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DNA SEQUENCING USING GRAPHENE NANOPORES

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ABSTRACT

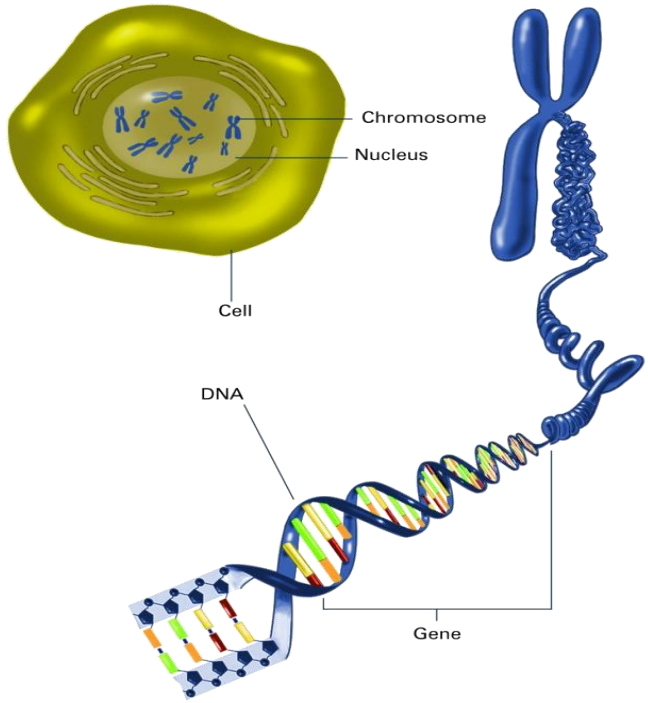
There is a worldwide race to develop fast and low-cost strategies to sequence DNA, that is, to read off the content of our genome. Particularly promising for the next generation of sequencing are devices where one measures on single molecules. Using electric fields, the tiny DNA strands are pushed through nanoscale-sized, atomically thin pores in a graphene nanopore platform for fast electronic sequencing of the four chemical bases of DNA based on their unique electrical signature. The pores, burned into graphene membranes using electron beam technology, provide with electronic measurements of the translocation of DNA. High resolution of graphene nanopore devices is expected because the thickness of the graphene sheet is smaller than the distance between two DNA bases. Here used a chemical vapor deposition, or CVD, method to grow large flakes of graphene and suspend them over a single micron-sized hole made in silicon nitride. An even smaller hole, the nanopore in the very center of the suspended graphene, was then drilled with an electron beam of a transmission electron microscope, or TEM [(13)].

INTRODUCTION

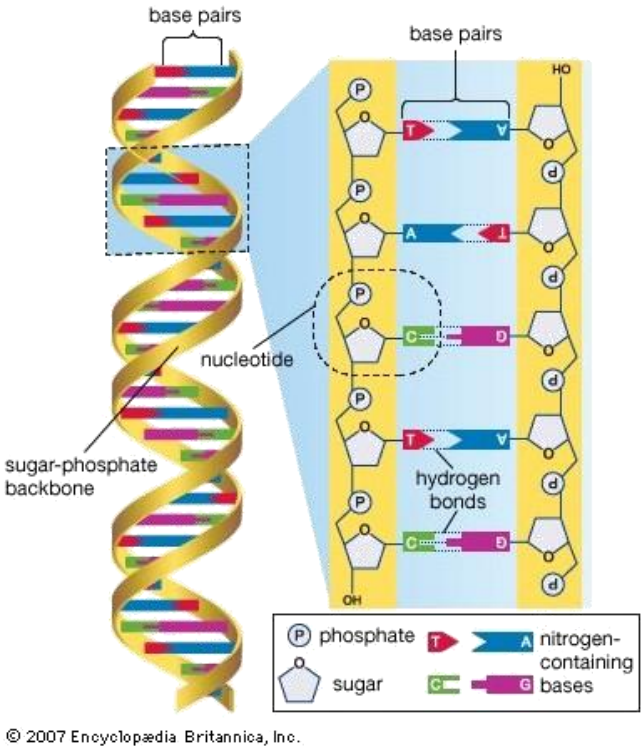
DNA base pairs

A genome is all of a living thing's genetic material. It is the entire set of hereditary instructions for building, running, and maintaining an organism, and passing life on to the next generation [12]. DNA is the hereditary material containing the genetic instructions used in the development and functioning of all known living organisms. The DNA segments carrying genetic information are called genes. The information in DNA is stored as 3 letter sequences called genetic code made up of: adenine (A), thymine (T), guanine (G) and cytosine (C) [(14)].

DNA PACKED IN CHROMOSOSME



The two strands of DNA run in opposite to each other [anti-parallel]. One of four types of bases is attached to each sugar. DNA bases pair up with each other, A with T and G with C, to form units called base pairs. Together, a base, sugar, and phosphate are called a nucleotide. Nucleotides are arranged in two long strands that form a spiral [double helix] with backbone made of sugar-phosphate groups joined by ester bonds. Double helix is like a ladder, with the base pairs forming the rungs and the sugar-phosphate backbone forming the vertical sidepieces [(14)].



NUCLEOTIDES IN DNA

Human DNA consists of about 3 billion bases, and about 99.9% are the same in all people [1/1500]. The genetic information in a genome is held within genes, and the complete set is called its genotype. A gene is the unit of heredity and is a region of DNA that influences a particular characteristic in an organism.

DNA sequencing [8]

DNA sequencing is the process of determining the precise order of the bases [A, T, G and C] in a molecule of DNA. Scientists will be able to detect the defective genes responsible for genetic diseases like Alzheimer's disease, cancer, diabetes etc and can replaced with the healthy ones. It also possible to provide patients precise personalized treatment developed on the basis of that patient's specific DNA sequence. The applications of DNA sequencing include:

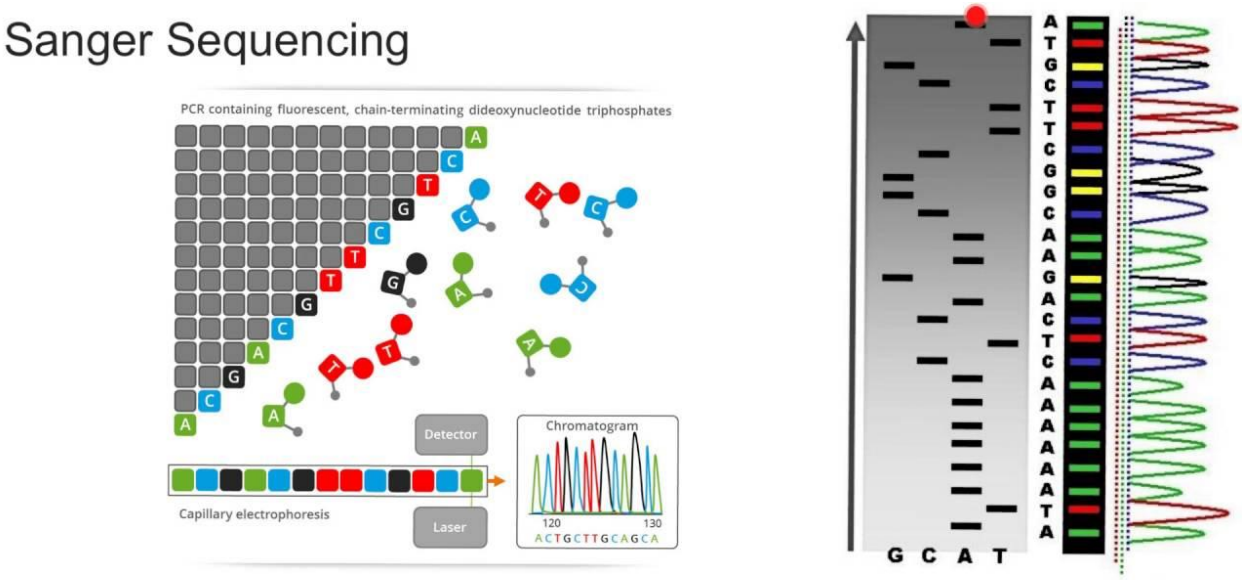
- Evolutionary biology: to study how different organisms are related and how they evolved.
- In crime forensics: to identify a body & solving a crime: height, skeletal and facial features, skin colour etc of a person can be determined.
- Determination of paternity.
- In medicine: to determine if there is risk of genetic diseases.

- In agriculture: to make pest resistant plants, to increase productivity and quality of milk & meat etc.

DNA sequencing has come a long way since 1970s, when the first technique was introduced by Frederick Sanger and colleagues in 1977; it was the most widely used sequencing method for approximately 40 years. The technique involved 4 steps:

- DNA amplification
- Sequencing reaction
- Separation and detection of the fragments
- Assembling of the sequenced parts of a gene

In this technique, the DNA is sequenced using an enzymatic method which polymerizes the DNA fragments complimentary to the DNA of interest. P ^{32} is used to label the synthetically designed primer that binds to the DNA template at a known sequence. The synthesis occurs with DNA polymerases and dNTPs (deoxynucleotide triphosphate) until a ddNTP (dideoxynucleotide triphosphate) is incorporated which terminates the reaction due to the absence of the deoxy-group. This is carried out in four reaction tubes containing the four nucleotides (A, T, G, and C) in the dideoxy- form. The starting point of synthesis is same but the 3' end is specific to the ddNTP attached. The fragments are run on a denaturing polyacrylamide gel on four different lanes. The gel pattern specifies the chain termination site and the sequence can be read on an autoradiograph.

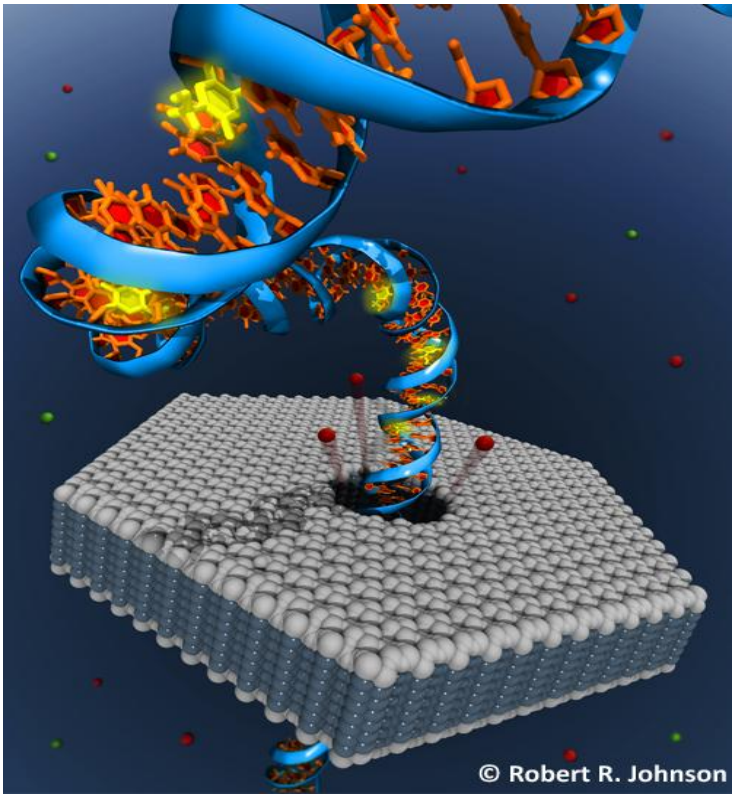


Sanger sequencing uses ddNTPs (dideoxynucleotide triphosphates) which do not have a free 3' OH mixed in with dNTPs
Whenever the DNA polymerase incorporates a ddNTP it won't be able to add any other nucleotides
Then gel electrophoresis is used to separate the DNAvv

Common challenges of DNA sequencing with the Sanger method include poor quality in the first 15-40 bases of the sequence due to primer binding and deteriorating quality of sequencing traces after 700-900 bases. It is also costly and time taking. More recently, higher volume Sanger sequencing has been supplanted by "Next-Gen" sequencing methods, especially for large-scale, automated genome analyses. However, the Sanger method remains in wide use, for smaller-scale projects.

Nanopore sequencing [7]

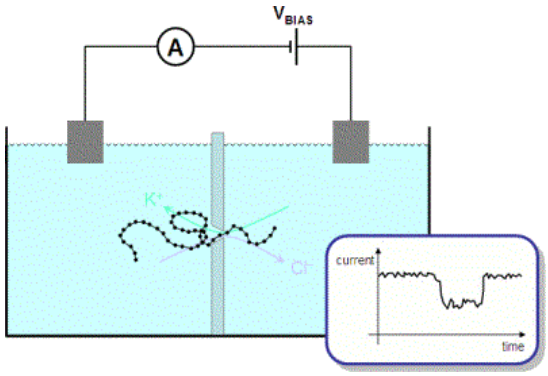
It's the latest fourth generation technique for real time sequencing of DNA. The DNA strand to be sequenced is passed through a membrane pore and is sequenced in real time. Using nanopore sequencing, a single molecule of DNA or RNA can be sequenced without the need for PCR amplification or chemical labeling of the sample. Nanopore sequencing has the potential to offer relatively low-cost genotyping, high mobility for testing, and rapid processing of samples with the ability to display results in real-time.



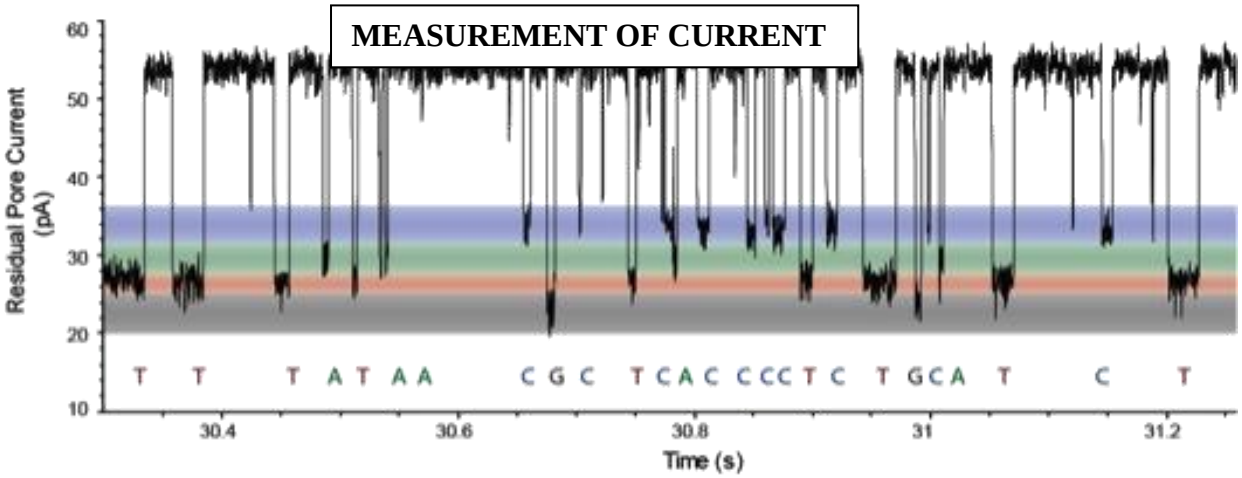
DNA PASSING THROUGH NANOPORE

DNA strands are electrical conductors. Different nucleotide bases should have different electrical characteristics. The measurement of these properties determines the DNA sequence. For this purpose, a tiny hole [nanopore] was made through a thin sheet of material and measures the

amount of current passes from one side of the sheet to another. Next, pull a DNA strand through the hole and measure the current again.



MEASUREMENT OF CURRENT



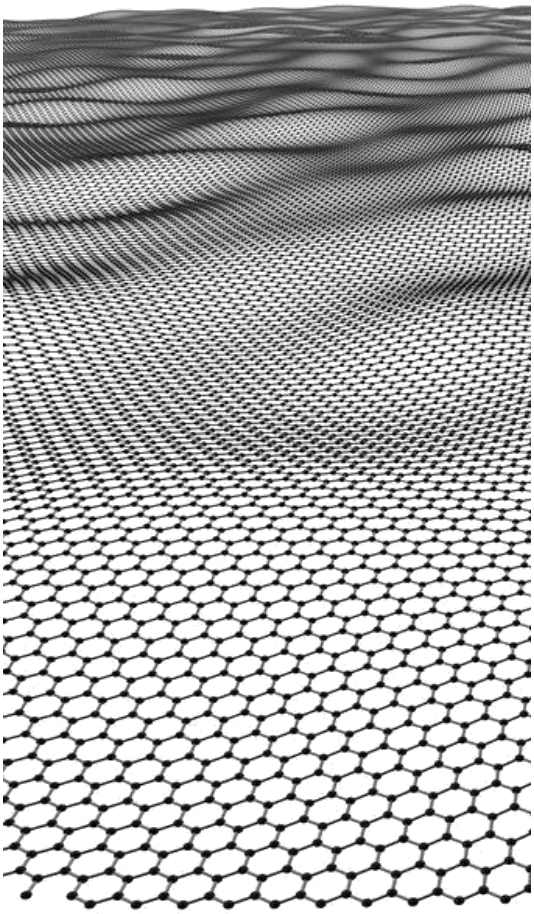
RESULT OBTAINED FROM NANOPORE SEQUENCING

So, measuring the changes in current gives the direct reading of nucleotide sequence in the strand. Biological protein pores such as α-hemolysin [α-HL] and Mycobacterium smegmatis porin A [MspA] were the first used nanopores. But, sensitivity of these to temperature, pH and applied voltage was a drawback. Solid state nanopores such as Silicon nitride [SiN], Aluminium oxide [Al_2O_3], Silicon oxide [SiO_2] were exciting alternatives as they are robust and possess electrical properties. But they are10-100 times thicker than the distance between 2 nucleotide bases. So, it is not a single nucleotide base that blocks the current flow at a particular time failing in obtaining single-nucleotide resolution. Therefore, the resolution is low.

Graphene nanopores [9]

Graphene is a unique and very special material, and yet widely available. Everyone has graphene at home: graphite is made of layers of graphene and occurs in for example the carbon of pencils, charcoal, or candle soot. But in this research, graphene is used because of that special property that one can make single-atom-thin monolayers of graphene.

Graphene is a one atom thick sheet of C-atoms, arranged in a honey comb [hexagonal] lattice. Its thickness is about 0.3nm. It is the thinnest, lightest, strongest, best heat and electricity conducting material ever discovered and is very flexible. It is continuous and exhibit high crystal quality. It was considered as a 'material that could not exist' since it was isolated for the first time in 2004.

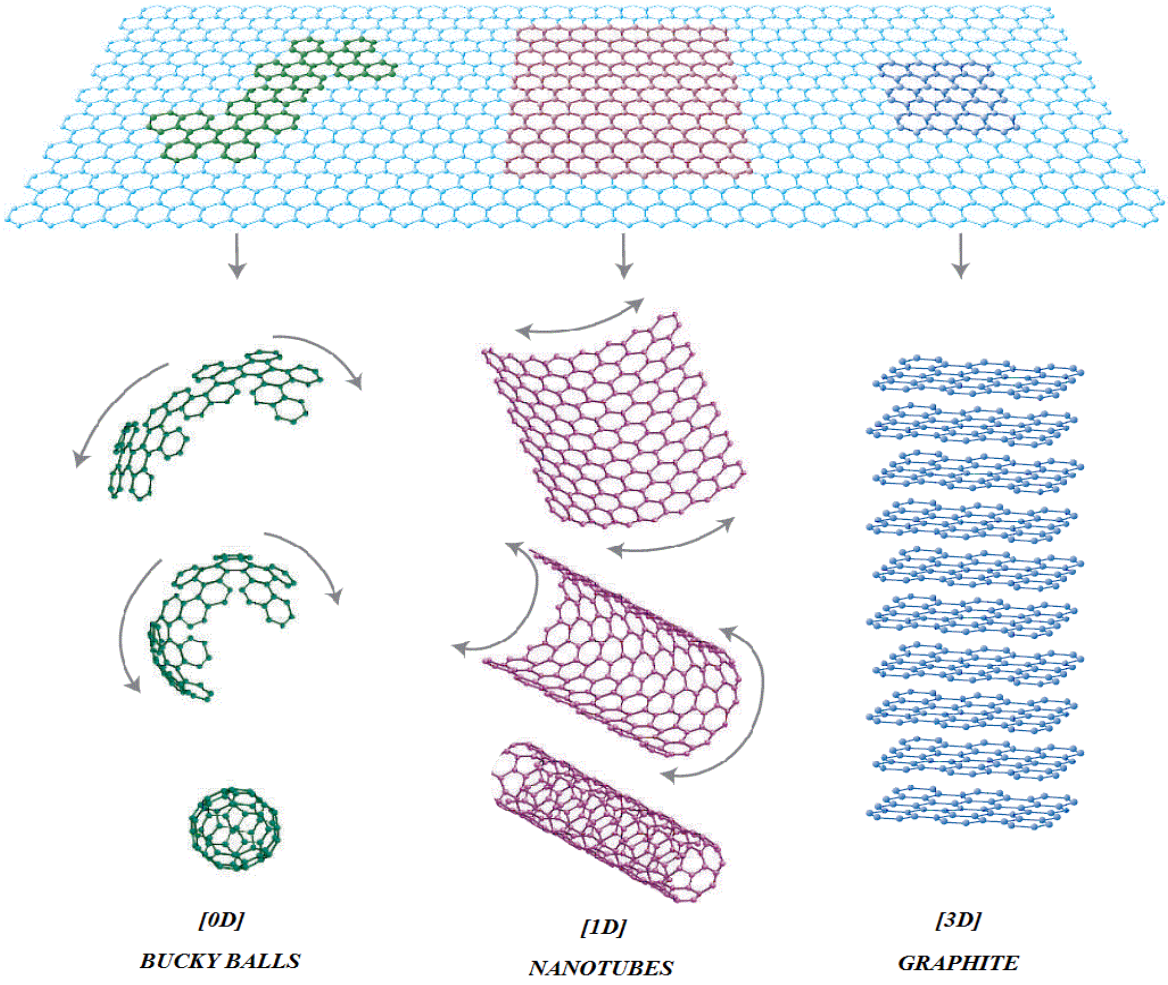


GRAPHENE MONOLAYER

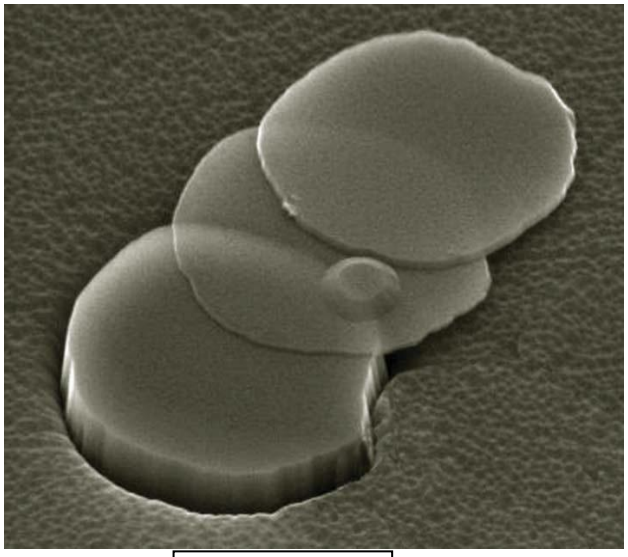
It is a great electrical conductor: Each atom is covalently bonded to three others; but since carbon has four valence electrons, one is left free – allowing graphene to conduct electricity. Electrons are able to flow as fast as 1/100th of the speed of light in vacuum. Graphene is transparent, cheap and plentiful. It is harder than diamond & 300 times harder than steel. It is stretchable up to 20% of its initial length.

Graphene is the mother of all other well-known graphitic forms of carbon [9]:

- graphite is a stack of graphene layers
- carbon nanotubes are rolled-up cylinders of graphene
- Buckminsterfullerenes (bucky balls) are molecules consisting of graphene balled into a sphere.



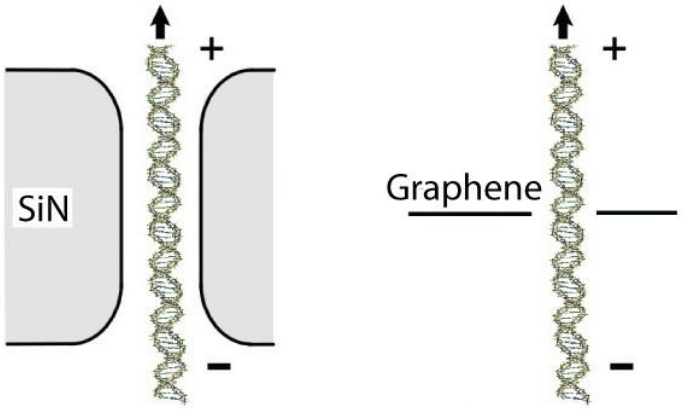
Basically there are two different approaches to preparing graphene. On the one hand graphene can be detached from an already existing graphite crystal, the so-called exfoliation methods; on the other hand the graphene layer can be grown directly on a substrate surface. The direct liquid-phase exfoliation of graphite to produce graphene is a convenient method for generating ideal graphene samples in large quantities.



GRAPHITE

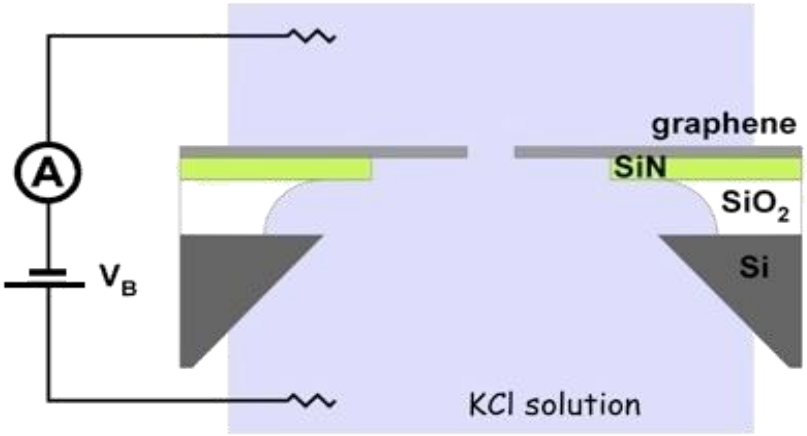
GRAPHENE LAYERS

Single layer of graphene is only 0.3nm thick, smaller than the distance between 2 DNA bases. When a DNA strand passes through it, a single nucleotide can block the pore at any particular moment. This makes graphene nanopore a promising device for DNA sequencing [6]. The study suggests that the method could identify about 66 billion bases (the smallest units of genetic information) per second with 99% accuracy and no false positives. Conventional sequencing involves separating, copying, labeling and reassembling pieces of DNA to read the genetic information.



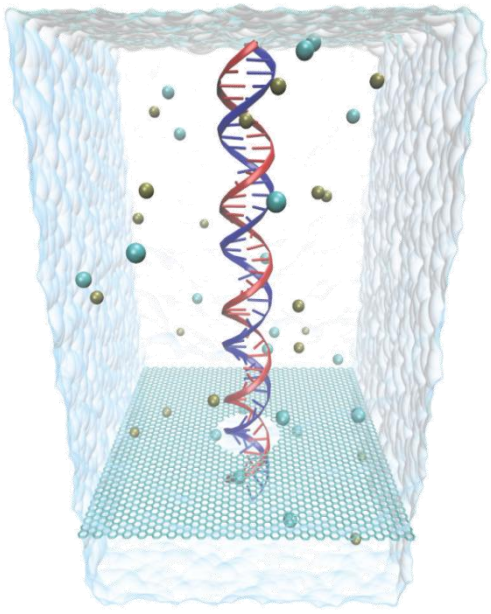
The pores are obtained by placing a graphene sheet over a 5µm sized hole in a SiN membrane and drilling a nanosized [about 5nm] hole in the graphene using highly focused electron beam of a Transmission Electron Microscope [TEM]. Then added a layer of Titanium oxide to the graphene membrane to make the hydrophobic graphene more wettable that allows the DNA to go through it more easily. Although graphene-only nanopores can be used for

translocating DNA, coating the graphene membranes with a layer of oxide consistently reduced the nanopore noise level and at the same time improved the robustness of the device.



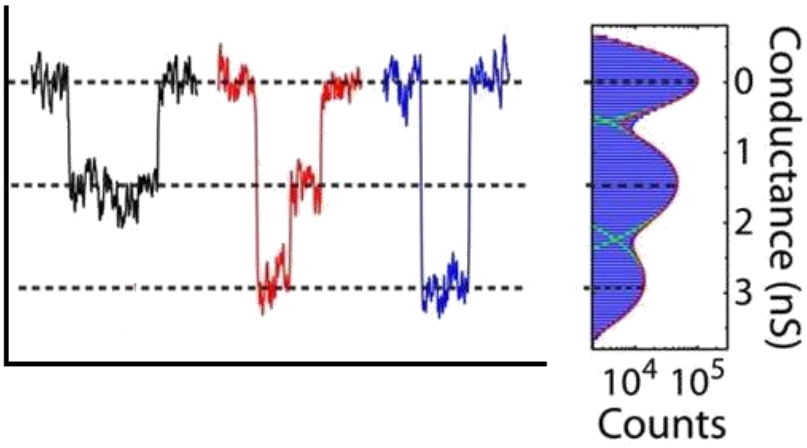
MICROFLUIDIC CELL FOR SEQUENCING

The layer is mounted into a microfluidic flow cell, added a 1M saline solution [1M KCl, pH-8.0] on both sides of the membrane. A voltage is applied across the membrane for which the current from ion transport through pore is measured. Single or double stranded DNA was driven electrophoretically through the nanopore as a long string.



DNA PASSING THROUGH GRAPHENE NANOPORE

Each nucleotide may obstruct the nanopore to a different characteristic degree and the amount of the current passing through the pore varies depending on the type of nucleotide. Each temporary drop in measured conductance arises from a single nucleotide that translocate through the pore.



In case of DNA double strands, A-T & G-C base pairs stretch to a different degree for a particular voltage because of their difference in number of H-bonding. Therefore the drop in conductance varies from each other.

Because of the ultrathin nature of the graphene pores, researchers were able to detect an increase in the magnitude of the translocation signals relative to previous solid state nanopores made in silicon nitride, for similar applied voltages.

Advantages of graphene as nanopore

- Excellent conductivity.
- Chemically inert & stable in all operational environments.
- Low thickness.
- Cheap.
- Fast sequencing, i.e, about 10 entire genome/day.
- Direct sequencing without the need of an intervening PCR amplification or a chemical labeling step or the need for optical instrumentation [lasers] to identify the chemical label.

CONCLUSION

The nanopore in graphene is the first nanopore thin enough to distinguish between two closely neighboring nucleobases [13]. This low-cost, ultra-fast & accurate DNA sequencing could revolutionize both healthcare and biomedical research, and lead to major advances in drug development, preventative medicine and personalized medicine. Several challenges still remain to be overcome including controlling the speed with which DNA threads through the pore. When

achieved, nanopore sequencing could lead to very inexpensive and rapid DNA sequencing and has potential to advance personalized health care. DNA sequencing could get a lot faster and cheaper and thus closer to routine use in clinical diagnostics.

REFERENCES

1. Eugene Paulechka, Tsjerk A Wassenaar, Kenneth Kroenlein, Andrei Kazakov, Alex Smolyanitsky, Nucleobase-functionalized graphene nanoribbons for accurate high-speed DNA sequencing. Nanoscale, 2016, **8**, 1861-1867.
2. Stephanie J Heerema, Cees Dekker, Graphene nanodevices for DNA sequencing. Nature Nanotechnology, 2016, 11,127–136.
3. Grégory F Schneider, Stefan W Kowalczyk , Victor E Calado , Grégory Pandraud , Henny W Zandbergen, Lieven M K Vandersypen, Cees Dekker, DNA Translocation through Graphene Nanopores**.** *Nano Letters*, 2010; 10(8):3163-7.
4. Chaitanya Sathe, Xueqing Zou, Jean-Pierre Leburton, Klaus Schulten, Computational Investigation of DNA Detection Using Graphene Nanopores. *ACS Nano*, 2011, 5 (11), 8842–8851.
5. Christopher A Merchant, Ken Healy, Meni Wanunu, Vishva Ray, Neil Peterman, John Bartel, Michael D Fischbein, Kimberly Venta, Zhengtang Luo, A T Charlie Johnson, Marija Drndi, DNA Translocation through Graphene Nanopores**.** *Nano Letters*, 2010, 10 (8), 2915–2921.
6. S Garaj, W Hubbard, A Reina, J Kong, D Branton, J A Golovchenko, Graphene as a subnanometre trans-electrode membrane**.** *Nature*, 2010; 467 (7312): 190-193.
7. Ben McNally, Alon Singer, Zhiliang Yu, Yingjie Sun, Zhiping Weng, Amit Meller, Optical Recognition of Converted DNA Nucleotides for Single-Molecule DNA Sequencing Using Nanopore Arrays**.** *Nano Letters*, 2010, 10(6): 2237–2244.
8. http://www.technologyreview.in/blog/arxiv/25252/
9. http://en.wikipedia.org/wiki/Nanopore_sequencing
10. http://users.ugent.be/~avierstr/principles/seq.html
11. http://www.edinformatics.com/interactive_molecules/graphene_molecule.htm
12. http://www.dnasequencing.org/
13. http://www.sciencedaily.com/
14. www.genomenewsnetwork.org/resources/whats_a_genome/http://www.scientistlive.com/European-Science-News/Genetics/Graphene_used_to_decode_DNA__/24968/.