

# Conformation dynamics and catalysis of aldolase studied by NMR

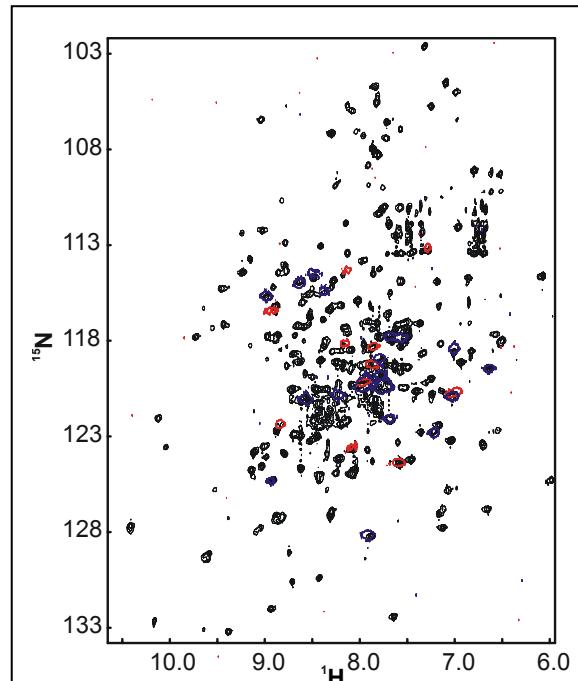
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## Introduction

Conformational changes appear vital for both enzyme catalysis and regulation, however these essential motions are poorly understood at present. NMR relaxation experiments allow residue specific characterization of these dynamic motions. *Escherichia coli* Class II fructose-1,6-bisphosphate aldolase (FBP-aldolase) provides an excellent system, as it has been extensively studied at Leeds University via X-ray crystallography and site directed mutagenesis, coupled with kinetic analysis. These studies have revealed the essential role played by the protein's flexible loops during catalysis. FBP-A adopts the  $(\alpha/\beta)_8$  barrel fold which is a common and versatile architecture, representing 10% of all known enzyme structures. Enzymes exhibiting an  $(\alpha/\beta)_8$  barrel fold have been found for 61 different types of E.C. number, including all primary classes, with the exception of ligases. However, at present, no high resolution dynamic studies have been completed for such systems. The flexible structure provides an excellent platform for evolving novel enzymes and a thermostable mutant has already been previously evolved in Leeds. This work will provide the first general information on dynamics during catalysis of a "large" enzyme by modern NMR methods, and will provide the basis for the development of further novel catalysts.

## Backbone amide resonance assignment

FBP-aldolase forms a 78kDa dimer, and, as such, its size represents a significant challenge to study by NMR. To date, there only is one example of a larger polypeptide chain that has been fully assigned. A combination of HNCA, HN(CO)CA, HNCO, HN(CA)CO and HSQC TROSY based experiments (Fig. 1) using an isotopically enriched [ $^2\text{H}$ ,  $^{15}\text{N}$ ,  $^{13}\text{C}$ ]-FBP-aldolase sample have been completed. This has resulted in a partial backbone amide assignment, which includes residues from the major  $\beta 5-\alpha 7$  flexible loop. This has been aided and confirmed via a number of selectively labeled samples including [ $^{15}\text{N}$ -Lys]-FBP-aldolase, [ $^{15}\text{N}$ -Glu]-FBP-aldolase, [ $^{15}\text{N}$ -Tyr]-FBP-aldolase and [ $^{15}\text{N}$ -Val]-FBP-aldolase samples. Residual dipolar coupling measurements have been measured on both [ $^2\text{H}$ ,  $^{15}\text{N}$ ,  $^{13}\text{C}$ ]-FBP-aldolase and [ $^{15}\text{N}$ -Lys]-FBP-aldolase aligned in a dilute crystalline media which, in conjunction with previously obtained crystal structures, will assist in further backbone assignment.

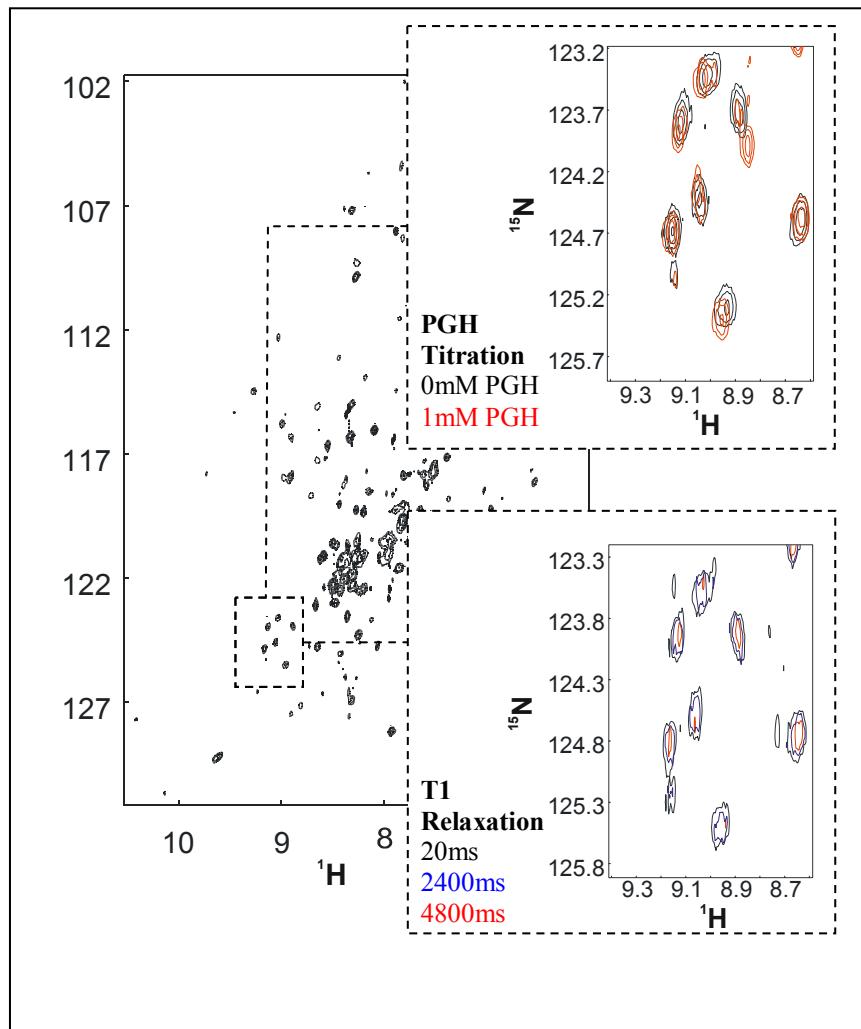


**Fig 1.** HSQC spectra of the amide resonances in [ $^2\text{H}$ ,  $^{15}\text{N}$ ,  $^{13}\text{C}$ ]-FBP-aldolase measured at 900MHz are shown in black. Overlaying resonances from [ $^{15}\text{N}$ -Lys]-FBP-aldolase and [ $^{15}\text{N}$ -Tyr]-FBP-aldolase are shown in blue and red respectively (measured at 750MHz).

## Characterization of nanosecond motions

T1, T2, and hetero-nuclear NOE relaxation rates have been attained and, for assigned residues, nanosecond motions have been quantified using Lipari-Szabo model-free formalism. This has confirmed the increased mobility of the  $\beta 5-\alpha 7$  loop with respect to other more stable regions of protein's architecture. Relaxation data has also been obtained in presence of a substrate analogue and inhibitor, phosphoglycolohydroxamate (PGH) (Fig. 2),

resulting in significant changes in both chemical shift and relaxation times. Relaxation data will also be obtained for natural substrate complexes, dihydroxyacetone phosphate, glyceraldehyde 3-phosphate, and FBP. This, coupled to a full assignment, will allow a complete description of enzyme motions during the catalytic cycle.



**Fig 2.** Example region of [<sup>2</sup>H, <sup>15</sup>N, <sup>13</sup>C]-FBP-aldolase HSQC spectrum which shows, top, changes in amide resonance chemical shift in the presence and absence of PGH and, bottom, differing longitudinal (T1) relaxation rates for the uncomplexed form. All data was collected at 750MHz.

## Funding

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