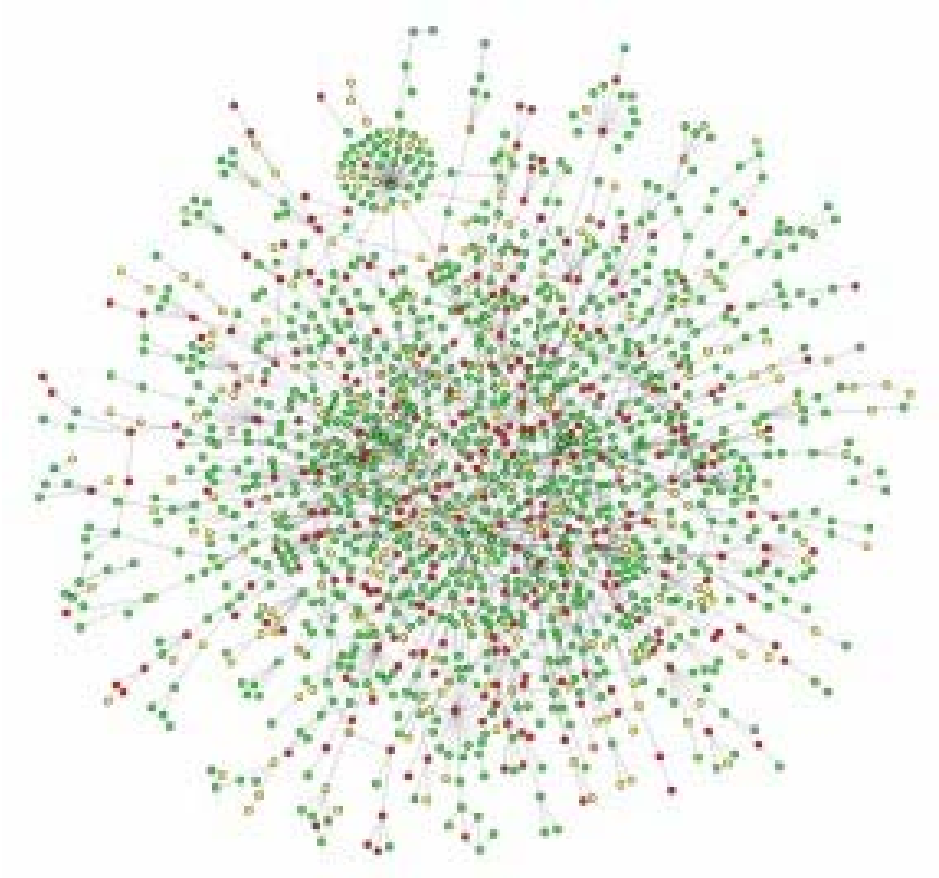
Determining Protein:Protein Interactions in Biology.

Niamh Moran

RCSI

Biological systems are controlled by protein complexes that associate into dynamic protein interaction networks



The protein-protein interaction network of yeast: a few proteins interact with a large number of other proteins, while most proteins have only one or two links.

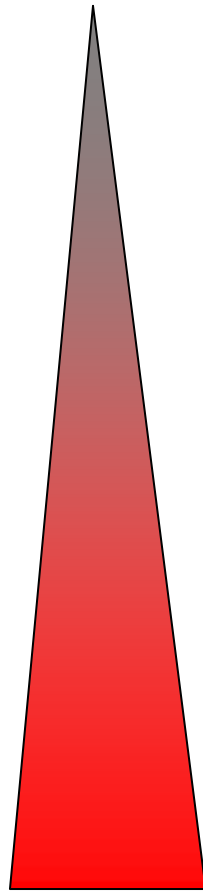
(from H. Jeong et al Nature 411, 41 (2001)).

Protein:Protein interactions

- to understand how proteins interact with each other in a dynamic cell system:
  - Any modification can alter the ability of a protein to interact with another
- Need to eliminate false positives
  - a typical experiment showing protein:protein interactions must be confirmed by at least two independent means

Types of question

- Do two proteins, X and Y, interact with each other?
  - Eg talin and integrin or Calreticulin and Integrin
- What proteins interact with ***my*** protein of interest?
  - eg Platelet Integrin αIIbβ3
- Can I develop a universal protein interaction discovery system?



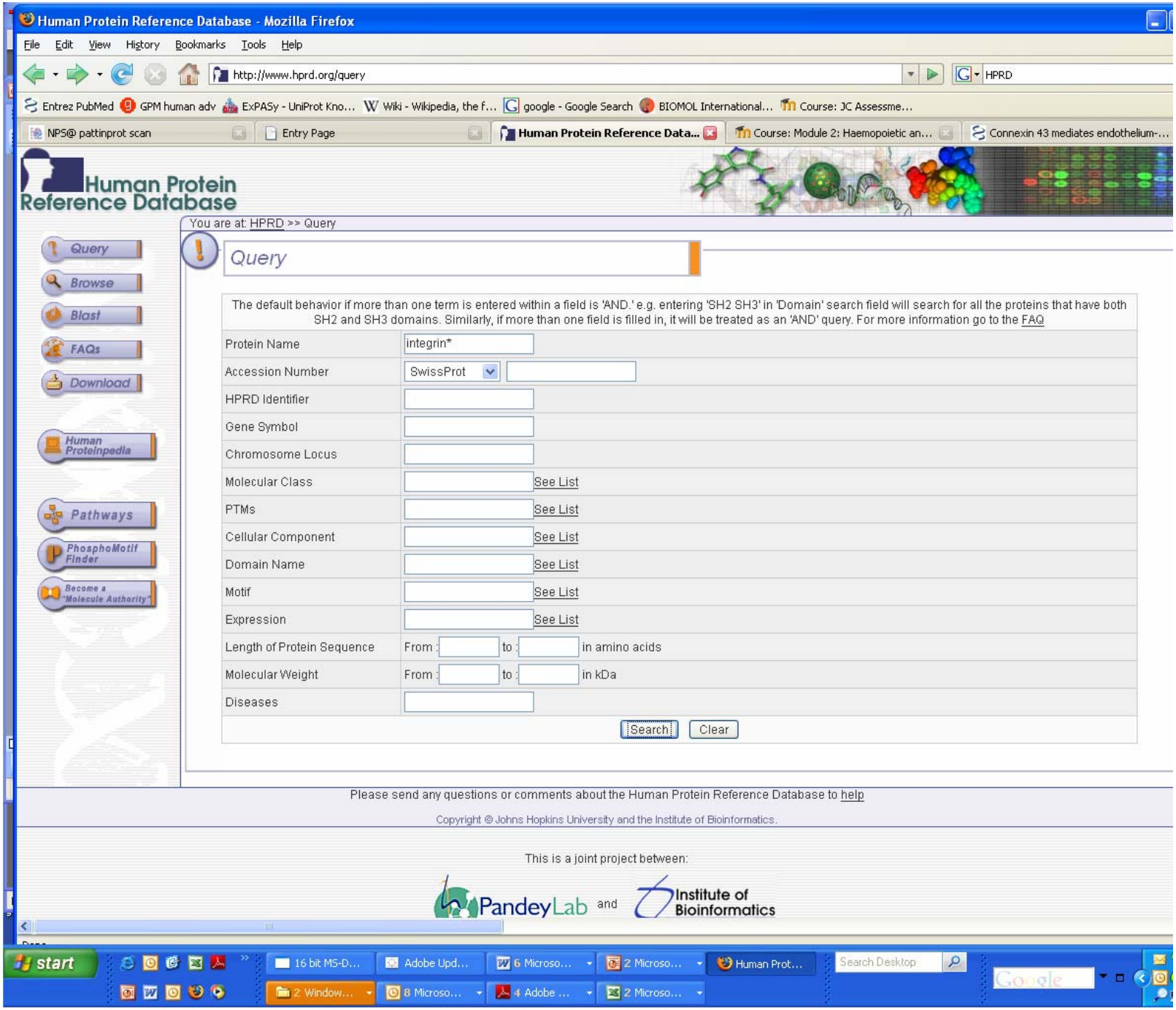
Types of Answers

- **Databases of protein:protein interactions**
  - http://www.himap.org/
  - **http://www.hprd.org/**
  - http://string.embl.de/
- **Experimental evidence**

– Affinity Chromatography

– Co-Immunoprecipitation
  - Far-Western Blot

– Confocal microscopy with labelled antibodies
  - FRET
  - Many related imaging techniques


Human Protein Reference Database - Mozilla Firefox
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http://www.hprd.org/query
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Human Protein Reference Database
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Query
Browse
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FAQs
Download
Human ProteinPedia
Pathways
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Become a "Molecule Authority"
Query
The default behavior if more than one term is entered within a field is 'AND.' e.g. entering 'SH2 SH3' in 'Domain' search field will search for all the proteins that have both SH2 and SH3 domains. Similarly, if more than one field is filled in, it will be treated as an 'AND' query. For more information go to the FAQ
Protein Name integrin*
Accession Number SwissProt
HPRD Identifier
Gene Symbol
Chromosome Locus
Molecular Class See List
PTMs See List
Cellular Component See List
Domain Name See List
Motif See List
Expression See List
Length of Protein Sequence From : to : in amino acids
Molecular Weight From : to : in kDa
Diseases
Search Clear
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This is a joint project between:
PandeyLab and Institute of Bioinformatics
start
16 bit MS-D... Adobe Upd... 6 Microso... 2 Microso... Human Prot...
2 Window... 8 Microso... 4 Adobe ... 2 Microso...
Search Desktop
Google

Integrin alpha 2B

Molecular Class	Cell surface receptor
Molecular Function	Receptor activity
Biological Process	Cell communication ; Signal transduction

ALTERNATE NAMES | DISEASES | PTMs & SUBSTRATES
SUMMARY | SEQUENCE | INTERACTIONS | EXTERNAL LINKS

General

Gene Symbol:	ITGA2B	Molecular Weight (Da) :	113377	Gene Map Locus:	17q21.32

Localization

Primary	Plasma membrane GO	Alternate	

Human Proteinpedia

Localization
Cytoplasm GO Cytoskeleton GO Nucleus GO

Domains and Motifs

Expression

Protein Interactions

PROTEIN INTERACTORS

Name of Interactor	Experiment Type	Type
Ancient ubiquitous protein 1	In Vivo ; In Vitro ; Yeast 2 Hybrid	Direct
CD36	In Vivo	Direct
Calcium and integrin binding protein	In Vitro ; Yeast 2 Hybrid	Direct
Calreticulin	In Vivo	Direct
Collagen, type I, alpha 2	In Vivo	Direct
Collagen, type II, alpha 1	In Vitro	Direct
Fibrinogen, alpha chain	In Vitro	Direct
Grb2	In Vivo ; In Vitro	Direct
Integrin beta 3	In Vivo ; In Vitro	Direct
Prothrombin	In Vitro	Direct
Von Willebrand factor	In Vivo ; In Vitro	Direct
CLNS1A	In Vivo ; In Vitro	Direct
Integrin beta 3 Junction adhesion molecule 1	In Vivo	Complex
Integrin beta 3 Talin	In Vitro	Complex
Integrin beta 3 Transglutaminase 2	In Vivo ; In Vitro	Complex
Integrin beta 3 CD47	In Vivo ; In Vitro	Complex
Integrin beta 3 Calcium and integrin binding protein	In Vitro	Complex
Cathepsin G Integrin beta 3	In Vitro	Complex

http://string.embl.de/

String: functional protein a...

Home · Download · Help/Info STRING

STRING - Search Tool for the Retrieval of Interacting Genes/Proteins

Enter your gene/protein of interest ...

identifier:

ITGA2b

e.g. 'trpB', 'ANP1_YEAST', ...
you may also upload a list

alternatively, paste an amino-acid sequence:

interactors wanted:

GO ! Reset COGs Proteins

What it does ...

STRING is a database of known and predicted protein-protein interactions.
The interactions include direct (physical) and indirect (functional) associations; they are derived from four sources:

Genomic Context	High-throughput Experiments	(Conserved) Coexpression	Previous Knowledge

STRING quantitatively integrates interaction data from these sources for a large number of organisms, and transfers information between these organisms where applicable. The database currently contains 736429 proteins in 179 species.

References / Info ...

STRING uses orthology information from the excellent COG database (Ref).
Up-to-date genomes and proteins are maintained at SWISSPROT and ENSEMBL
STRING references: von Mering et.al. 2005 / von Mering et.al. 2003 / Snel et.al. 2000.
Miscellaneous: Access Statistics, Robot Access Guide, Medusa Network Viewer, Supported Browsers.

EMBL
• BIOCOMPUTING
• BORK GROUP

Your Input:

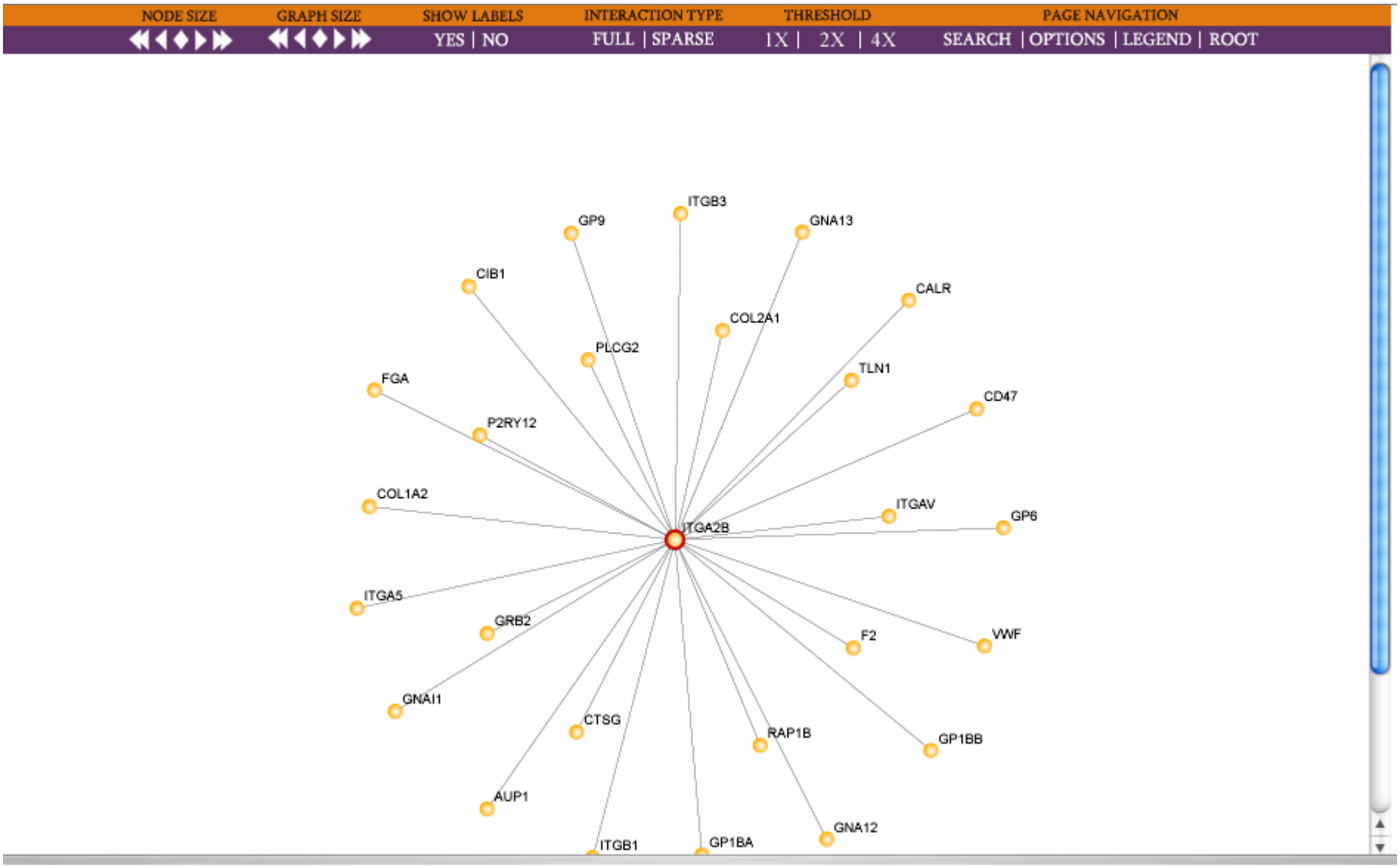
		Neighborhood	Gene Fusion	Cooccurrence	Coexpression	Experiments	Databases	Textmining	[Homology]	Score
ITGA2B	Integrin alpha-IIb precursor (Platelet membrane glycoprotein IIb) (GPa [...]									

Predicted Functional Associations:

ITGB3	Integrin beta-3 precursor (Platelet membrane glycoprotein IIIa) (GPIII [...]					●	●	●		0.999
FGA	Fibrinogen alpha/alpha-E chain precursor [Contains- Fibrinopeptide A] [...]					●	●			0.974
FGB	Fibr[...] [Contains- Fibrinopeptide B] (491 aa)						●	●		0.956
CIB1	Calcium and integrin-binding protein 1 (Calmyrin) (DNA-PKcs interactin [...]					●				0.935
CD36	Platelet glycoprotein IV (GPIV) (GPIIIB) (CD36 antigen) (PAS IV) (PAS- [...]					●		●		0.932
VWF	Von Willebrand factor precursor (vWF) [Contains- Von Willebrand antige [...]					●		●		0.924
FGG	Fibrinogen gamma chain precursor (PRO2061) (453 aa)						●			0.900
VTN	Vitronectin precursor (Serum spreading factor) (S-protein) (V75) [Cont [...]							●		0.861
TLN1	Talin 1 (2541 aa)					●		●		0.855
ITGB1	Integrin beta-1 precursor (Fibronectin receptor beta subunit) (CD29 an [...]							●		0.818
SLC4A1	Band 3 anion transport protein (Anion exchange protein 1) (AE 1) (CD23 [...]							●		0.805
GP1BA	Platelet glycoprotein Ib alpha chain precursor (Glycoprotein Ibalpha) [...]							●		0.787
GRN	Granulins precursor (Acrogranin) (Proepithelin) (PEPI) [Contains- Para [...]							●		0.766
CTSG	Cathepsin G precursor (EC 3.4.21.20) (CG) (255 aa)					●				0.747
CD47	Leukocyte surface antigen CD47 precursor (Antigenic surface determinan [...]					●				0.747
AUP1	Ancient ubiquitous protein 1 precursor (474 aa)					●				0.747
COL2A1	Collagen alpha 1(II) chain precursor [Contains- Chondrocalcin] (1418 a [...]					●				0.747
F2	Prothrombin precursor (EC 3.4.21.5) (Coagulation factor II) (625 aa)					●				0.747
CALR	Calreticulin precursor (CRP55) (Calregulin) (HACBP) (ERp60) (417 aa)					●				0.747
GRB2	Growth factor receptor-bound protein 2 (GRB2 adapter protein) (SH2/SH3 [...]					●				0.747
CD33	Myeloid cell surface antigen CD33 precursor (gp67) (Siglec-3) (364 aa)							●		0.744
GYPA	Glycophorin A precursor (PAS-2) (Sialoglycoprotein alpha) (MN sialogly [...]							●		0.742
HOXB6	Homeobox protein Hox-B6 (Hox-2B) (Hox-2.2) (HU-2) (224 aa)							●		0.738
ICAM2	Intercellular adhesion molecule-2 precursor (ICAM-2) (CD102 antigen) ([...]							●		0.679
CD34	Hematopoietic progenitor cell antigen CD34 precursor (324 aa)							●		0.662

ITGB3; click to see sequence

Views: Neighborhood | Fusion | Occurrence | Coexpression | Experiments | Databases | Textmining | Summary Network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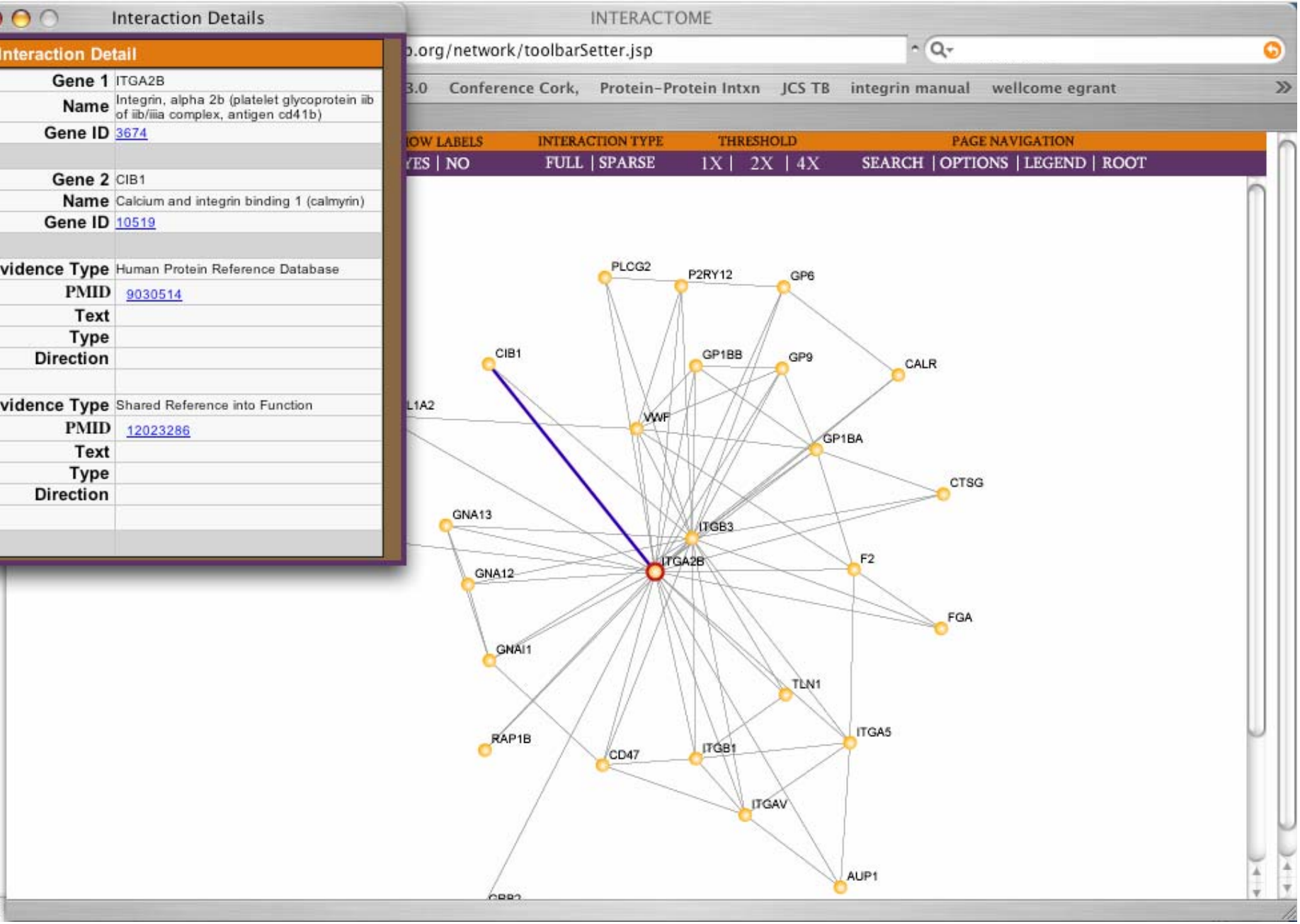
GRAPH SIZE
SHOW LABELS
YES | NO
INTERACTION TYPE
FULL | SPARSE
THRESHOLD
1X | 2X | 4X
PAGE NAVIGATION
SEARCH | OPTIONS | LEGEND | ROOT
ITGB3
GP9
GNA13
CIB1
CALR
COL2A1
PLCG2
TLN1
FGA
CD47
P2RY12
COL1A2
ITGAV
GP6
ITGA2B
ITGA5
GRB2
F2
VWF
GNAI1
CTSG
RAP1B
GP1BB
AUP1
GNA12
ITGB1
GP1BA


Interaction Details
INTERACTOME
Interaction Detail
Gene 1 ITGA2B
Name Integrin, alpha 2b (platelet glycoprotein iib of iib/iiia complex, antigen cd41b)
Gene ID 3674
Gene 2 CIB1
Name Calcium and integrin binding 1 (calmyrin)
Gene ID 10519
vidence Type Human Protein Reference Database
PMID 9030514
Text
Type
Direction
vidence Type Shared Reference into Function
PMID 12023286
Text
Type
Direction
.org/network/toolbarSetter.jsp
3.0 Conference Cork, Protein-Protein Intxn JCS TB integrin manual wellcome egrant
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/ES | NO FULL | SPARSE 1X | 2X | 4X SEARCH | OPTIONS | LEGEND | ROOT
PLCG2
P2RY12
GP6
CIB1
GP1BB
GP9
CALR
L1A2
VWF
GP1BA
CTSG
GNA13
ITGB3
ITGA2B
F2
GNA12
FGA
GNAI1
TLN1
ITGA5
RAP1B
CD47
ITGB1
ITGAV
AUP1

Caution!

- Some predictions are incorrect
- Not all protein : protein interactions occur in all tissues/ orgaisms
- Databases are still incomplete

Verifying protein:protein interactions in specific cells

- Affinity Chromatography
- Co-Immunoprecipitation
  - Far-Western Blot
- Confocal microscopy with labelled antibodies
  - FRET
  - Many related imaging techniques

Affinity Chromatography

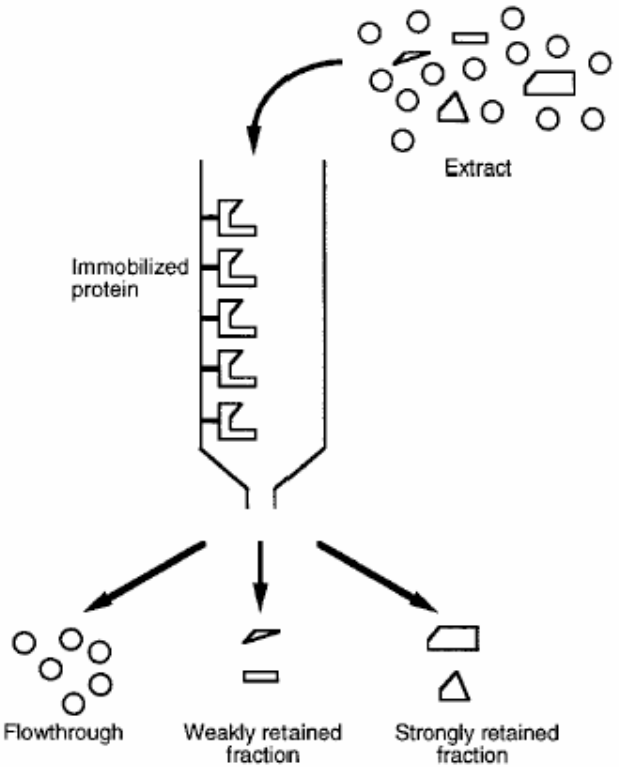
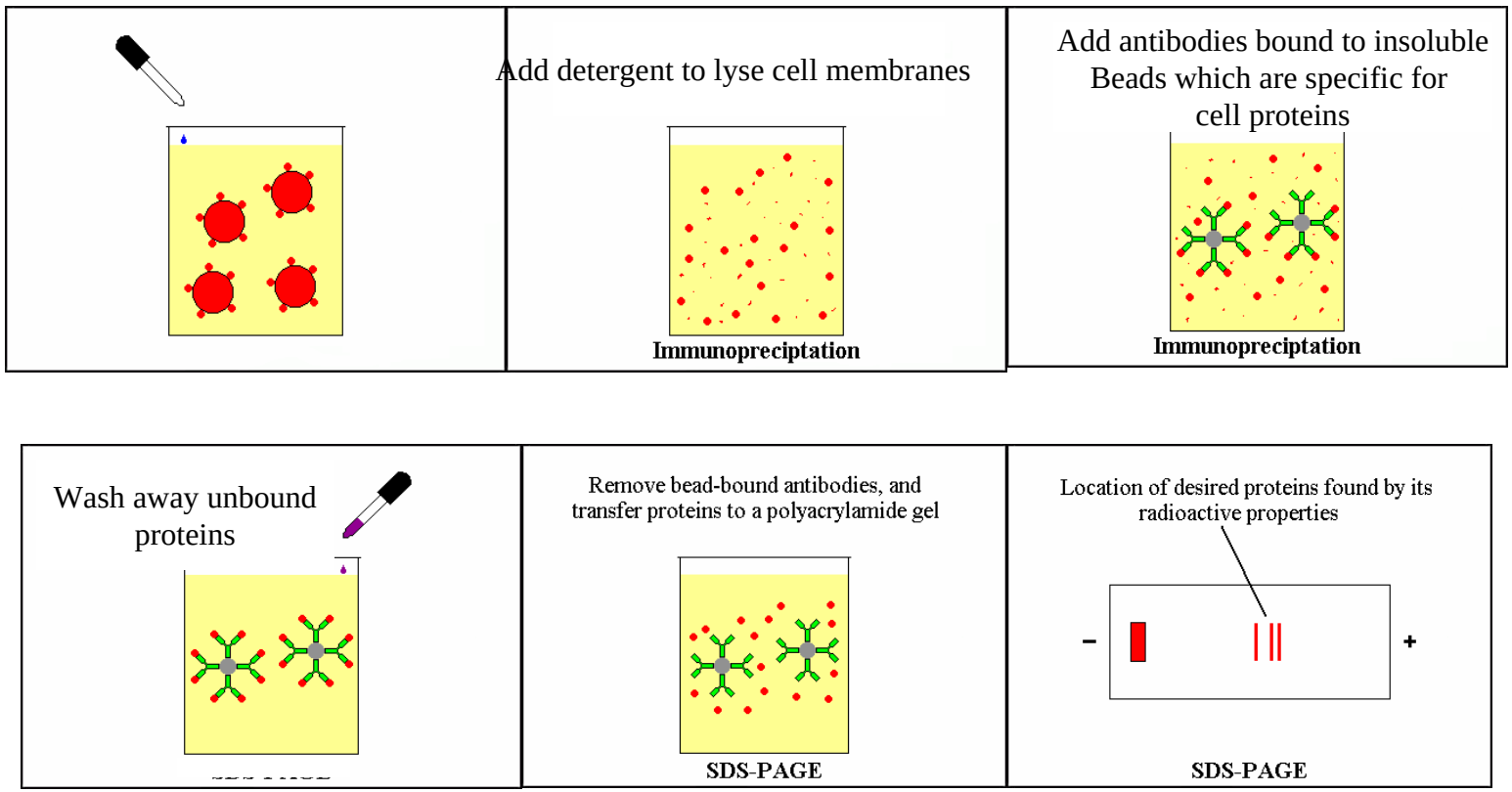


FIG. 1. Protein affinity chromatography. Extract proteins are passed over a column containing immobilized protein. Proteins that do not bind flow through the column, and ligand proteins that bind are retained. Strongly retained proteins have more contacts with the immobilized protein than do those that are weakly retained.

Immunoprecipitation

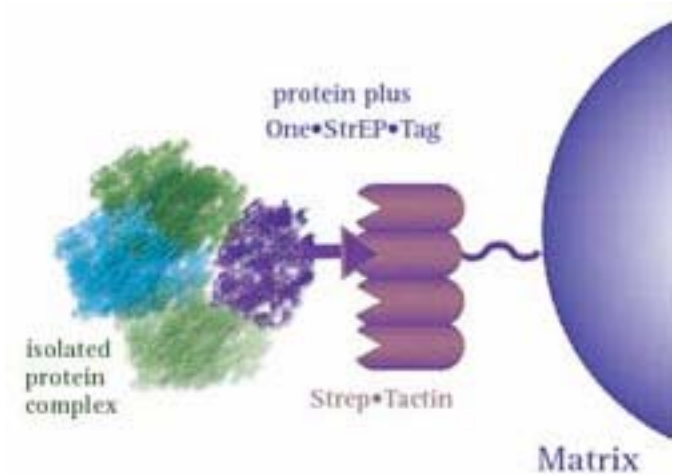


Co-immunoprecipitation

- Differs from immuno-precipitation in that the conditions of cell lysis and antibody interaction favour the co-association of macromolecular complexes.
- Thus, a protein immunoprecipitated by its specific antibody will co-precipitate its functionally associated proteins

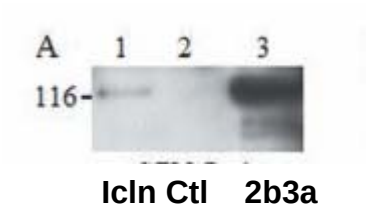
Co-Immunoprecipitation

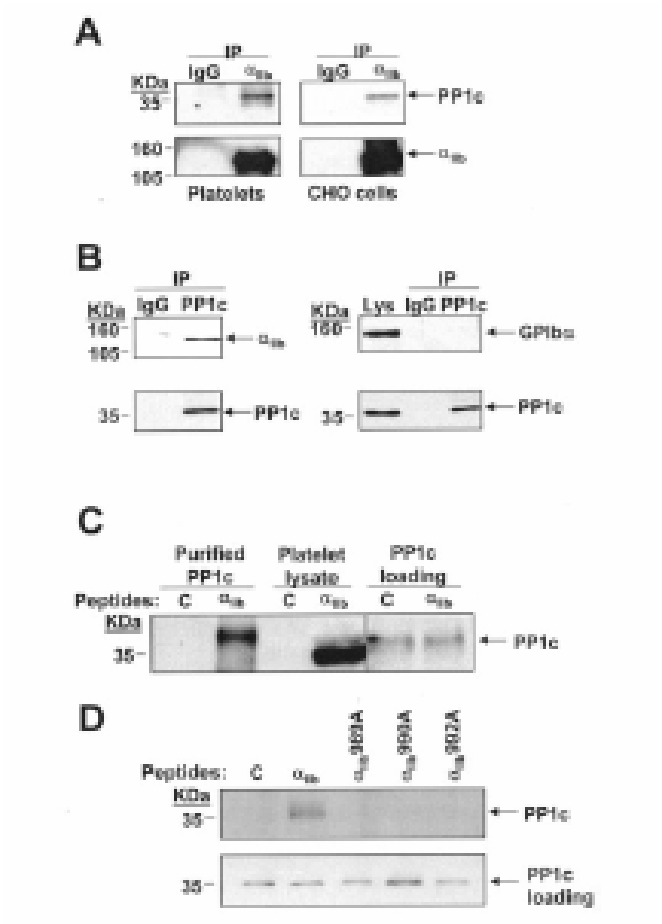
- immunoprecipitation
- Tagged protein pull-down
- Peptide pull-down



Types of Answers

- 2 Proteins, X and Y:
  - Co-Immunoprecipitation of 2 proteins with a known/suspect interaction

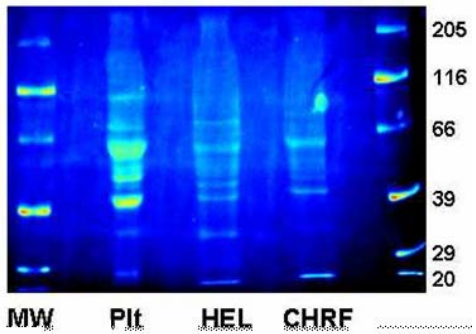




Larkin et al, Jbiol Chem Vol. 279, No. 32, Issue of August 6, pp. 33039–33042, 2004
Vijayen et al, Jbiol Chem Vol. 279, No. 32, Issue of August 6, pp. 33039–33039, 2004

Interaction of 2 known proteins X and Y

- Protein Overlay (Far Western Blot)
  - Run cell lysate containing protein X on SDS gel;
  - probe with tagged peptide/protein Y (or protein Y followed by antibody detection)



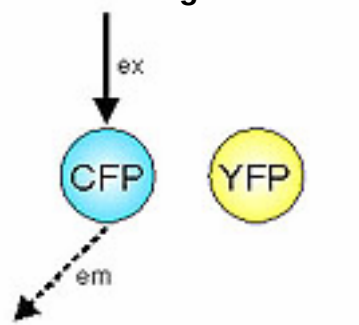
- FRET- Fluorescent resonance energy transfer
  - Imaging technique to visualize protein interactions

What is FRET?

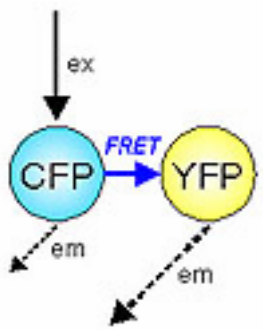
FRET is the non-radiative transfer of photon energy from an excited fluorophore (the donor) to another fluorophore (the acceptor) when both are located within close proximity (1-10 nm).

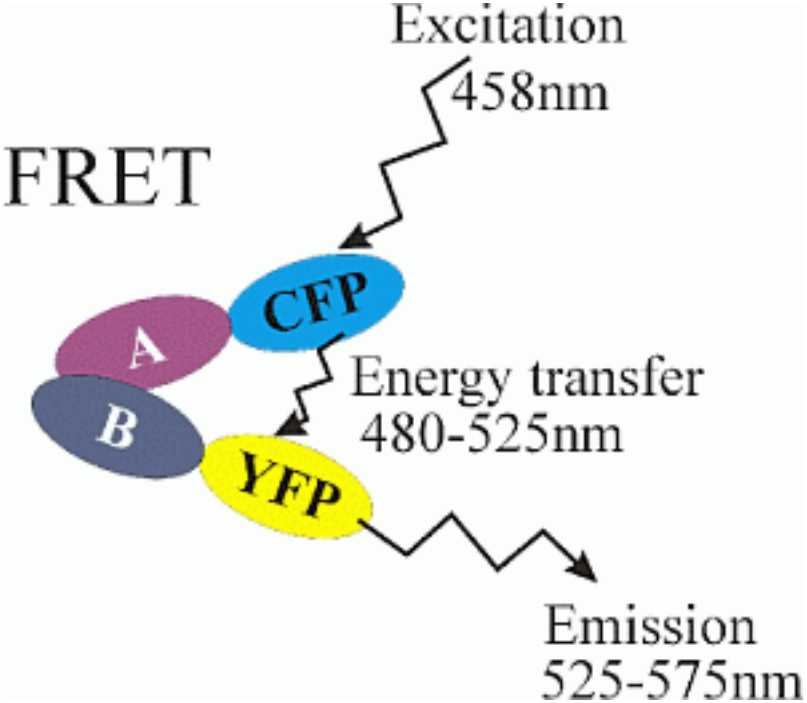
can resolve the relative proximity of molecules beyond the optical limit of a light microscope to reveal molecular interactions between two protein partners,

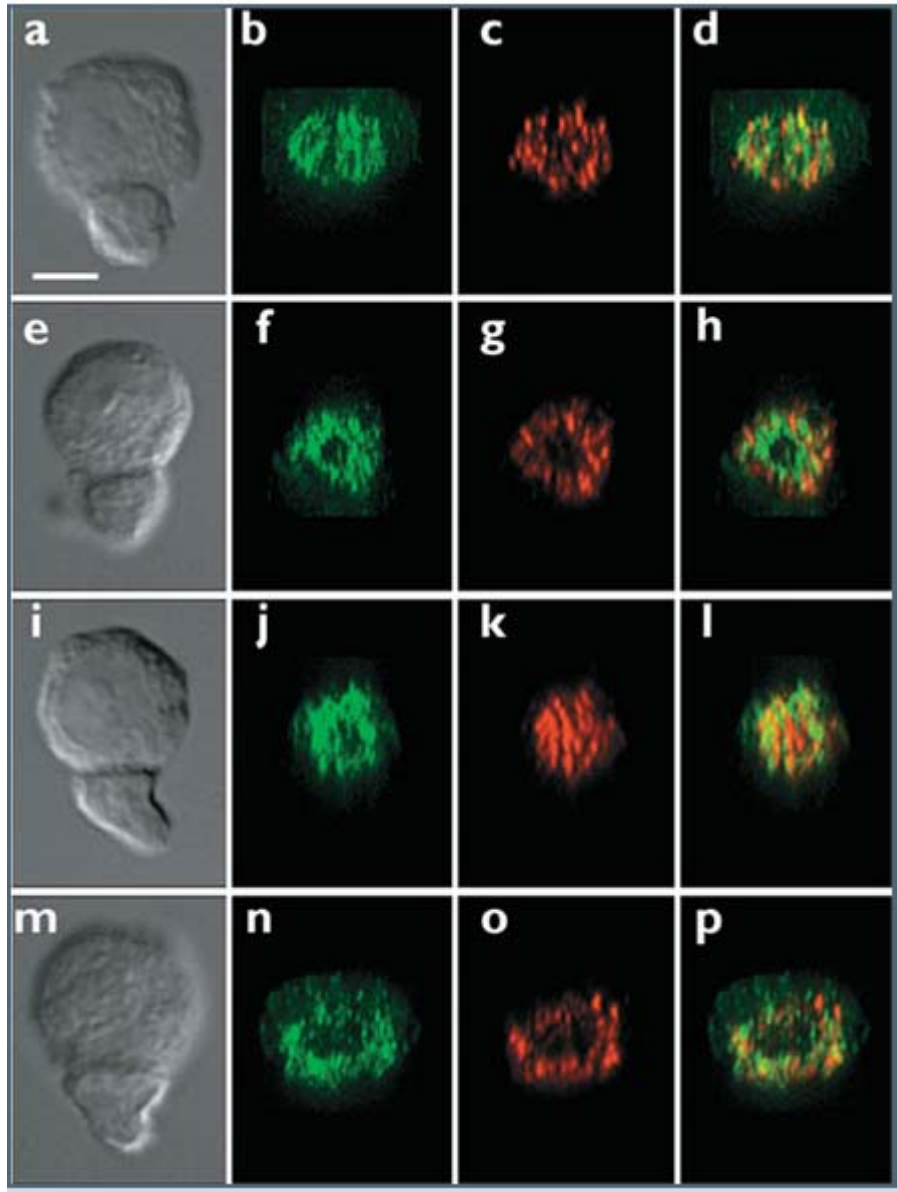
No FRET Signal



FRET Signal







- Tremuth et al. **Fluorescence Cell Biology Approach to Map the Second Integrin-binding Site of Talin to a 130-Amino Acid Sequence within the Rod Domain** J. Biol. Chem., Vol. 279,(21), 22258-22266, May 21, 2004

Seven Images Required for FRET Data Process

Symbol	Fluorophone or Sample	Excitation Filter Excitation Wavelength	Emission Filter Emission Wavelength	Meaning
a	Donor Only	Donor	Donor	Signal from a donor only specimen using donor excitation and donor emission filter set.
b	Donor Only	Donor	Acceptor	Signal from a donor only specimen using donor excitation and acceptor emission filter set.
c	Acceptor Only	Donor	Acceptor	Signal from an acceptor only specimen using donor excitation and acceptor emission filter set.
d	Acceptor Only	Acceptor	Acceptor	Signal from an acceptor only specimen using acceptor excitation and acceptor emission filter set.
e	Donor and Acceptor	Donor	Donor	Signal from donor-and-acceptor specimen using donor excitation and donor emission filter set.
f	Donor and Acceptor	Donor	Acceptor	Signal from donor-and-acceptor specimen using donor excitation and acceptor emission filter set
g	Donor and Acceptor	Acceptor	Acceptor	Signal from donor-and-acceptor specimen using acceptor excitation and acceptor emission filter set.

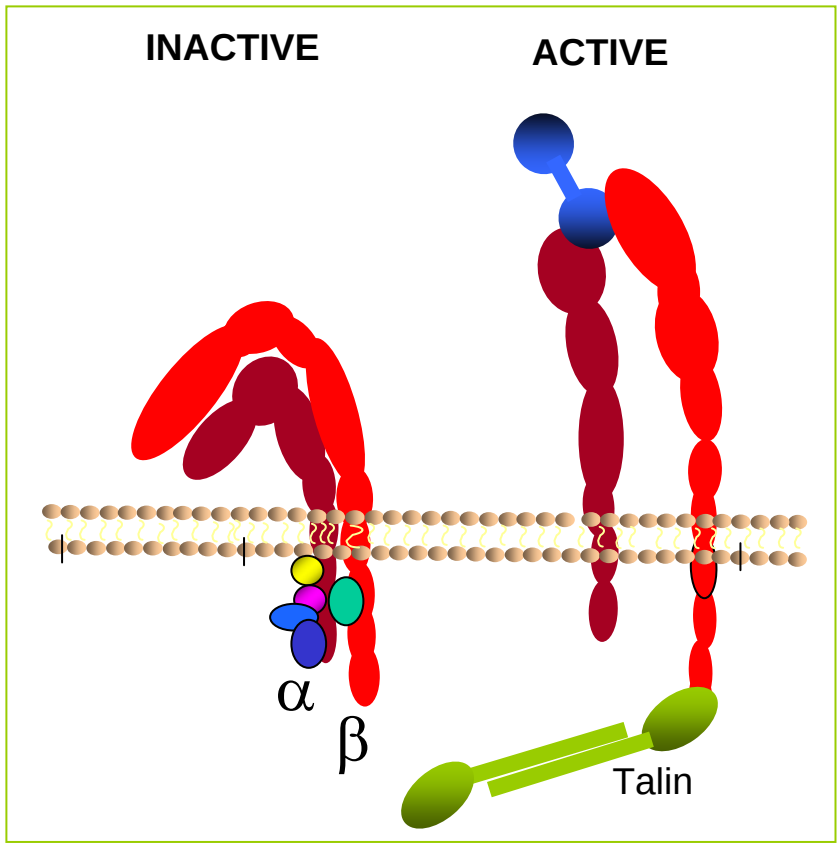
Caution!

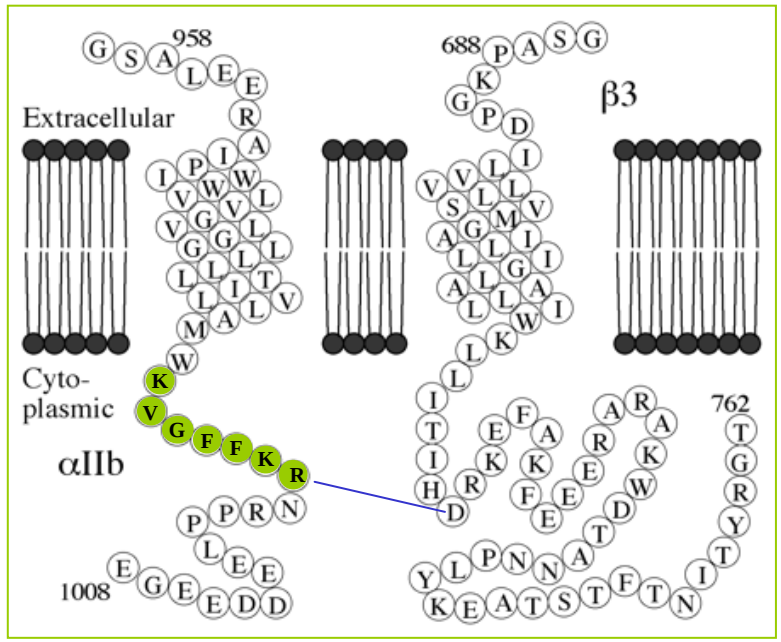
- Many verification techniques need specific antibodies for each interaction partner
  - Not always available
- False positives and false negatives can occur
  - Need for good controls

Types of question

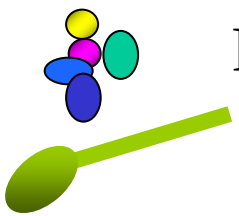
- Do two proteins, X and Y, interact with each other?
  - Eg talin and integrin or Calreticulin and Integrin
- What proteins interact with ***my*** protein of interest?
  - eg Platelet Integrin αIIbβ3
- Can I develop a universal protein interaction discovery system?

Integrin cytoplasmic tails control activation state



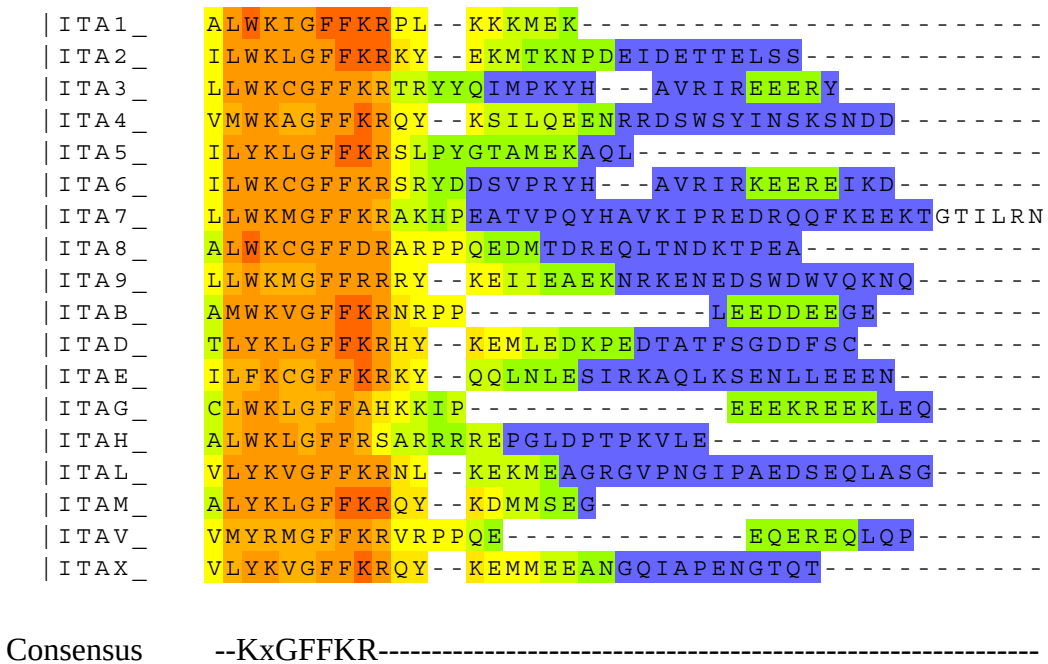


Li *et al*, 2001



Potential integrin binding proteins

Conservation of KxGFFKR Motif in all human alpha integrins (X=VIALCM)

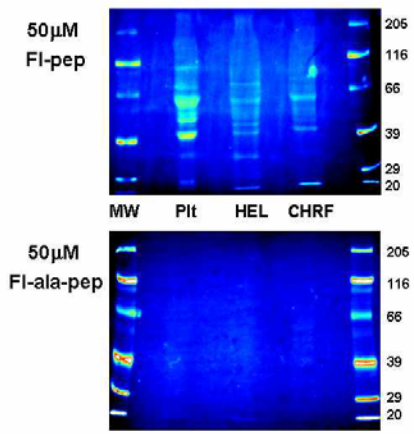


Current hypothesis

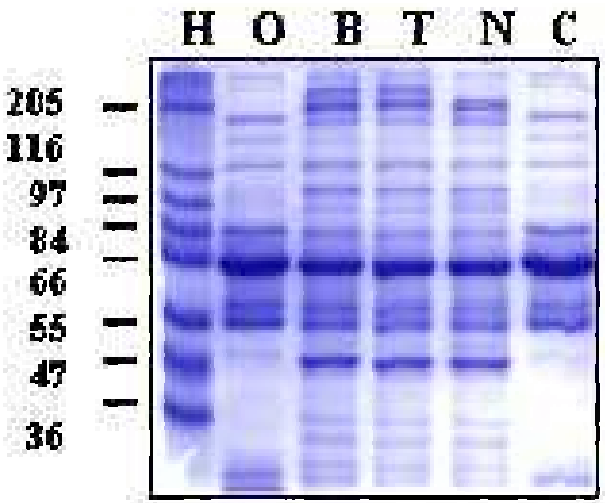
- KVGFFKR sequence co-ordinates signalling cascade that results in cytoskeletal rearrangement
- Regulated by dynamic interactions with cellular signalling proteins

Question: What is nature of the integrin binding protein(s) in the platelet?

- Strategies:
  - Literature review to identify potential integrin-associated proteins / Interactome web sites
  - Immunoprecipitate platelet integrin and hope that co-associated proteins will also precipitate. Identify by Western Blot/ Mass-Spec
  - Peptide-pull-down experiment with Biotin-KVGFFKR (+ control) followed by Western Blot/ Mass-Spec identification
  - Use FITC-tagged KVGFFKR peptide to identify platelet Proteins
    - On western blot->
    - On protein array

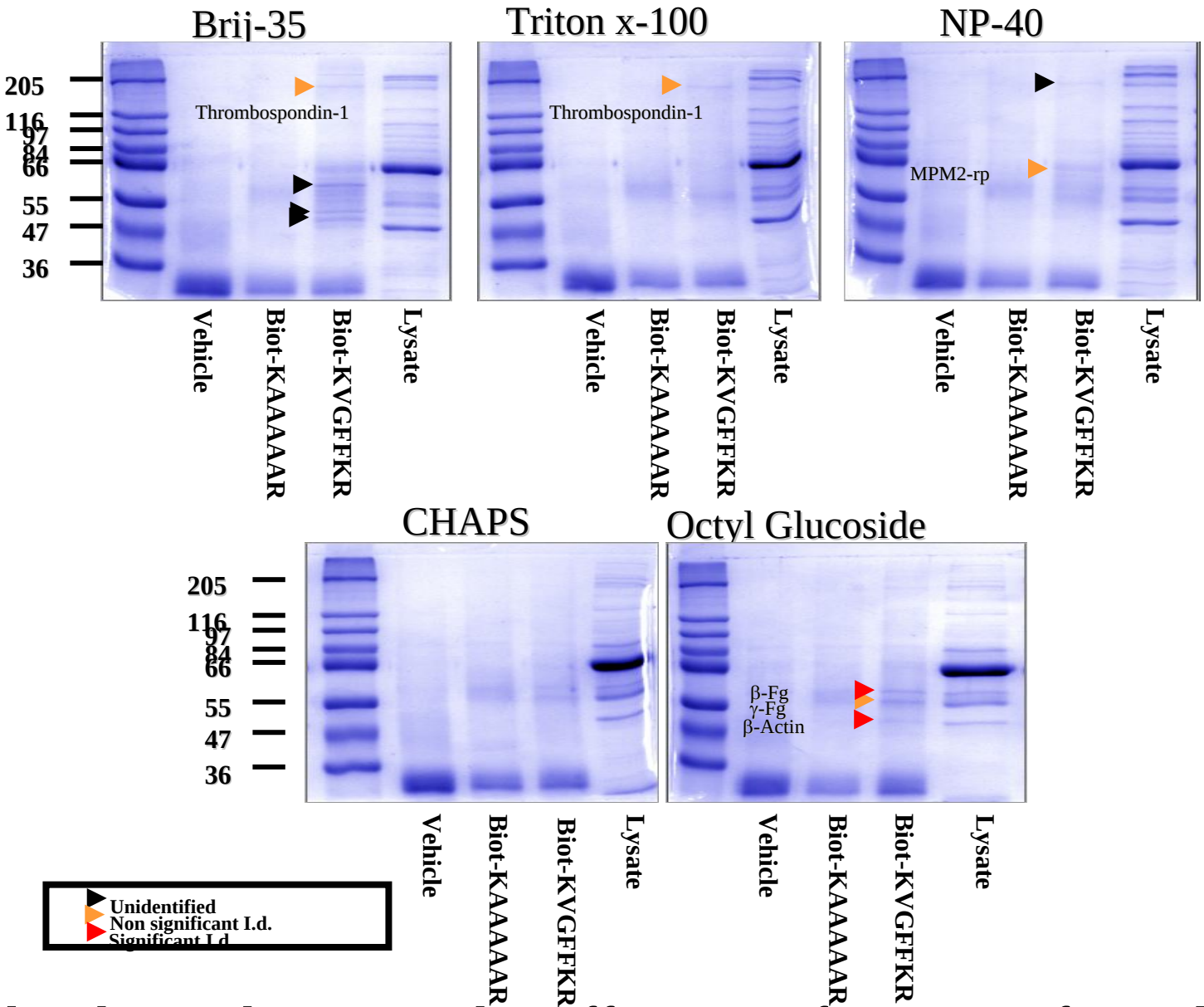


Which Detergent?

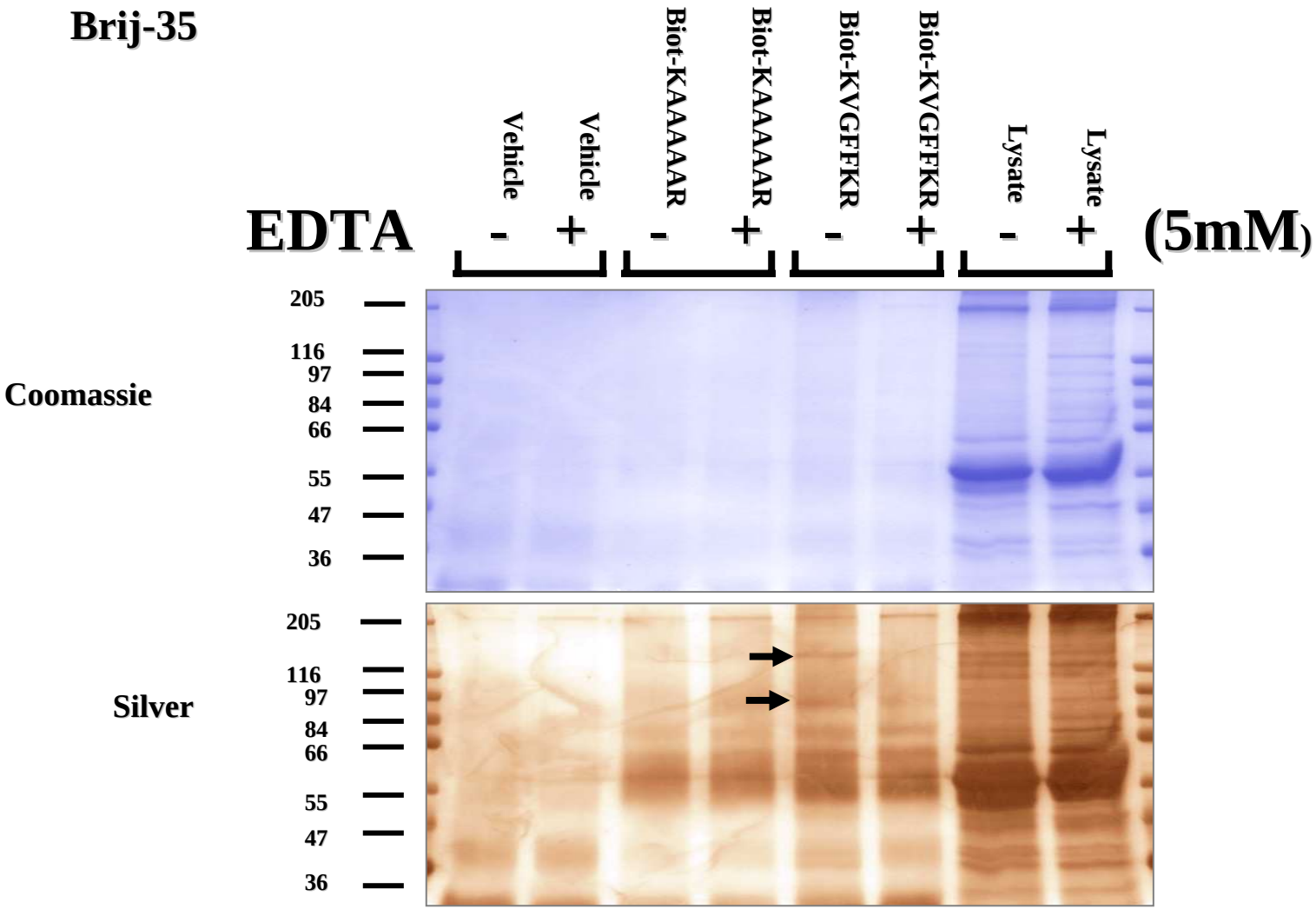


B = Brij-35
T = TritonX100
N = NP-40
C = CHAPS
O = Octyl Glucoside
- = control samples
L = Lysate +ve control
H = Markers

Platelets are lysed in Buffers containing 1% of the listed detergents. Solubilized proteins are separated on SDS PAGE and coomassie Stained.



Biotinylated peptides are used to affinity purify proteins from platelet Lysates.



Stringent washing was necessary to remove non-specific binding. Silver staining could still detect specifically-bound proteins.

Protein Arrays

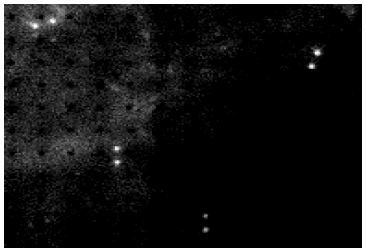
QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

- **RZPD Deutsches Ressourcenzentrum für Genomforschung GmbH**
- http://www.rzpd.de/products/proteinarrays/
- RZPD -> world's largest collection of arrayed proteins. Each Protein Array consists of up to 37,200 proteins, which are printed in duplicate onto 22 cm x 22 cm PVDF membranes.
- Individual expression verified cDNA clones are available for in-depth follow-up experiments.
- Similar available from Abnova
- **Prof Dolores Cahill, CHP RCSI/ Conway Inst UCD**

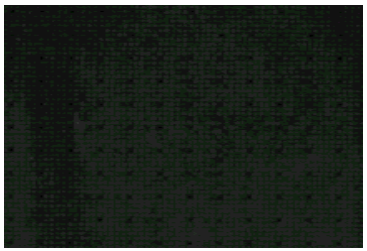
Use Tagged KVGFFKR To Identify Integrin Binding Proteins From Protein Arrays

- Synthesized Biotin KVGFFKR and control peptide Biotin KAAAAAR
- Optimized binding parameters on ‘known’ integrin binding partners (peptide concentration; time; blocking conditions; temperature)
- Tested for biotin-peptide binding to Protein arrays versus Biotin-control peptide.

A

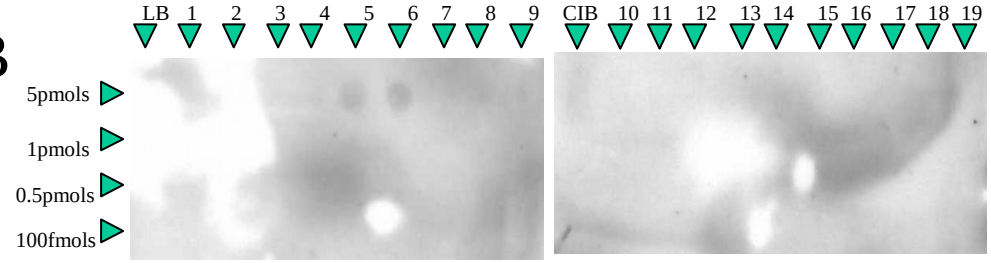


100µm Biot - KVGFFKR

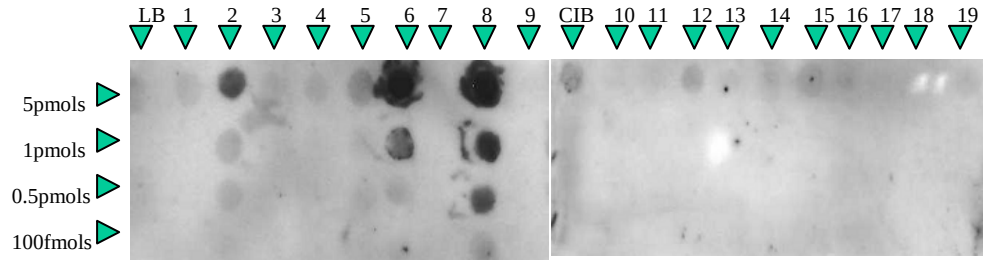


100µm Biot - KAAAAAR

B



100µm Biot - KAAAAAR



100µm Biot - KVGFFKR

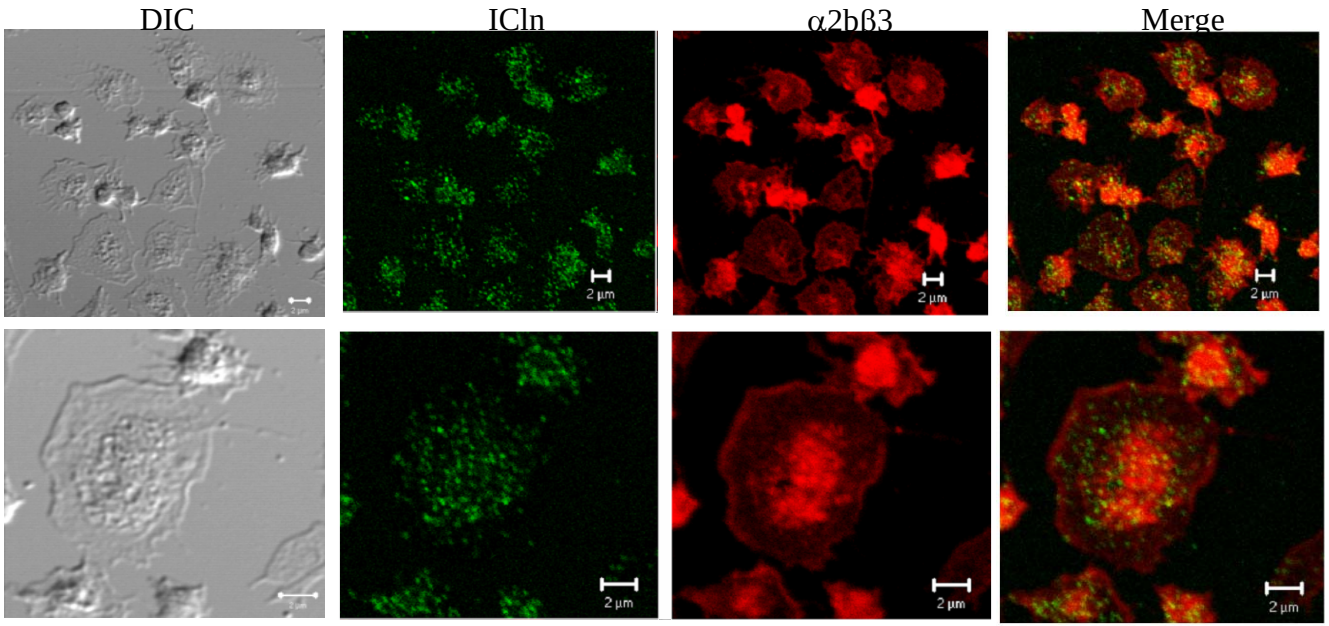
Figure 1

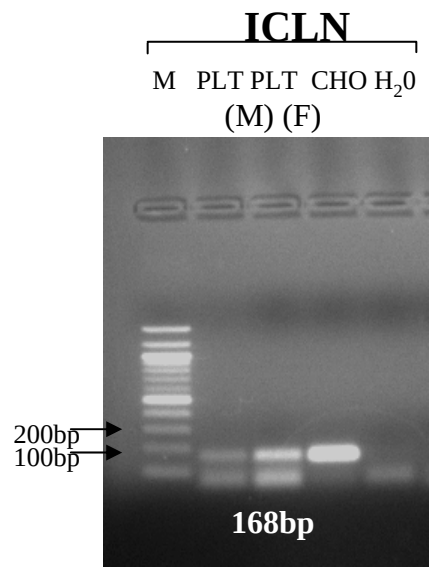
C

No.	Name	No.	Name
1.	Developmentally regulated GTB-binding protein 1	11.	Methyl-CpGbinding domain 3 (MBD3)
2.	Chloride channel (CLNS1A), I_{Cln}	12.	Myosin light chain 1 slow a (MLC1sa)
3.	Ras-related GTP-binding protein	13.	Myosin light chain 1 slow a (MLC1sa)
4.	Ribosomal Protein S5	14.	HMG domain protein HMGX1
5.	Ferritin heavy polypeptide 1	15.	Ribosomal protein L9
6.	Hypothetical Protein MGC: 3402, HSPC238	16.	Pyruvate carboxylase
7.	Ferritin heavy polypeptide 1	17.	Ribosomal Protein L9
8.	Chloride channel (CLNS1A), I_{Cln}	18.	Ferritin heavy polypeptide 1
9.	Mitochondrial Carrier Protein CGI-69	19.	Ferritin heavy polypeptide 1
10.	ADP-ribosylationfactor 1 (ARF-1)		

ICln Protein is present in platelets but at low abundance

B

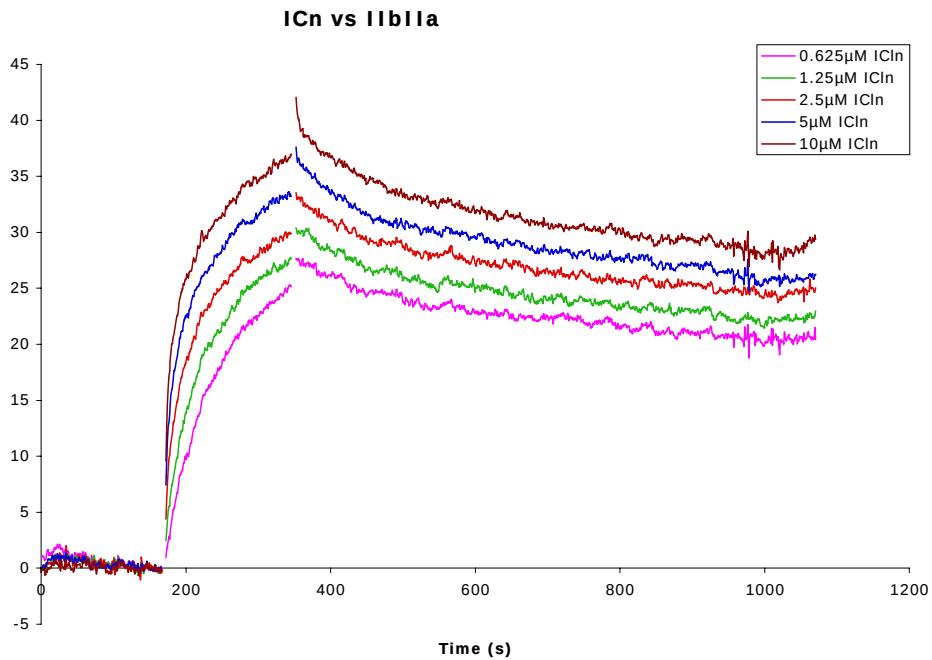


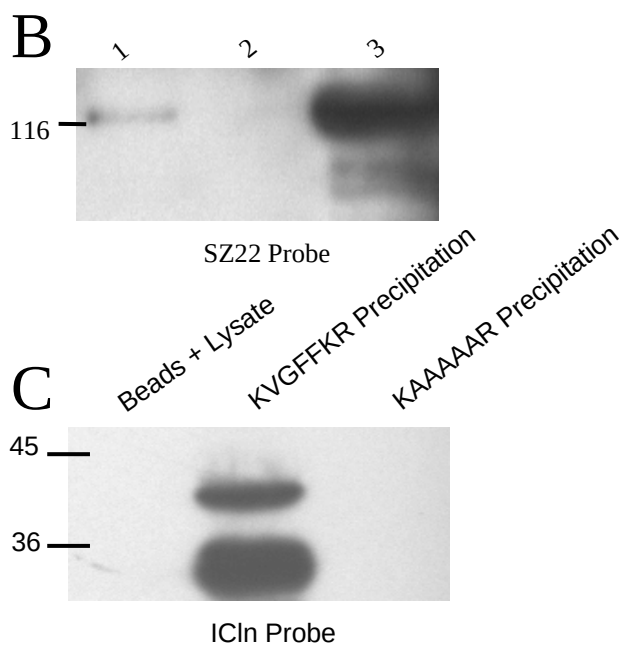


ICln co-association with the platelet integrin is confirmed by surface plasmon resonance and peptide-pull-down assays

Figure 3

Larkin et al





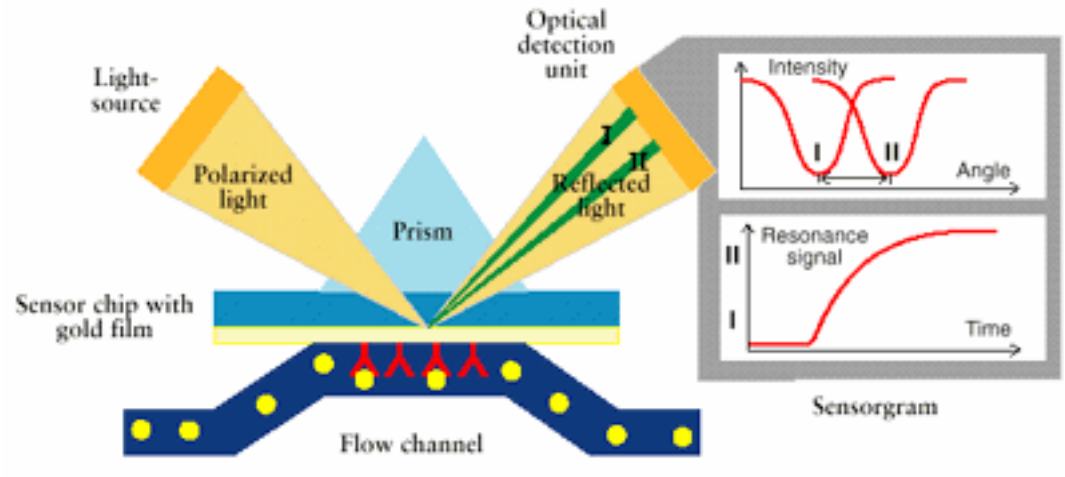
Techniques to confirm novel protein:protein interactions

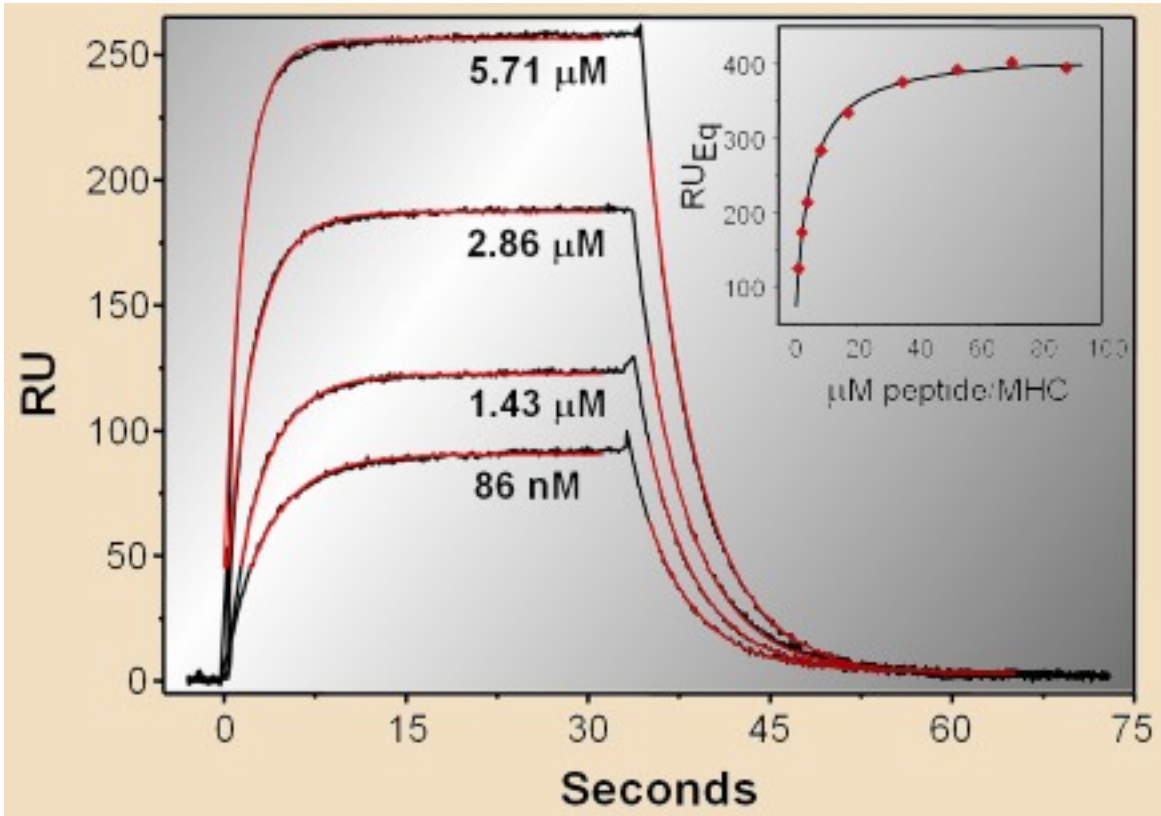
- Surface Plasmon resonance
- Isothermal Calorimetry

Biacore
(Surface Plasmon Resonance)

- a biosensing technique in which receptor-ligand interactions are detected by first immobilizing one biomolecule on one side of a metallic film.

- The refractive index of light reflecting off this surface is measured. When the immobilized biomolecules are bound by their ligands, an alteration in surface plasmons on the opposite side of the film is created that is directly proportional to the change in bound, or absorbed, mass.


Optical detection unit
Light-source
Polarized light
Prism
Reflected light
I
II
Sensor chip with gold film
Flow channel
Intensity
Angle
Resonance signal
Time
Sensorgram



Kinetic and steady-state equilibrium (inset) surface plasmon resonance (e.g. Biacore) experiment of a peptide/MHC complex binding to a T cell receptor complex. In this experiment, the receptor is immobilized on a sensor surface and the peptide/MHC is injected over it. As the peptide/MHC binds, the accumulated mass on the sensor surface increases, resulting in a change in refractive index detected by the instrument. (**Biomedical biosciences University of Notre Dame)**

Isothermal Calorimetrv

Yamniuk and Vogel

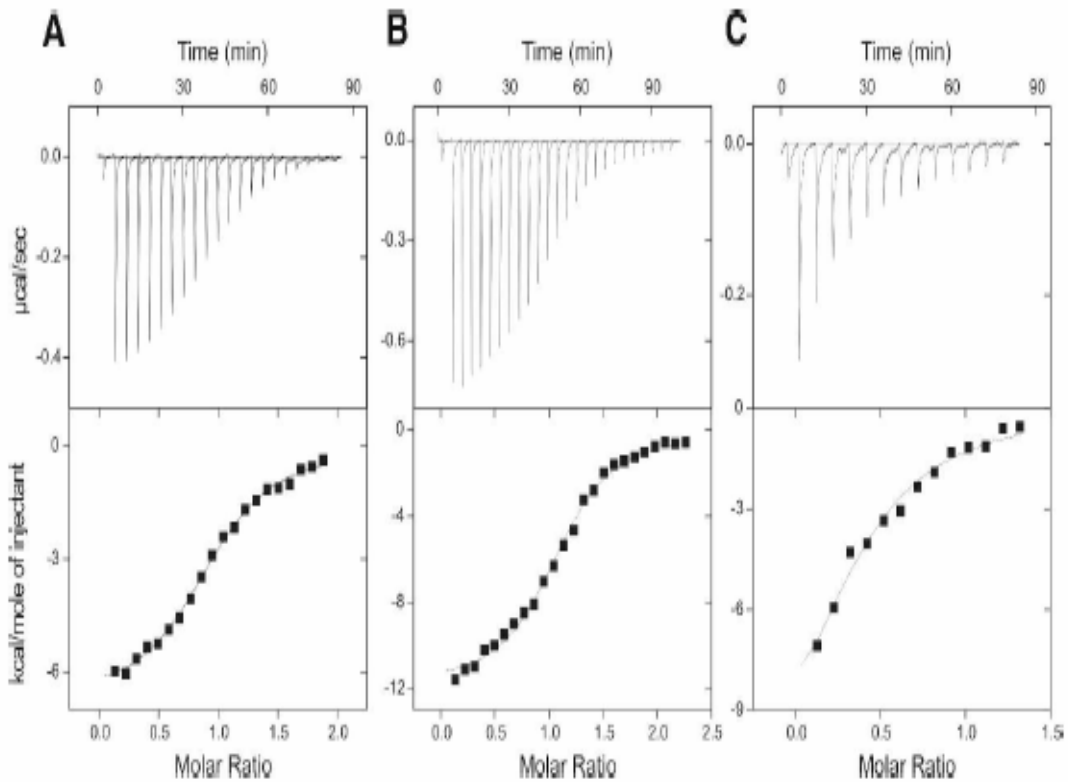


Figure 2. Representative isothermal titration calorimetry experiments for αIIb-L binding to Ca^{2+}-CIB (*A*), Mg^{2+}-CIB (*B*), and apo-CIB (*C*) in 20 mM HEPES, 100 mM KCl (pH 7.3) at 37°C. The *top* panels display the baseline corrected calorimetric titration data while the *bottom* panels display the derived binding isotherms for each experiment.

Caution!

- Need specialized equipment and trained personel
- Often available in core facilities

Types of question

- Do two proteins, X and Y, interact with each other?
  - Eg talin and integrin or Calreticulin and Integrin
- What proteins interact with ***my*** protein of interest?
  - eg Platelet Integrin αIIbβ3
- Can I develop a universal protein interaction discovery system?

Existing strategies for exploring novel protein: protein interactions on a large scale

- Protein array
- Yeast two hybrid
- Affinity chromatography (coupled to mass spectrometry)
- Tap-Tagging affinity methods (coupled to mass spectrometry)

Novel protein: protein interactions

Yeast-2-hybrid

(http://www.bioteach.ubc.ca/MolecularBiology/AYeastTwoHybridAssay/)

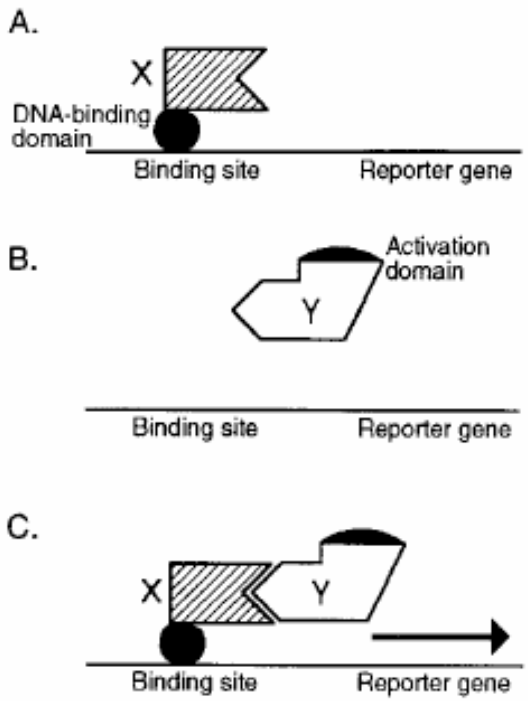
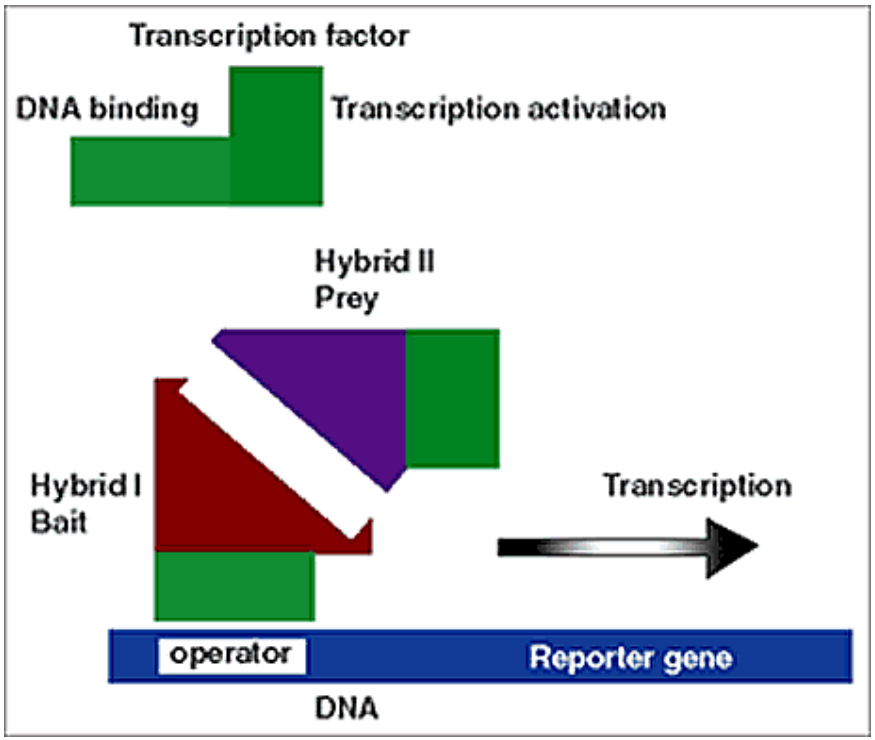


FIG. 6. The two-hybrid system. (A) The DNA-binding domain hybrid does not activate transcription if protein X does not contain an activation domain. (B) The activation domain hybrid does not activate transcription because it does not localize to the DNA-binding site. (C) Interaction between X and Y brings the activation domain into close proximity to the DNA-binding site and results in transcription.

Two hybrids are better than one

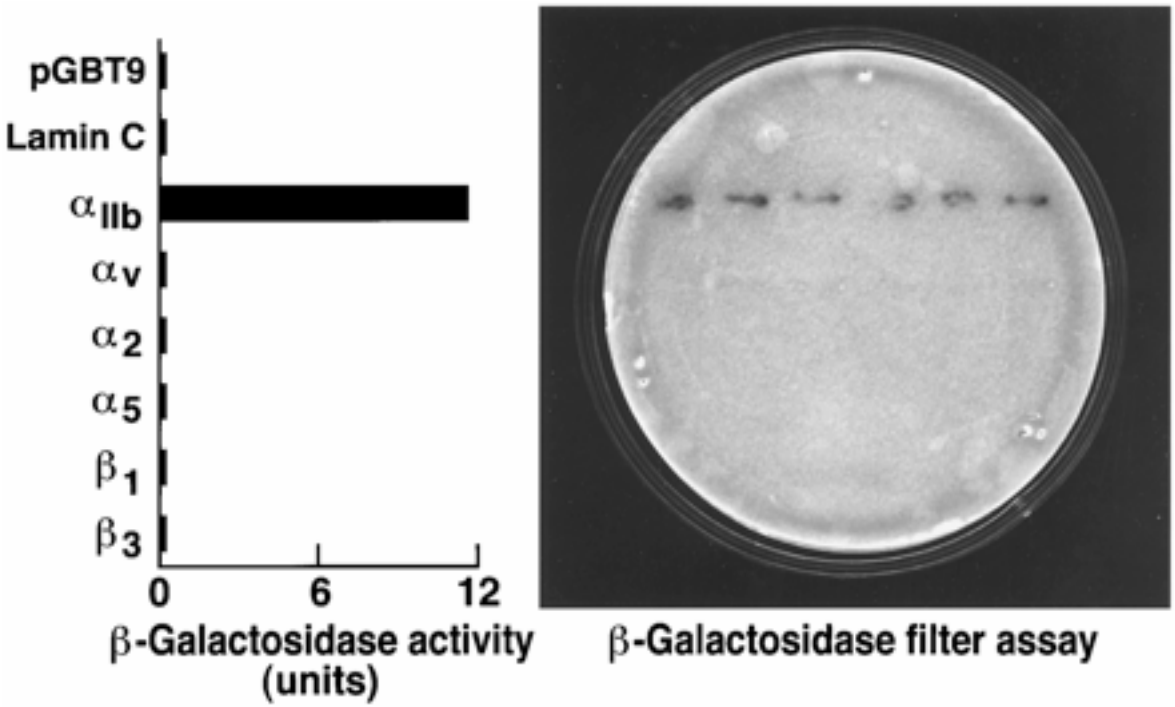
(Fields and Song, 1989) The original two-hybrid underwent many technical modifications but the original idea of reconstructing a functional factor (transcriptional, enzymatic, signal transduction) from two pieces that come close together, being in "hybrids" with proteins that interact specifically, is always present (see figure below).



If an interaction is established between the two hybrid proteins the transcription of a reporter gene is activated and one can select the cells by testing the activity of the reporter protein

Identification of a Novel Calcium-binding Protein That Interacts with the Integrin IIb Cytoplasmic Domain

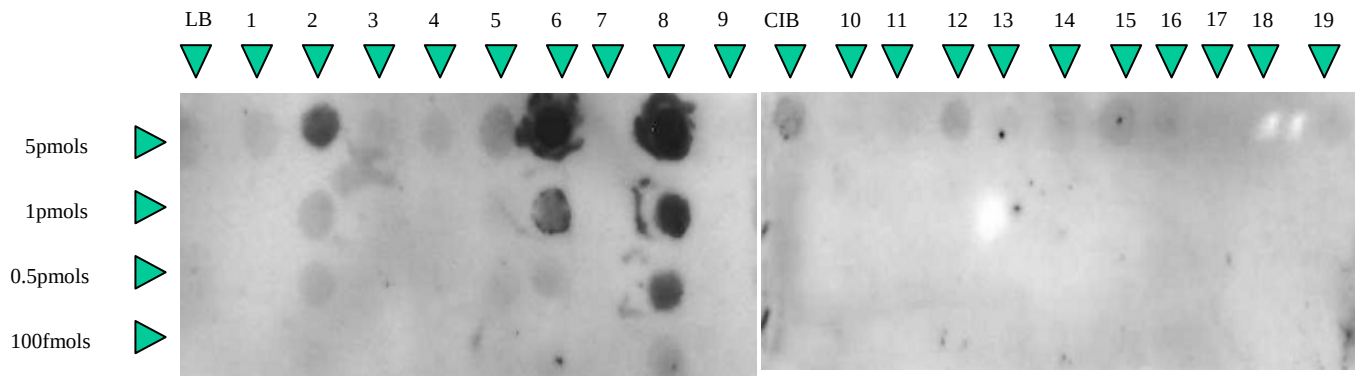
Ulhas P. Naik, UP, Patel M and PariseLV JBC Volume 272, Number 8, Issue of February 21, 1997 pp. 4651-4654



Specificity of interaction of the clone 8 protein with the IIb cytoplasmic domain. Clone 8 plasmid DNA was co-transformed in yeast SFY526 along with empty vector, plasmid encoding the unrelated protein lamin C, or plasmids encoding various integrin cytoplasmic domains as indicated, fused with the Gal4 DNA binding domain. The co-transformants were assayed for -galactosidase activity in both filter and liquid assays.

Protein Array Technology

- Peptide screening can identify specific interactions
- Must be relatively high affinity to withstand assay process
- More robust than the yeast-2-hybrid assay



100µm Biot - KVGFFKR

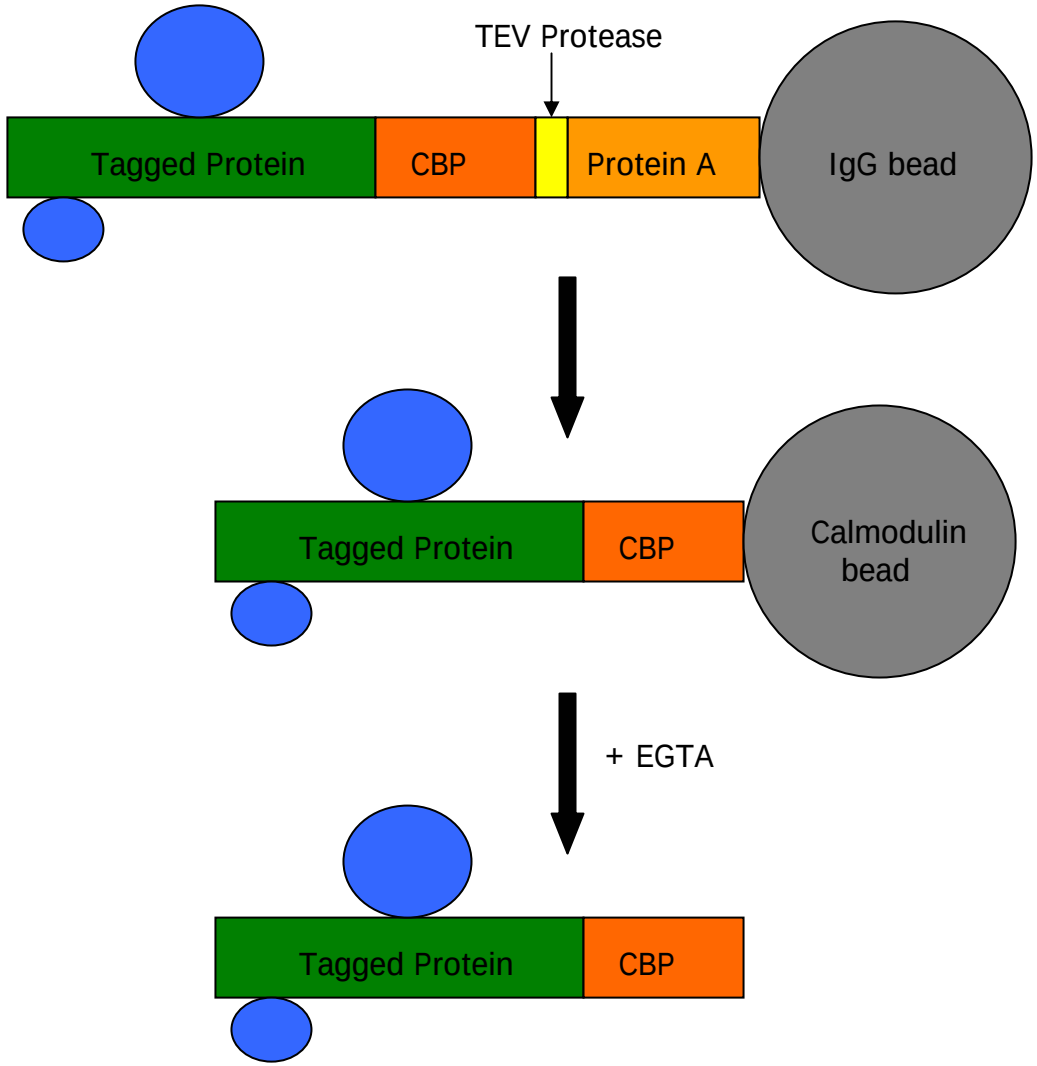
Novel protein: protein interactions

TapTag

Knuesel et al. Identification of Novel Protein-Protein Interactions Using A Versatile Mammalian Tandem Affinity Purification Expression System. *Molecular & Cellular Proteomics 2:1225-1233, 2003.*

Tandem affinity purification (TAP)

2. Purification



Affinity Chromatography

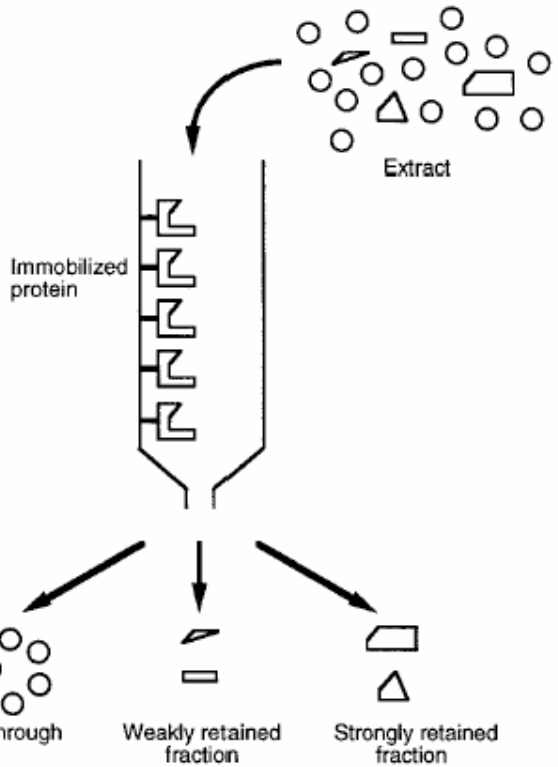
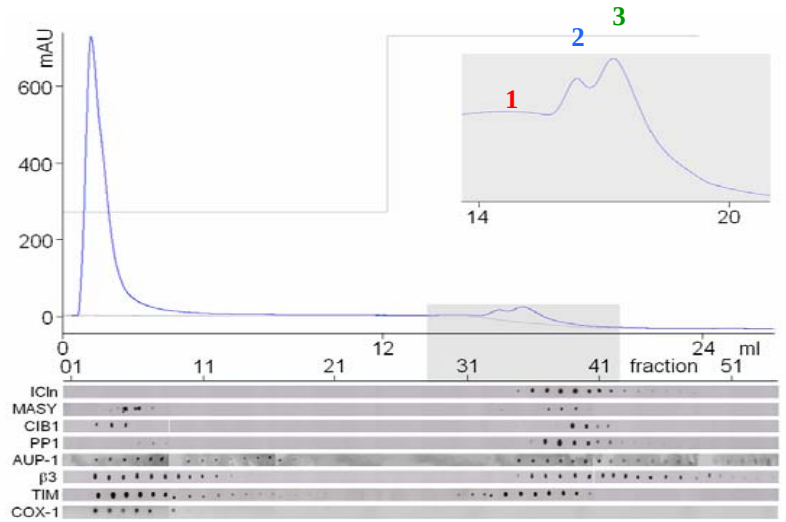
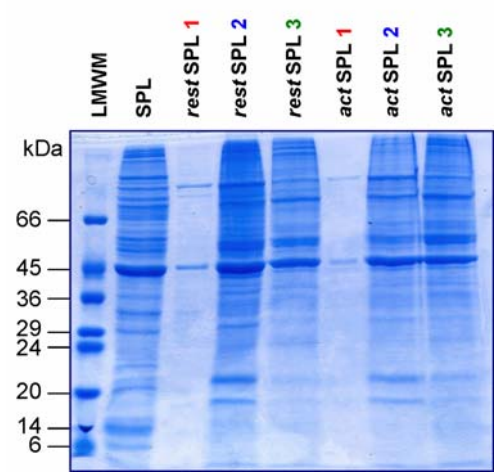


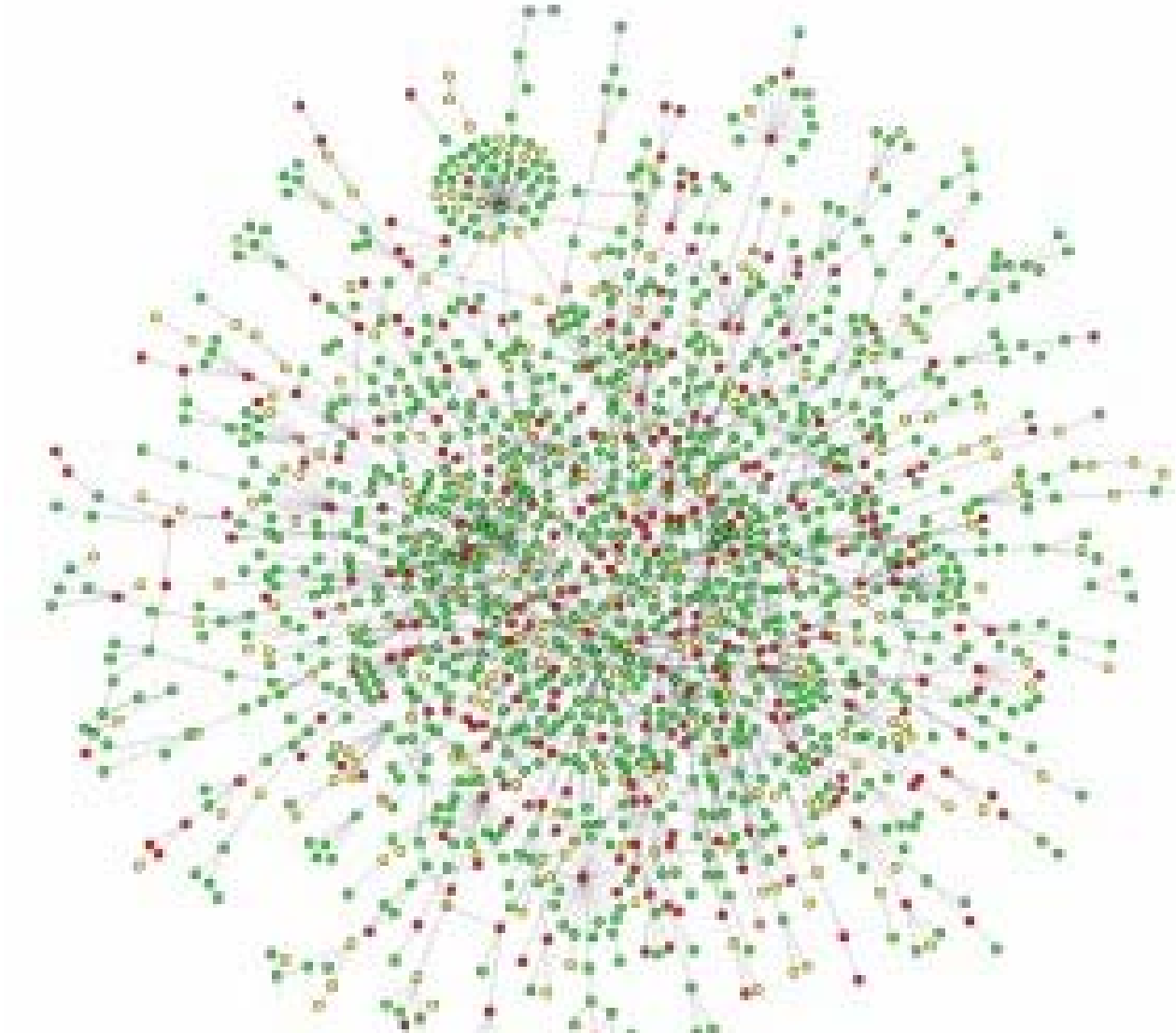
FIG. 1. Protein affinity chromatography. Extract proteins are passed over a column containing immobilized protein. Proteins that do not bind flow through the column, and ligand proteins that bind are retained. Strongly retained proteins have more contacts with the immobilized protein than do those that are weakly retained.





protein name	Ensemble ID	resting lysate	activated lysate
Bridging integrator-2	ENSP00000267012	x	x
Calcium and integrin-binding protein 1	ENSP00000333873	x	x
Integrin-linked protein kinase 1	ENSP00000299421	x	x
Platelet glycoprotein Ibα	ENSP00000329380	x	x
Platelet membrane glycoprotein IIIa	ENSP00000262017	x	x
Serine/Threonine protein phosphatase 1-γ	ENSP00000335084	x	x
Talin-1	ENSP00000373366	x	x
Gelsolin	ENSP00000340888	x	
Tripeptidyl-peptidase 1	ENSP00000299427	x	
Ubiquitin carboxyl-terminal hydrolase 5	ENSP00000229268	x	
Cell division control protein 42 homolog	ENSP00000337669		x
Four and a half LIM domains protein 1	ENSP00000359724		x
Guanine nucleotide-binding protein	ENSP00000367872		x
Platelet glycoprotein IX	ENSP00000303942		x
RAS-related protein (Rab-27B)	ENSP00000262094		x
Suppressor of T-cell receptor signaling 1	ENSP00000284273		x
Toll-interacting protein	ENSP00000314733		x

Table 1: Summary of the most interesting proteins present in the peak fractions of sonicated lysates of *resting* and/or *activated* platelets. Columns three and four are ticked under the condition that the respective protein is significantly enriched (enrichment ratio > 3.0) compared to whole lysate and log(e) value for protein identification is < -3.5.



The protein-protein interaction network of yeast: a few proteins interact with a large number of other proteins, while most proteins have only one or two links.

(from H. Jeong et al Nature 411, 41 (2001)).

Summary

- Co-immunoprecipitation
- FRET
- Surface Plasmon Resonance (Biacore)

- Verify

- Yeast-2-hybrid
- Protein arrays
- TAP-tag co-precipitation
- Affinity Chromatography

- Discover

Hunting for function

Interesting gene

Position in protein interaction maps

Functional analysis

Integration in functional pathway

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- **Pat Harriott,** QUB
- **Marc Devocelle** Peptide synthesis core

- **Dolores Cahill** protein arrays
- **Derek Murphy** Protein arrays

- **Achim Treumann** Mass Spec
- **Christian Kohler** Mass spec

Lab

- **Gillian Stephens**
- **Deirdre Larkin**
- **Dermot Reilly**
- **Heide Daxecker**
- **Markus Raab**
- **Emma Cummins**
- **Martha Cahill**

Surface Plasmon Resonance

- University of Reading
- Fred Kemp PhD