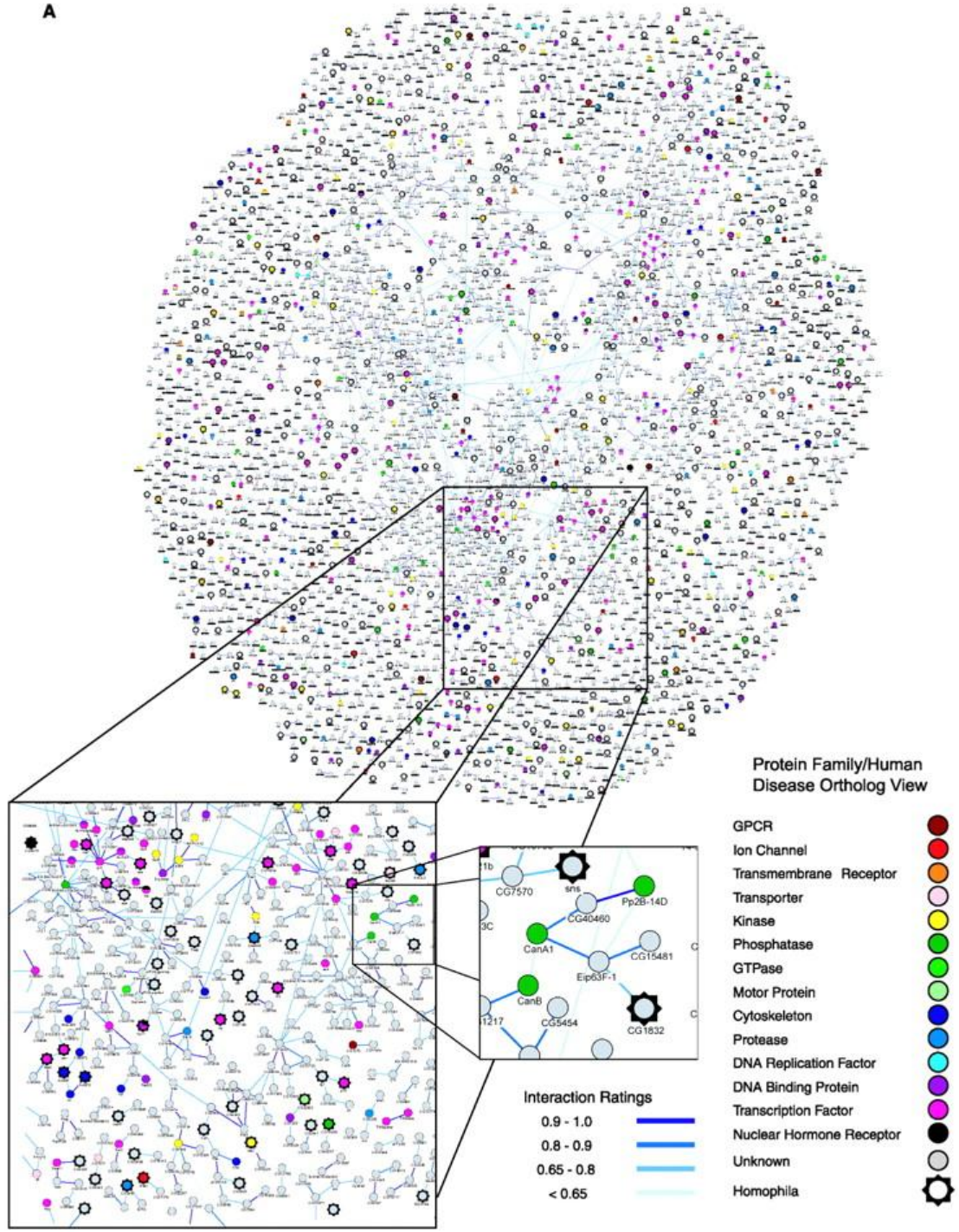
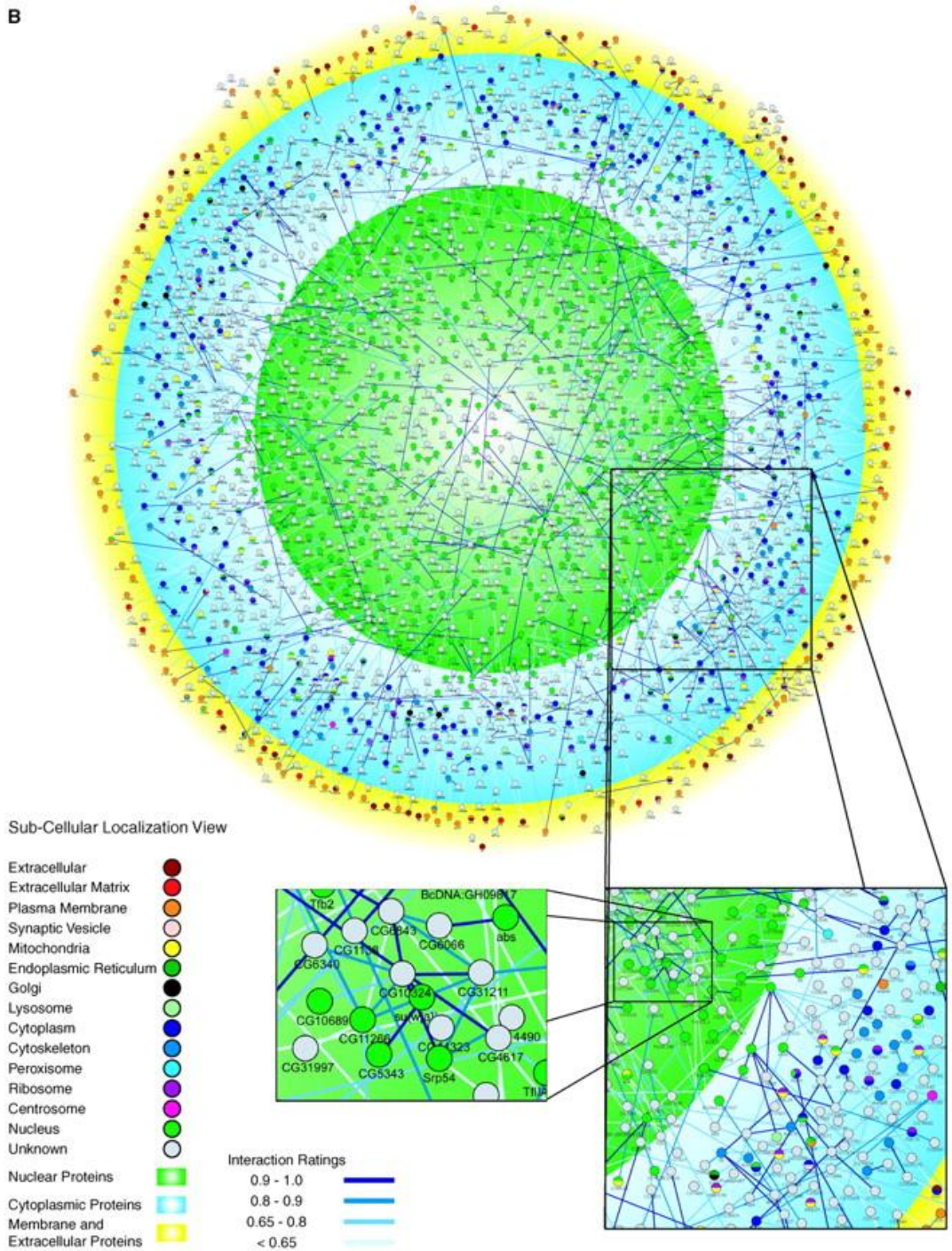
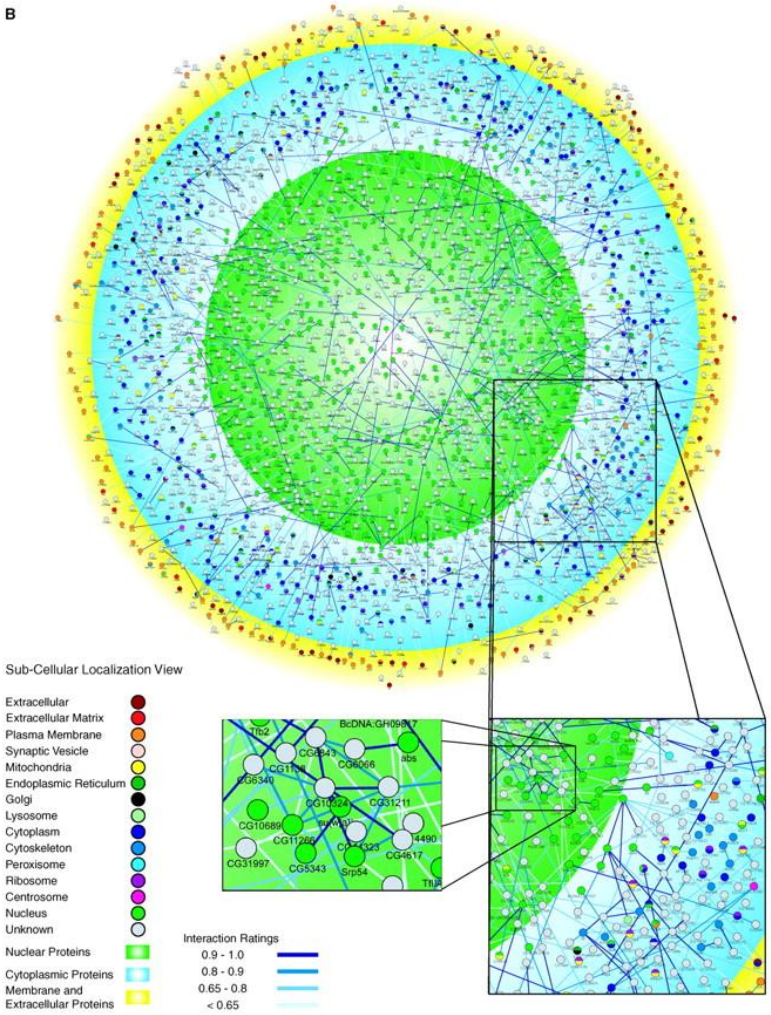
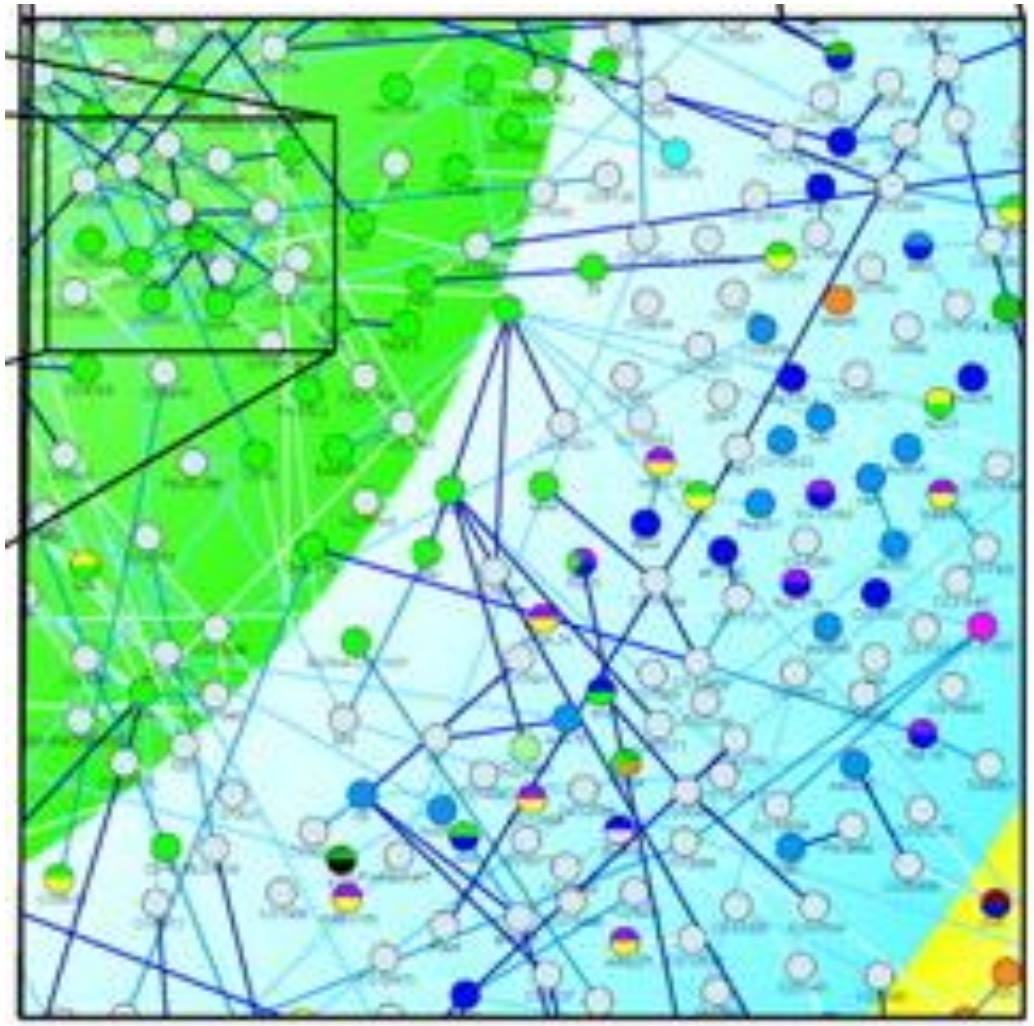
Discovering Macromolecular Interactions


A
CG7570
sns
Pp2B-14D
CG40460
CanA1
CG15481
Eip63F-1
CanB
CG5454
CG1832
Interaction Ratings
0.9 - 1.0
0.8 - 0.9
0.65 - 0.8
< 0.65
Protein Family/Human Disease Ortholog View
GPCR
Ion Channel
Transmembrane Receptor
Transporter
Kinase
Phosphatase
GTPase
Motor Protein
Cytoskeleton
Protease
DNA Replication Factor
DNA Binding Protein
Transcription Factor
Nuclear Hormone Receptor
Unknown
Homophila


B
Sub-Cellular Localization View
Extracellular
Extracellular Matrix
Plasma Membrane
Synaptic Vesicle
Mitochondria
Endoplasmic Reticulum
Golgi
Lysosome
Cytoplasm
Cytoskeleton
Peroxisome
Ribosome
Centrosome
Nucleus
Unknown
Nuclear Proteins
Cytoplasmic Proteins
Membrane and Extracellular Proteins
Interaction Ratings
0.9 - 1.0
0.8 - 0.9
0.65 - 0.8
< 0.65
Tfb2
CG6066
abs
CG6340
CG10689
CG31997
CG5343
Srp54
CG4617
4490

An experimental strategy for identifying new molecular actors in a process





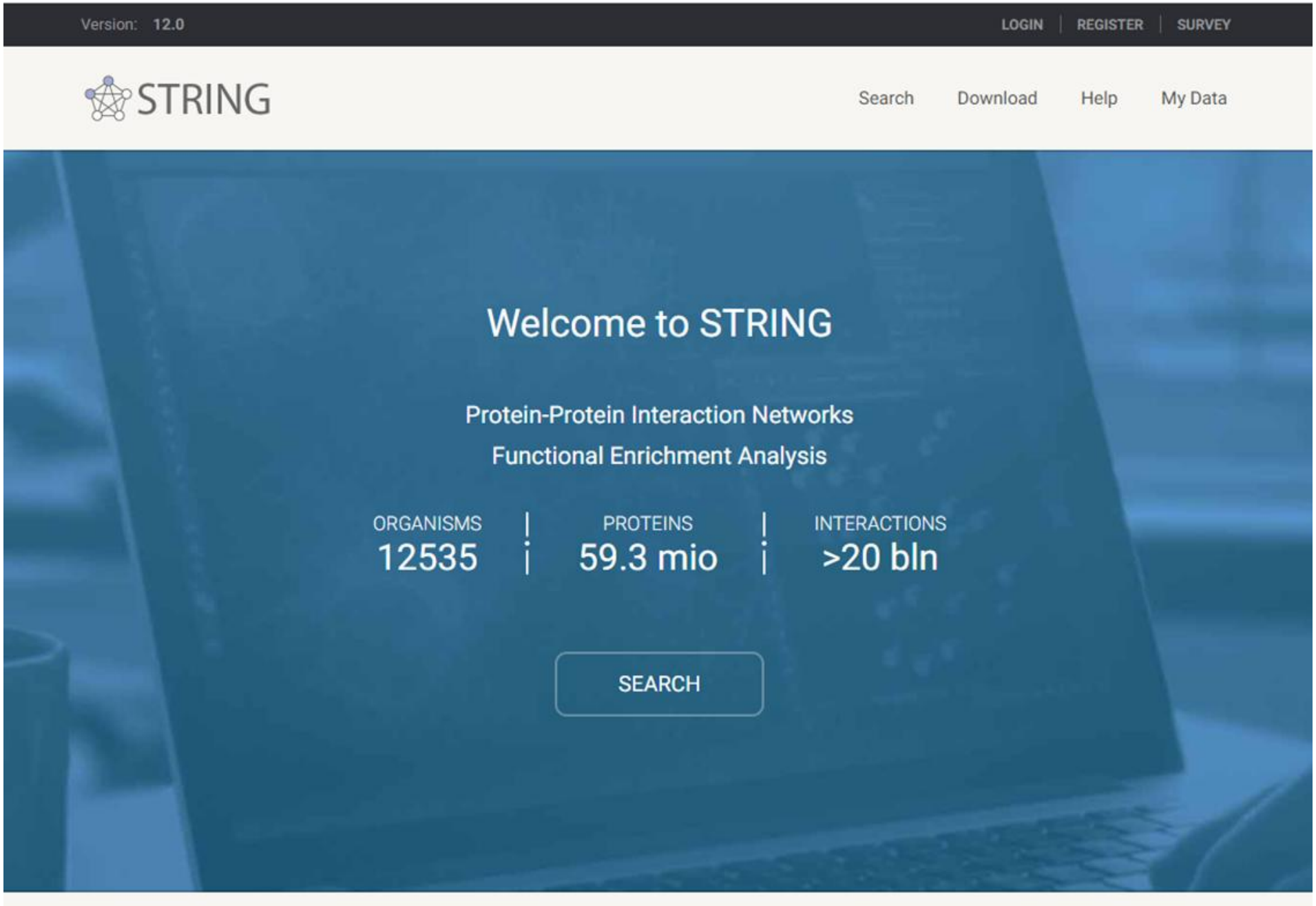
An experimental strategy for identifying new molecular actors in a process

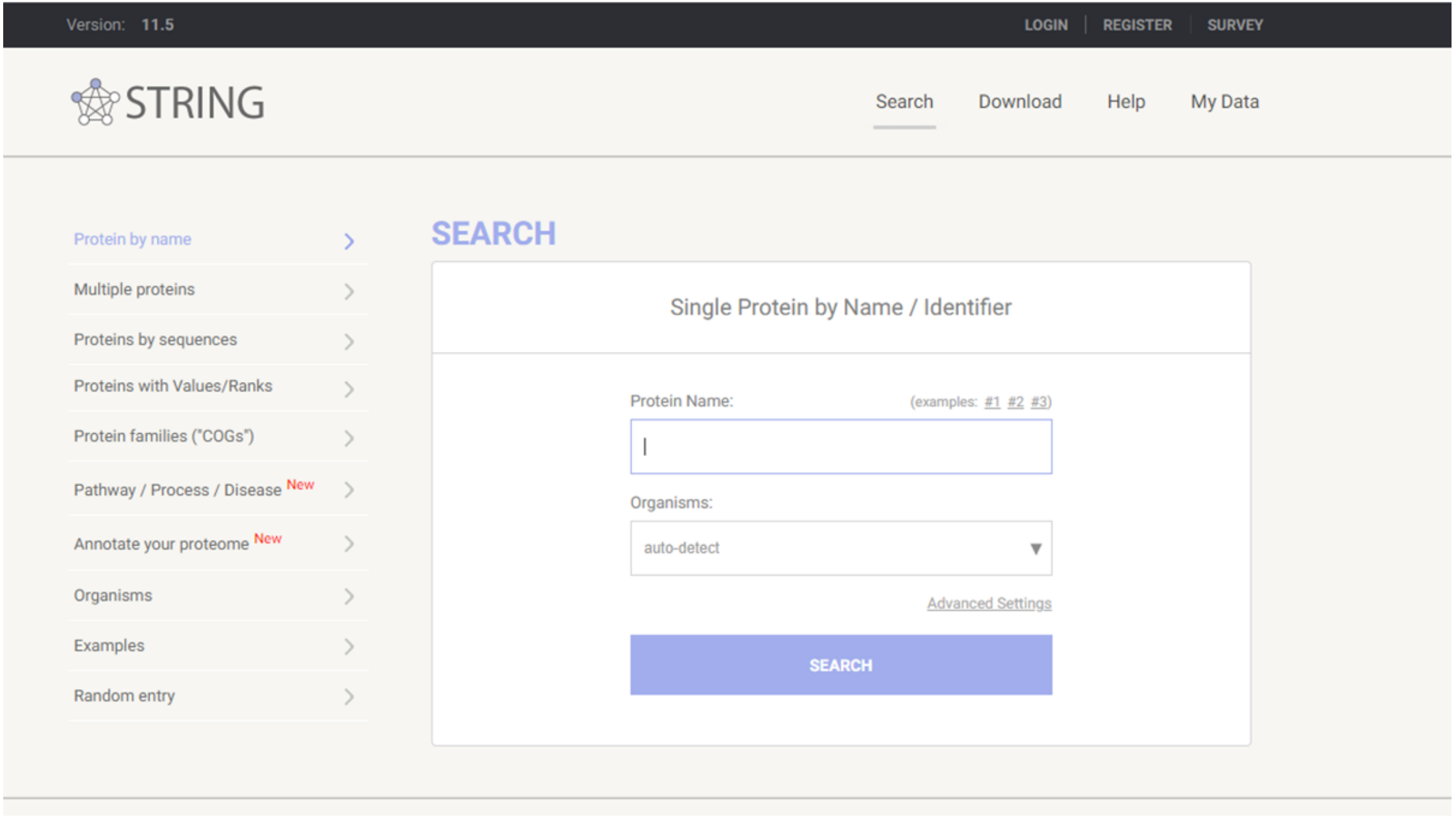
Consider the protein **Human Transglutaminase 2 (TGM2)**

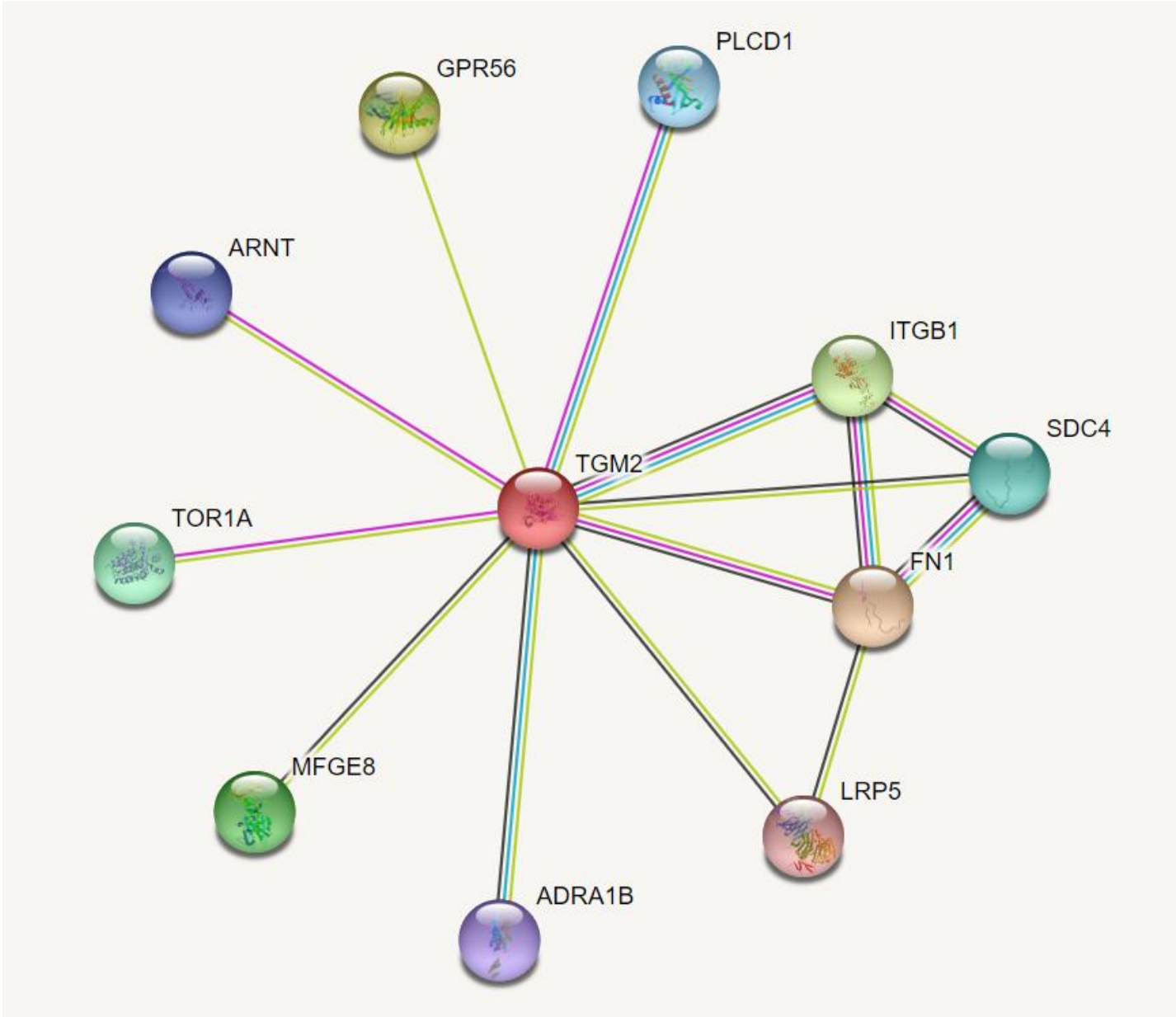
Biological question

?? Indentify its protein partners in the cell context ??

DATABASE search


Version: 12.0
LOGIN
REGISTER
SURVEY
STRING
Search
Download
Help
My Data
Welcome to STRING
Protein-Protein Interaction Networks
Functional Enrichment Analysis
ORGANISMS
12535
PROTEINS
59.3 mio
INTERACTIONS
>20 bln
SEARCH


Version: 11.5
LOGIN
REGISTER
SURVEY
STRING
Search
Download
Help
My Data
Protein by name
Multiple proteins
Proteins by sequences
Proteins with Values/Ranks
Protein families ("COGs")
Pathway / Process / Disease New
Annotate your proteome New
Organisms
Examples
Random entry
SEARCH
Single Protein by Name / Identifier
Protein Name:
(examples: #1 #2 #3)
Organisms:
auto-detect
Advanced Settings
SEARCH


PLCD1
GPR56
ARNT
ITGB1
SDC4
TGM2
TOR1A
FN1
MFGE8
LRP5
ADRA1B

BioGRID 5.0

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Welcome to our Database of Protein, Genetic and Chemical Interactions

BioGRID is a biomedical interaction repository with data compiled through comprehensive curation efforts. Our current index is version **5.0.256** and searches **88,077** publications for **2,943,480** protein and genetic interactions, **31,540** chemical interactions and **1,128,339** post translational modifications from major model organism species. All data are **freely** provided via our search index and available for download in many standardized formats.

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Search by Protein/Gene Identifiers ...

All Organisms

Submit Identifier Search

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Result Summary

tgm2

Homo sapiens

GO

BioGRID COVID-19 Coronavirus Curation Project

Search BioGRID for SARS-CoV-2 Protein Interactions | Download SARS-CoV-2 and Coronavirus-Related Interactions

TGM2

Homo sapiens

GALPHA8, GNAH, HEL-S-45, TG2, TGC, tTG-1054A22.2

transglutaminase 2

GO Process (3) | GO Function (2) | GO Component (4)

CHEMPM Database | OMIM | HGNC | Alliance of Genome Resources | Entrez Gene | RefSeq | UniprotKB | HPRD

Download Curated Data for this Protein

Network Statistics

Total Nodes	Total Edges
207	757

Physical Edges (736)

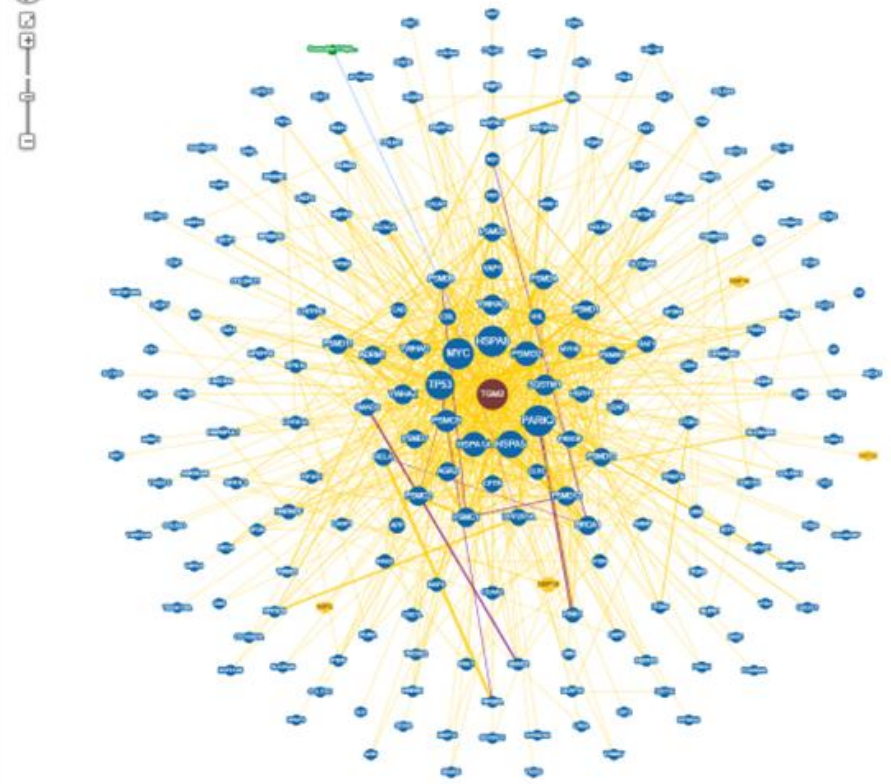
Physical/Genetic Edges (10)

Chemical Edges (1)

Switch to Node Stats

Switch View: Interactors 207 | Interactions 757 | Chemical Interactions 3 | Network | PTM Sites 24

FILE | FILTERS | 68.71% | MINIMUM EVIDENCE: 1 | LAYOUTS | RESOURCES | HELP



An experimental strategy for identifying new molecular actors in a process

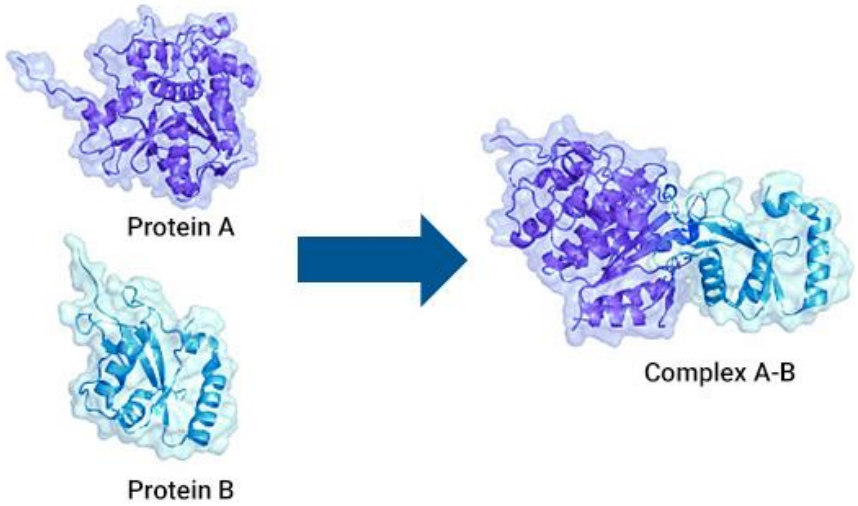
DATABASE search

Candidate approach

General screening

Types of Interactions

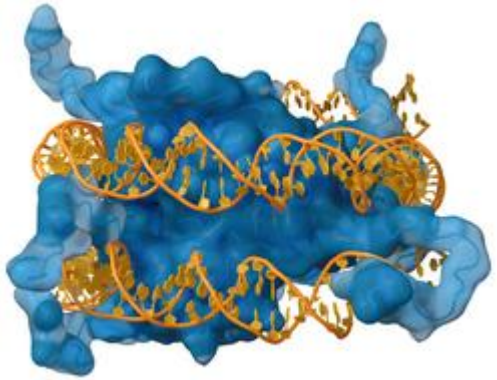
Protein / Protein



extracellular

intracellular

Protein / nucleic acid



DNA

RNA

Interaction detection Methods

- (co)-immunoprecipitation

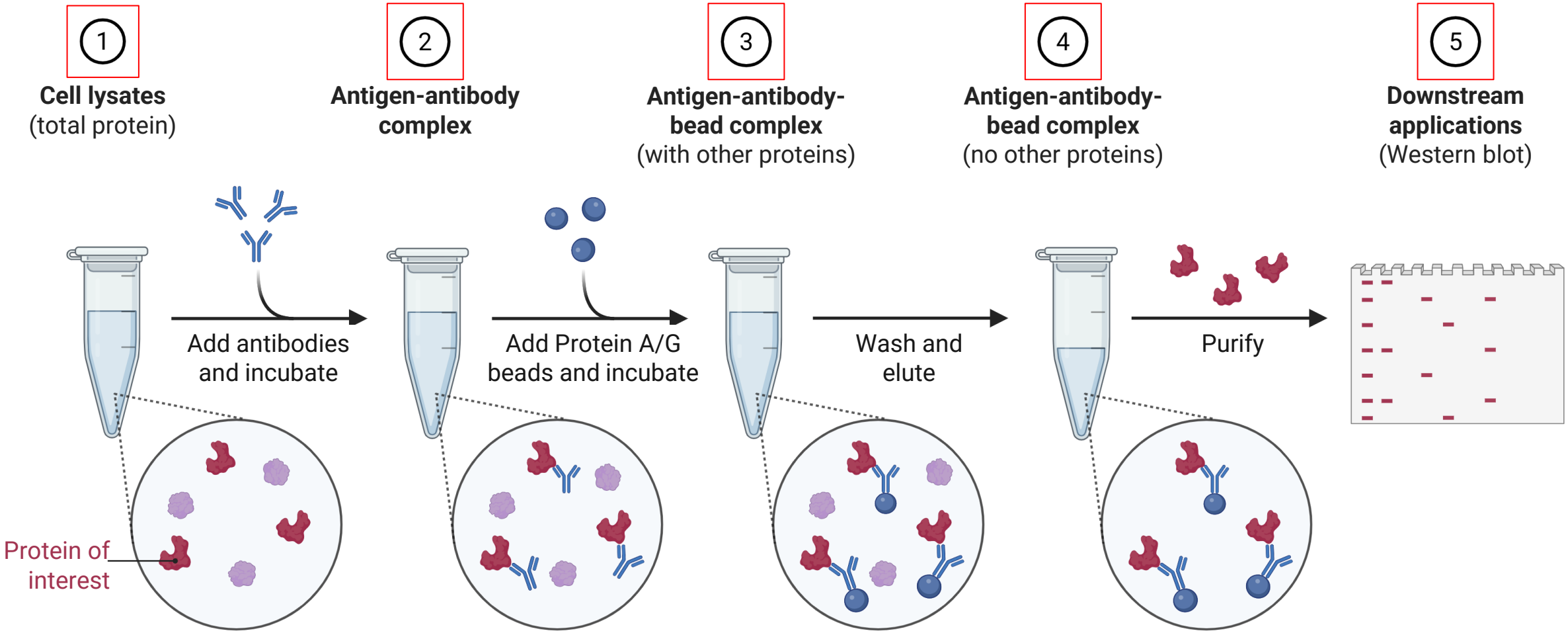
- glutathione-S-transferase (GST) pull down
- co-purification
  - chromatography, tandem affinity purification (TAP)
- yeast two hybrid
- phage display/expression libraries
- FRET
- solution binding- Scatchard analysis

(Co)- ImmunoPrecipitation (IP)

Immunoprecipitation (IP)
is a cellular and molecular biology method used in laboratories
- to isolate (or purify)
- a protein of interest
- via a solid phase (agarose or Sepharose™ resin or magnetic particles).

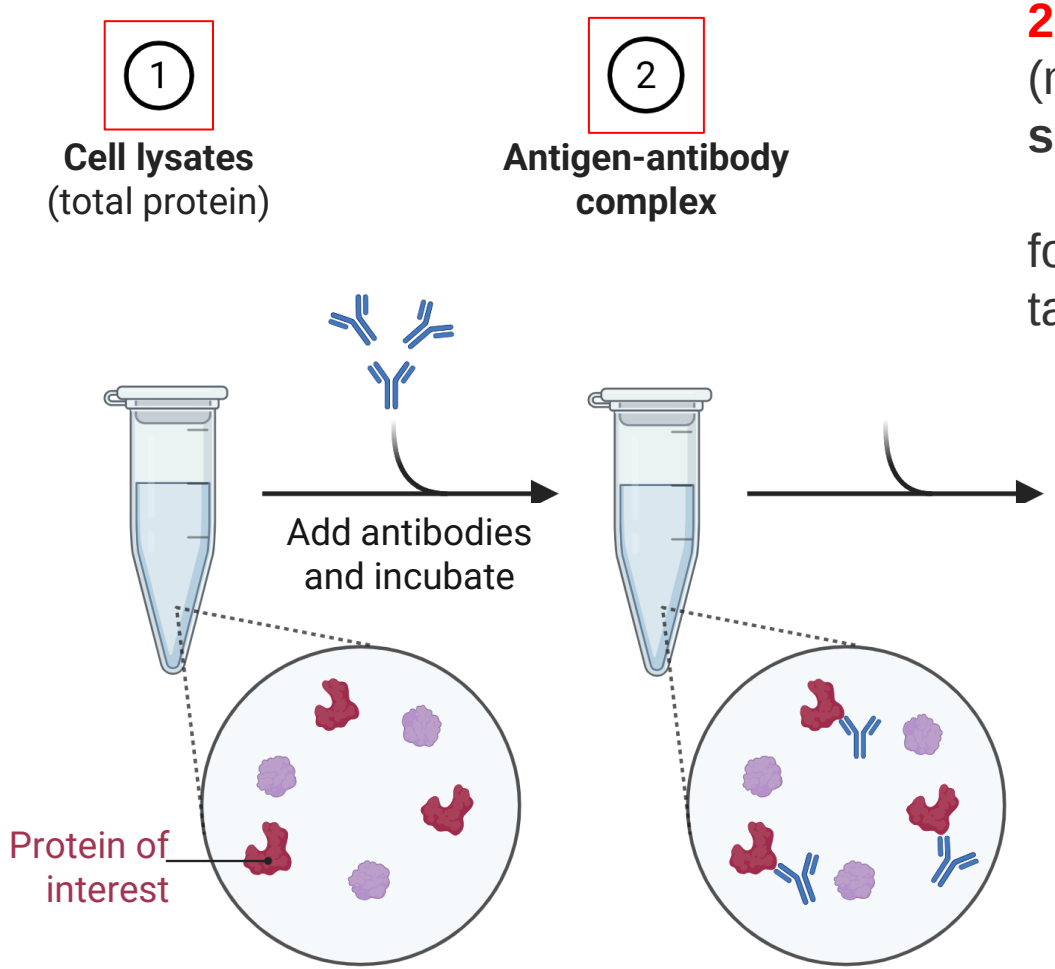
- Using an **antibody** to isolate and purify **a protein** from a **whole cell lysate**.
- Normally you will **"only"** purify the protein the antibody recognizes.
- Any additional proteins that **co-purify** are **candidates for interacting proteins.**

Immunoprecipitation Protocol



Immunoprecipitation Protocol

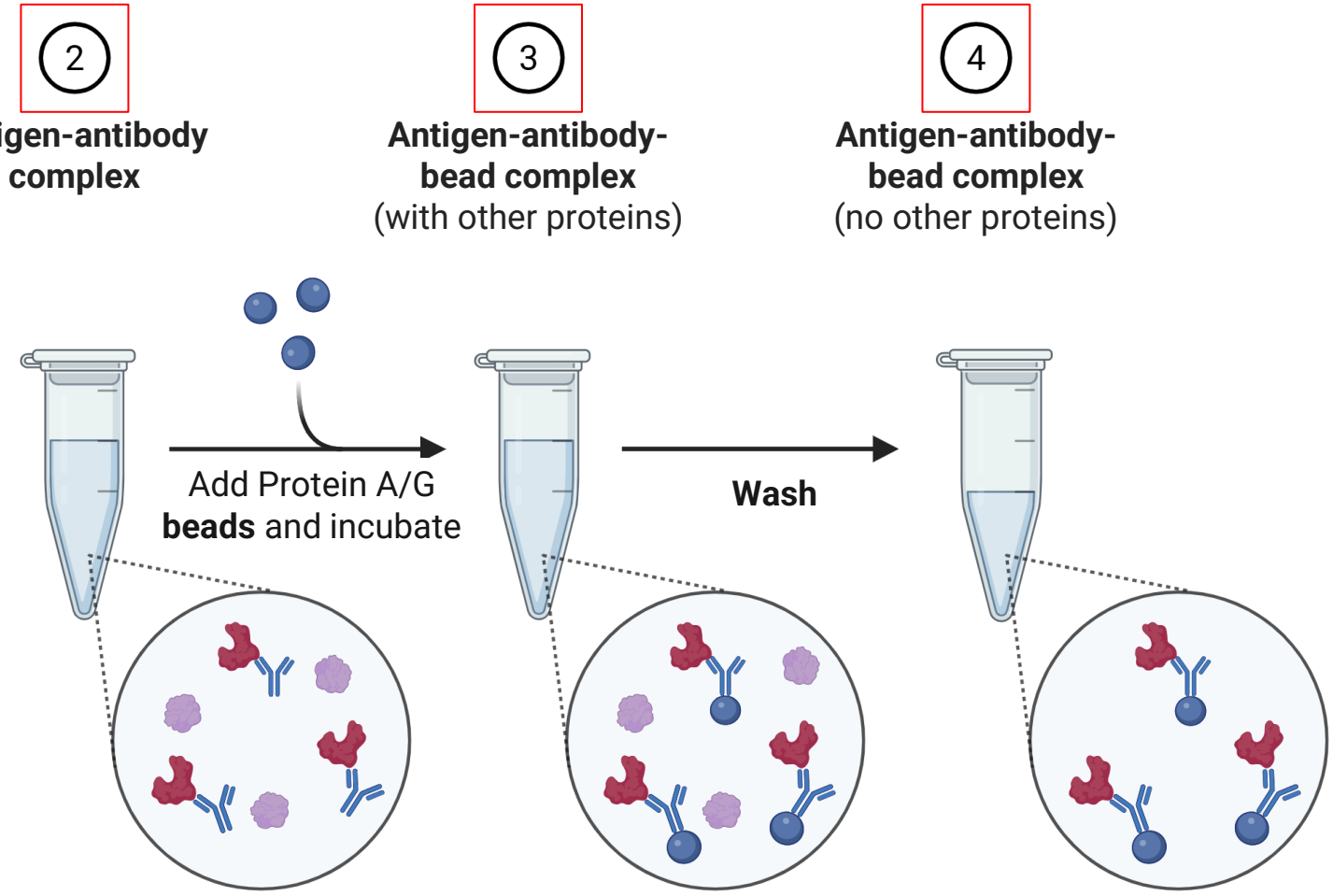
1- lysate cell



2- Add an antibody
(monoclonal or polyclonal) against **a specific target** protein

forms an **immune complex** with that target.

Immunoprecipitation Protocol



3- The immune complex is then **captured, or precipitated**, on a **beaded** support

to which an **antibody-binding protein is immobilized** (such as Protein A or G),

any proteins not precipitated on the beads are washed away.

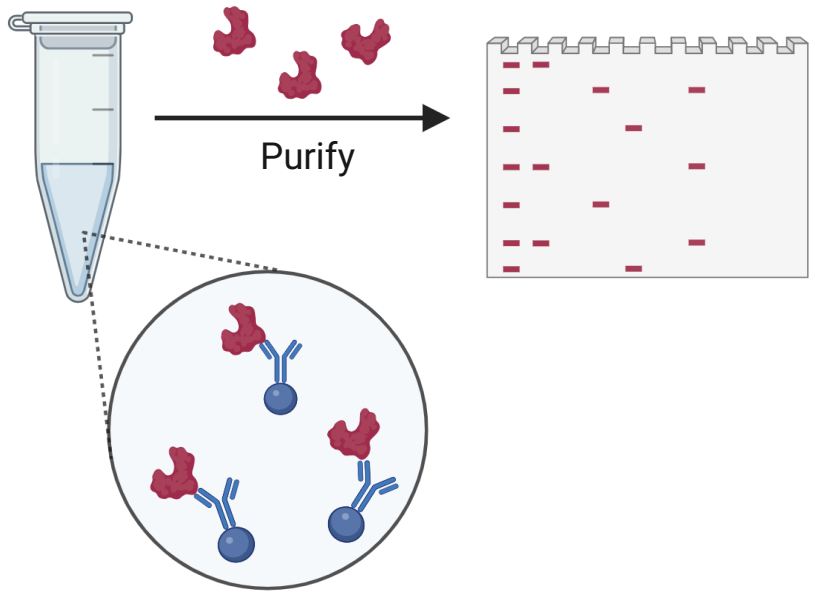
Immunoprecipitation Protocol

4

Antigen-antibody-bead complex
(no other proteins)

5

Downstream applications
(Western blot)

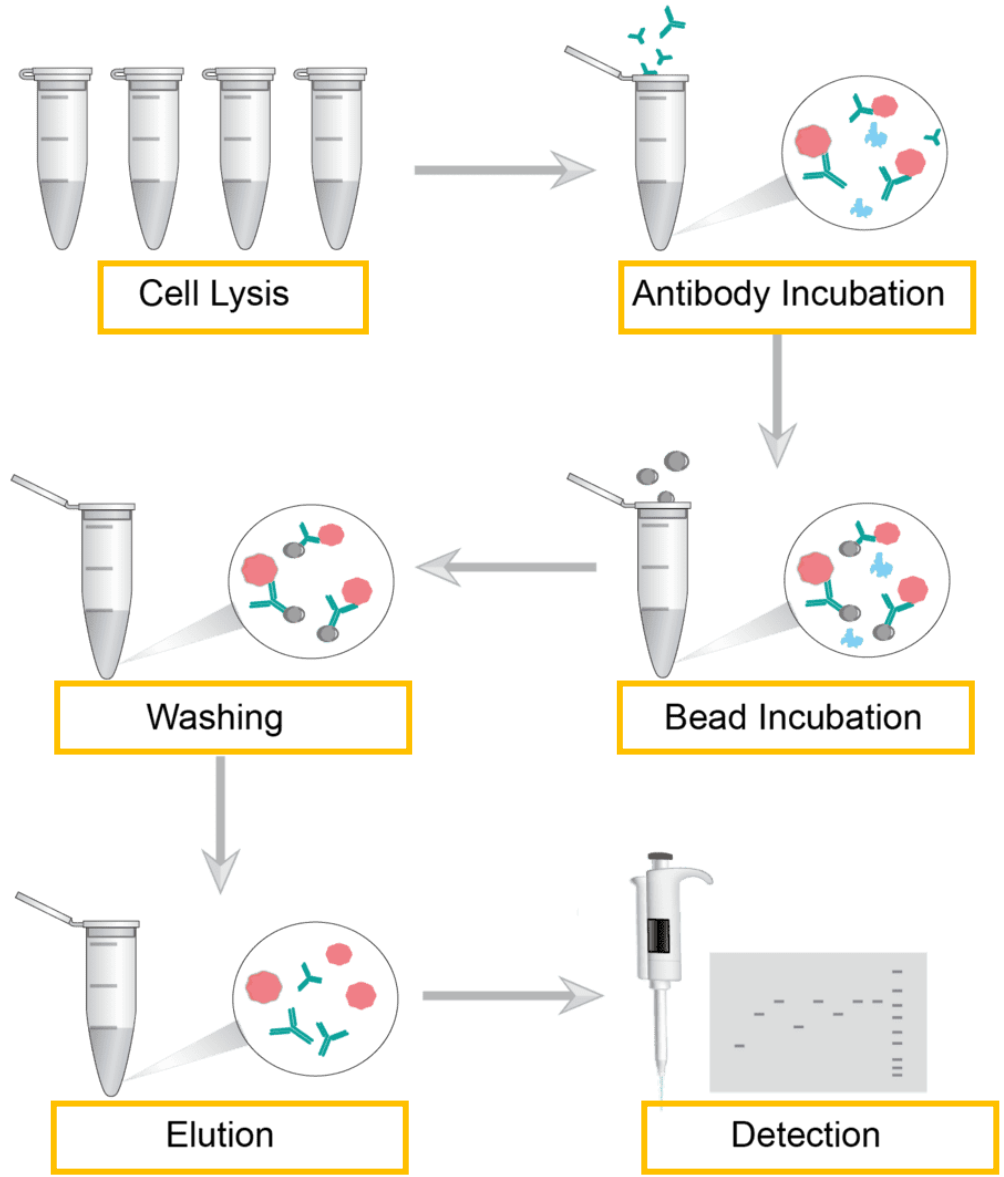


4- the antigen is eluted from the support
(and antibody, if it is not covalently attached to the beads and/or when using denaturing buffers)

5- The antigen is analyzed

- by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (**SDS-PAGE**),

- followed by **Western blot** detection to verify the identity of the antigen.


Cell Lysis
Antibody Incubation
Bead Incubation
Washing
Elution
Detection

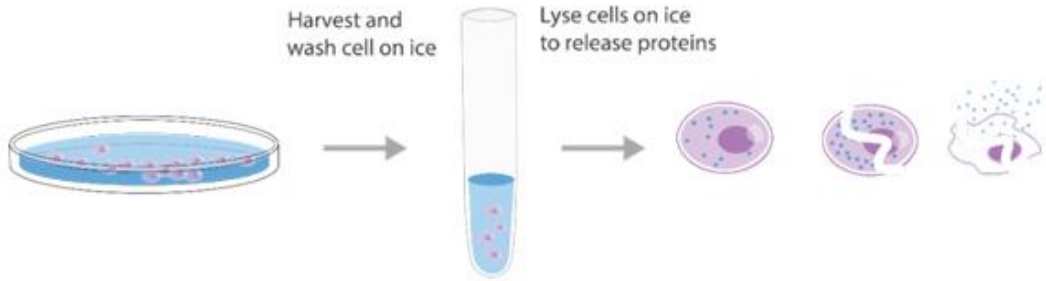
IP optimization

Empirical testing is nearly **always required to optimize IP conditions** to obtain the desired yield and purity of target proteins.

Lysis Buffers

The quality of the sample that is used for IP applications critically depends on the **right lysis buffer**, which

- stabilizes native protein conformation,
- inhibits enzymatic activity,
- minimizes antibody binding site denaturation
- maximizes the release of proteins from the cells or tissue.



The lysis buffer used for a particular application **depends on the target proteins** that will be immunoprecipitated, because the location of the protein in the cell (e.g., membrane, cytosol, nucleus) affects the ease of release during lysis.

NON DENATURING buffers

are used when
- the **IP antigen is detergent-soluble**
- **the antibody can recognize the native form of the protein**.

These buffers contain **non-ionic detergents**, such as NP-40 or Triton X-100.

DENATURING buffers

such as **radio-immunoprecipitation assay (RIPA) buffer**

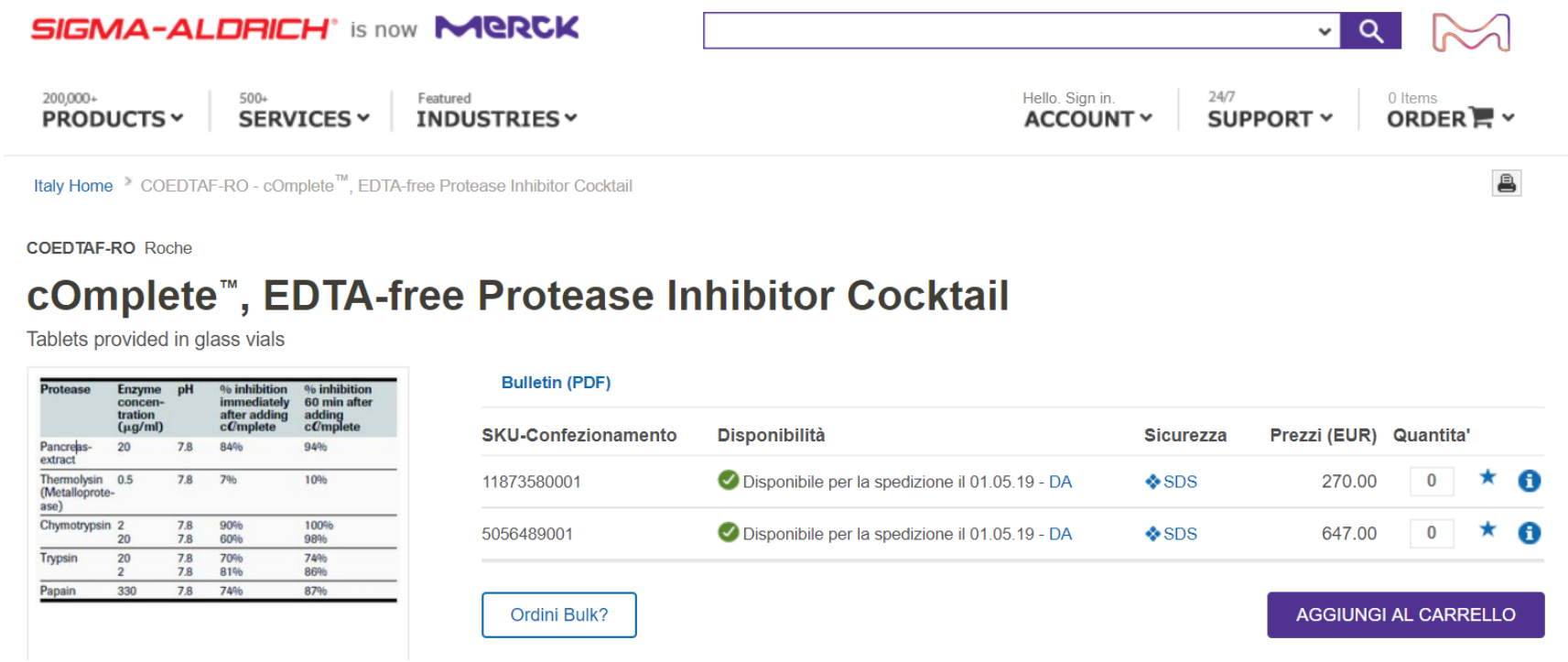
are more stringent than non-denaturing buffers because of the addition of ionic detergents like SDS or sodium deoxycholate.
While these buffers **do not maintain native protein conformation**, proteins that are difficult to release with non-denaturing buffers, such as **nuclear proteins**, can be released with denaturing buffers.

Both buffers contain NaCl and Tris-HCl and have a slightly basic pH (7.4 to 8).

** Because cell lysates also contain proteases and phosphatases that can modify or degrade the target protein, **most IP protocols are performed at 4°C**.

** **Protease (asomal) inhibitors**, such as PMSF, aprotinin and leupeptin are commonly added to the lysis buffer just prior to use, along with sodium orthovanadate or sodium fluoride as a **phosphatase inhibitor**.

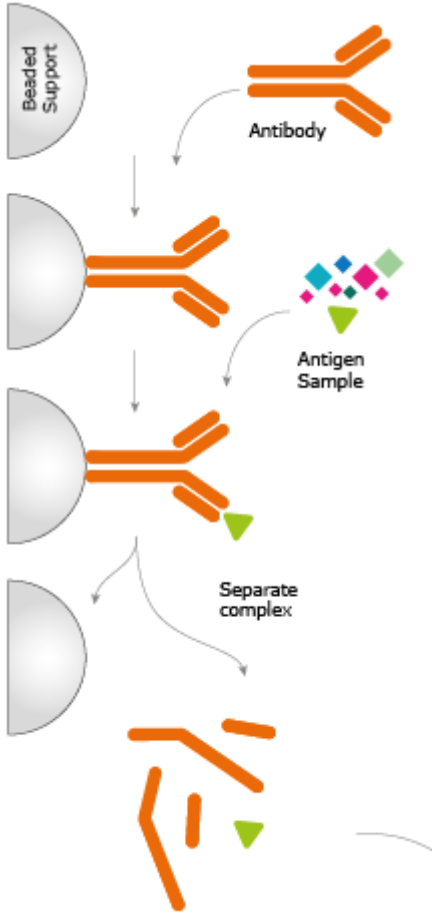
While these components can be added individually, commercial inhibitor cocktails are available that are higher quality and easier to use.

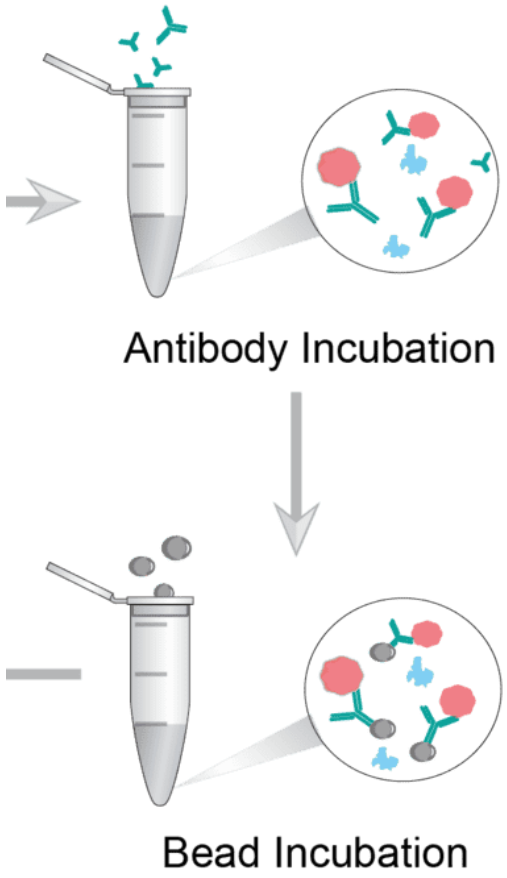


Protease	Enzyme concentration (µg/ml)	pH	% inhibition immediately after adding cOmplete	% inhibition 60 min after adding cOmplete
Pancreas-extract	20	7.8	84%	94%
Thermolysin (Metalloprotease)	0.5	7.8	7%	10%
Chymotrypsin	2	7.8	90%	100%
	20	7.8	60%	98%
Trypsin	20	7.8	70%	74%
	2	7.8	81%	86%
Papain	330	7.8	74%	87%

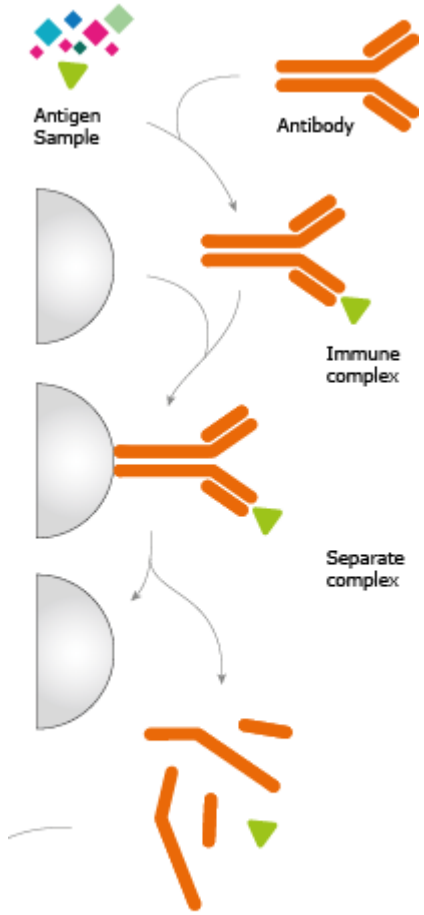
Diagram of immunoprecipitation (IP)

pre-immobilized antibodies.

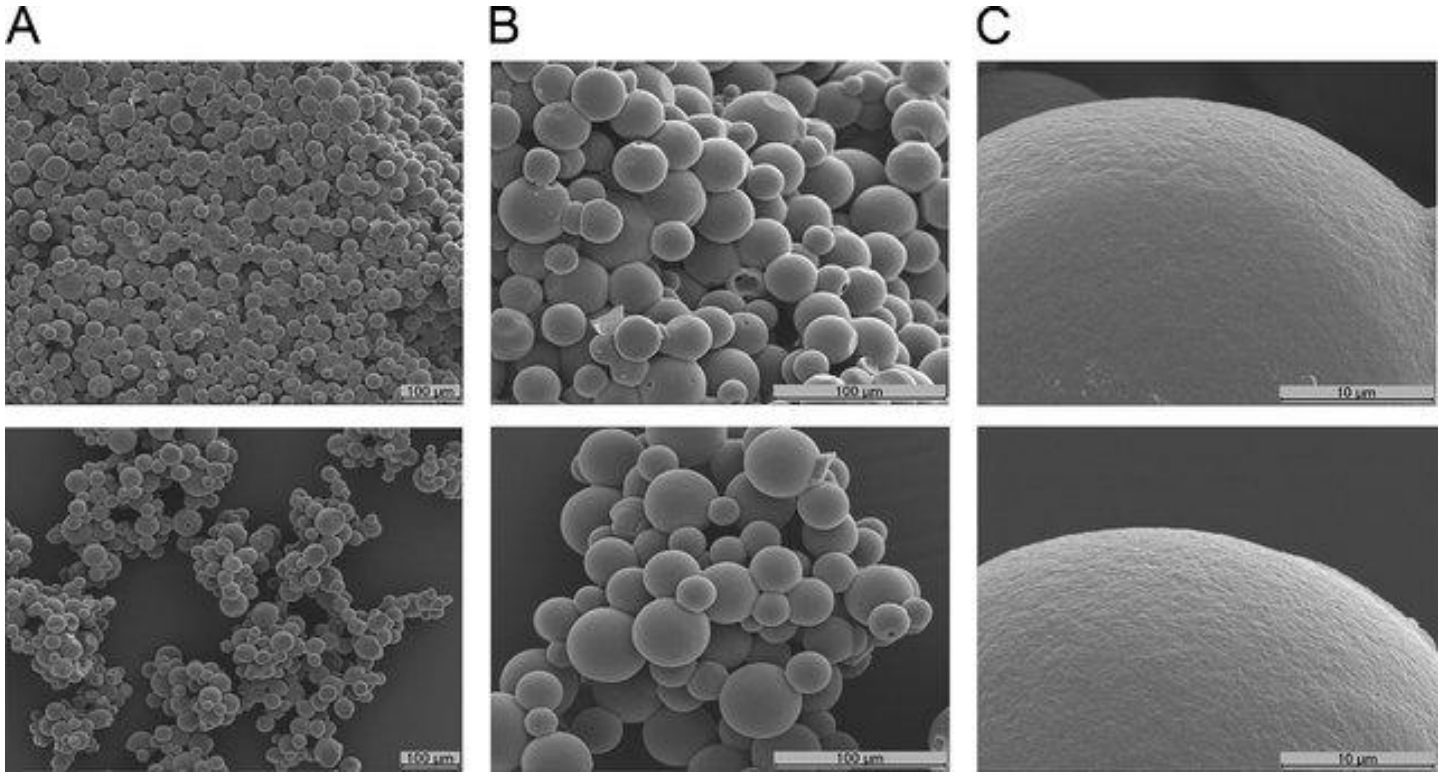




or **free** antibodies.



Because IP developed as an adaptation of column affinity chromatography, the IP technique was first done using small aliquots (10–25 µL) of **agarose resin** **in microcentrifuge tubes**.



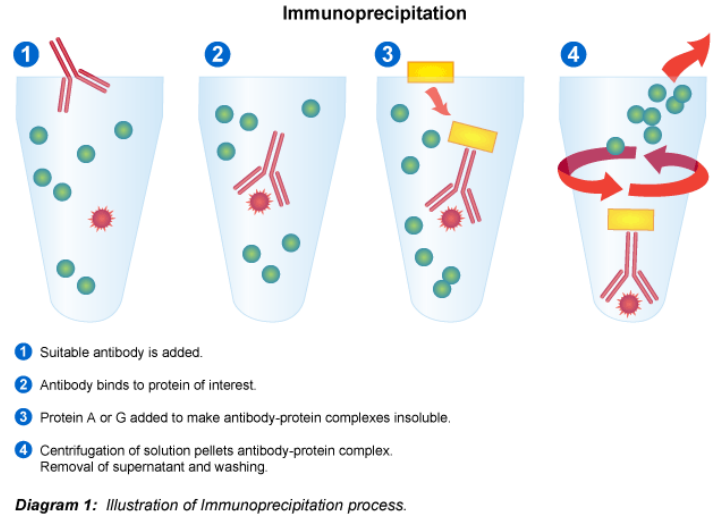
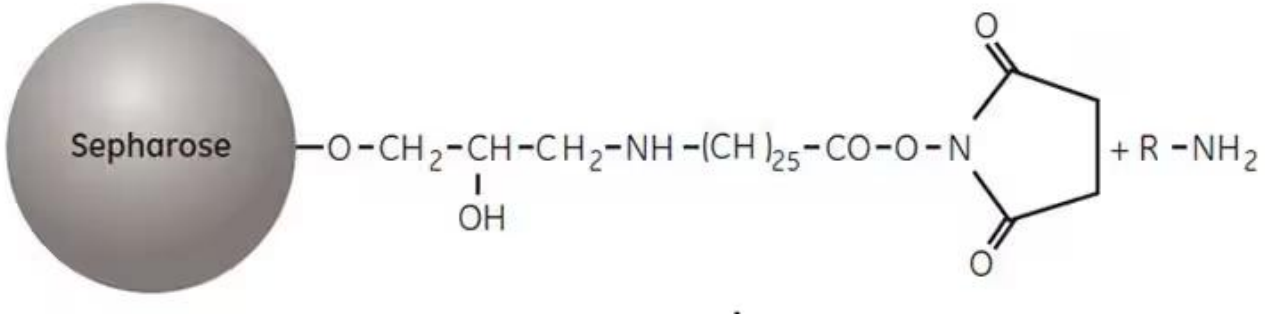


Diagram 1: *Illustration of Immunoprecipitation process.*



Magnetic particles, such as Dynabeads™ and Pierce™ magnetic beads, have **largely replaced agarose beads** as the preferred support for immunoprecipitation and other micro-scale affinity purification procedures.

Magnetic particles **are solid and spherical, and antibody binding is limited to the surface of each bead.**

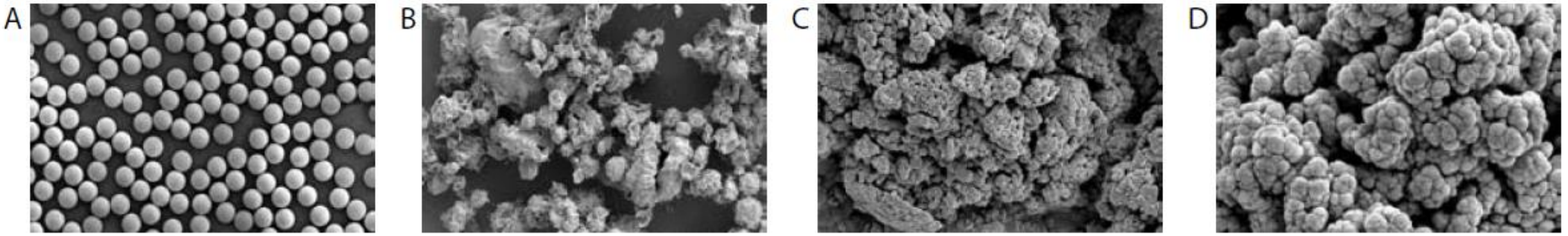


Figure 1—Monosized, superparamagnetic Dynabeads®. A. Manufactured with highly controllable product qualities, Dynabeads® are highly uniform within and between batches. **B–D.** Magnetic particles from alternative suppliers.

The advantages of magnetic beads for IP

Capacity and yield–, even if the antibody-binding **capacity is lower** compared to agarose, the final antigen **yield is often the same or greater** with magnetic beads.

Reproducibility and purity–magnetic beads generally provide higher reproducibility and purity compared to agarose. Pre-clearing is usually not necessary with magnetic beads.

Ease of use, speed, and automation an individual magnetic bead IP experiment can be completed in about 30 minutes.

Invitrogen™

Dynabeads™ Protein G for Immunoprecipitation

Catalog number: 10003D

Related applications: Antibody Purification | Chromatin Biology | Protein Assays and Analysis | Protein Purification & Isolation

Contact us for support ›

	Catalog number	Unit size	Price (EUR)	Qty
☆	10003D	1 mL	181,00	
☆	10004D	5 mL	690,00	
☆	10009D	50 mL	3.705,00	

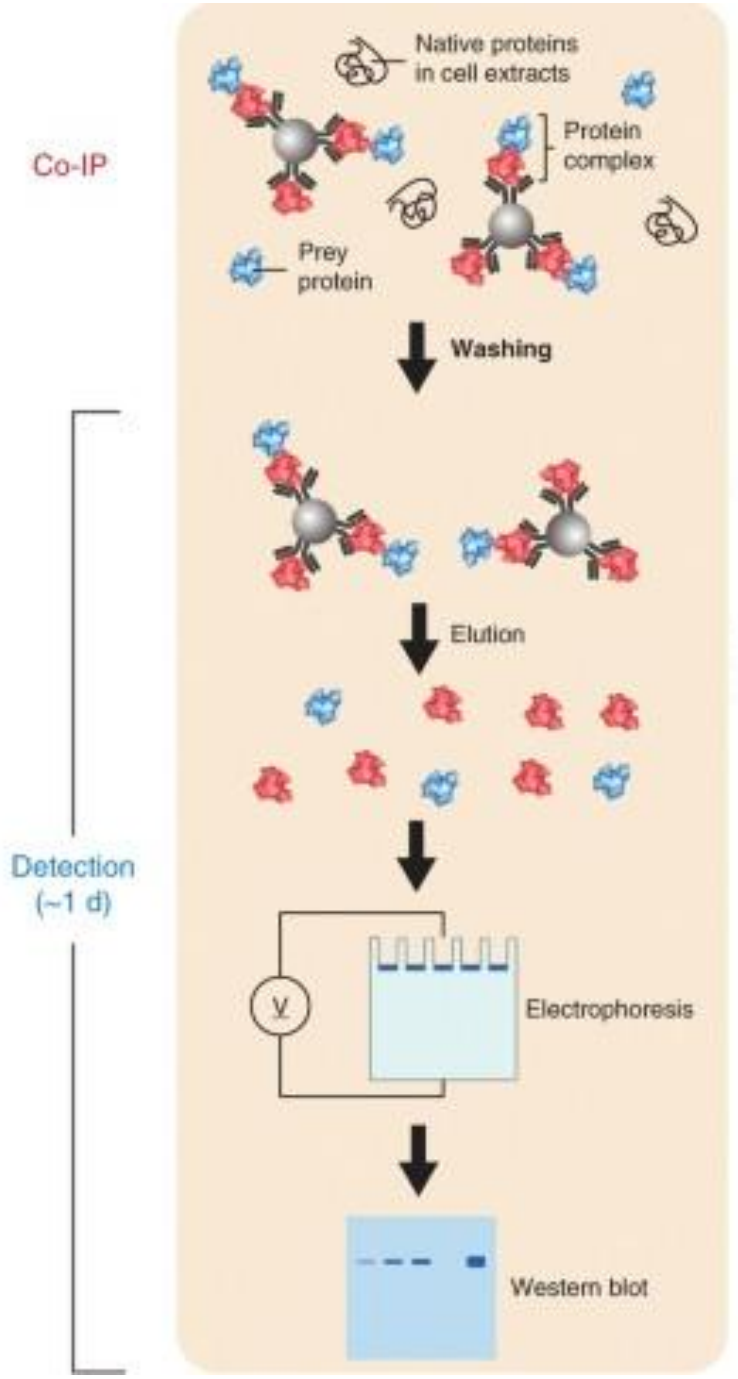
Check price & availability | Save to list | Add To Cart

Co-Immnunoprecipitation (Co-IP)

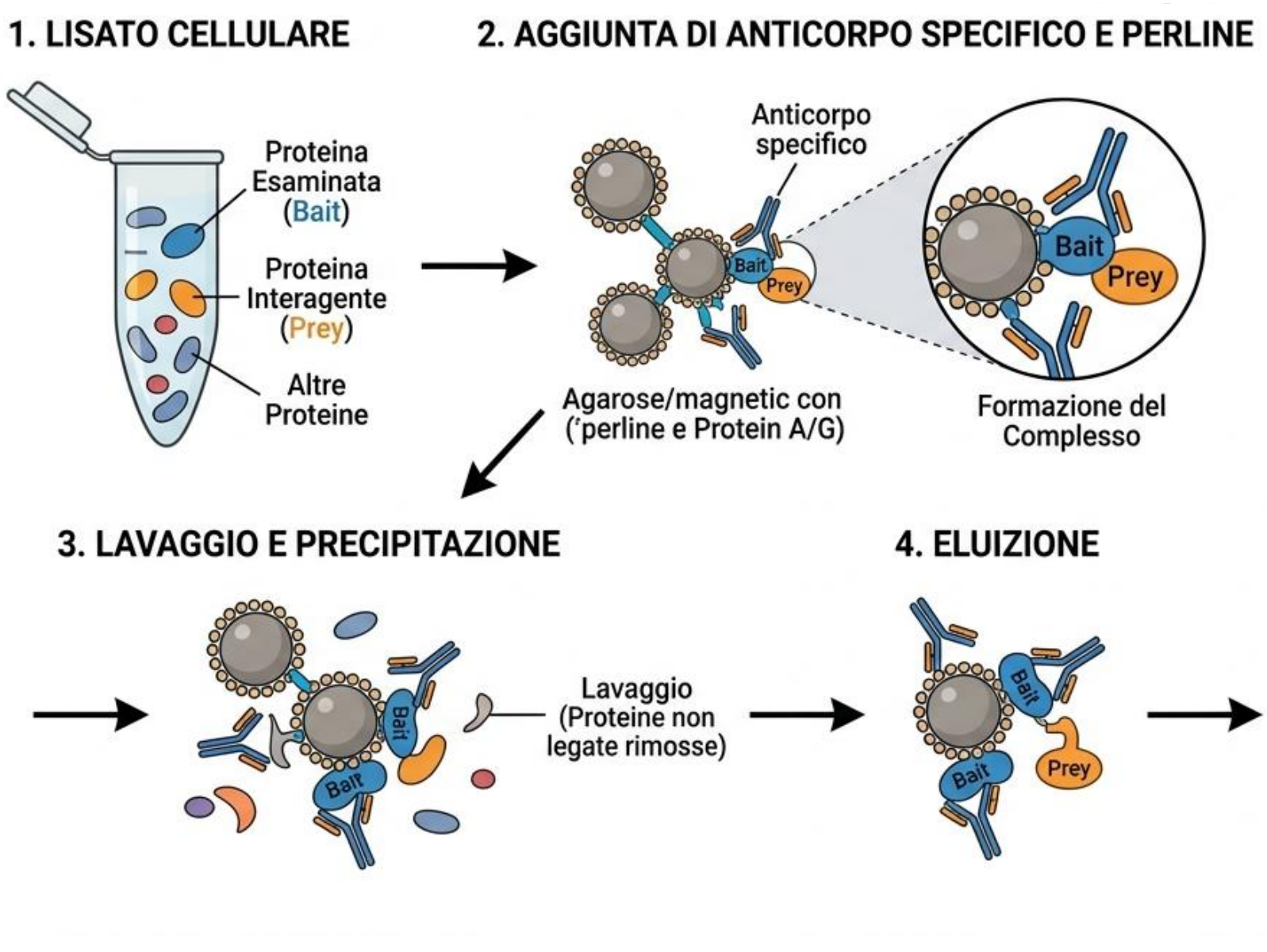
Co-immunoprecipitation **is an extension of IP** that is based on the potential of IP reactions to capture and purify the primary target (i.e., the antigen) **as well as other macromolecules that are bound t**o the target by native interactions in the sample solution.

Therefore, whether or not an experiment is called an IP or co-IP depends on **whether the focus of the experiment is:**

- **the primary target (antigen) -------> IP**
- **the secondary targets (interacting proteins) -------> co-IP**



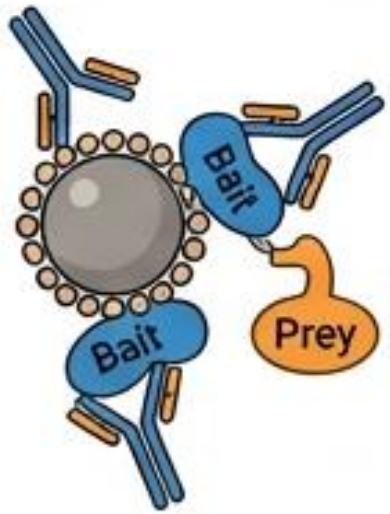
SCHEMA DI CO-IMMUNOPRECIPITAZIONE (Co-IP)

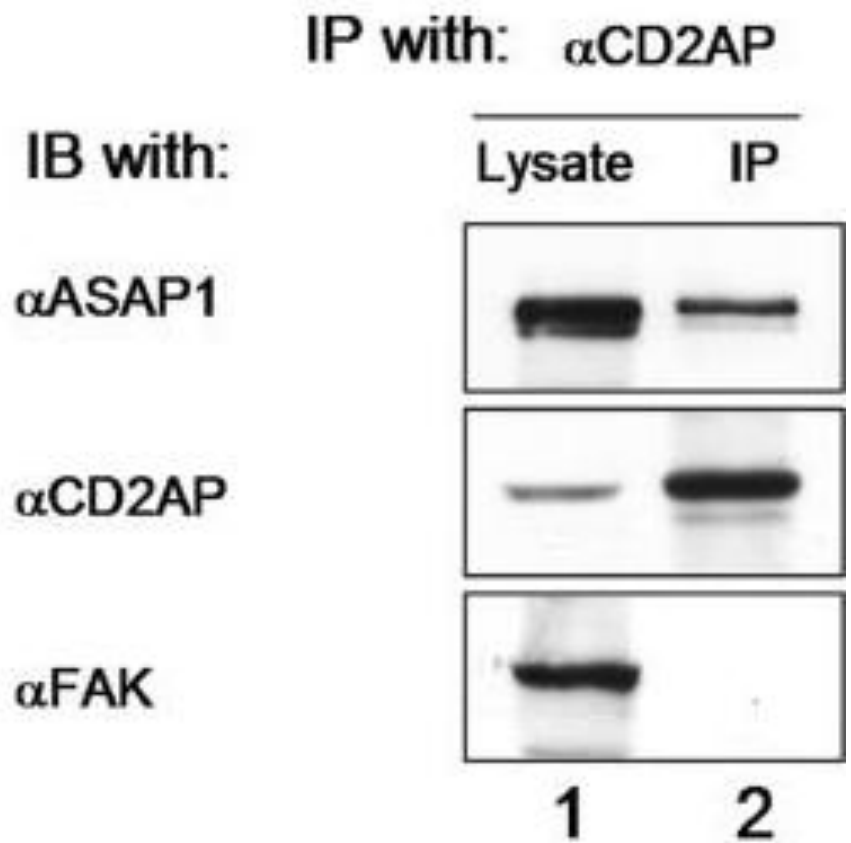


Co-Immunoprecipitation (Co-IP)

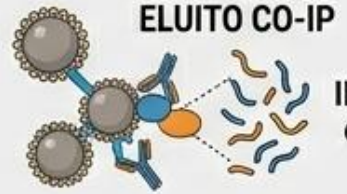
Advantages	1.Proteins that interact in Co-IP are **post-translationally modified** and conformationally natural. 2.Proteins that interact in Co-IP are in a **natural state**. 3.Interacting protein complexes in a natural state can be obtained.
Disadvantages	1.**Low-affinity** and instantaneous protein-protein interactions **may not be detected** in Co-IP. 2.Two proteins may not be **directly combined**. There may be some influence of another substance. 3.In order to select detecting antibody, researchers should predict the target protein. If the forecasting is incorrect, nothing will be obtained.

4. ELUIZIONE





2. SPETTROMETRIA DI MASSA (MS) PER IDENTIFICAZIONE GLOBALE



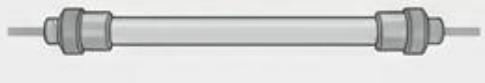
ELUITO CO-IP

DIGESTIONE TRIPTICA IN GEL O SOLUZIONE

IP: Anticorpo di Controllo (IgG)

IP: Anticorpo Specifico (anti-Bait)

SEPARAZIONE LC (CROMATOGRAFIA LIQUIDA)



ANALISI MS/MS
(SPETTROMETRIA DI MASSA TANDEM)

IDENTIFICAZIONE E BIOINFORMATICA

Proteina Bait
Proteina Prey
Nuovi Interattori Identificati

Cerca data

IDENTIFICAZIONE E BIOINFORMATICA

Controls are crucial for a successful Co-IP experiment

Negative	Positive
Beads only	Whole cell lysate
Beads + non-specific ab	Purified recombinant protein
Non-specific ab only	Known binding partner