An illustration of the expression of cooperative binding energy in arrays of non-covalent interactions

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An analysis of a thermodynamic cycle for the formation of ligand-bound dimers gives a simple illustration as to how a cooperative binding energy ($\Delta G°_{coop}$) can be expressed over a range of interfaces, rather than at just one of the interfaces within the array.

The phenomenon of cooperativity has been shown to be important in a variety of molecular recognition processes throughout Nature.[1] In such systems, cooperative enhancements to binding are important in conferring relatively large Gibbs free energies of association and also high specificities on binding processes. Different mechanisms for the operation of cooperative binding exist, including conformational changes in the receptors involved, or more subtle structure tightening which may occur without a significant change in receptor conformation.[2–7] This paper gives a simple illustration as to how cooperativity occurring without a significant conformational change can potentially result in large Gibbs free energies of binding and how binding to one part of a receptor can result in the strengthening of binding at other interfaces within that receptor.

In previous work, we have shown that the dimerisation of vancomycin group of antibiotics is cooperative with the binding of ligands (peptides terminating in the sequence –Lys-D-Ala-D-Ala).[8] That is, dimerisation constants are typically greater in the presence of cell wall precursor analogues than in their absence, and ligand binding constants to antibiotic dimers are greater than those to monomers. As a result, dimerisation, with the resultant formation of a tetrameric cooperative array (two ligands binding to an antibiotic dimer), is beneficial to antibacterial activity.[8,9]

Recently, we have identified correlations between the chemical shifts of particular antibiotic protons and the Gibbs free energies of ligand binding ($\Delta G°_{lig}$) and dimerisation ($\Delta G°_{dim}$).[10–12] In both cases, the parameters under consideration (chemical shift) were determined under limiting conditions, *i.e.* antibiotic fully bound by ligand or antibiotic fully dimerised. We have used these monitored proton resonances as microscopic probes of the local tightness of the ligand binding and dimerisation interfaces; when the association constant is large, the resonance related to that interface shows a greater chemical shift change relative to the unassociated state.[11] Using these microscopic probes, we have been able to analyse the interfacial origins of the cooperative binding energy expressed upon formation of a ligand-bound dimer for a number of vancomycin group antibiotics.[12] We describe here a new analysis of this extended cooperative array, based on a thermodynamic cycle, from which it is possible to illustrate in a simple way useful conclusions regarding the expression of cooperative binding energy.

The enhancements to dimerisation and ligand binding resulting from cooperativity can be represented in a thermodynamic cycle showing the formation of a fully ligand-bound dimer from the constituent elements of two antibiotic monomers and two ligand molecules (Fig. 1).[8] There are four binding events occurring in this cycle. The left-hand half of Fig. 1 shows the formation of a fully ligand-bound dimer through the formation of a ligand-free antibiotic dimer followed by the binding of two ligand molecules to that dimer (**A** → **B** → **D**/**E**). The right-hand half of Fig. 1 shows the formation of the same fully ligand-bound dimer, but through the formation of two ligand-bound antibiotic monomers followed by dimerisation of these two monomers (**A** → **C** → **D**/**E**). Two possible scenarios (**D** and **E**) are considered for the formation of the fully ligand-bound dimer. **D** is the hypothetical situation where there is no cooperativity between dimerisation and ligand binding. In this case, there are no net influences on ligand binding due to dimerisation, and *vice versa*, *i.e.* no net influences on dimerisation due to ligand binding. **E** is the situation for the majority of vancomycin group members where dimerisation and ligand binding are cooperative processes, and the resultant Gibbs free energy benefit due to cooperativity is defined as $\Delta G°_{coop}$. We can use this thermodynamic cycle to illustrate the means by which a measured cooperative binding energy can, in theory, be expressed at any of the interfaces within an extended array.

In Fig. 1, the local tightness of the respective ligand binding and dimerisation interfaces (as evidenced from NMR data[11,12]) is represented schematically simply by greater distances between associating entities when the association at that interface is looser. The experimental data show that locally looser interfaces correlate with smaller K_{dim} and K_{lig} (*i.e.* less negative $\Delta G°_{dim}$ and $\Delta G°_{lig}$) values.[11,12] If there is no cooperativity between ligand binding and dimerisation (**D**), then the dimerisation and ligand binding interfaces are antici-

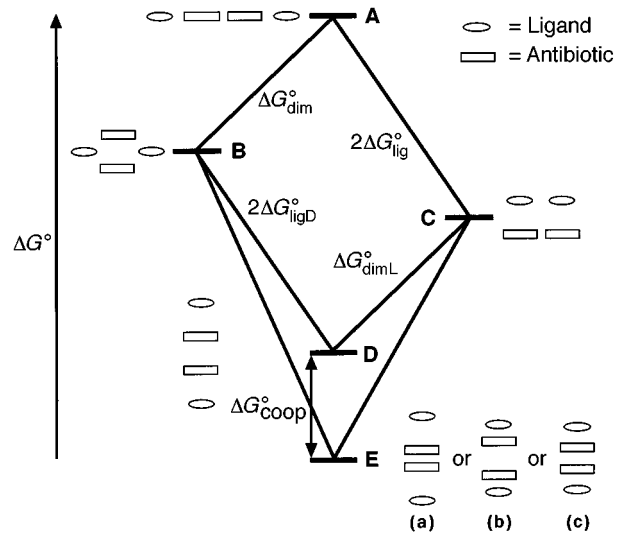

Fig. 1 Schematic thermodynamic cycle showing the formation of a fully ligand-bound antibiotic dimer from the constituent elements of two ligand molecules and two antibiotic monomers (**A**) *via* either a ligand-free antibiotic dimer (**B**) or two ligand-bound antibiotic monomers (**C**). The ligand-bound dimer at (**D**), is formed with no cooperativity between dimerisation and ligand binding. The ligand-bound dimers at (**E**) are formed with a cooperative Gibbs free energy ($\Delta G°_{coop}$) between dimerisation and ligand binding. For the two ligand binding events (**A** → **C** and **B** → **D**/**E**), the Gibbs free energies of binding are multiplied by 2 since each event involves the binding of two ligand molecules. See text for further analysis.

pated to be unchanged from those in **B** and **C**, respectively. If dimerisation and ligand binding are cooperative (**E**), however, then the cooperative binding energy ($\Delta G°_{coop}$) can, in theory, be expressed by tighter binding at either of the two interfaces, *i.e.* at the dimerisation interface and/or at the ligand binding interface.

There are two extreme states to consider for the expression of the cooperative binding energy, $\Delta G°_{coop}$. In **E(a)**, $\Delta G°_{coop}$ is expressed solely at the dimerisation interface and the tightness of the ligand binding interface remains the same as that in **C**. In **E(b)**, $\Delta G°_{coop}$ is expressed solely at the ligand binding interface and the tightness of the dimerisation interface remains the same as that in **B**. In practice, ligand-bound dimers are likely to express cooperative binding energy across both ligand binding and dimerisation interfaces as shown in **E(c)**. However, if we consider one of the extreme cases, *e.g.* **E(a)**, where all the cooperative binding energy is expressed at the dimerisation interface, then $\Delta G°_{coop}$ can still potentially be determined *via* measurement of either of two quantities: the ligand binding constant to dimer (**B** → **E**), or the dimerisation constant of ligand-bound antibiotic (**C** → **E**). For both measurements, $\Delta G°_{coop}$ is the same (**D** → **E**) and can be calculated from the increase in $\Delta G°_{dimL}$ over $\Delta G°_{dim}$, or from the increase in $2\Delta G°_{ligD}$ over $2\Delta G°_{lig}$. It is clear from this that in **E**(**a**), although $\Delta G°_{coop}$ is being expressed solely at the dimerisation interface and there is no increase in the tightness of the ligand binding interface, $2\Delta G°_{ligD}$ will show the same cooperative enhancement over $2\Delta G°_{lig}$ as will $\Delta G°_{dimL}$ over $\Delta G°_{dim}$. The same situation will exist even if $\Delta G°_{coop}$ is expressed solely at the ligand binding interface [**E(b)**]. In this case, although there will be no tightening of the dimer interface, $\Delta G°_{dimL}$ will still show an enhancement of $\Delta G°_{coop}$ over $\Delta G°_{dim}$. Thus, when $\Delta G°_{coop}$ is actually measured, *e.g.* by an increase in $\Delta G°_{dimL}$ over $\Delta G°_{dim}$, it is not possible to say, without further analysis of the complexes formed, at which interface the cooperative binding energy is expressed. (We have recently performed such an analysis of the partitioning of the cooperative Gibbs free energy between the dimerisation and ligand binding interfaces using the chemical shift of a proton at the dimer interface as a probe of interface tightness.[12])

Analogous diagrams would simply illustrate that in any system of weak interactions where there is a cooperative binding energy ($\Delta G°_{coop}$) expressed through the formation of an extended aggregate, then $\Delta G°_{coop}$ can potentially occur at *any* of the interfaces which go to make up that extended array. Also, if cooperativity is observed for a particular binding event in such an extended array, it does not necessarily follow that the bonding at the interface for that particular binding event has been improved.

One consequence of the above discussion is that the size of an extended array (number of non-covalent interactions making up the array) will affect the amount of cooperative binding energy ($\Delta G°_{coop}$) which can potentially be expressed. In the case of a fully ligand-bound antibiotic dimer, $\Delta G°_{coop}$ can be expressed over three binding interfaces whereas, if only one ligand was bound to the dimer, the cooperative binding energy could only be expressed over two binding interfaces. More generally, the greater the number of cooperatively-linked binding interfaces, the greater the scope for cooperative enhancements to binding. It thus follows that the greatest potential for expressing cooperative binding energies that would be expected in Nature are those involving the associations of large arrays of weak interactions, *e.g.* DNA duplexes, ligands binding to proteins.

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