Supramolecular Chemistry

Allosterically Driven Multicomponent Assembly**

Pall Thordarson, Ruud G. E. Coumans, Johannes A. A. W. Elemans, Paul J. Thomassen, Johan Visser, Alan E. Rowan,* and Roeland J. M. Nolte*

Cooperative interactions play a ubiquitous role in Nature and are utilized not only to construct discrete assemblies such as the tobacco mosaic virus (TMV), but also to transfer information, crucially exemplified in the binding of oxygen

[*] Dr. P. Thordarson, R. G. E. Coumans, Dr. J. A. A. W. Elemans, P. J. Thomassen, J. Visser, Dr. A. E. Rowan, Prof. R. J. M. Nolte
Department of Organic Chemistry, NSRIM Institute
University of Nijmegen
Toernooiveld, 6525 ED Nijmegen (The Netherlands)
Fax: (+31)24-3652929
E-mail: p.thordarson@chem.usyd.edu.au
rowan@sci.kun.nl

Dr. P. Thordarson
Australian Key Centre for Microscopy and Microanalysis
School of Chemistry, Madsen Building
The University of Sydney
NSW 2006 (Australia)
Fax: (+61)2-93517682

[**] This research was supported by the NRSCC, an EC Marie Curie Fellowship to P.T. (Contract No. HPMF-CT-2000-01053), and an NWO Vidi grant to A.E.R.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

 DOI: 10.1002/ange.200460398

to hemoglobin.[1,2] Cooperative interactions also play a critical role in gene transcription; for instance, cyclic AMP has a strong cooperative effect upon the binding of the gene-transcription-regulating cAMP receptor protein (CRP) to DNA.[3] In the latter two examples, the binding event at one site in a multivalent host causes a discrete, reversible alteration in the structure of the host which is transmitted to another binding site and is called an allosteric interaction.[4] In the case of hemoglobin, the binding of oxygen can be described as positively homotropic, whereas the allosteric interactions in the cAMP/CRP/DNA can be described as positively heterotropic.

The area of artificial allosteric interactions is of great general interest[5–7] not only because of its crucial role in Nature, but also because it may help in the construction of functional nanoscale objects. Towards understanding these complex processes, we recently developed a double-cavity porphyrin that displays very strong negative homotropic allosteric behavior.[8] As a continuation of this research, we present herein a positively heterotropic synthetic allosteric system based on a combination of host–guest and metal–ligand interactions.[9] This system was constructed from a monocavity-appended porphyrin host **ZnP**[10,11] by using various combinations of 4-*tert*-butylpyridine (tbpy), 1,4-diazabicyclo[2.2.2]octane (dabco), dimethylviologen (V), and *meso*-tetrakis(4-pyridyl)porphyrin (py_4por) as the ligands or substrates (Figure 1). The choice of ligands was based on

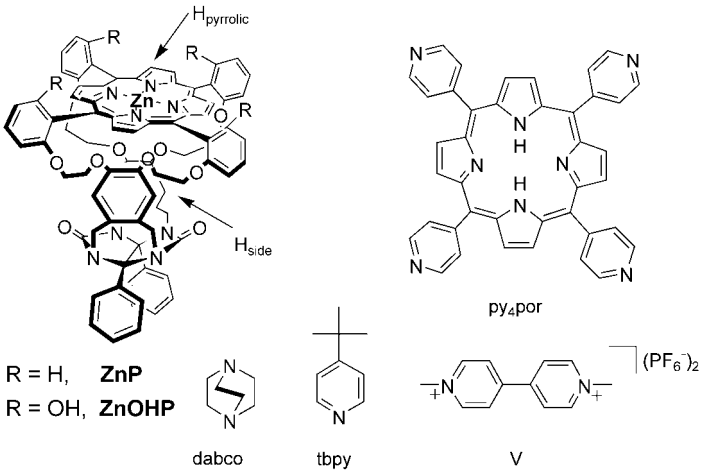

Figure 1. Molecules used in this study.

earlier work in which it was demonstrated that **ZnP** binds V very strongly in its cavity ($K_{ass} > 10^5\,M^{-1}$) and the bulky ligands tbpy and py_4por outside its cavity (Figure 2a).[12]

The binding between tbpy and **ZnP** was studied by a ^1H NMR titration (Figure 2b), both in the absence and presence of 1 equivalent of V as the allosteric substrate (Table 1). A clear positive heterotropic allosteric effect was observed: The K_{ass} for the binding of tbpy to **ZnP** is more than two orders of magnitude larger in the presence of V than in its absence. In a system in which a host interacts with two different guests, ligand L and substrate R (Figure 2a), the allosteric magnification $^{R}AM_{L}$ caused by R on binding of the ligand L to the host can be defined as:

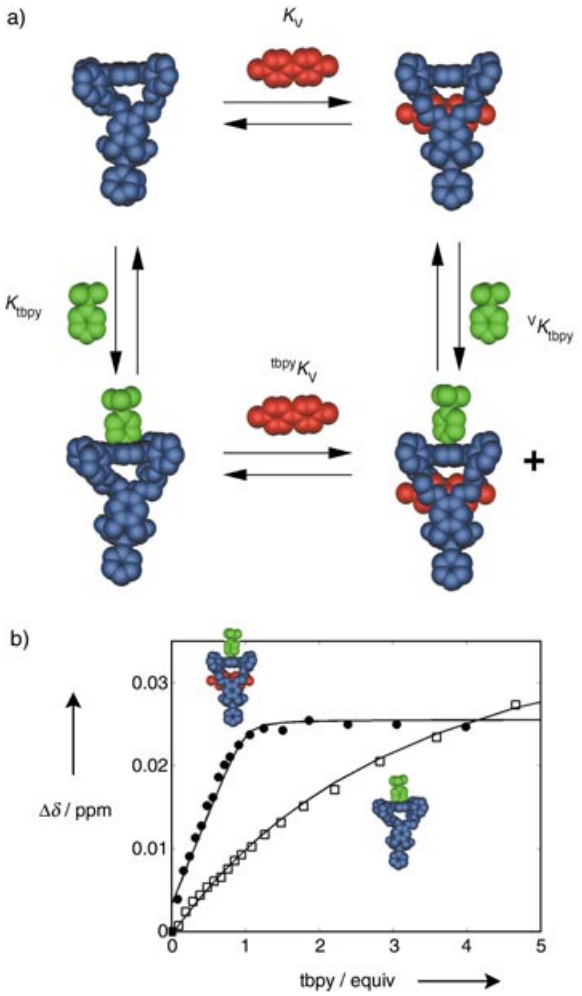

Figure 2. a) Schematic representation of the possible interactions between a host, ligand L, and substrate R, with **ZnP**, tbpy, and V, respectively, as an illustrative example. The + sign indicates the positive cooperativity between the neighboring ligands (guests). b) ^1H NMR titration of the **ZnP** host with the tbpy ligand showing the chemical shifts changes ($\Delta\delta$) for H_{side} (Figure 1) in the absence (□) and presence (●) of 1 equivalent of V. Also shown are the calculated binding isotherms (——) obtained by nonlinear regression (assuming a 1:1 binding model).

$$^{R}AM_{L} = \frac{K_{ass}\text{ for L in the presence of R}}{K_{ass}\text{ for L in the absence of R}} \qquad (1)$$

This results in a $^{V}AM_{tbpy}$ of 250 for the aforementioned system, and corresponds to a $\Delta\Delta G$ of $-14\,kJ\,mol^{-1}$. To investigate the reverse effect, that is, the effect of tbpy on the binding of V, a fluorescence titration was carried out in which V was added to **ZnP** both in the absence and presence of 500 equivalents of tbpy. An allosteric magnification $^{tbpy}AM_{V} = 3$ was observed (Table 1), revealing that the binding of tbpy also enhances the binding of V. It can be calculated that at the concentrations used ($\sim 10^{-6}$ M) about 17% of **ZnP** is bound to tbpy before the addition of V, but this portion rises to >98% after 1 equivalent of V has been added. In a perfect three-component system, as assumed here, the thermodynamic cycle has to follow Hess's law and balance: $^{R}AM_{L}$ should be equal to $^{L}AM_{R}$.[13] The observation that $^{V}AM_{tbpy}$ is not equal to $^{tbpy}AM_{V}$ highlights the fact that additional subtle effects also

Table 1: Association constants K [M^{-1}] and binding free energies ΔG [kJ mol^{-1}] for the complexation of **ZnP** and **ZnOHP** to various ligands L in the absence and presence of substrates R at 298 K.

Host	L	R	Without R		With R		$\Delta\Delta G$[b]	$^{R}AM_{L}$[c]
			K_1[a]	ΔG	K_1[a]	ΔG		
ZnP[d]	tbpy	V (1 equiv)	4×10^{2}	−15	1×10^{5}	−29	−14	250
ZnP[e]	V	tbpy (500 equiv)	9×10^{5}	−34	3×10^{6}	−37	−3	3
ZnP[f]	V	tbpy (500 equiv)	3×10^{5}	−31	1×10^{6}	−34	−3	3
ZnP[g]	dabco	V (10 equiv)	5×10^{4}	−27	4×10^{5}	−32	−5	8
ZnP[f]	V	dabco (10 equiv)	3×10^{5}	−31	9×10^{5}	−34	−3	3
ZnP[h]	py_4por	V (1 equiv)	2×10^{2}	−13	3×10^{3}	−20	−7	15
ZnOHP[i]	dabco	V (10 equiv)	n.d.	n.d.	2×10^{6}	−34	n.d.	n.d.

[a] Measurements in duplicate or triplicate. Estimated errors: 50%. [b] $\Delta\Delta G = (\Delta G$ for L with R$) - (\Delta G$ for L without R). [c] Defined as $^{R}AM_{L} = (K_1$ of L with R$)/(K_1$ of L without R). [d] In $CDCl_3/CD_3CN$ (4:1, v/v) measured by ^{1}H NMR titration. [e] In $CHCl_3/CH_3CN$ (4:1, v/v) measured by fluorescence titration. [f] In $CHCl_3/CH_3CN$ (1:1, v/v) measured by fluorescence titration. [g] In $CDCl_3/CD_3CN$ (1:1, v/v) measured by ^{1}H NMR (without R) and in $CHCl_3/CH_3CN$ (1:1, v/v) measured by UV/Vis titration (with R). An interaction cooperativity parameter α ($4K_2/K_1$) = 0.05–0.07 for dabco is used, see text for details. [h] In $CDCl_3/CD_3CN$ (1:1, v/v) measured by ^{1}H NMR titration. [i] In $CHCl_3/CH_3CN$ (1:1, v/v) measured by UV/Vis titration.

play a role. Inspection of the thermodynamic cycle shown in Figure 2a and Table 1 shows that the actual calculated free-energy gain in the anticlockwise direction is −52 kJ mol^{-1}, whilst in the clockwise direction the energy gain is −63 kJ mol^{-1}. The difference of only −12 kJ mol^{-1} is however significantly greater than the experimental errors and would appear to violate Hess's law, as in a complete thermodynamic cycle both directions should be equal. Our binding model, as used in the model studies, does not take into account a competitive effect from the solvent molecules. These third competitive species are the acetonitrile solvent or residual water molecules, (although extreme care was taken to avoid the latter solvent), both of which are known to bind strongly within the cavity. In a complete cycle, however, this competitive behavior should also balance out if all measurements are carried out at the same concentration. The discrepancy arises from the fact that the complete cycle is not measured at one concentration; owing to the extremely high association constant of the bipyridine within the host, binding studies were carried out at 10^{-6} M, whereas the binding of *tert*-butyl pyridine was measured at 10^{-3} M. The combination of the solvent effect and the different concentrations are thought to be responsible for the incomplete thermodynamic cycle. It is only when the whole thermodynamic cycle is measured that these additional factors become apparent.

We investigated whether the allosteric effect could be utilized in the directed self-assembly of a larger supramolecular complex, from a multicomponent solution consisting of a **ZnP** host and a tetradentate ligand py_4por, again with V as an allosteric cofactor (Figure 3). This system would be a very simple analogue to the natural cAMP/CRP/DNA allosteric system. **ZnP** and py_4por can form complexes with varying stochiometry. To calculate the K_{ass} values between **ZnP** and py_4por, each *meso*-4-pyridyl group on py_4por was treated as an independent ligand (the results from the NMR titrations showed no signs of cooperativity). In the presence of 1 equivalent of V per **ZnP** molecule a strong positive heterotropic allosteric effect was found: $^{V}AM_{py4por} = 15$, $\Delta\Delta G = -7$ kJ mol^{-1} (Table 1). From the K_{ass} values it can be calculated that in a mixture of **ZnP** and py_4por (4:1, [py_4por] = 1 mM) in the absence of V only 1% of the ligand is completely surrounded by four **ZnP** molecules, whilst in the presence of V about 34% of the py_4por ligands form a 1:4 complex with **ZnP**. The above results clearly illustrate how, in principle, allosteric magnification can drive complex equilibrium mixtures towards a more discrete architecture.

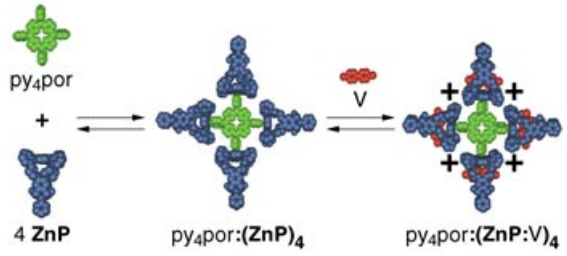

Figure 3. Schematic representation showing how 4 equivalents of **ZnP** assemble around the py_4pyr ligand either with or without V as a substrate, the + signs indicate the positive cooperativity between the neighboring ligands (guests).

The allosteric effect that V showed on the binding of the noncooperative ligands tbpy and py_4por was also investigated on the binding of the cooperative bidentate ligand dabco. Anderson and Taylor have shown[14] that dabco binds to zinc(II) porphyrins with negative cooperativity, with an interaction cooperativity parameter[15] ($\alpha = 4K_{2ass}/K_{1ass}$) that varies between 0.01 and 0.2, depending on the solvent. When **ZnP** was titrated with dabco, a similar negative cooperativity was observed. The cooperativity parameter α was estimated to be approximately 0.05, with the association constant $K_{1ass} = 5\times10^{4}$ M^{-1}.[16] Upon the addition of V, K_{1ass} was found to increase by one order of magnitude ($K_{1ass} = 4\times10^{5}$ M^{-1}) (Table 1, Figure 4). The differences in binding affinity of dabco for **ZnP** in the presence and absence of V were most clearly observed in the ^{1}H NMR spectra. The porphyrin resonances of **ZnP** bound and unbound to dabco underwent fast exchange on the NMR timescale when V was absent, but only slow exchange upon the addition of V. The latter observation allowed us to measure directly the ratio between the ternary V:**ZnP**:dabco complex ($\delta_{dabco} = 0.87$ and −2.88 ppm) and the pentameric V:**ZnP**:dabco:**ZnP**:V complex ($\delta_{dabco} = -4.74$ ppm) and thus measure the K_{1ass}/K_{2ass} ratios.[17] A cooperativity interaction parameter $\alpha = 0.07$, which is similar to the value found in the absence of V was calculated. The allosteric magnification observed in this case has a pronounced effect on the distribution of species present

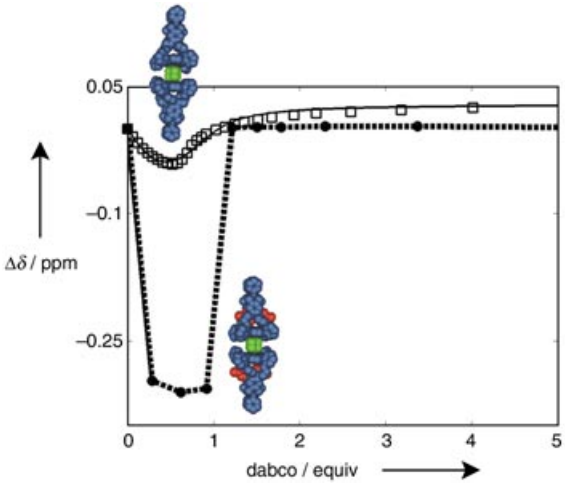

Figure 4. ^{1}H NMR titration of the **ZnP** host with the dabco ligand showing the chemical shifts changes ($\Delta\delta$) for $H_{pyrrolic}$ (Figure 1) in the absence of V (□) together with the calculated binding isotherm (——) obtained by nonlinear regression (assuming a 1:2 binding between **ZnP** and dabco and $\alpha = 0.05$). Also shown are the chemical shifts changes ($\Delta\delta$) for the same $H_{pyrrolic}$ in **ZnP** in the presence of 1 equivalent of V upon addition of dabco. In this case, the observed complexes do not undergo fast exchange, so that the data points shown (•••••) illustrate only the dominating species at any given point of the titration.

in the solution (Figure 5). Calculations with the measured association constants and with [**ZnP**] = 1 mM and [dabco] = 0.5 mM showed that the sandwich species **ZnP**:dabco:**ZnP** accounts for only 22% of **ZnP** present in solution. Upon the addition of V (and based on the assumption that **ZnP**, V:**ZnP**:dabco and V:**ZnP**:dabco:**ZnP**:V are the only species in the mixture), 57% of **ZnP** is calculated to be in the sandwich complex, thus illustrating how the binding of V promotes the assembly of **ZnP** around the dabco ligand. However, it does not drive the equilibrium through to the pentameric complex owing to the negative cooperativity of dabco.[18]

To form the desired pentameric architecture, a **ZnP** cavity was synthesized with 4 hydroxy functions positioned on the *meso* phenyl rings which might serve as additional interactive elements (**ZnOHP**, Figure 1).[8] As indicated by molecular modeling, the OH functions of **ZnOHP** are 3 Å apart when two molecules of this compound are self-assembled with dabco. These OH functions can form hydrogen bonds upon dimerization, possibly overcoming the negative cooperativity of dabco. Initial studies revealed that in the presence of V, the ditopic ligand dabco binds with $K_{1ass} = 2 \times 10^6\,M^{-1}$ and $K_{2ass} = 9 \times 10^6\,M^{-1}$, resulting in an α value of 17, which points to an exceptionally positive cooperative assembly process. A consequence of the cooperative process is that at concentrations of [**ZnOHP**] = 1 mM and [dabco] = 0.5 mM, a remarkable 98.5% of **ZnOHP** is involved in the pentameric sandwich complex (Figure 6). This indicates that the addition of an extra factor, namely, the OH-groups in **ZnOHP**, combined with the allosteric magnification that V exerts on the binding of dabco, results in the assembly of a single species from a complex equilibrium mixture.

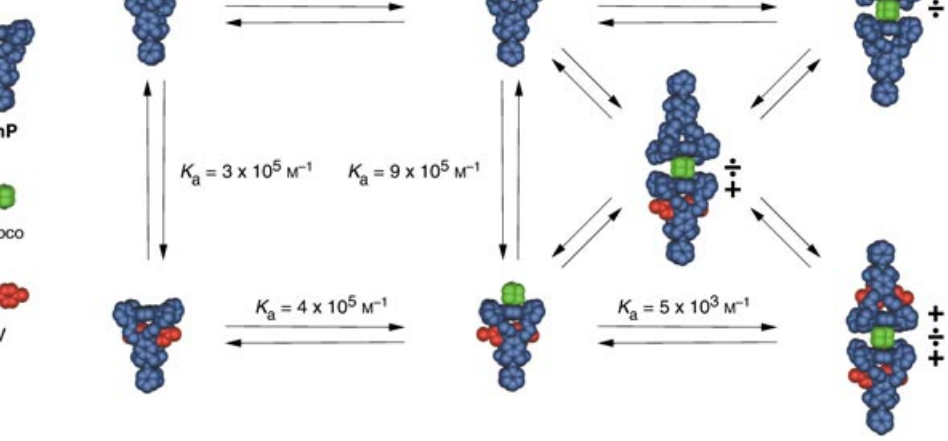

Figure 6. Molecular models of **ZnOHP** and the pentameric sandwich complex.

Figure 5. Schematic representation summarizing all the possible complexes of **ZnP** with dabco in the presence or absence of V, together with the relevant association constants obtained in this study. The +/÷ signs indicate the positive or negative cooperativity between the neighboring ligands (guests), respectively.

Allosterically controlled assembly, as demonstrated herein, opens up numerous possibilities for directing complex equilibrium mixtures to a

desired product that mimics the hierarchical self-assembly processes found in Nature. The manganese derivatives of the above-mentioned systems have the added property of catalytic activity.[12] Current research is directed at the construction of allosteric nanometer-sized assemblies with unique catalytic properties.

Received: April 21, 2004

Keywords: allosterism · cooperative phenomena · porphyrins · self-assembly · supramolecular chemistry

[1] A. Klug, *Angew. Chem.* **1983**, *95*, 579; *Angew. Chem. Int. Ed. Engl.* **1983**, *22*, 585.
[2] a) G. K. Ackers, M. L. Doyle, D. Myers, M. A. Daugherty, *Science* **1992**, *255*, 54; b) Y. Huang, M. L. Doyle, G. K. Ackers, *Biophys. J.* **1996**, *71*, 2094; c) M. L. Johnson, *Methods Enzymol.* **2000**, *323*, 124.
[3] J. G. Harman, *Biochim. Biophys. Acta* **2001**, *1547*, 1.
[4] J. Monod, J. Wyman, J. P. Changeux, *J. Mol. Biol.* **1965**, *12*, 88.
[5] a) J. Rebek, Jr., *Acc. Chem. Res.* **1984**, *17*, 258; b) I. Tabushi, *Pure Appl. Chem.* **1988**, *60*, 581; c) R. P. Sijbesma, R. J. M. Nolte, *J. Am. Chem. Soc.* **1991**, *113*, 6695.
[6] S. Shinkai, M. Ikeda, A. Sugasaki, M. Takeuchi, *Acc. Chem. Res.* **2001**, *34*, 494.
[7] M. Takeuchi, M. Ikeda, A. Sugasaki, S. Shinkai, *Acc. Chem. Res.* **2001**, *34*, 865.
[8] P. Thordarson, E. J. A. Bijsterveld, J. A. A. W. Elemans, P. Kasák, R. J. M. Nolte, A. E. Rowan, *J. Am. Chem. Soc.* **2003**, *125*, 1186.
[9] Positive heterotropic allosteric behavior has been demonstrated in synthetic ion-ligand systems: a) M. H. Al-Sayah, N. R. Branda, *Chem. Commun.* **2002**, 178; b) A. Lützen, O. Hass, T. Bruhn, *Tetrahedron Lett.* **2002**, *43*, 1807; c) examples listed in references [6] and [7].
[10] A. E. Rowan, P. P. M. Aarts, K. W. M. Koutstaal, *Chem. Commun.* **1998**, 611.
[11] J. A. A. W. Elemans, M. B. Claase, P. P. M. Aarts, A. E. Rowan, A. P. H. J. Schenning, R. J. M. Nolte, *J. Org. Chem.* **1999**, *64*, 7009.
[12] a) J. A. A. W. Elemans, E. J. A. Bijsterveld, A. E. Rowan, R. J. M. Nolte, *Chem. Commun.* **2000**, 2443; b) P. Thordarson, E. J. A. Bijsterveld, A. E. Rowan, R. J. M. Nolte, *Nature* **2003**, *424*, 915.
[13] E. Di Cera, Q. Dang, Y. Ayala, A. Vindigni, *Methods Enzymol.* **1995**, *259*, 127.
[14] P. N. Taylor, H. L. Anderson, *J. Am. Chem. Soc.* **1999**, *121*, 11538.
[15] a) K. A. Connors, *Binding Constants*, Wiley, New York, **1987**; b) K. A. Connors, A. Paulson, D. Toledo-Velasquez, *J. Org. Chem.* **1988**, *53*, 2023.
[16] Only binding of dabco to the "outside" of **ZnP** was observed, as seen for tbpy. We attribute this to the bulkiness of dabco, which cannot be easily accommodated within the cavity in **ZnP**.
[17] H. L. Anderson, C. A. Hunter, M. N. Meah, J. K. M. Sanders, *J. Am. Chem. Soc.* **1990**, *112*, 5780.
[18] It is proposed that a structural rearrangement of the host and/or electrostatic interactions occur upon binding of V and these alterations are responsible for the observed allosteric effect. Current investigations are focused on high-level molecular-modeling studies to obtain further insight into the processes reported herein.

