

Stretching the physical boundaries of DNA hybridization for mutation detection

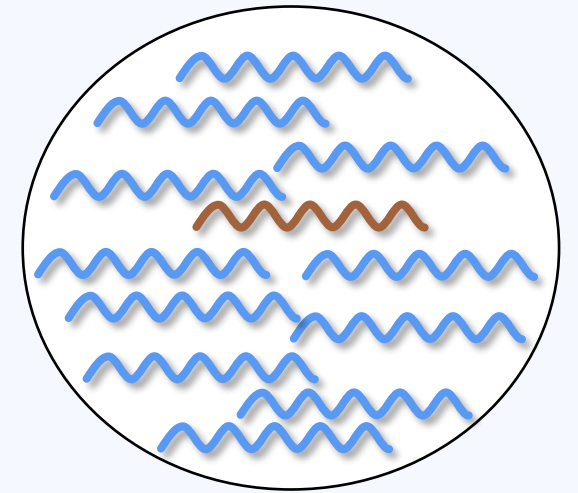
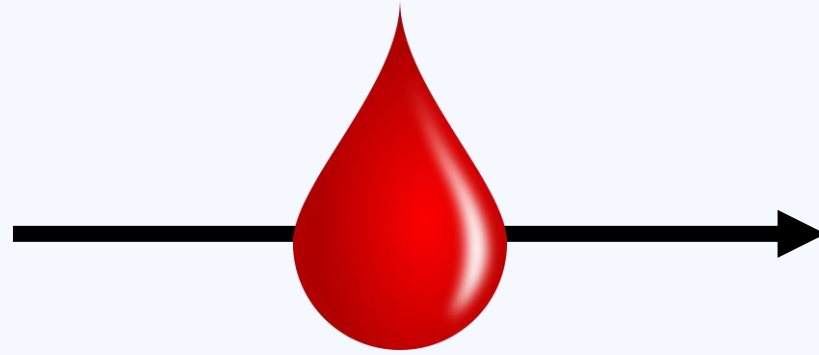
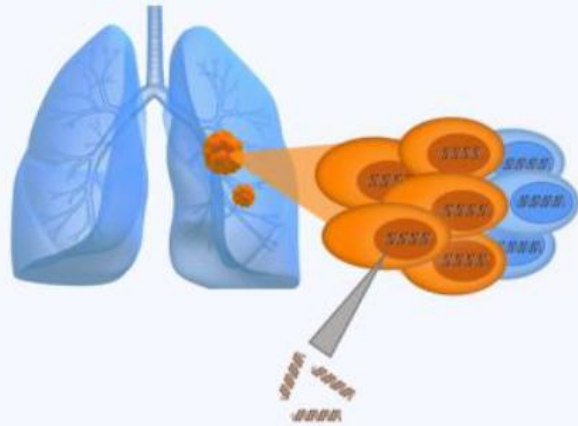
Jef Hooyberghs



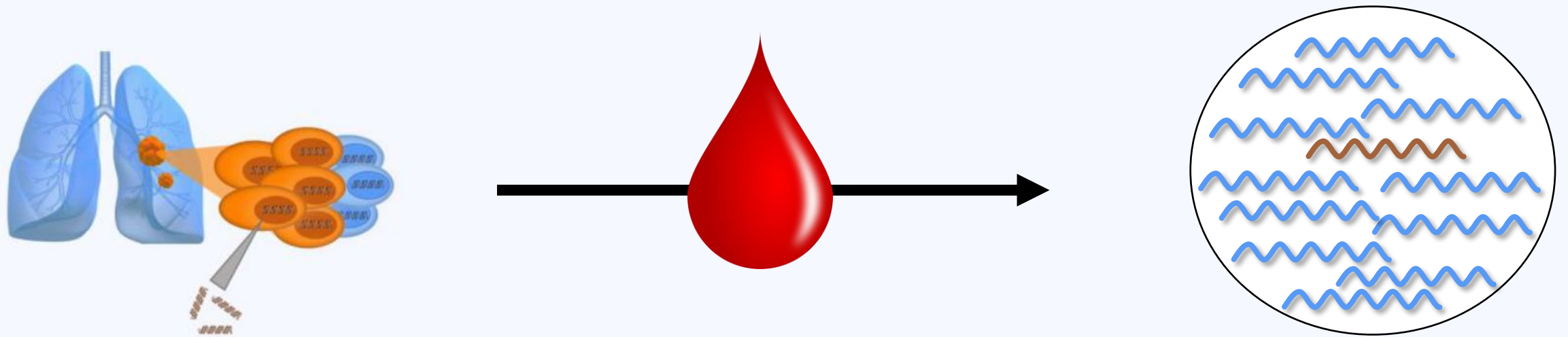
DNA from liquid biopsy



DNA from liquid biopsy




DNA from liquid biopsy




mutant

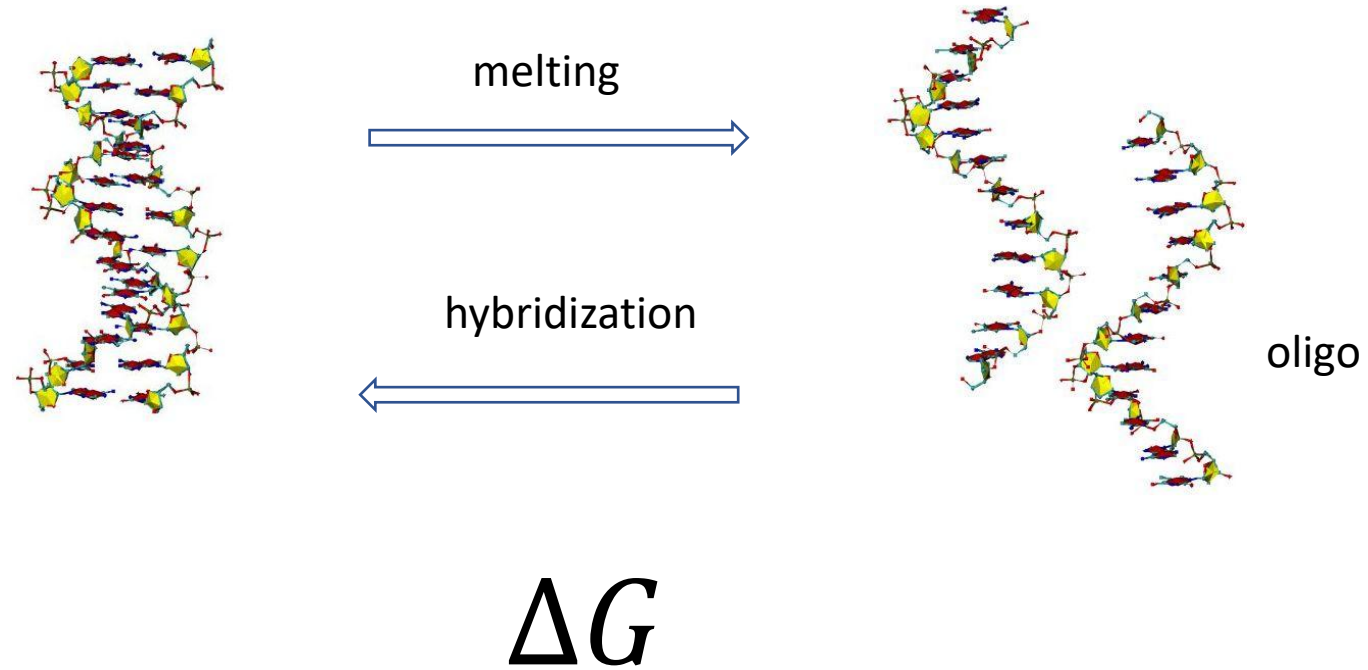
=


wild type

+ point mutation

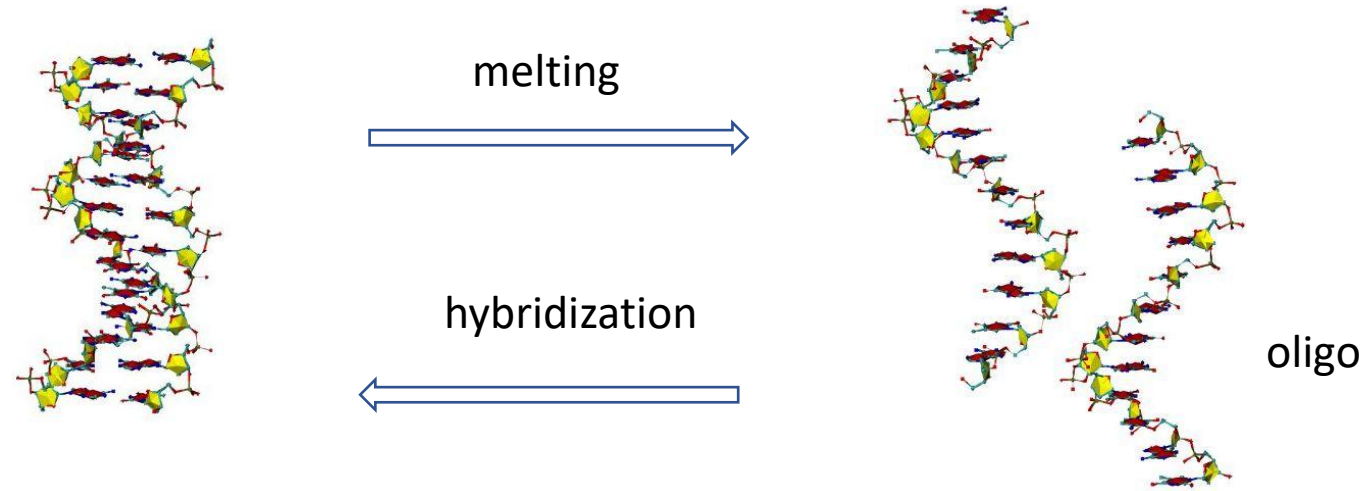
Statistical physics of DNA

E.g. Hybridization



Statistical physics of DNA

E.g. Hybridization



(Sequence dependence of) these processes for short DNA sequences

Non-trivial physics at this scale

High technological relevance: e.g.

Primers in PCR

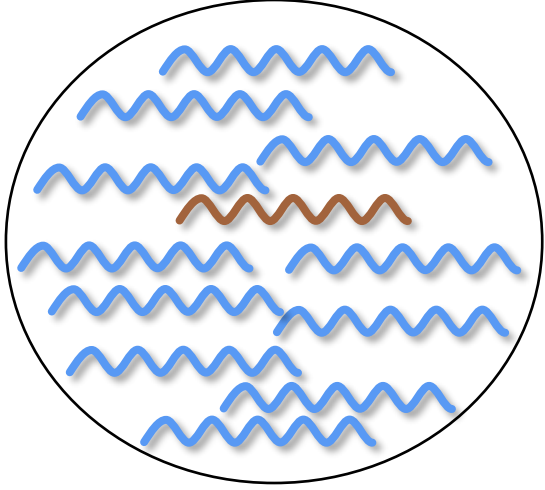
Probes in biosensors

DNA self-assembling structures

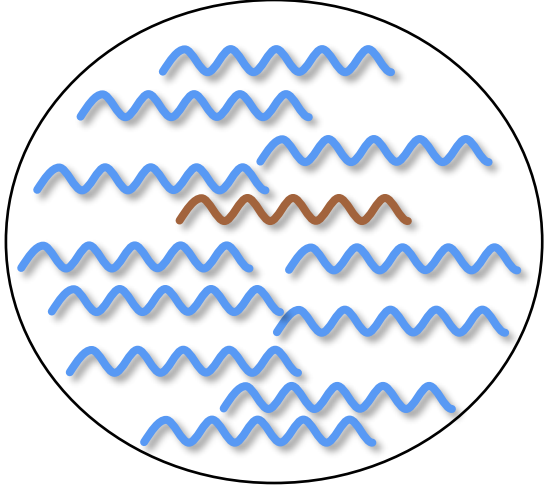
NA enrichment

...

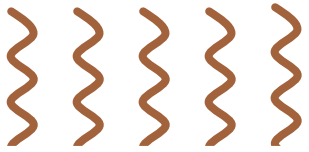
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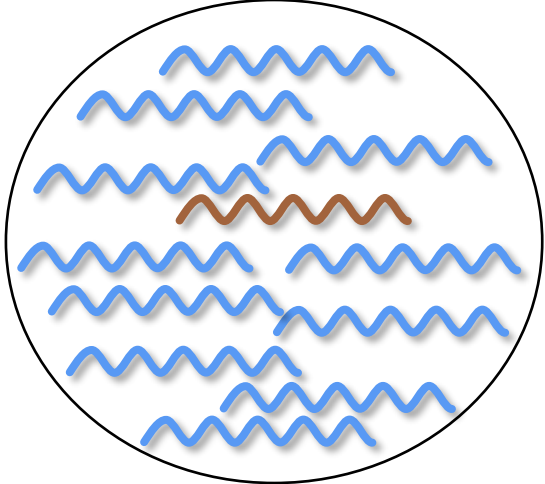
Target



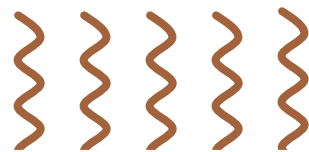
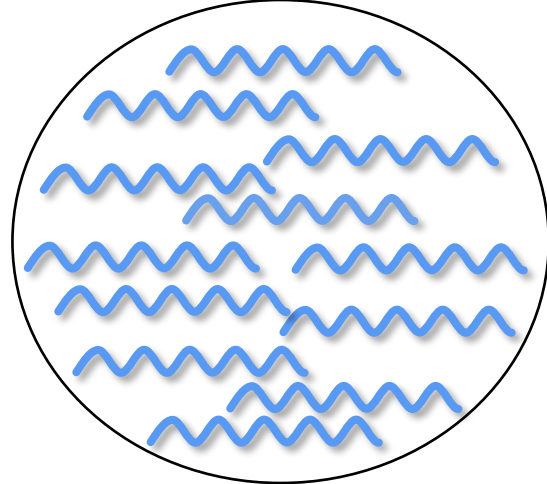
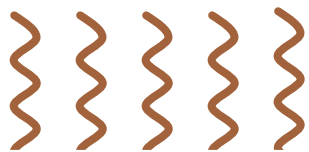
Probe



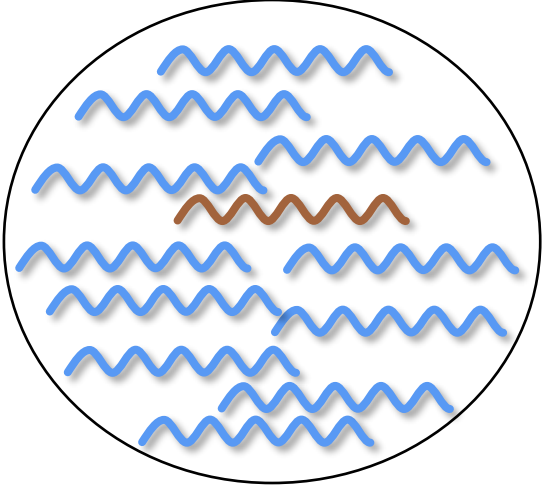
Target



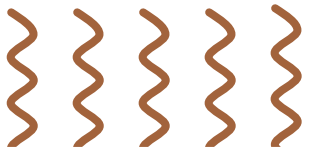
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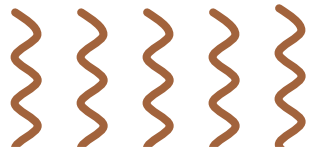
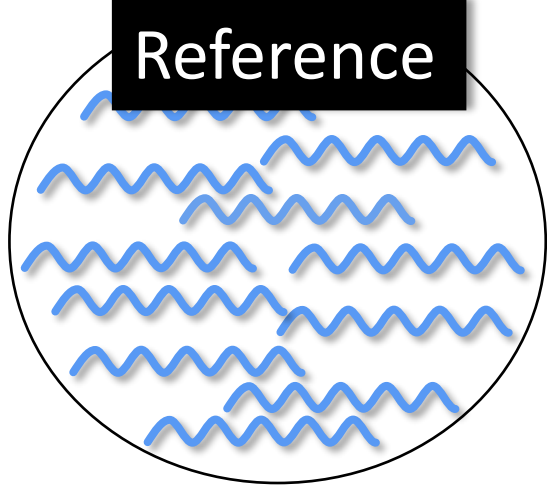
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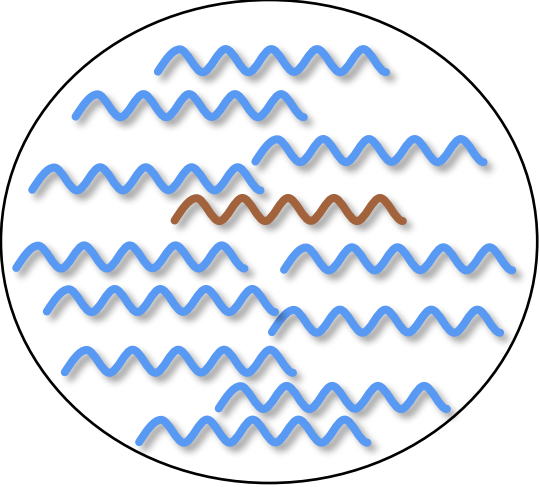
Probe



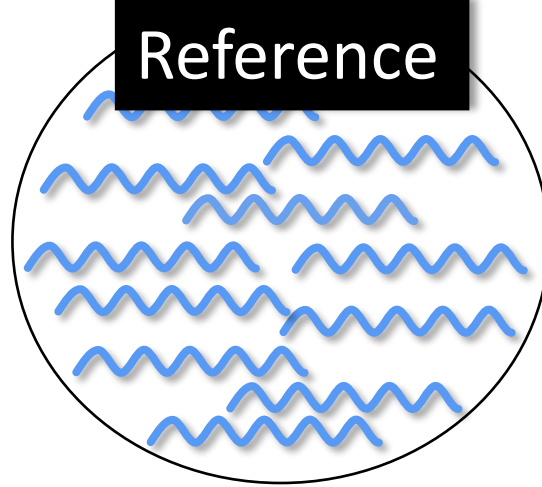
Reference



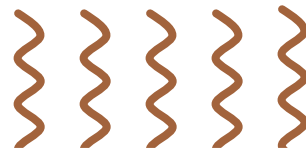
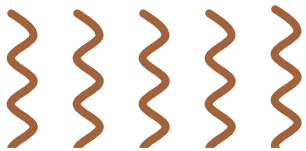
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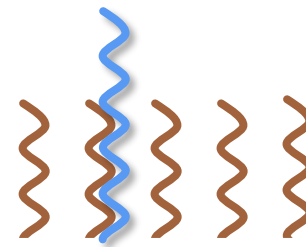
Reference



Probe



Hybridization



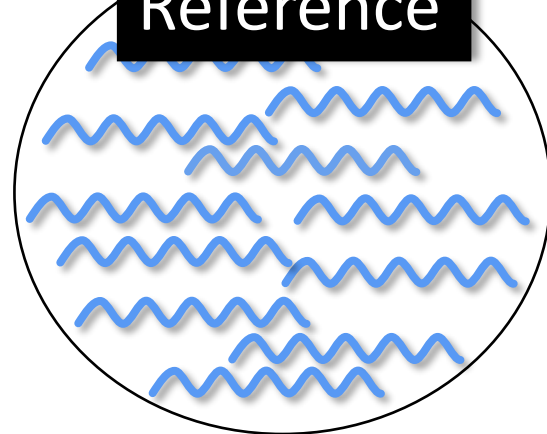
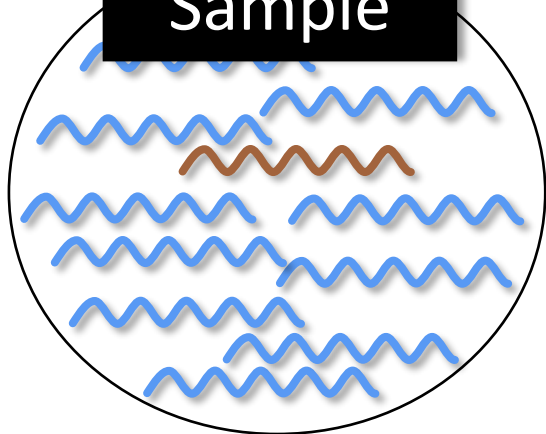
Readout Intensity

I_{ref}

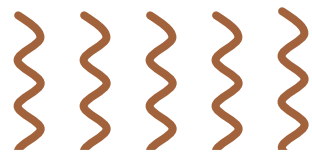
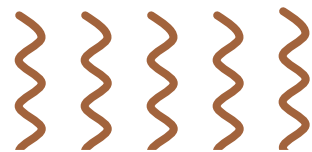
Sample

Reference

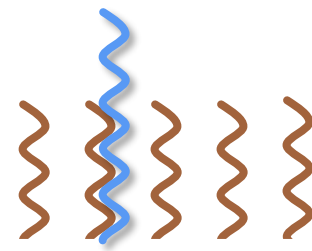
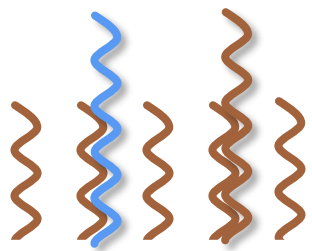
Target



Probe

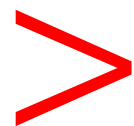


Hybridization



Readout Intensity

I_{sample}

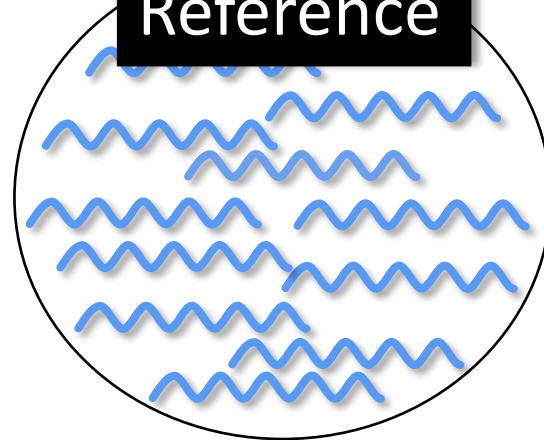
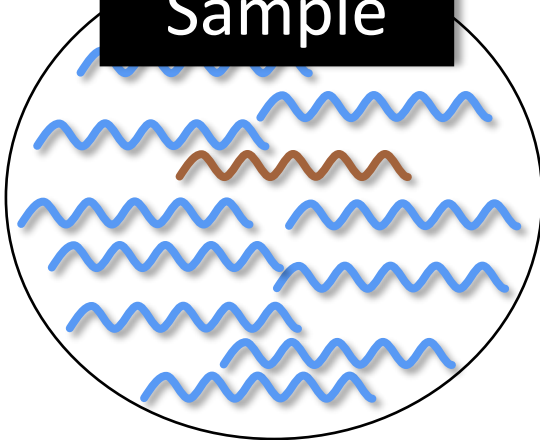


I_{ref}

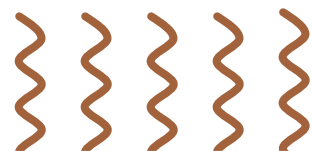
Sample

Reference

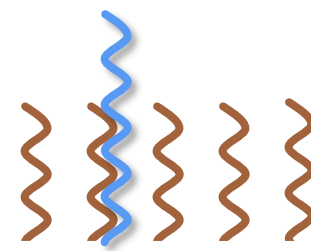
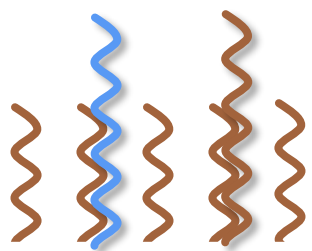
Target



Probe

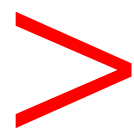


Hybridization



Readout Intensity

I_{sample}

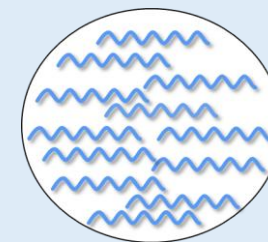


I_{ref}

Signalling quantity

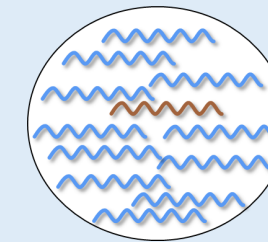
$$S = \log \left(\frac{I_{sample}}{I_{ref}} \right)$$

$S = 0$



No mutant

$S > 0$



Mutant

Signalling quantity

$$S = \log \left(\frac{I_{sample}}{I_{ref}} \right) = \log \left(1 + \frac{c_{mut}}{c_{wt}} e^{\Delta\Delta G/RT} \right)$$

Signalling quantity

$$S = \log \left(\frac{I_{sample}}{I_{ref}} \right) = \log \left(1 + \frac{c_{mut}}{c_{wt}} e^{\Delta\Delta G/RT} \right)$$

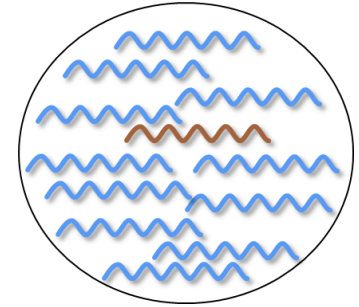
Concentrations

mutant

wild type

c_{mut}

c_{wt}



Signalling quantity

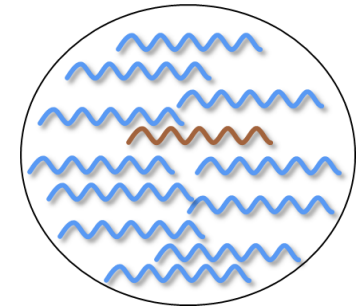
$$S = \log \left(\frac{I_{sample}}{I_{ref}} \right) = \log \left(1 + \frac{c_{mut}}{c_{wt}} e^{\Delta\Delta G/RT} \right)$$

Concentrations

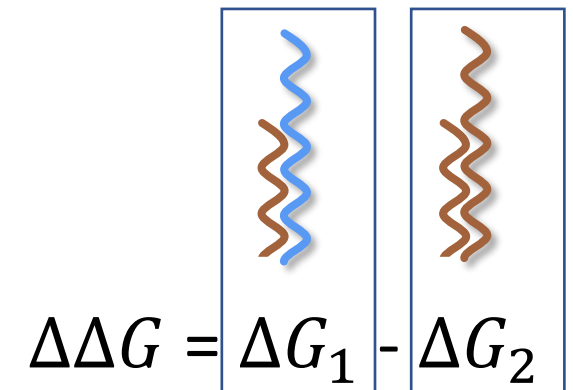
mutant wild type

c_{mut}

c_{wt}

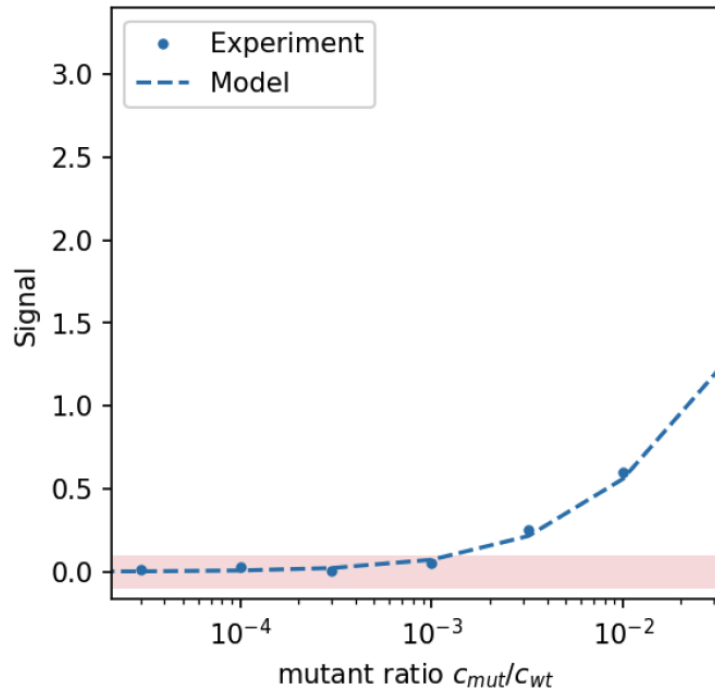


Free energy penalty



Signalling quantity

$$S = \log \left(\frac{I_{sample}}{I_{ref}} \right) = \log \left(1 + \frac{c_{mut}}{c_{wt}} e^{\Delta\Delta G/RT} \right)$$



Sensors & Actuators: B. Chemical 368 (2022) 132175

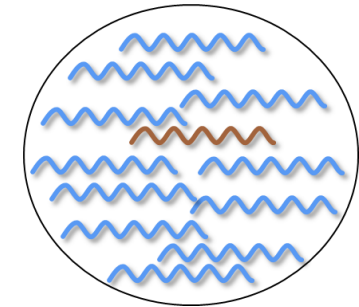
Rebekka Van Hoof^{a,b,c,1}, Michal Szymonik^{a,2,3}, Stefanos K. Nomidis^{a,c,4}, Karen Hollanders^{a,5}, An Jacobs^{a,6}, Inge Nelissen^{a,7}, Patrick Wagner^{c,8}, Jef Hooyberghs^{a,b,d,*,9}

Concentrations

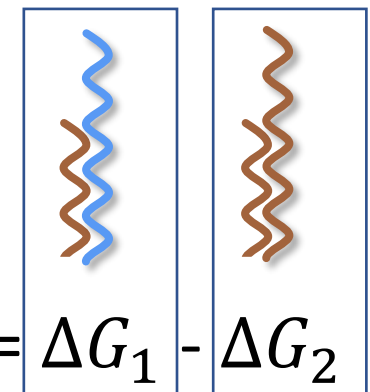
mutant wild type

c_{mut}

c_{wt}



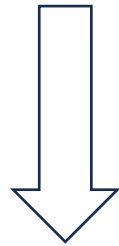
Free energy penalty



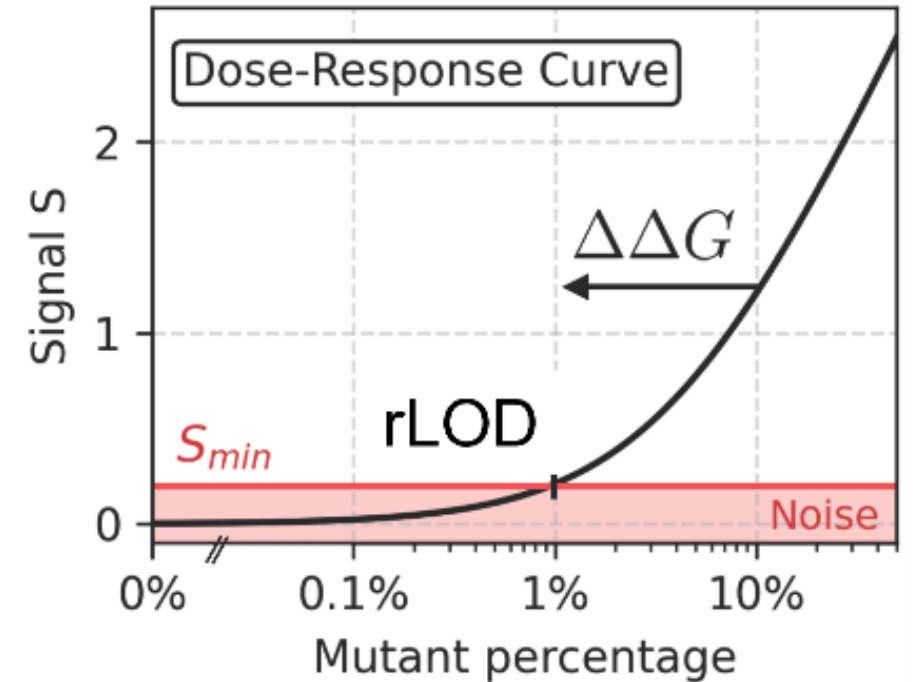
$$\Delta\Delta G = \Delta G_1 - \Delta G_2$$

Signalling quantity \rightarrow Relative Limit of Detection (rLOD)

$$S = \log\left(\frac{I_{sample}}{I_{ref}}\right) = \log\left(1 + \frac{c_{mut}}{c_{wt}} e^{\Delta\Delta G/RT}\right)$$



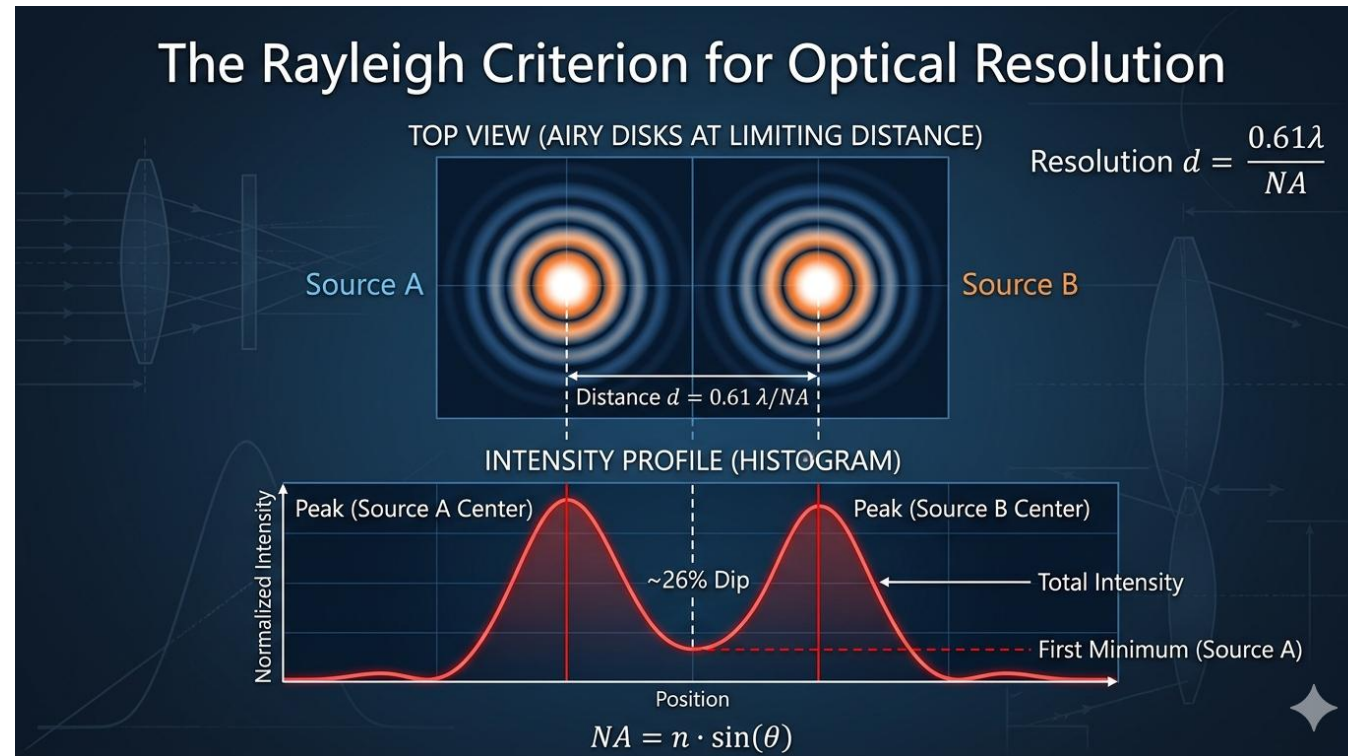
$$rLOD = S_{min} e^{-\Delta\Delta G/RT}$$



$$d = \left(\frac{0.61}{NA} \right) \lambda$$

Optical instrument

Physical property of light



Picture by Gimini

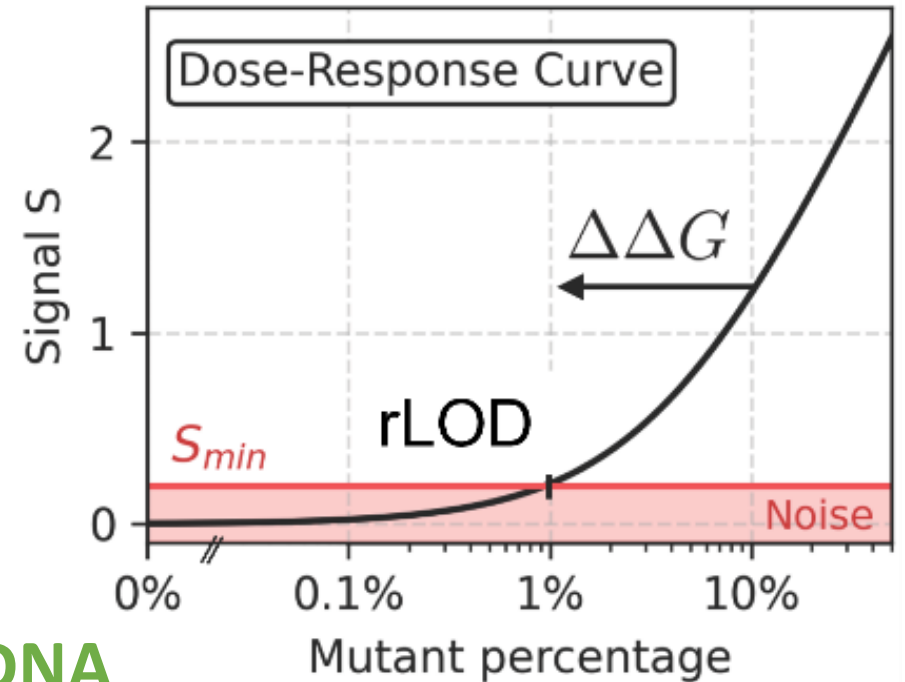
Signalling quantity -> Relative Limit of Detection (rLOD)

$$S = \log\left(\frac{I_{sample}}{I_{ref}}\right) = \log\left(1 + \frac{c_{mut}}{c_{wt}} e^{\Delta\Delta G/RT}\right)$$

$$rLOD = S_{min} e^{-\Delta\Delta G/RT}$$

Readout instrument

Physical property of DNA



$$rLOD = S_{min} e^{-\Delta\Delta G/RT}$$

Readout instrument

Physical property of DNA

$\Delta\Delta G = \text{physics}$

Is this a hard boundary?

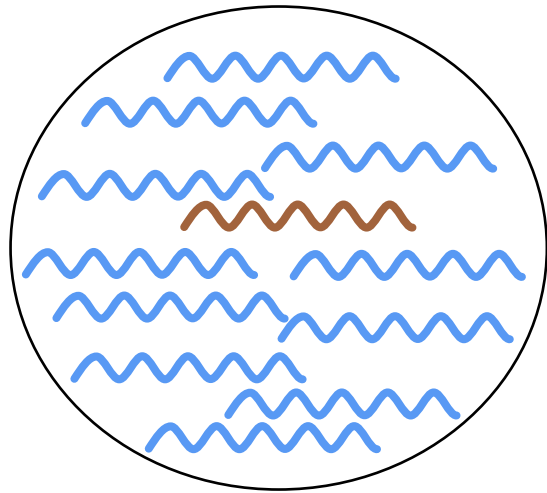
$\Delta\Delta G = \text{physics}$

Is this a hard boundary?

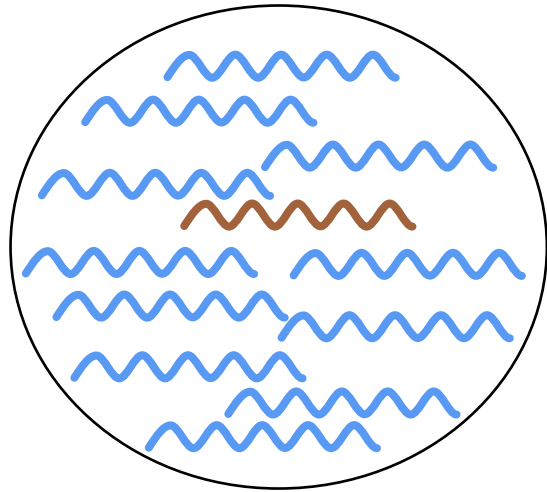
Stretching the physical boundaries
of DNA hybridization
for mutation detection

Option1: Depletion of wild type

Target



Target



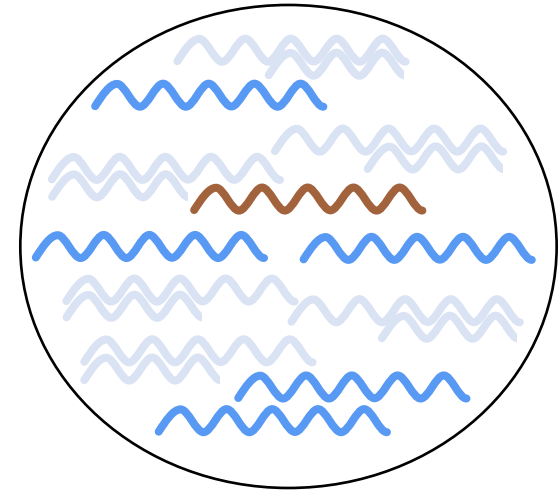
“Blocker”



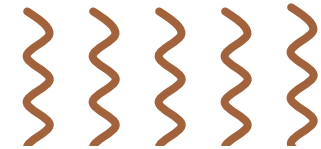
+

=

Depleted wild type



Probe



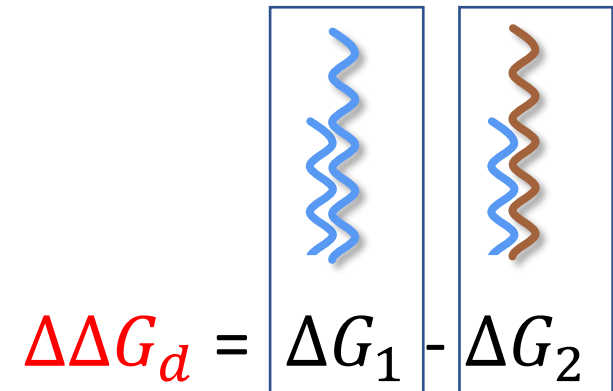
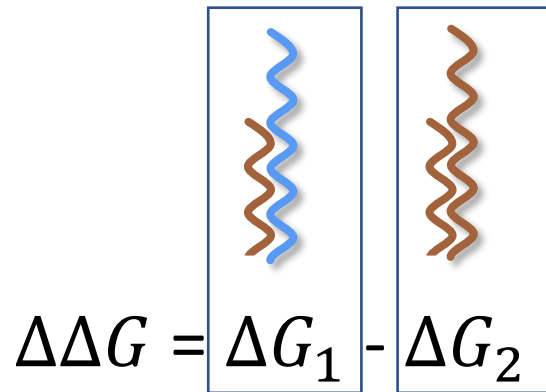
$$S = \log \left(1 + \frac{c_{mut}}{c_{wt}} e^{\Delta\Delta G/RT} \right)$$

$$S = \log \left(1 + \frac{c_{mut}}{c_{wt}} e^{(\Delta\Delta G + \Delta\Delta G_d)/RT} \right)$$



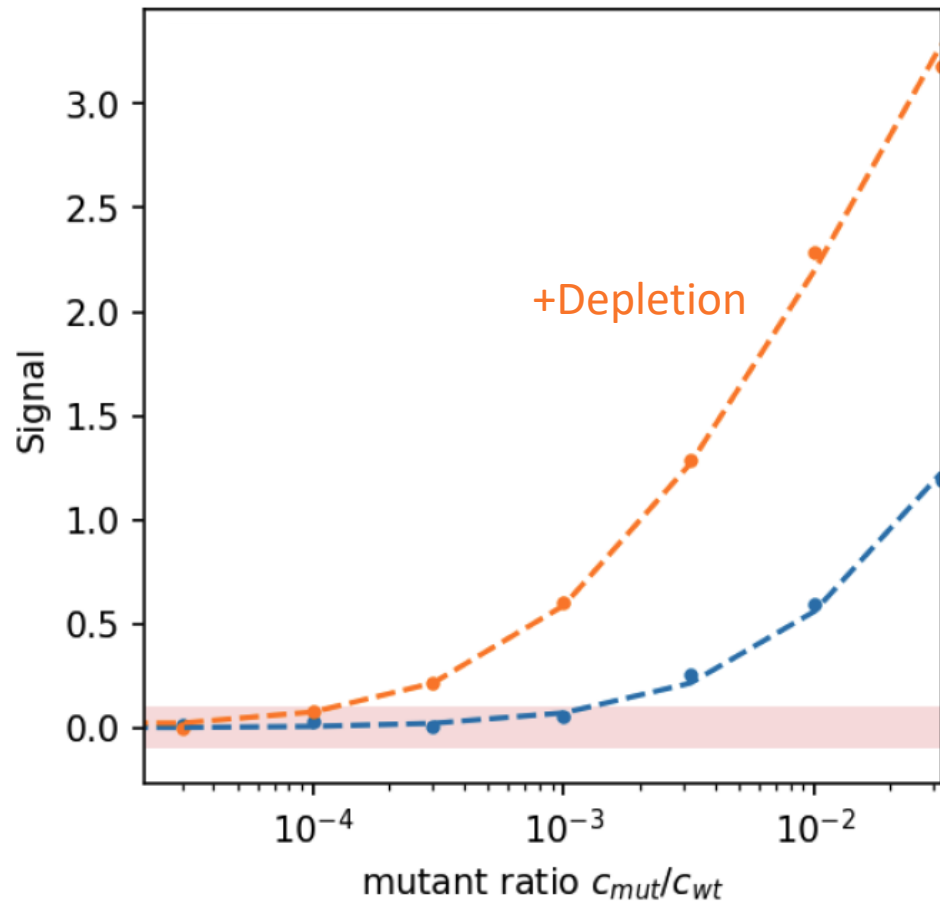
Depletion of wild type

Free energy penalty



$$S = \log\left(1 + \frac{c_{mut}}{c_{wt}} e^{\Delta\Delta G/RT}\right)$$

$$S = \log\left(1 + \frac{c_{mut}}{c_{wt}} e^{(\Delta\Delta G + \Delta\Delta G_d)/RT}\right)$$

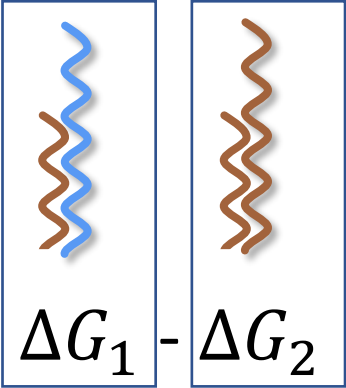


Confirmed on clinical samples

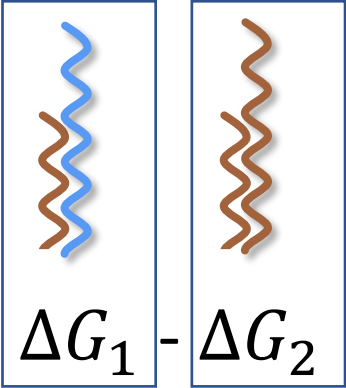
Biosensors and Bioelectronics 281 (2025) 117342

Yannick Stulens ^a, ¹, Rebekka Van Hoof ^{a,b,c}, ¹, Karen Hollanders ^b, Inge Nelissen ^b,
Michal Szymonik ^b, Patrick Wagner ^c, Guy Froyen ^{d,e}, Brigitte Maes ^{d,e},
Jef Hooyberghs ^a, *

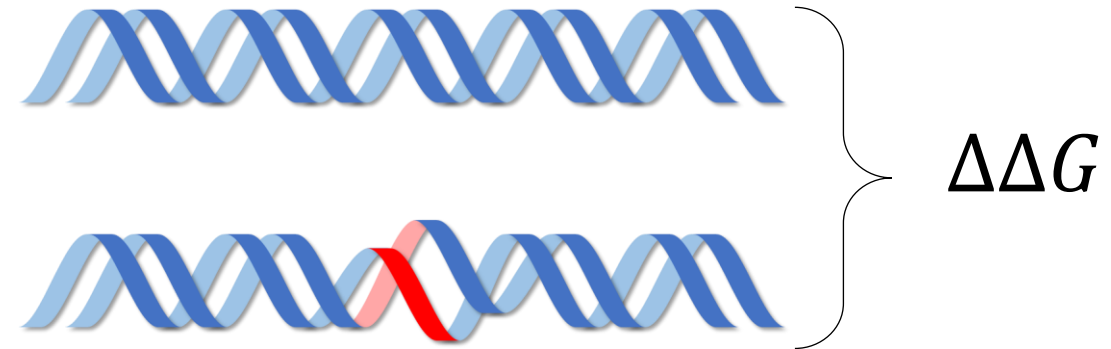
Option2: Literally stretching the boundaries

$$\Delta\Delta G = \Delta G_1 - \Delta G_2$$


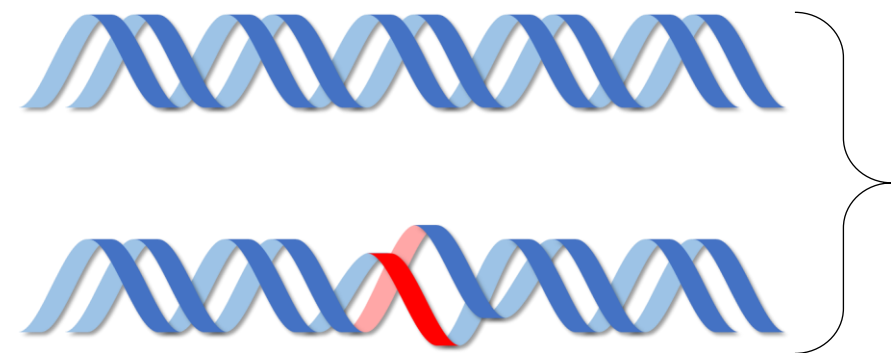
Free energy penalty
of **mutation**

$$\Delta\Delta G = \Delta G_1 - \Delta G_2$$


Free energy penalty
of **mutation**

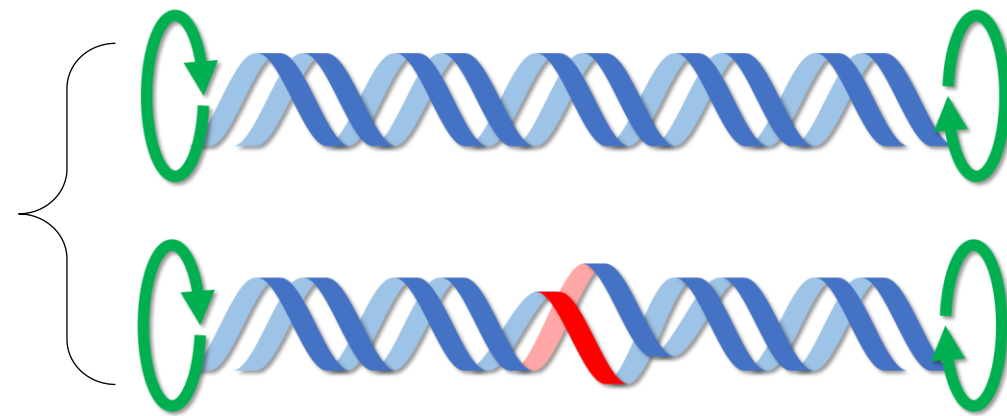


Free energy penalty
of **mutation**



No strain

$$\Delta\Delta G \neq \Delta\Delta G'$$



Strain

$$\Delta\Delta G' = \Delta\Delta G + \Delta\Delta G_s$$

$$S = \log\left(1 + \frac{c_{mut}}{c_{wt}} e^{\Delta\Delta G/RT}\right)$$

$$S = \log\left(1 + \frac{c_{mut}}{c_{wt}} e^{(\Delta\Delta G + \Delta\Delta G_d)/RT}\right)$$

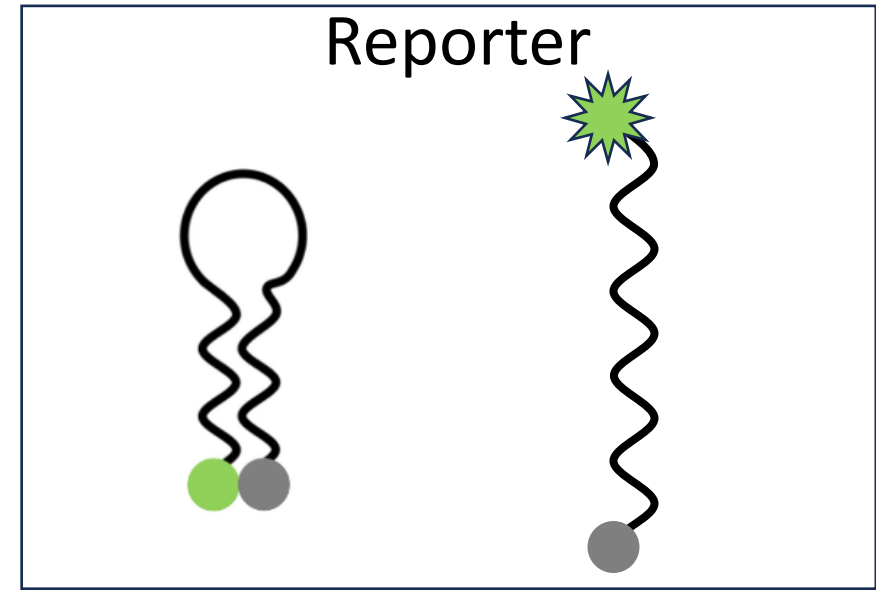
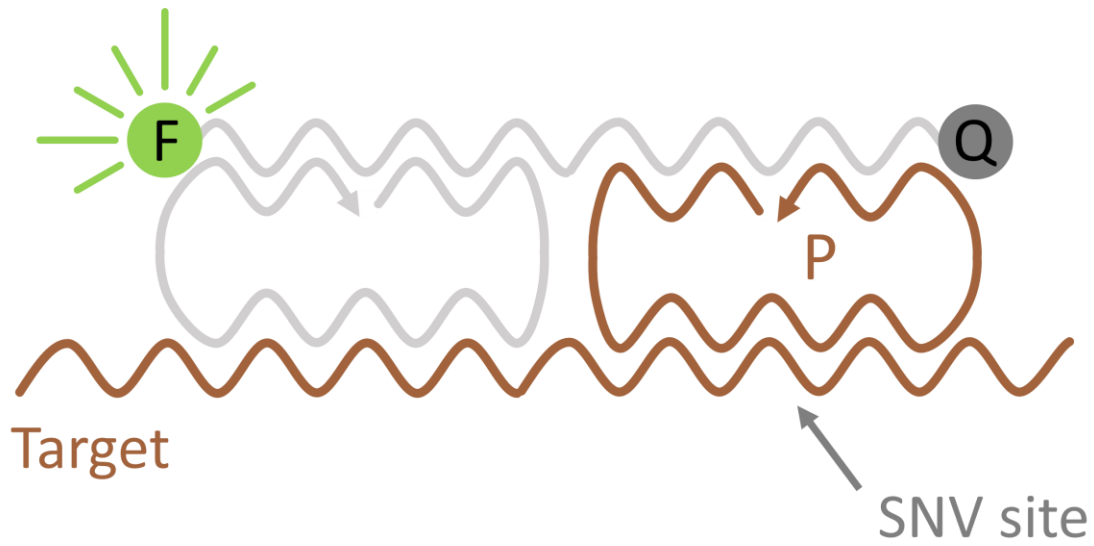
$$S = \log\left(1 + \frac{c_{mut}}{c_{wt}} e^{(\Delta\Delta G + \Delta\Delta G_d + \Delta\Delta G_s)/RT}\right)$$



Depletion of wild type



Strain

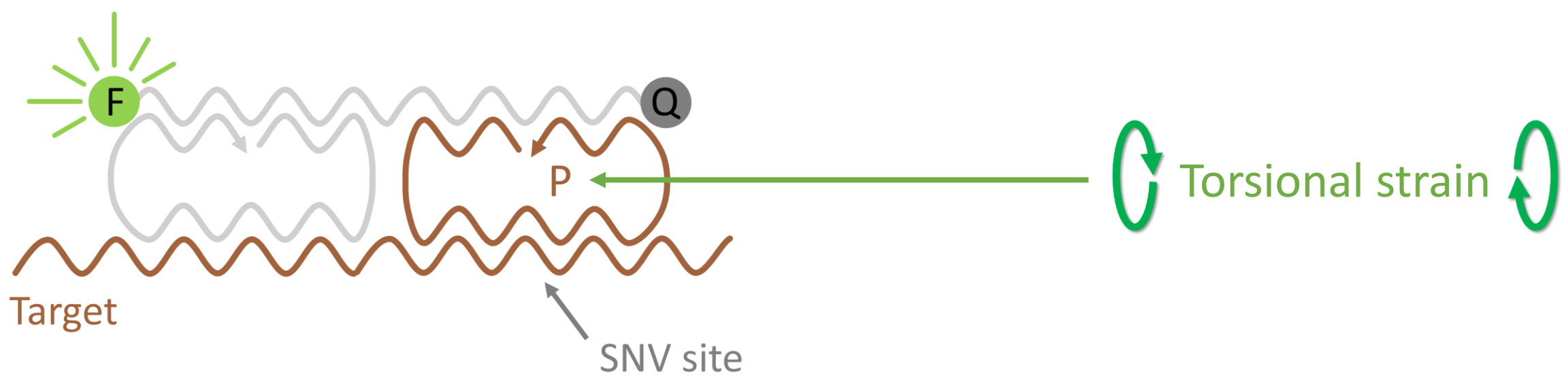


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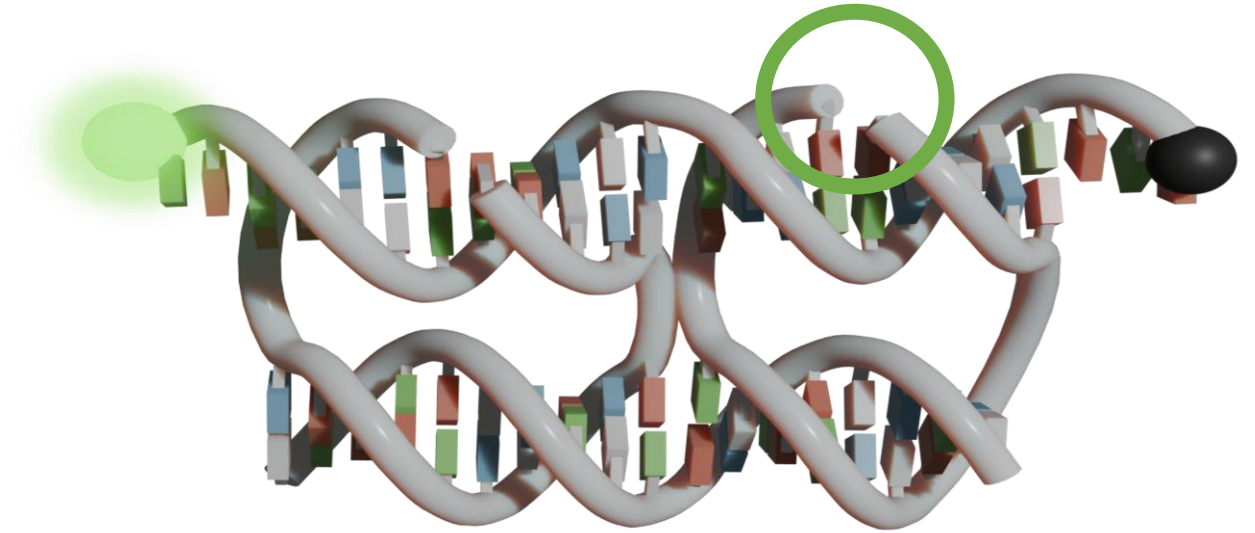
D. Kolpashchikov, B. Mueller

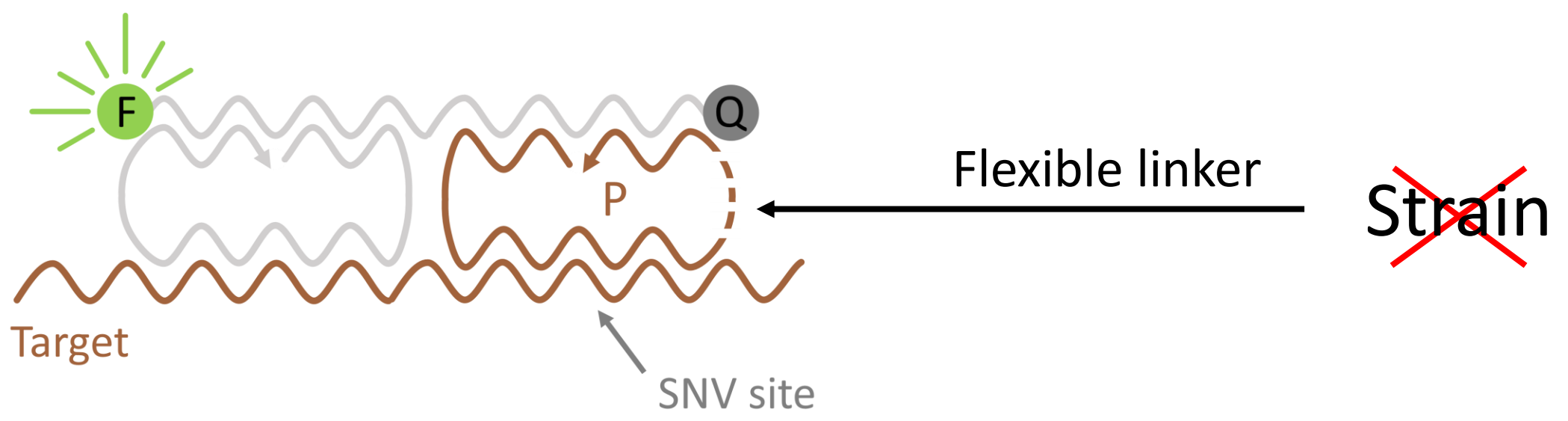


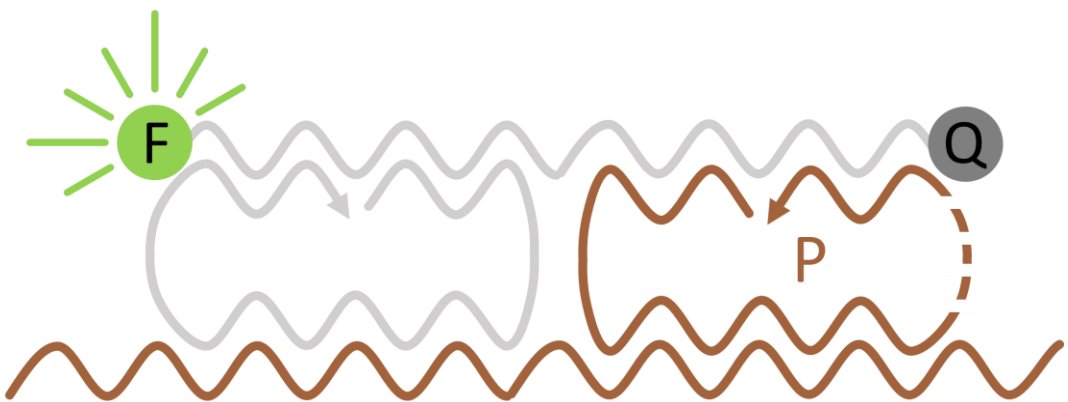
J. Hooyberghs, Y. Stulens



Locked ends

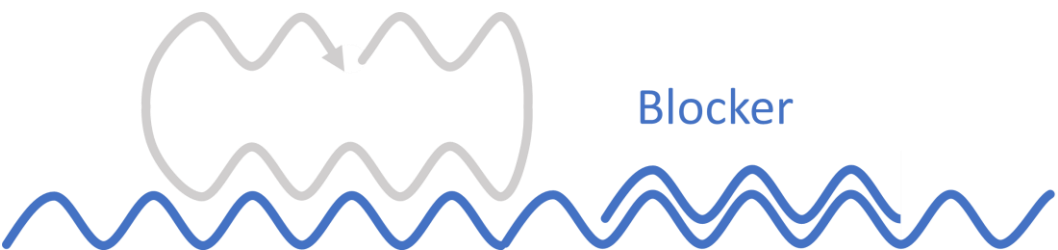






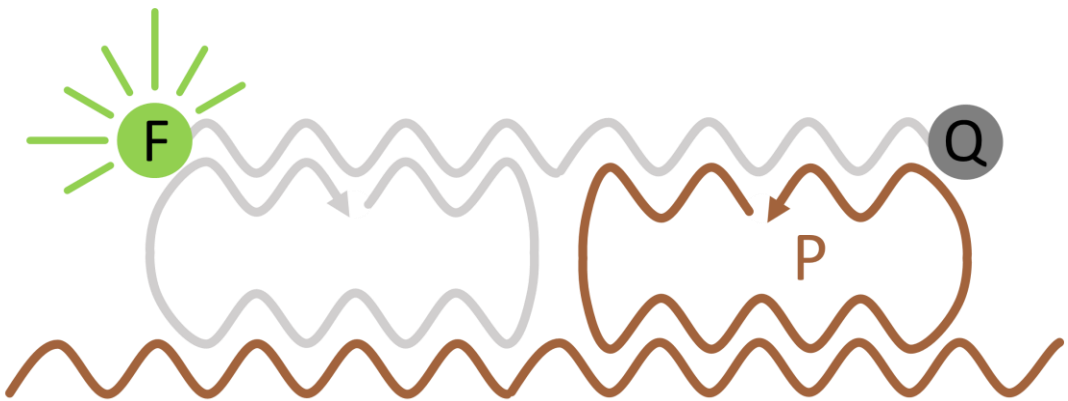
Standard

$$\Delta\Delta G$$



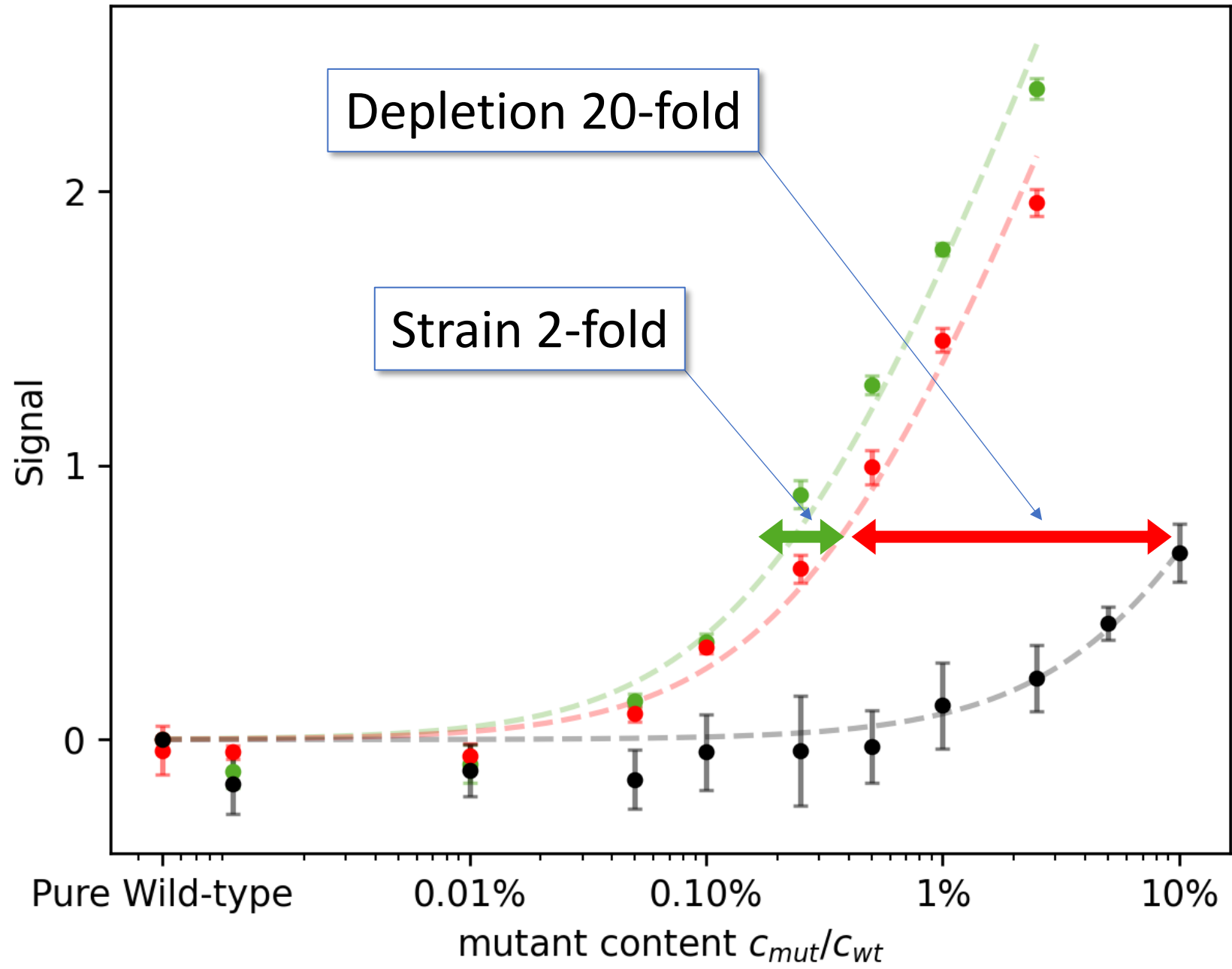
+ Depletion

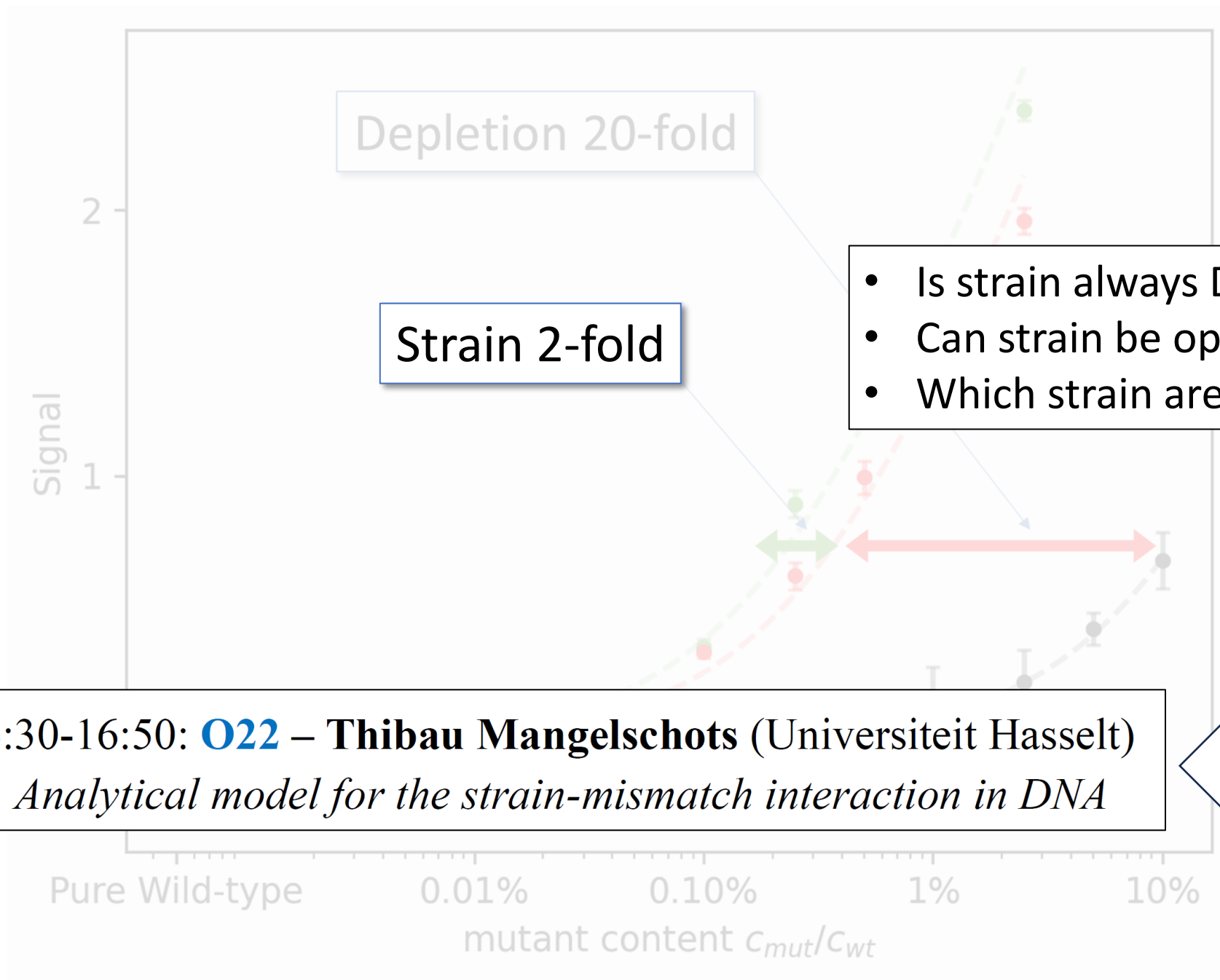
$$\Delta\Delta G + \Delta\Delta G_d$$



+ Strain

$$\Delta\Delta G + \Delta\Delta G_d + \Delta\Delta G_s$$





Depletion 20-fold

Strain 2-fold

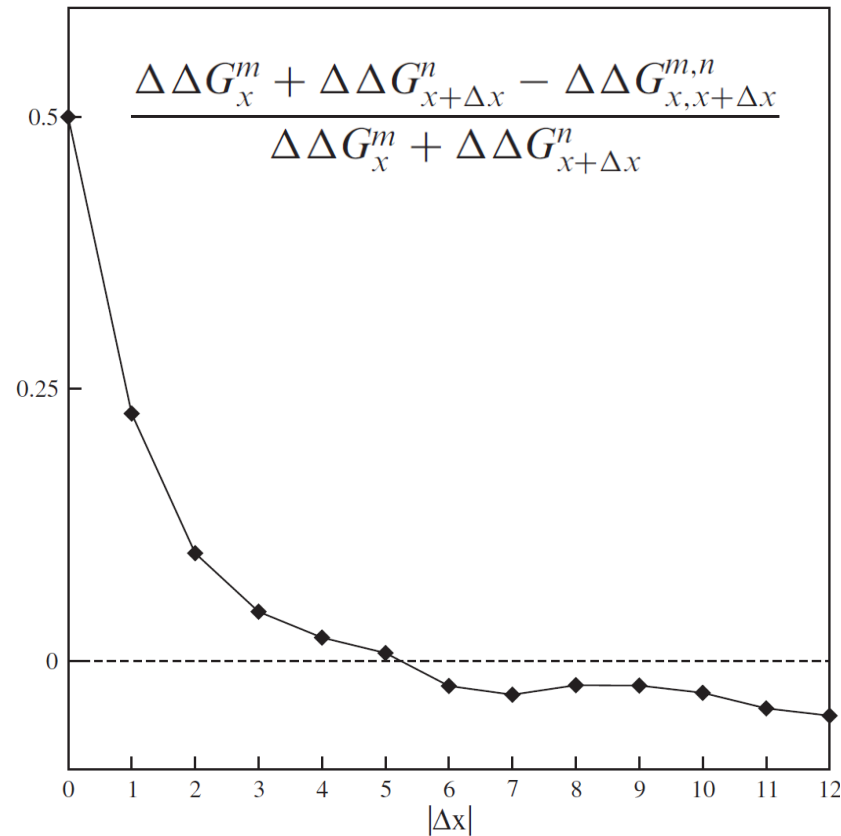
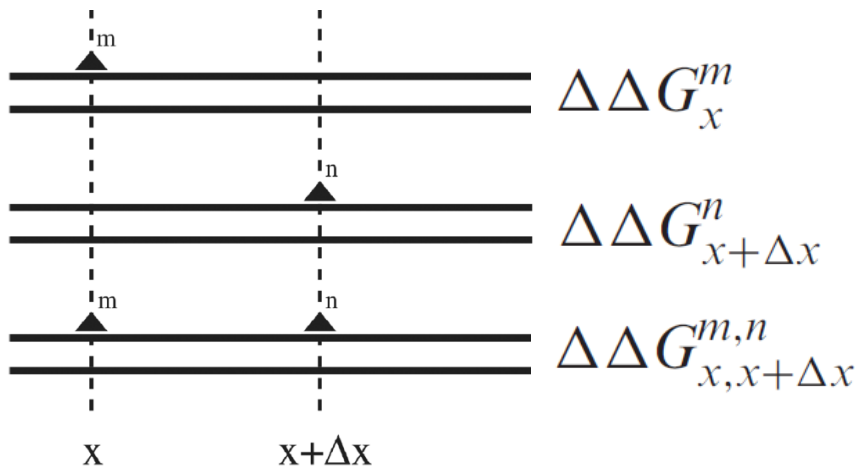
- Is strain always Destabilizing?
- Can strain be optimized?
- Which strain are we talking about?

16:30-16:50: **O22** – **Thibau Mangelschots** (Universiteit Hasselt)
Analytical model for the strain-mismatch interaction in DNA

- Option1: Depletion of wild type
- Option2: Strain-mismatch interaction
- Option3: ? Mismatch-mismatch interaction

Probing hybridization parameters from microarray experiments: nearest-neighbor model and beyond

W. W. Hadiwikarta^{1,2}, J.-C. Walter², J. Hooyberghs^{1,3} and E. Carlon^{2,*}



“On average”

Clearly stabilizing

Hence:

non-favorable

¹OncoRNALab, Department of Biomolecular Medicine, Ghent University, 9000, Ghent, Belgium

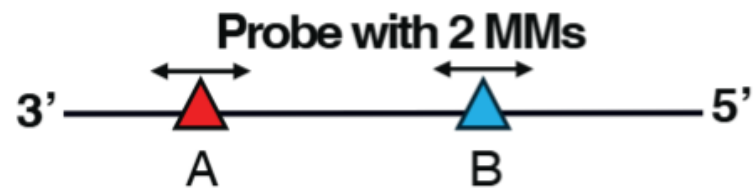
²UHasselt, Data Science Institute, Theory Lab, Agoralaan, 3590, Diepenbeek, Belgium

³Antwerp Engineering, Photoelectrochemistry and Sensing (A-PECS), Department of Bioscience Engineering, University of Antwerp, 2020, Antwerp, Belgium

⁴NANOlight Center of Excellence, University of Antwerp, 2020, Antwerp, Belgium

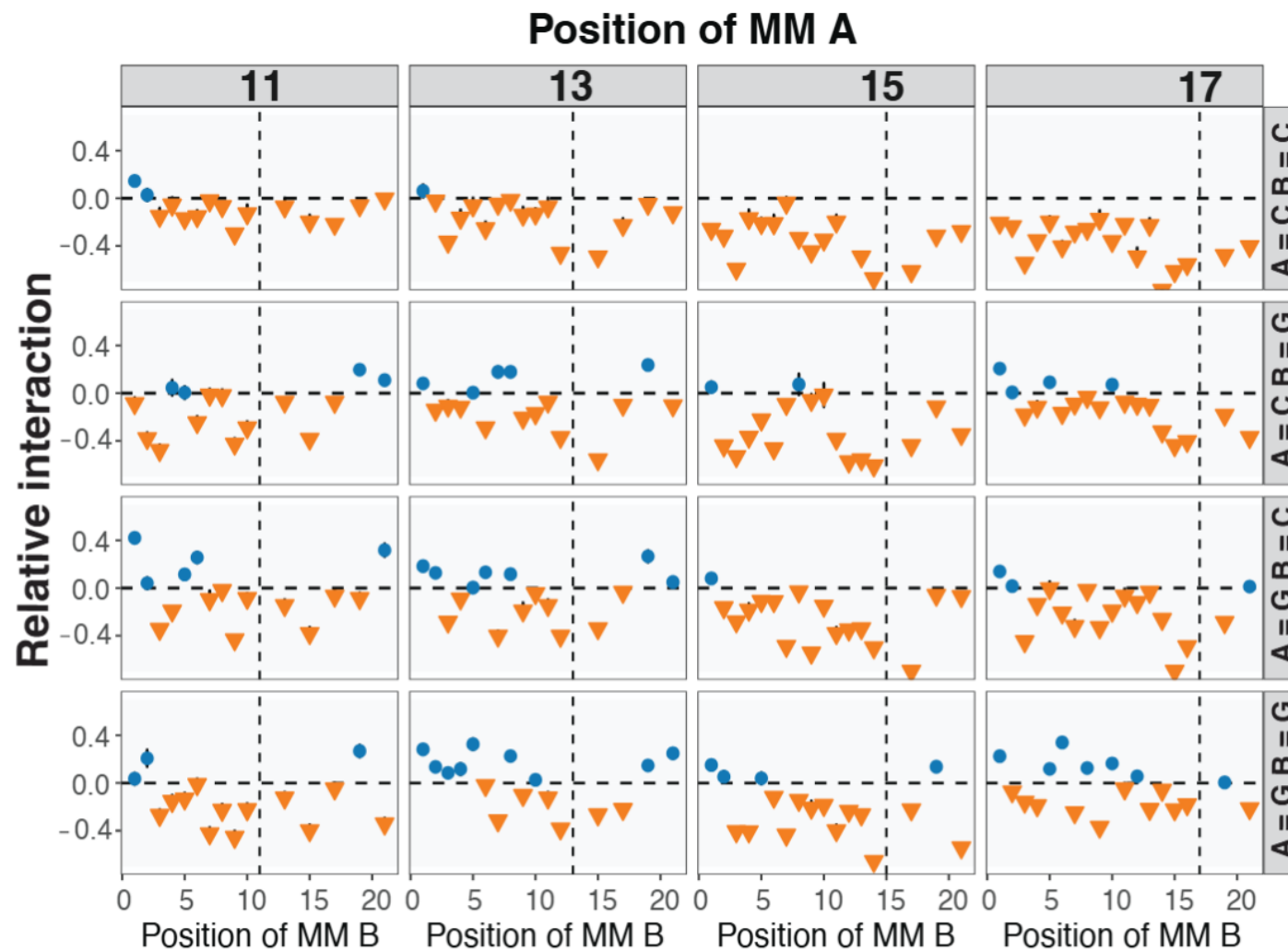
[†]Thijs Van der Snickt and Yannick Stulens contributed equally to this work

^{*}Corresponding authors. jef.hooyberghs@uhasselt.be and pieter.mestdagh@ugent.be



A = 21, 19, 17, 15, 13 or 11

B = every 5' end position from A



$$rLOD = S_{min} e^{-\Delta\Delta G/RT}$$

$\Delta\Delta G$ = physical property of DNA

This boundary can be stretched

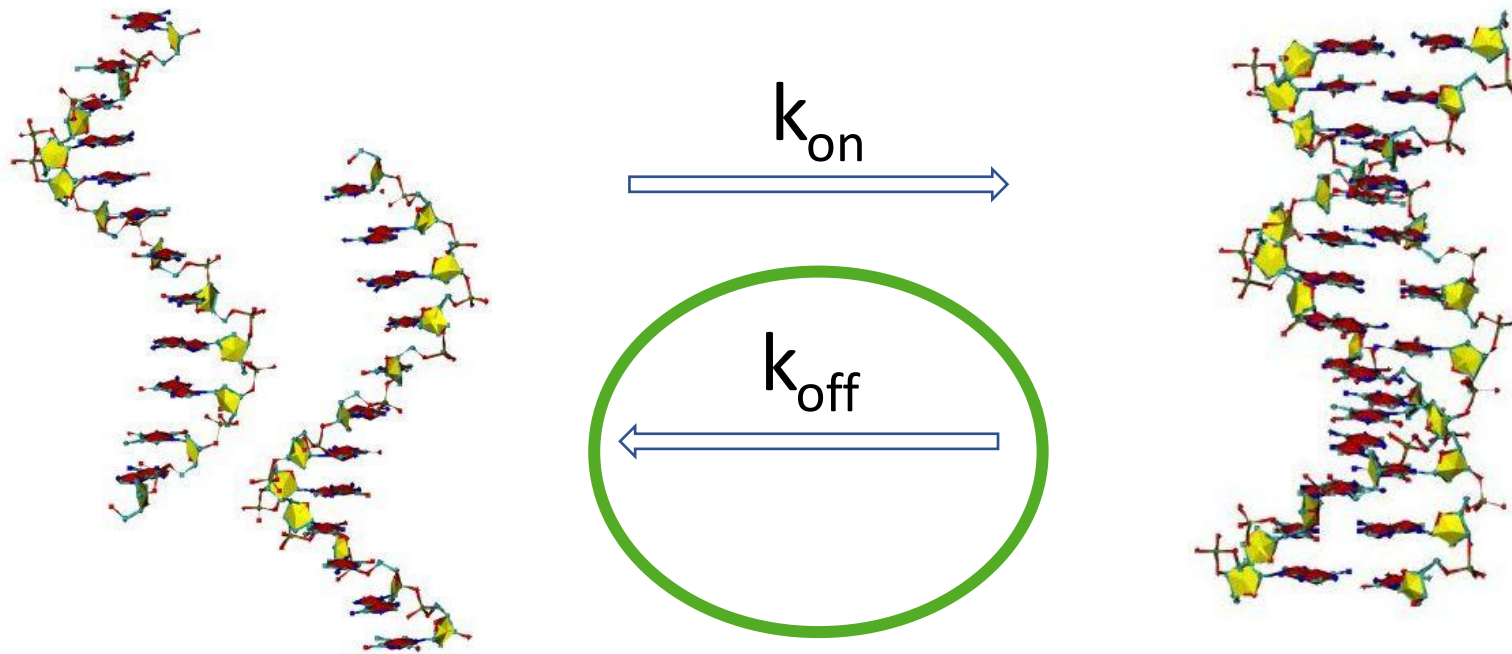
Option1: Depletion of wild type

Option2: Strain-mismatch interaction

Option3: Mismatch-mismatch interaction

What about kinetics, out-of-equilibrium ?

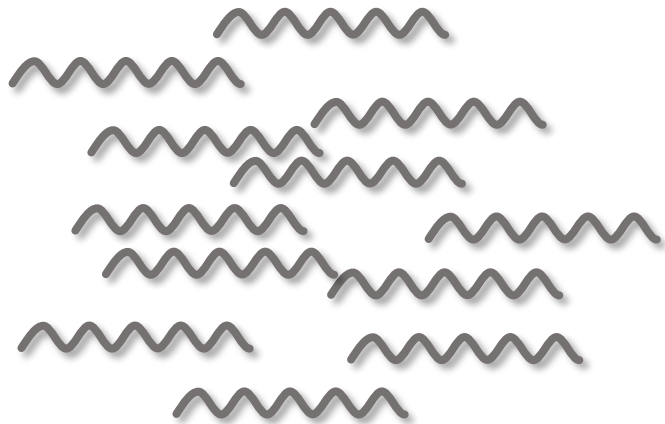
What about kinetics, out-of-equilibrium ?



What about kinetics, out-of-equilibrium ?

1. Step response

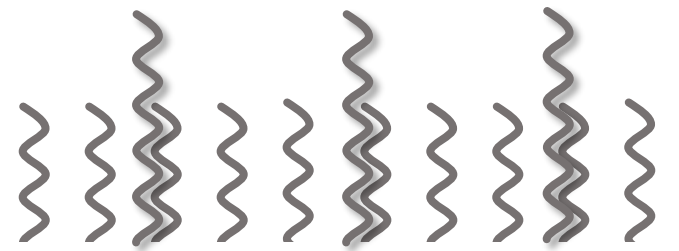
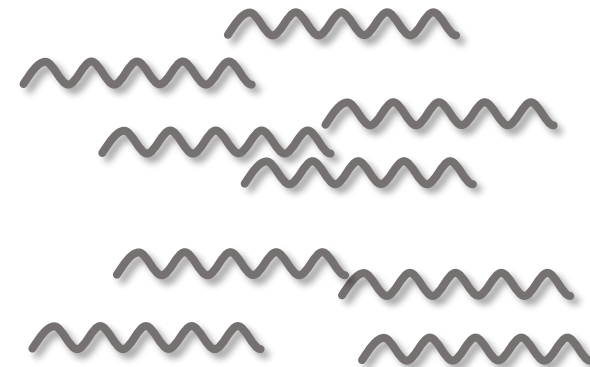
Targets



Time



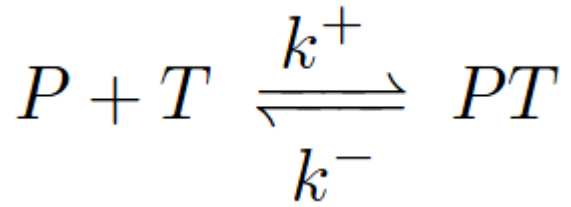
Probes



Duplex concentration = 0

Duplex concentration > 0

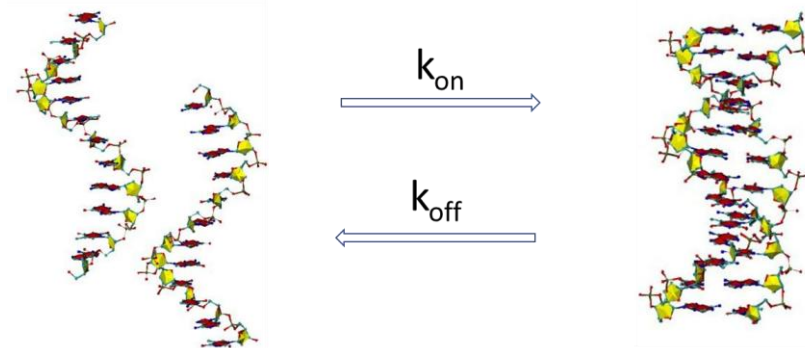
What about kinetics?



$$\frac{d[PT]}{dt} = k^+ [P][T] - k^- [PT]$$

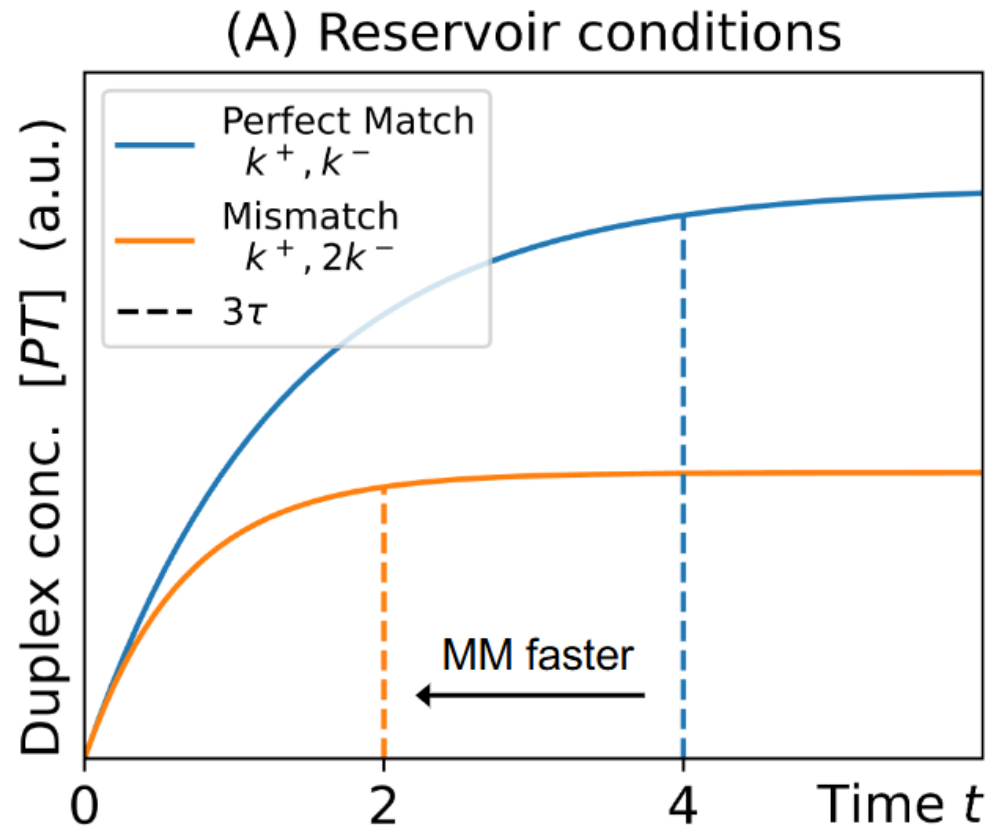
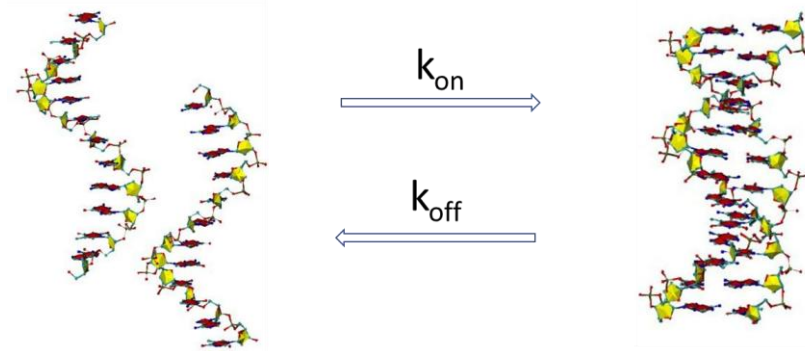
- $[P]_0 = a$ and $[T]_0 = c$
- Reservoir conditions

$$\frac{d[PT]}{dt} = k^+ c a - k^- [PT],$$

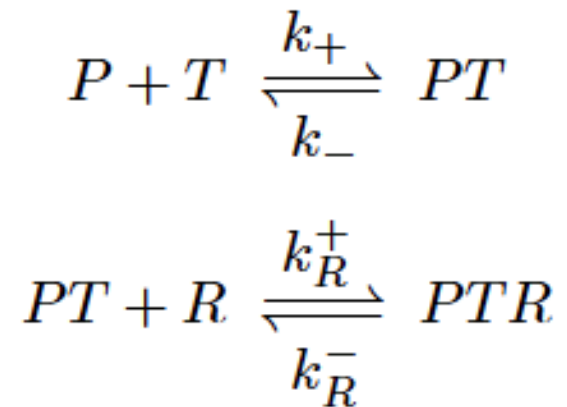


What about kinetics?

- Out-of-equilibrium

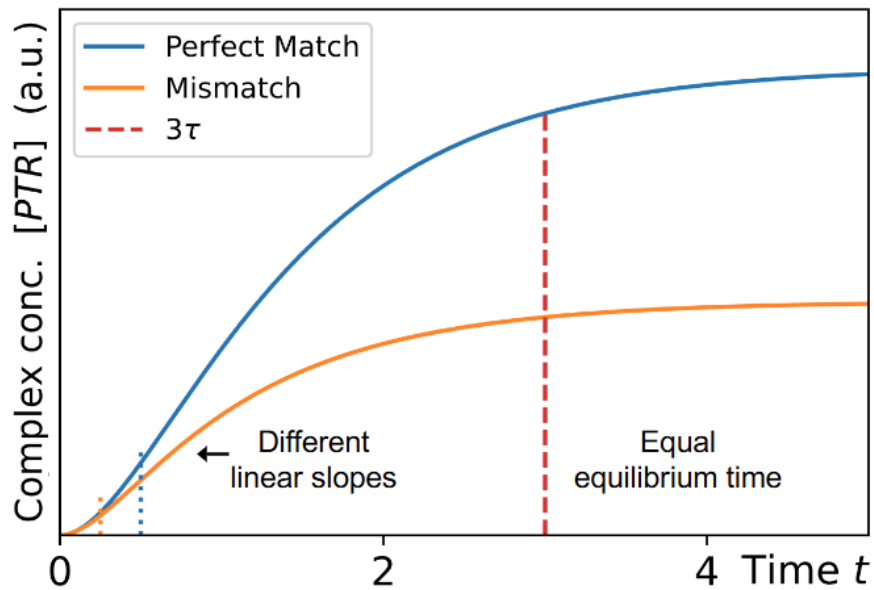


Two-step process with reporter

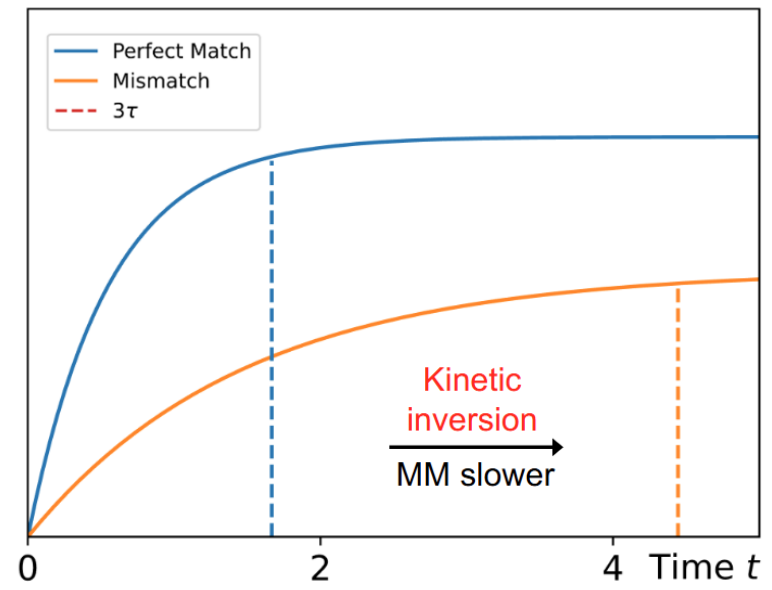


Kinetic discrimination of mismatch

Low reporter binding



Reporter saturation

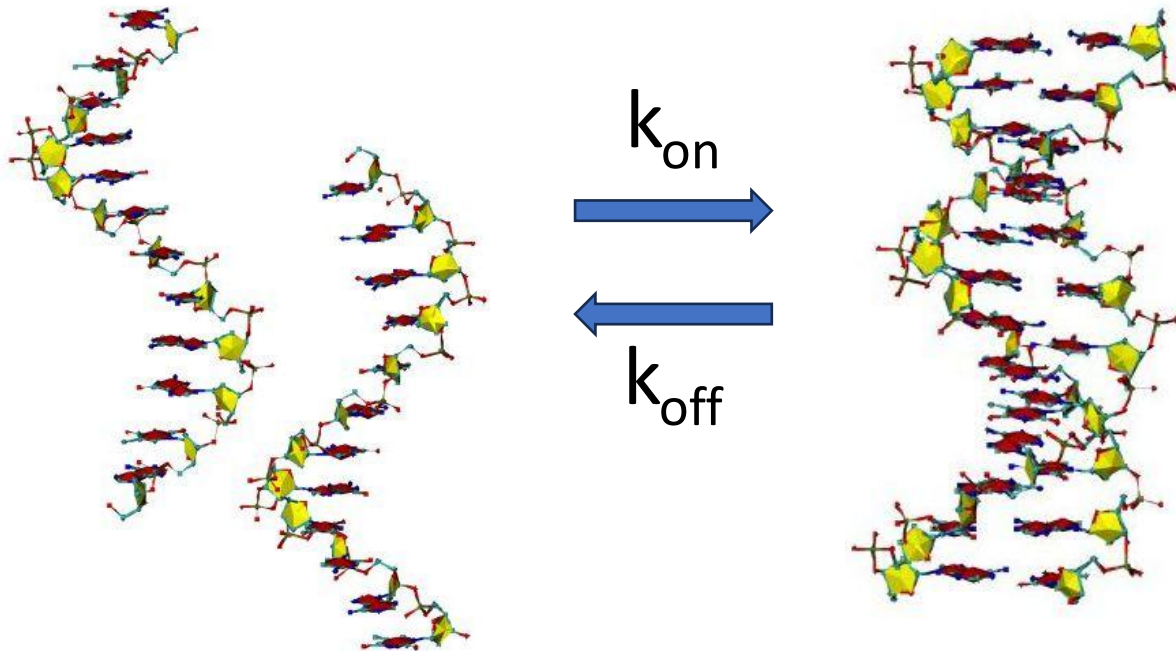


What about kinetics, out-of-equilibrium ?

1. Step response
2. Frequency response

Frequency response

$$\frac{k_{off}}{k_{on}} = e^{-\Delta G/k_B T}$$



Local in equilibrium:

transition rates
obey detailed balance wrt
instantaneous equilibrium distribution.

$$T(t) = T_{eq} + \delta T \sin(\omega t)$$

Frequency response

$$T(t) = T_{eq} + \delta T \sin(\omega t)$$

16:50-17:10: **O23** – **Robbe Van Haverbeke** (Universiteit Hasselt)

Characterizing biomolecular kinetics through out-of-equilibrium periodic temperature perturbations

What about kinetics, out-of-equilibrium ?

1. Step response
2. Frequency response
3. Fundamentally out-of-equilibrium

Fundamentally out-of-equilibrium

- Including an irreversible reaction step

Kinetic Proofreading: A New Mechanism for Reducing Errors in Biosynthetic Processes Requiring High Specificity

(protein synthesis/DNA replication/and

J. J. HOPFIELD

Department of Physics, Princeton University, Princeton, New Jersey 08540; and Bell Laboratories, Murray Hill, New Jersey 07974

Contributed by John J. Hopfield, August 6, 1974



Pepijn Moerman

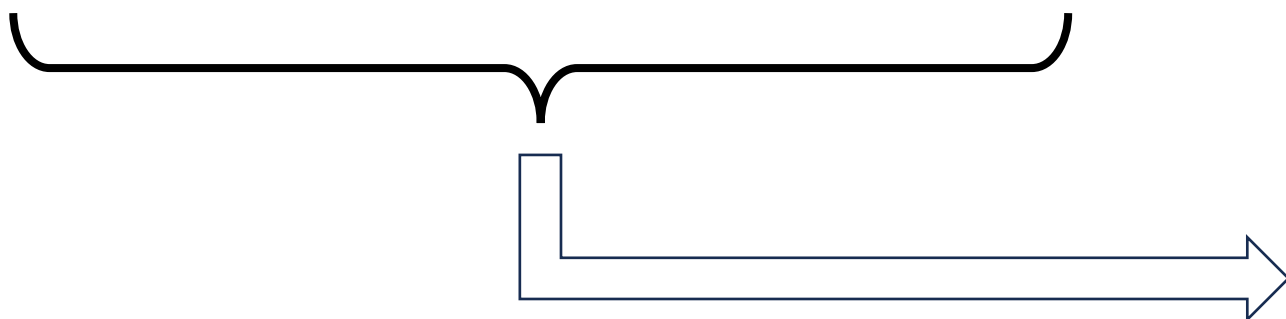


Jef Hooyberghs

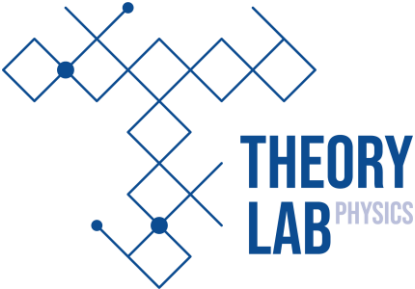


**Belgian Physical
Society general
scientific meeting**

2027



Thank you!



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