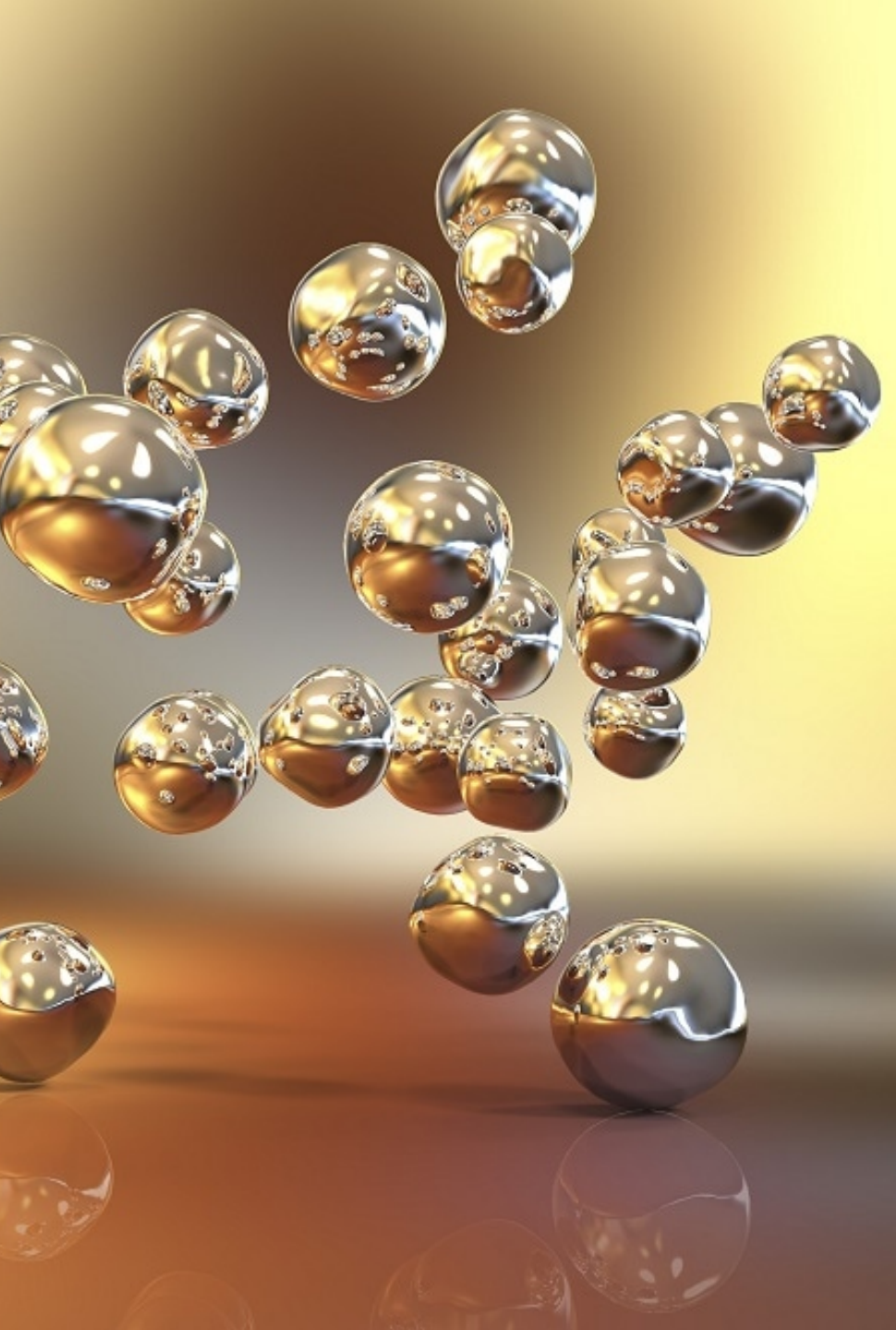COST ACTION CA 17140
NANO2CLINIC
CANCER NANOMEDICINE - FROM THE BENCH TO THE BEDSIDE

Steady-state fluorescence quenching method for evaluation of nano-bio interactions

## Rinea Barbir, mag.med.biochem.

Institute for Medical Research and Occupational Health, Zagreb, Croatia
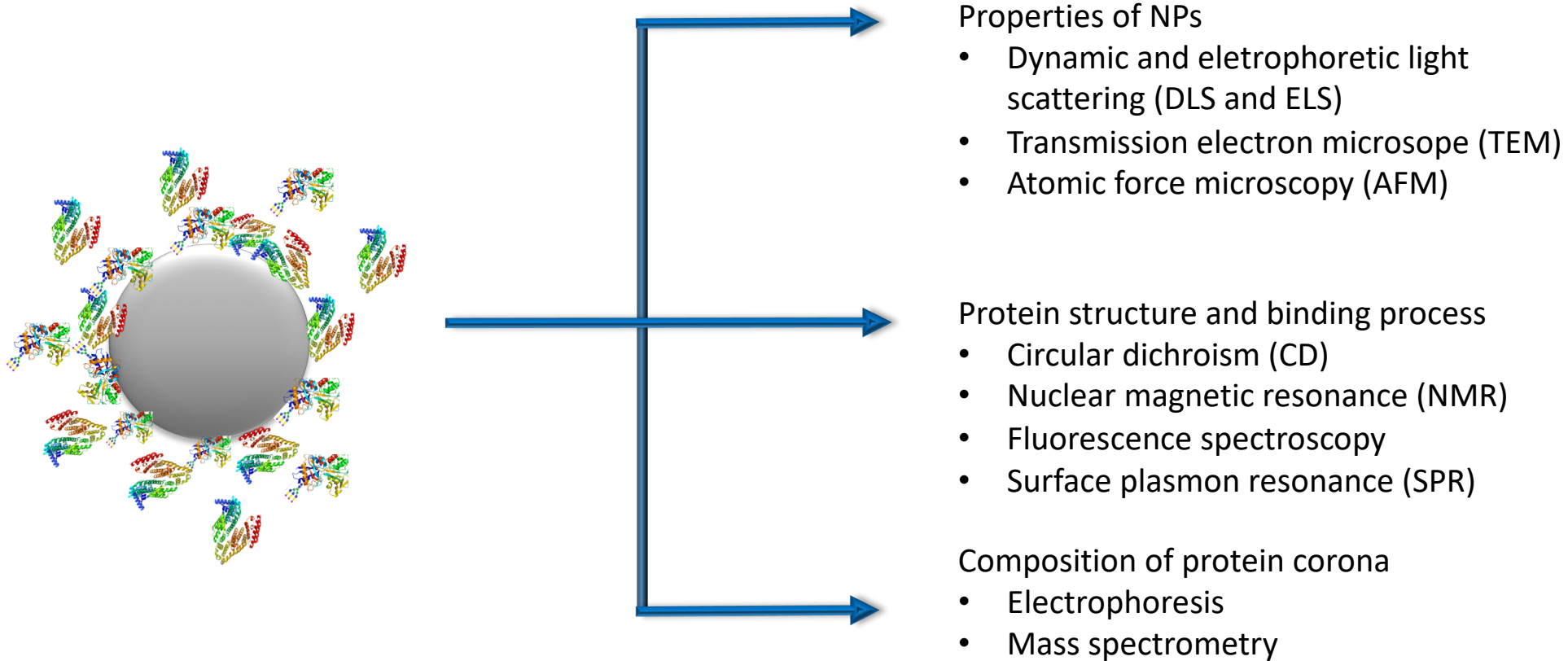
Objectives

- Protein corona
  - Meaning
  - Importance
- Methods for evaluation of protein corona
- Steady-state fluorescence quenching
  - Theoretical background
  - Stern-Volmer model
  - Interference – Inner filter effect
- Our research
  - Aim
  - Results
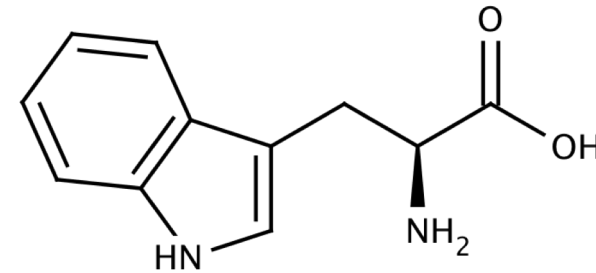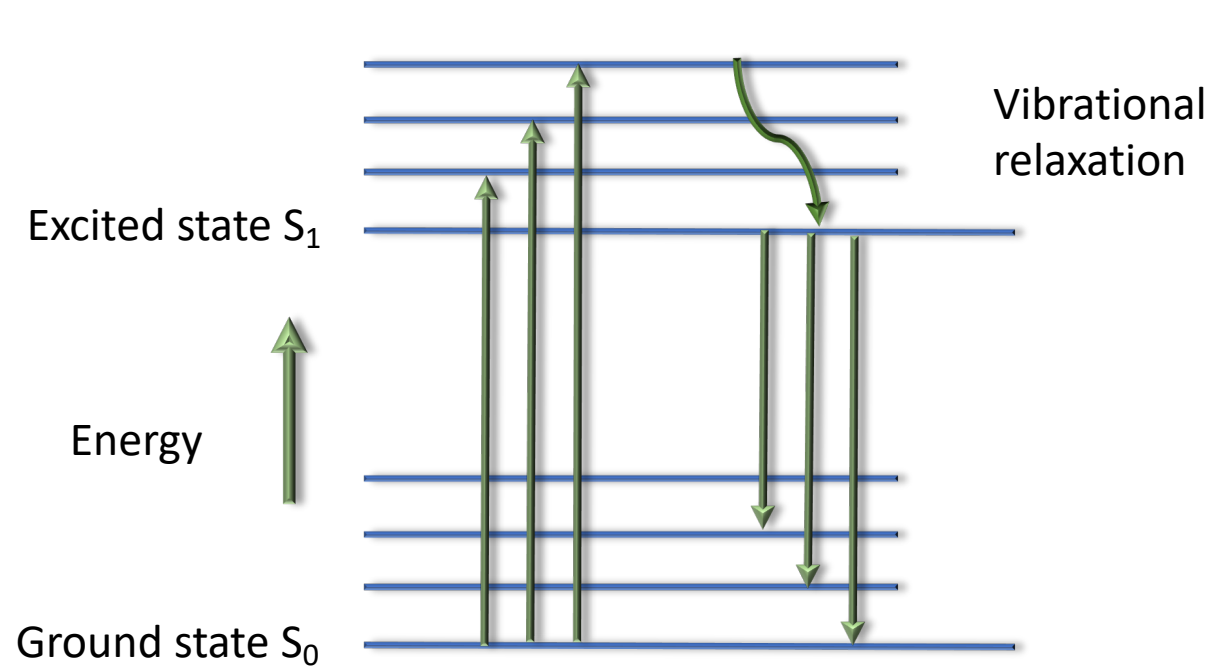- Conclusion

Protein corona (PC)
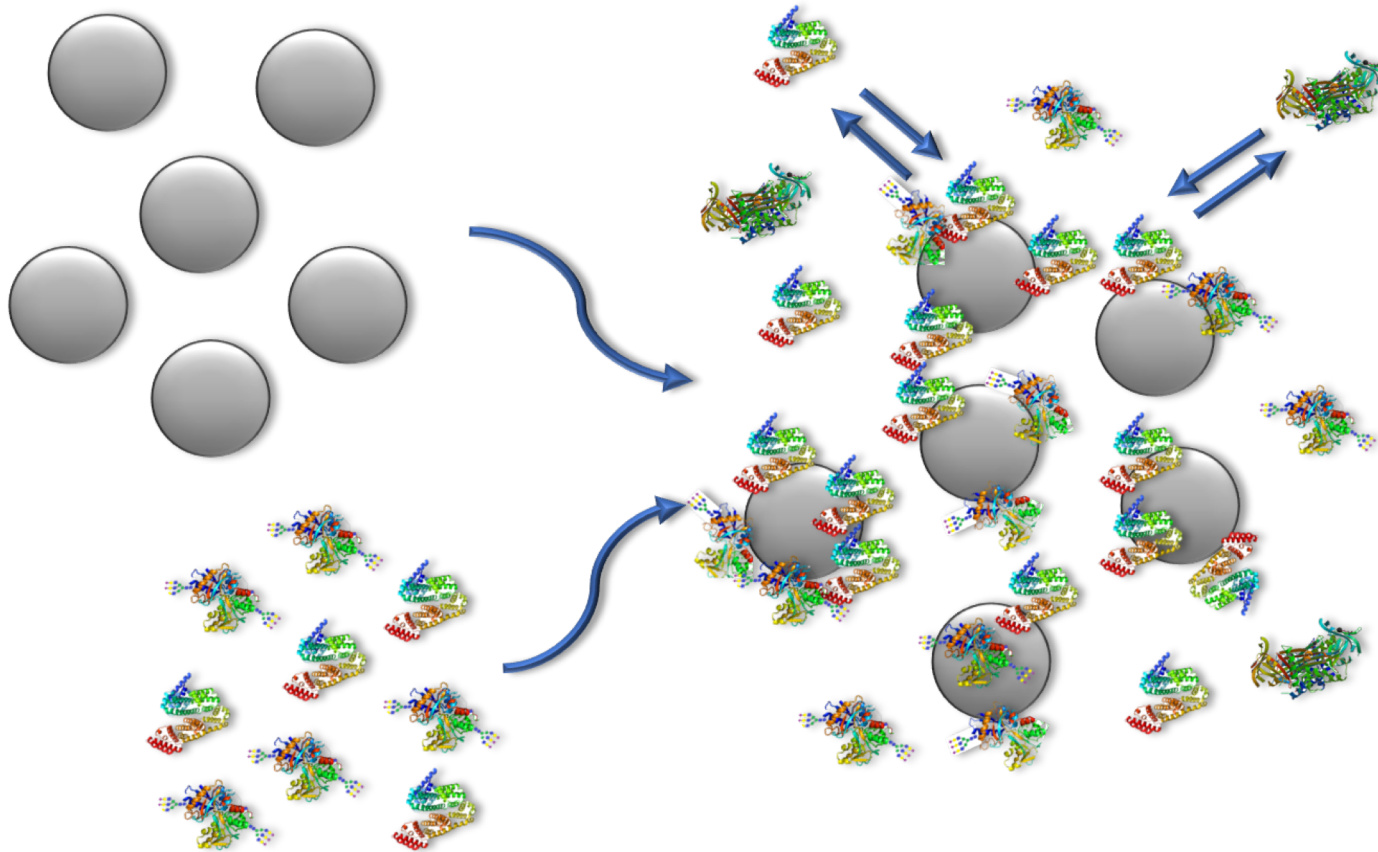
- Dynamic biomolecular layer
- Composition varies depending on the physicochemical properties of the nanoparticles
- Composition on the same type of NPs changes in cancerous vs. noncancerous patient samples
- Changes nanoparticles identity and conformation of proteins
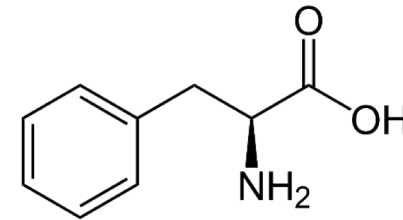- The mechanisms of biomolecular corona formation need to be elucidated

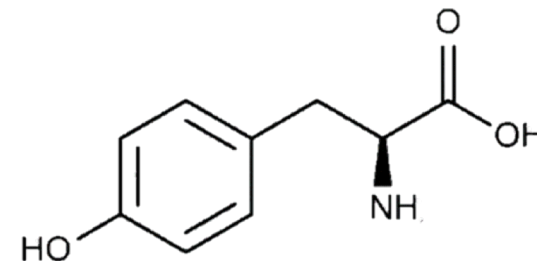# Analytical methods for evaluating nano-bio interactions

Properties of NPs
- Dynamic and eletrophoretic light scattering (DLS and ELS)
- Transmission electron microsope (TEM)
- Atomic force microscopy (AFM)

Protein structure and binding process
- Circular dichroism (CD)
- Nuclear magnetic resonance (NMR)
- Fluorescence spectroscopy
- Surface plasmon resonance (SPR)

Composition of protein corona
- Electrophoresis
- Mass spectrometry

Fluorescence

Vibrational relaxation

Excited state S_1

Energy

Ground state S_0

Tryptophan
λ_{ex} = 280 nm
λ_{em} = 348 nm

Phenylalanine
λ_{ex} = 257 nm
λ_{em} = 282 nm

Tyrosine
λ_{ex} = 274 nm
λ_{em} = 303 nm

Stern-Volmer model

Increasing NPs concentration
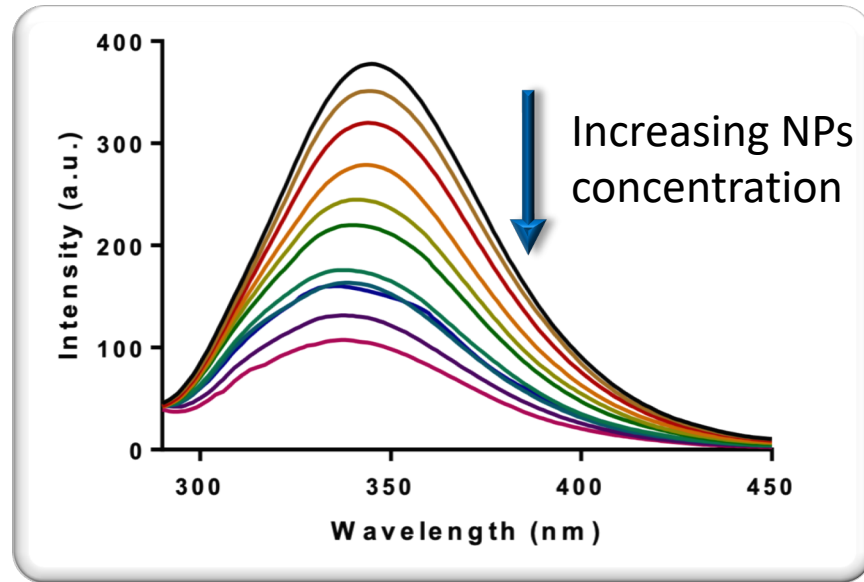
$$\frac{F_0}{F} = 1 + K_{SV}[\text{NPs}] = 1 + k_q\tau_0[\text{NPs}]$$
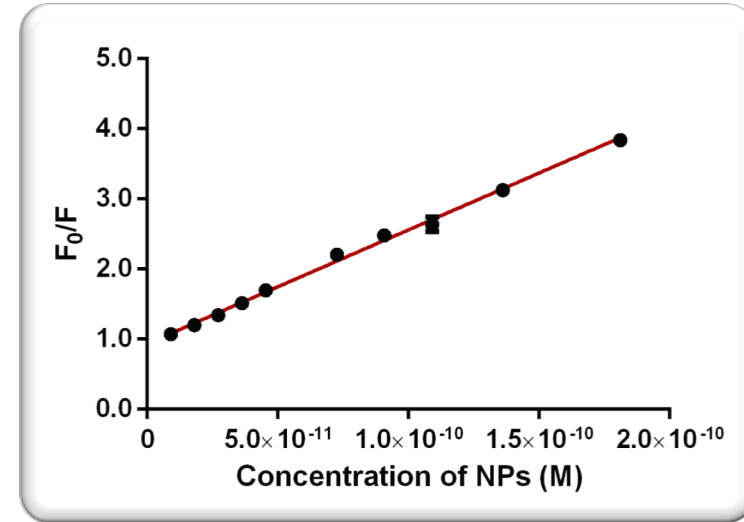
τ_0 = 5 ns

Dynamic quenching

$k_q < 2.0\times10^{10}$ M^{-1}s^{-1}

Static quenching
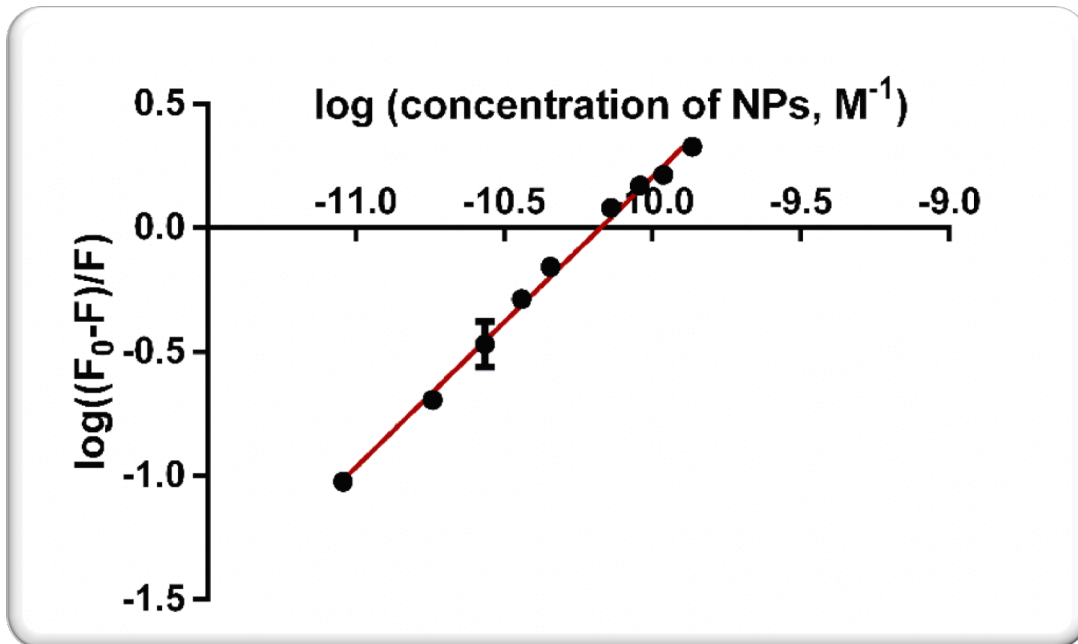
$k_q > 2.0\times10^{10}$ M^{-1}s^{-1}

Stern-Volmer model

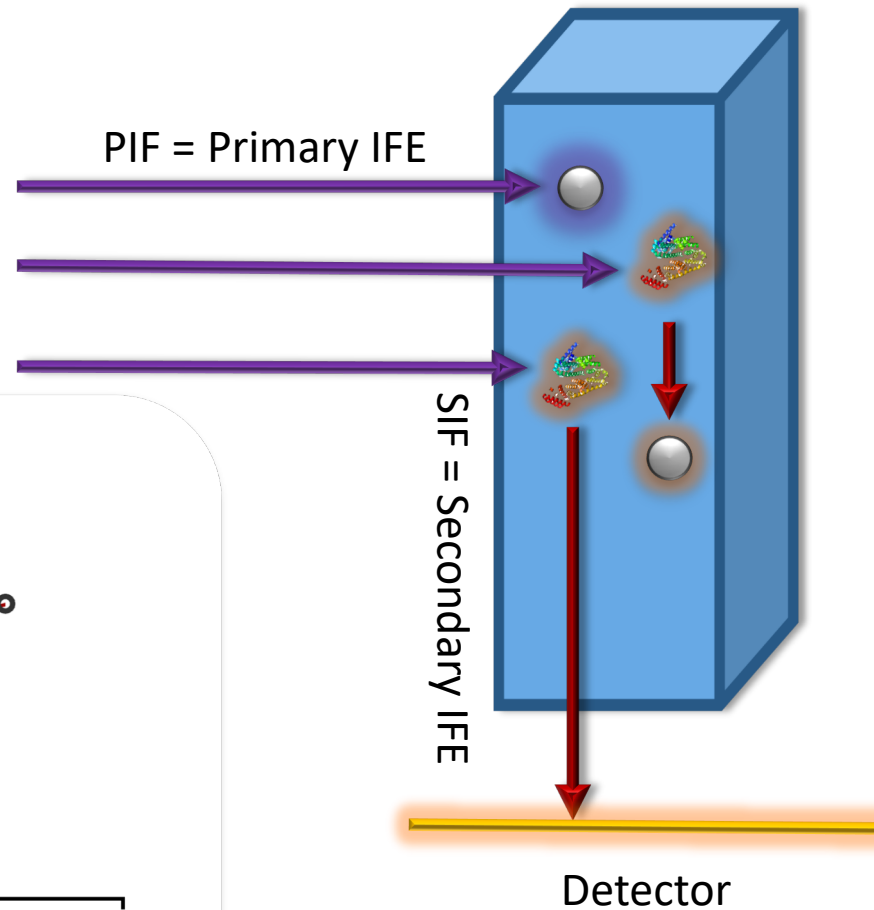$$\log\left(\frac{F_0 - F}{F}\right) = \log K_b + n\log[NP]$$
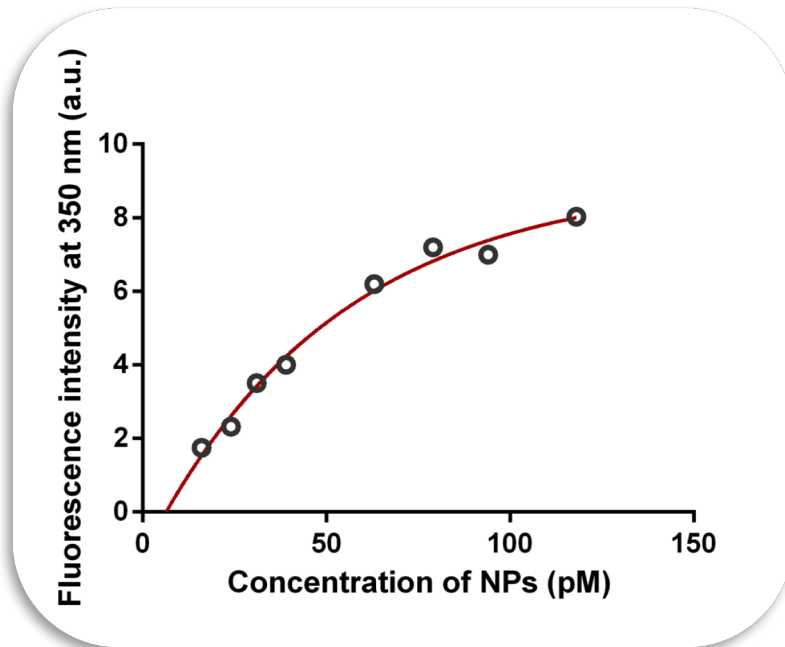

$\log K_b$ – logarithmic value of the binding constant

n value – Hills coefficient

$n > 1$ cooperative binding

$n < 1$ anti-cooperative binding

Inner-filter effect

PIF = Primary IFE

SIF = Secondary IFE

Detector

Fluorescence intensity at 350 nm (a.u.)

Concentration of NPs (pM)

Avoding inner filter effect:

- Small concentrations (A < 0.1)

- Reducing the path length of the light

- Changing the λ_{ex}

$$F_{corr} = F_{obs} \times e^{\frac{A_{ex}+A_{em}}{2}}$$

Our research - aim

- Development of nano-bio sensors for early cancer diagnostics (changes in glycan profile in patological conditions)

- to investigate the influence of glycosylation and different physicochemical properties of nanoparticles on the nano-bio interactions and formation of the protein corona
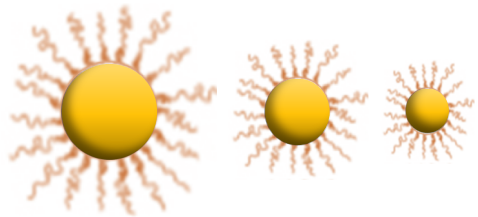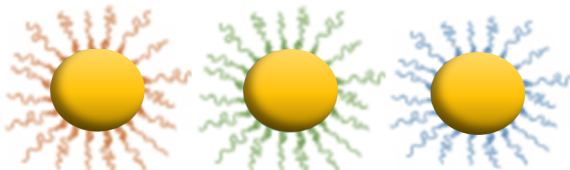
Citrate coated AuNPs
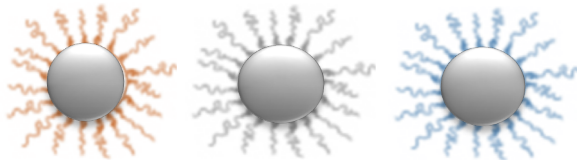
Differently coated AgNPs

Citrate   PVP   PEG
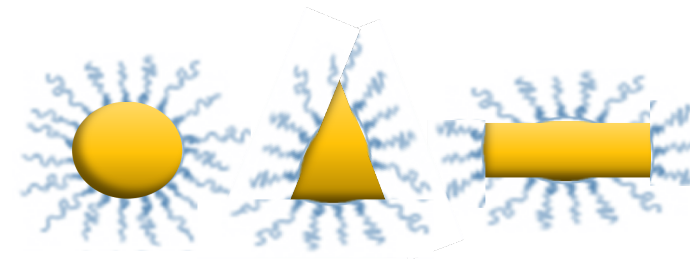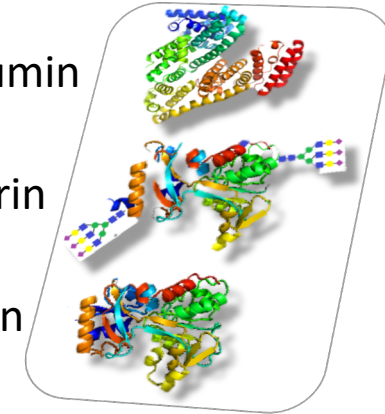
Differently coated AuNPs
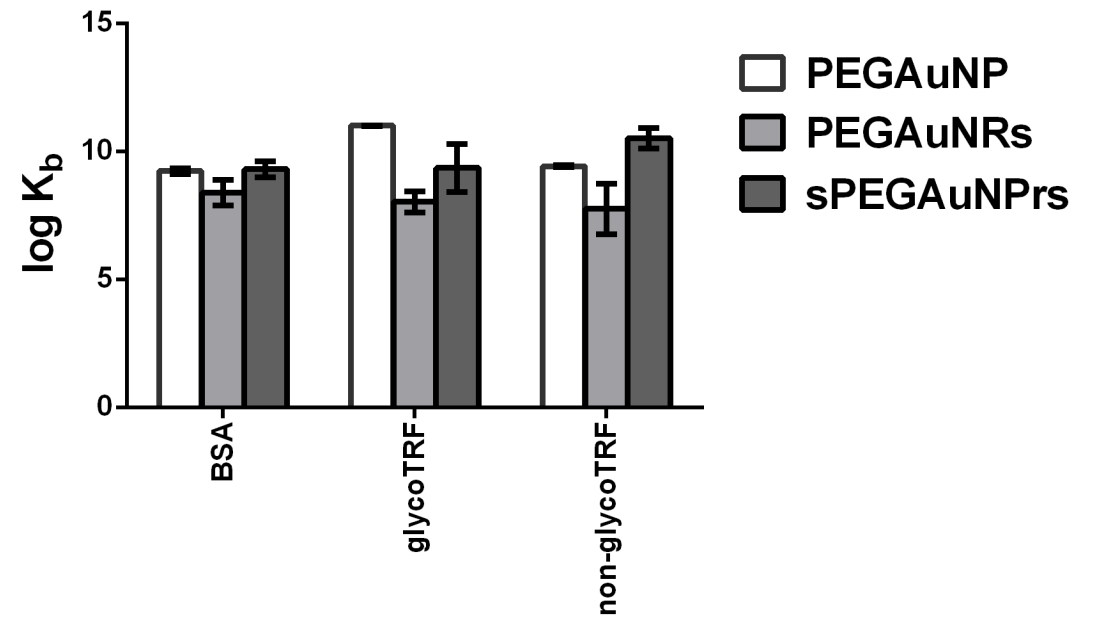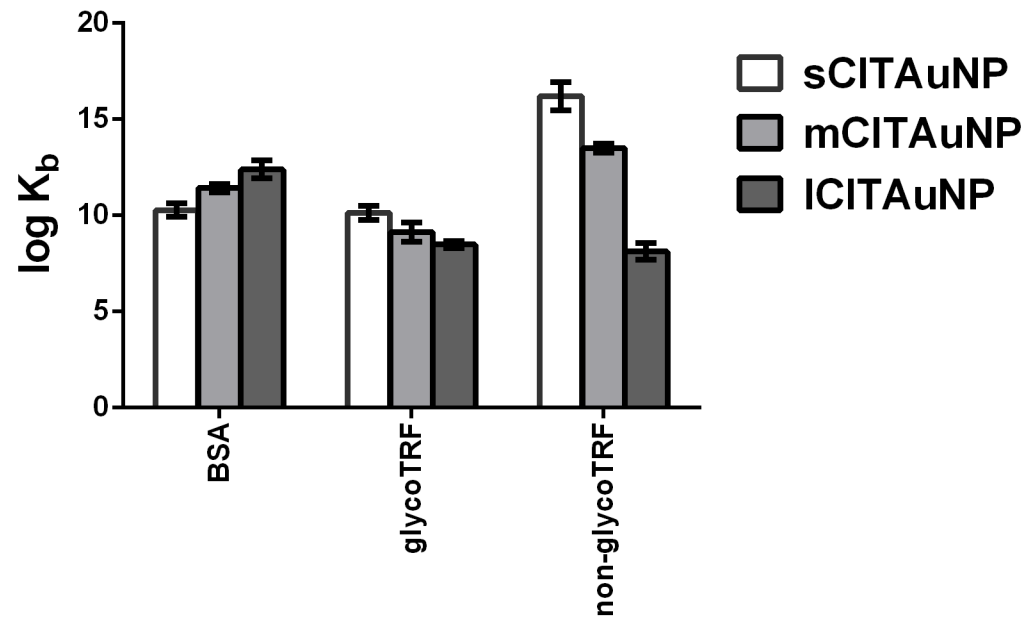
Citrate   GSH   PEG

Bovine serum albumin
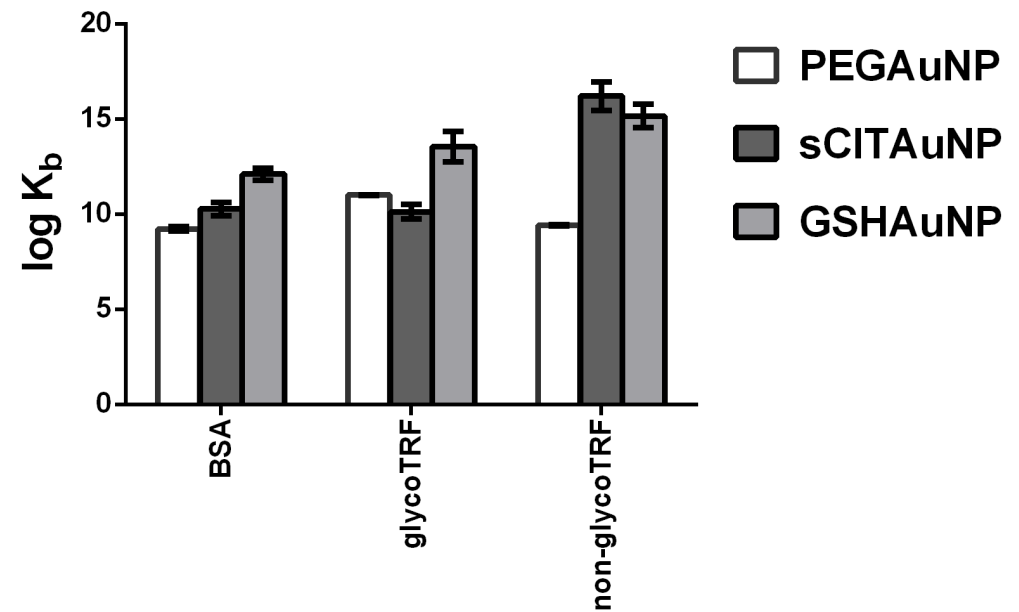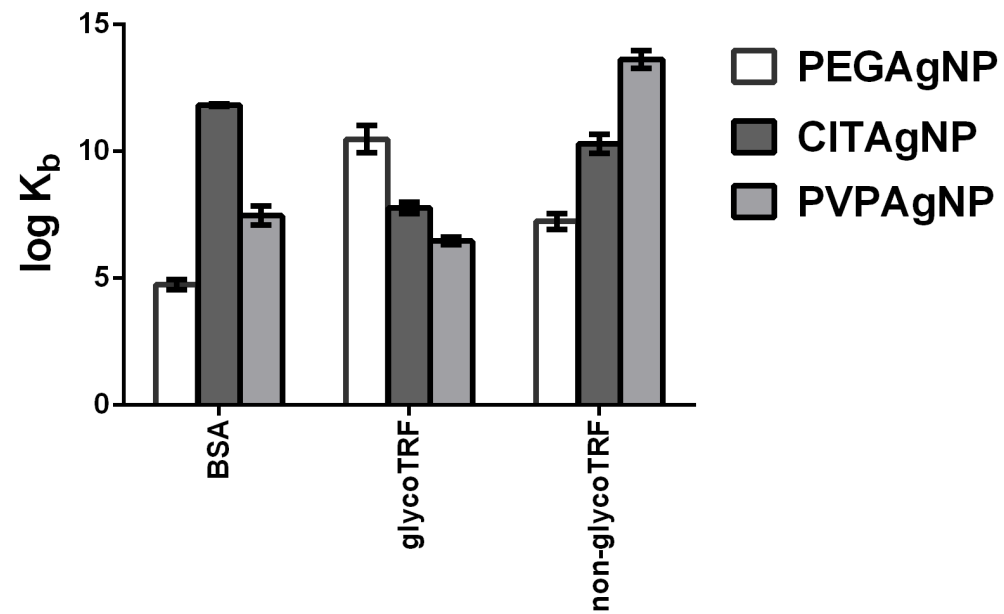
Glycosylated transferrin

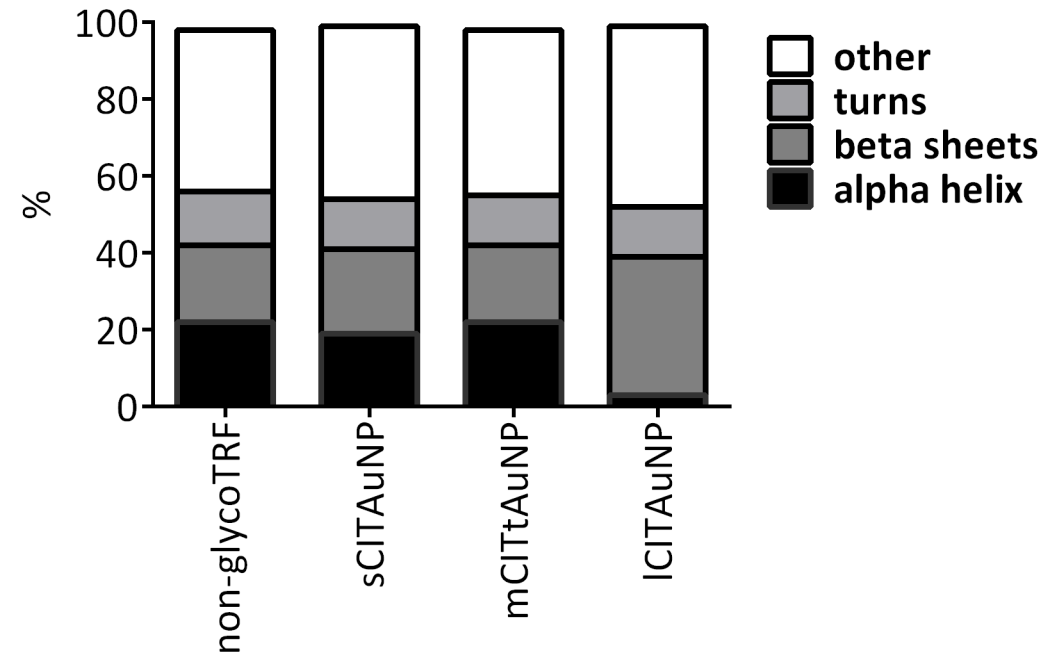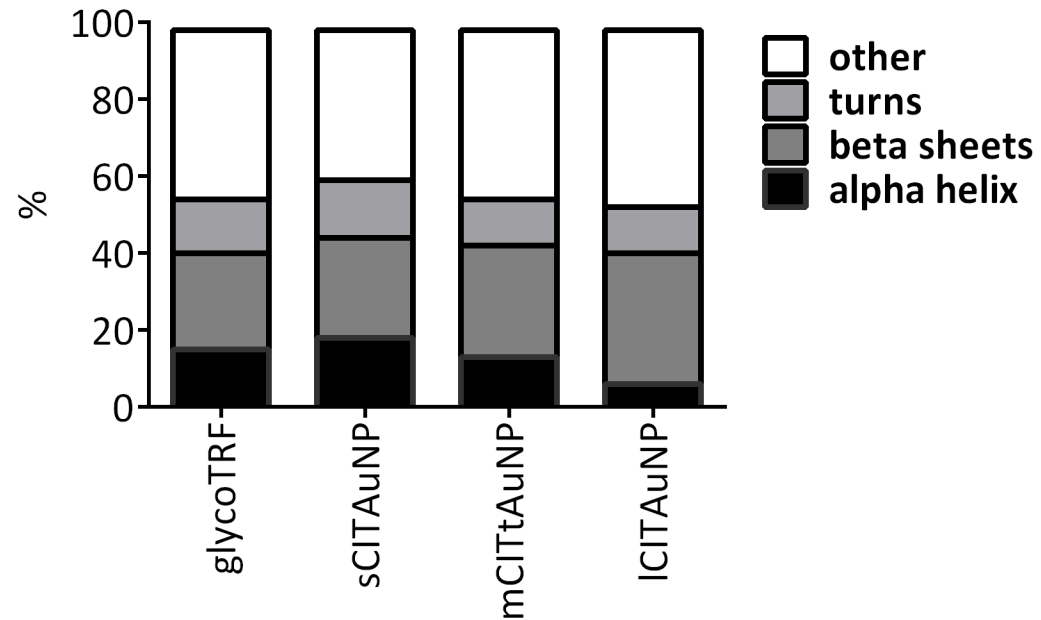Non-glycosylated transferrin

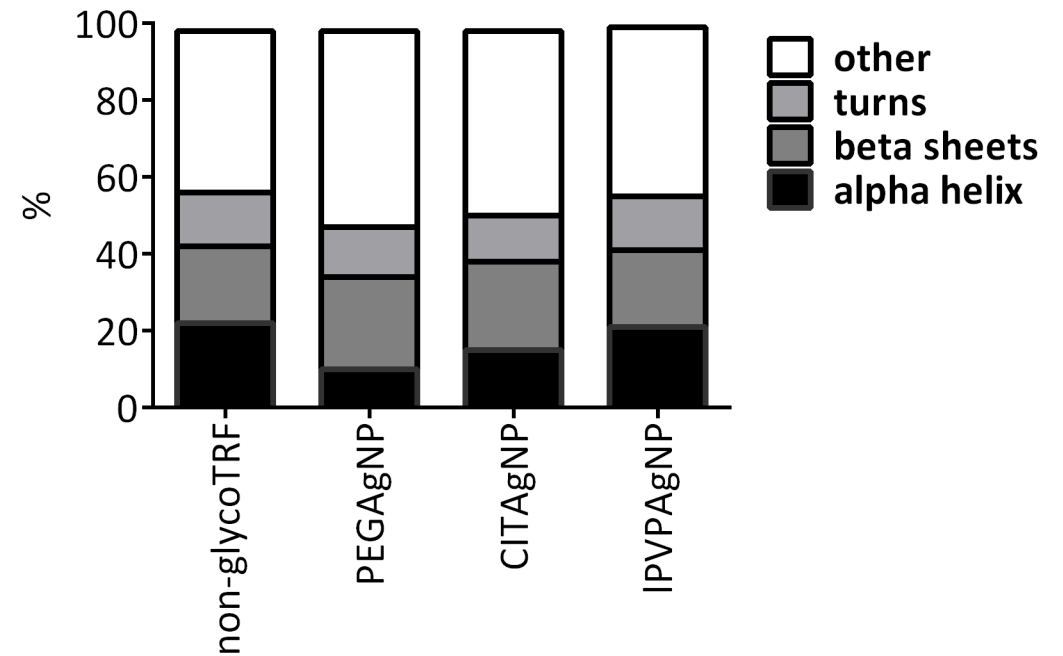PEG coated AuNP of different shapes
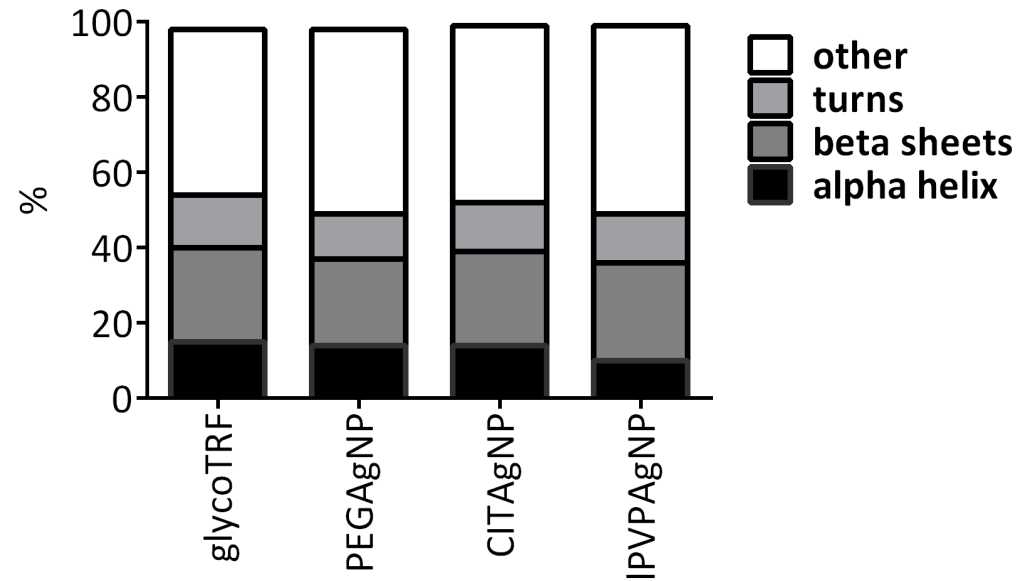
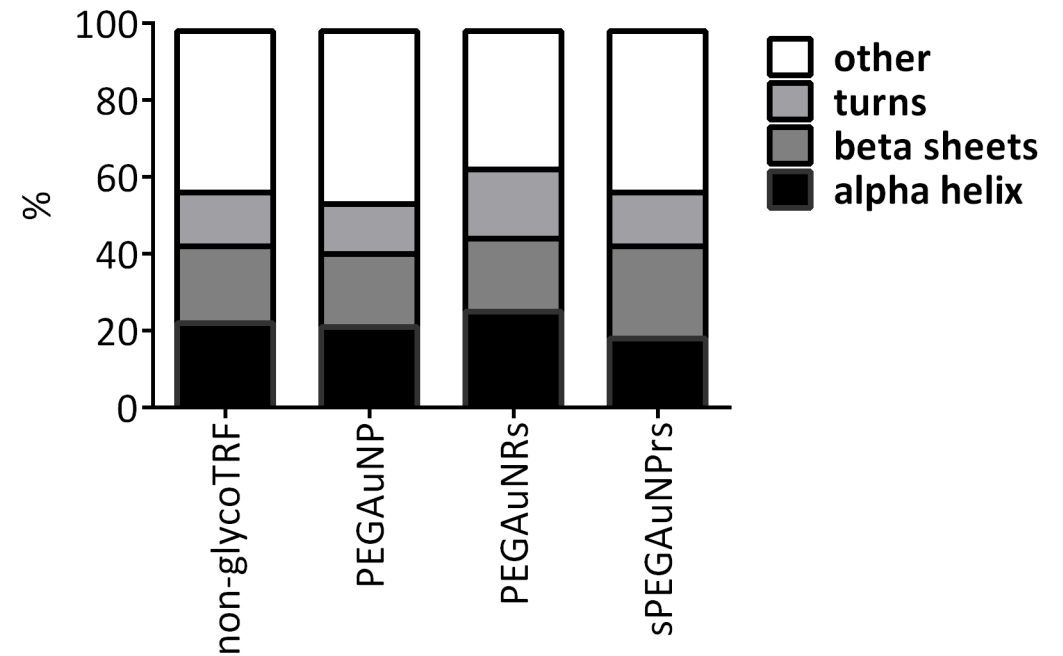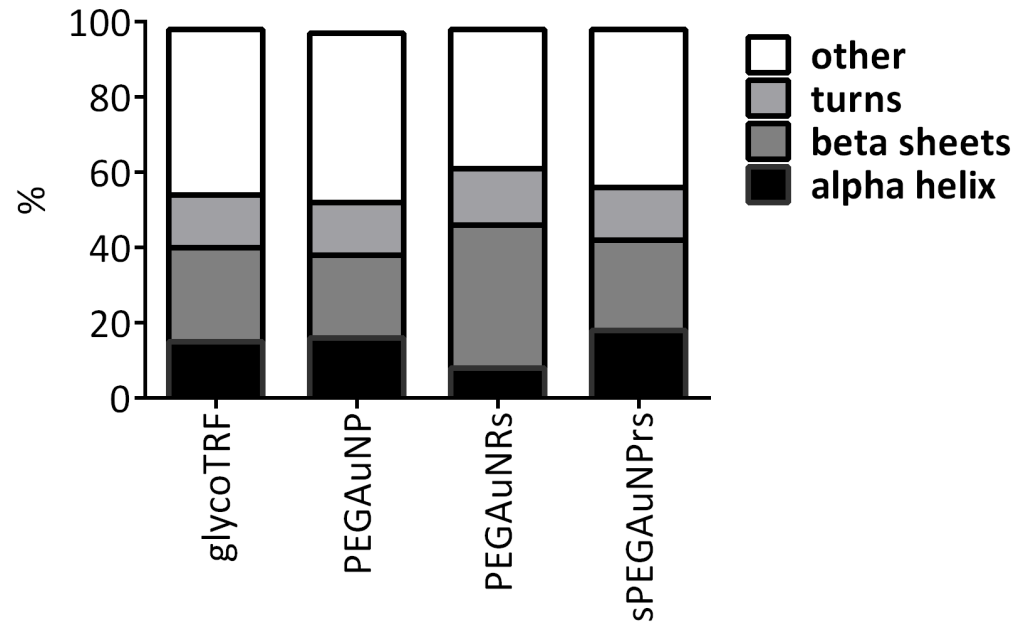# Results – influence of size and shape

Results – influence of coating

Circular dichroism (CD) - size

Circular dichroism (CD) - coating

Circular dichroism (CD) - shape

Future research


- Quantitative analysis of proteins around NP
- „Vroman effect"
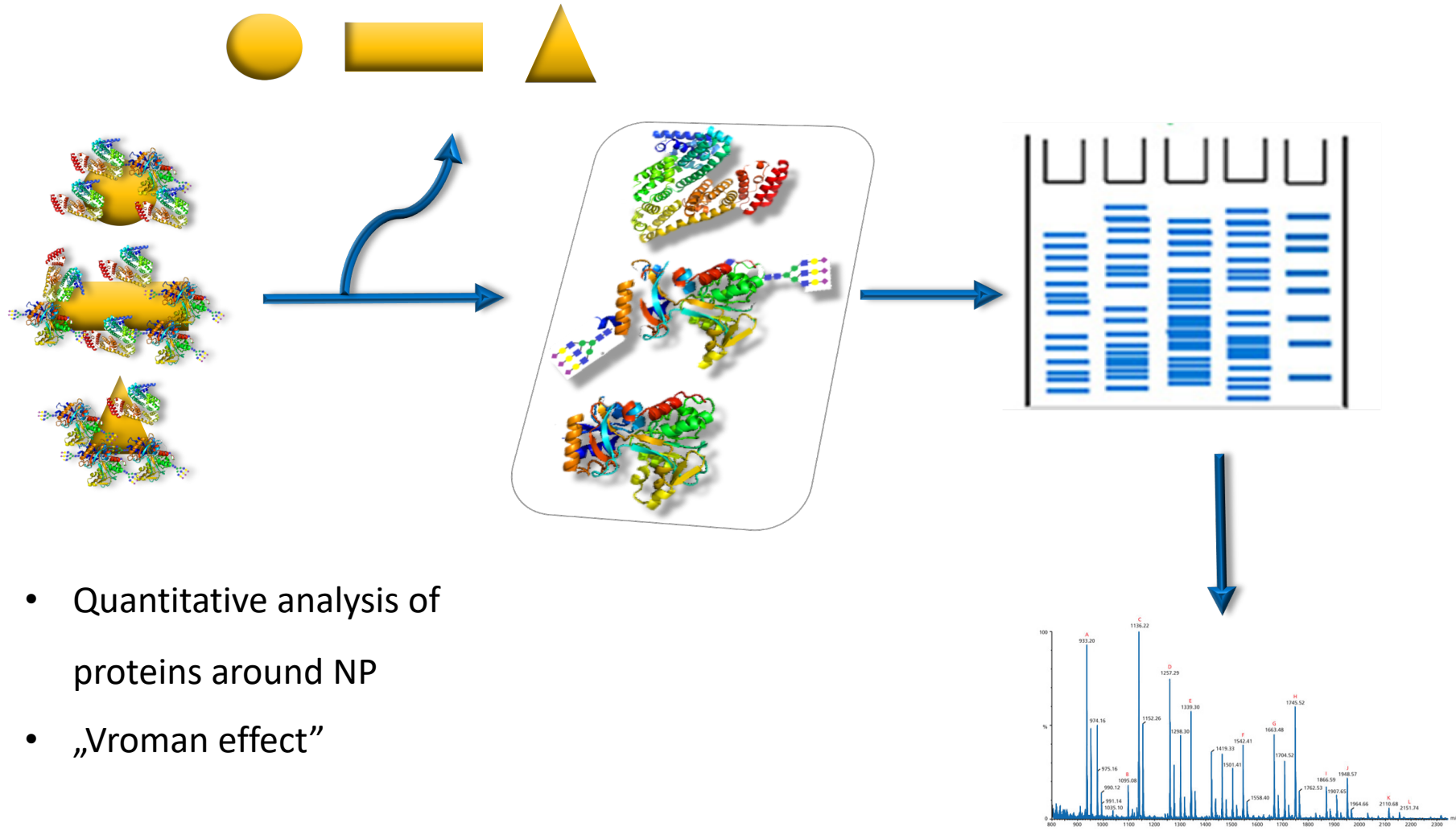
Conclusion

- first study which evaluated the influence of glycosylation status of proteins on the formation of biomolecular corona

- nano-bio interactions are highly dependent on multiple factors

- glycosylation can have a significant impact on biomolecular corona formation and thus on the biological fate of nanoparticles in the body

Acknowledgements
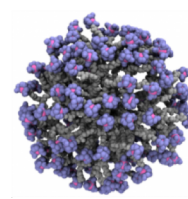
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