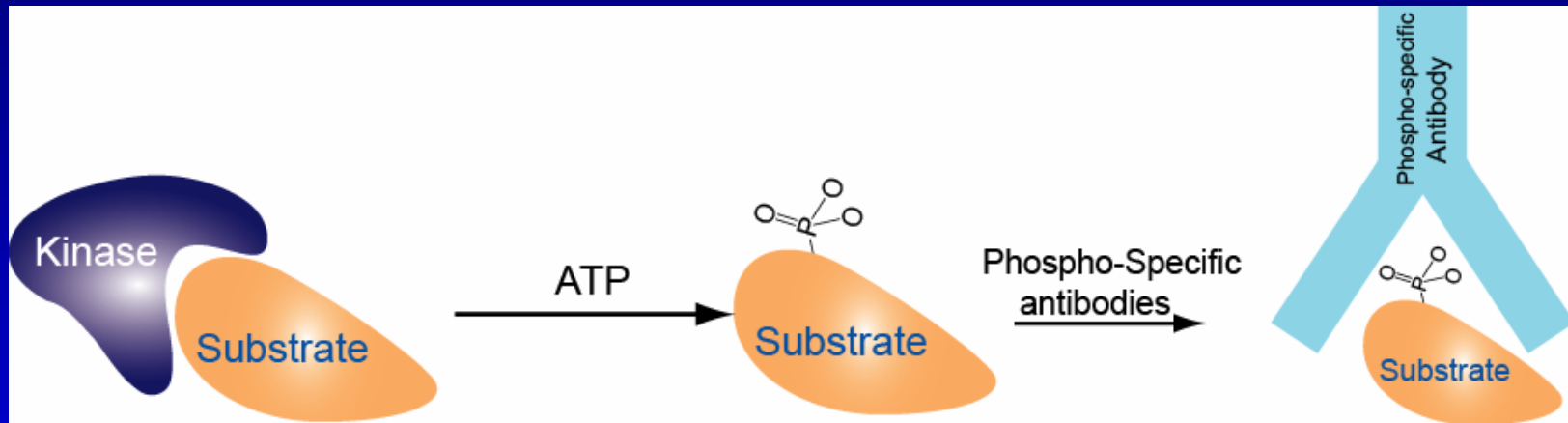


Phosphorylation and its Profiling

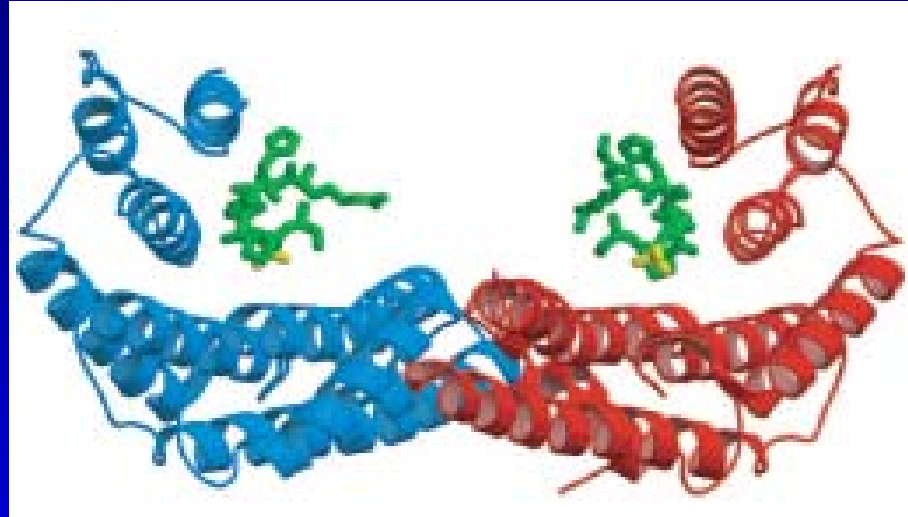


- Profile the phosphorylated proteins
 - As a pattern or “fingerprint” of the cells
 - Specific to cell types, or the presence of cell stimuli, i.e. small molecule
- Currently, most of the protein phosphorylation profiling are achieved
 - Through phospho-specific antibodies
 - With ELISA-like detection schemes (labeled)

Phospho-specific binding domains



14-3-3 Protein



D. Bridges, et al.
Sci. STKE 2005

- 14-3-3 is the first serine/threonine phosphorylation specific binding module
- Our lab has found a 14-3-3 binding motif SWTY as a novel membrane protein traffic signal

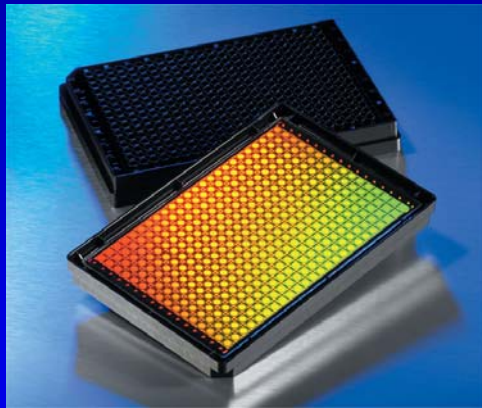
SWTY:
RGRSWTY-COOH

SW_pTY
RGRSW_pTY-COOH

S. Shikano, M. Li, et al.
Nature Cell biology 2005

Corning® Epic™ System as a high throughput label-free optical biosensor

A Platform for High-Throughput Label-Free Detection



Sensor plate

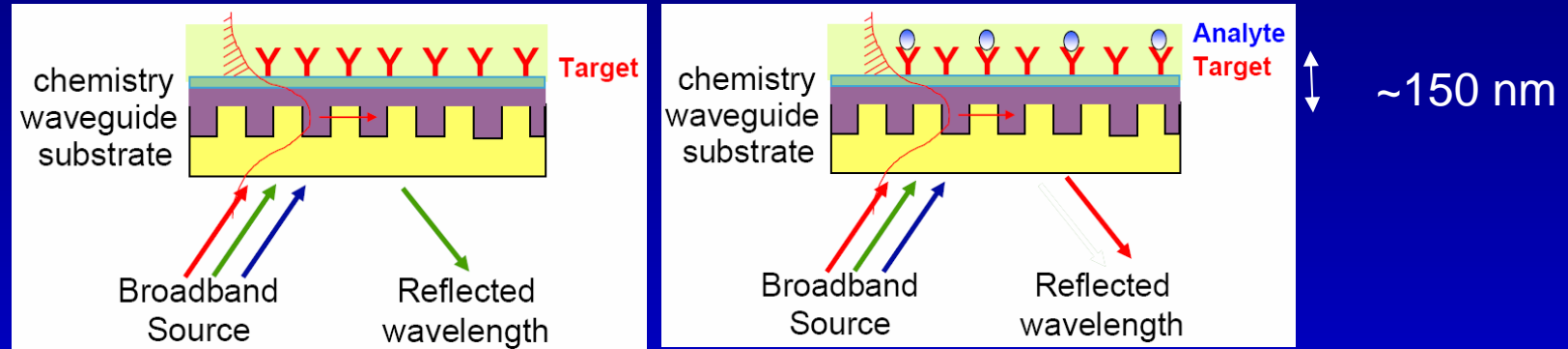


Beta



Latest version

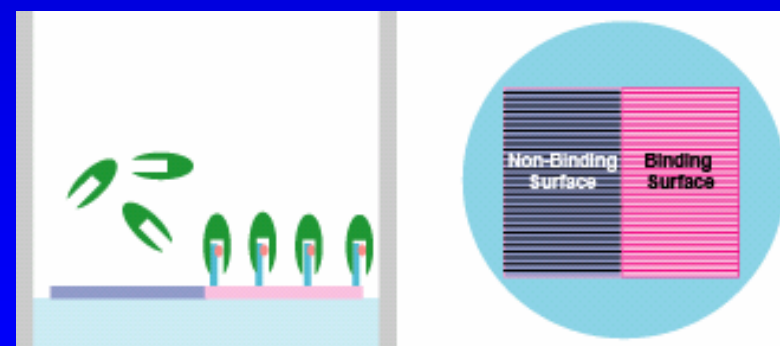
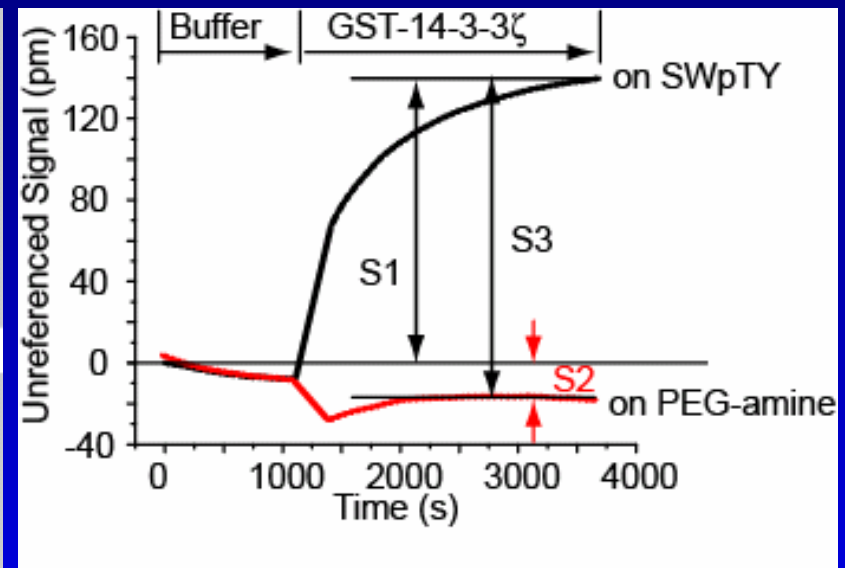
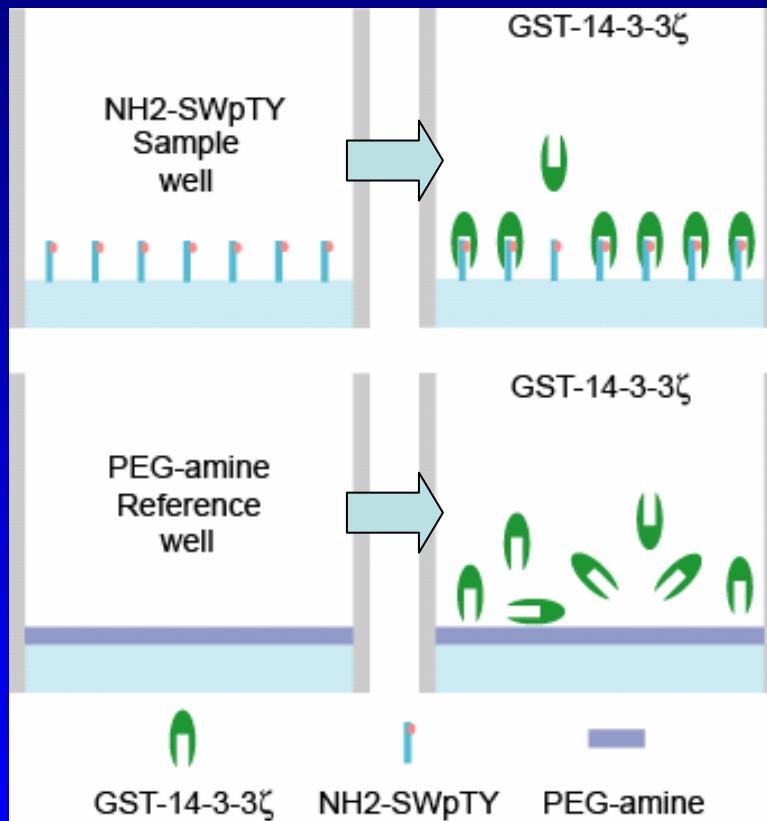
Epic™ System Detection Principle



Resonant Waveguide Grating (RWG) Sensor

- **Label free**
 - Built-in signal transduction on surface
- **Sensitive only to binding events within a 150 nm zone**
- **no need for washing**
 - High throughput compatible
- **High throughput, 384 well format**
 - High throughput capability with addition of different substrates in different wells

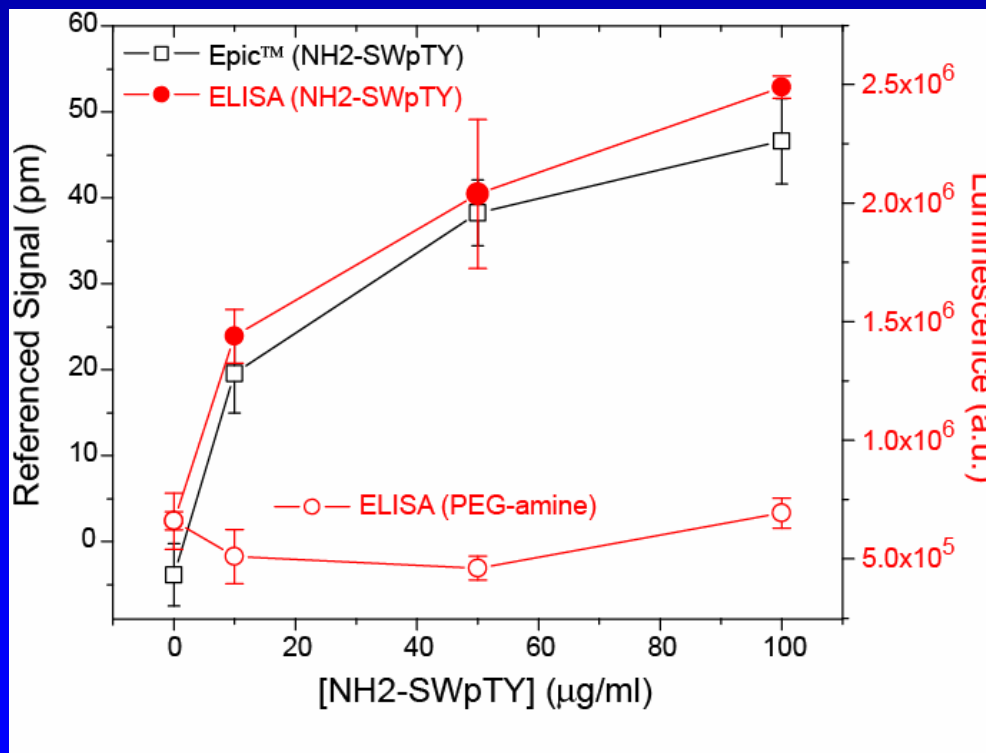
Scheme of Epic™ system detection



Self-reference plate: with the reference in the same well

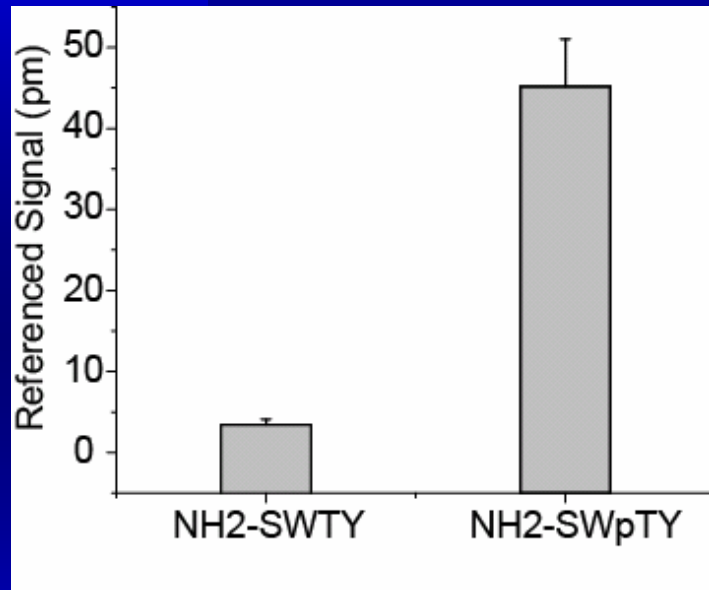
Binding detection of 14-3-3

- Validate that the signal from Epic™ is due to 14-3-3

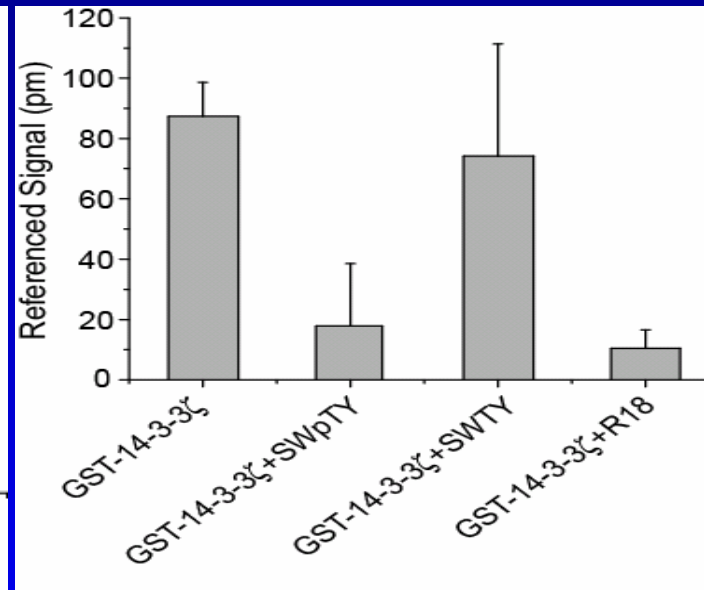


**In-situ
Anti-14-3-3
ELISA**

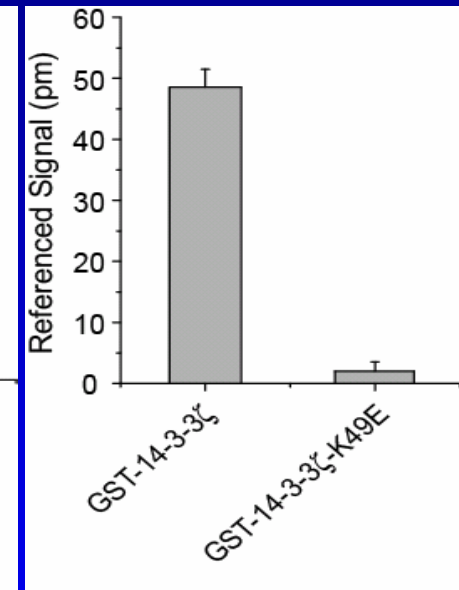
Specificity of detection



Specificity by different immobilized peptides



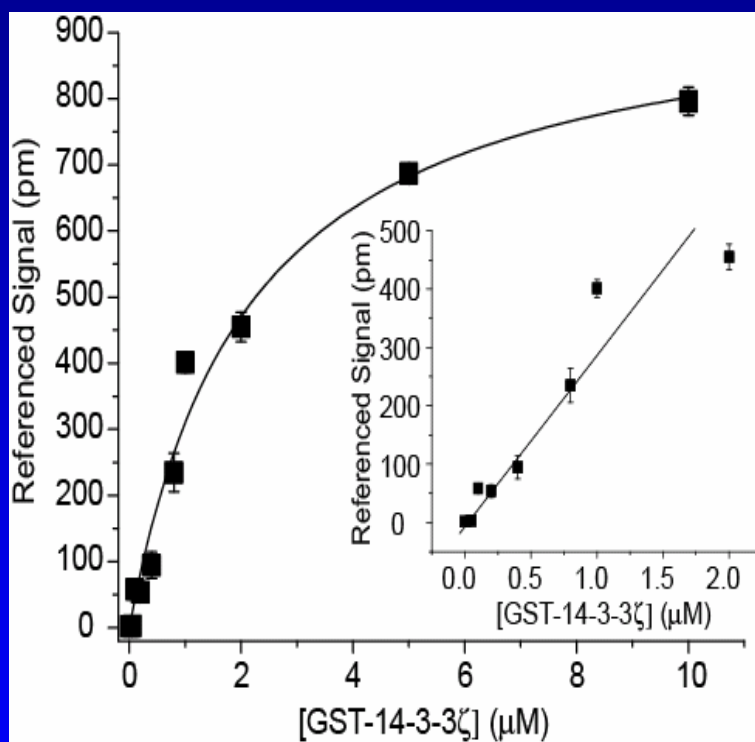
Specificity by competitors



Specificity by binding mutant

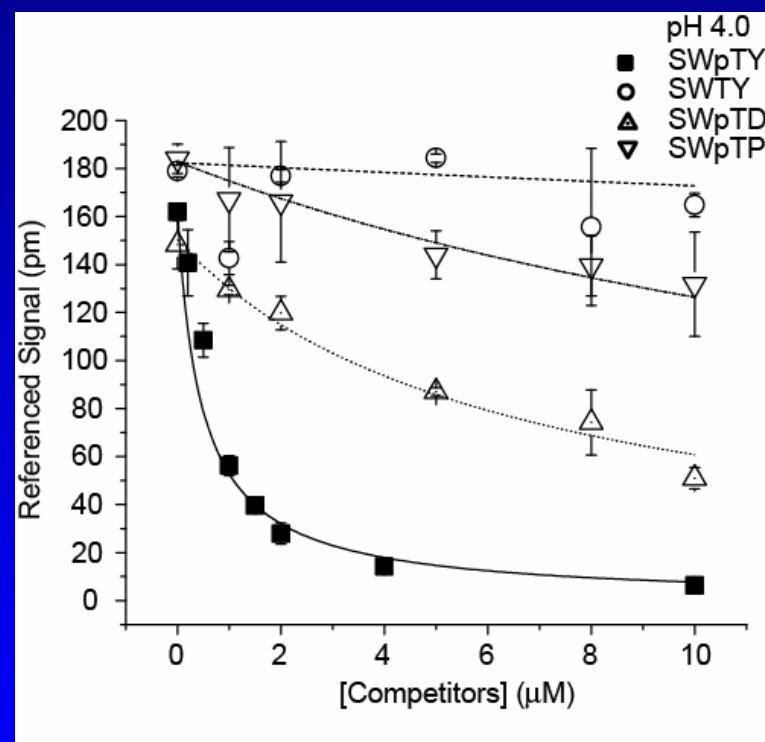
Quantification: Sensitivity and Affinity

- Competitive K_d determination



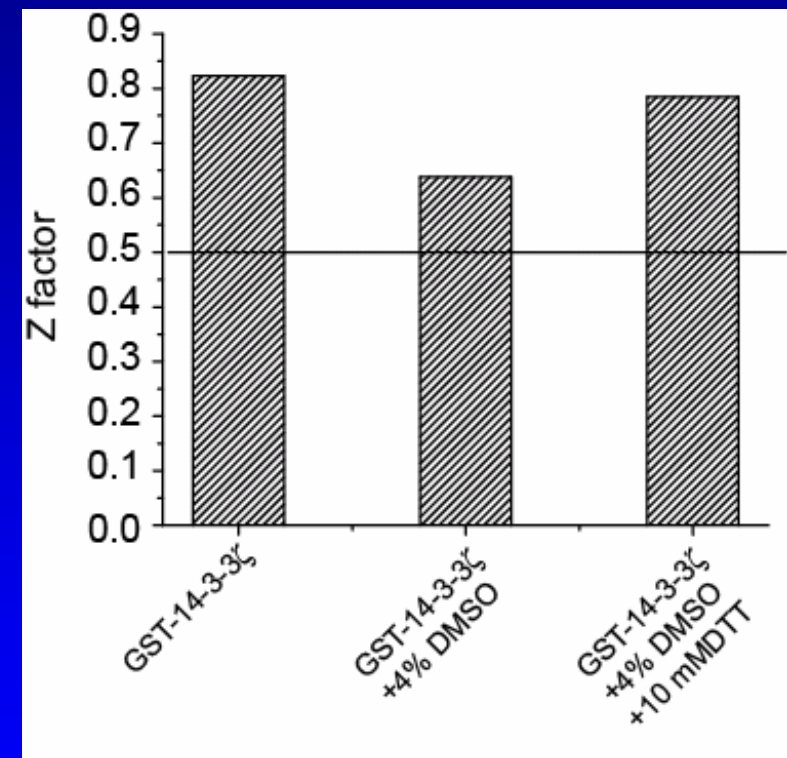
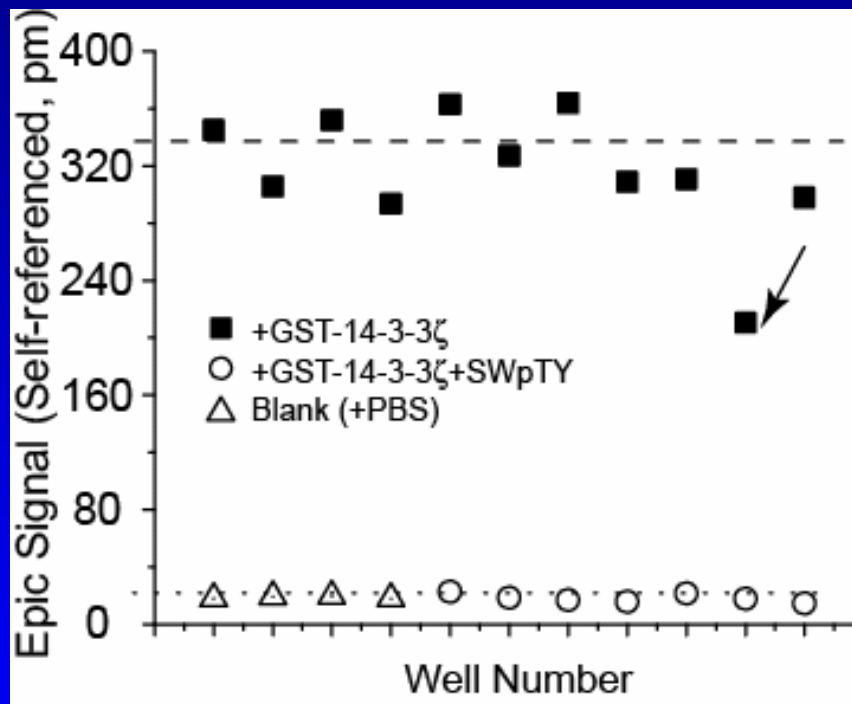
Limit of detection, LOD=38 nM,
Linear range, 0.038-2 μM

$$K_D = 2.1 \pm 0.4 \mu\text{M}$$



Affinity	SWpTY	SWTY	SWpTD	SWpTP
(μM, competitive)	0.12± 0.05	>50	1.75± 0.14	6± 1.67

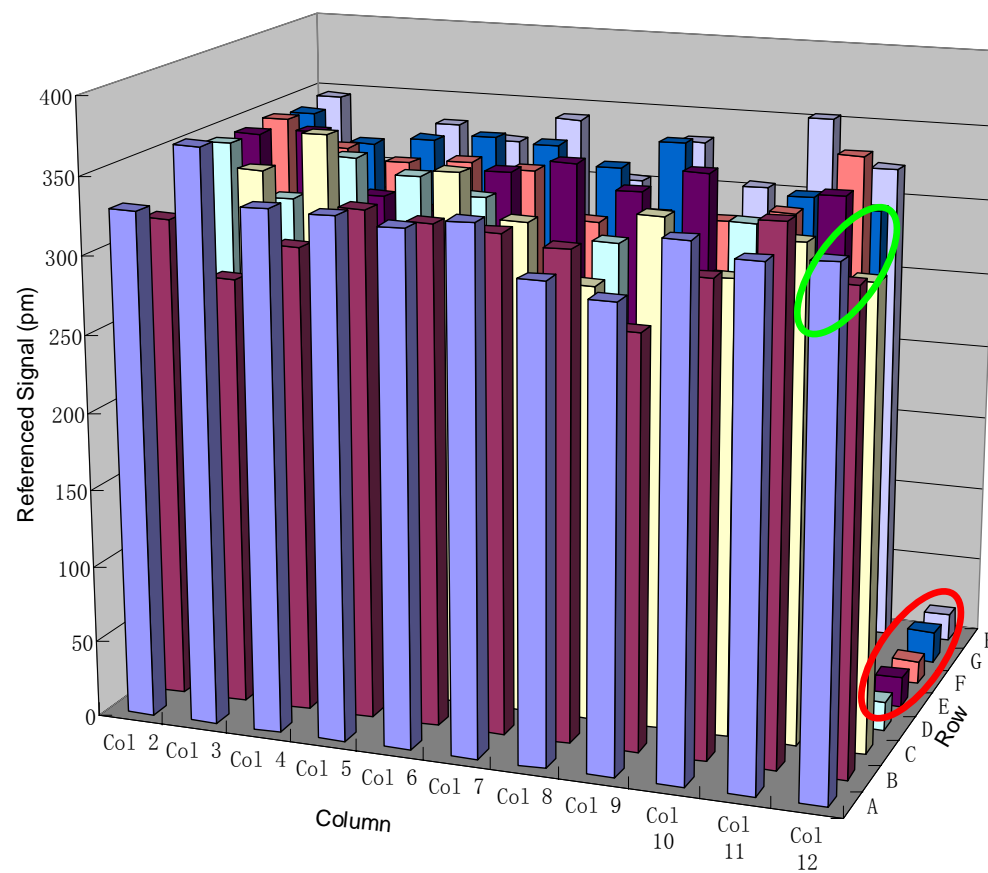
High throughput Screening – S/N and Z factor



S/N \approx 15, Z > 0.5

Small molecule screen trial

1 plate (80 compounds), each in quadruplicate, self-referencing plate



$Z'=0.81$
 $S/N=18$

Why Compare Epic™ system with Fluorescence Anisotropy

- Fluorescence anisotropy is considered as one of the most robust and sensitive HTS methods for studying protein-protein interactions

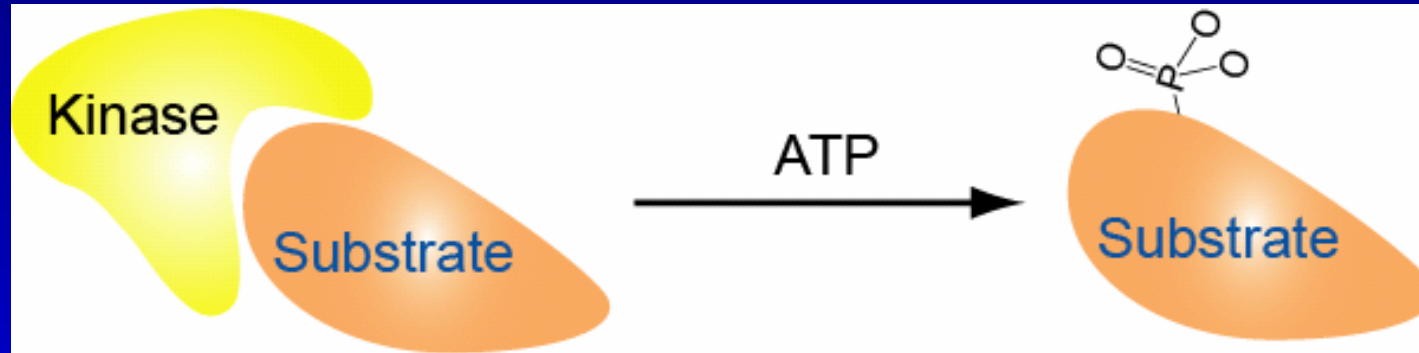
Roehrl, M. H. A., (2004) *Biochemistry*

- Epic™ is a new technology with great potential for the high throughput screening
- In addition, Epic™ is a label-free technology suitable for protein-protein interaction and drug discovery

Comparison of FA and Epic™ System for 14-3-3 assay

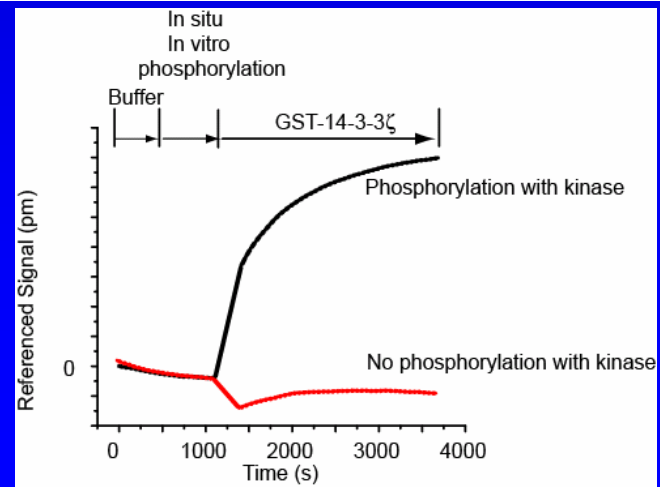
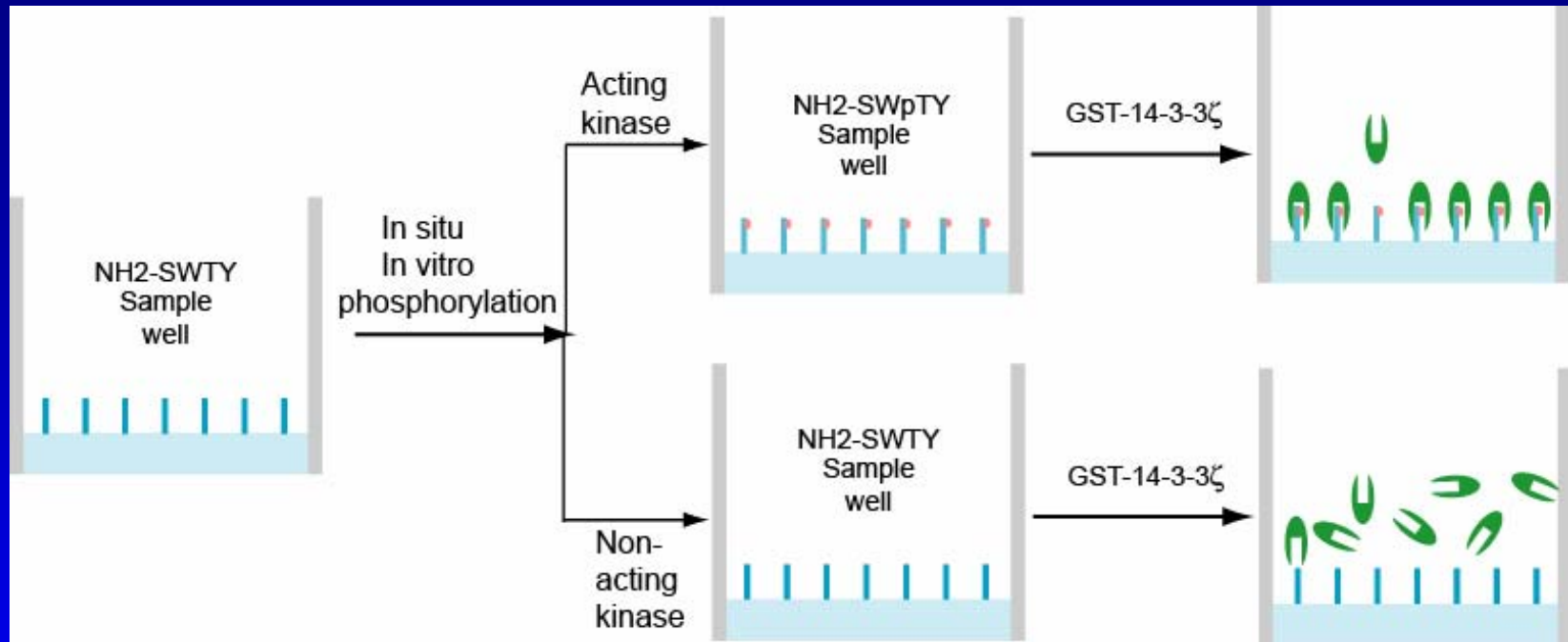
	Fluorescence Anisotropy				Epic™ System			
Sensitivity	16 nM				38 nM			
Linear range	16-700 nM				38-2000 nM			
Specificity	Good				Good			
Affinity (μM)	$K_D=1.7 \pm 0.3$ (GST-14-3-3ζ)				$K_D=2.1 \pm 0.4$ (GST-14-3-3ζ)			
Affinity (μM, competitive)	SWpTY	SWTY	SWpTD	SWpTP	SWpTY	SWTY	SWpTD	SWpTP
	0.17±0.04	>100	2.2±0.3	45±11	0.12±0.05	>50	1.75±0.14	6±1.67
DMSO tolerance	10%				10%			
Z factor	>0.5				>0.5			
S/N ratio	~8.4				~15			

Kinomics

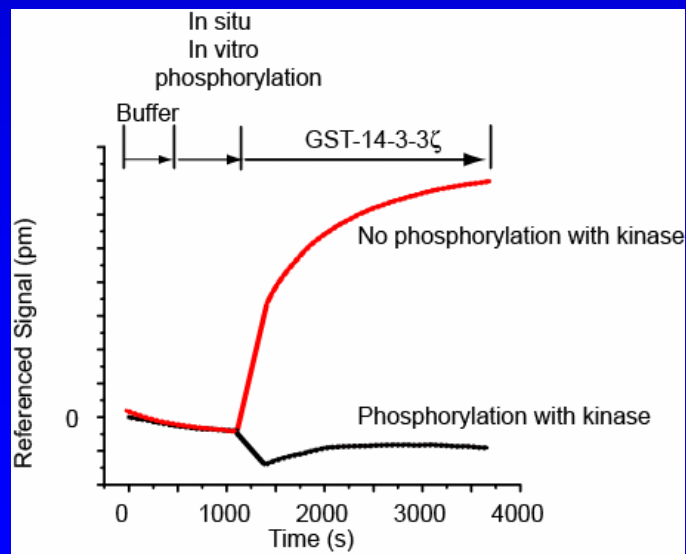
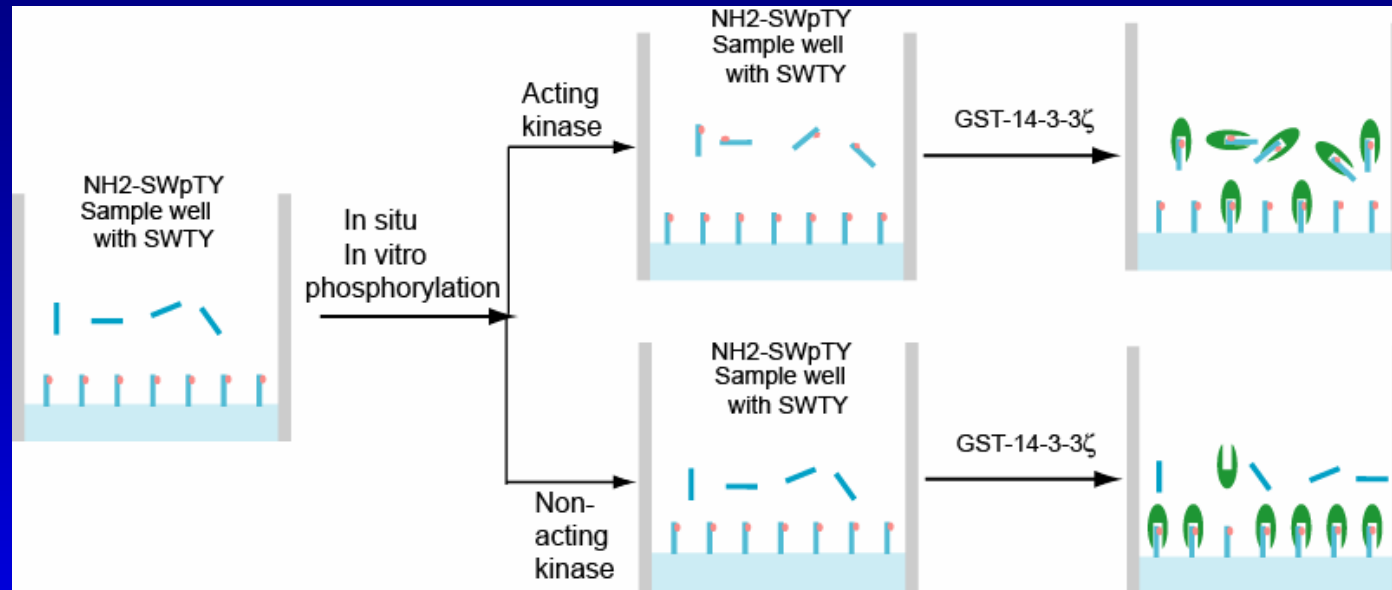


- Currently there is a great need to identify the kinases for the potential protein-protein interactions
- Human 518 kinases, Yeast 122 kinases
- EpicTM system can be used for the global approach for this purpose

Scheme



Scheme II Competitive



Summary

- Phospho-specific Assays have been developed
 - 14-3-3 assay
 - Anti-SWpTY antibody assay
 - Acting kinase Assay
- Assays on the label-free Epic™ system in a high throughput 384 format
- Epic™ system is an exciting label-free technology
 - For phosphorylation-specific protein-protein interactions
 - For the drug discovery

Acknowledgment

- Prof. Min Li
- Members of Li lab and ChemCore
 - Sojin Shikano
 - Haiyan Sun
 - Brian Coblitz
 - Alan Long
 - Jean-Ju Chung
 - Matt Spieker
- Corning Inc. for Epic™ system for β test
 - Anthony G. Frutos
 - Sunil Mukhopadhyay
 - Thomas H. Lynch
 - Ye Fang

CORNING

Epic™
system

Phosphorylation profiling - High throughput label-free detection of protein-protein interactions with the Epic™ system

Meng Wu

Department of Neuroscience and
High Throughput Biology Center (ChemCore),
School of Medicine,
Johns Hopkins University

