A Molecular Cable Car for Transmembrane Ion Transport

A molecular cable car for transmembrane ion transport

Alberto Credi

Abstract: *The controlled transport of molecular and ionic substrates across bilayer membranes is a fundamental task for the operation of living organisms. It is also a highly fascinating and demanding challenge for artificial molecular machines. The recent report of a synthetic transmembrane molecular shuttle that can transport potassium ions selectively down a gradient in a liposomal system makes a small but significant step forward towards this goal.*

The compartmentalization of space provided by biological membranes is one of the foundational elements of life.[1] Although membranes provide a barrier that define and protect cellular or subcellular structures, at the same time they need to allow the passage of ions and polar molecules necessary for the operation of the cell.

In general, the transport of ions across membranes is performed by species embedded in the bilayer, according to a *passive* or *active* mechanism. In the first case, the flow of ions is driven by a concentration gradient of ions across the membrane; thus, the transporter simply facilitates the system to relax to equilibrium (i.e., exhaust the gradient). Examples of passive transporters are ion carriers (Figure 1a) and channels (Figure 1b). In the active transport case, ions flow through the transporter against their concentration gradient by making use of the energy provided by an external source (for example, ATP hydrolysis). Transporters of this kind, namely ion pumps, operate away from chemical equilibrium and can transduce the input energy into a transmembrane chemical potential.

The development of synthetic molecular devices for regulating ion transport across membranes is a thriving research area in supramolecular chemistry,[2] motivated by the high basic science value and the potential for therapeutic applications, for example in the treatment of cancer and cystic fibrosis.[3] Realizing active transporters would be even more interesting because they could also lead to new ways for converting and storing energy (solar energy in the case of light-driven devices).[4]

Molecular machines[5] – i.e. molecular species characterized by non-trivial and controlled movements of their subcomponents – are promising candidates for the directed transport of substrates. Indeed, prototypes of artificial "molecular transporters" that operate in homogeneous solution have been recently reported,[6] and examples of man-made transmembrane molecular machines based on biomolecules are available.[7] Nevertheless, a case of a synthetic molecular machine that can carry ions across a membrane by exploiting its intercomponent motion was still missing. Very recently, a group at East China University of Science and Technology in Shanghai designed, synthesized and investigated an artificial molecular machine that can operate in a bilayer membrane and passively carry K^+ ions across it.[8]

The transporter is a molecular shuttle – that is, a rotaxane in which the mechanically interlocked ring and axle components can slide relatively to one another.[5] A species of this kind is indeed an interesting candidate to realize a passive transmembrane transporter, because (i) its chemical structure and one-dimensional nature could favour a perpendicular insertion in the bilayer, (ii) its length can be designed such that the thermally driven stochastic linear movement of the ring along the axle covers the membrane thickness, and (iii) its ring component can be equipped with a docking site for the cargo to be transported. From a functional point of view, the resulting species (Figure 1c) would possess some elements of both a carrier – in which the ion and the carrier move together through the membrane like cars on a ferry (Figure 1a) – and a channel – in which the ion moves past the membrane-anchored channel and follows a predetermined route like cars in a tunnel (Figure 1b). In the macroscopic analogy the shuttle transporter operates like a cable car that circulates continuously.

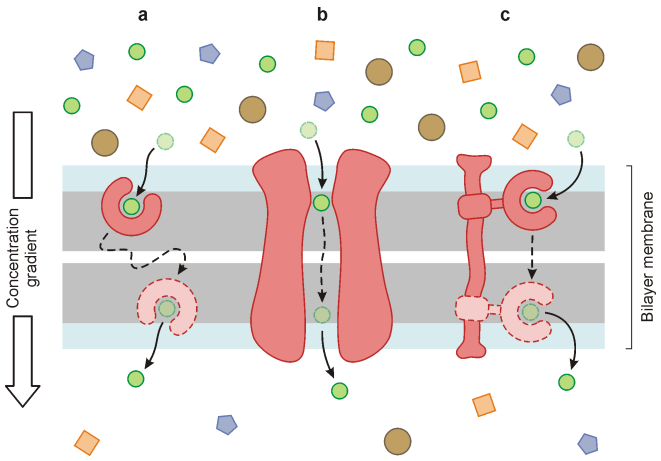

Figure 1. Schematic representation of passive selective ion transport across a membrane by a carrier (a), a channel (b), and a shuttle (c).

The investigated rotaxane (Figure 2) consists of: a symmetric axle comprising (a) two secondary ammonium ions as recognition sites for the ring component, which represent the terminal stations for the shuttle, (b) a central triazolium unit that acts as a weaker intermediate station for the shuttling motion, (c) alkyl chains as hydrophobic connectors between such units, and (d) hydrophilic end stoppers; a macrocyclic component comprising (e) a dibenzo[24]crown-8 (DB24C8) ring that can encircle the axle, tethered with (f) a benzo[18]crown-6 (B18C6) ring as a K^+ receptor. The amphiphilic structure of the axle and its length (~3.2 nm, comparable with the thickness of a typical phospholipid bilayer) are expected to stabilize a membrane-spanning arrangement of the rotaxane, as confirmed by molecular

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dynamics simulations and in agreement with the experimental findings.

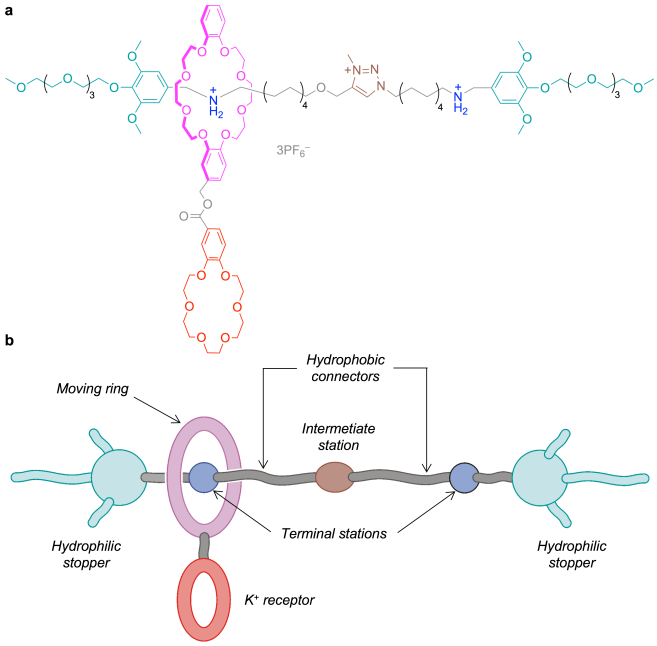

Figure 2. Structural formula of the rotaxane transporter (a) and a cartoon representation highlighting its modular design (b).

To study the ion transport phenomena, the rotaxane was incorporated in the bilayer of large unilamellar vesicles (LUVs, ~100 nm size) self-assembled from egg yolk phosphatidylcholine (EYPC) phospholipids.[9] LUVs are very interesting scaffolds for this purpose because they can be easily prepared and characterized by following well established methodologies, and provide a straightforward means for compartmentalizing an aqueous solution which, however, remains macroscopically homogeneous. The separated ring and axle components of the rotaxane, as well as other model rotaxanes, were also examined.

The change in the luminescence intensity of fluorescent guests entrapped within the LUVs was employed to assess both the stability of the rotaxane-doped bilayer and the extent of ion transport. In the key experiment, a pH gradient was applied by adding KOH in the extravesicular buffer; then the depletion of the transmembrane pH difference, caused by the influx of OH^- and/or the efflux of H^+, was monitored as a function of time by measuring the fluorescence changes of a pH-sensitive dye previously entrapped in the LUVs. The authors were able to show that the collapse of the pH gradient is related to K^+ transport (i.e., K^+/OH^- symport and/or K^+/H^+ antiport). Moreover, K^+ is transported more efficiently than other alkali metal ions, in an order of activity that reflects the affinity of the ions for B18C6 rather than the energetic cost of their dehydration. The linear correlation found between the observed rate constant and the concentration of the rotaxane indicates that the transport mechanism is unimolecular. Voltage clamp assays performed on a planar bilayer are in agreement with the formation of persistent channel-like structures across the membrane.

Compelling evidence that the K^+ transport is supported by the back-and-forth shuttling of the macrocyclic component along the axle (Figure 3) was gained with clever experiments carried out on other model rotaxanes. Specifically, if the intermediate triazolium station is replaced by an innocent triazole unit, the transport activity is decreased. It can be reasoned that in the absence of the intermediate station the barrier for shuttling between the two terminal stations is increased and the process is slowed down, as confirmed by NMR spectroscopy in organic solution. The transport activity can be further frustrated by disabling one of the two terminal ammonium stations by Boc-protection, a modification which prevents long-range shuttling.

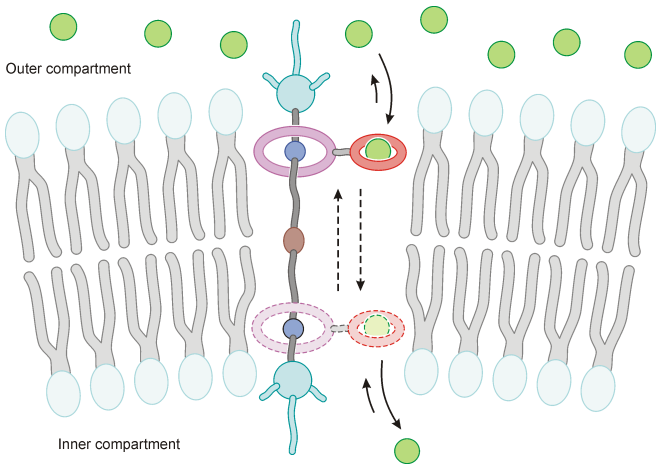

Figure 3. The transport of K^+ ions (green circles) across the lipid bilayer of a LUV through the thermally driven stochastic shuttling of a membrane-bound rotaxane (structure depicted in Figure 2).

Another insightful assay was performed on rotaxanes in which the terminal stations are switched off by deprotonation. The ^1H NMR spectra recorded in solution showed that in the deprotonated rotaxane the ring component is immobilized around the central triazolium station and no shuttling occurs. ^{23}Na NMR spectroscopy was employed to show that, consistently, the ion exchange across the LUV bilayer is blocked when the rotaxane is deprotonated. All these experiments nicely demonstrate the connection between ring shuttling and ion transport (Figure 3), and the effect of fine structural changes of the molecular machine on its membrane-mediated function.

Apart from the clever design and the intriguing mechanical aspects, one can wonder what is the advantage of the molecular shuttle in comparison with a much simpler K^+-selective molecular carrier. In fact, a huge amount of chemical information can be included in a molecular shuttle by design, which can result into modulation of the performance and functional diversity unreachable by a simple carrier. For example, earlier work has shown that the shuttling rate can be modulated by structural tweaking or by application of chemical, electrical and optical

stimuli.[10] Indeed, the shuttling process can be switched on/off with external control, thereby enabling gated ion transport.

A further significant development of the molecular shuttle approach is the progress from passive to active transport. The current version of the molecular cable car runs randomly back and forth, simply distributing the potassium ion crowd between the two terminals. It would be great if the cable car could be powered such that the ions could be moved directionally uphill. Indeed, rotaxane-type systems that can use chemical,[11] electrical[12] or light[13] energy to pump molecular components in solution and operate away from equilibrium have become available in the past few years, creating a promising basis for the construction of systems for active transmembrane transport.

In summary, the rotaxane described here can be seen as the forerunner of a next generation of molecular machines designed to operate in compartmentalized environments and capable of exploiting their motion to accomplish tasks that could not be achieved otherwise. In the long term, radically new solutions for medical therapy and energy conversion may emerge. Clearly, a significant research effort is yet necessary to understand how synthetic molecular machines can be successfully integrated with biological/biomimetic membranes, and operated in a controllable and reproducible manner.

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Keywords: crown ether • ion channel • mechanically interlocked molecule • molecular machine • rotaxane

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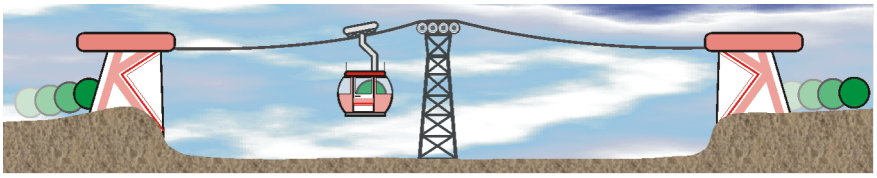

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Thanks for the lift, mate! A molecular machine based on a rotaxane, embedded in a lipid bilayer, can carry potassium ions across the membrane, taking advantage of the stochastic shuttling motion of its macrocyclic ring.