Recapturing and trapping single molecule with a solid –state nanopore

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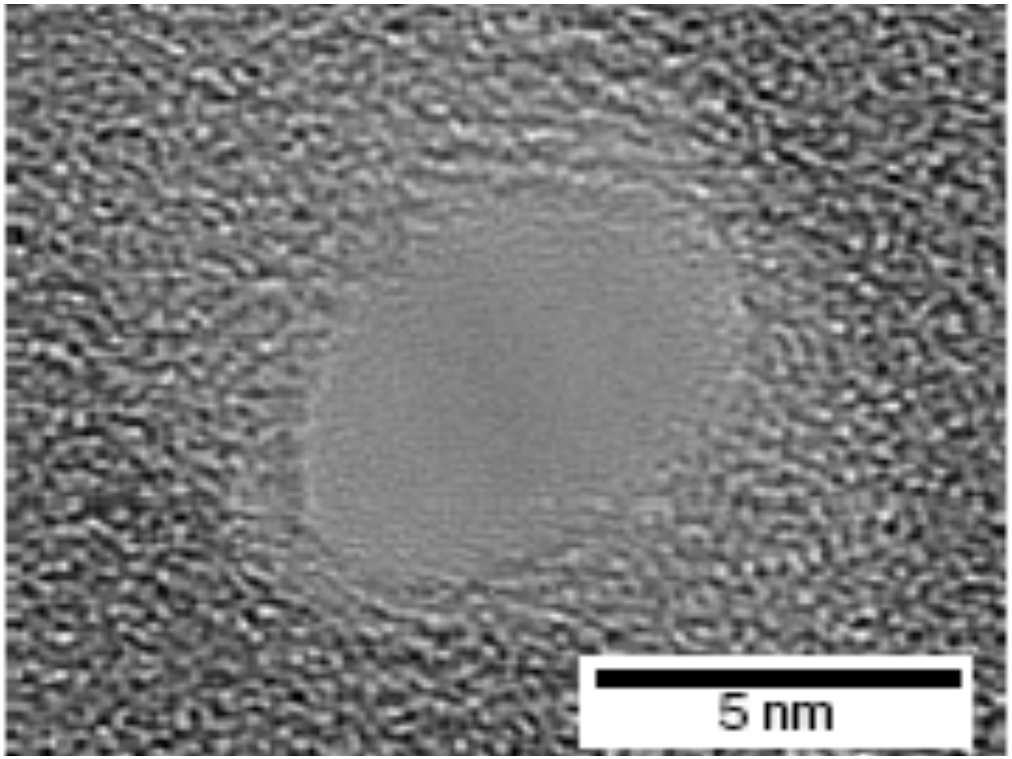
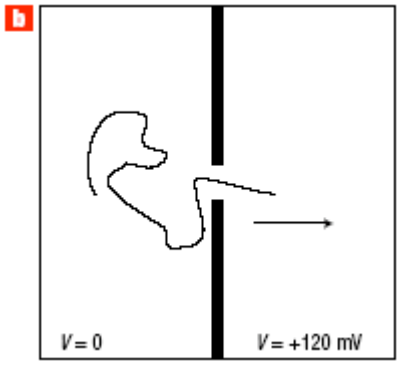
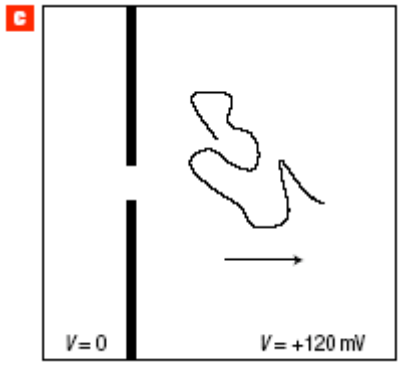
Introduction:-

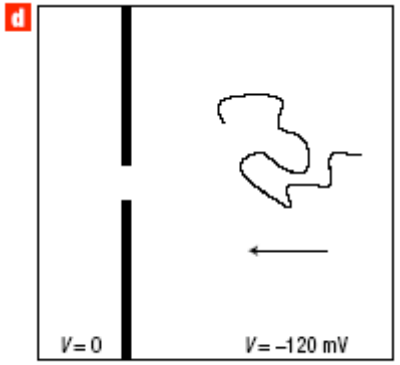
- Brief overview of how DNA passes through a nanopore.
- Overview of the recapture experiment
- Capture rate and recapture probabilities
- How to calculate characteristic distance up to witch recapturing is possible.

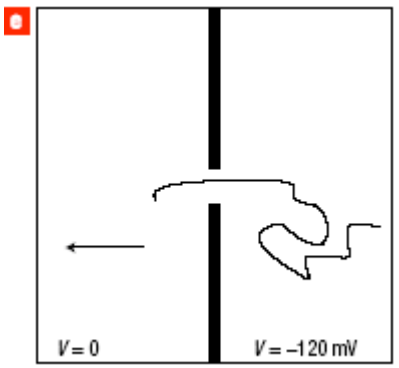


Nanopore were fabricated by TEM beam
Dimensions of nanopore 5nm x 7 nm , width 20nm


b
V = 0
V = +120 mV

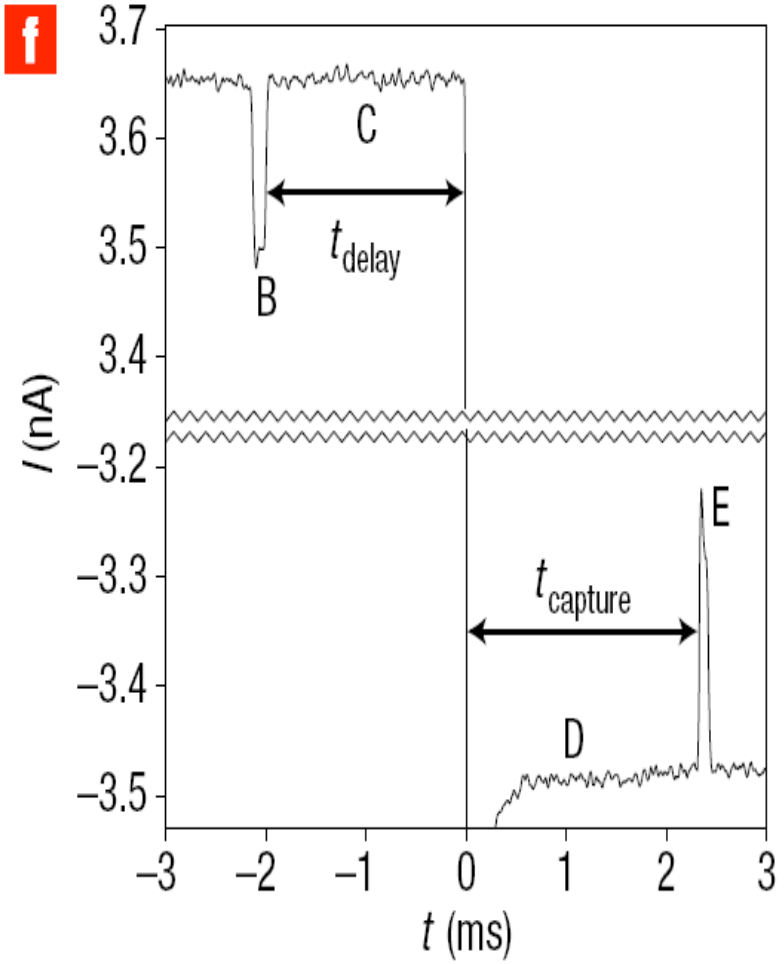

c
V = 0
V = +120 mV


d
V = 0
V = –120 mV


a
V = 0
V = –120 mV

Delay time t_{delay} and Capture time, $t_{capture}$

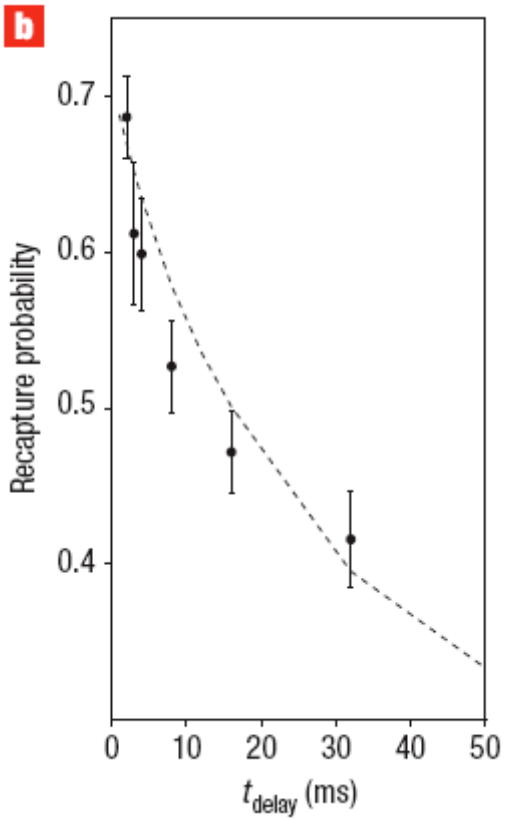
- t_{delay} is the time between first translocation and the voltage reversal
- $t_{capture}$ is the time until the molecule re enters the pore after voltage reversal.

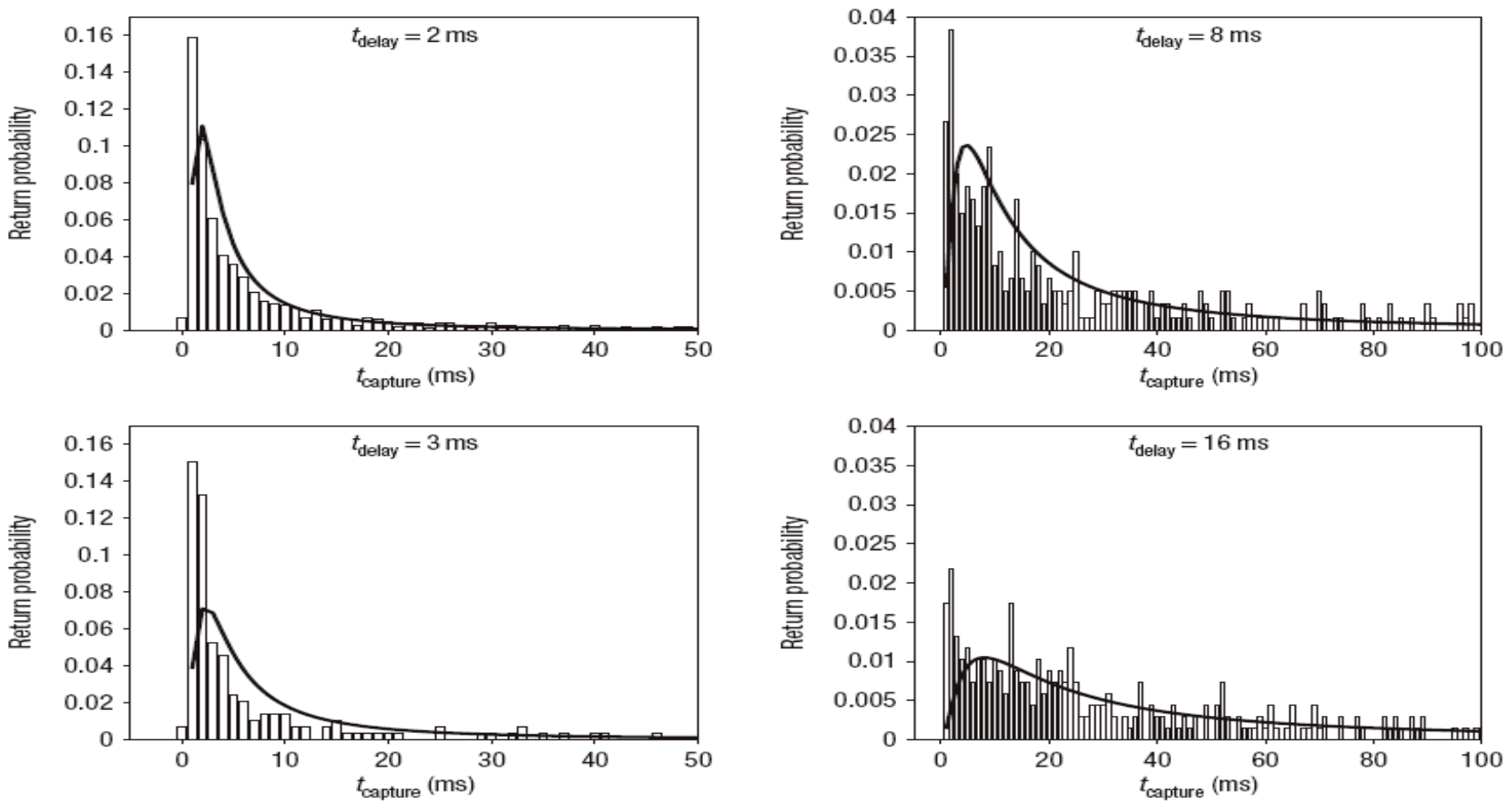


DNA Molecules recaptured in 500 ms of voltage reversal:-

✓ As the delay time increases recapturing probability decreases .

✓This is because with the time DNA molecule diffuses more and more so recapturing is less probable with the passage of time.



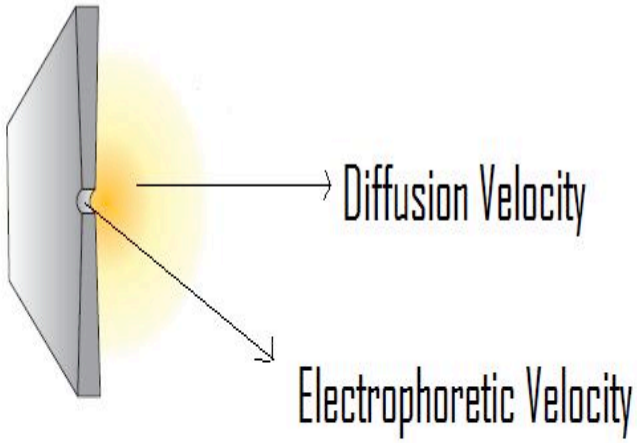


Graph shows variation in Returning probability with $t_{capture}$ at different values of t_{delay}

For $t_{delay} < 4$ ms most molecule arrives at the pore and are translocated through in less then 10ms

Region of different velocities in and around the nanopore:-

- In the pore the major effect is by the electric field(Diffusion force is neglected compare to electric force), the velocity of DNA(in the nanopore) due to this electric field is called electrophoretic velocity $V_{e.}$
- In the region outside the nanopore there is salt solution which is good conductor so electric field in this region is very week and velocity of DNA molecule is only due to diffusion of DNA molecule called Diffusion Velocity V_d



Two kind of forces acts on DNA molecule as:-

a. Diffusion force

b. Force due to electric field(Electrical Force)

a. Diffusion force

Diffusion velocity of DNA is defined as:-

$$V_d(r) = D/r$$

Here 'D' is called diffusion constant for DNA

It is defined as the diffusion of DNA in solution in unit area in unit time. So it has units as cm^2 / sec .

Here 'r' is the distance of DNA from the pore

b. Electrical Force

By Ohm's law $J = \sigma E$,

Here J is the current density and 'σ' is the electric conductivity of the ionic solution.

$$\text{And} \quad E = J / \sigma = (I * \mathbf{r}) / (2*\pi*r^{2*}\sigma)$$

'I' is current through the nanopore.

'r' is radius of the hemisphere(Electric field is assumed spherical symmetric).

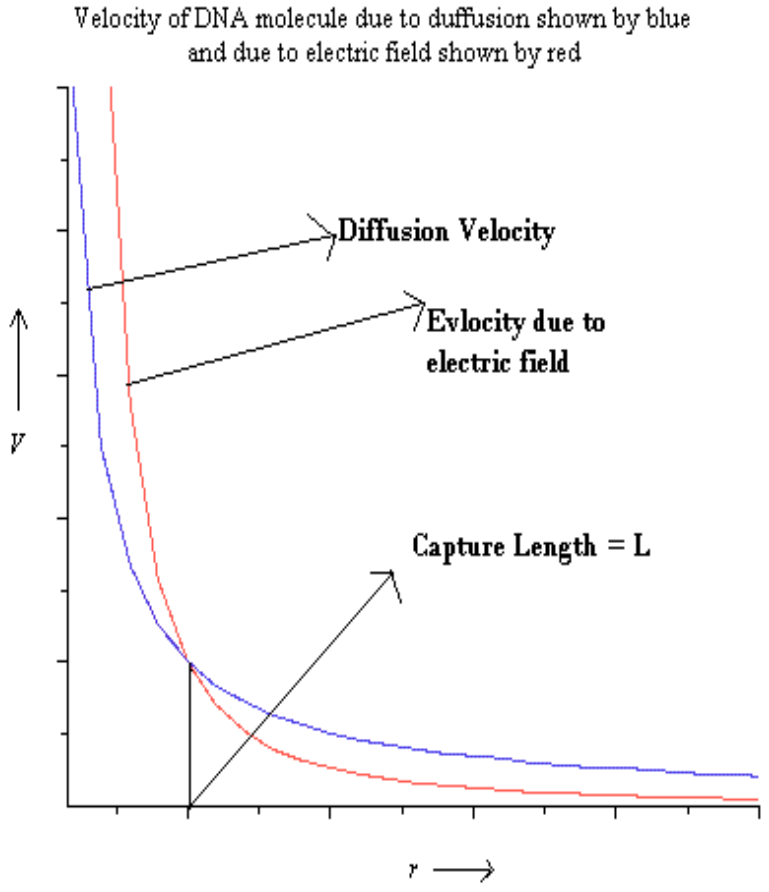
'**r**' is unit vector describing the direction of 'J'

So velocity of DNA due to this electrical force is

$$V_e = \mu*E = \mu*I / (2*\pi*r^{2*}\sigma)$$

To Compare V_e and V_d

- Diffusion velocity is directly proportional to 1/r
- Velocity due to electrified is proportional to $1/r^2$
- Capture length is defined as L.
- Recapturing is possible only if distance of DNA from the nanopore is less then L.



By Comparing V_d and V_e

Characteristic distance up to which recapturing is possible is

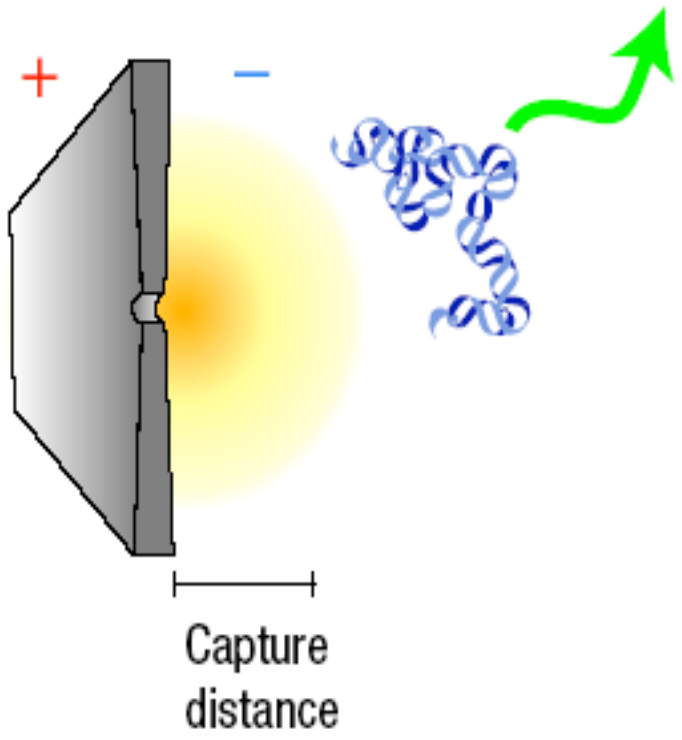
$$L = \mu * I / (2 * \pi * D * \sigma)$$

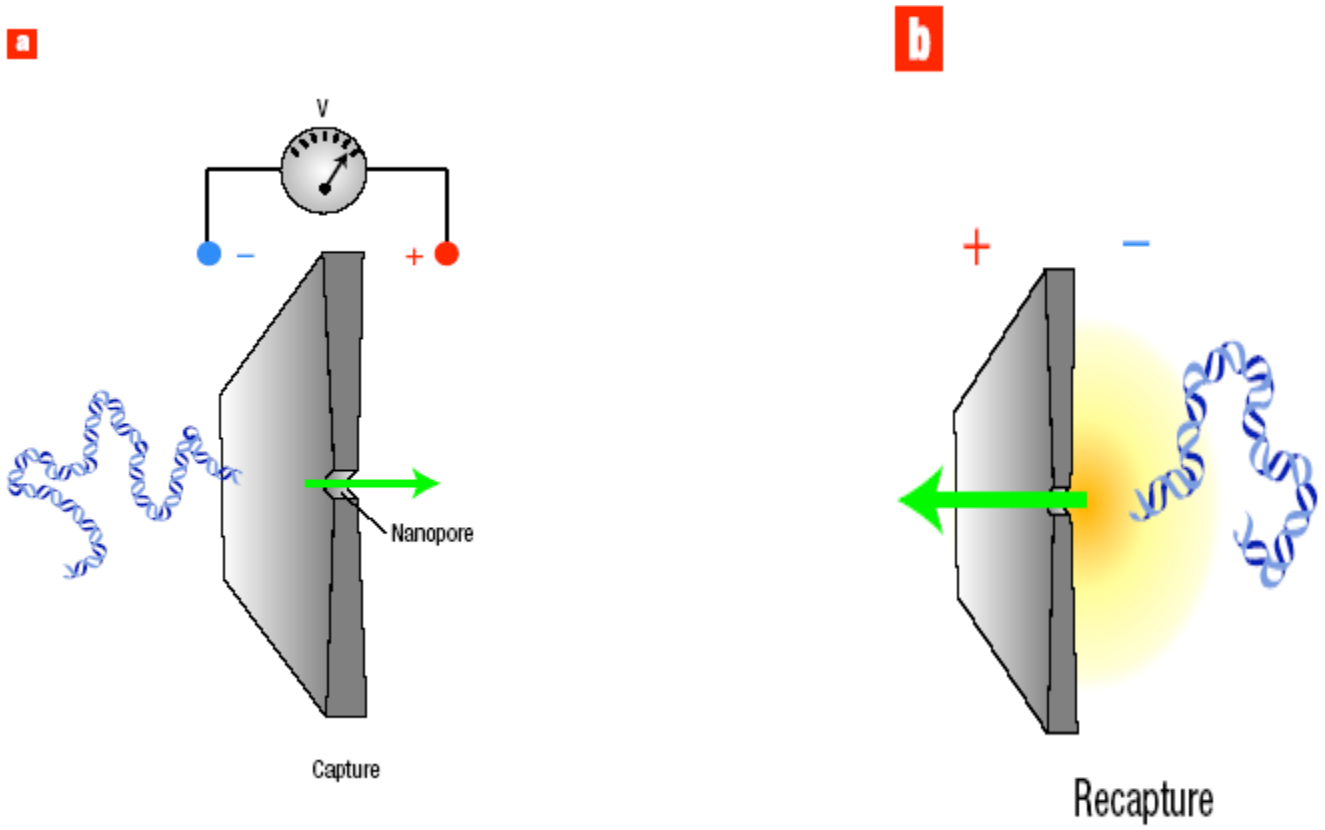
Here ‘μ’ is called electrophoretic mobility, it is constant of proportionality between Electric field and velocity of DNA in solution.

For this experiment:-

For 4 kbp dsDNA ‘L’= 940nm

For 6 kbp dsDNA ‘L’=1200nm



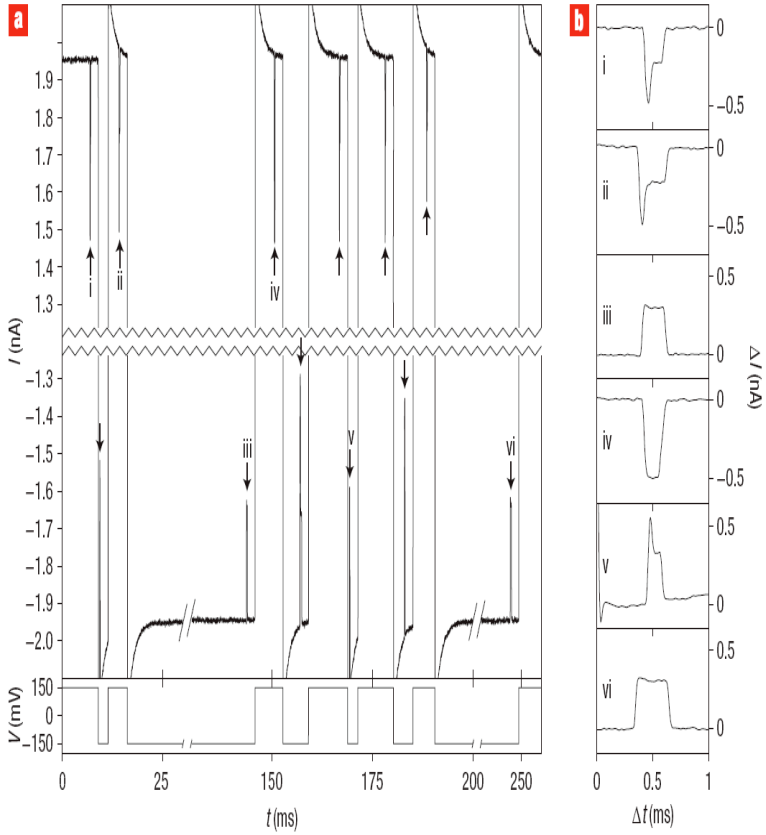

a
V
–
+
Nanopore
Capture
b
+
–
Recapture

Outcome for the 10 kbp dsDNA:-

Experiment performed with 10 kbp dsDNA molecule.

Here voltage biase is reversed after each t_{delay} = 2 ms interval.

- DNA molecule crosses the nanopore 12 times in 250ms.



Result:-

- Success rate for this experiment is 70%.
- This means 70 % of the DNA molecules can be recaptured.
- The reason for missing 30 % could be due either to molecules that stick briefly to the membrane surface or due to other approximations made in the experiment.

Advantages:-

- This method provides a way to distinguish molecular signal from the background noise.
- Recapturing of molecule would allow one to measure changes in molecules properties (such as charge, change in conformation) induced by passage through the nanopore.
- Biologically interesting molecules can be traped,detected and analysed in free solution without any chemical modification.
- This method can be used to confirm particular molecular confirmation.

References:-

- Gershow, M. and J.A. Golovchenko. 2007. Recapturing and trapping single molecules with a solid-state nanopore. Nature Nanotechnology 2: 775-779. (including a related New & Views article by Derek Stein).
- Qun Cai, Brad Ledden, Eric Krueger, Jene A. Golovchenko, Jiali Li. “Nanopore sculpting with noble gas ions.” Journal of Applied Physics 100, 024914 (2006).
- http://www.mcb.harvard.edu/branton/

Thanks