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This presentation:

- Introduction to technology
- Measuring Kinase Activities
- Biomarker research
- PamGene's PamAcademy

Small introduction into Pamgene

- Founded in 2000, LSP funded, Akzo Nobel spin off.
- Tech and biomarker IPR portfolio
- Services & Partnering in R&D Life Sciences
- Unique proprietary array-based platform for kinases & nuclear receptors for:
 - Lead Identification and Lead Optimization,
 - (Pre) clinical translational research
 - Biomarker development in Food & Pharma research



Pamgene Services & Research

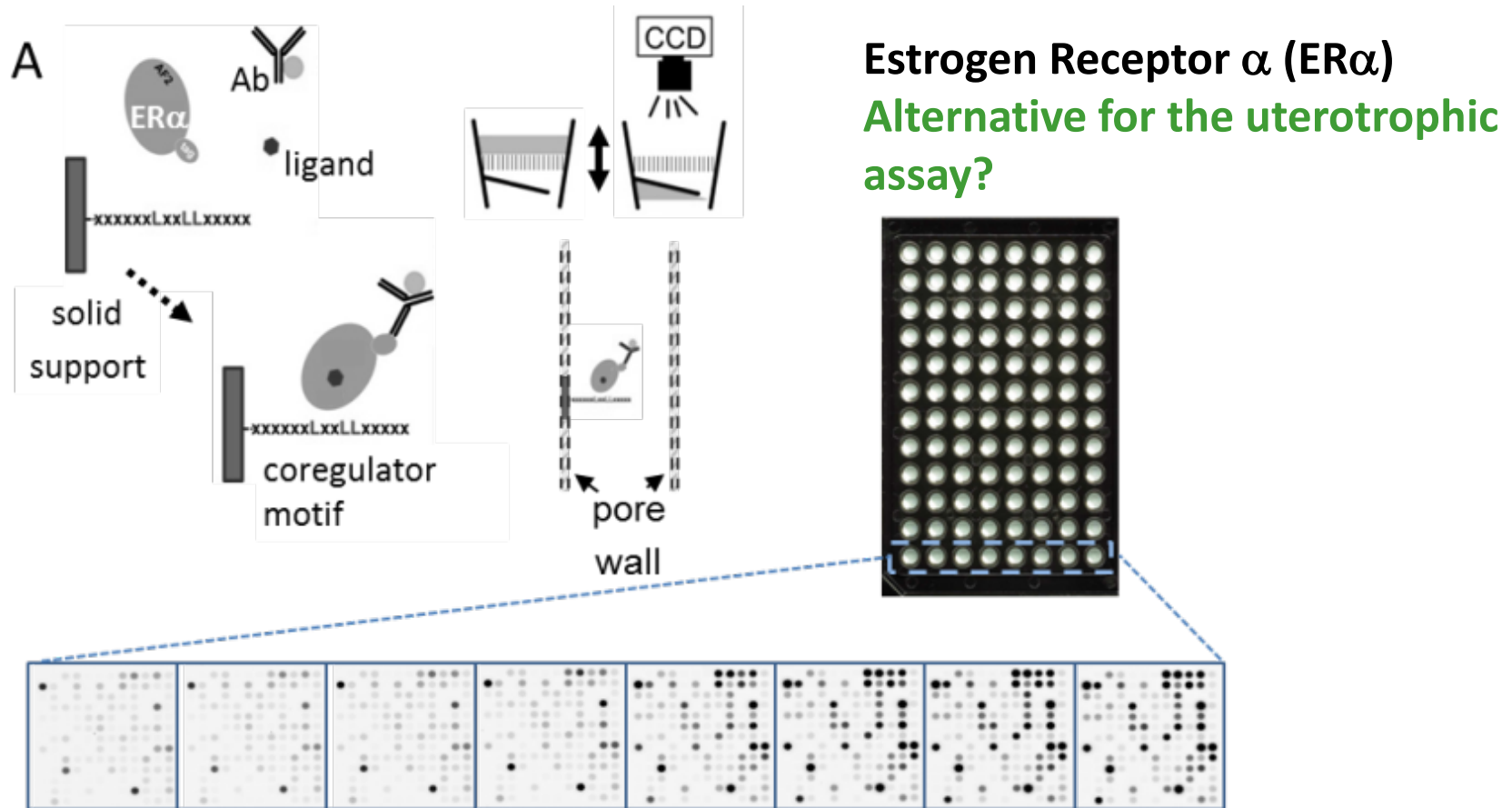
- Supporting Compound Development (Food,Pharma)
 - Lead Optimization through selectivity profiling of kinase inhibitors & NR-Food-coregulator interactions.
 - Develop selective kinase & NR modulators in cells and tissues (xenograft, PDTX, patient material, solid tumor).
 - MOA differentiation of Kinase and NR ligands at a functional and molecular level in cell & tissue models.
 - Patient stratification via biomarker profiling
 - Human Response prediction via biomarker profile.

How did we started in Wageningen

PamChip® Porous microarray, side view (Electron Microscope Image)

How did we started

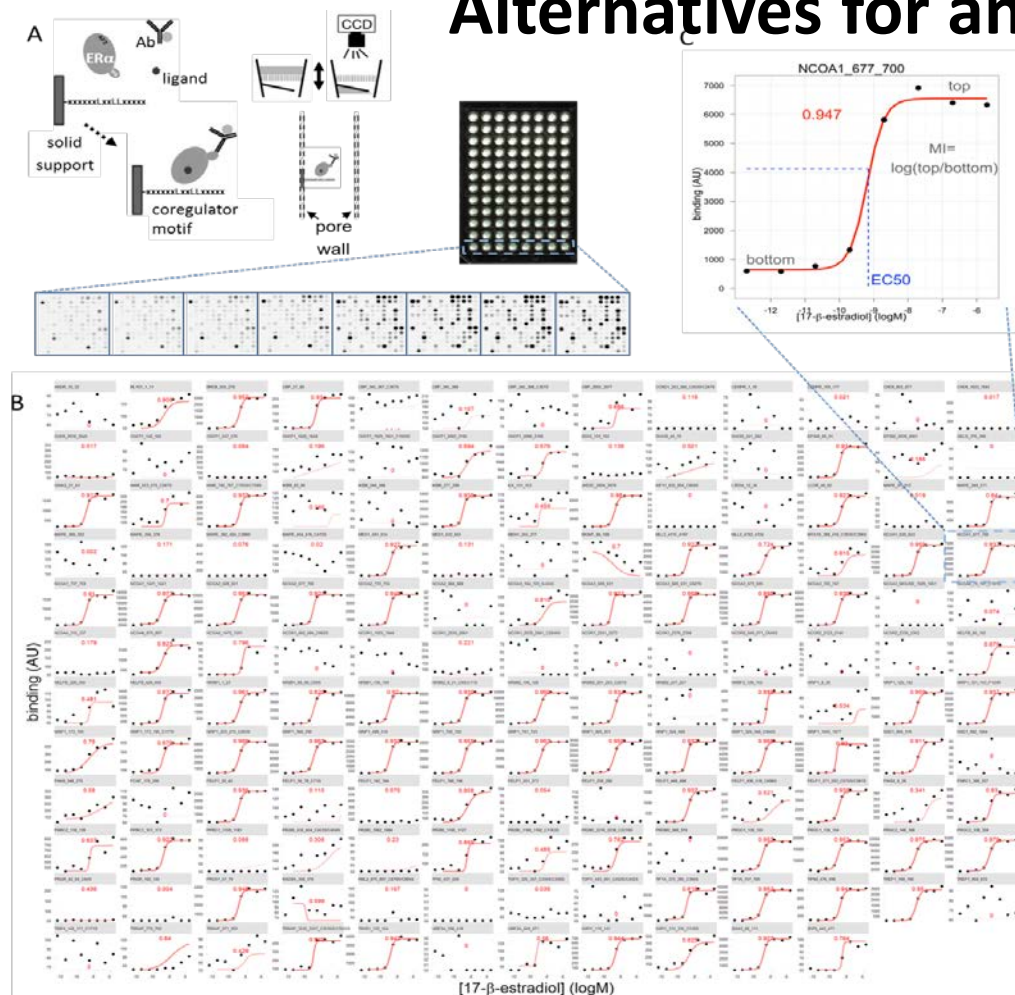
**Bridge to new WUR collaborations in NH receptors:
From reporter gene assays to coregulator binding assays**



Wang et al. Alternatives to Animal Experimentation (ALTEX) (2013)

How did we started II

Alternatives for animal testing



Estrogen Receptor α (ER α)
Alternative for the uterotrophic assay?

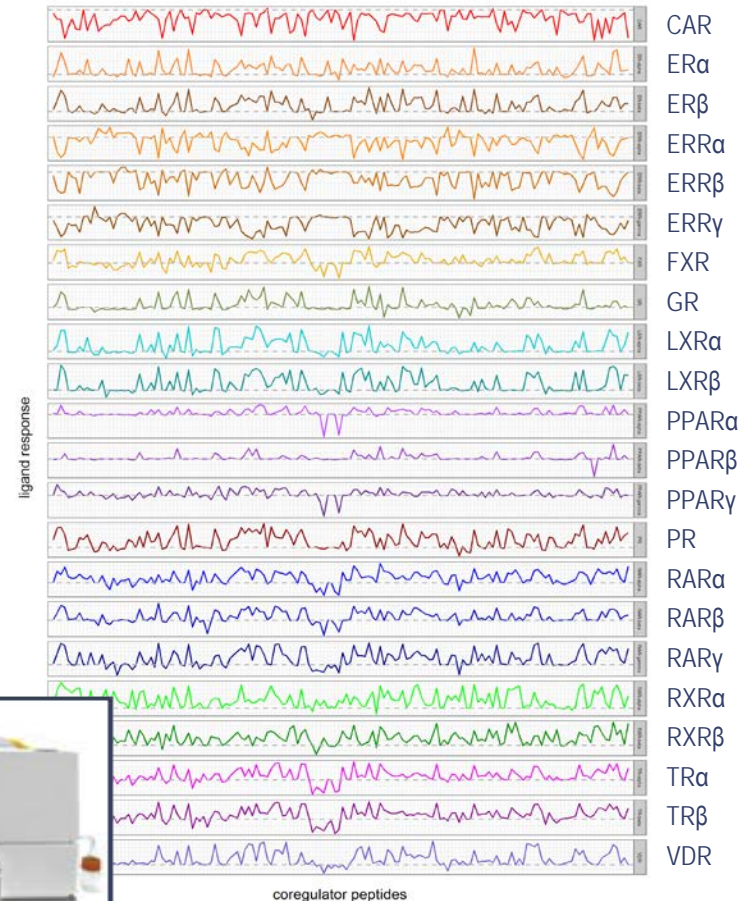
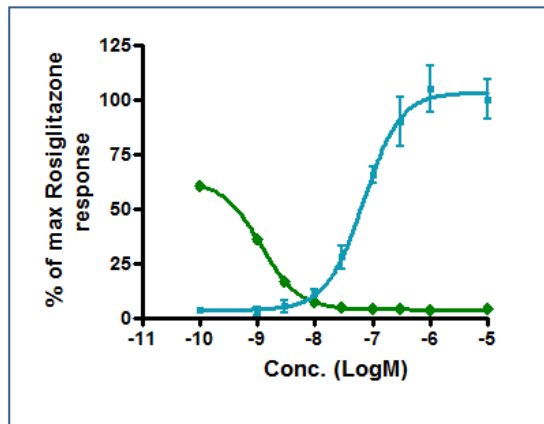
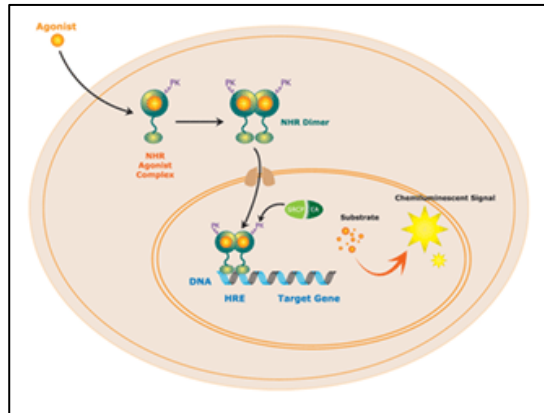
In one series:
 dose response curves and
 EC50 values
 for 155 coregulators determined
 for 18 model compounds
 for which data from
 uterotrophic assay
 are available

Wang et al. Alternatives to Animal Experimentation (ALTEX) (2013)



CAT Agro food: PamStation: nuclear receptors

From reporter gene assays to coregulator bindings assays



PamStation®12

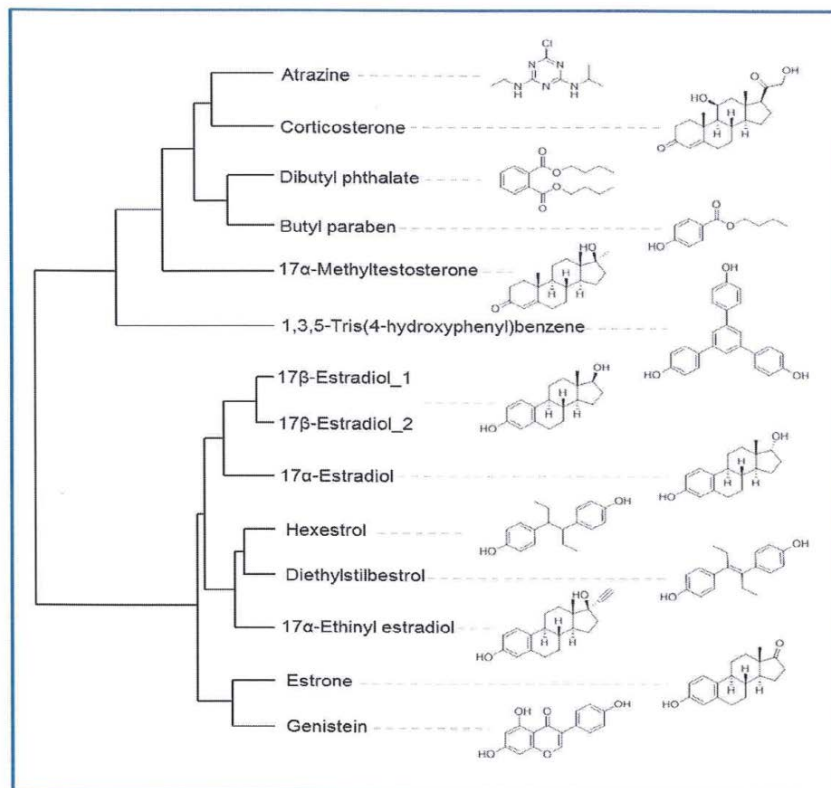
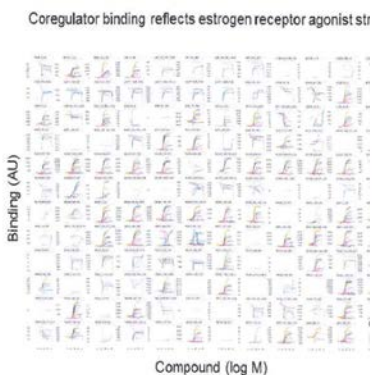
Wageningen, January 14th 2014

Slide 8

Alternatives for animal testing: Coregulator fingerprints for 14 estrogens



Hierarchical clustering



structurally related compounds cluster together
compounds having an aromatic A-ring were separated from those with a cyclohexene A-ring

assay reflects structural similarity of ER α agonists, indicating a potential to achieve identification and classification of ER α endocrine disruptors

Aarts et al. Chem Res Toxicol (2013)

MARCoNI Services

		MARCoNI silver	MARCoNI gold	MARCoNI platinum	MARCoNI diamond
Characteristic	Coregulators #	5	15	50	150
	Coregulator conc.	1	1	1	1-8
	Nuclear receptor*	LBD	LBD	LBD, FL	LBD, FL, Lysates
	Compound conc.	[1-3]	[1-3]	[1-3; IC ₅₀]	[1-3; IC ₅₀]
	Price-structure	← Per cmp + setup fee →		← Custom →	
	NHRs #	← 25+ nuclear receptors available →			Advanced bioinformatics →
MARCoNI Assays	Selectivity profiling** (up to 20 NHRs)				
	Safety-Panel (AhR, CAR, PXR, PPARα, FXR, LXR, TR and RAR)				
	Metabolic-Panel (PPARα, β/δ, γ, LXRs FXR)				
	Endocrine-Panel (ERα, PR, AR, GR, MR, VDR, TRs)				
	Custom			☑	☑
	Single			☑	☑

*LBD = Ligand Binding Domain; FL- Full length NHR protein; Lysates = Nuclear Receptor extracts from cell lysates

**The NHR panels have not been defined yet.

Kinase Activity Platform



Measuring Kinase Activity



Proteomics

2D Arrays

Mass Spec

Western blot

PamChips

Nuclear Hormone Receptor

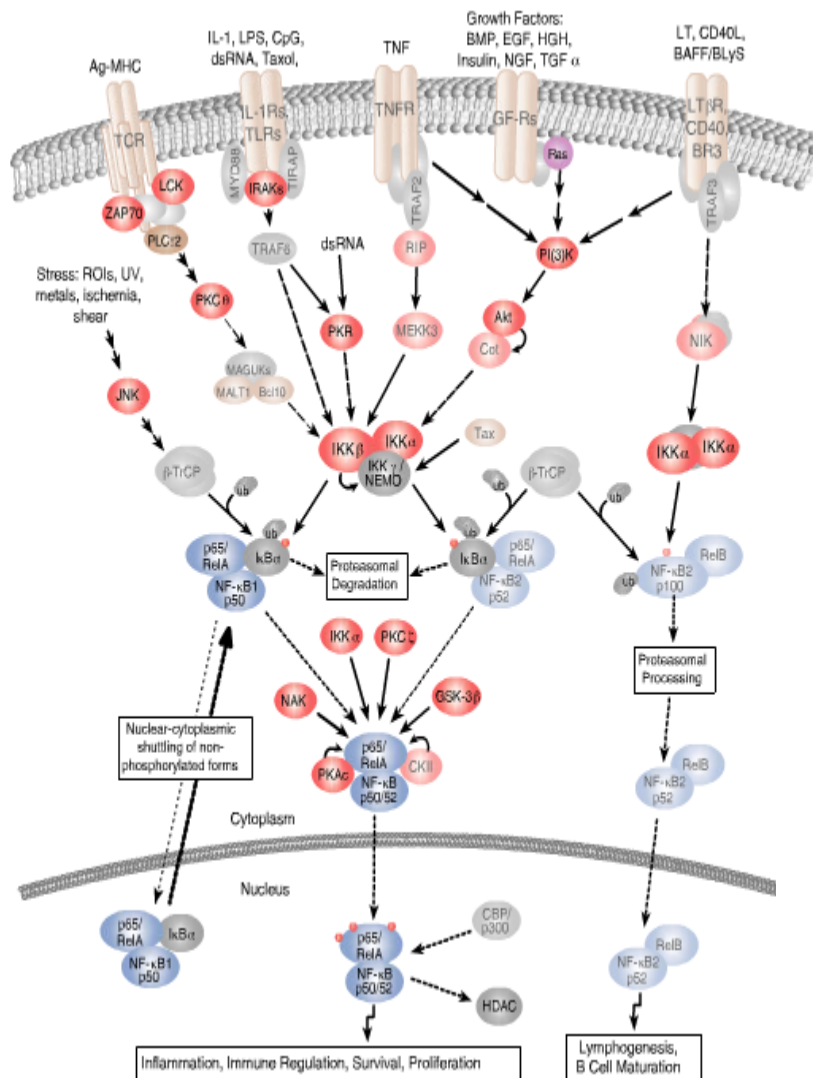
Kinase

Serine Threonine

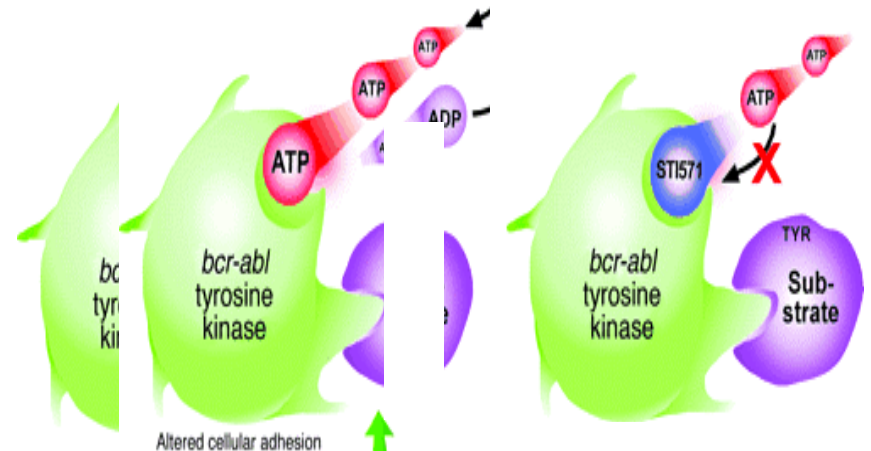
Ligand
Cellular lysates

Recombinant kinase
Yeast
Cell based
Xenograft
Mice
Zebrafish
Clinical samples

Signalling and Kinases

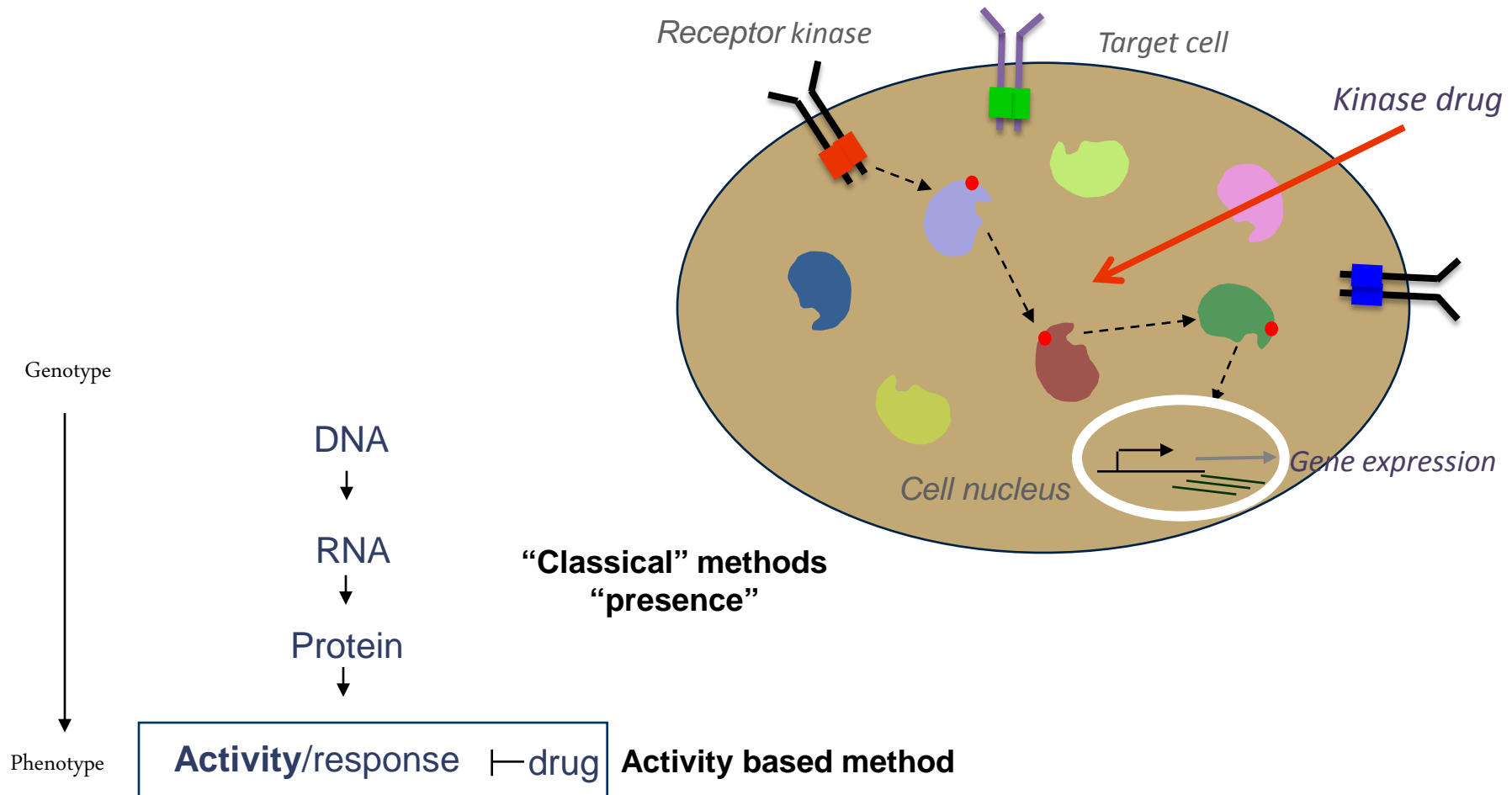


Most important signaling enzymes
phosphoryl transferases
2% of human genome
~ 30% of proteome phosphorylated
518-534 kinases
Main pharmaceutical drug target
PKI research: biased!



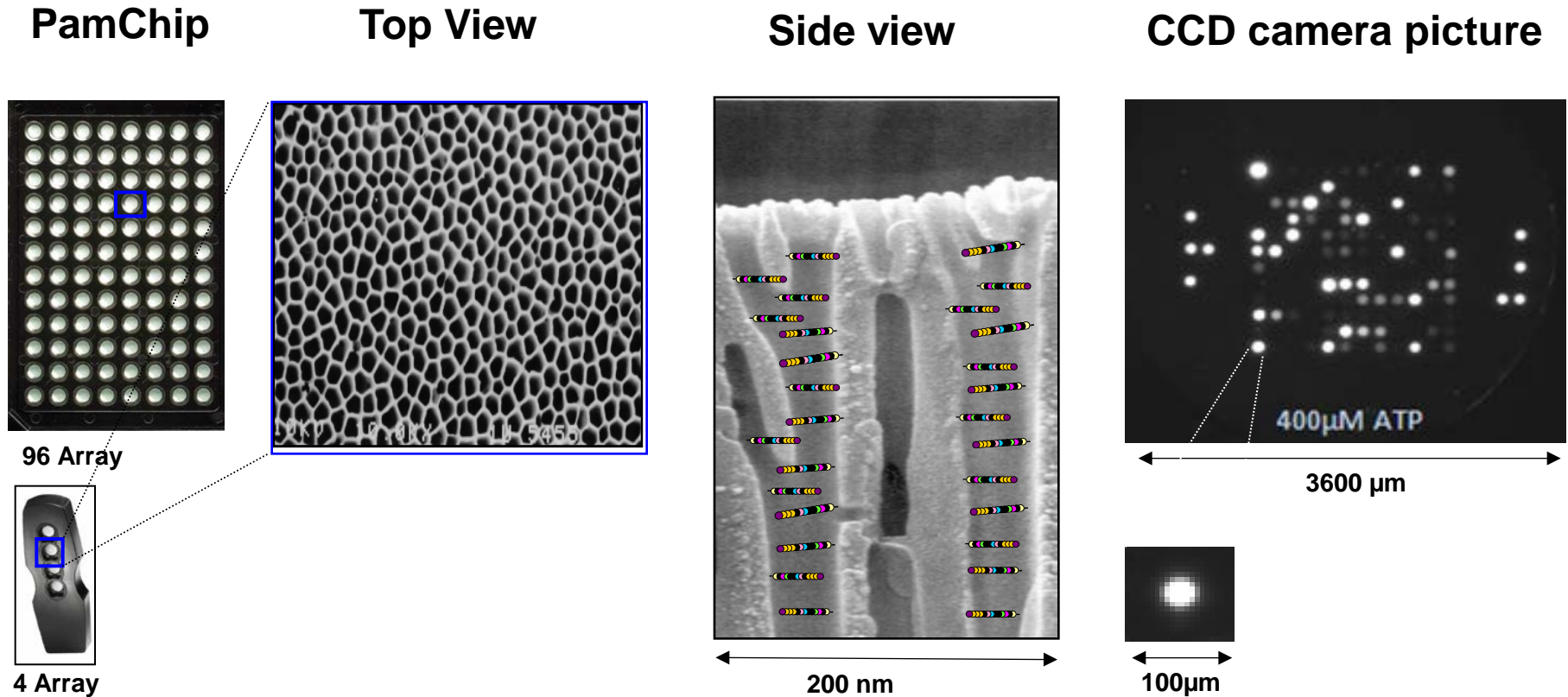
The Oncologist 2001;6:233-238 June 2001

Measuring kinase activity II



Missing: Technology bridging the gap between protein abundance and phenotype

Measuring Kinase Activity III



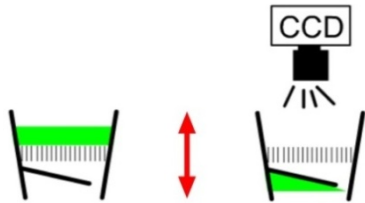
Different peptides are immobilized on the array:

Kinase application **144**

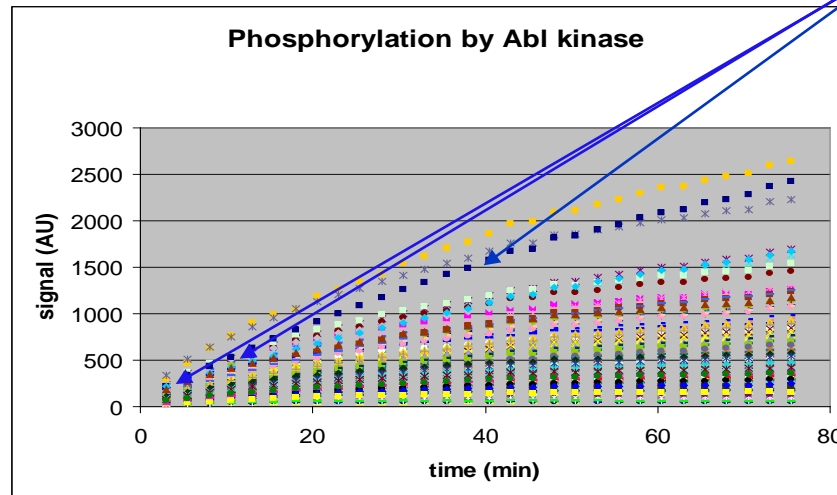
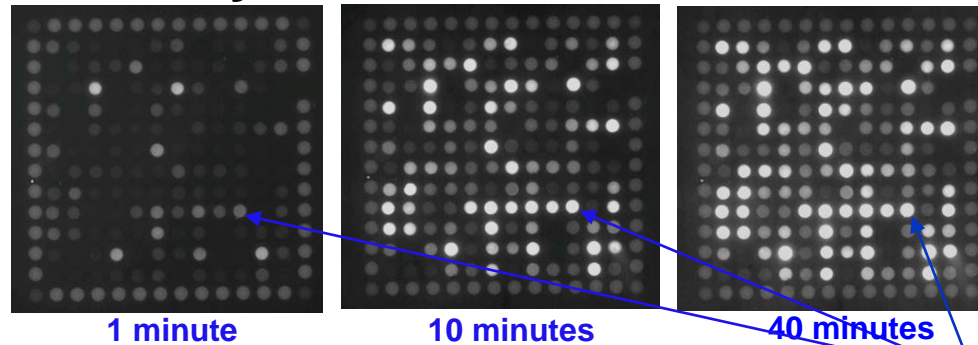
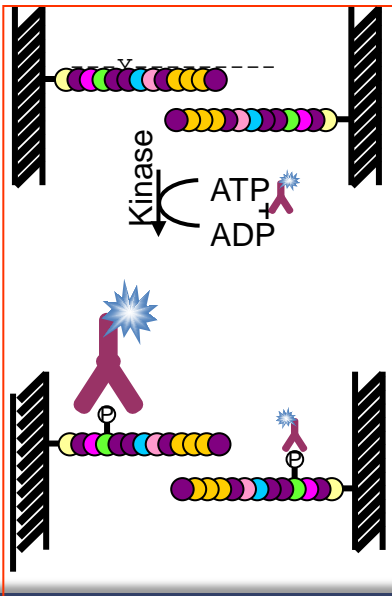
NHR application **53/155**

Measuring Kinase Activity IV

Measuring **Kinase Activity** with the assistance of the CCD camera



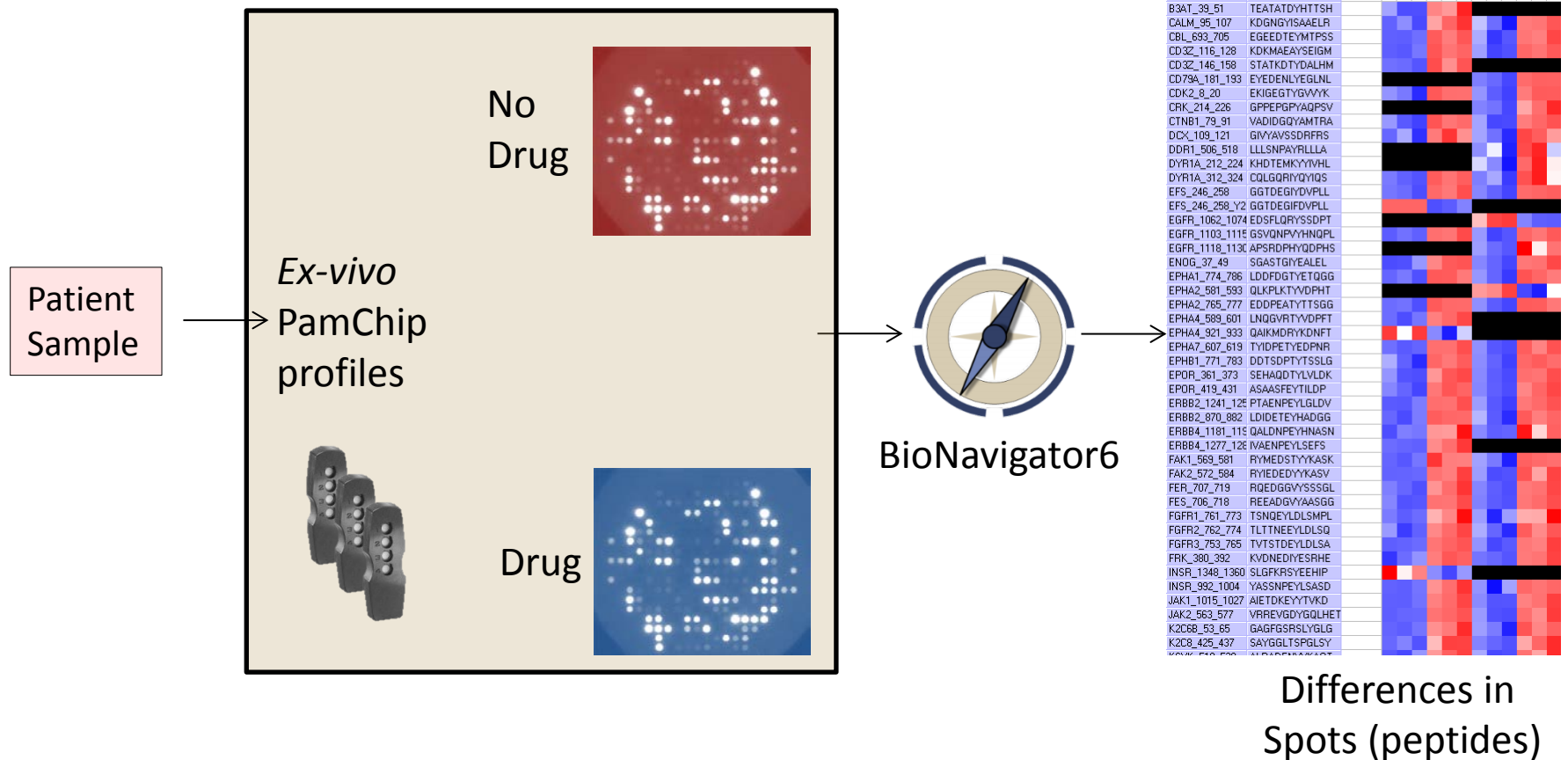
Sample is pulsed back and forth through the array



Every spot on the array or line on the graph is representing a peptide sequence that is phosphorylated on the array

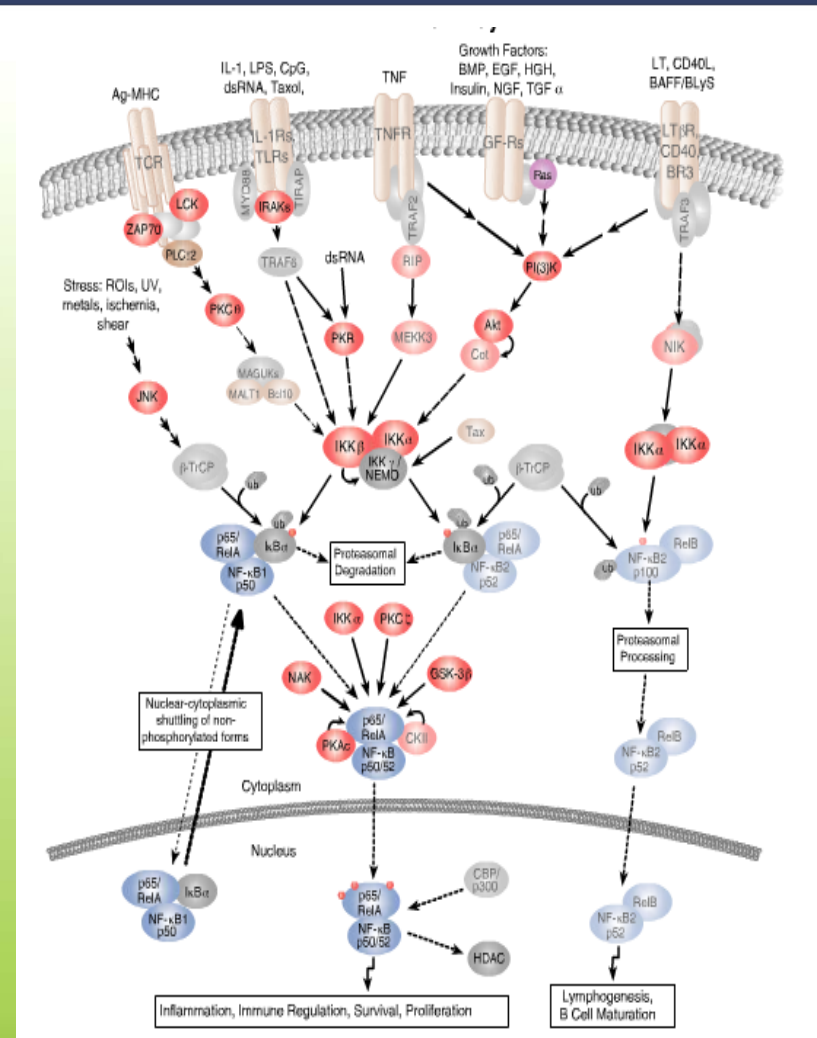
Measuring Kinase Activity V

Functional Proteomics on PamChip® arrays

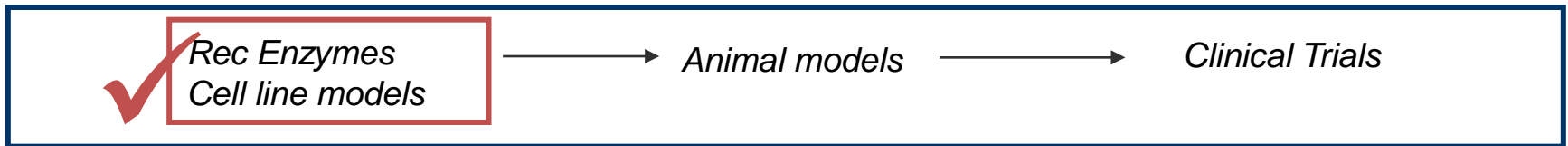


Towards kinase pathways

- From fingerprints to pathway
- Mechanism of action elucidation



Measuring Kinases: Cell based assays



Workflow cell lysate PamChip assay

“Lyse cells/tissue and profile”

1. Harvest cells
2. Spin down
3. Add lysis buffer
4. Lysis for 30' on ice
5. Apply on PamChip (1-10 ug prot)
6. Run incubation protocol
7. Analyse phosphorylation profiles



Real time data
within 40 minutes



Fast versus western, or gene
expression profiling

Mouse derived knock out T-cells

Purified mouse T-cells of WT and KO mice were stimulated with anti-CD3 for 0, 30 60 or 120 minutes.

Unstimulated control samples were provided for WT and KO for each time point.

Samples provided

	Time		Treatment	Protein µg / µl
1	0min	WT	unstimulated	1.2
2			+ anti-CD3	1.4
3		KO	unstimulated	1.1
4			+ anti-CD3	1.1
5	30min	WT	unstimulated	1.1
6			+ anti-CD3	0.8
7		KO	unstimulated	1.0
8			+ anti-CD3	0.8
9	60min	WT	unstimulated	0.7
10			+ anti-CD3	1.2
11		KO	unstimulated	1.0
12			+ anti-CD3	0.9
13	120min	WT	unstimulated	1.4
14			+ anti-CD3	4.0
15		KO	unstimulated	0.9
16			+ anti-CD3	0.9

- Samples were run on STK PamChips

- Samples were run on PTK PamChips

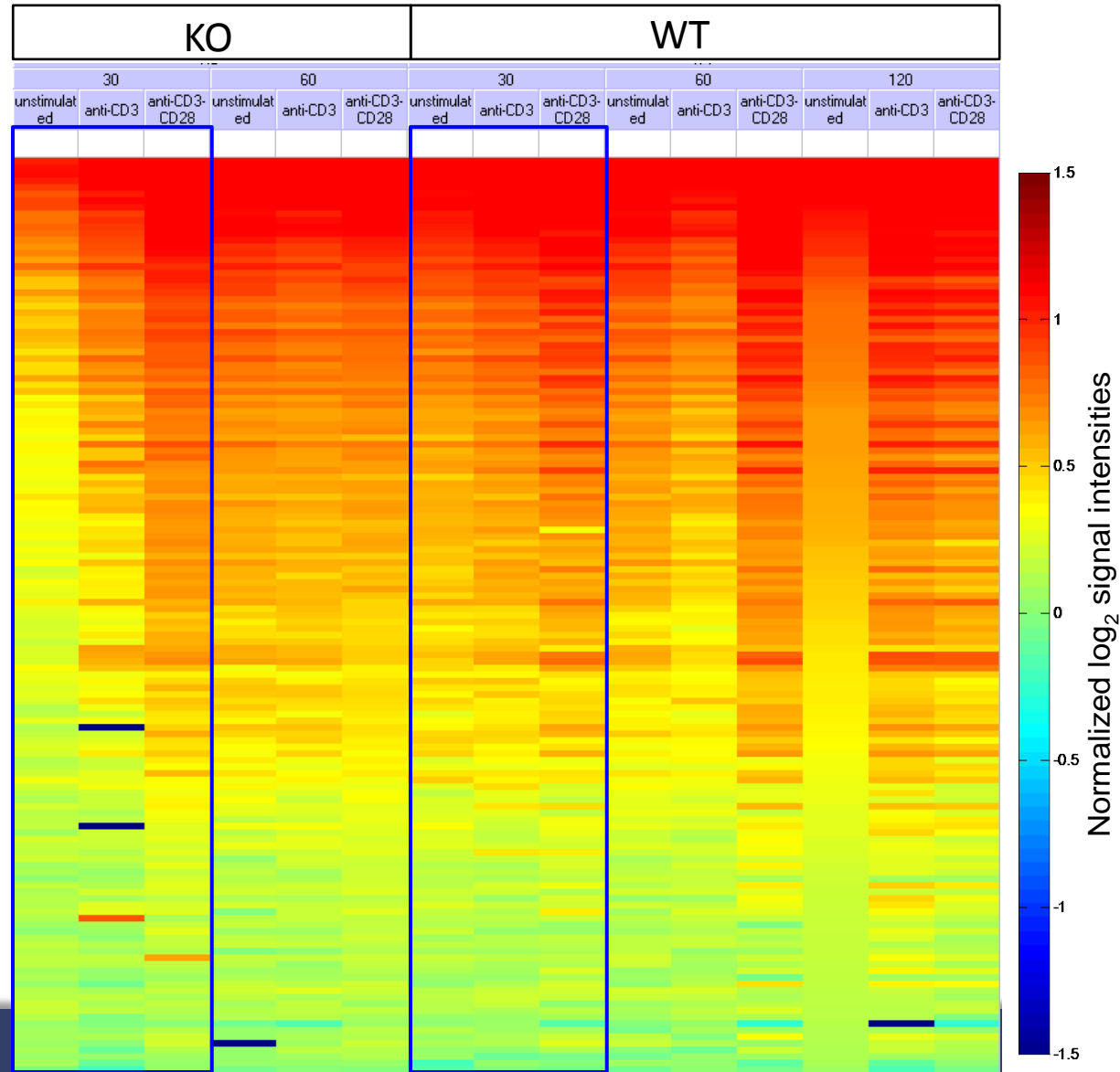
Protein Tyrosine Kinase profiling in T-cells

time series! →

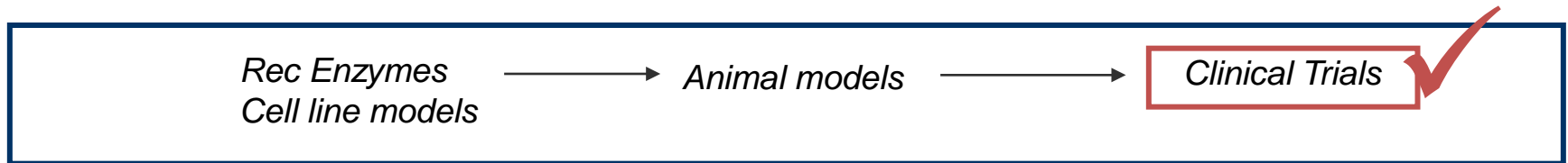
144 phospho-
rylations

(no WB/FACS
bias)

*Stimulation by anti-
CD3 treatment and
increased stimulation
when combined with
anti-CD28 in WT cells
at 30 min.*



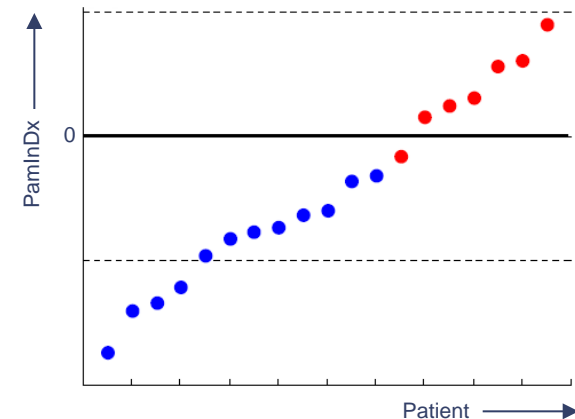
Measuring Kinases: Clinical Trials



Example:

Predicting Therapy Response in Rectal Cancer Patients

- Phase II clinical trial;
 - 67 patients treated with oxaliplatin and 5-FU in a Preoperative Chemoradiation (CRT) trial NCT00278694
- Goal:
 - Predict CRT prior to therapy using kinase activity profiling
- Conclusion:
 - 85% correct prediction



Sigurd Folkvord et al. Int J Radiat Oncol Biol Phys. 2010 Jul 31

Kinases Analysis Workflow: how does it work

Type of chips



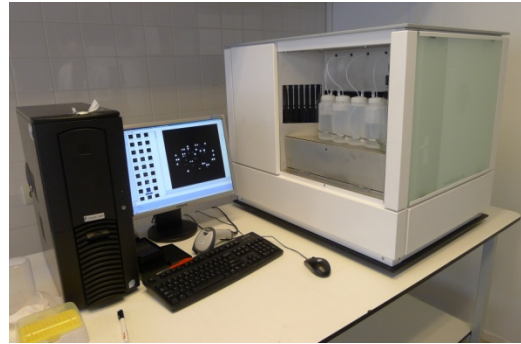
PamChip 96 array



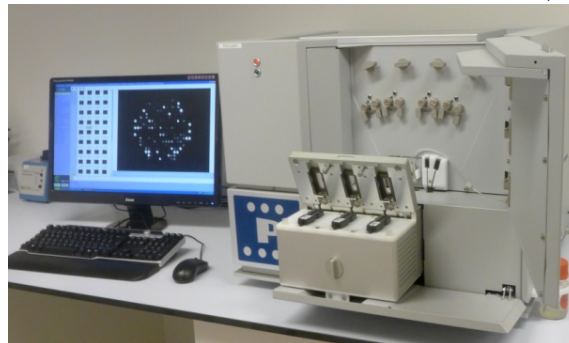
PamChip 4 array



Type of PamStations

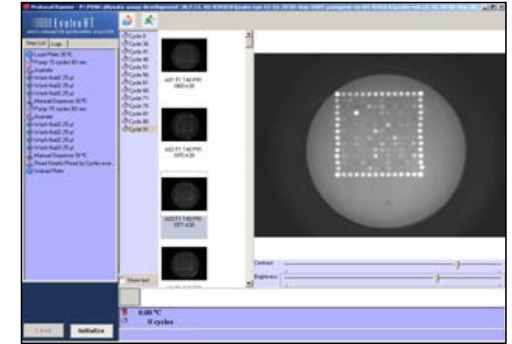


PamStation®96

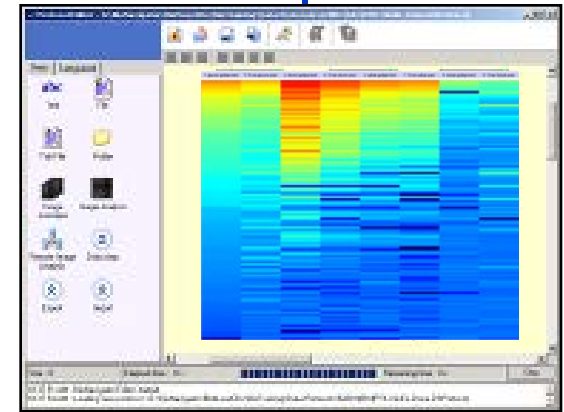


PamStation®12

BioNavigator



(controlling PamStation)

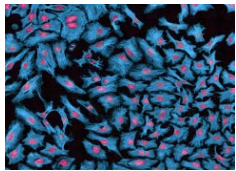


(software for data analysis)

Test materials

Small amount of tissues & cells

Per array 1-10 μ g total protein or 10,000 - 100,000 cells



Primary and cultured Cells (animal model and patient material)

White blood cells, blood platelets, PBMCs, Bone marrow

Primary cells e.g. liver

Culture cells, adherant or suspension cells



Primary tumors and biopsies (animal model and patient material)

Freshly frozen, archival tissues, up to 20 years old

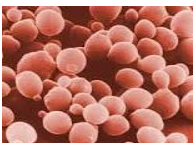
Tumor content >70% (>20% also used)

Different tissues (FNA) e.g. lung, liver, breast, brain, prostate, skin, thyroid, CSF



Laboratory animals

Zebrafish, rat, mouse tissue: xenograft tissue, pig, dog



Other

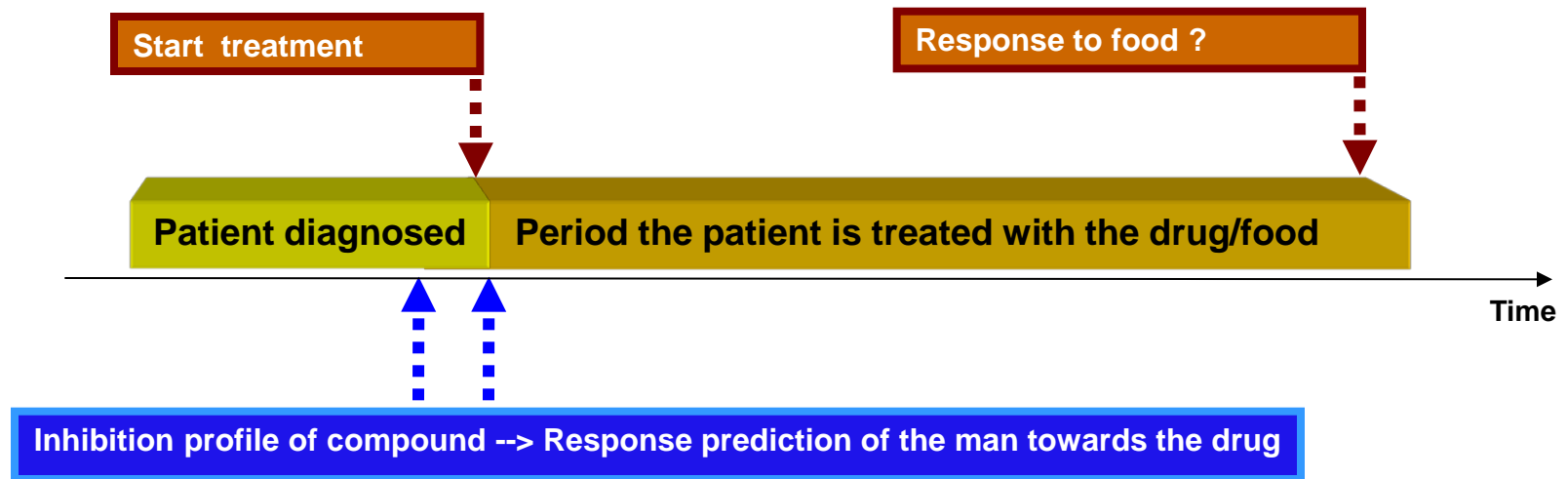
Yeast, Plant

Biomarker Research with PamGene



Joining the future with PamGene I

Dedicated Drug/Food treatment & selection for patients



Joining the future PamGene III

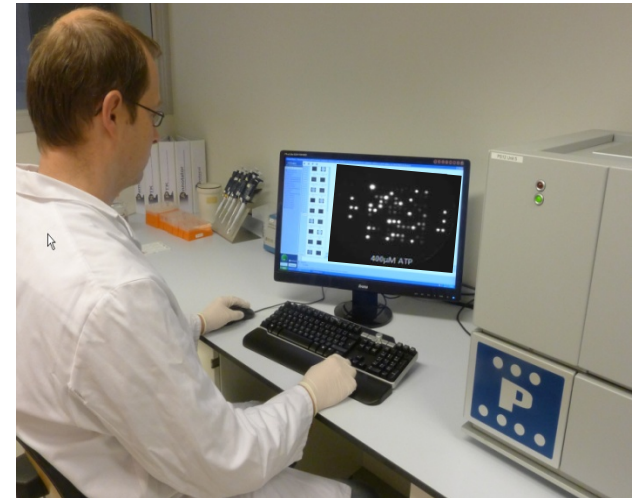
Actual work flow of the routine clinical practice



Step 1
Human Sample
(5 minutes)



Step2
Testing the sample of
the patient with a drug/food
(30 minutes)

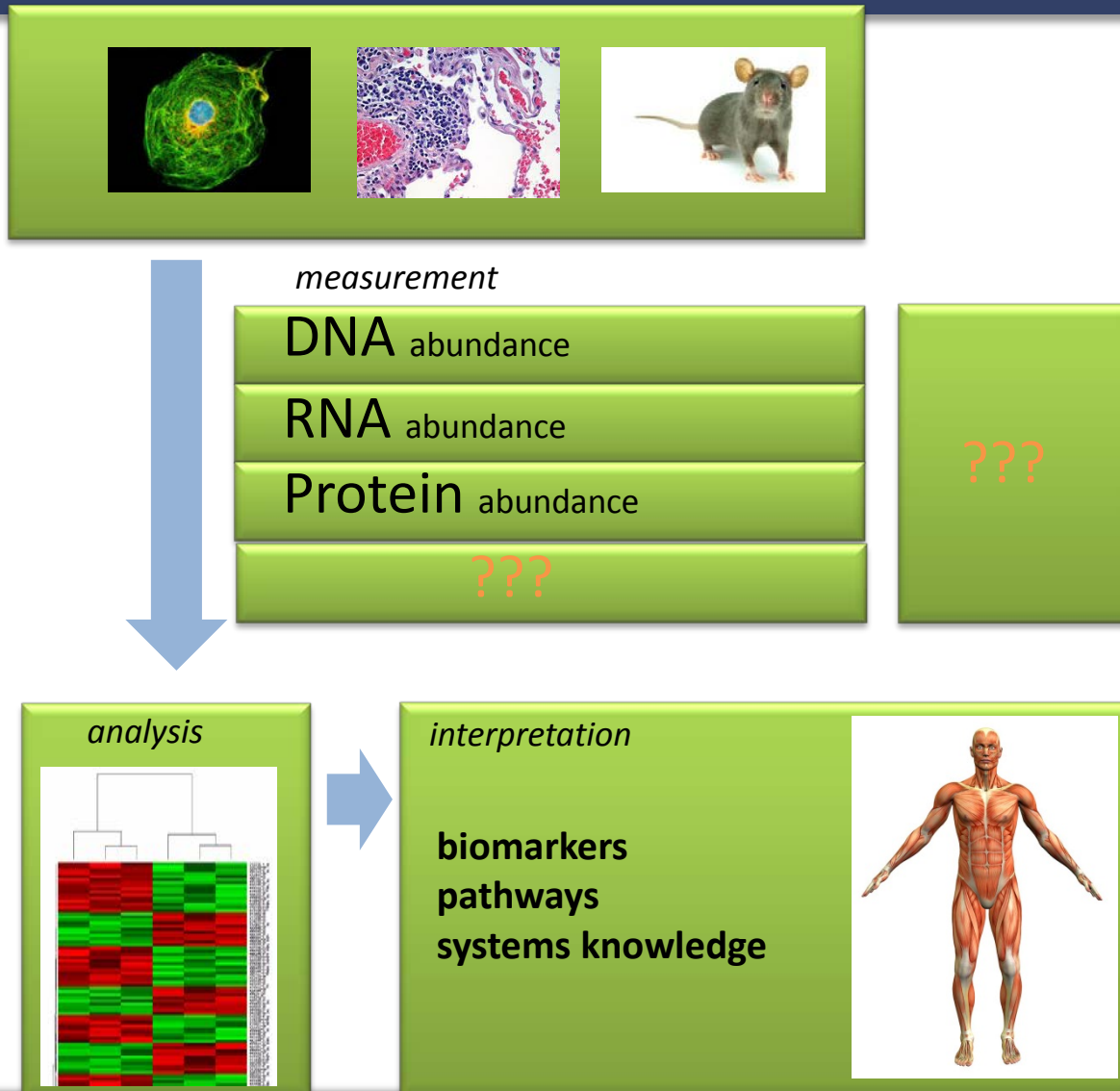


Step 3
Result of the patient
automatic analyst
(5 minutes)

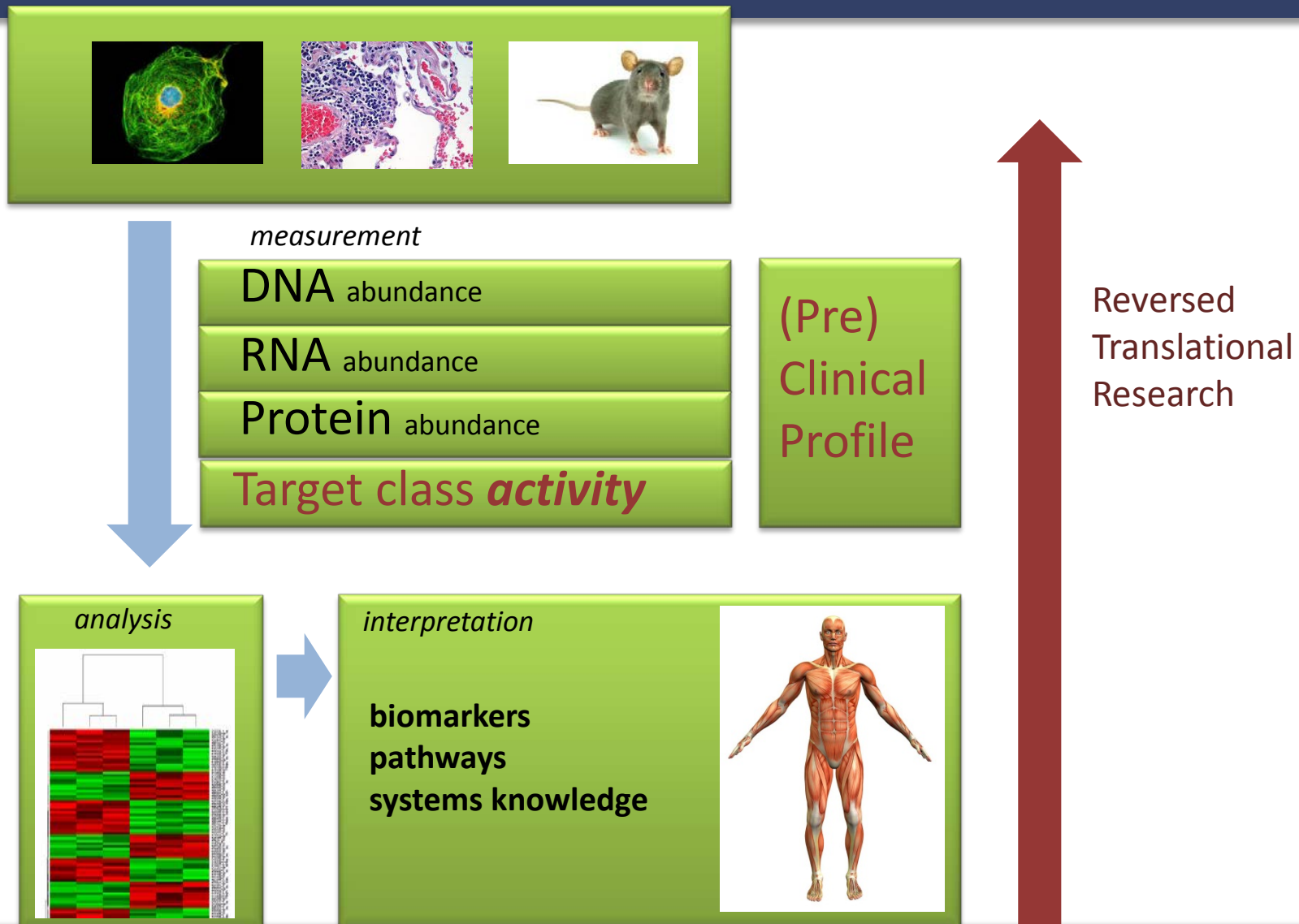
Take home message

- Dynamic **peptide microarrays**
- **Kinetics** → more knowledge
- **Kinase activity** (and nuclear **receptor function**) profiling of the full-length and fully decorated target proteins
- **Biomarker platform** for target and biomarker discovery and validation in **precision medicine**
- Drug specific profiles / biomarkers
- Bridging technology: abundance → activity → phenotype
- Translational platform: no platform changes in phase transitions
- Test inhibitory efficacy of drug or food *ex vivo*
 - to **predict clinical response**

Take home image



Take home image (II)



We would like to successfully support your research needs



PamGene's PamAcademy

How?



High sensitive measurements

Dedicated and proven software

Training programs in order to get people successful

Sharing 10 years of kinase activity expertise

Sharing knowledge also by using the PamCloud

Scientific publications

PamGene's PamAcademy II

Share your research question



PamGene's PamAcademy III



To continue your success we recommend

Create and handle samples according our protocol

Lyse the samples according to our protocol

Share your research problem, we have experience in creating scientific possibilities

Get trained with the PamAcademy

Use our powerful software tool to analyze loads of data





Explore next steps with CAT-AgroFood and PamGene:

- **Aim to continue your research needs**
- **Support you with high tech proteomic tools**
- **Train you and validate your skills**
- **Together creating new collaborations**