PRECONCENTRATION, RELEASE and DETECTION of microRNA by Combining Magnetic Hyperthermia and Electrochemistry Modules on a Microfluidic Chip

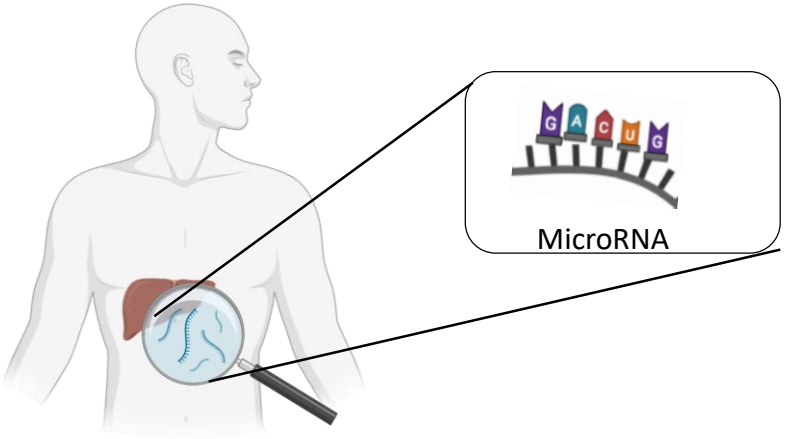
E-miRgency project

Djamila Kechkeche et Jean Gamby

10/12/2021

Why studying RNA is interesting ?
Why lab on chip device is developed ?

- Point-of-care testing for early pathology diagnosis



- small noncoding RNA
- Post-transcriptional regulation of gene expression

➔ **Biomarkers**[1]

1. Scand J Med Sci Sports. 2021;00:1–14.

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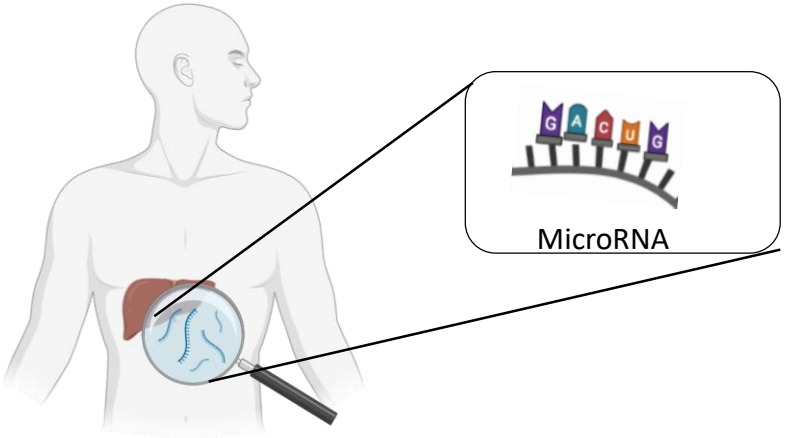

- small noncoding RNA
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➔ **Biomarkers**[1]

- What are the advantages of lab on chip ?

✓ **Detection within 30 minutes**

✓ **High sensisivity**

✓ **High specificity**

✓ **Low sample volumes**

✓ **Multiple sample matrices**

✓ **Small product**

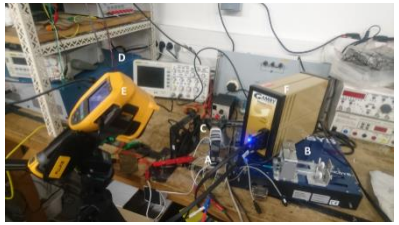
1. Scand J Med Sci Sports. 2021;00:1–14.

History of this project

Supervisor : Jean GAMBY

Marie Charlotte Horny, phD

Brevet PCT/EP2018077080
05/10/2018



2013-2017

2018

Funding AAP VALO NanoSaclay 2018
+ Pré-maturation CNRS AAP 2018

History of this project

Supervisor : Jean GAMBY

Claire Poujouly, phD
Pedro Gonzalez Losada,
post Doc

Déclaration Invention 2020
(suite brevet maître)

Marie Charlotte
Horny, phD

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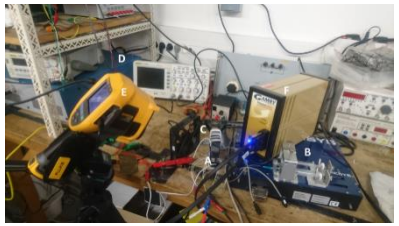

2013-2017 2018 2019 2020

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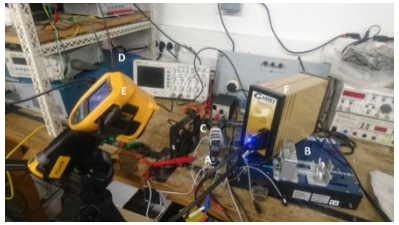

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DI2021-0031 Paris Saclay
01/06/2021

2013-2017 | 2018 | 2019 | 2020 | 2021 | 2022

Funding AAP VALO NanoSaclay 2018
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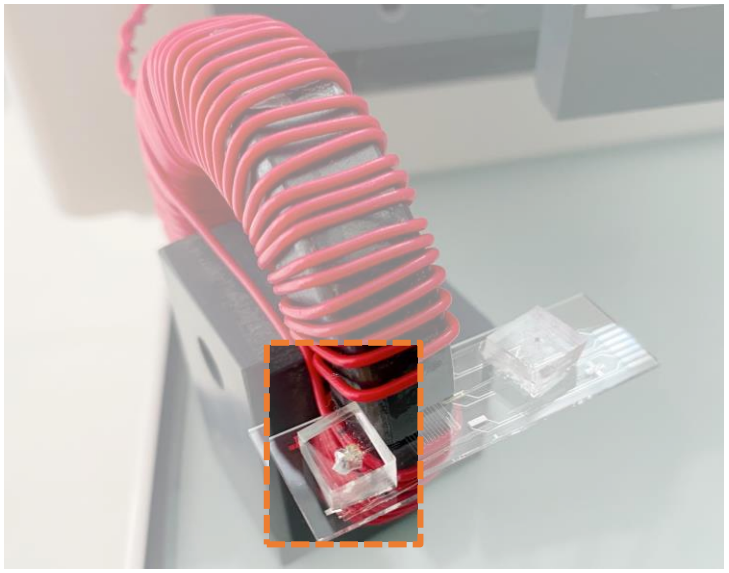
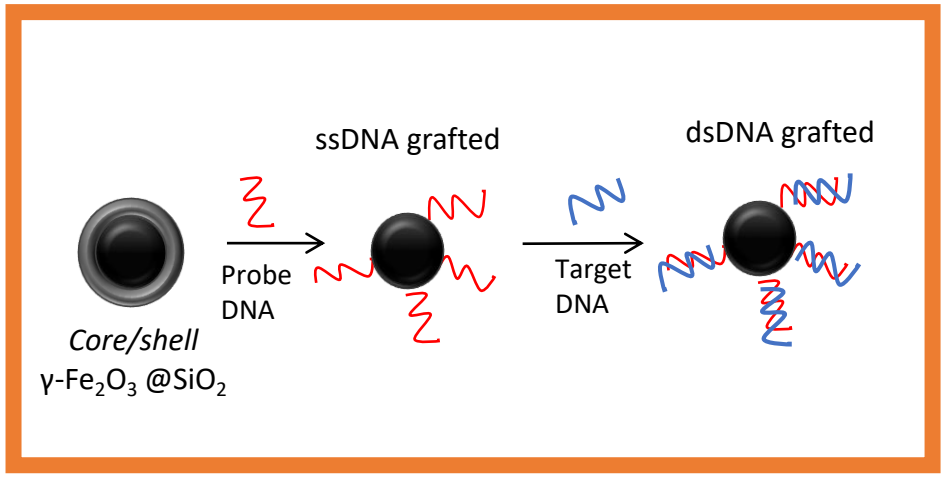
Funding Labex Nanosaclay 2020

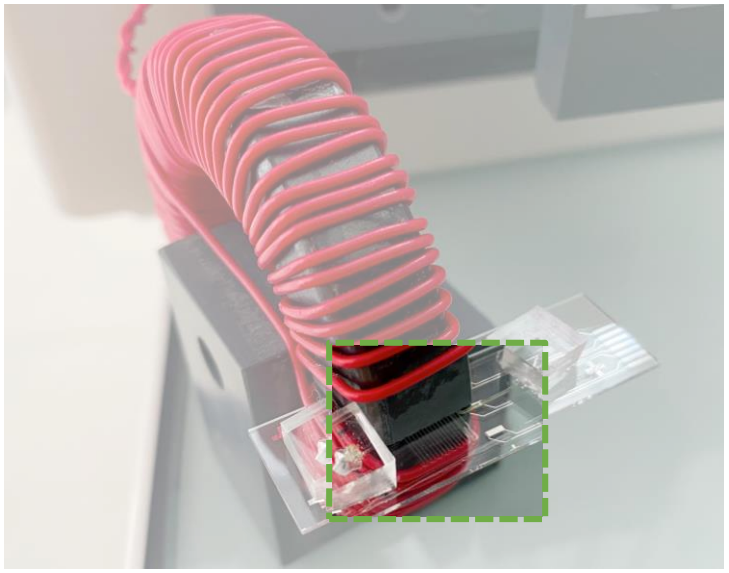
Preconcentration, Release and Detection of microRNA by Combining Magnetic Hyperthermia and Electrochemistry Modules on a Microfluidic Chip

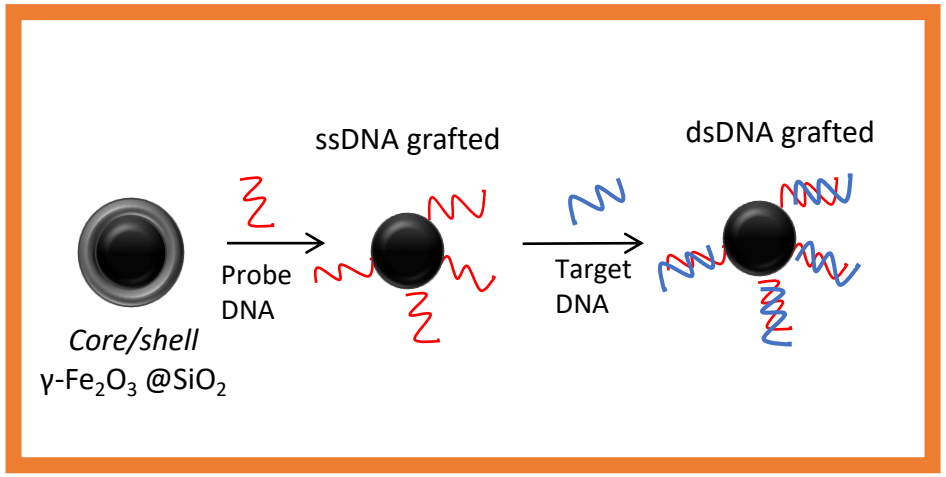




1. Preconcentration of DNA (off chip)

Preconcentration, Release and Detection of microRNA by Combining Magnetic Hyperthermia and Electrochemistry Modules on a Microfluidic Chip





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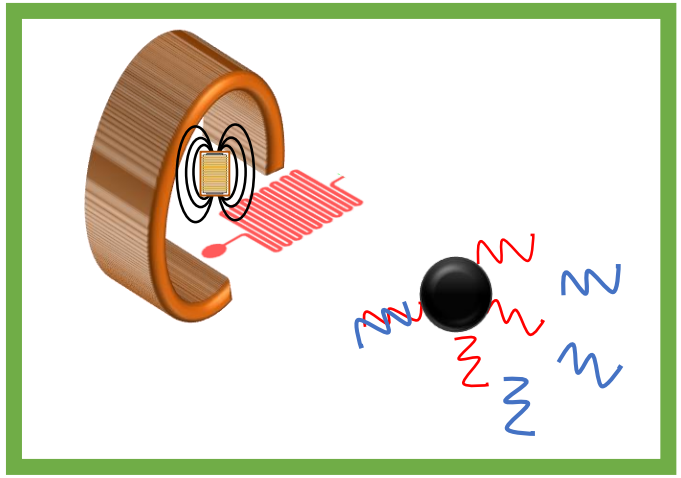

2. Release DNA by magnetic hyperthermia (on chip)

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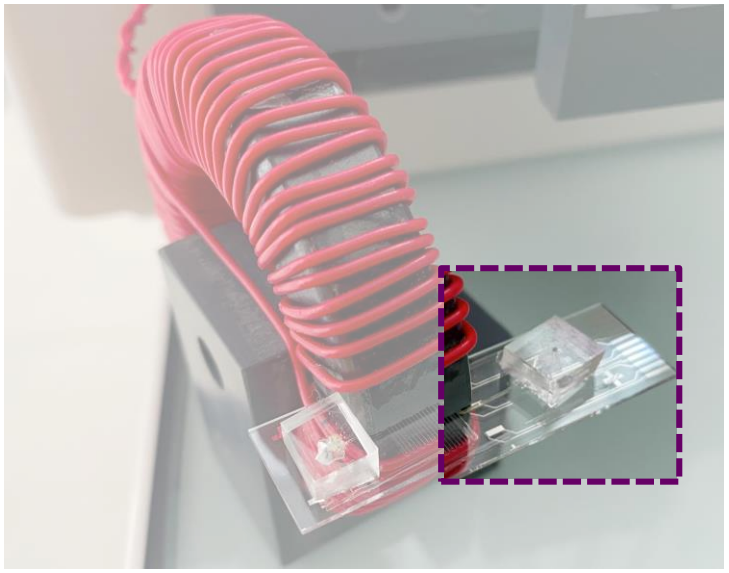

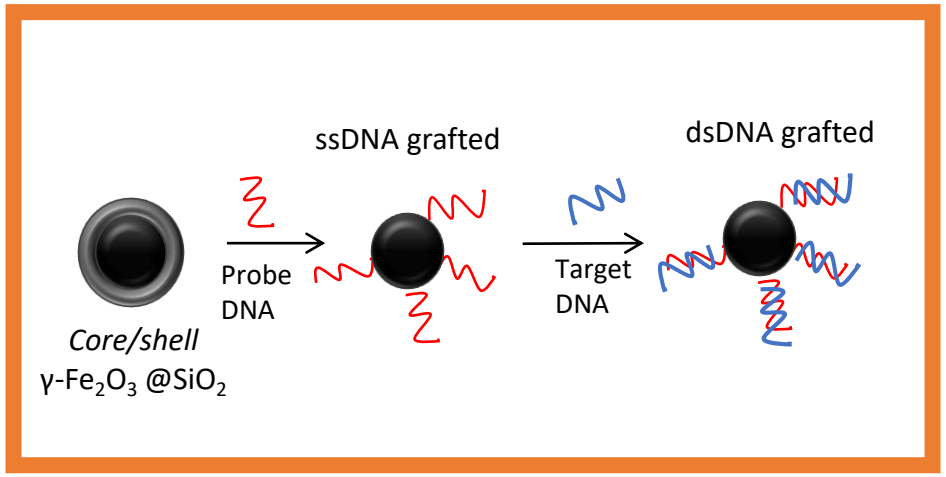

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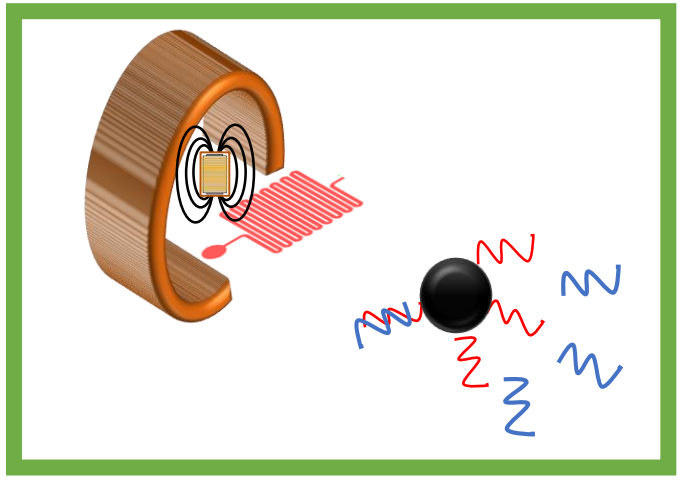

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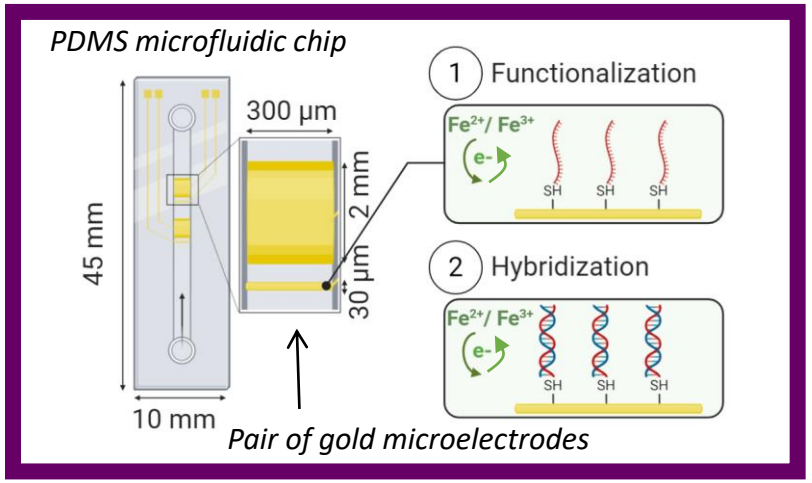

3. Electrochemical detection (on chip)

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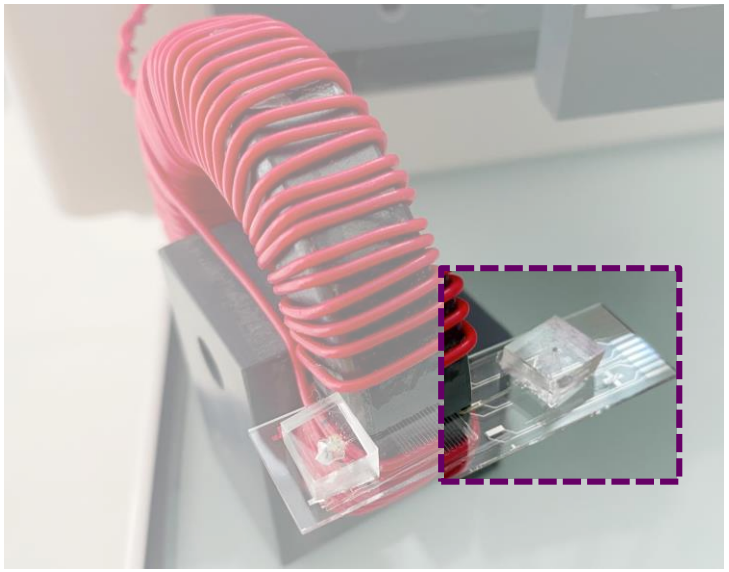

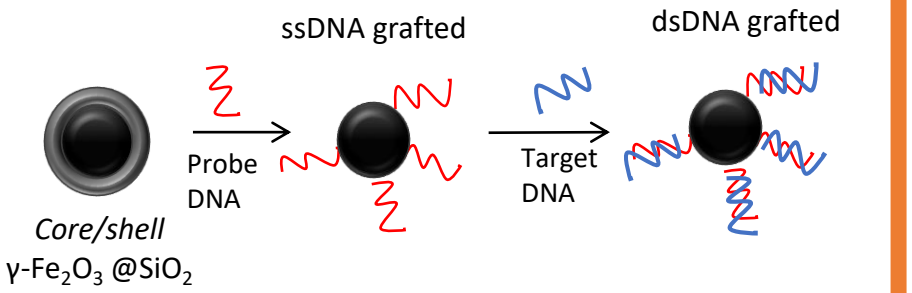

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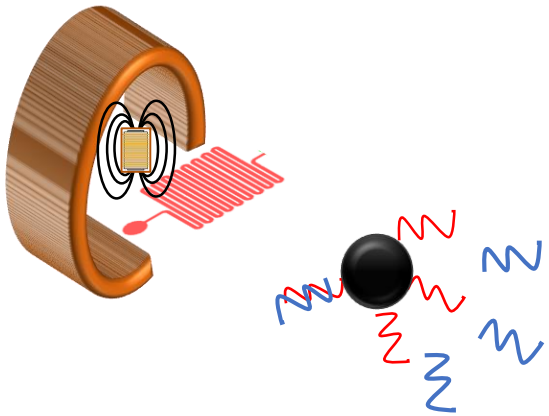
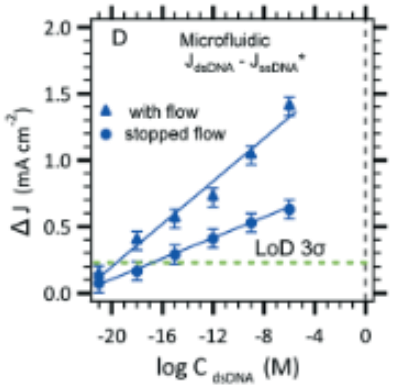


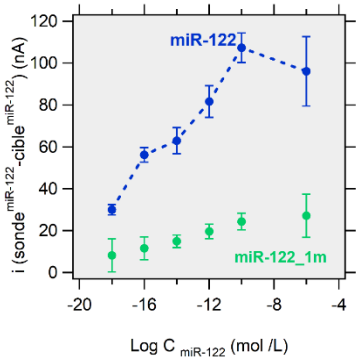
2. Release DNA by magnetic hyperthermia (on chip)

Marie charlotte Horny phD[2]
Claire Poujouly phD

High sensivity



High specificity



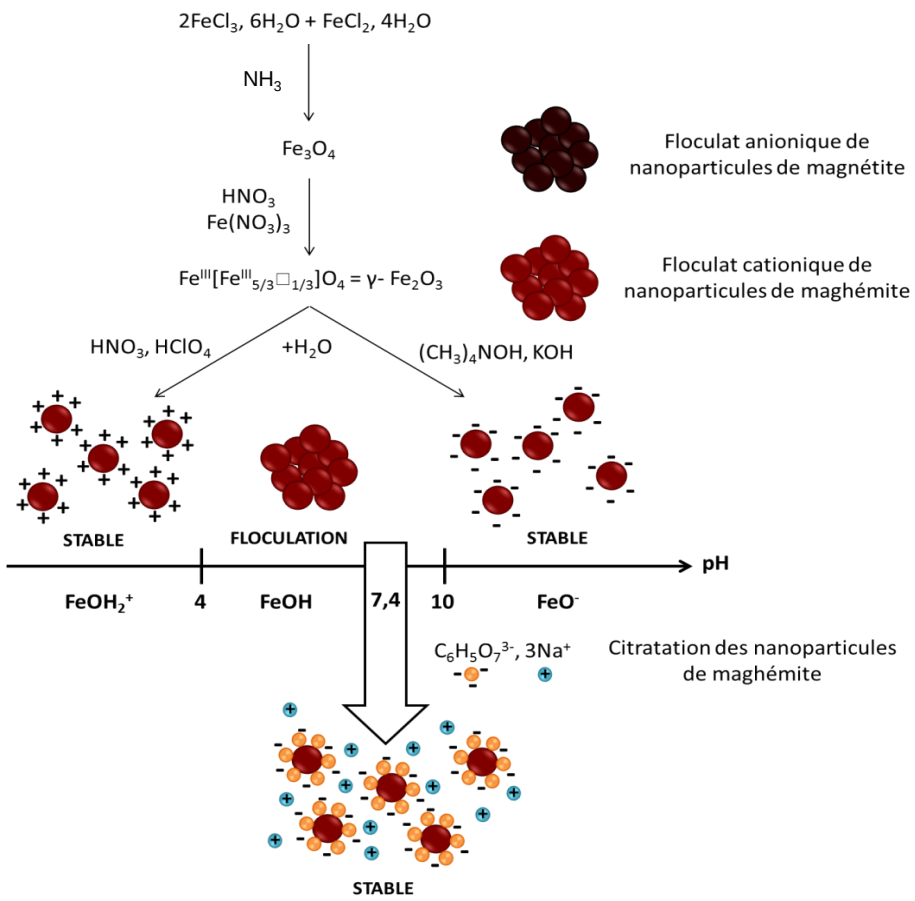
LOD = 10^{-18} M

2. Lab Chip,2016,16,4373

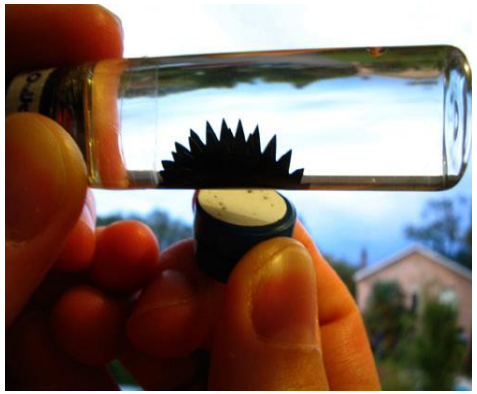
3. Electrochemical detection (on chip)

1. Synthesis of γ-Fe_2O_3 @SiO_2 Core-Shell Nanoparticles

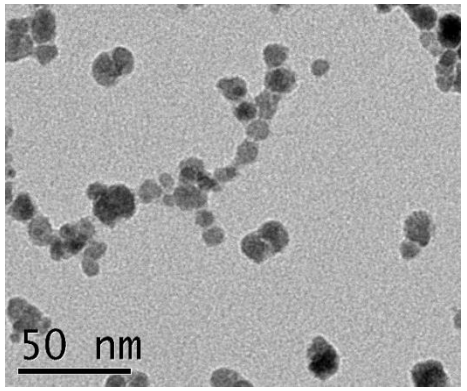
A. Co-precipitation procedure = Massart method[3]



- Several advantages :
  - Rapid, easy to synthesize
  - No organic solvent, no surfactants
  - Large quantities (1L, [Fe] = 1M)
  - Size sorting to reduce polydispersity



Ferrofluid

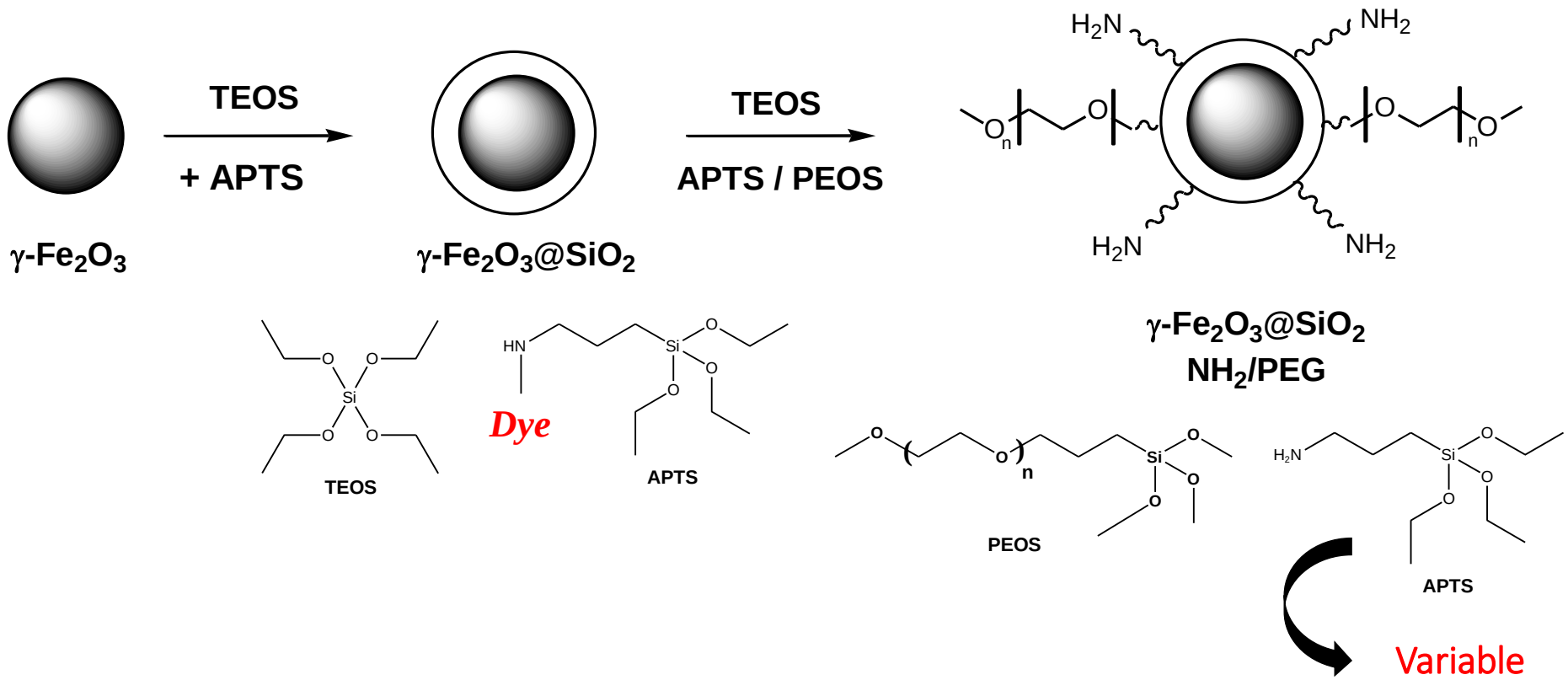


d_0 = 12 nm
σ = 0.35

3. J.of Magn and Magne Mater. 1995,149, 6-9.

1. Synthesis of γ-Fe_2O_3 @SiO_2 Core-Shell Nanoparticles

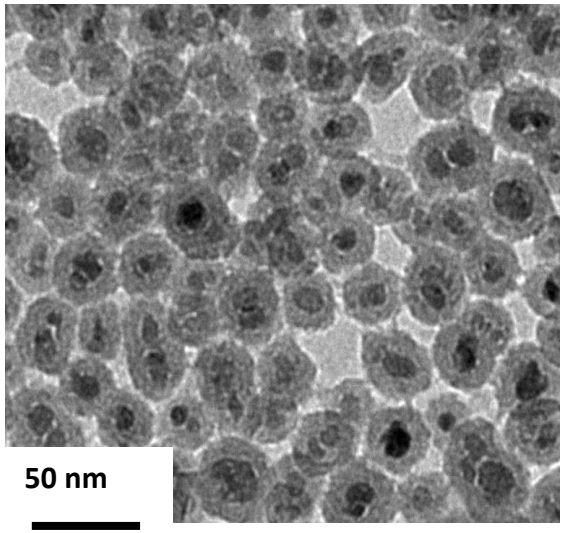
B. Silica coating process[4]

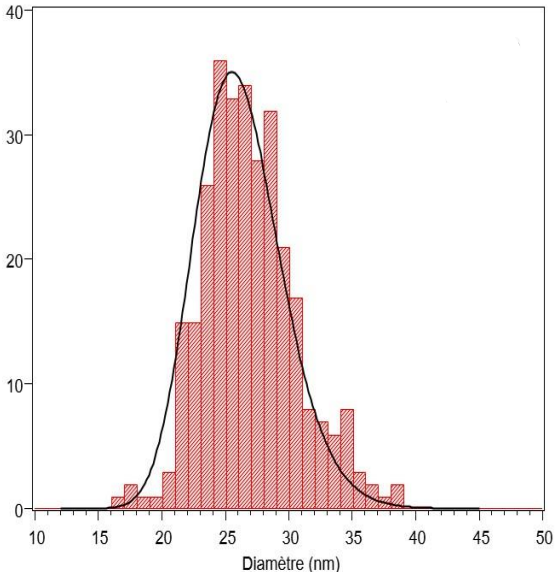


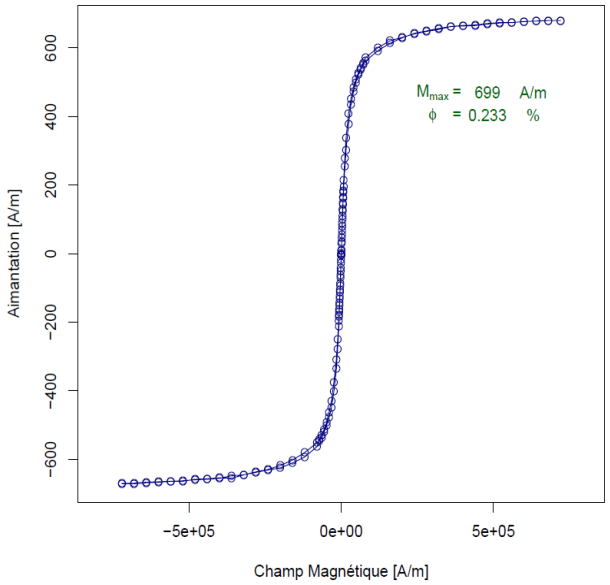
- Functionalization with PEG and NH_2 groups
  Various NH_2 groups surface densities

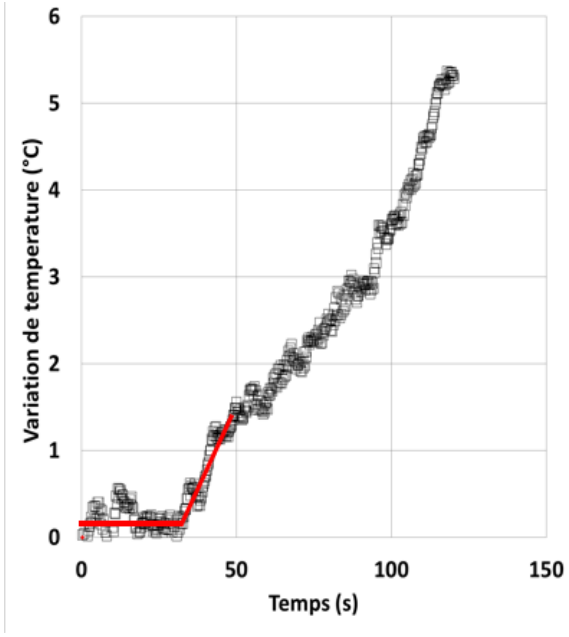
4. J.of Magn and Magne Mater. 2009,321, 1408-1413.

2. Characterization of γ-Fe_2O_3 @SiO_2 Core-Shell Nanoparticles









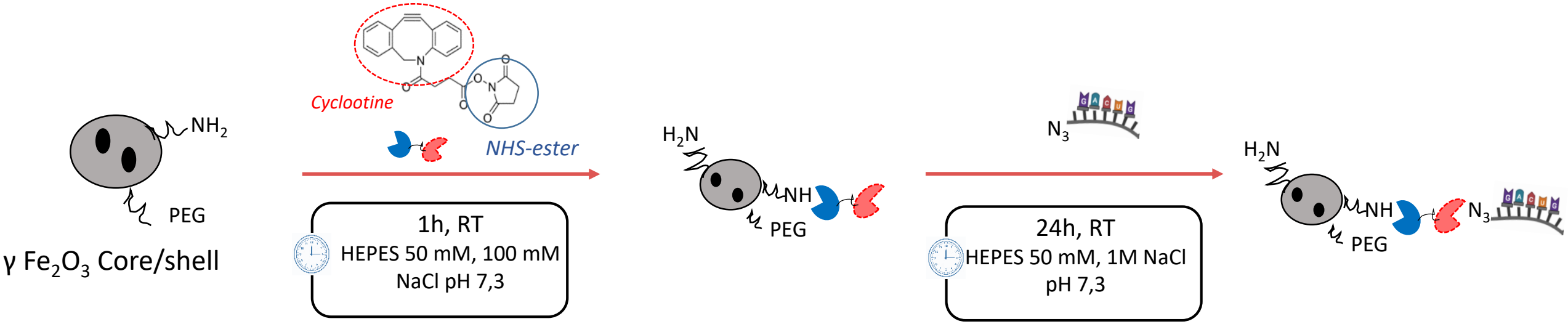
Diamètre TEM	30 nm
Amine à la surface/CC	12000/CC
Potentiel Zeta	20 mV
Aimantation à saturation Ms	60 emu/g
SLP à 535 kHz	33 W/g

3. Bioconjugation strategie

- In two steps using strain promoted click chemistry

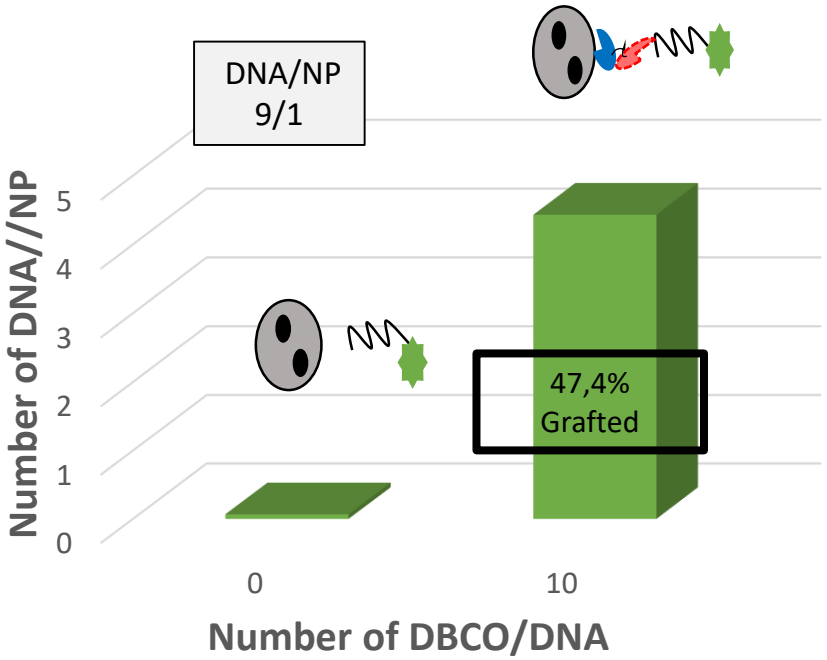
  - Introduction of alkyne groups onto the nanoparticle's surface

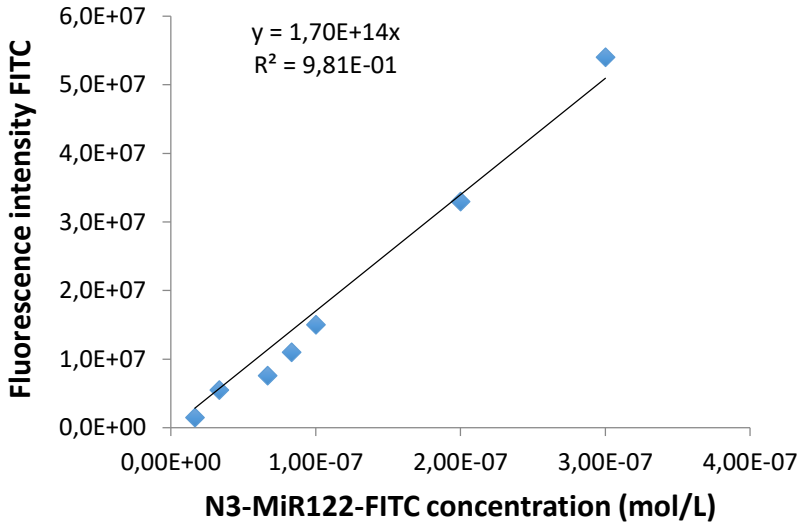
  - Copper free click chemistry with DNA-N_3



3. Bioconjugation strategie

- Grafting of single stranded DNA_{probes} (DNA modified with FITC)

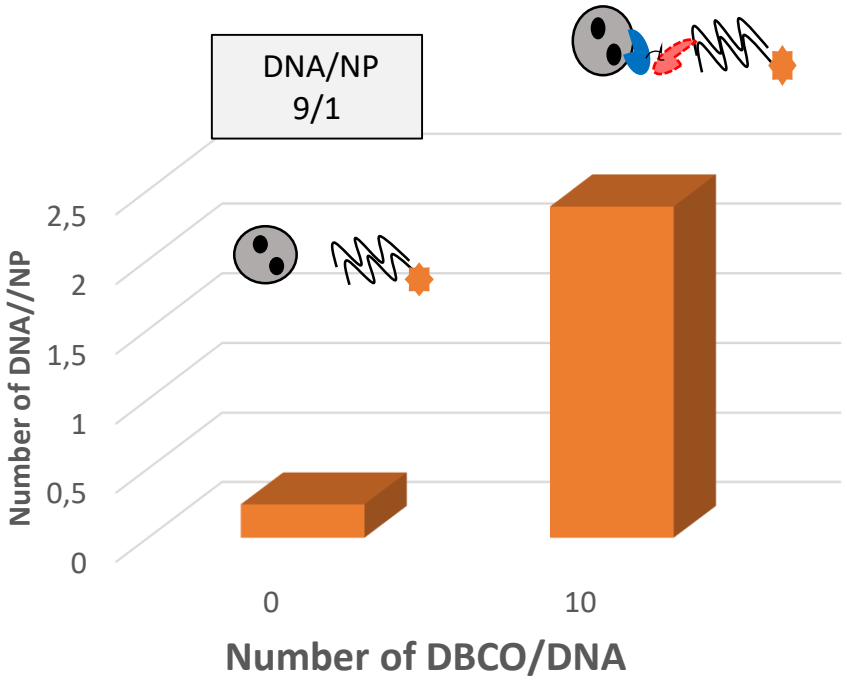


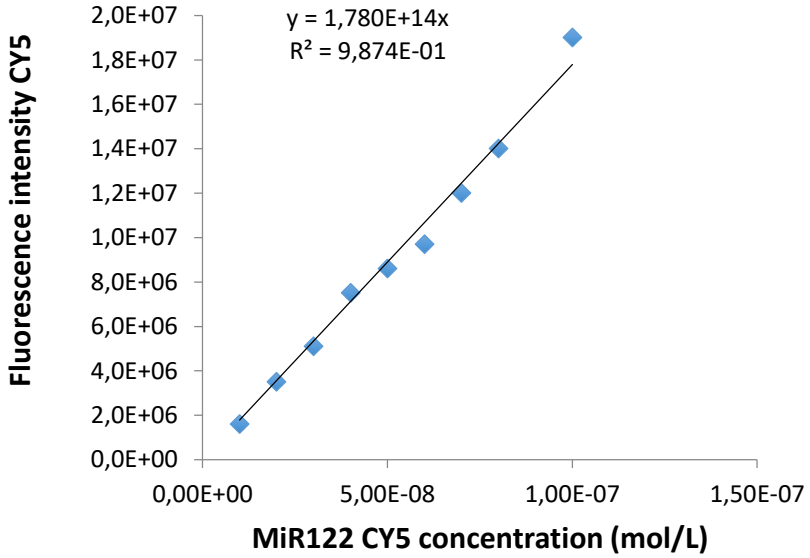


Calibration curve
Quantification of the probes DNA onto the nanoparticles

3. Bioconjugation strategie

- Capture of single stranded DNA_{target} (complementary DNA modified with CY5)





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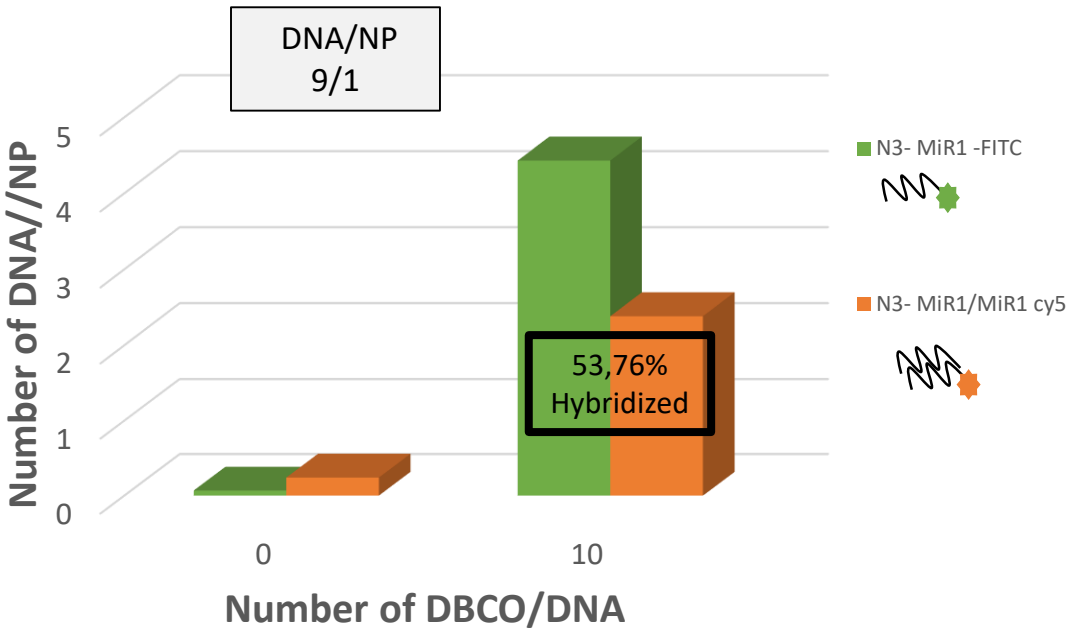
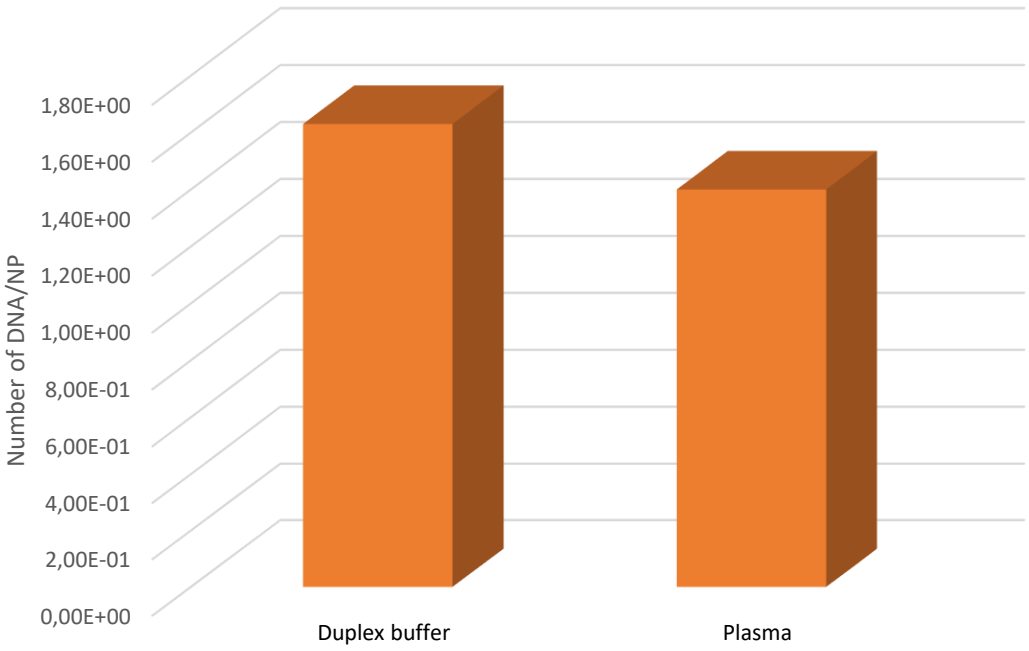


3. Bioconjugation strategie

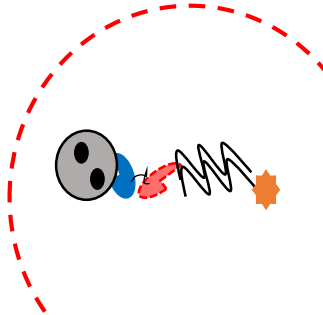
		ssDNA - Nanoparticles		ds DNA - Nanoparticles	
Disease	Name	ADN/NP	% grafted	ADN/NP	% hybridized
Skeletal/cardiac muscle	Mir 1	4,3	47,40	2,38	53,76
Liver	MiR 122	4,28	47,13	2,47	57,81
Skeletal/cardiac muscle	MIR 133a	3,9	42,97	1,93	49,62
Skeletal muscle	MiR 133b	4,86	53,55	2,78	57,11
Cardiac muscle	MiR 208a	3,82	42,13	2,19	57,47
Skeletal muscle	Mir 206	4,18	46,01	2,63	63

3. Bioconjugation strategie

- Biological sample



Duplex buffer	Biological media : plasma
1,63	1,4



Results

- Done :

  - Patent : DI2021-0031 Paris Saclay 01/06/2021

  - Article : Magnetic Hyperthermia on γ-Fe_2O_3 @SiO_2 Core-Shell Nanoparticles for multiple mi-RNA Detection *in process*

- To do :

  - Release microRNA captured by controlled magnetic hyperthermia on microfluidic chip

  - Multi-detection of microRNA on microfluidic chip

Funding

e-miRgency PROJECT

NanoSaclay
Laboratoire d'Excellence
en Nanosciences et Nanotechnologies

DIMELEC PROJECT

AGENCE NATIONALE DE LA RECHERCHE
ANR

C2N Centre DE Nanosciences & DE Nanotechnologies

Jean Gamby
Claire Poujouly

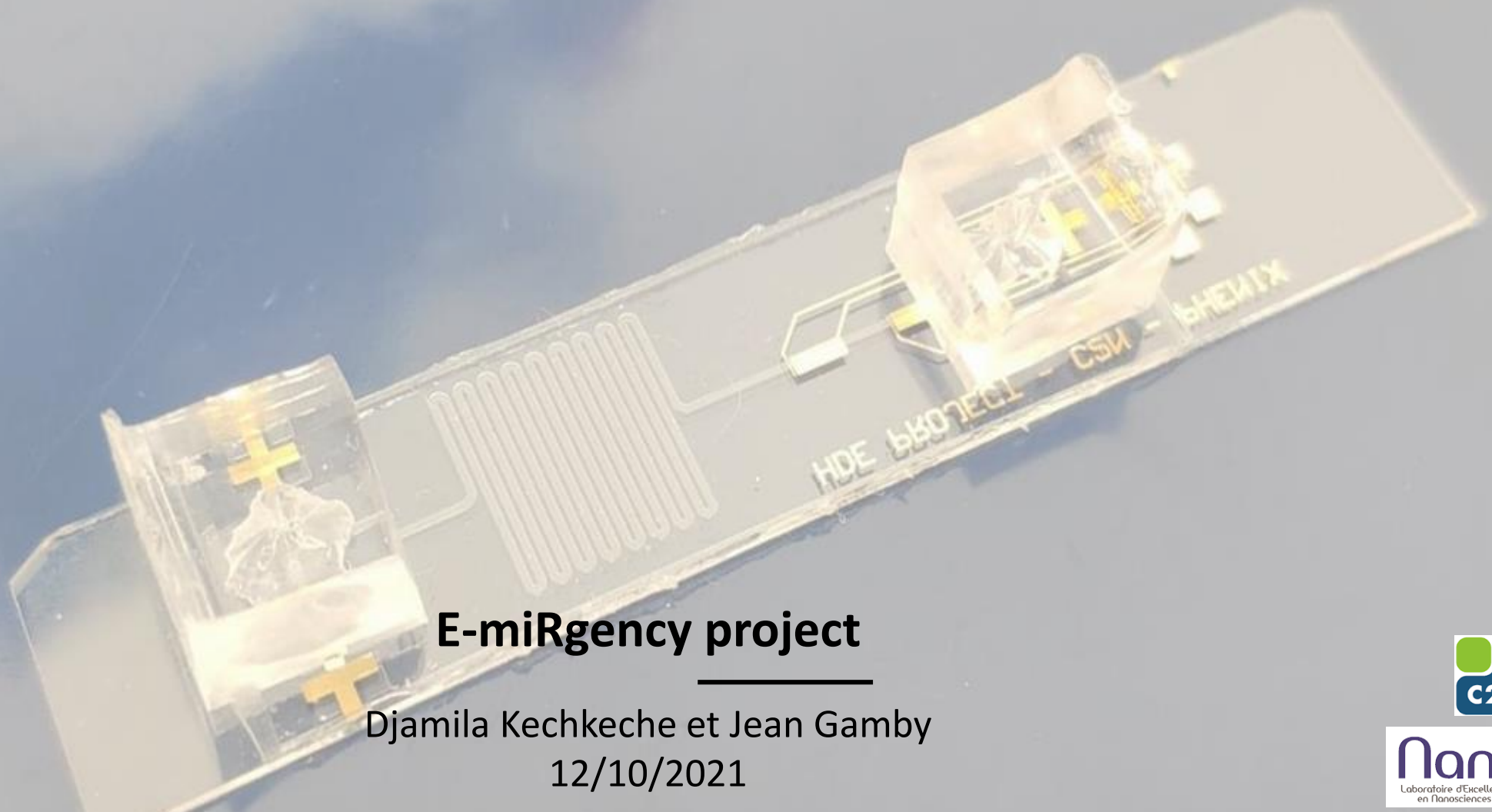
PHENIX

Jean Michel Siaugue
Emilie Secret
Sirine El Mousli

IRBA

Sébastien Banzet
Julien Siracuse

Thank you for your attention



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