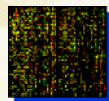


Planar Waveguides: How Nano Layers Enable to Detect Zepto Moles of Macro Molecules in Pico Liter Spots on Micro Arrays

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Zeptosens – A Division of Bayer Schweiz AG

Workshop Photonic Sensors
September 17, 2009

What are Biochemical Microarrays?



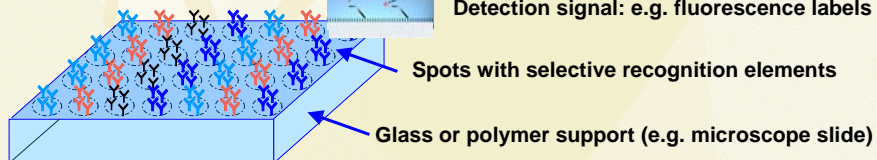
10'000 to 100'000 spots per cm^2 = "photo lithography" arrays



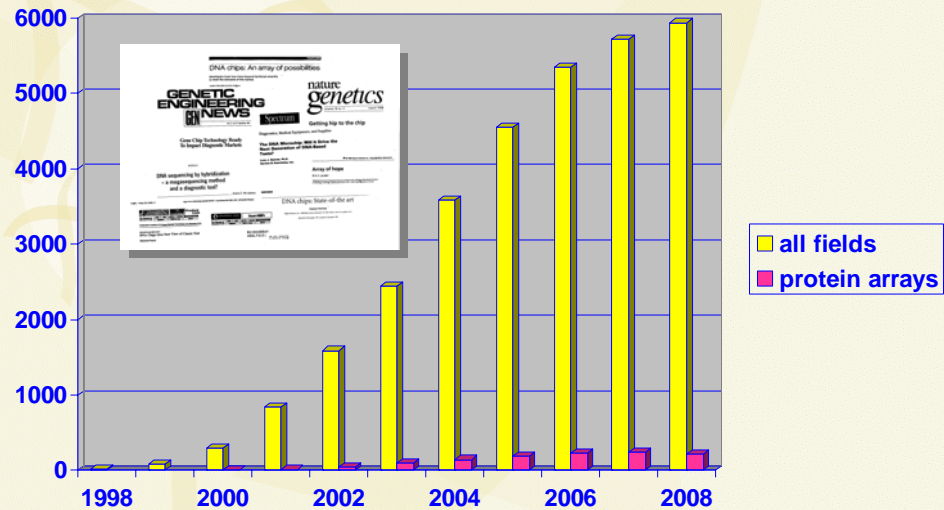
100 to 10'000 spots per cm^2 = spotted arrays



Each spot binds a specific analyte of
the sample solution
Detection signal: e.g. fluorescence labels

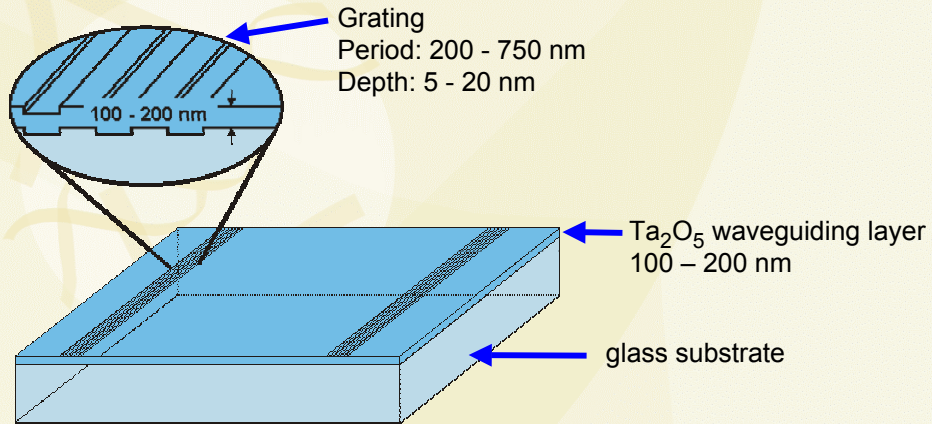


Entries in PubMed Database, Search Term „Microarray“

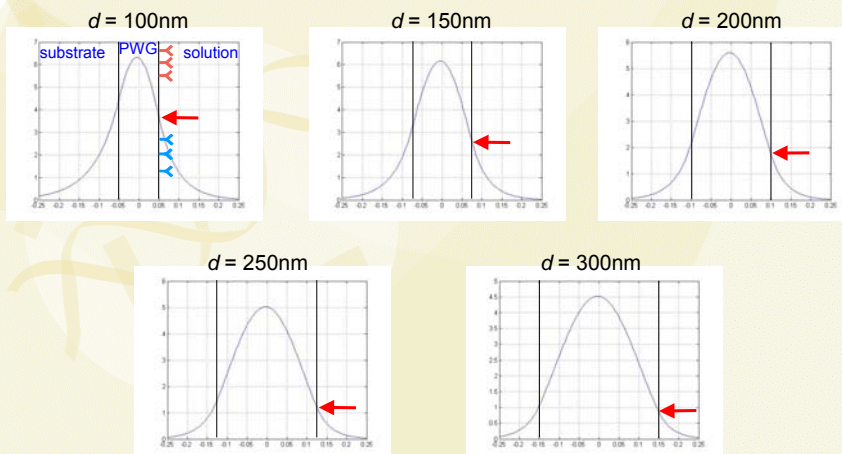


**Microarrays -
Small detection areas
Nanoliters of sample volumes:
Require high detection sensitivity**

Thin Film Planar Waveguide (PWG) Chip Design



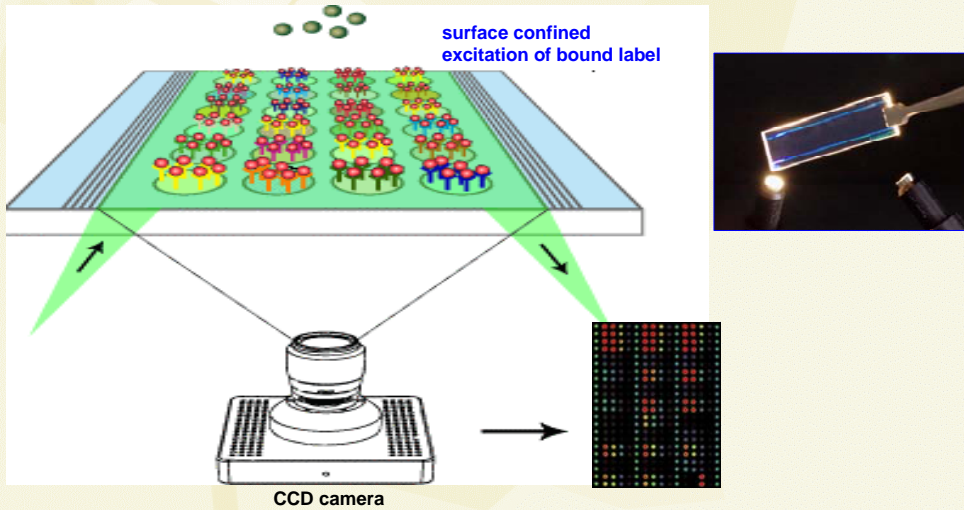
Light Intensity vs. Waveguide Thickness



Parameters:

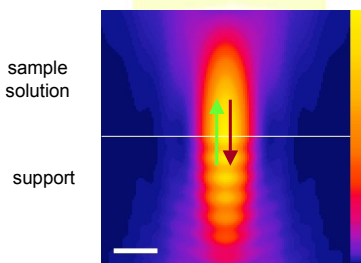
$$n_{\text{sub}}=1.52, n_{\text{sup}}=1.335, n_{\text{PWG}}=2.15, m=0, \lambda=635\text{nm}$$

Planar Waveguide Principle - High Sensitivity Fluorescence Microarray Detection



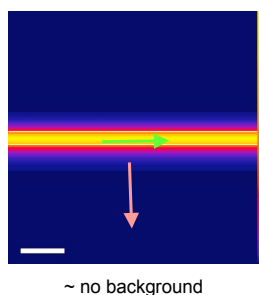
Advantages of Fluorescence Excitation on PWG

Conventional excitation



Confocal excitation:
 Focus depth ~ 2 μ m

ZeptoREADER™ - Evanescent excitation



Evanescent excitation:
 Depth ~ 100nm

- Separation of excitation and detection directions
- Ultimate sensitivity
- Fast time to result
- Less sample preparation
- Direct measurement in blood or serum

ZeptoREADER - Current "PWG Inside" Product



96-well plate format based system

- 32 samples per array (Reverse Protein Array format)
- Extended walk-away time using 360 array autoloader
- 120'000 data points in 6 hrs

ZeptoREADER Setup

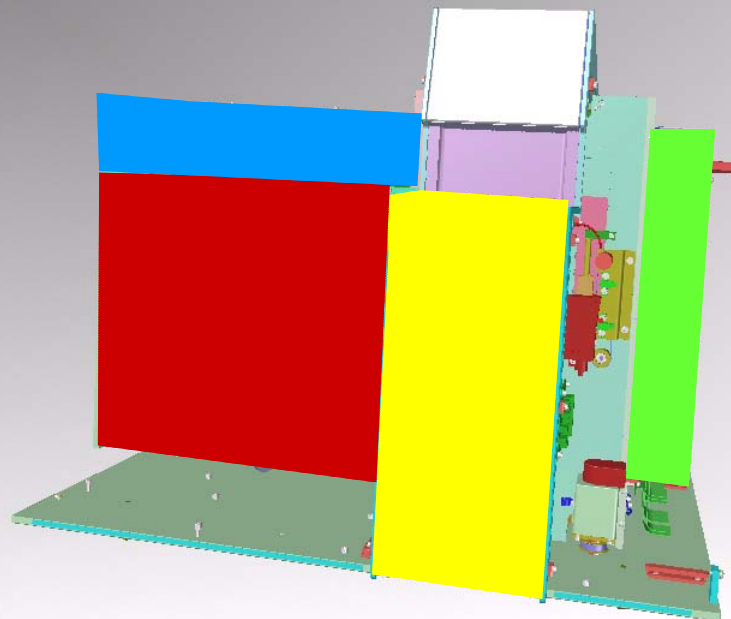
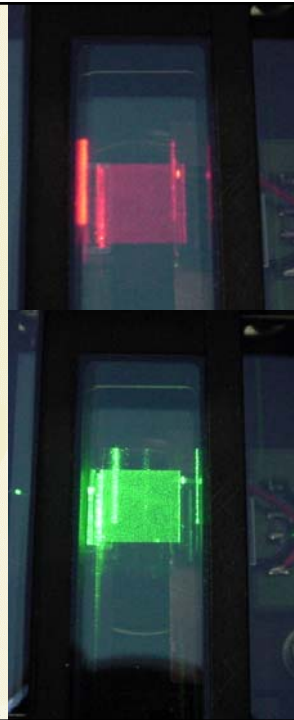
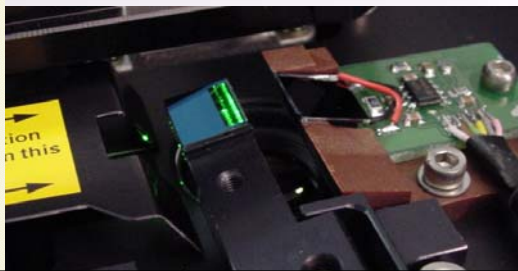
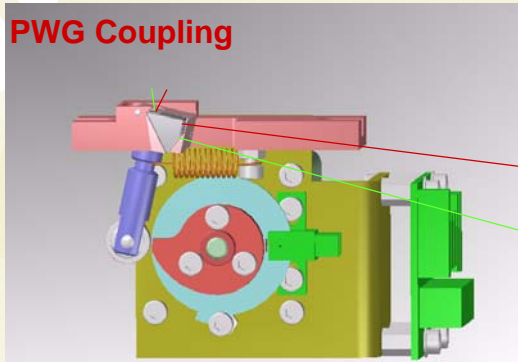


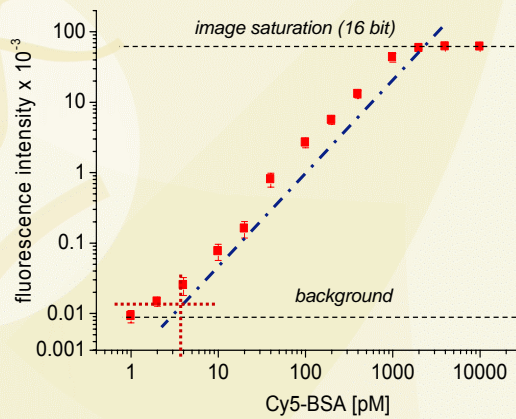
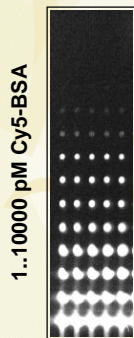
Plate Storage
Electronics
Optics
Measurement
Chamber

PWG Coupling



High Sensitivity of PWG Signal Detection

Dilution series

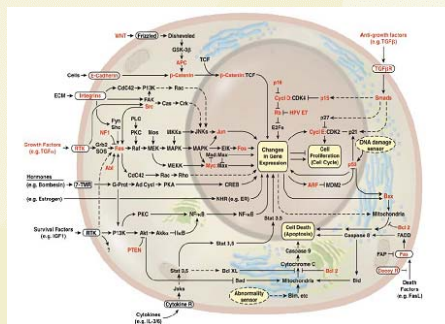


➔ **LOD = 1 zeptomol (600 proteins) per spot**

Why is it important to capture the action of a pharmaceutical compound in a cell?

Diseases as a Consequence of Malfunctioning Pathways

- About 350 genes repeatedly encountered in mutant forms in cancer
- Mutations impact cellular signaling pathways
- Malfunctioning pathways cause diseases



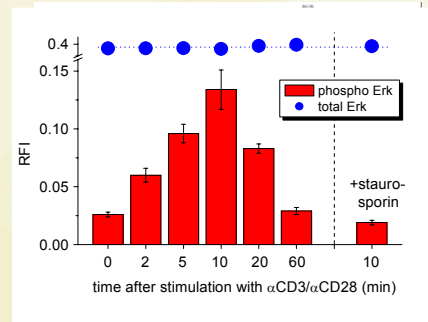
From D. Hanahan and R. A. Weinberg Cell (2000), 100, 57–70,

- Cure disease by influencing pathway activities
- Select compounds based on pathway activity profiles

Multidimensional Pathways Require Multidimensional Assays

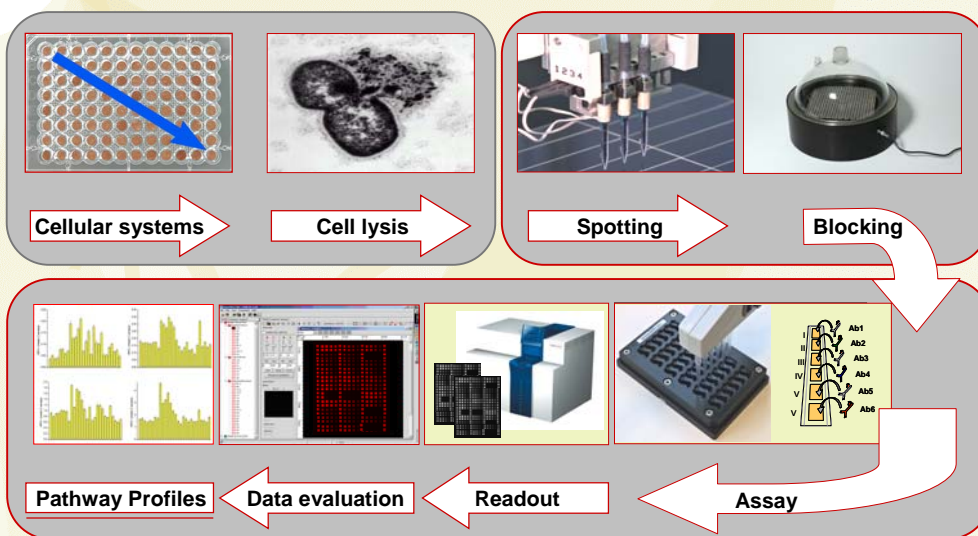
Signaling pathways are multi-dimensional

- Pathway branching
- Pathway crosstalk
- Feedback loops
- Activations occur in minutes
- Expression changes occur in days

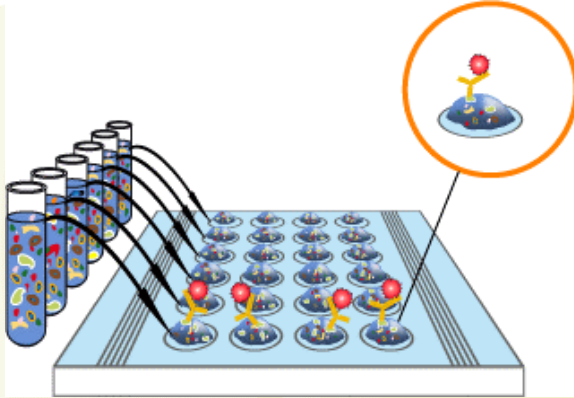


- Extensive mapping of pathway markers at different time points
- An array-based solution provides the scalability and throughput

ZeptoMARK Reverse Arrays – From Cells to Protein Profiles



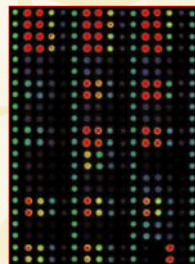
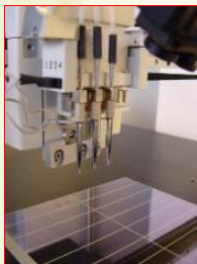
Reverse Protein Microarrays



Array of samples

- Samples are denatured
- Sample volume 400 pL
- Sample concentration is ~ 0.2 mg/ml
- Spot = ~1 cell (protein content)
- Specific detection with target-specific antibodies

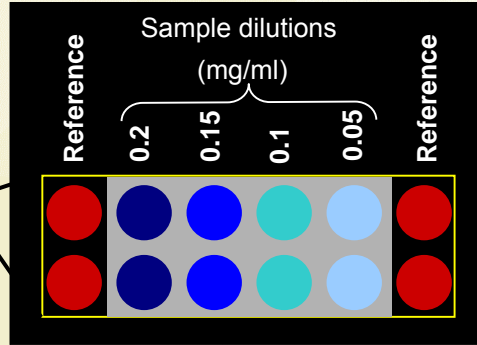
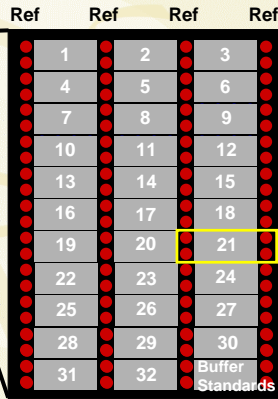
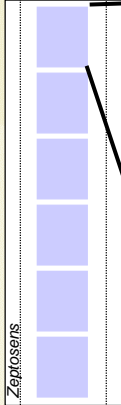
Spotting: Reproducibility & Quality



- 400pL sample volume
- Non-contact ink-jet spotting technology
- Up to 256 lysates/array or 1536 lysates/chip
- Reproducibilities of mean spot signals: CV's $\leq 2\%$

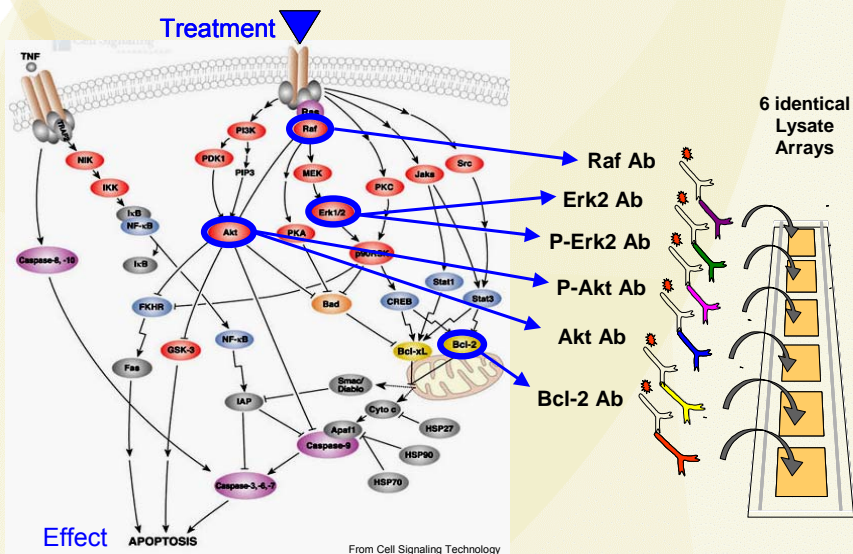
Chip and Array Layout

6 Arrays / Chip

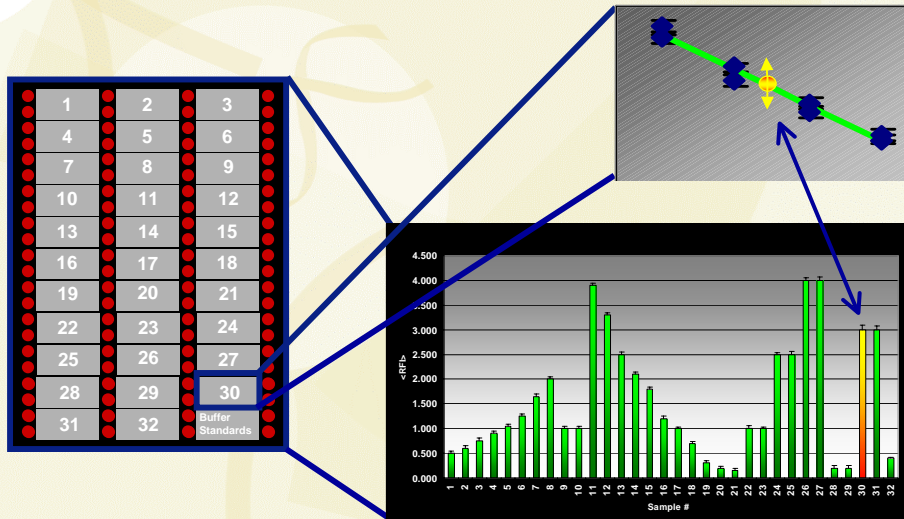


- (Ref) reference spots containing constant fluorescence
- 32 up to **32 samples** (4 dilutions, duplicates) **per array**
- up to **192 samples** (4 dilutions, duplicates) **per chip**

Highly Parallel Monitoring of Signaling Events



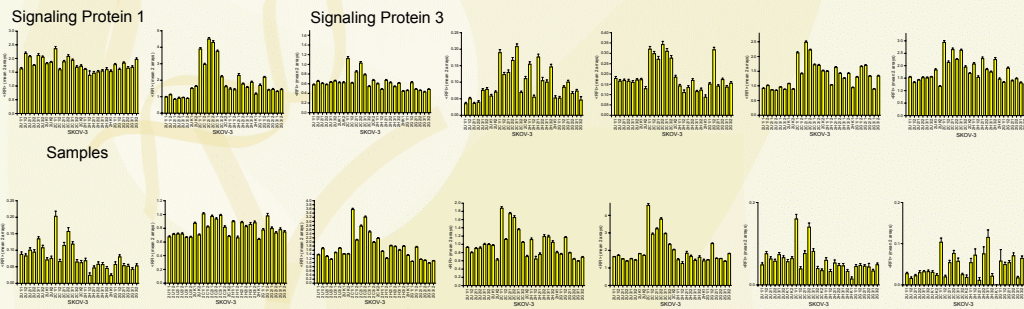
Reliable Data from the on-Array Dose Response Profile



Investigation of compound MODE OF ACTION

Pathway Screen – Detect Compound MOA

- Effect of 6 drugs at 3 to 5 concentrations on
- 60 signaling pathway markers tested in
- 7 different cell lines

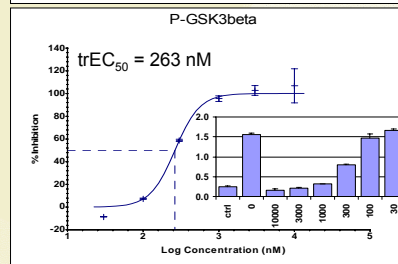
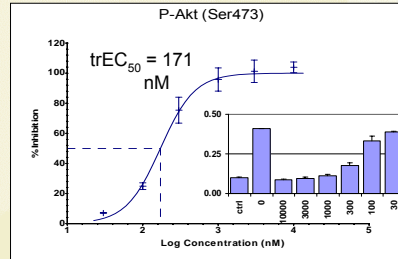
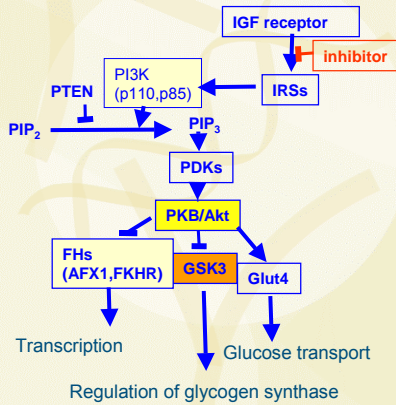


= Information that corresponds to 11'040 western blots (8 data-points each)
obtained in 1 week

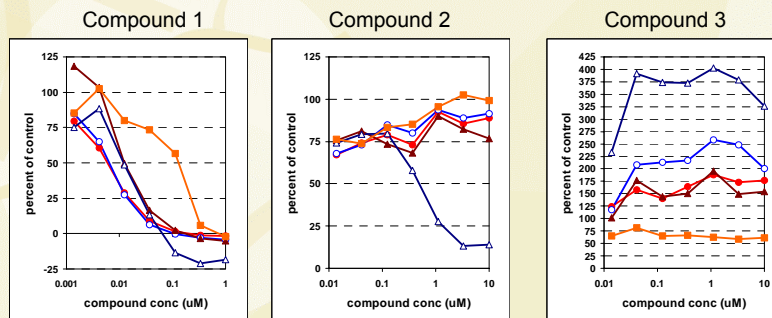
Selection of most effective and selective compounds

Compound Selection based on Pathway Profiles

"trEC50" (transduced EC50) curves of an IGF1R inhibitor at different pathway nodes



Identification of Leads Based on Pathway Selectivity



Broad Spectrum Inhibitor

Specific Inhibitor

Activator instead of Inhibitor

Summary Reverse Protein Array Technology

- ⇒ Robust and well-established process
- ⇒ Time course of stimulants on activation and expression of pathway proteins
- ⇒ Flexible application: free selection of protein profiling targets
- ⇒ Straight forward assay development
- ⇒ Economic technology
 - minimal amount of samples and antibodies (<1µL for 2 arrays) needed
 - short time to result: information corresponding to 100'000 Western blot lanes within 3 weeks
- ⇒ Sensitive
- ⇒ Results corroborated by well established technologies as e.g. WB, ELISA

Why Higher Sensitivity is Required to Detect Markers in Blood

	PSA	Interleukin 10
Molecular weight	33'000 Dalton	18'000 Dalton
LOD of diagnostic test	1 ng/mL 30 pMolar	0.6 pg/mL 33 fMolar
Molecules per mL	1.8×10^{10}	2.0×10^7
Molecules per nL	18'000	20
Molecules per pL	18	0.02

ZeptoMARK - The Powerful Reconnaissance Platform for
Complex Signaling Pathway Elucidation

