UNITE

INTRODUCTION TO BIOCHEMICAL METHODOLOGIES

I.C. **LABORATORY OF BIOCHEMICAL METHODOLOGIES AND EXPERIMENTAL MODELS**

- MODULE: **BIOCHEMICAL METHODOLOGIES**

BIOS-07/A

5 ECTS (CFU)



ABOUT ME

Sara Standoli, Ph.D.

Teaching Support – Chemistry & Biochemistry

RESEARCH & PUBLICATIONS

Research Focus

Endocannabinoid System (ECS)

Molecular mechanisms of neuroinflammation in murine microglial cells (BV2), cells (BV2), myocytes (C2C12) and neurospheres

Sphingosine-1-Phosphate (S1P)

ECS–S1P crosstalk; biomarker discovery and drug development targets targets

Recent Publications

New insights into the crosstalk between endocannabinoids and sphingosine-1-sphingosine-1-phosphate

J. Biol. Chemistry · 2025 · Rapino, **Standoli** et al.

Sphingosine Kinases at the Intersection of LPS and Endocannabinoid Signaling in BV2 Microglia

Int. J. Mol. Sci. · 2023 · **Standoli** et al.

Cannabinol modulates ECS and TRPV1-mediated anti-inflammatory properties in keratinocytes

Biofactors · 2024 · Di Meo, Tortolani, **Standoli** et al.

GET IN TOUCH
Where to Find Me



Email: sstandoli@unite.it



Location: 4th Floor Labs (Between the Yellow Bridge and the Blue Bridge)
Always reach out by email first



Office Hours: By appointment — email me in advance

COURSE INFORMATION

Credits (CFU)

[5 CFU] – [8 lectures + 6 lab hours]

Assessment

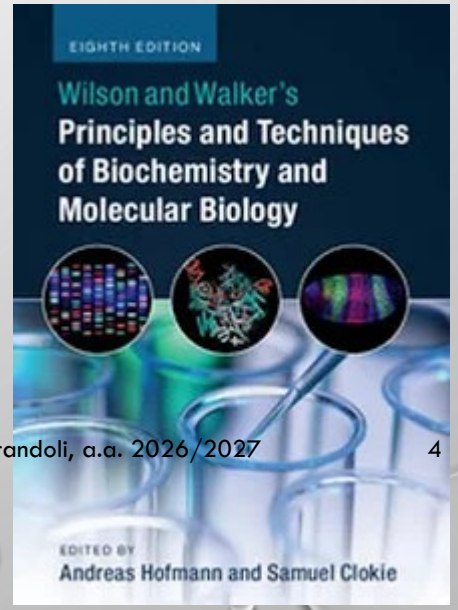
The examination consists of preparing laboratory practical reports, which will then be assessed during an oral exam

Course Materials

Moodle UniTE – [BICHBM26/27]

Suggested book: Wilson and Walker's Principles and Techniques of Biochemistry and Molecular Biology

✅ Lab access not required for report! Full materials will be shared with students without safety clearance — your exam reports are safe!



COURSE INFORMATION: SCHEDULE

Theoretical Lessons – Classroom 7

#	Date	Day	Time	Duration
1	15 October 2026	Thursday	16:30 – 18:30	2 h
2	22 October 2026	Thursday	16:30 – 18:30	2 h
3	29 October 2026	Thursday	16:30 – 18:30	2 h
4	5 November 2026	Thursday	16:30 – 18:30	2 h
5	12 November 2026	Thursday	16:30 – 18:30	2 h
6	19 November 2026	Thursday	16:30 – 18:30	2 h
7	26 November 2026	Thursday	16:30 – 18:30	2 h
8	3 December 2026	Thursday	16:30 – 18:30	2 h

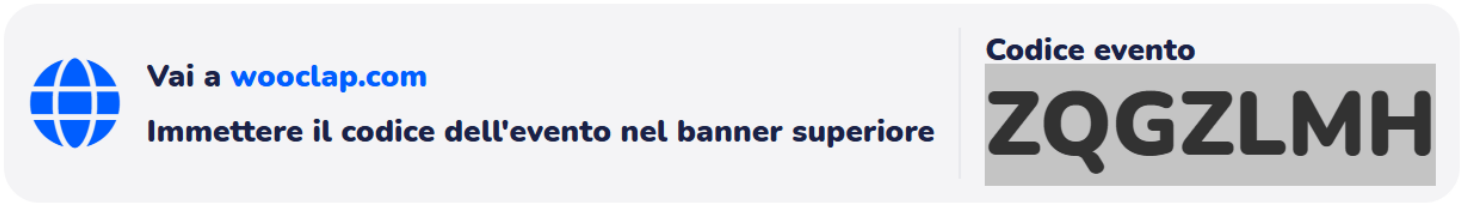
Laboratory Sessions – Lab Motti

#	Date	Day	Time	Duration
1	30 October 2026	Friday	16:00 – 18:00	2 h
2	2 November 2026	Monday	14:30 – 18:30	4 h
3	13 November 2026	Friday	10:30 – 15:30	5 h
4	27 November 2026	Friday	9:30 – 13:30	4 h
5	30 November 2026	Monday	9:30 – 13:30	4 h
6	4 December 2026	Friday	14:00 – 18:00	4 h

Note: for each session, students will be divided into 2 or more groups to be defined based on their lecture attendance. Group assignments will be communicated in advance.

file:///C:\Users\sara_\Downloads\wooclap_full_quiz_1.xlsx





KNOWLEDGE AND EXPECTATION

Go on wooclap.com and use the event code ZQGZLMH

BIOCHEMICAL AND BIOMOLECULAR INVESTIGATION

Application of separative analytical techniques

Methods and techniques form the tool set of an experimental scientist. They are applied in the context of studies which, in the life sciences address questions of the following nature:

- The structure and function of the total component of the cell (proteomics) and of all the small molecules in the cell (metabolomics);
- The mechanisms involved in the control of gene such expression;
- The identification of genes associated with a wide range of diseases and the development of gene therapy strategies for the treatment of diseases;
- The characterization of the large number of “orphan” receptors whose physiological role and natural agonist are currently unknown, present in the host and pathogen genomes and their exploitation for the development of new therapeutic agents;
- The identification of novel disease-specific markers for the improvement of clinical diagnosis;
- The engineering of cells, especially stem cells, to treat human diseases;
- The understanding of the functioning of the immune system in order to develop strategies for protection against invading pathogens;
- The development of our knowledge of the molecular biology of plants in order to engineer crop improvements, pathogen resistance and stress tolerance;
- The discovery of novel therapeutics (drugs and vaccines) to the nature and treatment of bacterial, viral and fungal diseases.

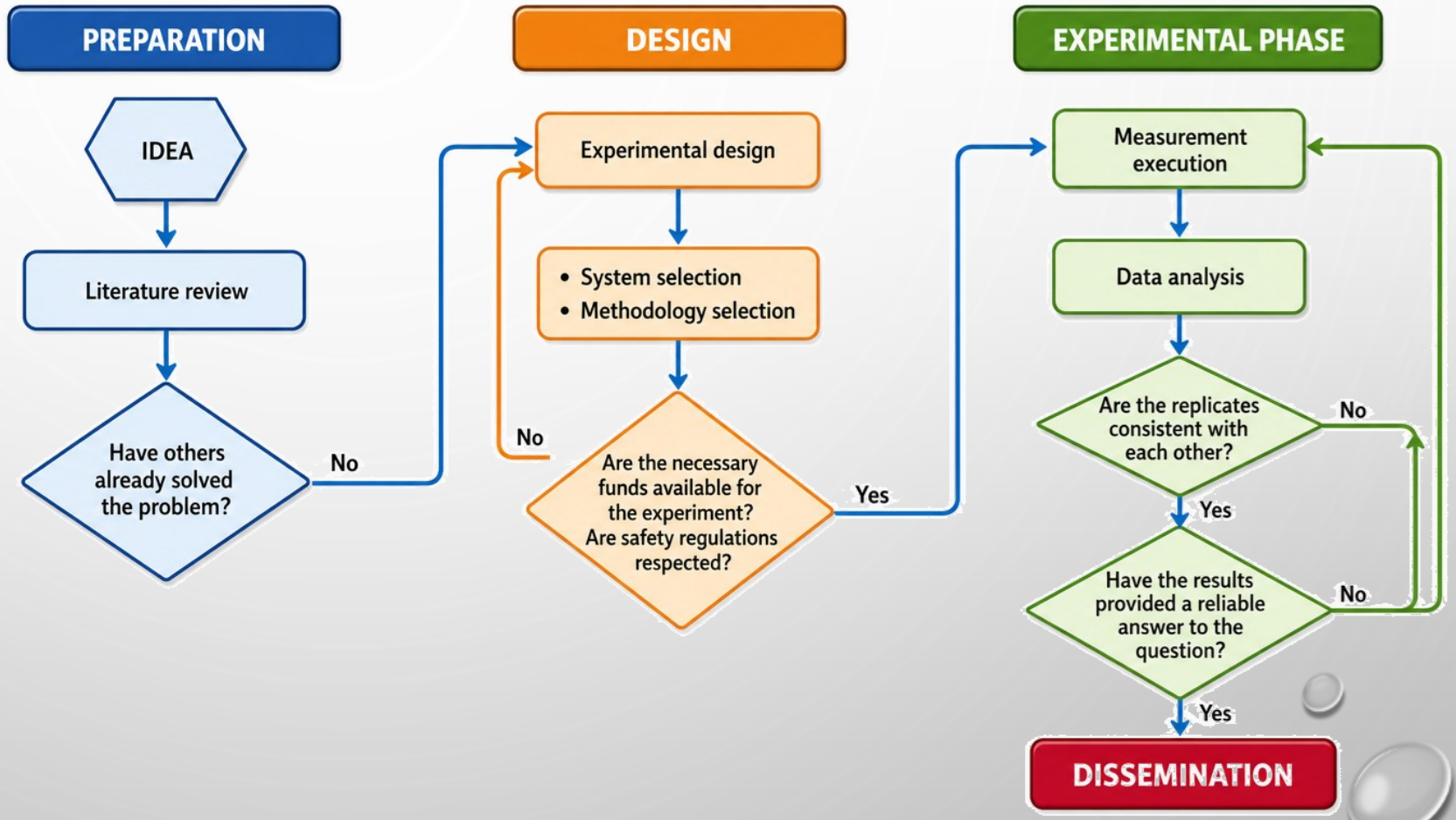
INTRODUCTION TO BIOCHEMICAL METHODOLOGIES

BY THE END OF THE LESSON, THE STUDENT WILL BE ABLE TO:

- Recall the main biological macromolecules and their role in life processes.
- *Identify and recognise the main laboratory instrumentation and its use.*
- Describe the purpose of biochemical and biomolecular investigation (diagnosis, markers, drugs, gene regulation, diseases).
- Recognise the fundamental SI units of measurement, together with derived units, multiples and submultiples, with examples relevant to biochemistry.
- Explain the concepts of error, sensitivity, uncertainty, precision and accuracy through simple examples.
- Understand the meaning of mean, standard deviation and uncertainty of the mean, and their use in presenting experimental data.
- Qualitatively relate different forms of dependence between variables (linear, exponential, hyperbolic, sigmoidal) to examples of biochemical techniques.



THE SCIENTIFIC METHOD



MEASUREMENTS AND SCIENTIFIC NOTATION

FUNDAMENTAL UNITS OF MEASUREMENT OF THE INTERNATIONAL SYSTEM (SI)

Quantity	Unit	Symbol
Length	metre	m
Mass	kilogram	kg
Time	second	s
Electric current	ampere	A
Thermodynamic temperature	kelvin	K
Amount of substance	mole	mol
Luminous intensity	candela	cd

- **SUPPLEMENTARY UNITS** for defining plane angles (degrees) and solid angles (steradians);
- **DERIVED UNITS** expressed as products of powers of the supplementary SI units, with a numerical factor of 1 [e.g., frequency in hertz (Hz), where 1 Hz = 1 s^{-1}];
- **DECIMAL MULTIPLES** and **SUBMULTIPLES** involve a prefix placed before the unit of measurement and have their own symbols [e.g., a nanometer (1 nm) equals 1×10^{-9} m: the order of magnitude of a peptide bond's dimensions].

Multiples and submultiples of units of measurement.

Multiple	Prefix	Symbol	Submultiple	Prefix	Symbol
10^{24}	yotta	Y	10^{-1}	deci	d
10^{21}	zetta	Z	**10^{-2}**	**centi**	**c**
10^{18}	exa	E	**10^{-3}**	**milli**	**m**
10^{15}	peta	P	**10^{-6}**	**micro**	**μ**
10^{12}	tera	T	**10^{-9}**	**nano**	**n**
10^{9}	giga	G	10^{-12}	pico	p
10^{6}	mega	M	10^{-15}	femto	f
10^{3}	kilo	k	10^{-18}	atto	a
10^{2}	hecto	h	10^{-21}	zepto	z
10^{1}	deca	da	10^{-24}	yocto	y

THE CAPACITY

The capacity of a liquid is measured using multiples and submultiples of the litre (L). Capacity and volume are used to refer to the same thing, as 1 litre corresponds to the capacity of 1 cubic decimetre:

$$1\ L = 1\ dm^3$$

The capacity of one millilitre corresponds to a volume of 1 cubic centimetre:

$$1\ mL = 1 \times 10^{-3}\ L = 10^{-3}\ dm^3 = 1\ cm^3$$

Performance Indicators of Analytical Methods

Characterise how a method performs under specified conditions — guides selection for each analytical application

Precision

imprecision · CV · variability

Reproducibility of repeated measurements on the same sample. High precision ≠ high accuracy — systematic errors can still bias the result.

Accuracy

trueness · bias

Closeness of the mean result to the true value. Assessed via reference methods, EQA schemes, or population statistics.

Detection Limit

★ Priority

LOD · clinical sensitivity

Lowest concentration distinguishable from zero at a defined confidence. Below this threshold, report as < LOD.

Analytical Range

★ Priority

linearity · dynamic range

Concentration interval measurable reproducibly. Lower bound = LOD; upper bound set by the calibration curve.

Specificity

★ Priority

selectivity

Extent to which interfering substances alter the result — e.g. glucose measured in the presence of other hexoses.

Anal. Sensitivity

slope of calibration curve

Change in instrument response per unit change in analyte quantity — expressed as the slope of the calibration curve.

Robustness

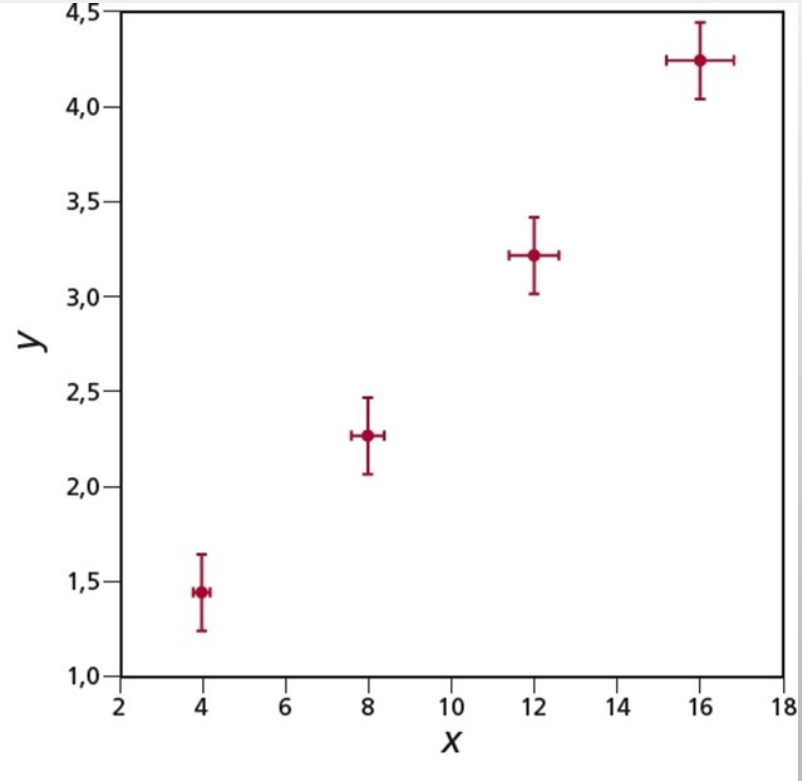
ruggedness · stability

Consistency of results despite small changes in pH, temperature, or reagent quantity — critical for routine labs.

ERROR

The difference between the obtained value and the "true" (absolute) value of the quantity.

The results of repeated measurements are reported as value ± error, along with the unit of measurement.



Graph showing the values of the measurements taken. Uncertainties are represented as error bars for both the variable on the x-axis (percentage error) and the variable on the y-axis.

There are two types of error: random error and systematic error

- **Random errors** vary unpredictably and arise from minor variations in the procedures involved in each experimental measurement;

- **Systematic errors** can be easily identified, eliminated, or reduced, as they depend on environmental conditions (ambient temperature, altitude, humidity levels) and the operator themselves. Furthermore, systematic errors include instrumental error, which reminds us that no instrument can yield an error-free measurement, as that would require it to be "infinitely precise."

Every instrument has an intrinsic uncertainty associated with the measurement.

SENSITIVITY AND INTRINSIC UNCERTAINTY

- **Sensitivity:** the smallest value the instrument can distinguish;

- **the uncertainty of a measuring instrument** is defined as 50% of its sensitivity.

  Example: a ruler—what is the error associated with a measurement made using this instrument?
  If we examine it with a scientific eye, we see that the sensitivity (the smallest division on the ruler) is 1 mm (1×10^{-3} m); therefore, the error associated with the instrument is half that amount: 0.5 mm (0.5×10^{-3} m $= 5 \times 10^{-4}$ m).

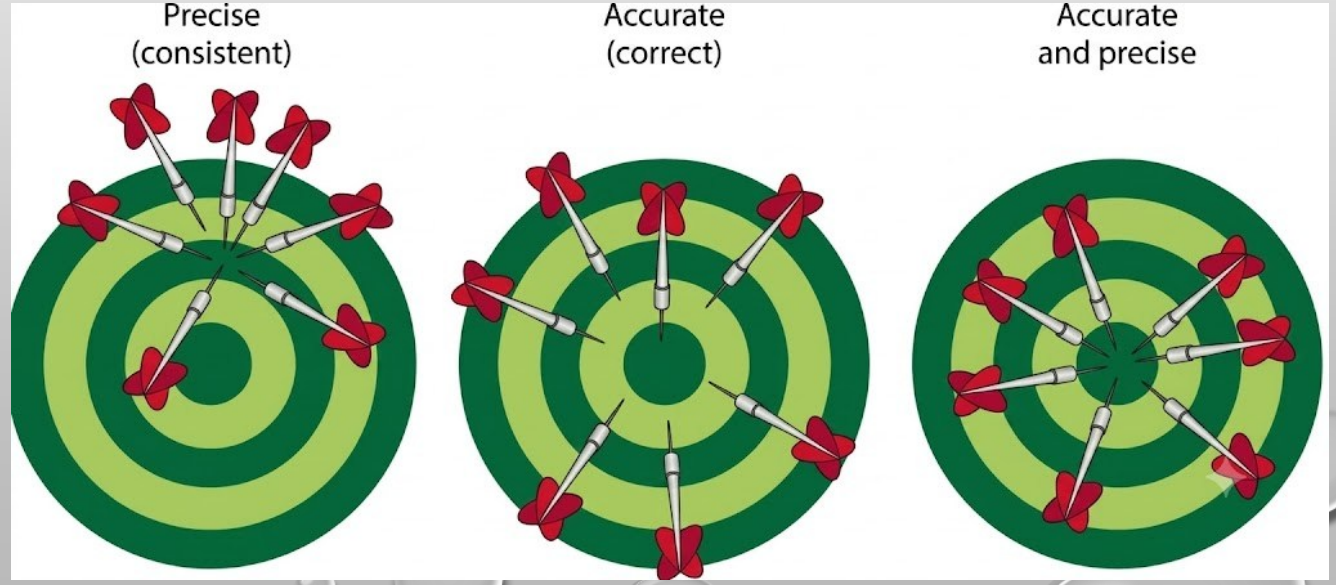
PRECISION AND ACCURACY

- The greater the sensitivity of an instrument, the **greater the precision** of the measurement it allows us to make; in other words, the values obtained from repeated measurements will all be **consistent** (close to one another).

- However, a precise instrument is not necessarily accurate; that is, the values obtained may not be correct if they are not centred around the expected value (or **true value**).

- Measurement uncertainty can be reduced by:
  a) repeating the tests multiple times (repeated trials);
  b) improving control over experimental conditions;
  c) verifying instrument accuracy through periodic calibration.

The classic example used to illustrate the difference between accuracy and precision is shooting at a target. 1) A **precise but inaccurate** archer will shoot arrows that are close to one another but far from the centre. 2) An **accurate but imprecise** archer will shoot arrows that are far apart but clustered around the centre. 3) An **accurate and precise** archer will always hit close to the centre—the Robin Hood of measurement!



STATISTICAL ANALYSIS

One number is never enough — we need replicates:

- Every measurement carries some error and biological variation.
- With one value you cannot tell a real signal from random noise.

So we repeat the measurement at least three times ($n \geq 3$). More replicates → a more reliable mean and a smaller uncertainty.

Biological replicates — these count for statistics

Different samples: separate cultures, animals, or preparations. They tell you whether the effect is real and general.

Technical replicates

The same sample measured several times. They check the precision of your method — not the biology.

MEAN, STANDARD DEVIATION AND SEM

Three numbers that summarise your replicates — in plain terms

Mean ($\bar{x}$)

$$\bar{x} = \Sigma\, x_i \,/\, n$$

i = number of each test
n = total number of tests performed
Your best estimate of the true value: add all the values and divide by how many there are.

Standard deviation (SD)

how spread the data are

Measures how far the individual values scatter around the mean.

Small SD = tight, consistent data.
Large SD = noisy data.

Use SD to DESCRIBE your data.

Standard error (SEM)

$$SEM = SD \,/\, \sqrt{n}$$

How precise your estimate of the mean is. It shrinks as n grows.

SEM is always smaller than SD — don't use it just to look more precise.

Always state which one your error bars show.

INFERENTIAL ANALYSIS

CALIBRATION CURVE: READING AN UNKNOWN

In biochemical investigations, a specific parameter to be measured is very often a function of another parameter, governed by a precise mathematical relationship. Generally, to determine the nature of the dependency between two variables, x and y, measurements of y are taken as a function of x at several points (at least three); statistical analysis software is then used to interpolate these points and derive the equation of the curve that best fits the data.

When one quantity depends on another by a known law, we fit a line and interpolate

The idea, in four steps

1 · The signal y depends on the quantity x by a known law.

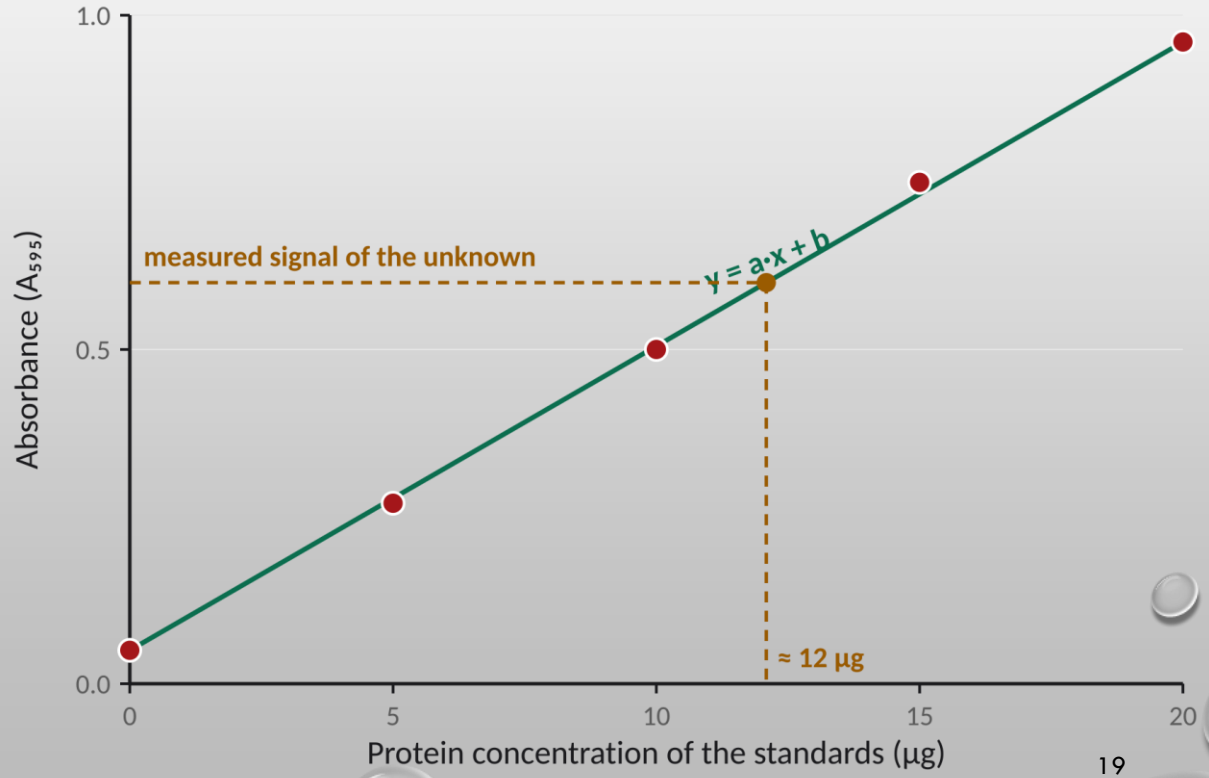
2 · Measure y for several known standards (at least 3 points).

3 · Fit the best line through the points (linear regression).

4 · For an unknown: measure its y, then read its x off the line.

$$y = a \cdot x + b$$

a = slope · b = intercept (y when x = 0)



Same principle in the lab: retention time vs log molecular size in size-exclusion chromatography, or log molecular weight vs relative mobility in SDS-PAGE.

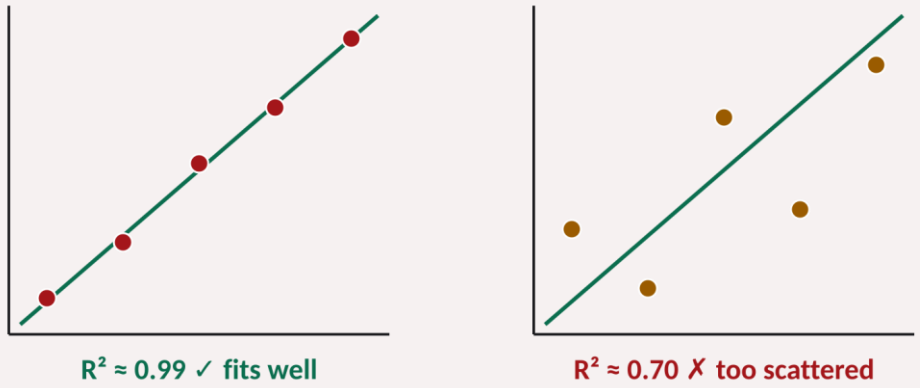
INFERENTIAL ANALYSIS

CALIBRATION CURVE: READING AN UNKNOWN

Two things that make or break a calibration

How well the line fits — and where you are allowed to read it

How good is the fit? R^2

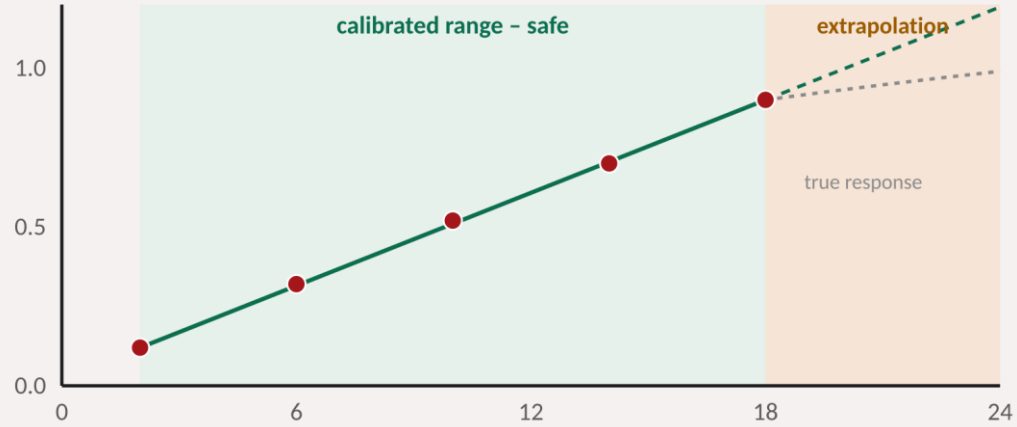


$R^2 \approx 0.99$ ✓ fits well

$R^2 \approx 0.70$ ✗ too scattered

R^2(Pearson correlation coefficient) goes from 0 to 1 and tells you how close the points sit to the line. **Close to 1 = the line describes the data; a low R^2 means the calibration is unreliable — recheck it before using it.**

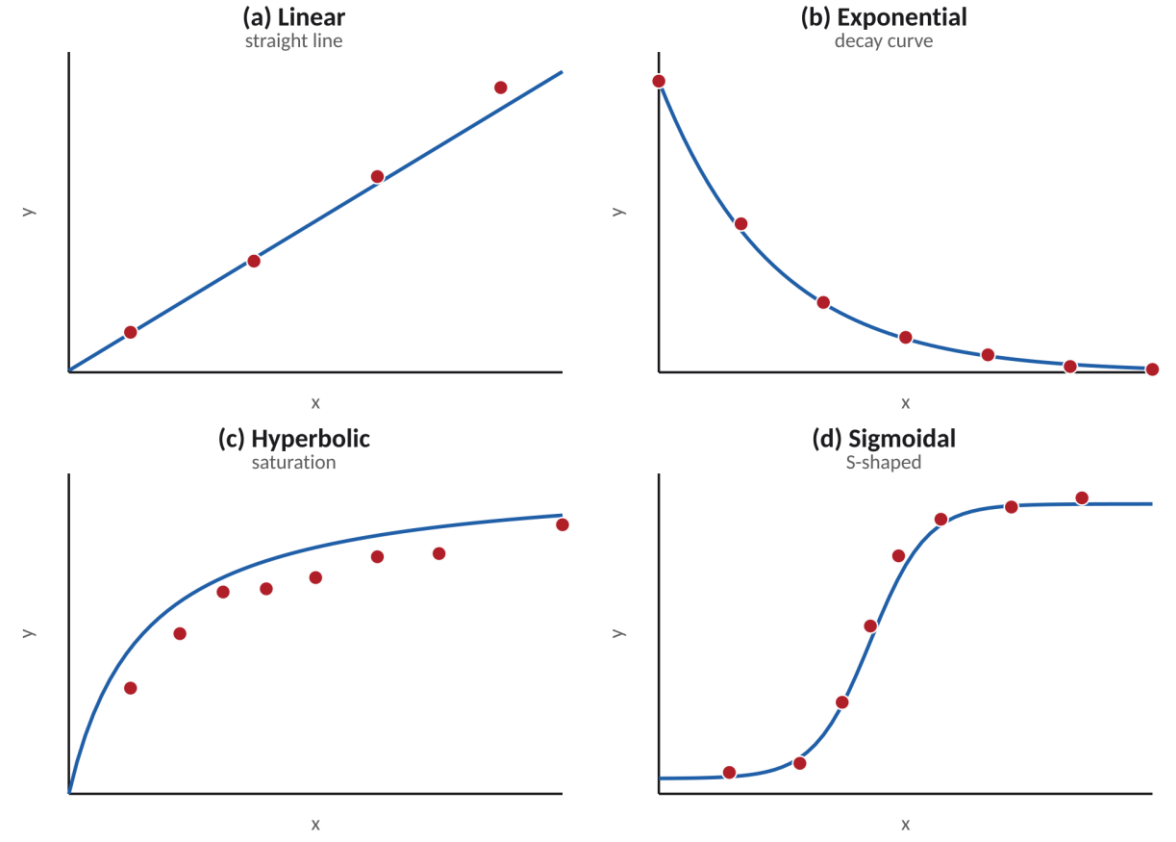
Stay within the calibrator range



Only read unknowns between your lowest and highest standard. **Beyond the last point the real response may bend, so extrapolating just invents numbers.**

TYPES OF DEPENDENCE BETWEEN VARIABLES

Measure y at several values of x, then fit the curve that best matches the points



How to read them

Each panel shows the same idea: real data points (red) and the fitted curve (blue).

The shape reflects the underlying law — linear, exponential, hyperbolic (saturating) or sigmoidal (S-shaped).

A number tells us how well the curve fits: R for a straight line, χ^2 for the others.

R — for a straight line

R is the correlation coefficient: it measures how well the points line up on a straight line.

|R| close to 1 → strong linear relationship. R close to 0 → no linear relationship.

R^2 (R squared) is the fraction of the variation in y explained by the line — e.g. $R^2 = 0.99$ means 99% explained.

Use it only when you expect a straight line.

χ^2 (chi-square) — for any curve

χ^2 adds up the gaps between each point and the curve (squared, and weighted by the measurement error).

Small χ^2 → the curve passes close to the points → good fit.

Large χ^2 → the points fall far from the curve → poor fit or wrong model[21]

It works for exponential, hyperbolic and sigmoidal curves too, not just lines.

WHERE THESE CURVES COME FROM

The same four shapes describe real biochemical laws

Exponential — decay

$y = y_0 \cdot e^{-ax}$

Radioactive decay.

y_0 = value of y at x = 0

α = decay constant

Hyperbolic — saturation

$y = (a \cdot x) / (b + x)$

Enzyme kinetics (Michaelis–Menten): product formation levels off.

a = maximum of y (y_max)

b = x where y = y_max / 2 (K_m)

Sigmoidal — S-shaped

$y = a + b / (1 + e^{-d(x-c)})$

Cooperative binding, competitive interactions, population growth (logistic).

a = minimum of y

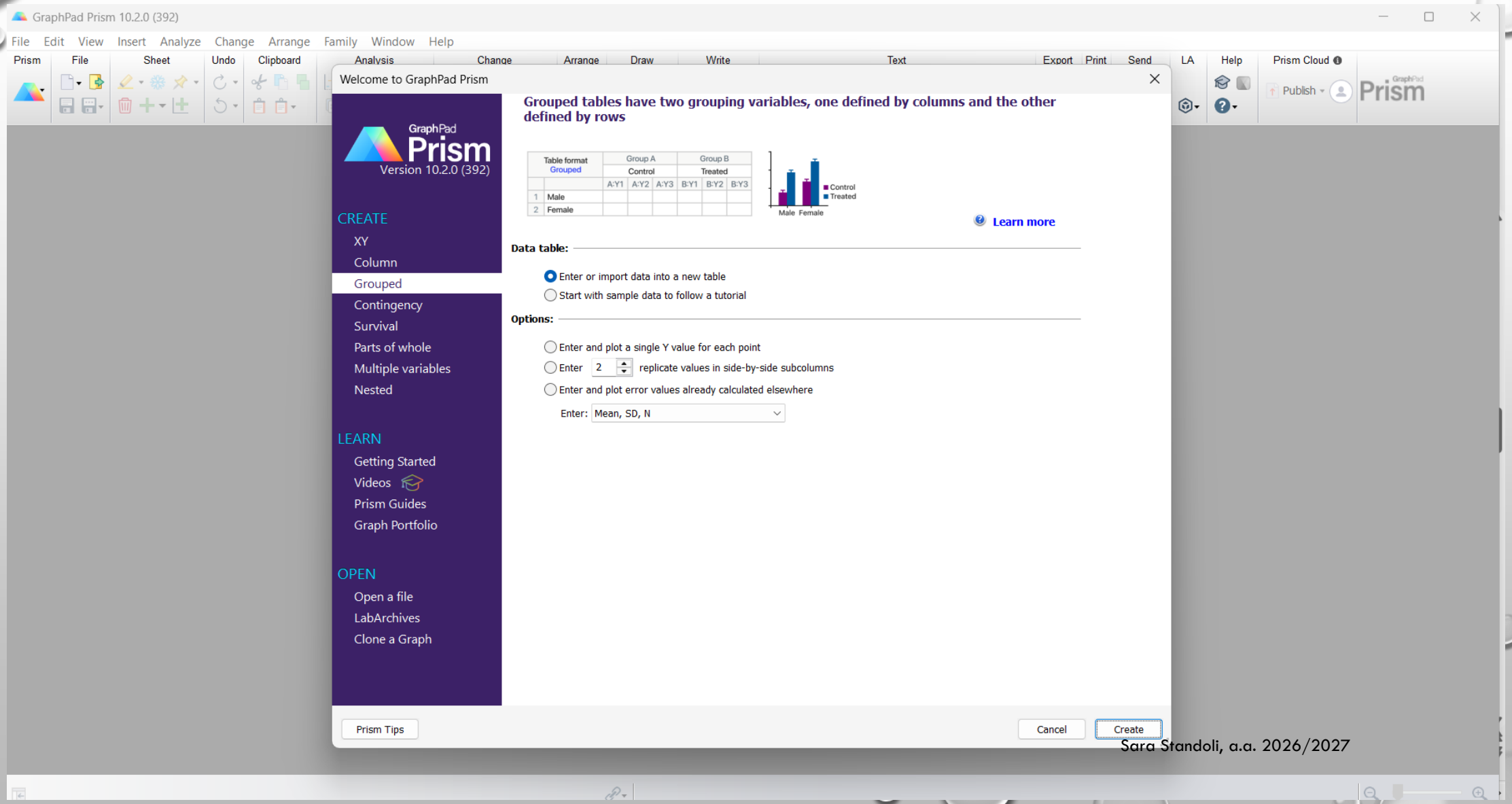
b = maximum − minimum

c = x at the midpoint

d = steepness of the rise

STATISTICAL ANALYSIS

How Do I Procede? Which Test Should I Use?



STATISTICAL ANALYSIS

How Do I Procede? Which Test Should I Use?

STEP 1 · Check whether each group is NORMAL Shapiro–Wilk test, backed up by a histogram or QQ-plot

Reading the Shapiro–Wilk test

The test asks: "Could this data come from a normal distribution?"

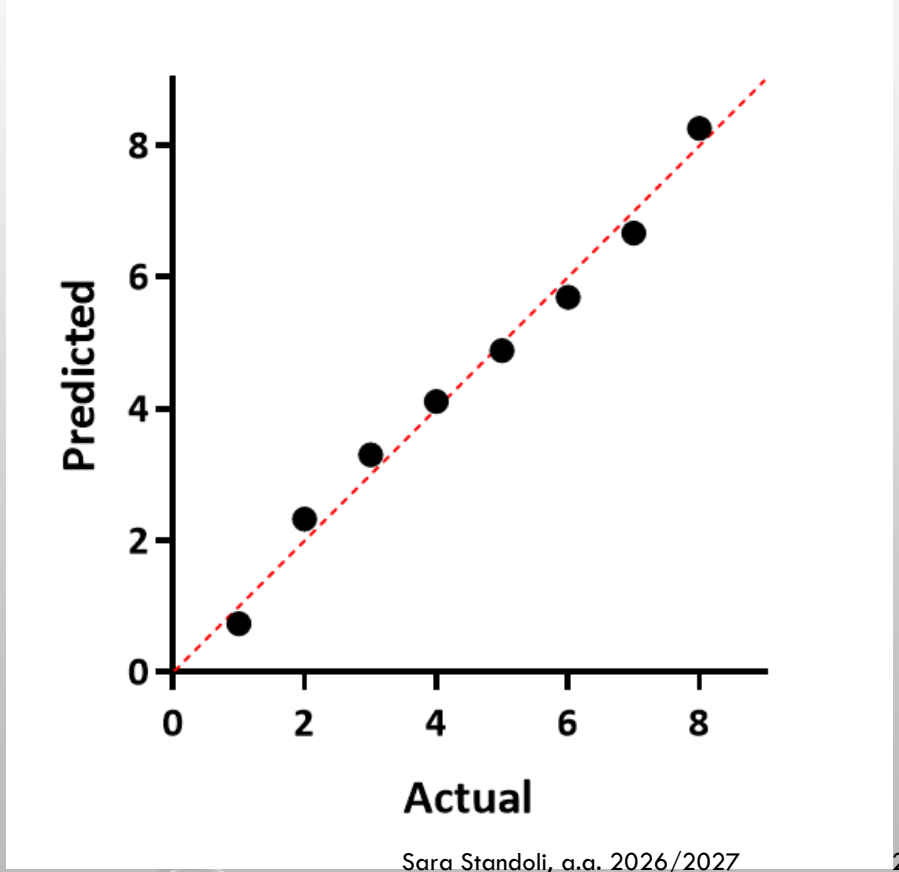
p > 0.05 → yes, treat it as normal → parametric tests.

p < 0.05 → not normal → non-parametric tests.

Two things to keep in mind:

With very few data the test is weak — always look at a histogram too.

Check each group separately, not all the data pooled together.



STATISTICAL ANALYSIS

How Do I Procede? Which Test Should I Use?

STEP 2 ·Test Selection

NORMAL data → PARAMETRIC tests

2 groups

t-test
unpaired (independent)
paired (same sample)

3 or more groups

one-way ANOVA
+ post-hoc
(Tukey / Šidák)

NON-NORMAL data → NON-PARAMETRIC tests

2 groups

Mann–Whitney (unpaired)
Wilcoxon (paired)

3 or more groups

Kruskal–Wallis
+ post-hoc
(Dunn)

Also remember: **check that the groups have similar variance; if they don't, use Welch's correction. "Paired" means the same subject measured twice (e.g. before / after).**

The test-choice table

Your experiment	Parametric (normal data)	Non-parametric
2 independent groups	unpaired t-test	Mann–Whitney test
2 paired groups	paired t-test	Wilcoxon test
3+ independent groups	one-way ANOVA + Tukey	Kruskal–Wallis + Dunn

After ANOVA or Kruskal–Wallis you must run a post-hoc test **to see WHICH groups differ: the main test only tells you that at least one difference exists.**

STATISTICAL ANALYSIS
Presenting your results

What to put in the figure — and how not to mislead

p-value and significance

The usual cut-off is 0.05; report the actual value, not just "<0.05".

```
*   p<0.05     **   p<0.01     ***   p<0.001     ****   p<0.0001
ns = not significant
```

Error bars: always say what they are

SD → how spread the data are.

SEM → how precise the mean is (smaller — don't use it just to look precise).

95% CI → the plausible range of the true value.

In the figure legend, state…

which statistical test (and post-hoc) you used;

the value of n, and whether they are biological or technical replicates;

what the error bars represent.

Statistical significance is not the same as biological importance: a tiny effect can still be "significant".

After ANOVA or Kruskal–Wallis you must run a post-hoc test to see WHICH groups differ: the main test only tells you that at least one difference exists.

STATISTICAL ANALYSIS
Bioinformatics

The term '**Bioinformatic**' describes the processing of biological data



But now-days is considered a stand-alone disciplines that includes methods on processing 'big data' coming from genomics, proteomics, and high throughput screening



Needs programs and tools (**programming**, often called 'coding'), like:

- **R** (https://www.r-project.org/)
- **Python** (https://www.python.org/)