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Introduction

Isotopic labelling of proteins, expressed in *E. coli* is a routine method in NMR supported structural genomics. Most widespread is the shaking culture on minimal medium M9 including required isotopes like ¹³C, ¹⁵N and/or ²H.

Beside manpower the costs for isotopes are the most expensive factor of that process. The literature given left hand side comprise the effort to reduce the amount of isotopes for protein labelling.

Ross et al. have introduced a fermentation strategy: biomass is generated in an unlabelled batch phase (3 l defined medium) and protein induction starts in a carbon-limited fed batch process with ¹³C-glucose.

According to the needs of structural genomics DASGIP developed a micro fermentation system (DASGIP's fedbatch-pro[®]) to cultivate up to 16 variants in parallel with that cost effective strategy.

The base unit consists of four 200 ml fermentation vessels (160 ml medium) with a sophisticated control unit allowing a high degree of automatization (see **screenshots** and **fermentation data**).

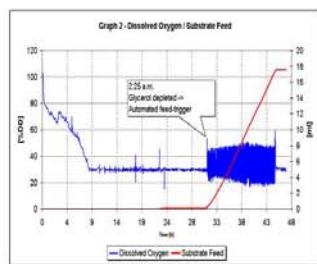
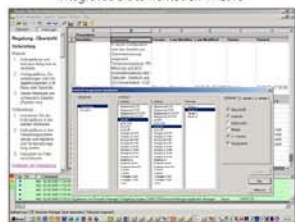
We have tested that novel high cell density fermentation system for growth and protein production of *E. coli* BL21. In a proof of principle experiment we have expressed a 14 kDa human protein domain under control of a T5 promoter in four isotopic variants (unlabelled, ¹⁵N, ¹⁵N/¹³C, ¹⁵N/²H).

The main culture starts with glycerol as sole carbon source. After depletion of glycerol (see **graph 2**, DO peak) the labelled feed with ¹³C-glucose and ¹⁵N-(NH₄)₂SO₄ is triggered followed by induction.

Intuitive Windows Software

User Definable Triggers

Integrated Documentation Wizard



Results

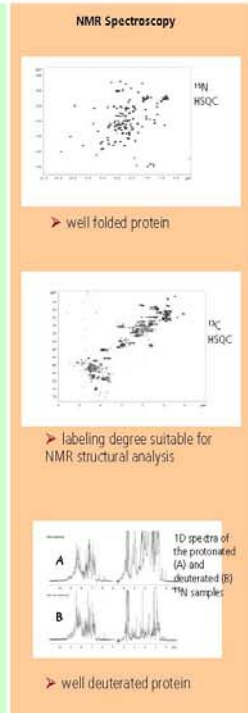
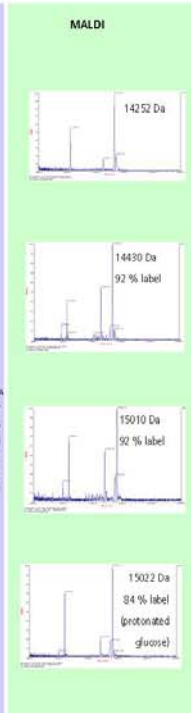
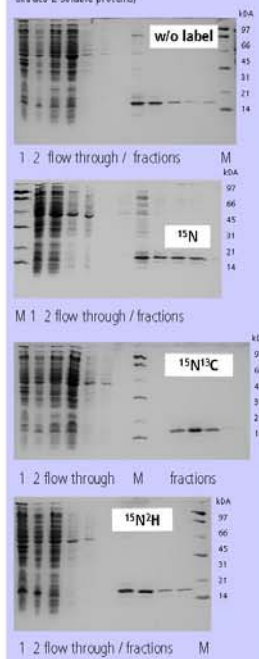
Recombinant protein from the biomass of the 4 set ups was purified via His-tag (see **SDS-PAGEs**). Resulting protein preparations were analyzed by **Maldi** and **NMR-spectroscopy**. Since the process was not optimized the duration of expression for the nondeuterated set ups was unfortunately too long to get the highest yields (**Table 1**, vessel 1-3). But in the case of the ¹⁵NH sample 80 mg of protein were purified from a 160 ml high cell density culture by 8 h of induction (**Table 1**, vessel 4).

The deuterated set up is the most critical one, therefore we expect at least 80 mg protein after protocol optimization for all labelling pattern. **Table 2** compares the isotope costs for shaking culture versus fed batch pro[®] system for experimental yields and extrapolated ones.

vessel	isotopes	amounts of isotopes	duration of batch phase in [h] / end OD 600nm	duration of expression in [h] / end OD 600nm	yield in mg
1	w/o		22 / 9	25 / 22	46
2	¹⁵ N	1,7 g	20 / 11	21 / 28	36
3	¹⁵ N / ¹³ C	1,7 g / 6 g	18 / 10	20 / 19	20
4	¹⁵ N / ² H	1,7 g / 200 ml	32 / 10	8 / 28	80

isotopes	conventional shaking culture			fed batch-pro [®]	
	price in € for isotopes per l M9	yield in mg / l	€ / mg protein	price in € for isotopes per set up	€ / mg protein (related to yield of 80 mg)
w/o					
¹⁵ N	11	25	0,5	38	1 (0,5)
¹⁵ N / ¹³ C	191	16	12	580	30 (7)
¹⁵ N / ² H	271	20	14	90	1

SDS-PAGE Analyses of Expression and Metal Chelate Purification (1 whole cell extract / 2 soluble proteins)



Summary / Outlook

The presented novel micro fermentation system proved to be suitable for the use in structural genomics for following reasons:

- even though the process hasn't been optimized with respect to induction point and duration we achieved **promising results** for labeling and yield;
- dissolved oxygen (DO) based feeding approves the **quantitative utilization** of the most expensive isotope ¹³C-glucose;
- moderate costs for ¹⁵N and ¹³C isotopes; **extremely low costs** (1/10) in case of deuteration (increasing importance for NMR-targets larger than 20 kDa);
- ideal for **medium or low expressed** proteins and for expression under **weak and tight promotor** control;
- **16 vessels** provide a broad spectrum of possibilities for
 - ✓ fast and cheap optimization of expression
 - ✓ parallel cultivation of different clones
 - ✓ parallel incorporation of different labeling patterns

Acknowledgement

We thank Kristina Rehbein and Martina Leidert (FMP) for technical assistance.

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