Comparison of optical and gravimetric methods for detection of chymotrypsin

Mgr. Ivan Piovarči

Supervisor: prof. RNDr. Tibor Hianik, DrSc.

Proteases

- Proteases are important in many different aspects of life
- Digestion system
- Coagulation system
- Immunity system
- Pharmacy, medicine, industry

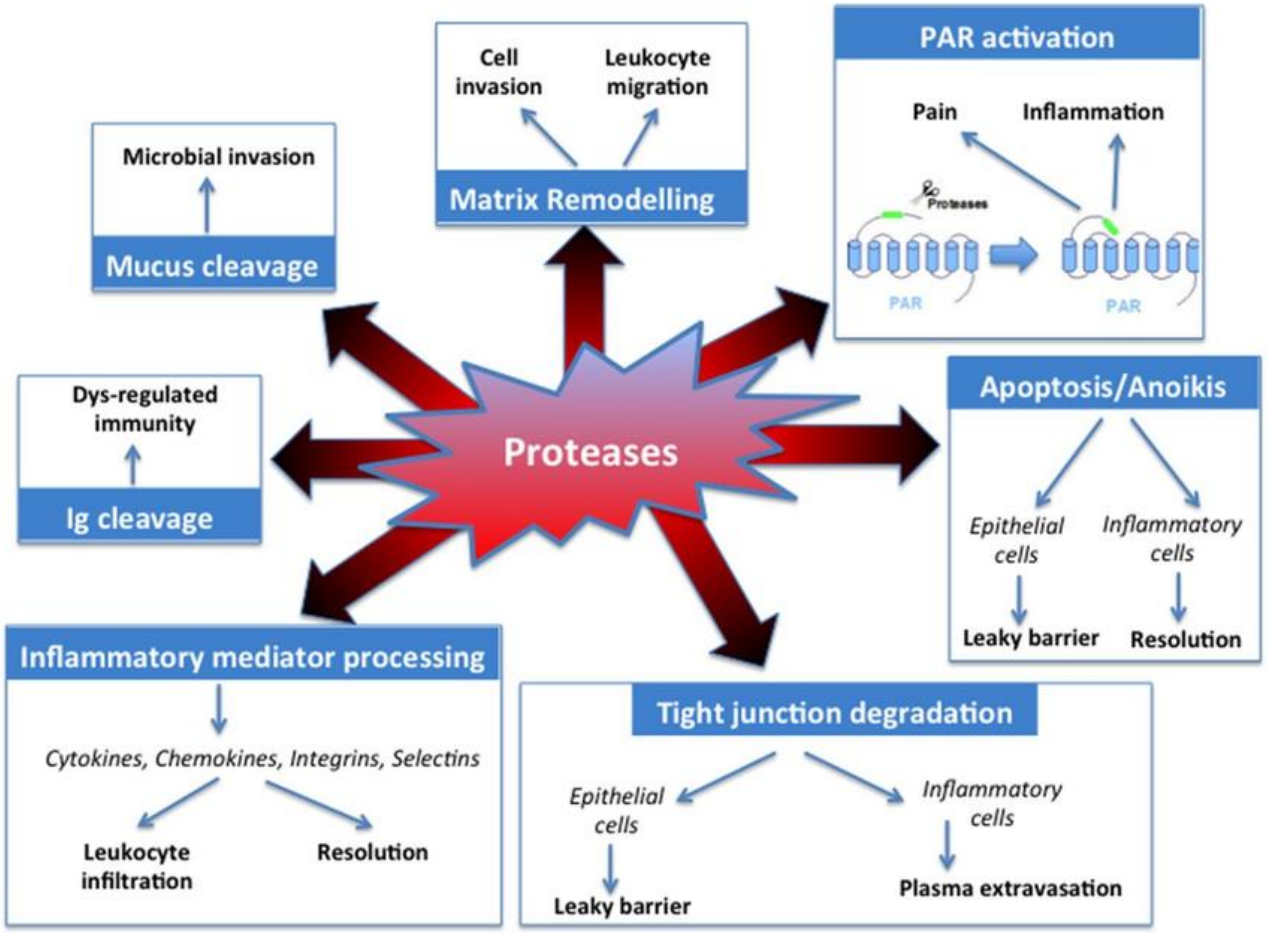

Fig 1. Examples of protease influence on gastro-intestinal diseases [1]

Chymotrypsin

- Serine endopeptidase
- Produced in pancreas
- Released in duodenum
- Cleaves peptide bond at hydrophobic amino acids (phenylalanine, tyrosine, tryptophane)
- Help in cataract surgeries and anti-inflammatory effects

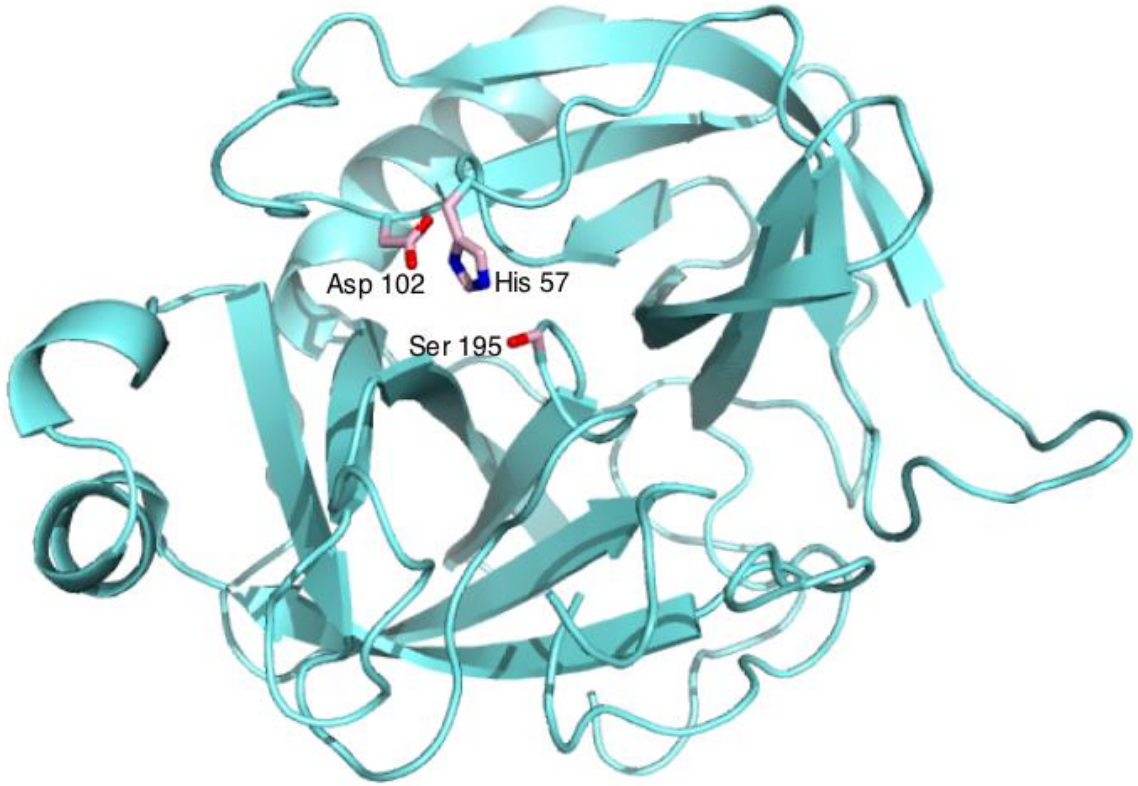

Fig 2. Chymotrypsin model with its cleavage site [2]

Biosensors

- Sensors based on
- Bioreceptor
- Transducer
- Electronic system

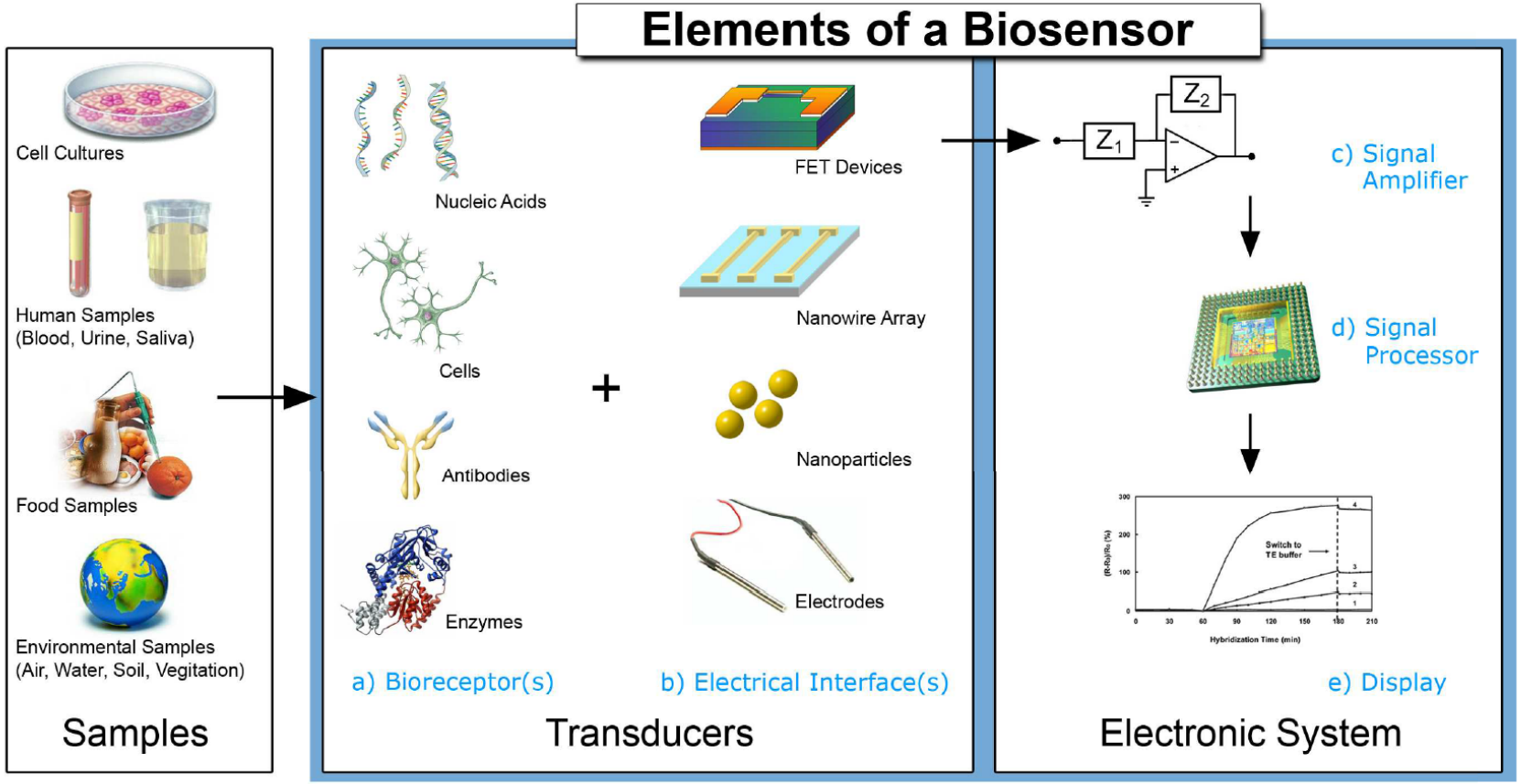

Fig 3. Biosensors scheme [3]

Acoustic biosensor

- Acoustic wave travels through crystal
- Depending on the cut – BAW (bulk acoustic wave) or SAW (surface acoustic wave)
- Measurement of changes of acoustic parameters (resonance frequency, resistance)

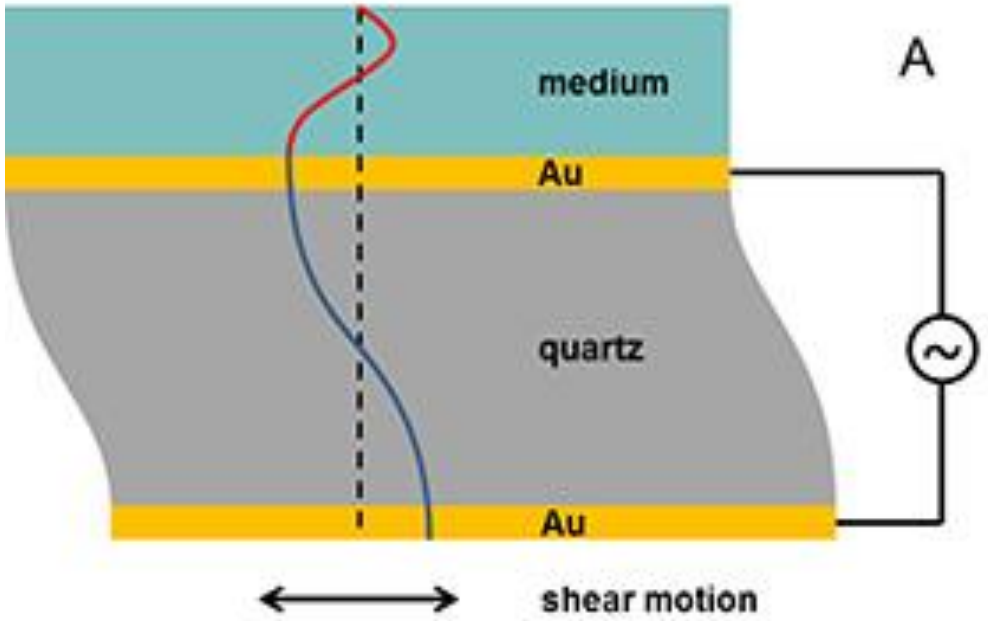

Fig 4. Scheme of TSM crystal [4]

$$\Delta f = \frac{-2 f_0^2}{\mathrm{A}\sqrt{\rho_q \mu_q}} \Delta m$$

Eq 1. Saubrey equation

Biosensor scheme

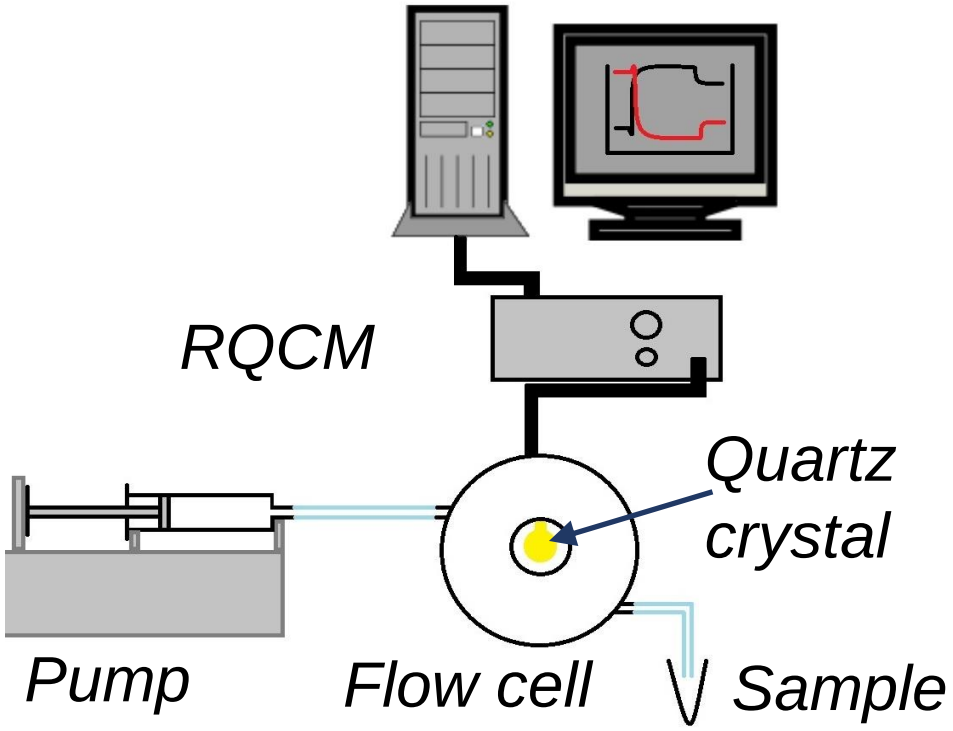

Fig. 5. Biosensor scheme

Preparation of casein layer

- 18 hour incubation of 2mM MUA
- 20 min EDC/NHS (20 mM/50 mM)
- 35 min 1 mg/ml β - casein

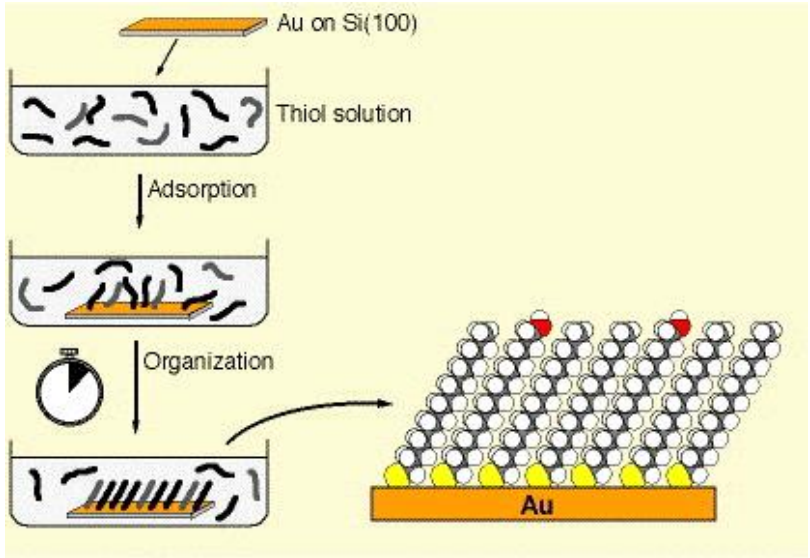

Fig 6. Adsorption of MUA [5]

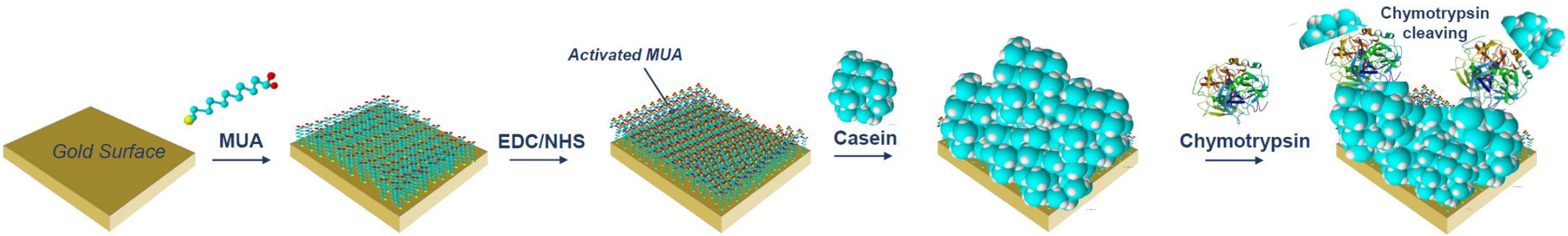

Fig 7. Scheme of preparation and cleavage of casein layer

Experiment example

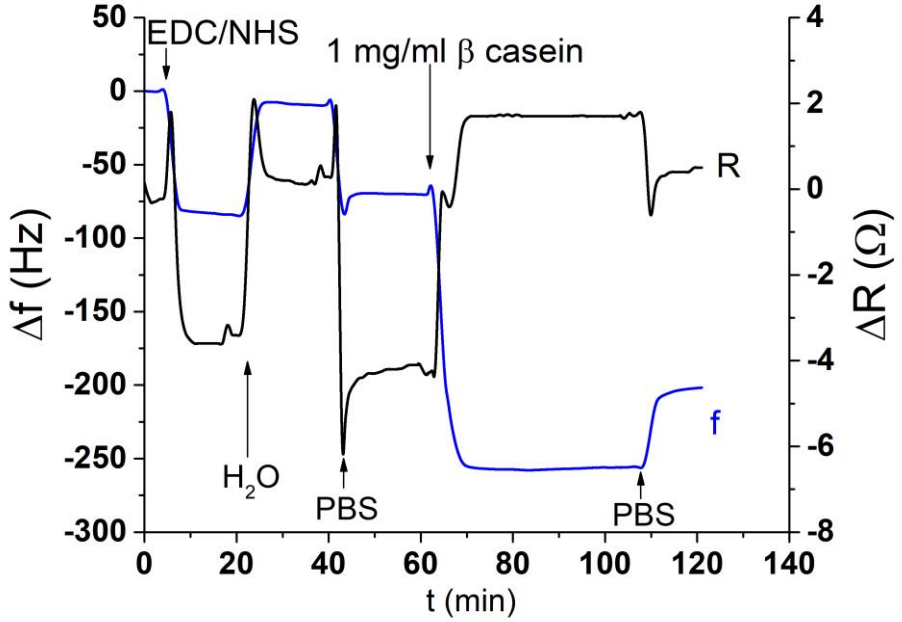

Fig 8. Change of resonance frequency and motional resistance during preparation of sensitive layer of biosensor

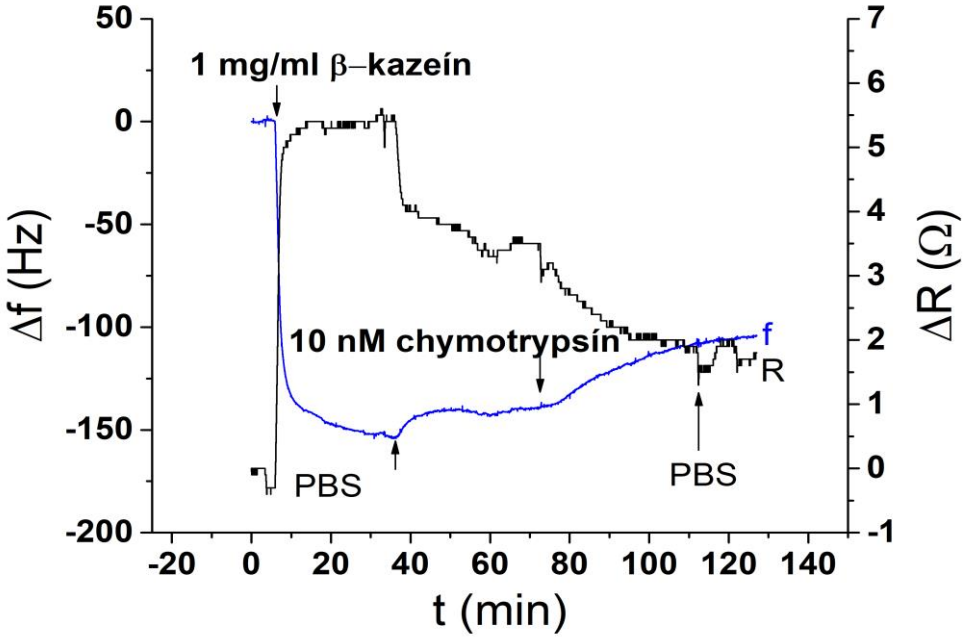

Fig 9. Change of resonance frequency and motional resistance during cleavage of 10 nM chymotrypsin

Calibration curve and limit of detection for gravimetric method

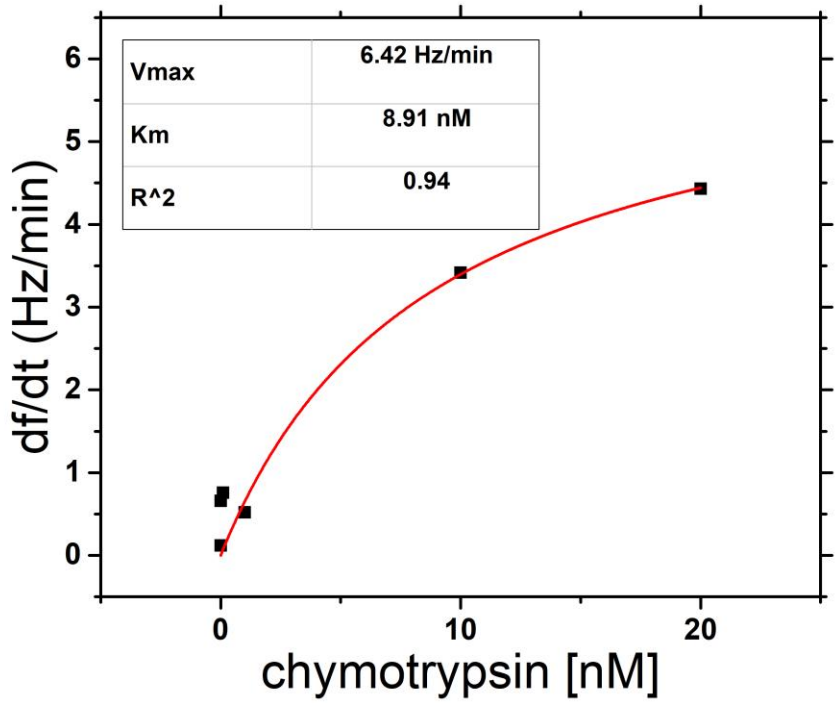

Fig 10. Calibration curve with reverse Michaelis-Menten model

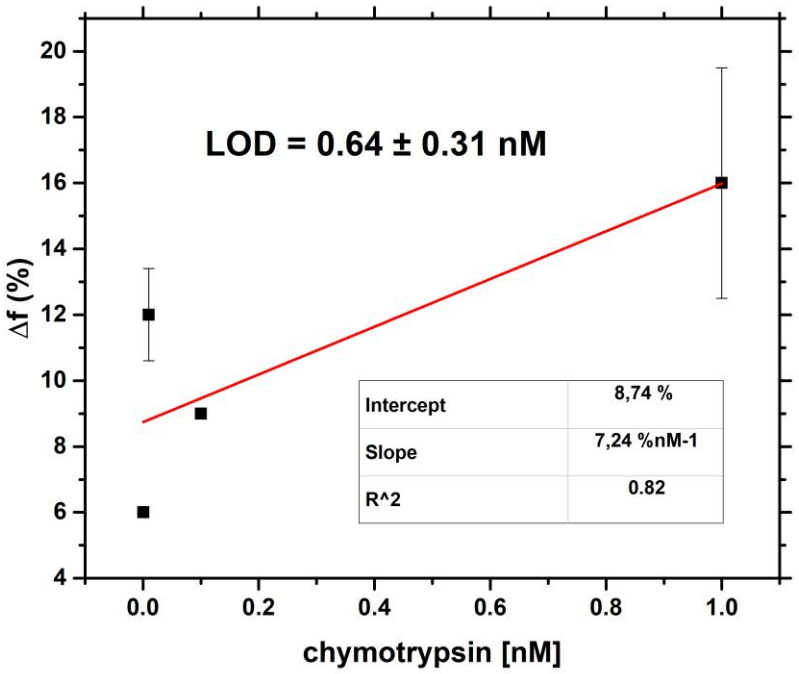

Fig 11. Linear part of calibration curve with calculated LOD

Gold nanoparticles

- Gold nanoparticles have many advantages in preparation of biosensors and medicine
- Different sizes 10-100 nm
- Biocompatibility
- High ability to functionalize (S-Au and N-Au bonds)

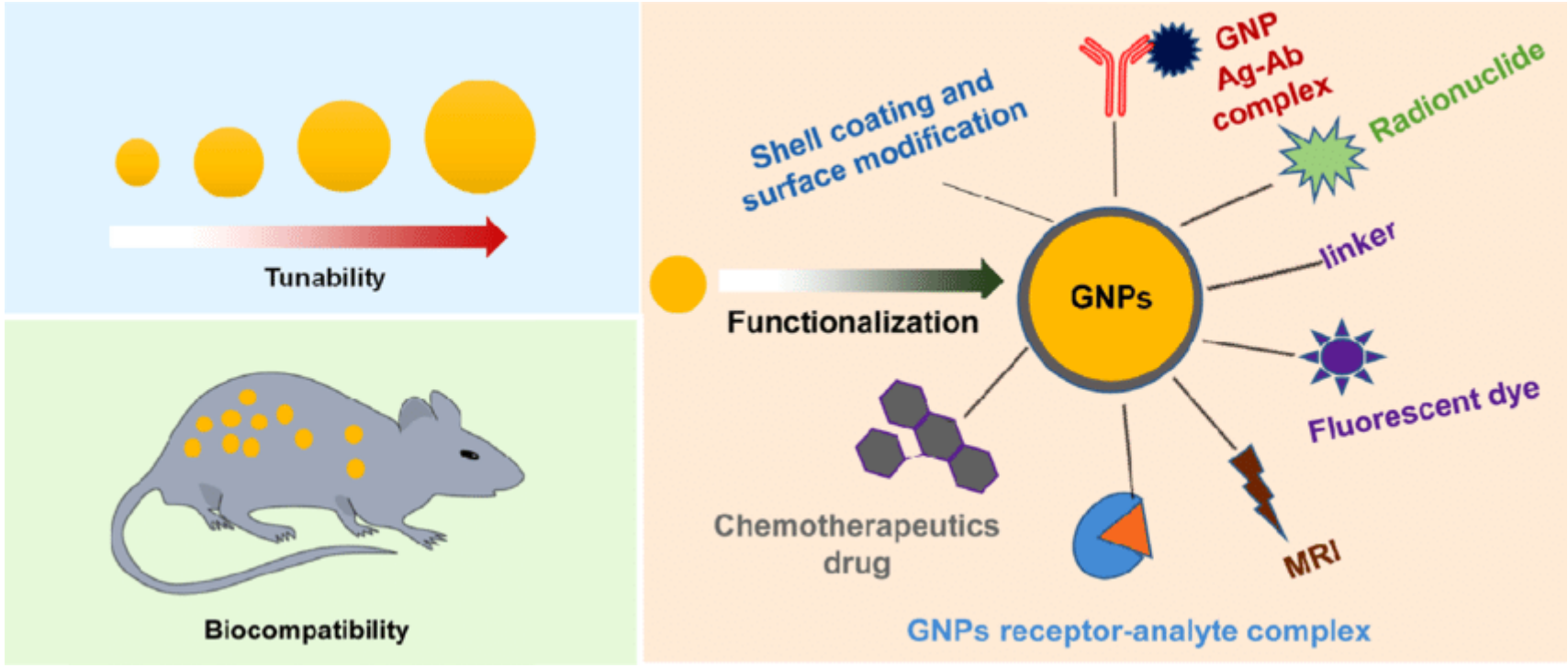

Fig 12 Gold nanoparticles and their uses for medicine and/or preparation of biosensors [6]

Surface plasmon resonance(SPR)

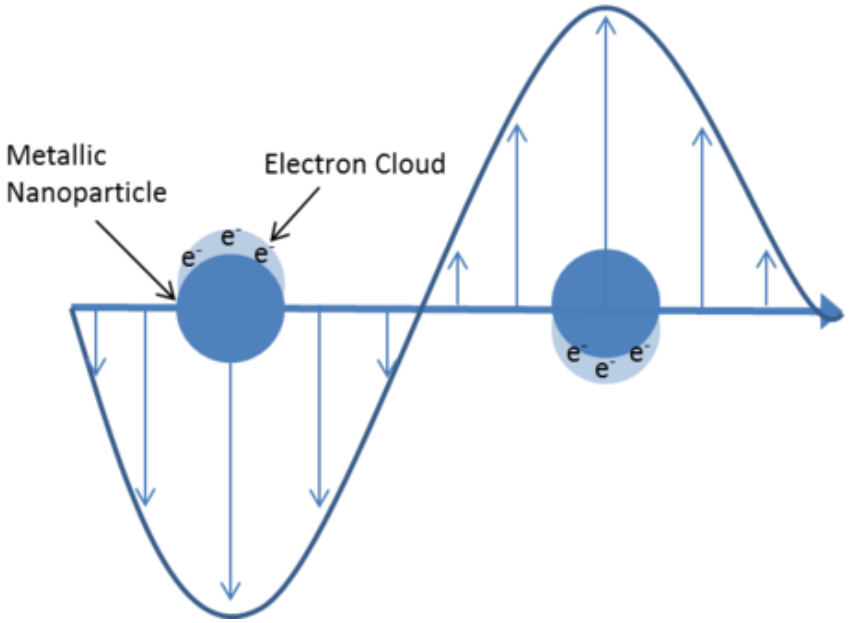

Fig 13. SPR in metallic nanoparticles [7]

Fig 14. Example of colloidal gold nanoparticles [8]

- Effect arises from the oscillation of free electron cloud in metallic particles induced by light
- Maximum amplitude can be seen in UV-VIS spectrophotometry
- Position and intensity depends on metal type, particle size, shape, structure, and dielectric constant of surrounding medium

Modification of gold nanoparticles

- 2 hours 0.1 mg/ml β-casein
- 18 hours MCH (1 mM)

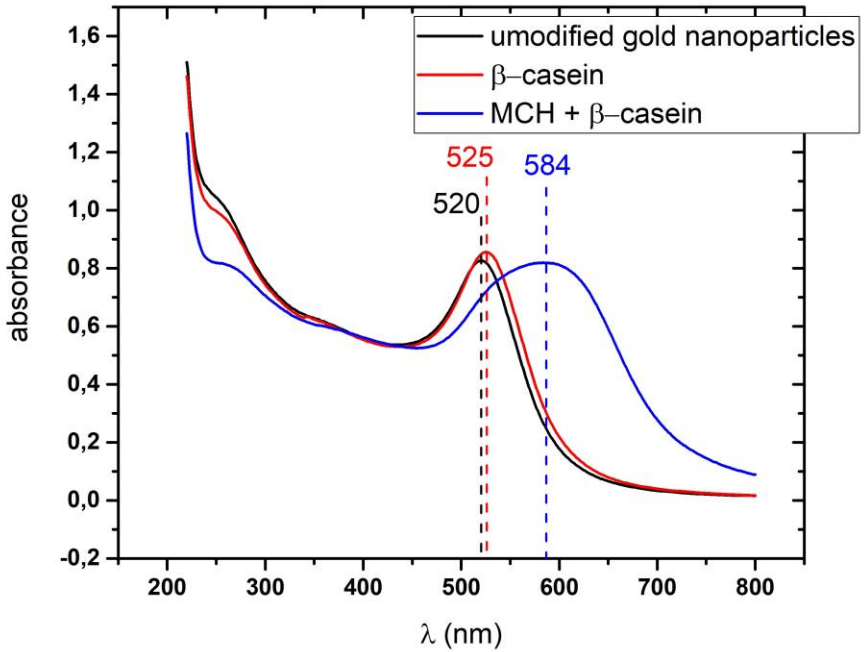

Fig 15. Change in absorbance spectra during modification of gold nanoparticles

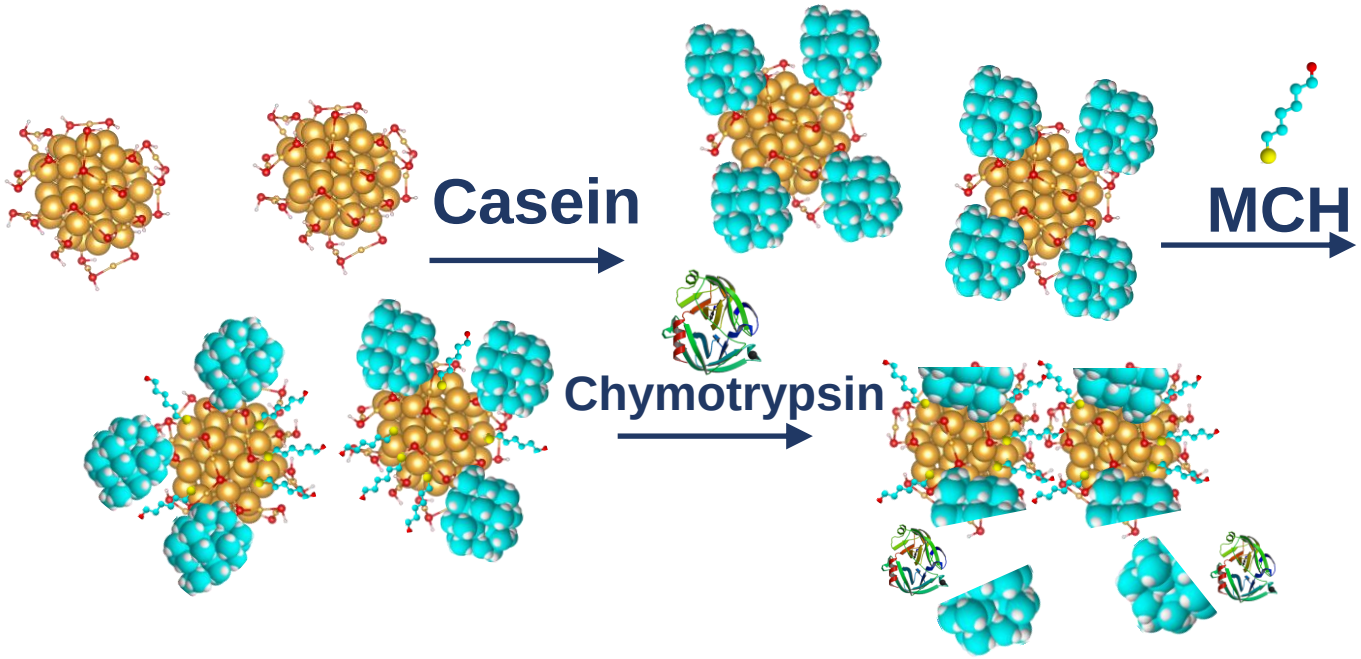

Fig 16. Scheme of modification of gold nanoparticles

Change in spectrophotometric parameters after cleavage with chymotrypsin

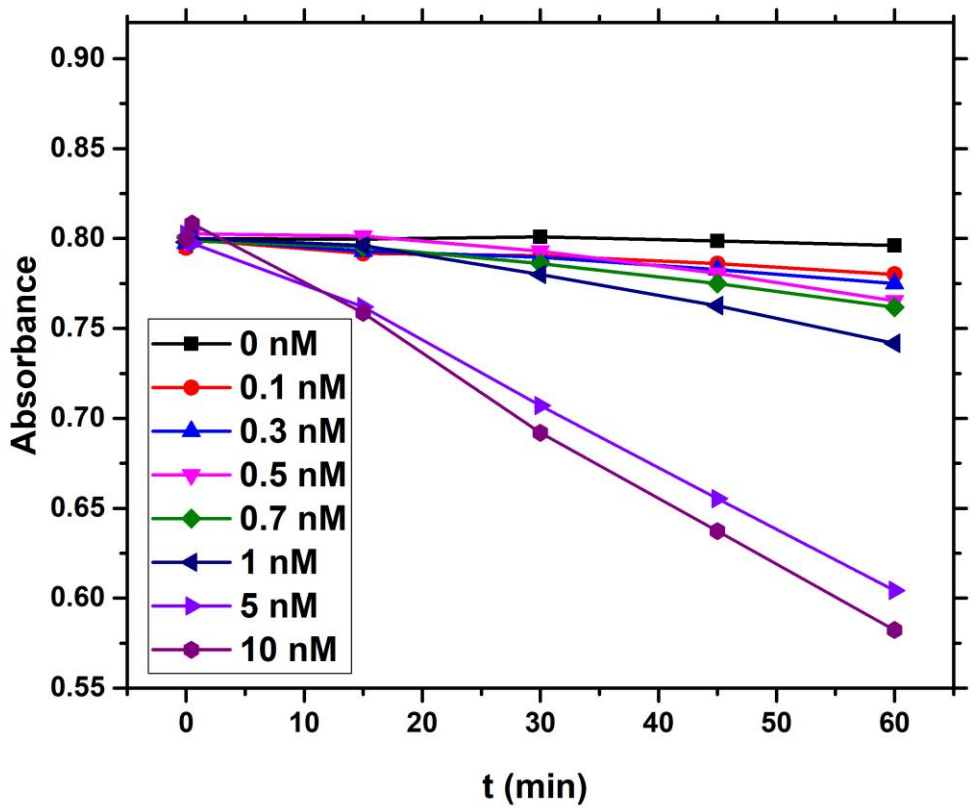

Fig 17. Absorbance change

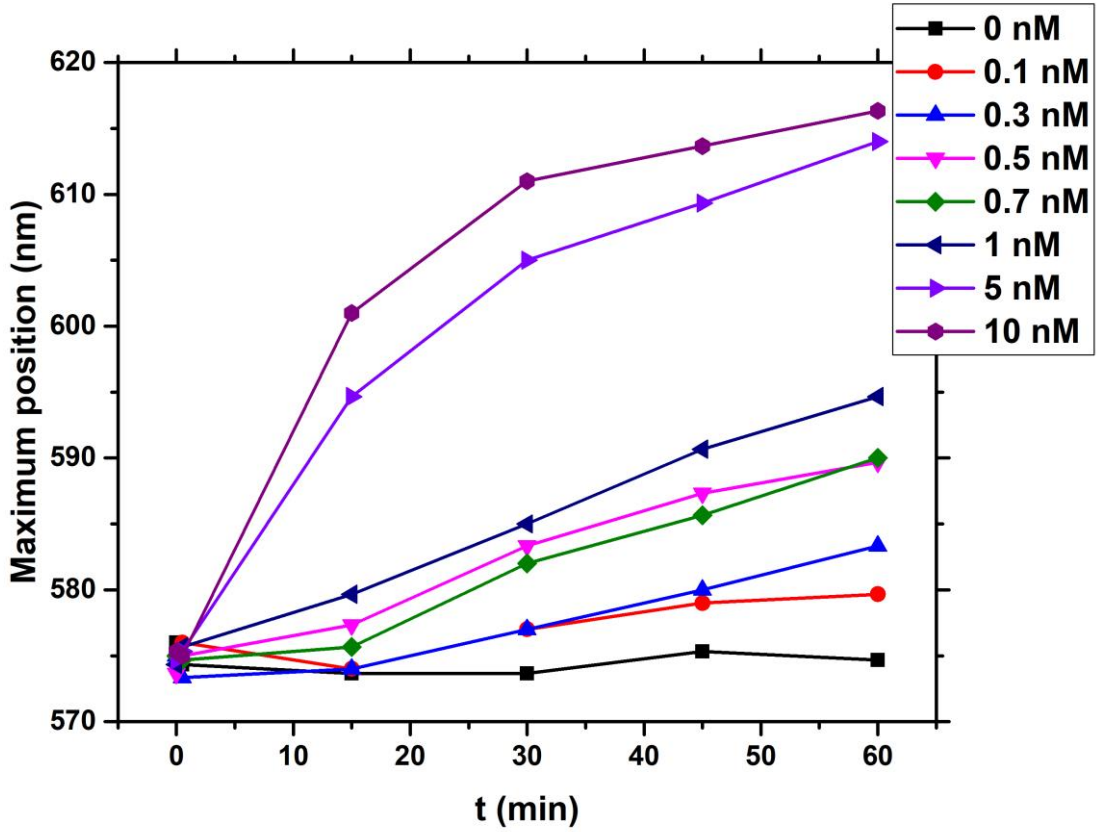

Fig 18. Change of maximum position

Calibration curve and limit of detection for optical method

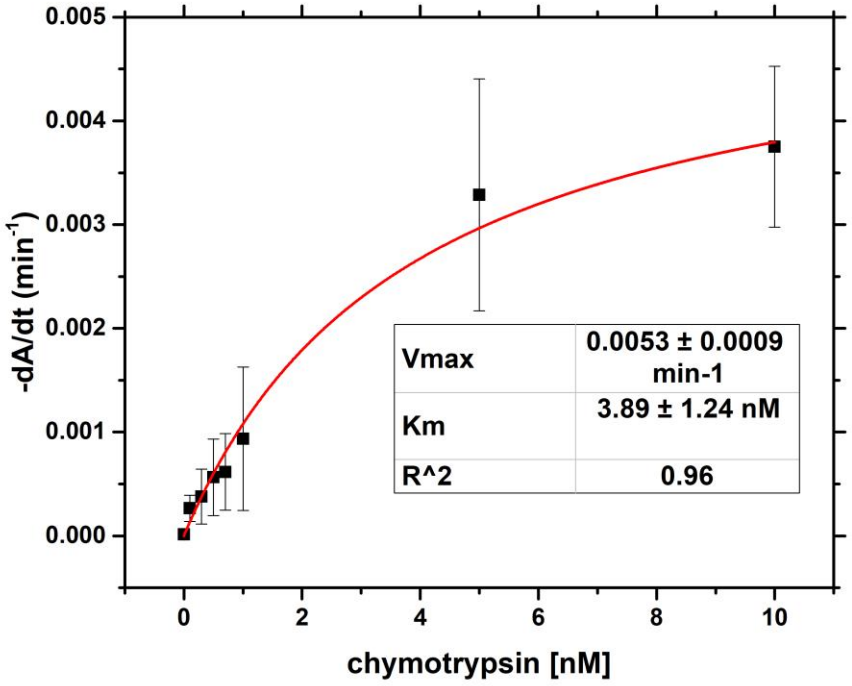

Fig 19. Calibration curve with reverse Michaelis-Menten model

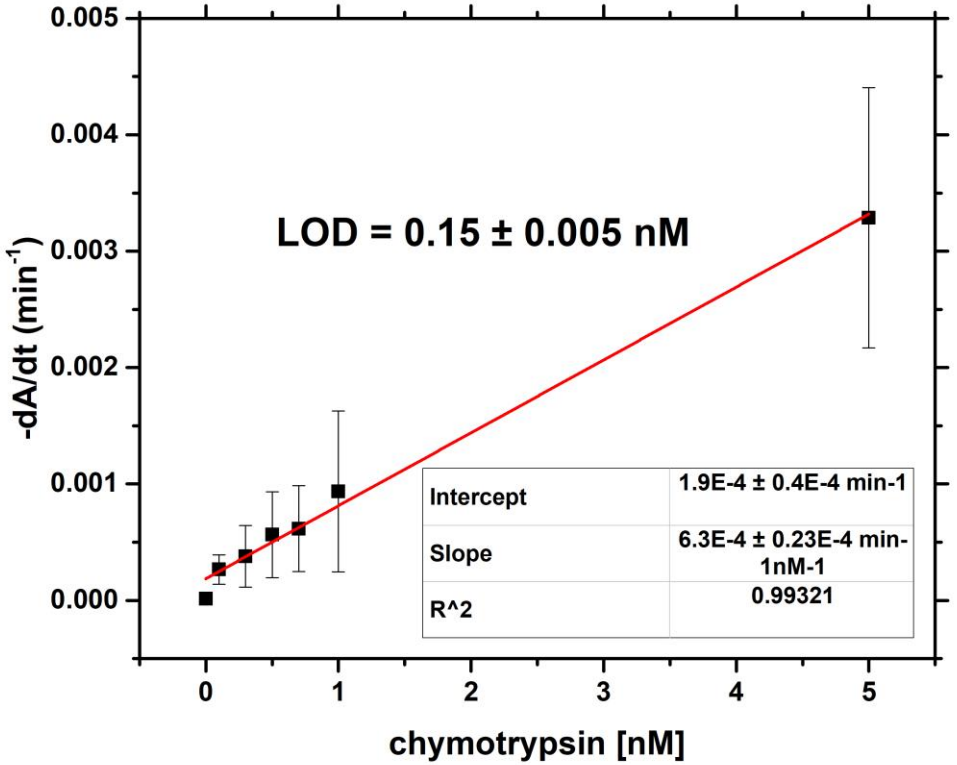

Fig 20. Linear part of calibration curve with calculated LOD

Conclusion

- LOD is better for optical rather than gravimetric method (0.15 nM vs 0.63 nM)
- Time of measurements are comparable between methods
- Preparation of biosensor is comparable
- Preparation of sample is more difficult for optical method
- Difference of K_M for optical (3.89 nM) and gravimetric (8.91 nM) might suggest chymotrypsin has better access to casein on AuNPs, which could also explain difference in LOD

Thank you for your attention

References

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