Temperature effects on Microarrays

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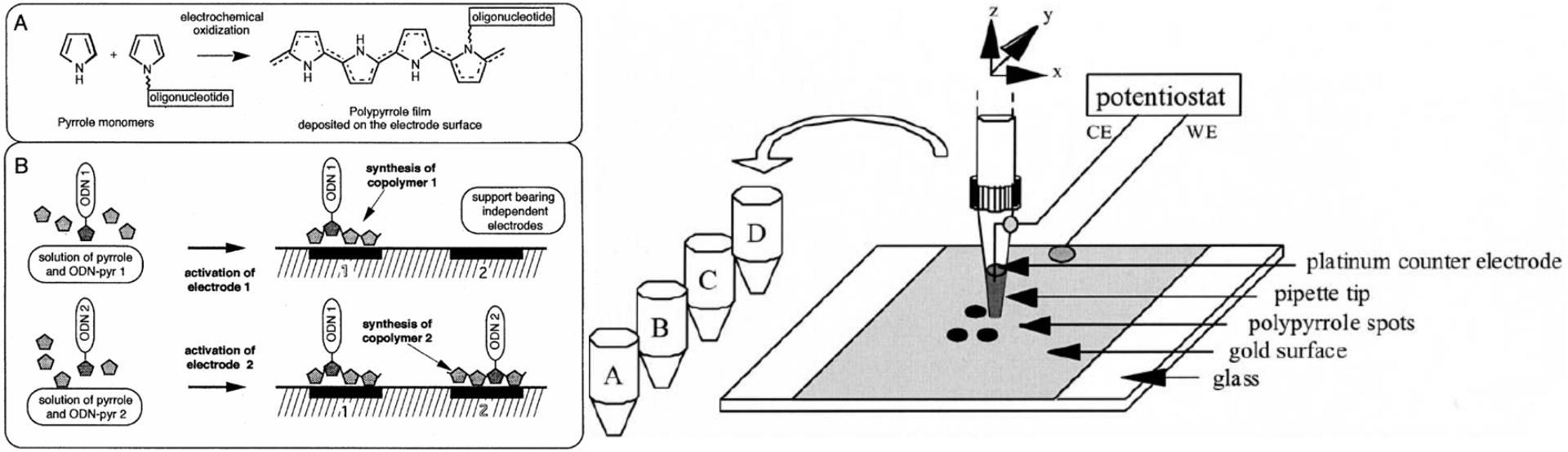
Outlook of the presentation

- Experimental Set Up
  - Grafting chemistry (electro-polymerization or SAMs)
  - Hybridization detection : Surface Plasmons Resonance Imaging
  - Temperature control (equilibrium and out-of-equilibrium scans)
- Electrostatic penalty
  - Equilibrium melting curves
  - Salt concentration effects
  - Confrontation to the model
- Potential applications
  - Single Nucleotide Polymorphism (SNP) genotyping
    - Homozygous case (pure targets)
    - Heterozygous case (mixed targets)
  - Low abundant somatic point mutation detection
    - Low temperature cooling hybridization
    - Temperature cycles
- Conclusion

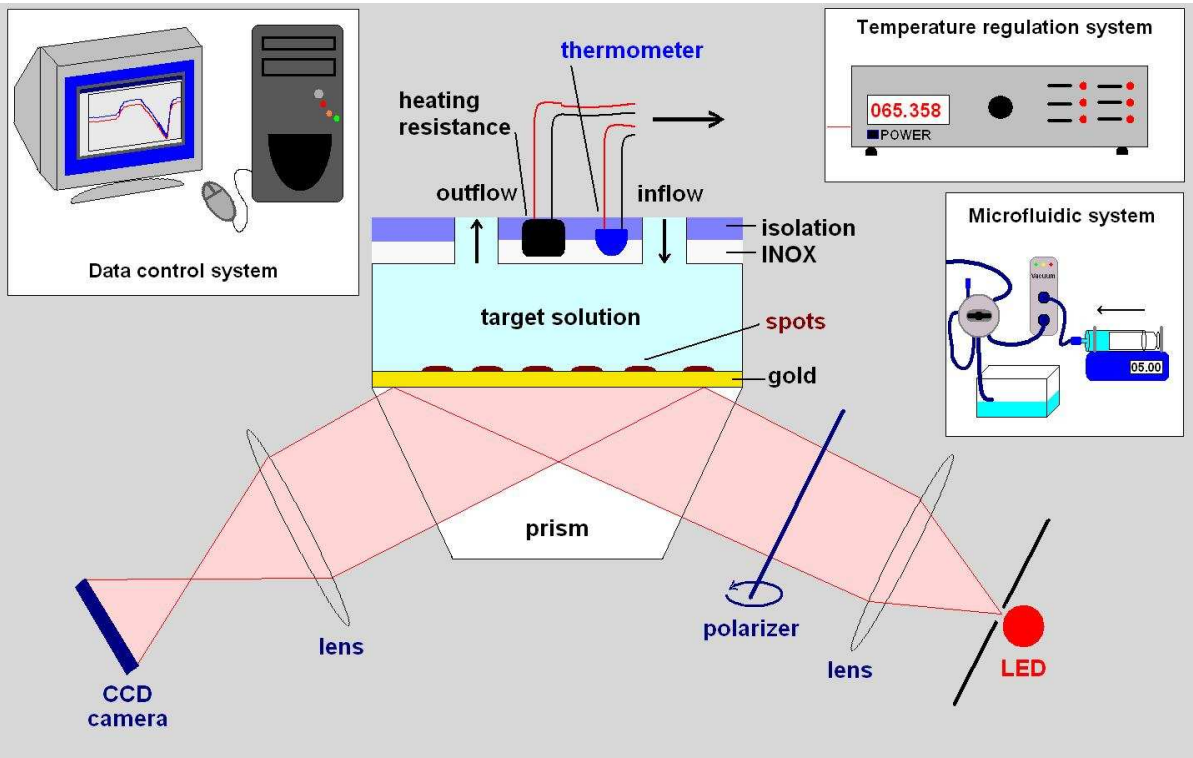
Microarray fabrication : Grafting chemistries

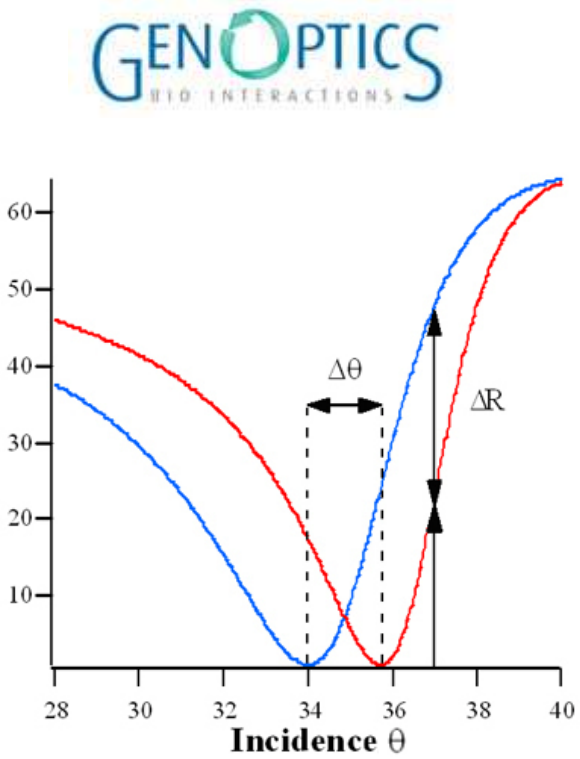
- Substrate : Gold surface on a glass prism for SPR imaging
- Spot Fabrication: Two grafting chemistries
  - Self Assembled Monolayer (SAM) of DNA-thiols
  - Electro-polymerization of pyrrole and DNA-pyrrole
- Relative advantages and drawbacks
  - Better accessibility of targets and SPR signal for thiol SAMs
  - Better stability (temperature and time) for poly-pyrrole



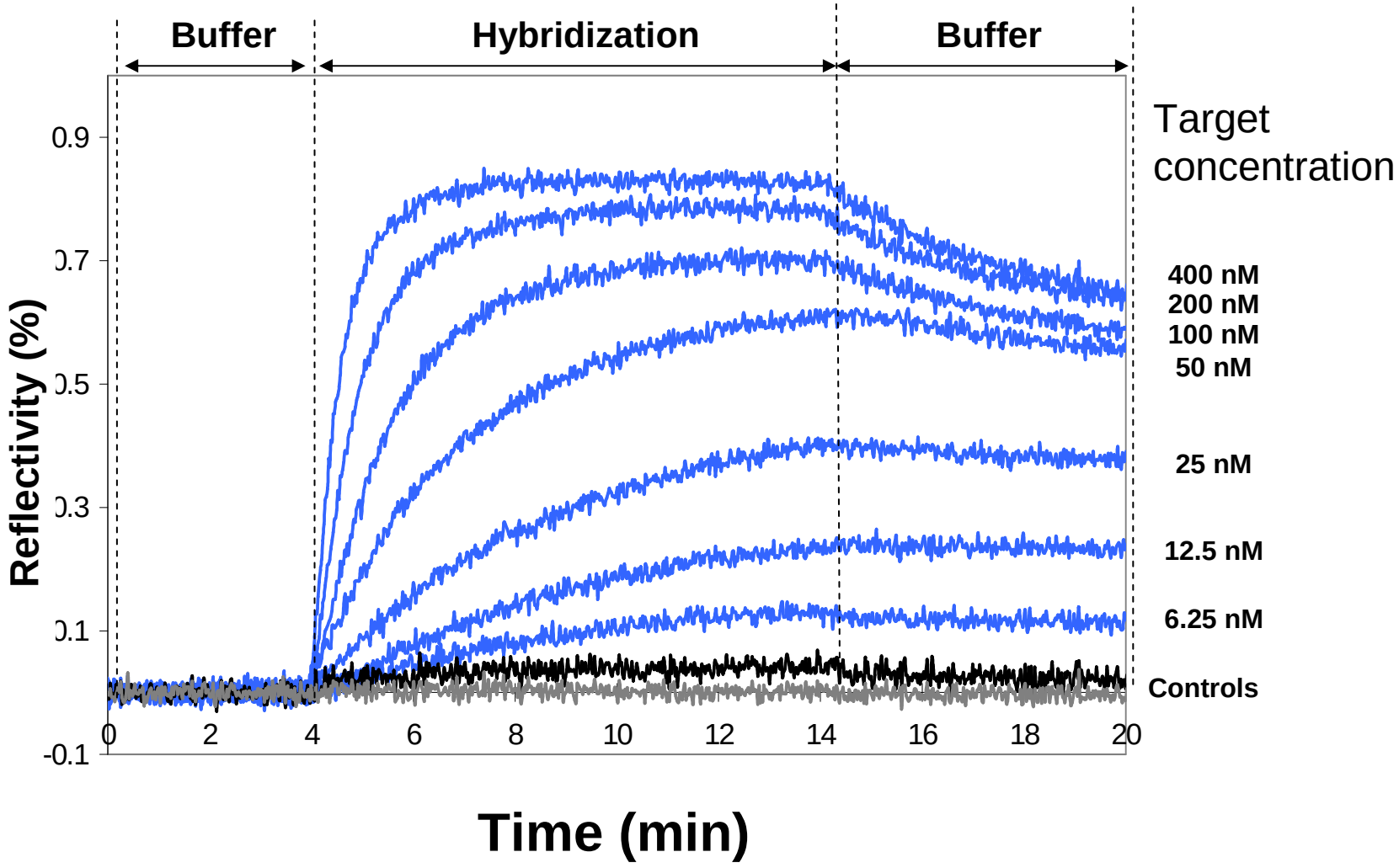
Signal detection : Surface Plasmon Resonance imaging

- Multi-spots detection : parallel (from few tens to thousands)
- Real time kinetics without label
- Hybridization cell of 4µL with fluidics
- Precise temperature control from 15 to 85℃ (home-made)
- Commercial apparatus by Genoptics (Horiba Jobin Yvon)





Kinetics of hybridization and denaturation



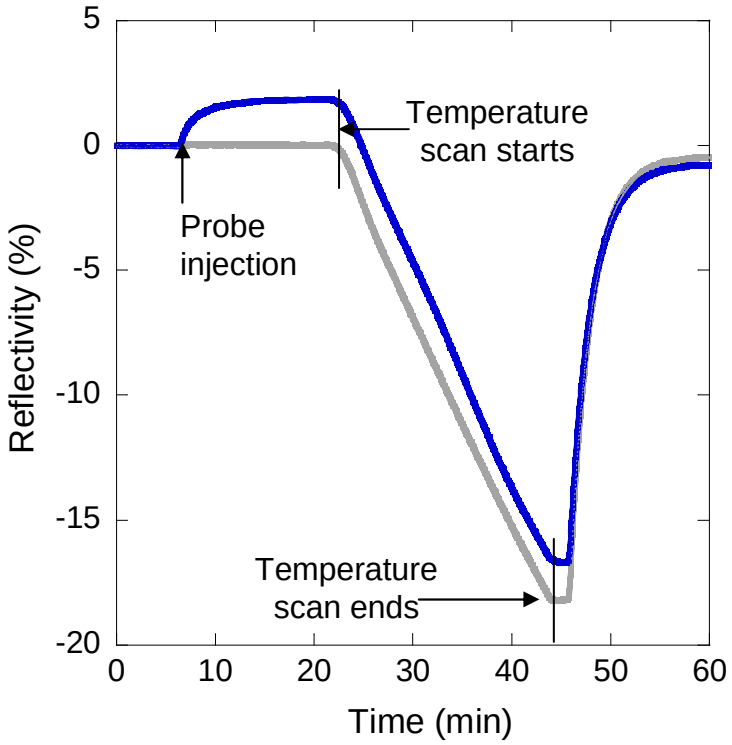
J.B. Fiche et al. **Biophys. J. 92**, 935 (2007)

Experimental set up : Temperature scan

Temperature effects on plasmon curves

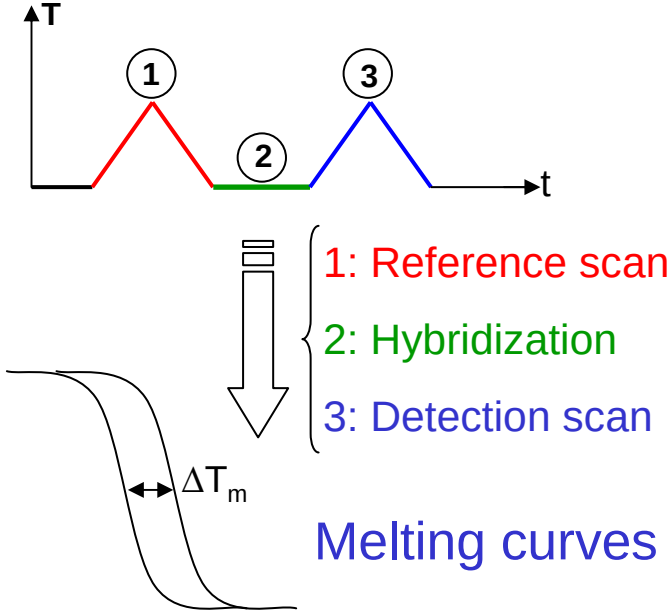
Temperature dependence of the water index leads to change of reflectivity

$\Delta T = 1℃ \Leftrightarrow \Delta R = 0.35\%$



Measurement protocol

Subtraction of a reference scan (1) from the detection scan (3) on each spot of the microarray:



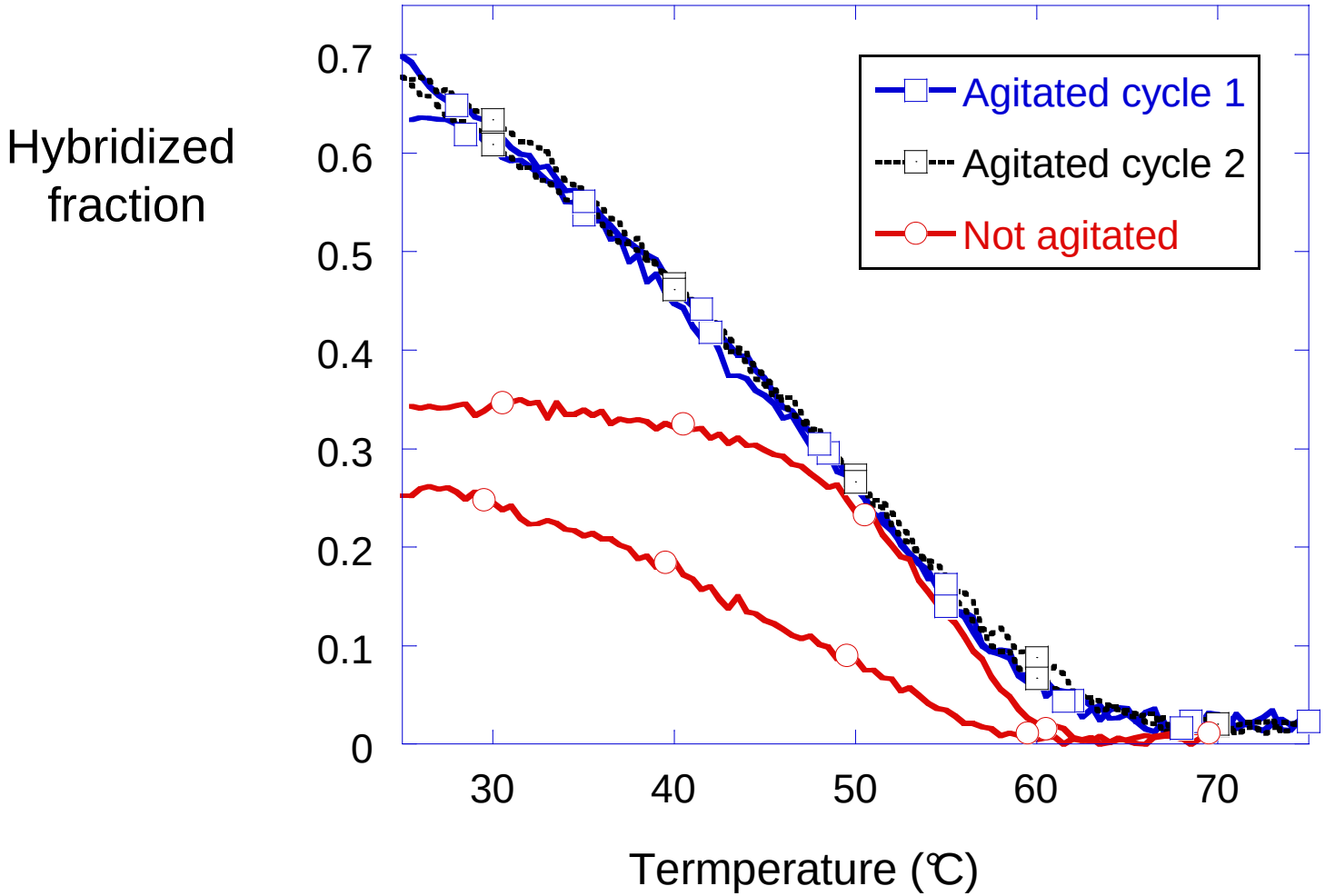
Melting curves

Condition: Precise control of the temperature

Applications: Melting curve analysis, SNP and/or somatic point mutation detection,…

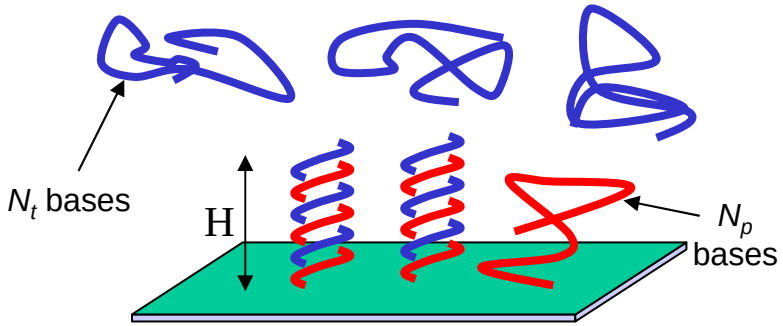
Equilibrium Melting Curves

Target concentration 250nM
Salt concentration 157mM

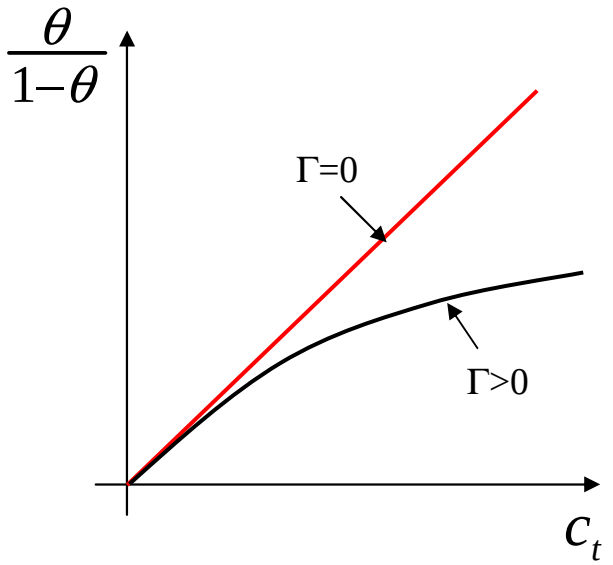


Model for electrostatic interactions

- DNA = Highly charged polymer
  - One charge per base
  - Spot = Charged surface
- Importance of Electrostatic Interactions
  - Effect of probe density σ
  - Effect of salt concentration c_s
- Hybridization
  - Increasing charge : $(N_p+N_t\theta)\,\sigma$
- Salt concentration
  - Screening effect increases with salt concentration c_s
- Hyp: Uniformly charged width H and $N_p=N_t$
- Modified Langmuir Model



Competition Free Experiment



$$\frac{\theta}{1-\theta} = c_t K_{pt} e^{-\Gamma(1+\theta)}$$

with $\Gamma = \frac{\sigma N_p}{H c_s} = \frac{c_p}{c_s}$

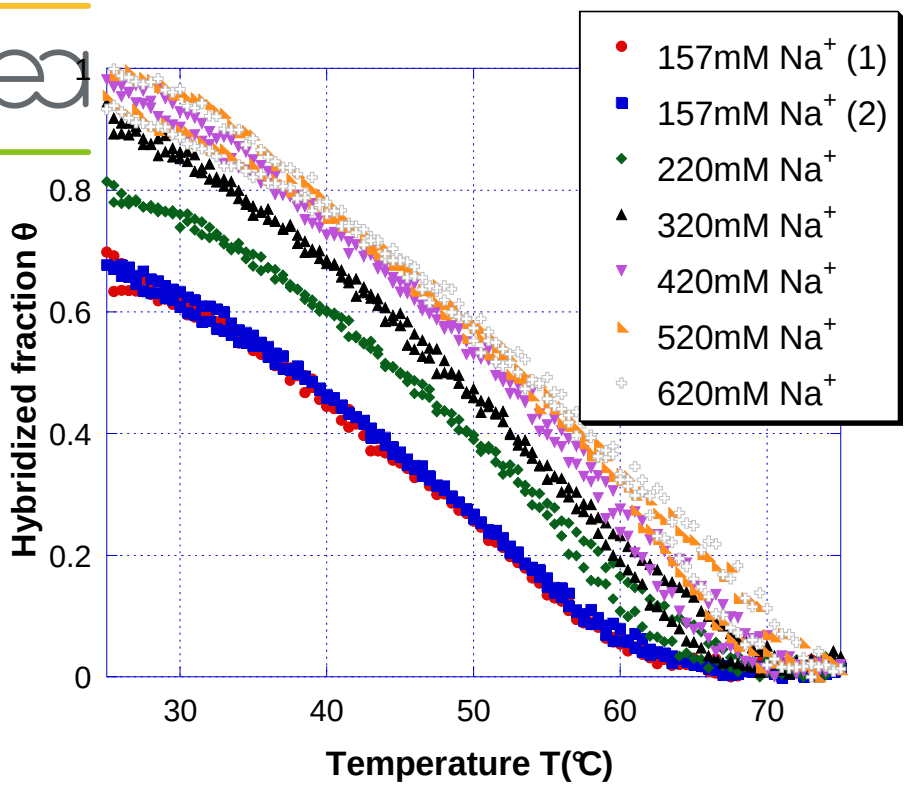
Note : at high salt $\Gamma \approx 0$

c_p probe base concentration

A. Halperin et al., **Biophys. J. 86**, 718 (2004)

Electrostatic interactions on microarrays

Salt concentration dependence of the melting curves



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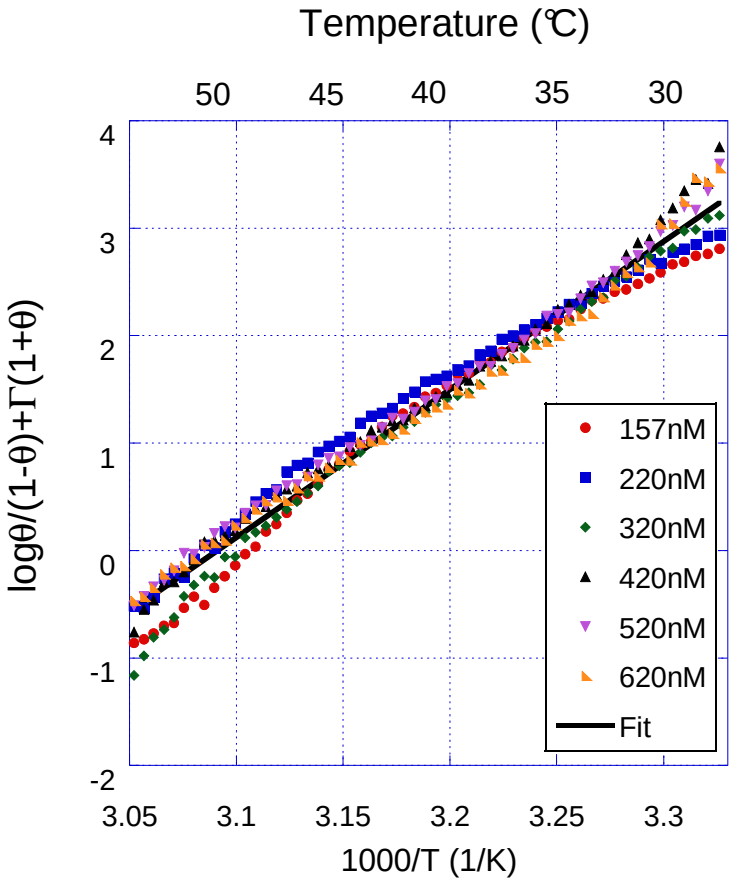
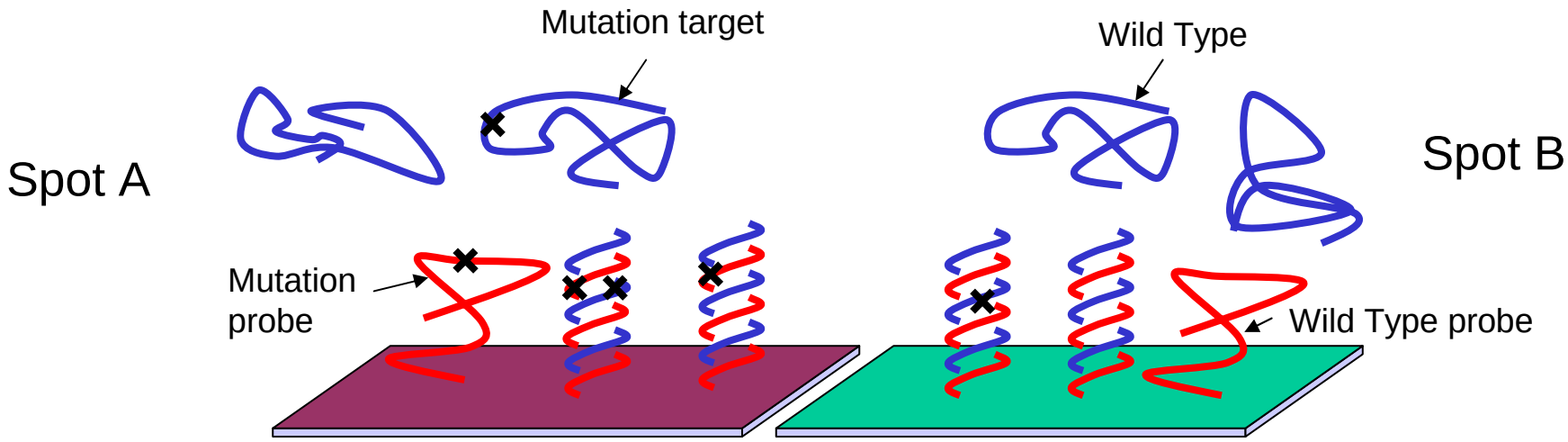


Collapse following the model with a single parameter

J. Fuchs et al. **Biophys. J. 99**, 1886 (2010)

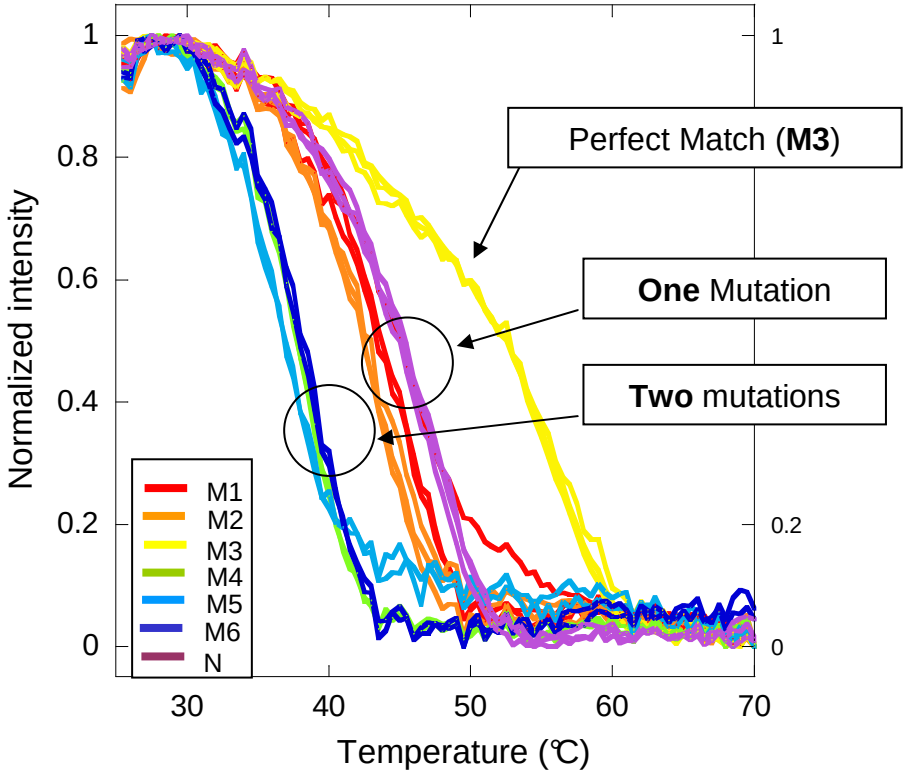
Point Mutation Detection through Melting Temperature

- Spot A grafted with mutation probes complementary to mutation targets
- Spot B grafted with probes complementary to wild type targets
- Hybridization on both spots with a single sequence target
- Both mutation and wild type targets hybridize to both spots
- Discrimination : Different melting curves and denaturation temperatures due to different stabilities between perfect match and mismatch sequences
- Analogue to solution phase method by Wittwer (LightCycler) :
  High Resolution Melting Curve Analysis



SNP genotyping through Melting Curve Analysis

Denaturation at 2℃/min after injection of M3* at 250nM

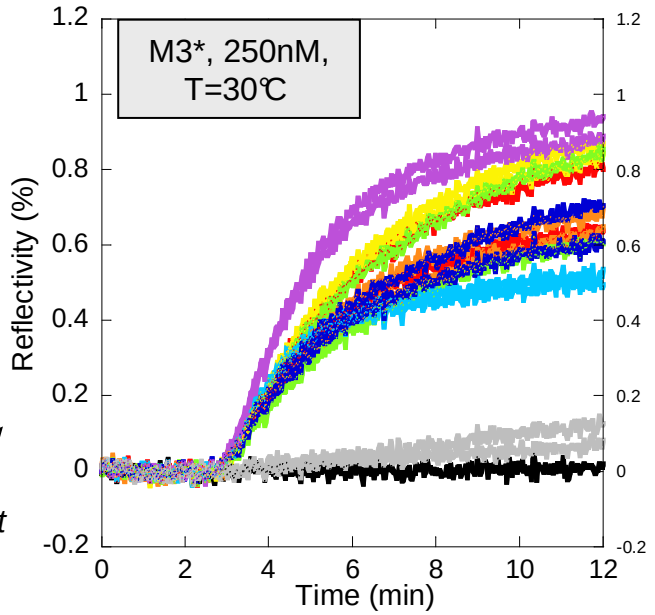


Target M3*: 3'- ACC TCG A**A**C ACC GCA -5'

M3: 5'-Py–(T_{10})- TGG AGC T**T**G TGG CGT -3'

X

N : 5'-Py–(T_{10})- TGG AGC T**G**G TGG CGT -3'
M1: 5'-Py–(T_{10})- TGG AGC T**A**G TGG CGT -3'
M2: 5'-Py–(T_{10})- TGG AGC T**C**G TGG CGT -3'



Homozygous case

Impossibility to distinguish perfect match from mismatch from the kinetics of hybridization.

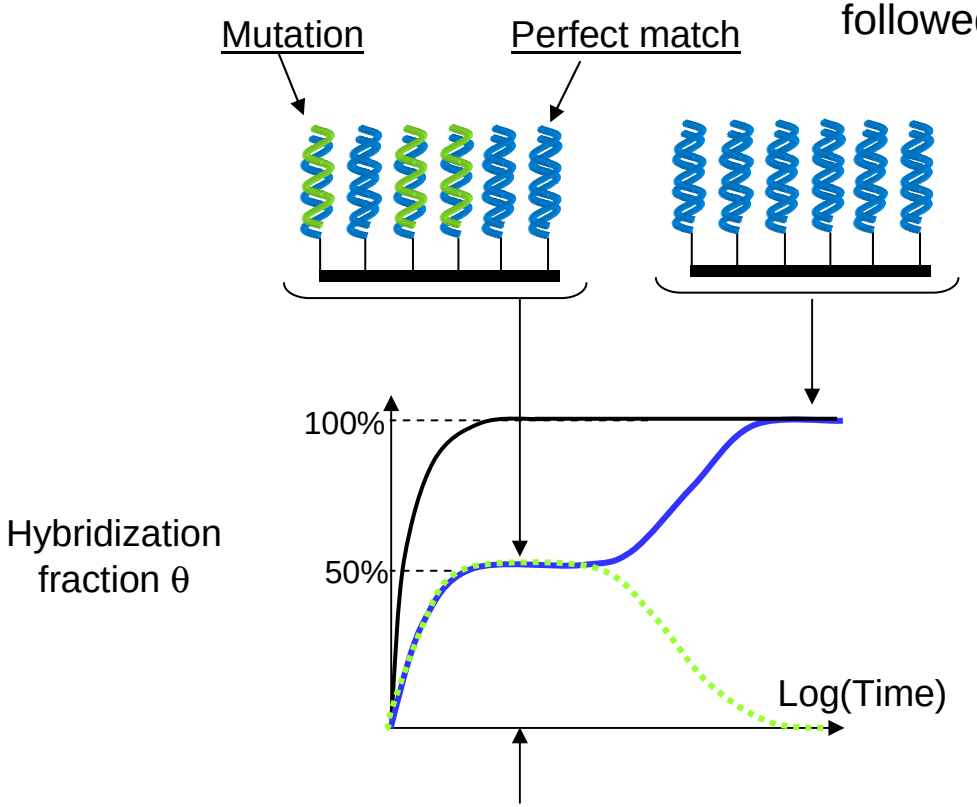
J.B. Fiche et al. **Anal. Chem. 80**, 1049 (2008)

SNP genotyping through Melting Curve Analysis

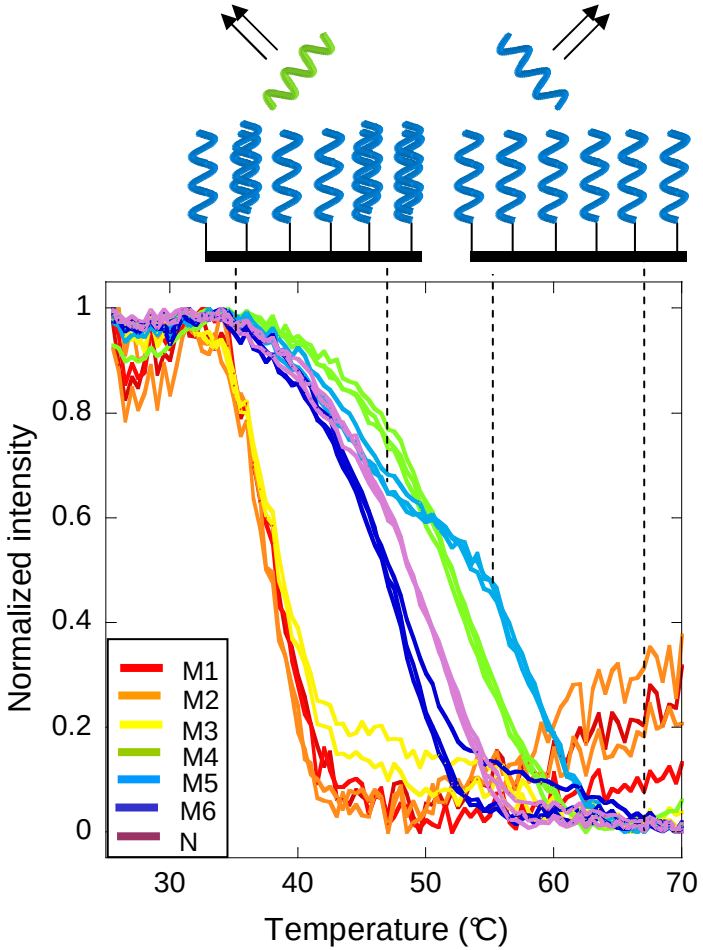
Heterozygous case : 50% wild type and 50% mutation

Hybridization kinetics

k_{on} DNA sequence and length independent
Limiting step : nucleation of a stable duplex followed by a fast zipping process

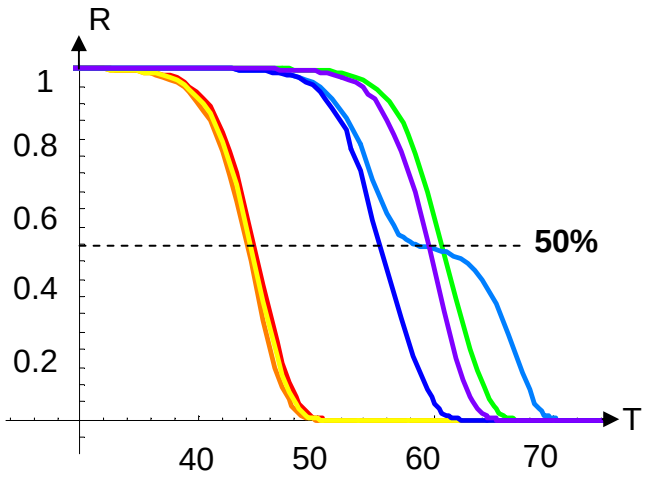


SNP genotyping through Melting Curve Analysis



Melting curves at 2℃/min after injection of M4* and M5* at 125nM each

Heterozygous case



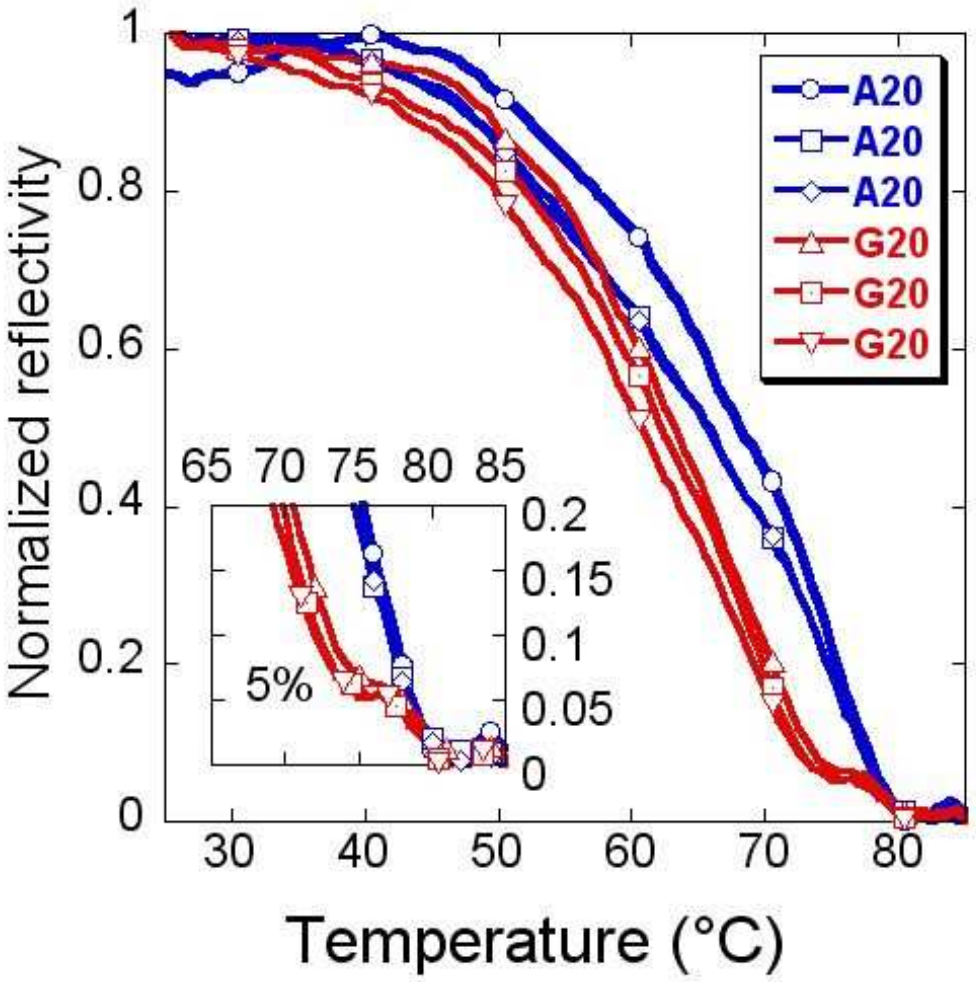
Theoretical simulation with reaction constants in solution

ΔT_m>7-8℃ sufficiently large to observe the two step denaturation

Low abundant mutation detection

- Somatic point mutations detection from tumor extract or even body fluids
- Low concentration of mutation vs wild type
- Detection limit = minimal percentage of mutation detected

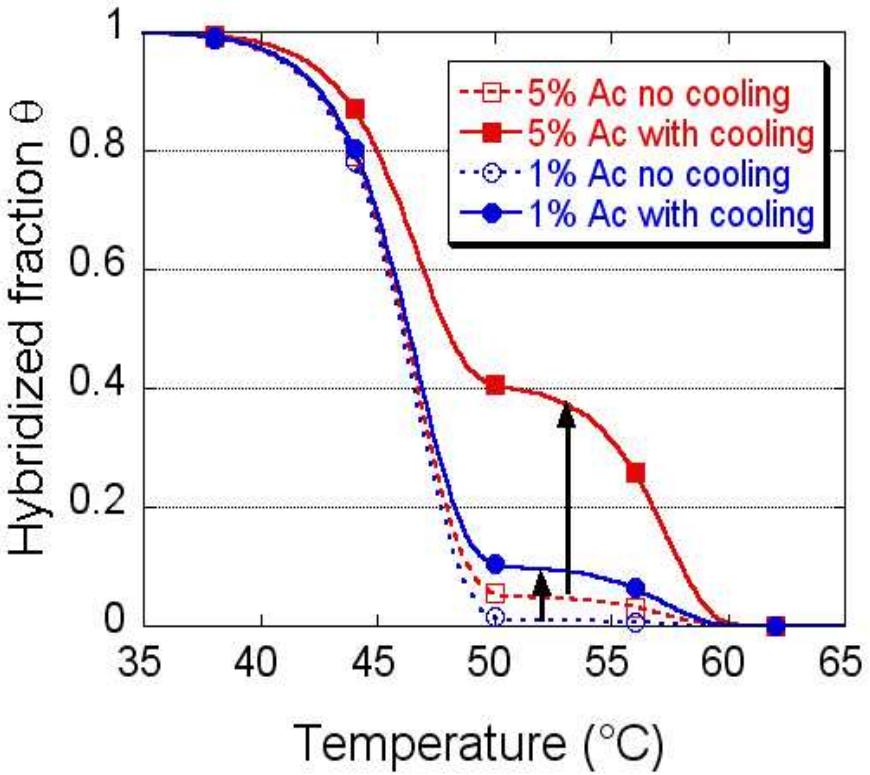
- Question: How to improve the detection level?



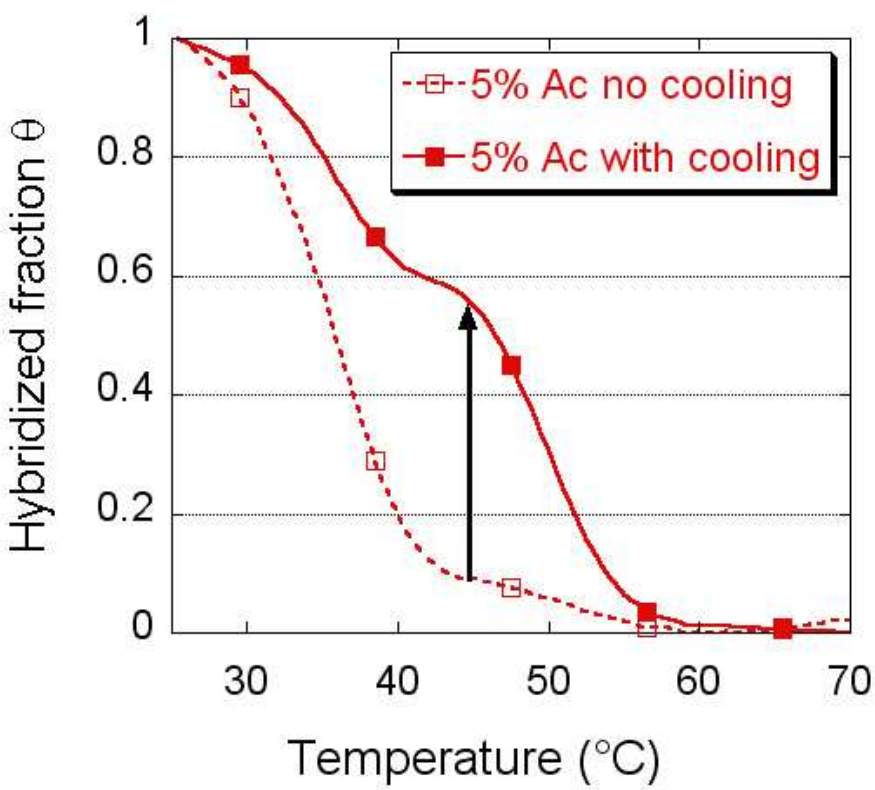
Low temperature cooling scans with targets

- Idea : perfect match more stable hybridize at higher temperature
- Result : Ten-fold increase of the plateau level

Simulation



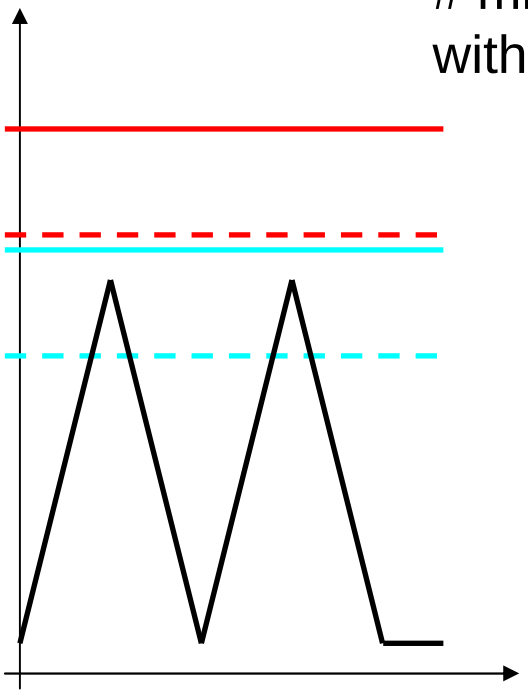
Experiment

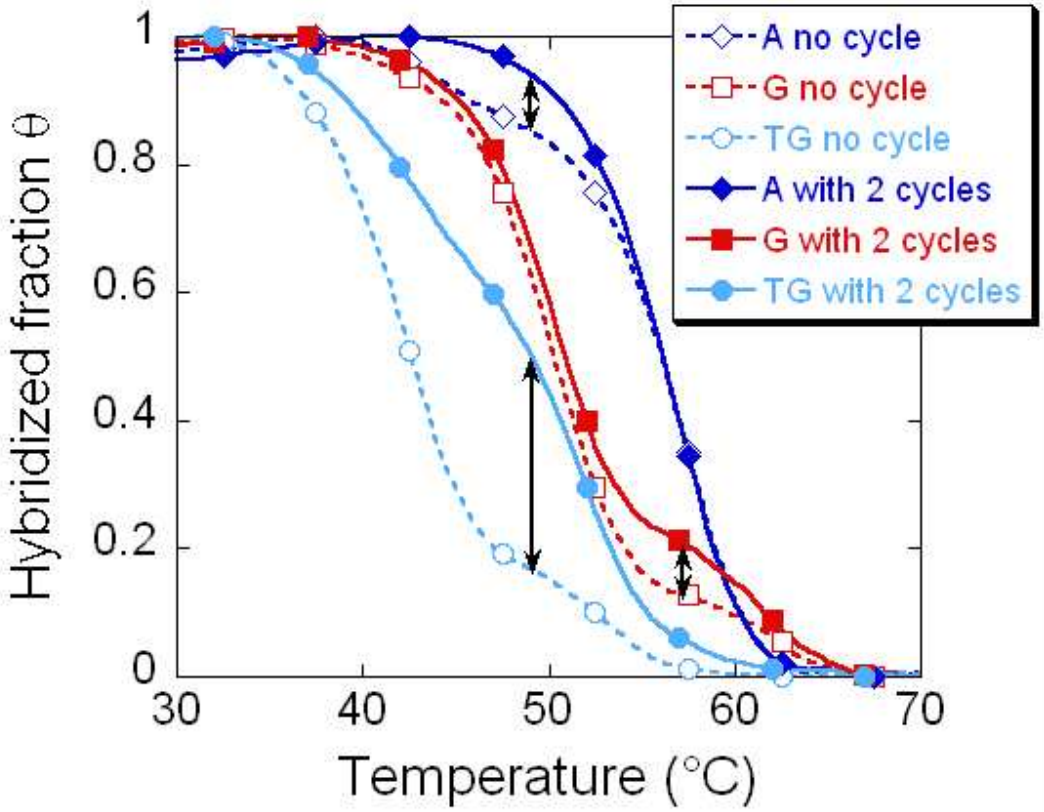


J. Fuchs et al. **Anal. Chem. submitted**

Temperature cycles with targets

- Idea : Successive improvements by multiple cycles
- Result : Improvement dependent on the maximal temperature
- Experimental result for T_{max} = 47°C





Conclusion

- Since hybridization is a thermodynamic reaction, temperature effects are important particularly on microarrays
- Temperature affects equilibrium as well as out-of-equilibrium melting curves and also kinetics
- Measuring equilibrium melting curves is experimentally possible
- It allows us to determine electrostatic effects, thermodynamic parameters and much more physico-chemical parameter effects on solid phase hybridization
- Possible applications : SNP genotyping or detection of somatic point mutations

- Drawbacks experienced for Fundamental Physico-Chemical studies
  - Grafting chemistry : none seems satisfying
  - Grafting density : independent determination difficult
  - Signal-to-noise ratio : low for precise confrontation to models
  - Dependence of the platform used (SPRi, microarrays fabrication)

Acknowledgements

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  - A. Halperin (Liphy, Grenoble)
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