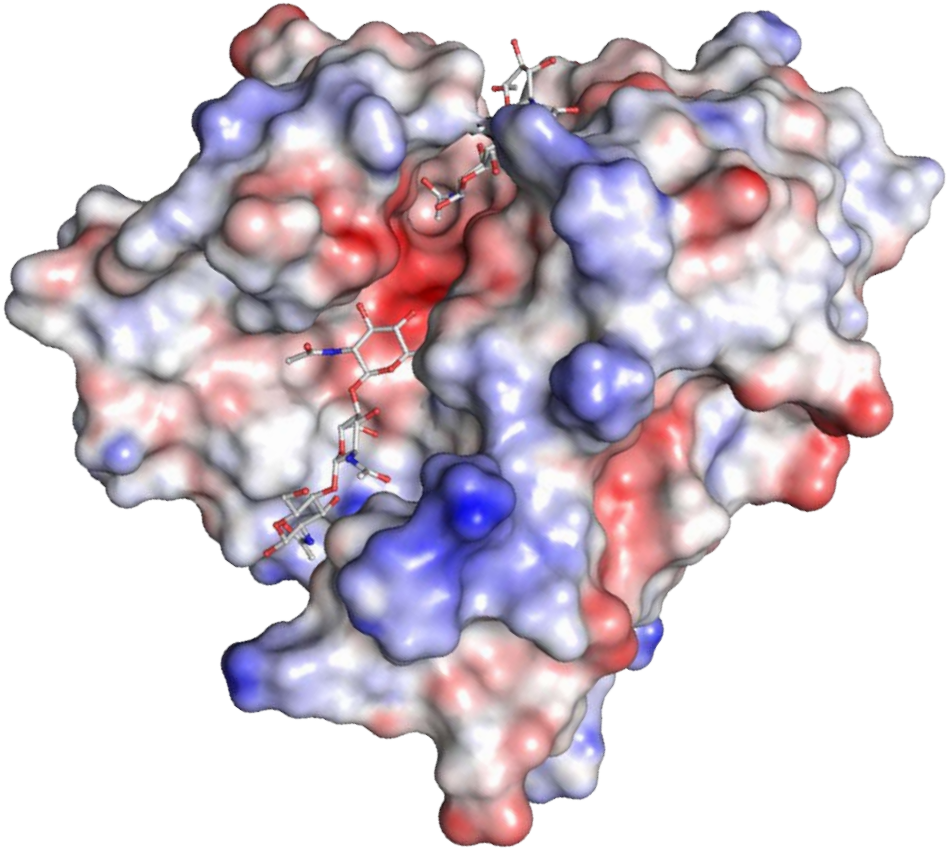
Some like it cold: Challenges with production of cold-adapted proteins



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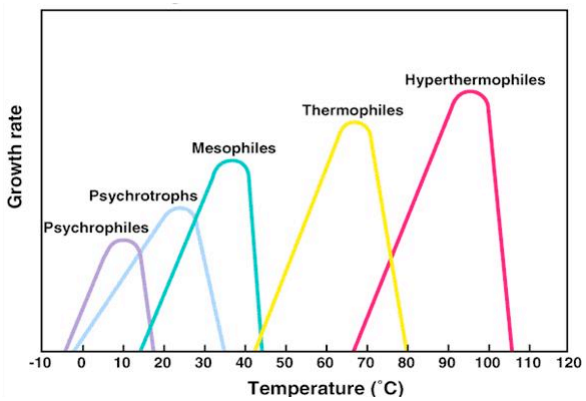
Department of chemistry
University of Tromsø

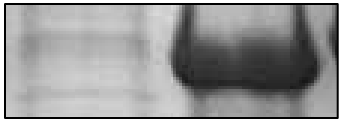
04.06.12

This presentation focuses on the development of cold-adapted proteins, applications, and challenges in production

Breifly, present the platform

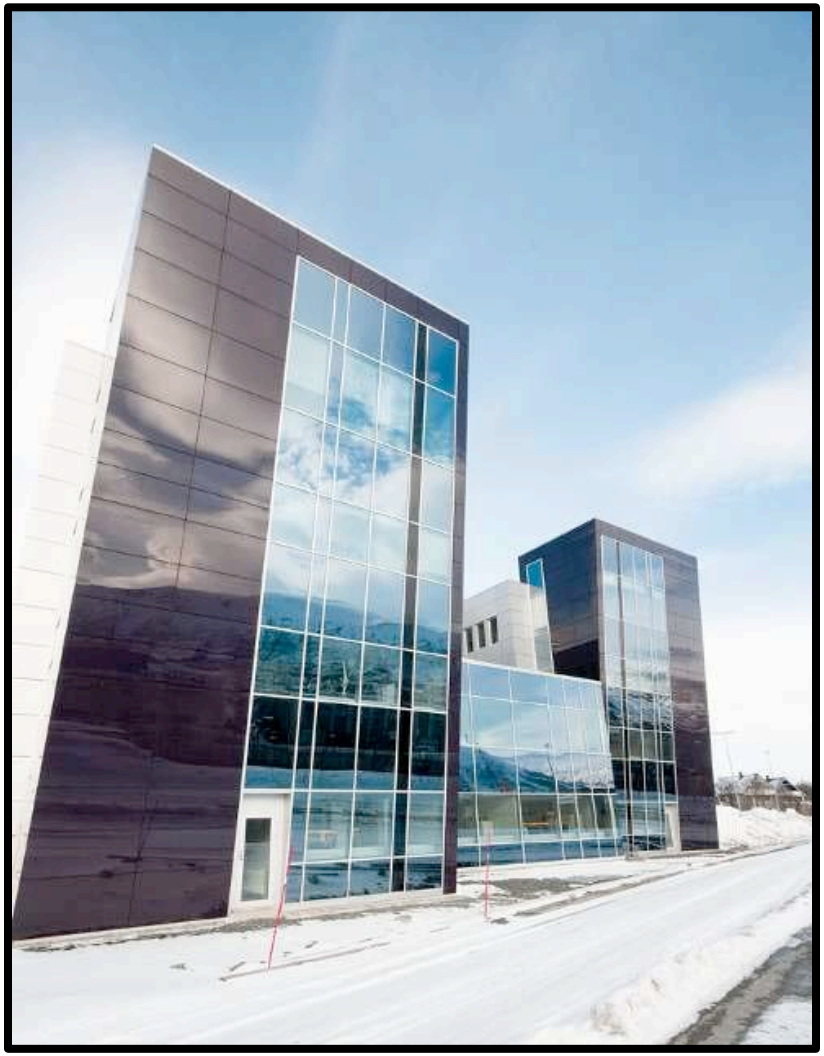


Cold-adaptation and application examples



Challenges with production

Norstruct is located in the sub-Arctic region of Norway



Norstruct is a national research and service centre

Three sub platforms

SP1: Protein production

SP2: Structure determination

SP3: Drug discovery and design

Internal research

Drug discovery and design

DNA interactions and modification

Host-pathogen interactions

Bioprospecting

National research courses

Visitor program

Research schools

MSB Research School

BioStruct Research School

Norstruct offer service on the pipeline from cloning to structure determination to drug discorvery


SP1
Protein Production



SP2
Structure Determination




SP3
Drug Discovery and Design

Target selection & bioinformatics

est expression and purification

ression a

Quality control and addit

stallization

d structure determination

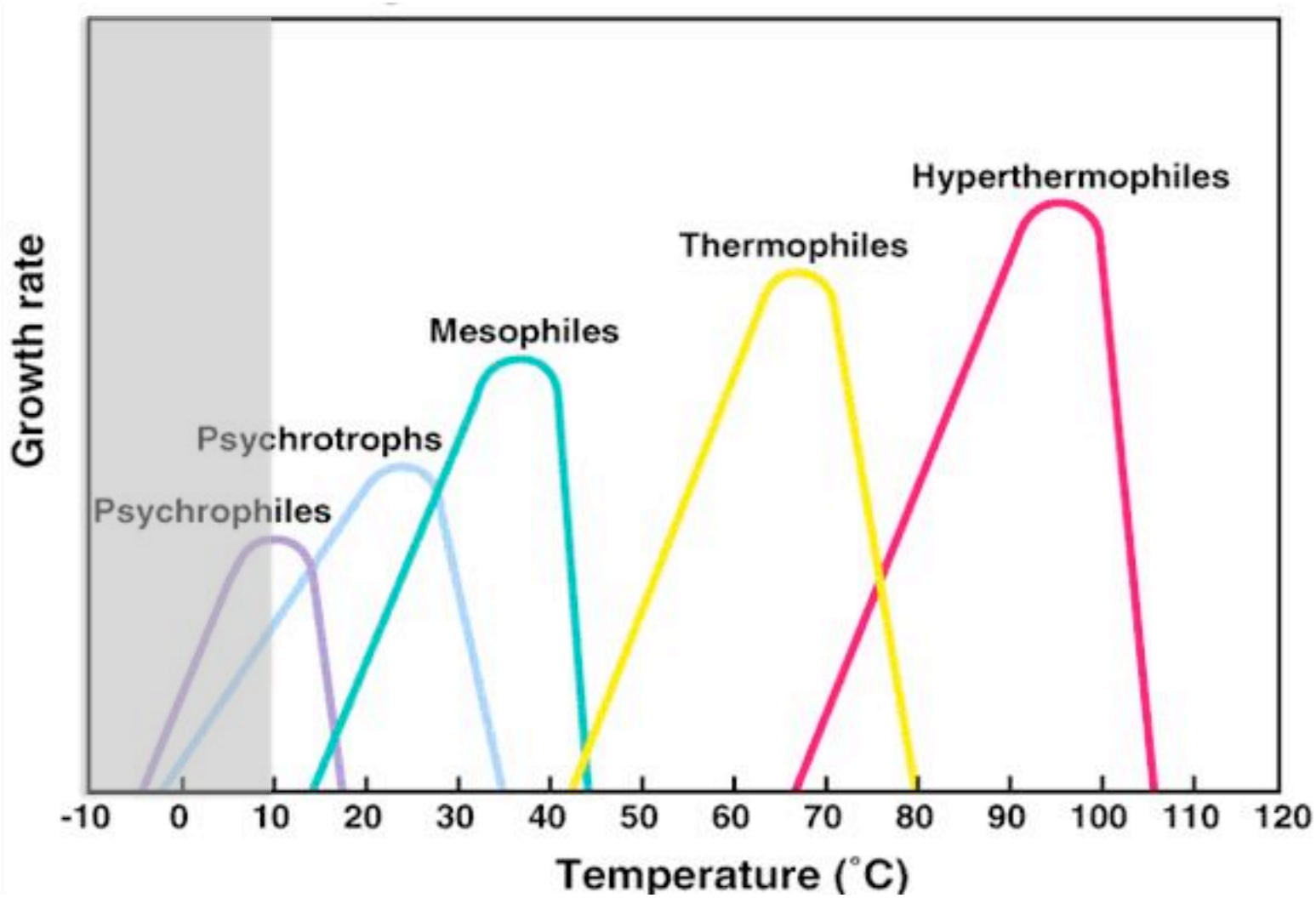
Protein-ligand complexes and off-target effects

Molecular modelling/docking and virtual screening

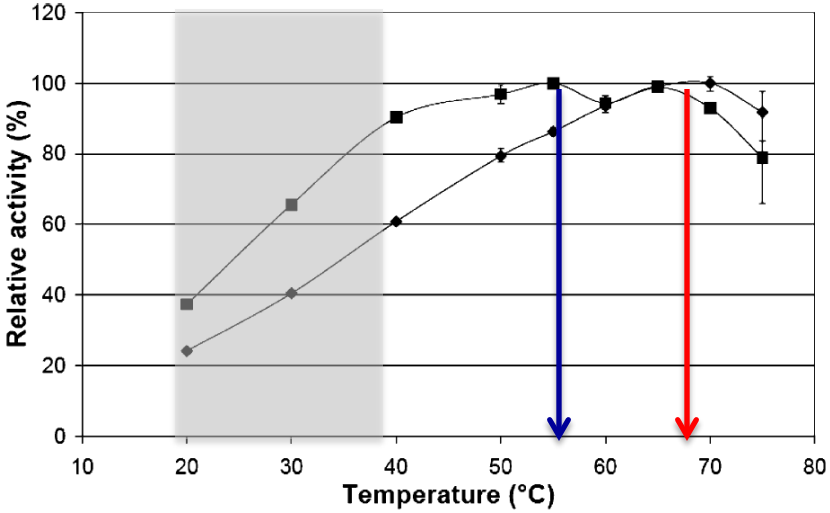
Protein-ligand thermodynamic studies

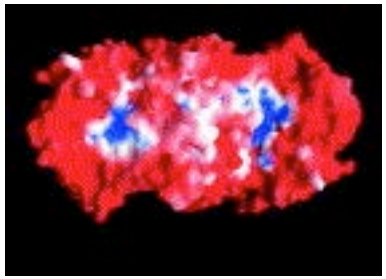
Psychrophiles represent an “unexplored” source for enzymes with novel properties

80% of Earth



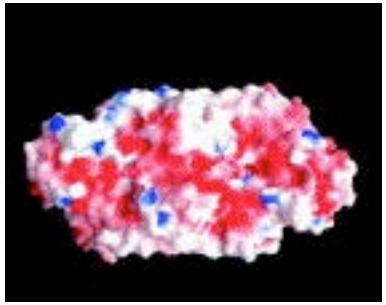
Cold-adapted proteins have a higher activity at low temperature compared to their mesophilic counterparts





Psychrophilic

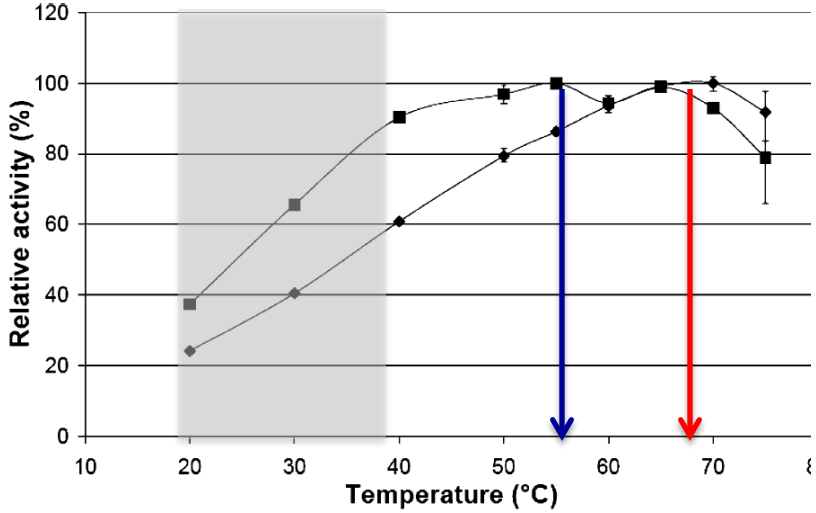
Mesophilic

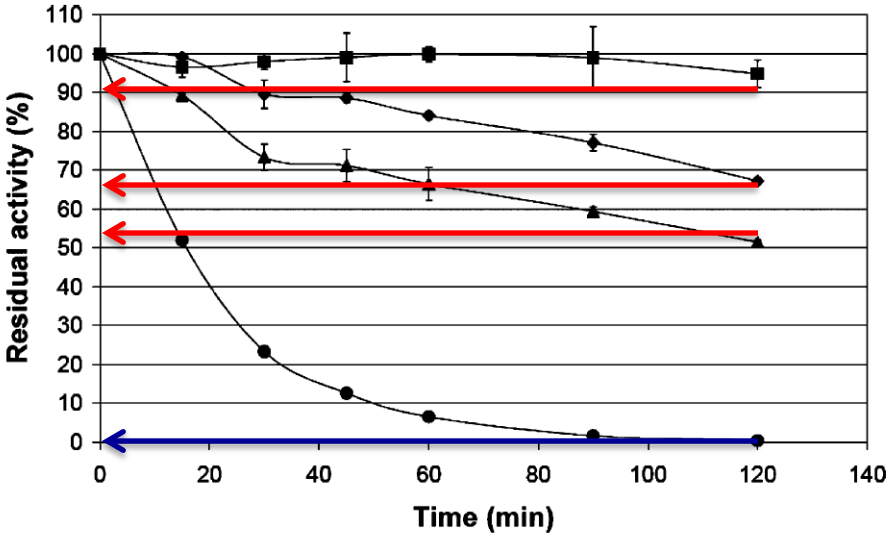


Backer et al, JBC, 2002

Larsen et al, FEBS, 2006

Cold-adapted proteins have a higher activity at low temperature compared to their mesophilic counterparts

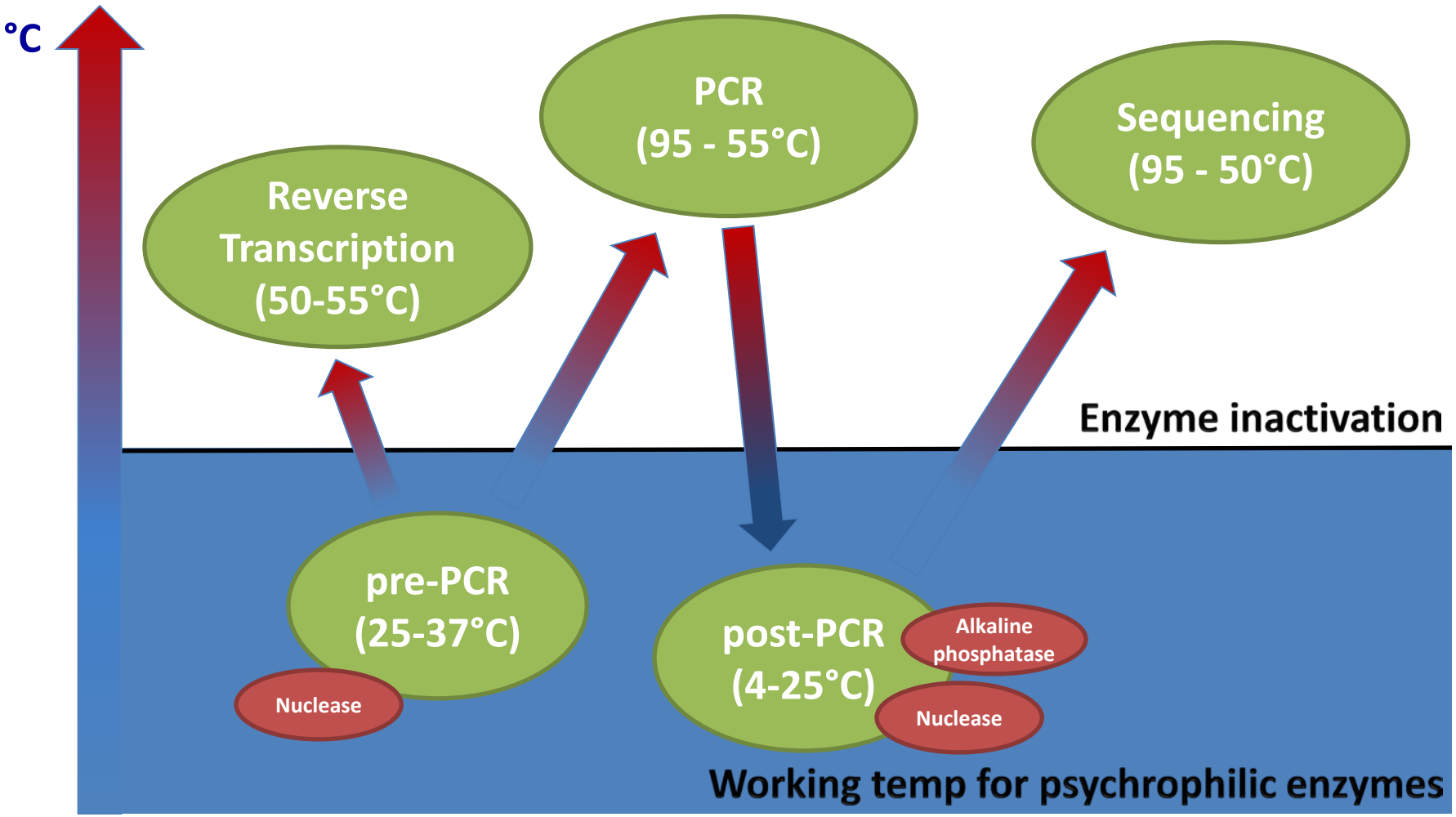




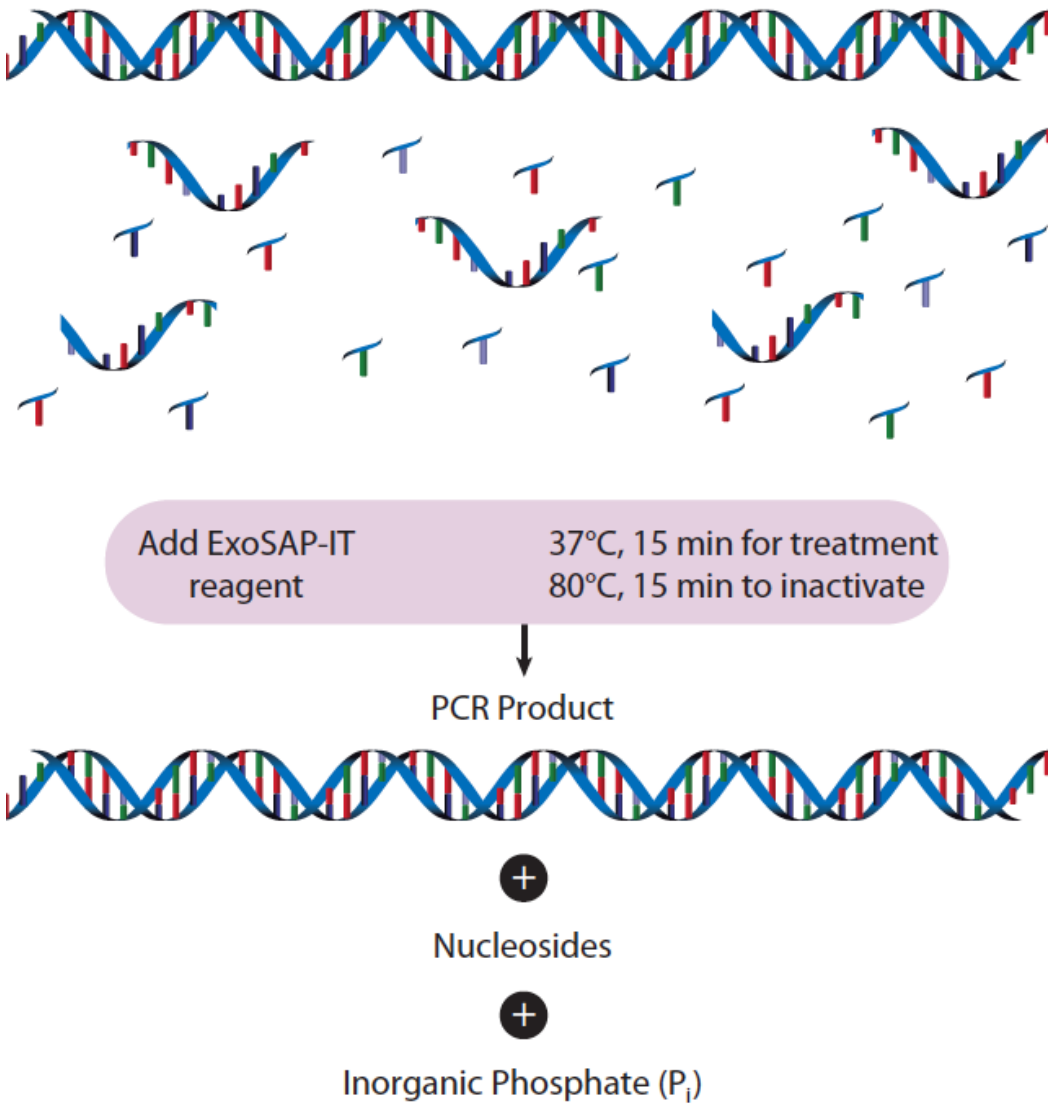
- and they are heat labile!

Larsen et al, FEBS, 2006

Heat-lability makes cold-adapted enzymes useful in biotechnology applications



Motivation example I: Shrimp alkaline phosphatase for PCR clean up is completely inactivated by heating

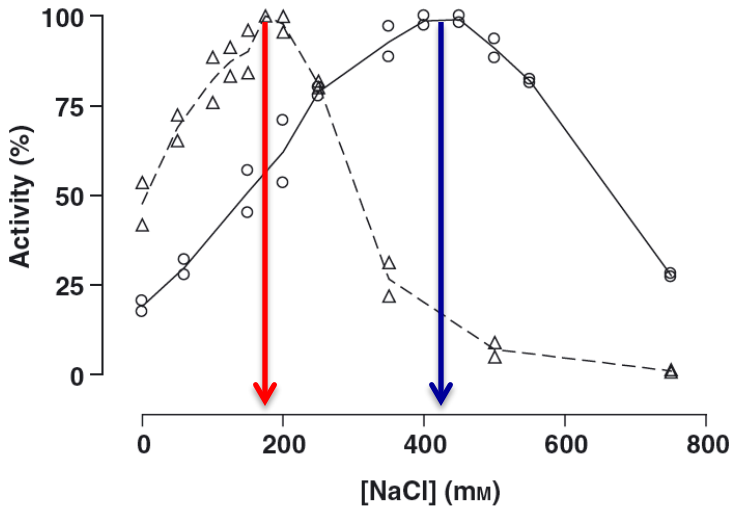


ExoSAP-IT is a one-step clean up method post-PCR.

Clean-up by the combination of an exonuclease for digestion of primers, and SAP to hydrolyse dNTPs. HTP sequencing or SNP analysis (genotyping).

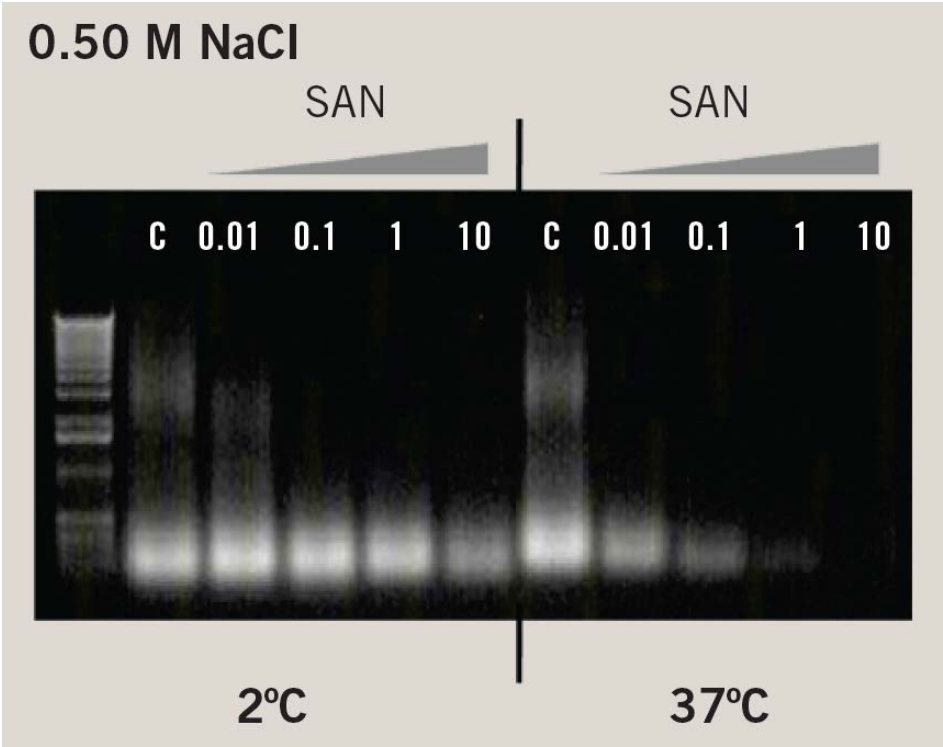
rSAP is heat-inactivated at 15 min incubation at 60°C.

Marine, cold-adapted and secreted enzymes are often more salt-tolerant compared to their mesophilic counterparts



Halophilic

Motivation example II: Salt active nuclease and heat labile mutant for removal of contaminating RNA and DNA



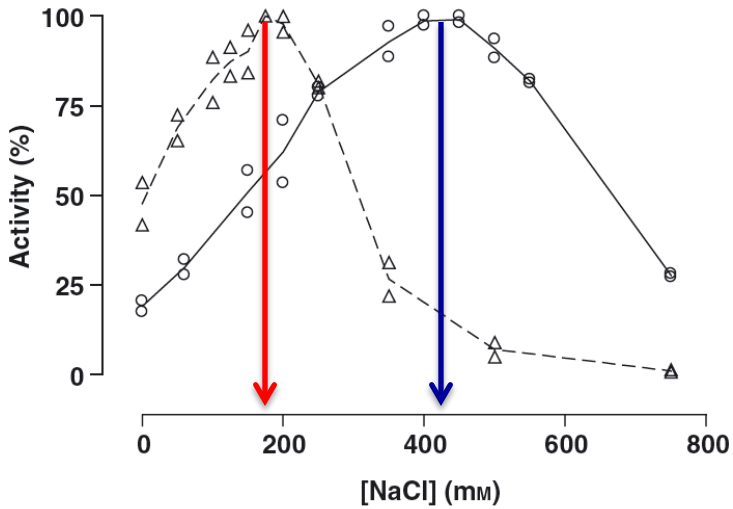
SAN for removal of contaminating DNA and RNA from protein preps and reagents etc.

HL-SAN Easily heat-inactivated after use. In combination with DTT a 15 min incubation at 40°C is sufficient.

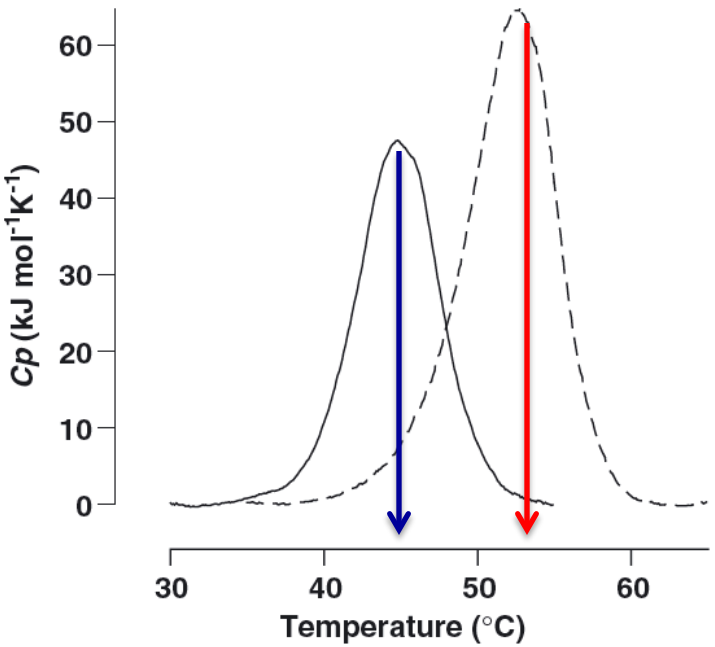
HL-SAN is ideal for use in HTP protein purification settings to remove DNA.

HL-SAN available 2nd half 2012

Increased flexibility leads to lower thermostability in cold-adapted enzymes

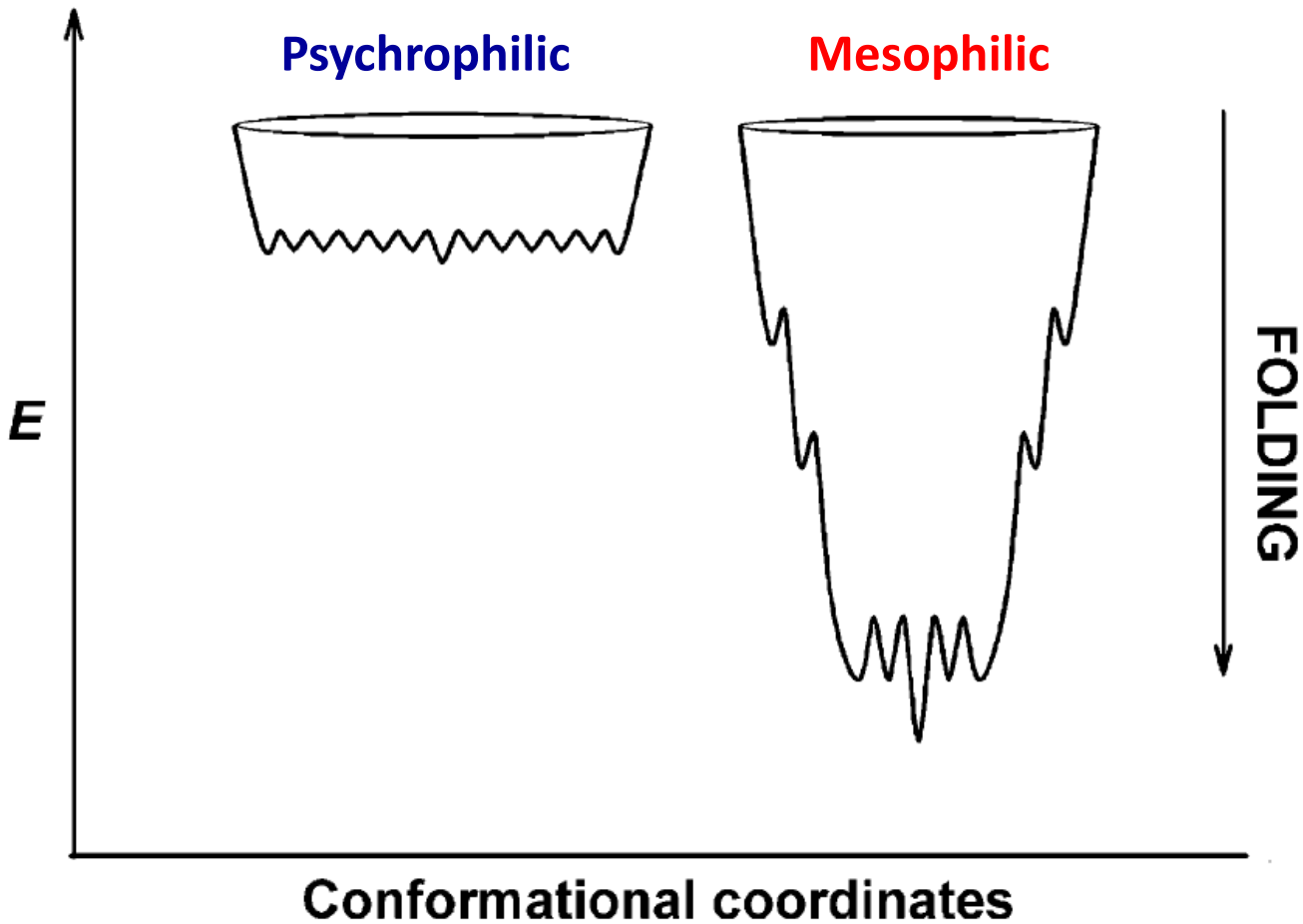


Halophilic



…but they are less stable!

High catalytic activity at low temperatures is associated with increased flexibility and reduced thermal stability

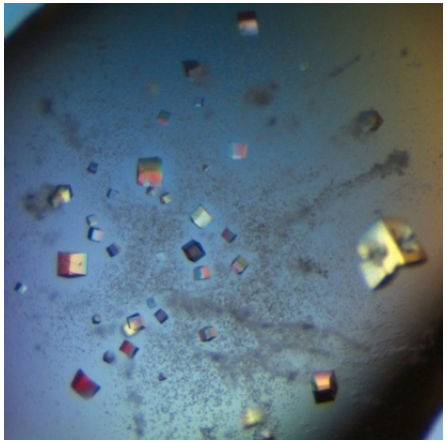


Solubility is the bottle neck of our production pipeline

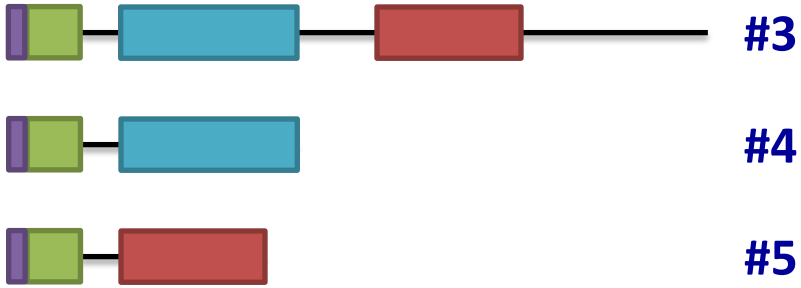
EC class/function	Primer design	PCR	Cloning	Expression	Solubility	Purification	Tag removal	Activity
Carboxylic Ester Hydrolases (EC 3.1.1.)	✓	✓	✓	✓	■			
Cyclic amide hydrolase (EC 3.5.2)	✓	✓	✓	✓	✓	✓	✓	✓
Cysteine endopeptidase II (EC 3.4.22)				✓	✓	✓		✓
Cysteine endopeptidases (EC 3.4.22)	✓	✓	✓	✓	✓	✓	✓	■
Cytokine	✓	✓	✓	✓	■			
Endodeoxyribonuclease (EC 3.1.22)				✓	■			
Endoribonucleases (EC 3.1.26)				✓	✓	✓		✓
Endoribonucleases II (EC 3.1.26)				■				
Glycosidase (EC 3.2.1)	✓	✓	✓	✓	■			
Glycosidase II (EC 3.2.1)	✓	✓	✓	✓	■			
Glycosidase III (EC 3.2.1)	✓	✓	✓	✓	■			
Ligase (EC 6.5.1)				■				
Linear amide hydrolase (EC 3.5.1)	✓	✓	✓	■				
Linear amide hydrolase II (EC 3.5.1)	✓	✓	✓	■				
Linear amide hydrolase III (EC 3.5.1)	✓	■						
Methyltransferase (EC 2.1.1)	✓	✓	✓	✓	■			
Methyltransferase II (EC 2.1.1)	✓	✓	✓	✓	■			
Nucleotidyltransferases (EC 2.7.7)				■				
Endodeoxyribonucleases: (EC 3.1.24)				✓	■			

Cool tips #1: Remove flexible regions, domains and tags

Trim sequence ends

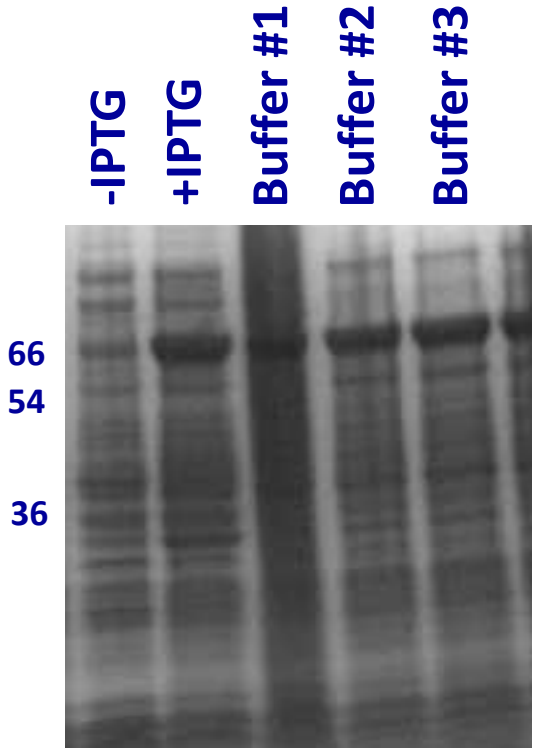


Trim domains

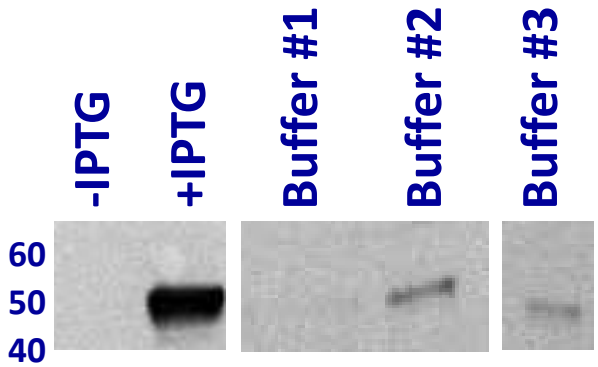


Trim tags

Cool tips #2: Co-expression w/chaperones



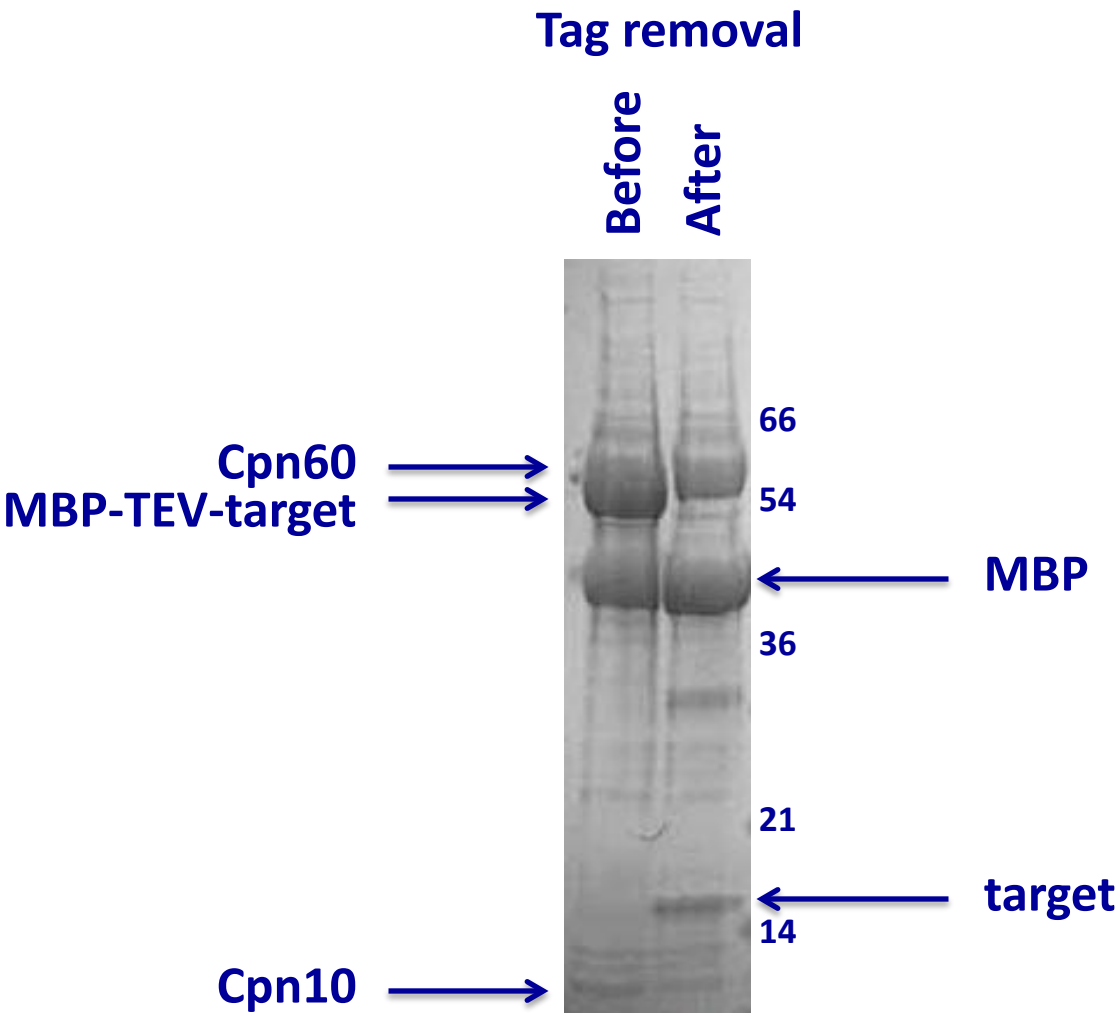
DnaK
GroEL
DnaJ
ClpB
GroES



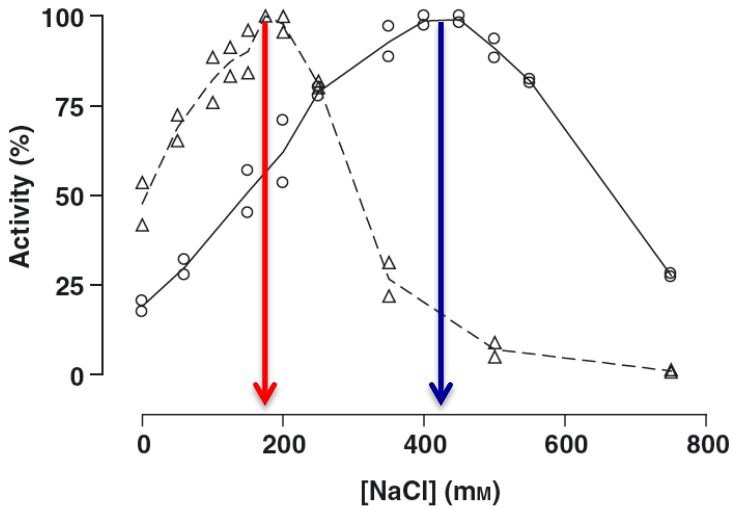
His-EK-target

DeMarco et al, BMC, 2007

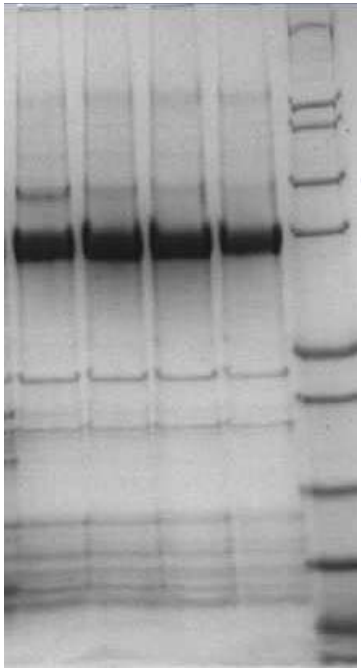
Cool tips #3: Co-expression w/cold-adapted chaperones



Cool tips #4: Salt-loving enzymes can't live without salt



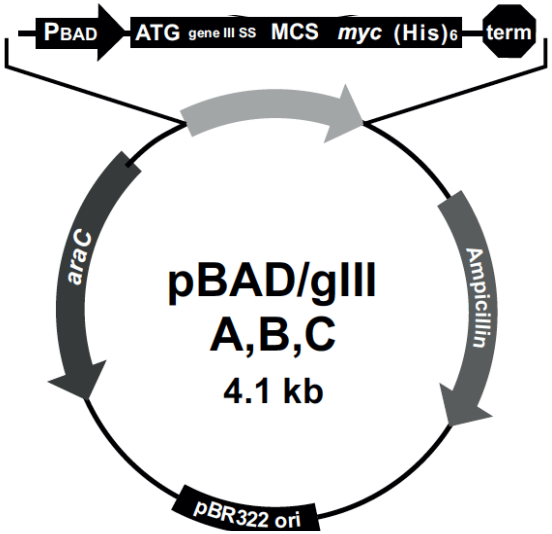
Halophilic

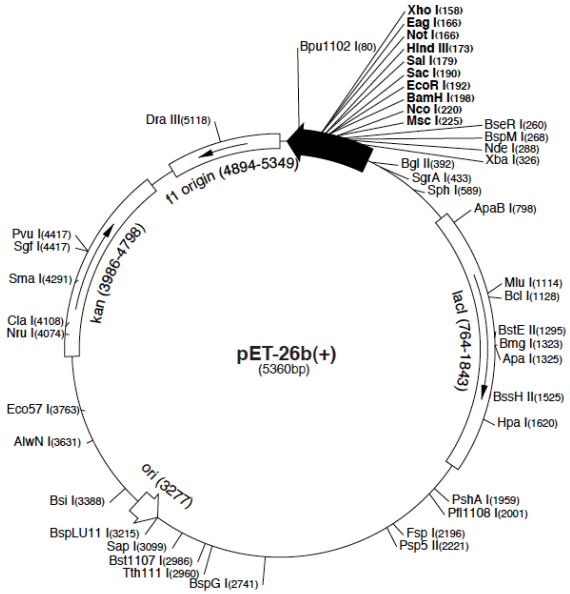


His-TEV-target

Grgic et al, unpublished
Pedersen et al, JM, 2010

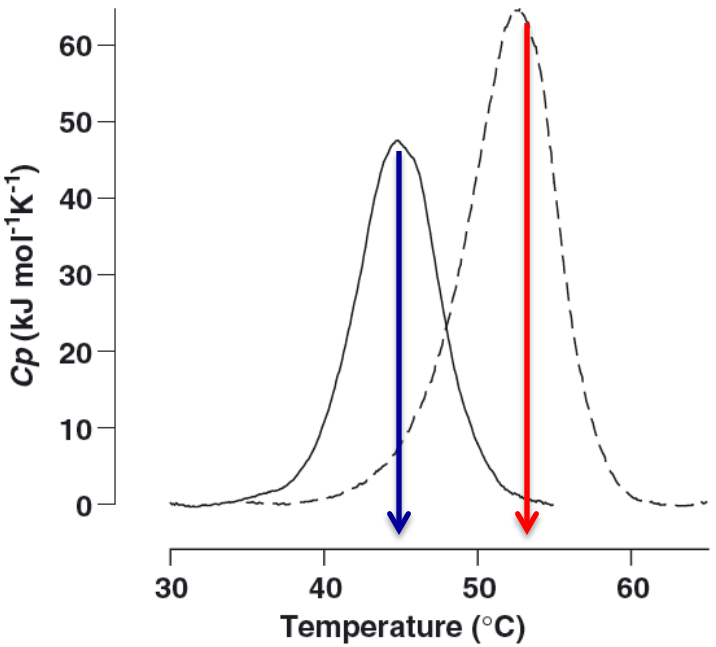
Cool tips #5: Periplasmic expression of secreted enzymes





Invitrogen/Novagen
Edvardsen et al, unpublished
Larsen et al, FEBS, 2006

The expression protocol for a given protein must be empirically determined



Acknowledgements

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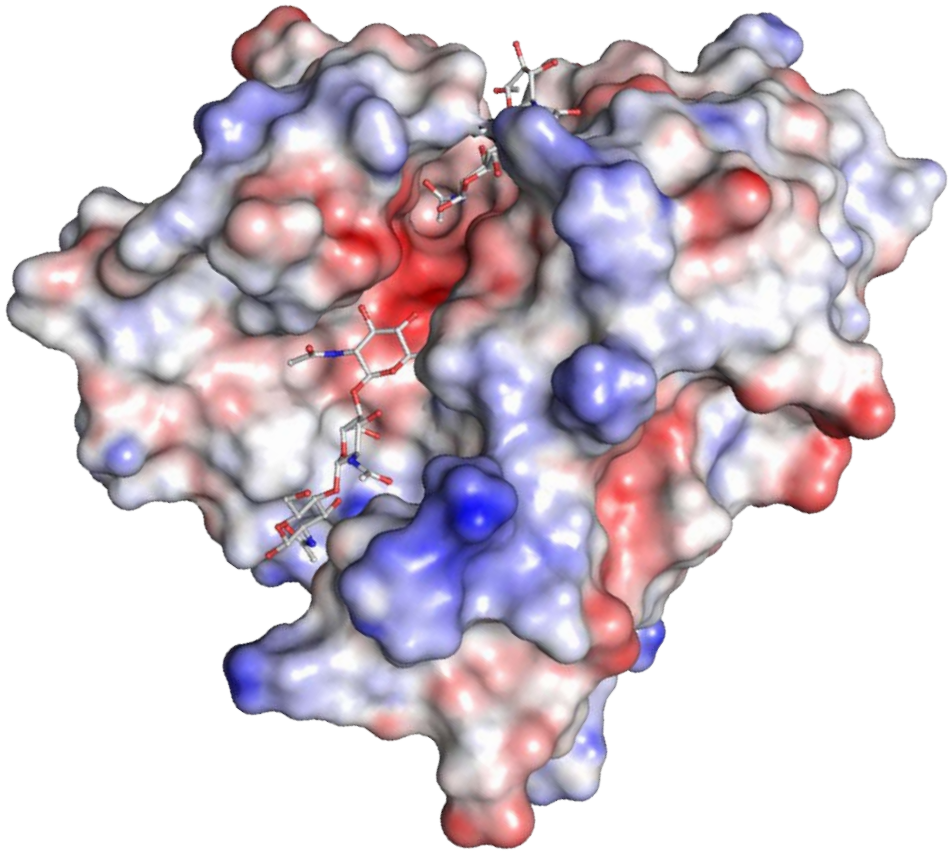
Nabin Malla
Susann Skagseth




In summary, cold-adapted enzymes have a great industrial potential, but are challenging to produce recombinantly



FUGE Large-scale Programme The Research Council of Norway

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Questions?