Thermodynamic Considerations for the Design of and Ligand Recognition by Molecularly Imprinted Polymers

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Thermodynamic considerations relevant to molecularly imprinted polymer (MIP) preparation and non-covalent interaction based ligand recognition are presented. Together with the description of a semi-quantitative approach to recognition analysis, adapted for MIPs, thermodynamically based principles for MIP design are discussed.

Molecular imprinting[1] is rapidly gaining acceptance as a technique for producing synthetic receptors as substitutes for traditional chromatographic[2,3] and immunological[4,5] recognition elements. The possibility of producing recognition sites of predetermined selectivity for a vast array of compound types makes molecular imprinting a technique of interest to industry, research and for gaining fundamental insights into molecular recognition. To date, however, no specific discussion has been forthcoming regarding the thermodynamic considerations underlying design of these systems, nor their potential for evaluating general factors influencing recognition phenomena. MIP preparation relies upon the formation of reversible interactions between functionalised monomers and template molecules. The subsequent polymerisation step captures the adducts to yield recognition sites of complementary steric and functional topography to the template species.[1]

The first stage, the so-called "prearrangement phase", prior to initiation of polymerisation, is a dynamic state. The extent of template complexation at equilibrium is governed by the change in Gibbs free energy for formation of each mode of template - functional monomer interaction. Thus, enthalpic and entropic factors determine the position of this equilibrium, *i.e.* the adduct stability. The polymerisation step captures not a single type of template - functional monomer adduct, but that of a distribution of complexes, the average nature of which reflects the position of the adduct formation equilibrium during the prearrangement step. This is manifested as site multiplicity, a continuum of sites from very high to very low template affinity, which may be compared to the recognition site distribution in a polyclonal antibody sample. This heterogeneity is reflected in the typical ligand binding curve profiles and chromatographic responses. Diffusion kinetics also influence such data. Thus, producing sites of enhanced regularity, and higher specific affinity, will yield more selective recognition systems. The effect of polymer chain growth on adduct conformation and stability is something yet to be examined.

Utilising the considerations of Page and Jencks,[6] Williams and colleagues proposed,[7,8] then extended,[9] a general factorisation of the energetic contributions to ligand-receptor interactions, equation 1. These terms are general to recognition phenomena and, therefore, apply equally well to both the formation of template - functional monomer solution adducts and to the recognition of a ligand by the MIP recognition sites.

$$\Delta G_{bind} = \Delta G_{t+r} + \Delta G_r + \Delta G_h + \Delta G_{vib} + \Sigma\Delta G_p + \Delta G_{conf} + \Delta G_{vdW} \quad \textbf{Equation 1.}$$

Where the Gibbs free energy changes are: ΔG_{bind}, complex formation; ΔG_{t+r}, translational and rotational; ΔG_r, restriction of rotors upon complexation; ΔG_h, hydrophobic interactions; ΔG_{vib} residual soft vibrational modes; $\Sigma\Delta G_p$, the sum of interacting polar group contributions; ΔG_{conf}, adverse conformational changes; and ΔG_{vdW}, unfavourable van der Waals interactions.

They concluded that for systems in which the complex formed between two species displays good molecular complementarity, and if the ligand-receptor interaction process takes place with each component at close to its global minimum energy conformation, this expression may be simplified, equation 2. In terms of MIP preparation, as the prearrangement phase is under thermodynamic control we may assume that the adduct population will possess, on average, no conformational strain, nor adverse van der Waals interactions. Furthermore, as the final recognition site topography reflects that of the solution adduct, and, granted the polymerisation and rebinding take place in similar solvents, no significant conformational compromise should be necessary for rebinding of ligand to MIP recognition sites. The high degree of cross linking in MIPs, 70%, suggests limited residual motion in the polymer matrix. Collectively these facts justify neglection of ΔG_{conf} and ΔG_{vdW} terms for molecular imprinting systems.

$$\Delta G_{bind} = \Delta G_{t+r} + \Delta G_r + \Delta G_h + \Delta G_{vib} + \Sigma\Delta G_p \quad \textbf{Equation 2.}$$

Most imprinting protocols and recognition studies reported thus far utilise non-polar solvents and polymers, *e.g.* styrene/divinyl benzene and ethylene glycol dimethacrylate cross linking. Under such circumstances the hydrophobic effect may be neglected. Thus equation 3 may be used for evaluating of MIP formation and subsequent ligand recognition. In recent studies on MIP water mediated recognition, however, the hydrophobic effect is of course significant.[3,5] Hydrophobic moiety selective functionalities, *e.g.* cyclodextrin and other water soluble captivand based functional monomers, will offer the possibility, of extending the range of the technique to include water soluble (organic insoluble) compounds.

$$\Delta G_{bind} = \Delta G_{t+r} + \Delta G_r + \Delta G_{vib} + \Sigma\Delta G_p \quad \textbf{Equation 3.}$$

The ramifications of equation 3 for the design of new imprinted polymer systems are manifold. The ΔG_{t+r} and ΔG_{vib} terms are functions of ligand/template size and temperature, as discussed elsewhere,[7] and are inherent to all recognition systems. Imprinting with suitable multi coordinating functional monomers should minimise the adverse effect, mainly entropic, of the ΔG_{t+r} term. With each reduction in the number of adduct components, the magnitude of the term decreases, in accordance with the three degrees of translational and three degrees of rotational free energy no longer having to be overcome upon complexation.[7,9] Polymerisable rigid peptide analogues, for ß-sheet mode recognition of peptides, and multi-hydrogen bonding monomers[10] utilise these principles, *i.e.* multiple simultaneous interactions between monomer and template species and reduction in internal rotation.

Selection of functional monomers which engage in stronger,

more specific interactions are advantageous, increasing the favourable (mainly enthalpic) contribution of the ΔG_p terms. Hydrophilic functional monomer - template interactions should be as stable as possible to induce adduct regularity. Reversible covalent linkages to functional monomers, and strongly coordinating functionalities, such as metal ions, offer much in this regard. The use of more stable template - monomer interactions reduces the need for excesses of functional monomers, in turn minimising non-specific interactions arising from random functional monomer residue orientation throughout the bulk polymer. Importantly, the intrinsic binding energy, ΔG_p, for each ligand functional group interaction with a MIP is a measure of the average strength of the ligand polar interactions with the polymer matrix under investigation. This encompasses the contribution of non-specific binding modes, *i.e.* hydrophilic interactions between ligands and functional monomer residues randomly oriented in the polymer matrix and with functionality present in the cross linking agent. Polymerisation solvents should be selected to optimise the strength of the polar interactions, rather than compete with the functional monomers for interaction with the template.

The energetic penalty associated with the freezing of a rotor, ΔG_r (entropic), has been the subject of some conjecture, though a figure of 4-6 kJ mol^{-1} ($T\Delta S$ at 300° K), corresponding to about one order of magnitude in binding constant, is currently considered a reasonable estimate.[8] The use of more rigid template structures will yield a more conformationally defined solution adduct population, through not having to contend with as broad a conformation distribution. This will, in turn, result in increased MIP site homogeneity. Indeed, the constraining of suitable rotors in ligands to improve binding affinity for biological receptors is well documented.[11] It is critical that a rigid template analogue must mimic the solution conformation of the desired ligand.

The entropically unfavourable impact of rotor freezing for binding is illustrated by recent results with relatively rigid structures, such as opioid alkaloids[5] and xanthines,[3] which showed significantly lower dissociation constants and higher selectivities for rebinding of their respective templates than in comparable less rigid systems. Polymers selective for opioid peptide derivatives, *e.g.* [Leu^5]-enkephalin, demonstrated consistently lower binding affinities than polymers specific for opioid alkaloids, *e.g.* morphine.[5] The opioid alkaloid's rigidity must contribute significantly to the superior binding, as relatively few polar binding features are present in morphine capable of contributing to the favourable $\Sigma\Delta G_p$ term. *Bait and switch* style strategies have proven useful in catalytic antibody work[12] and offer promise for use in molecular imprinting. By using equation 3, differences in the free energy of binding between closely related systems, $\Delta\Delta G_{bind}$, can be used to yield values for functional group - polymer intrinsic binding energies, ΔG_p, and internal rotation freezing, ΔG_r - thermodynamic values of utility in rational ligand design. The terms in equation 3 signify the importance of judicious functional monomer, cross linking agent, polymerisation solvent and template selection, and offers possibilities for examining fundamental molecular recognition factors using MIPs.

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