Determination of DNA concentration and purity

If the concentration of DNA in the sample is sufficient, it can be measured by **direct photometry** in the UV region. Due to the large number of purine residues, pure DNA has an absorption maximum at **260 nm**, with an absorbance of approximately 1 for a DNA solution with a concentration of 50 $\mu g \cdot ml^{-1}$.

At 260 nm, however, proteins also absorb, whose spectrum has a broad peak with a maximum at 280 nm due to tyrosine groups. In practice, DNA purity is often estimated by the **absorbance ratio** at 260 and 280 nm. Pure DNA has an A_{260}/A_{280} of approximately 1.8 (the higher the ratio, the purer the DNA).

When working with diluted DNA solutions, direct photometry is not sensitive enough. In that case, **intercalation fluorescent dyes** are most often used to determine its concentration. They are substances that contain several condensed aromatic nuclei, so they have a planar structure. Thanks to it, they can "wedge", **intercalate between the strands** of DNA double helices, while **increasing** their **fluorescence**. The prototype of intercalation dyes is ethidium bromide (3,5-diamino-5-ethyl-6-phenylphenanthridium bromide).