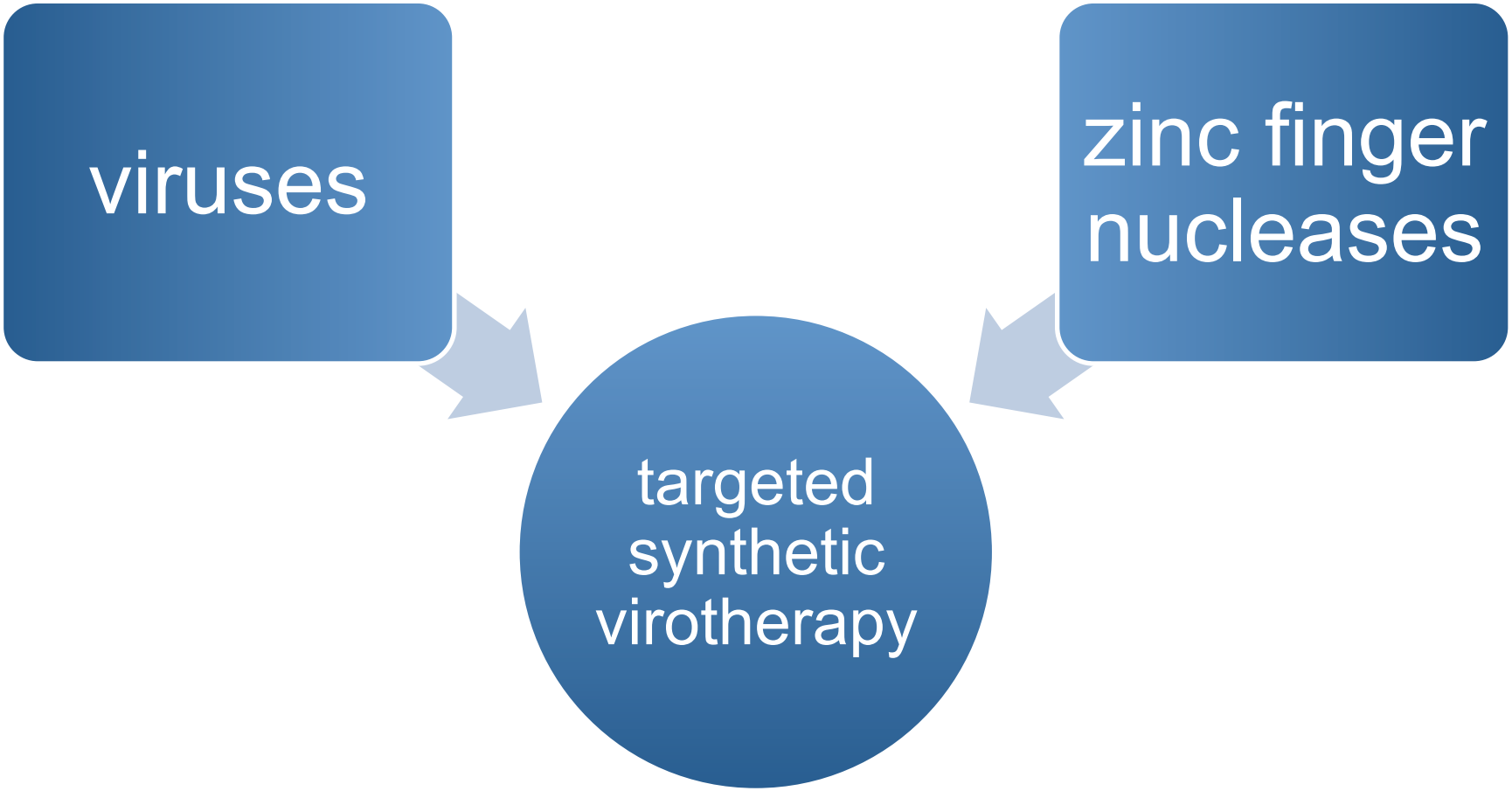
synergy project



structure

project overview

subteam “zinc finger nucleases”:
design for targeted gene correction

subteam “systems”:
virus assembly
1) cloning of adenoviral genes
2) assembly using “Biobytes”

subteam “devices”:
transcriptional targeting
1) promoter design (NFK-B 2009)
2) from analog promoters to digital threshold switches

subteam “parts”:
cell surface targeting
1) antibody-virus capsid fusions
2) cell directed virotherapy

subteam “modelling and simulation”:
predictions based on calculations

subteam “human practices and biosafety”:
can synthetic viruses be justified

reasons for viruses

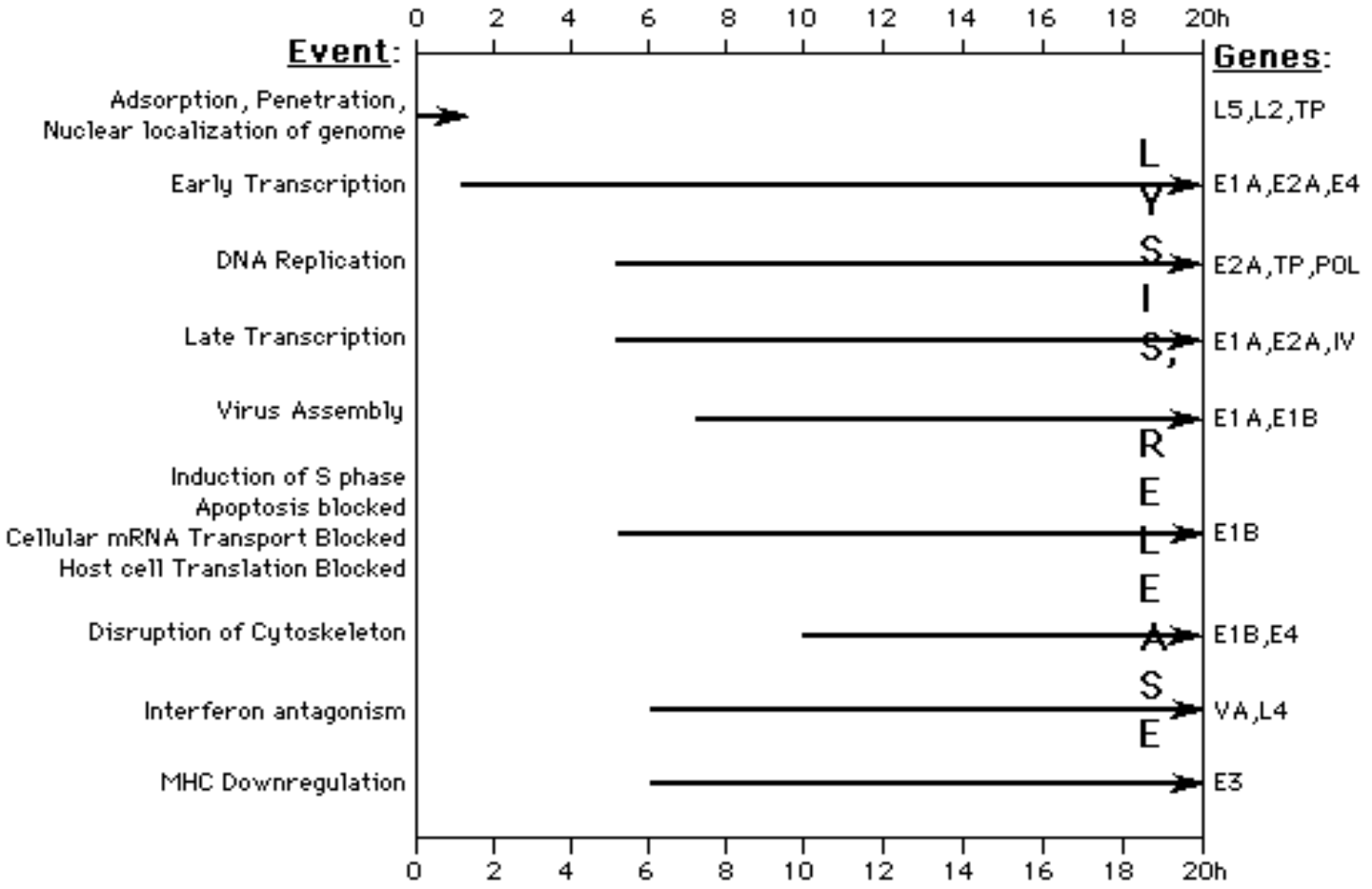
- efficient **mean** of drug delivery
- ability to **reprogram** mammalian cells
- simple yet powerful: understood on parts, devices and systems level
- sensible **extension** of Heidelberg 2009's project
- virotherapy has shown clinical **success**[1]
- virotherapy depends on “reprogramming” of viral systems
  - but: has **not yet** been attempted with a **synthetic biology** approach

“Synthetic viruses” will catch attention at the Jamboree!

[1] Liu, T.C. & Kirn, D., Gene therapy progress and prospects cancer: oncolytic viruses. *Gene Ther* 15 (12), 877-884 (2008).

synthetic virotherapy - systems

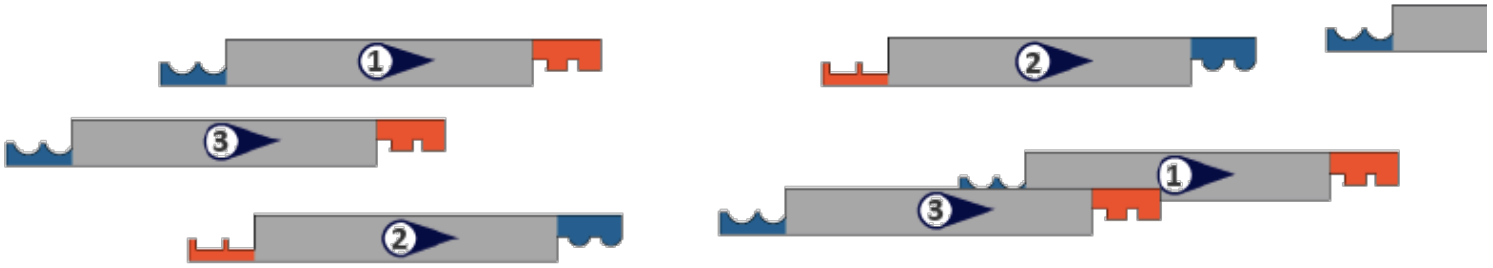
virus assembly



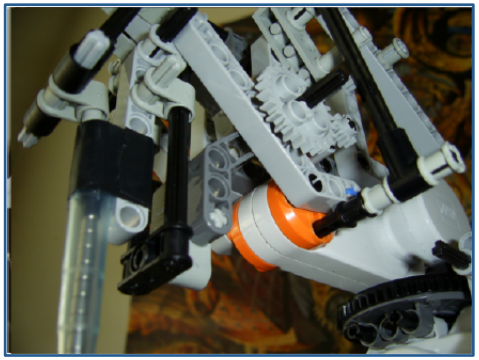
cloning and submission as parts

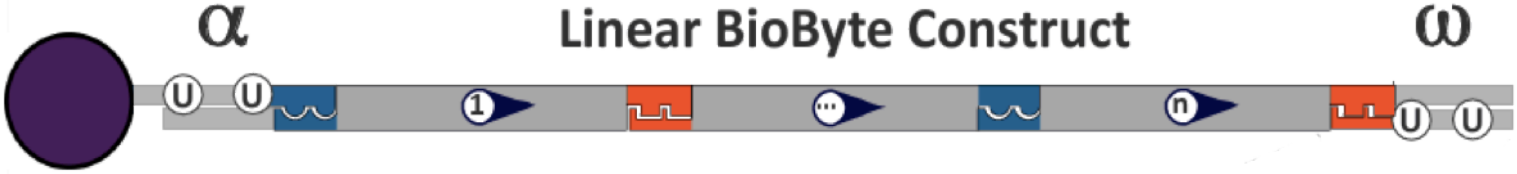
gene fusion

- usage of “BioBytes” (iGEM Team Alberta 2009)
  - allows rapid assembly of synthetic viral genomes

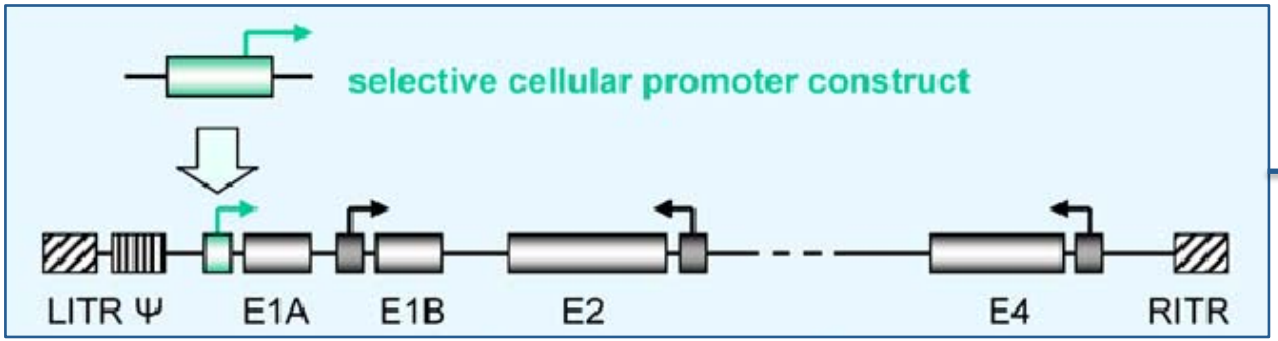


automatization:
LEGO mindstorms
pipetting system
(250€)



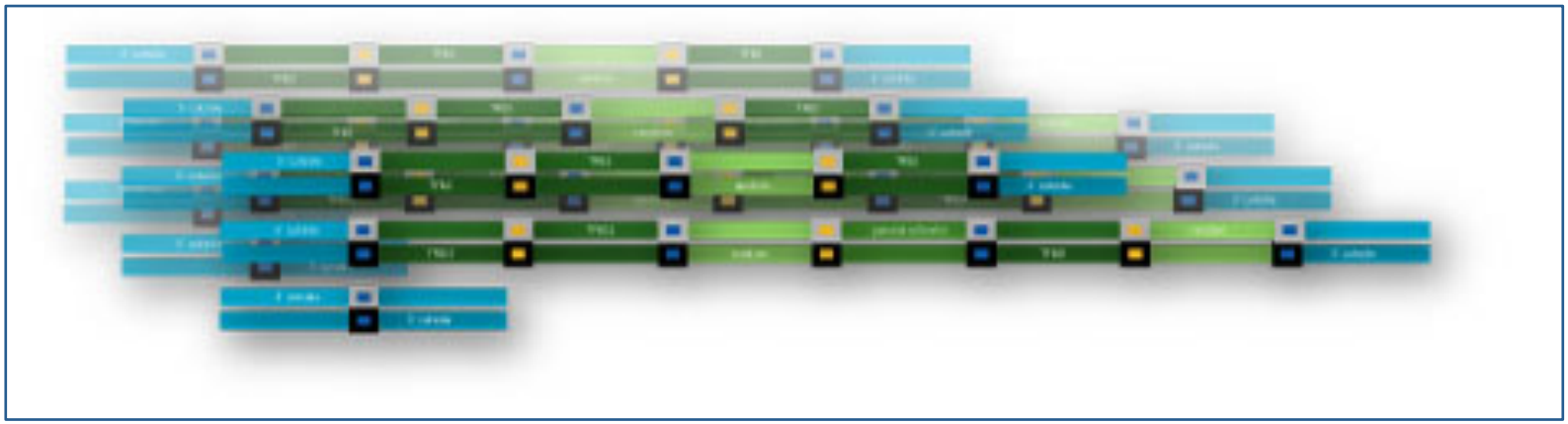


transcriptional targeting



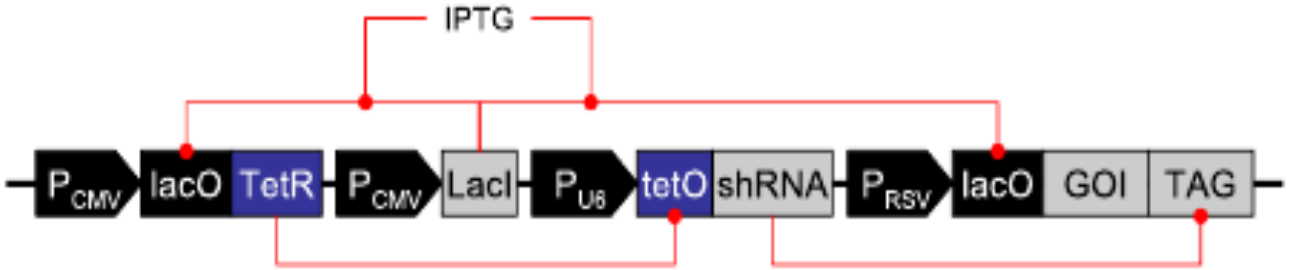
subteam "systems": **virus assembly**

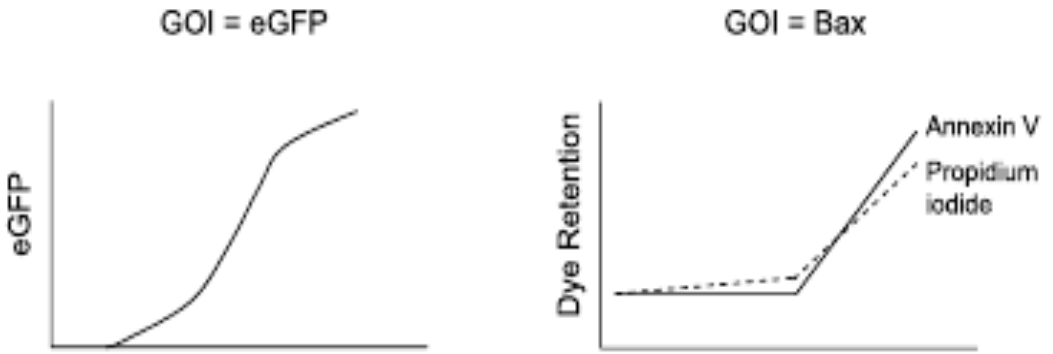
Dirk Nettelbeck, 2007



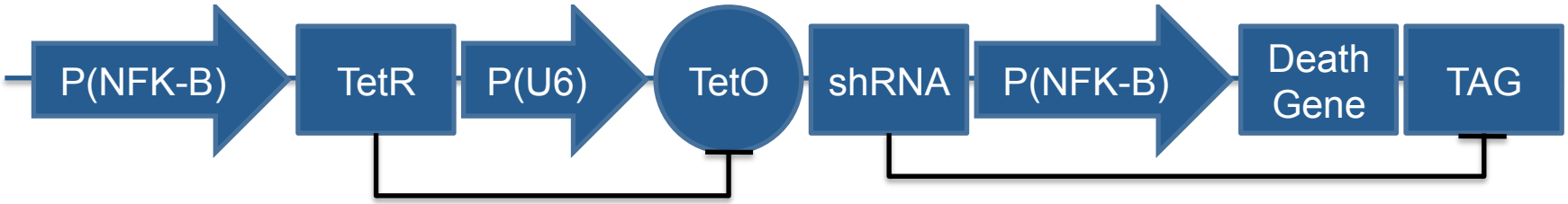
iGEM Team Heidelberg 2009: NFK-B responsive promoter

threshold devices



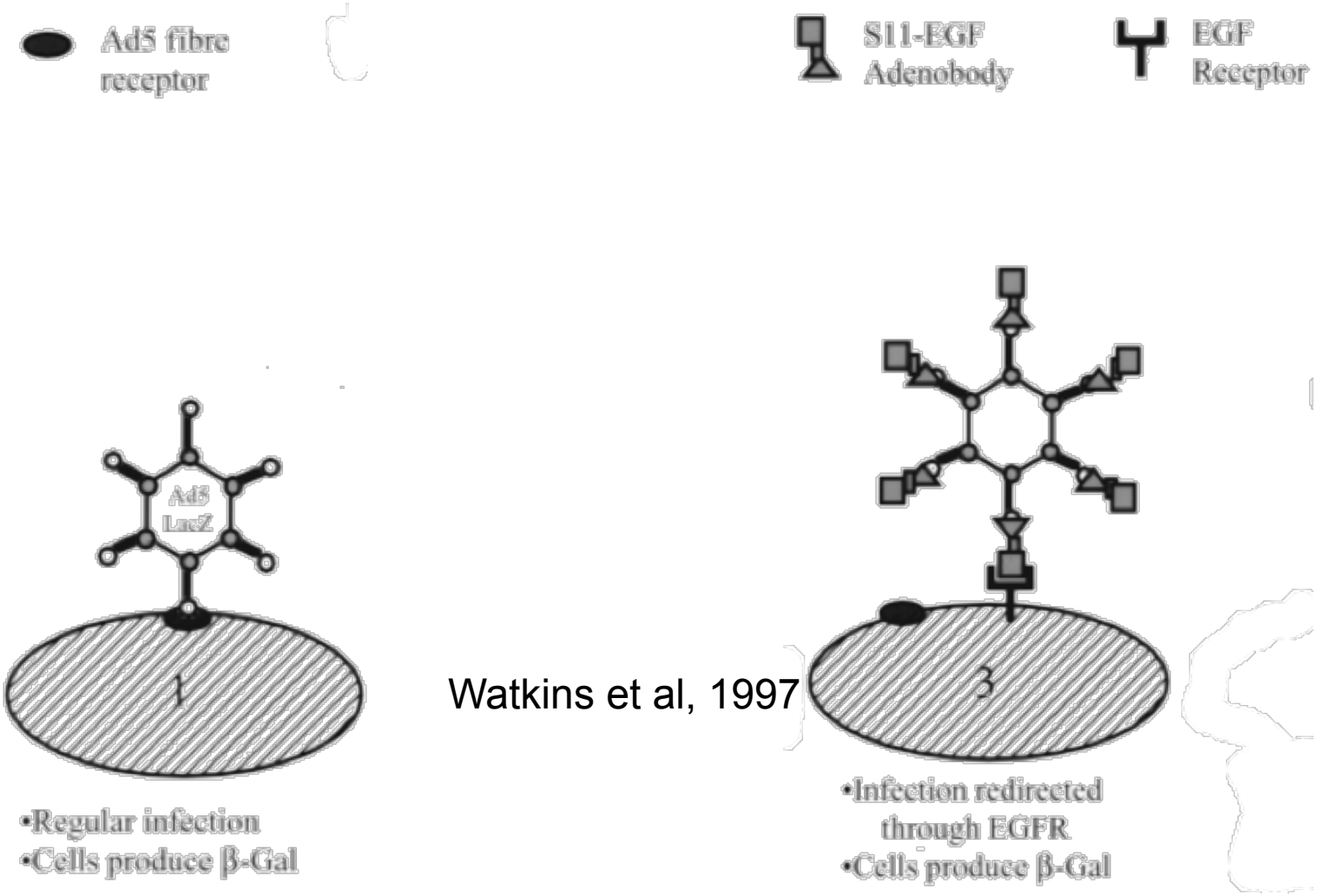


Fussenegger & Weber, 2009



- e. g. based on NFK-B specific promoter
- strategy: high-throughput screening of TetO/TAG repressors

cell surface targeting



different calculations

coupled but independent approaches: wet lab + dry lab

- certain attempts to predict immune response after viral infection[2]
  - simple mathematical models can ease understanding of drug therapy
- evaluation of virus assembly and zinc finger efficiency
  - intertwined theory and practice
- simulations for gene cluster interactions required[3]
  - target determination due to putative perturbation results
- engineering of genetic circuits for input-output regulation[4]
  - characterization of synthetic promoters

[2] Wang, Z. & Liu, X., A chronic viral infection model with immune impairment. *J Theor Biol* 249 (3), 532-542 (2007).
[3] Feng, X.J. *et al., Optimizing genetic circuits by global sensitivity analysis. Biophys J* 87 (4), 2195-2202 (2004).
[4] Voigt, C.A., Genetic parts to program bacteria. *Curr Opin Biotechnol* 17 (5), 548-557 (2006).

zinc finger nucleases (ZFN)

general facts

- **chimeric nuclease**: can be applied for targeted DNA restriction
  - 18 bp recognition sites: unique sequences in mammalian genomes
  - binding of two ZFN monomers for production of double-strand breaks[5]
    - one cleavage domain interacts with entire recognition site of one DNA strand

properties:

- **FokI**: containing endonuclease (typ II) domain for DNA cleavage
    - derived from ***F****lavobacterium* ***ok****eanokoites*
  - sequence specificity through N-terminal fusion of DNA binding domain

- **zinc finger**: interacting with specific DNA recognition sequences
    - discovered in 1985 by Miller et. al. in oocytes from in *Xenopus laevis*
  - α helix and β sheets coordinate Zn^{2+} by cystein and histidine residues

[5] Mani, M., Smith, J., Kandavelou, K., Berg, J.M., & Chandrasegaran, S., Binding of two zinc finger nuclease monomers to two specific sites is required for effective double-strand DNA cleavage. *Biochem Biophys Res Commun* 334 (4), 1191-1197 (2005).

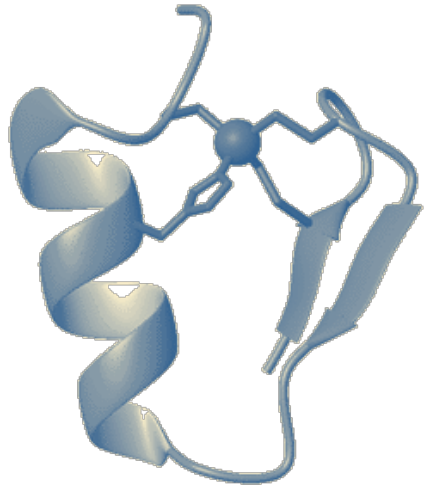
zinc finger databases

- access to (natural or engineered) motifs for known target sequences
- computational resources for new calculations

provided information online:

- database of zinc finger proteins and associated resources (ZifBASE)
  - http://web.iitd.ac.in/~sundar/zifbase
- Zinc Finger Consortium Database (ZiFDB)
  - http://bindr.gdcb.iastate.edu:8080/ZiFDB/controller/searchObjects
- including software tools for design (e. g. ZiFit)
  - http://www.zincfingers.org/software-tools.htm
- C2H2 zinc finger gene database (SysZNF)
  - http://epgd.biosino.org/SysZNF/

knowledge allows design



http://www.mmb.usyd.edu.au/mackay/images/uf9ribgif.gif

zinc finger nucleases (ZFN)

design and application

- creation of ZFN for **correction of gene defects**
  - potential to create sequence specific DNA double strand breaks
  - stimulated introduction of target genes by homologous recombination[6]

Figure[2]: Designed ZFN.
CMV – promoter, ATG – start codon, NLS – nuclear localization signal,
QQR – artificial zinc finger domain, L3 – three amino acids linker

- existing **protocols** for experimental workflow to design ZFN[7]
- established reporter system for **genome alteration** via ZFN *in vivo*[8]
  - exchange of mutated GFP* gene to restore its function
    - at least low frequency for stable incorporations into genotype

[6] Porteus, M.H. & Baltimore, D., Chimeric nucleases stimulate gene targeting in human cells. *Science* 300 (5620), 763 (2003).
[7] Carroll, D., Morton, J.J., Beumer, K.J., & Segal, D.J., Design, construction and in vitro testing of zinc finger nucleases. *Nat Protoc* 1 (3), 1329-1341 (2006).
[8] Urnov, F.D. *et al., Highly efficient endogenous human gene correction using designed zinc-finger nucleases. Nature* 435 (7042), 646-651 (2005).
* GFP = Green Fluorescent Protein

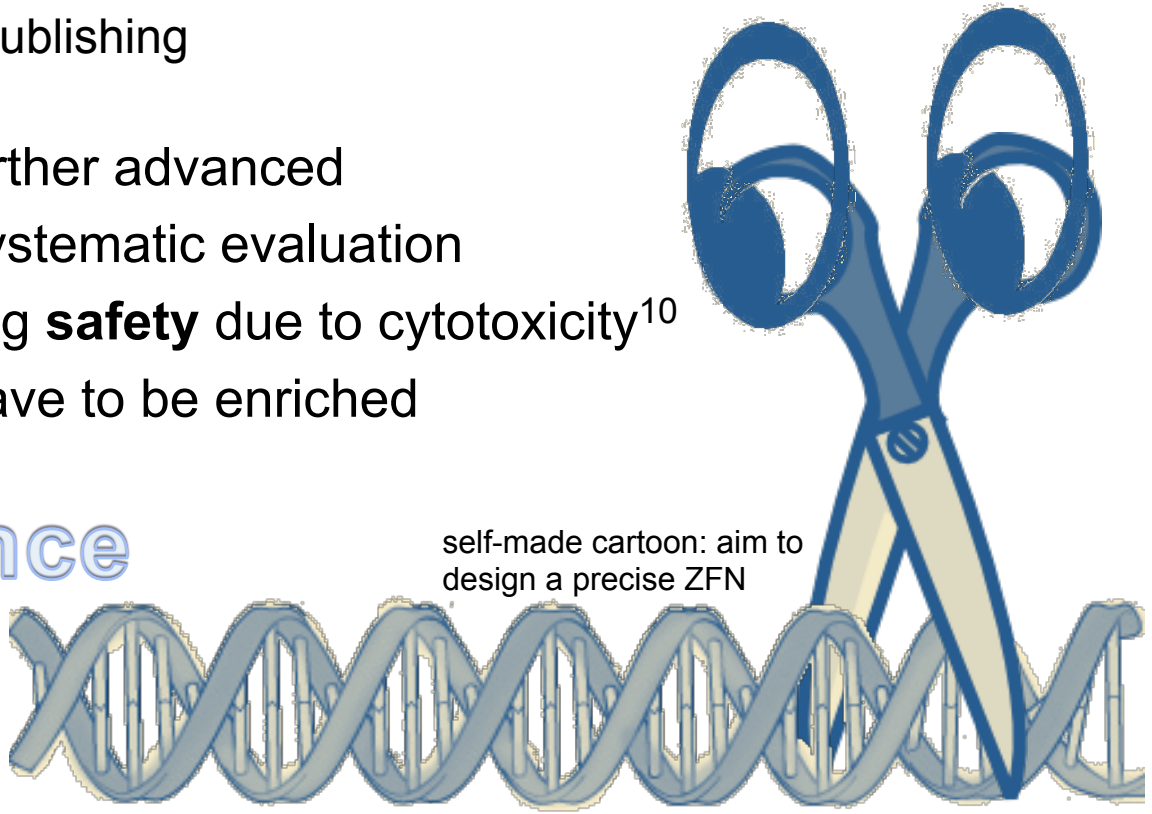
zinc finger nucleases (ZFN)

possibilities and problems

- over 1000-fold **increase** of directed homologous recombination[9]
- wide scope as a **tool** for molecular biology and biomedicine
  - ongoing research and publishing

- **insertions** have to be further advanced
- missing **standards** for systematic evaluation
- considerations concerning **safety** due to cytotoxicity[10]
- **transfection** methods have to be enriched

the iGEM chance



self-made cartoon: aim to design a precise ZFN

[9] Jasin, M., Genetic manipulation of genomes with rare-cutting endonucleases. *Trends Genet* 12 (6), 224-228 (1996).
[10] Pruett-Miller, S.M., Connelly, J.P., Maeder, M.L., Joung, J.K., & Porteus, M.H., Comparison of zinc finger nucleases for use in gene targeting in mammalian cells. *Mol Ther* 16 (4), 707-717 (2008).

partners

cooperation

- virotherapy: PD Dr. Dirk Nettelbeck (DKFZ)
  - "translate advances in basic oncology and virology research into innovative adenovirus cancer therapeutics"
  - "engineered oncolytic adenoviruses as agents for virotherapy by restricting their replication potential to cancer cells"

- zinc finger nucleases: D. Phil. Sehyo „Charley" Choe (DKFZ)

- „Targeted Genome Editing using Zinc Finger Nucleases" workshop
  - EMBL Heidelberg, 22nd – 24th June 2010
  - Scientific Organizers: Vladimir Benes, Rafael Camin, Sigma Aldrich
  - costs: approximately 800€ per participant; maybe for free

… to be fulfilled.

convincing aspects

- thoroughly planned project but still freedom of choice
  - proposal
- iGEM philosophy
  - characterization
  - standardization
  - free availability
- subteams
  - flexibility
  - no dependence
- feasible methods
- innovation

aroused interest