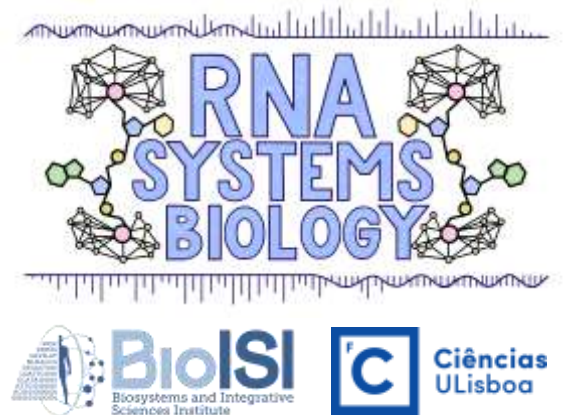


# Network biology approaches in the identification of novel pharmacological targets

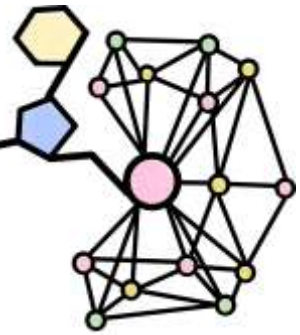
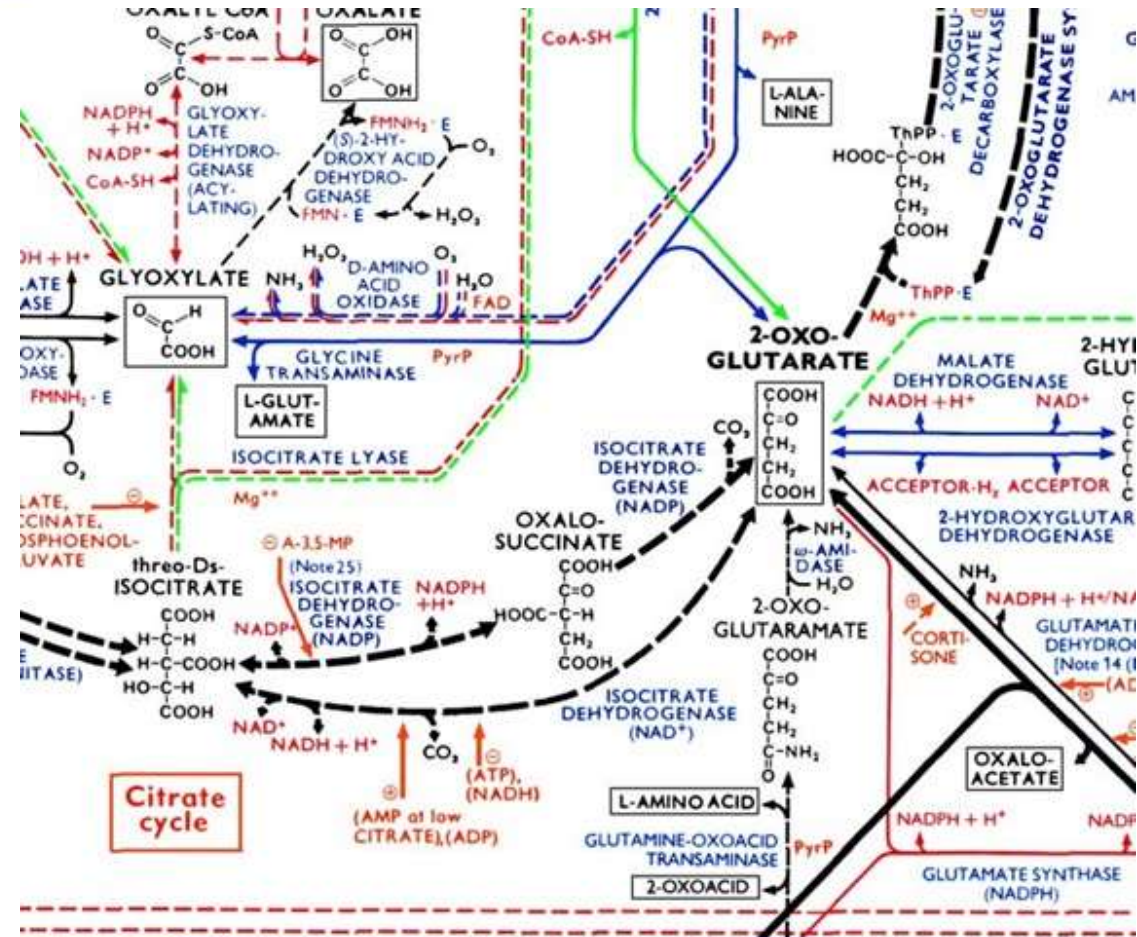


Francisco Rodrigues Pinto



# Biological Systems are complex

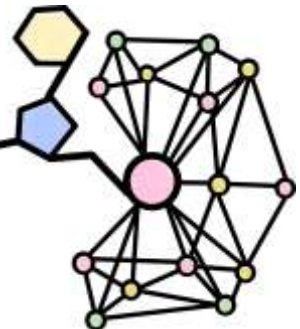
- High number of components and/or **interactions** between them
- Not possible to predict system behavior just knowing the behavior of individual components
- Computational approaches are needed to integrate available information



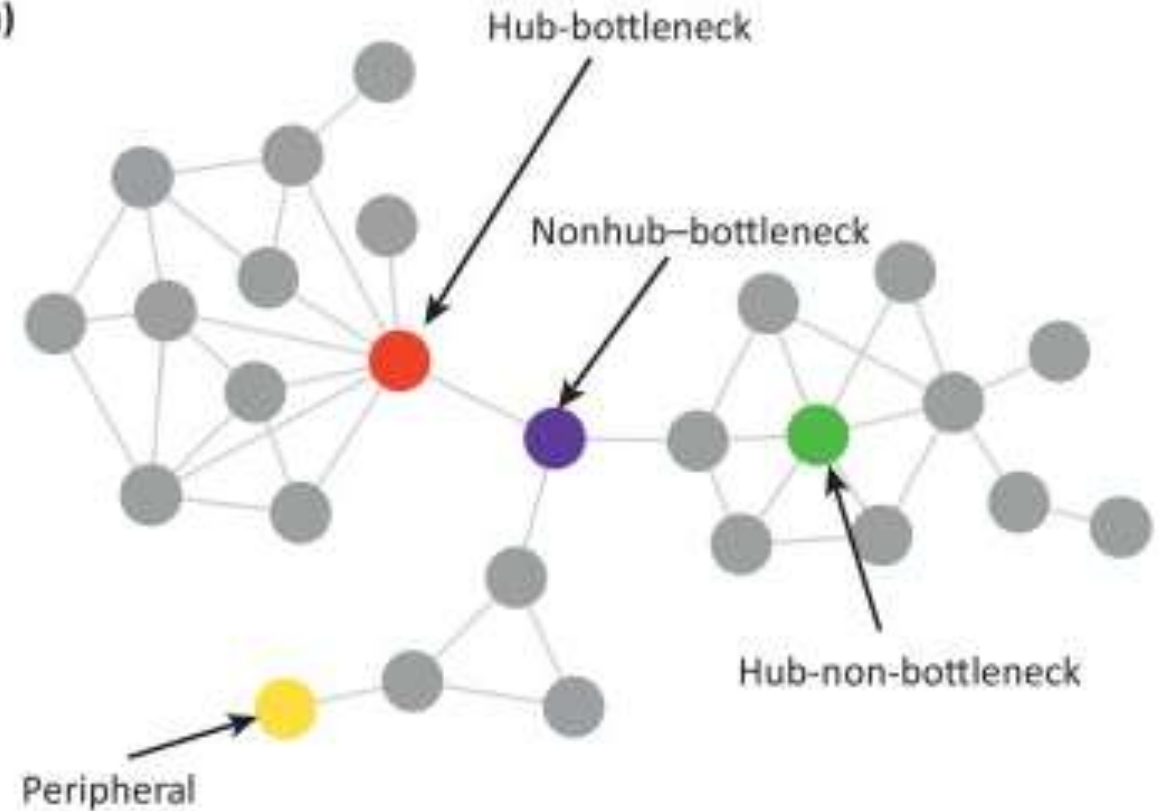
# Network Biology



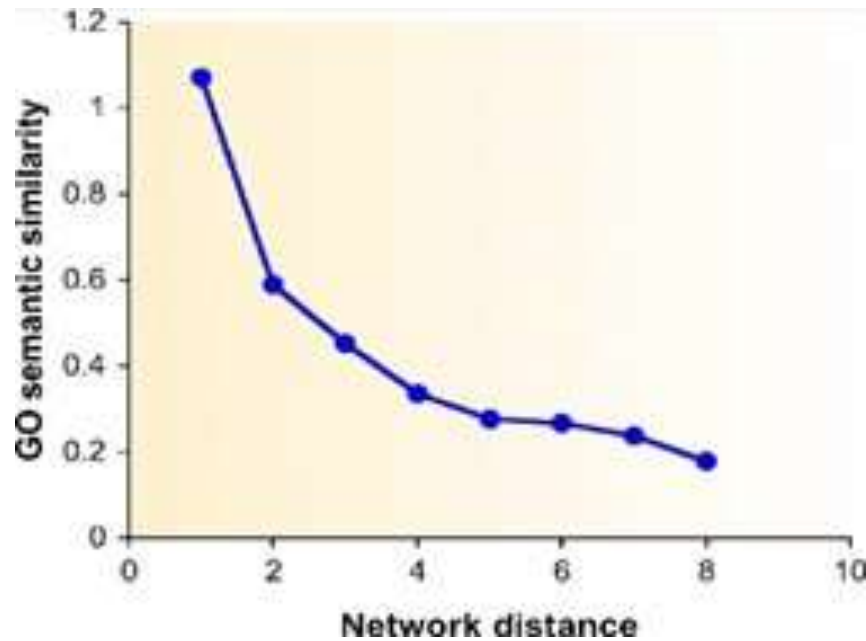
- Networks are good representations of complex systems
- Easy to analyze computationally
- Network theory measurements can:
  - Identify network modules
  - Identify relevant nodes



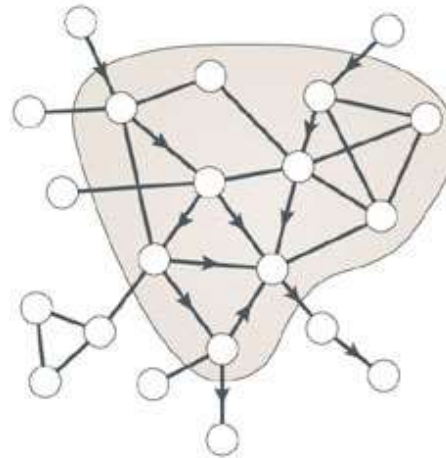
(a)



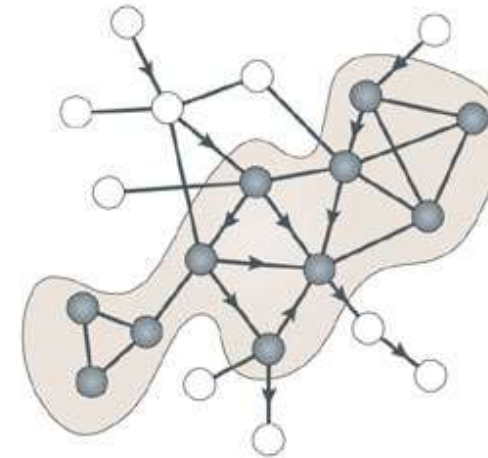
# Network Biology



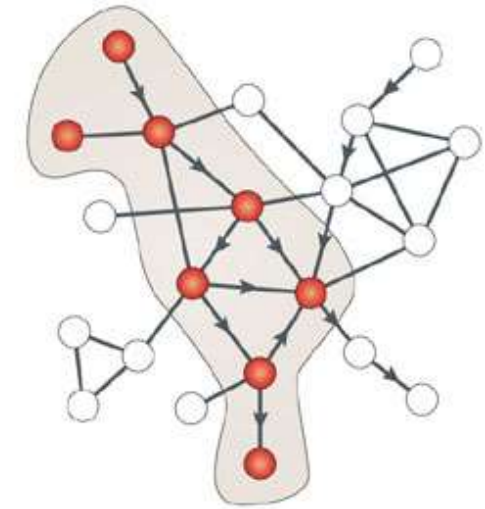
**a** Topological module



**b** Functional module



**c** Disease module



○ Topologically close genes (or products)

● Functionally similar genes (or products)

● Disease genes (or products)

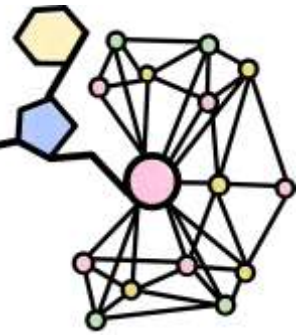
— Bidirectional interactions

→ Directed interactions

Nature Reviews | **Genetics**

Furlong LI (2013) Trends in Genetics

Barabási AL (2011) Nature Review Genetics





# Specific Betweenness



## SCIENTIFIC REPORTS

OPEN

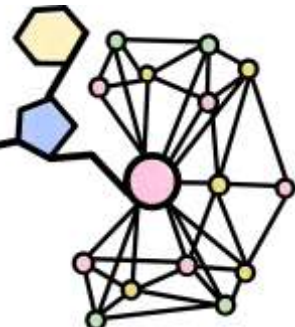
### Searching the overlap between network modules with specific betweenness (S2B) and its application to cross-disease analysis

Received: 28 February 2018

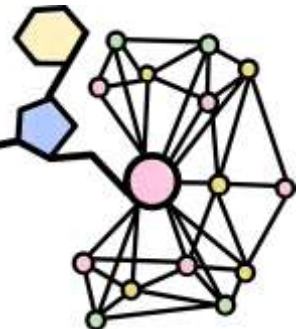
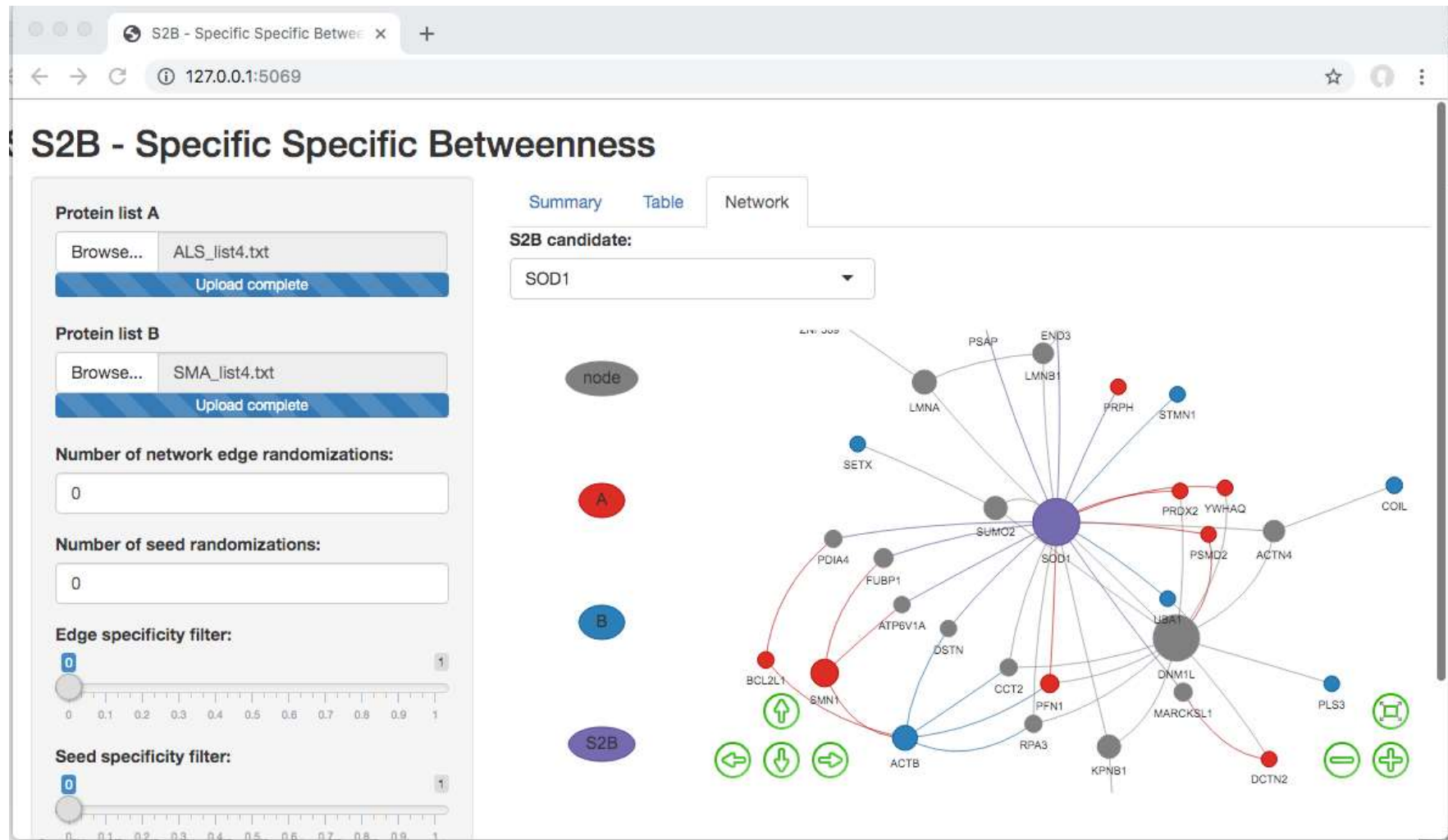
Accepted: 23 July 2018

Published online: 01 August 2018

Marina L. Garcia-Vaquero<sup>1</sup>, Margarida Gama-Carvalho<sup>1</sup>, Javier De Las Rivas<sup>1,2</sup> & Francisco R. Pinto<sup>1</sup>



# Specific Betweenness



# Network Biology of CFTR

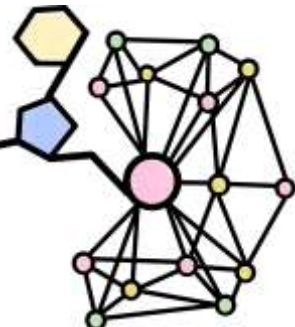


## Network Biology Identifies Novel Regulators of CFTR Trafficking and Membrane Stability

Cláudia Almeida Loureiro<sup>1,2†</sup>, João D. Santos<sup>1,3†</sup>, Ana Margarida Matos<sup>1,2†</sup>, Peter Jordan<sup>1,2†</sup>, Paulo Matos<sup>1,2†</sup>, Carlos M. Farinha<sup>1,3†</sup> and Francisco R. Pinto<sup>1,3\*</sup>

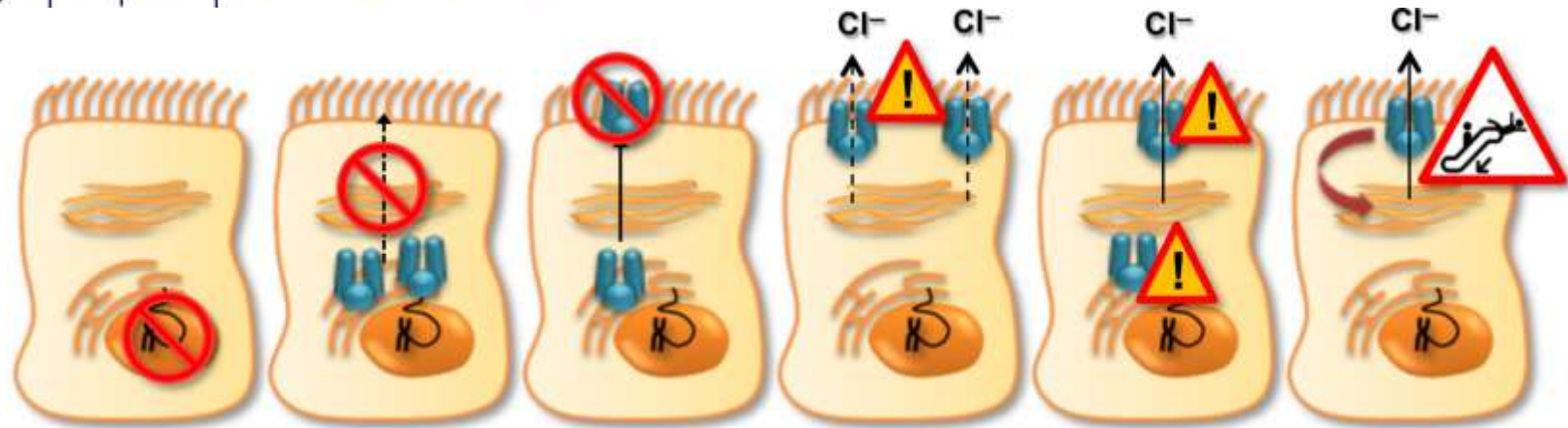
<sup>1</sup> BioISI—Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Lisbon, Portugal,

<sup>2</sup> Department of Human Genetics, National Health Institute "Dr. Ricardo Jorge," Lisbon, Portugal, <sup>3</sup> Department of Chemistry and Biochemistry, Faculty of Sciences, University of Lisbon, Lisbon, Portugal

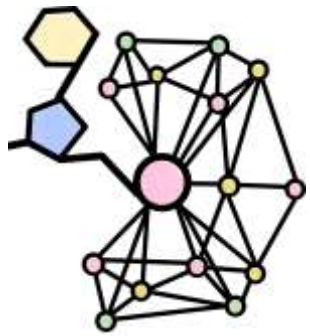




# Network Biology of CFTR



Mutation Class	I	II	III	IV	V	VI
Defect	Deficient synthesis	Deficient traffic	Impaired gating	Decreased conductance	Less protein (often splicing defects)	Reduced half-life at the PM
Example	G542X	F508del	G551D rF508del	R334W R347P	3272-26A>G	c.120del23 rF508del



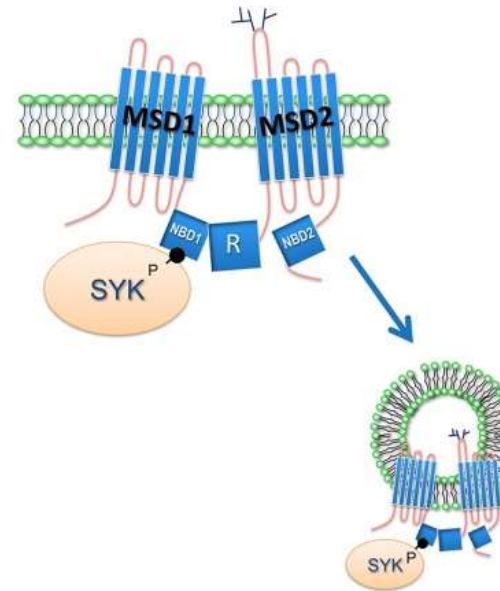


# CFTR stability modulators I

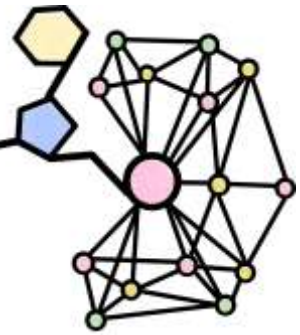
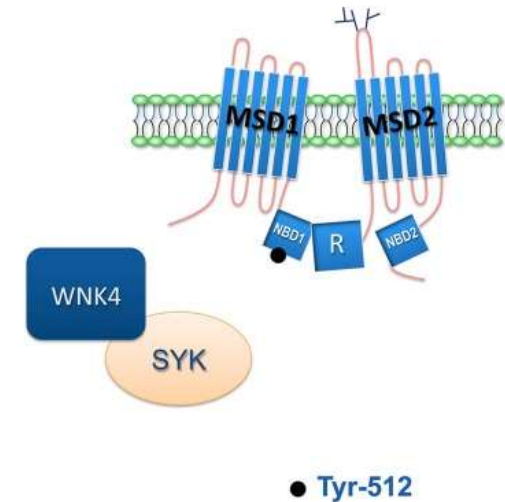


- SYK phosphorylates CFTR
- Promotes CFTR endocytosis
- Proteins interacting with phosphorylation site may modulate amount of CFTR at the plasma membrane

**A CFTR phosphorylation and endocytosis**

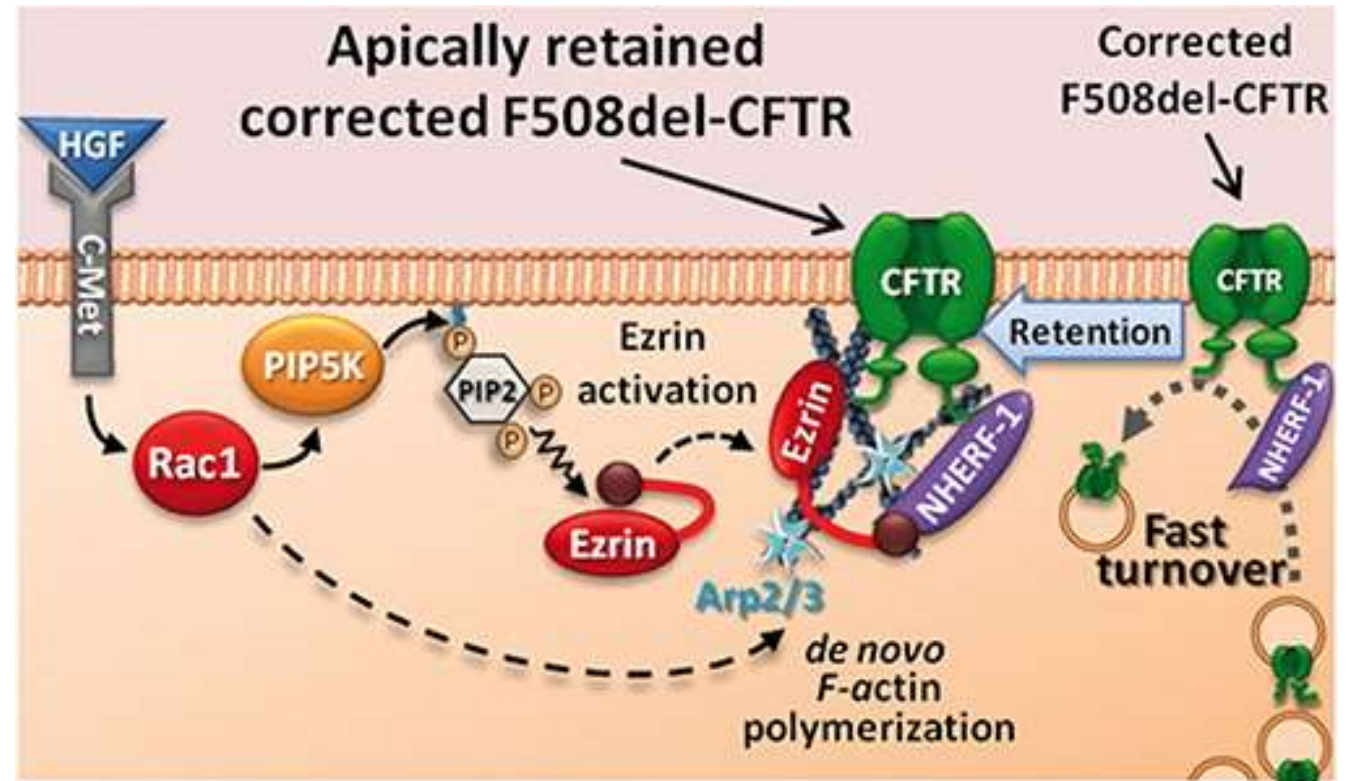


**B CFTR stabilization**



# CFTR stability modulators II

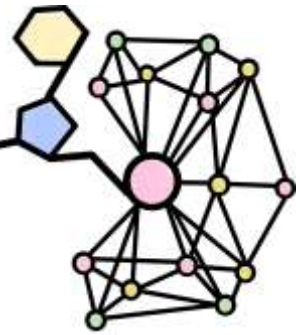
- NHERF-1 binds CFTR
- Activated Ezrin binds NHERF-1 and actin cytoskeleton



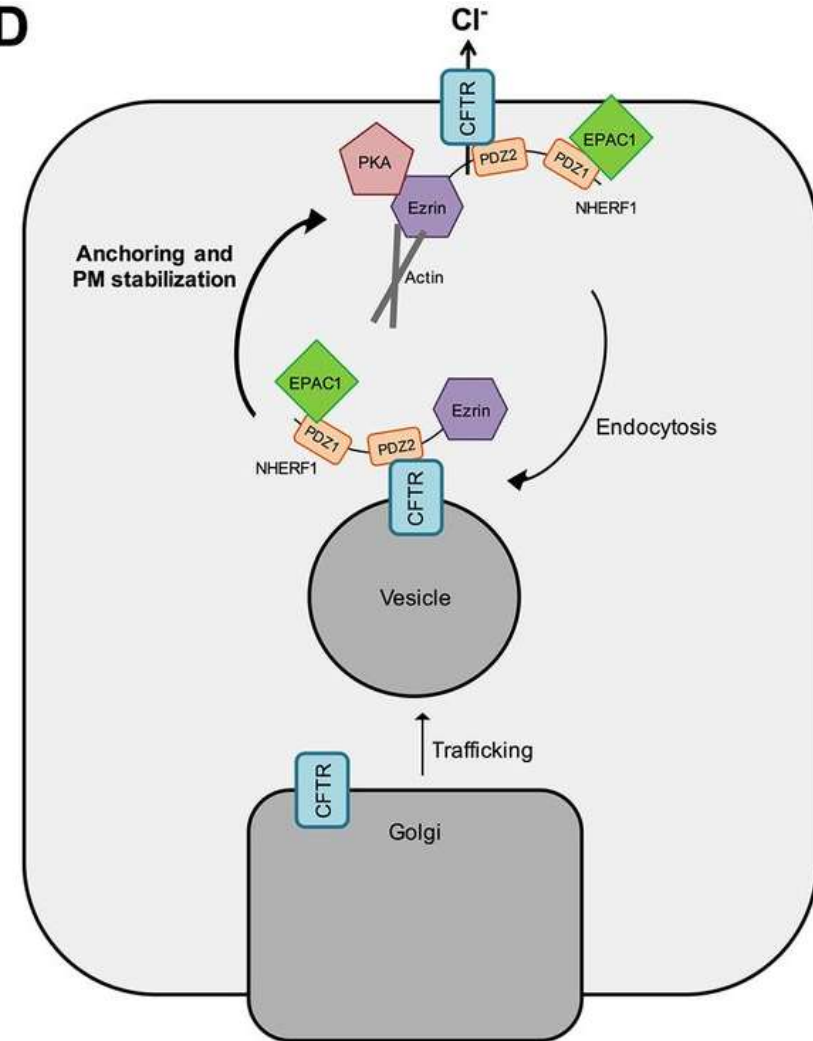
# CFTR stability modulators III



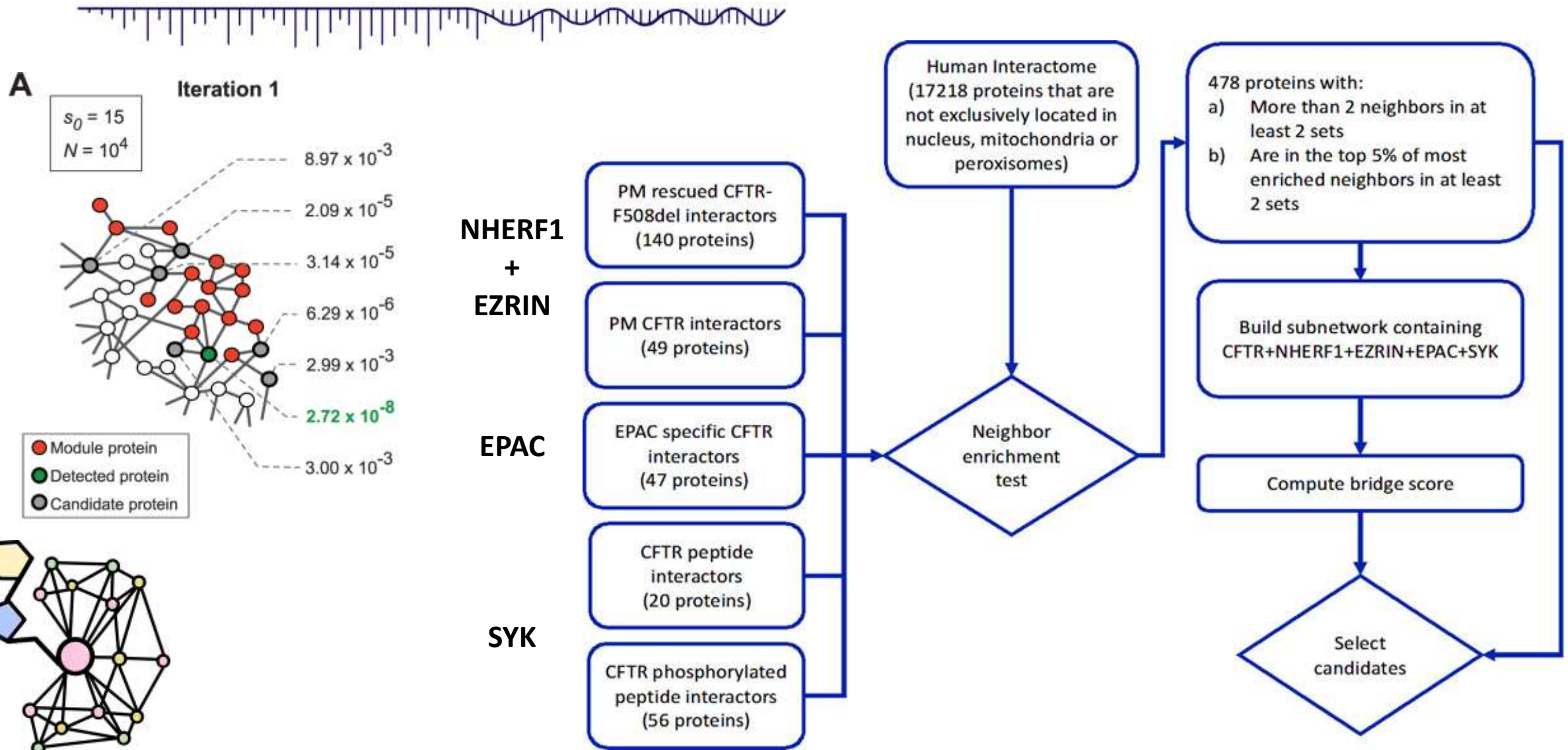
- cAMP signaling through EPAC1 promotes CFTR stabilization
- EPAC1 and CFTR colocalize and interact through NHERF1



D



# Specific neighbors of CFTR interactors

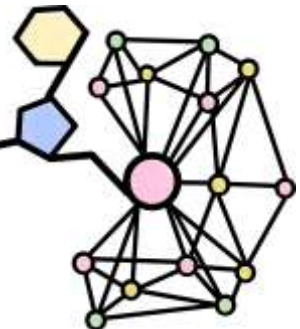
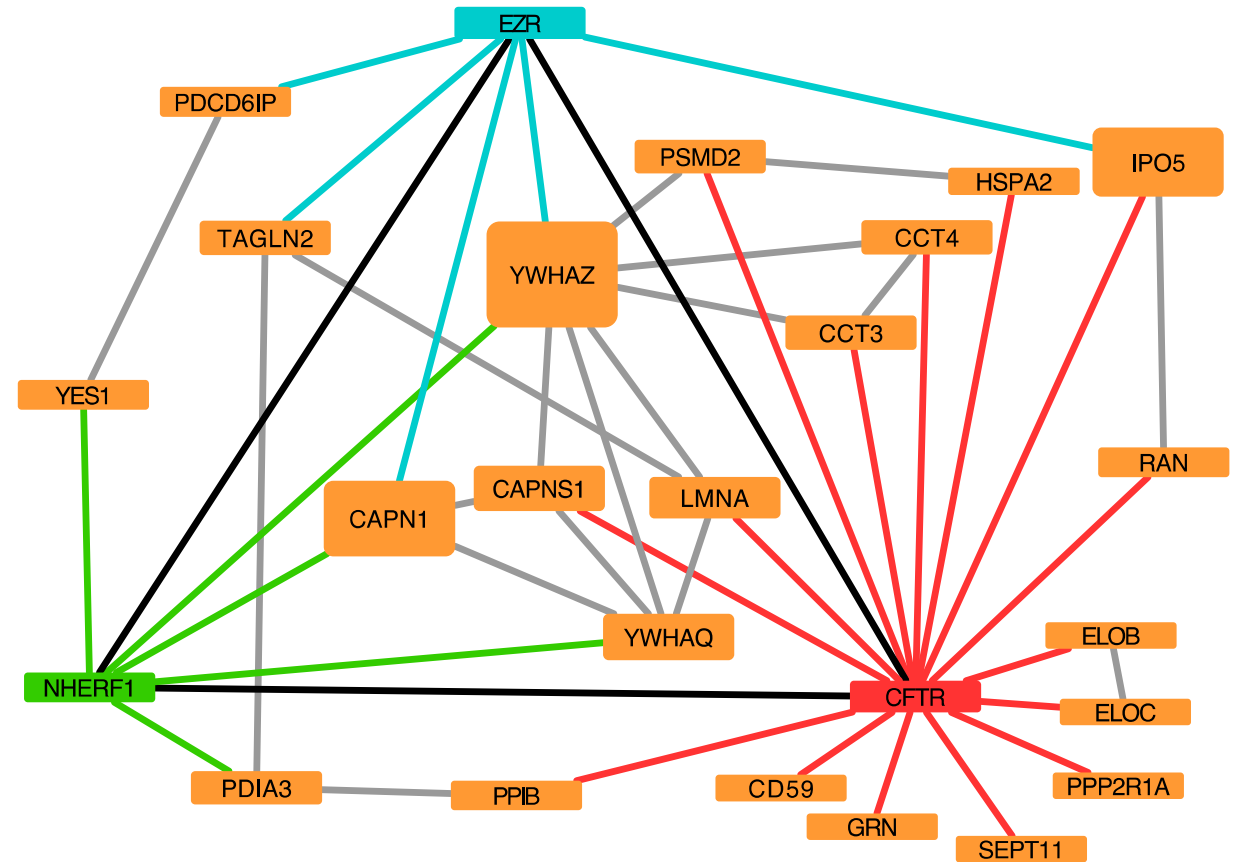




# Finding mediator proteins – Bridge Score



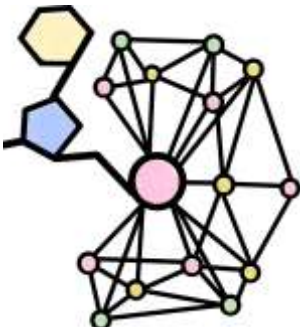
- List of paths linking interesting proteins
- Quantify fraction of paths that go through each candidate
- Decreasing weight according to path length



# Candidate selection

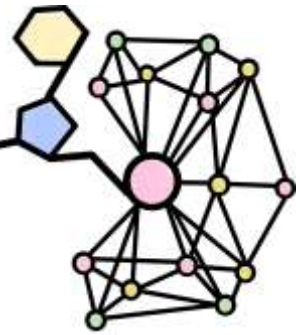
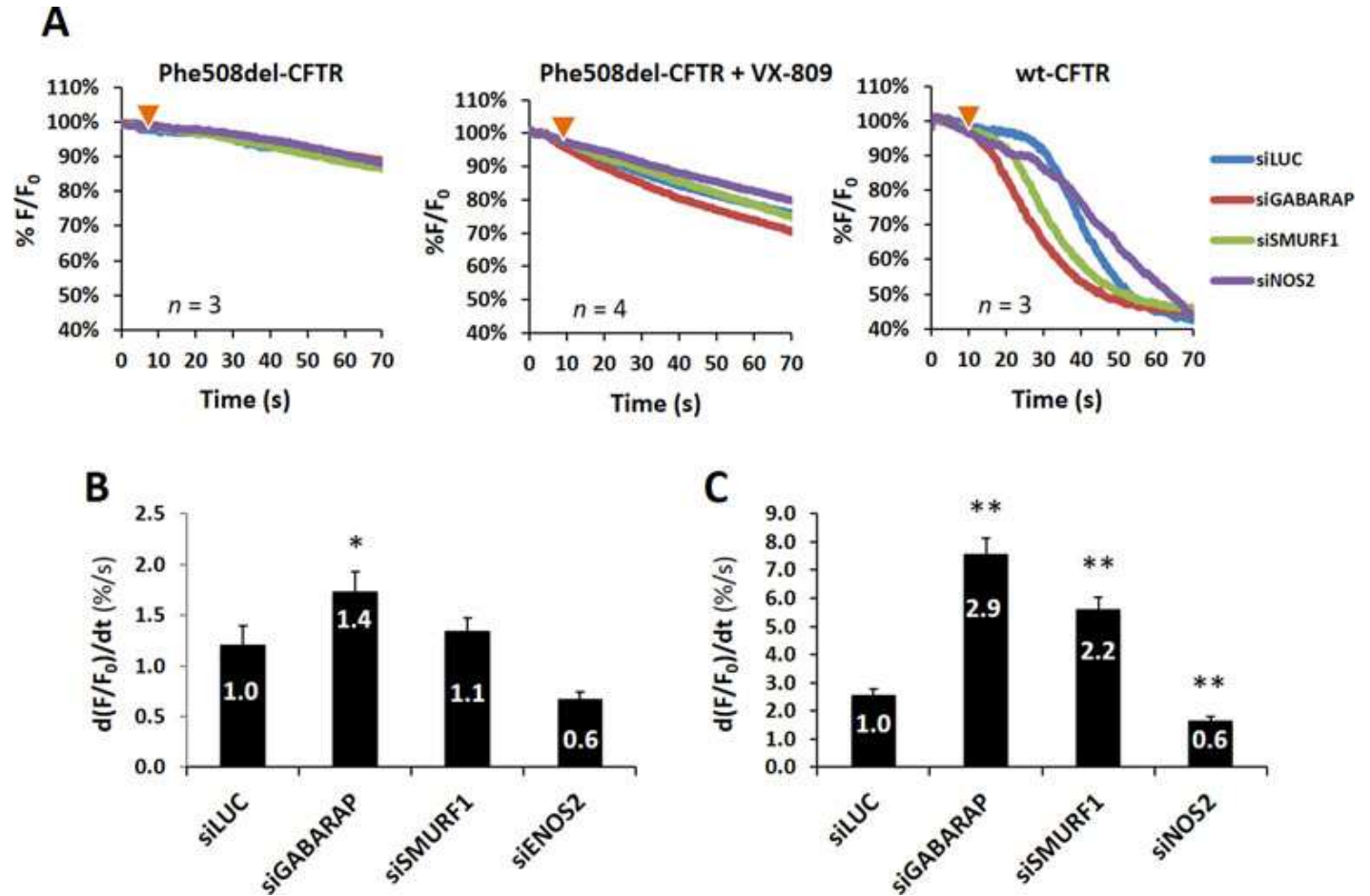


UniProt identifier	Gene	Protein	Specific neighbor of	Strong mediator of CFTR with
Q9H0R8	GABARAPL1	Gamma-aminobutyric acid receptor-associated protein-like 1 (Early estrogen-regulated protein) (GABA(A) receptor-associated protein-like 1) (Glandular epithelial cell protein 1) (GEC-1)	All sets	EZRIN, NHERF1, SYK
O95166	GABARAP	Gamma-aminobutyric acid receptor-associated protein (GABA(A) receptor-associated protein) (MM46)	All sets	NHERF1
P60520	GABARAPL2	Gamma-aminobutyric acid receptor-associated protein-like 2 (Early estrogen-regulated protein) (GABA(A) receptor-associated protein-like 2) (Glandular epithelial cell protein 2)	All sets	EPAC, EZRIN, NHERF1, SYK
P35228	NOS2	Nitric oxide synthase, inducible (EC 1.14.13.39) (Hepatocyte NOS) (HEP-NOS) (Inducible NO synthase) (Inducible NOS) (iNOS) (NOS type II) (Peptidyl-cysteine S-nitrosylase NOS2)	All sets	NHERF1
Q5S007	LRRK2	Leucine-rich repeat serine/threonine-protein kinase 2 (EC 2.7.11.1) (Dardarin)	All sets	EPAC, EZRIN, NHERF1, SYK
P35240	NF2	Merlin (Moesin-ezrin-radixin-like protein) (Neurofibromin-2) (Schwannomerlin) (Schwannomin)	All sets	EZRIN, NHERF1
Q13418	ILK	Integrin-linked protein kinase (EC 2.7.11.1) (59 kDa serine/threonine-protein kinase) (ILK-1) (ILK-2) (p59ILK)	All sets except pep-wt	
Q9HCE7	SMURF1	E3 ubiquitin-protein ligase SMURF1 (hSMURF1) (EC 2.3.2.26) (HECT-type E3 ubiquitin transferase SMURF1) (SMAD ubiquitination regulatory factor 1) (SMAD-specific E3 ubiquitin-protein ligase 1)	All sets except wt	NHERF1, SYK
Q8TF42	UBASH3B	Ubiquitin-associated and SH3 domain-containing protein B (EC 3.1.3.48) (Cbl-interacting protein p70) (Suppressor of T-cell receptor signaling 1) (STS-1) (T-cell ubiquitin ligand 2) (TULA-2) (Tyrosine-protein phosphatase STS1/TULA2)	All sets except wt	SYK
Q13618	CUL3	Cullin-3 (CUL-3)	All sets	EZRIN, NHERF1, SYK
P46940	IQGAP1	Ras GTPase-activating-like protein IQGAP1 (p195)	All sets except pep-py	EZRIN, NHERF1, SYK



# Experimental validation

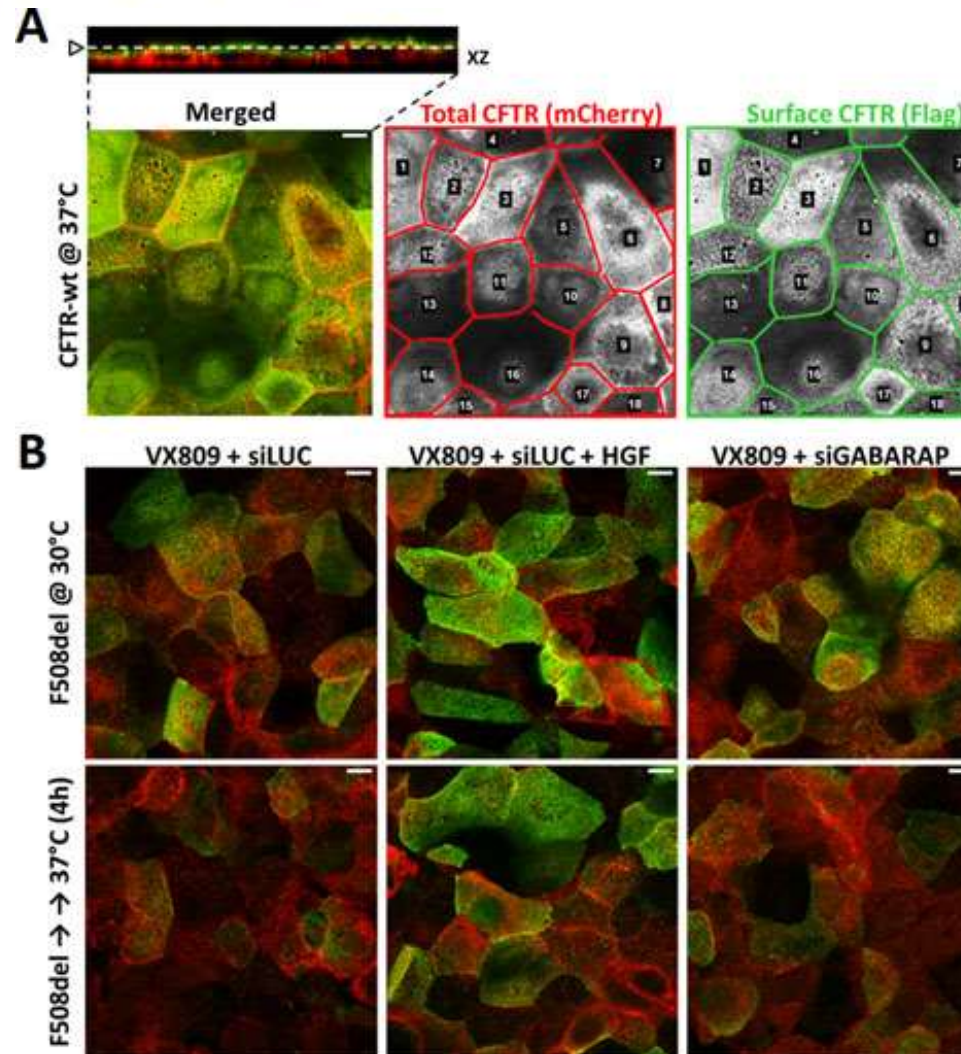
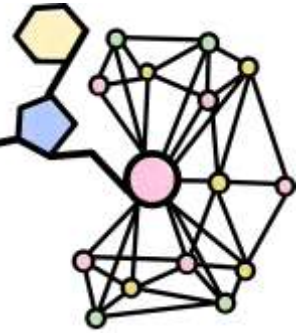
- KD of GABARAP, SMURF1 and NOS2
- Strong effects on wt-CFTR activity
- Moderate effects on F508del CFTR
- GABARAP keeps significant effect on F508del CFTR





# Experimental validation

- KD of GABARAP increases F508del CFTR abundance at the PM
- And increases surface protein stability

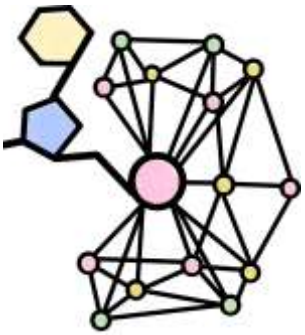




# Conclusions



- Experimental data only identifies a fraction of proteins involved in the process or disease of interest
- Network analysis allow us to reconstruct the full picture
- Specially powerful to find the missing links between related processes or diseases



# Acknowledgements



- RNA Systems Biology
  - Margarida Gama-Carvalho
  - Marina L. Garcia-Vaquero
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  - Jo o Santos
  - Ana Matos

- Peter Jordan
- Paulo Matos
- Carlos Farinha

