Jena Bioscience
Building Blocks of Life
www.jenabioscience.com

LEXSY
the protein expression platform based on
Leishmania tarentolae

Reinhard Breitling

České Budějovice, September 5th 2016

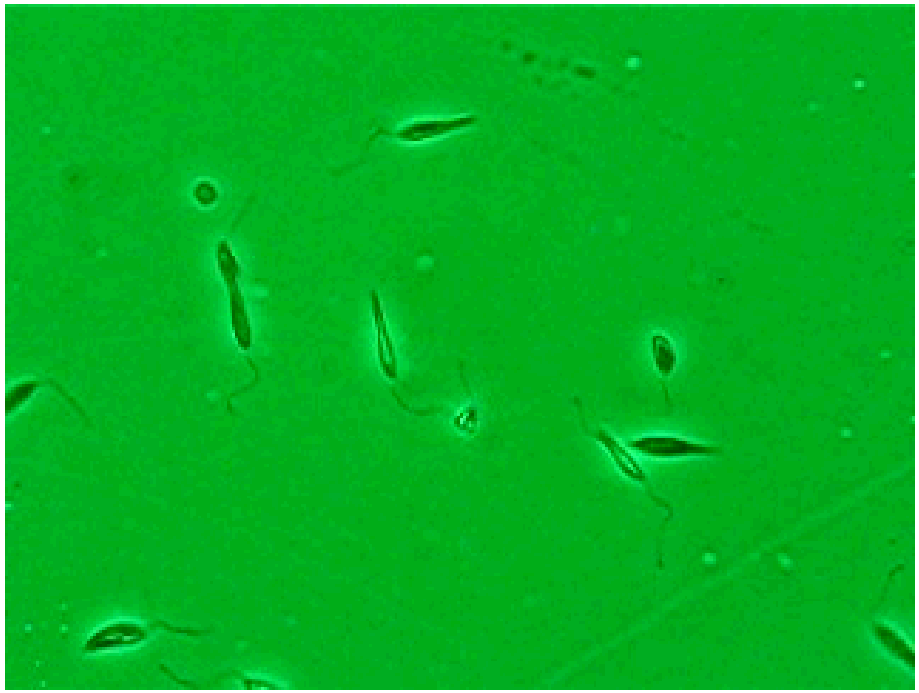
Jena Bioscience GmbH
Löbstedter Str. 71
07749 Jena, Germany

Tel.: +49-3641-628-5000
Fax: +49-3641-628-5100
e-Mail: info@jenabioscience.com

Leishmania tarentolae was chosen as expression host for production of recombinant proteins

Host: *Tarentola mauritanica*

- unicellular flagellated protozoa
- parasite of lizard (*Sauroleishmania*)
- not pathogenic for mammalians
- biosafety group 1 organism
- can be easily cultured *in vitro*
- genome sequence published 2012
- transcriptomic and proteomic data 2015



"LEXSY"

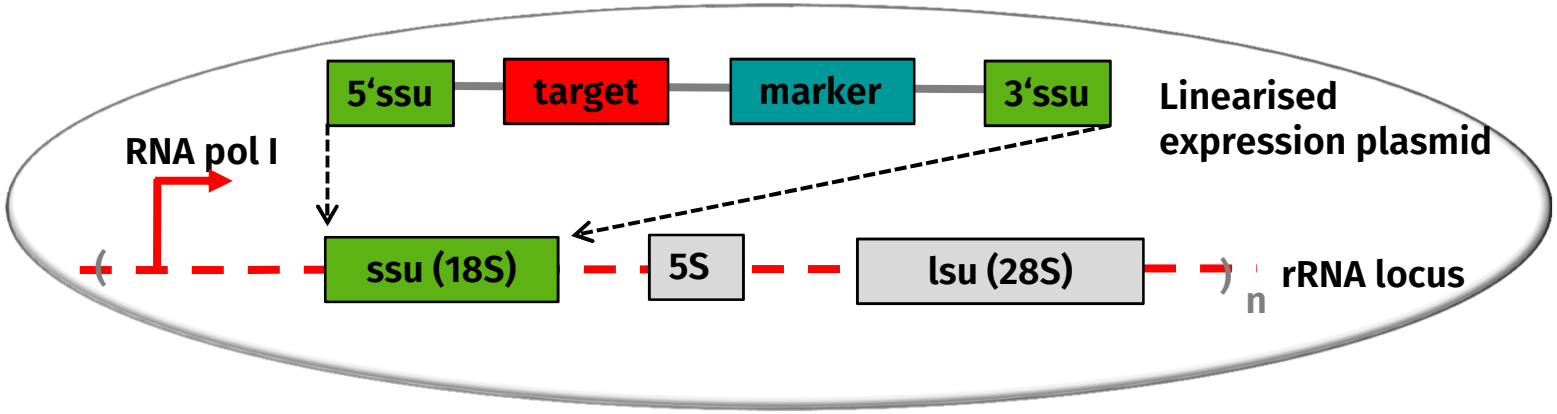
***L**eishmania*

Expression

System

Configuration 1: Constitutive expression

- *ssu* integration (multicopy 18S rRNA cluster)
- transcription by host RNA polymerase I

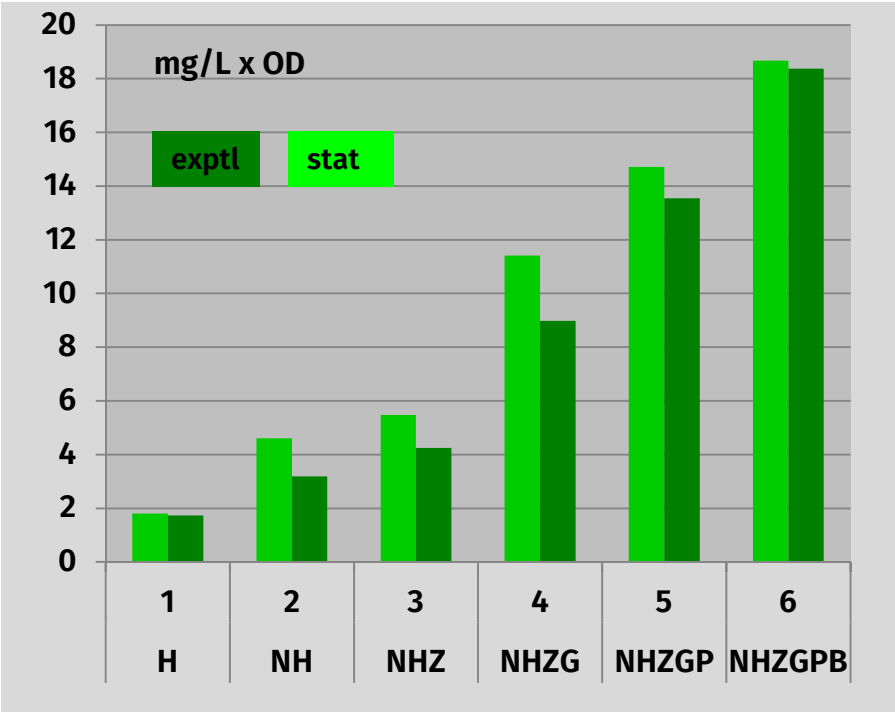


- RNA polymerase I: 10x stronger than RNA Polymerase II
- maximal activity during exponential growth (early product synthesis)
- six selection markers (Nourseothricin, Blasticidin, Bleomycin, Geniticin G418, Hygromycin & Puromycin)

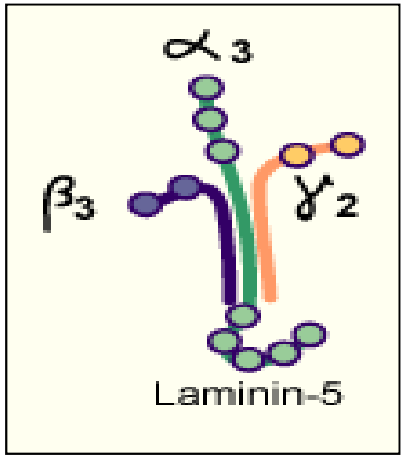
Worked also in *L. amazonensis, L. donovani, L. infantum, L. major, L. mexicana, Phytomonas serpens* ...

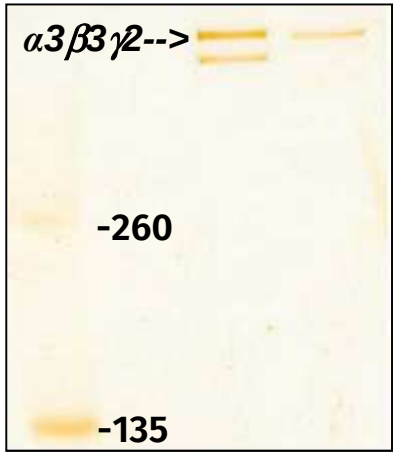
Co-integration of expression cassettes into *ssu* locus

Additive effect on protein expression



Expression of multi-subunit proteins

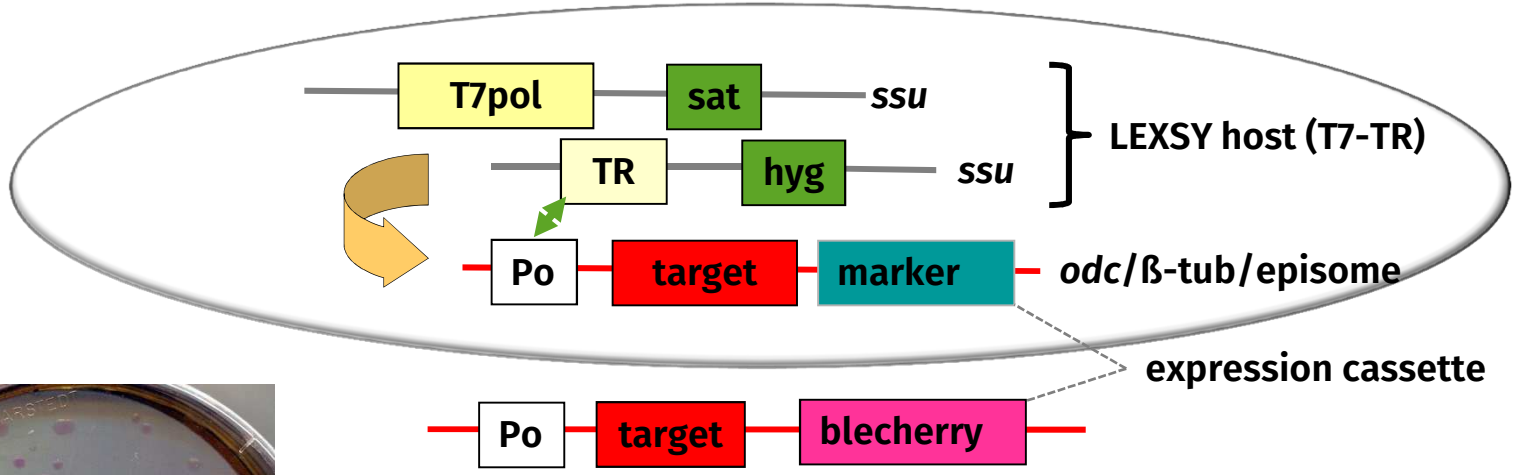




420 kDa Laminin-332 heterotrimer secreted from LEXSY
Phan *et al.* (2009) *Protein Expression and Purification* 68:79.

Configuration 2: Inducible expression

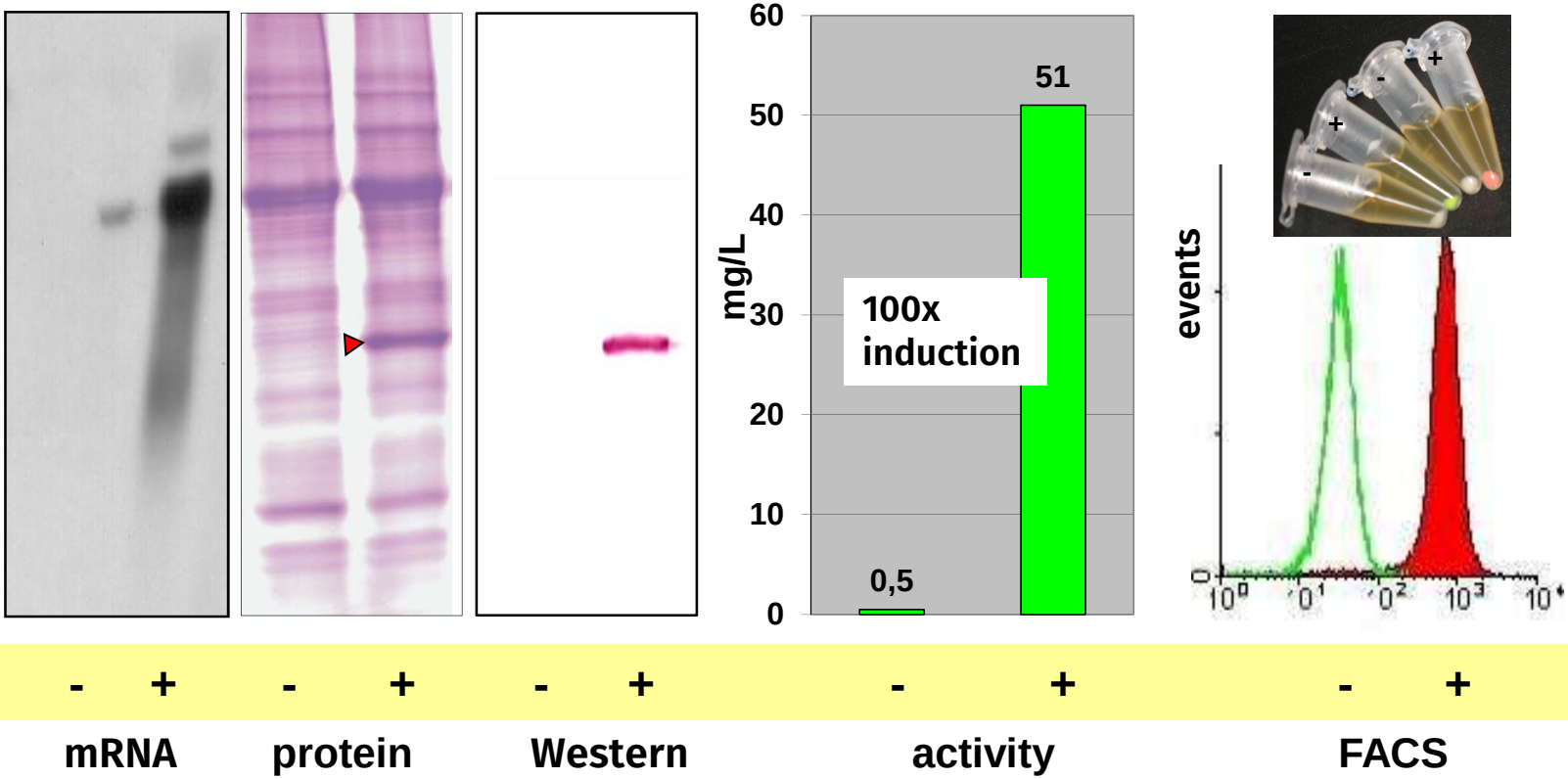
- transcription by heterologous T7 RNA polymerase
- control by TET repressor
- *odc (ß-tub)* integration or episomal propagation of target gene



- much stronger than RNA Pol I
- tetracycline induction at any time during culturing
- fluorescent marker (blecherry) for in-process monitoring

Was established also in *L. mexicana* (Kraeva *et al.* 2014)

Efficient expression control in inducible LEXSY from mRNA to protein



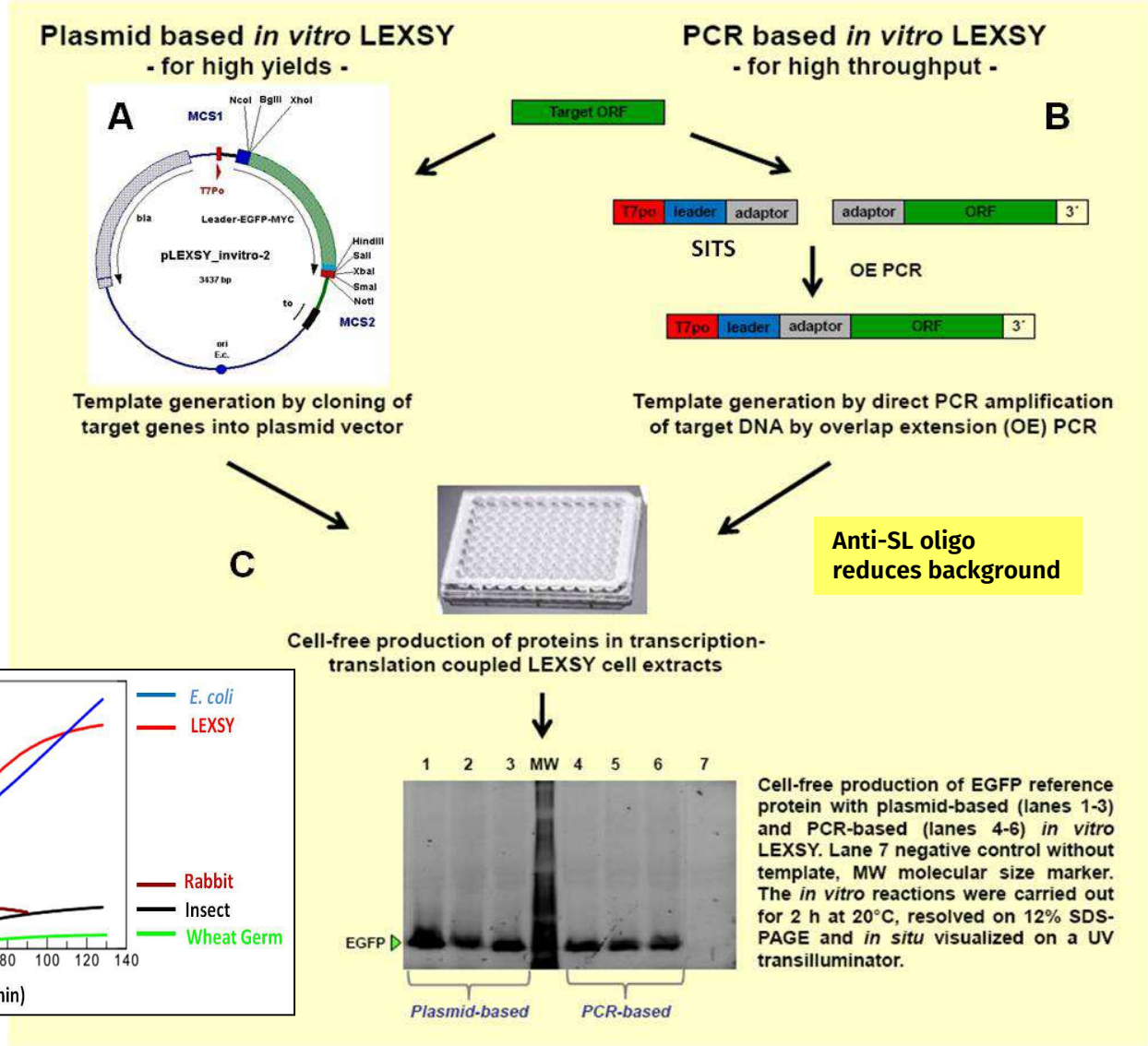
- induction profiles and yields stable over > 500 generations
- all cells induced

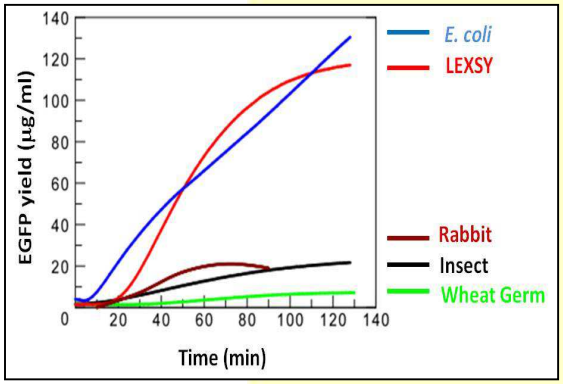
6

In Vitro LEXSY: Rapid cell-free protein production

Cloning required

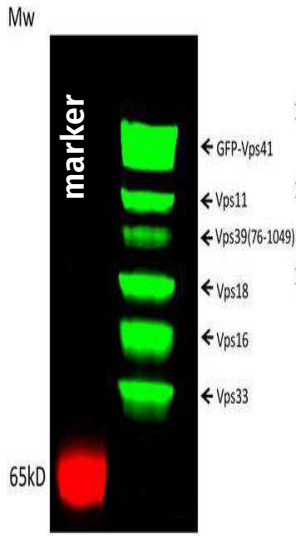
HTP compatible



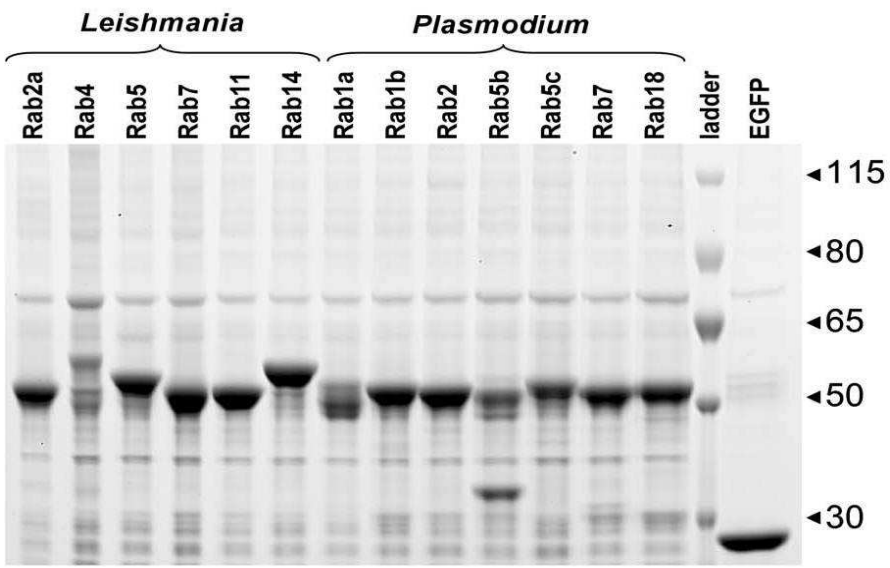


Versatility of In Vitro LEXSY

- **LEXSY cell-free production of to date largest multisubunit membrane protein complex[1]**
- **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] .



RabGTPases from *L. tarentolae* or *P. falciparum were expressed* in PCR-based *In Vitro* LEXSY. Coomassie stained SDS-PAGE gel loaded with EGFP-Rab GTPases eluted from GFP-Cap matrix (adapted from Kovtun *et al.* 2010).

(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 – work horse for parasitologists
due to phylogenetic relationship to other Kinetoplastids

Expression of kinetoplastid proteins in *L. tarentolae* e.g.

- Surface antigens of *T. brucei gambiense* ISG65, VSG LiTat 1.3 and VSG LiTat 1.5 (Rooney *et al.* 2015)
- Surface antigen PSA-38S of *L. amazonensis (Chamakh-Ayari et al.* 2014)
- α-N-acetylglucosaminyltransferase OGNT2 from *T. cruzi* (Heise *et al.* 2009)

Genetic manipulation of *Leishmania* species e.g.

- Fluorescent/luminiscent cells (Lang *et al.* 2005, Barak *et al.* 2005..... Sadlova *et al.* 2011, Bolhassani *et al.* 2011 , Rabhi *et al.* 2015, Taheri *et al.* 2015, García-Hernández *et al.* 2015)
- wt and mutant ferrous iron transporter LIT1 in *L. amazonensis* (Jacques *et al.* 2010)
- membrane acid phosphatase in *L. donovani* (Papadaki *et al.* 2015)

- functional characterization of parasite proteins
- investigation of parasite-host interactions
- *in vivo* and *in vitro* screening of anti-leishmanial drugs
- diagnostics and vaccines development

Collaborations

MPI molek. Physiologie Dortmund
Kirill Alexandrov
Susanna Kushnir
Ion Cirstea
Sergey Mureev

Univ. Queensland
Kirill Alexandrov
Sergey Mureev
Olexiy Kovtun
Wayne Johnston

Washington Univ. St. Louis
Stephen M. Beverley

Center for Infectious Disease Research Seattle
Peter J. Myler

University of Ghent
Nico Callewaert
Roland Contreras

Our LEXSY team at JBS


Reinhard Breitling


Andreas Licht


Larissa Consani Textor


Stefan Heiderich

expression@jenabioscience.com

References LEXSY applications for parasitologists

Fluorescent/luminiscent cells

- DsRed in *L. major* (Chagas *et al.* 2014, Rabhi *et al.* 2016)
- near-infrared *L. infantum* strains (Calvo-Álvarez *et al.* 2015, Balaña-Fouce *et al.* 2012)
- EGFP-LUC in *L. major* (Taheri *et al.* 2015)
- LUC bioluminescent *L. donovani* (García-Hernández *et al.* 2015)
- LUC bioluminescent *L. amazonensis* (Lang *et al.* 2005)
- eGFP and RFP in *L. donovani* (Sadlova *et al.* 2011)
- EGFP in *L. major* (Bolhassani *et al.* 2011, Gonzalez-Leal *et al.* 2014)
- EGFP in *L. donovani* (Barak *et al.* 2005)
- mCherry in *L. major* and *L. donovani* (Vacchina *et al.* 2014, Calvo-Alvarez *et al.* 2012)

References LEXSY applications for parasitologists

Antigens

- T. brucei gambiense surface ags ISG65, VSG LiTat 1.3 and VSG LiTat 1.5 (Rooney *et al.* 2015)
- Surface antigen PSA-38S L. amazonensis (Chamakh-Ayari *et al.* 2014)
- sand fly salivary antigen PpSP15-EGFP in L. tarentolae (Katebi *et al.* 2015)
- Polytope -PT-GFP (Seyed *et al.* 2015)
- Cysteine proteases cpa-cpb fusions (Taheri *et al.* 2014, Zahedifard *et al.* 2014, Saljoughian *et al.* 2013)

Functional studies

- membrane acid phosphatase in *L. donovani*, *Ld*MAcP (Papadaki *et al.* 2015)
- histone acetyltranferase HAT3 in *L. donovani* (Kumar *et al.* 2015)
- MAP kinase MPK4 *in L. major* (Dacher *et al.* 2014)
- Casein Kinase 1 in *L. donovani* (Rachidi *et al.* 2014)
- apurinic/apirimidinic endonuclease AP2 *T. cruzi* in *L. tarentolae* (Sepúlveda *et al.* 2014)
- LPG3 of *L.infantum* in *L. tarentolae* (Pirdel *et al.* 2012)
- *L. donovani* actin (LdACT) in *L. tarentolae* (Kumar *et al.* 2012)