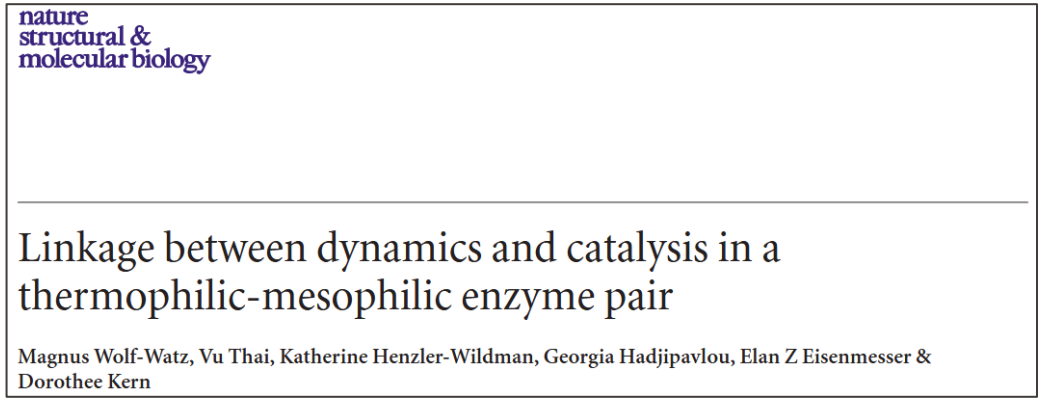
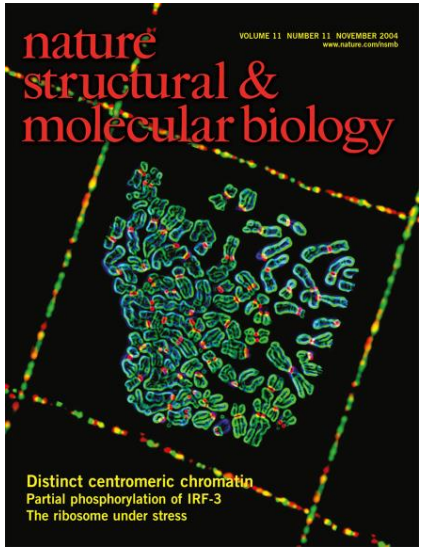
Linkage between dynamics and catalysis in a thermophilic-mesophilic enzyme pair

Vera Dias Gomes
Coralie Charpié
Coralie Reuse
Cédric Bärlocher



Introduction

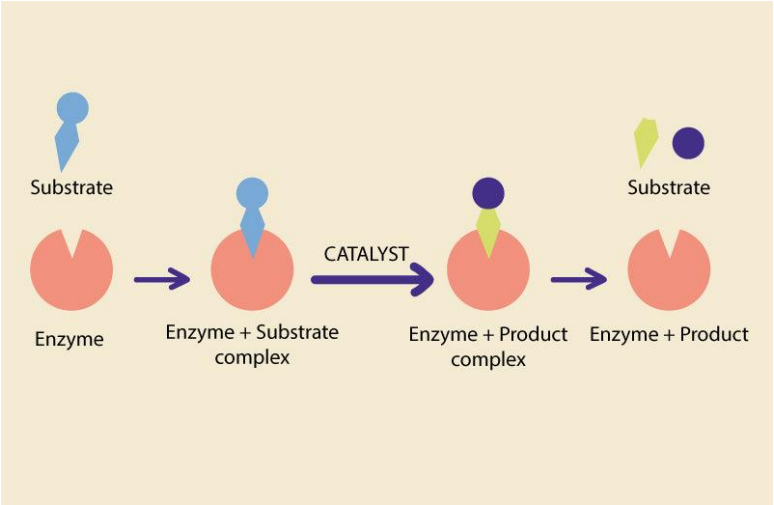
- Published in Nature structural & molecular biology
- 2004
- Brandeis University



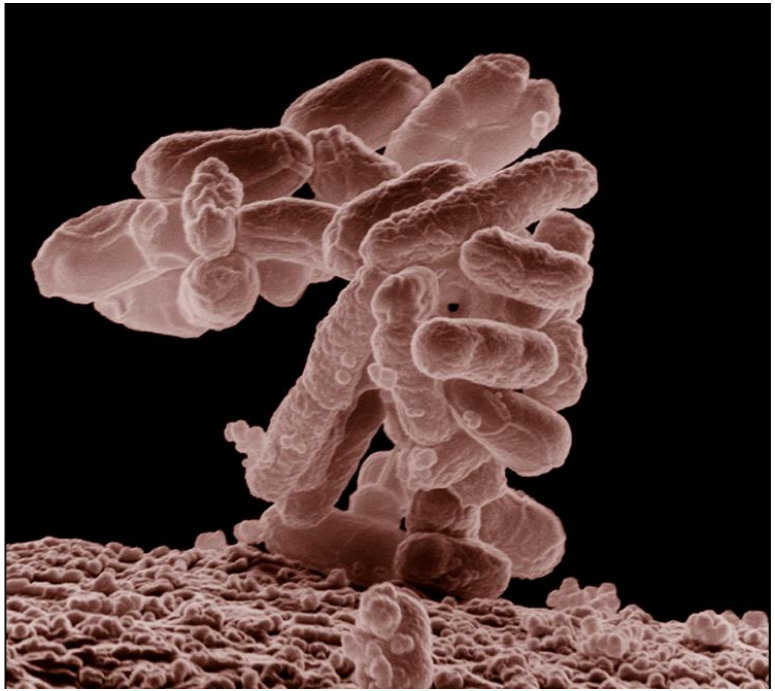
"Linkage between dynamics and catalysis in a thermophilic-mesophilic enzyme pair"

Introduction – Main issue

- What are main issues involved in enzymatic catalysis?
- 3D structure of enzymes
  - ➢Structural biology
- Internal dynamics
  - ➢Poorly understood



Introduction – definition

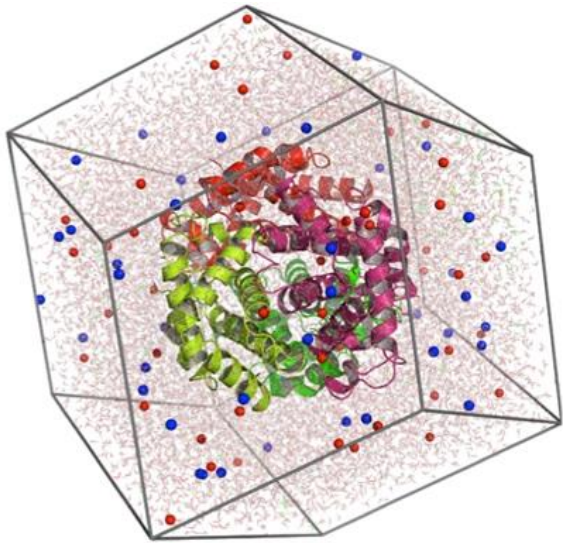


- **Thermophile**: organism living at high temperatures.
  - ➢contains very stable enzymes
- **Mesophilic** : organisms living in more moderate conditions
  - ➢Better catalytic activity

Intro – hypothesis

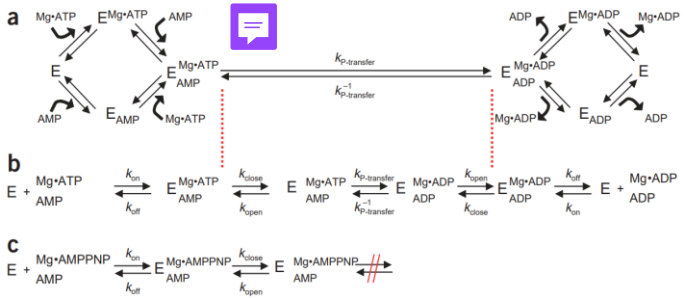
- The low activity of thermophilic enzymes at room temperature could be explained by reduced molecular dynamics

- Great stability ⟶ less flexibility
  - ➢ slow down of certain internal movements, essential for catalysis.



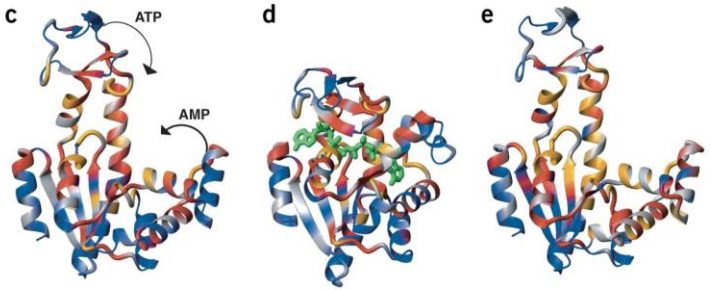
Introduction - research model

- Two homologs of the same enzyme, adenylate kinase (Adk)
  - **mesoAdk,** → mesophilic Escherichia coli,
  - **thermoAdk,** → thermophilic Aquifex aeolicus.

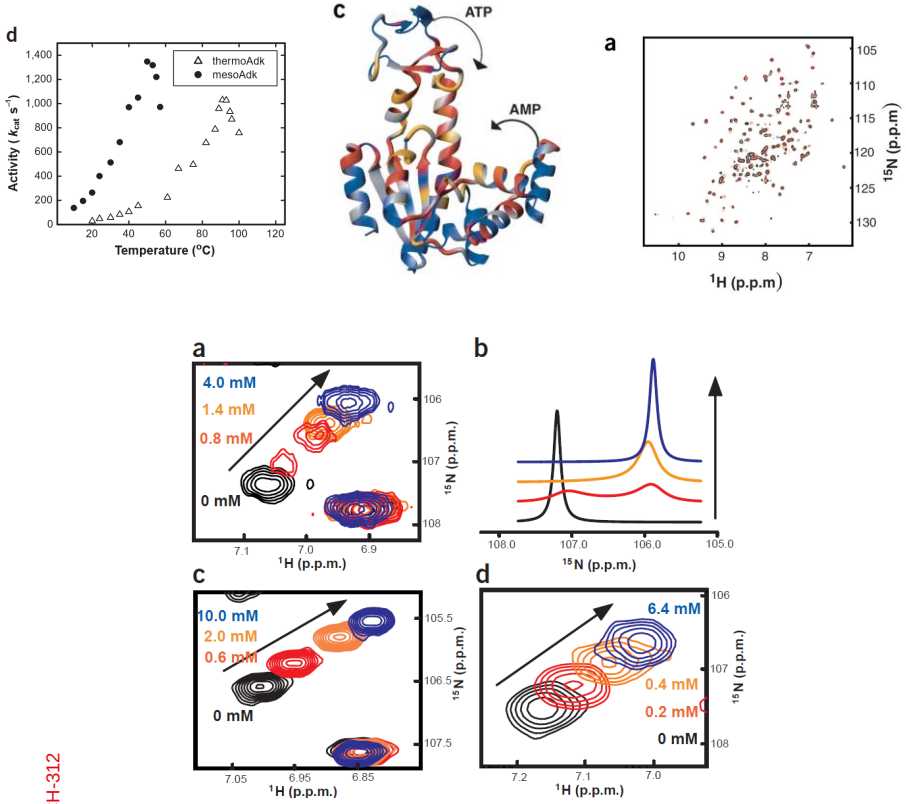


- Conversion of ATP and AMP into two molecules of ADP.

Introduction – approach



- Measurements of enzyme activity at different temperatures
- NMR measurements of dynamics
- Link the dynamic properties of the proteins to their catalytic capacity

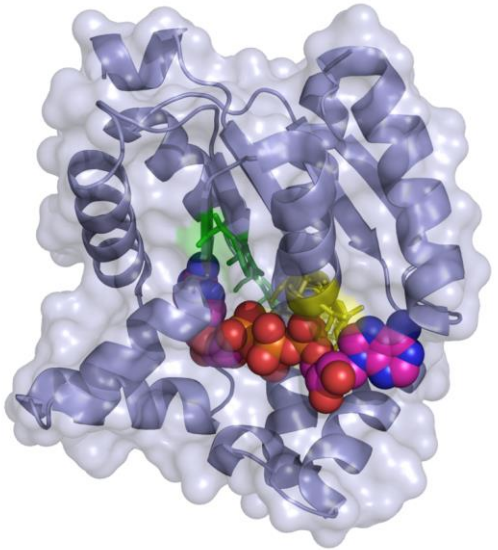


Experimental techniques and Results

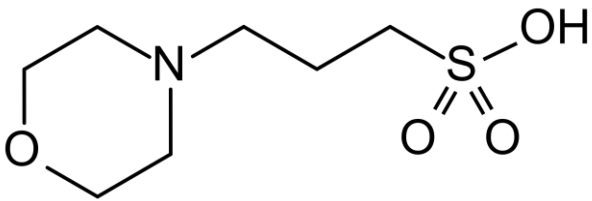
Study the catalytic activity and dynamics of the enzymes

Protein preparation

- MesoAdk and thermoAdk recombinantly expressed in E.coli.
  - ➢ Purified by chromatography.
- NMR : 15N-labeling, MOPS buffer.
  - ➢ Detect backbone amides.
- Preparation set up to mimic the conditions needed to monitor dynamics during catalysis.



Top : adenylate kinase (Adk)
Bottom : MOPS



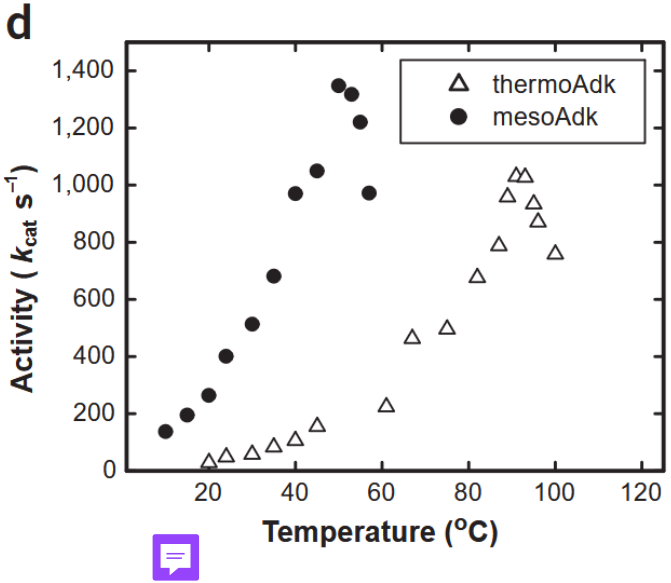
Coupled continuous spectroscopy assay

- Goal : compare how catalytic activity changes with temperature for the mesophilic Adk (mesoAdk) and the thermophilic one (thermoAdk).

- Tracks either ADP or ATP formation over time, by continuously monitoring the reaction.

- Coupling enzymes and nucleotides degrade at above 45°C…
  - ➢ couldn't use this continuous method for everything.

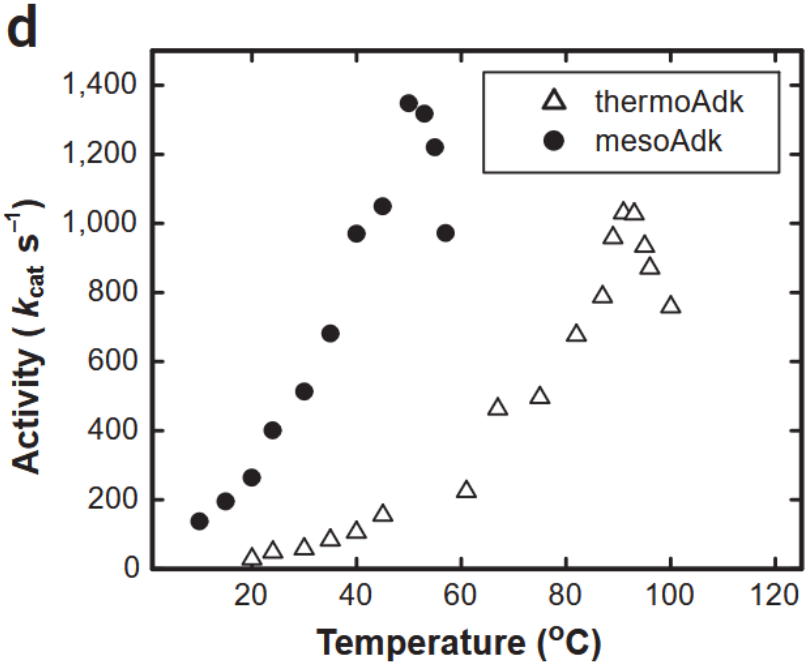
d : temperature dependence of turnover numbers in the direction of ADP formation



Activities of mesoAdk and thermoAdk as a function of temperature

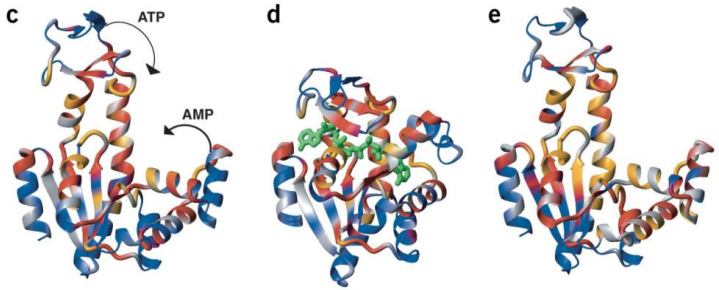
- Comparison of catalytic activities at different temperatures
- Quantification of kinetics parameters
  - Similar shapes
  - Optimal growth temperature
  - Same profiles for reverse process

d : temperature dependence of turnover numbers in the direction of ADP formation

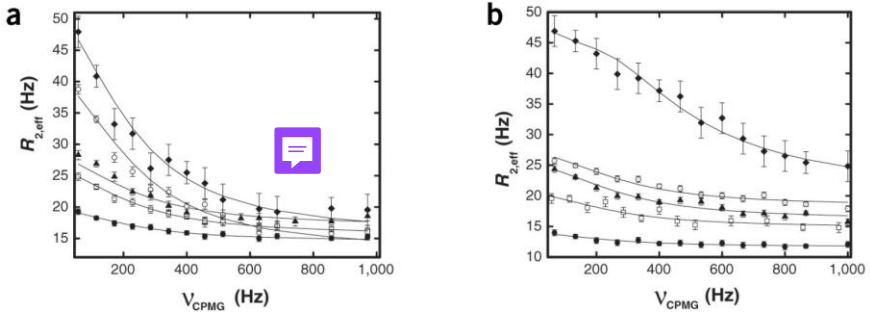


15N NMR relaxation dispersion experiments

- To detect how the protein moves between two conformations (open/closed).
- CPMG (Carr-Purcell-Meiboom-Gill) relaxation dispersion.
  - Good for two-state exchange.
- At 20°C : mesoAdk is active.
- Dynamics captured reflect the phosphotransfer step.



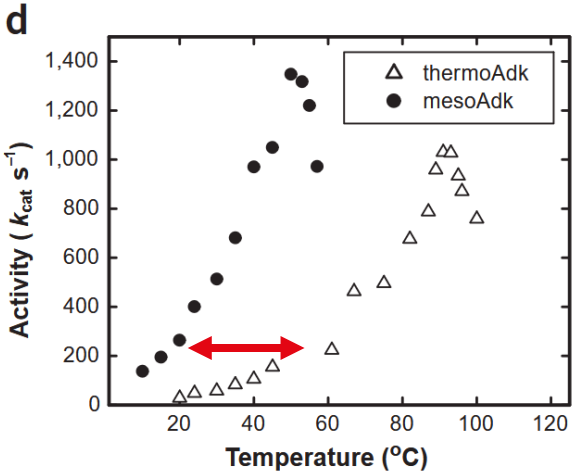
c : mesoAdk open state
d : mesoAdk closed state
e : thermoAdk open state



a : 15N NMR relaxation dispersion, globally fitted, curve for mesoAdk.
b : 15N NMR relaxation dispersion, globally fitted, curve for thermoAdk.

Backbone dynamics during turnover

- Measure of dynamic turnover using 15N NMR relaxation techniques
  - 20°C for mesoAdk
  - 50°C for thermoAdk
- Phosphotransfer step measurement
  - Wide distribution of the dynamic hotspots
- Single exchange rate constant:
  - 1660 ± 90 s^{-1} for mesoAdk
  - 1615 ± 100 s^{-1} for thermoAdk at 20°C



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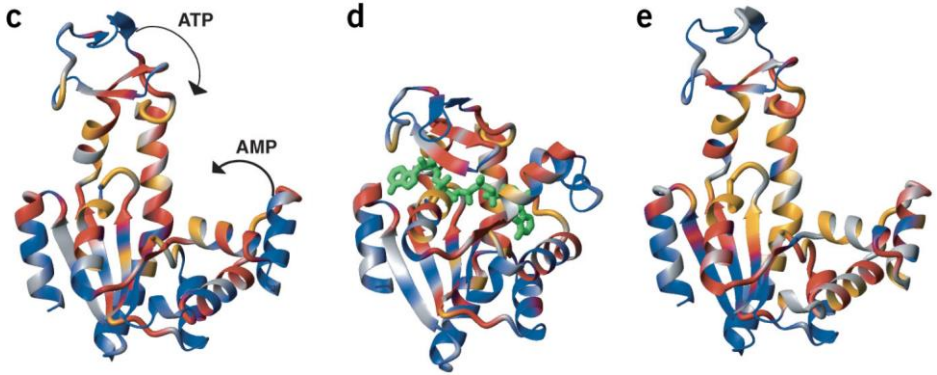
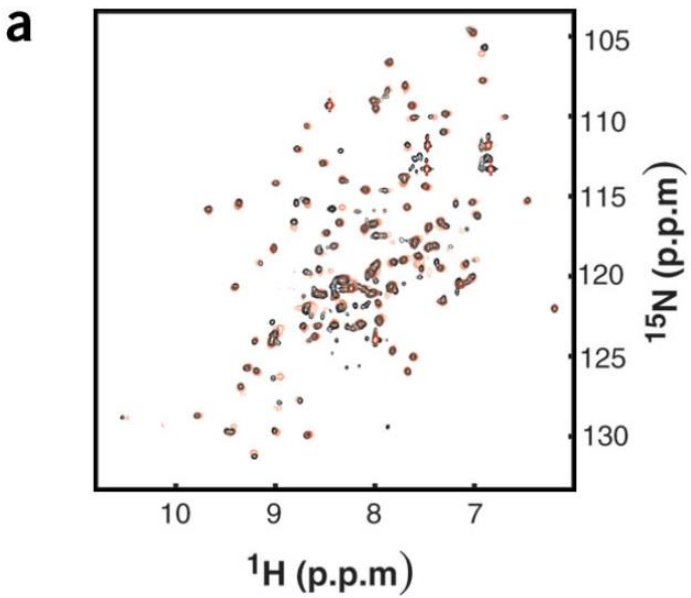


c : mesoAdk open state
d : mesoAdk closed state
e : thermoAdk open state

1H-15N correlation spectrum

- Looks at protein backbone dynamics in the presence of AMP and AMPPNP (non-reactive ATP analog).
  - blocks the phosphotransfer.

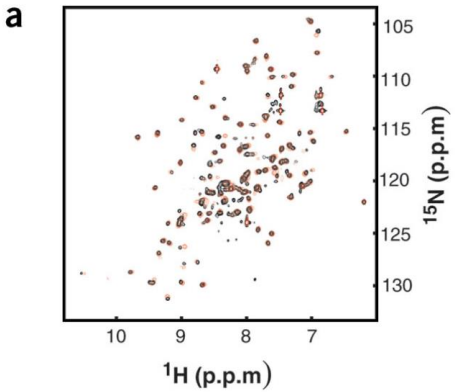
- Test if the motions observed are linked to lid movement rather than the chemical reaction.



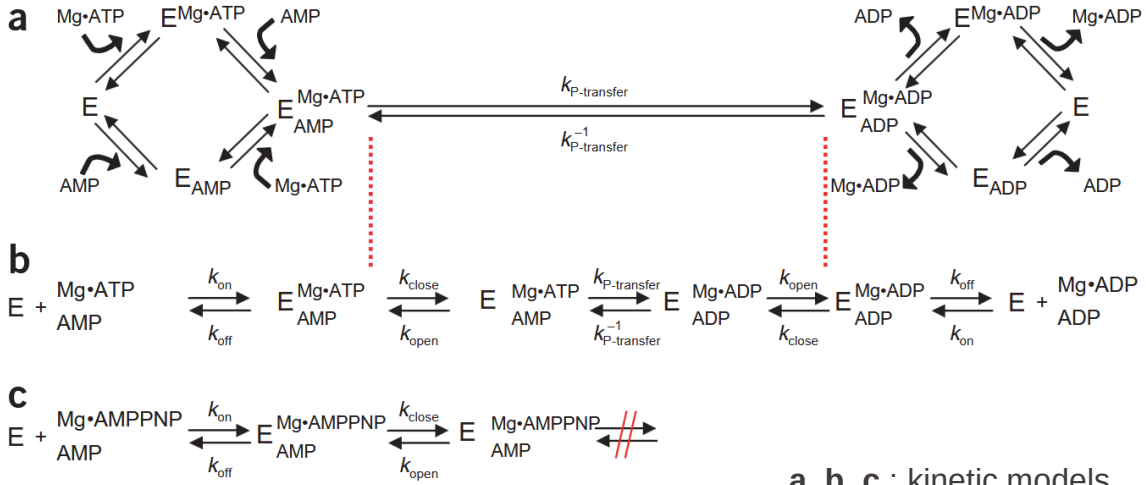
a : 2D 1H-15N correlation spectra of thermoAdk

Backbone dynamics in the presence of AMP and AMPPNP

- Measure of enzyme dynamics of thermoAdk with and without turnover
  - lid-opening and closing is detected in relaxation experiments during catalysis
- Fitting of 15N NMR
  - $k_{ex} = 1410 \pm 100\ s^{-1}$
  - same dynamic process with & without substrate



a : 2D 1H-15N correlation spectra

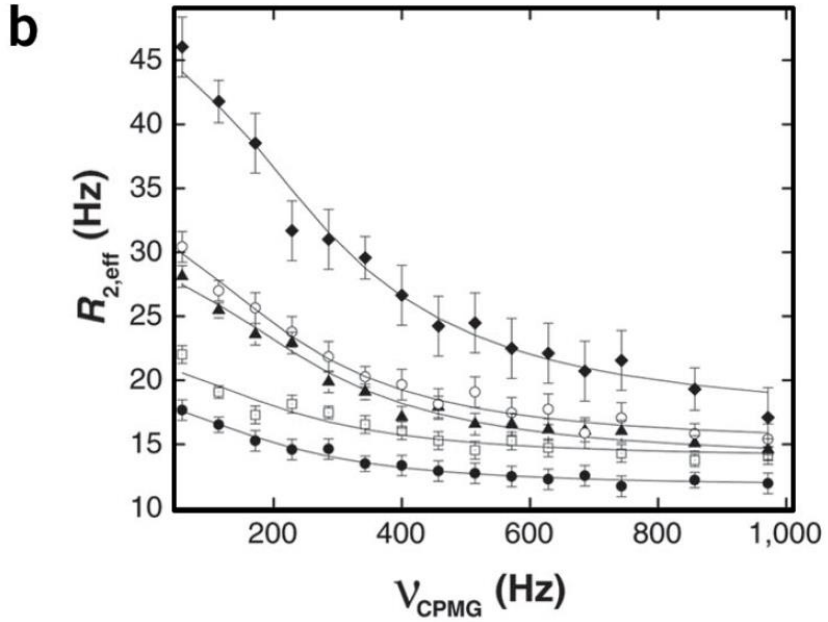


a, b, c : kinetic models

Intermediate NMR time regime

- The exchange between open and closed states happens at a similar rate to the frequency difference between the two states in NMR.

- Estimate how fast the lid opens and closes.
  - measurements at two different magnetic field strengths.

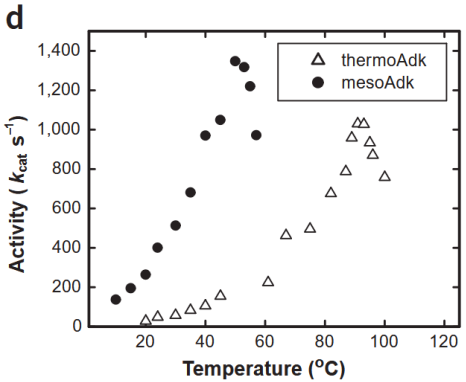


b : residues of 15N NMR relaxation dispersion profiles for thermoAdk (data globally fitted).

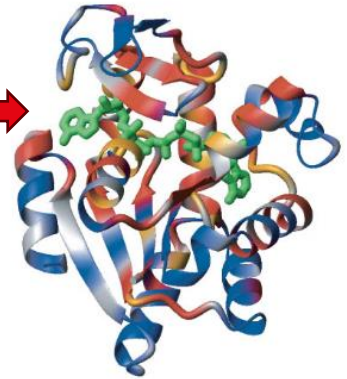
Determination of lid-closing and opening rates

- CPMG relaxation dispersion experiments

	thermoAdk	mesoAdk
k_{open}	44 ± 20 s^{-1}	286 ± 85 s^{-1}
k_{cat}	30 ± 10 s^{-1}	263 ± 30 s^{-1}
k_{close}	1571 ± 100 s^{-1}	1374 ± 110 s^{-1}

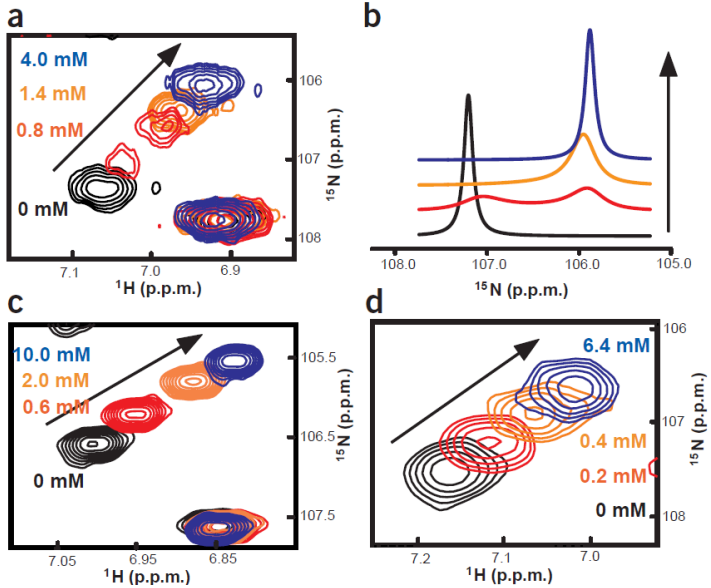


- Does the dynamic correspond to lid closure and opening?
  - similar chemical shifts during turnover and in the closed state
  - chemical shifts in relaxation dispersion and in open and close state are similar



Substrate titration experiments

- Gradually increased ligand concentration and monitored how the chemical shifts changed.
  - ➢ evidence for the slow dynamic process of thermoAdk
- 20°C : gradual shifts.
  - ➢ fast exchange.
- Intermediate ligand concentration: peak splitting.
  - ➢ slower opening of the lid.

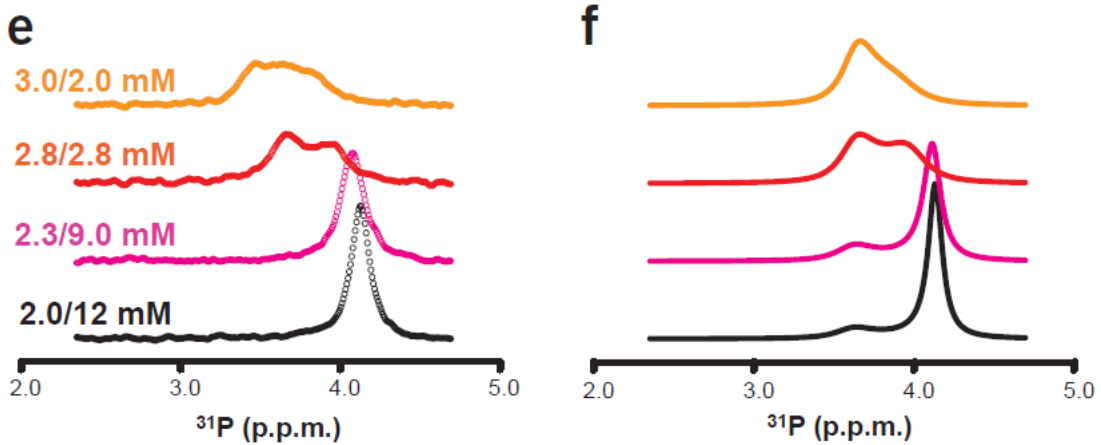


Chemical shifts of thermoAdk at :
- 20°C (**a**)
- 70°C (**c**)

b : lineshape simulation of **a**.
d : chemical shift of mesoAdk at 20°C.

31P NMR

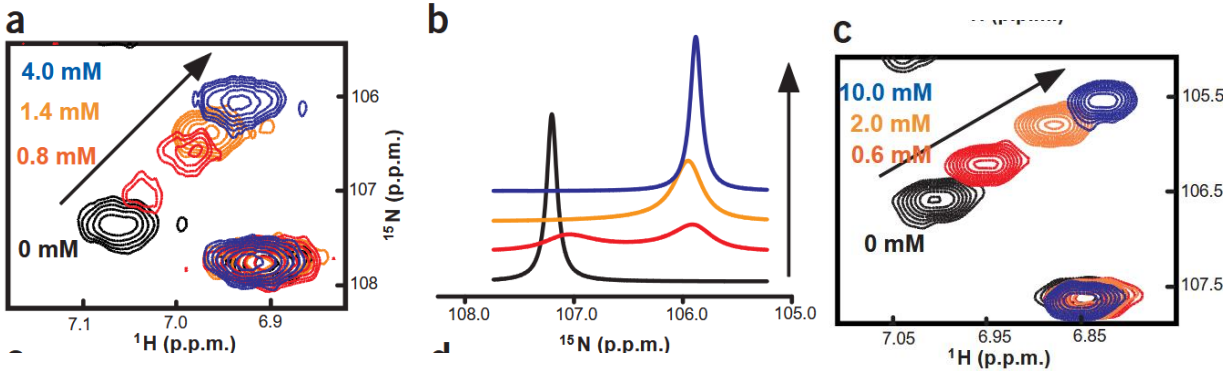
- To get more informations on the dynamics of thermoAdk at 20°C.



e : experimental 31P lineshape of AMP, with increasing thermoAdk concentrations
f : theoretical 31P lineshape of AMP, with increasing thermoAdk concentrations

Substrate-enzyme titration experiments

- Chemical shifts are due to conformational rearrangement



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- 20°C (**a**)
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