Jena Bioscience

HEC13
HEART OF EUROPE
BIO-CRYSTALLOGRAPHY MEETING

From gene to crystallization within two days

LEXSY cell-free protein production

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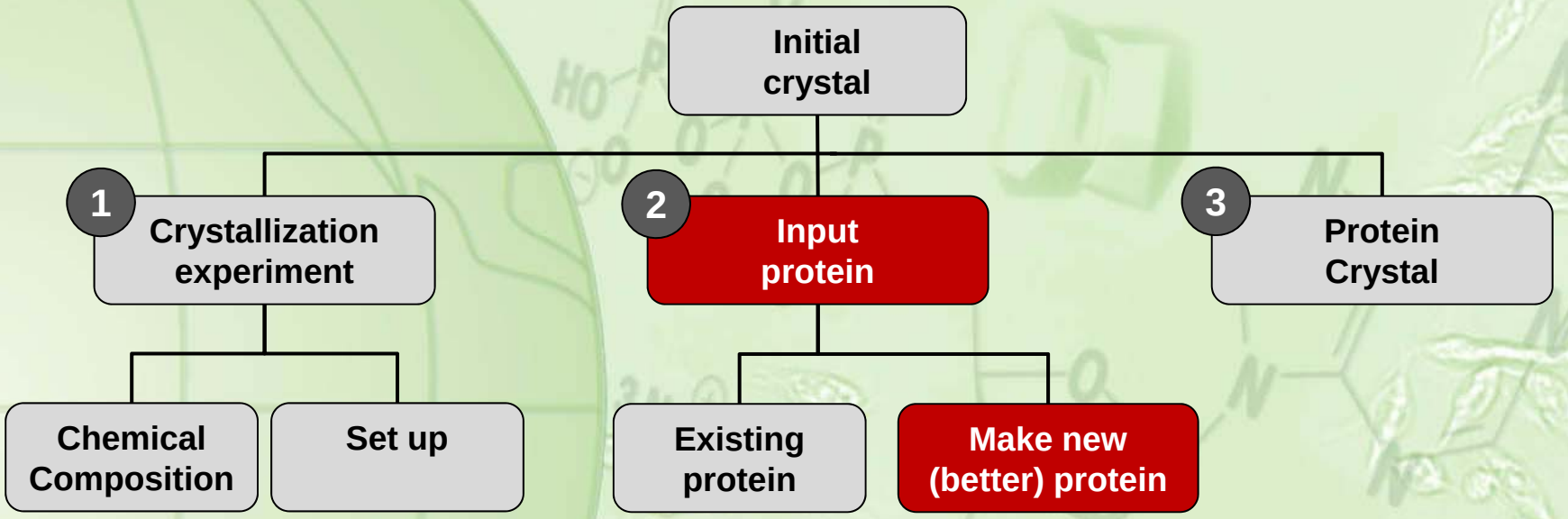
There are more options for optimising hits than adjusting pH

Protein sample equally important: No good protein ➔ no good crystal



- Buffer (pH)
- Precipitant(s)
- Additives
- Method of crystallization
- Temperature
- conc., purity, buffer
- Surface (Lys-methylation, …)
- Sequence (truncation)
- Sequence (mutagenesis)
- **Mode of production**
- Solvent content
- Re-annealing

E. coli **is major source for recombinant proteins for crystallization...**
...Why should one change anything there...?!?

Two major reasons why *E. coli* proteins often do not crystallize

E. coli in general not a good host for expression of „interesting“ proteins

Typical „symptoms“ with *E. coli* derived proteins		Underlying shortcoming of *E. coli*
• Insoluble / Inclusion bodies • Aggregate-forming ➔ precipitates over time and compromised biological activity • Low solubility, e.g. cannot be concentrated (< 10 mg/ml) • Unstable/fast degradation	1	Incorrect folding due to lack of chaperone folding system ➔ Applies to almost all proteins(1) of higher organisms expressed in *E. coli*
• Low expression yields (< 1 mg/L) • Unstable/fast degradation • Compromised biological activity and/or ligand binding • Low solubility	2	Incorrect processing due to lack of post-translational modification(2) machinery ➔ Applies to almost all proteins(1) of higher organisms expressed in *E. coli*

What should one do when low quality protein is obtained from *E. coli*...?

(1) >50% of human proteins are glycosylated, 10^6 protein variants present in humans (Walsh & Jefferis, 2006, Nat. Biotech. 24: 1241

(2) PTMs: glycosylation(1), disulfide bond formation, phosphorylation, acetylation, sulfation, amidation, prenylation, …)

Changing mode of protein production – a difficult trade-off

But often the better choice than „riding a dead horse[1]“

Being faithful to *E. coli*

Plenty of opportunities to optimize crystallization experiment, i.e. riding the dead horse[1]

Changing mode of production

Starting again from scratch

- **(Sub)cloning gene of interest**
- **Creation of expression strain**
- **Preparative cultivation/expression**
- **Protein harvest & purification**

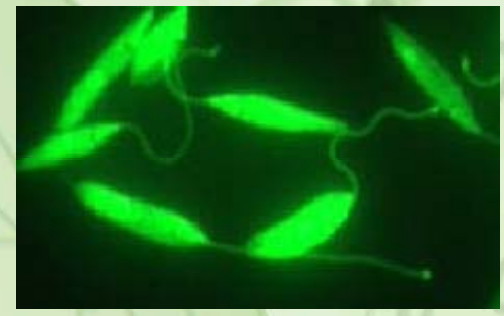
… or something completely different…

Changing mode of production may sometimes be the wiser strategy…

…and not as cumbersome as thought

(1) Dakota tribal wisdom, see http://www.tysknews.com/LiteStuff/riding_a_dead_horse.htm

May I introduce LEXSY: A Combination of robustness and eukaryotic features



Easy construction and rapid growth of LEXSY expression strains

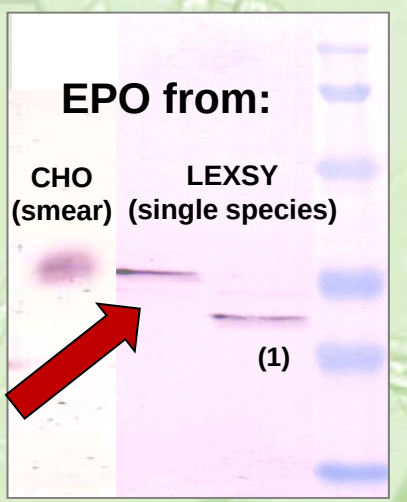
- Biosafety level 1 (as *E. coli)*
- Plasmid generation in *E. coli* shuttle vectors
- Cultivation in inexpensive media at 26°C
- Expression yields of up to 300 mg/l of culture

Eukaryotic protein folding and modification machinery

- Chaperones for folding (no inclusion bodies)
- Full range of PTMs (including exceptionally homogenous mammalian-type N-glycosylation)

Constitutive or inducible, intracellular or secretory versions

- Coexpression of up to 4 target genes



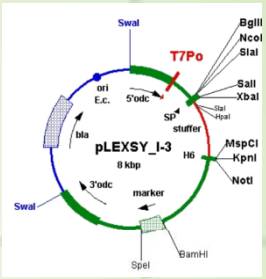
(1) Deglycosylated

Does it work for X-ray crystallography...?

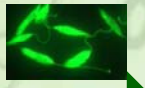
LEXSY-based human Cu/Zn SOD1 was crystallized and its structure solved[1]

4-6 weeks

1 Sub-cloning into LEXSY vector



2 Transfection & clonal selection (by color)



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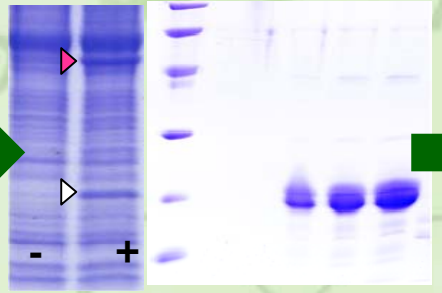
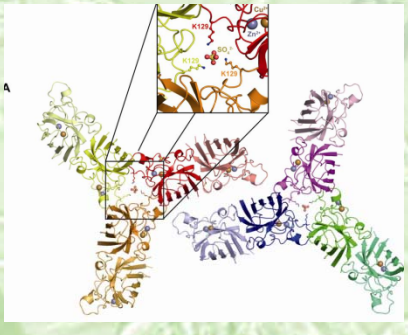
3 Expression and purification



4 Crystallization

5 High resolution structure[1]

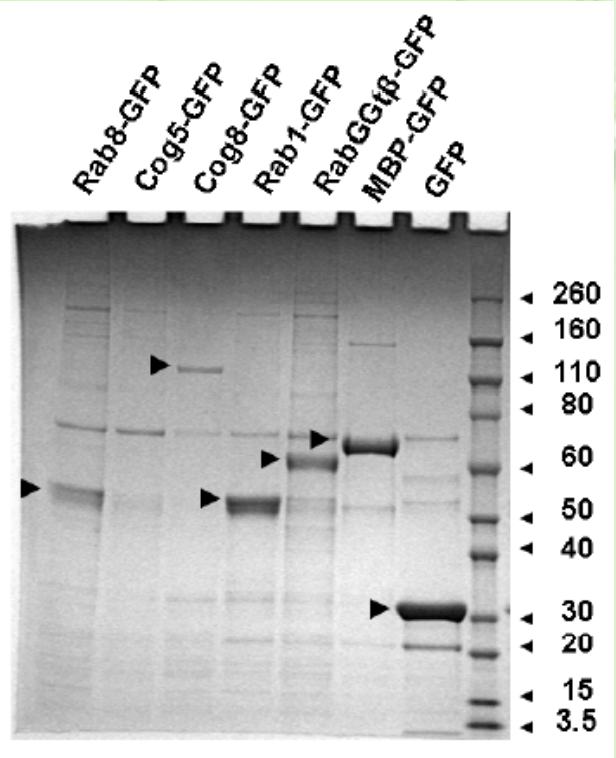


4-6 weeks is pretty good, but isn't there anything shorter...?

[1] Gazdag *et al.* (2010) Purification and crystallization of human Cu/Zn superoxide dismutase recombinantly produced in the protozoan Leishmania tarentolae. *Acta Cryst. F*66:871.

Yes there is: LEXSY *in vitro* expression kit now available

- **Based on LEXSY transcription-translation-competent cell extracts**
- **No cell culturing necessary**
- **Yields of 0.3 mg/ml of extract within 2 hours after initiation of *in vitro* expression**



Mureev *et al.* (2009) Species-independent translational leaders facilitate cell-free expression. *Nature Biotechnology* 27: 747.

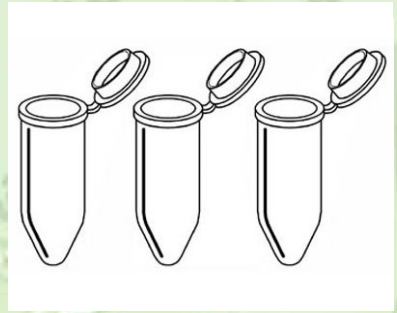
In vitro LEXSY protein synthesis involves three steps

Approximately 2 days

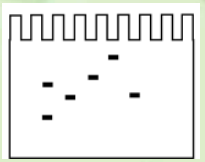
1 **Preparation of template DNA**

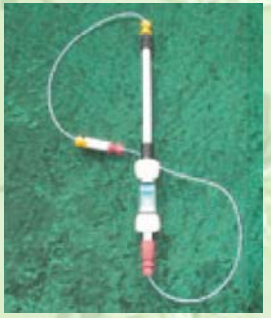
- **Subcloning into pLEXSY**
- **Crude PCR product** [1]

2 **Inoculation of cell extract with template DNA and *in vitro* translation**



3 **Detection & purification of recombinant protein**





????
Not yet shown

4 **Crystallization**

5 **High resolution structure**

Send a pre-print of an accepted paper to xtals@jenabioscience.com and receive the JBStructure Award worth EUR 2.500 in consumables from Jena Bioscience for the first published structure of an *in vitro* LEXSY derived protein!

(1) Under development, not yet commercially available

Summary: Two straightforward choices for skipping *E. coli*

In vivo expression

Programmable LEXSY (S1) cells (here: eGFP)

Yields up to 300 mg/L of culture

Proven for structure determination (SOD1, eGFP)

Advantages:

- **Eliminates all shortcomings of *E. coli***
- **Easy to handle**
- **No cell culture equipment necessary**

Disadvantage:

- **Requires going back to wet lab (cloning ➔ transfection & cultivation ➔ protein purification, 4-6 weeks total)**

In vitro expression (cell-free)

Programmable cell-extracts (*in vitro*)

Yields up to 0.3 mg/ml of cell extract

Not yet proven for structure determination

Advantages:

- **Eliminates all shortcomings of *E. coli***
- **Easy to handle**
- **Very fast results**

Disadvantage:

- **Lower yields (0.3 mg/ml of cell extract) and more expensive consumables**

➔ But 0.1 mg of protein allows crystal set ups in roughly 60 wells(1)

(1) Assuming a protein solution of 10 mg/ml and 150 nl per well

Acknowledgements

Kirill Alexandrov & group

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Reinhard Breitling

Astrid Rau

Thomas Billert

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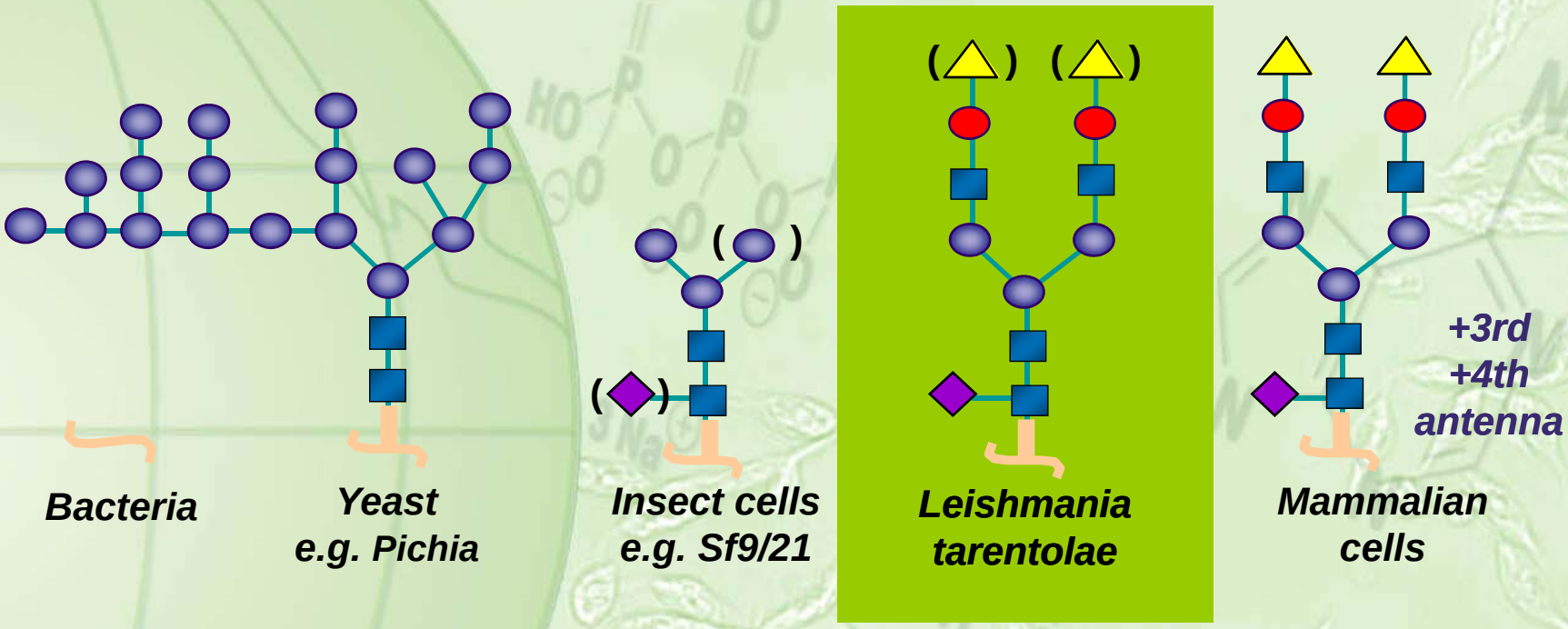
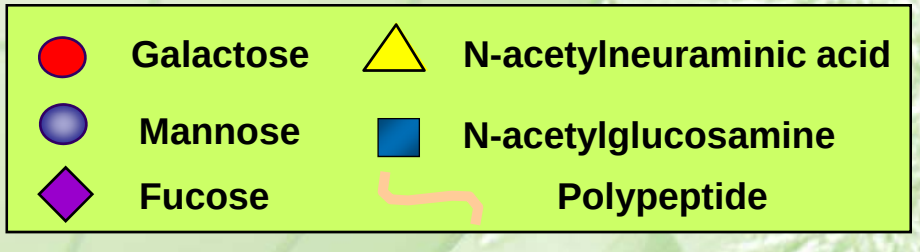
LEXSY N-Glycosylation pattern are of mammalian type





„JBStructure Award“: EUR 2.500 in consumables for first published structure of *in vitro* LEXSY protein

***In vitro* expression (cell-free)**

Programmable cell-extracts (*in vitro*)

Yields up to 0.3 mg/ml of cell extract

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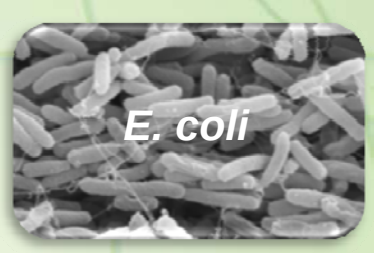
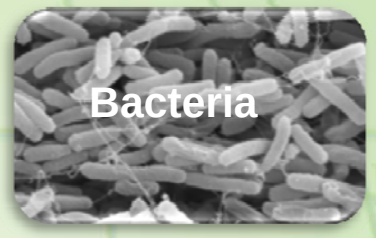
Again – commercially available cell-free systems have shortcomings



- Lack of chaperone based mechanisms
- Absence of post-translational modifications

- Difficult scale up
- Laborious and expensive preparation
- Difficult to manipulate genetically

Shortcomings of conventional expression systems require an alternative expression system



- Insufficient folding of complex proteins of higher organisms
- Lack of post-translational modifications
- Endotoxins

- Posttranslational modifications differ largely from mammalian cells (high mannose)
- Problematic cell disruption

- Laborious construction of over-expressing strains
- Expensive media
- Low growth rates
- Difficult scale-up

- Long developmental cycles
- Complex downstream processing
- Contamination problems

Current expression systems are not ready for proteomics era
Need eukaryotic machinery but prokaryotic robustness