

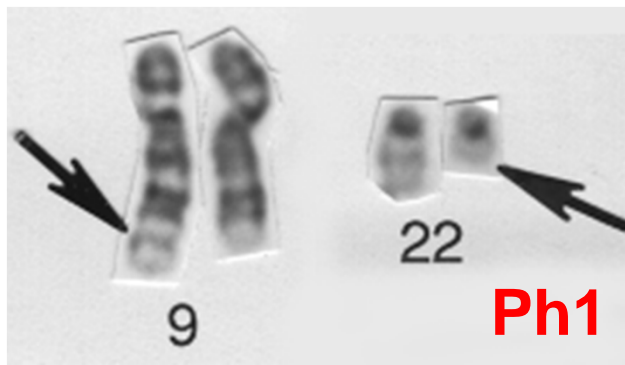
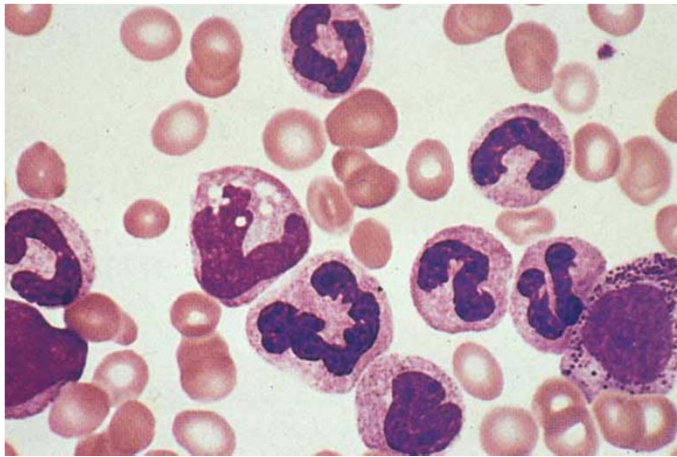
# Developing tests for Bcr-Abl activity and Gleevec resistance in CML patients

A progress report on IMAT R33 CA103235, "*Bcr-Abl  
kinase assays for STI571 sensitivity or response*"

Stephen J. Kron M.D.-Ph.D.  
The University of Chicago



# 1973: A chromosome translocation in CML



Janet Rowley M.D.  
U. Chicago  
Lasker Award 1998

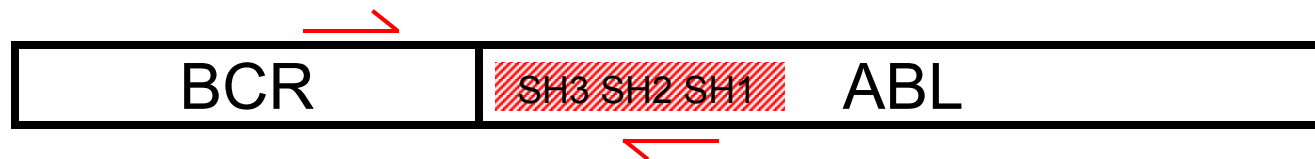
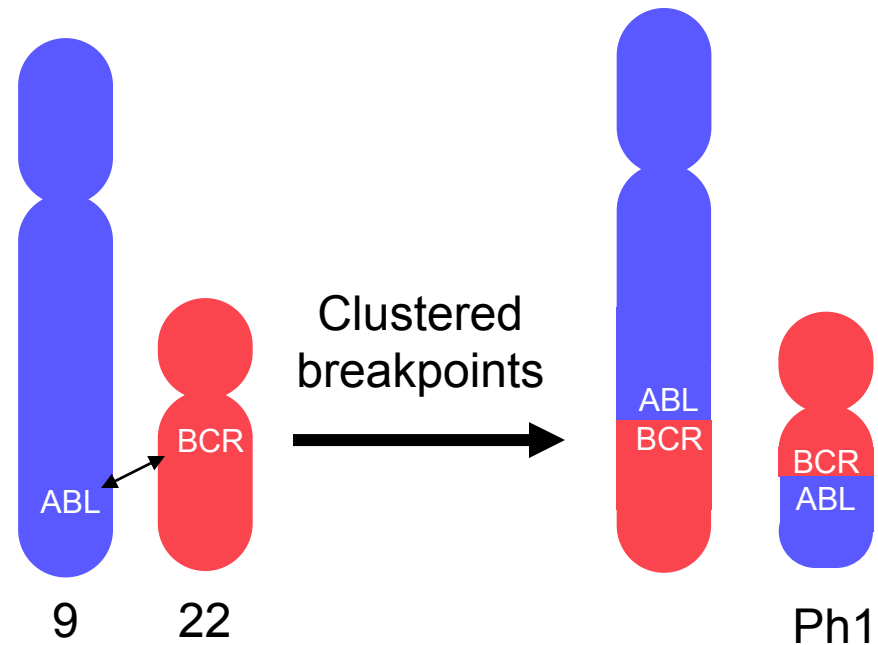
Rowley, J.D. (1973) A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature*, 243, 290–293.

Cytogenetic testing for molecular diagnosis, monitoring

# 1982: Ph1 chromosome encodes BCR-ABL

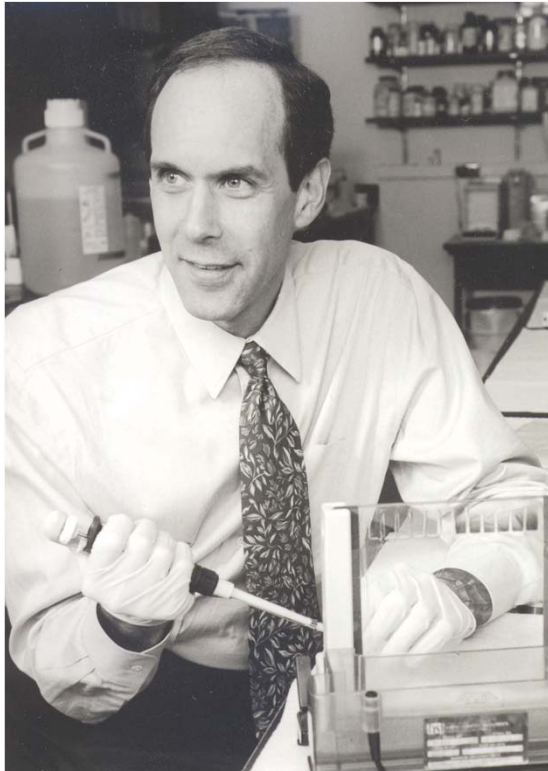


Owen Witte M.D.-Ph.D.  
UCLA

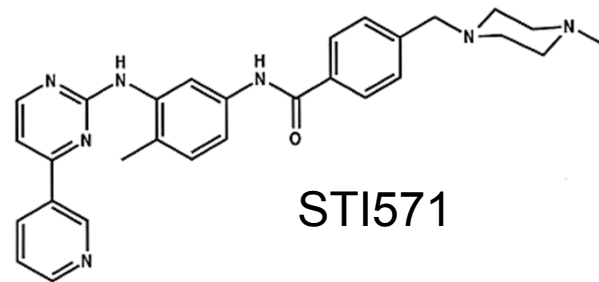


Molecular diagnosis and monitoring via unique transcript  
Tyrosine kinase enzyme: Active site = druggable target

# 1997: Bcr-Abl kinase blocker kills CML cells and "cures" chronic phase patients



Brian Druker M.D.  
Oregon Health Sci.  
Nobel Prize 200?



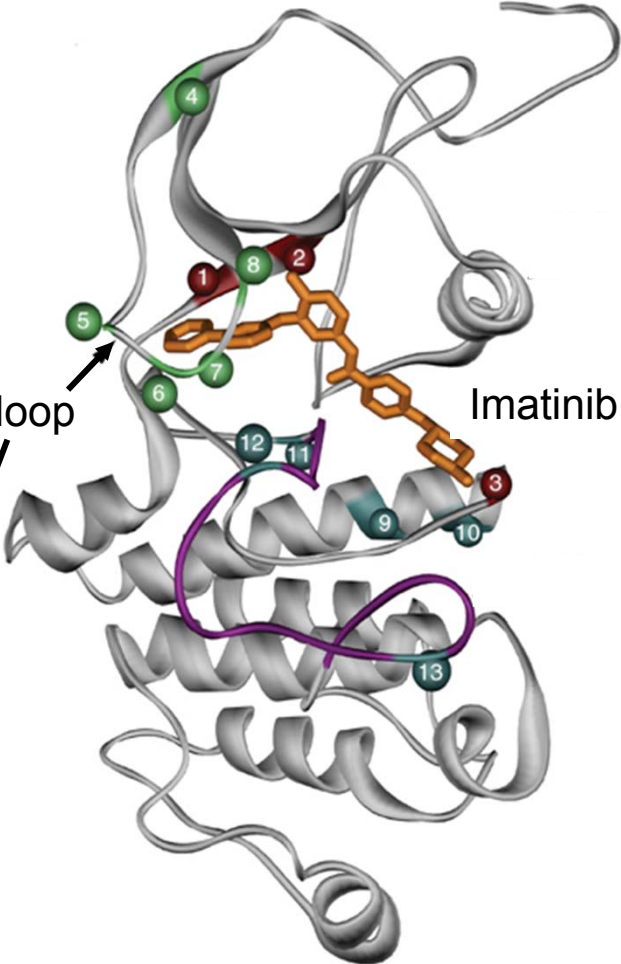
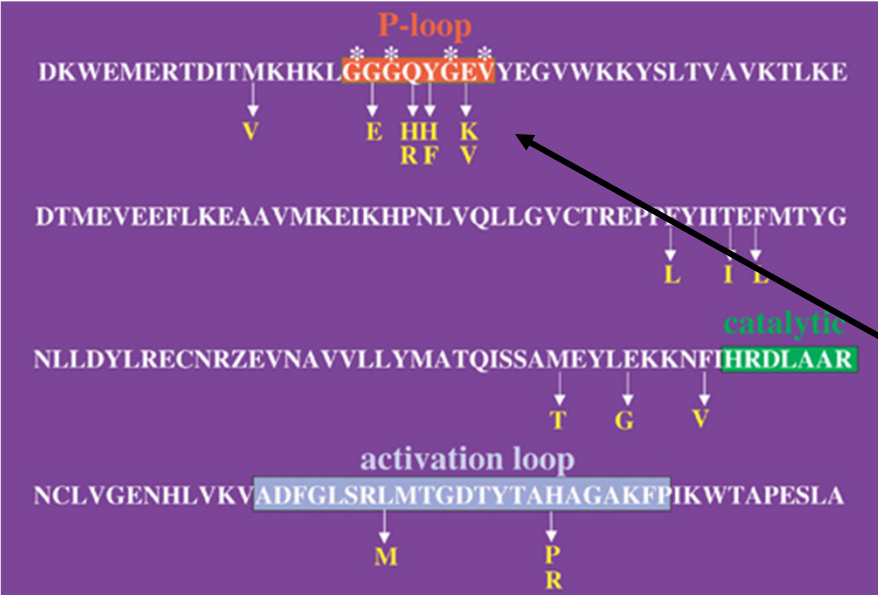
QuickTime™ and a  
Cinepak decompressor  
are needed to see this picture.

STI571+Abl  
J. Kuriyan

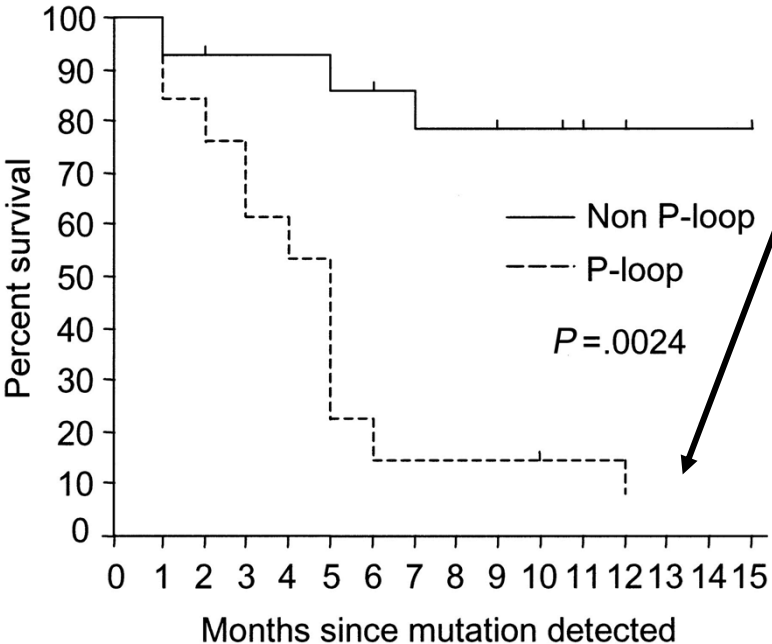
Imatinib mesylate  
Gleevec (Novartis)  
**\$2.2B in 2005**



# 2002: Imatinib resistant kinase mutations



- 1, F317L; 2, T315I; 3, F359; 4, M244; 5, G250; 6, Q252; 7, Y253; 8, E255; 9, M351; 10, E355; 11, V379; 12, L387; 13, H396



C. Sawyers and others

# 2006: A proliferation of new drugs, but no assays

## Cheaper, generic imatinib

USFDA approved

1<sup>st</sup> line treatment in CML

# Veenat

Imatinib 100 mg (as mesylate) Capsules

Tailor-made to attack cancer cells

Philadelphia chromosome positive Chronic Myeloid Leukemia (CML)

- > CML myeloid blast crisis
- > CML accelerated phase
- > CML in chronic phase prior/after Interferon- $\alpha$  therapy<sup>4,5</sup>

**Veenat**

The victory in cancer without collateral damage

## Newly approved & on the way

AMN107	Novartis	Nilotinib
BMS354825	Bristol-Myers	Dasatinib
CGP76030	Pfizer	
AP23464	Ariad	
AZD0530	Astra Zeneca	Phase I
SKI-606	Wyeth-Ayerst	
ON012380	Onconova	Phase I
VX-680	Merck	Phase I

and many more in development...

## New clinical challenges

Rapid testing for Imatinib resistance

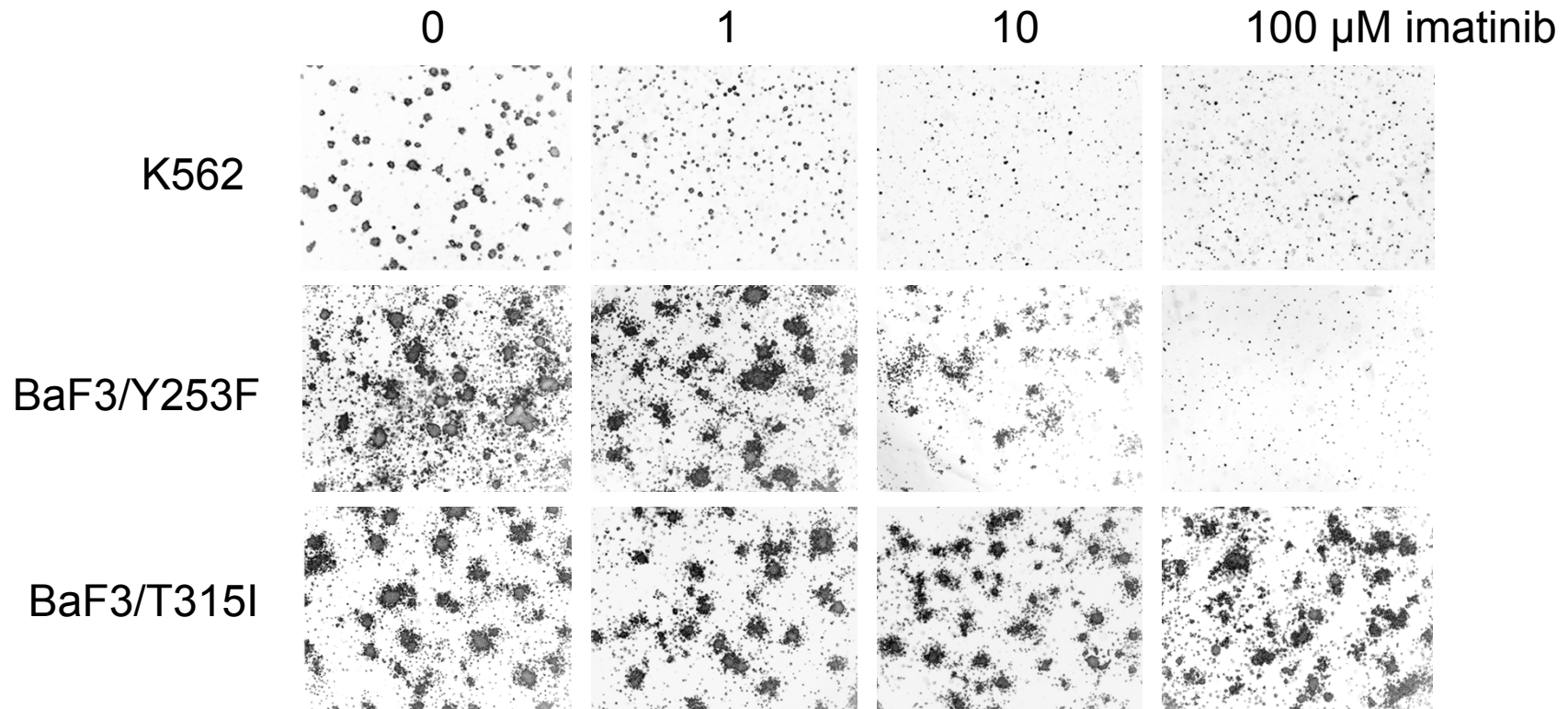
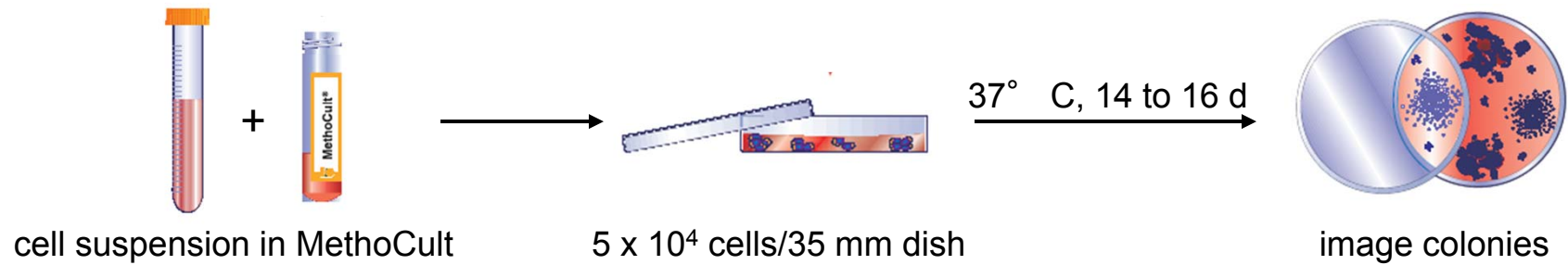
Selection of second-line therapy

Identifying effective dosage

Determining failure of STI therapy

# Methylcellulose assay for imatinib sensitivity

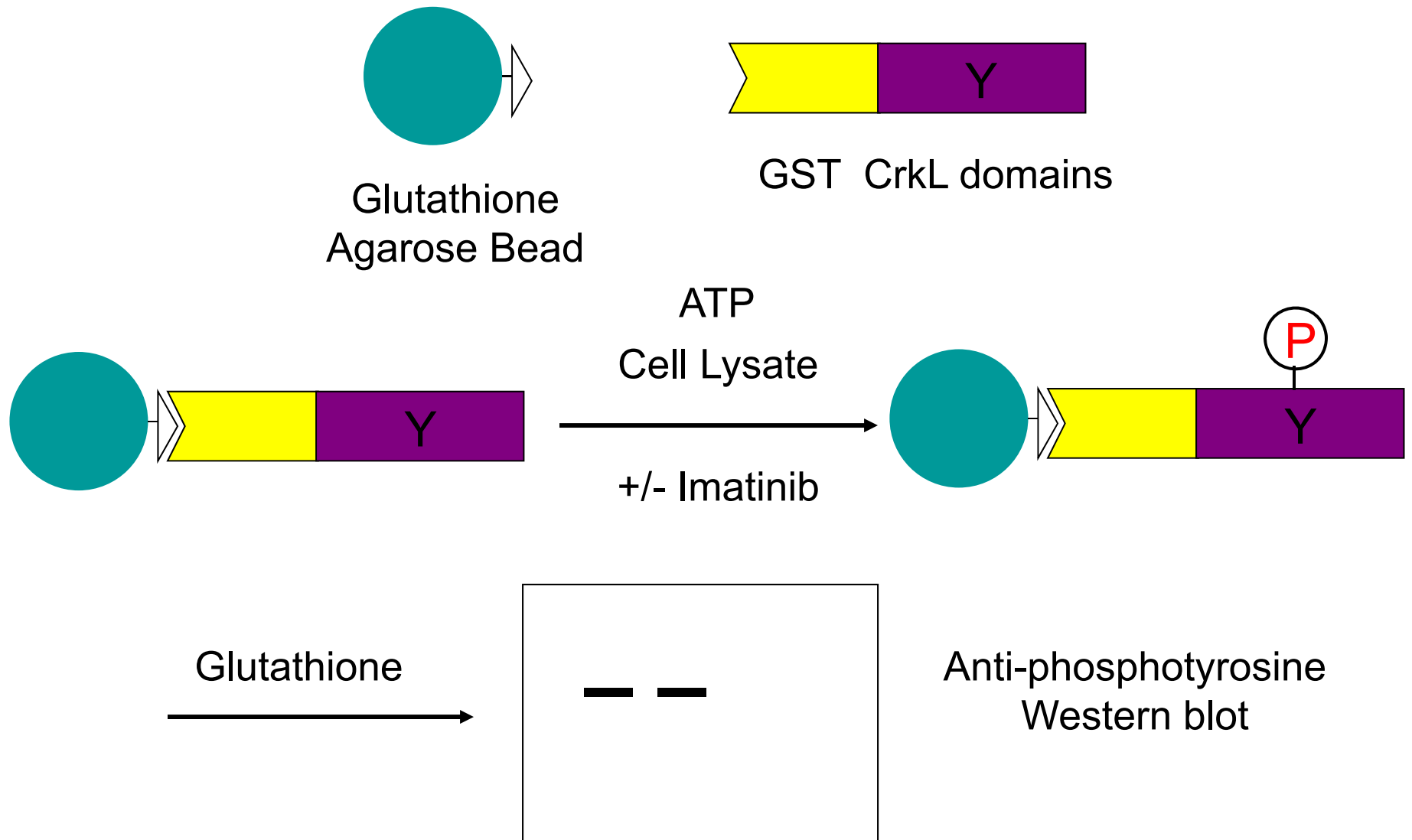
Semi-solid matrix, supplemented with growth factors, allows individual progenitors to form discrete colonies



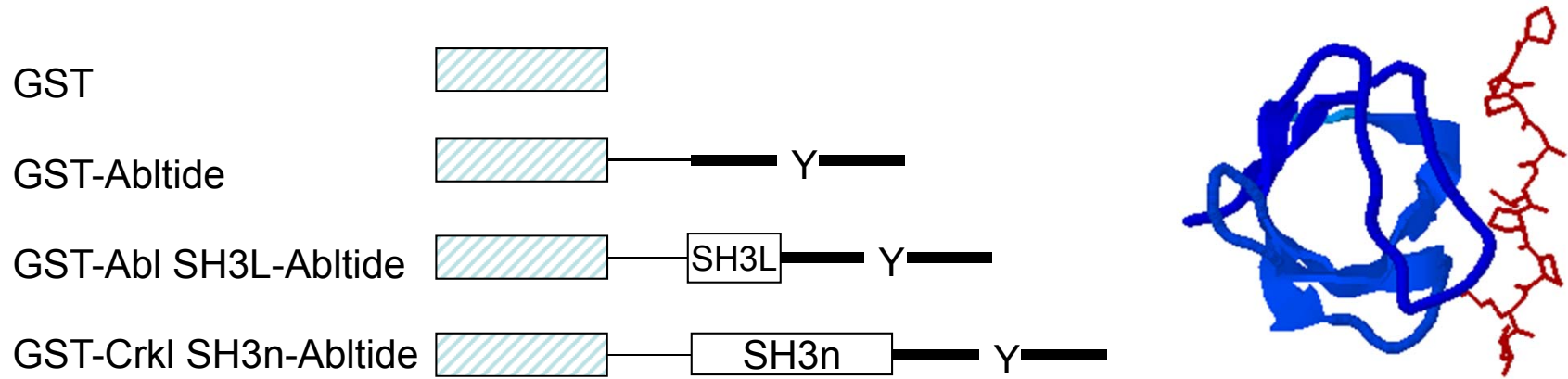




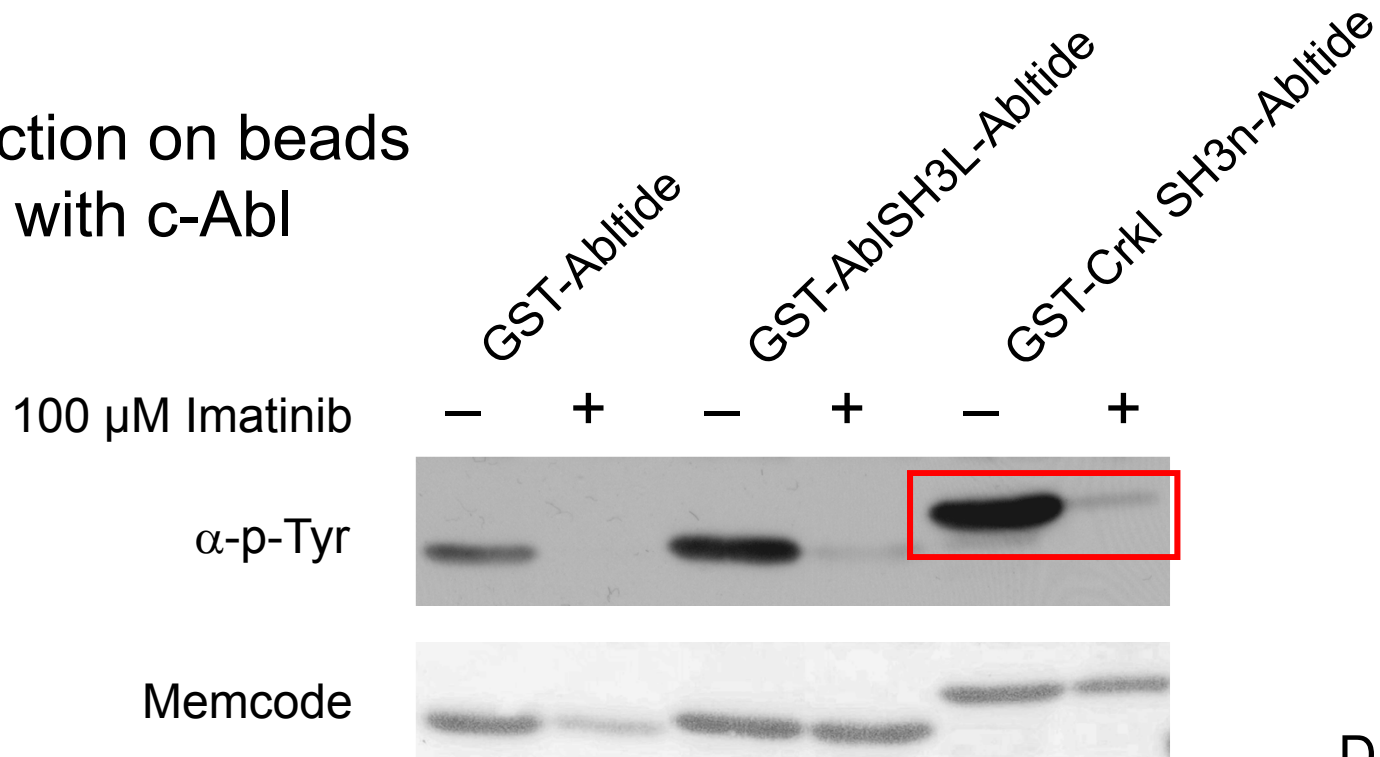
# Proof-of-principle Bead-based assay of Bcr-Abl in cell lysates



# High affinity substrates via Abl binding domain



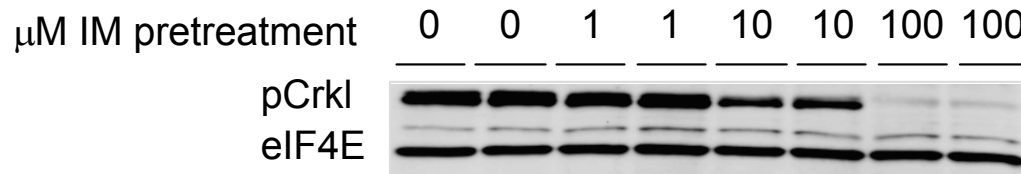
Reaction on beads  
with c-Abl



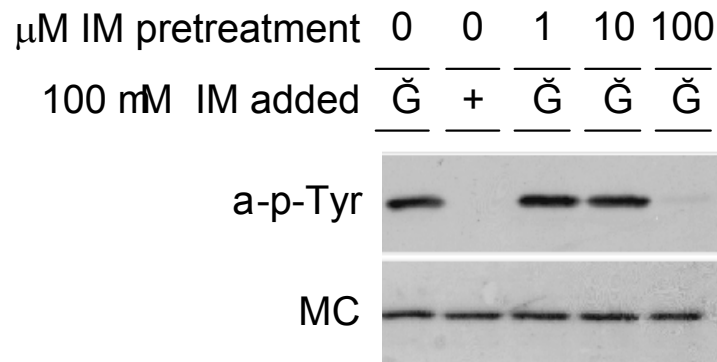
D. Wu et al.

# Bcr-Abl inhibition assay in K562 and CML cells

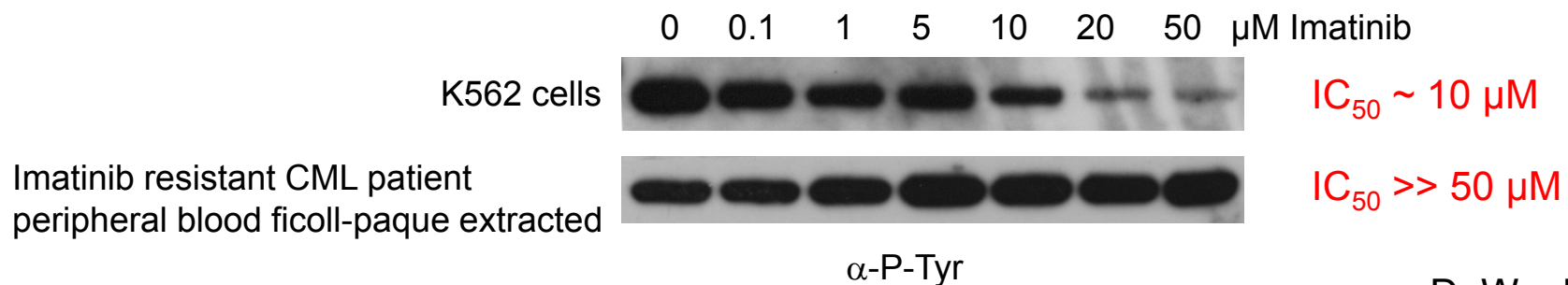
## Western blot -- K562 cell steady-state phosphorylation



## Bead assay -- K562 cell Bcr-Abl activity

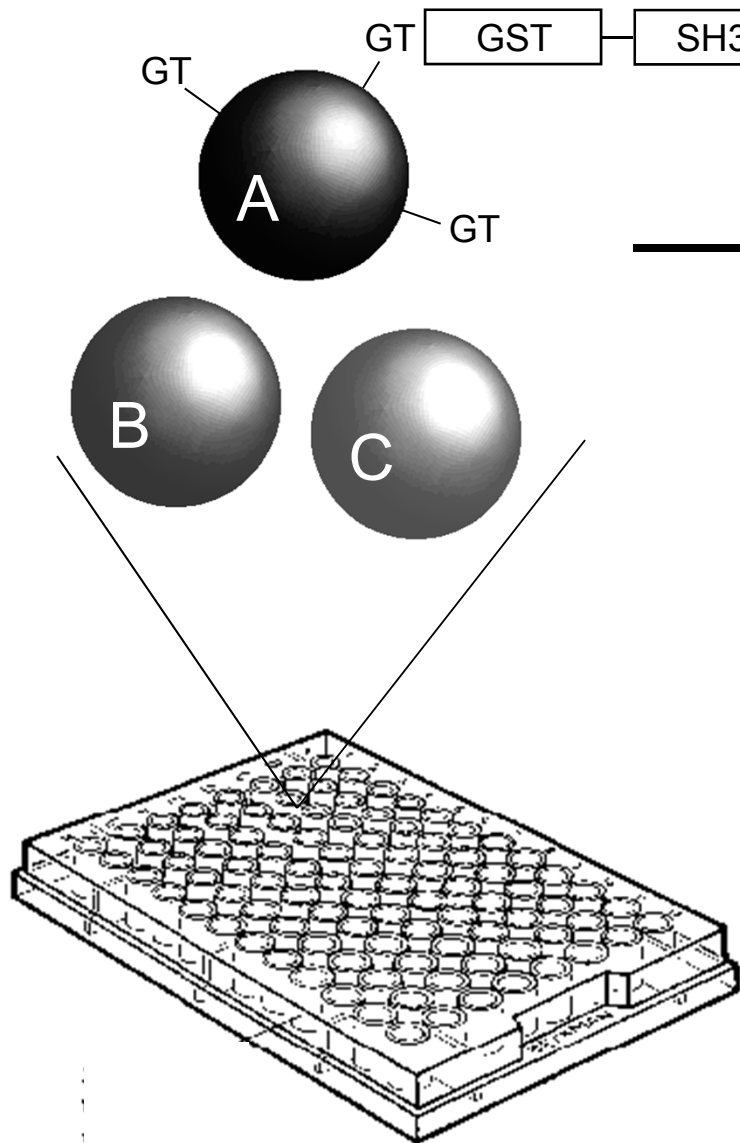


## Assay of Gleevec resistance in CML patient cells



# Rapid translation to clinic...

## Adapt kinase assay to Luminex technology

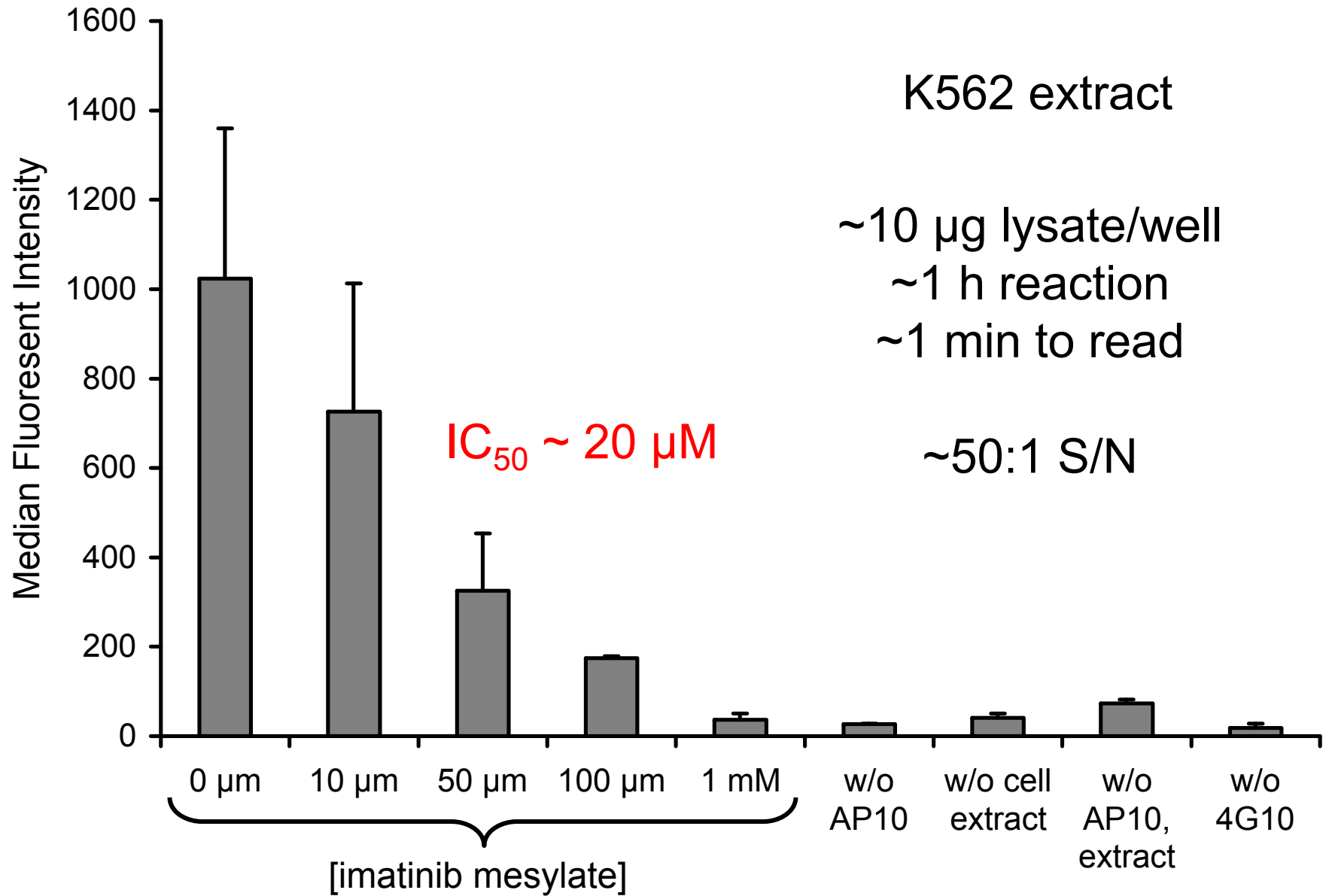


- 1) + cell extract, +ATP, +/- imatinib
- 2) anti-phosphotyrosine
- 3) anti-IgG-phycoerythrin



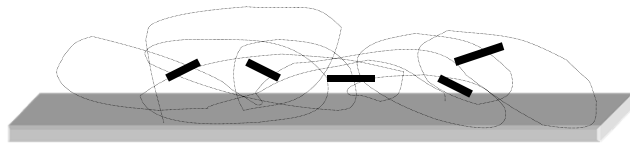
Well C2  
Bead A mean fluorescence  
Bead B mean fluorescence  
Bead C mean fluorescence

# Luminex bead assay for imatinib sensitivity



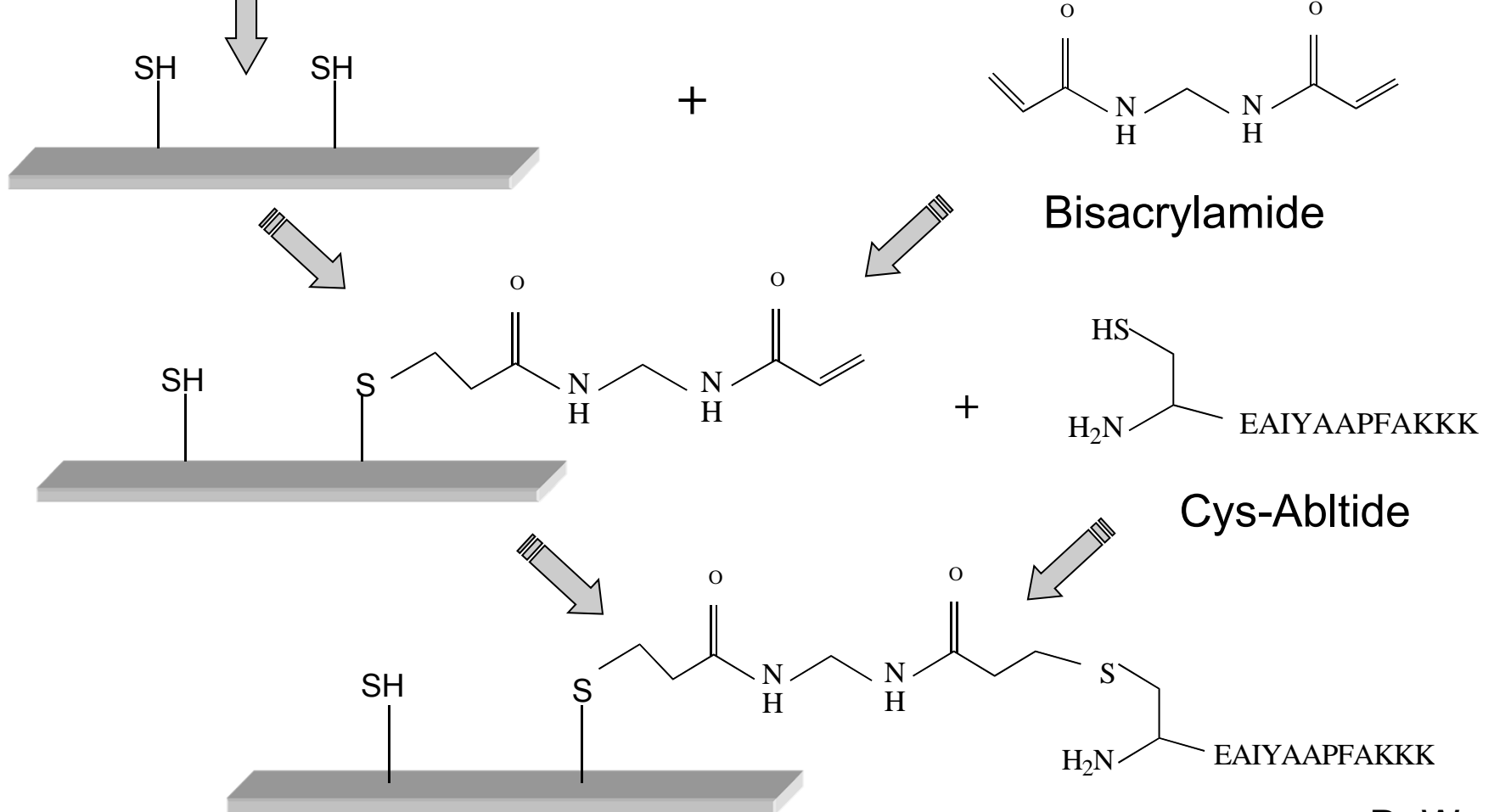
S. Petersen

# Chip based on ez-rays commercial hydrogel slide



ez-rays slides, multiwell plates (Matrix Technologies)

Activation  
by TCEP

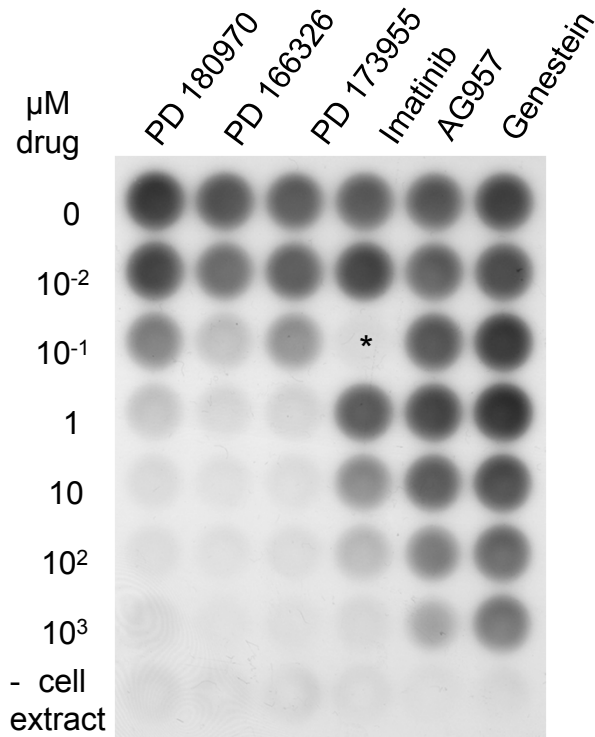


D. Wu

# High throughput Abl/Bcr-Abl activity assay

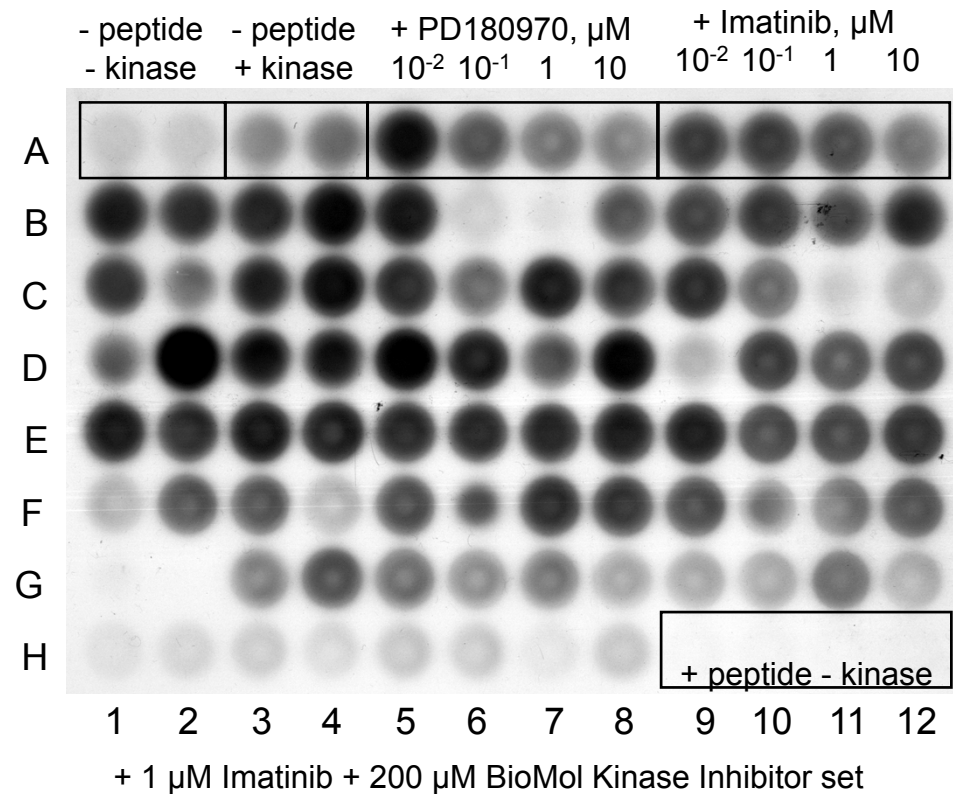
96 well ez-ray™ plates

K562 cell extract, Abltide  
10 μM ATP, 1 h @ 30° C



↓  
**IC<sub>50</sub> ~ 10 μM**

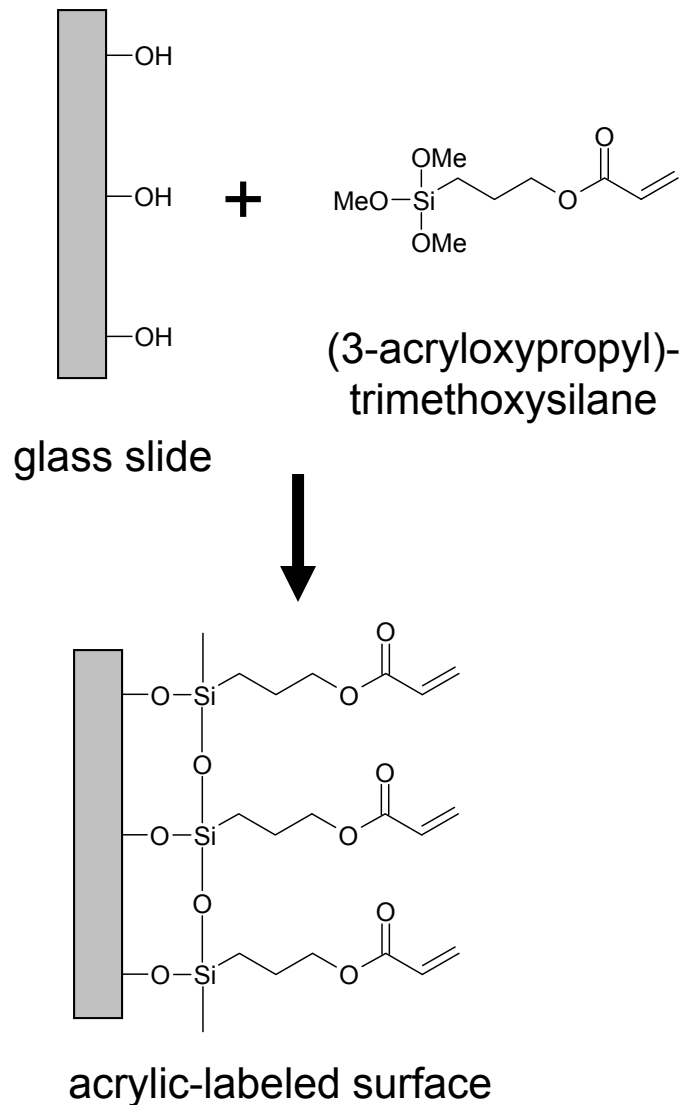
c-Abl, Abltide, 10 μM ATP, 1 h @ 30° C



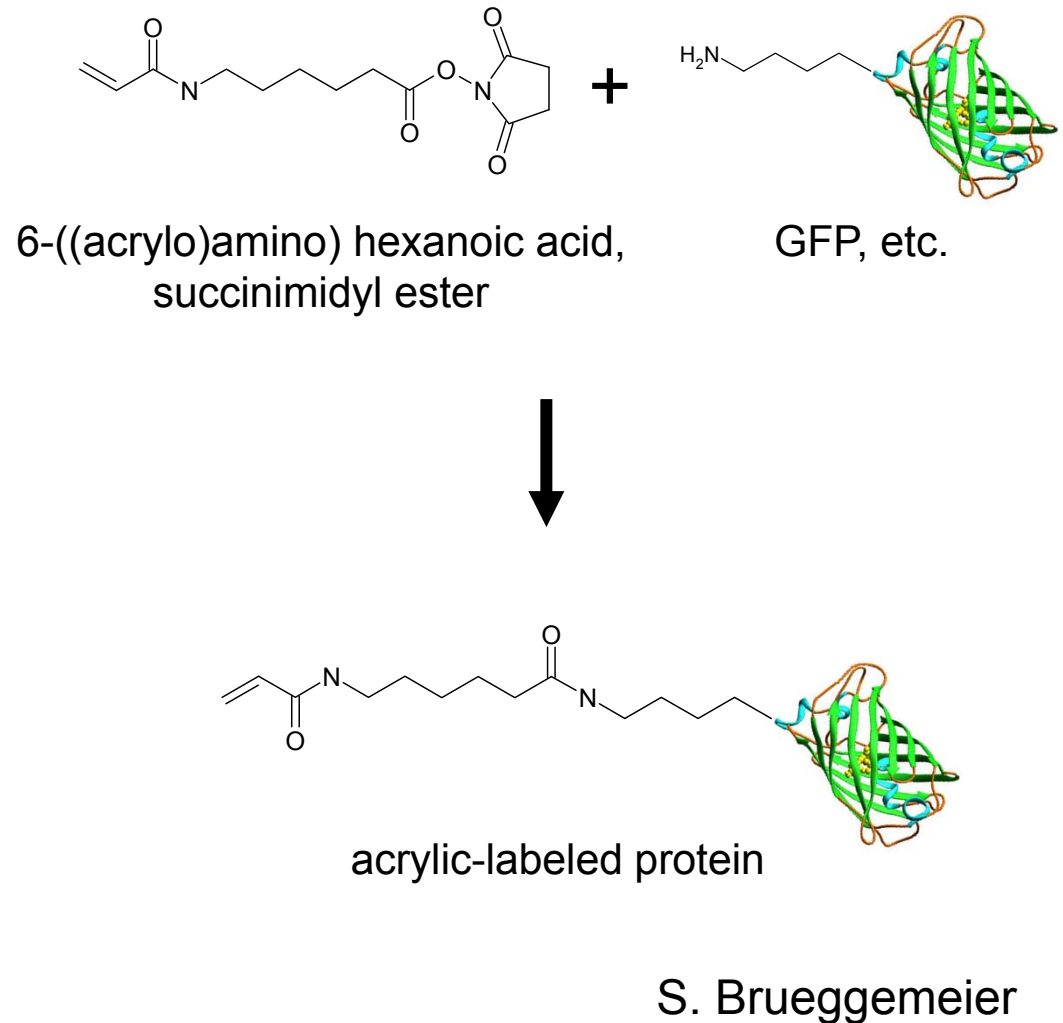
B6	Staurosporine	G1	Erbstatin analog	H2	Triciribine
B7	AG-494	G2	Quercetin dihydrate	H3	BML-257
C11	Piceatannol	G8	SP 600125	H4	SC-514
<b>C12</b>	<b>PP1</b>	G9	Indirubin	H5	BML-259
D9	Ro 21-8220	G10	Indirubin 3' monoxime	H6	Apigenin
F1	5-iodotubercidin	G12	Kenpaullone	H7	Erlotinib analog
<b>F4</b>	<b>PP2</b>	H1	Terreic acid	H8	Rapamycin

# Acrylic chemistry--Super glue for proteins

## Acrylated glass



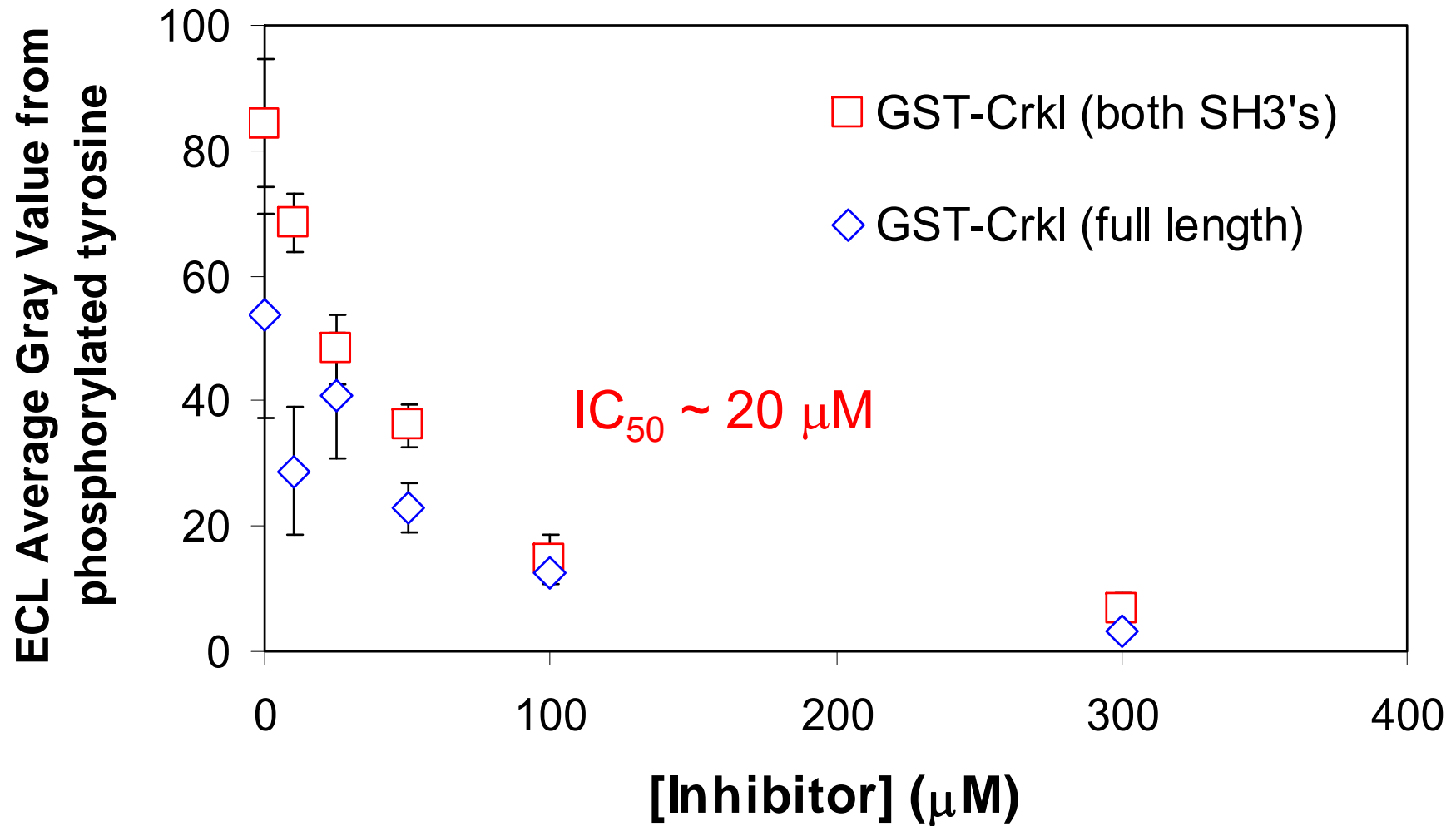
## Acrylated protein



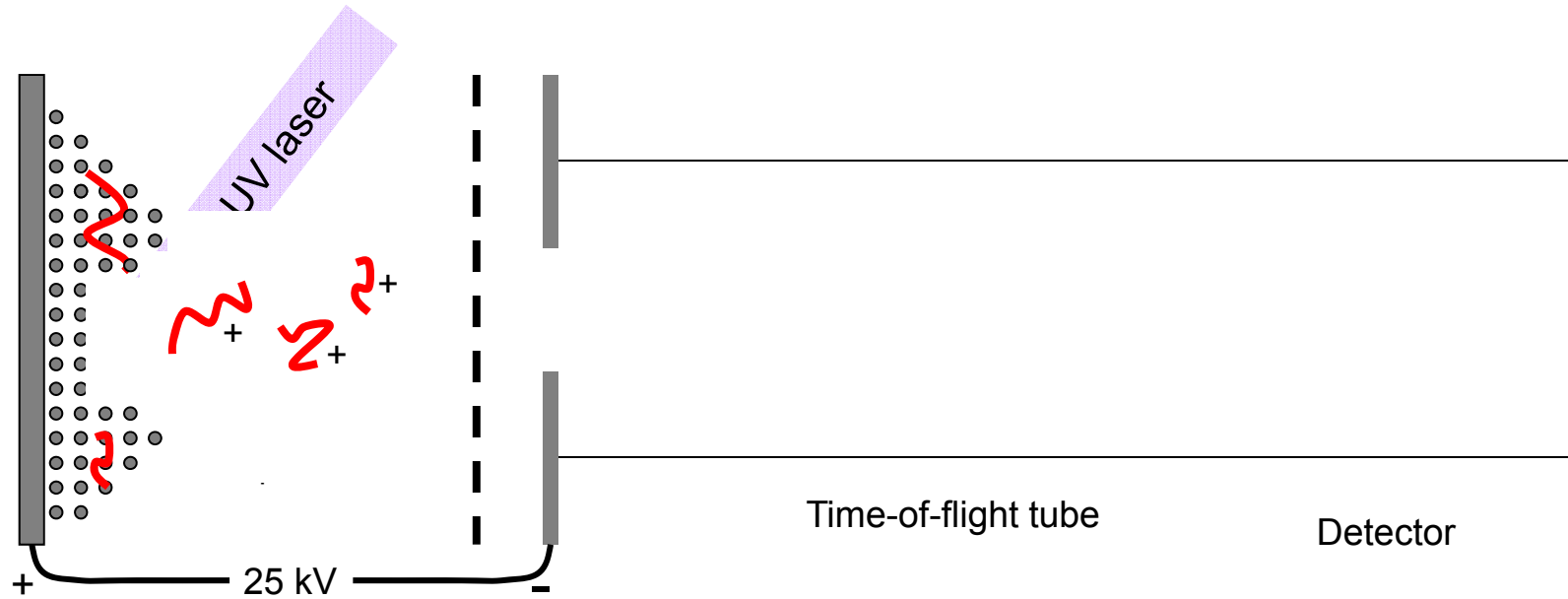




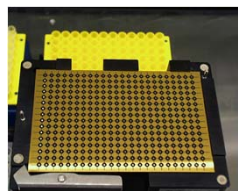
# Quantitative detection of Bcr-Abl inhibition by Imatinib



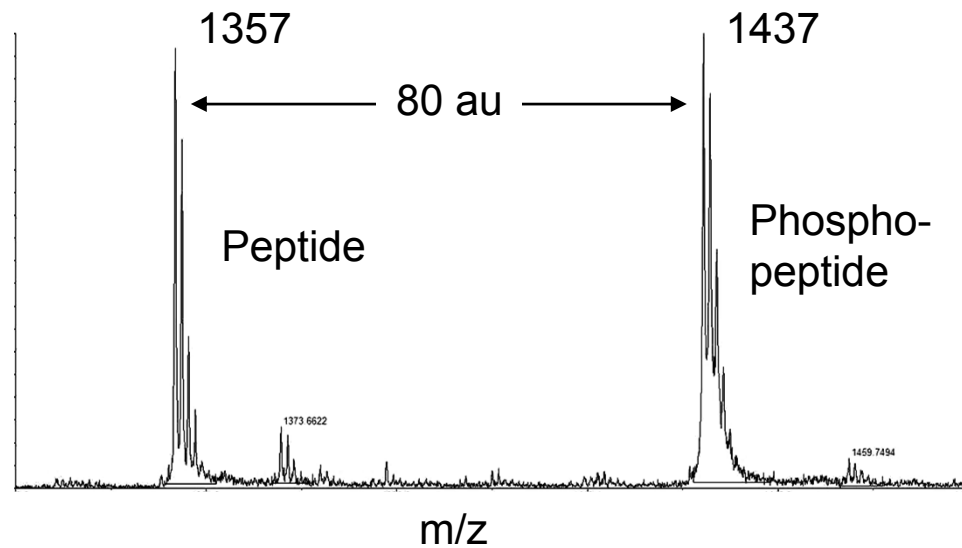
# Label-free detection of peptide phosphorylation



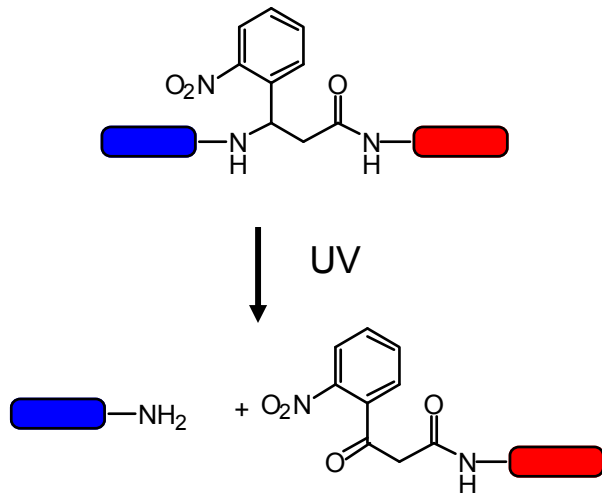
Matrix-assisted laser desorption ionization (MALDI) time-of-flight (TOF) mass spectrometry



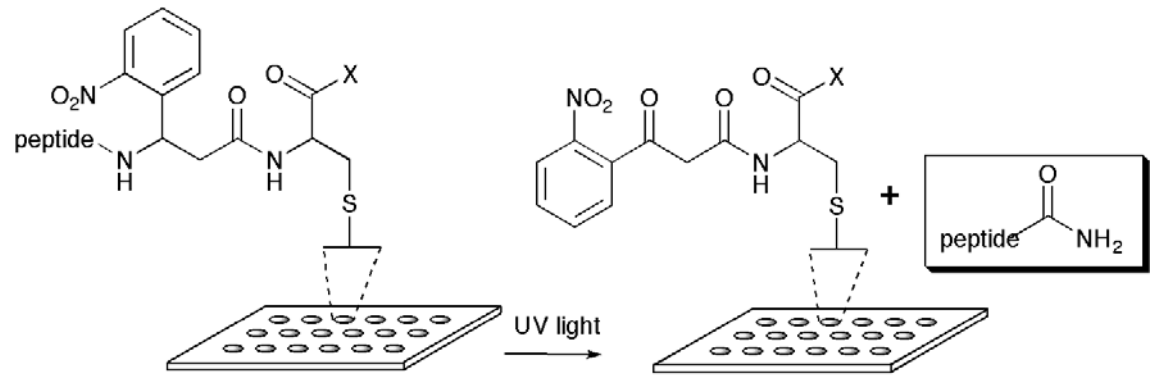
ABI 4700



# Photocleavable peptide array with MALDI read-off



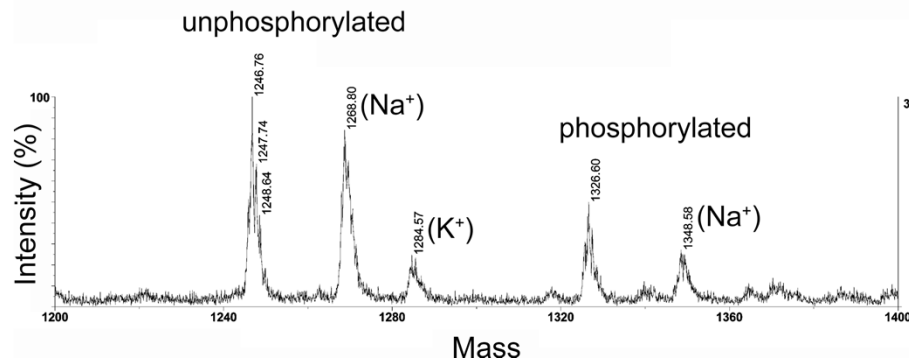
$\beta$ -NPA photolinker



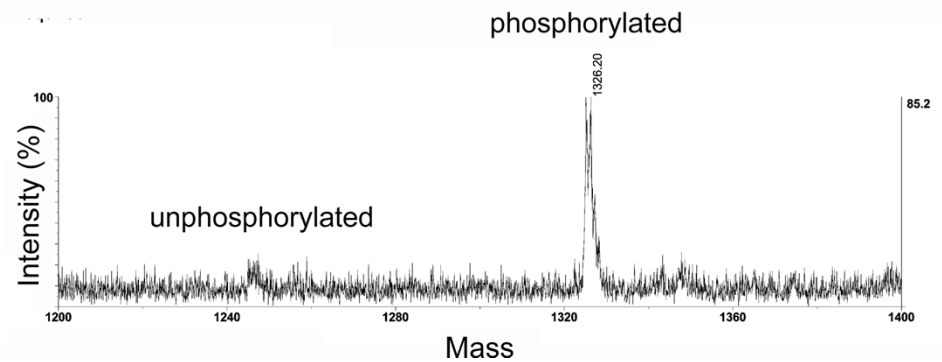
Photocleavable peptide arrays

1  $\mu$ l spot with 10  $\mu$ M Abltide, c-Abl, 1 h at 30 ° C

Linear positive



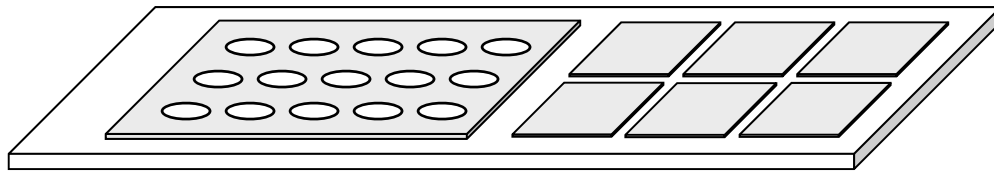
Linear negative



L. Parker et al.

# In development: Multiplexed "lawn format" assay

## Hydrogel pads in lawn or well geometry



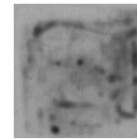
Polyacrylamide copolymer

Abltide EAIYAAPFAKKK- $\beta$ NPA-Cys-acrylamide

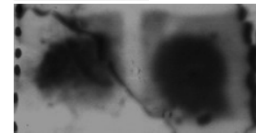
Srctide GEEPLYWSFPAKKK- $\beta$ NPA-Cys-acrylamide

etc..

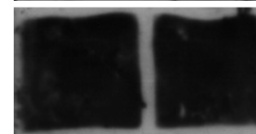
## Immunodetection



no Abltide-Cys  
+ cAbl



100  $\mu$ M Abltide-Cys  
+ cAbl



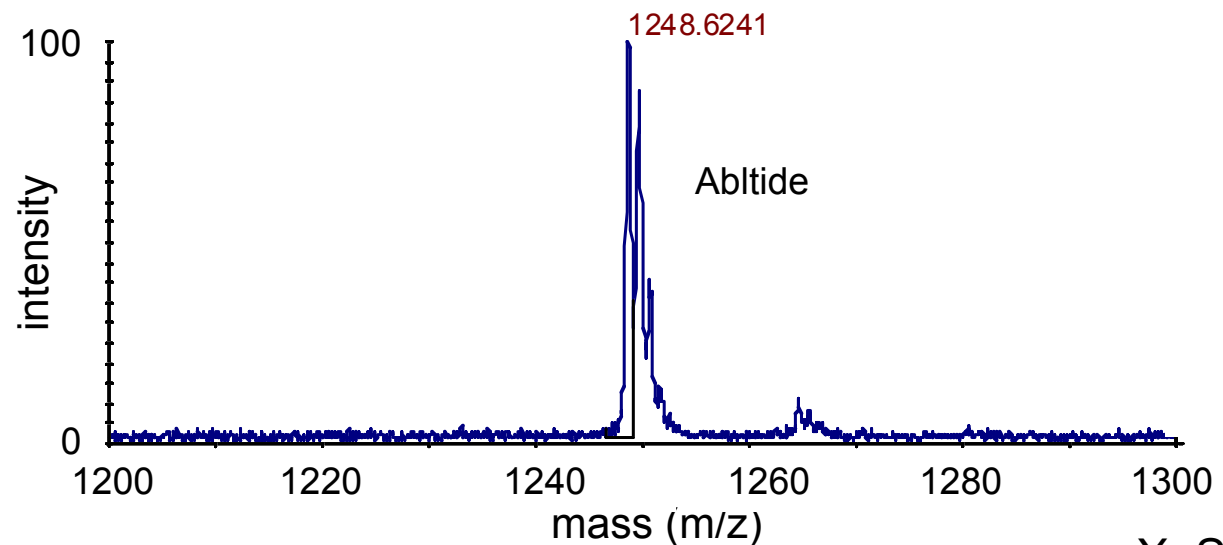
500  $\mu$ M Abltide-Cys  
+ cAbl

~1 cm

anti-pTyr "blot"

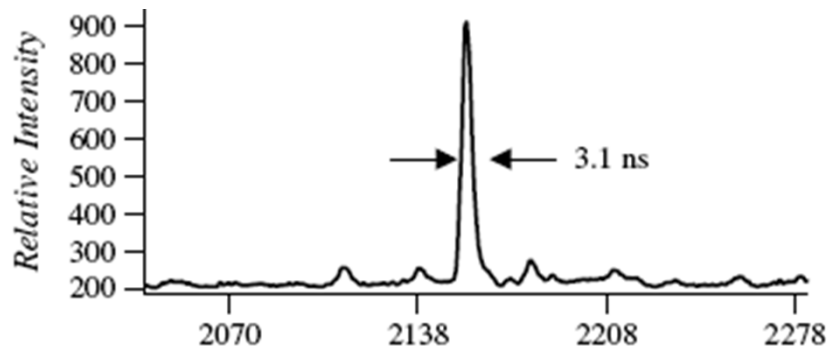
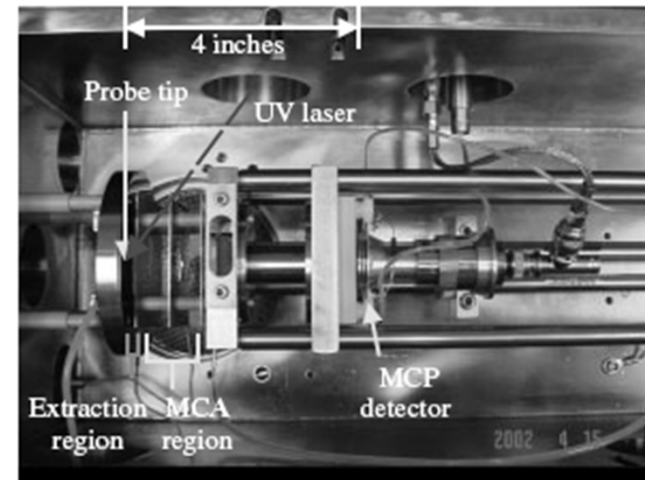
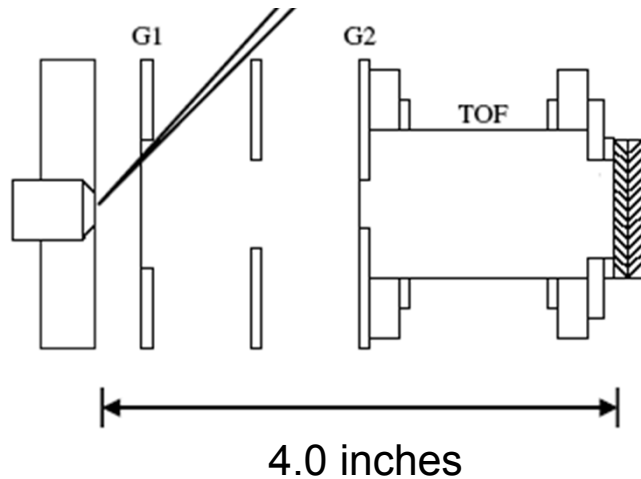
## MALDI detection from copolymerized pad

20  $\mu$ M Abltide- $\beta$ NPA-Cys  
CHCA matrix  
Linear positive mode



X. Shi

# Toward an integrated assay system: Cotter lab (JHU) mini-MALDI-TOF mass spec



Bench-top MALDI-TOF  
kinase activity microarray scanner?

# New assays, new geometries, new technologies

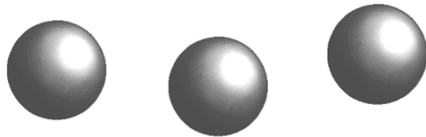
Plates



## **MethoCult methylcellulose colony forming cell assay**

Functional assay of growth inhibition by drugs  
Slow, low-throughput

Beads



## **Glutathione agarose/GST fusion phosphorylation assay**

Simple, sensitive, robust (Stratagene SignalScout)  
Low throughput

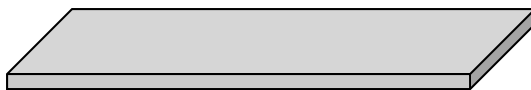
## **Luminex glutathione bead/GST fusion phosphorylation assay**

Simple, semi-quantitative, high throughput, easy multiplexing  
Dedicated reader, low sensitivity

## **Acrylamide copolymerization GST fusion phosphorylation assay**

Robust, high signal to noise  
Low sensitivity, difficult multiplexing

Chips



## **ez-rays 96 well hydrogel peptide phosphorylation assay**

Simple, semi-quantitative, medium throughput  
Low sensitivity, difficult multiplexing

## **Photocleavable peptide array with MALDI read-off**

Robust, semi-quantitative, high throughput, easy multiplexing  
Dedicated reader, low sensitivity

# The Team & Acknowledgements

## **Assays**

Steve Kron

Ding Wu

Xiangfu Shi

David Rhee

Jennifer Campbell

Shariska Petersen

## **Surface chemistry**

Sean Palecek

Shawn Brueggemeier

## **Funding**

**NIH NCI IMAT R33 CA103235**

NIH Roadmap R01 HG3864

NSF Chicago MRSEC

## **Cells/Patients**

Wendy Stock

Dorie Sher

Matthew Myers

## **Peptides/MALDI**

Steve Kent

Laurie Parker

Vivian Tien

## **Reagents**

A. Imamoto

J. Kuriyan

B. Druker

J. Groffen

C. Sawyers