Tag use in large scale protein purification

Tags in protein expression, detection and purification

May 10 - 14, 2010

Today's lecture notes are available at:

http://jeltsch.org/protein_tags_course

Protein Pharmaceuticals

- Jenner, 1796: First “protein vaccine” cow-pox
- Banting & Best, 1922: First protein pharmaceutical insulin
- Genentech, 1978: human insulin in E. coli (FDA approval in 1982)
- Genentech, 1987: FDA approval of recombinant Hepatitis B vaccine
- Today more than 200 approved peptide and protein pharmaceuticals on the FDA/EMA lists

Protein Pharmaceuticals

- List of all EMA-approved medicines (purification "details" in the scientific discussion
  http://www.ema.europa.eu/htms/human/epar/a.htm
- List of all FDA-approved medicines
  http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm
- (Detailed) description of purification is often not available ("trade secrets removed from publicly available documents")

No tags for protein pharmaceuticals

- The majority of protein pharmaceuticals is produced in mammalian cells
- Mostly Chinese Hamster Ovary (CHO) cells using methotrexate (MTX)-induced gene amplification
- The proteins need to be authentic (untagged) in the final product
- Conventional column chromatographic purification (ion exchange, gel filtration)

The FDA doesn't like tags

- The tag serves no purpose in the final product
- Potential of modifying the biological activity
- Potential of immune response (in the worst case scenario overriding tolerance)

→ Tag needs to be removed

Affinity tag		*ligand*	*typical binding capacity/ml*	*elution*	*Length (aa)*	*sequence*	*place-ment*
Histidine (His)	X	IDA-coordinated Me2+ (iminodiacetic acid)	10-40 mg	imidazol, low pH	5-15 (6)	HHHHHH	N, C, int
		NTA-coordinated Me2+ (nitrilotriacetic acid)		imidazol, low pH			
		CM-Asp-coordinated Me2+ (carboxymethylated aspartic acid)		imidazol, low pH			
Strep II	X	Strep-Tactin	3 mg	d-desthiobiotin	8	WSHPQFEK	
Strep III	X	Strep-Tactin	1.5 mg	d-desthiobiotin	28	WSHPQFEKGGGSGGGSGGGSWSHPQFEK	
Streptavidin binding protein (SBP)		streptavidin		biotin	38	MDEKTTGWRGGHVVEGLAGELEQLRARLEHHPQGQREP	
T7		mAb	0.3 mg	low pH	11/16 (11)	MASMTGGQQMG	N, int
FLAG	X	mAb	0.6 mg	low pH, FLAG peptide	8	DYKDDDDK	N, C, int
Ribonuclease S peptide (S)		Ribonuclease S protein, mAb		low pH	15	KETAAAKFERQHMDS	N, C
Softag1		Polyol-responsive mAbs		polyol	13	SLAELLNAGLGGS	
Softag2		Polyol-responsive mAbs		polyol	8	TKDPSRVG	
Elastin		none		temperature shift	18-320		
Haemagglutinine (HA)		mAb	2-3 mg	low pH	9	YPYDVPDYA	N, C
C-Myc	X	mAb		low pH	11	EQKLISEEDLN	N, C, int
V5		mAb		low pH	14	GKPIPNPLLGLDST	
Xpress		mAb		low pH	8	DLYDDDDK	N
Dihydrofolate reductase (DHFR)	X	Immobilized methotrexate (MTX)		MTX	171		N
Chitin binding domain (CBD)		Chitin	2 mg	DTT	51		N, C
Calmodulin binding domain (CBD)	X	Calmodulin	1 mg	EGTA, EDTA	26	KRRWKKNFIAVSAANRFKKISSSGAL	N, C
Immunoglobulin G Fc (IgGFc)	X	Protein A	50 mg	low pH, high salt, ethylene glycol	232		C
Protein A	X	Immunoglobulin G	2 mg	low pH, high salt, ethylene glycol	122		N
Cellulose binding domain (CBD)	X	Cellulose		H2O, high pH, urea, ethylene glycol, guanidinium HCl	27-129 (108)		N, C
Glutathion S-transferase (GST)	X	Glutathione, mAb	10 mg	glutathione	201-220		N
Maltose binding protein (MBP)		Amylose	6-8 mg	maltose	396		N, C
Octaarginine (Arg8)		Ion exchange resins		high salt	8	RRRRRRRR	N, C

Affinity tag		*kDa*	*Comments*	*Major supplier of medium*
Histidine (His)	X	0.84		GE Healthcare
				Qiagen
				Clontech/BD Biosciences
Strep II	X	1.06	modified streptavidin	IBA, Qiagen
Strep III	X	2.87	tandem strep II tag	IBA, Qiagen
Streptavidin binding protein (SBP)		4.3		Thermo Scientific/Pierce
T7		1.1	amino terminal 11 aa of the T7 gene 10 protein	Merck, Thermo Scientific/Pierce, Abcam
FLAG	X	1.01	Ca2+ dependend; mAbs recognize different placements of the tag (e.g. the M1 mAb recognizes only N-terminal FLAG)	Sigma
Ribonuclease S peptide (S)		1.75	derived from bovine pankreatic ribonuclease A by subtilisin cleavage	Bethyl Laboratories
Softag1		1.2	recognized by polyol-responsive mAbs	Neoclone, Lucigen?
Softag2		0.86	recognized by polyol-responsive mAbs	Neoclone, Lucigen?
Elastin			protein aggregation by temperature shift	
Haemagglutinine (HA)		1.1	influenza virus derived, more suitable for detection than for purification	Sigma, Roche, Babco
C-Myc	X	1.32		Sigma, Babco
V5		1.42	more suitable for detection than for purification	Abcam
Xpress		1	more suitable for detection than for purification	Invitrogen
Dihydrofolate reductase (DHFR)	X	19		
Chitin binding domain (CBD)		6	with the intein (self-cleavable tag) portion the tag size is 28 kDa (C-terminus) or 56 kDa (N-terminus)	NEB
Calmodulin binding domain (CBD)	X	2.96	binding is Ca2+ dependend	GE Healthcare
Immunoglobulin G Fc (IgGFc)	X	26	increases biological half-life, dimerizes the fusion partner	GE Healthcare, Thermo Scientific/Pierce
Protein A	X	14		GE Healthcare
Cellulose binding domain (CBD)	X	11	suitable for immobilization	Novagen
Glutathion S-transferase (GST)	X	26	enhances solubility	GE Healthcare, Thermo Scientific/Pierce
Maltose binding protein (MBP)		40	enhances solubility	NEB
Octaarginine (Arg8)		1.27		GE Healthcare, Toso

Histag is the only potential tag for pharmaceutical proteins

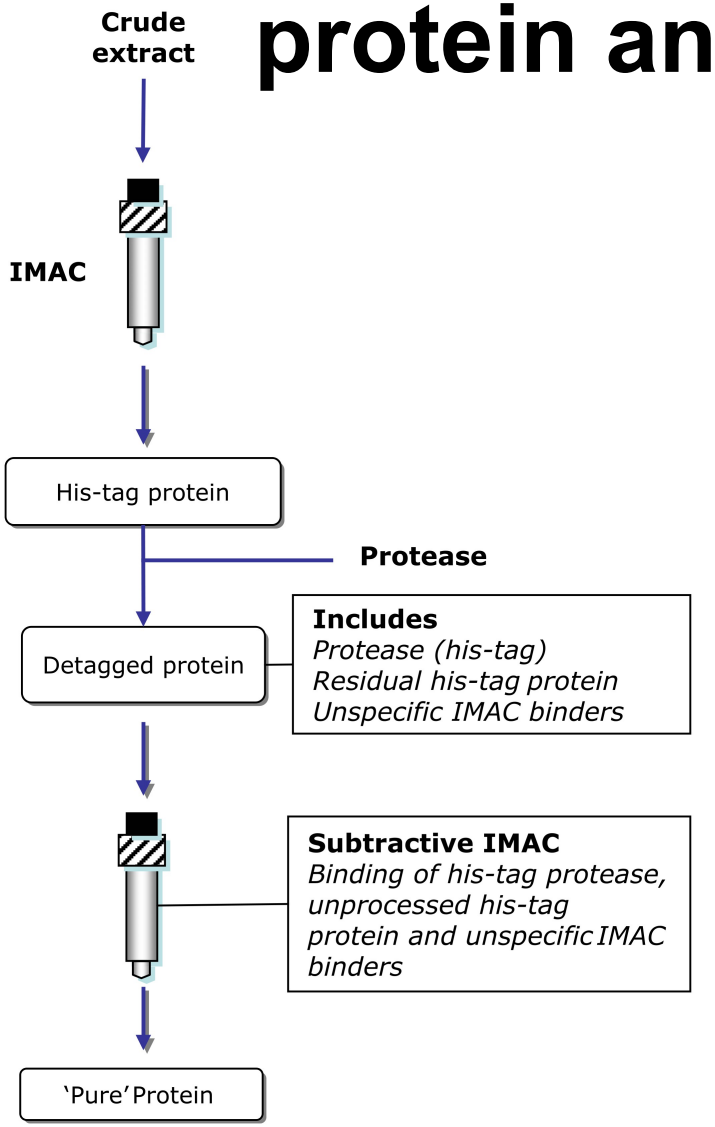
- Small tag size, “relatively” non-immunogenic
- Stability of the affinity resin towards high pH & detergents
- Lowest price/binding capacity ratio
- No patent restrictions anymore since 2005

Still too expensive

- Cost/binding capacity ratios: IEX 4-5¢/mg vs. IMAC 18¢/mg)
- 270$ vs. 1080$ media costs for a single typical Rituximab/MabThera* regimen (1x0.75g + 5x1g)
- Production in CHO cells, purification by Protein A affinity and AIEX chromatography

*Best-selling mAb of the last decade ~ 2 billion $/year, anti CD20 mAb for the treatment of non-Hodgkin's lymphoma, chronic lymphocytic leukaemia and rheumatoid arthritis

Requires enzymatic cleavage of protein and tag removal



- Additional affinity column
- Proteases are expensive and not very stable
- Impurities (Ni^{2+}, uncleaved and heterogenously cleaved protein, protease)

What is available from us

- http://mcblserver.hi.helsinki.fi/akta/
- http://tutoh-1.ltdk.helsinki.fi/akta