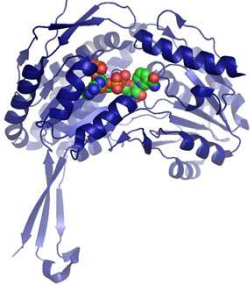





Les techniques de cinétique rapide et d'étude des interactions biomoléculaires en enzymologie



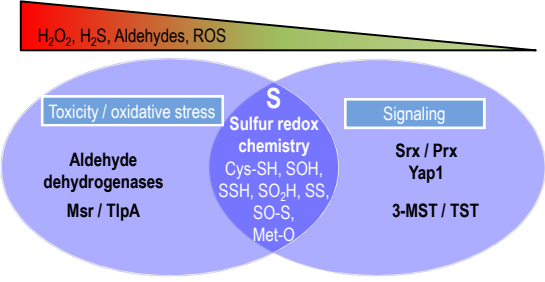
S. Rahuel-Clermont

Imopa UMR CNRS-UL 7365
Team 3
Molecular and Structural Enzymology

Imopa Team 3 - Sulfur Redox Enzymology Axis

Structure-function relationships studies of enzymes involved in redox reactions
Catalytic/chemical mechanism
Molecular recognition



Toxicity / oxidative stress

Aldehyde dehydrogenases
Msr / TipA

Sulfur redox chemistry
Cys-SH, SOH, SSH, SO₂H, SS, SO-S, Met-O

Signaling

Srx / Prx
Yap1
3-MST / TST

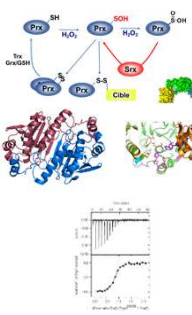
Imopa Team 3 - Sulfur Redox Enzymology Axis

Strategic approaches *in vitro*

Mechanism/Functional intermediates characterization
Thiol redox protein biochemistry
SDS PAGE
Chromatography RP GF (UPLC)
MS proteins (denaturing conditions)

Mechanism/molecular recognition
Protein protein non covalent interactions
Fluorescence based techniques
ITC
Native mass spectrometry
collaboration S. Cianferani, Strasbourg

Structural determinants of catalysis/reactivity
Structure/function analysis



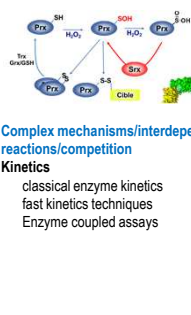
Imopa Team 3 - Sulfur Redox Enzymology Axis

Strategic approaches *in vitro*

Mechanism/Functional intermediates characterization
Thiol redox protein biochemistry
SDS PAGE
Chromatography RP GF (UPLC)
MS (non native conditions)

Mechanism/molecular recognition/complexes formation
Protein protein non covalent interactions
Fluorescence based techniques
ITC
Native mass spectrometry – large heterogenous Cx
collaboration S. Cianferani, Strasbourg

Complex mechanisms/interdependent reactions/competition
Kinetics
classical enzyme kinetics
fast kinetics techniques
Enzyme coupled assays

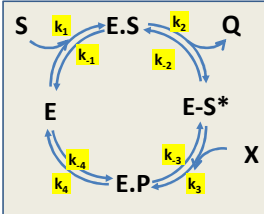


Pour catalyser une réaction chimique, une enzyme doit interagir avec son substrat

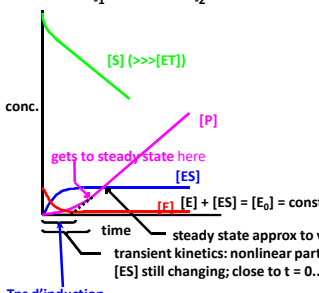
Etapes d'interactions non covalentes
Etapes de formation/rupture de liaisons covalentes

Cinétiques à l'état stationnaire
→ Signification de K_M , V_{max} ?

- Permet de proposer un mécanisme d'action plausible (ping-pong, hasard, ordonné, etc...), ordre de fixation et de départ des réactifs
- Quelle est l'étape cinétiquement limitante de la réaction?
- Ne rend pas compte des réactions entre fixation du(des) S et libération du(des) P



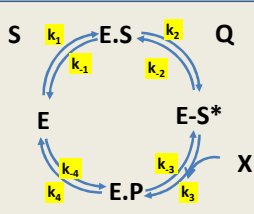
L'analyse des cinétiques à l'état préstationnaire permet de décomposer le mécanisme enzymatique

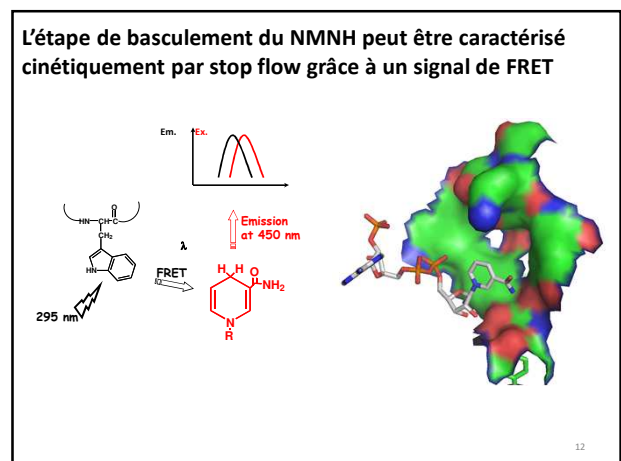
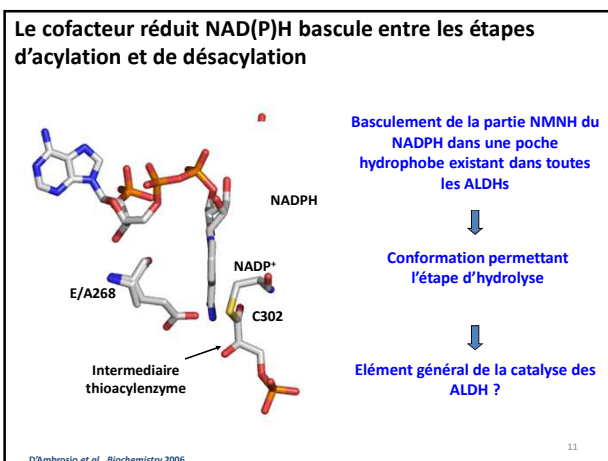
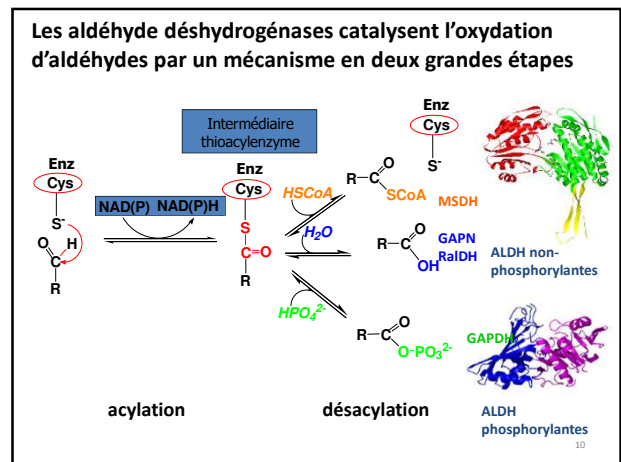
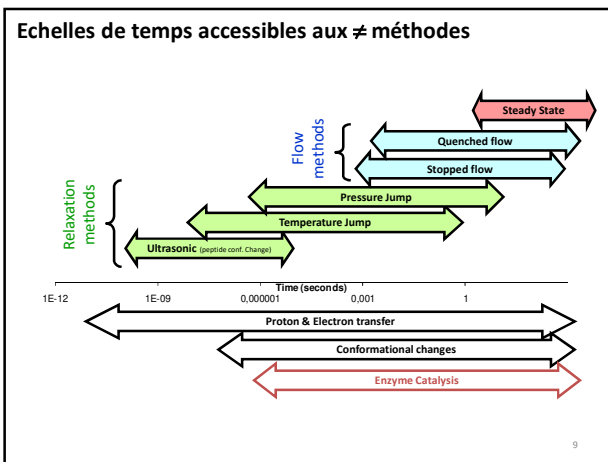
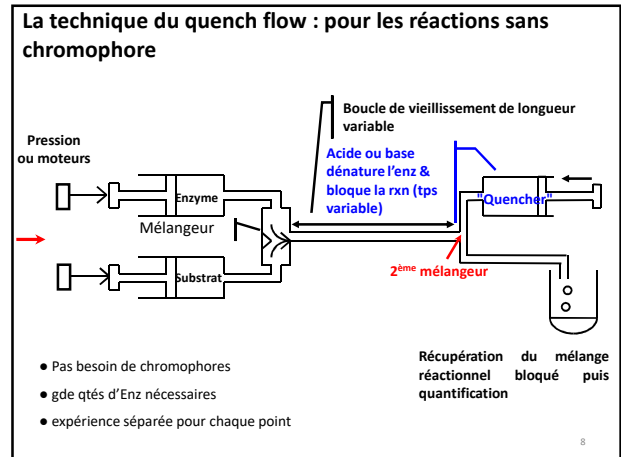
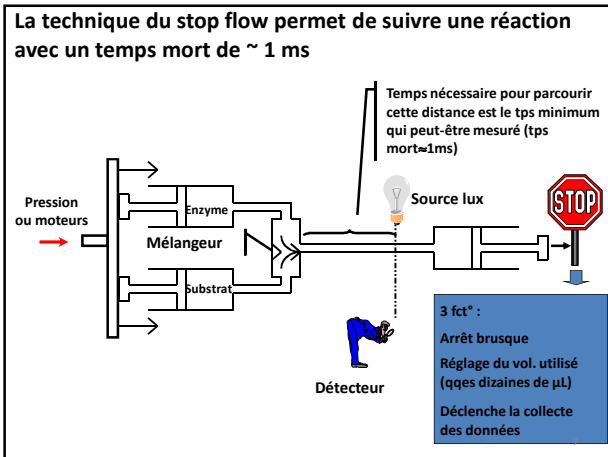
$$E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightleftharpoons[k_{-2}]{k_2} E + P$$


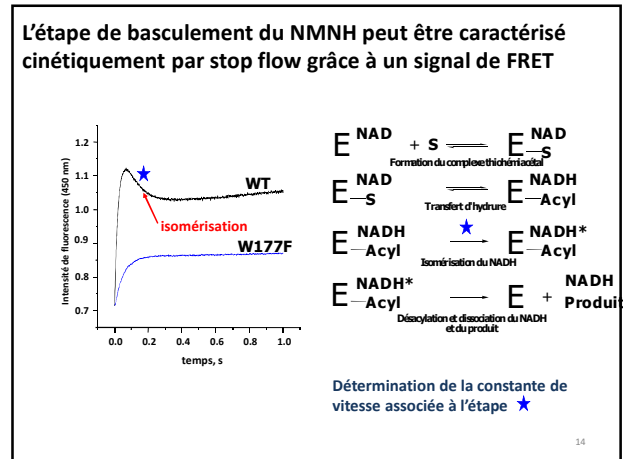
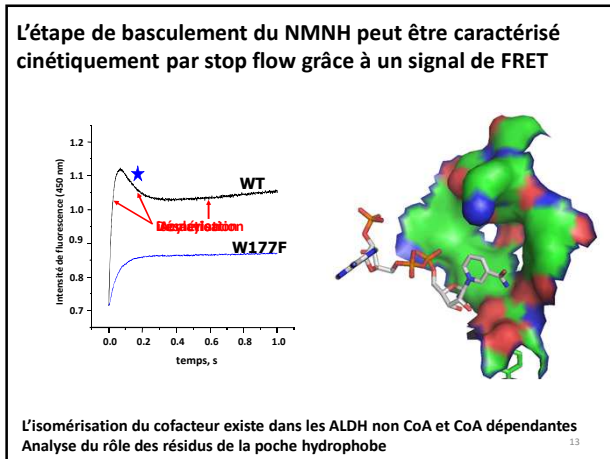
gets to steady state here [ES]

steady state approx to v_0
transient kinetics: nonlinear part, [ES] still changing; close to $t = 0...$

Tps d'induction







UNIVERSITÉ DE LORRAINE IMoPA

CNRS

I2BC

C22

The Yap1-binding protein, a scaffold protein that chaperones a cysteine-sulfenic acid in H₂O₂ signaling

UMR 7365 IMOPA
Structural and Molecular Enzymology
Nancy, France

Stress Oxydant et Cancer
IBITECS
I2BC, CEA Saclay
France

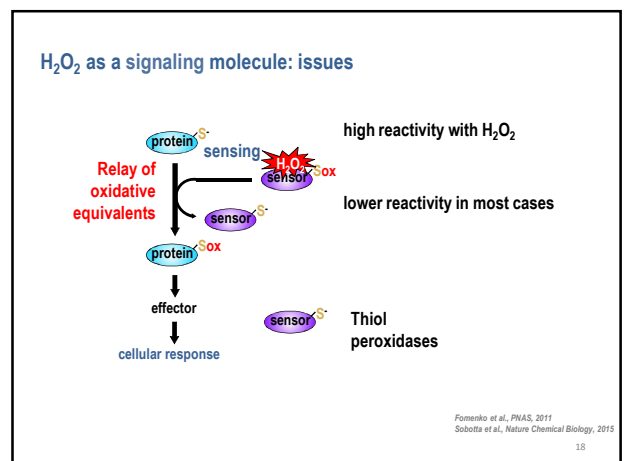
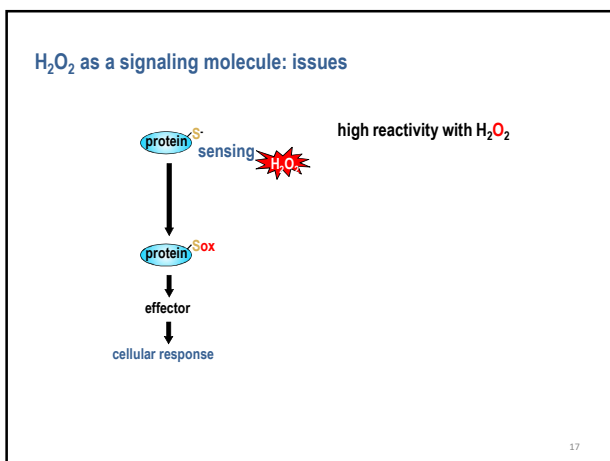
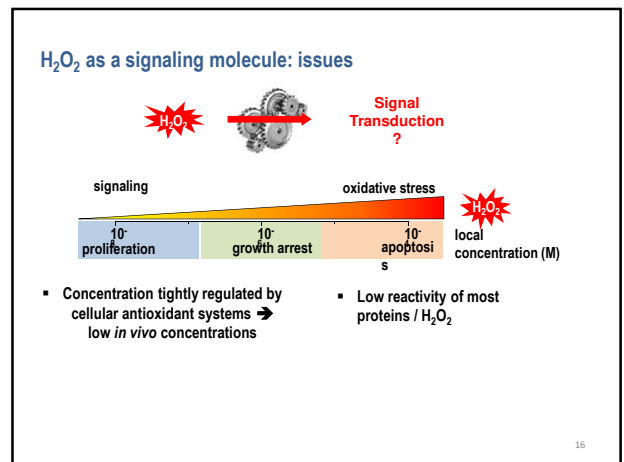
A. Bersweiler
H. Mazon
A. Kriznik
S. Rahuel-Clermont

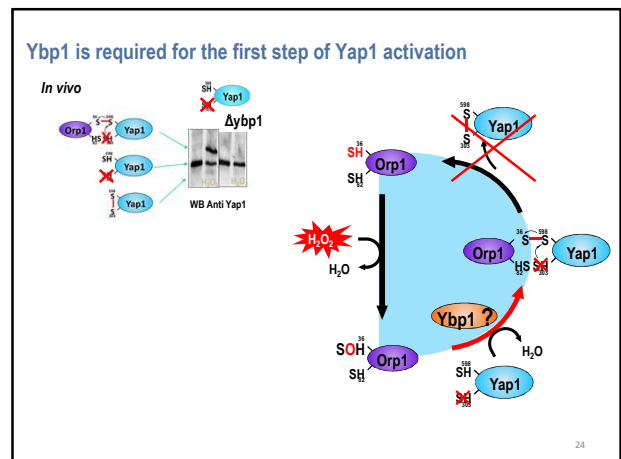
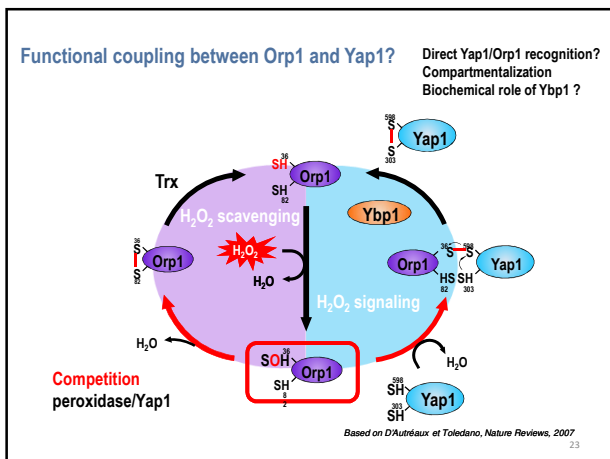
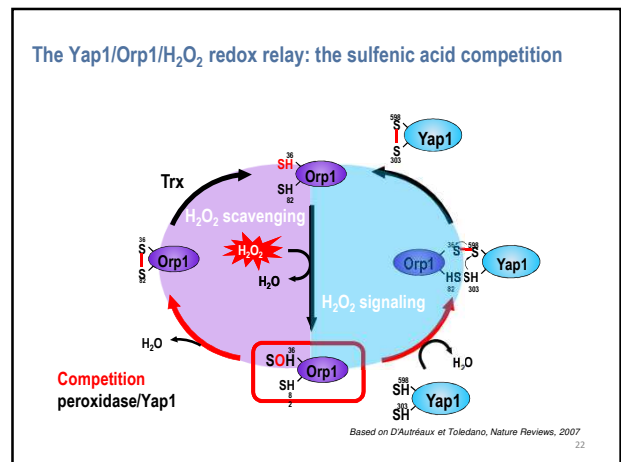
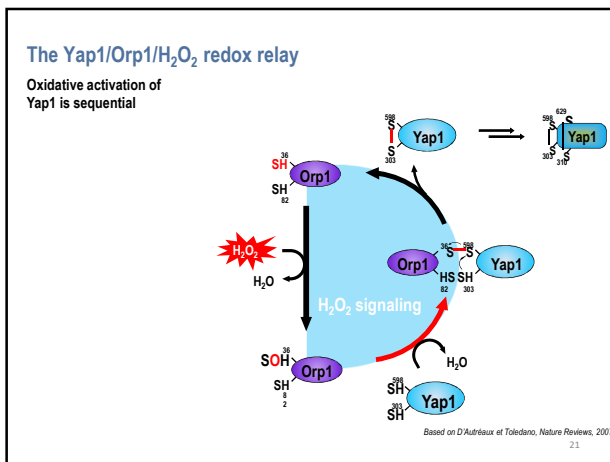
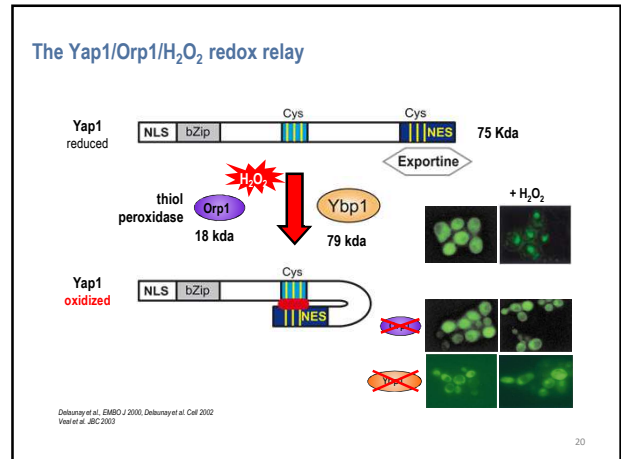
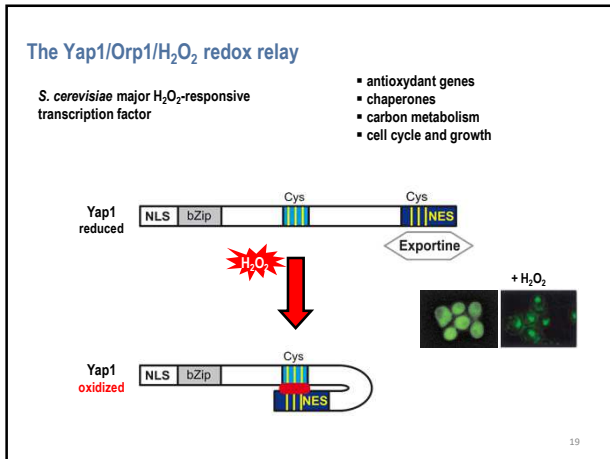
B. D'Autréaux
G. Belli
A. Delaunay-Moisan
M. Toledano

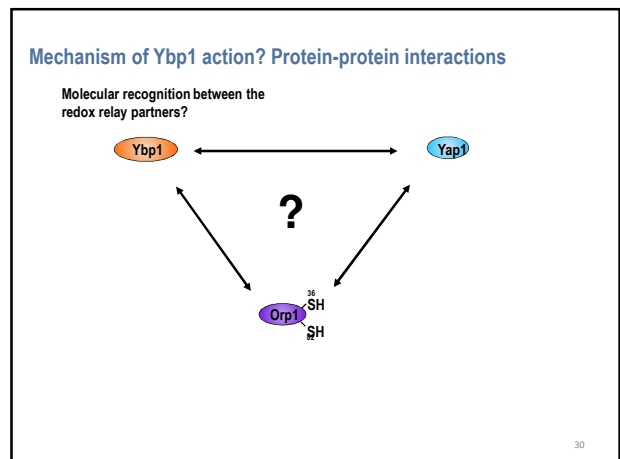
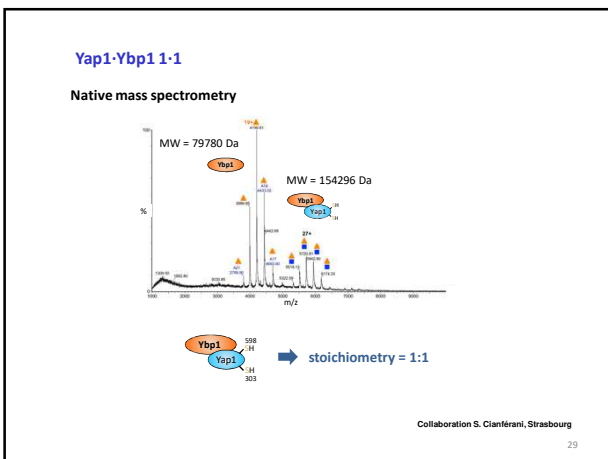
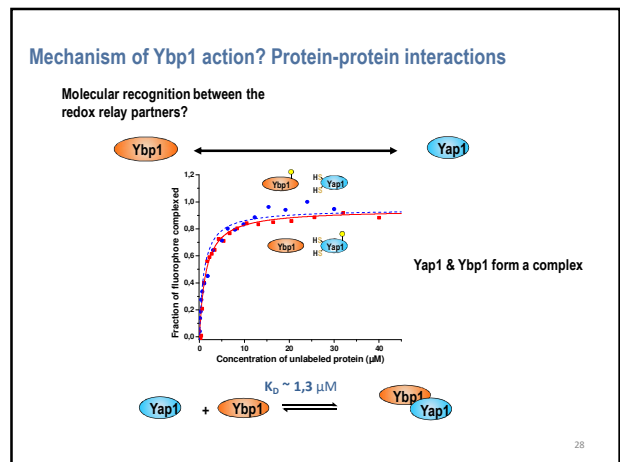
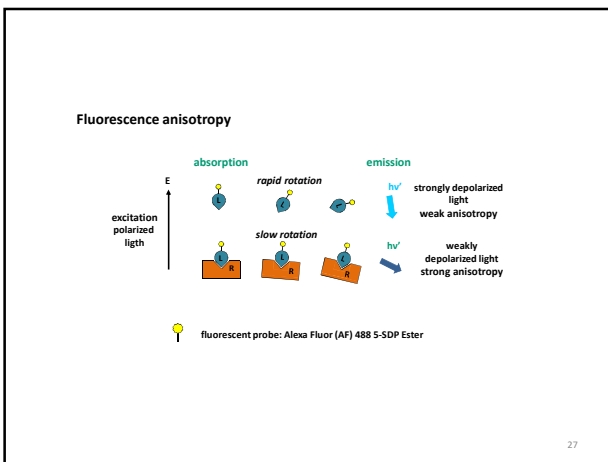
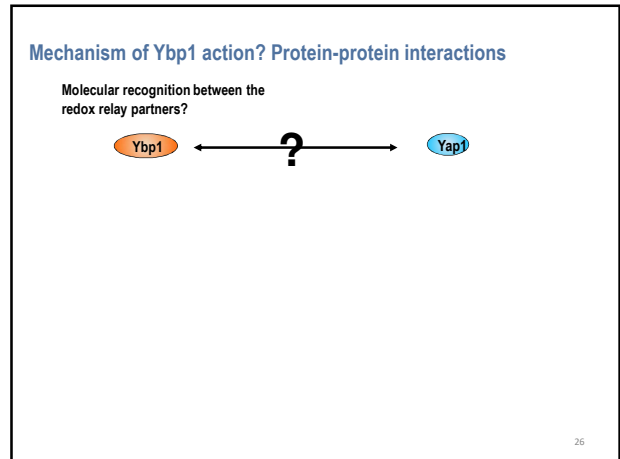
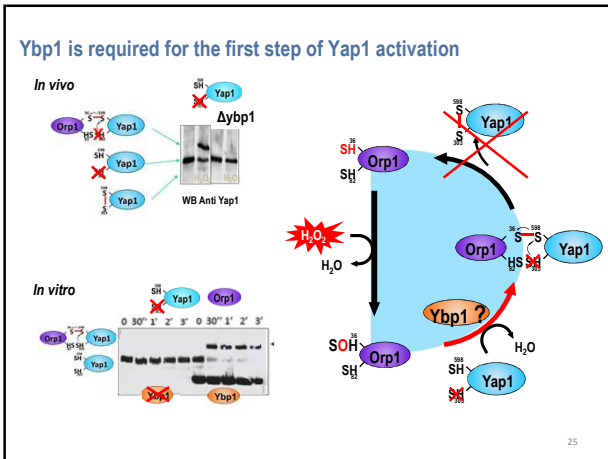
LA LIQUE

ANR

INSTITUT NATIONAL DU CANCER







Caractérisation d'un équilibre

Paramètres thermodynamiques

$$DG = -RT \ln K_d = RT \ln K_a$$

$$\Delta G = \Delta H - T\Delta S$$

Énergie libre disponible pour conduire la réaction vers l'équilibre

Facteur entropique Réorganisation structurale

Chaleur absorbée ou émise lors de la réaction

$\Delta G > 0$: réaction défavorable
 K_d élevé, faible affinité
 $\Delta G < 0$: réaction favorable
 K_d faible, forte affinité

Paramètres dynamiques

$$M + P \xrightleftharpoons[k_{on}/k_a]{k_{off}/k_d} MP$$

$$K_d = \frac{k_{off}}{k_{on}} = \frac{1}{K_a}$$

31

Etude des interactions biomoléculaires : biocapteur en phase solide – résonance plasmonique de surface

TRANSDUCTEUR
 Détecteur convertissant l'évènement de reconnaissance moléculaire en un signal analogique: variation d'une propriété de surface

- électrochimique
- acoustique
- thermométrique
- optique = phénomène SPR

Informations en temps réel
 → cinétiques

32

Principe de la résonance plasmonique de surface (système BIACORE)

Réflexion totale → résonance plasmonique (absorption d'énergie par les e⁻ libres du film métall) → chute d'intensité à un angle défini du faisceau réfléchi

Angle de la chute d'intensité (I, II) dépend de l'indice de réfraction du milieu liquide: proportionnel à la concentration massique de surface = accumulation de ●

Transducteur:
 - verre
 - film d'or
 - support d'immobilisation (dextran carboxyméthylé)

33

Surface plasmon resonance biosensor: principle of detection

Transducer:
 - glass
 - Gold film
 - Immobilization support

Integration on a microfluidic device = Sensor chip

34

Biacore 3000

Biacore T100

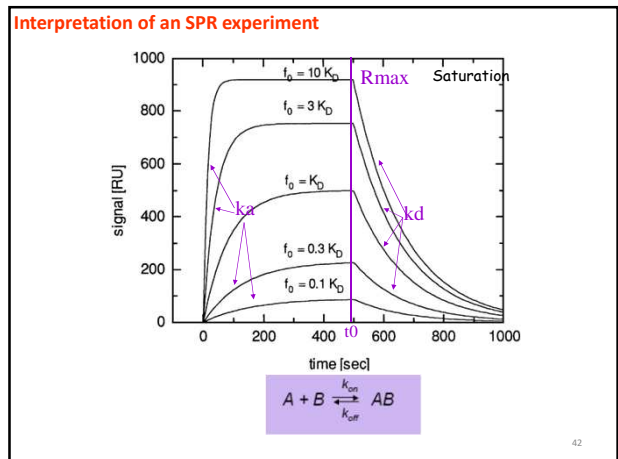
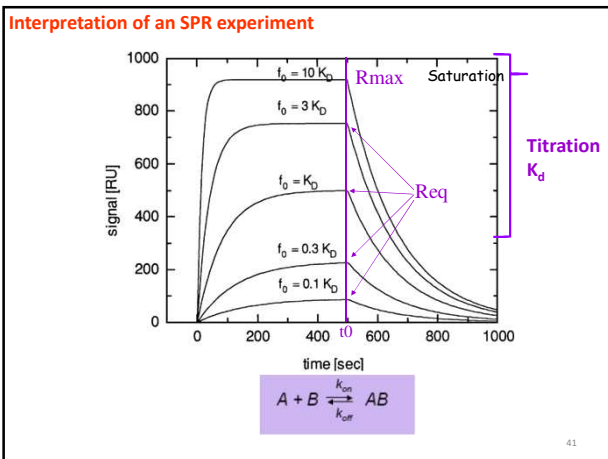
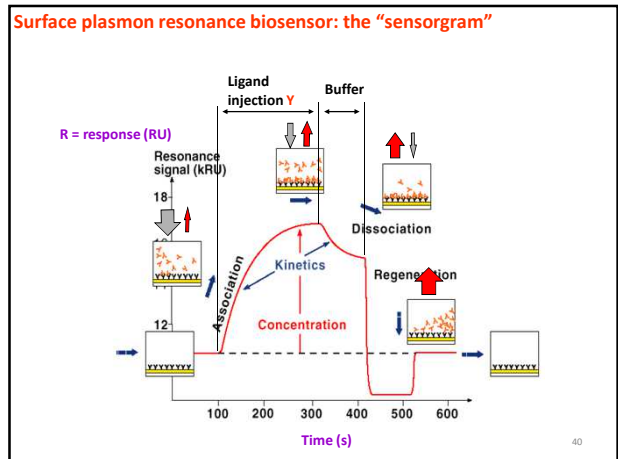
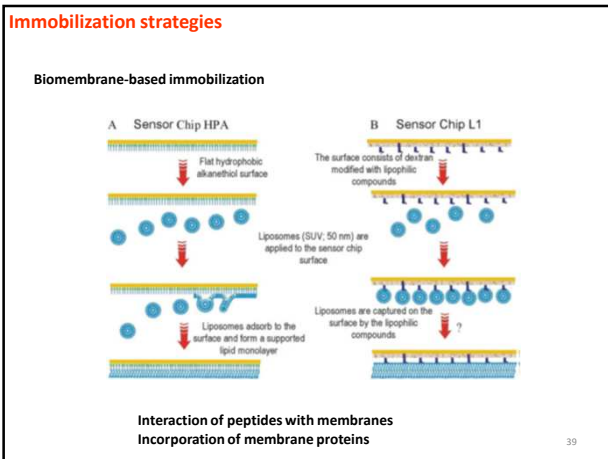
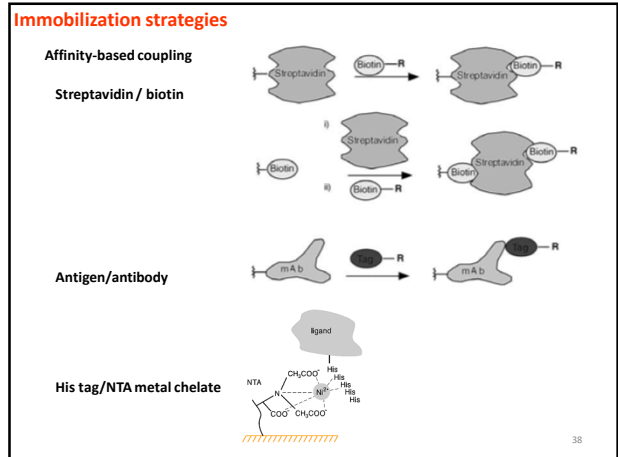
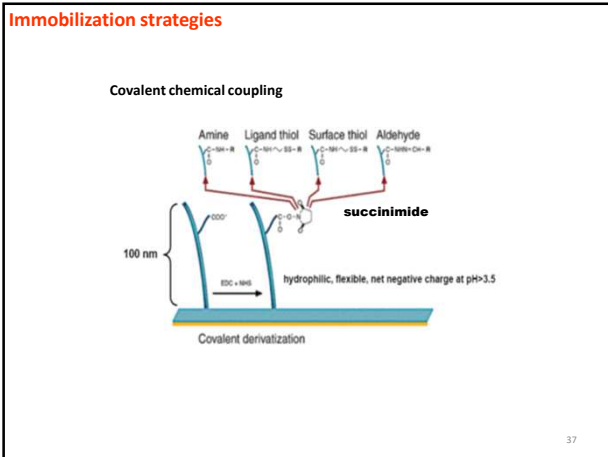
GE Healthcare Biacore
<http://www.biacore.com>

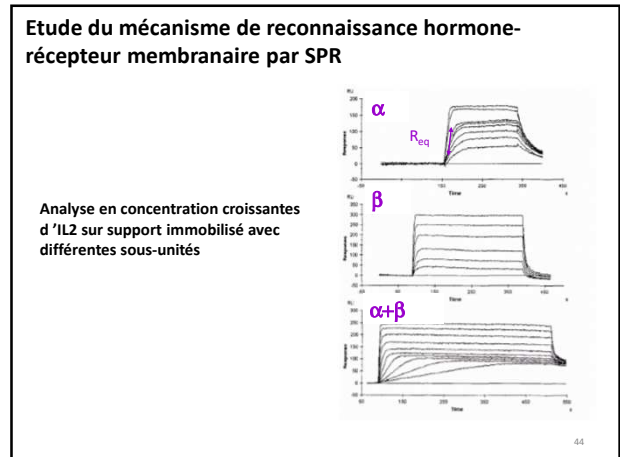
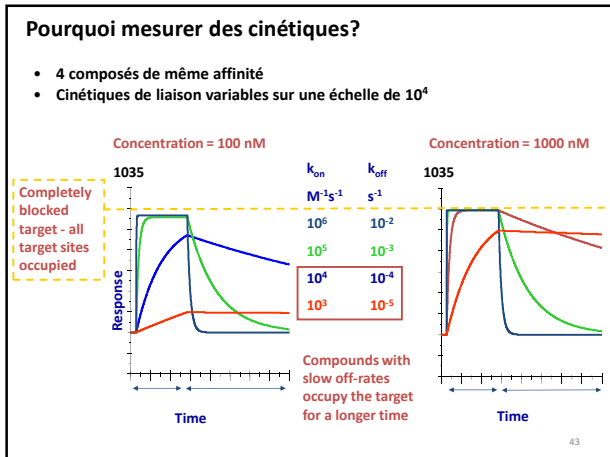
35

Immobilization strategies

- matrix : absence of non-specific interactions
 → Dextran (linear polymer of glucose) modified by carboxymethyl groups (-CH₂-COO⁻) for covalent coupling
 → very flexible, 97% H₂O (hydrogel)
- ligand: concentration, orientation
- coupling arm: length and chemical stability

36





Microcalorimétrie Ultrasensible

Mesure des échanges de chaleur associées aux réactions biologiques

- Changement de métabolisme chez les animaux
- Croissance bactérienne
- Interactions moléculaires

Equilibrium characterization : thermodynamics

$A + B \rightleftharpoons AB$

$\Delta G = RT \ln K_D = -RT \ln K_A$
 $\Delta G = \Delta H - T\Delta S$

Gibbs free energy available to drive the reaction to equilibrium: tells if the reaction is spontaneous or not

Entropic term
Structural or water reorganization "hydrophobic effect"

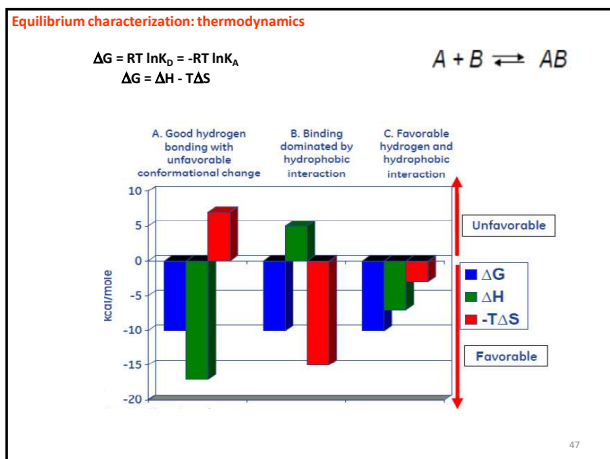
Heat absorbed or released upon complex formation
This is what is measured in ITC

$\Delta G > 0$: reaction non spontaneous
High K_D , low affinity
 $\Delta G < 0$: reaction favorable
Low K_D , high affinity

What do these energies physically mean?

- ΔS Interface desolvation
- ΔH vdW, H-bonds, ionic bonds
- ΔS Conformational change

$\Delta G^\circ = RT \ln K_D = \Delta H^\circ - T\Delta S^\circ$



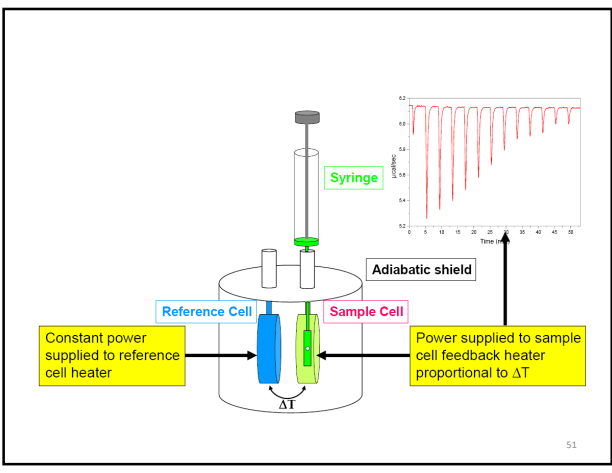
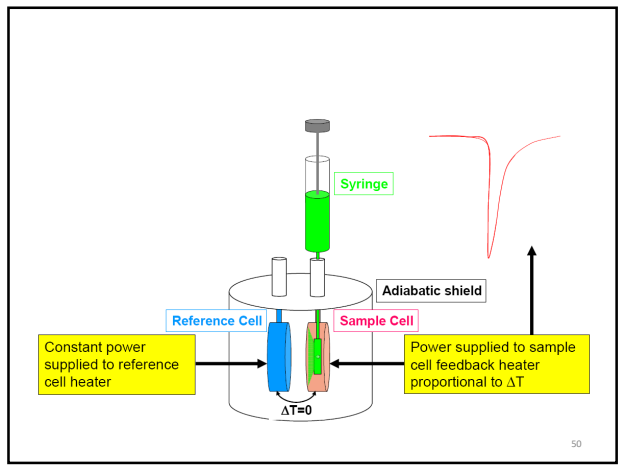
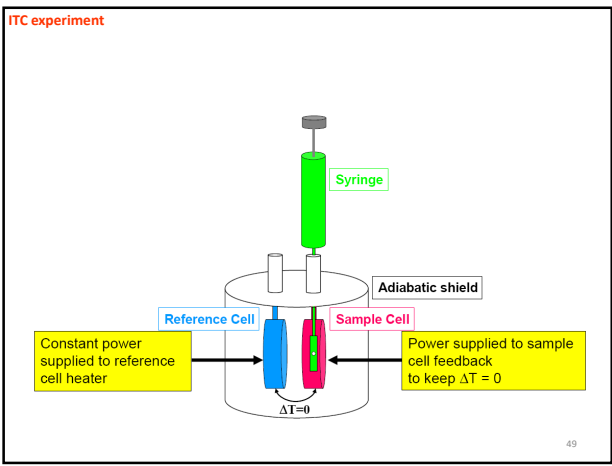
How does ITC work?

Measure of $\Delta P = P_{mes} - P_{ref}$

$\Delta P > 0 \rightarrow T_{mes} < T_{ref}$
 $\Delta P < 0 \rightarrow T_{mes} > T_{ref}$

$\Delta P > 0 \Leftrightarrow$ endothermic reaction
 $\Delta P < 0 \Leftrightarrow$ exothermic reaction

Power compensation design \rightarrow Higher sensitivity



ITC data analysis

Interaction of P (syringe) with M (cell) $M + P \rightleftharpoons MP$

$$v = \frac{[MP]}{[M]_T} = \frac{\frac{[P]}{K}}{1 + \frac{[P]}{K}}$$

Following each injection of P, the heat released is:

$$q_i = V \Delta H \Delta [P]_{lib}$$

Volume of cell Increase in the concentration of bound ligand P following this injection

Binding enthalpy Specific to the interaction

$$\sum q_i = V [MP] \Delta H \quad v = \frac{[MP]}{[M]_{total}} = \frac{\sum q_i}{V [MP] \Delta H}$$

52

ITC data analysis

Interaction of P (syringe) with M (cell) $M + P \rightleftharpoons MP$

$$v = \frac{[MP]}{[M]_T} = \frac{\frac{[P]}{K}}{1 + \frac{[P]}{K}}$$

Following each injection of P, the heat released is:

$$q_i = V \Delta H \Delta [P]_{lib}$$

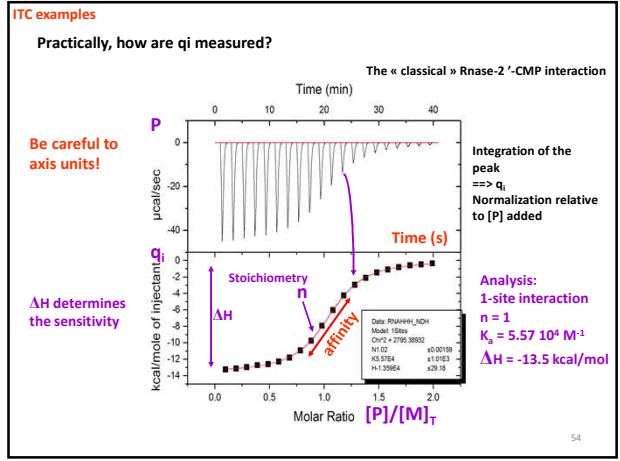
Volume of cell Increase in the concentration of bound ligand P following this injection

Binding enthalpy Specific to the interaction

Here the conditions: $[M] < K_D$ and $[P] \gg [M]$ then $[P] \sim [P]_{total}$ do NOT hold true

Either a direct solution exists $q_i = f([P]_{total}) \Rightarrow \Delta H, n, K_D$
Or must use a numerical solution $\Delta G = -RT \ln K_D$ et $T \Delta S = \Delta H - \Delta G$

53



Design of experimental conditions

The shape of the titration $q_i = f(P/M_i)$ depends on K_d and $[M]_{total}$

$c = [M]_i n / K_d$

Working window $1 < c < 1000$

55

Exemples d'applications de l'ITC

Protéine 50% active

Protéine 100% active

Mutant affinité 10%

Protéine native

56

Mechanism of Ybp1 action? Protein-protein interactions

Molecular recognition between the redox relay partners?

57

Ybp1 recruits Orp1 and Yap1 into a ternary complex

Orp1 does not interact with Yap1
Orp1 interacts with Ybp1

$K_D = 0,8 \mu M$

$N \sim 1$

$K_D = 0,7 \mu M$

$N \sim 1$

58

Orp1 interacts with Ybp1-Yap1

Two hypotheses

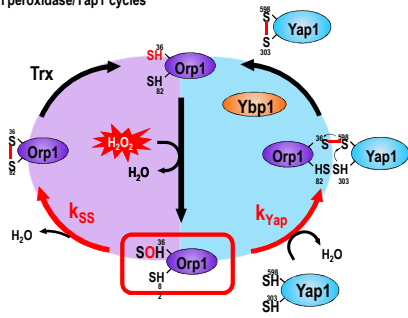
59

Ybp1 allows the formation of a ternary complex

60

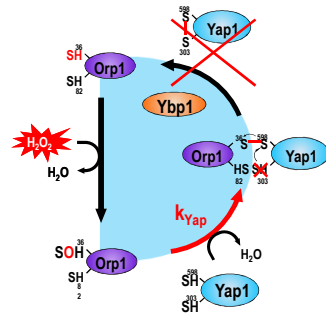
Competition in Yap1 pathway: a kinetic approach

Competition peroxidase/Yap1 cycles



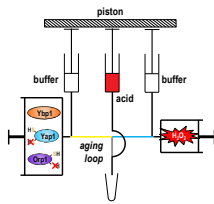
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Competition in Yap1 pathway: a kinetic approach



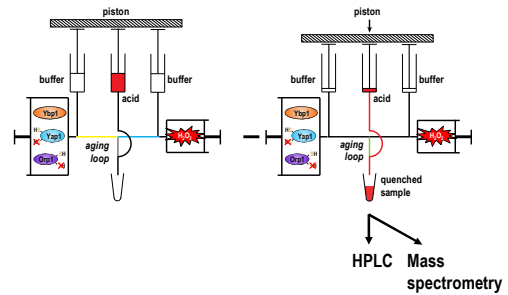
62

Method : principle of quenched-flow



63

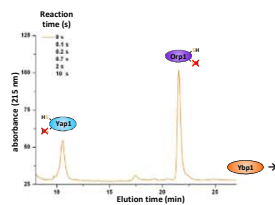
Method : principle of quenched-flow



64

k_{Yap} determination

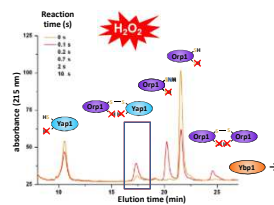
Orp1 C82S



65

k_{Yap} determination

Orp1 C82S



66

