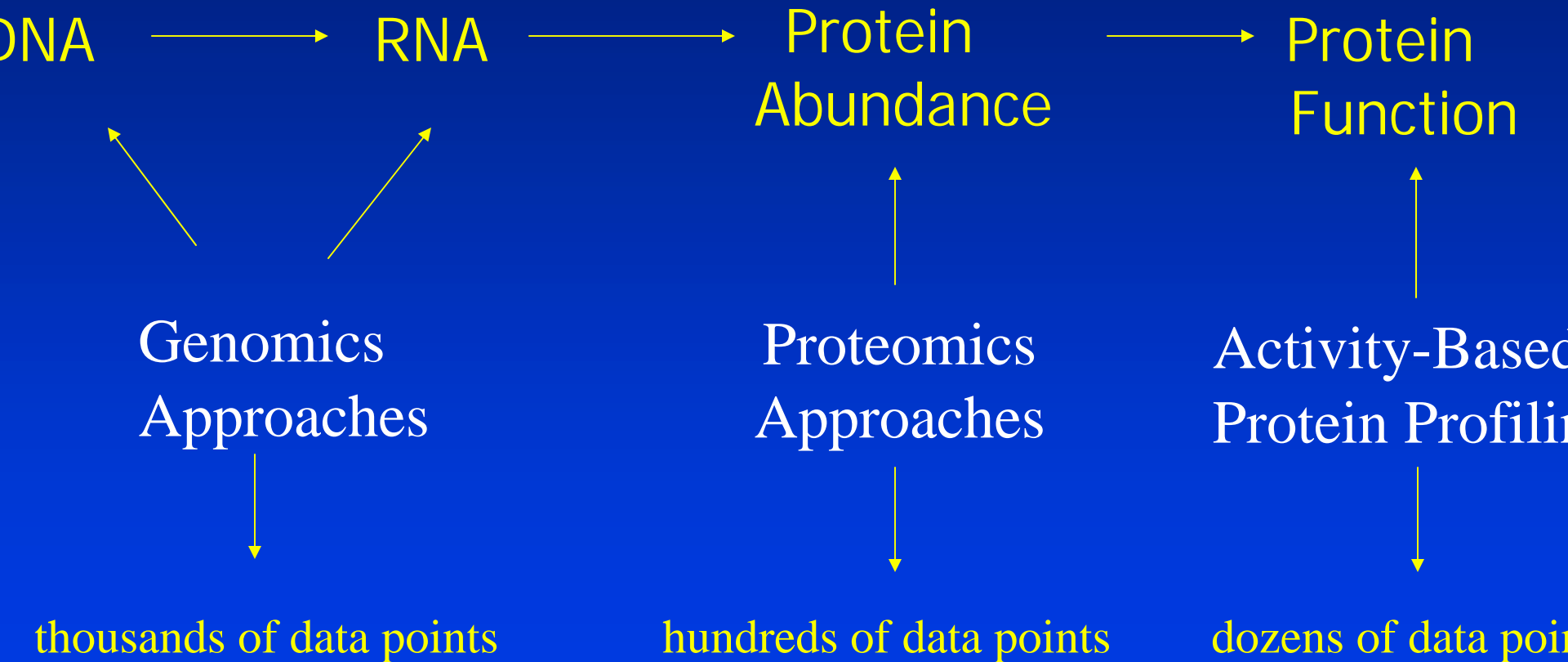


Protein Profiles in Breast Cancer - Why They are Important

**Benjamin F. Cravatt
The Scripps Research Institute**

What different profiling strategies measure



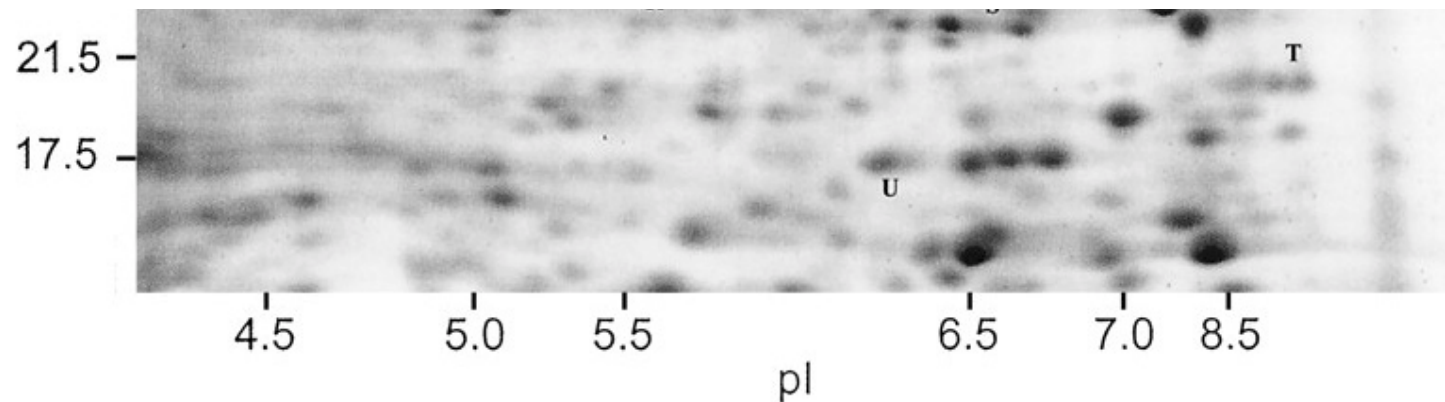
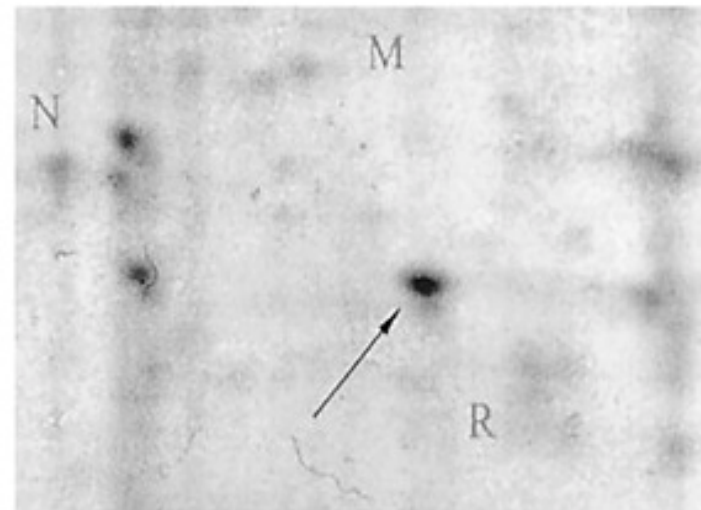
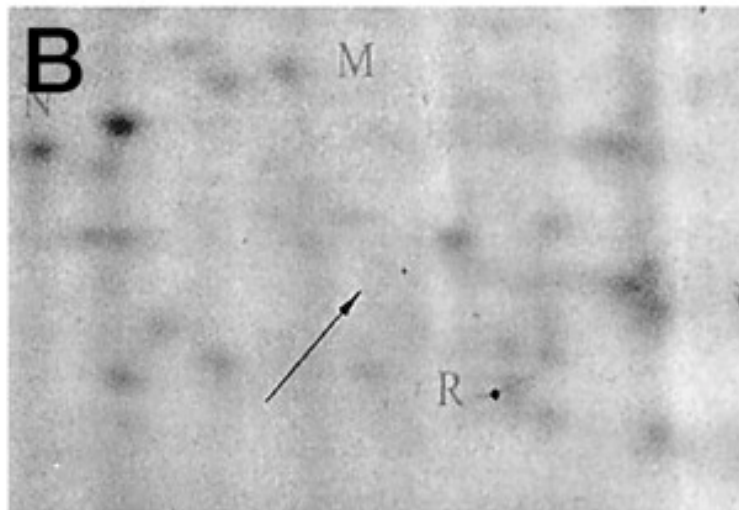
Why Proteins are Important to Measure in Breast Cancer

- 1) Biomarkers - proteins represent the primary source of diagnostic markers in fluids and tissues
- 2) Drug Targets - most of biochemical and cellular events that promote tumor growth are enacted by proteins

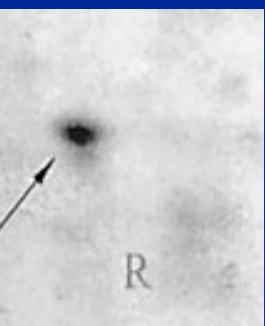
Abundance-Based Proteomics - 2D-Gel Profiles of Breast Cancer



Normal

DCIS



Abundance-Based Proteomics - 2D-Gels Coupled with Mass Spectrometry to Identify Breast Cancer-Expressed Proteins



 **NCBI** 

Your request has been successfully submitted and put into the Blast Queue

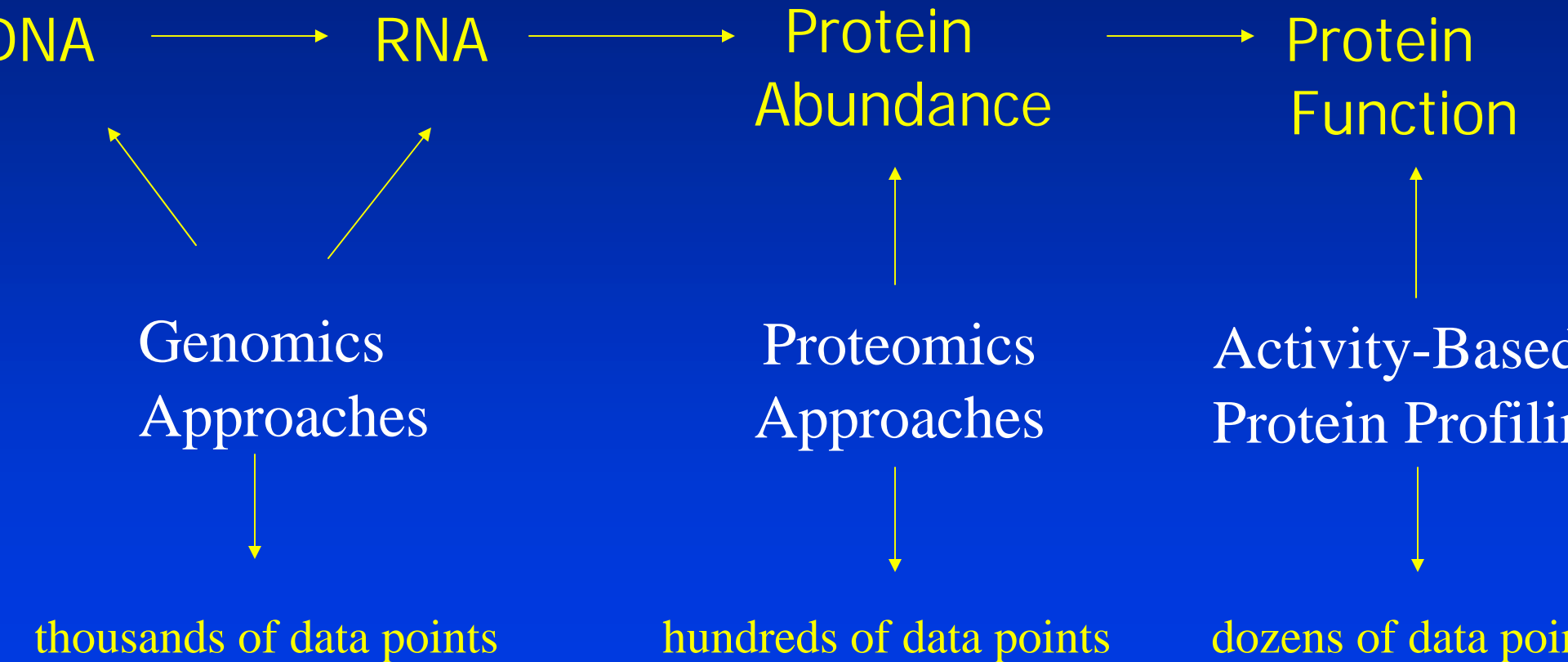
Query = (523 letters)

No putative conserved domains have been detected

sequences producing significant alignments:

Accession	Protein Name	Score (bits)
U01472.1	cytosolic sialic acid...	1642
U01473.1	hypothetical protein DSPEP741A51.1...	1638
U01474.1	cytosolic sialic acid...	325
U01475.1	sialic acid-specific 9-O...	243
U01476.1	cytosolic sialic acid...	231
U01477.1	receptor protein...	14
U01478.1	polyprotein (border...	11

What different profiling strategies measure

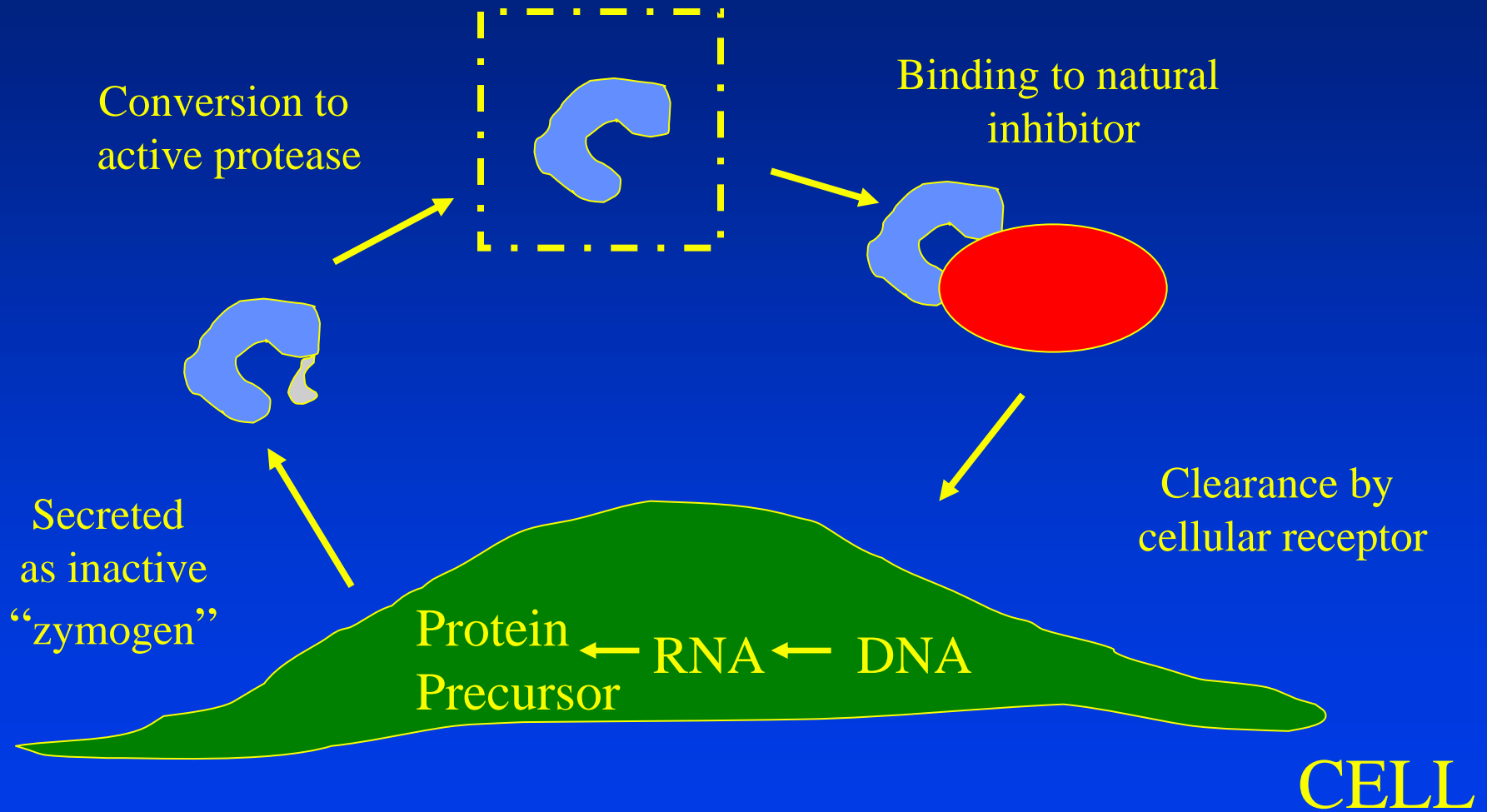


Is There a Need for Activity-Based Protein Profiling?

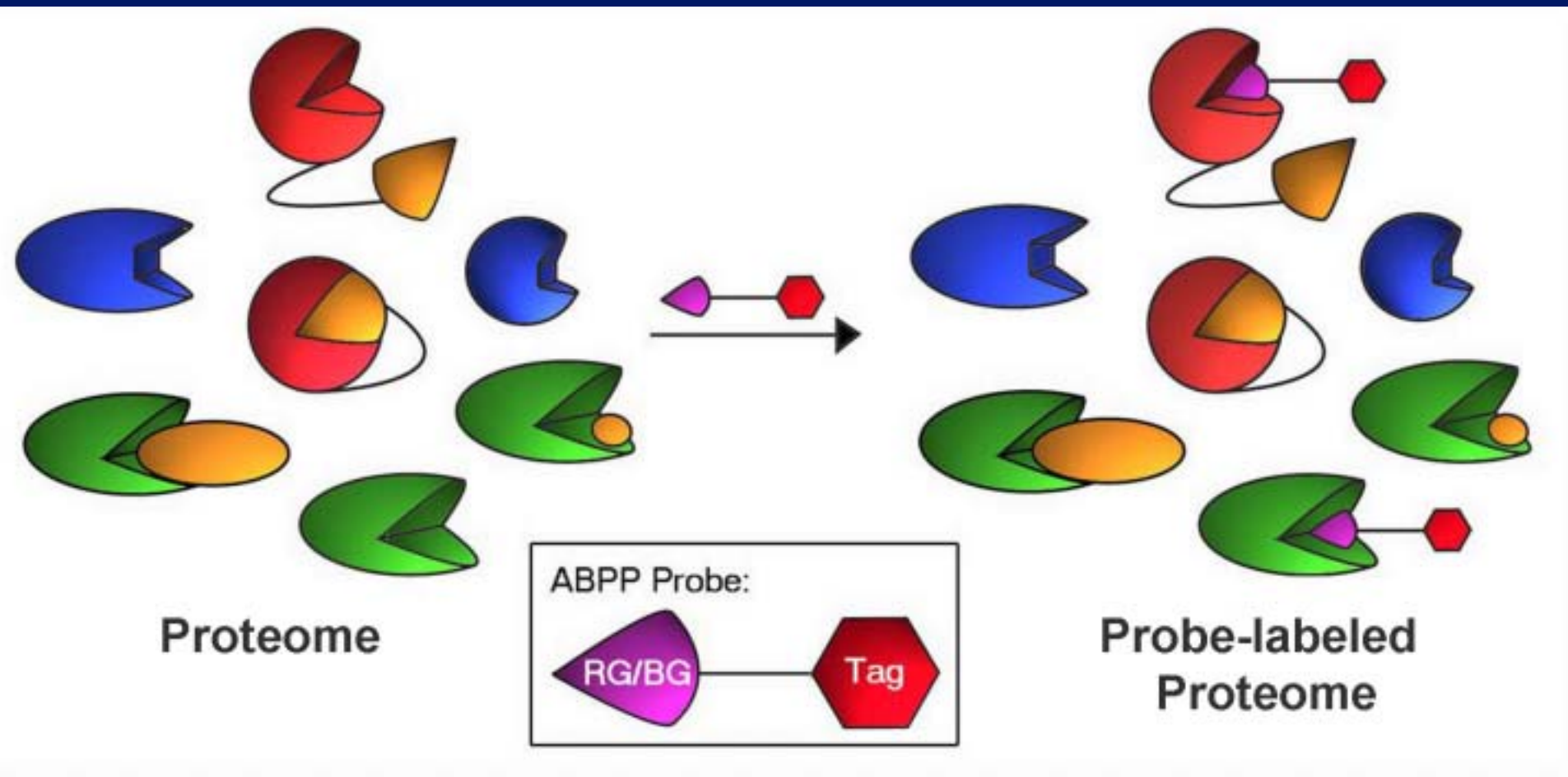
Enzyme activity is often regulated by post-translational mechanisms

- Active site-directed protein regulation (Kobe and Kemp, *Nature* 402, 373-376, 1999)
 - proteases: zymogens, endogenous inhibitors
 - kinases: autoinhibitory domains (*targets of Iressa, Gleevec*)
 - phosphatases: autoinhibitory domains, endogenous inhibitors
- *How do we define cellular functions for these enzymes using standard genomic and proteomic methods?*

Biochemical Life-cycle of a Protease



Chemical Probes for Activity-Based Protein Profiling (ABPP)



Current List of Enzyme Classes Targeted by ABPP Probes

proteases

lipases

esterases

reductases

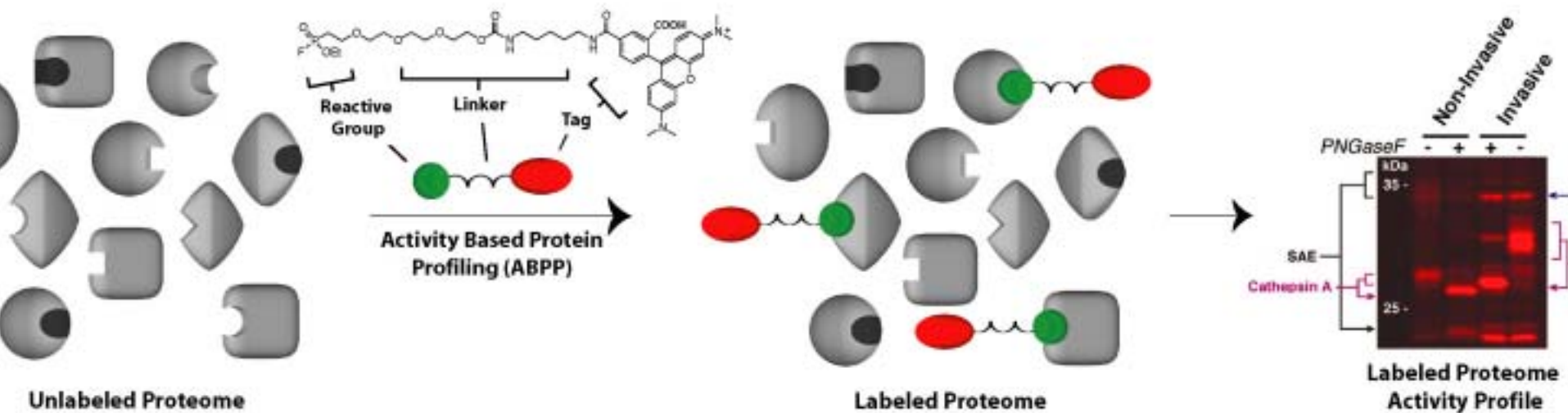
dehydrogenases

glutathione S-transferases

hydratases

transglutaminases

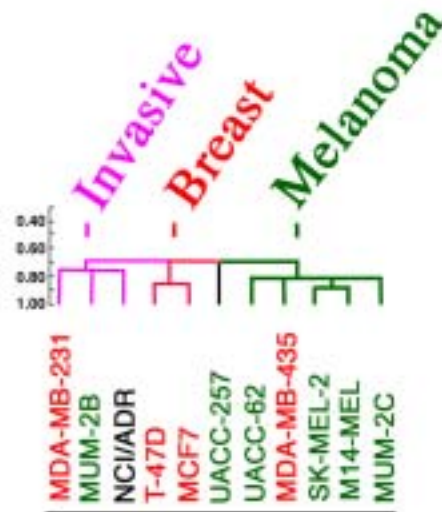
sugar kinases



Activity-Based Protein Profiling (ABPP) Applied to Cancer Cell Biology

- Experiment: a comprehensive analysis of serine hydrolase activities across a panel of human cancer cell lines
 - Cancer lines differ in: origin (breast/melanoma), hormone status, state of invasiveness, etc.
- Question: can ABPP provide proteomic information of sufficient quantity and quality to depict higher-order cellular phenotypes?

Cluster Analysis of Serine Hydrolase Activity Profiles



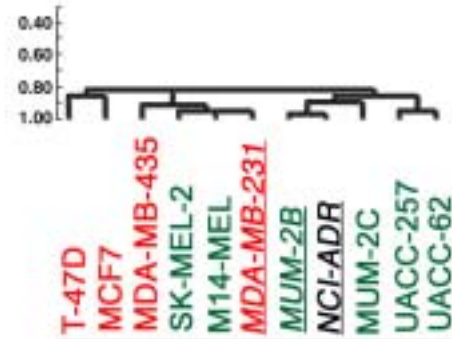
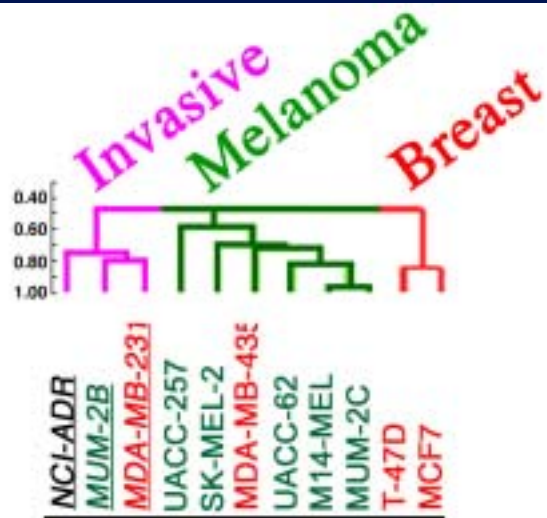
Cancer Lines Form
Three Major Clusters:

- **Breast**
- **Melanoma**
- **Invasive**

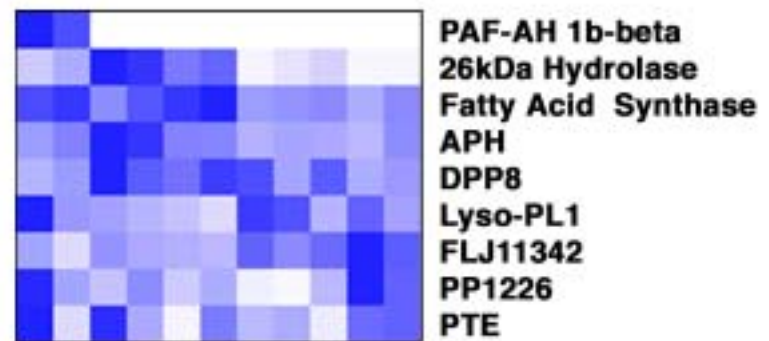


- Complement Component 1s
- PAF Acetylhydrolase isoform 1b, beta subunit (PAF-AH 1b-beta)
- Fatty Acid Amide Hydrolase (FAAH)
- Palmitoyl-Protein Thioesterase 2 (PPT-2)
- Butyrylcholinesterase (BCHE)
- 25kDa Unidentified Secreted Hydrolase (25kDa Hydrolase)
- Cathepsin A
- Phosphatidylserine-Specific Phospholipase 1 (PS-PL1)
- Urokinase Type Plasminogen Activator (uPA)
- Esterase D
- Membrane Amidase, Lower Glycosylated Form (KIAA Lower)
- Membrane Amidase, Upper Glycosylated Form (KIAA Upper)
- Platelet-Activating Factor Acetylhydrolase 2 (PAF-AH 2)
- 26kDa Unidentified Cytosolic Hydrolase (26kDa Hydrolase)
- Fatty Acid Synthase
- Acyl-Peptide Hydrolase (APH)
- Dipeptidyl Peptidase VIII (DPP8)
- Lysophospholipase 1 (Lyso-PL1)
- Soluble Alpha/Beta Hydrolase (FLJ11342)
- Soluble Alpha/Beta Hydrolase (PP1226)
- Peroxisomal Long-Chain Acyl CoA Thioesterase (PTE)
- Angiotensinase C

Cancer Clusters Are Driven by Secreted/Membrane Enzymes

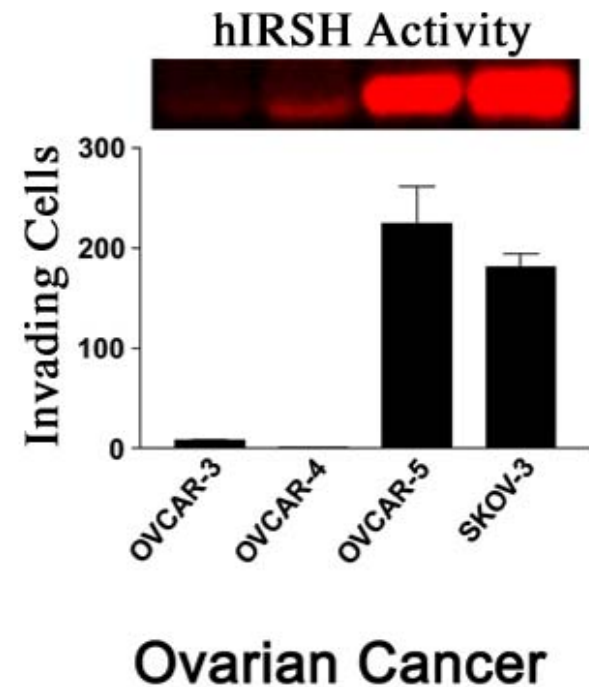
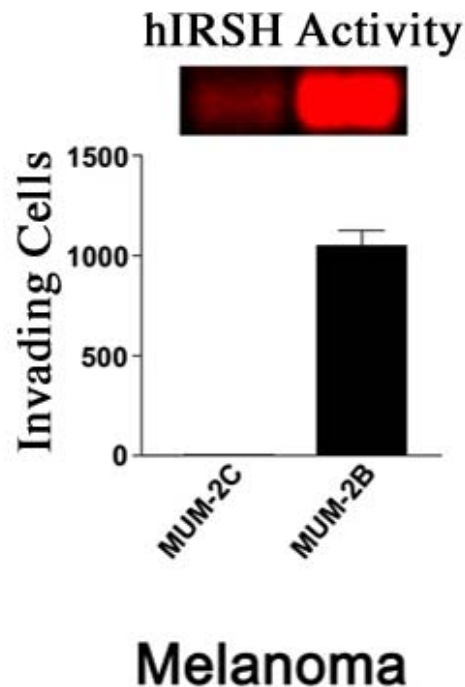
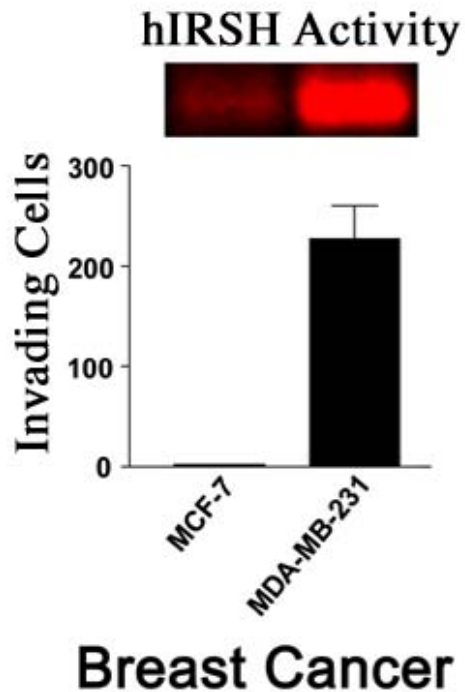


Secreted and Membrane Cluster



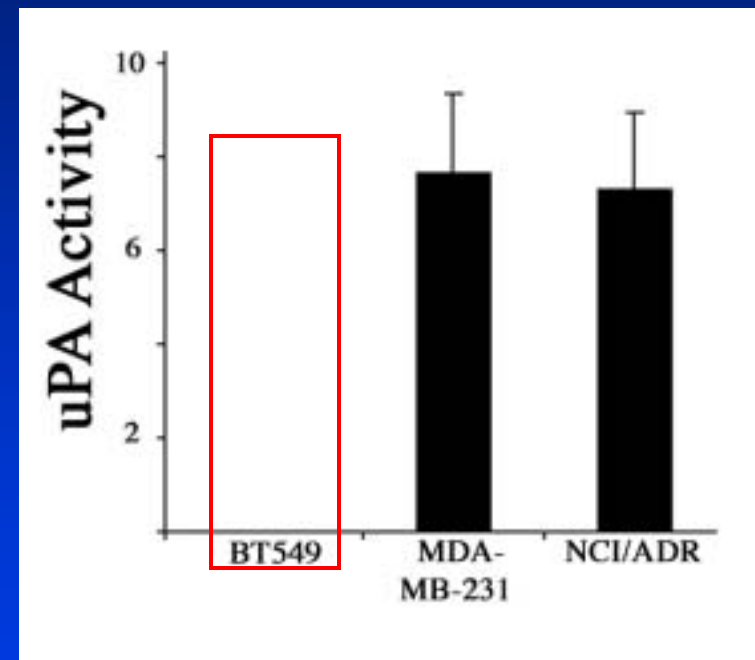
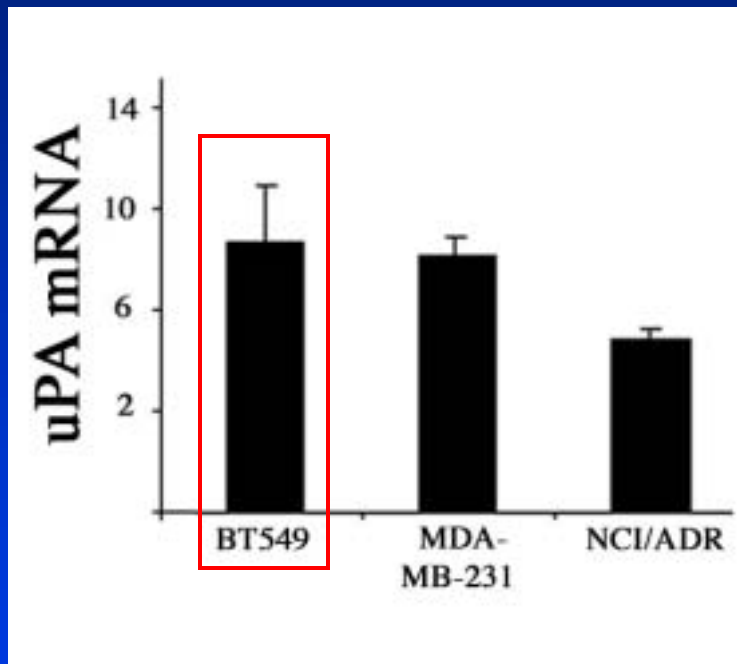
Soluble Cluster

A Novel Integral Membrane Hydrolase KIAA1363 That is Upregulated in Invasive Cancer Cells from Several Different Tumor Types



When Are mRNA Levels Not Correlated with Levels of Protein Activity?

- In a panel of human cancer lines, urokinase (uPA) mRNA levels do not correlate with urokinase activity



Conclusions and Future Directions

- Enzyme activity profiles can depict higher-order properties of cancer cells
 - Invasive and non-invasive cancers exhibited *nearly orthogonal* serine hydrolase activity profiles
 - Secreted and membrane hydrolases were *better predictors* of cancer phenotype than cytosolic hydrolases
-
- Do any of the enzymes play a *functional role* in cancer progression *in vivo*

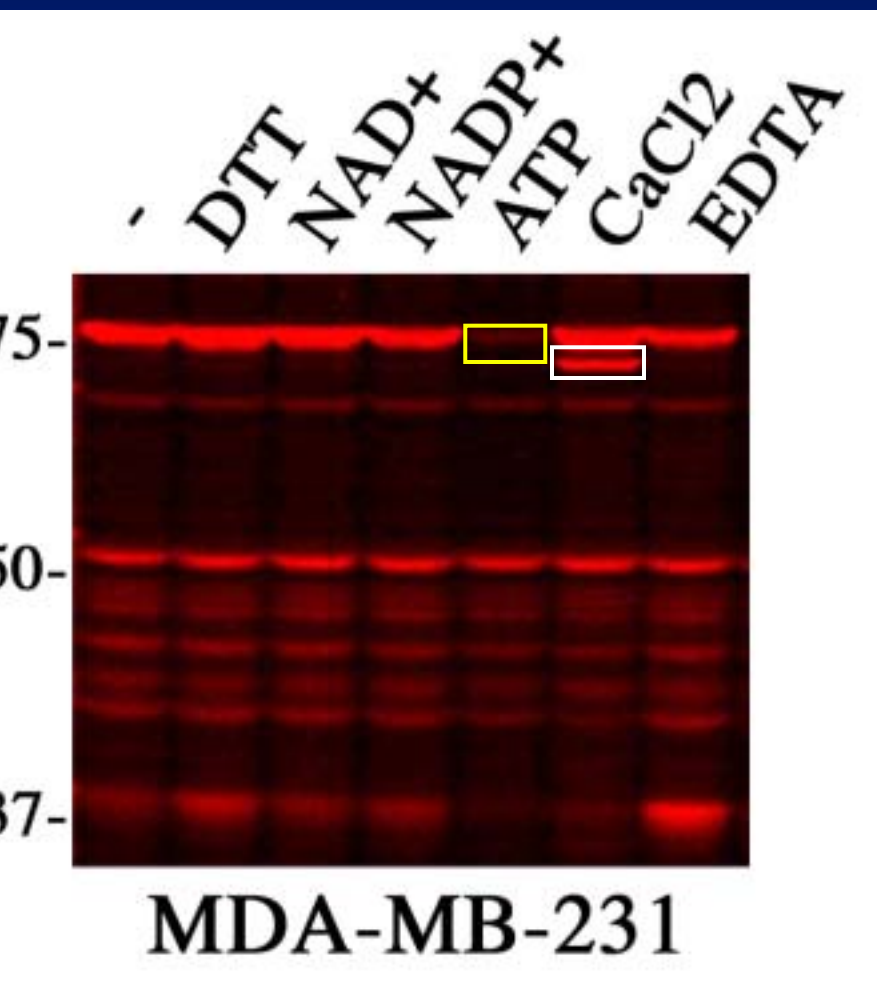
Efforts to Advance the Technology of ABPP

- 1) Expanding the proteome coverage of ABPP
- 2) Methods for conducting ABPP *in vivo*
- 3) Discovering potent and selective reversible enzyme inhibitors

Efforts to Advance the Technology of ABPP

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Context-Dependent Labeling Events in Whole Proteomes



- platelet-type phosphofructokinase
 - allosterically inhibited by ATP

- type II tissue transglutaminase
 - calcium-dependent activity

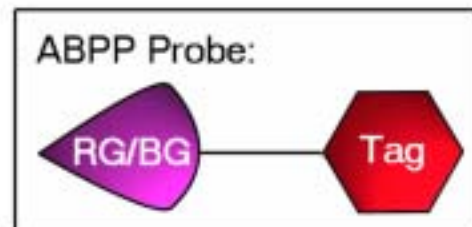
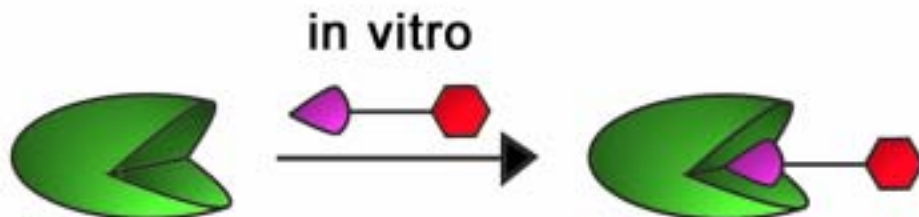
Potential Advantages of “Tag-Free” ABPP

Removal of bulky fluorophore/biotin tag should:

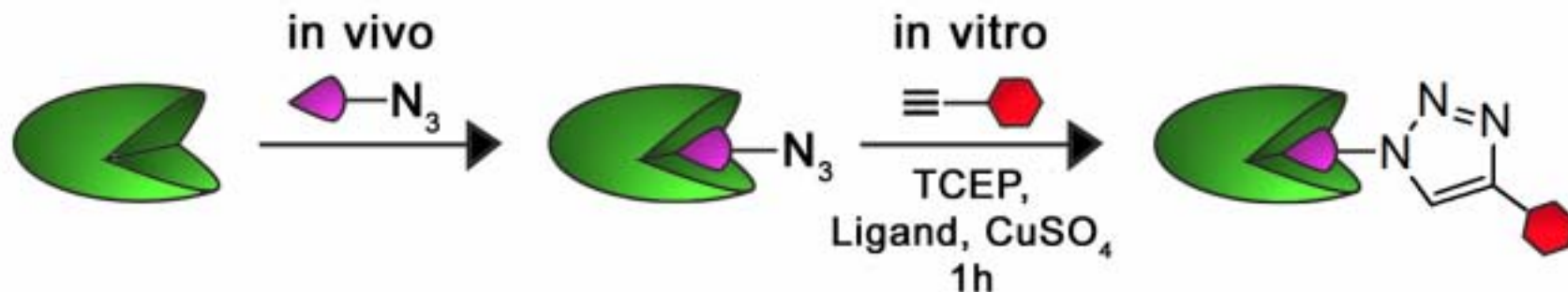
- 1) enhance cell permeability and distribution of probes
- 2) eliminate antagonistic effect on probe-protein interactions
- 3) streamline probe synthesis

“Tag-Free” Method for ABPP Using the *Bio-orthogonal* Azide-Alkyne Cycloaddition (Click Chemistry)

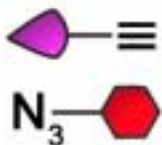
Standard ABPP:



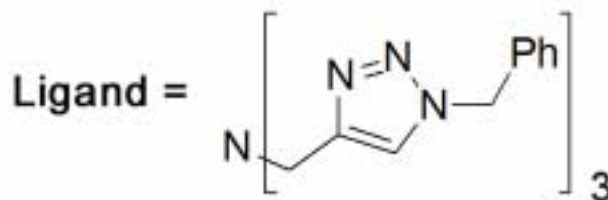
Click Chemistry ABPP:



Can switch click partners and use:

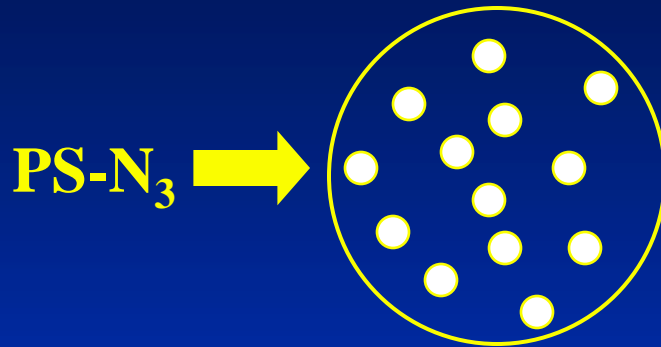


TCEP = Tris(carboxyethyl)phosphine



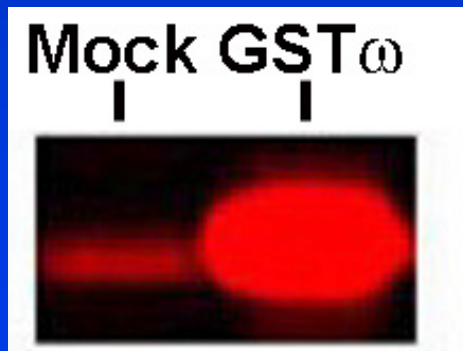
Profiling Enzymes *In Vivo* with Cycloaddition-Based ABE

In Cultured Cells



homogenize ↓

alkyne-Rh ↓



Profiling Enzymes *In Vivo* with Cycloaddition-Based ABE

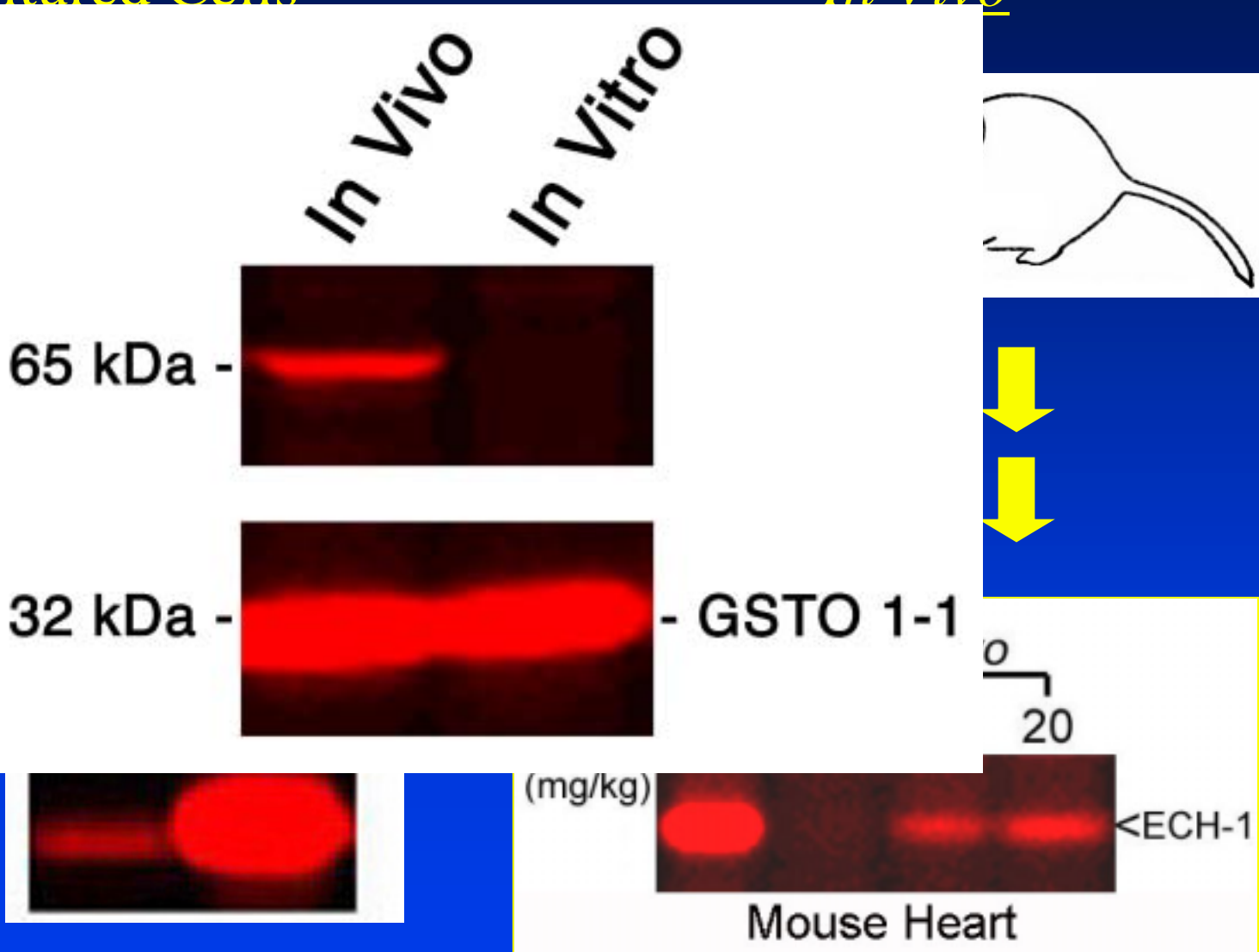
In Cultured Cells

In Vivo

PS-N₃ ■

homocysteine

alkylated

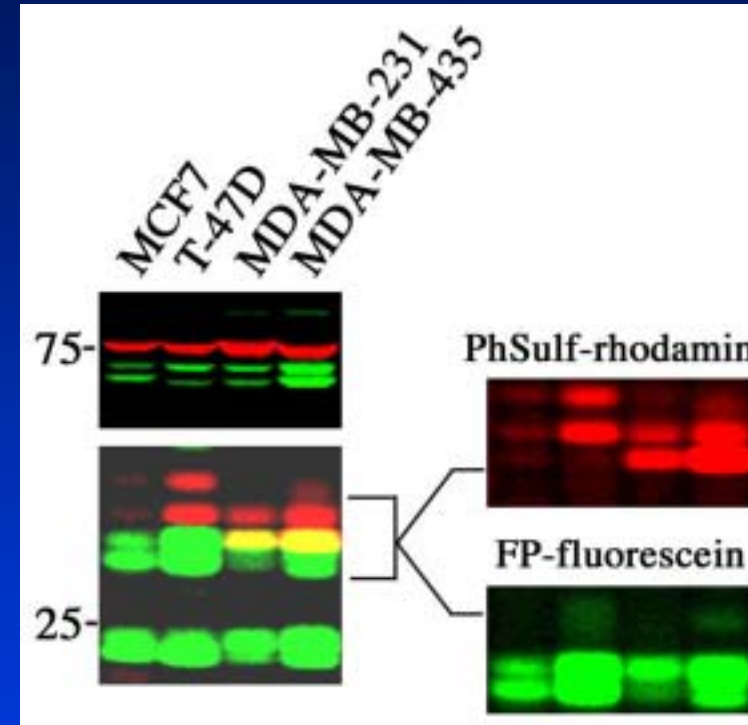


Challenges Facing the Application of Proteomic Methods to Breast Cancer

Can we detect all of the relevant proteins expressed in a cancer proteome?

How can we extract the most proteomic information out of the minimum amount sample?

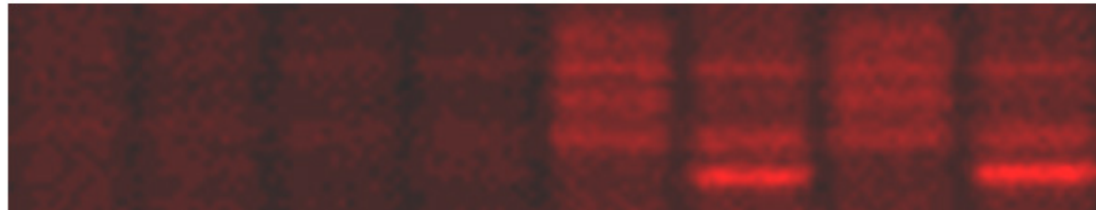
How can we accelerate the validation of candidate breast cancer-associated proteins as true diagnostic markers and/or therapeutic targets?



The Membrane-Associated Hydrolase KIAA1363 Is Highly Upregulated in Primary Breast Tumors

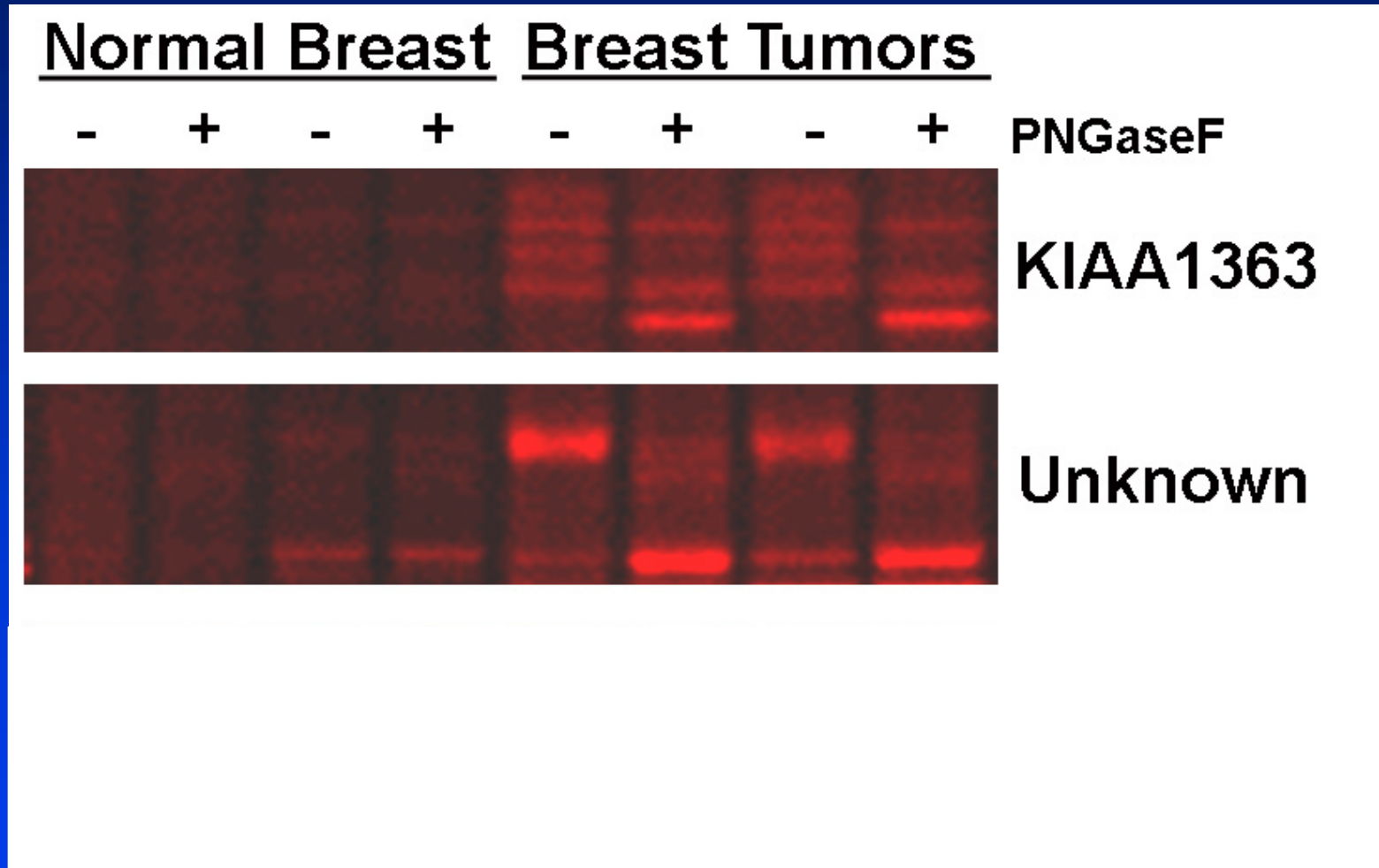
Normal Breast Breast Tumors

- + - + - + - + PNGaseF

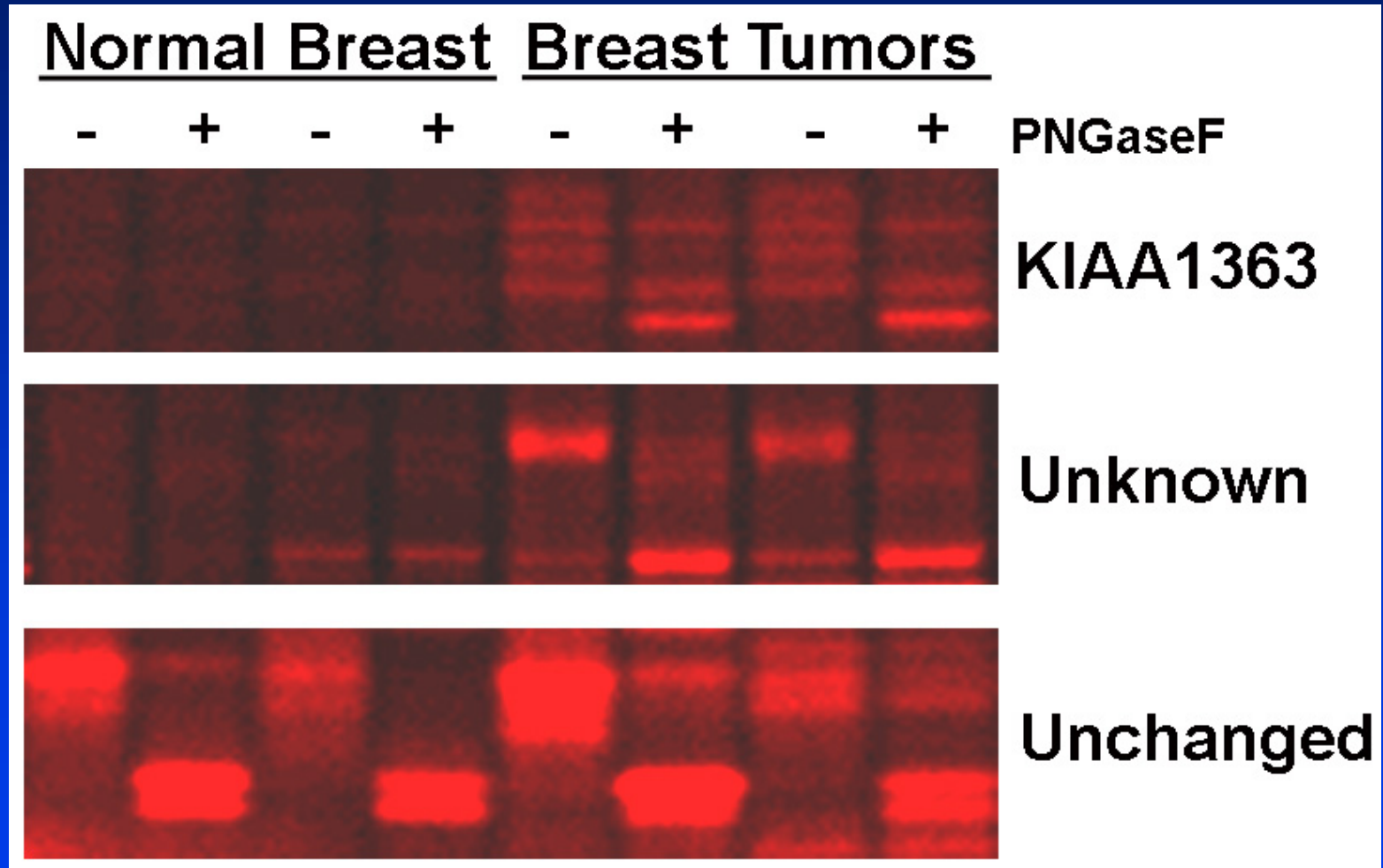


KIAA1363

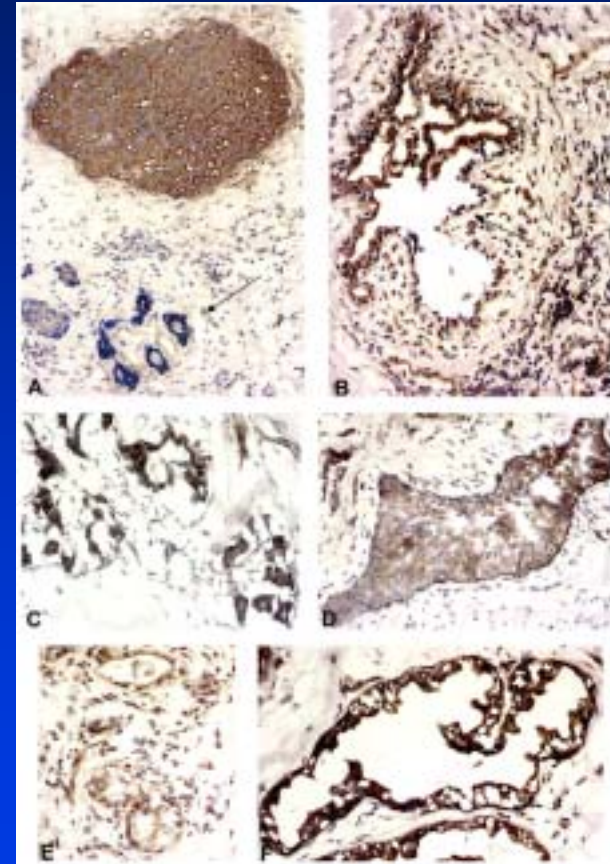
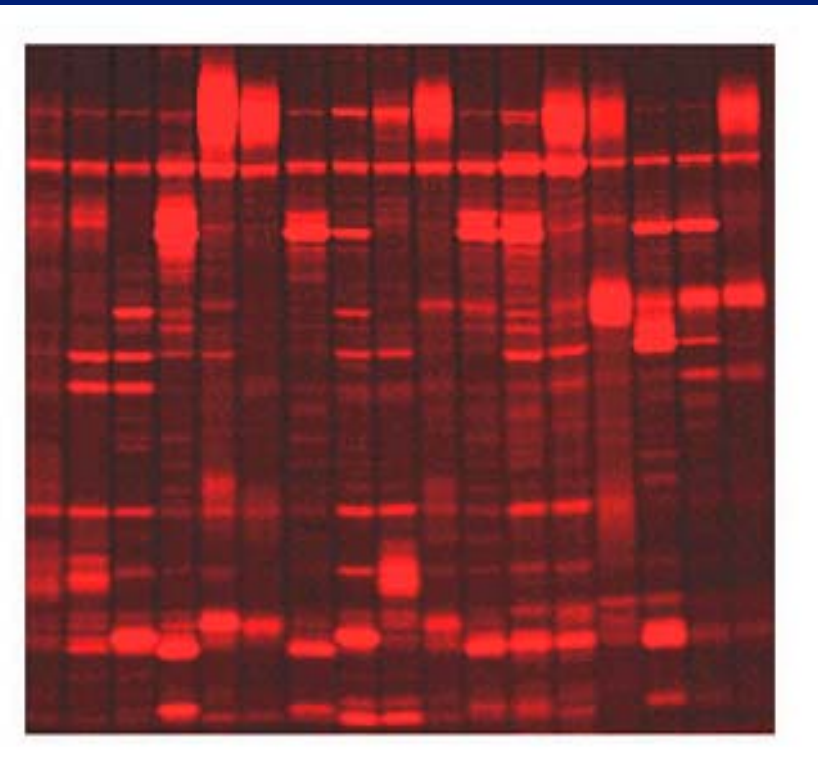
The Membrane-Associated Hydrolase KIAA1363 Is Highly Upregulated in Primary Breast Tumors



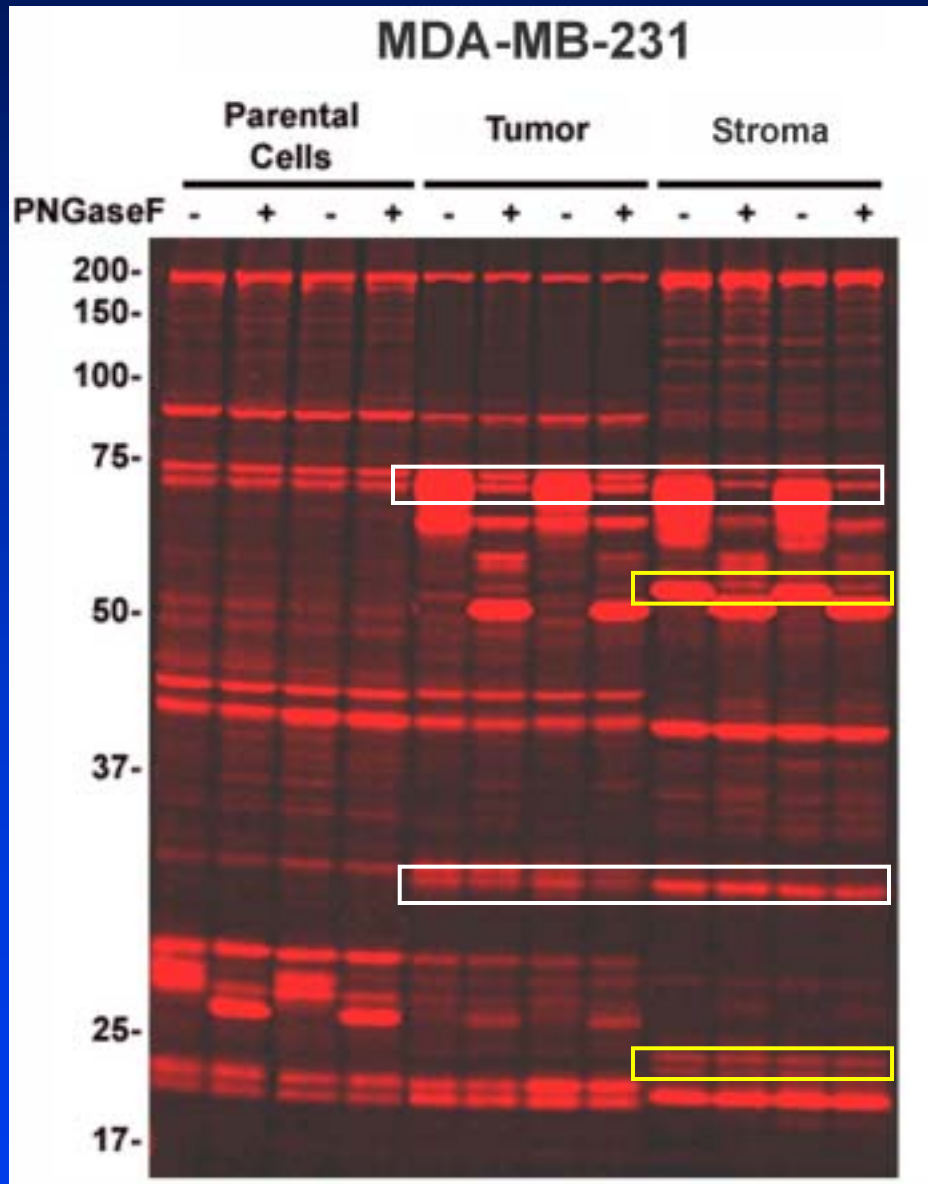
The Membrane-Associated Hydrolase KIAA1363 Is Highly Upregulated in Primary Breast Tumors



Validating Diagnostic Markers - Immunohistochemical Approaches to Breast Cancer



Activity-Based Profiles of *In Vivo* Models of Human Breast Cancer



- stromal enzymes *present* in tumor
- stromal enzymes *absent* in tumor

Acknowledgements

- Greg Adam
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- Nadim Jessani
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