Chemical Proteomics-based Approach for Drug Target Discovery in Living Systems

胡良海

吉林大学生命科学学院

E-mail: lianghaihu@jlu.edu.cn

Outline

1. Introduction drug target discovery

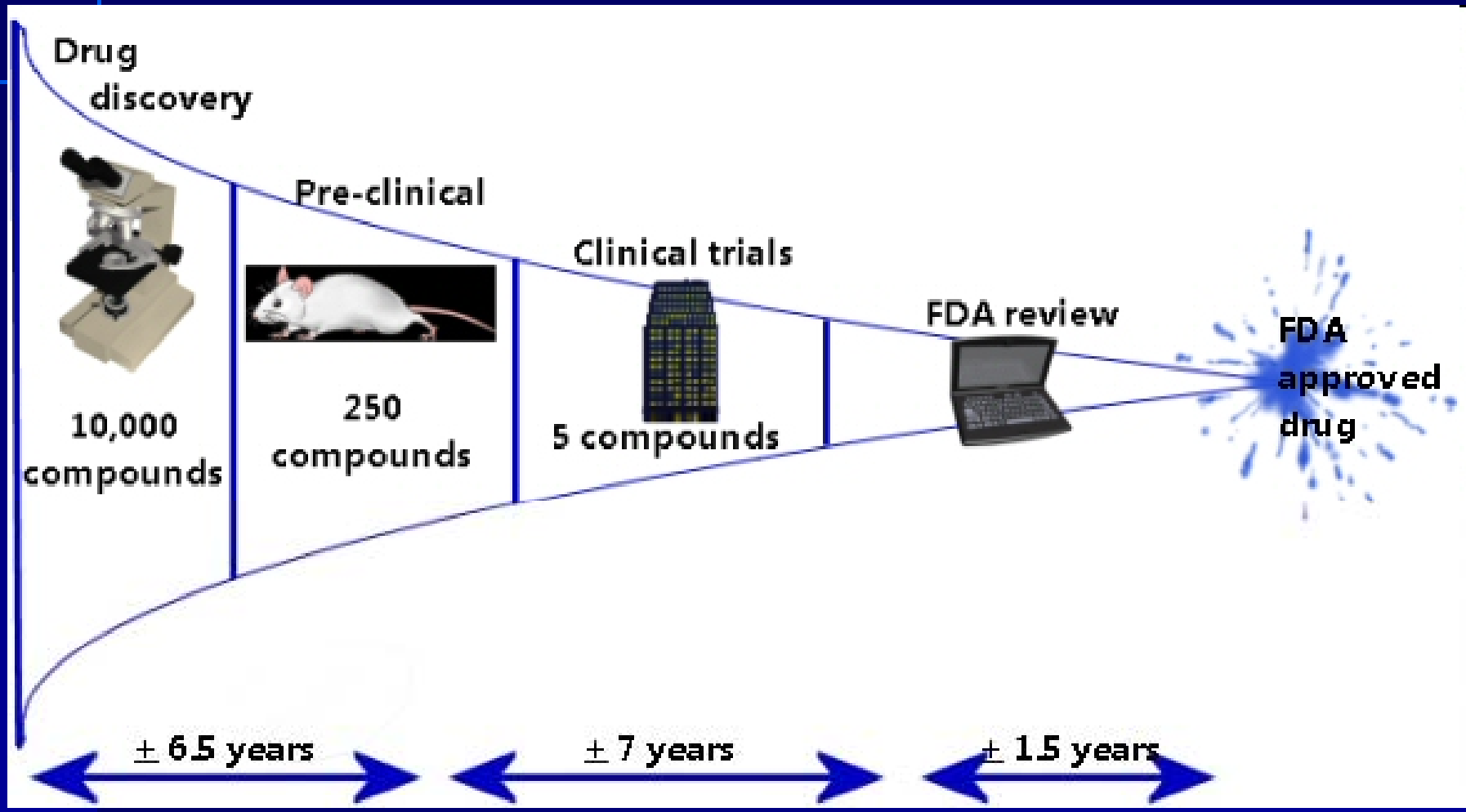
2. Identification of drug target in live cells by chemical proteomic approach

3. Tyrosine kinase/phosphatase substrate

Outline

1. Introduction drug target discovery
2. Identification of drug target in live cells by chemical proteomic approach
3. Tyrosine kinase/phosphatase substrate

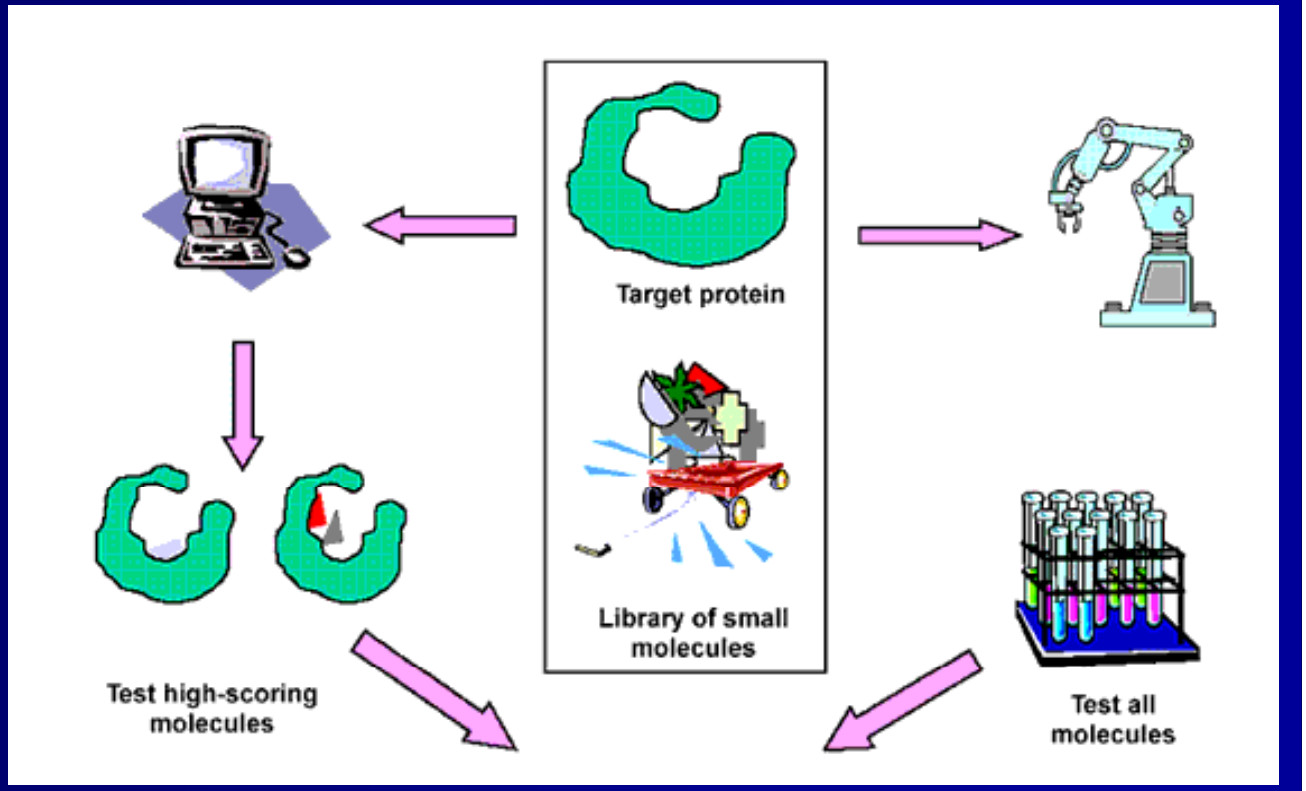
Drug Discovery Pipeline



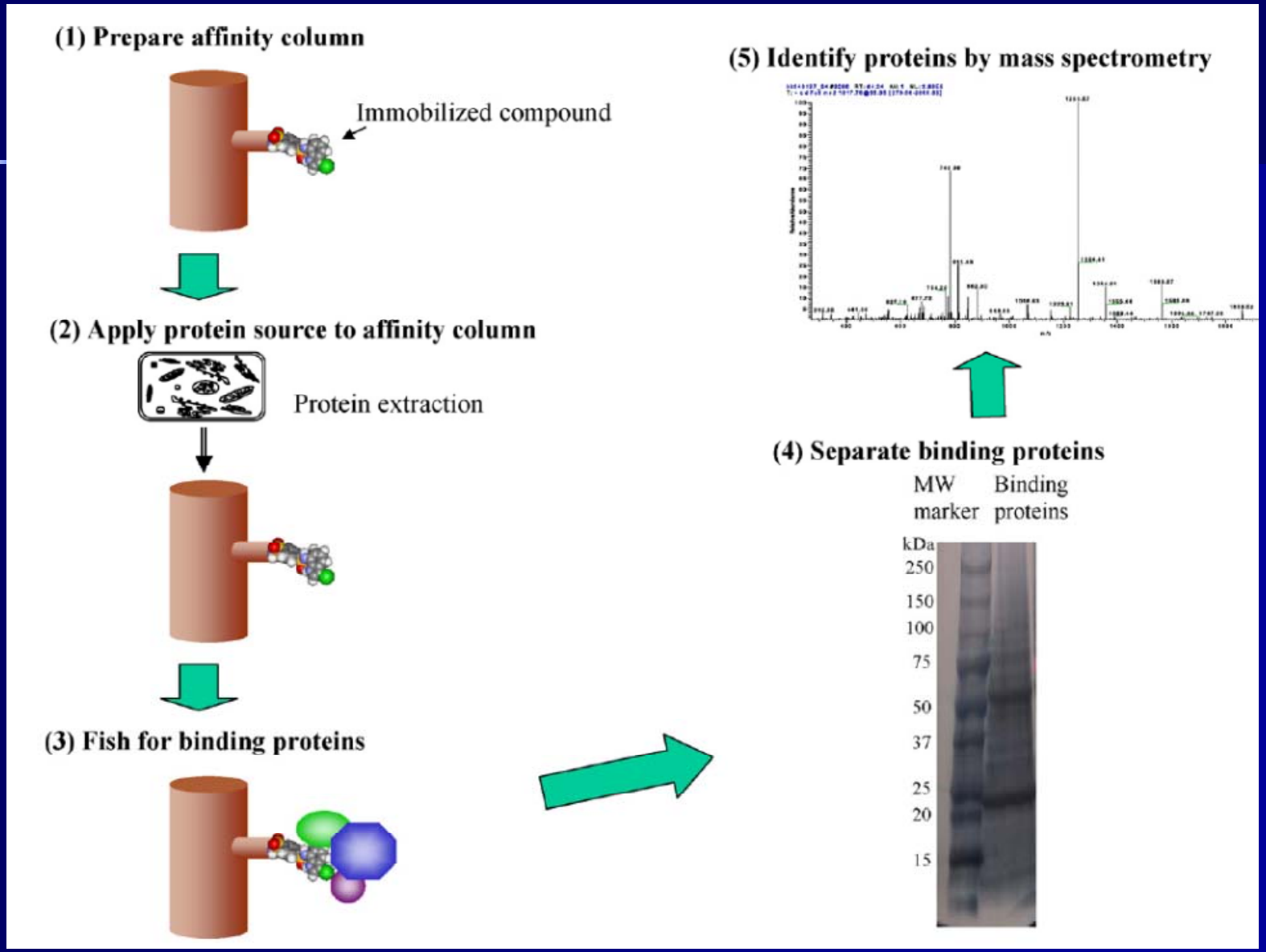
High-throughput Screening

The Nature of Drug Target

Who came first ?
Drug or target

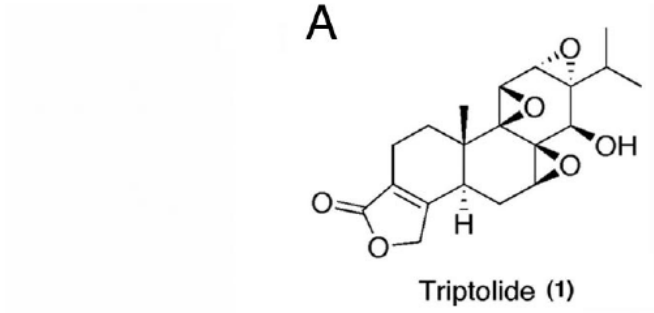


Bead-based Chemical Proteomics

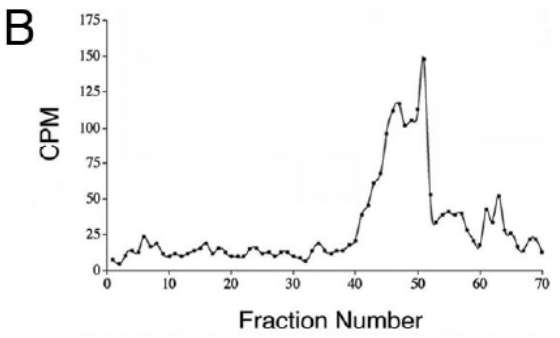


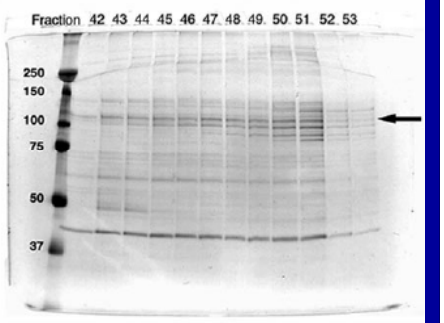
Why living systems ?

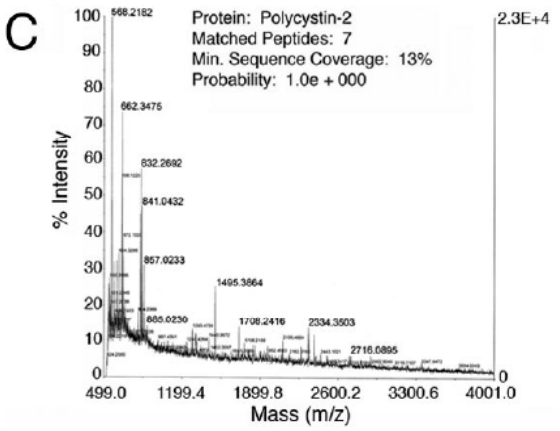
Triptolide is a traditional Chinese medicine-derived inhibitor of polycystic kidney disease

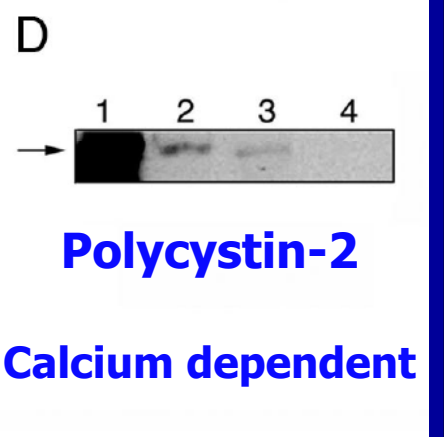


Triptolide









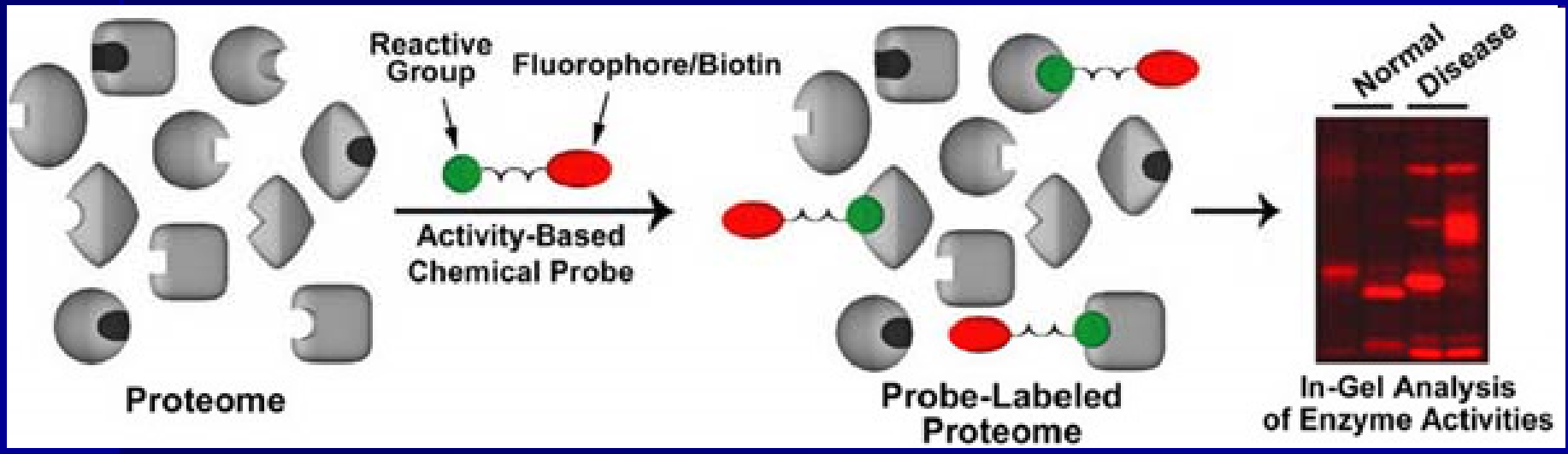
Polycystin-2

Calcium dependent

Chem Biol. **2005; 12(12): 1259-1268**
Nat Biotechnol. **2005; 23(10): 1303-1307**
Proc Natl Acad Sci USA. **2007; 104(11): 4389-4394**

Activity-Based Protein Profiling (ABPP)

Tag Linker Reaction group



Prof. Benjamin F. Cravatt, The Scripps Research Institute

Prof. Matthew Bogyo, Stanford University

Outline

1. Introduction to drug target discovery

2. Identification of drug target in live cells by chemical proteomic approach

3. Tyrosine kinase/phosphatase substrate

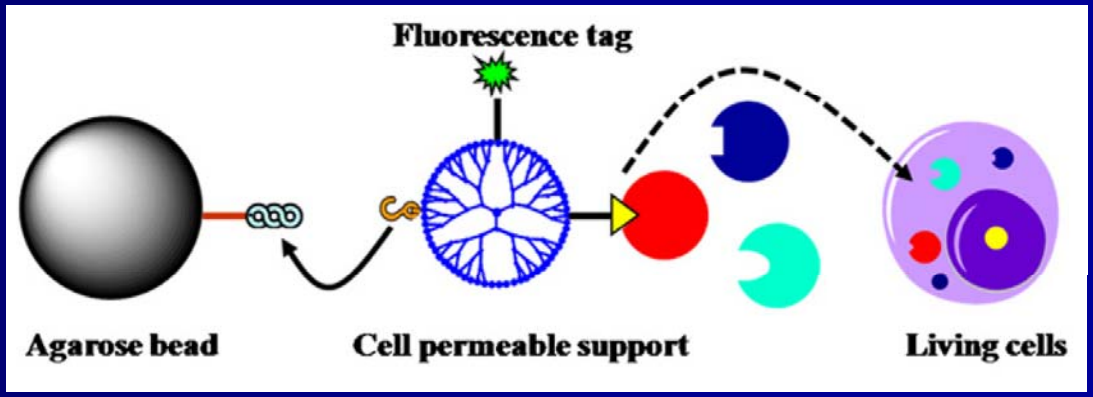
Intracellular protein target *in vivo*

Identification of Drug Targets In Vitro and in Living Cells by Soluble-Nanopolymer-Based Proteomics

Lianghai Hu, W. Andy Tao, et al.

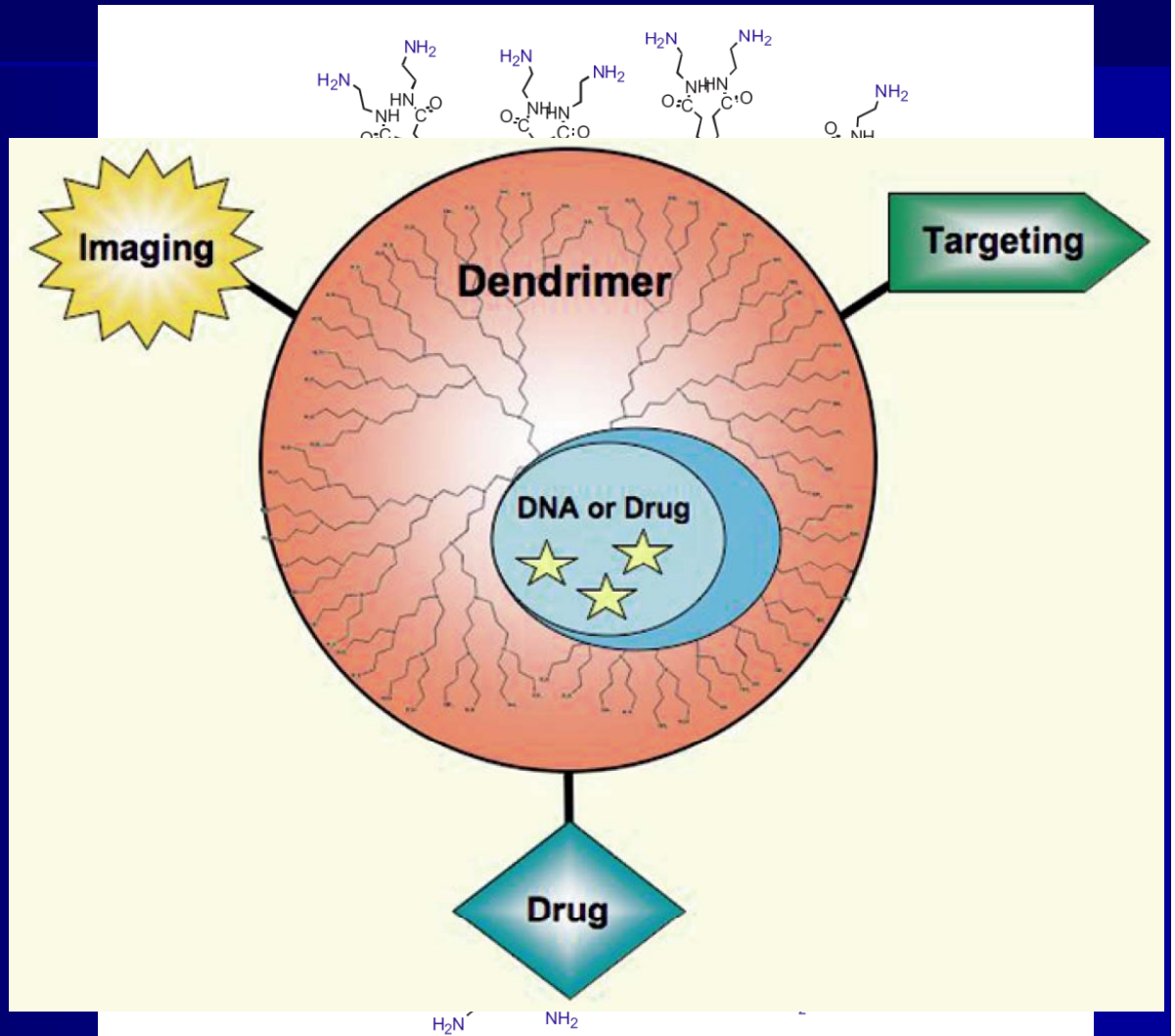
Angew. Chem. Int. Ed. 2011, 50(18):4133-4136

(Selected as "hot paper" by the editor)

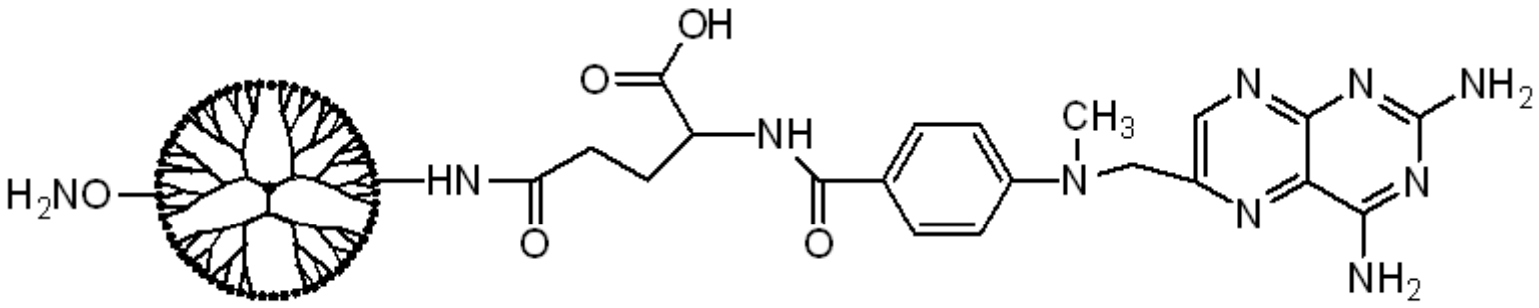


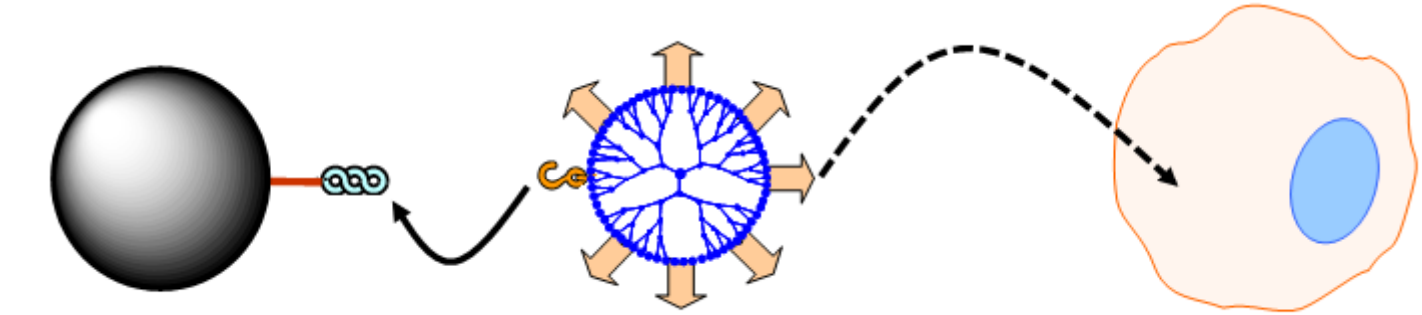
Dendrimer-based Nanomedicine

1. Excellent solubility
2. High structural/chemical homogeneity
3. Compact spherical shape
4. High branching
5. Controlled surface functionalities
6. Ability to permeate cells
7. Low cytotoxicity



In vivo characterization of protein targets

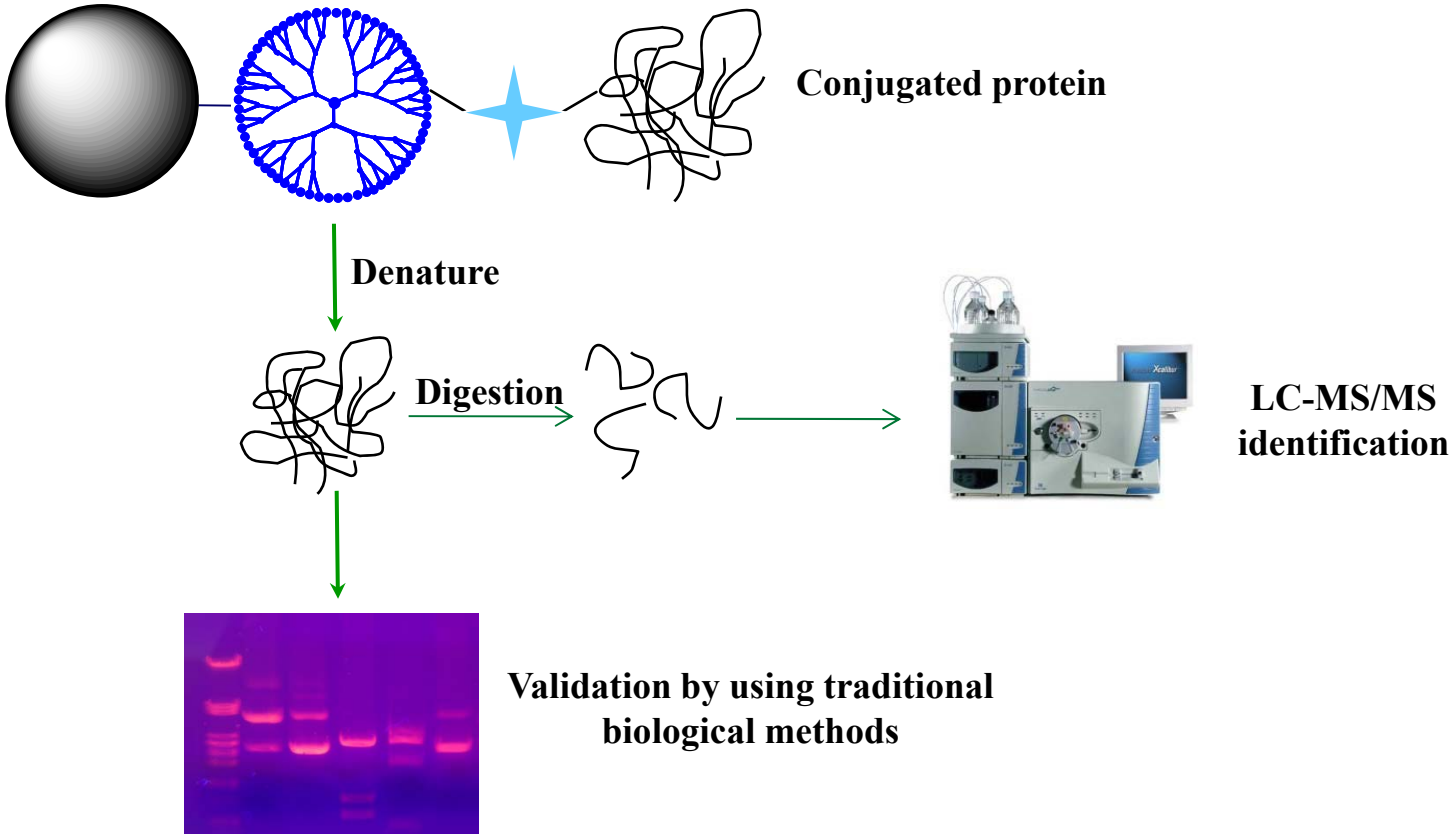




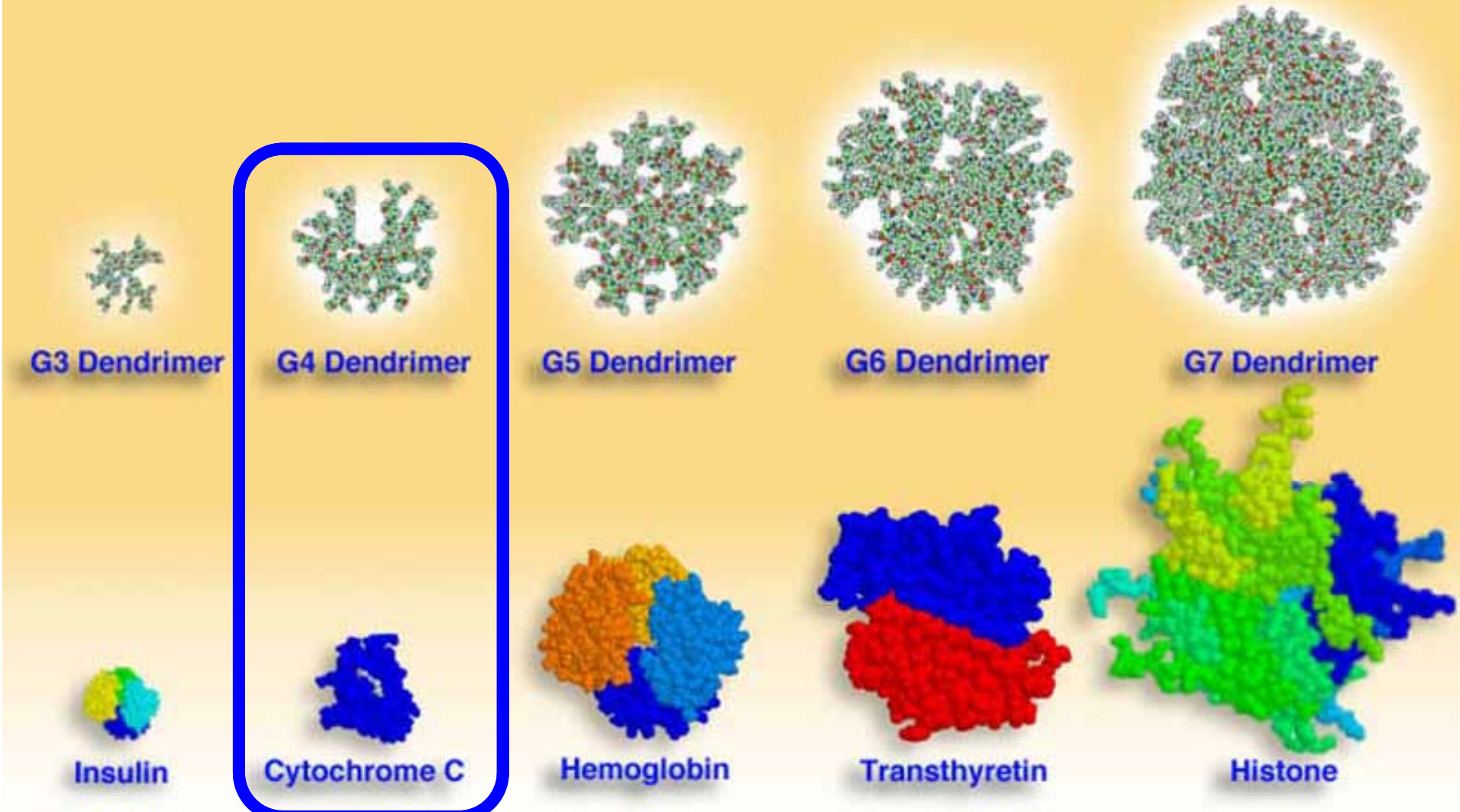
Hu L, Tao WA, et al. ***Angew. Chem. Int. Ed.***, , 50(18):4133-4136

(DOI:10.1002/anie.201006459, Selected as “Hot Paper” by the Editor)

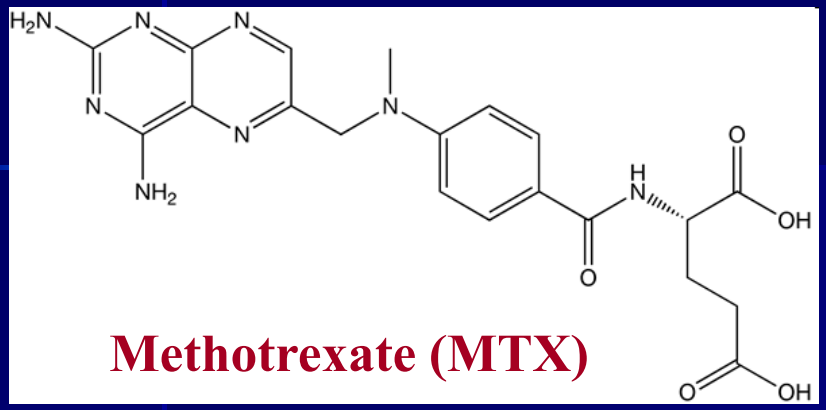
Identification of the therapeutic protein targets



Choose Dendrimer Generation



MTX-DHFR System: A Case Study

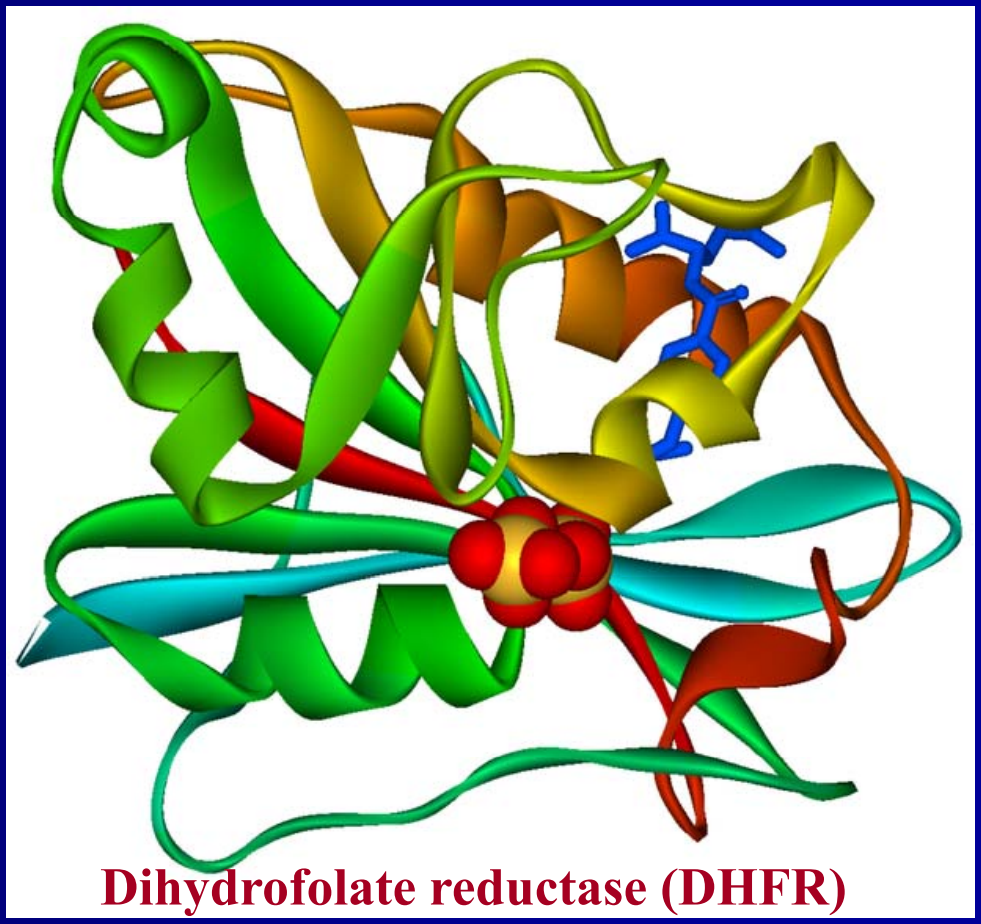


Methotrexate (MTX)

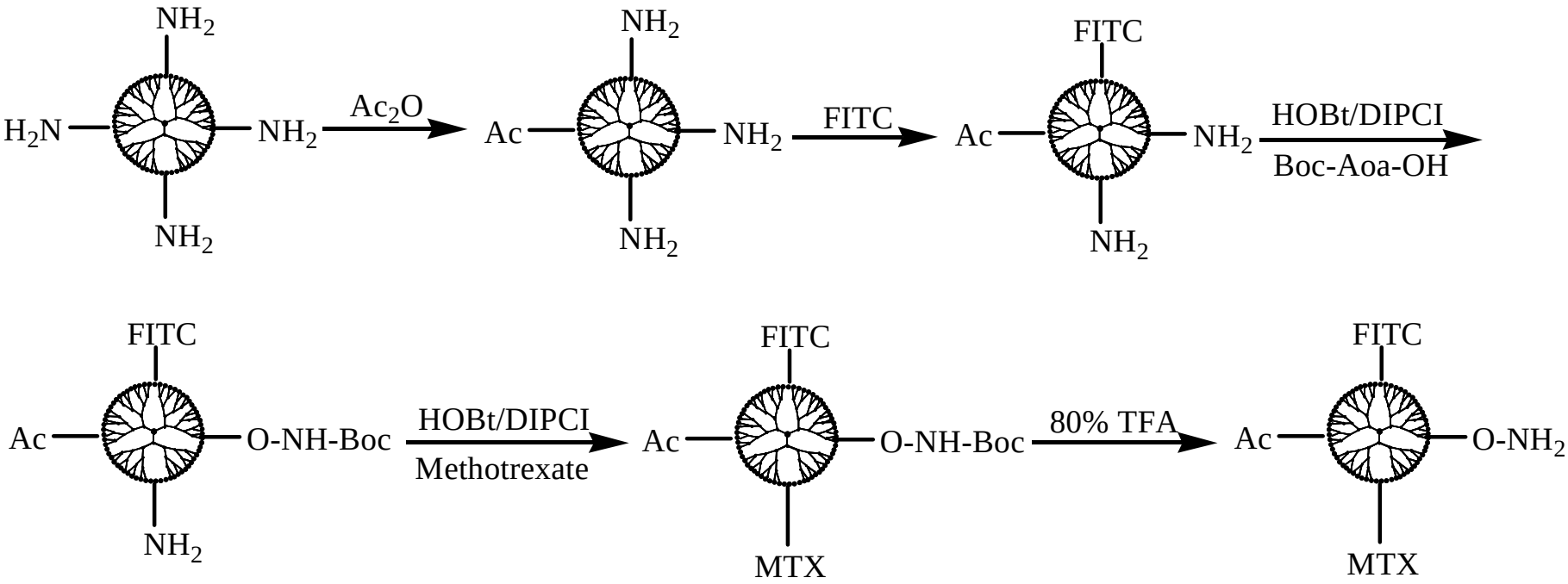
MTX: an antifolate drug in treatment of cancer by inhibiting the metabolism of folic acid

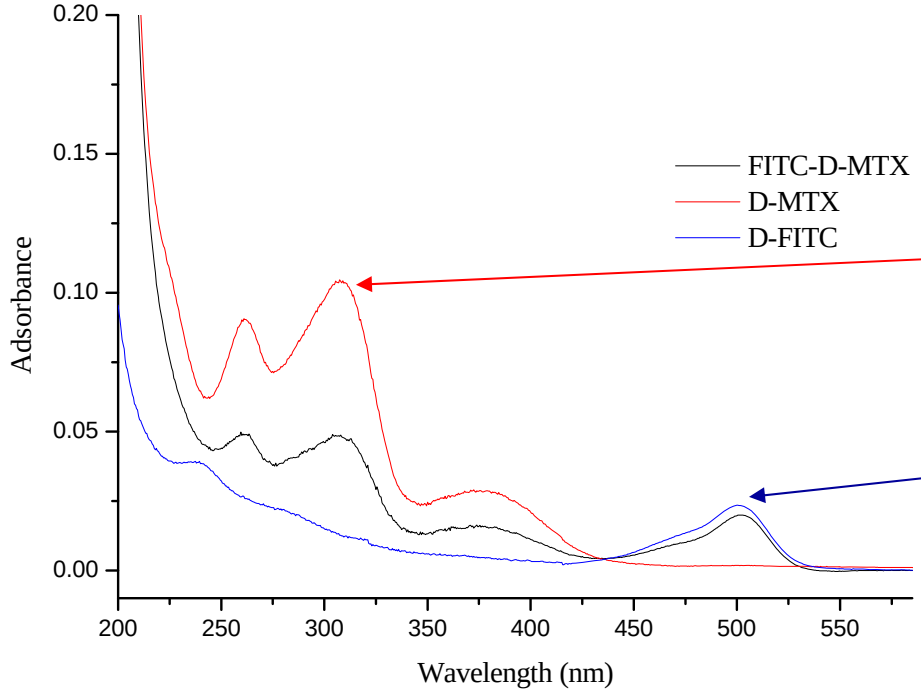
DHFR: required for the *de novo* synthesis of purines, thymidylic acid and certain amino acids

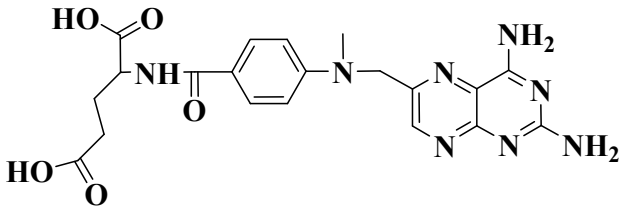
Deficiency of DHFR is linked to *megaloblastic anemia* disease`

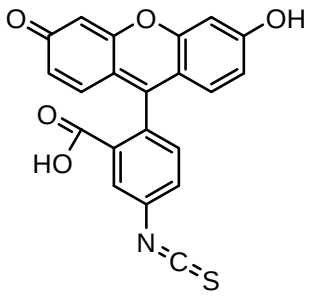


Dihydrofolate reductase (DHFR)

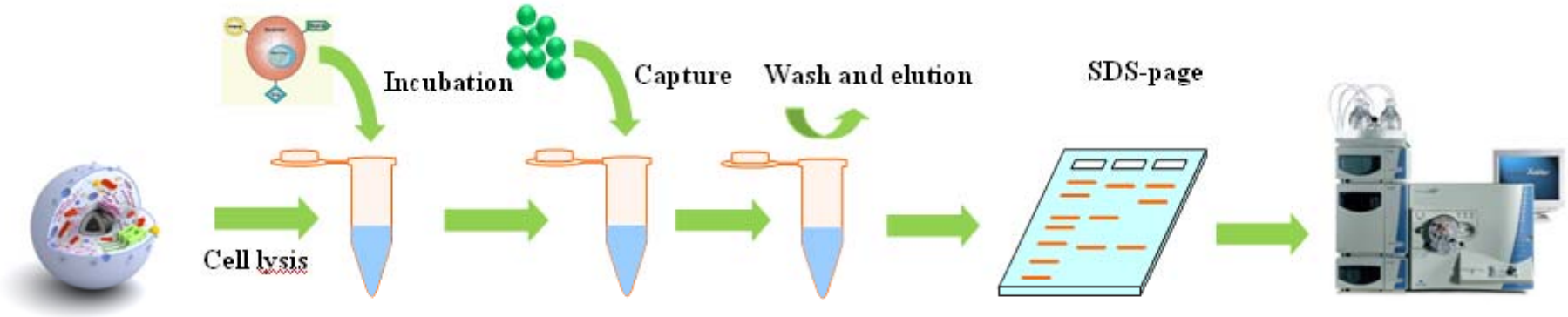

NH2
H2N
NH2
NH2
Ac2O
Ac
NH2
NH2
FITC
Ac
NH2
NH2
HOBt/DIPCI
Boc-Aoa-OH
FITC
Ac
O-NH-Boc
NH2
HOBt/DIPCI
Methotrexate
FITC
Ac
O-NH-Boc
MTX
80% TFA
FITC
Ac
O-NH2
MTX

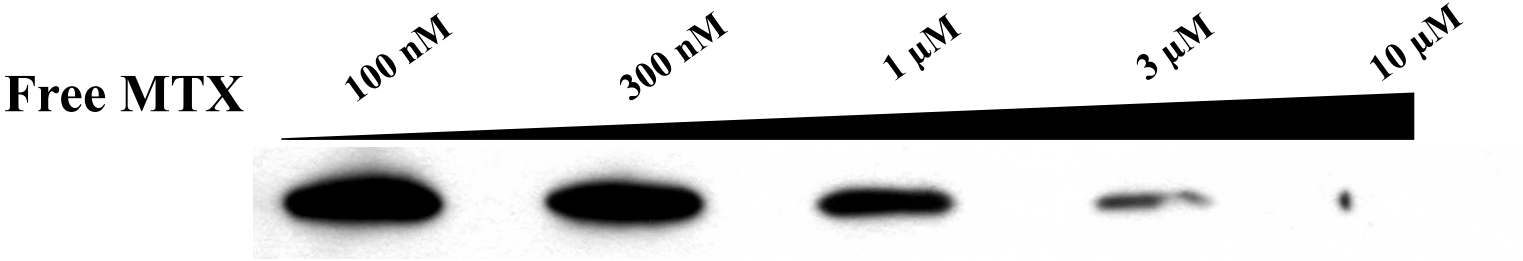

0.20
0.15
0.10
0.05
0.00
Adsorbance
200
250
300
350
400
450
500
550
Wavelength (nm)
FITC-D-MTX
D-MTX
D-FITC


HO
O
NH
O
N
NH2
N
N
N
N
NH2
HO
O

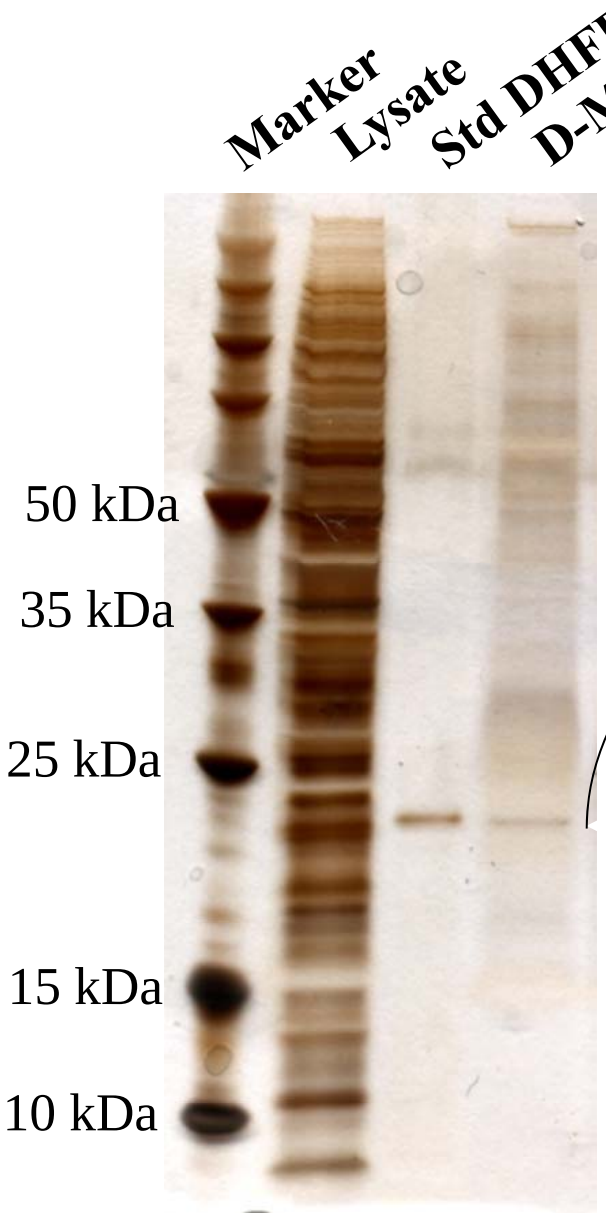

O
O
OH
O
HO
N
C
S

Capture of the endogenous DHFR *in vitro* from cell lysate using dendrimer-MTX





Identification of DHFR by MS/MS

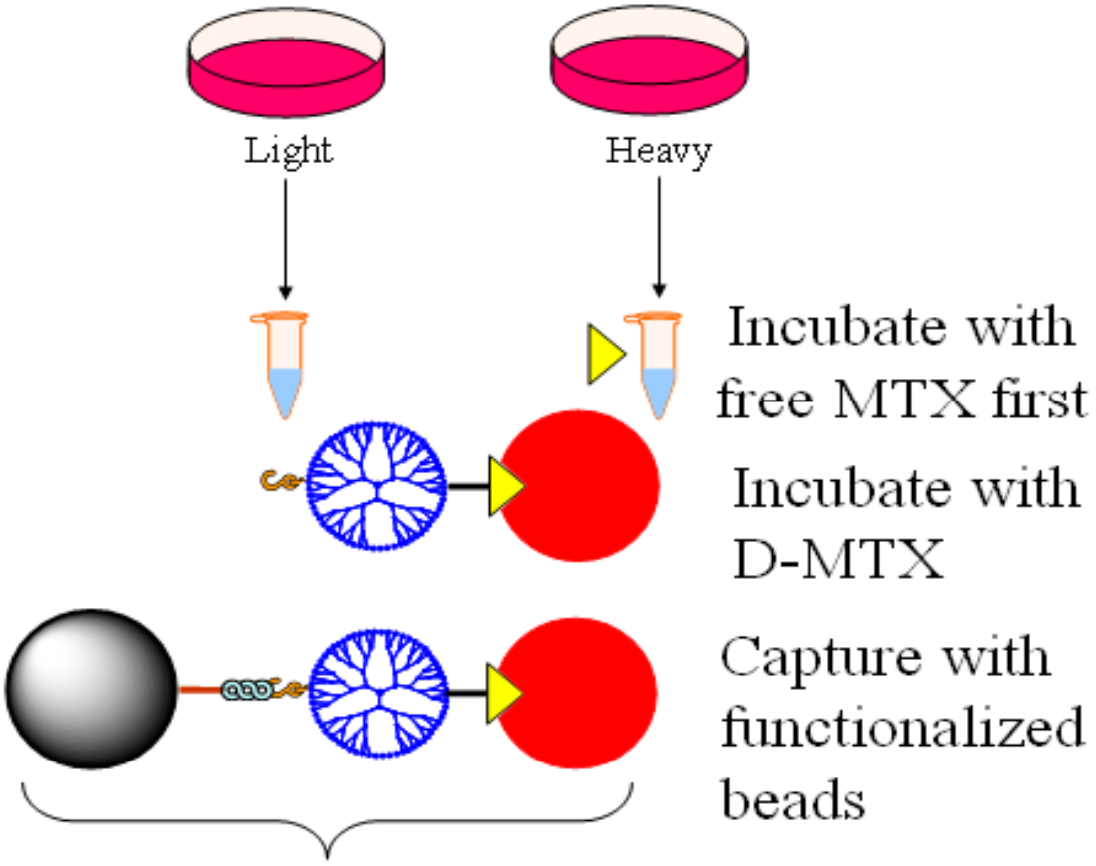


In-gel digestion for MS analysis

32.44% coverage of the whole sequence of DHFR

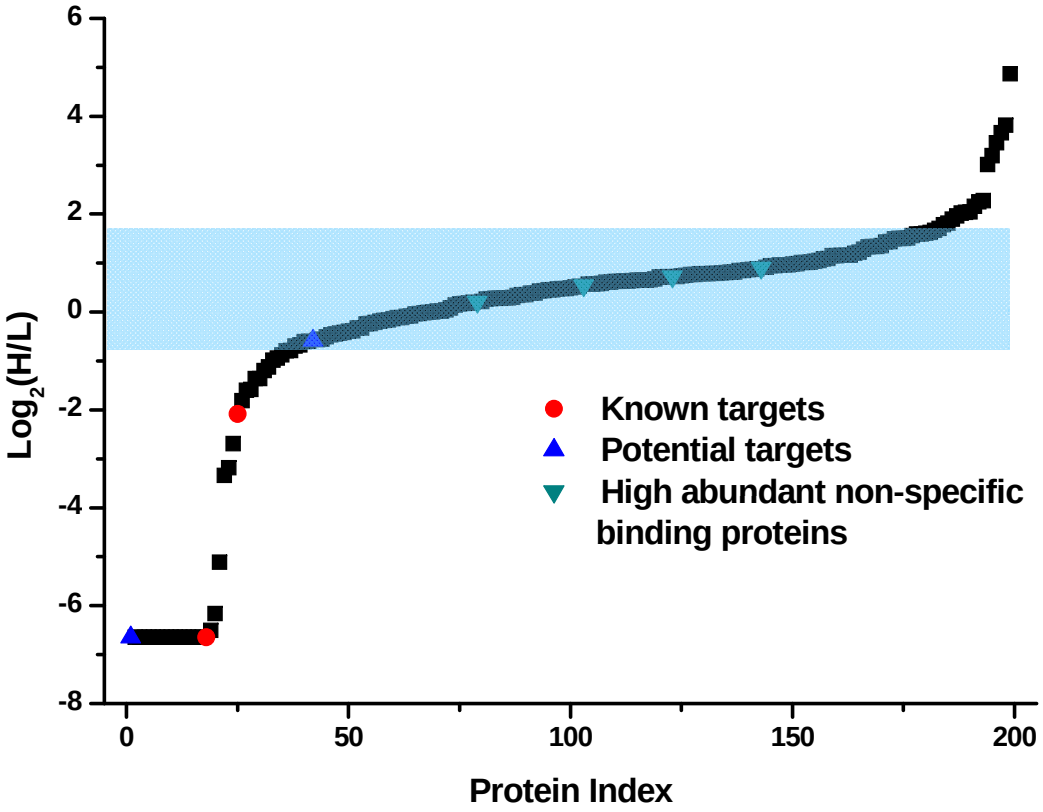
VDMVWIVGGSSVYK
LLPEYPGVLSDVQEEK
NGDLPWPPLR
LTEQPELANK
EAMNHPGHLK
TWFSIPEK
SLDDALK
MTTTSSVEGK

SILAC quantification for the differentiation of protein target and non-specific binding



Combine the heavy with light together and then on-bead digest for MS analysis

Classification of all the proteins identified by mass spectrometry

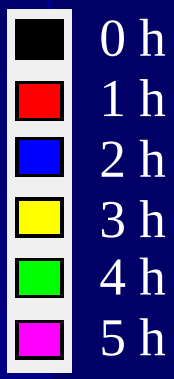


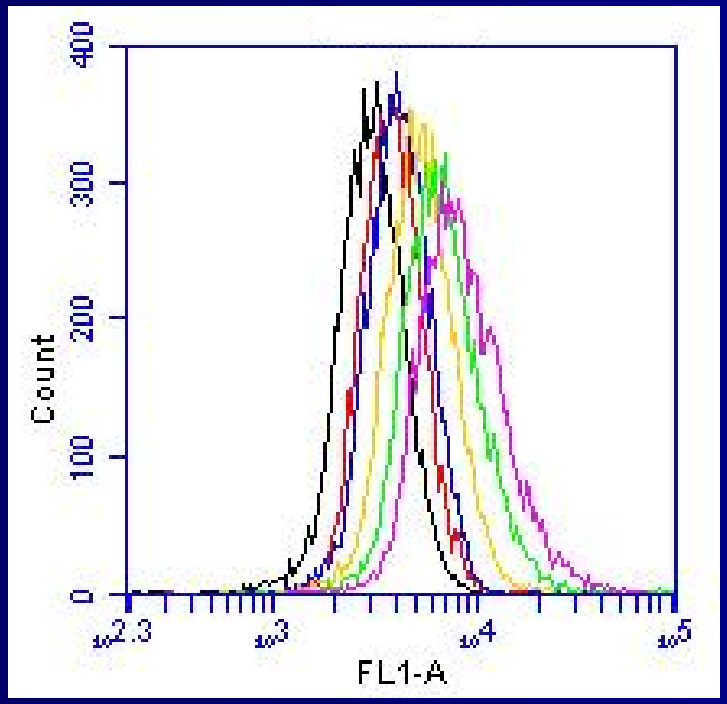
Known targets:
1. Dihydrofolate reductase
2. Deoxycytidine kinase

Potential targets:
1. Aspartate aminotransferase
2. Trifunctional purine biosynthetic protein adenosine-3

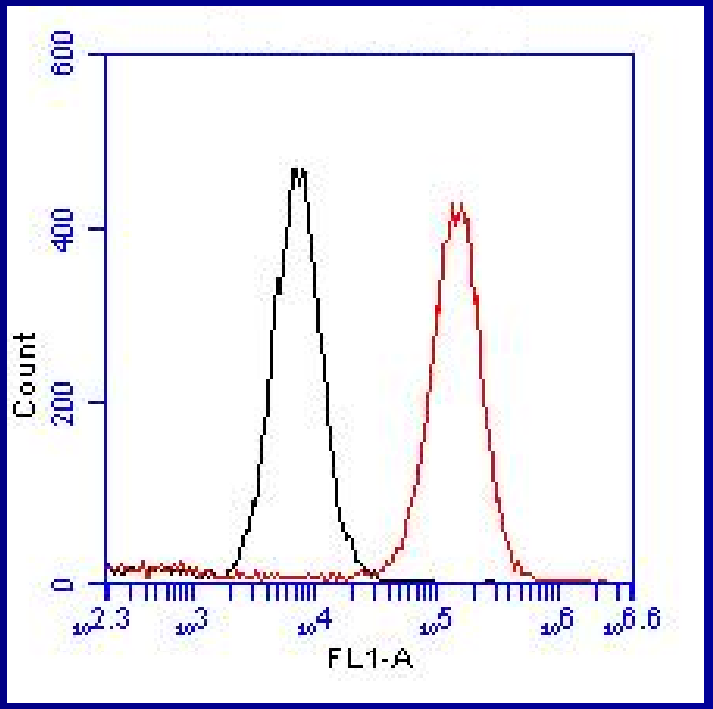
High abundant non-specific binding proteins:
1. Tubulin beta-2C chain
2. 40S ribosomal protein S3
3. Elongation factor
4. Heat shock protein

Flow cytometry for the deliver efficiency of the dendrimer reagent into living cells



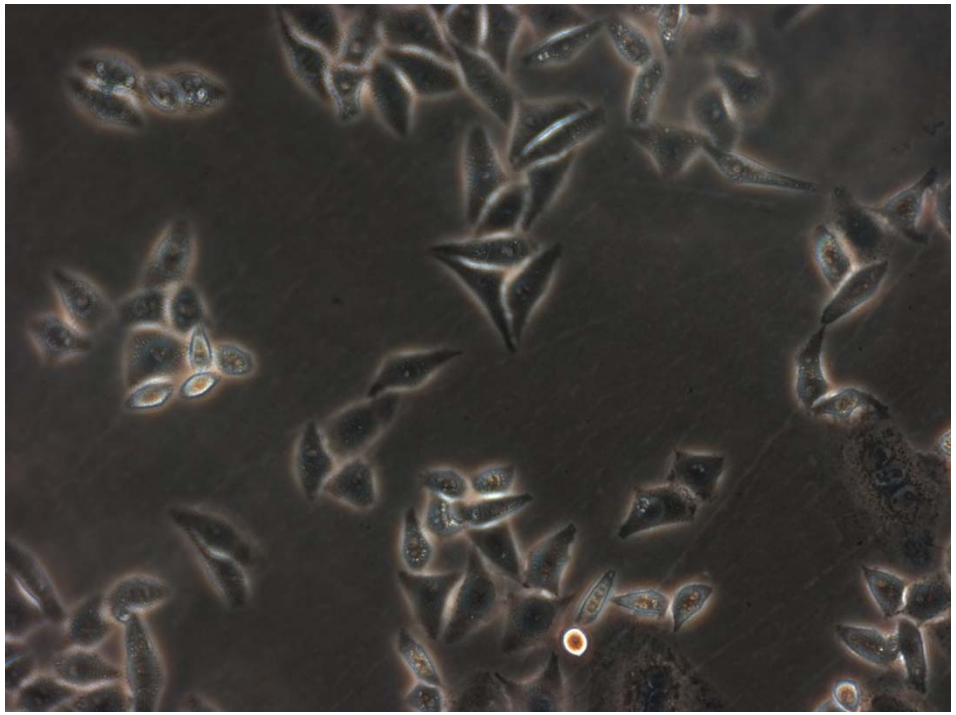


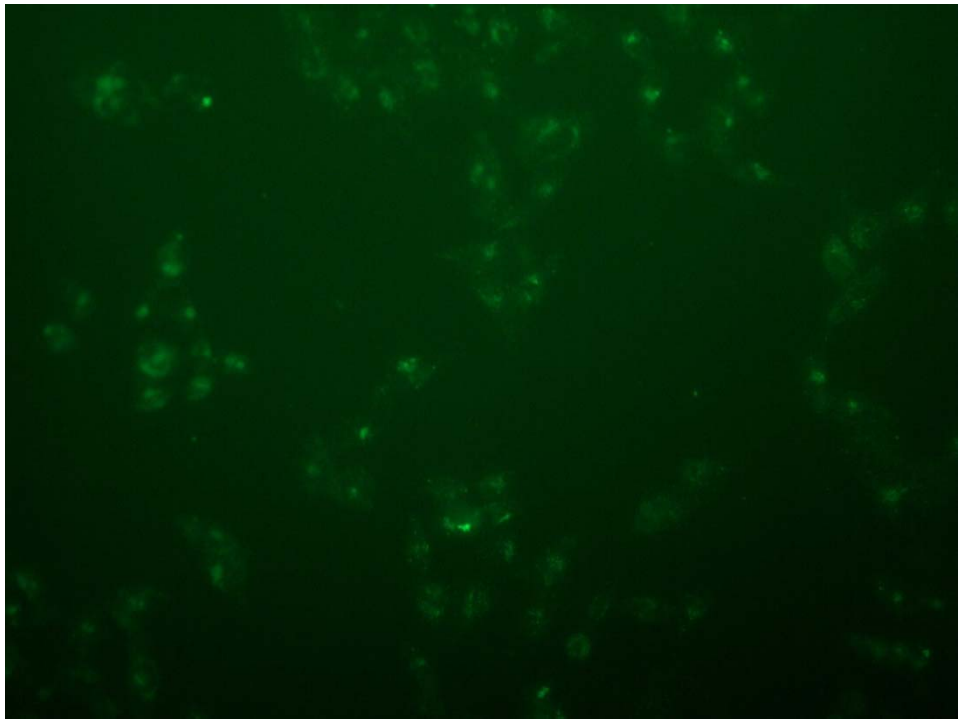
Human B cell-DG 75



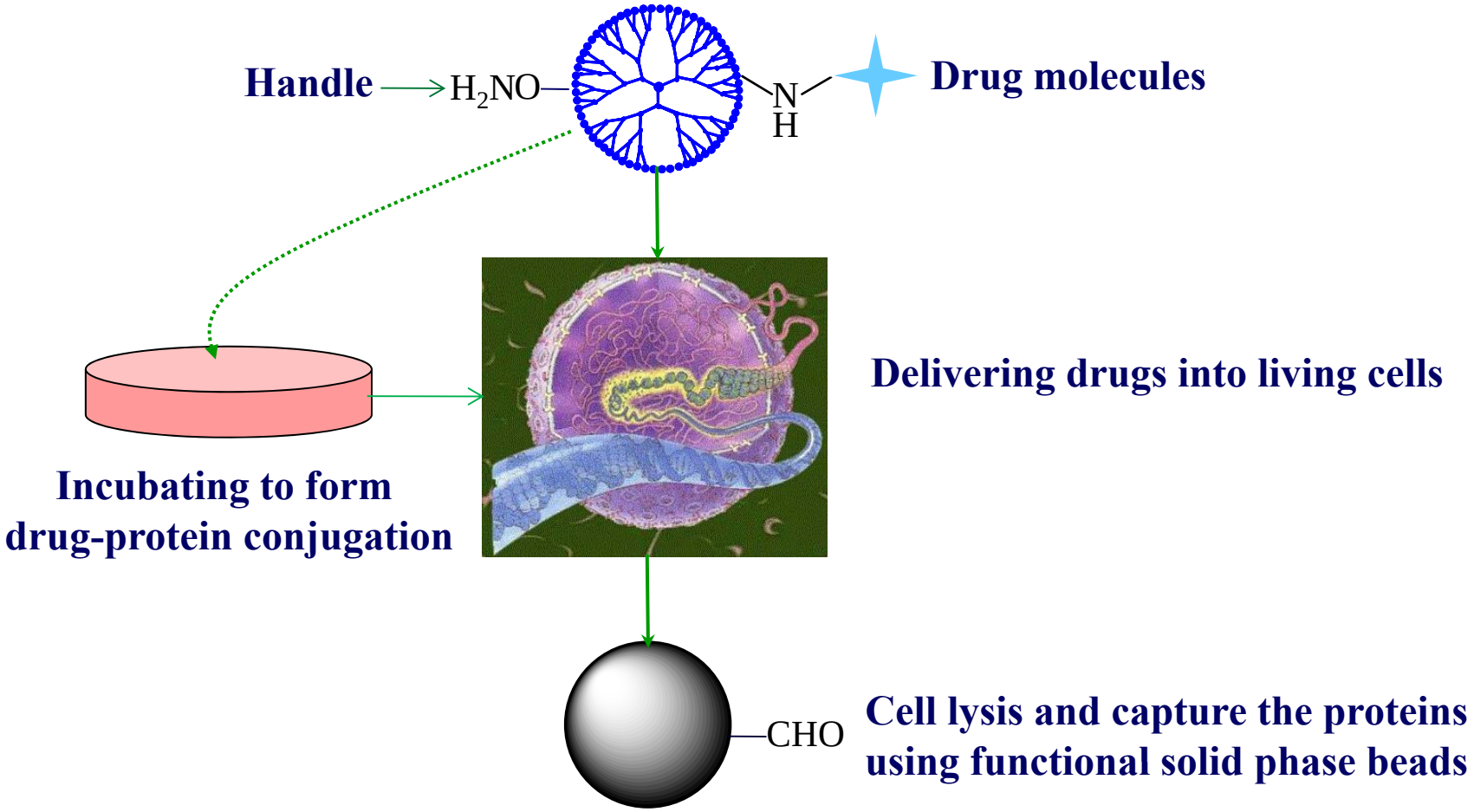
HeLa cell

Fluorescence microscopy imaging analysis

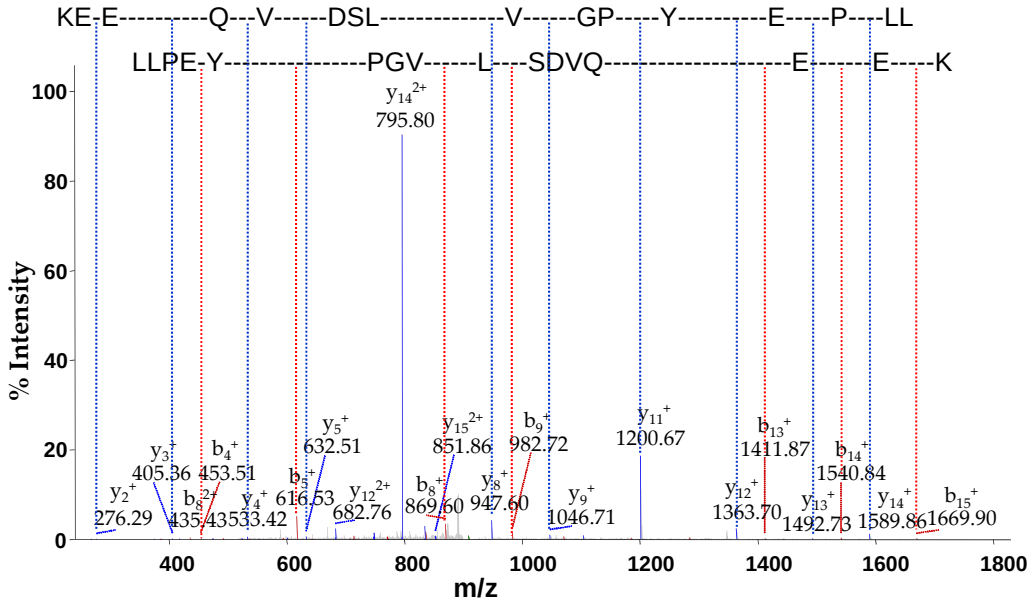




In vivo characterization of protein targets

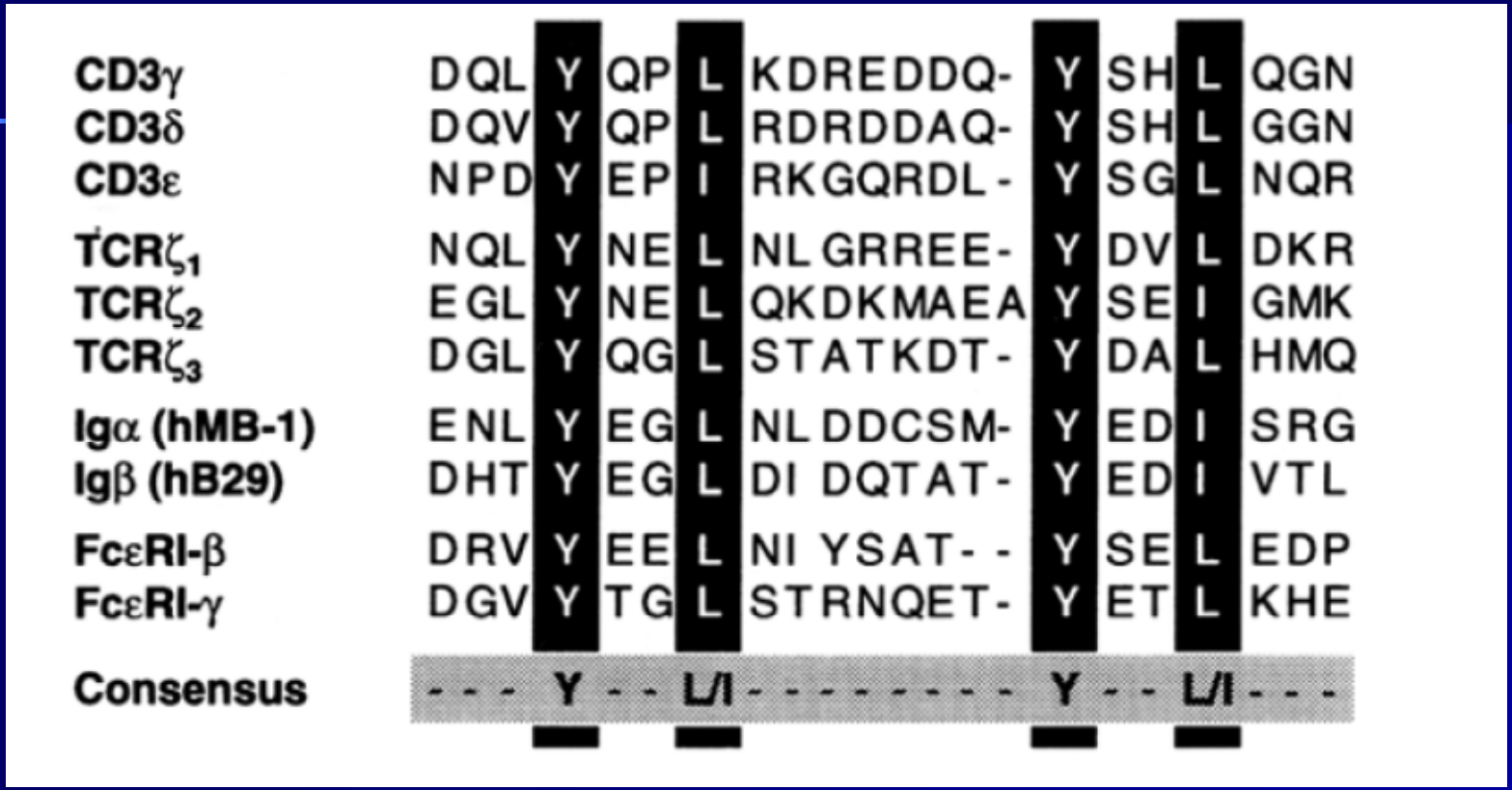


Two known target DHFR and Deoxycytidine kinase can be successfully identified from living cells



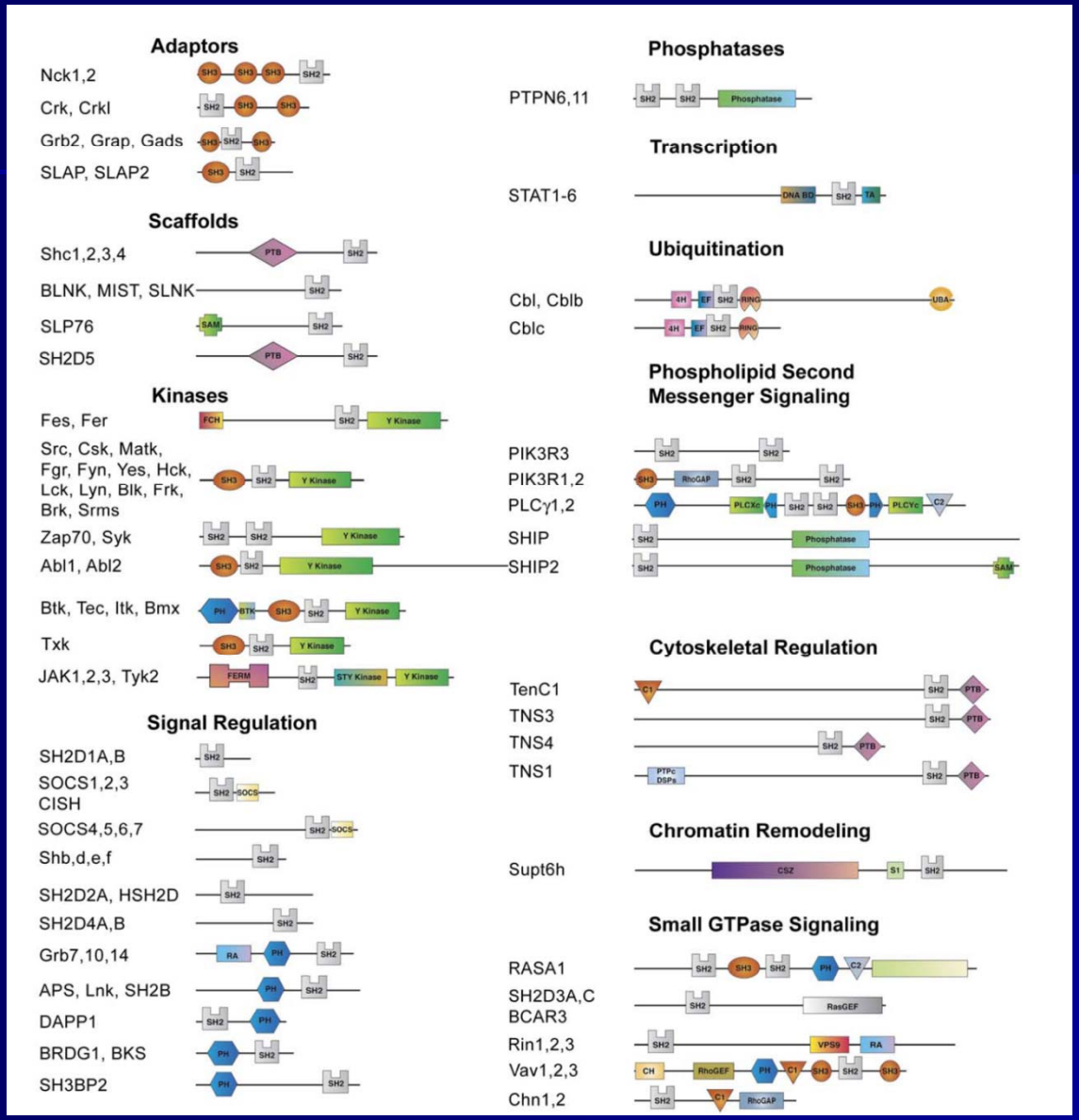
MS/MS spectrum of a peptide—LLPEYPGVLSDVQEEK from DHFR which is identified *in vivo*

ITAM: Immunoreceptor Tyrosine-based Activation Motif

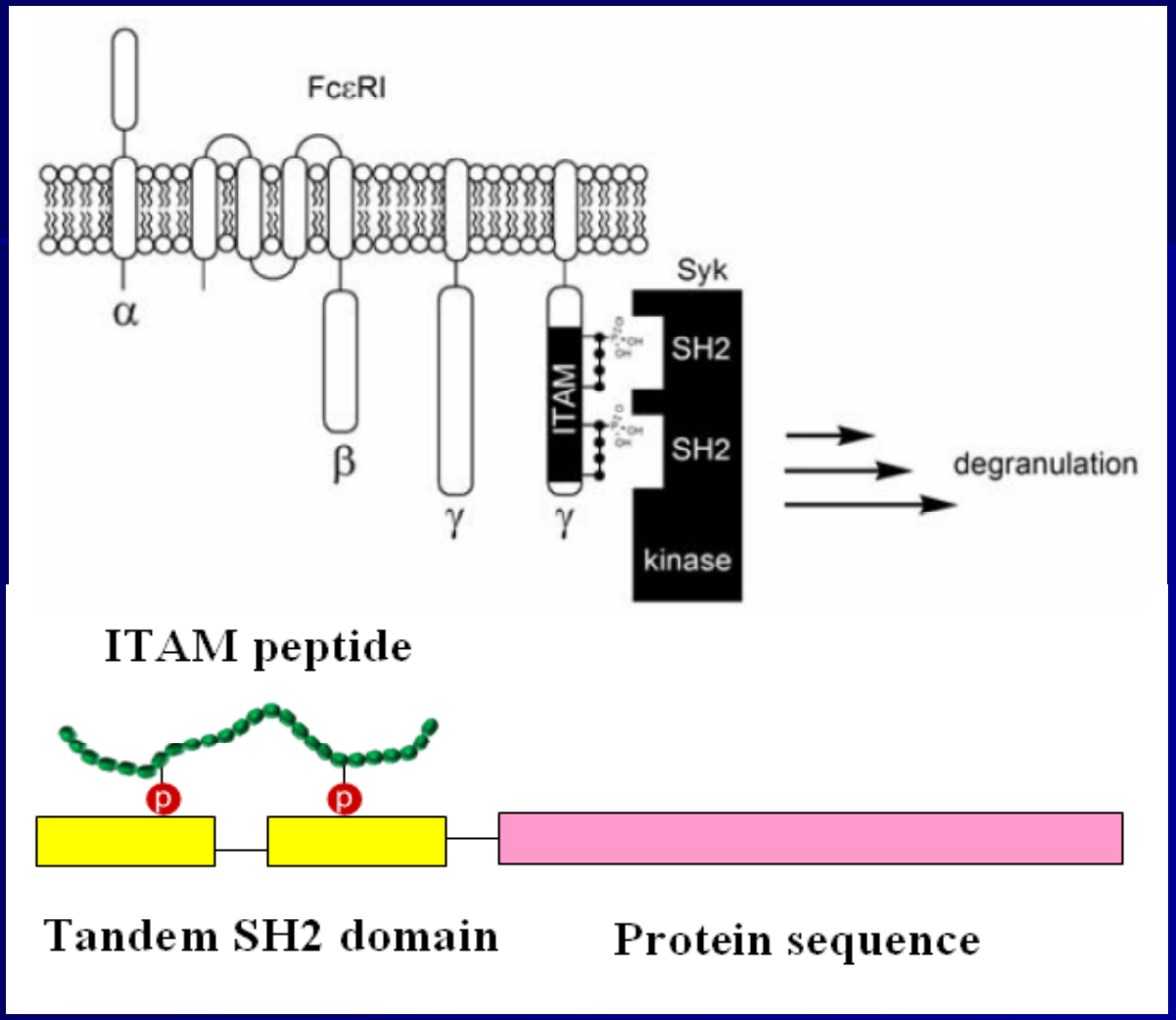


Tandem phosphorylated tyrosine residues of ITAM will bind to the Src Homology 2 (SH2) domain of other receptor proteins associated with activation, survival, and differentiation

Catalog of SH2-containing proteins



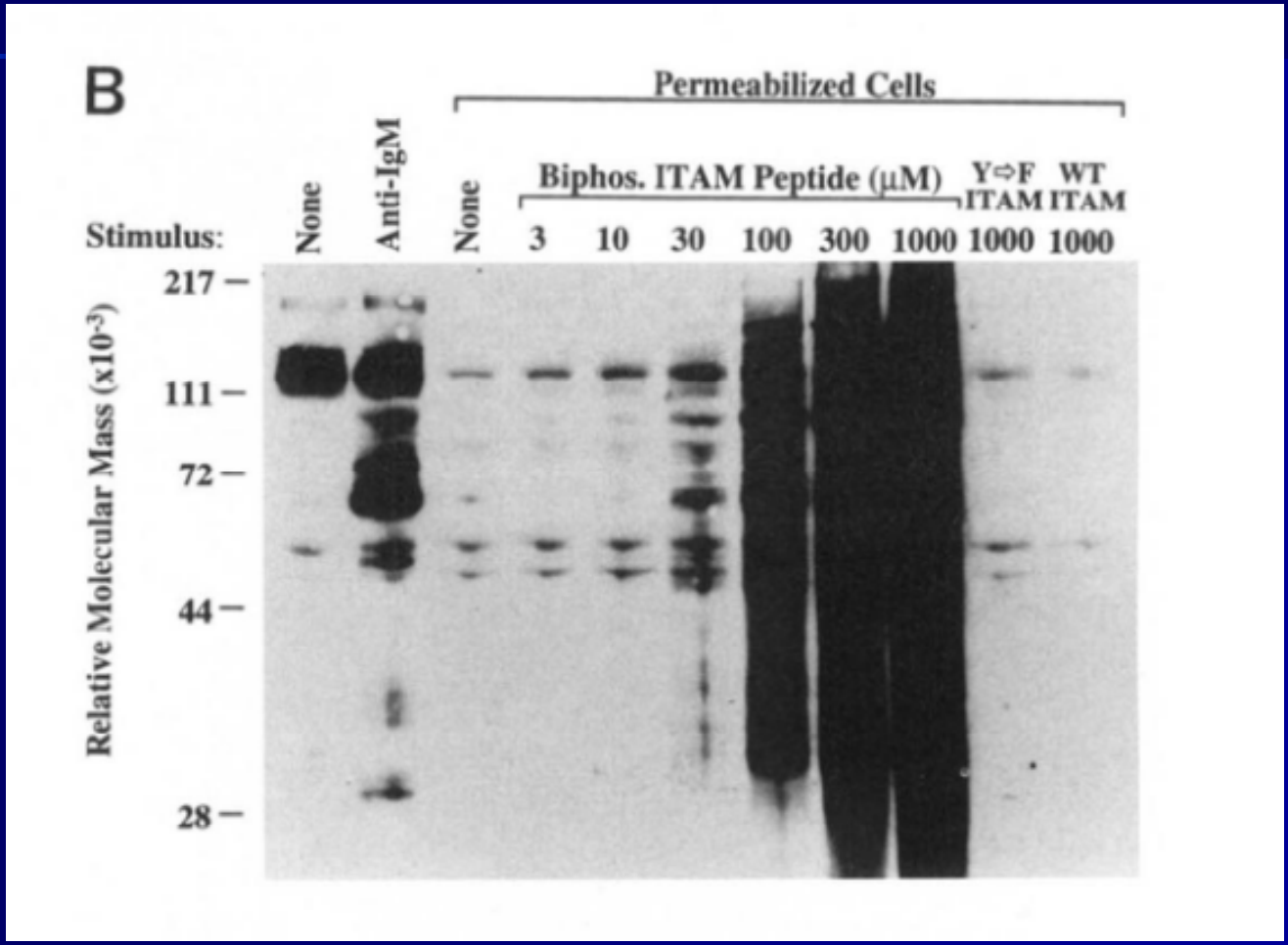
Syk (spleen tyrosine kinase) as a model study



Kuil J, et al. ***Adv. Exp. Med. Bio.***, 2009, 611, 81-82

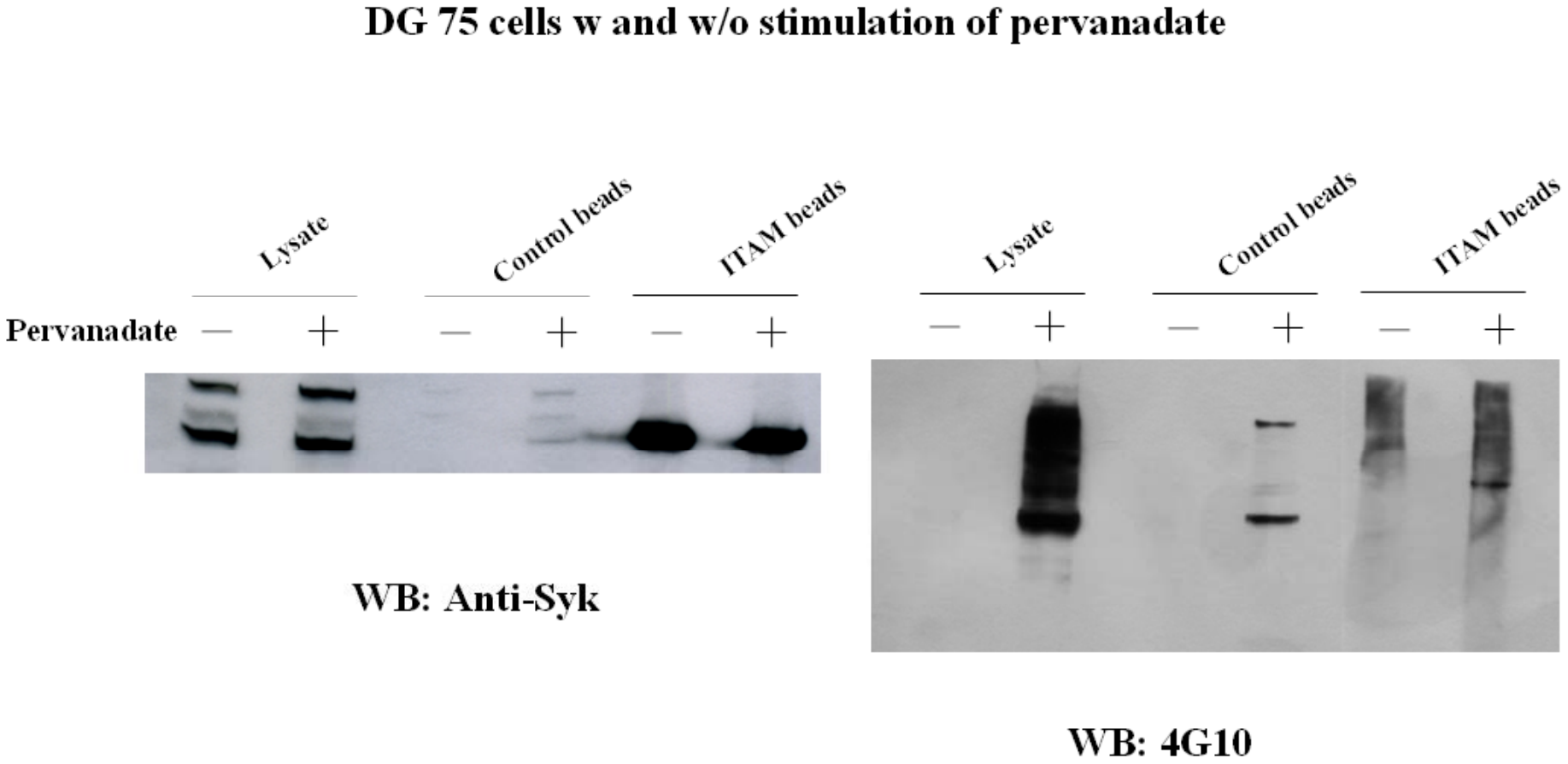
Recruitment of Syk to the diphosphorylated ɣ-ITAM of high affinity IgE receptor (FcεRI) results in activation of its kinase domain

Biphosphorylated ITAMs induce protein tyrosine phosphorylation in B cells

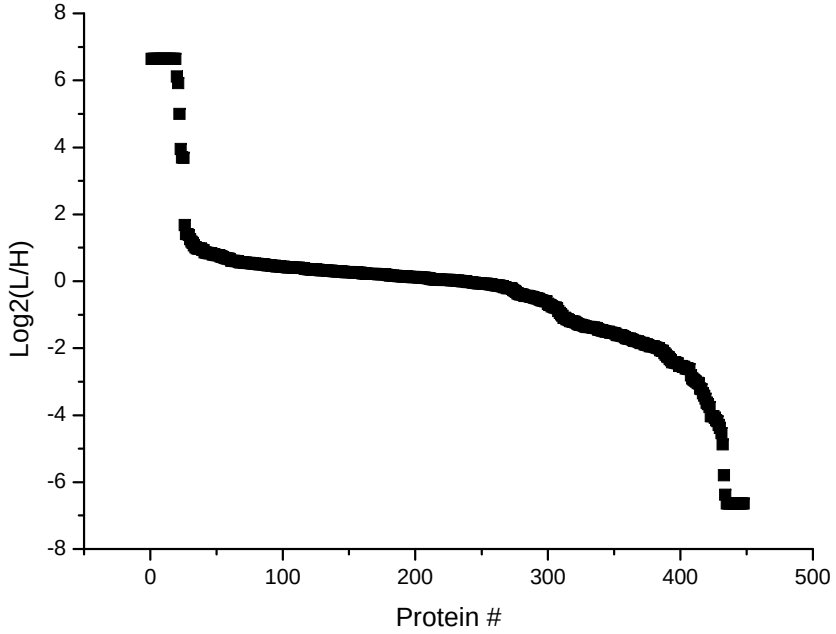


J Immunol. 1995 Nov 15;155(10):4596-603.

Affinity enrichment ability of ITAM peptide to Syk protein

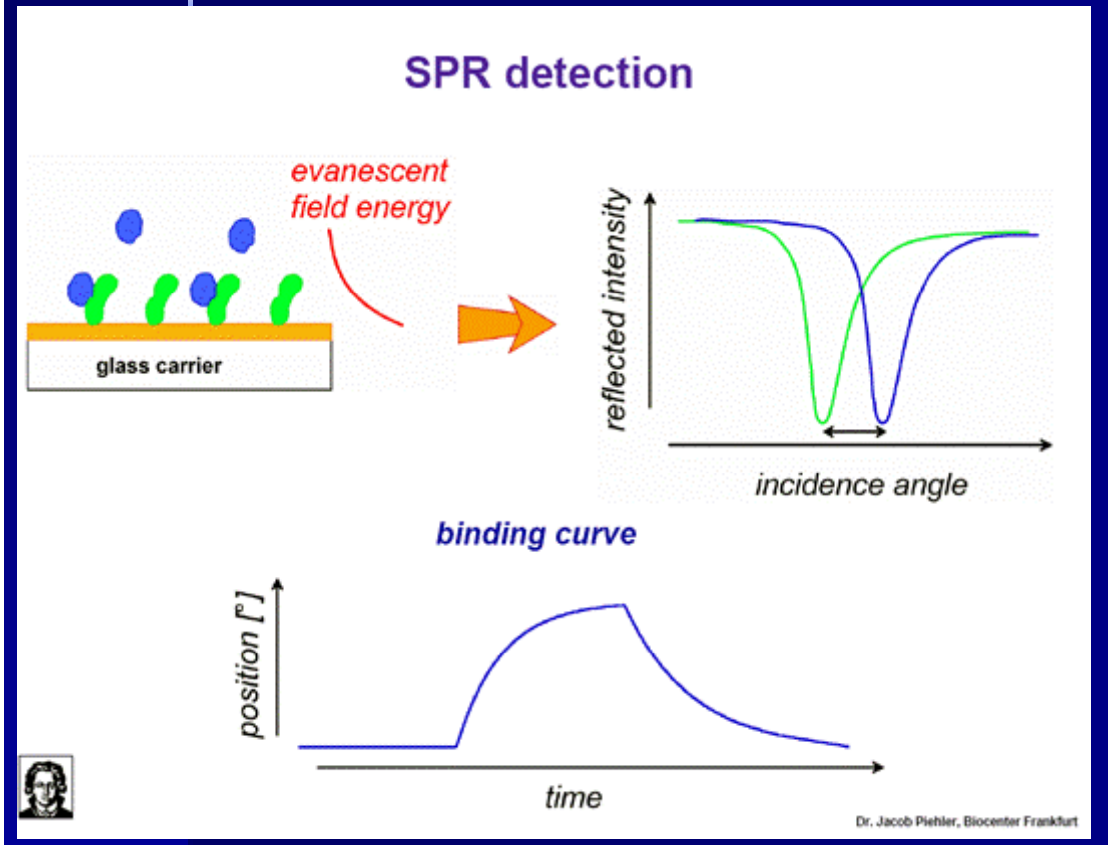


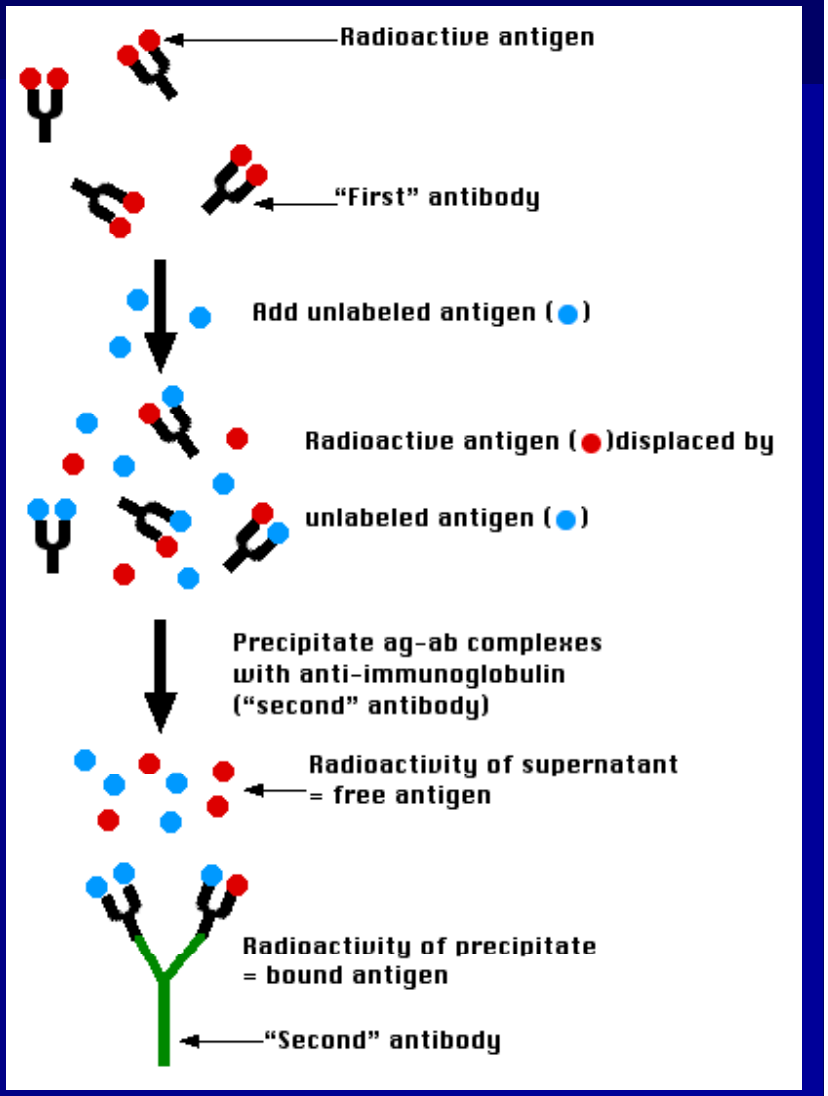
SILAC quantification for the differentiation of non-specific bindings and protein targets



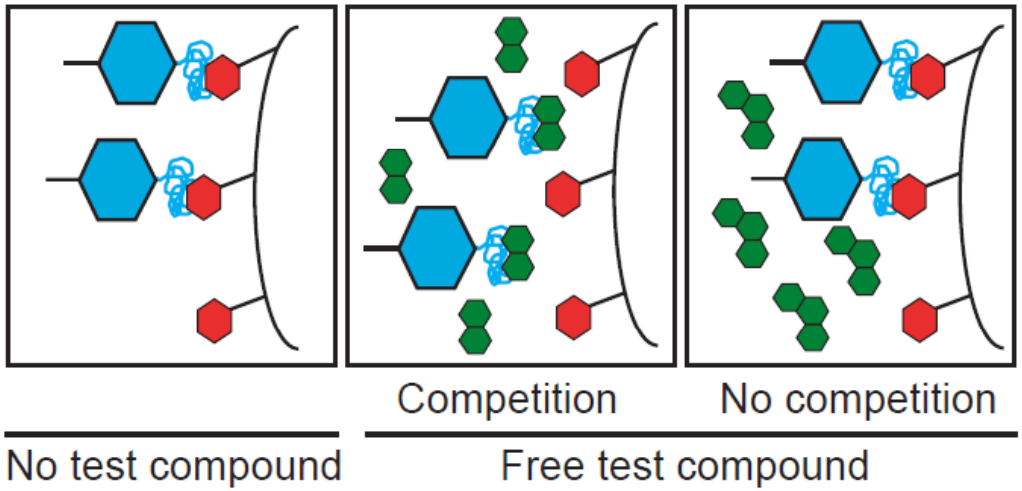
	Ratio(L/H)
Crk-like protein (coding a protein exhibiting the SH2 domain)	100
Tyrosine-protein kinase CSK	100
1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase gamma-1	100
Tyrosine-protein kinase SYK	100
Phosphatidylinositol 3-kinase regulatory subunit alpha	100
Tyrosine-protein kinase ZAP-70	100
Phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 1	100

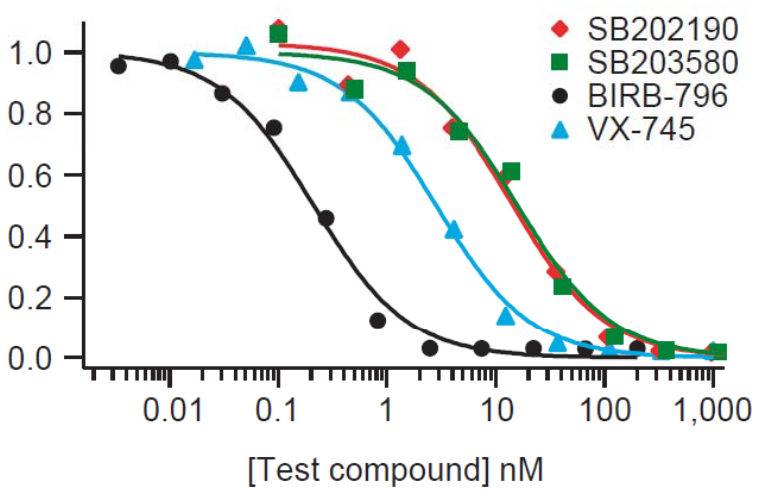
Traditional competitive assay





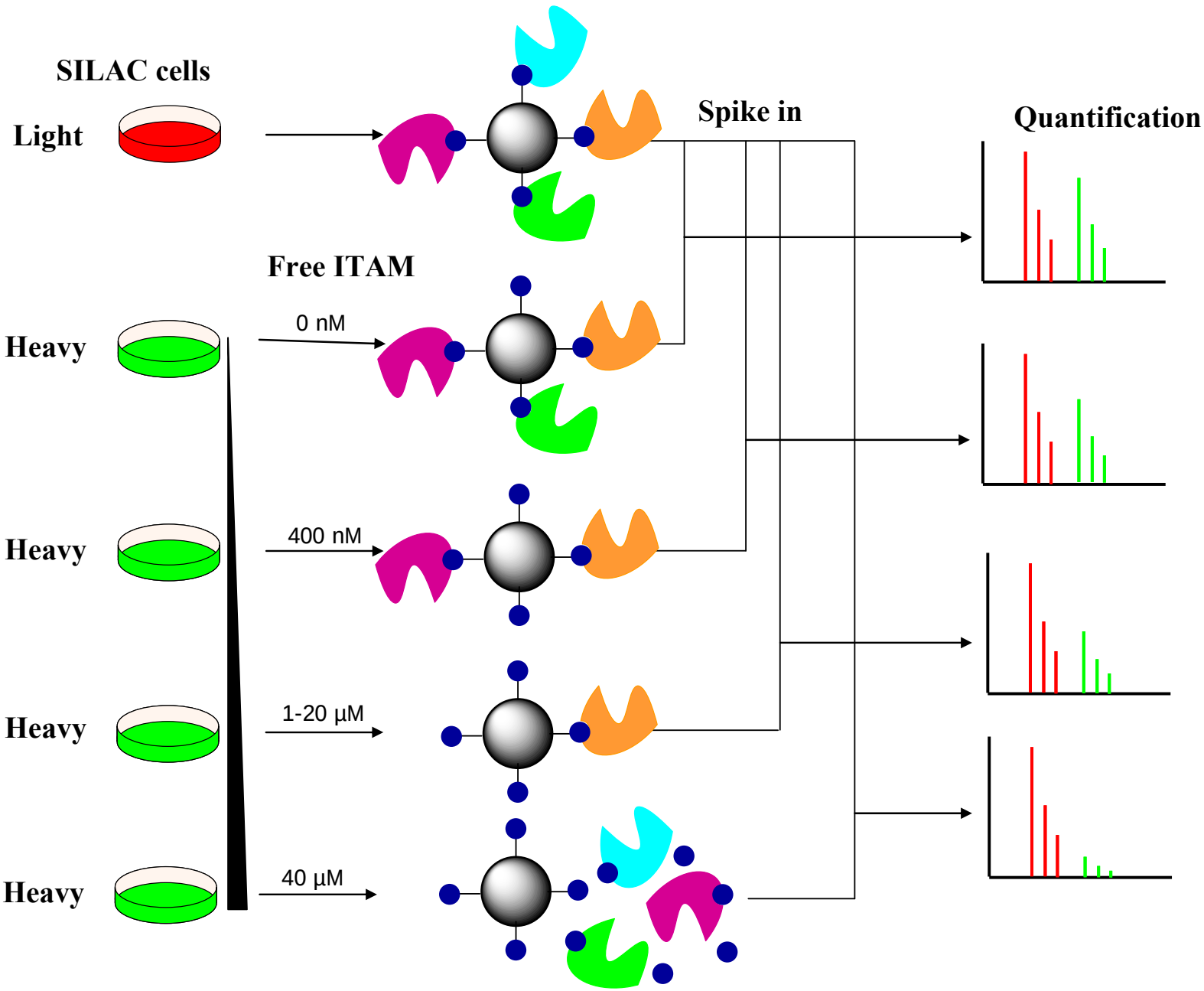
Quantitative proteomics for competitive binding assay by mass spectrometry



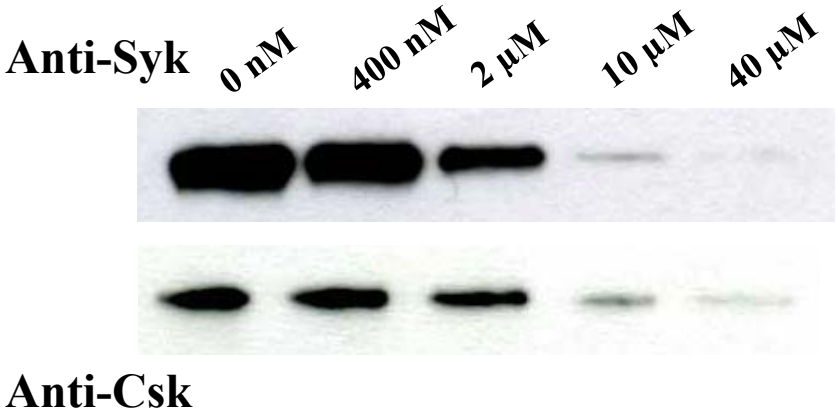


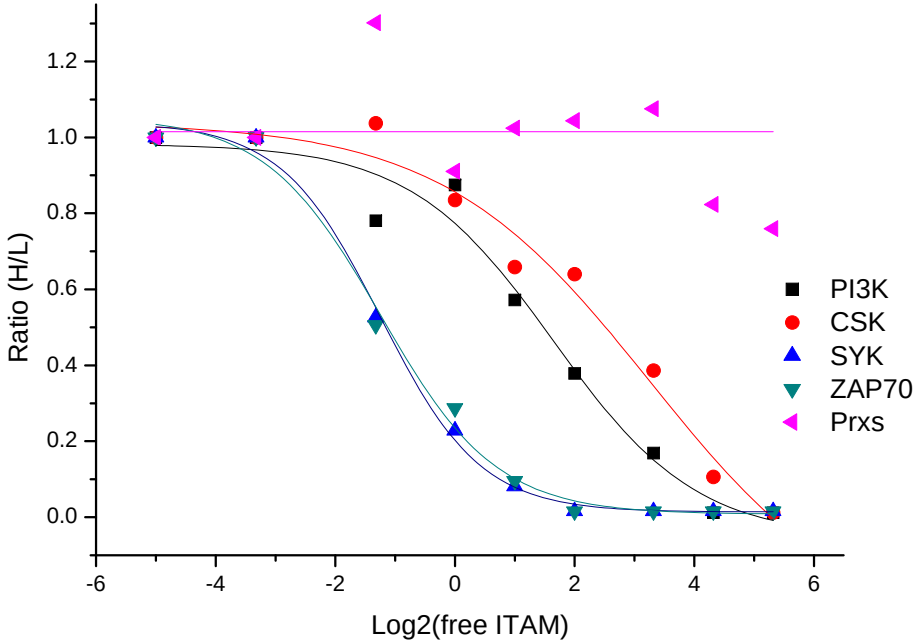
Physiological condition & high throughput

Protein digest as the internal standard



Determination of the binding affinity of ITAM peptide to multiple proteins



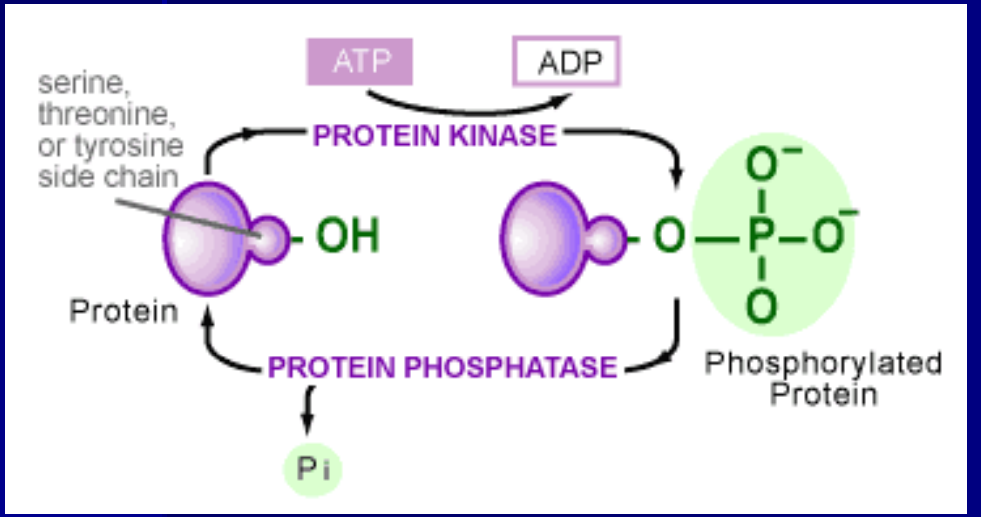


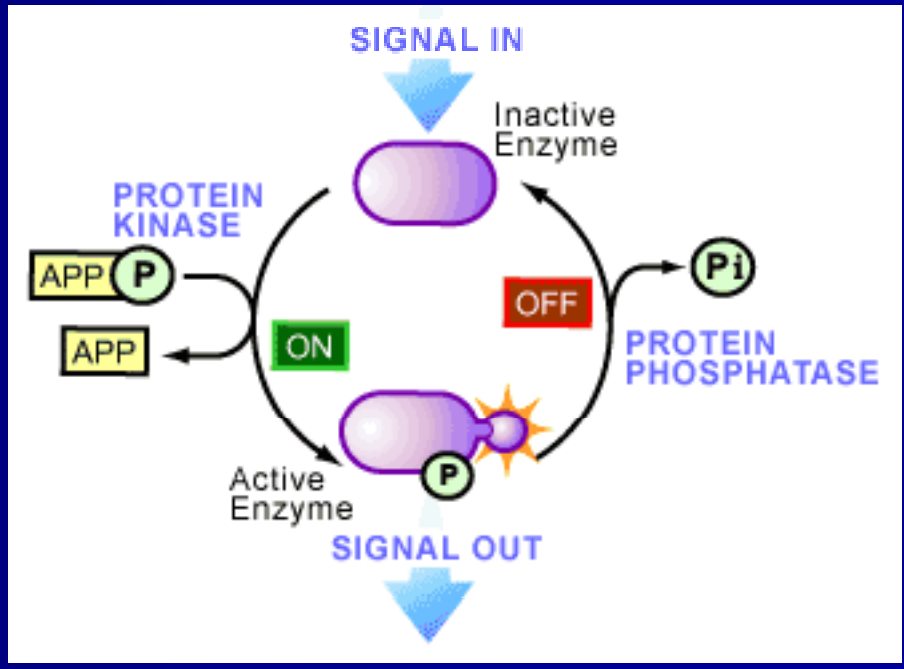
Relative binding affinity can be got by fitting the target protein concentration with different amount of free ITAM competitor

Outline

1. Introduction to drug target discovery
2. Identification of drug target in live cells by chemical proteomic approach
3. Tyrosine kinase/phosphatase substrate

Tyrosine kinase/phosphatase substrate





In vitro Kinase Substrate Screening

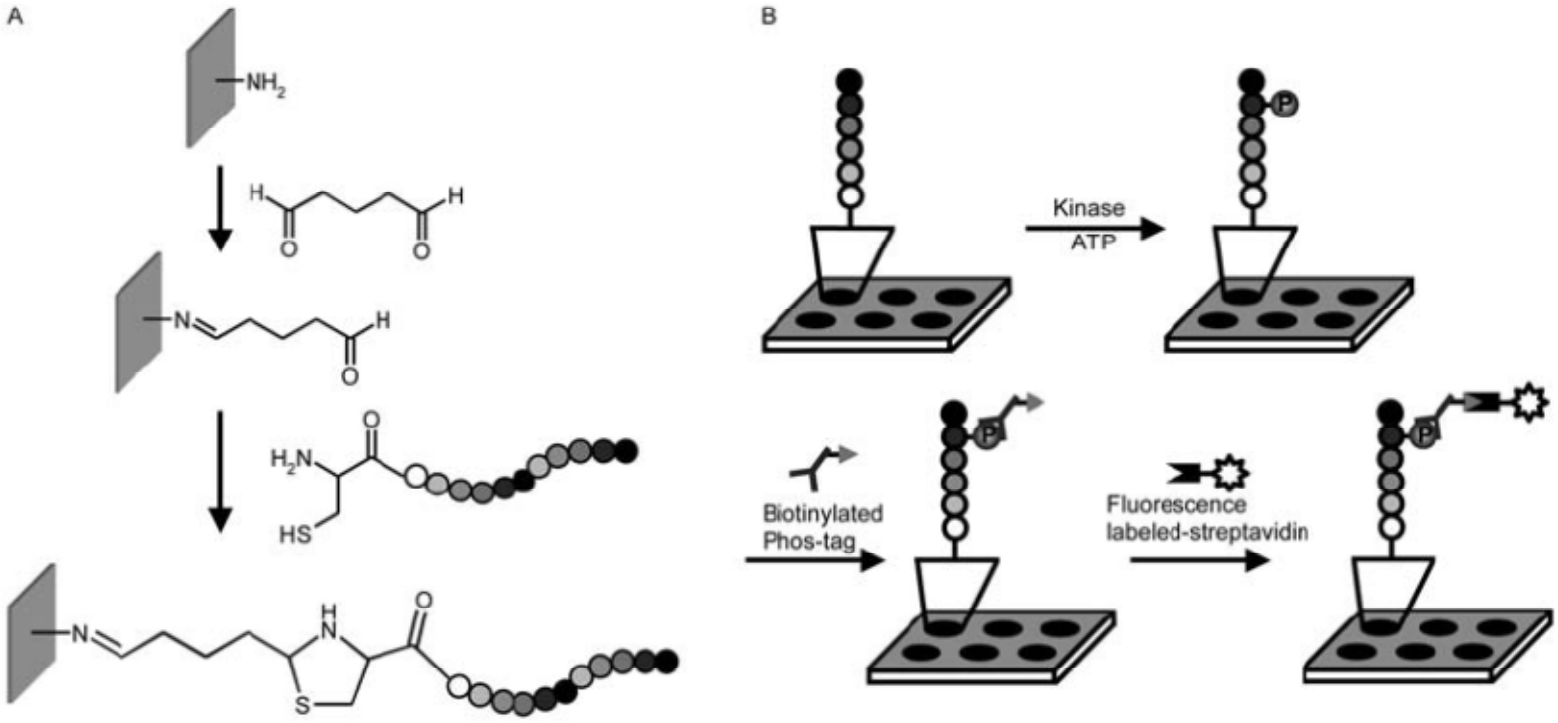
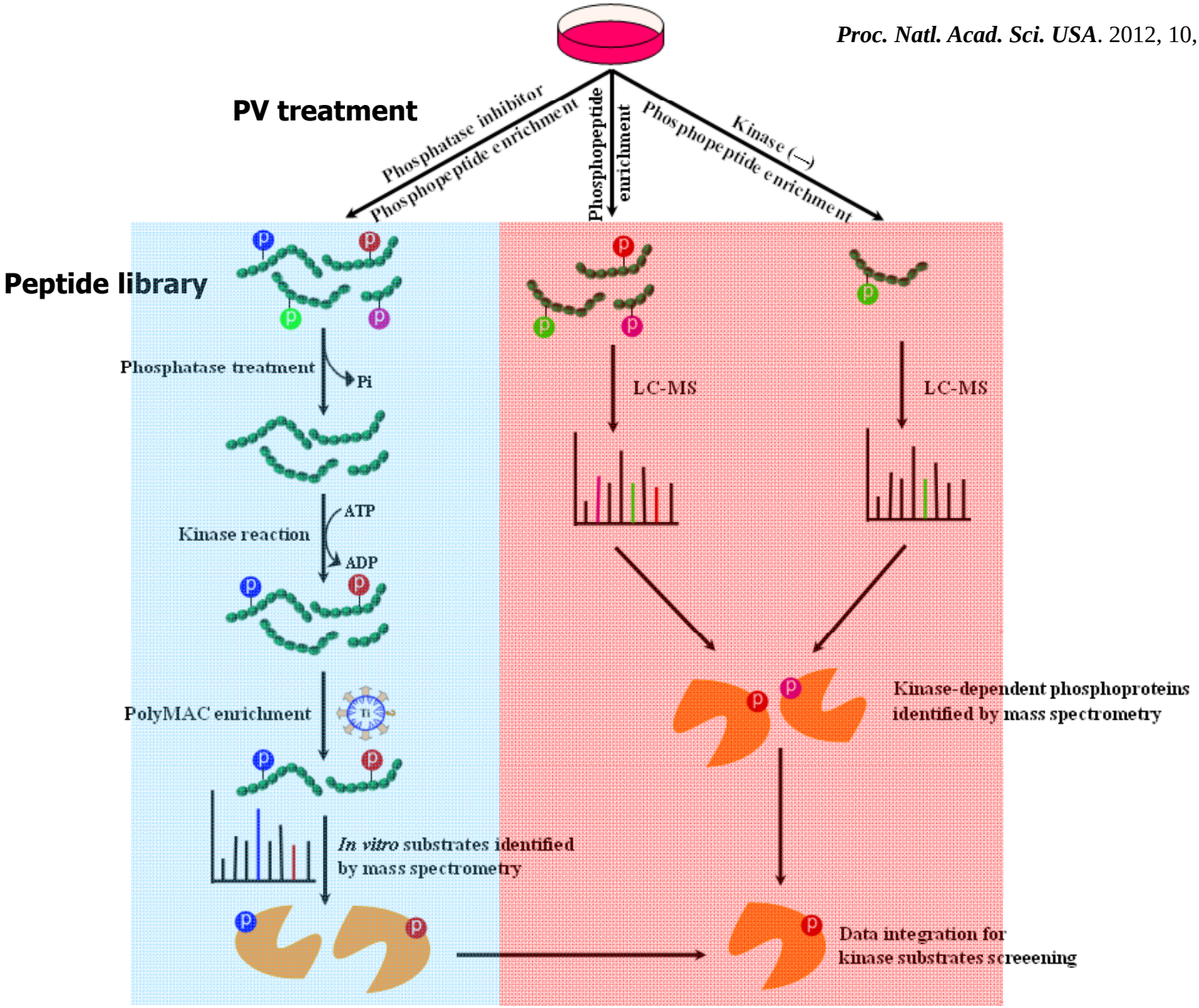
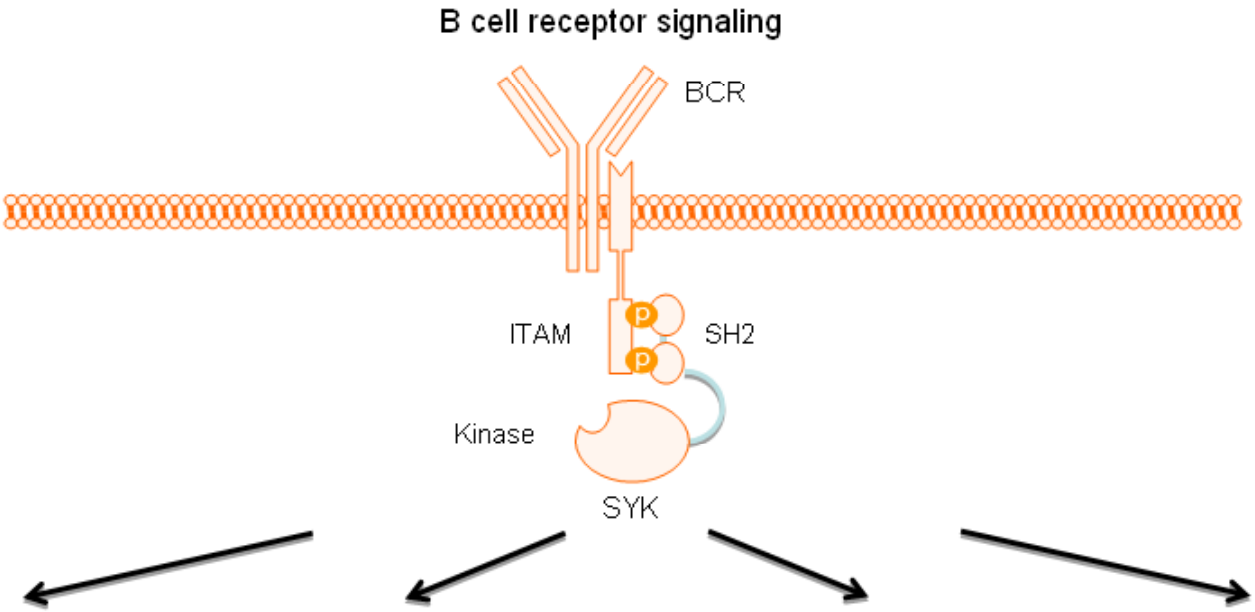


Fig. 1 Strategy for detections of on-chip phosphorylation. (A) Peptide immobilization method. (B) Outline of the detection of on-chip phosphorylation.

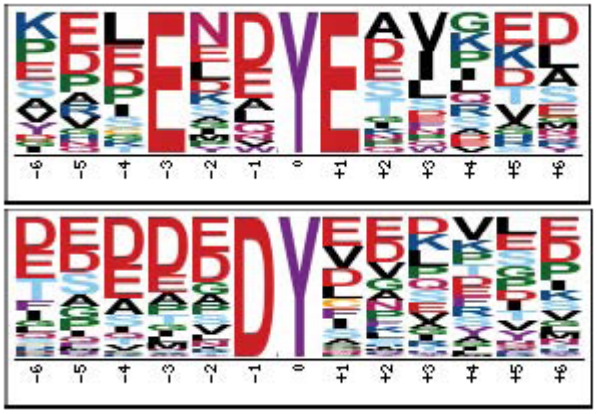
Proc. Natl. Acad. Sci. USA. 2012, 10, 5615-5620

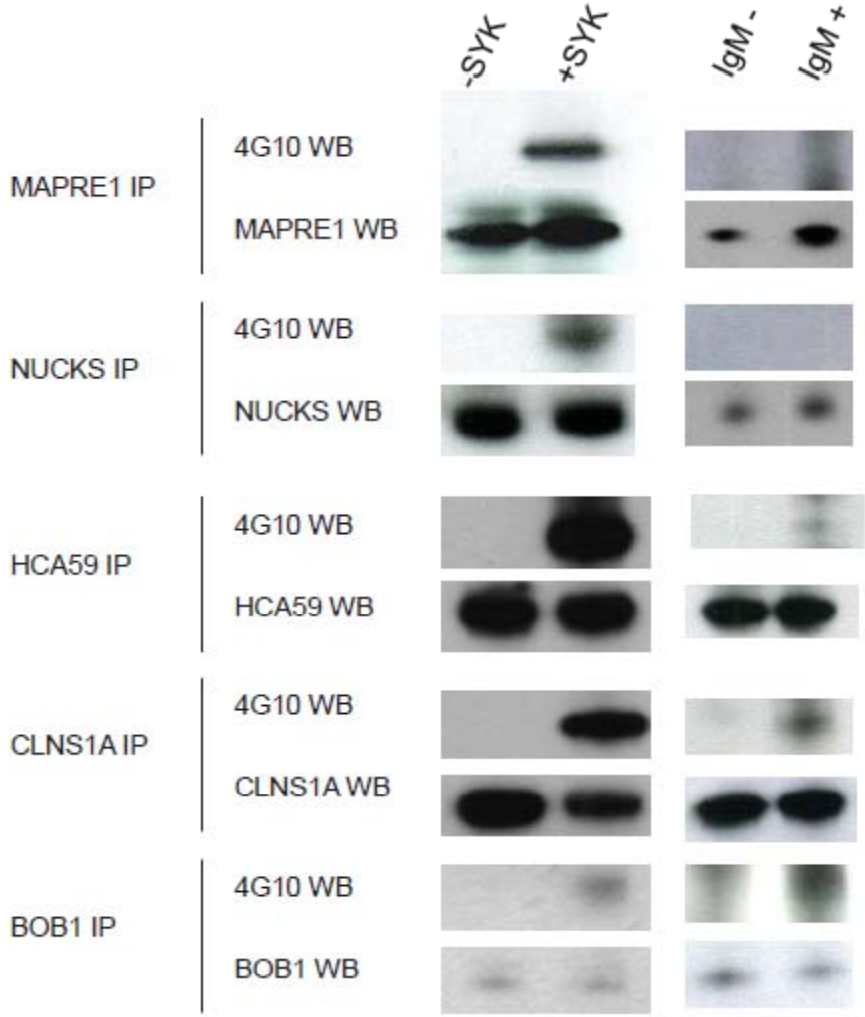




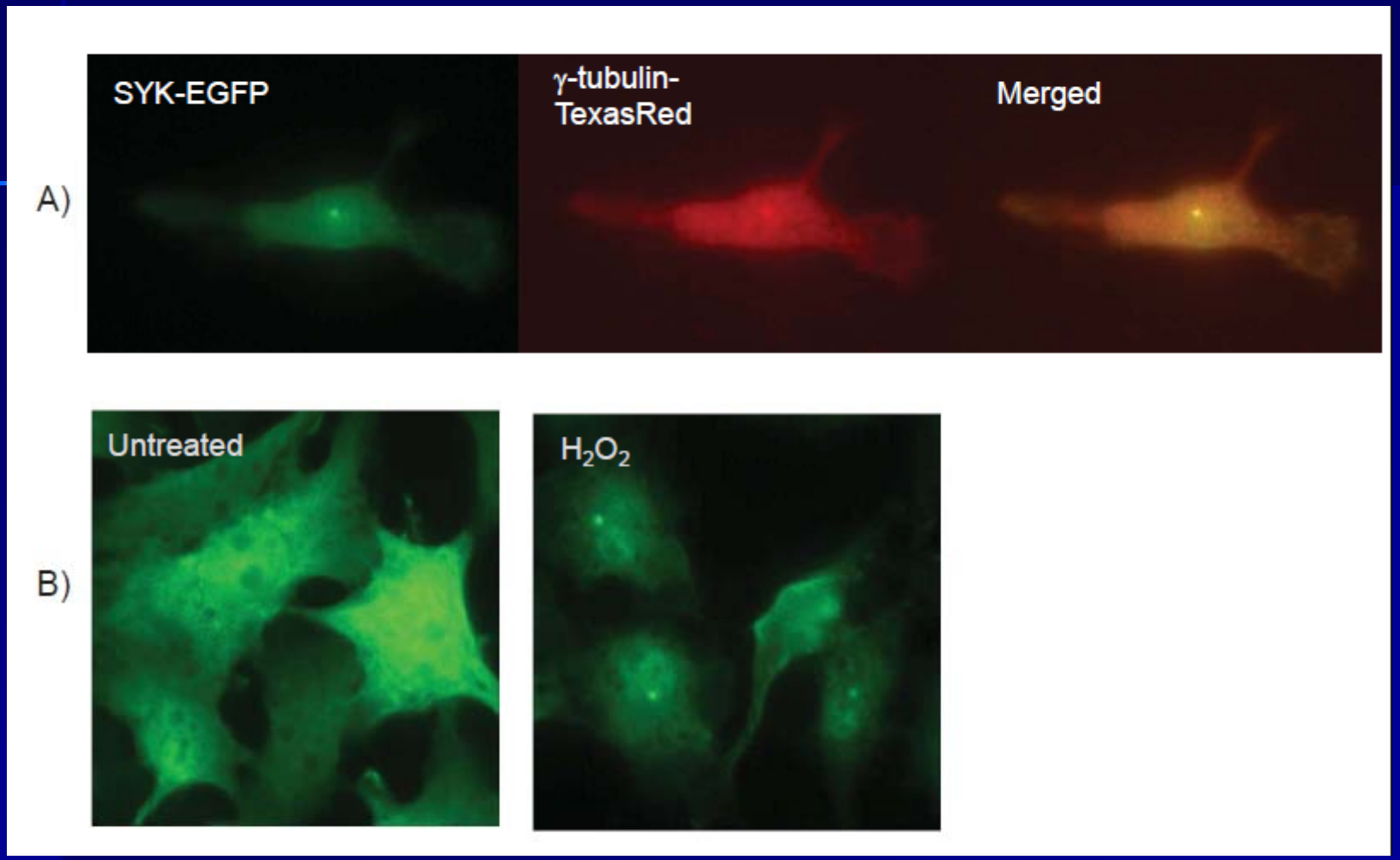
Substrates	ARHGDIA, BLNK, CAPN1, Ck2, DCTN2, DOK1, EZR, FGFR1OP, HCLS1, LAT2, MAP1B, NUCKS1, PAG1, PDAP1, PIK3R1, PRPF31, PTPN1, SET, STK4	HCA59, CALM1, CLNS1A, COPB2, DDX41, EEF2, HBS1L, LRBA, NFYA, PAWR, PJA2, PQBP1, SEC23IP, SFRS8, TACC3, TMEM49, TUBA1B, TUBA1C, ZRANB2	ARAP1, CDC5L, CLIC1, IK, PDCD4, POU2AF1, SF3A1, SF3B1, SLC4A1AP, TRIM28	DPF2, EIF3J, ERCC6L, NSFL1C, PDCL3, RAD21, TRIP4
Biological functions	Hematological System Development and Function, Inflammatory Response	Cell Death, Infection Mechanism, Cell Morphology	Infection Mechanism, Cellular Movement, Lipid Metabolism	Dermatological Diseases and Conditions, Cancer, Gastrointestinal Disease

141 tyrosine-phosphorylated peptides from 63 proteins in 3 mg of whole human B cell DG75 cell extract



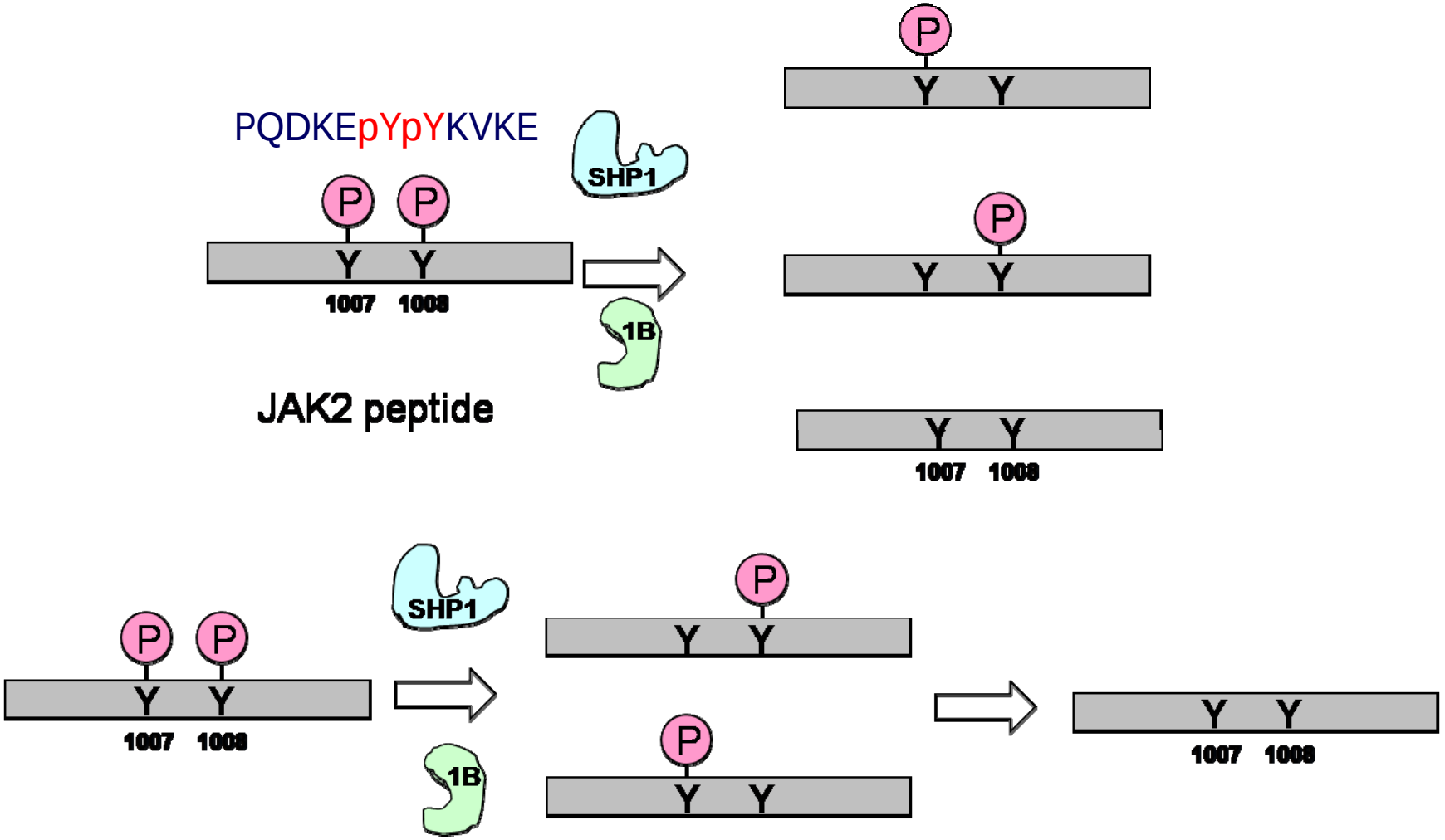


Confirmation of Syk kinase substrates

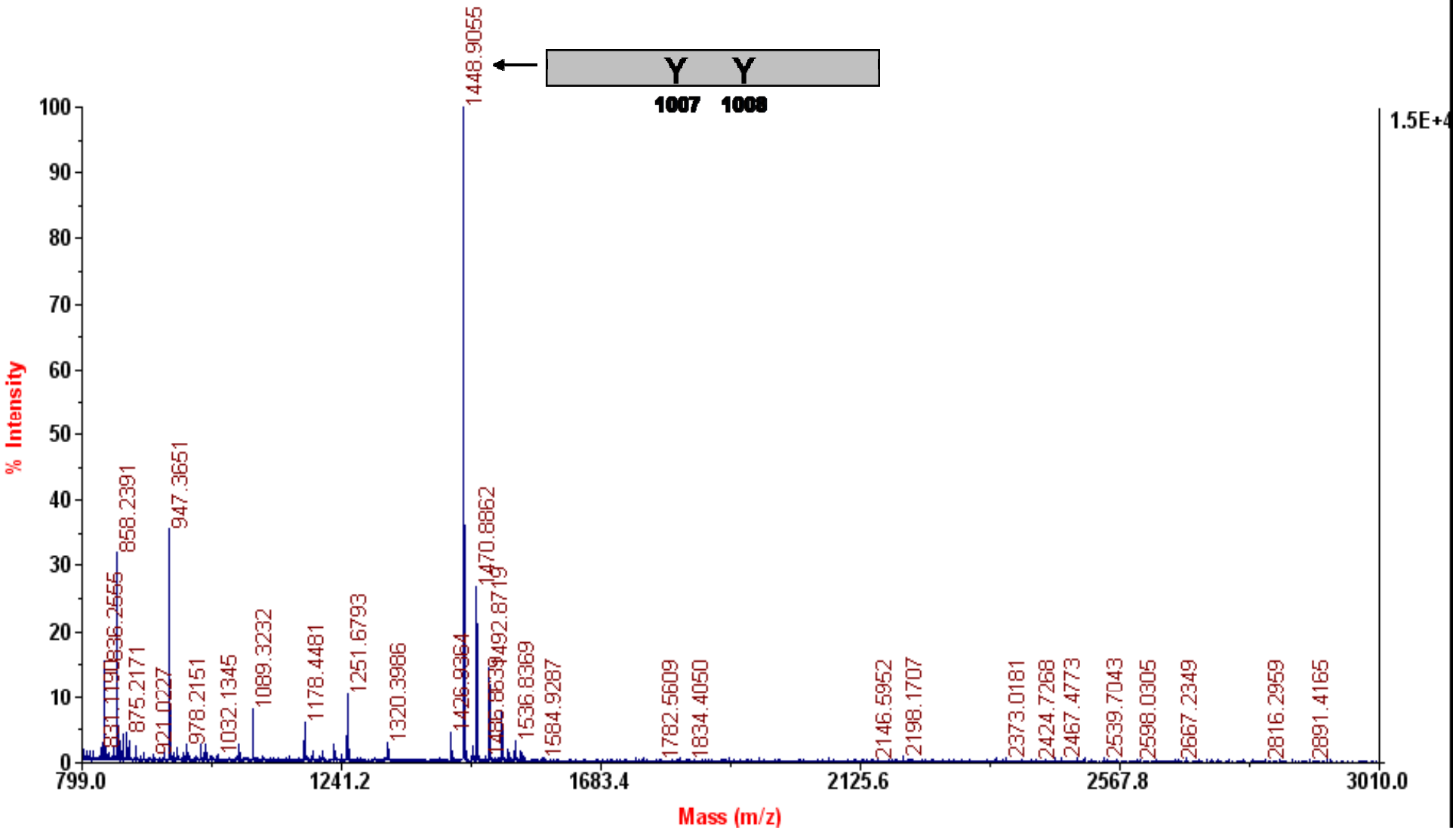


A) Centrosomal co-localization of GFP fused Syk with tubulin
B) The subcellular location of GFP-Syk fusion protein under oxidation stress.

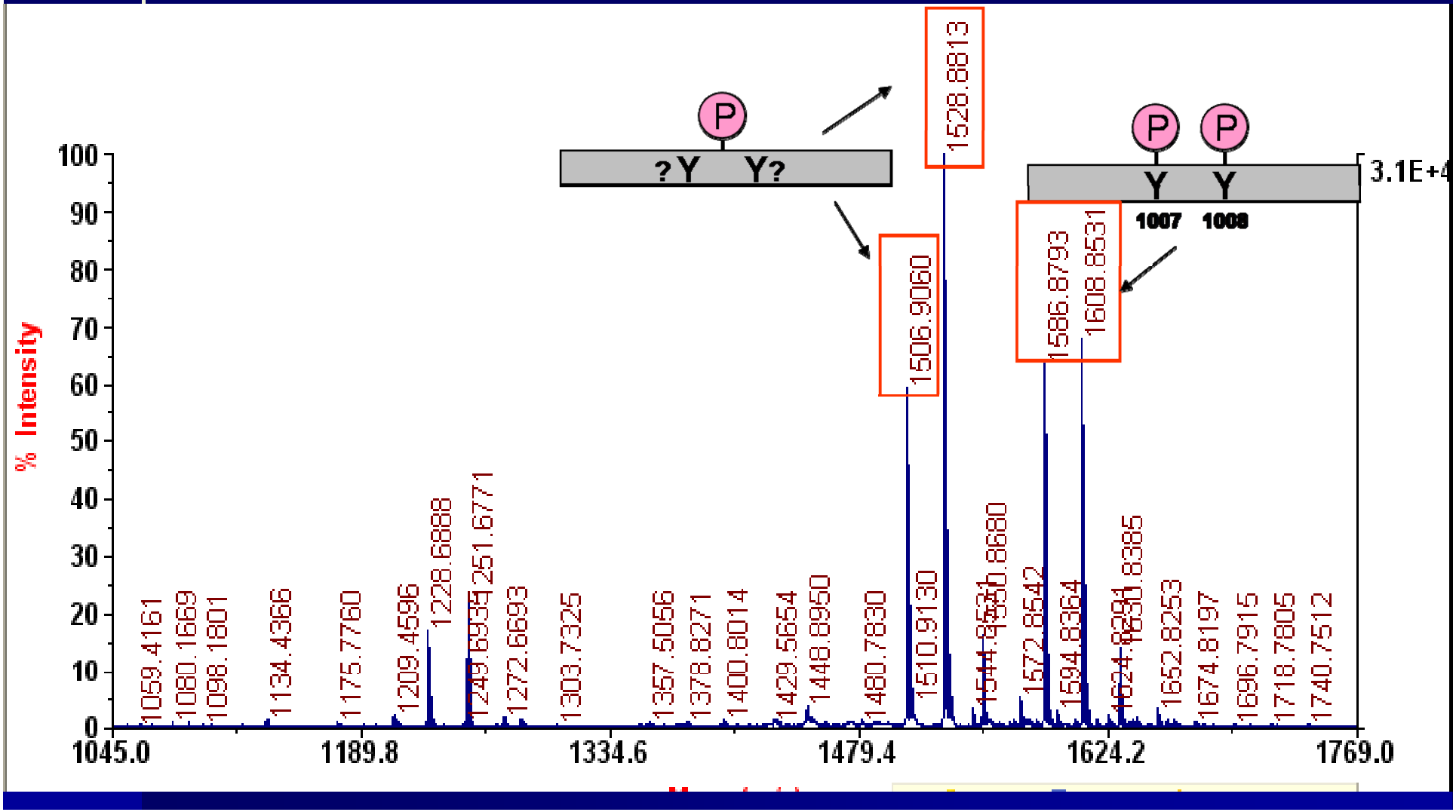
JAK2/PTP substrate



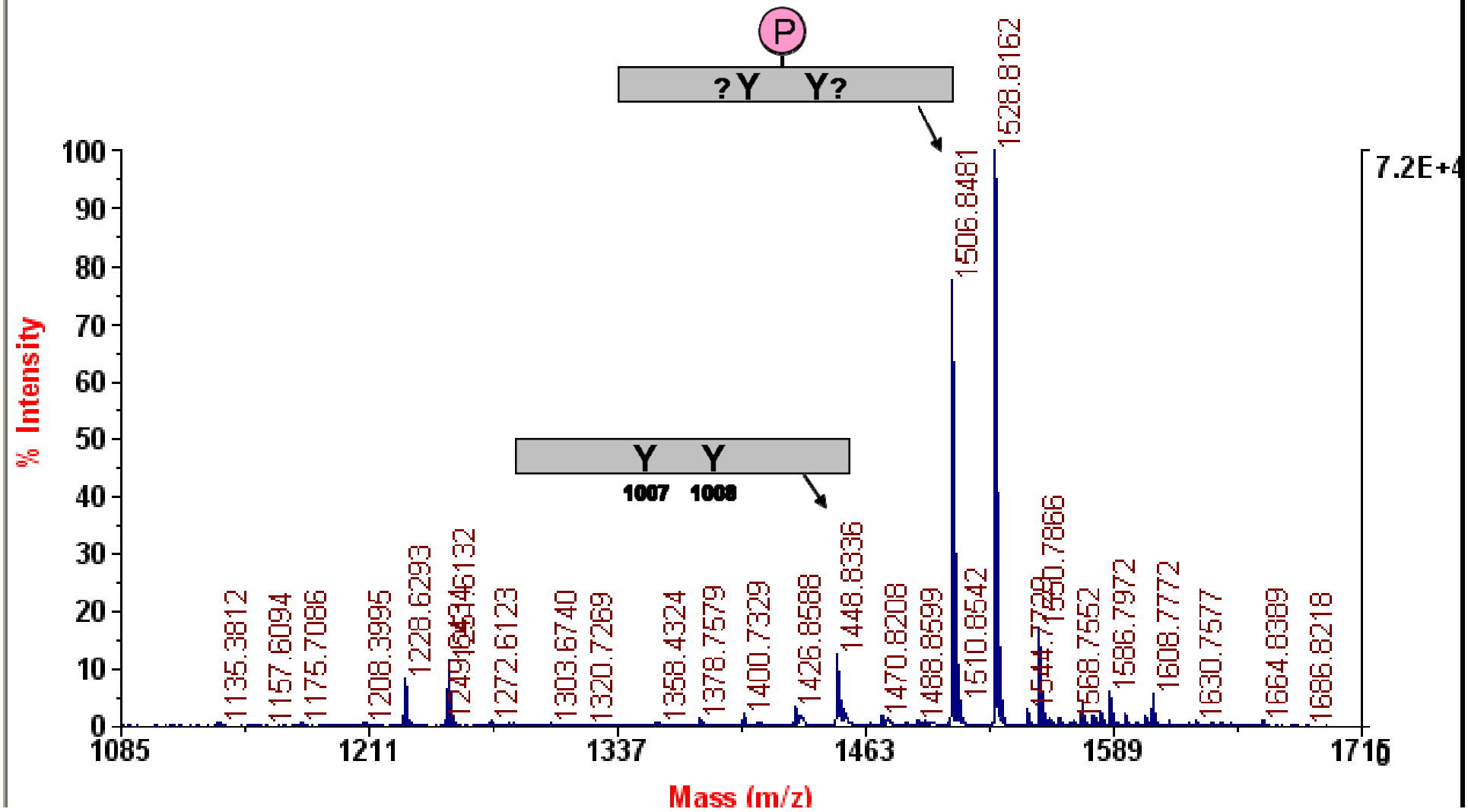
Both pY1007 and pY1008 can be dephosphorylated



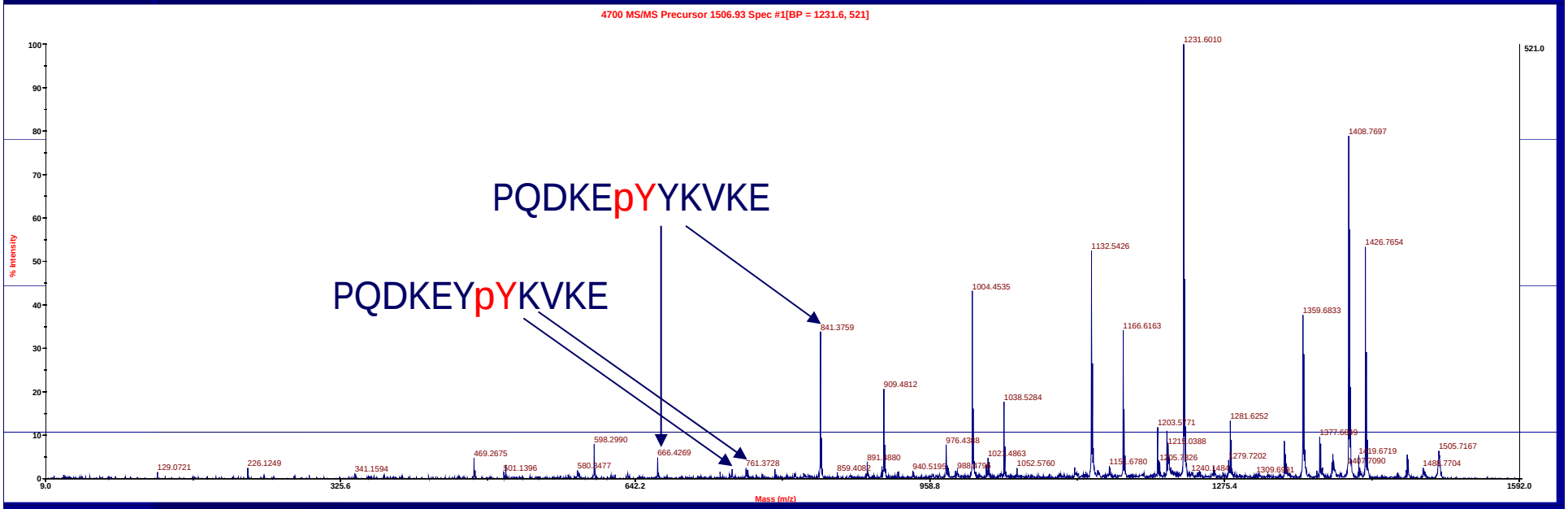
Single dephosphorylation site was found short time reaction



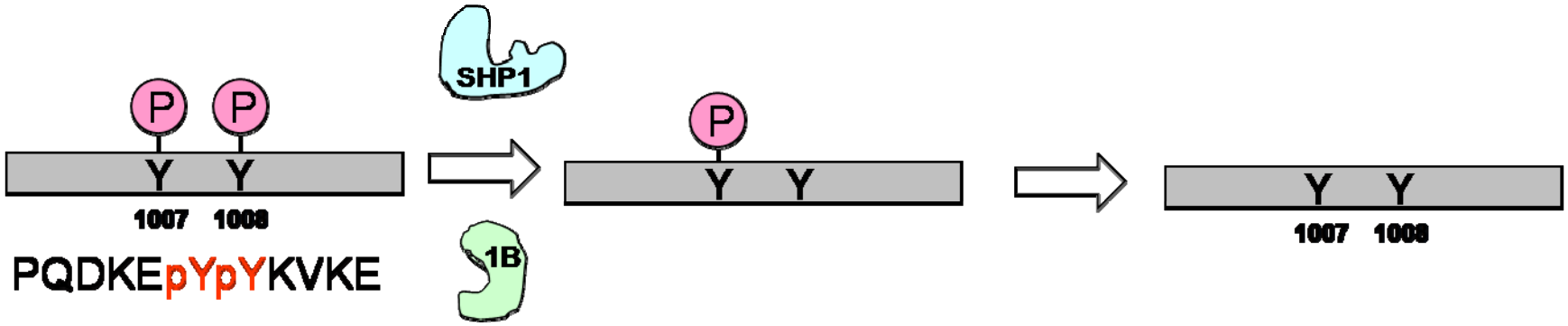
JAK2 peptide was dephosphorylated sequentially

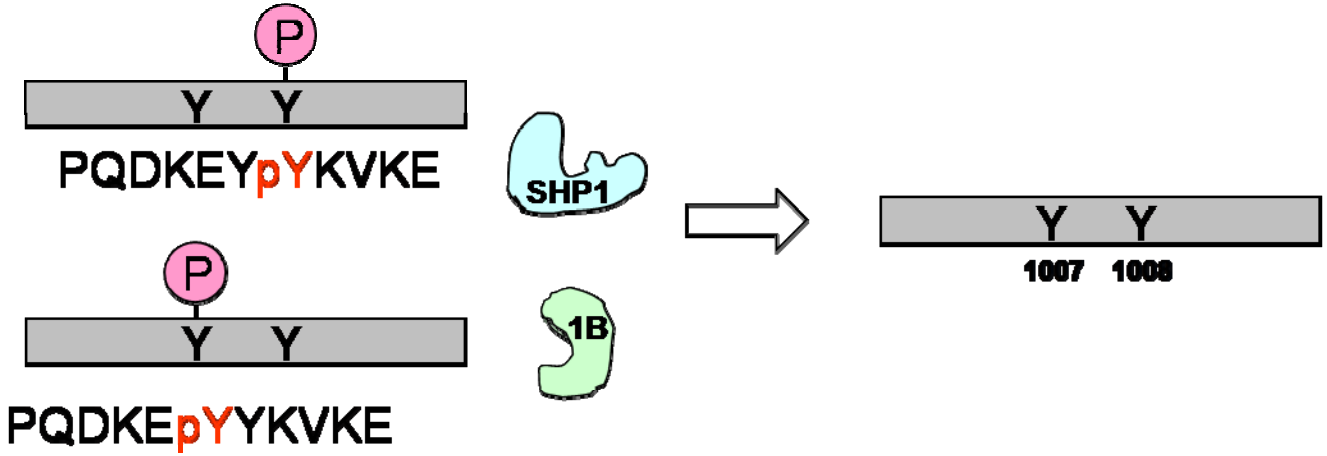


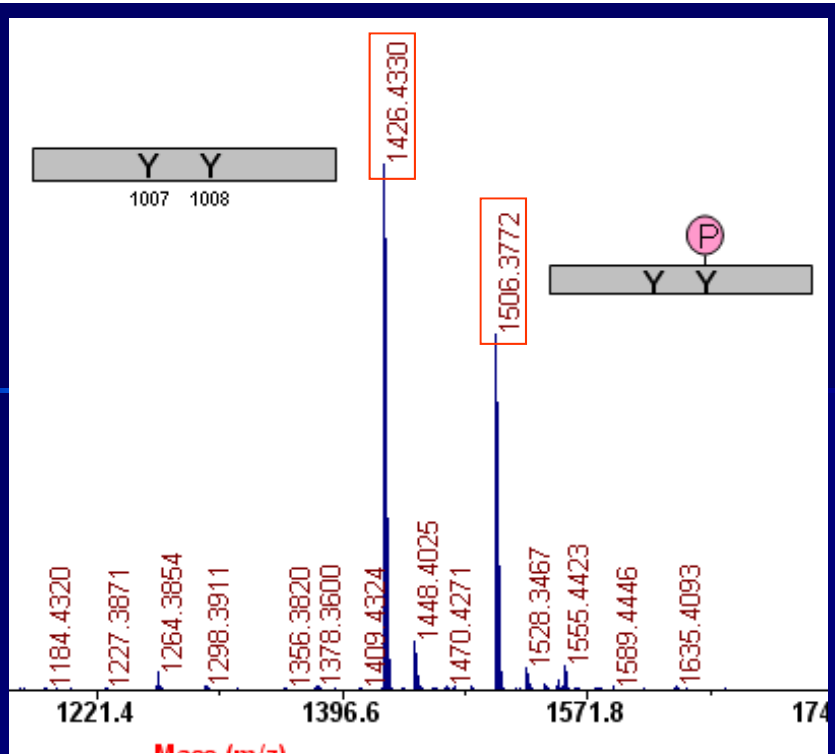
Identify the JAK2 peptide dephosphorylation site by MS^2

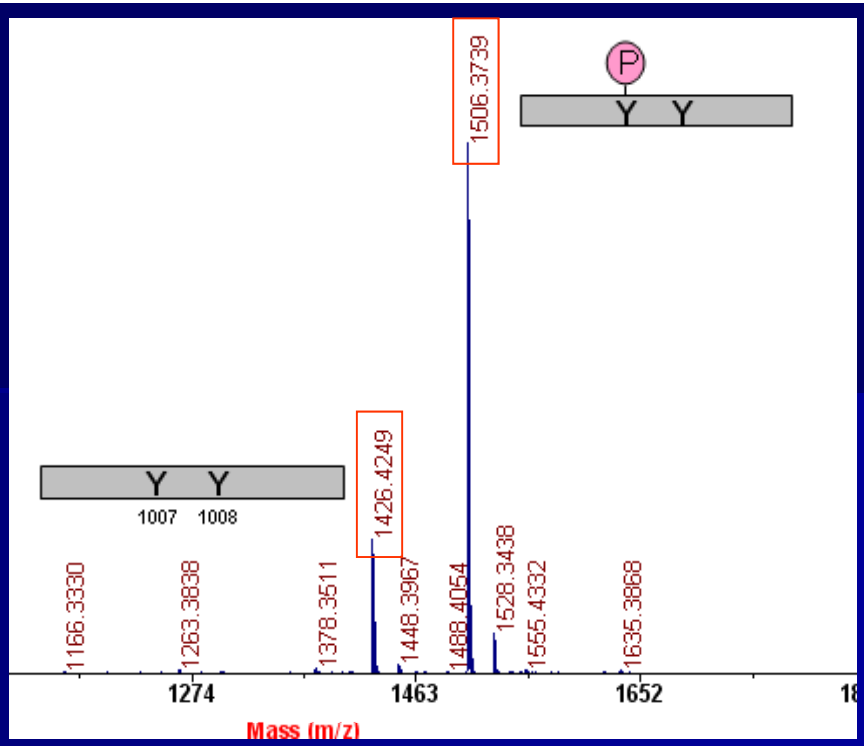


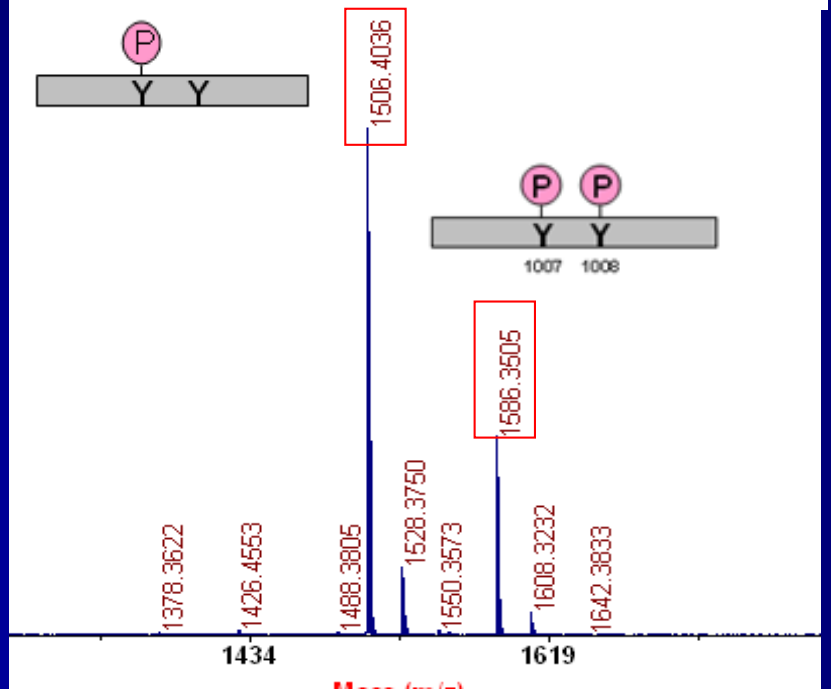
Does PTP recognize the phosphosite specifically ?



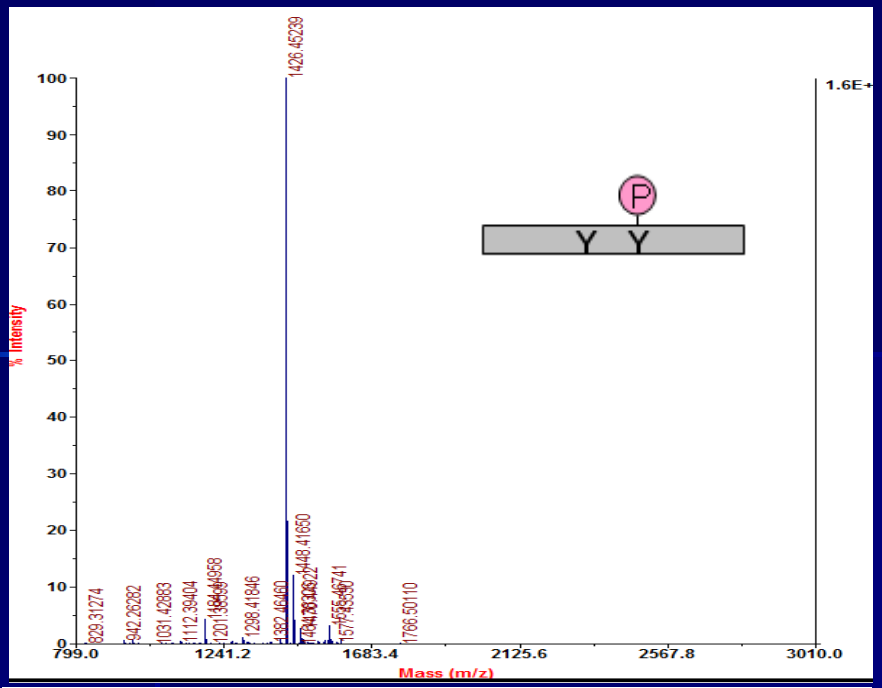


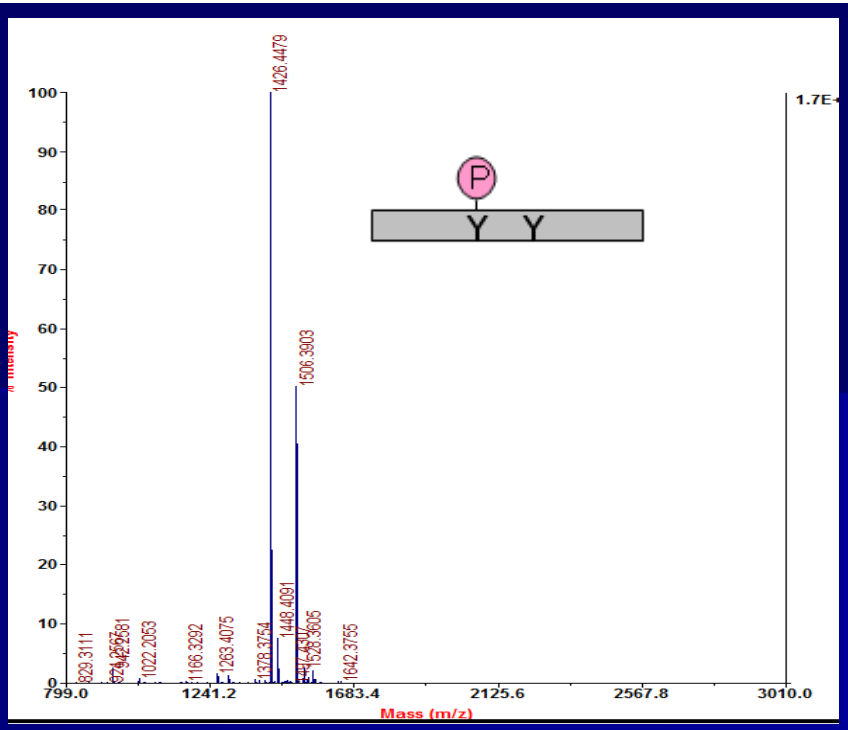


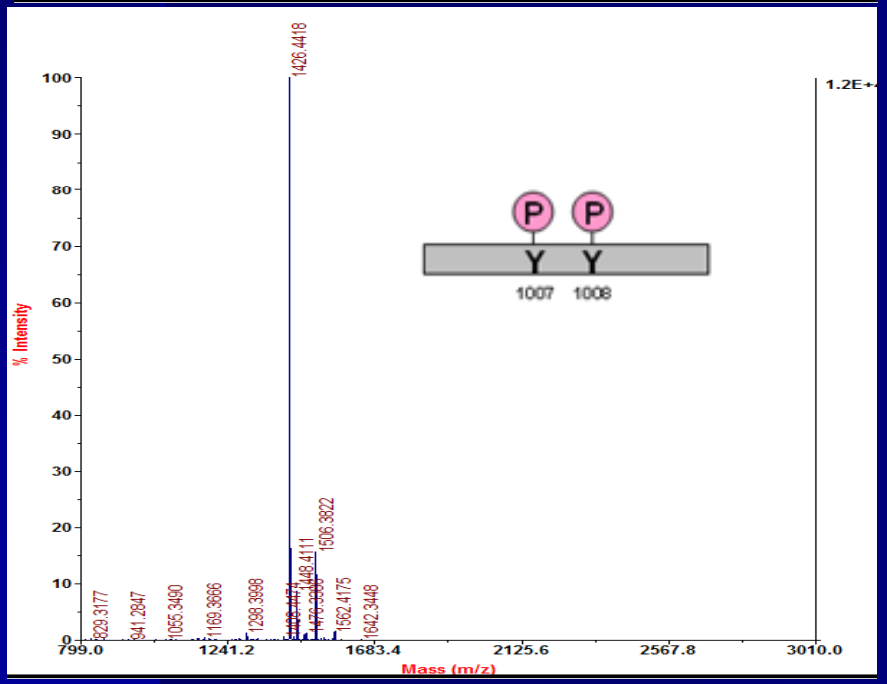




Compare the dephosphorylation efficiency of different phosphosites





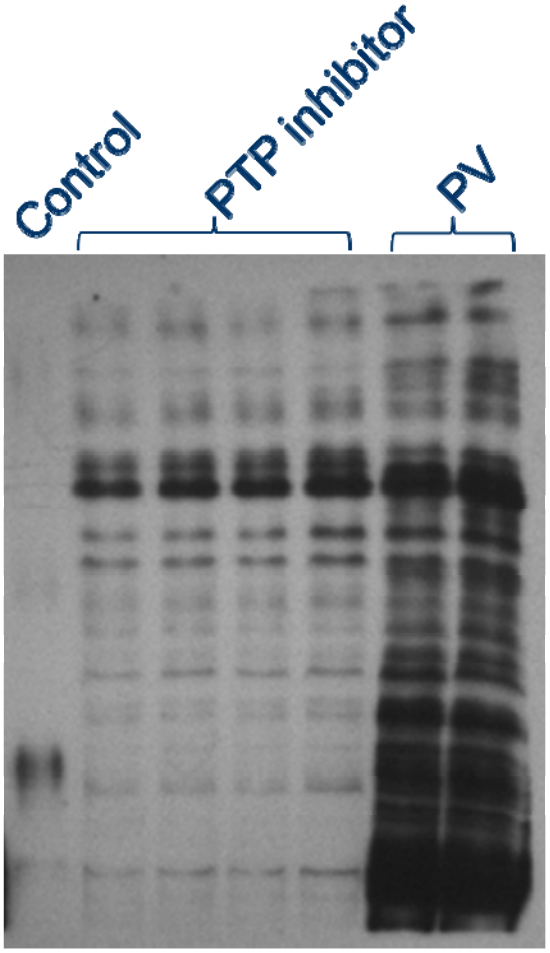


Dephosphorylation efficiency
1008>1007+1008>1007

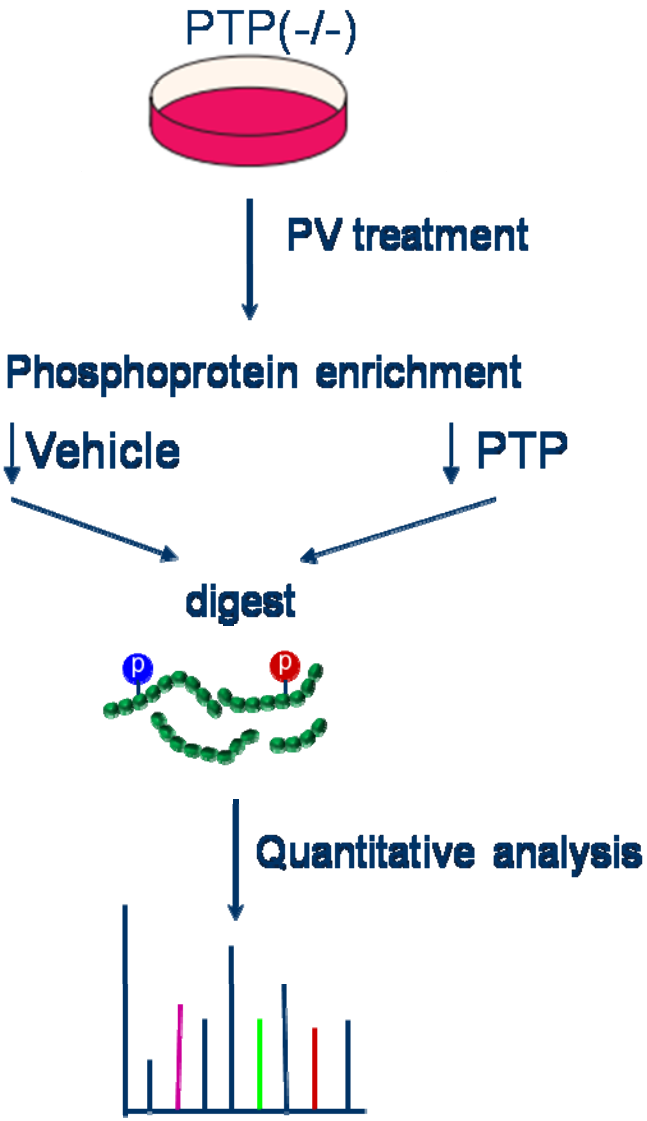
Summary

- PTP can dephosphorylate both pY1007 and pY1008.
- PTP prefer to catalyze the 1008 site.
- pY1008 enhance the dephosphorylation of pY1007 by binding to the PTP domain.

Investigation of PTP substrates by proteomics



pY WB



Thanks for your attention !