Allosteric Switches

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Signal-Amplifying Resonance Energy Transfer: A Dynamic Multichromophore Array for Allosteric Switching**

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Cooperativity is an important operation mode for regulatory proteins with multiple binding sites.[1–3] The interdependence of stepwise binding events in such multi-subunit receptors is manifested by a sigmoidal binding isotherm, in which a sharp change in the response curve relates the "on" and "off" states that are separated by a narrow concentration window (Figure 1a). Extreme cooperativity in homotropic allosterism results in a binary-function-like response curve that approaches that of genuine on–off switching (Figure 1b) and is difficult to reproduce with non-cooperative host–guest systems displaying a simple hyperbolic behavior (Figure 1c).[4–6] Undoubtedly, artificial systems with self-regulatory properties remain an engaging challenge.[3,5,7]

Despite elegant demonstrations of positive allosterism with selected synthetic receptors,[5,8] a genuine switching cycle, shown as reversible turn-on and turn-off scans in Figure 1a, is yet to be realized.[9] Herein, we describe a dynamic multichromophore array (DA_3), which transduces allosteric conformational changes of the energy-donor unit (D) into amplified fluorescence emission from the energy-acceptor units (A). The characteristic sigmoidal response function and signal buffering of this array convincingly demonstrate cooperativity in both the turn-on and turn-off switching cycles. Notably, the unusual nonlinear behavior observed in its resonance energy transfer (RET) process implicates orientation-dependent D–A coupling as a viable mechanism for signal amplification.[10]

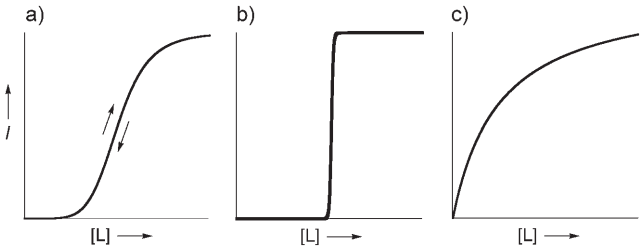

Figure 1. Response curves describing the cooperative binding of a ligand L to a receptor S with: a) $n=6$ and b) $n=100$ binding sites. A non-cooperative binding isotherm is shown in (c). These plots were constructed from the Hill equation with arbitrary binding constants (for comparative purposes).[4]

We recently showed that the photophysical properties of a two-dimensional (2D) electronic conjugation can be reversibly modified by structural folding and unfolding motions.[11a] The molecular C_3 symmetry of this first-generation compound **1** (Figure 2a) intrinsically suggested cooperativity in the stepwise assembly and disassembly of its cyclic hydrogen-bonding network as part of the conformational switching, but direct experimental evidences for this intriguing phenomenon could not be attained. To probe the solution dynamics of this emerging family of switchable 2D fluorophores,[11e] we turned our attention to the RET of multichromophore arrays.[10,12]

Our molecular design, as shown in Figure 2c, consists of three A units placed around a common D unit to cause RET from the donor excited state (D^*A_3) to an ensemble of acceptor excited states ($D(A_3)^*$). Unlike typical RET systems, in which the acceptor emission is modified by changing the D···A distance (while all other parameters are treated as constants),[10] this dynamic system operates by structural folding and unfolding, a procedure that reversibly tunes the donor quantum yield (Φ_D) to directly impact emission from the acceptor units (Φ_A) at essentially fixed D···A distances. This seemingly straightforward and arguably incremental structural modification has highly unexpected photophysical consequences, as described below.

For efficient RET with the tris(*N*-salicylideneaniline)[11] energy donor, a boron–dipyrrin (BODIPY)[13] derived energy acceptor was employed to prepare compound **2** in a highly convergent four-step synthetic sequence from readily available starting materials (Scheme 1).[14] The characteristic proton NMR signals of $N_{enamine}$–H (13.58 ppm) and C_{vinyl}–H (9.95 ppm) and the highly deshielded O–H (5.08 ppm) proton resonance signal of compound **2** match closely with the signals of structurally characterized analogues that have O–H···O–H···O_{ketone} hydrogen-bonding networks supporting the C_3 symmetric molecular core (Figure 2).[11a,b] Variable-temperature ^{1}H NMR studies of compounds **1** and **2** furnished comparable $\Delta\delta_{O-H}/\Delta T$ plots (see Figure S1 in the Supporting Information),[14] which were consistent with the conformational switching depicted in Figure 2c.

At 25 °C, compound **2** displays electronic transitions in CH_2Cl_2, which are associated with local chromophores resembling **1** and **3** (see Figure S2 in the Supporting Information).[14] The essentially orthogonal geometric relationship between the BODIPY plane and the aniline ring (Scheme 1) prevents direct electronic conjugation, thus minimizing the interchromophore interactions in the ground state. With restricted torsional motions around the $C_{aniline}$–C_{BODIPY} bonds, which are enforced by the BODIPY methyl groups pointing

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

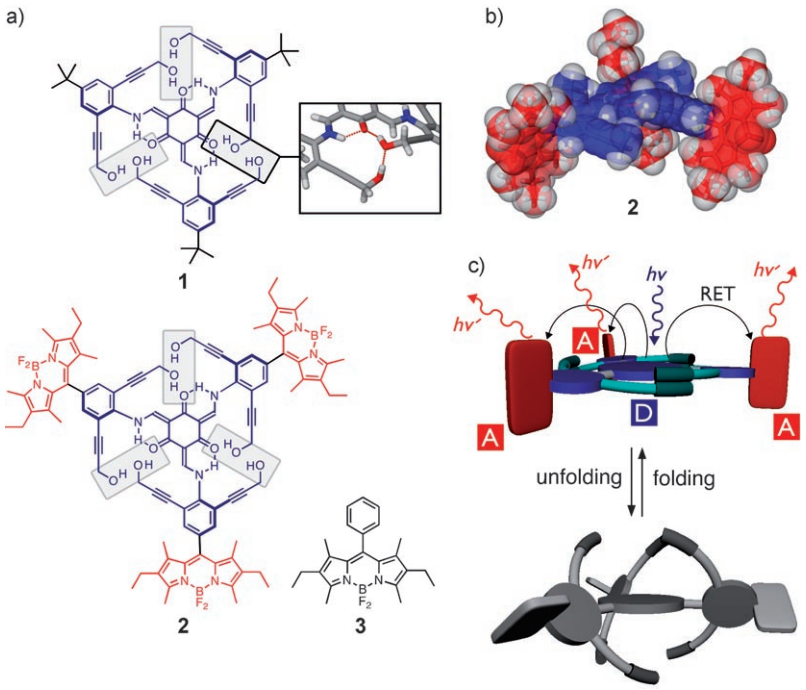
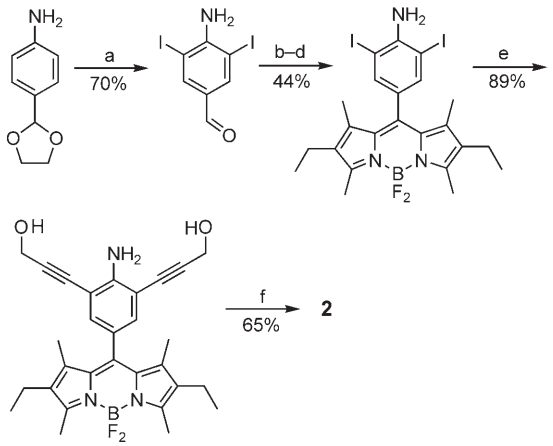


Figure 2. a) Chemical structures of compounds **1**–**3** with a close-up view of the $O_{hydroxy}$—H···$O_{hydroxy}$—H···$O_{carbonyl}$ hydrogen-bonding network (shown as capped-stick model).[11a,b] b) Energy-minimized (PM3) structure of compound **2** in which the tris(*N*-salicylideneaniline) and BODIPY units are color-coded with blue and red, respectively. c) Schematic representation of conformational switching between the emissive "folded" (top) and the nonemissive "unfolded" (bottom) states of compound **2** (D = energy donor; A = energy acceptor).



Scheme 1. Synthetic route to compound **2**.[15] a) Ag_2SO_4, I_2, EtOH, room temperature (RT); b) 3-ethyl-2,4-dimethylpyrrole (2.1 equiv), CH_2Cl_2, RT; c) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (1.1 equiv), CH_2Cl_2, RT; d) Et_3N (10 equiv), $BF_3{\cdot}Et_2O$ (15 equiv), CH_2Cl_2, △; e) $HCCCH_2OH$, $[PdCl_2(PPh_3)_2]$ (2 mol%), CuI (3 mol%), iPr_2NH/THF, RT; f) 1,3,5-triformylphloroglucinol, EtOH, △.

toward the aniline rings, the multichromophore array **2** with well-defined spatial relationships between the energy-donor and -acceptor units is established.

Upon excitation at 380 nm, compound **2** emits an intense yellow BODIPY fluorescence signal at $\lambda_{max,em} = 546$ nm with a quantum yield of 20%. The complete disappearance of the donor emission centered at 457 nm indicates an essentially quantitative RET (see Figure S2 in the Supporting Information).[14] Notably, this RET cascade resulted in an approximately fivefold enhancement of the overall emission quantum yield relative to that of the donor-only compound **1**. This enhanced dynamic range prompted us to use the emission from remotely located energy acceptors to probe structural folding and unfolding of the energy donor (Figure 2c).

In CH_2Cl_2, the fluoride ion, added as nBu$_4$NF, elicits a structural unfolding of the energy donor (compound **1**), which can be readily monitored by following the decrease in the fluorescence quantum yield (Φ_D).[11a] Under similar conditions, compound **2** undergoes an expected quenching of the energy-acceptor emission, but with an unexpected signal amplification towards the F^- ion. As shown in Figure 3, compound **1** exhibited a decrease of about 40% in its emission intensity (Φ_D) upon treatment with 6 equiv of F^- ions. In contrast, the fluorescence from the RET acceptor in compound **2** was essentially quenched (>90% decrease in Φ_A) under similar conditions. Control experiment with compound **3** ruled out direct interaction between BODIPY and the F^- ion in this process (Figure 3b).

The apparent 1:6 binding stoichiometry between compound **2** and the F^- ion (see Figure 3b) defined a narrow concentration window to probe the details of this molecular-recognition event. As shown in Figure 4a, the fluorescence intensity of compound **2** could be reversibly switched by the stepwise addition and removal of F^- species.[16] Notably, the turn-off and turn-on "scans" trace essentially superimposable sigmoidal isotherms reminiscent of that in Figure 1a. The rapid rise/drop in the response function, bracketed by signal-buffering zones ($dI/d[F^-] = 0$) on both sides, prevents undesired turn-on/off by noise-level

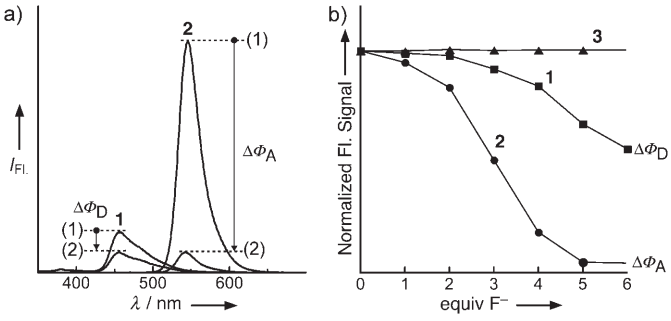


Figure 3. a) Emission spectra of compounds **1** and **2** normalized to the absorbance at the excitation wavelength (340 nm) 1) prior to and 2) after addition of F^- ions (6 equiv). b) Changes in the fluorescence intensity of compounds **1**–**3** as a function of the amount of F^-. For direct comparison, emission data have been normalized to the same value of integrated peak area at $[F^-] = 0$. $\lambda_{ex} = 340$ nm for compounds **1** and **2** and 480 nm for **3**. [**1**] = 1.0 μM, [**2**] = 1.0 μM, and [**3**] = 3.0 μM in CH_2Cl_2 (at 298 K). $I_{Fl.}$ = fluorescence intensity.

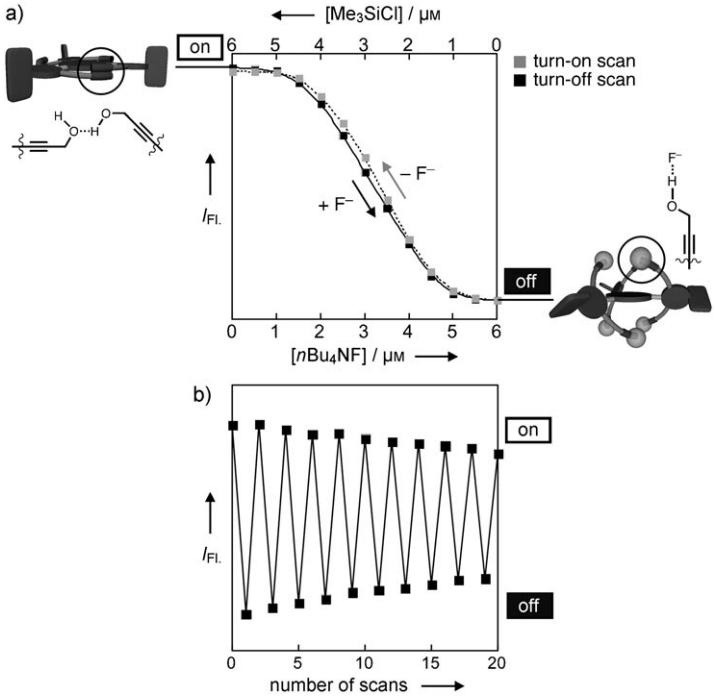


Figure 4. a) Sigmoidal fluorescence response curves obtained by titration of compound **2** with nBu$_4$NF and subsequent back-titration with Me$_3$SiCl ([**2**] = 1.0 μM in CH_2Cl_2 at 298 K). b) On–off switching cycles of compound **2** monitored by changes in the fluorescence intensity upon successive addition and removal of F^- ions (6 equiv), as described in (a). Corrections were made for dilution effects.

input, which is a critical requirement for genuine switching devices.[5–7] This switching cycle can be repeated over ten times without significant loss of reversibility (Figure 4b).

For a given n value, multiple isomeric **2**·$(F^-)_n$ species can exist which differ in the positional distribution of the fluoride ions among the n binding sites. The absence of hysteresis in the forward and backward scans shown in Figure 4a implicates a rapid establishment of equilibrium between these positional isomers to produce a population-averaged fluorescence signal that is independent of the reaction pathways followed to reach the same fluoride inventory, either by addition (turn-off scan) or by removal (turn-on scan) of F^- ions. The ^{1}H NMR spectrum of a mixture comprising compound **2** and nBu$_4$NF (6.5 equiv) in CD_2Cl_2 revealed the disappearance of the wing-tip hydroxy-proton resonances along with a significant broadening of the signals from adjacent methylene groups; this observation is consistent with a rapid chemical exchange of the O–H protons via a hydrogen-bonding interaction with the F^- ions.[17]

The self-accelerated reactivity of compound **2** can equally well be explained by invoking either the two-state concerted-switching model (MWC model) or the sequential conformational transition model (KNF model) of allosterism (see Schemes S1 and S2 in the Supporting Information).[1,2,14] The progressive structural unfolding of compound **2** should present an increasing number of OH groups, the interdependence of which is already preprogrammed in their symmetric arrangements in a tight hydrogen-bonded network (Figure 2a).

Key to the observation of this remarkable allosteric switching is the highly unusual signal-amplification behavior that we observed as part of the RET. Specifically, the normalized response profile shown in Figure 3b deviates significantly from the simple proportional relationship between the donor and acceptor emissions that would normally be anticipated from an RET with fixed D–A distance (R_{DA}), donor lifetime (τ_D), and spectral overlap integral (J), as described by Equation (1). The $J(\lambda)$ term expresses the degree of

$$\kappa_{RET} \propto \Phi_D \left[\frac{\kappa^2}{\tau_D R_{DA}^6}\right] J(\lambda) \qquad (1)$$

spectral overlap between the donor emission and the acceptor absorption, which remains constant in the absence of spectral shifts (see Figure 3a).[10] Preliminary lifetime measurements showed that the τ_D value also remains essentially independent of the fluoride concentration [F^-], which is characteristic of a static quenching mechanism. Therefore, the only remaining term that can be responsible for the experimentally observed nonlinearity is the orientation factor κ^2, which varies depending on the relative orientations of the electronic transition moments associated with D and A ($0 \le \kappa^2 \le 4$, see Figure 5a).[10,18]

As a consequence of the restricted rotations around the $C_{aniline}$–C_{BODIPY} bonds (Figure 2), the pairwise interactions between electronic transition moments residing on the tris(*N*-salicylideneaniline) and BODIPY planes in compound **2** are no longer subjected to conventional models of rapidly tumbling RET pairs in which κ^2 is simply treated as an orientation-averaged constant (Figure 5c).[10] In addition, it is reasonable to conceive that the correlated tilting motions of the three peripheral aryl rings, which are directly attached to the BODIPY groups, could assist in defining conformation-dependent finite trajectories of κ^2 (Figure 5b). The validity of this proposal needs to be tested against elaborate models that account for the probability distribution of κ^2 as a function of structural folding and unfolding and for the collective effects of the D–A

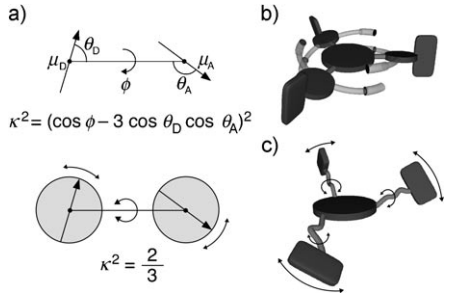


Figure 5. The orientation factor κ^2 depends on the three parameters θ_D, θ_A, and φ, which relate μ_D (the emission transition moment of the donor) and μ_A (the absorption transition moment of the acceptor), as defined in (a).[10] For D–A pairs that belong to a freely tumbling RET ensemble, such as that shown in (c), κ^2 becomes an orientation-averaged constant of 2/3.[10] For a system comprising spatially well-defined D–A pairs, such as in (b), a finite distribution of the κ^2 value is anticipated.

orientational relationships on the signal response (Figure 3).[18–20] Efforts are currently underway to understand and exploit this previously underutilized orientational aspect of RET as a viable amplification mechanism for signal transduction.

In summary, signal amplification in RET has enabled us to demonstrate the genuine allosteric switching of a dynamic multichromophore array, which operates in a perfectly reversible manner in response to a stepwise addition and removal of chemical input at a μM concentration level. The underlying molecular mechanism of the unique amplification behavior observed both in the recognition and the energy-transfer steps holds significant implications for molecular devices and materials used for information storage and processing.

Experimental Section

The synthesis and characterization of compounds **1**–**3** (including crystallographic data)[14] are described in the Supporting Information.

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[9] One challenge is to devise ingenious schemes to systematically modify the binding affinity of interdependent receptor sites toward the same ligand as a function of the degree of saturation (that is, the average number of bound ligands per receptor molecule).[5,8] Another and presumably more demanding challenge arises from the typically weak chemical forces involved in noncovalent interactions, which require a large excess of ligand L to drive the formation of $S{\cdot}L_n$ species from a multisite receptor S with *n* binding sites. To complete the switching cycle depicted in Figure 1a, this excessive amount of L needs to be quantitatively removed to regenerate free S. This is an operationally nontrivial task and has not been demonstrated before.

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[14] See the Supporting Information.

[15] CCDC-642093 contains the supplementary crystallographic data for the key precursor of **2** (BODIPY-appended aniline). The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. See also the Supporting Information.

[16] The stoichiometric reaction between Me_3SiCl and F^- affords Me_3SiF and Cl^-, neither of which reacts with the receptor molecule.

[17] The 1:1 binding between a F^- ion and the OH group (schematically drawn in Figure 4) is consistent with the experimentally observed 1:6 stoichiometry between compound **2** and $n Bu_4NF$, although alternative geometries can also be considered. See: a) G. A. Jeffrey, *An Introduction to Hydrogen Bonding*, Oxford University Press, New York, **1997**; b) T. Steiner, *Angew. Chem.* **2002**, *114*, 50–80; *Angew. Chem. Int. Ed.* **2002**, *41*, 48–76.

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[20] Additionally, one could also consider the orientation-dependent self quenching of BODIPY as another RET pathway leading to signal amplification. This mechanism is currently under investigation.

