Adenovirus-Host interactions

Possible implications for Thrombosis with Thrombocytopenia Syndrome

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Overview

- Characterization of the ChAdOx1 capsid structure.
- Determination of primary cell entry receptor usage.
- Investigating interactions with Platelet factor 4 (PF4).
- Computational modeling of ChAdOx1/PF4 interactions.
- Down stream implications.

1 Structural Characterization of ChAdOx1

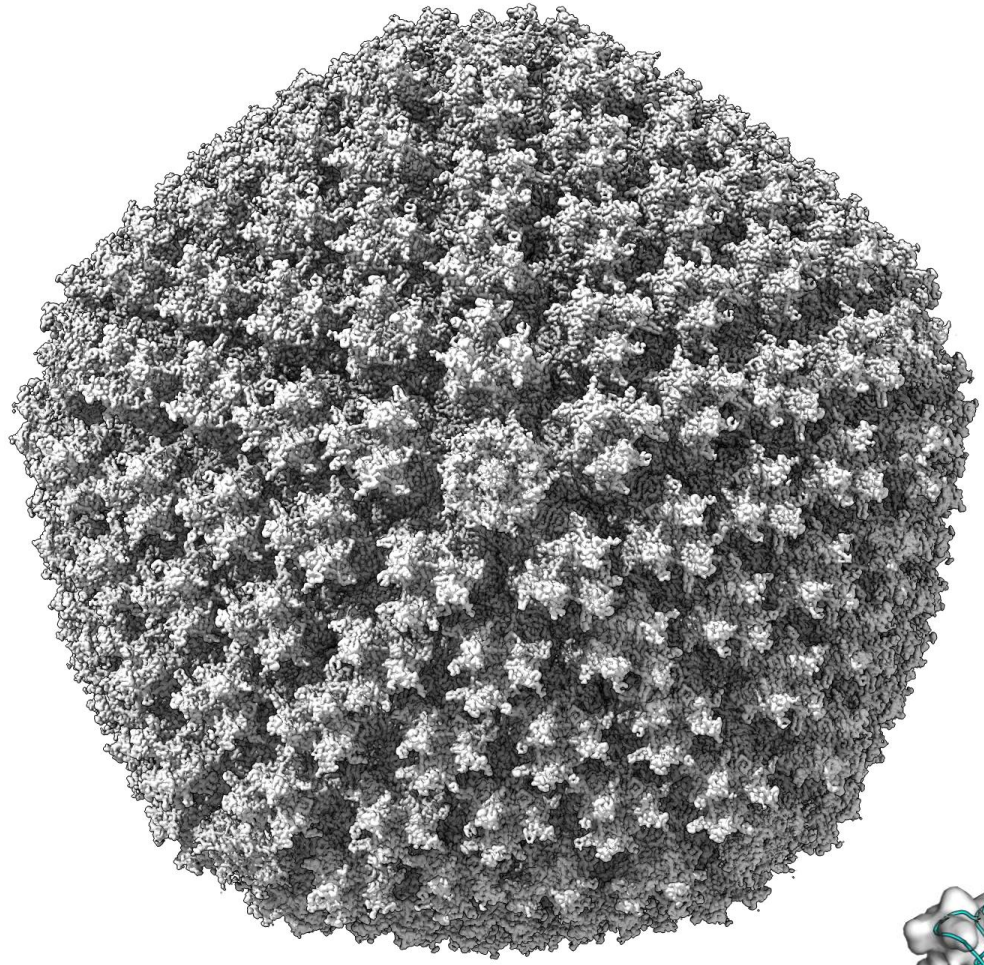
Modeling of ChAdOx1

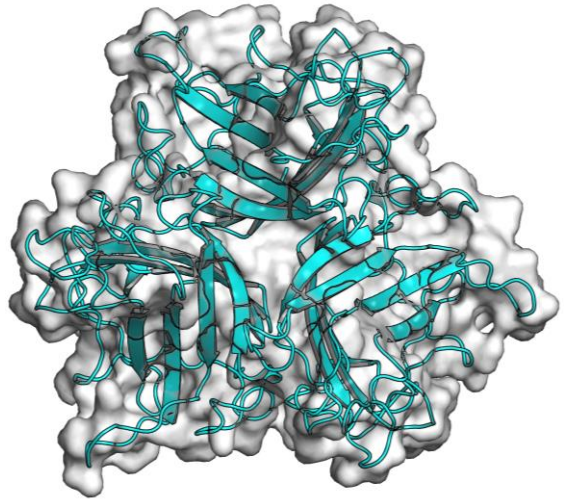
High Resolution Volume Data

Data sets enabled accurate capsid reconstruction

Interior (top) and exterior (bottom) capsid surfaces show density for all expected proteins

Integrative ‘all atom’ approach facilitates high confidence simulation of interfaces





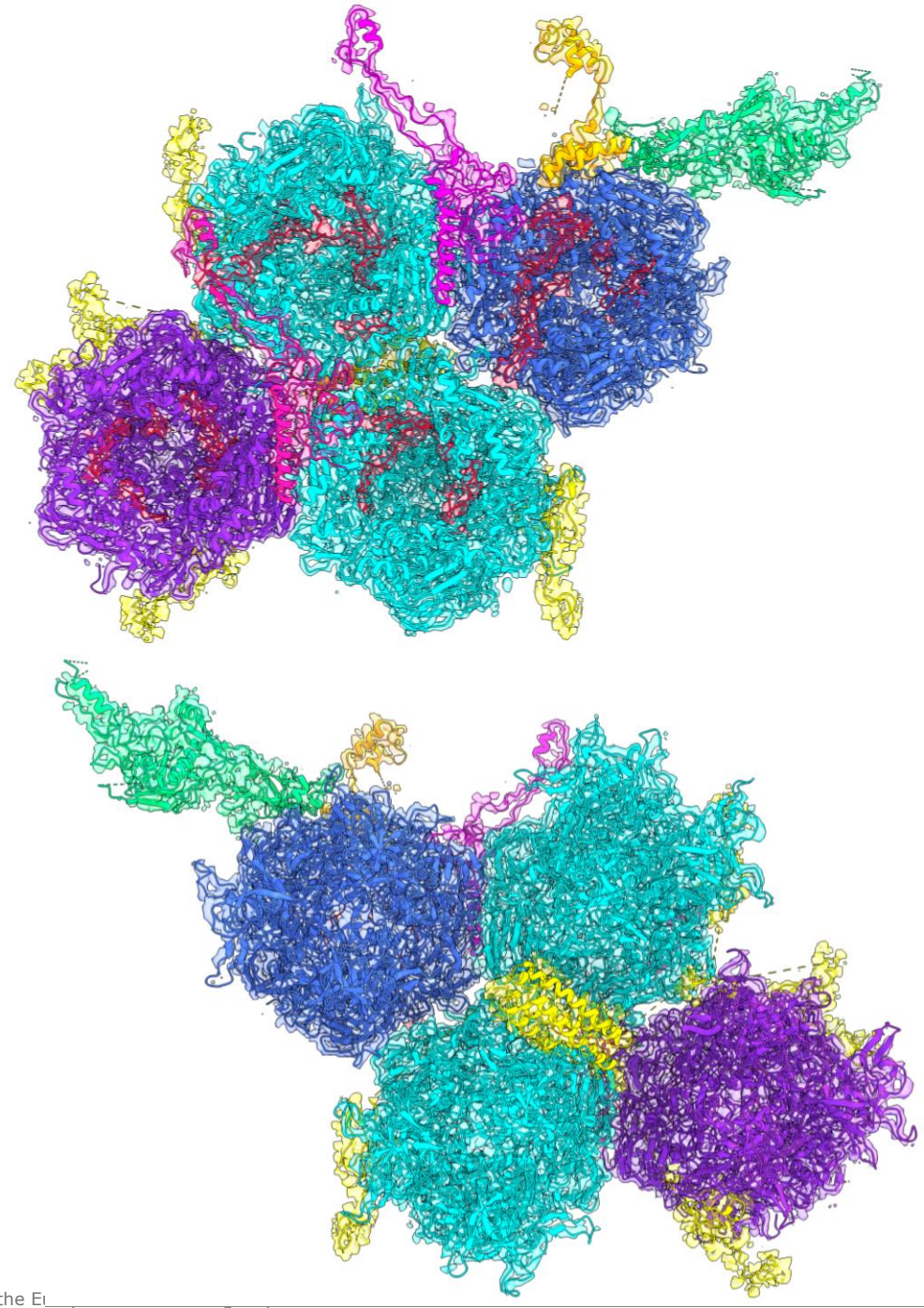
Modeling of ChAdOx1

Accurate atomic model placement

Data sets enabled accurate capsid reconstruction

Interior (top) and exterior (bottom) capsid surfaces show density for all expected proteins

Integrative ‘all atom’ approach facilitates high confidence simulation of interfaces



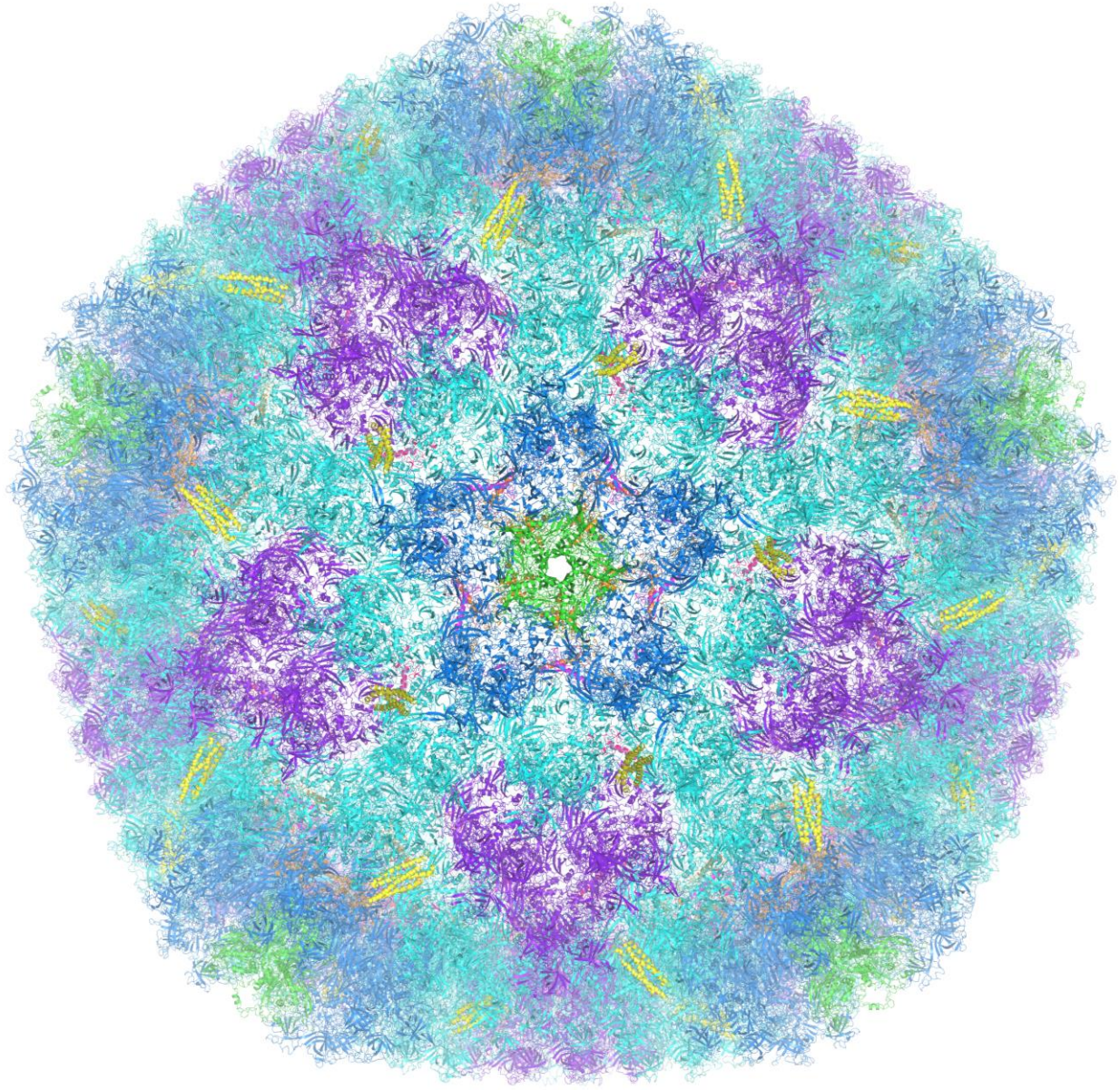
Modeling of ChAdOx1

Restrained MD simulations

Data sets enabled accurate capsid reconstruction

Interior (top) and exterior (bottom) capsid surfaces show density for all expected proteins

Integrative ‘all atom’ approach facilitates high confidence simulation of interfaces



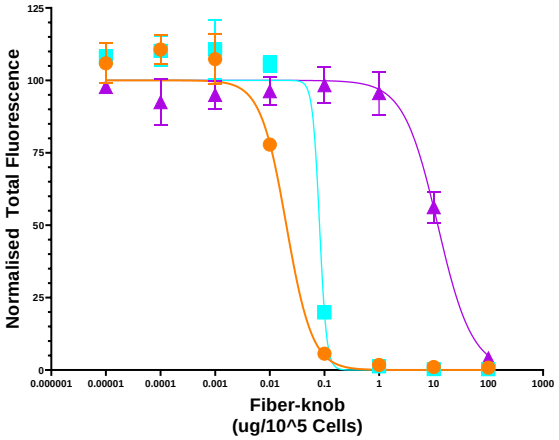
https://doi.org/10.1126/sciadv.abl8213

2 A Primary Receptor Tropism of ChAdOx1 is CAR

ChAdOx1 uses CAR as a primary receptor

New virus, same receptor

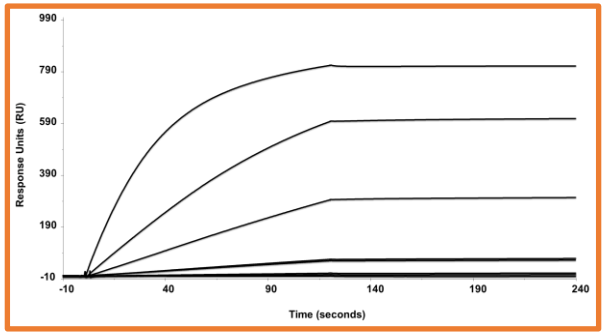
mAb Binding Inhibition Assay	CAR IC_{50} in CHO-CAR ($\mu g/10^5$ cells)	CD46 IC_{50} in CHO-BC1 ($\mu g/10^5$ cells)
HAdV-C5	0.01	NULL
HAdV-B35	11.86	$5.56x10^{-5}$
ChAdV-Y25/ChAdOX1	0.08	NULL

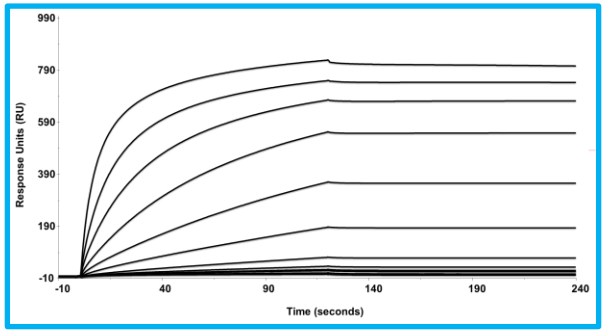


Cell based assays show similar displacement of anti-CAR mAb by ChAdOx1 and Ad5 fiber knob

SPR	Ligand	K_D (nM)	k_a (1/Ms)	K_d (1/s)
HAdV-C5	CAR	0.06±0.02	$1.56±1.07x10^6$	$1.09±0.7x10^{-4}$
ChAdOx1	CAR	7.16±1.92	$3.03±0.13x10^4$	$1.8±0.41x10^{-4}$

SPR shows the affinity of CAR is ~100X that of ChAdOx1





3 The ChAdOx1 Capsid Interacts with Platelet Factor 4

ChAdOx1 Binds to PF4

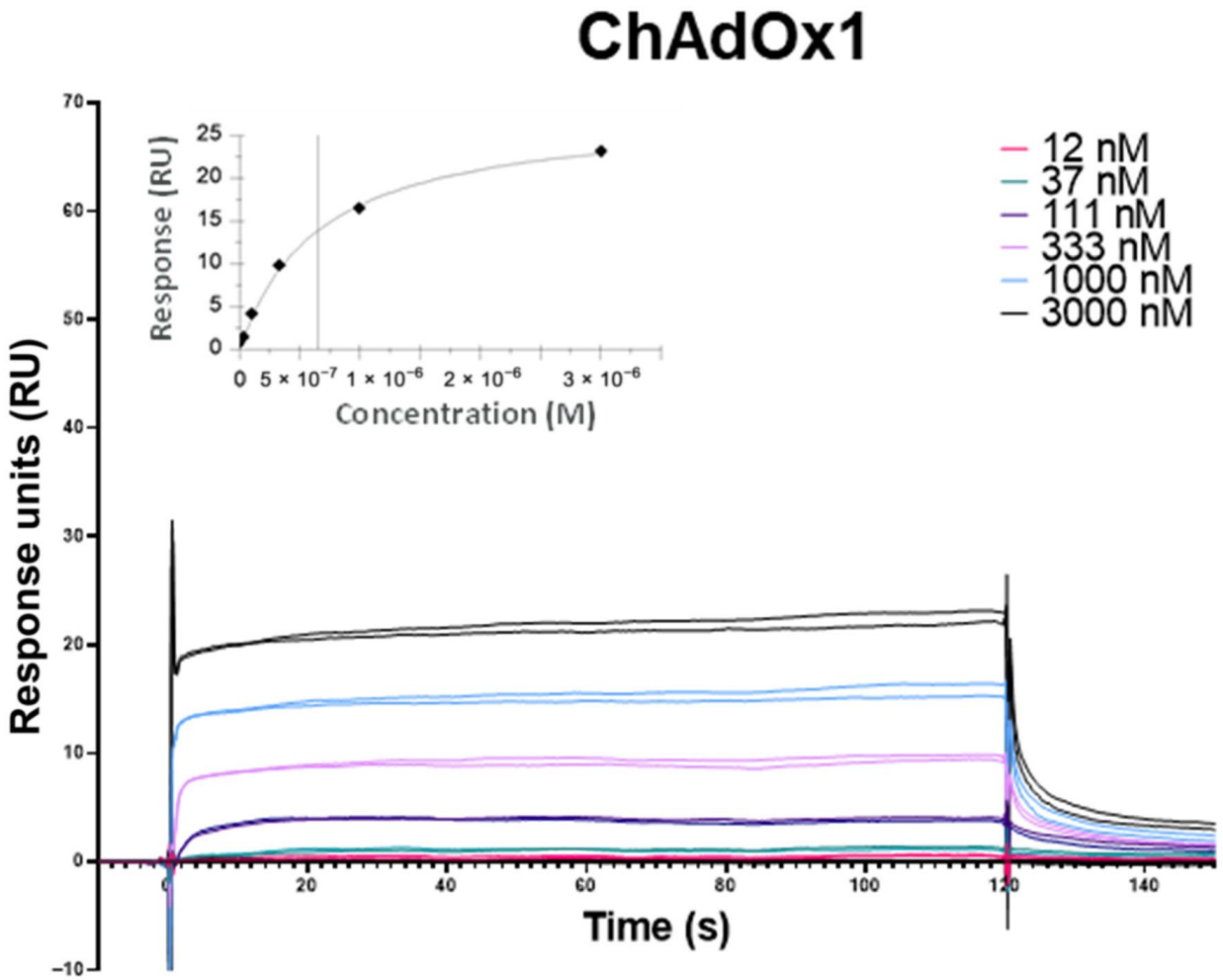
Dose dependent and above background

Dose dependent interaction between ChAdOx1 and PF4

Exhibited by 3 clinical adenovirus species

Complex is stable and specific

Complex formation is inhibited by the salt and heparin



ChAdOx1 Binds to PF4

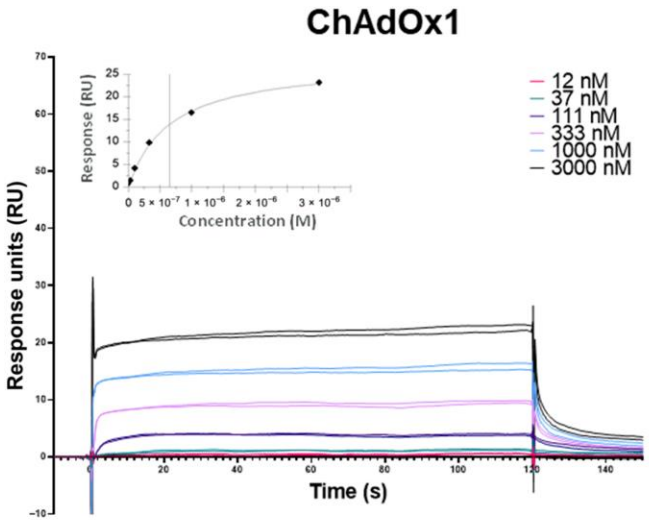
Conserved *and* incidental?

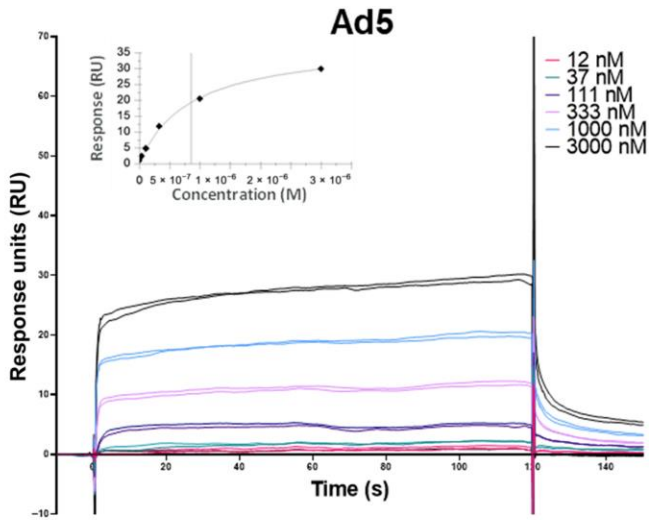
Dose dependent interaction between ChAdOx1 and PF4

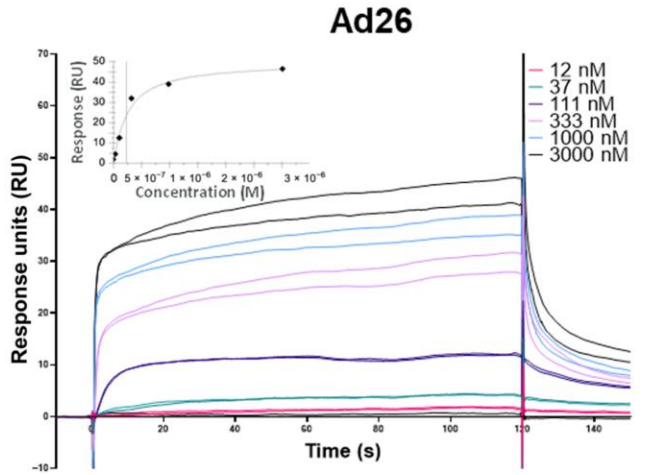
Exhibited by 3 clinical adenovirus species

Complex is stable and specific

Complex formation is inhibited by the salt and heparin







Virus	Average K_D (nM)
ChAdOx1	661 ± 53
Ad5	789 ± 137
Ad26	301 ± 67

ChAdOx1 Binds to PF4

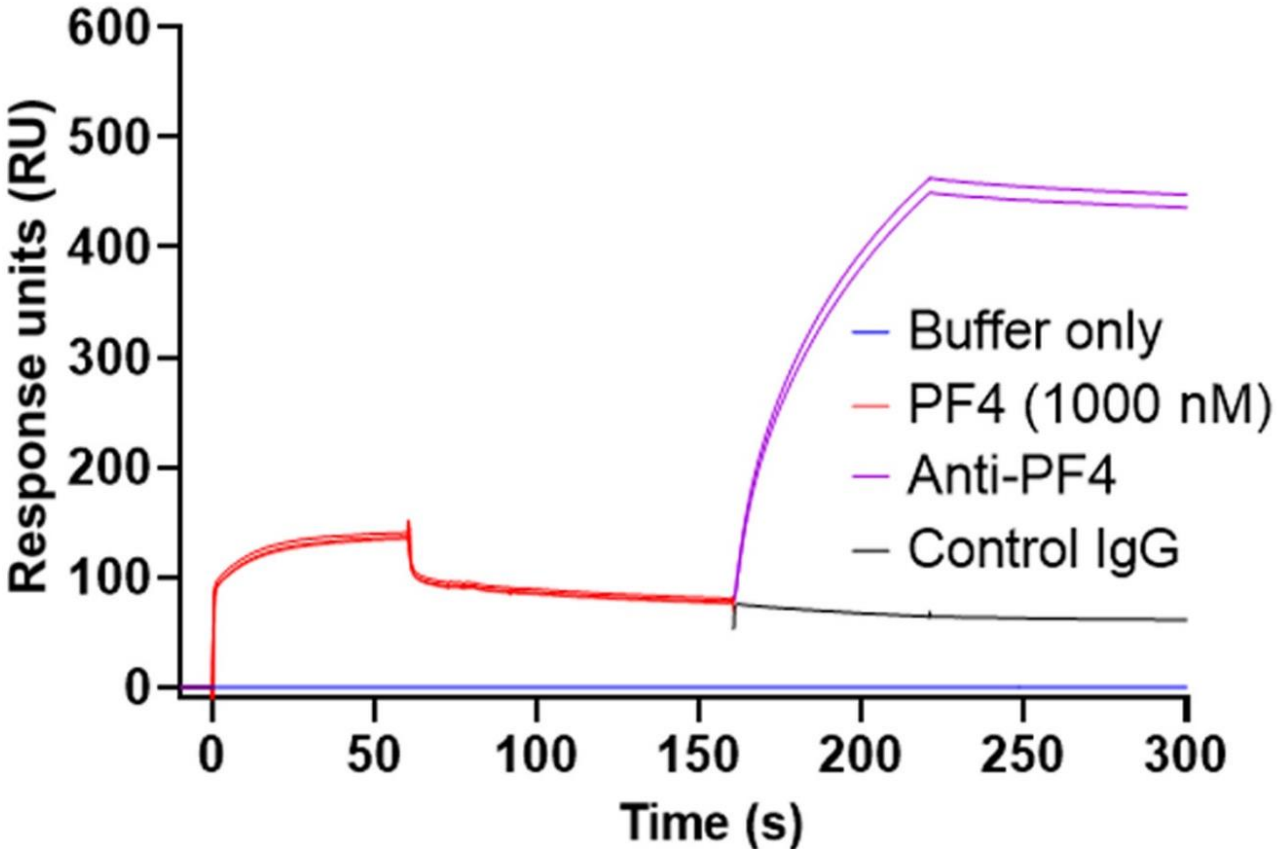
Capable of tertiary complex formation

Dose dependent interaction between ChAdOx1 and PF4

Exhibited by 3 clinical adenovirus species

Complex is stable and specific

Complex formation is inhibited by the salt and heparin



ChAdOx1 Binds to PF4

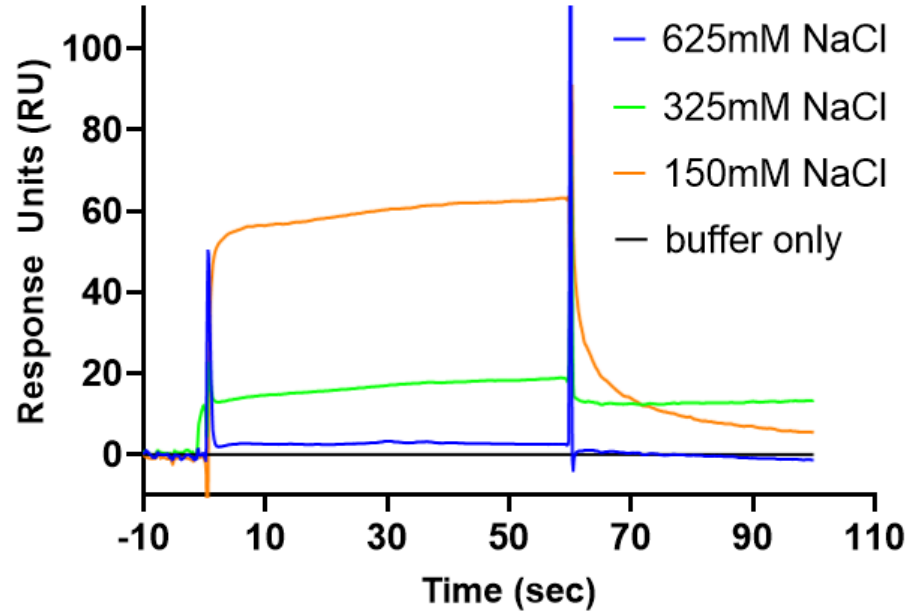
Charge dependent

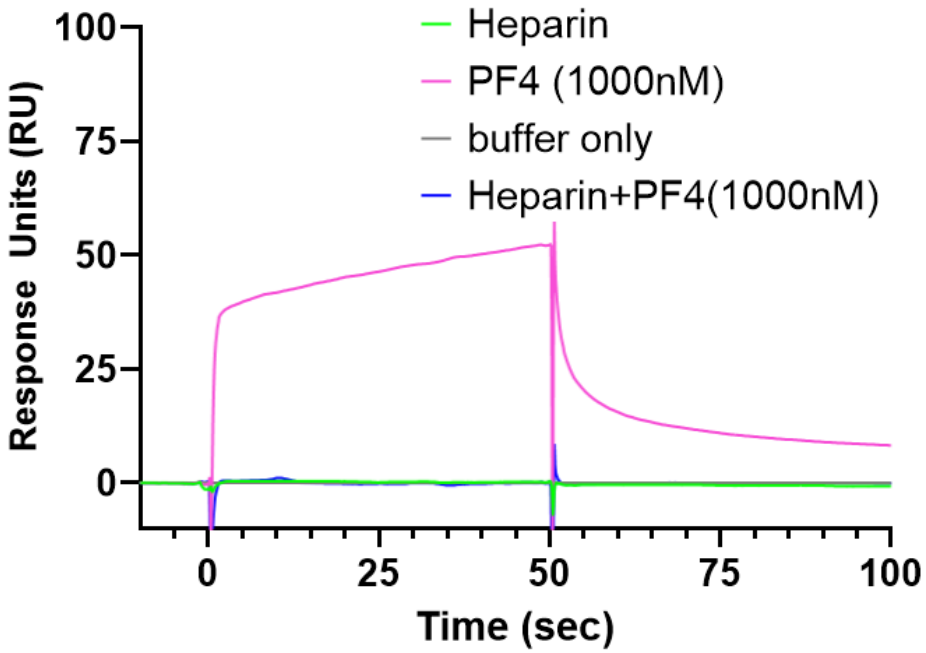
Dose dependent interaction between ChAdOx1 and PF4

Exhibited by 3 clinical adenovirus species

Complex is stable and specific

Complex formation is inhibited by the salt and heparin





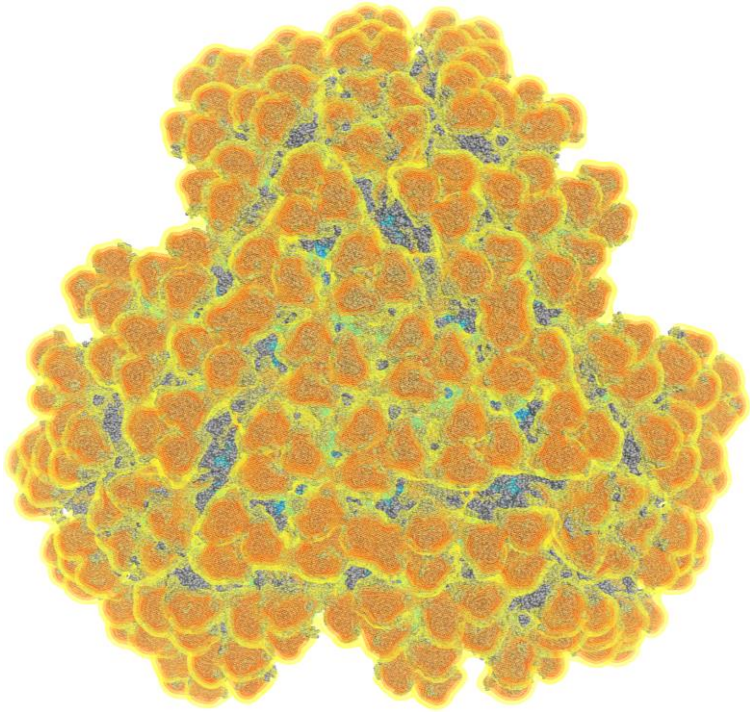
4 Mechanisms underpinning the adenovirus/PF4 interactions

Mechanism of Interaction

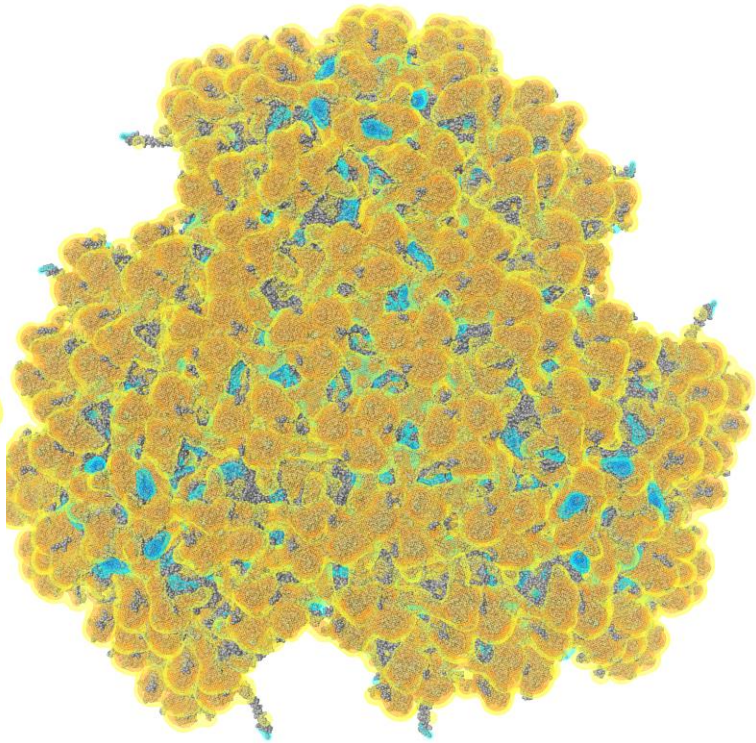
Opposites attract

Adenovirus capsids are negatively charged

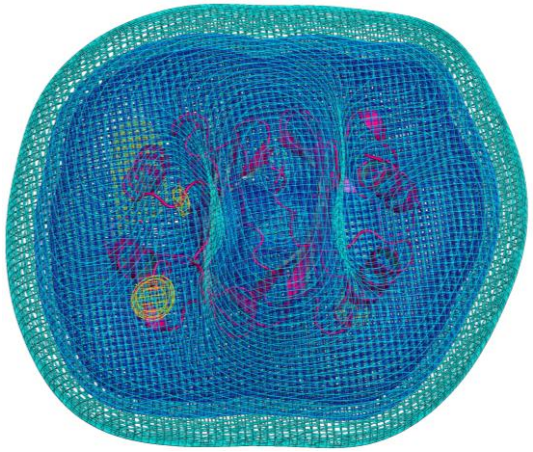
ChAdOx1



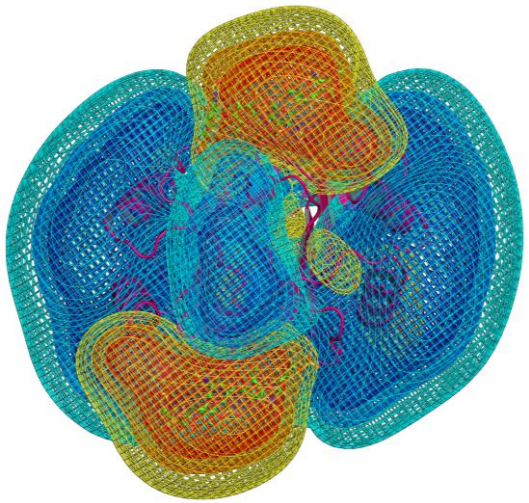
HAdV-D26



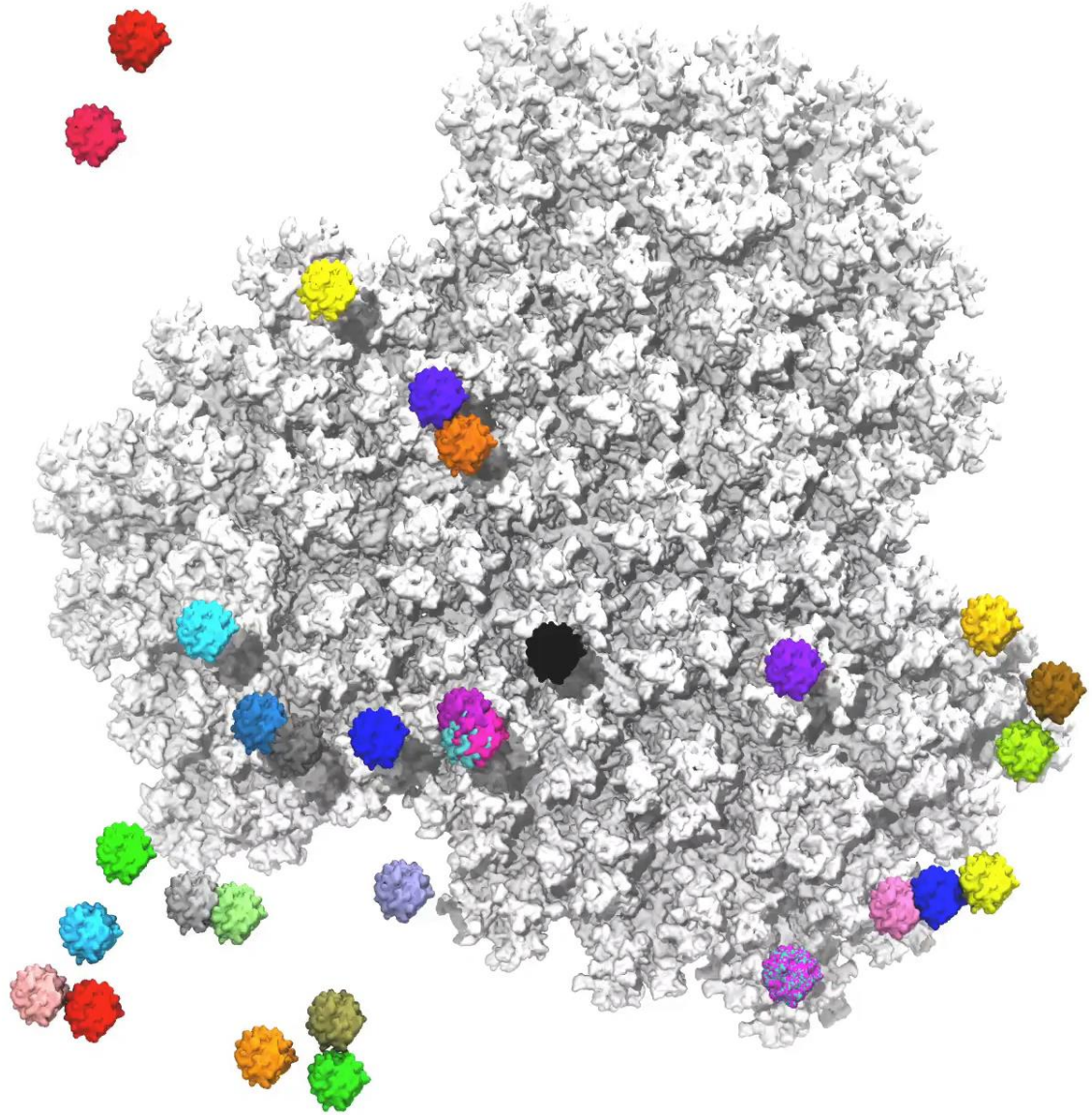
PF4 (Unbound)



PF4 (Heparin)



Brownian Dynamics (BD) simulations of platelet factor 4 (PF4) over ChAdOx1

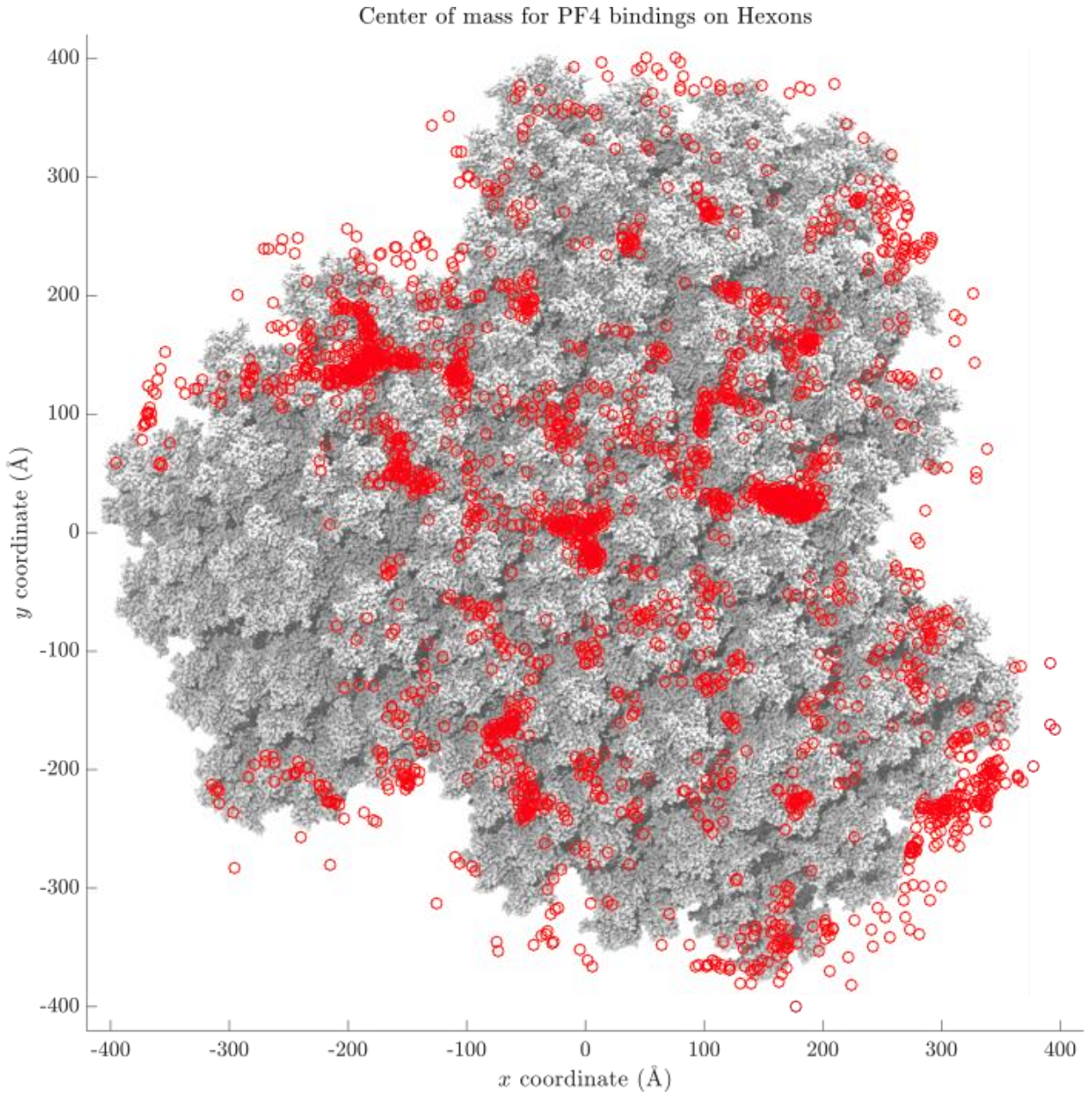


Mechanism of Interaction

Collisions are not randomly distributed

Adenovirus capsids are negatively charged

Most interactions are at the interfaces between hexons



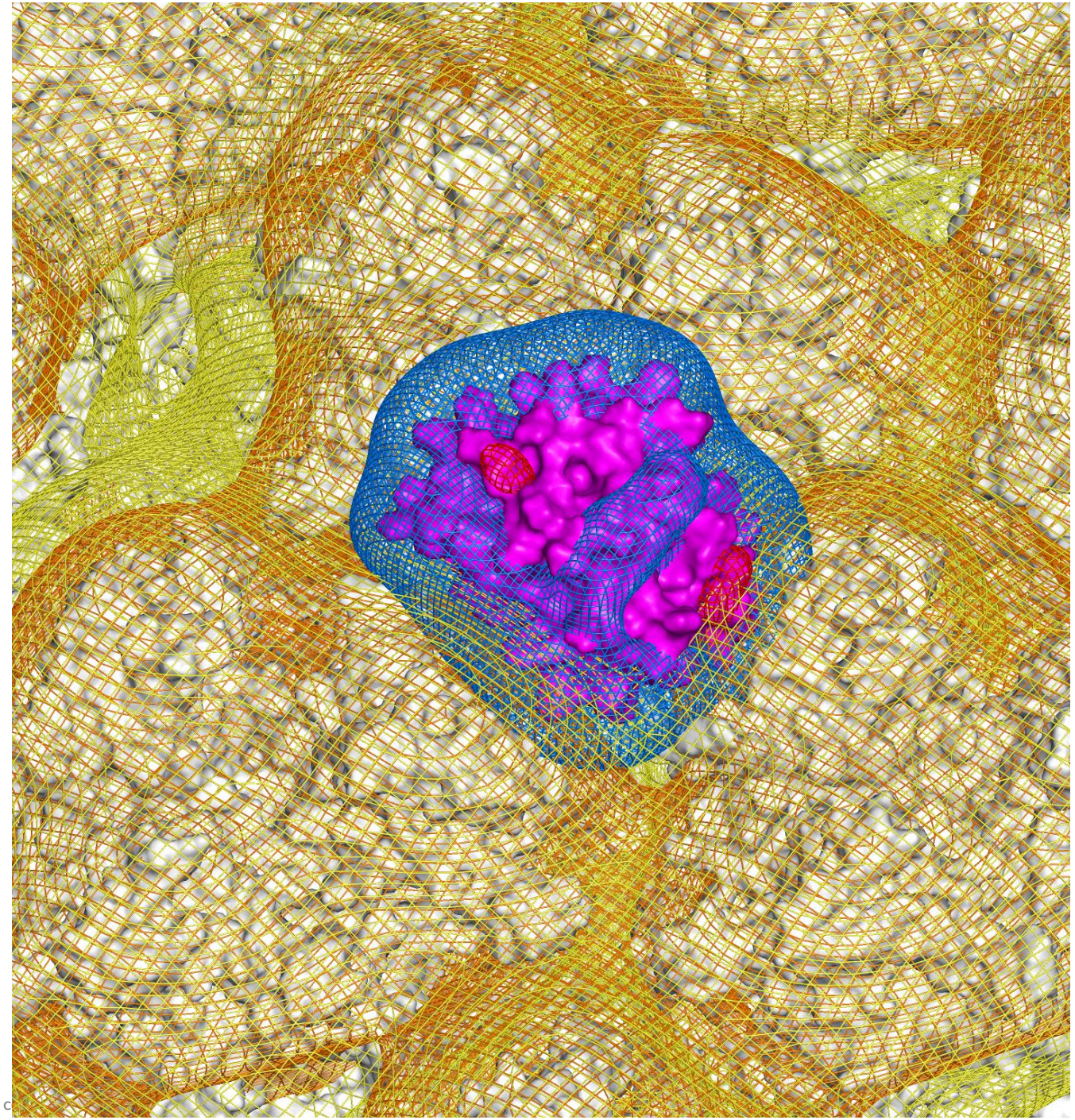
Mechanism of Interaction

Multiple factors are required

Adenovirus capsids are negatively charged

Most interactions are at the interfaces between hexons

Both charge and shape influence stability



5 Follow-up

Other information

Unpublished and/or preliminary

Questions raised:

1. To what extent is shape complementarity and electrostatic mechanism contributing to this interaction?

2. Is the frequency of interaction relevant to the mechanism of TTS in a biological context above a certain threshold of interaction?

3. Is a tertiary partner affecting the complex?

A hypothesis

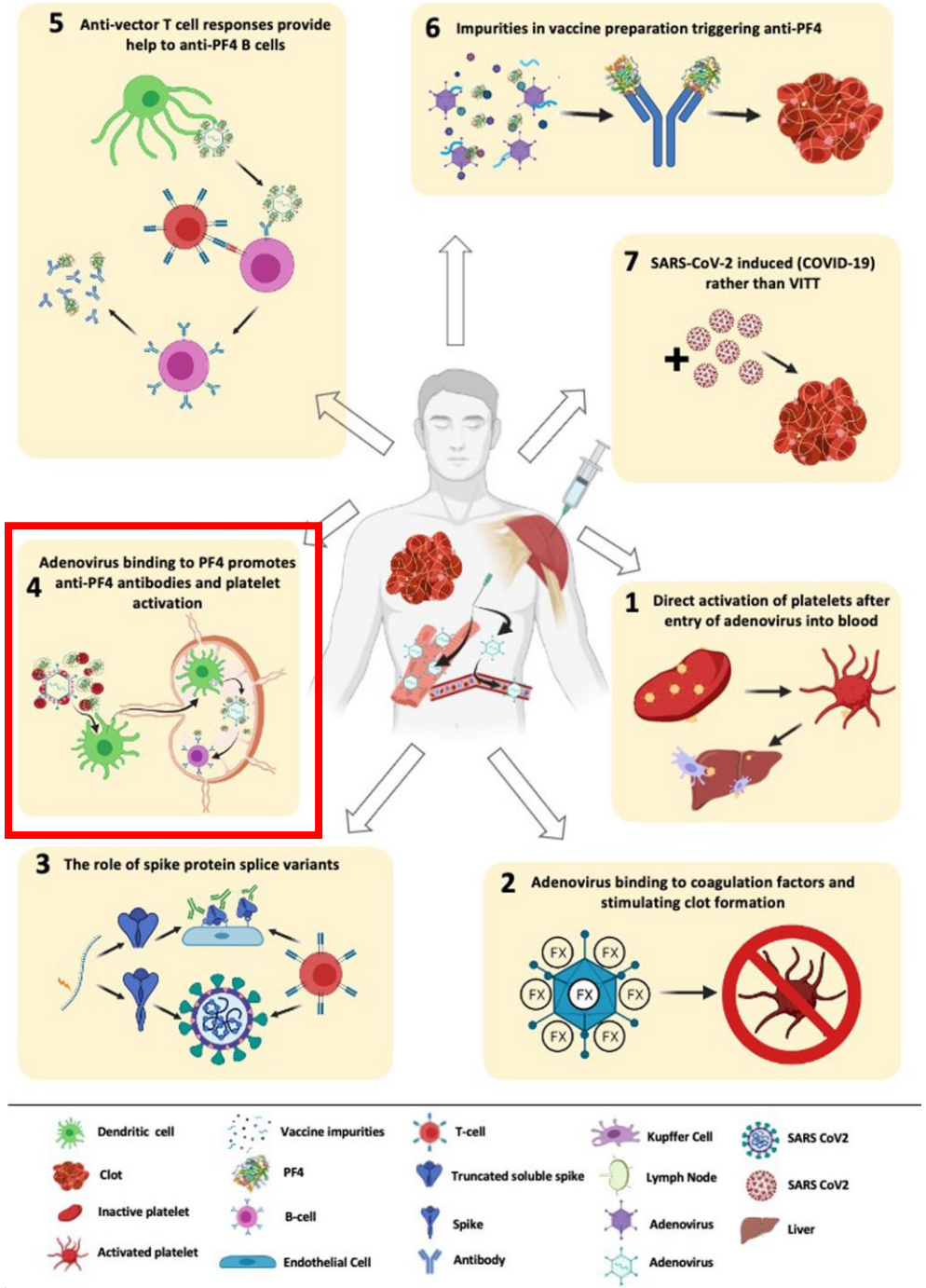
I. An Adenovirus-PF4 complex forms

II. The complex reaches the lymph

III. Stimulates proliferation of pre-existing anti-PF4 B-cells and antibody secretion

IV. Antibody-PF4-Platelet complexes form

V. Immuno-aggregates stimulate thrombosis, similar to what is observed in HIT



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