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pH-Responsive Molecular Tweezers

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Over the past three decades, significant progress has been made in the field of drug targeting.[1a] Although active compounds can be successfully delivered to some specific sites in the body, drug release from the carrier at the target site still remains a challenging step.[1b] Drug release is commonly achieved through the use of stimuli-responsive systems such as redox active functions or acid-sensitive linkage.[2a] The latter labile bonds undergo cleavage under a mildly acidic environment, such as those of the endosomes or the tumoral tissues.[2b−d] These systems, relying on pH-dependent chemical hydrolysis, are unfortunately often either too stable or too labile to offer adequate control over the release rate.[3] Alternatively, noncovalent interactions offer the possibility to form supramolecular synthetic receptors with tunable affinities and responsive properties.[4] In particular, dynamic molecular tweezers[5] are devices capable of switching from one conformation to another upon stimulation by an external trigger (light, pH, ions).[6] Although this characteristic has been largely exploited in molecular recognition and sensing,[7] it has yet to be explored for its potential use in drug delivery.

In this work, we report a pH-sensitive molecular tweezer prototype that could serve as a fast responding unit to manipulate the release rate of a substrate (Figure 1A) by a macromolecular carrier. The structure comprises three elements: (i) a poly(ethylene glycol) (PEG) backbone to provide water solubility to the carrier, (ii) two naphthalene arms to ensure binding to the substrate, and (iii) a switching unit, i.e. a methoxyphenylpyridinemethoxyphenyl triad, ***ortho*-Py**, to allow the tweezer to switch from the ***U***- to ***W***-shape conformations at acidic pH, thereby decreasing its affinity for the substrate (Figure 1B).

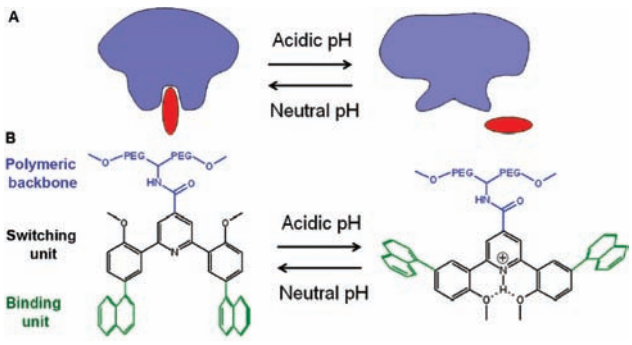

Figure 1. (A) Schematic representation of the concept. (B) Structure of the prototype pH-sensitive tweezer and impact of pyridine protonation on its conformation.

To validate the design of the switching unit itself, a simple, unsubstituted, ***ortho*-Py** triad was readily obtained from 2,6-dibromopyridine and 2-bromoanisole by a Kumada−Corriu−Tamao coupling.[8] At this step, the pH-triggered conformational change of ***ortho*-Py** (Figure 2A) was investigated by ^{1}H NMR spectroscopy and compared to its *para* counterpart ***para*-Py** (Figure 2B), which presented a single conformational state. Although, in both systems, protonation influenced the pyridine protons to the same extent, the behavior of the anisole protons differed. In the ***para*-Py** derivative, the *p*-Ho proton, facing the nitrogen atom in the neutral state, became shielded by the NH^+ group introduced in its close environment upon protonation (Figure 2C). Interestingly, this effect was not obvious for ***ortho*-Py**, suggesting a rotation around the $C_{Py}-C_{Ph}$ bond moving *o*-Ho away from the NH^+ proton. These results, together with 2D NOESY experiments, confirmed the expected conformational change. The pK_a of the system was estimated by ^{1}H NMR titration to be ∼6.3 (Supporting Information), which falls in the range of pH encountered in acidic cellular organelles.[2d]

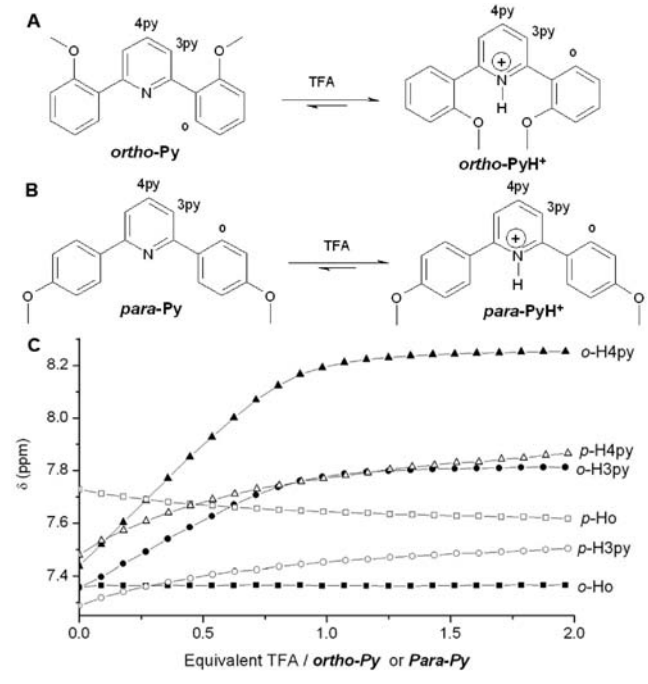

Figure 2. Representation of the influence of protonation of the pyridine ring on ***ortho*-Py** (A) and ***para*-Py** (B). (C) 400 MHz ^{1}H NMR experimental chemical shifts of significative protons of ***ortho*-Py** (10.9 mM, black) and ***para*-Py** (10.9 mM, white) upon addition of trifluoroacetic acid (TFA) in 1:1 $CDCl_3/CD_3OD$.

To function as a molecular tweezer with binding and release properties, the ***ortho*-Py** system was then flanked with two hydrophobic binding units. Naphthalene groups were chosen because of their flat aromatic structure and lower toxicity as

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compared to anthracene or pyrene.[9] ***ortho*-PyNaph** was therefore synthesized by two successive Suzuki coupling reactions. Its crystal structure confirmed the anticipated ***U***-shape (Supporting Information). ^{1}H NMR titration with trifluoroacetic acid showed that the switching properties were maintained in the presence of naphthalene groups (Figure 3). As far as the naphthalene protons were concerned, chemical shifts were spread over the aromatic region in the neutral form, significantly lower than that in free naphthalene (Figure 3B, bottom). This observation is in agreement with the mutual shielding of the aromatic groups, which face each other in the neutral state. Upon addition of TFA, all the naphthalene protons became deshielded and reached final chemical shifts in two clusters (n_3+n_4+n_7 and n_1+n_2+n_5+n_6), very similar to those of naphthalene itself (Figure 3B, top). This effect is consistent with a conformational change from a ***U***- to a ***W***-shape where each naphthalene unit no longer feels the electronic effect of its counterpart (Figure 3A).

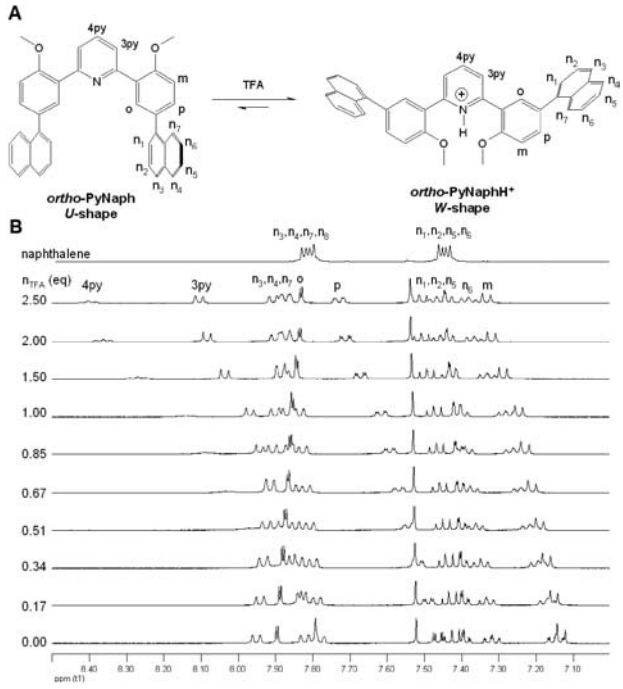

Figure 3. (A) Representation of the conformational change of ***ortho*-PyNaph** upon protonation of the pyridine ring. (B) 400 MHz ^{1}H NMR spectral modifications of ***ortho*-PyNaph** (5.9 mM) upon addition of TFA in 1:1 $CDCl_3$/CD_3OD and (top) chemical shifts of naphthalene in 1:1 $CDCl_3$/CD_3OD.

Finally, the tweezer structure was grafted to a branched PEG through position 4py of the pyridine to obtain a water-soluble carrier. This was achieved through peptide coupling, resulting in a 50% grafting efficiency (Supporting Information). The drug binding/release properties of the ***ortho*-PyNaph** tweezer were investigated with two substrates: quinizarin and mitoxantrone, an anticancer drug.[10] Both molecules possess an aromatic moiety that can interact with the two naphthalene units *via* aromatic stacking, donor/acceptor, as well as hydrophobic interactions (Figure 4). Quinizarin was selected because of its fluorescence properties and the absence of ionization in the studied pH range.[11] As illustrated in Figure 4, in aqueous conditions (pH 7.4 and 4.5), the addition of the free PEG slightly enhanced the fluorescence intensity of quinizarin (compare Dye alone and PEG), possibly due to the better dispersion (and hence poorer self-quenching) of the dye. In this context, it is particularly striking to notice that the **PEG-tweezer** actually led to an overall decrease of fluorescence of the dye, and so only in neutral medium (Figure 4A). This overall decrease in intensity at pH 7.4 therefore results from the competing effects of (i) fluorescence enhancement by the PEG portion and (ii) significant quenching by contact with the tweezing unit. In contrast, at pH 4.5, the **PEG-tweezer** did not quench quinizarin's fluorescence and the signal remained comparable to that obtained with the unconjugated PEG (Figure 4B). A similar phenomenon was also observed with mitoxantrone (Supporting Information). These findings point to a greater affinity of both substrates for the **PEG-tweezer** under neutral conditions, where it adopts a ***U***-shape, than at acidic pH, where the ***W*** conformation predominates.

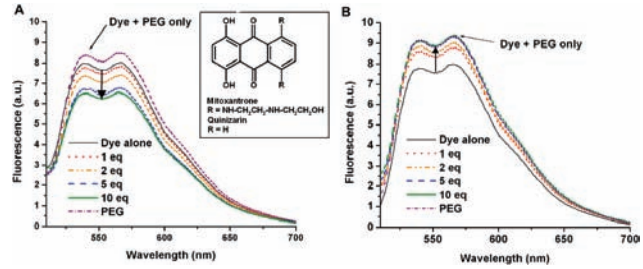

Figure 4. Emission fluorescence of quinizarin (5 μM, λ_{exc} = 490 nm) upon the addition of **PEG-tweezer** (0 to 10 equiv), at pH 7.4 (A) and 4.5 (B). Inset: structure of the substrates.

Overall, the **PEG-tweezer** reported herein, due to its water solubility and pH responsiveness, may have potential to control the release of drugs.

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Supporting Information Available: Supporting figures, experimental procedures, NMR, X-ray crystallographic data and mitoxantrone binding studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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