Molecular Walkers

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A Small Molecule that Walks Non-Directionally Along a Track Without External Intervention**

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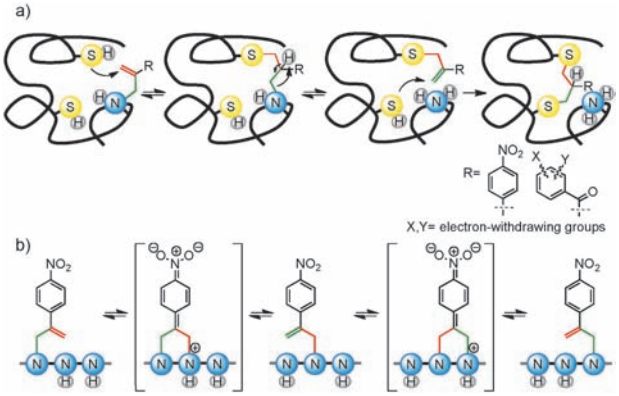
Kinesin, dynein, and some myosin motor proteins transport cargoes within the cell by "walking" along polymeric filaments, that is carrying out successive, repetitive, mostly directional changes of their point of contact with the molecular track without completely detaching from it.[1] These extraordinary biomolecules are inspiring scientists to mimic aspects of their dynamics to create artificial molecular transport systems.[2,3] Recently, the first small molecules that are able to walk down short molecular tracks were described.[2] However, external intervention (the addition of chemical reagents and/or irradiation with light) are required to mediate each step taken by the walker units in the non-DNA systems reported to date. Although migrations of submolecular fragments occur in many different types of chemical reaction,[4] few systems appear to offer the potential for multiple successive and cumulative transport necessary for developing small-molecule walkers.[5] An interesting exception are the so-called equilibrium transfer alkylating cross-linking (ETAC) reagents introduced in the 1970s by Lawton and co-workers for the dynamic cross-linking of biomolecules.[6,7] These reagents reversibly form covalent bonds between pairs of accessible nucleophilic sites on proteins through a series of inter- and intramolecular Michael and retro-Michael reactions until the most thermodynamically stable crosslink is located (Scheme 1 a).[6a] We wondered whether it would be possible to apply a similar concept, focusing instead on chemistry where the cross-linked products are less stable than those attached by a single covalent bond, to make synthetic small molecules that migrate with a high degree of processivity[8] along a linear molecular track.



Scheme 1. a) The migration of an ETAC[6] reagent between nucleophilic sites of a protein by Michael/retro-Michael reactions towards the most thermodynamically stable cross-linked product. b) Processive (intramolecular) migration of α-methylene-4-nitrostyrene along a polyamine track. Michael addition of a track amine group to the olefin of the "two-legged walker" results in a bridged intermediate (both "feet" attached to the track, shown in square brackets) that can subsequently undergo a retro-Michael reaction to either side, unmasking the double bond and leaving the walker attached to the track through a single covalent bond.

Herein we describe the attachment of α-methylene-4-nitrostyrene to oligoethylenimine tracks and the dynamics of its subsequent migration from amine group to amine group without fully detaching by a sequence of intramolecular Michael and retro-Michael reactions. In this way the α-methylene-4-nitrostyrene units move towards the most thermodynamically favored distribution of walkers on oligoamine tracks (Scheme 1 b).[9]

A model walker-track conjugate, **1**, was synthesized in which α-methylene-4-nitrostyrene was attached to an outer amine group of a triamine track and then allowed to exchange between the secondary amine footholds (Scheme 2 a; see the Supporting Information for experimental procedures and characterization data). The reaction was followed by ^{1}H NMR spectroscopy through the different chemical shift of vinyl protons ($H_{c/c'}$ and $H_{d/d'}$) of isomers **1** and **2** (Figure 1).

The kinetics of the amine-to-amine migration of the α-methylene-4-nitrostyrene unit ("walking") is highly solvent-dependent. Starting from pristine **1** (5 mM), no formation of **2** was observed in $CDCl_3$ or CD_2Cl_2 over 15 h at room temperature and the reaction only proceeded slowly in CD_3OD (< 10% conversion over 15 h) or CD_3CN (< 25% conversion over 15 h). However, the interconversion of **1** with **2** reached a close-to-1:1 steady-state ratio of **1**:**2** over 15 h in [D_7]DMF or 4.5 h in [D_6]DMSO (298 K, 5 mM). Increasing

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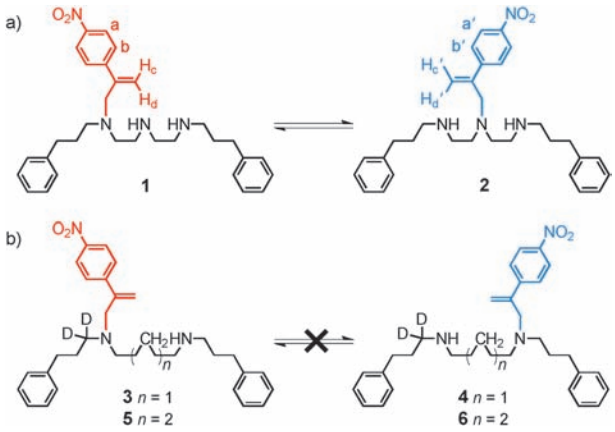


Scheme 2. Transfer of α-methylene-4-nitrostyrene between secondary amine groups through a) 1,4-*N*,*N*-migration and b) possible 1,7- or 1,10-*N*,*N*-migration. The experimental results show that under conditions ([D$_6$]DMSO, 298 K, 5 mM) where $t_{1/2} = 1.5$ h for (a), the double (1,7-) and triple (1,10-) "over-stepping" shown in (b) is not detectable over 48 h, suggesting that they would be rare events during walker migration along a poly(ethylenimine) track.

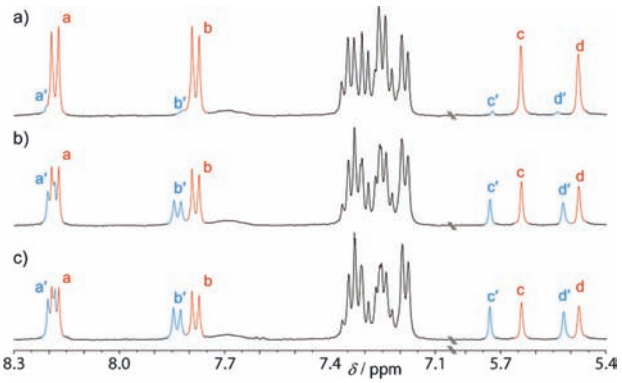


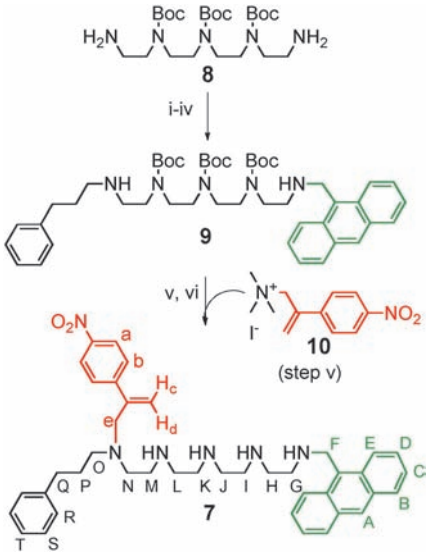
Figure 1. Partial ^{1}H NMR spectra (400 MHz, [D$_6$]DMSO, 5 mM, 298 K) of exchange between **1** and **2** at: a) $t = 5$ min, **1**:**2** ratio 1:0.06; b) $t = 2$ h, **1**:**2** ratio 1:0.6; c) $t = 15$ h, **1**:**2** ratio 1:0.9. The lettering corresponds to the proton labeling shown in Scheme 2.

the concentration of the starting material tenfold (to 50 mM **1**) gave no change in the rate constant of the reaction or the **1**:**2** isomer ratio. Partial ^{1}H NMR spectra of the exchange between **1** and **2** in [D$_6$]DMSO (298 K, 5 mM) are shown in Figure 1. A half-life $t_{1/2} = 1.5$ h was determined for the stepping process (see the Supporting Information).[10]

To determine the processivity of the migration reaction (in other words, the degree to which the reaction is intramolecular or intermolecular),[8] the exchange between **1** and **2** (Scheme 2a) was performed in the presence of a different walker-free track and the intermolecular migration monitored by mass spectrometry (see the Supporting Information for details). After 3 days, less than 6% of the walkers had detached from the original track or transferred to the different track. Accordingly, under these conditions, each α-methylene-4-nitrostyrene unit takes an average of 530 "steps" between amine groups before completely detaching from its track, which is several times the processivity of most wild-type kinesin motor proteins (typically mean step number 75–175).[11]

To determine how likely the walker is to take a double (1,7-) or triple (1,10-) step while migrating along the track, we prepared diamine tracks with five (**3**) or eight (**5**) methylene groups between the secondary amine sites (Scheme 2b).[12] The initial site of attachment of the α-methylene-4-nitrostyrene was deuterium-labeled to distinguish the walker position (that is, **3** or **4**; **5** or **6**) by ^{1}H NMR spectroscopy. Under conditions where single (1,4-) stepping occurs for **1**/**2** with a $t_{1/2} = 1.5$ h, no reaction was observed for either **3** or **5** over 48 h (see the Supporting Information). This suggests that on a longer polyamine track the walker should migrate predominantly through exchange between adjacent amine footholds. The large number of steps that the α-methylene-4-nitrostyrene walker takes on average before competing reactions occur (that is, over-stepping, completely detaching, or exchange with other tracks) is presumably a consequence of the relatively low-energy seven-membered-ring transition state for 1,4-N,N-migration.

Having established that an α-methylene-4-nitrostyrene walker can exchange between the amino groups of a di- or triamine track in a stepwise fashion with a high degree of processivity, we sought to demonstrate that the walker could migrate along a longer track through this mechanism and perform an observable task. A five-foothold walker–track conjugate **7**, incorporating an anthracene group situated at the far end of the pentaethylenimine track from the initial site of attachment of the walker, was prepared as shown in Scheme 3. Pentamine **8** was desymmetrized by reductive amination with 3-phenylpropionaldehyde and subsequent reaction with 9-anthraldehyde to give **9**. The α-methylene-4-nitrostyrene walker unit (**10**) was introduced exclusively to



Scheme 3. Synthesis of five-foothold walker–track conjugate **7**. i) 3-phenylpropionaldehyde, EtOH, RT, 72 h; ii) $NaBH_4$, RT, 24 h, 60% (two steps); iii) 9-anthraldehyde, EtOH, RT, 72 h; iv) $NaBH_4$, RT, 24 h, 55% (two steps); v) MeOH, *N*,*N*-diisopropylethylamine (DIPEA), 50°C, 72 h, 30%; vi) CH_2Cl_2, CF_3CO_2H, 5 h, quantitative. See the Supporting Information for details.

the amine furthest from the anthracene group. Subsequent deprotection gave compound **7** in which the walker was free to migrate along the five-foothold track from its original position.

Model compounds showed that the distance between the α-methylene-4-nitrostyrene unit and the anthrylmethyl moiety in the track influences its fluorescence.[13] Fluorescence lifetime measurements showed that static quenching by the nitrostyrene group quenches the anthracene fluorescence (see the Supporting Information). Dilution experiments confirmed that the quenching observed was from an intramolecular mechanism.

Molecular walker–track conjugate **7** was submitted to walking conditions (DMSO, 7.14×10^{-5} M, 298 K) and its fluorescence emission spectrum recorded periodically.[14] The fluorescence intensity diminished by 54% over 6.5 h, after which time the fluorescence intensity became almost invariant (Figure 2a).

The walker migration in **7** was also monitored by ^{1}H NMR spectroscopy, albeit under more concentrated conditions to give a suitable signal-to-noise ratio ([D_6]DMSO, 20 mM, 298 K, Figure 2b). The reaction was monitored every 0.5 h and, after 3 h, signals indicating that a proportion of the walker units had reached the fifth foothold of the track were observed (Figure 2b). After 6.5 h, no further changes were observed in the ^{1}H NMR spectrum until signals attributed to degradation of the anthracene moiety started to appear.[15] Accordingly, both ^{1}H NMR and fluorescence measurements (Figure 2) indicate that the walking of the α-methylene-4-nitrostyrene unit proceeds back and forth along the pentaethylenimine track, producing a steady distribution of walkers over the five-foothold track after 6.5 h.

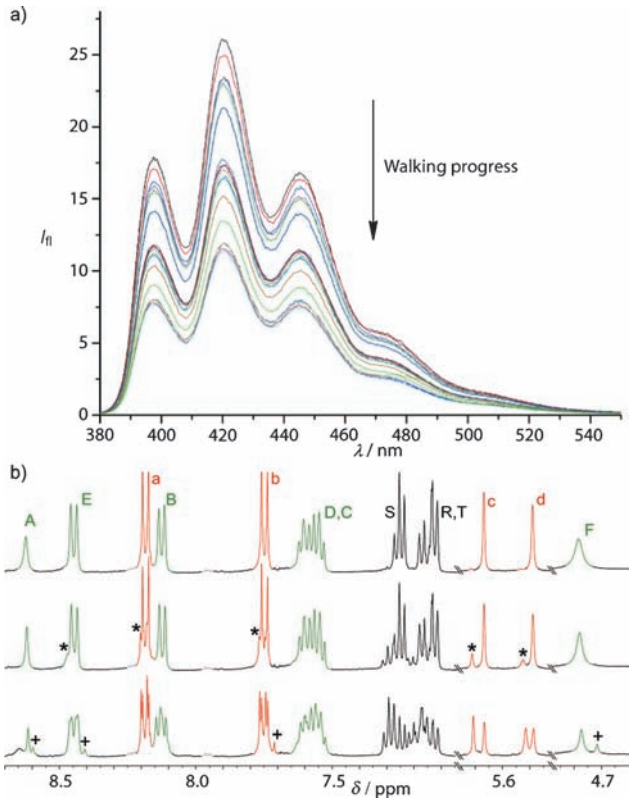


Figure 2. a) Fluorescence quenching ($\lambda_{exc}=366$ nm, $\lambda_{em}=413$ nm) in **7** (DMSO + 0.25% CF_3CO_2H, 1.78×10^{-5} M) as a result of migration of α-methylene-4-nitrostyrene along the oligoamine track. b) Partial ^{1}H NMR spectra (400 MHz, [D_6]DMSO, 20 mM, 298 K) of the reaction mixture at: $t=5$ min (top); $t=1$ h (middle); $t=6.5$ h (bottom). ⋆ Walkers attached to the inner three amine footholds, + walkers attached to the anthrylmethylamine group. The lettering and coloring corresponds to the proton labeling shown in Scheme 3.

In conclusion, we have described a system in which a small synthetic molecular walker migrates along oligoamine tracks without external intervention, moving towards an equilibrium distribution of walkers over all possible positions on the track. In terms of synthetic molecular machine properties, this walker–track system is reminiscent of a rotaxane-based molecular shuttle with degenerate stations:[16] The walker–track system uses a transferable covalent linkage between the α-methylene-4-nitrostyrene and the oligoethylenimine to ensure processivity and determine the preferred positions of the substrate on the track; in a rotaxane-based molecular shuttle a mechanical linkage confers the former property and attractive non-covalent interactions between a macrocycle and specific sites on the thread can be used to achieve the latter.

The small-molecule walking process is processive and takes place predominantly in a stepwise fashion by a Michael–retro-Michael addition mechanism between adjacent amines. The position of the walker can be precisely determined on short tracks by ^{1}H NMR spectroscopy, and on longer tracks the progress of walker migration can be inferred by performance of a simple task: quenching of the fluorescence of an anthracene group at one end of the track by the walker. Work towards developing walkers that consume a fuel to move directionally, and which carry cargoes along extended and branched tracks, is currently ongoing in our laboratory.

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