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UNIVERSITAS MASARYKIANA BRUNENSIS

S3002
Nanobiotechnology

Bioapplication of nanoparticles

Zdeněk Farka

Outline

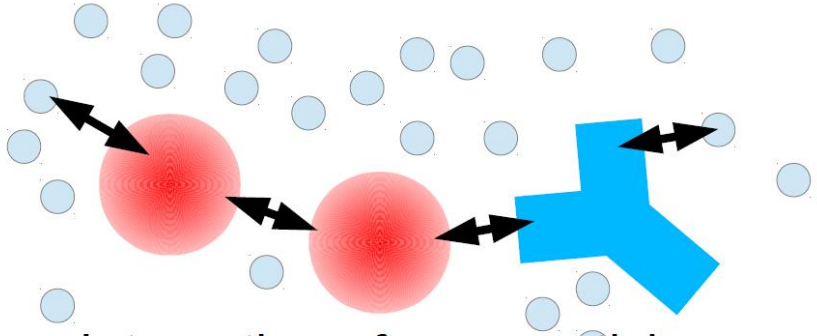
- Introduction to nanoparticles
- Conjugation of nanoparticles with biomolecules
- Characterization and purification
- Luminescent nanoparticles
  - Quantum dots
  - Photon-upconversion nanoparticles
- Biological applications of nanoparticles
  - Detection
  - Imaging

Nanoparticles

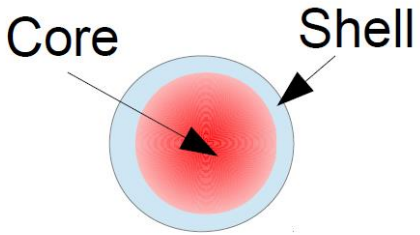
- Nano is the SI prefix of the multiples of 10^{-9}
  - Derived from the Greek νᾶνος – dwarf
- Nanoparticles are particles with size from 1 to 100 nm
  - (In each dimension)
  - Polymeric nanoparticles and covalent nanocrystals are commonly regarded as nanoparticles
  - In contrast, liposomes and polymersomes are usually not marked as nanoparticles, but rather referred as nanovesicles
- Alternative definition – at least one dimension is in the range from 1 to 100 (1000) nm
  - Also called nanofibers/nanoplates

Nanoparticles in liquid

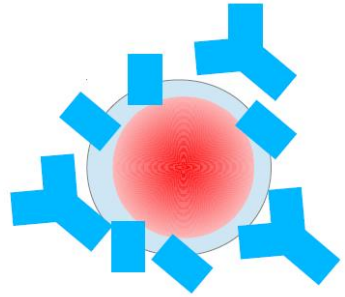
- We consider nanoparticles in dispersions (not in vacuum)
  - **Fluid**, gas, solid phase
- Nanoparticles interact with each other, with the surrounding liquid and with other components in the medium (proteins, other nanoparticles, immune system...)
  - Effect of surface chemistry
  - Interactions sometimes desirable and sometimes undesirable



Interaction of nanoparticles



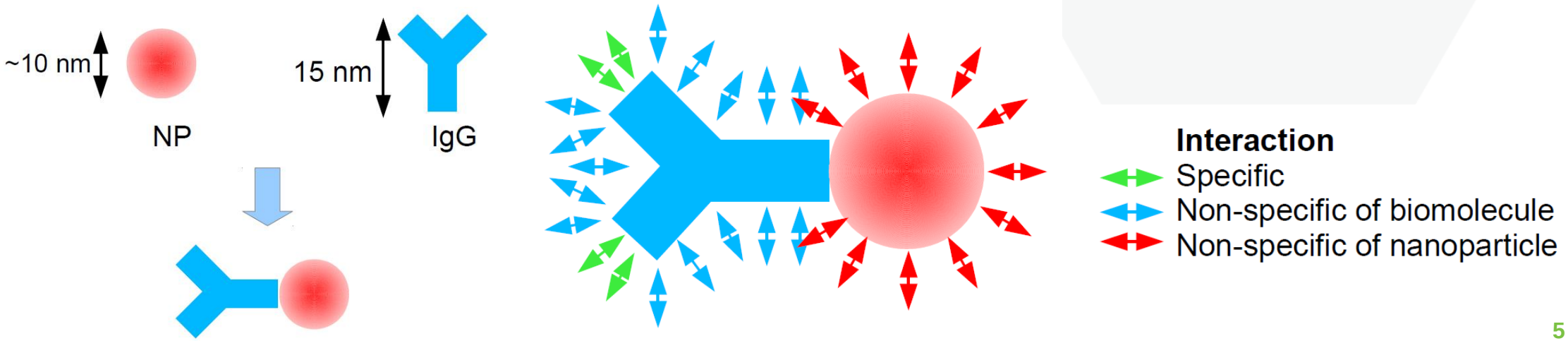
Surface modification



Protein corona

Nanobioconjugates

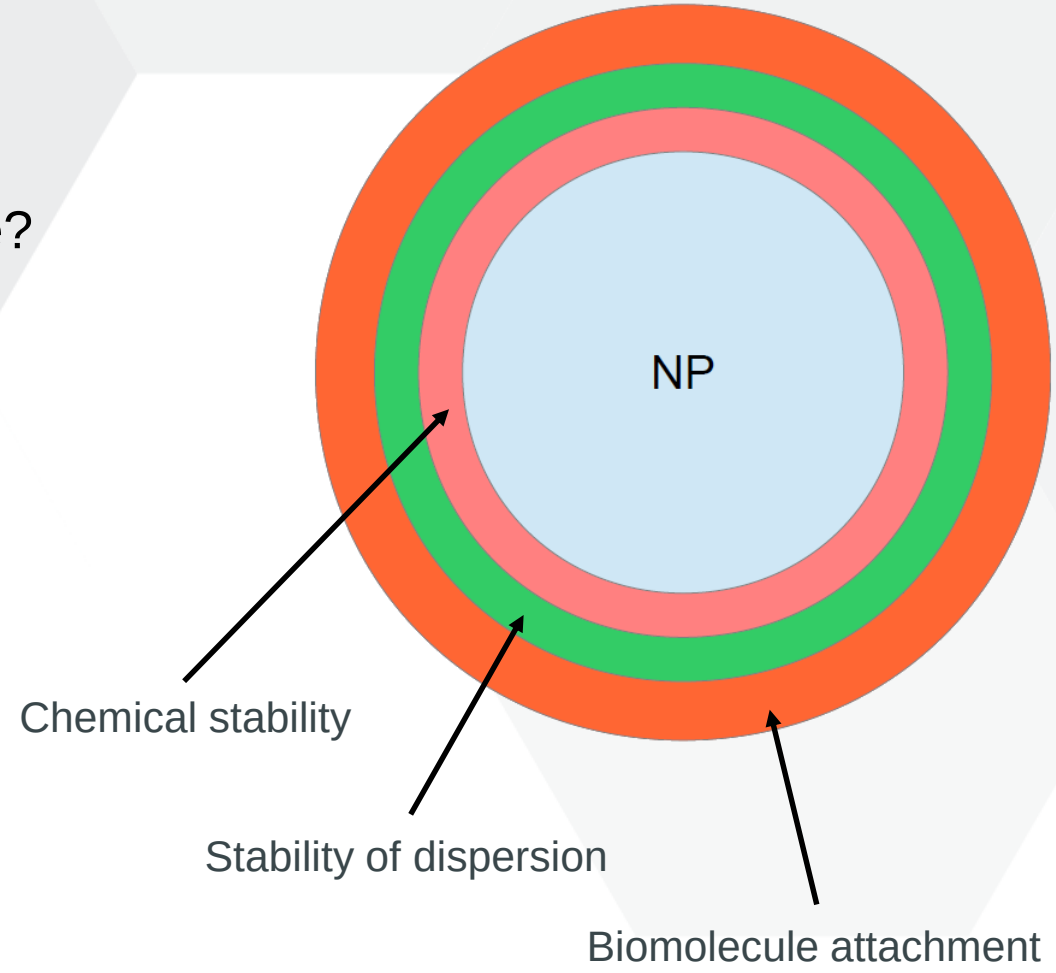
- Comparable size of nanoparticles and biomolecules
- Enables creation of nanostructures bearing biological function of the biomolecule (e.g. specific interaction of antibodies with antigen) and interesting properties of nanoparticles, which, e.g., facilitate their detection (luminescence, catalysis)
- Such structures are called bioconjugates of nanoparticles (nanobioconjugates)
- Wide applications in biology, medicine and analytical chemistry



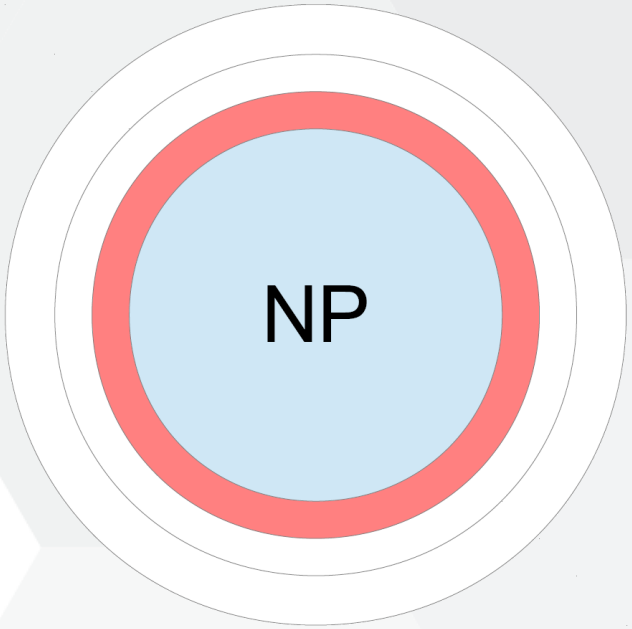
Stability and conjugation of nanoparticles

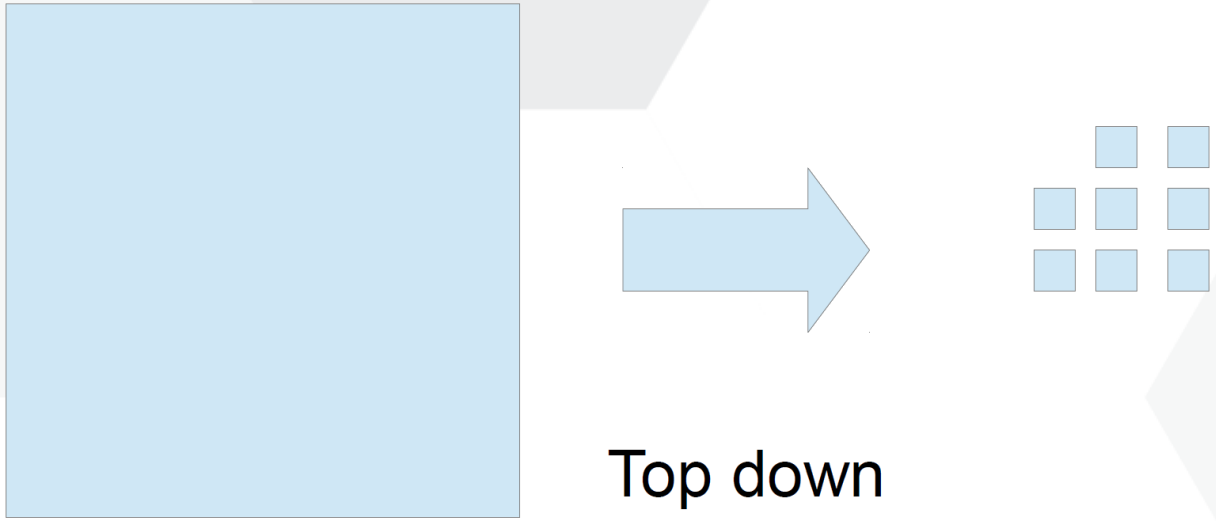
Surface of nanoparticles

- Chemical stability
  - What is the chemical structure of surface?
- Stability of dispersion
  - Avoid aggregation
- Biomolecule attachment
  - Biological applications



Chemical stability



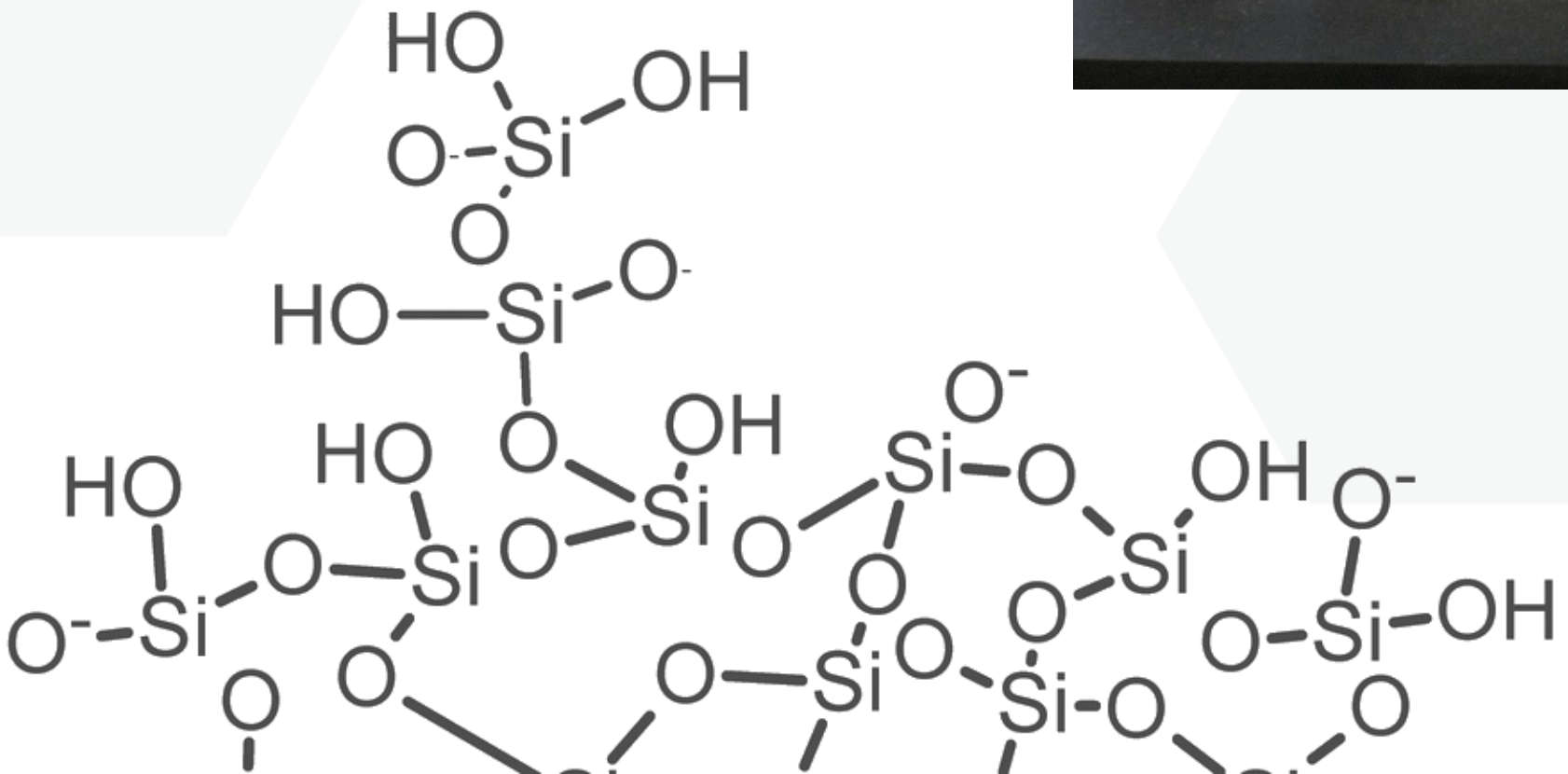


Top down

What is the chemical structure of surface?

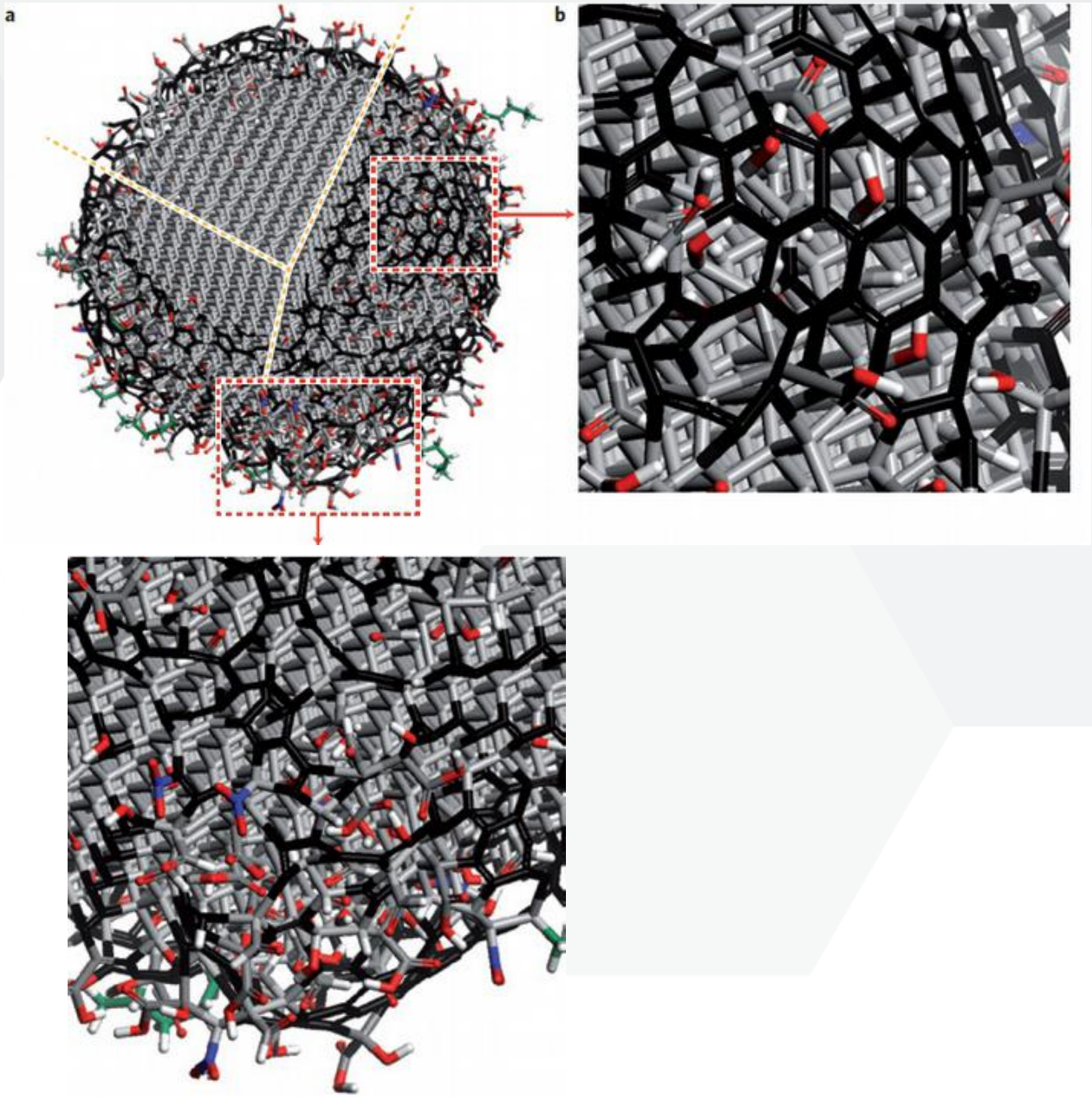
Silica nanoparticles

- SiO_2 nanoparticles
- High stability, low toxicity
- (Also – Silanization of other nanoparticles)



Nanodiamonds

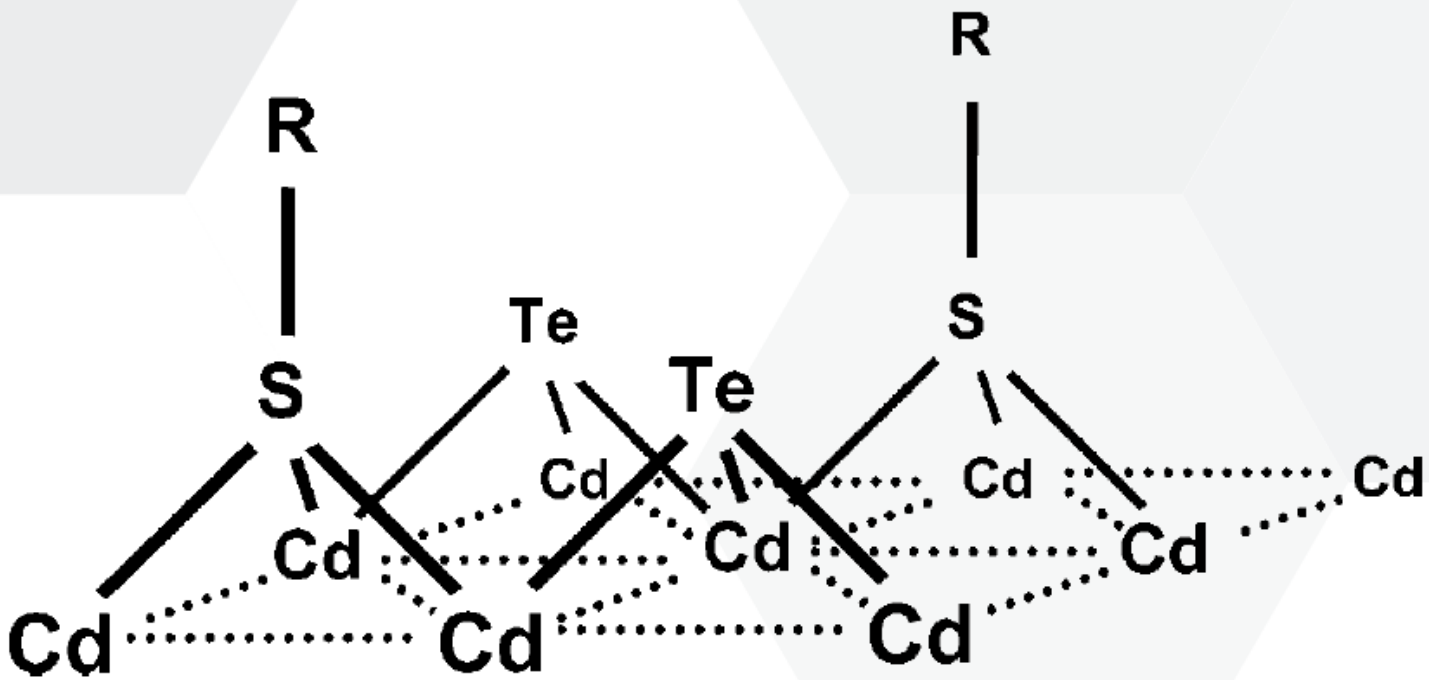
- Bare surfaces of cubic crystals exhibit structures similar to bulk diamond
- The surface of sp^3 clusters must be either stabilized through termination with functional groups or reconstructed into sp^2 carbon
  - Hydrogen termination
  - Onion-like shells with diamond cores (‘buckydiamond’)
  - Termination with oxygen-containing and nitrogen-containing functional groups



Mochalin, V.N., Shenderova, O., Ho, D., Gogotsi, Y. (2012) Nat. Nanotechnol., 7 (1), 11-23.

Quantum dots

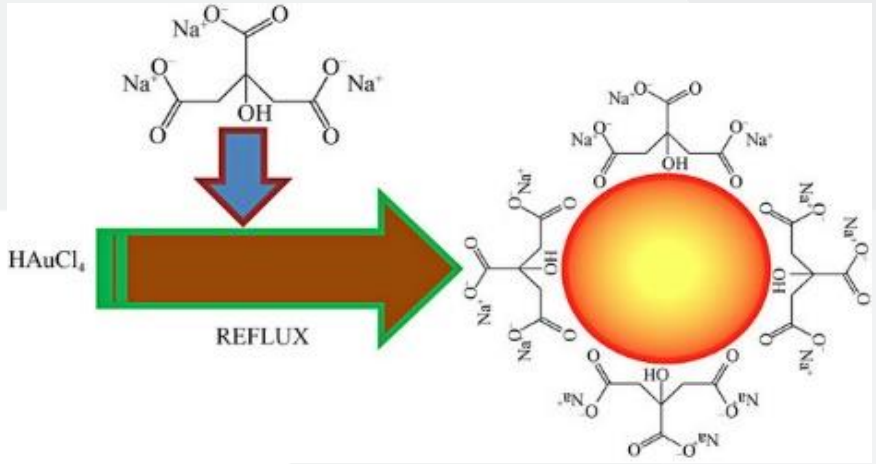
- Stabilization using thiols
  - Water solubility
  - Reactive group for functionalizations
  - E.g. mercaptopropionic acid (MPA)
- Coating by silica layer

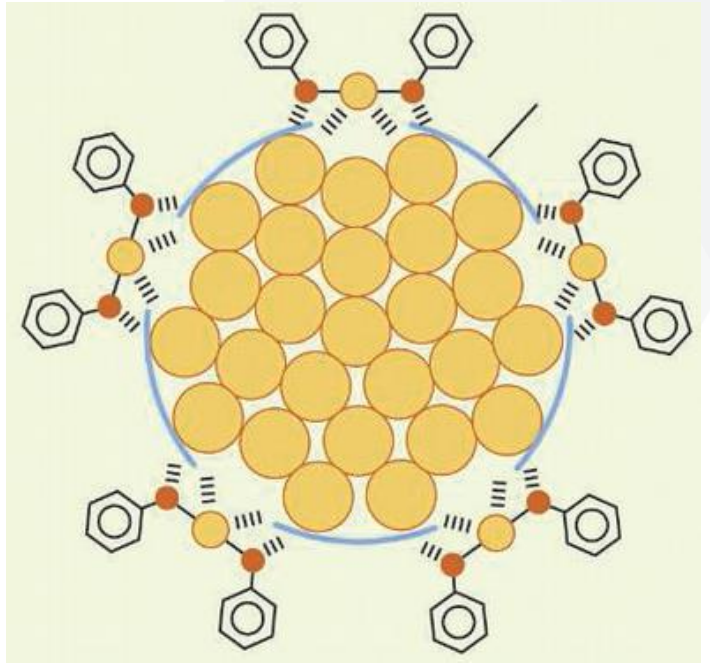


Borchert, H., Talapin, D.V., Gaponik, N., McGinley, C., Adam, S., Lobo, A., Möller, T., Weller, H. (2003) J. Phys. Chem. B, 107 (36), 9662-9668.

Gold nanoparticles

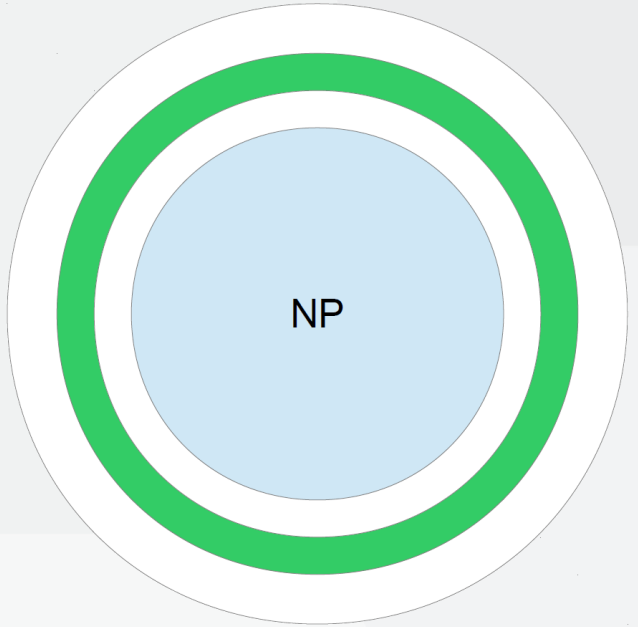
- Citrate capped Au NPs are produced by the most widespread Turkevich method
- Ligand exchange
  - Adsorption of thiols to Au
  - Stabilization
  - Introduction of reactive groups or biomolecules





Jadzinsky, P.D., Calero, G., Ackerson, C.J., Bushnell, D.A., Kornberg, R.D. (2007) Science, 318 (5849), 430-433.

Stability of dispersion



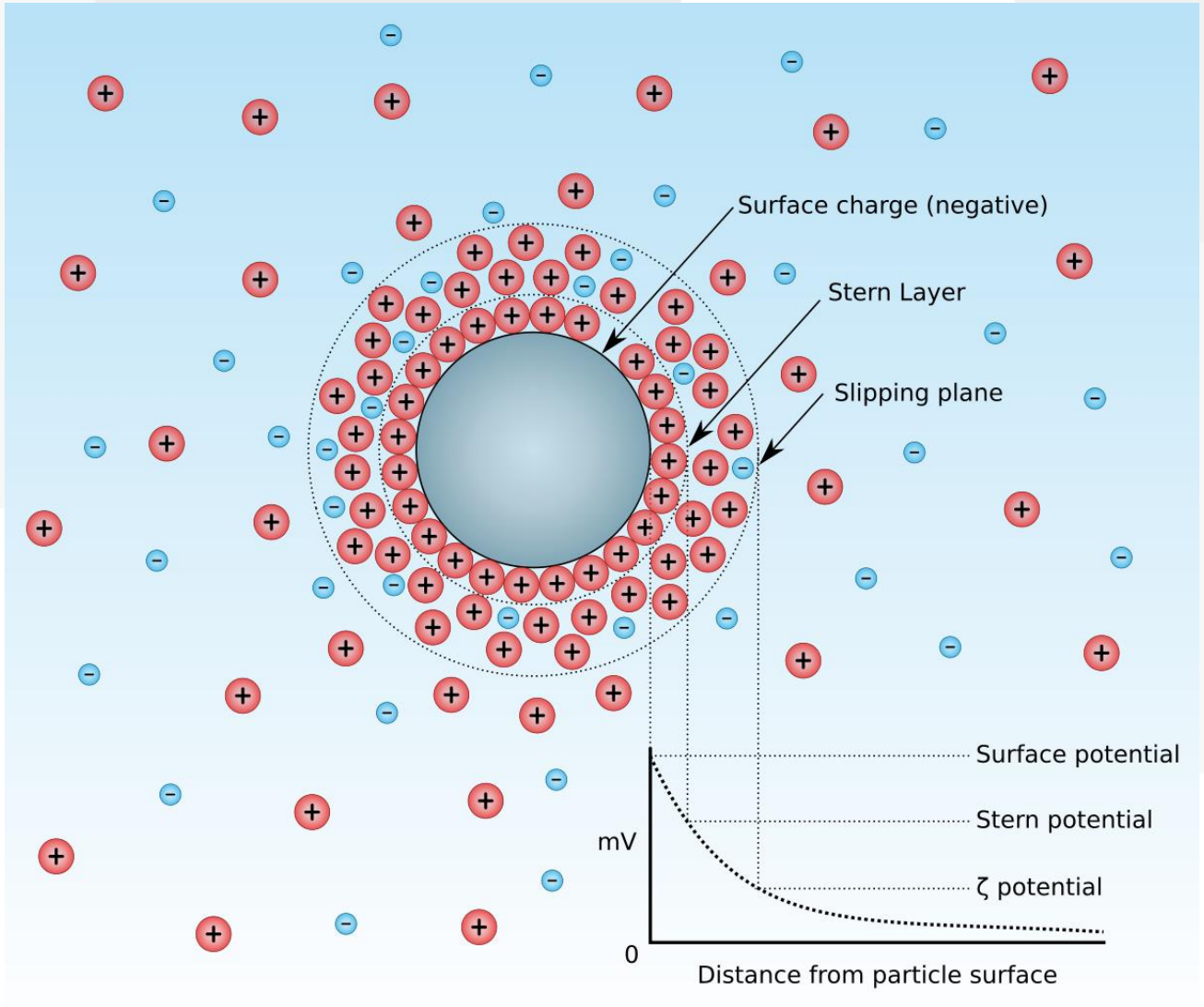
- Nanoparticles in liquid interact with the molecules of liquid, with each other, solid particles in the environment, the walls of the container...
- Interactions can be divided into several groups
  - Van der Waals interactions
  - Electrostatic interactions
  - Hydrophobic interactions
  - Solvation (hydration) interactions
  - Steric interactions
- These interactions are both attractive and repulsive
  - Attractive forces result in nanoparticle aggregation

Van der Waals interactions

- Attractive and repulsive interactions between molecules excluding covalent bonds, hydrogen bonds and electrostatic interactions
- Van der Waals interactions are divided:
  - Interactions between permanent dipoles (Keesom interaction)
  - Interactions between permanent and induced dipole (Debye interaction)
  - Interactions between induced dipoles (London's dispersion interactions)
- Short distances (~ 1 nm)
- Hamaker theory
  - Description of van der Waals interactions between nanoparticles, which are composed of many molecules
  - This theory considers additive nature of van der Waals interactions of the molecules that form the interacting system

Electrostatic interactions

- Effect of electrical double layer

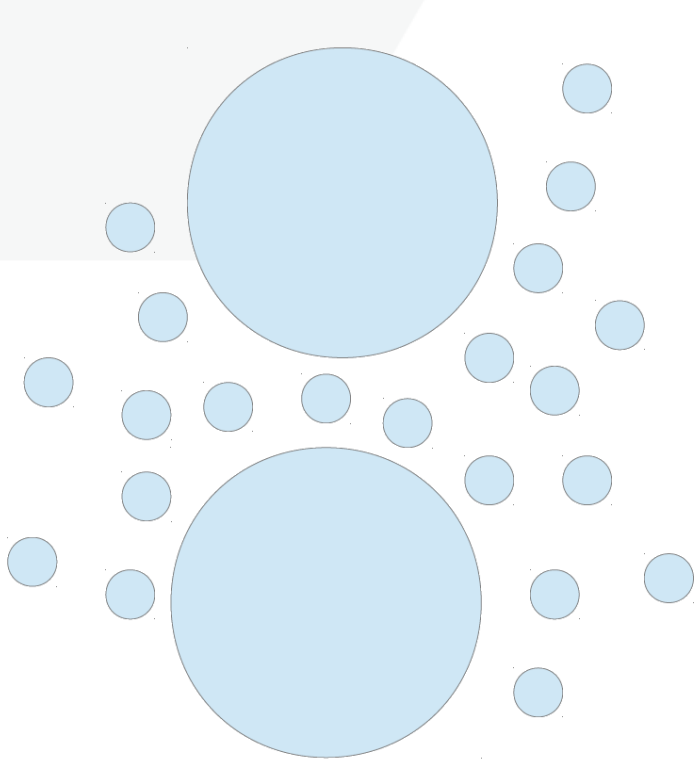


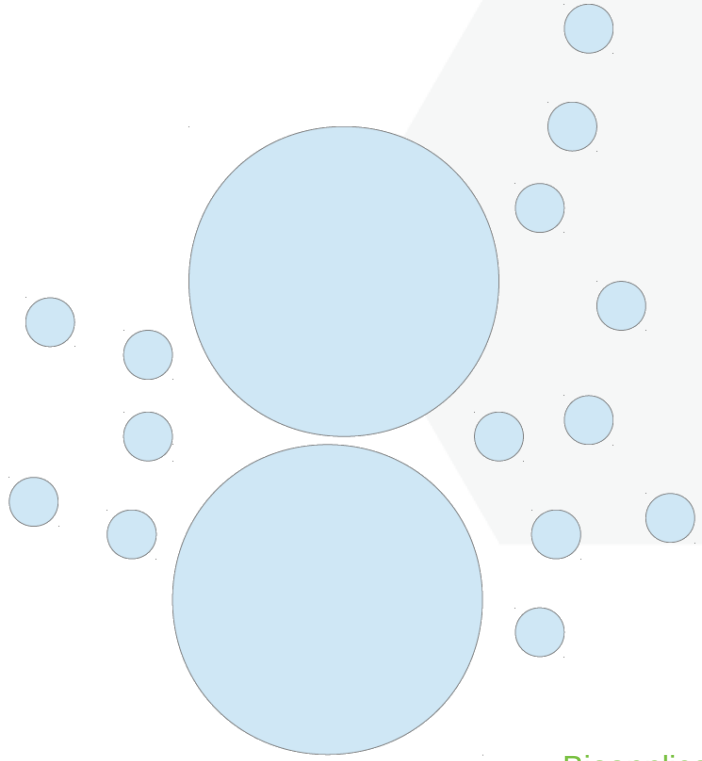
Electrostatic interactions

- Mutual repulsion of two negatively (positively) charged particles approaching each other, which thus prevents aggregation of these nanoparticles
- Nanoparticles carrying a high zeta potential (in absolute value) are sufficiently stabilized
  - From 0 to ± 5 unstable
  - From ± 10 to ± 30 slightly unstable
  - From ± 30 to ± 40 moderately stable
  - From ± 40 to ± 60 stable
  - More than ± 61 very stable
- The disadvantage of electrostatically stabilized nanoparticles is mainly unspecific adsorption onto oppositely charged surfaces and nanoparticles

Hydrophobic interactions

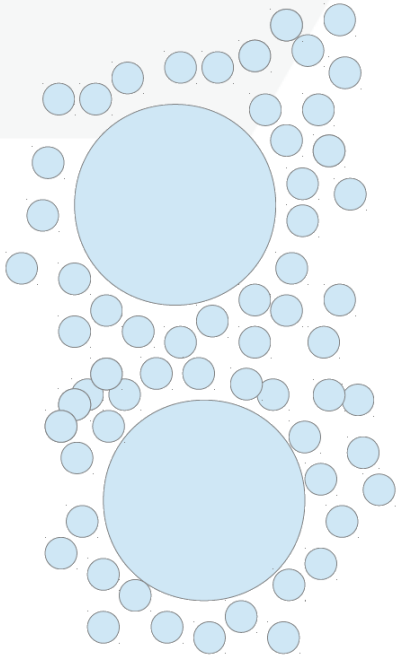
- Water molecules on the surface of hydrophobic molecules can not form hydrogen bonds with other molecules of water
- Hydrophobic interactions therefore lead to a reduction of hydrophobic surface → aggregation of hydrophobic nanoparticles in water



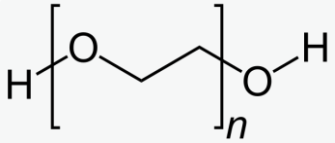


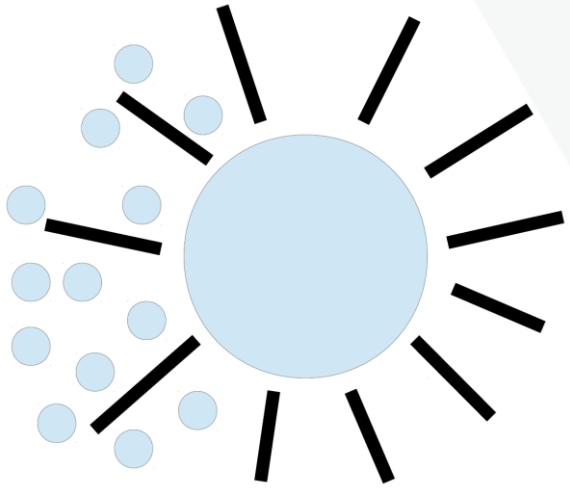
Solvation interactions

- Not fully understood
- Solvent forms a surface layer and hides the surface of nanoparticle
- An example may be the surface of particles coated with PEG or SiO_2
  - Adsorbed water molecules form a barrier that prevents aggregation



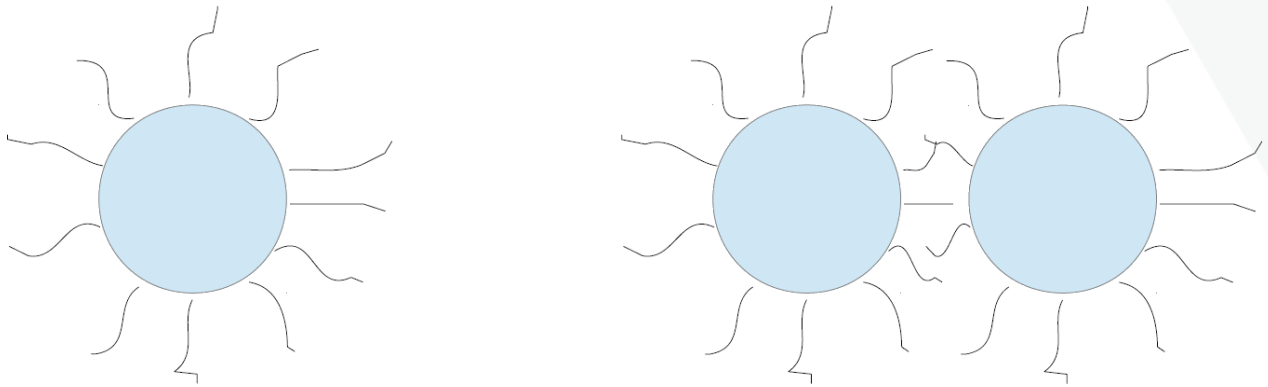
Polyethylene glycol



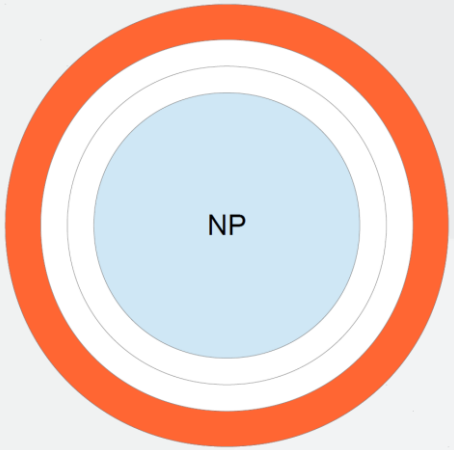


Steric interactions

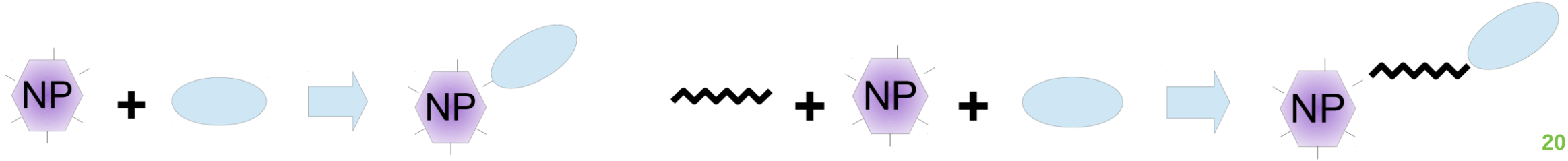
- Polymer coated nanoparticles (PEG)
- Polymeric chains protruding above the surface of the nanoparticles have a certain equilibrium conformation
- If this conformation is disturbed by compression or stretching, then the polymer chain tends to return to its original conformation
- Meeting of two nanoparticles leads to the deformation of polymer chains, repulsive interactions between nanoparticles prevents their aggregation



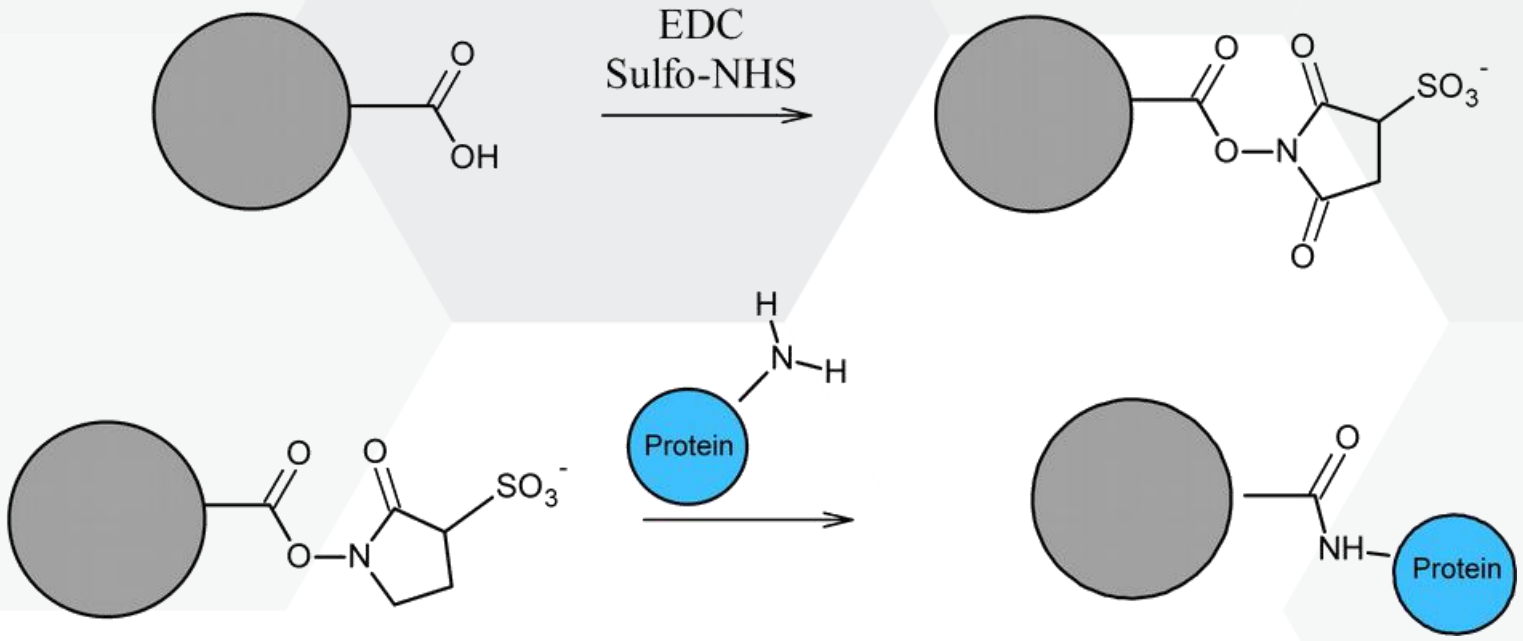
Reactive groups for bioconjugation

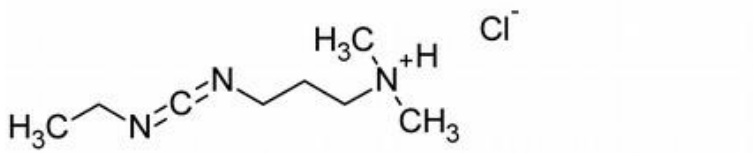


- Nanoparticle
  - Carboxyl -COOH
  - Amine -NH_2
  - Thiol -SH
  - Aldehyde -CH=O
  - Oxirane
  - Maleimide
  - Saccharide
  - Biotin
  - DNA
  - Bioorthogonal groups, click chemistry
- Biomolecule
  - Carboxyl -COOH
  - Amine -NH_2
  - Thiol -SH
  - Saccharide
  - DNA
  - Avidin/Streptavidin
  - Bioorthogonal groups, click chemistry



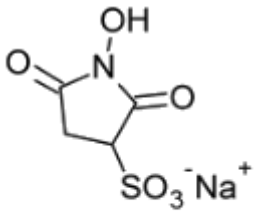
-COOH + -NH_2





N-(3-Dimethylaminopropyl)-N´-ethylcarbodiimide hydrochloride

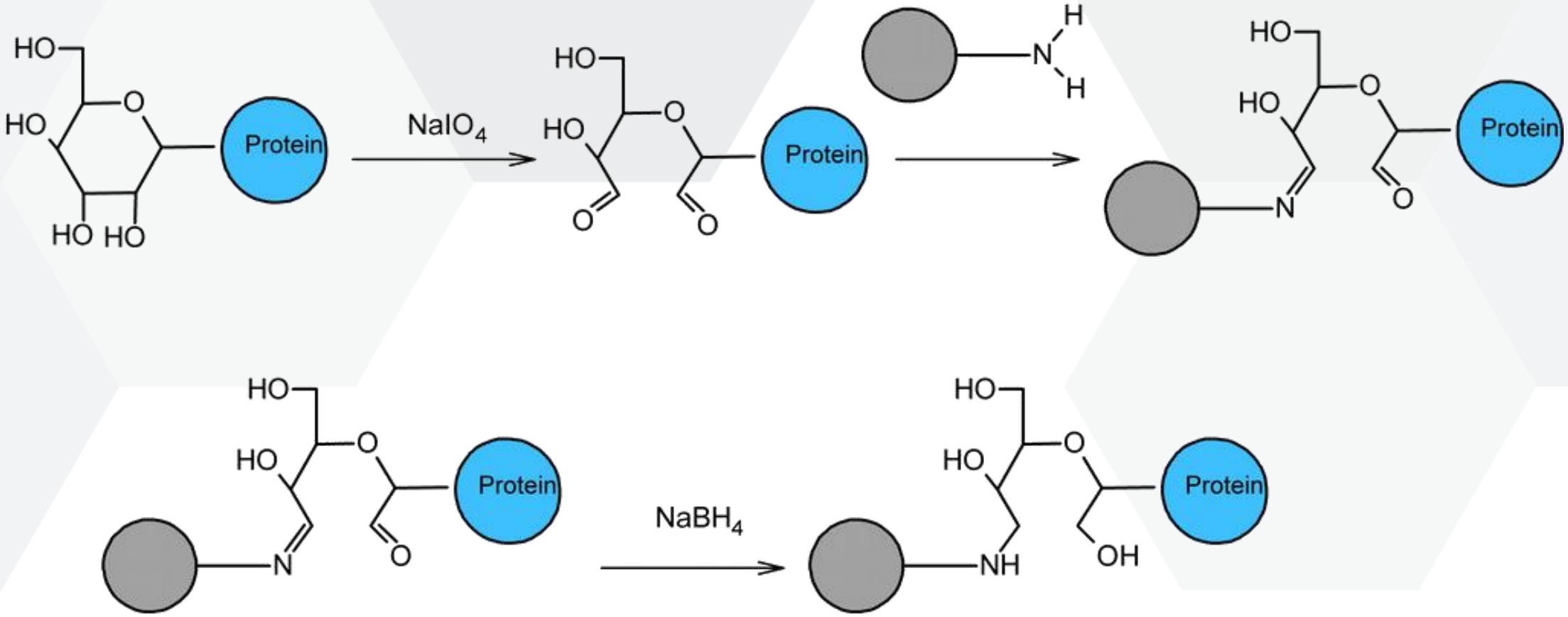
EDC



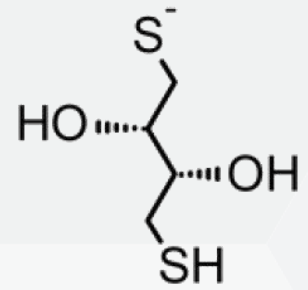
N-Hydroxysulfosuccinimide sodium salt

Sulfo-NHS

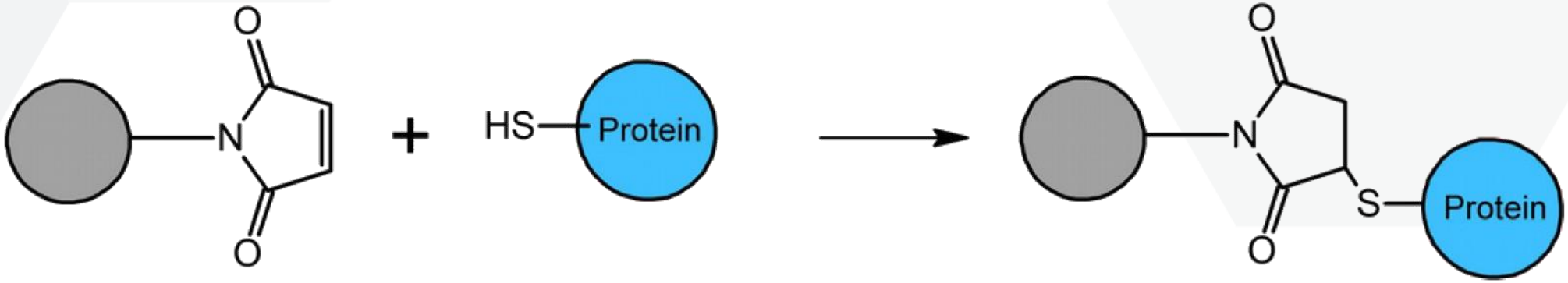
Glycated proteins + -NH_2



Maleimide group + -SH



Dithiothreitol
DTT



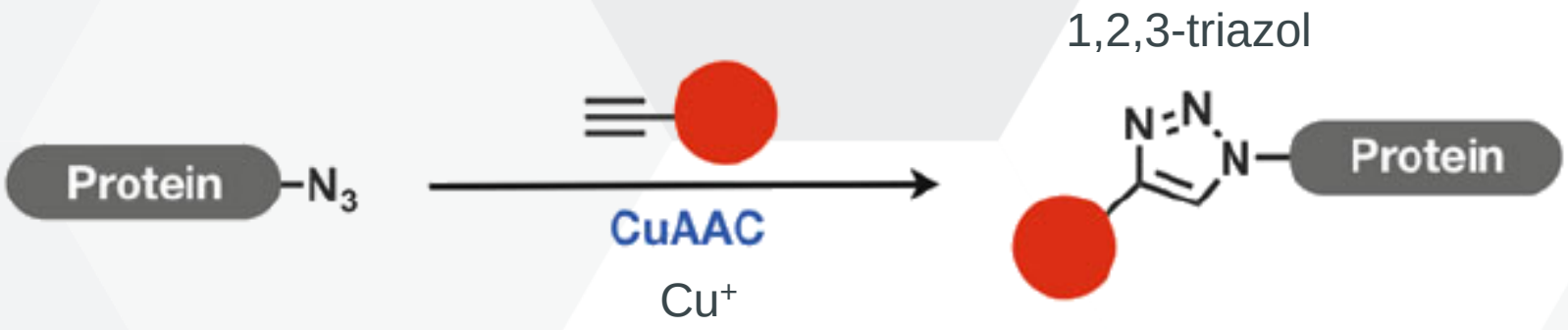
Click chemistry

- Bioorthogonal reaction
- Chemical reaction that can occur inside of living systems without interfering with native biochemical processes

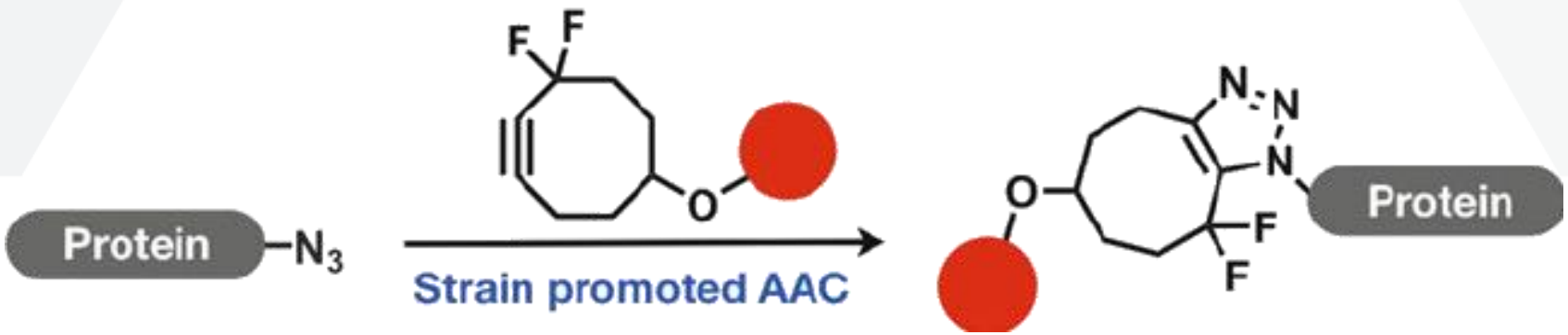
- Properties
  - High reaction specificity
  - High thermodynamic driving force that drives it quickly and irreversibly
  - High yield of a single reaction product

Azide-alkyne [3 + 2] cycloaddition

- Copper catalyzed cycloaddition

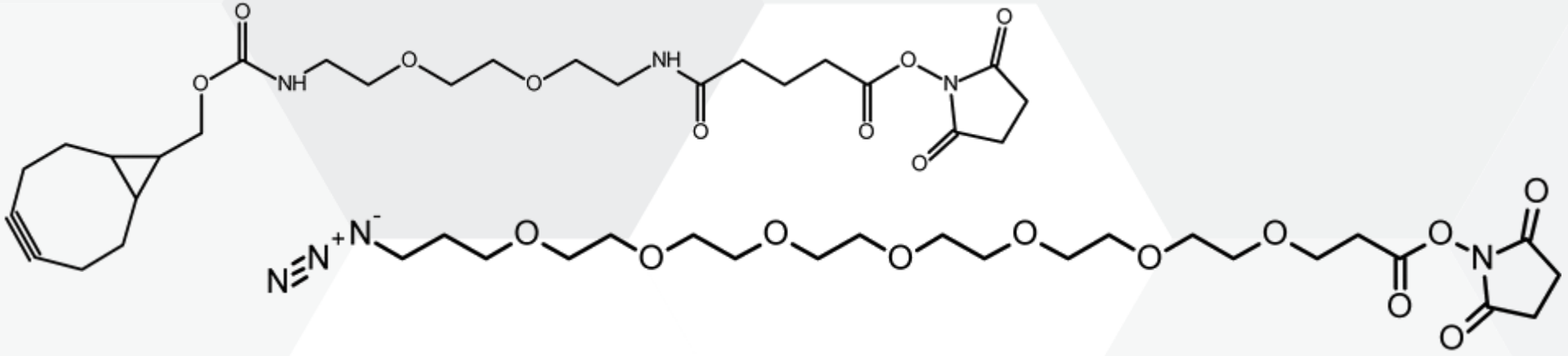


- Strain promoted cycloaddition

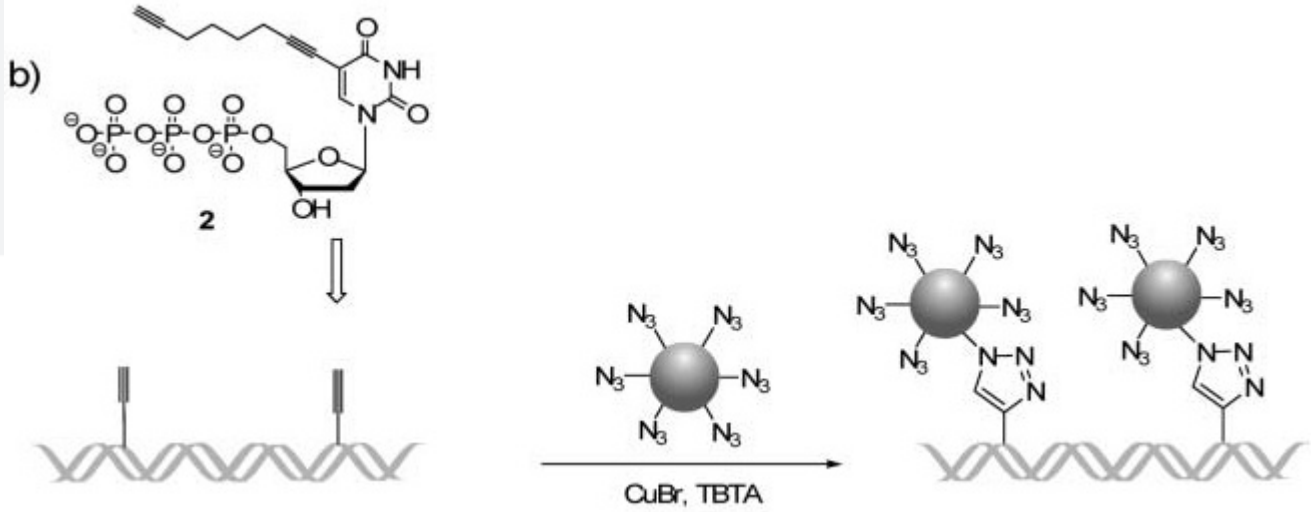


Azide-alkyne [3 + 2] cycloaddition

- NHS-esters

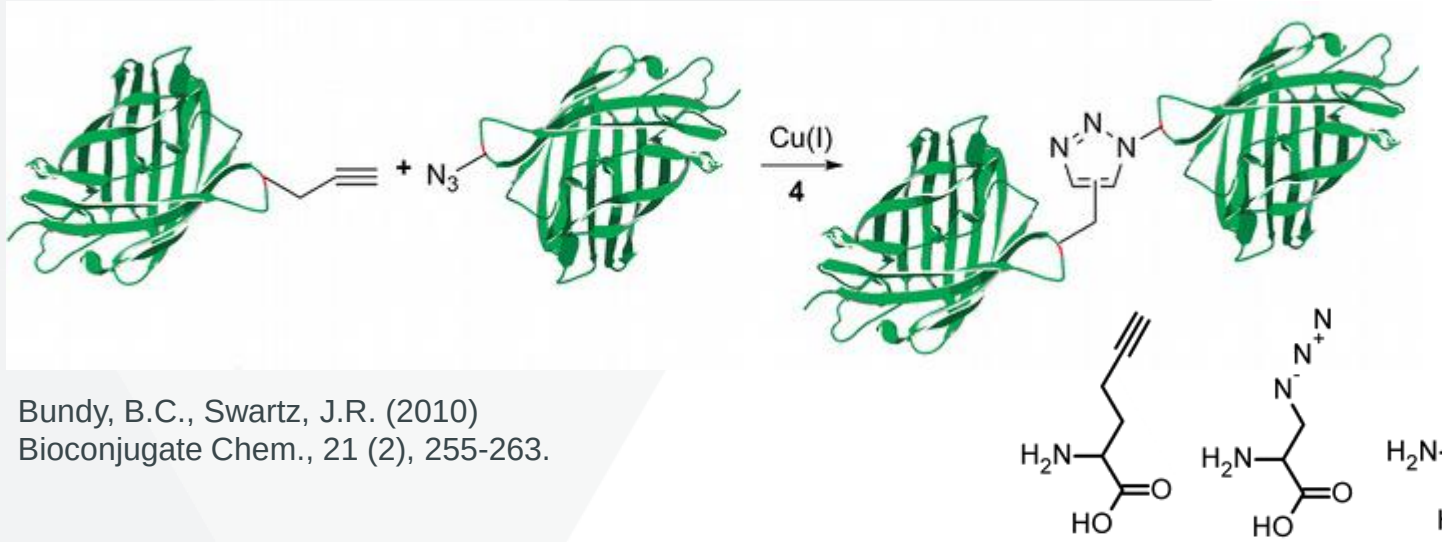


- Biomolecule building blocks derivatives – DNA



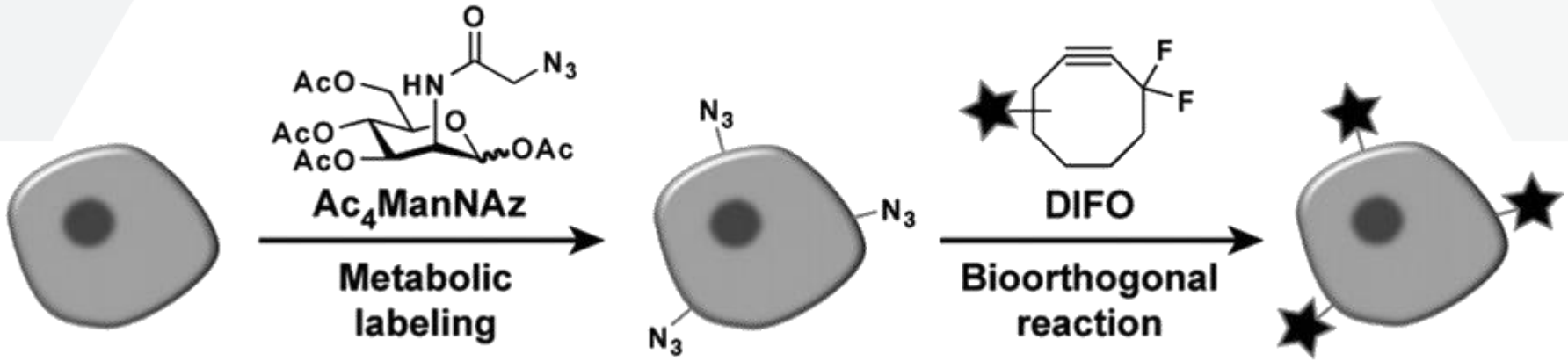
Azide-alkyne [3 + 2] cycloaddition

- Biomolecule building blocks derivatives – Proteins



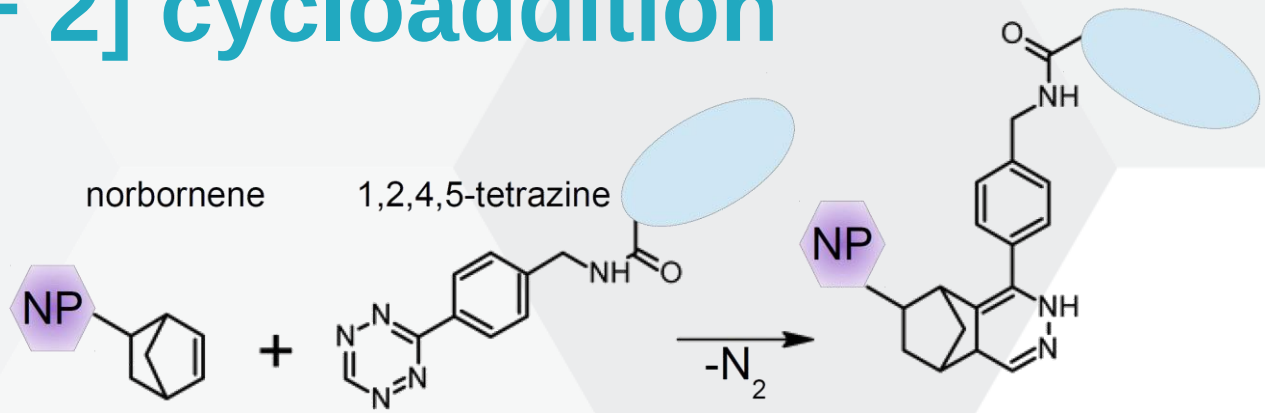
Bundy, B.C., Swartz, J.R. (2010)
Bioconjugate Chem., 21 (2), 255-263.

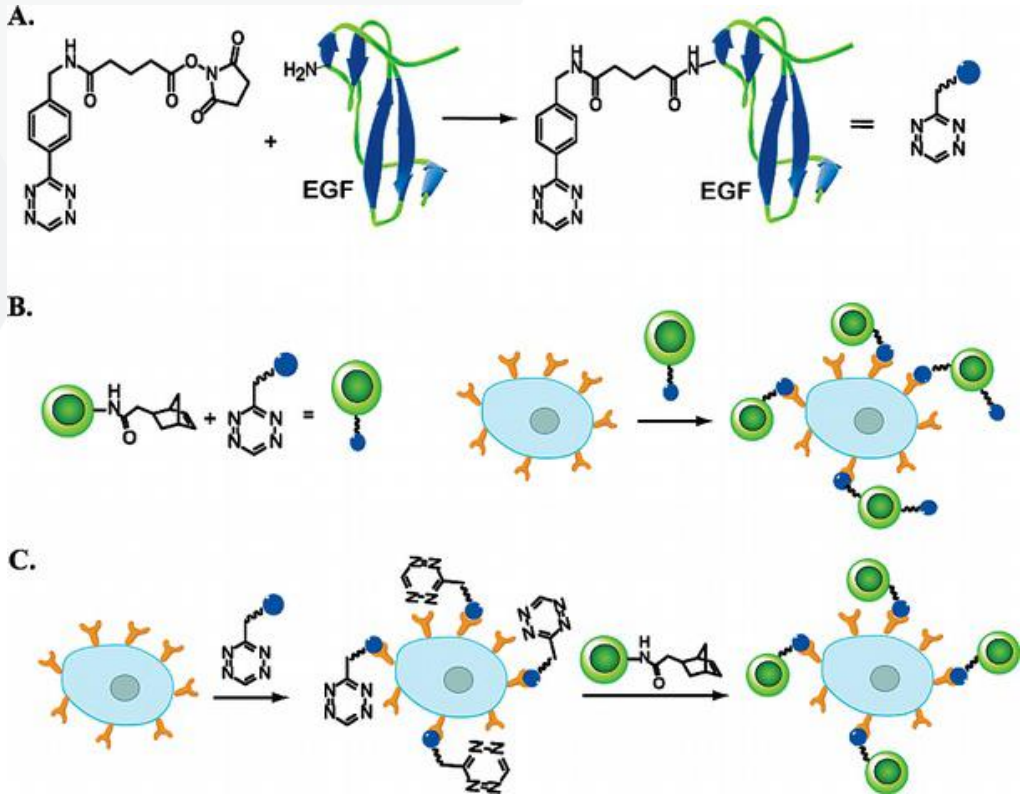
- Biomolecule building blocks derivatives – Carbohydrates

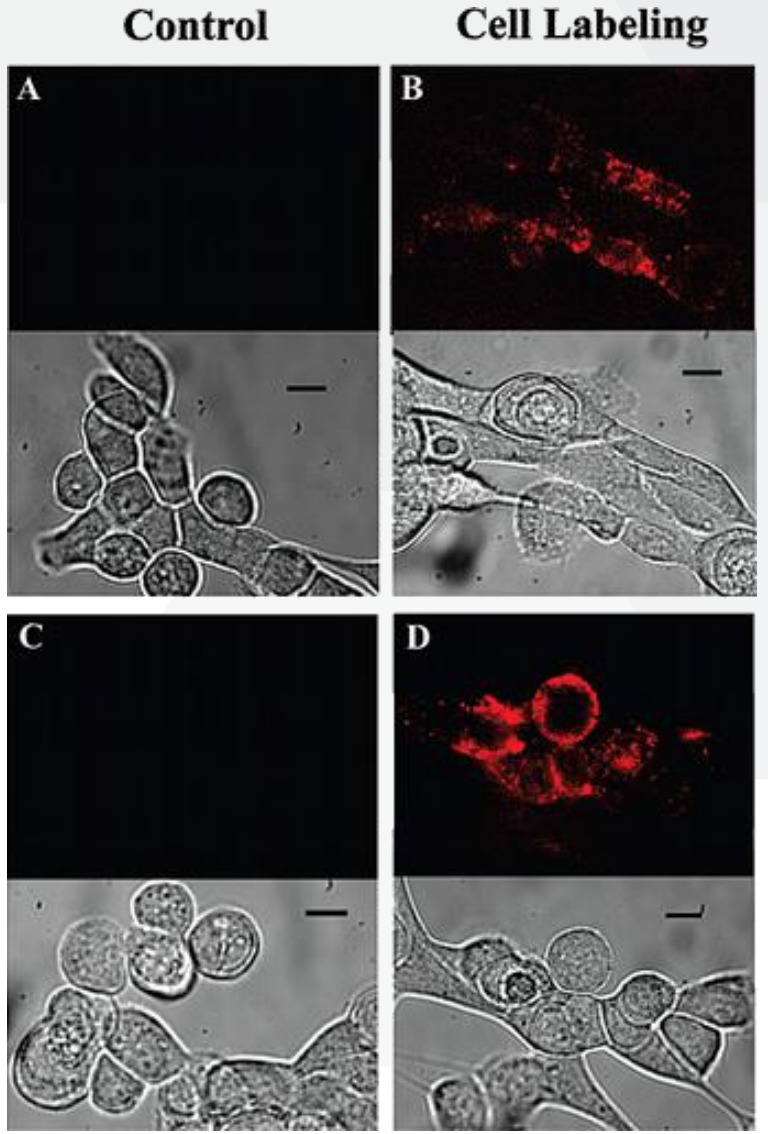


Baskin, J.M., Prescher, J.A., Laughlin, S.T., Agard, N.J., Chang, P.V., Miller, I.A., Lo, A., Codelli, J.A., Bertozzi, C.R. (2007) Proc. Nat. Acad. Sci. U. S. A., 104 (43), 16793-16797.

[4 + 2] cycloaddition



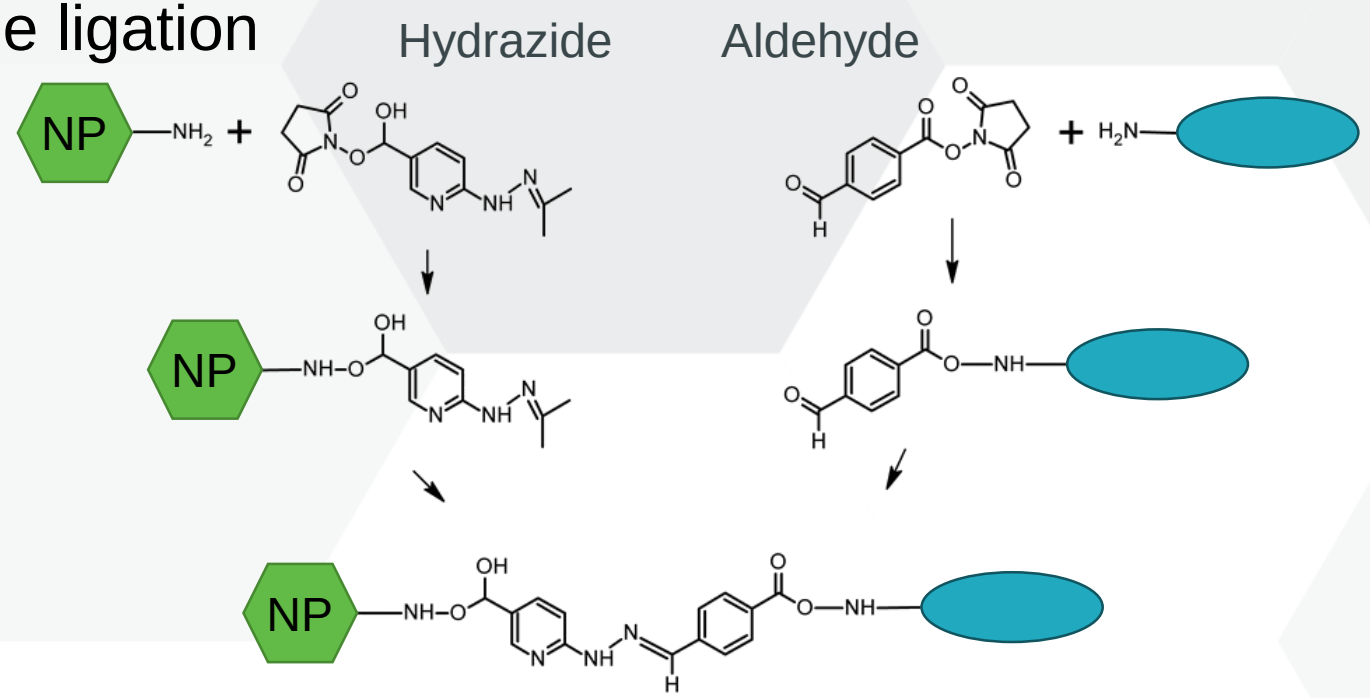




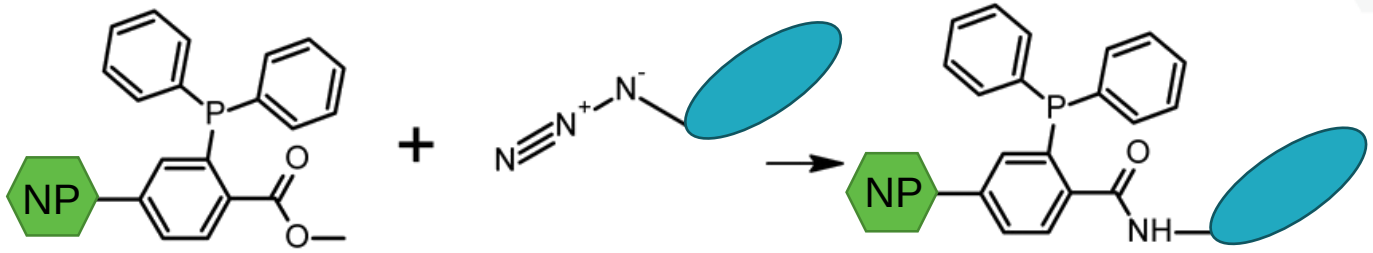
Han, H.-S., Devaraj, N.K., Lee, J., Hilderbrand, S.A., Weissleder, R., Bawendi, M.G. (2010) J. Am. Chem. Soc., 132 (23), 7838-7839.

Other reactions

- Hydrazone ligation



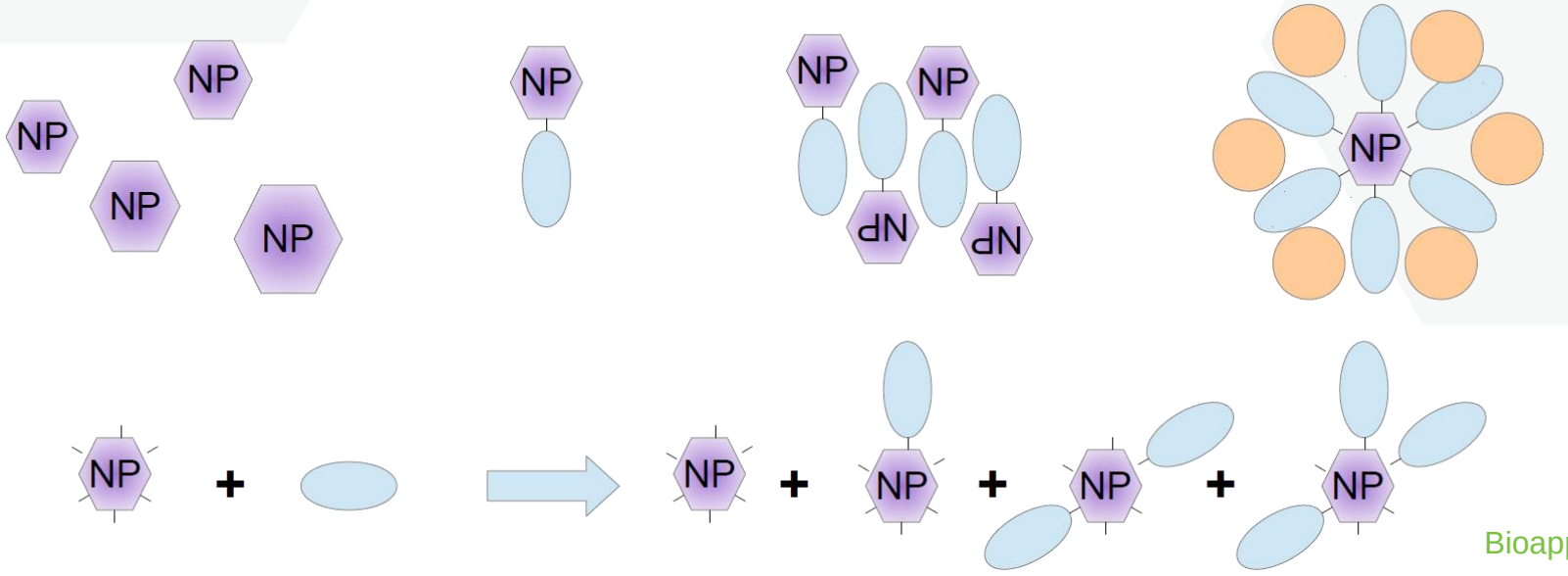
- Staudinger ligation



Characterization of nanoparticles

Nanobioconjugate characterization

- Separation techniques
  - Chromatography
  - Electrophoresis
  - Field flow fractionation
  - Centrifugation
- Other techniques
  - Microscopy
  - Spectroscopy
  - DLS, FCS, single particle tracking
  - Chemical methods
  - Thermogravimetric analysis
  - Simulation, modelling

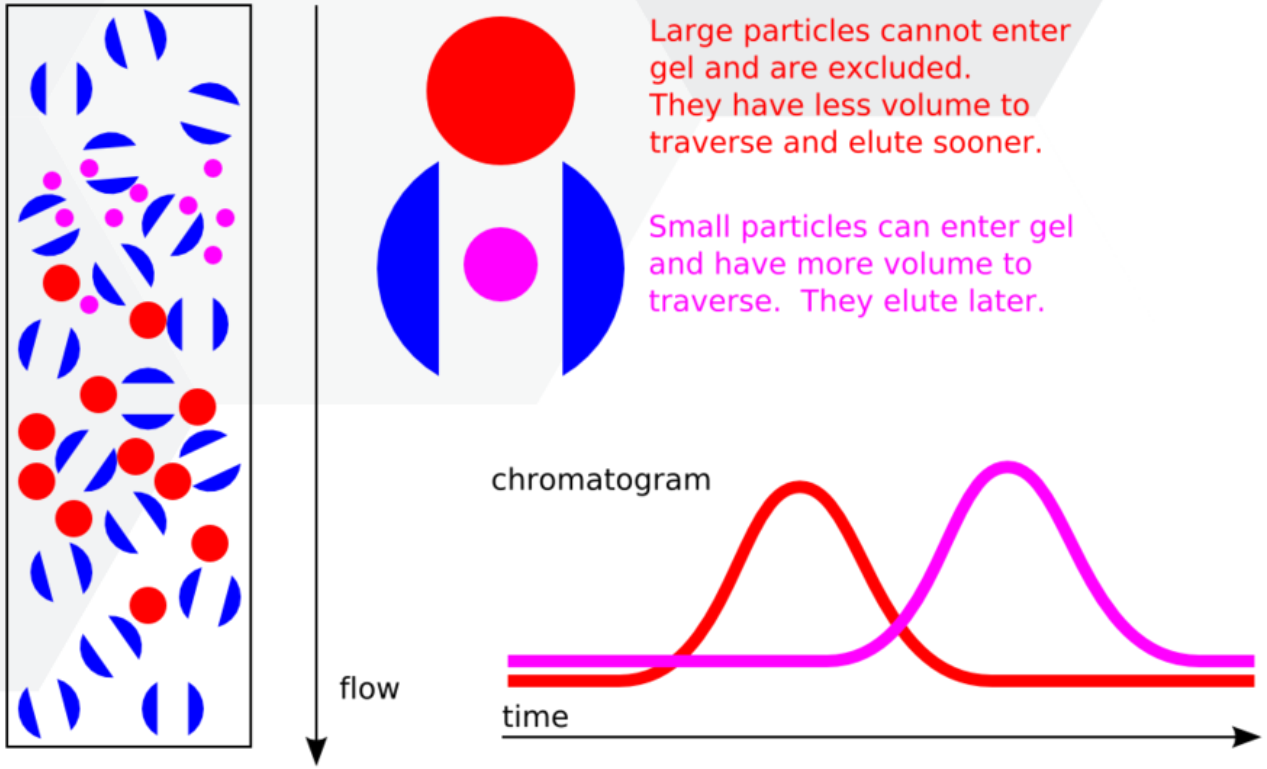


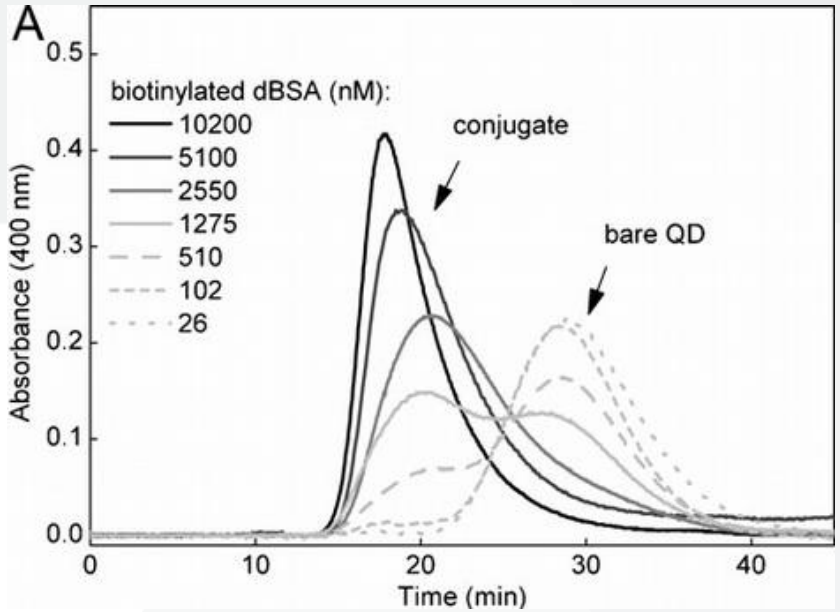
Separation techniques

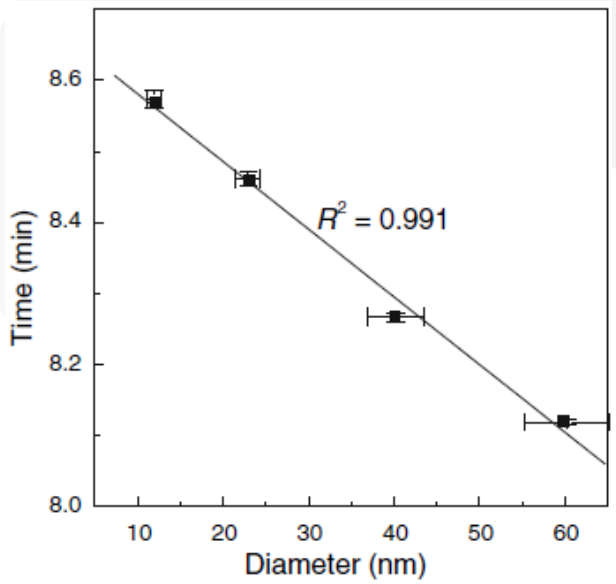
- Separation of sample into fractions followed by characterization using a suitable detector (UV / VIS, DLS, TEM, FT-IR, ...)
- Another possibility is the direct characterization (e.g. determining the size, density, electrical charge of nanoparticles in the individual fractions)
- Usually characterize the size, electric charge, polydispersity, degree of modification, presence of aggregates, specific / non-specific interactions with other nanoparticles and biomolecules
- Methods:
  - Chromatography
  - Electrophoresis
  - Field flow fractionation
  - Centrifugation

Size-exclusion chromatography

- Separation based on size



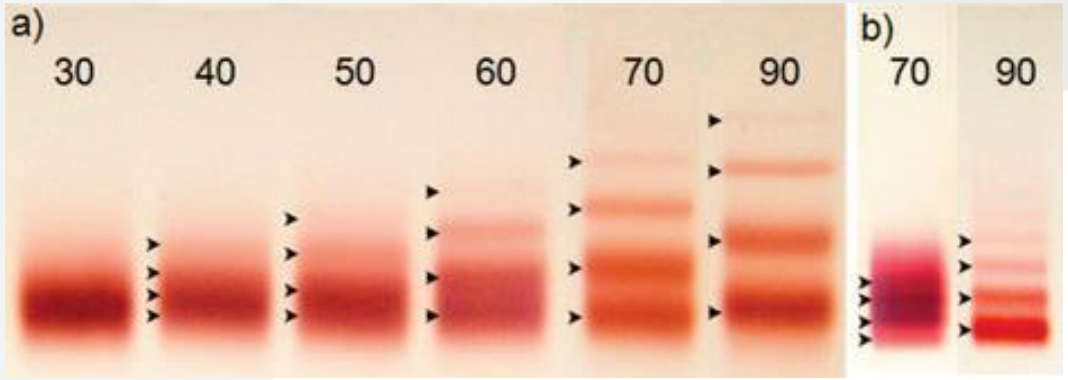




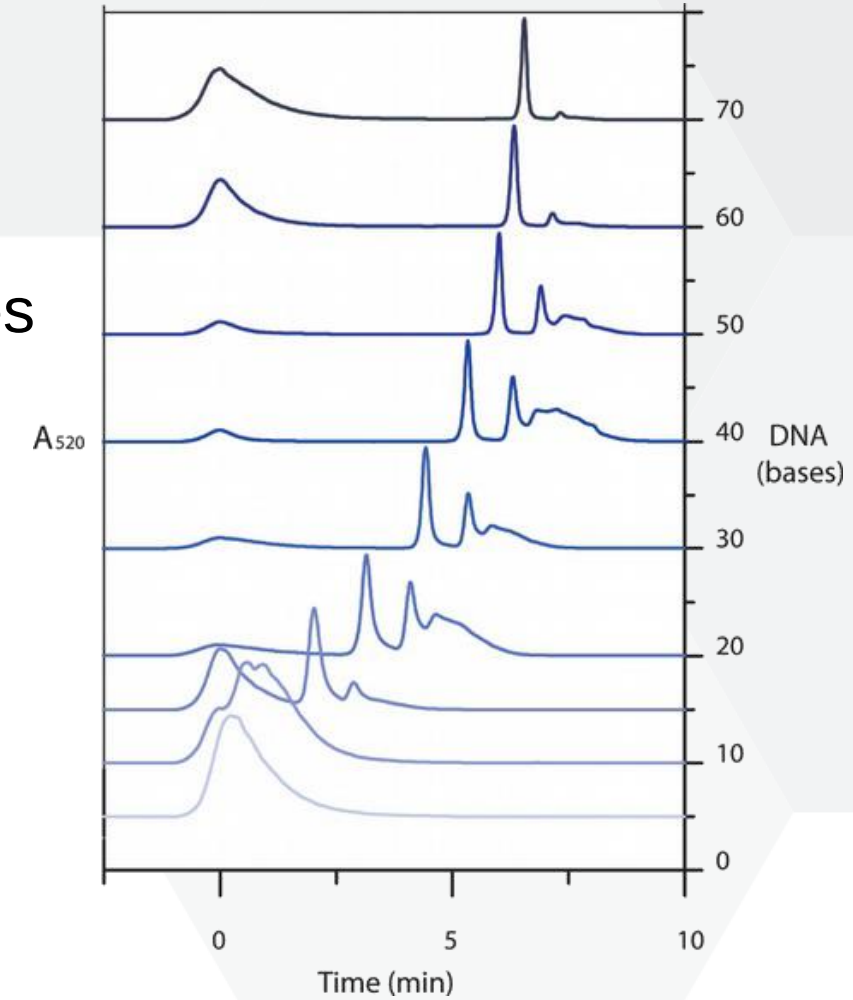
Hlaváček, A., Bouchal, P., Skládal, P. (2012) Microchim. Acta, 176 (3-4), 287-293.

Ion-exchange chromatography

- Electrostatic interactions
- E.g. separation of DNA-modified gold nanoparticles



Electrophoretic analysis of gold conjugated to thiolated DNA of varying lengths.
(a) 5 nm gold particles are conjugated to polyT DNA 30–90 bases in length. Black arrowheads indicate visible bands as a guide to the eye.
(b) 20 nm gold particles conjugated to 70- and 90-base polyT DNA.

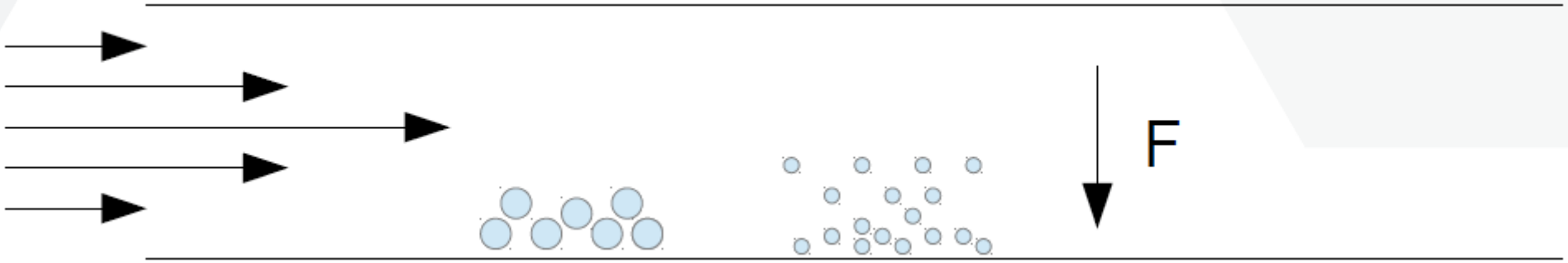


Elution profiles of varying lengths of polyT DNA conjugated to 20 nm AuNP. Leftmost peak is unconjugated gold. Monoconjugate peak migrates to longer retention times as DNA length is increased, with near-baseline separation achieved for monoconjugates of 15-base DNA.

Claridge, S.A., Liang, H.W., Basu, S.R., Fréchet, J.M.J., Alivisatos, A.P. (2008) Nano Lett., 8 (4) 1202-1206.

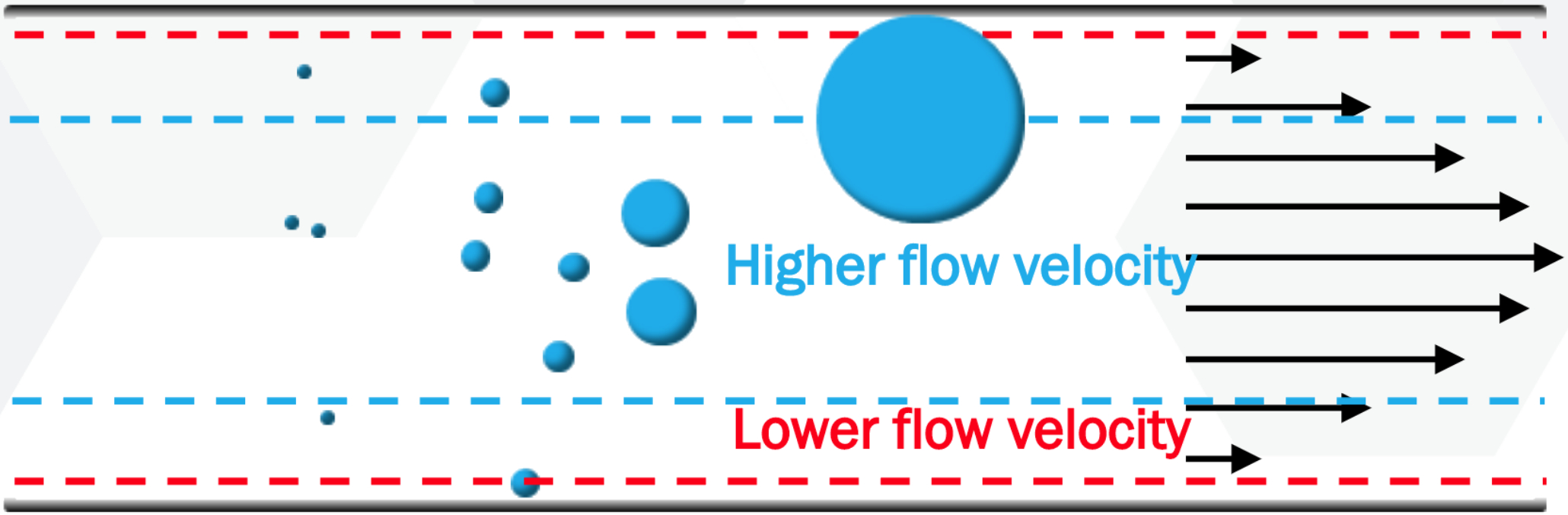
Field flow fractionation

- Separation in narrow channel
- Force is applied perpendicular to the flow direction
- Equilibration occurs between the diffusive movement of particles and particle migration induced by the force
- Flow velocity decreases towards the walls of the channel → particle separation; particles closer to the wall move slowly
- Different types of the acting forces, e.g. electric, magnetic, centrifugal (sedimentation) provides separation according to different properties of the nanoparticles



Hydrodynamic chromatography

- Separation in FFF can take place over a wide range of sizes, but from a certain threshold, the separation can switch to hydrodynamic chromatography

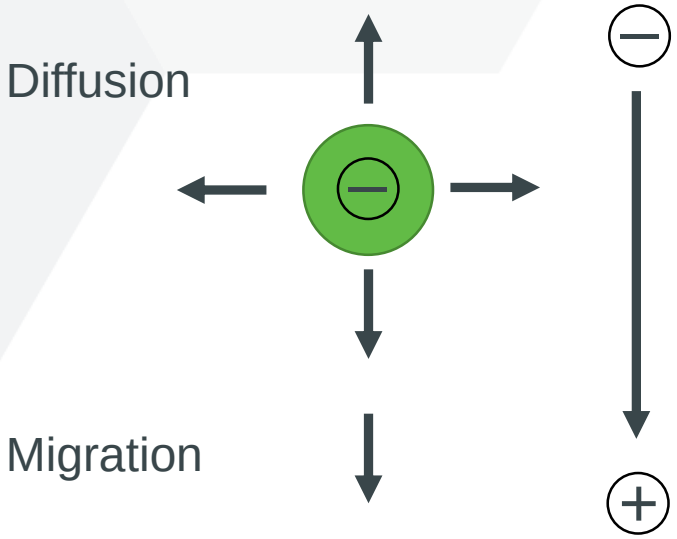


Electrophoresis

- Motion of nanoparticles in electric field
- Separation depends on nanoparticle size and charge

$$v = \frac{E\,q}{f} \qquad \mu = \frac{v}{E} \qquad \mu = \frac{Q\,D}{k\,T}$$

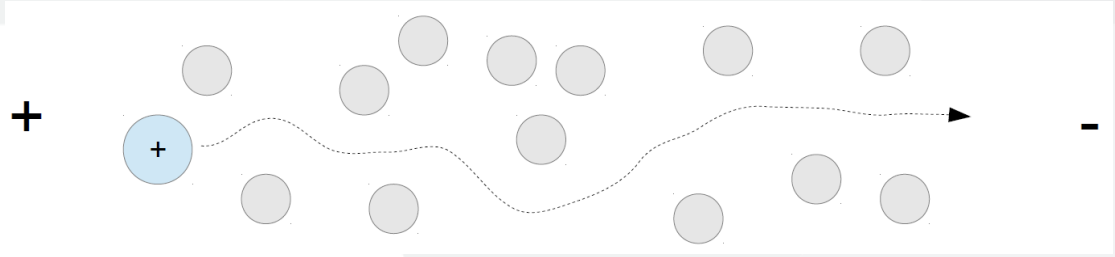
v...velocity
E...electric intensity
q...electric charge
f...frictional coefficient
μ...electrophoretic velocity
D... diffusion coefficient
k... Boltzman constant
T...thermodynamic temperature

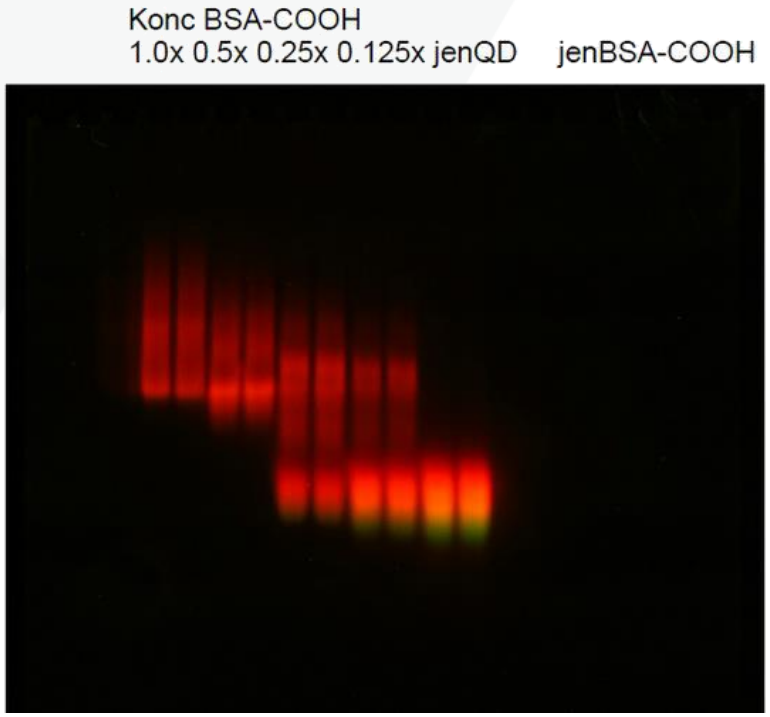


$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} - \mu E\frac{\partial c}{\partial x}$$

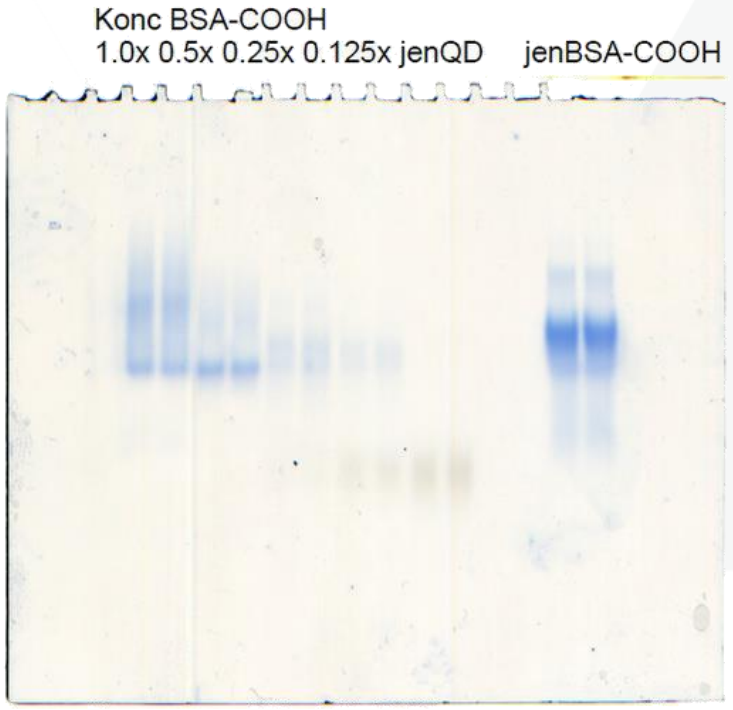
Gel electrophoresis

- Separation depends on the nanoparticle size
- Electrophoretic mobility shift assay
  - E.g. attachment of proteins on the surface of nanoparticles





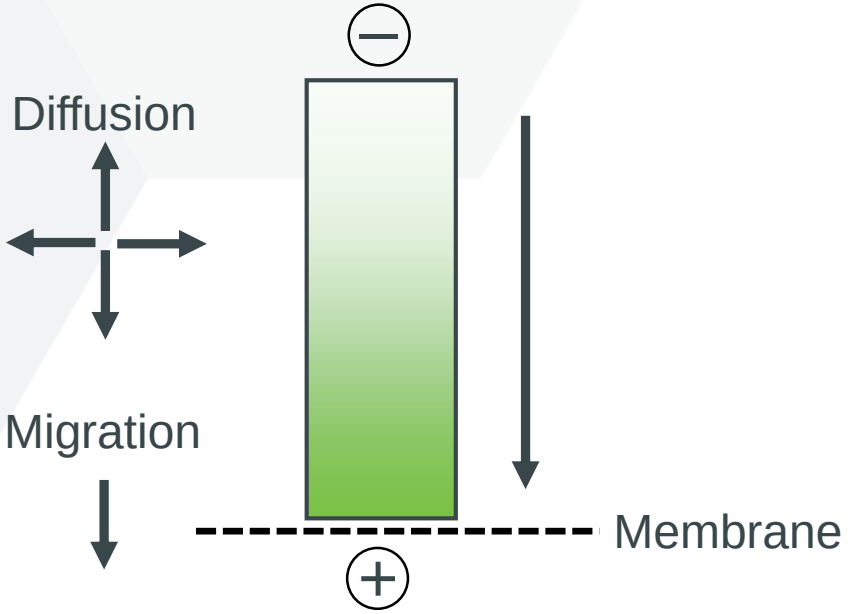
Fluorescence detection



Coomassie blue

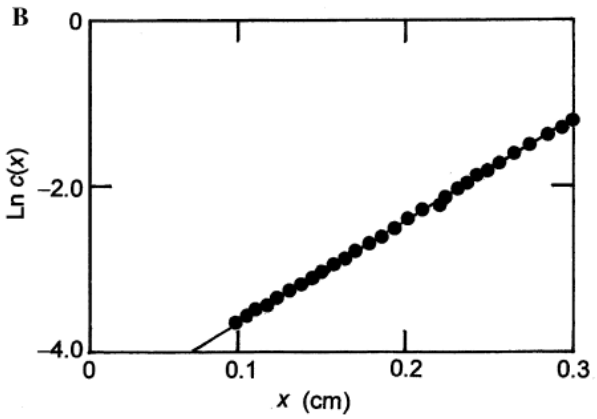
Equilibrium electrophoresis

- Sample is placed over the semipermeable membrane
- Equilibrium between the diffusion motion of nanoparticles and their electrophoretic migration is established after the application of an electrical field
- The surface charge of nanoparticles can be determined from the analysis of concentration gradient above the membrane.



$$\frac{d\ln(c(x))}{dx} = \varphi E / kT$$

c(x)...concentration in a position x
E...intensity of electric field
k...Boltzmann constant
T...thermodynamic temperature
φ ...electric charge of nanoparticle



Determination of protein valence using equilibrium electrophoresis. Horse-heart ferricytochrome c in 0.01 M acetate, pH 4.7. The charge was estimated to be 11.9×10^{-19} C.

Winzor, D.J. (2004) Anal. Biochem., 325 (1), 1-20.

Analytical ultracentrifugation

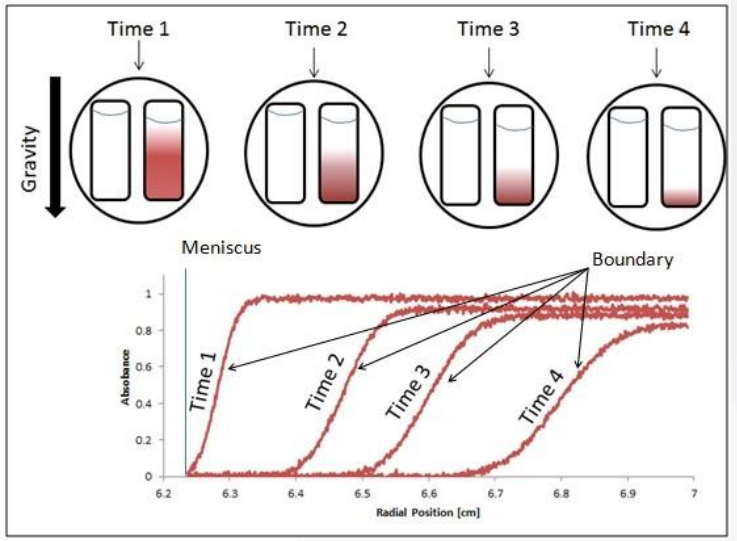
- Particle sedimentation in a centrifuge tube is observed
- Information about the size and weight of the particles

- Two approaches
  - Method of sedimentation equilibrium
  - Method of sedimentation velocity

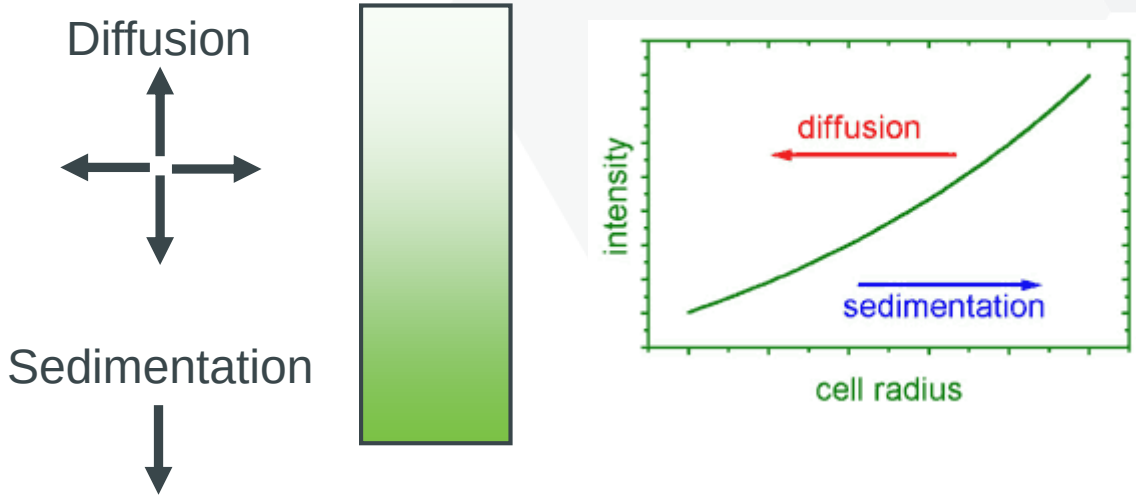
- Detection
  - Absorbance (190–650 nm)
  - Interference (different refractive indexes in cuvettes with pure solvent and the sample)
  - Fluorescence (analysis of very dilute samples 10^{-10} M)

Analytical ultracentrifugation

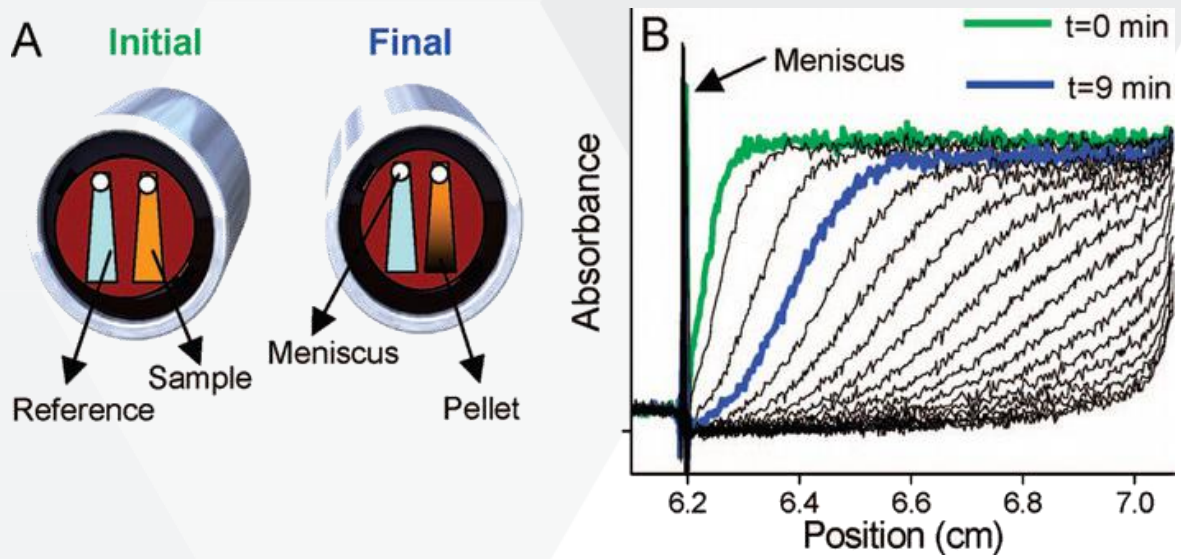
- Method of sedimentation velocity
  - Measurement of entire time-course of sedimentation
  - **Shape** and **molar mass** of the nanoparticles, as well as their **size-distribution**
  - High centrifugal fields



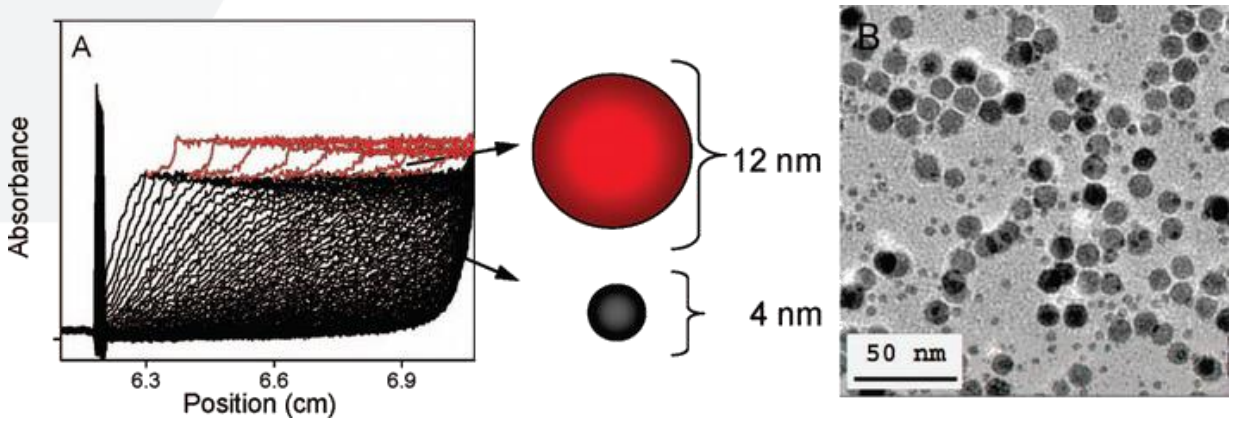
- Method of sedimentation equilibrium
  - Final steady-state of the experiment
  - Sedimentation is balanced by diffusion opposing the concentration gradients
  - **Molar mass** of the nanoparticles
  - Low centrifugal fields (hundreds and thousands of G)
  - Long equilibration (days)



Analytical ultracentrifugation
Method of sedimentation velocity



Overview of the analytical ultracentrifugation process
(A) Prior to sedimentation, the sample is uniformly dispersed in the cell. Once the centrifugation process starts, nanocrystals will sediment and ultimately form a pellet in bottom of the cell.
(B) The instrument scans the absorption spectrum of the entire cell from top to bottom during the process; this results in raw data which display at different time intervals the optical density of the sample along the length of the cell.

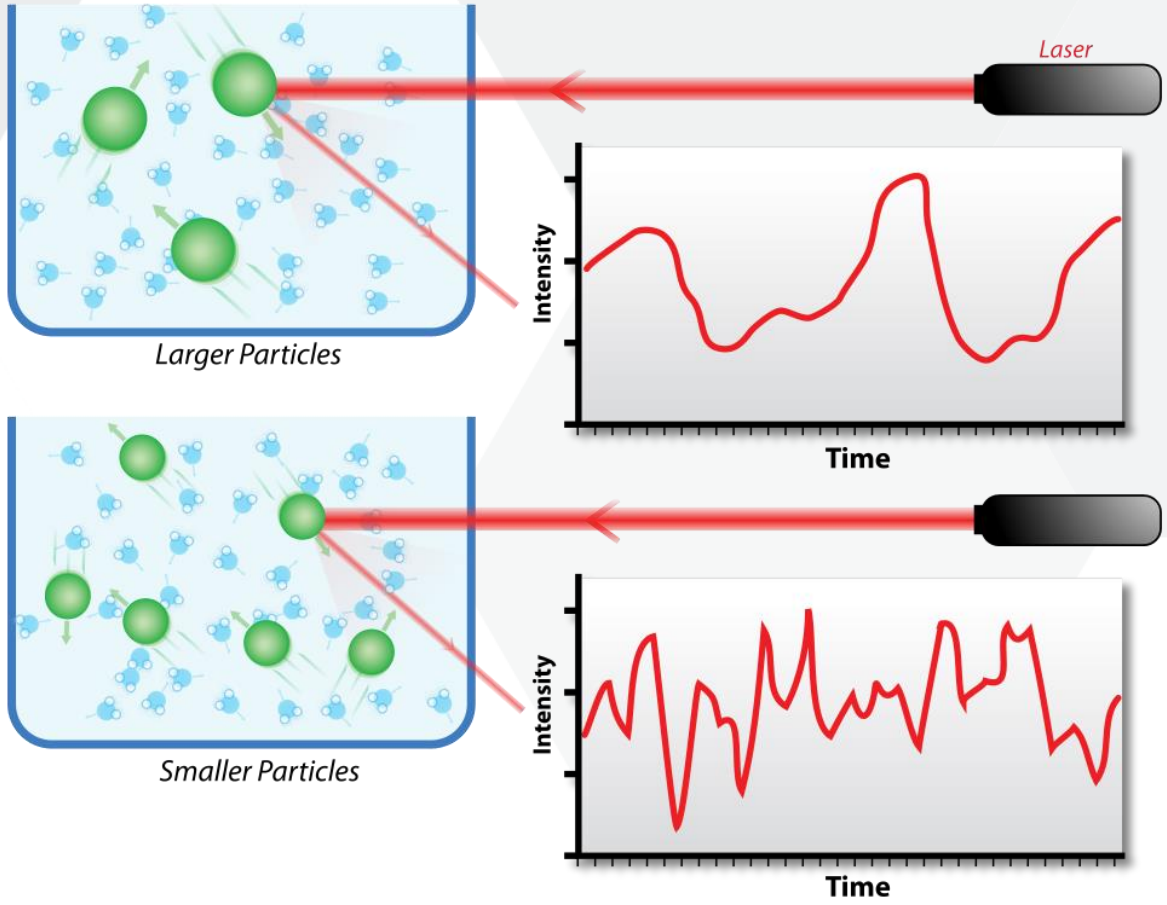


Bimodal magnetite nanocrystals sample run in hexanes
(A) The raw data (speed optimized for smaller nanocrystals) show two boundary regions, which is indicative of at least two species.
(B) TEM image of the sample.

Jamison, J.A., Krueger, K.M., Yavuz, C.T., Mayo, J.T., LeCrone, D., Redden, J.J., Colvin, V.L. (2008) ACS Nano, 2 (2), 311-319.

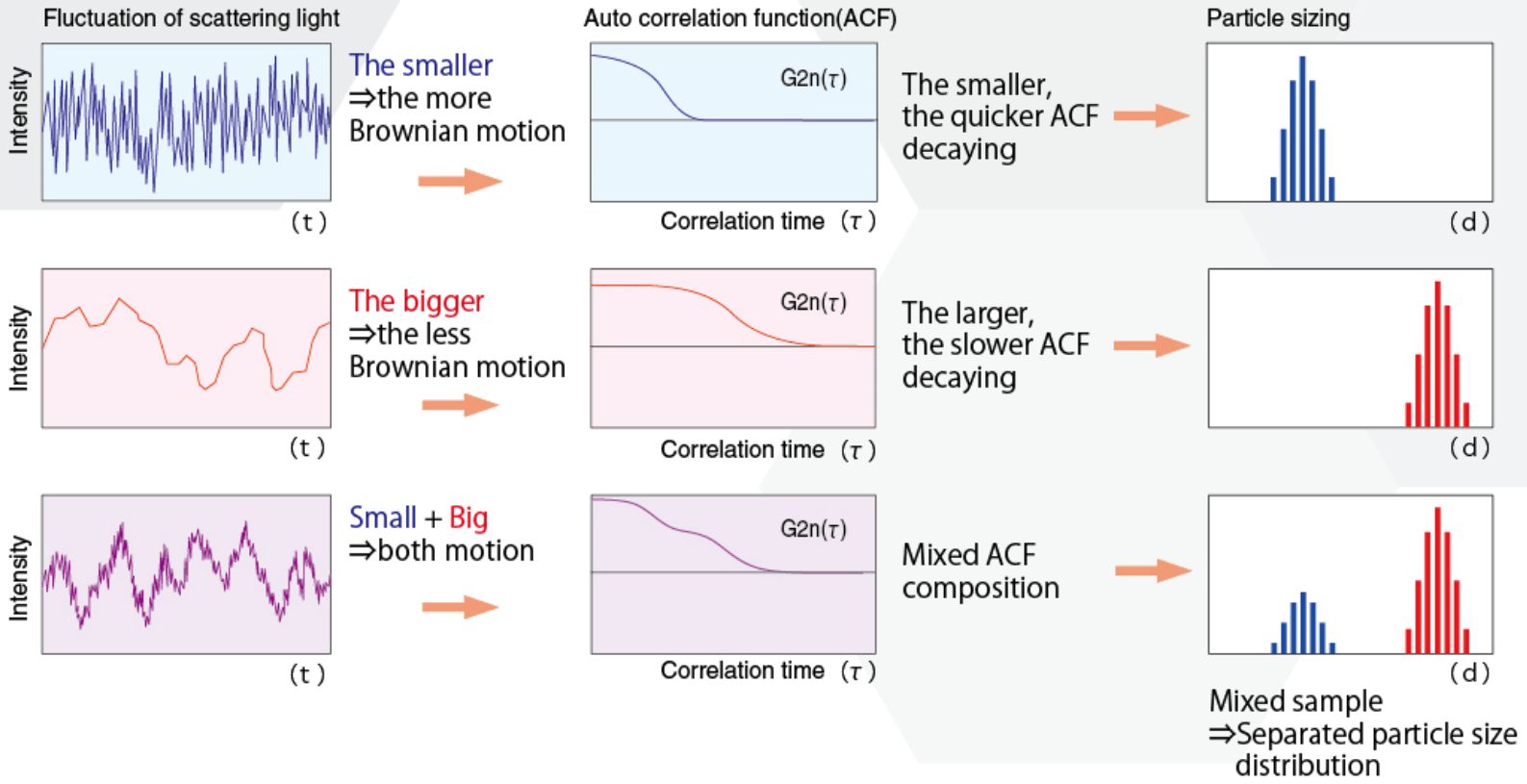
Dynamic light scattering

- One of the most common approaches for nanoparticle size determination
- Temporal fluctuations are analyzed by means of the intensity or photon auto-correlation function
- Autocorrelation function usually decays starting from zero delay time, and faster dynamics due to smaller particles lead to faster decorrelation of scattered intensity trace



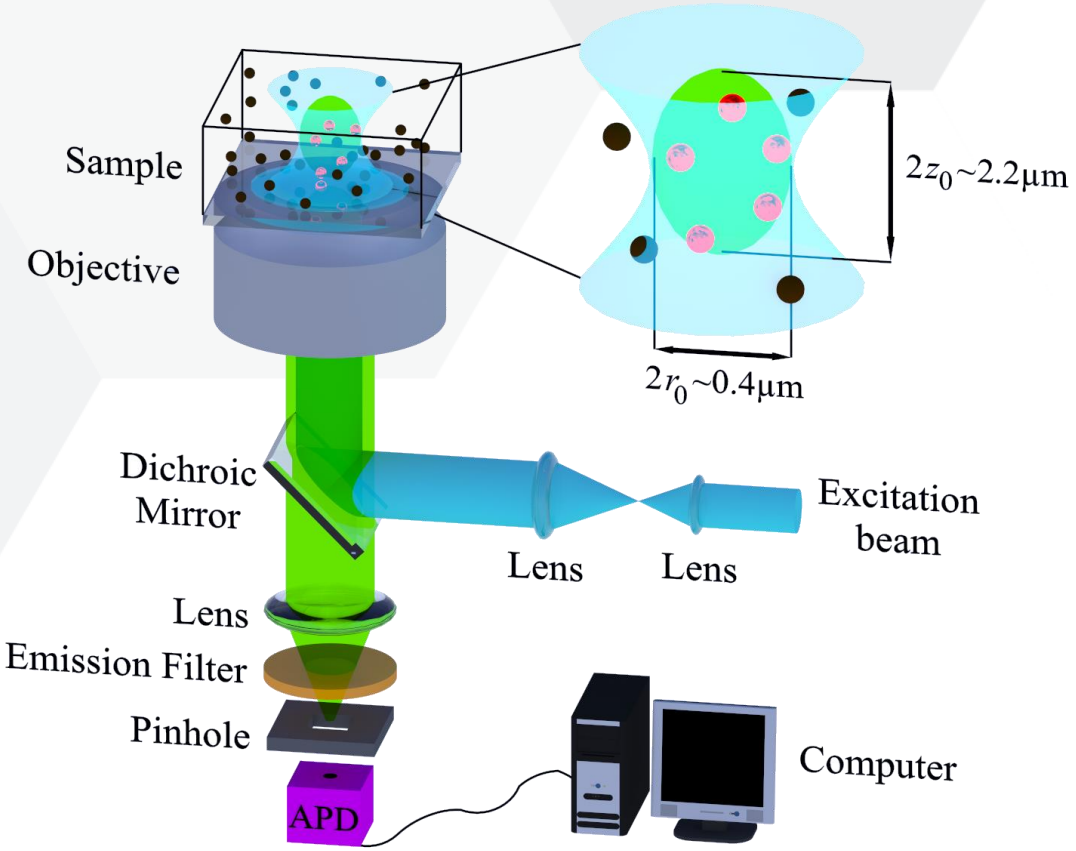
Dynamic light scattering

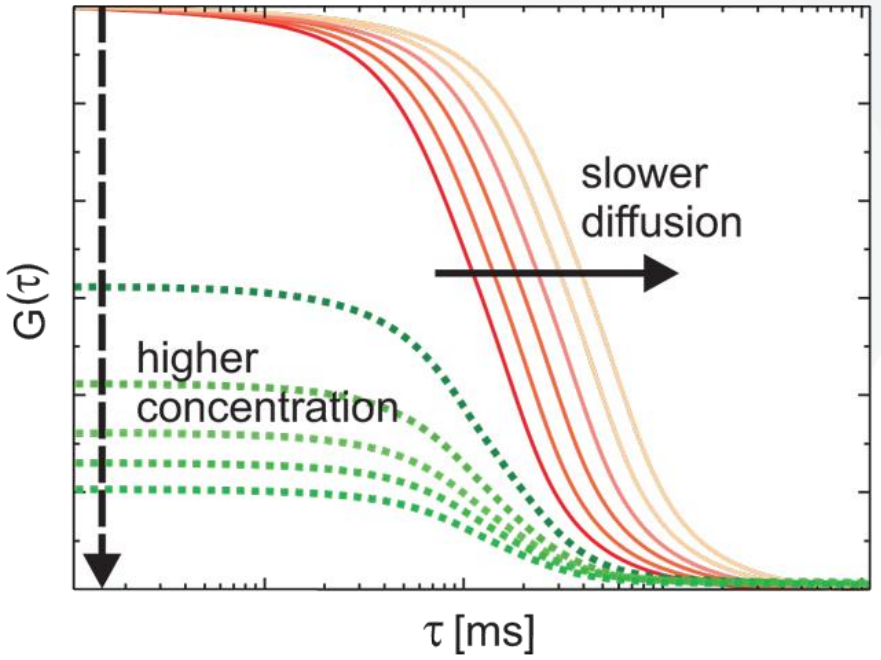
- 20 nm – up to several micrometers, CV < 20 %
- Intensity of the scattered radiation increases with R^6 (5 nm particle scatters 1,000,000 fewer photons than 50 nm particles)
- Aggregates disturb the measurement
- Small nanoparticles → measurements with a high concentration of nanoparticles



Fluorescence correlation spectroscopy

- Similar to DLS principle, fluorescence intensity fluctuations comes from small irradiated area of the typical volume of 1 fL containing 1–100 fluorescent molecules, which diffuse in and out of irradiated volume

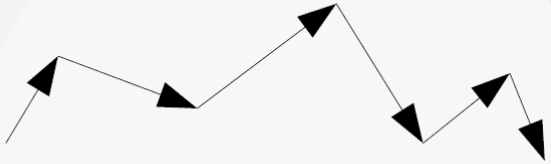




Single particle tracking

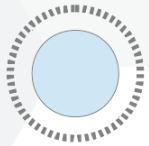
- Determination of diffusion coefficient of individual nanoparticles

Small nanoparticle

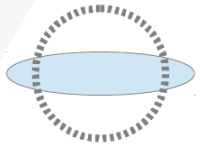


Large nanoparticle

Hydrodynamic radius...a



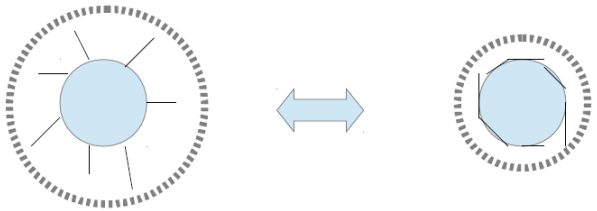
Electric double layer



nanorod

x,y; $\langle r^2 \rangle = 4\,D\,t$ x,y, z; $\langle r^2 \rangle = 6\,D\,t$

<average value>, **D...diffusion coefficient**, r...displacement in time...t

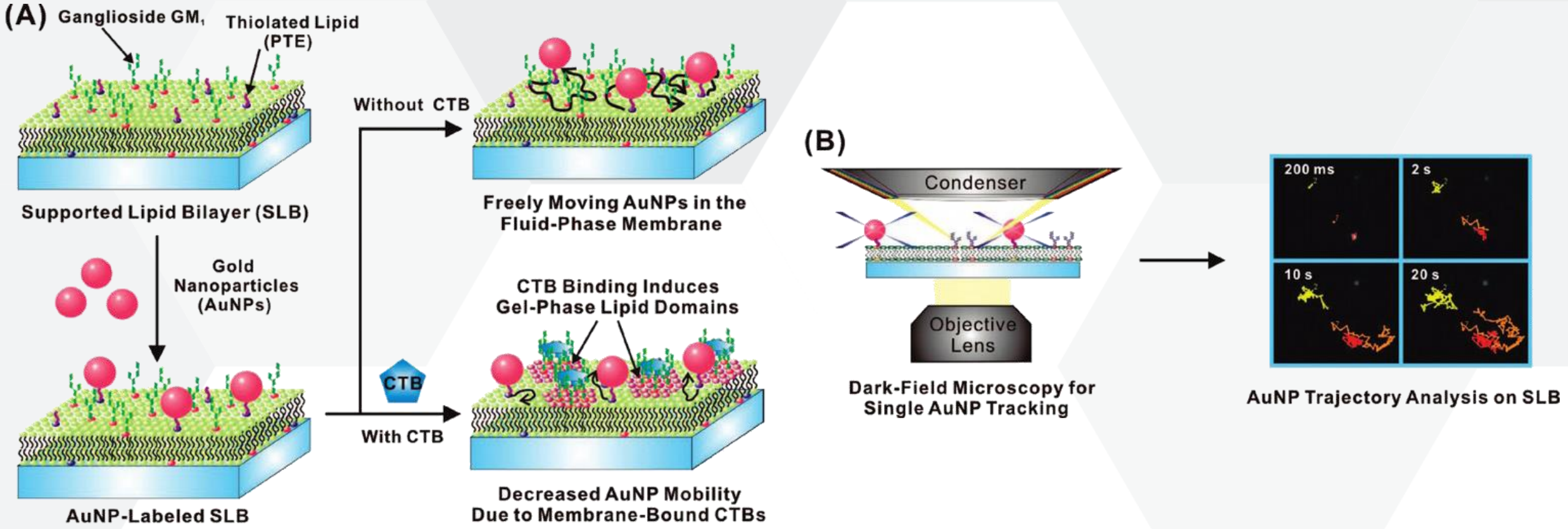


Polymeric chain conformation

$$D = \frac{k_B T}{6 \pi \eta a}$$

D...diffusion coefficient, k_B...Boltzmann constant, T...thermodynamic temperature, η...viscosity, **a...hydrodynamic radius**

Single particle tracking



Yang, Y.-H., Nam, J.-M. (2009) Anal. Chem., 81 (7), 2564-2568.

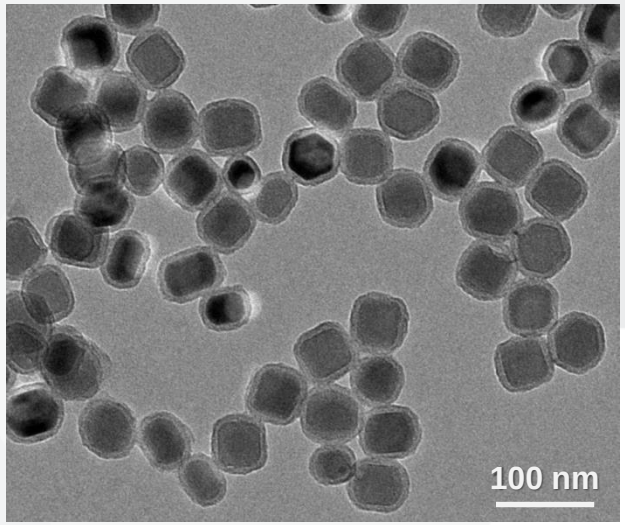
Electron microscopy

- Transmission electron microscopy
  - Electrons goes through the sample and after the impact on the detector produce an image of the sample
  - Biomolecules are typically observed after the staining by heavy atoms (e.g. uranium)
  - Higher resolution

- Scanning electron microscopy
  - Scanning of sample surface
  - Evaluation of composition
  - Lower resolution

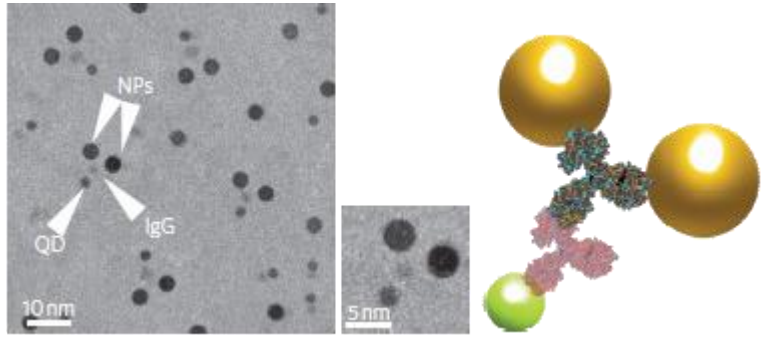
Transmission electron microscopy

Silica-coated upconversion NPs



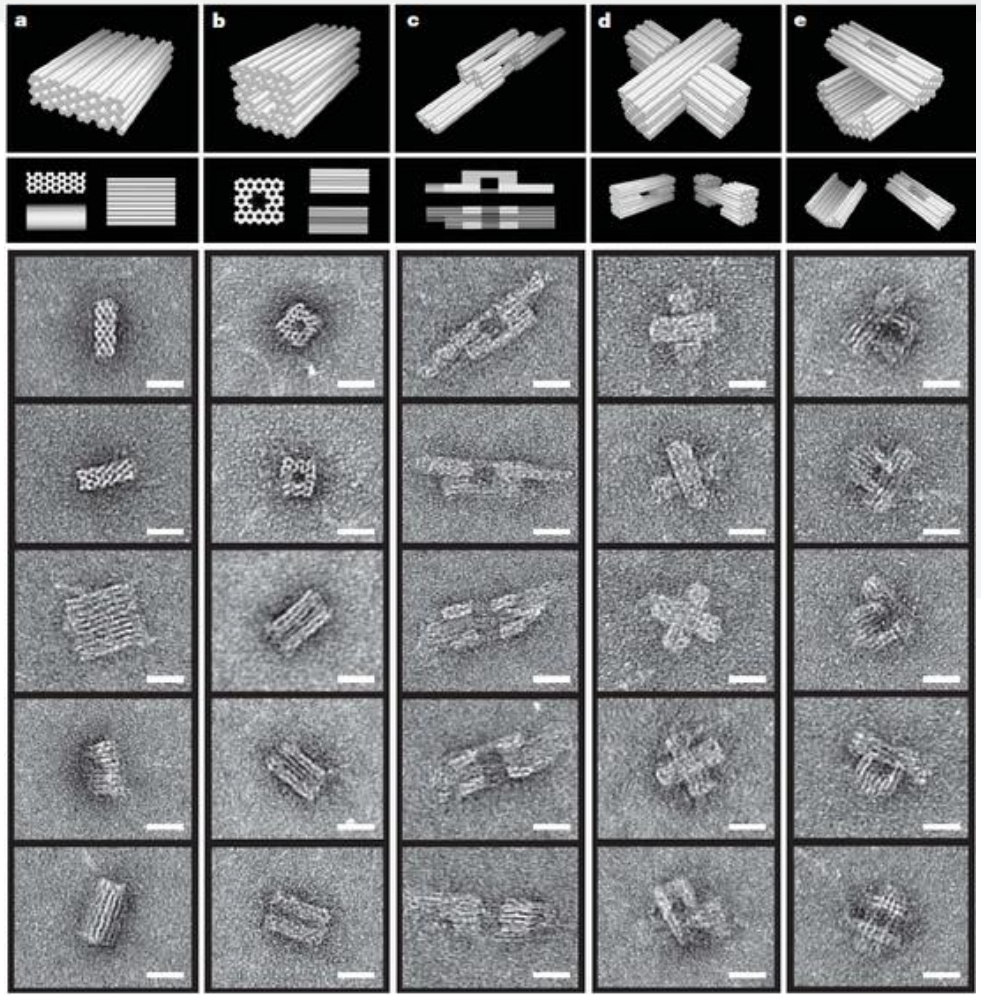
Hlaváček A., Farka Z., Hübner M., Horňáková V., Němeček D., Niessner R., Skládal P., Knopp D. Gorris H. H. (2016) Anal. Chem., 88 (11), 6011–6017.

Conjugate QD-Ab-Ab-GoldNPs



Chen, Y.-S., Hong, M.-Y., Huang, G.S. (2012) Nat. Nanotechnol., 7 (3), 197-203.

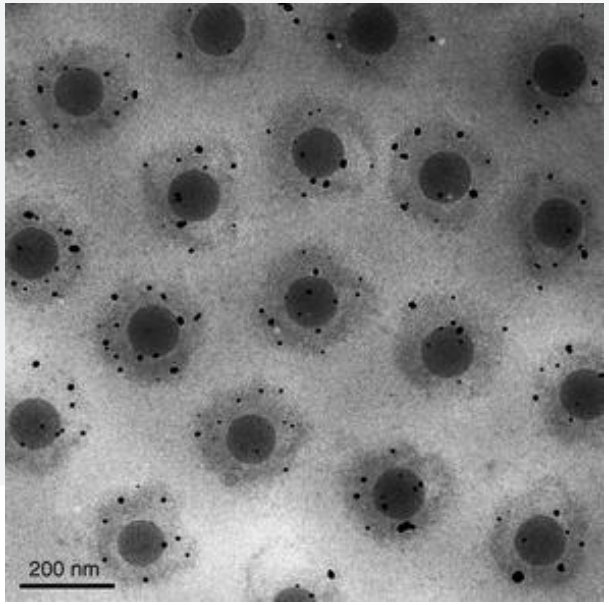
DNA nanoparticles, ~50 nm



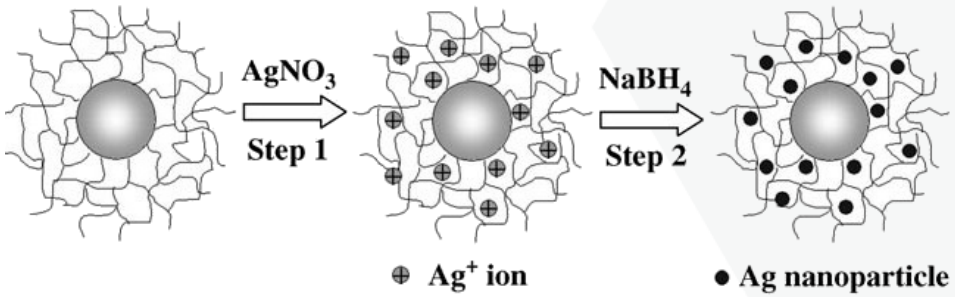
Douglas, S.M., Dietz, H., Liedl, T., Högberg, B., Graf, F., Shih, W.M. (2009) Nature, 459 (7245), 414-418.

Cryo-electron microscopy

- Developed as a method for structural biology to characterize protein complexes, virus particles, organelles and cell parts with about 1 nm resolution.
- The sample of interest dispersed in water is rapidly cooled to very low temperatures not forming crystals of water – vitrification



Thermosensitive network of poly(N-isopropylacrylamide) that surrounds a polystyrene core enables control over the catalytic activity of the NPs through a phase transition and leads to applications as a controllable nanoreactor.

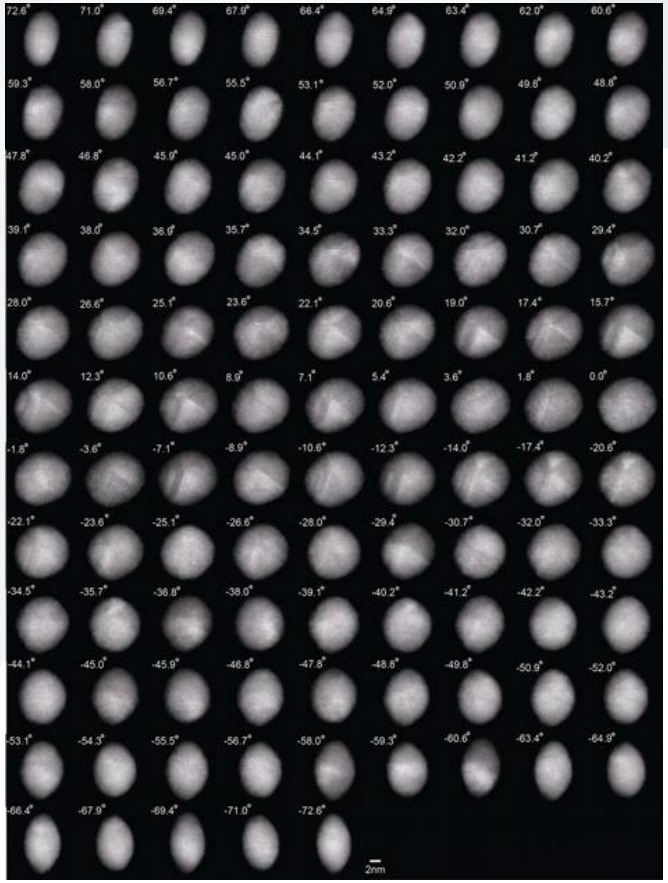


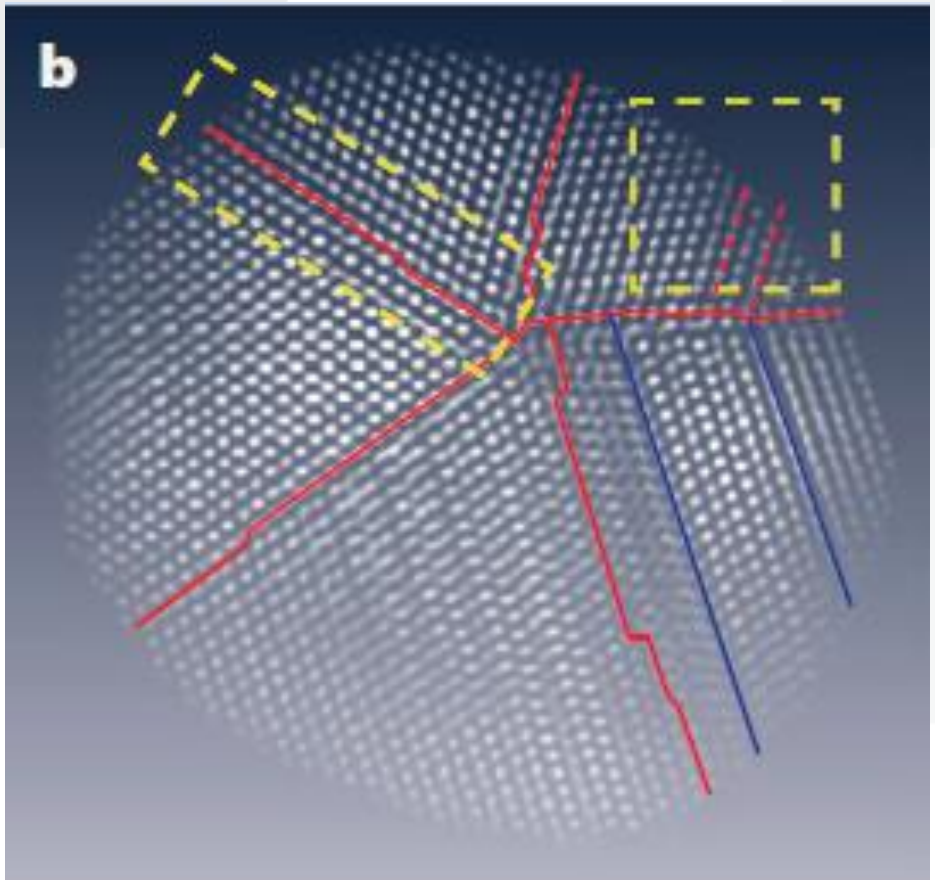
Formation of Ag NPs in the PS–NIPA core–shell system. The cross-linked PNIPA chains absorb Ag ions (step 1) which are reduced to produce Ag nanoparticles immobilized in the thermosensitive network (step 2).

Lu, Y., Mei, Y., Drechsler, M., Ballauff, M. (2006) Angew. Chem. Int. Ed., 45 (5), 813-816.

Electron tomography

- Sample placed in a microscope goniometer
- Specimen displayed at different angles creating 3D models

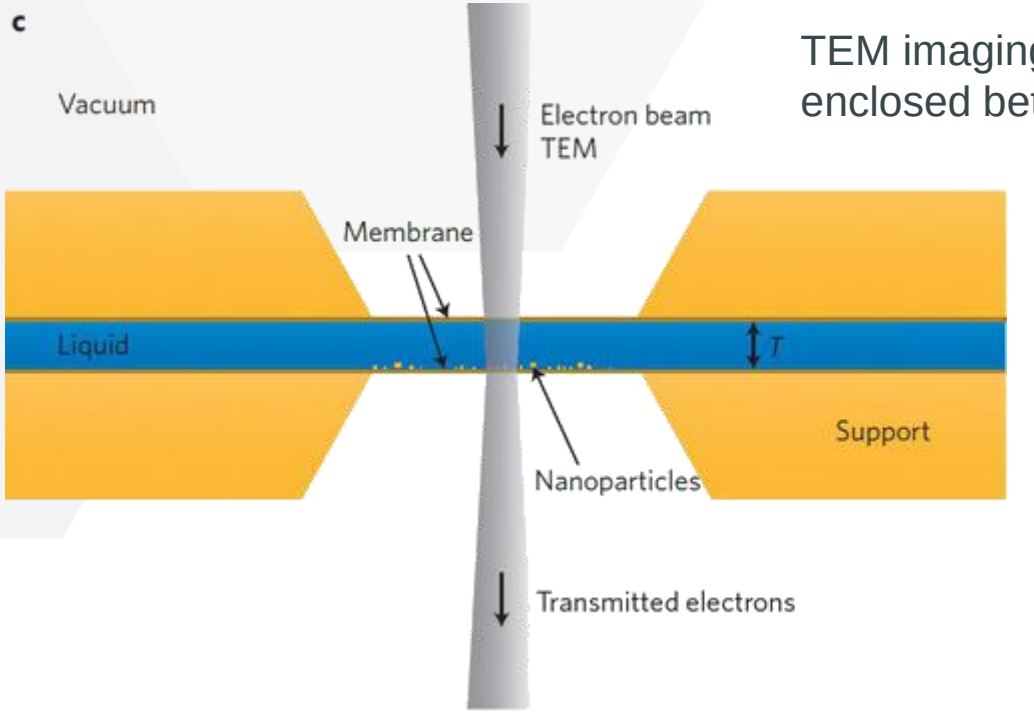




Chen, C.-C., Zhu, C., White, E.R., Chiu, C.-Y., Scott, M.C., Regan, B.C., Marks, L.D., Huang, Y., Miao, J. (2013) Nature, 496 (7443), 74-77.

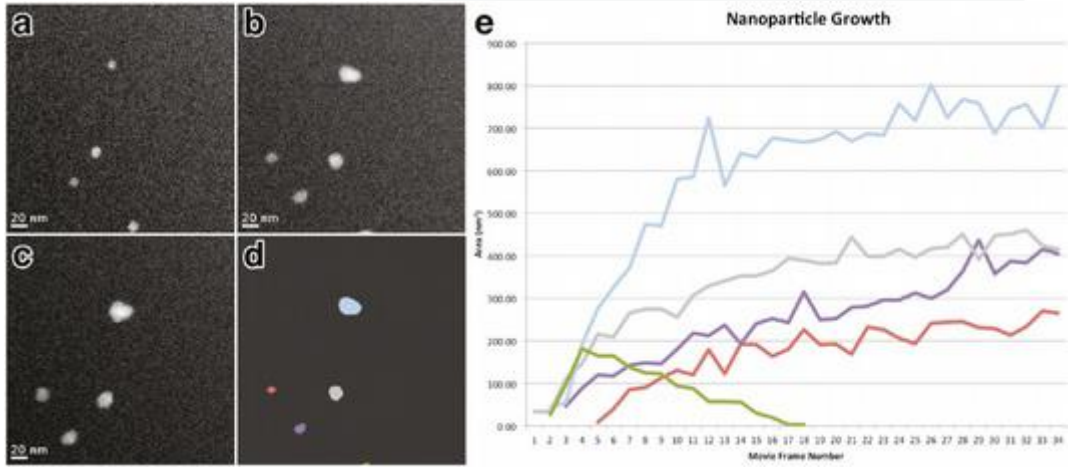
TEM in liquid

- It is possible to place a very thin chamber containing a dispersion of nanoparticles
- Study behavior of nanoparticles (e.g. growth, dissolution)



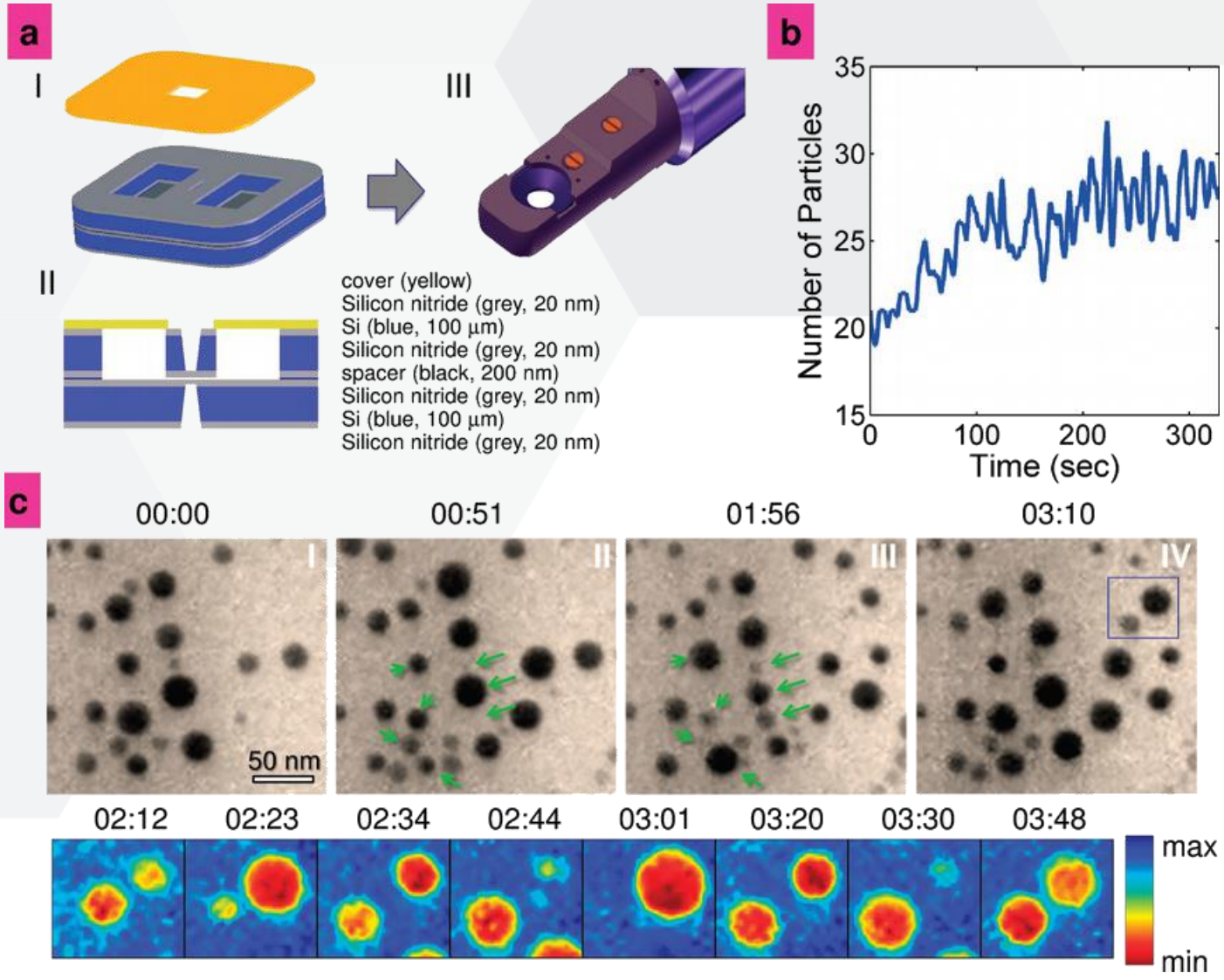
TEM imaging of nanoparticles in a liquid fully enclosed between electron transparent windows

Tracking PbS nanoparticle growth over time



Evans, J.E., Jungjohann, K.L., Browning, N.D., Arslan, I. (2011) Nano Lett., 11 (7), 2809-2813.

TEM in liquid



(a) Construction of a liquid cell that can fit into a commercial TEM heating holder. (I) 3D view and (II) cross-sectional view of a liquid cell; (III) a TEM heating holder that fits standard 3 mm samples.

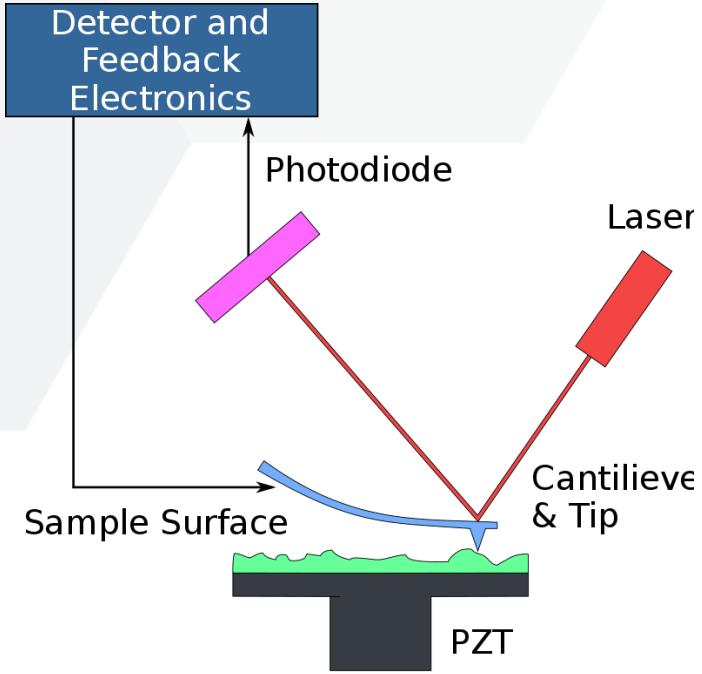
(b) Number of nanoparticles within the field of view as a function of time.

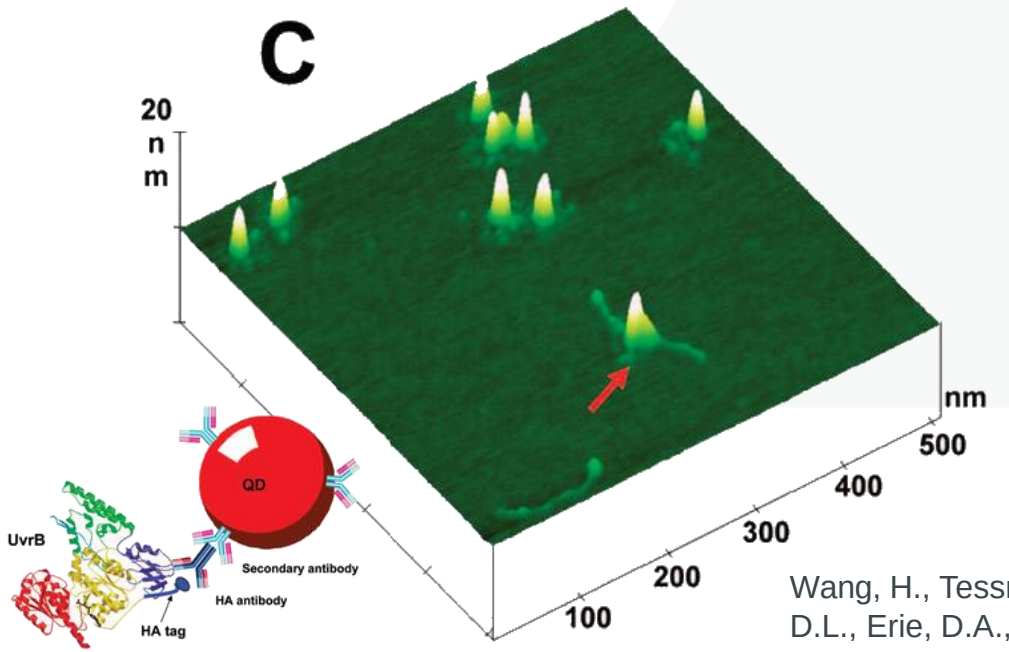
(c) Selected BF-TEM images of the in situ growth of Bi NPs and the corresponding color gradient maps showing oscilatory growth of a pair of nanoparticles.

Xin, H.L., Zheng, H. (2012) Nano Lett., 12 (3), 1470-1474.

Atomic force microscopy

- Measures interactions between probe (sharp tip) and sample
- Sample placed on flat substrate – visualization of topography (3D image)
- Advantages
  - Scanning in liquid
  - Interactions and forces between individual conjugates and molecules





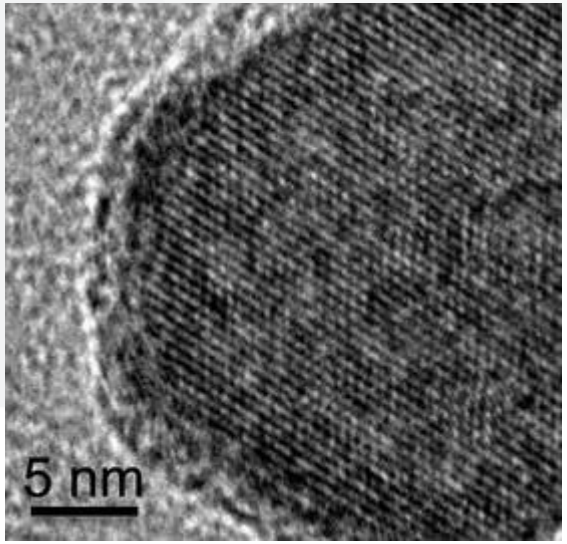
Wang, H., Tessmer, I., Croteau, D.L., Erie, D.A., Van Houten, B. (2008) Nano Lett., 8 (6), 1631-1637.

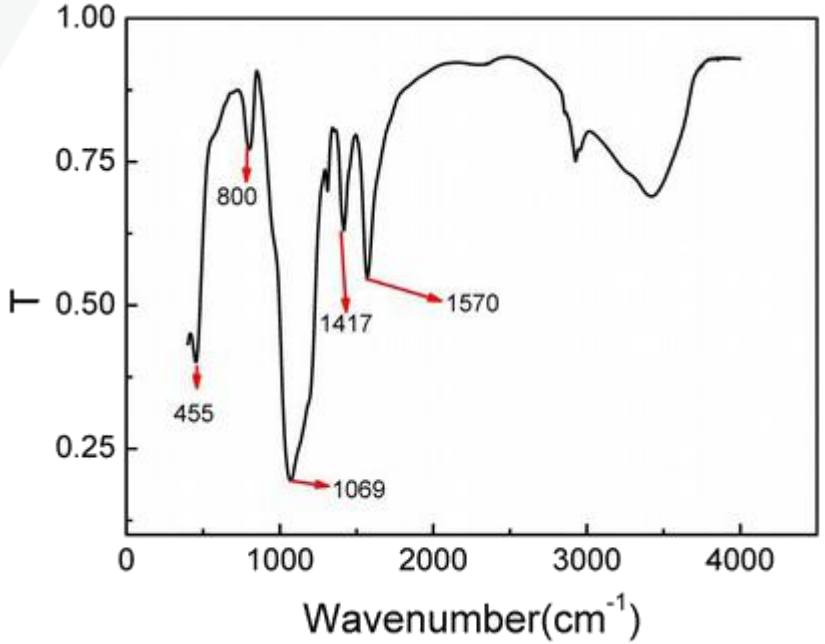
UV-VIS Spectroscopy

- Determination of nanoparticle concentration
- Spectrum changes upon binding of biomolecules on nanoparticle surface
  - Plasmonic nanoparticles (Au, Ag)
- Determining amount of bound biomolecules which exhibit distinguishable absorption band
  - May overlap with the absorption band of nanoparticles
  - E.g. proteins 280–290 nm, DNA 260 nm, cytochrome c heme at 410 nm...
- Indirect colorimetric detection
  - For the detection of biomolecules on the nanoparticle surface
  - E.g. Bradford reagent (Coomassie brilliant blue) and bicinchoninic acid
  - Nanoparticles may interfere with the colorimetric reaction → need to make reference experiments

IR Spectroscopy

- Measure the absorbance in the IR region, vibrational states of molecules
- Chemical characterization of molecules for modifying the surface of nanoparticles
- Detection of functional groups on the nanoparticle surface, evaluation of ligand exchange, molecular surface representation of various ligands, evaluation of bioconjugation – presence of biomolecule and conformation e.g. proteins



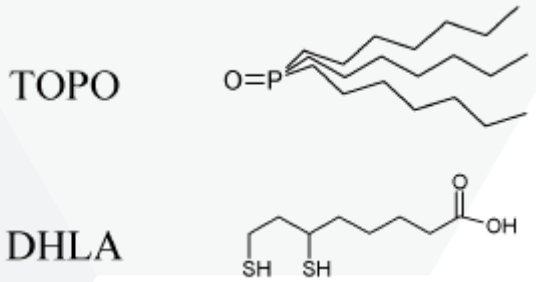


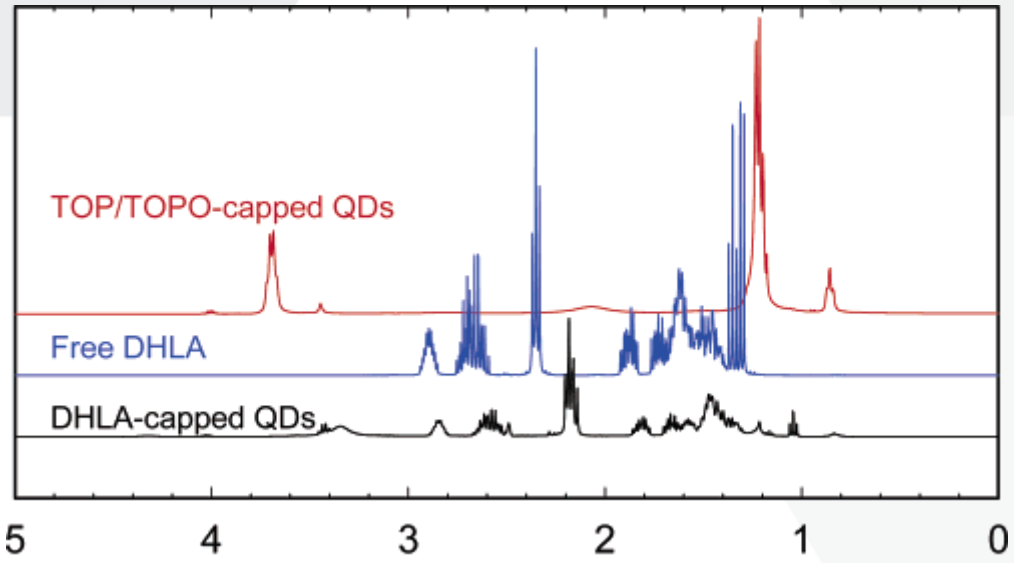
FTIR analysis of NaYF4 nanoparticles with surface coated by silica layer
1417 cm^{-1}, 1570 cm^{-1} carboxyl
455 cm^{-1}, 800 cm^{-1}, 1069 cm^{-1} SiO_2

Liu, F., Zhao, Q., You, H., Wang, Z. (2013) Nanoscale, 5 (3), 1047-1053.

Nuclear magnetic resonance

- Allows the chemical characterization of nanoparticles (presence of functional groups, ...) and conformation of biomolecules, usually measured spectra of 1H and 13C nuclei



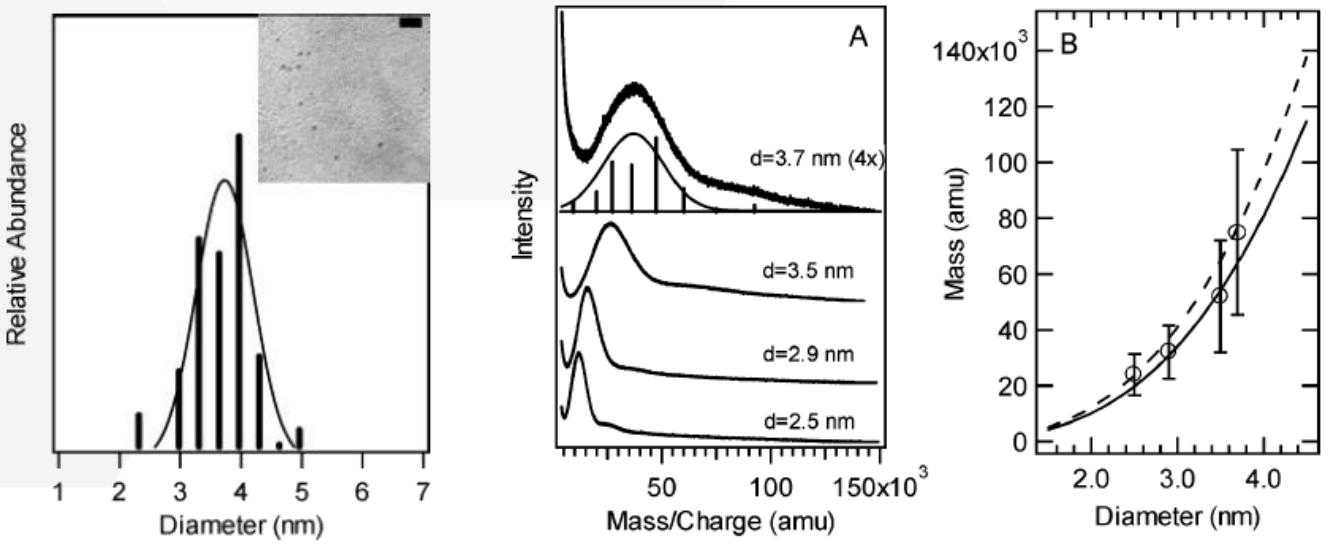


Characterization of the ligand exchange on the surface of quantum dots
Cap exchange of TOP/TOPO with DHLA and DHLA-PEG ligands was verified using 1H NMR and probing changes in the thiol resonances of the free ligand, before and after cap exchange and removal of excess unreacted ligand.

Pons, T., Uyeda, H.T., Medintz, I.L., Mattoussi, H. (2006) J. Phys. Chem. B, 110 (41), 20308-20316.

Mass spectrometry

- Measures mass to charge ratio (m/z)
- MALDI-TOF; detection of binding of small molecules to biomolecules (e.g. binding fluorophores, biotin, bioorthogonal groups on biomolecules)
- It is possible to ionize nanoparticles and directly measure the molecular weight

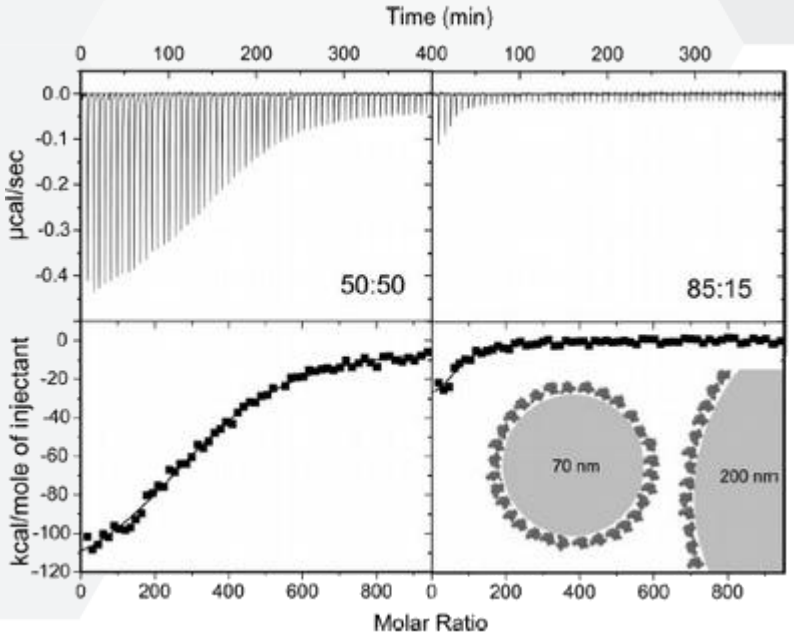


Measurement of weight ZnO nanoparticles
Size distribution obtained from TEM image analysis of ZnS-HDA nanocrystals.
Inset shows one of micrographs used for the measurements.
(A) MALDI-MS spectra of ZnS-HDA nanocrystals.
(B) Nanocrystal masses projected from MS data assuming +2 species vs masses calculated by assuming spherical (solid line) and prolate (dashed line) nanocrystal morphologies

Khitrov, G.A., Strouse, G.F (2003) J. Am. Chem. Soc., 125 (34), 10465-10469.

Calorimetry

- Determination of thermodynamic parameters of the binding of biomolecules on the surface of nanoparticles
- ITC – Isothermal titration calorimetry

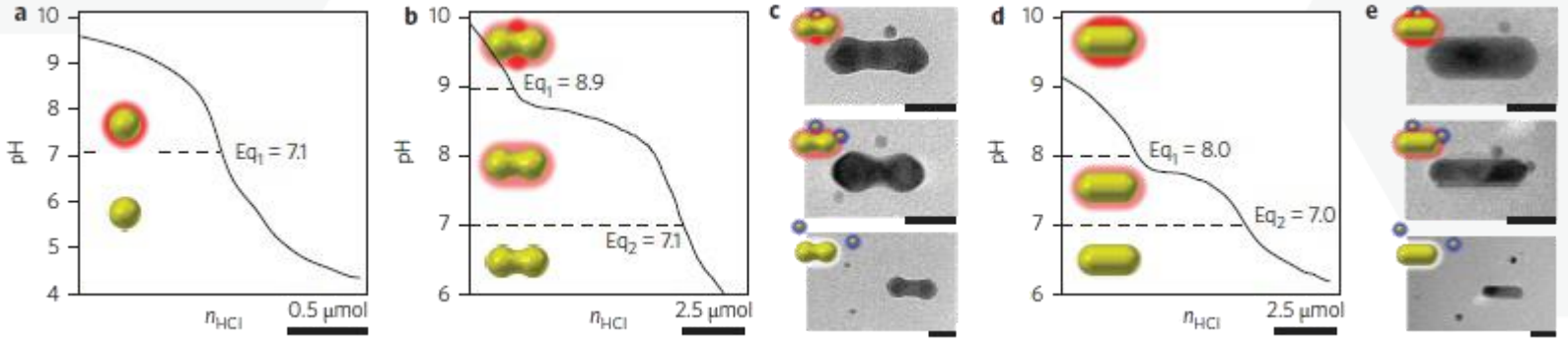


ITC Reveals a Dependence on Particle Hydrophobicity and Size (Radius of Curvature).
ITC was investigated for its potential to assess the stoichiometry, affinity and enthalpy of protein-nanoparticle interaction. Protein is injected into a nanoparticle solution in the sample cell and the difference in heat that needs to be added to the sample and reference cells to keep both cells at the same temperature is monitored. Data from multiple injections provide information on the number of protein molecules bound per particle, the apparent affinity, and the enthalpy change.
The negative injection signals imply an exothermic process. A larger number of protein injections are needed to reach saturation of the more hydrophobic particles, implying that the number of protein molecules bound (stoichiometry) increases with the particle hydrophobicity.

Cedervall, T., Lynch, I., Lindman, S., Berggård, T., Thulin, E., Nilsson, H., Dawson, K.A., Linse, S. (2007) Proc. Nat. Acad. Sci. U. S. A., 104 (7) 2050-2055.

„Chemical“ methods - Titration

- Determining the amount of functional groups on the surface of nanoparticles
- Absolute method, calibration is not needed
- Acid-base titration
  - Determination of carboxyl groups on the surface of nanoparticle
  - Shape and size of nanoparticles determines the curvature of the surface, the curvature in turn influences the chemical properties of chemical groups on the NP surface, e.g. acidity of carboxyl groups; can be proved by acid-base titration



Walker, D.A., Leitsch, E.K., Nap, R.J., Szleifer, I., Grzybowski, B.A. (2013) Nat. Nanotechnol., 8 (9) 676-681.

a) Titration of spherical nanoparticles features a single equivalence point.
b,d) NPs with varying regions of curvature have multiple equivalence points
c,e) To help visualize which regions of the particles are negatively charged, TEM images were collected of assembly between larger NPs and small spherical NPs of opposite charge.

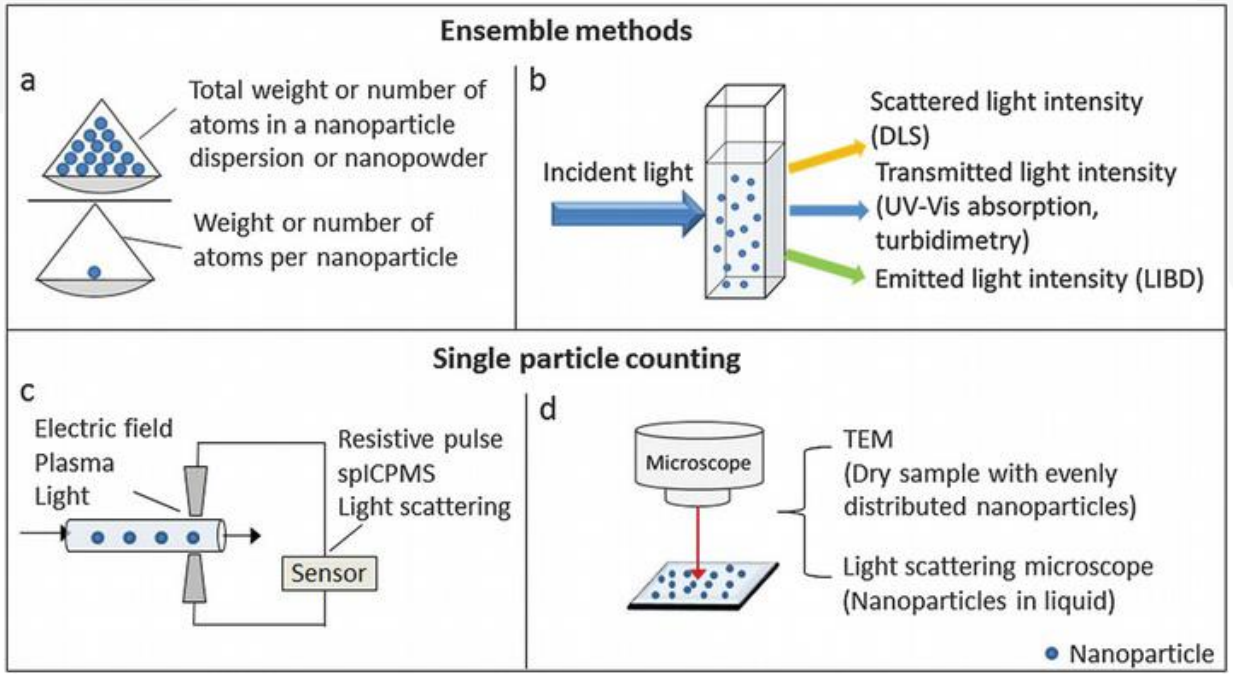
Estimation of nanoparticle concentration

- Molar and mass concentration

$$x = \frac{m}{V} \qquad c = \frac{m}{M\,V}$$

x... mass concentration, m... weight of nanoparticles in sample, V... sample volume

c... molar concentration (useful for chemical reactions of nanoparticles (bioconjugation, surface modification), M... molar mass of nanoparticles



Shang, J., Gao, X. (2014) Chem. Soc. Rev., 43 (21) 7267-7278.

Gravimetry

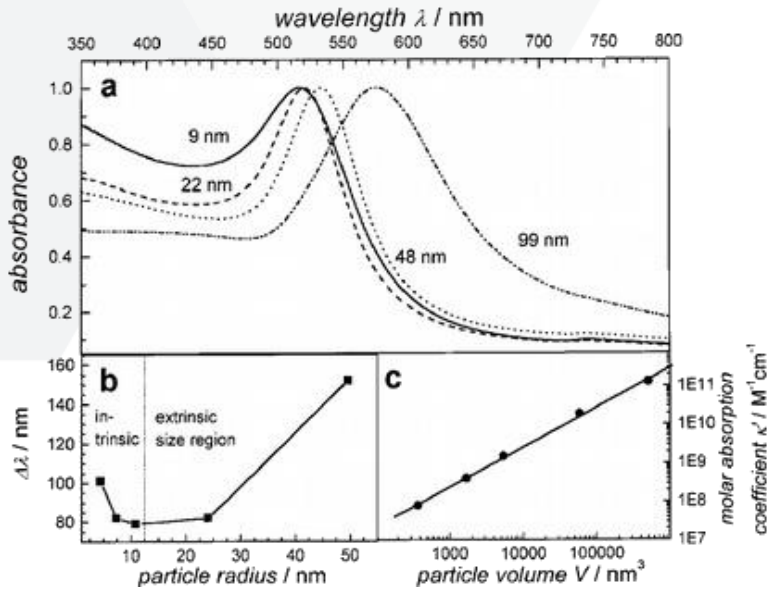
- Determination of the total weight of nanoparticles
  - From quantity of reactants assuming 100 % yield
    Can be used for instance for gold NPs prepared from $HAuCl_4$ and NPs prepared by coprecipitation reaction (CdTe NPs, $NaYF_4$…)
  - Determination of concentrations of components in the sample of prepared and purified NPs
    Element composition and concentration of ions in samples e.g. CdSe, PbSe, and Fe_3O_4 may be determined by AAS, AES, ICP-MS or titration.
  - Determination of the total weight after solvent evaporation
    Mass may be influenced by the presence of buffer ions and surface ligands
- Determination of molar mass of nanoparticles
  - Assuming regular shape of nanoparticles and known values of their density
  - $M = M_{NP}. N_A$
  - $M_{NP} = 4.3\ \pi.r^3.\rho$ (for spherical nanoparticles)
- Radius of nanoparticles is typically estimated by electron microscopy (TEM and SEM)
- Density of nanoparticles is usually assumed the same as the density of the bulk material
  - Can be used e.g. for gold NPs, polymeric NPs
  - Deviations due to the surface structure of nanoparticles, e.g. PbSe nanoparticle surface layer contains multiple atoms of lead, which are not specified by chemical formula → density difference

UV-Vis spectroscopy

$$c = \frac{A}{\varepsilon\, l}$$

A... absorbance, ε... molar extinction coefficient,
l... optical path length, c... molar concentration

- Necessary to know the molar extinction coefficient ε for the determination of nanoparticle concentration
- ε is known for several types of nanoparticles including various shapes of Au NPs and quantum dots



Size effects on the surface plasmon absorption of spherical gold nanoparticles.
The UV-Vis absorption spectra of colloidal solutions of gold nanoparticles with diameters between 9 and 99 nm show that the absorption maximum red-shifts with increasing particle size.

Link, S., El-Sayed, M.A. (1999) J. Phys. Chem. B, 103 (40), 8410-8426.

Turbidimetry

- $\tau = \frac{1}{4} \pi K d^2 C$
  τ... turbidance, K... scattering coefficient (function of wavelength, refractive index and particle size), d... diameter of nanoparticles, C... nanoparticle concentration (number per volume)
- Scattering coefficient K is known for some nanoparticles, e.g. silica NPs, latex NPs, and some other polymer particles; generally the determination of K is a complex problem

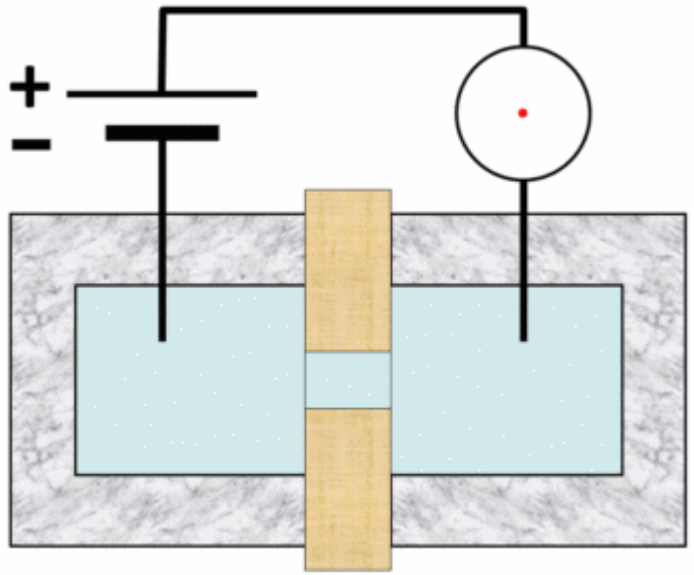
Dynamic light scattering (DLS)

- The intensity of scattered light is directly proportional to the NP concentration
- Calibration curve can be measured to determine the concentration dependence
- Standards must be the same material with a suitable size range

Single-particle counting

- (In principle) no need for calibration

- **Resistive-pulse sensing**
  - Change in electrical conductivity of the pore in the membrane through which nanoparticles flow
  - Since fifties (Coulter counter), for the measurement of microparticle concentration
  - Usually necessary to calibrate with known concentration of NPs
  - Possible to determine the concentration without calibration (calculated volume of solution transferred) when the pore geometry is precisely known



Single-particle counting

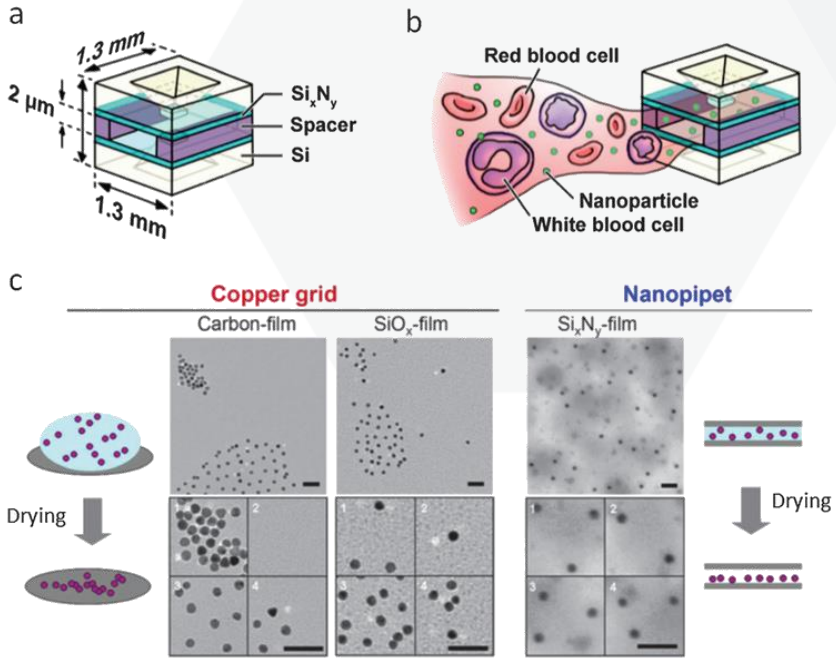
- **Single particle ICP-MS**
  - Fluid is introduced into the ICP-MS at short intervals
  - Discrete pulses of ions from individual nanoparticles are detected, the number of pulses is proportional to the concentration
  - Calibration required due to losses of liquid during ionization
  - Used for nanoparticles comprising metal ions, e.g. Au, Ag, TiO_2, Al_2O_3 and ZrO_2

- **Optical detection of individual nanoparticles in a flow cell**
  - Measurement of fluorescence and scattered light in a narrow channel
  - Molar concentration can be estimated directly without calibration
  - For example, determination of the concentration of Au NPs with small diameter (24 nm)

Single-particle counting

- **Single particle tracking**
  - Direct observation of the scattered radiation from nanoparticles in optical microscope
  - Polymeric nanoparticles of 40 nm in diameter, plasmonic nanoparticles from ~10 to 15 nm can be analyzed
  - Calibration not necessary, however, it not suitable for polydisperse samples (larger particles scatter too much radiation)

- **Transmission electron microscopy**
  - Aggregation of nanoparticles can complicate the experiment



Elsaesser, A., Barnes, C.A., McKerr, G., Salvati, A., Lynch, I., Dawson, K.A., Howard, C.V. (2011) Nanomedicine, 6 (7) 1189-1198.

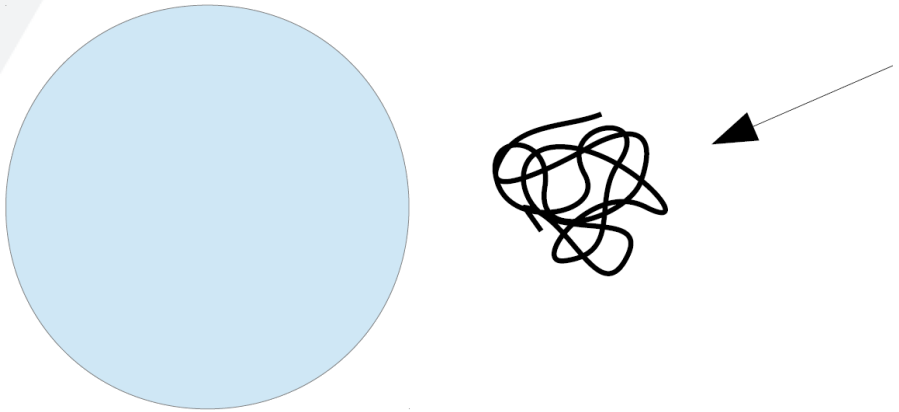
Estimation of nanoparticle concentration

Methods	Types of nanoparticles	Size limitation (diameter)	Prerequisites
Gravimetric measurement (ensemble quantity/quantity per nanoparticle)	Nanoparticles with known density and regular shape	No restriction	Precise measurement of average weight or number of atoms per nanoparticle
UV-Vis spectroscopy	Strong extinction on UV-Vis spectra (*e.g.*, gold, silver and cadmium selenide)	No minimal limitation	Accurate determination of the molar extinction coefficient of nanoparticles
Turbidimetry	Non-absorbers at the detection wavelength (*e.g.*, polystyrene at 350 nm)	$\geq$70 nm[21]	Accurate determination of the scattering coefficient of nanoparticles
Dynamic light scattering (DLS)	No absorbance/fluorescence at the laser wavelength	Ultimately sub-nanometer[54]	Calibration with a standard nanoparticle solution
Laser-induced breakdown detection (LIBD)	No specific restriction	1–2 nm[31]	Powerful laser system; calibration with a standard nanoparticle solution
Resistive-pulse sensor	No specific restriction	Izon qNano: $\geq$50 nm[41]	Calibration with a standard nanoparticle solution
Single particle inductively coupled plasma mass spectrometry (spICPMS)	Metal nanoparticles	Depends on the type of nanoparticles: Gold nanoparticles: $\geq$15 nm[45] Silver nanoparticles: $\geq$20 nm[44]	Calibration with a standard nanoparticle solution
Optical sensing (light scattering particle counter)	Nanoparticles with strong scattering	24 nm for gold nanoparticles[48]	Calibration with a standard nanoparticle solution, or accurate determination of sample volume and detection efficiency
Laser-illuminated light scattering microscopy (Nanosight)	Any type of nanoparticles with as high scattering coefficient	Depends on the type of nanoparticles: Organic nanoparticles: $\geq$50 nm Metal and semiconductor: $\geq$10 nm[49]	Optimize nanoparticle concentrations to ensure that all particles are analyzed by the NTA software
Transmission electron microscopy (TEM)	High electron density (metal nanoparticles); organic nanoparticles with staining	Depends on image contrast, many inorganic nanoparticles can be imaged at sub-nanometer sizes	Uniform distribution on TEM grids and unbiased counting

Shang, J., Gao, X. (2014) Chem. Soc. Rev., 43 (21), 7267-7278.

Modelling

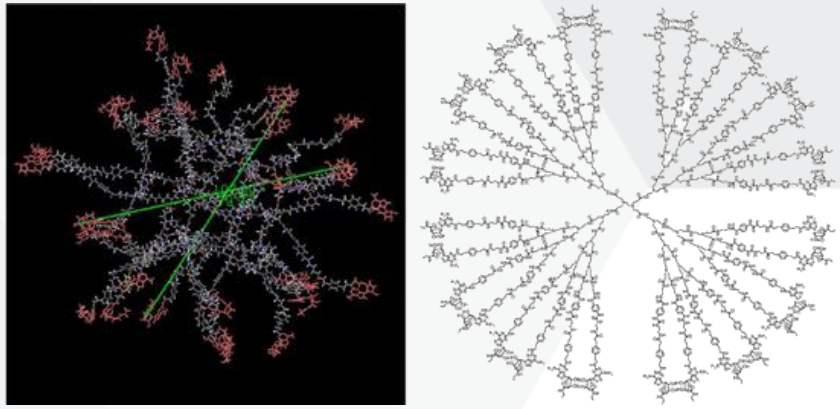
- As in other areas of chemistry, models and simulations are used for the characterization of nanoparticles
  - Ab-initio method for accurate computing
  - Quantum mechanical models
  - Molecular dynamics
  - In practice, even simple models are often useful
    e.g. the determining of the concentration of nanoparticles, the calculation of the quantity of biomolecules, which can be attached on the surface of nanoparticle



How many biomolecules fit on the surface of nanoparticle?

Modelling

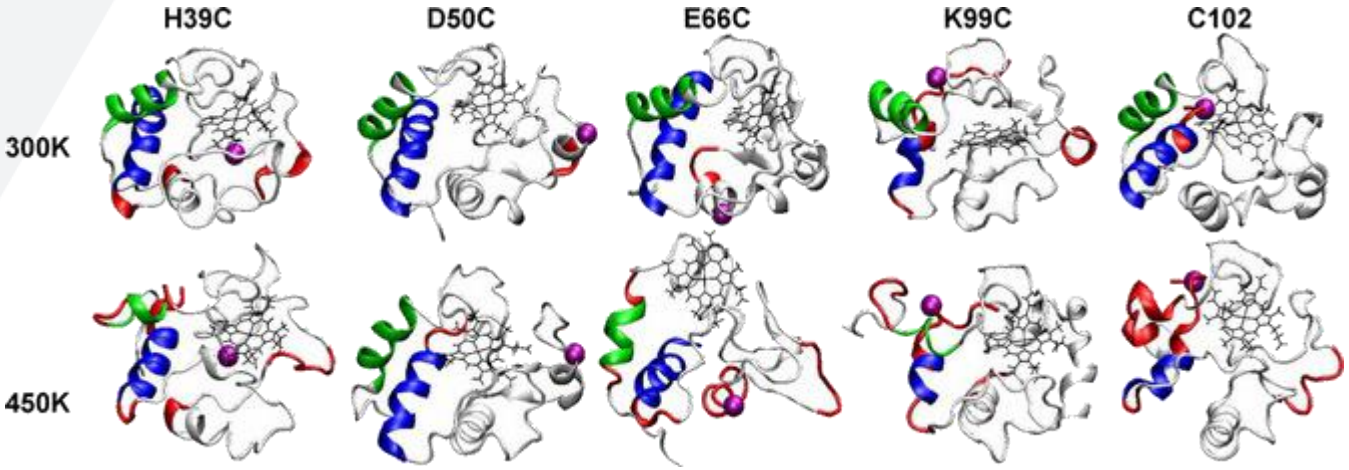
- Calculate nanoparticle structure by potential energy minimization (using a force field), e.g. polymeric nanoparticles modified nucleosides



G3-PAMAM dendrimer with chemically attached nucleosides

Kim, Y., Klutz, A.M., Hechler, B., Gao, Z.-G., Gachet, C., Jacobson, K.A. (2009) Purinergic Signalling, 5 (1) 39-50.

- Interaction of protein with NP

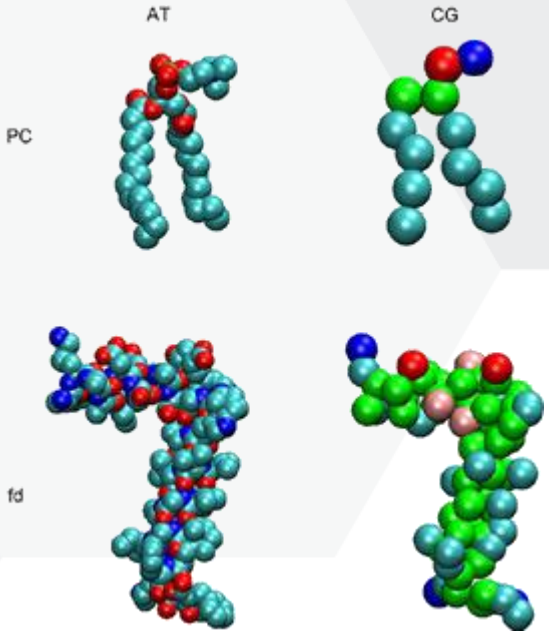


Structures of cytochrome c in dependence on the point of connection to the surface of 1.5 nm gold particles

Aubin-Tam, M.-E., Hwang, W., Hamad-Schifferli, K. (2009) Proc. Nat. Acad. Sci. U. S. A., 106 (11), 4095-4100.

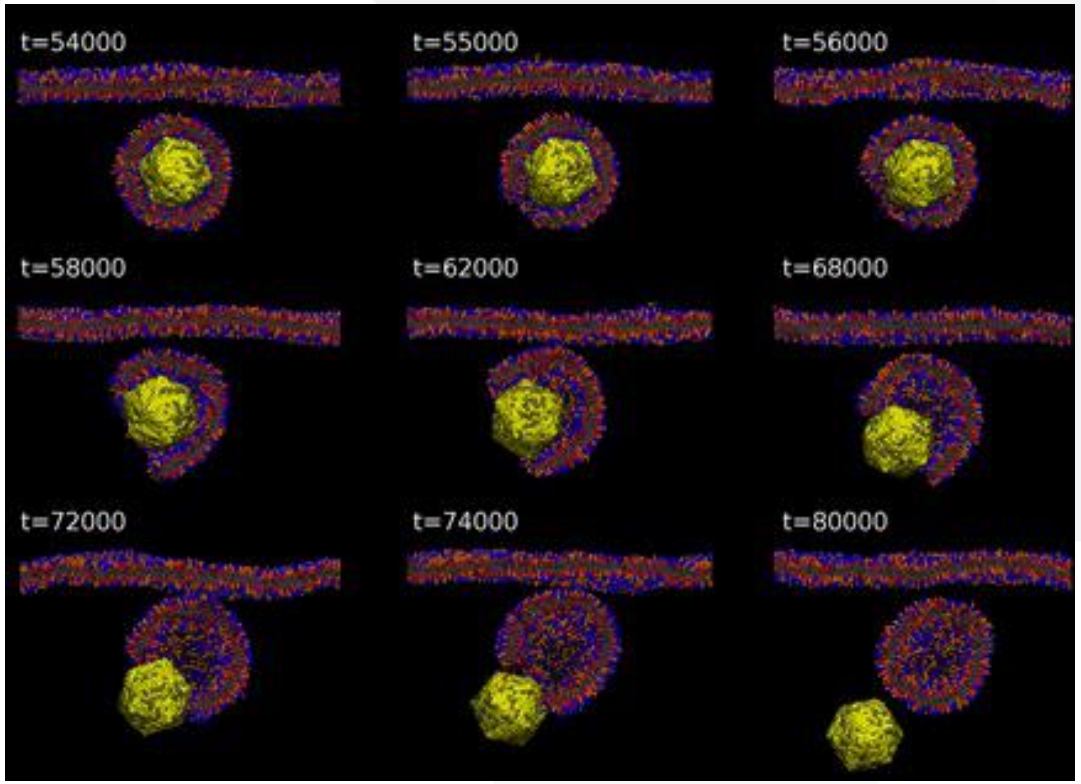
Coarse-grained molecular dynamics

- Does not simulate the atom by atom but larger units



Atomistic (AT) vs. coarse-grained (CG) representations of phosphatidyl choline (PC) and fd phage coat protein. In the AT models the atoms are colored using the CPK convention. In the CG models the particles are colored according to the following scheme: green, mixed polar/nonpolar particle; cyan, hydrophobic particle; red/blue, negative/positive, charged particle; and pink, polar particle.

Bond, P.J., Holyoake, J., Ivetac, A., Khalid, S., Sansom, M.S.P. (2007)
J. Struct. Biol., 157 (3), 593-605.



Several representative snapshots of a MD trajectory where the nanoparticle is released from the enclosing vesicle.

Vácha, R., Martinez-Veracoechea, F.J., Frenkel, D. (2012)
ACS Nano, 6 (12), 10598-10605.

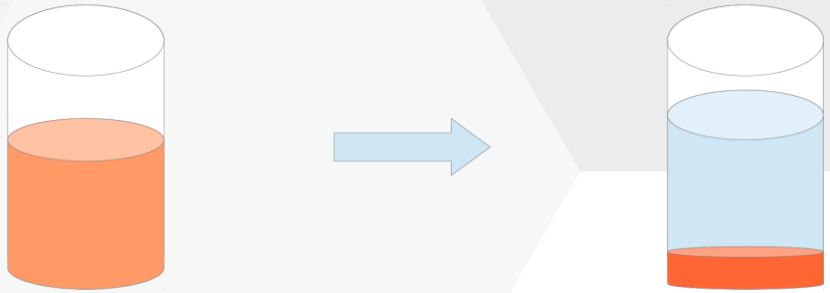
Purification of nanoparticles

Nanoparticle purification

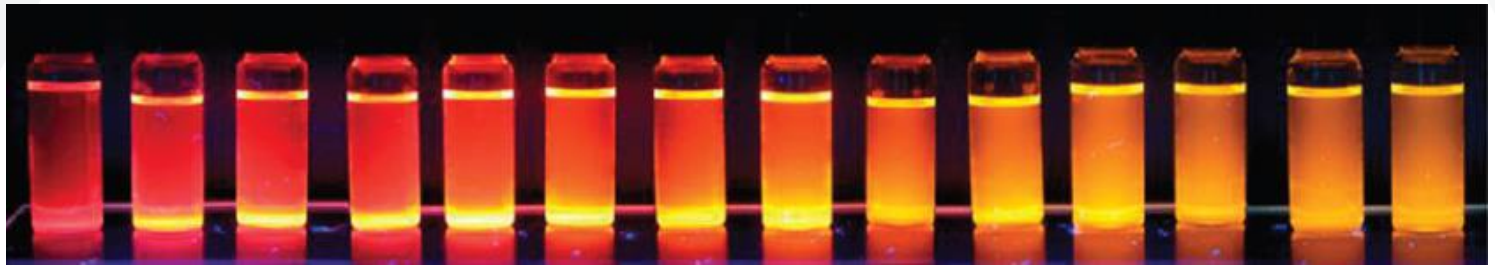
- Isolation of just prepared nanoparticles
- Improving the properties of nanoparticles (separation by shape, size, ...)
- Separation of excess modifying agents (surface ligands, small molecules introducing functional groups) or biomolecules in the preparation of bioconjugates
- Purification bioconjugates (e.g. according to the degree of modification)
- Methods
  - Precipitation
  - Extraction
  - Dialysis
  - Chromatography
  - Filtration
  - Centrifugation
  - Electrophoresis and isotachoforesis

Precipitation

- Typical as a first step of NP purification
  - Addition of antisolvent, salt, gas



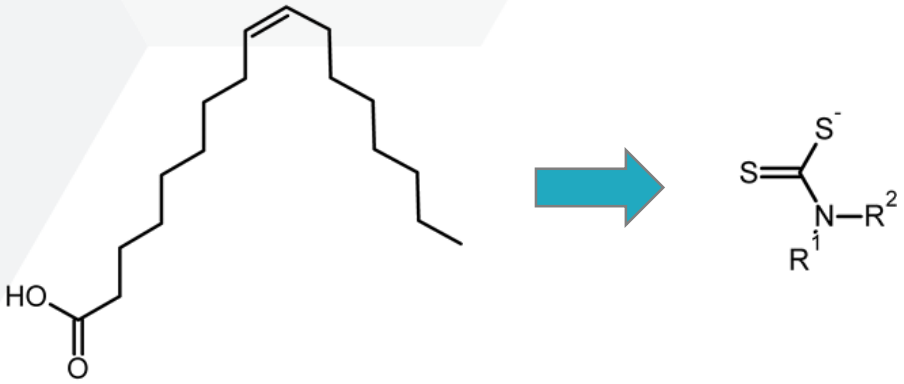
- Size-selective precipitation
  - It is usually necessary to add more precipitant for the precipitation of small nanoparticles → separation by size

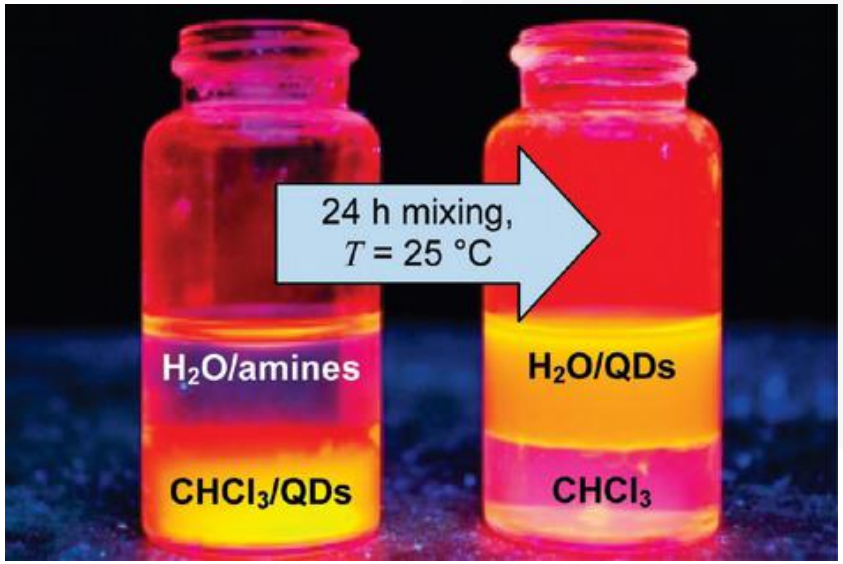


Mastronardi, M.L., Maier-Flaig, F., Faulkner, D., Henderson, E.J., Kübel, C., Lemmer, U., Ozin, G.A. (2012) Nano Lett., 12 (1), 337-342.

Extraction and ligand exchange

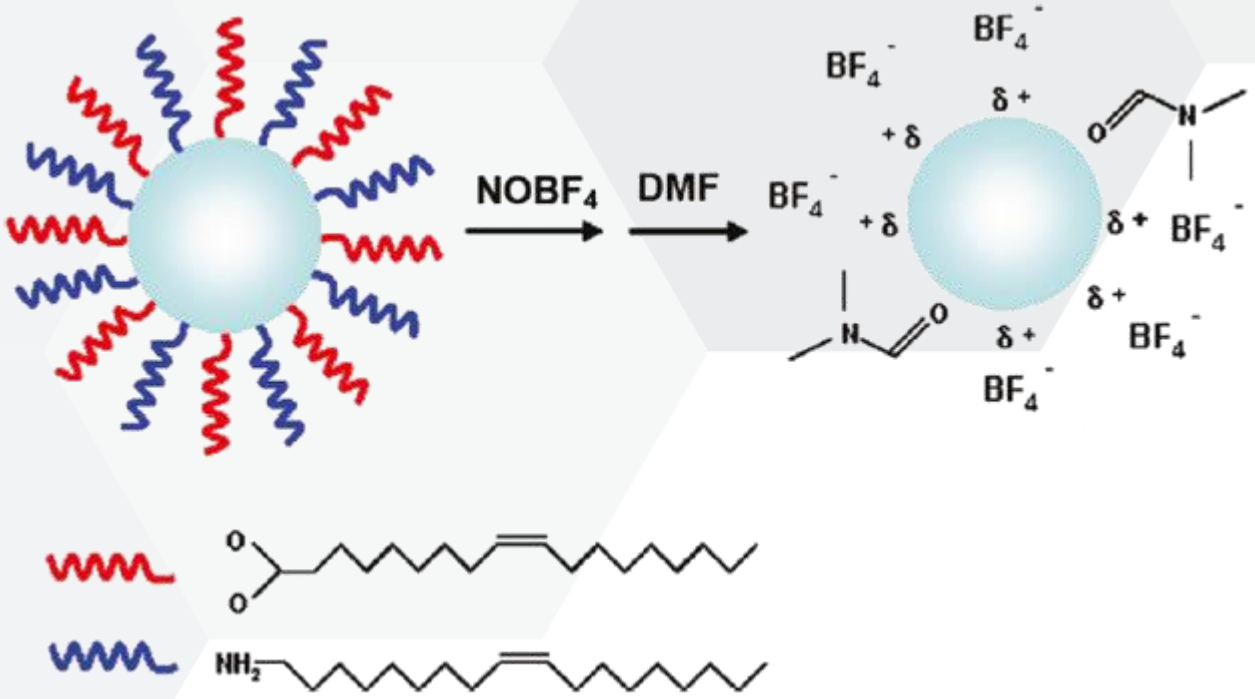
- Extraction of nanoparticles into another solvent is often connected with replacement of stabilizing molecules on the nanoparticle surface
- E.g. nanocrystals stabilized in organic solvent (toluene, chloroform, cyclohexane) via hydrophobic ligands (stearic acid, oleic acid, ...) can be extracted into aqueous phase containing a stabilizing ligands (mercaptoacetic acid, dithiocarbamates, ...)





Zhang, Y., Schnoes A.M., Clapp, A.R. (2010) ACS Appl. Mater. Interfaces, 2 (11), 3384-3395.

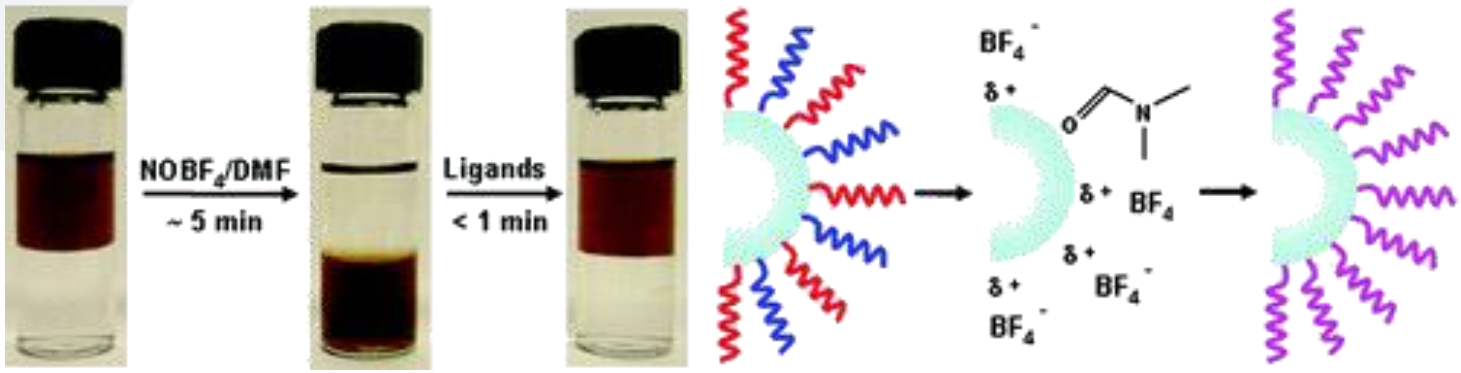
Extraction and ligand exchange



Nitrosonium tetrafluoroborate ($NOBF_4$) is used to replace the original organic ligands attached to the NC surface, stabilizing the NCs in various polar, hydrophilic media such as N,N-dimethylformamide for years, with no observed aggregation or precipitation.

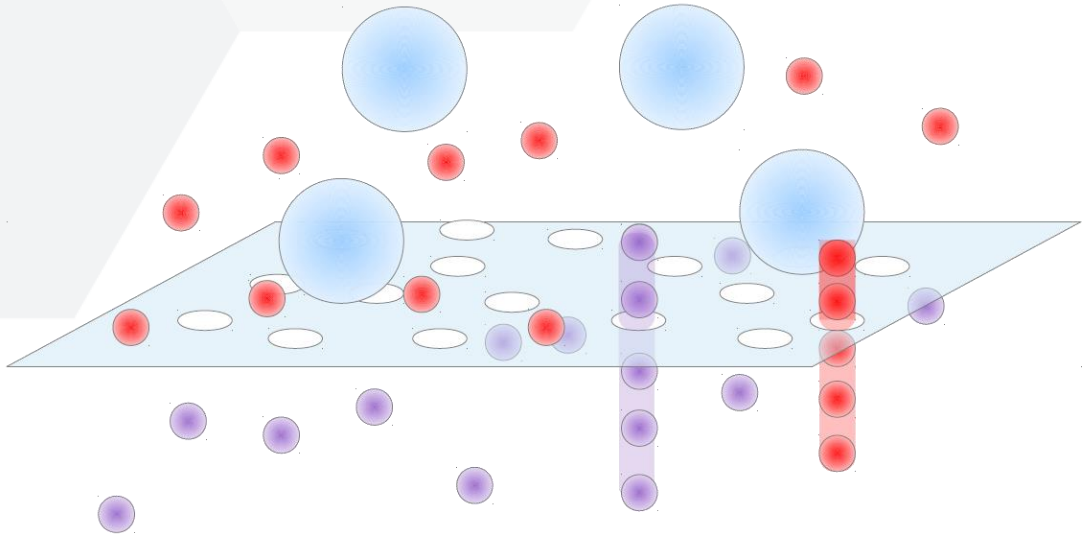
This approach is applicable to various NCs (metal oxides, metals, semiconductors, and dielectrics) of different sizes and shapes. The hydrophilic NCs obtained can subsequently be further functionalized using a variety of capping molecules.

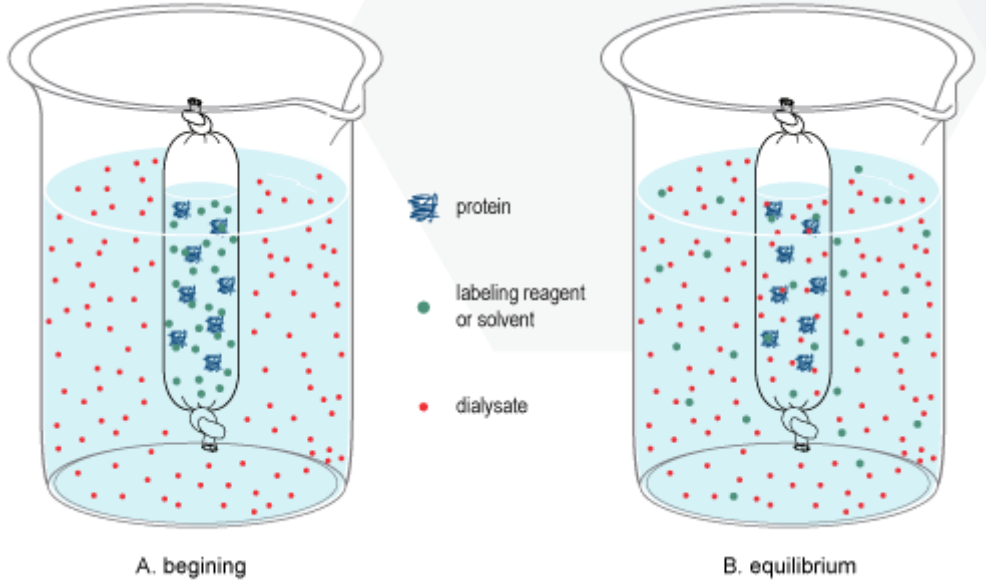
Dong, A., Ye, X., Chen, J., Kang, Y., Gordon, T., Kikkawa, J.M., Murray, C.B. (2011) J. Am. Chem. Soc., 133 (4), 998-1006.



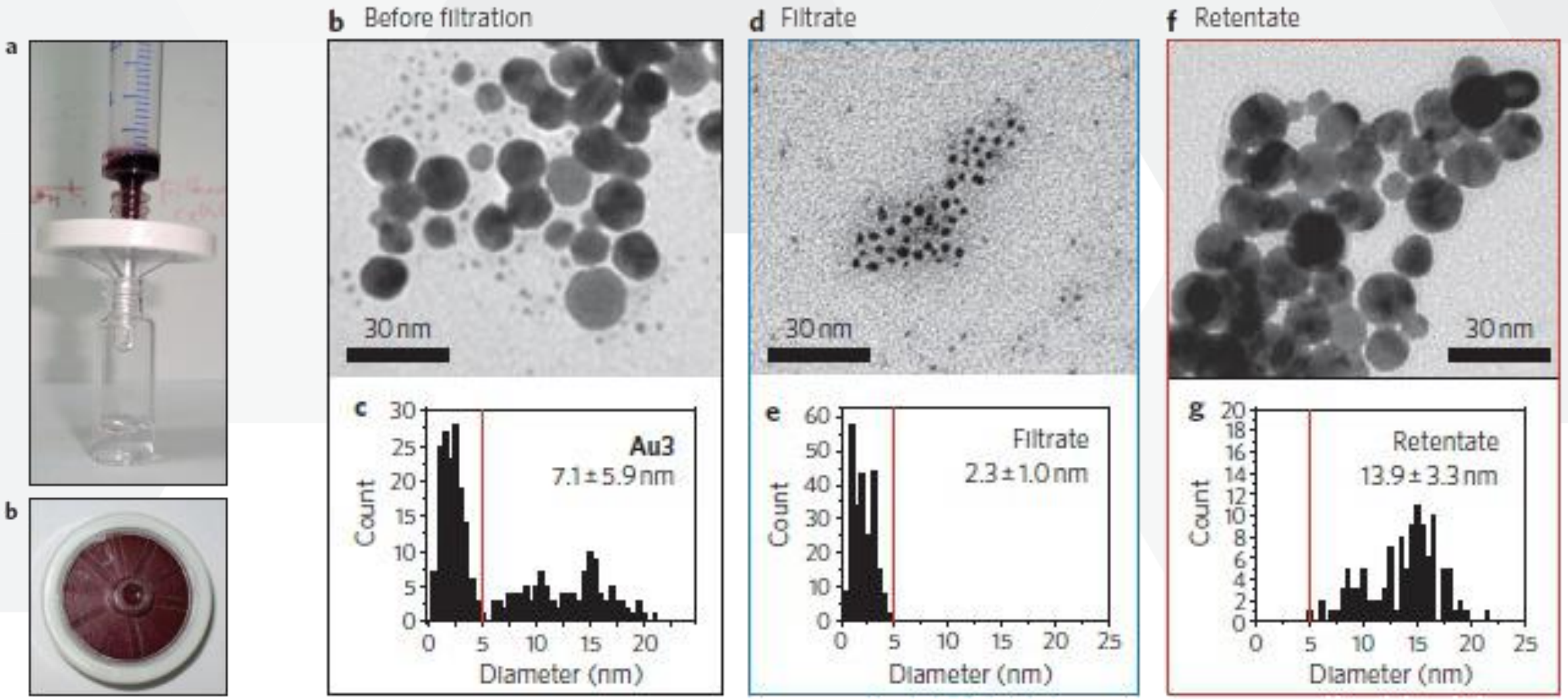
Dialysis

- Typically used to remove the excess of small molecules (stabilizing ligands, reagents) and exchange solvents and buffers
- Dialysis speed can be increased by increasing the ratio of the area of the dialysis membrane and dialyzed solution and stirring
- Equilibrium dialysis can be used to determine dissociation constants
- Can be used for sample concentration (removing solvent)





Filtration



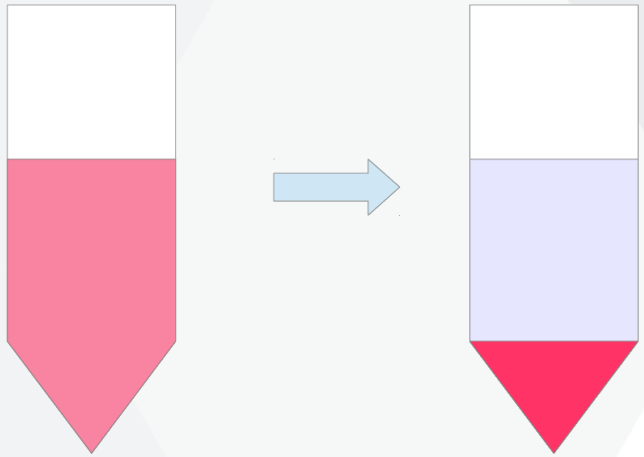
Krieg, E., Weissman, H., Shirman, E., Shimoni, E., Rybtchinski, B. (2011) Nat. Nanotechnol., 6 (3), 141-146.

Centrifugation

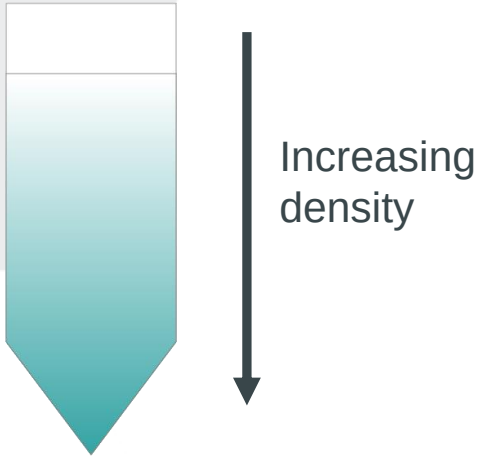
- Sedimentation of nanoparticles and their bioconjugates in centrifugal field
- Forces are exerted on nanomaterial during the centrifugation:
  - Centrifugal force (rotation speed)
  - Buoyancy force (density difference of nanoparticles and the surrounding liquid)
  - Frictional force (the shape of nanoparticles and fluid viscosity)
- Centrifugation often combined with purification by precipitation
- Differential centrifugation
  - Easy separation of nanoparticles from biomolecules, replacing buffer, removing excess modifying agents...
- Density gradient centrifugation
  - Isopycnic (equilibrium of densities of nanoparticles and liquid)
  - Zonal (nonequilibrium, nanoparticles creates zones)

Centrifugation

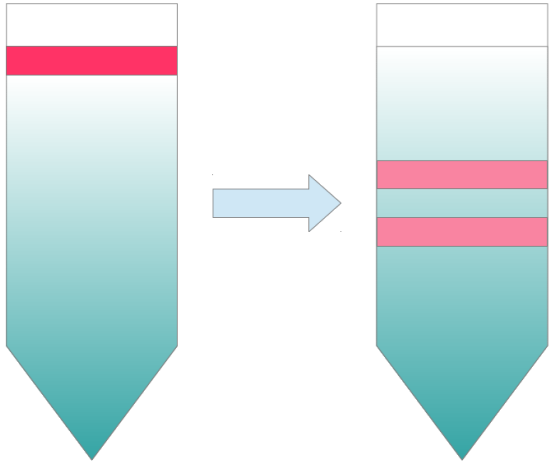
Differential centrifugation



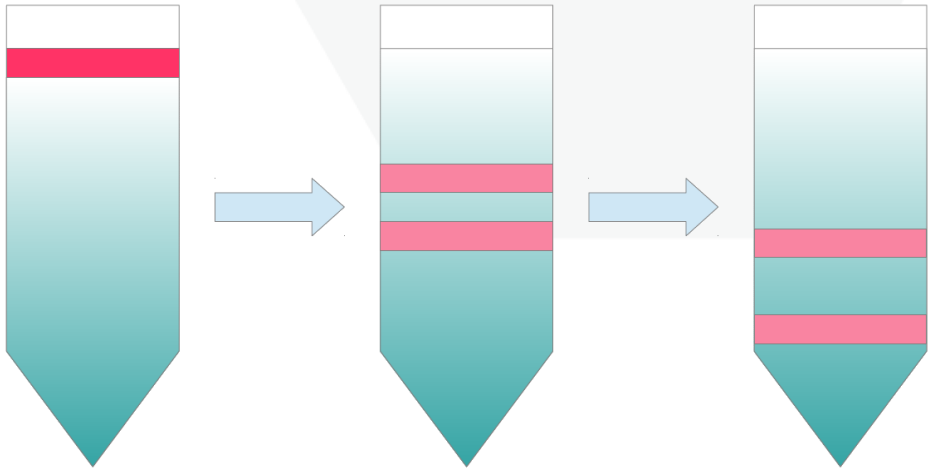
Density gradient



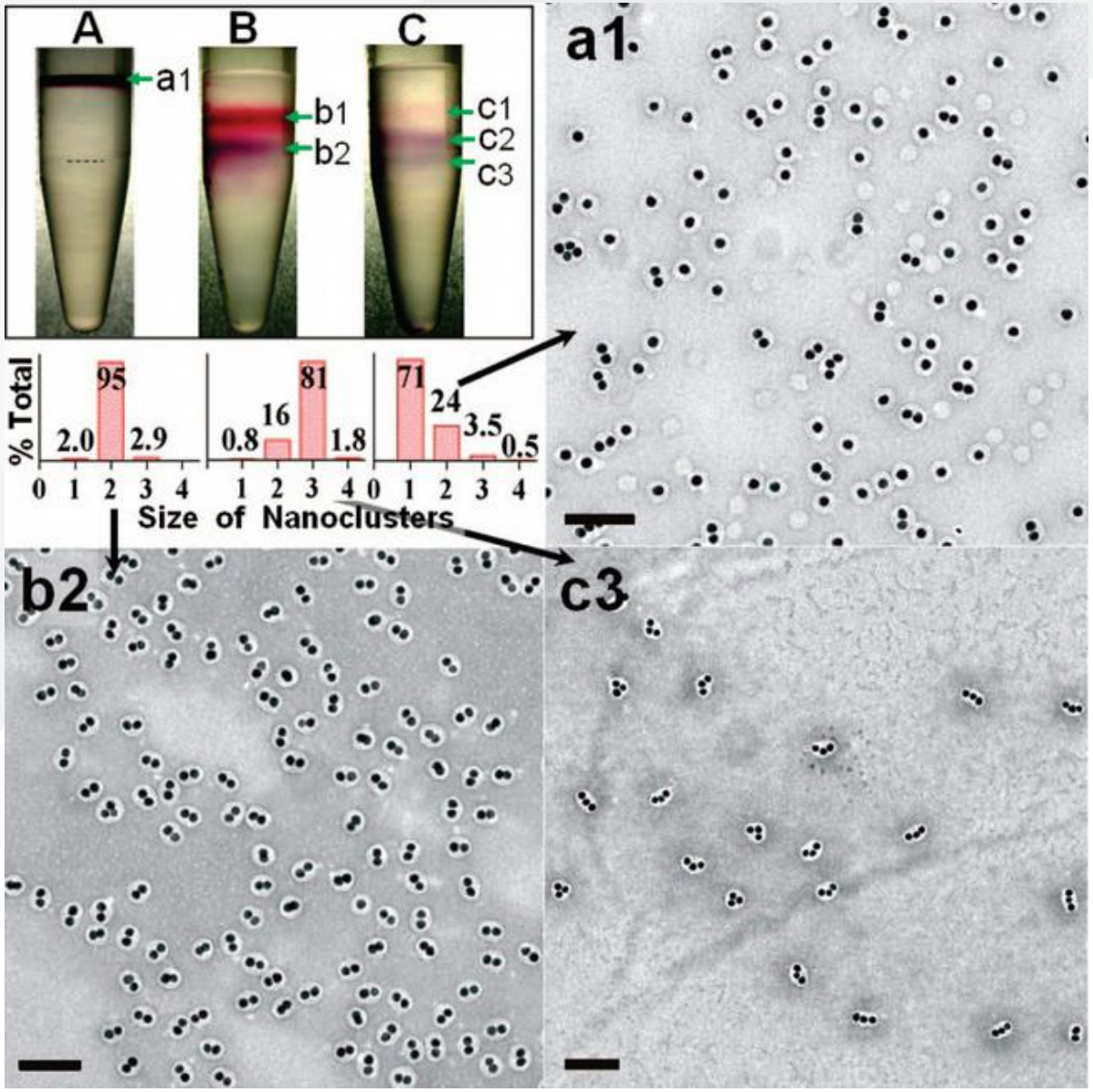
Isopycnic centrifugation



Zonal centrifugation

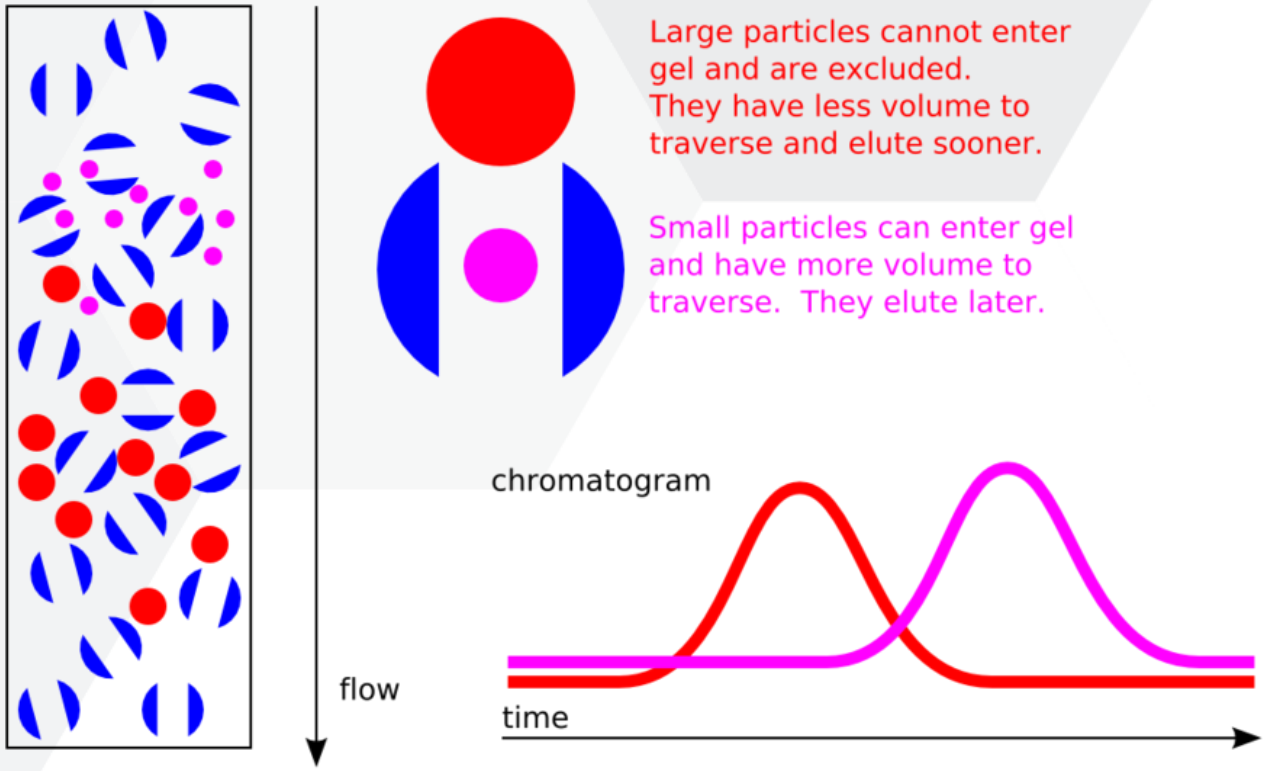


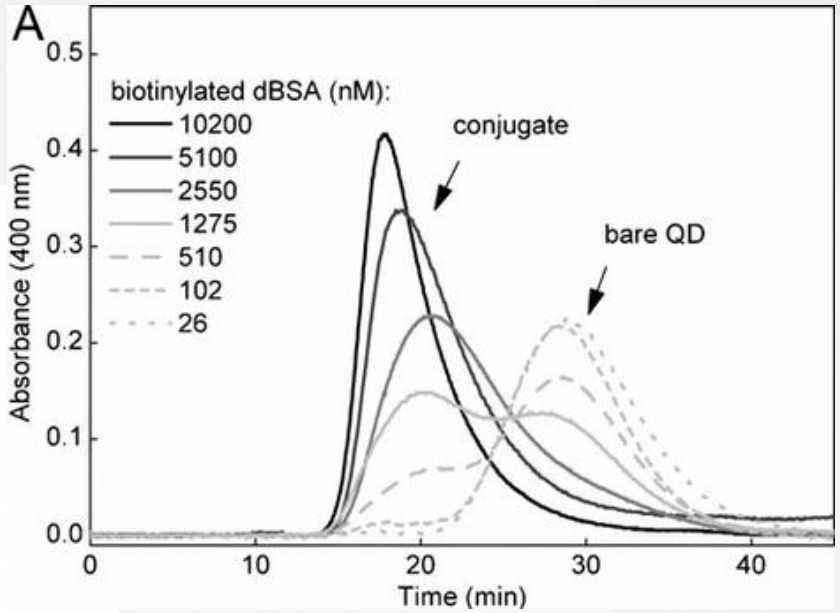
Zonal centrifugation in viscosity gradient

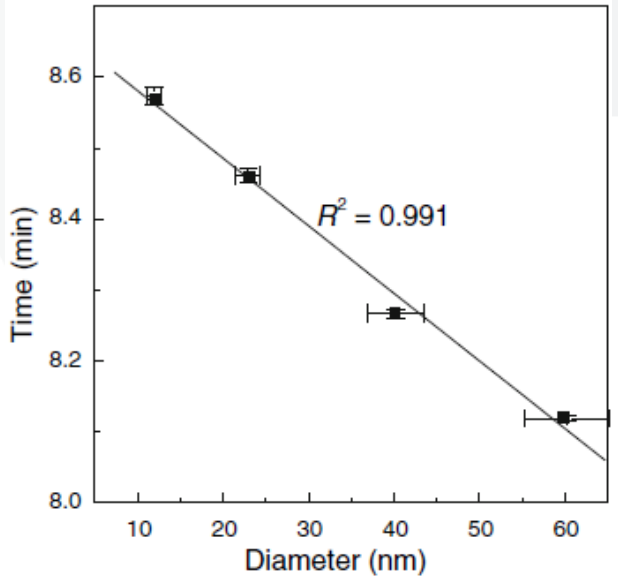


Chen G., Wang, Y., Tan, L.H., Yang, M., Tan, L.S., Chen, Y., Chen, H. (2009) J. Am. Chem. Soc., 131 (12), 4218-4219.

Size-exclusion chromatography



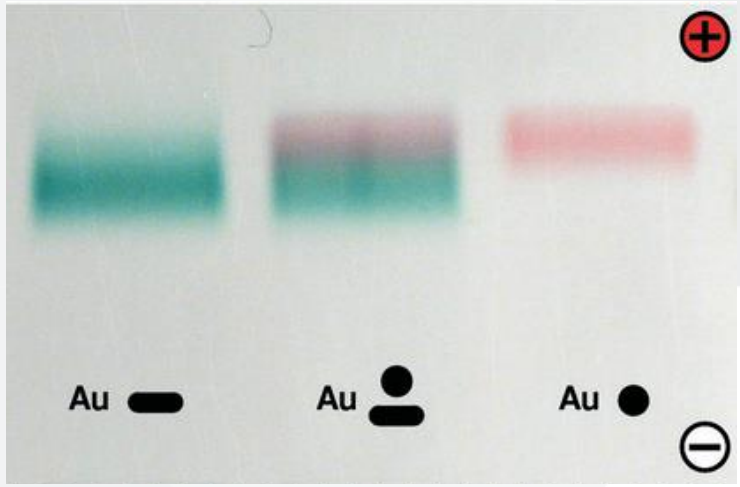




Hlaváček, A., Bouchal, P., Skládal, P. (2012) Microchim. Acta, 176 (3-4), 287-293.

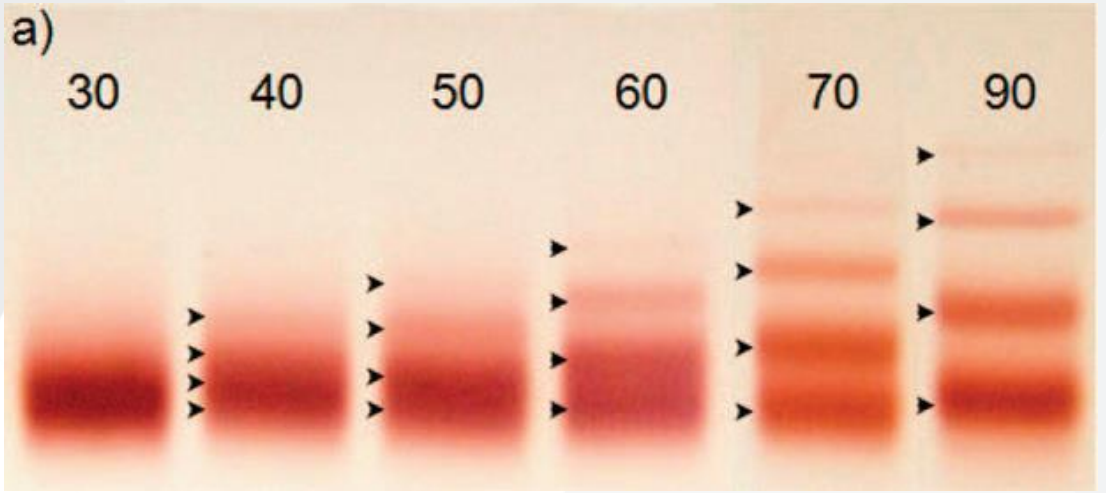
Gel electrophoresis

Shape separation of Au NPs

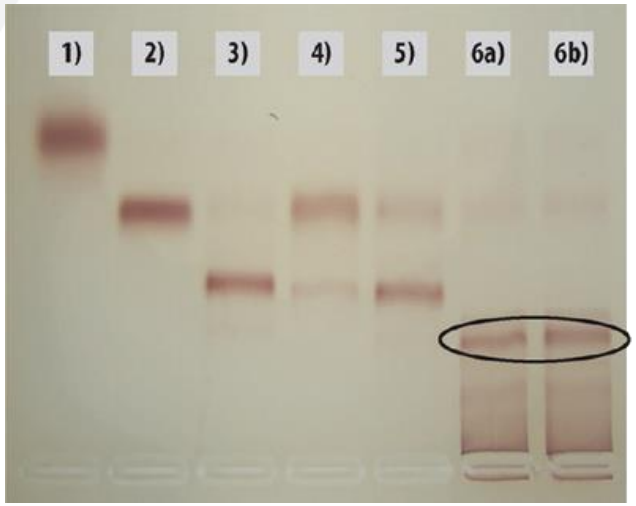


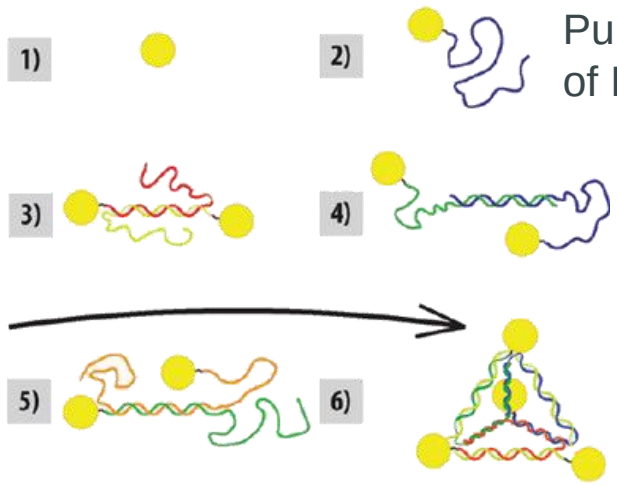
Hanauer, M., Pierrat, S., Zins, I., Lotz, A., Sönnichsen, C. (2007) Nano Lett., 7 (9), 2881-2885.

Separation of 5 nm nanoparticle binding different number of DNA strands of different lengths (30–90 bp)



Claridge, S.A., Liang, H.W., Basu, S.R., Fréchet, J.M.J., Alivisatos, A.P. (2008) Nano Lett., 8 (4), pp. 1202-1206.



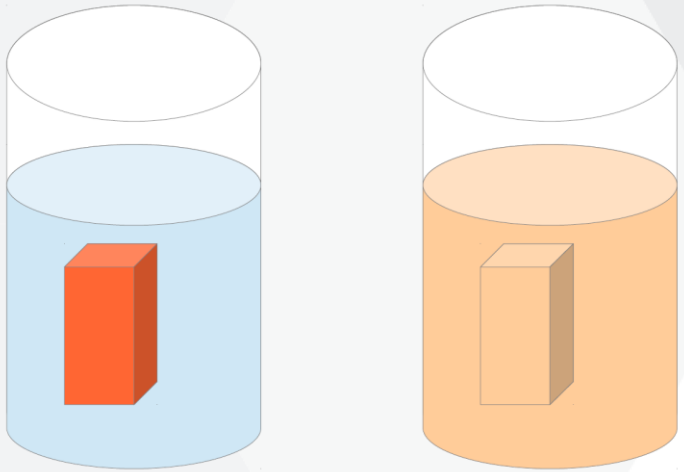


Purification nanostructures composed of DNA and gold particles

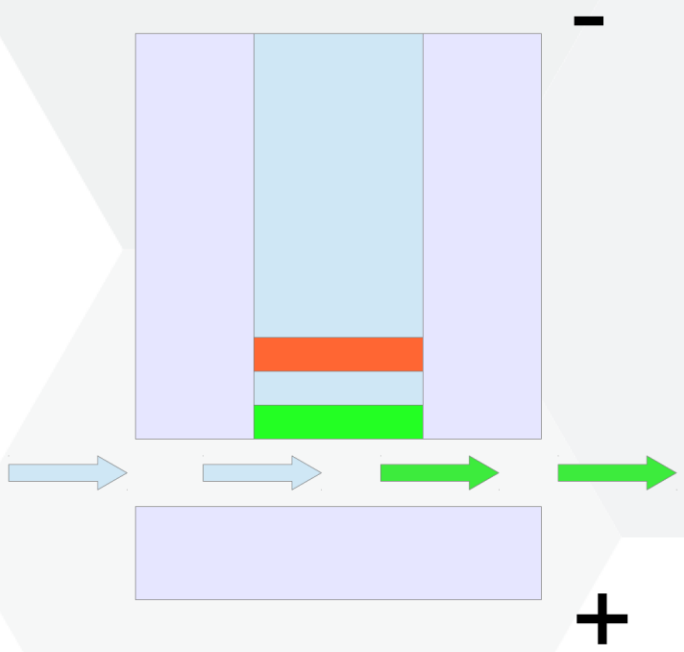
Mastroianni, A.J., Claridge, S.A., Alivisatos, A.P. (2009) J. Am. Chem. Soc., 131 (24), 8455-8459.

Gel electrophoresis – Elution

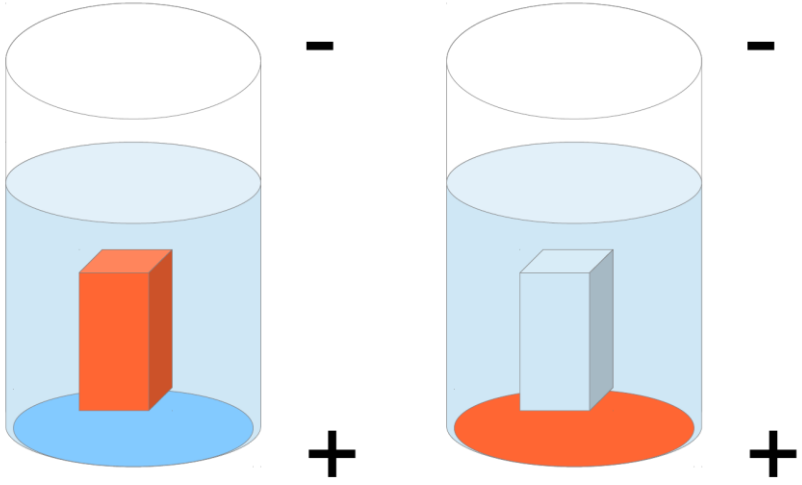
Diffusion



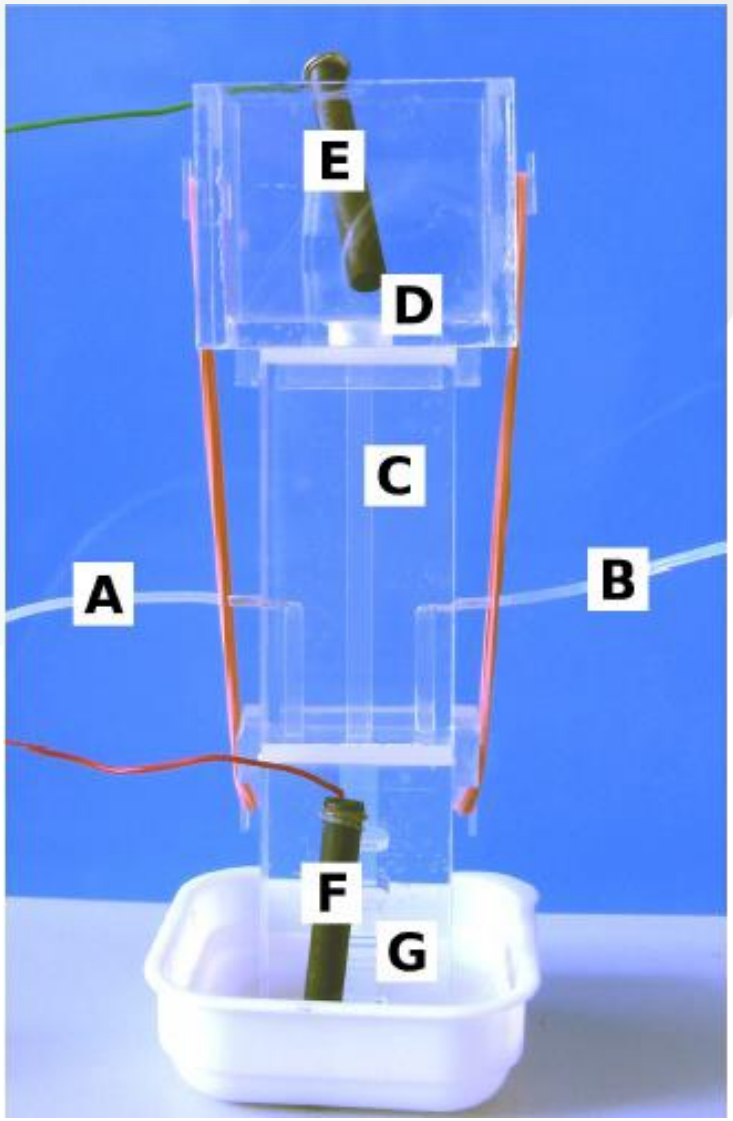
Continuous elution

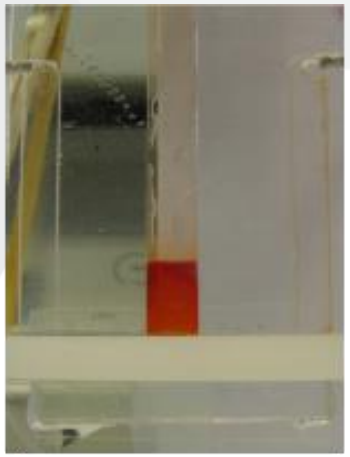


Electroelution

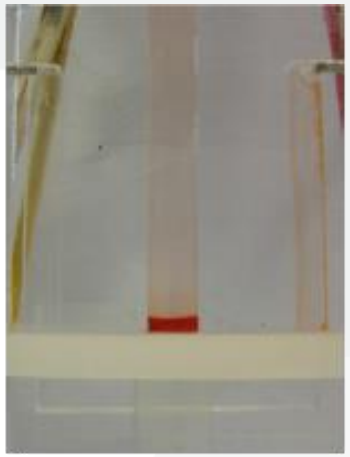


Gel electrophoresis – Continuous elution





1

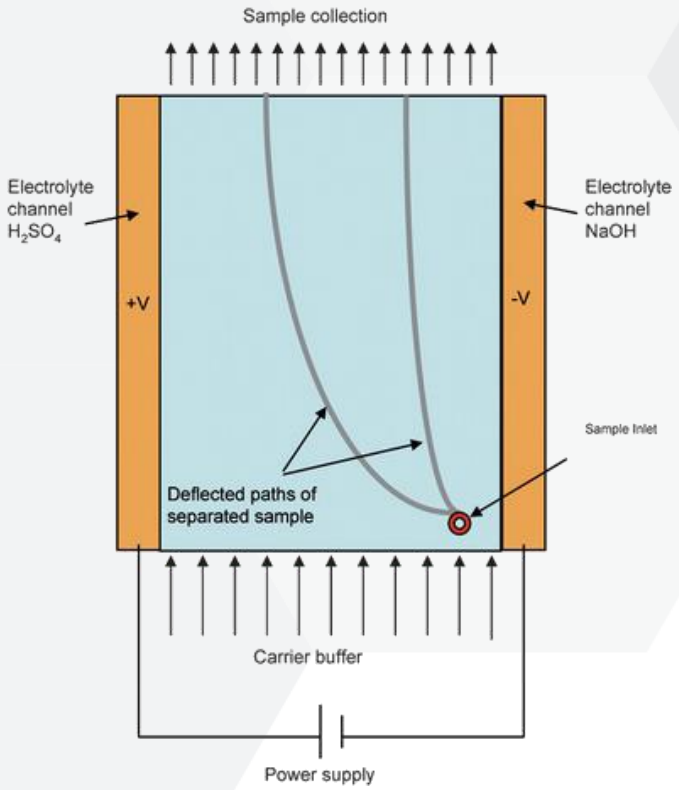


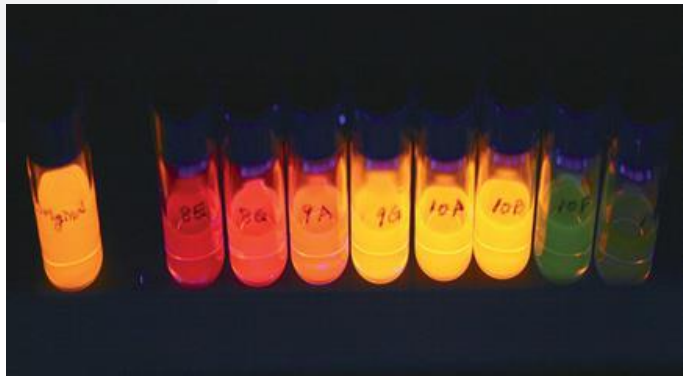
2

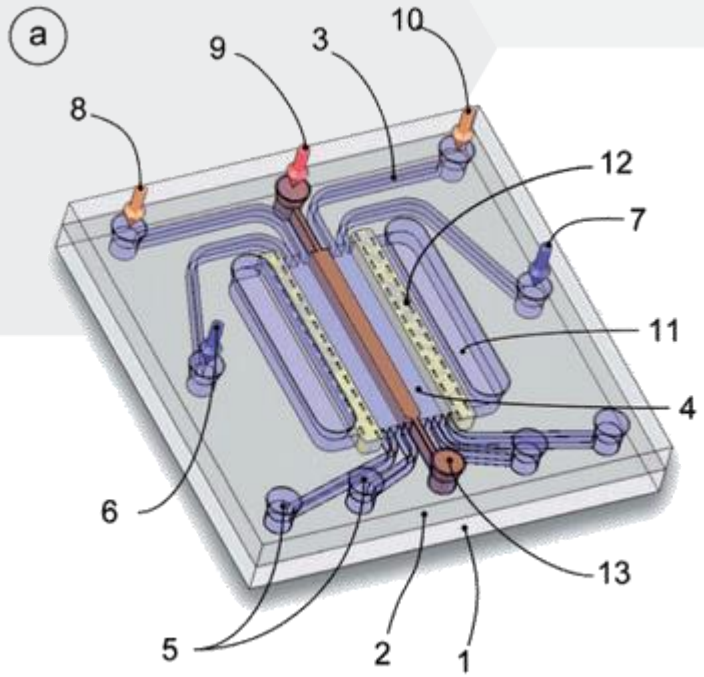
3

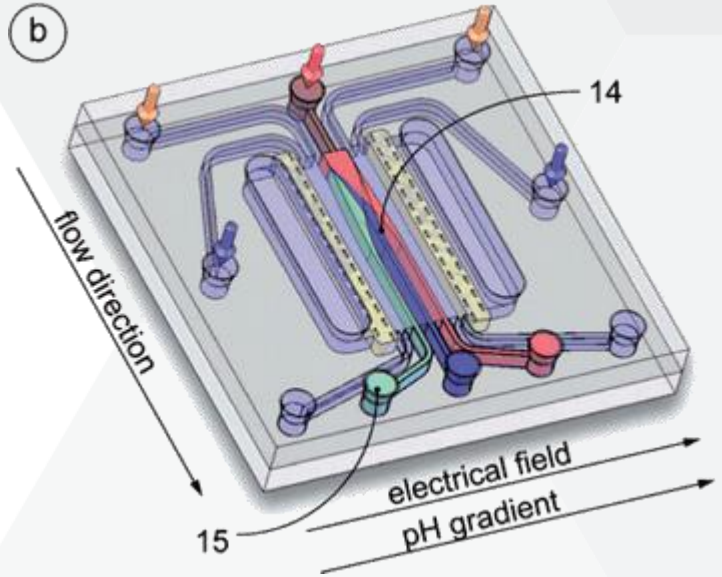
4

Free flow electrophoresis







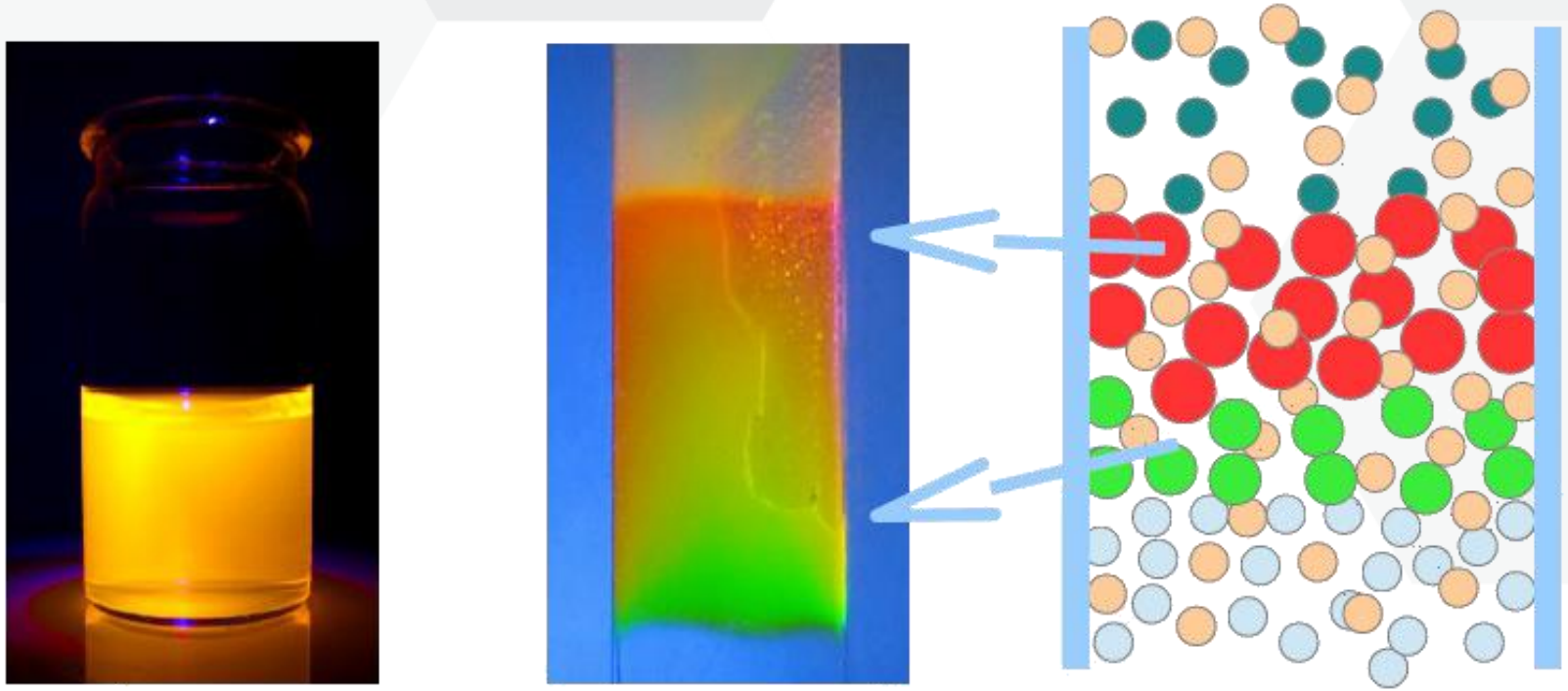


FFIEF chip layout and working principle
(a) no voltage applied; no separation **(b)** voltage applied, IEF of three components. **1** bottom chip plate; **2** top chip plate; **3** microfluidic channel; **4** separation chamber; **5** outlets; **6** low-pH sheath flow inlet; **7** high pH sheath flow inlet; **8** ampholytes 1 inlet; **9** ampholytes 2 + sample inlet; **10** ampholytes 3 inlet; **11** electrode compartment; **12** conductive membrane; **13** not separated sample; **14** focused sample; **15** collected sample.

Kohlheyer, D., Eijkel, J.C.T., Schlautmann, S., Van Den Berg, A., Schasfoort, R.B.M. (2007) Anal. Chem., 79 (21), 8190-8198.

Isotachophoresis

- Electric field produces sharply bounded zone containing the separated nanoparticles.
- Can be used for purification and concentration of nanoparticles

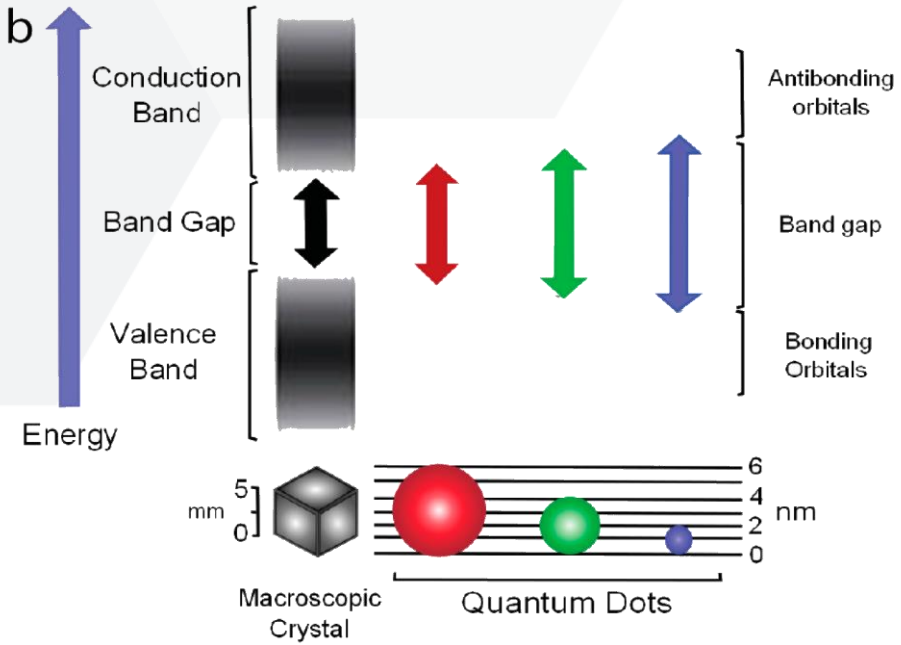


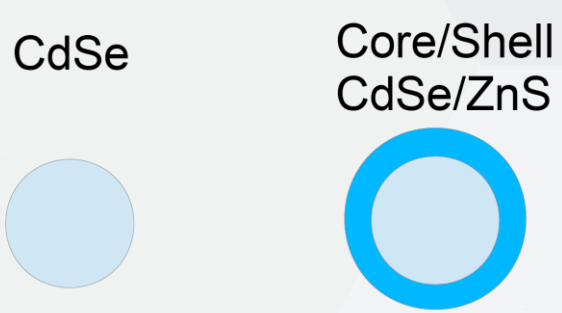
Hlaváček, A., Skládal, P. (2012) Electrophoresis, 33 (9-10), 1427-1430.

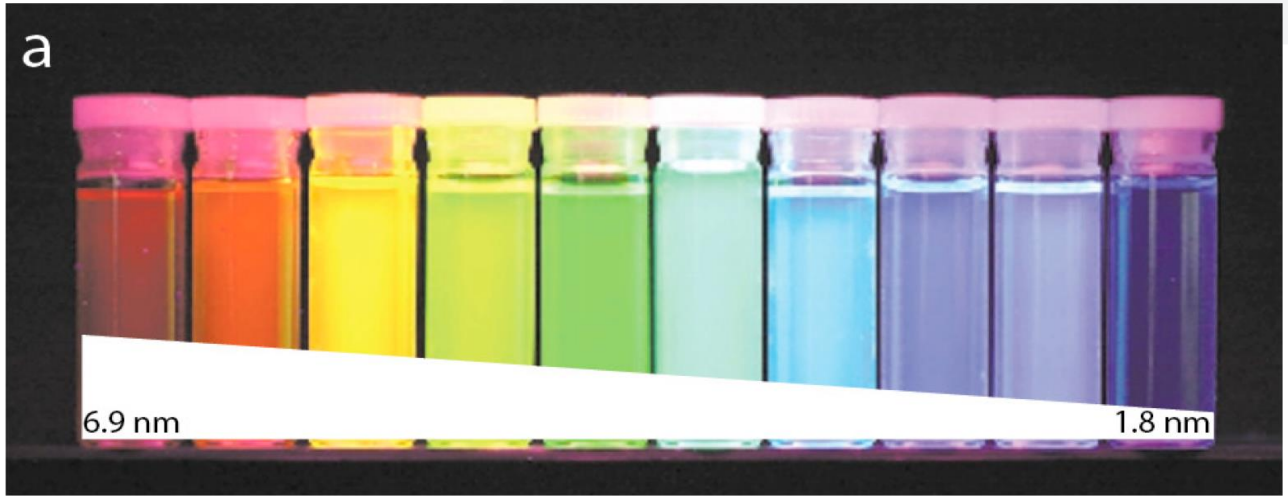
Luminescent nanoparticles

Quantum dots

- Semiconductor nanoparticles (small size of 2–10 nm)
- Typically prepared from group II and VI elements (e.g. CdSe) and group III and V elements (e.g. InP, InAs)
- Size-dependent fluorescence properties
- Often core-shell structure (e.g. CdSe/CdS)

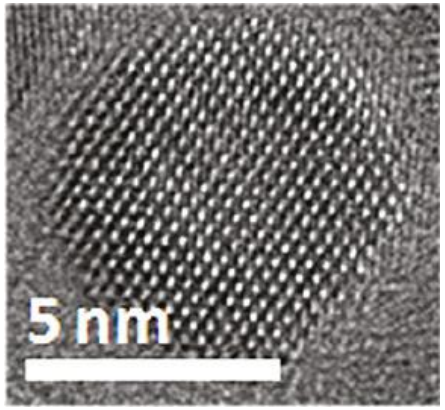


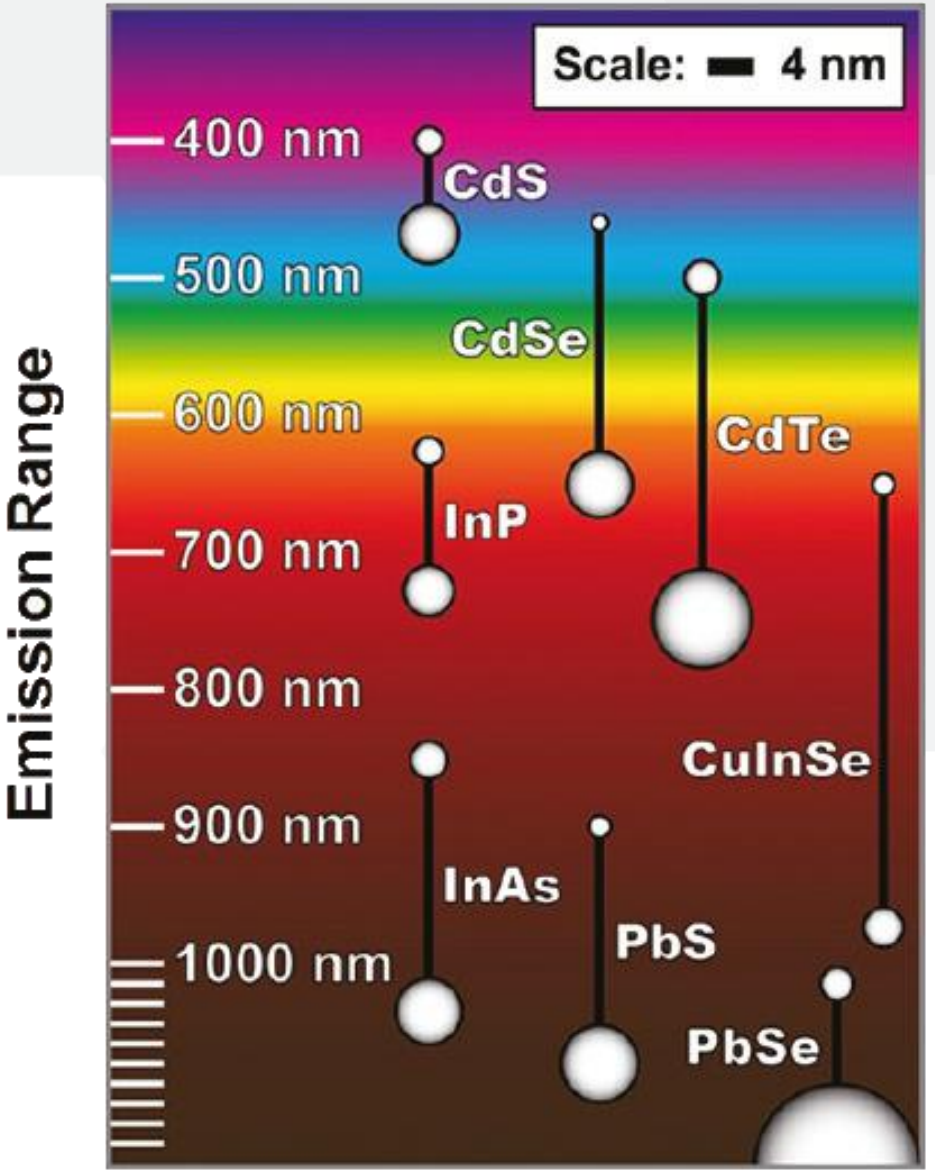




Quantum dots

- Emission between 400 and 4000 nm
  - Different materials and sizes
- High photostability
- Excitation of different particles by single wavelength
  - Single excitation for multiplexed detection
  - Large Stokes shift (Reduction of fluorescence background)
- High absorption coefficients and quantum yields
  - 20–30× higher brightness than organic fluorophores





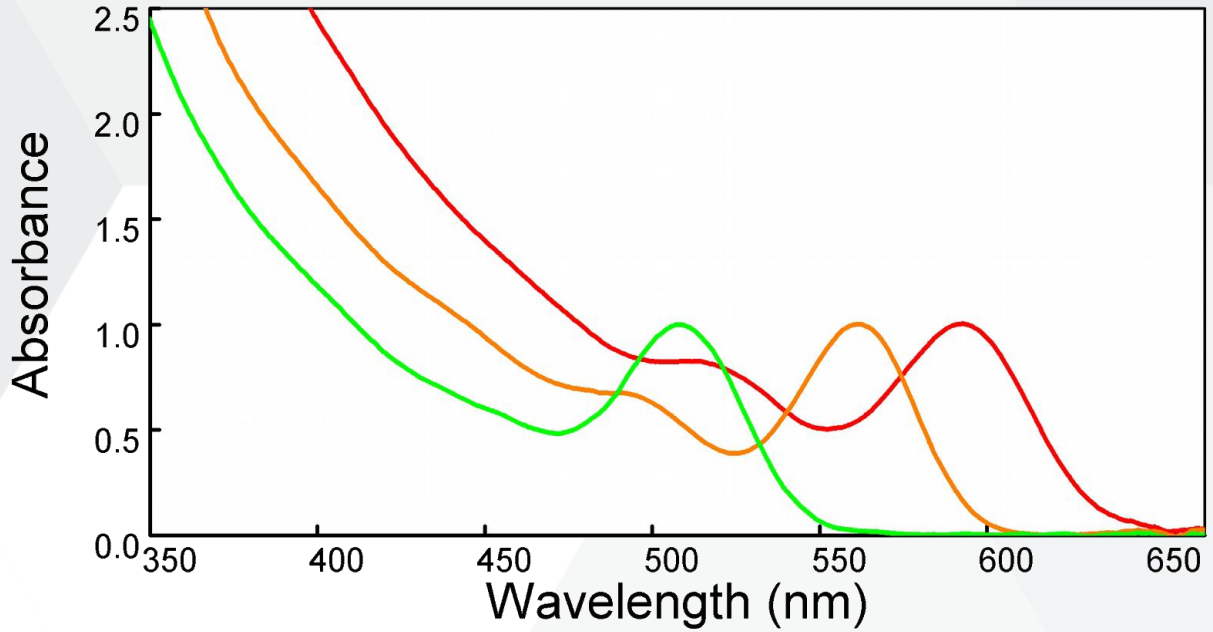
Algar W.R., Susumu K., Delehanty B., Medintz I.L. (2011) Anal. Chem. 83 (23) 8826-8837.

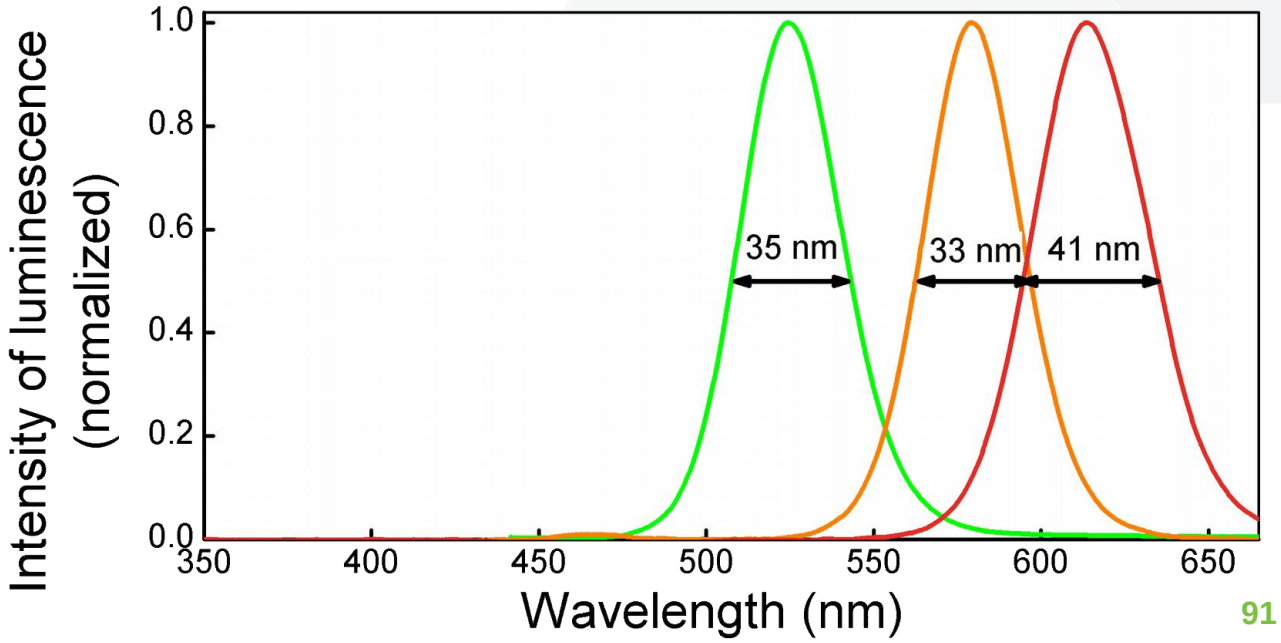
Quantum dots

CdTe
⌀ 2.5 nm

CdTe
⌀ 3.0 nm

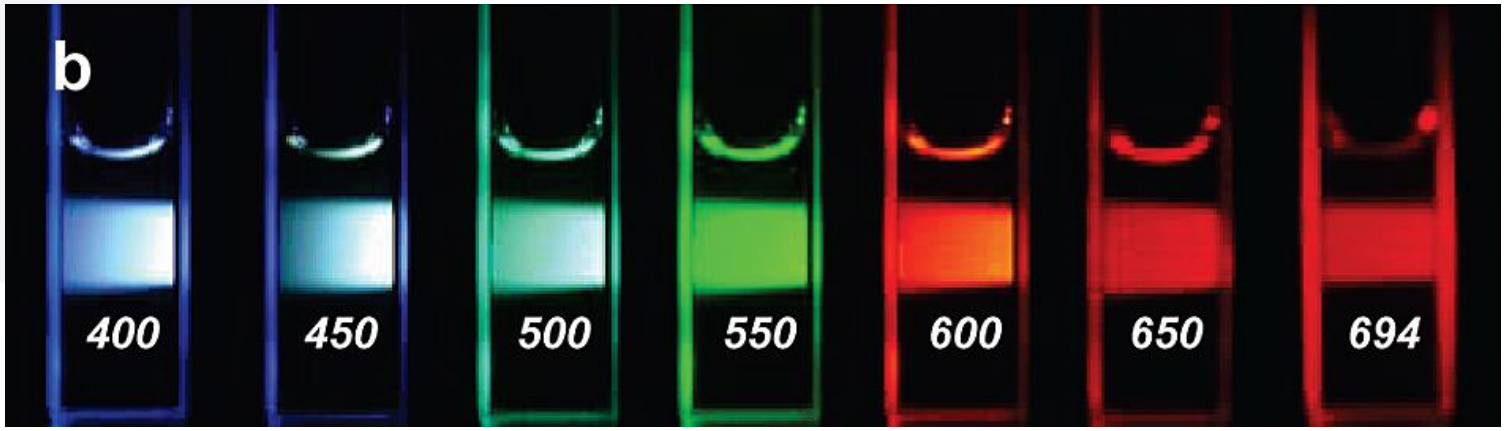
CdTe
⌀ 3.4 nm

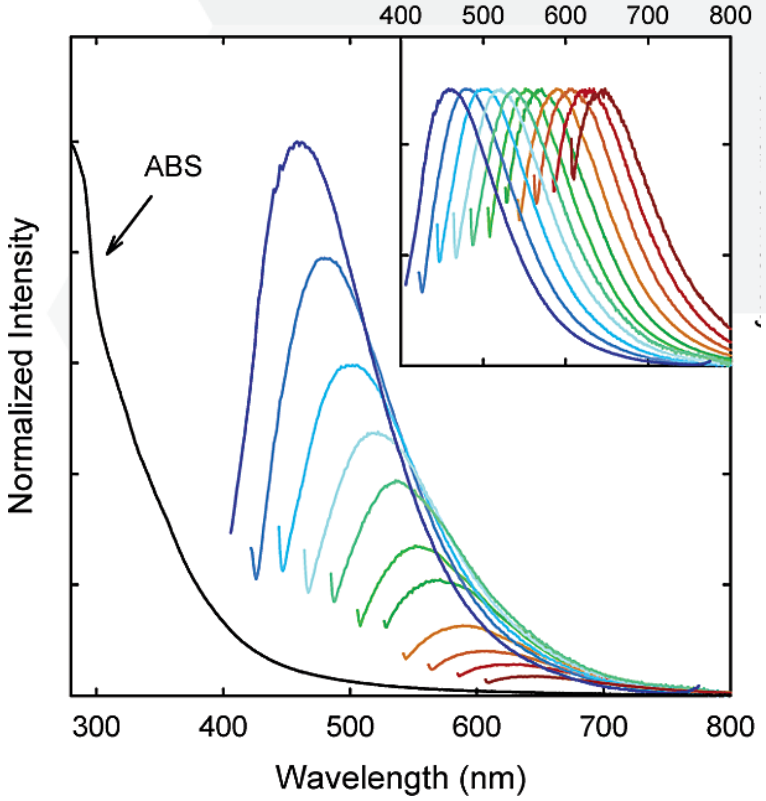




Carbon dots

- Carbon-based nanomaterial
- Low toxicity, high stability, good conductivity
- Size approximately 10 nm
- Surface covered by –COOH groups
  - Solubility, biocompatibility
  - Bioconjugations





Sun Y.P., Zhou B., Lin Y., Wang W., Fernando K.A.S., Pathak P., Meziani M.J., Harruff B.A., Wang X., Wang H., Luo P.G., Yang H., Kose M.E., Chen B., Veca L.M., Xie S.Y. (2006) J. Am. Chem. Soc. 128 (24) 7756-7757.

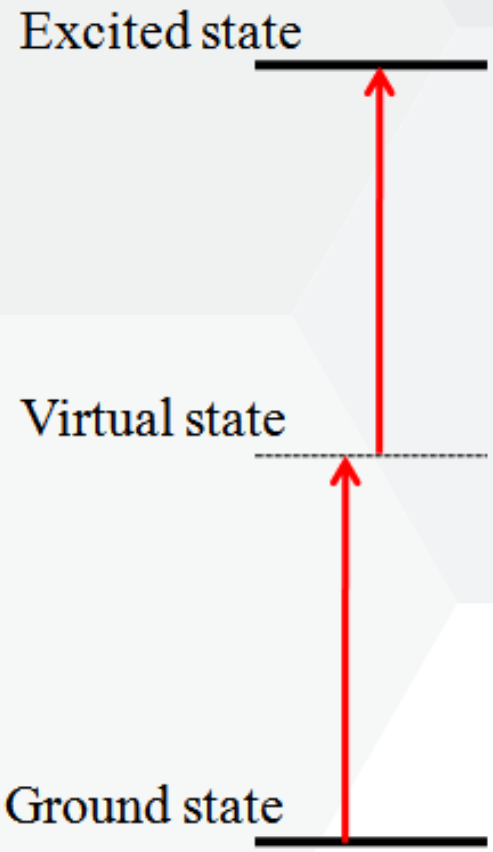
Anti-Stokes luminescence

- Organic fluorophores can be excited by light with shorter wavelengths
- Part of energy is dissipated by irradiative processes, rest can be emitted as light with longer wavelength
- The energy of emitted light is directly proportional to excitation energy

- Some processes, however, can transform light with linger wavelength to shorter wavelength
- Energy of multiple photons is added up and emitted as one photon with higher energy
- These processes are called nonlinear, because emitted intensity depends on higher powers (typically I^2) of excitation intensity
- Processes
  - Multi-photon luminescence
  - Second harmonic generation
  - Photon-upconversion

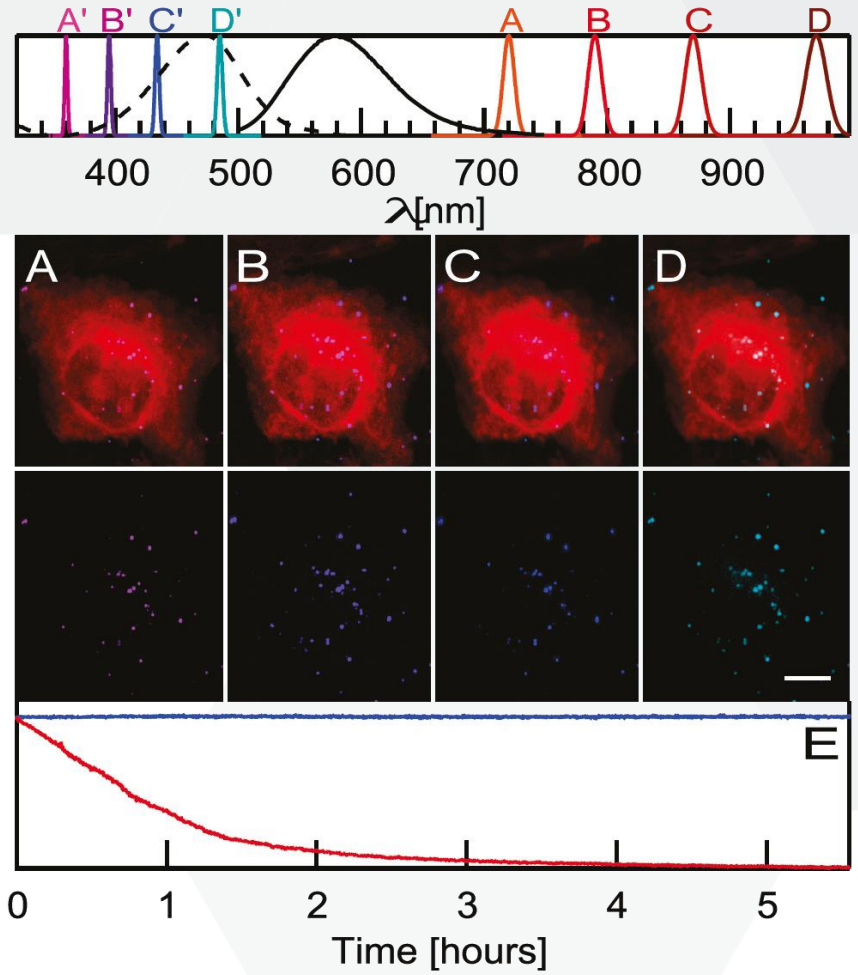
Two and multi-photon luminescence

- First described in 1931, first observed in 1961 (short after development of first laser)
  - Greater development with discovery femtosecond lasers (1990)
- Simultaneous interaction of several photons with one molecule
  - Organic fluorophores as well as nanocrystals
- High intensity of radiation is required
- Two-photon fluorescence microscopy
  - Typically uses near-infrared excitation
  - Reduction of scattering in the tissue
  - Suppression of background signals
  - Increased penetration depth



Second harmonic generation

- Increase in the energy of photons that pass through a material with certain properties
- Output radiation has a double frequency than the radiation entering
- Discovered in 1961 (allowed by discovery of laser)
- Intensity of emitted radiation is proportional to the second power of excited radiation
- Nanocrystals of $KNbO_3$, $LiNbO_3$, $BaTiO_3$, ZnO
- Application in fluorescence microscopy
  - No radiation degradation – suitable for long-term experiments
  - Freely variable excitation/emission wavelength
  - Very narrow emission peaks
  - Emitted radiation is coherent

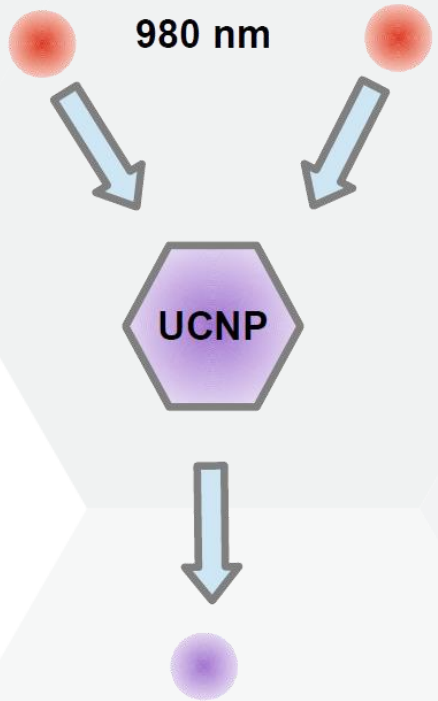


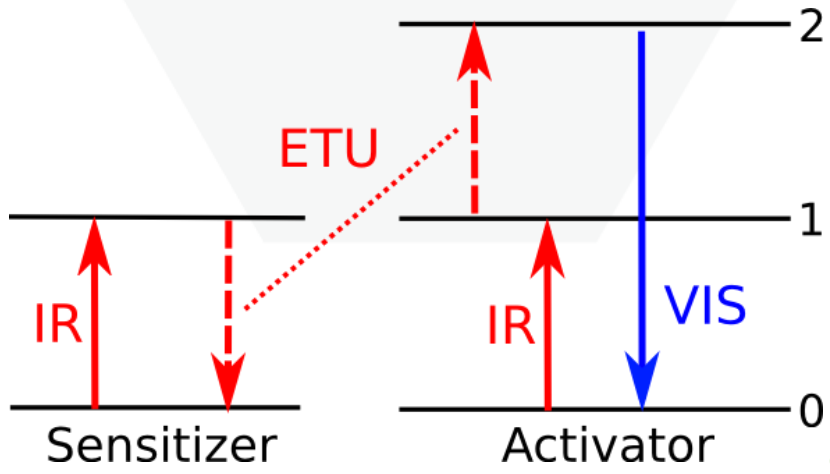
Top: Excitation (A, B, C, D) and emission (A‘, B‘, C‘, D‘) spectra of $KNbO_3$ nanoparticle and organic fluorophore FM 1-43
Middle: Cell membrane labelled by FM 1-43 and $KNbO_3$ NPs excited under various wavelengths
Bottom: Comparison of fluorescence decrease (degradation) of FM 1-43 and $KNbO_3$ NPs

Staedler, D., Magouroux, T., Hadji, R., Joulaud, C., Extermann, J., Schwung, S., Passemard, S., Kasparian, C., Clarke, G., Gerrmann, M., Dantec, R.L., Mugnier, Y., Rytz, D., Ciepielewski, D., Galez, C., Gerber-Lemaire, S., Juillerat-Jeanneret, L., Bonacina, L., Wolf, J.-P. (2012) ACS Nano, 6 (3), 2542-2549.

Photon-upconversion

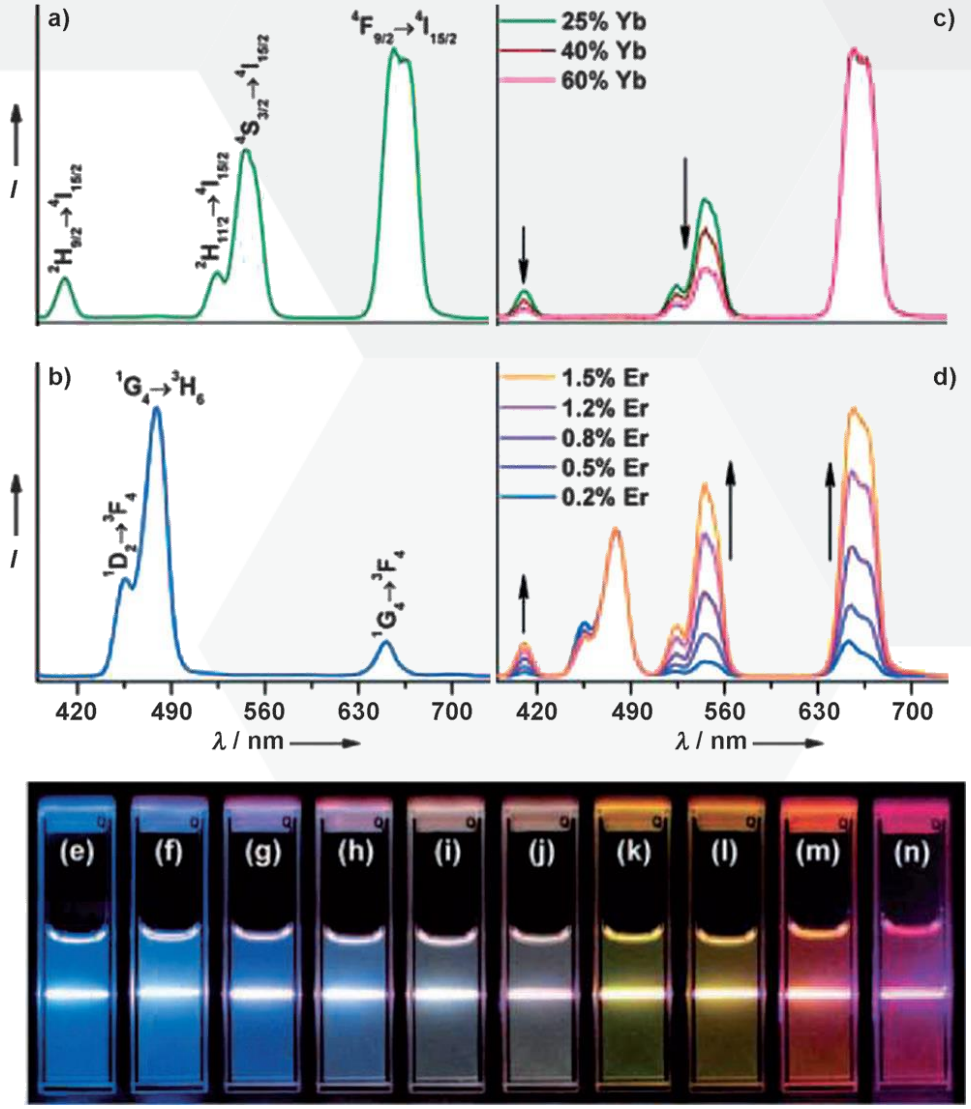
- Gradual absorption of several photons
  - Intensity of the emitted radiation is again proportional to the higher powers of the excitation radiation
  - Lower excitation intensities compared to multi-photon luminescence harmonic generation
  - Excitation using normal semiconductor laser
- Upconversion was observed in materials doped with some d elements (e.g. Ti, Ni, Mo, Os) and actinoids
- High upconversion exhibited by nanoparticles of materials doped by rare earth metals
  - Crystalline $NaYF_4$ matrix doped with various lanthanides (e.g. Yb^{3+} and Er^{3+} or Yb^{3+} and Tm^{3+})





Photon-upconversion nanoparticles

- Modification of UCNP surface typically by silanization
- Advantages
  - Low autofluorescence and light scattering
  - No optical background, high signal to noise ratio
  - High stability
- Applications
  - Luminescent labels for immunoassays and microscopy
  - Luminescence resonance energy transfer
  - Photodynamic therapy
  - Temperature sensing



Wang, F., Liu, X. (2008) J. Am. Chem. Soc., 130 (17), 5642-5643.

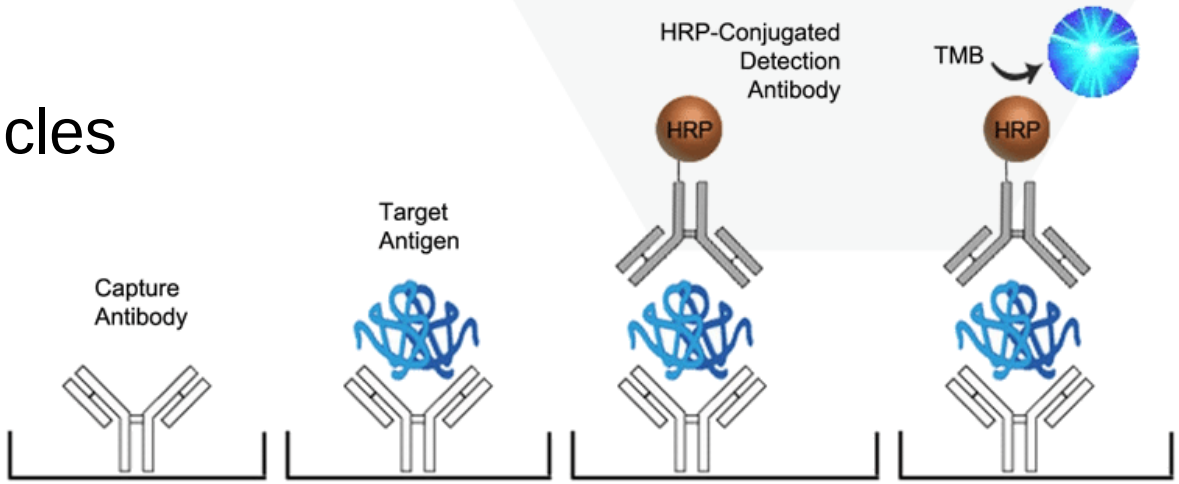
Biological applications of nanoparticles

Biological applications of nanoparticles

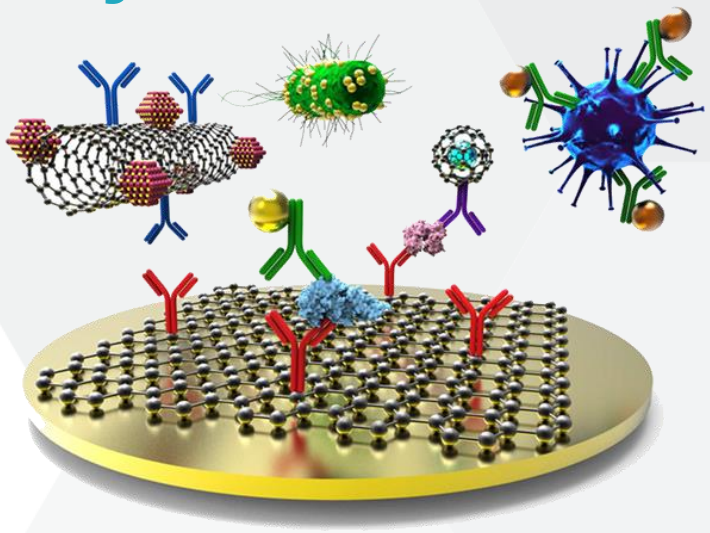
- Detection and signal multiplexing
  - Assays
  - Biosensors
- Biological imaging
  - Luminescent labels
  - Magnetic resonance imaging
- Nanosensors
  - Imaging of chemical composition

Enzyme-linked immunosorbent assay (ELISA)

- Widely used analytical method for detection of various analytes
  - Assay in microtiter plate
  - Specificity provided by antibody
  - Signal amplification provided by enzyme label
- Disadvantages
  - Limited stability of enzymes
  - Time-consuming signal development
  - High enzyme production costs
- Replacement of enzyme by nanoparticles to enhance assay properties



Application of nanoparticles in immunoassays and immunosensors

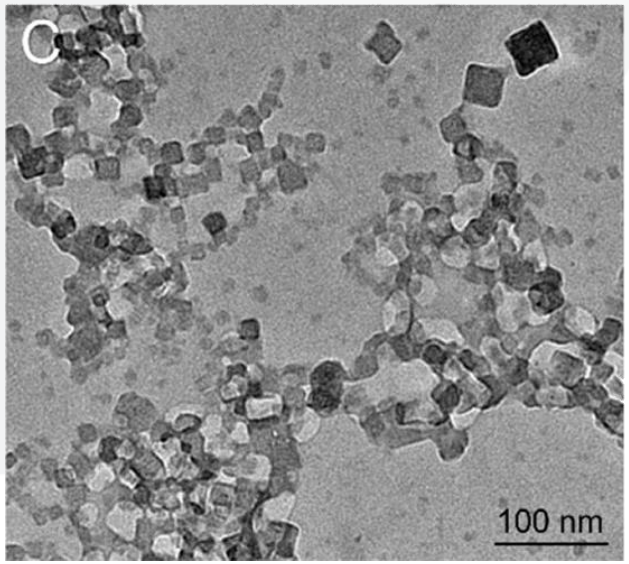


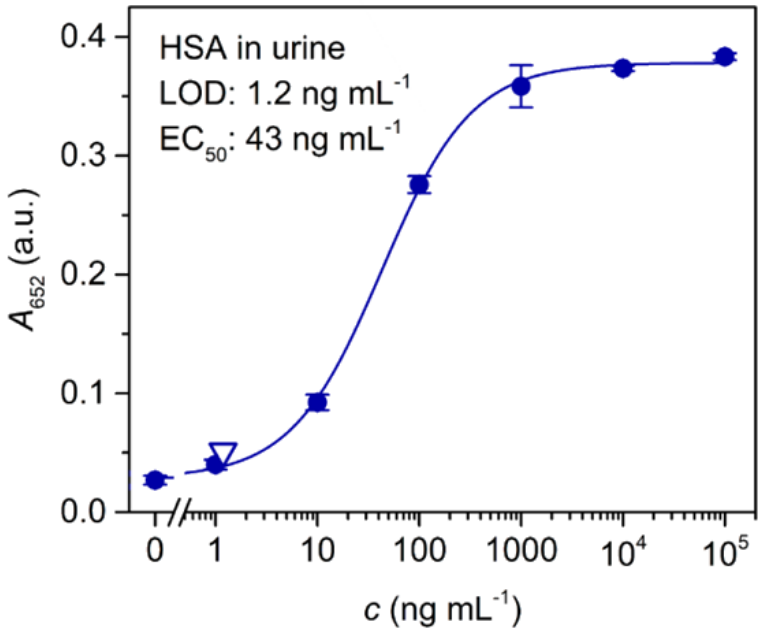
- Sample preconcentration
  - Magnetic nanoparticles
- Enhancement of electrochemical signal (amperometry, electrochemical impedance spectroscopy)
  - Carbon nanotubes, graphene, quantum dots
- Optical detection
  - Quantum dots, photon-upconversion nanoparticles
- Plasmonic properties (surface plasmon resonance, surface enhanced Raman spectroscopy)
  - Gold and silver nanoparticles

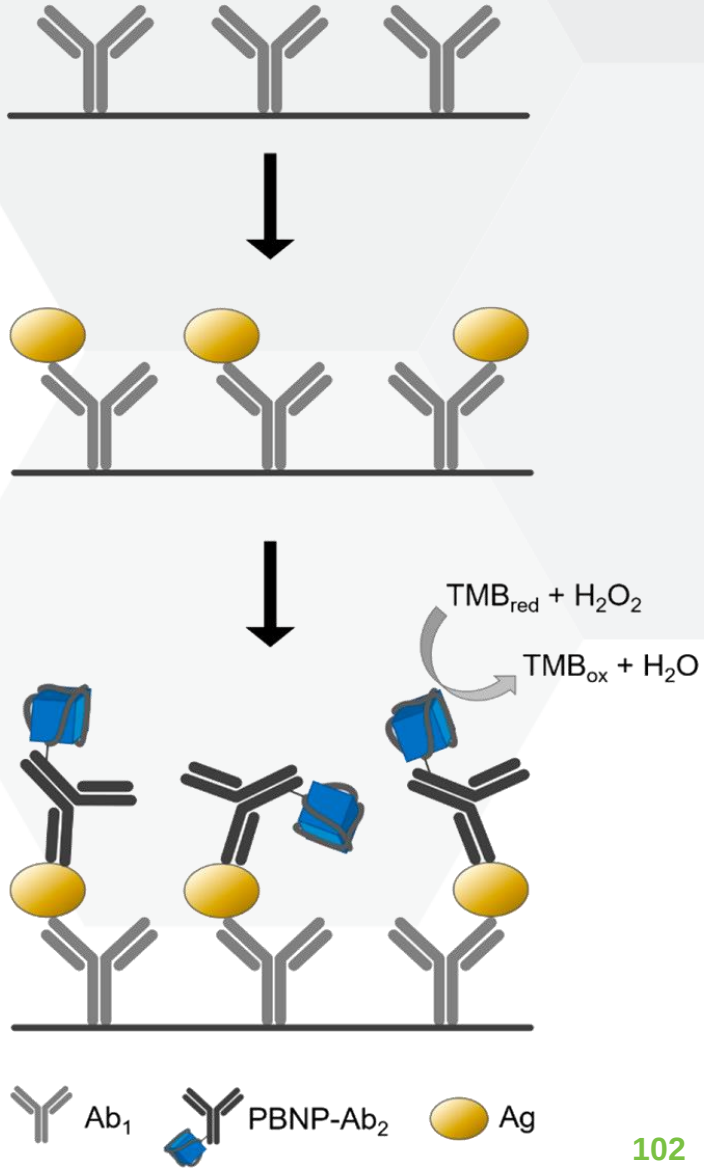
Farka Z., Juřík T., Kovář D., Trnková L., Skládal P. Chem. Rev. 2017, 117(15), 9973–10042.

Nanozyme-linked immunosorbent assay

- Prussian blue nanoparticles
  - $Fe_4[Fe(CN)_6]_3$
- Catalytic activity (oxidation of TMB)
- Higher stability than enzymes



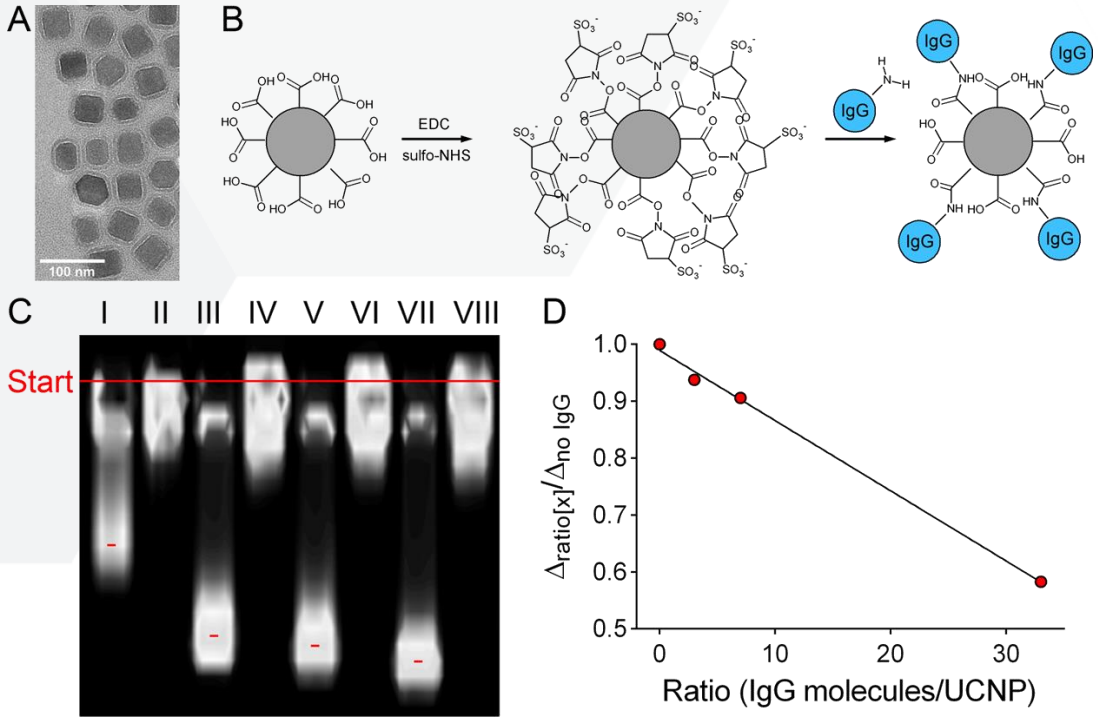


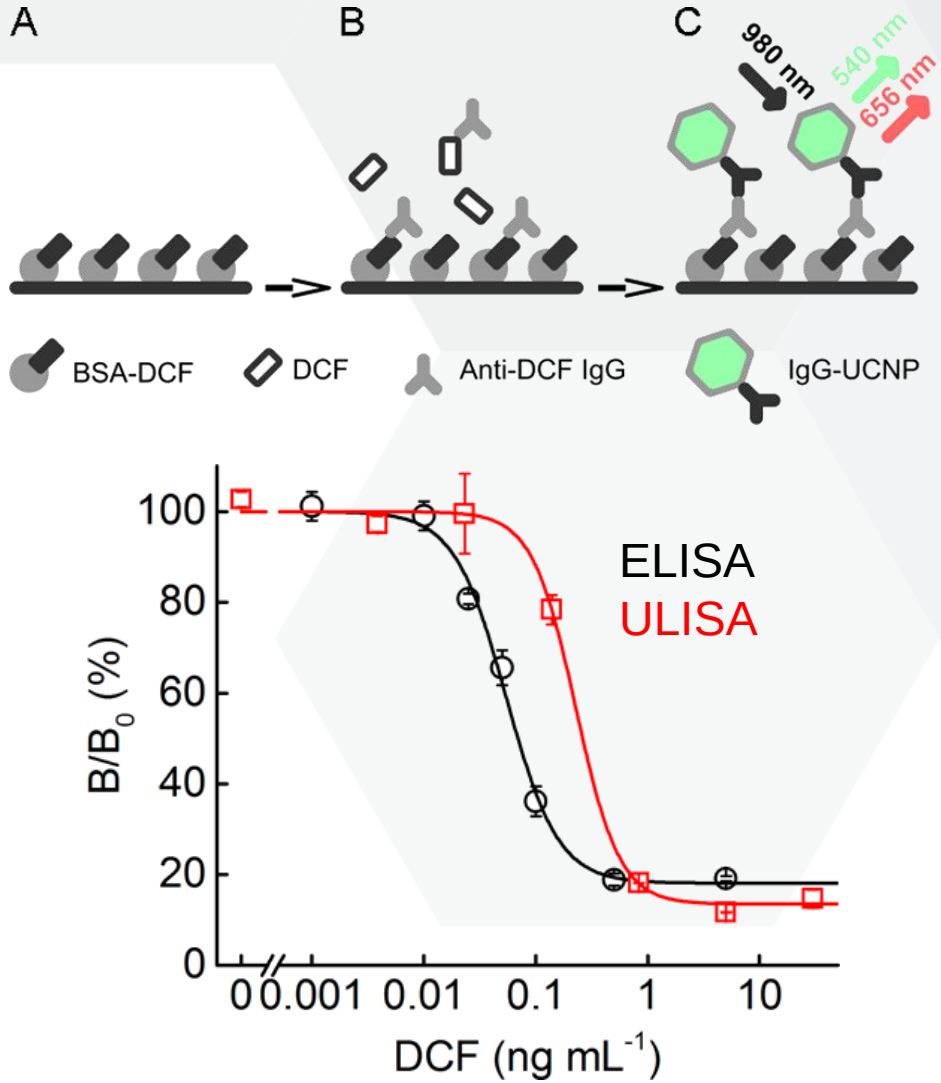


Farka Z., Čunderlová V., Horáčková V., Pastucha M., Mikušová Z., Hlaváček A., Skládal P. Anal. Chem. 2018, 90(3), 2348–2354.

Upconversion-linked immunosorbent assay

- Detection of pharmaceutical diclofenac
- Competitive assay
- High sensitivity, low background

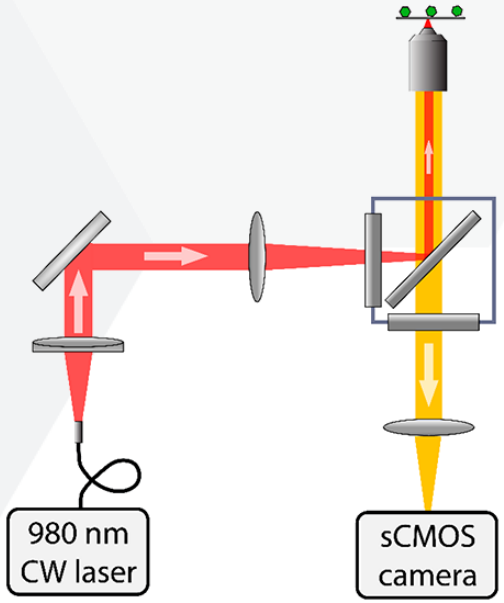


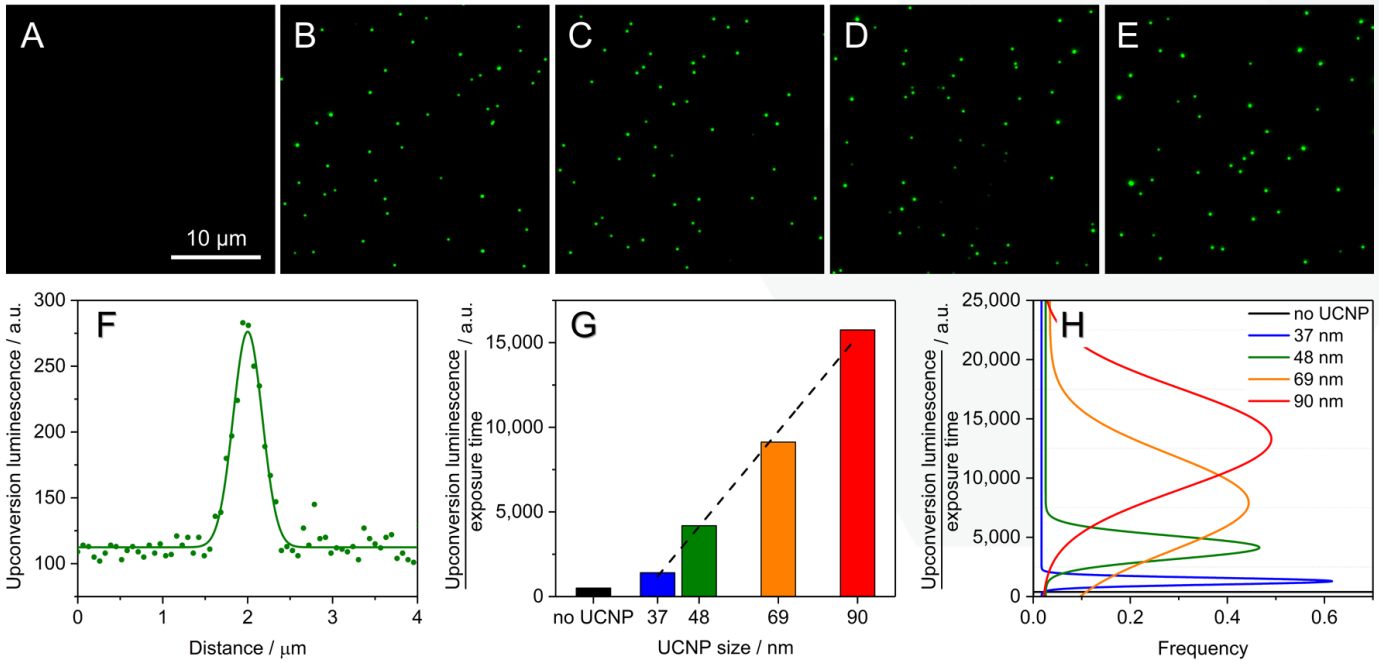


Hlaváček A., Farka Z., Hübner M., Horňáková V., Němeček D., Niessner R., Skládal P., Knopp D., Gorris H. H. Anal. Chem. 2016, 88(11), 6011–6017.

Single-molecule detection

- Low background of UCNPs allows visualization of individual particles by upconversion microscopy
  - 980 nm excitation
  - Individual UCNPs appear as diffraction-limited spots (~ 400 nm in diameter)

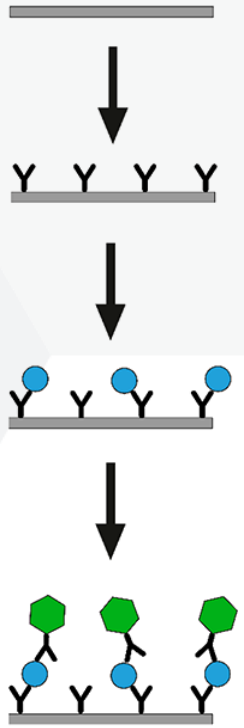


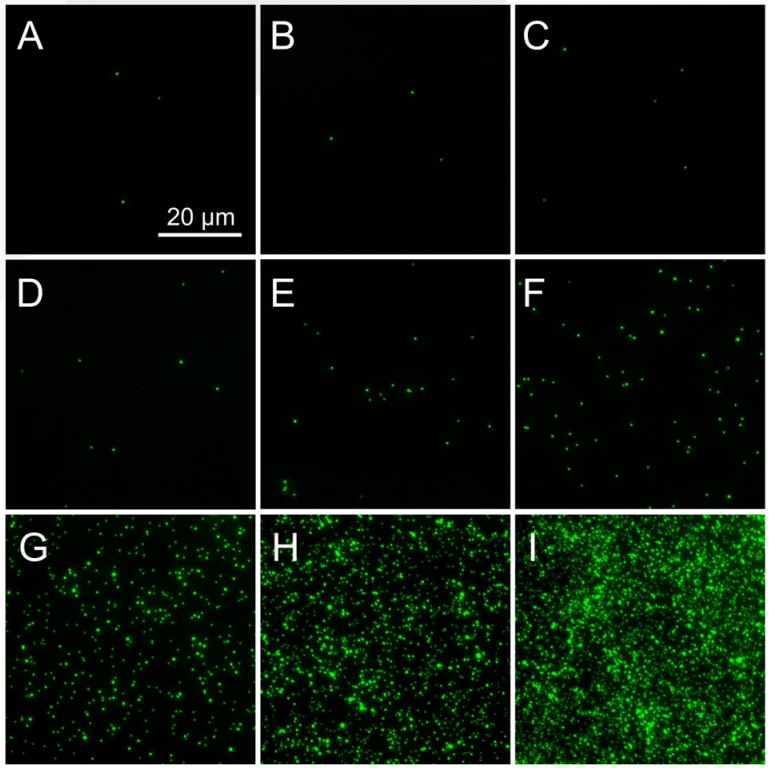


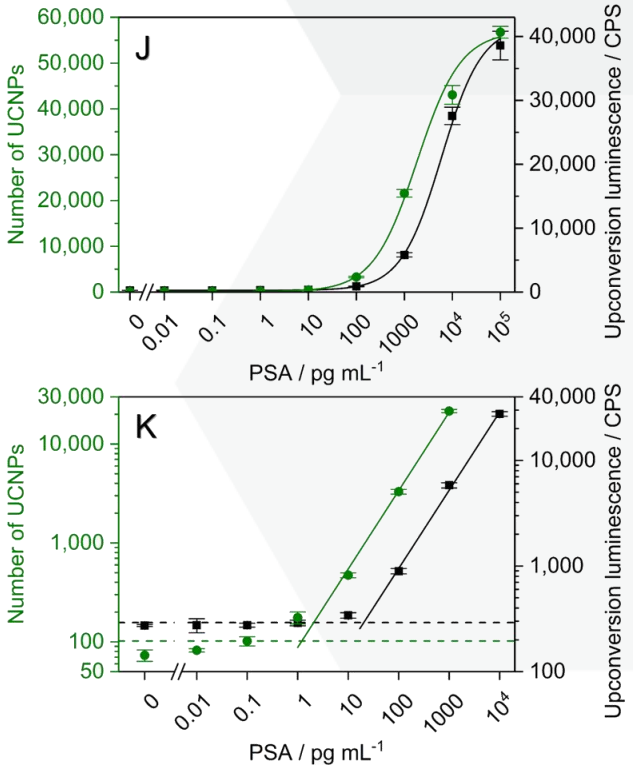
Farka Z., Mickert M. J., Hlaváček A., Skládal P., Gorris H. H.
Anal. Chem. 2017, 89(21), 11825–11830.

Single-molecule detection

- Sandwich immunoassay for prostate specific antigen (PSA)
- Counting of individual immunocomplexes



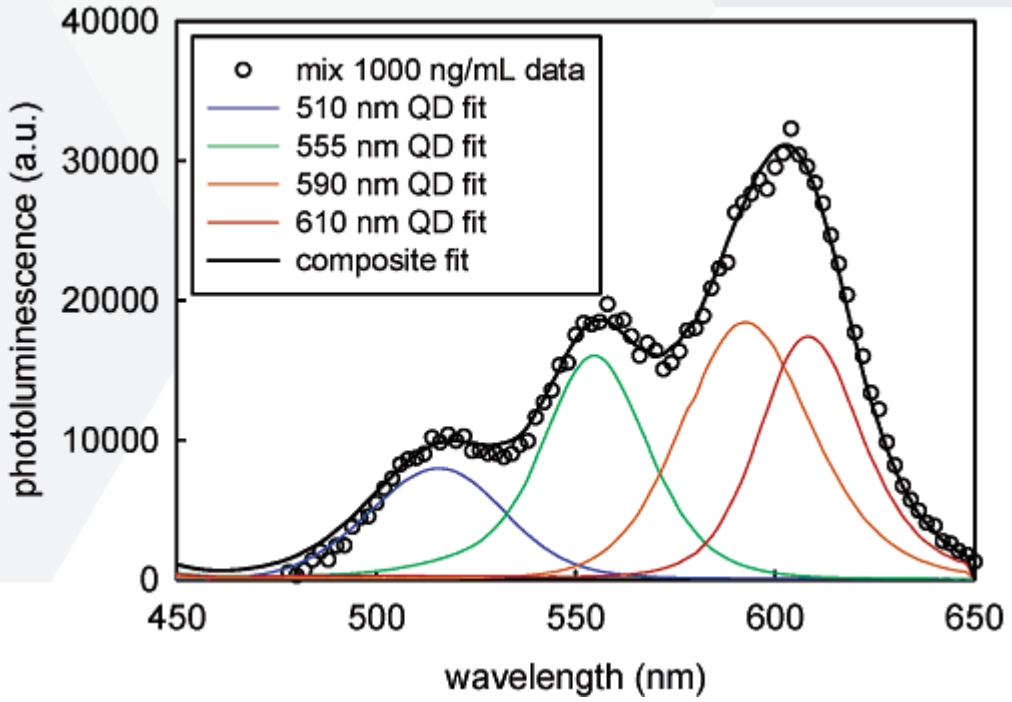


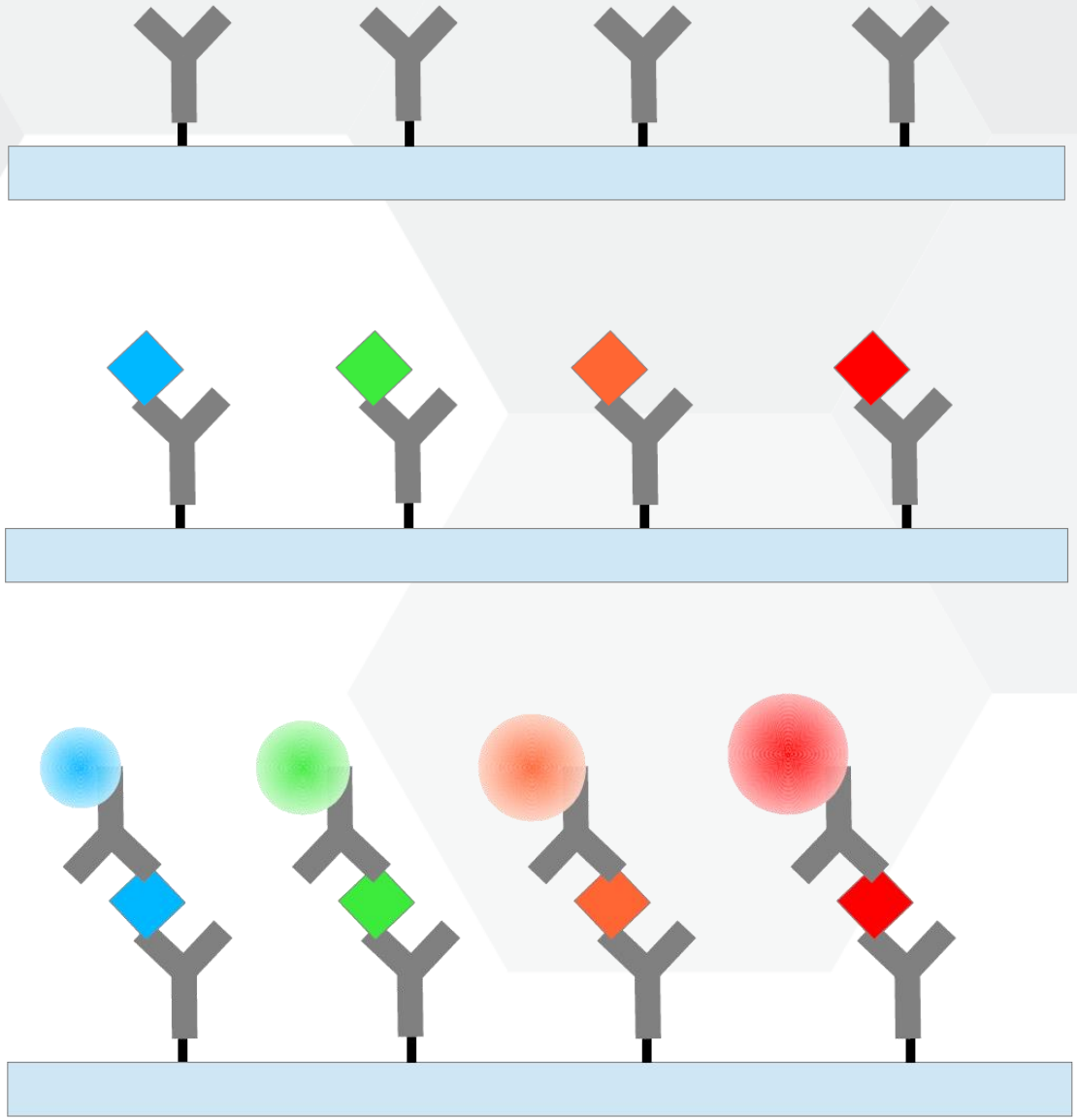


Farka Z., Mickert M. J., Hlaváček A., Skládal P., Gorris H. H.
Anal. Chem. 2017, 89(21), 11825–11830.

Multiplexed detection

- Different antibodies conjugated with QDs of different sizes
- Can be performed in microtiter plate

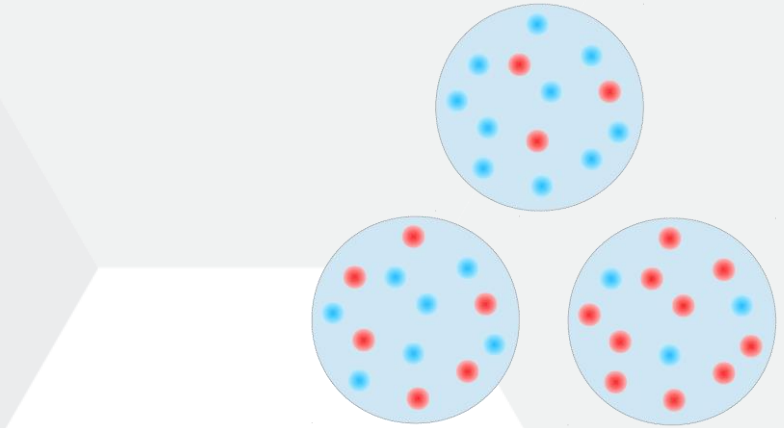


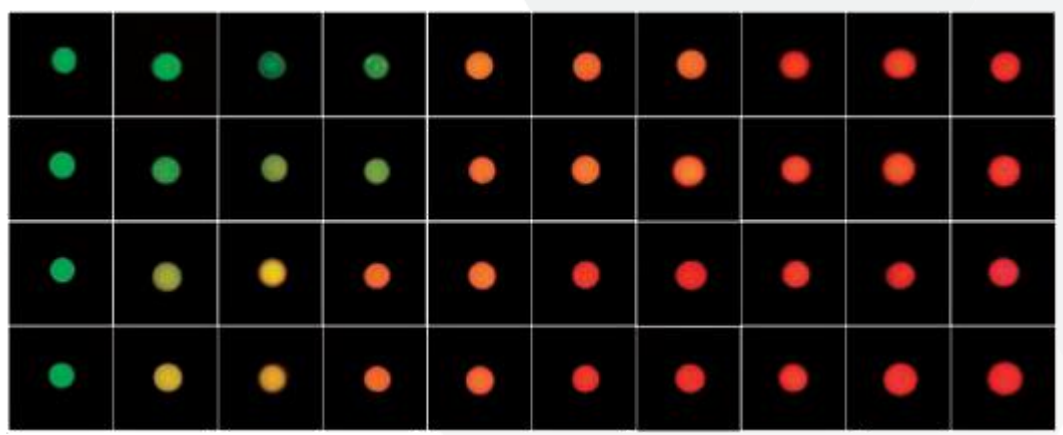


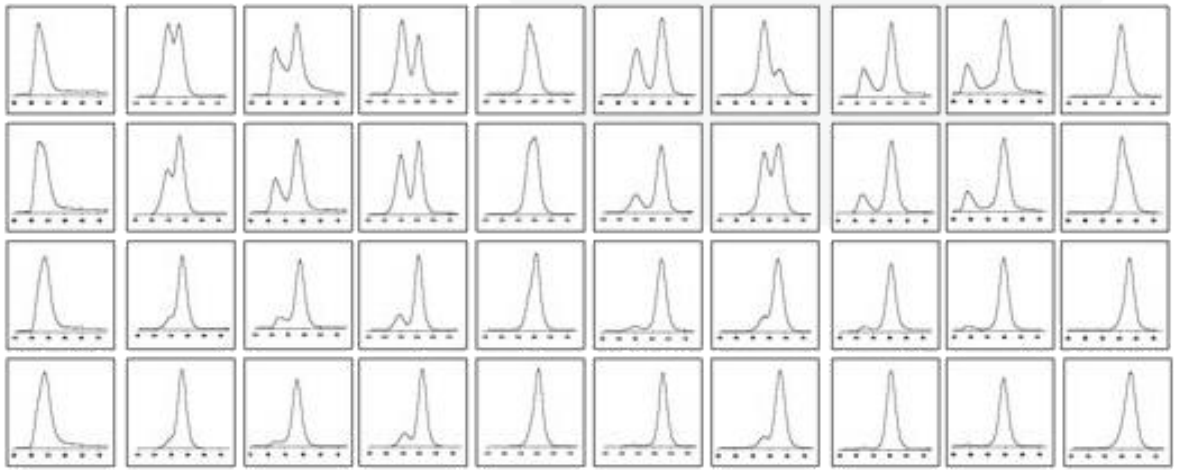
Goldman E.R.,Clapp A.R., Anderson G.P., Uyeda H.T., Mauro J.M., Medintz I.L., Mattoussi H. (2004) Anal. Chem. 76, 684-688.

Microparticle barcoding

- Detection of large number of molecules in complex mixtures
- Microparticles encoded by mixture of QD with different wavelengths
  - Varying ratio or QDs
- Detection of hundreds of analytes in parallel
  - Antibody-antigen
  - DNA-DNA
- Readout using device analogical to flow-cytometer

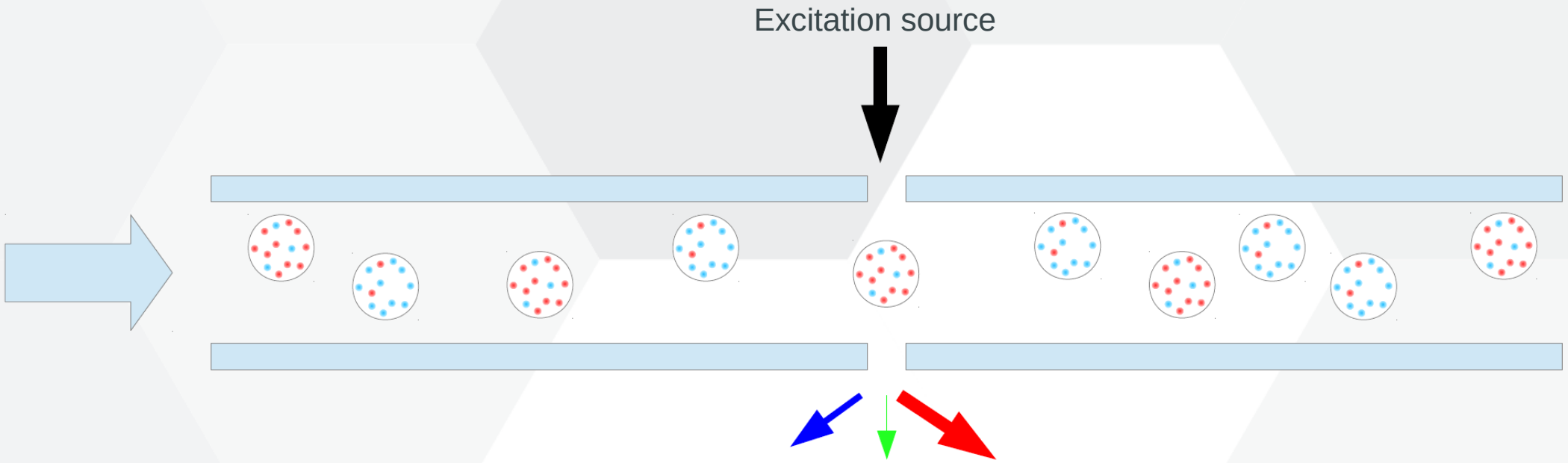




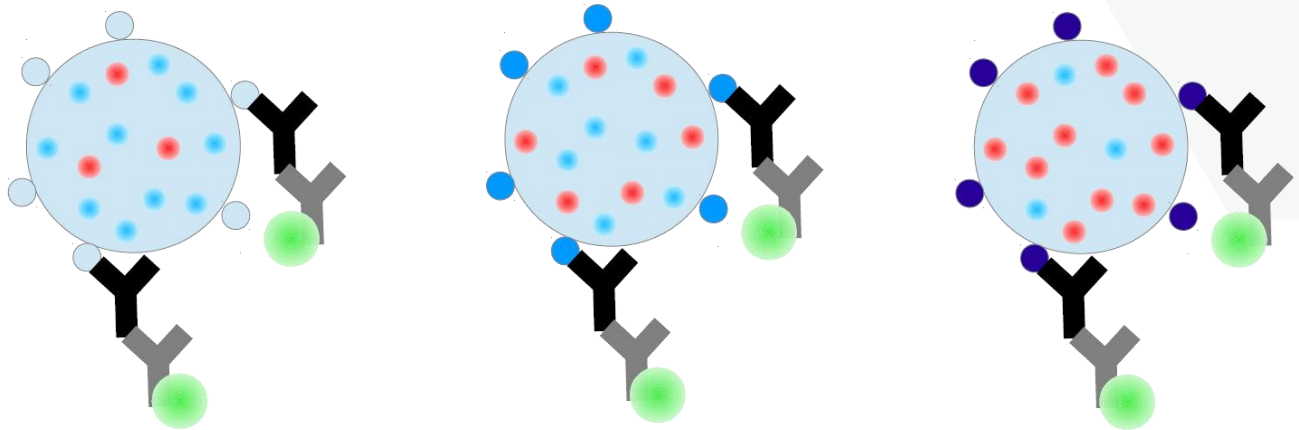


Fournier-Bidoz S, Jennings TL, Klostranec JM, Winnie Fung, Rhee A, Li D, Chan WCW (2008) Angew. Chem. Int. Ed. 47, 5577–5581.

Microparticle barcoding

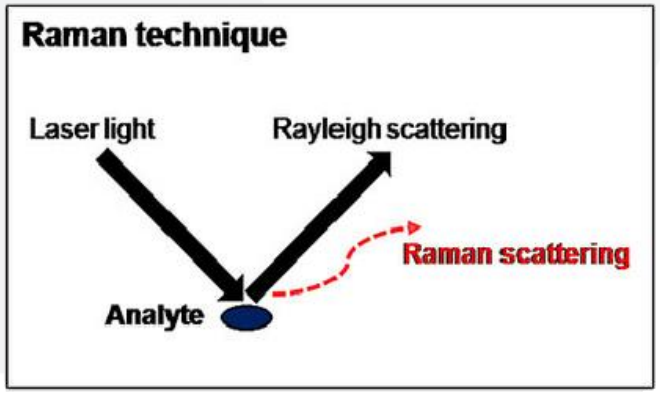


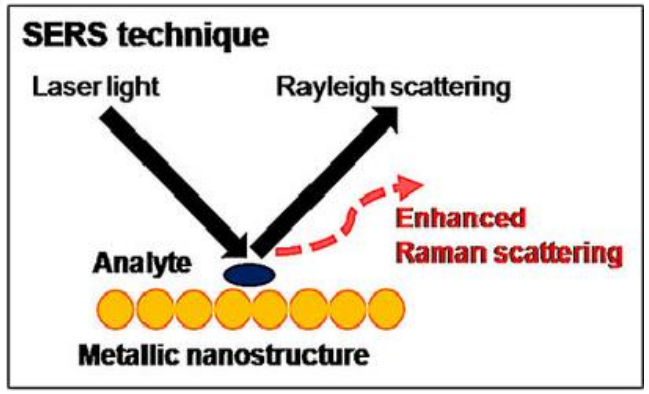
Specificity is determined by the fluorescence code of microparticle.
Binding is detected by fluorescent label, which is the same for all analytes.



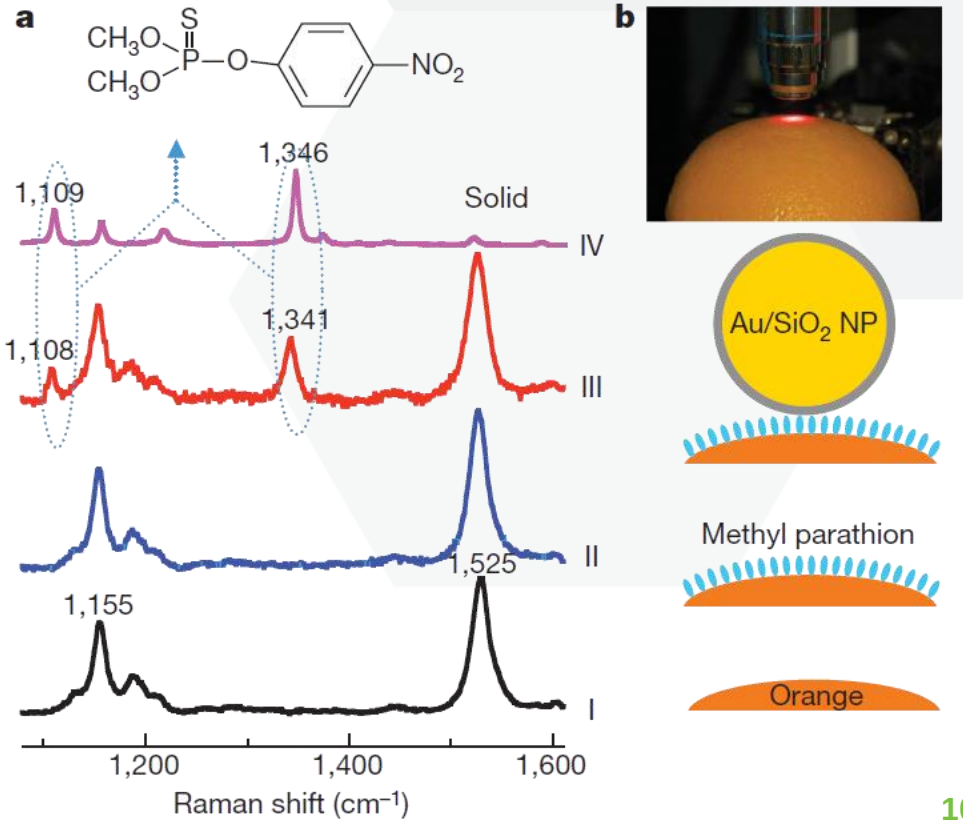
Surface-enhanced Raman spectroscopy

- Enhancement of Raman scattering by adsorption of molecules on rough metal surfaces or by nanostructures
- Typically Au or Ag
- Enhancement factor up to 10^{10}–10^{11}
- Label-free technique





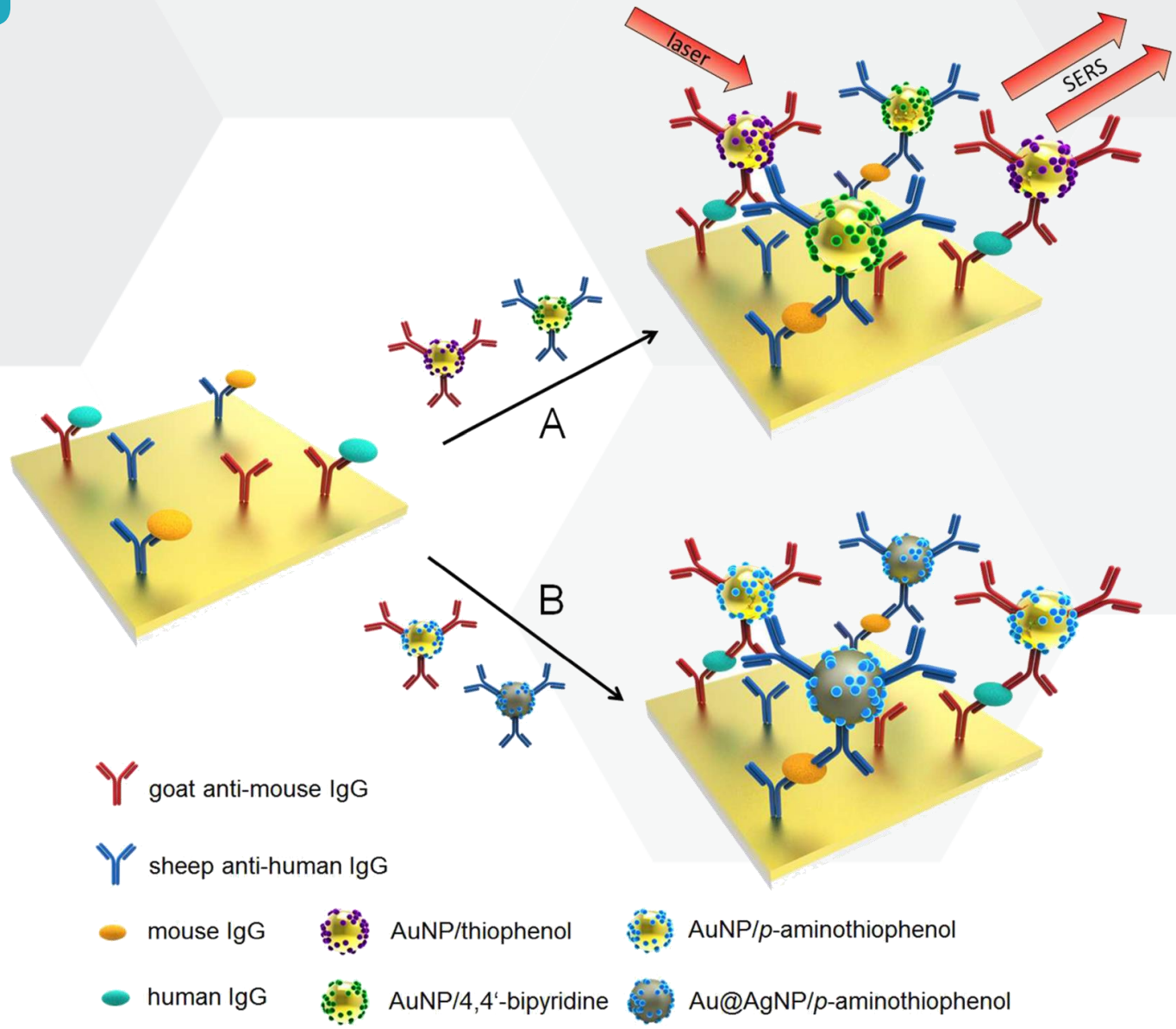
Detection of parathion



Li, J.F., Huang, Y.F., Ding, Y., Yang, Z.L., Li, S.B., Zhou, X.S., Fan, F.R., Zhang, W., Zhou, Z.Y., Wu, D.Y., Ren, B., Wang, Z.L., Tian, Z.Q. (2010) Nature, 464 (7287), 392-395.

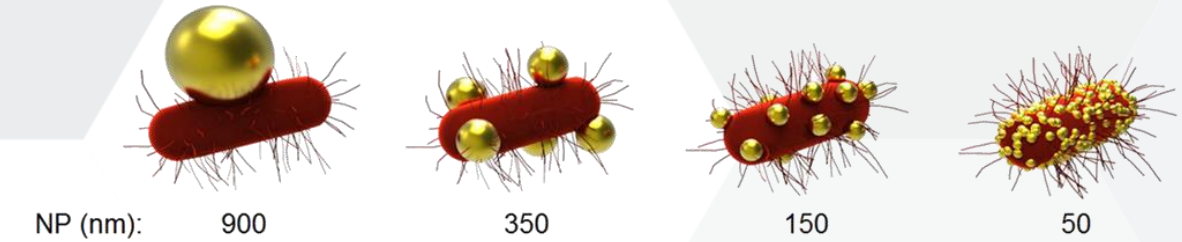
SERS immunosensing

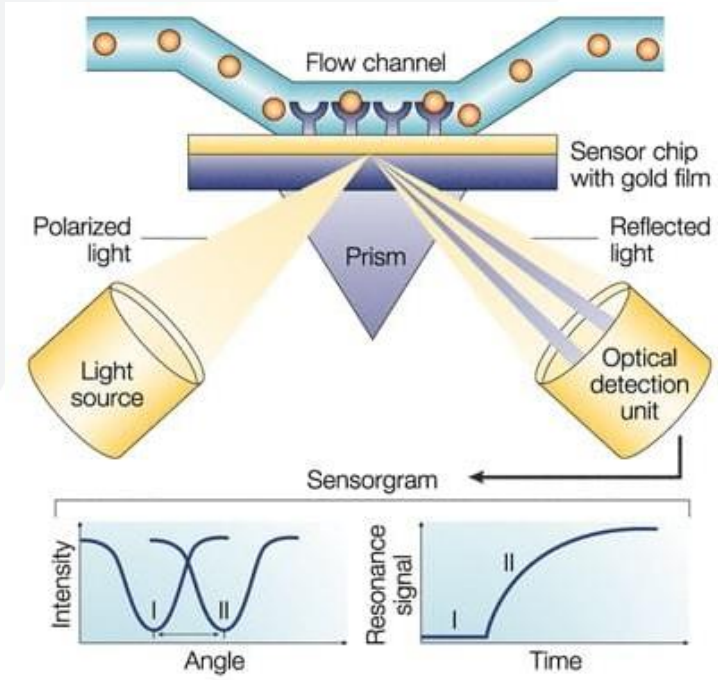
- Typically label-based technique
- SERS nanotag
  - Plasmonic nanoparticle
  - Raman reporter molecules
  - Biorecognition element (antibody, DNA)
- Allows multiplexed detection
- SERS microscopy
  - Direct imaging of fingerprints
  - Utilization of nanotags
- Label-free operation
  - Frequency shift due to analyte binding

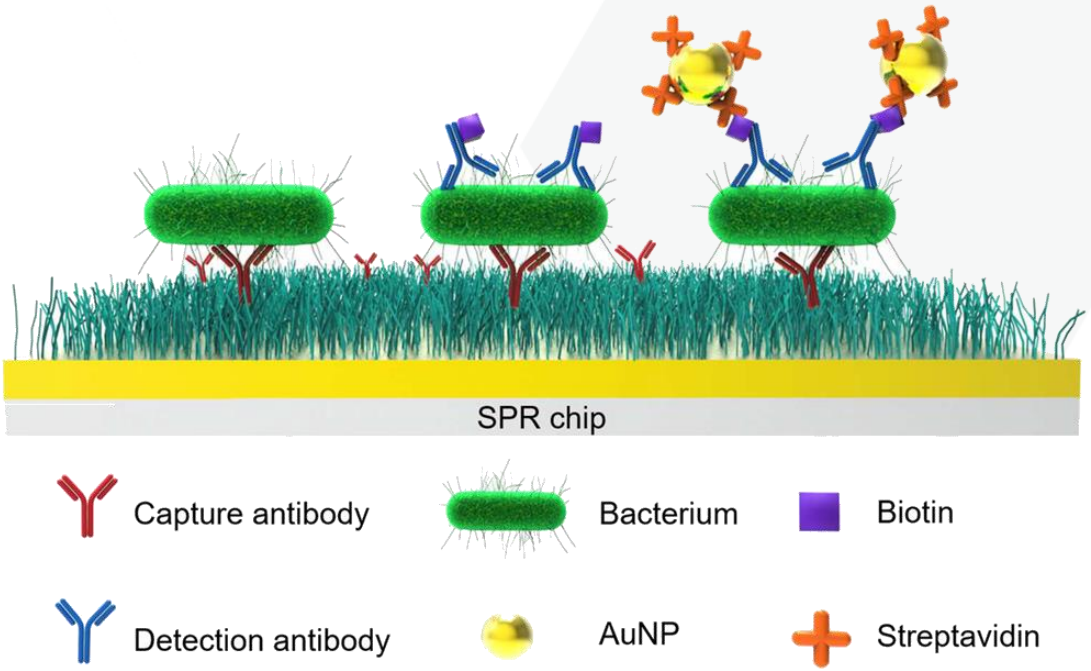


Surface plasmon resonance

- In principle allows label-free operation and does not require any nanoparticles
- Easy operation, fast analysis
- Enhancement by nanoparticles
  - Nanostructured surfaces
  - Nanoparticle labels

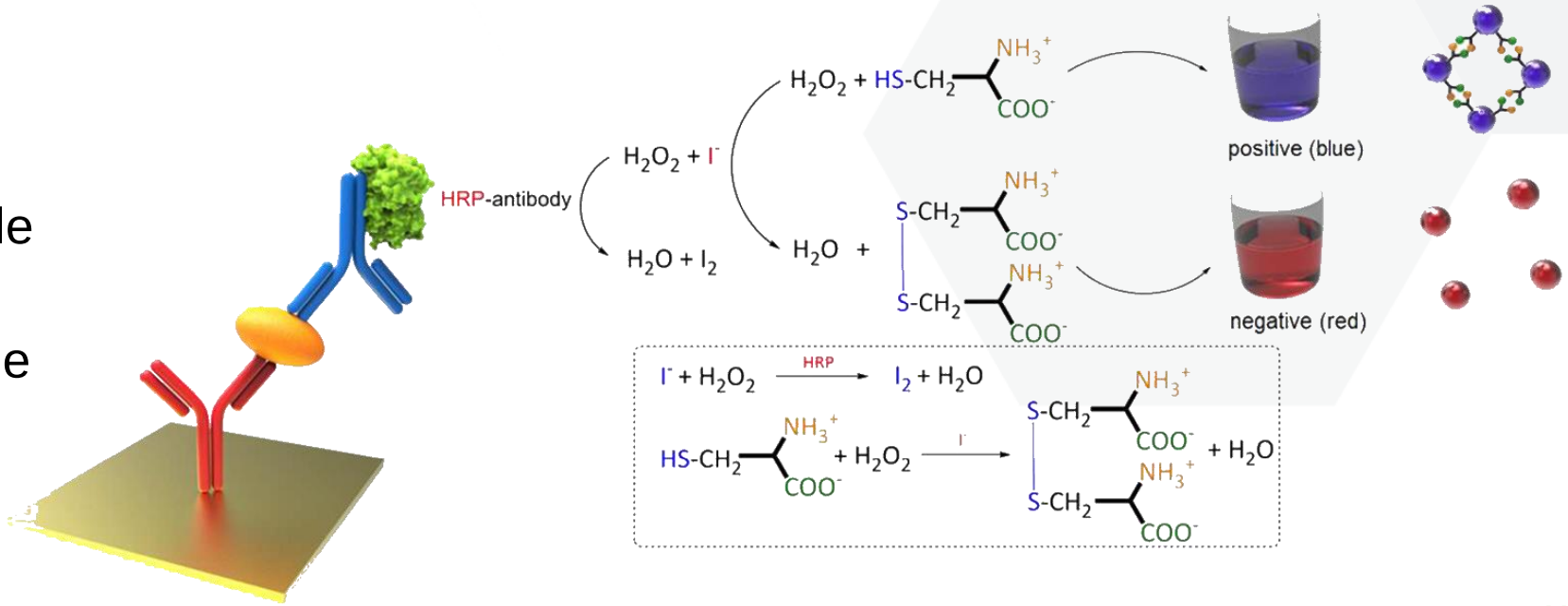






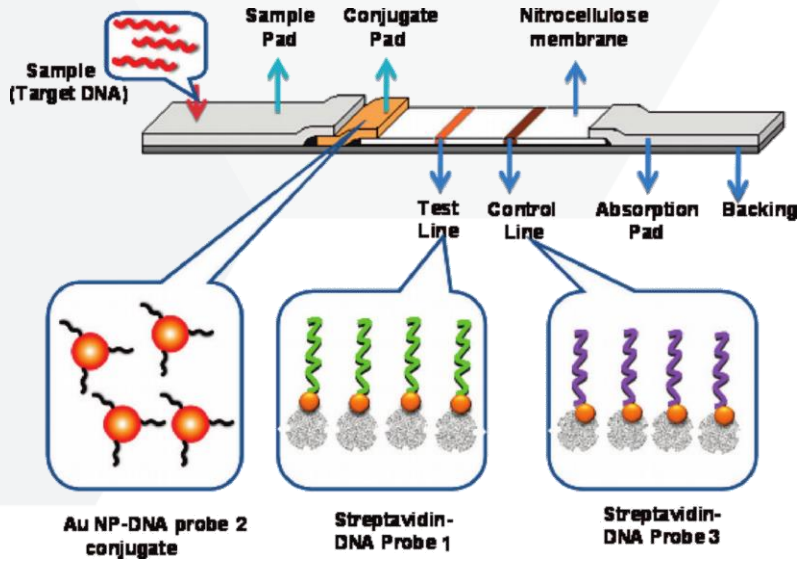
Localized surface plasmon resonance

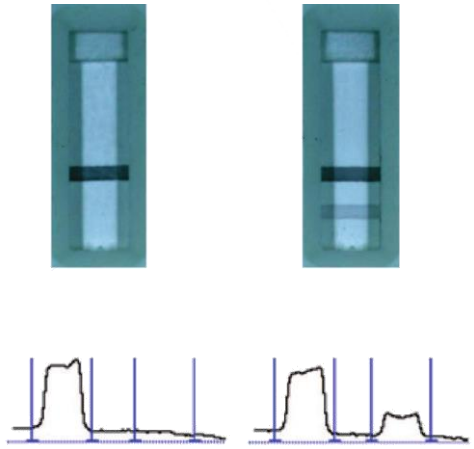
- Au and Ag NPs
- Change of plasmonic properties upon presence of analyte
  - Change of wavelength due refractive index change (analyte binding)
  - Aggregation/growth of nanoparticles due to analyte presence
- Often enables naked-eye readout
- Variants
  - Solution-based LSPR
  - Surface-based LSPR
  - Plasmonic nanoparticle assemblies
  - Controlled nanoparticle growth

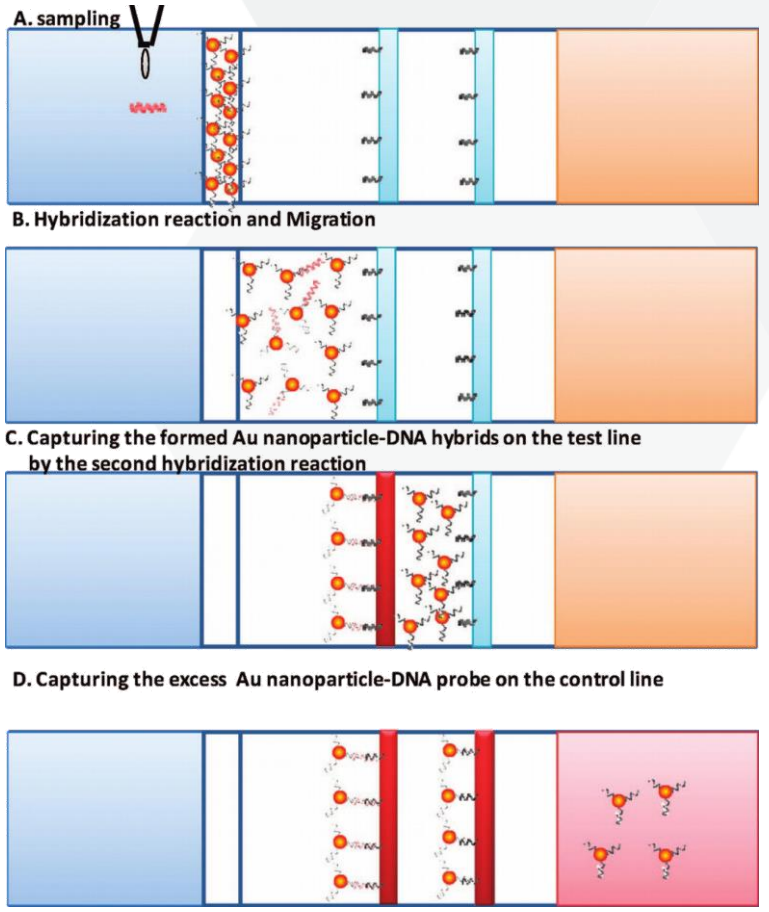


Lateral flow immunoassay

- Particles with high absorbance (e.g. Au NPs) adsorbed to surfaces can be observed by naked eye
- Antibody-antigen interaction, DNA-DNA interactions

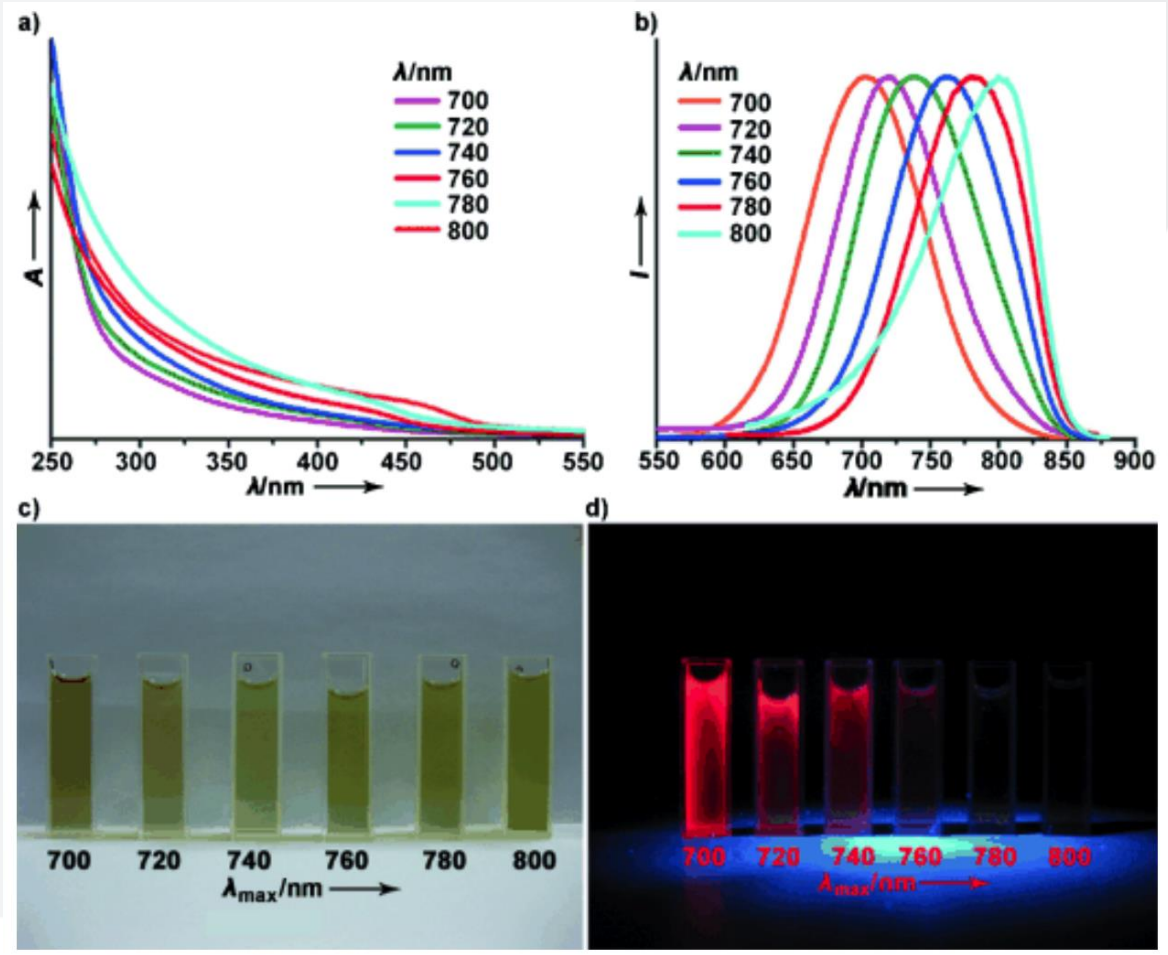


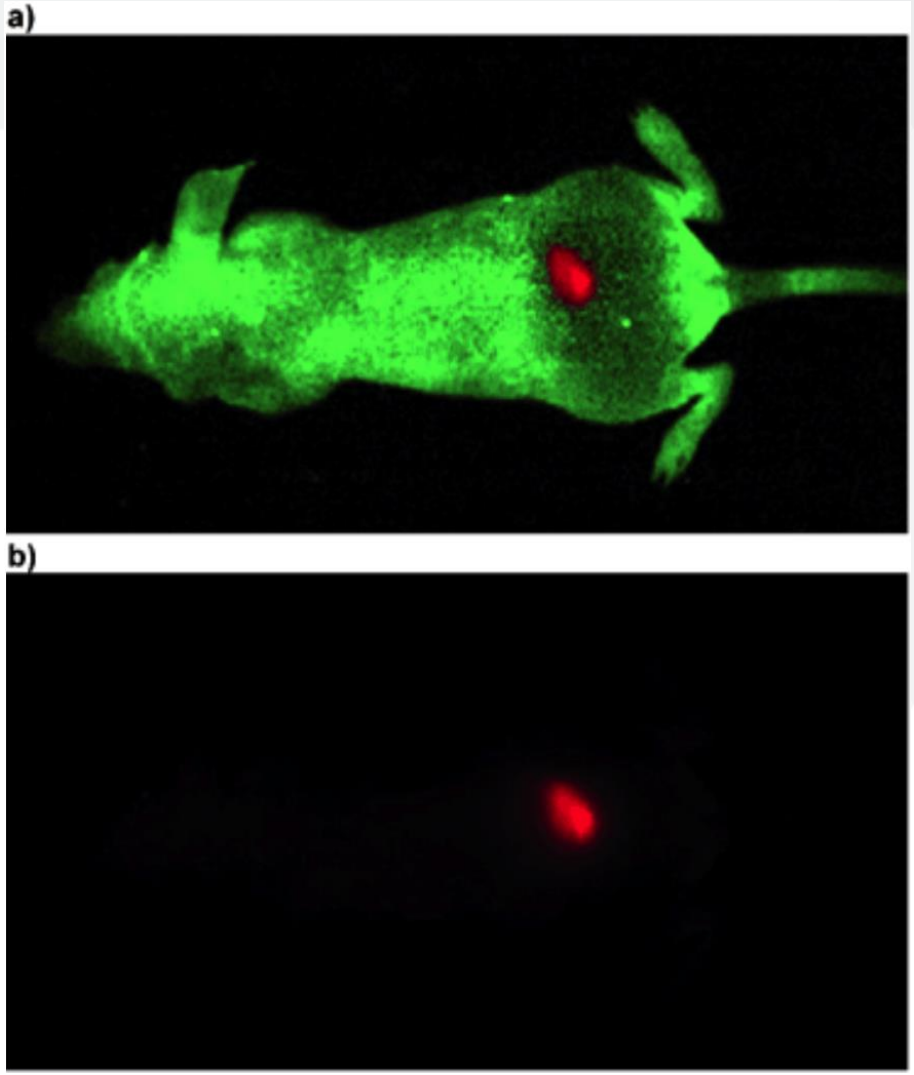




Mao, X., Ma, Y., Zhang, A., Zhang, L., Zeng, L., Liu, G. (2009) Anal. Chem., 81 (4), 1660-1668.

Biological imaging

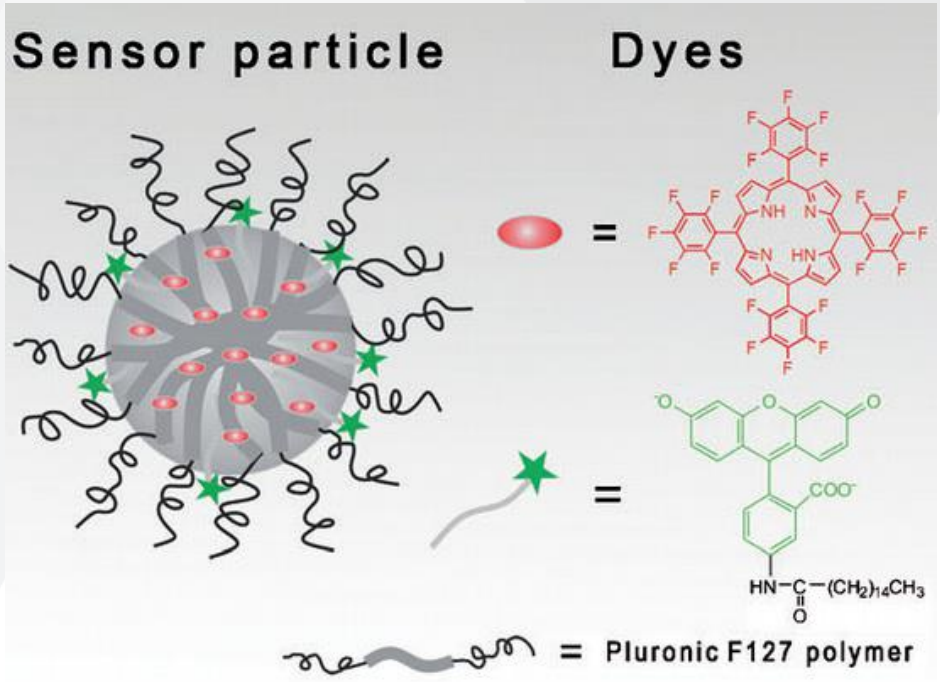


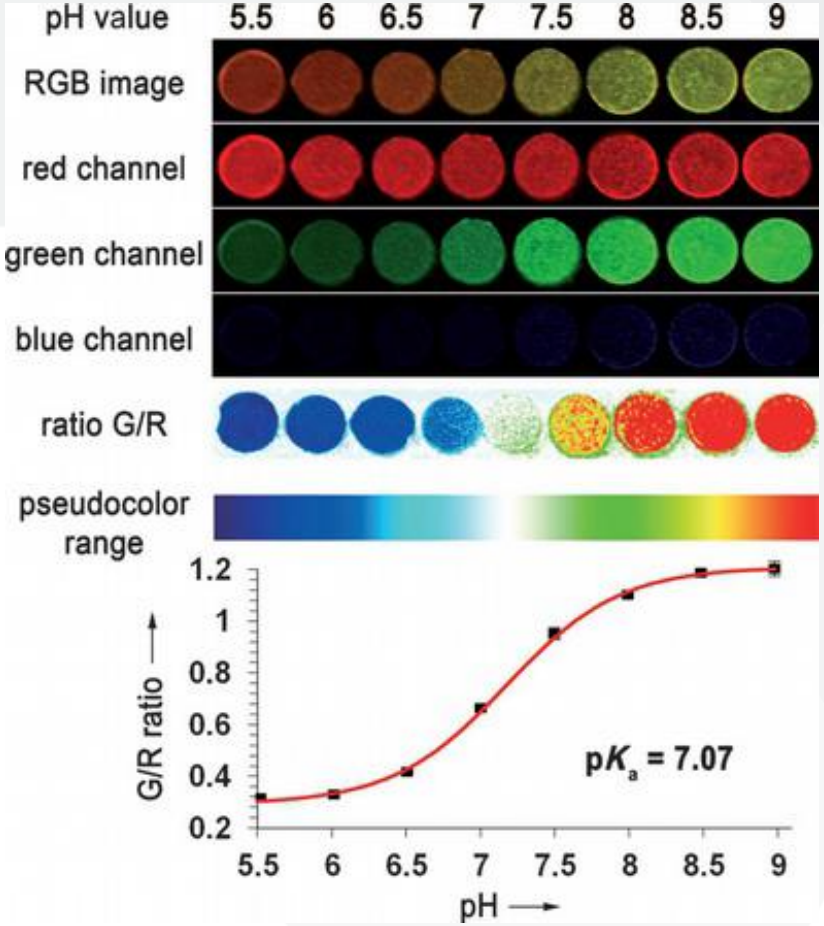


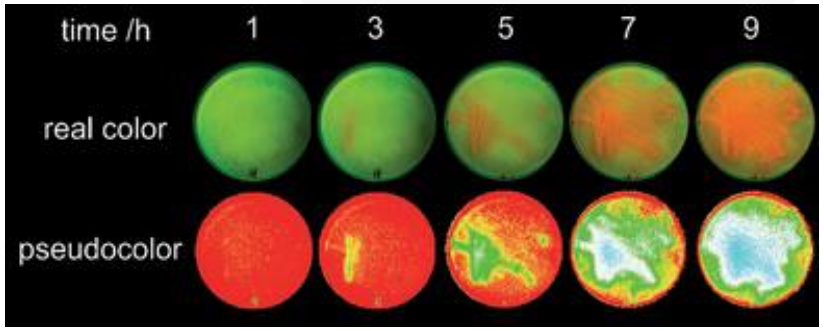
He Y., Zhong Y., Su Y., Lu Y., Jiang Z., Peng F., Xu T., Su S., Huang Q., Fan C., Lee S.T. (2011) Angew. Chem. Int. Ed., 50 (25), 5695-5698.

Nanosensors

- Ratiometric nanosensors
- pH imaging



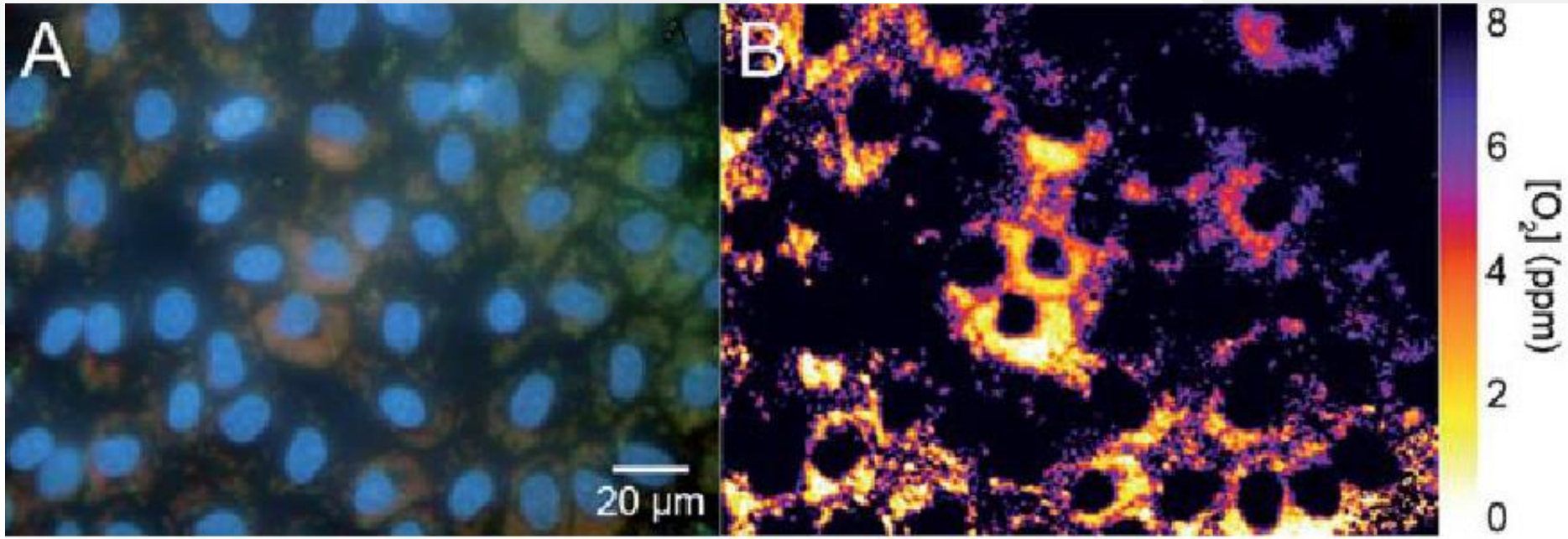




Wang X.D., Meier R.J., Wolfbeis O.S. (2013) Angew. Chem. Int. Ed., 52 (1) 406-409.

Nanosensors

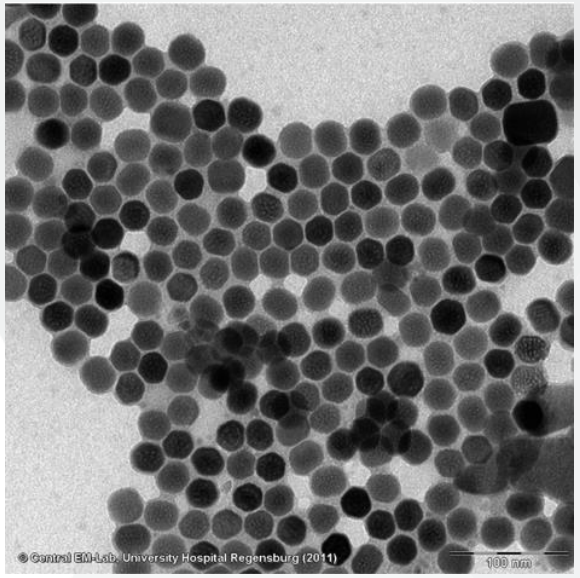
- Oxygen imaging in cells

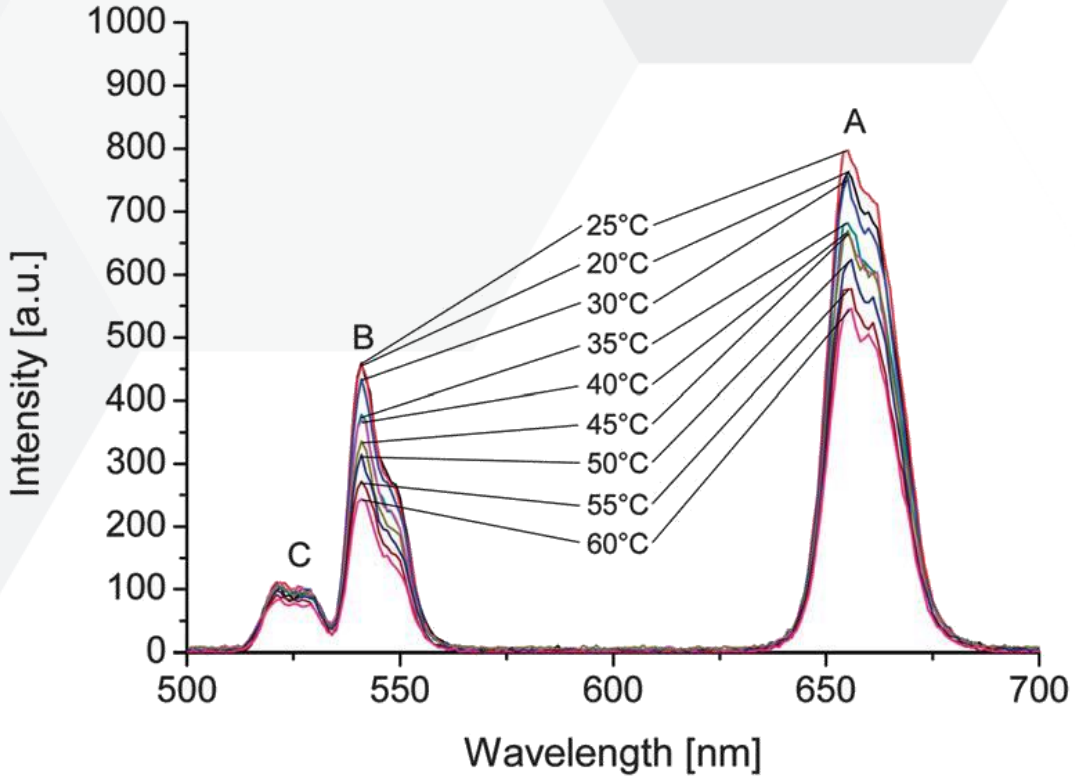


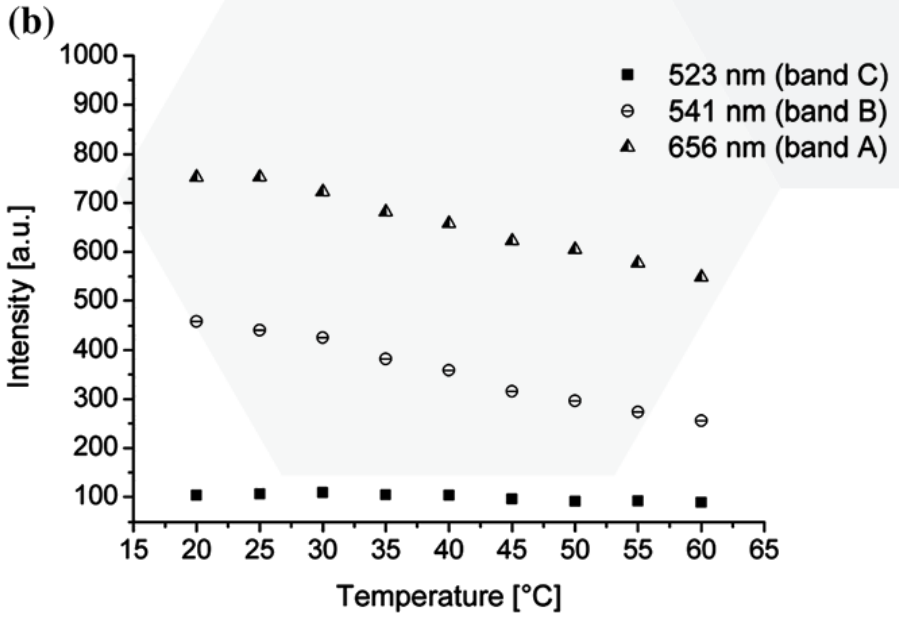
Wang X.D., Gorris H.H., Stolwijk J.A., Meier R.J., Groegel D.B.M., Wegener J., Wolfbeis O.S. (2011) Chem. Sci. 2 (5) 901-906.

Nanosensors

- Temperature sensing
  - Photon-upconversion nanoparticles



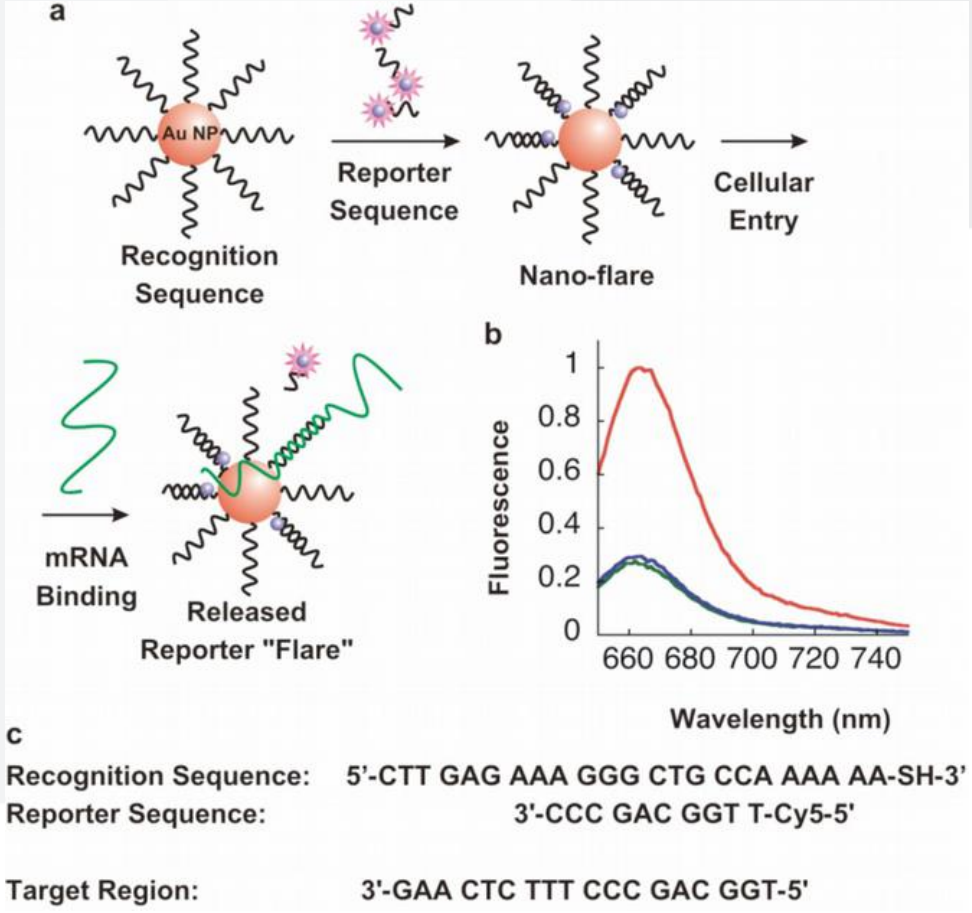


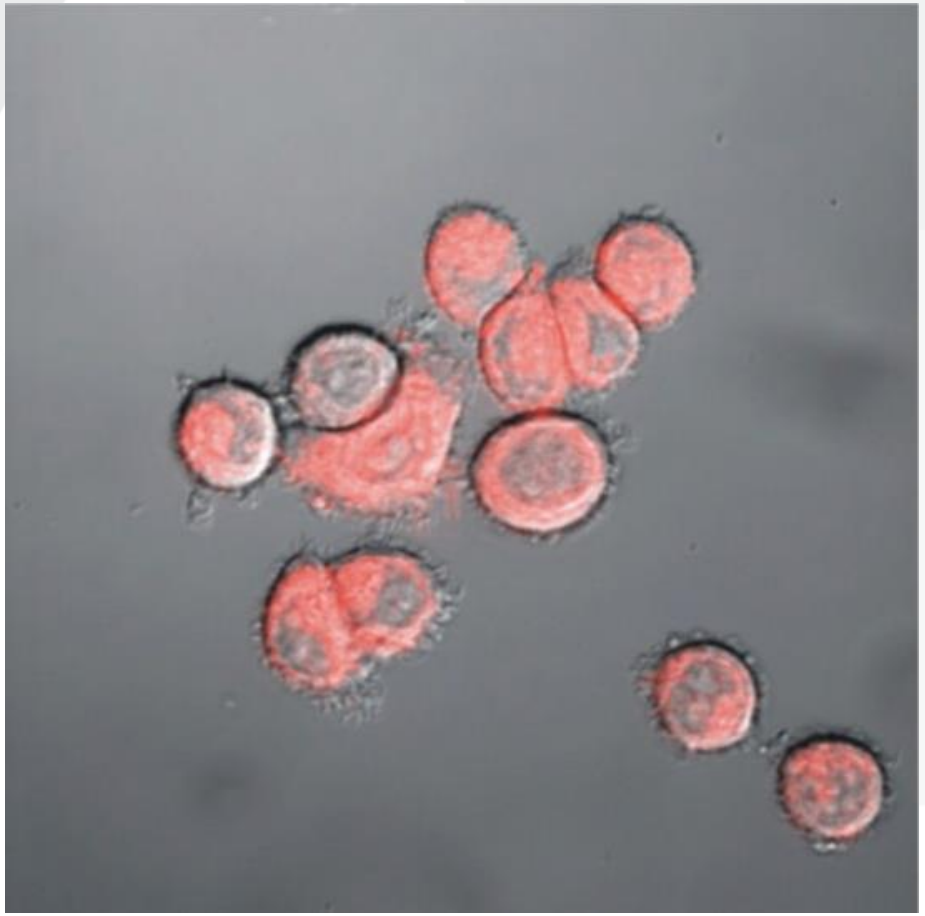


Sedlmeier A., Achatz D.E., Fischer L.H., Gorris H.H., Wolfbeis O.S. (2012) Nanoscale 4, 7090-7096.

Nanosensors

- mRNA imaging

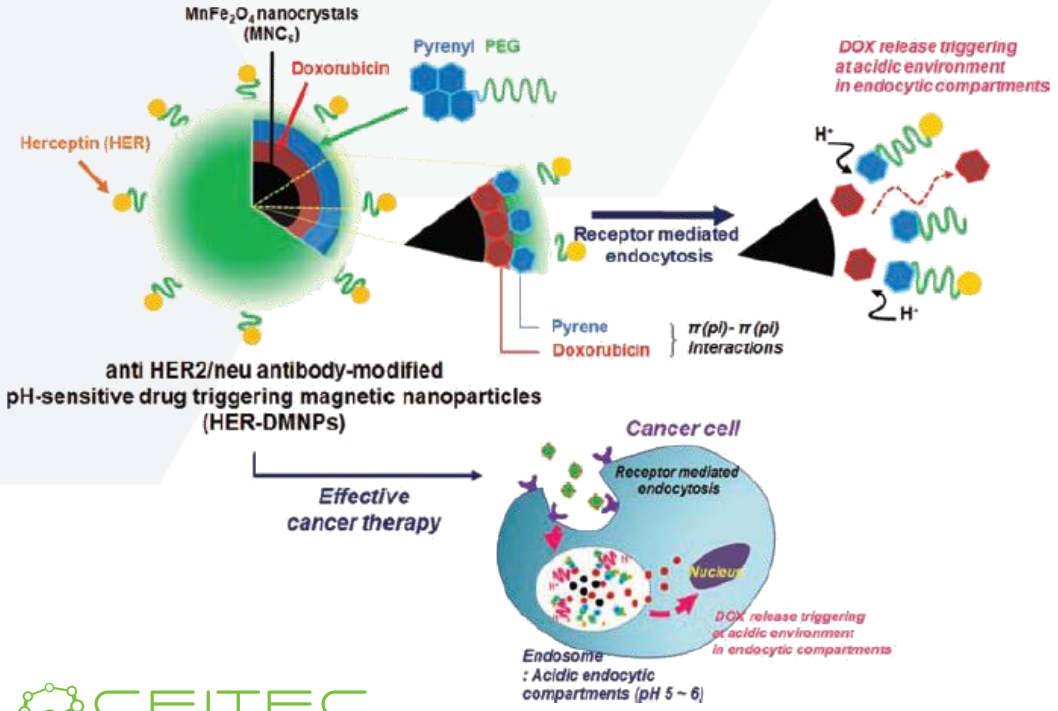


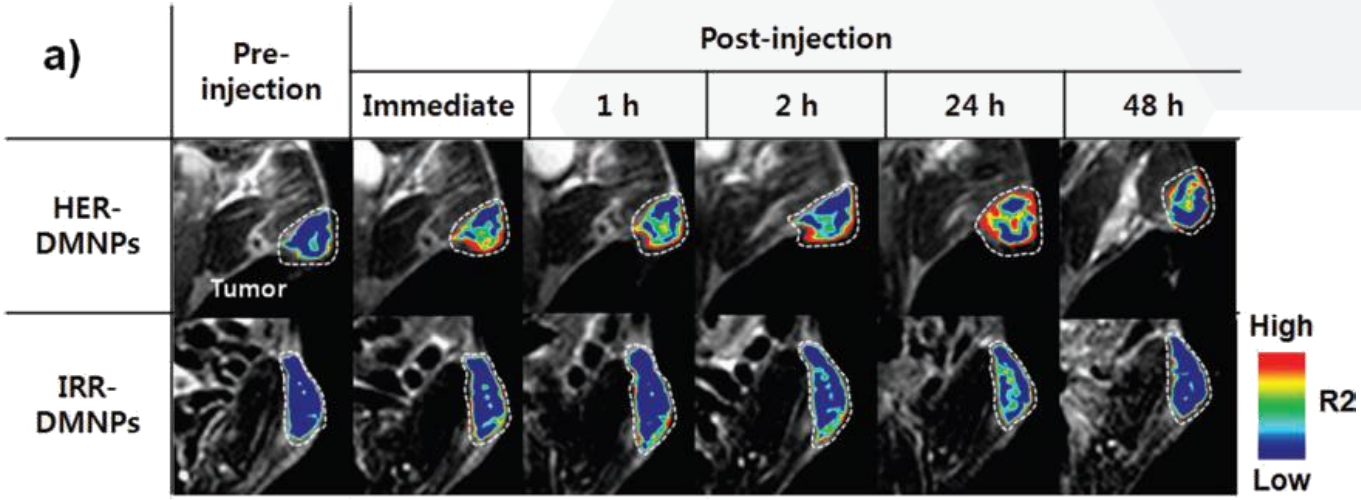


Seferos, D.S., Giljohann, D.A., Hill, H.D., Prigodich, A.E., Mirkin, C.A. (2007) J. Am. Chem. Soc., 129 (50), 15477-15479.

Magnetic resonance imaging

- Superparamagnetic nanoparticles
- Contrast agent
  - High relaxivity
  - Targeting ability

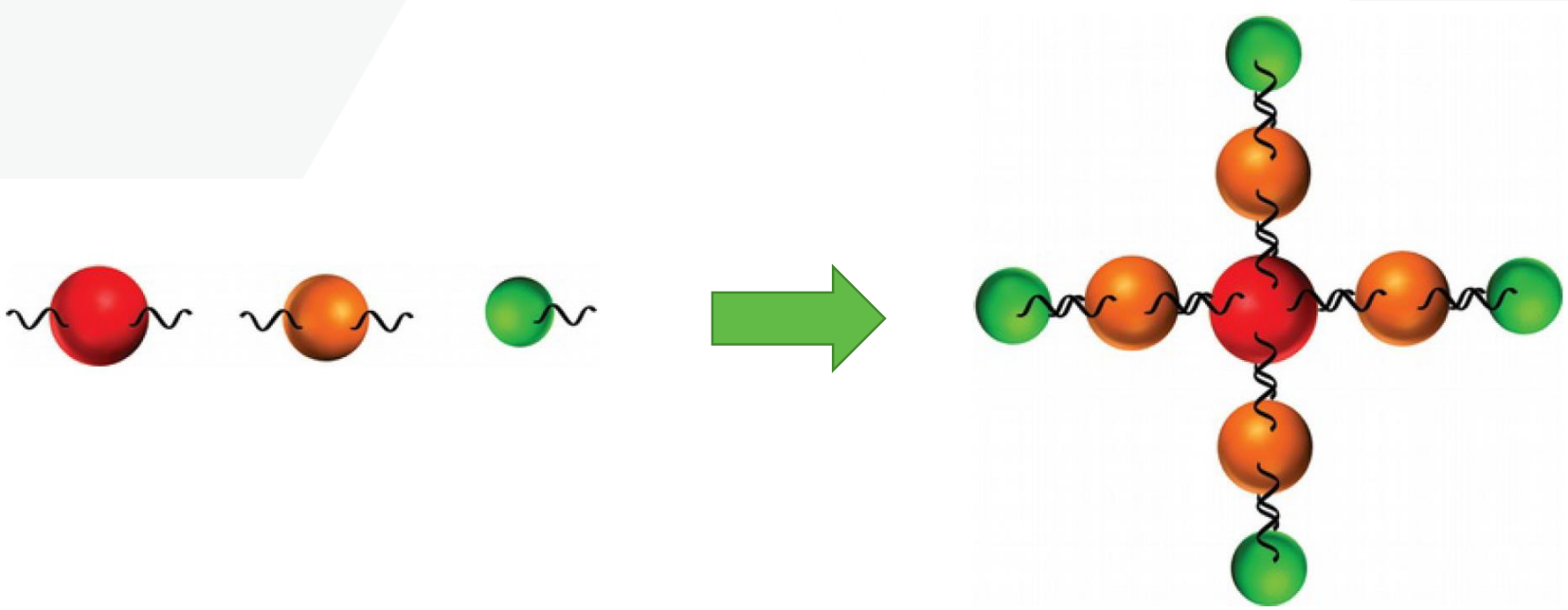




Lim, E.-K., Huh, Y.-M., Yang, J., Lee, K., Suh, J.-S., Haam, S. (2011) Adv. Mater., 23 (21), 2436-2442.

Self-assembly

- Nanoparticles can be considered as artificial building blocks of matter – atoms
- Recent studies consider new possibilities of artificial bond formation and the creation of artificial molecules



Tikhomirov, G., Hoogland, S., Lee, P.E., Fischer, A., Sargent, E.H., Kelley, S.O. (2011) Nat. Nanotechnol., 6 (8), 485-490.

Drug delivery

- Properties of drugs can be enhanced by using suitable carrier
  - Use of hydrophobic drugs
  - Control of drug release rate
  - Reduction of degradation and excretion from organism
  - Targeted transportation
- Various nanomaterials used as carriers
  - Liposomes, polymerosomes
  - Polymers
  - Porous inorganic materials (mesoporous silica, zeolites)
- Theranostic nanoparticles
  - **Thera**py and diag**nostics**
  - Carry therapeutic and imaging agent
  - Often possibility to specifically find target molecules and release the drug uder certain conditions (changes in pH in/around tumor cell, presence of proteases, irradiation)

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Thank you for your attention