Kód předmětu: C8980

MASARYKOVA UNIVERZITA

Protein expression and purification

- V. Protein expression

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Radka Dopitová, Jozef Hritz and Adam Norek

Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.

EVROPSKÁ UNIE

MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY

OP Vzdělávání pro konkurenceschopnost

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

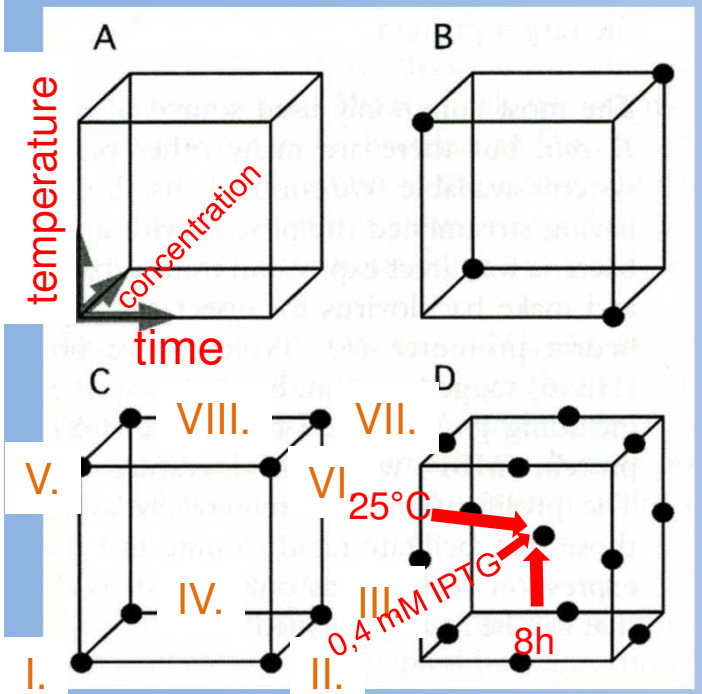
5.1. Designing experiments for high-throughput protein expression

High-throughput platform requires:

- Automation
- Miniaturization
- Quantitative management tools (to identify trends and relationships)

Experimental design:

- An ill-defined experiment will often produce ambiguous results and fail to reach any conclusion.
- Analysis of quantitative response allows the experimenter to optimize conditions critical to production of a soluble protein.
- Performing one-factor-at-a-time experiments raises the risk of locating a local maximum (missing the actual best conditions).



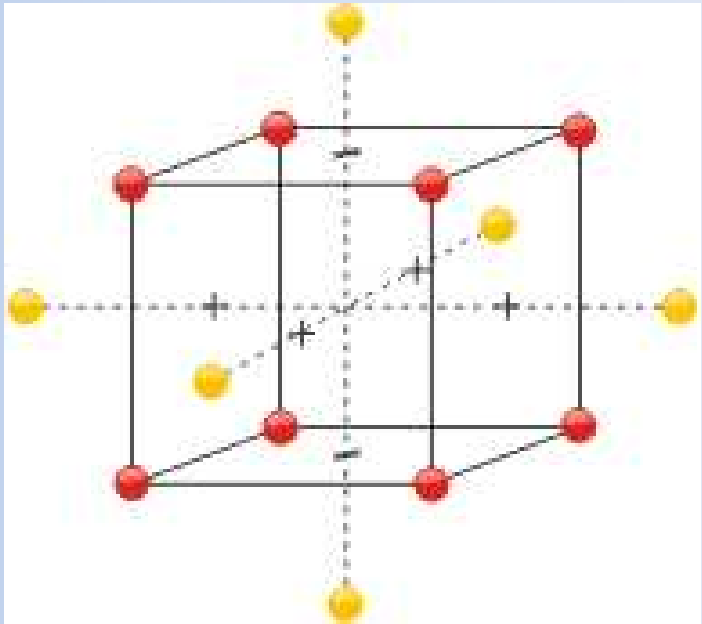
A: One factor at a time
B: Fractional factorial
C: Full factorial
D: Response surface model (Box-Behnken design for three factors)

I. 15°C/1h/0,1mM IPTG
II. 15°C/24h/0,1 mM IPTG
III. 15°C//24h/1mM IPTG
IV. 15°C/1h/1mM IPTG
V. 37°C/1h/0,1 mM IPTG
VI. 37°C/24h/0,1 mM IPTG
VII. 37°C/24h/1mM IPTG
VIII. 37°C/1h/0,1 mM IPTG

Experimental design (commonly referred to as DOE) is a useful complement to multivariate data analysis because it generates “structured” data tables, i.e. data tables that contain an important amount of structured variation. This underlying structure will then be used as a basis for multivariate modeling, which will guarantee stable and robust models.
More generally, **careful sample selection** increases the chances of extracting useful information from the data. When one has the possibility to actively perturb the system (experiment with the variables), these chances become even greater. The **critical part** is to decide 1) **which variables to change**, 2) **the intervals for this variation**, and 3) **the pattern of the experimental points**.

1) Temperature
2) 15°C-37°C
3) About 1°C; 5°C or 10°C

Temperature	15°C-37°C~23 culture conditions
IPTG	0,1 – 1,0 mM ~10 culture conditions
Time	1h-24h ~25 culture conditions
	23x10x25=5750 culture conditions



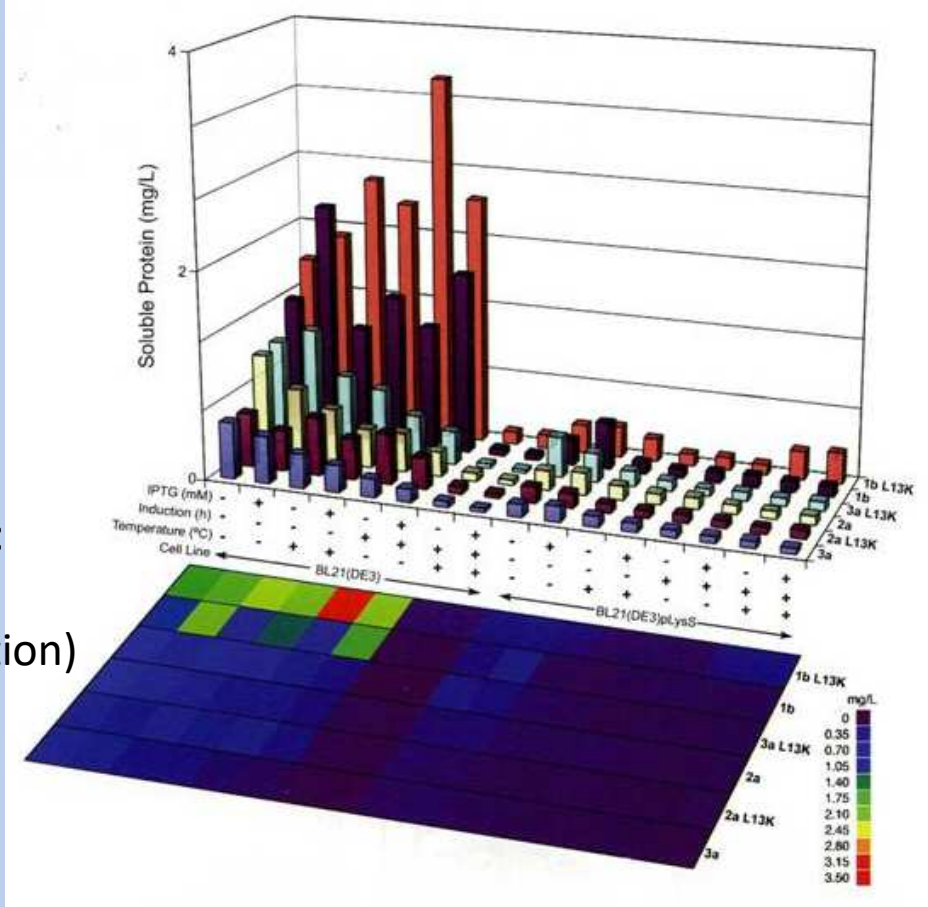
5.1. Designing experiments for high-throughput protein expression

Factors affecting expression:

- Construct - Cat
- Expression system and vector - Cat
- Cell line (host strain) - Cat
- Temperature and time - Cont
- Media - Cat
- Additives - Cat

Full factorial design (16 conditions per construct):

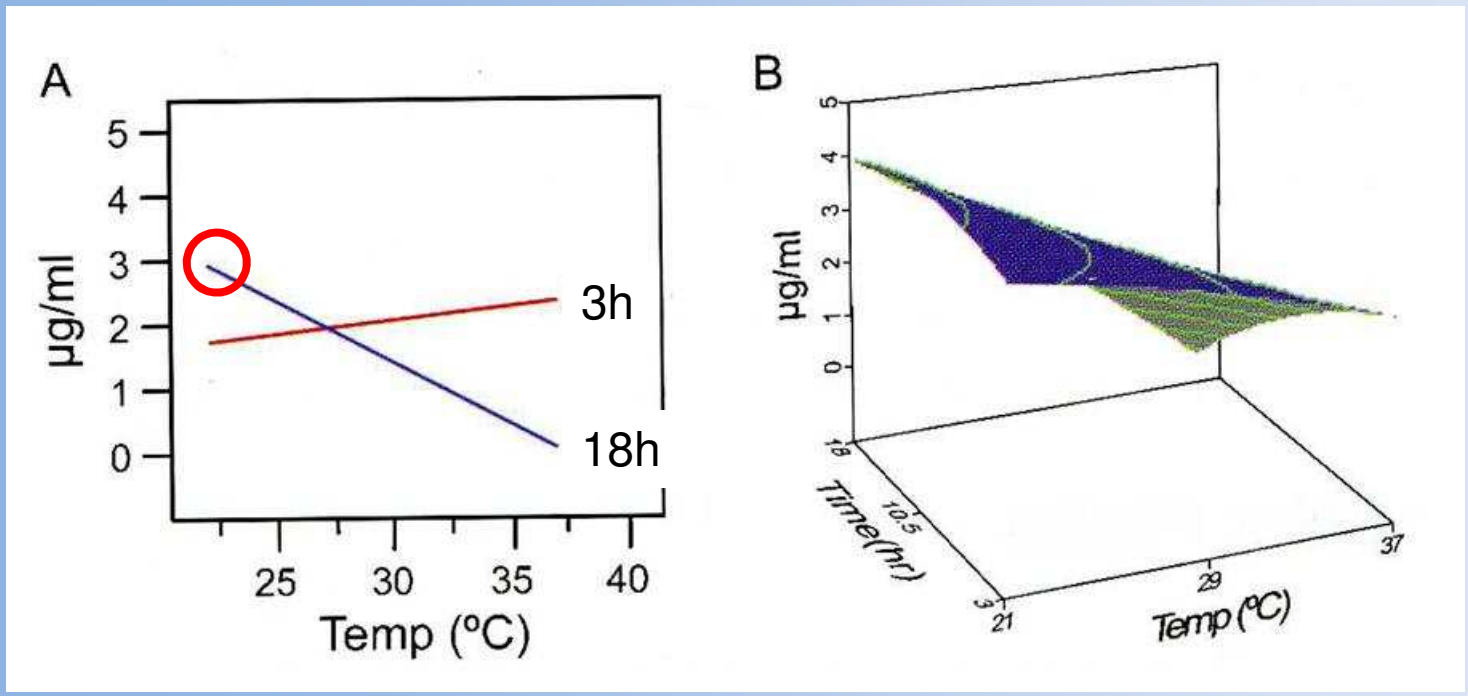
- three continuous factors (temperature, time, IPTG concentration)
- one categorical (host strain)



5.1. Designing experiments for high-throughput protein expression

Response surface model:

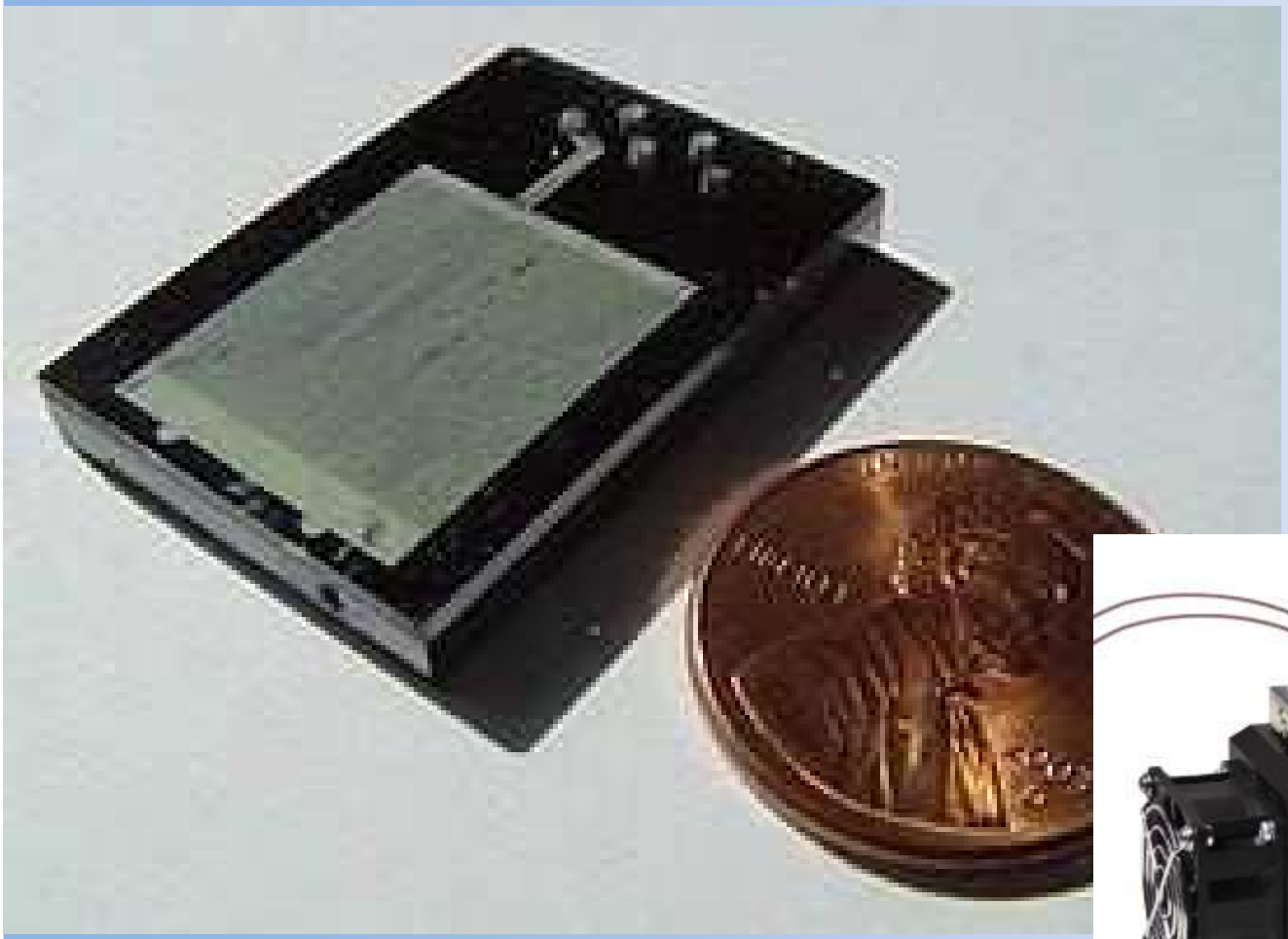
- fine-tunes the conditions
- capable to identify minimum or maximum



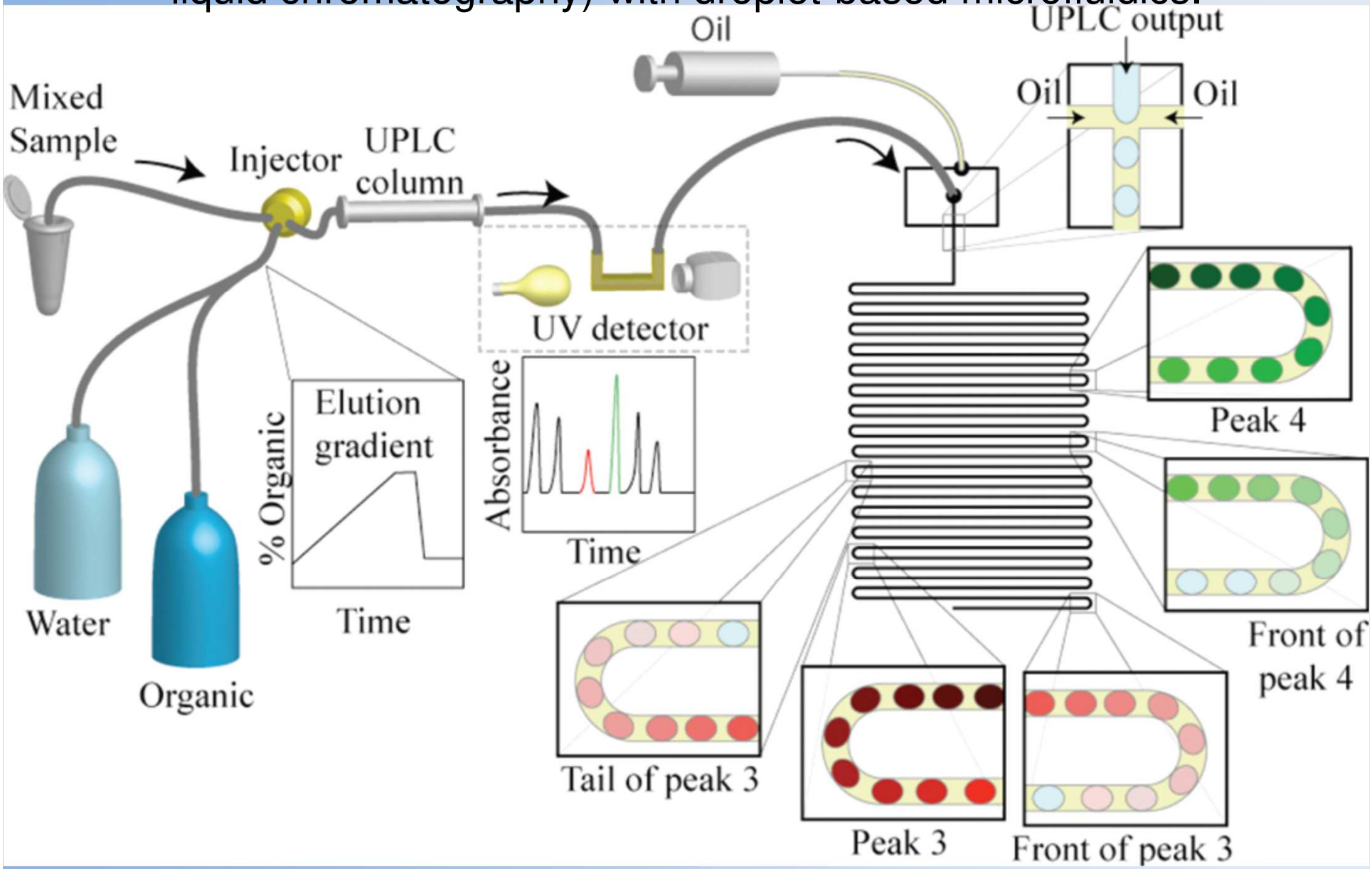
Design of experiment is merely a statistical tool, a means to an end.

It does not guarantee success and cannot replace technical expertise or creativity in experimental work.

Microreactor technologies developed at LLNL use micromachining techniques to miniaturize the reactor design.



Schematic diagram of integration of nano-UPLC (ultra performance liquid chromatography) with droplet-based microfluidics.



Intuition, experience and good judgment

5.2. Approaches for efficient protein production

I. Genetic approach x ***protein knowledgebase*** (biochemical approach)

II. Expression density x ***functional activity***

III. Expression system x ***medium engineering***

IV. Troubles with removing tag fusion proteins x ***less convenient purification with classical chromatography***

4.2. The key questions before DNA cloning

4.2.1. DNA-protein analysis

4.2.1.2. Secondary structure prediction

www.expasy.ch jpred3

```
MASTDSESETRVKSVRTGRKPIGNPEDEQETSKPSDDEFLRGKRVLVVDDNFISRKVATG
--EEEEEE----EEEEEEEEEE--------------------EEEEEE--HHHHHHHHH

KLKKMGVSEVEQCDSGKEALRLVTEGLTQREEQGSVDKLPFDYIFMDCQMPEMDGYEATR
HHHH----EEEEE--HHHHHHHHHH----------------EEEEEE------HHHHH

EIRKVEKSYGVRTPIIAVSGHDPGSEEARETIQAGMDAFLDKSLNQLANVIREIESKRH
HHHH---------EEEEEE-----HHHHHHHHHH-----E---HHHHHHHHHHHHH---
```

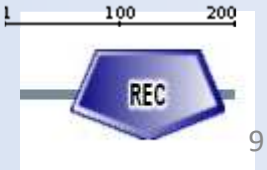
4.2.1.3. Domains detected by SMART

www.expasy.ch SMART

```
KRVLVVDDNFISRKVATGKLKKMGVSEVEQCDSGKEALRLVTEGLTQREEQGSVDKLP
FDYIFMDCQMPEMDGYEATREIRKVEKSYGVRTPIIAVSGHDPGSEEARETIQAGMDA
FLDKSLNQLANVI
```

Confidently predicted domains, repeats, motifs and features:

Name	Begin	End	E-value
REC	43	171	$1.19e^{-26}$



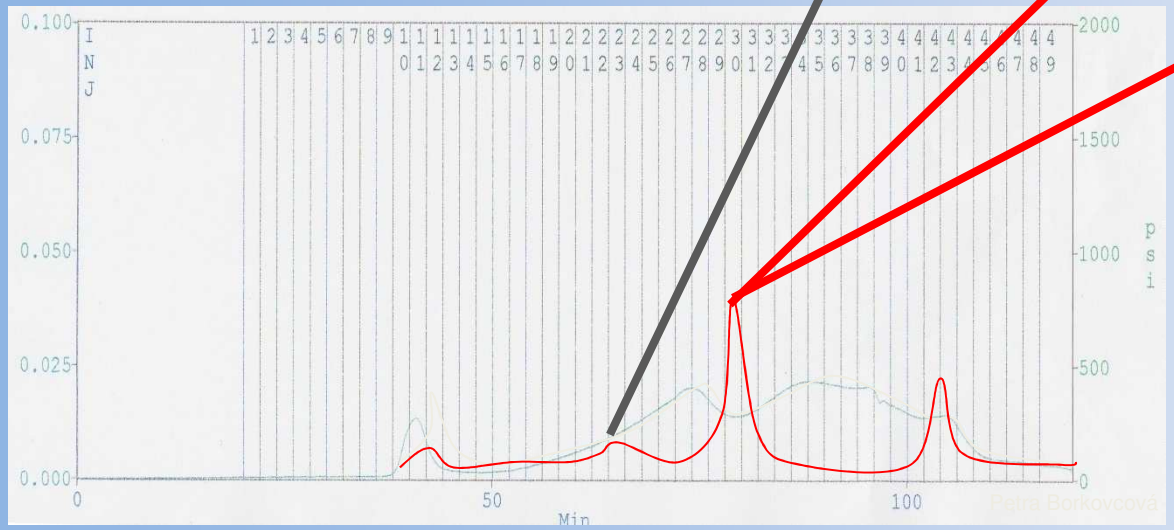
5.2. Approaches for efficient protein production

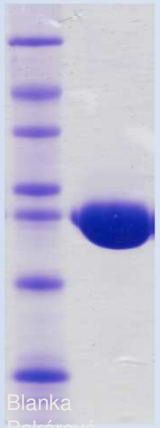
5.2.1. Genetic approach x ***protein knowledgebase***

Histidine kinase from *A. thaliana* - CKI 1

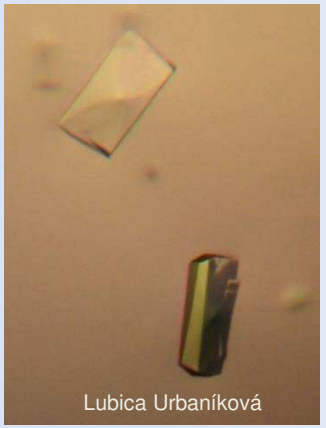
Three constructs of receiver domain.

pET 28





Blanka Pekárová

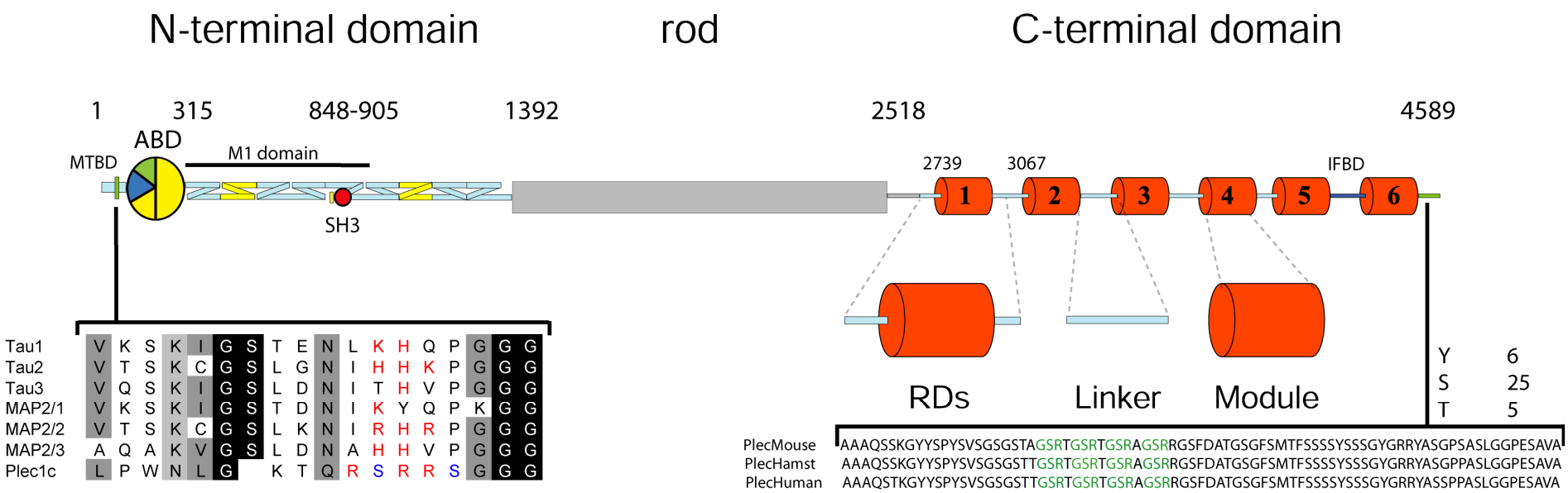


Lubica Urbaníková

5.2. Approaches for efficient protein production

5.2.1. Genetic approach x ***protein knowledgebase***

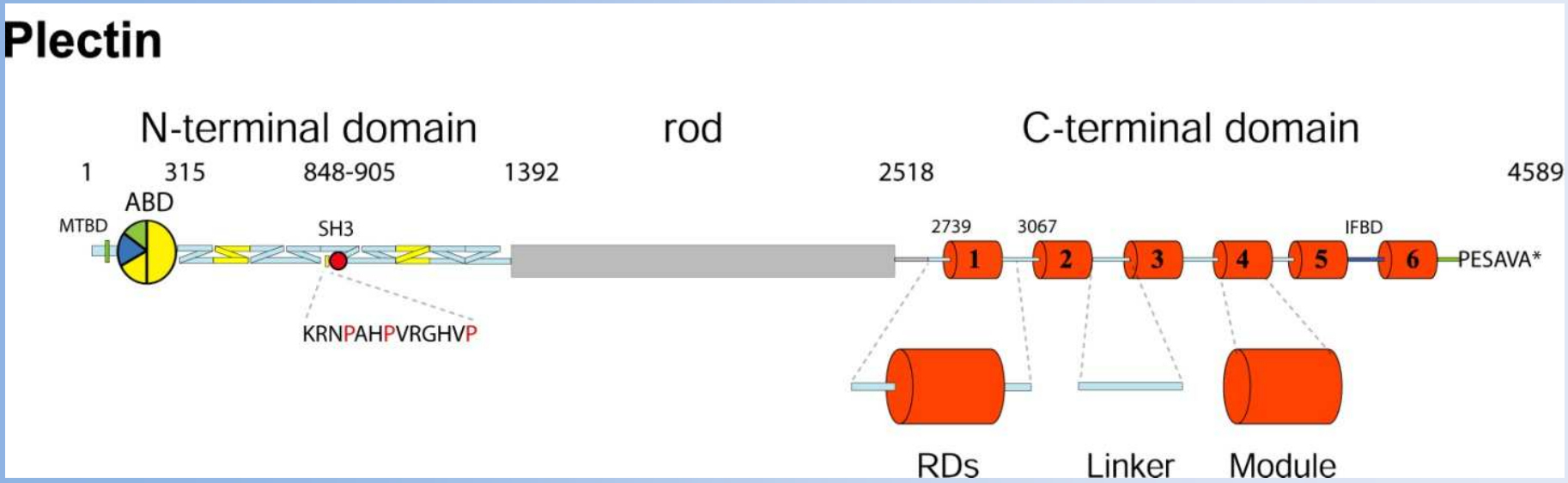
Plectin

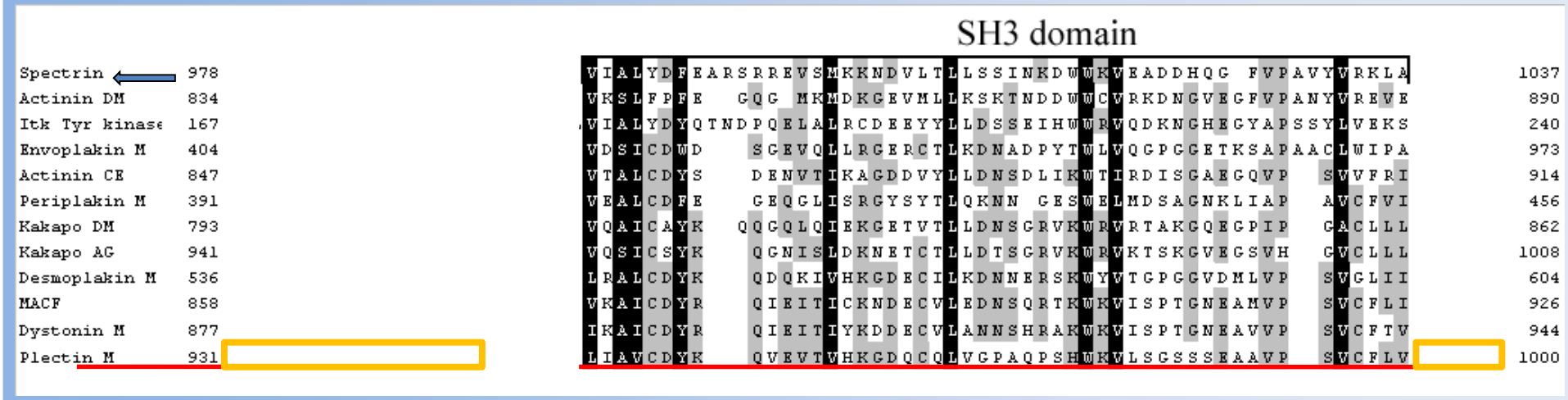


Kd of Plectin (Ex 1-24) for actin	320 nM	(Ex 2-8) 25uM
Kd of Plectin (R5) for vimentin (IF)	100 nM	
Kd of Plectin (Ex 2-8) for integrin beta 4	170 nM	
Kd for microtubules in case of MAP2	1-3 uM	

5.2. Approaches for efficient protein production

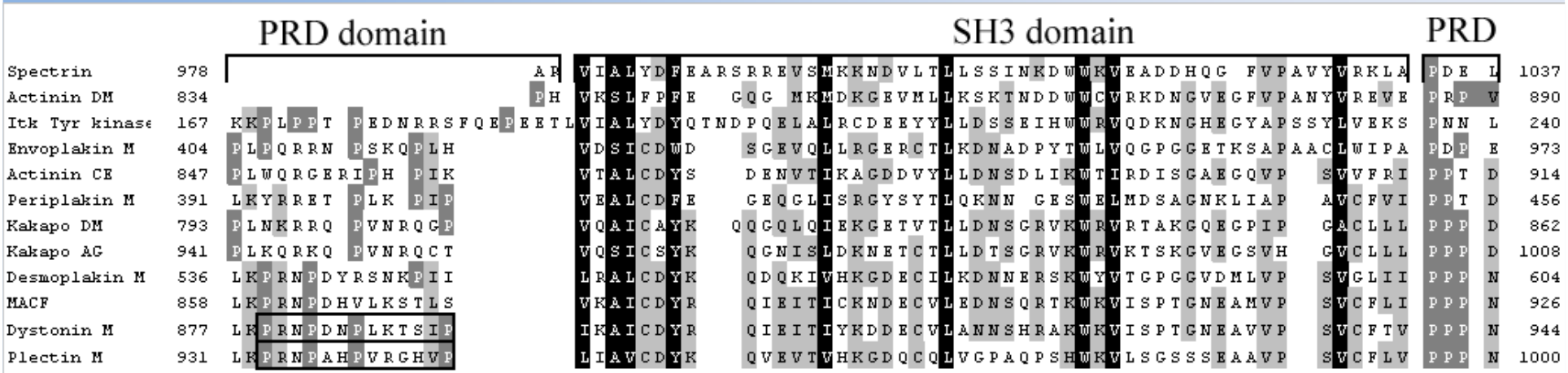
5.2.1. Genetic approach x ***protein knowledgebase***

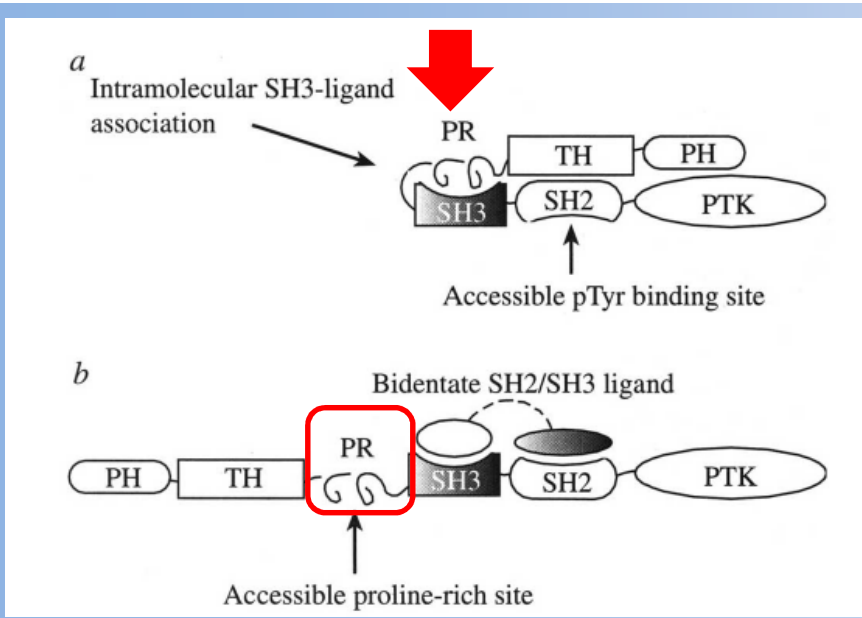




5.2. Approaches for efficient protein production

5.2.1. Genetic approach x ***protein knowledgebase***





a) Model of observed intramolecular interaction showing the observed interaction between the Itk proline-rich region and SH3 domain.

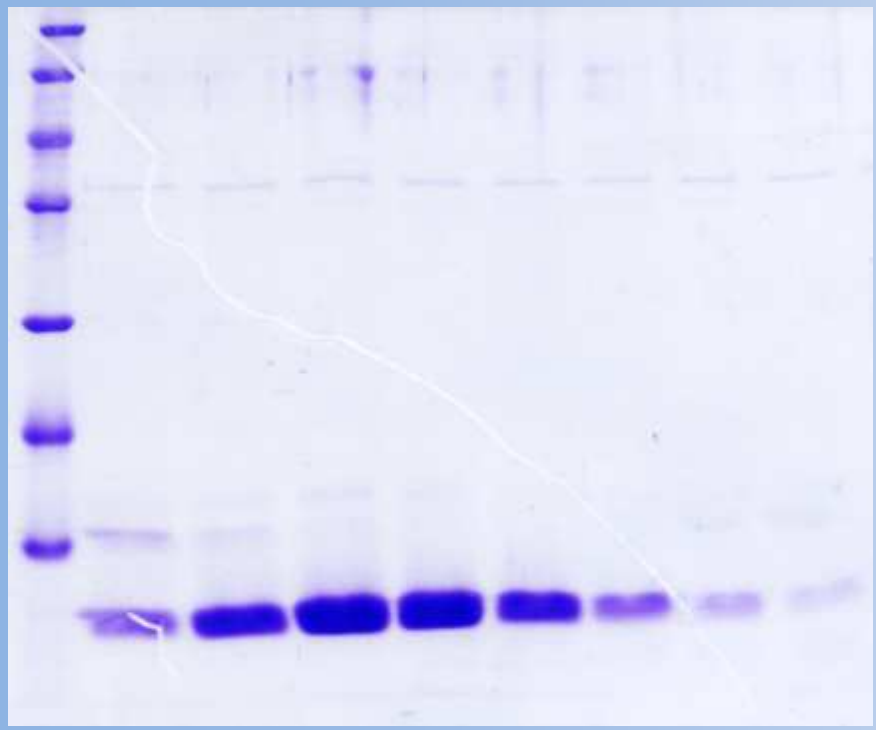
b) Model of the opening of the intramolecular complex by interaction with bidentate ligand for the Itk SH3 and SH2 domains.
(Andreotti et al., Nature 1996)

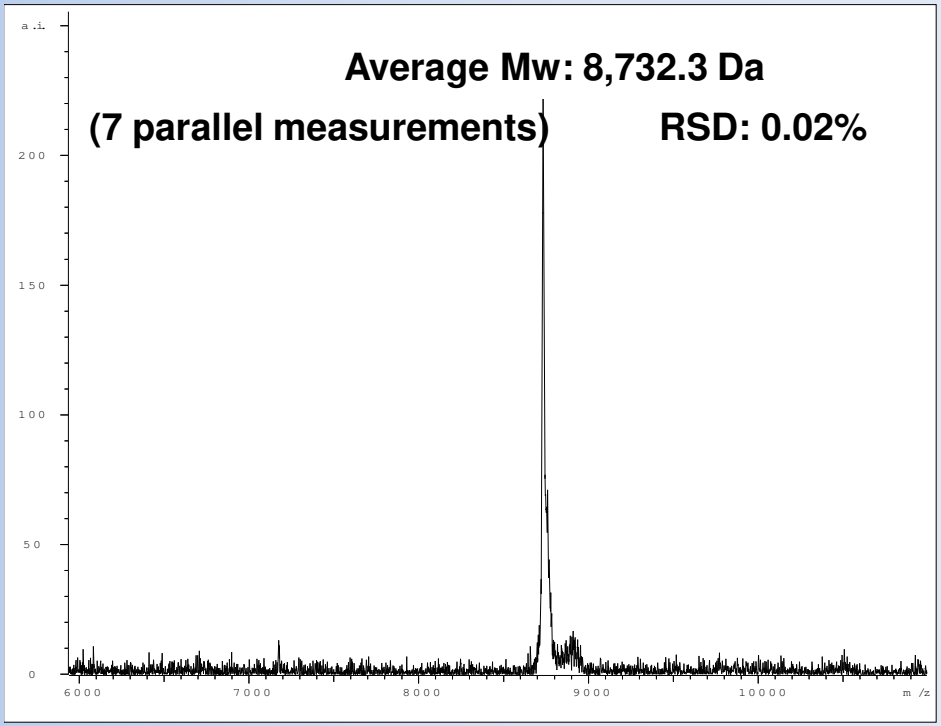
5.2. Approaches for efficient protein production

5.2.1. Genetic approach x ***protein knowledgebase***

SH3 domain of plectin with surrounding proline rich regions

(Sarc homology domain soluble in citrate buffer of pH 3.5)

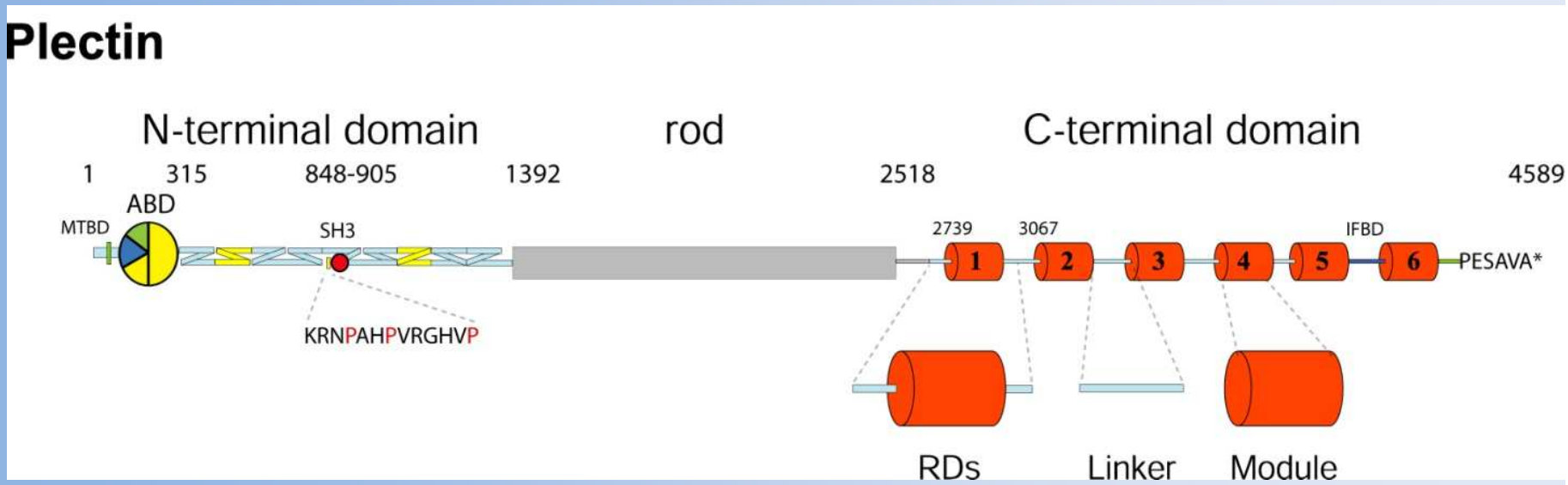




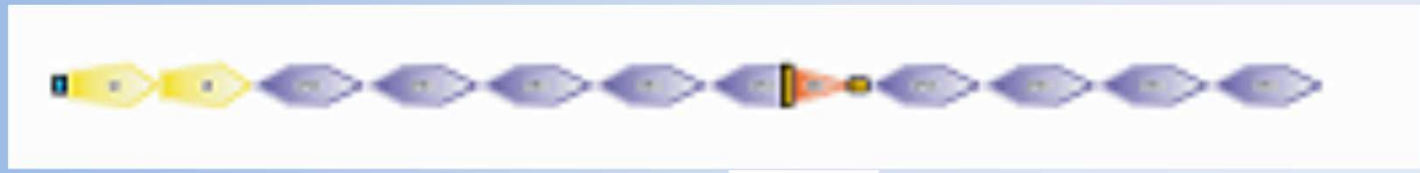
Theoretical pI/Mw (average) for the protein sequence

Theoretical pI/Mw: 7.78 / **8,726.11**

mefKAIVQLKPRNPAHPVRGHVPLIAVCDYKQVEVTVHKGD
QCQLVGPAQPSHWKVLSGSSSEAAVPSVCFLVPPPNQEf



Exon 1d-30



Exon 20-21

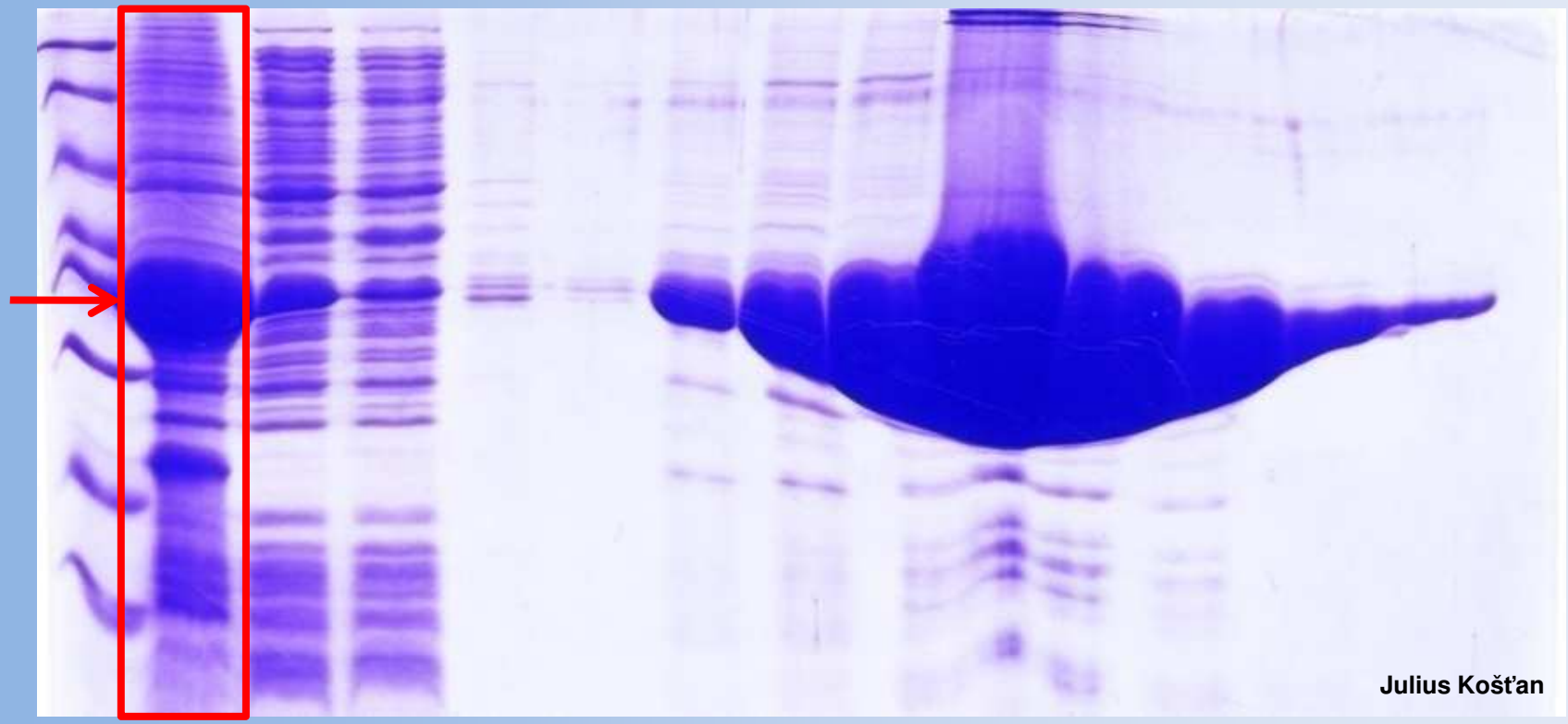
Exon 16-24

Exon 16-21

5.2. Approaches for efficient protein production

5.2.2. Expression density x ***functional activity***

Expression and purification of plectin's ABD (Actin Binding Domain) in three isoforms.



5.2. Approaches for efficient protein production

5.2.2. Expression density x ***functional activity***

Maize recombinant β–glucosidase produced in *E. coli.*

Cultivation condition	***Yield (mg)***	***Specific activity (nkat/mg) /(total activity nkat)***
LB medium	*380*	*1.9* (966 nkat)
TB medium - pH 6	*230*	*3.8* (874 nkat)
TB medium – pH 7	*230*	*4.2!* (966) nkat)
TB medium – pH 8	*410*	*2.8* (1,148 nkat)
Additive of cellobiose (LB medium)	*400*	*2.7!* (1,080 nkat)

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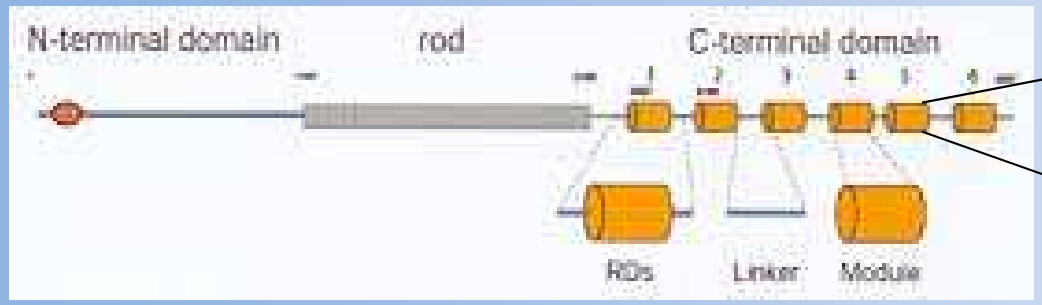
Result: TB medium (pH 7.0) supplemented by cellobiose shows 3.1 x higher β-glucosidase specific activity than in common LB medium.

5.2. Approaches for efficient protein production

5.2.2. Expression density x ***functional activity***

The cytolinker protein:
plectin

Plectin is one of the main linker proteins for the cytoskeleton.



Plectin R5

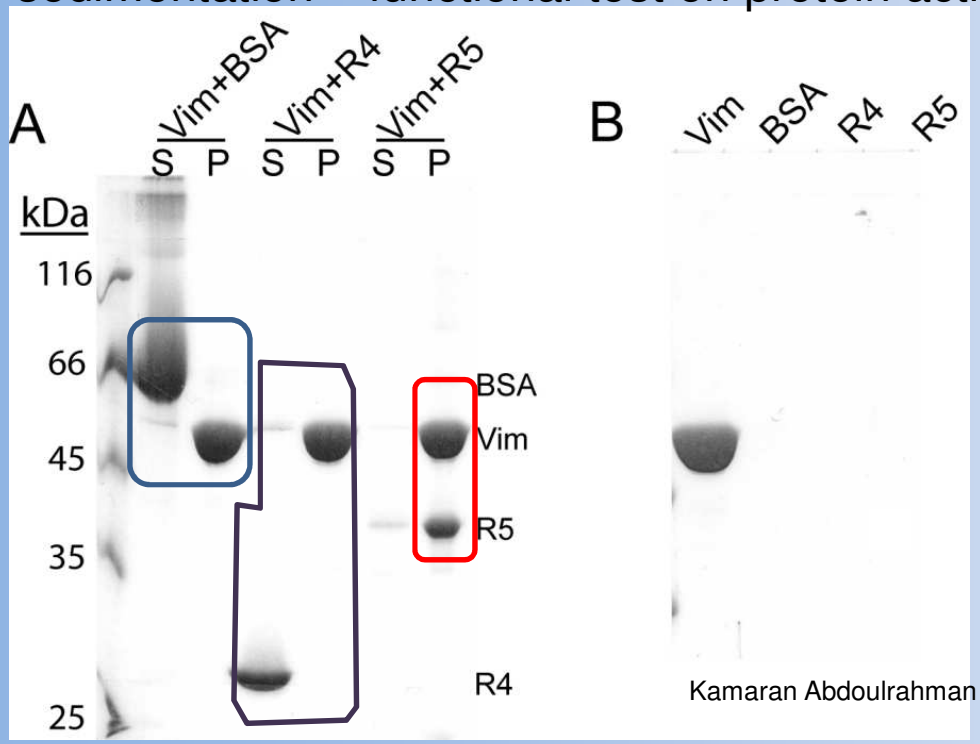
5.2. Approaches for efficient protein production

5.2.2. Expression density x ***functional activity***

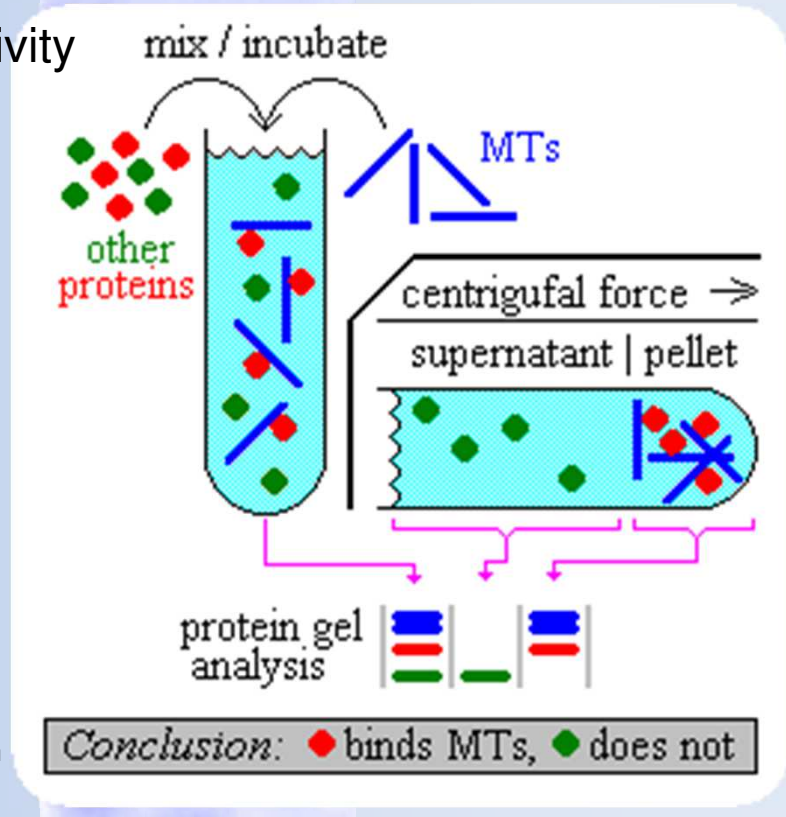
Converted pET 15b + IF binding domain of plectin

• R5 d. plectin (pH 7.9)	Insoluble form
• R5 d. plectin (pH 7.9, urea, dialysis)	Func. act.(45%)
• R5 d. plectin (pH 7.9, urea, refolding HR)	Func. act.(60%)
• R5 d. plectin (pH 11, purification pH 9.0)	Func. act. (≥95%)

Co-sedimentation – functional test on protein activity



Kamaran Abdoulrahman



5.2. Approaches for efficient protein production

5.2.3. Expression system x ***medium engineering***

Examples of *E. coli* expression systems and web pages for further information

	Vector system	Promoter/ induction method	Special host strains required	Protein tag	Source (website)
	Pinpoint	*tac*/IPTG or *T7* IPTG	Yes	Biotin binding domain	www.promega.com
*	pET	*T7* IPTG	Yes	His_6, T7 gene	http://www.merckbiosciences.co.uk
*	pGEX	*tac*/IPTG	No	GST	www.amershambiosciences.com
	pBAD	*ara*BAD	Yes	His_6, GFP	www.invitrogen.com
	pLEX	P_L/trp	Yes		
*	pRSET	*T7* IPTG	Yes	His_6, T7	
	pPROTet	P_{Ltet}/anhydrotetracycline	No	His_6	www.clontech.com
	pTYB	*T7* IPTG	Yes	Chitin binding domain	www.neb.com
*	pMAL	*tac*/IPTG	Yes	Maltose binding domain	
	pQE	*T5*/IPTG	Yes/TOPP	His_6	www.qiagen.com
	pCAL	*T7*/IPTG	Yes	Calmodulin binding peptide	www.stratagene.com
	pFLAG	*tac*/IPTG	Yes		www.sigmaaldrich.com

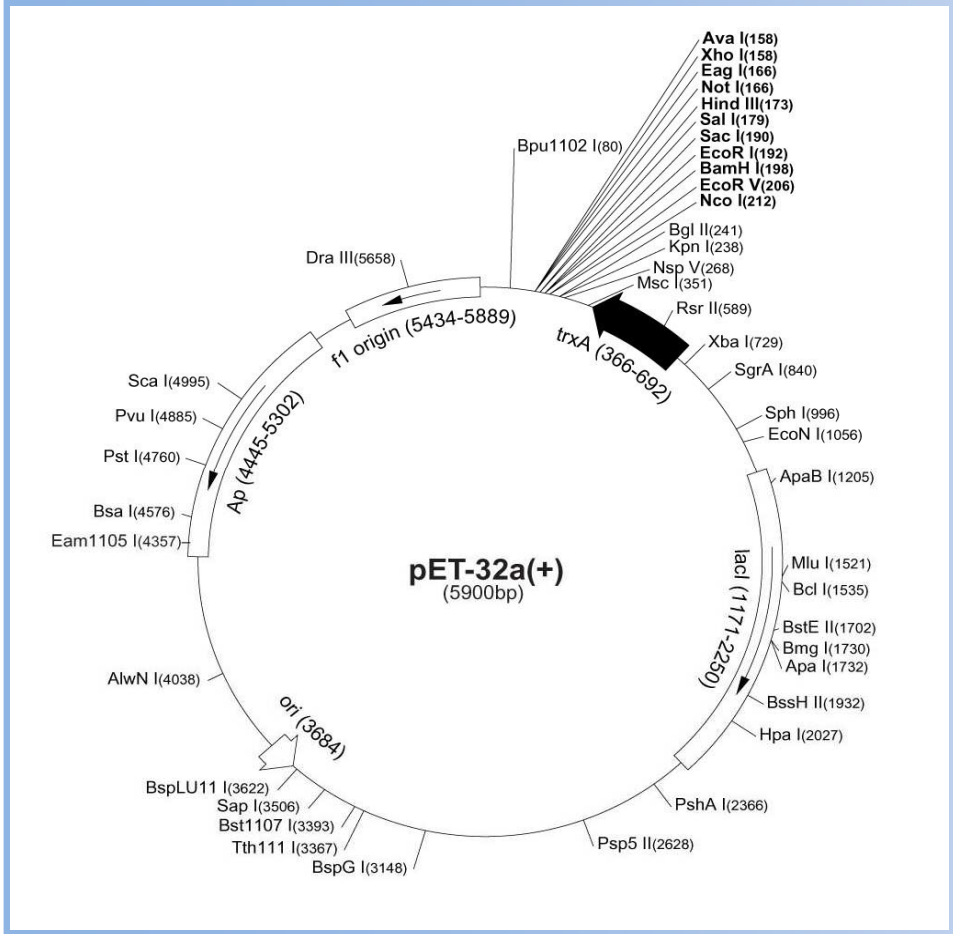
5.2. Approaches for efficient protein production

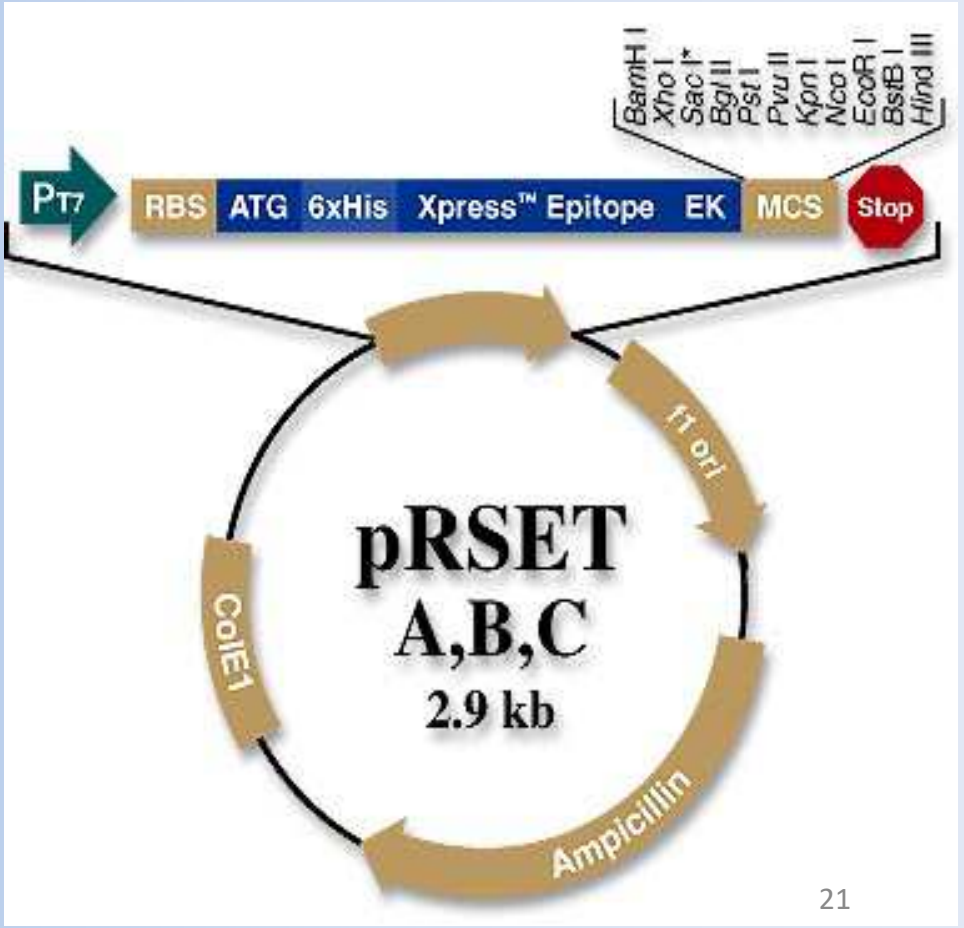
5.2.3. Expression system x ***medium engineering***

pET32a::AHP1
pET32a::AHP5

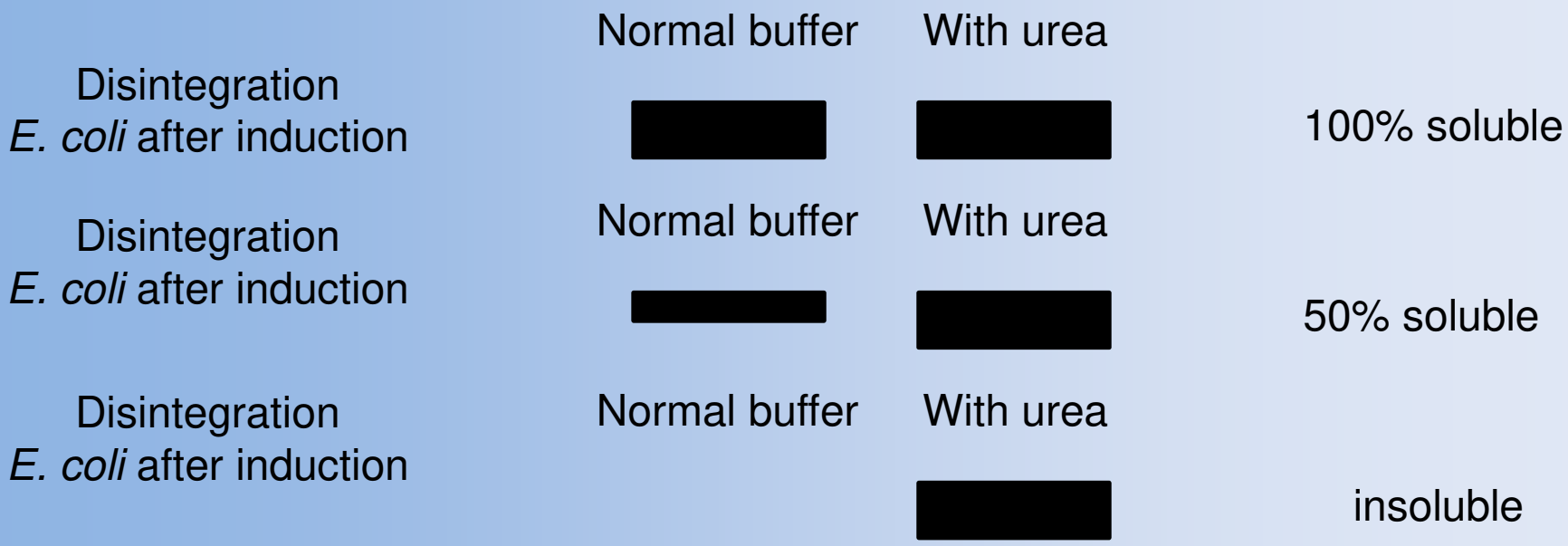
AHP – phosphotransfer protein in cytokinin signalling pathway of *A. thaliana*

pRSETB::AHP1
pRSETB::AHP5





Bands of protein of interest on SDS-PAGE



- **Disintegrate** *E. coli* in native buffer and divide into two same parts.
- **Denaturate** second part of the crude extract by chaotropic compounds (urea).
- Sediment both extracts and load on **SDS-PAGE**
- **Scan** the gel after staining and subsequent de-staining.
- Evaluate differences between signals from protein denaturated by chaotropic compounds and protein signal from native buffer.

5.2. Approaches for efficient protein production

5.2.3. Expression system x ***medium engineering***

5.2.3.1. Temperature

Expression system

AHP1

Plasmid	pET32a+		pRSETB	
Temperature (°C) growth/induction	Soluble form (%)	Insoluble form (%)	Soluble form (%)	Insoluble form (%)
22°C/22°C	62%	38%	71%	29%
37°C/22°C	0%	100%	82%	18%
37°C/28°C	0%	100%	8%	92%

AHP5

Plasmid	pET32a+		pRSETB	
Temperature (°C) growth/induction	Soluble form (%)	Insoluble form (%)	Soluble form (%)	Insoluble form (%)
22°C/22°C	78%	22%	76%	24%
37°C/22°C	67%	33%	81%	19%
37°C/28°C	61%	39%	81%	19%

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5.2. Approaches for efficient protein production

5.2.3. Expression system x ***medium engineering***

5.2.3.1. Temperature

Production of soluble AHP proteins using *E. coli* expression vector pRSET at different temperatures (%)						
Temperature (°C) growth/induction	AHP1	AHP2	AHP3	AHP4	AHP5	AHP6
37°C/28°C	8%	85%	100%	0%	76%	0%
37°C/22°C	82%	73%	100%	0%	81%	51%
22°C/22°C	71%	78%	100%	30%	81%	73%

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5.2. Approaches for efficient protein production

5.2.3. Expression system x ***medium engineering***

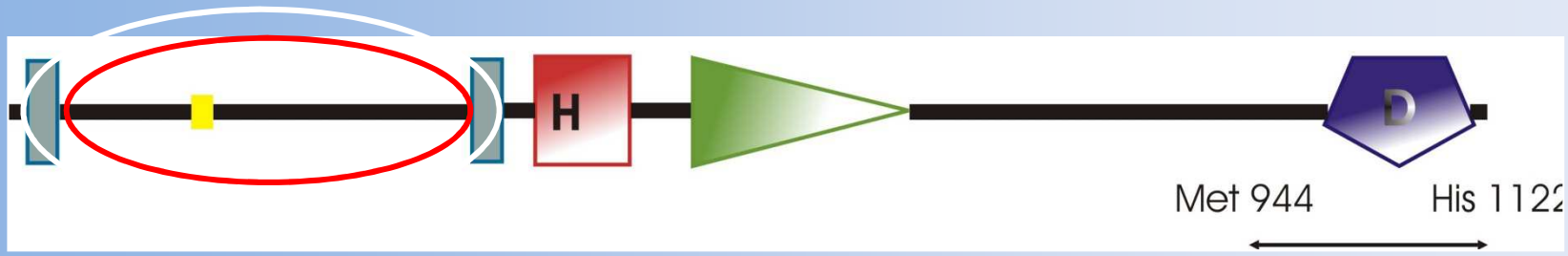
5.2.3.2. Medium pH

Production receiver domain of plant histidine kinase AHK4 in *E. coli* by *pET161DEST*			
pH	6.0	7.0	8.0
Soluble fraction	35%	89%	100%

- **Disintegrate** *E. coli* in native buffer and divide into two same parts.
- **Denaturate** second part of the crude extract by chaotropic compounds (urea).
- Sediment both extracts and load on **SDS-PAGE**
- **Scan** the gel after staining and subsequent de-staining.
- Evaluate differences between signals from protein denaturated by chaotropic compounds and protein signal from native buffer.

5.2. Approaches for efficient protein production

5.2.3. Expression system x ***medium engineering***



pDEST17::CKI1ex1 – 371 AA, Mw = 42 kDa
pDEST17::CKI1ex2 – 419 AA, Mw = 47 kDa

5.2. Approaches for efficient protein production

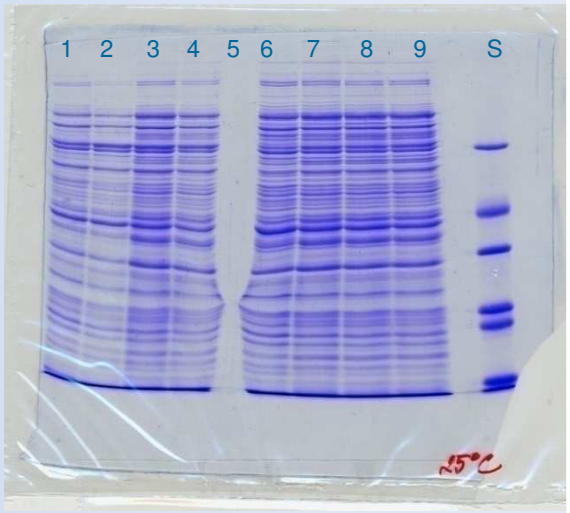
5.2.3. Expression system x ***medium engineering***

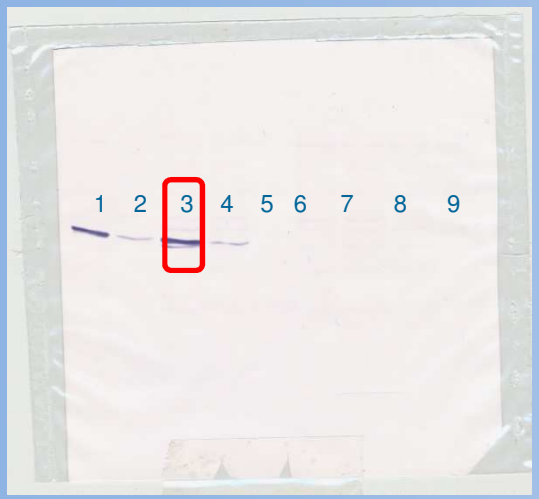
Growth temperature
37°C, expression 28°C

1: BL21 – 1 h after induction = OD 0.5
2: 4 h after induction = OD 0.5
3: 1 h after induction = OD 2
4: 4 h after induction = OD 2
5: -
6: C43 – 1 h after induction = OD 0.5
7: 4 h after induction = OD 0.5
8: 1 h after induction = OD 2
9: 4 h after induction = OD 2

S: 14–66 kDa

Growth and expression 25°C





1: BL 21 before induction
2: 1 h after induction = OD 0.5
3: 3 h after induction = OD 0.5
4: 2 h after induction = OD 2
5: -
6: C43 before induction
7: 1 h after induction = OD 0.5
8: 3 h after induction = OD 0.5
9: 2 h after induction = OD 2

S: 14–66 kDa

5.2. Approaches for efficient protein production
5.2.3. Expression system x ***medium engineering***

Expression *E. coli BL21 (DE3)$_{RIL}$*

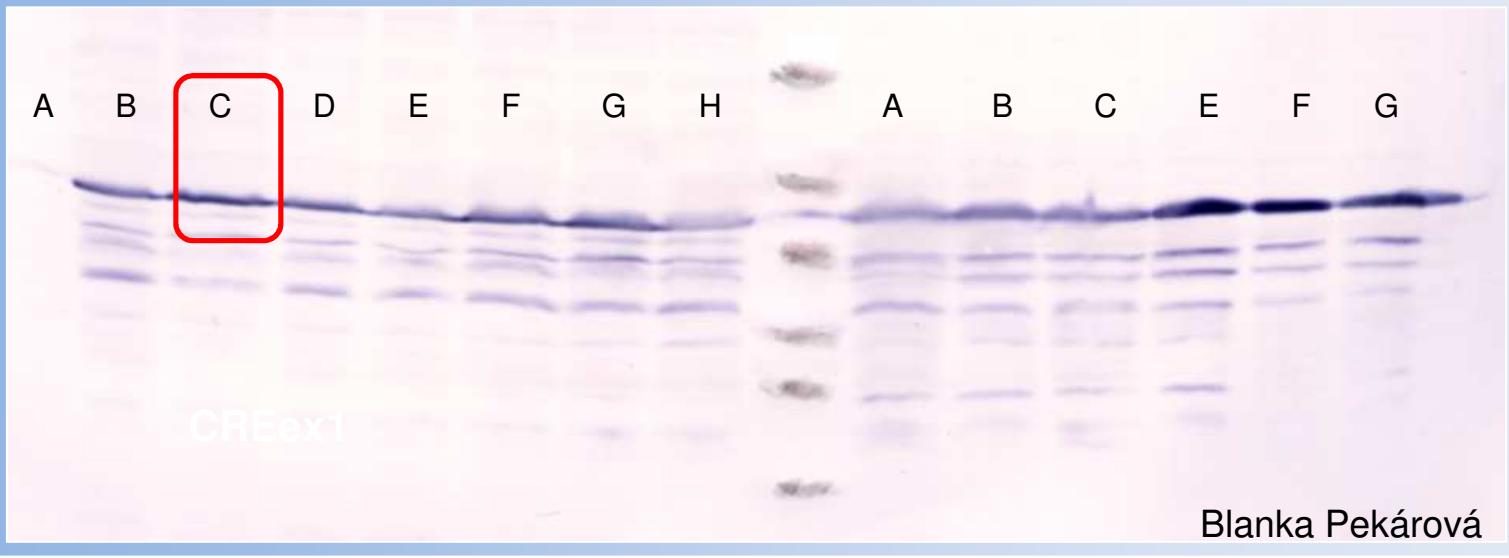
TB medium: pH 6.0, pH 7.0,

growth → induction
growth 25°C → induction 22°C, 13.5 h

Lysis buffers:

A. Citrate b., pH 3.6, Triton X-100
B. Tris b., pH 7.9, Triton X-100
C.
D. Tris b., pH 7.9, Triton X-100
E. Tris b., pH 7.9, CTAB
F. Tris b., pH 7.9, NONIDET P-40
G. Tris b., pH 7.9, SDS
H. Tris b., pH 7.9

CKI1ex1



Blanka Pekárová

5.2. Approaches for efficient protein production

5.2.3. Expression system x ***medium engineering***

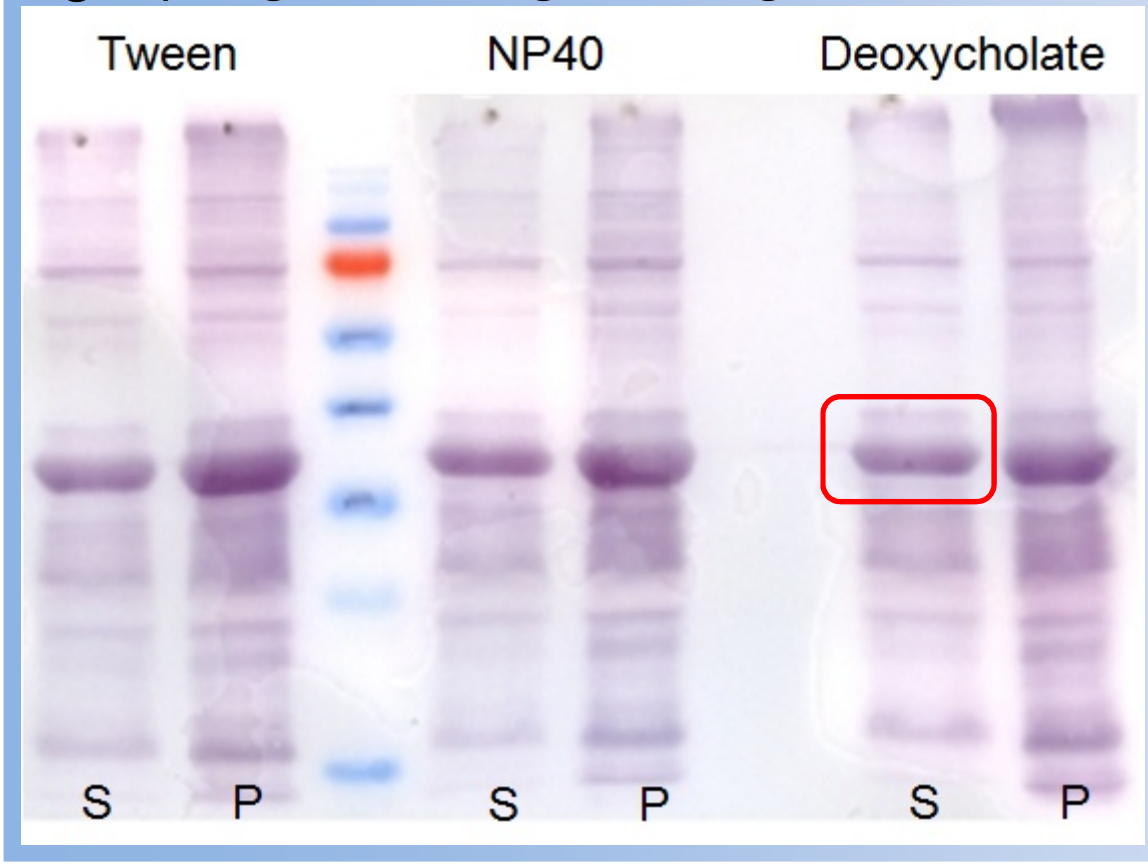
E. coli BL21(DE3) Arctica

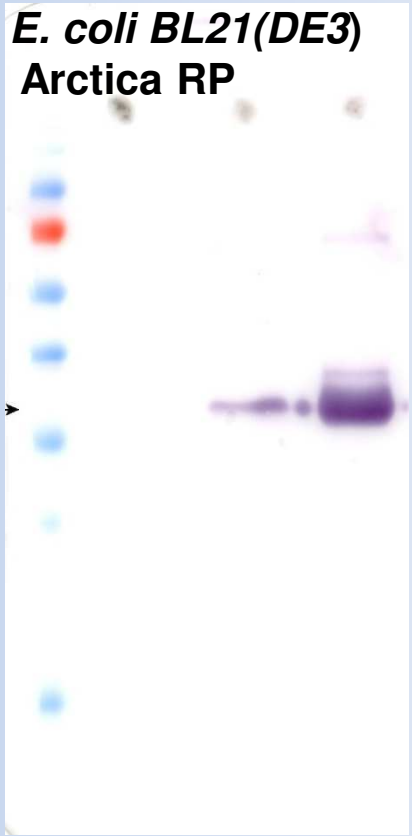
Buffer engineering
1) pH
2) detergent

Tris Glycine Tris Glycine pH10,6

S P S P S P S P

Western blot
detected by poly- His antibodies



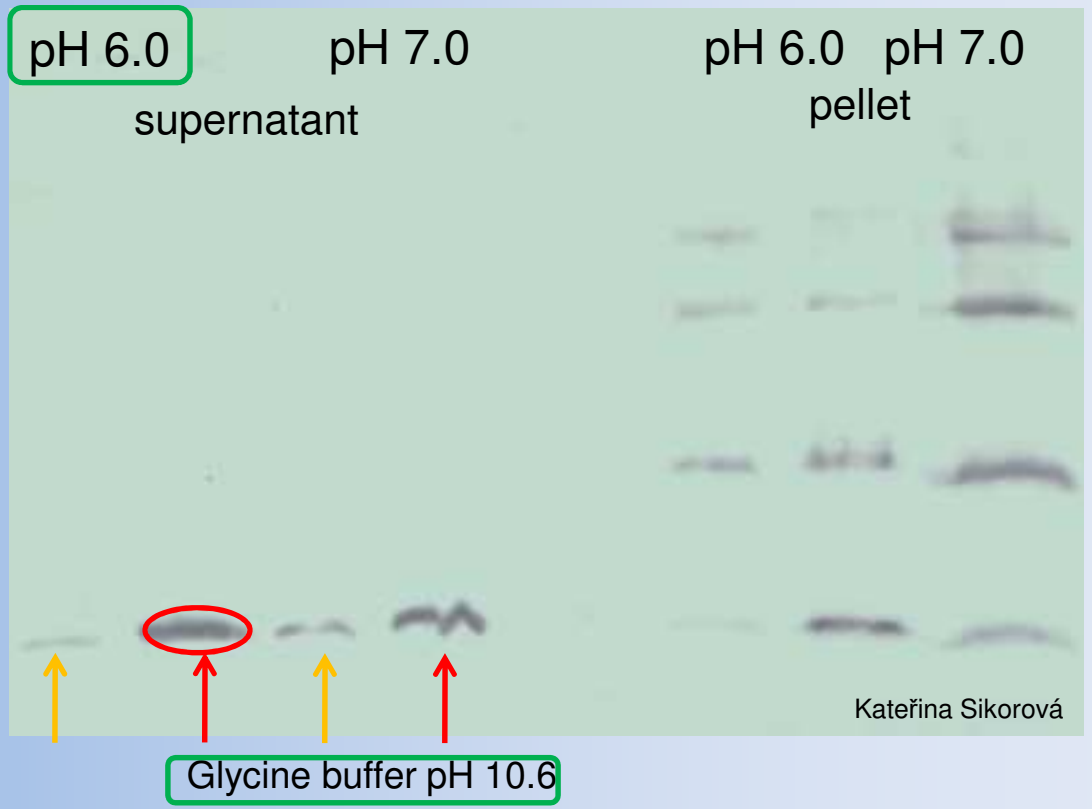


5.2. Approaches for efficient protein production

5.2.3. Expression system x ***medium engineering***

5.2.3.3. Buffer for desintegration (pH)

LTP-2 (non-specific lipid transporting protein from wheat)



Screening for solubility

- Optimum Solubility Screen
  - Optimizing protein solubility and stability with salts and buffers prior to crystallization can improve crystallization results
  - Microbatch experiments can identify salt and buffer conditions that increase a protein's solubility

Acta Cryst. (2006) D**62**, 833 - 842. Izaac, C. A. Schall and T. C. Mueser. Assessment of a preliminary solubility screen to improve crystallization trials: uncoupling crystal condition searches.

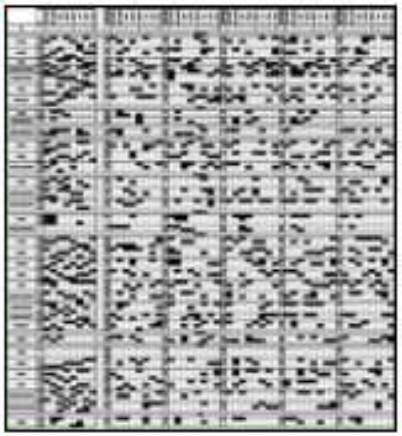
Acta Cryst. (2004) D**60**, 1674 -1678. B. K. Collins, S. J. Tomanicek, N. Lyamicheva, M. W. Kaiser and T. C. Mueser. A preliminary solubility screen used to improve crystallization trials: crystallization and preliminary X-ray structure determination of Aeropyrum pernix flap endonuclease-1

Acta Cryst. (2004) D**60**, 1670 -1673. J. Jancarik, R. Pufan, C. Hong, S.-H. Kim and R. Kim. Optimum solubility (OS) screening: an efficient method to optimize buffer conditions for homogeneity and crystallization of proteins.

Incomplete factorial: 950 cocktails

35 Salts and 8 Buffers

Salt	Occurances (# cocktails)
NH_4Br	28
NH_4Cl	29
NH_4NO_3	27
$NH_4H_2PO_4$	32
$(NH_4)_2HPO_4$	26
$(NH_4)_2SO_4$	30
$Ca(C_2H_3O_2)_2$	12
$CaCl_2$	13
LiBr	32
LiCl	30
$Mg(C_2H_3O_2)_2$	17
$MgCl_2$	26
$MgSO_4$	27
MnCl2	22
$KC_2H_3O_2$	30
KBr	33
K_2CO_3	34
KCl	31
KNO_3	32
KH_2PO_4	30
KSCN	29
RbCl	28
NaBr	28
NaCl	28
Na_2MoO_4	28
$NaNO_3$	30
NaH_2PO_4	23
$Na_2S_2O_3$	23
$Zn(C_2H_3O_2)_2$	22
K_2HPO_4	25
$CoSO_4$	24
Li2SO4	27
K_3PO_4	28
NH_4SCN	29
$MnSO_4$	14
$Mg(NO_3)_2$	23
TOTAL	**950**



Buffer	pH	Occurances (# cocktails)
Citrate	4.2	116
Acetate	5	144
MES	6	124
MOPS	7	124
HEPES	7.5	104
Tris	8	134
TAPS	9	109
CAPS	10	95
TOTAL		950

Joseph R. Luft

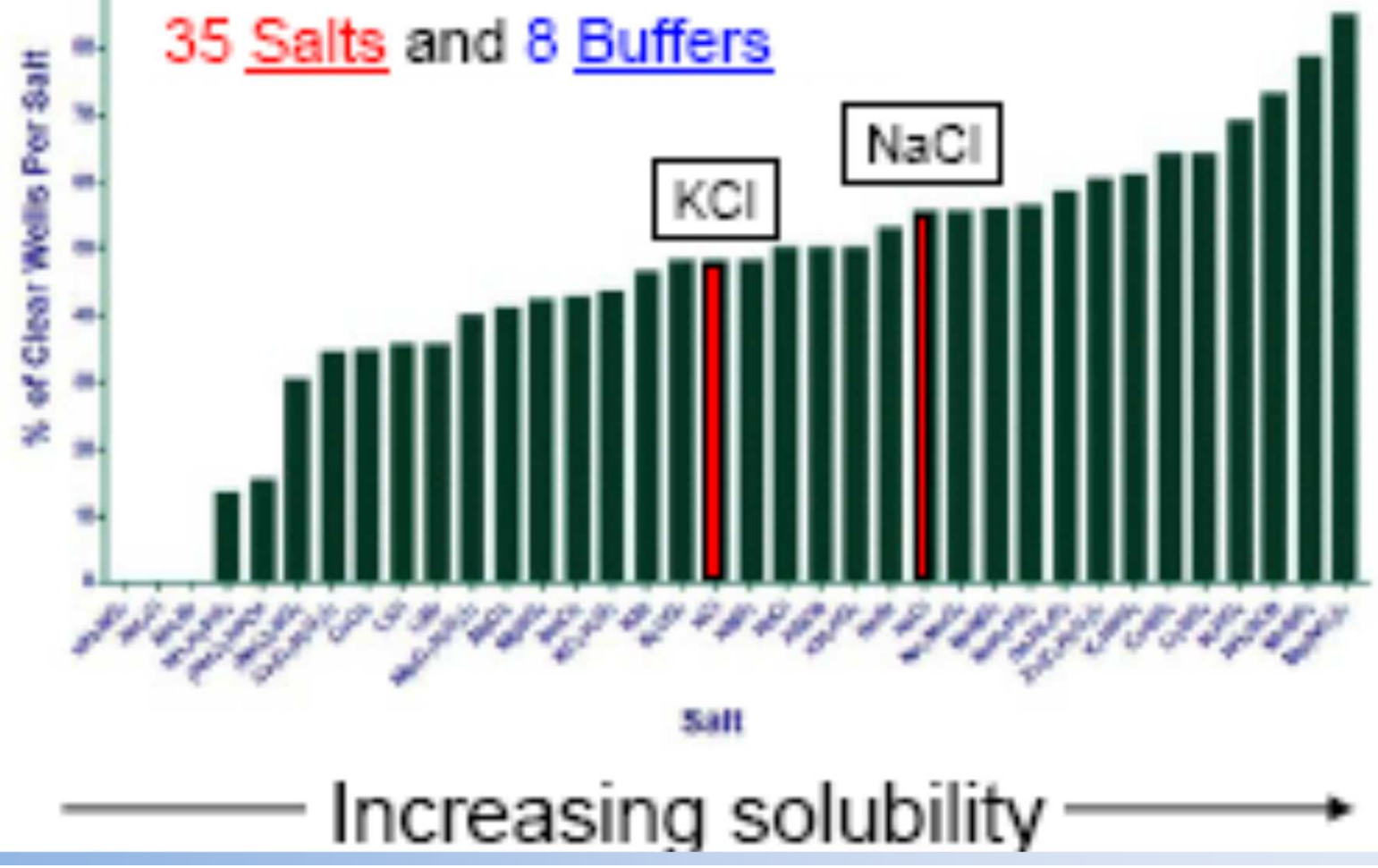
Data to calculate this for every protein

Statistically significant data

Incomplete factorial:

950 cocktails

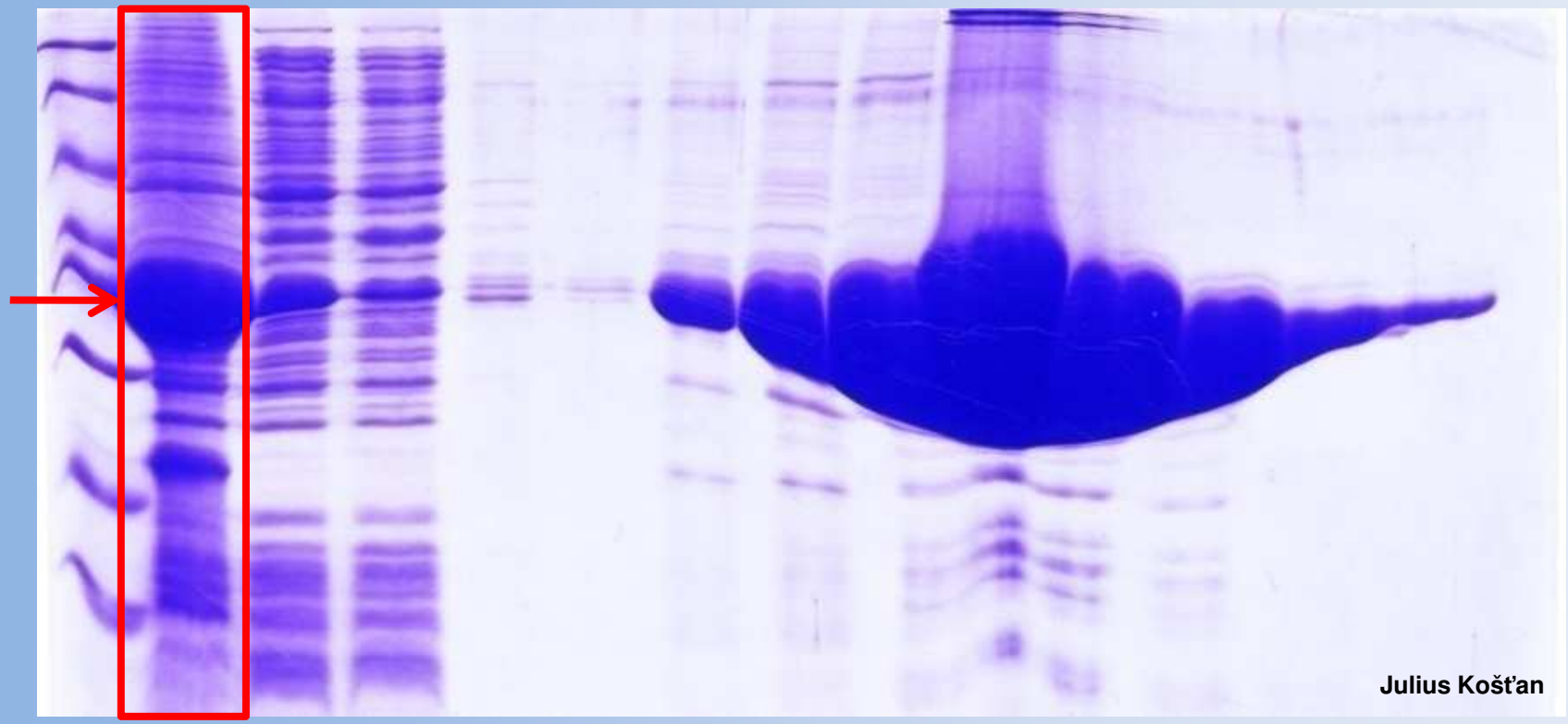
35 Salts and 8 Buffers



5.2. Approaches for efficient protein production

5.2.3. Expression system x ***medium engineering***

Expression and purification of plectin's ABD (actin binding domain) in three isoforms.

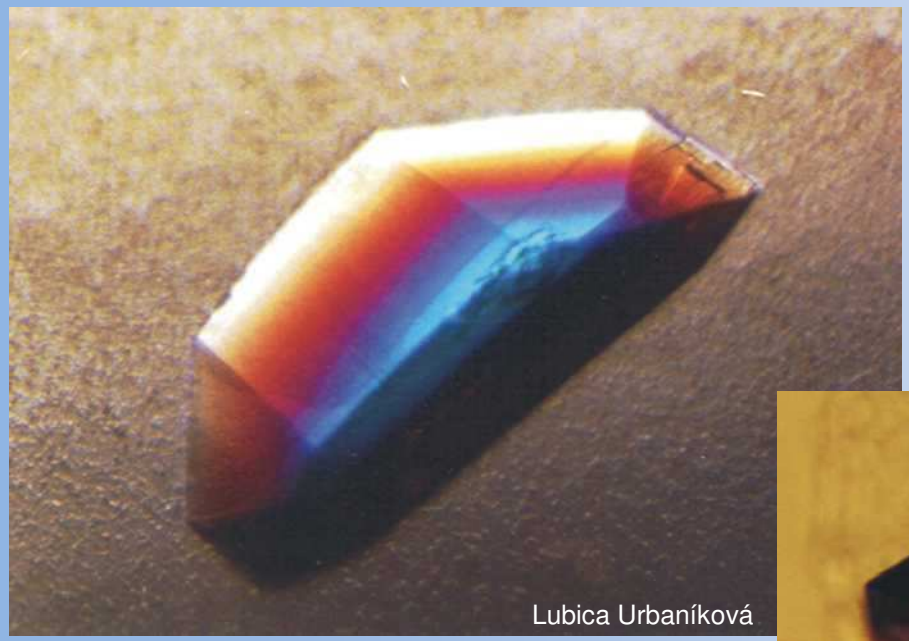


5.2. Approaches for efficient protein production

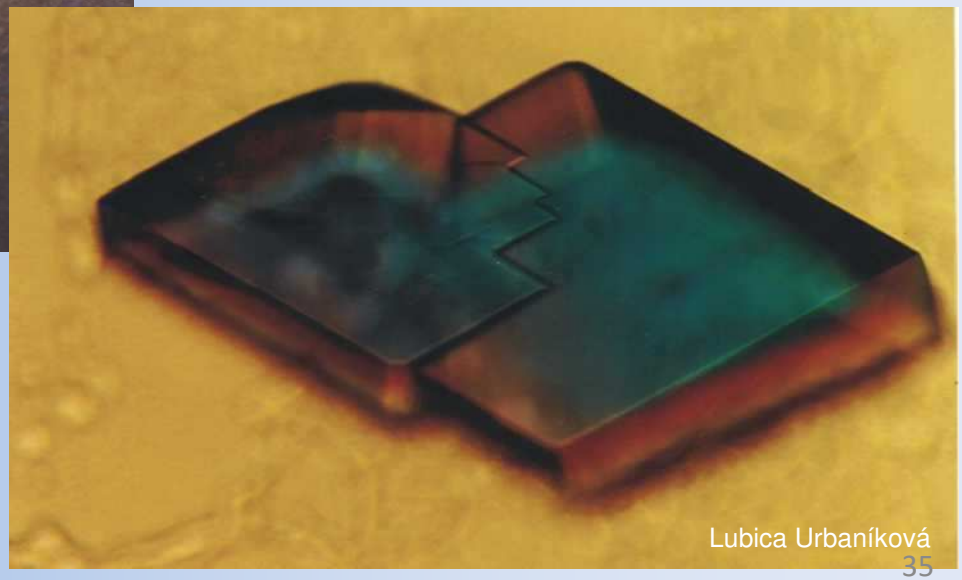
5.2.3. Expression system x ***medium engineering***

Monoclinic crystals of plectin ABD

Precipitant solution: 0.1 M TRIS buffer pH 8.5
10% PEG 4000



2% dioxane



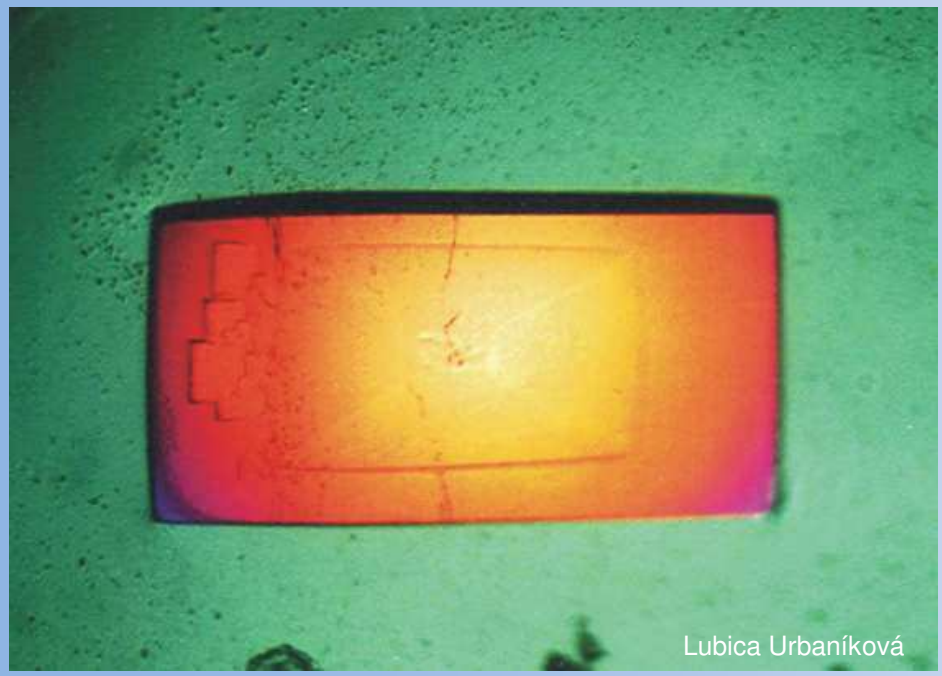
Space group $P2_1$
2 molecules in asym. unit

5.2. Approaches for efficient protein production

5.2.3. Expression system x ***medium engineering***

Orthorhombic crystals of plectin ABD

Precipitant solution:



0.1 M Cacodylate buffer pH 6.5
6–8% PEG 8000
0.2 M Ca acetate
2% dioxane

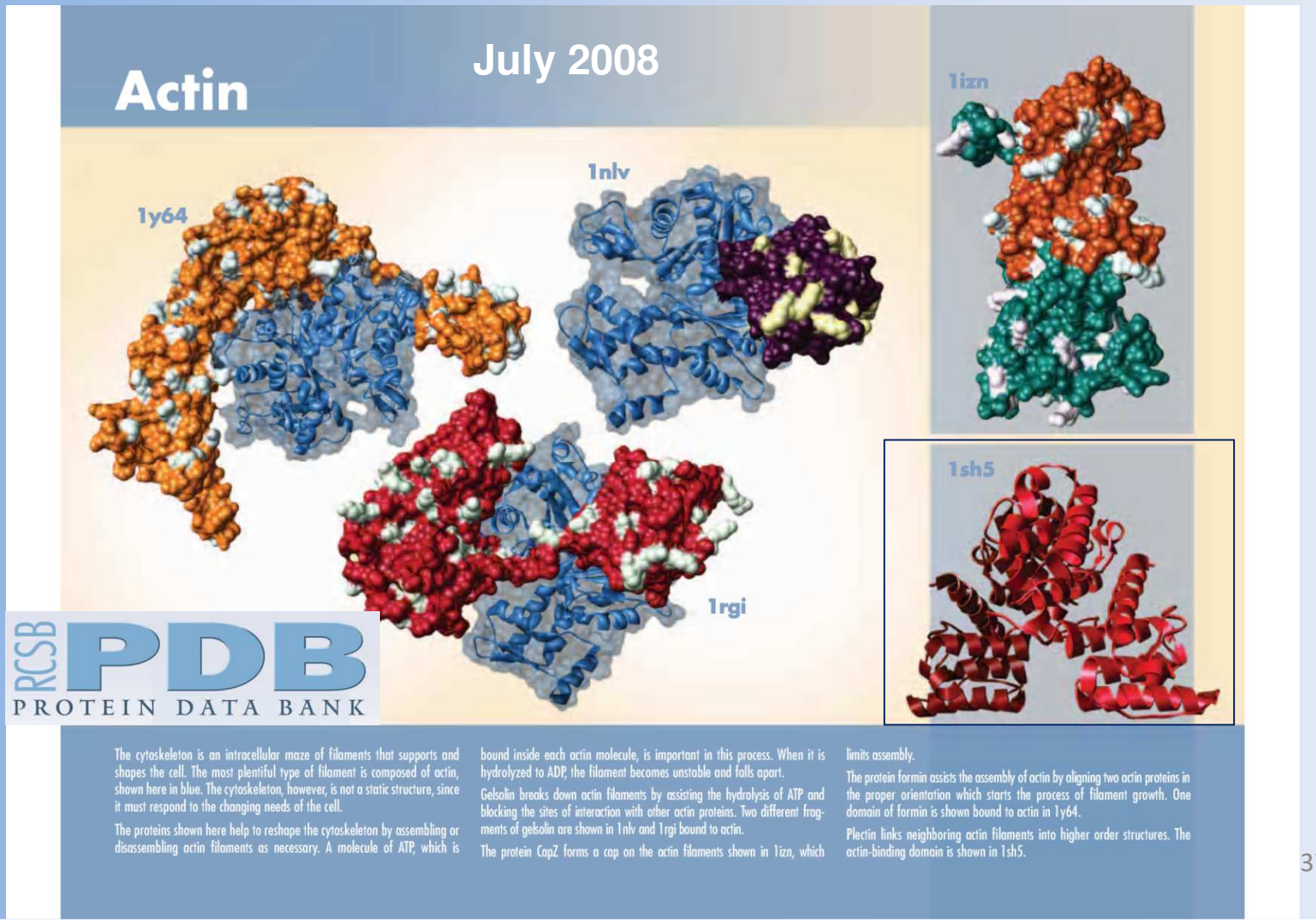
Space group $P2_12_12_1$
1 molecule in asym. unit
2.0 Å resolution

1nlv: S.M.Vorobiev, B. Strokopytov, D.G. Drubin, C. Frieden, S. Ono, J. Condeelis, P.A. Rubenstein, S.C. Almo. The structure of non-vertebrate actin: Implications for the ATP hydrolytic mechanism (2003). *Proc.Natl.Acad.Sci. USA* ***100:5760-5765.***

1rgi: L.D.Burtnick, D. Urosev, E. Irobi, K. Narayan, R.C. Robinson (2004). Structure of the N-terminal half of gelsolin bound to actin: roles in severing, apoptosis and FAF. *EMBO J.* **23:2713-2722.**

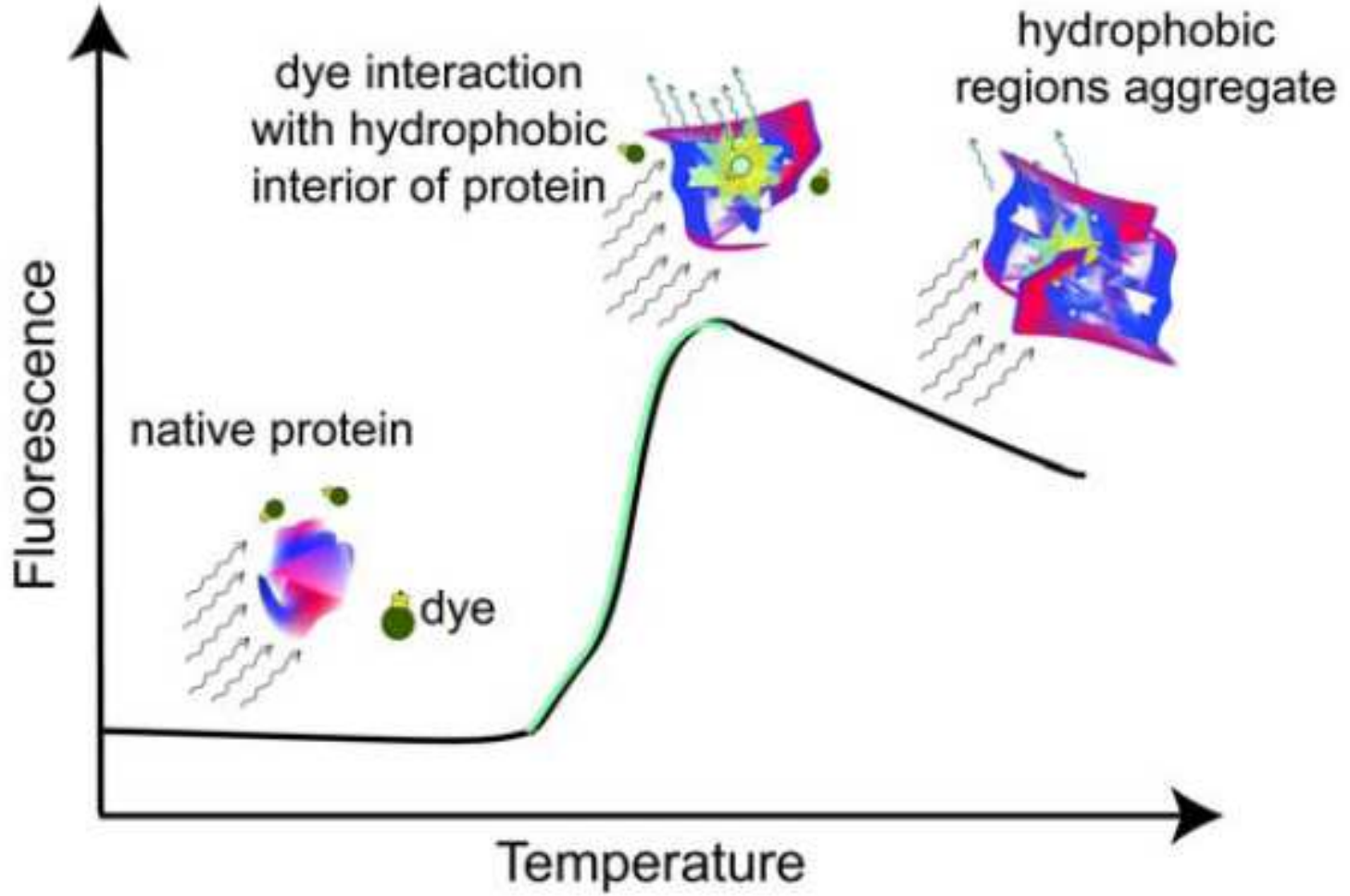
1izn: A.Yamashita, K. Maeda, Y. Maeda (2003). Crystal structure of CapZ: structural basis for actin filament barbed end capping. *EMBO J.* **22:1529-1538.**

1sh5: J. Sevcik, L. Urbanikova, J. Kostan, L. Janda, G. Wiche (2004). Actin-binding domain of mouse plectin: crystal structure and binding to vimentin. *Eur.J.Biochem.* ***271:873-*** **1884**.



ThermoFluor® assay

CD - spectroscopy



ThermoFluor® assay

- No data in the literature to support the prediction of crystallization conditions from T_m values
- Literature reports ThermoFluor® can identify ligands that stabilize macromolecules to improve crystallization outcomes

Ericsson U.B., *et al*. Thermofluor-based high-throughput stability optimization of proteins for structural studies. (2006) Analytical Biochemistry **357**(2) 289-298.

Vedadi M, *et al*. Chemical screening methods to identify ligands that promote protein stability, protein crystallization, and structure determination. (2006) *PNAS*, **103**(43):15835-40.

5.2. Approaches for efficient protein production

5.2.5. Maximizing target protein recovery

- **Protein expression**
  - The knowledge applied in constructing expression vectors;
  - Changes in cultivation conditions:
    - protein induction at different temperature
    - media pH
    - various additives;
- **Protein purification**
  - choice of buffer for disintegrating cells (all buffers should be degassed,
  - The availability of a test for protein activity as a supplement to the solubility test;
  - The use of expression vectors without affinity fusion proteins, which one should not be afraid to do.

5.3. Expression system

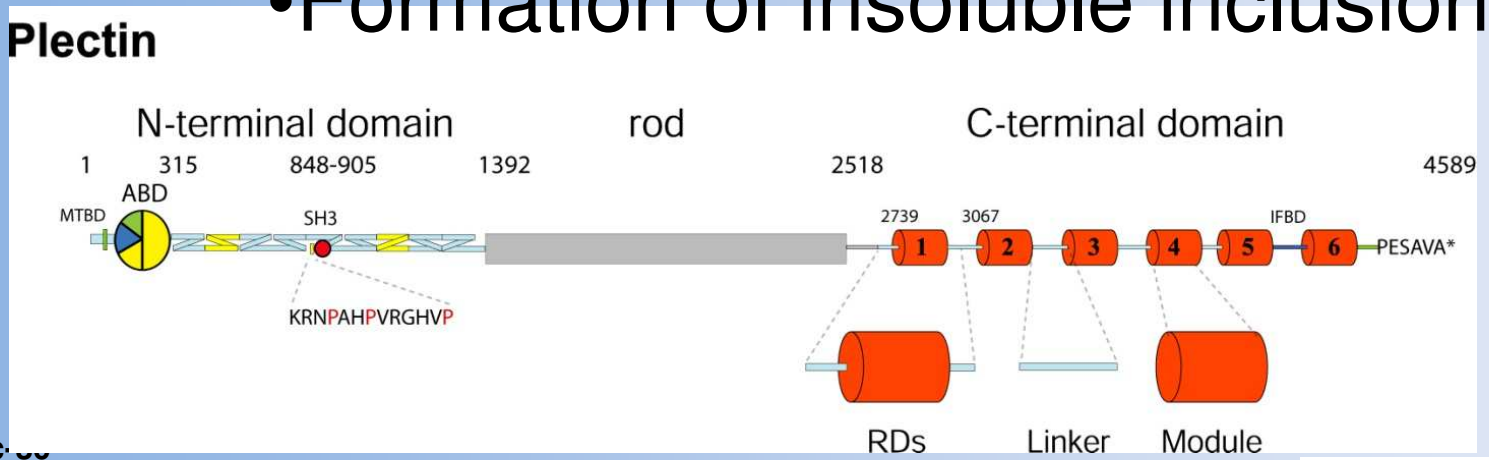
5.3.1. *E. coli* expression system

Advantages and Disadvantages of *E. coli*

- Ease of gene manipulation
- Availability of reagents
- Easy of producing quantities of protein
- Speed
- Low cost
- Adaptability of the system

- Formation of insoluble inclusion bodies
- Size of the protein
- Post-translational modification

- Size of the protein
- Formation of insoluble inclusion bodies

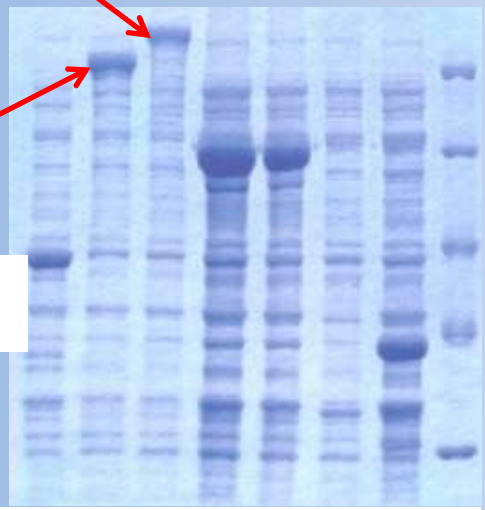


1c-
MEFHMSGEDS ... TLRRMEEEEF
:
pI/Mw: 6.37 / **160,602.22**

MW 160,602.22 Da

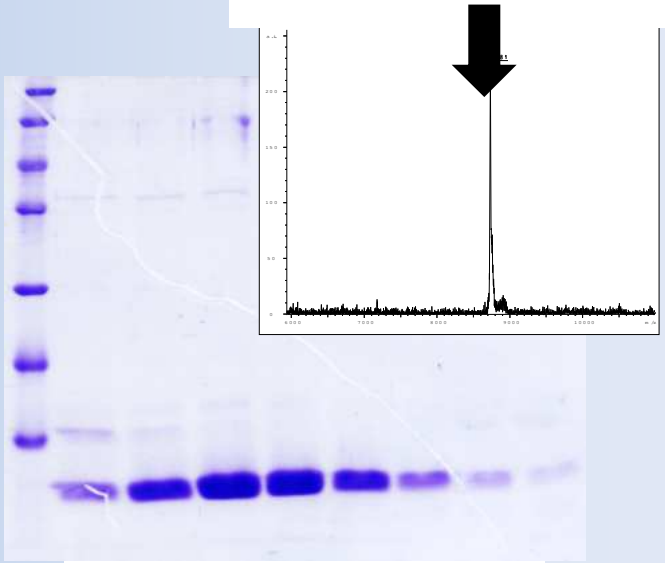
1c-24
MEFHMSGEDS ...
CISELKDIEF :
pI/Mw: 6.38 / **119,799.86**

MW 119,799.86 Da



N-terminal plakin domain of mouse

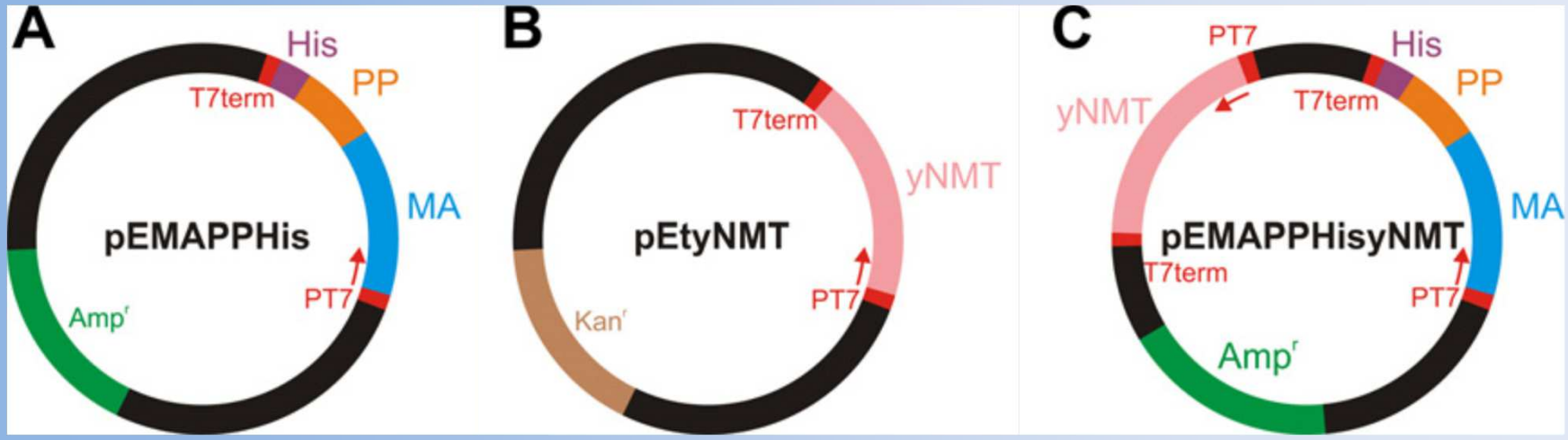
Mw: 8,732.3 Da



SH3 domain of mouse plectin

(A) The sequence encoding the matrix protein and downstream 18 amino acids of phosphoprotein (PP) was introduced to the pET22b vector (Novagen). The resulting vector pEMAPPHis was verified by restriction cleavage and by sequencing.

(B) The vector pETyNMT for the production of yeast N-myristoyltransferase was constructed by inserting the gene encoding the enzyme to the pET29b vector



(C) The plasmid carrying both MAPPHis and NMT genes constructed on the basis of the pET19b and pET11b vectors used in the single-plasmid system.

(Prchal et al., 2011)

5.3. Expression system

5.3.2. Baculovirus protein expression system

- HT-bacmid propagation
- HT-suspension-based insect cell transfection
- Methods of recombinant viral titer determination
  - GFP co-expression, titration assay using Alamarblue, Cedex cell counter
- HT-miniaturized deep-well block insect cell expression
- Transient insect cell expression

5.3. Expression system

5.3.2. Baculovirus protein expression system

Overview on commercially available baculovirus expression systems

Baculovirus expression kits and vendors	Compatible transfer vectors	Methodology for cloning foreign gene into transfer vector	Transfer of foreign gene into Baculovirus genome	Selection/ Recombination efficiency
BacPAK™ (Clontech)	Based on homologous recombination at polyhedrin locus	Ligase dependent	Homologous recombination in insect cells	≥90%
Bac-to-Bac™ (Invitrogen)	Based on site-specific transposition	Ligase-dependent, Gateway™ adapted	Site-specific transposition in bacterial cells	Selection of recombinants by blue-white selection on agar plates
BaculoDirect™ (Invitrogen)	Based on site-specific recombination	Gateway™ adapted	Site-specific recombination in Eppendorf tube	Antibiotic selection of transfectants in insect cells
flashBac™ (OET/ NextGen Sciences)	Based on homologous recombination at polyhedrin locus	Ligase dependent	Homologous recombination in insect cells	100%
BacVector™ 1000, 2000, 3000 (EMD/ Novagen)	Based on homologous recombination at polyhedrin locus	Ligase dependent	Homologous recombination in insect cells	≥ 95%
BaculoGold™ (Cloentech)	Based on homologous recombination at polyhedrin locus	Ligase dependent	Homologous recombination in insect cells	≥ 95%
DiamondBac™ (Sigma-Aldrich)	Based on homologous recombination at polyhedrin locus	Ligase dependent	Homologous recombination in insect cells	≥ 95%

5.3. Expression system

5.3.3. Cell-free protein expression system

Simple open system which influences:

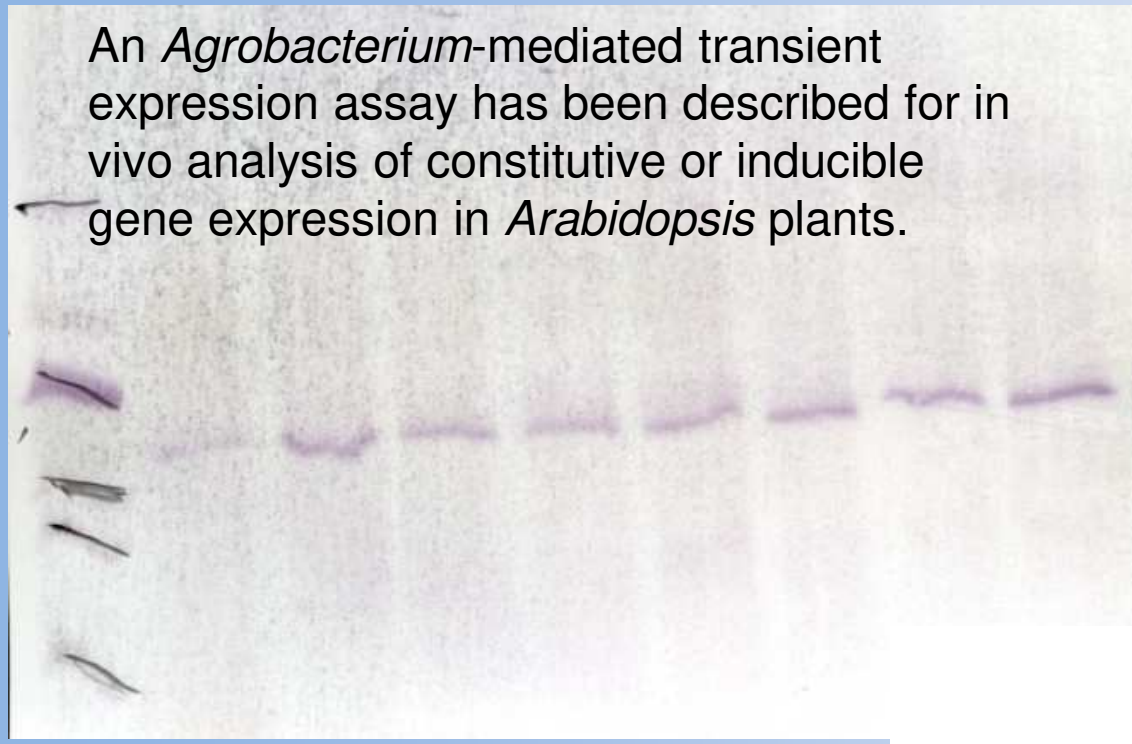
- Protein folding
- Disulfide bond formation
- Incorporation of unnatural amino acids
- Protein stability
- Expression of toxic proteins

Use the machinery of *E. coli* S30

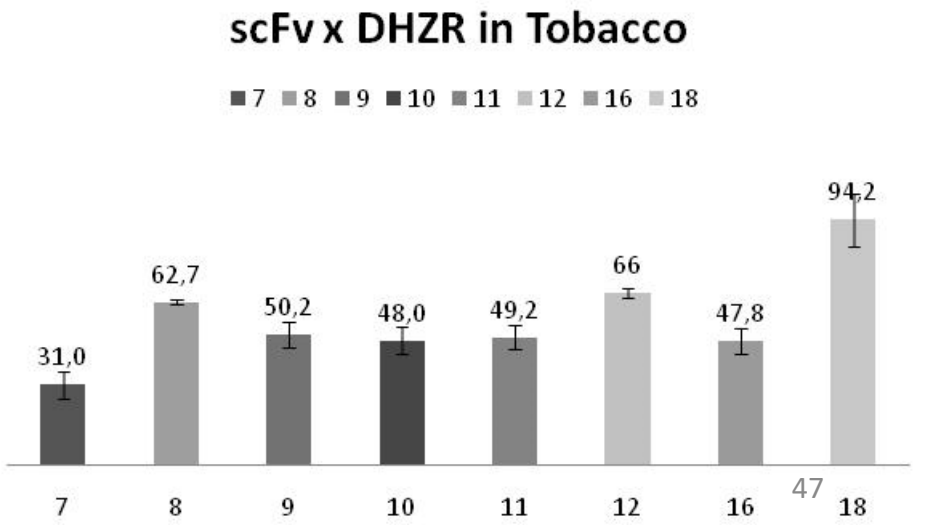
5.3. Expression system

5.3.4. Transient protein expression in tobacco leaves

An *Agrobacterium*-mediated transient expression assay has been described for in vivo analysis of constitutive or inducible gene expression in *Arabidopsis* plants.



- Plant number: ca 30
- Weight of tobacco leaves: 7–10 g
- Number of tobacco leaves: 12–15
- Total: ca 3.5 kg~12–15 g protein~120–150 mg scFv



Before expression

- Host strain (E. coli, Yeast, mammalian cell, plant)
- Expression vector (plasmid)
- Gene construct:
  - domain and secondary structure prediction
  - codon usage
  - stability
  - toxicity
- Buffer options for disintegration