ESPCI Paris   Biophysics 3rd year lecture   January-April 2020

Microarrays and DNA-based nanotechnology

1 PCR and mutation detection

2 Electronic detection of biomolecules

3 DNA-based nanotechnology

Part 1

Polymerase Chain Reaction

Mutation detection

Effect of a frame-shifting point mutation: example of a 35delG mutation in the CX-26 gene

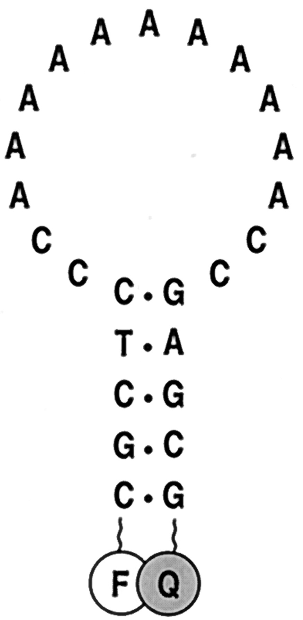
Leucine Glycine Glycine Glutamic Acid

WT: ...CTG GGG GGT GTG A...

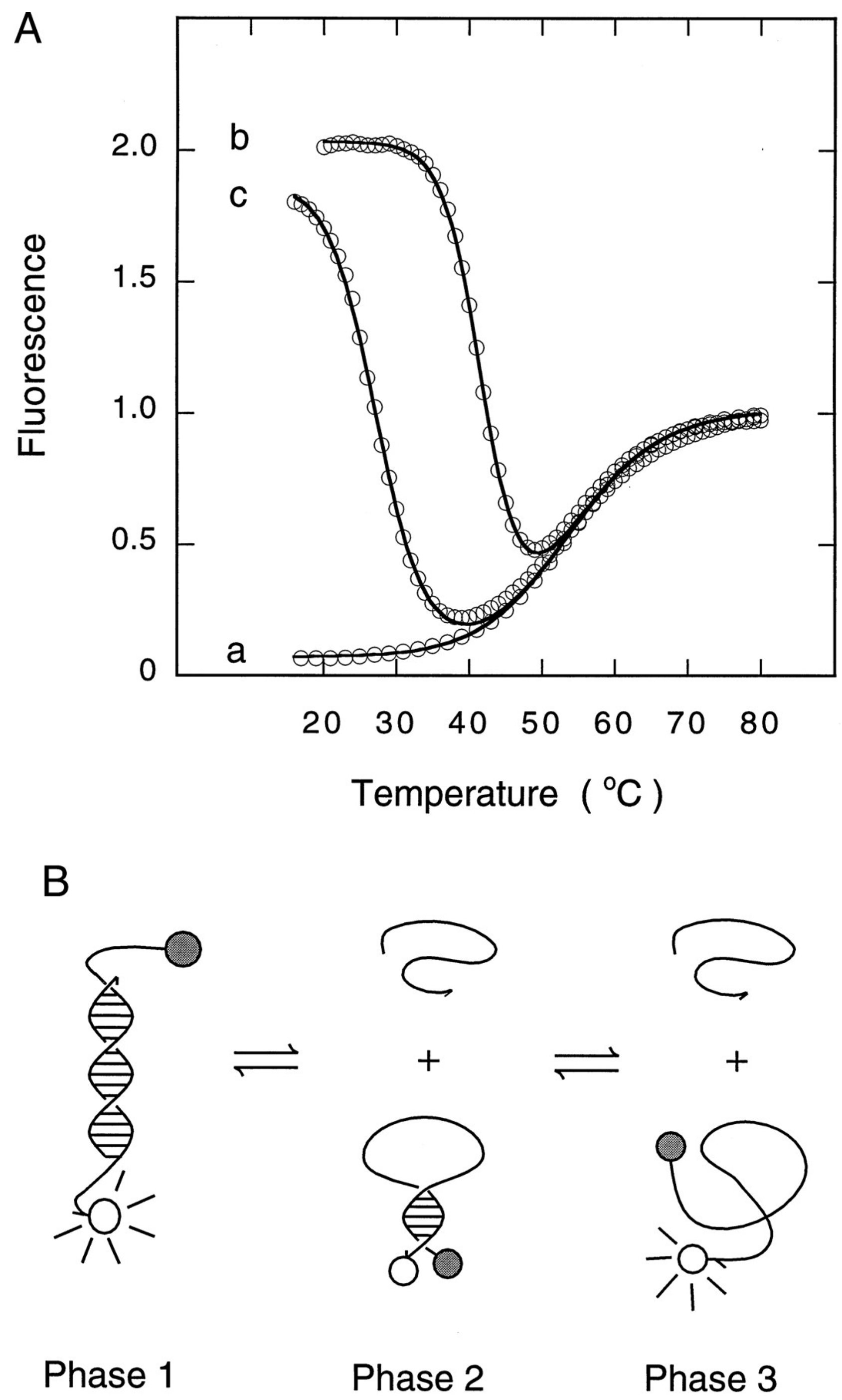
MUT: ...CTG GGG GTG TGA ...

Leucine Glycine Glutamic Acid STOP

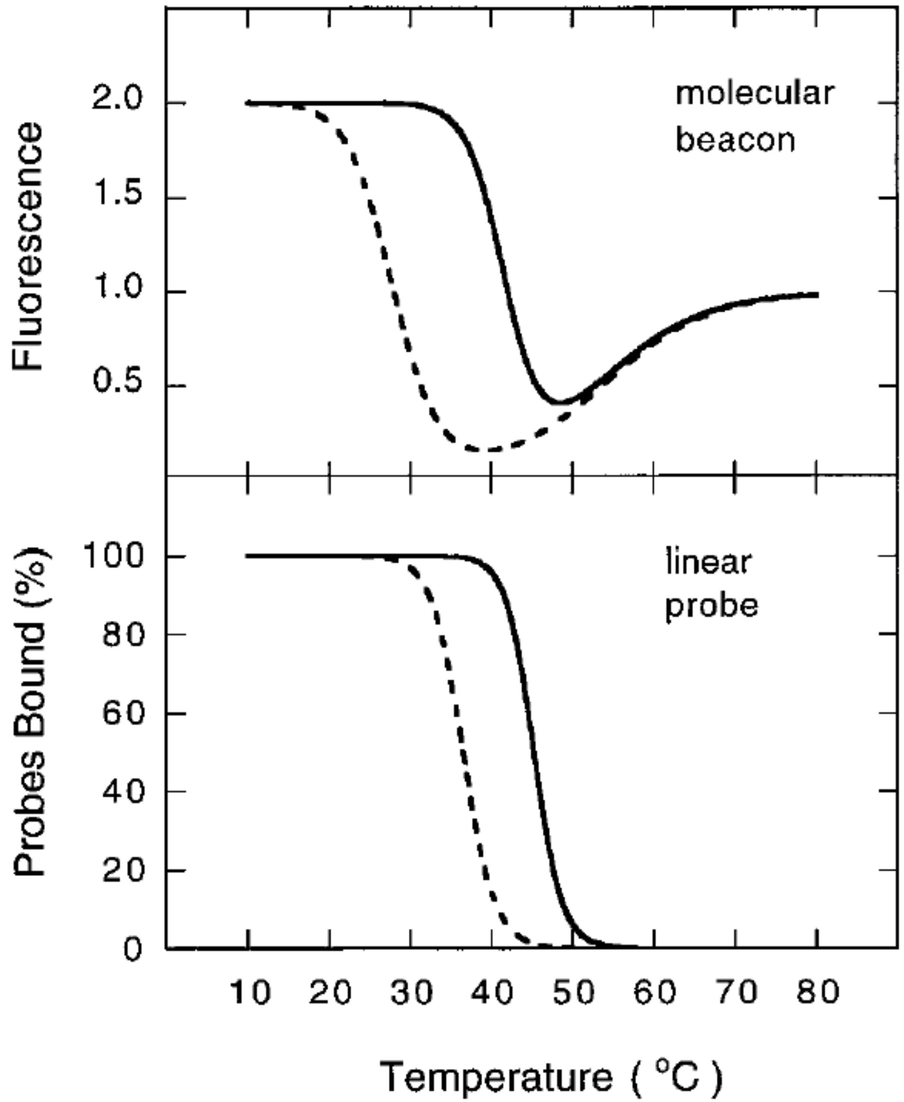
« Enhanced specificity of molecular beacon probes »



Bonnet, Tyagi, Libchaber and Kramer,
PNAS 96, 6171 (1999)

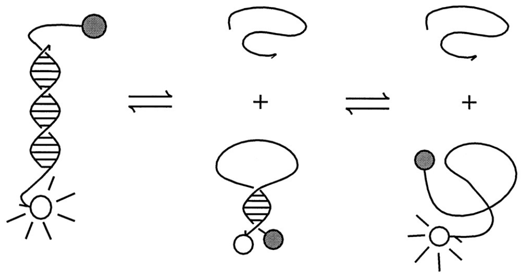


General result: the molecular beacon exhibits higher specificity.
Why?



Energy diagram derived from measured thermodynamic parameters:

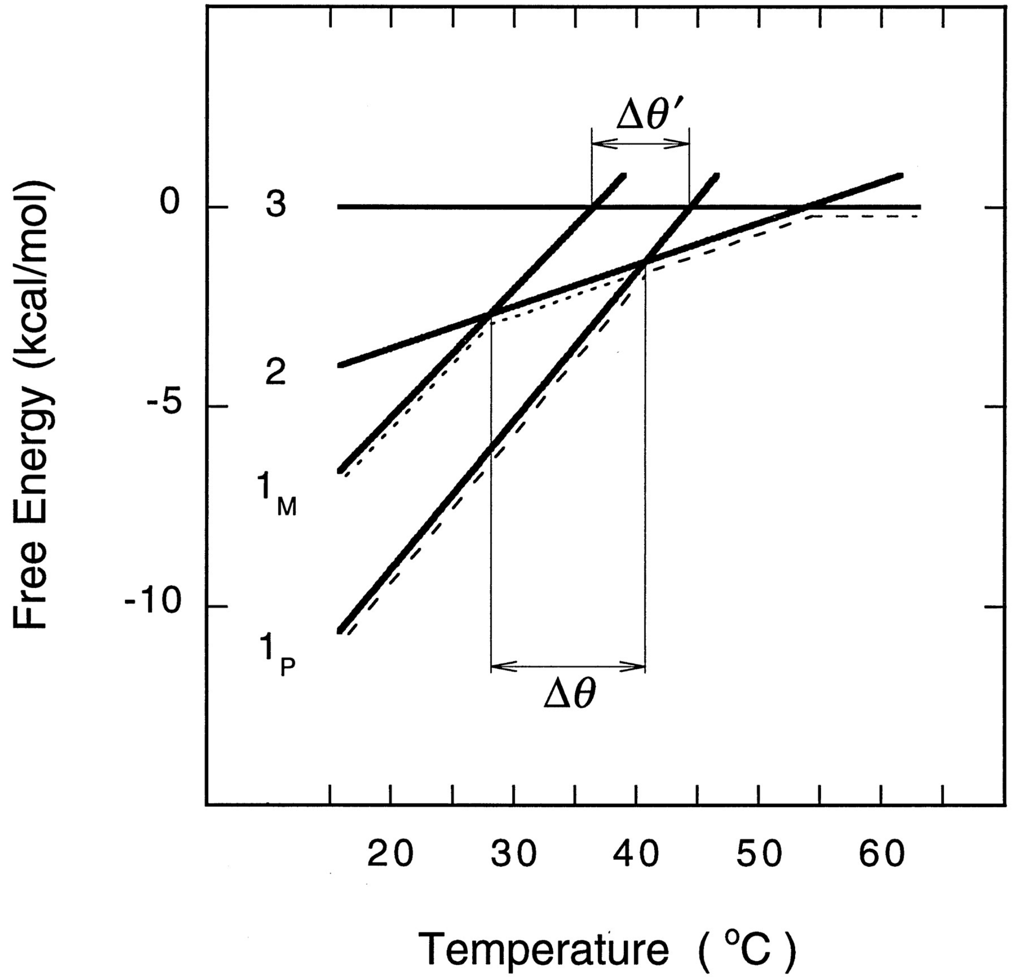
$\Delta G = \Delta H - T\Delta S$



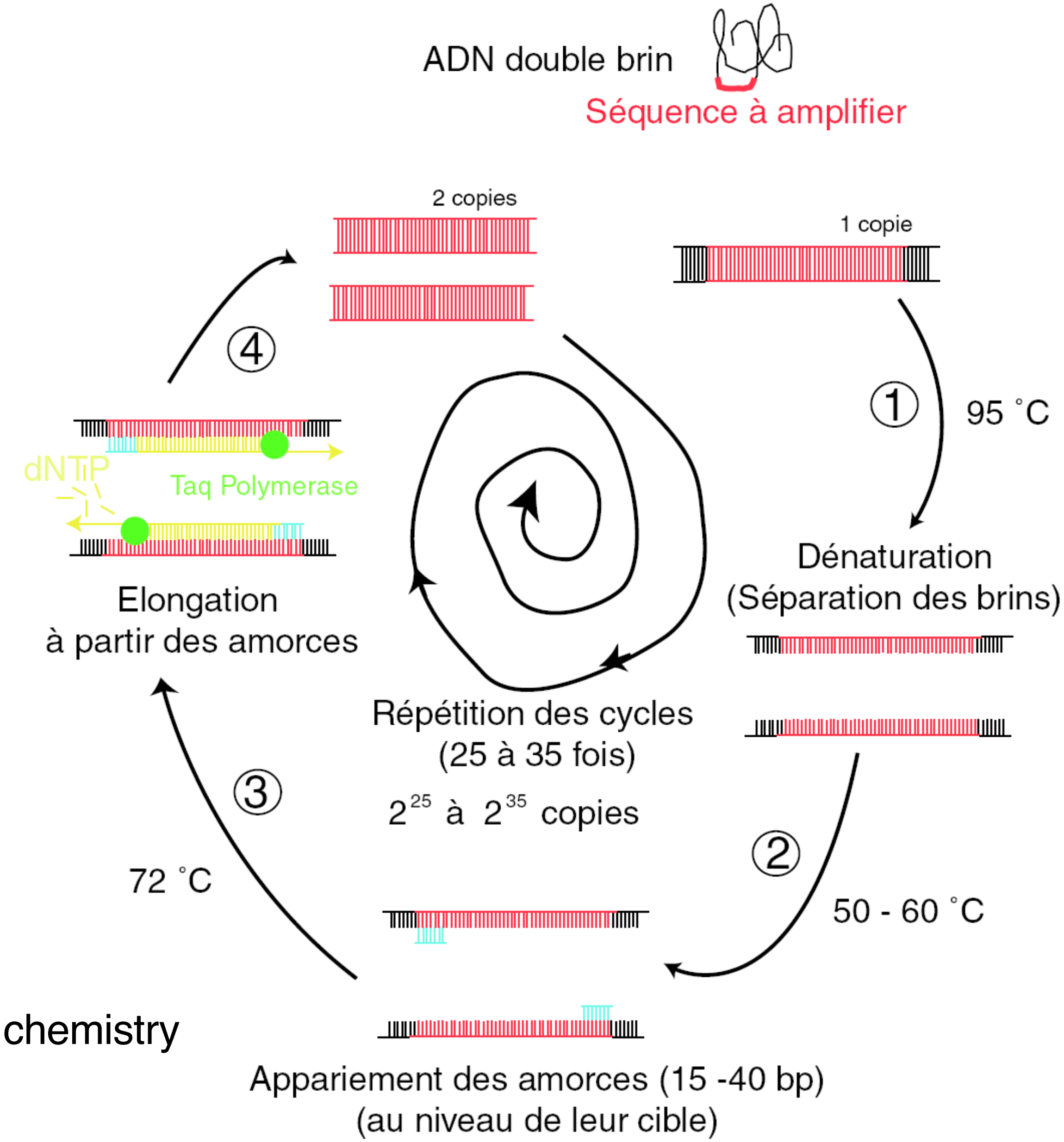
$\Delta H_{1p} < \Delta H_{1m} < \Delta H_2 < \Delta H_3$

$\Delta S_{1p} < \Delta S_{1m} < \Delta S_2 < \Delta S_3$

Zero-energy state.

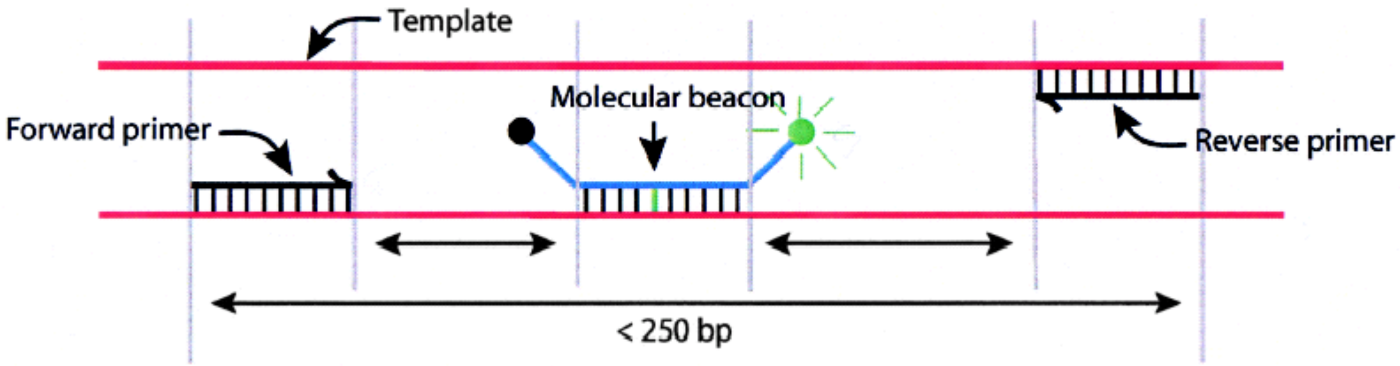


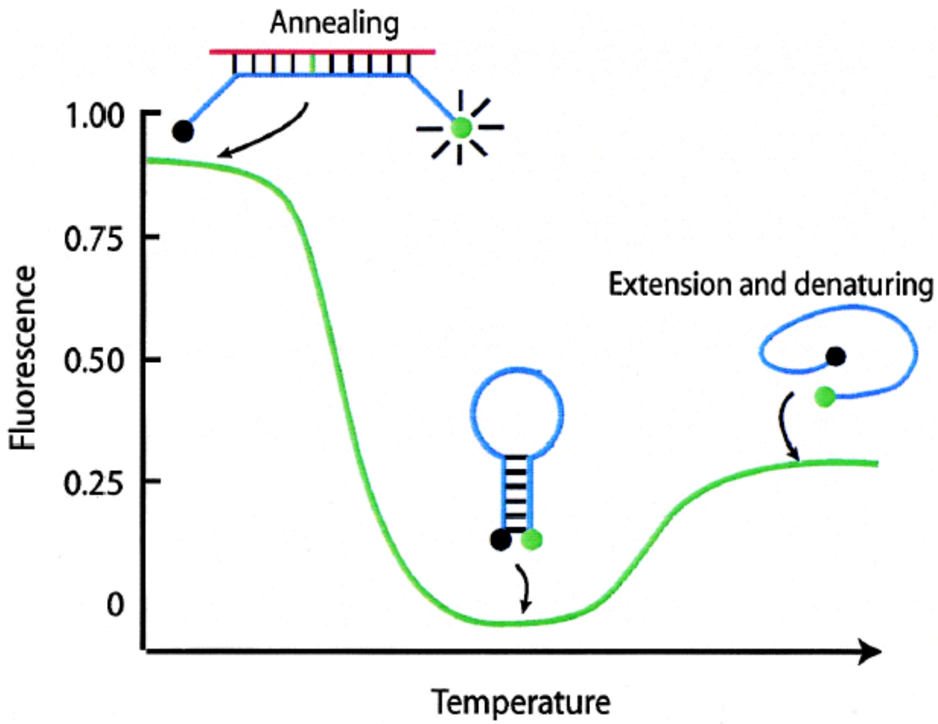
Polymerase Chain Reaction



Kary Mullis,
1993 Nobel prize in chemistry

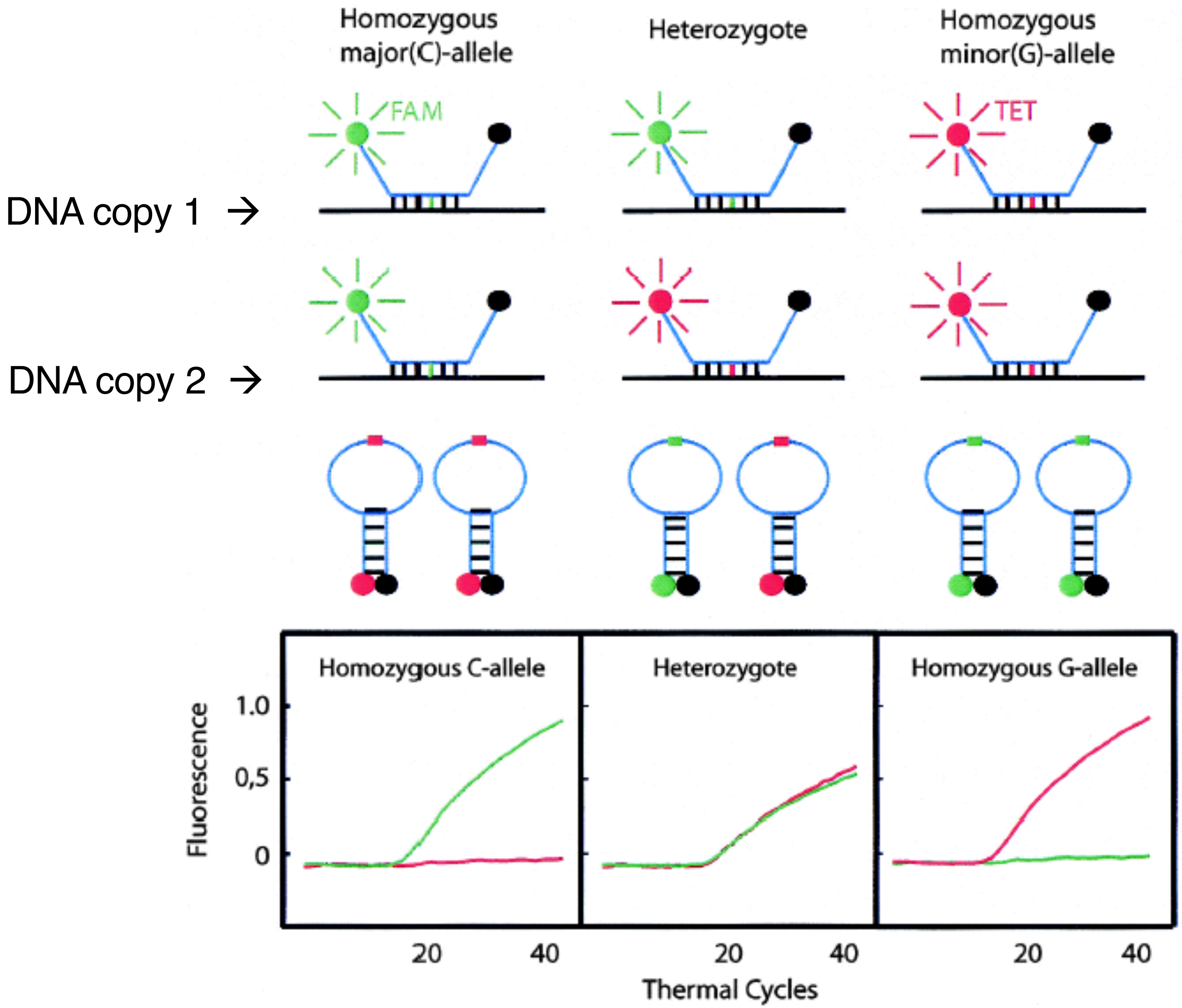
Mutation detection by real-time PCR



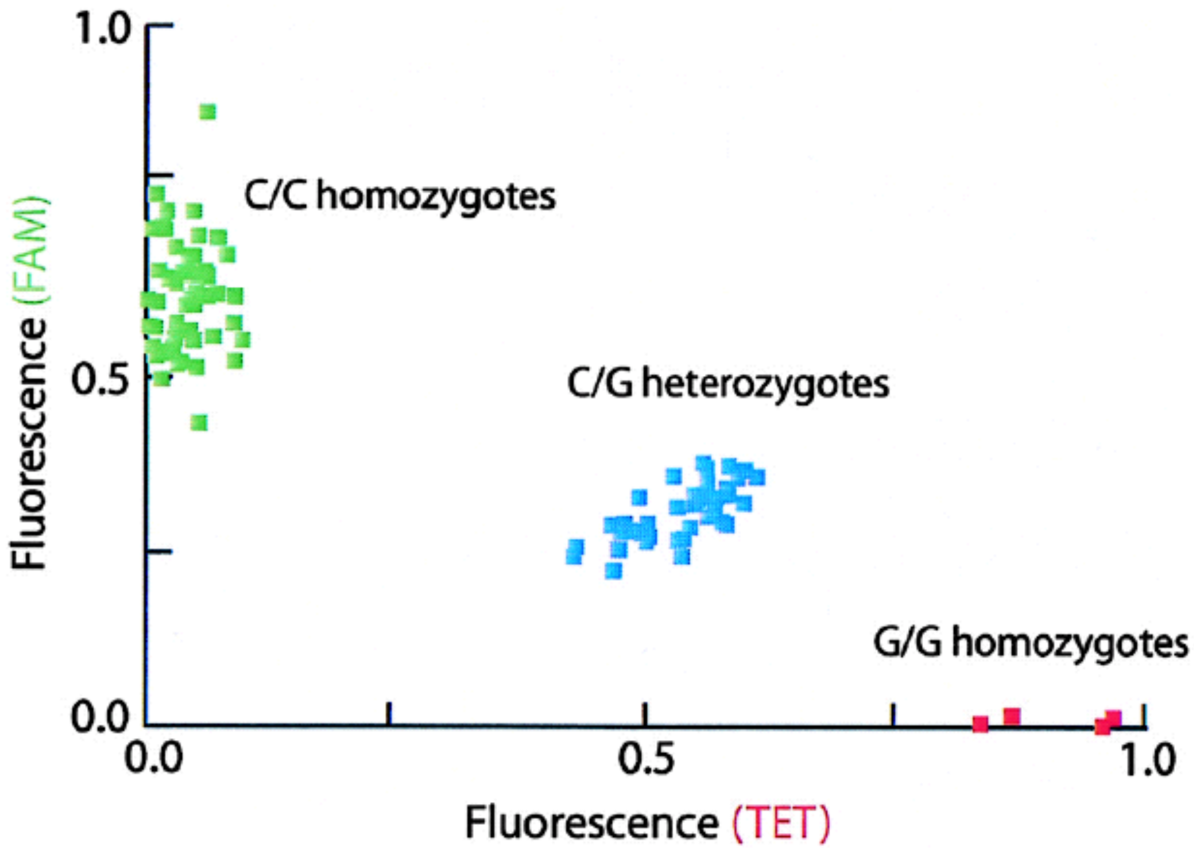


M. M. Mhlanga and L. Malmberg
Methods 25, 463 (2001)

Flurorescence signals measured during real-time PCR

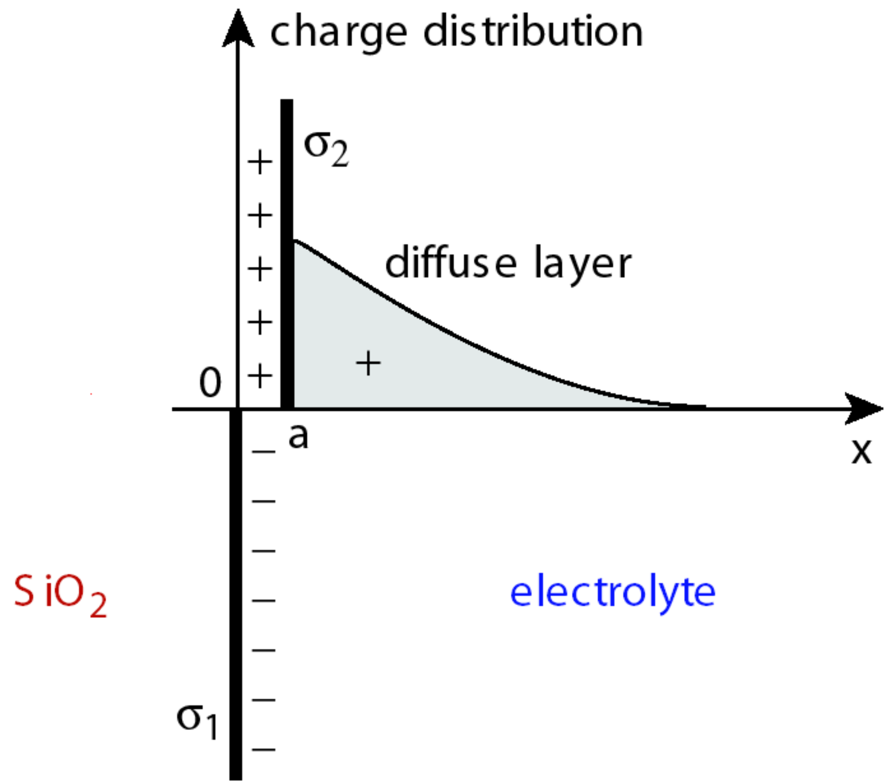


Statistics for this mutation detection



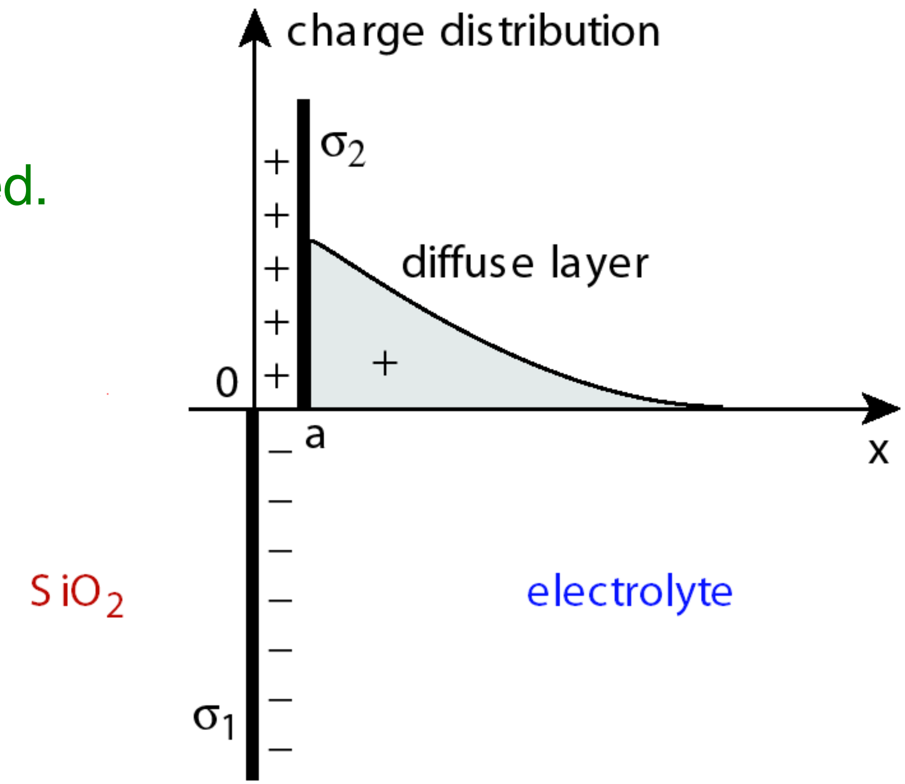
Part 2

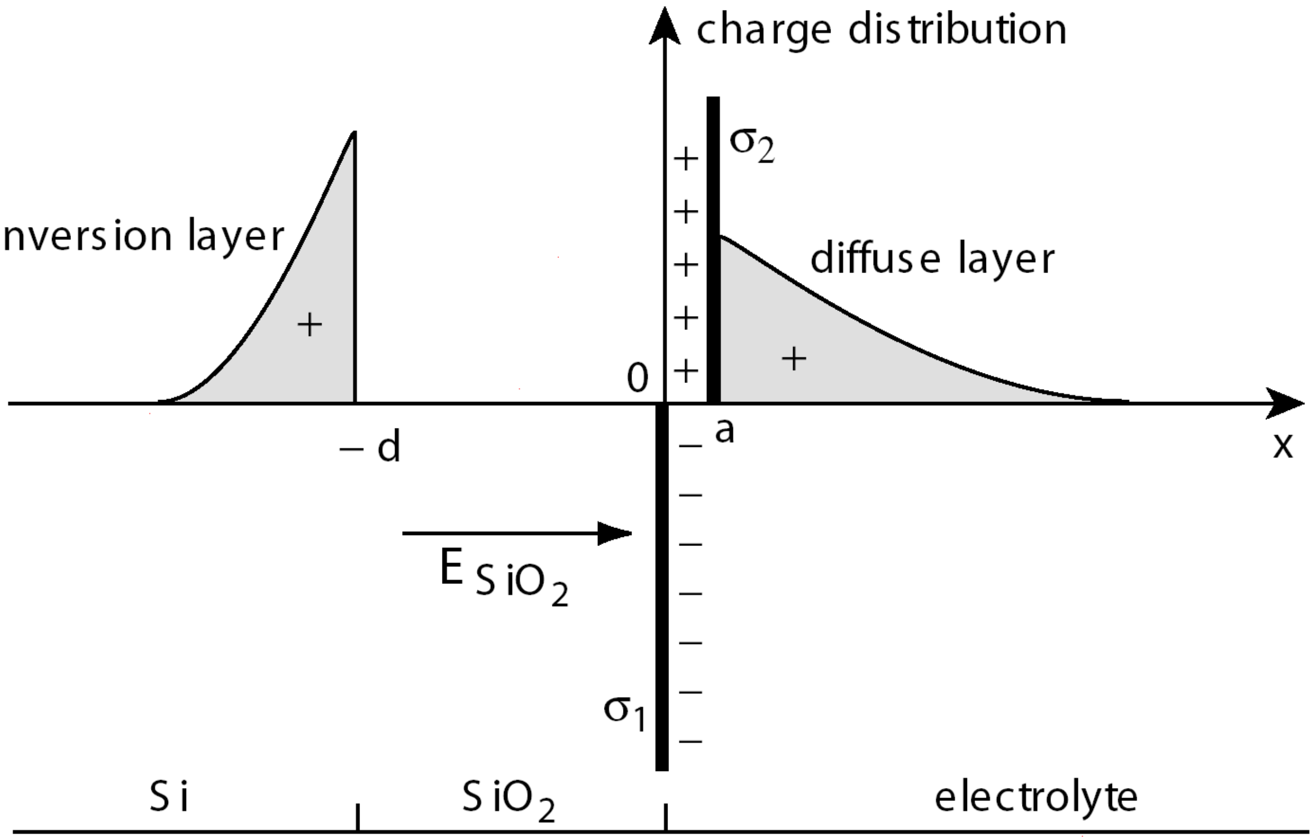
Electronic detection of biomolecules



charge distribution
σ2
diffuse layer
+
0
a
x
SiO2
electrolyte
σ1

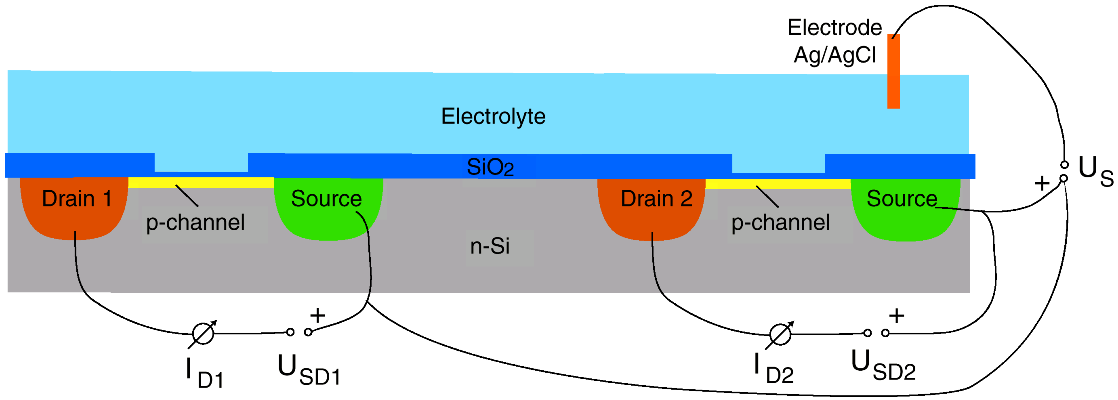
Biomolecules are charged.
Direct detection ?

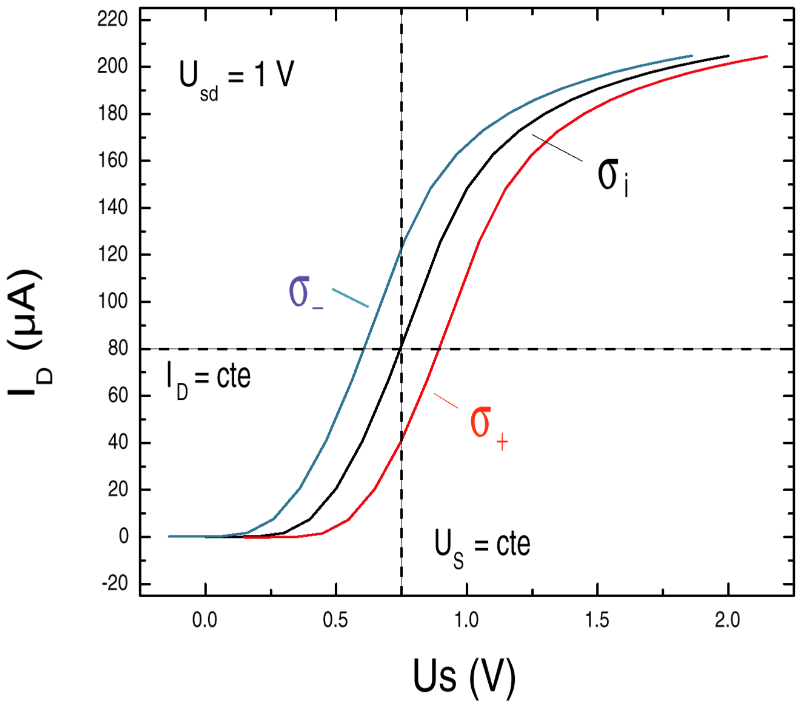




charge distribution
σ2
inversion layer
diffuse layer
0
a
x
− d
E SiO2
σ1
Si
SiO2
electrolyte

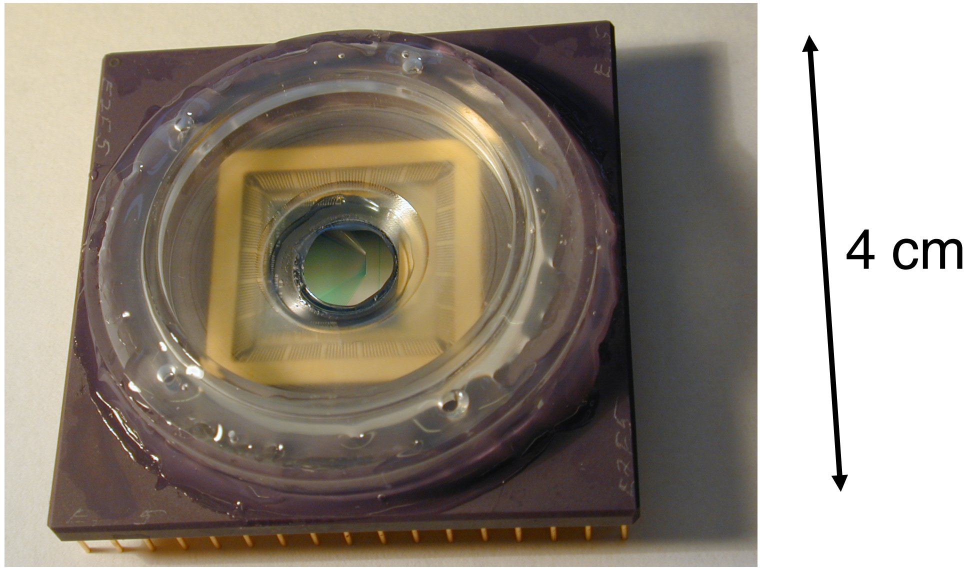
The charging state of the surface determines the drain current of the field-effect transistor

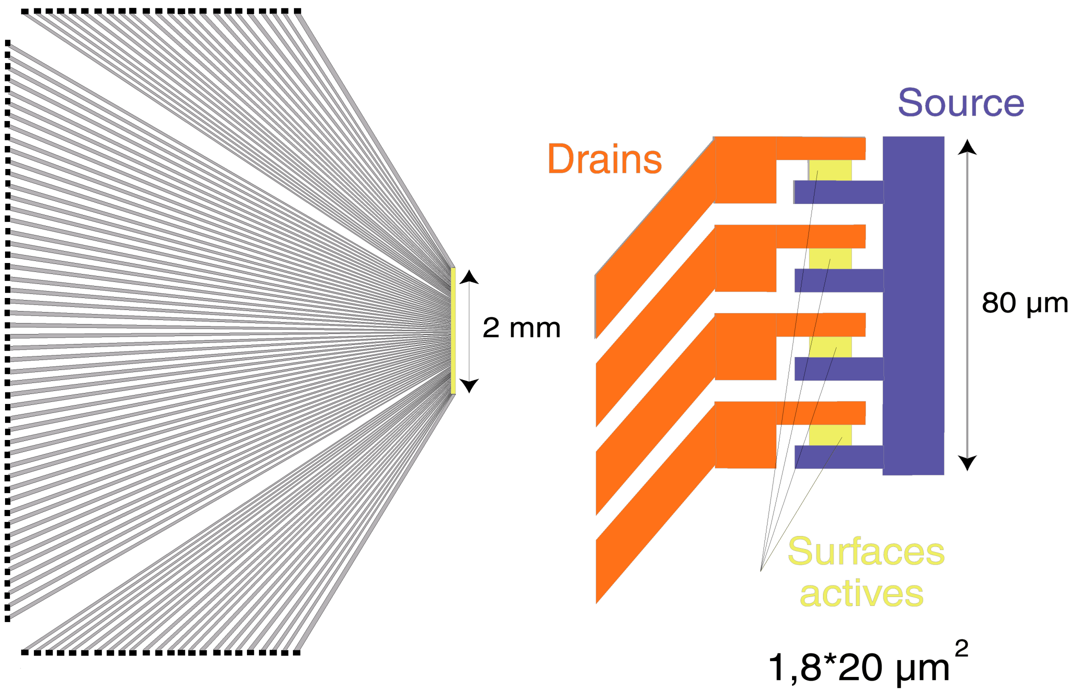




Transistor arrays

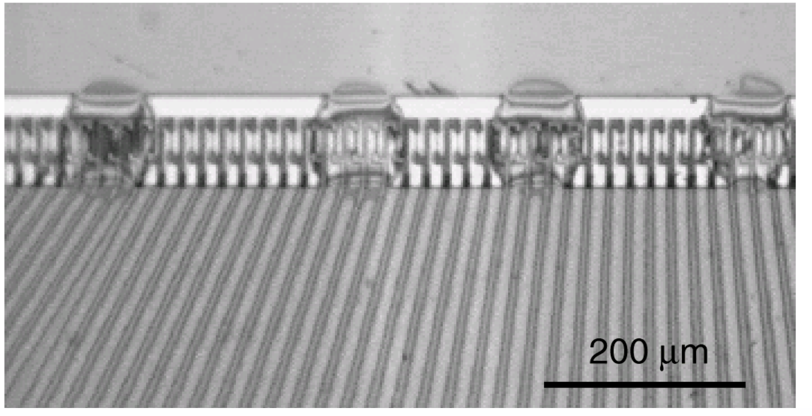
B. Straub, G. Zeck, P. Fromherz
Max Plack Institut für Biochemie,
Martinsried (Germany)

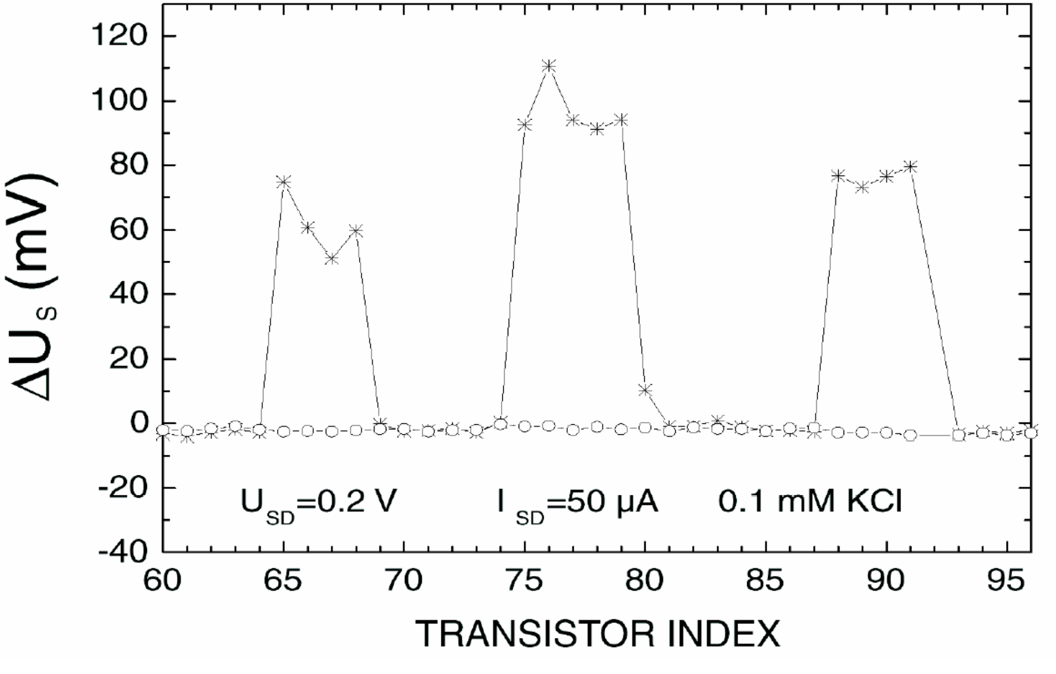




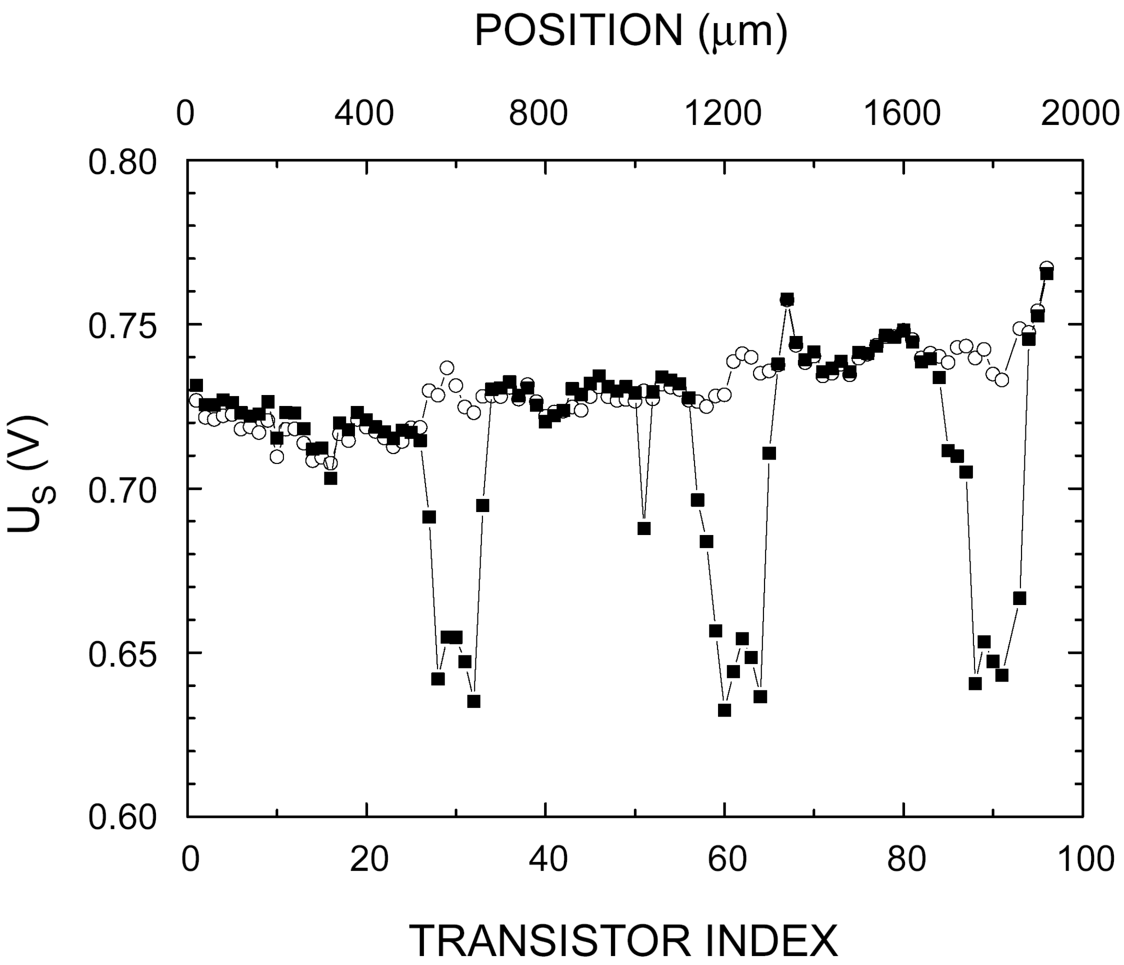
Local deposits and differential measurements

Poly(L-lysine) 0.01% (w/v) in PBS buffer (0.1X, pH7)

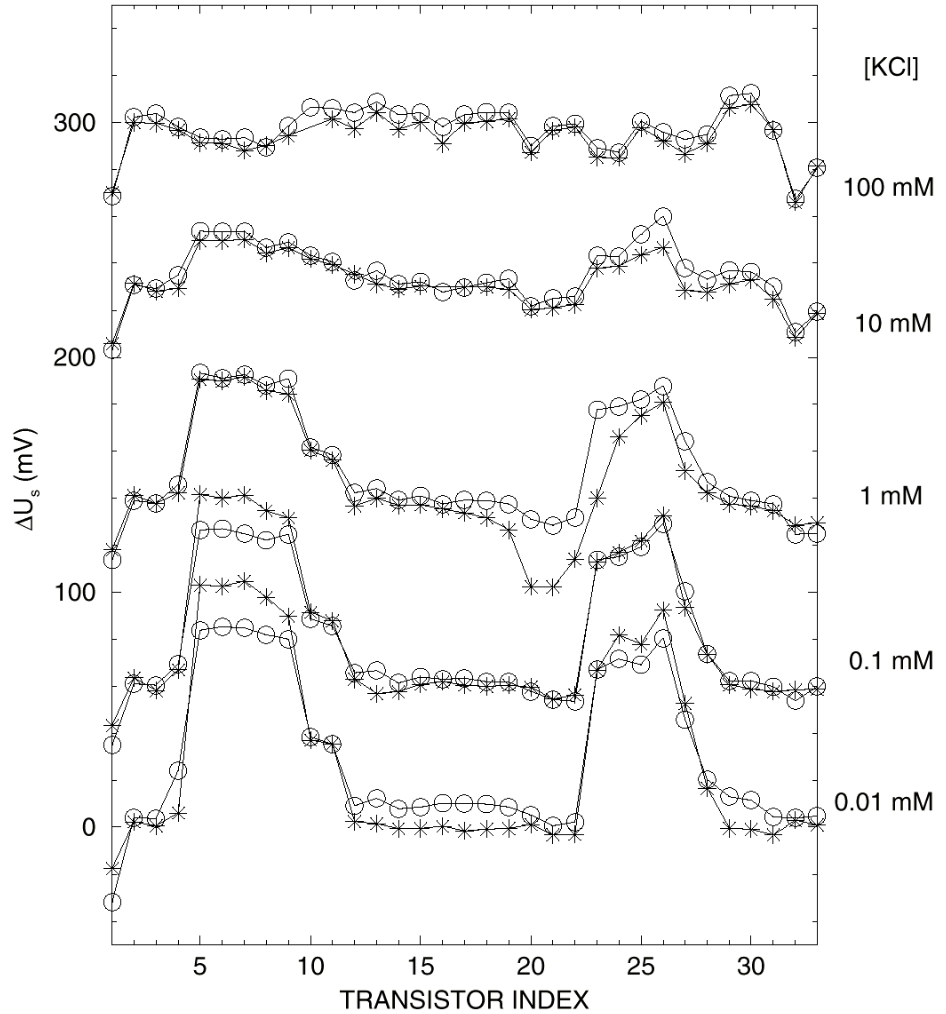




Electronic detection of DNA



Increasing the salt-concentration in the electrolyte: screening by mobile ions



λ_D
0.95 nm
3 nm
9.5 nm
30 nm
95 nm

U_{SD} = 1.2 V I_D= 50 μA

F. Pouthas, C. Gentil, D. Côte, G. Zeck, B. Straub, U. Bockelmann, Phys. Rev. E 70, 031906 (2004)

35 delG mutation, connexin-26 gene defect, prelingual nonsyndromic deafness

(in collaboration with Pasteur institute Paris)

Leucine Glycine Glycine Glutamic Acid

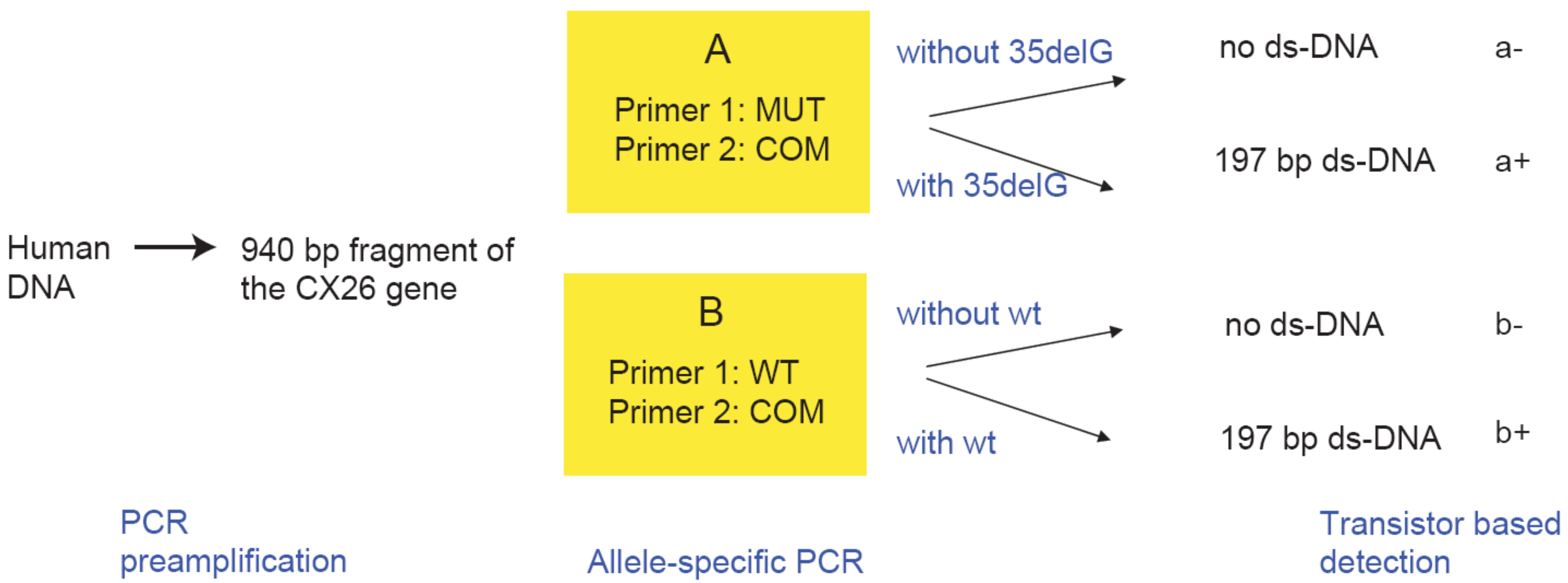
WT: ...CTG GGG GGT GTG A...

MUT: ...CTG GGG GTG TGA ...

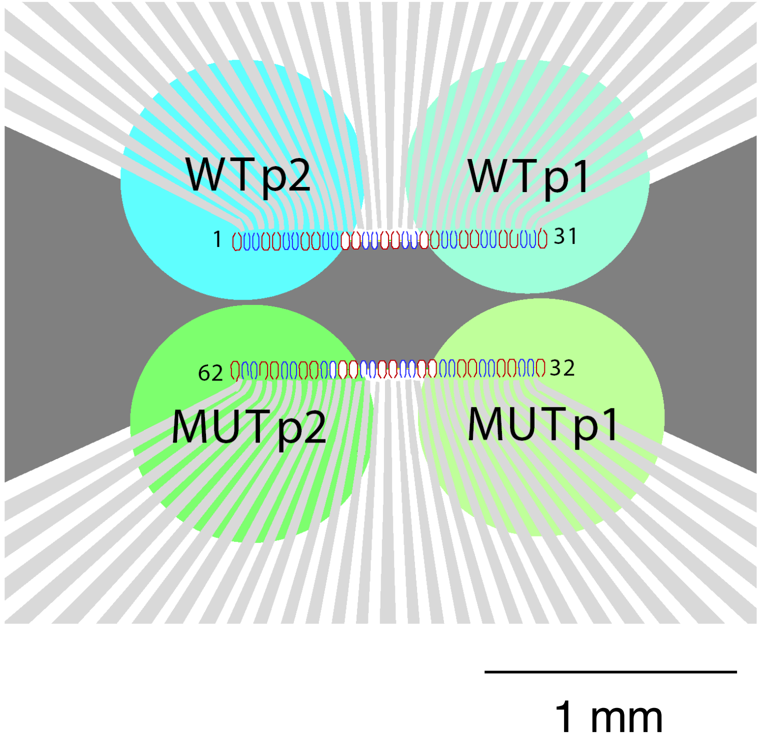
Leucine Glycine Glutamic Acid STOP

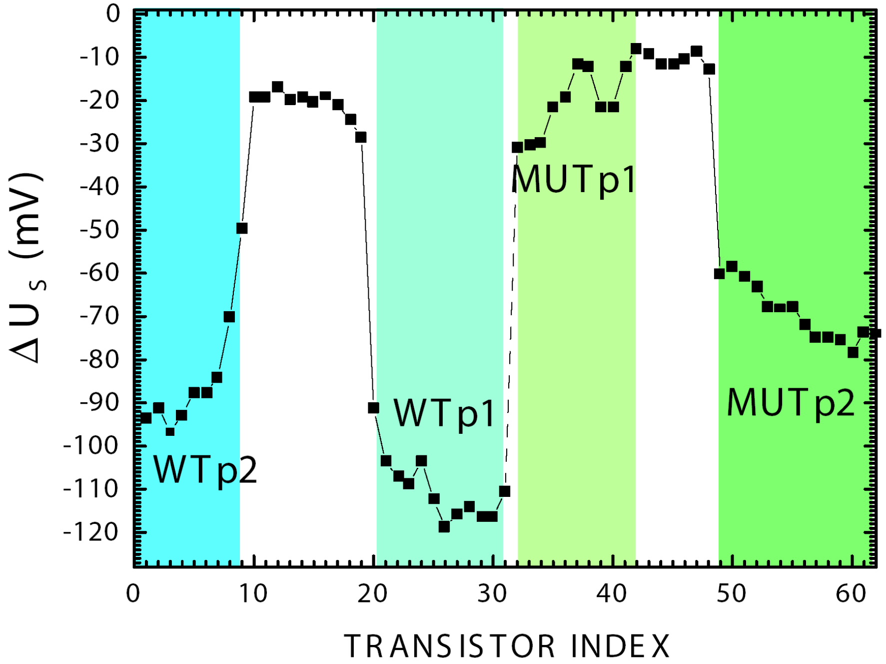
Possible results:

a- b+ homozygous normal
a+ b+ heterozygous
a+ b- homozygous in 35delG



Genotyping two different patients



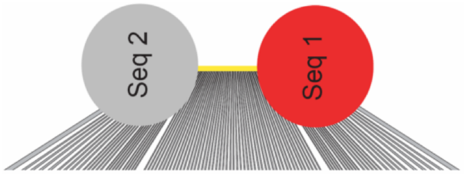


F. Pouthas, C. Gentil, D. Côte, U. Bockelmann, Appl. Phys. Lett. 84, 1594 (2004)

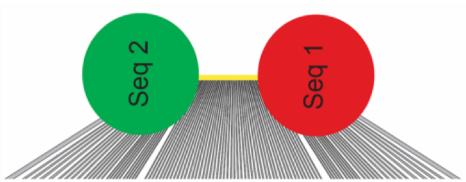
Hybridization detection with buffer change for signal amplification

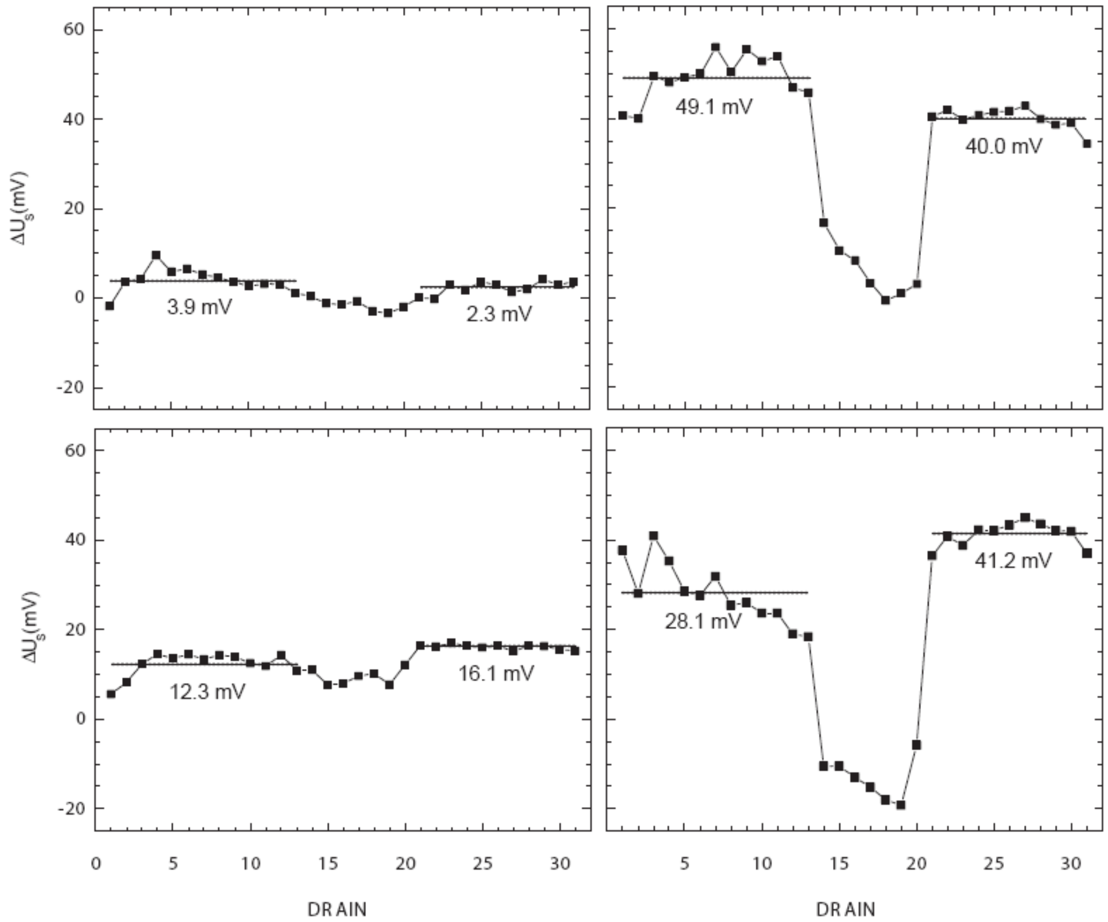
50 mM KCl 0.01 mM KCl

First hybridization



Second hybridization

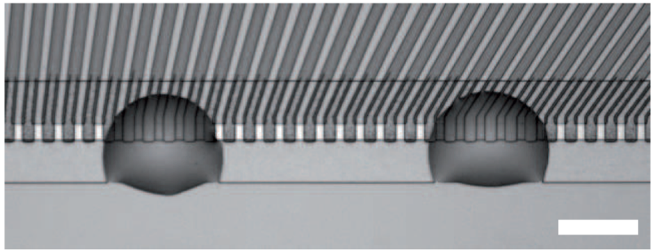


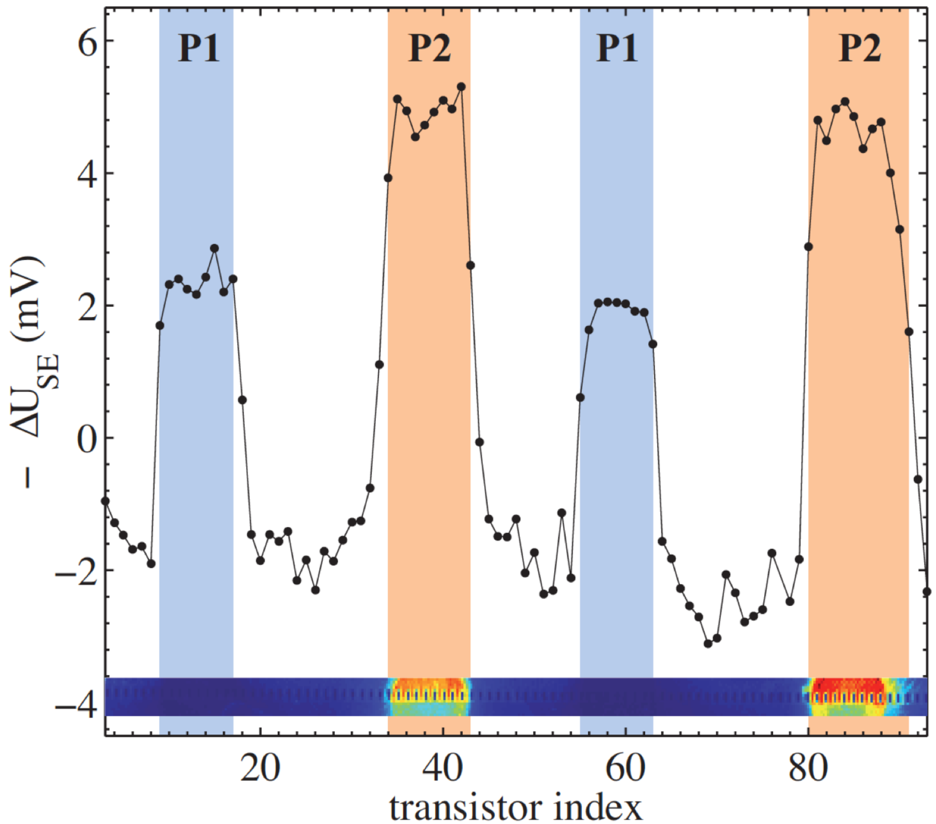


C. Gentil, G. Philippin, U. Bockelmann, Phys. Rev. E 75, 011926 (2007).

Electronic detection of DNA hybridization with FET arrays

For the first time with micro-spotted DNA probes.

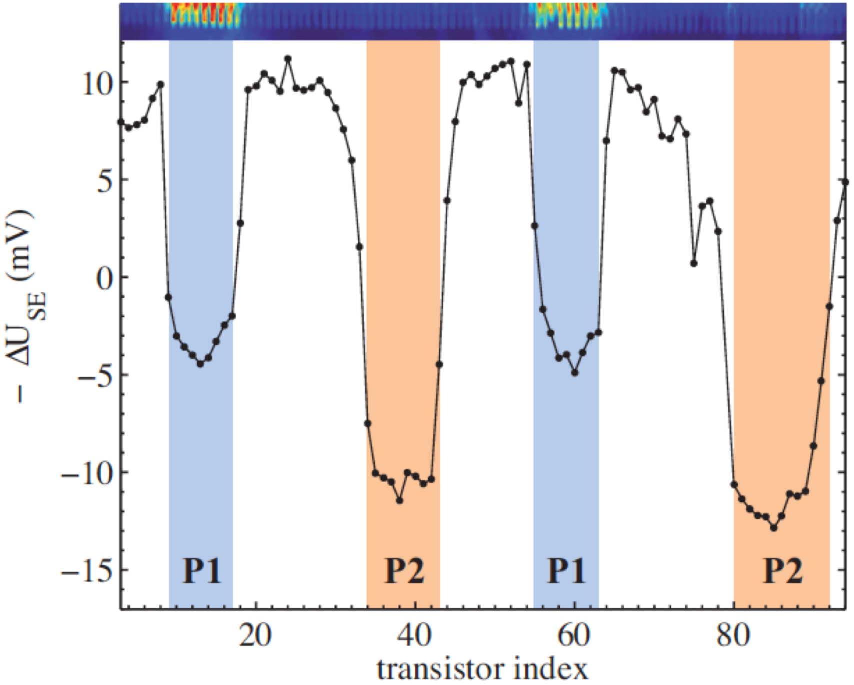




Hybridization and electronic detection at 25 mM KCl

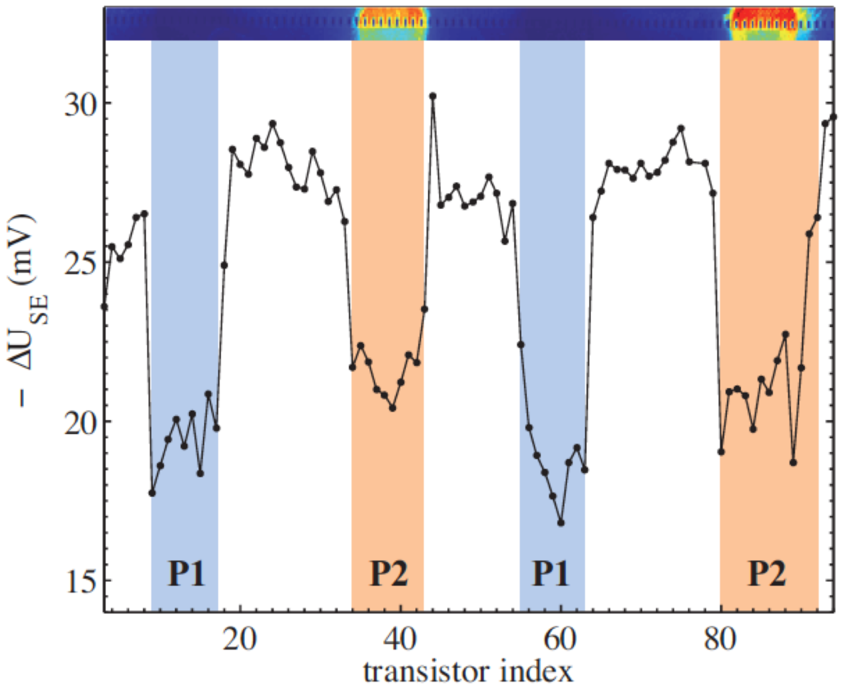
A. Blin, I. Cissé and U. Bockelmann, Scientific Reports 4, 4194 (2014)

T1 cy3



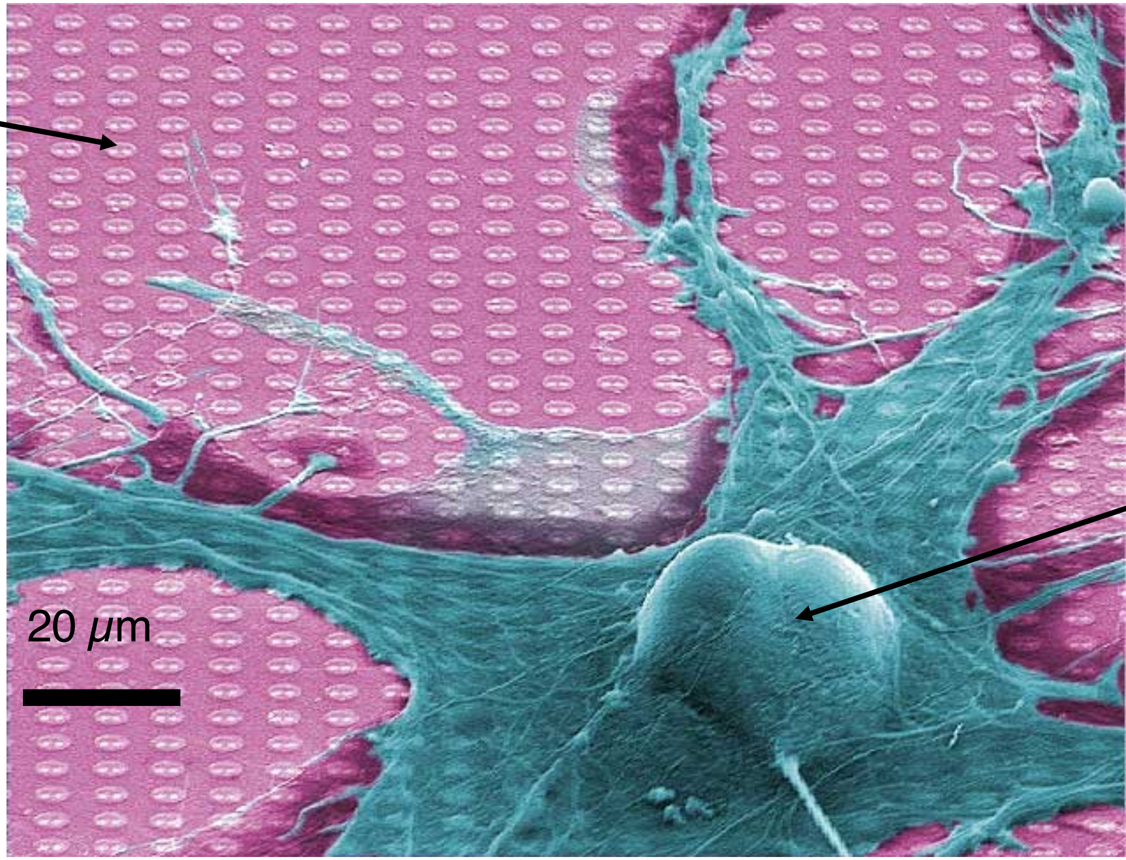
Electronic detection at 0.01 mM KCl

T2 cy5



A 128 x 128 CMOS Biosensor Array for Extracellular Recording of Neural Activity
B. Eversmann et al, IEEE J. Solid-State Circuits 38, 2306 (2003)

Collaboration: Fromherz (MPI) – Thewes (Infineon)



Snail neuron on sensor chip in culture.

Part 3

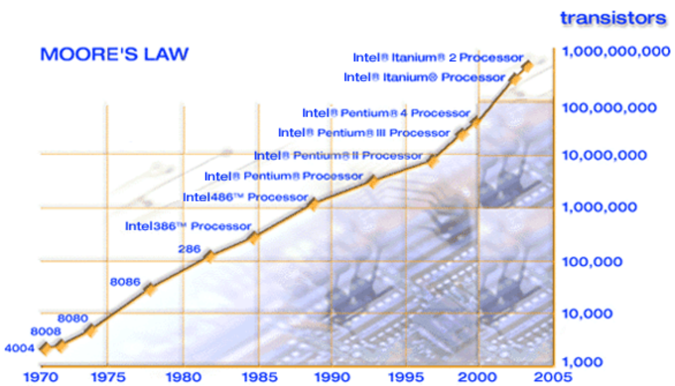
DNA-based nanotechnology

a) Nano-structures

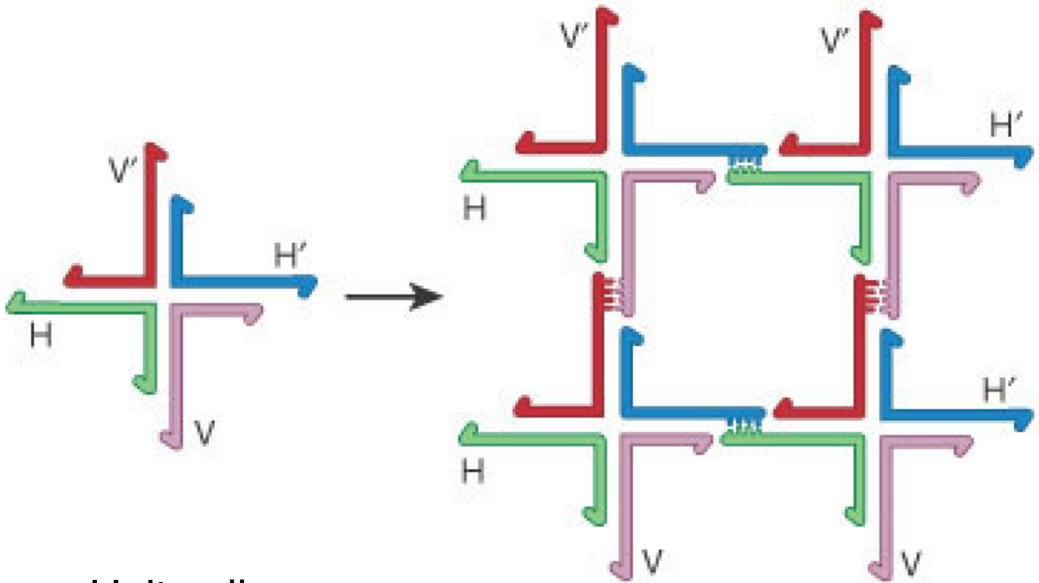
b) Nano-machines

4a) Nanostructures

A 2D crystal formed by self-assembly of DNA



Motivation of the microelectronics industry:
Moore's law: « Chip density doubles every 18 months ».
Bottom up approach.



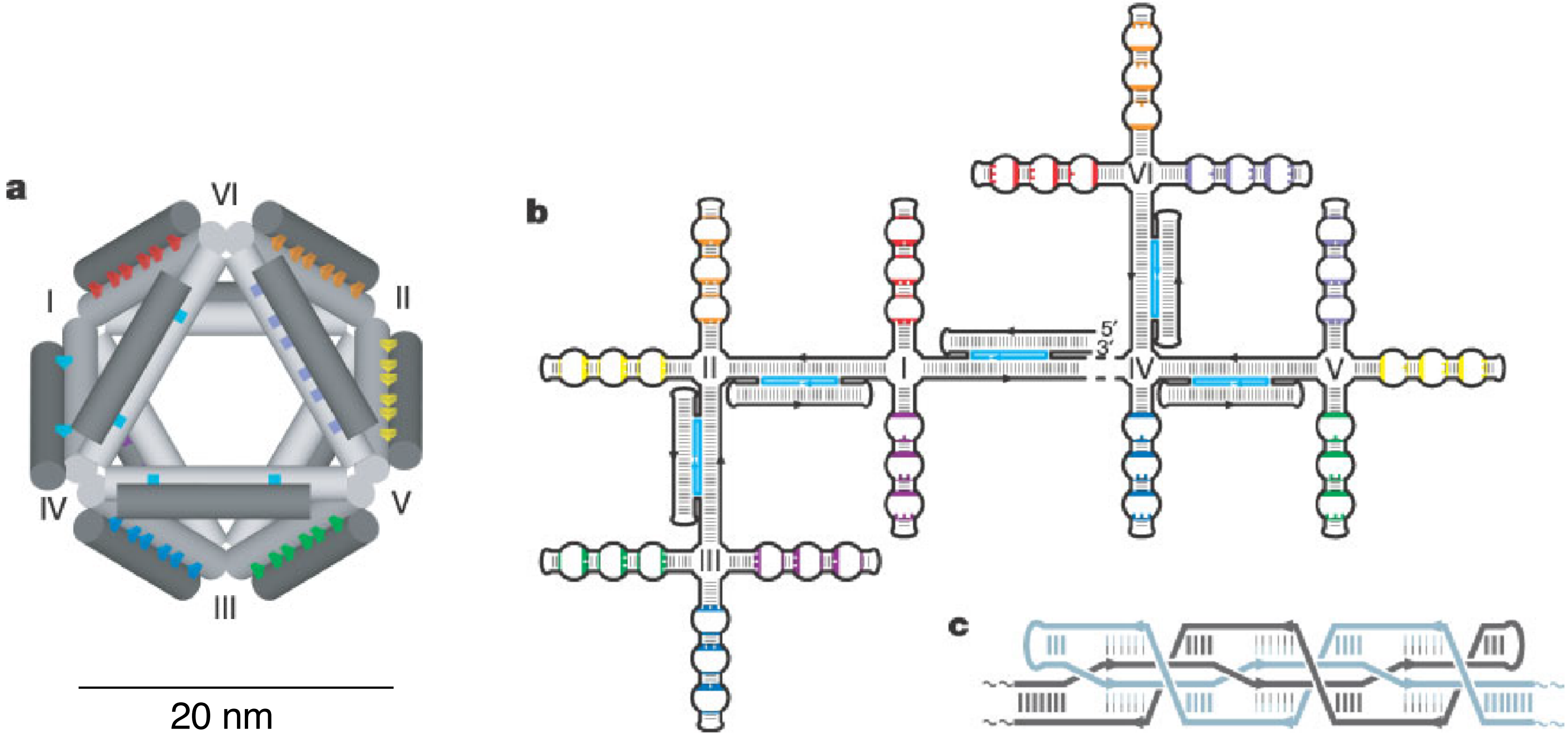
Unit cell

2D crystal

Self-assembly of a 3D nanostructure: octahedron

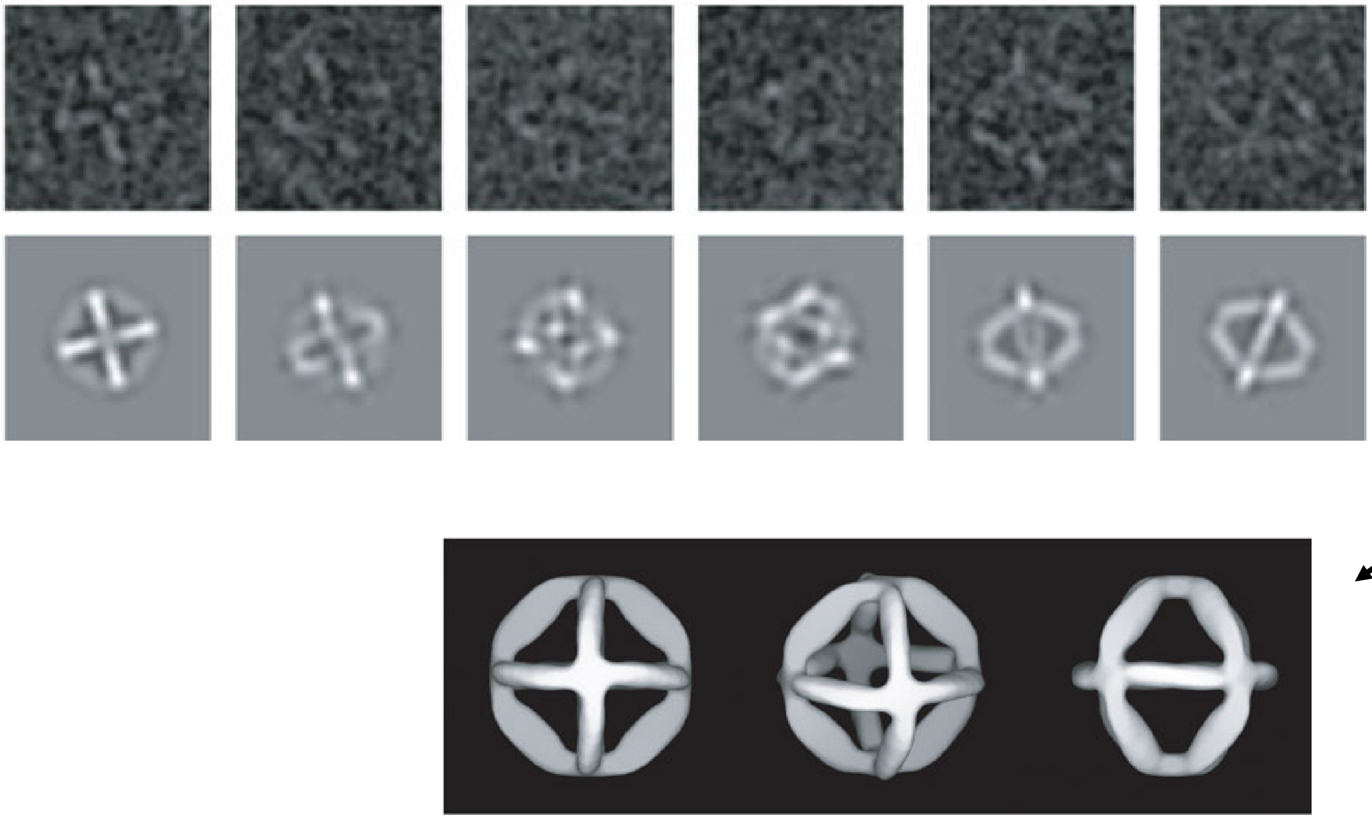
1 single-stranded DNA molecule with 1669 nucleotides prepared by PCR and cloning
5 single-stranded DNA oligonucleotides (40 mer each)

Hybridization in solution. Rigid double-stranded parts and flexible single-stranded parts.
Uses two different types of specific interactions between DNA helixes (5+7).



W. M. Smith, J. D. Quispe, G. F. Joyce, Nature 427, 618 (2004)

Images of nanostructures obtained by cryo-electron microscopy



Raw images

Numerical reconstitution

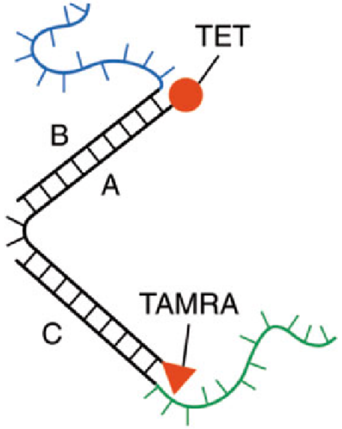
W. M. Smith, J. D. Quispe, G. F. Joyce, Nature 427, 618 (2004)

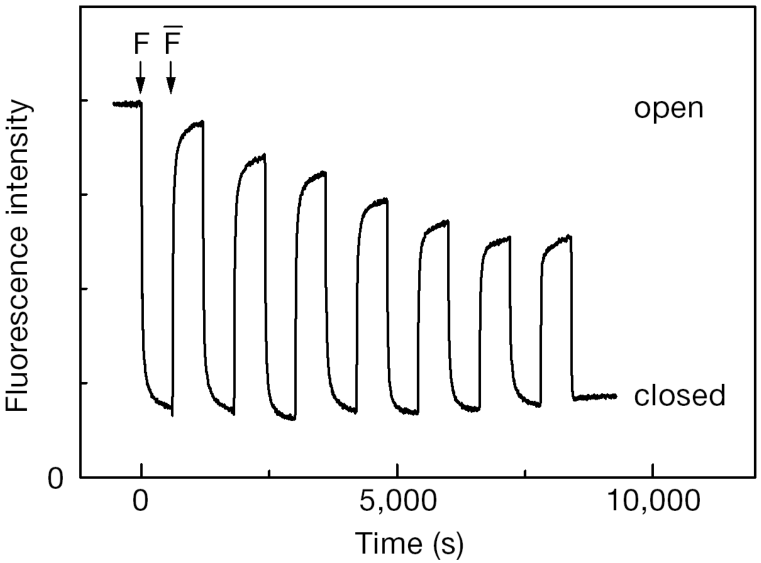
4b) Nanomachines

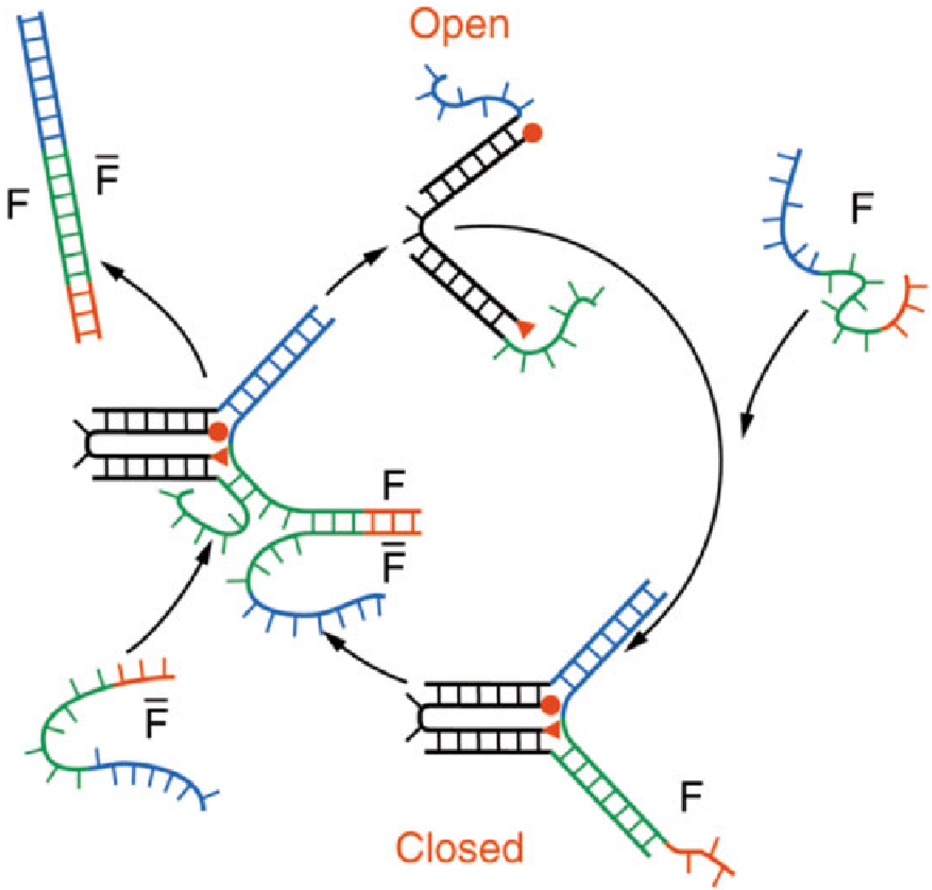
Molecular tweezers that use DNA as fuel

5 oligonucleotides:
Tweezers: A, B, C
Fuel: F, F

One FRET pair:
TET/TAMRA







B. Yurke et al, Nature 406, 605 (2000)

SUMMARY

Measuring the hybridization equilibrium of short oligonucleotides as a function of temperature.

Data analysis based on a two-state model.

Model with first-neighbour interactions (12 parameters) to predict enthalpy and entropy as a function of base sequence. Numerical tool to choose base sequence.
Extension to mispairing: mutation detection
Extension to loops: predicting secondary structures

Applications: Understanding biological roles of RNA
Optimising enzymatic amplifications, like PCR
Detecting mutations
Designing DNA microarrays and sensors
Designing RNA and DNA aptamers
DNA nanotechnology
…