

# **Thermodynamics of binding of 2-methoxy-3-isopropylpyrazine and 2-methoxy-3-isobutylpyrazine to the major urinary protein**

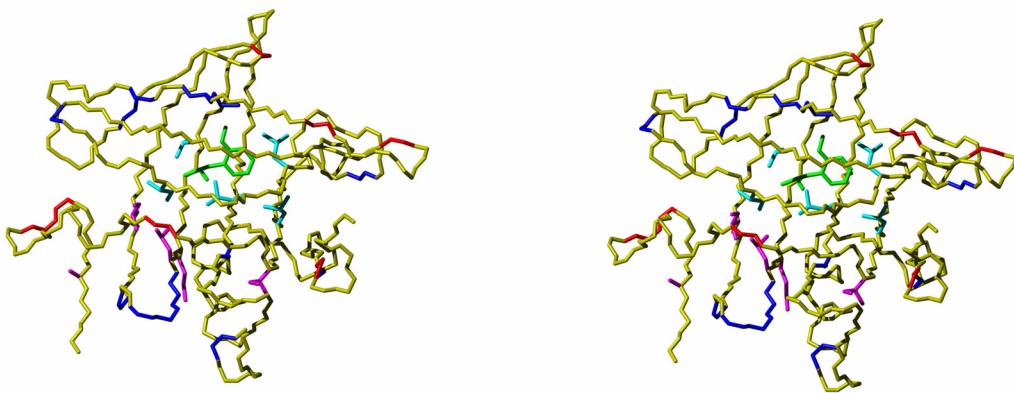
Richard J. Bingham, John B. C. Findlay, Shih-Yang Hsieh, Arnout P. Kalverda, Alexandra Kjellberg, Chiara Perazzolo, Simon E. V. Phillips, Kothandaraman Seshadri, Chi H. Trinh, W. Bruce Turnbull, Geoffrey Bodenhausen and Steve W. Homans

## **Introduction**

Protein-ligand interactions are of fundamental importance in a great many biological processes. However, despite enormous advances in the speed and accuracy of the three dimensional structure determination of proteins and their complexes, our ability to predict binding affinity from structure remains severely limited. One reason for this dilemma is that affinities are governed not only by energetic considerations concerning the precise spatial disposition of interacting groups, but also by the dynamics of these groups, in addition to solvent effects. Thus, in order to predict accurately the affinity of a protein for a given ligand, it is essential to have prior knowledge of both the enthalpy of binding,  $\Delta H_b^\circ$  and the entropy of binding  $\Delta S_b^\circ$ . A quantitative measure of the elusive  $\Delta S_b^\circ$  component is notoriously difficult, since it depends on the dynamics of the complex (including solvent) over all degrees of freedom of the system. Isothermal titration microcalorimetry experiments offer the possibility to measure thermodynamic binding parameters including  $\Delta S_b^\circ$ , but since the derived parameters are global in nature, it is difficult to separate contributions from protein, ligand and solvent. In principle, characterisation of the internal dynamics of a protein in the absence and presence of ligand should enable measurement of  $\Delta S_b^\circ$  values associated with the internal degrees of freedom of the protein. In particular, NMR relaxation-time measurements offer scope for the measurement of  $\Delta S_b^\circ$  on a per-residue basis, and this approach has been pioneered by a number of workers. As a model study, we examine the entropies of binding of two related ligands, namely 2-methoxy-3-isopropylpyrazine (IPMP) and 2-methoxy-3-isobutylpyrazine (IBMP), to the major urinary protein MUP-I, using a combination of isothermal titration calorimetry (ITC), X-ray crystallography and NMR backbone  $^{15}\text{N}$  and methyl side-chain  $^2\text{H}$  relaxation measurements.

## **Binding thermodynamics**

Global thermodynamic data derived from ITC indicate that binding is driven by favourable enthalpic contributions, rather than the classical entropy-driven hydrophobic effect. Unfavourable entropic contributions from the protein backbone and side-chain residues in the vicinity of the binding pocket are partially offset by favourable entropic contributions at adjacent positions, suggesting a ‘conformational relay’ mechanism, whereby increased rigidity of residues on ligand binding are accompanied by increased conformational freedom of side-chains in adjacent positions. The principal driving force governing ligand affinity can be attributed to solvent-driven enthalpic effects from desolvation of the protein binding pocket. Interestingly, the specificity of MUP-I for the two ligands cannot be explained by a difference in solvation of the binding site in the complex, but can be explained in terms of an entropic contribution from ligand desolvation.



**Fig. 1.** Stereo view of structural details of residues that contribute to the entropy of binding of 2-methoxy-3-isobutylpyrazine (coloured green) to MUP-I. Backbone residues that exhibit an unfavourable entropic contribution to binding are coloured blue, while those that exhibit a favourable contribution are coloured red. Similarly, residues whose methyl-containing side-chains exhibit an unfavourable contribution are coloured light blue, whereas those that exhibit a favourable contribution are coloured magenta.

### Collaborators

Geoffrey Bodenhausen and Chiara Perazzolo, ENS, Paris and EPFL, Lausanne; ProSpect Pharma Inc. (USA).

### Publications

Chaykovski, M.M., Bae, L.C., Cheng, M-C., Murray, J.H., Tortolani, K.E., Zhang, R., Seshadri, K., Findlay, J.B.C., Hsieh, S-Y., Kalverda, A.P., Homans, S.W. & Miles Brown, J. (2003) Methyl side chain dynamics in proteins using selective enrichment with a single isotopomer. *J. Am. Chem. Soc.* **125**, 15767-15771.

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