Basic Protein Methods

（Notes for proteomics course）

Protein properties used during purification.

Protein property	Technique
Charge	Ion exchange (IEX)
Size	Gel filtration (GF)
Hydrophobicity	Hydrophobic interaction (HIC), Reversed phase (RPC)
Biorecognition (ligand specificity)	Affinity (AC)
Charge, ligand specificity or hydrophobicity	Expanded bed adsorption (EBA) follows the principles of AC, IEX or HIC

Protein properties and their effect on development of purification strategies.

Sample and target protein properties	Influence on purification strategy
Temperature stability	Need to work rapidly at lowered temperature
pH stability	Selection of buffers for extraction and purification Selection of conditions for ion exchange, affinity or reversed phase chromatography
Organic solvents stability	Selection of conditions for reversed phase chromatography
Detergent requirement	Consider effects on chromatographic steps and the need for detergent removal. Consider choice of detergent.
Salt (ionic strength)	Selection of conditions for precipitation techniques, ion exchange and hydrophobic interaction chromatography
Co-factors for stability or activity	Selection of additives, pH, salts, buffers
Protease sensitivity	Need for fast removal of proteases or addition of inhibitors
Sensitivity to metal ions	Need to add EDTA or EGTA to buffers
Redox sensitivity	Need to add reducing agents
Molecular weight	Selection of gel filtration media
Charge properties	Selection of ion exchange conditions
Biospecific affinity	Selection of ligand for affinity medium
Post translational modifications	Selection of group-specific affinity medium
Hydrophobicity	Selection of medium for hydrophobic interaction chromatography

Common sample extraction processes.

Extraction process	Typical conditions	Protein source	Comment
Gentle Cell lysis (osmotic shock)	2 volumes water to 1 volume packed pre-washed cells	erythrocytes, E.coli periplasm: intracellular proteins	lower product yield but reduced protease release
Enzymatic digestion	lysozyme 0.2 mg/ml, 37 °C, 15 mins.	bacteria: intracellula proteins	lab scale only, often combined with mechanical disruption
Hand homogenisation	follow equipment instructions	liver tissue	
Mincing (grinding)	"	muscle	
Moderate Blade homogeniser	follow equipment instructions	muscle tissue, most animal tissues, plant tissues	
Grinding with abrasive e.g. sand	"	bacteria, plant tissues	
Vigorous Ultrasonication or bead milling	follow equipment instructions	cell suspensions: intracellular proteins in cytoplasm, periplasm, inclusion bodies	small scale, release of nucleic acids may cause viscosity problems inclusio bodies must be resolubilised
Manton-Gaulin homogeniser	follow equipment instructions	cell suspensions	large scale only
French press	follow equipment instructions	bacteria, plant cells	
Fractional precipitation		extracellular: secreted recombinar proteins, monoclonal antibodies, cell lysates	precipitates must be resolubilised

Common substances used in sample preparation.

Substances	Typical conditions for use	Purpose
Tris	20 mM, pH 7.4	maintain pH, minimise acidification caused by lysosomal disruption
NaCl	100 mM	maintain ionic strength of medium
EDTA	10 mM	reduce oxidation damage, chelate metal ions
Sucrose or glucose	25 mM	stabilise lysosomal membra-nes, reduce protease release
Detergents ionic or non-ionic detergents		extraction and purification of integral membrane proteins solubilisation of poorly soluble proteins
DNase and RNase	1 μg/ml	degradation of nucleic acids, reduce viscosity of sample solution
Protease inhibitors* PMSF	0.5 - 1 mM	Inhibits serine proteases
APMSF	0.4 - 4 mM	serine proteases
Benzamidine-HCl	0.2 mM	serine proteases
Pepstatin	1 μM	aspartic proteases
Leupeptin	10 - 100 μM	cysteine and serine proteases
Chymostatin	10 - 100 μM	chymotrypsin, papain, cysteine proteases
Antipain-HCl	1 - 100 μM	papain, cysteine and serine proteases
EDTA	2 - 10 mM	metal dependent proteases, zinc and iron
EGTA	2 - 10 mM	metal dependent proteases e.g. calcium
Reducing agents 1,4 dithiothreitol, DTT	1 - 10 mM	keep cysteine residues reduced
1,4 dithioerythritol, DTE	1 - 10 mM	"
Mercaptoethanol	0.05%	"
Others Glycerol	5 - 10%	for stabilisation, up to 50% can be used if required

PMSF - Phenylmethylsulfonyl fluoride APMSF - 4-Aminophenyl-methylsulfonyl fluoride PMSF is a hazardous chemical. Half-life time in aqueous solution is 35 min. PMSF is usually stored as 10 mM or 100 mM stock solution (1.74 or 17.4 mg/ml in isopropanol) at - 20 °C. * Protease inhibitors are available in pre-made mixes from several suppliers. Details taken from Protein Purification, Principles and Practice, R.K. Scopes. 1994, Springer., Protein

Examples of ionic and non-ionic detergents.

Sodium dodecyl sulphate	0.1 - 0.5%	denatures proteins, used for SDS-PAGE use non-ionic detergents to avoid denaturation
Triton™ X-100	0.1 %	non-ionic detergent for membrane solubilisation. Note: may absorb strongly at 280 nm!
NP-40	0.05 - 2%	"
Dodecyl β D-maltoside	1%	"
Octyl β D-glucoside	1 - 1.5%	"
Polyethyleneimine	0.1% w/v	
Protamine sulphate	1% "	

Examples of denaturing agents.

Denaturing agent	Typical conditions for use	Removal/comment
Urea	2 - 8 M	remove using Sephadex G-25
Guanidine hydrochloride	3 - 8 M	remove using Sephadex G-25 or during IEX
Triton X-100	2%	”
Sarcosyl	1.5%	”
N-octyl glucoside	2%	”
Sodium dodecyl sulphate	0.1 - 0.5%	exchange for non-ionic detergent during first chromatographic step, avoid anion exchange chromatography
alkaline pH	> pH 9, NaOH	may need to adjust pH during chromatography to maintain solubility

Examples of precipitation techniques

Precipitation agent	Typical conditions for use	Sample type	Comment
Ammonium sulphate		>1mg/ml proteins especially immuno-globulins	stabilizes proteins, no denaturation, super-natant can go directly to HIC
Dextran sulphate		samples with high levels of lipoprotein, e.g ascites	precipitates lipoprotein
Polyvinylpyrrolidine	Add 3% (w/v), stir 4 hours, centrifuge, discard pellet	"	alternative to dextran sulphate
Polyethylene glycol (PEG, M.W. >4000)	up to 20% wt/vol	plasma proteins	no denaturation, super natant goes direct to IEX or AC. Complete removal may be difficult
Acetone	up to 80% vol/vol at 0 ℃	useful for peptide precipitation or con-centration of sample for electrophoresis	may denature protein irreversibly
Polyethyleneimine	0.1% w/v		precipitates aggregated nucleoproteins
Protamine sulphate	1%	"	