Jena Bioscience

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LEXSY taking off:
Selected examples for protein production with *Leishmania tarentolae*

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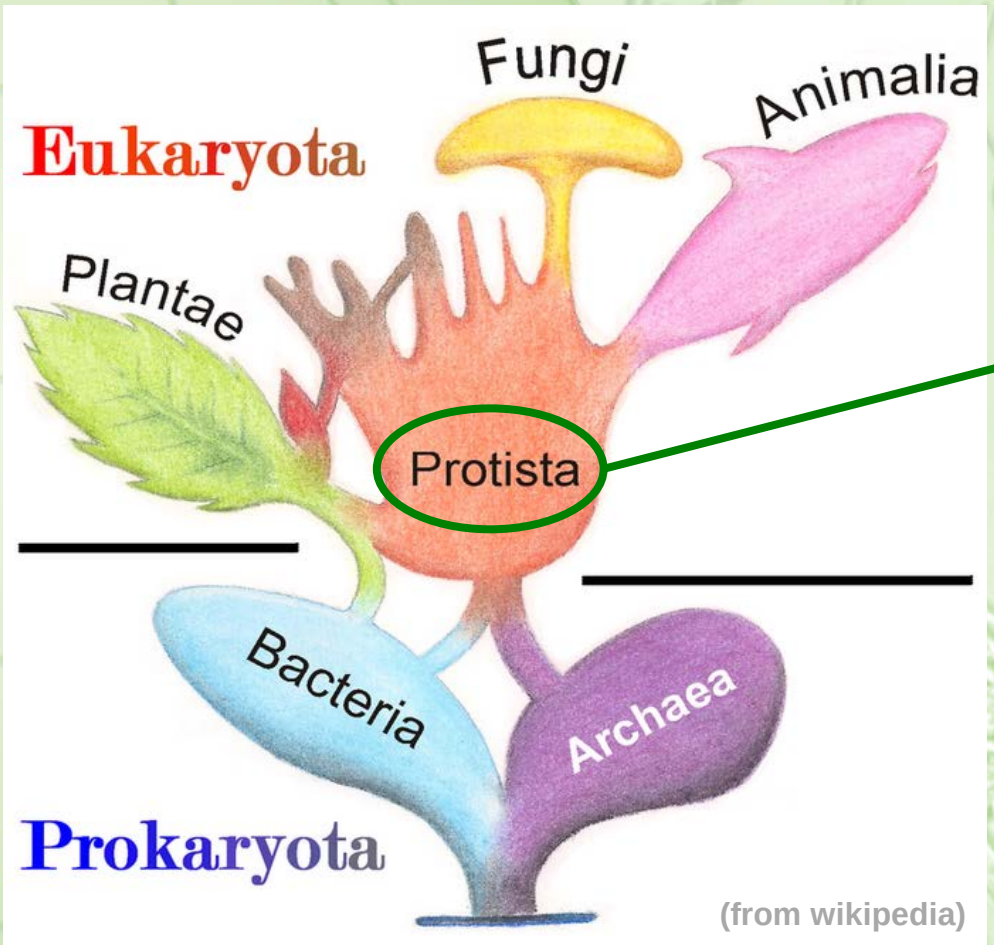
Time warp to 1998

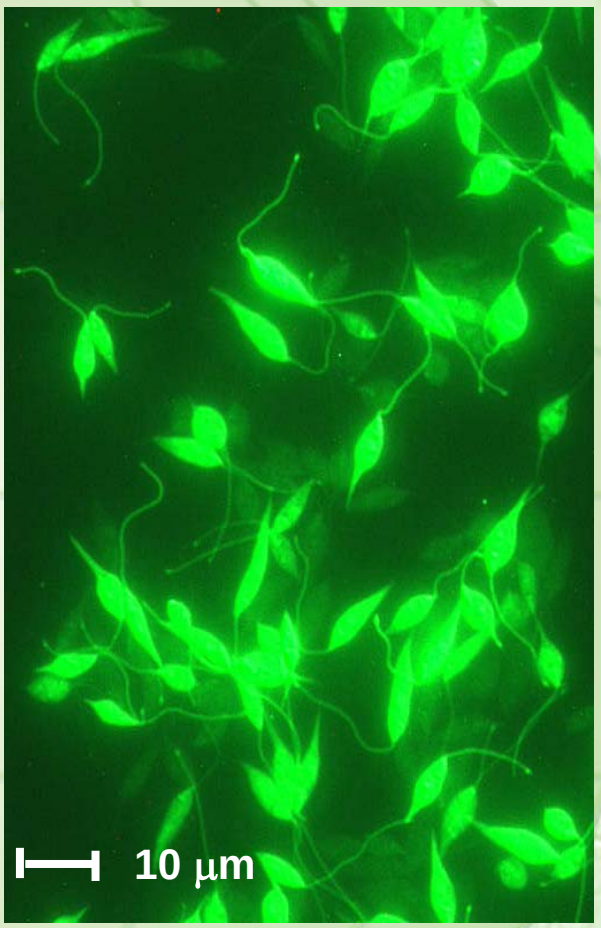
Why not an expression system that combines prokaryotic easiness with eukaryotic capabilities?



- Large, diverse kingdom of organisms
- Many didn‘t look very promising…
- **…but one did:**

Leishmania tarentolae

So *Leishmania tarentolae* became LEXSY



Leishmania tarentolae

- Nonpathogenic to mammals (S1-clearance)
- Very happy in culture (flasks and fermenters)
- Complete eukaryotic protein synthesis / folding / modification machinery (PTMs(1))

Not even 10 years of hard lab work…

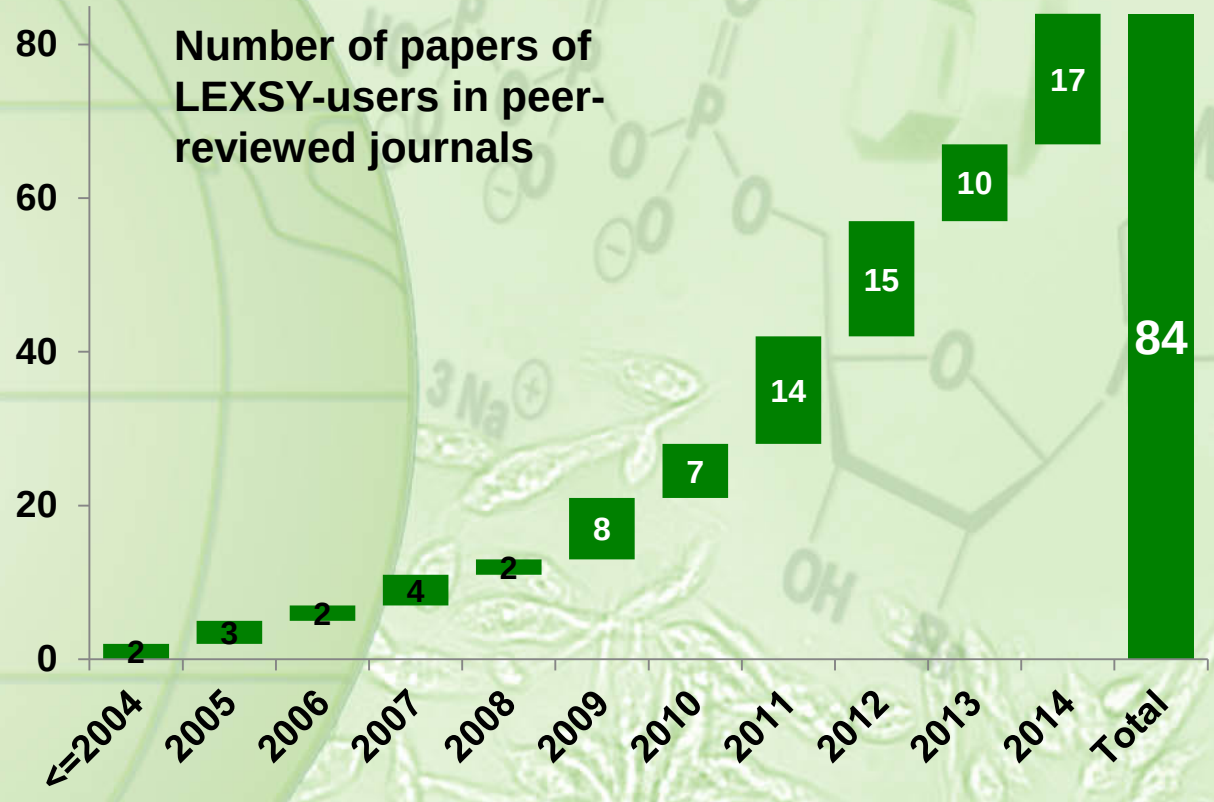
LEXSY

- Suitable for many *types* of proteins including membrane, cytosolic, nuclear and secreted proteins
- Approx. 80% positive expression projects with yields up to 500 mg per litre of culture
- Cell-free version (*in vitro* translation with LEXSY extracts)

(1) PTMs = post-translational modifications such as mammalian-type glycosylation, phosphorylation, glypiation (GPI ancoring), acetylation, prenylation, myristoylation, ADP-ribosylation, proteolytic processing, oligomerisation…

Soon more and more people started using LEXSY

To date, LEXSY is established in roughly 200 labs worldwide



Exponential take-off…? Last few minutes some selected LEXSY examples

LEXSY makes antigens, EPO, interleukin and interferon

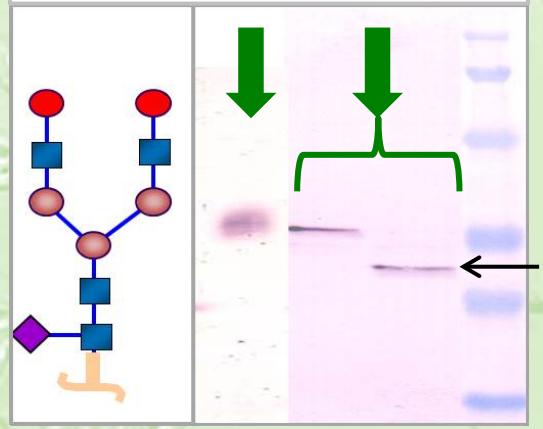
Vaccines

- Production of recombinant viral antigens (influenza[1], Hepatitis [2], Papilloma Virus[3])
- Production of recombinant parasite antigens (Leishmaniasis[4])
- Whole LEXSY cells as live vaccine delivery vector in tumor models[5,6]

Cytokines

- Human interferon gamma (IFNγ) with yields of 10 mg/L[8]
- N-glycosylated, antivirally active human interleukin 29 (IL-29[9])
- Mammalian-type N-glycosylated <u>homogeneous</u> biologically active human EPO[7]

EPO from: CHO, LEXSY



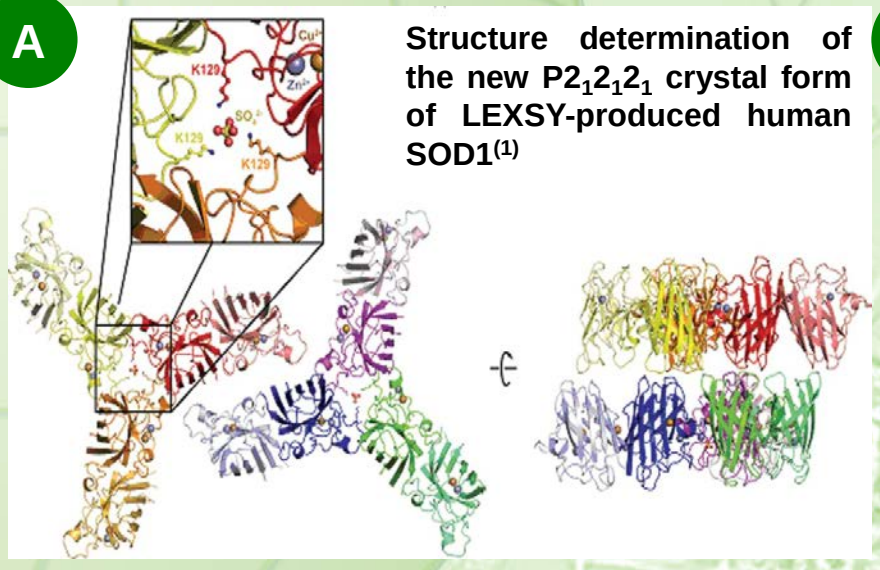
(1) Pion *et al.* (2014) *Vaccine* 32: 5570
(2) Baechlein *et al.* (2013) *Journal of Virological Methods* 193: 238
(3) Hosseinzadeh *et al.* (2013) *Drug Delivery* 20: 190
(4) Chamakh-Ayari *et al.* (2014) *PLOS ONE* 9: e92708
(5) Saljoughian *et al.* (2013) *PLoS Neglected Tropical Dis.* 7: e2174
(6) Salehi *et al.* (2012) *Immunotherapy* 4: 1107
(7) Breitling *et al.* (2002) *Prot Expr and Purification* 25: 209
(8) Davoudi *et al.* (2011) *World J. Microbiol. Biotechnology* 27: 1893
(9) Taromchi *et al.* (2013) *Iranian Journal of Biotechnology* 11: 168

LEXSY-proteins can be crystallized and NMRed

Structural biology

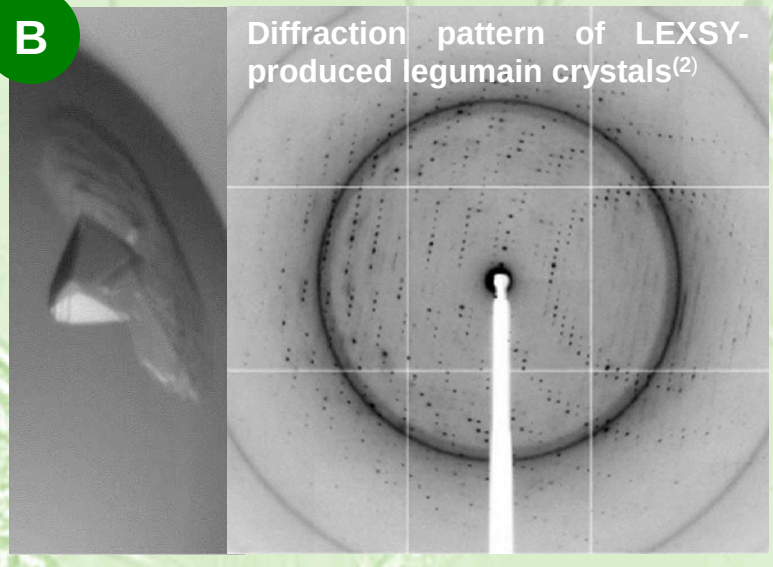
- Crystal structure of LEXSY-made antioxidant human Cu/Zn superoxide dismutase SOD1[(1)] (A)
- LEXSY-made legumain (cyst. protease) crystals diffract to 2.5 A[(2)] (B)
- ^{15}N-HSQC NMR yields assignment of 18x ^{15}N-Val in a 28 kDa protein[(3)]

A



Structure determination of the new $P2_12_12_1$ crystal form of LEXSY-produced human SOD1[(1)]

B



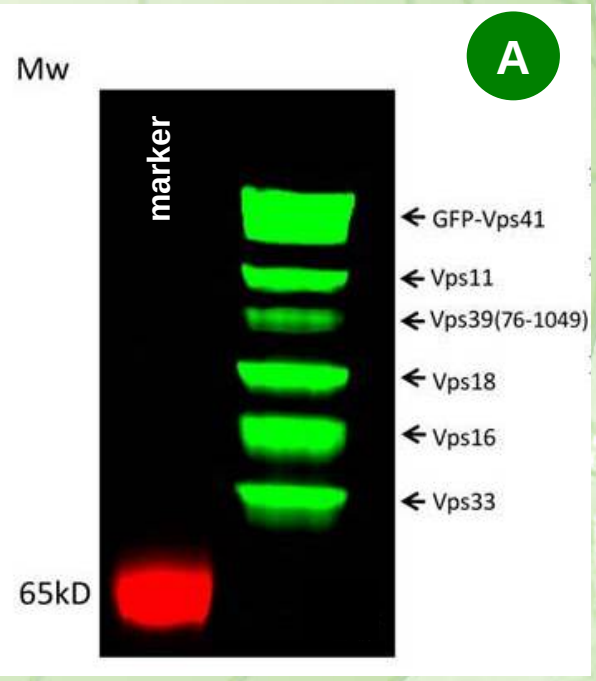
Diffraction pattern of LEXSY-produced legumain crystals[(2)]

(1) Gazdag *et al.* (2010) *Acta Crystallographica* F66: 871
(2) Dall & Brandstetter (2012) *Acta Crystallographica* F68: 24
(3) Niculae *et al.* (2006) *Protein Expression and Purification* 48: 167

In vitro LEXSY (cell-free expression based on LEXSY cell extracts) is an alternative to traditional wheat germ or RRL systems

In vitro **LEXSY**

- **LEXSY cell-free production of the to date largest multisubunit membrane protein complex[1] (A)**
- **Fluorescence-based protein interaction studies without purification[2,3,4]**
- **Fast high throughput screening[5,6,7]**



Active HOPS membrane tethering complex (600 kDa) reconstituted *in vitro* from all six subunits co-expressed by *In vitro* LEXSY[1] .

(1) Guo *et al.* (2013) *PLoS ONE* 8: e8153
(2) Brooks *et al.* (2014) *Science* 344: 1249783
(3) Gambin *et al.* (2014) *eLife* 3: e01434
(4) Han *et al.* (2014 *Journal of Biological Chemistry* 289: 7764
(5) Mureev *et al.* (2009) *Nature Biotechnology* 27: 747
(6) Kovtun *et al.* (2010) *PLOS one* 5: e14388
(7) Kovtun *et al.* (2011) *Methods* 55: 58

LEXSY-expressed pharmaceutically relevant enzymes and diagnostics tools

Pathogen diag-nostics

- LEXSY-made surface antigens SAG1 and SAG2 of *Toxoplasma gondii* for ELISA kits[(1)]
- LEXSY-made PTX toxin for diagnostics of fungal-caused wheat disease[(2)]

Antibodies

- Expression and purification of IgG based scFc fusions[(3)] from LEXSY
- LEXSY-expression and characterization of scFv collection[(4)]

Pharma research

LEXSY-made

- Proprotein convertase PC4 (subtilisin kexin) for inhibitor design[(5)]
- Human liver serine protease Coagulation Factor VII[(6)]
- Modified human Tissue-Plasminogen Activator (t-PA) that shows >100x higher activity than *E. coli* t-PA[(7)]
- Glycosylated Amyloid Precursor Protein sAPPα involved in Alzheimer disease[(8)]

(1) Ebert *et al.*, not published
(2) JBS, not published
(3) Jørgensen *et al.* (2014) *Microbial Cell Factories* 13: 9
(4) Klatt *et al.* (2012) *Microbial Cell Factories* 11: 97
(5) Basak *et al.* (2008) *Protein Expression and Purification* 60: 117
(6) Mirzaahmadi *et al.* (2011) *Journal of Biomedicine Biotechnology* 2011: 873874
(7) Nazari *et al.* (2011 *Biotechnology Letters* 33: 503
(8) Klatt *et al.* (2013 *Journal of Proteome Research* 12: 396

Our LEXSY group…

Dr. Reinhard Breitling „The LEXSYest Man Alive“

Dr. Andreas Licht

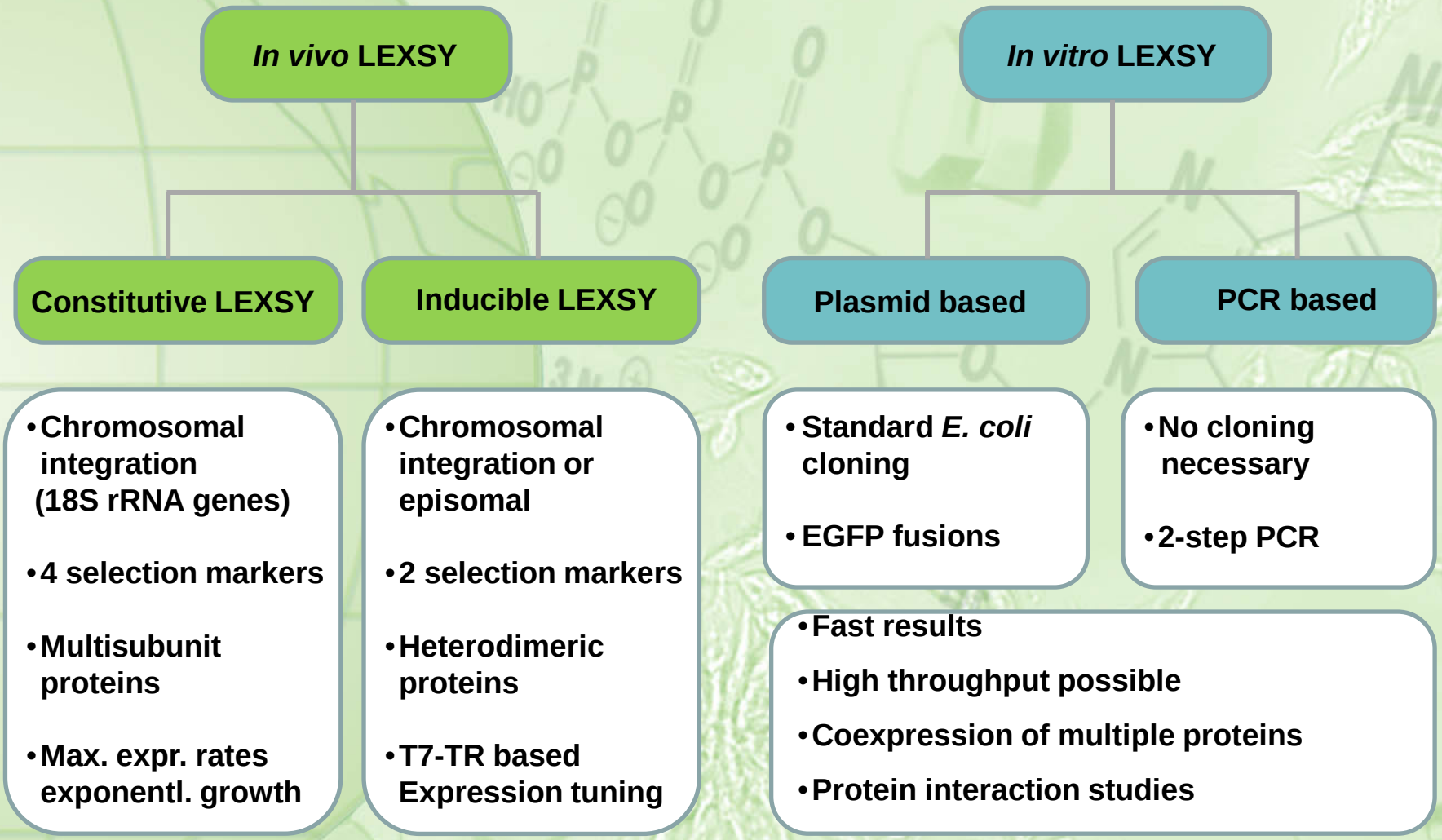
Dr. Larissa Consani Textor

Stefan Heiderich

…answers any question when contacted at expression@jenabioscience.com

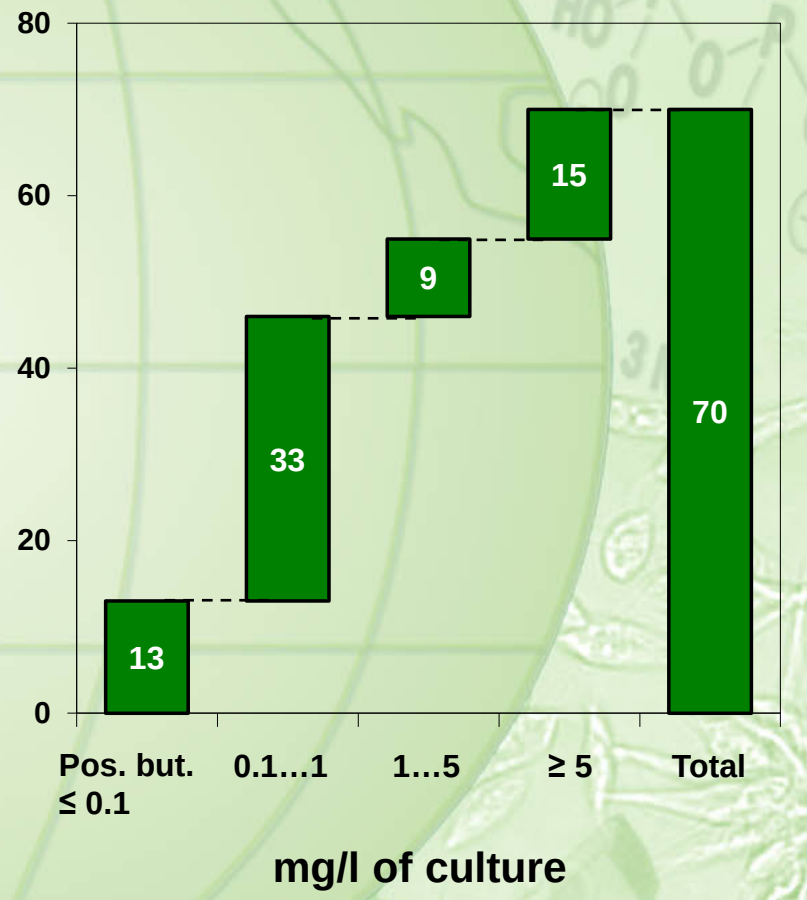
backups

Flexibility of LEXSY expression configurations
provides solutions for your needs



80% successful (> 0.1 mg/l) expression projects

Random selection of 70 targets that gave unsatisfactory results in other expression systems

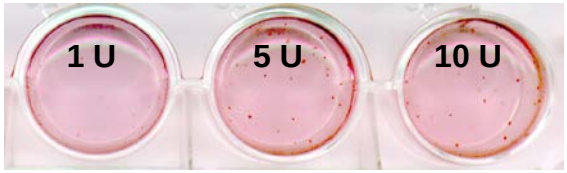


Target protein	Size kDa	Yield mg/L
Cytoplasmic proteins		
EGFP	28	300
SOD1	16	30
SPEE	35	30
p85 of PI3 kinase	85	3
smmyHC	154	1
Nuclear proteins		
T7 RNA Pol	100	1
Secreted proteins		
MHC II-β	30	500
CRP	23	44
SAG1&2	15/31	10
Fc fusion	39	10
MDP1	45	6
Laminin 332	420 (150+135+135)	0.5
Membrane proteins		
EGFP-Rab7 (mb-associated)	52	12
PDM9 (Type I)	43	0.5
BkrB2-GST (Type III TM7)	55	0.1

Case study

Homogeneously glycosylated human Erythropoietin from LEXSY

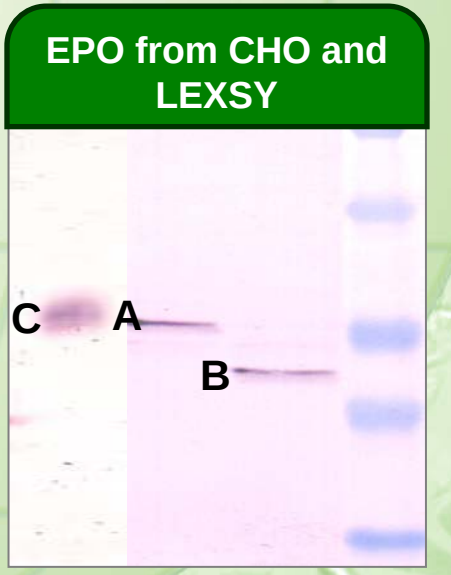
- Completely secreted to the culture medium
- Natively processed at the N-terminus
- Biologically fully active



1.2 x 10^5 U/mg

- Exceptionally homogenously & mammalian-type N-glycosylated

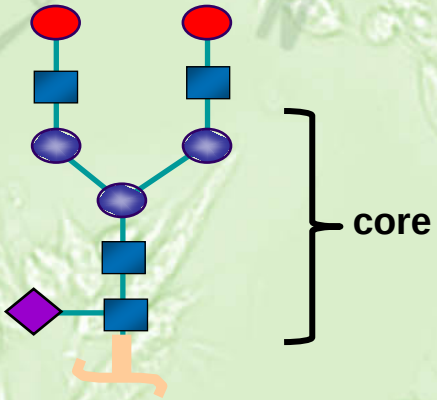
(biantennary fully galactosylated $Man_3GlcNAc_2$core-α-1,6-fucosylated structure)



A: homogenously glycosylated EPO from LEXSY

B: N-deglycosylated EPO from LEXSY

C: heterogenously glycosylated EPO from CHO



- Breitling *et al.* (2002) Non-pathogenic trypanosomatid protozoa as a platform for protein research and production. *Protein Expression and Purification* **25**: 209

Full repertoire of eukaryotic PTMs in LEXSY

PTM	Homol. Targets *Leishmania*	Heterol. Targets LEXSY	Selected references
Disulfide bond formation (folding)	**PMGP46**	**t-PA**	Kahl *et al.* 1987, Hong *et al.* 2008 , Soleimani *et al.* 2007,
Multimerization	**RNAP II, RNAP III**	**Laminin-322 SOD1, TETR**	Phan *et al.* 2009, Gazdag *et al.* 2010, Kushnir *et al*. 2005, Martínez-Calvillo *et al.* 2007
Proteolytic processing -Signal peptide cleavage - Pro-region processing - Furin like processing	**Gp63, 3'NT/NU CPB2.8**	**EPO, IFNγ,SAG1 rPC4 sortilin**	Breitling *et al.* 2002 and unpublished, Brooks *et al.* 2000, Basak *et al.* 2008 P. Madsen, not published
Glycosylation - N-Glycosylation - O-Glycosylation - Proteoglycan formation	**gp63, sAP, 3'NT/NU sAP, KMP11 sAP**	**EPO, IFNγ,SAG1**	Breitling *et al.* 2002 and unpublished, Debrabant *et al.* 2000, Ellis et al. 2002, Ilg *et al.* 1994, Lippert *et al.*1999, McGwire *et al.* 1996, Olafson *et al*. 1990, Stierhoff *et al.*1998 Weise et al. 2000
Glypiation (GPI ancoring)	**Gp63 Proteophosphoglycans MBAP^{GPI}**	**rPC4 SAG1**	McGwire *et al.* 1996, Ellis *et al.* 2002, Foth et al. 2002, Weise et al. (2000) , Basak *et al.* 2008, Breitling *et al.* unpublished,
Prenylation	**LmLRAB, RAS-CVIM 7 non-assigned 14-140 kDa**	**Rab7**	Chenik *et al.* 2006, Gillespie et al. 2007, Hasne *et al*. 1999, Alexandrov *et al.* not publ.

ARL-1 small G protein ADP-Ribosylation Factor-Like protein 1 of *L. donovani* , **CPB2.8** Cysteine proteinase of *L. mexicana,* **EPO** hu Erythropoietin**, Gp63** most abundant surface glycoprotein, GPI ancored and N-glycosylated HExxH Zn metalloproteinase (leishmanolysin), **H2A** Histone 2A of *L. donovani*, **HSP83-3** Heat shock protein of *L. donovani,* **HSP60** Heat shock protein of *L. donovani,* **IF3** Translation initiation factor 3 subunit of *L. donovani*, **IFNγ** hu intereron gamma, **KMP11** Kinetoplastid membrane protein of *L. donovani,*, **LIP2** 60S acidic ribosomal protein P2 of *L. donovani,* **LmLRAB** RAB GTPase of *L. major,* **Lt1200** 1200 kDa Cytoskeletal Giant Protein of *L. tarentolae,* **MBAP^{GPI}** artificially GPI ancored acid phosphatase of *L. mexicana,* **3'NT/NU** Surface Membrane 3'-Nucleotidase/ Nuclease of *L. donovani,* **OADC** bacterial oxaloacetate decarboxylase in *L. mexicana amazonensis,* **PMGP46** promastigote membrane glycoprotein of *L. mexicana amazonensis,* **Rab7** geranylgeranyl transferase component A of *Rattus norvegicus,* **Rbp16** RNA-binding protein of *L. donovani,* **RNAP II** RNA polymerase II of *L.major,* **RNAP III** RNA polymerase III of *L.major,* **rPC4** rat proprotein convertase 4, **S10 & S18** 40S ribosomal proteins of *L. donovani,* **SAG1** surface antigen 1 of *Toxoplasma gondii,* **sAP** secreted acid phosphatase (N- and O-glycosylated and phosphoglycosylation) of *L. mexicana* and *L.donovani,* **SOD1** hu superoxide dismutase, **TETR** tetracycline repressor, **t-PA** hu tissue plasminogen activator, **VG7A5** amastigote spec. Protein of *L. mexicana mexicana*

Full repertoire of eukaryotic PTMs in LEXSY

PTM	Homol. Targets *Leishmania*	Heterol. Targets LEXSY	Selected references
Methylation	**α-Tubulin (D329) Carboxypeptidase (E53) Rbp16 (R104), H2A (S97) …**		Rosenzweig *et al.* 2008, Jardim *et al*. 1995
Phosphorylation	**Lt1200 (S,T), HSP83-3 (T) RNA helicase II (S) …**		Baqui *et al.* 2000, Rosenzweig *et al..*2008
Acetylation - N-terminal - internal	**LIP2 (M1), KMP11 (A2), S10 (S2), IF3 (T2) … S18 (S15) , HSP60 (E293) …**	**SOD1 (A2)**	Rosenzweig *et al.* 2008, Gazdag *et al.* 2010, Thomas *et al.* 2009
Myristoylation	**ARL-1**		Sahin *et al.* 2008
Amidation	**VG7A5**		Liu *et al.* 2000
Glutathionylation	***Tb* mono-Cys-glutaredoxin 1**		Melchers *et al.* 2007
ADP-ribosylation	**TGN-Lysosome trafficking**		Sturm *et al.* 1998 , Sahin *et al.* 2008
Biotinylation (rec. strain)	**-**	**OADC AVI-EGFP**	Detke *et al.* 2007 Konthur *et al.* 2009

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unused

References

Baqui *et al.* (2000) In Vivo and In Vitro Phosphorylation and Subcellular Localization of Trypanosomatid Cytoskeletal Giant Proteins. *Cell Motility and the Cytoskeleton* **47:** 25

Basak *et al.* (2008) Recombinant proprotein convertase 4 (PC4) from *Leishmania tarentolae* expression system: Purification, biochemical study and inhibitor design. *Protein Expression and Purification* **60:** 117

Breitling *et al.* (2002) Non-pathogenic trypanosomatid protozoa as a platform for protein research and production. *Protein Expression and Purification* **25:** 209

Brooks *et al.* (2000) Processing and trafficking of cysteine proteases in *Leishmania mexicana.* Journal of Cell Science **113:** 4035

Chenik *et al. (*2006) Identification of a new developmentally regulated Leishmania major large RAB GTPase. *Biochem Biophys Res Commun*. **341:** 541

Debrabant *et al.* (2000) Dissection of the Functional Domains of the *Leishmania* Surface Membrane 3'-Nucleotidase/Nuclease, a Unique Member of the Class I Nuclease Family. *Journal of Biological Chemistry* **275:** 16366

Detke *et al. (*2007) Leishmania mexicana amazonensis: development of a peptide tag useful for labeling and purifying biotinylated recombinant proteins. *Exp Parasitol*. **115:** 221

Ellis *et al.* (2002) Processing and Trafficking of *Leishmania mexicana GP63. Journal of Biological Chemistry* **277:** 27968

Foth *et al.* 2002 Leishmania major proteophosphoglycans exist as membrane-bound and soluble forms and localise to the cell membrane, the flagellar pocket and the lysosome. *Int J Parasitol.* 32:1701

Gazdag *et al.* (2010) Purification and crystallization of human Cu/Zn superoxide dismutase recombinantly produced in the protozoan *Leishmania tarentolae*. *Acta Crystallographica* F66: 871

Gillespie *et al.* (2007) C-terminal proteolysis of prenylated proteins in trypanosomatids and RNA interference of enzymes required for the post-translational processing pathway of farnesylated proteins**.** *Mol Biochem Parasitol*. 2007 **153:** 115

Hasne *et al*. (1999) Characterization of prenylated protein methyltransferase in *Leishmania. Biochem. J.* **342:** 513

Hong *et al. (*2008) Identification and enzymatic activities of four protein disulfide isomerase (PDI) isoforms of *Leishmania amazonensis*. *Parasitol. Res.* **102:** 437

References

Ilg *et al.* (1994) 0- and N-Glycosylation of the Leishmania mexicana-secreted Acid Phosphatase. *Journal of Biological Chemistry* **269:** 24073

Jardim *et al*. (1995) Isolation and structural characterization of the *Leishmania donovani* kinetoplastid membrane protein-11, a major immunoreactive membrane Glycoprotein. *Biochem. J*. **305:** 307

Kahl *et al*. (1987) Structural and antigenic characterization of a species- and promastigote-specific *Leishmania mexicana amazonensis* membrane protein. *J. Immunol*. **138:**1587

Konthur *et al.* 2009 *In Vivo* Biotinylation of Recombinant Proteins in the Protozoan Host *Leishmania tarentolae*. PEPTalk 2009 January 11 - 16, Coronado, CA

Kushnir *et al.* (2005) Development of an inducible protein expression system based on the protozoan host *Leishmania tarentolae*. *Protein Expression and Purification* **42:** 37

Lippert *et al.(*1999) Phosphoglycosylation of a secreted acid phosphatase from *Leishmania donovani*. *Glycobiology.* **9:** 627

Liu *et al. (*2000) Isolation and analysis of a new developmentally regulated gene from amastigotes of *Leishmania mexicana mexicana. Parasitol Res.* **86:** 140

Martínez-Calvillo *et al. (*2007) Characterization of the RNA polymerase II and III complexes in *Leishmania major. Int J Parasitol.* **37:** 491

McGwire *et al.* (1996) Posttranslational Regulation of a *Leishmania HEXXH* Metalloprotease (gp63). *Journal of Biological Chemistry* **265:** 12240

Melchers *et al.* 2007 Glutathionylation of trypanosomal thiol redox proteins. *J Biol Chem.* **282:** 8678

Olafson *et al*. (1990) Structures of the N-linked oligosaccharides of Gp63, the major surface glycoprotein, from *Leishmania mexicana amazonensis. Journal of Biological Chemistry* **269:** 24073

Phan *et al.* (2009) The production of recombinant human laminin-332 in a *Leishmania tarentolae* expression system. *Protein Expression and Purification* **68:** 79

Rosenzweig *et al. (*2008) Post-translational modification of cellular proteins during *Leishmania donovani* differentiation. *Proteomics*. **8:** 1843

Sahin *et al. (*2008)The *Leishmania* ARL-1 and Golgi Traffic. PLoS One. **3:** e1620

References

Soleimani *et al.* (2007) Expression of human tissue-type plasminogen activator (t-PA) in *Leishmania tarentolae. Biotechnology & Applied Biochemistry* **48:** 55

Sturm *et al.* (1998) Characterization of a GTP-binding protein in the ADP-ribosylation factor subfamily from Leishmania tarentolae. *Biochim Biophys Acta*. **1442:** 347

Stierhoff *et al.* (1998) Structure of a filamentous phosphoglycoprotein polymer: the secreted acid phosphatase of Leishmania mexicana. *J. Mol. Biol.* **282:** 137

Thomas *et al. (*2009) Histone acetylations mark origins of polycistronic transcription in *Leishmania major. BMC Genomics* **10:** 152

Weise *et al.* (2000) Distribution of GPI-anchored proteins in the protozoan parasite *Leishmania,*based on an improved ultrastructural description using high-pressure frozen cells. *Journal of Cell Science* **113:** 4587