

Team members: Dr. biol. hum. Uwe Jandt,

Supervisor: Prof. Dr. An-Ping Zeng

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Description:

The majority of relevant pharmaceutical proteins are produced via **transfection** of the corresponding genes into mammalian cells. Establishing a stable and efficient cell line can take up to 12 months. More rapid expression of small amounts of proteins (e.g. for evaluation purposes) can be achieved by **transient expression**, i.e., without incorporation of the DNA into the cell's genome. Conventionally, viral vectors are utilized to deliver genetic material into the cells. Viral vectors are very efficient. However, **non-viral vectors** are more desirable in order to reduce the risk of producing toxic byproducts and to avoid viral replication. Examples for non-viral vectors are calcium phosphate, modified PEI or peptides. However, their efficiency is considerably lower.

This project aims at a quantitative understanding and optimization of the gene delivery processes during transient transfection in HEK293s cells (Fig. 1). Recently developed replicating and non-integrating minicircles (Broll et al., 2010) are used for transfection. In the first part of the project, a stochastic and spatio-temporally resolved simulation model has been developed (Jandt et al., 2011). The most relevant parameters have been identified and are currently adapted to the model cell line, based on experimental data of time-lapse confocal microscopy and quantitative PCR.

References:

- Broll S, Oumard A, Hahn K, Schambach A, Bode J. 2010. Minicircle performance depending on S/MAR nuclear matrix interactions. *J Mol Biol* 395(5):950–965.
- Jandt U, Shao S, Wirth M, and Zeng AP. 2011. Spatiotemporal modeling and analysis of transient gene delivery. *Biotech Bioeng: In print.*

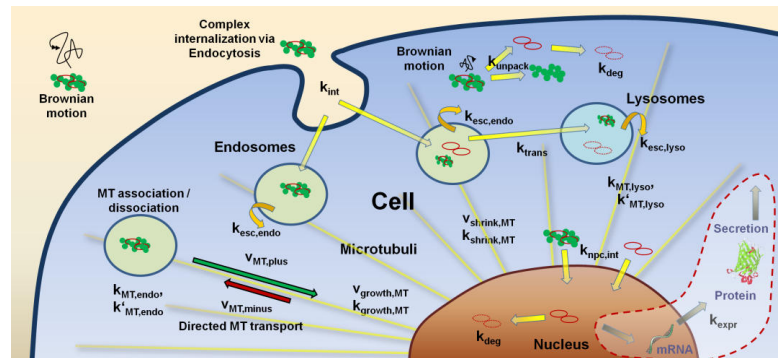


Fig. 1: Implemented transient transfection pathways. Genetic material is introduced into the cell using non-viral vectors. The DNA is incorporated into the nucleus. Pharmaceutical or reporter proteins are expressed via transcription, mRNA export and translation and secreted.

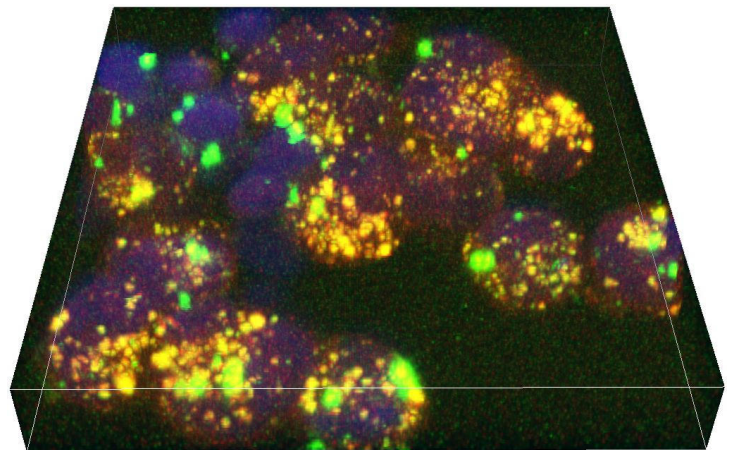


Fig. 2: 3D image from confocal microscopy of HEK293 cells. Transfected plasmids are stained red, endosomes and lysosomes in green. Overlay of plasmids and vesicles is consequently denoted yellow. The nuclei are stained blue.

Contact: Prof. Dr. An-Ping Zeng

Institute of Bioprocess and Biosystems Engineering, Hamburg University of Technology.
Denickestrasse 15, D-21071 Hamburg, Germany.

Phone: +49-40-42878-4183 Email: aze@tu-harburg.de Web: www.tu-harburg.de/ibb