A general and versatile molecular design for host molecules working in water: a duplex-based potassium sensor consisting of three functional regions†

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A general and versatile molecular design for host molecules was proposed based on the structural motif of antibodies. A water-soluble potassium sensor was developed by this molecular design, which consists of three functional regions, benzo-15-crown-5 ether, DNA, and pyrene as guest-binding, dimerising, and signaling sites, respectively. The molecular sensor emitted the monomer fluorescence of the pyrene fluorophore predominantly when existing as a random structure, while it formed a duplex upon recognition of K^+ to bring about monomer–excimer emission switching.

Molecular design plays a major role in the development of a variety of host molecules, self-assembled molecules, molecular devices, *etc.* In host–guest chemistry, most of well-sophisticated host molecules have been created from the viewpoint of "pre-organization" concept for targeting a specific guest molecule.[1,2] Macrocycles, such as cyclophanes, based on this concept are representative host structures that reduce entropic disadvantages upon recognition of guest molecules in contrast to the cases for acyclic ones.[3] However, such pre-organization approach lacks versatility for recognizing a series of guest molecules. On the other hand, molecular recognition events *in vivo* are mainly due to an "induced-fit" mechanism. To provide a new approach for designing a variety of host molecules, especially in water, we noted the structure of immunoglobulin, an antibody (Fig. 1A).[4] Antibodies show versatile recognition abilities for many antigens, taking advantage of their structural feature consisting of two regions, *i.e.*, variable and constant regions: the former responds to the binding with antigens and the latter mediates the effector functions. Here, we propose a novel design technique for molecular sensors working in water, inspired from the structural characteristics in antibodies (Fig. 1B).

The unique point in the designed host is that dimerisation of the monomer units results in the recognition process, accompanying an additional function of signaling. To realise our molecular design, a water-soluble potassium cation sensor **1** (**1a** + **1b**) was developed (Fig. 2).[5] Thus, each monomer consists of three functional regions, crown ether (corresponding to a variable region), DNA (a constant region), and pyrene (an additional constant region) from top to bottom in Fig. 1B. The crown ether of the recognition site can be replaced by various host molecules such as cyclodextrins, cyclophanes, and synthetic peptides, and a hetero-type host combination of two different recognition sites is also possible, *e.g.*, crown ethers and cyclodextrins. Water solubility of the host molecules is provided by the DNA site that simultaneously works as a hinge for dimerisation of the monomer units. Furthermore, the DNA site should be used to regulate a monomer–dimer equilibrium of host molecules at will by simply adjusting the number and types of base pairs in its DNA. The hydrophobic pyrene moiety possesses signaling ability based on its photochemical property of monomer–excimer emission switching, and it additionally acts like a base pair to enhance the stability of the duplex.[6]

The two complementary monomers **1a** and **1b** were prepared by labeling the corresponding 7-mer oligonucleotides with pyrene and benzo-15-crown-5 ether. A single-stranded 7-mer DNA possessing a C3-alkylamino linker (3′ end) was coupled with the pyrene-based phosphoramidite **2**[6] at the 5′ end on solid-phase DNA synthesis and released from the support. The obtained 5′-functionalized DNA was further tethered to the crown-based succinimidyl ester **5** at the amino terminal of the 3′-linker to give **1a**. In the case of **1b**, the complementary 7-mer oligonucleotide was modified with the crown-based **3** and the pyrene-based **4**.[6] The reference monomers **1a′** and **1b′** lacking in the crown ether moieties were similarly prepared with **2** (5′ end) and **4** (3′ end), respectively (Scheme 1).

To test the abilities of **1** as a molecular sensor, fluorescence titration experiments were performed with K^+ in an aqueous buffer solution (Fig. 3). Addition of KCl to a solution of **1a/1b** ([**1a**] = [**1b**] = 200 nM) caused an increase in the excimer intensity ($I_{500\ nm}$) and a decrease in the monomer one ($I_{380\ nm}$), illustrating monomer–excimer switching on the pyrene moieties. The presence of the isoemissive point at 448 nm means that only two types of fluorescent species exist in the solution, *i.e.*, the single-stranded **1a** (and **1b**) and the duplex **1**, as expected. We must, however, consider the possibility for the stabilisation of the duplex structure by interaction between the phosphates in the DNAs and the added metal cations.[7] Therefore, control experiments were performed in order to shed light on this aspect. Fluorescence changes were measured in each case of four sets of duplexes (**1a/1b**, **1a/1b′**, **1a′/1b**, and **1a′/1b′**) on titration with KCl at 380 nm (monomer emission) and 500 nm (excimer emission). The chemical structure of **1a′** (or **1b′**) is the same as that of **1a** (or **1b**) except for the absence of the crown moiety. The increasing order of the ratio ($I_{500\ nm}/I_{380\ nm}$) against KCl concentration is as follows:

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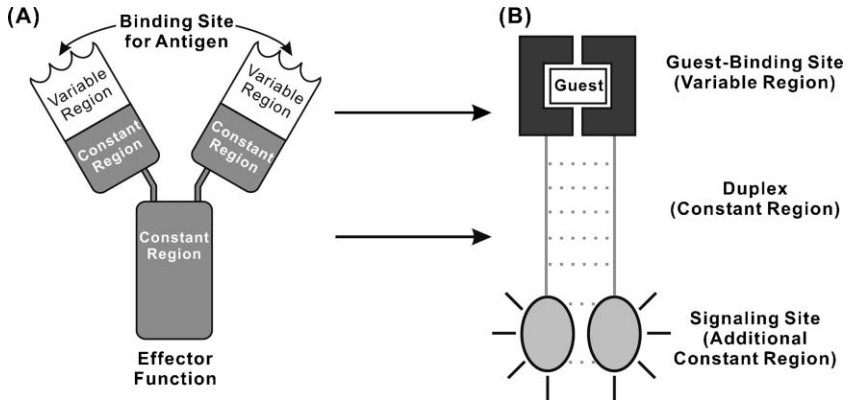
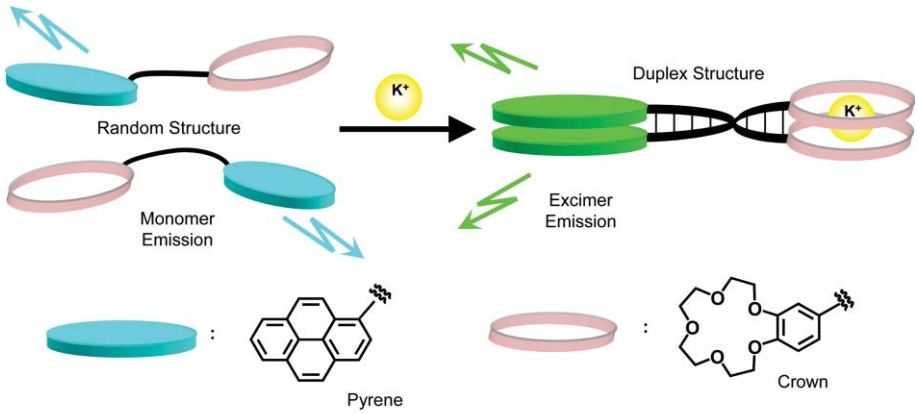
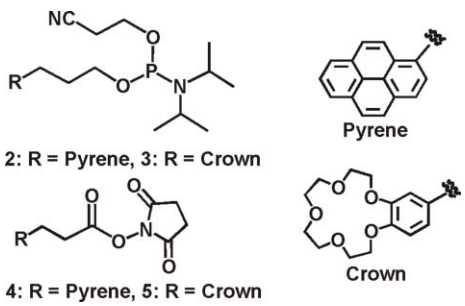


Fig. 1 The structural motif of antibodies (A) and the molecular design for host molecules working in water (B).



1a : 5′-*Pyrene*-$(CH_2)_3$-GACTGAC-$(C_3NHCOC_2H_4)$-*Crown*-3′
1b : 3′-*Pyrene*- $(C_2H_4CONHC_3)$- CTGACTG-$(CH_2)_3$-*Crown*-5′
1a′ : 5′-*Pyrene*-$(CH_2)_3$-GACTGAC-3′
1b′ : 3′-*Pyrene*- $(C_2H_4CONHC_3)$- CTGACTG-5′

Fig. 2 A schematic representation of a duplex-based potassium sensor **1** (**1a** + **1b**) with monomer–excimer switching, and the chemical structures of **1** and **1′** (**1a′** + **1b′**).



Scheme 1

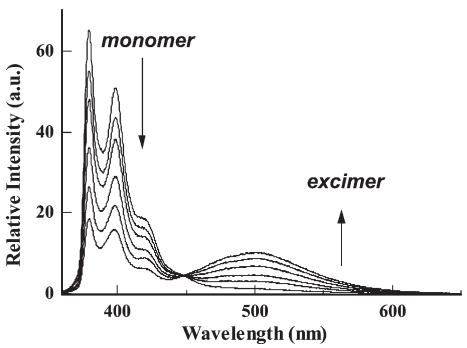


Fig. 3 Fluorescence titration spectra of **1** ([**1a**] = [**1b**], 200 nM; [Tris-HCl], 20 mM, at pH 8.0) on titration with KCl (5–100 mM). Excitation wavelength was 350 nm.

1a′/1b′ < **1a/1b′** ≃ **1a′/1b** < **1a/1b** (Fig. 4). In **1a′/1b′**, slight increase of that value was observed, probably depending on the stabilisation of the duplex simply by the increase of the ionic strength. The values for **1a/1b′** and **1a′/1b** were at an intermediate extent between those for **1a/1b** and **1a′/1b′**. Thus, the florescence change of **1**, at least partially, might be driven by the sandwich-type binding of K^+ with the aid of a pair of the crown ethers in **1**.

Since *in vivo* ubiquitous monovalent metal cations are Na^+ and K^+, the selectivity of **1** between them was investigated. As compared with the results for K^+, the fluorescence change for **1** by the addition of Na^+ was small (Fig. 4). The extent of this change was similar to those for **1a/1b′** and **1a′/1b** on titration with K^+,

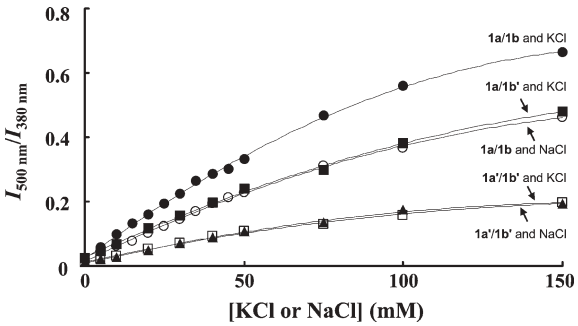


Fig. 4 Plots of the intensity ratio ($I_{500\ nm}/I_{380\ nm}$) against the concentration of KCl and NaCl ([**Host**], 200 nM; [Tris-HCl], 20 mM, at pH 8.0). ●:**1a/1b** and KCl, ■:**1a/1b**′ and KCl, ○:**1a/1b** and NaCl, □:**1a**′**/1b**′ and KCl, and ▲:**1a**′**/1b**′ and NaCl. Plot for **1a**′**/1b** and KCl was almost identical to that for **1a/1b**′ and KCl.

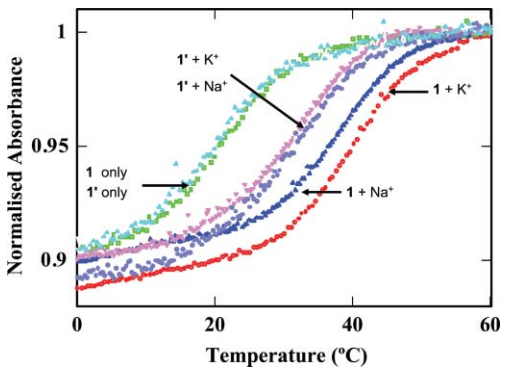


Fig. 5 Normalised melting curves at 260 nm. Melting curves were normalized to the absorbance at 60 °C. The solution for the measurements contained **1** or **1**′ (1 μM) and 20 mM Tris-HCl (pH 8.0) in the presence or absence of alkali-metal chloride (100 mM).

certifying the selectivity of **1** for K^+. It is well-known that 15-crown-5 ethers prefer 1 : 1 binding with Na^+ and 2 : 1 binding in sandwich-type complexes with K^+.[8] Thus, these data reveal that the selective binding of K^+ by the crown moieties occurs cooperatively with the duplex formation between **1a** and **1b**. To obtain further evidence that the crown ethers in **1** contribute to the binding with K^+, melting points (T_m) of **1** (1 μM) in the presence or absence of KCl (100 mM) were measured on the basis of the electronic absorption spectra (Fig. 5). The T_m values decreased in the following order: 41 °C (**1** + K^+) > 32 °C (**1**′ + K^+) > 21 °C (**1** only). The fluorescence titration and the T_m measurement demonstrated that a pair of the crown ethers in **1** played a key role for the selective binding with K^+. Unfortunately, it was impossible to accurately determine the binding constants of **1** for K^+ and Na^+ because the guest cations can interact not only with the crown ethers of **1** but also with the phosphate anions in the DNA sites nonstoichiometrically.

We developed a duplex-based potassium sensor working in water by means of a novel molecular design technique, in which three functional regions integrated on the host architechure can act cooperatively for sensing guest molecules. Recently, a few studies for sensing K^+ in water have been reported, utilizing G-quartet structures of DNAs, cryptands, and diaza-crown ethers as a binding component.[9–11] Although the sensitivity for K^+ in the present system is not remarkably high, this design technique will be general and versatile for creating various host molecules working in water and may have a great practicability. In future investigations, other recognition components will be introduced not only homogeneously but also heterogeneously into the architecture to produce a variety of water-soluble host molecules.

This paper is dedicated to our colleague, Haruna Teraoka, deceased August, 2004.

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