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Voltage addressable nanomemories in DNA?

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Abstract Short single-stranded DNA (or RNA) molecules can be *designed* to have several long lived (> hours) secondary structures. In principle, such molecules could be used as nanomemories if they could be *easily* induced to switch between trapped states. We propose here that the necessary work required to drive the molecule into one particular trapped state can be provided by *its own synthesis*. Following this idea, we argue that a low voltage (< 1 V) may induce a bistable DNA molecule to switch structure at will, by forcing it to thread through a nanopore and refold alternatively from either of its ends. ***To cite this article: H. Isambert, C. R. Physique 3 (2002) 391–396.*** © 2002 Académie des sciences/Éditions scientifiques et médicales Elsevier SAS

nanomemory / nanopore / DNA synthesis

Nanomémoires adressables en ADN ?

Résumé Il est possible de concevoir des courtes séquences d'ADN simple-brin présentant plusieurs structures secondaires stables (> heures). En principe, de telles molécules pourraient être utilisées comme des nanomémoires si elles pouvaient être *facilement* passées d'un état piégé à un autre. Nous proposons ici que le travail nécessaire pour placer la molécule dans un état piégé particulier peut être fourni par sa propre synthèse. Prolongeant cette idée, nous montrons qu'une faible tension (< 1 V) pourrait induire le basculement structural d'une molécule bistable à volonté, en la forçant à passer au travers d'un nanopore et à se replier alternativement ensuite à partir de l'une ou l'autre extrémité. ***Pour citer cet article : H. Isambert, C. R. Physique 3 (2002) 391–396.*** © 2002 Académie des sciences/Éditions scientifiques et médicales Elsevier SAS

nanomémoire / nanopore / synthèse de l'ADN

Version française abrégée

Alors que l'idée d'assembler chimiquement des nanocircuits suscite un intérêt croissant [1], le besoin de mémoires moléculaires facilement adressables apparaît de plus en plus nécessaire [2–4].

Il est aujourd'hui facile de synthétiser de courtes molécules d'ADN simple-brin (ADNsb) avec à peu près n'importe quelle séquence (longueur < 150 bases). Comme les molécules d'ARN simple-brin, ces ADNsb adoptent des structures secondaires stables (à température ambiante et dans des conditions physiologiques) en appariant des régions complémentaires au sein de leur propre séquence pour former des bouts d'hélice

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Watson–Crick. Il est en particulier possible de concevoir des molécules présentant plusieurs structures très stables à partir de longues hélices mutuellement incompatibles.

Inspirés par plusieurs études montrant que le repliement d'une telle molécule peut être guidé efficacement par sa propre synthèse [9,10], nous proposons dans cette note qu'un moyen de reproduire à volonté la synthèse d'une molécule d'ADNsb (ou d'ARN) est de la forcer à passer au travers d'un nanopore trop étroit pour laisser passer ses sections hélicoïdales, Fig. 1(b). Une tension appliquée d'environ un volt devrait suffire à diriger cette molécule vers le nanopore puis à la faire passer au travers [14,17], en reproduisant ainsi une synthèse artificielle à chaque inversion de tension. Nous montrons sur un exemple générique simple que le travail ainsi fourni peut permettre de faire basculer alternativement une séquence artificielle bistable d'une structure à une autre en la « synthétisant » alternativement à partir de l'une ou l'autre de ses extrémités. La séquence générique considérée est constituée de l'arrangement suivant de quatre bouts différents d'ADNsb (avec deux répétitions) :

$$5'\text{–A–B–A–}\overline{\text{B}}\text{–}\overline{\text{A}}\text{–}\overline{\text{B}}\text{–}3',$$

où les bouts de 6–8 bases de long $5'\cdot A\cdot 3'$ et $5'\cdot\overline{A}\cdot 3'$ (respectivement $5'\cdot B\cdot 3'$ et $5'\cdot\overline{B}\cdot 3'$) sont complémentaires et peuvent s'hybrider en $A\bullet\overline{A}$ (respectivement $B\bullet\overline{B}$). A l'équilibre, cette molécule adopte deux structures en épingle à cheveux différentes quoique ressemblantes, que l'on pourrait distinguer par exemple à l'aide d'un couple de fluorophore/quencher [5], Fig. 1(a).

Grâce à son extrémité $3'$ libre, la structure 1 devrait conduire à une synthèse artificielle de $3'\rightarrow 5'$ qui favorise un nouveau repliement dans la structure 2 de l'autre coté du nanopore puisque la région hélicoïdale $\overline{A}\bullet A$ devrait nucléer en premier dès que les bases initiales $3'$–$\overline{B}$–$\overline{A}$–$\overline{B}$–A$\cdots$ deviennent disponibles, Fig. 1(b). Inversement, nous nous attendons ensuite à rebasculer la séquence dans sa structure 1 en inversant la tension électrique appliquée avant que la molécule n'ait le temps de relaxer spontanément (des résultats expérimentaux préliminaires [7] montrent que cette relaxation à l'équilibre thermodynamique peut être retardée pour plusieurs heures voire plusieurs jours avec un choix approprié de séquence).

Ceci suggère qu'une telle molécule d'ADNsb bistable pourrait être utilisée *en principe* comme une *nanomémoire adressable électriquement* (avec la nécessité d'être périodiquement rafraîchie).

En fin de compte, il pourrait aussi s'avérer intéressant de greffer chimiquement deux groupes volumineux terminaux aux extrémités de la courte molécule d'ADN, pour l'empêcher de sortir complètement du nanopore et bloquer stériquement ses structures secondaires de chaque coté du pore (figure non dessinée). Ceci permettrait en outre de facilement remettre à jour l'état mémoire d'une molécule qui a relaxé.

1. Introduction

As the idea of chemically assembling nanocircuits attracts more and more interest [1], it becomes clear that easily addressable molecular switches are in high demand [2–4].

Short single-stranded DNA (ssDNA) molecules can nowadays be *easily* synthesized with nearly any desired sequence (length < 150 bases). Like single-stranded RNA (ssRNA), such ssDNA molecules adopt (at room temperature and physiological conditions) stable secondary structures by base pairing complementary regions within their own sequence, thereby forming internal stretches of stable Watson–Crick helices.

With a typical timescale for a helical region to form around 10^{-5} s [5], and a stacking energy between consecutive base pairs of the order of $(1-3)kT$ per bp, we can roughly estimate that the *minimum* length n for a helix to spontaneously dissociate *at most* once per day (that is once every 10^5 s) is of the order $n\sim\ln(10^{10})/(1-3)\sim 8-23$ bp long, depending on its G + C versus A + T contents.

For short random sequences, typical secondary structures have usually 5–6 bp long internal helices, which do not make them suitable candidates to display longlived traps. Still, it is possible to *design* short sequences with long mutually incompatible helices that compete for common bases. In principle, such molecules can exhibit several distinct longlived structures that can sometimes be separated using standard native gel electrophoresis techniques [6,7].

Designing DNA sequences that exhibit several longlived secondary structures is however far from providing an easily addressable nanomemory! Clearly, some work has to be used to *first* drive the molecule into a specific trapped configuration while avoiding the other stable structures, and *then* to switch structure at will in order to change the stored information. This is not an easy task, and early attempts [8] have proposed to use *additional* oligonucleotides to trigger structural changes via a competition between intra- and intermolecular hybridizations. Although appealing, such approaches have the inconvenience of requiring (i) the successive addition *and* removal of extra molecules and (ii) the need to vary temperature to switch configuration by alternatively hybridizing and melting away helical regions.

In this short note, we purposely focus the discussion on methods which would *not* require *additional* molecules to induce the desired structural switch.

In principle, large variations in heating and cooling rates could lead on their own to statistical differences in the distribution of trapped states. However, we expect that such average collective behavior under either rapid quench or slow annealling would unlikely be selective enough to become useful in reliably addressing the different possible secondary structures of *individual* ssDNA molecules.

Following, instead, inspiring results on the *sequential* folding kinetics of the RNaseP RNA molecule [9] and our recent study of the hepatitis delta virus ribozyme folding pathways [10], we propose that the actual synthesis of the molecule can potentially drive a (single) molecule to selectively fold into a *specific* trapped structure [7]. We first illustrate this idea with a simple hypothetical bistable molecule before discussing the concomitant use of an external electric field *and* a transmembrane nanopore to induce alternative switches between its two stable structures.

2. Synthesis driven selective folding

Because of the local asymmetry of their phosphate–sugar–phosphate bonds, ssDNA (and ssRNA) molecules are globally oriented. Thus, all DNA (and RNA) sequences – even perfect palindromes – have two different ends, usually referred to as the $5'$ and $3'$ ends. *Chemical synthesis* of ssDNA usually proceeds from their $3'$ end towards their $5'$ end. Conversely, biological *transcription* into RNA (or *replication* of DNA) *always* proceeds from the $5'$ end to the $3'$ end, with typical processing rates varying from 5 to 500 bases per second. Since helical base paired regions can form much more rapidly than the synthesis process itself, RNA molecules start folding *while* their downstream sequence is still being transcribed. In principle, this allows for a genetic encoding of folding pathway information that translates into a succession of stable intermediate structures 'conspiring' to efficiently guide the molecule into a specific, possibly trapped configuration. As suggested by the analysis of the hepatitis delta virus ribozyme folding pathways [10], such cascading folding scenarios rely on the stability exchange between consecutive transient helices as new bases of the downstream sequence become available.

We consider here the simplier and more generic case of an artificial sequence consisting of the following arrangement of four different ssDNA pieces (with two repetes):

$$5'\text{–A–B–A–}\overline{\text{B}}\text{–}\overline{\text{A}}\text{–}\overline{\text{B}}\text{–}3',$$

where the 6–8 base long stretches $5'\cdot A\cdot 3'$ and $5'\cdot\overline{A}\cdot 3'$ (respectively $5'\cdot B\cdot 3'$ and $5'\cdot\overline{B}\cdot 3'$) are complementary and can hybridize as $A\bullet\overline{A}$ (respectively $B\bullet\overline{B}$). At equilibrium, this molecule adopts two distinct, though

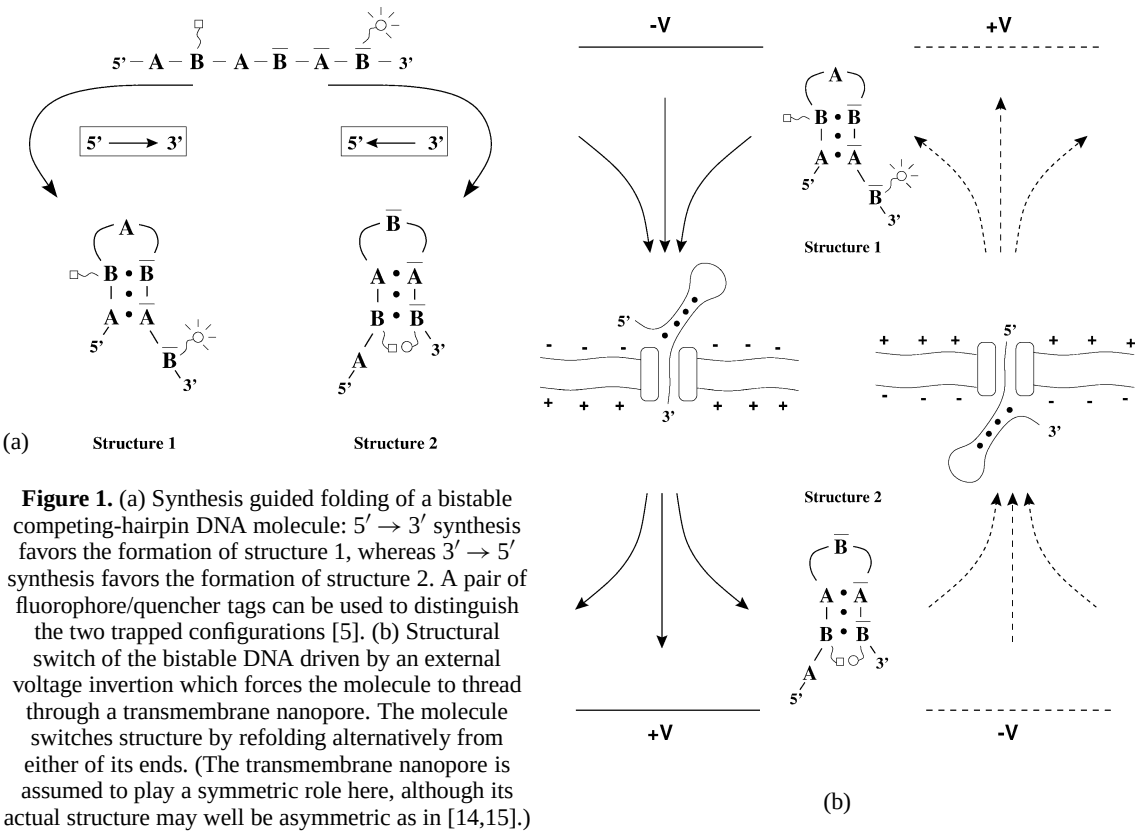

Figure 1. (a) Synthesis guided folding of a bistable competing-hairpin DNA molecule: $5' \to 3'$ synthesis favors the formation of structure 1, whereas $3' \to 5'$ synthesis favors the formation of structure 2. A pair of fluorophore/quencher tags can be used to distinguish the two trapped configurations [5]. (b) Structural switch of the bistable DNA driven by an external voltage invertion which forces the molecule to thread through a transmembrane nanopore. The molecule switches structure by refolding alternatively from either of its ends. (The transmembrane nanopore is assumed to play a symmetric role here, although its actual structure may well be asymmetric as in [14,15].)

Figure 1. (a) *Repliement d'une molécule bistable guidé par sa synthèse* : *la synthèse de* $5' \to 3'$ *favorise la formation de la structure* 1, *alors que celle de* $3' \to 5'$ *favorise la formation de la structure* 2. (b) *Le basculement structural de la molécule ADN bistable est induit par une inversion de tension externe qui force la molécule à passer au travers d'un nanopore transmembranaire. La molécule change de structure en se repliant alternativement depuis l'une ou l'autre de ses extrémités.*

resembling hairpin structures, which could for instance be distinguished with the help of a pair of fluorophore/quencher tags [5], Fig. 1(a).

For generality, we will not refer to a specific sequence in this short note, assuming also that all other alternative structures can be neglected by a careful choice of the 6–8 base long regions $5'\cdot A\cdot 3'$ and $5'\cdot B\cdot 3'$ (and hence $5'\cdot\overline{A}\cdot 3'$ and $5'\cdot\overline{B}\cdot 3'$) [7].

Considering first a $5' \to 3'$ direction of synthesis, we expect structure 1 (Fig. 1(a)) to be preferentially formed since the helical region $B\bullet\overline{B}$ can nucleate as soon as the $5'-A-B-A-\overline{B}\cdots$ initial bases become available. Conversely, if we could synthesize the same molecule in the $3' \to 5'$ direction, we would expect to preferentially form structure 2 (Fig. 1(a)) since the helical region $\overline{A}\bullet A$ should now nucleate first as soon as the $3'-\overline{B}-\overline{A}-\overline{B}-A\cdots$ initial bases would become available.

Hence, this hypothetical bistable molecule should be *selectively* driven by its own synthesis into *one or the other* stable state depending on the direction of synthesis. Naturally, on long enough timescales we still expect this trapped bistable molecule to equilibrate into its two available structures, thereby loosing the initially stored information. However, this eventual relaxation may be retarded for several hours or even days with an appropriate sequence choice as early experimental results show [7].

It remains that the actual biological or chemical synthesis being performed *de facto* only once, such molecular system would actually correspond to a *mere* erasable nanomemory.

3. Electrophoretic ssDNA translocation through a nanopore

Reviewing the possible means to couple secondary structure changes with the effects of external perturbations (excluding again here the use of extra molecular species), it is important to keep in mind the typical force F_c that is necessary to break open long stretches of double-stranded regions which are required to stabilize trapped secondary structures. This has been independently measured by several different groups using various micromechanical setups to unzip dsDNA [11] or RNA [12] molecules. All agree on a critical force around $F_c = 10$–20 pN.

As DNA (or RNA) molecules are usually highly charged (one net negative charge per base), it is instructive to evaluate the typical electric field E_c which should be used in an attempt to reach such critical force F_c without resorting to an elaborate micromechanical setup. The electric force experienced by n elementary charges under an electric field E being $F_e = neE$, this roughly implies $E_c \sim (10^6/n)$ V/cm (a more precise estimate should also consider the local dissipation associated with the counterion motion [13]). For small n, E_c is much too large to be applied in a bulk aqueous solution without serious side effects both at the electrodes and in bulk! Still, such electric fields are routinely applied across insulating bilayer membranes acting as 5 nm-thick *local* capacitors within much more conductive aqueous solutions. Indeed, artificial and cellular membranes can withstand up to nearly 1 volt across their bilayer before induring an irreversible perforation, known as *electroporation*. The corresponding *local* electric fields (upto 10^6 V/cm across the membrane) can potentially generate 10–100 pN forces on transmembrane charged nano-objects (with $n = 1$–10 elementary charges).

Actually, this phenomenon has already been used to induce voltage-driven translocations of ssRNA and ssDNA molecules through single transmembrane proteic nanopores [14,15], a phenomenon also studied theoretically in detail [16].

In those remarkable experiments, individual ssDNA (or ssRNA) molecules are efficiently conducted through a single nanopore by the (small) electric current, and hence electric field, channeling them from the bulk solution towards, and then through, the narrow passage. Once engaged into the nanopore the ssDNA is translocated at a typical rate of $V_{DNA} \sim 10^{-4}$ m/s (i.e., one base every 10 µs), which is considerably slower than the conduction of the small K^+ and Cl^- ions from the 1M electrolyte solution inside the non-obstructed nanopore, i.e., $V_{K\,Cl} \sim 10$ m/s. Hence, in practice, the translocating ssDNA acts as an insulating 'nanoplug' which blocks the conduction of the small ions through the nanopore (measured currents typically drop by more than 10-fold from ~ 70 pA to ~ 5 pA). Note, that this phenomenon likely explains the observed trend of faster translocation for ssDNA *shorter* than the length of the 5.2 nm-long nanopore [15], since a shorter insulating nanoplug implies a locally enhanced electric field for a fixed voltage drop (when we further assume that the friction inside the pore is roughly proportional to the ssDNA length < 5.2 nm and translocating velocity).

4. Oriented electrophoretic extrusion of DNA secondary structures through a nanopore

In fact the nanopore can be so narrow (1.5–1.8 nm) that a 2.3–2.5 nm-wide double helix cannot translocate through it [17]. However, the reported electric force [15,16], as well as the crude estimate above, suggest that it is likely sufficient to *extrude* helical regions through the nanopore by forcing them to *first unfold* and then *sequentially refold* on the other side of the nanopore, reproducing *de facto* at will an *artificial* synthesis of the molecule each time the latter is forced to pass through the nanopore. Early experimental results on short helices suggest that it is indeed the case [17]. Most likely, this can only occur *if* a sufficiently long single-stranded dandling end is available to penetrate the nanopore, hence transmitting a strong initial mechanical force on the first base pair of the blocking helix.

Interestingly, this provides us with a way to control the orientation of extrusion, and hence the direction of the new artificial synthesis, with the shape of the entering structure.

Returning now to our hypothetical bistable molecule of Fig. 1(a), we see on Fig. 1(b) that extruding such a molecule through a nanopore may provide a voltage controllable molecular switch. Indeed structure 1

is expected to be extruded from its $3' \to 5'$ ends which induces a new artificial synthesis favoring the formation of structure 2 (see Fig. 1(a)), while the reverse switch from state 2 to state 1 is predicted when starting with structure 2 (see Fig. 1(a)). Inverting the external applied tension should then drive the molecule to switch back into its initial state by forcing it to thread through the nanopore in the opposite direction, *before* it has time to spontaneously relax into its two avalaible states.

This suggests that such bistable ssDNA molecules could *in principle* be used as *voltage addressable nanomemories* (with the need of being periodically refreshed like electronic RAM chips) but this, of course, still needs to be experimentally demonstrated!

On the theoretical side of the concret design of an *ad hoc* DNA sequence, it is well possible that other bistable DNA structures prove more appropriate than the *generic* competing hairpin example used to illustrate this Note.

On the experimental side, we can also anticipate serious challenges, such as the need to control the extruding velocity in order to optimize selective refolding into the desired trapped secondary structure.

Ultimately, it could also prove more convenient to chemically graft two terminal caps on the short DNA molecule to prevent it from escaping out of the nanopore and to sterically block its secondary structure on either side of the pore (figure not drawn). This would in particular enable an easy reset of the memory state of a molecule at equilibrium (that is a molecule whose current structure is unknown).

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