Solid-state Nanopore: A Nanoelectronic Sensor for Single-Molecule Diagnostics

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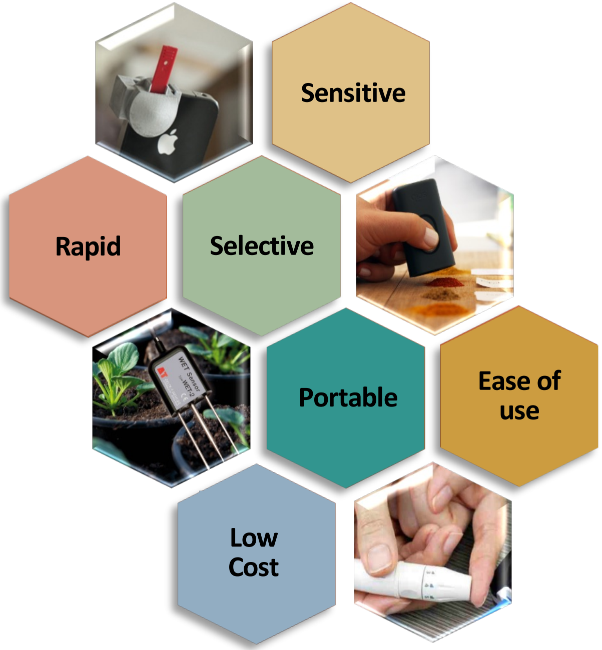
Research School of Physics
Australian National University

Development of Sensing Platforms

Pharmaceutical quality assurance

Water and soil quality


Sensitive

Rapid

Selective

Portable

Ease of use

Low Cost

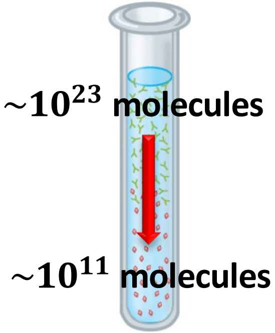
Disease diagnosis

Food quality

Nutriphone
SCIO

delta-t devices
www.thehealthsite.com

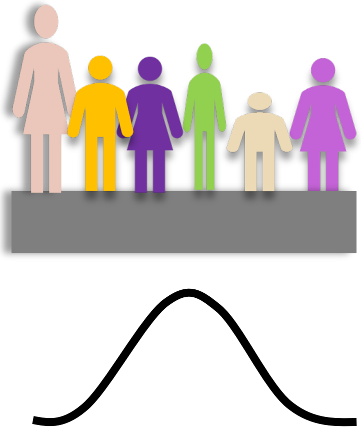
Single-Molecule Sensing



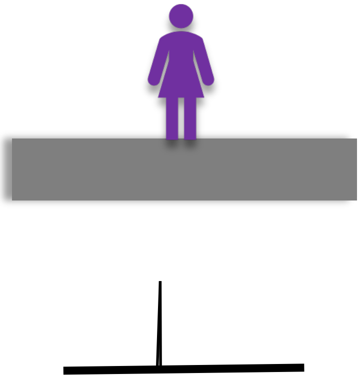
$\sim 10^{23}$ molecules

$\sim 10^{11}$ molecules

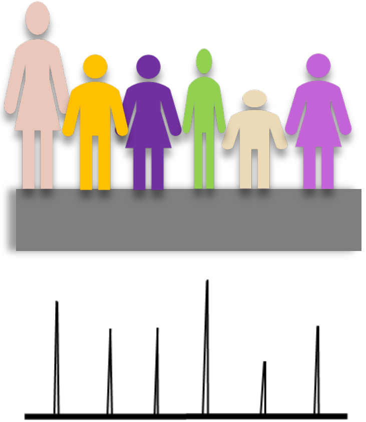
1 molecule



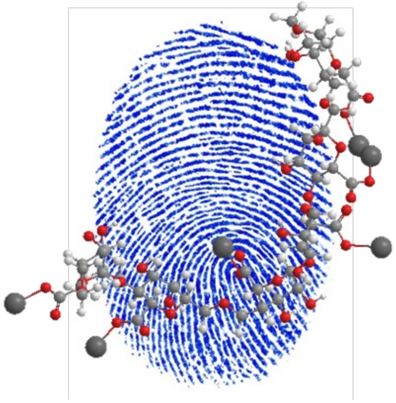
Conventional Sensing
Many people one signal



Single Molecule Detector
One person-one signal



Single Molecule Sensor
Many people-many single molecule signals



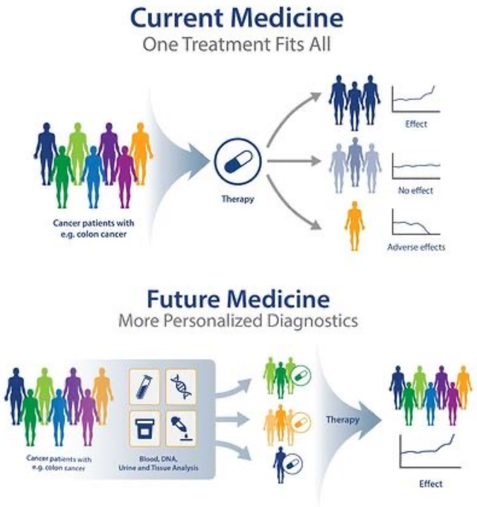
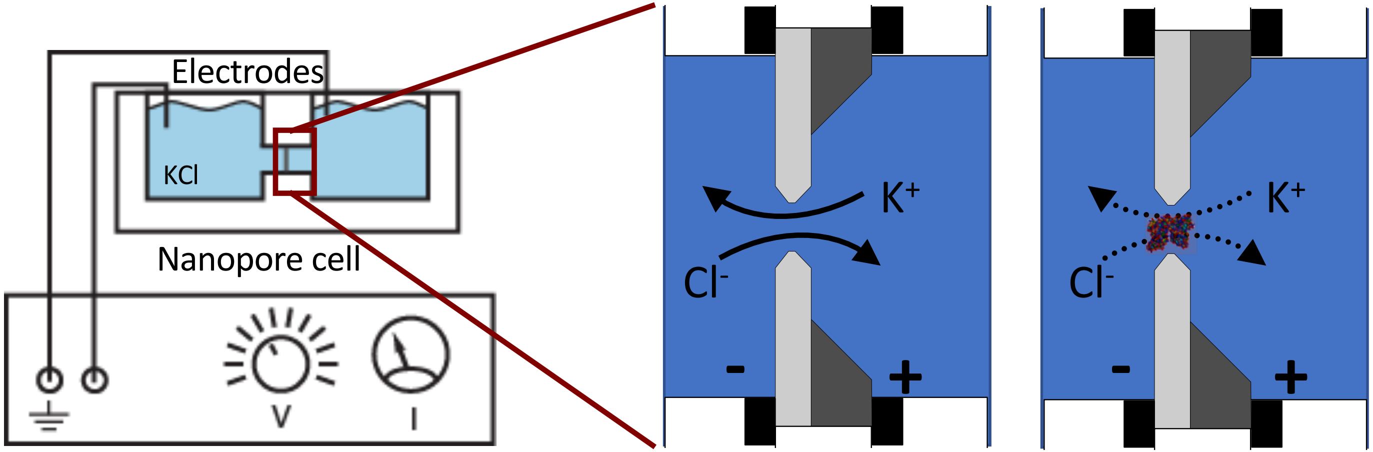
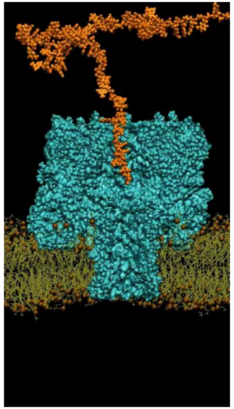

Current Medicine
One Treatment Fits All
Cancer patients with e.g. colon cancer
Therapy
Effect
No effect
Adverse effects
Future Medicine
More Personalized Diagnostics
Cancer patients with e.g. colon cancer
Blood, DNA, Urine and Tissue Analysis
Therapy
Effect

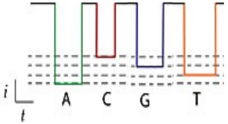
Image Source: https://courses.lumenlearning.com
Image Source: https://blog.crownbio.com/pdx-personalized-medicine

Nanopore Operation and Detection

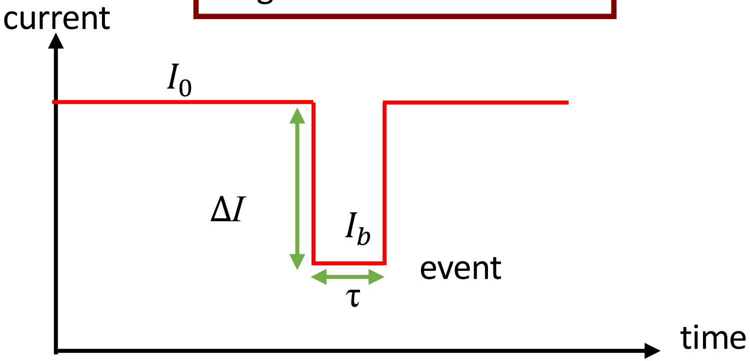

Electrodes
KCl
Nanopore cell
V
I
K$^+$
Cl$^-$
-
+
K$^+$
Cl$^-$
-
+

Patch clamp amplifier

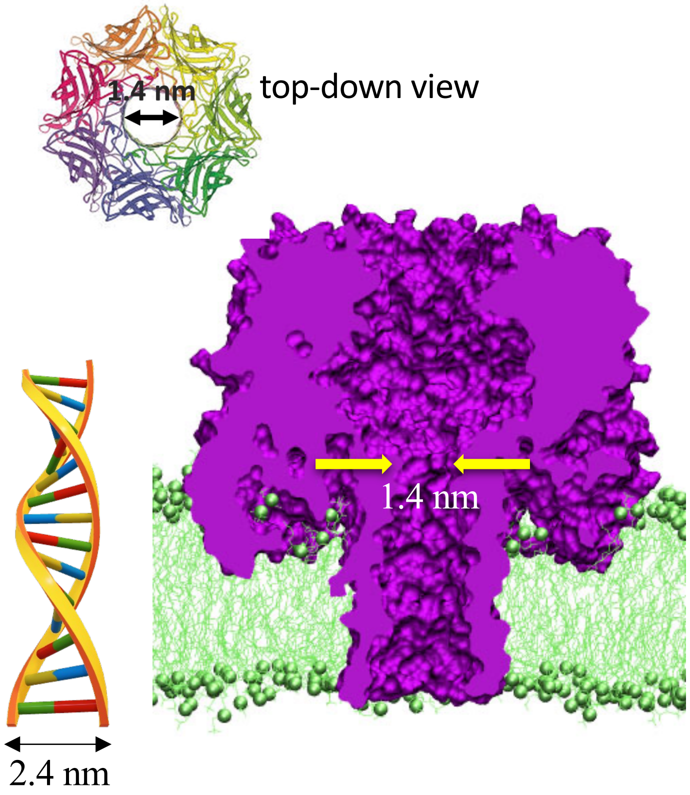




single molecule detection



Why Solid-state Nanopores?


1.4 nm
top-down view
1.4 nm
2.4 nm

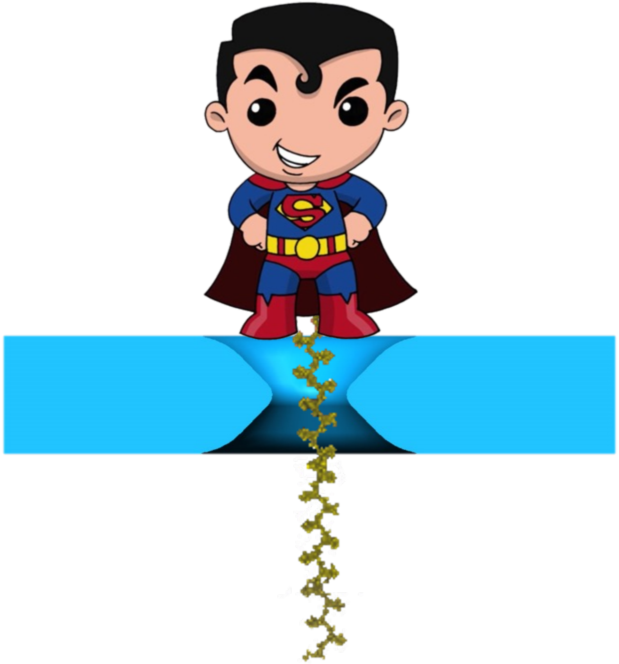
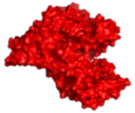


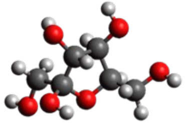
Figure Ref: crystallography365.wordpress.com
Figure Ref: www.ks.uiuc.edu

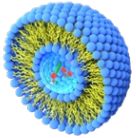
A Nanoelectronic Sensor Going Big

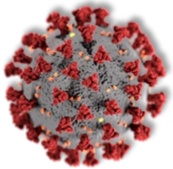

protein

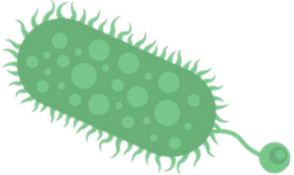
antibody

DNA/RNA


metabolites

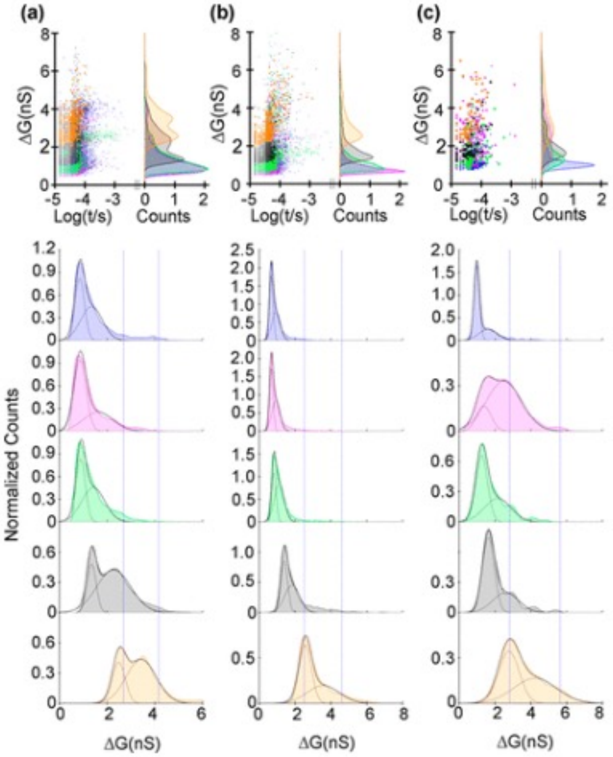

liposomes


virus


bacteria

- structural characterization/sequencing
- discrimination
- charge characterization
- folding-unfolding electrokinetics
- pH response
- electrodeformation
- bond strengths

Protein unfolding

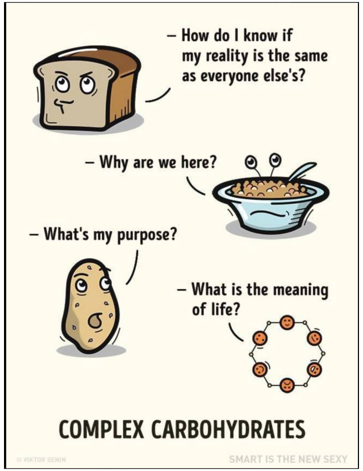


Voltage increase

ACS Nano 2019, 13, 4, 4246–4254

CRICOS PROVIDER #00120C

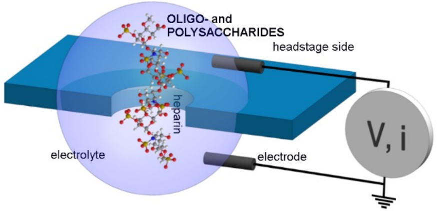
Heparin Contamination

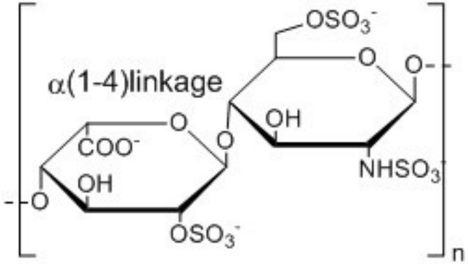

– How do I know if my reality is the same as everyone else's?
– Why are we here?
– What's my purpose?
– What is the meaning of life?
COMPLEX CARBOHYDRATES
© VIKTOR SENIN
SMART IS THE NEW SEXY

NOT for LOCK FLUSH
HEPARIN
5,000 USP units/mL
NOT for LOCK FLUSH
HEPARIN
Sodium Injection, USP
5,000 USP Units/mL
FOR IV OR SC USE
Heparin Sodium Inj., USP

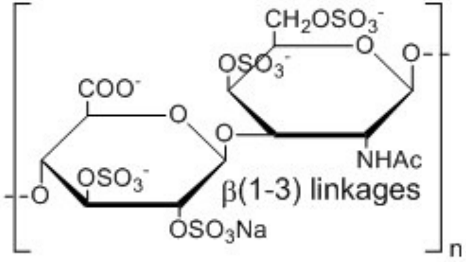
Heparin Picture Ref: www.101nursing.com/

Anticoagulant
drug


OLIGO- and POLYSACCHARIDES
headstage side
heparin
electrolyte
electrode
V, i

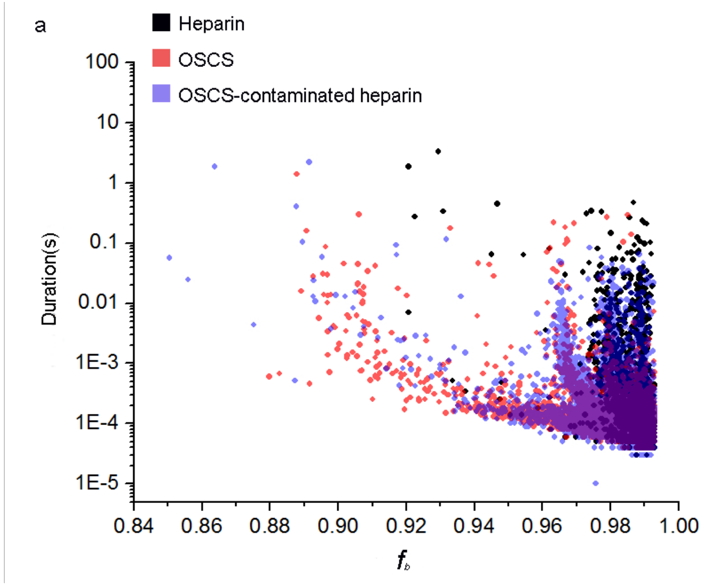

α(1-4)linkage
COO⁻
OH
OSO3⁻
OSO3⁻
OH
NHSO3⁻
n

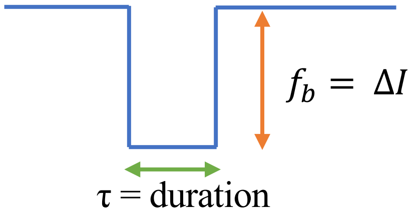
Heparin
polymer of 2-O-sulfo-iduronic acid and 6-O-sulfo-N-sulfo-glucosamine


CH2OSO3⁻
OSO3⁻
COO⁻
OSO3⁻
NHAc
β(1-3) linkages
OSO3Na
n

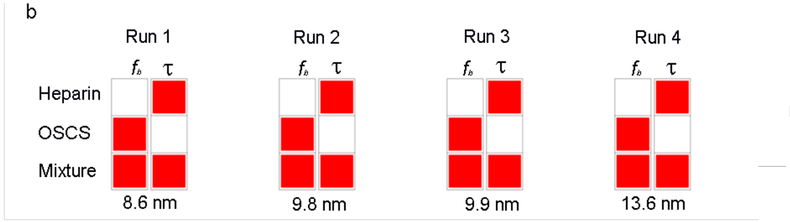
Oversulfated Chondroitin Sulfate
polymer of D-glucuronic acid and N-Acetyl-galactosamine-4 or 6-sulfate

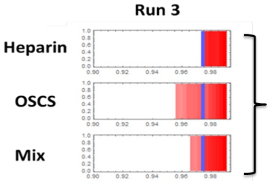
OSCS Contaminant Detection in Heparin



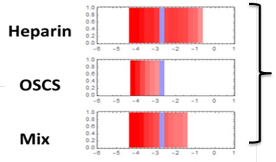


https://ahdc.vet.cornell.edu/sects/coag/test/heparin.cfm





$$f_b \lesssim \mathrm{mode}\left(f_{b,\mathrm{USP\ heparin}}^{\mathrm{binned}}\right) - 3\sigma\left(f_{b,\mathrm{USP\ heparin}}^{\mathrm{binned}}\right)$$

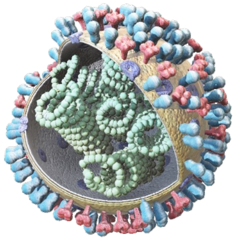


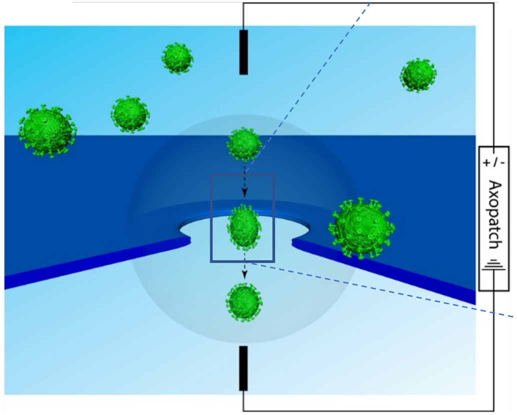
$$\tau \gtrsim \mathrm{mode}\left(\left(\log_{10}\tau_{\mathrm{USP\ OSCS}}\right)^{\mathrm{binned}}\right) - 3\sigma\left(\left(\log_{10}\tau_{\mathrm{USP\ OSCS}}\right)^{\mathrm{binned}}\right)$$

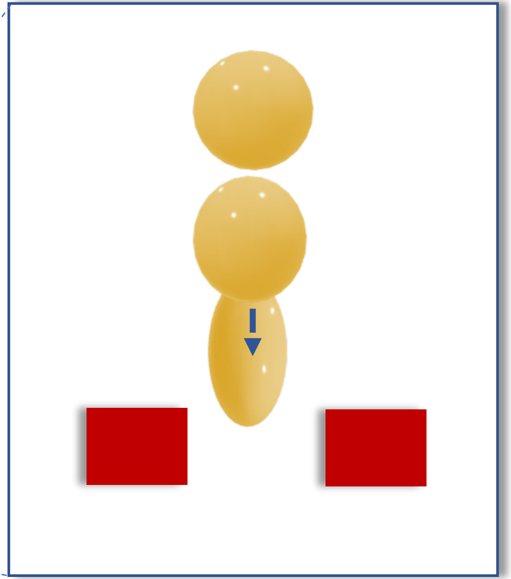
Nature Communications, volume 9, 3278 (2018)

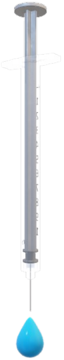
Someone sneezes in 2019:
“Bless you”
Someone sneezes in 2020:
IndyaSpeak

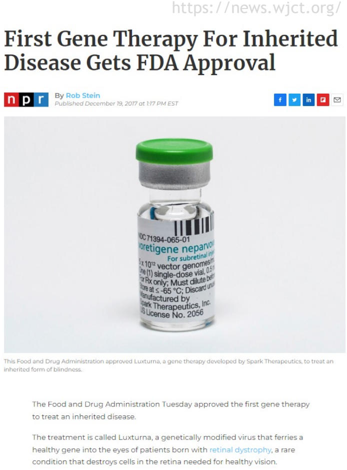
Discriminating Virus with Different Therapeutic Cargo










NDC 71394-065-01
voretigene neparvo
For subretinal
vector genomes
single-dose vial
Rx only; Must dilute
Store at ≤ -65 °C; Discard
Manufactured by
Spark Therapeutics, Inc.
US License No. 2056

https://news.wjct.org/

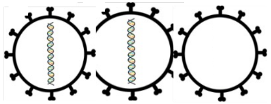
First Gene Therapy For Inherited Disease Gets FDA Approval

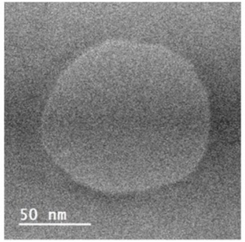
npr By Rob Stein
Published December 19, 2017 at 1:17 PM EST

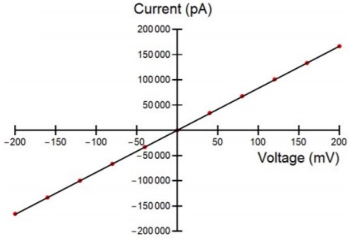
This Food and Drug Administration approved Luxturna, a gene therapy developed by Spark Therapeutics, to treat an inherited form of blindness.

The Food and Drug Administration Tuesday approved the first gene therapy to treat an inherited disease.

The treatment is called Luxturna, a genetically modified virus that ferries a healthy gene into the eyes of patients born with retinal dystrophy, a rare condition that destroys cells in the retina needed for healthy vision.

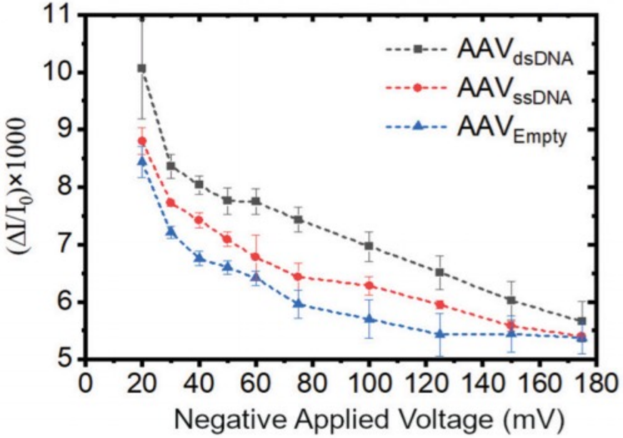


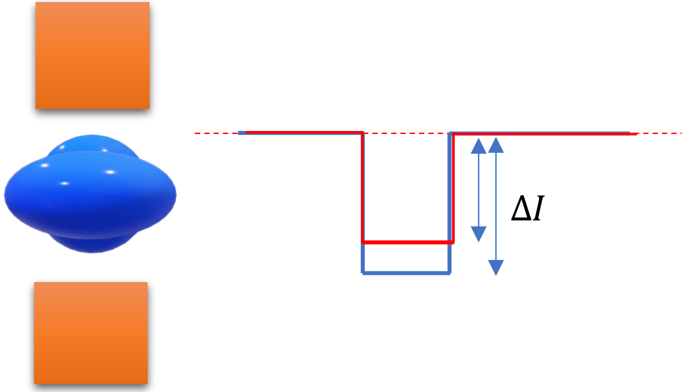


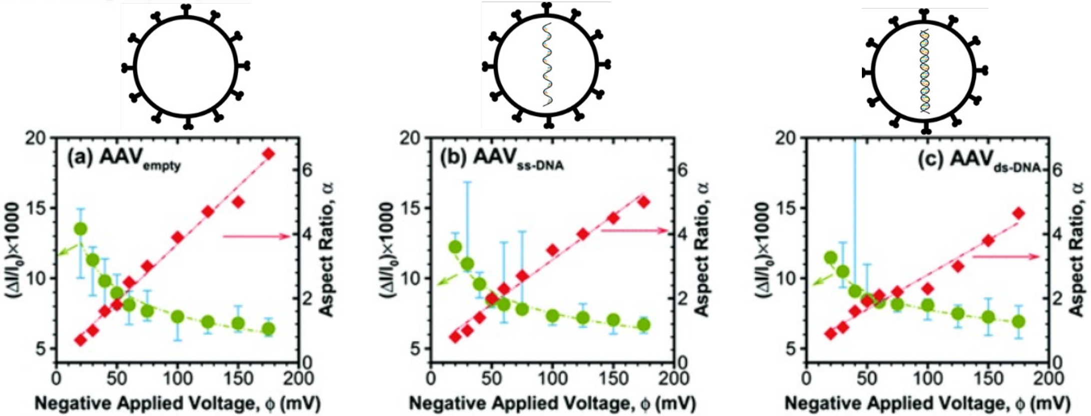


Nanoscale, 2020, 12, 23721-23731

Characterising Virus with Different Genetic Cargo

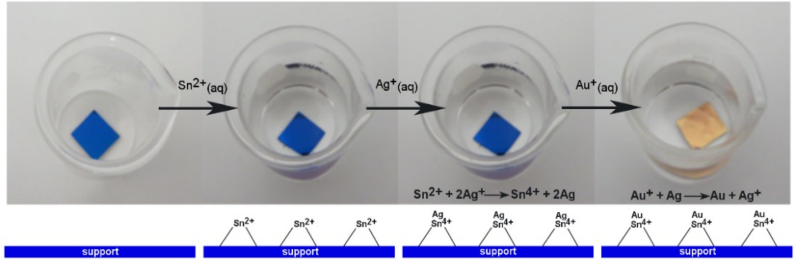


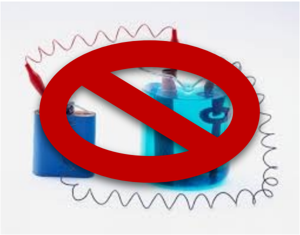


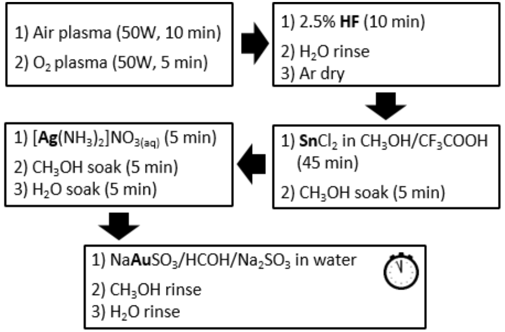


Nanoscale, 2020, 12, 23721-23731

Surface Modification by Electroless Gold Plating


$Sn^{2+}_{(aq)}$
$Ag^{+}_{(aq)}$
$Au^{+}_{(aq)}$
$Sn^{2+} + 2Ag^{+} \rightarrow Sn^{4+} + 2Ag$
$Au^{+} + Ag \rightarrow Au + Ag^{+}$
Sn^{2+}
Ag
Sn^{4+}
Au
Sn^{4+}
support
support
support
support





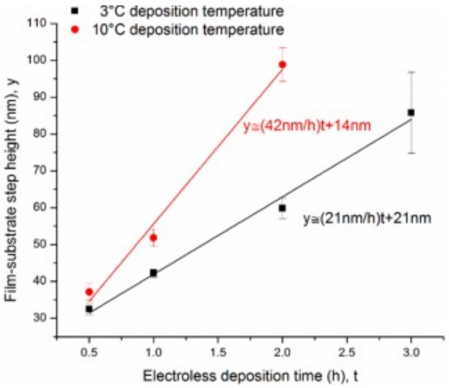
1) Air plasma (50W, 10 min)
2) O_2 plasma (50W, 5 min)

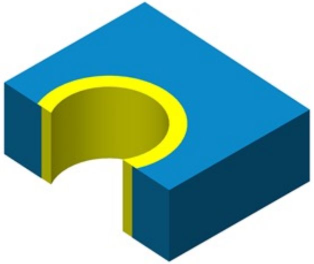
1) 2.5% **HF** (10 min)
2) H_2O rinse
3) Ar dry

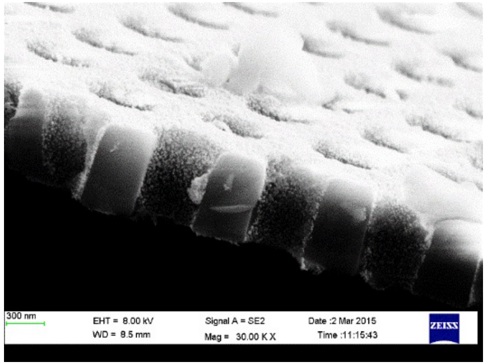
1) **Sn**Cl_2 in CH_3OH/CF_3COOH (45 min)
2) CH_3OH soak (5 min)

1) [**Ag**$(NH_3)_2$]$NO_{3(aq)}$ (5 min)
2) CH_3OH soak (5 min)
3) H_2O soak (5 min)

1) Na**Au**$SO_3/HCOH/Na_2SO_3$ in water
2) CH_3OH rinse
3) H_2O rinse





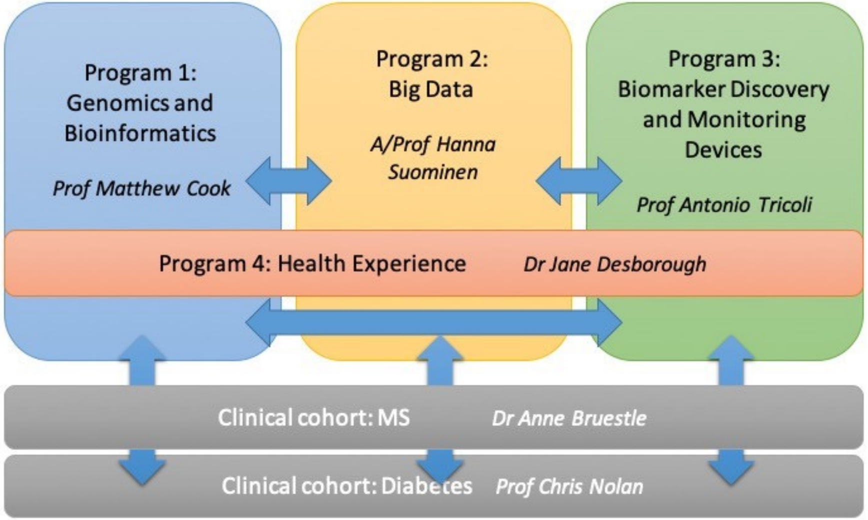

300 nm
EHT = 8.00 kV
WD = 8.5 mm
Signal A = SE2
Mag = 30.00 K X
Date :2 Mar 2015
Time :11:15:43
ZEISS

ACS Appl. Mater. Interfaces 2014, 6, 10952–10957

OHIOH Grand Challenge

MULTIPLE SCLEROSIS

PANCREAS
CELLS
RESISTANCE
METABOLISM
GLUCOSE
INSULIN
STAGES
ISLETS
INJECT
SENSITIVITY
MONITOR
WEIGHT
CONGENITAL
NERVE
ENDOCRINE
ACUTE
WELL
DIABETES
TYPE
HEALTHCARE
SYMPTOMS
HYPERGLYCEMIA
KETOACIDOSIS
SUGAR
RESPOND
COMPLICATIONS
INSULIN
PANCREAS
MELLITUS
INJECT
ADULTS



Program 1:
Genomics and Bioinformatics

Prof Matthew Cook

Program 2:
Big Data

A/Prof Hanna Suominen

Program 3:
Biomarker Discovery and Monitoring Devices

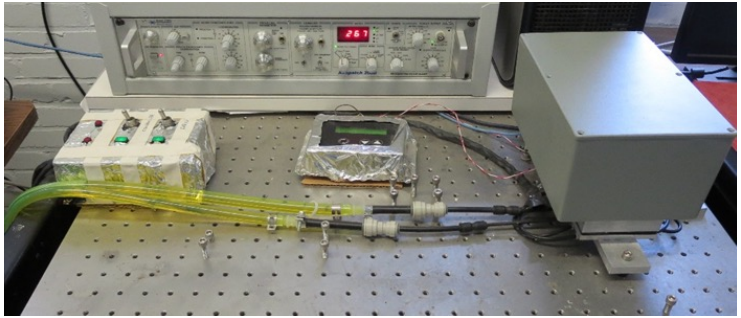
Prof Antonio Tricoli

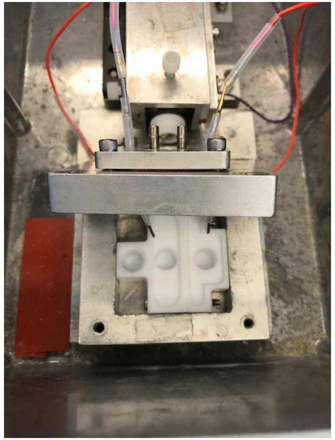
Program 4: Health Experience *Dr Jane Desborough*

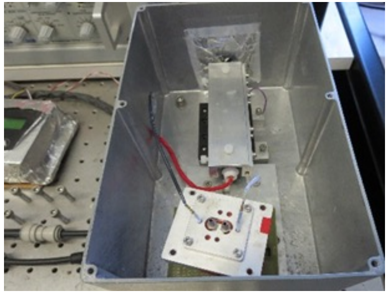
Clinical cohort: MS *Dr Anne Bruestle*

Clinical cohort: Diabetes *Prof Chris Nolan*

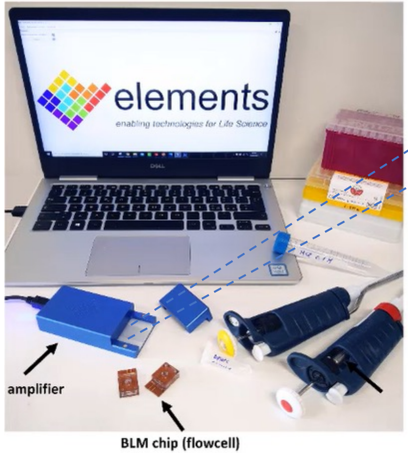
Nanopore Instrumentation



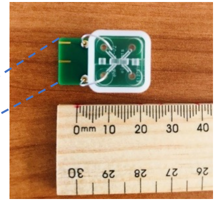




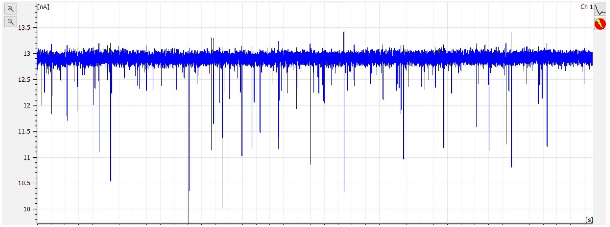
- table-top instruments
- not portable


elements
enabling technologies for Life Science
amplifier
BLM chip (flowcell)

Flow cell


0mm 10 20 30 40

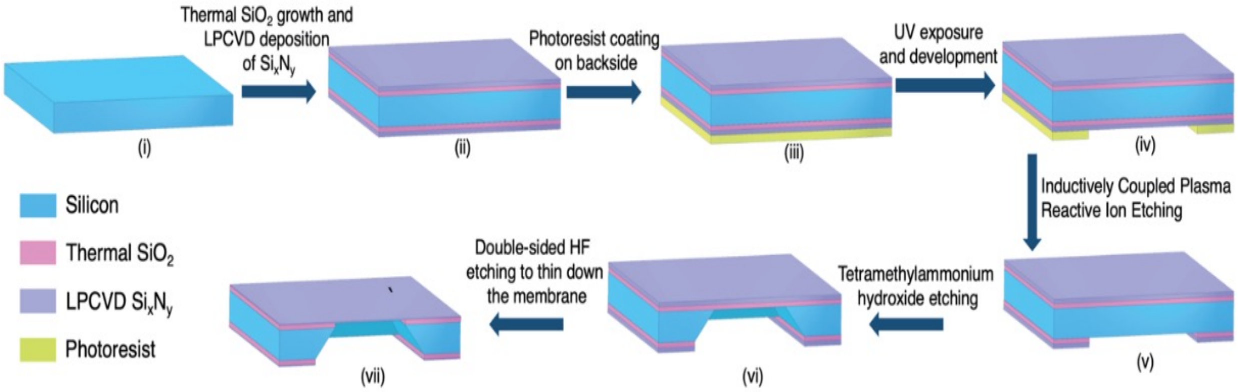
- miniaturized
- portable

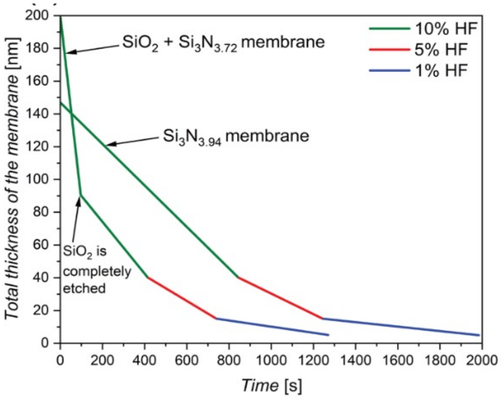


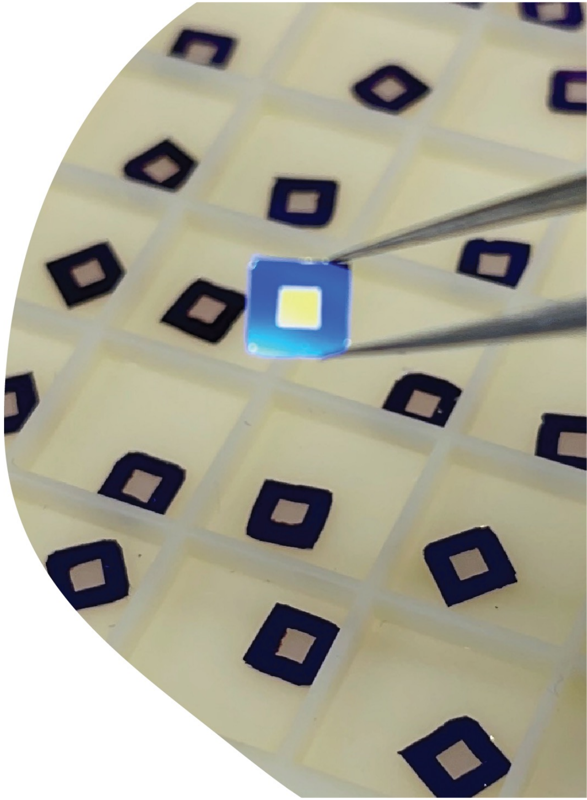
Pictures: Dwyer Lab, URI

https://elements-ic.com/nanopore-reader/

Thin Nanopore Membranes

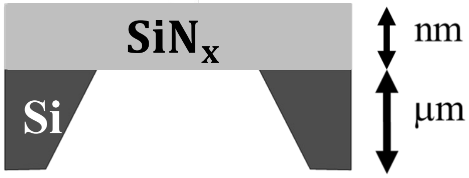

Thermal SiO_2 growth and LPCVD deposition of Si_xN_y
Photoresist coating on backside
UV exposure and development
Inductively Coupled Plasma Reactive Ion Etching
Tetramethylammonium hydroxide etching
Double-sided HF etching to thin down the membrane
(i)
(ii)
(iii)
(iv)
(v)
(vi)
(vii)
Silicon
Thermal SiO_2
LPCVD Si_xN_y
Photoresist

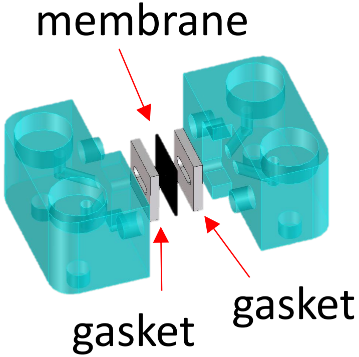


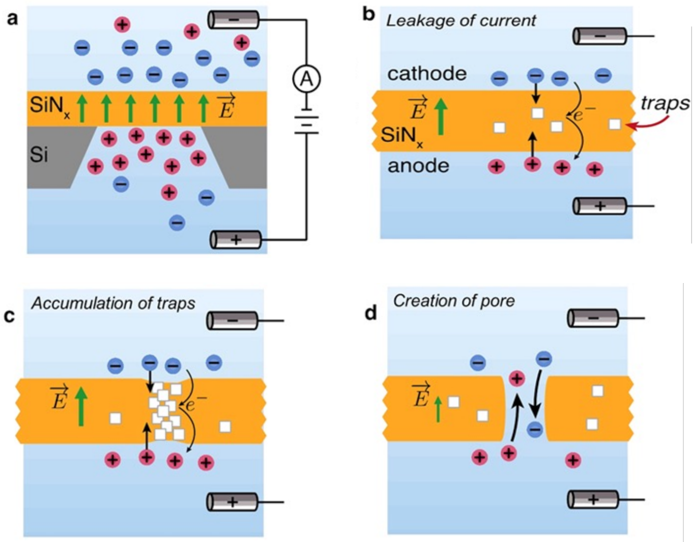


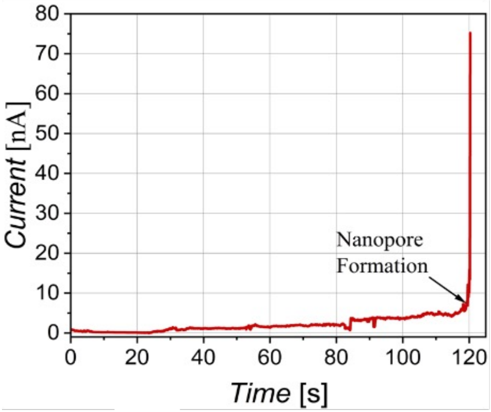
arXiv:2201.09024

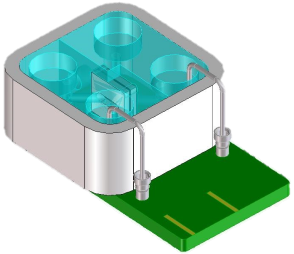
Silicon Nitride Nanopore Fabrication


SiN_x
Si
nm
µm


membrane
gasket
gasket

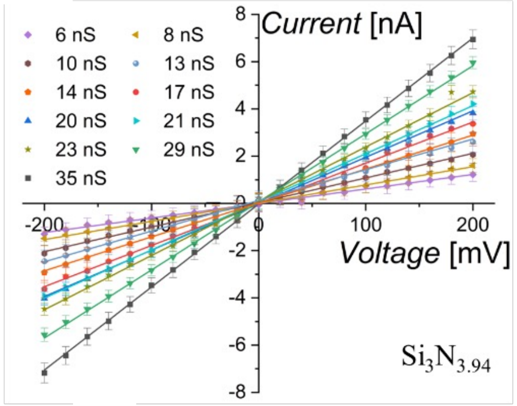

a
SiN$_x$
$\vec{E}$
Si
A
b
Leakage of current
cathode
$\vec{E}$
SiN$_x$
e^-
traps
anode
c
Accumulation of traps
$\vec{E}$
e^-
d
Creation of pore
$\vec{E}$

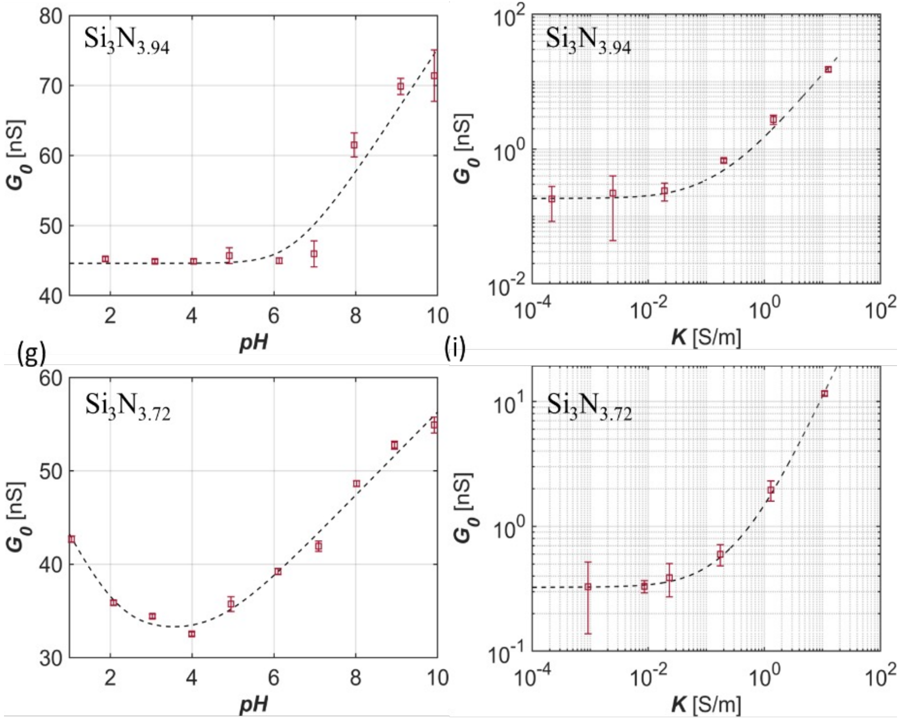




V.T Cossa et al, PLOS ONE, March 2014, 9, 3, e92880
Nanotechnology 31 (33), 335707

Sensor Surface





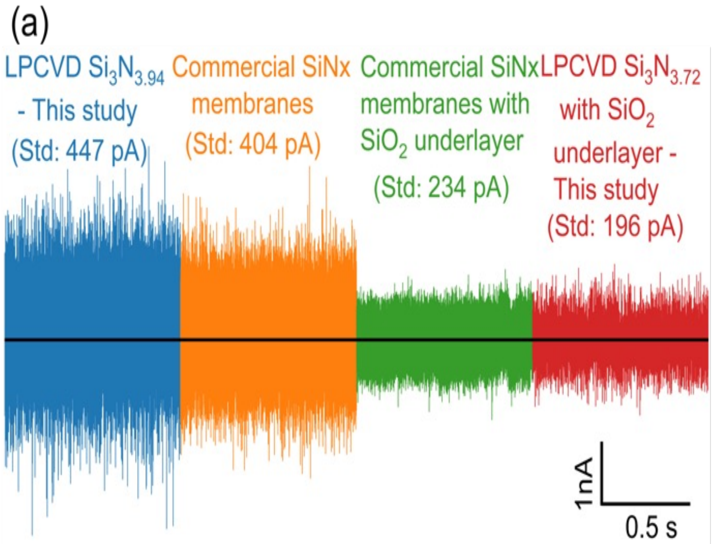
$$\mathrm{G}=\frac{I}{V}=\left(\frac{1}{G_{\mathrm{bulk}}+G_{\mathrm{surface}}}+\frac{1}{G_{\mathrm{access}}}\right)^{-1}$$

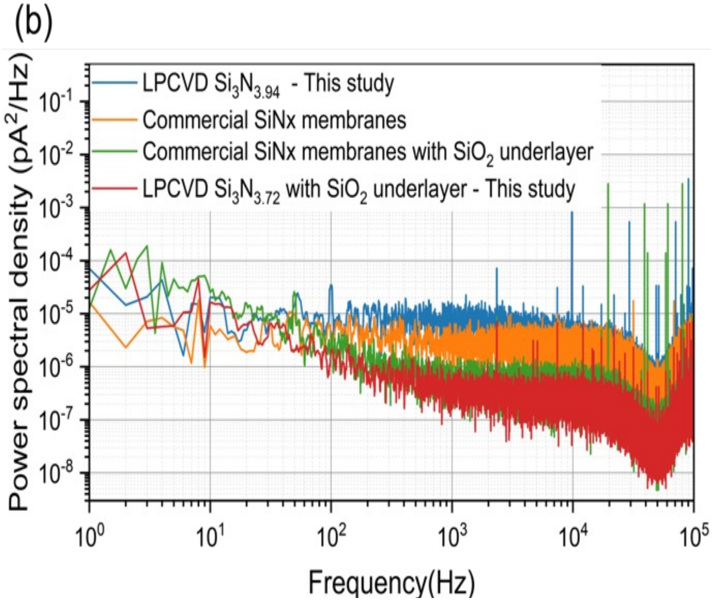
$$G=K\left(\frac{1}{\frac{\pi r_0^2}{L}+\frac{\mu|\sigma|}{K}\cdot\frac{2\pi r_0}{L}}+\frac{2}{\alpha\cdot 2r_0+\beta\cdot\frac{\mu|\sigma|}{K}}\right)^{-1}$$

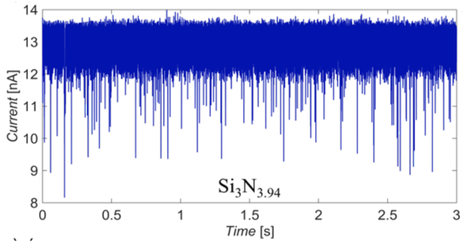
$$|\sigma|\cong\frac{C_{\mathrm{eff}}}{\beta e}W\left(\frac{\beta e}{C_{\mathrm{eff}}}\exp\big((pH-pK_a)\ln(10)+\ln(e\Gamma)\big)\right)$$

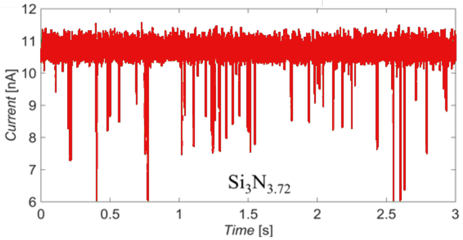
arXiv:2201.09024

Noise Levels of Nanopore Sensors



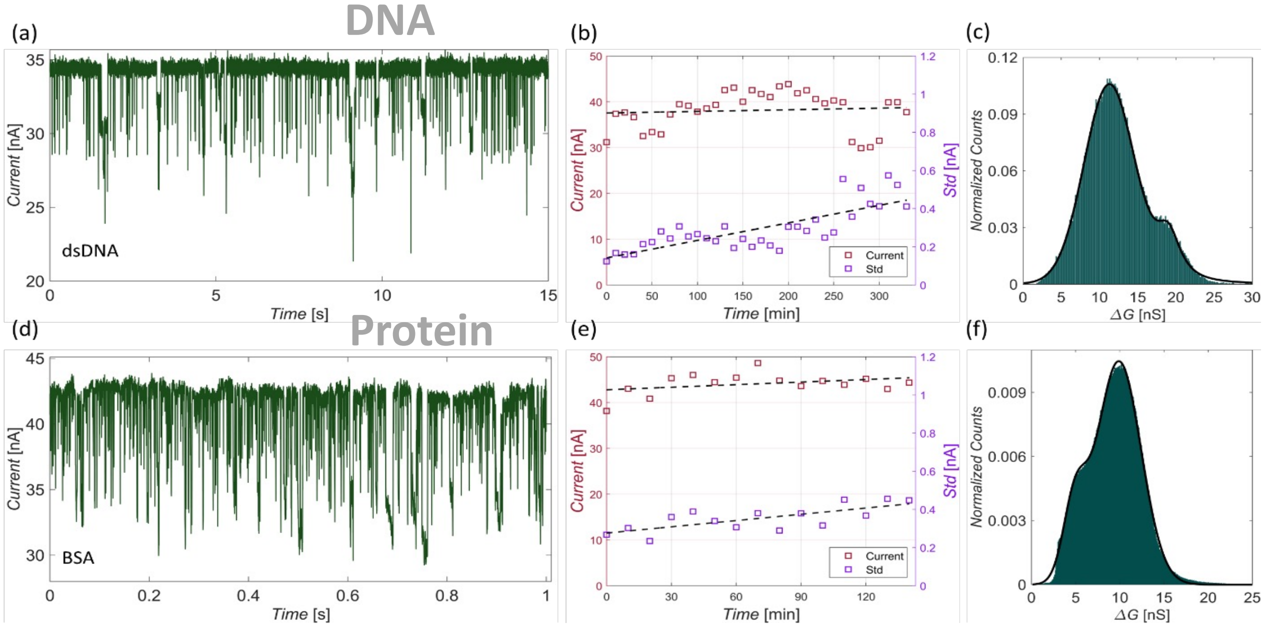






arXiv:2201.09024

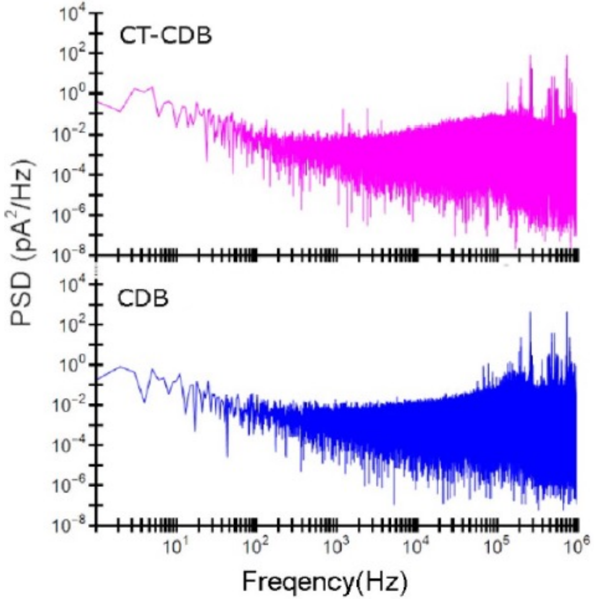
Over Million Events

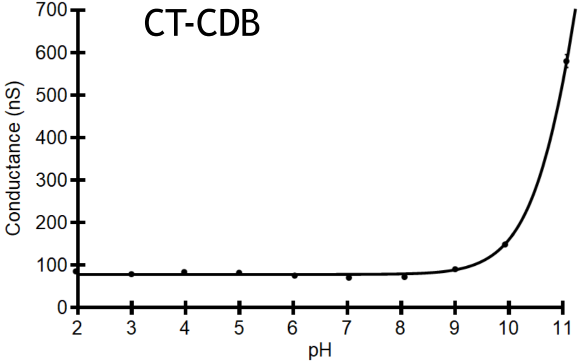


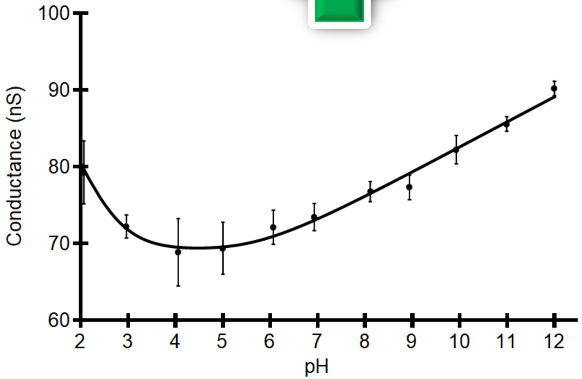
1 MILLION DOLLARS
I MEANT EVENTS..EVENTS
imgflip.com

CT-CDB and beyond

2:9 bleach:1 M KCl (with ~10 mM HEPES at pH ~7)





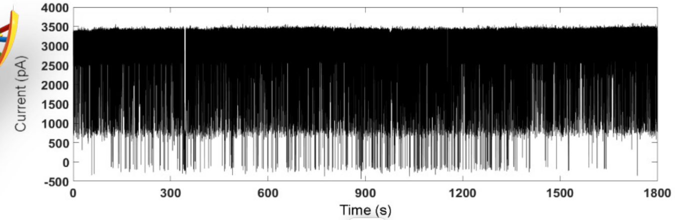


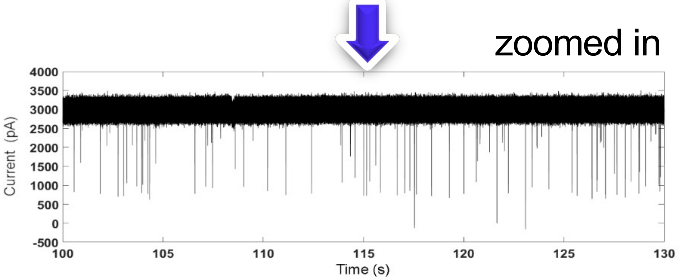
nanopore surface chemistry seems to be drastically different

Nanotechnology, 2020, 31, 33

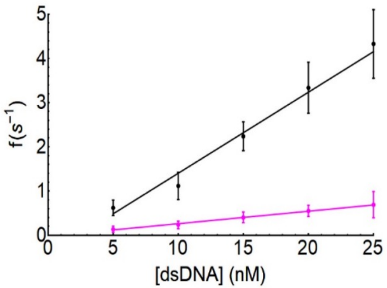
CT-CDB and beyond

DNA



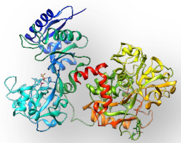


zoomed in



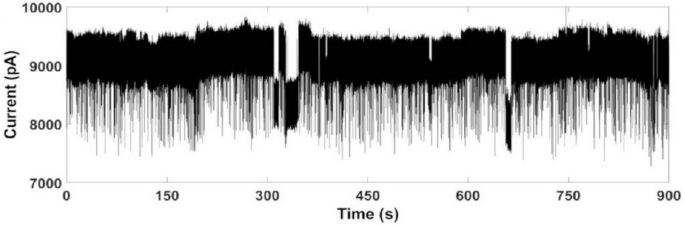
CT-CDB

CDB

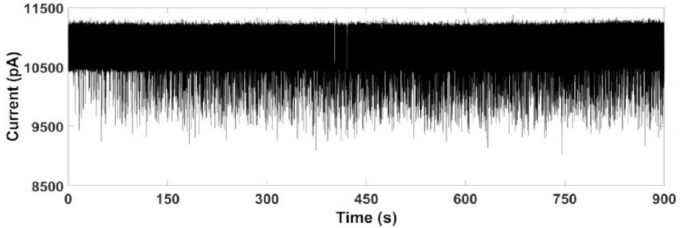


hSTf
Protein

CDB



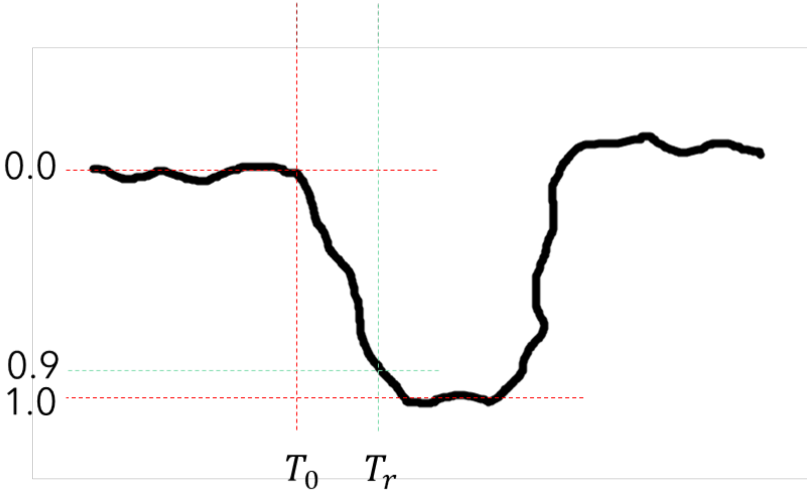
CT-CDB



- Stable baseline over a long time
- Higher frequency in DNA events
- Minimum pore clogging
- Self-correcting ability

Nanotechnology, 2020, 31, 33

High Bandwidth Studies



Attenuated events

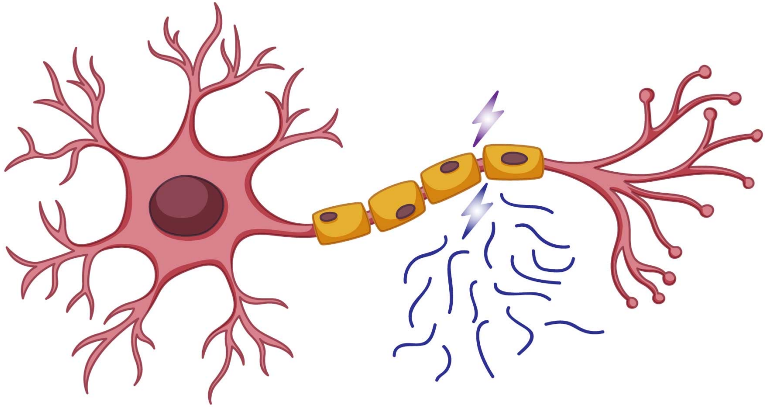
$$T_r = 0.35/BW$$

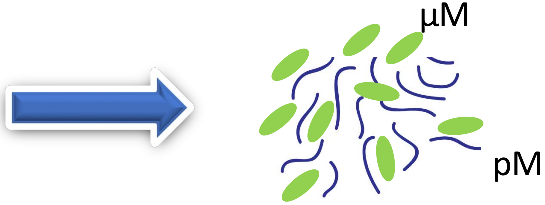
$$event\ duration < \ 2T_r$$

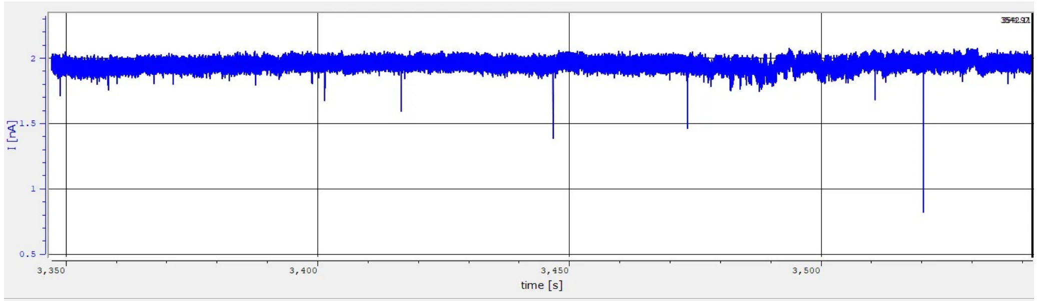
100 kHz

10 MHz

Neurofilament Sensing: Semi-complex Samples







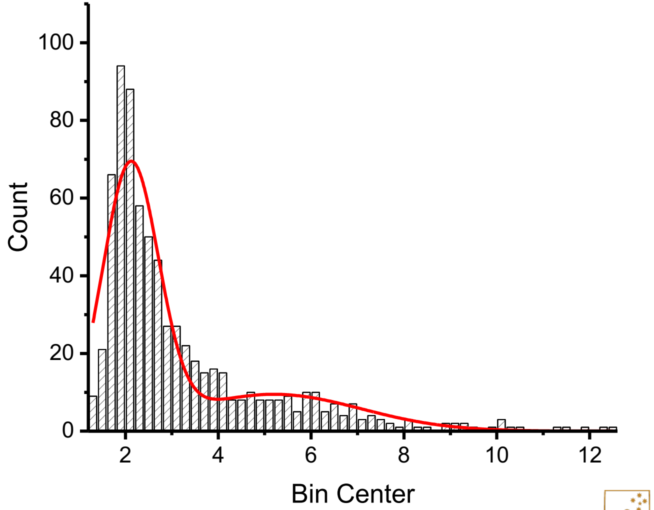
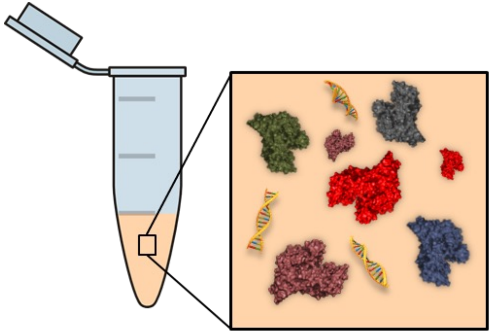


Image: https://multiplesclerosisjourney.com/

Machine Learning for Complex Samples



Develop

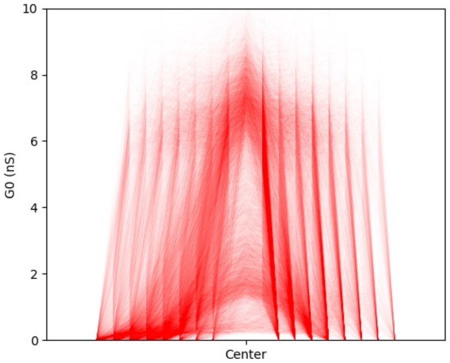
- Develop algorithms to differentiate between different protein signals.

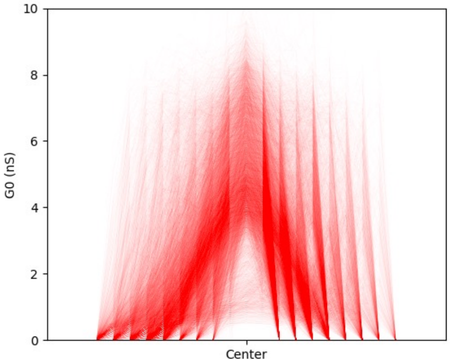
Train

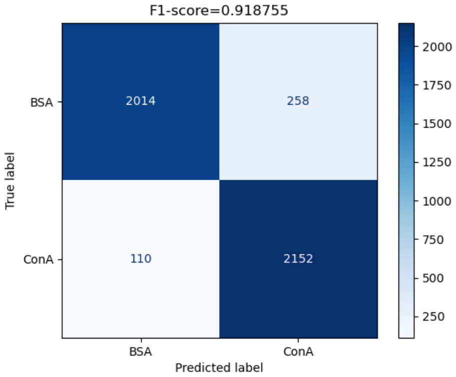
- Train the algorithms with each protein and then with a mixture of different proteins.

Identify

- Identify the presence and concentration of NfL in blood.







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ANFF

OHIOH

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Research School of Physics

Australian National University

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ARC Centre of Excellence for Transformative Meta-Optical Systems (TMOS)

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