

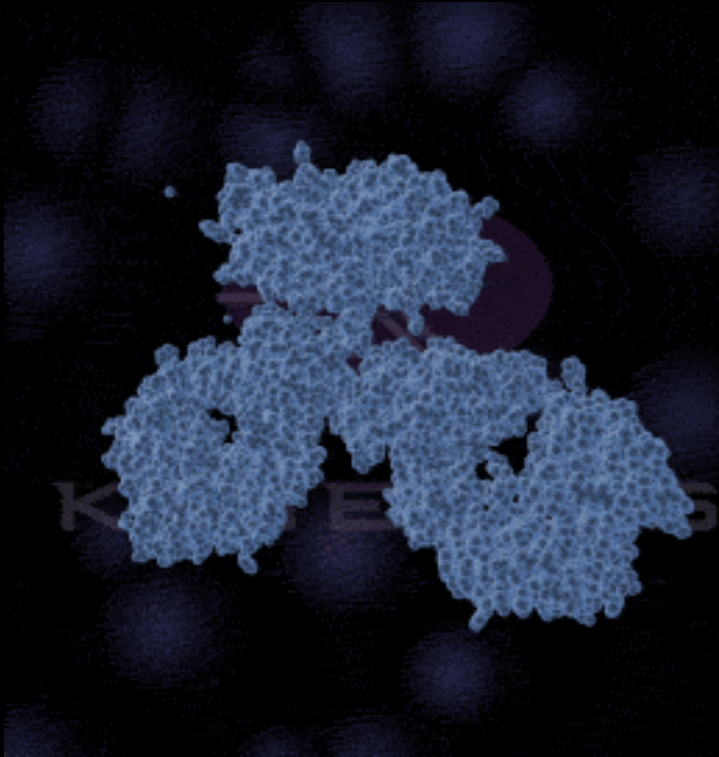
# Navigating Human Phosphorylation Networks with the SigNET Suite of On-line Knowledge Bases

Presented by Steven Pelech, Ph.D.

Professor, Department of Medicine,  
University of British Columbia  
President & CSO, Kinexus Bioinformatics Corp.



Presented at the MAP Kinase II Meeting at the Life Sciences Baltics  
2014 Conference in Vilnius, Lithuania, 2014 September 10



- ◆ Kinetworks™ and Kinex™ proprietary analyses uses over 1000 of the world's best antibodies cherry-picked from over 26 vendors and validated in-house
  - ◆ Targets low abundance cell signalling proteins
  - ◆ Quantifies protein expression and phosphorylation
  - ◆ Kinetworks™ multi-immunoblotting is more accurate and quantitative than competing methods
  - ◆ Kinex™ antibody microarray is more comprehensive and sensitive than other methods with unfractionated cell/tissue lysates



- ◆ Open access Kinetworks™ multi-immunoblotting database
- ◆ Features results from >10,000 immunoblots that track protein expression and phosphorylation from data generated with over 330 antibodies
- ◆ Over 200,000 protein measurements
- ◆ Searchable by protein, treatment or cell/tissue type
- ◆ Over 95% unpublished data



- ◆ Open access Kinex™ KAM antibody microarray database
- ◆ Features results from >2,500 microarrays that track protein expression and phosphorylations data generated with 650 to 850 antibodies
- ◆ Over 3 million protein measurements
- ◆ Searchable by protein, treatment or cell/tissue type
- ◆ Over 99% unpublished data



- ◆ Open access human phosphorylation site database
- ◆ >177,000 confirmed and 790,000 additional predicted phosphorylation sites in >21,500 human proteins
- ◆ Functional information for ~1% of the phosphosites
- ◆ Evolutionary analyses in 20 other species
- ◆ Kinase specificity prediction



### Info Box

S473 is phosphorylated by the following protein kinases in vitro: ATM, ILK1, mTOR, PDK1, PKC-beta1, PRK2

Click the coloured buttons below for further information in the Info Box, or click the orange buttons for links.

### Phospho-Sites

	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	Expt. conf.	Effect	Kinase	PPase	Ref.	Evol.	Kinase Pred.	
Site 1	S122	E	E	E	M	D	F	R	S	G	S	P	S	D	N	S	■	■	■	■	■	■	■
Site 2	S124	E	M	D	F	R	S	G	S	P	S	D	N	S	G	A	■	■	■	■	■	■	■
Site 3	S126	D	F	R	S	G	S	P	S	D	N	S	G	A	E	E	■	■	■	■	■	■	■
Site 4	S129	S	G	S	P	S	D	N	S	G	A	E	E	M	E	V	■	■	■	■	■	■	■
Site 5	S246	L	S	R	E	R	V	F	S	E	D	R	A	R	F	Y	■	■	■	■	■	■	■
Site 6	S473	R	P	H	F	P	Q	F	S	Y	S	A	S	G	T	A	■	■	■	■	■	■	■
Site 7	T308	K	D	G	A	T	M	K	T	F	C	G	T	P	E	Y	■	■	■	■	■	■	■
Site 8	T34	F	L	L	K	N	D	G	T	F	I	G	Y	K	E	R	■	■	■	■	■	■	■
Site 9	T450	T	A	Q	M	I	T	I	T	P	P	D	Q	D	D	S	■	■	■	■	■	■	■
Site 10	T479	F	S	Y	S	A	S	G	T	A	-	-	-	-	-	■	■	■	■	■	■	■	■
Site 11	T72	T	E	R	P	R	P	N	T	F	I	R	C	L	Q	■	■	■	■	■	■	■	■
Site 12	Y175	K	E	K	A	T	G	R	Y	Y	A	M	K	I	L	K	■	■	■	■	■	■	■
Site 13	Y176	E	K	A	T	G	R	Y	Y	A	M	K	I	L	K	K	■	■	■	■	■	■	■
Site 14	Y253	S	E	D	R	A	R	F	Y	G	A	E	I	V	S	A	■	■	■	■	■	■	■
Site 15	Y272	H	S	E	K	N	V	V	Y	R	D	L	K	L	E	N	■	■	■	■	■	■	■
Site 16	Y315	T	F	C	G	T	P	E	Y	L	A	P	E	V	L	E	■	■	■	■	■	■	■
Site 17	Y326	E	V	L	E	D	N	D	Y	G	R	A	V	D	W	W	■	■	■	■	■	■	■
Site 18	Y38	N	D	G	T	F	I	G	Y	K	E	R	P	Q	D	V	■	■	■	■	■	■	■
Site 19	Y437	T	S	E	T	D	T	R	Y	F	D	E	E	F	T	A	■	■	■	■	■	■	■
Site 20	Y474	P	H	F	P	Q	F	S	Y	S	A	S	G	T	A	-	■	■	■	■	■	■	■


### Legend

- Confirmed in Mammals
- Confirmed in Related Proteins or Other Species
- Predicted by Kinexus Algorithm
- No Data/Link Available
- Link Available



### PHOSPHONET Evolution

Human Phospho-Site KnowledgeBase

SIGNET KNOWLEDGEBANK + KINETICA ONLINE + KINEXUS 

Search by protein name, UniProt number, IPI number, or 15 AA P-site sequence.

Search

[Kinexus Home](#) | [Contact](#) | [Credit](#)

Want to screen cells/tissues for altered protein expression and phosphorylation?  
Our Antibody Microarray Services track over 500 signalling proteins.

#### Phospho-Site

Human Protein: **AKT1**  
Human Site: **S473**  
UniProt: **P31749**

#### Conservation Score

All Species: **34.55**  
Identified Species: **69.09**  
Number Species: **11**

#### Phospho-Site Substitution

Charge Score: **0**
























### Conservation

Percent  
 Protein Identity:  
 Protein Similarity:  
 P-Site Identity:  
 P-Site Similarity:

																
Human	Chimpanzee	Rhesus Macaque	Dog	Cat	Mouse	Rat	Wallaby	Platypus	Chicken	Frog	Zebra Danio	Tiger Blowfish	Fruit Fly	Honey Bee	Nematode Worm	Sea Urchin
100	81.5	99.5	93.9	N.A.	98.1	98.1	N.A.	73	39.7	93.1	39.3	N.A.	49.5	N.A.	52.5	N.A.
100	92.1	99.5	95.2	N.A.	98.7	99.1	N.A.	80.4	56.8	96.2	57.5	N.A.	61.7	N.A.	67.8	N.A.
100	73.3	100	100	N.A.	100	100	N.A.	0	26.6	93.3	26.6	N.A.	46.6	N.A.	26.6	N.A.
100	73.3	100	100	N.A.	100	100	N.A.	0	40	93.3	46.6	N.A.	53.3	N.A.	53.3	N.A.

Percent  
 Protein Identity:  
 Protein Similarity:  
 P-Site Identity:  
 P-Site Similarity:

					
Poplar Tree	Maize	Rice	Thale Cress	Baker's Yeast	Red Bread Mold
N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
N.A.	N.A.	N.A.	N.A.	N.A.	N.A.



## PHOSPHONET Kinase Predictor

Human Phospho-Site KnowledgeBase

SIGNET KNOWLEDGEBANK + KINETICA ONLINE + KINEXUS

Search by protein name, UniProt number, IPI number, or 15 AA P-site sequence.

[Kinexus Home](#) | [Contact](#) | [Credit](#)

Want to visualize the locations of proteins and phosphorylation reactions in cells?  
Check out our Custom Immunohistochemistry Services

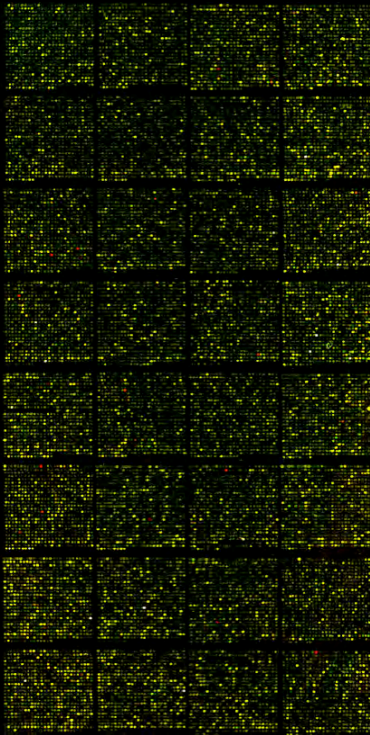
### Phospho-Site

Human Protein: AKT1  
Human P-Site: S473  
P-Site Sequence: RPHFPQF**S**YSASGTA  
UniProt: P31749  
Intern. Prot. ID: IPI00012866

### Protein Kinase Match

	Human Kinase Short Name	Human UniProt. ID	Prediction Score
Kinase 1:	mTOR/FRAP	P42345	228.49
Kinase 2:	ILK	Q13418	158.34
Kinase 3:	SRPK1	Q96SB4	118.15
Kinase 4:	SRPK2	P78362	117.84
Kinase 5:	MSSK1 (STK23)	Q9UPE1	117.65
Kinase 6:	ATM	Q13315	94.18
Kinase 7:	ERK1	P27361	86.16
Kinase 8:	ERK2 (MAPK1)	P28482	86.10
Kinase 9:	DNAPK	P78527	76.92
Kinase 10:	GPRK5	P34947	76.15
Kinase 11:	GPRK6	P43250	75.50
Kinase 12:	GPRK7	Q8WTQ7	75.37

- ◆ Prediction of kinases is based in silico screening with phospho-site specificity matrices for 500 human protein kinase catalytic domains
- ◆ Each matrix is generated with a proprietary algorithm that uses the primary amino acid structure of the kinase for prediction



- ◆ Gene microarrays permit measurement of mRNA levels
- ◆ NCBI Gene Expression Omnibus (GEO) Database features gene chip data from >50,000 studies
- ◆ Kinexus developed TranscriptoNET to consolidate and normalize human gene expression data from 6000 normal and tumour cells and tissues to permit comparisons across studies



- ◆ Open access human mRNA expression knowledgebase
- ◆ >600 human control and cancer tissues and cell lines
- ◆ ~21,000 human genes covered
- ◆ Contains data from over 125 million measurements
- ◆ Identifies highly, commonly and uniquely expressed genes in human tissues and cells
- ◆ Identifies differentially expressed genes in about 50 types of human cancers
- ◆ Identifies genes that are highly co-expressed
- ◆ Shows tissue and cell relatedness



KINEXUS

Human Cancer Protein KnowledgeBase

[www.onconet.ca](http://www.onconet.ca)

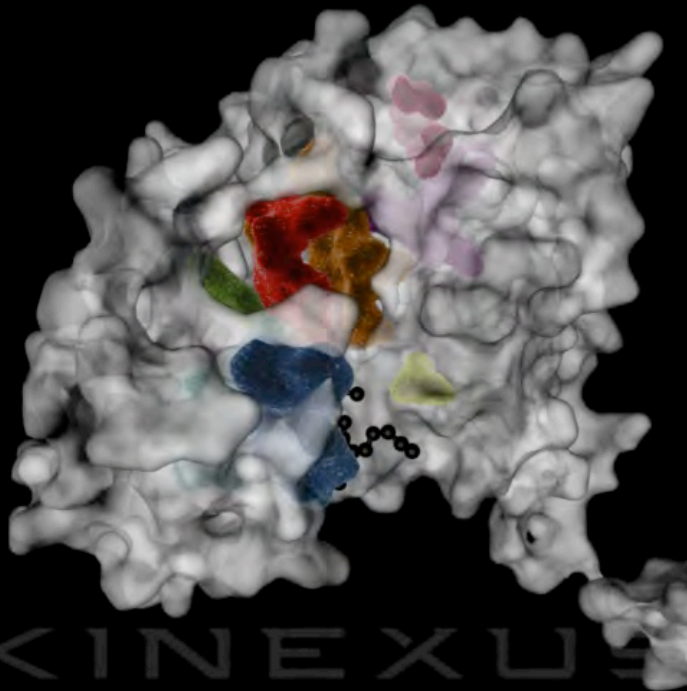


- ◆ Open-access human cancer gene knowledgebase
- ◆ Beta-version currently available on-line
- ◆ Features mRNA expression and mutation information on 3000 proteins previously linked to cancer
- ◆ Classifies oncoproteins (OPs), tumour suppressor proteins (TSPs), and tumour-requiring proteins (TRPs)



## Approved Kinase Drugs and Those in Development

▫



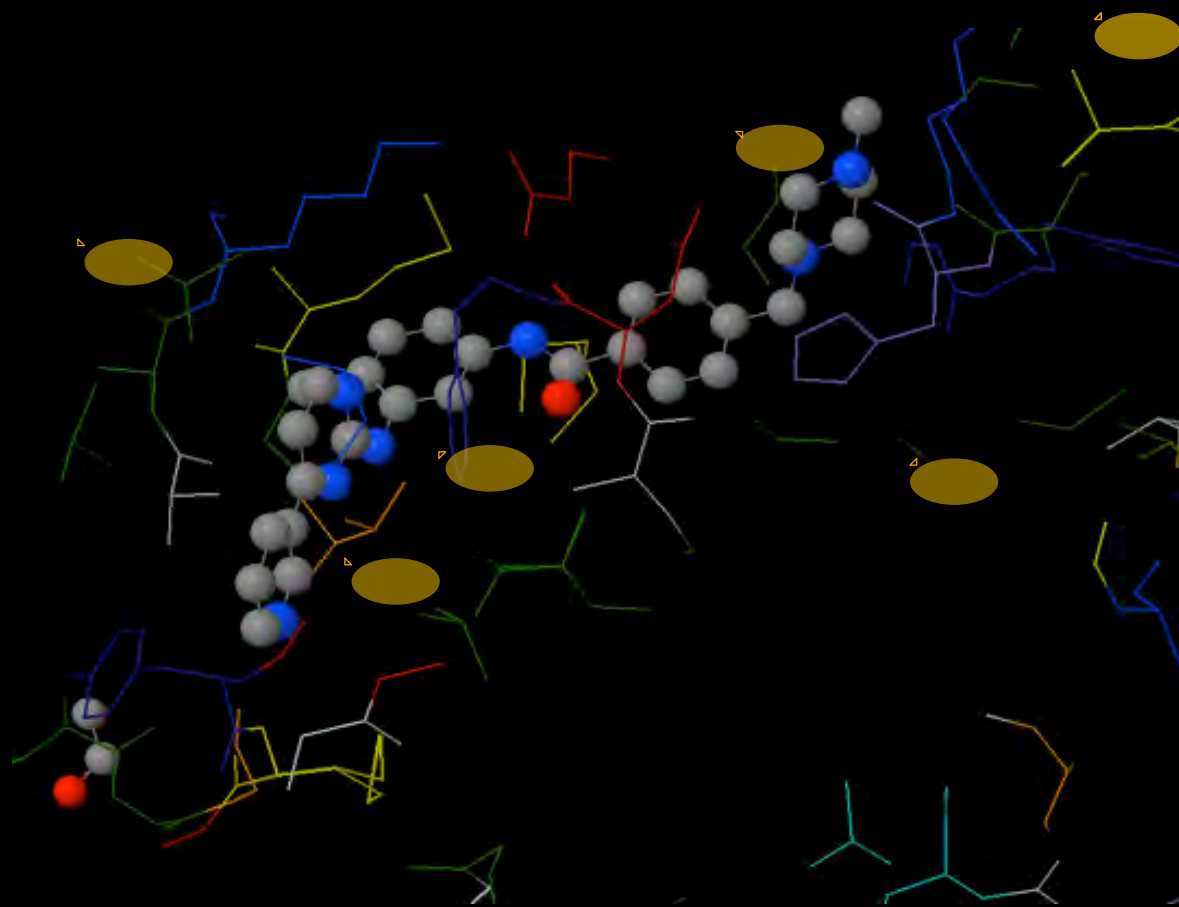
EGF receptor 3D structure with kinase inhibitor drug Tarceva

- ◆ >19 drugs approved
- ◆ >150 in clinical trials
- ◆ >500 in pre-clinical trials



- ◆ Human protein kinase drug knowledgebase
- ◆ Features experimental results for over 105,000 kinase drug pairs (400 kinases, >850 compounds that inhibit kinases)
- ◆ Features predictive data for another 200,000 kinase-drug pairs with 500 kinases
- ◆ Information and links to other websites provided about each drug

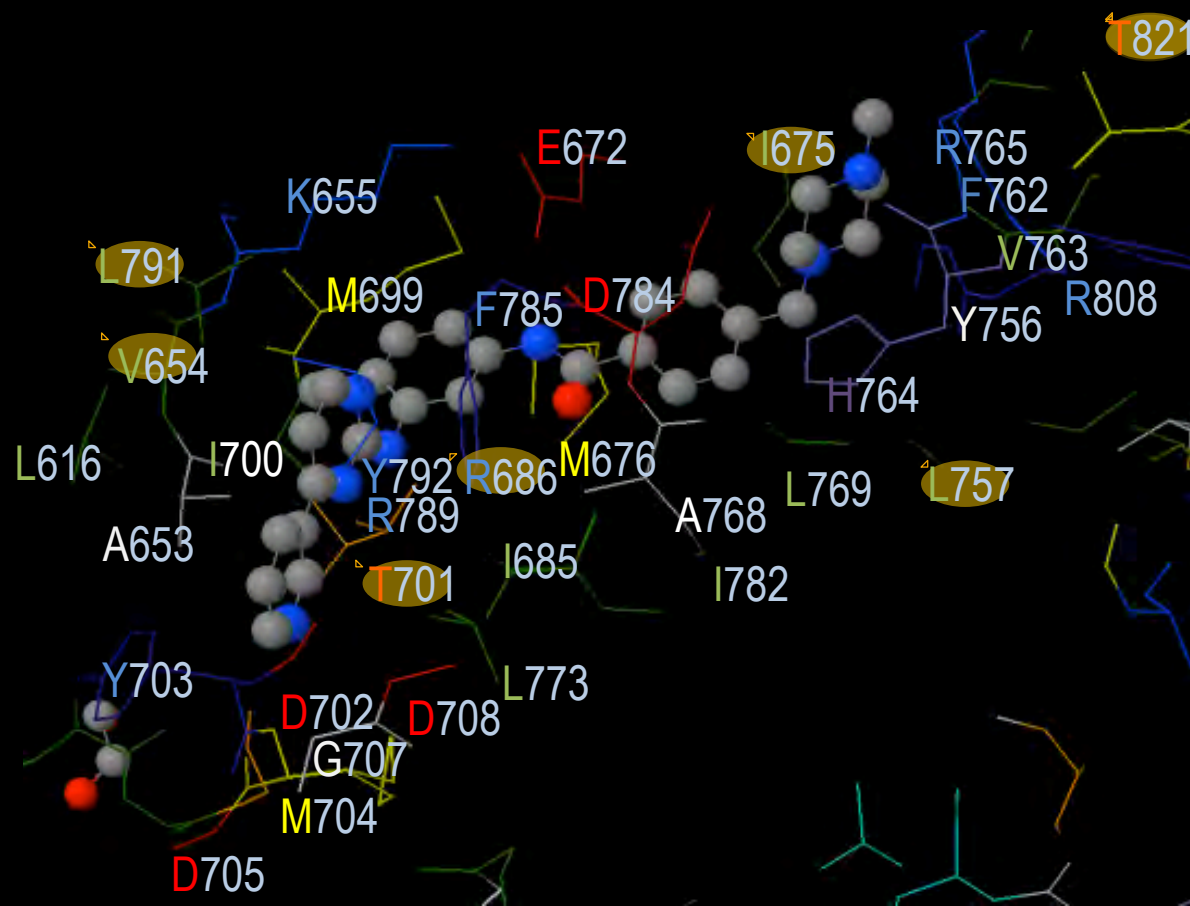
## Identified Imatinib Interactions with DDR1 Amino Acid Residues



- ◆ DrugKiNET identified amino acids are indicated in orange ovals



## Identified Imatinib Interactions with DDR1 Amino Acid Residues



- ◆ DrugKiNET identified amino acids are indicated in orange ovals
- ◆ Similar analyses may define biomarkers for drug sensitivity



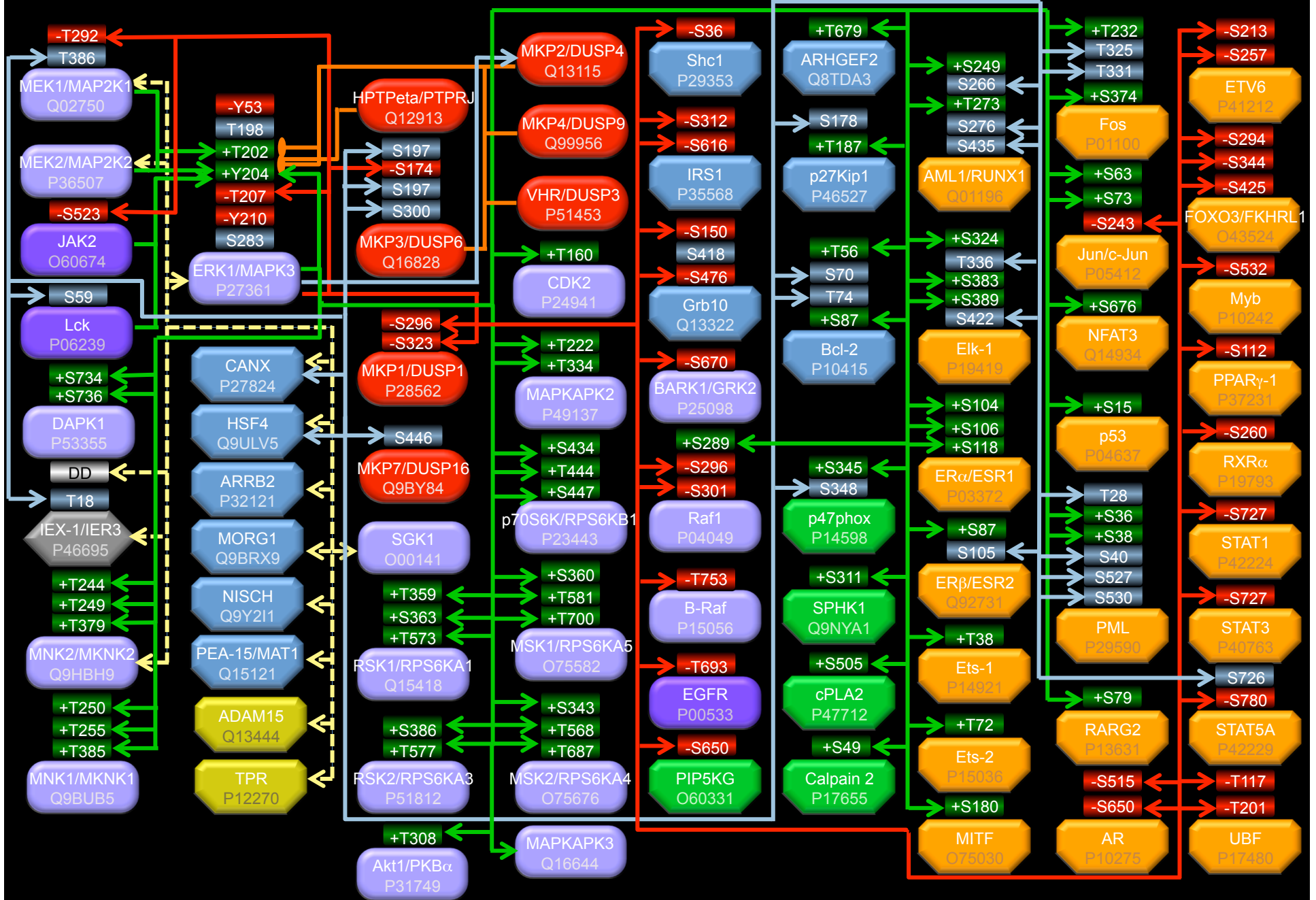
Two mapping initiatives are currently underway at Kinexus

- ◆ Kinnections Maps

- ◆ Static, hand-annotated mini-maps that are target-centric with immediate upstream and downstream direct and functional interactions tracked
- ◆ To be provided with Kinex™ KAM- 880 Antibody Microarray analyses – An MS-Excel data table is generated with each map based on the data obtained from the comparison of two cell or tissue lysates
- ◆ Will be available as MS-PowerPoint downloads

# Kinections Map P27361

# Extracellularly Regulated Kinase 1



- ◆ KinATLAS KnowledgeBase
  - ◆ Dynamic, customizable tissue/cell-specific maps of protein substrate and drug interactions with kinases based on experimental and predictive data
  - ◆ Identifies direct protein-protein interactions
  - ◆ Constructed from PhosphoNET, TranscriptoNET and DrugKiNET data and hyperlinks to these and other websites
  - ◆ Generates new maps with associated protein as the hub



Select type of query desired

[Click to view options](#)

[Home](#) | [Contact](#) | [Credits](#)

### Query Type

#### Single Target Protein Phosphorylation Interaction

This query identifies the top phosphorylation interactions involving protein kinases and their substrates around a selected protein of interest. The target protein appears in the center of the generated interaction map that is cell, tissue or organ-specific. The stronger the kinase-substrate connection as calculated with our Kinase Substrate Predictor Version 2.0 Algorithm, the closer the associated protein. Apply the following filters to narrow down the most relevant interactions to your individual specifications.

### Filters

#### Step 1 - Target Protein Identification

Provide the gene name, protein name, UniProt ID, RefSeq or IPI ID as a search term.

#### Step 2 - Human Cell/Tissue Specification

Use one of the two pull-down menus to specify the cell, tissue or organ type of interest.

#### Step 4 - Generation of Protein Phosphorylation Map

Click on boxes below to produce a custom map or reset parameters. The map will appear in a new window.

Want to compare your Kinexus proteomics data with thousands of other studies? Query our KINET Immunoblotting DataBase online with free access.

#### Step 3 - Selection of Display Parameters

Boxes that are marked are activated to display. Click on boxes to change status.

- Show experimentally-derived interactions
- Show predicted interactions
- Show upstream inputs
- Show downstream outputs
- Show stimulatory phosphosites
- Show inhibitory phosphosites
- Show non-defined phosphosites
- Show protein kinases
- Show protein phosphatases
- Show transcription factors
- Show regulatory proteins
- Show metabolic proteins
- Show structural proteins
- Show uncharacterized proteins
- Show black background
- Show icons with uniform size
- Show UniProt ID



### Figure Legend

Protein Type

- Tyr Kinase
- Ser/Thr Kinase
- Phosphatase
- Transcription
- Regulatory
- Metabolic
- Structural
- Unknown

Phosphosite Type

- Inhibitory
- Stimulatory
- Unknown

Interaction Type

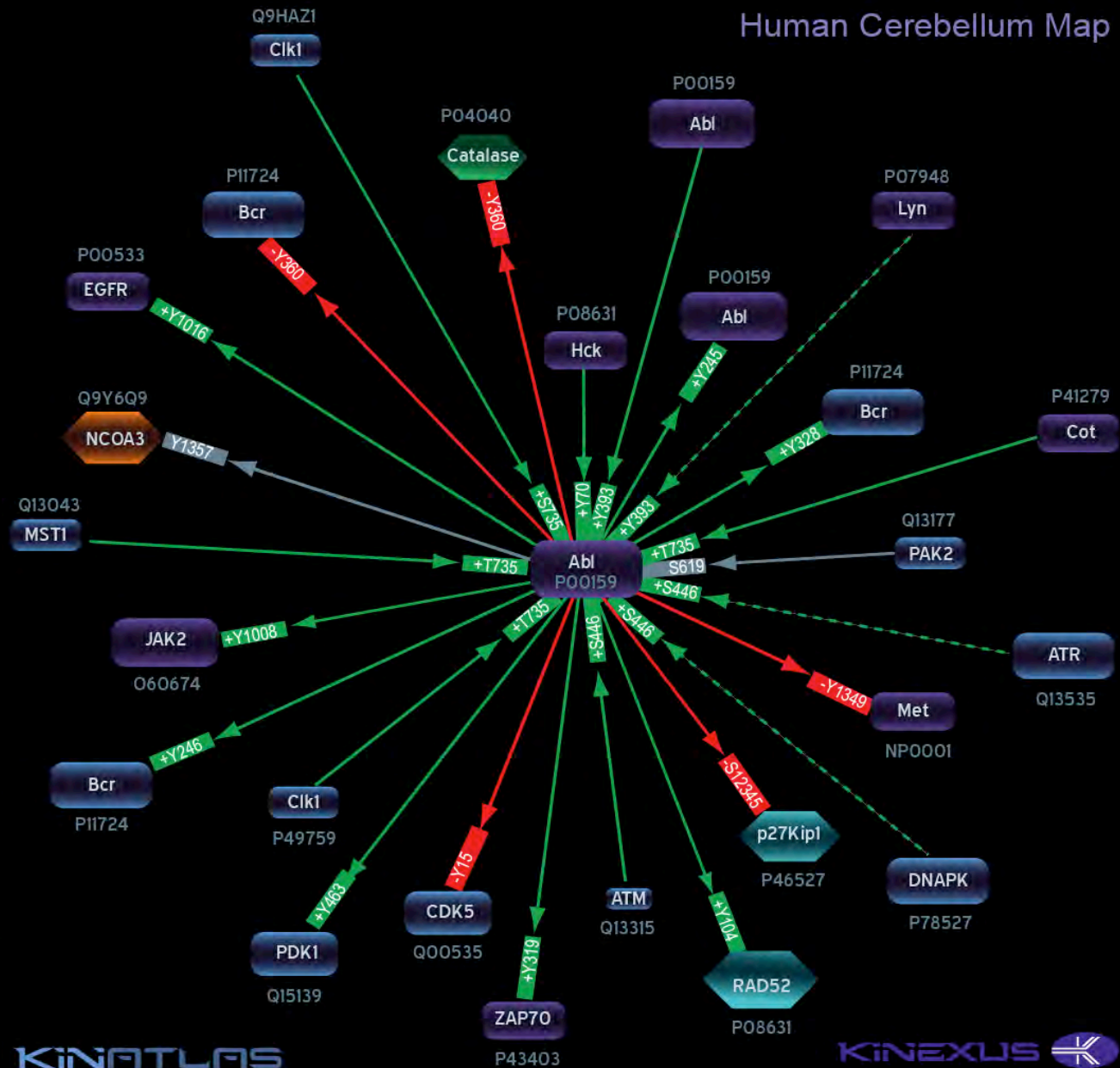
Experimentally Confirmed Phosphorylation

- Inhibitory
- Stimulatory
- Unknown

Predicted Phosphorylation

- Inhibitory
- Stimulatory
- Unknown

### Human Cerebellum Map











**Figure Legend**

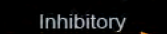
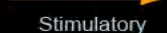
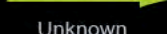
## Protein Type

-  Tyr Kinase
-  Ser/Thr Kinase
-  Phosphatase
-  Transcription
-  Regulatory
-  Metabolic
-  Structural
-  Unknown

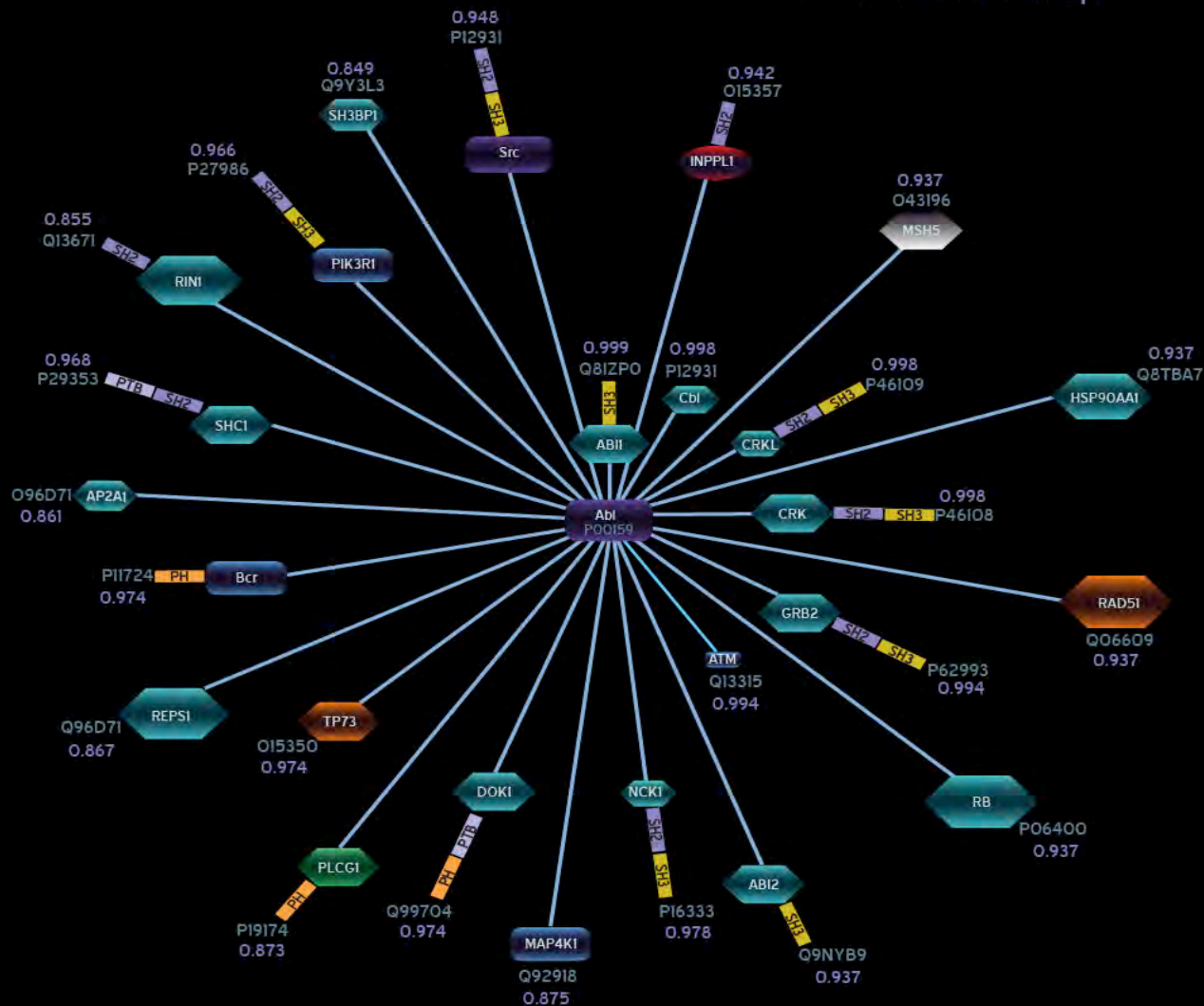
## Domain Type

-  CaMB Calmodulin-binding
-  DD Death
-  DED Death Effector
-  PDZ PDZ
-  PH Pleckstrin Homology
-  PTB P-Tyr Binding
-  SH2 Src Homology 2
-  SH3 Src Homology 3

## Interaction Type


-  Inhibitory
-  Stimulatory
-  Unknown

## Human Liver Map



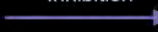
### Figure Legend

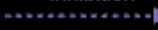
Protein Type

 Tyr Kinase

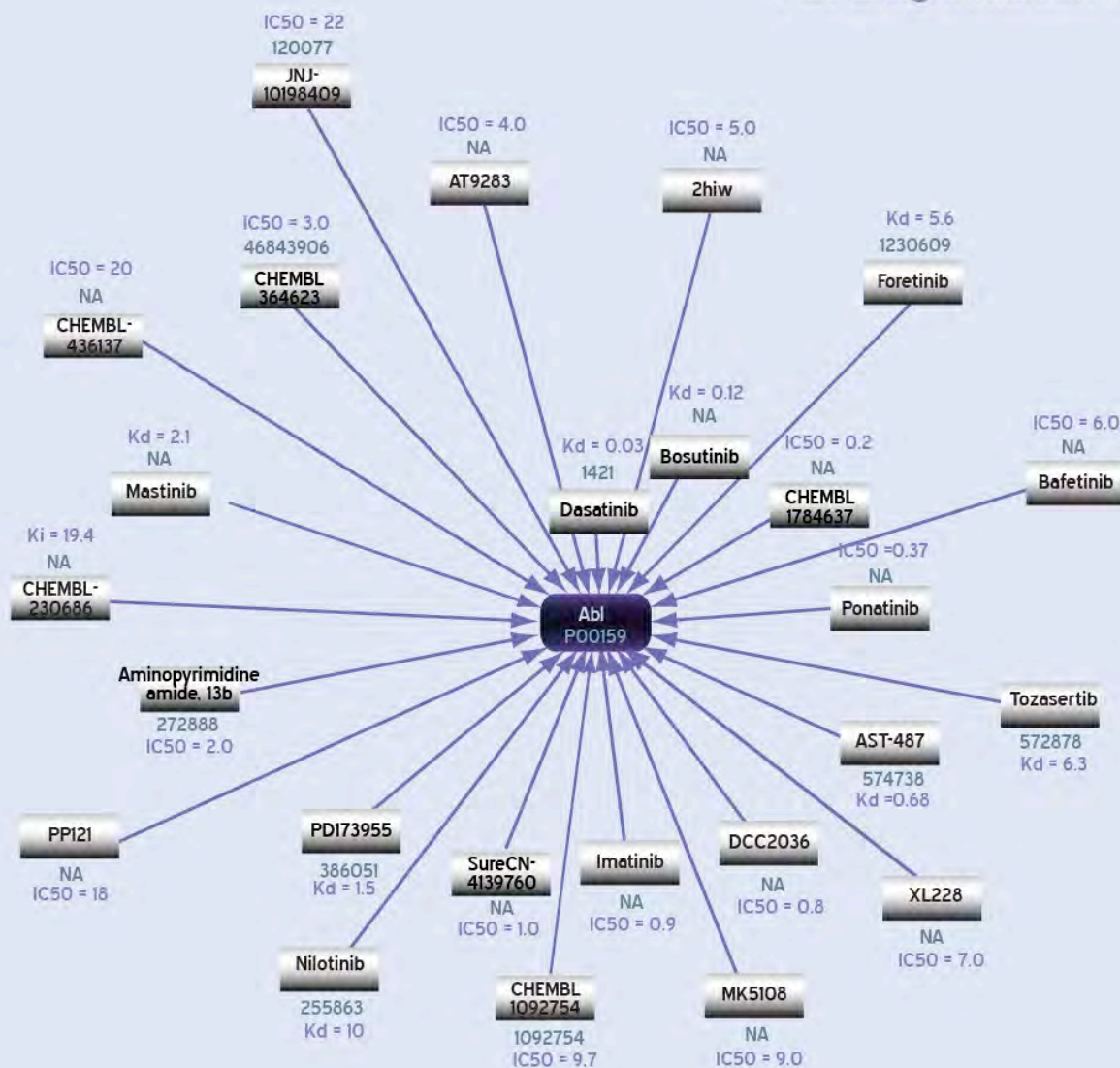
 Ser/Thr Kinase

Interaction Type

Experimentally Confirmed Inhibition  


Predicted Inhibition  


## Abl Drug Inhibition Map







### University of British Columbia

Javad Safaei, Dr. Jano Manuch, Shabab Hossein, Alireza Davoodi, Shenshen Lai, Sehyun Cho, Stephen Yiu, Chuen Choi, Pier-Luc Clemont, Christine Eisner, Cameron Rogers, Scott Wilder, Leon, Chew, Erandika Gunaretnam, Anna Isphording, Christopher Laver, Asher Mendelson, Steven Pham, Cecilia Sjoestroem, and Jovian Wat

### Simon Fraser University

Dr. Ladislav Stacho

### Kinexus Bioinformatics Corporation

Dr. Steven Pelech, Winnie So, Catherine Sutter, Dr. Hong Zhang, Dr. Dirk Winkler, Dr. Jane Shi, Dr. Arthur Yee, Dr. Rod Verdie, Cameron Bowyer, Litsa Blanis, Angie Chu, Mandy Chung, Pringle Comia, Aneesa Din, Nova Do, Bryanna Grace, Eileen Hu, Iris Juan, Joanna Kam, Stephanie Lam, Adam Leigh, Raymond Leung, Sara Marrello, Charrise Pagarigan, Andrew Park, Michael Peabody, Sabrina Rayworth, Ryan Saranchuk, Kevin Sha, Ellen Sung, Sharan Swarup, Amy Tam, Ryan Whitehead, Christine Yu, and Sarah Zaidi

### British Columbia Institute of Technology

#### PhosphoNET ([www.phosponet.ca](http://www.phosponet.ca))

Joe Hu, Jonathan Jose, Ysabel Lago, David Liao, John Liao, Christine Livingstone, Melissa Manalac, Travis Nicholson, Kevin Odger, Ryan Pattinson, Nevin Petersen, Hannah Rosellon, Janice Sargent, Brandon Wong, and Alvin Yip

#### TranscriptoNET ([www.transcriptonet.ca](http://www.transcriptonet.ca))

Isan Chen, Keegan Kelly, Aly Jamani, and Colin Nguyen

#### DrugKiNET ([www.drugkinet.ca](http://www.drugkinet.ca))

James Chen, Andy Chow, Sunju Christine Jeong, and Dan Stephenson

#### OncoNET ([www.onconet.ca](http://www.onconet.ca))

Kyle Li, Theo Mutia, Travis Ryder, and Clarence Sng

#### KiNET-AM ([www.kinet-am.ca](http://www.kinet-am.ca))

Simon Ho, Nicholas Tagle, Fausto Faioli, Igor Kozlov, and Justin Ma