SPP 2330

New Concepts in Prokaryotic Virus-host Interactions – From Single Cells to Microbial Communities

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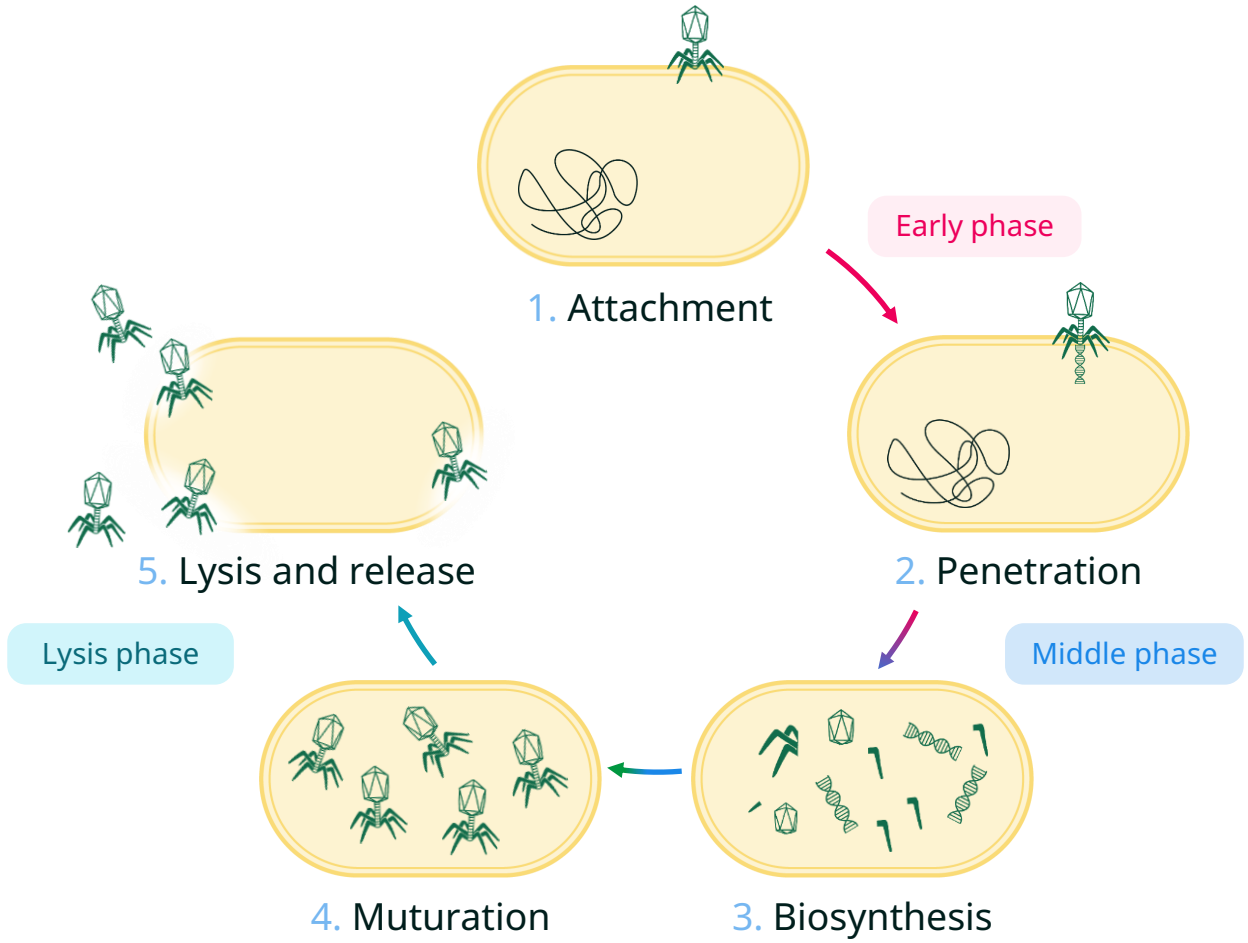
New Concepts in Prokaryotic Virus-host Interactions – From Single Cells to Microbial Communities

PROTEIN – PROTEIN Interaction

Why ?

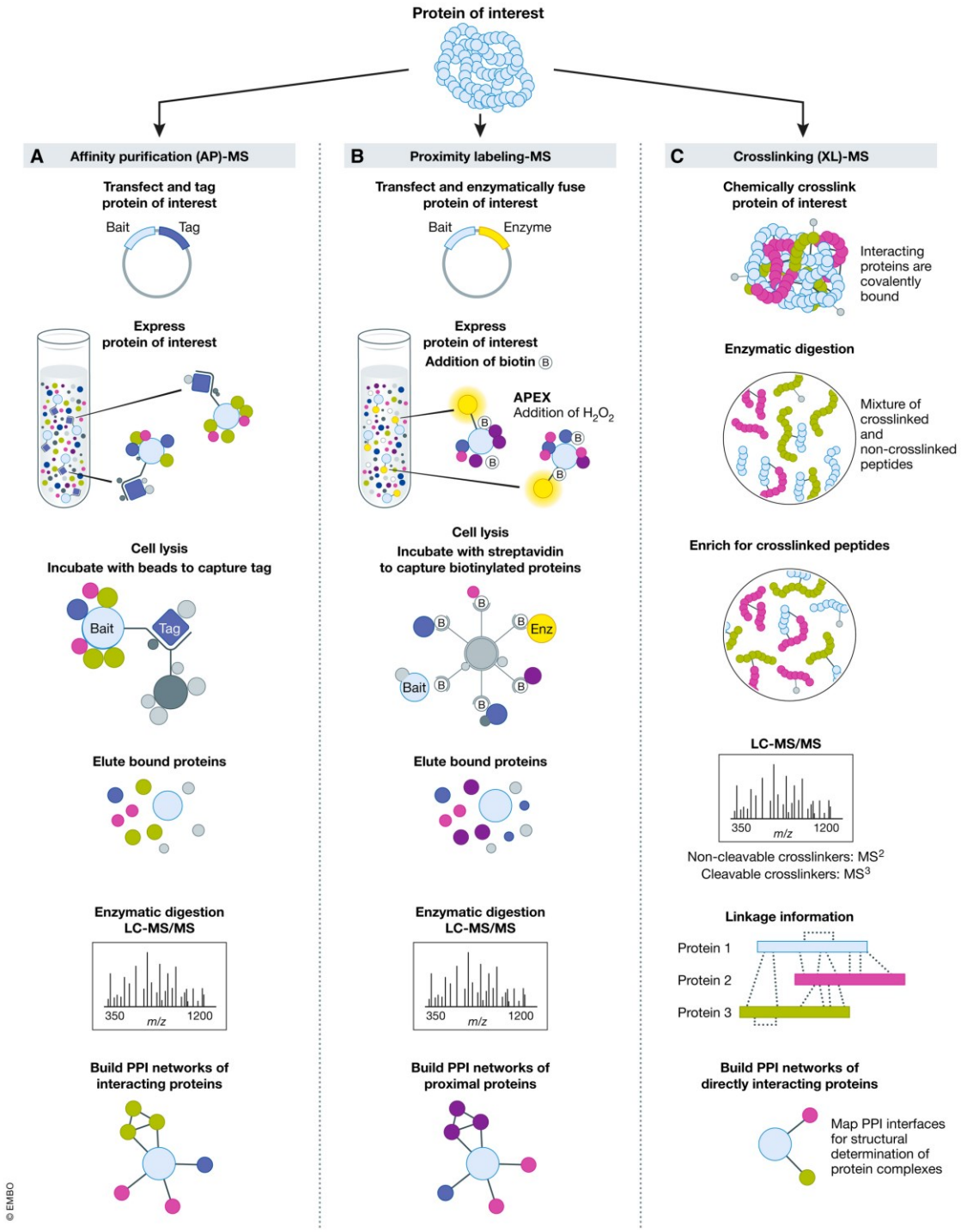
- Understanding phage infection mechanisms
- Identify interaction partners



How?

Technologies:

- Co-IP
- Proximity labelling
- Crosslinking-MS



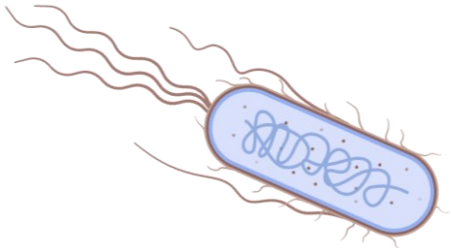
Mol Syst Biol (2021) 17: e8792
https://doi.org/10.15252/msb.20188792

Today

Proximity labelling

* Theoretical background of BioID
* Cloning strategy for BioID constructs
* Explanation of BioID pipeline
* What's next ?!

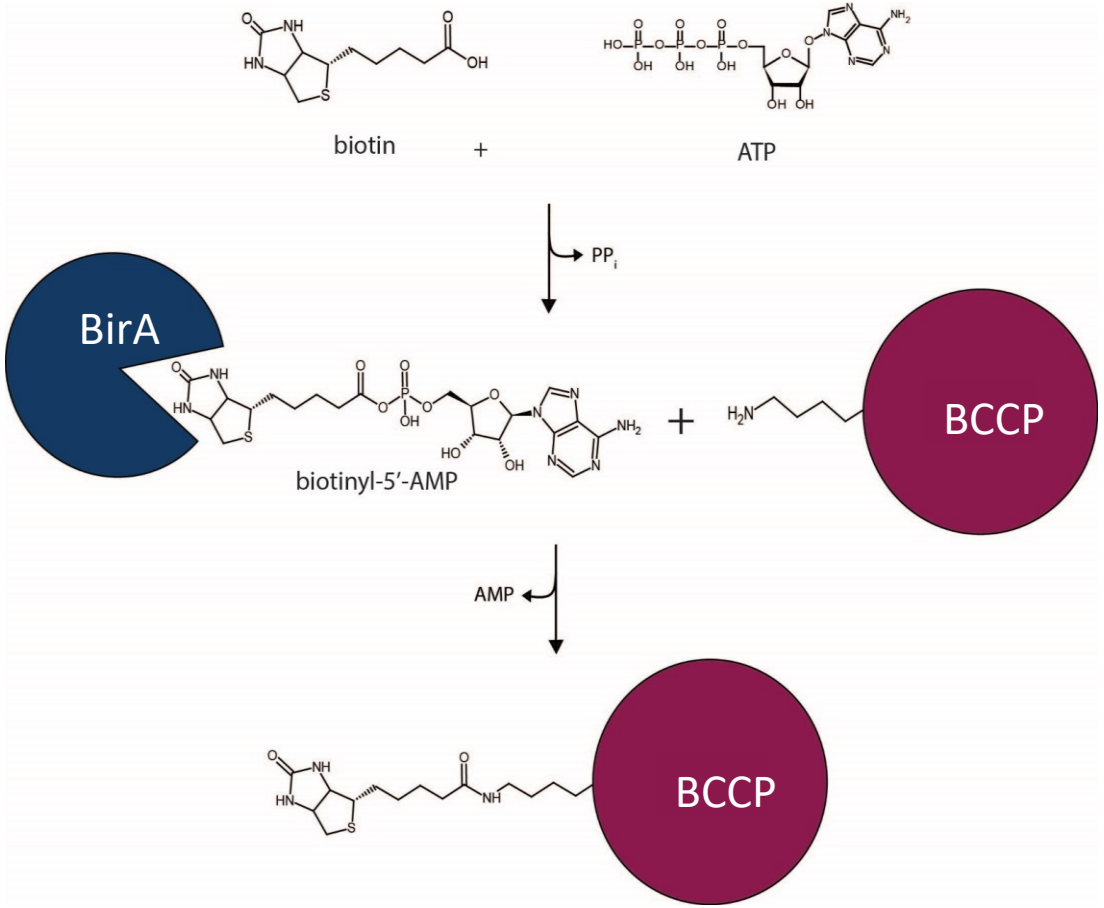
BioID Proximity dependent Biotin IDentification“



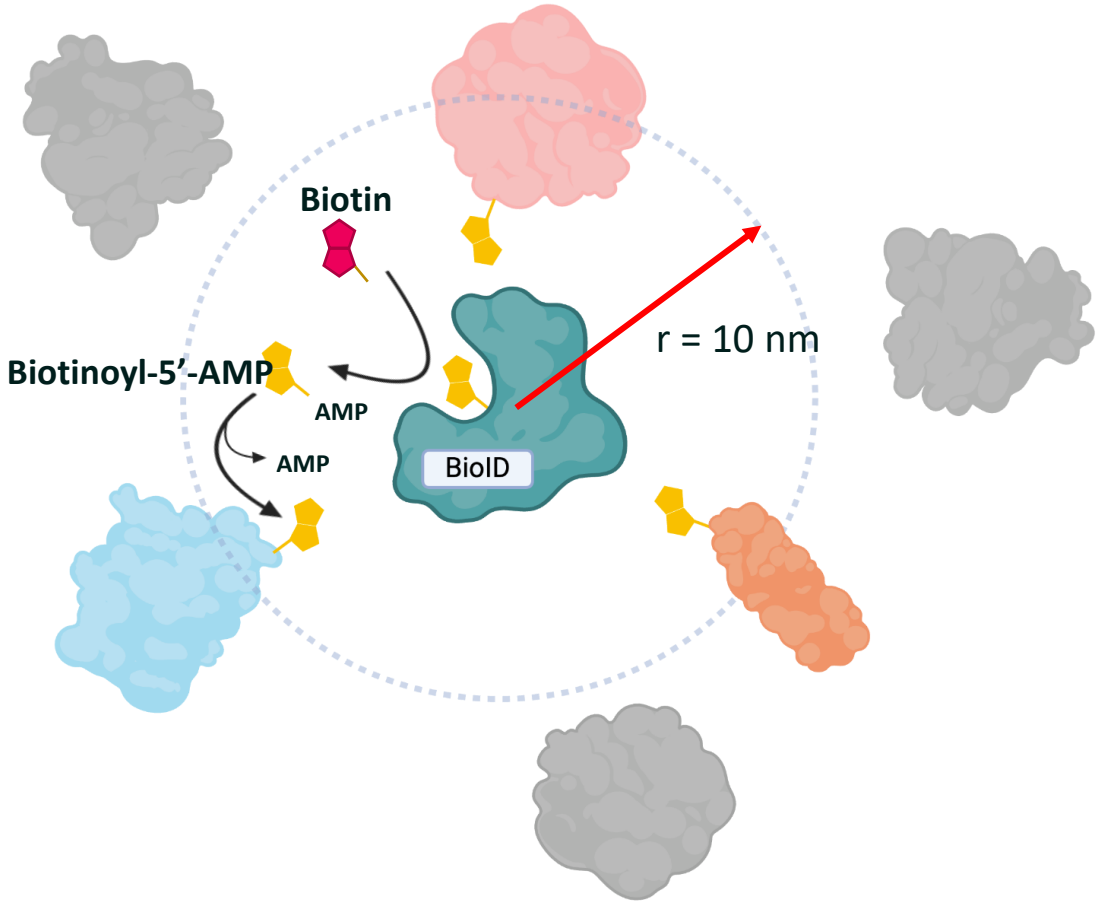
Enzyme: BirA (*E. coli*)

Reaction: Biotinylation

Target: biotin carboxyl carrier protein (**BCCP**) subunit of the acetyl-CoA-carboxylase



BioID unspecifically biotinylates proximate proteins



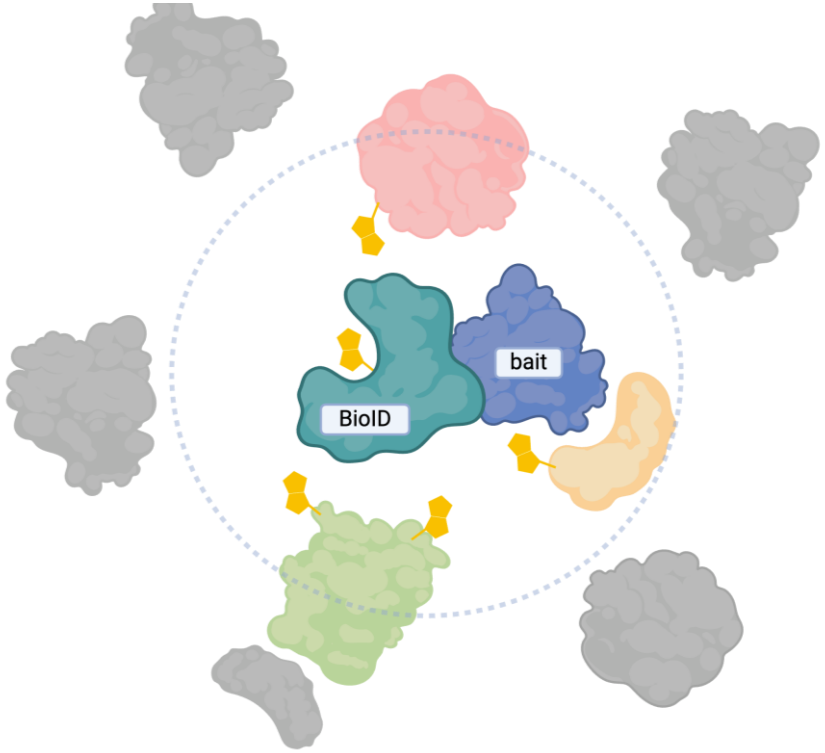
BirA_R118G (BioID)

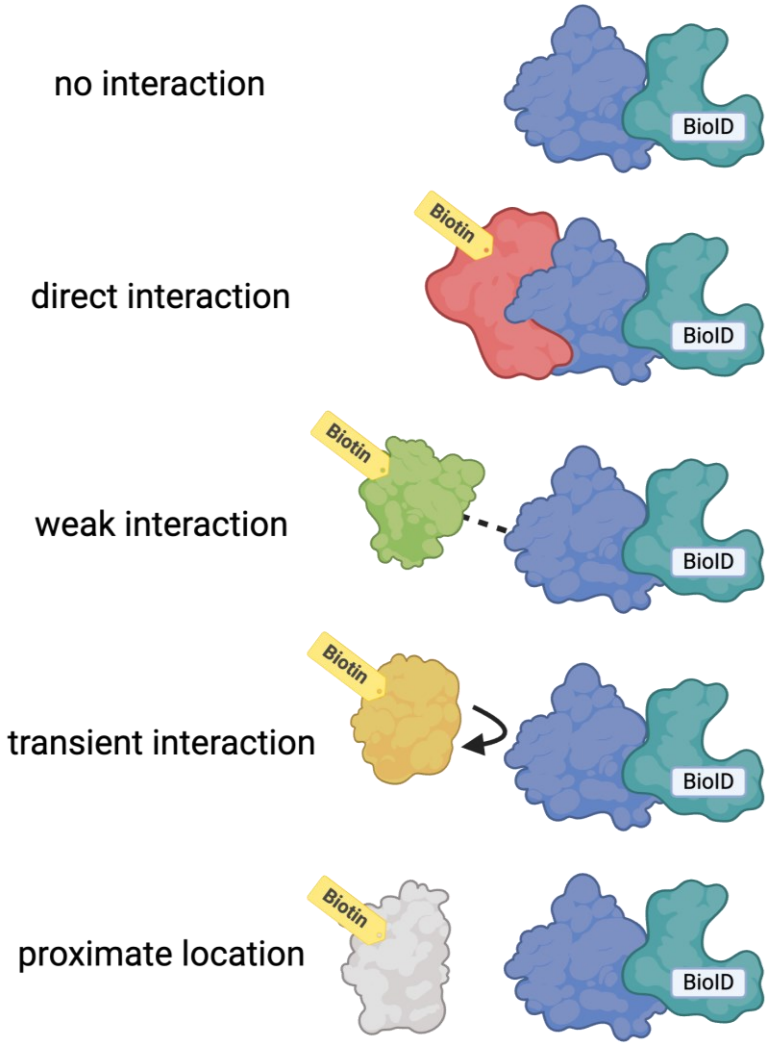
Premature release of biotinyl-5’-AMP

⬇

unspecific biotinylation of proximate protein

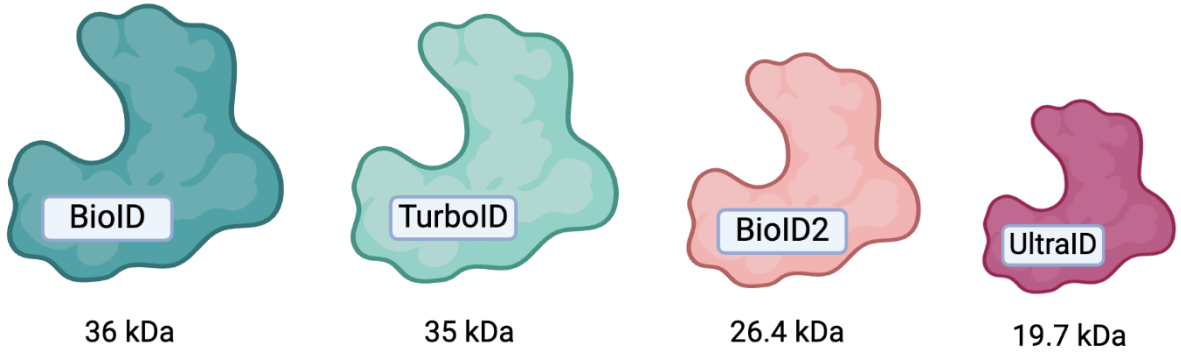
Interactome analysis through BioID





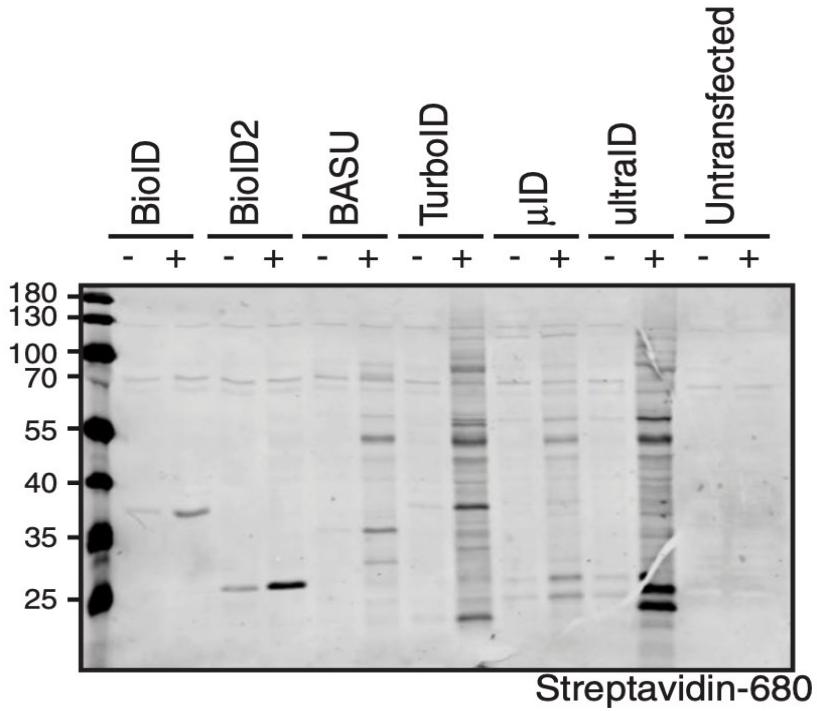
Proteins in the proximity of the POI (bait) are biotinylated

UltraID is a highly efficient and small biotin ligase



Big
Inefficient (>12 h)

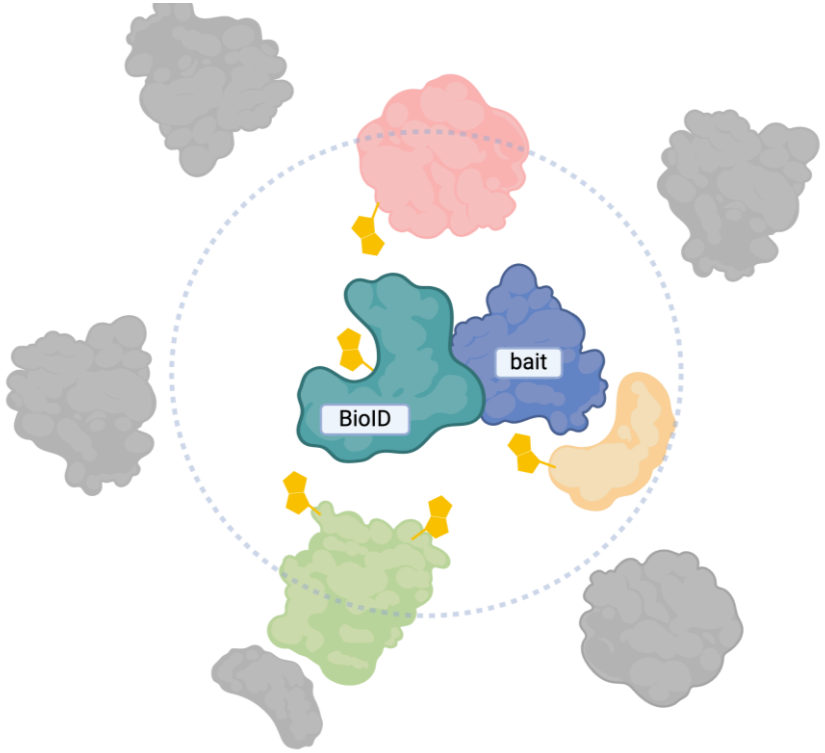
- ✓ smaller
- ✓ faster
- ✓ lower background biotinylation

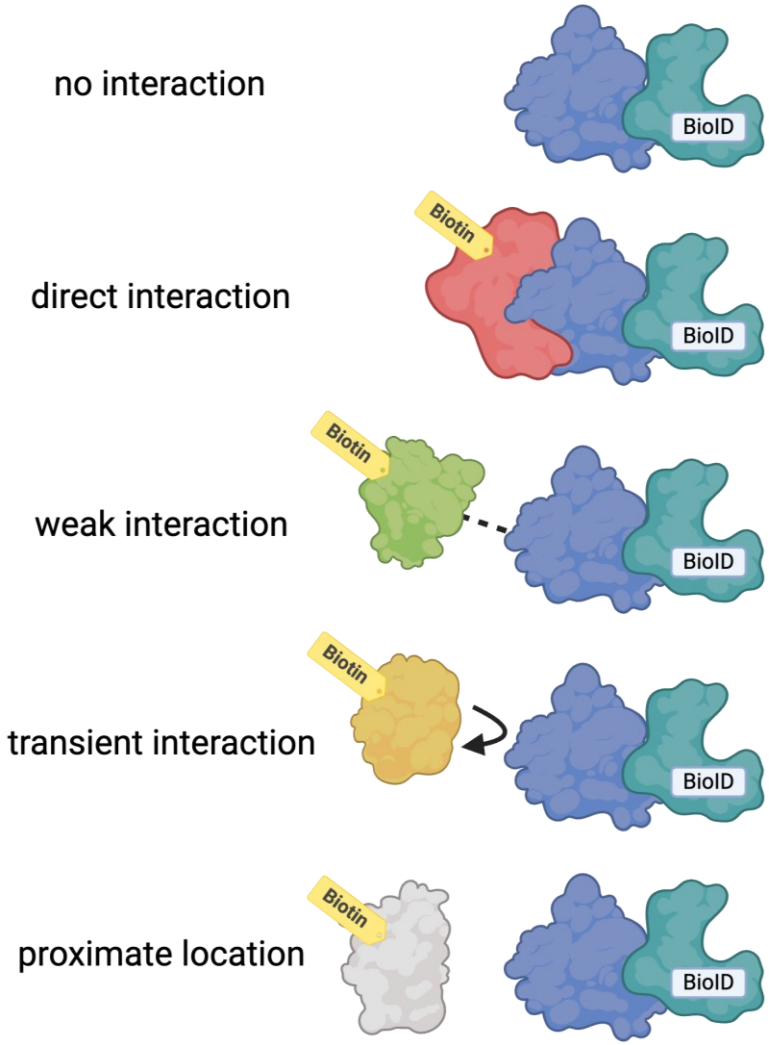


10 min

Kubitz et al., 2022. *Communications biology*

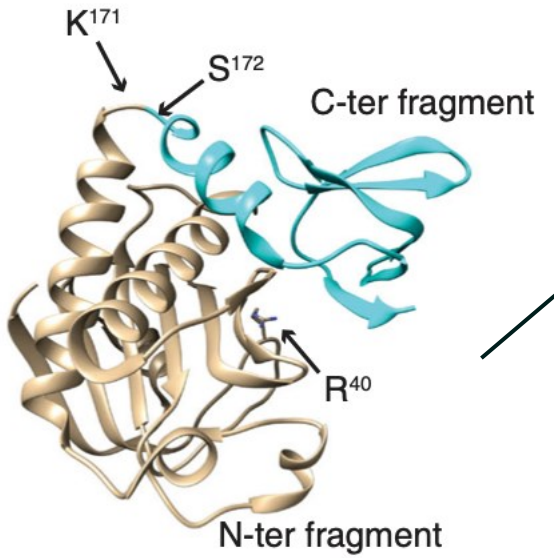
Interactome analysis through BioID





Proteins in the proximity of the POI (bait) are biotinylated

Development of MicroID and UltraID



A. aeolicus BirA

(= BioID2)

truncation

MicroID

N-terminal fragment of BioID2

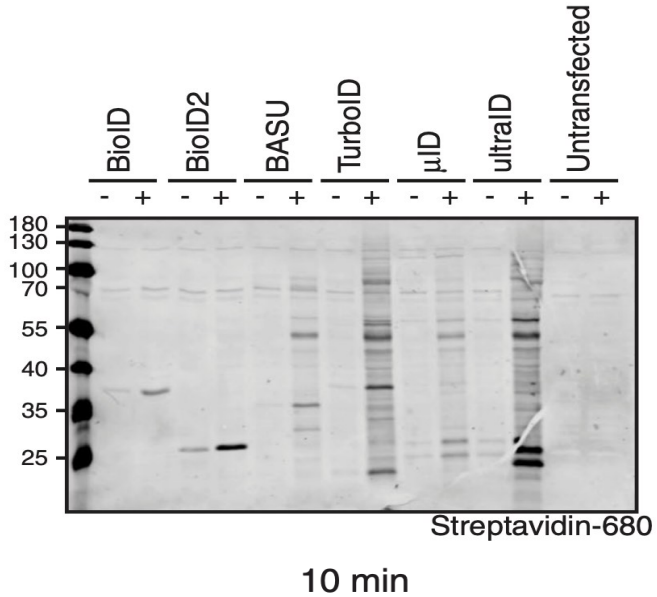
Only 19.7 kDa (instead of 26.4 kDa)

Site-directed mutagenesis

UltraID

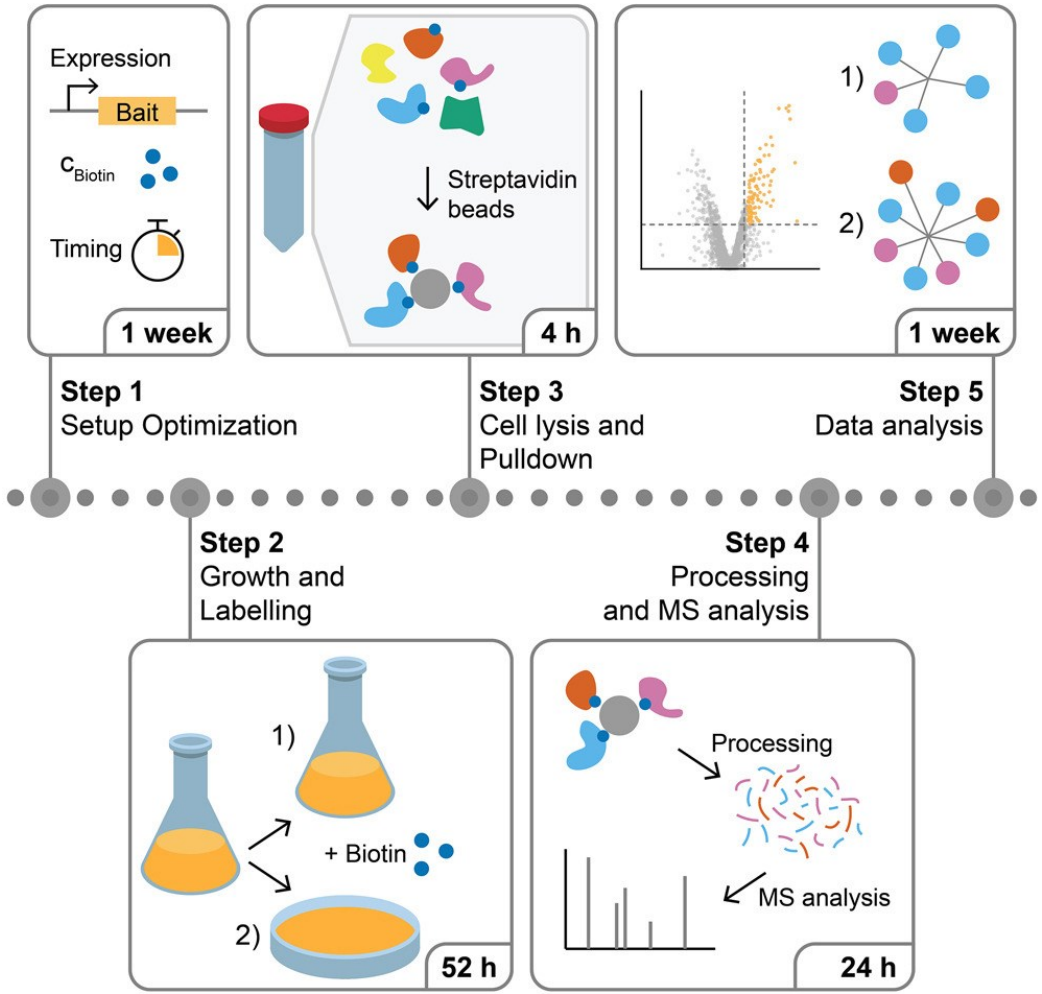
= microID-R40G/L41P

Enhanced activity, same thermal stability

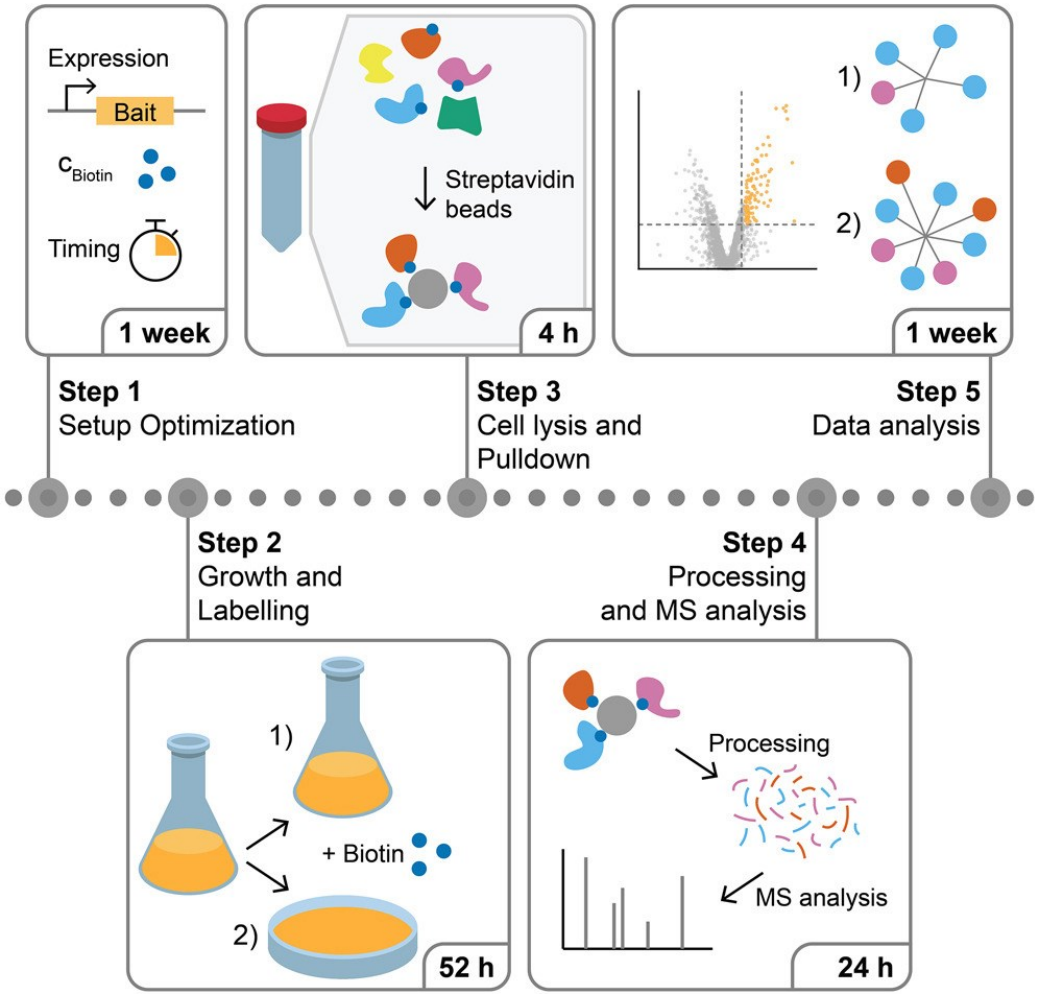


Kubitz et al. (2022)
https://doi.org/10.1038/s42003-022-03604-5

Workflow BioID

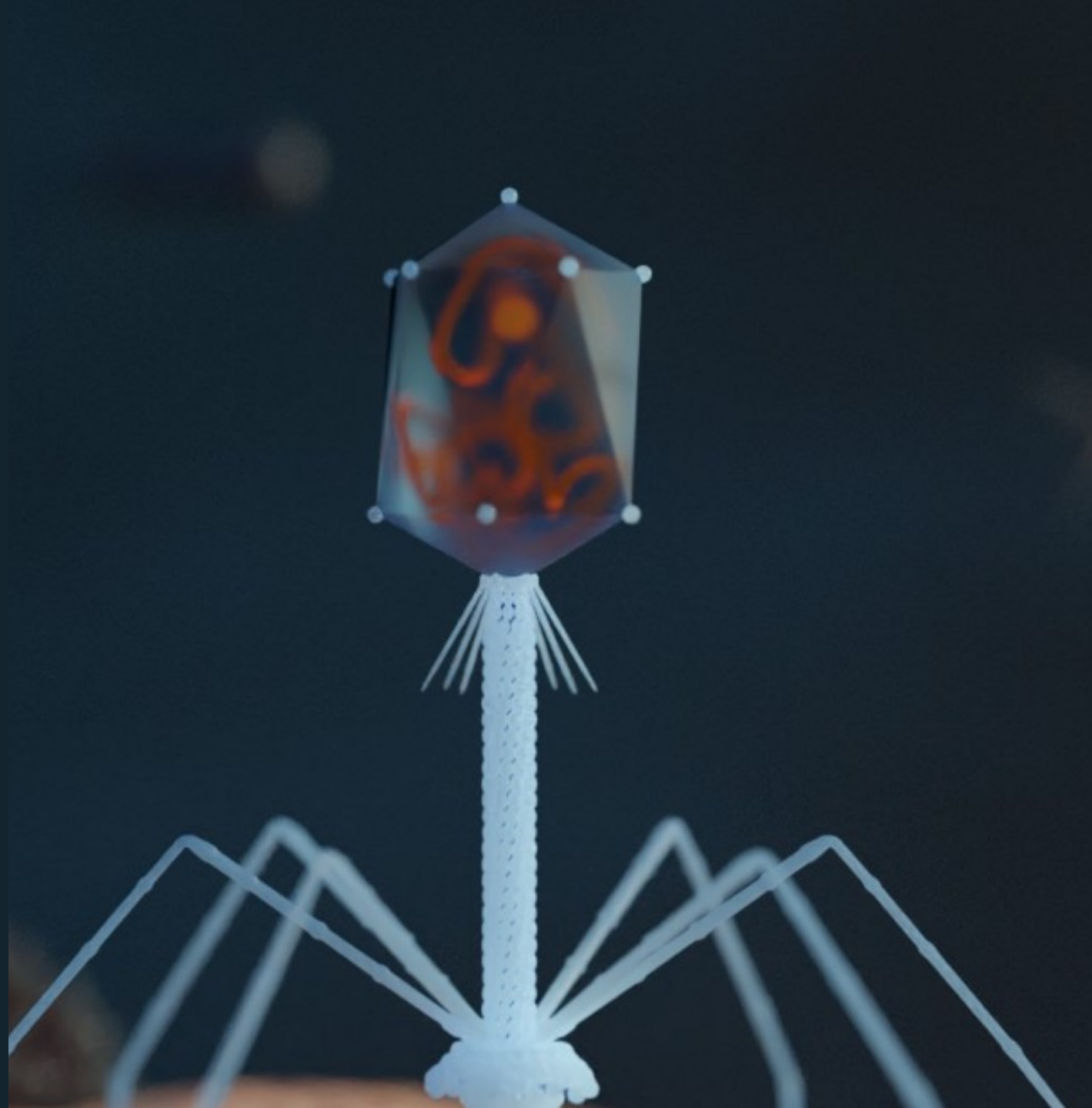


Workflow BioID



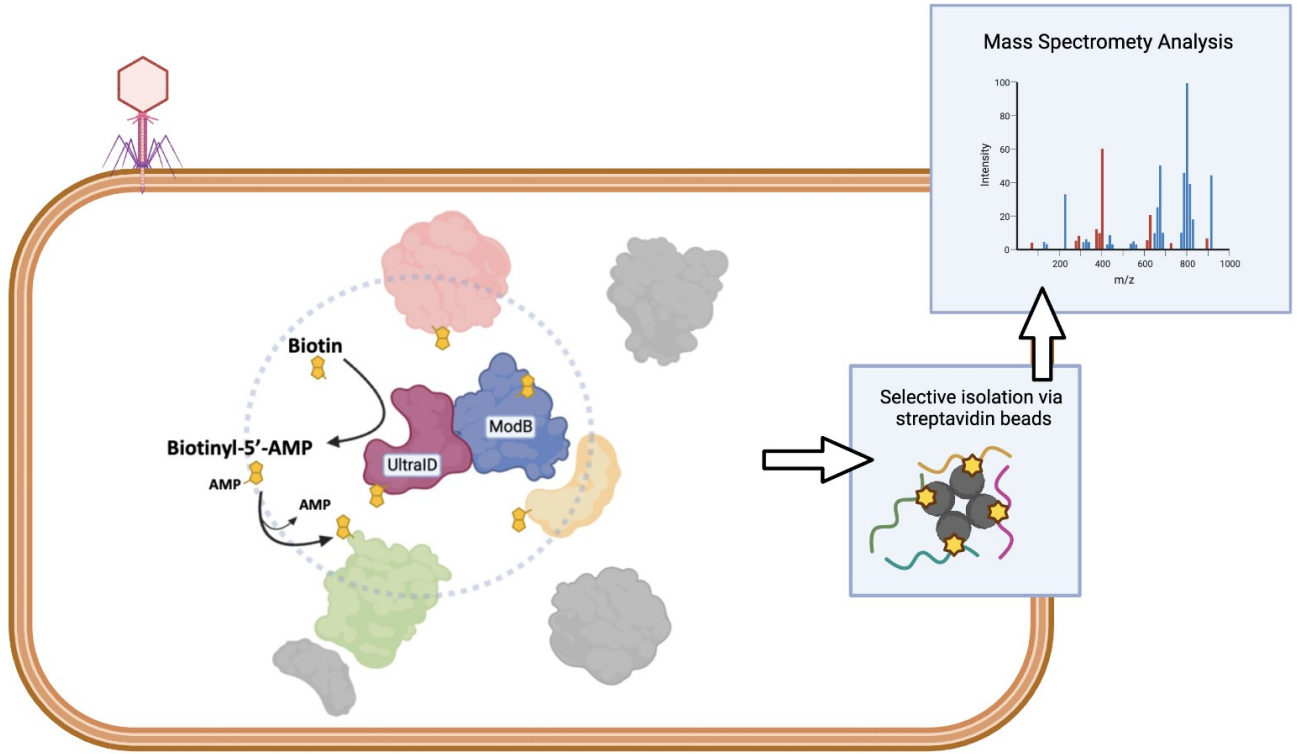
Herfurth, M., Müller, F., Søgaard-Andersen, L., & Glatter, T. (2023). A miniTurbo-based proximity labeling protocol to identify conditional protein interactomes in vivo in Myxococcus xanthus. *STAR Protocols*, *4*(4), 102657. https://doi.org/10.1016/j.xpro.2023.102657

How to apply BioID to analyze protein-protein interaction during infection?



Limitations

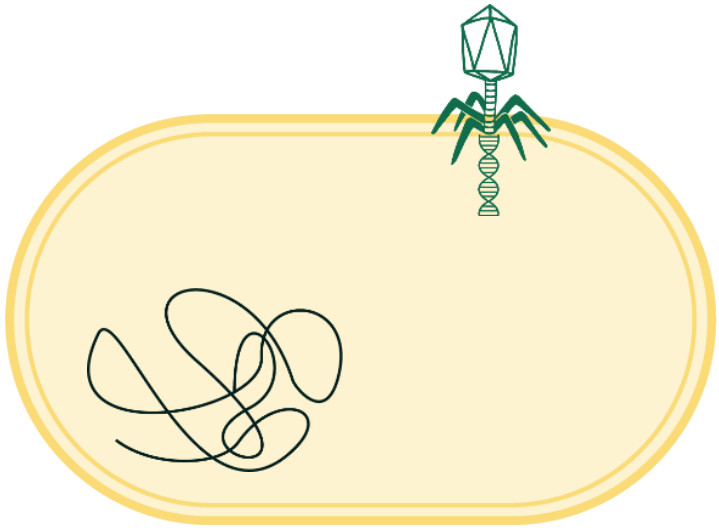
- Phage infections are fast
- Low amount of phage proteins
- Genetics of host and phage to express BioID fusions
- Intracellular biotin concentration
- Fusions can change activity/binding behavior of the POI



Example

T4 – *E. coli*

- Biological question



What are the interaction partners of ModB that influence target specificity?

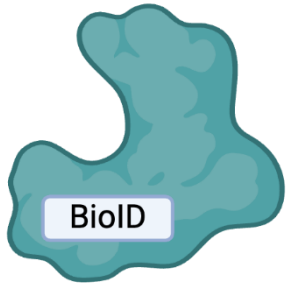
Example

T4 – *E. coli*

ModB interactome

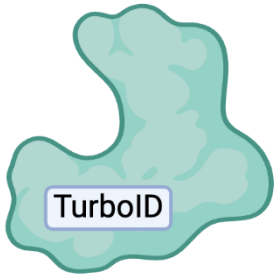
- Cloning strategy

Which BioID system is the best?

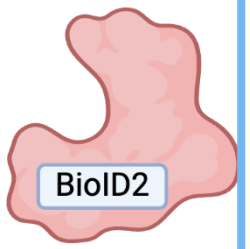


36 kDa

Big
Inefficient (>12 h)



35 kDa



26.4 kDa

19.7 kDa

- ✓ smaller
- ✓ faster
- ✓ lower background biotinylation

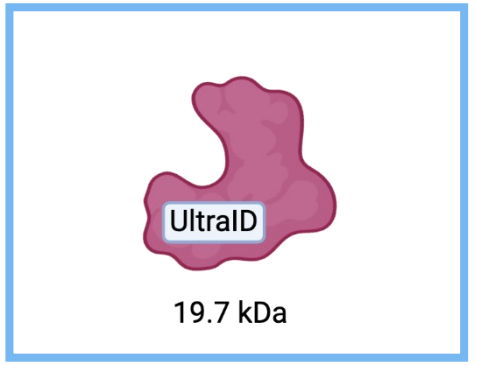
Example

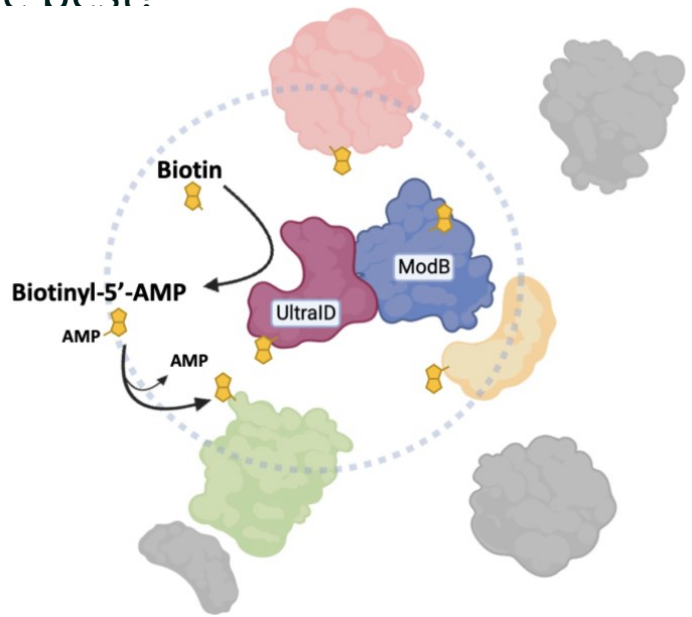
T4 – *E. coli*

ModB interactome

- Cloning strategy

Which BioID system is the best?





UltraID – ModB – His_6-tag	N-terminal fusion WT
ModB – UltraID – His_6-tag	C-terminal fusion WT
UltraID – His_6-tag	„background“ – neg. control

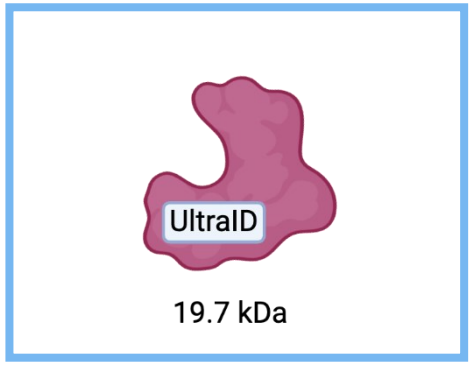
Example

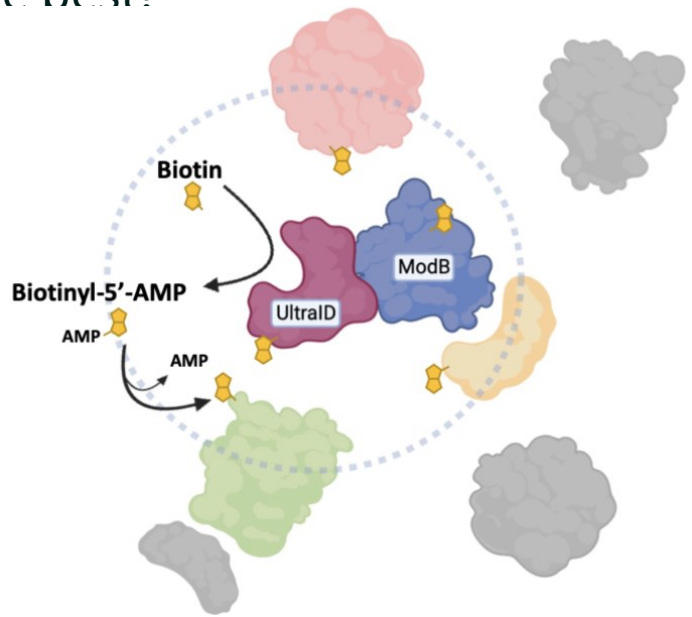
T4 – *E. coli*

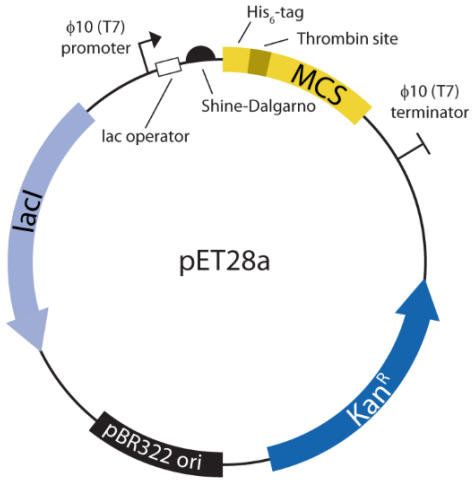
ModB interactome

- Cloning strategy

Which BioID system is the best?







- Cloning in vector that have inducible expression system of the BioID-fusion protein

or

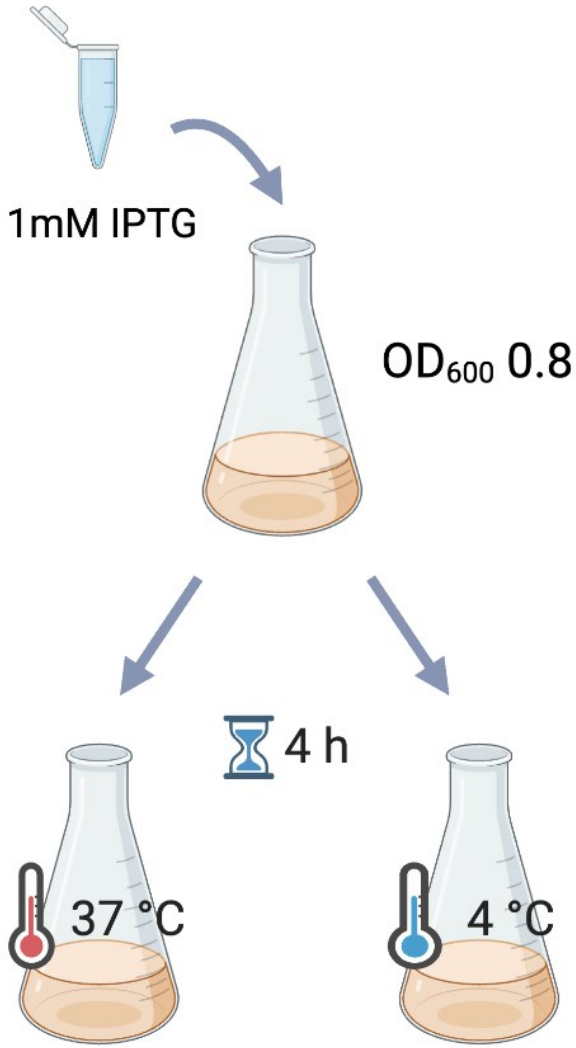
- Integration into chromosome

Example

T4 – *E. coli*

ModB interactome

- Cloning strategy
- Activity test

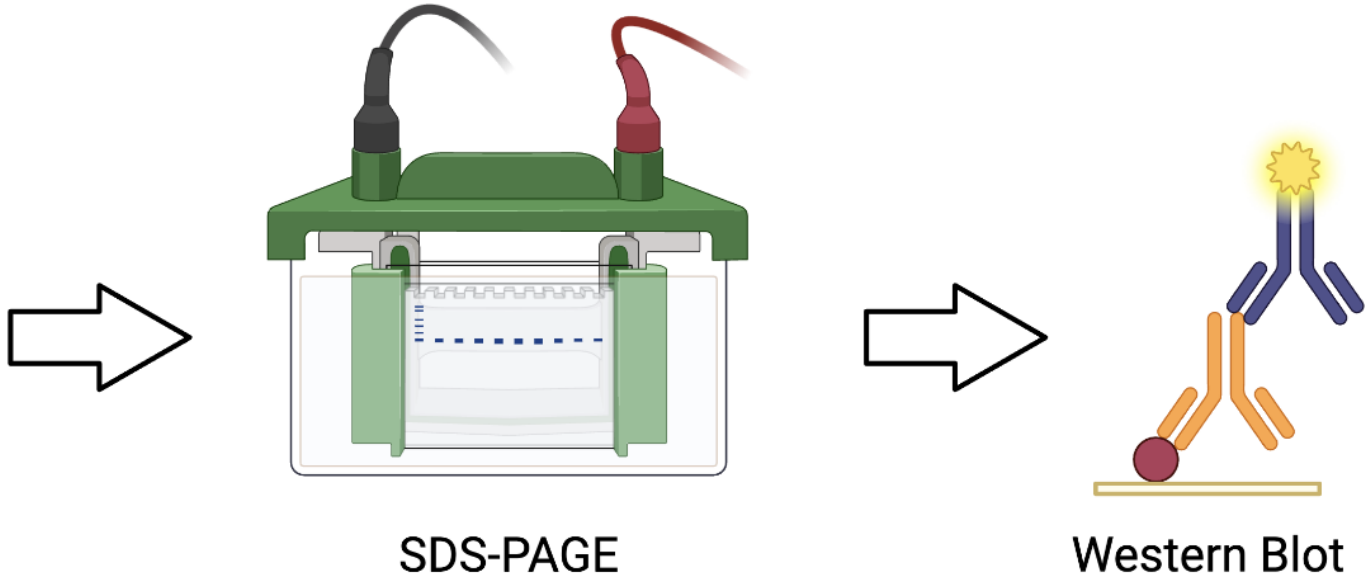


Example

T4 – *E. coli*

ModB interactome

- Cloning strategy
- Activity test

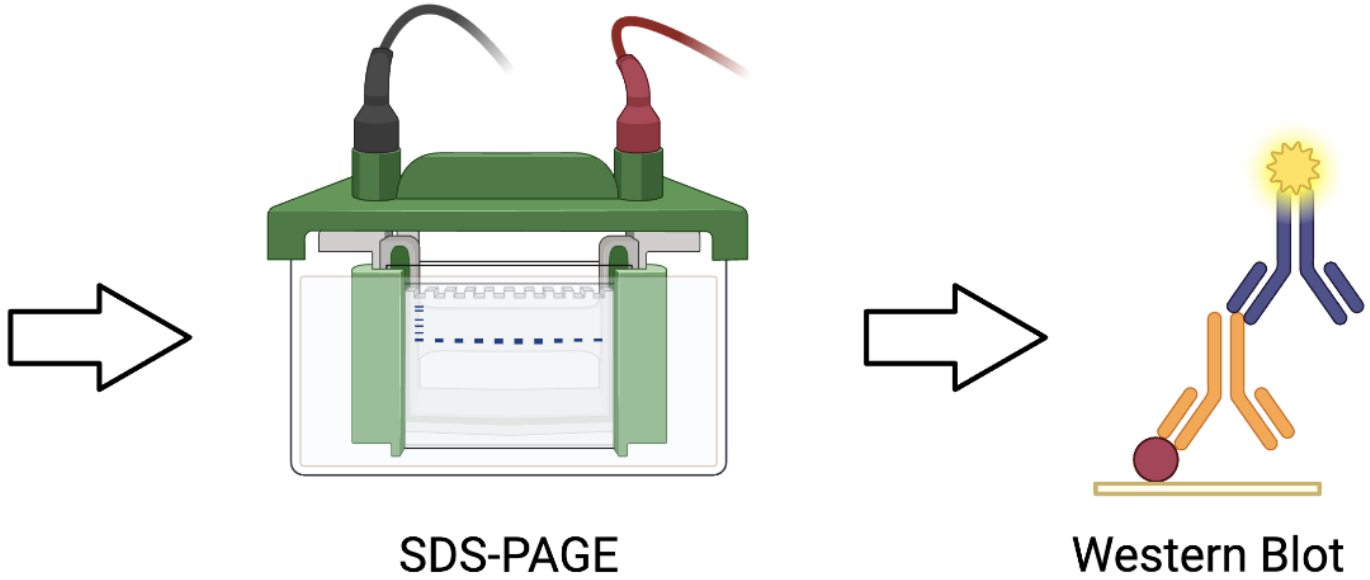


Example

T4 – *E. coli*

ModB interactome

- Cloning strategy
- Activity test

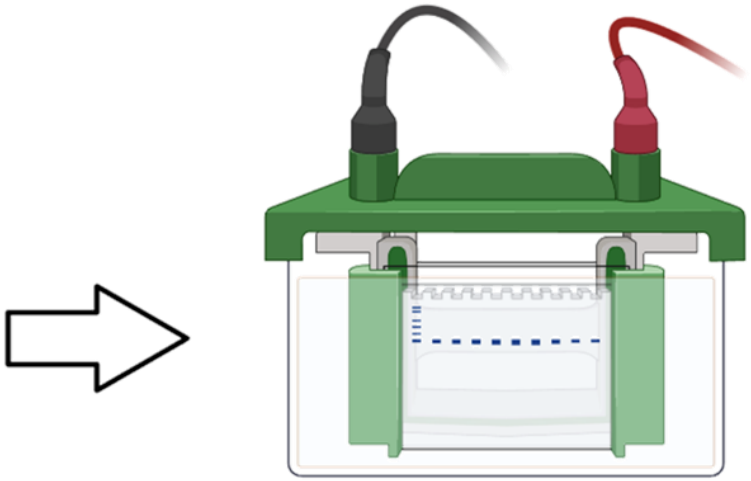


Example

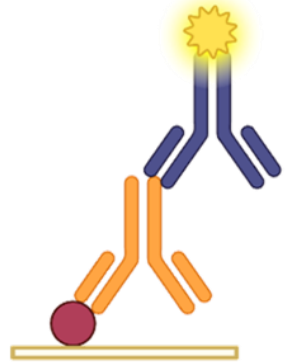
T4 – *E. coli*

ModB interactome

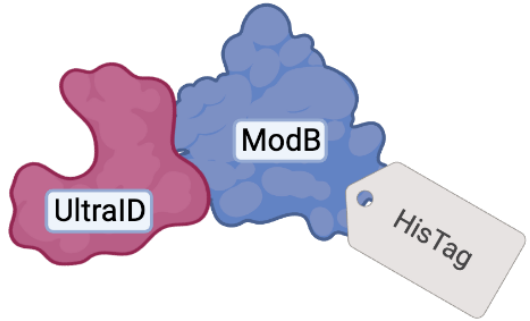
- Cloning strategy
- Activity test



SDS-PAGE



Western Blot



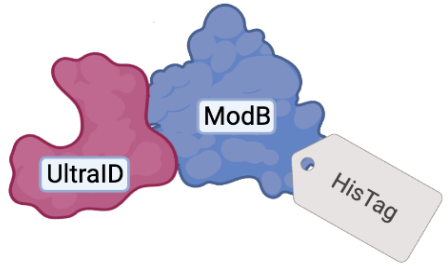
Biotinalytion ADP-ribosylation

Example

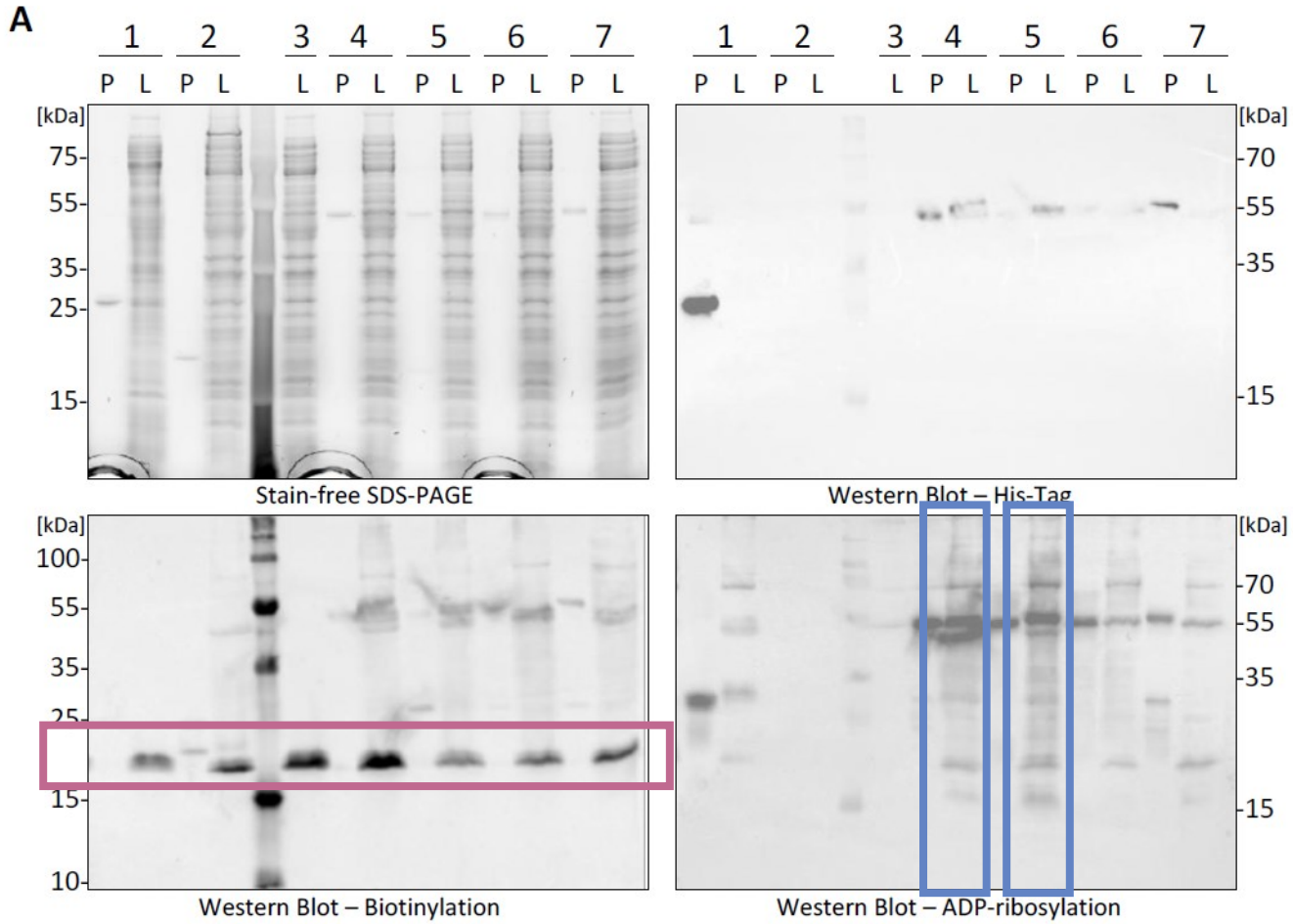
T4 – *E. coli*

ModB interactome

- Cloning strategy
- Activity test



Biotinalytion ADP-ribosylation

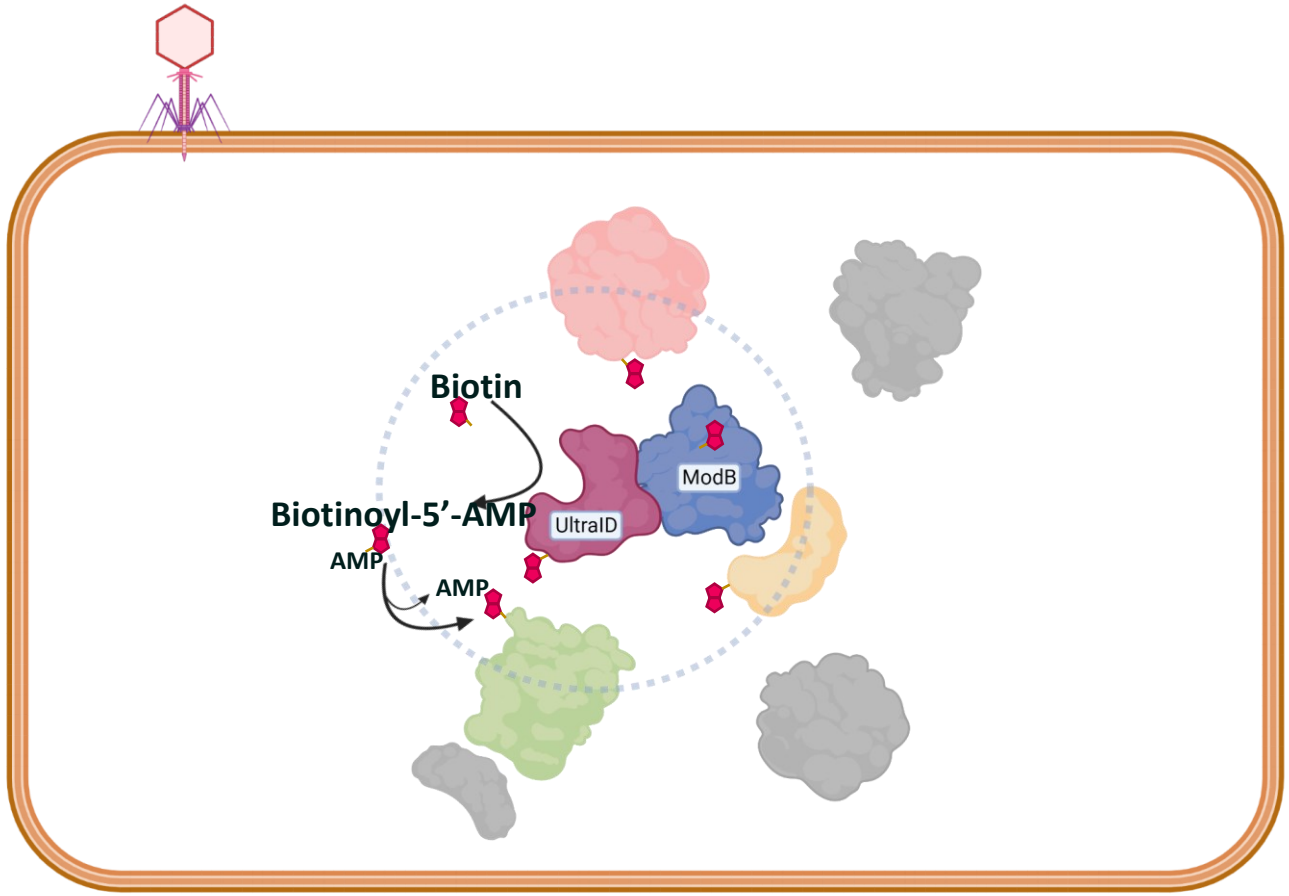


Example

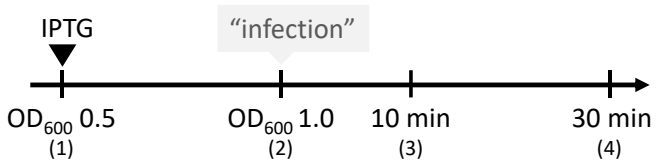
T4 – *E. coli*

ModB interactome

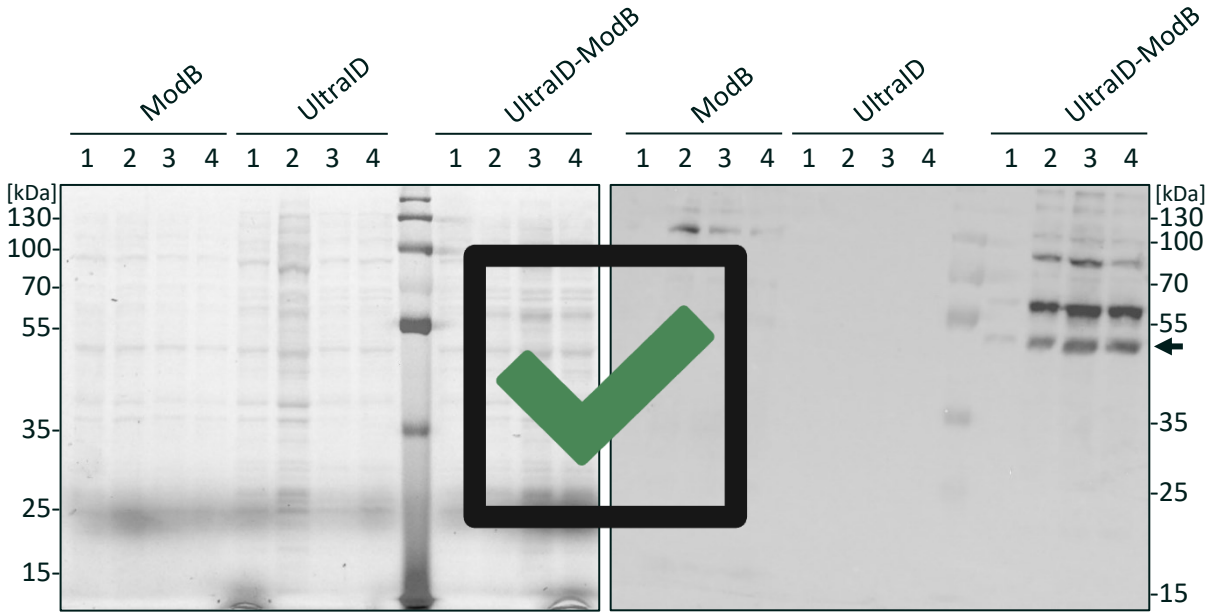
- Cloning strategy
- Activity test
- Infection



What is the ideal timepoint of protein induction for T4 phage infection?



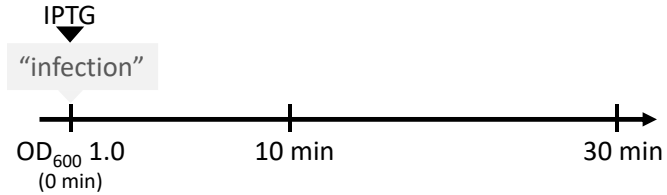
successive



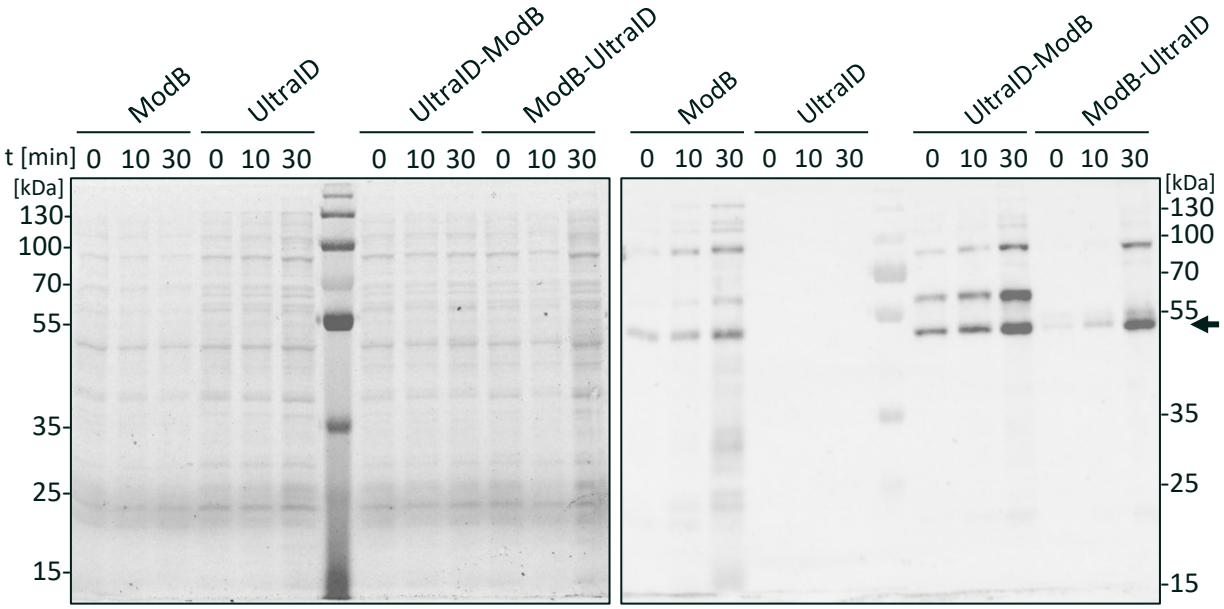
Stain-free SDS-PAGE
Auto exposure

Western Blot

ADP-ribosylation
Chemiluminescence
SignalFire ECL standard
50 s exposure
MABE1016



simultaneous



Stain-free SDS-PAGE
Auto exposure

Western Blot

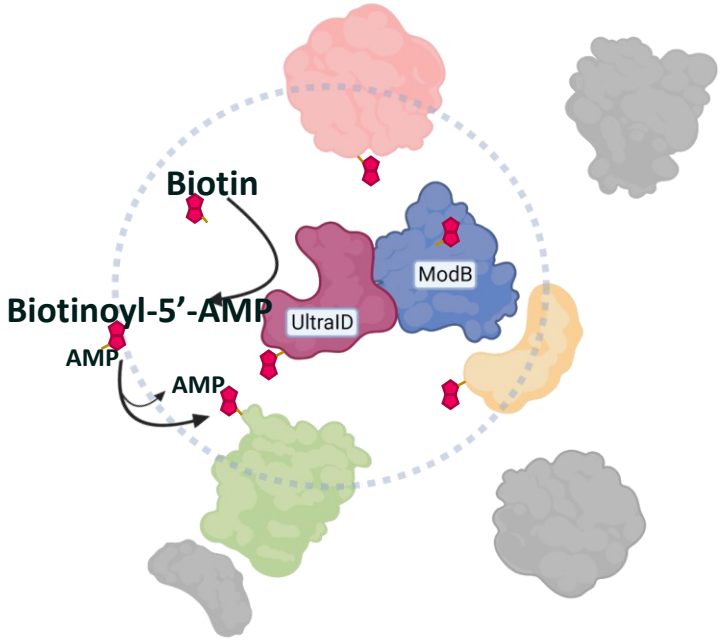
ADP-ribosylation
Chemiluminescence
SignalFire ECL standard
50 s exposure
MABE1016

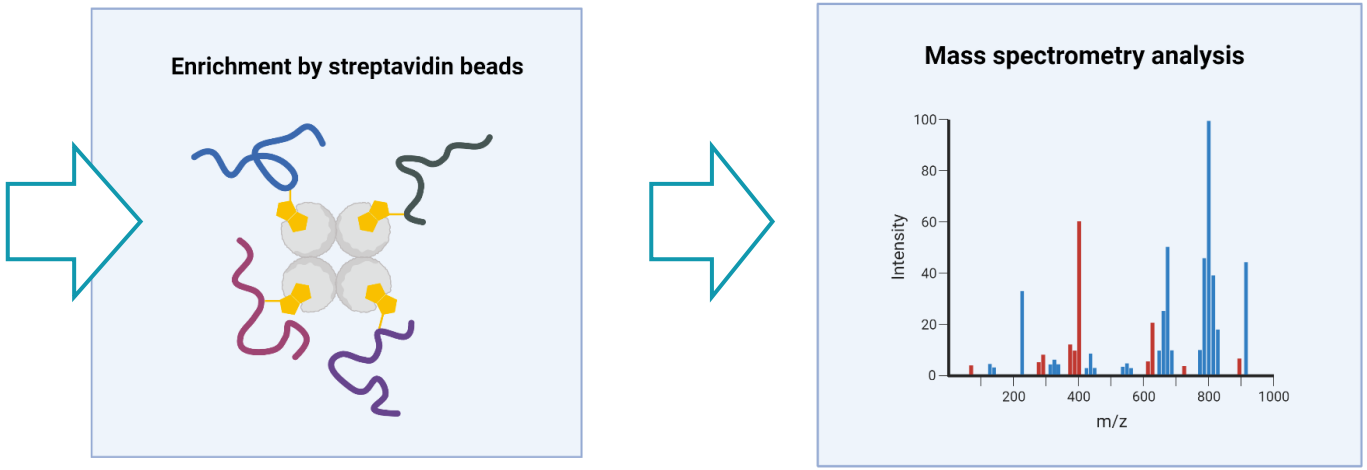
Example

T4 – *E. coli*

ModB interactome

- Cloning strategy
- Activity test
- Infection
- Enrichment and MS



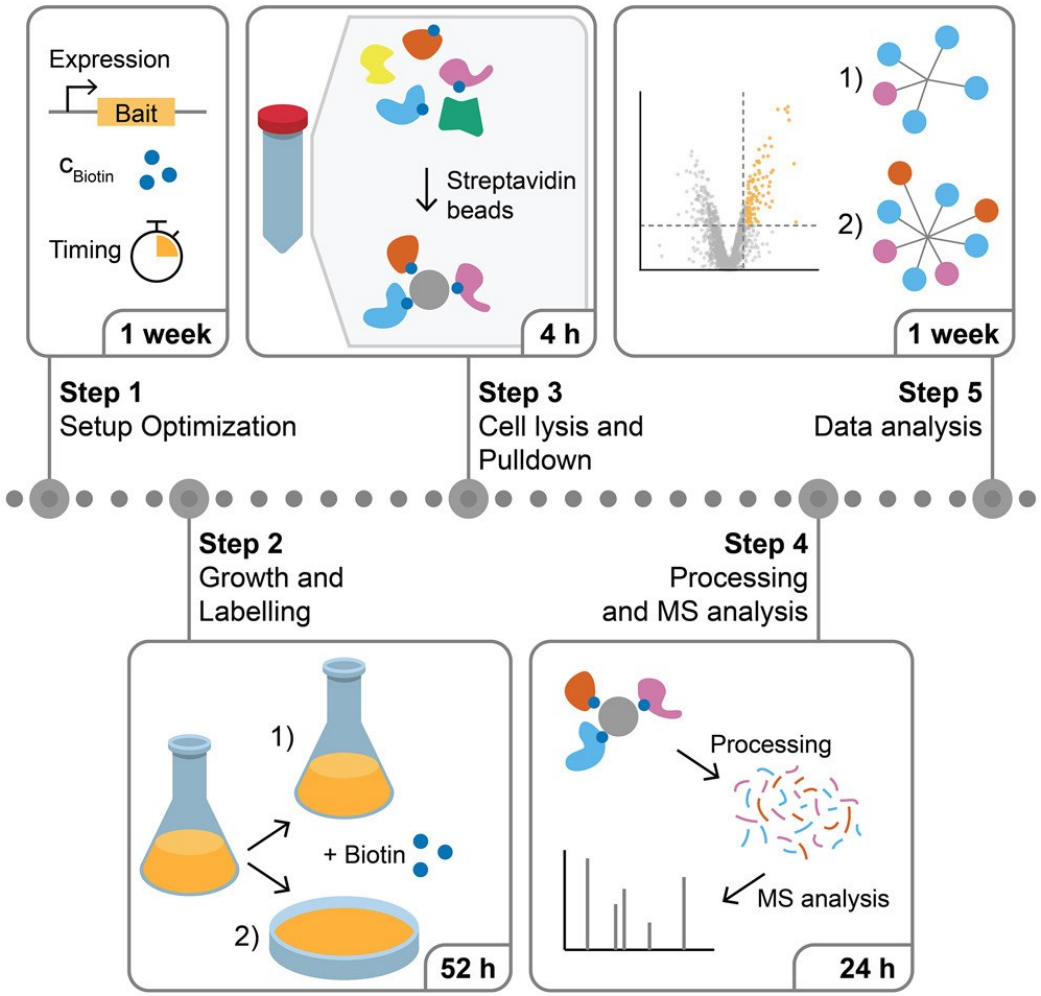


Example

T4 – *E. coli*

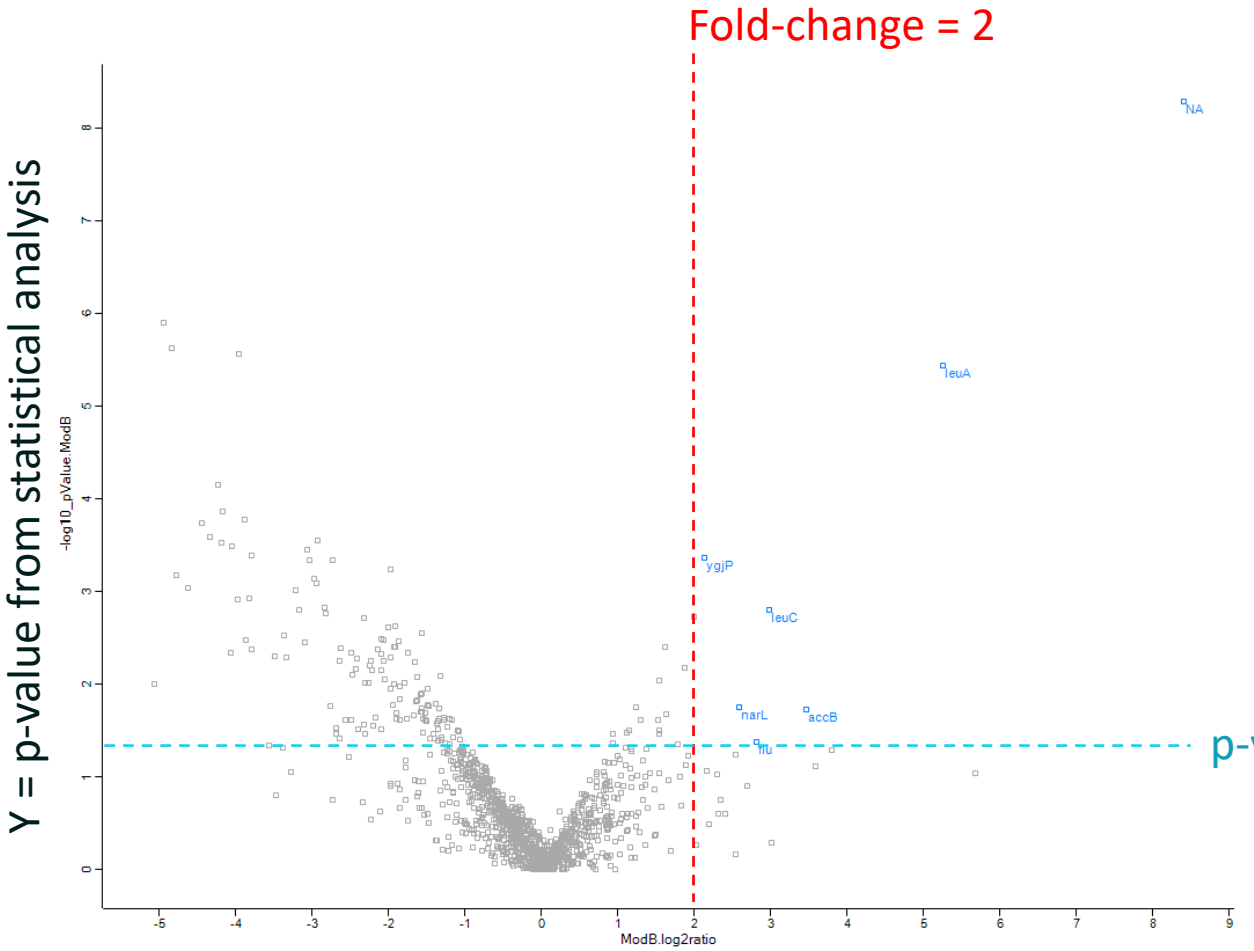
ModB interactome

- Cloning strategy
- Activity test
- Infection
- Enrichment and MS
- Data analysis



Data analysis

Proteomics analysis



Data set: data from 4 biological replicates

Proteins considered:

Fold-change ≥ 2 (set to UltraID = background)

Statistical significance p-value ≤ 0.05

Example

T4 – *E. coli*

ModB interactome

- Cloning strategy
- Activity test
- Infection
- Enrichment and MS
- Data analysis
- Verification of hits

What are the interaction partners of ModB that influence target specificity?

What's next!?

Proximity labelling

* Theoretical background of BioID
* Cloning strategy for BioID constructs
* Explanation of BioID pipeline
* What's next ?!

What's next!?

Proximity labelling

* What's next ?!

- **Online Q&A Session**
- **Sharing of plasmids (from Höfer lab)**
- **Hands on workshop (Sample prep MS)**