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# Tuneable pseudorotaxane formation between a biotin–avidin bioconjugate and CBPQT$^{4+}$†

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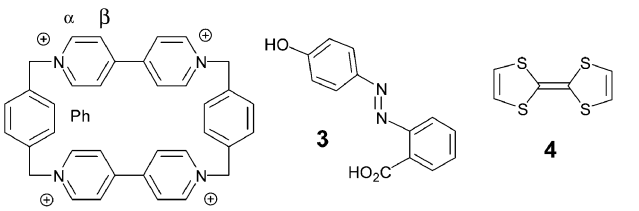
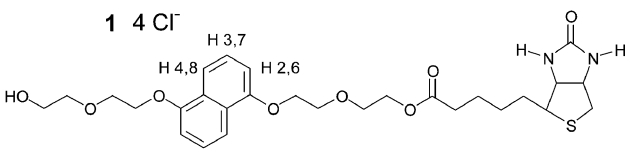
**A biotinylated 1,5-dialkoxynaphthalene derivative has been shown to have the ability to bind strongly to avidin and thus act as an artificial binding site for cyclobis(paraquat-*p*-phenylene) thereby facilitating the formation of a tuneable pseudorotaxane-based bioconjugate.**

The popularity of avidin–biotin supramolecular formulations is largely due to the ability of biotin to bind to the four binding sites of the avidin subunits with very high affinity, thereby affording a convenient model for probing protein–ligand interactions.[1] This phenomena has led to a number of biological applications including: clinical diagnostics,[2] tumour pre-targeting[3] and drug delivery.[4] More recently, materials science applications have also emerged including surface engineering[5] and synthetic polymer bioconjugates.[6] In the broader sense, the non-covalent modification of proteins in this fashion offers an attractive strategy for conveniently allowing the production of reversible stimuli-responsive bioconjugates.[7]




**2**

The tetracationic cyclophane cyclobis(paraquat-*p*-phenylene) (CBPQT$^{4+}$, **1**) has emerged as an important host molecule for electron-rich guests.[8] This is primarily due to: the ability to synthesize this cyclophane in reasonable quantities;[9] its ability to have its recognition properties tuned by redox processes;[10] its ability to form complexes in both organic and aqueous media[11] and its formation of guest–specific coloured complexes that conveniently allows guest exchange reactions to be monitored using UV-Vis spectroscopy.[12] Here, we report a biotinylated naphthalene derivative **2** that has the propensity to bind to avidin strongly. The electron-rich naphthalene moiety of **2** is a proven effective guest for **1**,[12] thereby allowing the creation a bioconjugate possessing an artificial binding site for CBPQT$^{4+}$, and thus facilitating the formation of a pseudorotaxane.[13]

Compound **1** was prepared as reported previously.[9] Compound **2** was conveniently obtained by EDCI catalysed coupling of 1,5-bis[2-(hydroxyethoxy)ethoxy]naphthalene and biotin (see ESI†). The ethylene glycol groups were included in structure **2** to increase the solubility in aqueous media and improve the biocompatibility of the naphthalene moiety. Complexation of **1** and **2** in aqueous media was confirmed by $^1$H NMR (Fig. 1), NOESY and COSY (see ESI†) spectroscopy. A mixture of $D_2O$–EtOH-$d_6$ (70/30 v/v) was used as solvent to aid the solubility of **2**. A 1 : 1 mixture of **1** and **2** gave rise to a purple solution, and large upfield shifts in the $^1$H NMR signals for the naphthalene protons (compared to **2** alone), which are consistent with those reported for a unmodified 1,5-dialkoxynaphthalene derivative upon complexation

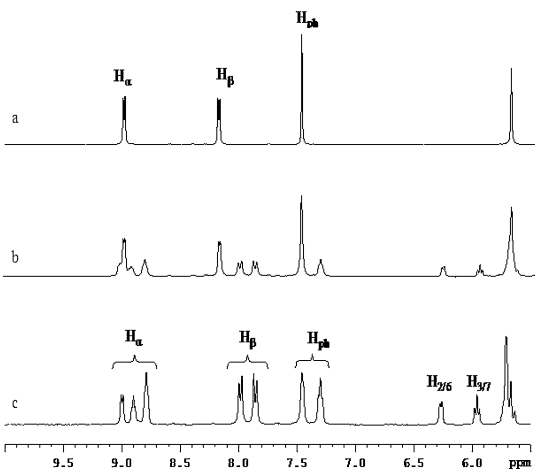



**Fig. 1** Partial $^1$H NMR spectra recorded in $D_2O$/EtOH-$d_6$ (70 : 30) showing: (a) spectrum of **1**, (b) upon the addition of 0.5 equivalents of **2**, (c) 1 : 1 complex of **1** and **2**. $H_\alpha$, $H_\beta$ and $H_{ph}$ refer to the hydrogens of **1** whereas $H_{2/6}$ and $H_{3/7}$ refer to the hydrogens of the naphthalene moiety of **2**.

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† Electronic supplementary information (ESI) available: Synthesis of **2**, NMR and ITC data. See DOI: 10.1039/b803856b

 

with **1** in aqueous conditions.[11] Complexation of **1** and **2** resulted in minimal changes in the proton resonances for the biotin moiety, indicating that this unit is not interacting with **1** to any significant extent. The unsymmetrical nature of **2** imposes different environments on the α-pyridinium hydrogens of **1**, as reflected in the $^1$H NMR spectrum by an unsymmetrical splitting pattern (see Fig. 1 and ESI†). 2D NOESY experiments performed on a 1 : 1 admixture of **1** and **2**, clearly show dipolar correlations between the $H_{4/8}$, $H_{3/7}$ protons of **2** and the $H_{Ph}$ and $H_{\beta}$ protons of cyclophane **1**, respectively. Thus, the NMR data are consistent with pseudorotaxane formation between **1** and **2** under the conditions investigated.

The binding between avidin, **1** and **2** was investigated using isothermal titration microcalorimetry (ITC) (Fig. 2 and ESI).[11g] Titration of **2** into a solution of avidin gave a strong exothermic response with $K_a = 7.3 \times 10^6$ M$^{-1}$ ($\Delta H = -23$ kcal mol$^{-1}$) (see Fig. 3(i)). A binding stoichiometry of four suggests that **2** has the ability to complex with all four available binding sites of avidin. A solution of **1** was then titrated into the precomplexed **2** and avidin. Again a strong exothermic response was observed with $K_a = 4.7 \times 10^4$ M$^{-1}$, ($\Delta H = -12$ kcal mol$^{-1}$). This is in accordance with complex formation between **1** and the naphthyl unit of avidin-bound **2**. As it has previously been shown that **1** has the propensity to weakly bind to electron rich amino acids,[13] control experiments were undertaken by titrating **1** into a solution of avidin which showed negligible complexation compared to data obtained when the naphthalene unit was pre-complexed with avidin (Fig. 3(ii)).

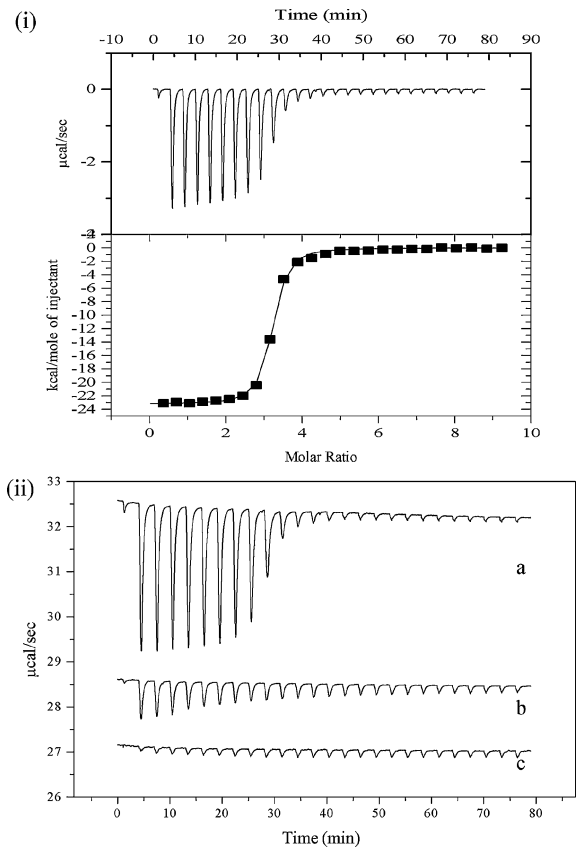


**Fig. 2** Isothermal titration calorimetry recorded in water–ethanol (70 : 30, v/v) showing plots of: (i) heat *vs.* molar ratio of guest **2** added to avidin; (ii) heat *vs.* time plots for: (a) the titration of **2** into avidin, (b) the titration of **1** into an avidin-**2** complex, (c) the titration of **1** into an avidin solution.

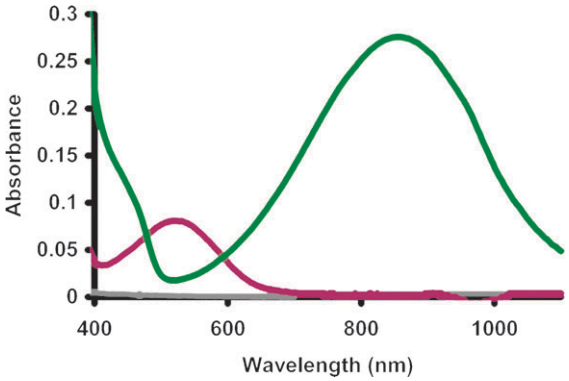


**Fig. 3** UV-Vis spectra recorded in water–ethanol (70 : 30, v/v) of: avidin (~$6 \times 10^{-4}$ M) + **2** (~$6 \times 10^{-5}$ M) (grey line); avidin (~$6 \times 10^{-4}$ M) + **2** (~$6 \times 10^{-5}$ M) + **1** (~$6 \times 10^{-5}$ M) (purple line), avidin (~$6 \times 10^{-4}$ M) + **2** (~$6 \times 10^{-5}$ M) + **1** (~$6 \times 10^{-5}$ M) + **3** (~$2 \times 10^{-4}$ M) (green line).

Further evidence to prove that compound **2** successfully binds to avidin was achieved by performing competition experiments between the complex of 2-(4-hydroxyphenylazo)-benzoic acid (HABA, **3**) and avidin. Compound **3** binds moderately strongly to the same binding site on avidin as biotin, resulting in the formation of a red coloured complex, thereby allowing the displacement of **3** upon the addition of **2** to be monitored colorimetrically (see ESI†).[14] The addition of **2** to a solution of the avidin–**3** complex resulted in the immediate disappearance of the peak at $\lambda \sim 500$ nm and the formation of a peak at $\lambda \sim 353$ nm characteristic of free **3**. Thus, these experiments are consistent with the ITC measurements and show that **2** has a good affinity for avidin.

Having shown by ITC that the biotin moiety of **2** has the ability to bind strongly to avidin and then subsequently to complex with the **1**, we next investigated whether it is possible to disrupt the complex using a competing guest for the cavity of the cyclophane. We have exploited the well-documented interchangeability of guest species within the cavity of **1**.[15] In particular, to achieve this we have exploited the ability of tetrathiafulvalene **4** to disrupt complexation with naphthalene based guests in aqueous media. An attractive feature of this host exchange process is the associated colour change (purple to green) that accompanies this process, thereby allowing convenient monitoring using UV-Vis spectroscopy (Fig. 3).

Addition of **1** into a solution of avidin dissolved in water–ethanol (70 : 30) resulted in no observable colour change of the solution, further supporting the ITC data indicating negligible complexation occurs. However, the addition of **1** to a solution containing **2** and avidin resulted in the formation of a purple solution ($\lambda \sim 520$ nm) characteristic of pseudorotaxanes formed between **1** and naphthalene guests.[11] The addition of **4** resulted in two major changes to the UV-Vis spectrum. First, the absorption around $\lambda \sim 520$ nm is significantly reduced and a new absorption appears around $\lambda \sim 856$ nm

giving rise to an emerald green solution that is characteristic of a pseudorotaxane formed between **1** and **4**.[15] Thus, the data are consistent with the ability of **4** to successfully compete for the binding site of **1**, thereby disrupting the complex formed between the avidin–**2** conjugate and cyclophane **1**.

In summary, we have successfully introduced an artificial binding site onto avidin using biotin derivative **2**. This bioconjugate facilitated the formation of a complex with cyclophane **1**. We have then shown that the complex can be disrupted upon the addition **4**, which successfully competes for the cavity of **1**. These studies pave the way for the fabrication of tuneable avidin–biotin conjugates for diagnostic applications. Furthermore, the study lays the foundations for the development of other tuneable protein–pseudorotaxane conjugates. Our endeavours in these areas will be published in due course.

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