HHS Public Access

Author manuscript

Gene Ther. Author manuscript; available in PMC 2011 May 01.

Published in final edited form as:

Gene Ther. 2010 November ; 17(11): 1384–1389. doi:10.1038/gt.2010.81.

Surface immobilization of hexa-histidine-tagged adeno-associated viral vectors for localized gene delivery

Jae-Hyung Jang[1,3], **James T. Koerber**[1], **Kunal Gujraty**[4], **Sridhar R. Bethi**[4], **Ravi S. Kane**[4], and **David V. Schaffer**[1,2,*]

[1]Department of Chemical Engineering and Bioengineering, University of California, Berkeley, CA 94720-1462

[2]Department of Helen Wills Neuroscience Institute, University of California, Berkeley, CA 94720-1462

[3]Department of Chemical and Biomolecular Engineering, Yonsei University, Seoul, Korea 120-749

[4]Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180

Abstract

Adeno-associated viral vectors, which are undergoing broad exploration in clinical trials, have significant promise for therapeutic gene delivery due to their safety and delivery efficiency. Gene delivery technologies capable of mediating localized gene expression may further enhance AAV's potential in a variety of therapeutic applications by reducing spread outside of a target region, which may thereby reduce off-target side effects. We have genetically engineered an AAV variant capable of binding to surfaces with high affinity via a hexahistidine-metal binding interaction. This immobilized AAV vector system mediates high efficiency delivery to cells that contact the surface and thus may have promise for localized gene delivery, which may aid numerous applications of AAV delivery to gene therapy.

Keywords

adeno-associated virus; localized gene delivery; substrate-mediated gene delivery; hexa-histidine

Introduction

Adeno-associated virus is a non-pathogenic parvovirus that depends on the presence of a helper virus, such as adenovirus, to replicate. It has a single-stranded 4.7-kb genome contains two open reading frames (ORFs), *rep* and *cap*, which encode proteins that mediate

*Address correspondence to: David V. Schaffer, University of California, Berkeley, Department of Chemical Engineering, 274 Stanley Hall, Berkeley, CA 94720-1462, Phone: 510 – 643 – 5963, Fax: 510 – 642 – 4778, schaffer@berkeley.edu.

There is no conflict of interest to disclose.

replication of the viral genome and form the viral capsid, respectively. Recombinant AAV vectors, in which a transgene is inserted in place of the viral genome, have the capacity to deliver genes to both dividing and non-dividing cells in numerous tissues, such as muscle 1, brain 2, and retina 3. Furthermore, AAV has enjoyed recent clinical success, including in clinical trials that employed AAV2-mediated gene delivery to the retina for the treatment of Leber's congenital amaurosis and resulted in significant improvements in sight for numerous patients 4, 5. Despite these numerous advantages, a number of challenges remain in engineering AAV gene delivery systems, such as achieving targeted and/or localized gene delivery to specific cells and tissues.

Gene delivery typically involves the direct injection of a vector in solution; however, this mode of administration is accompanied by its local or systemic spread away from the injection site 6. Such spreading can reduce vector levels at the target site, lead to possible side effects in off-target regions, and potentially enhance immune responses against the vector. As an alternative, immobilizing gene delivery vectors onto material surfaces followed by implantation into target regions, i.e. substrate-mediated gene delivery, is a strategy that can potentially yield effective and localized gene expression while preventing systemic vector spread 7, 8. Specifically, substrate-mediated delivery places the vectors into sustained contact with target cells to facilitate subsequent cellular internalization, and in some systems it has been shown to overcome mass transfer barriers or limitations to gene transfer 9. Additionally, substrate-mediated delivery has the potential to reduce the vector quantities required for high level gene expression, and in the cases of adenoviral vectors and non-viral vehicles, the use of lower doses in substrate-mediated delivery can result in reduced cellular toxicity, which is typically caused by the initial burst of vector upon direct injection *in vivo* 10–12. Due to these advantages, a variety of substrate-mediated gene delivery systems have been employed, primarily involving non-viral vectors and biomaterials 12, 13. Although viral vectors can have significantly higher gene transfer efficiencies than non-viral vectors, few studies have investigated viral vector delivery from substrates, presumably due to the lack of moieties on the viral surfaces that can specifically interact with biomaterials compared to engineered non-viral vectors 8, 14–16. However, developing substrate-mediated delivery approaches for viral vectors may yield systems that combine cell contact-mediated delivery with high efficiency. Due to an increasing understanding of its capsid, AAV vectors have the potential to be readily modified to interact with a substrate, thereby mediating "localized" AAV delivery to cells that come into contact with the substrate.

Results and Discussion

We have developed a strategy to specifically immobilize AAV vectors directly onto a substrate to which cells subsequently adhere, thus concentrating the virus for direct contact with the cell. Specifically, we had previously inserted a hexa-histidine (6xHis) onto a physically-exposed loop of the AAV2 (i.e., amino acid 587 position) (Figure 1a) and AAV8 capsids, which enabled an efficient, single step viral purification via immobilized metal affinity chromatography (IMAC) 17. The resulting tagged virus was able to mediate high efficiency gene delivery *in vivo* and elicited a macrophage and T-cell immune response equivalent to that of a phosphate buffer control injection 17. Here, the same 6xHis tag was

harnessed to immobilize AAV onto a material surface that presents nickel ions chelated by biotin-nitrilotriacetic acid (biotin-NTA) moieties bound to a streptavidin-coated surface (Figure 1b). Once the AAV vectors were bound to the surface, various cell types – including HEK293T, CHO, HeLa, and B16F10 cells – were plated on these substrates, and the subsequent gene delivery was analyzed and compared to gene expression achieved via the direct addition (i.e., bolus delivery) of AAV vector (with either wild type AAV2 or 6xHis tag capsids) to the cell culture medium.

The presence of histidine residues on each of 60 capsid protein subunits likely leads to the binding of a single virus to multiple Ni-NTA groups, resulting in potentially strong multivalent binding to the surface 18. Tuning virus binding levels and strength could thus potentially optimize downstream transduction.

To modulate the interactions between histidine residues and multiple Ni-NTA groups, chimeric 6xHis-AAV vectors were generated by mixing AAV packaging plasmids encoding the 6xHis mutant (pXX2 His6) or the wild type capsid 2 (pXX2Not) at various mass ratios (i.e., 100%, 75%, 50%, 25%, and 0% (w/w) pXX2 His6). In the case of 100% pXX2 His6 plasmid, all sixty viral protein subunits would display histidine residues on the capsid 17, and as the proportion of pXX2 His6 is reduced, the number of 6x histidine residues exposed on the viral shells is anticipated to decrease (Figure 2a) 19. This reduced presence of histidine tags on the virus likely decreases the extent of multivalent interaction between a single vector and Ni-NTA, thereby decreasing the overall affinity of the virus for the surface. We anticipate that the capsids should contain sufficient levels of histidine to become effectively immobilized to the surfaces; however, overly strong interactions may inhibit subsequent vector release from the surface and thereby reduce gene delivery. Therefore, low or intermediate histidine levels should both adsorb effectively and subsequently be released in close proximity to adjacent cell surfaces to mediate gene delivery. It is also possible that the binding of the immobilized virus to its cellular receptors may aid vector desorption from the surface.

We first analyzed the extent of AAV immobilization to surfaces as a function of histidine content. Interestingly, low levels of 6xHis in the vector resulted in effective AAV immobilization to the surface, and the amount of bound virus decreased with increasing histidine. That is, maximal surface binding occurred for vectors packaged with 25% of 6xHis and 75% wild type AAV2 capsid helper plasmids (i.e., 25% 6xHis AAV vectors, Figure 2b). The reduced binding with higher levels of 6xHis may be due to free capsid proteins not incorporated into viral particles competing with assembled capsids for binding to the surface. In addition to modulating the capsid, changing the concentration of biotin-NTA on the streptavidin substrate was a major factor that modulated the amount of immobilized 6xHis AAV vector (Figure 2b). Viral binding increased substantially as the level of biotin-NTA was elevated up to 10 pmol, but higher concentrations of biotin-NTA (i.e., 100, 1000 pmol) did not further enhance AAV binding. This binding saturation at 10 pmol was observed for all 6xHis AAV formulations. The increased viral binding with higher biotin-NTA levels may be due to a progressive increase in the number of sterically accessible NTA groups for the virus to bind.

The capacity for localized gene delivery to cells that come into contact with a vector-loaded substrate vector requires that the surfaces not prematurely release the vector. To assess desorption in the absence of cells, we incubated the AAV-laden substrates with cell-culture medium. Approximately 6–14% of bound vector initially dissociated from the surface, but for the subsequent 6 days no additional vector desorbed under any conditions (Figure 2c). To investigate the extent to which cells internalize virus introduced by direct addition vs. substrate-mediated delivery, cell-internalized AAV vector was quantified as a function of time and the concentration of biotin-NTA after plating HEK293 cells on the virus using a previously published approach 20. Approximately 15–20% of the surface-bound 6xHis AAV vector was associated with cells by 2 days, and the level progressively increased through day 6 (Figure 3a). In contrast, the majority of wild type AAV2 vector and 25% 6xHis AAV vector directly added to the medium was internalized into cells within 2 days of exposure to HEK293T (Figure 3a). The difference in cellular internalization between wtAAV2 and 25% 6xHis AAV upon direct addition may represent differences in the affinities of each vector for cell surface receptors, presumably heparan sulfate proteoglycans (HSPG) 21, as our previous study indicated that insertion of 6x histidine into aa587 resulted in a slightly reduced affinity for a heparin column compared with wtAAV2 17. Regardless, the cellular internalization results for the immobilized vector importantly indicate cell contact with the bound vector can lead to localized vector uptake.

To analyze substrate-mediated gene delivery, 293 cells were incubated on surfaces bound with AAV at a number of 6xHis formulations, and after 2 days luciferase gene expression was assayed. The 25% 6xHis formulation, which yielded optimal vector binding to the surface (Figure 2a), resulted in the highest substrate-mediated gene expression. This result indicates that the binding capacities of the 6xHis AAV vector are likely an important determinant of gene delivery efficiency (Figure 3b)

We next analyzed whether surface-immobilized AAV could mediate transduction of several additional cell types, including CHO, HeLa, and B16F10 cells. HEK293T and HeLa cells, which are highly permissive to AAV2, were utilized as positive controls, and Chinese hamster ovary (CHO) cells and B16F10 human melanoma cells were chosen to assess delivery to cells known to be non-permissive to AAV2. Infection with AAV carrying a luciferase reporter gene varied as a function of both the levels of histidine residues in the vector and biotin-NTA on the surface, demonstrating the potential for modulating localized gene delivery though engineering and tuning the virus-substrate interactions. Luciferase gene expression following bolus vs. substrate-mediated delivery of 25% 6xHis AAV and wtAAV2 was examined at day 2, 4, and 6. Note that, to enable a comparison with bolus delivery, the level of AAV directly added to the medium was fixed at 1×10^7 viral genomes, equal to the levels of vector immobilized to the substrate prior to addition of cells based on viral binding results (Figure 2).

The onset of gene expression following bolus infection was more rapid and could be detected by 2 days; however, luciferase expression mediated by substrate-mediated delivery reached that of bolus delivery over time (Figure 4). Substrate-mediated delivery to 293 cells demonstrated similar or slightly improved gene transfer capabilities as compared with direct addition method. One interesting and important aspect of this substrate-mediated delivery is

that comparable gene expression could be obtained even with significantly reduced quantities of internalized vector as compared with bolus delivery (~70% less in Figure 3a), indicating that substrate-mediated delivery may somehow alter intracellular processing of the vector. In addition to 293 cells, substrate-mediated delivery to HeLa and CHO cells was comparable or slightly reduced compared to bolus delivery. Finally, delivery to B16F10 cells, a human melanoma cell line reported to be non-permissive to AAV transduction 22, was equivalent or slightly higher for substrate-mediated delivery vs. bolus addition.

In conclusion, this study developed a novel AAV delivery system to mediate local gene delivery, with comparable gene transfer efficiency to a bolus delivery for a variety of cell types. Since one possible rate limiting step for AAV transduction is limitations in binding to the cell surface, we hypothesized that maintaining high local concentration of AAV vectors within the cell microenvironment, as well as increasing the physical contact time with the target cell types, may be a promising approach to mediate localized and efficient AAV vector gene delivery. Importantly, this system yielded comparable gene expression with significantly reduced internalized viral quantities compared to bolus or direct addition to medium. Furthermore, we anticipate that the level of gene expression can be tuned by controlling binding capacities (e.g., bound quantity, strength of binding, etc.), which implies that “smart” gene delivery devices can be developed for controlled release of vector. Finally, since the substrate can be potentially “upgraded” to three dimensional scaffolds, this system may have future application in tissue engineering and regenerative medicine efforts. The AAV vector can be incorporated into such a material or device as the final step, such that complex scaffold fabrication processes would not affect the activity of surface-bound AAV. The development of systems with the capacity for local, efficient gene transfer therefore represents an additional gene delivery mode with the potential for application to a number of disease therapies.

Materials and Methods

Cell culture

HEK293T and B16F10 were cultured in Dulbecco’s modified Eagle medium (DMEM; Mediatech, Herndon, VA), HeLa in Iscove’s modified Dulbecco’s medium (IMDM, Mediatech, Herndon, VA), and CHO in DMEM/F-12 (1:1) (Invitrogen) with 10% fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA) and 1% penicillin and streptomycin (Invitrogen) at 37°C and 5% CO_2. All cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA). For viral packaging, AAV293 cells (Stratagene, La Jolla, CA) were grown in DMEM with 10% FBS and 1% penicillin and streptomycin at 37°C and 5% CO_2.

Production and purification of 6xHis AAV vectors

Construction of 6x-histidine coding plasmids (pXX2 His6) and pXX2Not are described in a previous study 17, and histidine residues were inserted at 587 amino acid sequence, corresponding to physically exposed loop domain: - LQRGNLGHHHHHHSRQA- (wt AAV2: ^{583}LQRGNRQA-). Hexa-histidine-AAVs (6x His-AAV) were packaged with variable total mass ratios of 6x-histidine coding plasmids to pXX2Not. Recombinant AAV

encoding luciferase was produced by methods described in a study 23. Briefly, viral vectors were packaged using calcium-phosphate transient transfection of pAAV CMV luc, pHelper, pXX2Not, and pXX2 His6, and were harvested as previously described 24. The levels of hexahistidine-presenting capsid proteins within a preparation were changed by varying the relative amounts of pXX2 His6 and pXX2Not: 100% (7 μg: pXX2His6, 0 μg: pXX2Not), 75% (5.25 μg: 1.75 μg), 50% (3.5 μg: 3.5 μg), 25% (1.75 μg: 5.25μg), and 0% (0 μg: 7 μg). Subsequently, 1 volume of cell lysate was mixed with 0.5 volume of binding buffer (10 mM Tris [pH 8.0], 300 mM NaCl, and 20 mM imidazole) and 500 μL of 50% Ni-NTA agarose beads (Qiagen, Valencia, CA). This mixture was agitated gently overnight at 4°C, then loaded onto a plastic column (Kontes, Vineland, NJ). The loaded mixture was washed twice with 5 mL of wash buffer (10 mM Tris [pH 8.0], 50 mM imidazole), and viral vectors bound to the Ni-NTA beads were eluted with 2–3 mL of elution buffer (10 mM Tris [pH 8.0], 500 mM imidazole). The eluted virus was then concentrated using Microcon spin columns (Millipore, Billerica, MA) according to the manufacturer's instructions and buffer exchanged into phosphate-buffered saline (PBS) / 0.01% Tween.

Immobilization of 6xHis AAV vectors

Hexa-Histidine-tagged AAV vectors were immobilized onto the surface by varying the concentration of biotin-NTA and the quantity of histidine residues on the viral surfaces. Prior to immobilizing the 6xHis AAV vectors, the streptavidin-coated polystyrene surfaces (Roche, Pleasanton, CA) were pre-washed with PBS/0.01% Tween according to the manufacturer's instructions, and a mixture (100 μL) of nickel chloride (100 mM) with biotin-NTA (0, 0.1, 1, 10, 100, 1000 pmol) was agitated gently on the surface at room temperature for 4 hours. The surface was then washed three times with PBS/0.01% Tween to remove unbound Ni-NTA-biotin. Finally, purified viral vector (10^7 genomic particles) was added onto the surface and agitated gently at 4°C overnight. The surface was rinsed three times with PBS/0.01% Tween to remove unbound AAV, and each cell type (5,000 cells/well, 200 μL) was then seeded onto the surface.

Quantification of surface-bound and dissociated AAV vectors

For quantification of the surface-bound 6xHis AAV, viral genomic titers were determined by quantitative PCR. Surface-bound AAV vectors were incubated with cell-culture media without cell-seeding. At each time point, cell-culture medium was collected, and the surface was rinsed twice with PBS, which was also collected for quantification. Subsequently, viral vectors were quantified using QPCR. For quantification of the surface-dissociated 6xHis AAV, immobilized 25% 6xHis AAV vectors were incubated with cell-culture media without cell-seeding for each time point (4hrs, 1, 2, 4, 6 days). As with quantifying surface-bound AAV, both cell culture medium and rinsed buffers were collected at each time point, and viral vectors were quantified using QPCR.

Quantification of vector internalization

Viral vectors associated with cells were quantified to examine each delivery mechanism (bolus vs. substrate-mediated delivery). Note that percentages for each delivery mechanism were calculated from the initial quantities that were added to the medium (bolus) and to the surface for binding (substrate). After a 2, 4, or 8 day incubation at 37°C, the media were

removed, and the surface was rinsed twice with PBS. Cells were subsequently trypsinized and collected by centrifugation 20. To prevent the immediate association of surface-bound AAV vectors on the detached cells during the trypsinization, the cultures were incubated and blocked with heparin (30 μg/mL) at 37°C for 30 minutes prior to trypsinization. Vectors in the collected buffers were harvested and quantified using QPCR to estimate the amount of cell-internalized AAV vector.

Transduction assay

At several time points (2, 4, and 6 days), luciferase levels were measured using a luminometer (Turner Biosystems), which was set for a 2s delay with signal integration for 10s, using the luciferase assay system (Promega, WI), with levels normalized to the total amount of protein, which was measured using a BCA™ Protein Assay Kit (Pierce, Rockford, IL). For bolus delivery, 10^7 genomic particles of wt AAV2 or 25% 6xHis AAV were directly added to the cell culture medium (5,000 cells / well).

Acknowledgments

This work was supported by NIH R01HL081527. In addition, JJ was supported by a grant from the California Institute for Regenerative Medicine (Training Grant Number T1-00007). The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of CIRM or any other agency of the State of California.

References

1. Fisher KJ, Jooss K, Alston J, Yang Y, Haecker SE, High K, et al. Recombinant adeno-associated virus for muscle directed gene therapy. Nat Med. 1997; 3:306–312. [PubMed: 9055858]
2. Kaplitt MG, Leone P, Samulski RJ, Xiao X, Pfaff DW, O'Malley KL, et al. Long-term gene expression and phenotypic correction using adeno-associated virus vectors in the mammalian brain. Nat Genet. 1994; 8:148–154. [PubMed: 7842013]
3. Flannery JG, Zolotukhin S, Vaquero MI, LaVail MM, Muzyczka N, Hauswirth WW. Efficient photoreceptor-targeted gene expression in vivo by recombinant adeno-associated virus. Proc Natl Acad Sci U S A. 1997; 94:6916–6921. [PubMed: 9192666]
4. Maguire AM, Simonelli F, Pierce EA, Pugh EN Jr, Mingozzi F, Bennicelli J, et al. Safety and efficacy of gene transfer for Leber's congenital amaurosis. N Engl J Med. 2008; 358:2240–2248. [PubMed: 18441370]
5. Bainbridge JW, Smith AJ, Barker SS, Robbie S, Henderson R, Balaggan K, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. N Engl J Med. 2008; 358:2231–2239. [PubMed: 18441371]
6. Selkirk SM. Gene therapy in clinical medicine. Postgrad Med J. 2004; 80:560–570. [PubMed: 15466989]
7. Hu WW, Wang Z, Hollister SJ, Krebsbach PH. Localized viral vector delivery to enhance in situ regenerative gene therapy. Gene Ther. 2007; 14:891–901. [PubMed: 17344901]
8. Stachelek SJ, Song C, Alferiev I, Defelice S, Cui X, Connolly JM, et al. Localized gene delivery using antibody tethered adenovirus from polyurethane heart valve cusps and intra-aortic implants. Gene Ther. 2004; 11:15–24. [PubMed: 14681693]
9. Jang JH, Houchin TL, Shea LD. Gene delivery from polymer scaffolds for tissue engineering. Expert Rev Med Devices. 2004; 1:127–138. [PubMed: 16293016]
10. Mizuguchi H, Hayakawa T. Targeted adenovirus vectors. Hum Gene Ther. 2004; 15:1034–1044. [PubMed: 15610604]
11. Barnett BG, Crews CJ, Douglas JT. Targeted adenoviral vectors. Biochim Biophys Acta. 2002; 1575:1–14. [PubMed: 12020813]

12. Jang JH, Bengali Z, Houchin TL, Shea LD. Surface adsorption of DNA to tissue engineering scaffolds for efficient gene delivery. J Biomed Mater Res A. 2006; 77:50–58. [PubMed: 16353173]
13. Lei P, Padmashali RM, Andreadis ST. Cell-controlled and spatially arrayed gene delivery from fibrin hydrogels. Biomaterials. 2009; 30:3790–3799. [PubMed: 19395019]
14. Yue TW, Chien WC, Tseng SJ, Tang SC. EDC/NHS-mediated heparinization of small intestinal submucosa for recombinant adeno-associated virus serotype 2 binding and transduction. Biomaterials. 2007; 28:2350–2357. [PubMed: 17298843]
15. Fishbein I, Alferiev IS, Nyanguile O, Gaster R, Vohs JM, Wong GS, et al. Bisphosphonate-mediated gene vector delivery from the metal surfaces of stents. Proc Natl Acad Sci U S A. 2006; 103:159–164. [PubMed: 16371477]
16. Mei L, Jin X, Song C, Wang M, Levy RJ. Immobilization of gene vectors on polyurethane surfaces using a monoclonal antibody for localized gene delivery. J Gene Med. 2006; 8:690–698. [PubMed: 16634113]
17. Koerber JT, Jang JH, Yu JH, Kane RS, Schaffer DV. Engineering adeno-associated virus for one-step purification via immobilized metal affinity chromatography. Hum Gene Ther. 2007; 18:367–378. [PubMed: 17437357]
18. Vance D, Martin J, Patke S, Kane RS. The design of polyvalent scaffolds for targeted delivery. Adv Drug Deliv Rev. 2009; 61:931–939. [PubMed: 19596034]
19. Arnold GS, Sasser AK, Stachler MD, Bartlett JS. Metabolic biotinylation provides a unique platform for the purification and targeting of multiple AAV vector serotypes. Mol Ther. 2006; 14:97–106. [PubMed: 16624620]
20. Duan D, Yue Y, Yan Z, Yang J, Engelhardt JF. Endosomal processing limits gene transfer to polarized airway epithelia by adeno-associated virus. J Clin Invest. 2000; 105:1573–1587. [PubMed: 10841516]
21. Summerford C, Samulski RJ. Membrane-associated heparan sulfate proteoglycan is a receptor for adeno-associated virus type 2 virions. J Virol. 1998; 72:1438–1445. [PubMed: 9445046]
22. Girod A, Ried M, Wobus C, Lahm H, Leike K, Kleinschmidt J, et al. Genetic capsid modifications allow efficient re-targeting of adeno-associated virus type 2. Nat Med. 1999; 5:1052–1056. [PubMed: 10470084]
23. Koerber JT, Maheshri N, Kaspar BK, Schaffer DV. Construction of diverse adeno-associated viral libraries for directed evolution of enhanced gene delivery vehicles. Nat Protoc. 2006; 1:701–706. [PubMed: 17406299]
24. Maheshri N, Koerber JT, Kaspar BK, Schaffer DV. Directed evolution of adeno-associated virus yields enhanced gene delivery vectors. Nat Biotechnol. 2006; 24:198–204. [PubMed: 16429148]

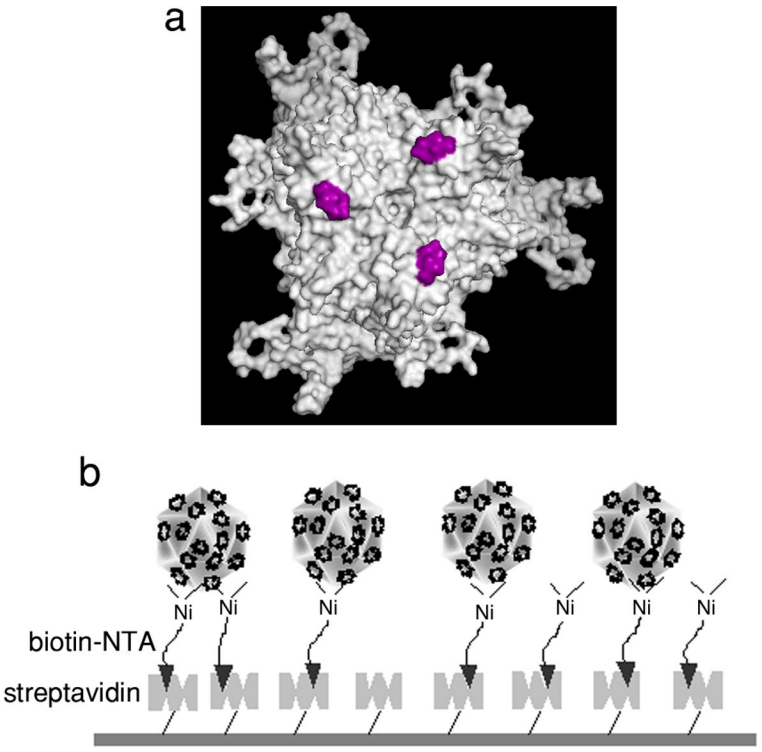

Figure 1.

Schematic illustration of 6xHis AAV vectors and immobilization of the vectors onto the surface. **(a)**: A view of the trimer at the 3-fold axis of symmetry in the capsid (Rasmol). The heparan sulfate proteoglycan (HSPG) binding site, where the 6xHis insertion occurs, is shaded in black. **(b)**: Binding of 6xHis AAV vectors on Ni-NTA-biotin conjugated on the streptavidin surface.

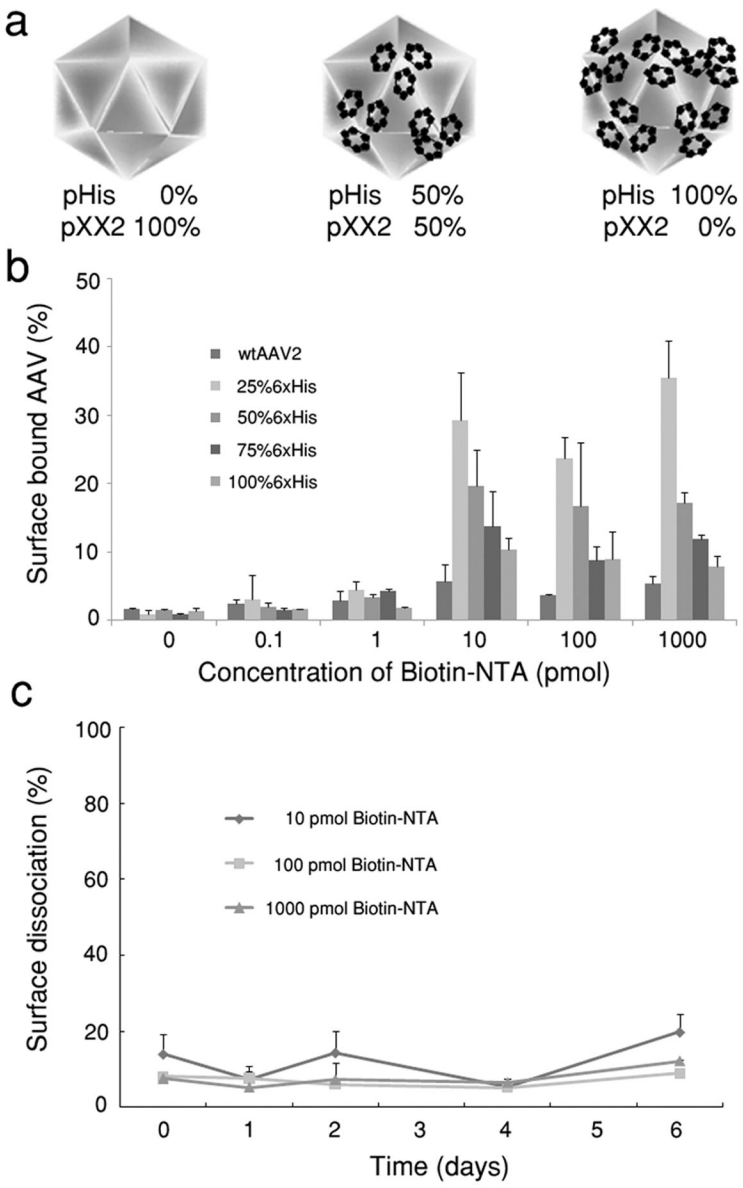

Figure 2.

(a): Schematic illustration of 6xHis AAV vectors formed by varying the mass ratio of pHis to pXX2. **(b)**: Surface-bound quantity of hexa-histidine tagged AAV as a function of both the concentration of biotin-NTA on the surface and the fraction of histidine residues on the viral surface. **(c)**: the amount of 25% 6xHis AAV vectors dissociated from the surface containing no cells.

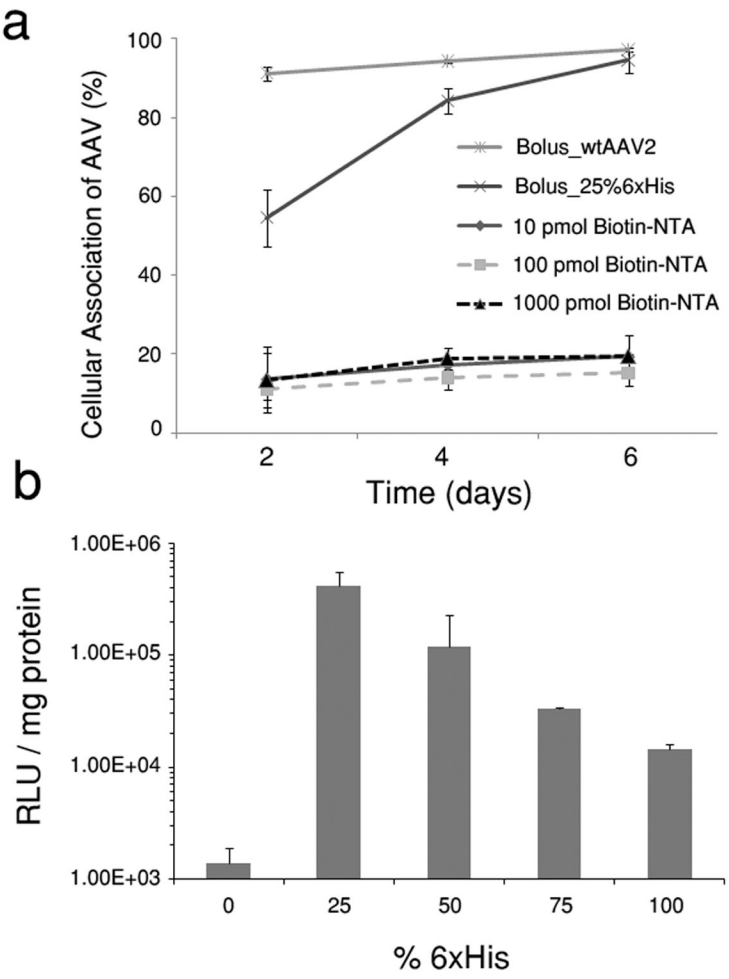

Figure 3.

(a): Quantification of vector internalization into cells after Ni-NTA surface mediated delivery or 25% 6xHis AAV or bolus delivery of vectors. **(b)**: HEK293T cell infection and luciferase gene expression by substrate-bound AAV vectors, which were formulated with different ratios of pHis6/pXX2.

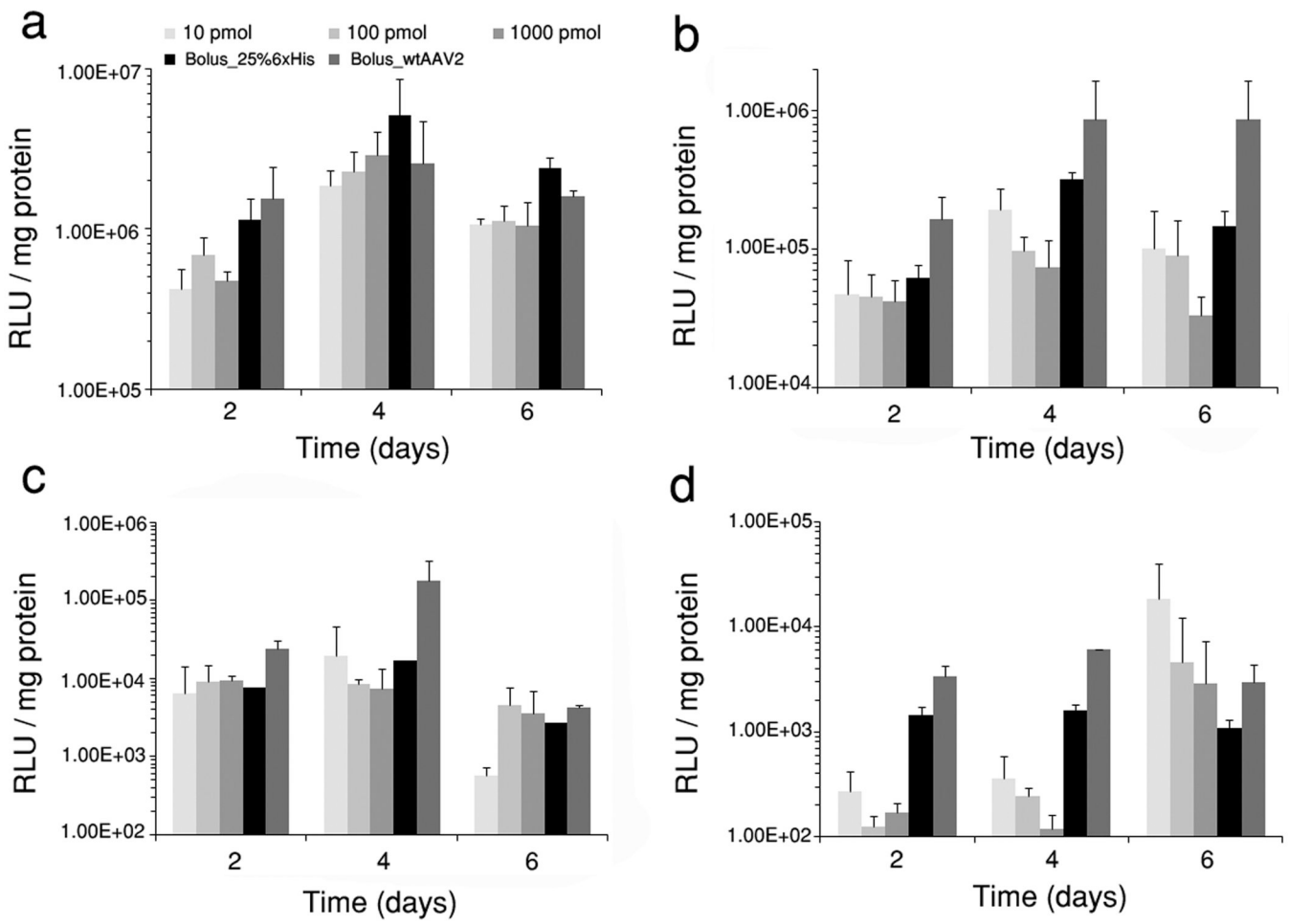

Figure 4.

Luciferase expression following substrate-mediated and bolus gene delivery. Luciferase expression for various cell types – including **(a)** HEK293T, **(b)** HeLa, **(c)** CHO, and **(d)** B16F10 cell lines – were investigated.