

# Quantitative Methoden in der Molekularbiologie

## *2. Protein quantification*

# Outline

## 1. Analytical methods

- Spectrophotometric quantification
- Mass spectrometry

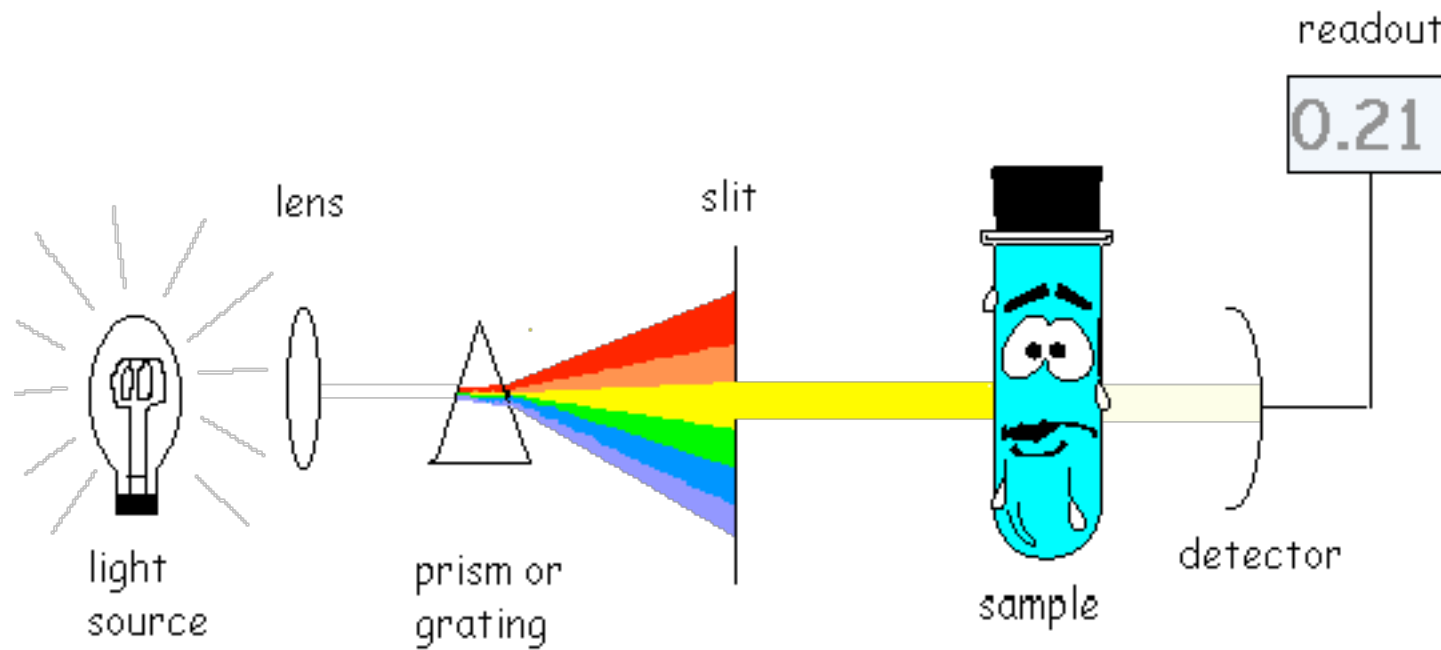
## 2. Data analysis

- Sampling
- Normal distribution
- Probabilities
- Variability
- Regression

# Spectrophotometric quantification

- Intrinsic absorbance
  - Absorbance at 280nm
  - Absorbance at 205nm
- Colorimetric assays
  - Lowry
  - Bradford
  - ...

# Spectrophotometer



# Lambert Beer law

$$E_{\lambda} = \lg\left(\frac{I_0}{I_1}\right) = \varepsilon_{\lambda} \cdot c \cdot d$$

$E_{\lambda}$  Extinction at wave length  $\lambda$

$I_0$  Signal intensity for empty buffer

$I_1$  Signal intensity for protein sample diluted buffer

$\varepsilon_{\lambda}$  specific extinction coefficient at wave length  $\lambda$

$c$  concentration

$d$  thickness of sample

# Homework reading: proteinassays.pdf

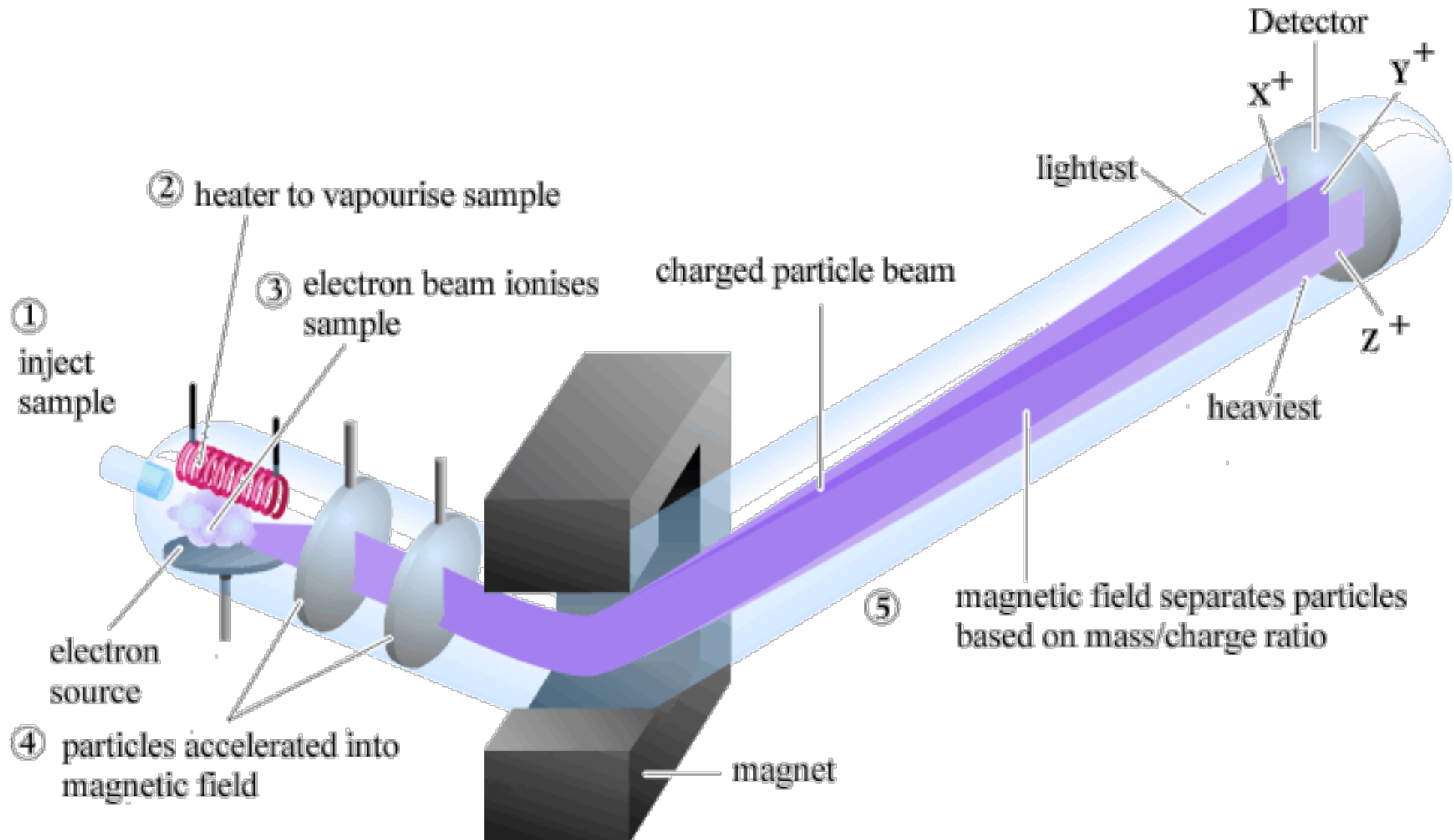


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# Mass spectrometry

1. Sample -> MS instrument -> vaporization
2. Sample ionization -> charged particles
3. Separation of ions by mass-to-charge ratio in an analyzer
4. Detection of ions (quantitative)
5. Signal processing -> Mass spectrum

# Mass spectrometer





# Homework reading: ms.pdf



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## **Protein Quantitation Using Mass Spectrometry**

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### **Abstract**

Mass spectrometry is a method of choice for quantifying low-abundance proteins and peptides in many biological studies. Here, we describe a range of computational aspects of protein and peptide quantitation, including methods for finding and integrating mass spectrometric peptide peaks, and detecting interference to obtain a robust measure of the amount of proteins present in samples.