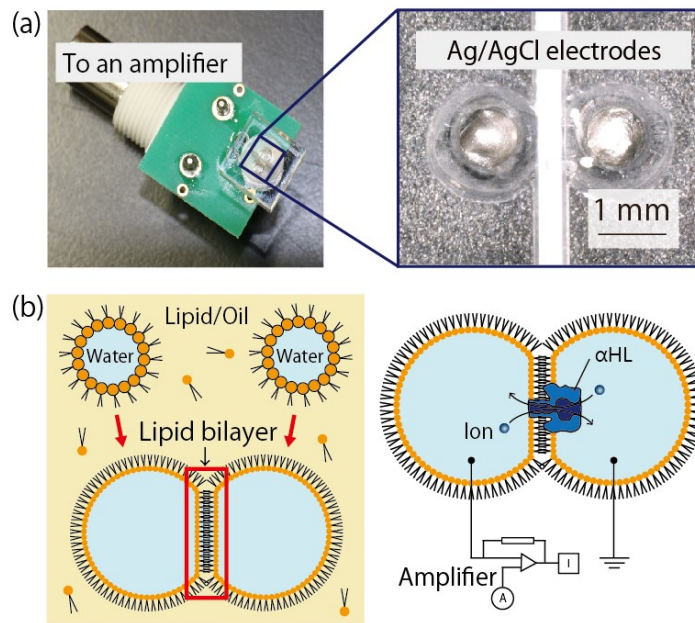Supplementary Information

Nanopore Decoding for a Hamiltonian Path Problem

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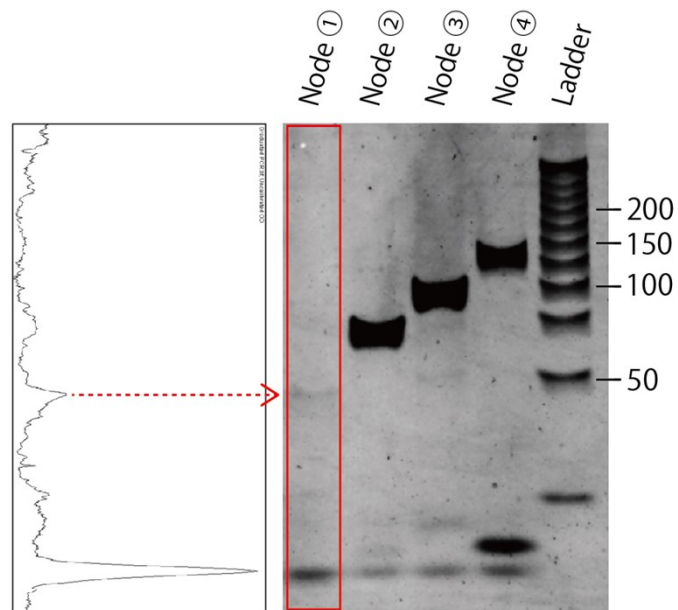Technology, Tokyo, 184-8588, Japan

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Fig. S1 (a) A photograph of the device for measuring channel currents. (b) Lipid bilayers are prepared by the droplet contact method and αHL is reconstituted in the lipid bilayer.
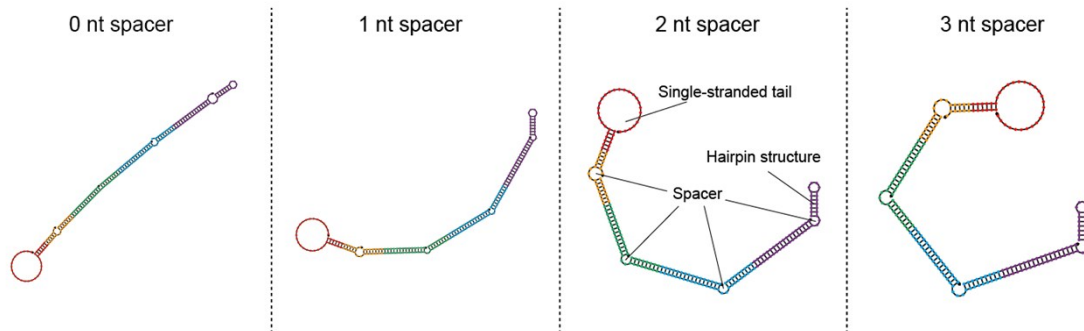
Fig. S2 The result of NUPACK simulation of the duplex with different numbers of spacer nucleotides.

Fig. S3 Detailed analysis of the result of the graduated PCR using Image J 1.52 (National Institutes of Health, Bethesda, Maryland, USA). This figure is the same data as **Fig. 2b** but the UV intensity is different, and the band corresponding to Node 1 was confirmed. We considered that the amplification efficiency of short fragments like Node 1 was lower than the others, resulting in the detection of weak fluorescence intensity.
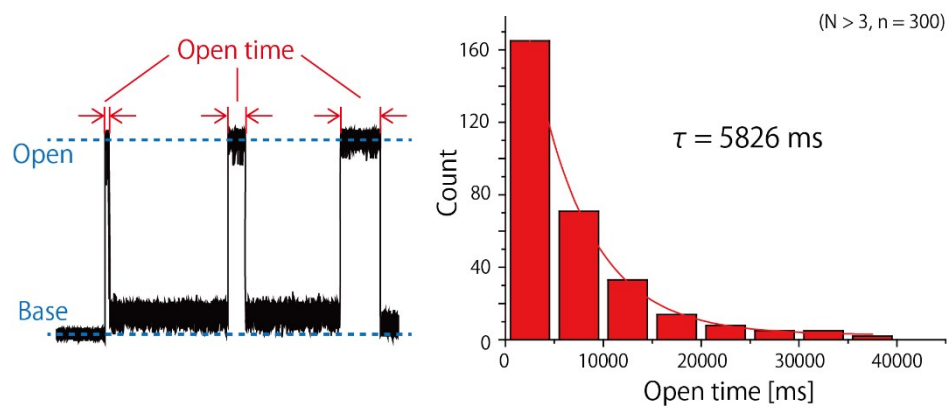
Fig. S4 Open time analysis to calculate the event frequency. The open time indicates the time until the output duplex to be inserted into the nanopore. The inverse value of the open time should be equal to the event frequency, resulting in 0.17 s^{-1} in this case (N is the number of αHL nanopore and n is the number of unzipping signal).
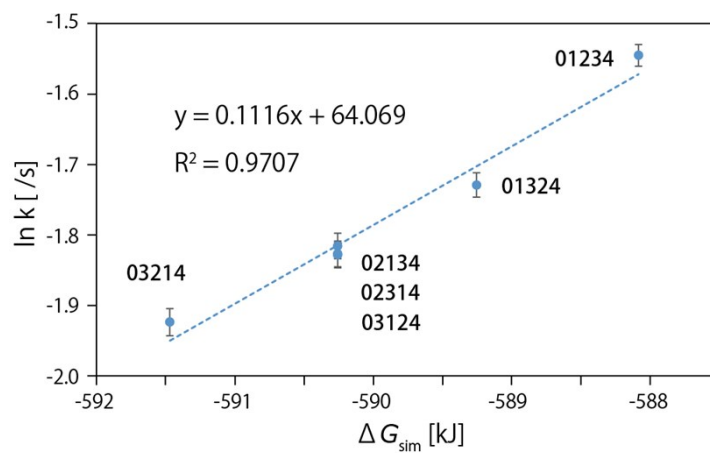
Fig. S5 The relationship between the first order rate constant and the simulated free energy of hybridization (ΔG_{sim}).
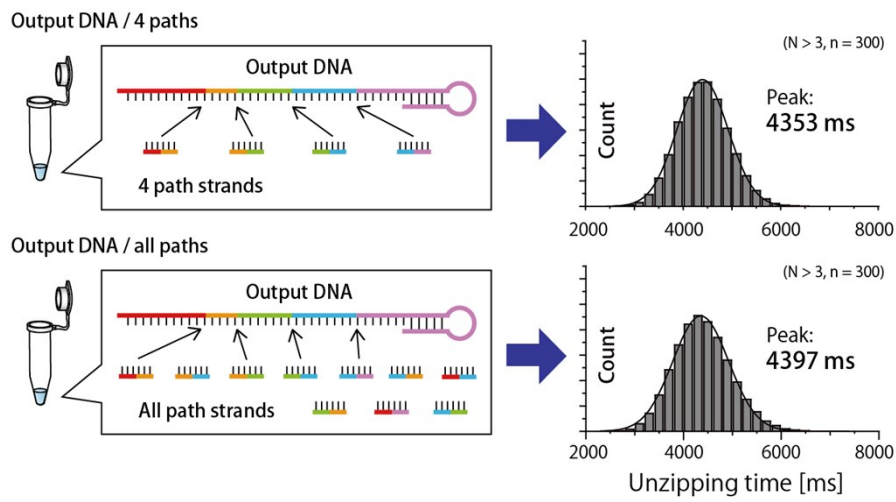
Fig. S6 Result of nanopore measurement under 2 conditions: using the output DNA with the 4 corresponding kinds of path strands, and with all path strands (N is the number of αHL nanopore and n is the number of unzipping signal).
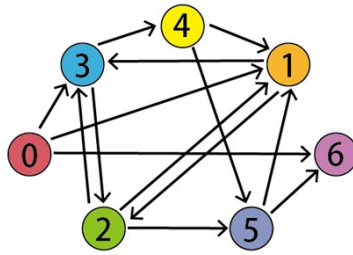
Fig. S7 A previous directed graph encoding a Hamiltonian path problem.

Table S1. The free energy [kJ/mol] of each duplex depending on the spacer length.

	0 nt spacer	1 nt spacer	2 nt spacer	3 nt spacer
0 → 1 → 2 → 3 → 4	-580.6	-585.7	-588.1	-588.1
0 → 1 → 3 → 2 → 4	-581.9	-586.9	-589.3	-589.3
0 → 2 → 1 → 3 → 4	-582.9	-587.9	-590.3	-590.3
0 → 2 → 3 → 1 → 4	-582.9	-587.9	-590.3	-590.3
0 → 3 → 1 → 2 → 4	-582.9	-587.9	-590.3	-590.3
0 → 3 → 2 → 1 → 4	-584.0	-589.1	-591.5	-591.5

Table S2. DNA sequences as each node in the graph.

Node	DNA sequence
0	5' GGTAAACCTCTGTCACCCCTCTTTCGTCG 3'
1	5' CATCGCCAATAC 3'
2	5' AGGAGGCCGCTCA 3'
3	5' TACGATTCCGTGGTA 3'
4	5' CAACCAGTCCACTCCGT 3'
5	5' CGCAATGCTCCGGCTCAAT 3'
6	5' GTAGAACTTTTGTTCTACCCGTCGAGGTA 3'

Table S3. Comparison of the ΔG.

Route	Free energy [kJ/mol]
0 → 1 → 2 → 3 → 4 → 5 → 6	-567.69
0 → 5 → 4 → 3 → 2 → 1 → 6	-565.68