Supplementary information

A tensegrity driven DNA nanopore

*O. Mendoza, P. Calmet, I. Alves, S. Lecomte, M. Raoux, C. Cullin and J. Elezgaray**

CBMN, UMR5248, 33600 Pessac, France

Sequences of the strands forming H-pore and C-pore.

All the unmodified DNA single strands were purchased from Sigma-Aldrich (desalted purification). Strands with cholesteryl, Cy5 or BHQ2 modifications (HPLC purification) were purchased from Eurogentec.

Strands forming H-pore and C-pores are derived from the original design of ref. [1]. Two groups can be distinguished: scaffold strands (7 to 12) span double helices. Linking strands switch from one helix to the other, providing with the necessary structural stability. The original design was modified in several ways. First, in the H-pore configuration, we added two single stranded over-hangs (part of the sequences shaded in yellow, see below table S1) , designed to hybridize to a ssDNA with two cholesteryl modifications. Second, a 30nt long sequence (green shaded) was added to link strands 9 to 12. Sequences are given in table S1.

The design of C-pore is different: instead of using a single strand modified in 5' and 3' with cholesteryl, we used four strands modified either at 5' or 3' with cholesteryl (list of staples is in table

S2, the corresponding scheme in Figure S2). Although the number of cholesterol moities is four in both cases, the design of H-pore appears topologically more convolved, thus we wanted to check that this design does not hinder the insertion into the bilayer.

Table S1: sequence of staples participating in the formation of H-pore.

Name	Sequence H-pore
Handle-2chol	Chol – TATCAACTCCAACT - Chol
2	5′-TGTTCCAAATAGCCAAGCGGT-3′
handle_13f_1	5' - AGGGTGGGAATCGGACAAGAG TTTTT AGTTGGAGTTGATA TTTTT GCGGGGAGCGTATTAGAGTTG -3'
3	5′-AGTGAGATGTCGTGACGTGGA-3′
4i	5' – ATCGGCATTAAAGACCAGCTG -3'
handle_4f_13init	5' - CATTAATTTTTTCTCCTTCAC TTTTT AGTTGGAGTTGATA TTTTT CCACGCTCCCTGAGGGGCGCC -3'
5	5′-CAACAGCATCCTGTTTCCGAA-3′
6	5′-TCCACTAAAATCCCCCCAGCAGGCGAAATGATTGCTTTCACC-3′
7	5′-TCCACGTTCTTTAATAGTGGA CTCTTGTTCCAAACTGGAACA-3′
8	5′-GGCTATTCTTTTGATTTATAA GGGATTTTGCCGATTTCGGAA-3′
10	5′-CAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATCAGCTGTTG-3′
11	5′-TCTCACTGGTGAAAAGAAAAA CCACCCTGGCGCCCAATACGC-3′
12-loop30-9	5' - TCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACA GCGTAAGACCCACAATCCAATTTCCCTCGC ACAGGATTTTCGCCTGCTGGGGCAAACCAGCGTGGACCGCTT - 3'
14	5' - CGCCTGGGGTTTGCTTATAAA TCAAAAGGTTTGGACCAACGC -3'

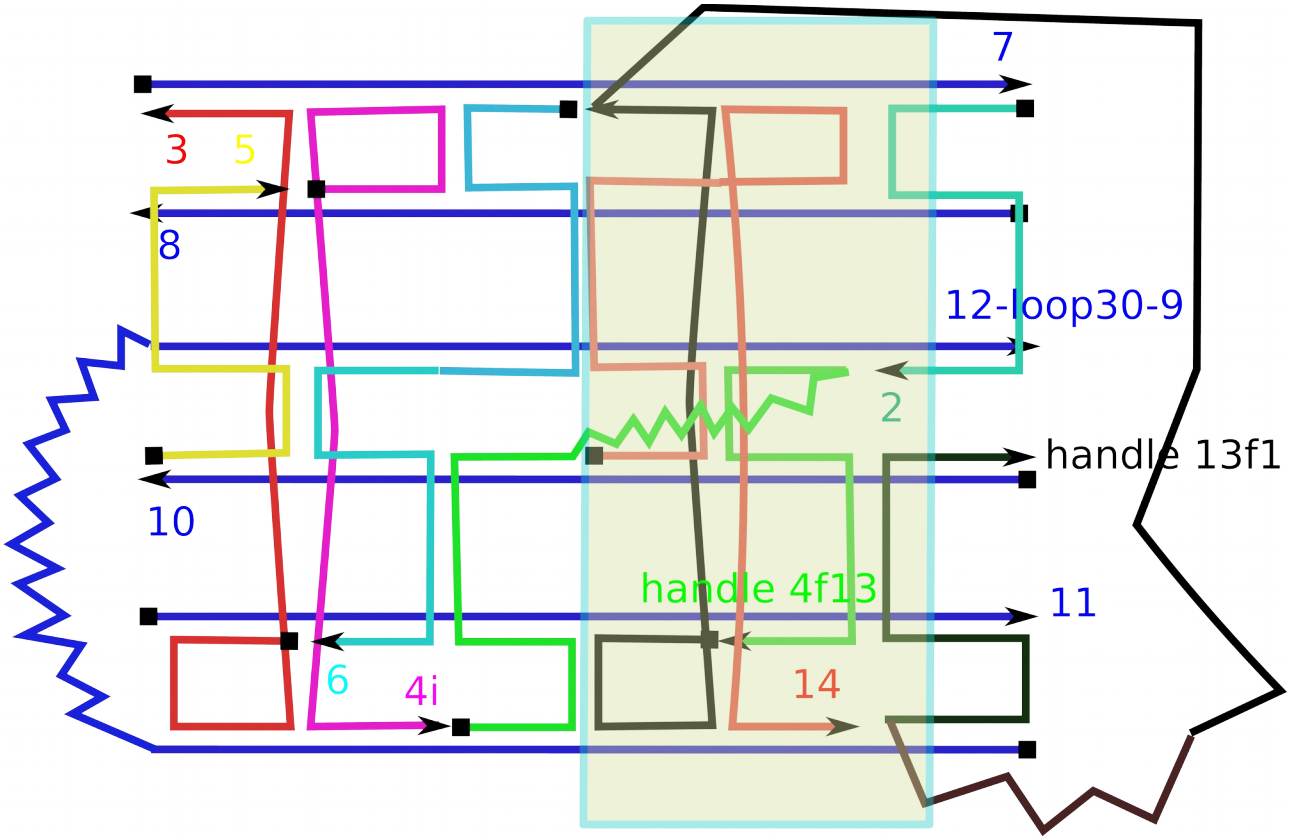

Figure S1: *Schematic representation of staples needed to form the H-pore. 5' ends are represented by a black square, 3' end with an arrow. Zigzag segments correpond to ssDNA handle or lock ssDNA . The shaded area corresponds to the region expected to insert into the lipid bilayer. The name of each staple and the staple itself share the same color.*

Name	Sequence C-pore
1_chol	Chol – GCGGGGAGCGTATTAGAGTTG -3'
2	5′-TGTTCCAAATAGCCAAGCGGT-3′
13f_chol	5' - AGGGTGGGAATCGGACAAGAG - Chol
3	5′-AGTGAGATGTCGTGACGTGGA-3′
4i	5' – ATCGGCATTAAAGACCAGCTG -3'
4f_chol	5' - CATTAATTTTTTCTCCTTCAC - Chol
5	5′-CAACAGCATCCTGTTTCCGAA-3′
6	5′-TCCACTAAAATCCCCCCAGCAGGCGAAATGATTGCTTTCACC-3′
7	5′-TCCACGTTCTTTAATAGTGGA CTCTTGTTCCAAACTGGAACA-3′
8	5′-GGCTATTCTTTTGATTTATAA GGGATTTTGCCGATTTCGGAA-3′
10	5′-CAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATCAGCTGTTG-3′
11	5′-TCTCACTGGTGAAAAGAAAAA CCACCCTGGCGCCCAATACGC-3′
13i_chol	Chol – CCACGCTCCCTGAGGGGCGCC - 3'
12-loop30-9	5' - TCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACA GCGTAAGACCCACAATCCAATTTCCCTCGC ACAGGATTTTCGCCTGCTGGGGCAAACCAGCGTGGACCGCTT - 3'
14	5' - CGCCTGGGGTTTGCTTATAAA TCAAAAGGTTTGGACCAACGC -3'

Table S2: sequence of staples participating in the formation of the C-pore.

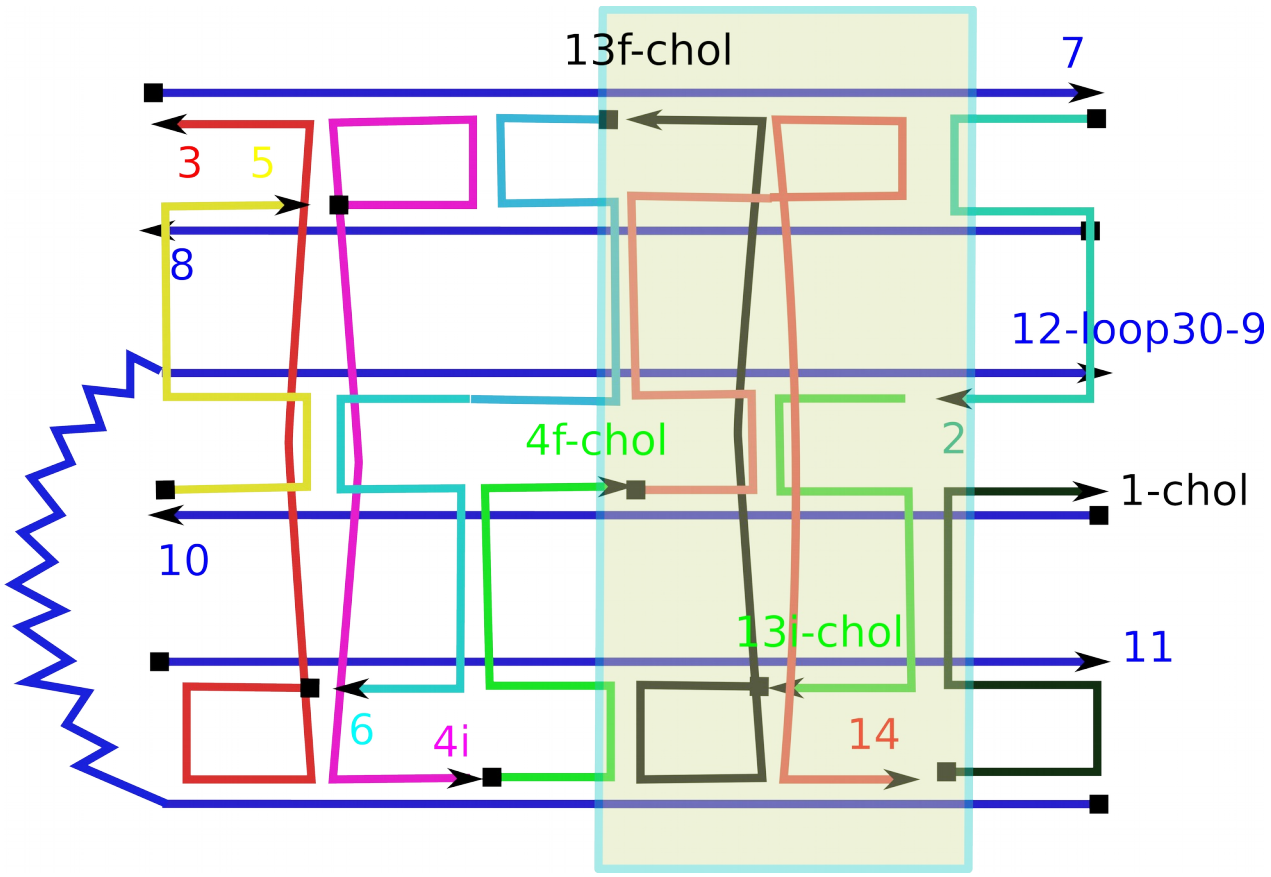

Figure S2: *Schematic representation of staples needed to form the C-pore. 5' ends are represented by a black square, 3' end with an arrow. Zigzag segments correpond to ssDNA handle or lock ssDNA . The shaded area corresponds to the region expected to insert on the lipid bilayer. The name of each staple and the staple share the same color.*

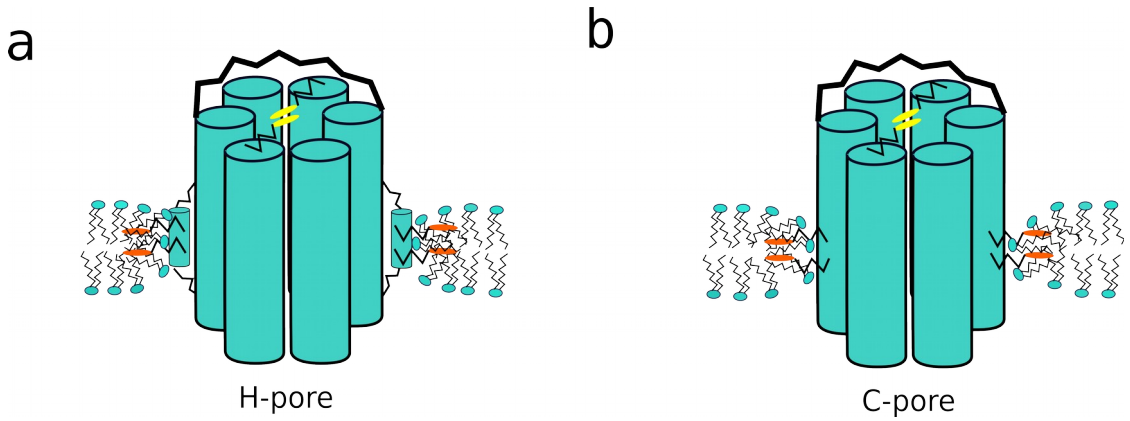

Figure S3: *Comparison between H-pore and C-pore structures. Both structures bear four cholesteryl moieties (orange ellipses) as hydrophobic anchors in addition to two cholesteryl moieties (yellow ellipses) which form a hydrophobic barrier on one side of the nanopore. (a) In the case of H-pores, a 14nt long single strand (Handle-2chol) with two cholesteryl modifications is (partially) complementary to two 24nt long overhangs, with common central sequence AGTTGGAGTTGATA. Hybridization of strand Handle-2chol to the two flanking overhangs results in two short (14bp long) helices, each bearing two cholesteryls. (b) In the case of C-pores, four strands (1_chol, 4f_chol, 13i_chol, 13f_chol, cf. Table S2) have been modified with a single cholesteryl. As in H-pores, a total of four cholesteryls ensure the insertion into the membrane.*

Coarse-grained simulations.

In order to get a microscopic view of the open-close transition, we used the oxDNA coarse-grained model [2]. In this model, each nucleotide is a 3D rigid body. Pairs of nucleotides interact through a number of pairwise effective interactions representing chain connectivity, excluded volume, hydrogen-bonding and stacking interactions between bases. There is no explicit representation of

solvent or ion species. The model is quite effective in the modeling of thermodynamic and mechanical properties of oligonucleotides at high salt concentrations. Here, we use oxDNA to simulate the close to open transition of H-pores. In doing so, we explicitly discard the influence of lipids and particularly the effect of membrane tension.

We performed molecular dynamics simulations, 500ns long, using the Langevin Dynamics algorithm at T = 300K. In this approach, the effect of implicit solvent is included by augmenting the Newtonian equations with drag and random noise forces. Initial configurations of the open and closed stated were relaxed for 10ns. Typical configurations of the open and closed states are represented in Figure 1c of the manuscript. For each of the six helices that form the pore, Figure S4 represents the position of the terminal nucleotide for open and closed configurations.

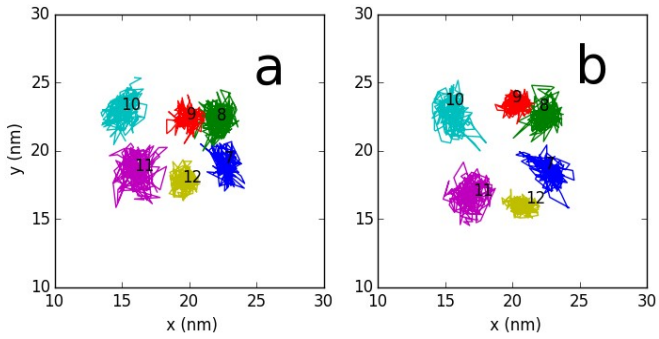

Figure S4: *Closed to open transition. For each helix (numbered from 7 to 12 according to the nomenclature of figures S1 and S2) is represented the position of the 5' nucleotide (helices 7, 9 and 11) (respectively 3' nucleotide, helices 8, 10 and 12) along the oxDNA simulation. (a) Closed conformation. (b) Open conformation.*

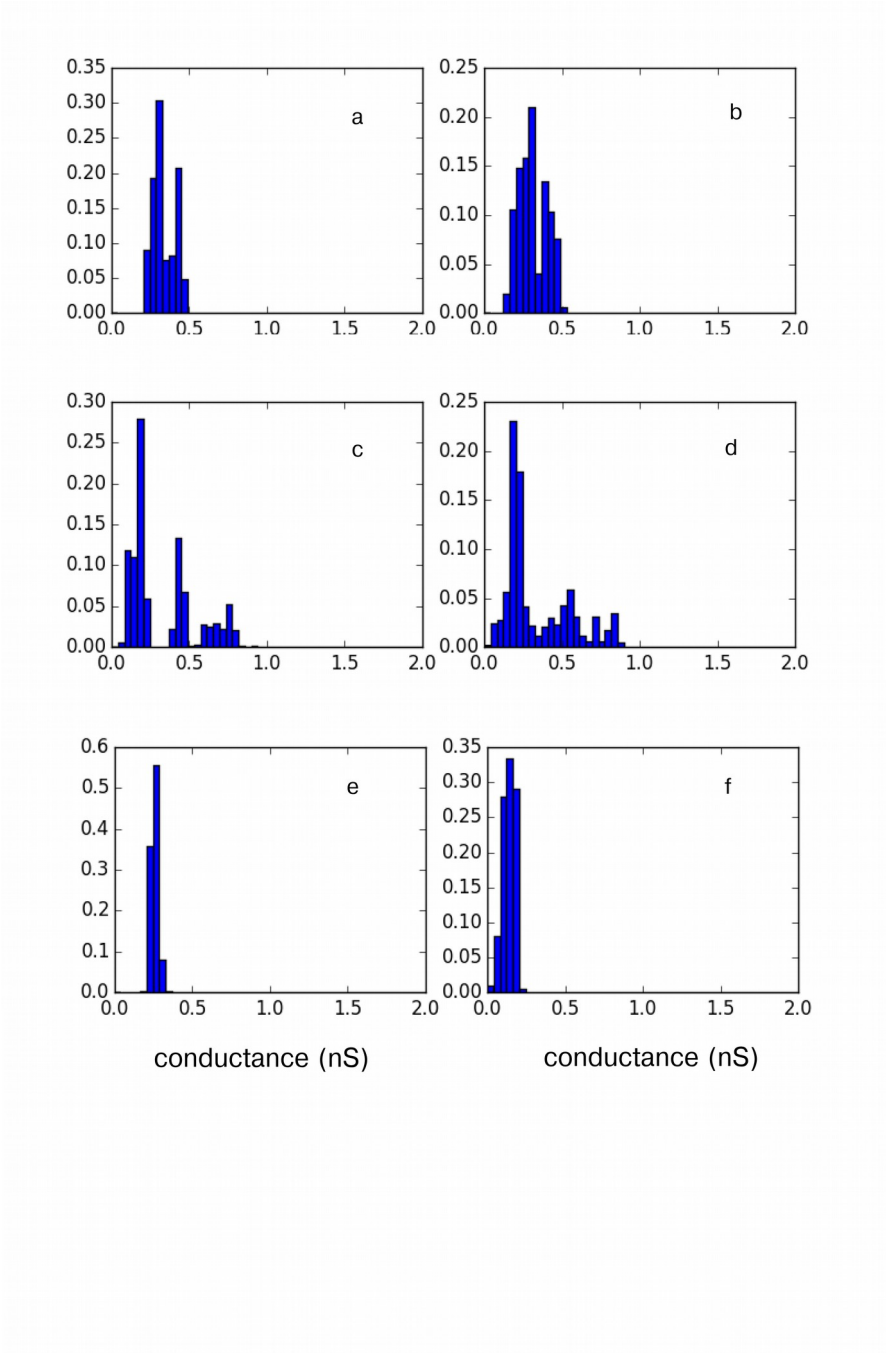

Figure S5: *All-point histogram of conductance for H-pores in the closed state (a and b for negative and positive potentials, respectively) open state (c and d for negative and positive potentials, respectively) and with no cholesteryl modifications (e and f or negative and positive potentials, respectively) The conductance value of each point of these histograms was obtained by dividing the measured current by the applied voltage (-80mV, -60mV, -40mV, 40mV, 60mV or 80mV). Data corresponding to high (±100 mV) or low (±20mV) voltage values were excluded.*

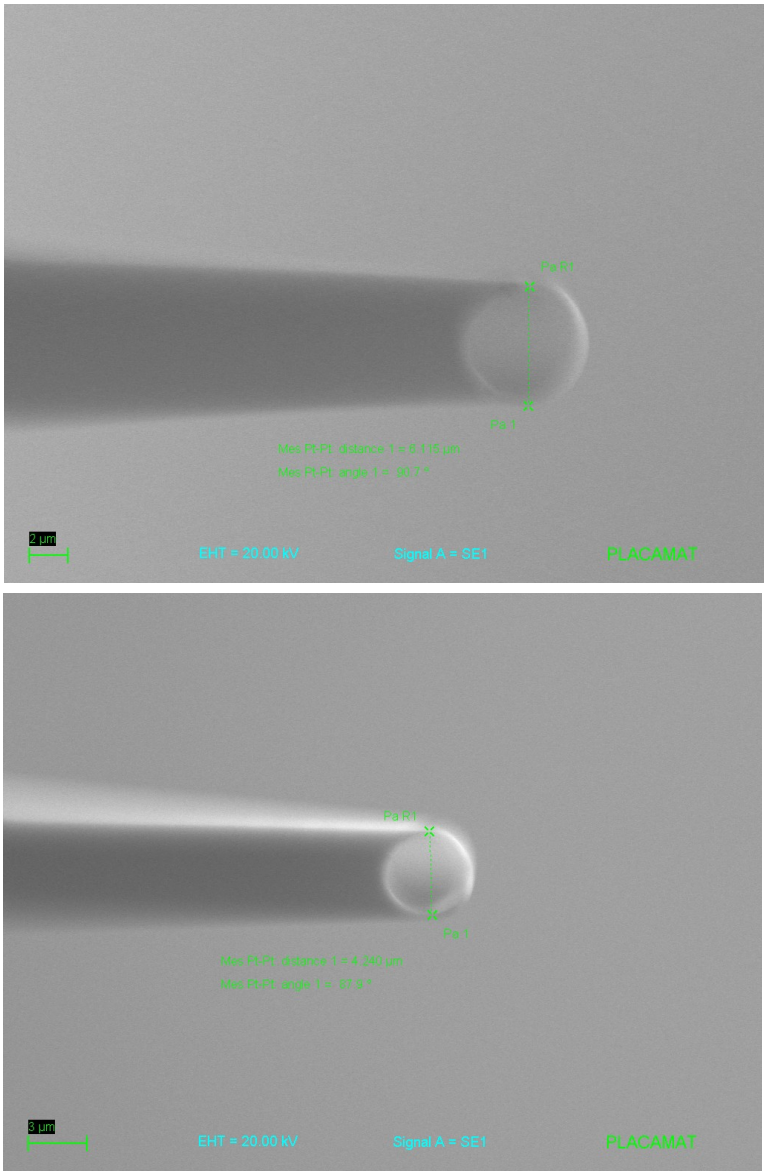

Figure S6: *SEM images of the tip of capillaries used in the patch-clamp experiments. The upper image shows a ~6µm diameter, the lower a ~4.2µm diameter.*

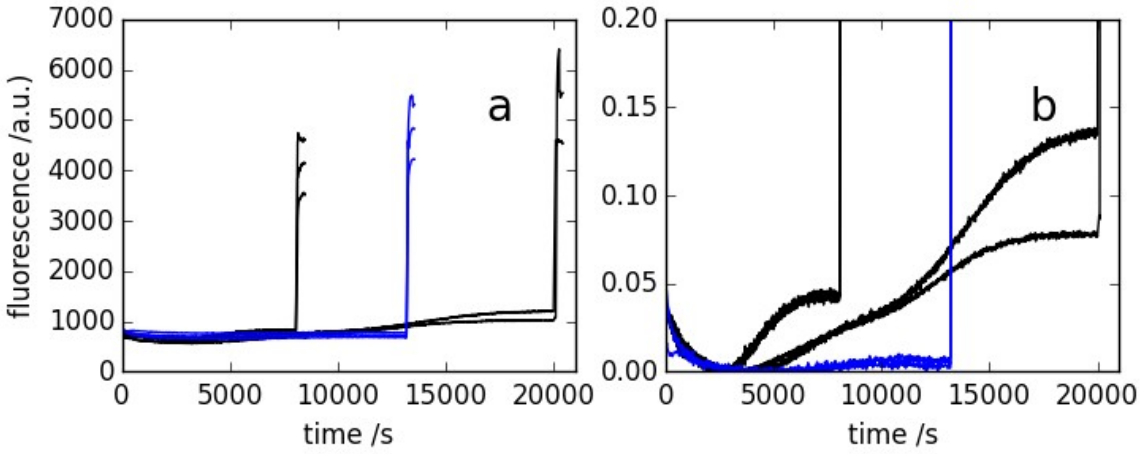

Figure S7: *Sulphorhodamine-B leakage from LUV's. Black (resp. blue) traces correspond to assays done in presence of H-pore without (resp. with) lock mechanism. Temperature held to 35C. (a) Raw data. (b) Data have been normalized by the maximal fluorescence signal obtained after disruption of vesicles by Triton X-100 (10%). Initial decrease of fluorescence (0 < t < 2500s) corresponds to a transient regime where temperature is stabilized and LUV's partially adsorb to the plastic surface of multiwell plates.*

Gel electrophoresis:

H-pores, with and without cholesterol, were analyzed using 1.0 % agarose gel electrophoresis,

much in the same way it was done in ref. [1]. We used standard TBE buffer (45mM Tris, 45 mM boric acid, 1mM EDTA) with additional 11 mM $MgCl_2$. Varying concentrations of H-pores were mixed with 2 μL loading buffer, then loaded into the wells. The gel was run at 80 V for 30 minutes at 4°C. Band visualization was performed with SYBR Safe staining (1/10000 concentration) and blue light illumination. A geneRuler 1kb DNA Ladder was used as reference standard for migration.

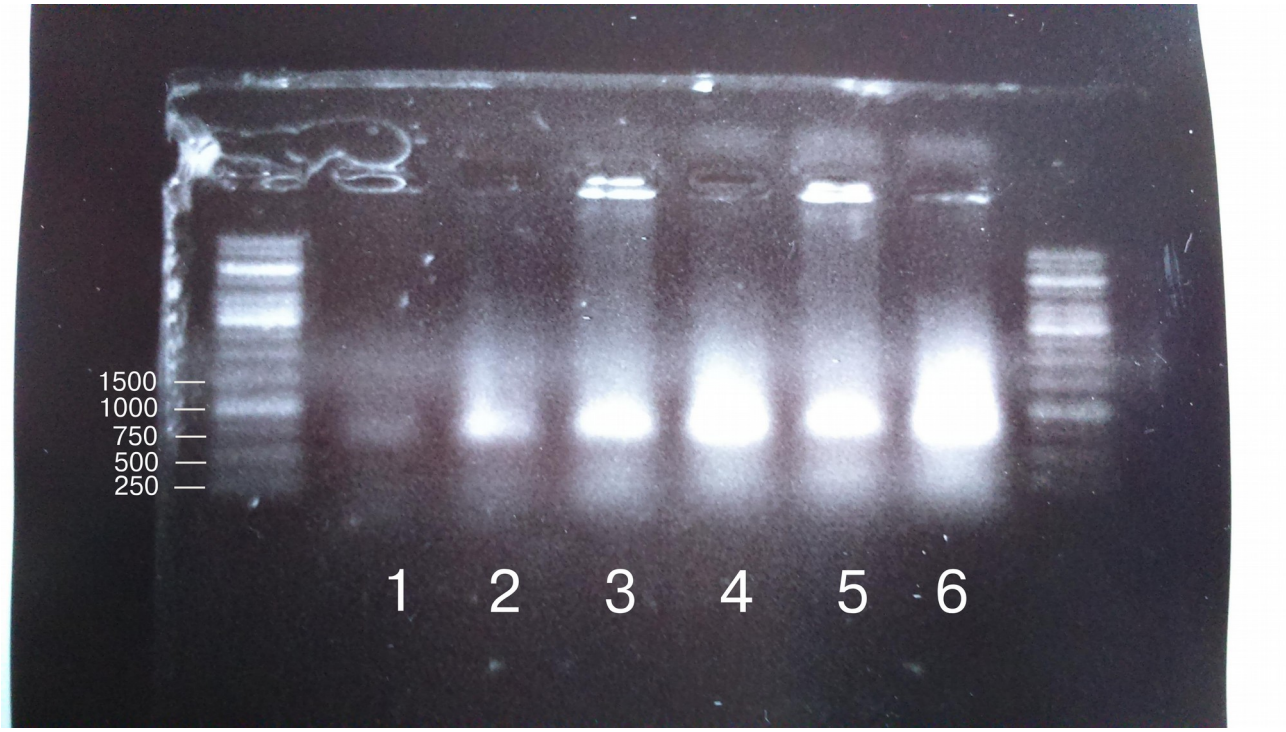

Figure S8: Electropherogram of DNA H-pores, without (lanes 2, 4, 6) or with cholesteryl modifications (lanes 1, 3 and 5). Lanes before lane 1 and after lane 6 correspond to the 1kb DNA reference ladder. If the initial DNA concentration of lane Li is noted [Li], then [L6] = [L5] = 2x[L4] = 2x[L3] = 4x[L2] = 4x[L1]. Cholesteryl free nanopores migrate as a sharp band, indicating correct formation under gel electrophoretic conditions. Nanopores containing cholesteryl also show a well defined band, slightly slower than the cholesteryl-free nanopores. Fluorescence in the initial well points to the existence of aggregation in presence of cholesteryl.

Atomic force microscopy:

AFM images of H-pores with no cholesterol modifications were obtained by first adsorbing them onto freshly cleaned mica (typically a drop of 3uL was deposited at room temperature, imaging started few minutes after drop deposition). Images were acquired with a Dimension FastScan (Bruker) in peak-force mode (500pN force threshold) and liquid conditions using FastScan-C cantilevers. Images were analyzed using gwyddion (http://gwyddion.net). H-pores, as observed here, seem to be less 'compressed' (cross sections are higher and thinner) than previously reported (ref. [1]) measurements on similar structures.

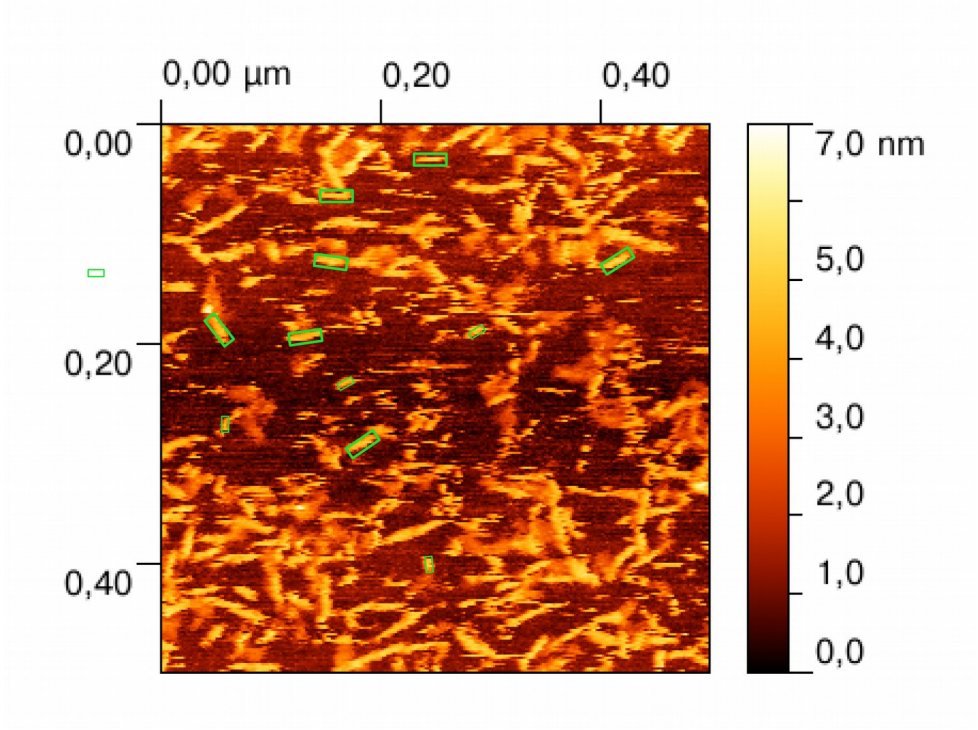

Figure S9: *AFM images of H-nanopore, without cholesteryl modifications, final concentration 100nM, buffer TAEx1. As a guide of the eye, a few representative objects have been marked off with green rectangles of size 30 nm x 10 nm and 15nm x 10nm, corresponding respectively to theoretical sizes of dimeric and monomeric nanopores.*

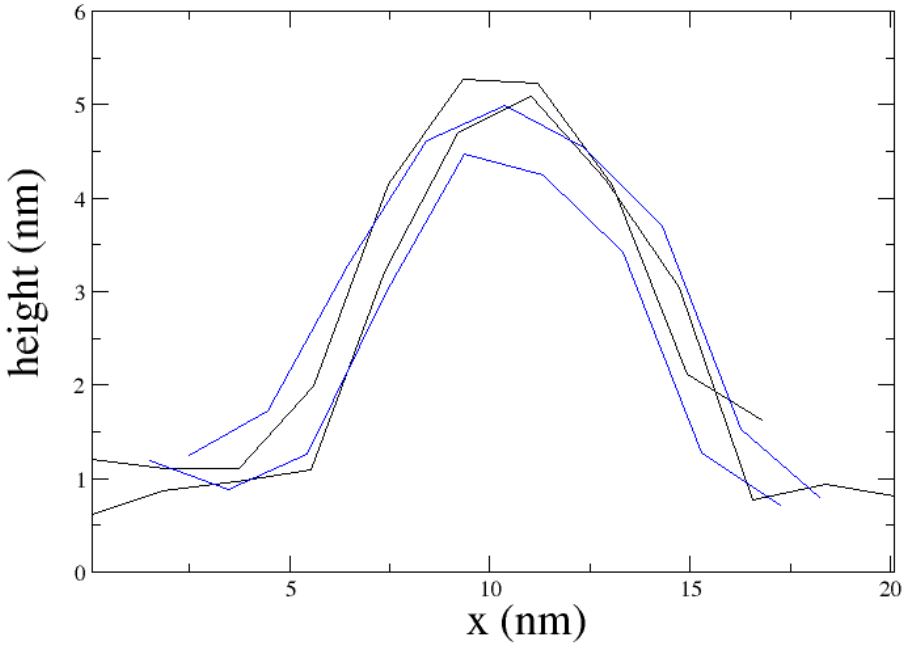

Figure S10: Cross section, normal to the long axis, performed on two dimeric (black line) (respectively monomeric, blue line) H-pores.

References:

[1] Burns, J. R.; Stulz, E.; Howorka, S. Self-Assembled DNA Nanopores That Span Lipid Bilayers. *Nano Lett.* **2013**, 13, 2351−2356.

[2] Ouldridge,T.E., Louis,A.A. , Doye, J.P.K. Structural, mechanical and thermodynamic properties of a coarse-grained DNA model. *J. Chem. Phys.* **2011**, 134, 085101.