A chemical switch for controlling viral infectivity†

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Chemically triggered molecular switches for controlling the fate and function of biological systems are fundamental to the emergence of synthetic biology and the development of biomedical applications. We here present the first chemically triggered switch for controlling the infectivity of adeno-associated viral (AAV) vectors.

Chemically triggered molecular switches to control biological processes represent a cornerstone technology in the recent emergence of synthetic biology. Such chemical switches allow nowadays to control at will biological processes on every level of cellular signaling cascade such as receptor activation, modulation of signaling pathways, gene expression or protein degradation. The time-resolved chemical control of cellular function has provided unmatched insight into pathological and physiological processes and has opened novel opportunities in drug discovery, manufacturing of biopharmaceuticals, gene therapy and tissue engineering.

So far, such chemically controlled synthetic biological switches have been reported which prior introduction of transgenes into the cell either by non-viral or viral vector means. However, no system has been described to date, in which a conditionally controlled transgene transfer itself was used as a control point for the expression of a (therapeutic) target gene. Controlling transgene transfer to cells in a switchable manner could be used to avoid off-target expression and thus lower the risk of side effects. Due to their high efficiency and amenability to genetic engineering, viral vectors are preferred vehicles for transferring foreign DNA into mammalian cells. In particular, vectors based on the adeno-associated virus (AAV) have become popular and have recently been approved as the first gene therapeutics in the EU. In this study we describe a novel strategy to control infectivity. Specifically, we report – using AAV vectors as an example – the first small molecule-triggered chemical switch that defines viral infectivity.

The chemically switchable AAVSWITCH is based on an engineered serotype 2 viral capsid and on an adapter protein mediating inducible infectivity. The engineered capsid contains two modifications: (i) the capsid’s natural infectivity was eliminated by mutating two key residues for AAV’s primary receptor binding on target cells, a heparin sulfate proteoglycan (HSPG) structure (mutations R585A and R588A in the viral capsid proteins VP1, VP2 and VP3) and (ii) the human FK-binding protein (FKBP) was fused at the amino-terminal end to the viral capsid protein VP2 since it was previously shown that large insertions are tolerated at this position. The adapter protein consists of a modified FKBP–rapamycin binding (FRB) domain of mTOR fused to the fluorescent protein mCherry (for visualization) and a designed ankyrin repeat protein (DARPin) specific for the human epidermal growth factor receptor (DARPinEGFR). In this configuration, addition of the non-immunosuppressive and commercially available rapamycin structural analog AP21967 induces heterodimerization of the FRB–FKBP domains and thus the recruitment of cell-binding DARPinEGFR to the viral capsid surface, thereby restoring binding of the capsid to the cell surface and subsequent infection. However, in the absence of AP21967 the capsid cannot bind to the cell surface, thus preventing infection (Scheme 1).

For the synthesis of the adapter protein, a bacterial expression vector was constructed encoding (from N- to C-terminus) the FRB domain (FRB consists of amino acids 2021–2113 of the human FRAP18 and FRAP18) fused via a glycine–serine
linker to the red fluorescent protein mCherry and to DARPinEGFR. A hexahistidine tag was fused to the carboxy terminus for purification. The protein was produced in E. coli (titer: ~100 mg protein per 1 L culture) and purified by immobilized metal ion affinity chromatography (IMAC, see Fig. S1, ESI†). The functionality of the DARPinEGFR domain was confirmed by incubating the adapter protein with the EGFR-overexpressing human epidermoid carcinoma A-431 cell line for 1 h and subsequent visualization of cell-bound mCherry via fluorescence microscopy and flow cytometry (Fig. S2, ESI†). Confocal microscopy analysis revealed strong red fluorescence at the membrane and partly in the cytoplasm likely caused by EGFR internalization.19 However, cells treated with an adapter protein variant lacking the DARPinEGFR domain did not show red fluorescence thereby confirming specific DARPinEGFR-mediated cell binding (Fig. S2a, ESI†). Quantitative analysis of adapter-cell interaction by flow cytometry confirmed the microscopy observations and revealed its dose-dependency (Fig. S2b, ESI†).

In order to confirm the functionality of the FRB domain in the adapter protein, we evaluated its AP21967-dependent binding to an FKBP protein fused to the yellow fluorescent protein mVenus (for synthesis of the FKBP-mVenus protein, see the ESI† and Fig. S1). To this aim, equimolar amounts of the adapter and FKBP–mVenus were mixed, incubated with or without 5-fold molar excess of AP21967 for 1 h and subsequently analyzed by size exclusion chromatography (Fig. S3, ESI†). In the absence of AP21967, FRB–mCherry–DARPinEGFR and FKBP–mVenus were both separated as monomers, while in the presence of AP21967 both proteins eluted as heterodimers thereby confirming functionality of FRB (Fig. S3, ESI†).

Viral vector particles with an engineered capsid were produced by the adenovirus helper-free AAV serotype-2 packaging system (Fig. 1a).20 In order to eliminate natural infectivity, arginines 585 and 588 of the viral capsid proteins VP1, VP2 and VP3 were mutated to alanine (HSPG-knockout mutation).11 Furthermore, a silent mutation was introduced within the VP2 start codon T138 in order to prevent expression of wildtype VP2. Plasmid pCMVgfp harbors a self-complementary AAV genome22 encoding green fluorescent protein (sc-GFP) under the control of the CMV promoter. Plasmid pHHelper provides adenoviral genes E2A,23 E424 and VA25 required for the production of functional AAV vector particles. (b) Production and characterization of FKBP–AAV/HSPG-ko particles. FKBP–AAV/HSPG-ko were produced by transfecting the plasmids shown in (a) into HEK-293T cells. Following purification by density gradient centrifugation, viral vector particles were analyzed by SDS-PAGE and visualized by Western blot analysis. As control, AAV-2 vectors with wildtype (wt) capsid were used. Calculated molecular sizes: VP1 and VP1/HSPG-ko: 82 kDa; VP2: 67 kDa; FKBP–VP2/HSPG-ko: 79 kDa; VP3 and VP3/HSPG-ko: 60 kDa. Abnormal mobility of AAV capsid proteins in SDS-PAGE is in accordance with previous observations26 and the reduced mobility of the R585A and R588A containing capsid proteins was already shown.13 ITR, inverted terminal repeat; M, molecular weight marker; p5, p19, p40, adeno-associated viral promoters; pCMV, human cytomegalovirus promoter.
transfected into human embryonic kidney cells (HEK-293T) together with an adenovirus-derived helper plasmid (pHelper), and viral vector particles were isolated by cell lysis and subsequent purification by ultracentrifugation on an iodixanol density gradient. The viral titer was determined by quantitative genomic PCR and revealed 4.1 × 10^{11} genomic copies per mL vector stock.

Incorporation of the engineered proteins into the viral capsid was analyzed by SDS-PAGE and Western blotting revealed the expected presence of the VP1 and VP3 HSPG-ko variants and of the FKBP–VP2/HSPG-ko fusion protein (Fig. 1b).

Based on the successful synthesis and characterization of the adapter protein and the engineered viral capsid, we evaluated the functionality of AAVSWITCH. To this aim, 4.8 × 10^4 A-431 cells were seeded per well (volume: 600 μl), incubated overnight and subsequently supplemented with 10^{-7} M adapter protein, 3.3 × 10^4 viral vector particles per cell (based on genomic copy number) and optionally with 1 μM AP21967. After 48 h, infection was analyzed by visualizing the expression of the vector-encoded reporter gene gfp by confocal microscopy (Fig. 2a). Under both conditions (with and without AP21967) binding of the adapter protein to cells was confirmed by membrane- and cytoplasm-localized red fluorescence. However, only in the presence of AP21967, green fluorescent cells were observed indicating the functionality of the chemically switchable viral vector AAVSWITCH (Fig. 2a).

The performance of the chemically switchable infection was quantified by flow cytometry in the absence and presence of the adapter protein and using different vector doses (Fig. 2b and Fig. S4, ES). In the absence of the adapter, only background GFP levels were detected reflecting the efficacy of the HSPG-ko mutation to ablate natural viral tropism. In the presence of the adapter protein and the viral vector particles but without AP21967, GFP levels similar to those of the adapter-lacking controls were observed. However, upon addition of the chemical inducer AP21967 (1 μM), a more than 50-fold increase in GFP levels was observed thereby confirming the previous qualitative microscopy analysis (Fig. 2a) and the functionality of the AAVSWITCH system. The relative gene-delivery efficiency of AAVSWITCH in the AP21967-induced state was in the same range (75–85%, Fig. S5, ES) as that of AAV-2 vectors with unmodified capsids using the same number of viral vector particles per cell.

To show that AAVSWITCH is specific for EGFR-overexpressing cells, the system was tested with five other cell lines reported to express EGFR at different levels (Fig. S5 and S6, ES). It could be observed that the AAVSWITCH system infects cells with high (A-431) or medium (A549, HeLa, MDA-MB-231) EGFR expression in an AP21967-dependent manner while cell lines with low (MCF7) or absent (CHO-K1) EGFR expression are not transduced.

Fig. 2  Chemically switchable viral infection of mammalian cells. (a) Chemically switchable infection. 9.6 × 10^4 A-431 cells cultivated in 600 μl medium were supplemented with FRB–mCherry–DARPinEGFR (final concentration 10^{-7} M) and 3.2 × 10^3 FKBP–AAV/HSPG-ko particles (based on genomic copies (gc), MOI (genomic particles per cell): 3.3 × 10^4). Cells were cultivated in the absence or presence of 1 μM AP21967 for 48 h prior to confocal imaging of GFP and mCherry. Cell nuclei were visualized by DAPI staining. Scalebar, 10 μm. (b) Quantitative characterization of the chemically switchable infection. 1.6 × 10^4 A-431 cells cultivated in 100 μl medium were supplemented with FRB–mCherry–DARPinEGFR (final concentration 10^{-7} M) and 1.1 × 10^8 or 5.3 × 10^8 FKBP–AAV/HSPG-ko particles (MOI: 6.6 × 10^3 or 3.3 × 10^4). The samples were cultivated for 48 h in the absence or the presence of 1 μM AP21967 prior to flow cytometry analysis for quantifying GFP and mCherry fluorescence. A minimum of 23 000 cells were analyzed per condition. Data are means ± standard deviation (n = 3). *P < 0.005 (two-sided t-test).
In this study we describe the first viral system with chemically switchable infectivity. This system perfectly complements previously developed synthetic biological switches for controlling intracellular processes. While all these switches focus on transgenes systems already installed into the cell, AAVSWITCH allows for the first time the direct chemically switchable control of the gene transfer process beyond the described specific implementation of AAVSWITCH we assume that the here-presented approach is likely generically applicable to different viral vector systems, target cell lines and chemical inducers by modularly exchanging the dimerizer modules or the cell-binding domain. The concept presented in this study thus represents a blueprint for obtaining a time-resolved control of the cell specificity of viral vectors in order to precisely target molecular interventions in future synthetic biological devices and gene therapy scenarios.

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Notes and references

9. (a) C. S. Hackett, A. M. Geurts and P. B. Hackett, Genome Biol., 2007, 8(suppl 1), S12; (b) P. Maier, C. von Kalle and S. Laufs, Future Microbiol., 2010, 5, 1507.