LEXSY taking off:
Selected examples for protein production with *Leishmania tarentolae*
Why not an expression system that combines prokaryotic easiness with eukaryotic capabilities?

Time warp to 1998

- 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\(^{(1)}\))

**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)

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\(^{(1)}\) PTMs = post-translational modifications such as mammalian-type glycosylation, phosphorylation, glypiation (GPI anchoring), 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

Number of papers of LEXSY-users in peer-reviewed journals

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 (IFN\(_\gamma\)) with yields of 10 mg/L\(^{(8)}\)
- N-glycosylated, antivirally active human interleukin 29 (IL-29\(^{(9)}\))
- Mammalian-type N-glycosylated homogeneous biologically active human EPO\(^{(7)}\)

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(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
LEXSY-proteins can be crystallized and NMRed

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

(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

- 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\(^{(1)}\).

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\(^{(1)}\) 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


\(^{(6)}\) 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 diagnostics**
- 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 sAPP\(_\alpha\) 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

**In vivo LEXSY**
- Constitutive LEXSY
  - Chromosomal integration (18S rRNA genes)
  - 4 selection markers
  - Multisubunit proteins
  - Max. expr. rates exponential growth
- Inducible LEXSY
  - Chromosomal integration or episomal
  - 2 selection markers
  - Heterodimeric proteins
  - T7-TR based Expression tuning

**In vitro LEXSY**
- Plasmid based
  - Standard *E. coli* cloning
  - EGFP fusions
- PCR based
  - No cloning necessary
  - 2-step PCR
  - Fast results
  - High throughput possible
  - Coexpression of multiple proteins
  - Protein interaction studies
80% successful (> 0.1 mg/l) expression projects
Random selection of 70 targets that gave unsatisfactory results in other expression systems

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

- Completely secreted to the culture medium
- Natively processed at the N-terminus
- Biologically fully active
- Exceptionally homogenously & mammalian-type N-glycosylated
  (biantennary fully galactosylated Man$_3$GlcNAc$_2$core-α-1,6-fucosylated structure)

**EPO from CHO and LEXSY**

A: homogenously glycosylated EPO from LEXSY

B: N-deglycosylated EPO from LEXSY

C: heterogenously glycosylated EPO from CHO

# Full repertoire of eukaryotic PTMs in LEXSY

<table>
<thead>
<tr>
<th>PTM</th>
<th>Homol. Targets Leishmania</th>
<th>Heterol. Targets LEXSY</th>
<th>Selected references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disulfide bond formation (folding)</td>
<td>PMGP46</td>
<td>t-PA</td>
<td>Kahl et al. 1987, Hong et al. 2008 , Soleimani et al. 2007,</td>
</tr>
<tr>
<td>Proteolytic processing</td>
<td>Gp63, 3'NT/NU CPB2.8</td>
<td>EPO, IFNγ, SAG1 sortilin</td>
<td>Breitling et al. 2002 and unpublished, Brooks et al. 2000, Basak et al. 2008 P. Madsen, not published</td>
</tr>
<tr>
<td>Prenylation</td>
<td>LmLRAB, RAS-CVIM 7 non-assigned 14-140 kDa</td>
<td>Rab7</td>
<td>Chenik et al. 2006, Gillespie et al. 2007, Hasne et al. 1999, Alexandrov et al. not publ.</td>
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**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*, **HSP60** Heat shock protein of *L. donovani*, **HSP83-3** Heat shock protein of *L. donovani*, **IF3** Translation initiation factor 3 subunit of *L. donovani*, **IFNγ** hu interferon 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** 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*. 
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<tbody>
<tr>
<td>Methylation</td>
<td>α-Tubulin (D329)</td>
<td></td>
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<tr>
<td></td>
<td>Carboxypeptidase (E53)</td>
<td></td>
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<tr>
<td></td>
<td>Rbp16 (R104), H2A (S97)</td>
<td></td>
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<tr>
<td>Phosphorylation</td>
<td>Lt1200 (S,T), HSP83-3 (T)</td>
<td></td>
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<tr>
<td></td>
<td>RNA helicase II (S)</td>
<td></td>
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<tr>
<td>Acetylation - N-terminal</td>
<td>LIP2 (M1), KMP11 (A2),</td>
<td>SOD1 (A2)</td>
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</tr>
<tr>
<td></td>
<td>S10 (S2), IF3 (T2)</td>
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<tr>
<td></td>
<td>S18 (S15), HSP60 (E293)</td>
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<tr>
<td>Acetylation - internal</td>
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<tr>
<td>Myristoylation</td>
<td>ARL-1</td>
<td></td>
<td>Sahin et al. 2008</td>
</tr>
<tr>
<td>Amidation</td>
<td>VG7A5</td>
<td></td>
<td>Liu et al. 2000</td>
</tr>
<tr>
<td>Glutathionylation</td>
<td>Tb mono-Cys-glutaredoxin 1</td>
<td></td>
<td>Melchers et al. 2007</td>
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<tr>
<td>ADP-ribosylation</td>
<td>TGN-Lysosome trafficking</td>
<td></td>
<td>Sturm et al. 1998 , Sahin et al. 2008</td>
</tr>
<tr>
<td>Biotinylation (rec. strain)</td>
<td>-</td>
<td>OADC AVI-EGFP</td>
<td>Detke et al. 2007, Konthur et al. 2009</td>
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unused
References

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


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