Analysis of soil microbial communities

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Methods of analyses of soil microbial communities

- Cultivation – determination of Colony Forming Units
  + covers viable microorganisms
  - only 1-2% of soil microbes cultivable
- **Activity – determination of microbial activities**
  – enzyme activities, respiration, production / consumption of chemicals…
  + covers viable microorganisms
  - dependent on conditions

Methods of analyses of soil microbial communities

- Genetic analyses – extraction of DNA / RNA, sequencing, comparison with databases
  - \+ detailed information (taxonomy, abundance of taxons, metabolic potential, transcriptomics – expressed genes, stress genes…)
  - \- costly and not as spread equipment
  - \- sometimes too detailed data (limited database data, laborious evaluation)

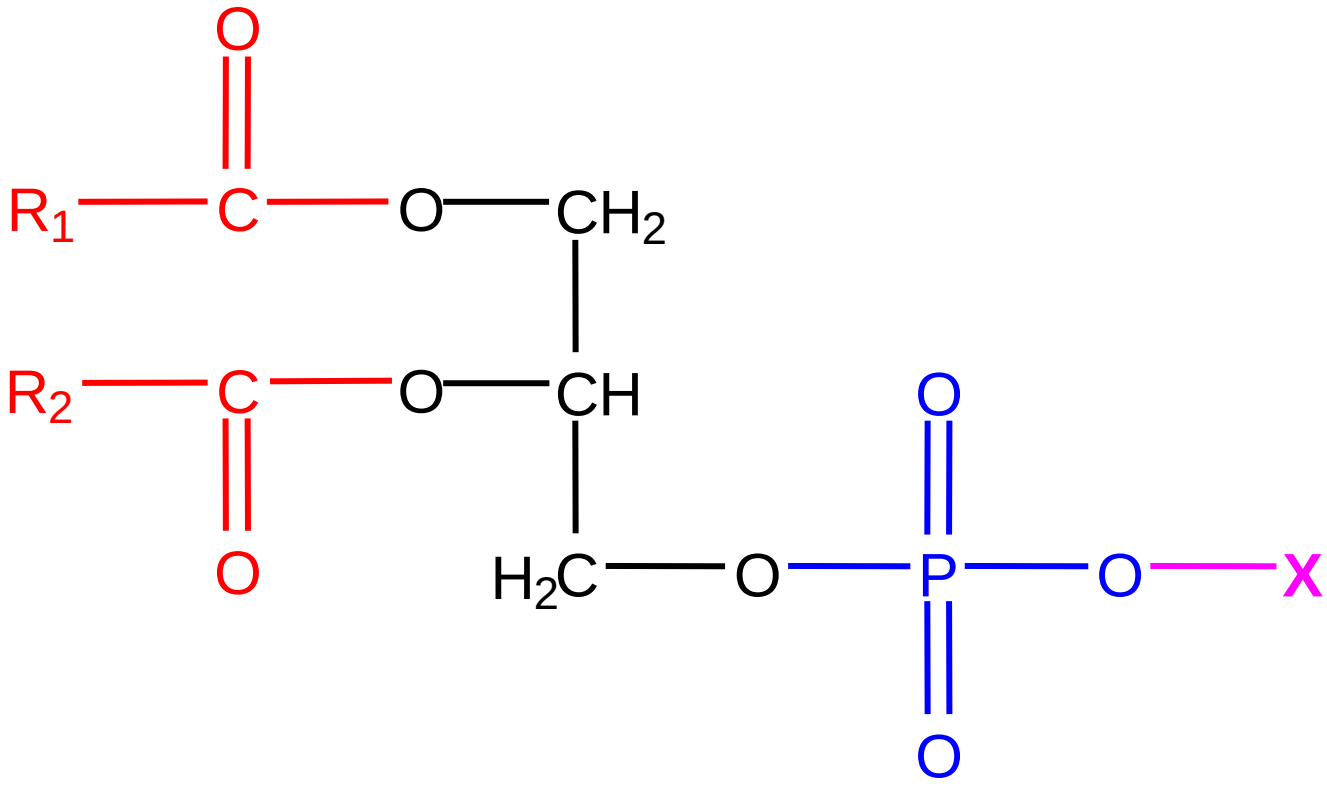
Methods of analyses of soil microbial communities

- Chemical analyses – determination of biomarker molecules / profiles
  - \+ in general simpler
  - \+ widespread and cheaper equipment
  - \- not as detailed information
  - \- possible interferences – need of careful interpretation

Biomarker molecules

- Respiration isoprenoids – chinons, length of side chain
- Polysacharides – surface, sheat
- Polyamines
- Mycolic acids
- Sterols – eucaryotic membrane (ergosterole in fungi)
- **Membrane lipids – especially phospholipid fatty acid profiles**
- …

Phospholipids



R_1, R_2 – fatty acid acyls

X – hydrophilic groups

Phospholipids

- *In vivo* in membranes only
  - **cytoplsmatic**
  - outer membrane of G- bacteria
  - inner membranes of eukaryotes
- Never storage compounds → ~proportional content to biomass
- Fast decomposition after cell death → estimation of living biomass

Phospholipid fatty acids (PLFA)

- Composition of membrane PLFA depends on
  - species (taxonomy)
  - temperature
  - physiological state (stress detection)
  - nutrition

Utilization of PLFA analyses in soil ecology

- **Quantification of living microbial biomass (total PLFA) – fast decomposition after cell death – non-cultivation technique**
- **Quantification of microbial groups**
  - **fungi / bacteria ratio**
  - G+/G- ratio
  - abundance of other groups (actinobacteria, methanogenes, anaerobes…)
- **Monitoring of stress and soil disturbation**

Basic PLFA extraction

- Extraction of total lipids
  - **single-phase** mixture MeOH+chloroform+phosphate buffer
- Separation of lipid fractions
  - solid-phase extraction – silica columns
- Derivatization
  - Usually alcalic methanolysis
- GC-MS
  - usually polar column

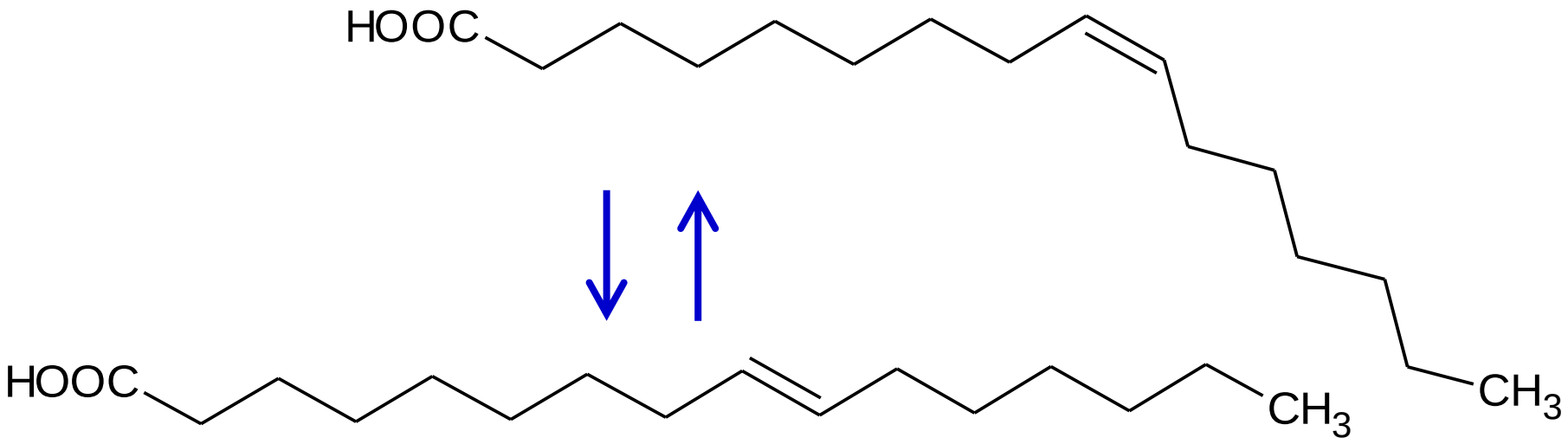
Community biomarker fatty acids

Group	subgroup	Biomarker fatty acids
Bacteria	G+	i14:0, i15:0, a15:0, i17:0, a17:0
	G-	cy17:0, cy19:0, 18:1w7
	Actinobacteria	10Me-16:0, 10Me-17:0, 10Me-18:0
	Other	16:1ω7t, 16:1ω7, 16:1ω9,
Fungi		18:2ω6,9
Protozoa		20:4ω6

Physiological indicators

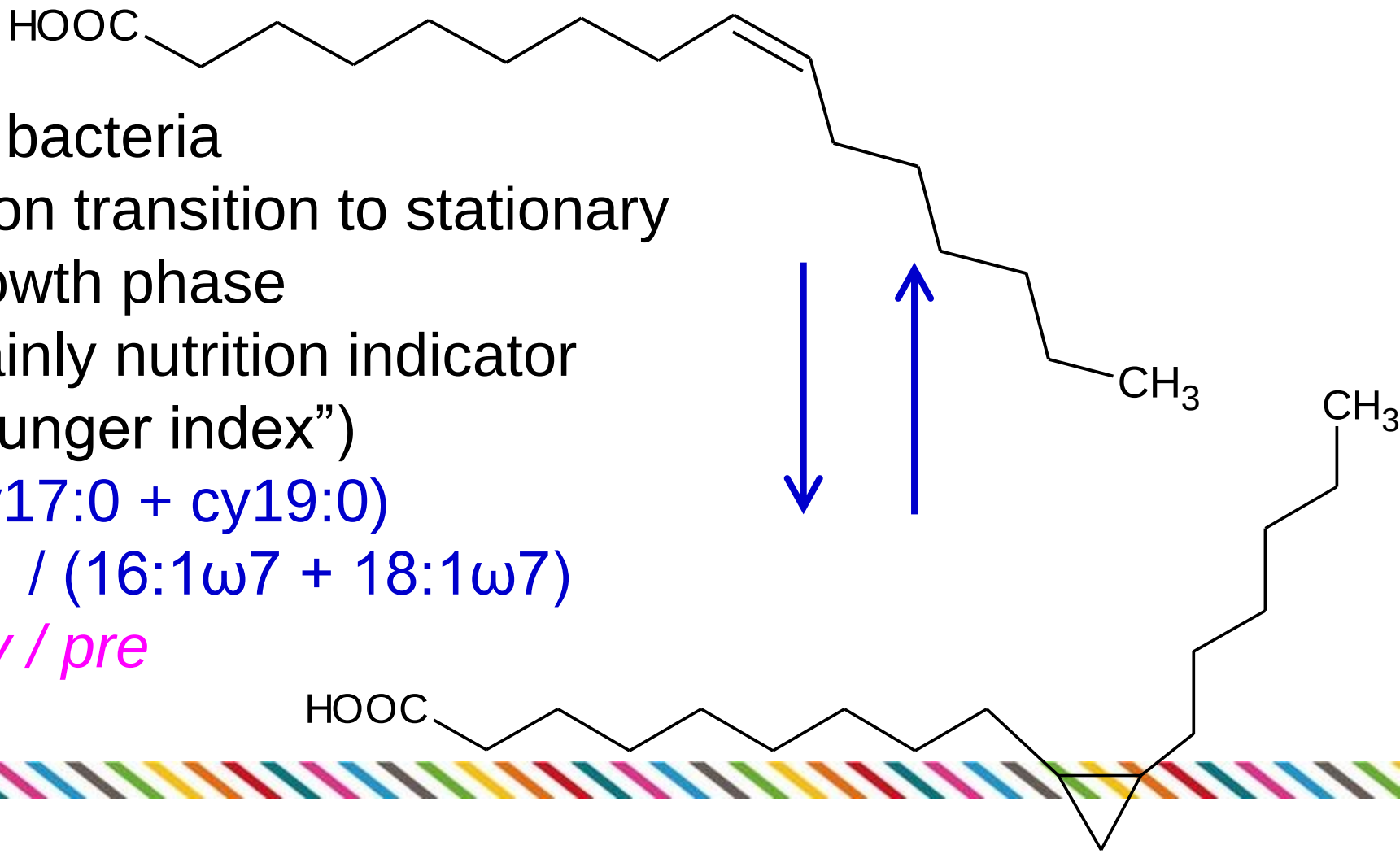
- Biochemical + empiric knowledge
- Only changes affecting membranes
- A series of published variants

cis / *trans* isomeration



- Bacteria
- Changes directly in membrane
- *trans* / *cis* index
- general stress indicator
- (18:1ω7+16:1ω7) / (16:1ω7t+18:1ω7t)
- >0.1 → soil disturbation and stress

Cyclization of monounsaturated FA



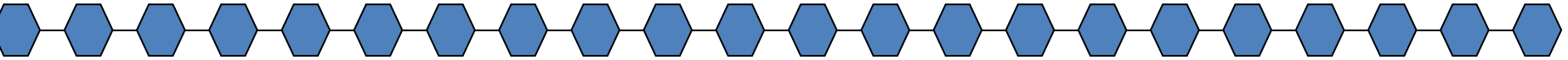
- G- bacteria
- upon transition to stationary growth phase
- mainly nutrition indicator (“hunger index”)
- (cy17:0 + cy19:0) / (16:1ω7 + 18:1ω7)
- *cy / pre*

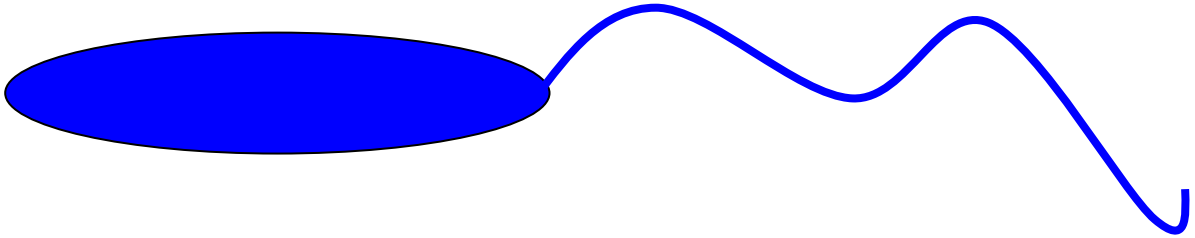
Determination of microbial activities

- Completes biomass data with indication of activities
  - comparison of living biomass vers. activities gives useful information about overall state of community
- Activity of extracellular enzymes – decomposition of polymers
- Respiration – analyses of O_2 consumption or CO_2 production

Determination of microbial activities

- Activity of extracellular enzymes – decomposition of polymers





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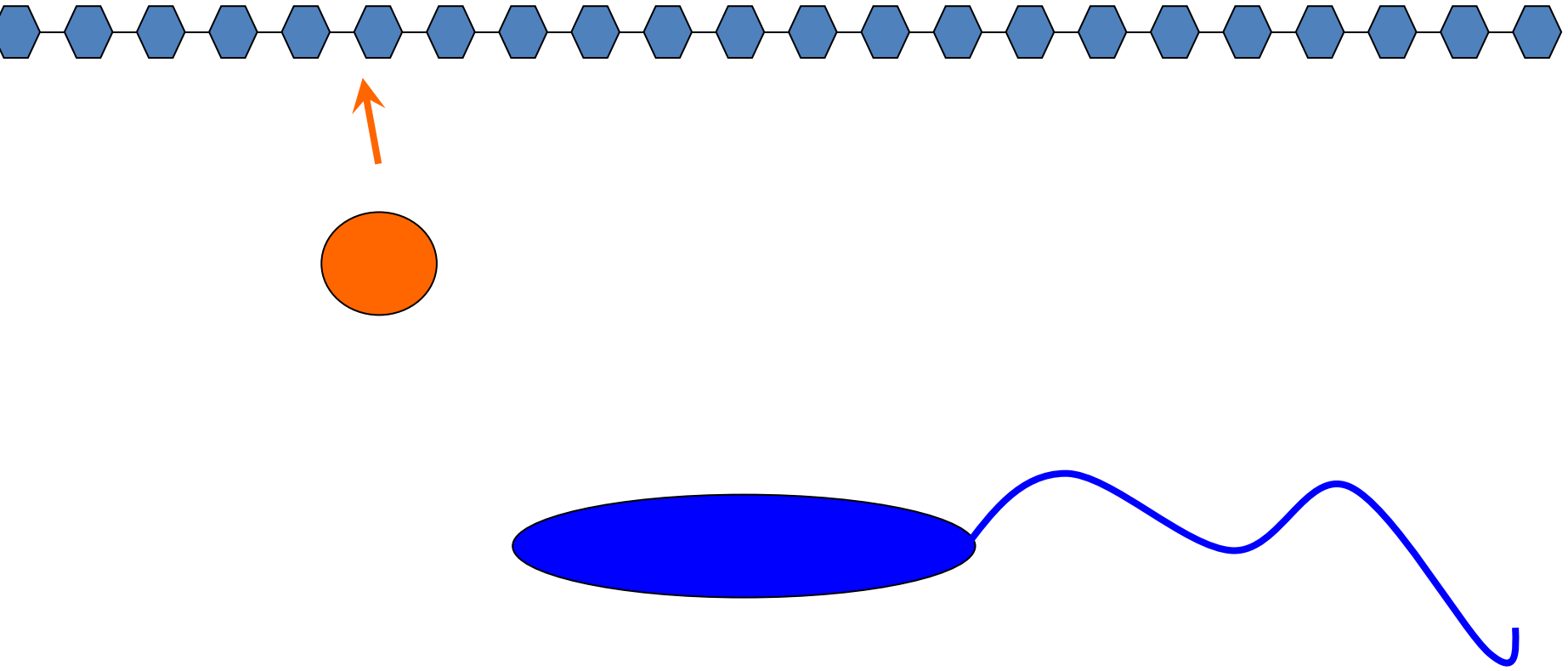

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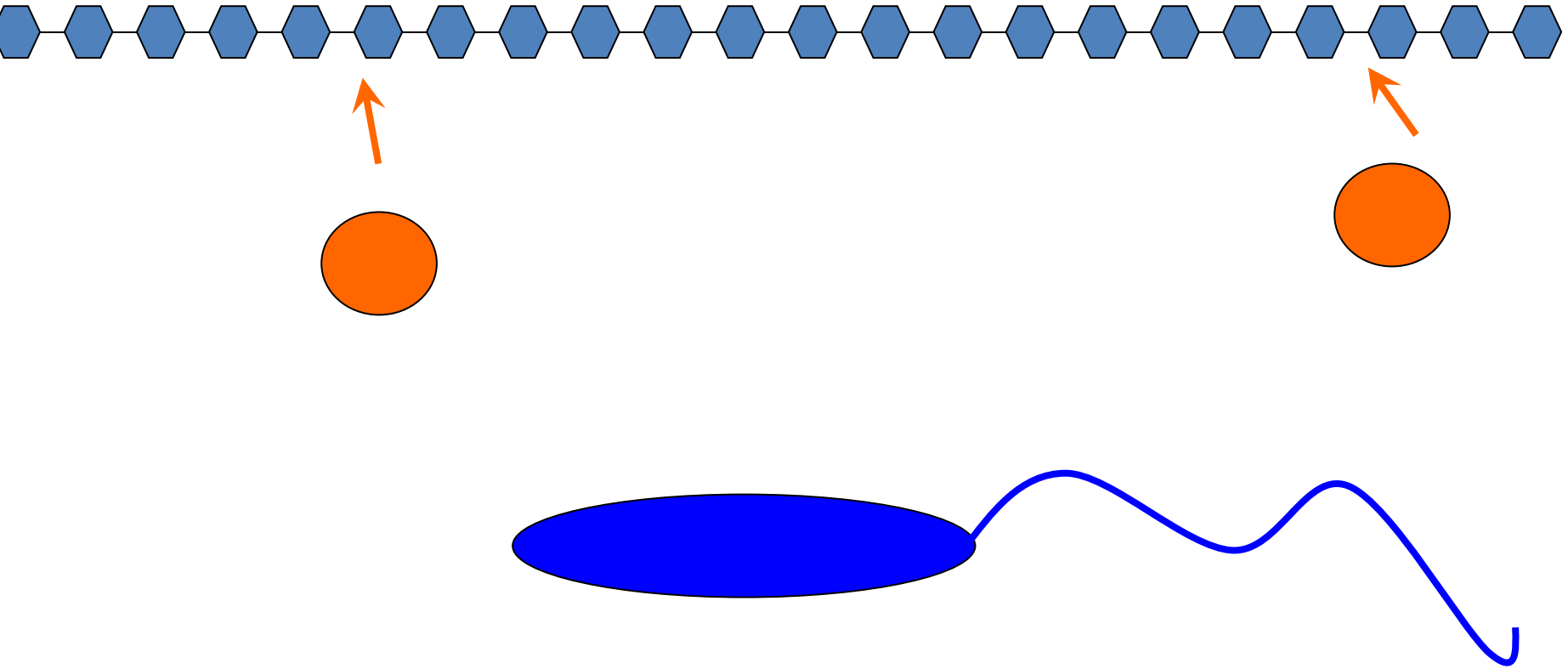

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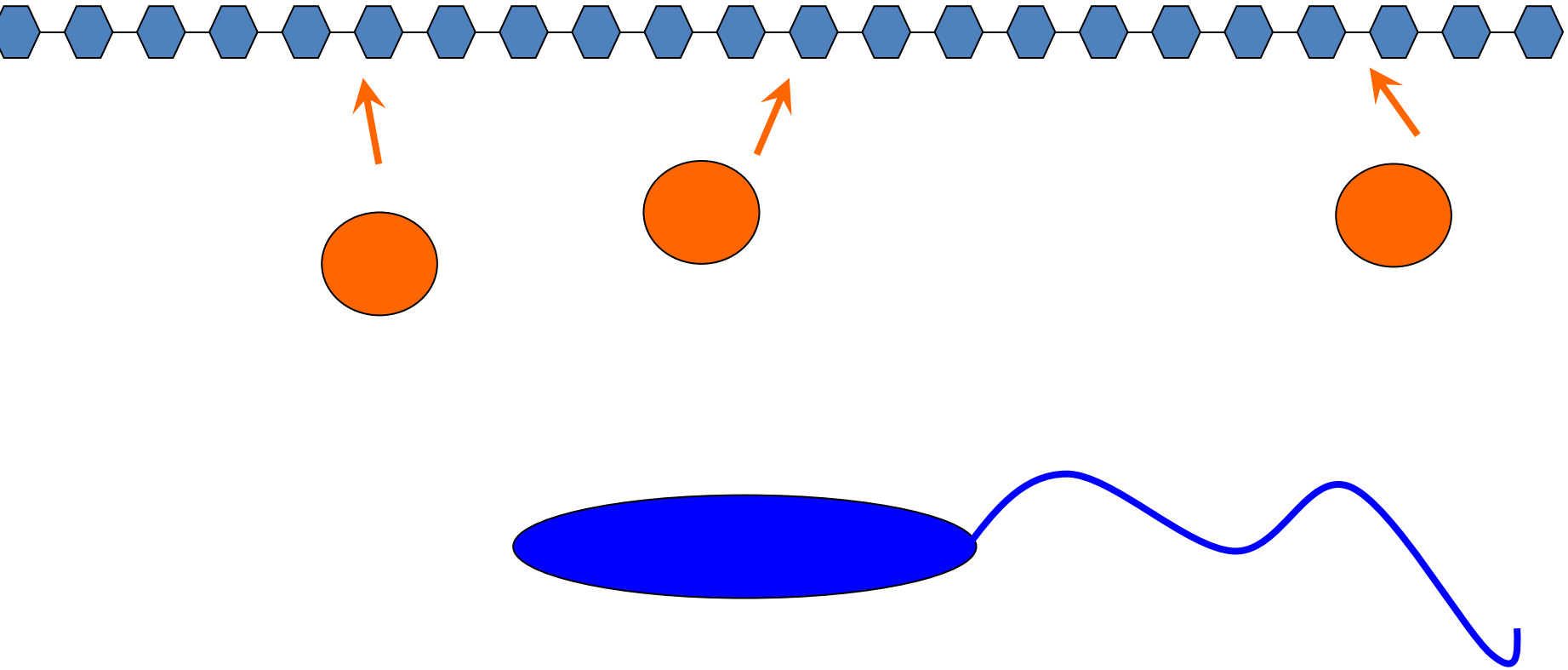

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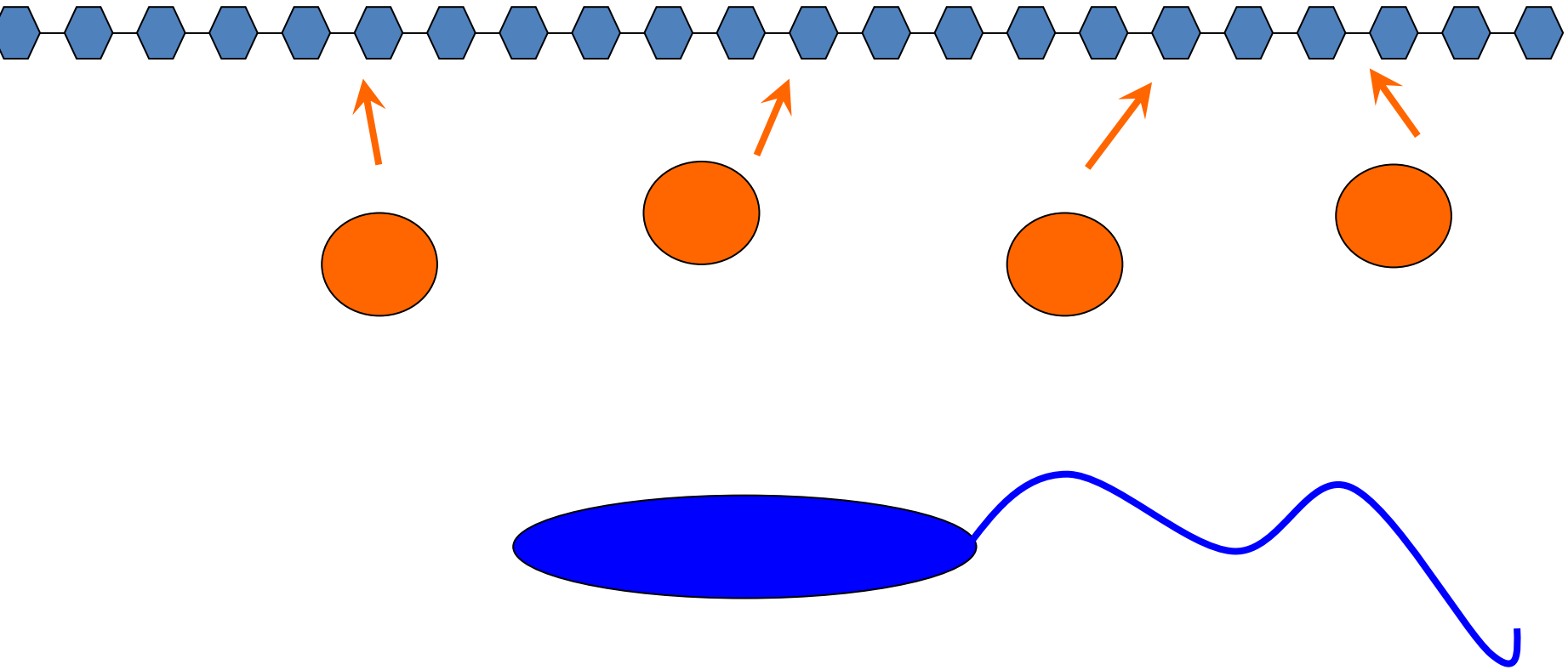

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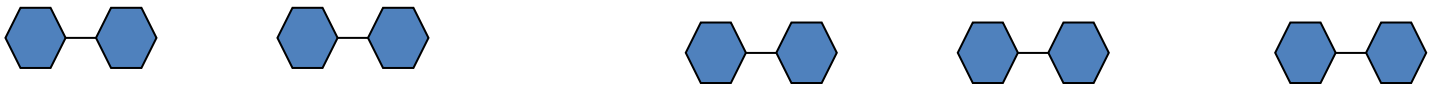
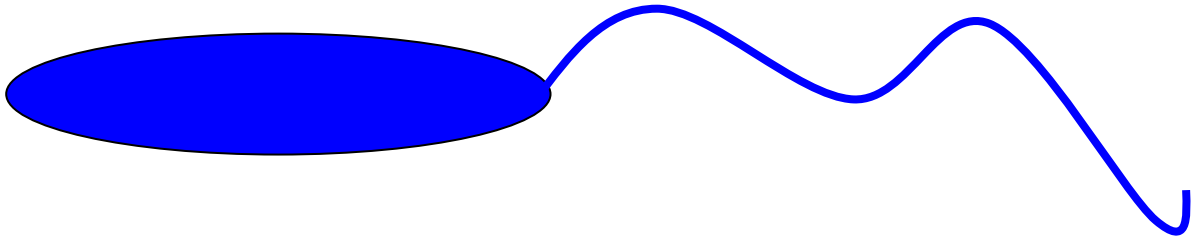


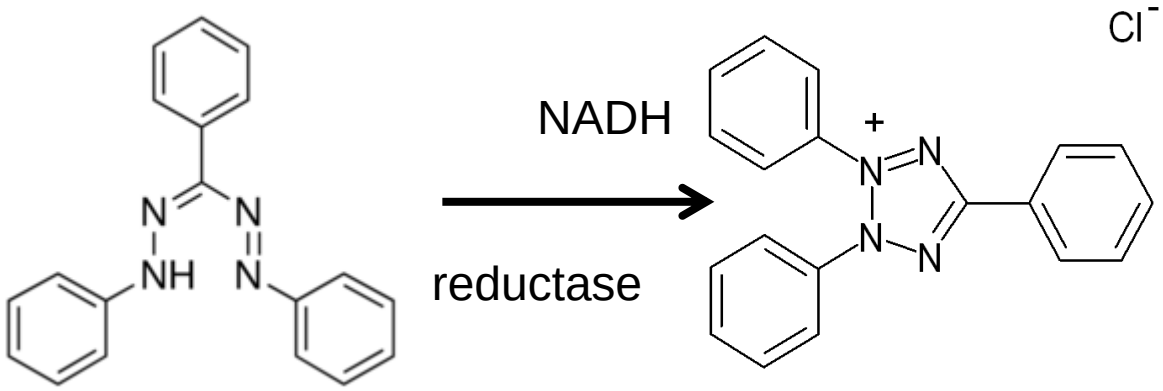


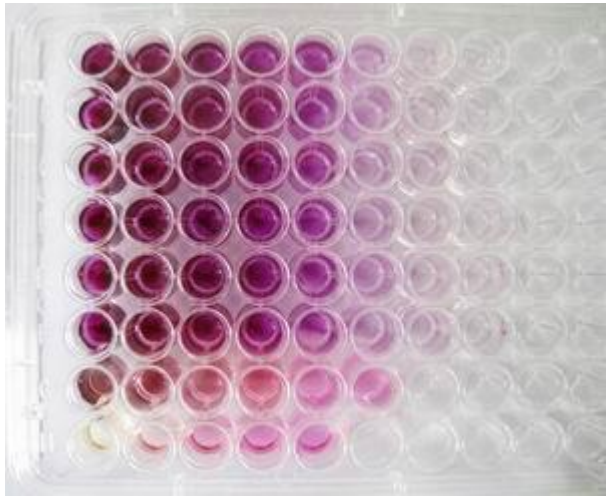
Activity of enzymes

- Artificial enzyme substrates – change to easily determined compounds
- Many assays – spectrophotometric or fluorometric
- High / low specifity

Tetrazolium assay

- Based of reduction of triphenyltetrazolium to triphenylphormazan
  - pink – spectrophotometric determination at 546 nm)
- Substrate of many oxidoreductases

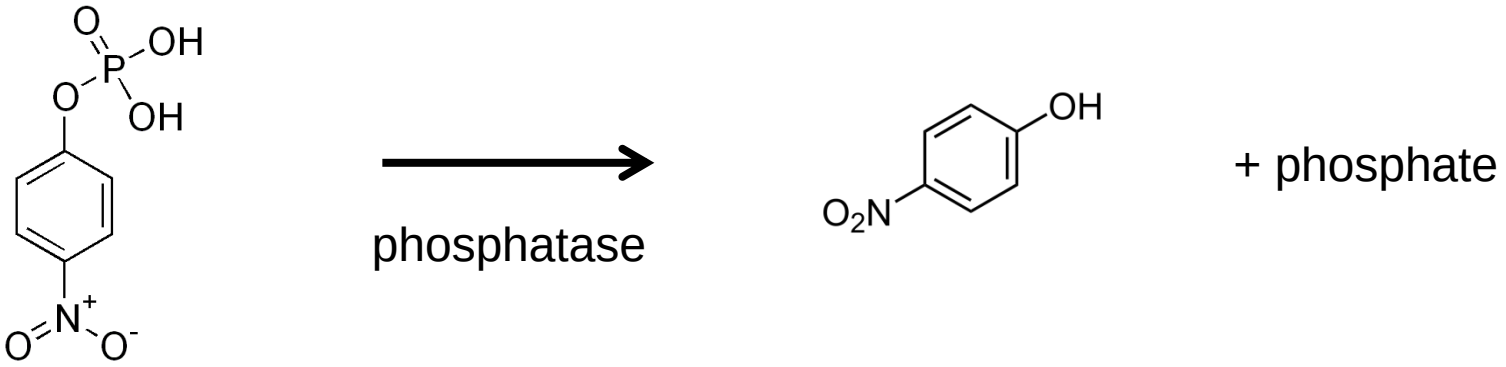




- determines overall activity of soil or sludge

pNP assay

- Hydrolysis of pNP-X to p-nitrofenyl phosphate and X
- Determination of yellow pNP (pH >7, 400 nm)
- Many variants – phosphatases, sulphatases, proteases, glucosidases, chitinases…



Respiration

- Determination of the rate of
  - O_2 consumption
  - CO_2 production
- Simple titration
  - $CO_2 + OH^- \rightarrow HCO_3^-$
- Advance respirometers

- Variants:
  - optimization of humidity
  - addition of subtrate (glucose, polysacharides…)