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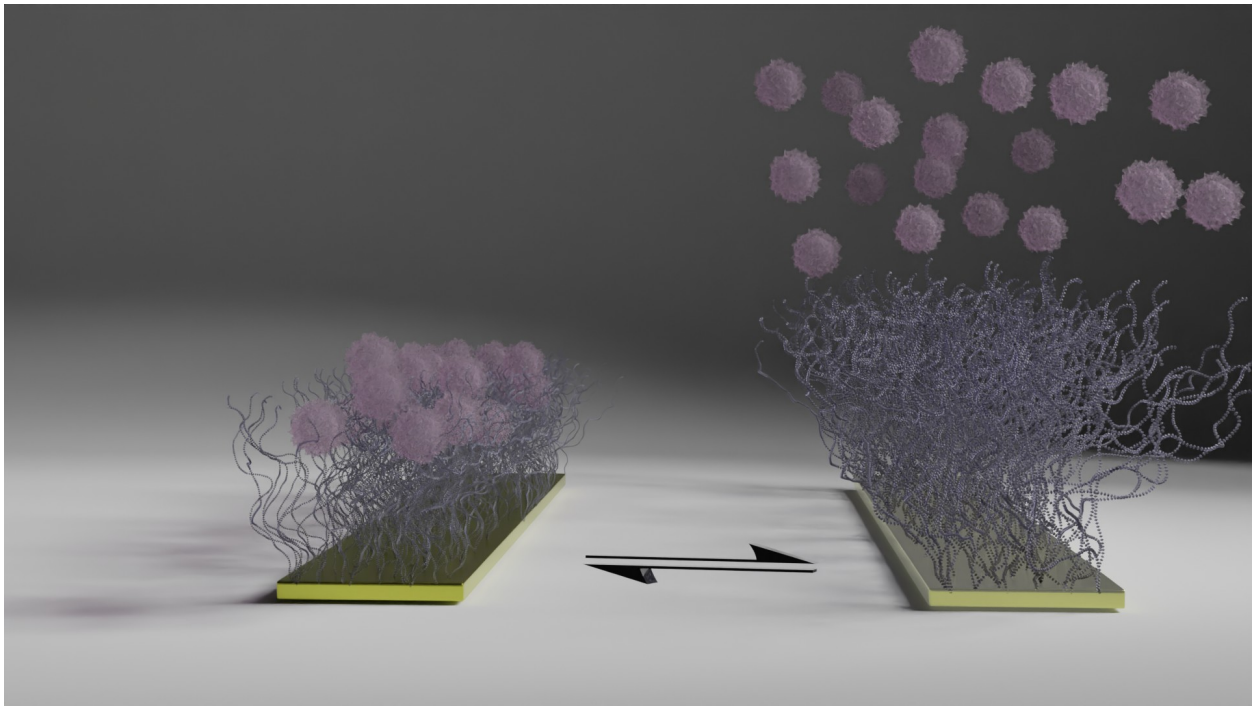

Purification of Adeno-Associated Viruses Using an Electrochemically Controllable Polyelectrolyte Brush

Separation of Genome-Filled Adeno Associated Viruses from Non-Genome-Corporated Virus Capsids

Master's thesis in Biotechnology

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CHALMERS UNIVERSITY OF TECHNOLOGY
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Cover: Visualization of the capture and release mode of polyelectrolyte bushes[1].

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Abstract

Adeno-associated Viruses (AAVs) are a promising gene therapy vector due to their low toxicity, long-term gene expression, versatility in transducing various cell types, and site-specific integration into the chromosome. However, several challenges remain in AAV production, such as low yield and difficulties in gene incorporation, which necessitate a thorough purification process that is both labor-intensive and expensive. Conventional techniques, that rely on chromatography column resins, face severe limitations, especially with diverse AAV serotypes.

This thesis explores a novel purification approach using an electrochemical controllable polyelectrolyte brush (PE) brush. The PE brush serves as an adhesive surface for biomolecules, with high protein-binding capacity, and electrochemical signals induce controlled release. The electrochemical method avoids the drawbacks of conventional pH changes and hence, offers the potential to resolve impurity challenges.

The objective was to validate the electrochemical PE brush system for AAV purification. Three milestones were established: confirming immobilization and elution, evaluating product purity and quantity, and optimizing the system. Results demonstrate successful immobilization and electrochemical elution using both PAA and PDEA brushes. The system shows the potential to up-concentrate capsids and enhance the percentage of filled capsids relative to the total amount of capsids. Recirculation further improves binding efficiency.

Even though this study shows great potential to improve purification, the need for further purity enhancement needs to be acknowledged to unlock the advancement of the method in the aspect of AAV purification. Further refinements can put this innovative approach in a competitive position with conventional purification methods for AAVs.

Keywords: Adeno-associated virus, Bio-molecules, Polyelectrolyte, Purification, Separation, Selectivity, Electrochemistry, Protein purification.

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Last but not least, I want to express my gratitude to my advisor, Professor Andreas Dahlin, your guidance, and invaluable insights have been crucial in shaping the direction of my work.

This master's thesis journey has been a challenging yet fulfilling experience, and I am grateful to have had such an incredible network of support. Thank you all for being part of this significant chapter in my academic and personal growth.

Hanna Tranchell, Gothenburg, Month 2023

List of Acronyms

Below is the list of acronyms that have been used throughout this thesis, listed in alphabetical order:

AAV	Adeno-associated viruses
CV	Cyclic Voltammetry
HCP	Host Cell Proteins
PAA	Poly(acrylic acid)
PDEA	Poly(2-(diethylamino)ethyl methacrylate)
PE	Polyelectrolyte
pI	Isoelectric Point
VG	Vector Genome
VP	Viral Particles

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1
Introduction

Adeno-associated Viruses (AAVs) are deactivated virus particles used, by the biopharmaceutical industry, as vehicles to transport recombinant genes into the nucleus of specific cells where the gene is transduced (e.g. the delivering of DNA from the virus to the cell) [3] [4]. Due to AAVs wide range of cell targets, low toxicity, long-term gene expression, and site-specific integration into the chromosome, AAVs are showing promising results as an attractive vector for gene therapy. Furthermore several FDA approved AAV based gene therapies, such as Roctavian, a biomedicine treating severe haemophilia A, and Luxturna, a treatment of patients with inherited retinal disease, put AAVs as a gene vehicle at the forefront of gene therapy research [5] [6]. However, difficulties regarding the production of AAVs, such as low yield and problems with gene incorporation in the viruses, cause impurities and do therefore create a need for a labor-intensive, time-consuming, and expensive purification method with the current purification techniques [3] [7].

The virus consists of a shell of several proteins in a complex three-dimensional structure and current purification techniques, such as affinity- and anion exchange chromatography, rely on chromatography column resins with the ability to attach to specific proteins in the shell [8] [9]. However, depending on the targeted cell type the AAV aims to deliver the gene to, different variants of AAVs are used, so-called serotypes [10]. Different AAV serotypes consist of different proteins and/or structure and thus creates a need for tailored purification chromatography resins for each product [8]. These resins are usually very expensive and short-lived and hence drive a need for new purification techniques.

The work by Ferrand-Drake et al. have resulted in a new method for selectively capturing and releasing proteins and biomolecules [2]. In this technique, a polyelectrolyte (PE) brush with a high binding capacity for proteins and biomolecules is used to selectively capture targeted proteins and biomolecules on demand. By changing the pH in the PE brush environment the properties of the PE brush change and cause the proteins and biomolecules to release. It's well known since before that PE brushes have the ability to adhere proteins and biomolecules as well as release them upon changed pH [11]. However, chemical usage and pH changes could be invasive for biomolecules and proteins and also cause a risk of further impurities in need to be removed and an extra purification and polishing step might therefore be needed [8]. With the aid of electrochemistry electron transfer to the electrolyte can be enabled, which efficiently forms an electrochemical-induced, local, and temporary pH gradient, and thus overcomes the need for excessive chemicals or long-term

exposure of invasive pH to the biomolecules and proteins. The result is a new type of electro-responsive PE brush, that can be used to capture and selectively release biomolecules and proteins on demand.

In this thesis, a PE brush combined with electrochemistry was used to investigate if AAVs can be purified from impurities in a solution using this brand-new technique. The focus of the thesis was to validate that AAVs bind to the PE brush and that gene-filled virus particles could be separated from non-gene-incorporated virus impurities by using different electrochemical signals. This thesis represents one step towards an increased knowledge of PE brush combined with electrochemistry which is an important step for science and technology since it enables the development of new devices to be used in several applications in the biotechnology industry, such as protein purification, drug delivery, and nanoscale scaffolds for enzyme cascade reactions.

1.1 Objective

The main objective of this thesis was to validate the function of an electrochemical controllable PE brush to capture and release biomolecules and proteins in the scope of purifying AAV. To verify the function of the electrochemical purification system three milestones were put into place. First and foremost the system was validated by confirming sufficient immobilization and elution was achieved. Secondly, the system was evaluated by determining the purity and quantity of the product. Finally, an attempt to optimize the system in terms of improving quality, quantity, and overall effectiveness. In summary, this project aimed to validate the named system's utility for immobilizing and releasing AAVs, assess its purification capabilities, and ultimately enhance the system's performance in terms of quality, purity, and overall efficiency.
The overall hypothesis was that using electrochemistry to control a capture and release system of AAVs on a PE surface can aid the production of these carriers. To validate that this type of system could be used to purify AAVs the following aspect was considered;

- Capacity for the PE brush to immobilize AAVs.
- The ability to separate genome-filled virus particles from empty ones, by electrochemical elution.

Method validation and proof of concept are needed as a step towards the direction of commercializing this process as well as developing a plug-and-play system compatible with current technologies, such as a chromatography system, to enable an easy-to-use method.

2
Theory

Electronically controllable PE brushes can be utilized to purify a wide variation of biomolecules. Successful exploration of the purification of monoclonal antibodies, enzymes, and proteins from serum has been done with this method prior to this thesis and as the development of the process proceeds more biomolecules will be explored [12][13]. At the time of writing this thesis the development of the method is focused on the purification of biomolecules in the production of biopharmaceuticals. In this early development stage, this method has shown great capacity for immobilization and therefore, other immobilization applications could also be considered, such as drug delivery systems and nanoscale scaffolds for enzyme cascade reactions. However, this thesis focuses on using the method to purify AAVs, and the following chapter aims to cover what AAVs are, why they are needed, and how the traditional production is made. This alternative purification method will, thereafter, be presented by first describing the mechanism behind the method followed by the interaction between the AAVs and the surface.

2.1 Adeno-Associated Viruses

AAVs are small viral particles, ca 20-25 nm in diameter, that are widely used as gene-delivering carriers in gene therapy applications [14] [7]. They have a shell of proteins, called capsids, and have the ability to hold a single-stranded DNA, called transgene [15]. These capsids can cross membranes which are utilized to deliver the therapeutic gene cargo to the nucleus of a cell [16].
AAVs capsids exhibit different variations of structure, called serotypes [14]. Different serotypes are able to target certain tissues or cell types, which enables specificity and selectivity in the treatment. Other advantages such as a high safety profile and long-term gene expression make them promising viral vectors for gene therapy usage [17] [18] [16]. Furthermore, several AAV-based gene therapy products have already been approved by regulatory bodies such as FDA and EMA [15]. Notable examples of such approvals include Luxturna, An approved treatment for a rare genetic form of blindness, and Zolgensma, an approved treatment for spinal muscular atrophy. These are some of the driving factors behind the increasing demand for AAVs.

AAVs are produced by transfecting a cell culture with a plasmid containing the AAV genome, helper genes, and the gene of interest and give the culture the right environment to initiate the growth of AAVs in the cells [15]. After harvesting, which includes cell lysis and clarification to release the AAVs and remove cell debris, a

solution containing host-cell proteins (HCP), and virus capsids remains. However, only a small part of the capsids produced will incorporate the transgene, these capsids are referred to as filled capsids, while those capsids missing the whole or parts of the transgene are referred to as empty capsids [7].
Impurities such as empty capsids affect the efficiency and safety of the treatment since the risk of immunogenicity and transduction inhibition increases. Thus A pure AAV product is required for gene therapy applications.
A highly concentrated and pure product requires thorough purification which is typically achieved by following the conventional purification steps seen in Figure 2.1 [9].

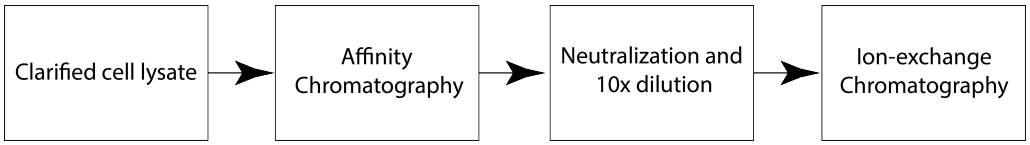

Figure 2.1: Conventional downstream process for AAV

The conventional purification process includes chromatography columns. In a typical approach, an affinity chromatography step is used to separate the viral particles from other impurities [7][15]. However, this method is not able to distinguish filled from empty capsids. The filled and empty capsids are similar in size and charge and do therefore contribute to one of the most challenging parts of the manufacturing. The most common way to separate the filled from empty capsids is by ion-exchange chromatography. This method is based on the different charge properties of the filled and empty capsids. AAVs, being proteins, carry a net charge that varies with the surrounding pH. The pH at which the capsid changes from net positive charge to net negative charge is called the isoelectric point (pI) and is approximated to 5.9 and 6.3 for filled and empty capsids respectively, for the AAV5 serotype [8] [9] [15] [7][19]. However, this approach usually requires extreme pH and high salt-concentrated buffers which can be invasive for the capsids. Moreover, achieving a high purity can compromise the yield.

In summary, the column resins used in the AAV purification process are expensive, short-lived, and serotype-specific [15]. Moreover, a several-step purification process takes a lot of time and creates a need for a lot of hands-on work and the downstream process of AAVs is therefore considered to be one of the costliest part of AAV manufacturing. New robust purification techniques are desired to overcome these challenges.
Separating AAVs through electrochemically controlled interfaces functionalized with polymers could challenge the conventional purification methods by decreasing the chromatography column cost, broadening the specificity to use on several serotypes, and decreasing the number of steps in the downstream process [8].
This innovative early-on method has shown great results in capturing and releasing biomolecules before [20]. In this thesis, an electrochemical controllable capture-release system was used to purify AAVs in aspect to explore the separation of filled

from empty capsids, by letting AAVs interact with the surface through electrostatic attraction and repulsion.

2.2 Electrochemically Controllable Polyelectrolyte Brushes

In this thesis, AAVs are purified by using an electrochemically controllable capture-and-release system. This system consists of a PE brush attached to a conductive surface which is used as the working electrode in an electrochemical cell. The PE brush serves as an adhesive surface to capture AAVs while electrochemistry serves to release the desired compound, on-demand, by locally and temporarily altering the pH in the environment close to the brush.
The following chapter aims to provide the information needed to get a comprehensive picture of the system. Firstly, by describing the protein-adhesive surface used, or more specifically the PE brushes, in chapter 2.2.1, followed by a description of how to control the PE brush by using electrochemistry, in chapter 2.2.2. Finally, a combined picture of the system will be given in chapter 2.2.3.

2.2.1 Polyelectrolyte Brushes

PE brushes are chains of polymers with a functional group that is attchaed, with one end, to a two-dimensional surface [21]. When the polymers attach to the surface at high density they create a three-dimensional, brush-like morphology with stretched polymers at the surface.

The functional groups on the PE create the ability to change charge depending on the pH of the surrounding environment [2]. PE exists in two classes: strong and weak PE, in the aspect of this project only the latter is used, more specifically poly(acrylic acid) (PAA), and poly(2-(diethylamino)ethyl methacrylate) (PDEA).

Weak PE can go back and forth from uncharged to charged state in an aqueous solution depending on the surrounding pH in the environment [2] [21]. The acid dissociation constant (pKa) indicates the pH where a specific PE has an equal number of monomers in the charged and uncharged state. The pKa is dependent on the ion concentration in the surrounding environment, which in this thesis will correspond to a salt concentration of 10 mM [2]. In this salt concentration, the PE brush consisting of PAA has a pKa at around 7, and the equilibrium reaction for this PE is shown in Figure 2.2. In the same salt concentration, the PDEA PE brush has a slightly lower pKa, and this equilibrium reaction can be seen in Figure 2.3.

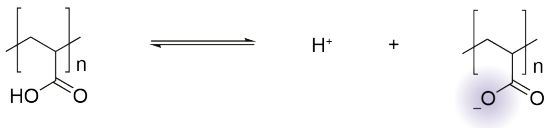

Figure 2.2: The reactions for the uncharged and charged state of the PAA polyelectrolyte [2].

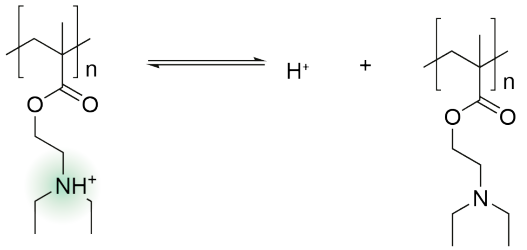

Figure 2.3: The reactions for the uncharged and charged state of the PDEA polyelectrolyte [2].

In the charged state, the PE brush will interact with the aqueous solution and hence, result in a swollen conformation of the PE brush, while an uncharged state of the brush causes a more hydrophobic behavior [21][13].
It is well known that the unique properties of the PE brushes create an effective tool to immobilize proteins and biomolecules [21][22][12]. Proteins are attracted to the PE through various types of interactions, depending on protein and PE used [13][21][20]. Hydrogen bonds contribute to the interaction by adhering proteins to the hydrogen in the functional group of PAA brush and the collapsed conformation of the brush can adhere to proteins by hydrophobic interactions. However, the main motivation for using a PE brush is the electrostatic interactions created between the charged brush and proteins. When the state of the brush changes the proteins will be released from the brush, either by electrostatic repulsion or by simply losing the interaction. To achieve effective protein immobilization, it's essential to understand the tunable dynamics of both the brush and the protein and how to adjust pH in the surrounding environment to control the interactions.

2.2.2 Electrochemistry

The electrochemistry technique used in this thesis is cyclic voltammetry (CV), an experimental method used to cyclically apply two different potentials to a system, as described by Elgrishi et al. [23]. When a CV is employed in an aqueous electrolyte, it induces a redox reaction, making it capable of locally and temporarily altering the pH of the solution.

For successful cyclic voltammetry, an electrolytic cell is essential [23]. This cell can be configured as either a two or three-electrode system, with the focus in this thesis

being on the latter. In the three-electrode system, components include a working electrode, a counter electrode, a reference electrode, and a power supply, as illustrated in Figure 2.4 [24]. To connect the electrodes an electrolyte composed of an aqueous solution is used.

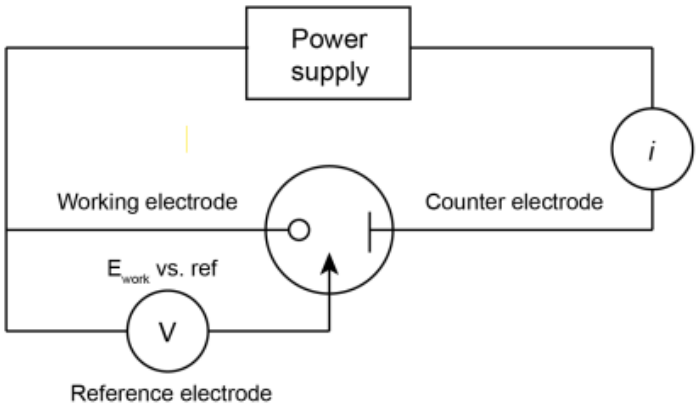

Figure 2.4: A schematic figure of a three-electrode electrolytic cell set-up [2]

This thesis will mainly focus on increasing the pH at the surface of the working electrode. This could be done by applying a negative potential, this chapter will therefore only cover the mechanism of applying a negative potential [20]. Applying a negative potential to the electrolytic cell results in a current flow from the counter electrode to the working electrode, as explained by Elgrishi et al. [23]. The excess electrons at the working electrode lead to a reduction of the electrolyte, represented by reaction 2.1 and 2.2 [24][13]:

$$O_2 + 4\mathrm{H}^+ + 4\mathrm{e}^- \leftrightarrow 2H_2\mathrm{O} \tag{2.1}$$

$$O_2 + 2\mathrm{H}^+ + 2\mathrm{e}^- \leftrightarrow 2H_2\mathrm{O}_2 \tag{2.2}$$

The power supply (potentiostat) generates a voltage that causes electrical current between the working and counter electrodes [23]. The electrolyte serves to connect the working electrode to the counter electrode and typically consists of an aqueous solution that conducts an electrical current. The electrolyte consists of a solvent containing ions that carry the charge within the solvent [24]. On the working and the counter electrode, electrons are transferred from the electrode surface to a redox-active molecule in the electrolyte [23]. The redox-active molecule is losing or gaining electrons and thus either oxidizes or reduces, this is called heterogeneous electron transfer.

A reference electrode is utilized to maintain precise and repeatable control over the current difference between the counter and working electrodes [2] [24]. If the reference is not used, the so-called two-electrode setup, variations of the potential at the counter electrode during the experiment may introduce inaccuracies in potential values.

2.2.3 Combined Picture

To enable a PE brush to be controlled with electrochemistry the polymers are polymerized onto a golden-plated surface which serves as the working electrode in a three-electrode system, accordingly to the protocol in the work of Ferrand-Drake et al. [20]. When introducing a protein to the system and by creating an environment favoring adherence, the brush will immobilize proteins by interacting with them. When negative potentials are applied to the system, the pH in the environment near the PE-coated conductive surface will temporarily increase, and the behavior of the brush changes. As mentioned before, knowledge of both the protein and the PE brush behavior is needed for proper control of the system. The following section will therefore provide the necessary information regarding the interaction between AAVs and the PE brushes used in this thesis, more specifically PAA and PDEA.

2.2.3.1 AAV Interaction with Polyelectrolyte Brushes

As mentioned previously, the AAV capsids consist of protein which carries a net charge. The net charge of the capsids is dependent on the pH in the environment and the pI defines the equilibrium between the net charge [14]. The pI of filled capsids is lower than the pI of empty capsids.

PAA brushes have a pKa at around 7 in an environment corresponding to 10 mM ion concentration [2]. A schematic illustration of the AAV interaction with the PAA brush in different pH values can be seen in Figure 2.5 When introducing AAVs to the brush in a pH environment lower than the pKa of the brush, favorable conditions for non-electrostatic interactions between the capsids and the brush are achieved [21]. Successively increasing the pH will result in an increasing negative charge in the brush [2]. Simultaneously, the capsids become a net negative which causes an anionic electrostatic repulsion. The capsids with the lower pI, filled capsids, will get net negative at slightly lower pH values than the empty capsids [14]. Hence, a separation of the filled and empty capsids could be achieved.

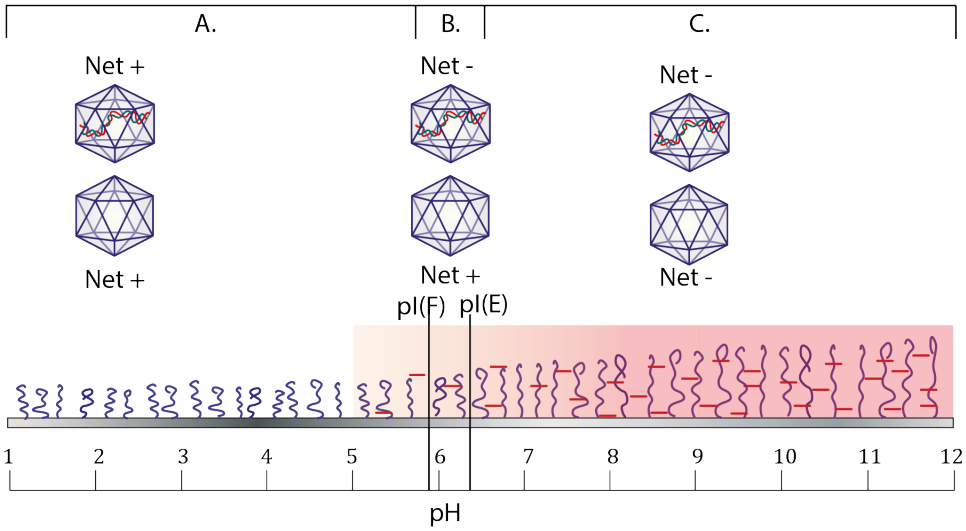

Figure 2.5: Schematic picture of the interaction between PAA brushes and virus capsids in different pH values. A: The immobilization phase where favorable binding conditions are achieved between the AAV capsids and the PAA brush. B: Illustrate the phase where genome-filled AAV capsids start to release from the brush and C: illustrate the phase where full elution is achieved

The interaction between the PDEA brush and the AAV capsids can be seen in Figure 2.6. The PDEA brush has a slightly lower pKa and opposite to the PAA brush, the PDEA brush is positively charged at pH environments lower than the pKa[2]. In a pH environment above the pI of the capsids, the PDEA brush could contribute by electrostatic interaction with proteins and biomolecules. In an increased pH environment above the pKa the PDEA dissociates its protons resulting in an uncharged state and distracting the electrostatic interaction to the attached protein and biomolecules causing them to release. The filled capsids have a stronger net negative charge than the empty ones [14]. Hence, filled capsids could adhere stronger to the brush and release later than the empty ones.

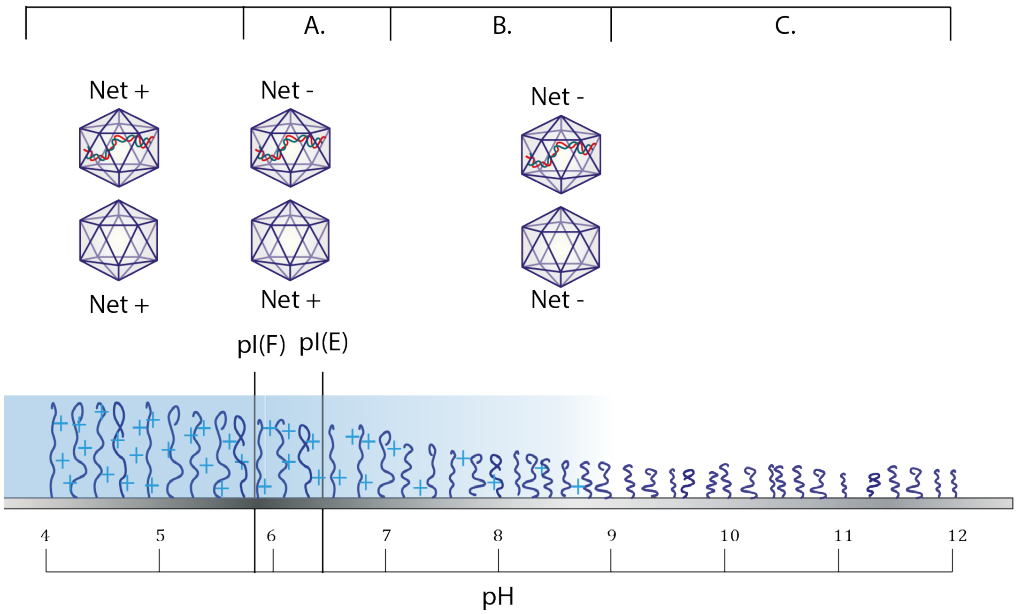

Figure 2.6: Schematic picture of the interaction between PDEA brushes and virus capsids in different pH values. A: Illustrate the immobilization phase where favorable binding properties are achieved between the AAV capsids and the PDEA brush. B: Illustrate the phase where empty AAV capsids start to release from the brush and C: illustrate the phase where full elution is achieved

3
Experimental

The purification of AAV obtained from cell culture involved the use of an electrochemically controllable PE brush. A chromatography-compatible plug-and-play flow cell, housing the PE brushes, was utilized, and its details are outlined in Chapter 3.2. Monitoring and control of the experiments were achieved through a chromatography system (ÄKTA^{TM}), with a UV sensor measuring the absorption at 255 nm and 280 nm wavelengths throughout the experiment. The experimental process began with loading the sample onto the brush. Electrochemical signals, provided by an external potentiostat (Gamry^{TM} instruments or Nyctea Technologies), were then applied to the electrodes to release the immobilized AAVs. To confirm the effectiveness of the electrochemical pulses in releasing AAVs, the experiment concluded with a pH buffer elution. Fractions collected during the experiment were subsequently analyzed and the purification was evaluated as detailed in Chapter 3.5. Chapter 3.3 provides information on the materials used in the experiments, while details about the AAV samples, including different serotypes, capsid concentration, and purity, are presented in Chapter 3.3.1.
Prior to this project, the molecular interaction was evaluated and during the course of the project the method developed as a deeper understanding of the function was achieved, chapter 3.1 gives a supportive outline of this development.

3.1 Method development

Prior to this work, the molecular interactions between the AAVs and the PE brush were explored in a surface plasmon resonance (SPR) sensor, see appendix A.1. The results of these investigations contributed to a first draft of the parameters used in the chromatography experiment.
Three experiments will be provided in this thesis, the first experiment (Named: Cationic electrochemical ion exchange purification) was performed to obtain the immobilization of AAVs on a PAA surface, electrochemical elution was carried out by applying a range of different electrochemical signals to evaluate the potential window for release of captured material in the brush. The second and third experiments (named: Anion electrochemical ion exchange purification and PDEA recirculation respectively) were performed on a PDEA brush. In the second experiment, a solution containing AAVs was used to benchmark the separation of filled and empty capsids compared to conventional ion exchange chromatography. The third and last experiment was performed to enhance the immobilization of AAVs by recirculating the sample for a longer time period.

3.2 Description of the flow-cell

A 3D printed chromatography compatible flow-cell with a volume capacity of around 2 mL was used to flow the sample through the PE brush coated meshes (see Appendix A.2). The capsule is formed as a cylinder with two screw-on lids on the top and bottom of the flow cell. The flow cell contains counter electrodes, stainless steel meshes with pore sizes from 1-10 μm, and working electrodes, golden-plated stainless steel meshes coated with PE brushes. Three pins are inserted into the flow cell, one pin on one side and two on the other side. The single pinned side connects the working electrode to the power source. On the opposite side, one pin connects the counter electrode to the power source, and the other one is a silver wire that serves as a reference electrode, which measures the current flowing through the electrodes.

3.3 Materials and preparation

Prior to this project, PE brushes, consisting of PAA and PDEA, were prepared on gold-coated stainless steel meshes by atom transfer radical polymerization, by technology developer Maria Kyriakidou, following the protocol [13].
Buffers were prepared as described in the Table 3.1 below.

Chromatographic buffer	
Purpose	Buffer solution
Wash	Milli-Q
Binding and elution	PBS: 1 mM Phosphate, 10 mM NaCl
Clean-in-place	NaOH 0.1 M

Table 3.1: Description of chromatographic buffers and their respective purpose.

Depending on the polymer used, the pH buffer for binding and elution was set accordingly to Table 3.2. The pH was adjusted with HCL 1M and NaOH 1M.

Chromatographic buffer pH setting		
Polymer	Binding buffer pH	Elution buffer pH
PDEA	pH 7	pH 11.5
PAA	pH 5	pH 8

Table 3.2: pH settings for the chromatographic buffers for respective samples.

3.3.1 Adeno-associated viruses

Two different serotypes were used. AAV5 which was supplied by Stuns Life Science and produced at Testa Center, and AAV9 provided by PhD student Brian Ladd from the department of biotechnology at KTH. Samples with different purity and capsid concentration were used and details about the sample used for each experiment are provided in Table 3.3 and 3.4. To keep track of the experiments they are numbered

according to the order represented in the result, corresponding to chronological order. The pH of the sample was set to match the binding buffer for each experiment, see Table 3.2.

Experiment chart		
Experiment name	Sample content	PE type
PAA immobilization	AAV5s and HCP	PAA
PDEA immobilization	AAV5s solution	PDEA
PDEA recirculation	AAV9s and HCP solution	PDEA

Table 3.3: Sample content and PE brush used for each experiment

Sample Concentration chart		
Experiment nr	Concentration (Cap/mL)	Percentage of filled capsids
PAA immobilization	3,6*$10^1$1	7
PDEA immobilization	3,6*$10^1$1	6
PDEA recirculation	8,3*10^8	26

Table 3.4: Total AAV concentration and viral genome (VG) percentage in the sample used for each experiment.

3.4 Purification Process

The experiments were conducted to an ÄKTA^{TM} chromatography (Pure 25 or Explorer 100) system for the capture of biomolecules. A UV sensor measured the 255 nm and 280 nm wavelength absorption, and in-line sensors measured the pH and conductivity throughout the whole experiment. For the PAA and PDEA immobilization experiments, the sample size of 2 mL was exposed to the PE above 1 h to immobilize the AAVs of the biomolecules. In the recirculation experiment a 30 mL sample was exposed to the surface for above 16 h by recirculating the sample through the brush with a flow rate of 0.5mL/min
The electrochemical elution was carried out using a potentiostat (Gamry^{TM} or Nyctea^{TM}), a CV is used as the electrochemical method and is applied to the three-electrode set-up. details of the electrochemistry method can be seen in Table 3.5, and Figure 3.1 gives an overview of how the potential changes over time for a CV scan. The specific potential limits differ for each experiment and will therefore be given for each experiment, in chapter 4.
Each experiment ended by applying an elution buffer for around 10 mL to confirm sufficient elution.

Electrochemistry Details	
Electrochemical method	Cyclic voltammetry
Scan rate	1000 mV/sec
Scan time	180 sec/pulse
Starting position	0 mV

Table 3.5: Method details for the electrochemical elution

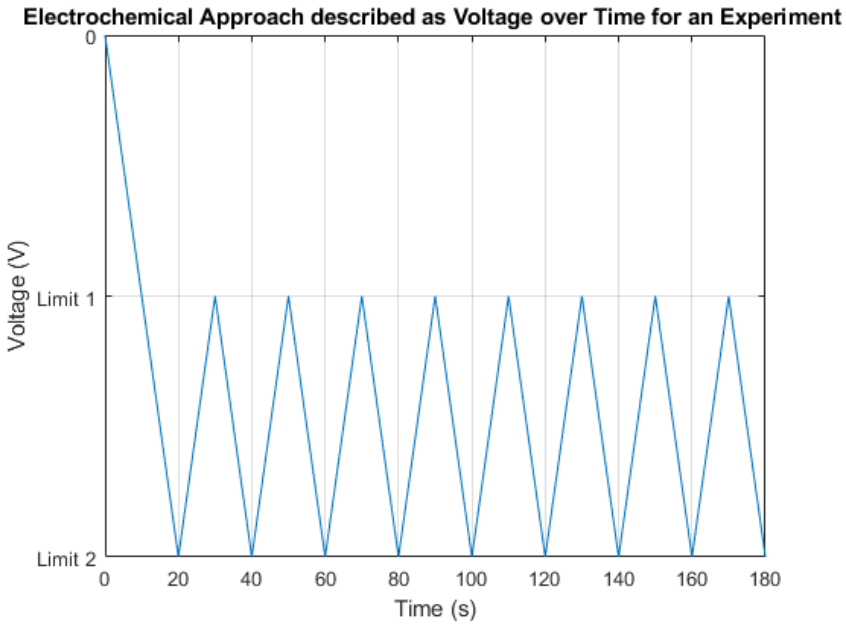

Figure 3.1: Electrochemical approach for each CV scan viewed in voltage over time.

3.5 Analysis

The purification process given in this thesis was evaluated with regard to the quantity and purity of the product. The quantity of the immobilized AAVs was determined by determined by ELISA AAV5 and AAV9 (Progen) following the protocols [25][26]. The result of the ELISA concludes the number of viral particles (VP) in a ml. The product's purity was encompassed by the method's ability to separate the filled capsids from the empty ones. To evaluate the filled and empty capsid separation qPCR was conducted to quantify the number of viral genome (VG), by following the protocol [27]. The genome titer supports how many of the capsids contain the gene of interest. The result of ELISA and qPCR was thereafter used to calculate the percentage of filled capsids relative to total amount of capsids was thereafter calculated using the equation below.

$$Filledcapsidspercentage = \frac{NumberofVP(ELISA)}{NumberofVG(qPCR)}$$

4

Results and Discussion

In this chapter, three experiments conducted in the project will be presented. The first experiment, called Cationic Electrochemical Ion Exchange Purification, was performed with a PAA brush to obtain the immobilization of AAVs on a PAA surface and to evaluate the percentage of filled virus particles in relation to the total amount of virus particles as a function of the potential window. The second experiment, called Anionic Electrochemical Ion Exchange Purification, was performed with a PDEA brush, a purer sample than the other experiments was used, and aimed to benchmark the separation of filled and empty capsids compared to conventional ion exchange chromatography. The third and last experiment, called PDEA recirculation aimed to enhance the immobilization of AAVs by recirculating the sample through the brush for a longer period of time. For each experiment, the potential magnitude for release will be given along with a figure showing the chromatogram from the experiment and a bar chart showing the result of the ELISA and q-PCR analysis. The result will be followed by a short discussion for each experiment. It needs to be stressed that not all material has been analyzed in either of the following experiments, furthermore, a loss of sample is common during the injection of sample. It is therefore not possible to conduct a complete mass-balance of fed material, captured material, and released material.

Figure 4.1 shows the result of an experiment performed with a PAA PE brush with a clarified cell lysate sample, with a percentage of 7% filled virus capsids concerning the total amount of virus particles, and in 4.2 the result of an experiment performed to purify AAVs from a pre-Capto Q solution, with a percentage at 5% of genome filled particles using a PDEA PE brush. The top graphs show the chromatogram with UV absorbance over volume flowthrough (mL), the middle graphs show the relation between total AAV capsid titer and viral particle (VP) titer, and the lower graphs show the pH variations throughout the experiment. Table 4.1 and 4.3 show the corresponding potentials applied as electrochemical signals for the respective experiment.

4.1 Cationic Electrochemical Ion Exchange Purification

Potential Magnitude	
Peak number	Potential applied (V)
1	-0.4 to -0.3
2	-0.5 to -0.4
3	-0.6 to -0.5
4	-0.7 to -0.6
5	-0.8 to -0.7
6	-0.9 to -0.8

Table 4.1: Electrochemical signals (V) applied to the PAA polyelectrolyte brush during purification AAVs from a clarified cell lysate.

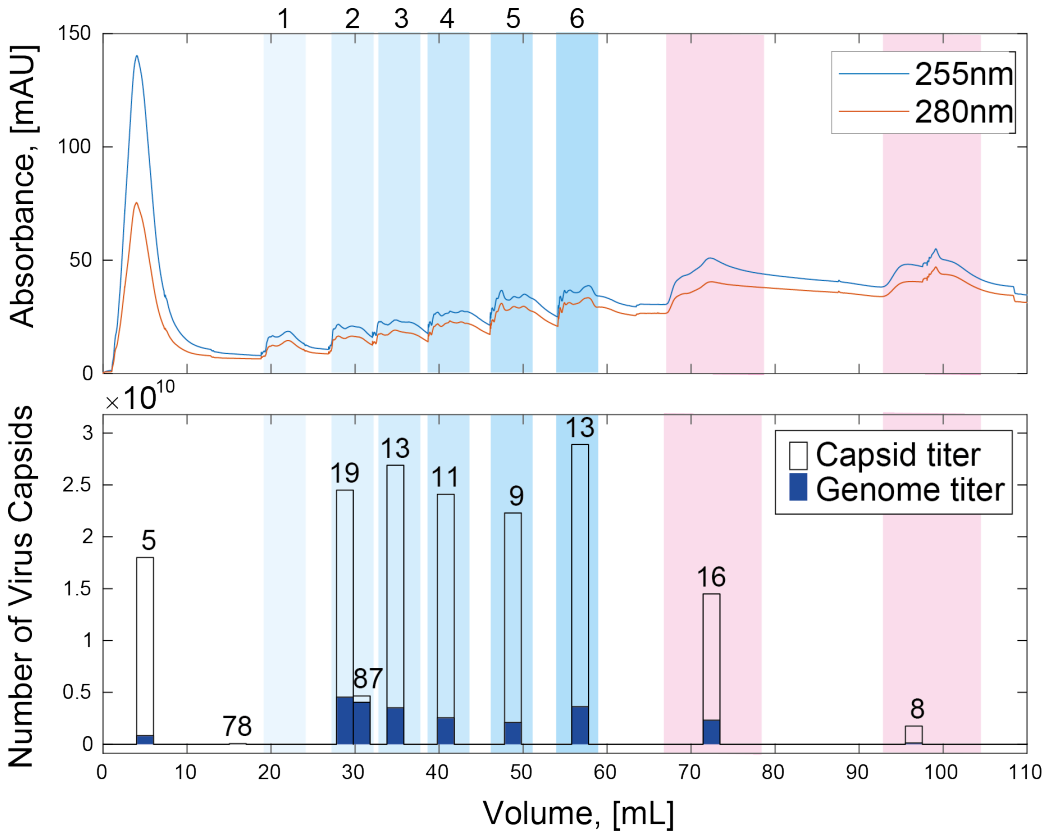

Figure 4.1: Result of AAV purification performed with a PAA polyelectrolyte brush. **Top Graph:** Shows the UV absorbance in chromatogram sensor per mL flow. **Middle Graph:** Shows the total numbers of virus capsids in collected samples (total bar height), the numbers of genome-filled virus capsids (blue part of the bar), and the percentage of genome-filled virus particles relative to the total number of virus particles (above the bar). The y-axis represents the amount of virus capsids and the x-axis represents the flow (mL). **Bottom Graph:** Shows pH detected over the flow (mL).

Figure 4.1 shows the result of an experiment performed with a PAA PE brush with a clarified cell lysate sample containing HCP and AAV5s, with 7% of VG in relation to VP. The electrochemical signals create a pH shown in the lower graph. When the pH gradient increases visible peaks are shown in the UV-absorbance spectrum indicating that the substance is released from the surface of the PE brush. The analyzed virus-capsid and genome titer bars in the middle graph further confirm that the viruses were immobilized and released upon electrochemical signal since the highest titer bars correspond to when the electrochemical signals are applied.
Since the titer bars corresponding to the immobilization and pH elution are smaller than those corresponding to the electrochemical signals, an assumption can be drawn that most of the capsids have interacted with the PAA brush and are eluting during the electrochemical signals.
Furthermore, the pH gradient created upon the electrochemical signals is dependent on the signal intensity since the pH increase is bigger when stronger signals are applied. The graph also confirms that the pH gradient produced upon the signals

is temporary and rapidly returns when the signal is removed.
As expected for the PAA brush, the titer bar trend shows a higher percentage VG relative to VP upon weaker electrochemical signals, implying that, upon electrochemical signals, the pH can be increased to the extent that VP capsids release while the empty capsids remain. However, looking at the titer bars for the second peak, the VG percentage changes radically within the electrochemical scan where most of the filled VP elutes first at the second half of the electrochemical pulse. The cause of this fluctuation needs to be further explored and learned how to be controlled. The percentage of filled VP, from the analyzed material throughout this experiment, is still quite low and there is room for improvement to remove a sufficient amount of empty capsids. However, since this experiment is conducted on a crude sample the result shown in this experiment is still impressive for this early development of the method.
The results from the Cationic Electrochemical Ion Exchange Purification experiment, described in this section, inspired the following experiments, namely Anionic Electrochemical Ion Exchange Purification and PDEA Recirculation experiments, which were performed with three electrochemical pulses, one pulse at a potential at -0.4V to -0.3V where the pH gradient was between the pI of filled and empty AAVs, one at -0.75V to -0.65V, which creates a pH gradient slightly above the pI of both filled and empty virus capsids and one at -1V to -0.5V creating a pH gradient strong enough to release all interaction to the PE.

4.2 Anionic Electrochemical Ion Exchange Purification

Potential Magnitude	
Cycle number	Potential applied (V)
1	-0.5 to -0.4
2	-0.75 to -0.65
3	-1 to -0.9

Table 4.2: Electrochemical signals (V) applied to the PDEA polyelectrolyte brush during purification of AAVs from a pre-Capto Q solution.

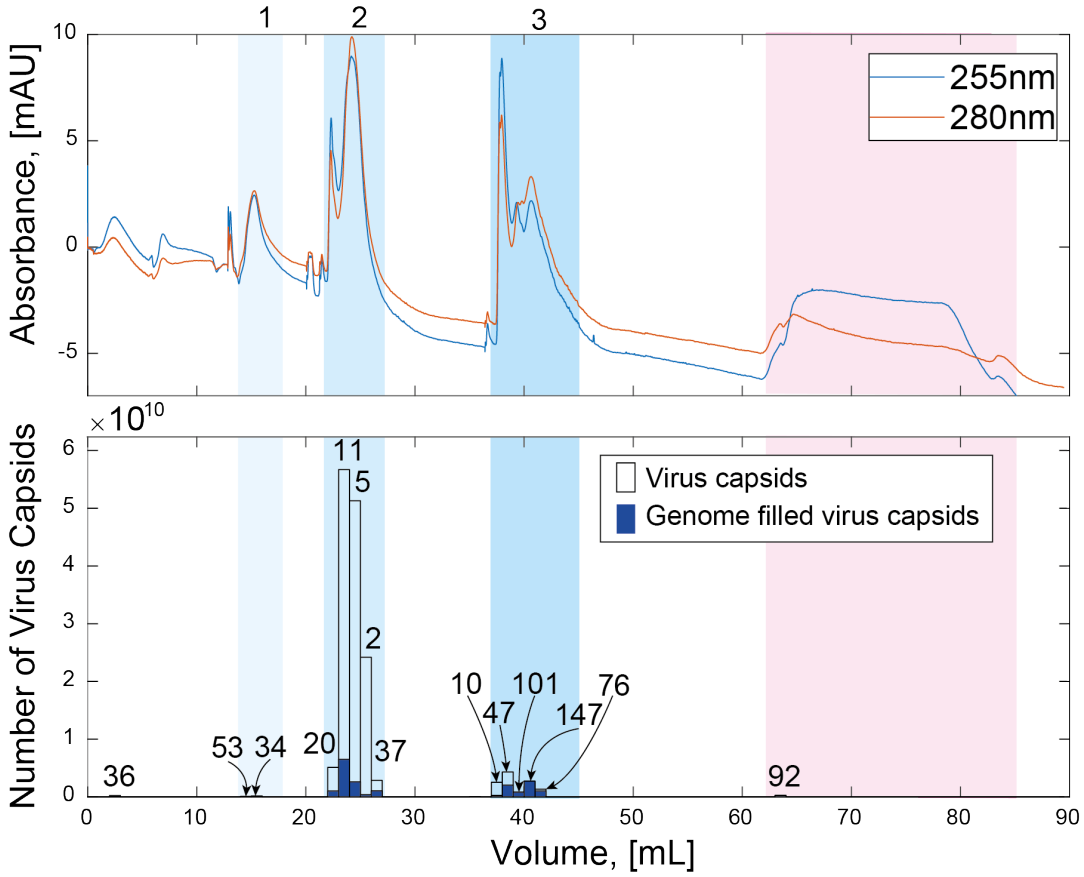

Figure 4.2: Result of an AAV purification performed with a PDEA polyelectrolyte brush. **Top Graph:** Shows the UV absorbance in chromatogram sensor per mL flow. **Bottom Graph:** Shows the total numbers of virus capsids in collected samples (total bar height), the numbers of genome-filled virus capsids (blue part of the bar), and the percentage of filled VP in relation to the total amount of VP (above the bar). The y-axis represents the amount of virus capsids and the x-axis represents the flow (mL).

In Figure 4.2 the result of an experiment performed using a PDEA PE brush to purify AAV5s. The sample used was, prior to this experiment, purified from excessive HCP using an affinity chromatography technique. The percentage of filled VP relative to the total amount of VP of the sample was 5%. In this experiment, a more sensitive UV-absorbance unit was used to detect the absorbance from the small AAV particles.

Similar to the previous experiment the titer bars, in the lower graph, that correspond to the immobilization and the pH elution are low, indicating a good binding capacity of the brush as well as sufficient potentials applied for elution. As expected with a PDEA PE brush the percentage of VG in relation to VP was higher in higher pH values, which was generated by applying the stronger potential pulses. However, the strongest pulse has a higher genome titer than the capsid titer. This could be due to burst capsids as a result of intense potentials or that the titer is too low to get accurate results.

The 255:280 nm wavelength ratio exceeds 1 at the beginning of the second and third electrochemical pulse (appears around 25 mL respective 40 mL) and is lower than 1 at the end of the electrochemical pulses. Similar to the previous experiment, this could indicate that mostly genome-filled capsids are released at the beginning of the electrochemical pulse. This phenomenon is seen both in the second and the third electrochemical pulse. However, the third pulse has a bigger peak at the region where the wavelength ratio exceeds 1, confirming more release of filled capsids at higher potentials. This trend can not be confirmed by looking at the bar plots, which is probably caused by a delay between the UV sensor and the fraction collector.

4.3 PDEA Recirculation

Potential Magnitude	
Peak number	Potential applied (V)
1	-0.5 to -0.4
2	-0.75 to -0.65
3	-1 to -0.9

Table 4.3: Electrochemical signals (V) applied to the PDEA polyelectrolyte brush during purification of AAVs from a pre-Capto Q solution with duration time and flow rate for each signal.

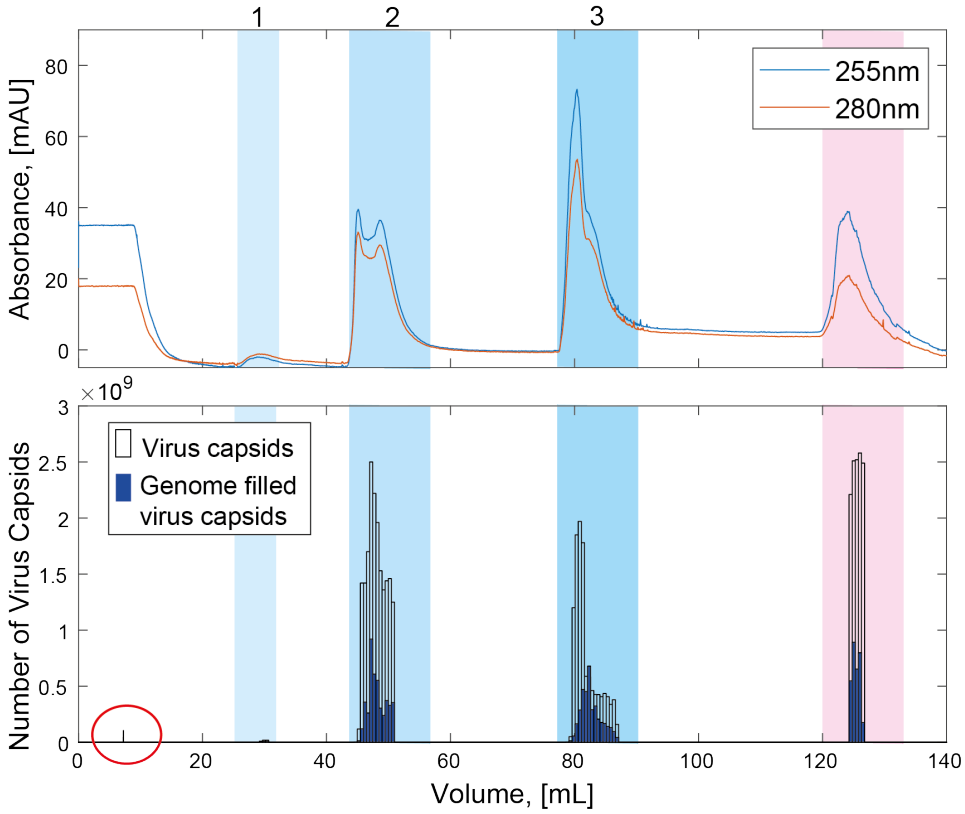

Figure 4.3: Result of an AAV purification with a PDEA brush. **Top Graph:** Shows the UV absorbance in chromatogram sensor per mL flow. **Lower Graph:** Shows the total numbers of virus capsids in collected samples (total bar height), the numbers of genome filled virus capsids (blue part of the bar). The y-axis represents the amount of virus capsids and the x-axis represents the flow (mL).

Figure 4.3 shows the result of an experiment conducted to purify AAV9s from a sample containing HCP and capsids, with a VG percentage of 26% among total number of VP. The experiment was performed on a PDEA brush and the sample was recirculated through the sample for over 16 h in an attempt to enhance binding of AAVs to the brush.

The titer bar of the remaining sample after recirculation, marked in the red circle in Figure 4.3, is very low compared to the rest of the bars, indicating a great binding capacity of the brush. Furthermore, comparing the capsid titer of the starting material at 8,3*10^8 cap/mL to the peaks from the elution shows that the sample has been up-concentrated which contributes to the conclusion that recirculation may enhance the binding.
Similar to the previous experiment, the lower electrochemical pulse (appears around 30 mL) led to almost no release of capsids, and this potential range may be excluded from future experiments. The high capsid titer bars from the pH gradient elution (appears around 125 ml) show that a significant amount of capsids were unable to be released during the electrochemical elution. This could be due to differences in pI values for different serotypes and indicates that exploration of potential ranges

might be needed for every serotype specifically.
Equal to the previous experiment the higher potential magnitude results in a higher VG percentation in relation to total amount of VP, as expected for the PDEA brush. Total percentage of VG relative to the amount of VP were calculated for all values analyzed in each peak and the result can be viewed in Table **??**, this was not done in previous experiments since too few fractions were analyzed. As expected the bars corresponding to the larger potential magnitude resulted in the highest percentage of filled VP in relation to total amount of VP. However, this value is still insufficiently low and significantly lower than in the previous experiment, which further indicates the need for tweaking the potential magnitude when working with different serotypes.

Whole Peak percentage of VG over VP		
-0.75V to -0.65V	-1V to -0.9V	pH Elution
24%	32%	25%

Table 4.4: The total percentage of filled VP over the total amount of VP for each peak measured for PDEA recirculation experiment.

4.4 Discussion

This project has focused on validating the use of the electrochemistry controllable PE brushes in a chromatography set-up for purifying certain AAVs, however, other aspects and influencing factors of the method remain to be explored. First and foremost, there is a significant risk that HCP is captured by the PE brush and released at the same time as the AAVs. However, due to time limits, this was not explored during the project. Secondly, even though only two different types of PE (PDEA and PAA) were used, different batches of brushes were used throughout the project. Not surprisingly batch variations for the brushes exist and do highly affect the results. The differences in the batches could be due to variations in grafting density but also due to batch differences in the diazonium salt used to anchor the PE to the conductive surface. Grafting density highly affects the binding capacity of the surface, making it hard to determine if the high binding was due to the PE type used or batch variations, more experiments are needed to validate this. Not only do batch variations affect the binding capacity of the brush they could also affect the life span. This effect was experienced during the project, where the functionality of the brush seemed to decrease over time. Other brushes did, however, last until the end of the project. Commercialization of this technique requires more even results.

4.4.1 Impact of Flow-Cell Design

The pipe-shaped flow-cell is flexible since it is small and mobile, allows assembling to a chromatography system, and is easy to use and reassemble if the content needs to be changed. It also contributes to advantages including allowing continuous flow at different rates, increasing the attachment surface for the polymer, and enhancing turbulence in the flow.

Even with the advantages of this type of capsule, some challenges remain. A common issue is that air bubbles get stuck in the capsule and disturb the UV sensor if transported downstream. Questions are also raised as to whether the liquid distributes equally on the meshes containing the PE brush and if the flow runs through the mesh or bypasses it. These challenges enlighten the need for further investigations to shed light on potential parts of the capsule where the liquid is completely still.
One aspect of the capsule's shape and size is that it's fitted for lab-scale use, holding a total volume of 1-2 mL. To overcome these challenges one could imagine different types of capsules and PE brush surfaces, such as a batch variation or a serpentine flow where the PE brush is dispersed on solid plate material.

During this project, two types of loading techniques were explored to bind the AAVs to the surface. Firstly, a one-time flow technique where the sample was filtered through the brush once, and secondly, a recirculating technique where the sample was filtered through the capsule multiple times for a longer time period. The advantage of the one-time flow technique is that it is an effective and fast technique to load the AAVs to the brush. However, a low flow rate (0.01-0.1 mL/min) is needed to ensure a sufficient exposure time, this could cause a laminar flow through the capsule which could lead to less distribution of liquid on the meshes. The recirculation technique supports overcoming the issue of using low flow rates by looping the sample, however, this technique causes more hand on workload and is less time-efficient than the one-time technique.

4.5 Environmental, industrial and social impact

Commercial separation and purification methods constitute one of the most expensive parts of AAV production, new purification methods create possibilities to increase cost and time efficiency for the bio-pharmacy industry and decrease chemical usage in production[28]. A lowered production cost of AAVs does not only open opportunities to increase the availability of treatment globally it also decreases a large barrier to develop new treatment. In the last decades' the development of life science technology has accelerated and commercialization of this method could speed up the development process even more since fewer resources are put into the downstream process.

As the biotechnology industry is moving fast toward new and unexplored areas there is a big ethical debate about using therapy techniques involving gene editing. Even if the development of new purification techniques is not involved in the research regarding finding new therapeutic methods it is important to consider that the commercialization of this new technique would make it more accessible to produce gene therapies both due to the decreased cost and also due to the gentleness and potential effectiveness of the method. It's important to consider that cost and accessibility are two important considerations when regulations are put into place. One could argue that cost and accessibility are natural regulations. If the manufacturing price and accessibility were to change quickly new regulations would need to

be put into action to eliminate the risk of unwanted and dual-use research.

During this thesis, an elution buffer has been used to confirm that electrochemical pulses were sufficient to elute the bounded compound from the brush. However, commercialization of this new method aims to only use electrochemical pulses for elution. Hence, no need for hazardous chemicals will be needed for the purification. This creates a possibility of an environment-friendly AAV purification, without excessive chemical use such as used in conventional bind and elute chromatography today.

5
Conclusion

In this study a successful demonstration of the feasibility of immobilization and electrochemical elution was achieved, employing both PAA and PDEA brushes. Electrochemically controllable PE brushes were for the first time used to purify AAVs and the findings from the study do not only emphasize the immobilization of AAVs but also the up-concentration of capsids and enhanced percentage of filled capsids concerning the total amount of capsids.

Furthermore, the research has shed light on the potential to improve binding efficiency by implementing a recirculation strategy, demonstrating a significant enhancement of bounded material in the brush.

While the achieved results showcase promising advancements in AAV purification through this innovative method, acknowledging the need for further refinement is essential. Specifically, enhancements in product purity remain an ongoing area for development. In future studies, it's essential to gain a deeper understanding in regard to the pH dynamics during electrochemical pulses and clarify the variations in AAV release within these pulses is crucial for refining the purification process. An exploration of alternative potential ranges to further improve the percentage of filled capsids is also recommended.

While this project focused primarily on AAV purification, an essential aspect left unexplored was the identification of potential impurities, such as HCP. A comprehensive purification assessment should include a thorough examination of AAV samples to ensure the elimination of impurities, contributing to a more refined and reliable purification process.

Additionally, an intriguing pathway for future exploration involves post-modification of the brush to incorporate an affinity AAV ligand, equal to those utilized in conventional purification columns. This innovative approach holds promise for further enhancing the overall efficiency and purity of the AAV purification process.

Nevertheless, considering that this study marks the initial success in applying this method to achieve AAV purification, there is a strong foundation for its future potential as a competitive alternative to conventional purification methods for AAVs.

Bibliography

[1] Gustav Ferrand-Drake Del Castillo. Nyctea Technologies Homepage. URL https://nycteatechnologies.com/[2024-03-12].

[2] Gustav Ferrand-Drake Del Castillo, Gustav Emilsson, and Andreas Dahlin. Quantitative Analysis of Thickness and pH Actuation of Weak Polyelectrolyte Brushes. *Journal of Physical Chemistry C*, 122(48):27516–27527, 2018. ISSN 19327455. doi: 10.1021/acs.jpcc.8b09171.

[3] E. Kiwanuka, F. Hackl, D. Nowinski, and E. Eriksson. Molecular and gene therapies for wound repair. In *Advanced Wound Repair Therapies*, chapter 15, pages 395–415. Woodhead Publishing, david farrar edition, 2011. ISBN 9781845697006.

[4] M. Carter, R. Essner, N. Goldstein, and M. Iyer. *Guide to Research Techniques in Neuroscience: Gene Delivery Strategies*. Academic Press, third edition edition, 2022. ISBN 9780128186466.

[5] K S. Hanlon, B P. Kleinstiver, S P. Garcia, M P. Zaborowski, A. Volak, S E. Spirig, A. Muller, A A. Sousa, S Q. Tsai, N E. Bengtsson, C. Lööv, M. Ingelsson, J S. Chamberlain, David P. Corey, Martin J. Aryee, J K. Joung, X O. Breakefield, C A. Maguire, and B György. High levels of AAV vector integration into CRISPR-induced DNA breaks. *Nature Communications*, 10(1), 12 2019. ISSN 20411723. doi: 10.1038/s41467-019-12449-2.

[6] C. Kempler. FDA Approves First Gene Therapy for Adults with Severe Hemophilia A, 6 2023. URL https://www.fda.gov/news-events/press-announcements/fda-approves-first-gene-therapy-adults-severe-hemophilia[2024-03-12].

[7] T Li, T Gao, H Chen, Z Demianova, F Wang, J Luo, H Yowanto, and S Mollah. Determination of Full, Partial and Empty Capsid Ratios for Adeno-Associated Virus (AAV) Analysis. Technical Report 10, SCIEX, 2020. URL https://sciex.com/content/dam/SCIEX/pdf/tech-notes/all/AAV-Full-Partial-Empty.pdf[2024-03-12].

[8] N Singh and C L Heldt. Challenges in downstream purification of gene therapy viral vectors. *Current Opinion in Chemical Engineering*, 35, 2022. ISSN 22113398. doi: 10.1016/j.coche.2021.100780. URL https://doi.org/10.1016/j.coche.2021.100780.

[9] Cytiva life science. Optimizing capture and polishing steps in an rAAV purification process, 6 2023. URL https://www.cytivalifesciences.com/en/us/solutions/cell-therapy/knowledge-center/resources/enhanced-aav-downstream-processing[2024-03-12]].

[10] Adeno-associated Virus (AAV) Guide, 2023. URL https://www.addgene.org/guides/aav/[2024-03-12].

[11] Z Lei, J Gao, X Liu, D Liu, and Z Wang. Poly(glycidyl methacrylate-co-2-hydroxyethyl methacrylate) Brushes as Peptide/Protein Microarray Substrate for Improving Protein Binding and Functionality. *ACS Applied Materials and Interfaces*, 8(16):10174–10182, 4 2016. ISSN 19448252. doi: 10.1021/acsami.6b01156.

[12] Gustav Ferrand-Drake del Castillo, Rebekah L. N. Hailes, and Andreas Dahlin. Large Changes in Protonation of Weak Polyelectrolyte Brushes with Salt Concentration—Implications for Protein Immobilization. *The Journal of Physical Chemistry Letters*, 11(13):5212–5218, 7 2020. ISSN 1948-7185. doi: 10.1021/acs.jpclett.0c01289. URL https://pubs.acs.org/doi/10.1021/acs.jpclett.0c01289.

[13] G. Ferrand-Drake del Castillo, R. L. N. Hailes, Z. Adali-Kaya, T. Robson, and Andreas Dahlin. Generic high-capacity protein capture and release by pH control. *Chemical Communications*, 56(44):5889–5892, 2020. ISSN 1359-7345. doi: 10.1039/D0CC01250E. URL https://pubs.rsc.org/en/content/articlelanding/2020/cc/d0cc01250e.

[14] Carmela Zincarelli, Stephen Soltys, Giuseppe Rengo, and Joseph E. Rabinowitz. Analysis of AAV serotypes 1-9 mediated gene expression and tropism in mice after systemic injection. *Molecular Therapy*, 16(6):1073–1080, 2008. ISSN 15250024. doi: 10.1038/mt.2008.76.

[15] A Srivastava, K.M.G. Mallela, N Deorkar, and G Brophy. Manufacturing Challenges and Rational Formulation Development for AAV Viral Vectors. *Journal of Pharmaceutical Sciences*, 110(7):2609–2624, 7 2021. ISSN 15206017. doi: 10.1016/j.xphs.2021.03.024.

[16] Garrett Edward Berry and Aravind Asokan. Cellular transduction mechanisms of adeno-associated viral vectors. *Current Opinion in Virology*, 21:54–60, 12 2016. ISSN 18796265. doi: 10.1016/j.coviro.2016.08.001.

[17] Ana Fajardo-Serrano, Alberto J. Rico, Elvira Roda, Adriana Honrubia, Sandra Arrieta, Goiaz Ariznabarreta, Julia Chocarro, Elena Lorenzo-Ramos, Alvaro Pejenaute, Alfonso Vázquez, and José Luis Lanciego. Adeno-associated viral vectors as versatile tools for parkinson's research, both for disease modeling purposes and for therapeutic uses. *International Journal of Molecular Sciences*, 22(12), 6 2021. ISSN 14220067. doi: 10.3390/ijms22126389.

[18] Lin Kang, Shilin Jin, Jiayi Wang, Zhongyue Lv, Chengqi Xin, Chengcheng Tan, Mengke Zhao, Liang Wang, and Jing Liu. AAV vectors applied to the treatment of CNS disorders: Clinical status and challenges. *Journal of Controlled Release*, 355:458–473, 3 2023. ISSN 18734995. doi: 10.1016/j.jconrel.2023.01.067.

[19] Jihad El Andari and Dirk Grimm. Production, Processing, and Characterization of Synthetic AAV Gene Therapy Vectors. *Biotechnology Journal*, 16(1), 1 2021. ISSN 18607314. doi: 10.1002/biot.202000025.

[20] Gustav Ferrand-Drake del Castillo, Maria Kyriakidou, Zeynep Adali, Kunli Xiong, Rebekah L N Hailes, and Andreas Dahlin. Electrically Switchable Polymer Brushes for Protein Capture and Release in Biological Environments**. *Angewandte Chemie International Edition*, n/a(n/a):e202115745, 3 2022. ISSN 1433-7851. doi: https://doi.org/10.1002/anie.202115745. URL `https://doi.org/10.1002/anie.202115745`.

[21] Zhenyuan Qu, Hong Xu, and Hongchen Gu. Synthesis and biomedical applications of poly((meth)acrylic acid) brushes. *ACS Applied Materials and Interfaces*, 7(27):14537–14551, 7 2015. ISSN 19448252. doi: 10.1021/acsami.5b02912.

[22] Wei Liang Chen, Roselynn Cordero, Hai Tran, and Christopher K. Ober. 50th Anniversary Perspective: Polymer Brushes: Novel Surfaces for Future Materials. *Macromolecules*, 50(11):4089–4113, 2017. ISSN 15205835. doi: 10.1021/acs.macromol.7b00450.

[23] Noémie Elgrishi, Kelley J. Rountree, Brian D. McCarthy, Eric S. Rountree, Thomas T. Eisenhart, and Jillian L. Dempsey. A Practical Beginner's Guide to Cyclic Voltammetry. *Journal of Chemical Education*, 95(2):197–206, 2018. ISSN 19381328. doi: 10.1021/acs.jchemed.7b00361.

[24] Slobodan Petrovic. *Electrochemistry Crash Course for Engineers*. Springer, Happy Valley, 2021. ISBN 978-3-030-61561-1. doi: https://doi.org/10.1007/978-3-030-61562-8.

[25] PROGEN. AAV5 Titration ELISA Manual. Technical report, PROGEN Biotechnic GmbH, Heidelberg, 2018.

[26] PROGEN. AAV9 Titration ELISA Manual. Technical report, PROGEN Biotechnic GmbH, Heidelberg, 2018.

[27] Takara Bio Inc. AAVpro® Titration Kit (for Real Time PCR) Ver.2 For Research Use v202001Da. Technical report. URL `http://www.takara-bio.com`.

[28] Wellcome IAVI. *Expanding access to monoclonal products: A global call to action*. Wellcome, 2020. URL `https://wellcome.ac.uk/sites/default/files/expanding-access-to-monoclonal-antibody-based-products-executive-summary.pdf`.

A

Appendix 1

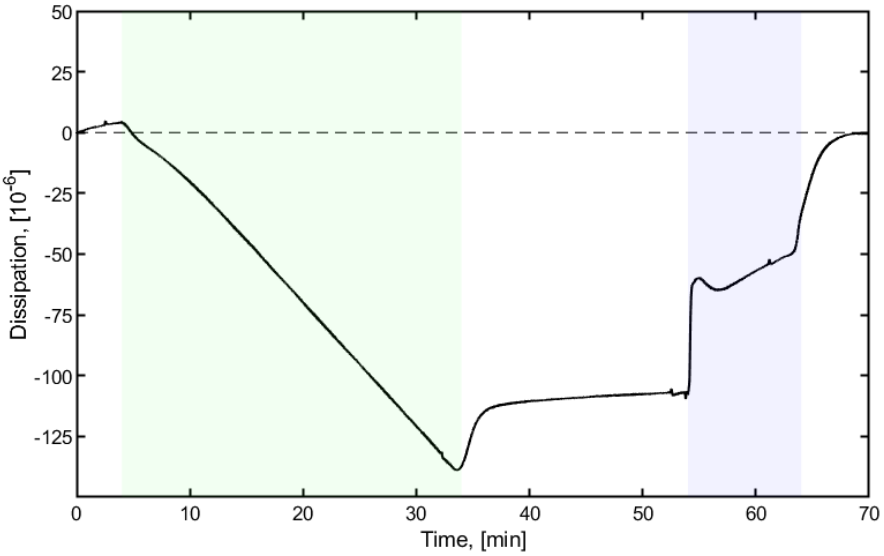

Figure A.1: SPR result from AAV9 performed by Maria Kyriakidou.

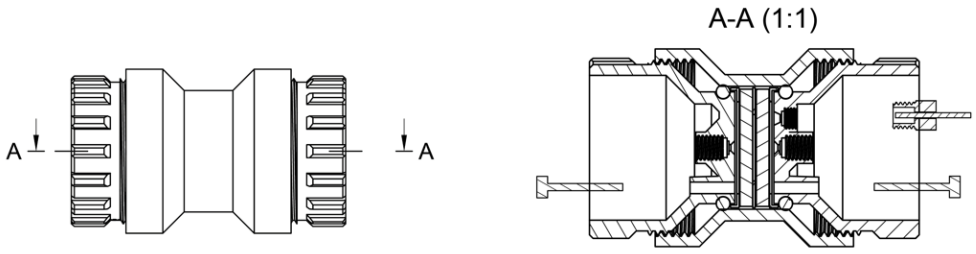

Figure A.2: A schematic figure and cross-section of the capsule, created by Gustav Ferrand-Drake Del Castillo.

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