Electrochemical DNA Hybridization Sensors

Shenmin Pan

Bioanalytical Chemistry 395
Instructor: Prof. Rusling

Outline

- Biosensors

- Electrochemical DNA Hybridization Biosensor
  - Four different pathways
  - Three types of DNA sensors

- Conclusion and challeges
- References

Biosensors

- **What is biosensors:**
- a device for the detection of an analyte that combines a biological component with a physicochemical detector component.

- **Components of biosensors**
- the sensitive biological element
- the transducer in between (associates both components)
- the detector element (optical, electrochemical, thermometric, or magnetic)

Electrochemical DNA biosensor

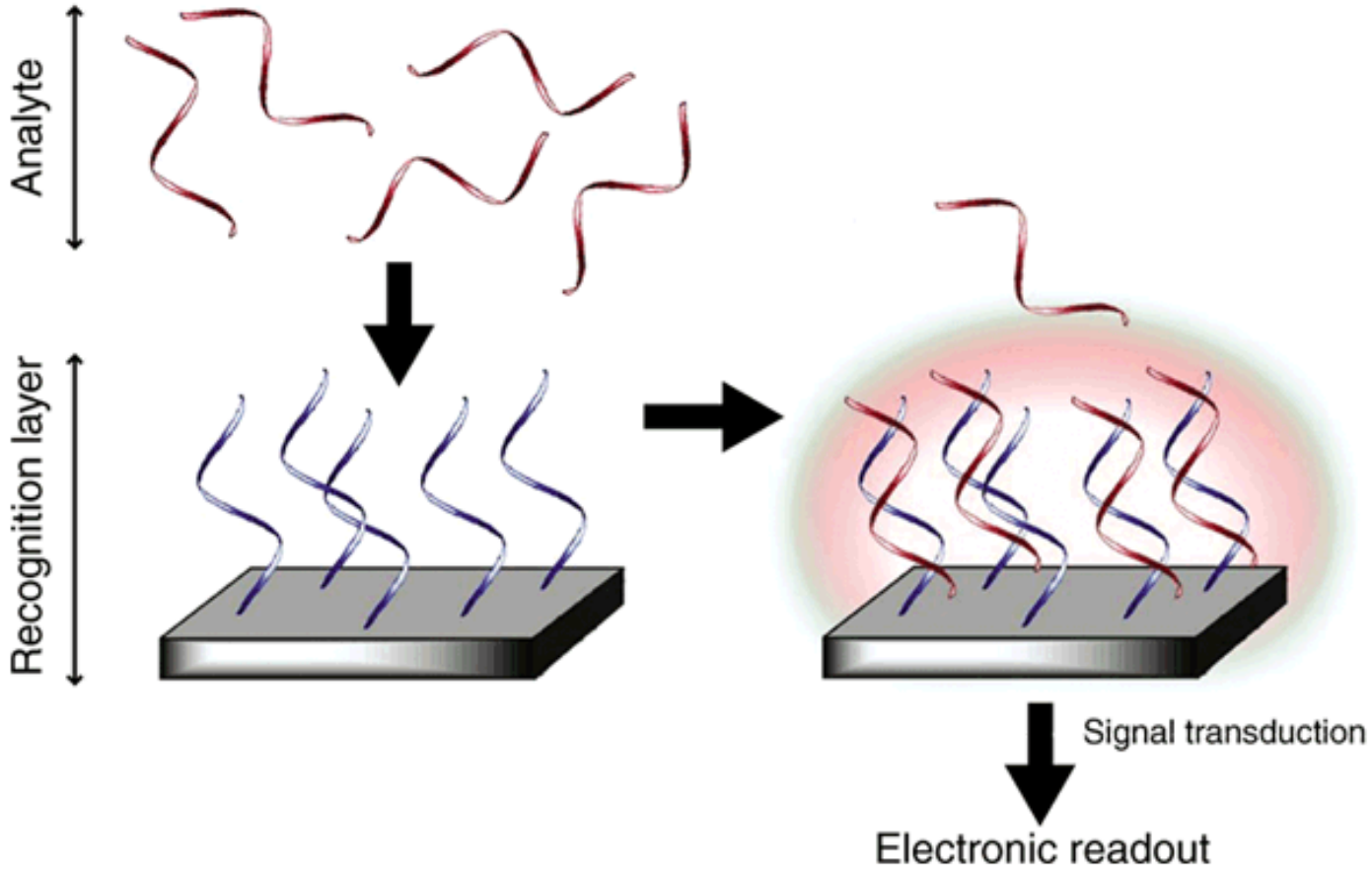
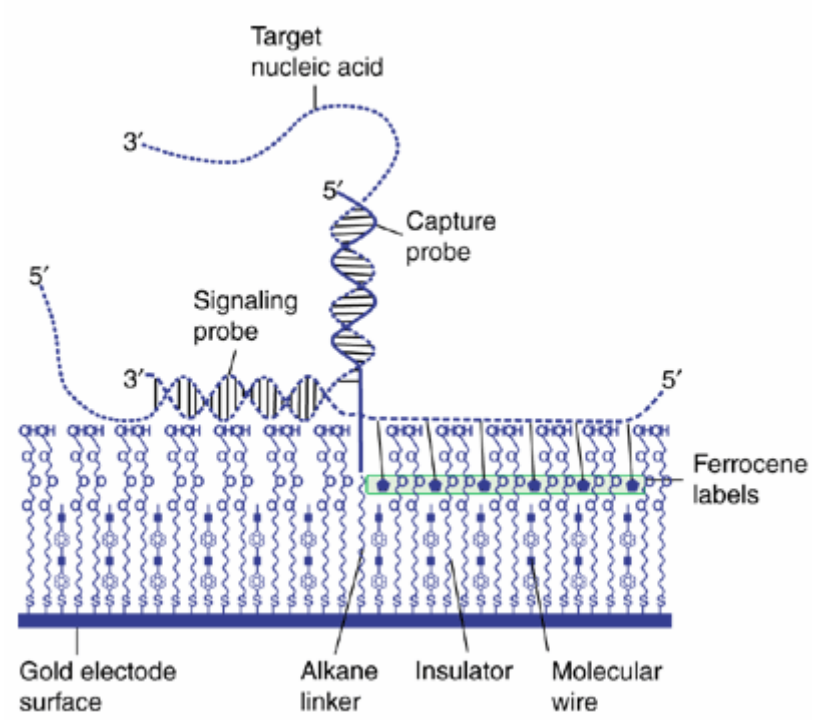
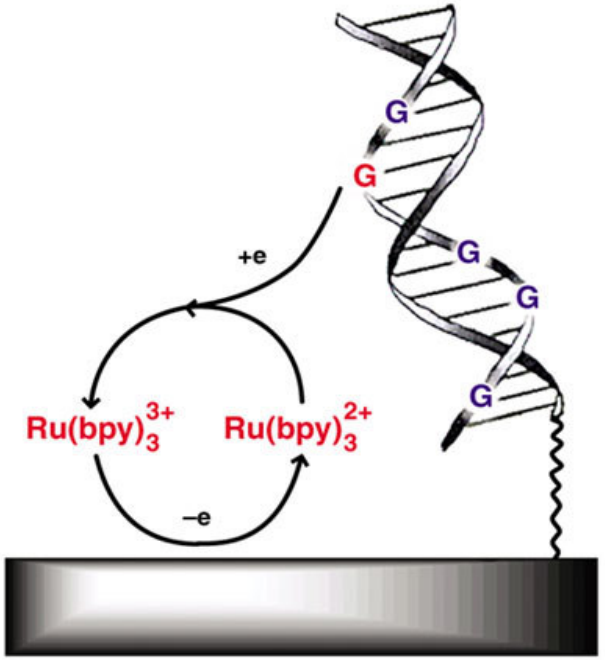


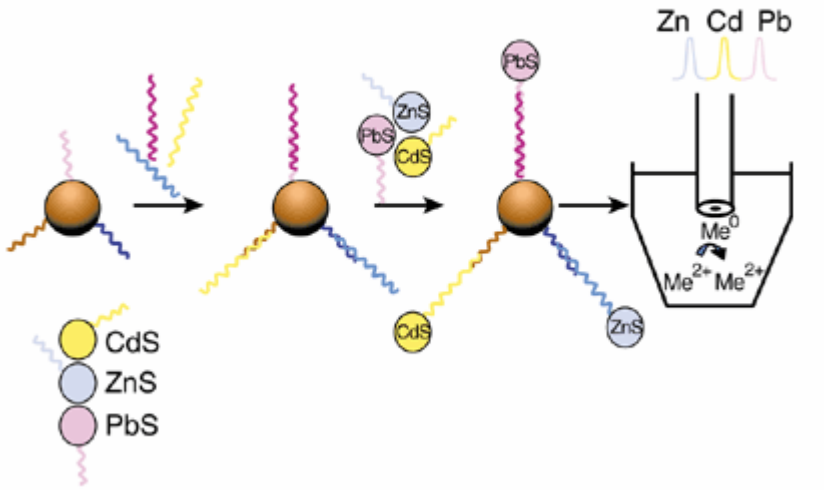
Fig. 1 General DNA sensor design

DNA Hybridization Sensor

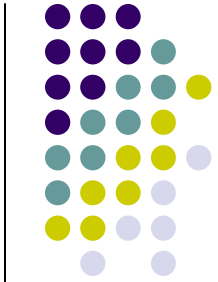
- Four pathways:
- A ⇧ / ⇩ in the ox./red. i_p of the label which selectively binds with ds-DNA/ss-DNA
- A ⇧ / ⇩ in the ox./red. i_p of electroactive DNA bases such as guanine or adenine
- The S. of the substrate after hybridization
- The S. of the nanoparticle probe attached after hybridization with the target


Target
nucleic acid
3′
5′
Capture
probe
5′
Signaling
probe
3′
5′
Ferrocene
labels
Gold electode
surface
Alkane
linker
Insulator
Molecular
wire


G
G
G
G
G
+e
Ru(bpy)3 3+
Ru(bpy)3 2+
–e


Zn Cd Pb
PbS
ZnS
PbS
CdS
Me0
Me2+ Me2+
CdS
ZnS
CdS
ZnS
PbS

Focus: three types



- DNA-specific redox indicator detection
- Nanoparticle-based DNA detection
- Intercalator-based DNA detection

DNA-specific redox indicator detection

- Osmetech eSensor®
-

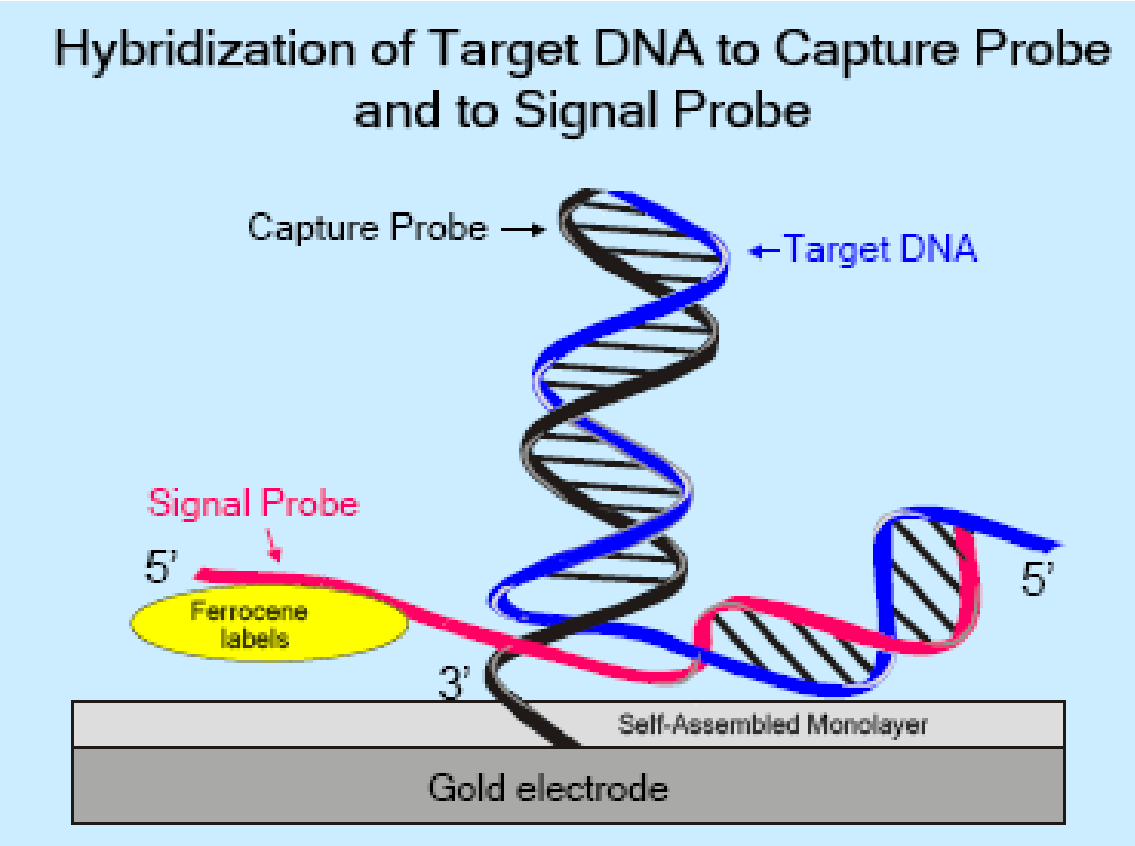


Fig.3 Schematic representation of eSensor

Outline of Basic Steps of Assay

gDNA from patient sample (blood)

↓

PCR Amplification

↓

Generation of single-stranded DNA using Lambda Exonuclease

↓

Hybridization Solution Preparation

↓

Preparation of Cartridges

↓

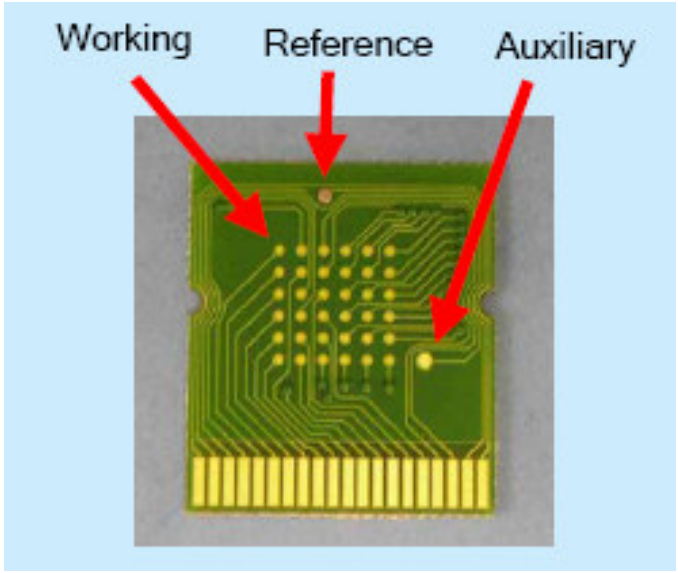
Hybridization of Cartridges

↓

Data Acquisition

↓

Review of Results

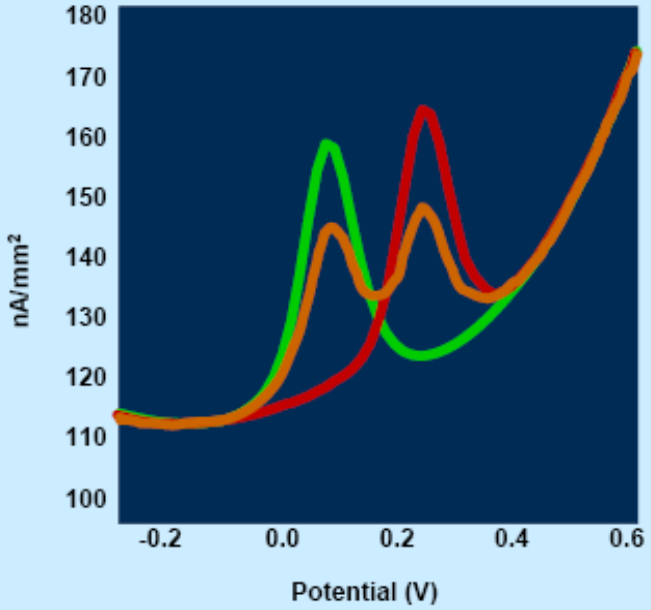


Genotyping Signature Waves

Wild type

Mutant

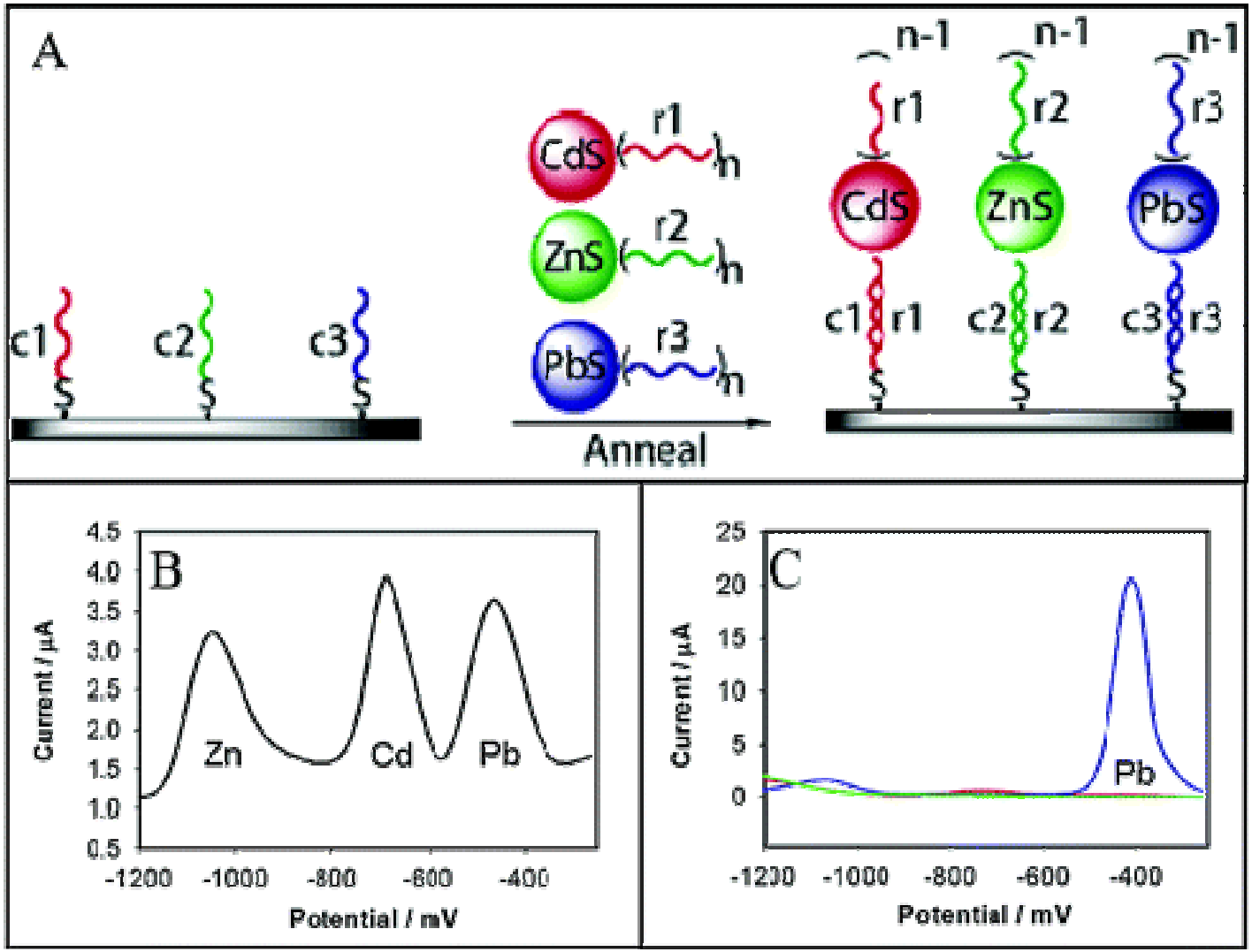
Heterozygote



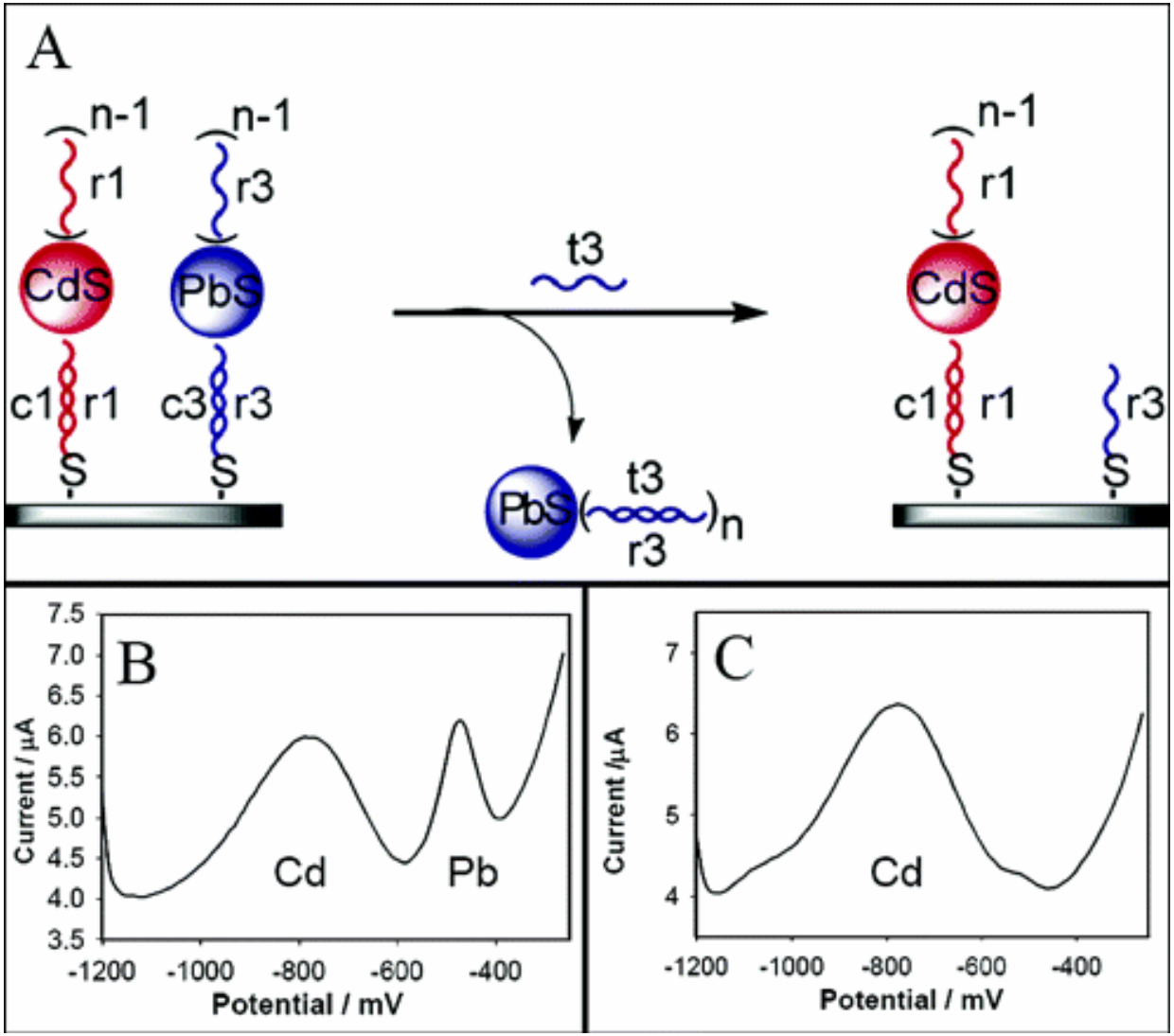
Nanoparticle-based DNA detection

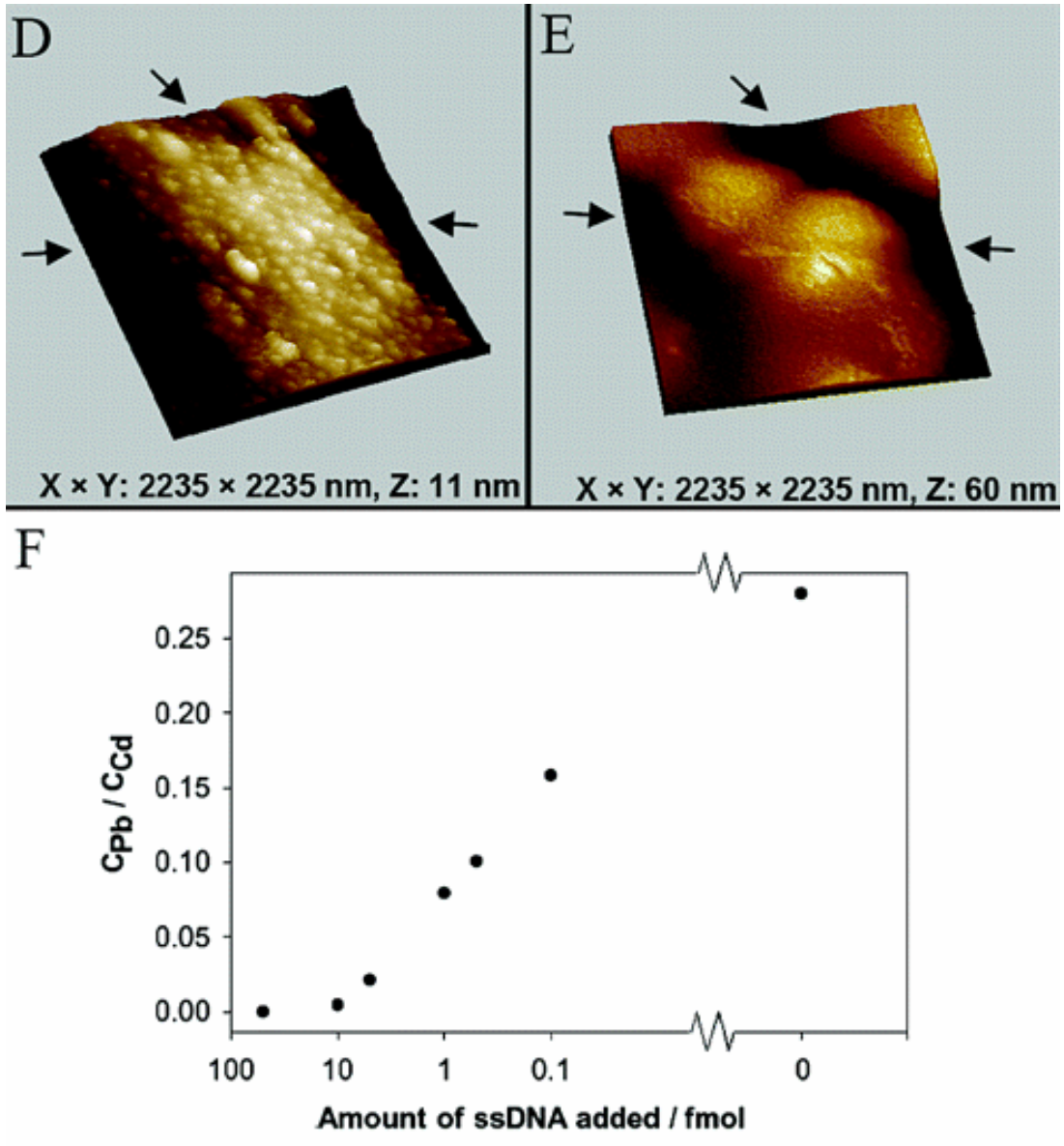
- Example: fm detection of DNA using metal sulfide nanoparticles
- 5’-thiolated capture sequence DNA c1, c2, c3 on the gold substrate
- CdS, ZnS, PbS nanoparticles (3nm, 5nm)
- Conjugated with 5’-thiolated DNA reporter sequences r1, r2, r3

Multi-target Detection



Competitive Binding




D
X × Y: 2235 × 2235 nm, Z: 11 nm
E
X × Y: 2235 × 2235 nm, Z: 60 nm
F
0.25
0.20
0.15
0.10
0.05
0.00
C_{Pb} / C_{Cd}
100
10
1
0.1
0
Amount of ssDNA added / fmol

Intercalator-based DNA detection

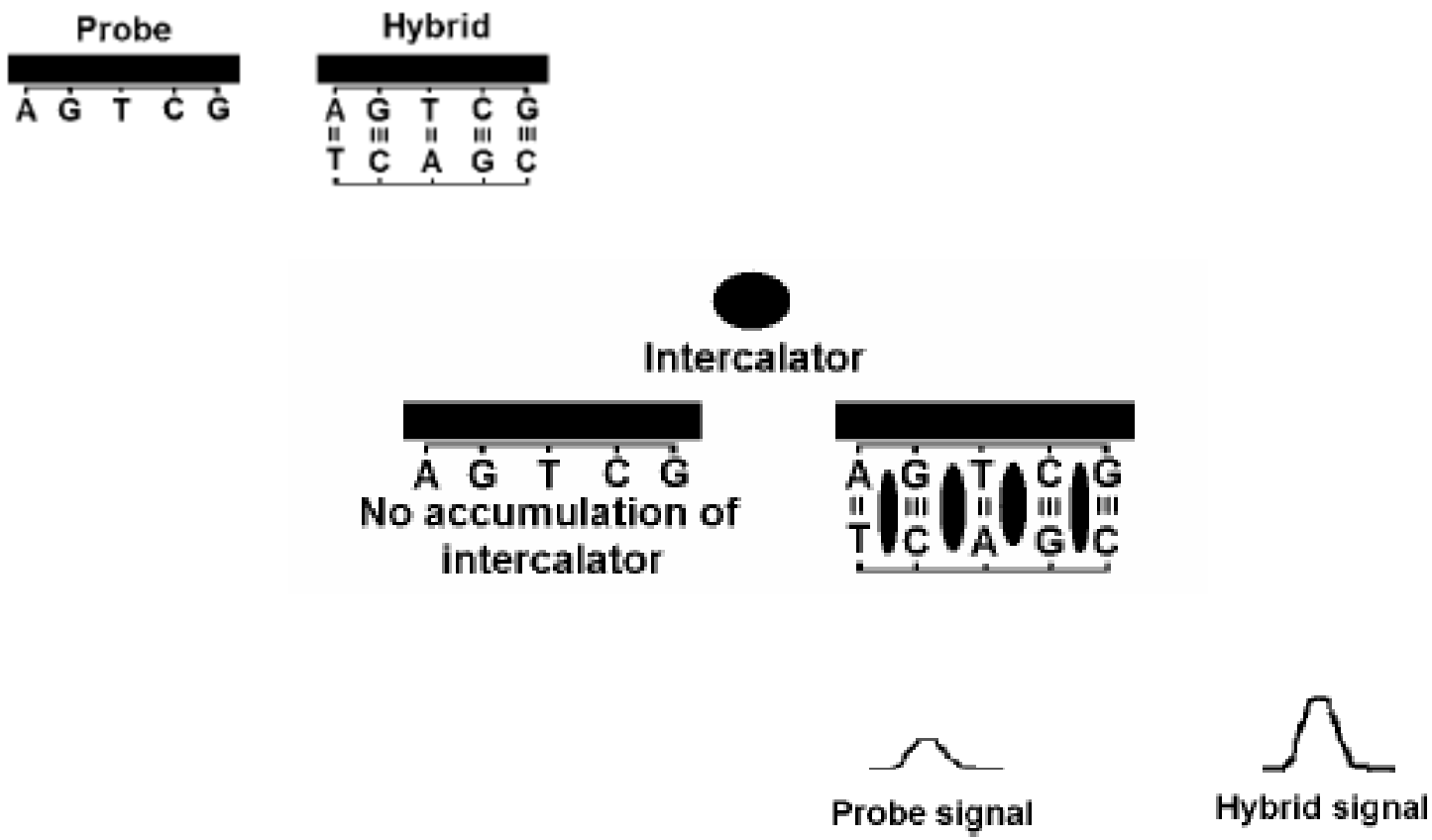


Fig. 4. Basic principles of intercalative probes

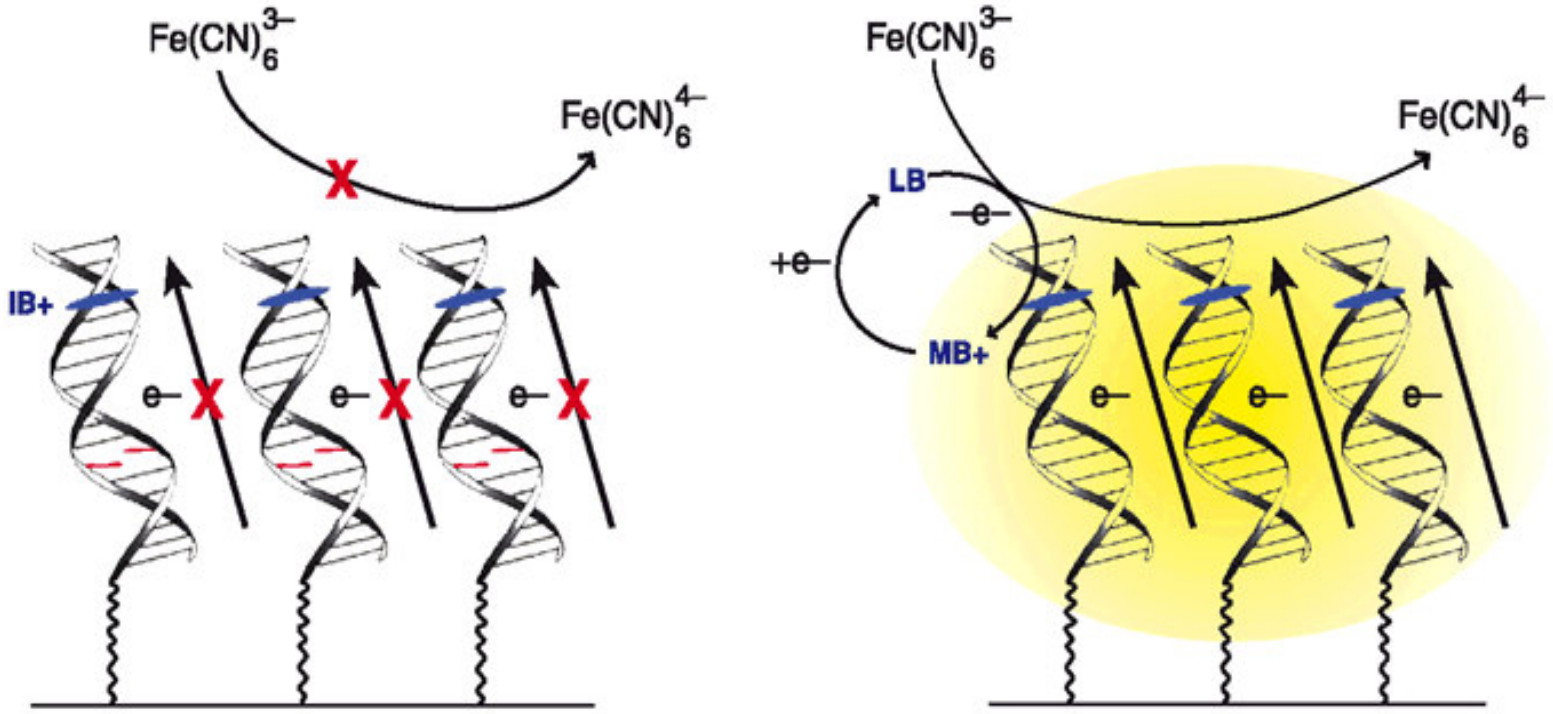


Fig. 5. electrochemical assay for mismatches through DNA-mediated charge transport

Comparison of the methods

Sensor type	Ad.	Disad.
DNA-specific redox	Good sens. Sample remain unaltered	Labeling step required
Nano-based amplification	very good sens. Well suited for multi	Preparation; reliability
DNA-mediated charge transport	Highly sens. Suited for mismatch det.	Preparation of target sample

Conclusion

- Low cost, small size, inherent sensitivity, relatively simple in data processing
- Most used are metal nanoparticles, photoelectrochemical detection of DNA hybridization of these metal sulfide
- Carbon nanotubes

Challenges

- Desirable Electrode Surface (Polymer layer electrical conductivity, amenability to probe immobilization, prevent nonspecific binding)
- Fabrication into large scale and useful arrays
- Biological complexity of a genomic DNA sample. Real biological sample and detection (inherent complexity: purification and isolation)

references

1. Drummond, T.G.; Gill, M.G. Nat. Biotechnol. 2003, 21, 1192
2. Kerman, K.; Kobayashi, M.; Tamiya, E. Meas. Sci. and Technol., 2004, 15, R1
3. http://www.osmetech.com/products/esensor/
4. Hansen, J. A.; Mukhopadhyay, R.; Hansen, J.; Gothelf, K.V. J. Am. Chem. Soc., 2006, 128, 3860
5. Tang, X.; Bansaruntip, S.; Nakayama, N.; Yenilmez, E.; Chang, Y.; Wang, Q. Nano Lett. 2006, 6, 1632


Thank you!