LEXSY
Eukaryotic Protein Expression with Leishmania tarentolae
- Overview -
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
Leishmania – a closer relative than one may think
Full eukaryotic protein folding and modification machinery
LEXSY: Combination of robustness and eukaryotic features

- Easy construction and rapid growth of LEXSY expression strains
  - expression construct generation in *E. coli* shuttle vectors
  - selection and cultivation in inexpensive media at 26°C
  - high cell densities in suspension cultures > 10^8 cells/ml
  - extremely stable over hundreds of generations
  - animal-free complex media or fully synthetic media available
  - compatible with common fermentation technology (biosafety level 1)

- Constitutive or inducible, intracellular or secretory versions
  - coexpression of up to four target genes
  - simple purification from supernatants or easy to lyse cells

- Eukaryotic protein synthesis and modification machinery
  - exceptionally homogenous mammalian-type N-glycosylation

- *In vivo* protein labeling for structural studies (*15N-HSQC NMR*)
  - auxotrophy for 11 amino acids
  - complete assignment of 18 *15N* Val demonstrated with 27 kDa protein
In six weeks from gene to protein

Gene of interest

Expression plasmid construction
Easy cloning in E. coli
Versatile LEXSY expression vectors

Transfection & selection
Reliable electroporation protocols
Clonal or polyclonal selection

Expression evaluation
Constitutive or inducible
Intracellular or secretory

Scale-up
Fully adapted to common fermentation technology
Up to 100 litres tested

Protein purification
One-step affinity purification
Conventional techniques

Soluble active POI

1 week
2 weeks
1 week
1 week
1 week
High success rate of LEXSY protein expression

76% of proteins tested, expressed at > 0.1 mg/L of culture. Yields > 300 mg/L of culture obtained in laboratory cultivation.

50 proteins in total (5 membrane proteins, 13 secretory proteins, 32 cytosolic proteins), in alphabetical order: Amylase hu (SPepo); BkrB2-GST (Tm7 protein); Bradykinin receptor B2 hu (SPepo); CFP-Tev-YFP fusion protein - processed; CHK2-GST; CNAk; CNB; Cu/Zn SOD human; Cyt28 ExSegment human (native SP); EGFP-N1; EPO human (native SP, SPImsap); Epocl-GST; Epocl-GST; GST yeast; GST-Myc human; GST-Rab7 human; hCRP; IFNy human (native SP); Intein-Myc human; LBH9 (native SP); Luciferase (SPImsap); MAK-HC (SPImsap); MAK-LC (SPImsap); Max-2 human; MDP1hu (SPImsap); Miz-1 human; MnSOD human; MPP1 human; Myc-Intein; NNMT hu; p85alpha; PC4; PC4 truncated; PDM9 (Typ1 mbp, N out) PFTa; PFTb; proBNP hu (SPImsap); PROC human; Rab7 human; recBSA (SPImsap); Rep rat; SCA (SPImsap, SPsak); smmyHC human; SOD1-GST; SPEE hu; β-galactosidase; T7 RNA polymerase; TET repressor; WASP human
Four convenient architectures

**Cytosolic expression**
- Two-step purification from lysed cells to purity > 95%
- Protein A

**Secretory expression**
- Protein of interest efficiently secreted to culture medium
- Protein B

**Inducible**
- Up to 100 fold induction by addition of tetracycline
- Protein D

**Constitutive**
- Upon induction protein of interest becomes major component of cell culture medium
- Protein E

*Leishmania tarentolae*
Inducible LEXSY yielded high homogeneity of induced and uninduced cultures

A: EGFP in cell extracts of expression clone 3d post induction, based on rfu

- no tetracycline added
+ 5 μg/ml tetracyline added

C: Cell pellets from 1 ml of cultures inducibly expressing EGFP and DsRed fluorescence proteins.

Induction profiles and yields were stable over > 500 generations in continuous suspension culture!
One-step NiNTA purification of secretory target yielded highly pure protein

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NiNTA purification

- 1 cell extract
- 2 conc. SN
- 3 flow through 10x conc.
- 4 wash 10x conc.
- 5 eluate 1
- 6 eluate 2
- 7 prestained MW marker
- 8 eluate 3
- 9 eluate 4
- 10 beads

Deglycosylation

- 1 imidazol eluted POI (-20°C)
- 2 20x conc. POI in PBS
- 3 prestained MW
- 4 N-Glycosidase F (138 ng = 250U) 36 kDa
- 5 POI in deglycosylation buffer
- 6 POI + N-Glycosidase F
- 7 imidazol eluted POI (4°C)

- two glycoforms consistent with EPO (→)
- shift to one band
LEXSY N-Glycosylation pattern are of mammalian type

- **Bacteria**:
  - Galactose
  - N-acetylneuraminic acid
  - Mannose
  - N-acetylglucosamine
  - Fucose
  - Polypeptide

- **Yeast e.g. Pichia**

- **Insect cells e.g. Sf9/21**

- **Leishmania tarentolae**

- **Mammalian cells**

+3rd +4th antenna
Exceptional homogeneity of N-glycosylation in LEXSY

Recombinant hu EPO from LEXSY was

- biologically fully active
- natively processed at the N-terminus
- mammalian-type N-glycosylated

The N-glycosylation profile was exceptionally homogenous with a biantennary fully galactosylated Man₃GlcNAc₂core-α-1,6-fucosylated structure

Human recombinant EPO was exceptional homogenously N-glycosylated

A: homogenously N-glycosylated EPO from LEXSY
B: N-deglycosylated EPO from A
C: heterogenously glycosylated EPO from CHO

Exceptional homogeneity of N-glycosylation will be of advantage for structure analysis of glycosylated recombinant proteins isolated from LEXSY!

LEXSY in vivo labeling enables protein structure studies

Full assignment of all 18 Val was achieved by $^{15}$N-HSQC NMR analysis of $^{15}$N-Val labeled EGFP

Isotopic labeling of recombinant proteins expressed in the protozoan host Leishmania tarentolae.
Protein Expression and Purification 46: 167-172.
LEXSY is compatible with common fermentation technology.

Constitutive protein production parallels growth of expression culture. 

>120 mg/L of culture reached at 8 x 10^8 cells/ml.
Major LEXSY features published and patented

Non-pathogenic trypanosomatid protozoa as a platform for protein research and production

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Development of an inducible protein expression system based on the protozoan host *Leishmania tarentolae*

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