Protein Storage & Handling

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What makes proteins go bad?

- Aggregation, precipitation
- Chemical modifications:
  - oxidation
  - hydrolysis (non-enzymatic & enzymatic)
  - deamidation
  - disulfide exchange
- Adsorption

Protein aggregates

- Can be soluble (no precipitation, detection by gel filtration)
- Nucleation threshold (protein solutions go bad within a few hours or days after been stored for a prolonged period)
- Dependend on protein concentration (the higher the concentration the higher the aggegation rate)
- Do form under ”physiological” conditions

What promotes protein aggregation?

- Interface contacts (gas-liquid: mixing; liquid-solid: filtration)
- Unsuitable pH
- Freezing-thawing
- High temperatures

Precipitation

- Preceipitation does not necessarily inactivate a protein
- Precipitation can result from: aggregation, exceeding the solubility constant (”salting out”), denaturation

Chemical modifications

- Oxidation (Met, aromatic aa, unpaired Cys) Is used in the physiological regulation of enzymes; products: e.g. disulfide-linked oligomers.
- Non-enzymatic hydrolysis (both side-chain & backbone) Both acid- & base-catalyzed.
- Enzymatic hydrolysis Purity problem! Occurs usually early during storage.
- Deamidation of side-chain residues (Asn-Gly) High pH, phosphate ion-catalyzed.
- Disulfid exchange Rare, base-catalyzed.

Low temperature, but not -20°C!

- All physical & chemical modifications are temperature-dependend
- Store at lowest possible temperature, but not at -20°C!
  - At -20°C the solution is not completely frozen:

$$H_2O \rightarrow HPO_4^{2-} \text{ -> } H_2PO_4^-$$

↑

pH drops to ~4

Why not -20°C?

- Much of the protein stays in the liquid phase at low pH and high salt concentration
- Actual temperature in the freezer fluctuates around -20°C: Constantly changing pH & repeated freeze-thawing cycles
- PBS is NOT a good storage buffer

Frozen or non-frozen?

Non-frozen

- Short term storage (less than a week): 0°C
- Intermediate storage (a few months): -20°C with anti-freezing agents, e.g. 50% glycerol, sucrose, PEG

Frozen

- Long term storage: -70°C (months – a few years), -150°C (decades), addition of cryo-protectants (glycerol, sucrose, citrate)

How to freeze?

Freezing

- Flash-freezing is better than slow freezing
- Rarely inactivates proteins (partial loss of activity due to aggregation)
- Avoid repeated freeze-thawing cycles! Aliquot!
- VEGF-C is resistant to activity loss due to freezing-thawing (10 cycles => >90% activity)
- Activity loss depends on protein concentration (protein acts as its own cryoprotectant)

Freeze-drying

Freeze-drying/Lyophilization

- Last resort due to technical difficulties
- A very good vacuum is needed (increase temperature at the end of the process to 37°C to evaporate the last tracer of water)
- If correctly done, proteins can be stored at RT for centuries
- Due to technical limitations in academic labs, lyophilized proteins shoud be stored at 4°C or lower

Adsorption

- Protein doesn't go bad, but simply seems to disappear
- Proteins do stick to virtually every surface
- ng protein/cm^2 surface: NBS (<2.5) < mPET () < PET (100?) < PP (300-440) < PC/PS (300-500) < HB-PS/”Maxisorp” (up to 650)

Adsorption

- When pipetting, first saturate the platic surface by pipetting up and down a few times (a 200µl PP pipette tip adsorbs about 70 ng of protein)
- Don't dilute protein solutions below 0.1mg/ml without adding carrier proteins (0.1%BSA in PBS, cell culture medium with 10% FCS)
- ng protein/cm^2 surface: NBS (<2.5) < mPET () < PET (100?) < PP (300-440) < PC/PS (300-500) < HB-PS/”Maxisorp” (up to 650)