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VST FACULTY OF PROCESS AND SYSTEMS ENGINEERING

Multidimensional Monte Carlo Cell Population Dynamics in Virus Replication Systems

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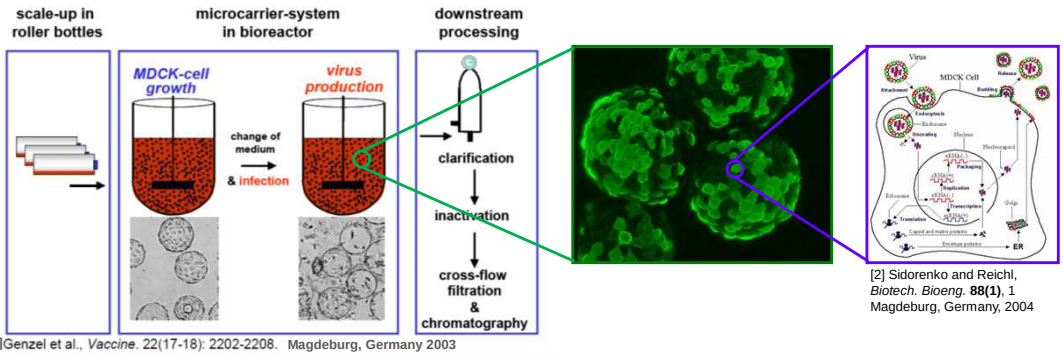
Motivation

Influenza virus infection poses an increasingly serious, maybe even deathly, **human health risk**. As globalization goes on and virus spread cannot be controlled in reliable ways the risk of infection increases every year. Mutation of new virus types may lead to more dangerous and easy spreading types with epidemic or even pandemic scenarios. **Preventing** the influenza virus infection **by yearly immunization** is common practise in many countries all over the world, but global immunization will need vaccines for billions. Today there are well-known **limitations on the production**, especially when **based on egg replication**. The development of alternative production methods based on **mammalian cell population cultivation** and virus replication inside these cells is important to overcome current vaccine production limitations. The **optimization** of the cell population based **virus replication** can be successful accompanied **by modelling and simulation.**

Process Basics

Mammalian cells (Madin-Darby canine kidney – MDCK) are **pre-cultivated** in roller bottles, transferred into a bioreactor and **grown** as adherent cells on microcarriers. They are **infected** by influenza virus and start replicating virus inside and release it to the medium. After certain time all cells **degrade** and the process ends. Now all virus is **harvested** from the medium by several separation steps [1].



[1] Genzel et al., *Vaccine*. 22(17-18): 2202-2208. Magdeburg, Germany 2003

[2] Sidorenko and Reichl, *Biotech. Bioeng.* **88(1)**, 1 Magdeburg, Germany, 2004

Model Formulation and Simulation Details

- Each cell *i* is simulated separately with internal properties based on the virus replication cycle [2] (*i=1–N)*
- Virus replication is based on the dynamical evolution of three main units inside every cell *i* :
  - viral genomic RNA (vRNA) … M_{vRNA}
  - viral messenger RNA (vmRNA) … M_{vmRNA}
  - viral complementary RNA (cRNA) … M_{cRNA}
- Replication of cRNA depends on the presence of vRNA and on the variable replication probability … p_{cRNA}
- Transcription of vmRNA depends on the presence of vRNA and on the variable transcription probability … p_{vmRNA}
- Replication of vRNA depends on the presence of cRNA and on the variable transcription probability … p_{vRNA}

- Increase or decrease of every unit number inside cell *i* is carried out at time step *k* with a constant cell variation of p_{var}=0.5:

1. Replication of cRNA: if(rand>p_{var}) $M_{cRNA}(i,k+1) = M_{cRNA}(i,k) + p_{cRNA} \cdot M_{vRNA}(i,k)$
2. Transcription of vmRNA: if(rand>p_{var}) $M_{vmRNA}(i,k+1) = M_{vmRNA}(i,k) + p_{vmRNA} \cdot M_{vRNA}(i,k)$
3. Replication of vRNA: if(rand>p_{var}) $M_{vRNA}(i,k+1) = M_{vRNA}(i,k) + p_{cRNA} \cdot M_{cRNA}(i,k)$

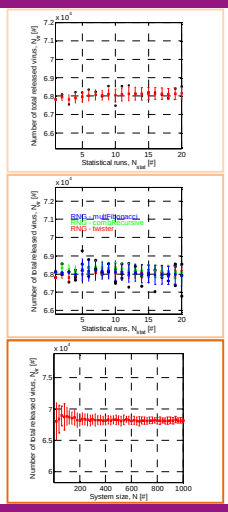
- Change of virus number in solution N_{vir} when release threshold $N_{release}$ is reached for $M_{vRNA}(i,k)$ of a cell *i* with random variation *r* :

4a. Virus release: if($M_{vRNA}(i,k) > N_{release}$) $N_{vir}(k+1) = N_{vir}(k) + ceil\,[r \cdot (M_{vRNA}(i,k) - N_{release})]$
4b. Unit decrease: if($M_{vRNA}(i,k) > N_{release}$) $M_{vRNA}(i,k+1) = M_{vRNA}(i,k) + ceil\,[r \cdot (M_{vRNA}(i,k) - N_{release})]$

- Change of unit number $M_{vmRNA}(i,k)$ and $M_{cRNA}(i,k)$ in each cell *i* by degradation probability p_{deg} with random variations *r'* and *r''*:

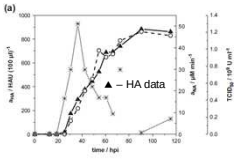
5a. Unit decrease: if($M_{vRNA}(i,k) > N_{release}$) $M_{vmRNA}(i,k+1) = M_{vmRNA}(i,k) - ceil\,[r' \cdot p_{deg} \cdot M_{vmRNA}(i,k)]$
5b. Unit decrease: if($M_{vRNA}(i,k) > N_{release}$) $M_{cRNA}(i,k+1) = M_{cRNA}(i,k) - ceil\,[r'' \cdot p_{deg} \cdot M_{cRNA}(i,k)]$

- Model implementation in MatLab:
  - Statistical analysis with mean and errors by repeated simulation runs (approx. m = 1 – 20)
  - Use of different random number generators to avoid hidden systematics (twister, combRecursive, multFibonaccil)
  - Variation of total cell numbers for finite size analysis (N = 100 – 100.000)

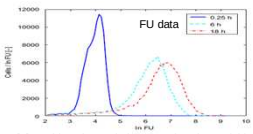


Experiment vs. Simulation

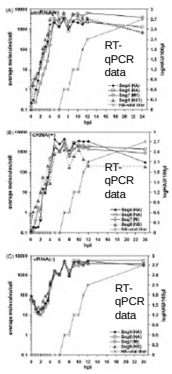
Experimental data from hemagglutination assay (HA, [3]), fluorescence microscopy units (FU, [4]) and quantitative real time reverse transcriptase polymerase chain reaction assay (RT-qPCR, [5]).



[3] Kalbfuss, Knöchlein, Kröber, Reichl: *Biologicals* **36** (2008) 145–161.



[4] Y. Sidorenko, Schulze-Horsel, Voigt, Reichl, Kienle: *Chemical Engineering Science* **63** (2008) 157 – 169.



[5] Vester, Lagoda, Hoffmann, Seitz, Heldt, Bettenbrock, Genzel, Reichl: *Journal of Virological Methods* **168** (2010) 63–71

Simulation results of **average** data for the virus number **in solution** (compare to HA, left-top), of **distributed** data of average molecule numbers **inside** the whole cell **population** (compare to FU, left-bottom) and of **averaged** data of selected molecule units **inside cells** from the complete cell population (compare to RT-qPCR, middle), as well as **distributions** of molecule unit numbers from the cell population (right).