Protein Expression for structure determination by NMR
Protein expression group

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Protein Expression group

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94793 310
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CV

Protein expression group

- Leipzig University (1980 - 1985) certificate as Biochemist
- scientific coworker at Research Center of Biotechnology Berlin (1985 - 1993)
  - Purification and characterisation of extracellular hydrolases
- PHD at Technical University Berlin (1993 - 1998)
  - Quinoprotein Ethanol Dehydrogenase of *P. aeruginosa*
- Postdoc at Max - Delbrück-Center Berlin (1998 - 2000)
  - Complex of IF2 and initiator t-RNA
- Postdoc at FMP since 2000
  - Protein expression and purification for NMR
  - HTP techniques
Our Requirements for NMR

- Proteins, as targets for NMR must have a known primary structure.
- Soluble well folded expression in *E. coli*
- NMR structure is calculated from magnetic properties of several nuclei, therefore
- Labelling of proteins with stable isotopes 15N 13C 2H
- Result: a set of probably 3D structures

Structure of SH3 1AEY solution NMR
Structural genomics projects - all over the world

in Germany
Die Proteinstrukturfabrik
PSF
(http://www.proteinstrukturfabrik.de/)

we are a part of the PSF
- Genomes are sequenced
- thousands of targets are available
- many genes with unknown function and requirements
- expression of stable, correct folded hypothetical proteins is a challenge
- the gene products are unified by one or more tags
- affinity purification
structural genomics will turn protein structure discovery into a factory production-line process
Large Scale Protein Production for NMR

- Cultivation 2 x 500ml M9 with labeled C and/or N-Source / 2L flask
- French press
- Centrifugation and sterile filtration
- Chromatographie at Workstation Vision (PE Biosystems)
  - Tandem Column Configuration
    - MC Poros 20 (1.7 ml) / StrepTactin-Sepharose (10 ml)
- Analysis (SDS-Page)

Duration: 2 days
Workstation Vision
Protein expression group

Purification of His-SH3-StrepII

in less than 1 hour

MW in kDa

15% SDS Gel

applied crude extract

flow through fractions

fractions with protein of interest

His-SH3-StrepII

MV in kDa

25.02.03
First success using this system

Müller et al. Rapid Structure Determination of a Protein Carrying Two Small Terminal Affinity Tags: Potential for Automated Protein Structure Analysis in press

Lajos Nyasik

Uwe Müller

Taged SH3-Domain of Spectrin
Protein expression group

His-SH3-Strep

NMR/Ricardo Pires

X-ray Uwe Müller
High Throughput Approaches

- **Cloning / Expression**
  - TOPO Cloning
  - multiple choice vector / host systems

- **Protein production**
  (96 well)
  - growth / cell lysis
  - affinity purification
Protein expression group

HTP Solubility screening
Protein expression group

Solubility screening

- Cloning into expression vectors
- Transformation into expression host
- Use of the transformation setup for inoculation of a preculture for an expression test
- No single colony check at that point
Inoculation of the 5ml Expression Culture

From an over night culture 96 well plate to 4 x 24 well plates for expression

Matrix
Impact2 pipette
Inoculation of the 5ml Expression Culture

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Impact2 pipette
Search for soluble Protein-Expression by Automated Expression-Clone Screening

Ni-NTA Superflow 96 BioRobot Kit

induced

Overnight cultures

Harvest

Resuspend

Lyse Transfer Overlay with ethanol

Clear lysates

Vacuum

TurboFilter

QIAfilter – Ni-NTA Superflow

Bind

Channeling Block

Vacuum

Wash

Channeling Block

Vacuum

Elute

High-purity 6xHis-tagged proteins

96 protein minipreps — 150 min
Protein expression group

BioRobot 9600 used for plasmid preps and protein purification
BioRobot 9600

Protein expression group
Protein expression group

BioRobot 9600

Eluation of the target plasmids or proteins into an UV suitable Microplate reader TECAN
Protein expression group

Microplate Reader

all types of plates
Growth etc.
Protein estimation
DNA estimation

FMP

25.02.03
Output of the microplate reader
XXL-Gel System (CBS)

amount of samples  102 per gel / two gels per run
multi-channel sample application
time 4-5 h
Protein expression group

<10% soluble expressed

40% expressed

Whole cell extracts after NiNTA

XXL-Gels
Protein production
Label introduction

- Expression host *E. coli*
- plasmid coded recombinant proteins
- shaking cultures
- IPTG induction
- M9 minimal medium
  - defined N and C source, replaceable by 15N 13C
  - labeled amino acids
Protein expression group

Methods to introduce selective labels
Methods to introduce selective labels

- *in vitro* translation with a mixture of labeled and unlabeled aa
- Expression host grows in minimal medium with all aa labeled or unlabeled as wanted
  - repression and inhibition of aa metabolism
Protein expression group

15N HSQC SH3

sample purified

SH3 with 15N glycine
INTERCONVERSION OF SERINE AND GLYCINE. Serine hydroxymethyltransferase

15N HSQC SH3
M9 15N NH4Cl
sample purified

15N Gly HSQC SH3
M9 AA 40-160 mg/l
Ala 13C 200 mg/l
Gly 13C2 15N 200 mg/l
sample purified
Fast selective labelled NMR sample production

- inoculation of 5 ml LB,
- before induction - exchange of the medium (M9 with labelled and unlabeled AA)
- collection of the cells
- lysis of the cells
- buffer exchange of the supernatant
- HSQC without prior protein purification
15N HSQC SH3

15N Gly HSQC SH3
M9 AA 40-160 mg/l
Ala 13C 200 mg/l
Gly 13C2 15N 200 mg/l

5 ml scale prior induction
than M9

minimized scrambling
compared with the big prep
Sample Preparation for solid state NMR method development
Sample Preparation for solid state NMR method development

application fields:

- structure determination
  - of membrane proteins
  - proteins, which give only microcrystals

precondition

- ordered state of the proteins in the preparation
Sample Preparation for solid state NMR method development

- Uniform 15N 13C labelled sample
- for reduction of signals
  - two further samples were made
  - opposite label pattern

carbon-source:

[(1,3-^{13}C) glycerol]

[(2-^{13}C) glycerol]
The carbons are labelled only in particular positions

Usually directly bonded $^{13}$C nuclei are not labelled

The reduced labelling allows detection of long-range correlations
SH3- Microcrystals for solid state NMR

First approach: microcrystals with precipitate

Improved conditions: microcrystals without precipitate
SH3- structure by ss-NMR

- **Method:** NMR, 12 Structures

Summary

- Expertise in protein production and purification
  - solution and solid state NMR
  - established protocols for introducing different labels into proteins
  - started to use HT-methods

- flexible react on new demands from the NMR method developers