Genetically engineered Tags
Recombinant proteins
Specific Proteases
Affinity Chromatography (AC)

- Affinity Tags and Fusion Proteins
- Chelating, Strep-tag, GST, MBP, SUMO, C-terminal, etc
- Cleavage sites – Proteases
- Parameters for development optimization
- Troubleshooting
- Examples
Affinity Tags - I

- Small stretches of amino acids added to the N-terminal or C-terminal end of a protein with a high affinity for a specific biological or chemical ligand
- Allow purification and detection of the expressed protein
- Enables different proteins to be purified using a common method (HTPS)
- Most popular: His-tag, comprised of six histidine that provide specific binding to metal chelate resins
- Polyarginine or polyaspartic acid tags can be used to alter binding of a protein on ion-exchange resins
Affinity Tags - II

- The *Strep*-tag II (8 amino acids, WSHPQFEK), which binds with high selectivity to Strep-Tactin.
- The tag may be placed at either end of the protein or in a region with appropriate surface exposure to allow binding or recognition.
- To enable removal of the tag, a linker region is typically included between the tag and the native protein sequence.
- The linker contributes to increased accessibility of the affinity tag and is often required for effective endoprotease cleavage.
Fusion proteins
Solubility-enhancement tags (SETs)

- Use to overcome some of the problems of bacterial expression: protein aggregation, poor expression levels and difficult purification.
- The most popular fusion systems employ maltose-binding protein (MBP), glutathion S-transferase (GST), thioredoxin (TRX), SUMO and NusA. These genes are well expressed and the proteins are highly soluble and provide specific characteristics to aid purification.
- Typically, the gene of interest is inserted “in frame” at the 3’ end of the carrier protein, in place of the termination codon.
- To enable removal of the fusion protein, a linker region is typically included between the fusion protein and the native protein sequence.
- Specific recognition of the linked regions with specific proteases: TEV, Prescission, SUMO protease, Enterokinase
IMAC: Immobilized metal affinity chromatography
Chelating-Chromatography for poly-His fusion proteins

- The most widely used.
- It’s small in size. Less immunogenically active
- It does not need to be removed for many downstream applications
- Availability of a large number of expression vectors
- Tag may be placed at either the N or C terminus
- A protease cleavage site allows the tag to be removed after purification
- The interaction of the tag with the Ni$^{2+}$ column does not depend on the tag structure, making it possible to purify otherwise insoluble proteins using denaturating conditions (8 M urea or 6 M GuHCl)
- Avoid use of chelating: EDTA, DTT, etc. (cell chelating molecules in low expression)
Immobilized metal ion affinity chromatography (IMAC) – for poly-His fusion proteins

- The basis of the purification is the interaction of the imidazole moiety of the poly His with the metal (Nickel in most cases).
- The metal is immobilized to a support through complex formation with a chelate that is covalently attached to the support.

- In some resins we can use Co, Cu, Zi or Fe instead of Ni and obtain different results.
- Ni-NTA, Co resins, etc.

Figure 5. Chemical structures of chelating ligands used in IMAC. Binding groups are colored purple. SP = spacer, M = matrix.
IMAC

Binding Conditions:
- 20 mM phosphate or TrisHCl buffers
- + (0.5 M NaCl) or low imidazol concentrations (10-20mM) to avoid non-specific binding of contaminants to the resin.
- Buffers may include 8 M urea or 6 M GuHCl when purifying inclusion bodies solubilised proteins.

Elution Conditions:
- Usually Imidazol (Histidine analogue) or low pH (~4.5)
- Alternatives: EDTA, Histidine
The Strep-tag II is a short peptide (8 amino acids, WSHPQFEK), which binds with high selectivity to Strep-Tactin, an engineered streptavidin.

This technology allows one-step purification of almost any recombinant protein under physiological conditions, thus preserving its bioactivity (elution with 2.5mM desthiobiotin).

The Strep-tag system can be used to purify functional Strep-tag II proteins from any expression system including baculovirus, mammalian cells, yeast, and bacteria.

The Strep-tag/Strep-Tactin interaction is compatible with a variety of reagents (detergents, reducing agents, etc.) making the system attractive for purifying metallo- and membrane proteins, large proteins and protein complexes.
Strep-tag/Strep-Tactin system

According to Expression and purification of proteins using Strep-tag and/or 6xHis-tag. A comprehensive manual from IBA GmbH
**Strep/6 x Histidine system (double-tag)**

*IBA and QIAGEN*

- Useful for full length recombinant proteins purification at high purity under standardized and non-denaturative conditions (Imidazol and desthiobiotin elution)

- Especially useful to eliminate “difficult to resolve” protease cleavage fragments of target protein.

- Recombinant proteins that carry 6 x His-tag at the N-terminus and Strep tag II at the C-terminus (or vice versa).

- Efficiently expressed in *E. coli*, yeast, insect, or mammalian cells.

- **Recommendation:** use IMAC as first capture step. No buffer exchange is required for the second purification step: *Strep*-Tactin resin.
His-STREPPER - His/Strep-tag®II Adapter

$I^B_A$

✓ *Strep*-tag®II (SA-WSHPQFEK) conjugated with a nickel charged trisNTA

✓ Tightly binds to 6xHis-tag converting a 6xHis-tag fusion protein to a *Strep*-tag®II fusion protein without the need for cloning

✓ Applied to the cell lysate or to the His-tag eluate (after complete removal of imidazole)

✓ Purification with *Strep*-Tactin® column
### Commonly used affinity tags

**Preparative Purification of Recombinant Proteins: Current Status and Future Trends**  

<table>
<thead>
<tr>
<th>Tag</th>
<th>Size [amino acids or kDa]</th>
<th>Ligand or separation method</th>
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<tr>
<td>Polyhistidine</td>
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<td>Calmodulin binding peptide</td>
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**A panel of commonly used affinity tags selected for purification of recombinant fusion proteins**
A small 4 amino acid peptide tag: E-P-E-A (glutamic acid - proline - glutamic acid - alanine) that binds to a 13 kDa Camelid antibody fragment affinity matrix.

The CaptureSelect C-tag affinity matrix purifies C-terminal tagged proteins with high affinity and selectivity, even in the presence of Urea and Guanidine HCl.

Mild elution conditions at neutral pH can be applied using 2M magnesium chloride or 50% propylene glycol, which ensures high activity recoveries of pH sensitive target proteins; or more specifically with 2mM S-E-P-E-A peptides.

The affinity resin recognizes the E-P-E-A tag sequence when fused either directly to the C-terminus of a protein or through linkers between the C-terminus and the E-P-E-A tag.
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- Specific recognition of the linked regions with specific proteases: TEV, Prescission, SUMO protease, Enterokinase
Advantages / disadvantages of fusion proteins

In several cases

- Facilitate protein refolding / increase solubility
- Facilitate protein purification
- Improve protein production (high expression)
- Prevent proteolysis

But in other cases

- Target is not soluble after cleavage
- Target bounds to the protein tag by non-covalent forces
- Extra work if you need to cleave
- Loss of protein yield at expenses of the fusion protein
- Alteration in biological activity
- Change in protein conformation and undesired flexibility in structural studies

There is no single fusion tag which is the “best” in every case
SUMO (Small Ubiquitin-like MOdifier) and NusA fusion tags dramatically outperform glutathiones transferase (GST), maltose binding protein (MBP), thioredoxin (TRX), and ubiquitin (Ub). The protein target tested in this study is GDF-8, a growth/differentiation factor. UN: un-induced, IN: induced, S: soluble, IB: inclusion body.

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<th></th>
<th>UN</th>
<th>IN</th>
<th>S</th>
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<td>GDF8 ALONE</td>
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<tr>
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<tr>
<td>NUS A-GDF8</td>
<td></td>
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</table>
Glutathione S-transferase or GST-fusion proteins

- **Matrix**: Glutathione Sepharose 4B, pre-packed
- **Binding**: PBS (+ 1% Triton X-100)
- **Elution**: 5-10 mM Glutathione, 50 mM Tris.HCl, pH 8.0

**GST**: 26kDa cytoplasmic protein. Binds specifically to glutathione

- Highly use in immunoprecipitation
- Less use for increasing solubility
- Commercial vectors containing either factor Xa, Prescision or a thrombin recognition site to allow cleavage and removal of the GST.
- Useful for dimerization
**Maltose-binding protein pMgL™**

- **MBP**: a 43kDa secreted protein from *E. coli*, binds specifically to maltose
- Purification: Maltose or Amylose agarose columns and Dextrin Seph (most recommended)
- Can be secreted to the periplasm
- Or expressed without the signal peptide in the cytoplasm.
- Very effective for solubility enhancement
- TEV protease cleavage site
- Sometimes additional 6His in N-terminal
- Could be used to aid crystallization of target protein
MBP serves as a passive participant in the folding process; passenger proteins either fold spontaneously or with the assistance of chaperones.

Chaperones and/or chaperonins seem to come into play after a passenger protein has been rendered soluble by MBP. MBP serves primarily as a “holdase”, keeping the incompletely folded passenger protein from forming insoluble aggregates until either spontaneous or chaperone-mediated folding can occur.

A third class of passenger proteins is unable to fold and exists in an incompletely folded state and typically precipitate after they are cleaved.

Recently, MBP has also been used to maintain proteins that contain disulfide-bonds in a soluble state in the E. coli cytoplasm so that they could be acted upon by appropriate redox enzymes that were co-expressed in the same cellular compartment.
SUMO (small ubiquitin-like modifier)

- Increased expression
- Increased solubility
- Acidic protein ~10kDa
- Both the tag and the protease have 6xHis tags
- SUMO Protease leaves no unwanted residues on the N-terminus
- SUMO Protease extremely efficiently (1:500)
Thioredoxin (Trx)

✓ **Thioredoxin**: 12 kDa intracellular *E. coli* protein.

✓ Very soluble, and highly over-expressed

✓ Periplasmic or cytoplasmatic expression

✓ Robust folding (some chaperone activity)

✓ **Most popular to increase disulfide bonds**

✓ 6His Tag can be add to the N terminal of TRX for IMAC purification

✓ Does not work well with larger MW target proteins

➢ Since TRX is thermostable, and some heat-stable fusion proteins can be purified by thermal denaturation of contaminants by thermo-osmotic shock (osmotic shock coupled with heat-treatment)  

*According to Q.-R. Guo et al. / Protein Expression and Purification 49 (2006) 32–38*
IMPACT™-CN System (NEB)

- Intein Mediated Purification with an Affinity Chitin-binding Tag protein purification system.

- Use inducible self-cleavage activity of a protein splicing element (intein) to separate the target protein from the affinity tag without the use of a protease.

- A target protein is fused to a tag consisting of the intein and the chitin binding domain.

- In the presence of thiols such as DTT, β-mercaptoethanol or cysteine, the intein undergoes specific self-cleavage which releases the target protein from the chitin-bound intein tag.
Biotinylation in vivo
PinPoint™ Xa from Promega

- In vivo Biotinylated Affinity Tags: biotinylation reaction in E. coli through biotin ligase holo-enzyme

- Fusion purification tag with a single biotin specifically on one Lys residue

- The system use a monomeric avidin (Soft Release Avidin Resin): allows protein elution with a non-denaturing 5mM biotin buffer.

- Tag cleavage with Factor Xa
Comparison of Affinity tag technologies

According to J.J. Lichty et al. / Protein Expression and Purification 41 (2005) 98–105

<table>
<thead>
<tr>
<th>Tag</th>
<th>Size(aa)</th>
<th>Resin</th>
<th>Eluting agent</th>
<th>Source</th>
<th>Capacity</th>
<th>Cost</th>
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<td>MBP</td>
<td>396</td>
<td>Amylose</td>
<td>Maltose</td>
<td>Biolabs</td>
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<td>6</td>
<td>Talon</td>
<td>Imidazole</td>
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<td>Ni–NTA</td>
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<td>Qiagen</td>
<td>5–10mg/ml</td>
<td>$257/25ml</td>
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<td>Amersham</td>
<td>10 mg/ml</td>
<td>$396/25ml</td>
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<td>EGTA</td>
<td>Stratagene</td>
<td>2mg/ml</td>
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<td>Strep-Tactin</td>
<td>Desthiobiotin</td>
<td>IBA</td>
<td>50–100 nmol/ml</td>
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<td>$293</td>
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<td>FLAG</td>
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<td>Anti-FLAG M2</td>
<td>FLAG peptide</td>
<td>Sigma</td>
<td>0.6mg/ml</td>
<td>$1568/25ml</td>
<td>$1045</td>
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<td>HPC</td>
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<td>Anti-Prot.C Ab</td>
<td>EDTA</td>
<td>Roche</td>
<td>2–10 nmol/ml</td>
<td>$299/1ml</td>
<td>$4983</td>
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</table>

By starting a new project cost is one of the most commonly confronted decisions.
Fusion protein cleavage methods

- Chemicals cleavage is effective but often requires extreme conditions (low pH or high temp.), and is often non-specific (cyanogen bromide, hydroxylamine, etc).

- **Enzymatic digestion is the method of choice** for soluble fusion protein cleavage. Reaction is carried out under relative mild conditions.

- Commonly used endo-proteases: thrombin, factor Xa, enterokinase

- **Best sellers: TEV, SUMO and rhinovirus 3C protease (Prescision)**

- Exoprotease: TAGZyme, carboxypeptidase A (C-terminal tag)

- Intein cleavage by reduction agents such as DTT, or low pH
Cleavage Sites

• **Thrombin** Amersham-Biosciences, Novagen, SIGMA, Roche
  
  Leu-Val-Pro-Arg▼Gly-Ser
  
  Protease Capture: Benzamidine-Agarose
  

• **Factor Xa** Amersham-Biosciences, NEB, Roche
  
  Ile-Glu/Asp-Gly-Arg▼
  
  Protease Capture: Benzamidine-Agarose
  
  Will not cleave if followed by proline and arginine. Secondary cleavage sites following Gly-Arg sequences.

• **Enterokinase** NEB, Novagen, Roche
  
  Asp-Asp-Asp-Asp-Lys▼
  
  Protease Capture: Trypsin Inhibitor-Agarose
  
  The site will not cleave if followed by a proline residue. Secondary cleavage sites at other basic residues, depending on conformation of protein substrate. Active from pH 4.5 to 9.5 and between 4°C and 45°C.

• **TEV protease** Invitrogen – Life Technologies
  
  Glu-Asn-Leu-Tyr-Phe-Gln▼Gly
  
  Protease Capture: Ni-NTA (6His recomb. TEV)
  
  Seven-residue recognition site, making it a highly site-specific protease. Active over a wide range of temperatures. Protease available as a His-tag fusion protein, allowing for protease removal after recombinant protein cleavage.

• **PreScission** Amersham-Biosciences
  
  Leu-Glu-Val-Leu-Phe-Gln▼Gly-Pro
  
  Genetically engineered form of human rhinovirus 3C protease with a GST fusion tag, allowing for facile cleavage and purification of GST-tagged proteins along with protease removal after recombinant protein cleavage. Enables low-temperature cleavage of fusion proteins containing the eight residue recognition sequence.

• **TAGZyme** Qiagen: His-tag removal by Exoproteolytic Digestion
  
  Protease Capture: Ni-NTA (6His recomb. enzyme)
  
  The TAGZyme System is an efficient and specific solution for the complete removal of small N-terminal His tags and other amino acid tags by the use of exopeptidases. These recombinant enzymes contain a C-terminal His tag and can therefore be bound to Ni-NTA matrices.

• **Intein Site** (dithiothreitol cleavage) NEB
  
  DTT elimination by dialysis
  
  Uses self-cleavable affinity tags. Even after cleavage un-natural termini are present on the protein of interest.
Incubation of HLT-435aaCtermBP2 with TEV-protease

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<tr>
<th>Protein µl</th>
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<td>TEV-pr. µl</td>
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<td>0.2</td>
<td>1</td>
<td>2.5</td>
<td>5</td>
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<td>4</td>
<td>22</td>
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</tr>
</tbody>
</table>

- Protease / protein ratio
- Protease quantity
- Protein concentration (volume)
- Temperature
- Time
- Cleavage site exposure
- Buffer
- Presence of aggregates

Shahar Rotem, Assaf Friedler
Purification of Fusion Proteins

TROUBLESHOOTING

Protein target and fusion tags are difficult to separate:

- Increase competition (Imidazol)
- Reduce non-covalent interaction: detergents, chaotropics, conductivity, etc
- Separation by charge
- Separation by mass/radius
- Hydrophobic separation

Chromatography

Best option

RPC for easy to refold low MW proteins or peptides
Advantages / disadvantages of Proteolytic Cleavage

- Spurious, non-specific proteolytic cleavage of the fusion protein
- Extra amino acids in the N-terminal after cleavage for some proteases
- Non-covalent forces maintain proteins connected after cleavage: separation problems
- Enterokinase need elevated temperatures (25-37°C) for efficient cleavage: can denature or cause aggregation of the fusion protein
- Incomplete cleavage, which reduces the yield and/or introduces heterogeneity to the purified protein
- Buffer and additive restriction (mainly detergents)
- $ and time: The need for additional steps to separate the cleaved fusion protein from the fusion tag, remove the protease, and exchange buffer or desalt
- Increase purity by using negative column (retains impurities) or other chromatographic procedure
- Targets are mainly C-terminal of the solubility protein (exoproteases as carboxypeptidase A)
The Trip Adviser guide to the protein science world: a proposal to improve the awareness concerning the quality of recombinant proteins

Mario Lebendiker, Tsafi Danieli and Ario de Marco

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Parameters for optimization during binding and washings- I

- If protein does not bind to the resin, there are several options to choose

- Check the quality of the resin (use an His-tag control protein) or check for the presence of reagents that avoid binding

- Partial binding: use more resin, or bind for longer time (BUT: the longer the duration of purification, the greater the risk of protein degradation). Use unbound material for a new purification.

- Try additives to prevent interaction with other proteins: glycerol, detergents, low [chaotropics]: urea, GuHCl, or more kosmotropics as NaCl, AmmSO₄ (soluble aggregates??)

- Tag is inaccessible: try purification under denaturating conditions (for poly-His fusion proteins)

- Check by western-blot if the tag has been degraded; if this is the case, try to work all the time at 4°C and use more protease inhibitors during lysis

- Construct a new vector with the tag in the opposite end of the protein

- Presence of contaminants (chelants for IMAC or biotin in Streptag)
Parameters for optimization during binding and washings - II

- **If multiple proteins bands are seen in the elution try:**
- Increase the washing step volume.
- **Protein degradation** (you can check previously with western blot) try to work all the time at 4°C and use protease inhibitors during lysis.
- **Use more stringent competitive conditions during binding and washing** (example: use low Imidazole concentrations during binding and washings to increase competition for the same sites on the resin)
- **Decrease resin volume** (allows higher competition between fusion protein and contaminants for the same sites on the resin)
- **If contaminants are associated with the tagged protein**, try to disrupt the non-specific interaction by adding to the wash buffer before elution additives as β-ME, glycerol up to 50%, detergents as Triton X-100, NP40 or Tween 20 up to 2% or increase ionic strength up to 1.5M NaCl or KCl.
- **Consider an additional purification step before or after purification.**
- **Consider the use of a pre-column** of beads without ligand to adsorb proteins that bound to beads non-specifically.
Parameters for optimization during elution

- Optimize resolution
- Adjust gradient slope/shape, or step elution with increasing competitor concentration
- Include additives in buffers
- Adjust flow rate
- Adjust column volume
- Bead size and quality of the resin
- If the protein slightly elutes or does not elute from the column
  - Use higher competitor concentration (Example: up to 1M Imidazol for chelating columns), or additives
  - Reduce elution flow-rate
  - Change elution conditions, consider elution under denaturating conditions
Parameters for optimization during protease cleavage and purification

Cleavage depends on:

- Target/protease ratio
- Reaction volume
- Temperature
- Time
- Buffer
- Others

- If possible try to cut at low temperature
- Cleavage can be done inside dialysis bags under dialysis to prepare protein to the next step
- If necessary add additives in buffers to avoid aggregation of the target after cleavage
- High aggregation can affect cleavage. So consider SEC before cleavage
- Some targets slightly bound to the IMAC resin in the negative step: increase competition (Imidazol, etc)
- Cleavage is OK, but proteins elute together because of other interactions. Try to reduce these interactions with additives (high salt, detergents, etc)
- Alternatives to negative affinity: IEX, HIC, GF
Optimization during binding *pDest 17 (His tag) cc-stop of ARNO*

- Lysis of 10ml culture
- Bound and washed (in the presence of 10, 20, 30 mM imidazol) to nickel resin
- Elution was with 300 mM imidazol

Each well contains 10 micro liter of the desired step (from 40 microliters )+ 5 micro liter sample buffer.

Each of the marker proteins is 2 micro-grams.
### P38 CAPTURE - Optimization

<table>
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<th>10</th>
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<td>Elution number</td>
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<td>2</td>
<td>3</td>
<td>1</td>
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<td>MW</td>
<td>1</td>
<td>2</td>
<td>3</td>
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</table>

- **Cells lysis from 250ml culture.**
- **Spin 30min 20000g 4°C.**
- **Divide supernatant in 4 fractions,**
  **add to each one different [Imidazol].**
- **Incubate each fraction (batch binding) 60min 4°C with 150μl Ni-NTA (equilibrated with different [Imidazol]).**
- **Spin 3min 3000rpm 4°C. Discharge unbound material.**
- **Wash resin 6x1ml washing buffer + different [Imidazol].**
- **Elution 3x300μl elution buffer (250mM Imidazol). Run 12%PAGE-SDS of each elution.**
100gr cells (6L culture). Cell disruption with Mountain Goulin in 900ml lysis buffer. Batch binding to 15ml Ni-NTA 90min 4°C in the presence of 20mM Imidazol. Wash with 20mM Imidazol buffer and elution with 5cv gradient 20-250mM Imidazol.
P38 INTERMEDIATE PURIFICATION: Anion Exchange

60 OD 280nm P38 after Ni-NTA and dialysis ON vs buffer A. Load on Resource Q-30 6ml column. Wash with 10cv 50mM NaCl buffer. Elute with gradient 5cv 50-300mM + 40cv 300-750mM + 5cv 750-1000mM NaCl
P38 FINAL POLISHING: SEC

35 OD280nm P38 after Ni-NTA, Res.Q & ultrafiltration cut-off 10000. Load on Superdex 75 60x1.6cm column (2 runnings).

Elution 1ml/min
P38 After Affinity
Highly resolutive anion exchange
Resource 15Q 29 x 1cm ~23ml column

1st run main peak of Affinity column
2nd run: main peak of previous run
IMAC purification: Low Imidazol step washing before elution

Case study: AbrB - Collaboration with A. Kaplan & D. Schatz

Load + 35 cv 0% B + 8 cv 4% B + 8 cv 10% B + 5 cv 10-100% B + 10 cv 100% B (500 mM Imidazol)
HLT-p53CT- Affinity
start with pellet of 1.5L culture
Ni-Sepharose FF 14ml

Ronen Gabizon – Assaf Friedler Group

Load + 10cv 0%B + 3cv 8%B + 4cv 15%B + 4cv 100%B

POOL 17-22: 3.5OD x 35ml ~ 276mg
HLT-p53CT- Cation Exchange after TEV protease cleavage ON 4°C SP-Sepharose FF 5ml
HLT-p53CT- GF after Cation Exchange Sephacryl S100 FF 500ml

POOL 7-14

5/6/2018
**GST-FliD : Glut.Aggar, TEV protease ON & split to SEC or AEIX**

Aviv Vronen – Assaf Friedler Group

**GST-TEV-1-747-FliDPAO1**: aa:708, MW:76689.1, pI: 5.92, 4Cys, 4Trp, Ext.55030, Abs:0.718(ox),

**1-747-FliDPAO 1 (noGST, but including Glycine)**: aa:474, MW:49375.4, pI: 6.72, 0Cys, 0 Trp, Ext.coef:10,430, **Abs:0.211** Cys (C) 0

**GST**  Number of amino acids: 23  Molecular weight: 27331.7  Theoretical pI: 5.7

**Purification:**
- GST-Agarose
- TEV protease + dialysis
- Negative GST-Agarose
- Negative IMAC (eliminate TEV)
- SEC
- AEIX
- Dialysis and concentration

**SEC**: Superdex 75 ~200ml
GST-FliD: Glut.Agarr, TEV protease ON & split to SEC or AEIX

Aviv Vronen – Assaf Friedler Group

Next time do not freeze after TEV

AEIX: MonoQ 1ml dilution before loading
MBP fusion protein
Capture: combined IMAC and Dextrine-Sepharose Affinity Chromatography

Load lysate on IMAC column

Disconnect IMAC column

Start Maltose elution of dextrin column

Tandem connection of dextrin column after IMAC column. Start Imidazol elution

Start Maltose elution of dextrin column

Pool 21-32

Fraction 7
MBP fusion protein
Intermediate purification: Gel Filtration

Add 50 mM Na glutamate and 50 mM L-arginine, **immediately** load on Sephacryl S200 FF column 97 x 2.6 cm ~ 500ml
MBP fusion protein

Optimization of Size Exclusion Chromatography

Superdex 75 columns connected in tandem (60 x 1.6 cm and 95 x 1.6 cm, total ~320 ml)

BSA dimer