

# Properties of nanofabricated biosensors based on DNA aptamers

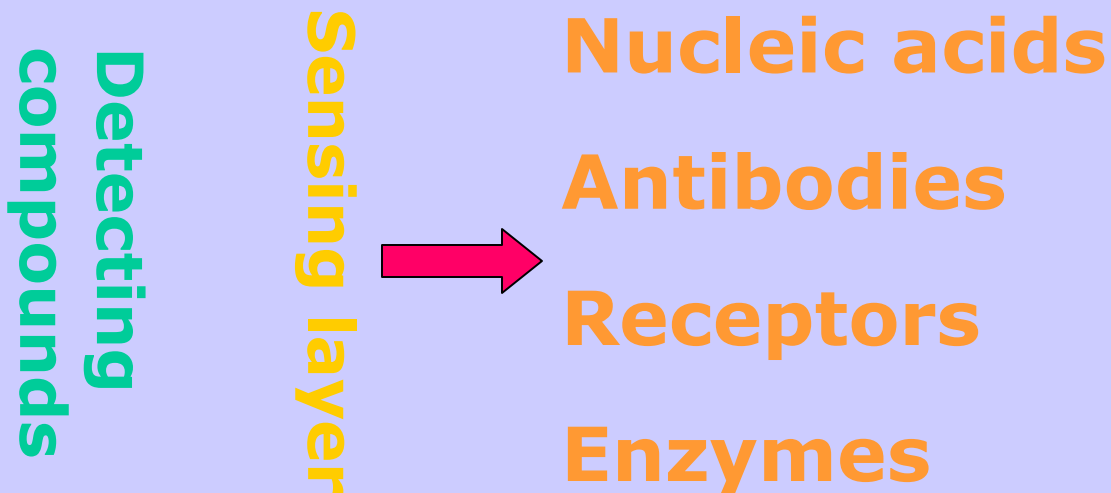
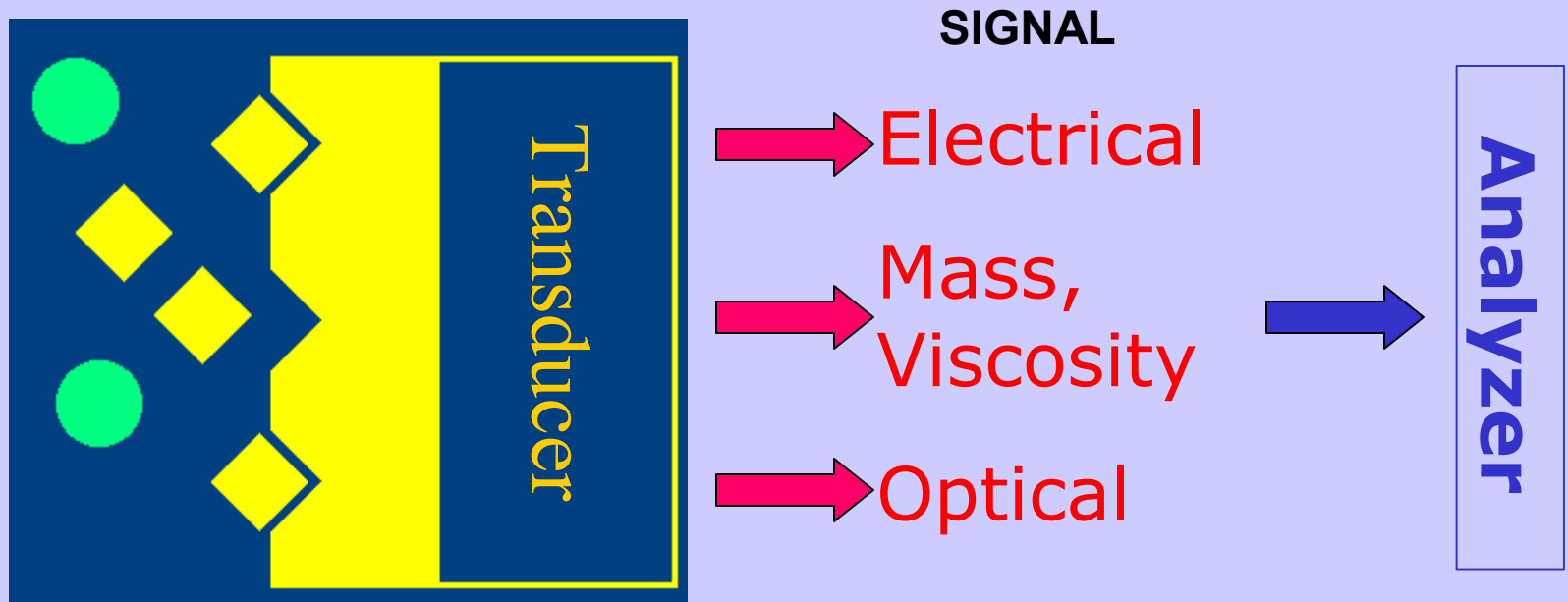
**Tibor Hianik**

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Comenius University,  
Bratislava, Slovakia*

# Content of presentation

- Introduction – Concept of biosensors
- Structure of DNA aptamers
- Immobilization of aptamers at various surfaces
- Methods of detection of aptamer-ligand interactions
- Properties of the surfaces with immobilized aptamers and the affinity interactions
- Future perspectives

# Scheme of biosensor



# Advantages of Biosensors

(in comparison with traditional analytical methods)

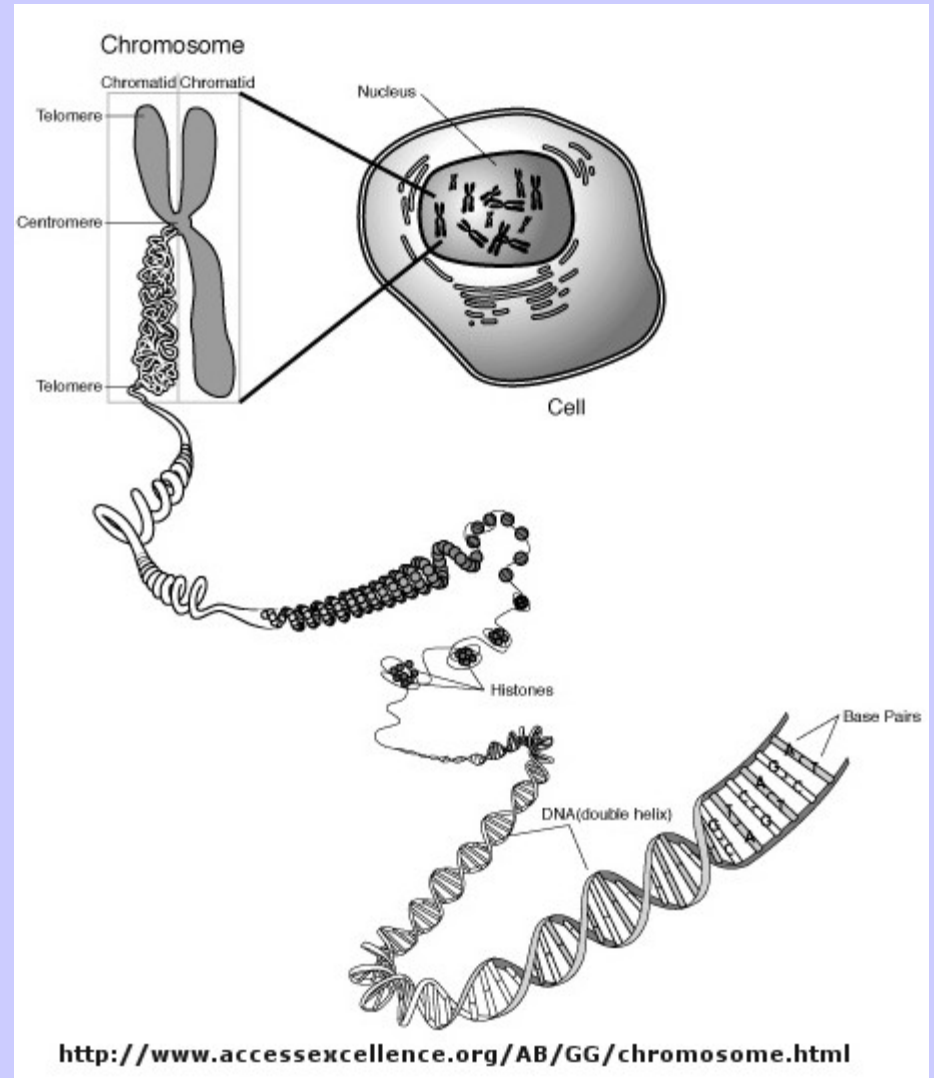
- **Fast detection (minutes)**
- **Fast response (seconds)**
- **High sensitivity (typically nM, improved sensitivity with nanoparticles pM and better)**
- **High selectivity**
- **Easy preparation and operation**
- **Miniature**
- **Reusable**
- **Low cost (Typically less than 10 EUR/sensor)**

# Nucleic acids as sensing elements

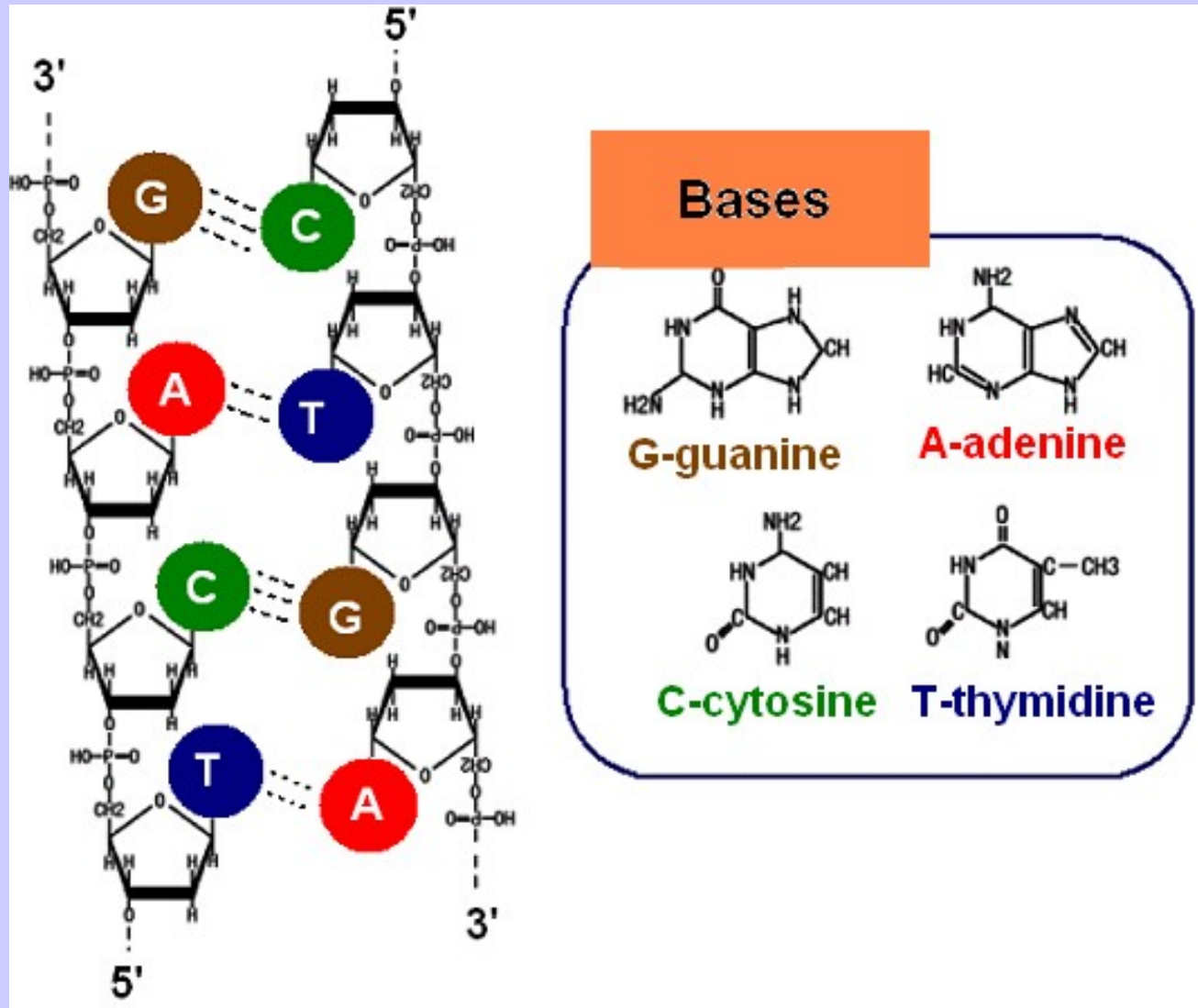
The main role of DNA – storage of genetic Information

DNA is composed of two complementary chains

B-form DNA – double helix



# Each DNA chain is composed of 4 types of bases



**Heating unwinds the double helix.  
Single DNA chains could adopt various  
conformations in a solution**

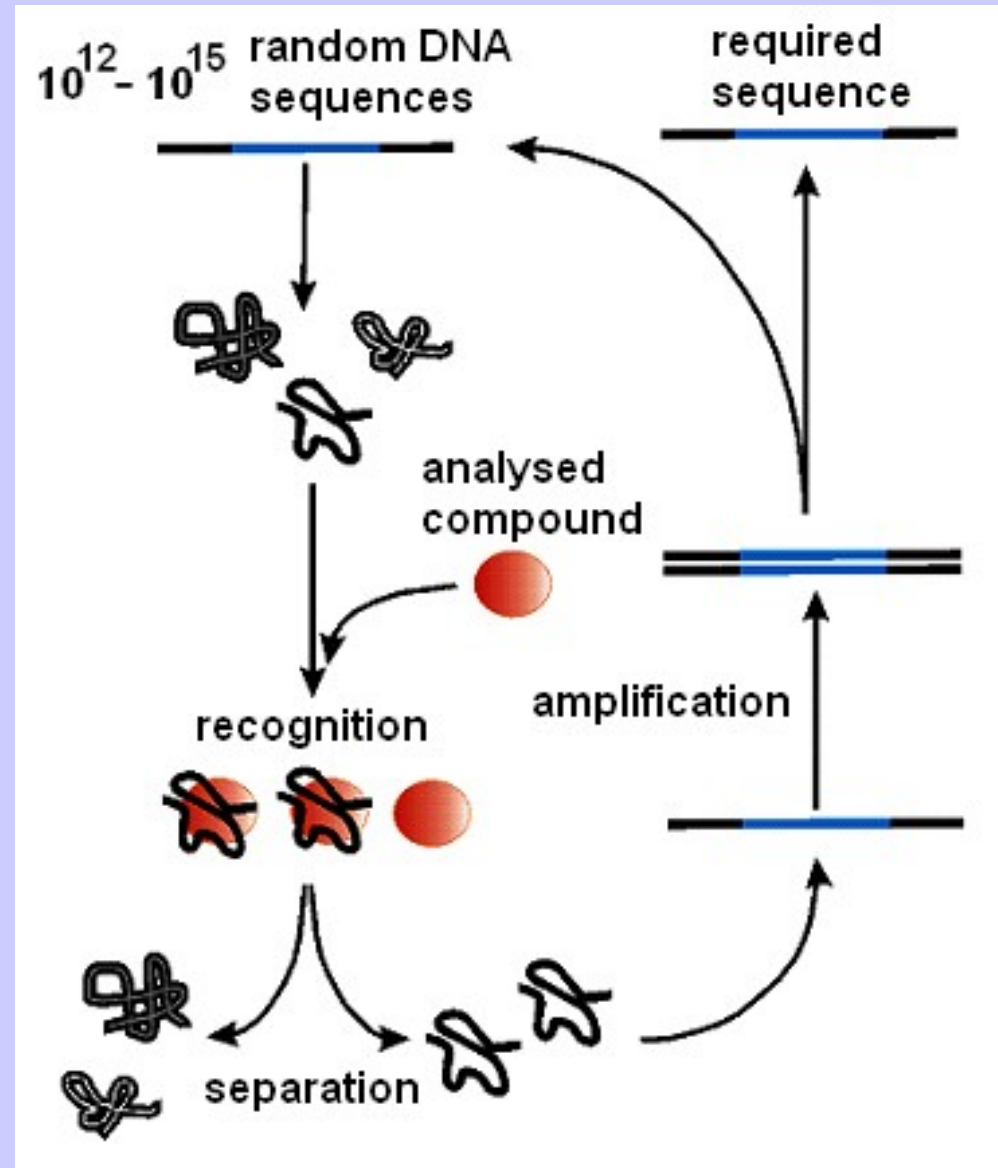


# Some of single stranded DNA sequences (15-60 nucleotides) can specifically bind proteins or other compounds - SELEX

Robetson and Joyce, 1990

Tuerek and Gold, 1990

Elington and Szostak, 1990

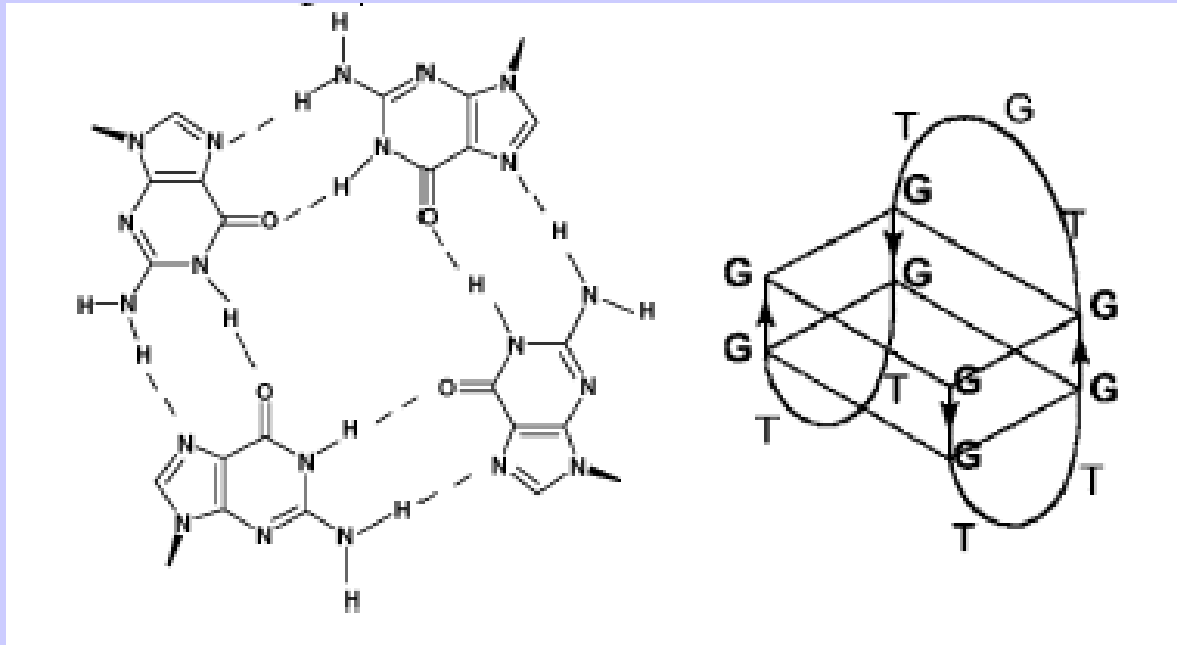


# APTAMERS

- are single stranded RNA or DNA with high affinity to proteins or other compounds
- comparable affinity with antibodies
- aptamers can recognize protein isoforms
- aptamers can be chemically modified by thiol groups or biotin, that allowing them to attach to the solid surface
- This system can be used as a biosensor for detection proteins in complex biological liquids
- 1992 – first aptamer for human thrombin (Bock et al., Nature 353 (1992) 564).



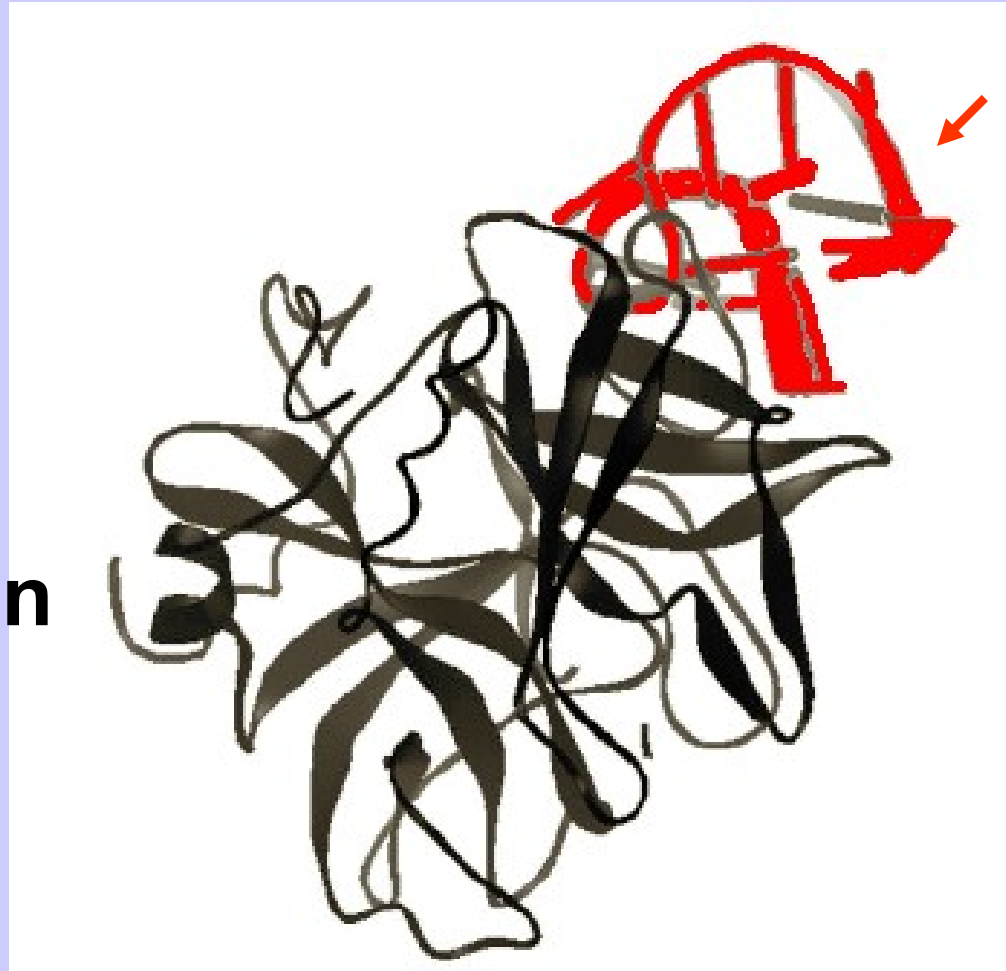
# Antithrombin DNA aptamer



Thrombin aptamer consists of sequence of 15 nucleotides that create specific binding site

# Example of three-dimensional structure of aptamer-thrombin complex

**Thrombin**



**Aptamer  
binding site**

**Thrombin is serine protease, that play important role in blood coagulation**



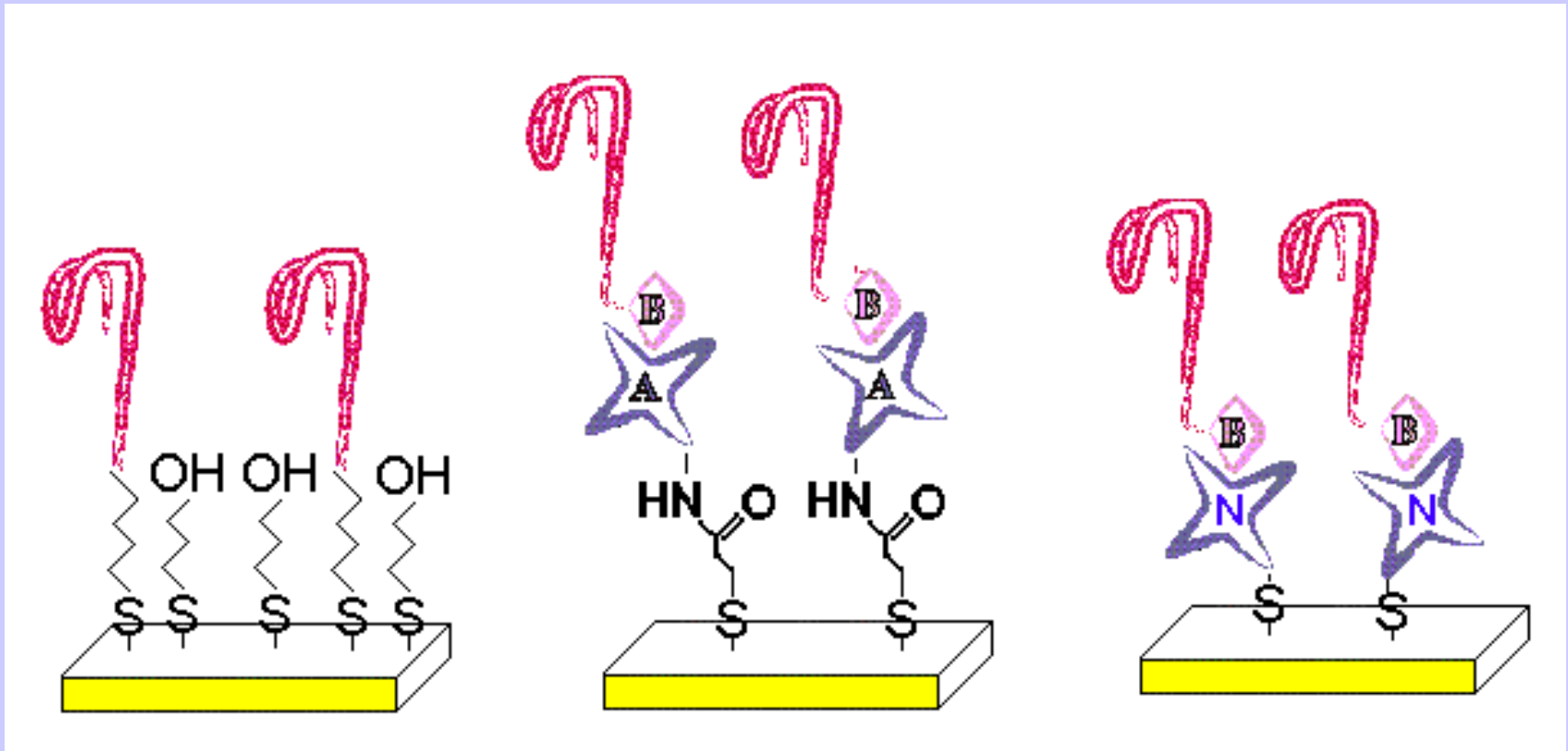
# PROBLEMS

- **Immobilization of aptamers to a solid support in order to provide conformational flexibility**
- **Selection of optimal structure of aptamer**
- **Selection of best physico-chemical conditions (pH, ionic strength, temperature)**
- **Elimination of interferences with other proteins and cells**
- **Methods of detection protein-aptamer interactions in complex biological liquids**

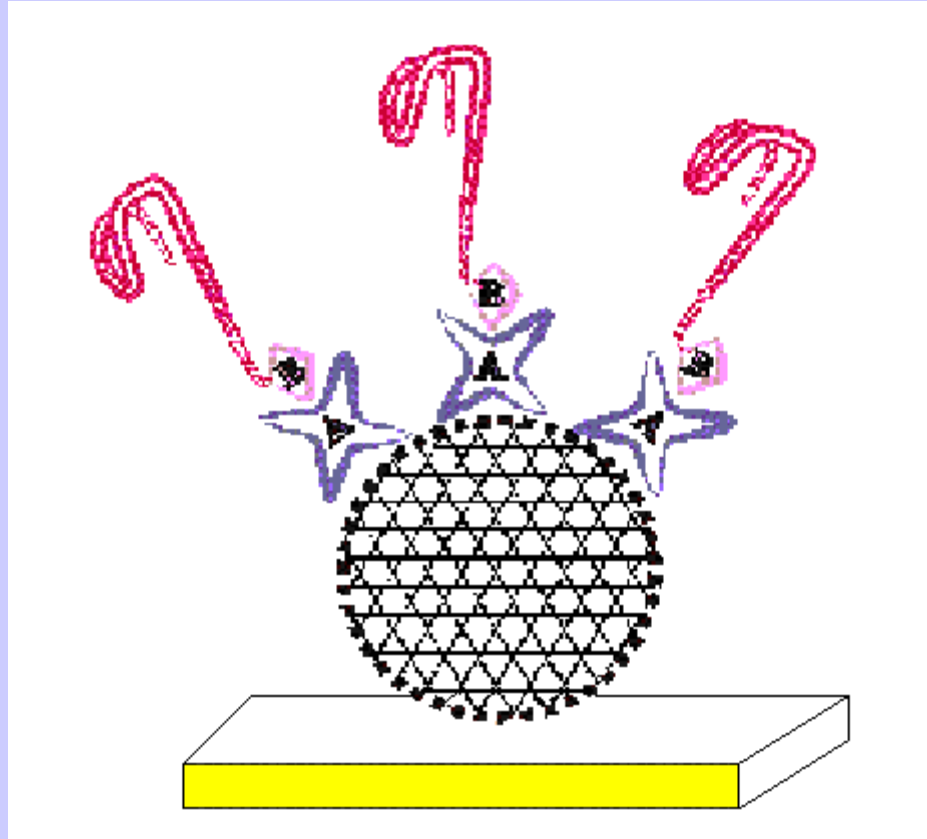
# Methods of immobilization of aptamers at surfaces

1. Thiolated DNA - gold
2. Avidin -biotin
3. Neutravidin -biotin
4. Streptavidin - biotin
5. Dendrimers – avidin-biotin
6. Self assembled monolayers
7. Conducting polymers
8. Carbon nanotubes

# Aptamer immobilization by chemisorption and by means of avidin or neutravidin-biotin binding

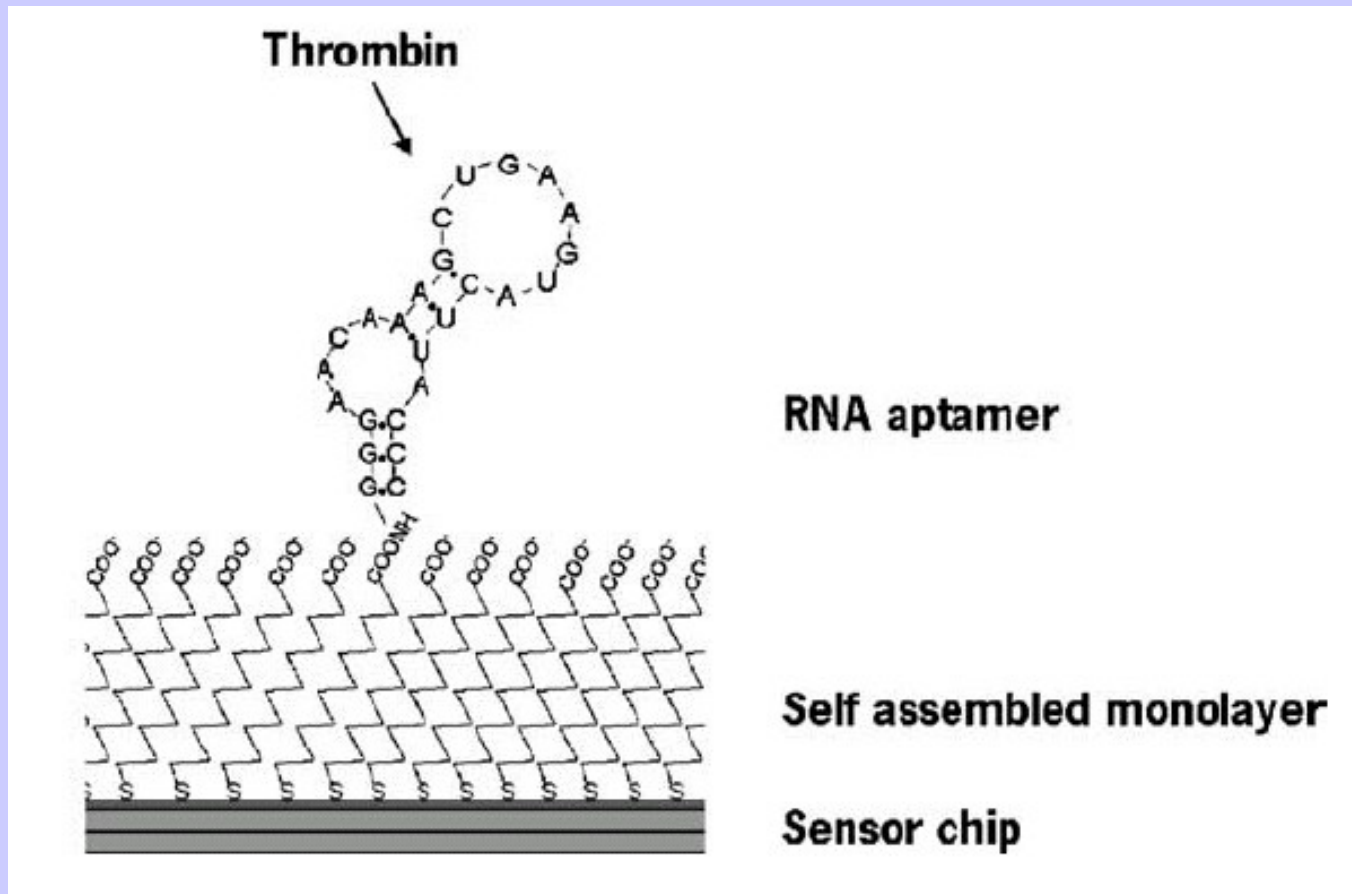


# Dendrimers



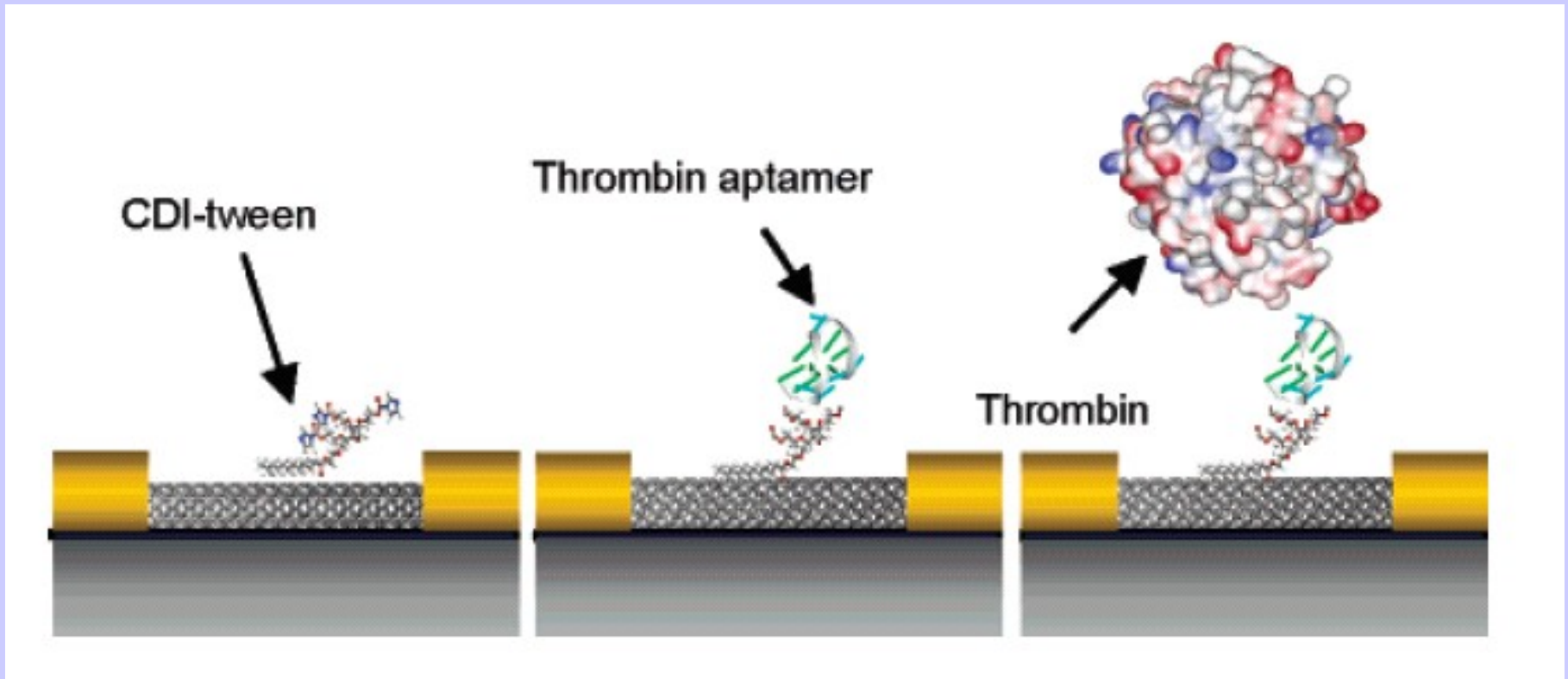
**Dendrimers** are highly branched structures that allowing to Immobilize large number of molecules

# Immobilization of aptamers on self assembled monolayers



# Immobilization of aptamers on carbon nanotubes

So et al. *J. Am. Chem. Soc.*, 127 (2005) 11906



CDI – tween: carbodiimidazole-activated Tween 20

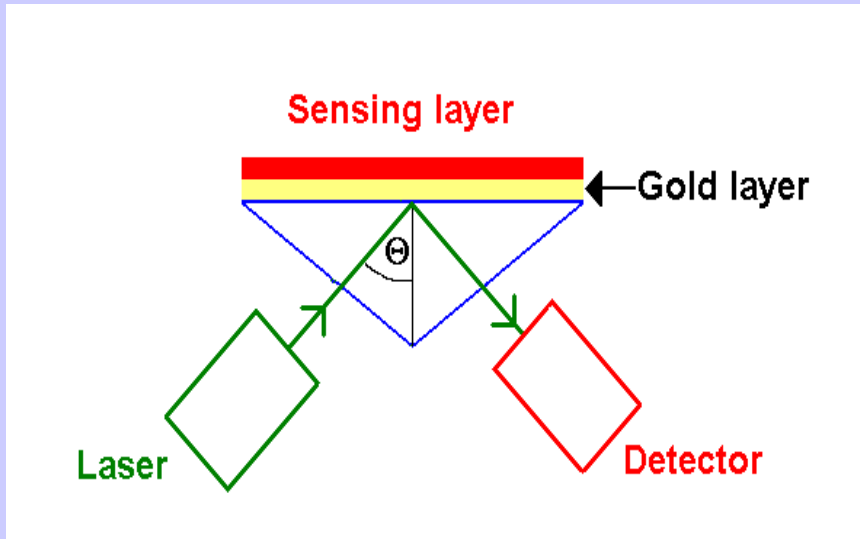
# Methods of detection of protein-aptamer interactions

- Quartz crystal microbalance (QCM)
- Thickness shear mode method (TSM)
- Electrochemical indicators
- Impedance spectroscopy
- Surface plasmon resonance (SPR)
- Fluorescence method utilizing DNA beacons

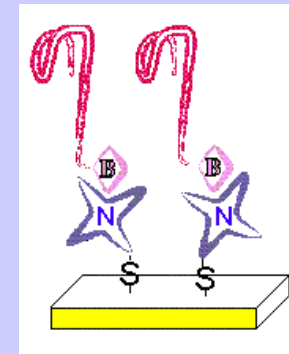
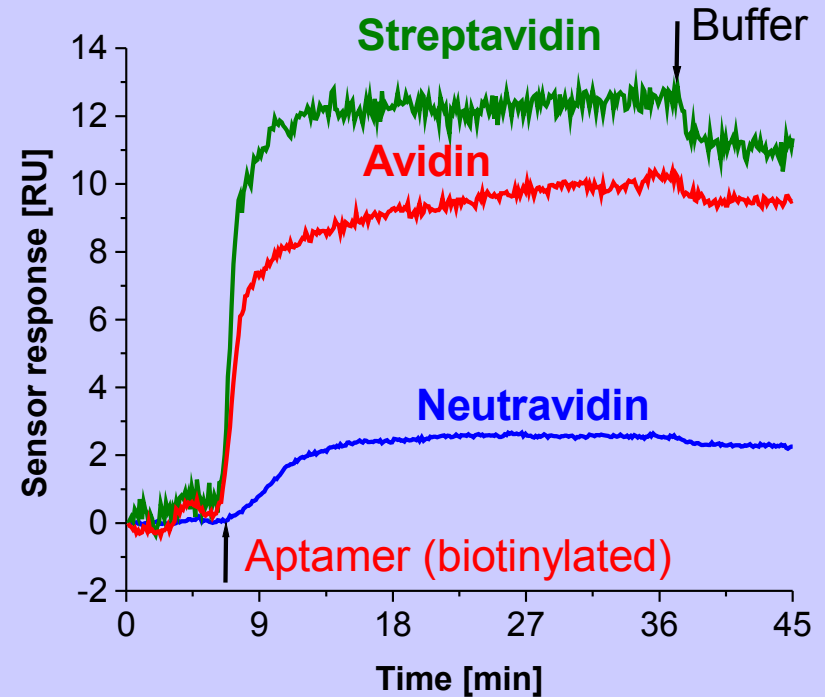
## Methods used in this study

- **Surface plasmon resonance (SPR)**
- **Quartz crystal microbalance (QCM)**
- **Thickness shear mode method (TSM)**

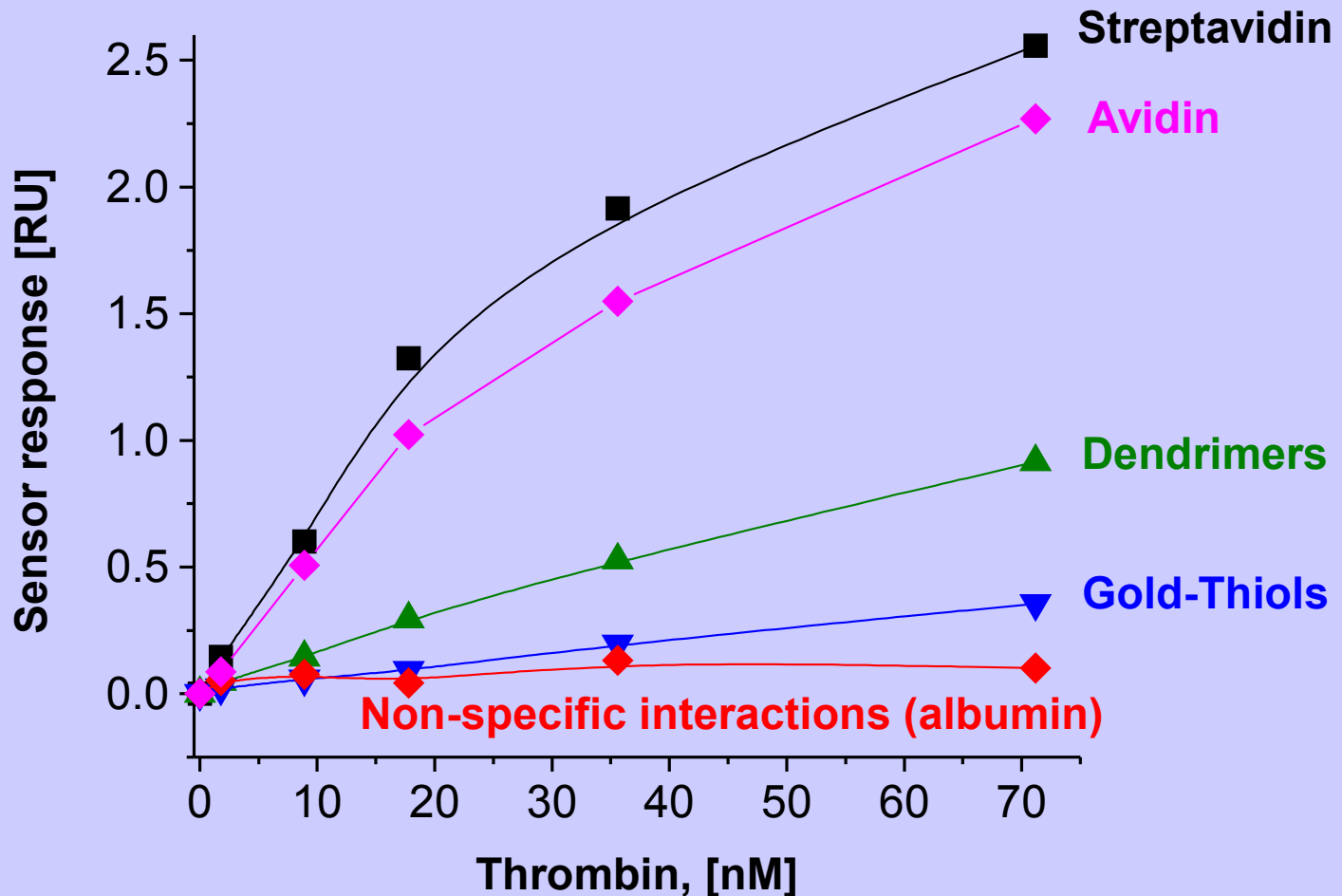
# Optimalisation of aptamer immobilisation using surface plasmon resonance (SPR)



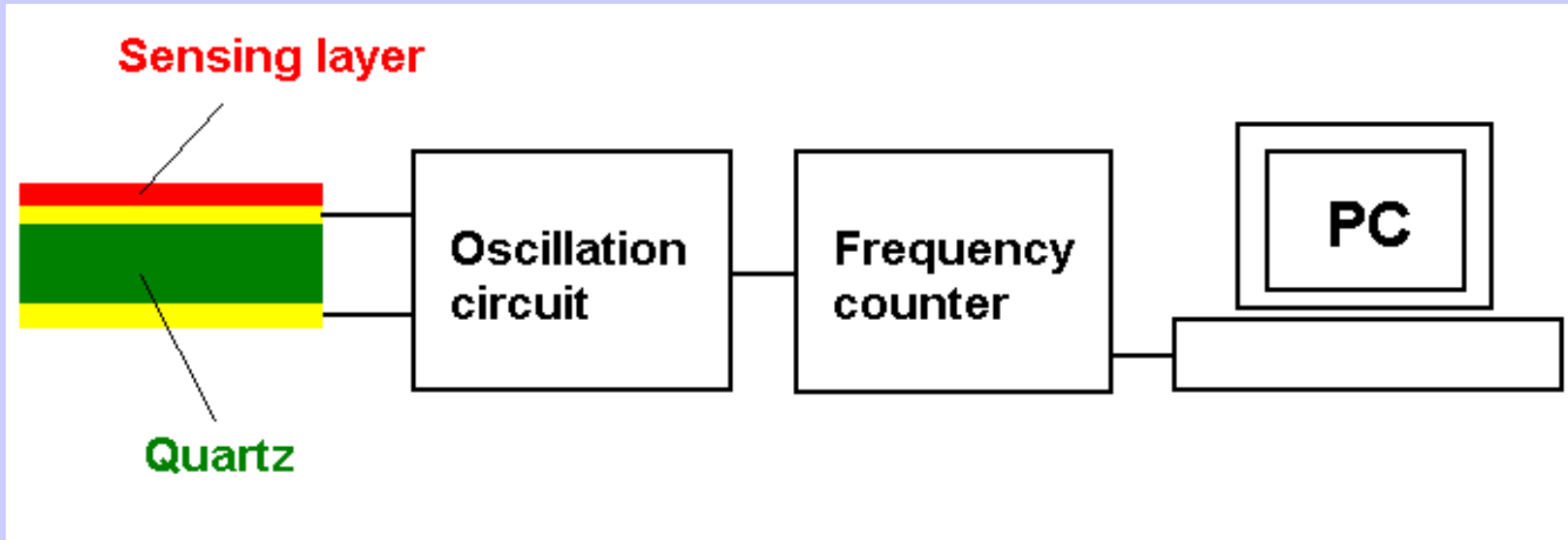
Surface **plasmons** are excited by polarised laser beam at certain angle  $\Theta$ . The intensity of reflected light is measured



# SPR detection of thrombin by aptamers immobilized at various surfaces



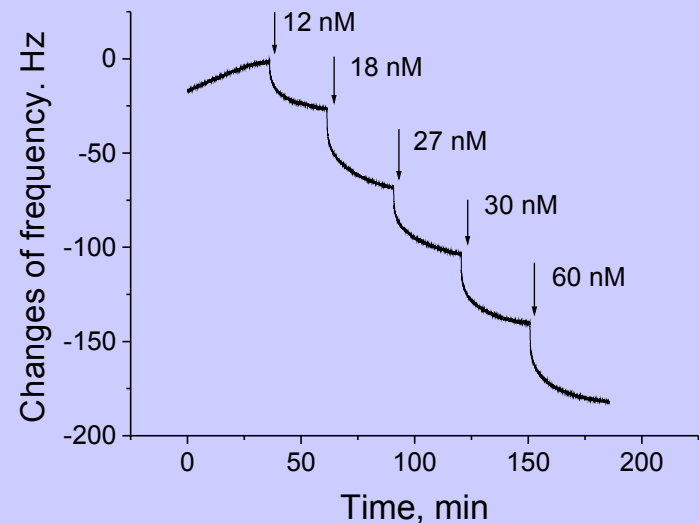
# Kinetics of thrombin-aptamer binding can be studied by QCM



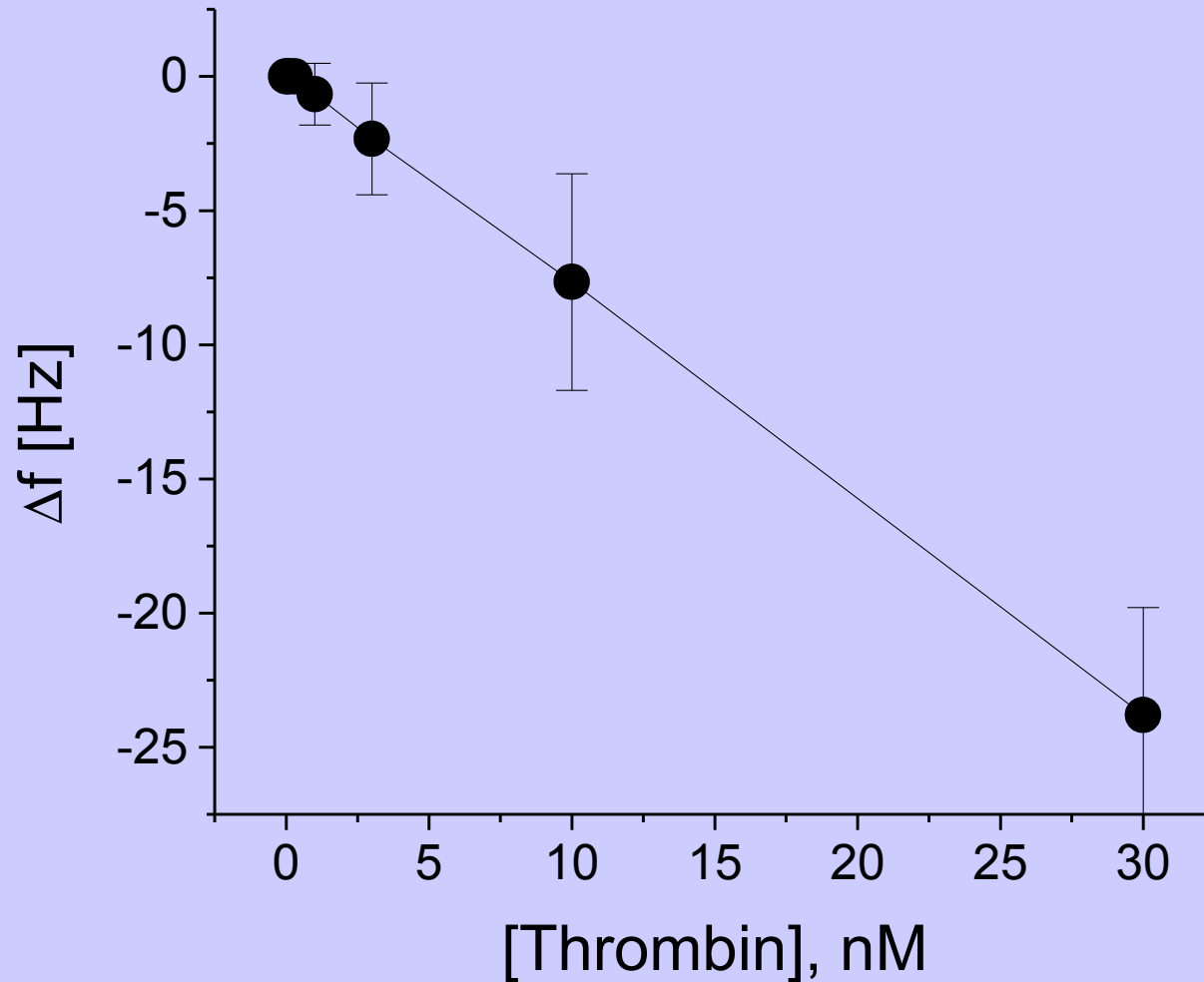
Frequency of the oscillation of the quartz is proportional to the mass of the crystal (Sauerbrey, 1959) :

$$\Delta f = -2.26 \times 10^{-6} f_0^2 (\Delta m/A)$$

Frequency decreases with increasing of the mass.

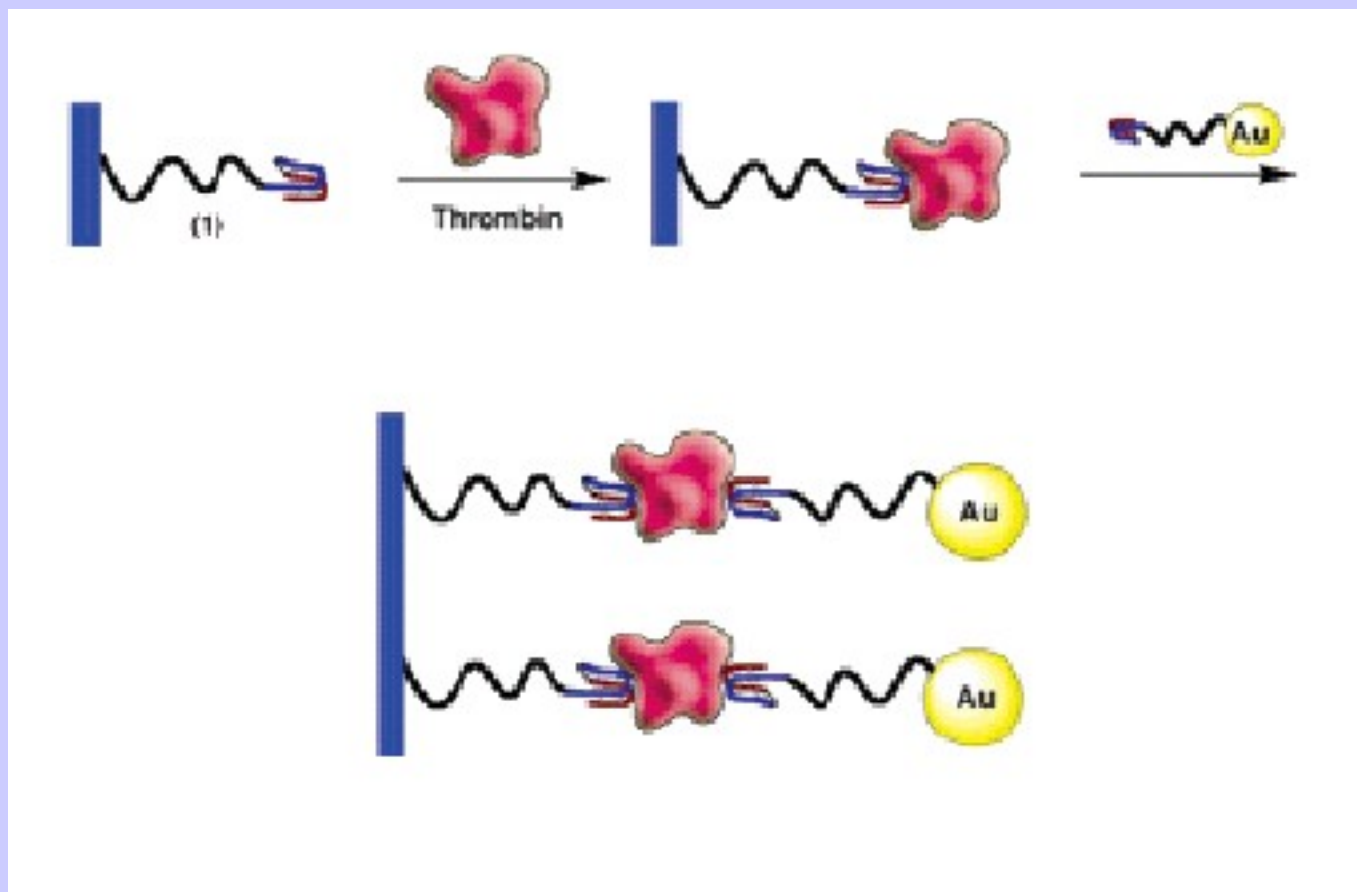


# Example of calibration curve



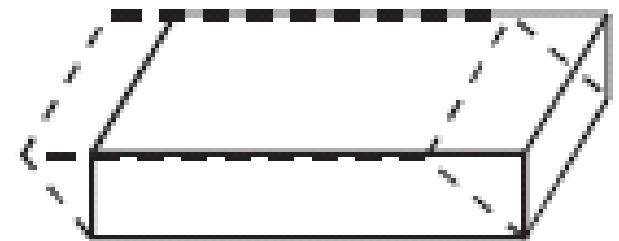
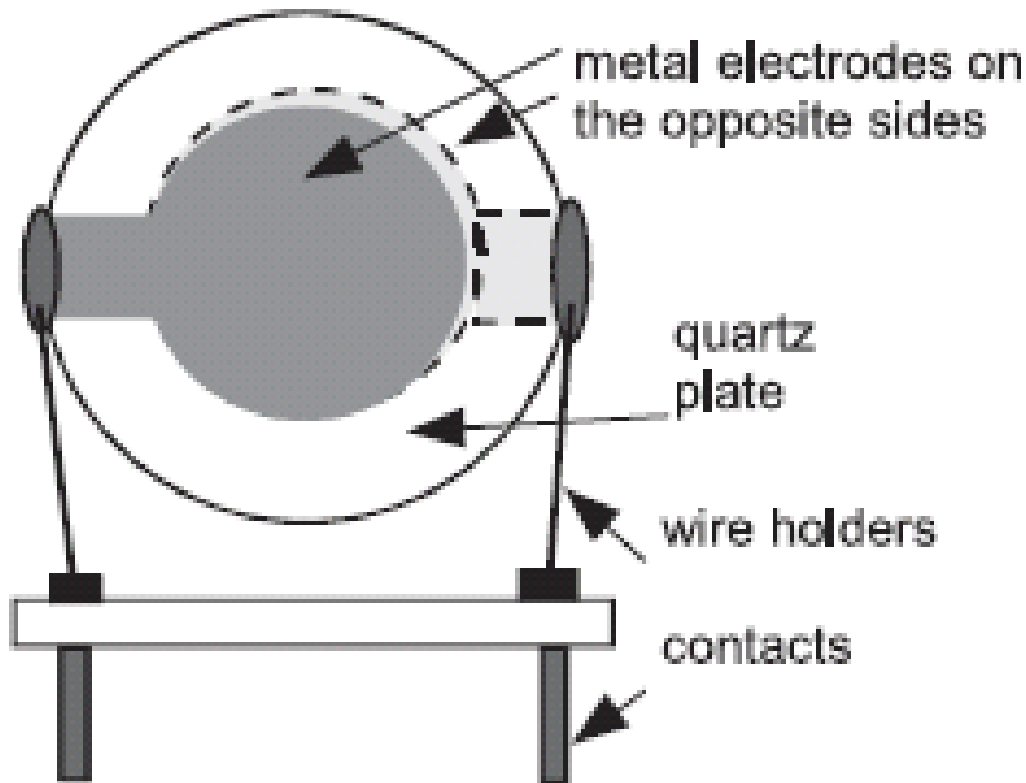
Detection limit approx. 5 nM – sufficient for diagnostics

# Sensitivity of QCM method can be increased using nanoparticles



Pavlov et al., JACS, 126 (2004) 11768

# Piezoelectric crystal and scheme of oscillations



thickness shear vibration of the quartz crystal plate

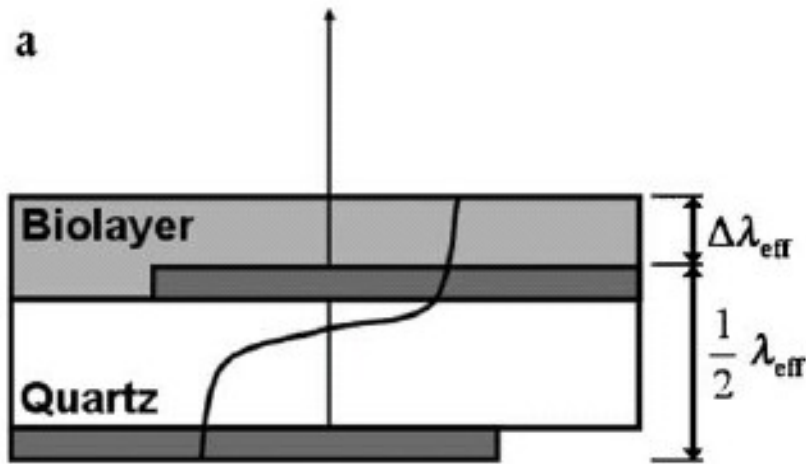
# Contribution of viscosity to the frequency changes

$$\Delta f = -f_0^{3/2} \sqrt{\frac{\rho_{Liq} \eta_{Liq}}{\pi \mu_q \rho_q}}$$

(Kanazawa and Gordon, 1985)

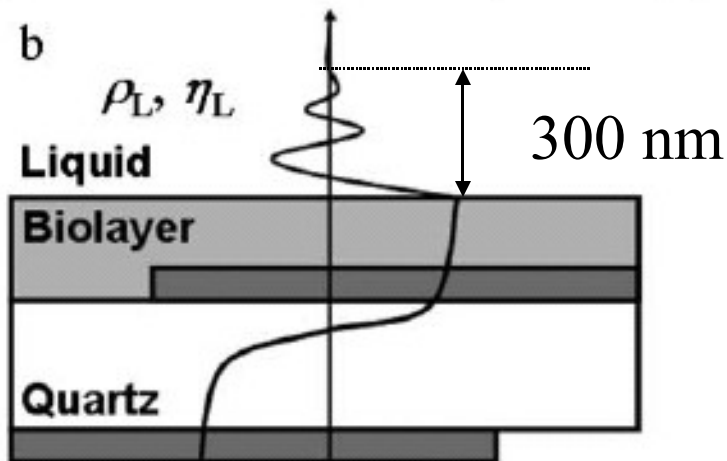
**Using frequency changes it is not possible to distinguish effect of mass and viscosity on frequency of oscillations**

# Propagation of acoustic wave inside the crystal and at sensor surface



## a) Biolayer in an air

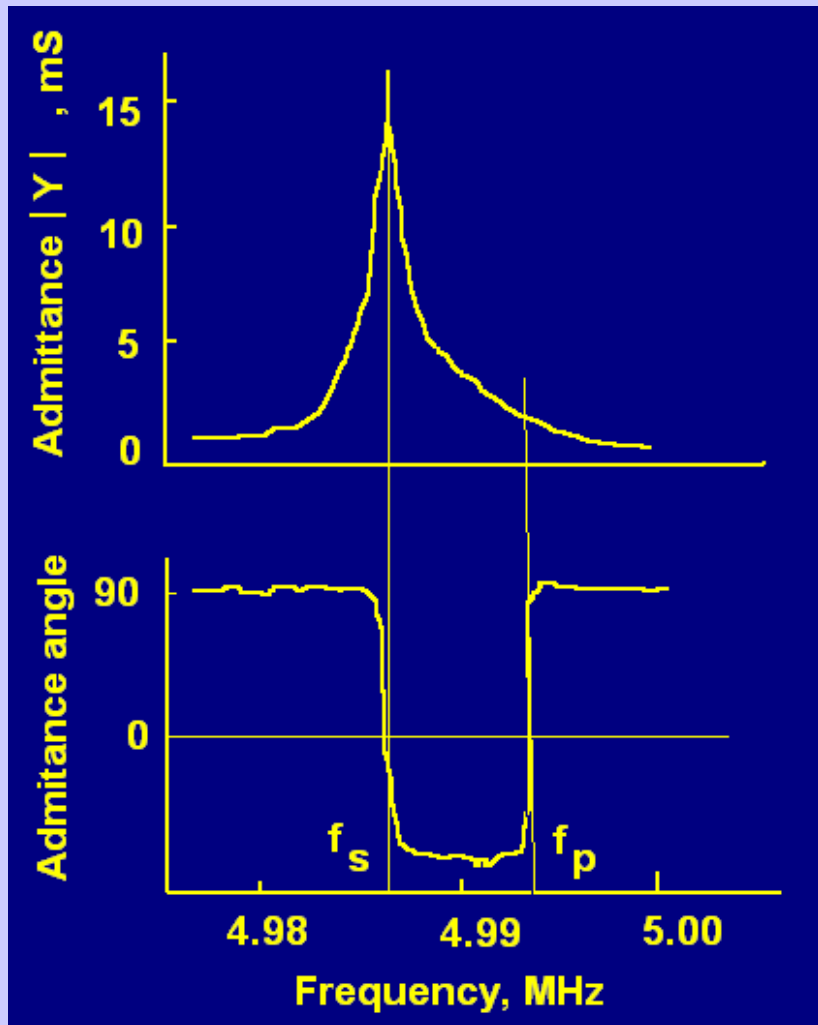
Only partial dissipation of the energy of acoustic wave



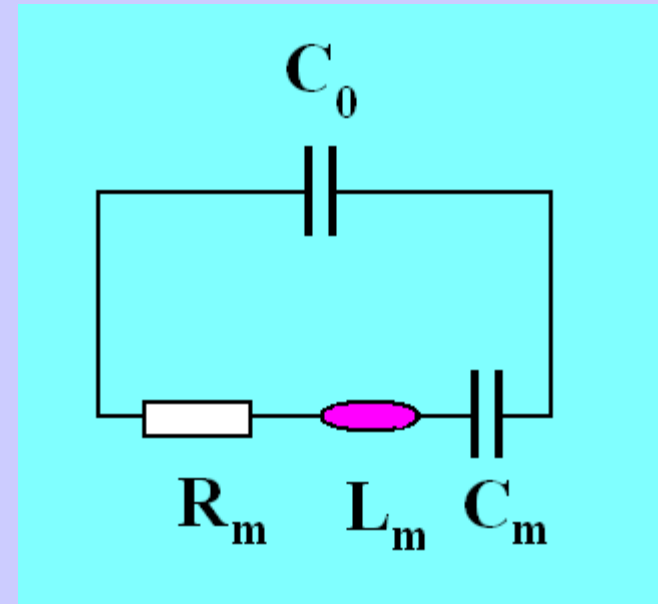
## b) Biolayer in a liquid

Substantial dissipation of the energy of acoustic wave due to viscosity.

# Analysis of complex impedance of the crystal oscillation using network analyzer



## Equivalent circuit

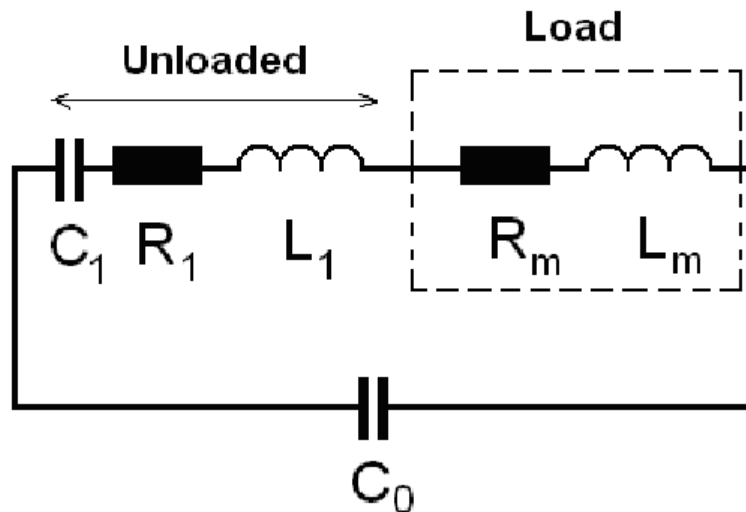
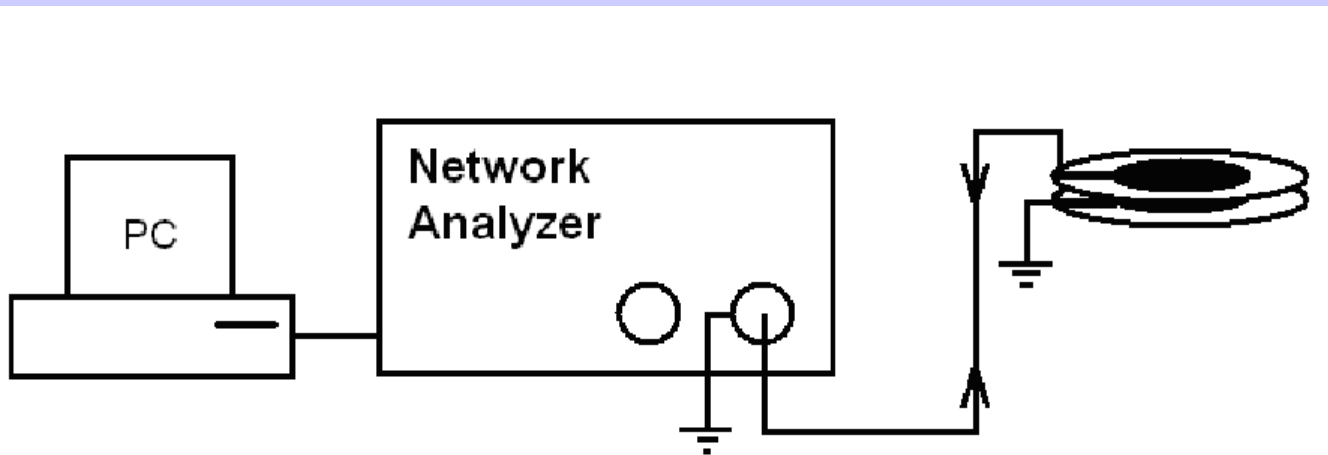


$R_m$  – viscosity

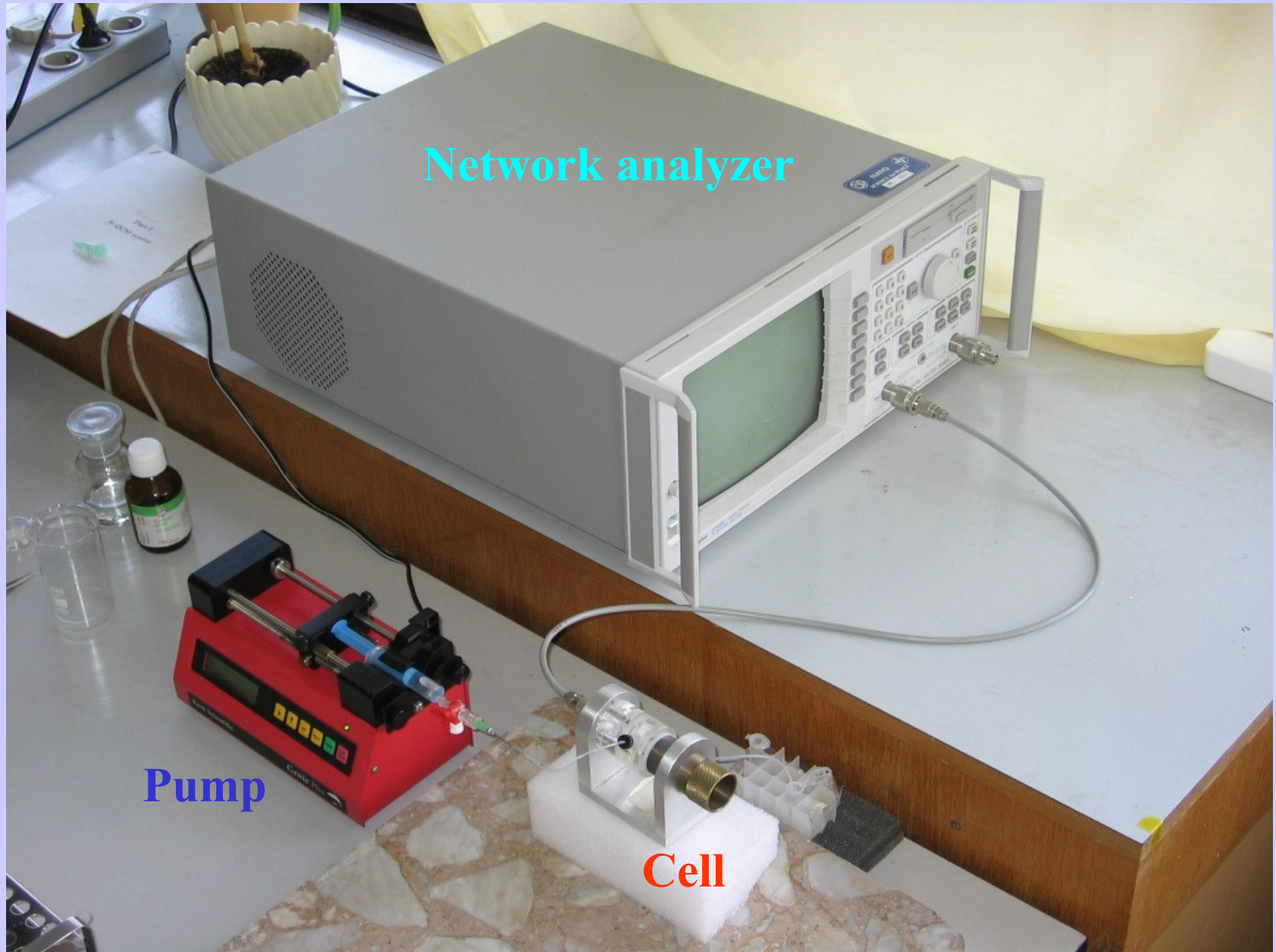
$L_m$  – mass

$C_m$  - elasticity

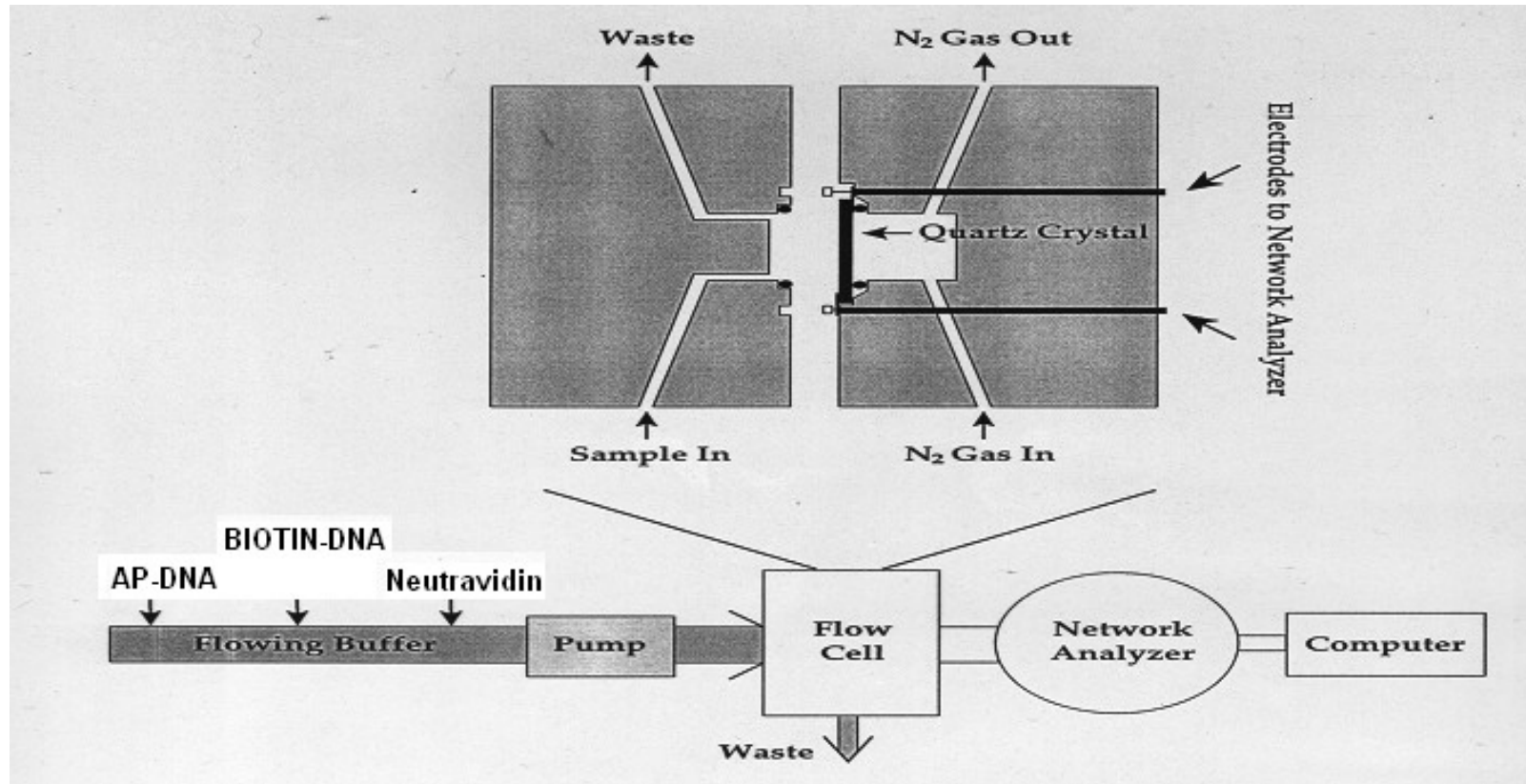
# Experimental setup and analysed equivalent circuit



# Measuring system

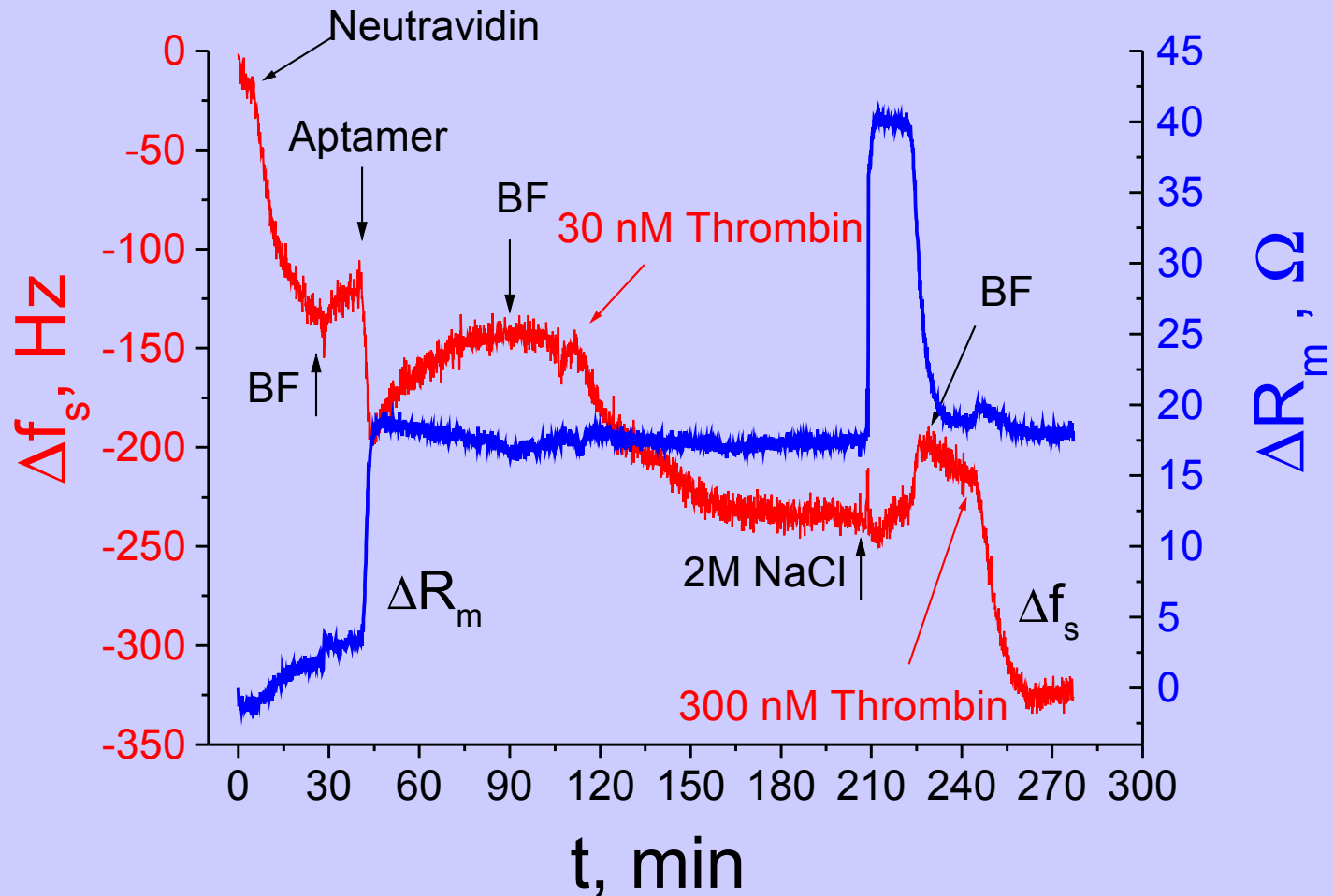


# Measuring cell



B.A. Cavic, M. Thompson, *Anal. Chim. Acta*, 469 (2002) 101-113

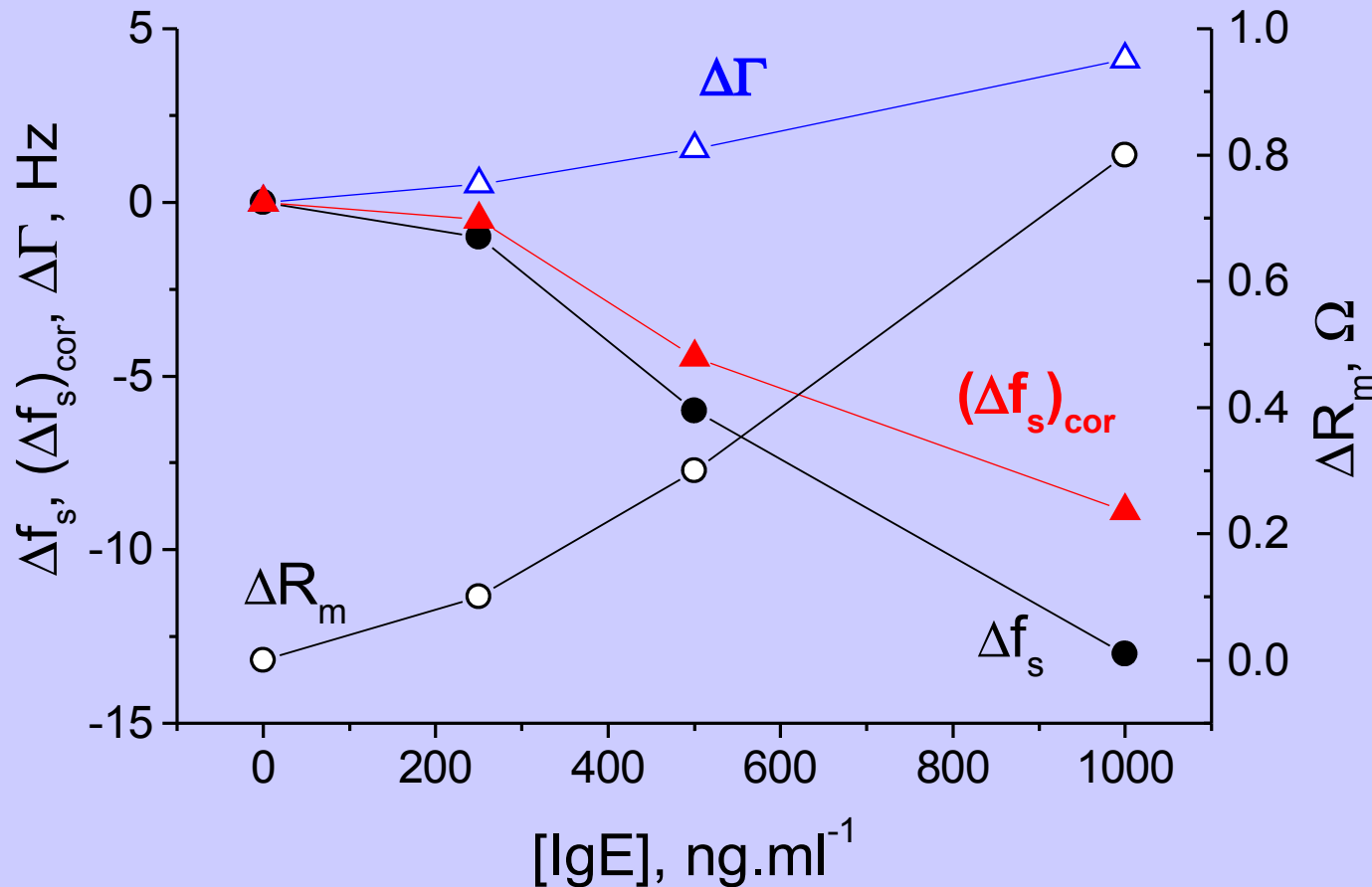
# Changes of frequency and motional resistance following binding events at the crystal surface



# Measurement of motional resistance allows to determine contribution of viscosity to the frequency changes

$$\Delta\Gamma = 8K^2C_0f_0^2\Delta R_m/\pi$$

$$(\Delta f_s)_{\text{cor}} = \Delta f_s + \Delta\Gamma$$



# Comparison of detection limits for thrombin aptasensors

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<b>Method</b>	<b>Detection limit, nM</b>
<b>Electrochem. indicators</b>	<b>10</b>
<b>Fluorescence</b>	<b>10</b>
<b>QCM</b>	<b>5</b>
<b>SPR</b>	<b>5</b>
<b>EQCM (carbon nanotubes)</b>	<b>0.5</b>

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# Conclusions

- **Aptamer and immuno-sensors provides comparable sensitivity**
- **Aptasensors are more stable than immuno sensors and can be reusable after surface regeneration**
- **Aptasensors are suitable for protein detection in complex biological samples, for example in blood**

# Future perspectives

- **Carbon nanotubes and conducting polymers could improve aptasensor properties**
- **Selection of nanoparticles for improvement aptasensor selectivity**

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