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# Species-Specific Binding Affinities of Ad5 Fiber Protein to CAR Receptors: Implications for Adenoviral Vector Design in Drug Delivery and Gene Therapy

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**ABSTRACT:**

Adenoviruses play a critical role in molecular biology and gene therapy due to their interaction with coxsackie and adenovirus receptors (CAR). Human adenovirus type 5 (Ad5) has been widely used in the design of gene vectors for therapeutic applications, particularly in drug delivery and gene therapy. This study aims to compare the structural and molecular interactions between Ad5 fiber protein and CAR receptors from humans and mice to enhance the design of adenoviral vectors for drug delivery systems and gene therapy. Amino acid sequences of the Ad5 fiber protein and CAR receptors from humans and mice were obtained from the Protein Data Bank (PDB) and UniProt database. These structures were refined using molecular dynamics simulations and analyzed using the I-TASSER system and GalaxyRefine server. Quality assessments included Ramachandran plot analysis and ProSA-web Z-score calculations. Molecular docking analysis was performed using ClusPro 2.0 to compare the binding affinities. Refinement of hCAR and mCAR models showed significant structural improvements, with residues in favorable areas increasing to 80.3% for hCAR and 78.3% for mCAR. The Z-scores also improved, indicating better structural stability. Molecular docking revealed that mCAR had a stronger binding affinity to the Ad5 fiber protein than hCAR, suggesting species-specific differences in adenoviral infection mechanisms. This study highlights the importance of species-specific CAR receptor interactions in the design of adenoviral vectors. The stronger binding affinity of mCAR for Ad5 fiber protein suggests distinct drug and gene delivery mechanisms in mice compared to humans. These insights are crucial for developing more effective adenoviral vectors for targeted therapeutic applications.

**Keywords:** Adenovirus type 5; Fiber protein; Coxsackievirus and adenovirus receptor (CAR); In silico analysis; Molecular docking; Gene therapy; Adenoviral vectors.

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## 1. Introduction

Adenoviruses are non-enveloped DNA viruses. Although considered major pathogens, they are extensively used as molecular models in biology and microbiology. Recently, adenoviruses have garnered significant attention as viral carriers in gene therapy [1, 2]. About 120 serotypes of adenoviruses have been discovered in humans, mammals, birds, and reptiles. This virus causes disease in people with immune disorders, but most diseases are seen in animals. The approximate size of adenoviruses ranges from 65 to 90 nm. These viruses are classified into six groups with multipart structures, and their genomes are 26 to 48 Kb in length. After infection, the transcripts of this virus are classified into three groups: early, middle, and late [3]. Many hexons on the surface of adenoviruses contain specific antigens, α and ε. Among their other features, the fiber contains a main antigen called γ, responsible for type characteristics, cell binding, and hemagglutination. This protein facilitates the entry of the virus into host cells and causes pathogenic infections [4]. Based on their different

species, such as A, C, D, E, and F, adenoviruses have different binding receptors on the surface of the host. HAdV C is known for its ability to cause persistent and latent infections in lymphoid organs such as the tonsils and adenoids. Ad5, a member of subspecies C, has been widely used as a non-replicating recombinant vector for vaccine development [5, 6]. Ad5 has been purified from E. coli bacteria. This species contains 196 amino acid residues and has a molecular weight of 279 kDa. Ad5-based vectors have found widespread application as gene transfer vehicles. Ad5 vectors are considered for many applications, including vaccine production and gene therapy [7].

The Ad5 fiber protein is homotrimeric, consisting of three identical subunits. A globular knob domain at the end of the fiber binds to the coxsackie virus and adenovirus receptor (CAR) on the surface of host cells. This interaction is the primary determinant of Ad5 tropism and is essential for initiating infection [8]. Evidence of CAR binding to adenoviruses of different species, including 2, 5, 9, 12, 19, 31, 37, and 41, shows that CAR is a critical receptor for the entry of both Coxsackie B virus and adenovirus into host cells. CAR is a 46 kDa protein, the primary receptor for coxsackievirus B (CVB) and adenovirus (Ad). This cell surface receptor plays a significant role in CVB and Ad entry into host cells [9]. CAR establishes adhesion at the cell surface through cellular junctions. The Ad5 fiber protein binds to CAR with a higher affinity than CAR, suggesting that Ad5 may disrupt cell junctions by weakening CAR interactions. Ad5 uses its ability to competitively bind to CAR to disrupt junctions, releasing the virus from infected cells and further spreading the infection. Therefore, Ad5 directly connects to CAR by disrupting cell junctions such as β-catenin and relocating [10].

Gaining insight into the binding interactions between Ad5 fiber proteins and cellular receptors in various species is essential for enhancing the design of adenoviral vectors. Human adenoviruses, such as Ad5, mostly use CAR as the receptor for cell entrance. However, murine adenoviruses have distinct preferences for receptors. For instance, murine adenovirus type 1 (MAV-1) does not use CAR as its major receptor, suggesting a difference in receptor utilization that might impact the applicability of animal research to human applications.

Examining the adenovirus fiber protein's structure, specifically the handle domain offers a valuable understanding of the molecular processes involved in receptor identification and binding. The architectures of fiber knob domains at high resolution have shown both shared and unique characteristics that play a role in determining receptor selectivity. The knowledge gained from these structural insights is beneficial for developing adenoviral vectors with modified tropism, which allows for the precise delivery of genes to particular tissues or cell types.

In this context, in silico methods offer a powerful approach to investigate and compare the interactions between the Ad5 fiber protein and CAR receptors from different species. In this study, we aim to clarify the in-silico comparison of human and mouse Ad5 receptor binding with the Ad5 fiber protein using computational procedures. By analyzing the structural and molecular interactions, we can gain a deeper understanding of the tropism and entry mechanism of Ad5 in both human and mouse cells. Furthermore, this knowledge may contribute to developing more effective and targeted gene therapy and vaccine approaches using Ad5 vectors.

## *2.* Materials & Methods

### 2.1 Protein Structure Retrieval and Preparation

The amino acid sequences of the Ad5 fiber protein and its receptors, CAR, in humans, and its murine counterpart were obtained from the Protein Data Bank (PDB) (complex IDs: PDB-CPX-136677, PDB-CPX-160223, and PDB-CPX-161326) and the UniProt database. Subsequently, the structures underwent energy minimization using molecular dynamics simulations to ensure stability and accuracy for further studies.

### 2.2. Structural Modeling and Refinement Human (h)CAR

The I-TASSER system was used to predict five models for the hCAR. The C scores for models 1 to 5 were -1.89, -2.43, -3.0, -4.12, and -3.15, respectively. The model with a C score of -1.89, referred to as Model 1, was chosen for modification. The chosen model underwent refinement using the GalaxyRefine server, resulting in the generation of five refined models. Model 4 was chosen based on a range of qualitative criteria, including GDT-HA, RMSD, MolProbity score, collision score, weak rotamers, and Ramachandran areas of interest.

#### 2.2.1 Mouse (m)CAR

The I-TASSER service was used to forecast five models of the mCAR. The C scores for models 1 to 5 were -2.66, -3.09, -3.58, -3.53, and -3.86, respectively. The change was performed on Model 1, with a C score of -2.66.

The chosen model underwent refinement using the GalaxyRefine server, resulting in the generation of five refined models. Model 4 was chosen using the same qualitative criteria as for hCAR.

In this study, the selection of final models for human and mouse CAR was guided by considerations of structural stability and alignment with known biological characteristics of CAR, ensuring that our final models closely reflect available experimental data on CAR structures. Initial models with the highest C-scores from I-TASSER were selected, as these scores indicate higher confidence in the predicted structures. Following refinement with GalaxyRefine, we finalized the model selection based on quality metrics, including Ramachandran plot distribution, MolProbity scores, and ProSA-web Z-scores, demonstrating substantial improvements in structural quality and stability. These enhancements reinforce the robustness and biological relevance of the chosen models, addressing the feedback provided by reviewers.

### 2.3. Quality Assessment

The quality of the main and refined structures was assessed using the following tools:

#### 2.3.1. Analysis of the Ramachandran plot

The SAVES v6.0 server was used to assess the dispersion of dihedral angles in protein structures.

#### 2.3.2 Calculation of the Z-score

The ProSA-web tool assessed the overall quality and stability of protein structures. Lower Z ratings imply superior structural quality.

### 2.4. Molecular Docking

The binding affinity of hCAR and mCAR to Ad5 fiber protein was compared using ClusPro 2.0 server via molecular docking analysis. During the docking procedure, 30 clusters were formed for each receiver. The optimal cluster was determined by considering both the lowest energy and the number of members.

## 3. Results & Discussion

### 3.1. Refinement and quality assessment of hCAR

The initial hCAR model generated by I-TASSER had a C-score of -1.89. Model 4 was chosen after being refined using GalaxyRefine, based on its qualitative attributes. The proportion of residues in favorable areas rose from 57.1% to 80.3%, as seen by the Ramachandran plots. The Z-score increased from -3.5 to -4.06, showing an improvement in structural quality (Table 1 and Figure 1).

### 3.2. Refinement and Quality Assessment of mCAR

The initial mCAR model generated by I-TASSER had a C-score of -2.66. Based on its qualitative characteristics, model 4 was chosen after undergoing refinement using GalaxyRefine. The proportion of residues in favorable areas significantly rose from 57.5% to 78.3%, as seen by the Ramachandran plots. The Z-score increased from -3.98 to -4.75, showing an improvement in structural quality (Table 2 and Figure 1).

### 3.3. Molecular Docking Analysis

Molecular docking analysis using ClusPro 2.0 for hCAR with Ad5 fiber protein produced 30 clusters. Cluster 0, with the lowest energy of -852.7 and 115 members, was selected as the best. For mCAR with fiber protein Ad5, cluster 0 had the lowest energy of -977.2 and 124 members, indicating a stronger binding affinity than hCAR (Figure 2).

### 3.4. Comparison of Binding Affinities

The docking details indicate that the mCAR protein has a stronger binding affinity to the Ad5 fiber protein than hCAR. This is supported by the lowest energy value and a higher number of members in the best cluster for mCAR. These findings indicate that the mCAR protein forms a more enduring combination with the Ad5 fiber protein than the hCAR protein.

**Table 1.** Quality Scores of Refined hCAR Models by GalaxyRefine.

| Model | GDT-HA | RMSD | MolProbity | Clash score | Poor rotamers | Rama favored |
|---|---|---|---|---|---|---|
| Initial | 1.0000 | 0.000 | 3.445 | 13.7 | 17.2 | 67.2 |
| Model 1 | 0.9226 | 0.478 | 2.601 | 22.6 | 1.6 | 87.6 |
| Model 2 | 0.9219 | 0.486 | 2.528 | 25.9 | 0.6 | 86.8 |
| Model 3 | 0.9075 | 0.510 | 2.425 | 21.1 | 0.9 | 87.6 |
| Model 4 | 0.9219 | 0.493 | 2.513 | 20.9 | 1.2 | 86.8 |
| Model 5 | 0.9164 | 0.496 | 2.601 | 23.3 | 1.6 | 88.2 |

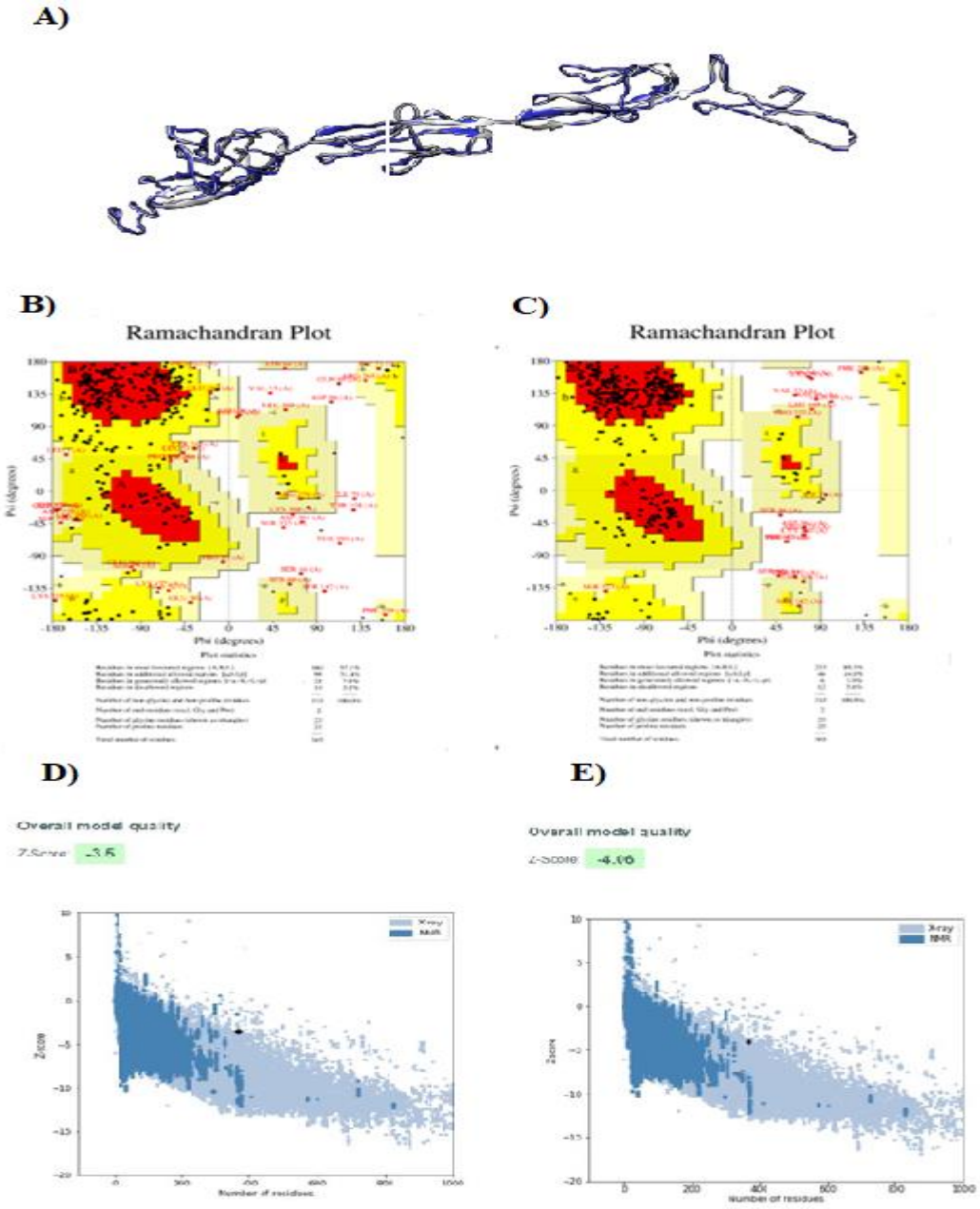


**Figure 1.** Structural and quality assessment of the refined hCAR model (A) 3D structures on the initial and refined hCAR models. The original model is shown in gray, while the improved model is illustrated in blue. (B) The original hCAR model's Ramachandran plot displays the distribution of dihedral angles phi (Φ) and psi (Ψ). The graph illustrates that 57.1% of the residues are located in places that are considered beneficial, while 31.4% are found in locations that are authorized. Additionally, 7.9% of the residues are in generous areas, and 3.5% are in non-permitted areas. (C) The modified hCAR model's Ramachandran plot demonstrates an enhanced distribution, with 80.3% of residues located in favorable areas, 14.0% in permitted regions, 1.9% in generous regions, and 3.8% in prohibited regions. (D) The original hCAR model's Z-score was derived from ProSA-web. A Z-score of -3.5 suggests that the first model's overall quality is consistent with the scores often seen for natural proteins of comparable size. (E) The ProSA-web analysis yielded the Z-score of the revised hCAR model. The enhanced Z-score of 4.06 indicates that the revised model exhibits superior structural quality and stability when compared to the original model.

**Table 2.** Quality Scores of Refined mCAR Models by GalaxyRefine**.**

| Model | GDT-HA | RMSD | MolProbity | Clash score | Poor rotamers | Rama favored |
|---|---|---|---|---|---|---|
| Initial | 1.0000 | 0.000 | 3.385 | 11.4 | 17.9 | 67.5 |
| Model 1 | 0.9137 | 0.498 | 2.498 | 24.5 | 0.6 | 87.1 |
| Model 2 | 0.9178 | 0.496 | 2.521 | 25.2 | 0.6 | 86.5 |
| Model 3 | 0.9205 | 0.496 | 2.498 | 24.5 | 0.6 | 87.1 |
| Model 4 | 0.9205 | 0.488 | 2.498 | 24.1 | 0.6 | 86.8 |
| Model 5 | 0.9274 | 0.492 | 2.480 | 23.8 | 0.3 | 87.3 |

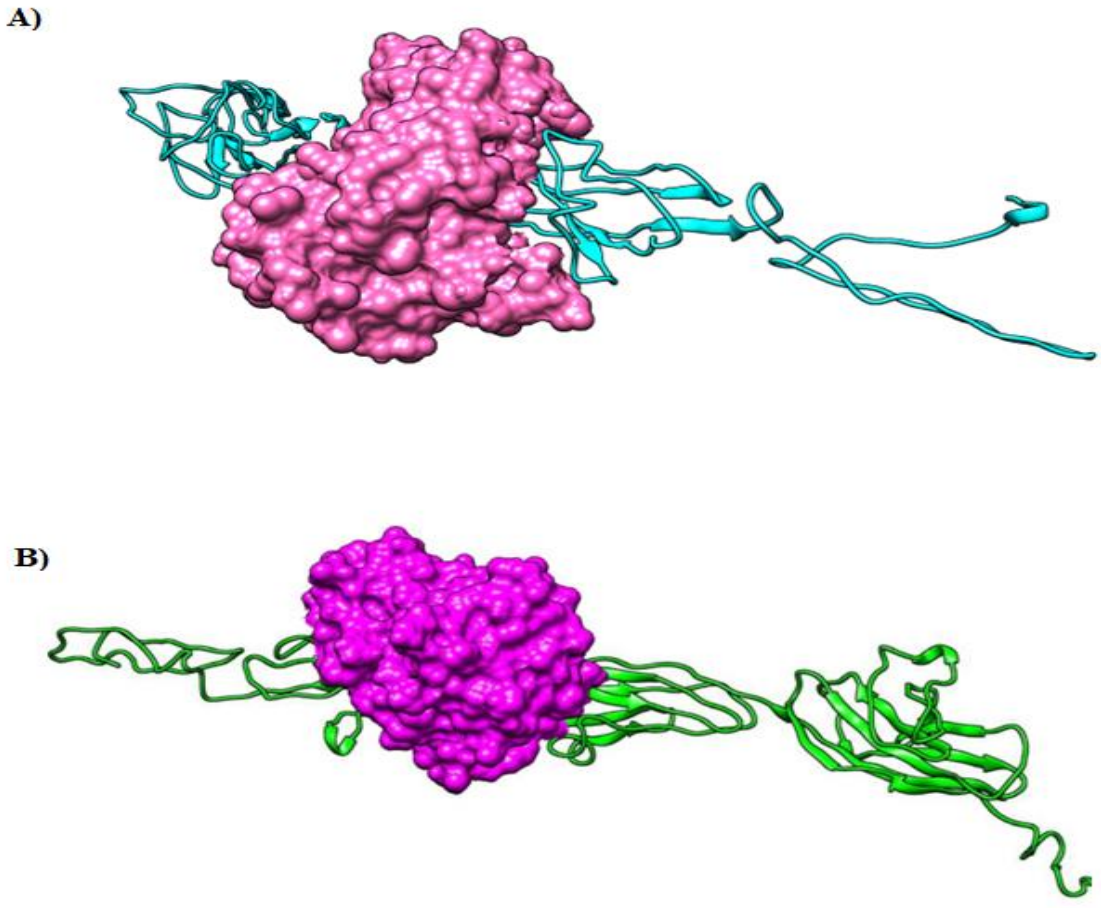


**Figure 2.** The docked complex of CAR and adenovirus fiber protein 5. (A) Human protein is shown in ribbon format and cyan color, and fiber protein in surface format and purple color. (B) mCAR protein is shown in ribbon format and green color, and fiber protein in surface format and purple color.

To assess the stability of the bound complexes more thoroughly, it is advisable to conduct molecular dynamics simulations.

This study aimed to analyze the receptor binding affinities of human and murine adenovirus type 5 (Ad5) receptors and the Ad5 fiber protein using silico methods. This work compares and analyzes the structural and interaction dynamics that cause species-specific alterations in adenoviral infection. These differences are important for improving adenoviral vectors for medical treatments.

The refining process greatly enhanced the structural integrity of both the human CAR (hCAR) and mouse CAR (mCAR) models. The selected refined models

exhibited more residues in favorable regions and demonstrated superior overall stability, as evidenced by the Ramachandran plot and Z-score analysis. The percentage of residues in favorable locations for hCAR rose from 57.1% to 80.3%, while the Z-score showed improvement from -3.5 to -4.06. In the case of mCAR, the proportion of residues in favorable regions rose from 57.5% to 78.3%, and the Z-score showed improvement from -3.98 to -4.75. The enhancements demonstrate that the revised models are now more precise and dependable for future binding investigations. The significance of the Ramachandran plot and Z-score analysis in assessing the protein structure quality is well acknowledged. A significant proportion of residues located in the favorable portions of the Ramachandran plot signifies excellent stereochemical quality. Conversely, lower (more negative) Z-scores suggest that the protein conformation is more similar to native-state conformations [11, 12]. The combination of these factors provides strong evidence for the enhanced dependability of the hCAR and mCAR models.

Analysis of molecular binding demonstrated considerable disparities in the binding affinities between hCAR and mCAR for Ad5 fiber protein. The most optimal binding cluster for hCAR exhibited a minimum energy of -852.7, including 115 members. Similarly, the most optimal cluster for mCAR had the lowest energy of -977.2, consisting of 124 members. The increased number of members and lower energy value in the best mCAR cluster suggests a more robust binding affinity between mCAR and Ad5 fiber protein than hCAR. The results align with the current body of research, which documents differences in the use of adenovirus receptors among different species. Research has shown that human red blood cells produce CAR, a sequestration mechanism to defend against systemic Ad5 infection. In contrast, mouse red blood cells do not exhibit CAR [13]. This disparity is likely to contribute to the observed variations in graft binding and might potentially impact the effectiveness of adenoviral transmission across various animals.

The observed variations in the affinities of receptor binding have important implications for the development and use of adenoviral vectors in both preclinical and clinical environments. Adenoviral vectors are often used in gene therapy research to assess the effectiveness and safety of treatments using animal models. Nevertheless, the heightened affinity of mCAR for Ad5 fiber protein implies that adenoviral vectors may display distinct tropism and transduction effectiveness in mice compared to humans.

In order to resolve these inconsistencies, it could be essential to alter adenoviral vectors in a manner that more accurately replicates human receptor interactions in mice models. One possible approach is to modify the fiber protein via engineering to adjust its ability to bind to the human CAR, thereby enhancing the therapeutic significance of research results. Moreover, comprehending these mutual interactions might facilitate the advancement of adenoviral vectors with enhanced selectivity and diminished unintended impacts, eventually resulting in a safer and more efficient gene therapy [14].

The research emphasized the significance of adapting adenoviral vectors to address CAR deficiency in malignancies. Huang et al. (2022) showed that using DOTAP-folate liposomes containing adenovirus substantially enhanced transfection effectiveness and tumor-killing capability in CAR-deficient cell lines [15]. This suggests that receptor-targeting alterations can potentially enhance the efficacy of adenoviral vectors.

Subsequent research should examine the bound complexes' molecular dynamics to validate the stability and interaction dynamics found in this work. Molecular dynamics simulations provide an intricate understanding of the conformational alterations and interaction energies that transpire throughout the binding process. Moreover, it is essential to conduct biochemical tests and infectivity investigations in cell culture and animal models to validate these results and verify the in-silico predictions experimentally. This process will also help improve the design of adenoviral vectors.

Although the present study sheds valuable light on the species-specific interactions between the fiber protein of the Ad5 virus and the CAR receptors, there are some limitations to the study that should be acknowledged. First and foremost, our investigation is based on predicting binding affinities and structural interactions by computational modeling and molecular docking. Whereas silico analyses represent a powerful starting point, they cannot fully mimic conditions in vivo or in vitro. Therefore, experimental validation using least-binding affinity assays and cell-based transduction studies should be performed to confirm our findings.

Moreover, although our data suggest differences in the receptor binding affinity between human and mouse CAR, the absence of corresponding in vivo or in vitro experiments limits our appreciation of how such differences may impact viral transduction efficiency and therapeutic efficacy in relevant settings. Again, while proposing that receptor tropism might influence immune response profiles, this assumption remains untested in this study. Immune responses to adenoviral vectors are a multivariate system influenced by such variables as receptor interactions, host immunity, and vector alterations; thus, further studies will be needed in an immune-competent model to evaluate this interaction properly.

By focusing our study on CAR as the primary receptor of entry for Ad5, the generalization of the findings may also

be blunted since adenoviruses interact with alternative receptors in a tissue-specific or conditional manner. Indeed, this narrow scope might affect the applicability of our findings because other interactions of receptors might play roles in the tropism and efficiency of Ad5 across diverse cell types. Finally, the structural models were refined with assistance from molecular dynamics simulations. However, predictions based on this might vary due to simulation parameters like force fields, simulation time, and environment settings. Experimental validation will be necessary to assume stability and reliability for these refined models.
Overcoming such limitations in future studies would substantially enhance our findings' translational relevance and clinical applicability, subsequently allowing for the design of more effective and targeted adenoviral vectors in gene therapy applications.

## 4. Conclusion

This study highlights the significance of species-specific receptor interactions in the design of adenoviral vectors. The higher affinity of mCAR for Ad5 fiber protein compared to hCAR underscores the need to thoroughly evaluate the use of this receptor in therapeutic models. With this understanding, scientists may create more efficient adenoviral vectors customized for various therapeutic purposes.

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None.

## Conflict of interest

There is no conflict of interest.

## Ethics

None.

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