Combining isothermal titration calorimetry, fluorescence anisotropy and kinetic approaches to decipher the molecular mechanisms of the redox activation of the Yap1 transcription factor in *S. cerevisiae*

UMR 7365 IMOPA
Structural and Molecular Enzymology
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Dr M. Toledano

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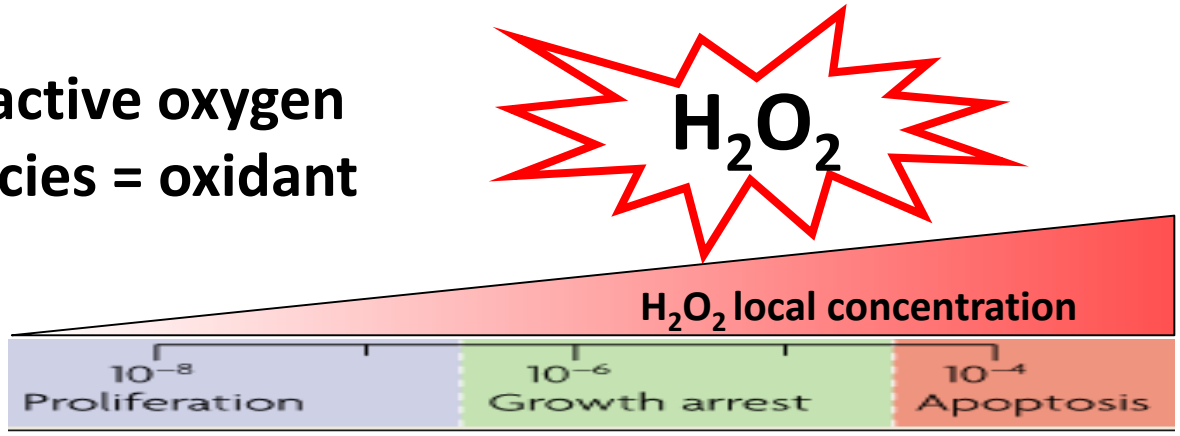
H_2O_2 as a signaling molecule

Reactive oxygen species = oxidant



Cellular messenger

Toxic

Normal physiological conditions

Enzymes/transcription factors activation

Oxidative stress

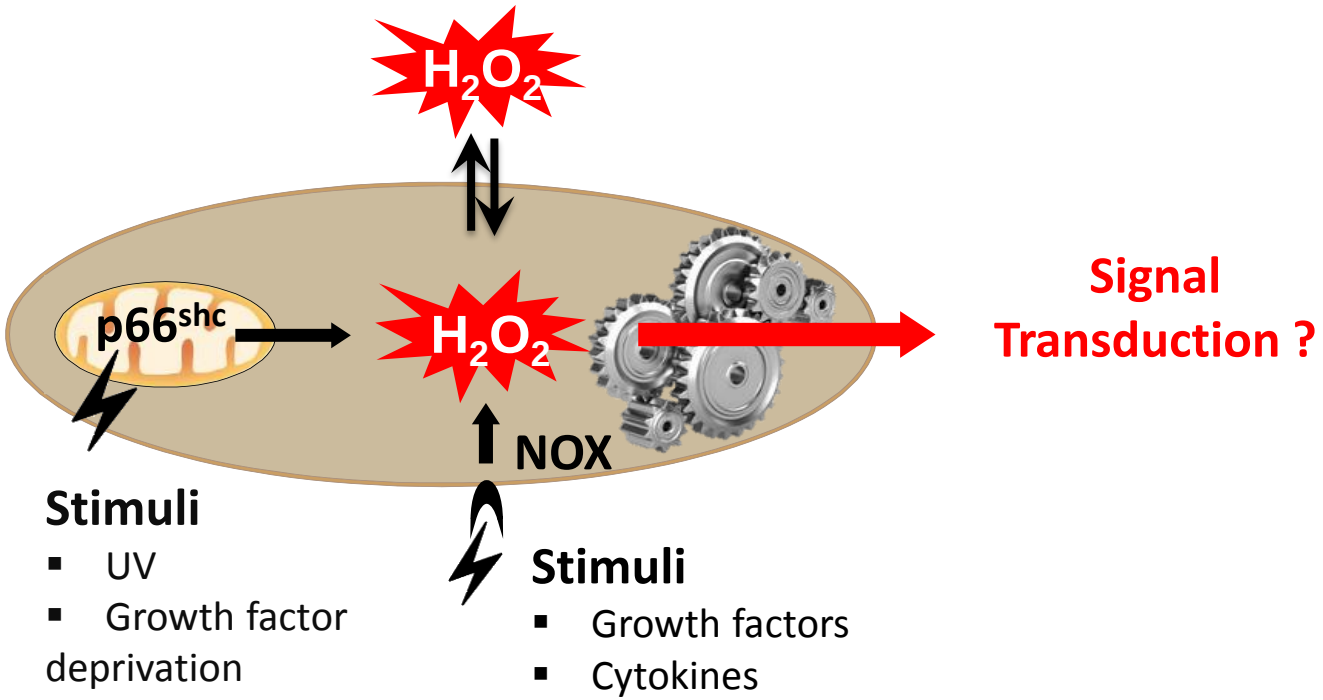
Stress proteins
Chaperones

Alteration of biomolecular function

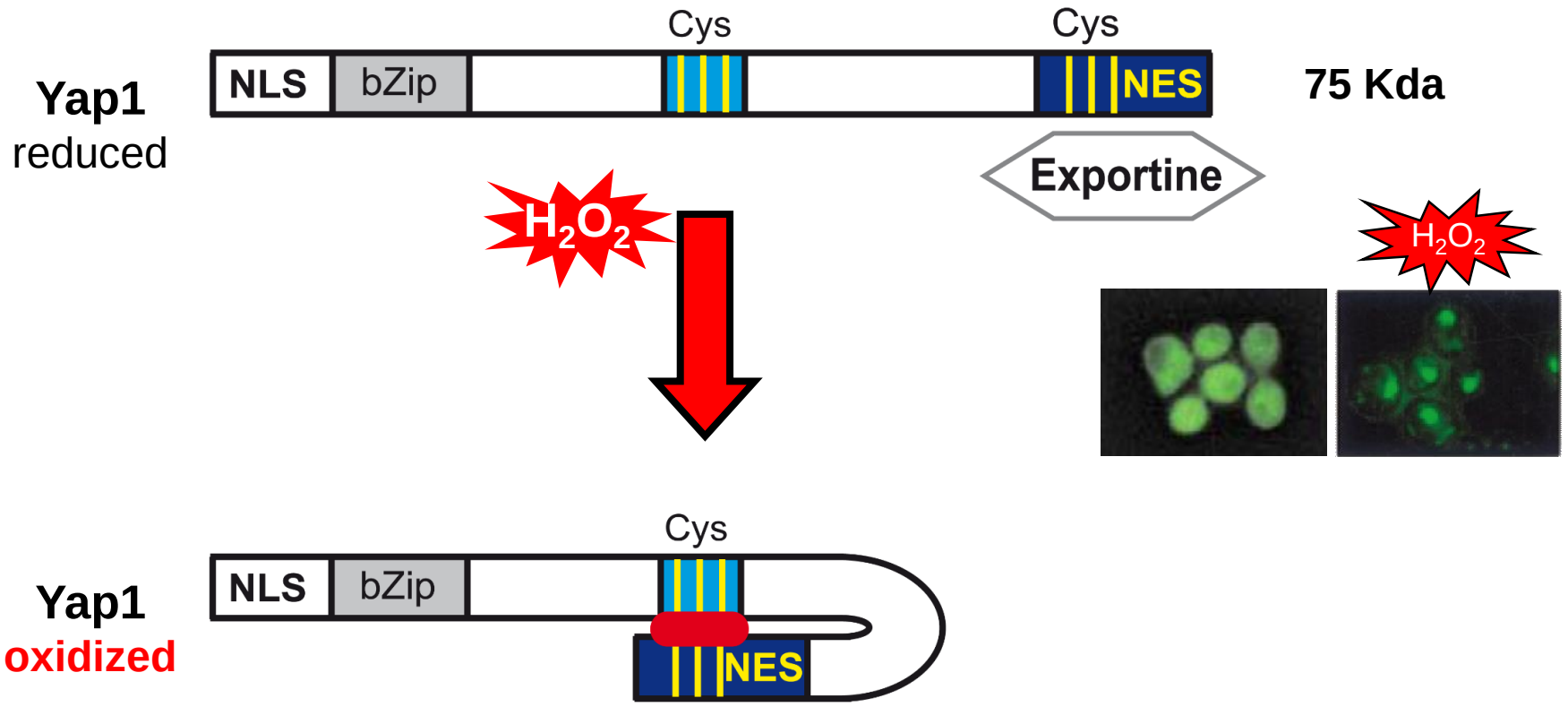
H_2O_2 as a signaling molecule: issues

- **Concentration tightly regulated by cellular antioxidant systems**
  ➔ low *in vivo* concentrations
- **Specificity**

Molecular mechanisms that generate specificity in the transmission of oxidative stress signals or redox signals ?



Yap1: redox activation of transcription factor by thiol peroxidase



Delaunay et al., EMBO J 2000, Delaunay et al. Cell 2002
Veal et al. JBC 2003

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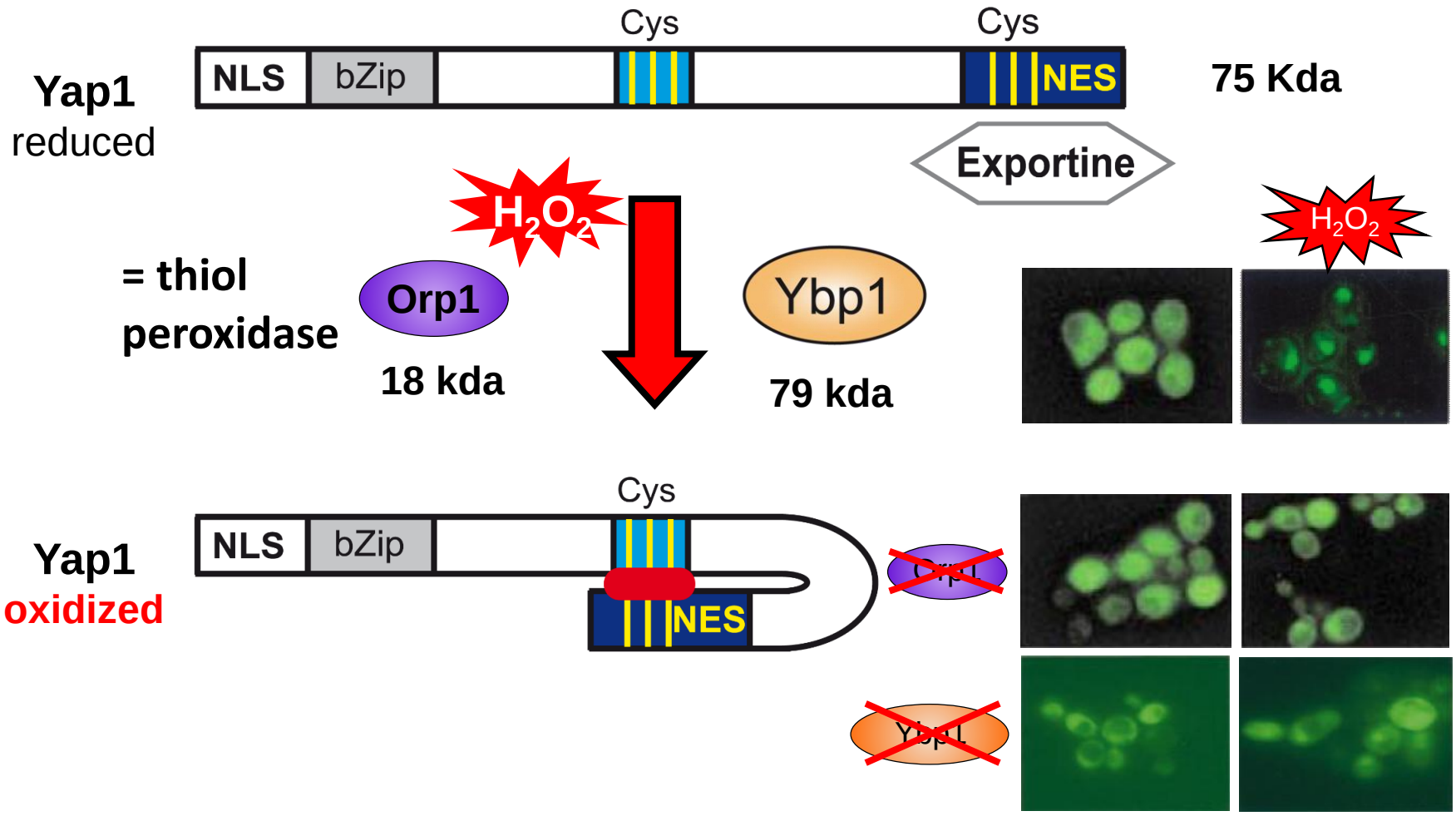
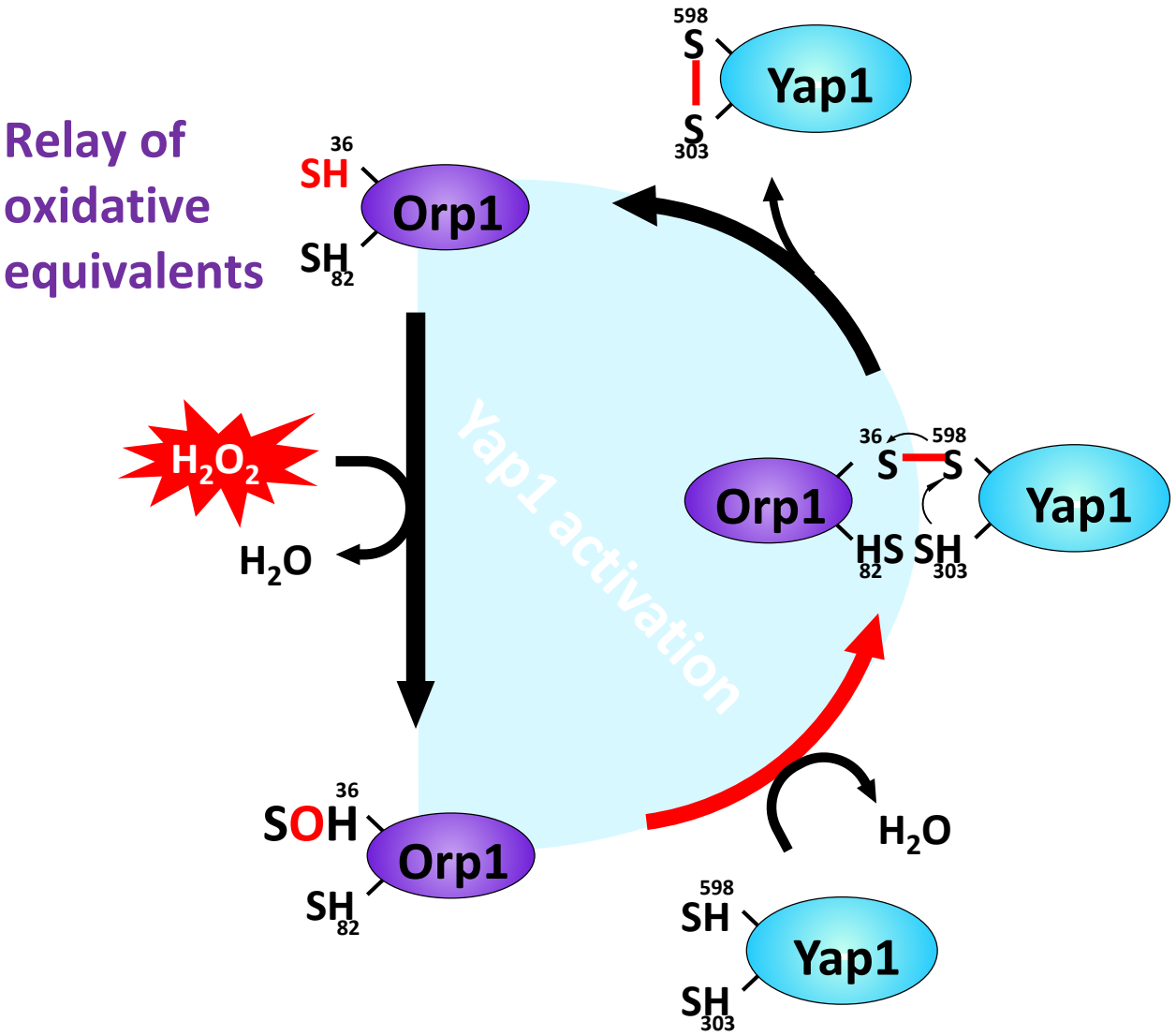


Delaunay et al., EMBO J 2000, Delaunay et al. Cell 2002
Veal et al. JBC 2003

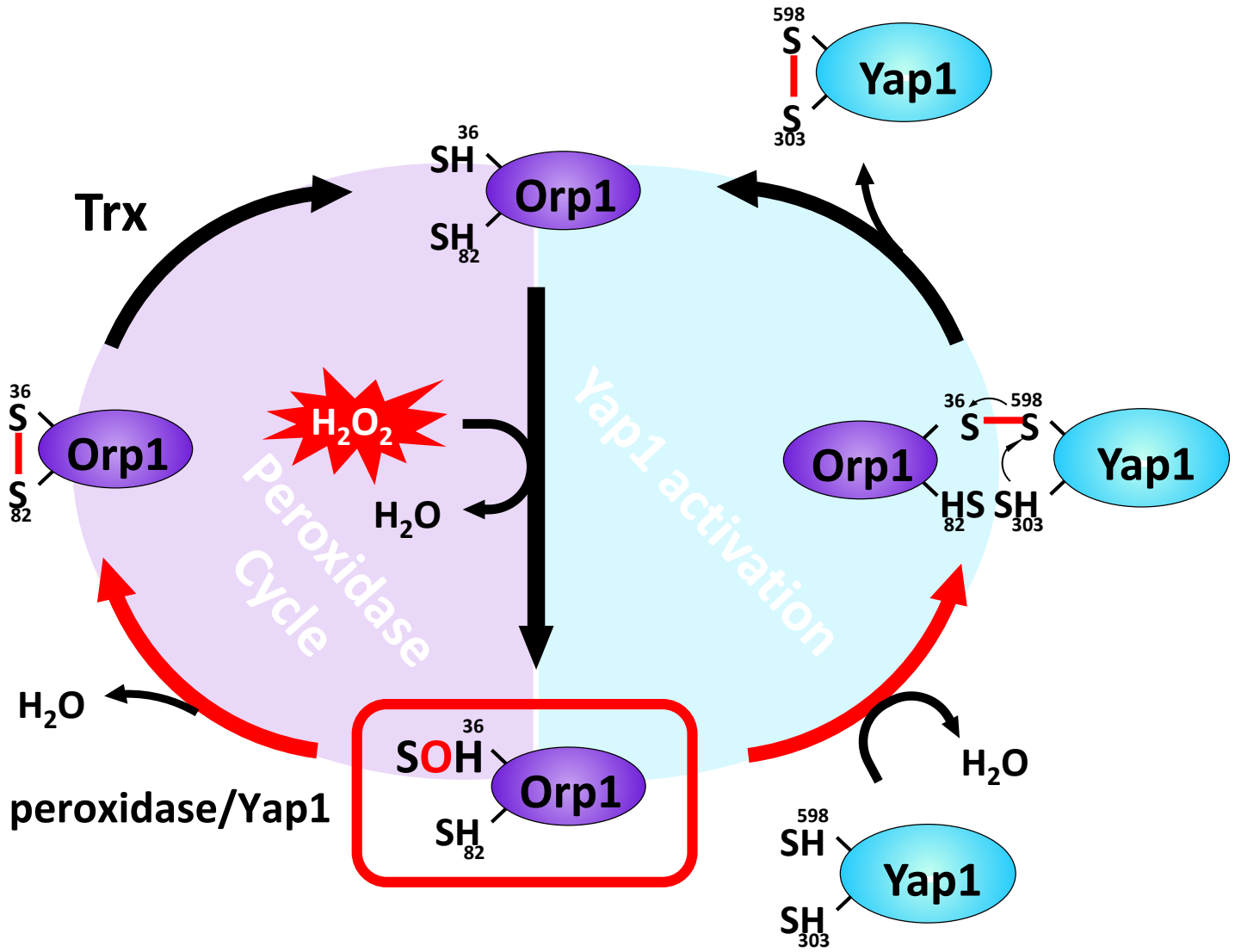
Yap1: the redox relay
1st step of activation pathway

Oxidative activation of Yap1 is sequential



Based on D'Autréaux et Toledano, Nature Reviews, 2007

Yap1: Specificity?



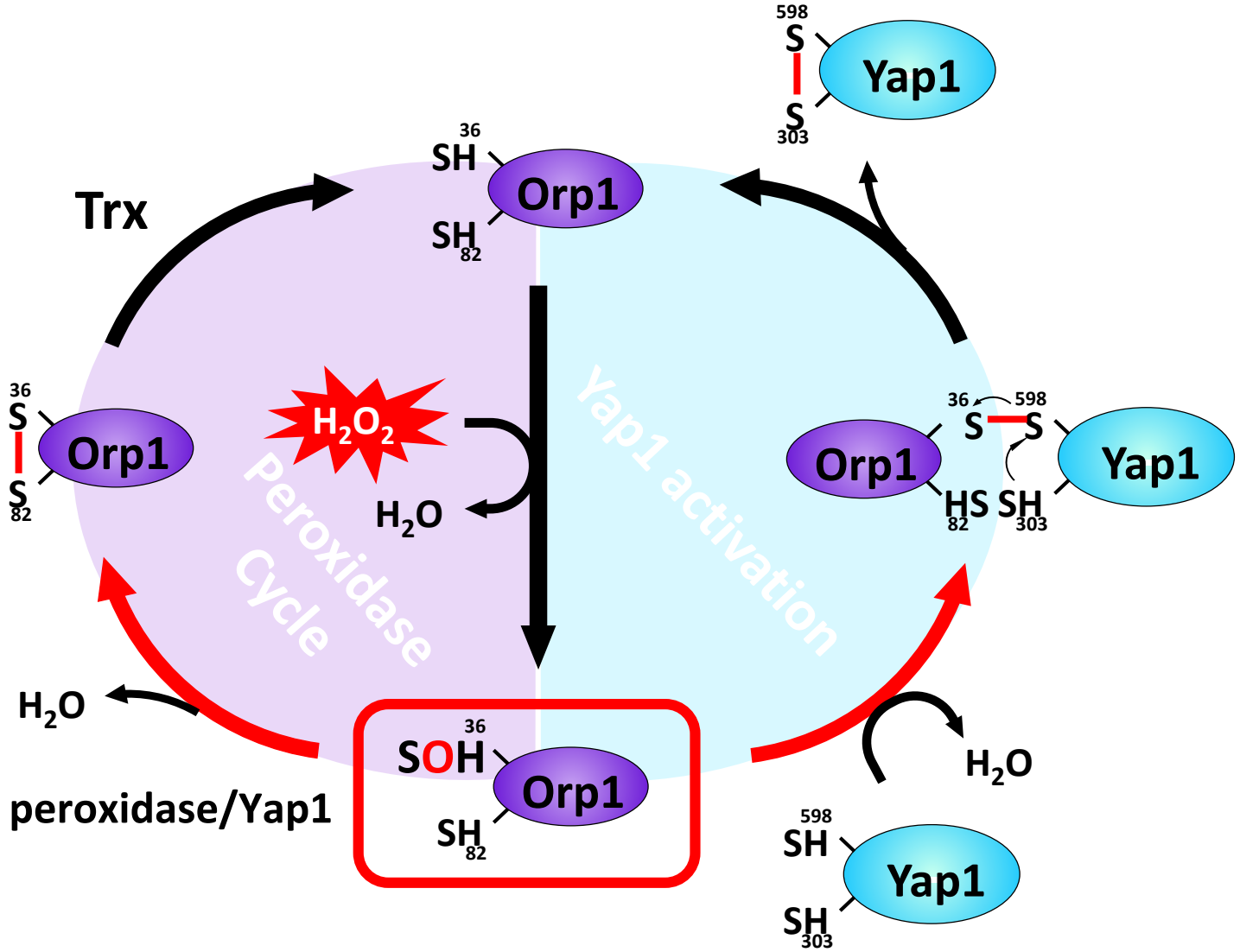
Competition peroxidase/Yap1

Based on D'Autréaux et Toledano, Nature Reviews, 2007

Yap1: Specificity?

Direct recognition Orp1/Yap1?

Functional coupling between Orp1 and Yap1?



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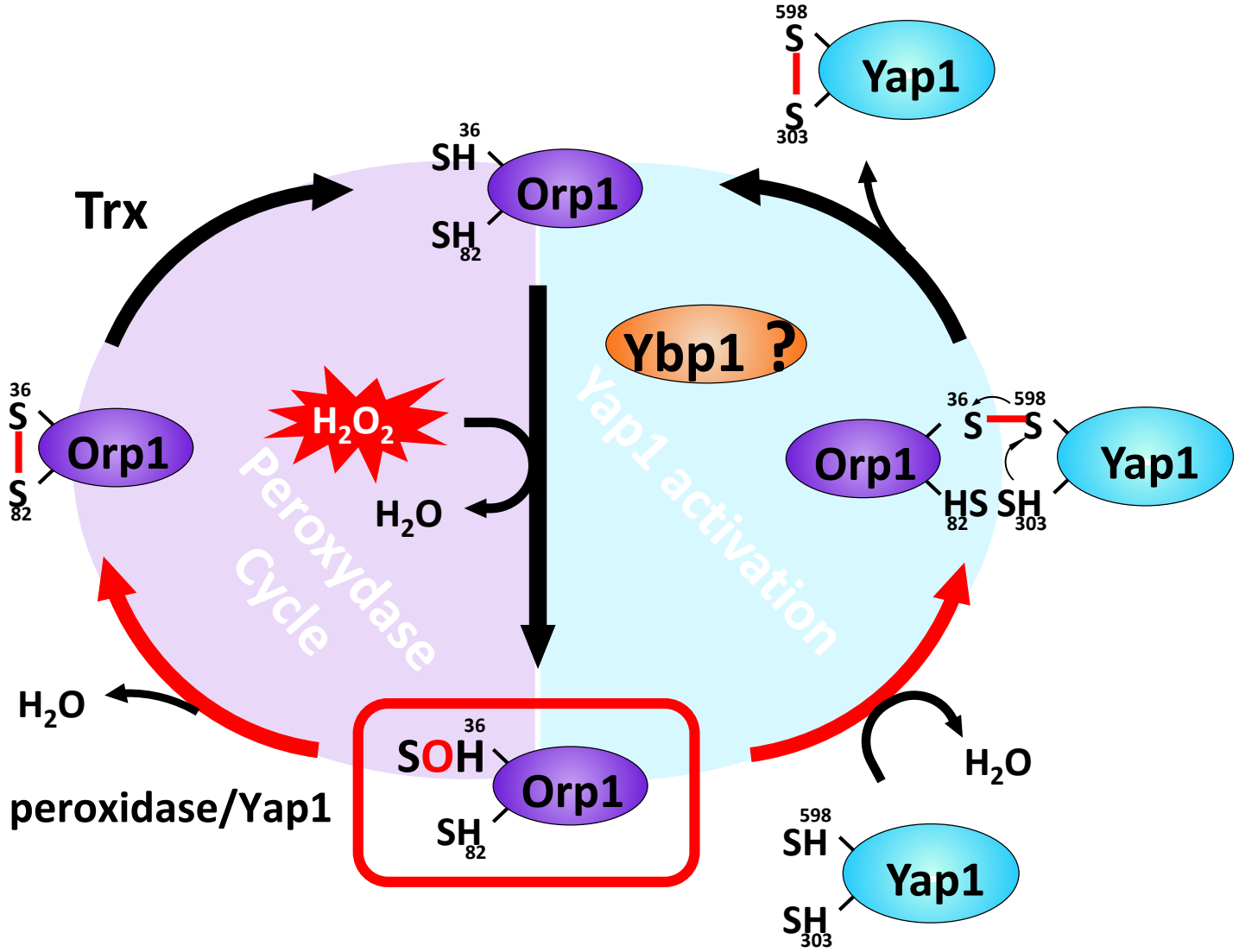
Yap1: Specificity?

Functional coupling between Orp1 and Yap1?

Direct recognition Orp1/Yap1?
Role of Ybp1 ?



Competition peroxidase/Yap1

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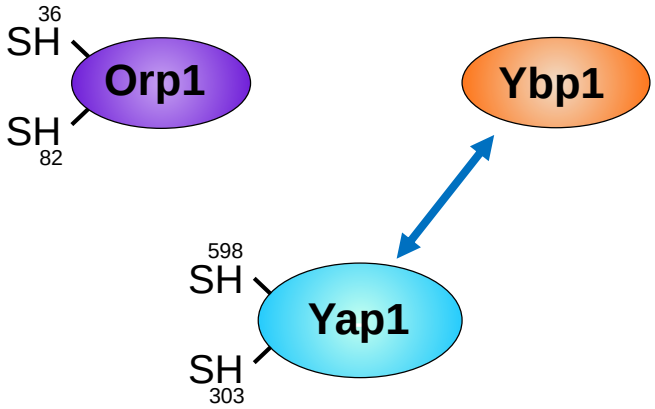
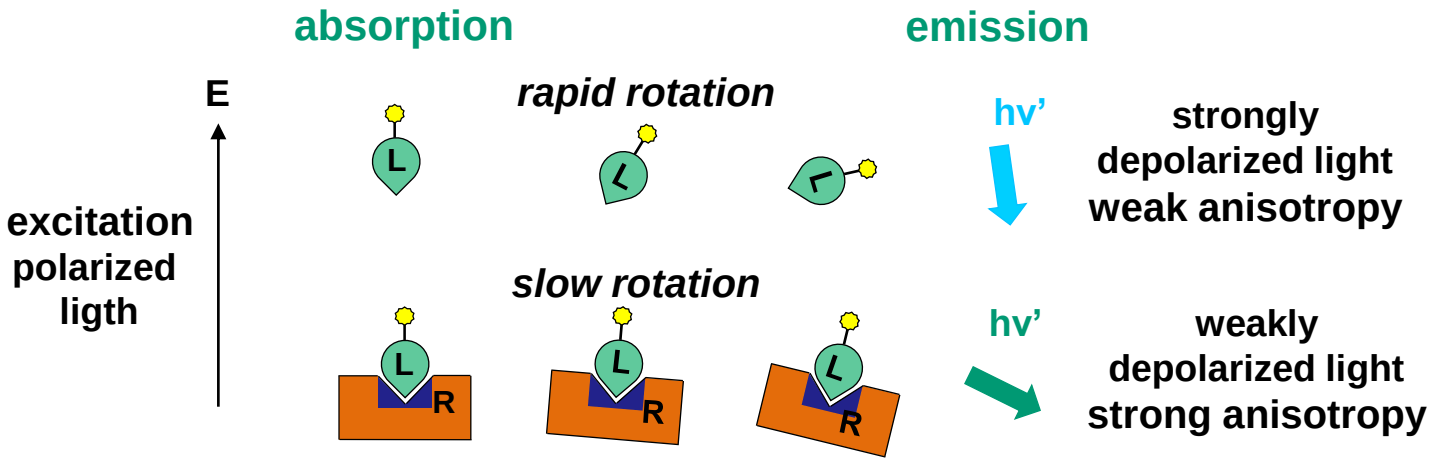
Mechanism of Ybp1 action? Protein-protein interactions

Molecular recognition between the redox relay partners?



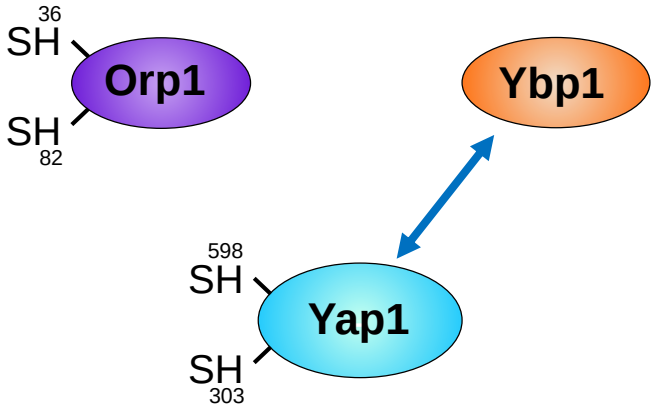
Fluorescence anisotropy

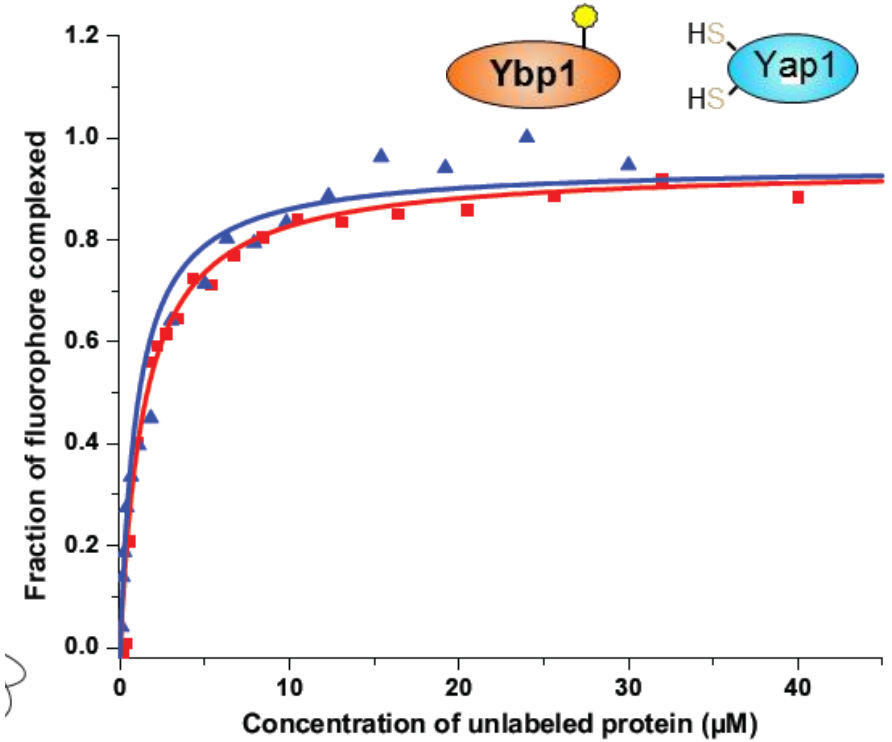


fluorescent probe: Alexa Fluor (AF) 488 5-SDP Ester

Mechanism of Ybp1 action? Protein-protein interactions

Molecular recognition between the redox relay partners?





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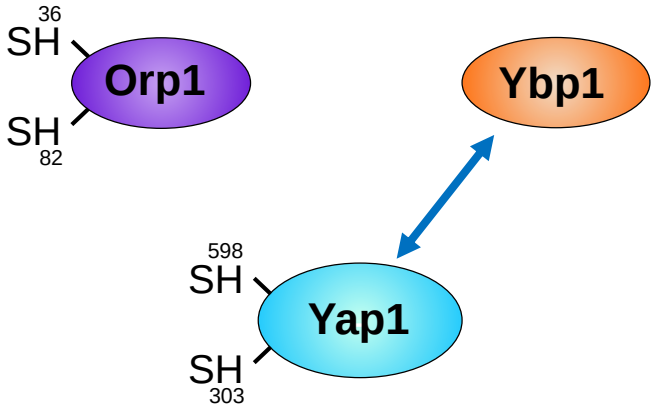
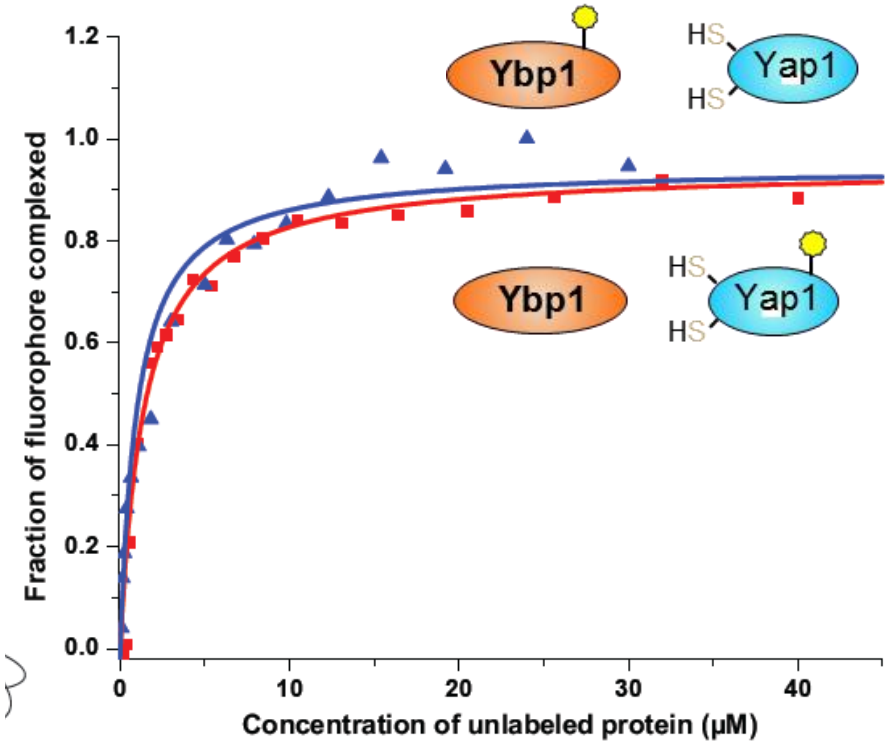
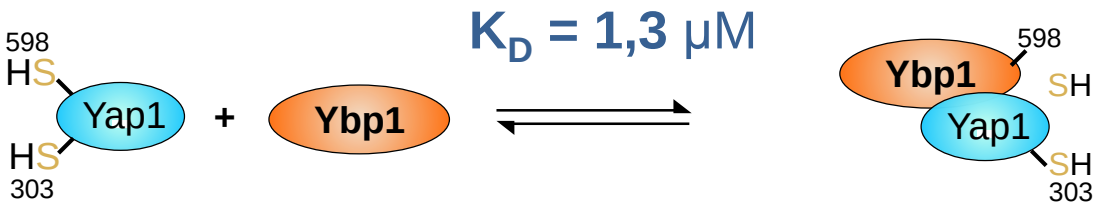
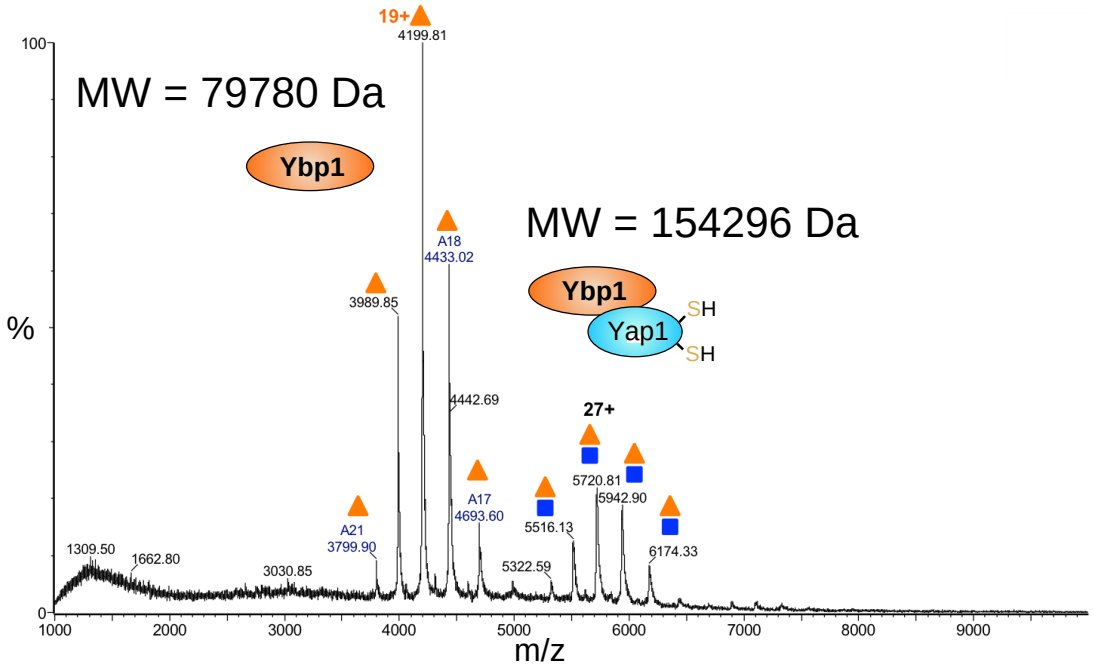


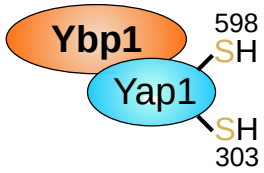




Yap1·Ybp1 1·1

Native mass spectrometry

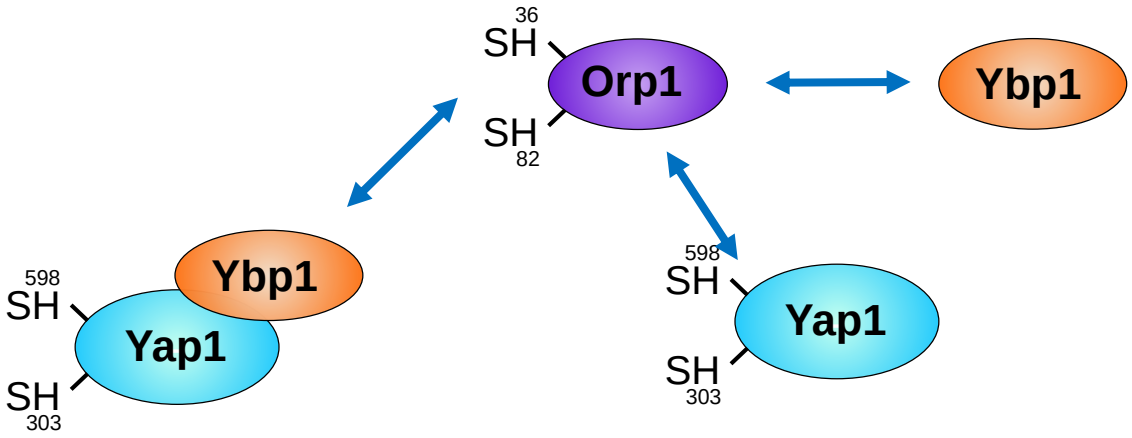




stoichiometry = 1:1

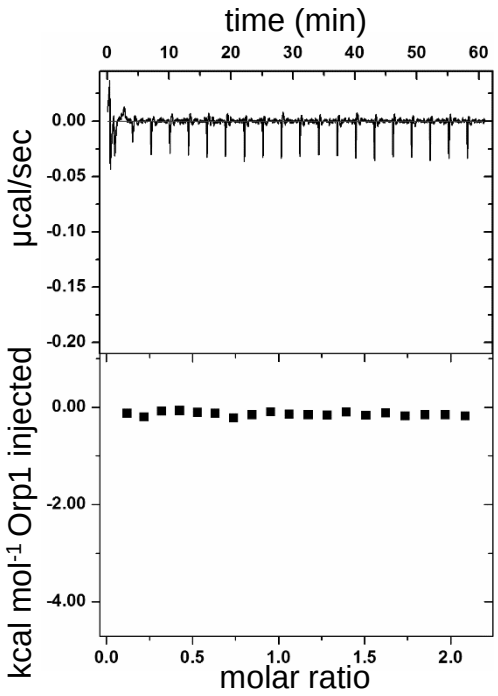
Collaboration S. Cianférani, Strasbourg

Ybp1 recruits Orp1 and Yap1 into a ternary complex

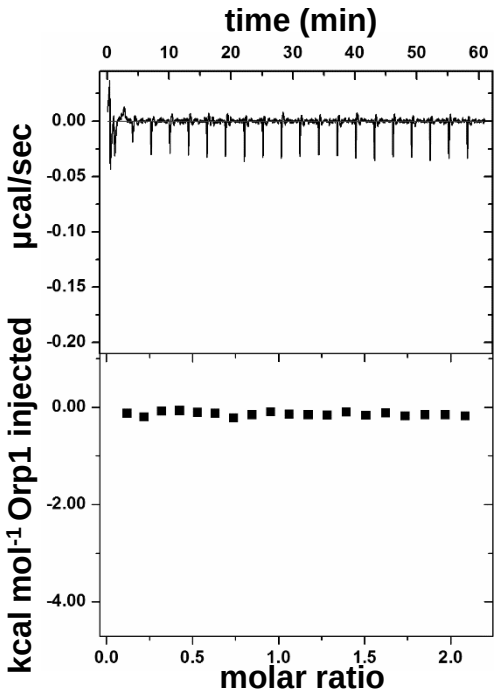


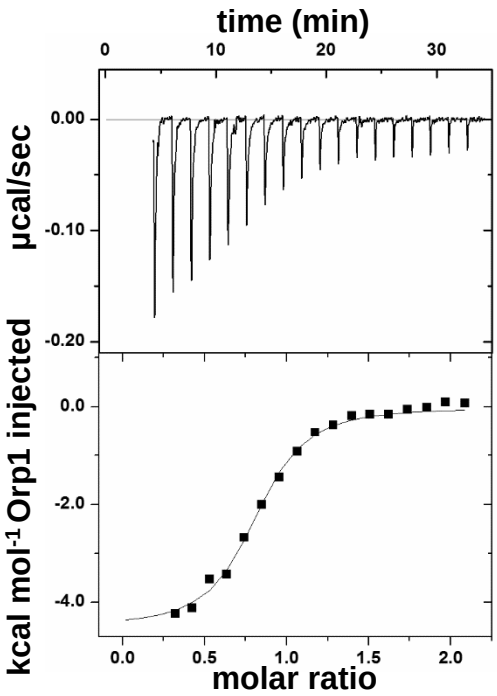
ITC titration of Yap1, Ybp1 and Yap1-Ybp1 by Orp1

Orp1 and Yap1 do not interact



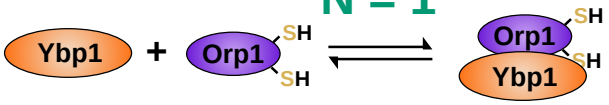
Ybp1 and Orp1 form a 1·1 complex



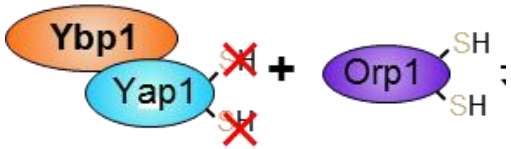


K_D = 0,8 µM

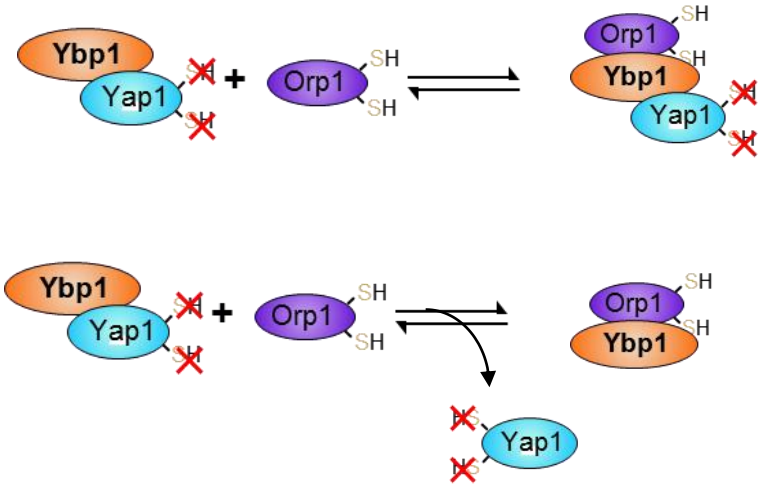
N = 1

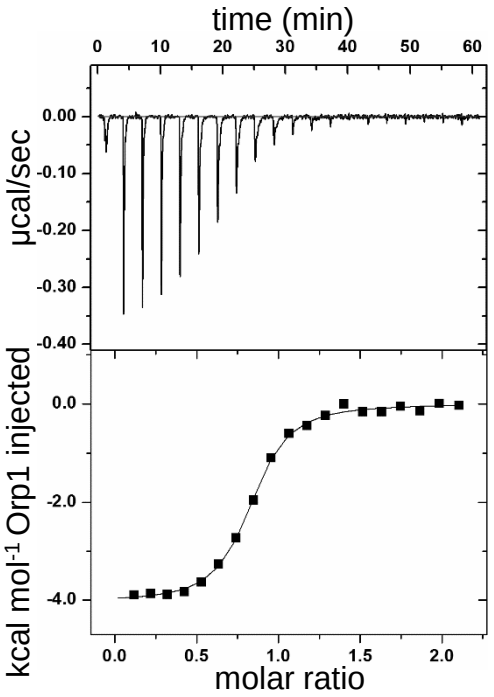


Orp1 interacts with Ybp1·Yap1



Two hypotheses





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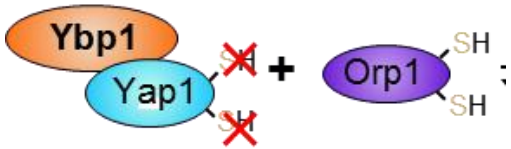
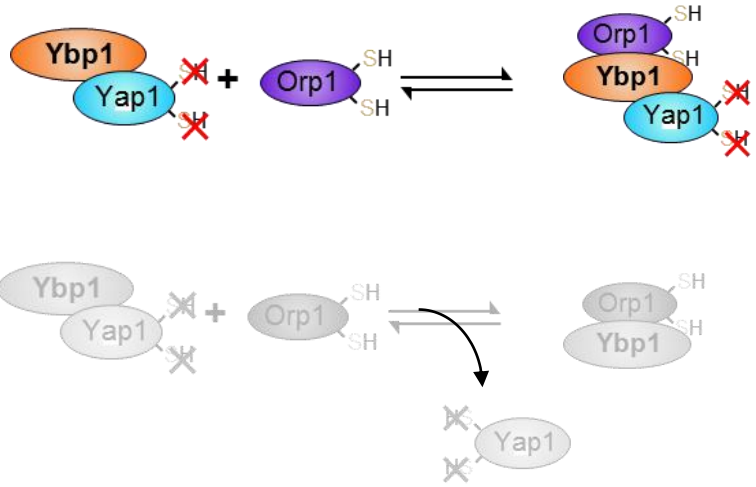
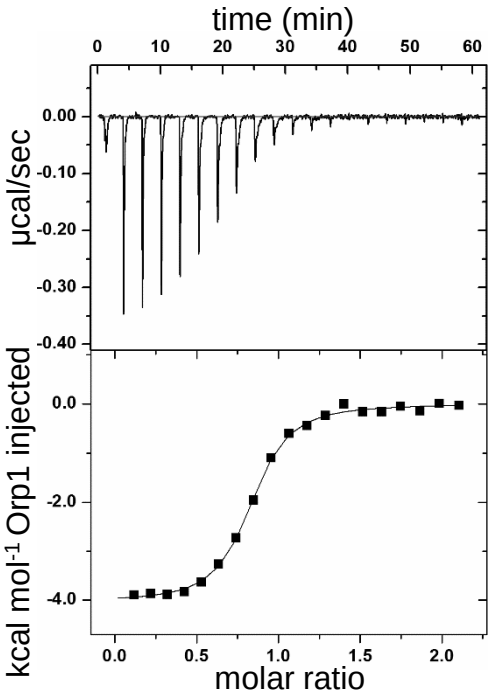


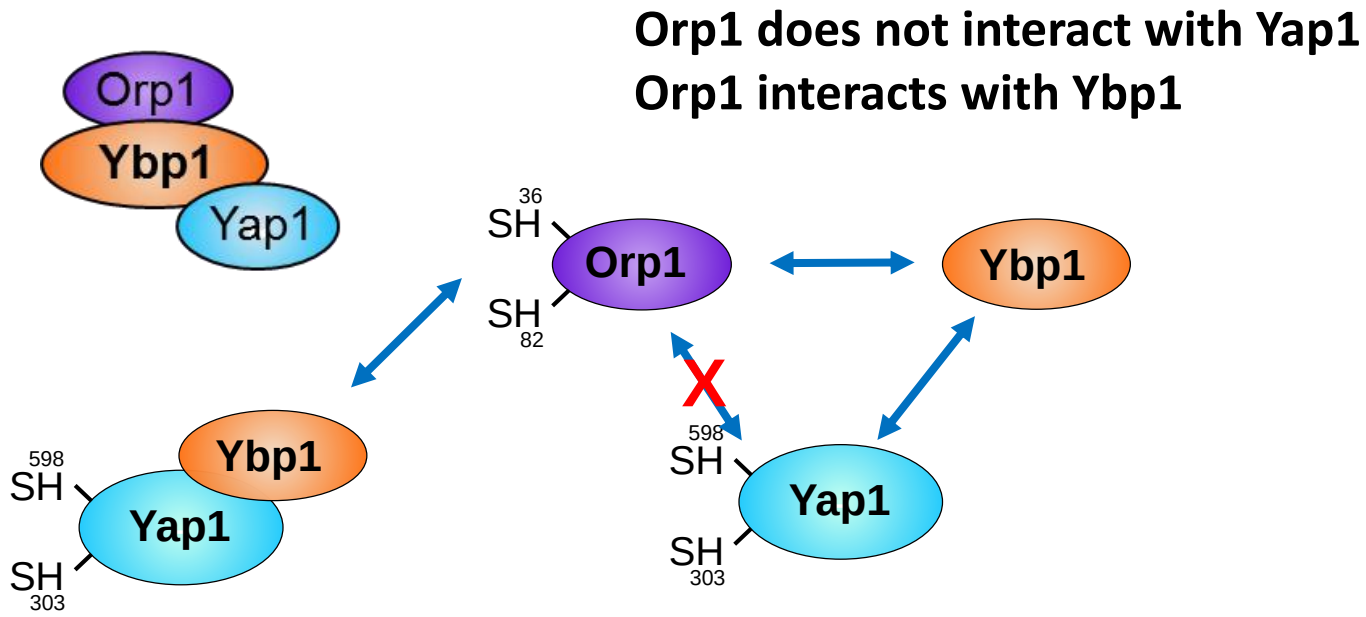
Two hypotheses

K_D = 0,7 µM
N = 1



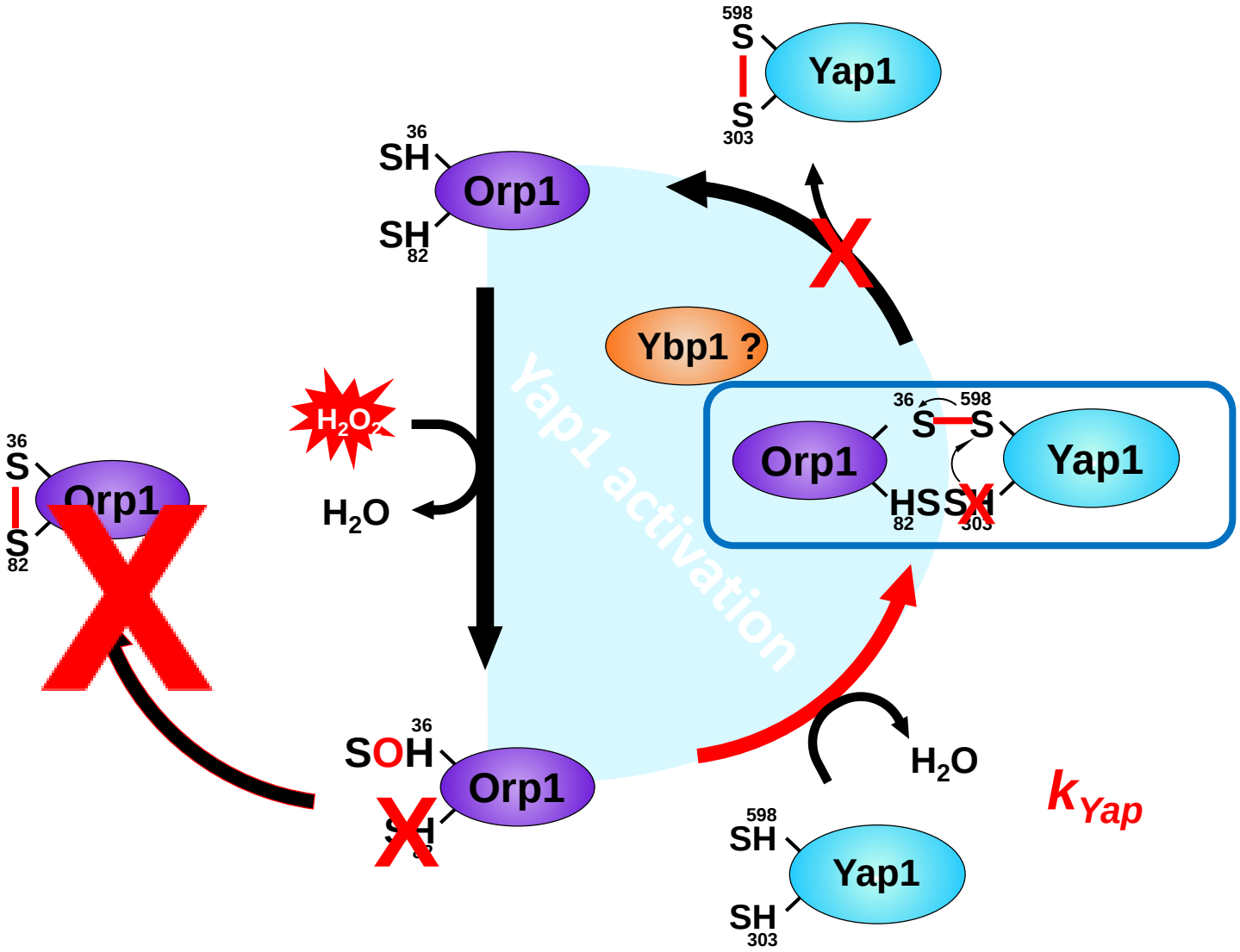


Ybp1 recruits Orp1 and Yap1 into a ternary complex

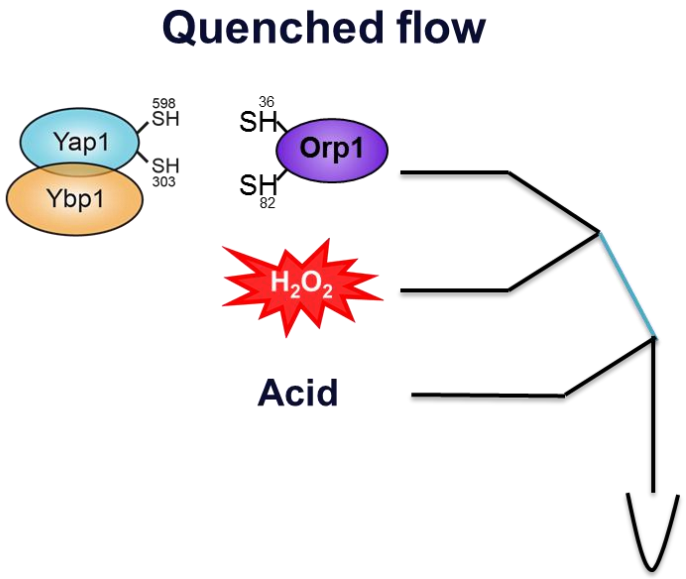


Rate of the first step of Yap1 activation pathway

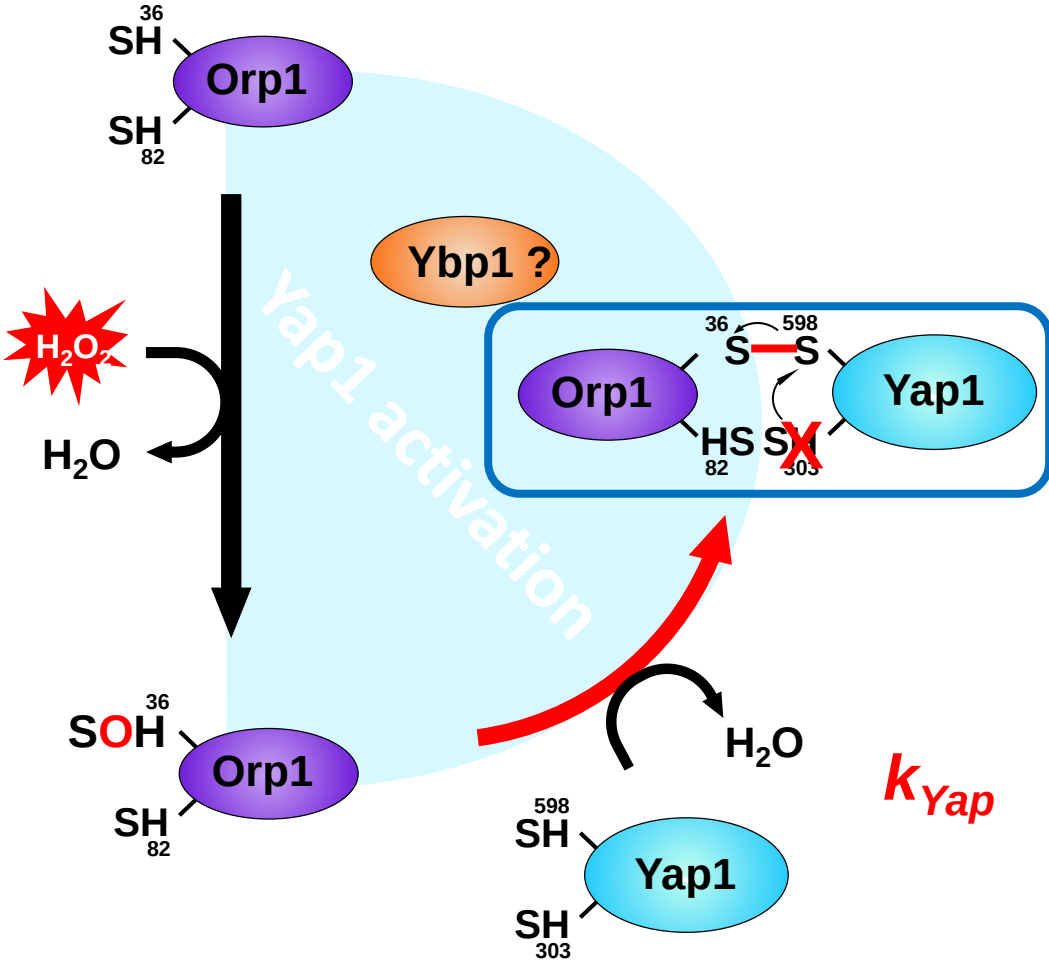
Role of ternary complex in Yap1 oxidation mechanism?



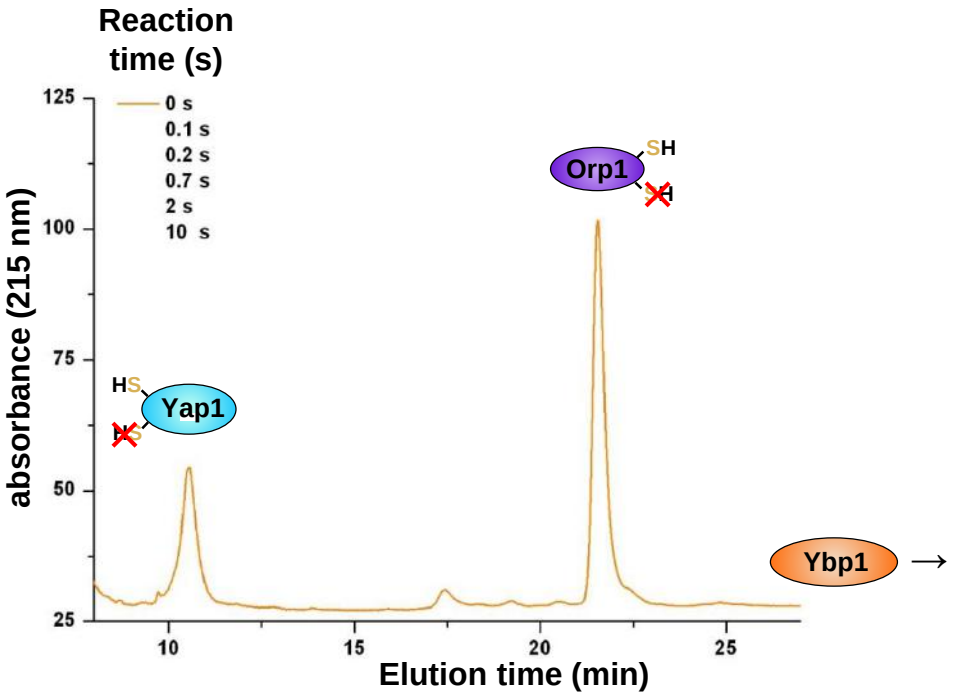
Reconstitution of first step of activation pathway *in vitro*



ms to min incubations
→ Reverse phase chromatographic analysis (C8)



k_{Yap} determination



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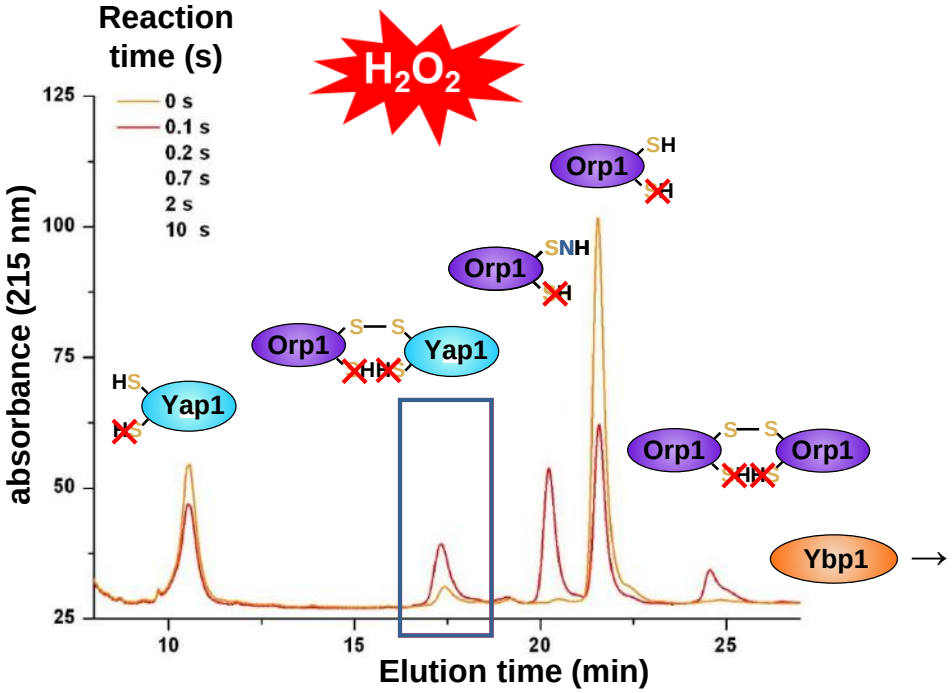

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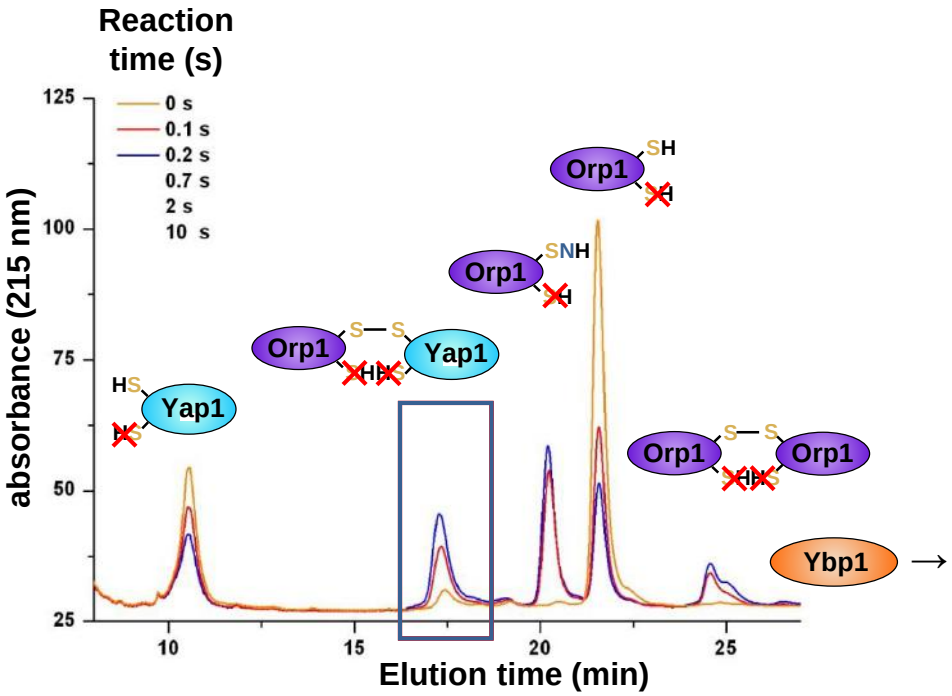

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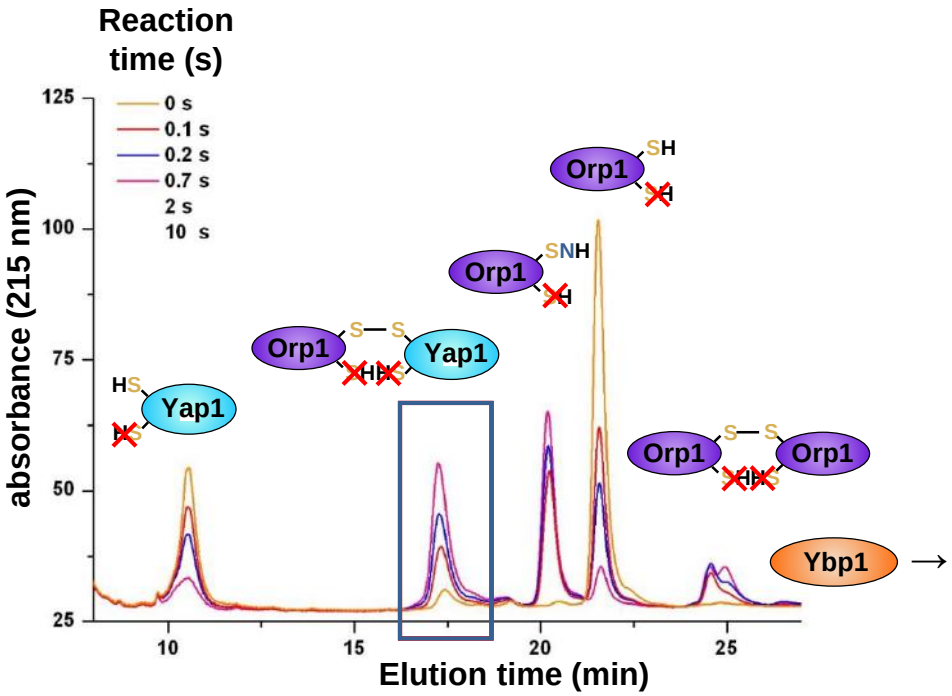

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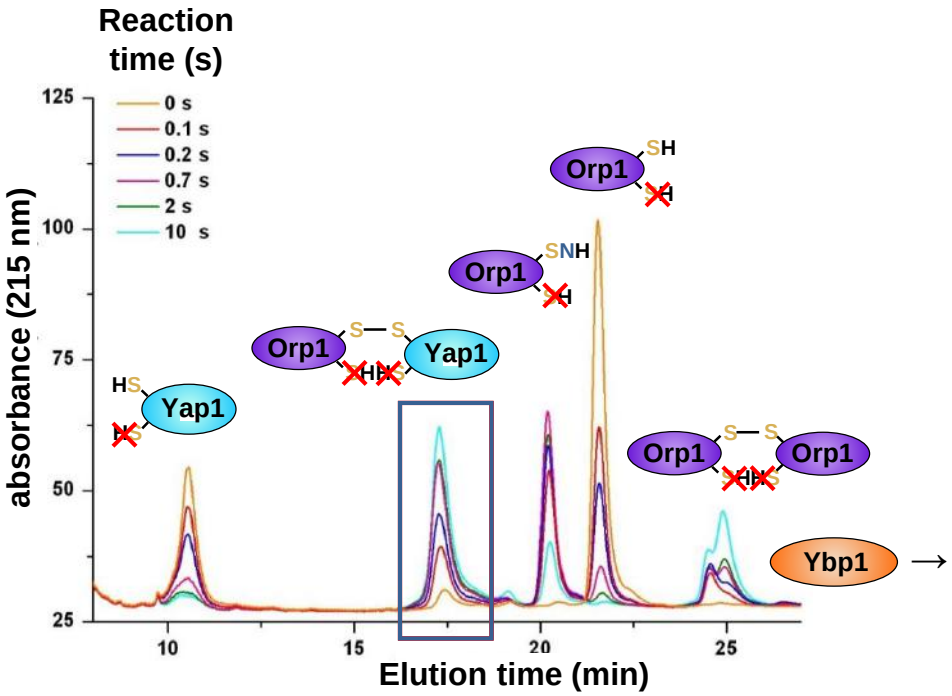
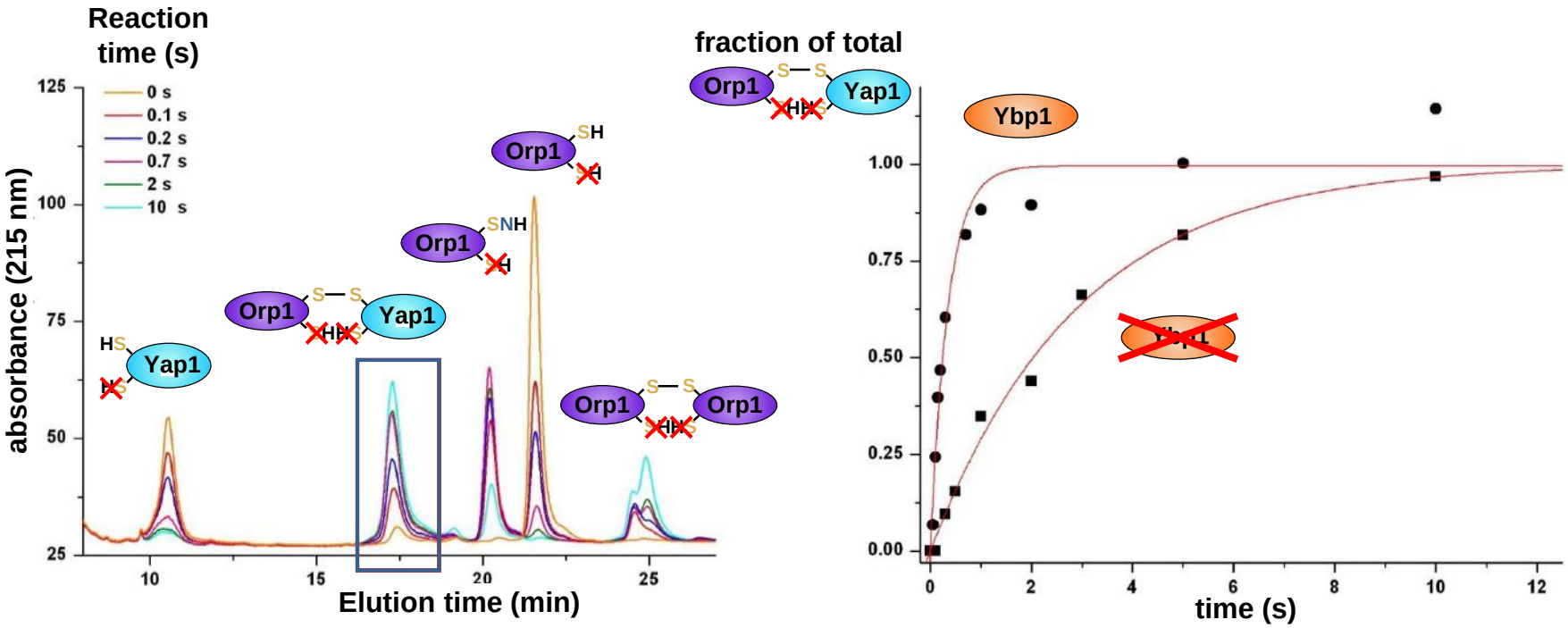


Ybp1 accelerates the reaction Orp1-SOH + Yap1



Ybp1 observed rate constant = 2.6 s^{-1}

observed rate constant = 0.3 s^{-1}

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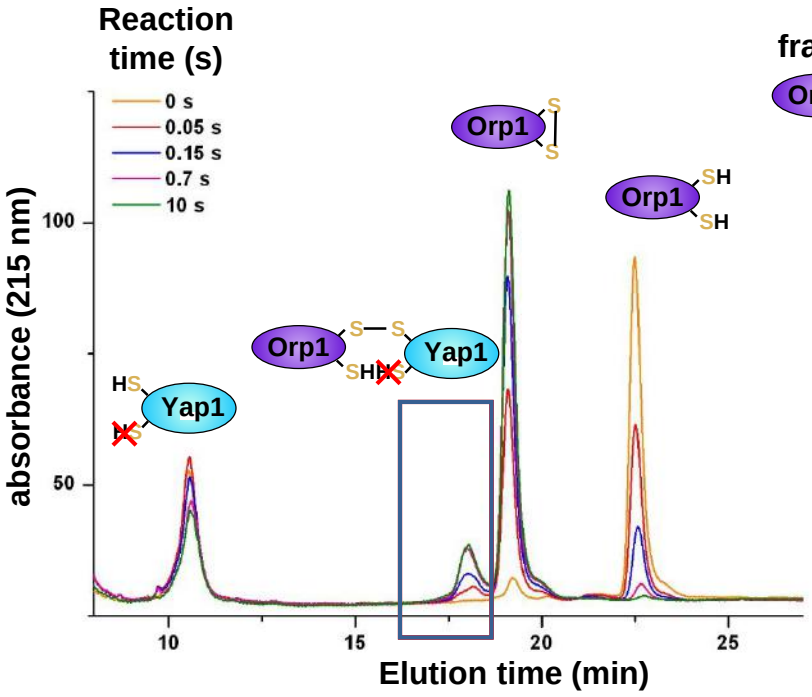
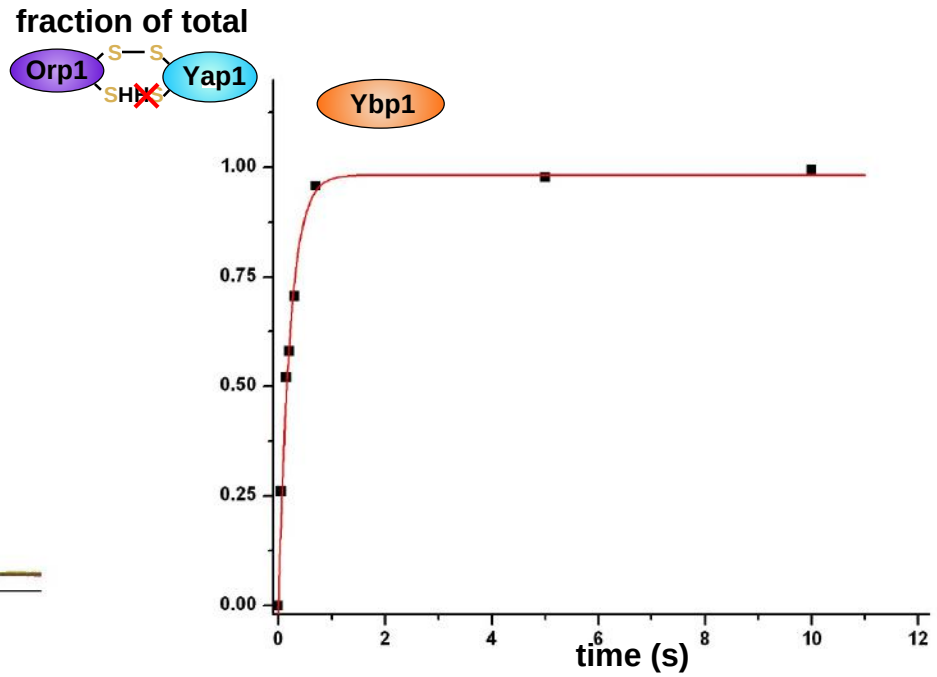


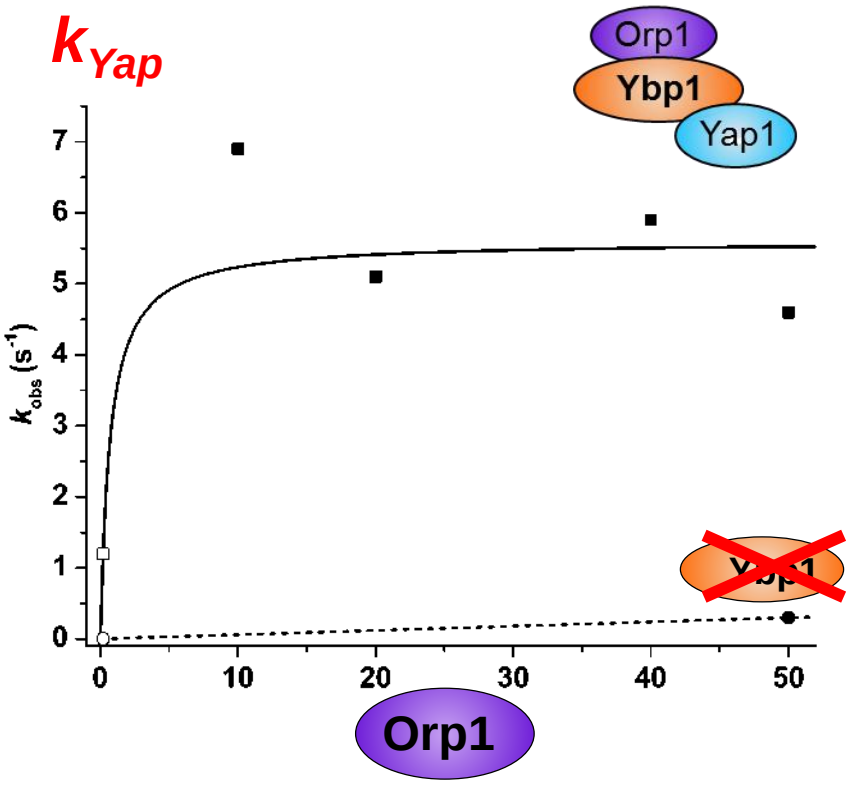


Ybp1 observed rate constant = 2.6 s^{-1}

Wild type Orp1 = 5 s^{-1}

Yap1 observed rate constant = 0.3 s^{-1}

Role of ternary complex in Yap1 oxidation mechanism?



I*n vivo* Orp1 concentration = 0,5 µM
Experiments @ 50 µM

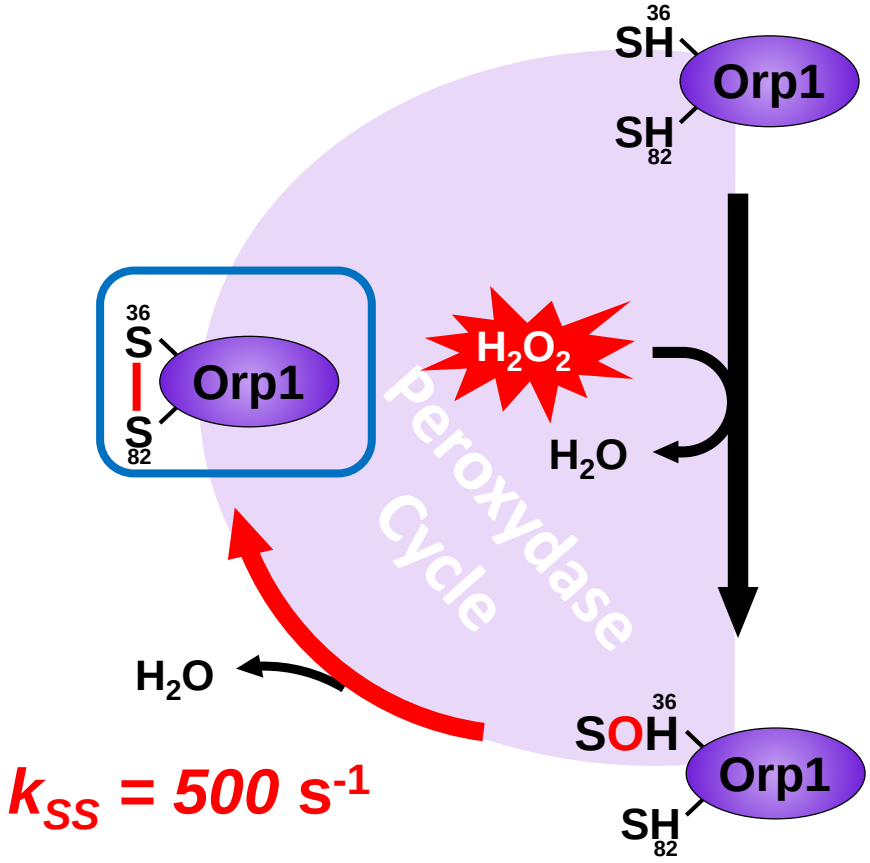
➔ **k_{Yap} = *Cste* suggests that the reaction occurs within the ternary complex**

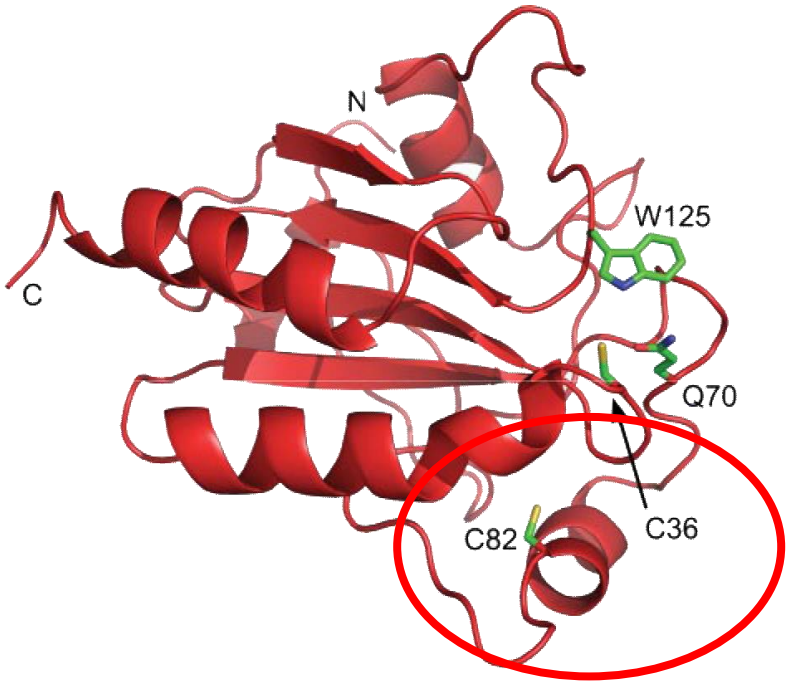
➔ **Simulation of k_{Yap} = f(Orp1) based on K_D of 0,7 µM**

➔ **In the absence of Ybp1 the reaction is bimolecular**

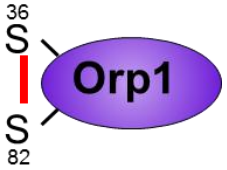
➔ **Real effect of complex formation = 600 fold**

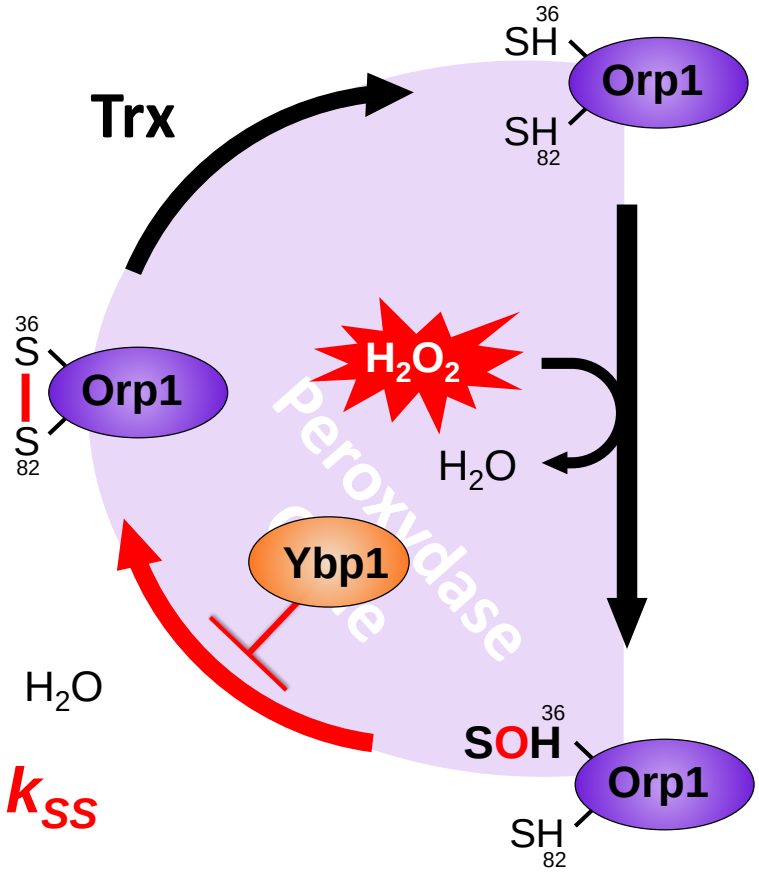
Intramolecular disulfide formation in Orp1 is very fast

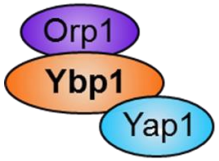




Ma et al. JBC 2007

Does Ybp1+Yap1 inhibit  formation?

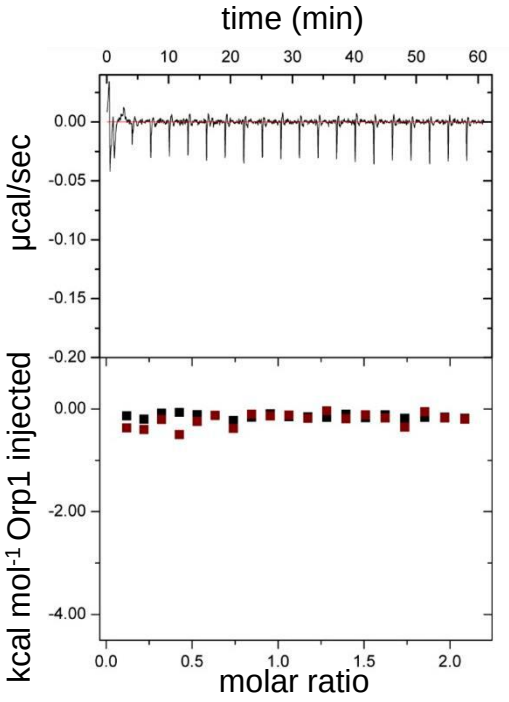


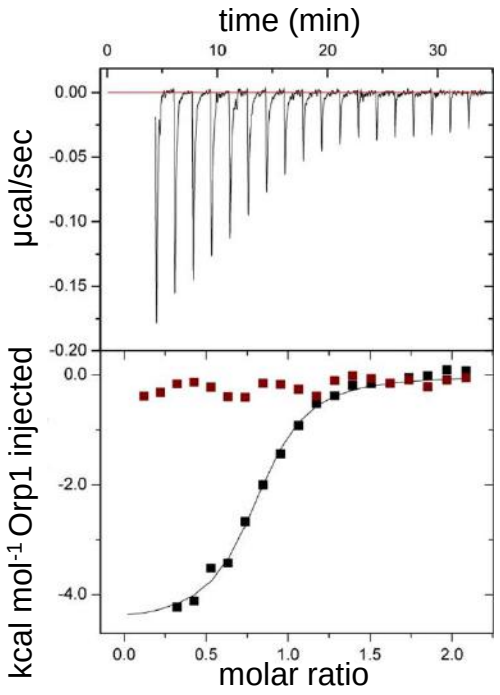
Within 

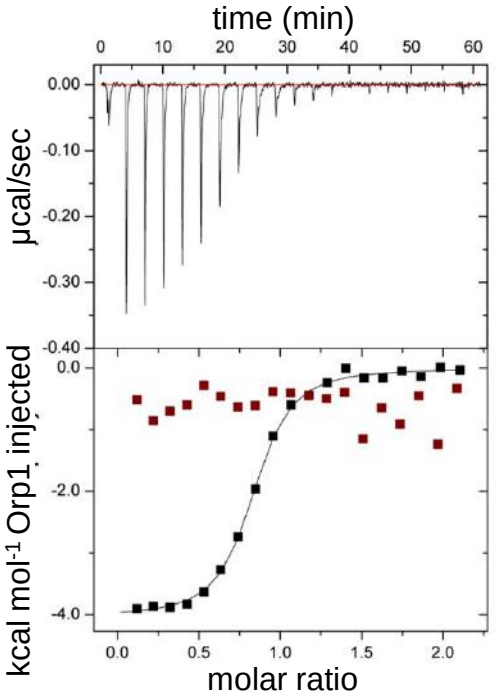
k_{ss} decreased from 500 to 6 s^{-1}

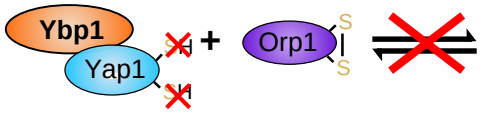
- ➔ **Reduced competition with Yap1**
- ➔ **Reaction between Orp1 and Yap1 is possible**

The disulfide form of Orp1 doesn't interact with Ybp1 and Yap1·Ybp1

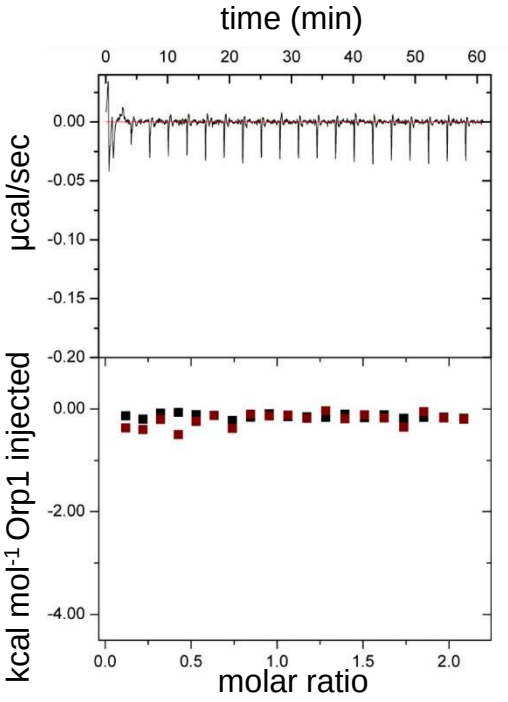


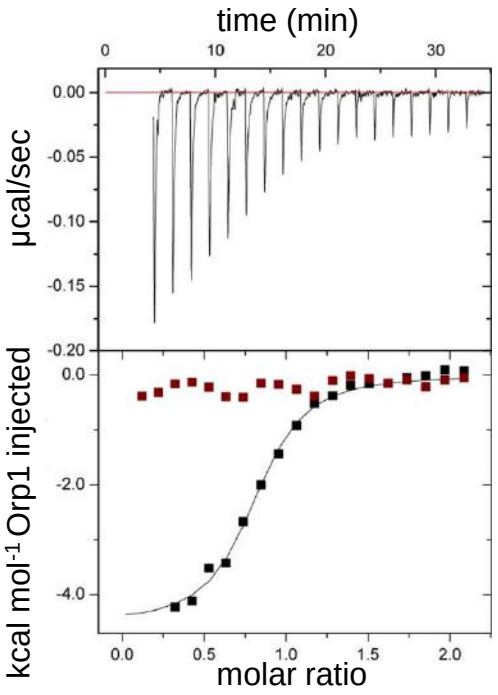


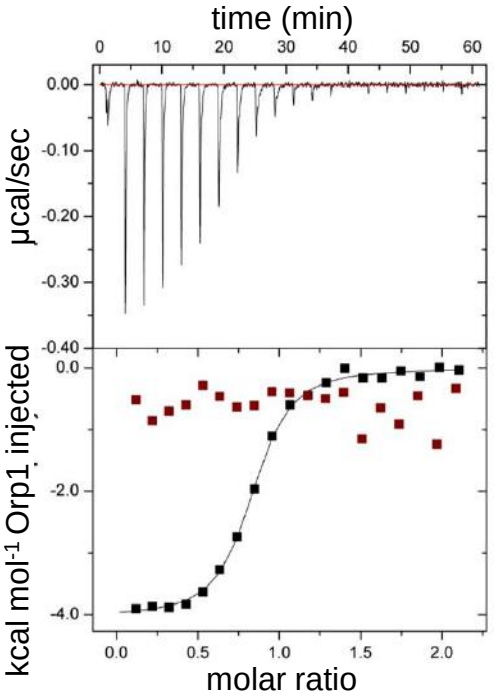


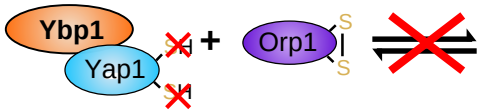


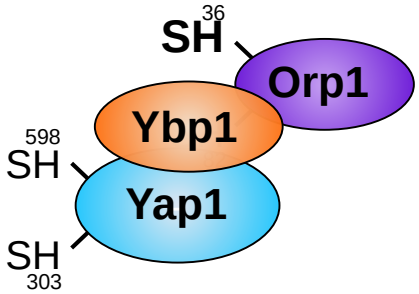
The disulfide form of Orp1 doesn't interact with Ybp1 and Yap1·Ybp1





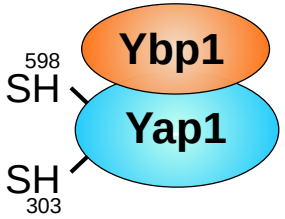


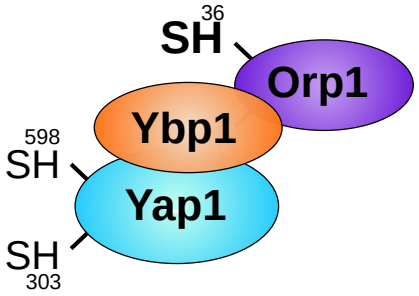




Yap1 activation mechanism

Ybp1 recruits Orp1 (ITC)

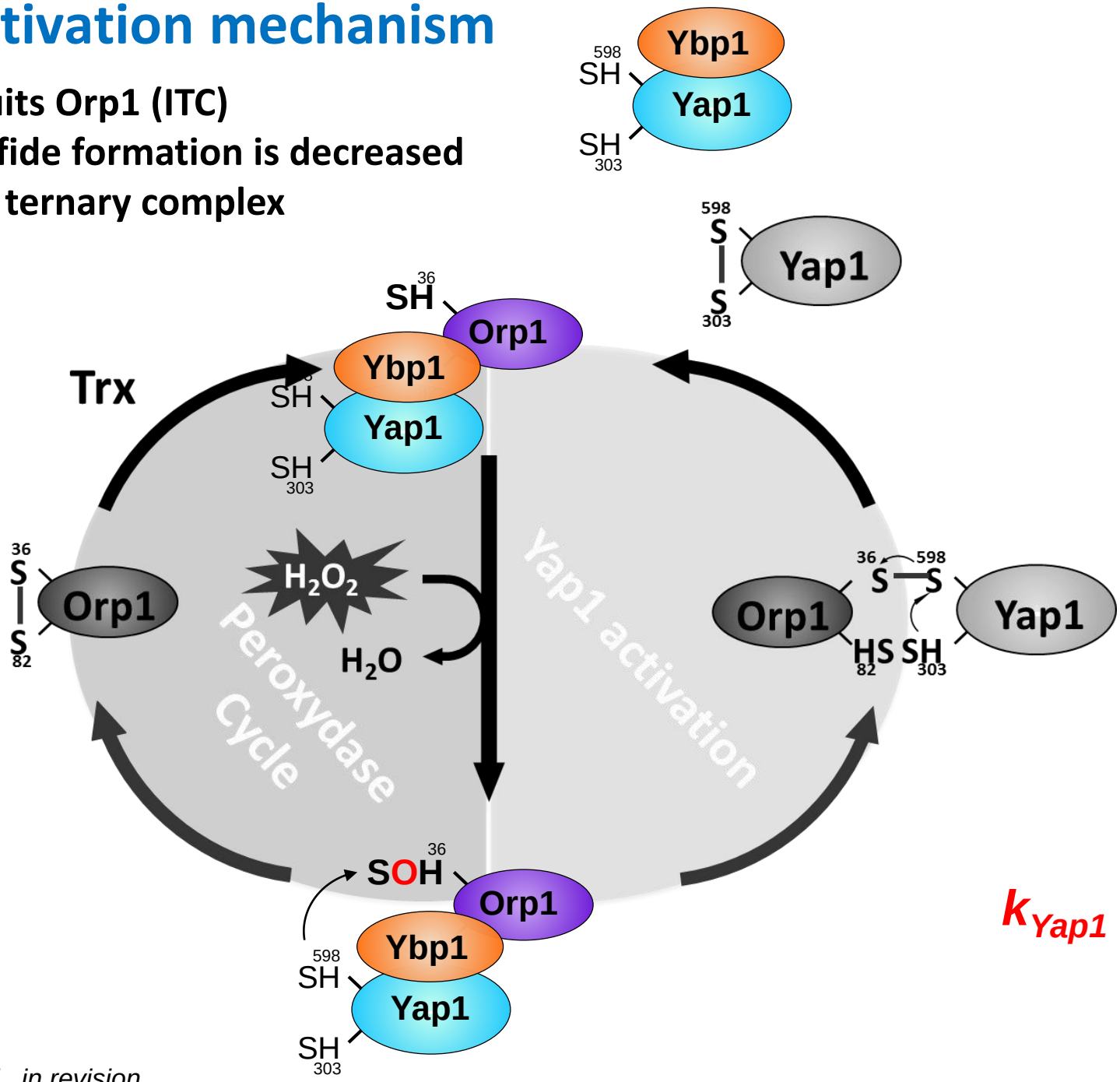




Bersweiler et al., in revision

Yap1 activation mechanism

Ybp1 recruits Orp1 (ITC)
Orp1 disulfide formation is decreased within the ternary complex



Bersweiler et al., in revision

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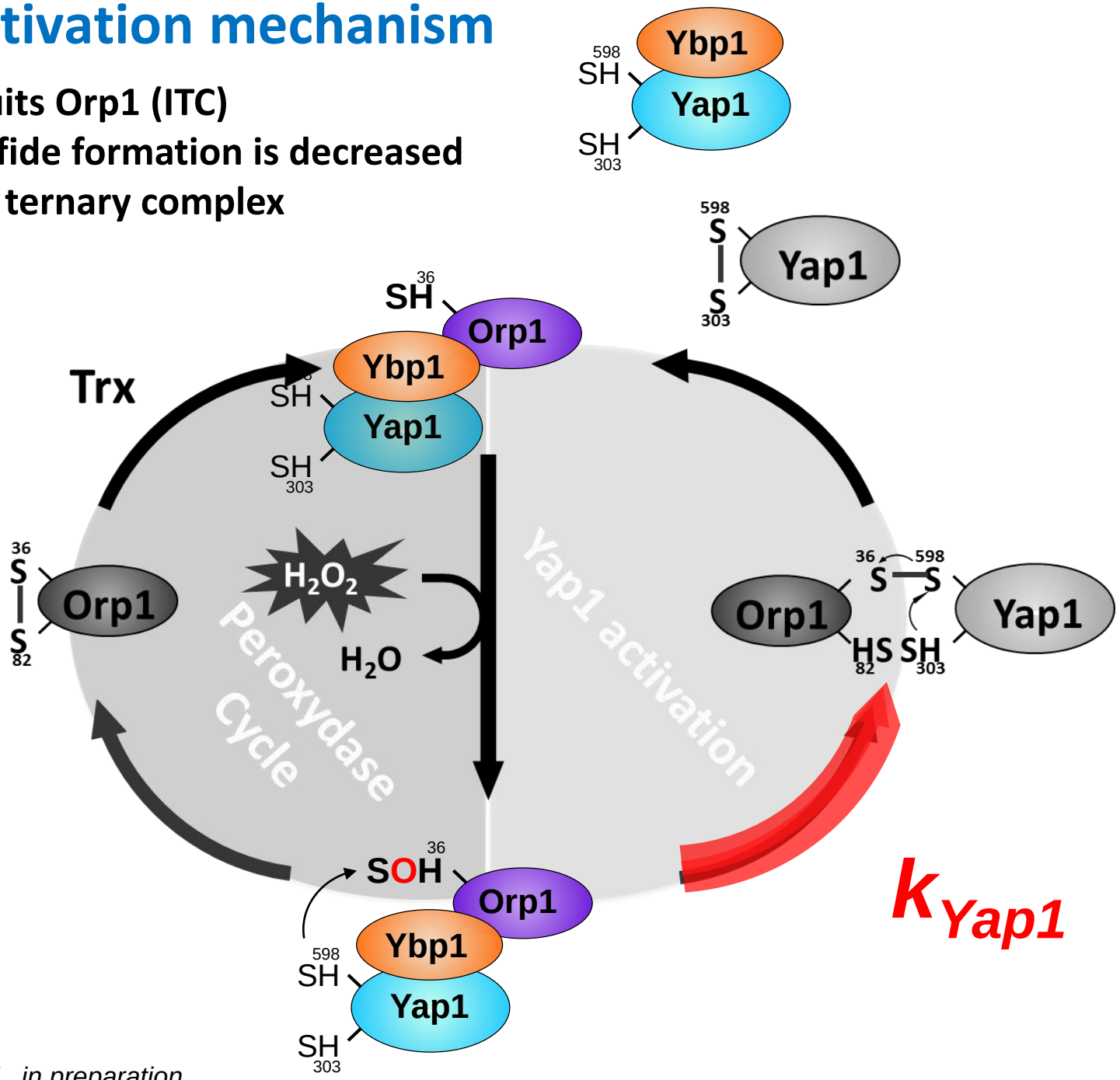

Bersweiler et al., in preparation

Yap1 activation mechanism

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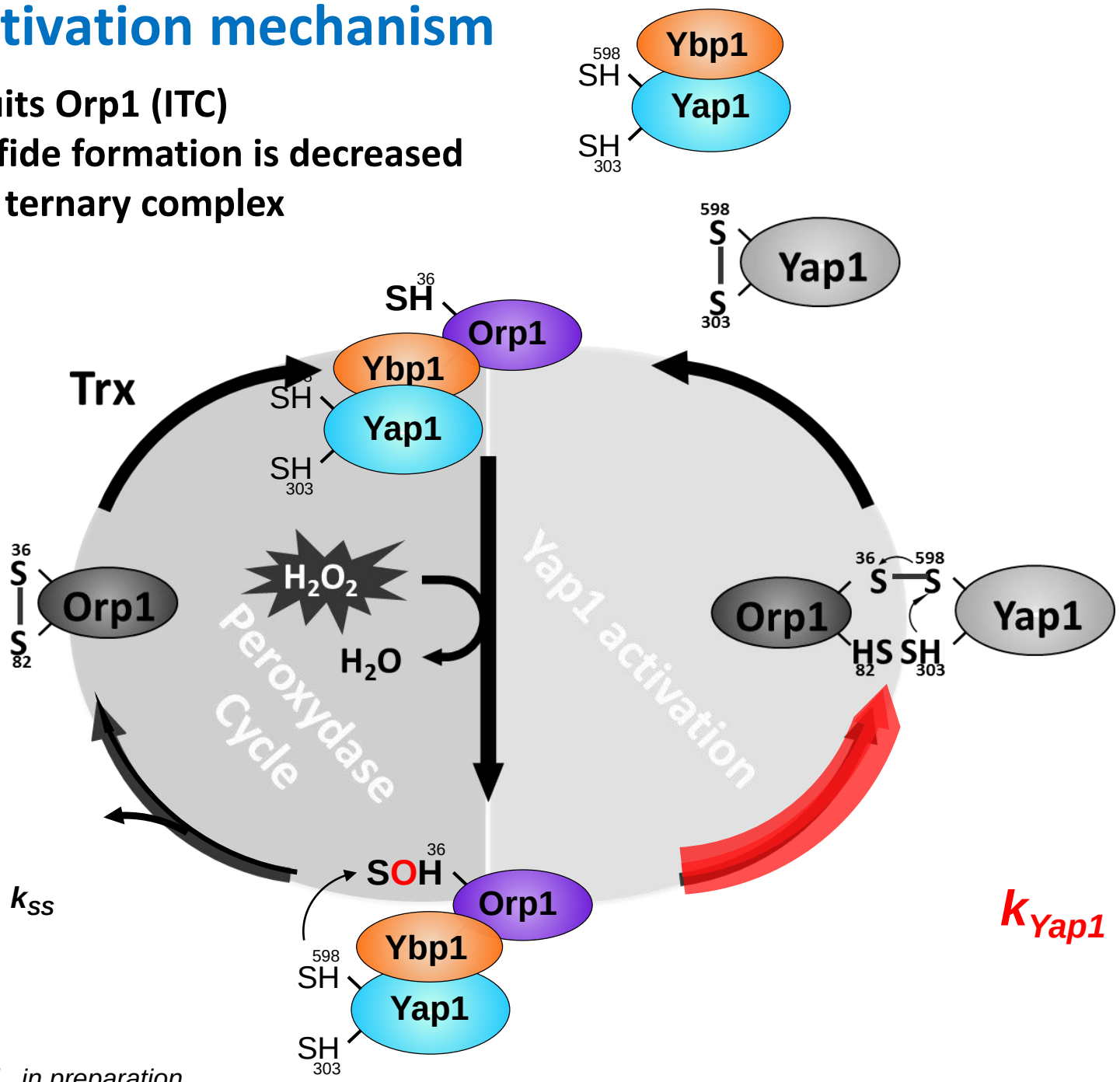
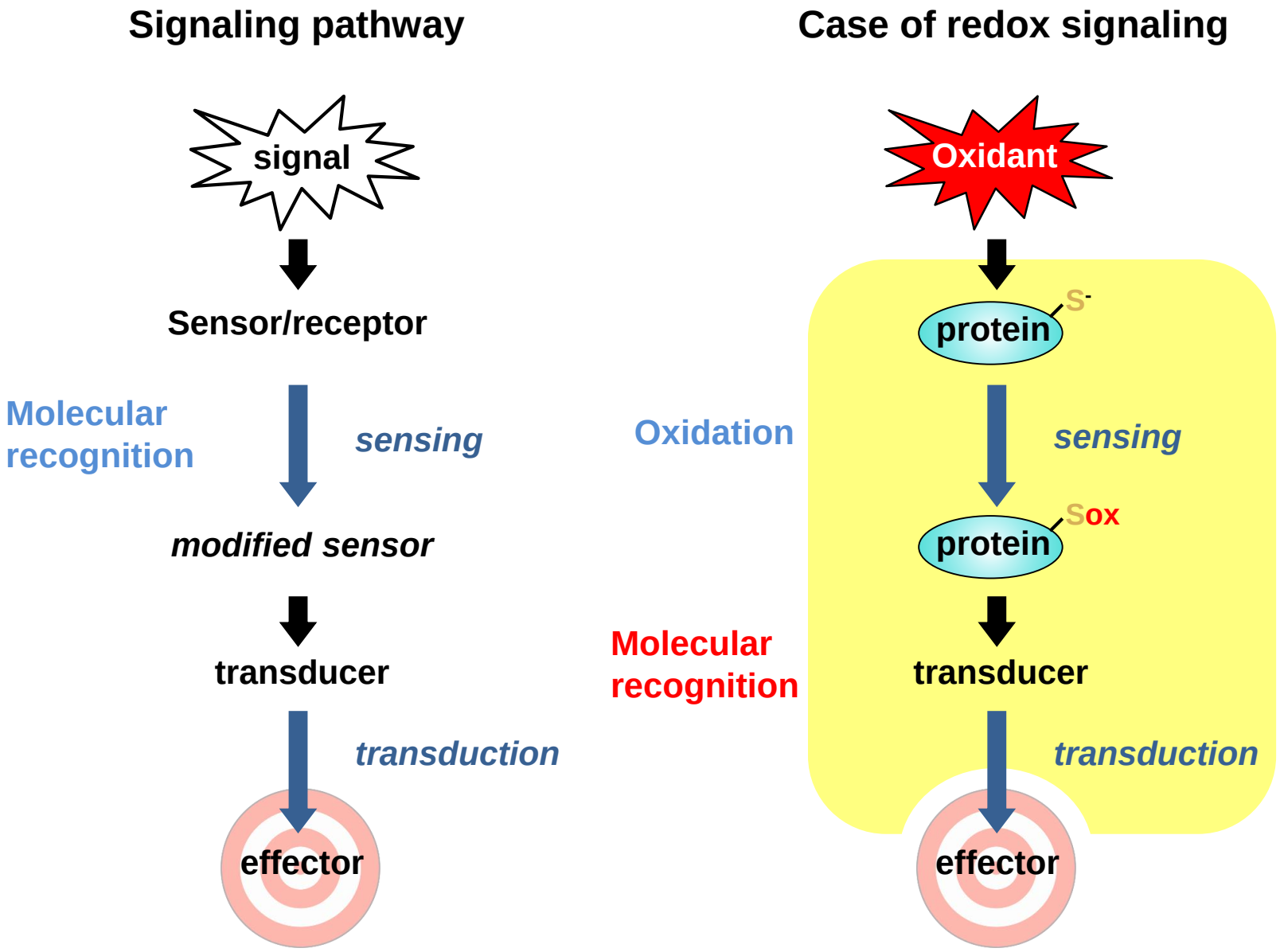


Bersweiler et al., in preparation

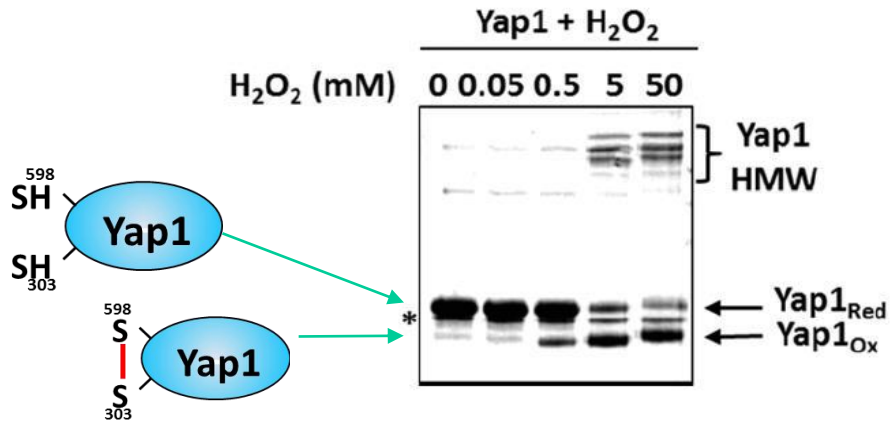
Cellular signaling pathways



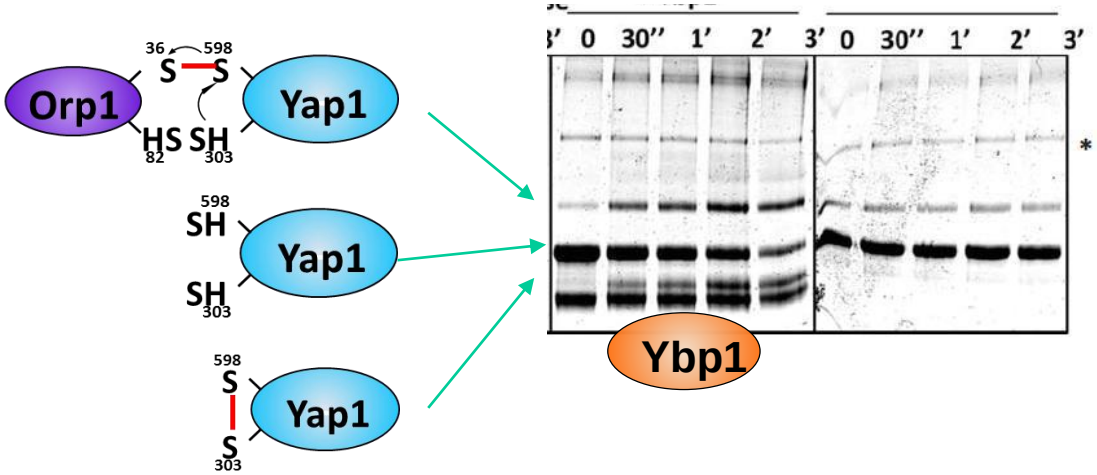
Thank you for your attention

In vitro reconstitution of the redox relay

Recombinant purified proteins



High H_2O_2

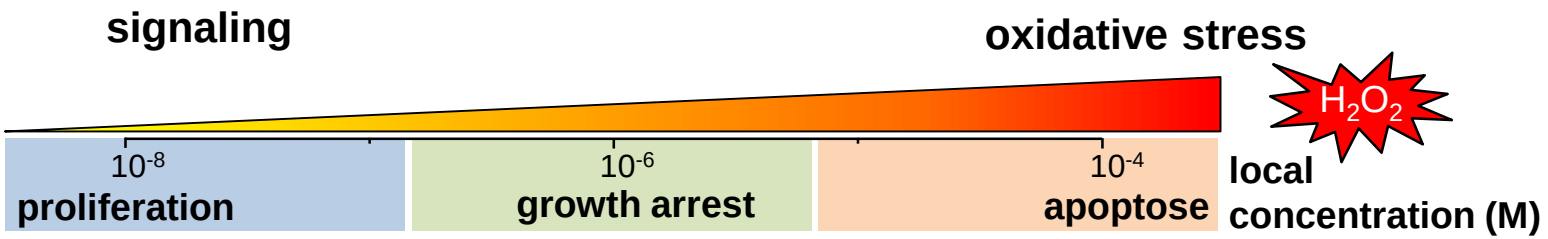


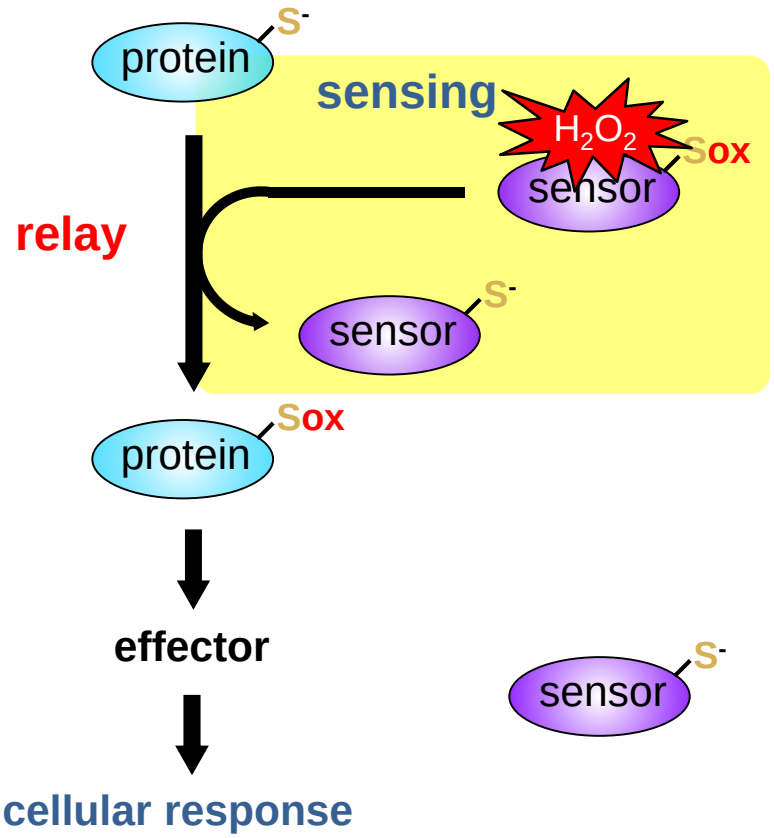
Low H_2O_2

Conclusion: requirement of both Orp1 and Ybp1 in efficient and sensitive Yap1 oxidation by H_2O_2

Collaboration M. Toledano

Direct/indirect sensing of the H_2O_2 signal





high reactivity with H_2O_2

OxyR

lower reactivity in most cases

Prx2/Stat3

= Enzyme family
Thiol peroxidase

Sobotta et al., Nature Chemical Biology, 2015

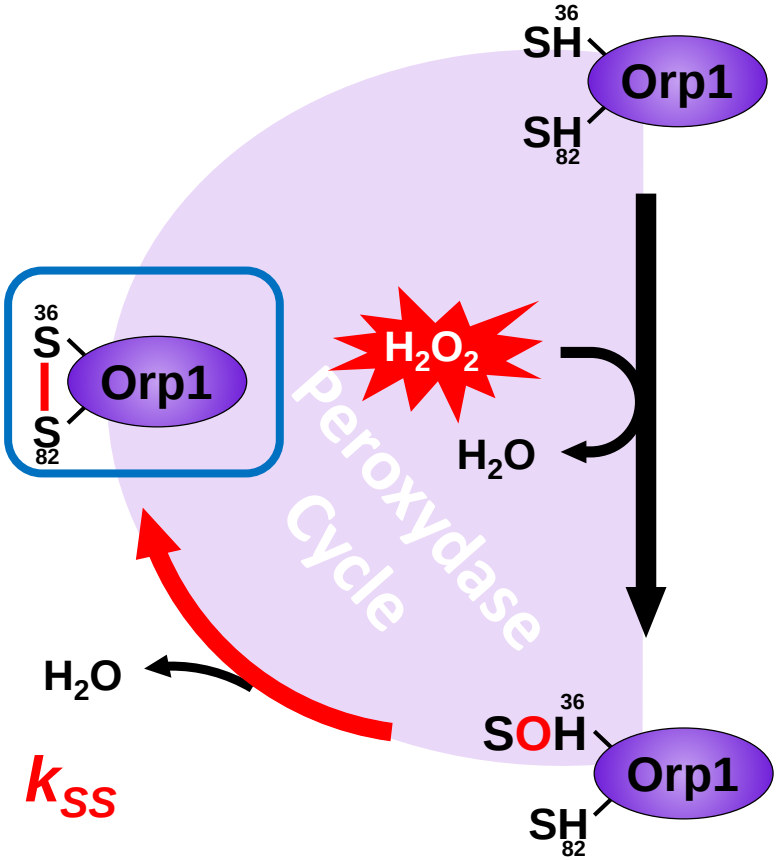
Table 1. Binding and thermodynamic parameters of Orp1 binding to Ybp1 and the Yap1$^{SSS\ SSS}$·Ybp1 complex deduced from ITC titrations[a]

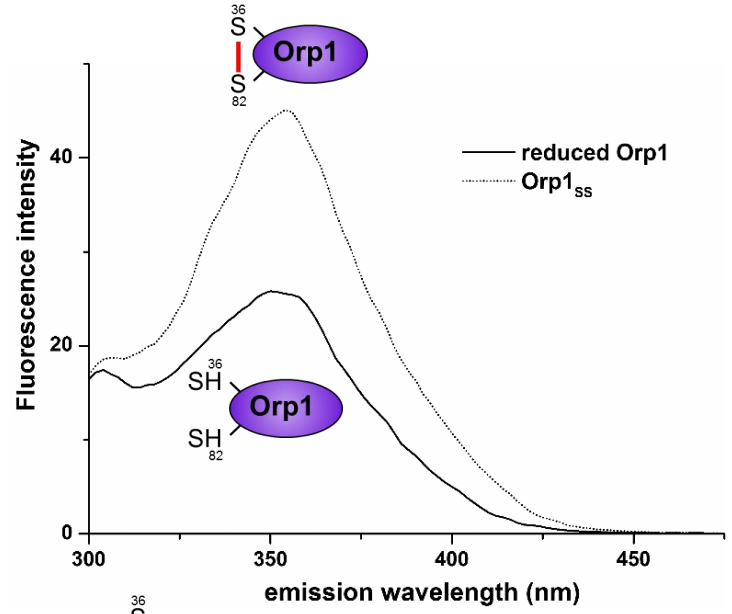
	Dissociation constant (µM)	Stoichiometry	ΔH cal mol^{-1}	-TΔS cal mol^{-1}
Ybp1	0.8 ± 0.1	0.8 ± 0.1	- 4890 ± 400	- 3430
Yap1$^{SSS\ SSS}$·Ybp1	0.7 ± 0.2	0.8 ± 0.1	- 4210 ± 240	- 4235

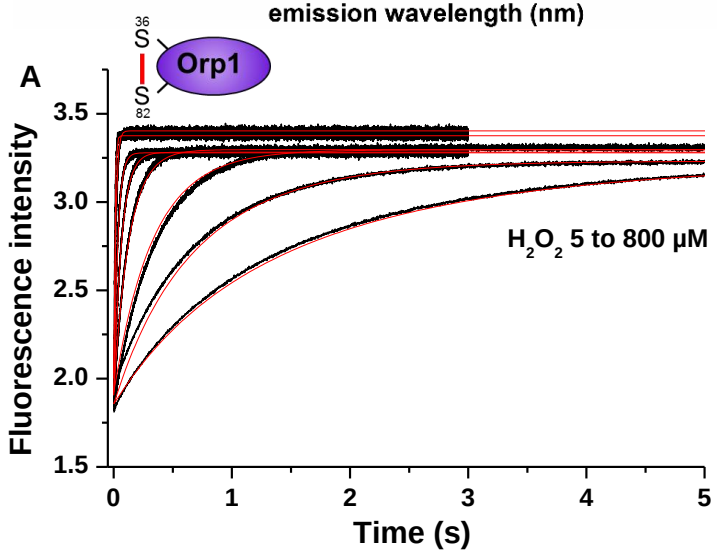
[a] Parameters deduced from the analysis of binding isotherms shown Fig. 3C

Intramolecular disulfide formation in Orp1 is very fast

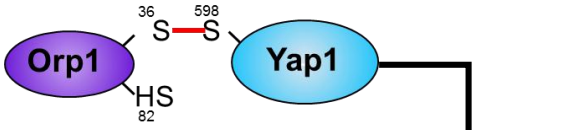
$k_{SOH} = 1.5\ 10^5\ M^{-1}s^{-1}$
$k_{SS} = 500\ s^{-1}$

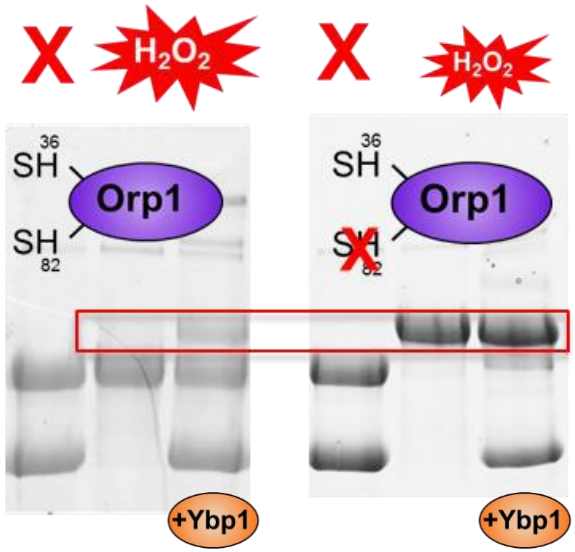






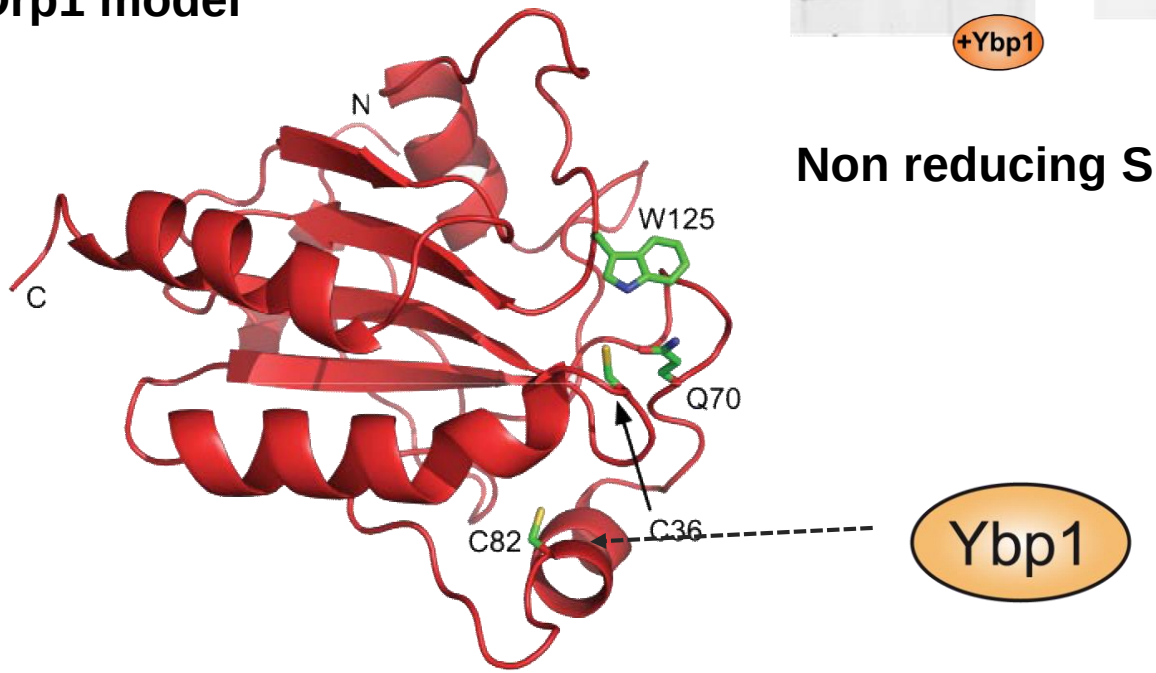
Ybp is required for the reaction WT Orp1-SOH + Yap1





Non reducing SDS-PAGE

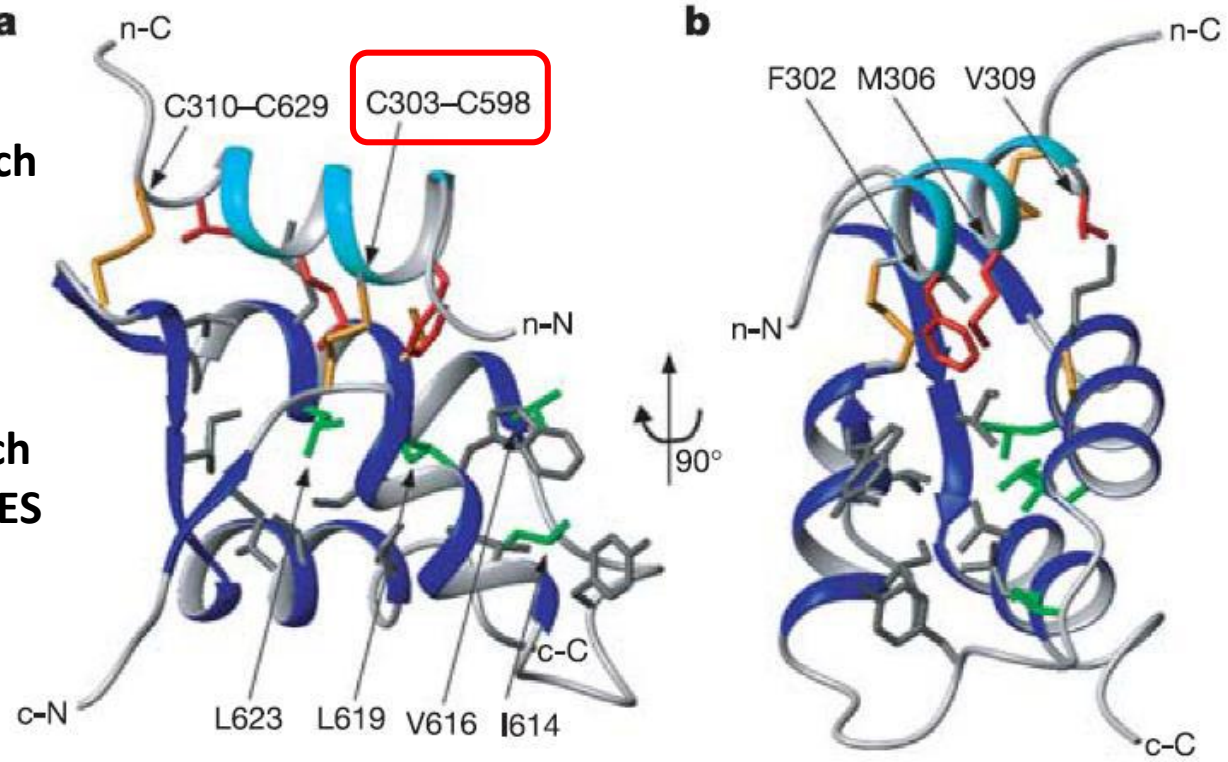
Orp1 model



Ma et al. JBC 2007

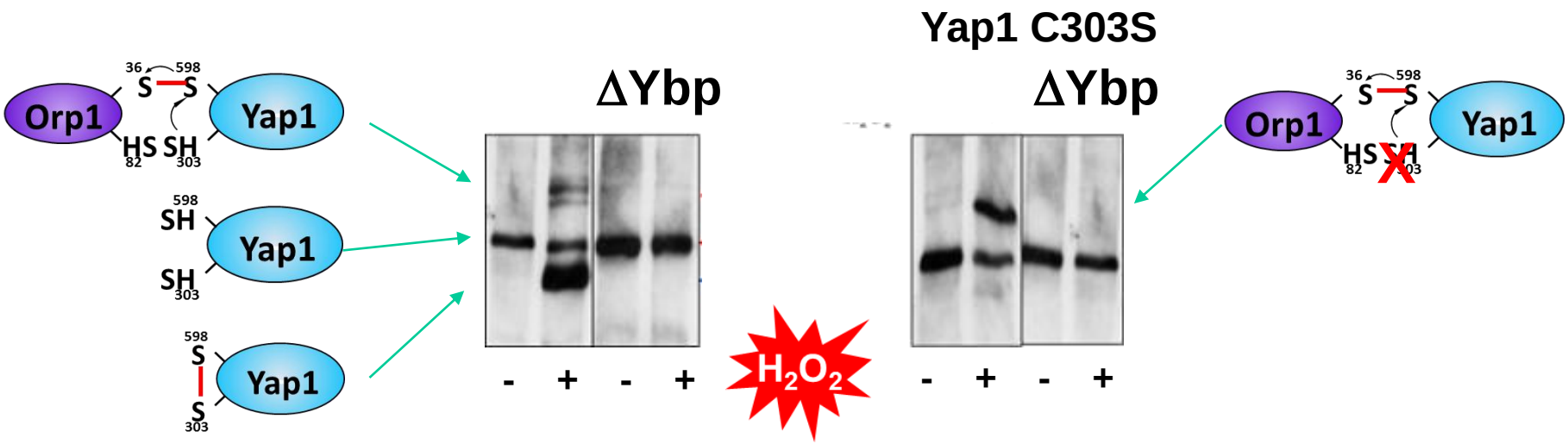
N-ter Cys rich domain

C-ter Cys rich domain / NES



Wood et al., Letters to Nature 2003

Ybp1 required *in vivo* for the first step of Yap1 activation



Mechanism of Ybp1 action?

Collaboration M. Toledano