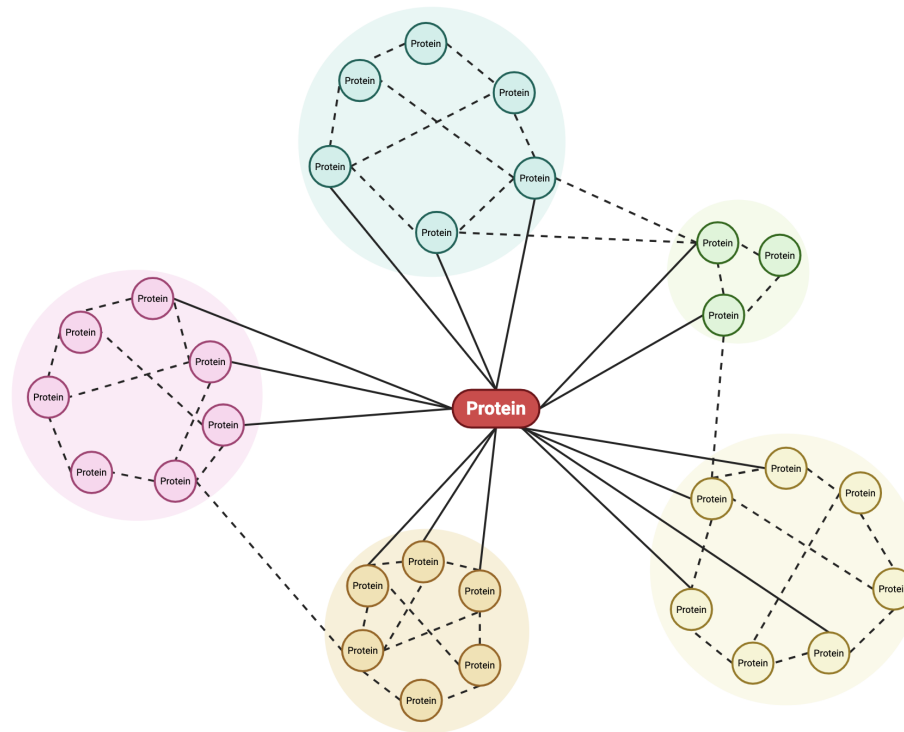


CdS in Scienze e Tecnologie Biologiche

Corso di Biotecnologie Cellulari 2025-26

Lezione 8



Analisi dell'interazione proteina-proteina

ESPERIMENTO VIRTUALE:

- a) Sovraesprimere separatamente le ORFs VIRALI nel modello cellulare**
- b) Analizzare l'INTERATTOMA (proteine cellulari associate) delle proteine virali**

APPROCCIO SPERIMENTALE:

Utilizzo di tecniche che permettono di isolare proteine endogene associate alla proteina «ESCA» sovraespressa

Immobilizzare la bait su una fase solida

FASE
SOLIDA

"Bait"

+

Incubate

Lisato cellulare

"Prey"

FASE
SOLIDA

"Bait"

"Prey"

Wash

FASE
SOLIDA

"Bait"

"Prey"

Elute

FASE
SOLIDA

"Bait"

"Prey"

"Bait" only

Sample



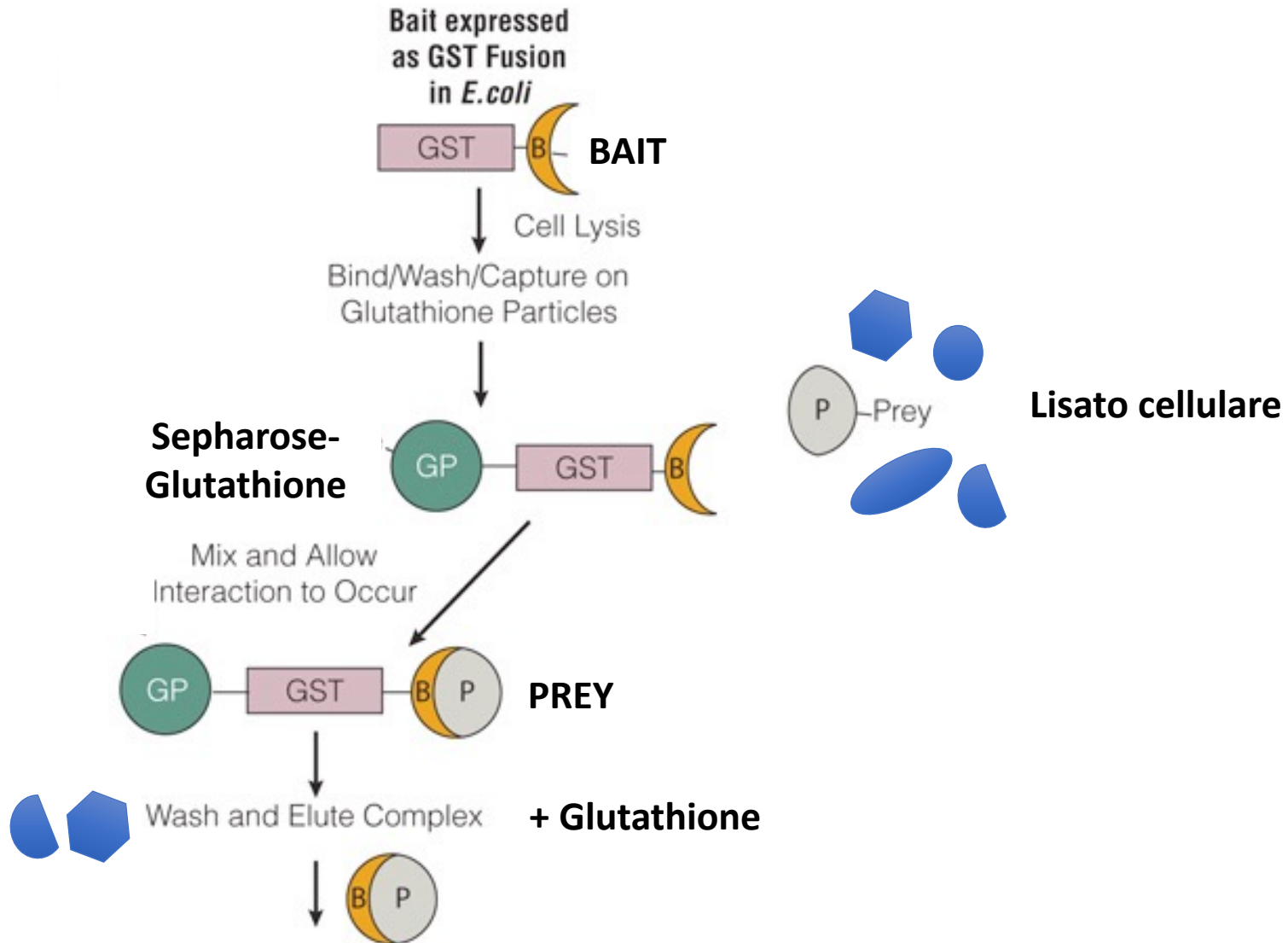
- "Bait"

- "Prey"

Detect

Espressione di bait come proteina di fusione in batteri

Es. GST-pulldown



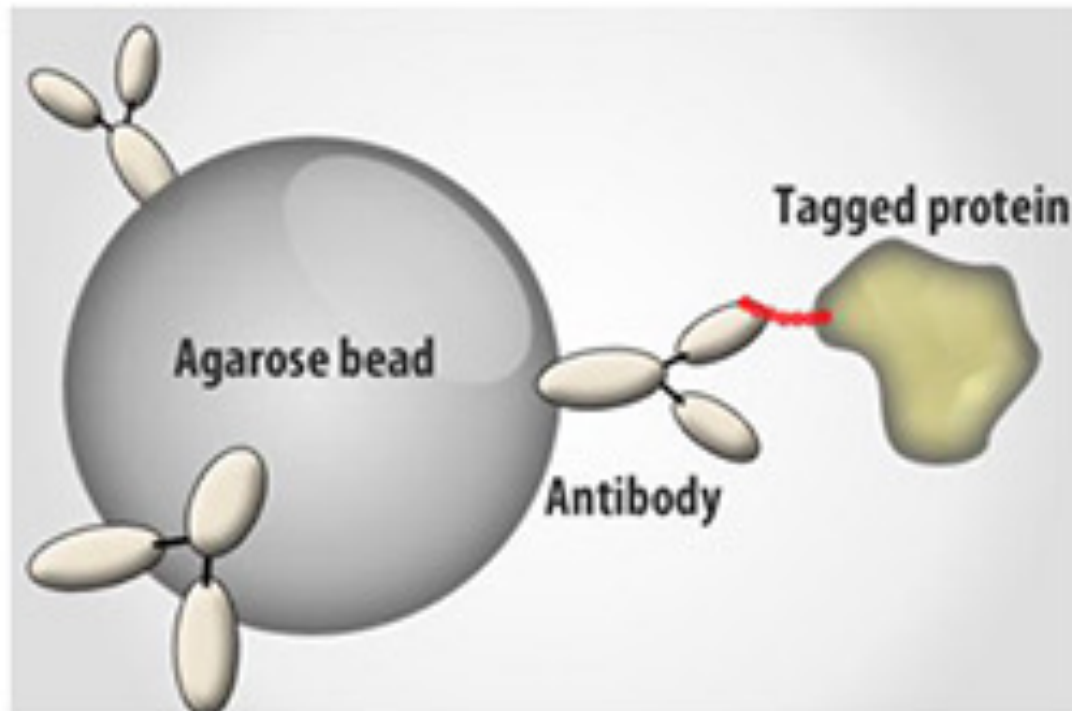
**Limite dell'approccio: le interazioni NON avvengono tra
proteine della cellula**

Approccio alternativo:

**PURIFICAZIONE di proteine SOVRAESPRESSE (in fusione) o
ENDOGENE**

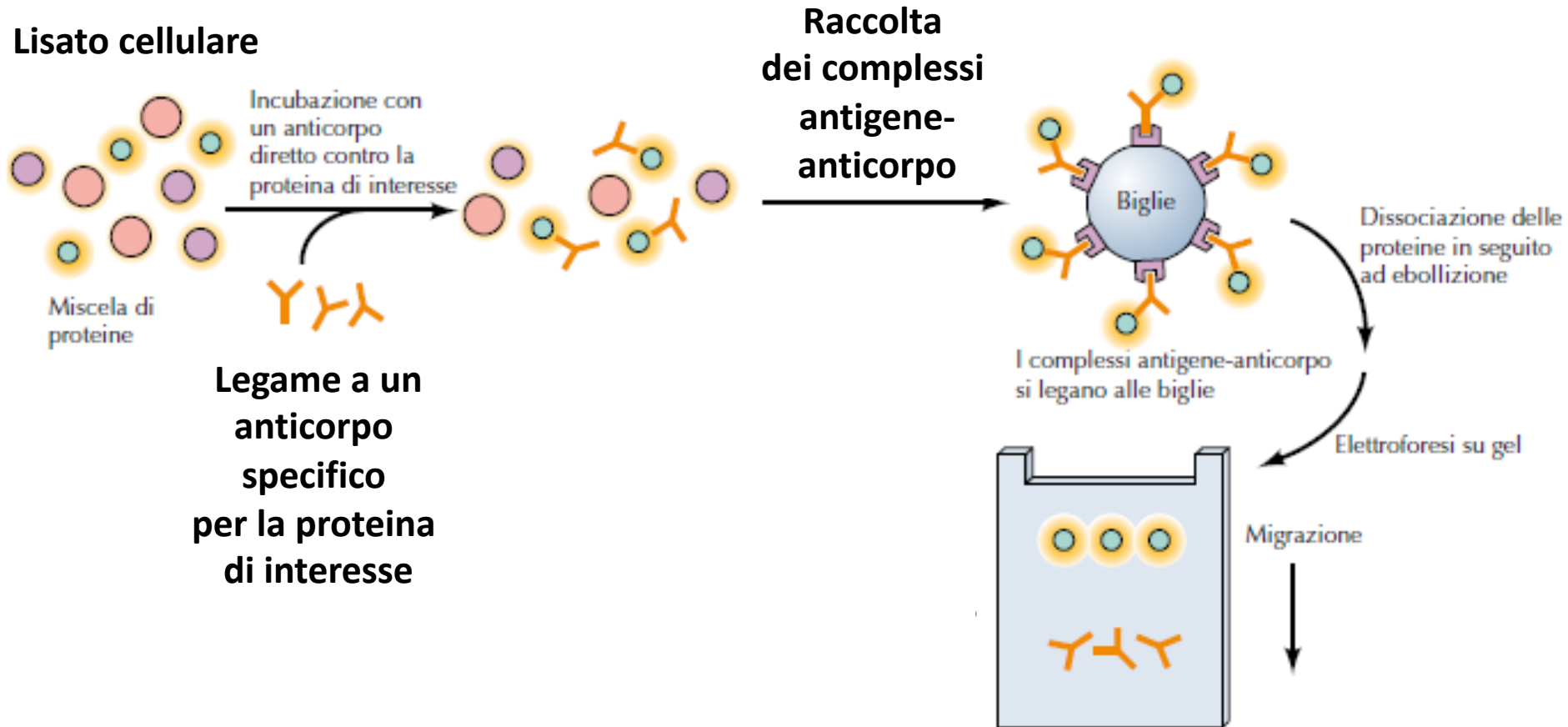
isprese in cellule di mammifero e analisi dell'interattoma

IMMUNOPRECIPITAZIONE: **purificazione di proteine mediante anticorpi specifici**



Vantaggio: si può applicare anche a proteine ENDOGENE

Immunoprecipitazione

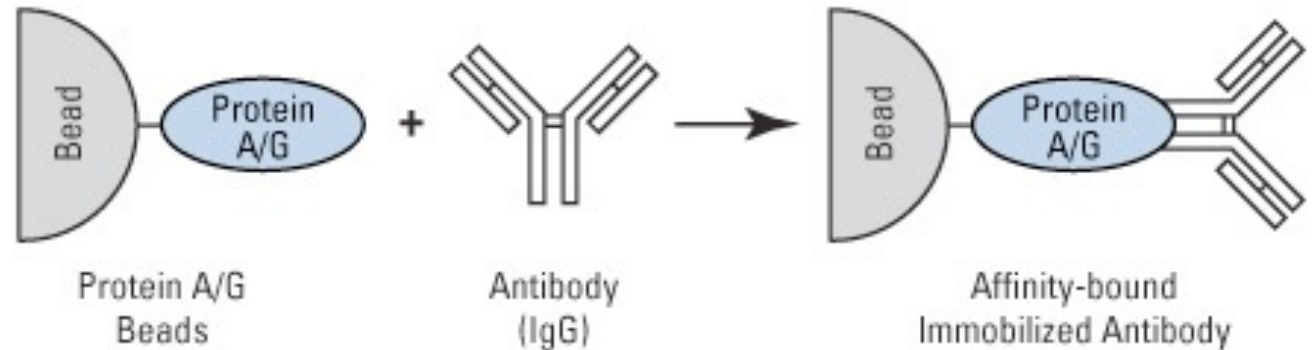


Immobilizzazione degli anticorpi sulla resina

https://www.cd-bioparticles.com/t/Protein-Isolation_48.html

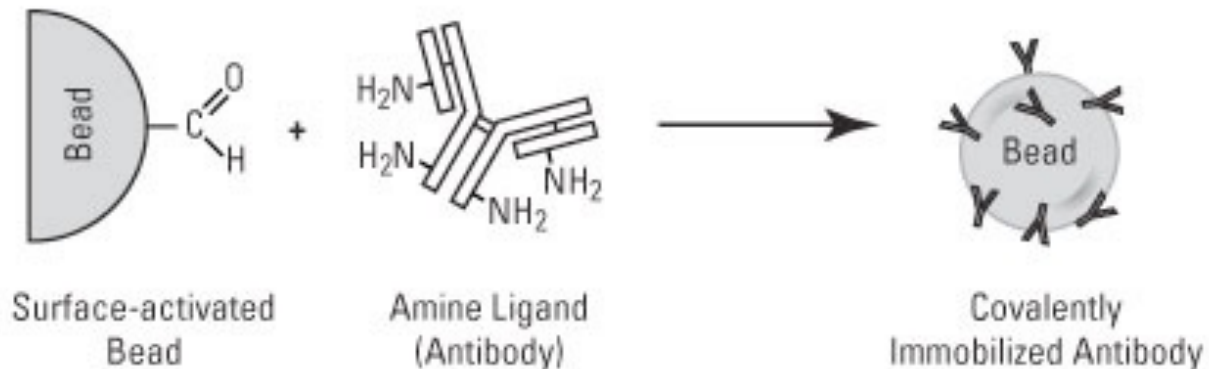
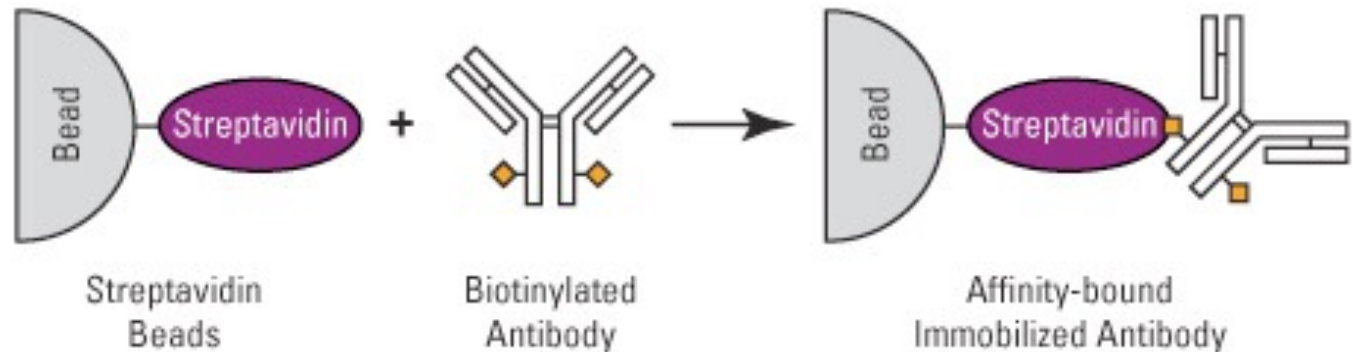
IgG binding proteins

Protein A
Staphilococcus



Protein G
Streptococcus

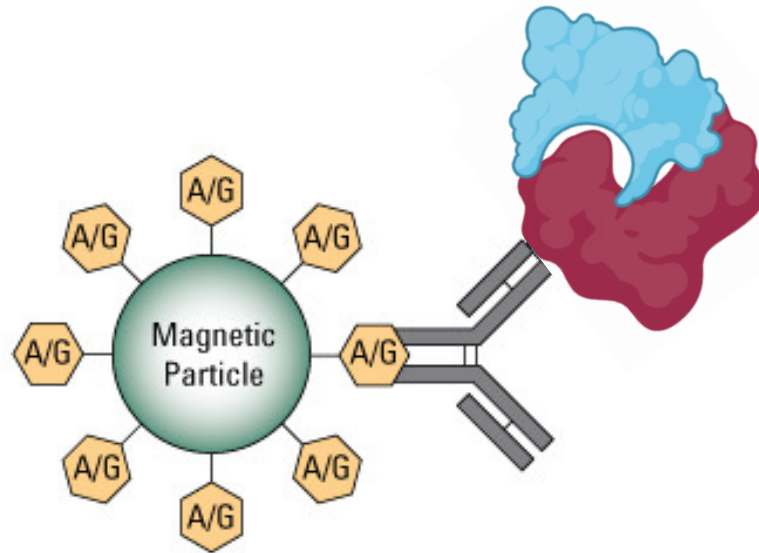
Protein A/G
Recombinant fusion protein



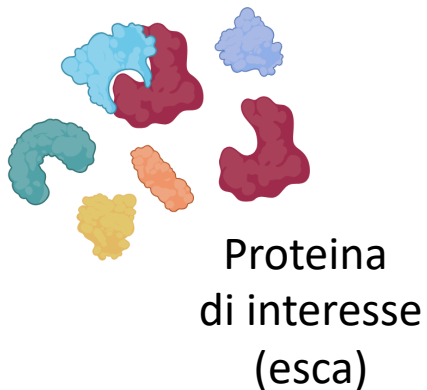
Proteine «taggate» sovraespresso o proteine endogene?

Comparison of Co-IP with endogenous proteins versus tagged proteins		
	Endogenous proteins	Tagged proteins (pull-down assay)
Main advantages	Protein complexes are isolated in a relatively natural state.	An <i>N</i> - or <i>C</i> -terminal tag is likely available for antibody binding after complex formation. Antibody binding is unlikely to interfere with complex formation.
Issues to consider	The epitope may be buried upon complex formation. Antibody binding may interfere with complex formation.	The expression levels of recombinant proteins are substantially higher than those of their endogenous counterparts, which may result in artifactual results.

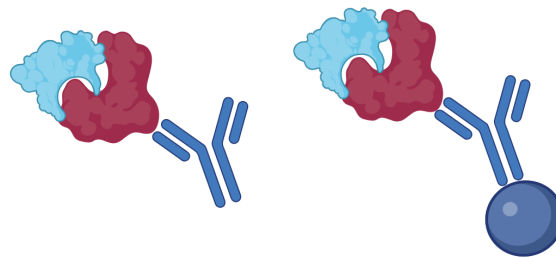
Identificazione degli interattori della proteina esca



1) Lisato cellulare



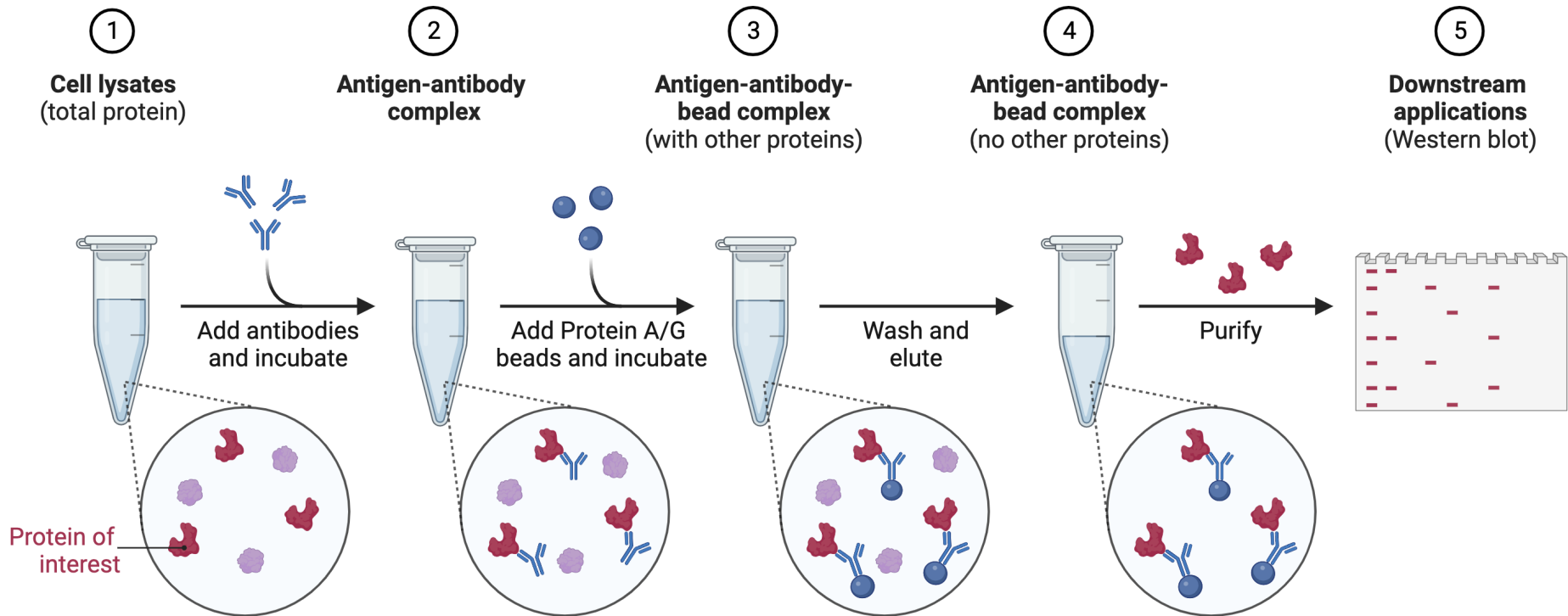
2) Purificazione dell'esca



3) Analisi delle proteine co-purificate

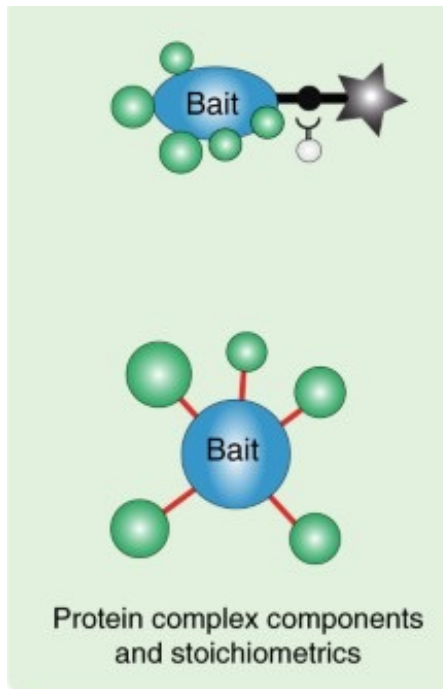
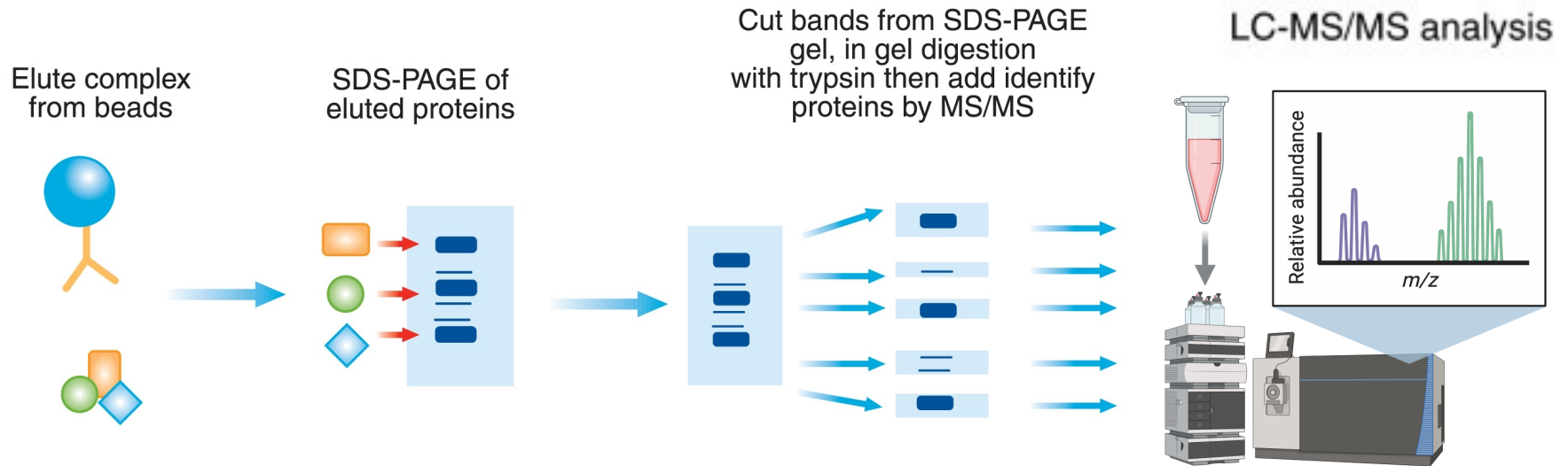


SCELTA DEI SAGGI PER L'ANALISI DELL'INTERATTOMA #1



Se le proteine partner sono **candidati noti**,
si possono analizzare mediante **Western Blot** con specifici anticorpi

SAGGI E STRUMENTI PER L'ANALISI DELL'INTERATTOMA #2



Analisi dell'interattoma mediante spettrometria di massa

Se le proteine partner **NON sono note**, si sottopongono le bande proteiche dell'interattoma a digestione e successiva analisi mediante **spettrometria di massa**.

ANALISI DEI RISULTATI

DELL'INTERATTOMA DELLE PROTEINE DI SARS-COV-2 IN CELLULE UMANE

Article

Nature | Vol 583 | 16 July 2020 | 459

A SARS-CoV-2 protein interaction map reveals targets for drug repurposing

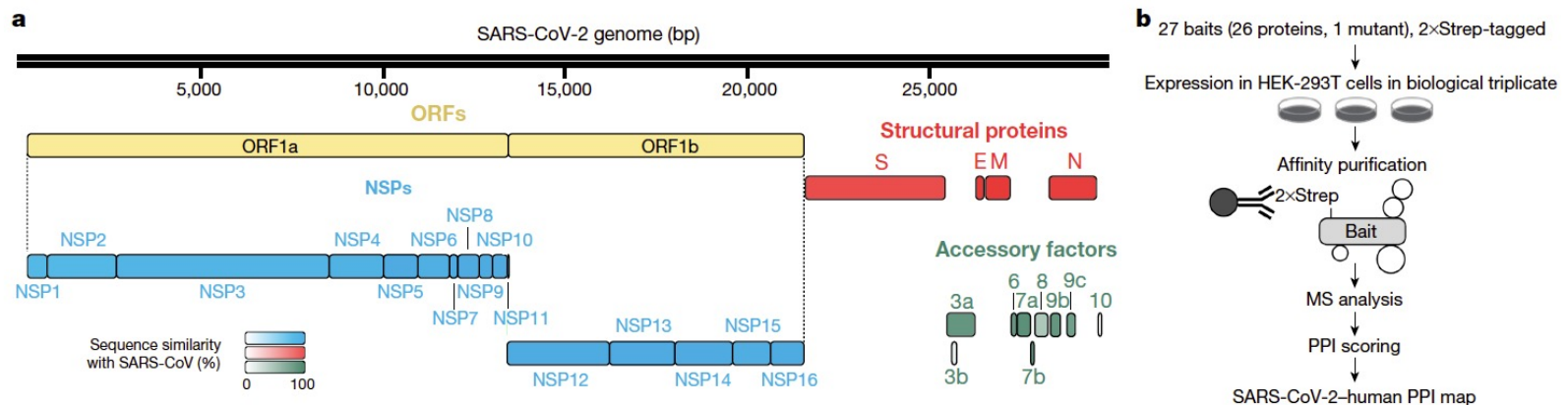


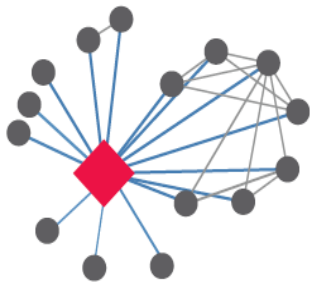
Fig. 1 | AP-MS workflow for the identification of SARS-CoV-2-host protein-protein interactions. **a**, SARS-CoV-2 genome annotation. The colour intensity is proportional to the protein sequence similarity with SARS-CoV homologues

(when homologues exist). $n = 4$ structural proteins; $n = 16$ NSPs; $n = 9$ accessory factors. **b**, Experimental workflow for AP-MS studies. MS, mass spectrometry; PPI, protein-protein interaction.

5) ANALISI DEI RISULTATI
DELL'INTERATTOMA DELLE PROTEINE DI
SARS-COV-2 IN CELLULE UMANE

a

26 SARS-CoV-2 proteins

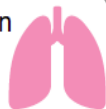


332 human
proteins

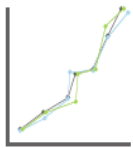
GO and Pfam enrichments



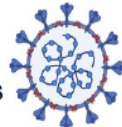
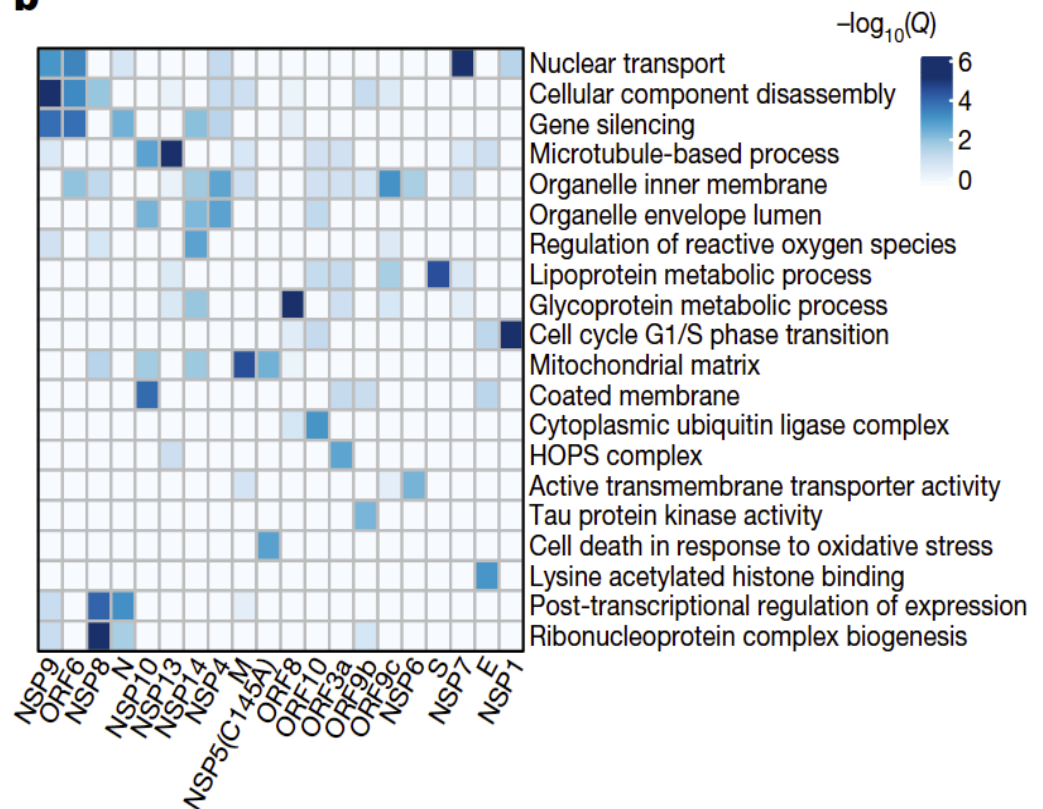
Tissue expression
(protein and
mRNA)



Response to infection



Comparative viral networks

**b**

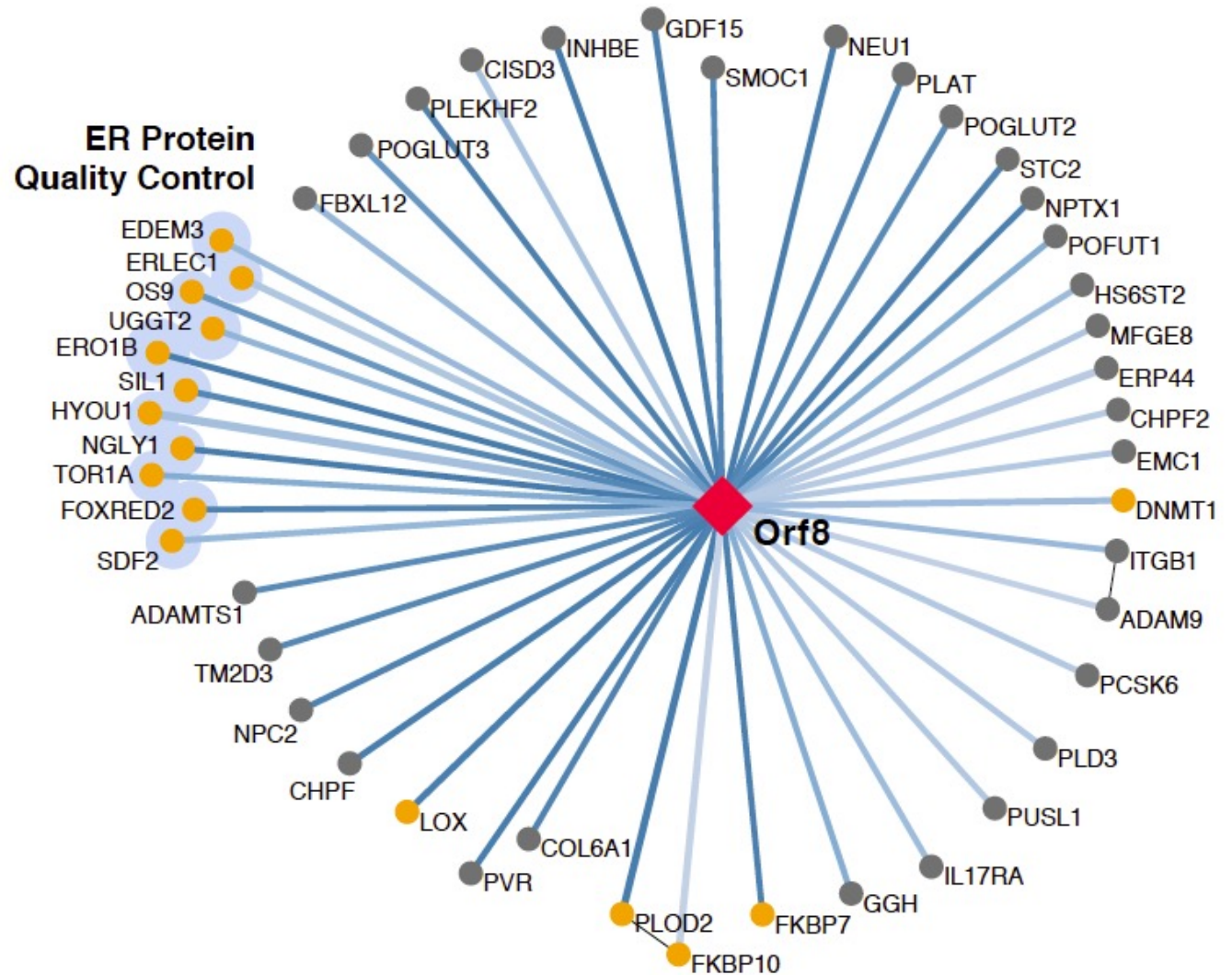
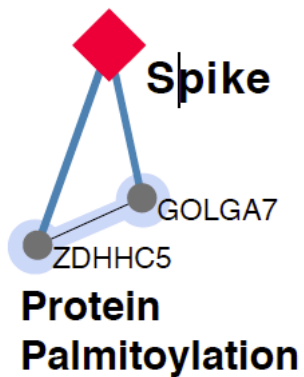
ANALISI DELL'INTERATTOMA DELLE PROTEINE DI SARS-COV-2 IN CELLULE UMANE

25% Nucleus
24% Secretory pathway
15% cytosol
13% Plasma membrane
13% mito
6% ECM
2% endosome
2% centrosome

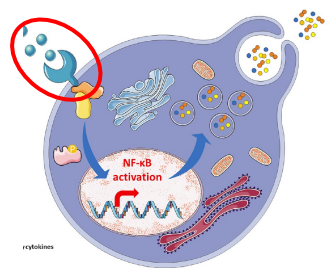
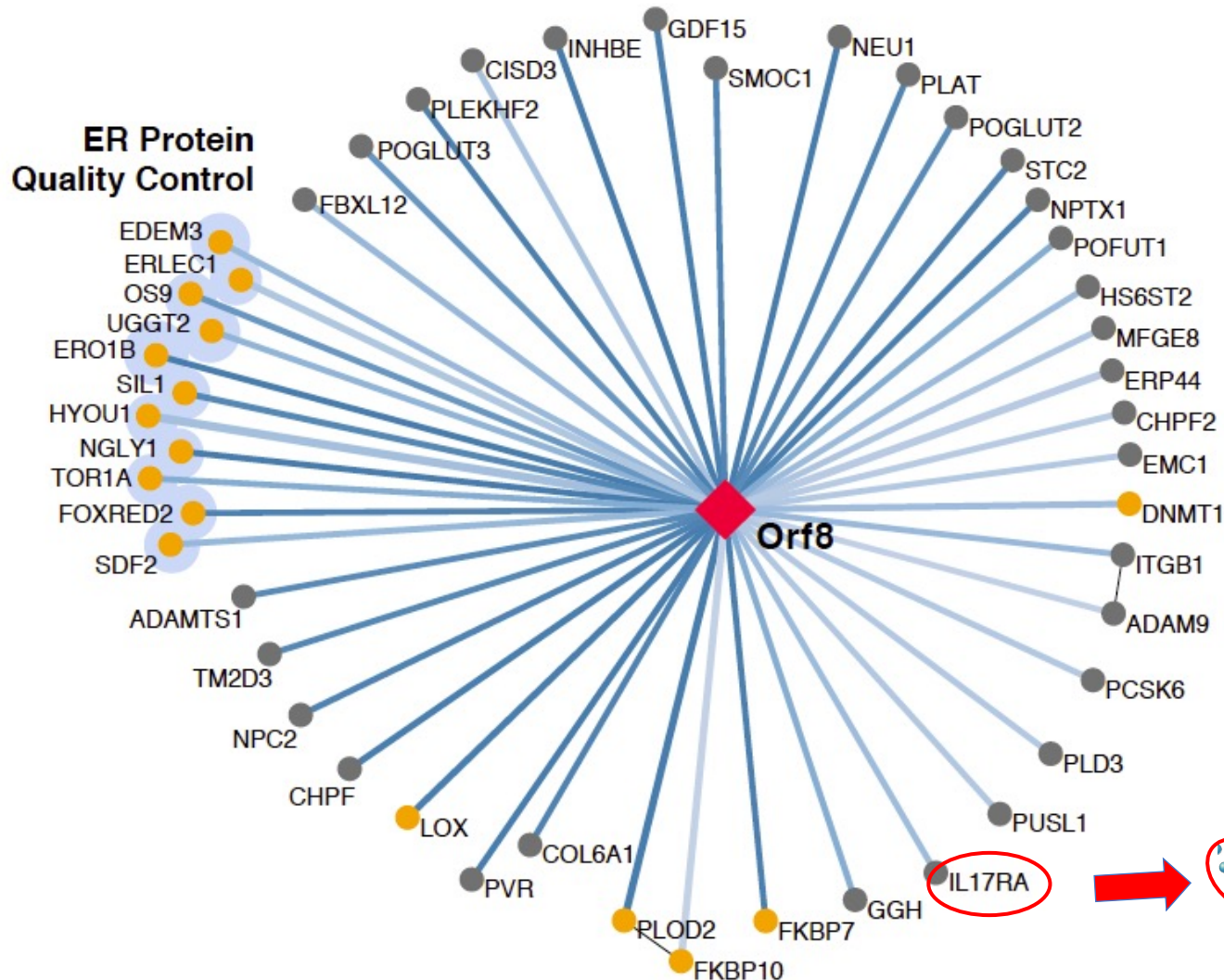


47% ER membrane
28% Golgi
17% ER lumen
8% ERGIC

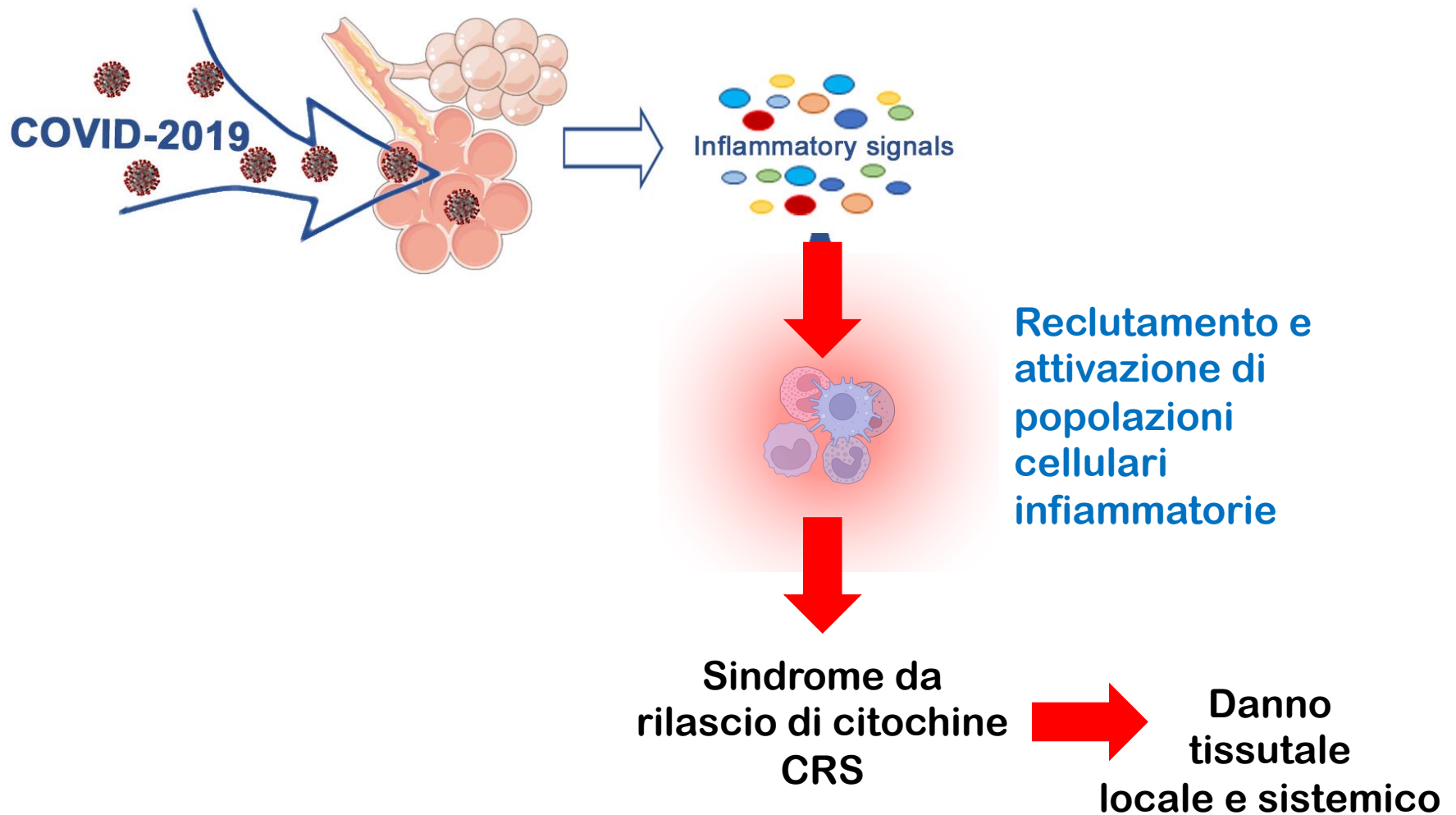
Rappresentazione dell'interattoma



Interattoma di ORF8

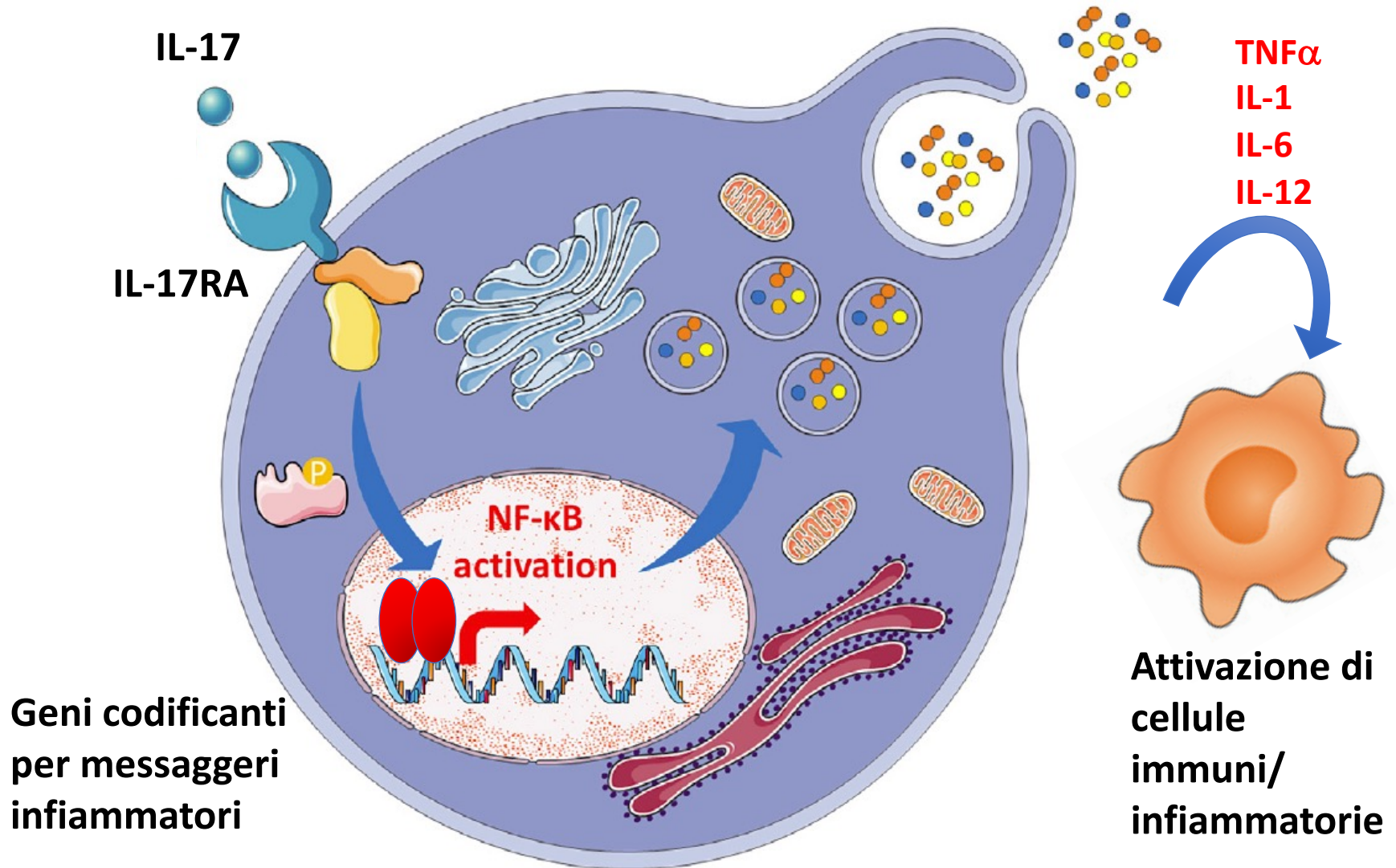


La patologia COVID severa è causata una **risposta infiammatoria forte/cronica** che è scatenata nell'ospite dall'interazione tra specifici **fattori virali e fattori cellulari**

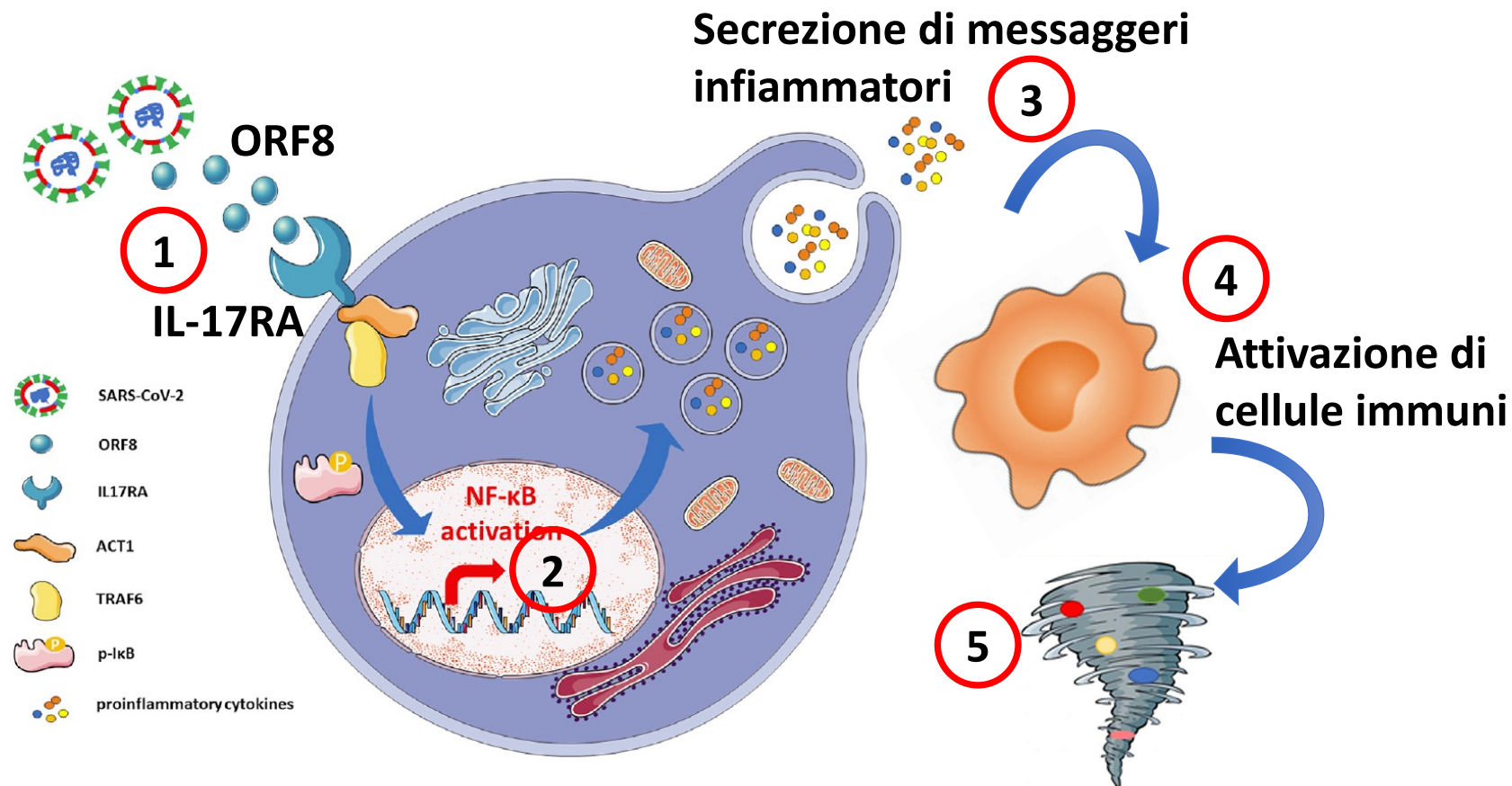


IL-17 RA è un recettore coinvolto nell'inflammatione

Secrezione di messaggeri infiammatori



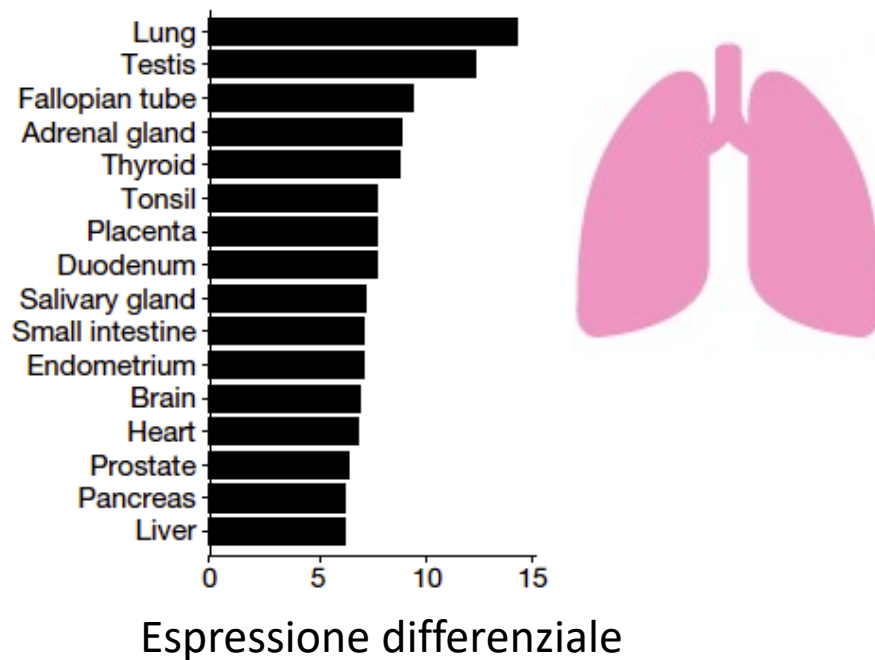
IPOTESI: ORF8 lega e attiva il recettore IL-17RA innescando una risposta infiammatoria



**Cytokine release syndrome CRS
= infiammazione persistente
e tossicità tissutale**

È plausibile che IL-17 RA sia implicato nell'infiammazione indotta da SARS-COV2?

Analisi dell'espressione di IL-17 RA (mRNA e proteina) in diversi tessuti

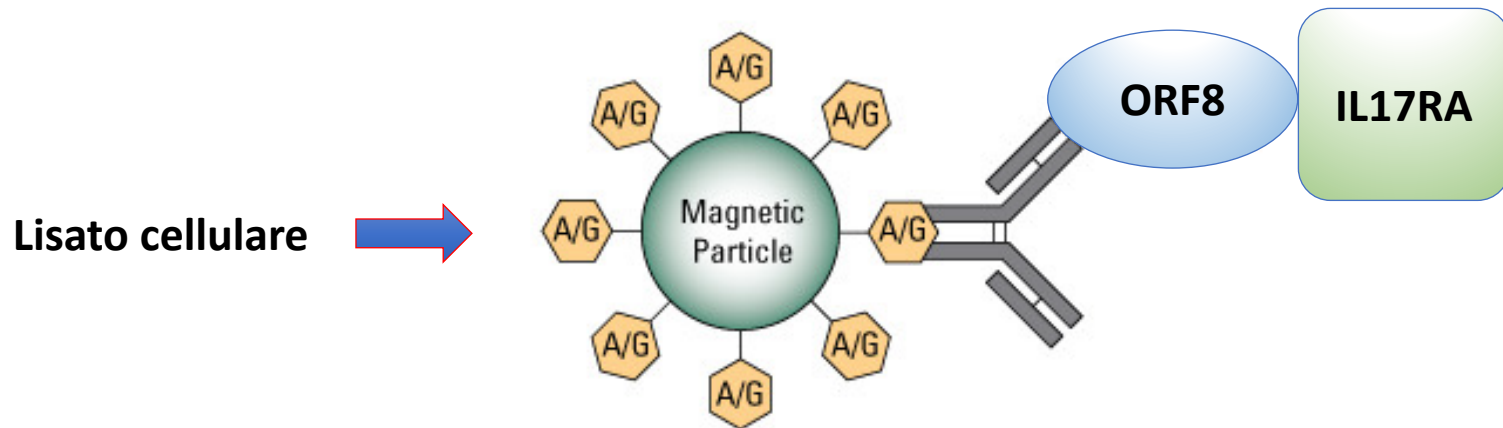


**È plausibile che IL-17 RA sia implicato
nell'infiammazione indotta da SARS-COV2?**

**È necessario una conferma (validazione)
dell'interazione tra le proteine ORF8 e IL17RA
di tipo sia biochimico che biologico**

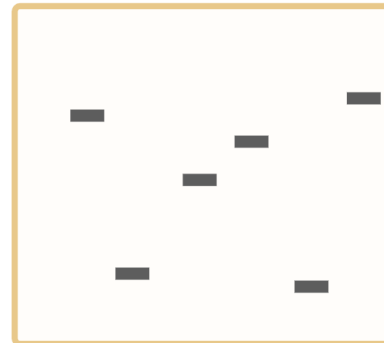
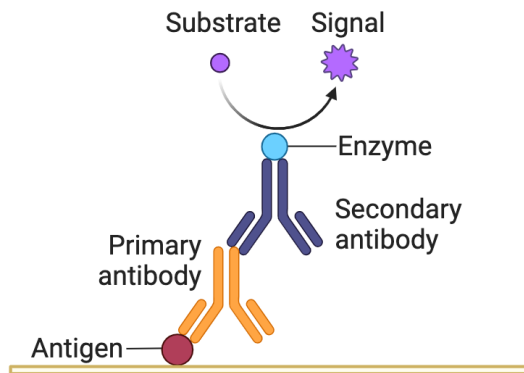
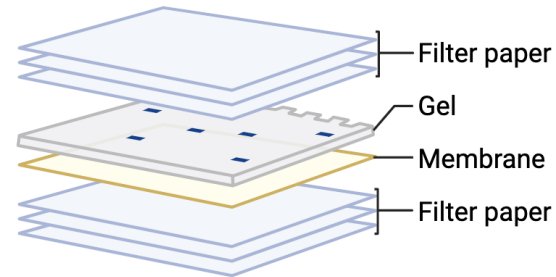
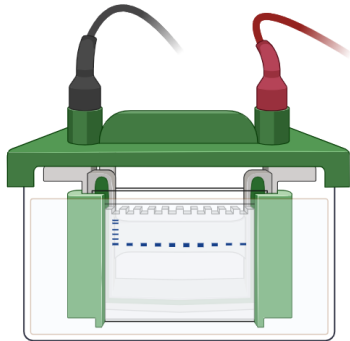
Validazione delle interazioni (1): co-IP oppure AP e western blotting

È utile anche per effettuare una MAPPATURA dei domini proteici coinvolti
nell'interazione
e per stabilire in QUALI CONDIZIONI essa avviene

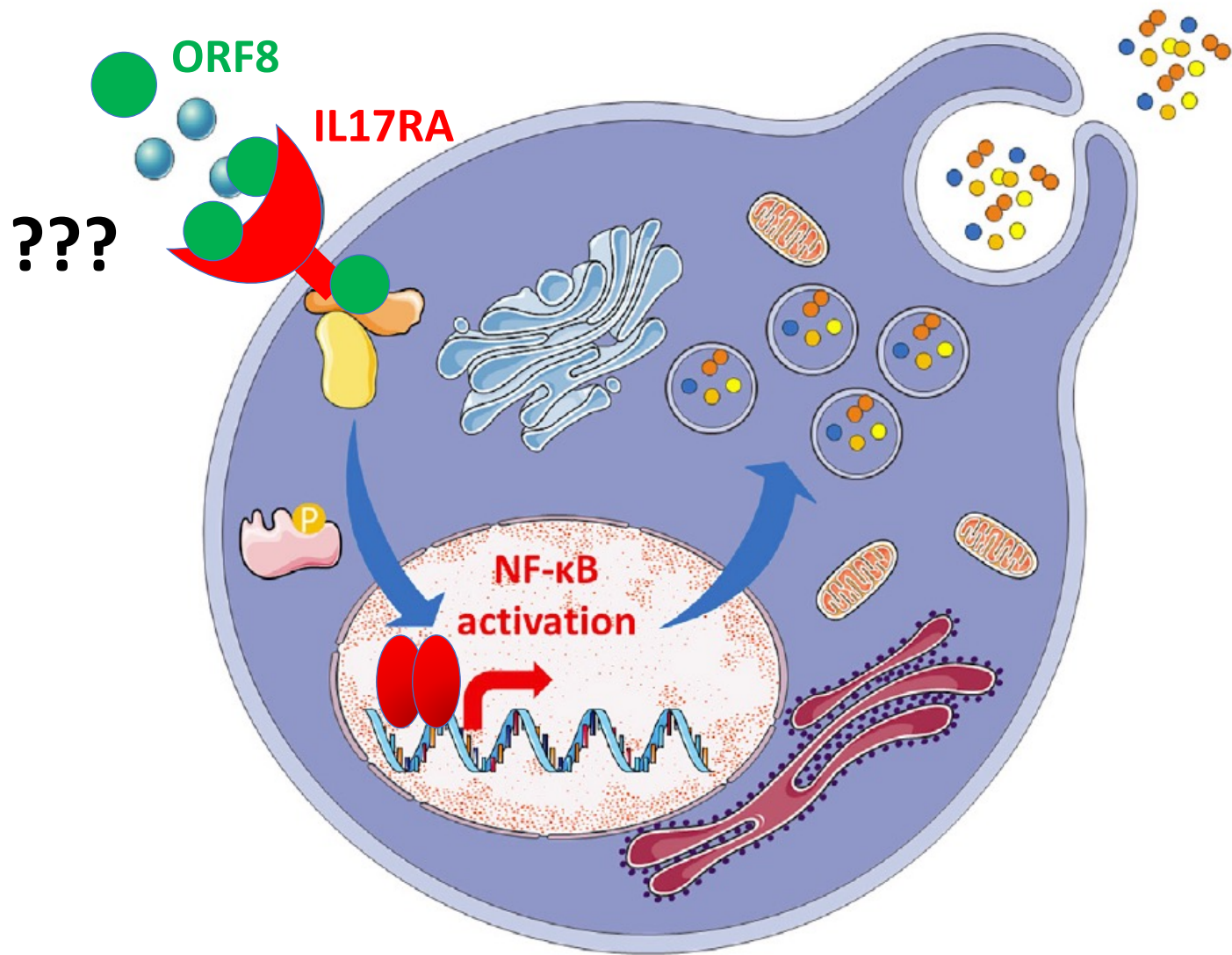


Le proteine partner sono candidati noti,
quindi si possono analizzare mediante WB

Validazione delle interazioni (1): co-IP e western blotting



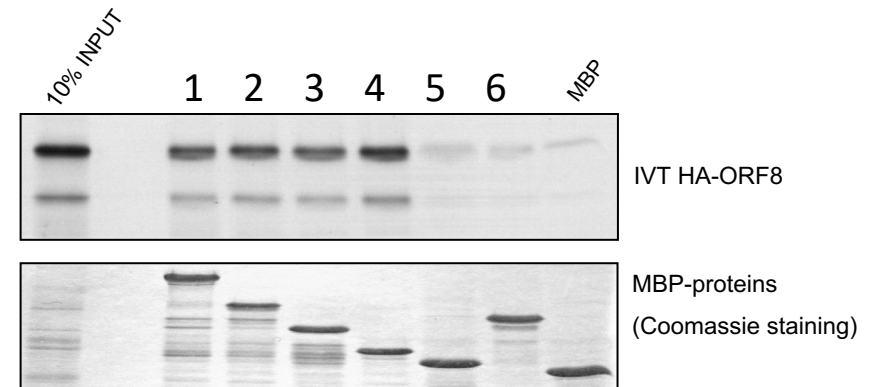
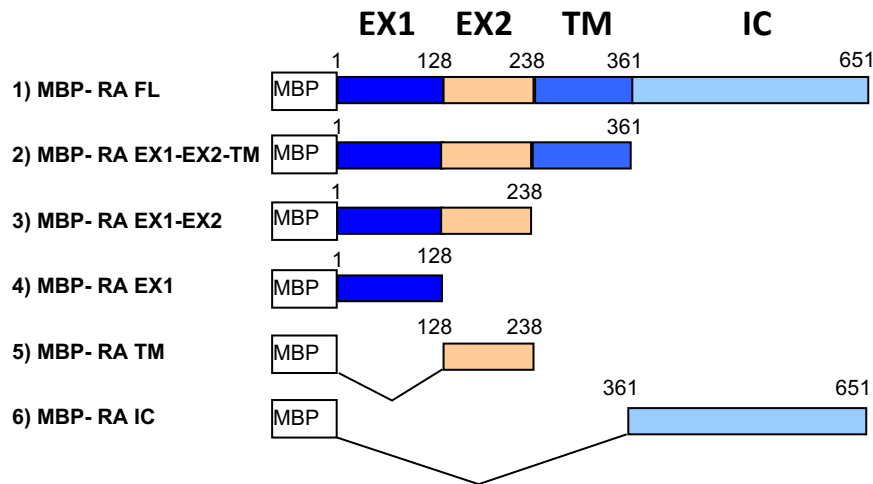
MAPPATURA DELLE INTERAZIONI



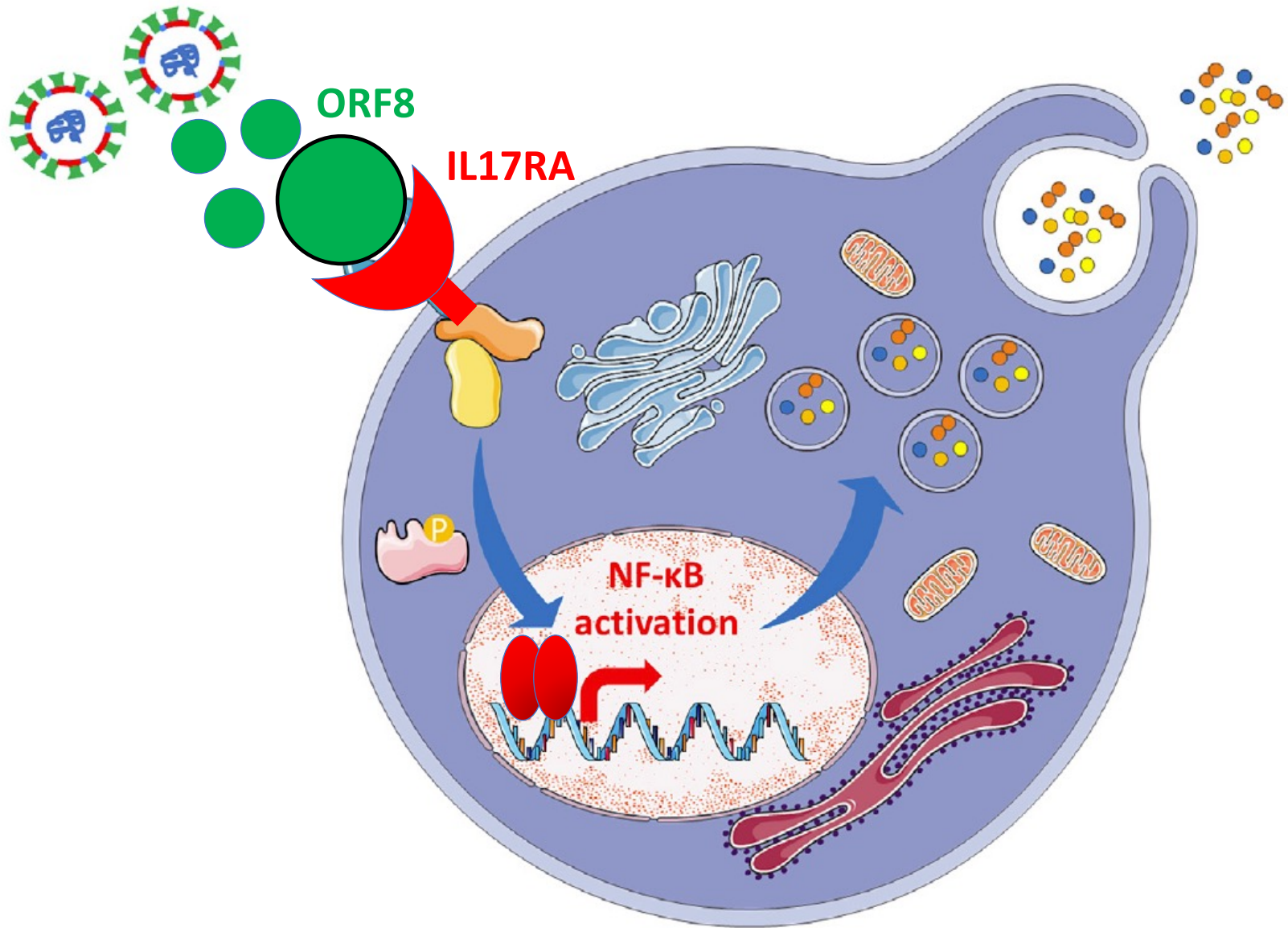
MAPPATURA DELLE INTERAZIONI

Mediante CO-IP/AP e WB/FAR-WESTERN con PORZIONI
(mutanti di delezione) delle proteine

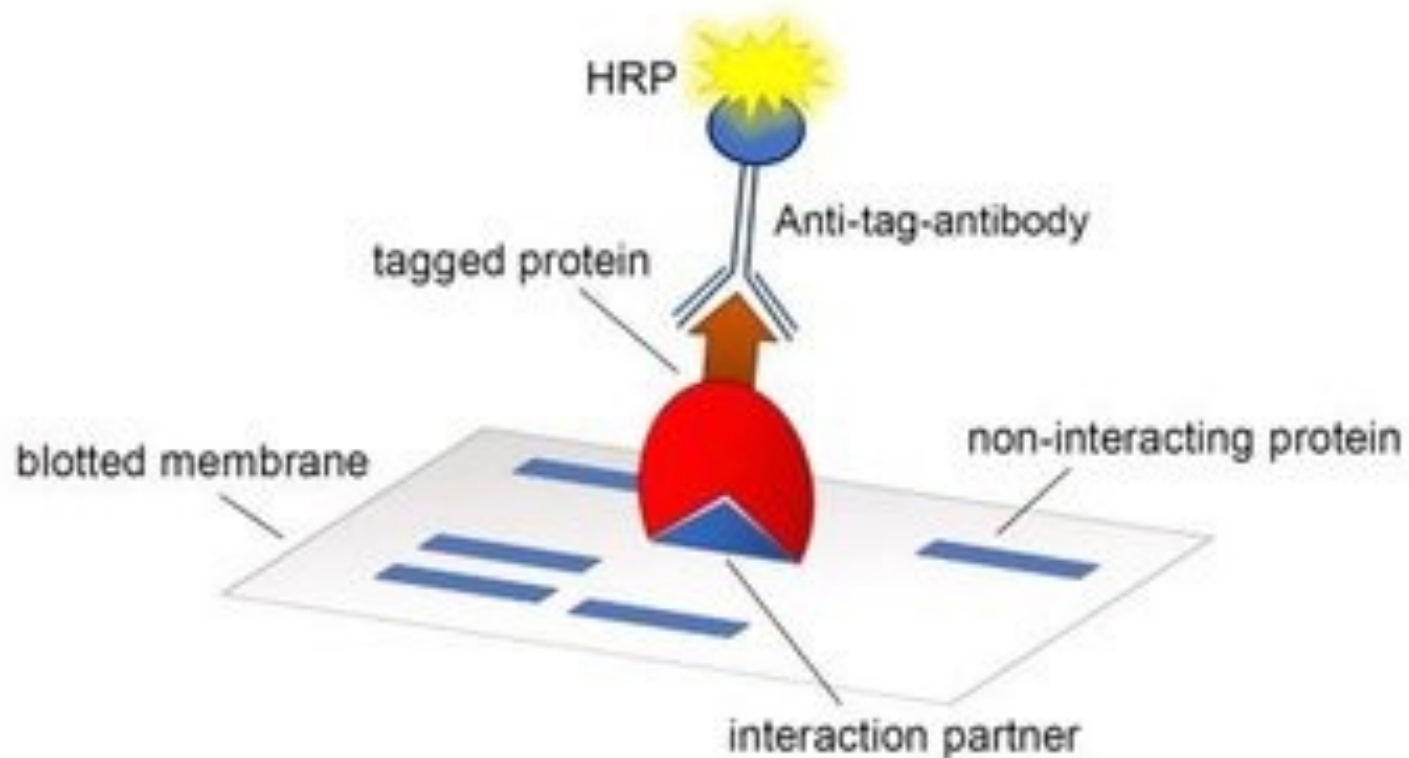
Pulldown of MBP-RA deletion proteins with IVT HA-ORF8



ORF8 lega la porzione extracellulare del recettore IL-17

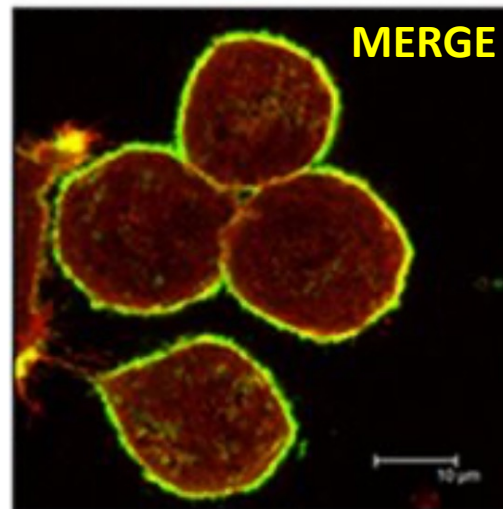
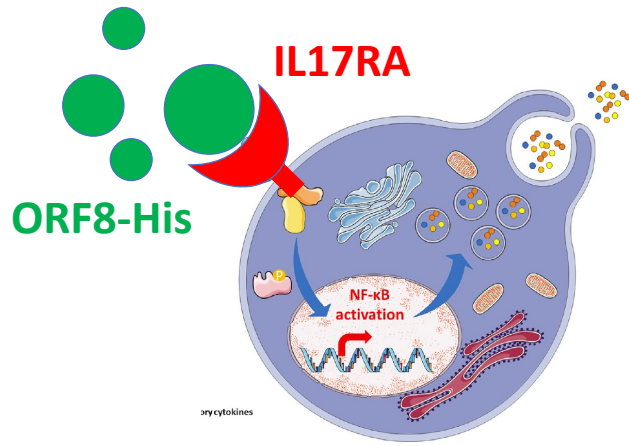


VALIDAZIONE DELLE INTERAZIONI (2): Analisi di interazione DIRETTA proteina-proteina mediante FAR WESTERN:



VALIDAZIONE DELLE INTERAZIONI (3): immunofluorescenza e microscopia confocale

- Aggiunta di ORF8-His alla coltura di cellule H1299

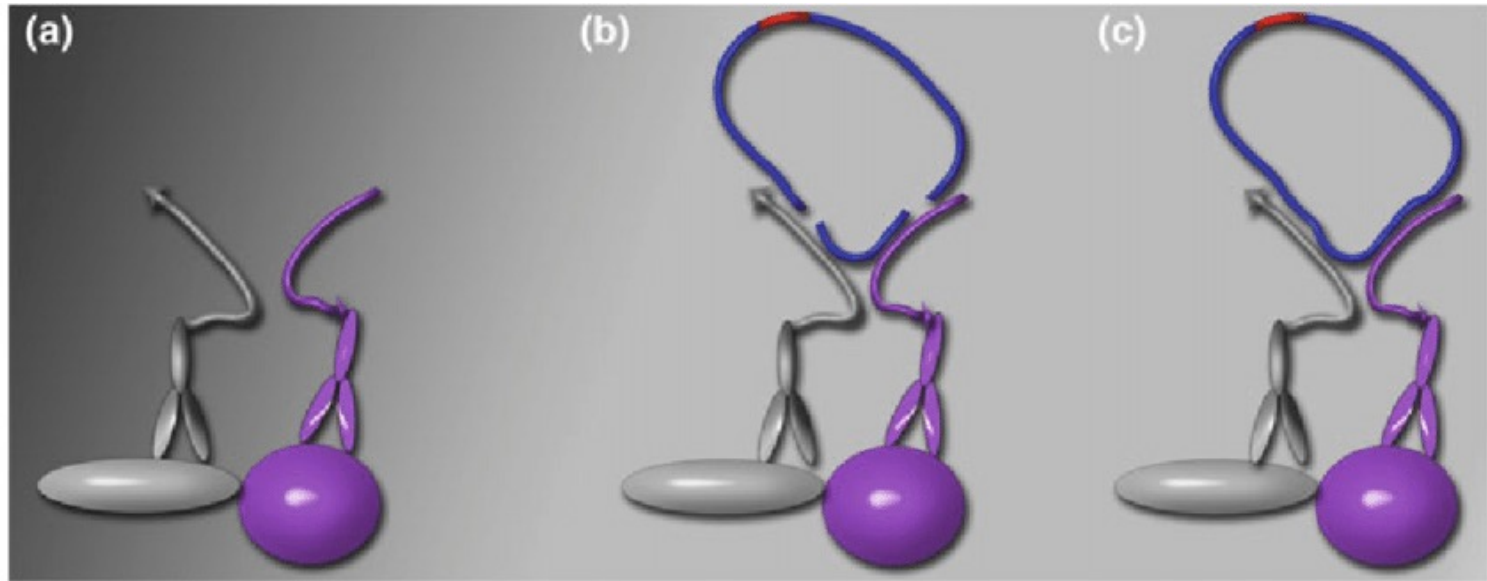


VALIDAZIONE DELLE INTERAZIONI (4)

**Analisi dell'interazione diretta proteina-proteina
IN SITU:**

Proximity Ligation Assay PLA

IN SITU Proximity Ligation Assay PLA

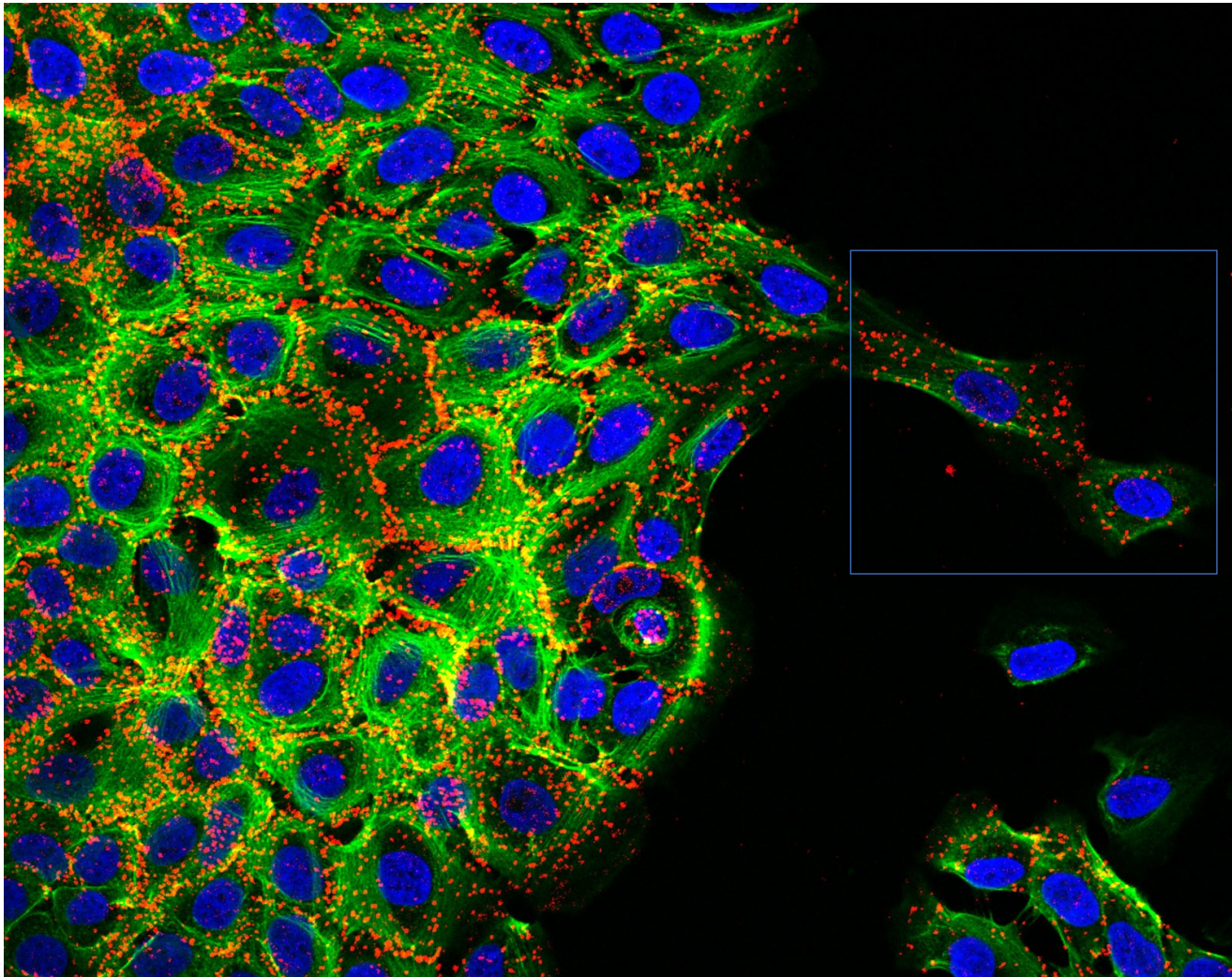


Secondary antibodies
conjugated to
oligonucleotides

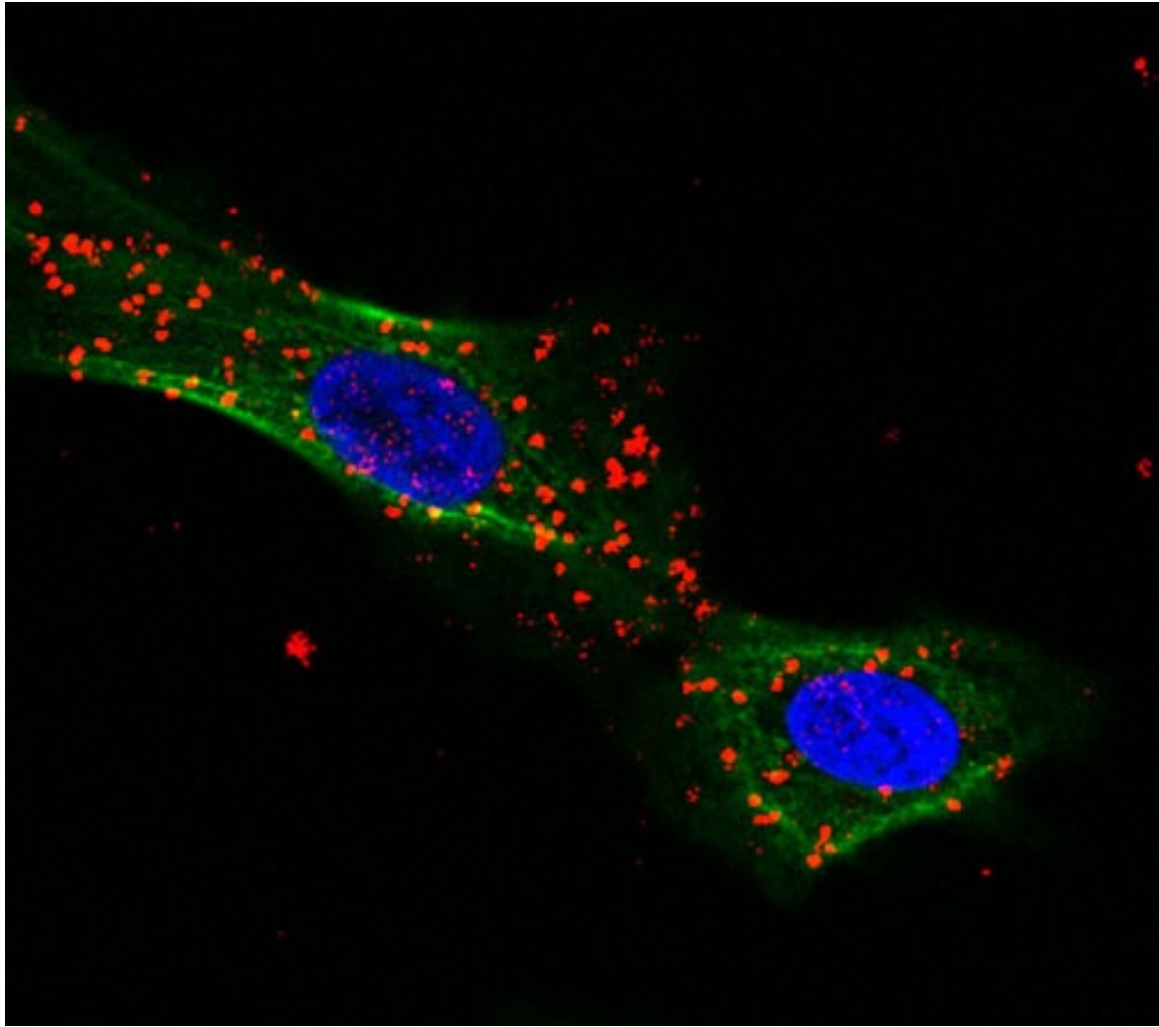
used as templates for
the joining of linear
oligos into a DNA circle

DNA amplification
hybridize with
fluorescent probe

NB il saggio evidenzia la **PROSSIMITA'**: le proteine devono essere distanti meno di **40 nm**



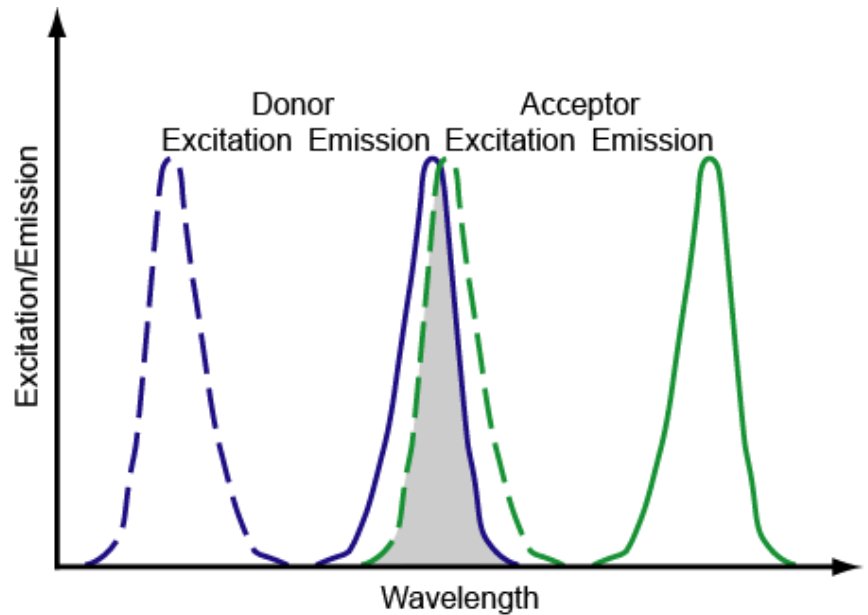
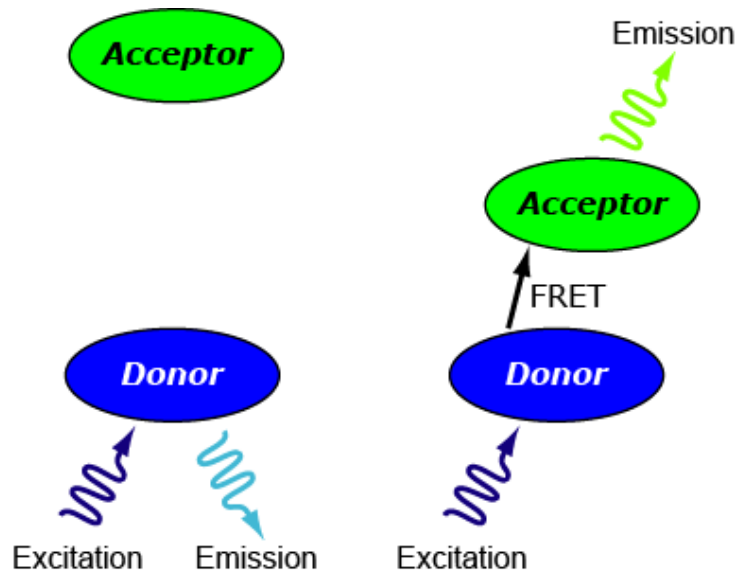
In situ proximity ligation assay for E-cadherin and p120ctn (red) in MCF10A-ER-Src cells stained with Phalloidin to mark actin filaments (green) and DAPI



VALIDAZIONE DELLE INTERAZIONI (5)

**Analisi dell'interazione proteina-proteina IN SITU
mediante FRET
Fluorescence (Förster) Resonance Energy Transfer**

Förster Resonance Energy Transfer



Emission (donor) and excitation (acceptor) spectra must significantly overlap

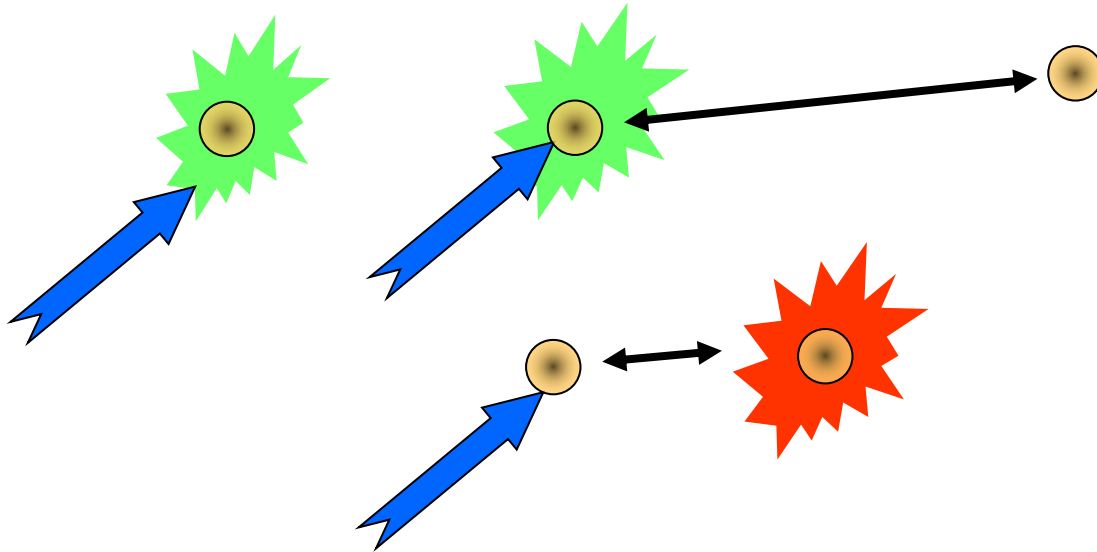
Es. CFP/YFP

Cy3/Cy5

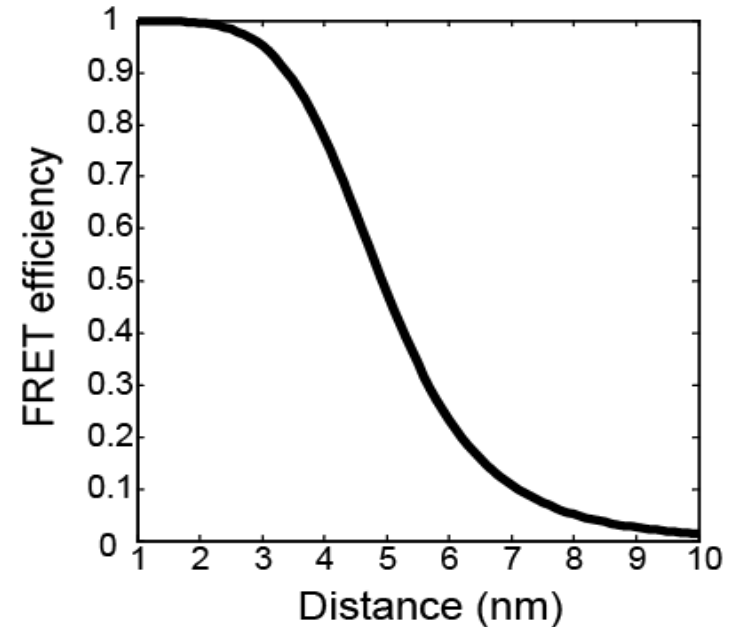
La FRET è efficiente su piccole distanze

Isolated donor

Donor distance too great



Donor distance correct



For CFP-YFP,
50% transfer at $R_0 = 4.9$ nm

È efficiente per **distanze inferiori a 10 nm**
minore del limite di risoluzione del microscopio a fluorescenza!