

# Thermodynamics of binding of small hydrophobic ligands to the major urinary protein

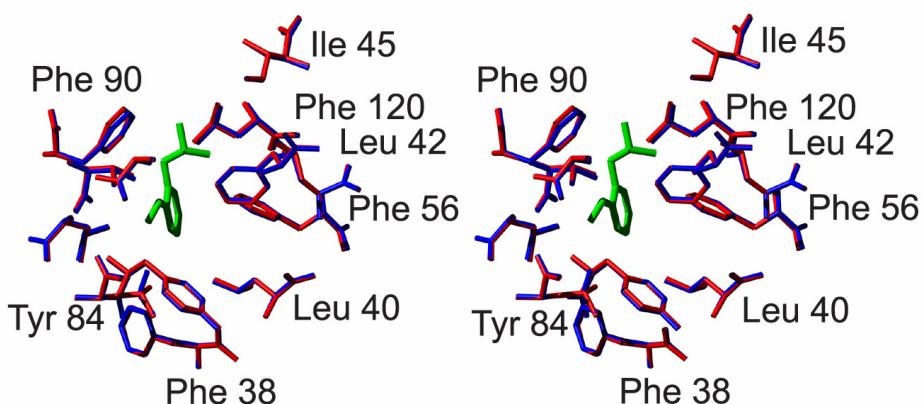
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## Introduction

An understanding of protein ligand interactions is vital to our understanding of many biological processes. Isothermal titration microcalorimetry has long been used to study these interactions. However, this technique gives values for the overall changes in the energetics of a system upon binding of a ligand to a protein molecule, but can tell us nothing of the processes involved in causing these energetic changes. The Major Urinary Protein (MUP) (Fig. 1) is used as a model protein to study the processes involved in the binding of small, hydrophobic ligands to proteins. A great deal is already known about this protein, including the crystal structures of the apo form, as well as in the presence of many natural and synthetic ligands. NMR relaxation data has also been acquired to give an idea as to what happens in terms of molecular motions upon ligand binding. A combination of this data has allowed inferences to be made as to the role of individual residues. By using a combination of site-directed mutagenesis, isothermal titration microcalorimetry, X-ray crystallography, NMR spectroscopy and computational molecular modelling, it is hoped that we can further understand the contribution to the overall binding energetics of particular amino-acid residues and ligand protein interactions in this model system.

## Hydrogen bonding

The binding of ligand to MUP is an enthalpically driven process. Factors which affect the enthalpy of binding include intrinsic factors, and solvation effects of both the protein and ligand. The MUP Y120F mutant was produced to probe the contribution to the binding energetics of the single hydrogen bond present in the MUP binding site. This hydrogen bond is produced by the interaction between the hydroxyl group of residue Tyr 120 and the ring nitrogen of the pyrazine ligands of MUP. Removal of this hydrogen bond would be expected to make the ligand binding reaction less favourable.



**Fig. 1.** Stereo view of the superimposition of the ligand binding sites observed in crystal structures of uncomplexed Y120F MUP (blue) and Y120F MUP (red) in complex with 2-isobutyl-3-methoxypyrazine (green). No ordered water molecules are observable within the binding site in either structure.

The ITC data recorded for this mutant showed the enthalpic contribution of binding to be less favourable in the Y120F mutant than in wild-type MUP, however overall ligand binding is still an enthalpically driven process. X-ray crystallography, along with molecular modelling showed there to be very little, if any, water in the MUP binding site in the absence of ligand. As a result of this, the only solvent re-organisation occurring when ligands bind to MUP is the

re-organisation of the water surrounding the ligand molecule. Therefore solvent reorganisation plays only a minor role in binding of ligand to MUP. This has been confirmed by the measurement of ITC data in deuterated solvent. This leaves the dominant interaction driving the binding of pyrazines to MUP as van der Waals interactions.

### **Van der Waals interactions**

In order to examine the effect of van der Waals interactions on ligand binding, a number of mutants have been made where leucine and isoleucine residues in the MUP binding site have been substituted for alanines. A combination of all the techniques previously mentioned should give an insight into the role of van der Waals interaction on the thermodynamics of binding of pyrazines to MUP.

### **Collaborators**

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### **Publications**

Bingham, R., Bodenhausen, G., Findlay, J. B.C., Hsieh, S-Y., Kalverda, A.P., Kjellberg, A., Perazzolo, C., Phillips, S.E.V., Seshadri, K., Turnbull, W.B. and Homans, S.W. (2004) Thermodynamics of binding of 2-methoxy-3-isopropylpyrazine and 2-methoxy-3-isobutylpyrazine to the Major Urinary Protein. *J. Am. Chem. Soc.* **126**, 1675-1681

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