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Multivalent recognition of bis- and tris-Zn-porphyrins by *N*-methylimidazole functionalized gold nanoparticles

Gianluca Fantuzzi,[a] Paolo Pengo,[a] Rosa Gomila,[b] Pablo Ballester,[b] Christopher A. Hunter,[c] Lucia Pasquato*[a] and Paolo Scrimin*[a]

[a] *Dipartimento di Chimica Organica and CNR ITM Sezione di Padova, Università di Padova, via Marzolo 1, I-35131 Padova, Italy. E-mail: lucia.pasquato@unipd.it; paolo.scrimin@unipd.it; Fax: +39 049 8275239; Tel: +39 049 8275255*

[b] *Departament de Química, Universitat de les Illes Balears, 07071 Palma de Mallorca, Spain*

[c] *Centre for Chemical Biology, Krebs Institute for Biomolecular Science, Department of Chemistry, University of Sheffield, Sheffield, UK S3 7HF*

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_N_-Methylimidazole-functionalized gold nanoparticles behave as multivalent ligands for porphyrin arrays with an increase in binding strength of up to three order of magnitude with respect to a monovalent system.

In nature the specificity and the selectivity of a recognition process are in many cases mediated by multivalent interactions such as antibody–antigen interactions or cell–cell recognition. These multiple, simultaneous events have unique collective properties that are qualitatively and quantitatively different from the properties displayed by their constituents, which interact monovalently. This is reflected in binding constants orders of magnitude larger than those based on a monovalent interaction.[1] In recent years, many scientists have focused their research on the rational design of multivalent ligands which may take advantage of the same principle and can be used as inhibitors of receptor–ligand interactions or as activators of signal transduction pathways.[1,2] To this aim different templates have been synthesized such as polymers,[3] dendrimers[4] or small clustered ligands[5] having multiple ligand sites able to elicit substrate recognition through simultaneous multiple interactions.[6]

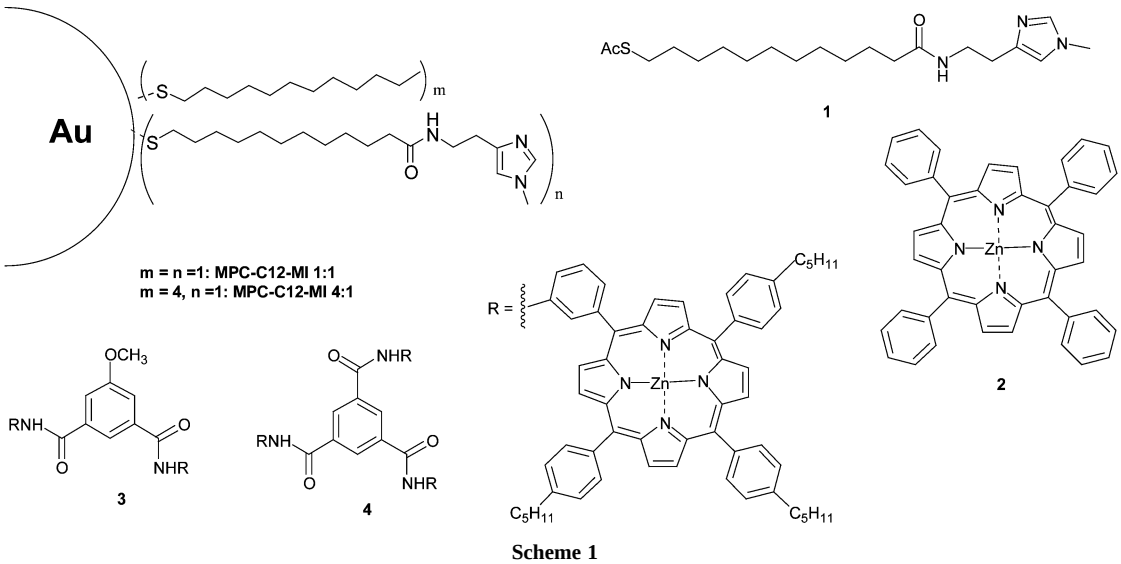
A subtle role in polyvalent interactions is played by factors controlling enthalpy and entropy. For instance, if the geometry of the polyvalent entity fits perfectly into the binding sites without suffering distortion, this event should not be entropically costly. Conformational flexibility increases the entropic cost of the association, but increases the likelihood that all ligand–receptor interactions can occur without energetic strain.

Monolayer protected gold nanoclusters (MPCs) may provide a simple, self assembled model of a multivalent entity. They present a collection of functional groups on their periphery and may constitute an excellent and easily accessible system to test the relevance of multivalent interactions. Indeed, examples of multivalent systems based on MPCs are emerging in the chemical literature.[7]

To assess the quantitative significance of multiple interactions, we have exploited MPC with a mixed monolayer containing different loadings of *N*-methylimidazole as ligands for the recognition of discrete porphyrin arrays. In particular, we used MPC-C12-MI 1:1 and MPC-C12-MI 4:1 which have a mixed monolayer composed of a 1:1 and 4:1 mixture of dodecanethiolates and thiolates functionalized with *N*-methylimidazole (Scheme 1). These nanoparticles of 2.2 nm gold cores have been prepared as previously described[8] using the procedure developed by Brust and Shiffrin[9] and fine tuned by Murray.[10] Compound **1** was used as the monomeric ligand for reference purposes. The porphyrin arrays used were ZnTPP **2**, bis-porphyrin† **3** and tris-porphyrin **4**.

They show typical UV-Vis spectra with red shifted Soret bands upon binding of an apical ligand. This allows the easy determination of the affinity constant and, in the present case, the quantification of the multivalent interaction between MPC and the porphyrins as their number increases from 1 to 3 in the array.

By plotting the increase of absorbance at the bound wavelength (*ca* 430 nm) for the three porphyrin-based systems (**2–4**) upon changing the concentration of ligand **1**, MPC-C12-MI 1:1 and MPC-C12-MI 4:1, we could determine the binding



Scheme 1

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constants reported in Table 1.‡ § The following conclusions can be drawn from the data. i) The binding constant of model methylimidazole derivative **1** to monoporphyrin **2** is slightly larger than that measured for the two clusters, suggesting some steric hindrance when the porphyrins approach the MPC surface. ii) The apparent binding constant to the nanoclusters increases by up to three order of magnitude for the tris-porphyrin-based system, and there is no significant dependence on the concentration of methylimidazole functions on the MPC surface. This is likely due to the fact that the experiments have been carried out using an excess of nanoclusters, so that there is only one porphyrin array per MPC, and all ligands on the surface are available for binding.¶ iii) As the number of porphyrins increases, the relative gain in binding constant (β) and effective molarity (EM) for intracomplex binding become lower. This is highlighted by the plot of Figure 1 where the dotted line represents the binding constant expected on the basis of an additive and independent contribution by each porphyrin. By extrapolation of this trend, one may speculate that upon increasing the number of porphyrins in the array the binding constant will reach a limiting value with no further improvement on increasing the number of recognition sites in the multivalent system. This might be explained by the poor fitting of the porphyrin array to the curved surface of the MPCs or to the accumulation of adverse interactions with the surface, mentioned in point i) above.

However, it must be pointed out that the values of β and EM as well, observed for tris-porphyrin **4**, are slightly higher than those reported by us using a tripodal methylimidazole derivative whose structure was complementary to that of **4**.[12] This indicates that the cooperative binding of the methylimidazoles on the nanoparticles to the tris-porphyrin is as good as (or better than) that of the tripodal ligand. This positive comparison implies that, due to the flexibility of the terminal part of the chain on the MPC surface, the mobility of the methylimidazole thiolates compensates for the lack of complementarity without paying a significant entropic price.

Table 1 Binding constant of Zn(TPP), bis-porphyrin **3** and tris-porphyrin **4** to model ligands **1**, MPC-C12-MI 1: 1 and 4:1 in CH_2Cl_2 at 25 °C

Porphyrin	Ligand	logK_b[a]	$\beta^c = \frac{(K_b)_{poly}}{(K_b)_{mono}}$	EM[d] (mM)
2	**1**	4.49 ± 0.02		
	MPC-C12-MI 1:1	4.04 ± 0.04	1	—
	MPC-C12-MI 4:1	4.15 ± 0.05	1	—
3	**1**	4.61 ± 0.06		
	MPC-C12-MI 1:1	6.01 ± 0.05	93	9
	MPC-C12-MI 4:1	6.04 ± 0.10	78	5
4	**1**	[b]		
	MPC-C12-MI 1:1	7.05 ± 0.05	1023	1
	MPC-C12-MI 4:1	7.10 ± 0.20	891	0.8

[a] Binding constants are in mol^{-1}. [b] Not determined. [c] See reference 1a for its definition. [d] See reference 11 for its definition.

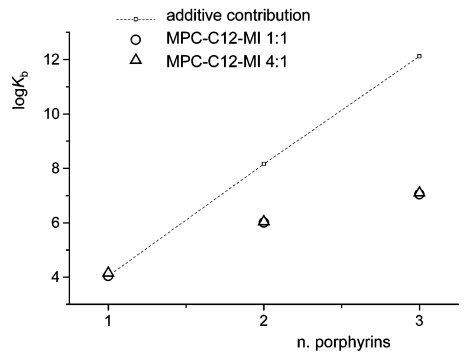

Fig. 1 Graphical representation of log K_b as a function of the number of porphyrins in the array for the two clusters (○) MPC-C12-MI 1:1 and (Δ) MPC-C12-MI 4:1. The dotted line is the expected value for log K_b considering an additive, independent contribution for each porphyrin.

In conclusion, we have reported a compelling example of multivalent recognition based on functional gold nanoparticles where the single contribution of each binding event could be dissected. As pointed out by Whitesides,[1a] with the notable exception of hemoglobin (allosteric binding), "There are presently no convincingly characterized examples of positive cooperativity for polyvalent systems in the literature." The present system does not constitute an exception.

The complexation of porphyrins on the external surface of functional nanoparticles may allow one to obtain new functional materials. The study of the properties of these and similar supramolecular aggregates are in progress in our laboratory.

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Notes and references

† All new compounds gave the expected ^{1}H- and ^{13}C-NMR spectra and the correct elemental analyses (C, H, N).

‡ The binding isotherms were fitted using the program Scientist, MicroMath Scientific Software.

§ With porphyrin **3** the spectra indicate the formation of stacked porphyrins at very low cluster concentration (i.e. when [porphyrin] > [methylimidazole] on the cluster). This does not hamper the correct determination of the binding constant.

¶ Preliminary experiments carried out by saturating the monolayer surface with phorphyrin **2** indicate that *ca.* 70% of the imidazoles of MPC-C12-MI 1:1 is available for binding.

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