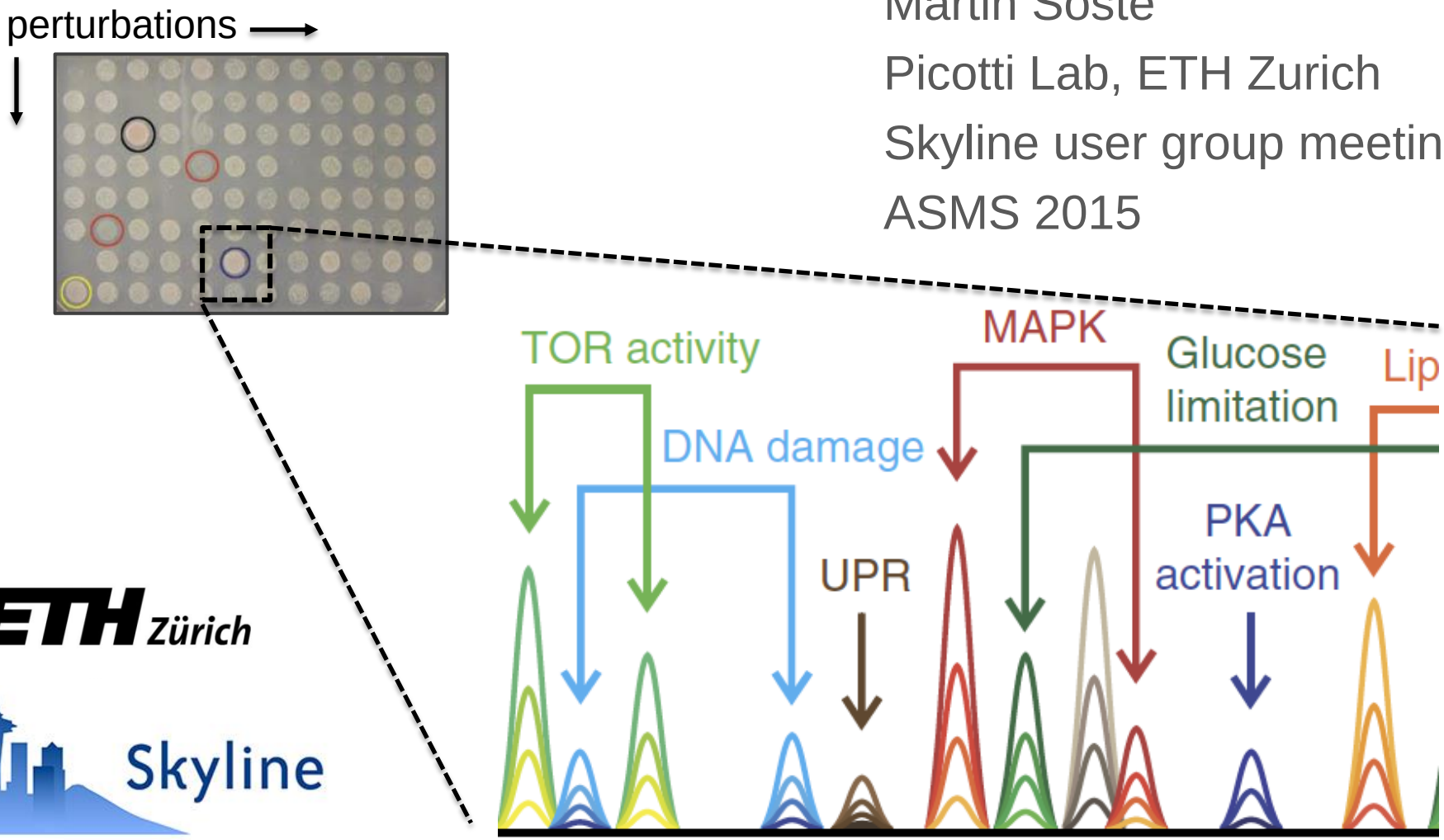
A sentinel protein assay for simultaneously quantifying cellular processes

Martin Soste
Picotti Lab, ETH Zurich
Skyline user group meeting
ASMS 2015

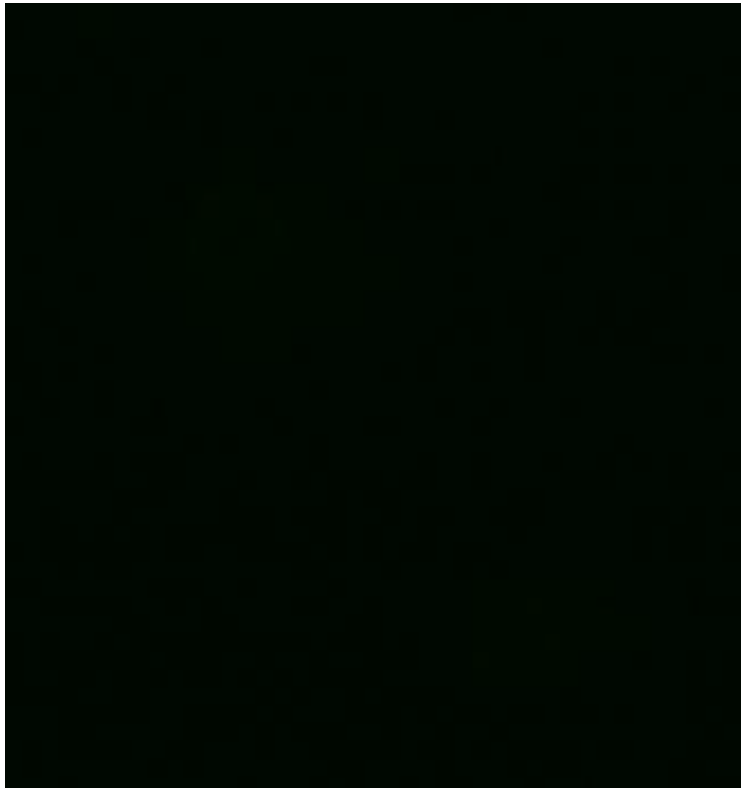


ETH Zürich

Skyline

α-Synuclein induced cytotoxicity

1 copy α-Syn



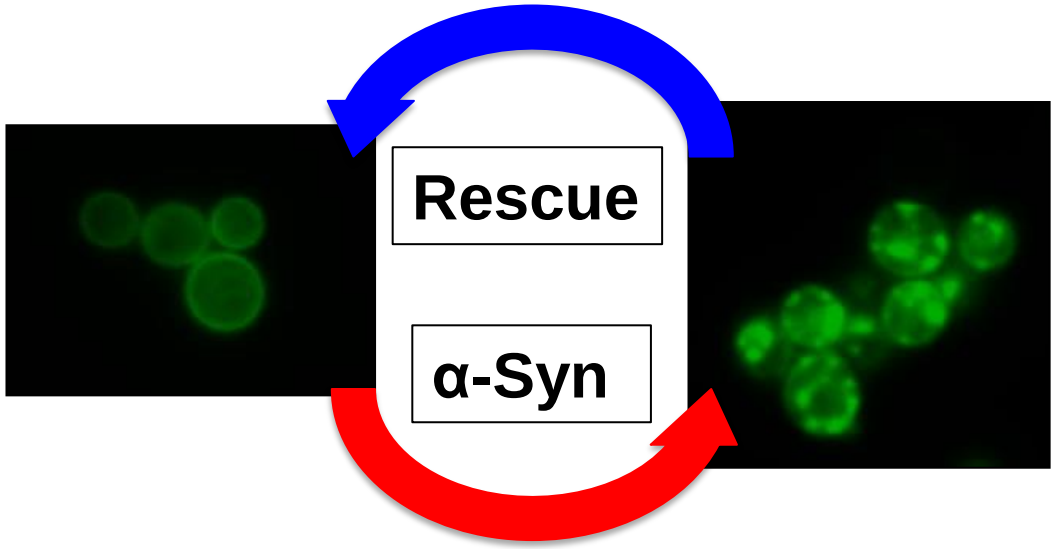
2 copies α-Syn

⇨ Established model for protein aggregation disease

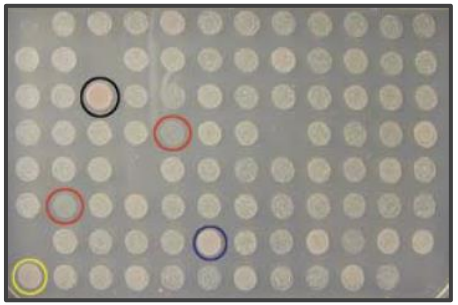
collaboration with Lindquist lab

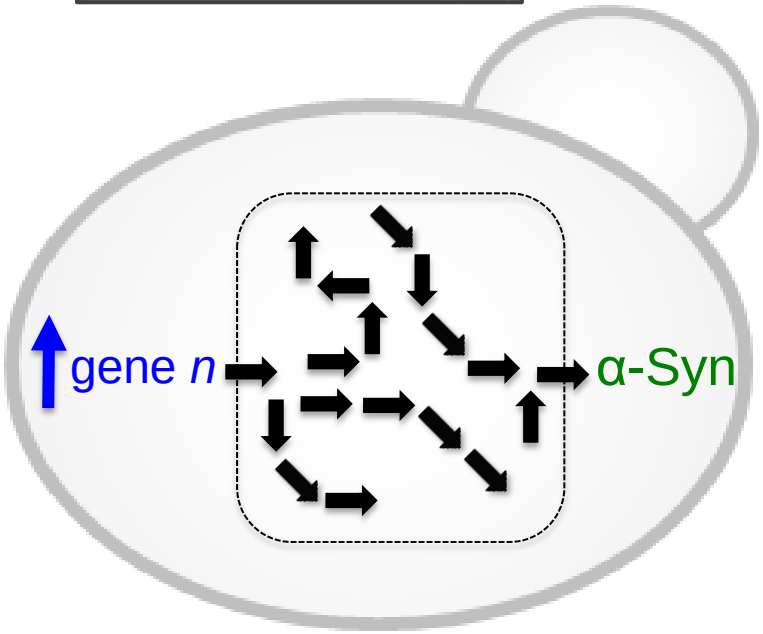
100 protective genes

Genetic modulator (1…100)



α-Syn + gene *n*





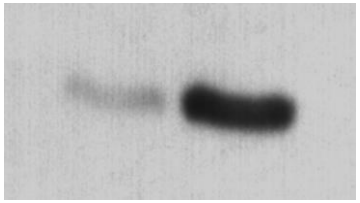
What are the genetic modulators doing to rescue the yeast?

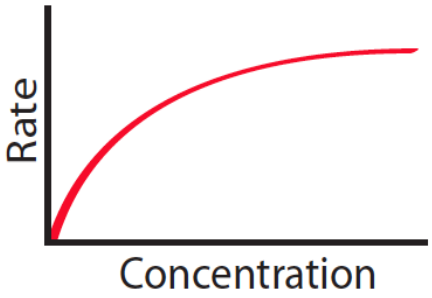
Willingham *et al.* Science. 2003 Cooper *et al.* Science. 2006 Gitler *et al.* Nat Genet. 2009 Yeger-Lotem *et al.* Nat Genet. 2009

How to find activated processes?

effects of 100 genes, triplicates = 300 samples

Cell biology & biochemistry

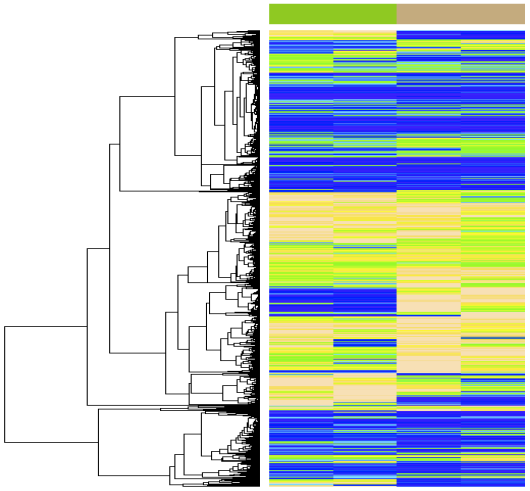




✓ functional activity

x time and labour

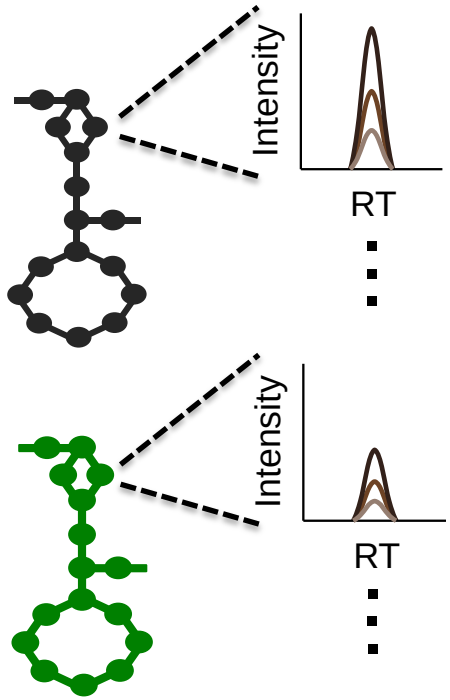
Omics



✓ unbiased

x not necessarily activity

Targeted proteomics



✓ quantitative

x biased

x not necessarily activity

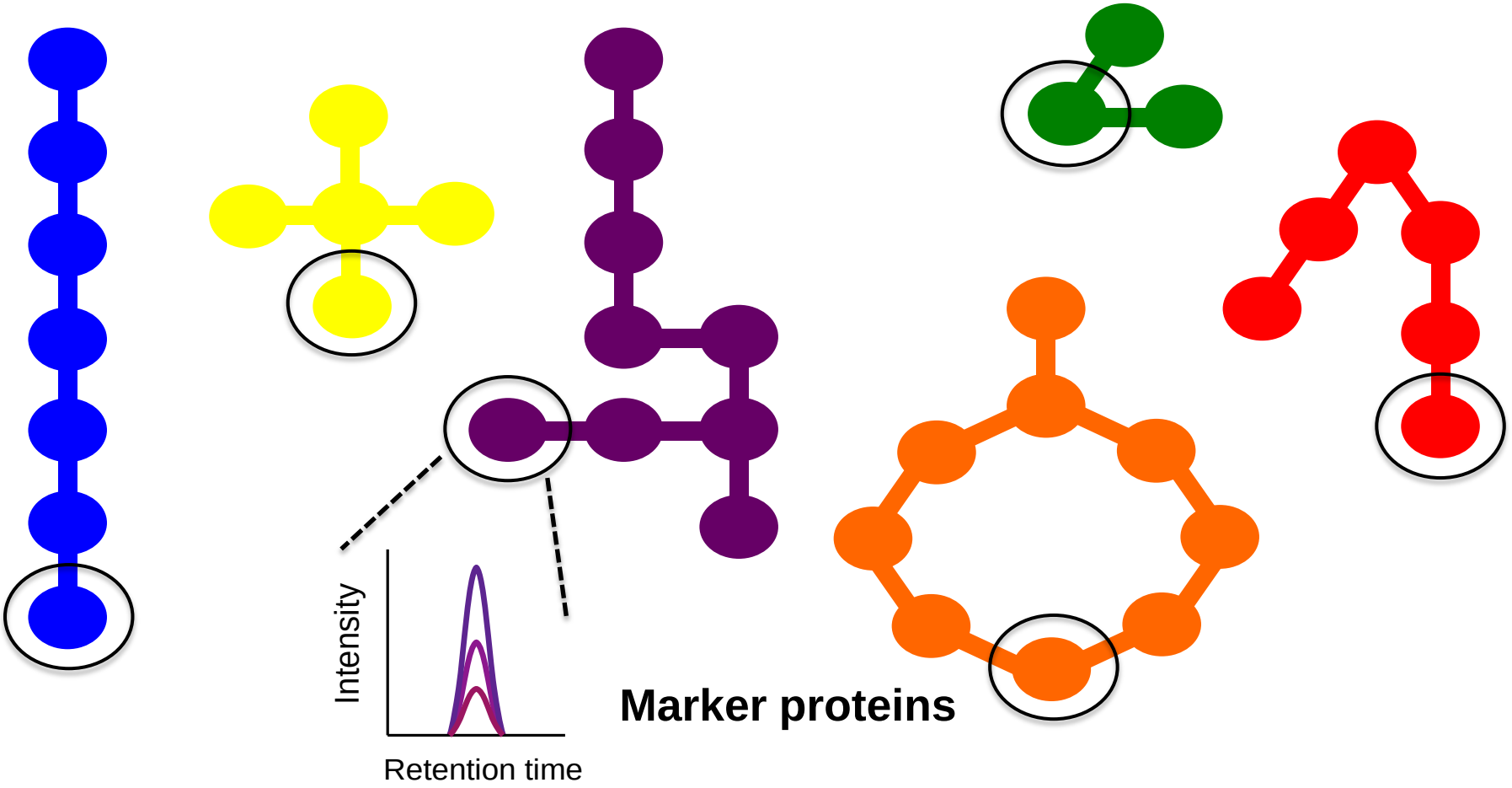
Methodological aim

- unbiased screening for activity with targeted proteomics

✓ unbiased

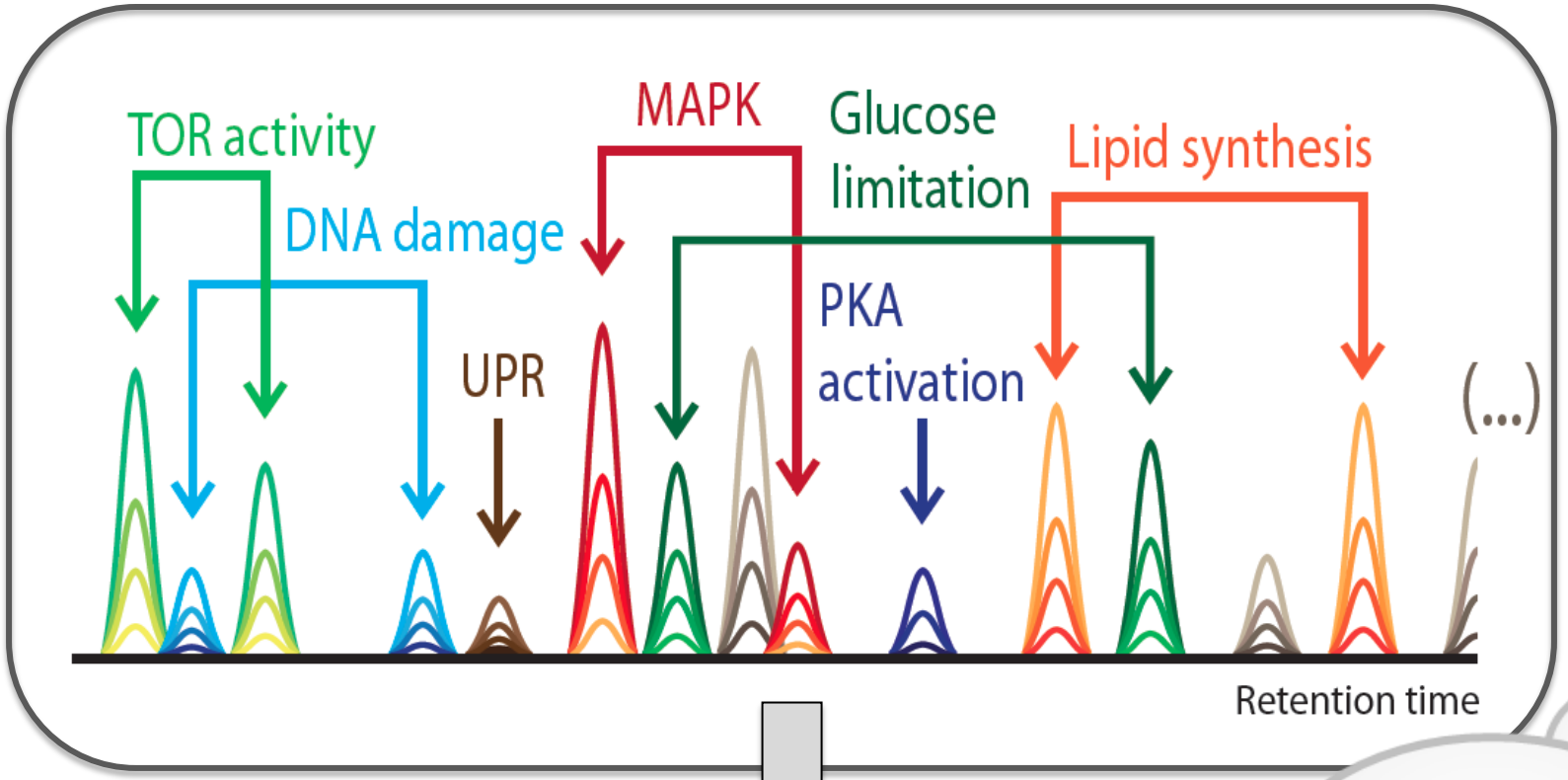
✓ functional activity

✓ quantitative

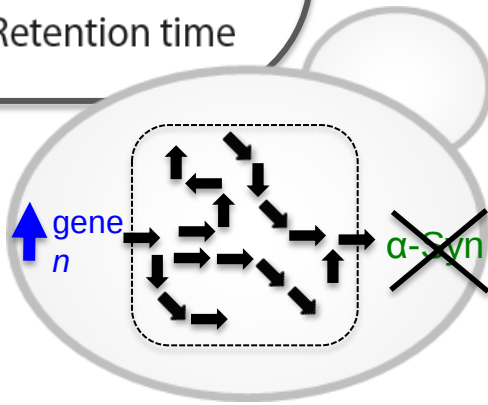


The sentinel protein assay

- A fingerprint for activity



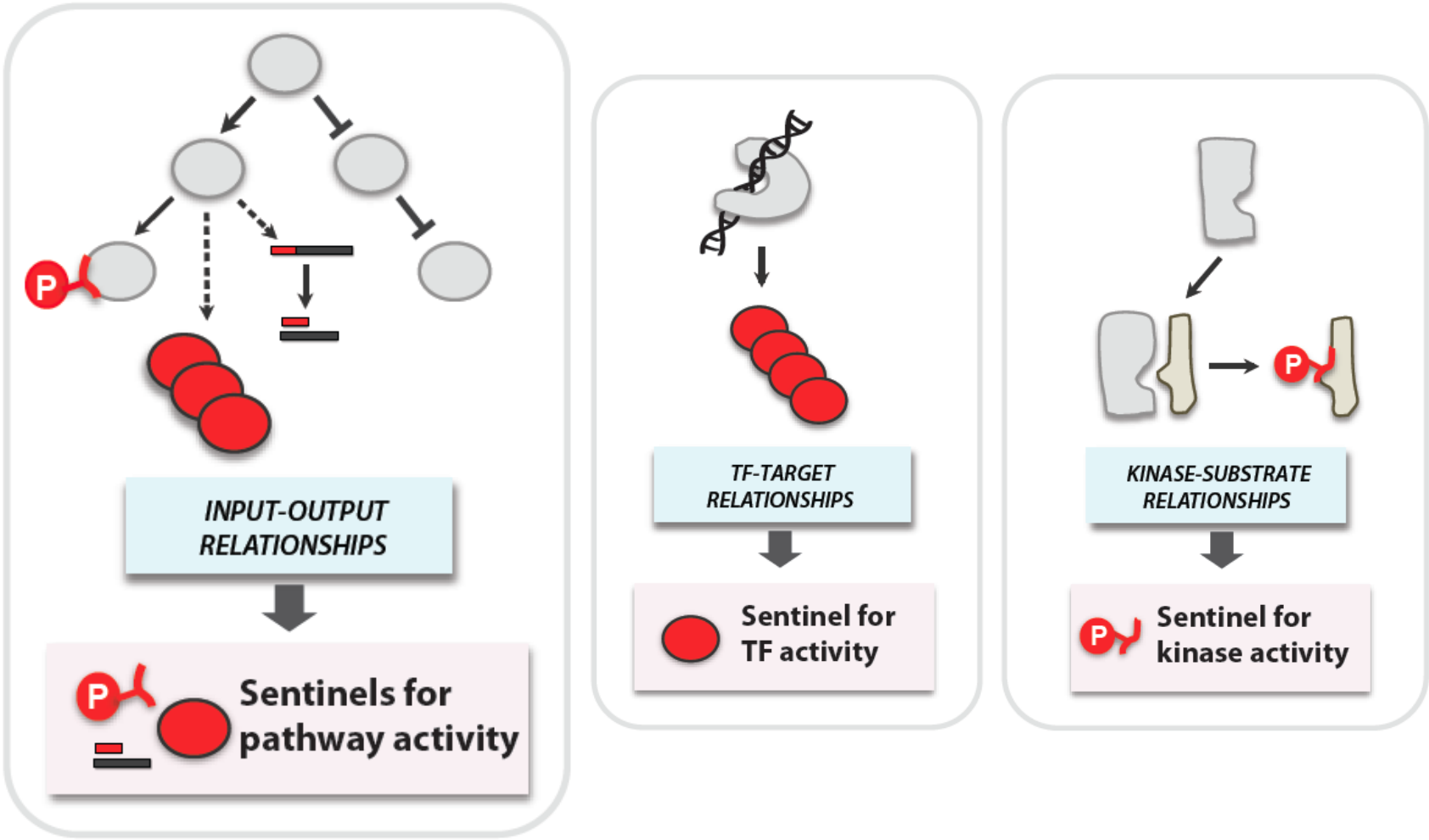
Effectors of protective genes?



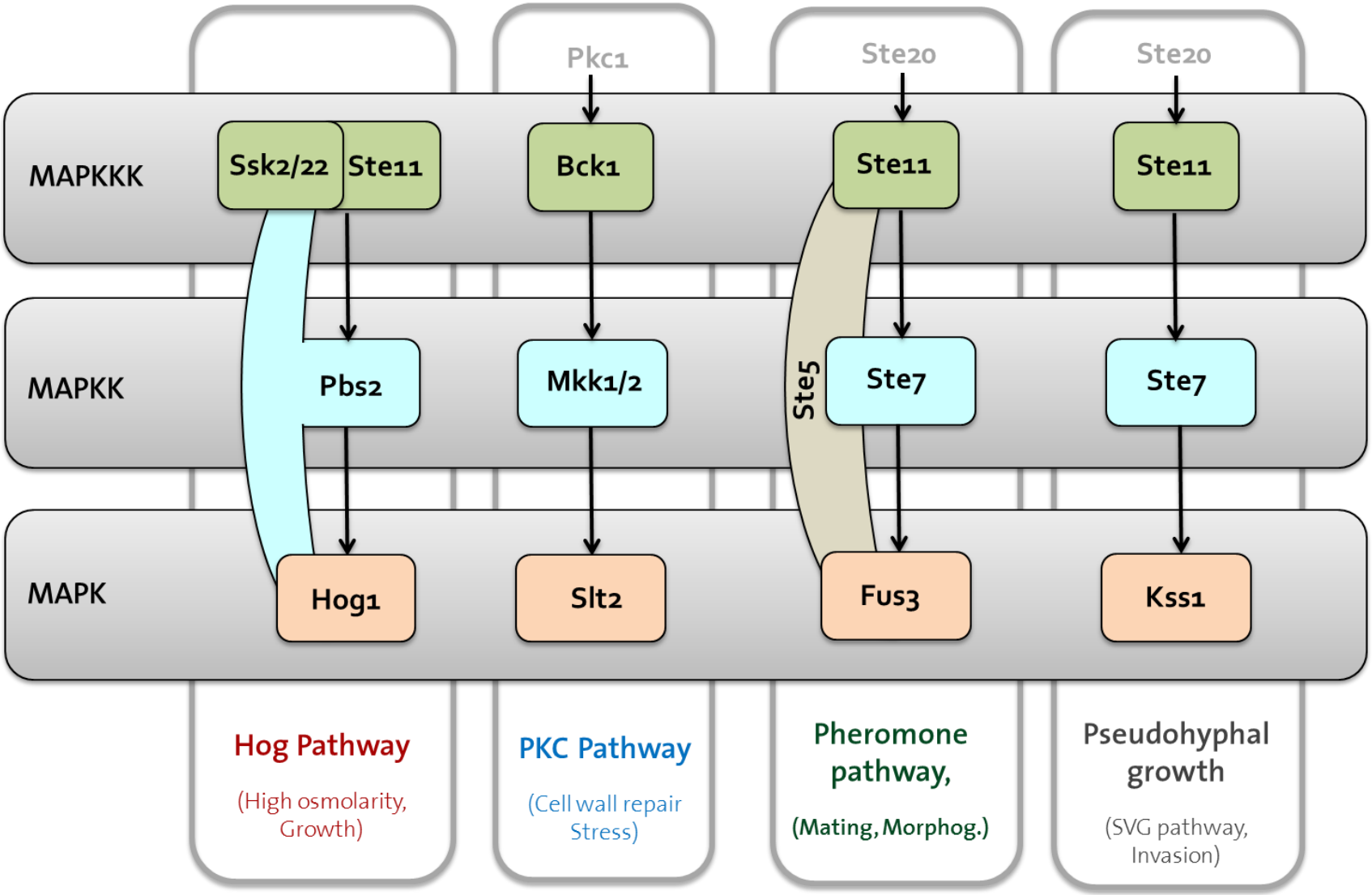
Sentinels:

Markers that report cellular process activation

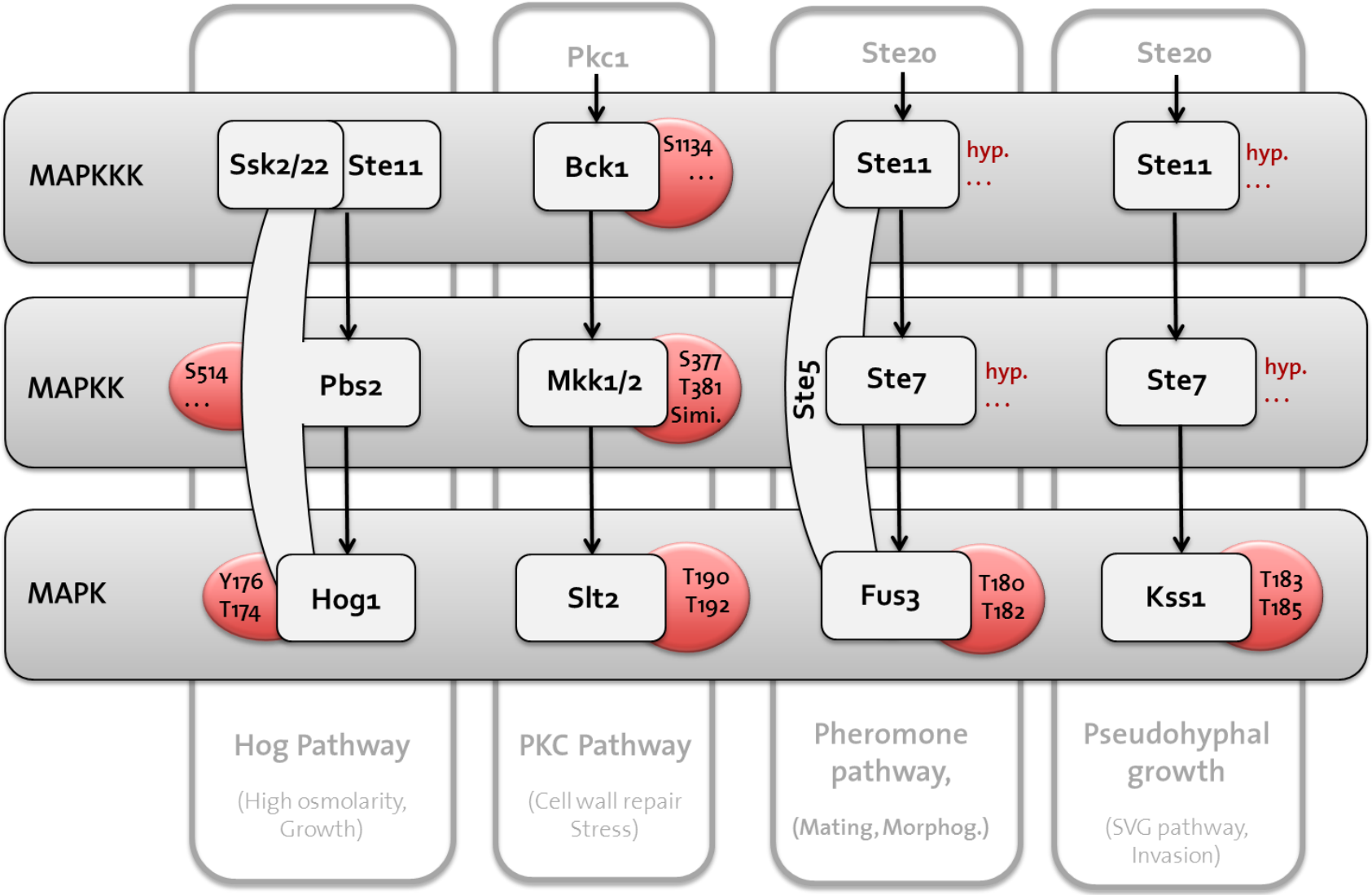
Sentinels are proteins, phosphorylation sites or degradation products



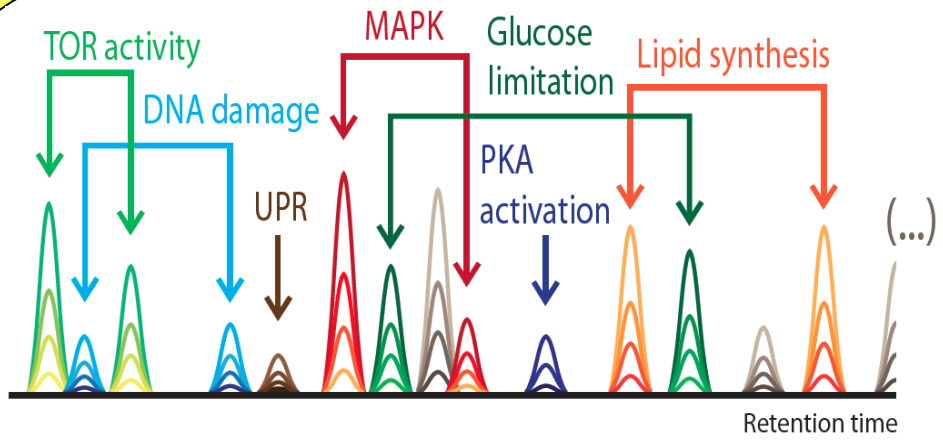
Example: phosphorylation-based sentinels



Example: MAPK activation



Identified functionally validated sentinels



Fingerprint assay:

- 157 proteins

- 152 P-sites

SRM assays

Skyline

- Proteotypic peptides
  PROT – 300, PHOS – 152
- Top 3–6 transitions
  PROT – 1174, PHOS – 807
- iRT-based scheduling

BIOGNOSYS
PROTEOMICS – NEXT GENERATION

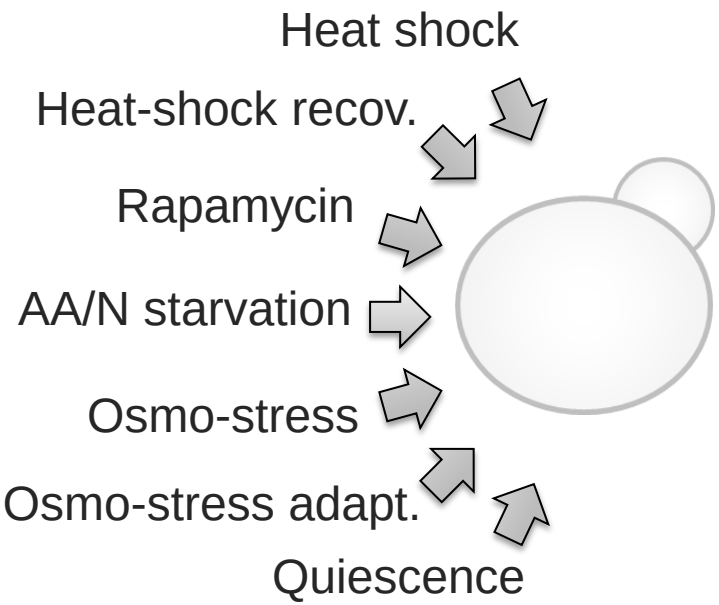
- Synthetic heavy peptide confirmation

SENTINEL			
ATG1			
ATG13			
IRE1			
HOG1			
FUS3			
SLT2			
CKI1			
MAF1			
SCH9			
YPK1			
IGO1			
MIG1			
SUI2			
DUN1			
SLX4			
TLG1			
TLG1			
SIP1			
CTK1			
ADR1			
SEC63			
CDC23			
CDC24			
CDH1			
CDH1	P-site	CDC28 - KINASE ACTIVITY	Phosphorylated by CDC28, in S, G2 and M phase
Bcy1	P-site	YAK1 - KINASE ACTIVITY	Phosphorylated by YAK1 in response to glucose star
YPK1	P-site	PKH1 - KINASE ACTIVITY	Phosphorylated by Pkh1
NPR1	P-site	NITROGEN METABOLISM	Dephosphorylated upon nitrogen limitation
RCK2	P-site	HOG1 - KINASE ACTIVITY	Phosphorylated by HOG1 after osmotic stress.
(.....)		(.....)	(.....)

188 processes

Testing the sentinel protein assay

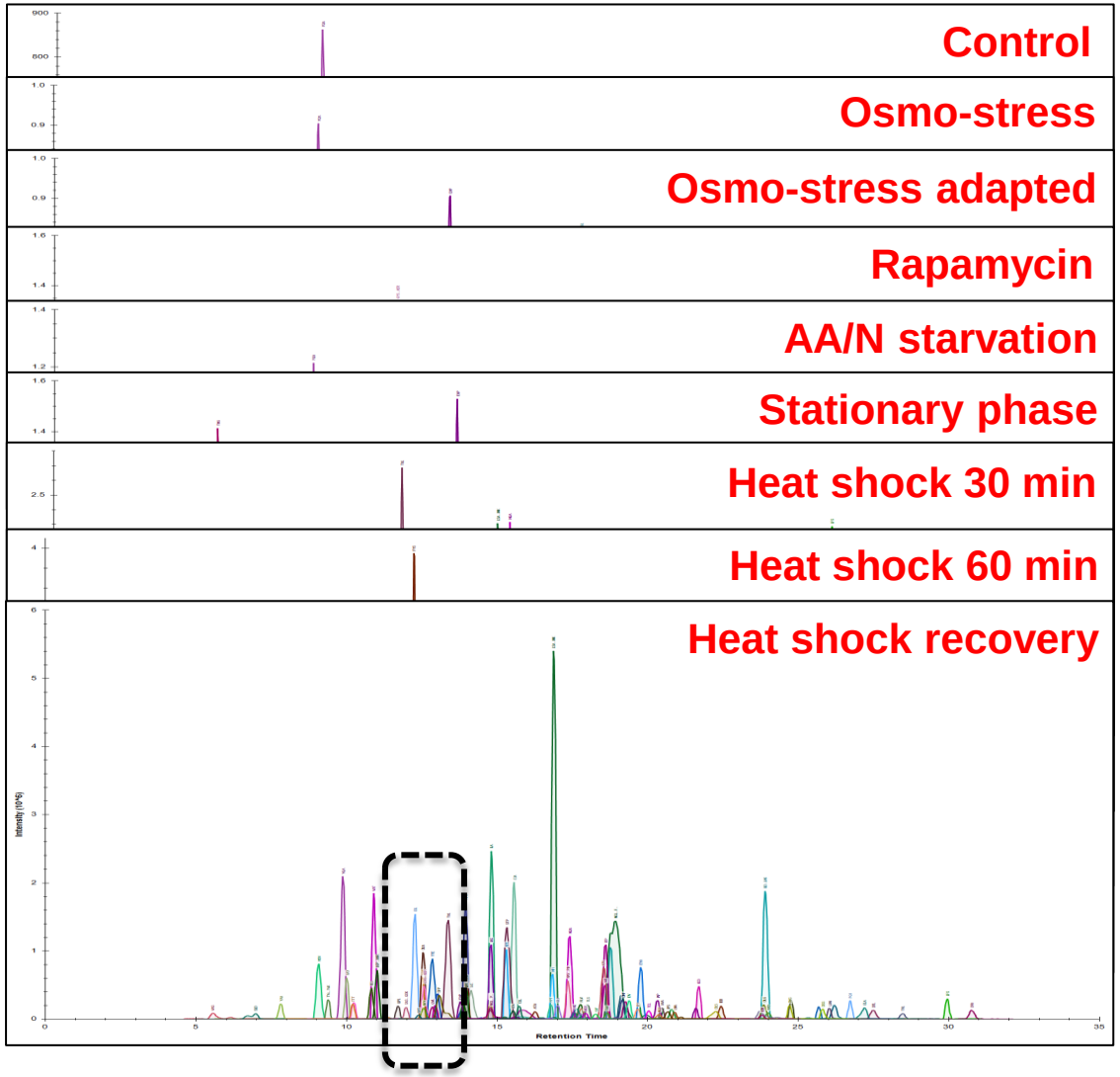
Well-characterized perturbations



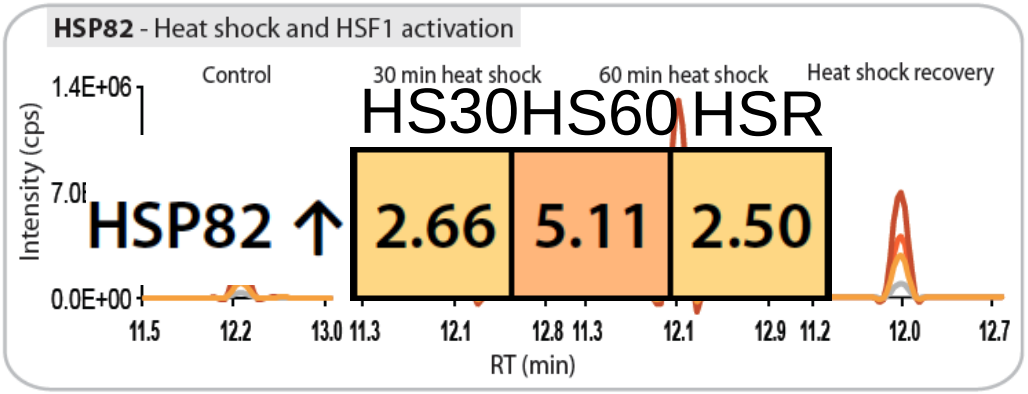
Skyline

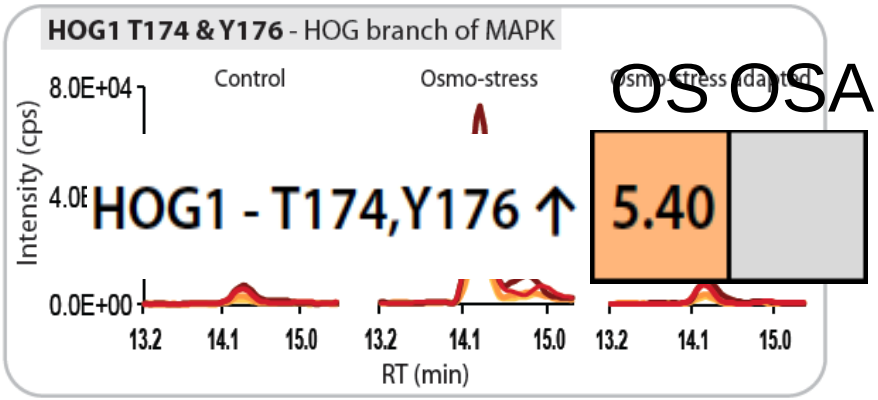
Multi-peptide chromatograms

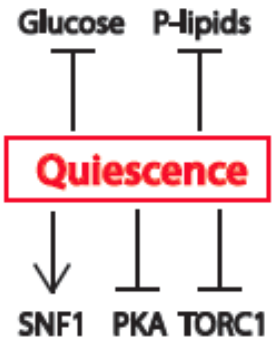
"Sentinel Fingerprints"

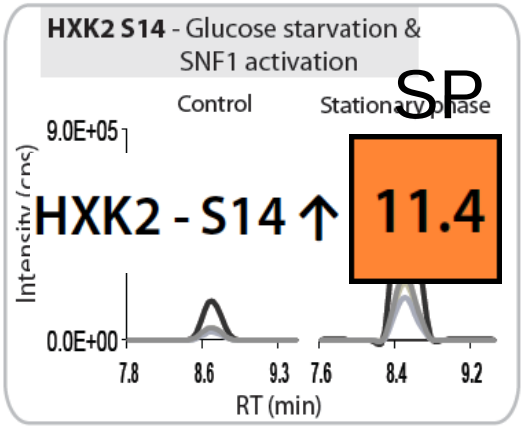


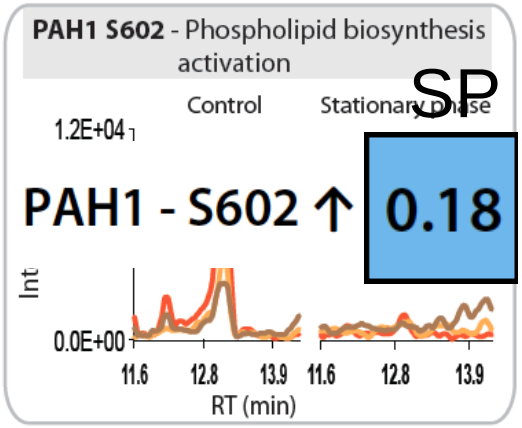
Validation from expected responses

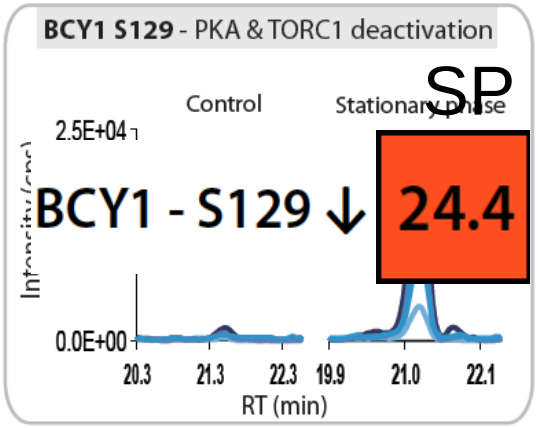




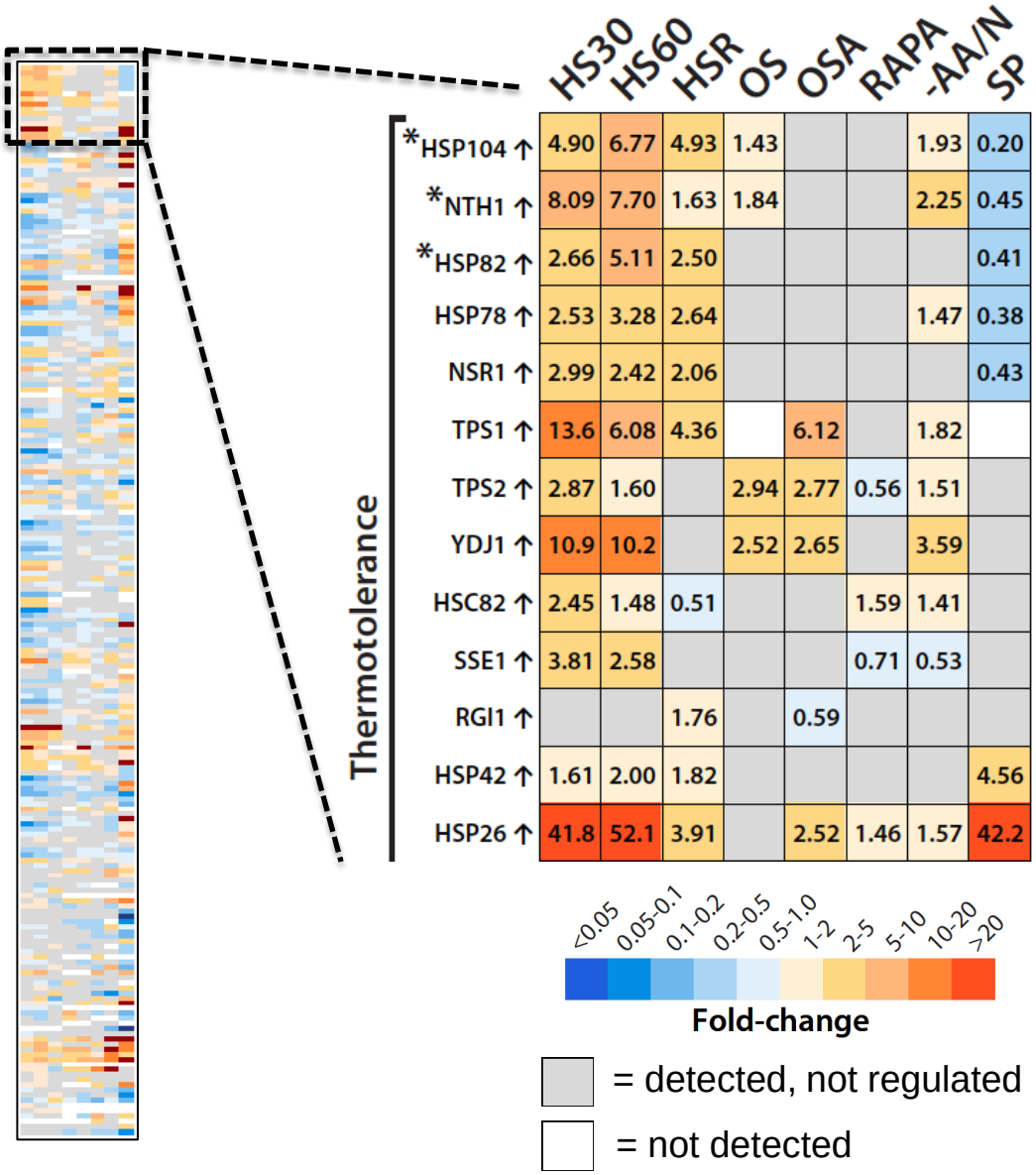








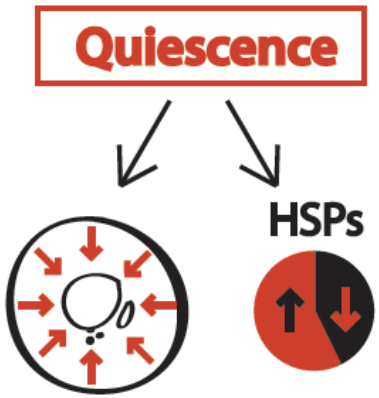
Simultaneous and large-scale coverage



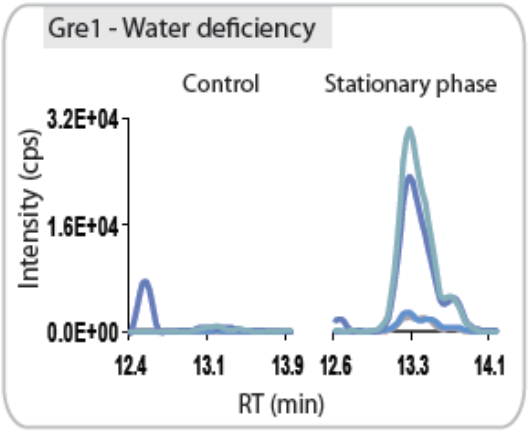
- Snapshots of yeast physiology
- Report of 202 markers for activity
- Success rate = 74%

Soste *et al.* Nat Methods. 2014

Novel responses in stationary phase

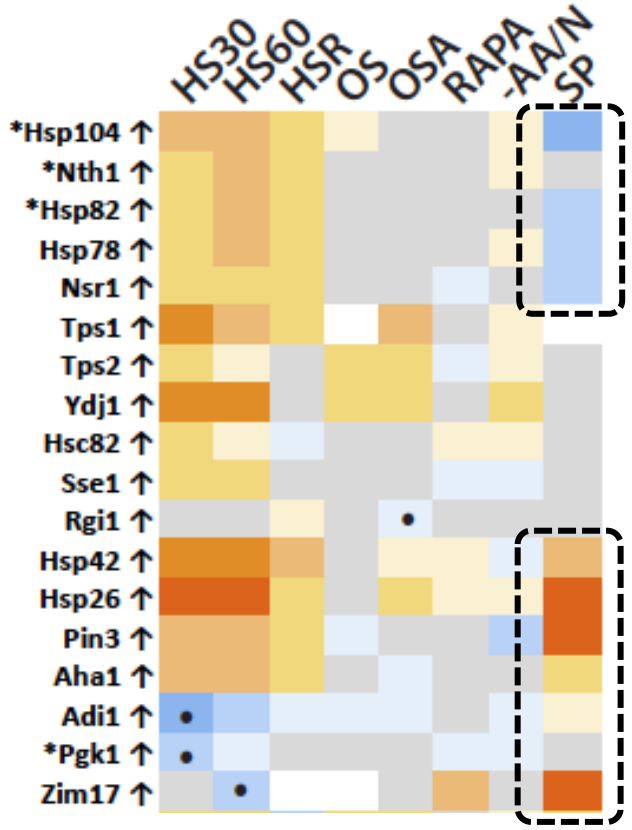


Concentration of cytosol



- Water deficiency marker up

Coordinated response of HSPs

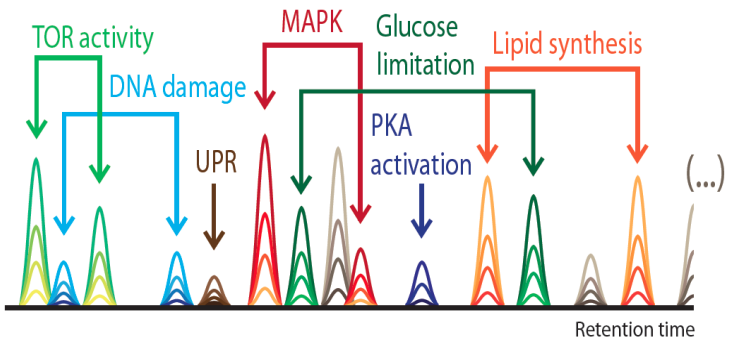


- Subset of heat-shock proteins up
- Typical markers of heat-shock down

Summary: sentinel assay

Advantages:

- Captures behaviour of many pathways
- Probes activation, not merely abundance
- Targeted yet unbiased (“discovery SRM”)
- Fast and interpretable readout of cell status



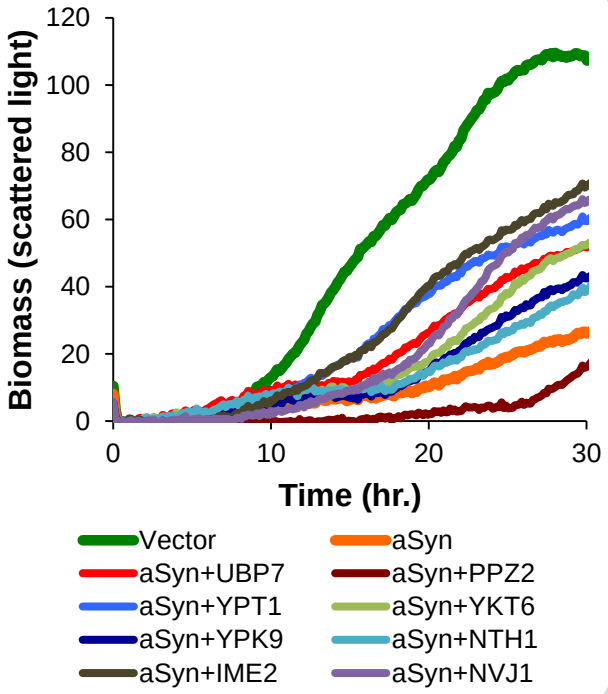
⇨ Apply to any perturbation of yeast
(e.g. large set of genetic manipulations or drugs)

Limits:

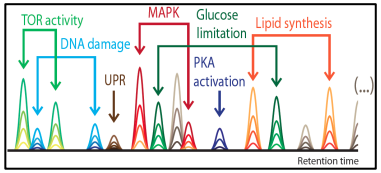
- Cannot identify a perturbed pathway if a marker is not included
- Influence of pathway crosstalk

Outlook: fingerprints of rescue from α-Syn

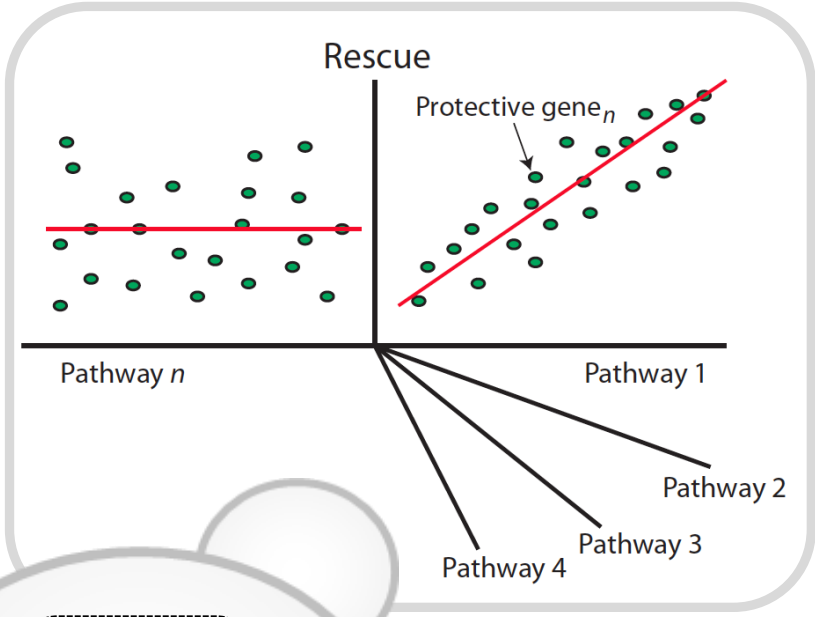
Protective genes (~100)

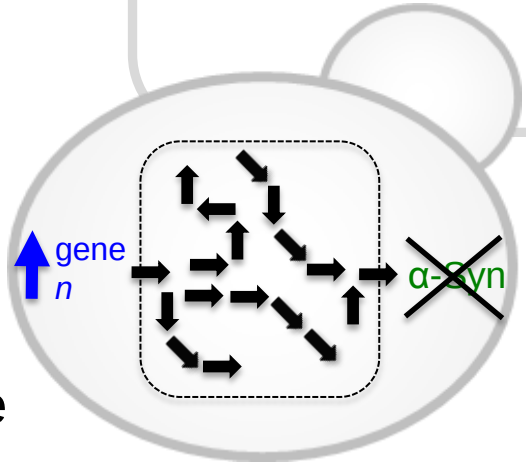


Sentinels



Multivariate regression





- Identify key processes in rescue
- Modulate key processes in higher organism models of α-Syn toxicity

Acknowledgements

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Picture on title slide
from Cooper *et al.* 2006

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