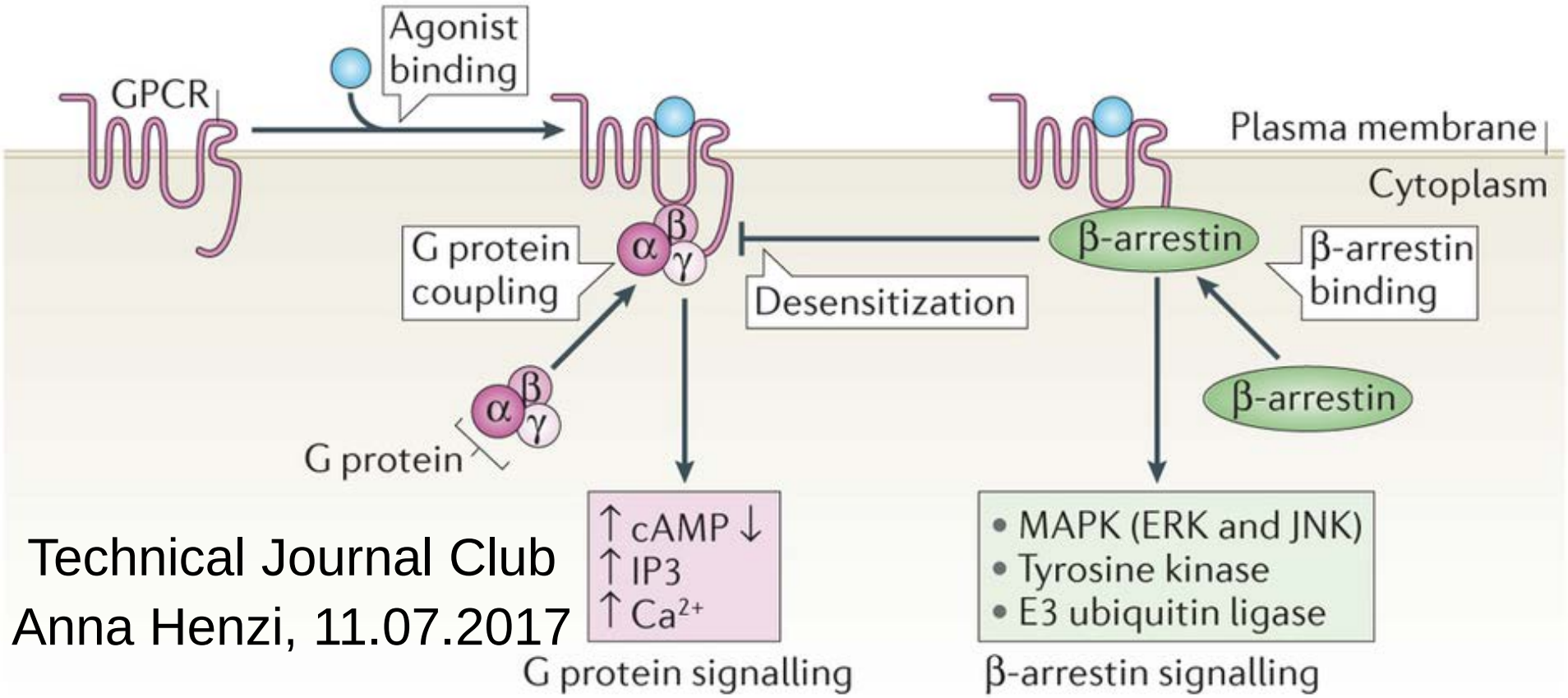
Multidimensional Tracking of GPCR Signaling with Proximity-Labeling



Technical Journal Club
Anna Henzi, 11.07.2017

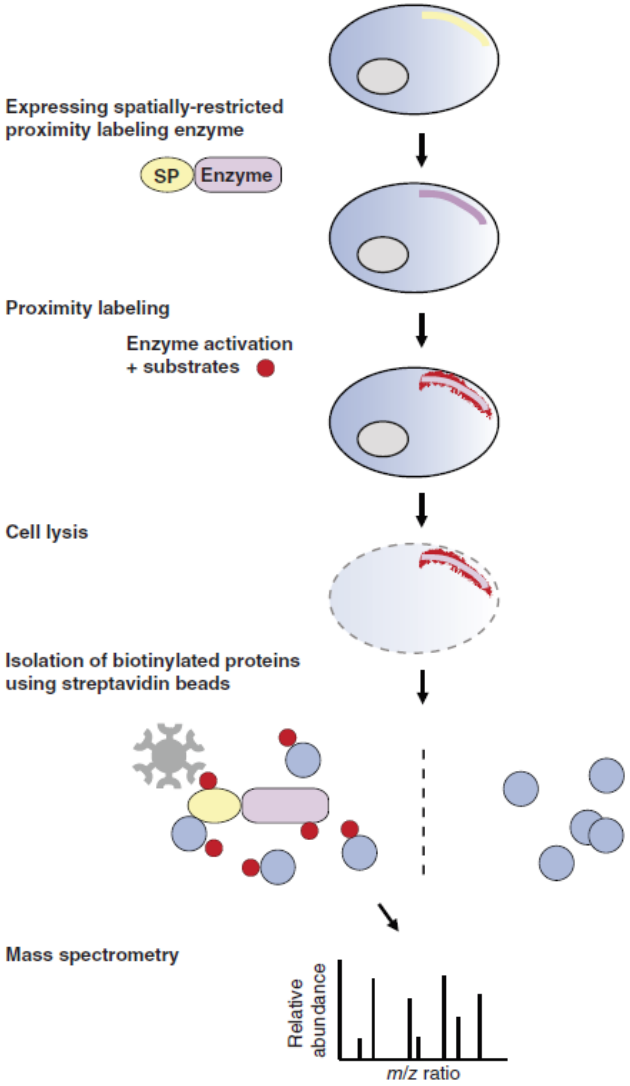
Proximity-dependent labeling methods

- Enzymes create radicals
  - Proximity-dependent biotin identification (BioID)
  - Horseradish peroxidase (HRP)
  - Ascorbate peroxidase (APEX)

➢ covalently tag neighbouring proteins with biotin

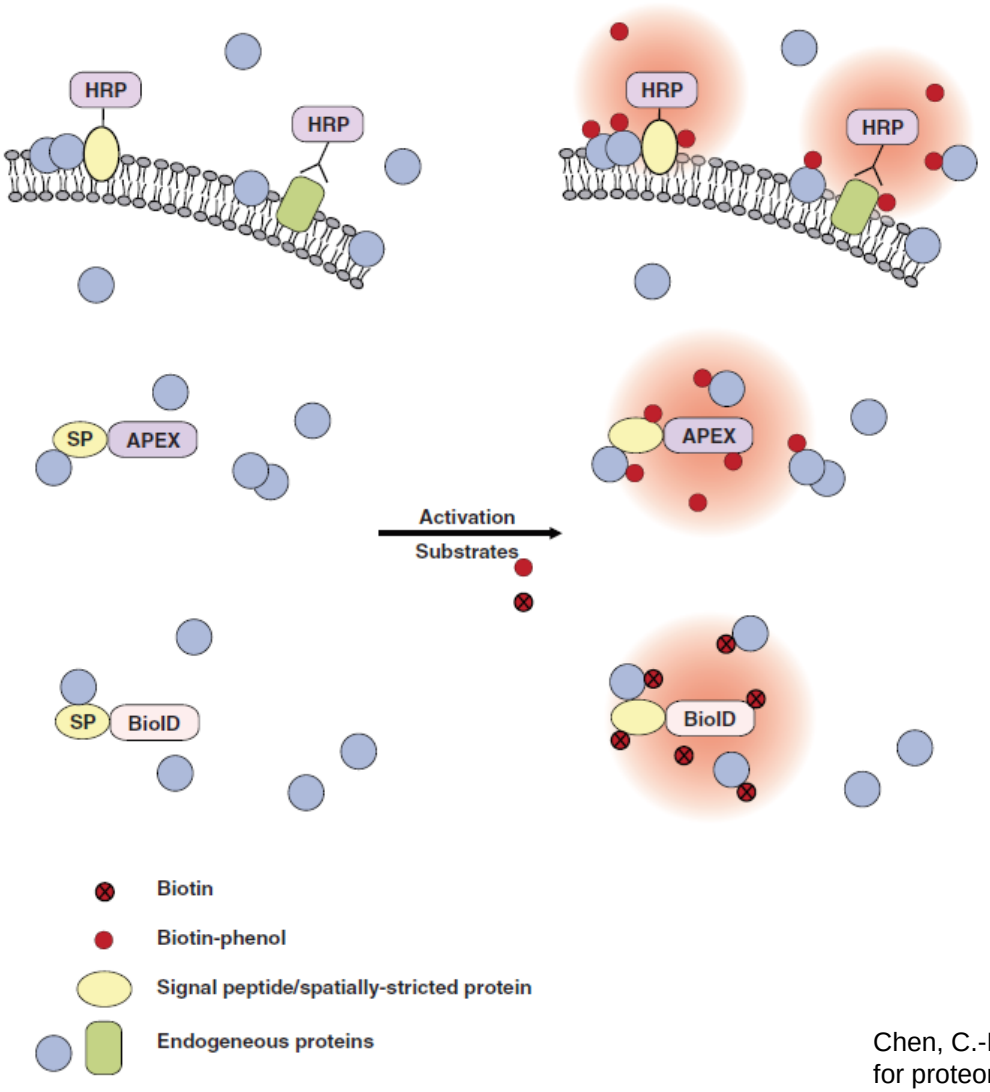
- identify labeled proteins by mass spectrometry (MS)

- Cells remain intact for labeling



Chen, C.-L. and Perrimon, N. (2017), Proximity-dependent labeling methods for proteomic profiling in living cells. WIREs Dev Biol, e272. doi:10.1002/wdev.272

Enzymes for proximity labeling



- Horseradish peroxidase (HRP)
- Ascorbate peroxidase (APEX)
- Proximity-dependent biotin identification (BioID)

Chen, C.-L. and Perrimon, N. (2017), Proximity-dependent labeling methods for proteomic profiling in living cells. WIREs Dev Biol, e272. doi:10.1002/wdev.272

Enzymes for proximity labeling

TABLE 1 | Comparison of the Different Proximity Labeling Methods

Enzyme	BioID	HRP	APEX
Enzymatic activity	Biotin ligase based	Peroxidase based	Peroxidase based
Labeling target	Lysine	Electron-rich amino acids	Tyrosine and potentially other electron-rich amino acids
Size (kD)	35	44	27
Labeling time	15–24 h	5–10 min	1 min
Incubation time with substrate	15–24 h	5–10 min	30–60 min
Activation by H_2O_2	No	Yes	Yes
Substrates for protein labeling	Biotin	Biotin-phenol (and biotin- or fluorescein-acylazide)	Biotin-phenol
Half-life of generated radicals	Mins	<1 ms	<1 ms
Active region	Intracellular	Extracellular, secretory pathway (inactive in cytosol)	Intracellular
Available variants	BioID2 (27 kD)	Split HRP	APEX2
Note	Reduced activity below 37 degrees	Can be used as an EM tag; HRP-conjugated antibodies available	Can be used as an EM tag
Organisms	Mammalian cells, xenograft tumors in mice, *Trypanosoma brucei*, *Toxoplasma gondii*, *Dictyostelium discoideum*, *Plasmodium berghei*	Mammalian cells	Mammalian cells, *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae*, *Drosophila melanogaster*

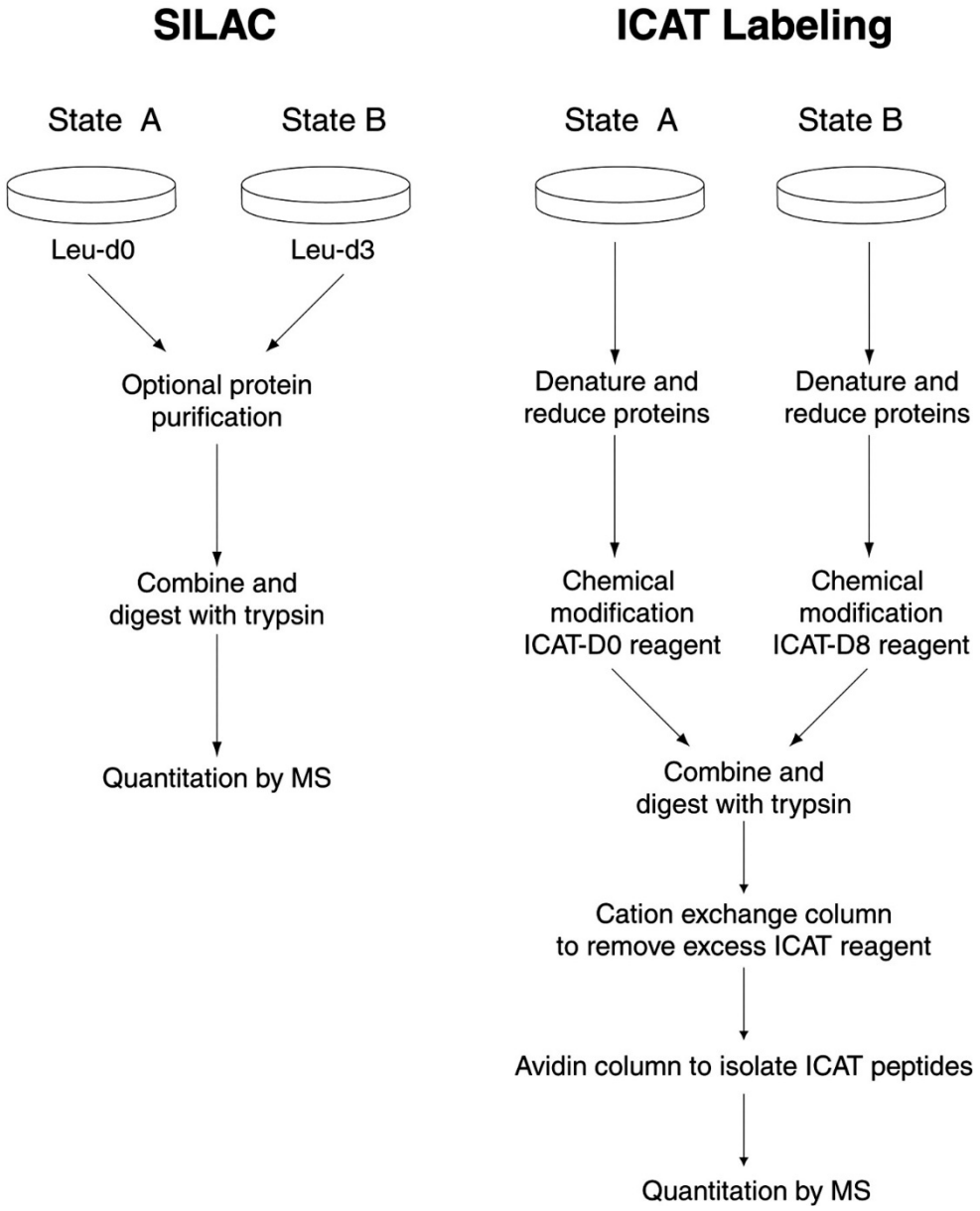
APEX, engineered ascorbate peroxidase; BioID, proximity-dependent biotin identification; EM, electron microscopy; HRP, horseradish peroxidase.

Chen, 2017

SILAC

(**S**table **I**sotope **L**abeling by **A**mino Acids in **C**ell Culture)

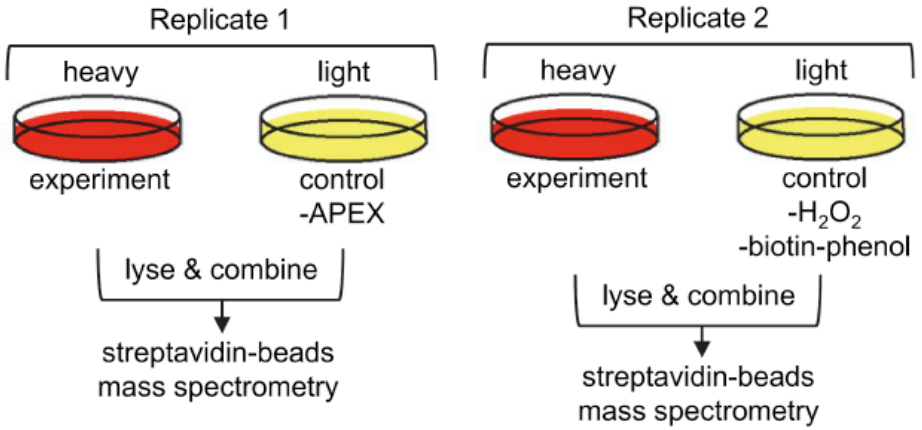
- Approach for expression proteomics: simultaneous identification and quantitation of protein mixtures
- Non-radioactive isotopically labelled amino acids in cell culture medium



Shao-En Ong et al. Mol Cell Proteomics 2002;1:376-386

Proteomic Mapping of Mitochondria

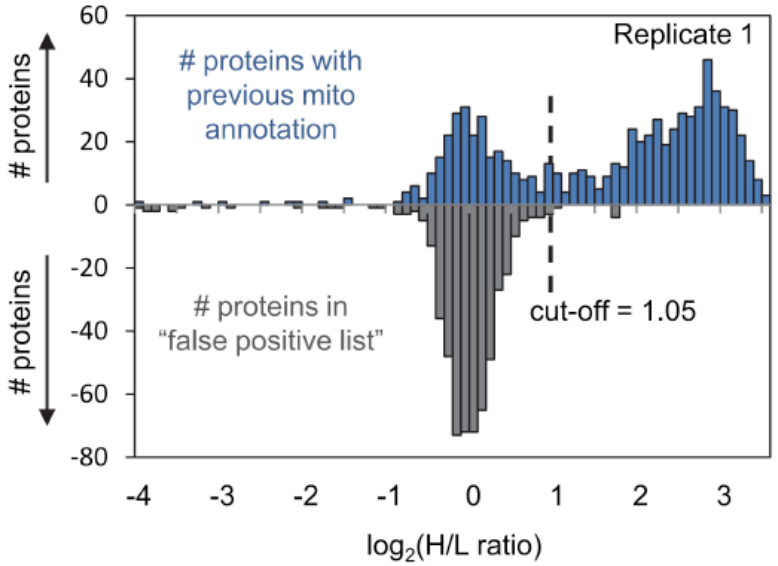
A



B

	Rep1	Rep2
# detected peptides	50032	54744
overlap	39947	
# detected proteins	3430	3599
overlap	3147	
# enriched proteins	527	579
overlap	495	

C



- 94% with mitochondrial annotation
- Coverage 80-90%
- 31 orphans

Rhee H-W, Zou P, Udeshi ND, et al. Proteomic Mapping of Mitochondria in Living Cells via Spatially-Restricted Enzymatic Tagging. *Science (New York, NY)*. 2013;339(6125):1328-1331. doi:10.1126/science.1230593.

Article

Cell

Multidimensional Tracking of GPCR Signaling via Peroxidase-Catalyzed Proximity Labeling

Paek et al, 2017

GPCR

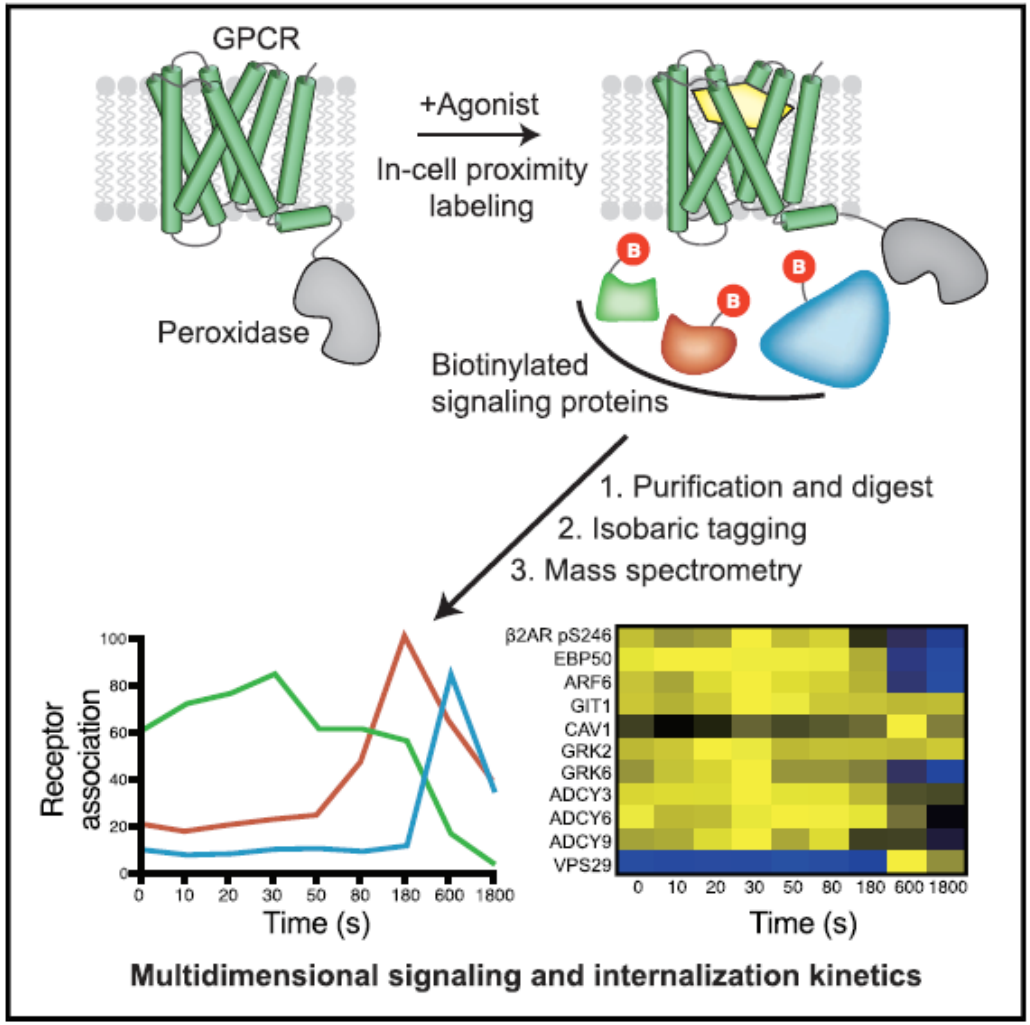
- Many GPCR targeted drugs
- Many aspects of signalling incompletely characterized

GPCR signaling assays: limitations

- Single pathway readout
- Limited time resolution
- Overexpression or modification of effectors

Multidimensional Tracking of GPCR Signaling via Peroxidase-Catalyzed Proximity Labeling

Graphical Abstract



GPCR-APEX

Peroxidase-based proximity labelling

Isobaric tagging and MS

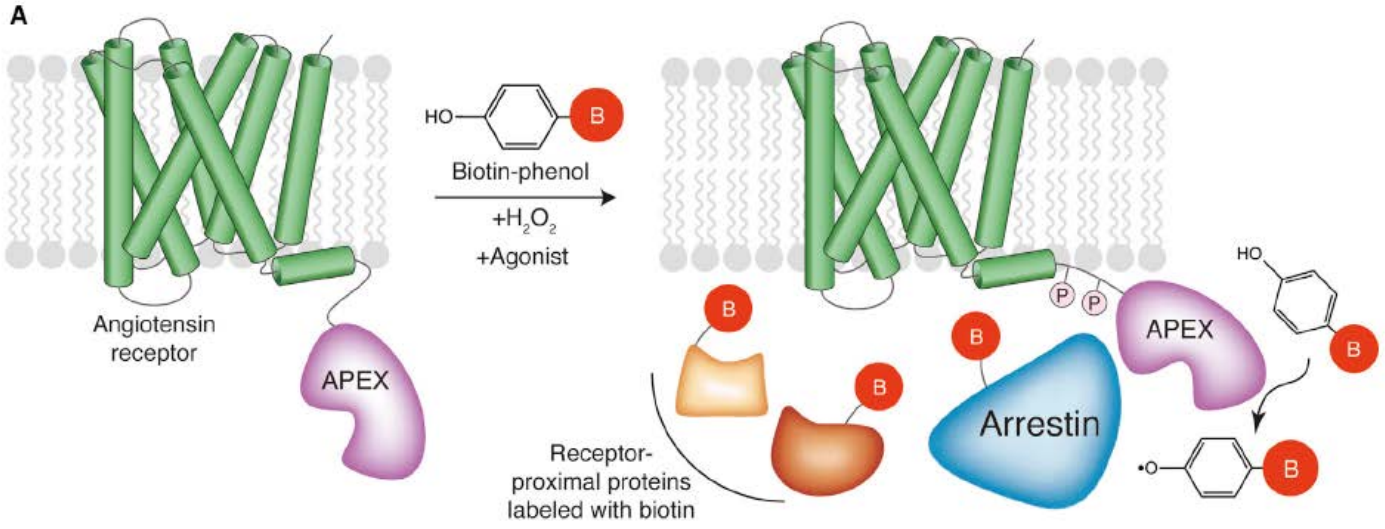
- quantitative, time-resolved measurement of GPCR agonist response
- Parallel quantification of all interacting effectors
- Native cellular environment, endogenous expression level

Multidimensional Tracking of GPCR Signaling via Peroxidase-Catalyzed Proximity Labeling

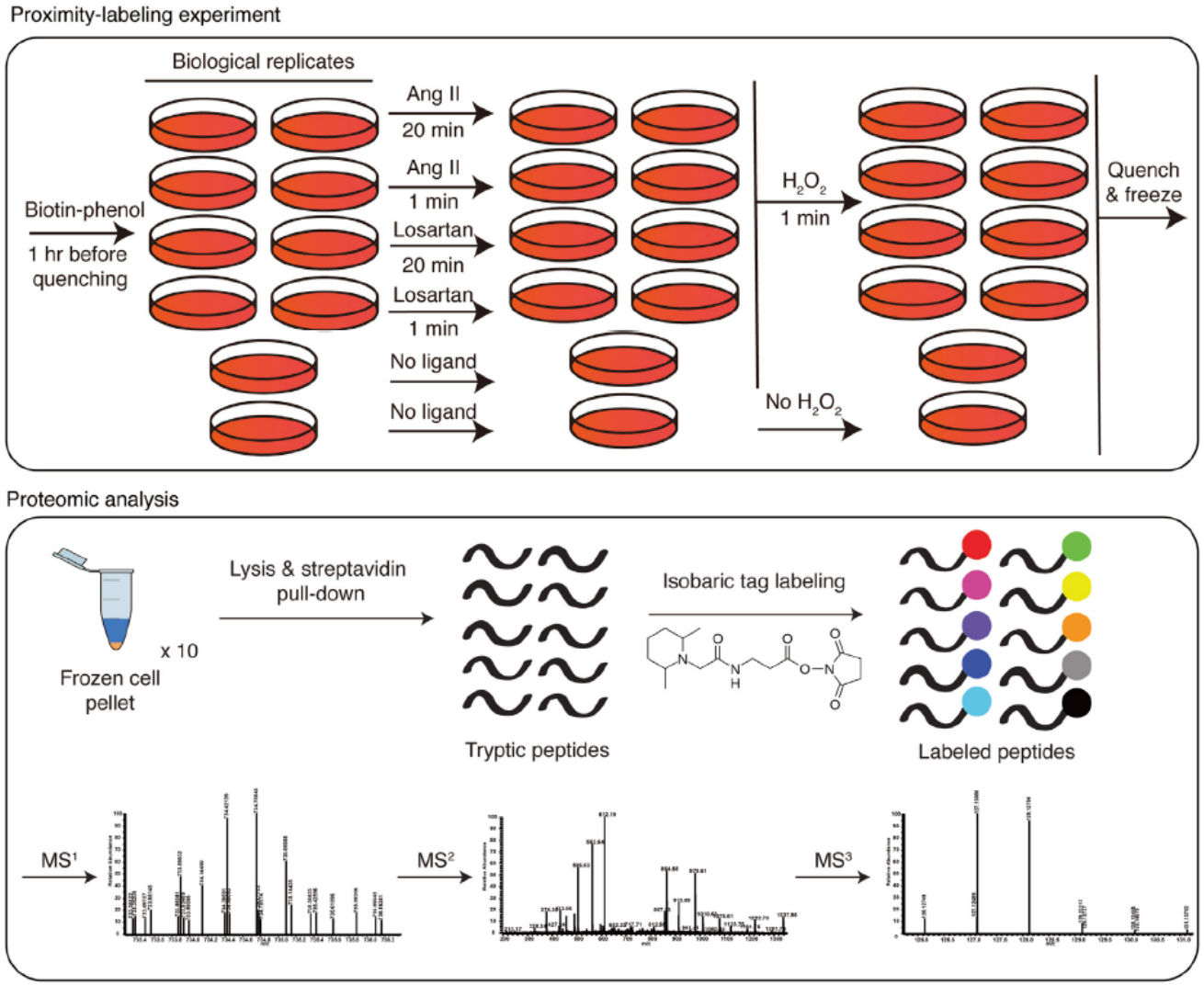
APEX2

- Biotin-phenoxy radicals (< 1ms)
- Labeling radius 20nm

Angiotensin II type 1 receptor (AT1R)

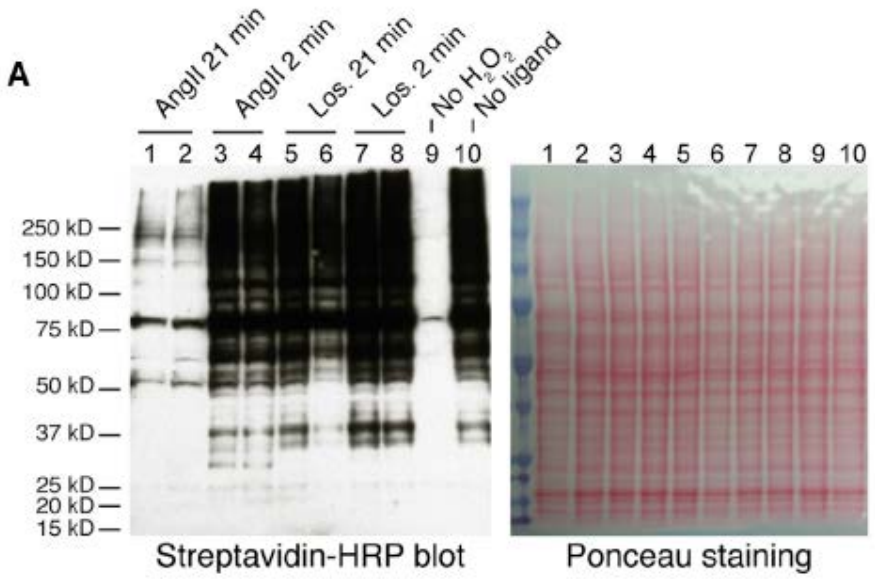


Multidimensional Tracking of GPCR Signaling via Peroxidase-Catalyzed Proximity Labeling

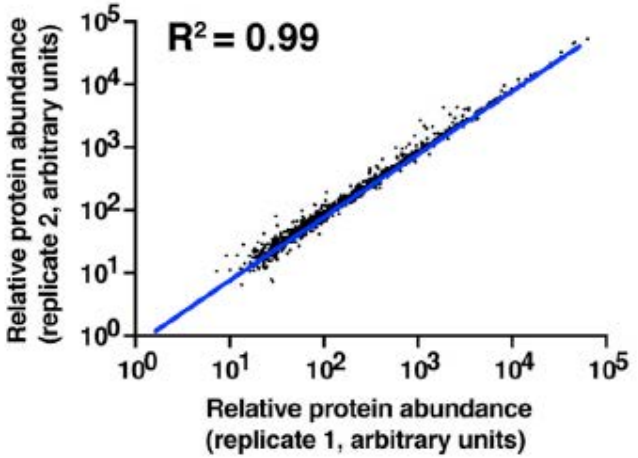


Multidimensional Tracking of GPCR Signaling via Peroxidase-Catalyzed Proximity Labeling

Western blot



Quantification by MS



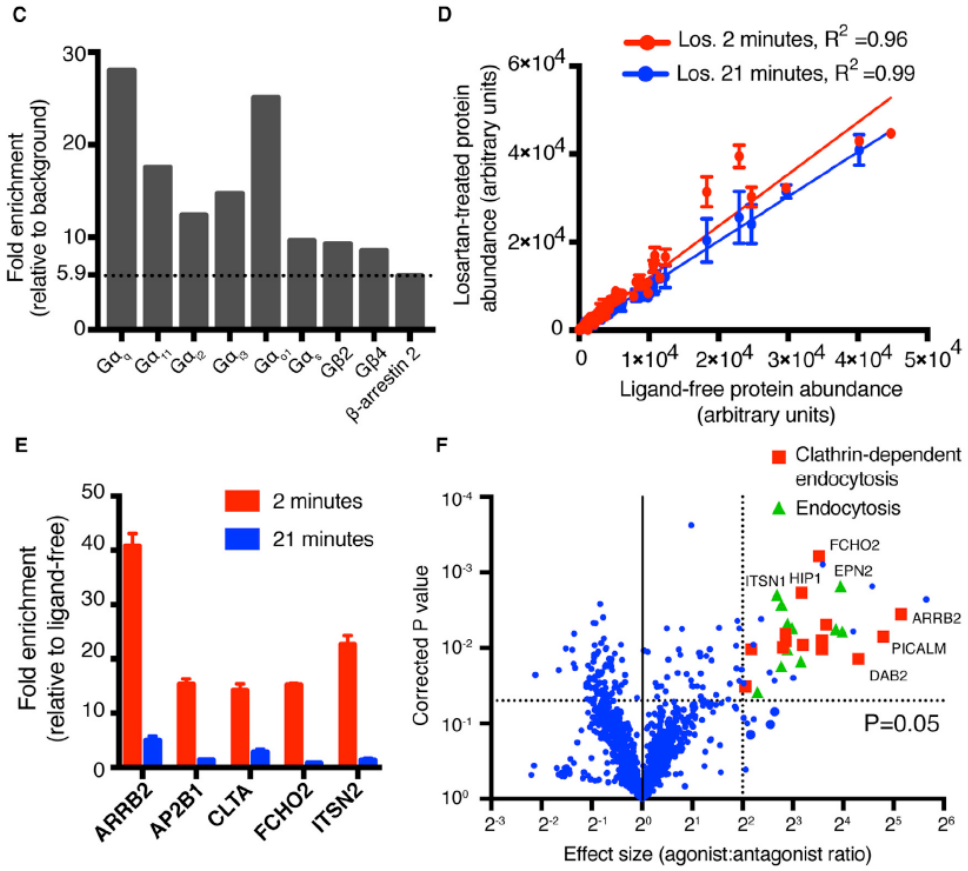
Multidimensional Tracking of GPCR Signaling via Peroxidase-Catalyzed Proximity Labeling

Characterize local milieu of AT1R

Enrichment of G-proteins

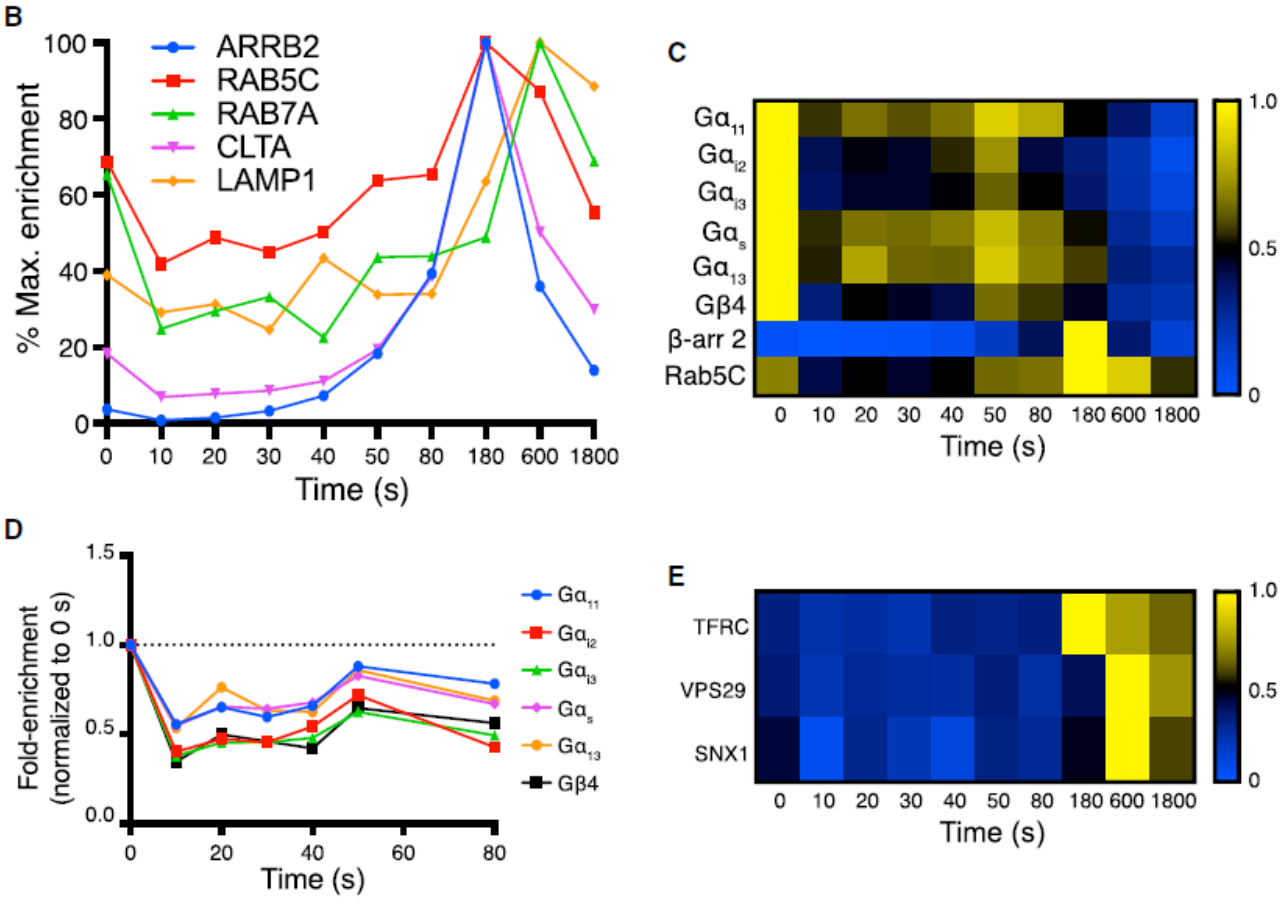
No difference for antagonist treatment (losartan)

Differential enrichment of endocytosis-related proteins after agonist treatment (ATII)



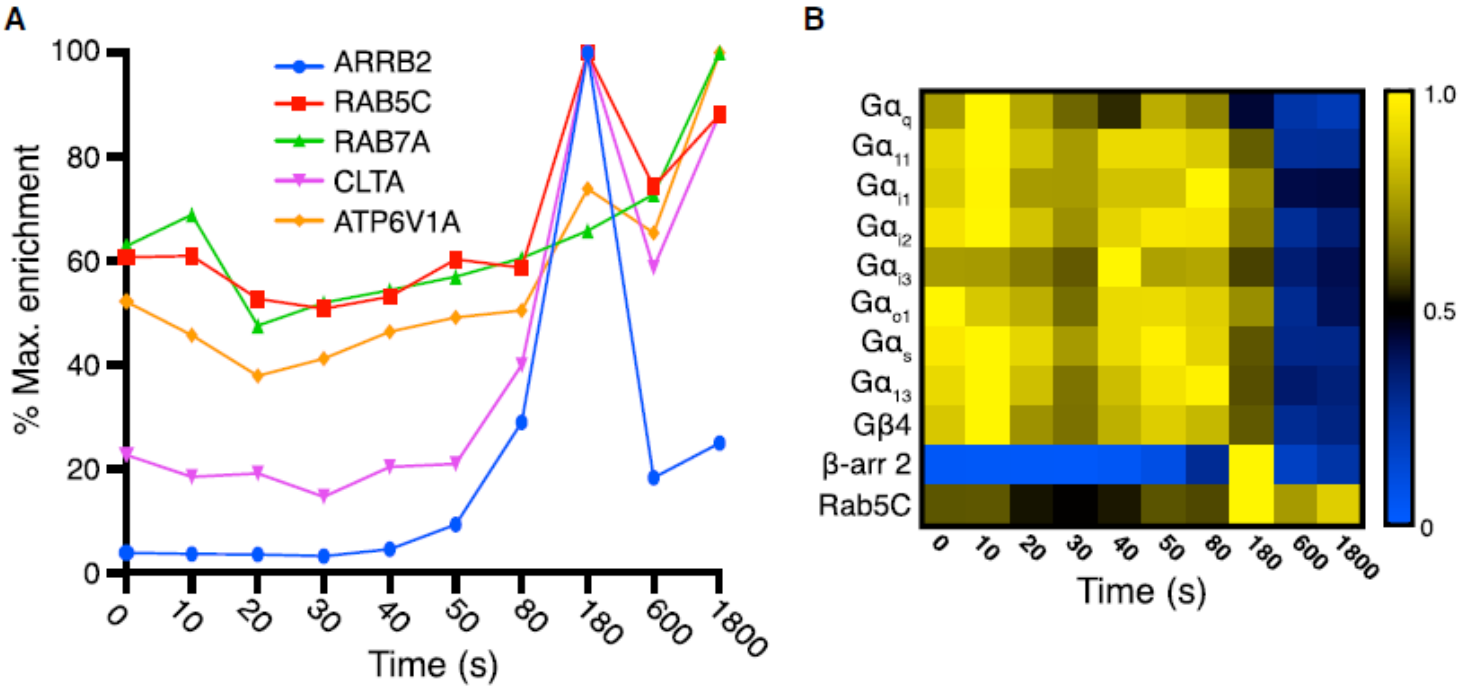
Multidimensional Tracking of GPCR Signaling via Peroxidase-Catalyzed Proximity Labeling

Time resolution



Multidimensional Tracking of GPCR Signaling via Peroxidase-Catalyzed Proximity Labeling

biased agonist TRV027

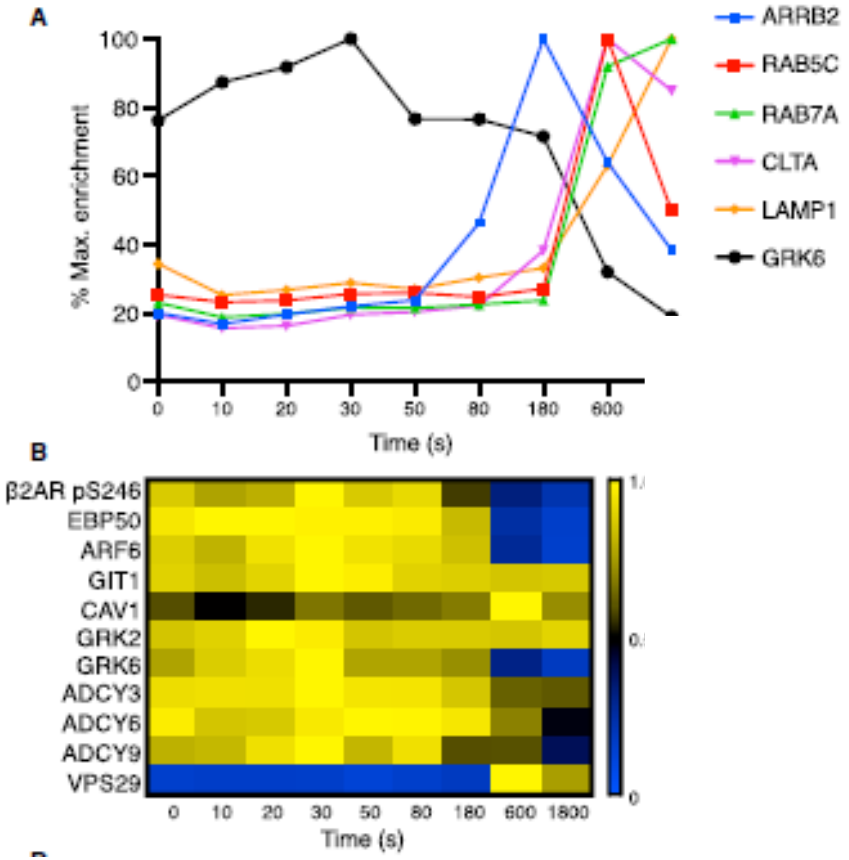


Multidimensional Tracking of GPCR Signaling via Peroxidase-Catalyzed Proximity Labeling

Generalizability?

β2 adrenergic receptor (β2AR)

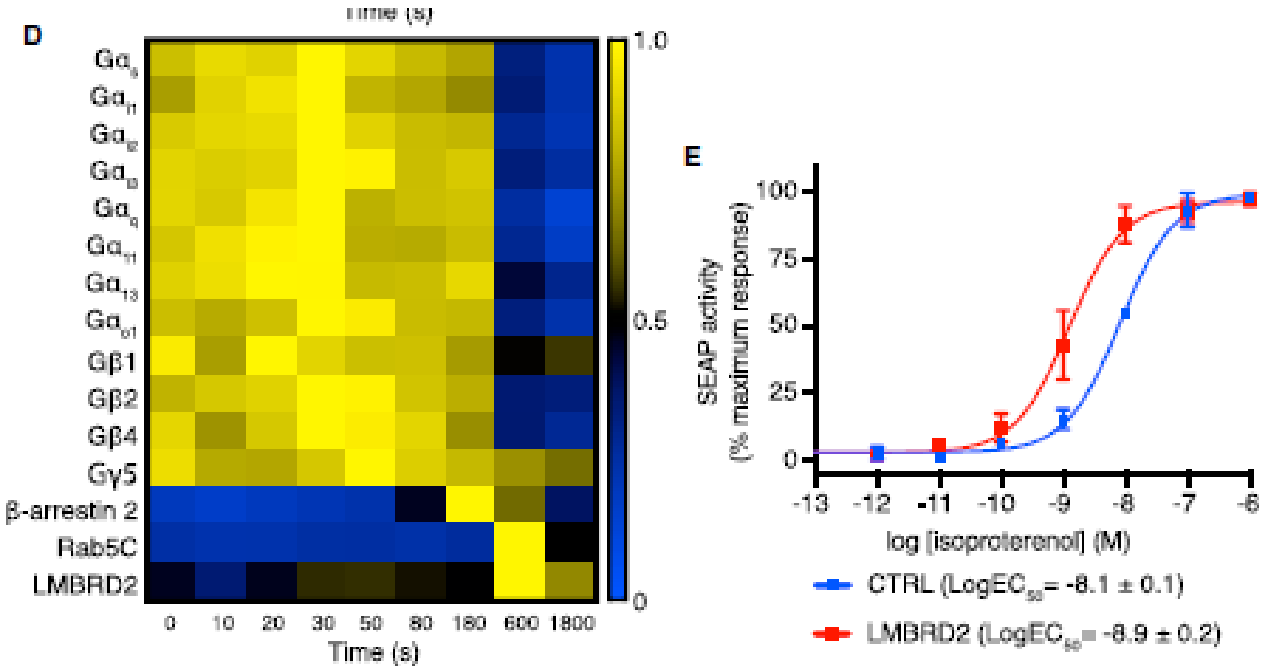
Agonist: BI167107



Multidimensional Tracking of GPCR Signaling via Peroxidase-Catalyzed Proximity Labeling

Potential to discover novel mediators of GPCR signalling:

LMBRD2



Multidimensional Tracking of GPCR Signaling via Peroxidase-Catalyzed Proximity Labeling

Summary

- Local milieu of GPCR in ligand-free state
- G proteins separated from activated receptor during internalization, but not adenylyl cyclase 3 (B2AR)
- Differences in internalization kinetics

- Bystander proteins

Resource

Cell

An Approach to Spatiotemporally Resolve Protein Interaction Networks in Living Cells

Lobingier et al, 2017

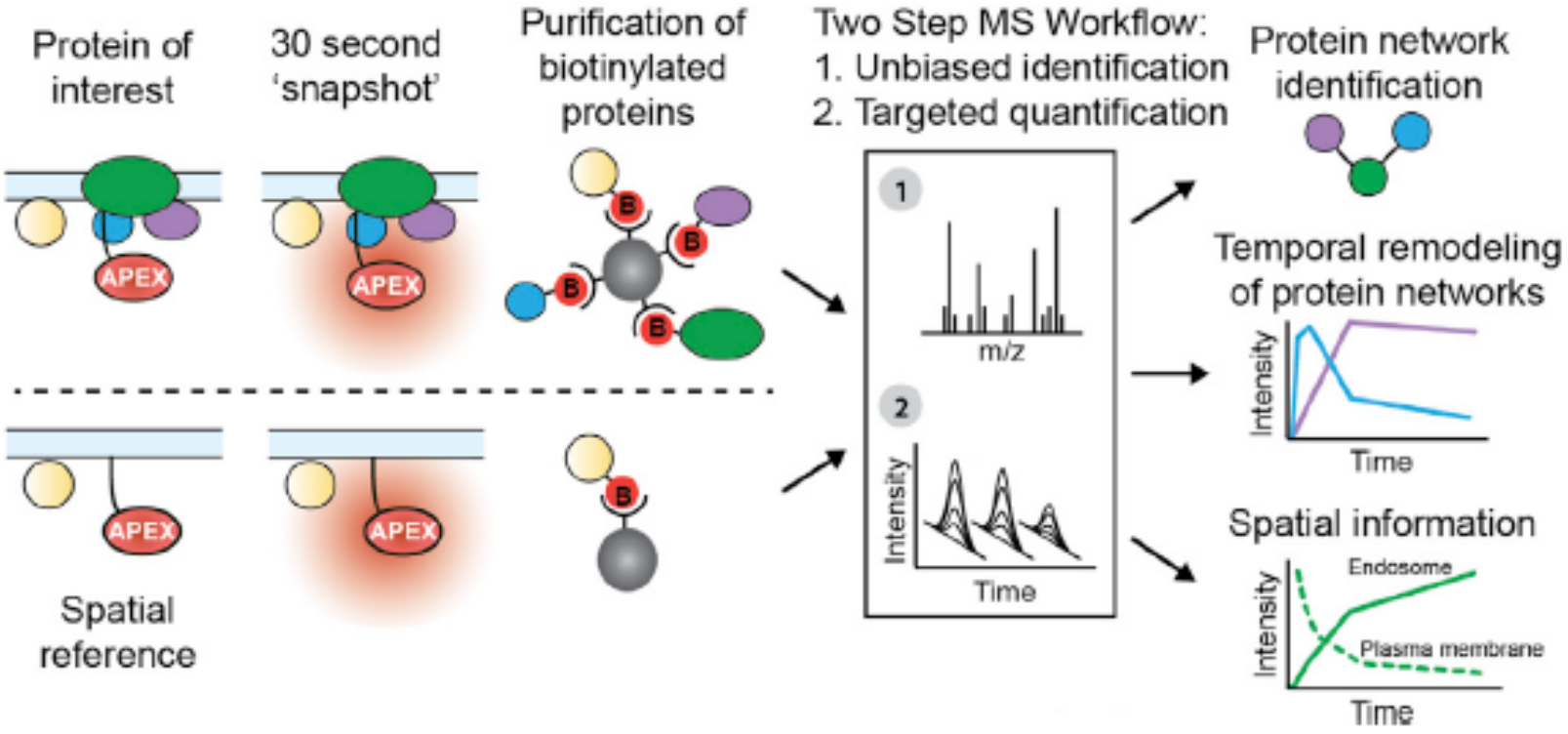
Simultaneously capture spatial and temporal context

High background by APEX proximity labelling

> deconvolve: **timing, location and interactions**

Spatiotemporally Resolve Protein Interaction Networks in Living Cells

GPCR Protein Network: B2AR



Spatiotemporally Resolve Protein Interaction Networks in Living Cells

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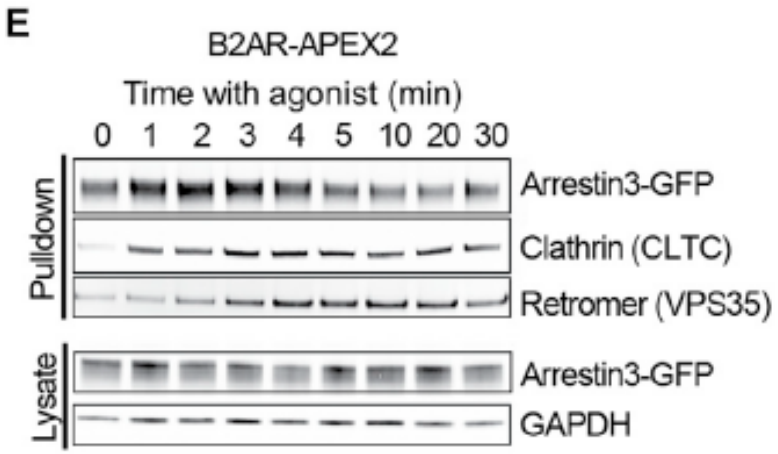
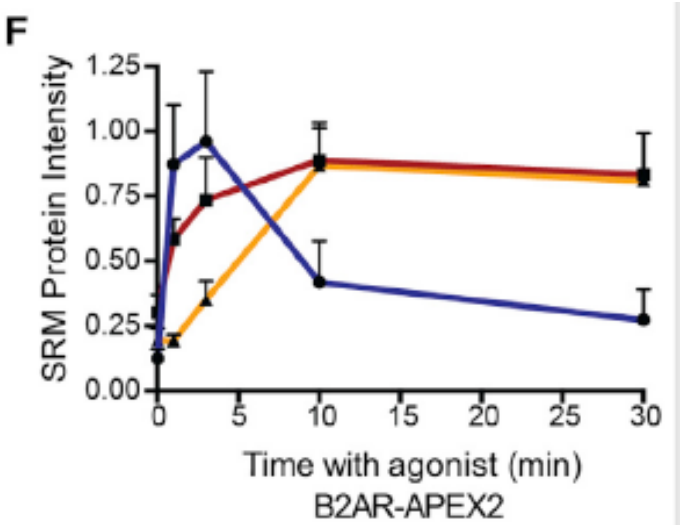
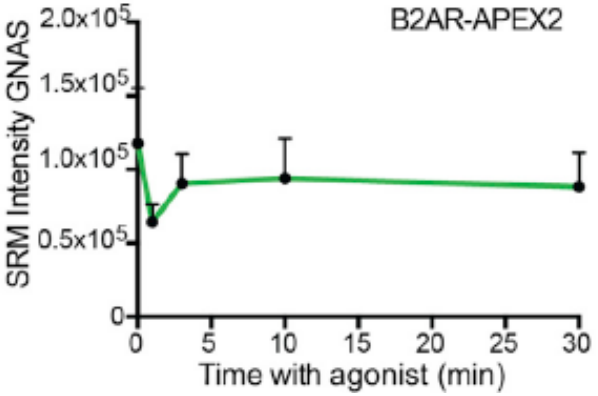




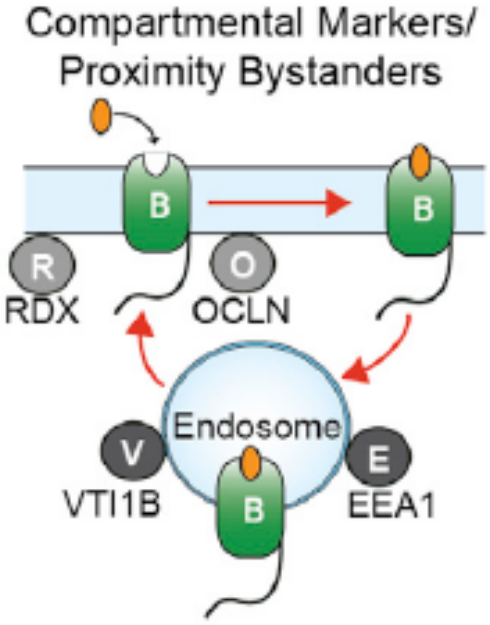


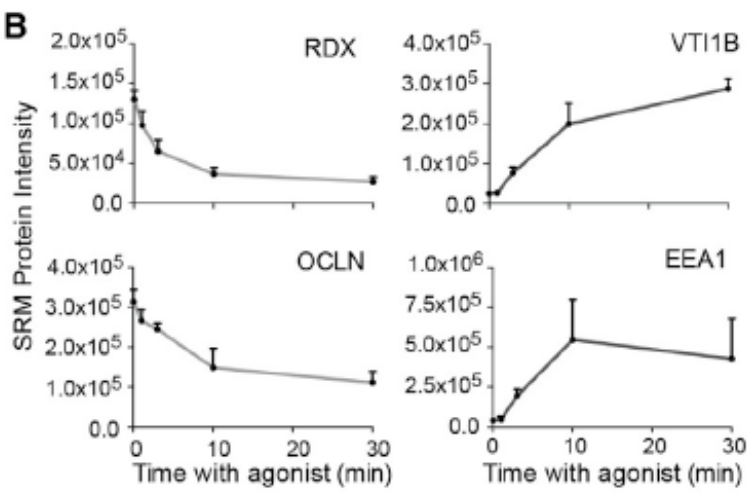
Arrestin3
CLTL (clathrin heavy chain)
VPS35 (retromer complex)

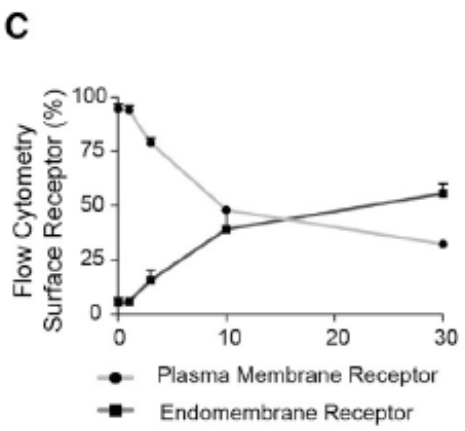
Spatiotemporally Resolve Protein Interaction Networks in Living Cells

"bystanders" as compartmental markers

- Plasma membrane: radixin (RDX), occluding (OCLN)
- Endosomes: early endosomal antigen 1 (EEA1), VTI1B



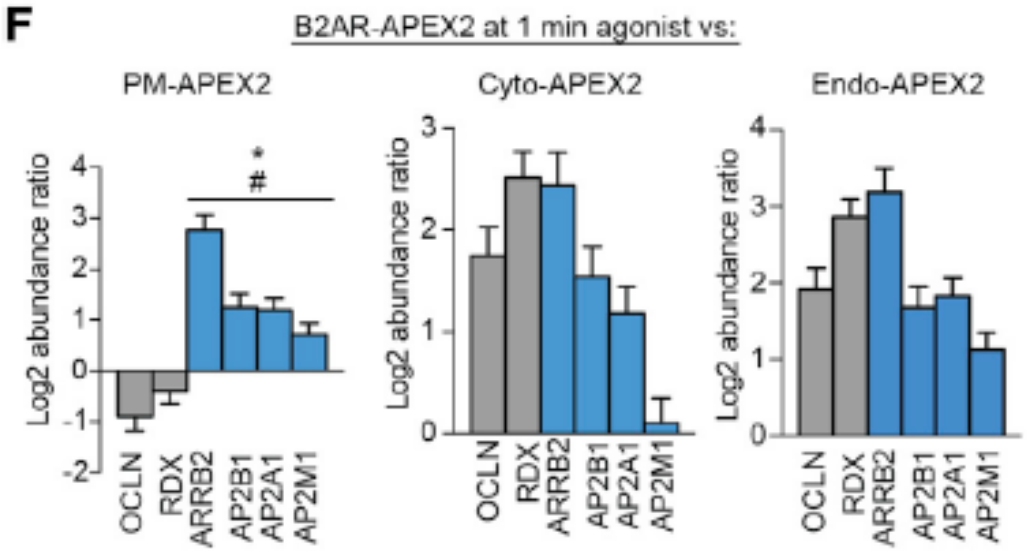




Spatiotemporally Resolve Protein Interaction Networks in Living Cells

Generate spatial APEX references

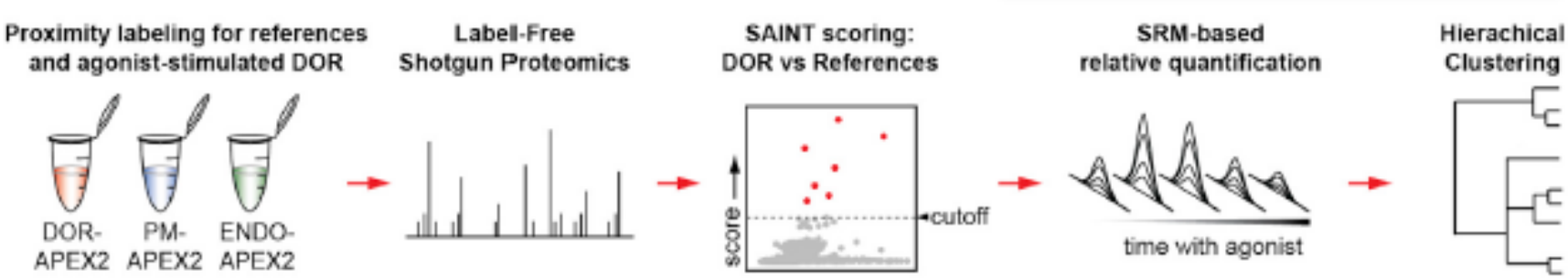
APEX profiles from same subcellular compartment quantitatively similar (bystander), but differ for interacting proteins



Spatiotemporally Resolve Protein Interaction Networks in Living Cells

Identify unknown interacting proteins?

delta-opioid receptor (DOR): mechanisms of downregulation



Spatiotemporally Resolve Protein Interaction Networks in Living Cells

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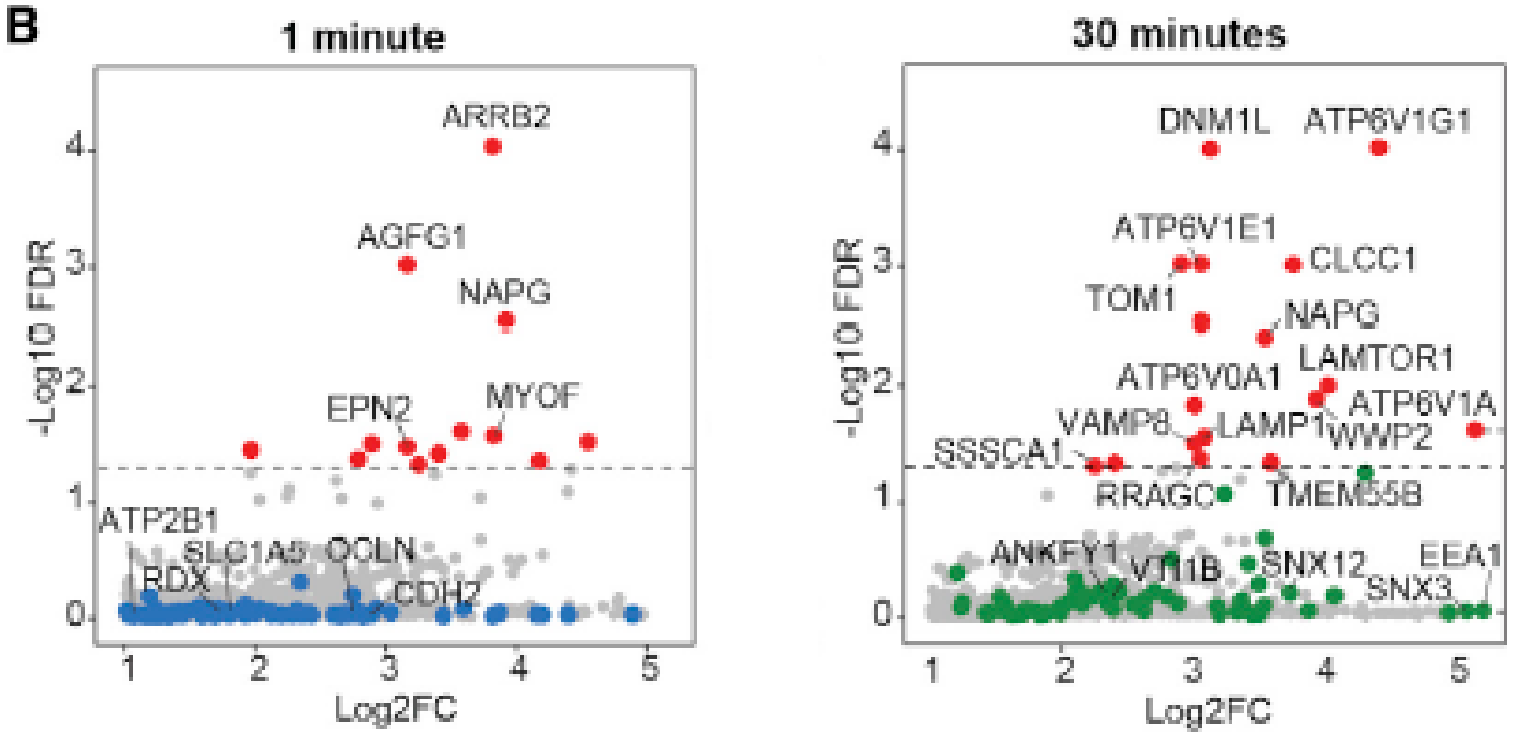
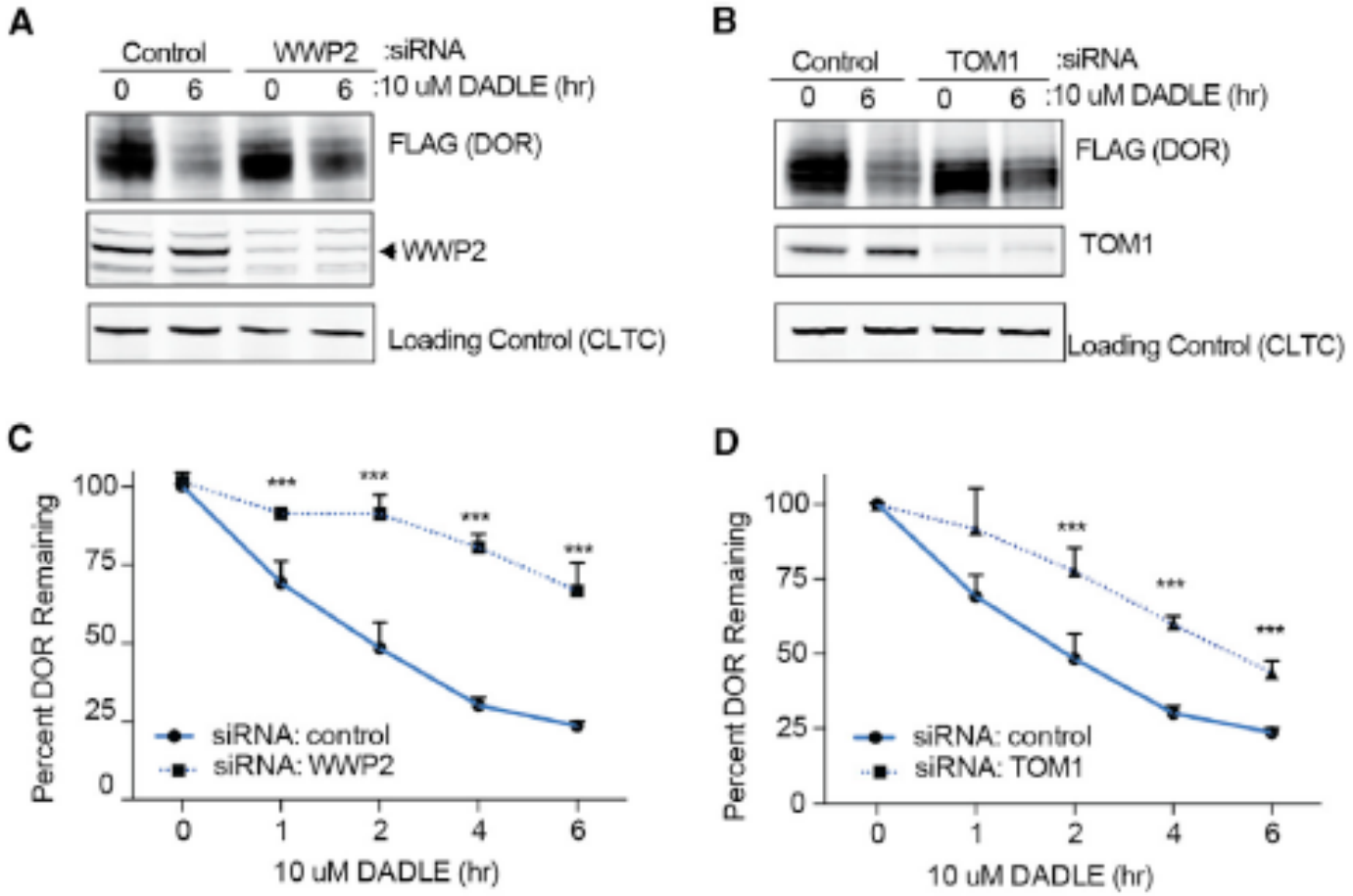


Spatiotemporally Resolve Protein Interaction Networks in Living Cells



Spatiotemporally Resolve Protein Interaction Networks in Living Cells

- Allows resolving of protein networks according to locating and timing
- Established using GPCR, validated with capture of known binding partners
- Discovery of new components

Proximity labeling

Advantages

- No need for organelle purification
- Labeling in intact cells > preserve weak/transient interactions, reduce false positives
- Labeling in living cells
- Proteomic analysis of other subcellular regions

Limitations

– Limitations for labelling

– Cannot distinguish direct binding vs proximity

– Further fine-tuning by split APEX, faster kinetics