McMaster Centre for Transfusion Research

Michael G. DeGroote SCHOOL OF MEDICINE

Disease mechanisms in Vaccine-Induced Immune Thrombotic Thrombocytopenia

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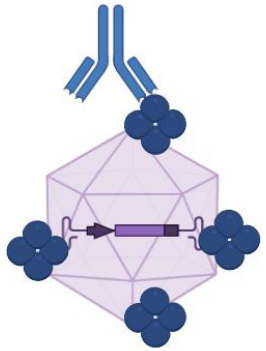
Objectives

- How do viral vector-based COVID-19 vaccines lead to VITT?
  - PF4 and ChAdOx1 interaction – molecular mapping

- How do the clotting events happen in VITT?
  - Molecular and biochemical characteristics of VITT antibodies

Viral vector-based COVID-19 vaccines and VITT

- Vaccine-induced immune thrombotic thrombocytopenia (VITT)
- Thrombosis and thrombocytopenia following vaccination with adenovirus-based COVID-19 vaccines:
  - ChAdOx1 nCoV-19/AZD1222 (Oxford-AstraZeneca)
  - Ad26.COV.2.S (Johnson & Johnson)
- Associated with IgG class antibodies against platelet factor 4 (PF4)

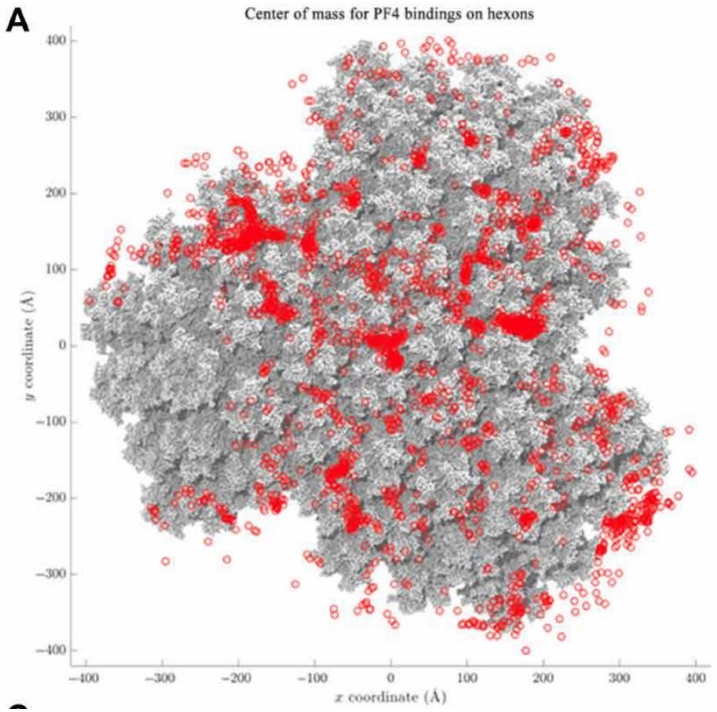


PF4-ChAdOx1-IgG complex

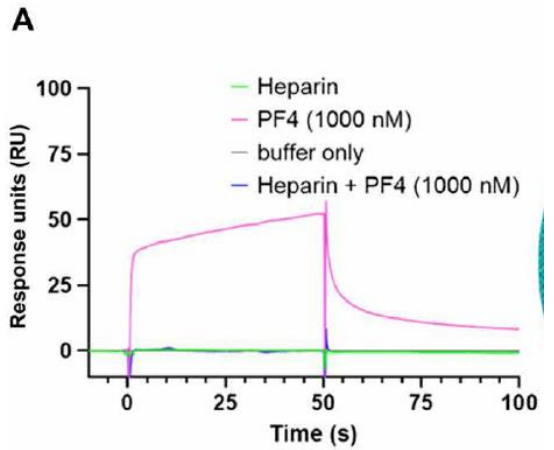
CORONAVIRUS

ChAdOx1 interacts with CAR and PF4 with implications for thrombosis with thrombocytopenia syndrome

Alexander T. Baker[1,2,3,4,5,6]*†, Ryan J. Boyd[5,7]†, Daipayan Sarkar[7,8,9]‡, Alicia Teijeira-Crespo[6]‡, Chun Kit Chan[10]‡, Emily Bates[6], Kasim Waraich[11], John Vant[5,7], Eric Wilson[5,7], Chloe D. Truong[5,7], Magdalena Lipka-Lloyd[12], Petra Fromme[5,7], Josh Vermaas[8,9], Dewight Williams[13], LeeAnn Machiesky[14], Meike Heurich[15], Bolni M. Nagalo[1,2,4]§, Lynda Coughlan[16,17], Scott Umlauf[14], Po-Lin Chiu[5,7], Pierre J. Rizkallah[18], Taylor S. Cohen[19]*, Alan L. Parker[6]*, Abhishek Singharoy[5,7]*, Mitesh J. Borad[1,2,3,4]*



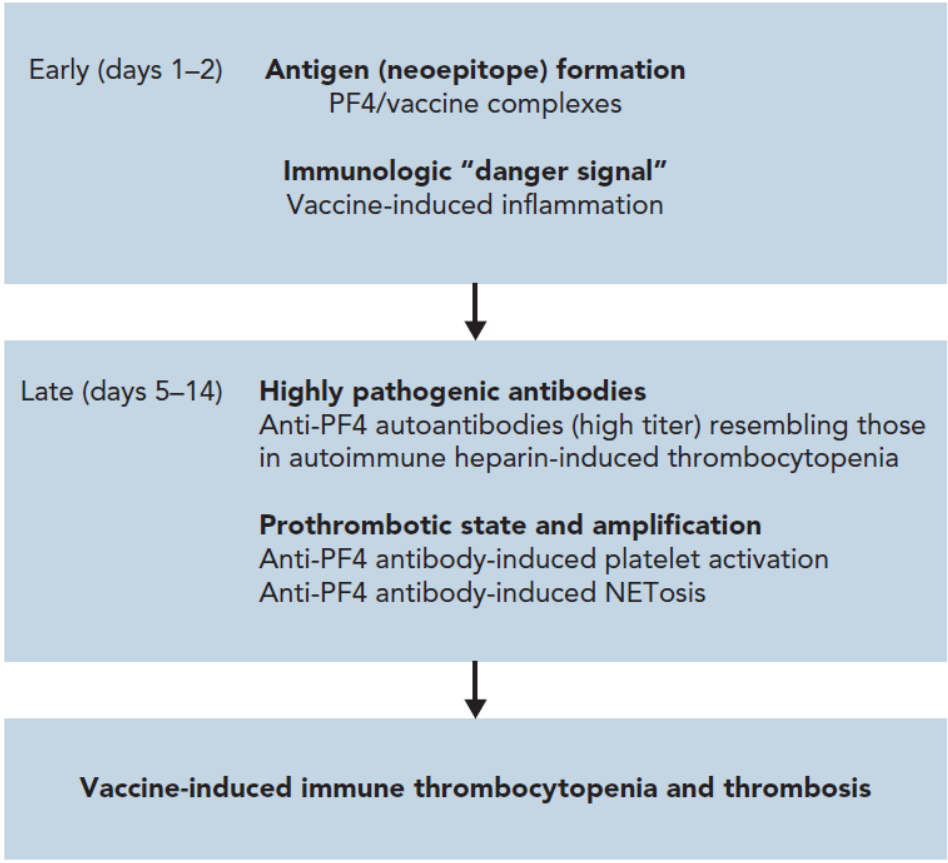
- ChAdOx1 binds to PF4 at the Heparin binding site – inhibition assays
- Charge-dependent electrostatic interaction occurring mostly at the inter-hexon spaces



Insights in ChAdOx1 nCoV-19 vaccine-induced immune thrombotic thrombocytopenia

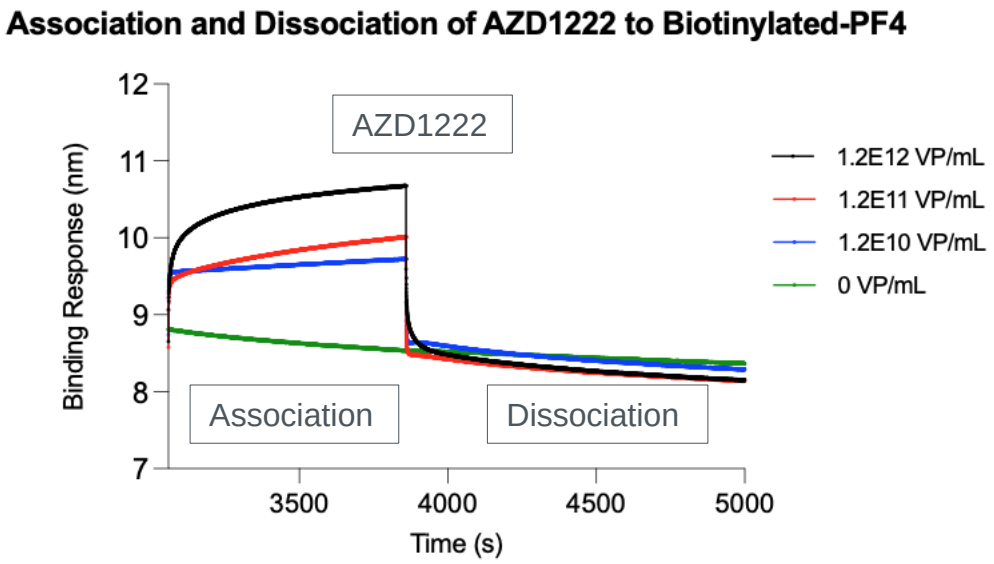
Andreas Greinacher,[1] Kathleen Selleng,[1] Raghavendra Palankar,[1] Jan Wesche,[1] Stefan Handtke,[1] Martina Wolff,[1] Konstanze Aurich,[1] Michael Lalk,[2] Karen Methling,[2] Uwe Völker,[3,4] Christian Hentschker,[3] Stephan Michalik,[3] Leif Steil,[3] Alexander Reder,[3] Linda Schönborn,[1] Martin Beer,[5] Kati Franzke,[6] Andreas Büttner,[7] Boris Fehse,[8,9] Evi X. Stavrou,[10,11] Chandini Rangaswamy,[12] Reiner K. Mailer,[12] Hanna Englert,[12] Maike Frye,[12] Thomas Thiele,[1] Stefan Kochanek,[13] Lea Krutzke,[13] Florian Siegerist,[14] Nicole Endlich,[14,15] Theodore E. Warkentin,[16,17] and Thomas Renné[12,18]

- Immunofluorescence microscopy revealed complexes between:
  - ChAdOx1-derived hexon particles & PF4
  - ChAdOx1, PF4 & VITT anti-PF4 IgG on platelet surface
- Vaccination with ChAdOx1 induces proinflammatory responses
  - Presence of T-Rex HEK293-origined proteins and EDTA in the vaccine
- B cells produce anti-PF4 IgGs, which further lead to platelet activation



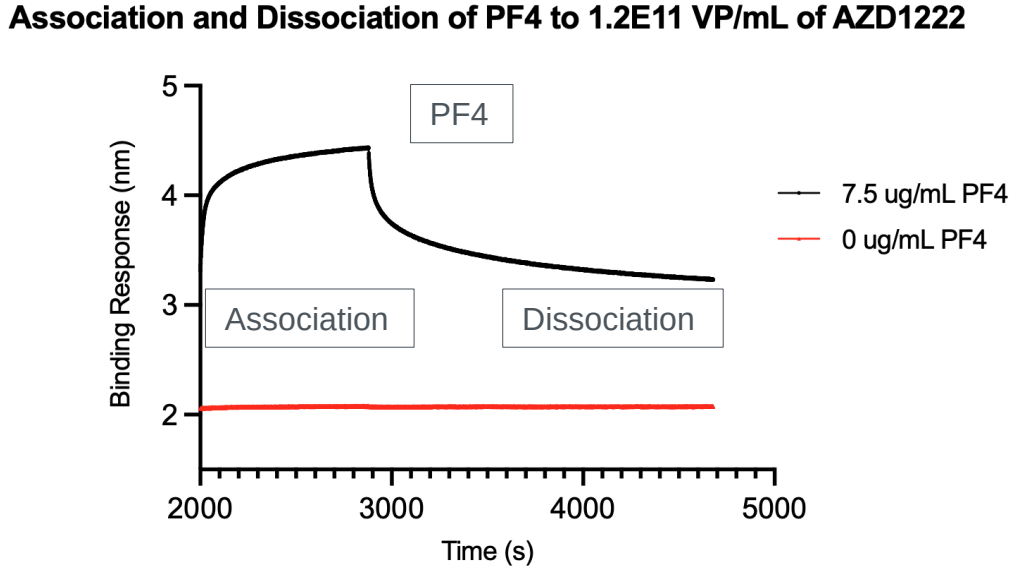
PF4 binding to ChAdOx1 using Biolayer Interferometry

Streptavidin Sensor



[AZD1222] (VP/mL)	Response (nm)	K_D (M)	K_D error
1.2E12	1.29	4.20E-09	4.40E-09

Amine Coupling Sensor



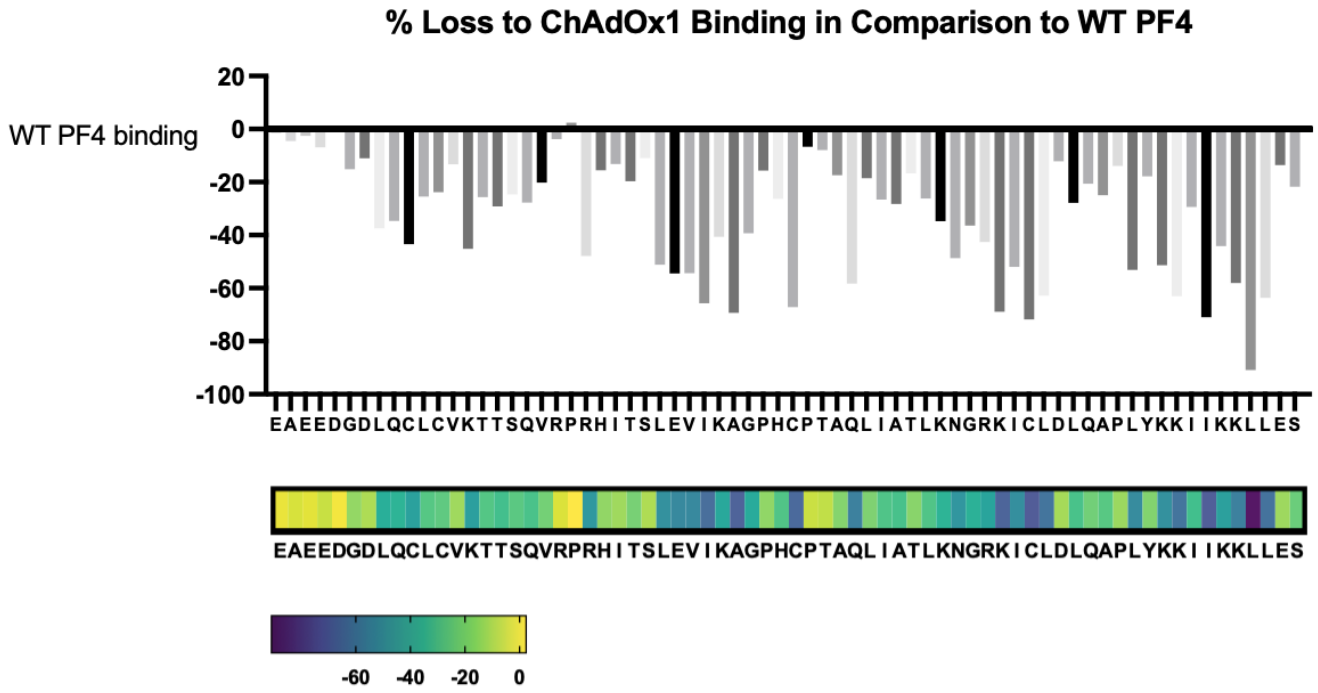
Response (nm)	K_D (M)	K_D error
2.04	1.08E-09	4.06E-11

Alanine Scanning Mutagenesis technology

Wild-type PF4 (70 amino acids)	EAEEDGDLQCL	.....	QAPLYKKIIKKLLES
Mutant E1A	**A**AEEDGDLQCL	.....	QAPLYKKIIKKLLES
Mutant A2V	E**V**EEDGDLQCL	.....	QAPLYKKIIKKLLES
Mutant E3A	EA**A**EDGDLQCL	.....	QAPLYKKIIKKLLES
Mutant L68A	EAEEDGDLQCL	.....	QAPLYKKIIKKL**A**ES
Mutant E69A	EAEEDGDLQCL	.....	QAPLYKKIIKKLL**A**S
Mutant S70A	EAEEDGDLQCL	.....	QAPLYKKIIKKLLE**A**

Molecular mapping of PF4 binding to ChAdOx1 using BLI

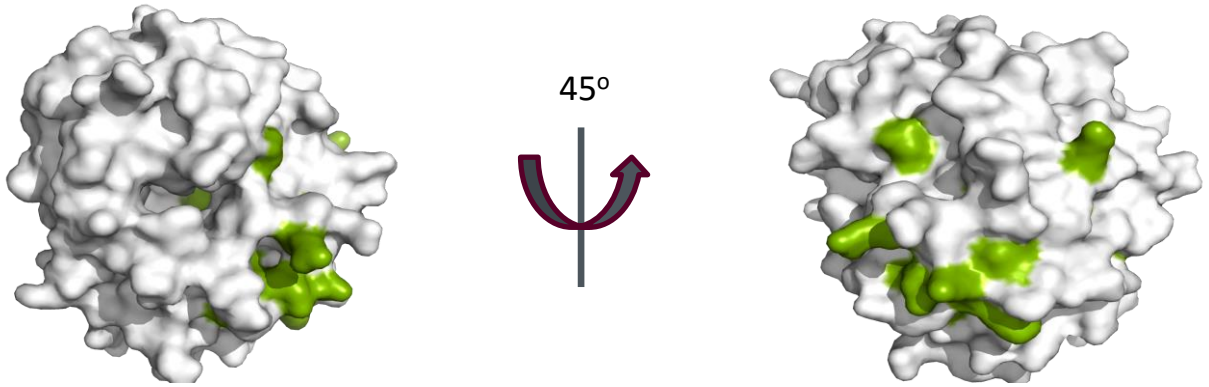
- Coupled ChAdOx1 to BLI sensors
- Measured > 45% loss in binding of all PF4 mutants compared to wildtype PF4
- Identified multiple amino acids involved in the binding of PF4 and ChAdOx1
- ChAdOx1 binding site overlaps with the heparin site
- there are 5aa in common between the two binding sites



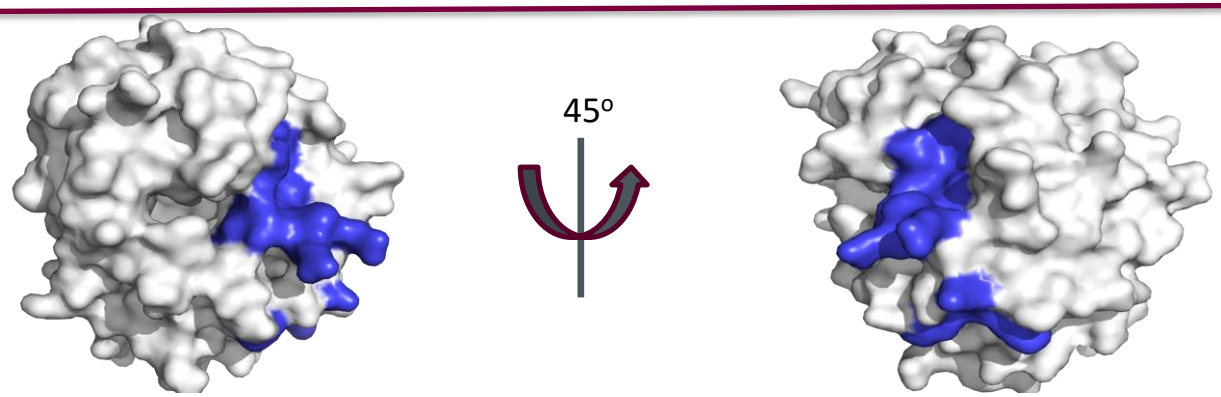
Molecular Mapping of PF4 binding to ChAdOx1

ChAdOx1 site

(≥ 45% loss of binding comparing to WT PF4)



Heparin site



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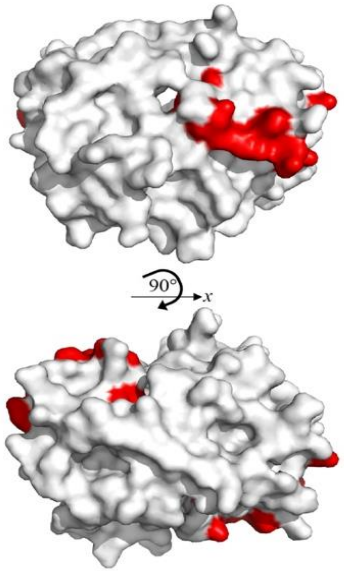
Antibody epitopes in vaccine-induced immune thrombotic thrombocytopaenia

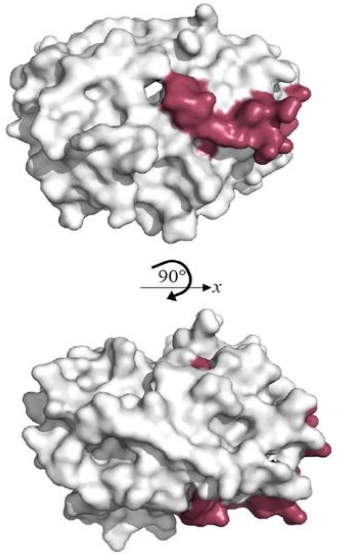
Angela Huynh, John G. Kelton, Donald M. Arnold, Mercy Daka & Ishac Nazy

VITT antibodies bind to a distinct and restricted site on PF4, which overlaps the heparin binding site

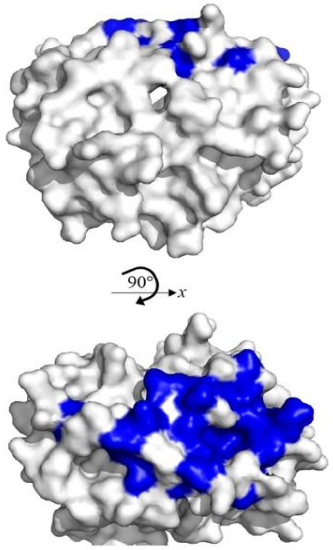
A) VITT (n = 5)



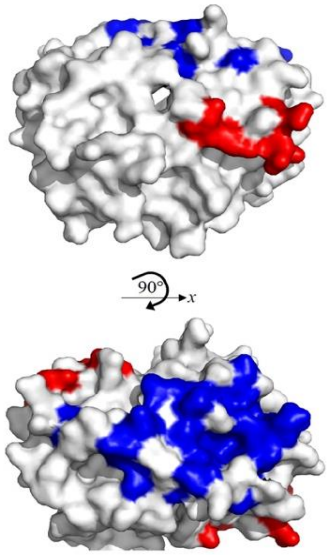
B) Heparin binding site



C) HIT (n = 4)



D) HIT (n = 6)



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Characteristics of VITT antibodies in patients vaccinated with Ad26.COV2.S

Tracking no: ADV-2022-007336R2

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VITT # 1 (J&J) VITT # 2 (J&J) VITT # 3 (J&J)