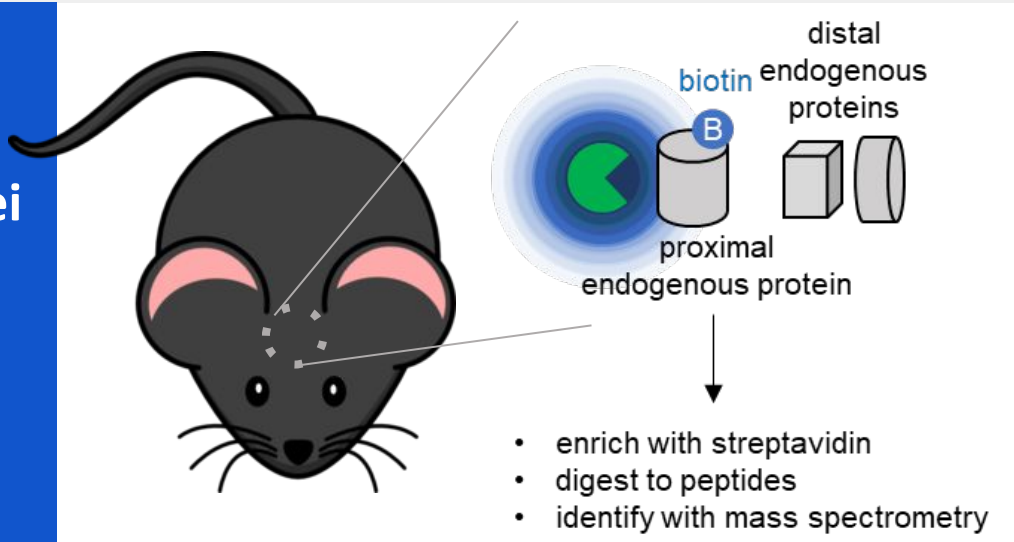
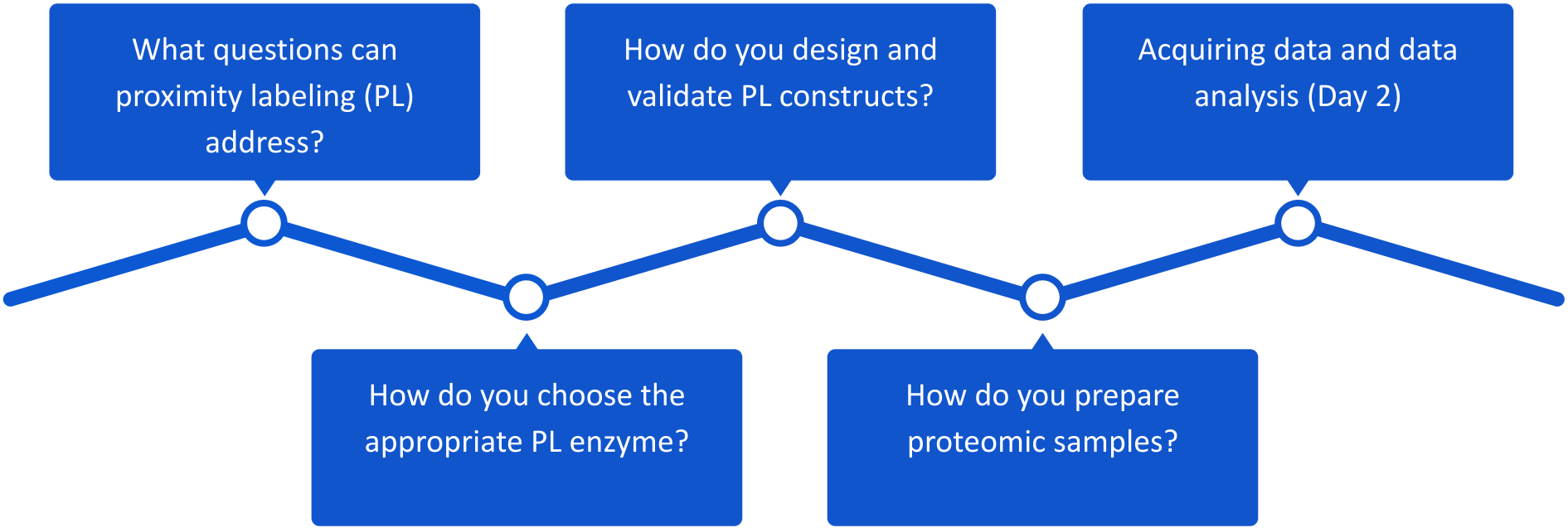
Neuro-omics Week 2:
Design and execution of proximity labeling experiments

Tess Branon, Shuo Han, Kelvin Cho, Wei Qin, and Jiefu Li

Stanford University and UC Berkeley



Outline


What questions can proximity labeling (PL) address?
How do you choose the appropriate PL enzyme?
How do you design and validate PL constructs?
How do you prepare proteomic samples?
Acquiring data and data analysis (Day 2)

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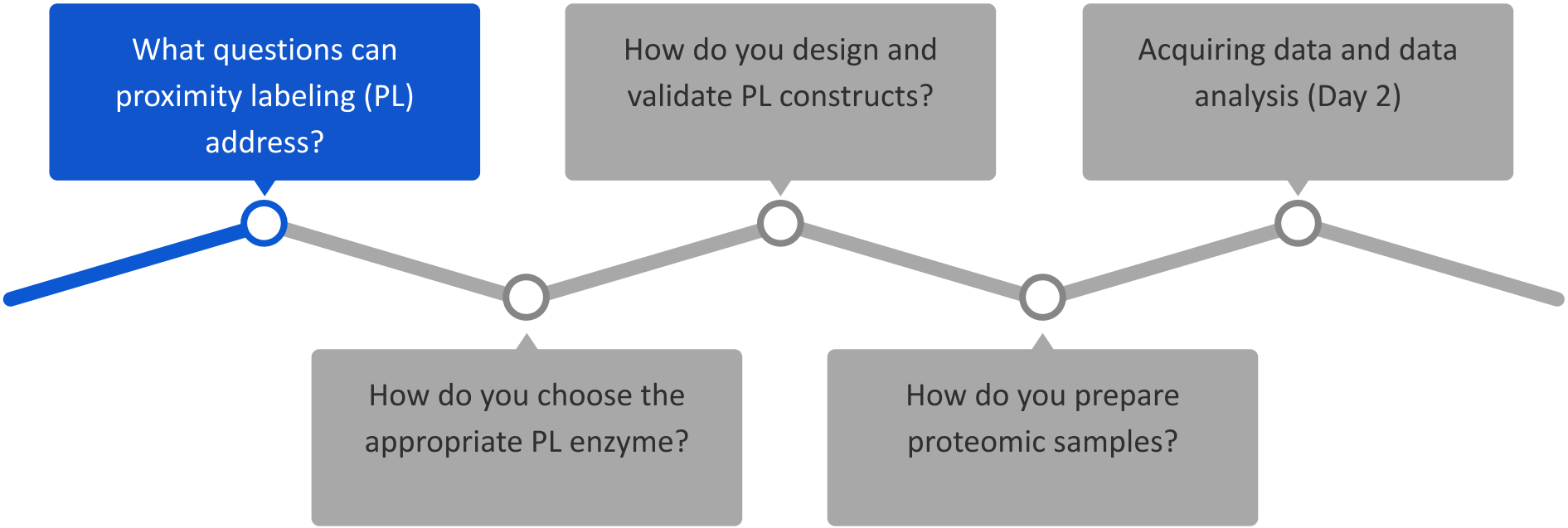
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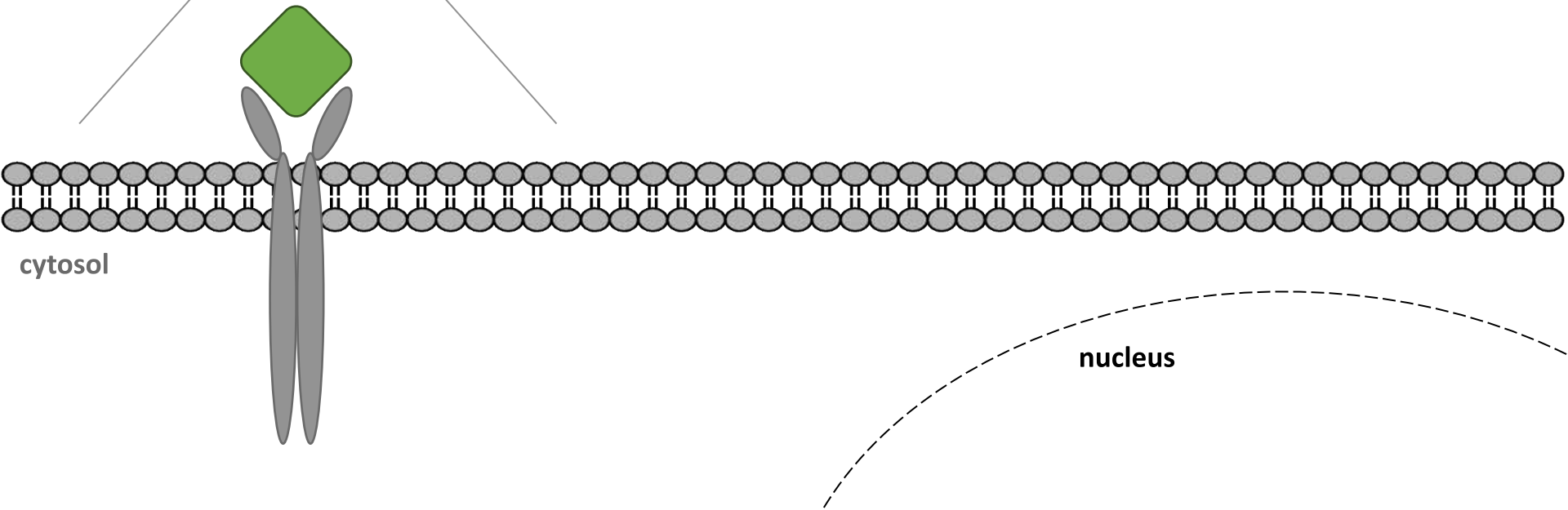
- **Protein chemical function**
  Kinase? Receptor? ligand?



- **Protein chemical function**
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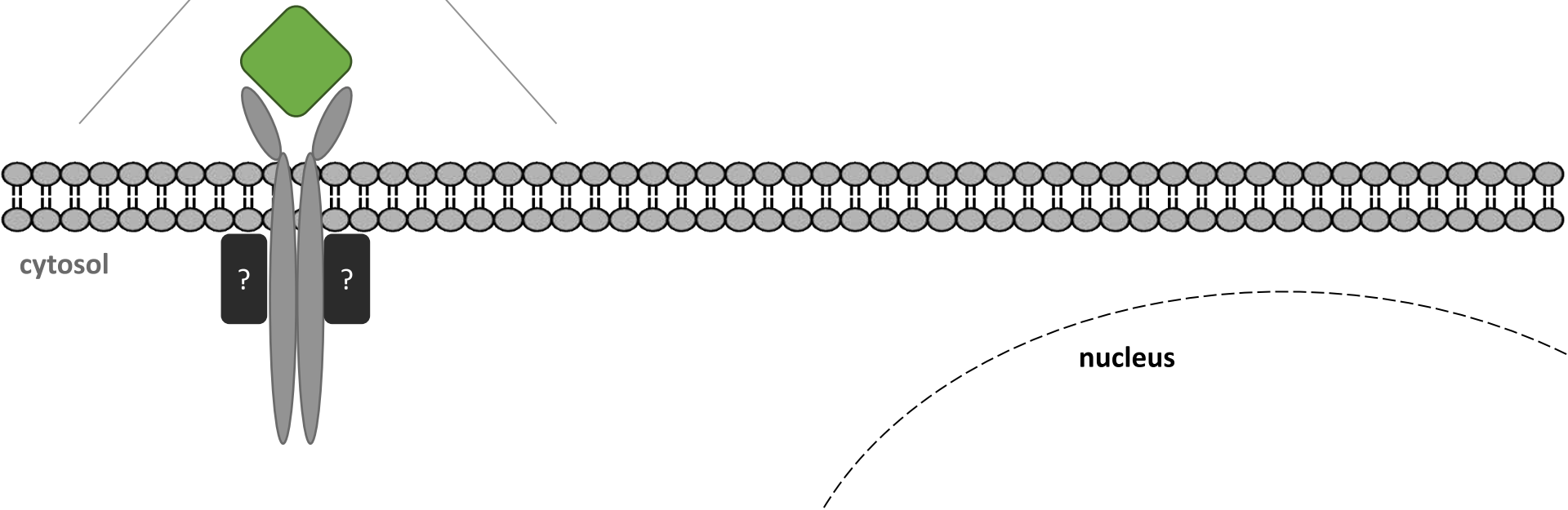
- **Protein biological function**
  Protein-protein interactions?



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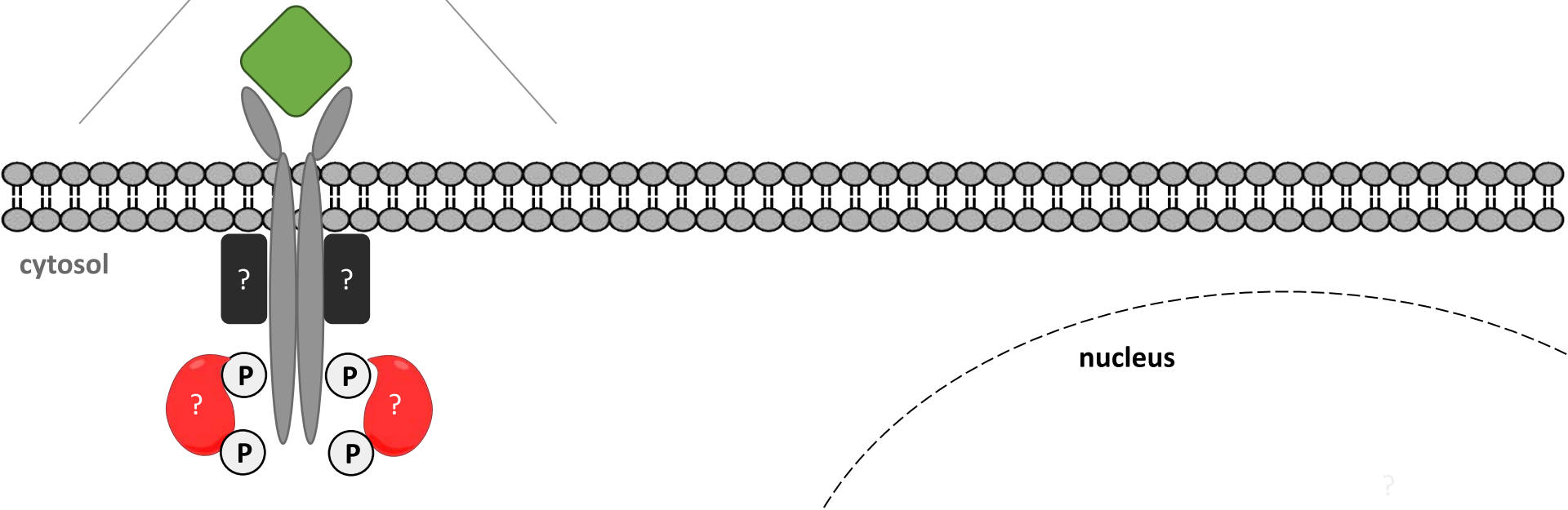
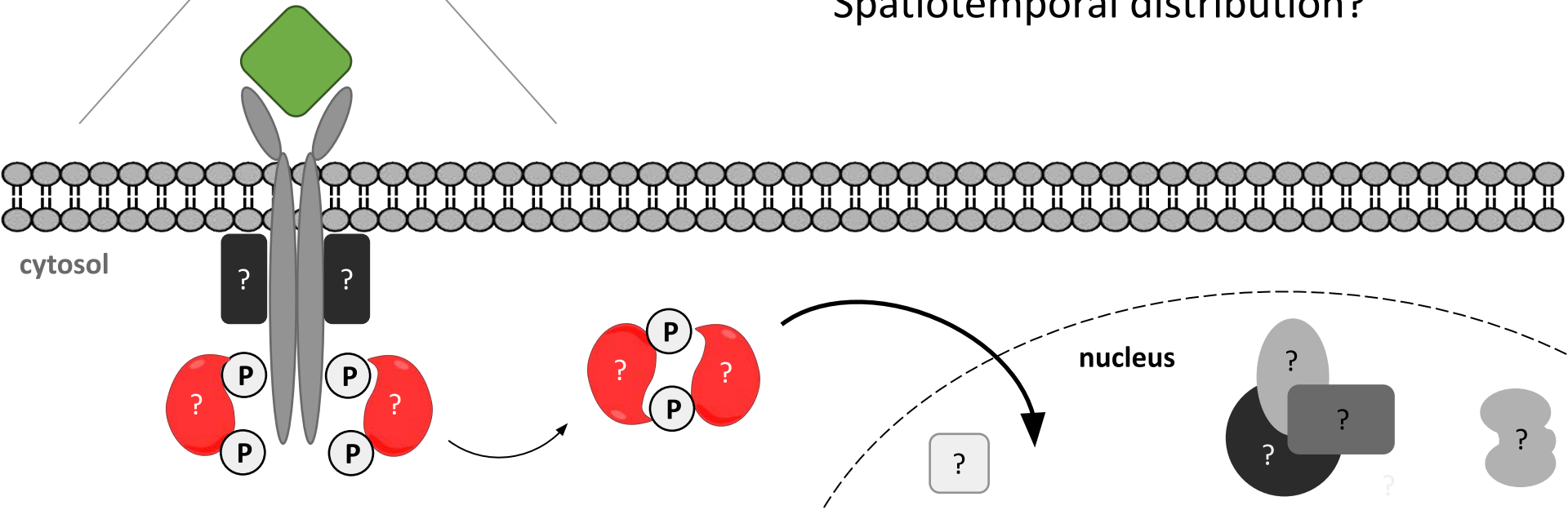


- **Protein chemical function**
  Kinase? Receptor? ligand?

- **Protein biological function**
  Protein-protein interactions?
  Spatiotemporal distribution?



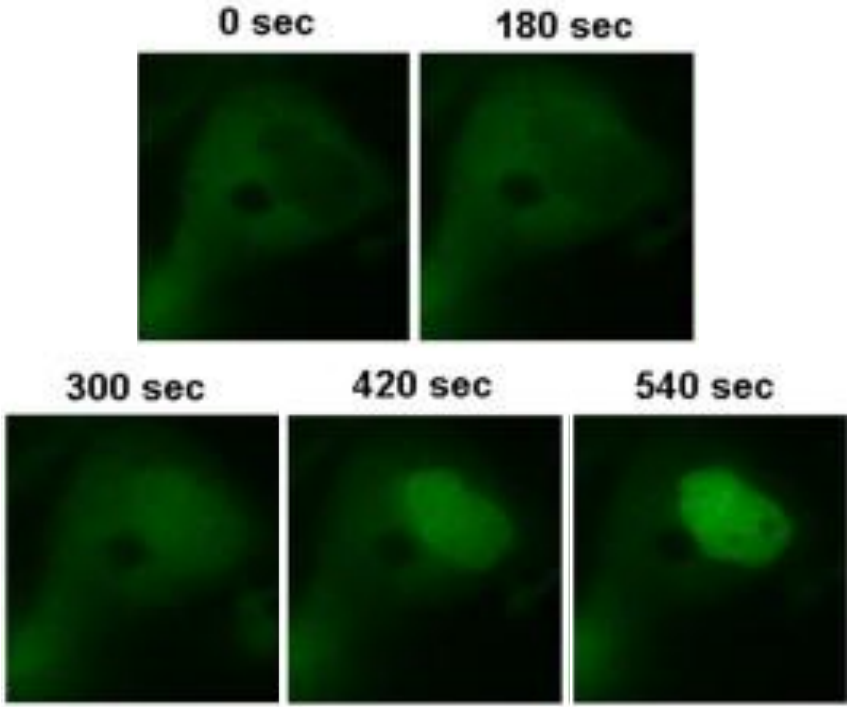
Imaging-based techniques

✓ Spatiotemporal info in live cells

✓ Single-cell information

x Recombinant proteins

x Cannot multiplex



Kwon et al. *Biochim. Biophys. Acta.* 2008

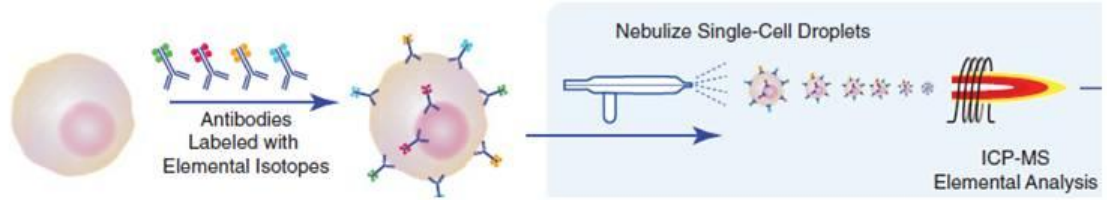
Flow cytometry-based techniques

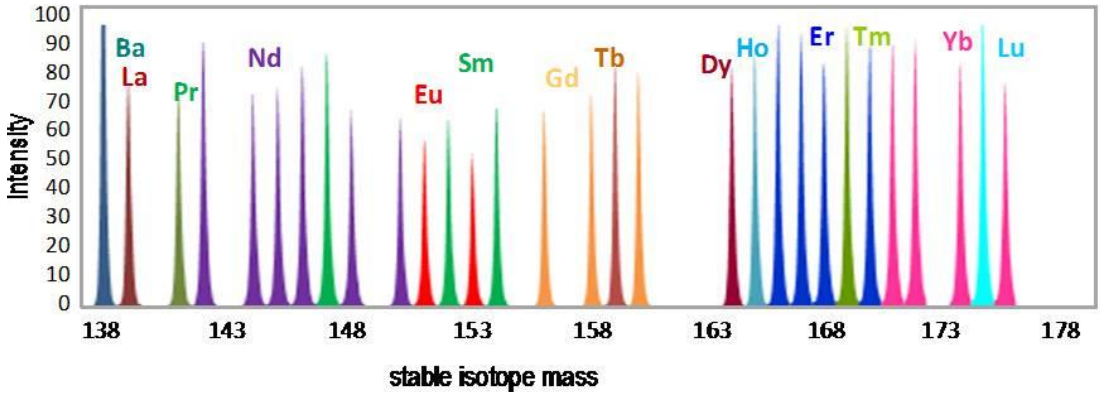
✔ Info on endogenous proteins

✔ Single-cell information

x Limited by antibody availability/quality

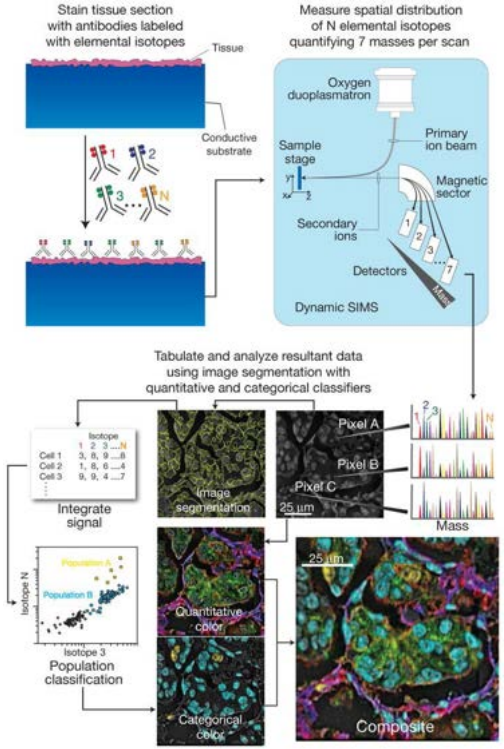
x No subcellular information





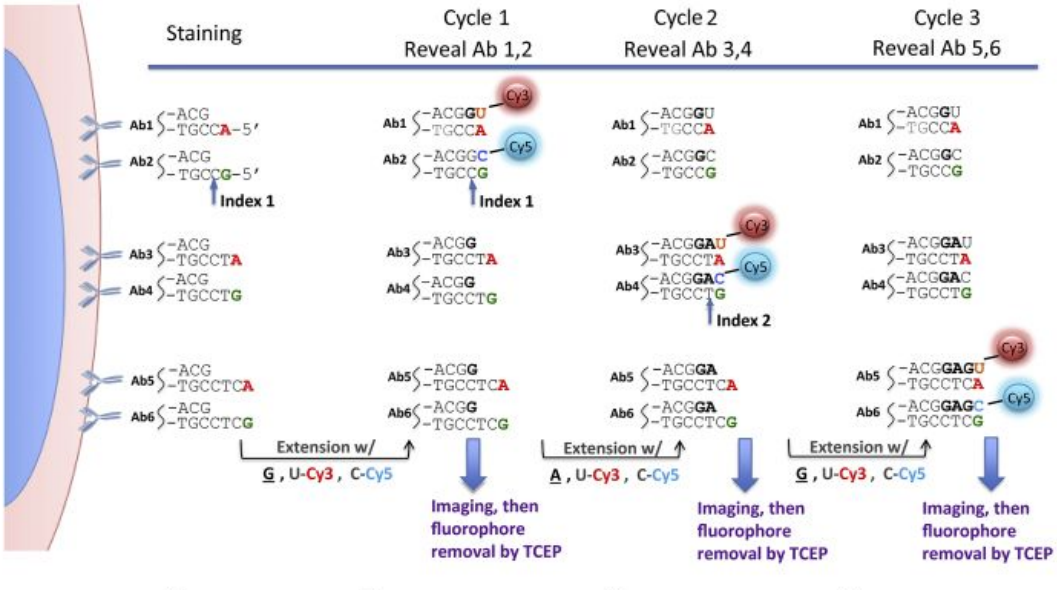
New innovations in antibody-based imaging

MIBI



Angelo et al. *Nat. Med* 2014

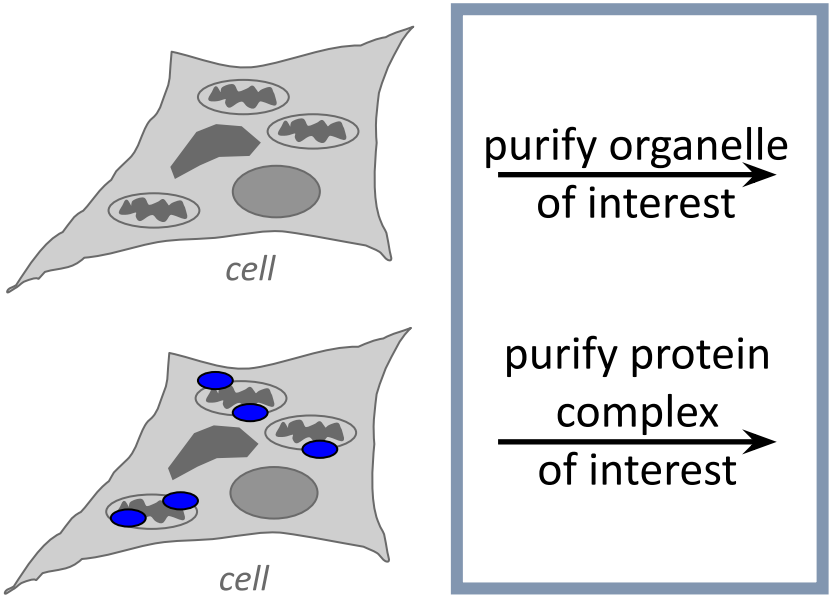
CODEX



Goltsev et al. *Cell* 2018

Fractionation-based techniques

Two major classes:



Differential centrifugation

Affinity purification

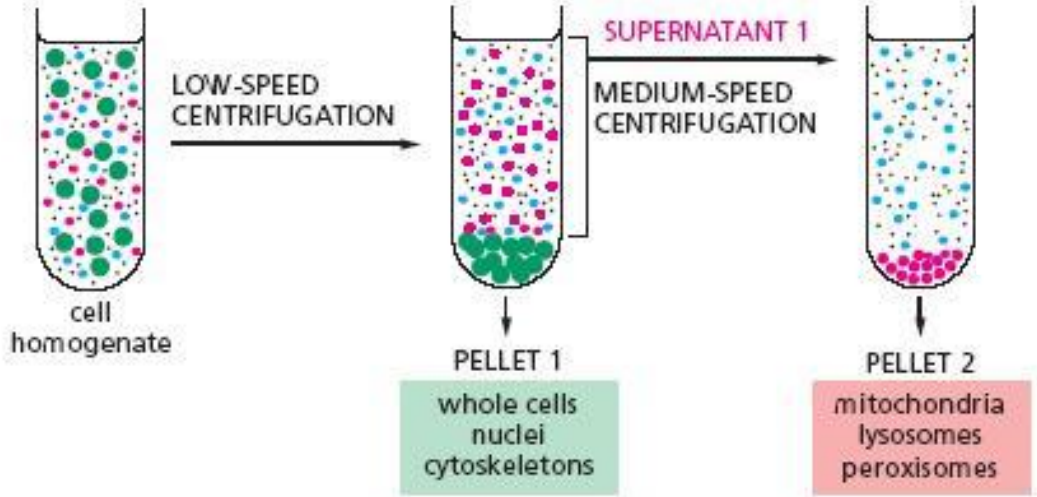
Differential centrifugation

✔ Multiplex coverage of whole proteome

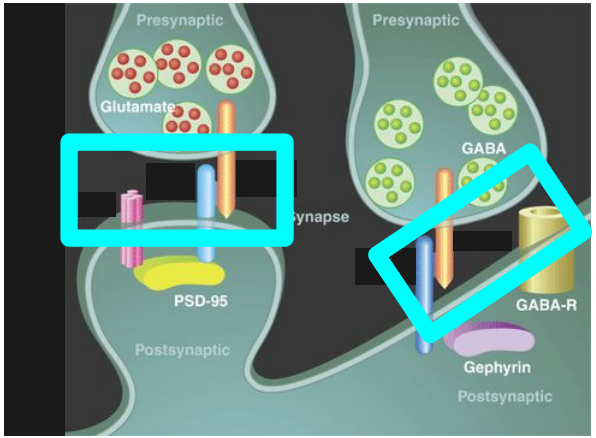
✔ Endogenous proteins

x Cell lysis disrupts compartments and complexes

x Many compartments and complexes cannot be purified

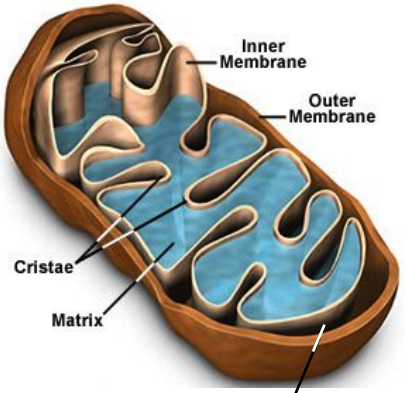


Many cellular regions cannot be purified



neuronal synaptic cleft

- stress granules
- P-bodies
- other membrane-less organelles
- nuclear lamina
- nuclear envelope
- nucleolus
- outer mito membrane
- mito-ER junctions
- mito cristae junctions



mitochondrial intermembrane space

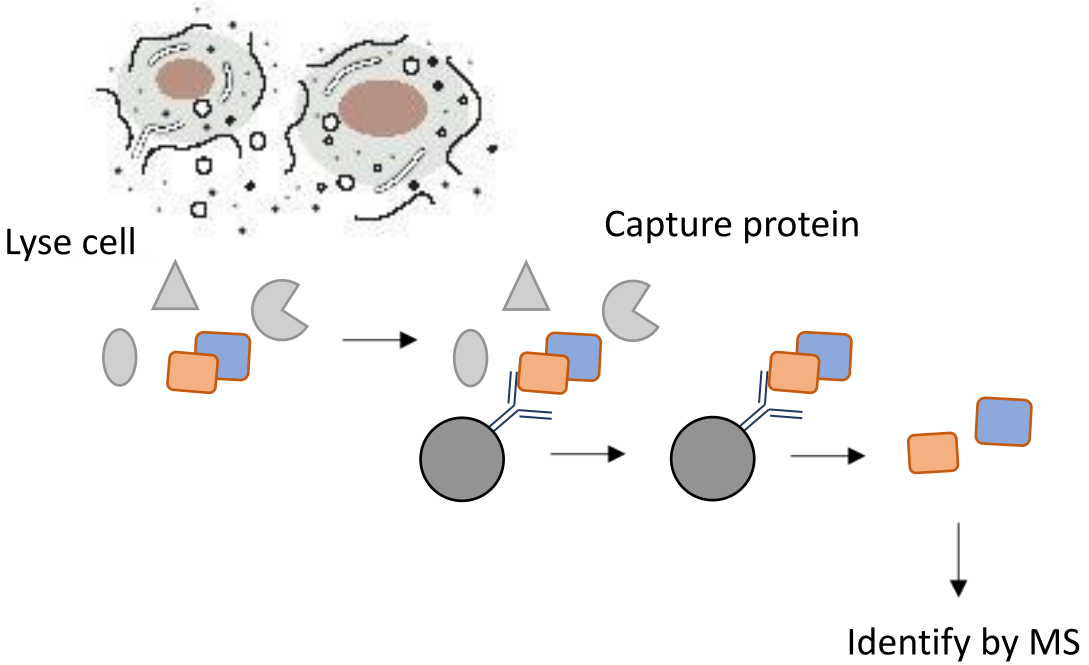
- pre-synaptic active zone
- inhibitory post-synaptic density
- axon initial segment
- transport vesicles
- autophagosomes
- centrosome
- cilia
- specific genomic loci
- many more....

Immunoprecipitation-mass spectrometry (IP-MS)

✓ Identify protein-protein interactions

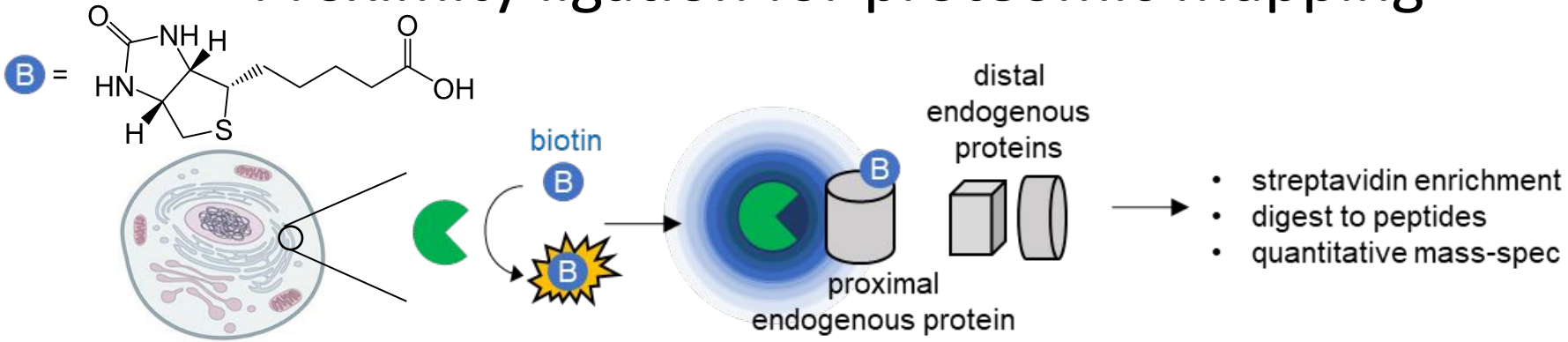
x Miss transient and weak interactions

x False positives from non-specific binders

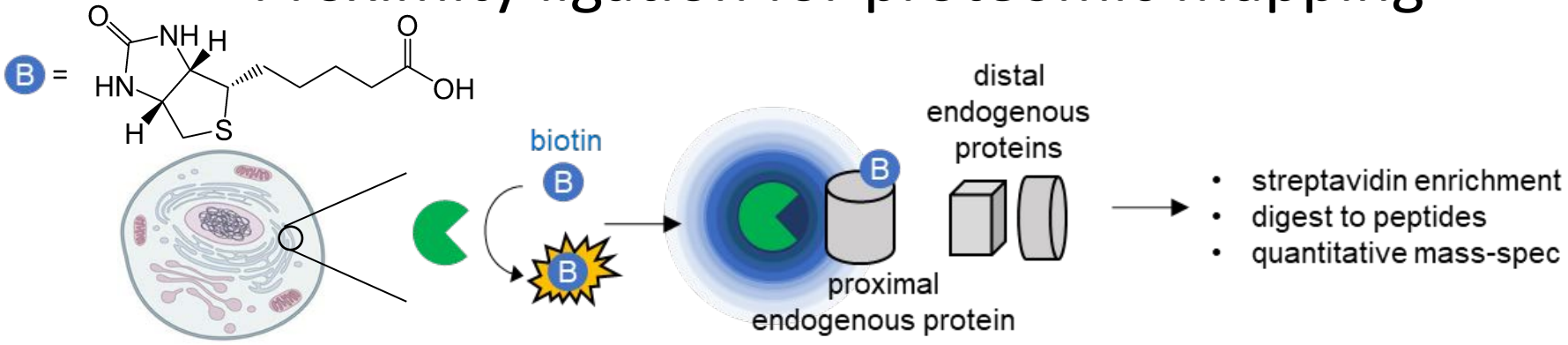


Imaging Techniques	Flow Techniques	Fractionation	Proximity labeling
✓ Single-cell info	✓ Single-cell info	x No single-cell info	x No single-cell info
✓ Spatiotemporal info in live cells	x No spatial info	x Limited spatial info	✓ Spatiotemporal info in live cells
x Recombinant proteins/requires abs	✓ Endogenous proteins	✓ Endogenous proteins	✓ Endogenous proteins
x Limited by antibody availability	x Limited by antibody availability	x Limited by antibody availability	✓ Does not depend on antibodies
x Limited multiplexability*	x Limited multiplexability	✓ Multiplexed	✓ Multiplexed
x Cannot ID PPIs	x Cannot ID PPIs	✓ Identify protein-protein interactions	✓ Identify protein-protein interactions
*Much improved with new techniques like MIBI and CODEX		x Miss transient and weak interactions	✓ ID transient and weak interactions
		x False positives from non-specific binders	✓ Low false positive

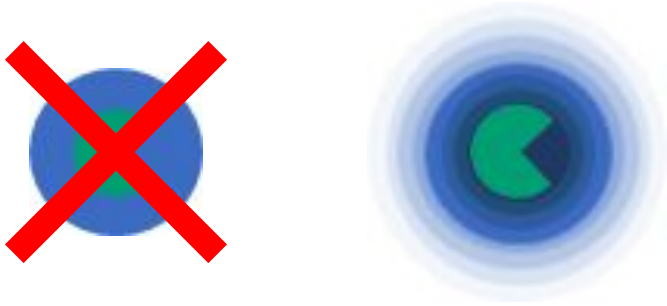
Proximity ligation for proteomic mapping



Proximity ligation for proteomic mapping

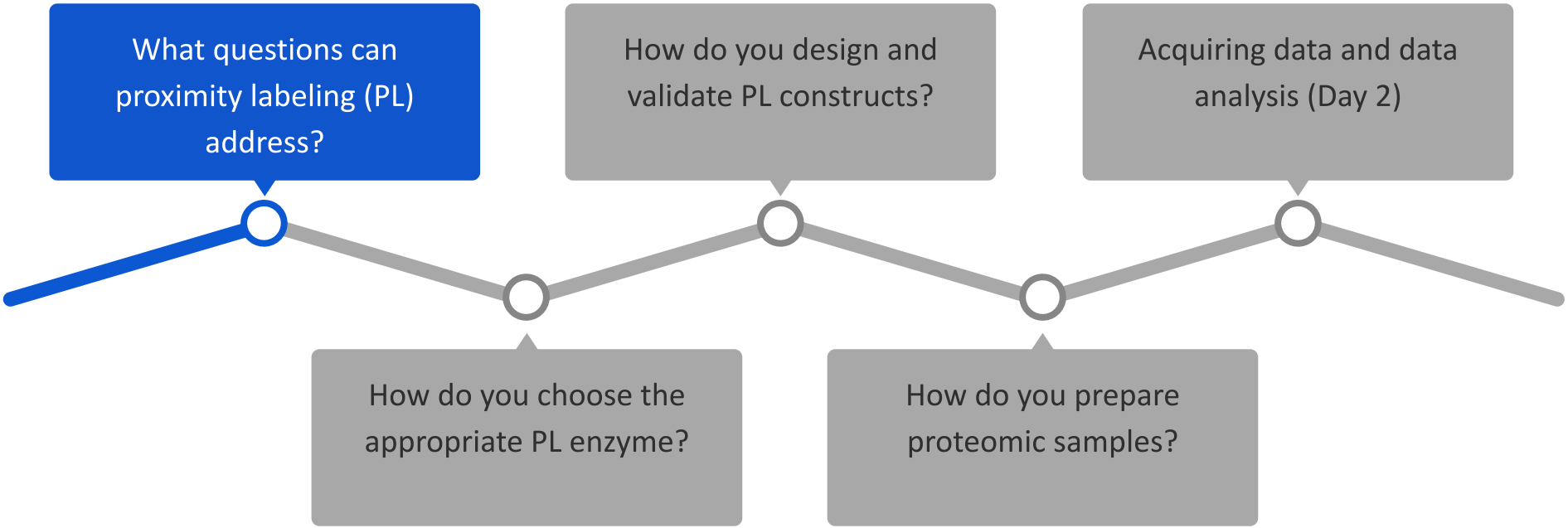


Labeling radius?



Labeling radius of most proximity labeling enzymes are on the order of nanometers (1-10 nm)

Outline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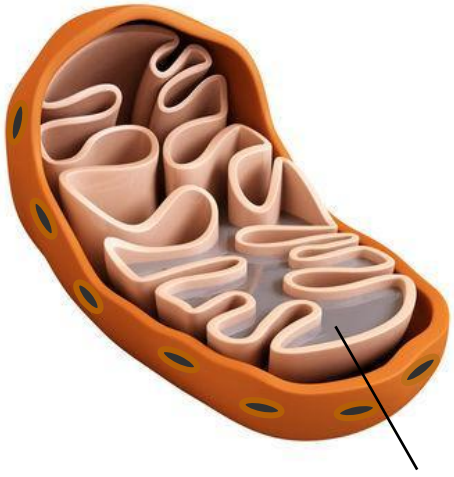
Review article discussing PL applications

W. Qin*, K. F. Cho*, P. E. Cavanagh*, and A. Y. Ting. Deciphering molecular interactions by proximity labeling. *Nature Methods* 2020, in press.

Proteomic mapping of closed subcellular compartments



Mitochondrial matrix

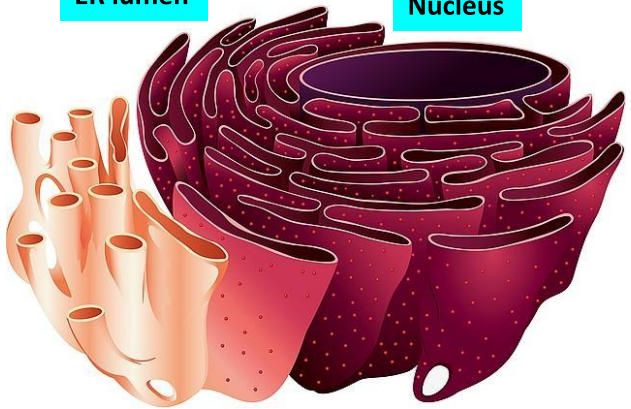
Rhee et al. *Science* 2013 (APEX)
Branon et al. *Nat. Biotech.* 2018 (TurboID)

(APEX)

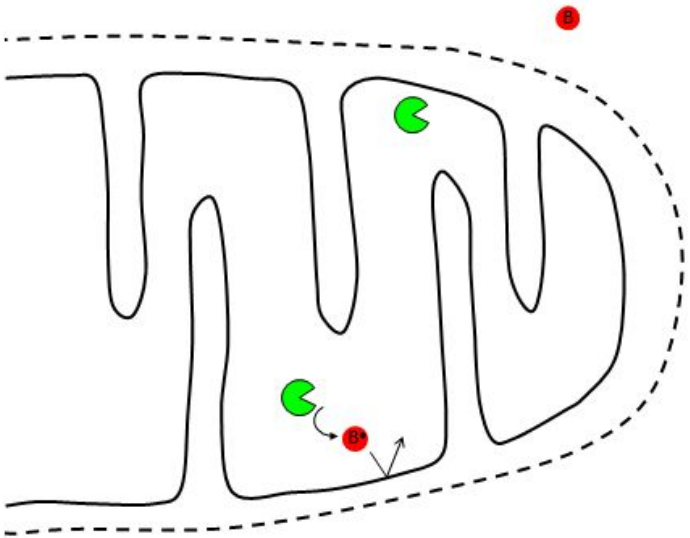
Branon et al. *Nat. Biotech.* 2018 (TurboID)

ER lumen

Nucleus



Reactive species generated by PL enzymes cannot cross tight membranes



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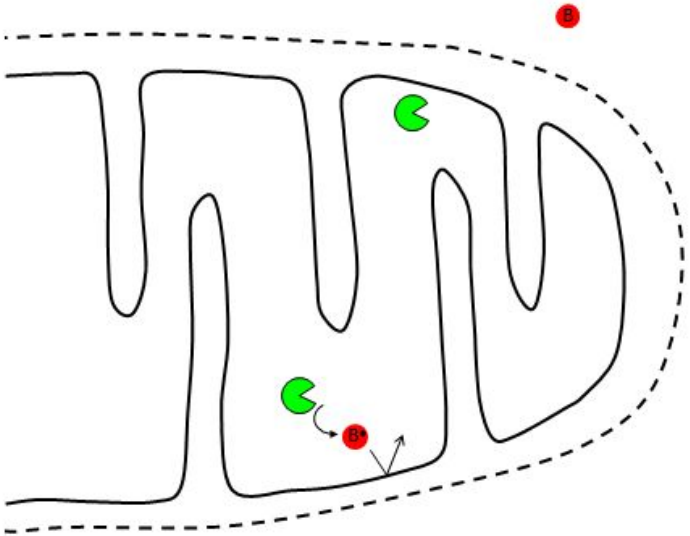
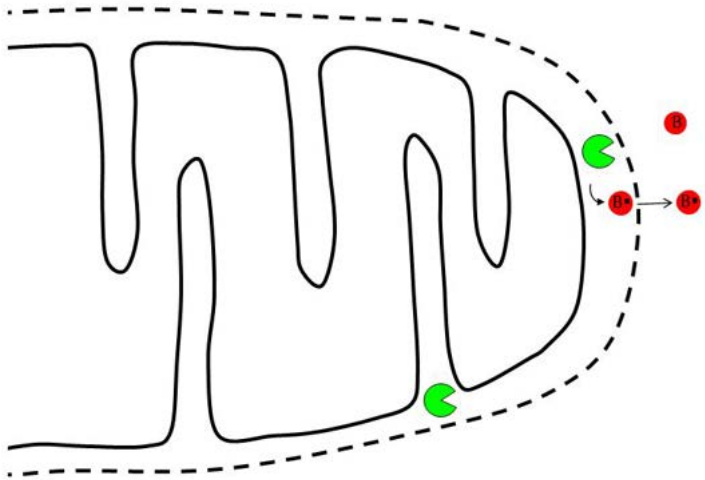
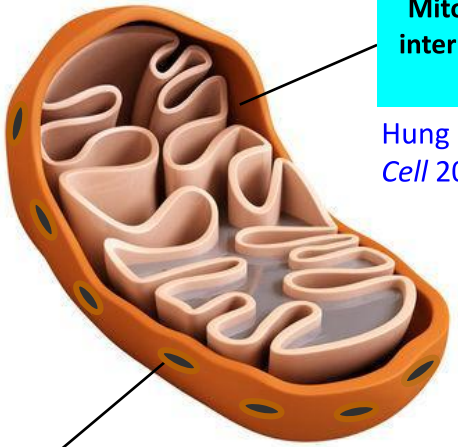




Proteomic mapping of open subcellular compartments



Mitochondrial intermembrane space

Hung et al. *Mol. Cell* 2014 (APEX2)

Mitochondrial outer membrane facing cytosol

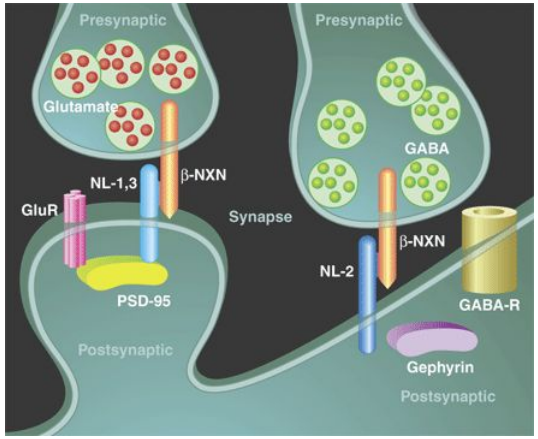
Hung et al. *eLife* 2017 (APEX2)

ER membrane facing cytosol

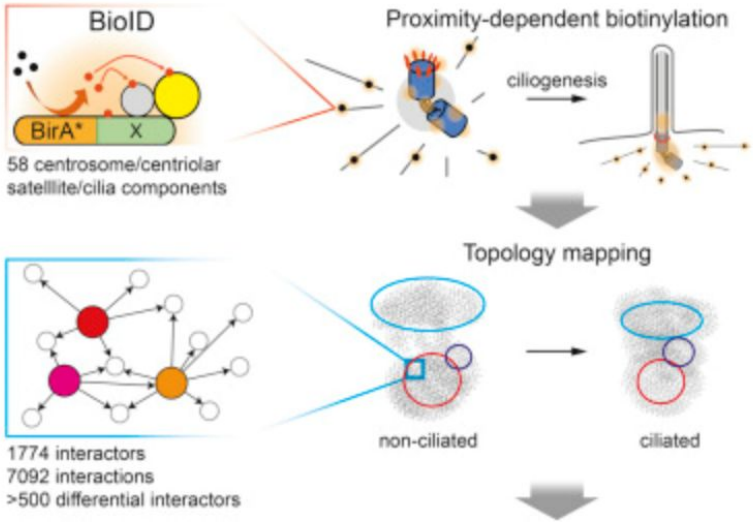
Hung et al. *eLife* 2017 (APEX2)
Branon et al. *Nat. Biotech.* 2018 (TurboID)

Loh et al. *Cell* 2017 (HRP)

Excitatory synapse **Inhibitory synapse**

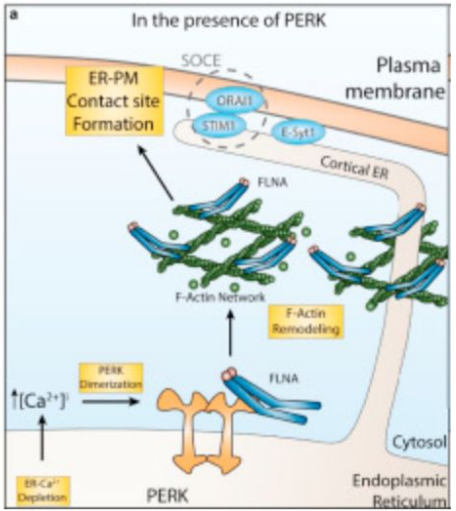


Proteomic mapping of a subcellular structure



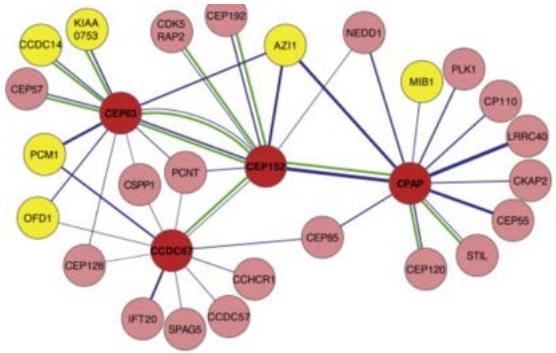
Centrosome, cilium and their interface

Gupta et al. *Cell* 2015 (BioID)



ER-PM interface

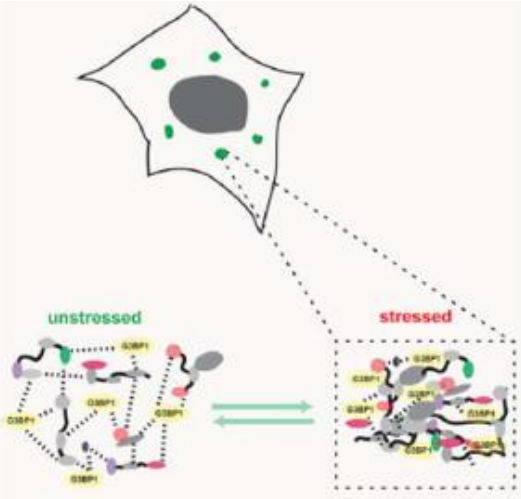
Vliet et al. *Mol. Cell* 2017 (BioID)



Centrosome proteome

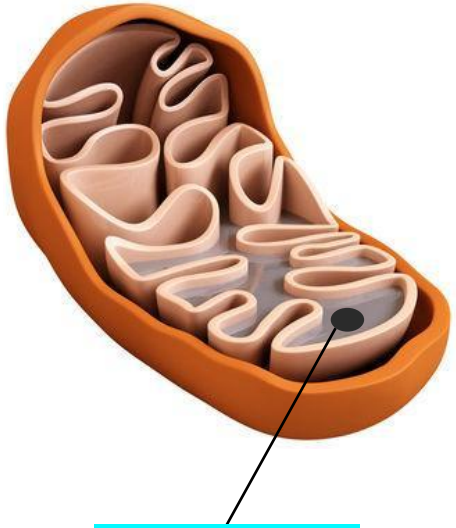
Firat-Karalar et al. *Curr. Biol.* 2014 (BioID)

Proteomic mapping of a macromolecular complex



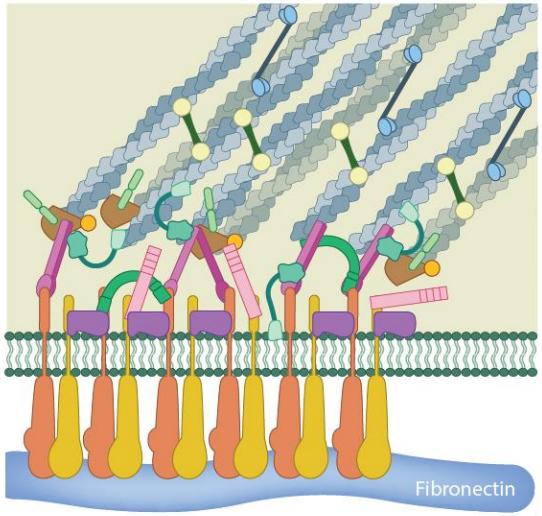
Stress granules

Markmiller et al. *Cell* 2018 (APEX2)



Mitochondrial nucleoid (mtDNA)

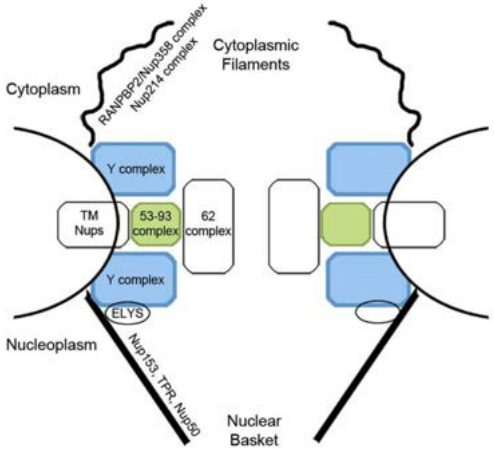
Han et al. *Cell Chem. Biol.* 2017 (APEX2)



Focal adhesion complex

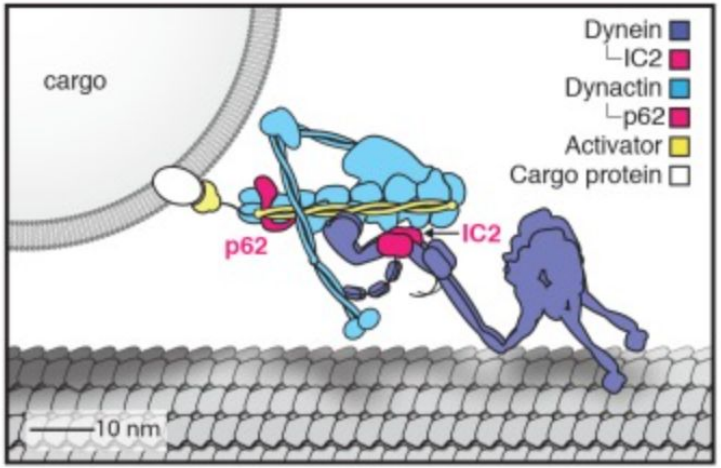
Dong et al. *Sci. Signal.* 2016 (BioID)

Proteomic mapping of a macromolecular complex



Nuclear-pore complex

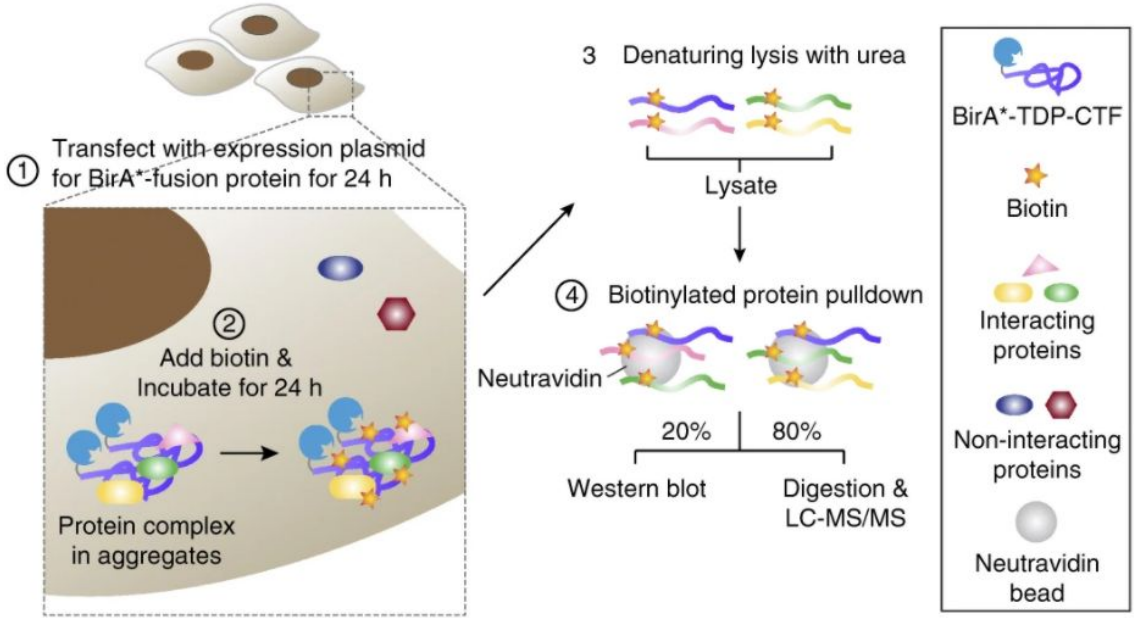
Kim et al. *PNAS* 2014 (BioID)



Dynein complex

Redwine et al. *eLife* 2017 (BioID)

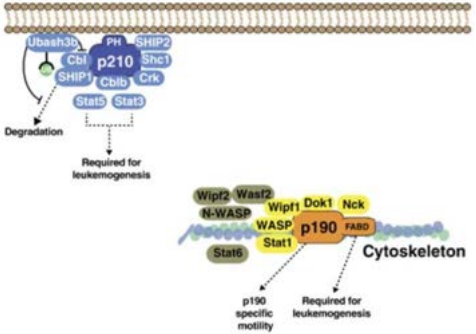
Proteomic mapping of insoluble complexes



Protein aggregates

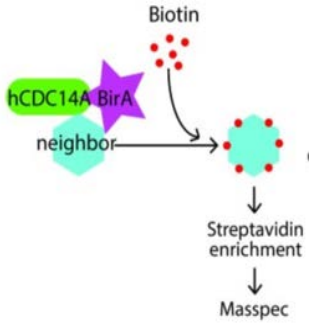
Chou et al. *Nat. Neurosci.* 2018 (BioID)

Interactome mapping of enzyme-substrate interactions



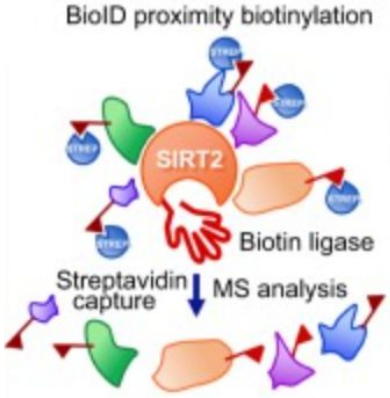
Kinases

Cutler et al. *Leukemia* 2017 (BioID)



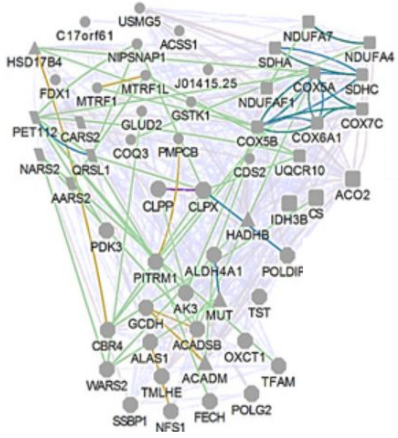
Phosphatases

Chen et al. PNAS 2017 (BioID)



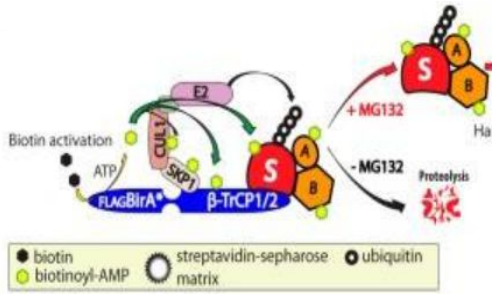
Deacetylases

Kaufmann et al. *Journal of Cell Science* 2016 (BioID)



Proteases

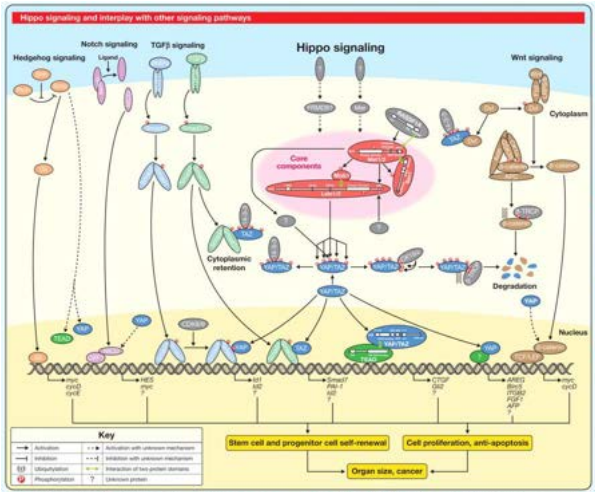
Cole et al. *Cancer Cell* 2015 (BioID)



E3 ligases

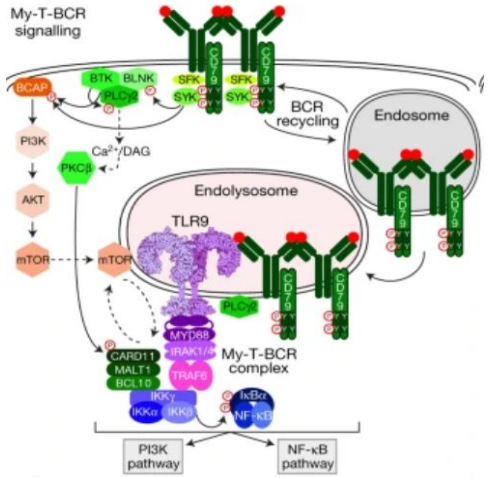
Coyaud et al. *Mol. Cell Proteom.* 2015 (BioID)

Interactome mapping of signaling pathways



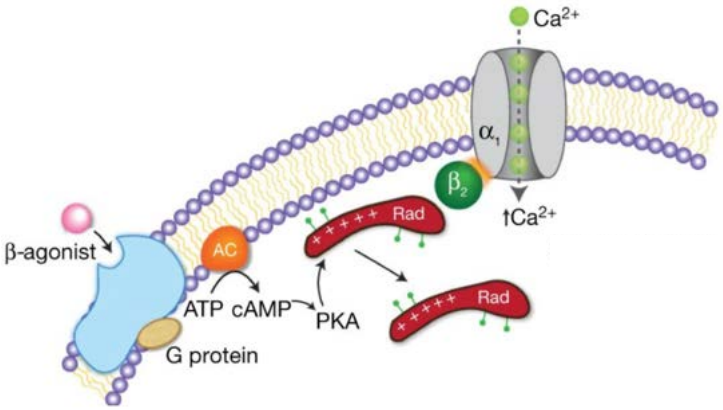
Hippo pathway

Couzens et al. *Sci. Signal.* 2013 (BioID)
Couzens et al. *Mol. Cell Proteom.* 2017 (BioID)



TLR signaling

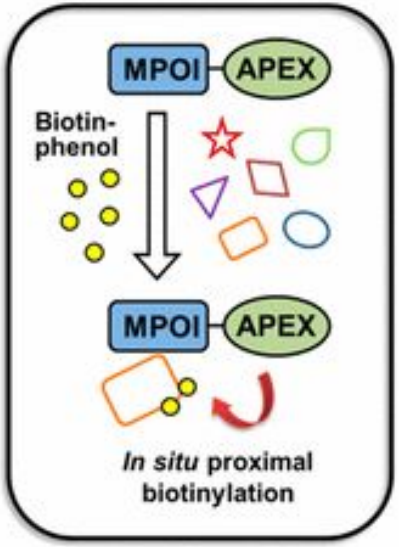
Phelan et al. *Nature* 2018



Voltage-gated calcium channels

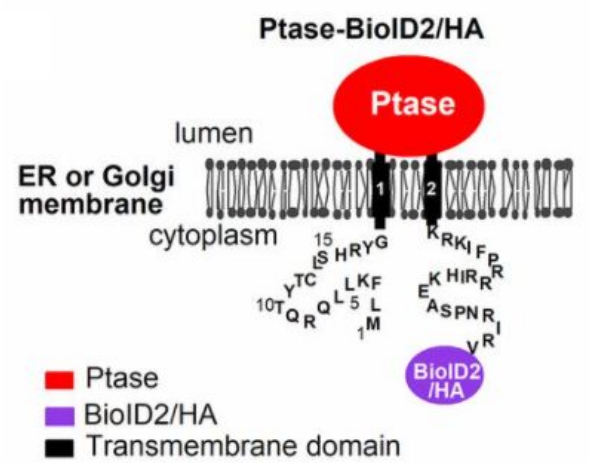
Liu et al. *Nature* 2020

Interactome mapping of other PPIs



Microproteins

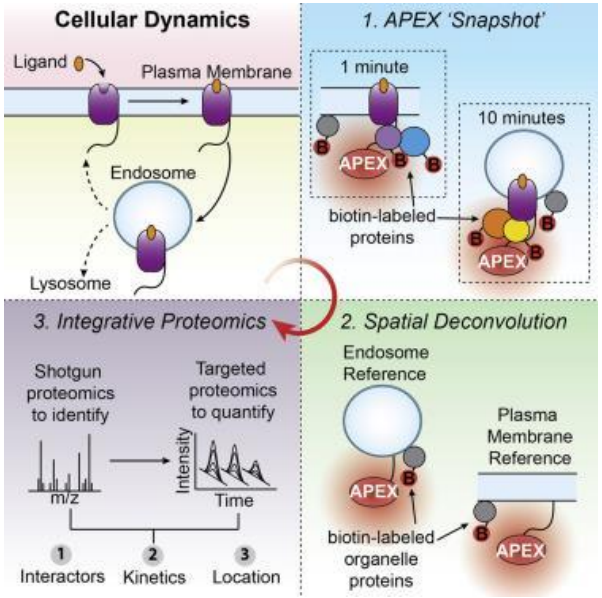
Chu et al. *Biochemistry* 2017 (APEX2)
Rathore et al. *Biochemistry* 2018 (APEX2)



Trafficking/sorting machinery

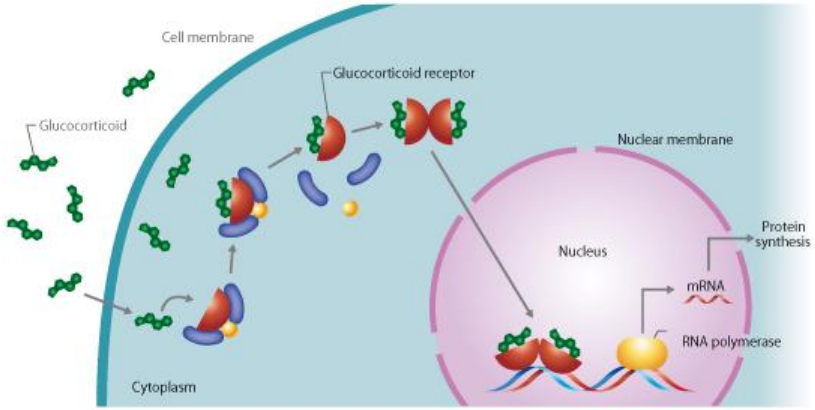
Liu et al. *PNAS* 2018 (BioID2)
Shin et al. *Nat. Cell Biol.* 2017 (BioID)
Liao et al. *Cell* 2019 (APEX2)

Measure dynamic proteomic changes before/after stimulus



GPCR signaling dynamics

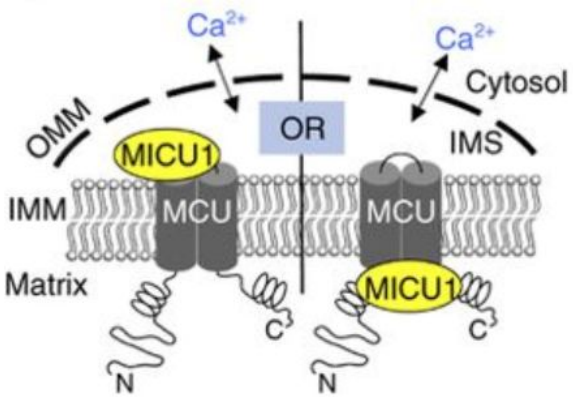
Paek et al. *Cell* 2017 (APEX2)
Lobingier et al. *Cell* 2017 (APEX2)



Hormone receptor signaling dynamics

Lempiäinen et al. *Molecular and Cellular proteomics* 2017 (BioID)

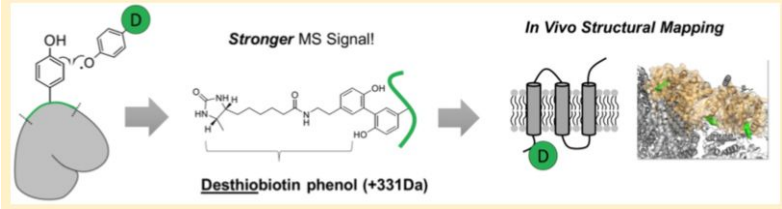
Membrane topology mapping



MCU topology determination

Lam et al. *Nature Methods* 2015 (APEX2)

Improved methods for topology mapping

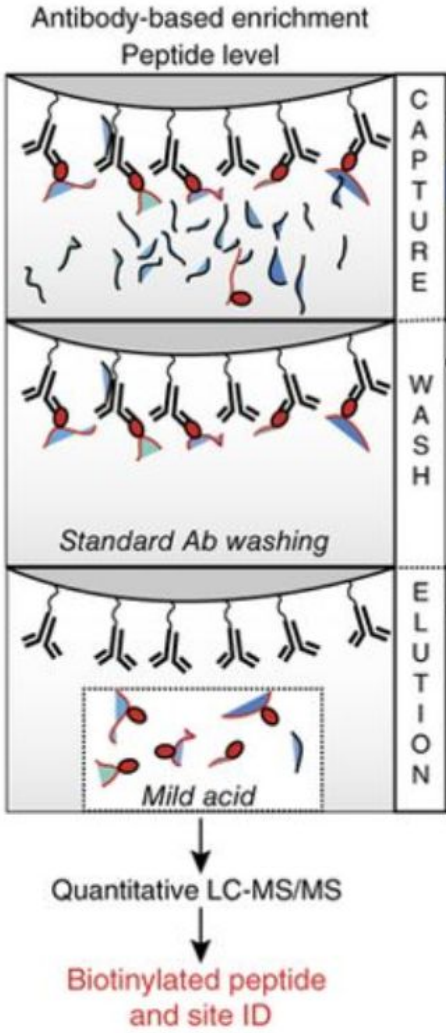


Desthiobiotin probe

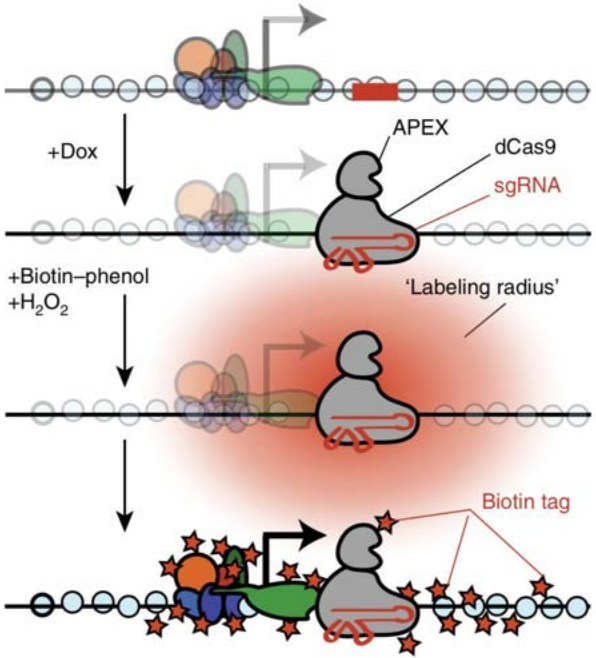
Lee et al. *JACS* 2017

Anti-biotin antibody

Udeshi et al. *Nature Methods* 2017

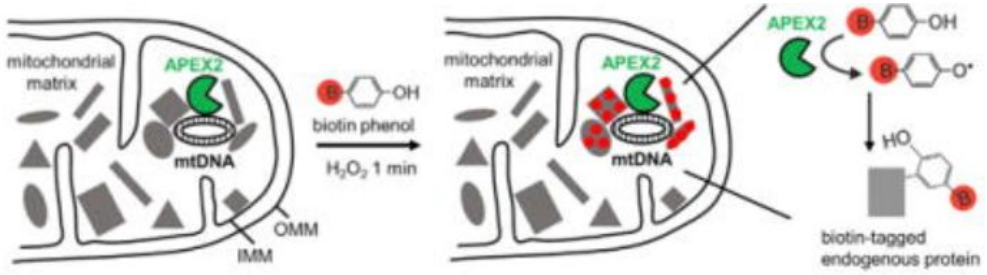


Mapping proteins associated with DNA



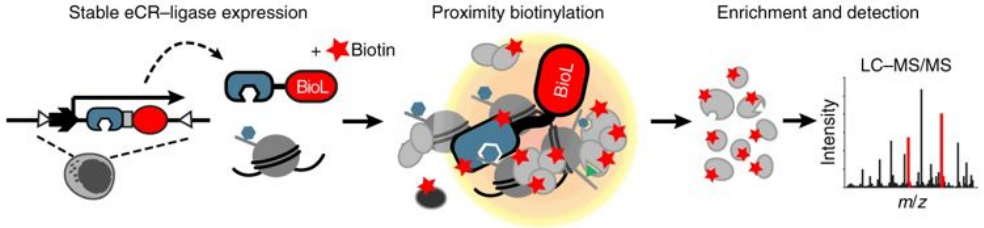
Protein interactomes around genomic loci guided by dCas9

Myers et al. *Nature Method* 2018 (APEX2)
Gao et al. *Nature Method* 2018 (APEX2)



Protein interactomes around mtDNA guided by DNA-binding proteins

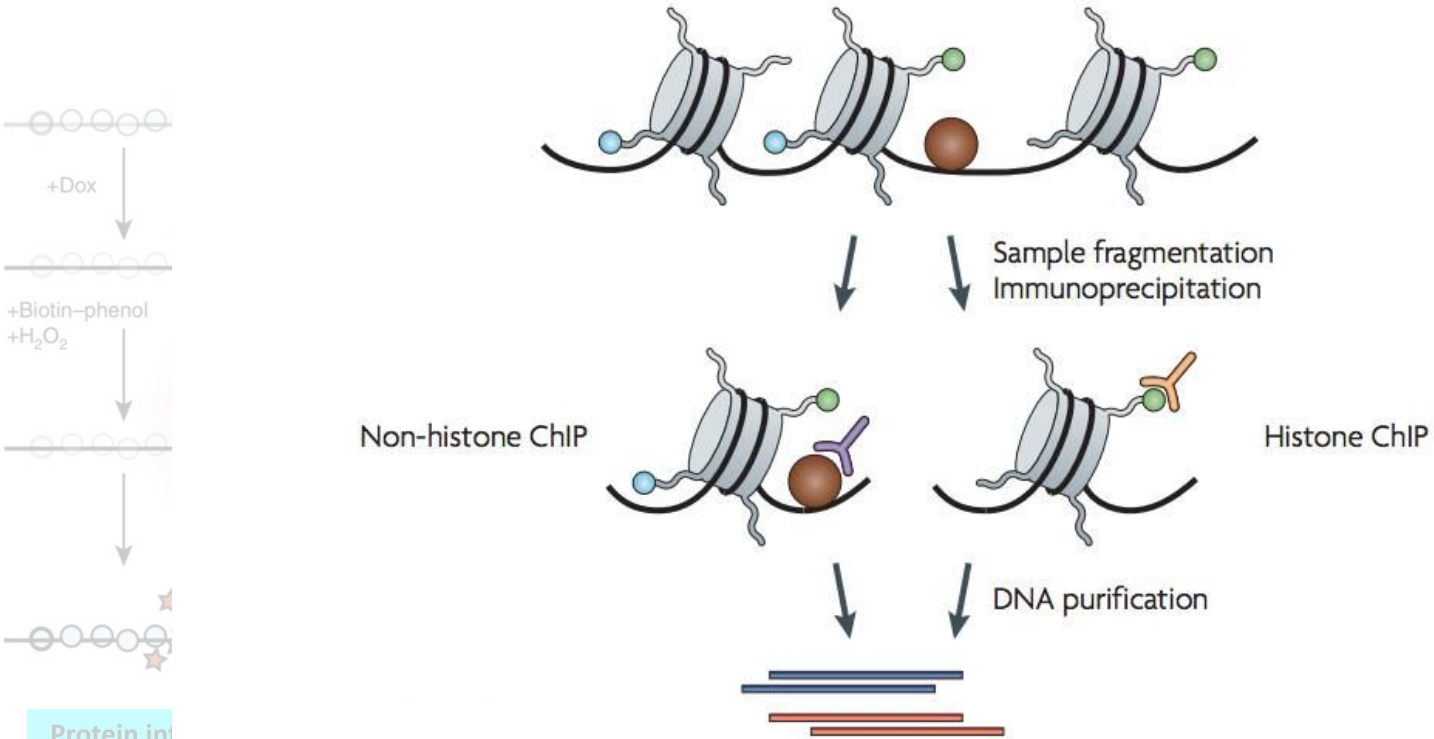
Han et al. *Cell Chem. Biol.* 2017 (APEX2)



Protein interactomes around chromatin marks

Villaseñor et al. *Nature Biotechnology* 2020 (BASU)

Mapping proteins associated with DNA



inding proteins
2)

hment and detection

Protein int
guided by dCas9

arks

Myers et al. *Nature Method* 2018 (APEX2)
Gao et al. *Nature Method* 2018 (APEX2)

Villaseñor et al. *Nature Biotechnology* 2020 (BASU)

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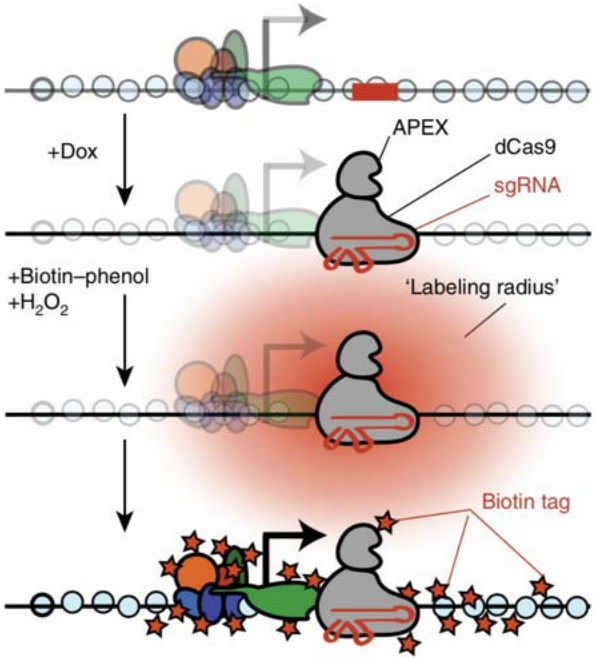

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Myers et al. *Nature Method* 2018 (APEX2)
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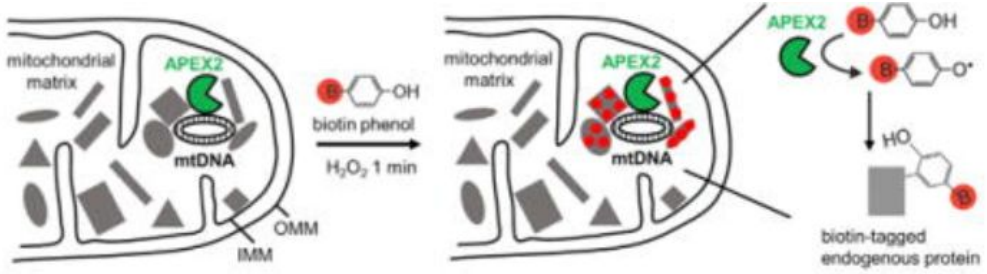
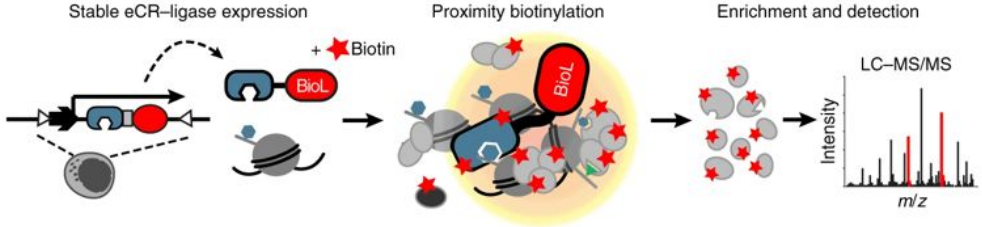


Protein interactomes around mtDNA guided by DNA-binding proteins

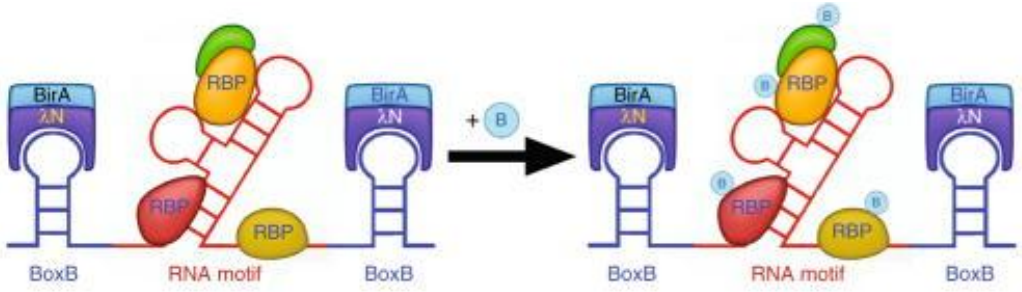
Han et al. *Cell Chem. Biol.* 2017 (APEX2)



Protein interactomes around chromatin marks

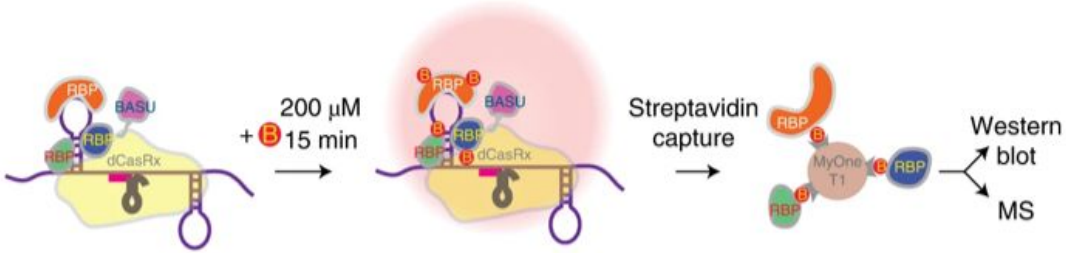
Villaseñor et al. *Nature Biotechnology* 2020 (BASU)

Mapping proteins associated with RNA



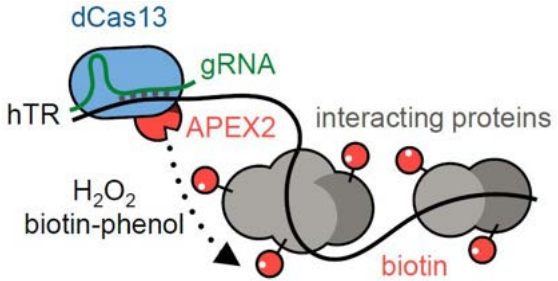
Protein interactomes around RNA motifs guided by BoxB/λN or MS2/coat protein

Ramanathan et al. *Nature Method* 2018 (BASU)
Mukherjee et al. *PNAS* 2019 (BioID)
Han et al. *PNAS* 2020 (APEX2)



Protein interactomes around RNA motifs guided by dCasRx

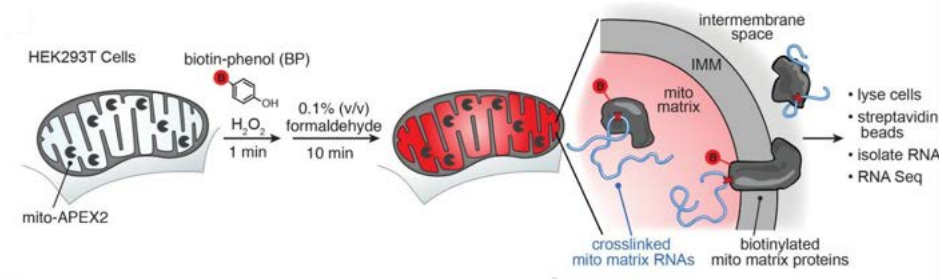
Yi et al. *Nature Method* 2020 (BASU)



Protein interactomes around RNA motifs guided by dCas13

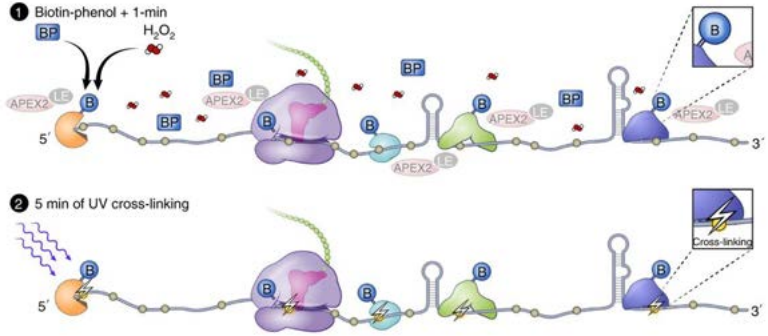
Han et al. *PNAS* 2020 (APEX2)

PL for spatial transcriptomics



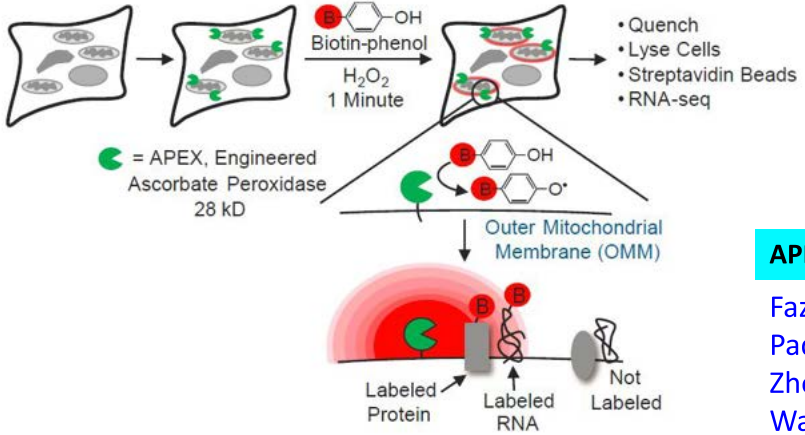
APEX-RIP (RNA IP after FA crosslinking)

Kaewsapsak et al. *eLife.* 2017



Proximity-CLIP (IP after UV crosslinking)

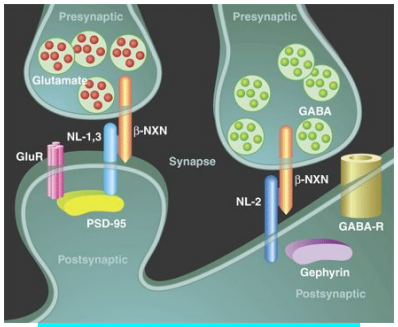
Benhalevy et al. *Nature Methods.* 2018



APEX-seq (direct biotinylation)

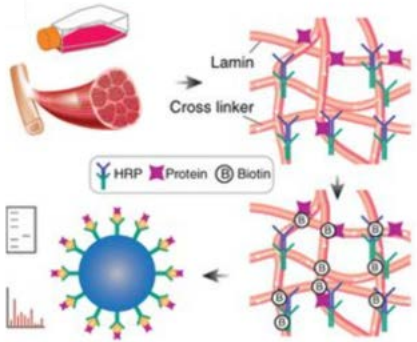
Fazal et al. *Cell.* 2019
Padron et al. *Mol. Cell* 2019
Zhou et al. *Angew. Chemie - Int. Ed.* 2019
Wang et al. *Nat. Chem. Biol.* 2019

Application in different cell type/tissue/organism



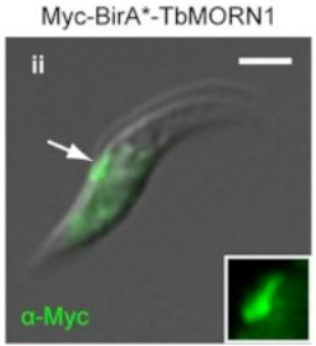
Primary neuron culture

Loh et al. *Cell* 2017 (HRP)



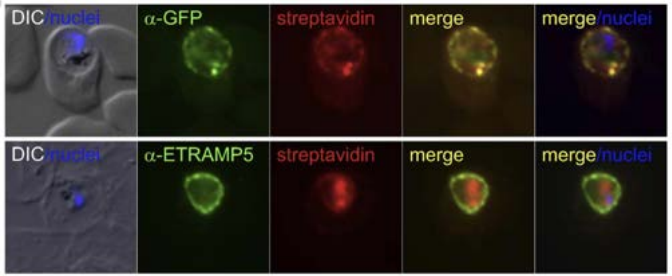
Fixed tissue samples

Bar et al. *Nat. Method.* 2017 (HRP-conjugated secondary antibodies)



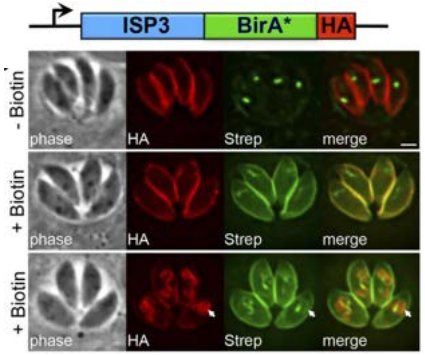
Trypanosoma brucei

Morriswood et al. *Eukaryot. Cell* 2013 (BioID)
McAllaster et al. *Mol. Biol. Cell* 2015 (BioID)



Plasmodium falciparum

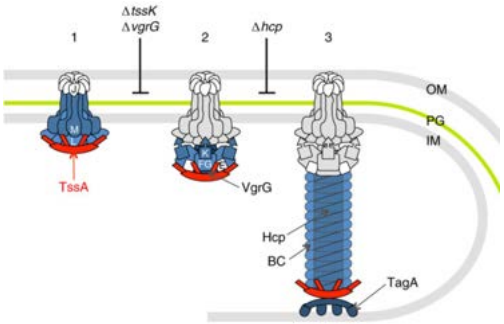
Khosh-Naucke et al. *Int. J. Med. Microbiol.* 2018 (BioID)



Toxoplasma

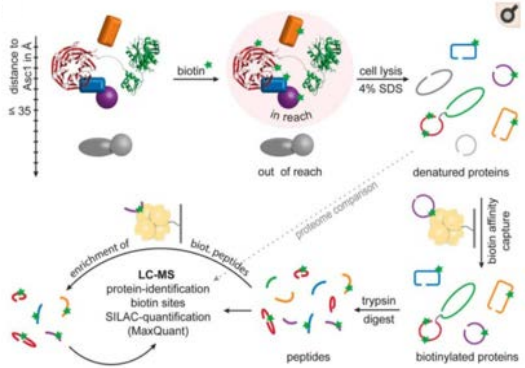
Chen et al. *mBiol* 2015 (BioID)
Tu et al. *mBio* 2015 (BioID)

Application in different cell type/tissue/organism



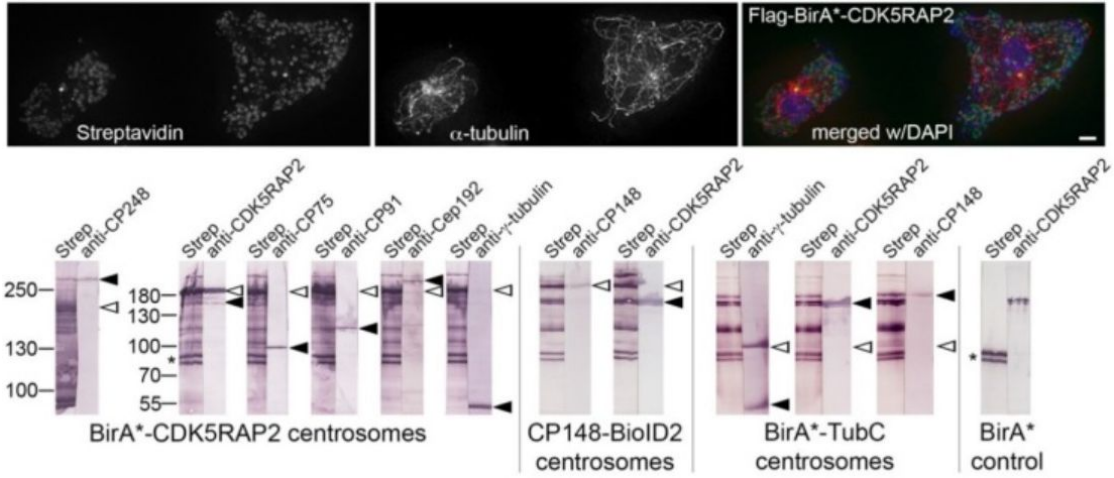
Living Bacteria

Santin et al. *Nature Microbiology.* 2018 (APEX2)
Branon et al. *Nat. Biotech.* 2018 (TurboID)



Living yeast

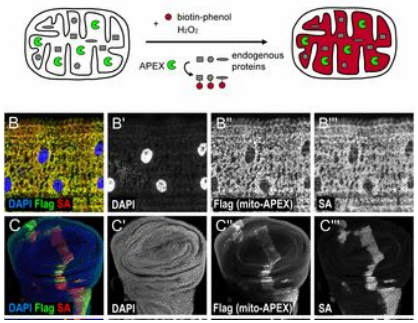
Opitz et al. *Mol Cell Proteomics.* 2017 (BioID)
Branon et al. *Nat. Biotech.* 2018 (TurboID)
Larochelle et al. *J. Cell Sci.* 2019 (TurboID)



Dictyostelium

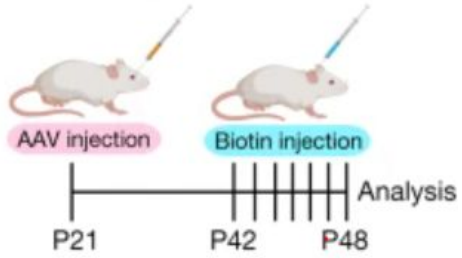
Pitzen et al. *Cells* 2018 (BioID)
Batsios et al. *Meth. Enzymol.* 2016 (BioID)

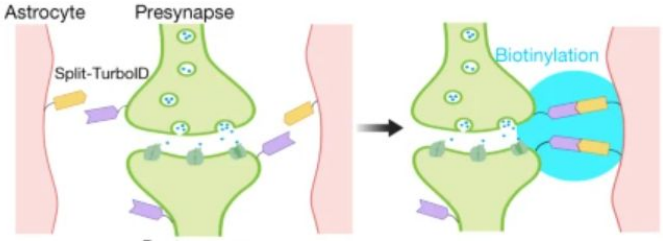
Application in different cell type/tissue/organism



Live drosophila and *ex vivo* tissue

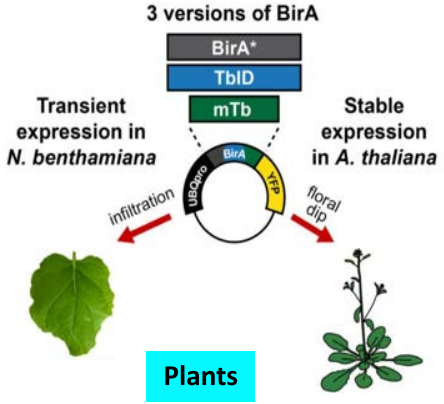
Chen et al. *PNAS* 2015 (APEX)
Branon et al. *Nat. Biotech.* 2018 (TurboID)





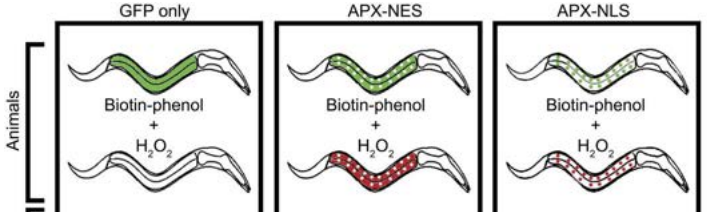
Living mouse brain

Takano et al. *Nature* 2020 (TurboID)
Uezu et al. *Science.* 2016 (BioID)



Plants

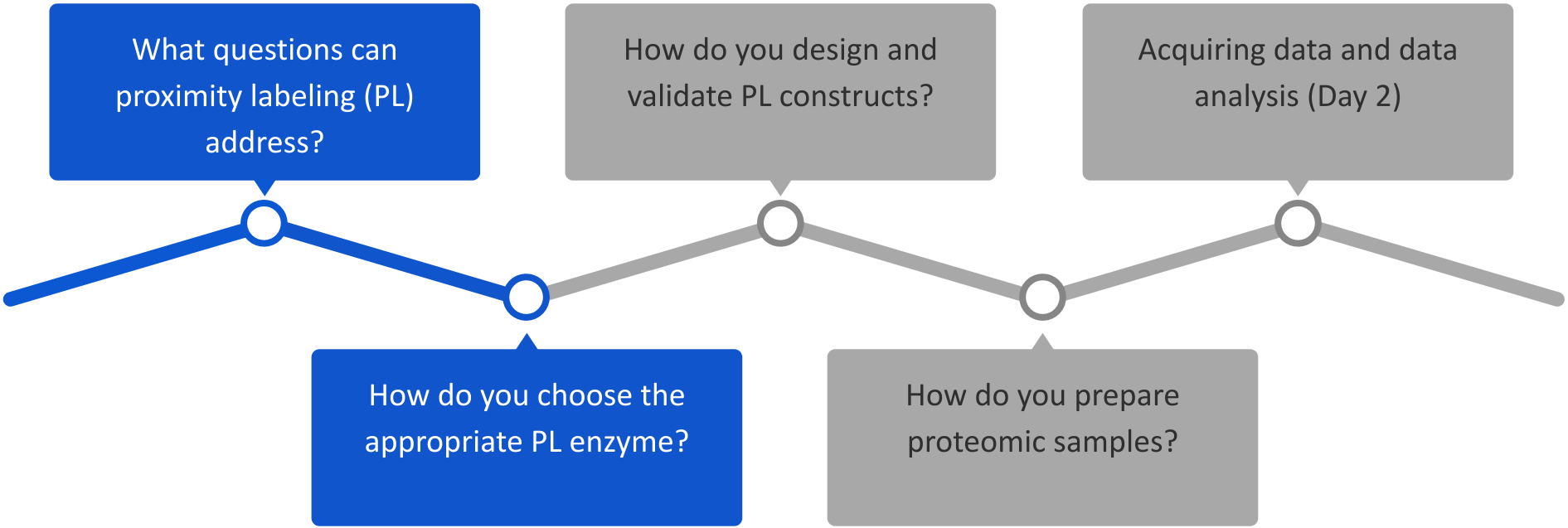
Mair et al. *eLife* 2019 (TurboID)
Zhang et al. *Nat. Comm.* 2019 (TurboID)
Khan et al. *Sci. Rep.* 2018 (BioID)
Conlan et al. *Front. Plant Sci.* 2018 (BioID)



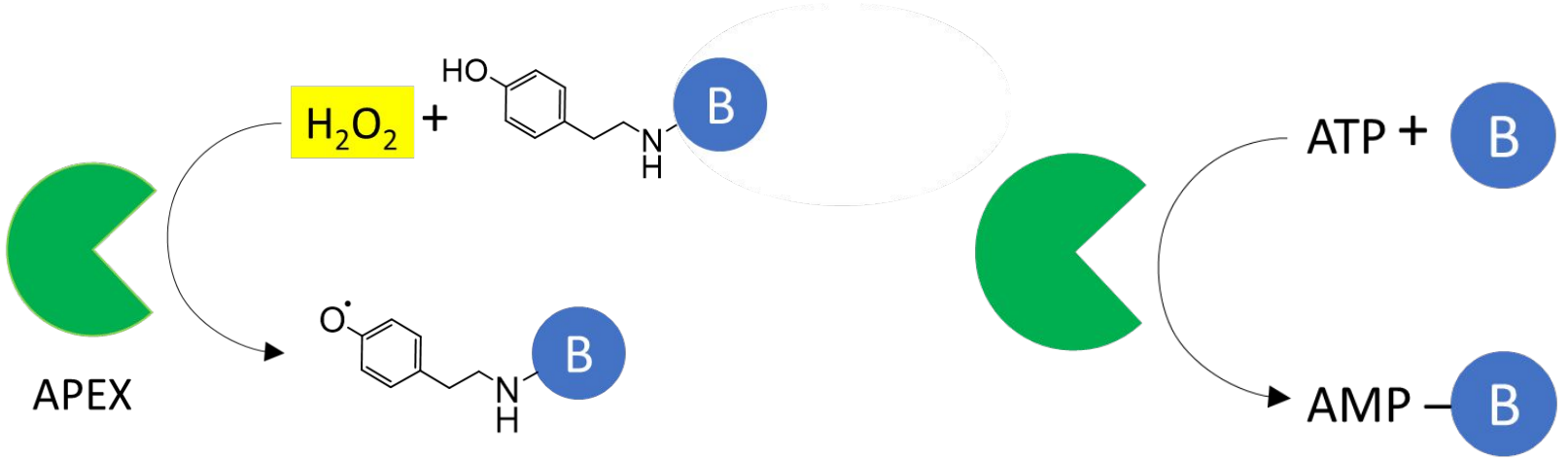
Living C. elegans

Reinke et al. *Sci. Adv.* 2015 (APEX2)
Branon et al. *Nat. Biotech.* 2018 (TurboID)

Outline


What questions can proximity labeling (PL) address?
How do you choose the appropriate PL enzyme?
How do you design and validate PL constructs?
How do you prepare proteomic samples?
Acquiring data and data analysis (Day 2)

Proximity labeling enzymes for proteomic mapping

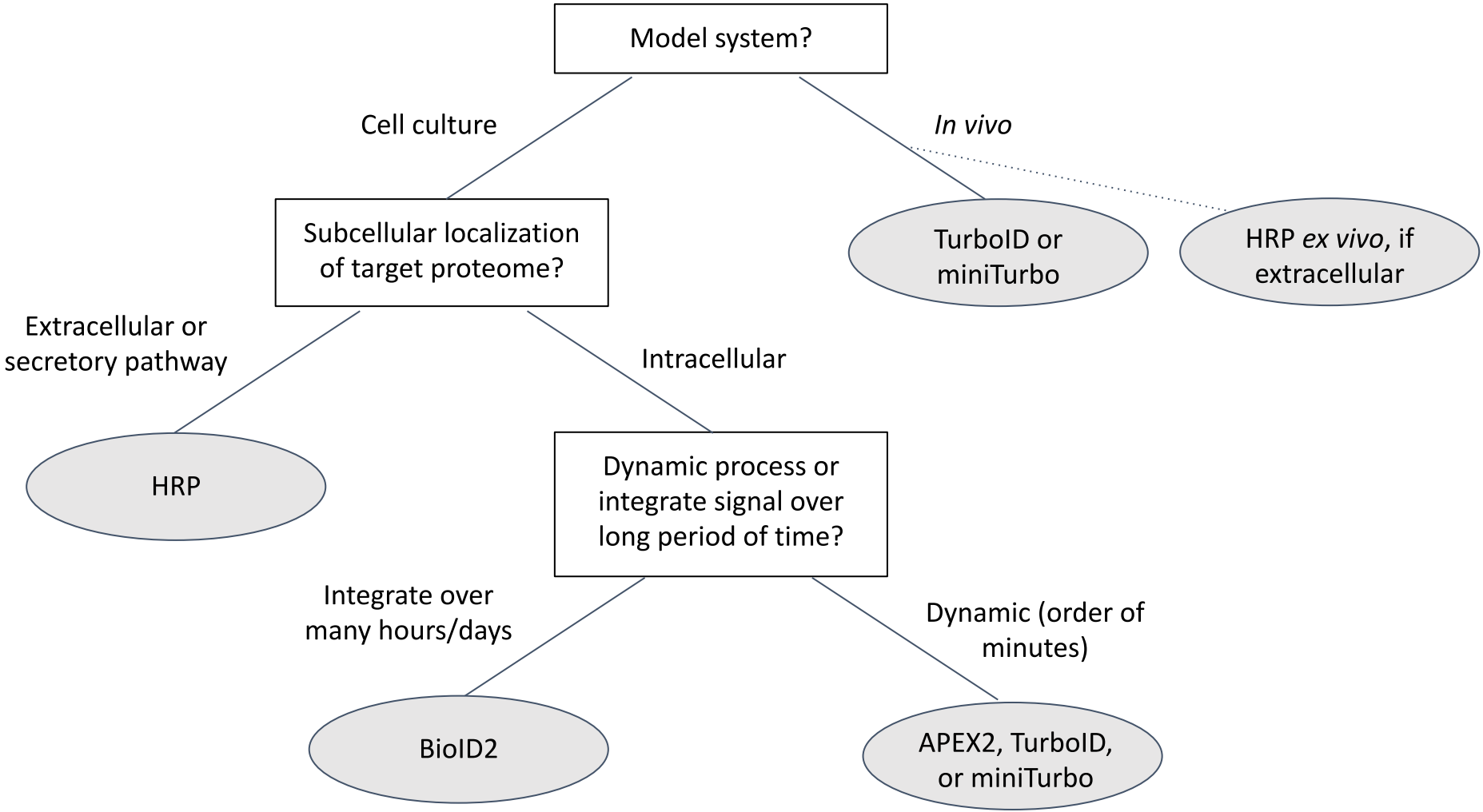


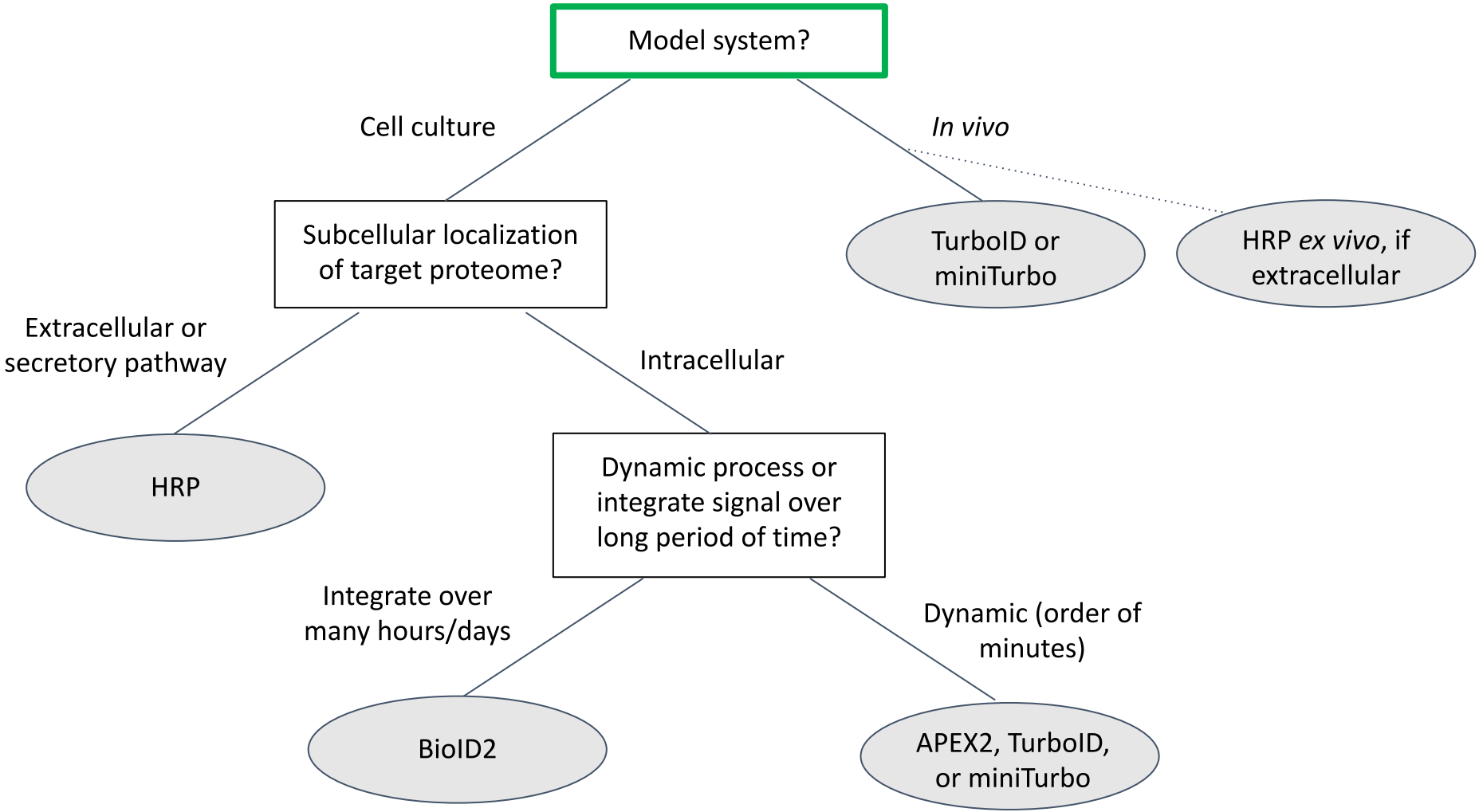
Peroxidases

(APEX2, HRP)

Biotin ligases

(BioID, BioID2, BASU, TurboID, miniTurbo)


Model system?
Cell culture
In vivo
Subcellular localization of target proteome?
TurboID or miniTurbo
HRP ex vivo, if extracellular
Extracellular or secretory pathway
Intracellular
HRP
Dynamic process or integrate signal over long period of time?
Integrate over many hours/days
Dynamic (order of minutes)
BioID2
APEX2, TurboID, or miniTurbo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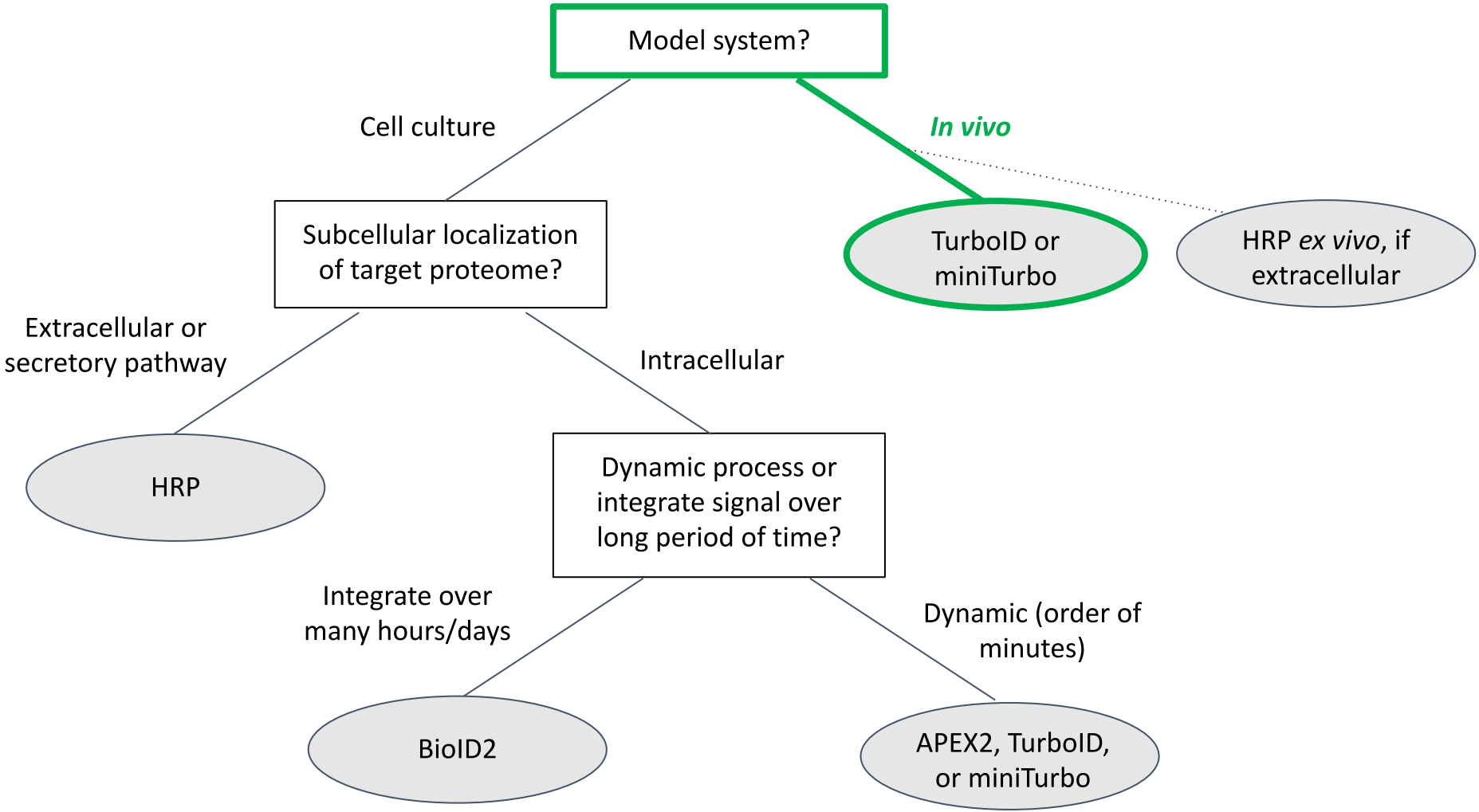
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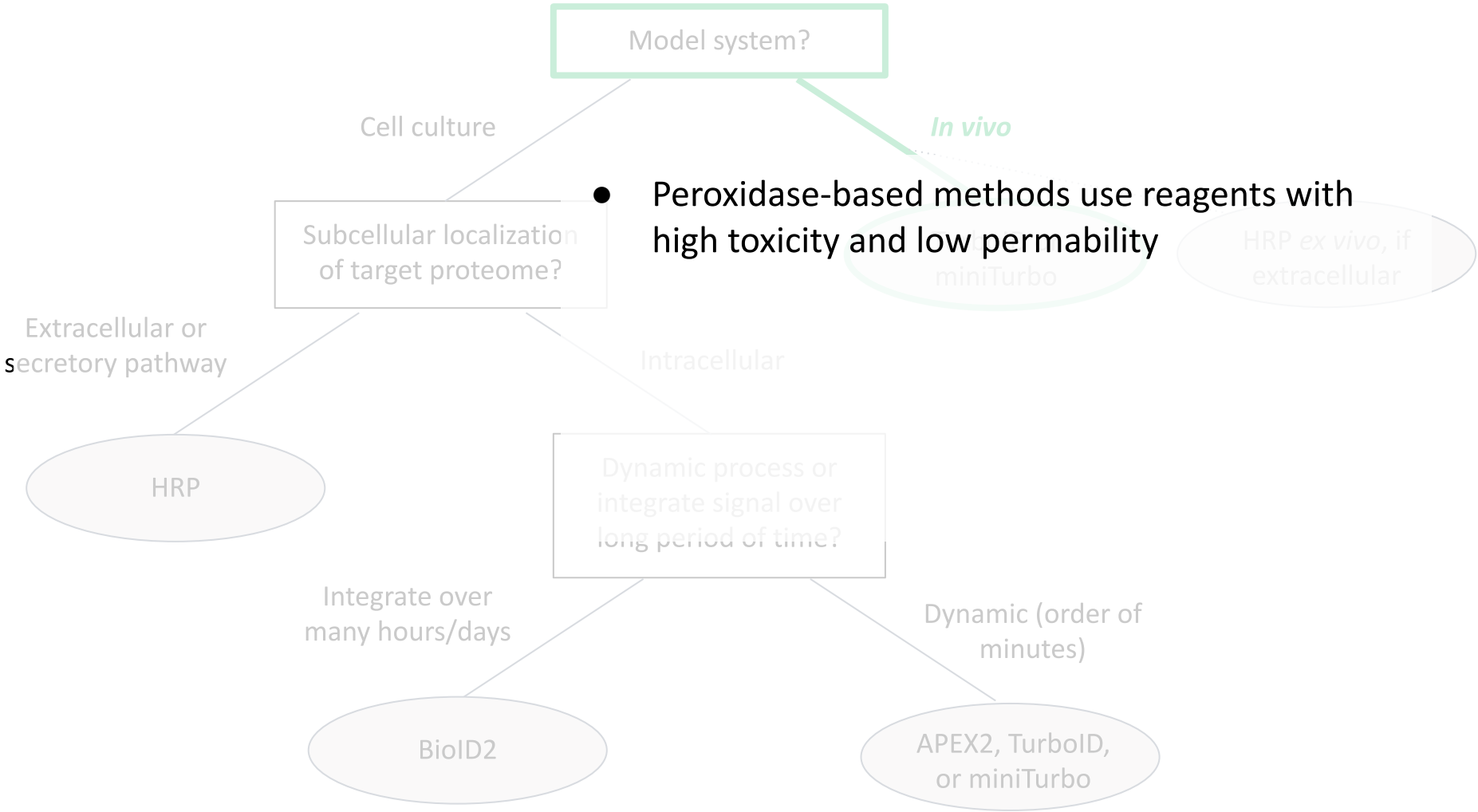
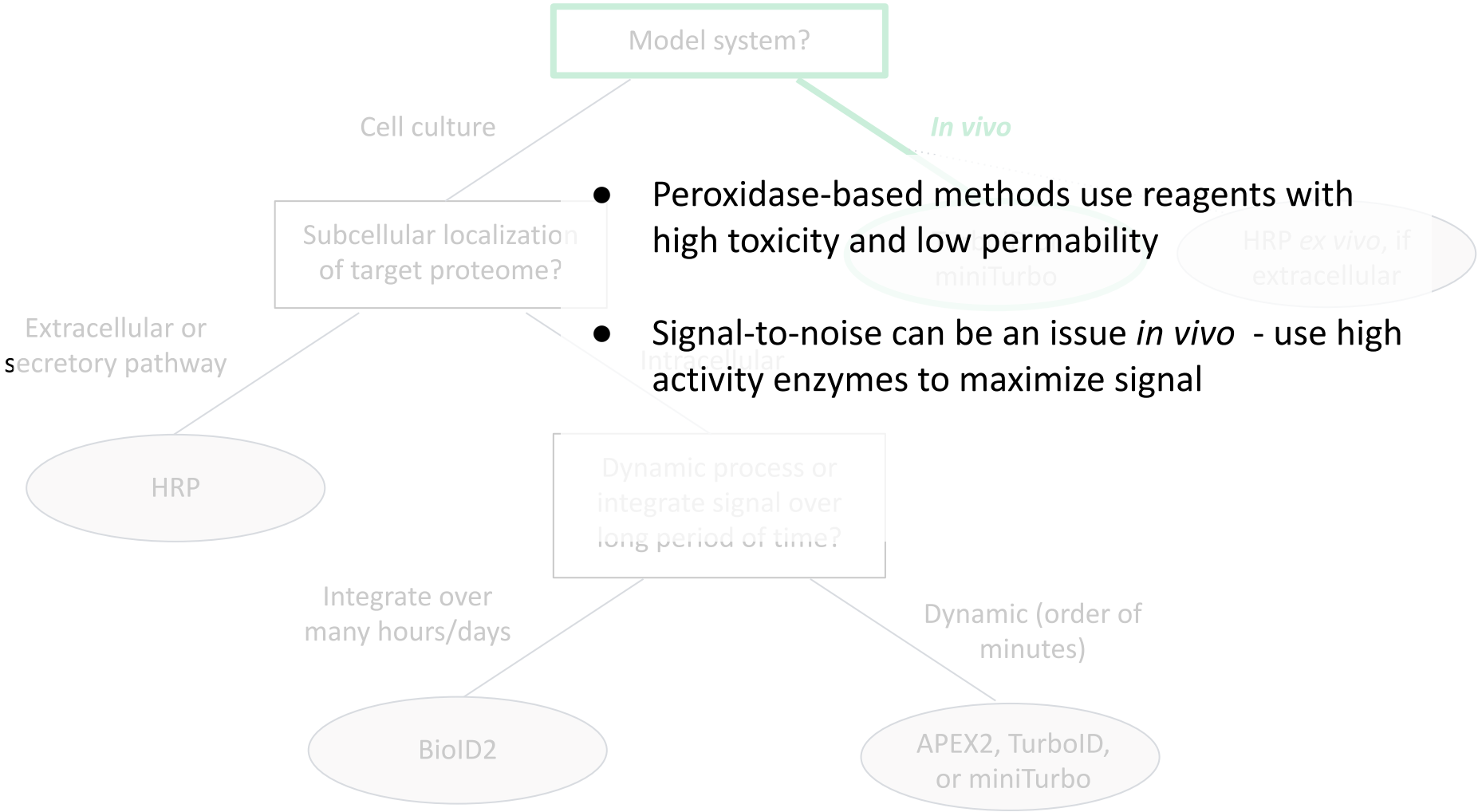
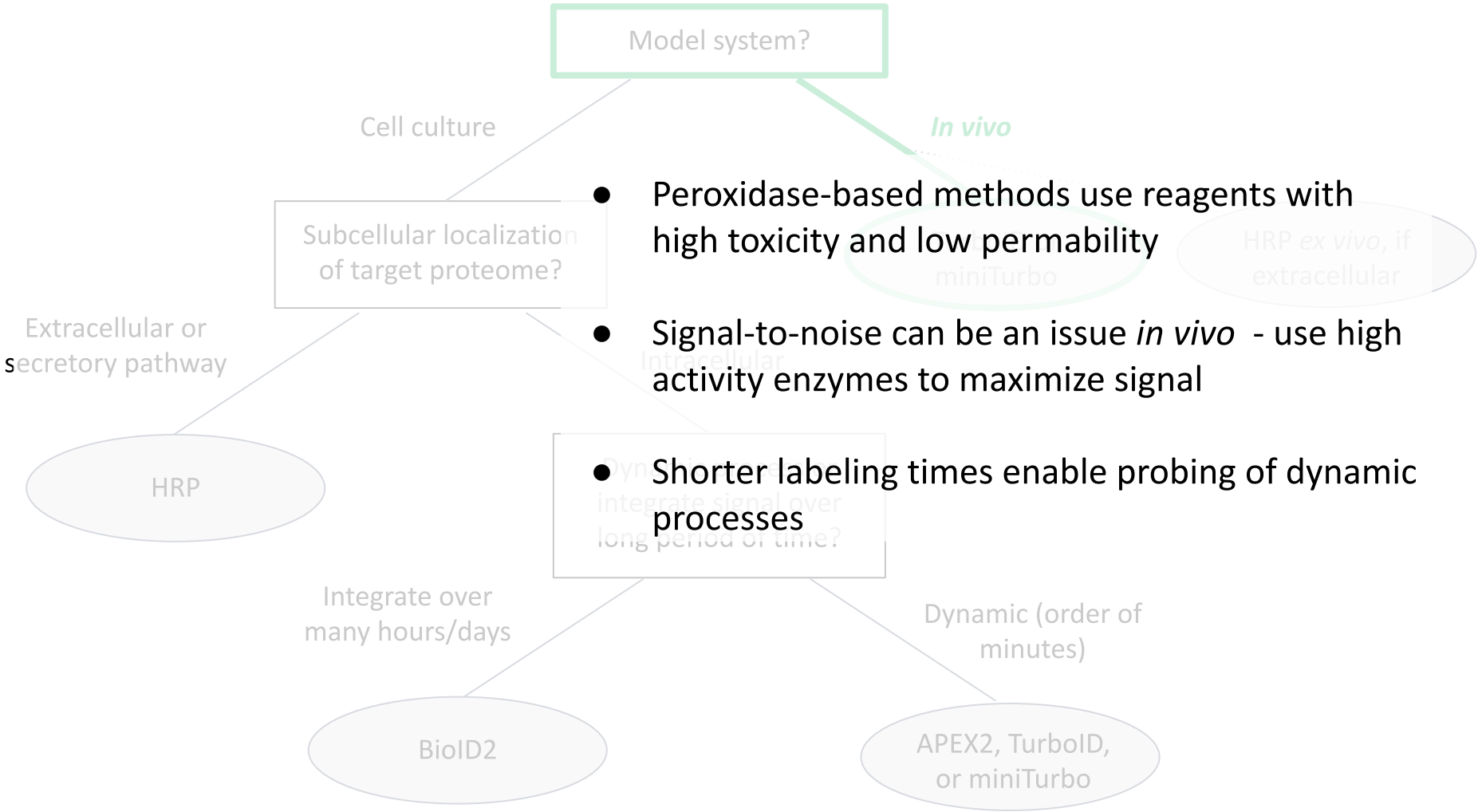


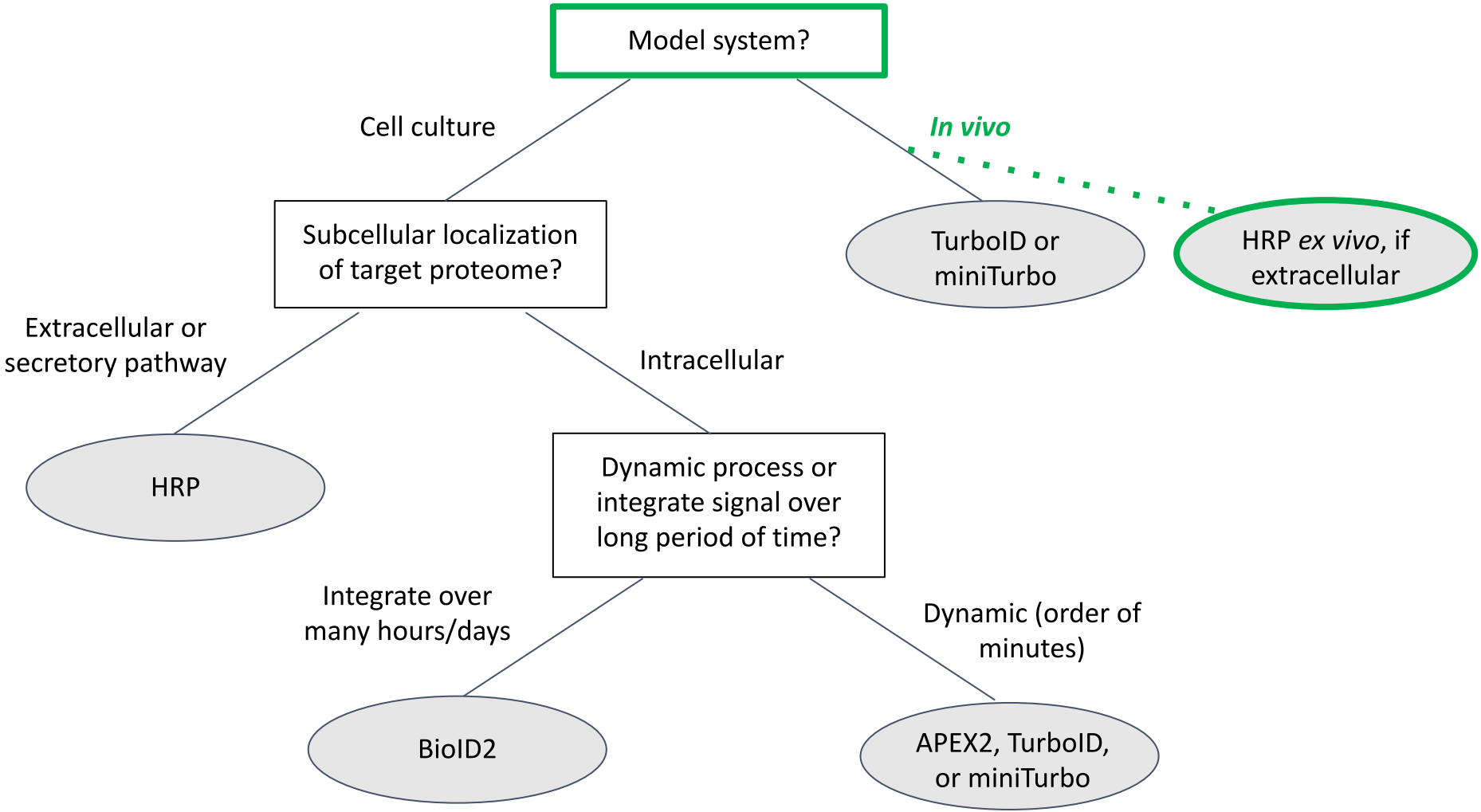
- Peroxidase-based methods use reagents with high toxicity and low permability

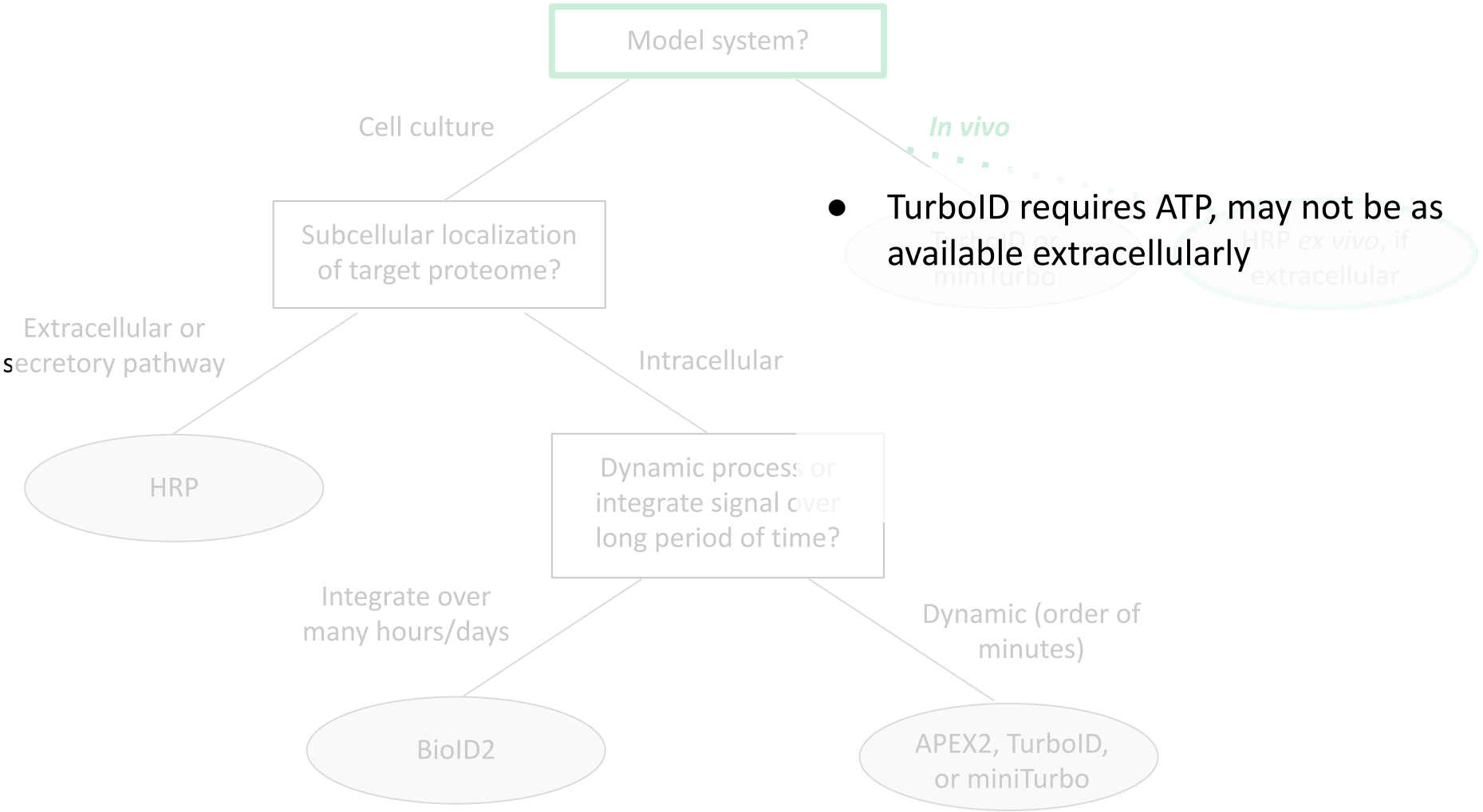


- Peroxidase-based methods use reagents with high toxicity and low permability
- Signal-to-noise can be an issue *in vivo* - use high activity enzymes to maximize signal

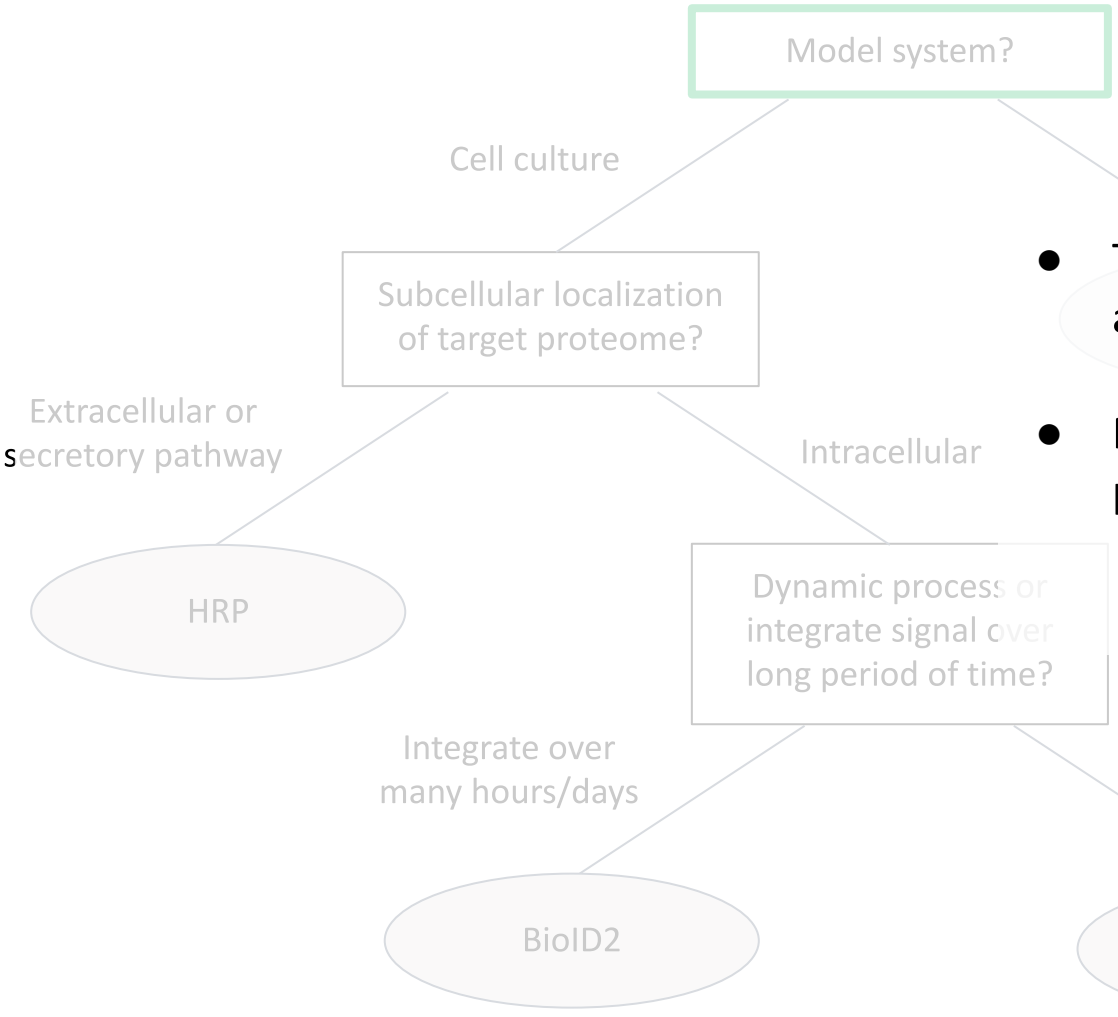


- Peroxidase-based methods use reagents with high toxicity and low permability
- Signal-to-noise can be an issue *in vivo* - use high activity enzymes to maximize signal
- Shorter labeling times enable probing of dynamic processes

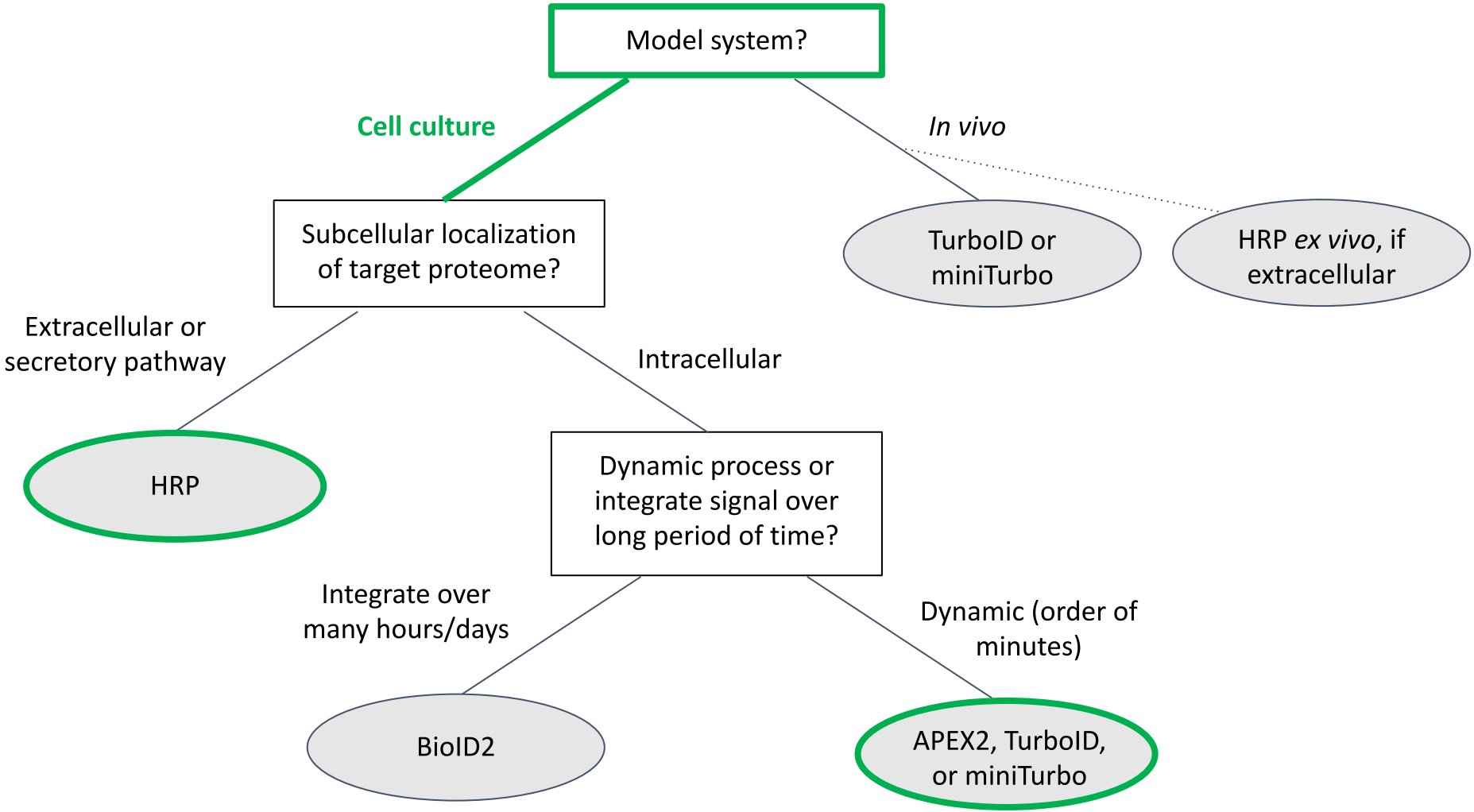

Model system?
Cell culture
In vivo
Subcellular localization of target proteome?
TurboID or miniTurbo
HRP ex vivo, if extracellular
Extracellular or secretory pathway
Intracellular
HRP
Dynamic process or integrate signal over long period of time?
Integrate over many hours/days
Dynamic (order of minutes)
BioID2
APEX2, TurboID, or miniTurbo



- TurboID requires ATP, may not be as available extracellularly



- TurboID requires ATP, may not be as available extracellularly
- Membrane impermeable BxxP probe:
  - Loh et al. Cell 2017
  - Li et al. Cell 2020


Model system?
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In vivo
Subcellular localization of target proteome?
TurboID or miniTurbo
HRP ex vivo, if extracellular
Extracellular or secretory pathway
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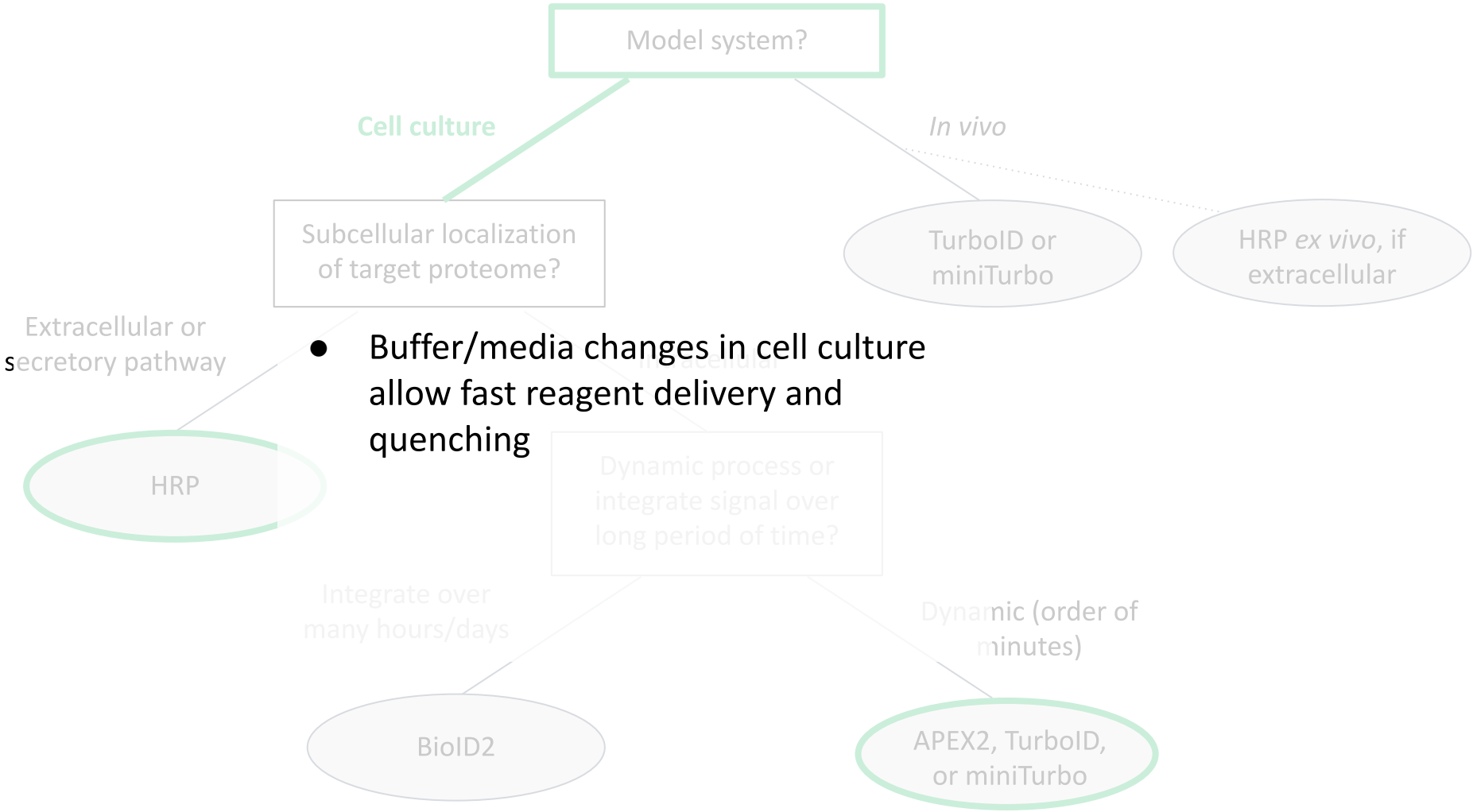
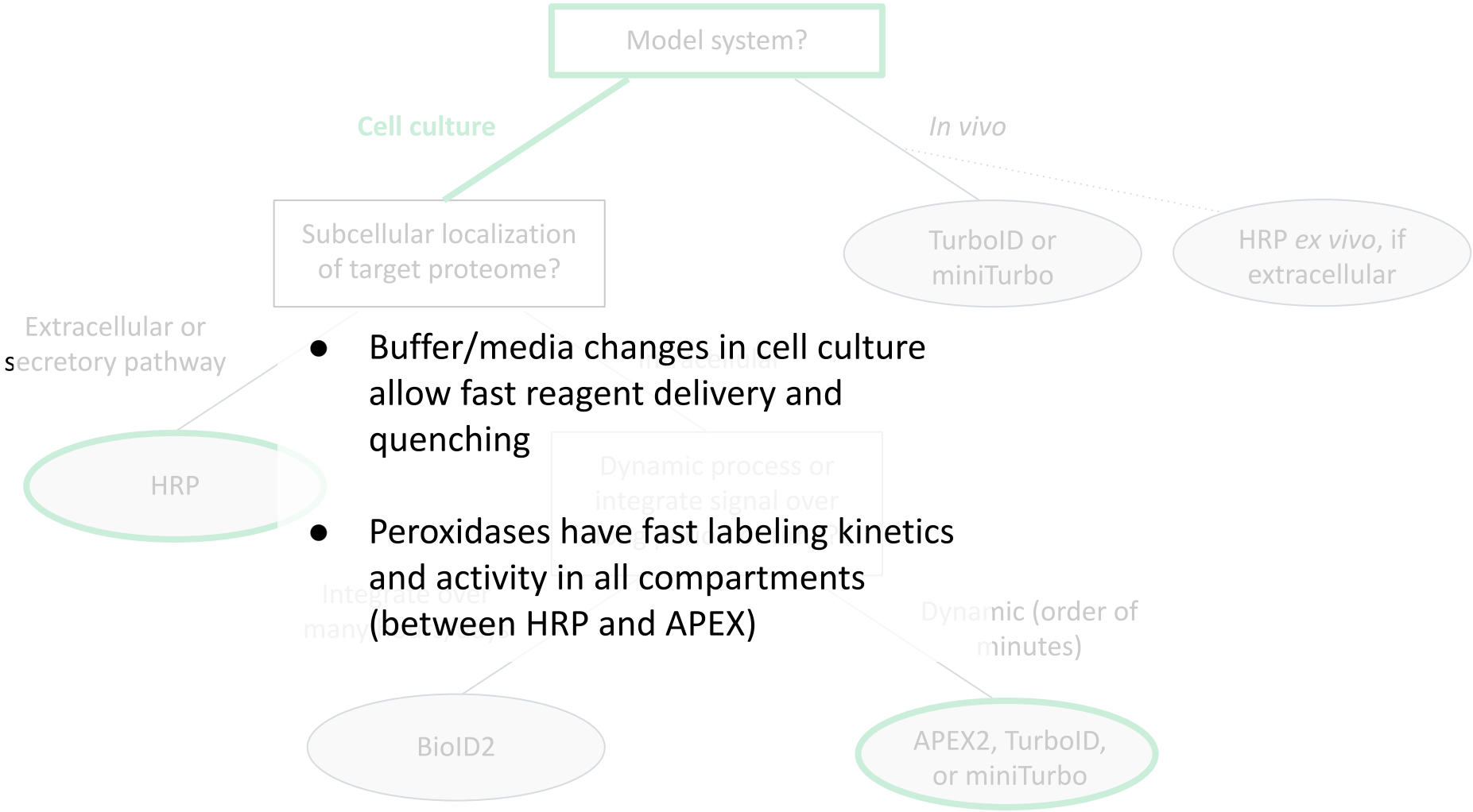
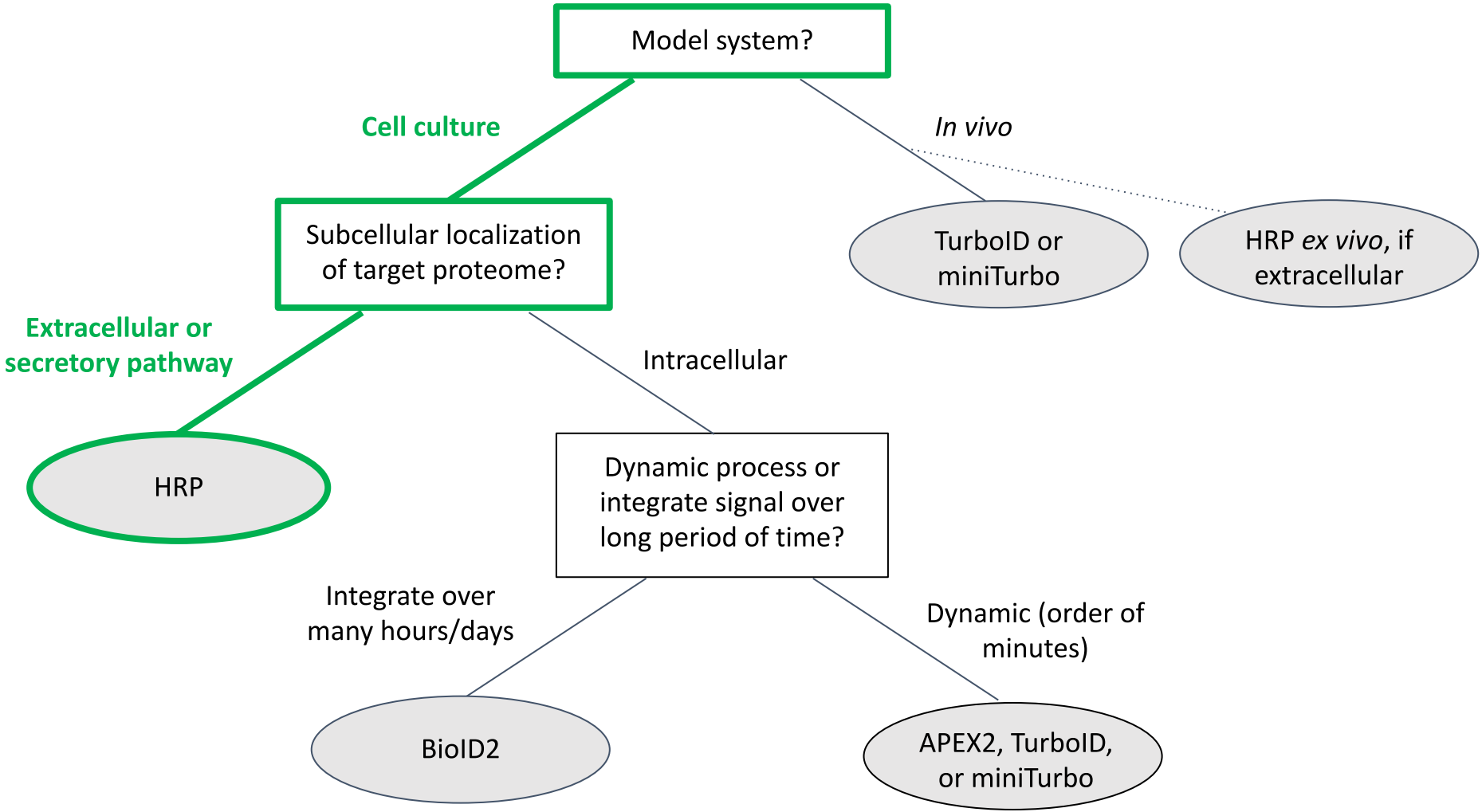
Dynamic process or integrate signal over long period of time?
Integrate over many hours/days
Dynamic (order of minutes)
BioID2
APEX2, TurboID, or miniTurbo



- Buffer/media changes in cell culture allow fast reagent delivery and quenching



- Buffer/media changes in cell culture allow fast reagent delivery and quenching
- Peroxidases have fast labeling kinetics and activity in all compartments (between HRP and APEX)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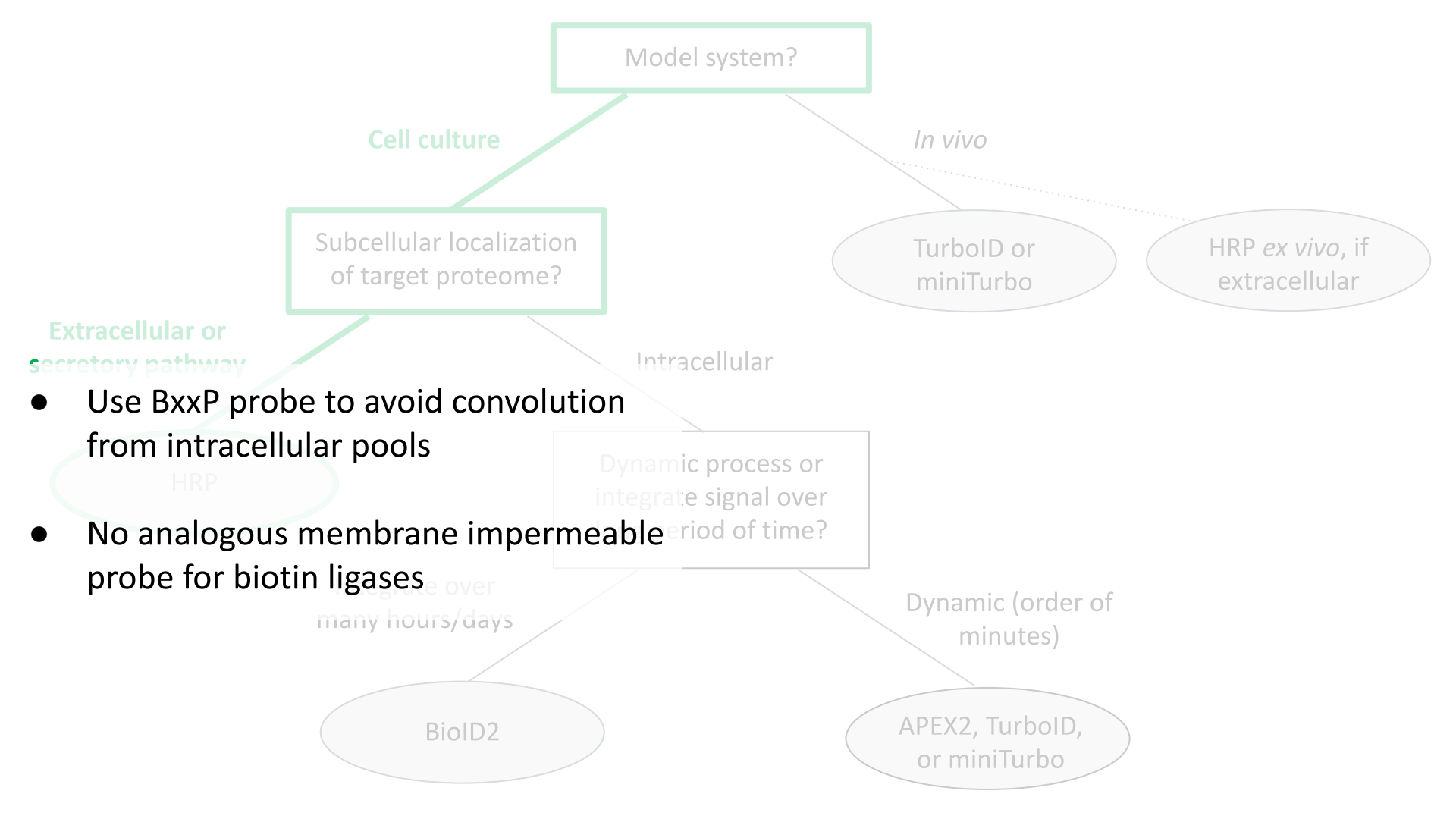
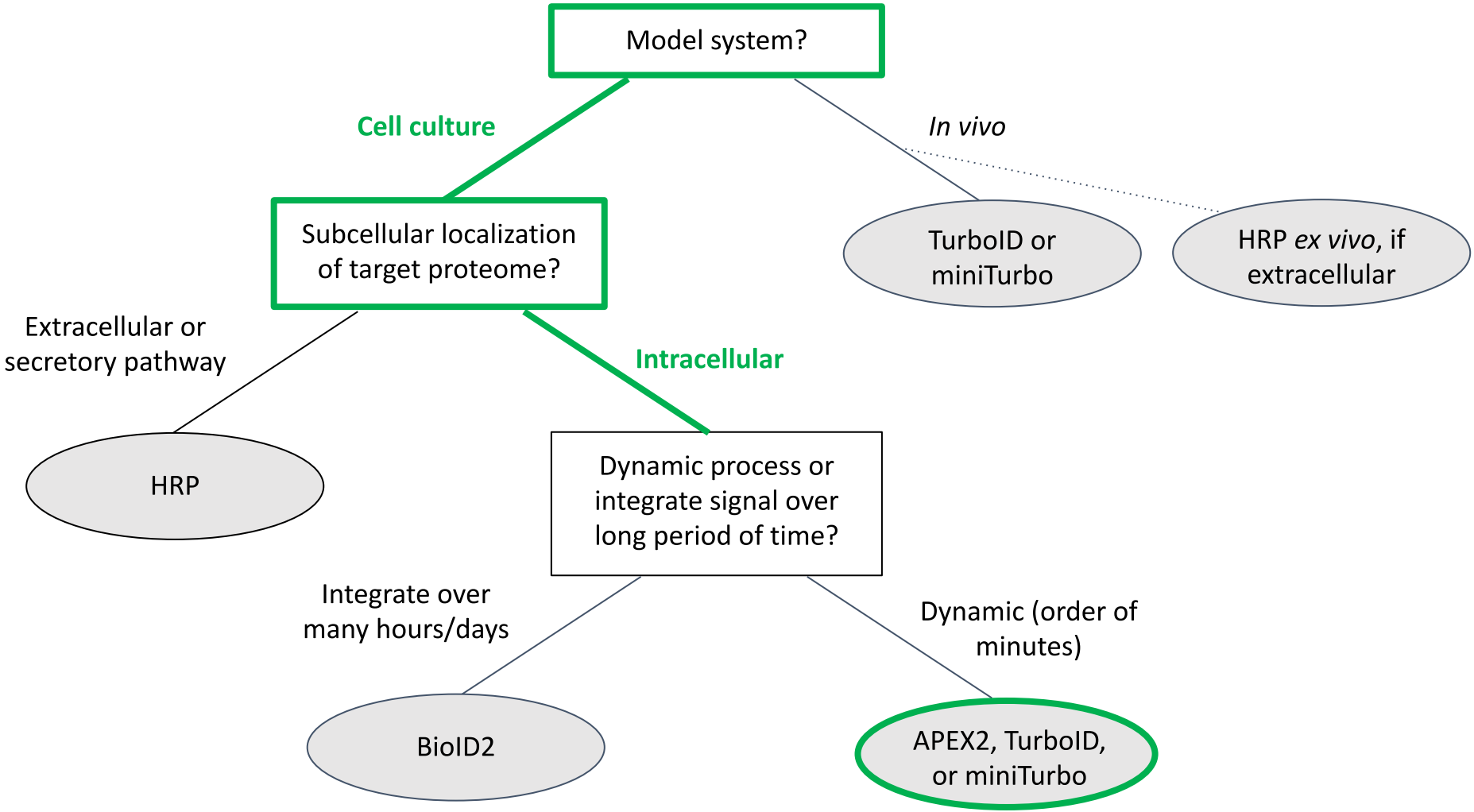


- Use BxxP probe to avoid convolution from intracellular pools
- No analogous membrane impermeable probe for biotin ligases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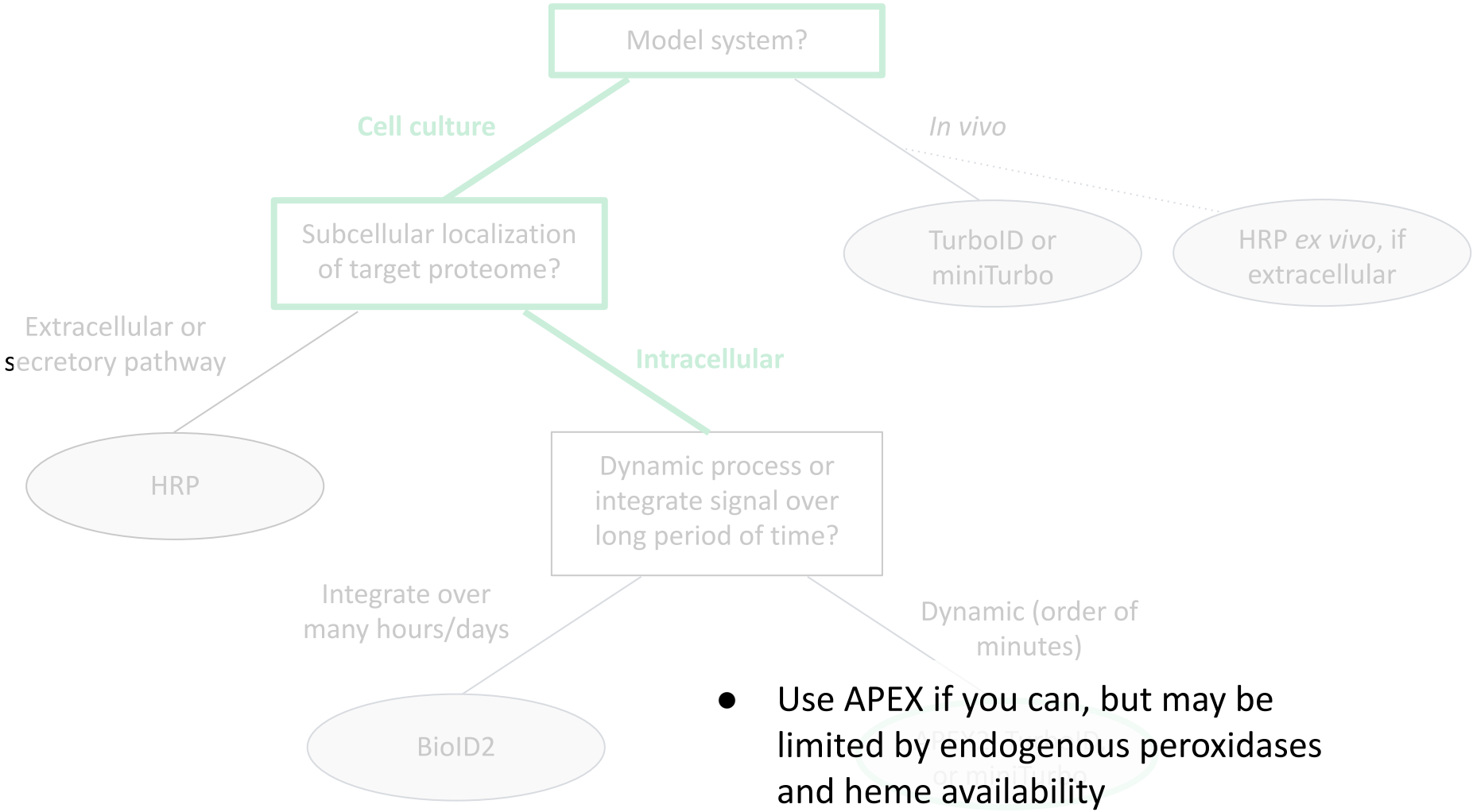
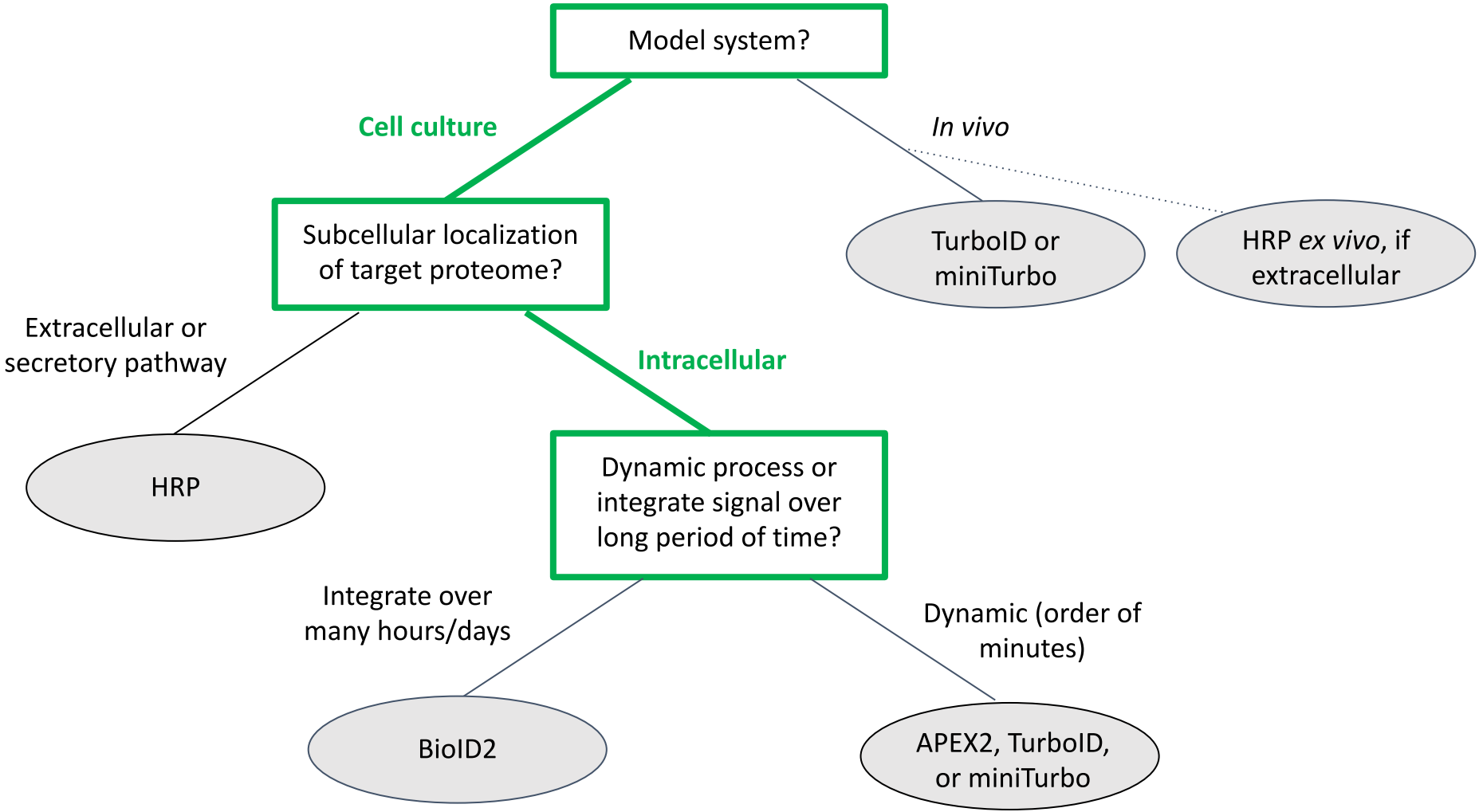


- Use APEX if you can, but may be limited by endogenous peroxidases and heme availability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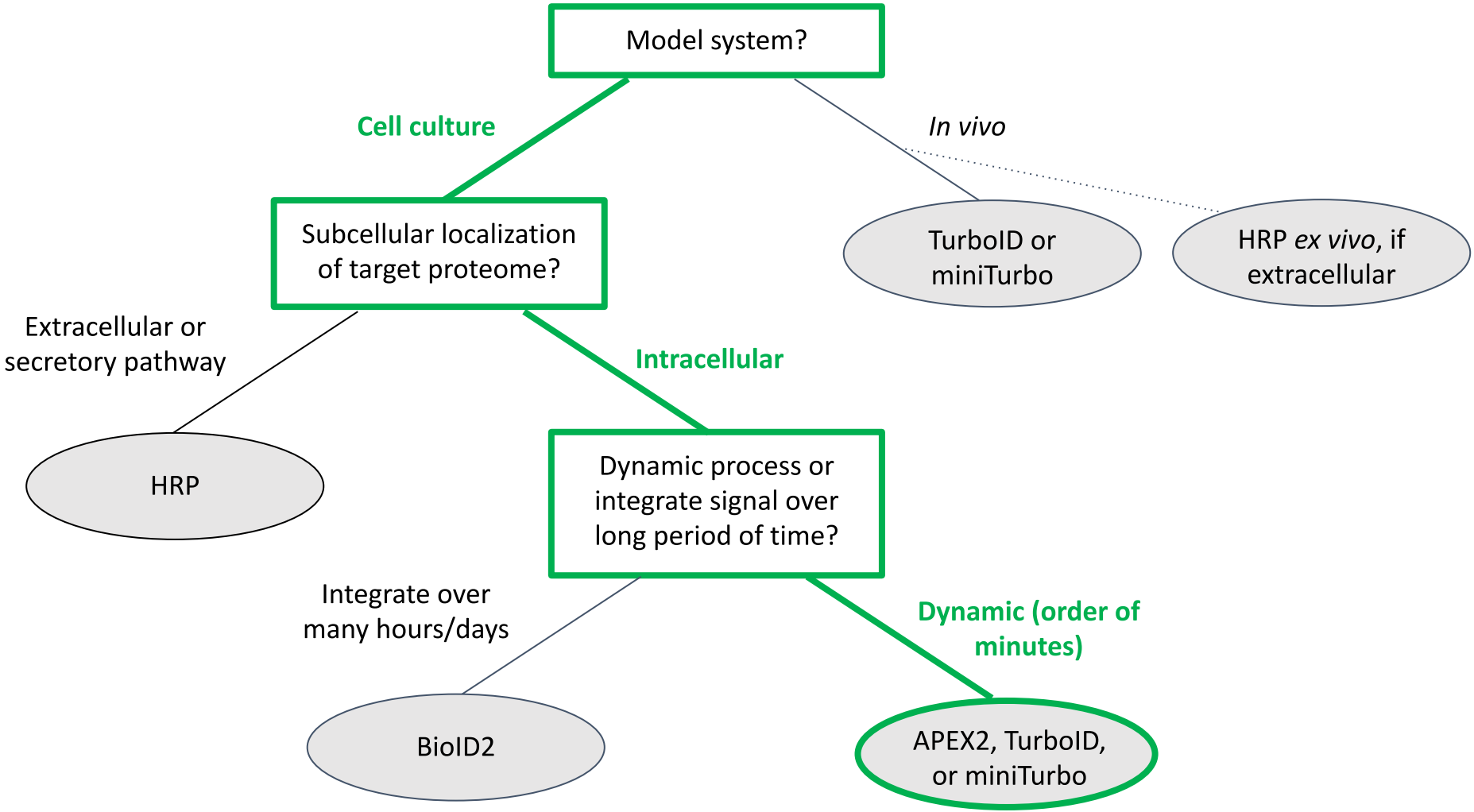
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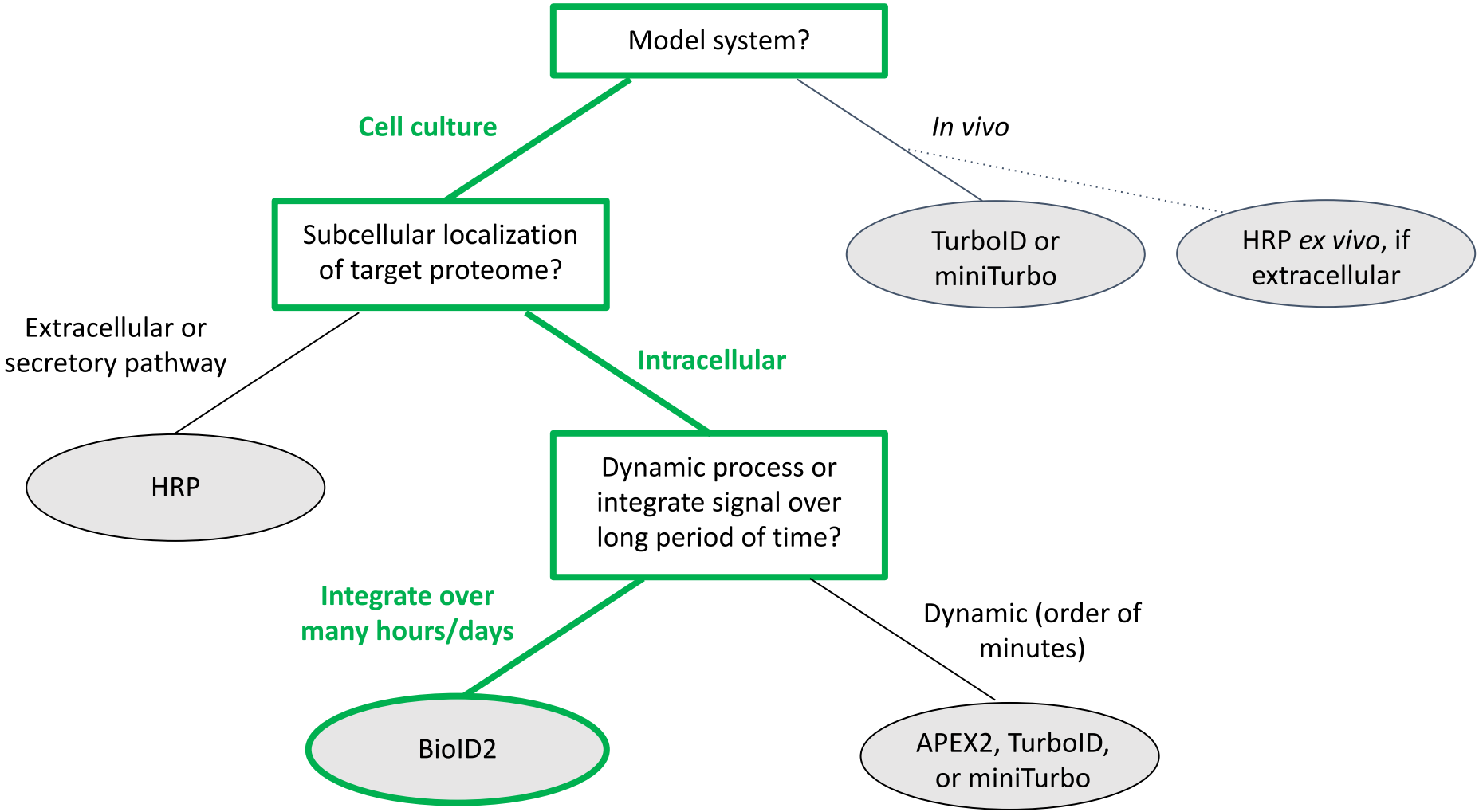
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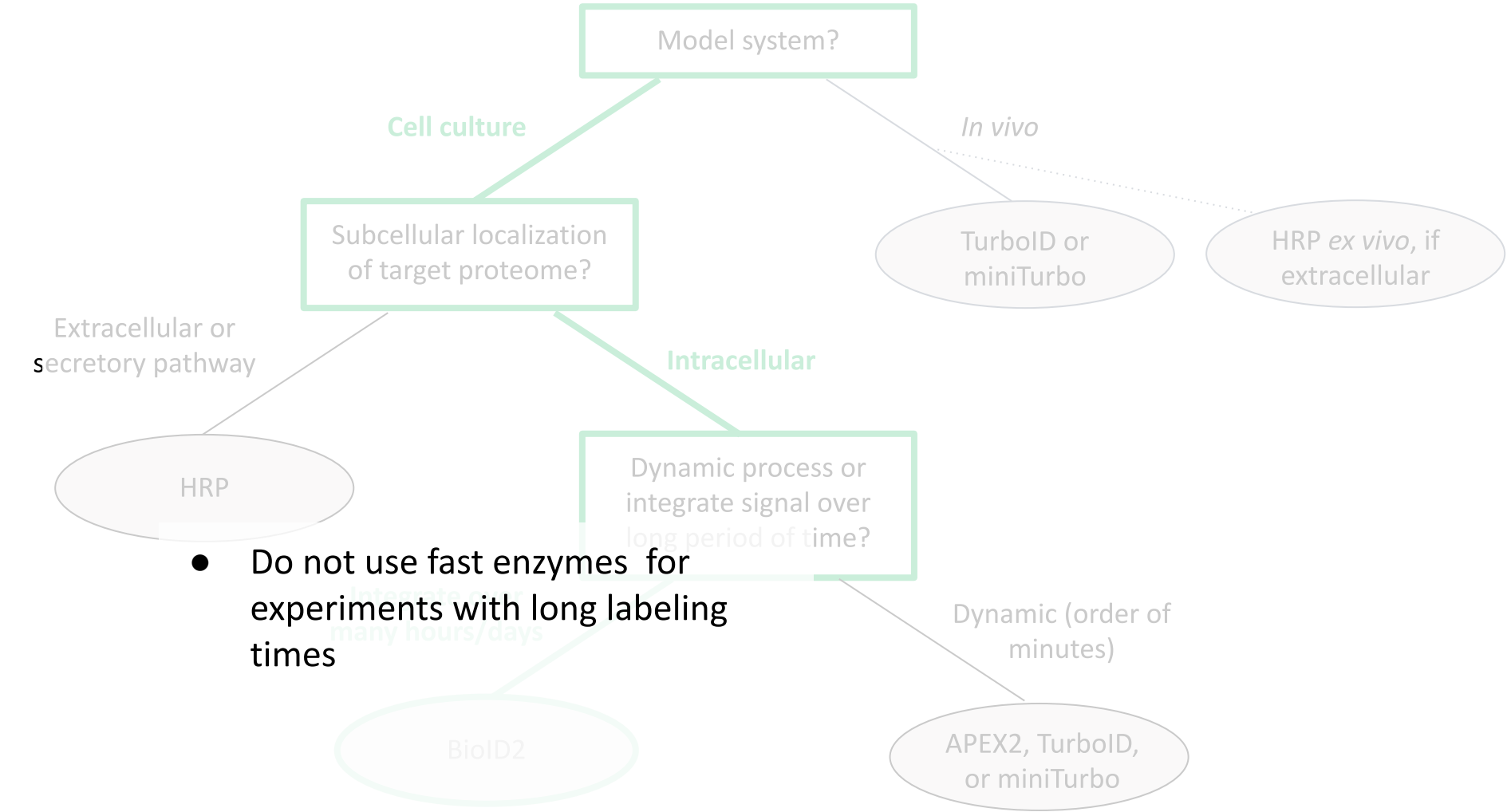
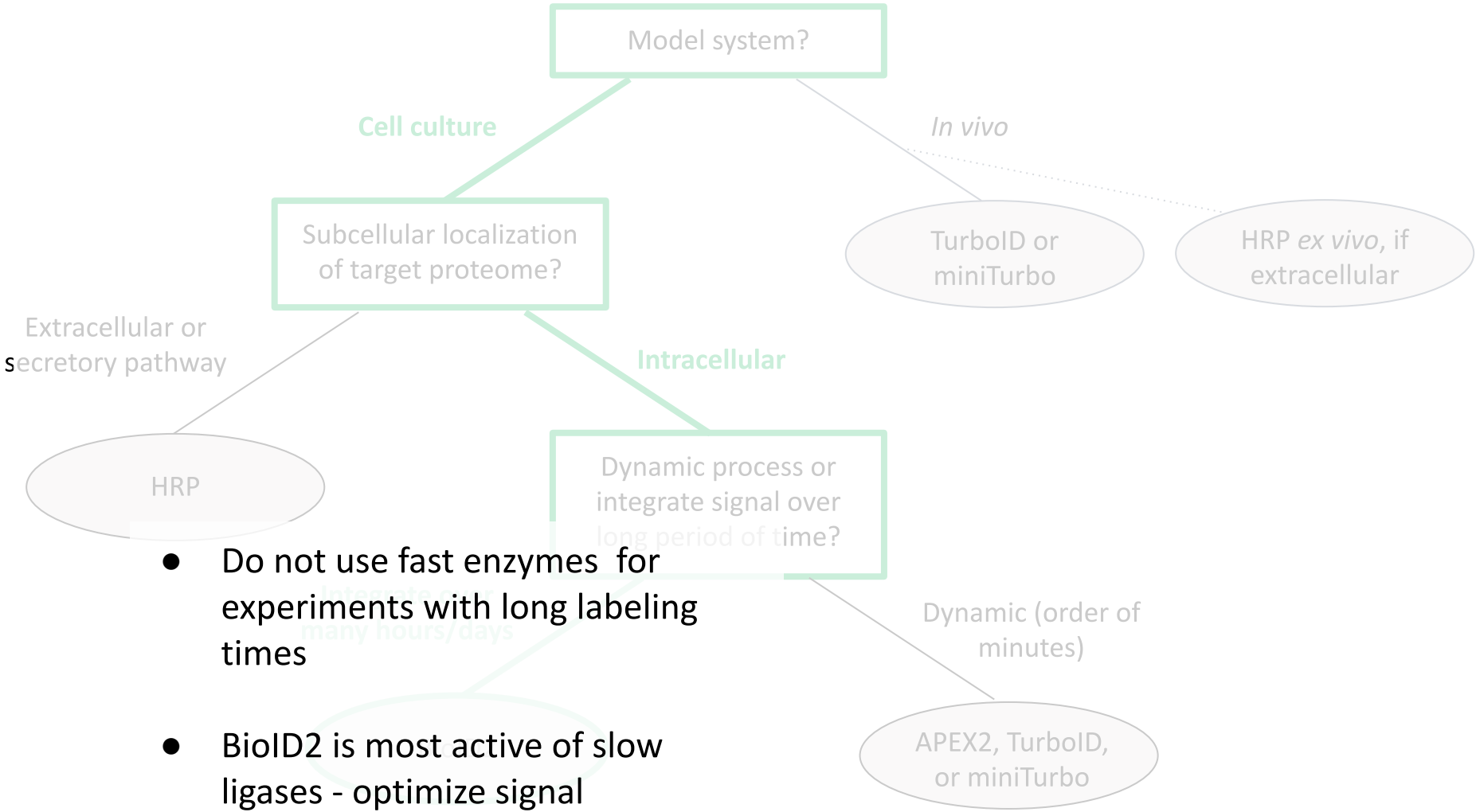


- Do not use fast enzymes for experiments with long labeling times



- Do not use fast enzymes for experiments with long labeling times
- BioID2 is most active of slow ligases - optimize signal

Additional considerations

- Only exposed, reactive residues will get tagged
  - Peroxidases: mainly tyrosines
  - Biotin ligases: N-termini and lysines

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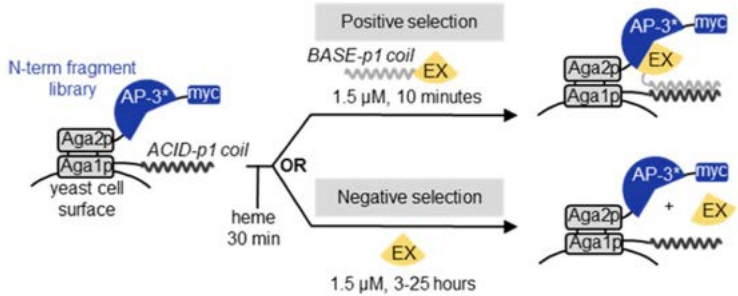
- Only exposed, reactive residues will get tagged
  - Peroxidases: mainly tyrosines
  - Biotin ligases: N-termini and lysines

- Consider cellular environment and its effect on labeling chemistry
  - Biotin ligases will not work as well in acidic environments,
  - HRP only active in oxidizing environments, e.g. secretory pathway
    - Biotin ligases have reduced activity in ER
    - TurboID DOES work in ER

Comparison of proximity labeling enzymes

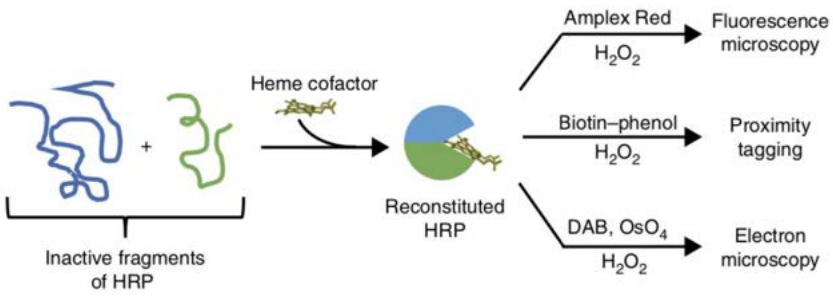
Enzyme	Labeling time	Substrates	Advantages	Limitations
APEX	≤1 minute	Biotin phenol, hydrogen peroxide	High temporal resolution, high activity in most compartments	Limited *in vivo* applications due to requiring hydrogen peroxide
HRP	≤1 minute	Biotin phenol, hydrogen peroxide	Highest activity, but only works extracellularly or in secretory pathway	Limited *in vivo* applications due to requiring hydrogen peroxide; only works in oxidative environments
TurboID	≤10 minutes	Biotin, ATP	Highest activity promiscuous biotin ligase	May exhibit background labeling due to high affinity for biotin
miniTurbo	≤10 minutes	Biotin, ATP	High temporal resolution	Lower activity and stability than TurboID
BioID2	18 hours	Biotin, ATP	Higher activity than BioID; stable at higher temperatures	Low activity; poor temporal resolution
BioID	18 hours	Biotin, ATP	Non-toxic labeling conditions	Low activity; poor temporal resolution
BASU	18 hours	Biotin, ATP	Higher activity than BioID	Low activity; poor temporal resolution

Split proximity labeling enzymes for increased spatial specificity



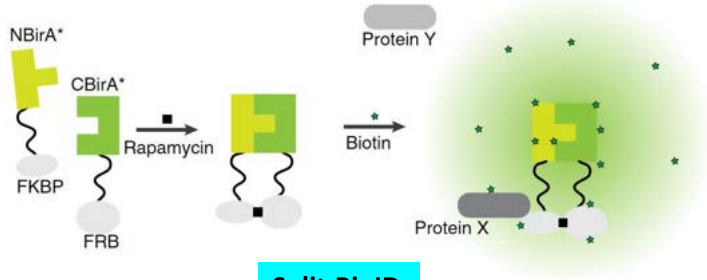
Split-APEX

Han et al. *ACS Chem. Biol.* 2019



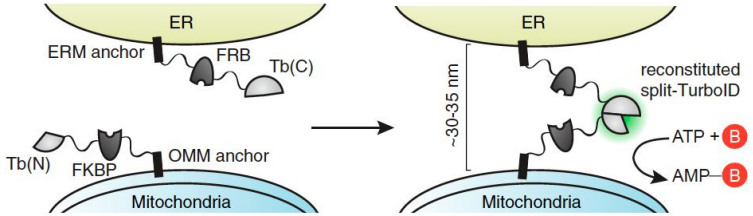
Split-HRP

Martell et al. *Nature Biotech.* 2016



Split-BioID

De Munter et al. *FEBS Lett*. 2017
Schopp et al. *Nature Comm.* 2017
Kwak et al. *PNAS*. 2020

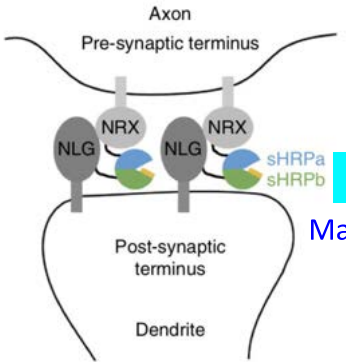


Split-TurboID

Cho et al. *PNAS.* 2020

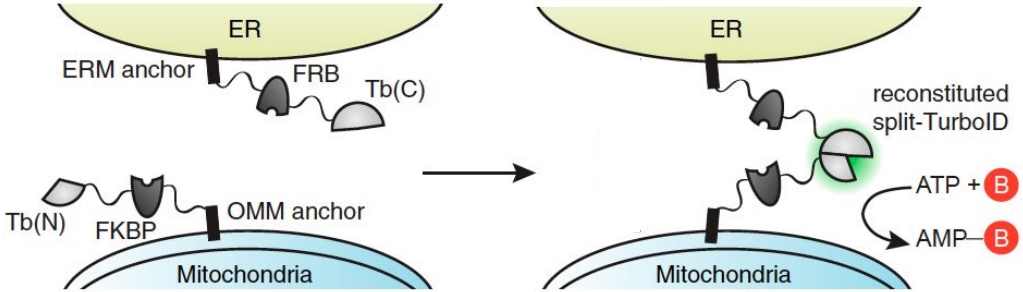
Split proximity labeling enzymes for increased spatial specificity

Split PL enzymes can offer improved specificity for subcellular compartments previously difficult to access



Visualizing neuronal synapses

Martell et al. *Nature Biotech.* 2016



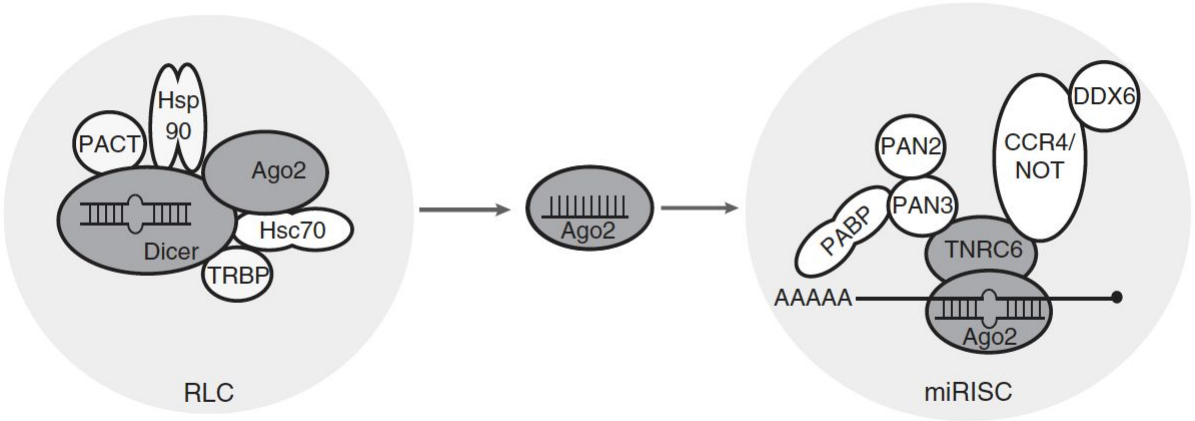
ER-mitochondria contact sites

Cho et al. *PNAS.* 2020

Kwak et al. *PNAS*. 2020

Split proximity labeling enzymes for increased spatial specificity

Split PL enzymes can offer improved specificity for interactome mapping of proteins that participate in multiple subcomplexes

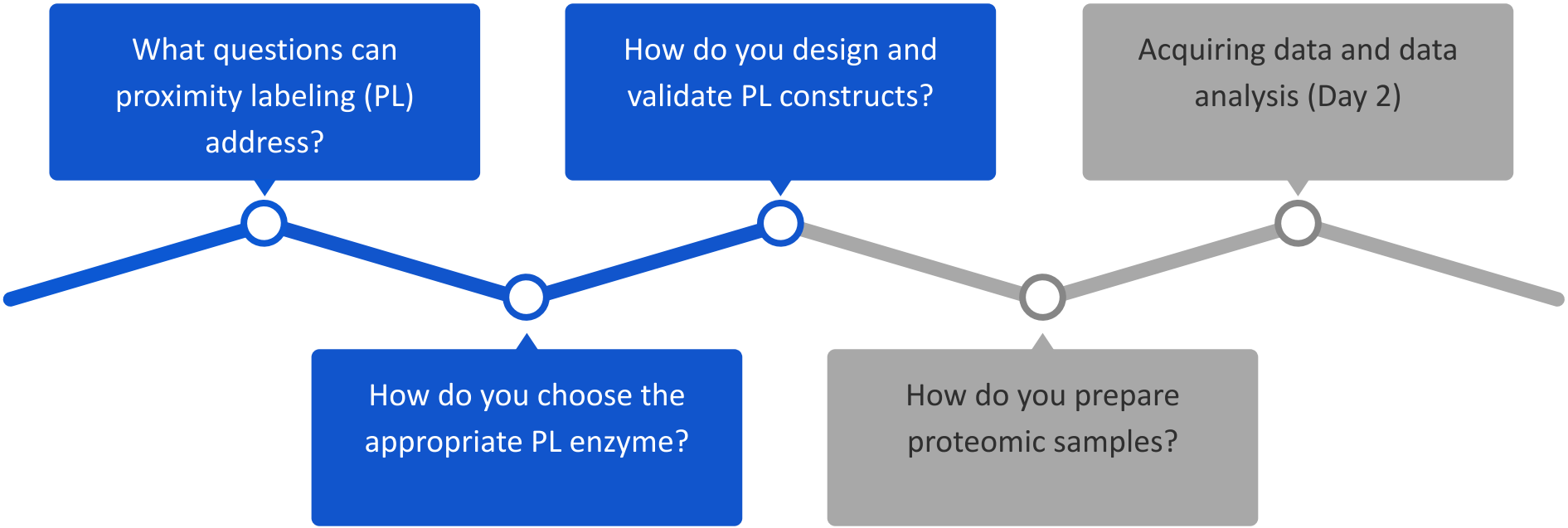


miRNA-mediated gene silencing

Schopp et al. *Nature Comm.* 2017

Dark grey proteins tagged with split-BioID fragments
(Dicer, Ago2, TNRC6)

Outline


What questions can proximity labeling (PL) address?
How do you choose the appropriate PL enzyme?
How do you design and validate PL constructs?
How do you prepare proteomic samples?
Acquiring data and data analysis (Day 2)

Designing PL constructs for proteomics

- For organelle mapping, use the minimal signal sequence necessary for proper subcellular targeting
  - Avoid full length proteins if possible

Designing PL constructs for proteomics

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Region	Sequence (Branon et al. *Nat. Biotech.* 2018)
Cytosol	Nuclear export signal: LQLPPLERLTLD
Nucleus	Nuclear localization signal: DPKKKRKVDPKKKRKVDPKKKRKV
Mitochondrial matrix	From COX4: MLATRVFSLVGKRAISTSVCVRAH
Outer mito membrane	From MAVS: RPSPGALWLQVAVTGVLVVTLLVVLYRRRLH
ER lumen	From Ig K-chain: METDTLLLWVLLLWVPGSTGD; retention sequence: KDEL
ER membrane	From cytochrome P450: MDPVVVLGLCLSCLLLLSLWKQSYGGG
Cell surface	Ig K-chain ss: METDTLLLWVLLLWVPGSTGD; transmembrane domain of PDGF receptor from pDisplay vector (Invitrogen)

Designing PL constructs for proteomics

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- For interactome mapping, full protein fusion is necessary: the best approach is to base design off other protein fusions in previous literature (i.e. GFP)

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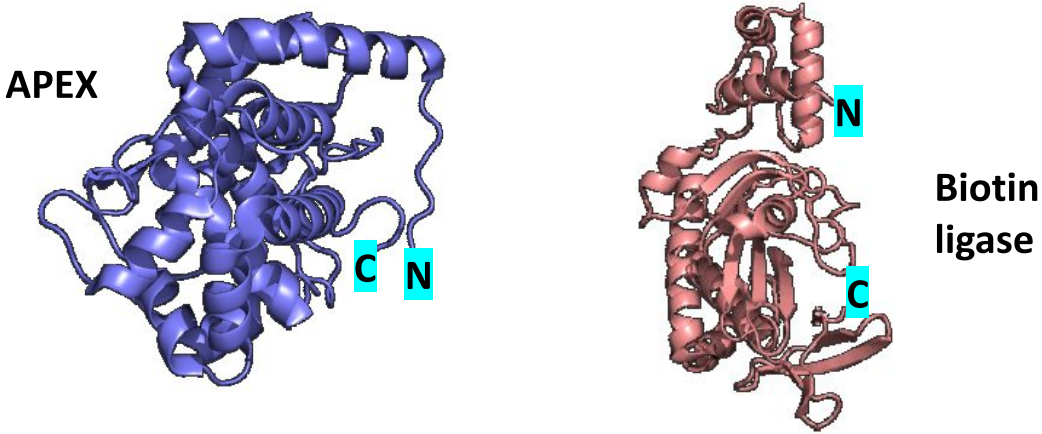
  - Internal fusions can be made in your protein of interest



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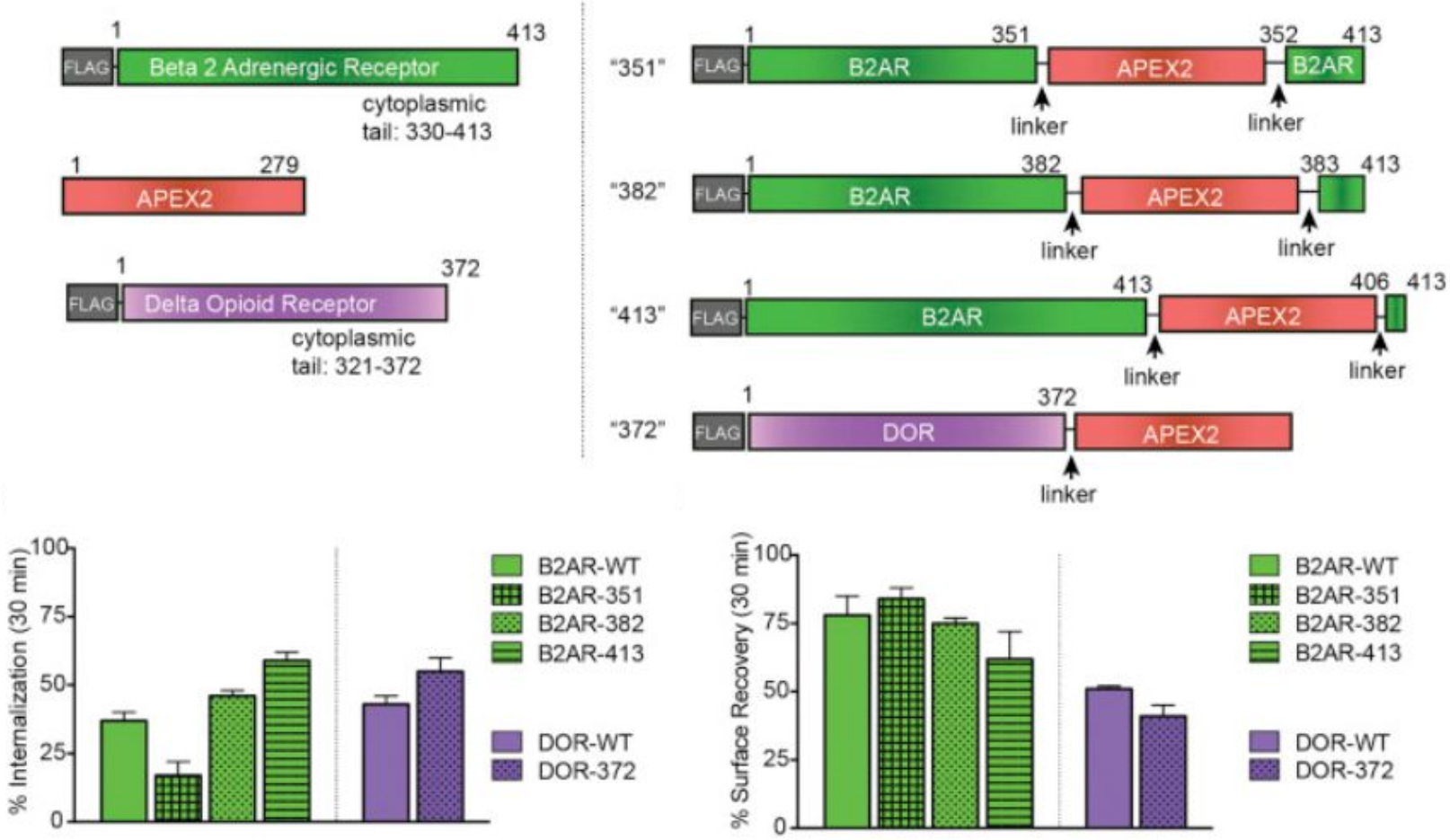
  - Internal fusions can be made in your protein of interest
  - Very important to assess any potential perturbation of your protein of interest's localization, function, interactions, etc.



Lobingier et al. *Cell* 2017

Designing PL constructs for proteomics

- For organelle mapping, use the minimal signal sequence necessary for proper subcellular targeting
- For interactome mapping, full protein fusion is necessary: the best approach is to base design off other protein fusions in previous literature (i.e. GFP)
- Both N- and C-terminal fusions of peroxidases and biotin ligases will retain activity

Designing PL constructs for proteomics

- Linkers are not always necessary, but may help maintain proper folding/function/targeting of fusion construct
  - Typically start with 10 aa flexible linker (e.g. GGGGSGGGGS), optimize as needed

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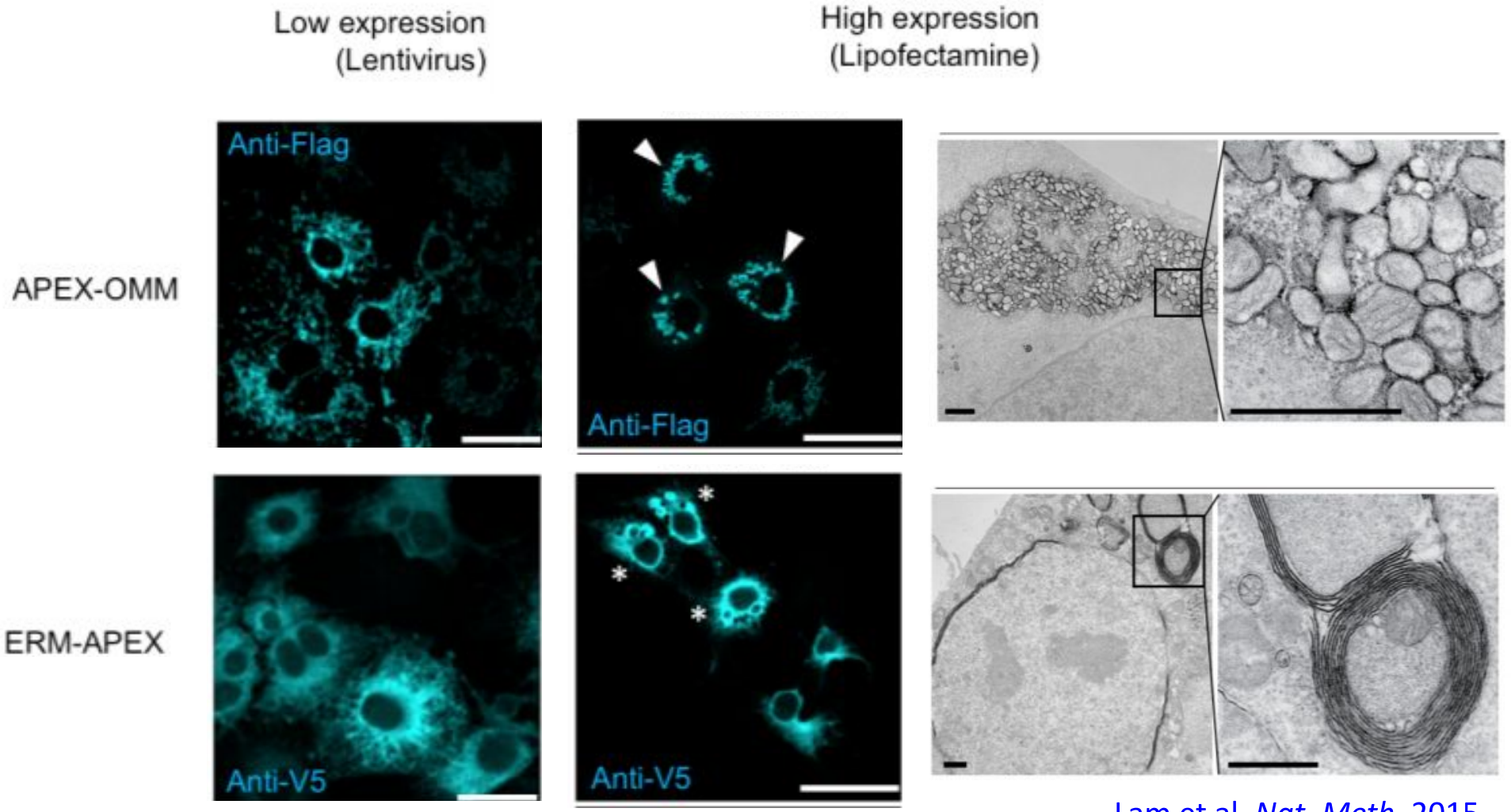
- Include epitope tags!
  - For biotin ligases, avoid lysine-rich epitope tags
    - FLAG (DYKDDDDK)
  - For peroxidases, avoid tyrosine-rich epitope tags
    - HA (YPYDVPDYA)

Expressing PL constructs for proteomics

- For cell culture, start with transient transfection then move to transduction or stable expression if necessary



Lam et al. *Nat. Meth.* 2015

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Expressing PL constructs for proteomics

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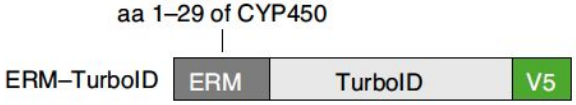
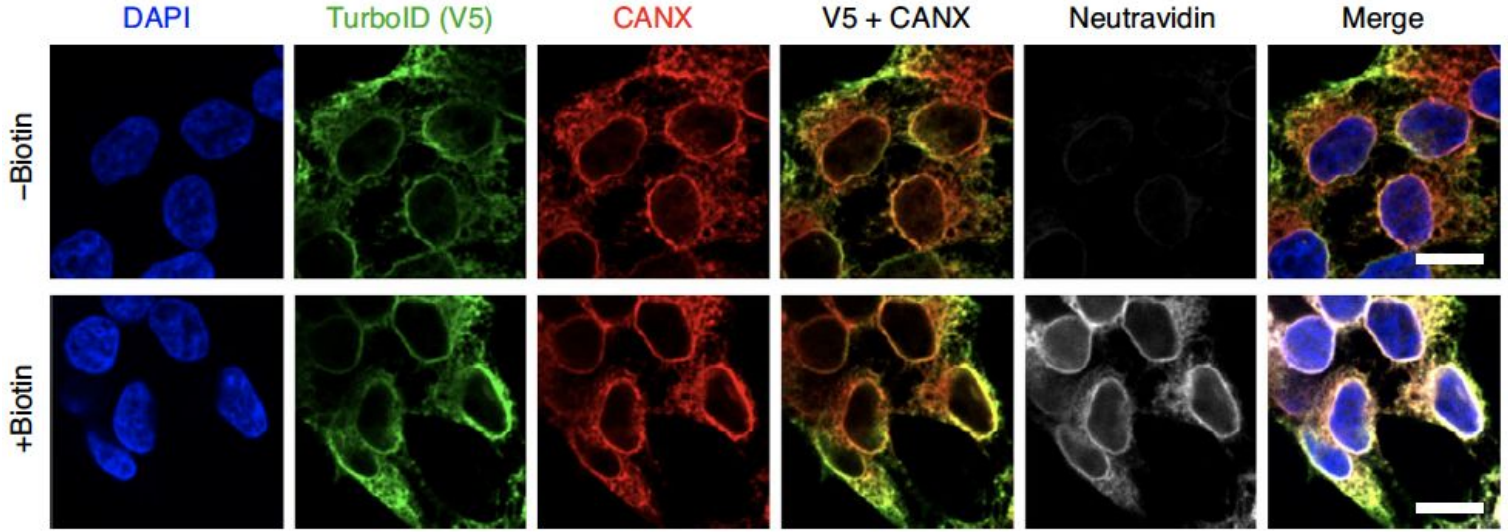
- Tradeoff between increasing signal:noise while minimizing perturbation
- Both constitutive and inducible promoters can be used, can also try different strength promoters
- In cell culture, you want at least 50% of cells expressing the fusion construct
  - Unlikely that you can achieve this in vivo
    - Can do pre-enrichment (e.g., synaptosome prep), longer labeling time, etc.

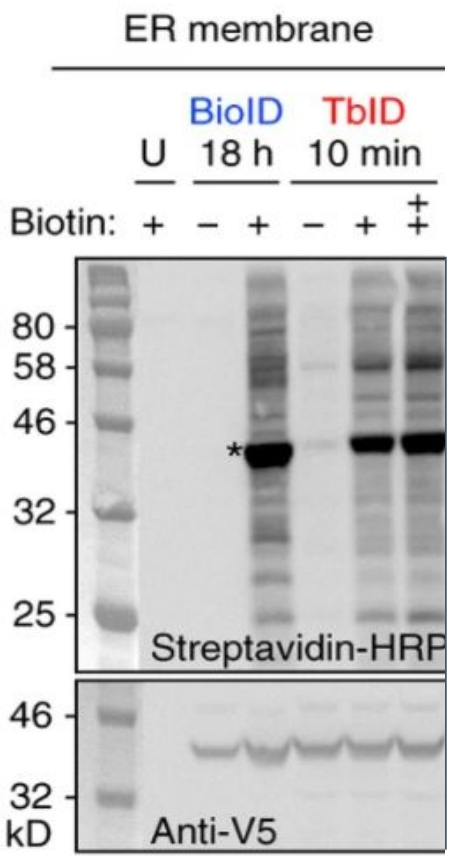
Quality check #1: validate construct by imaging



- Verify that the localization of the construct and resulting biotinylated proteins colocalize with known markers
- Calnexin used here as an ER marker



Quality check #2: validate construct by streptavidin blotting



- Assess biotinylation activity in whole cell lysates
- Assess banding pattern: there should be numerous bands indicating biotinylation of multiple protein species
- Intensity of bands labeled from PL enzymes should be greater than that of endogenously biotinylated proteins

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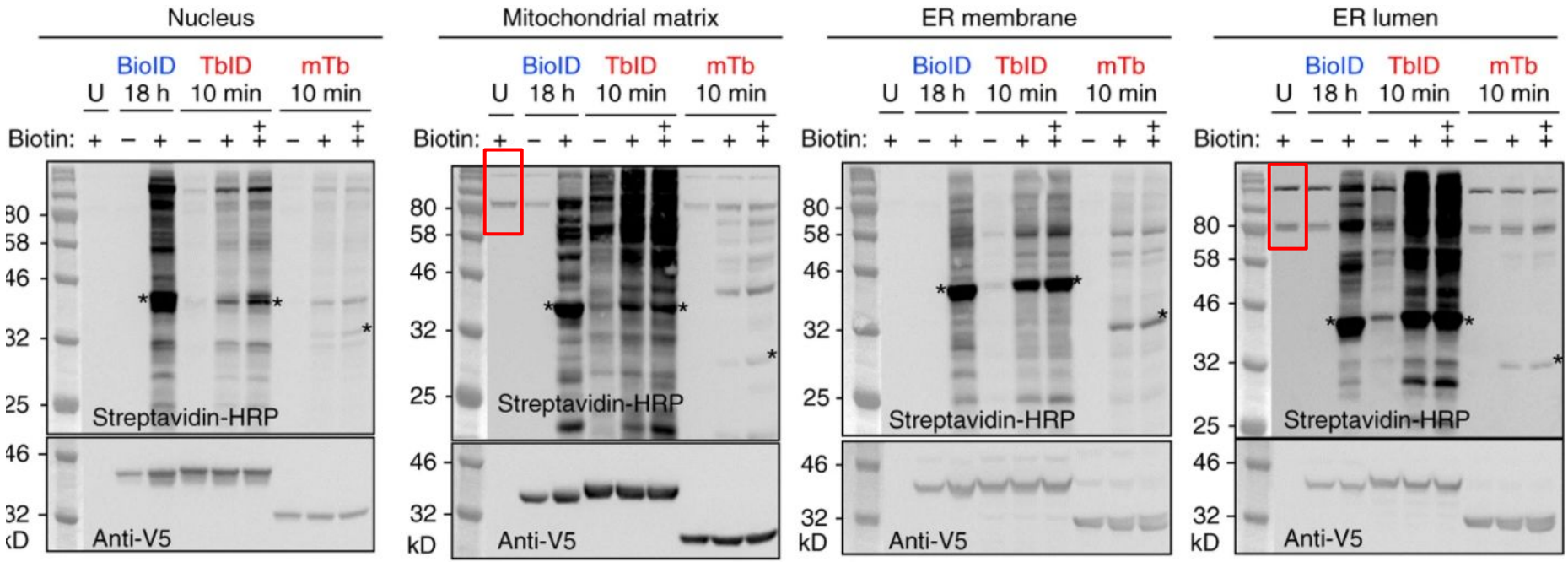

Branon et al. *Nat. Biotech.* 2018

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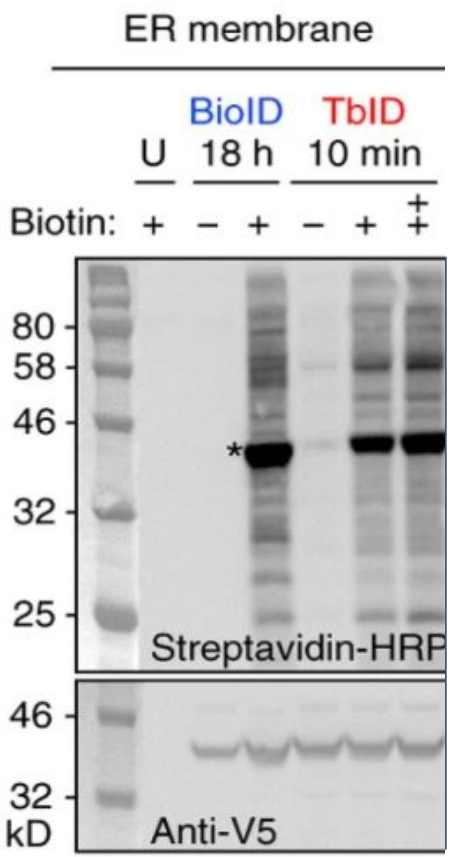

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- Intensity of bands labeled from PL enzymes should be greater than that of endogenously biotinylated proteins
- From this information, optimize labeling conditions: shortest time necessary for adequate signal:noise to maintain specificity
- Immunostaining against epitope tag can tell you expression level and and any degradation

Quality check #3: validate enrichment protocol

- Detailed protocols available from Hung et al. *Nature Protocols* 2016 and Cho et al. *Nature Protocols* 2020

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- IMPORTANT to follow protocol closely!!
  - Label samples and generate whole cell lysates as previously
    - May need additional steps to prepare lysates from *in vivo* samples
  - Start with 25 µL streptavidin beads (use Thermo Fisher Scientific cat. No. 88817), wash twice with RIPA lysis buffer
  - Incubate with ~300 µg protein in 500 µL RIPA for minimum 1h at 4°C with rotation

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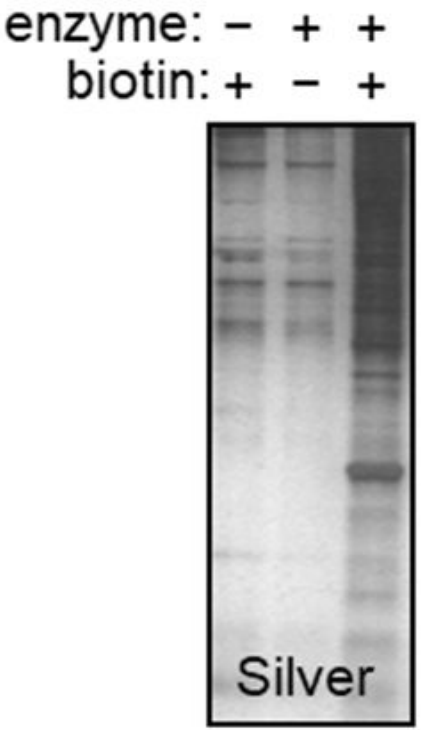
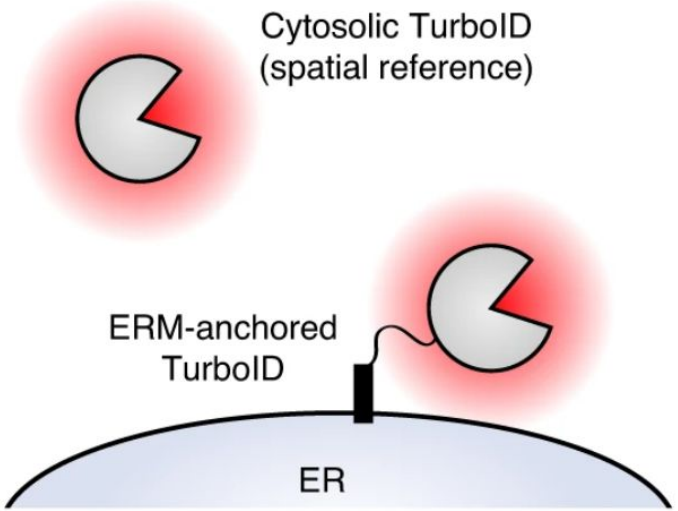
  - Elute enriched material by boiling in 30 µL 3x protein loading buffer + 2mM biotin and 20mM DTT for 10 min at 95°C
  - Collect eluate and analyze enrichment by Coomassie and/or silver stain

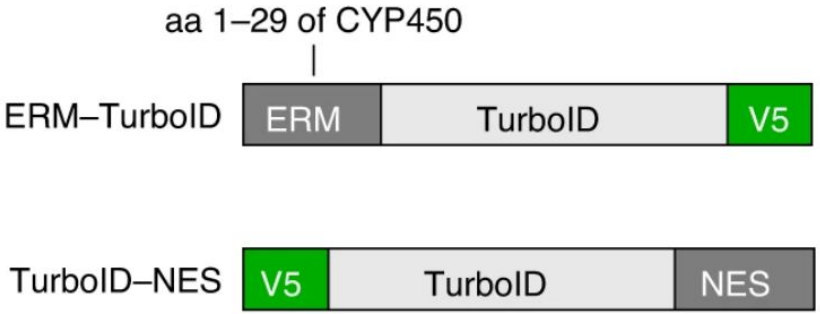
Quality check #3: validate enrichment protocol



- Goal is to observe enrichment of proteins either by Coomassie or silver stain
- Should see much higher signal in experimental conditions compared to negative controls
- May need to optimize the amount of beads used
- While not necessary, western blotting for known positive control proteins in enriched samples can be performed for additional confidence

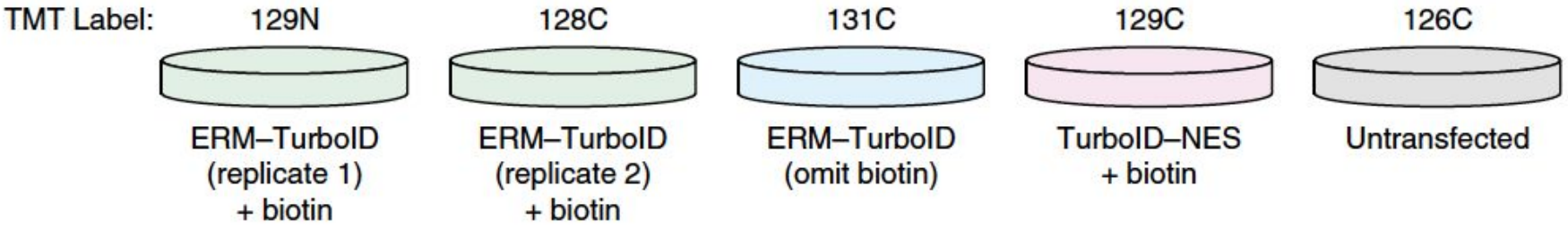
Designing quantitative proteomic experiments





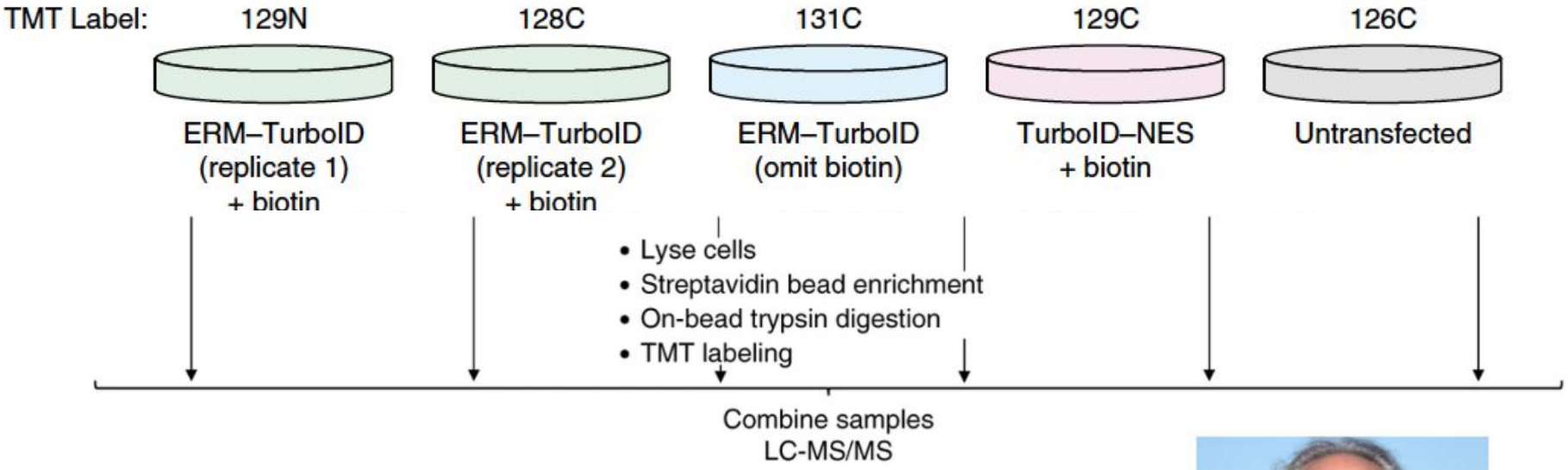
- Use quantitative MS approach e.g. TMT
- Include omit substrate negative control and spatial specificity control

Designing quantitative proteomic experiments



- Include at least 2 replicates of experimental samples
  - If you have room, have replicates of controls (TMT can take up to 11 samples)
- Scale up according to optimized enrichment conditions

Designing quantitative proteomic experiments



- MS typically done with collaborators or core facilities

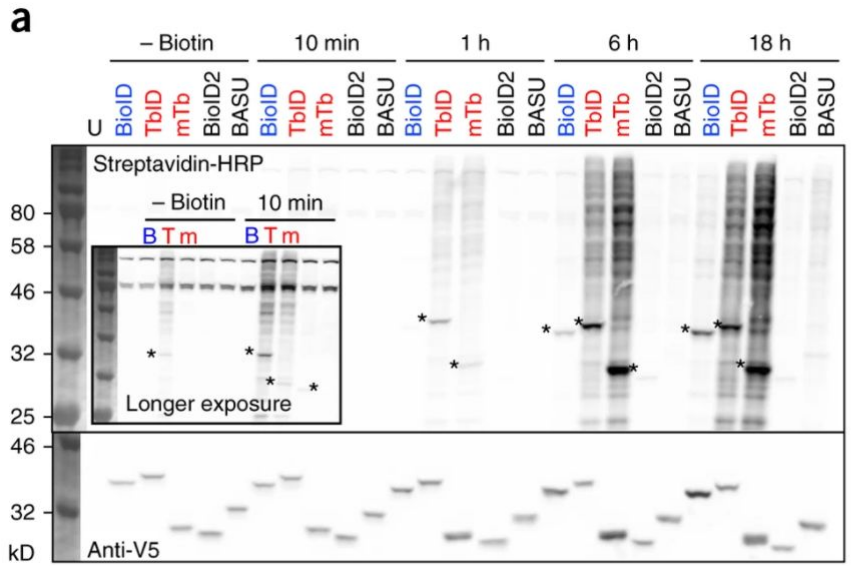
Steven A. Carr

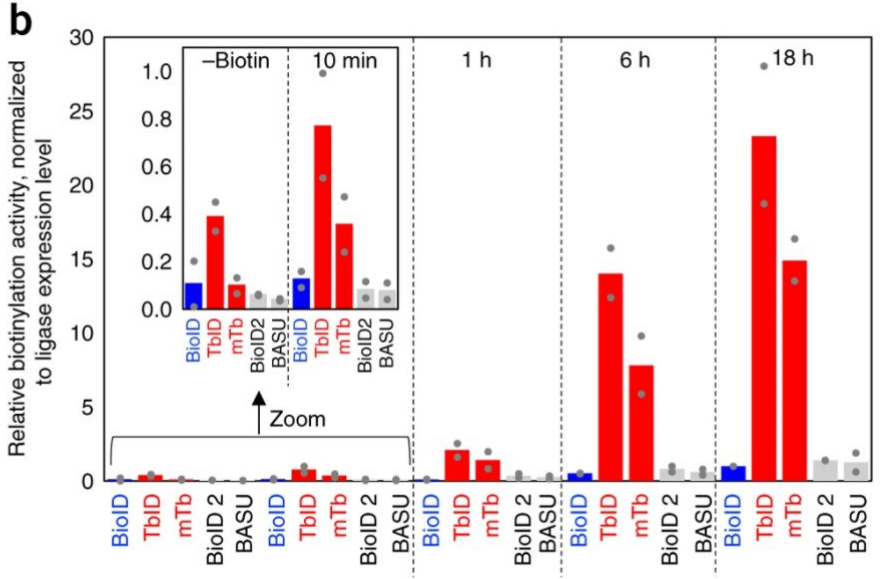
Summary: proximity labeling for proteomics

- Proximity labeling can be used to identify protein-protein interactions and determine spatiotemporally resolved proteomes
- Different PL enzymes each have pros and cons, choosing between them depends on the application
- Fusion constructs should be tested and validated by imaging and streptavidin blotting
- Small scale enrichment and optimization should be performed before scaling up for proteomics

Questions?

Comparison of biotin ligase enzyme activities





Branon et al. *Nat. Biotech.* 2018