Analytical applications of fluorescence

Fluorescence methods in life sciences

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Determination of concentration

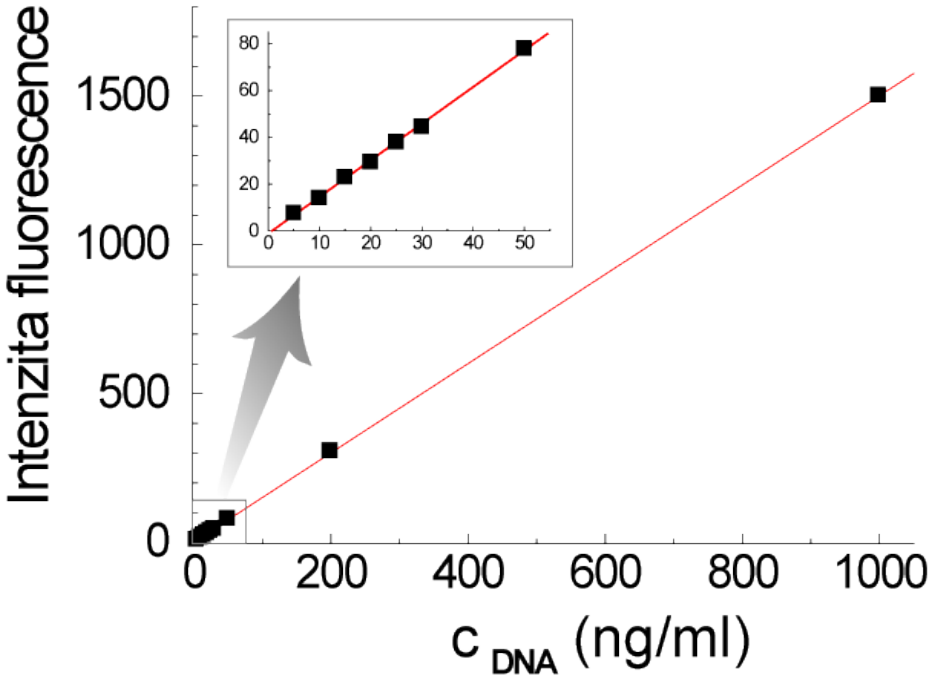
- How to determine quantity of DNA and protein solution?

- How to determine quality of biomolecules?

Determination of DNA concentration

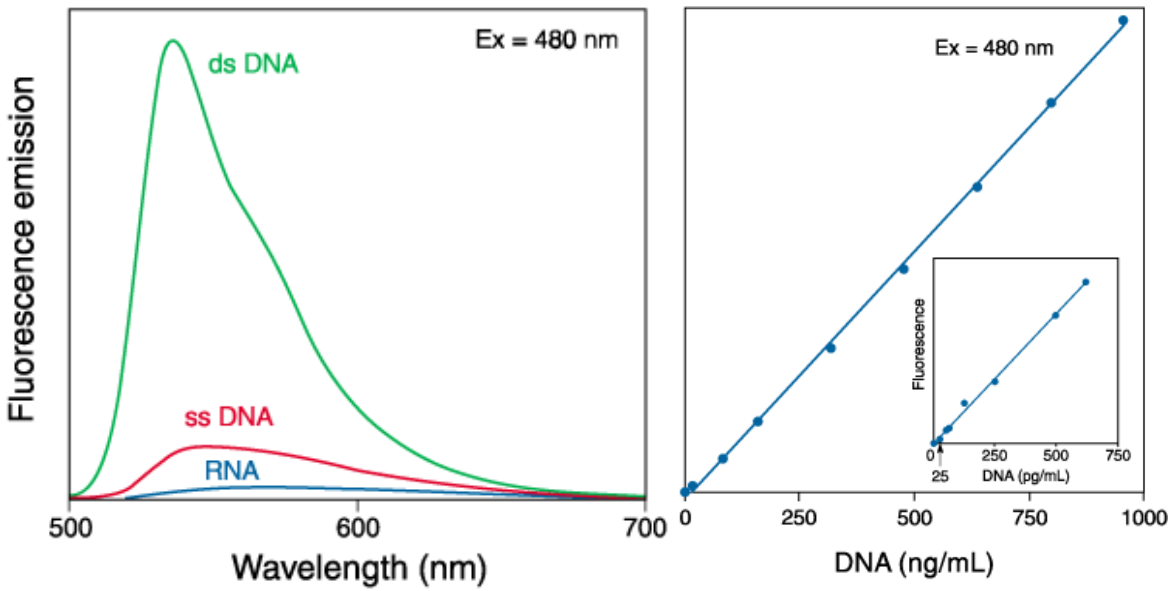
- Using UV absorption spectroscopy it is possible to determine accurately the concentration down to the limit 1 μg/ml; the accuracy is about 1%
- The use of fluorescent probe allows to reduce the detection limit 1000-times - to **1 ng/ml!**
- When using fluorescent probes for measuring the concentration of complex samples (cell lysate, blood plasma), the effect of the presence of proteins (absorb at 260nm), nucleotides and short oligonucleotides is less pronounced.
- The measuring accuracy is about 5%

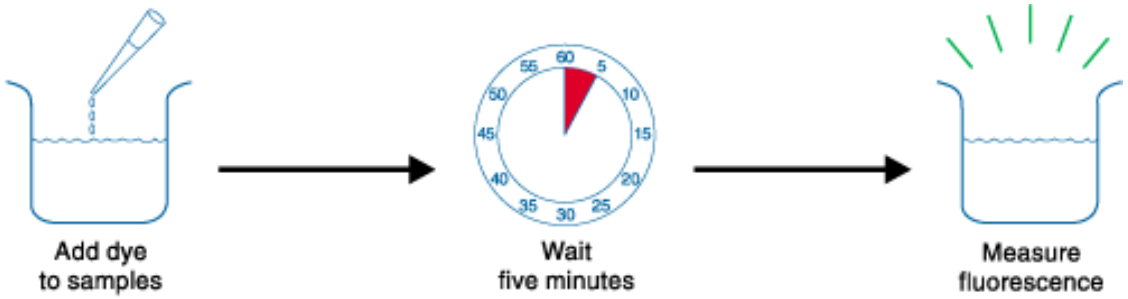
Determination of DNA concentration using Hoechst 33258



- Standard - DNA solution from 5 – 1000 nM
- Mixed with a solution Hoechst (200 ng/ml) in ratio 1:1
- Ex/Em 350/455 nm
- Measured in TE buffer
- Sample consumption ~ 50 ng DNA

Determination of DNA concentration using PicoGreen





- Detection **25 pg/ml**
- Ex/Em 480/520 nm
- Wide dynamic range – it can determine the concentration of 0.025 -1000 ng/ml

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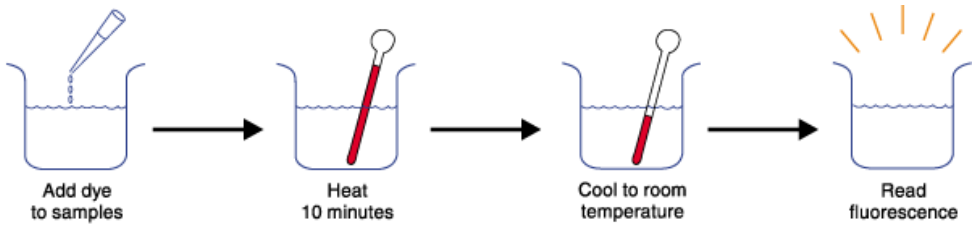
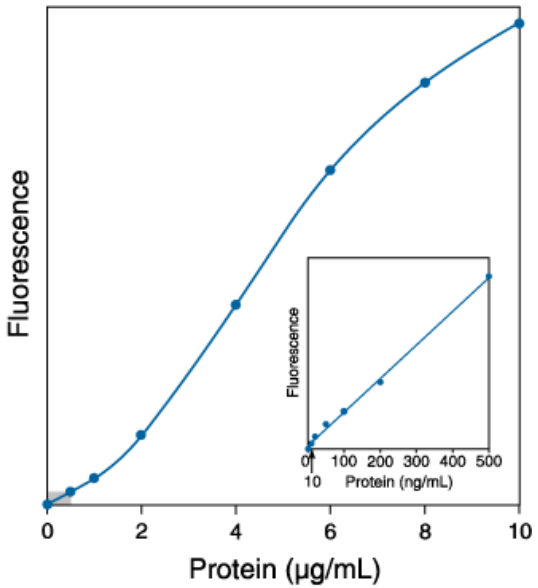
Determination of protein concentration

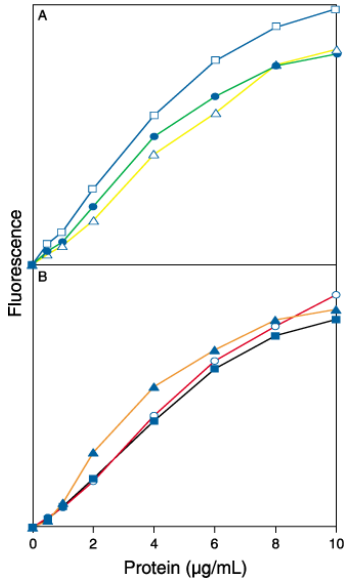
- **UV absorption** - determination based on the absorption of aromatic AA - dependent on their occurrence in the sequence (1 mg/ml)
- **Bradford method**– based on the change in color after binding to the protein
(0.25 mg/ml)
- **Fluorescent methods** (10 ng/ml)

Fluorescence determination of protein concentration





BSA



A) BSA, trypsin, carbonic anhydrase
B) IgG, streptavidin, RNase A

- When using fluorescent probe, NanoOrange is needed to compensate differences in protein structure, which would affect the interaction - they should be denatured
- Sensitivity 10ng/ml
- Dynamic range 1000
- Stable for 6 hrs
- Small dependence on the protein type
- The method is not sensitive to contamination by other molecules than lipids
- To quantify in the presence of lipids or lipoproteins, CBQCA kit can be used

Determination of the quality and amount using densitometry of gels

- Simultaneous information about the quality and quantity can be obtained in the analysis of molecules in the gel
- The amount of absorbed / emitted light is measured depending on the position of the 2D
- Determination of the concentration of DNA and protein after gel staining with Coomassie, silver or fluorescently (SYBR Green, SYPRO Ruby)