TÉCNICO LISBOA

Nanotechnology

Detection of Antibiotic-Resistant Bacteria (P2, Topic 1)

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General state-of-the-art (classical approaches)

- Sample preparation is time-consuming, cost of analysis is high

1. Phenotypic methods

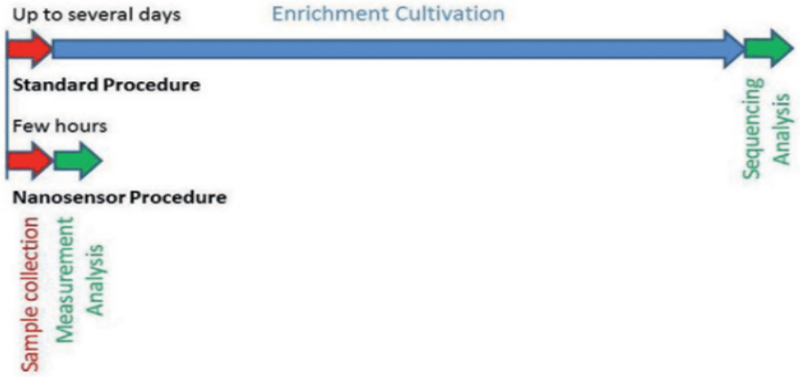
a) Broth dilution;
b) Disc diffusion;

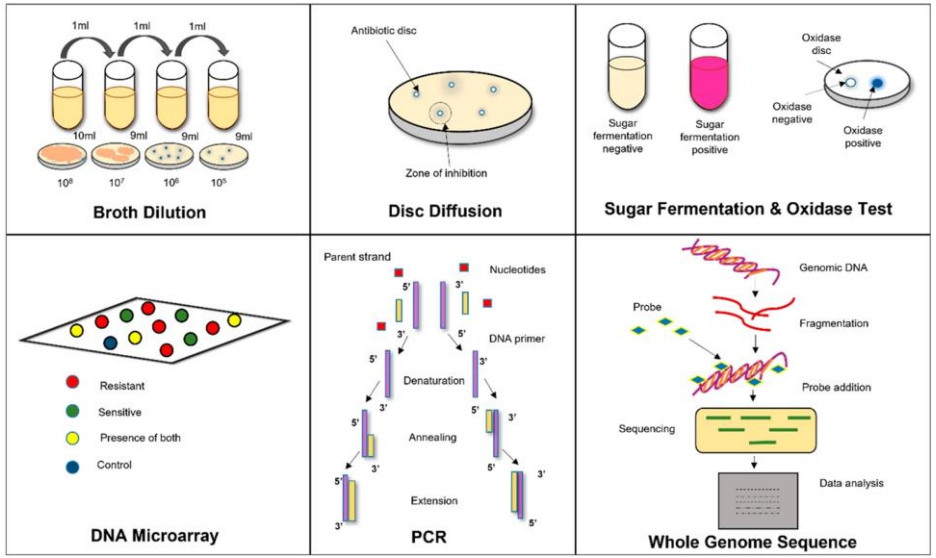
2. Biochemical methods

a) pH indicators;
b) Metabolic test;

3. Molecular diagnostic methods

a) PCR;
b) DNA microarrays;
c) Whole Genome Sequencing (WGS).





Nanotechnology approaches for AMR diagnostics

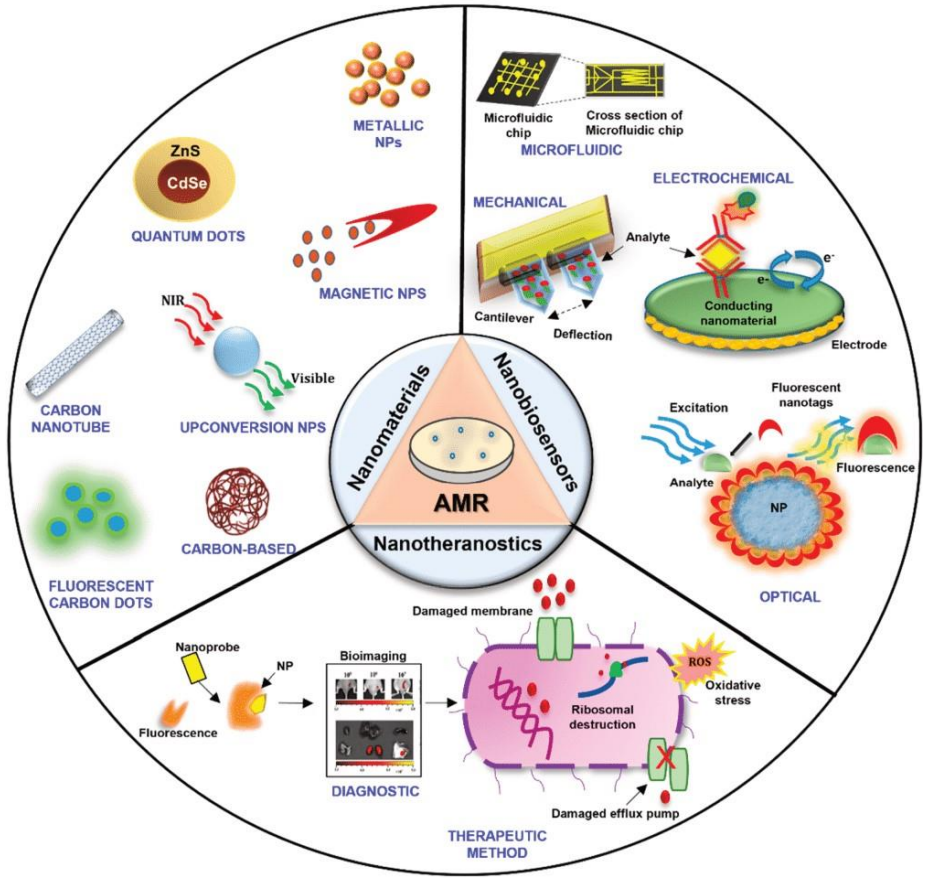
- Rapid detection, high sensitivity, more affordable, semi-skilled user, portable

1. Nanomaterial-based nanosensors

a) Metallic nanoparticles;
b) Quantum dots and fluorescent nanoparticles;
c) Magnetic nanoparticles;
d) Upconversion nanoparticles;
e) Carbon-based nanomaterials;
f) Nanoparticle-based POC rapid diagnostics;

2. Nanoparticle-based biosensors

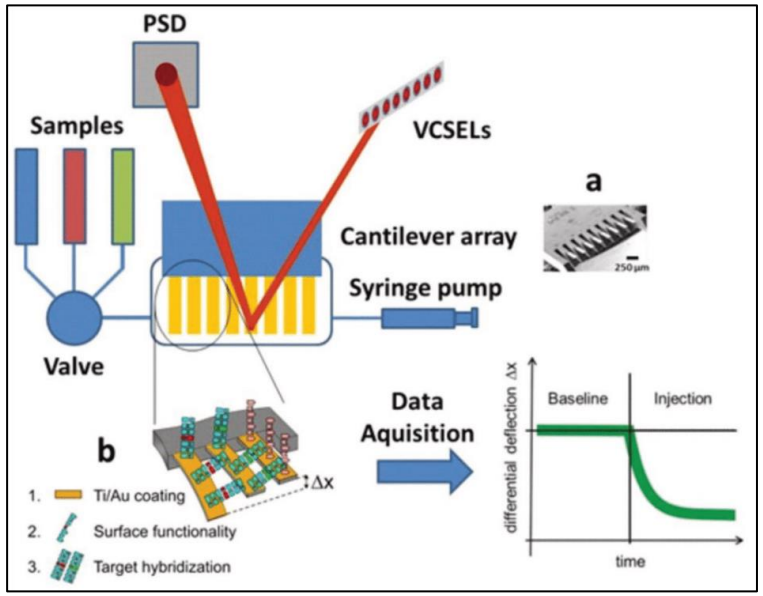
a) Optical biosensors;
b) Mechanical biosensors;
c) Electrochemical biosensors;
d) Microfluidic-integrated biosensors;
e) Other nanoscale biosensing techniques.



Proposed solution (1)

Rapid and Ultrasensitive Detection of Mutations and Genes Relevant to Antimicrobial Resistance in Bacteria

François Huber, Hans Peter Lang, Daniela Lang, Daniel Wüthrich, Vladimira Hinić, Christoph Gerber, Adrian Egli, and Ernst Meyer*



- ➔ Each array with 8 identical silicon cantilevers;
- ➔ diluted RNA injected sequentially (syringe pump and multiway valve);
- ➔ measurements at 28 °C in temperature controlled box;
- ➔ PSD for beam deflection readout of each cantilever (accuracy 0.1nm).

- Each nanosensor coated with probes for molecular recognition;
- binding of bacterial RNA sequence mechanically transduced (**bending of the cantilever**);
- external factors such as nonspecific interactions and thermal drift eliminated by calculating differential responses of probe and reference sensors.

Different SNPs highlighted by capitalized letters

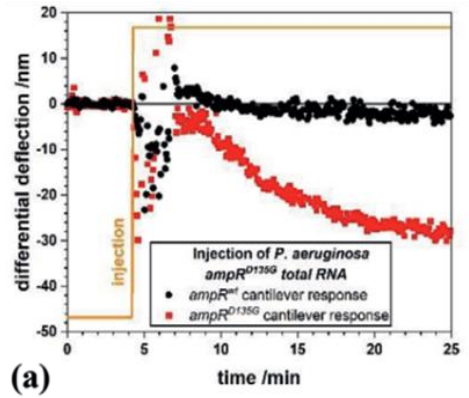
Designation	Sequence	Position range	Position	T_m [a]
$ampR^{135wt}$	gcggcgatg**T**cgacgcggt	394–413	404	27.3 °C
$ampR^{D135G}$	gcggcgatg**C**cgacgcggt	"	"	28.0 °C
$ampR^{135ref}$	gcggcgatg**A**cgacgcggt	"	"	27.1 °C
$ampR^{154wt}$	cctcggtgc**C**gtgccaggc	452–470	460	28.1 °C
$ampR^{G154R}$	cctcggtgc**G**gtgccaggc	"	"	27.9 °C
$ampR^{154ref}$	cctcggtgc**A**gtgccaggc	"	"	27.1 °C

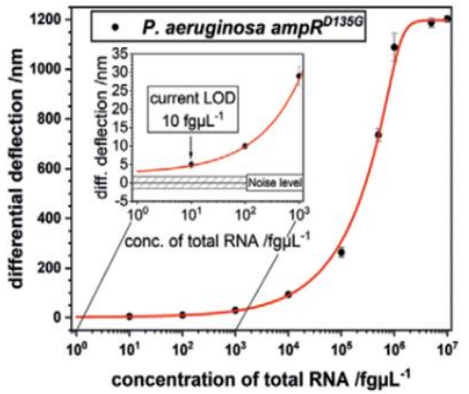
[a] T_m stands for melting temperature.

Differential responses to injection of RNA

(a) mutation and **reference** cantilever responses;

(b) limit of detection (LOD), sensitivity of detection of merely 10 bacterial cells.





Proposed solution (2)

➔ To analyse the genes that may code for antibiotic resistance, two possible methods:

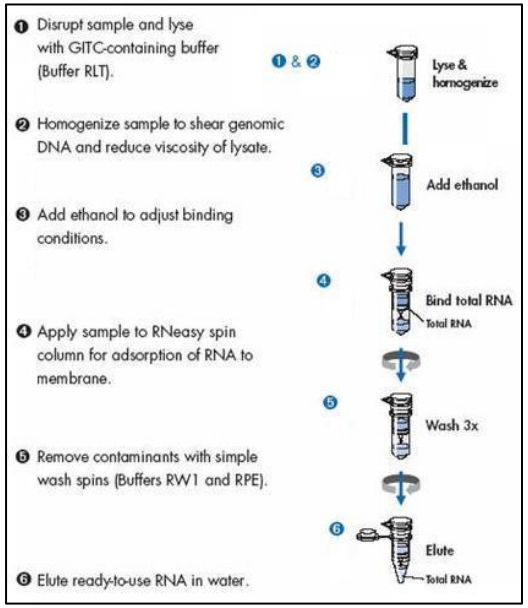
Direct bacterial mRNA analysis

- doesn't require any preprocessing;
- RNA already represents individual genes;
- genes of interest may not be active at time of analysis, thus no RNA available to be analysed;
- more fragile than DNA.

Quality control:

➔ benchtop fluorometer

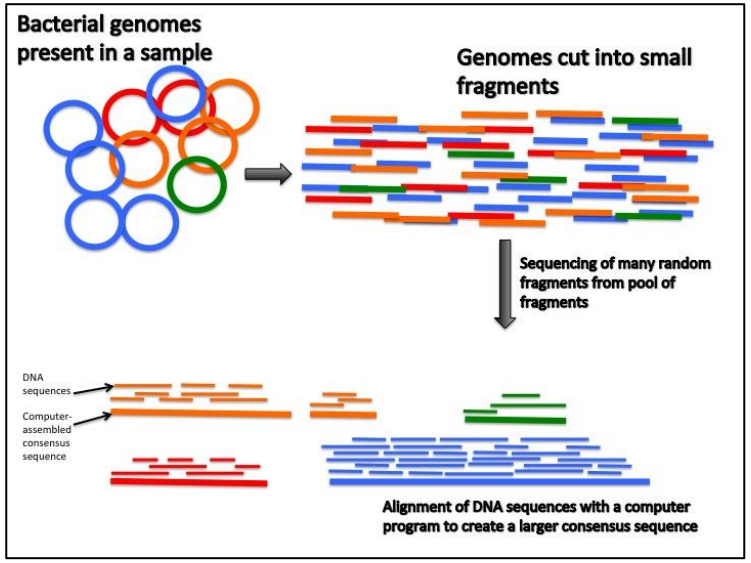
➔ spectrophotometer



RNeasy Mini Kit (Qiagen)

Shotgun analysis of DNA

- randomly chops up bacterial DNA;
- guarantees that whole genome is available for analysis;
- may cleave relevant genes, thus lowering efficiency.



Proposed solution (3)

- Thinner and longer cantilevers → higher sensitivity;
- upper sides of cantilevers coated with 3nm layer of Ti, followed by 20nm thick Au layer;
- nanosensor arrays functionalized using modified ink-jet spotting;
- data acquisition hardware, temperature regulation and syringe pump controlled by LabView software.

➔ **Bending of cantilevers** detected by **reflection of external laser beam** focused at cantilever apex;

➔ monitoring deflection of 8 sensors in parallel in time-multiplexed manner.

➔ **Antibiotic resistance** mechanisms commonly in clinics;

➔ SNPs detected using probes that cover location of mutation;

➔ very high **sensitivity** and **specificity**;

➔ **rapid response time in the order of 5 min;**

➔ low detection limit → potential to **diagnose directly** from patient;

➔ feasibility of bacterial RNA isolated from complex samples, such as blood.

Potential cost: ≤10€

Alternative → capacitive sensing

Piezoresistive	Capacitive	Electromagnetic
Simple fabrication	Simple mechanical structure	Structural complexity varies
Low cost	Low cost	Complex packaging
Voltage or current drive	Voltage drive	Current drive
No need for circuits	Requires electronic circuits	Simple control circuits
Low output impedance	Susceptible to EMI	Susceptible to EMI
High temperature dependence	Low temperature dependence	Low temperature dependence
Small sensitivity	Large dynamic range	Sensitivity ∝ magnetic field
Insensitive to parasitic resistance	Sensitive to parasitic capacitance	Insensitive to parasitic inductance
Open loop	Open or closed loop	Open or closed loop
Medium power consumption	Low power consumption	Medium power consumption