



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

# Workshop v rámci semináře: Cholinesterasy a jejich využití v konstrukci biosenzorů

Ing. Skaličková – Quartz Crystal Microbalance & application for nucleic acid analysis

pátek 18. října 2013, od 10.00 hod v přednáškové posluchárně Ústavu chemie a biochemie (budova D, učebna D06)

Akce je realizována v rámci klíčové aktivity 02 „Interdisciplinární vzdělávání pracovníků výzkumu a vývoje projektu

EXCELENCE DOKTORSKÉHO STUDIA NA AF MENDELU  
PRO NAVAZUJÍCÍ EVROPSKOU VĚDECKO - VÝZKUMNOU KARIÉRU

CZ.1.07/2.3.00/20.0005

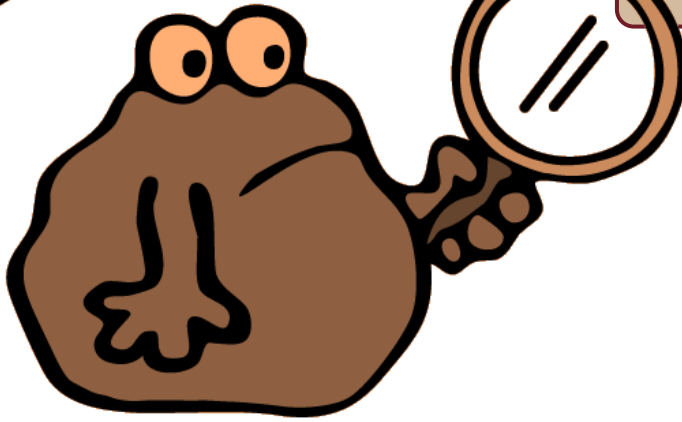
# Quartz Crystal Microbalance

& application for nucleic  
acid analysis

# Content

???

LOOKING FOR  
SOMETHING?



Principle of QCM

Instrumentation

Nucleic acid analysis

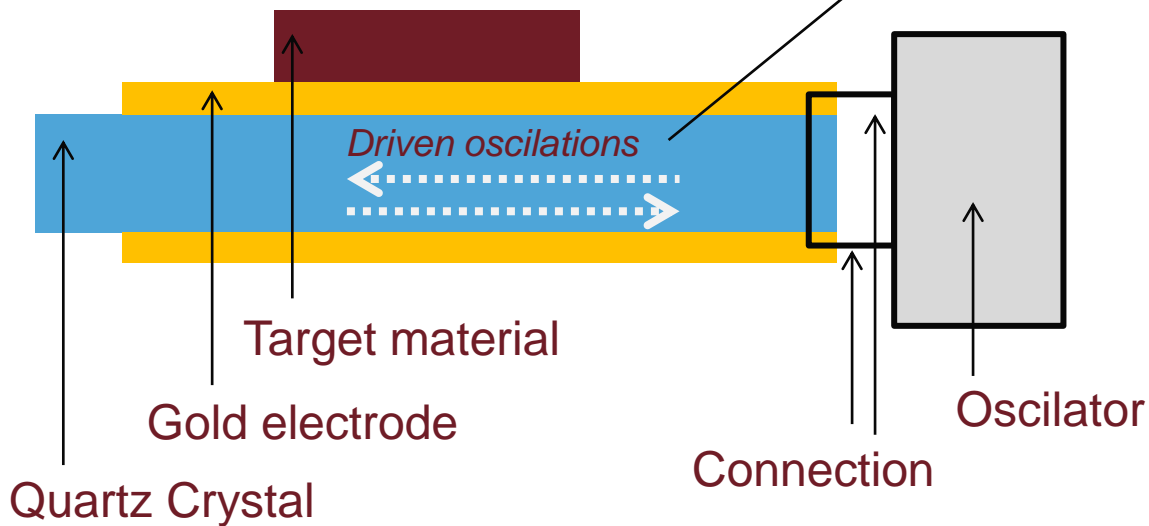
Experiment in progress

Conclusions

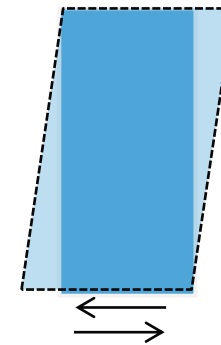
# Principle of QCM

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- Piezoelectric effect
  - Crystals become mechanically deformed under the influence of an electric field
- Quartz crystal
  - Frequency 5 – 20 Hz
  - Width influence of crystal ( $\mu\text{m}$ )

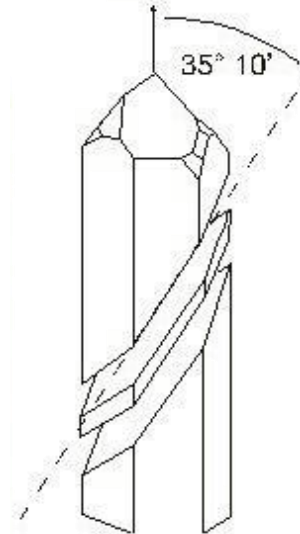


*Driven oscillations*



optical axis

35° 10'



# Principe of QCM

- Measuring
  - vacuum, gas phase, liquid enviroment
- Sensitivness
  - 0.1-1 ng/cm<sup>2</sup>
- Sauerbrey equation
  - $\Delta f$  (Hz) frequency change
  - $f_0$  (Hz) resonant frequency
  - $2,26 \cdot 10^6$  shear modulus of crystal
  - $\Delta m$  (g) mass change
  - $A$  (cm<sup>2</sup>) surface area of the electrode
- $\Delta f$  depends on pressure, temperature, humidity of enviroment, viscosity

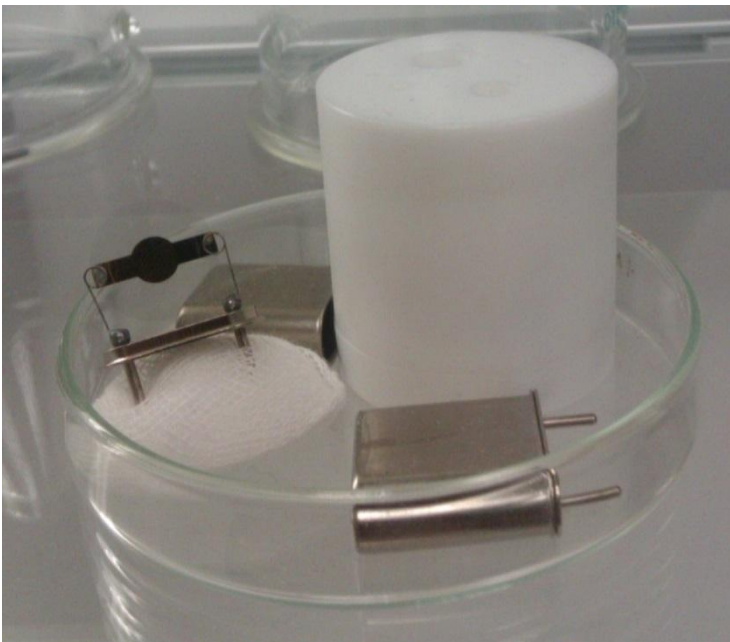


$$\Delta f = -2,26 \cdot 10^6 f_0^2 \frac{\Delta m}{A}$$

# Instrumentation

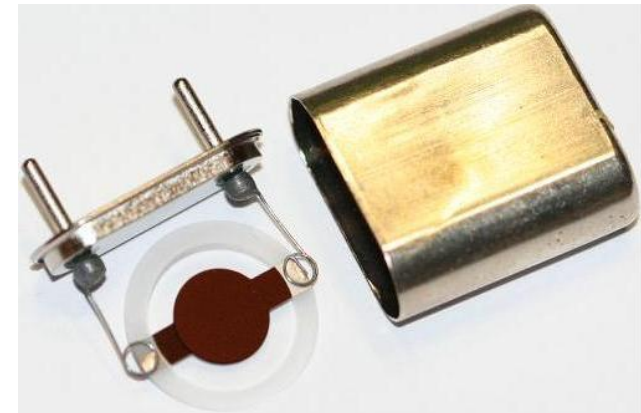
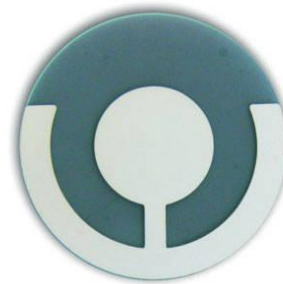
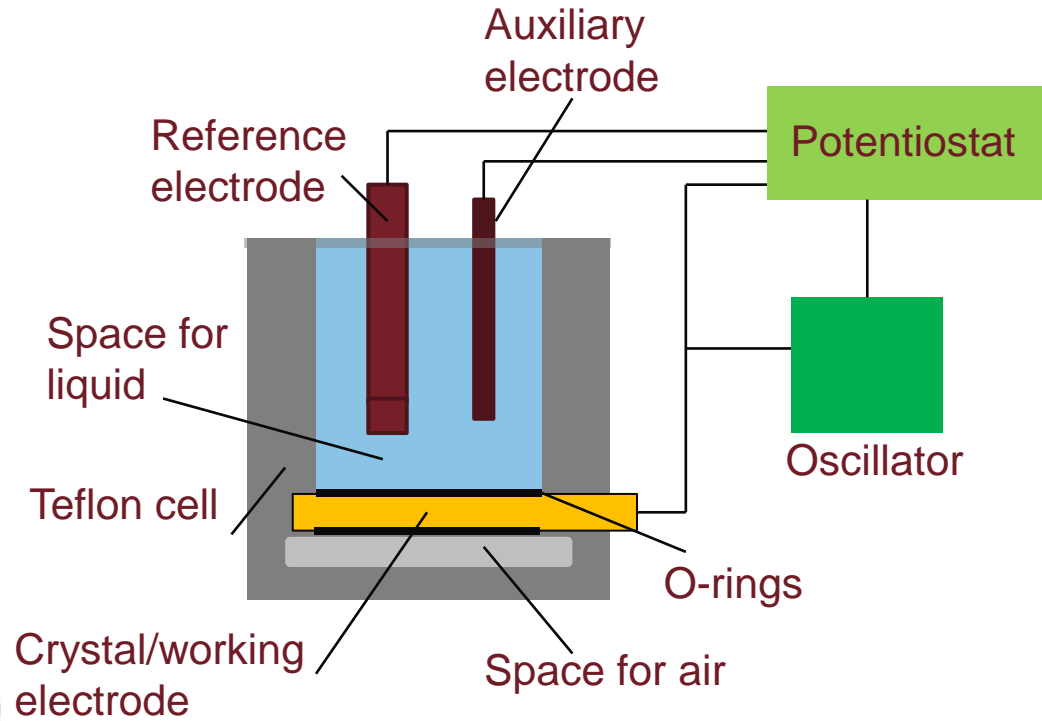
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- **Stationary systems**
- **Flow systems**
- QCM-D
  - interfacial acoustic sensing
  - sensing of oscillations when the voltage is off
  - characterization of layer's softness
- EQCM



# Instrumentation

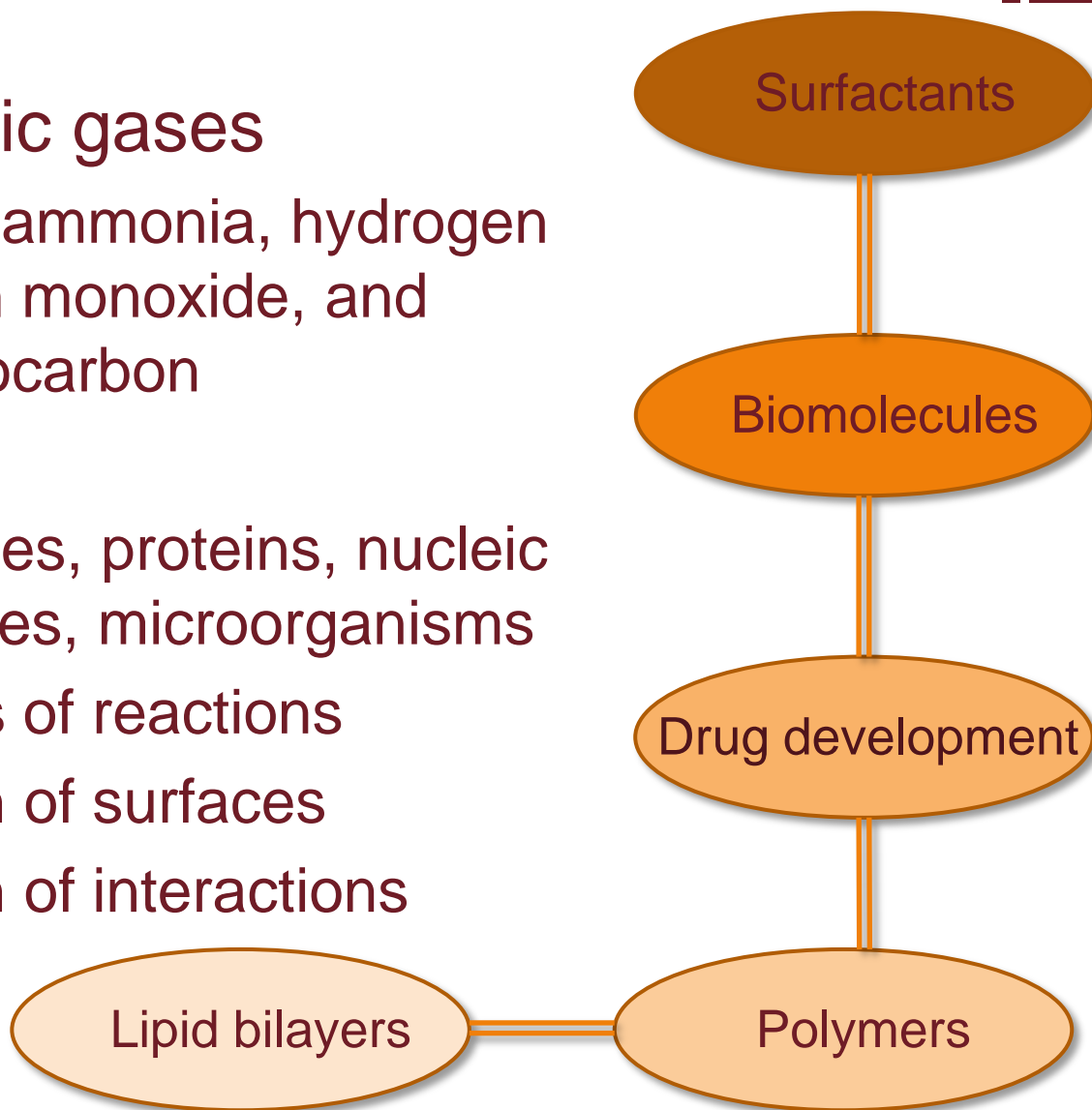
- Types of quartz crystals:
  - application
  - materials
  - designs
  - thickness



# Applications

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- Detection of toxic gases
  - sulfur dioxide, ammonia, hydrogen sulfide, carbon monoxide, and aromatic hydrocarbon
- Biosensors
  - ions, metabolites, proteins, nucleic acids, antibodies, microorganisms
- Study of kinetics of reactions
- Characterization of surfaces
- Characterization of interactions





# Nucleic acid analysis

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- QCM analysis of NA in 1989
- cleavage of NA
- duplex of DNA formation
- virus detection
- detection of S. Aureus
- analysis of telomerase activity
- kinetics of hybridization chain reaction

**2004** – Pavlov:  
Amplified detection of  
telomerase activity  
using EQCM

**2008** – Xia: Detection  
of Staphylococcus  
epidermidis by a QCM  
Nucleic Acid

**2010** - Chomean: QCM-  
based biosensor for the  
detection of alpha-  
thalassemia 1 (SEA deletion)

**2012** – Wang: QCM  
Studies on Surface-  
Initiated DNA  
Hybridization Chain  
Reaction

**1998** – Wang:  
Real-time  
monitoring of  
enzymatic  
cleavage of NA  
using QCM

**2000** – Sato:  
Electrochemical  
responses of Cyt  
c on Au  
electrodes  
modified with NA  
bases

**2001** – Pope:  
Probing DNA  
duplex formation  
and DNA-drug  
interactions by  
the QCM

**2002** – Eun:  
Detection of two  
orchid viruses  
using QCM  
based DNA  
biosensors

**1989** – Fawcett:  
The QCM as a  
detector for  
nucleic acid  
hybridization

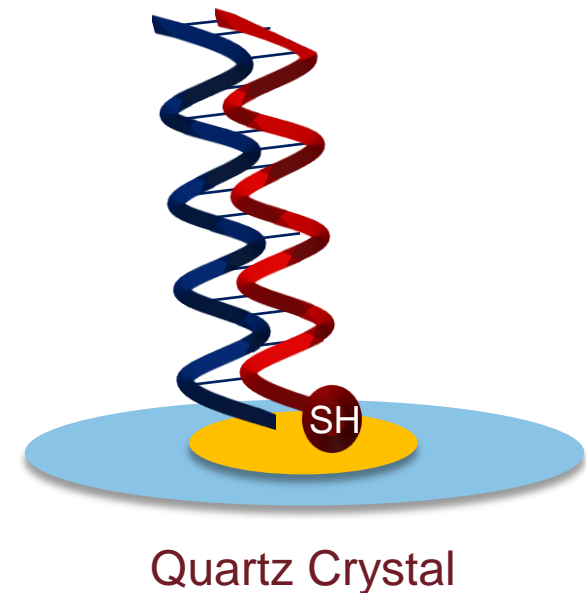
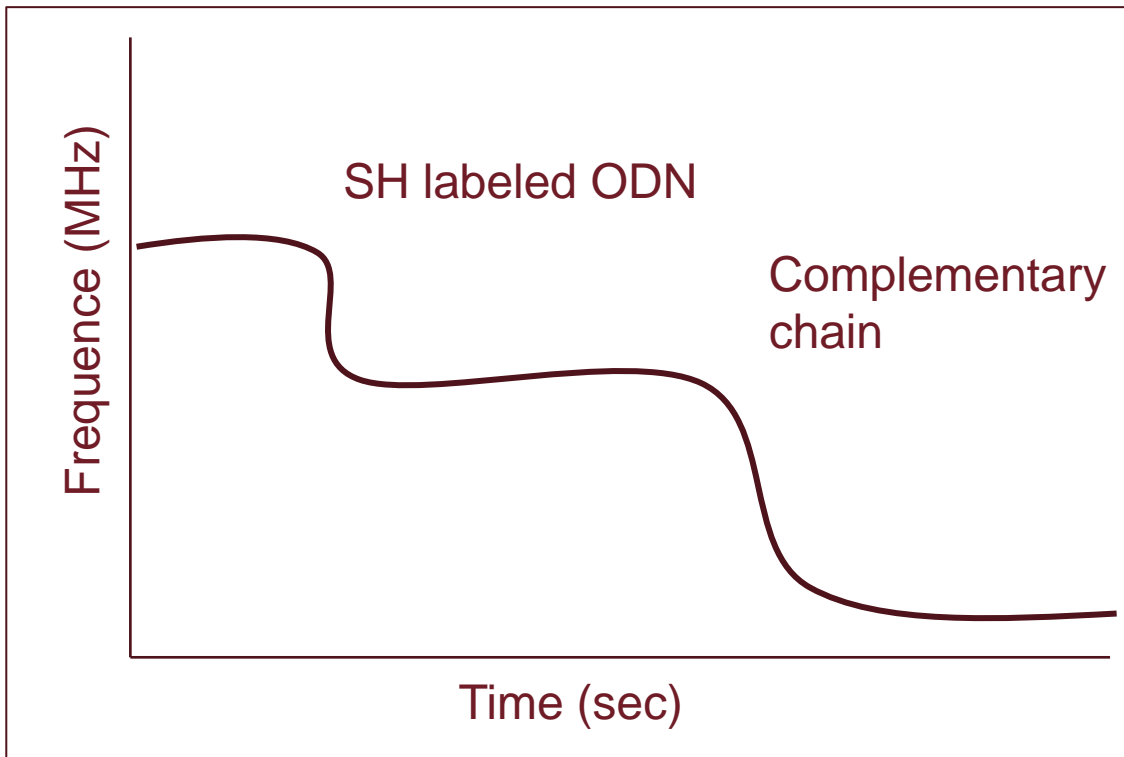


# Nucleic acid analysis

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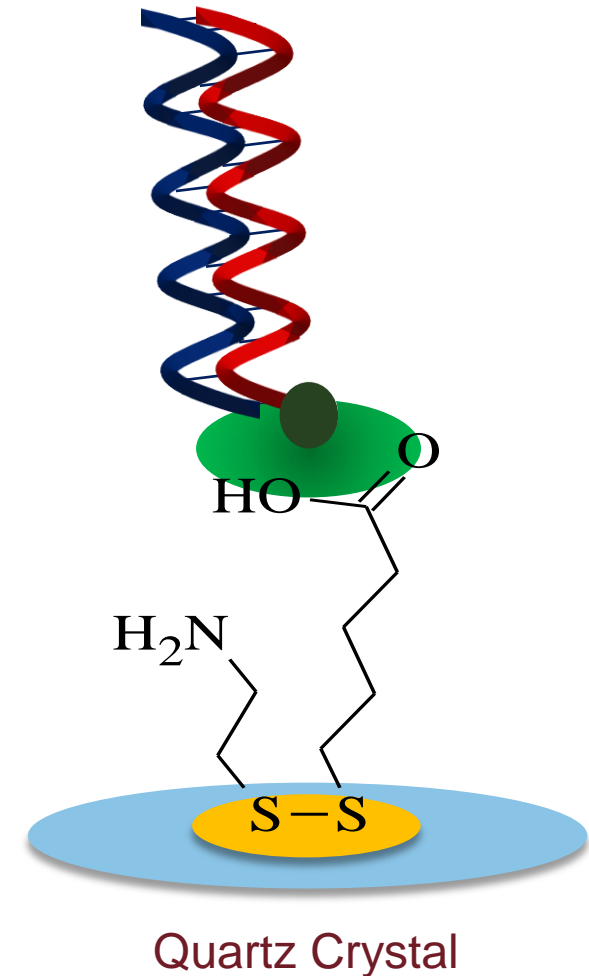
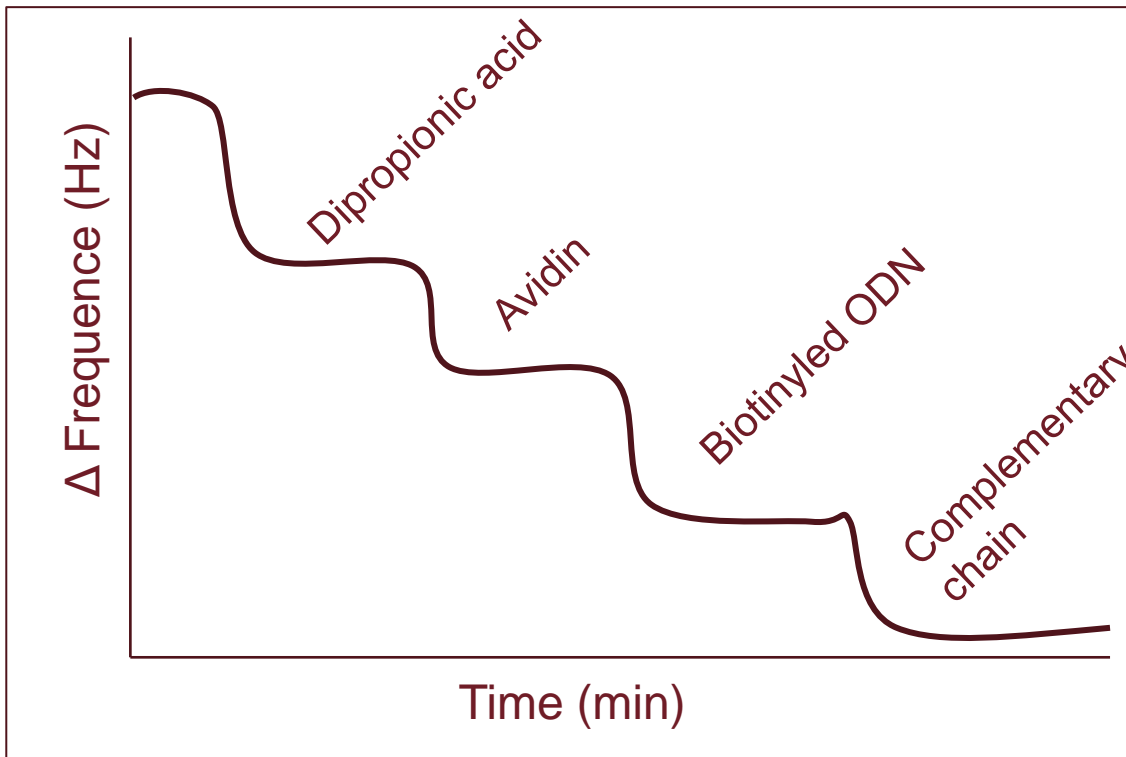
- SH linked NK is bonded to the Au surface
- Binding of complementary chain



# Nucleic acid analysis

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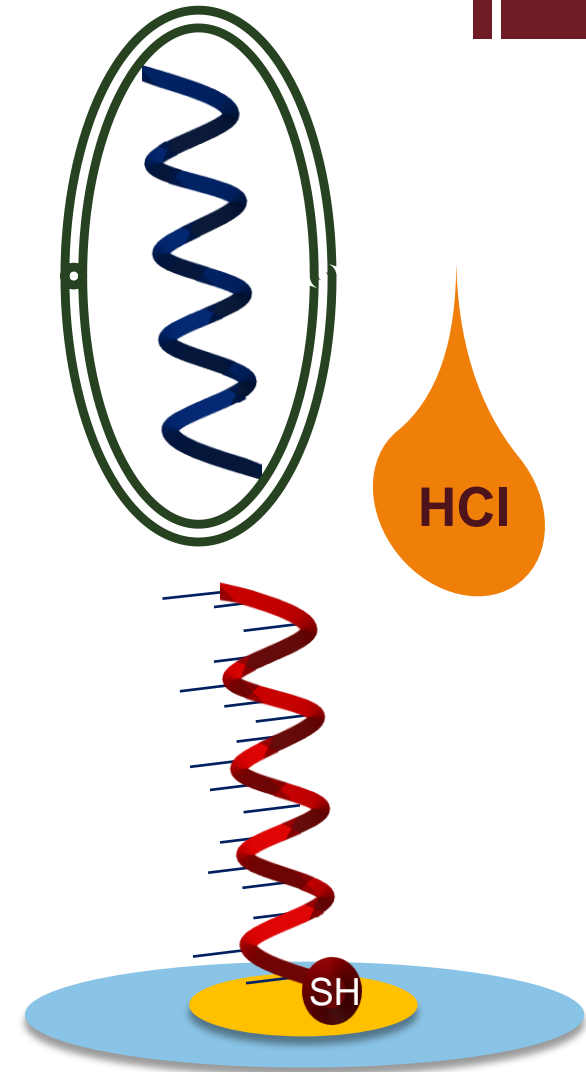
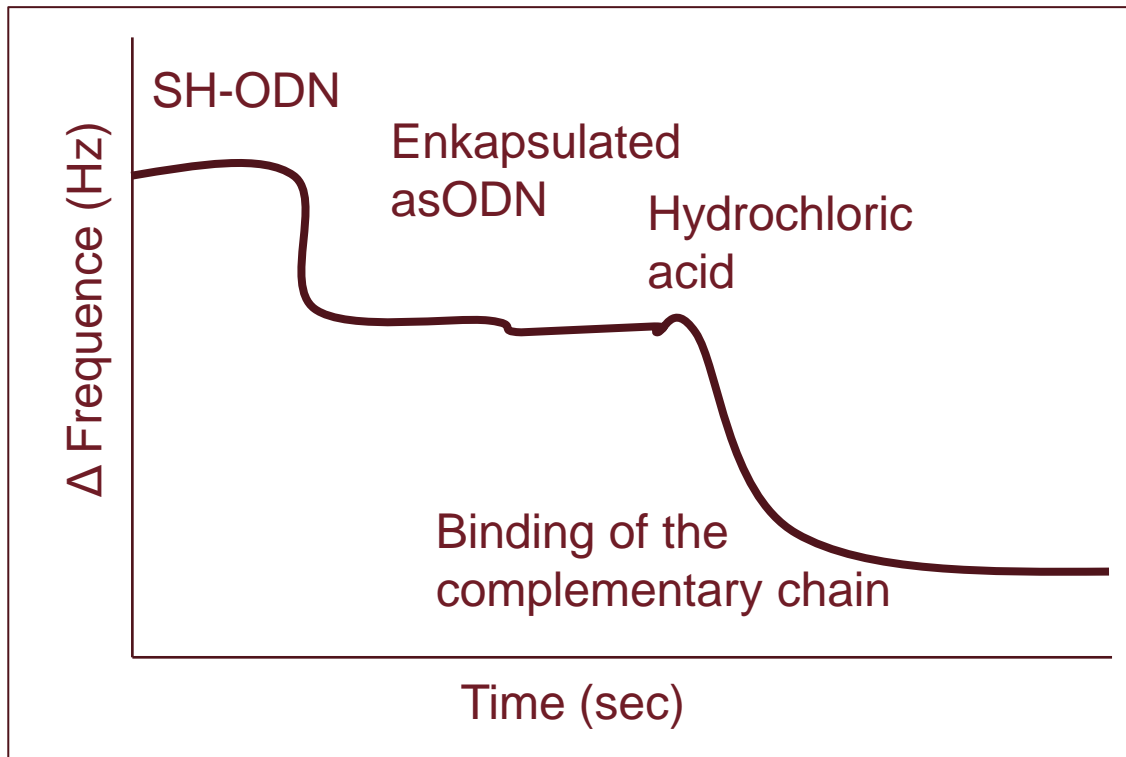
- The Au is modified by Avidin
- Binding of biotinylated NK
- Binding of complementary chain



# Experiment in progress

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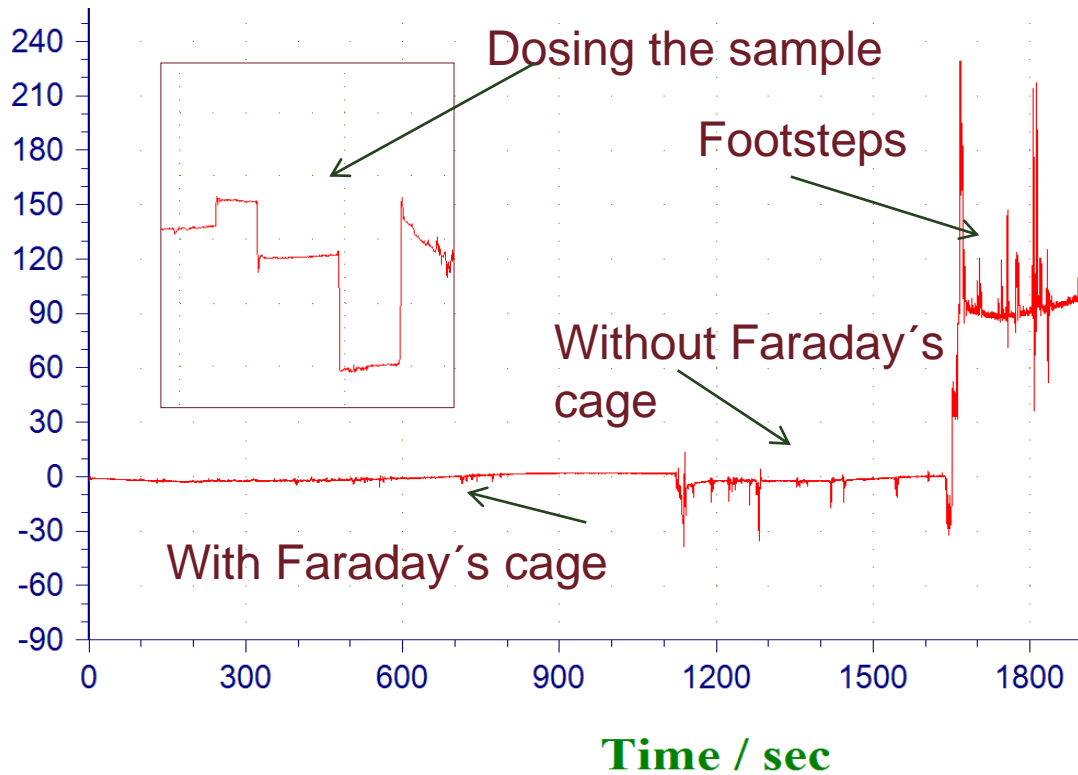
- Antisense therapy against influenza virus
- Interaction of SH-ODN with encapsulated asODN



# Experiment in progress

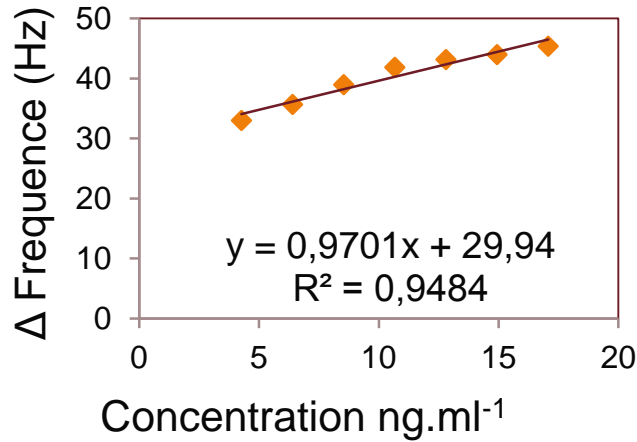
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- Done:
  - Optimization of QCM measuring
    - Brick & Strainer & Total volume 4 ml

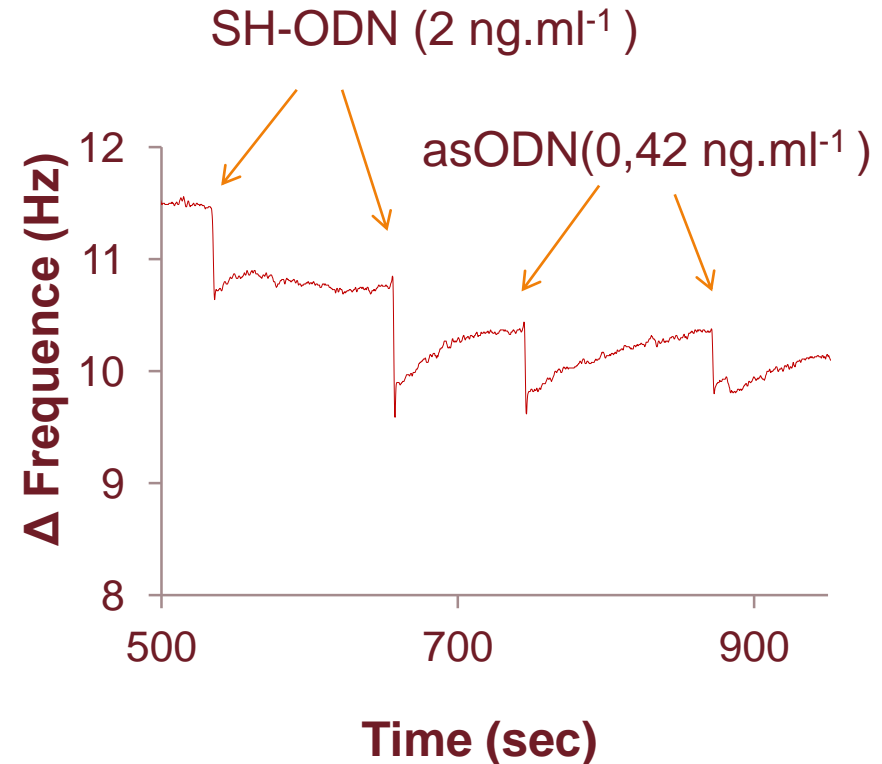
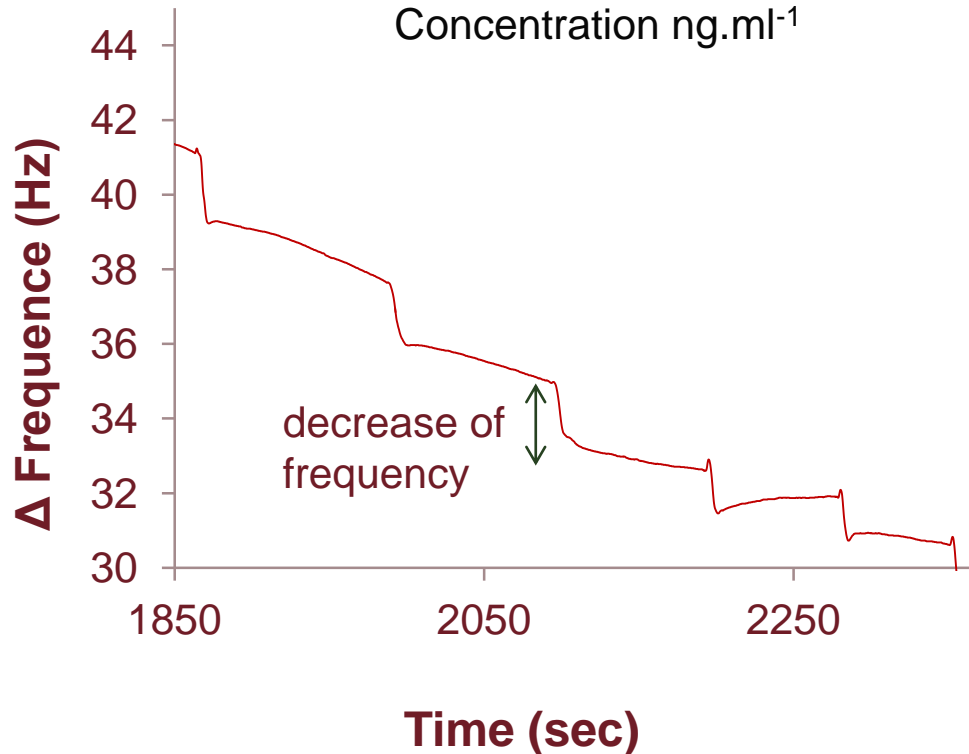


# Experiment in progress

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- Addition of sample:  $4 \mu\text{l}$
- Cell volume  $4,5 \text{ ml}$



# Experiment in progress

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- **Troubleshooting**
  - baseline decreasing/increasing
  - temperature fluctuation
  - need heating
  - cleaning the crystal surface  
(pirahna solution, acetone)

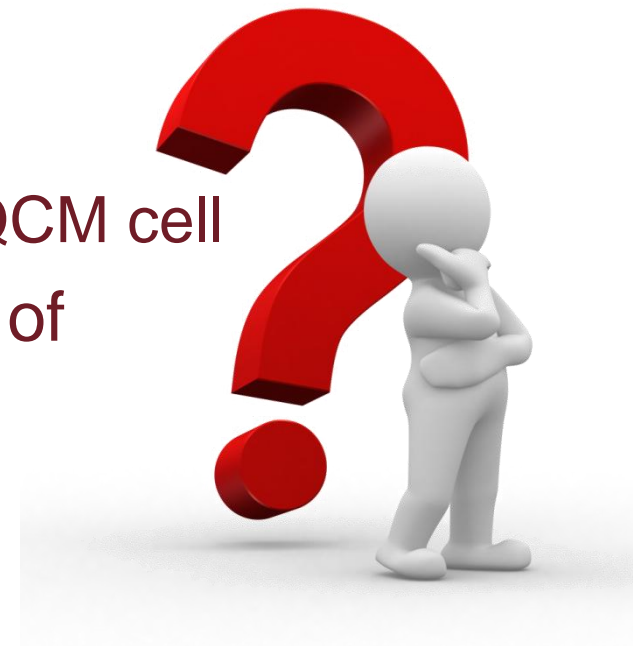


# Experiment in progress

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## ■ To be continued...

- Testing the strenght of interaction SH-ODN and asODN
- Selection of a nanotransporter
  - apoferritin
  - liposome→pH opening, no interacting with QCM cell
- Electrochemical characterization of nanotransporters using EQCM





# Conclusion

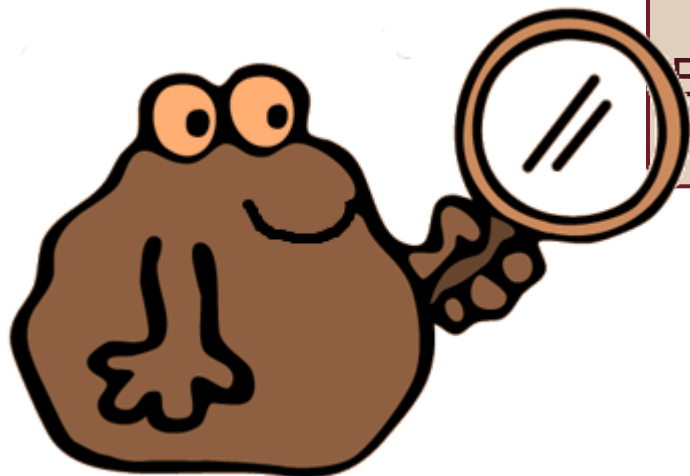
QCM is very sensitive device

Need a robust instrumentation

Broad spectrum of applications

Usable for molecular chemistry,  
nanotransporters, NA analysis

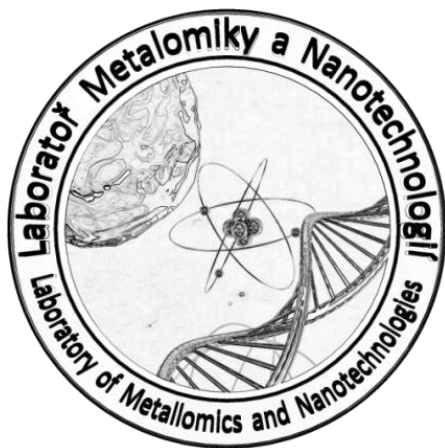
**You already know**



# Acknowledgement

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- Ondřej Zítka
- David Hynek
  
- Laboratory of electrochemistry



**Electrochemical scientist**

# Thank you for your attention<sup>19</sup>

