

BIO-479 Exercise 2

Lecture 2 Recapitulation

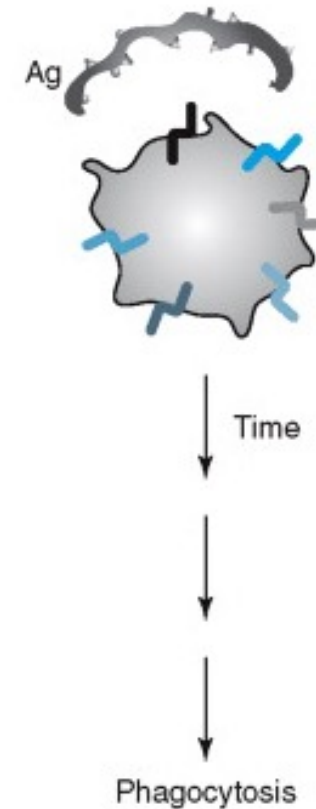
Soluble Effector Molecules of Innate Immunity

- Which statement(s) about soluble effector molecules of innate immunity below are **NOT** correct?
- 1, they can bind to microbes and act as opsonins, enhancing the ability of phagocytes to ingest pathogens
- 2, they recognize and neutralize viral nucleic acids in the cytoplasm, preventing viral replication
- 3, they promote inflammatory responses by recruiting innate immune cells to the sites of infection
- 4, complement proteins, collectins, pentraxins and ficolins are some of their components
- 5, they are mainly produced by neutrophils during infection

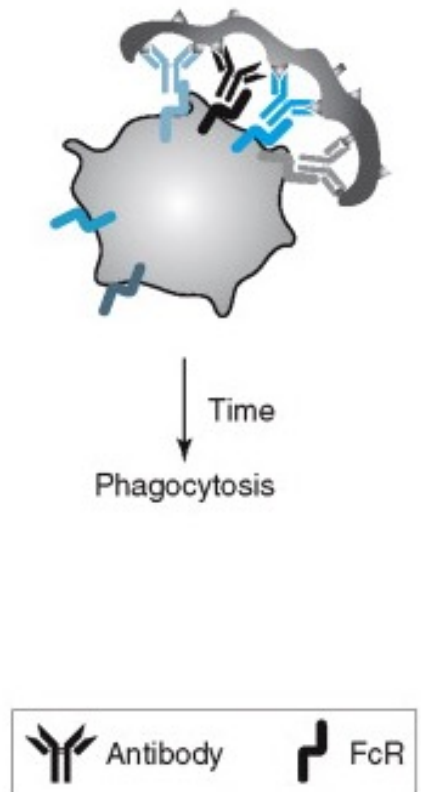
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A. Without opsonin

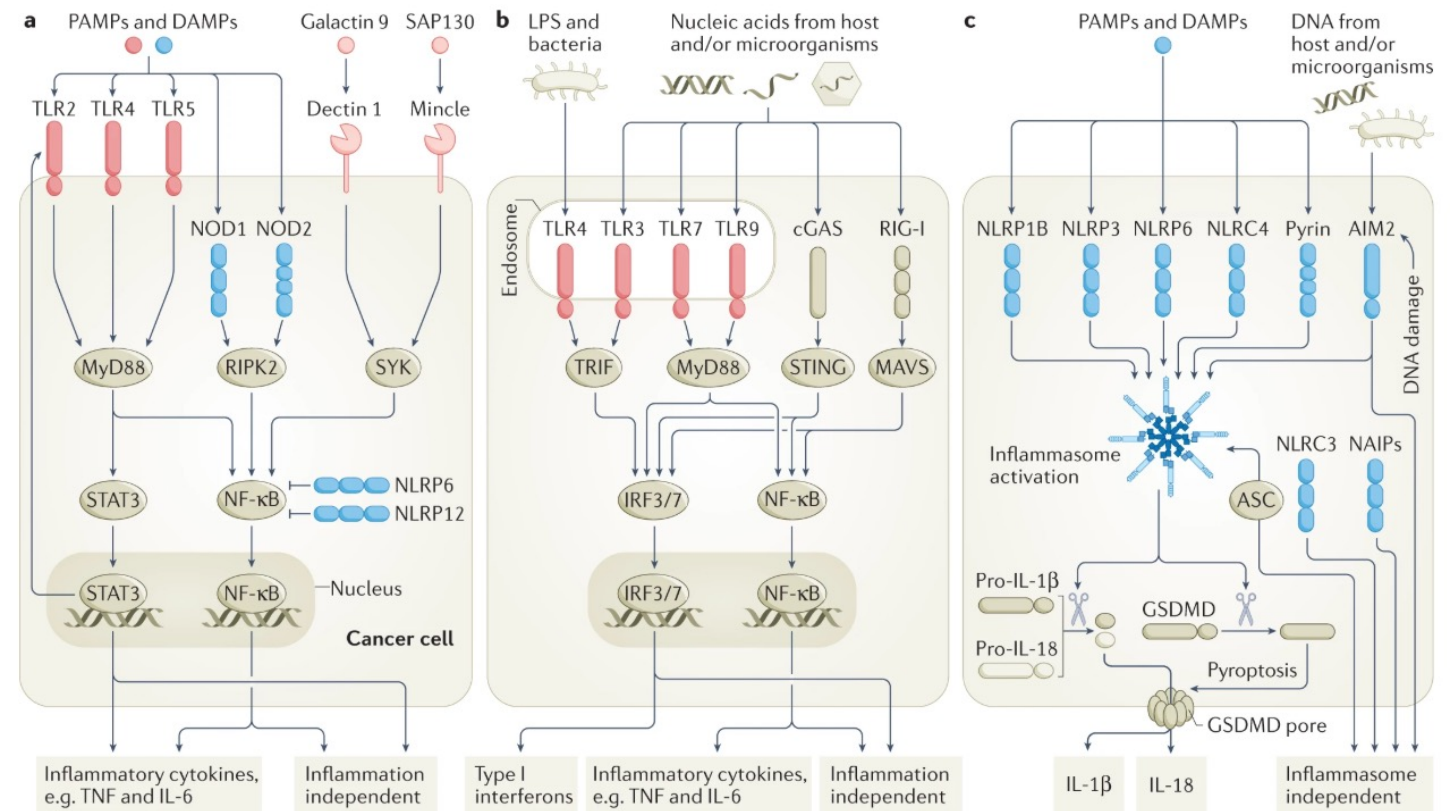


B. With opsonin



Soluble Effector Molecules of Innate Immunity

- Which statement(s) about soluble effector molecules of innate immunity below are **NOT** correct?
- 2, they **DO NOT** recognize and neutralize viral nucleic acids in the cytoplasm, preventing viral replication



Soluble Effector Molecules of Innate Immunity

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- 3, they promote inflammatory responses by recruiting innate immune cells to the sites of infection
- 4, complement proteins, collectins, pentraxins and ficolins are some of their components
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The Complement System is a collection of soluble proteins mainly produced in the liver.

Collectins – liver, intestinal mucosa, some lung epithelial cells

Pentraxins – mainly liver

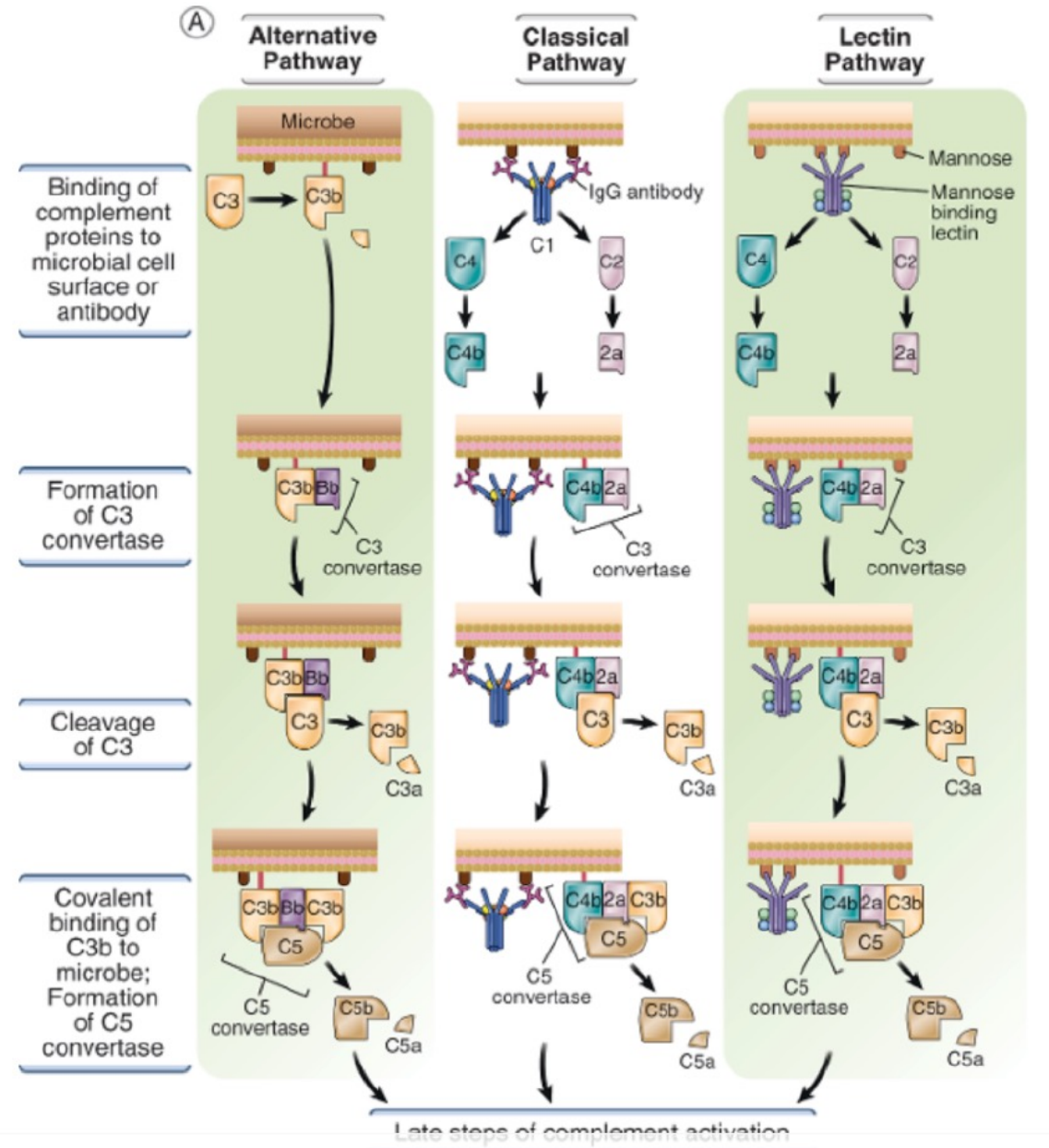
Ficolins – monocyte, liver, etc.

Complement System

- Which statement(s) below are **NOT** correct?
- 1, the complement system is a collection of more than 30 soluble proteins mainly produced in liver and circulate as inactive precursors.
- 2, there are three main pathways of complement activation: classical, alternative and lectins
- 3, activation of complement leads to cleavage of central components like C3 and C5, producing fragments such as C3b for opsonization and C3a/C5a for anaphylatoxins
- 4, the main function of the complement system is to promote B cell proliferation and antibody class switching during adaptive immune responses.
- 5, The complement system enhances opsonization, recruits immune cells, and can directly lyse microbes via the membrane attack complex

Complement System

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Complement System

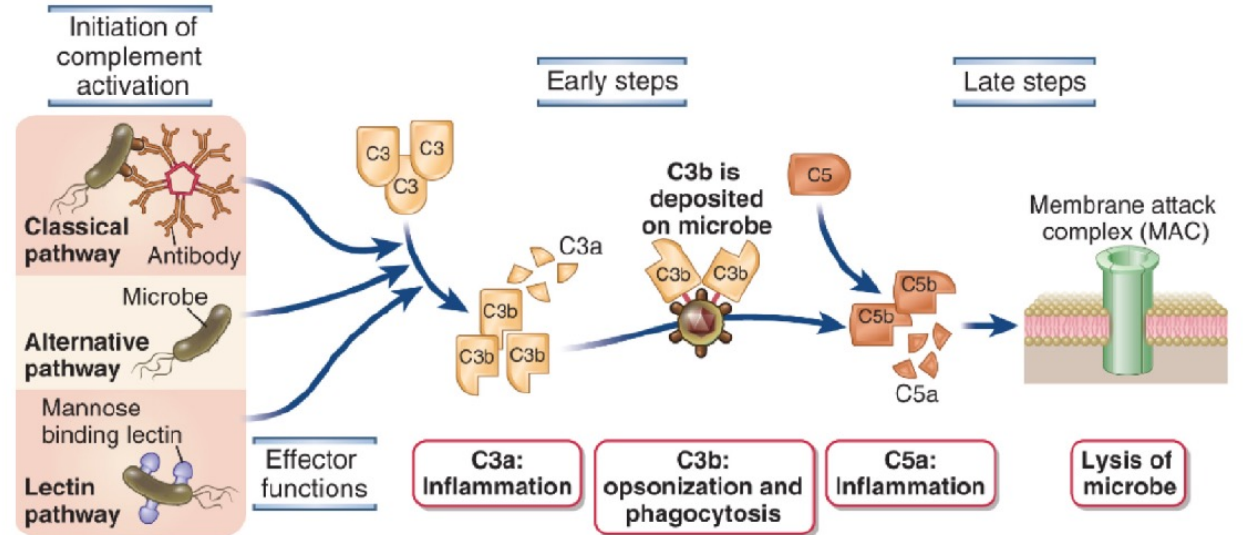
- Which statement(s) below are **NOT** correct?
- 4, the main function of the complement system is to promote B cell proliferation and antibody class switching during adaptive immune responses.



Mainly by CD4+ T cells

Complement System

- Which statement(s) below are **NOT** correct?
- 5, The complement system enhances opsonization, recruits immune cells, and can directly lyse microbes via the membrane attack complex



Inflammation

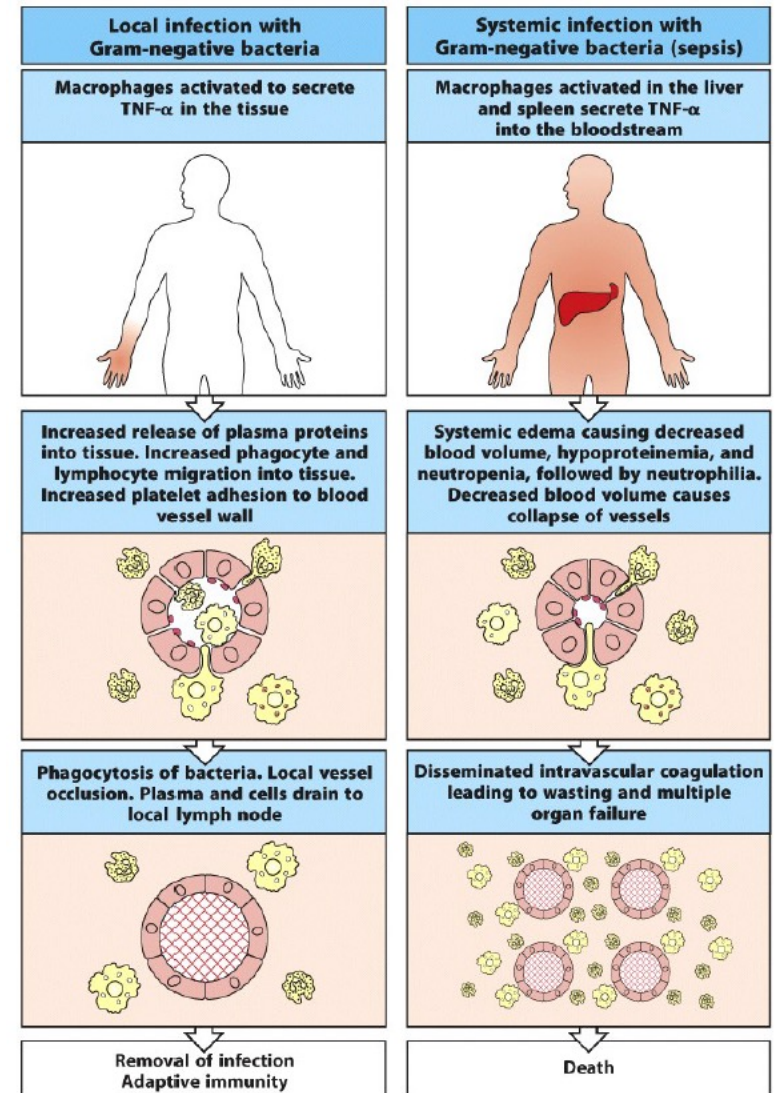
- Which statement(s) below are correct?
- 1, generally speaking, extracellular bacteria can recruit neutrophils, monocytes and complement, intracellular bacteria can activate phagocytes, and viruses can induce type I interferons and NK cell activity
- 2, the four characteristics of inflammation are redness, warmth, swelling, and pain, which result from increased vascular permeability, and stimulation of nerve endings
- 3, acute inflammatory reactions are initiated by cytokines, chemokines, and small-molecule mediators released by sentinel cells (such as macrophages and dendritic cells) upon detection of MAMPs and DAMPs.
- 4, generally, inflammation does not require adaptive immunity to initialize but proinflammatory cytokines can contribute to innate and adaptive immunity

Tumor Necrosis Factor and Other Important Cytokines and Chemokines

- Which statement(s) below are **NOT** correct?
- 1, TNF receptors are expressed on most cells and activate multiple signaling pathways that regulate inflammation and immunity
- 2, controlled TNF- α production can stimulate leukocyte extravasation, promote local clotting, and support dendritic cell migration and maturation. It is beneficial in this manner
- 3, excessive systemic TNF- α leads to harmful effects, such as septic shock, and disseminated intravascular coagulation (DIC)
- 4, as a pathologic effects, TNF, IL-1 and IL-6 can induce fever in Brain, and leukocyte production in bone marrow
- 5, Liver is responsible for acute phase response induced by IL-1 and IL-6. The relevant proteins include C-reactive protein, Mannose-binding lectin and surfactant proteins

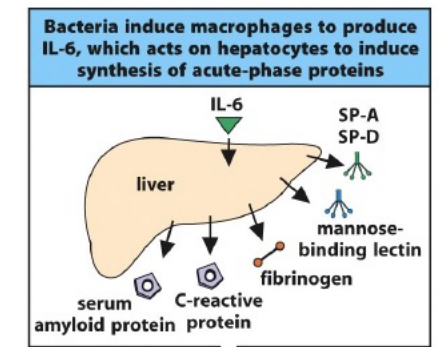
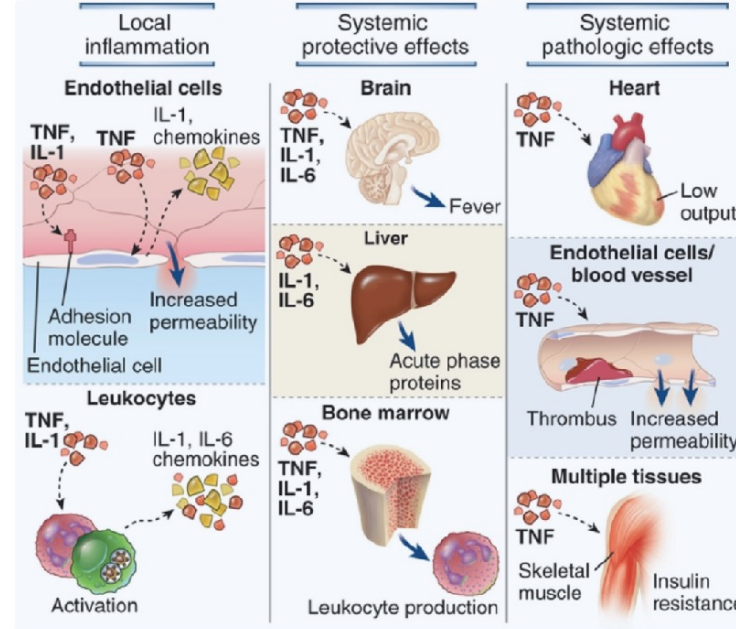
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Tumor Necrosis Factor and Other Important Cytokines and Chemokines

- Which statement(s) below are **NOT** correct?
- 4, as a **PROTECTIVE** effects, TNF, IL-1 and IL-6 can induce fever in Brain, and leukocyte production in bone marrow
- 5, Liver is responsible for acute phase response induced by IL-1 and IL-6. The relevant proteins include C-reactive protein, Mannose-binding lectin and surfactant proteins



1) CRP

- C-reactive protein (pentraxin family)
- CRP: multipronged PR molecule, that binds to bacterial/fungal cell wall components
 - > opsonizing of pathogens
 - > activation of the complement

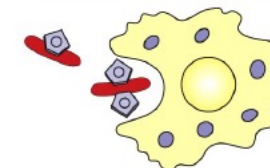
2) MBL (mannose-binding lectin)

- opsonin, activation of the complement cascade

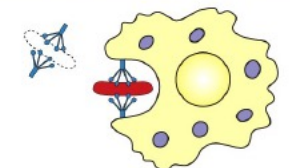
3) SP-A/SP-D (surfactant proteins)

- opsonin for *Pneumocystis jirovecii*

C-reactive protein binds phosphocholine on bacterial surfaces, acting as an opsonin, and also activating complement



Mannose-binding lectin binds mannose residues on bacterial surfaces, acting as an opsonin, and also activating complement

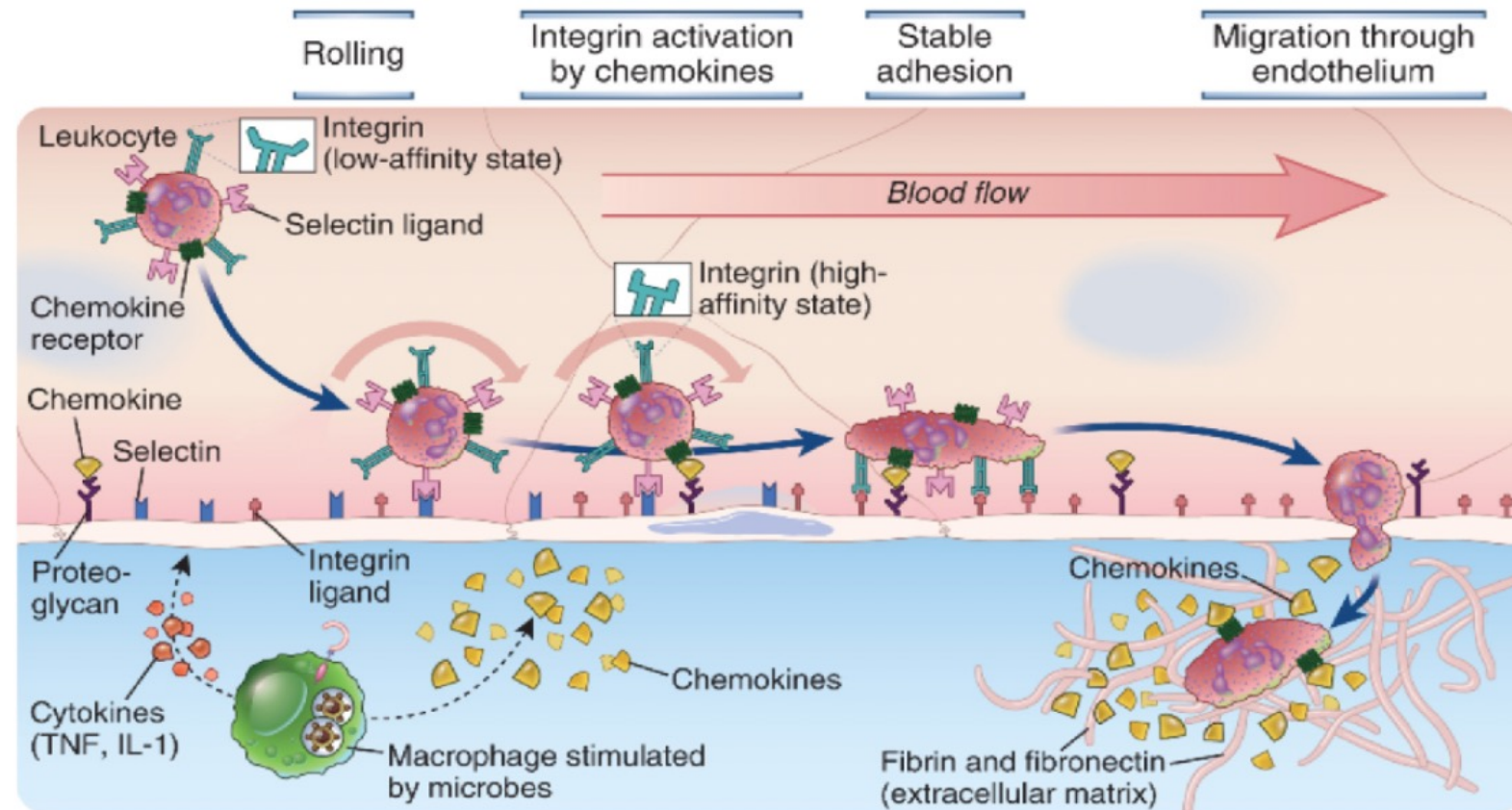


Leukocyte Migration and Adhesion Molecules

- Which statement(s) are **NOT** correct?
- 1, selectins are expressed on activated endothelial cells, bind to carbohydrate ligands like sialyl-Lewis X on leukocytes, and mediate rolling adhesion
- 2, selectins mediate firm adhesion by directly binding to ICAM molecules on endothelial cells once leukocytes have been activated by chemokines
- 3, integrins on leukocytes exist in inactive and active states; chemokines activate integrins, allowing strong binding to ICAMs on endothelial cells for firm adhesion
- 4, intercellular adhesion molecules (ICAM-1 and ICAM-2) are induced on endothelial cells during infection and serve as ligands for leukocyte integrins such as LFA-1 and CR3
- 5, diapedesis (transmigration) occurs when leukocytes squeeze between endothelial cells, a process supported by adhesion molecules like CD31 and extracellular matrix remodeling

Leukocyte Migration and Adhesion Molecules

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- 1, selectins are expressed on activated endothelial cells, bind to carbohydrate ligands like sialyl-Lewis X on leukocytes, and mediate rolling adhesion
- 2, selectins mediate **LOOSE** adhesion by directly binding to ICAM molecules on endothelial cells once leukocytes have been activated by chemokines



Phagocytosis

- Which statement(s) below are **NOT** correct?
- 1, phagocytosis involves recognition of microbial surface structures, internalization into a phagosome, and fusion with lysosomes to form a phagolysosome
- 2, engagement of phagocytic receptors triggers assembly of the NADPH oxidase complex, producing reactive oxygen species that contribute to microbial killing
- 3, inducible nitric oxide synthase (iNOS) generates nitric oxide (NO), and lysosomal proteases (e.g., elastase, cathepsins) degrade microbial proteins in the phagolysosome
- 4, phagocytosis requires prior opsonization by antibodies or complement; phagocytic receptors cannot mediate direct recognition and uptake of non-opsonized microbes
- 5, neutrophil extracellular traps (NETs) release is the primary method for killing microbes that have already been internalized within phagosomes

Interferons

- Which statement(s) below are **NOT** correct?
- 1, type I interferons are produced by many cell types upon viral infection, with plasmacytoid dendritic cells being a major specialized producers
- 2, their production can be triggered by recognition of viral nucleic acids through TLRs and intracellular sensors
- 3, type I interferons downregulate MHC class I molecules on host cells, promoting recognition by cytotoxic T lymphocytes and protecting against autoimmunity
- 4, type I interferons induce an “antiviral state” by up-regulating interferon-stimulated genes (ISGs) that inhibit viral replication
- 5, type I interferons promote differentiation of naïve T cells into Th1 cells

BIO-479 Seminar 2

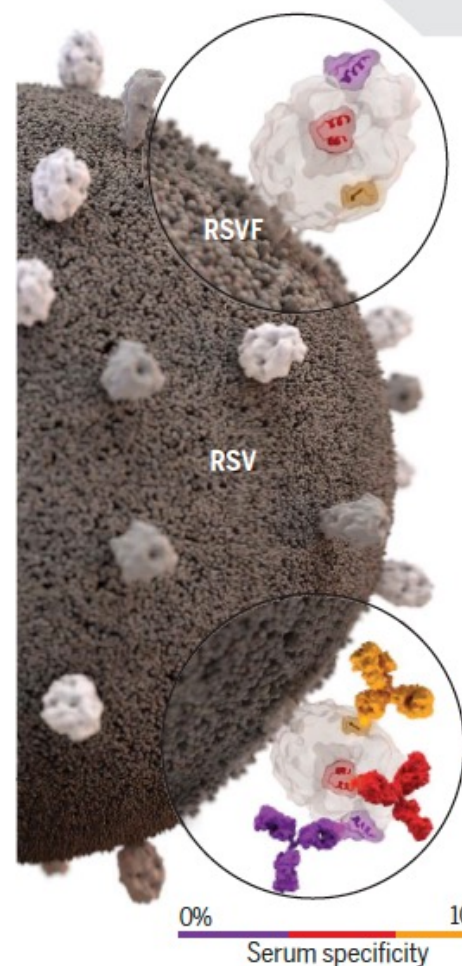
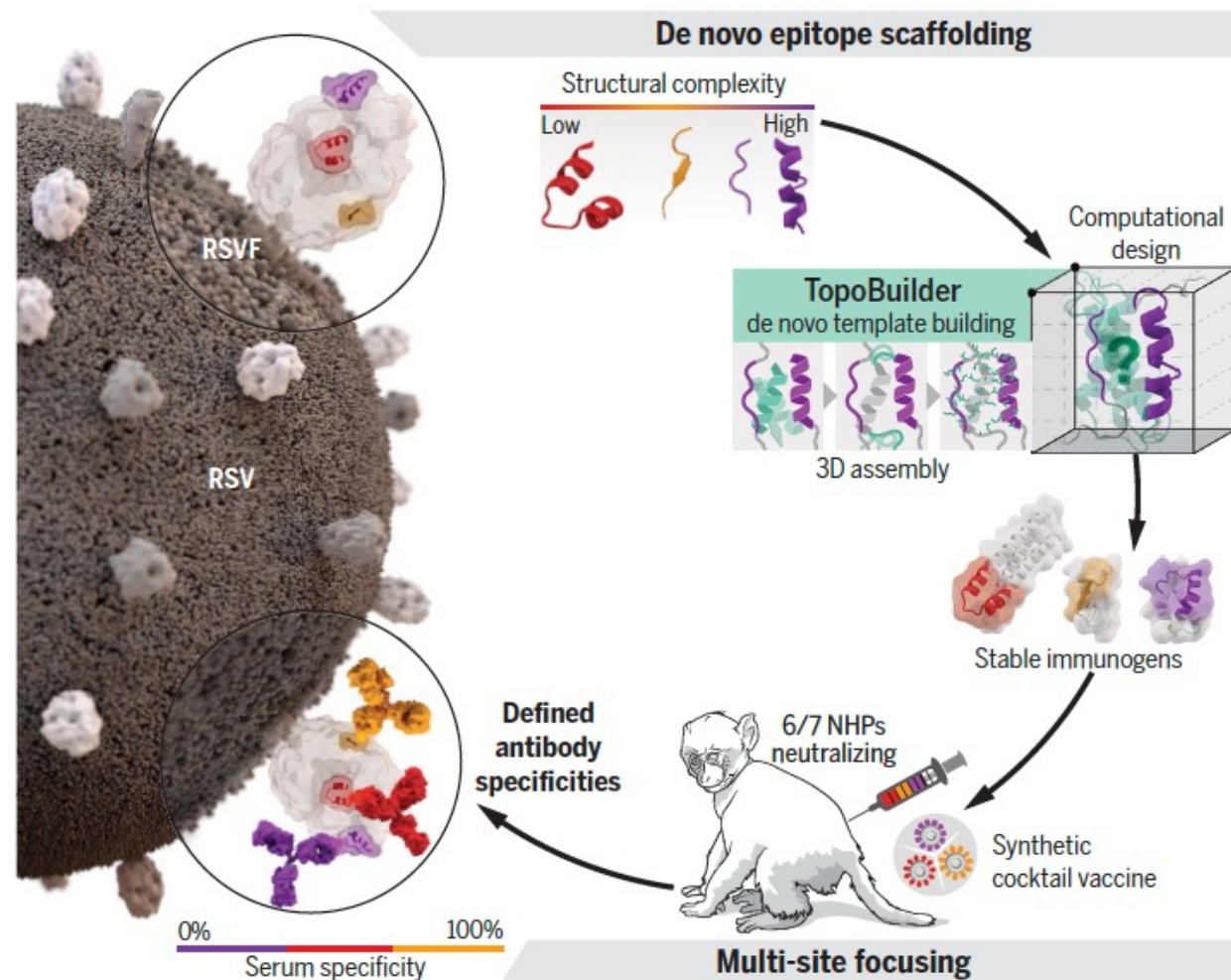
Computational Design for Immunological Components – Vaccines and Antibodies

RESEARCH ARTICLE

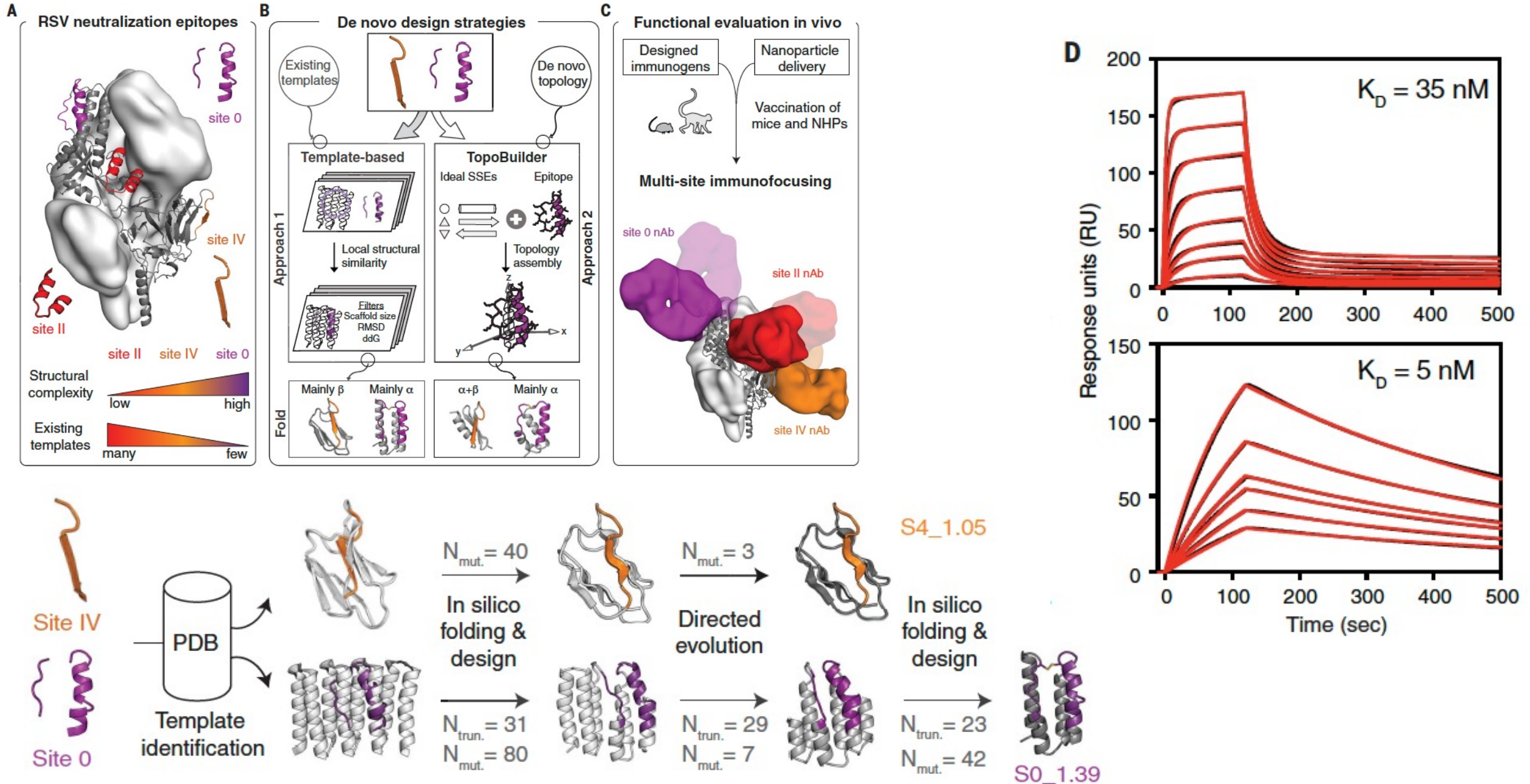
VACCINOLOGY

De novo protein design enables the precise induction of RSV-neutralizing antibodies

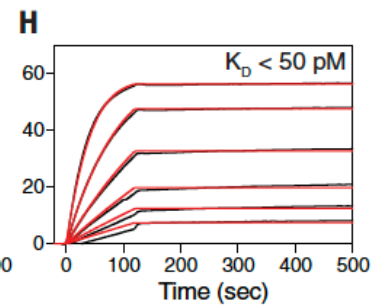
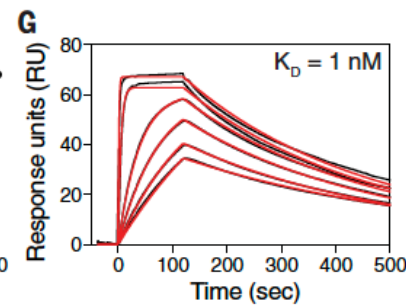
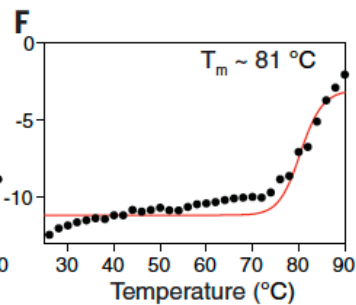
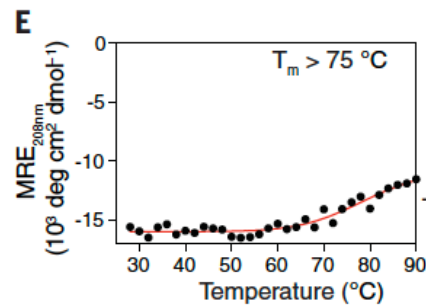
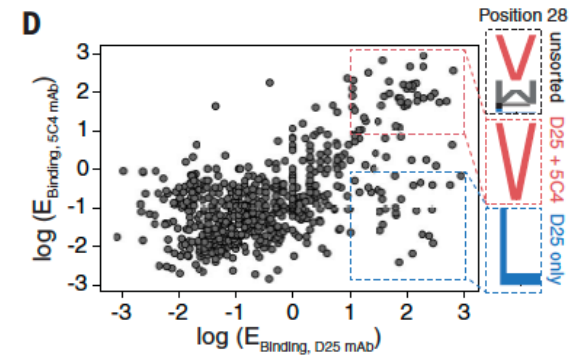
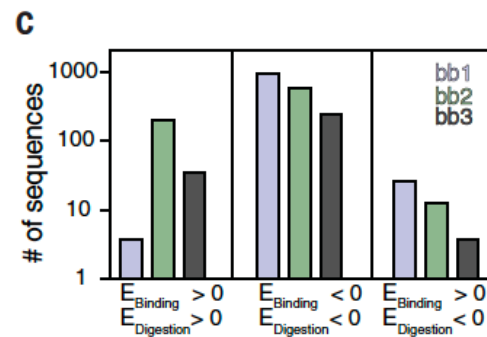
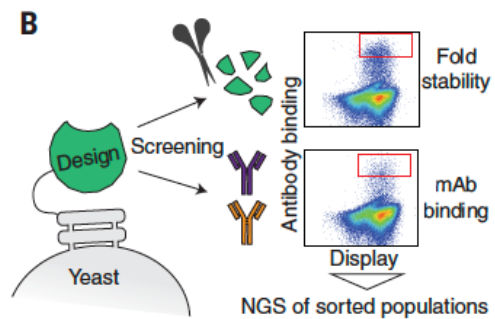
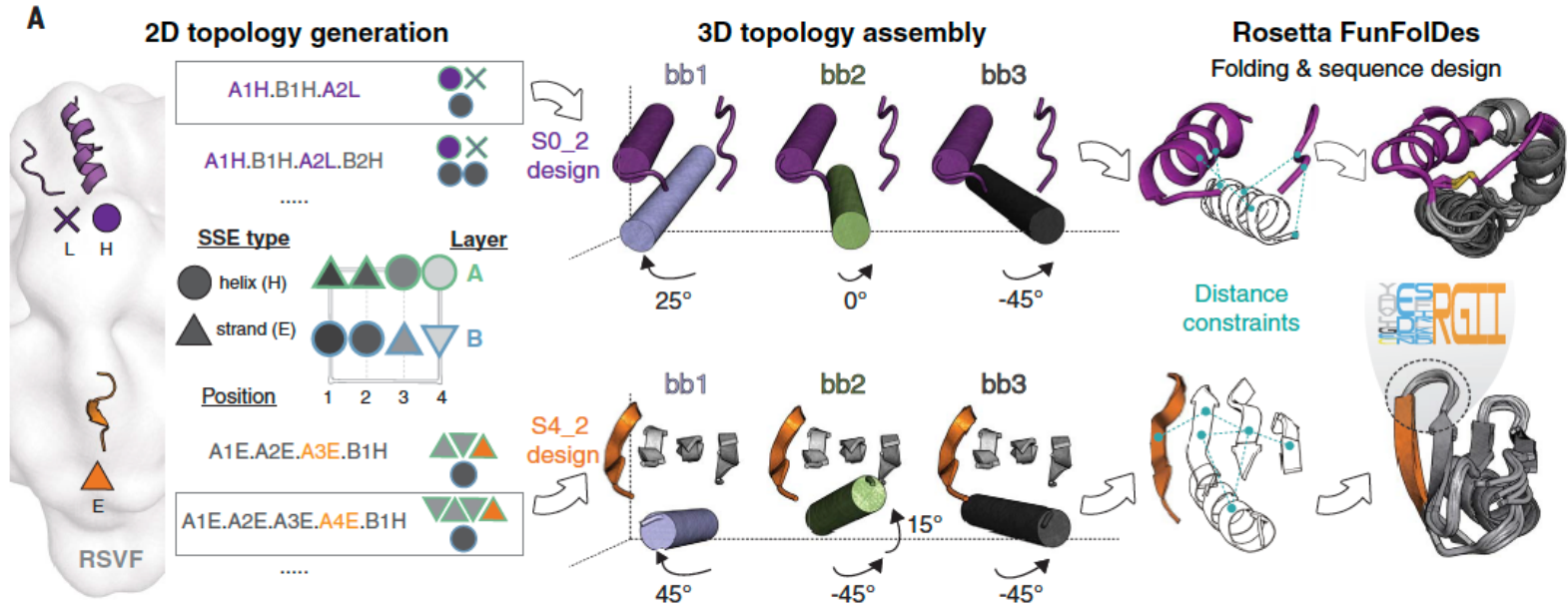
Fabian Sesterhenn^{1,2*}, Che Yang^{1,2*}, Jaume Bonet^{1,2†}, Johannes T. Cramer^{3†}, Xiaolin Wen⁴, Yimeng Wang⁵, Chi-I Chiang⁵, Luciano A. Abriata^{1,2}, Iga Kucharska^{6,7}, Giacomo Castoro³, Sabrina S. Vollers^{1,2‡}, Marie Galloux⁸, Elie Dheilly⁹, Stéphane Rosset^{1,2}, Patricia Corthésy^{1,2}, Sandrine Georgeon^{1,2}, Mélanie Villard^{1,2}, Charles-Adrien Richard⁸, Delphine Descamps⁸, Teresa Delgado¹⁰, Elisa Oricchio⁹, Marie-Anne Rameix-Welti¹¹, Vicente Más¹⁰, Sean Ervin¹², Jean-François Eléouët⁸, Sabine Riffault⁸, John T. Bates¹³, Jean-Philippe Julien^{6,7}, Yuxing Li^{5,14}, Theodore Jardetzky⁴, Thomas Krey^{3,15,16,17,18}, Bruno E. Correia^{1,2§}



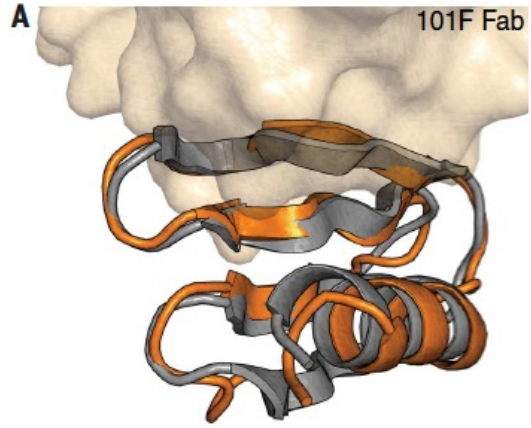
➤ Pre-AlphaFold *de novo* design



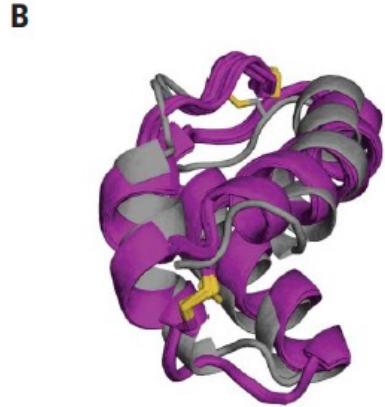
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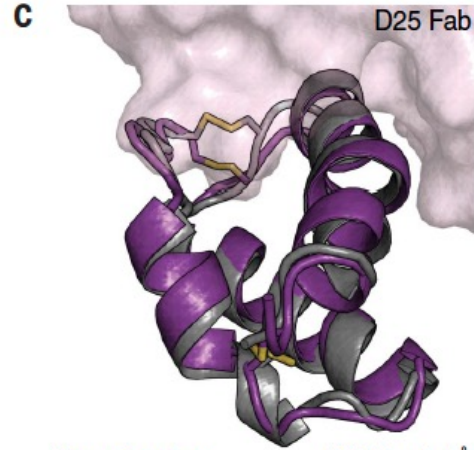
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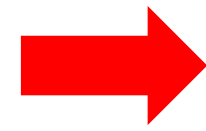
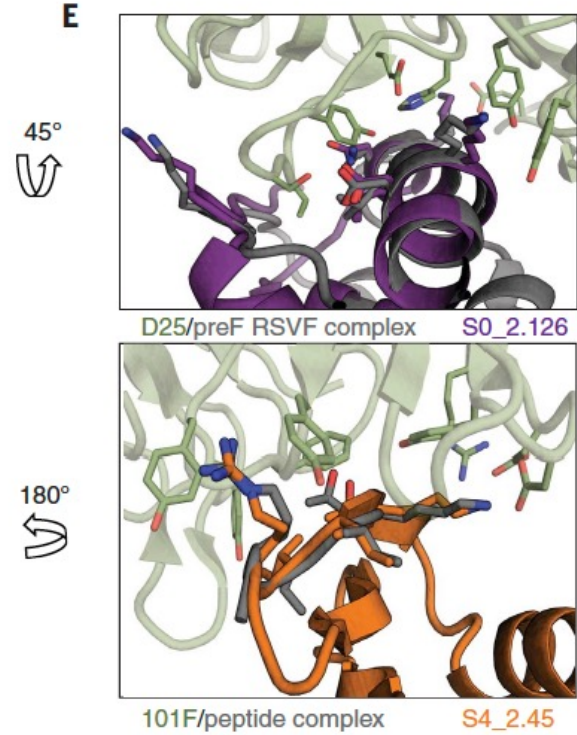
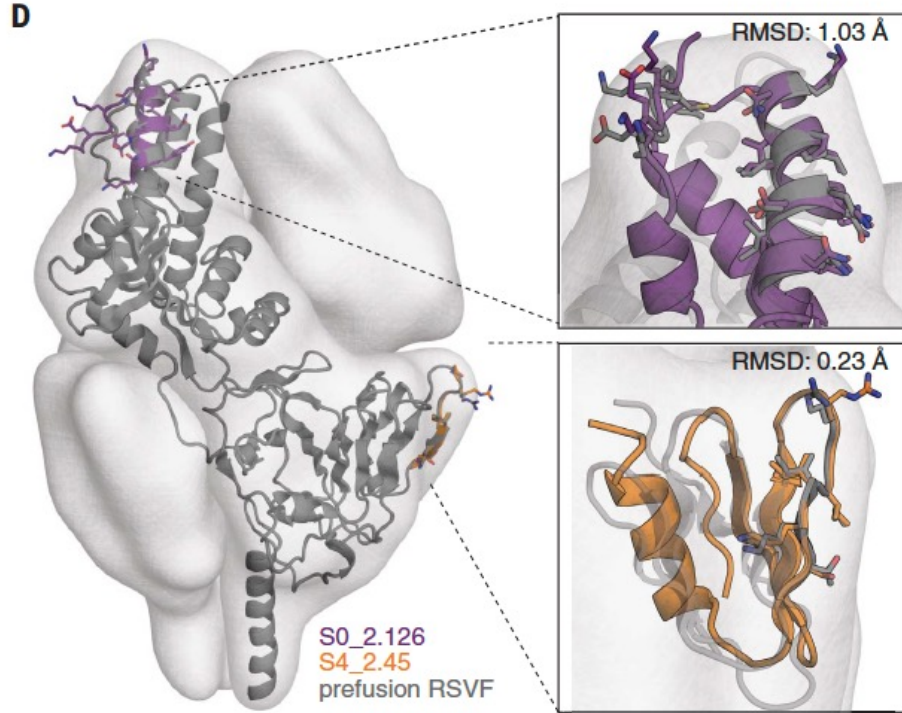
Xray structure RMSD = 1.5 Å
 Computational model E value = 4.8



NMR ensemble RMSD = 2.9 Å
 Computational model

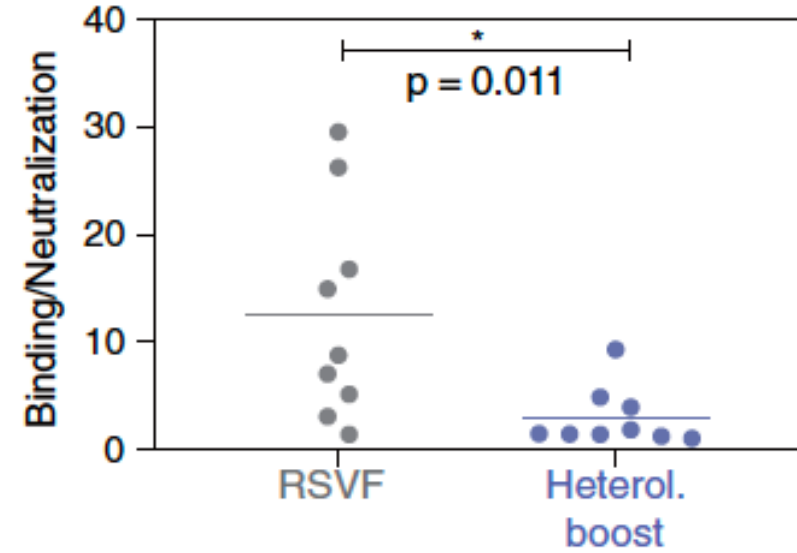
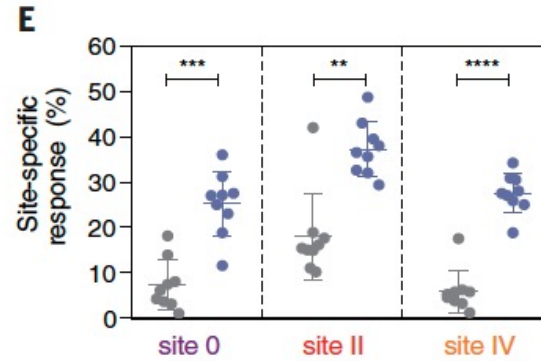
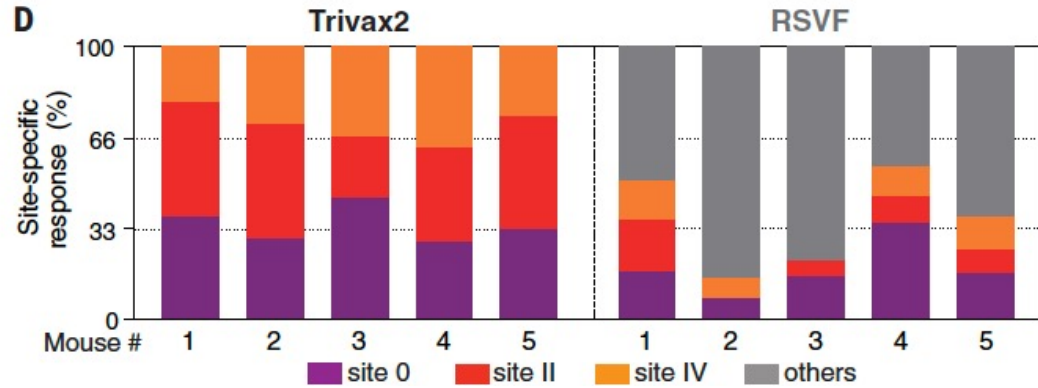
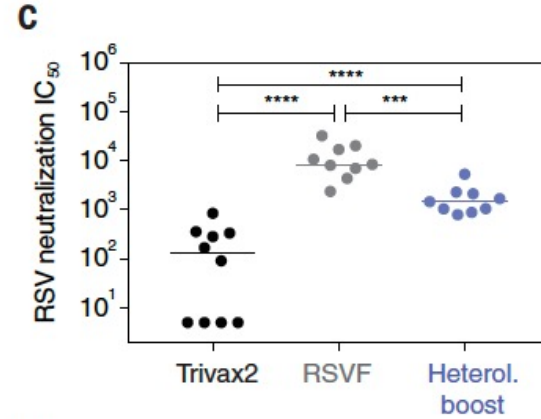
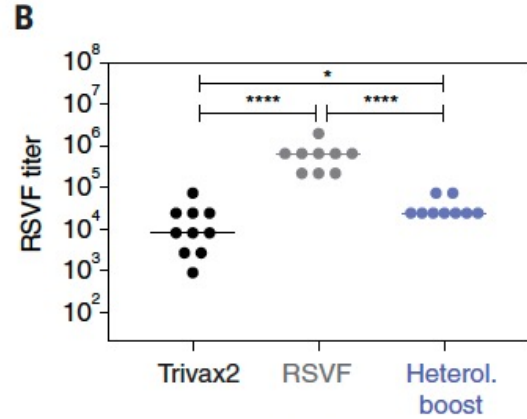
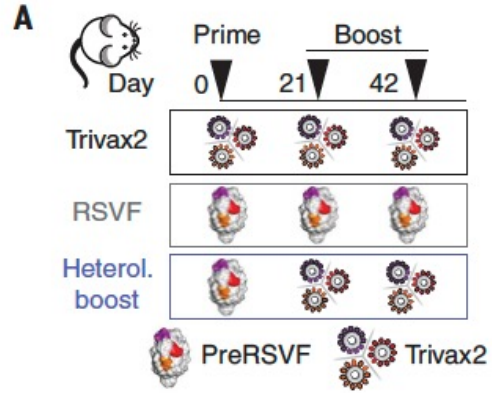


Xray structure RMSD = 1.4 Å
 Computational model E value = 0.11

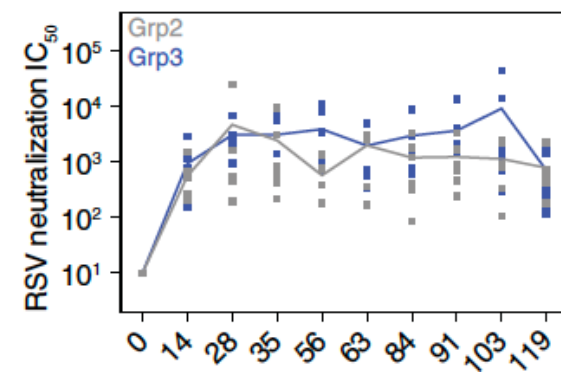
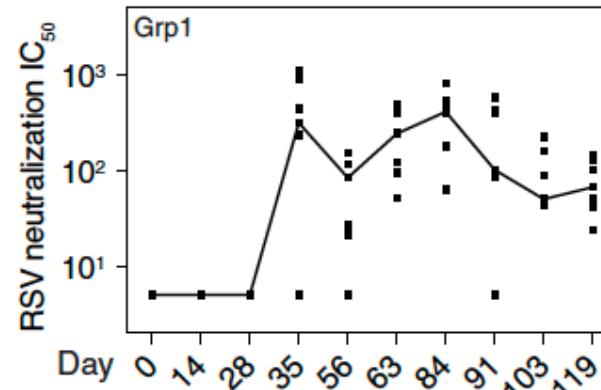
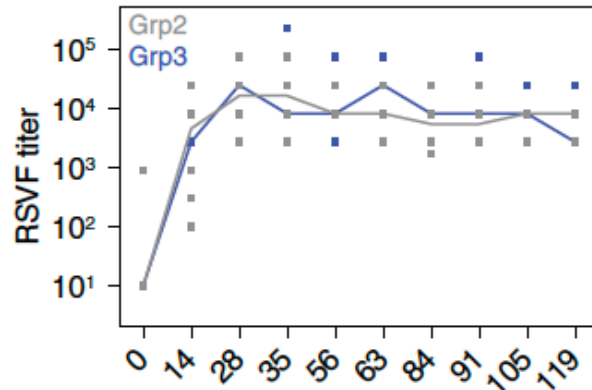
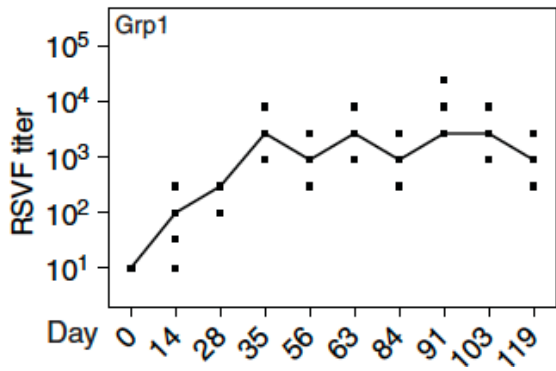
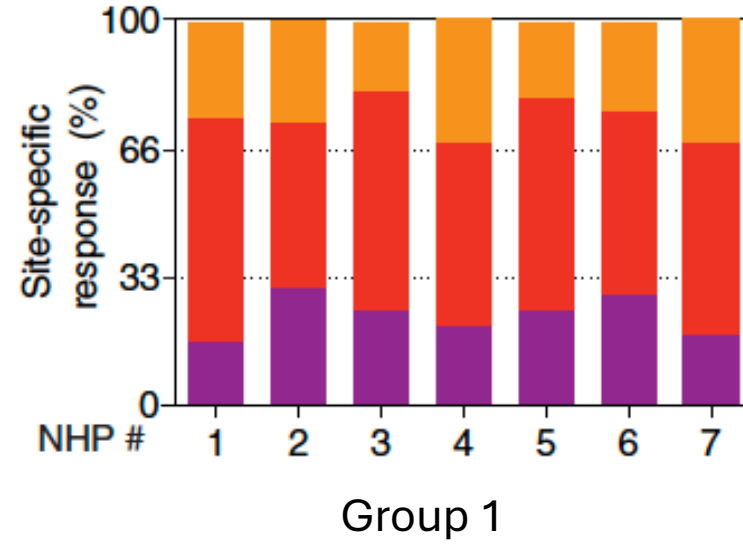
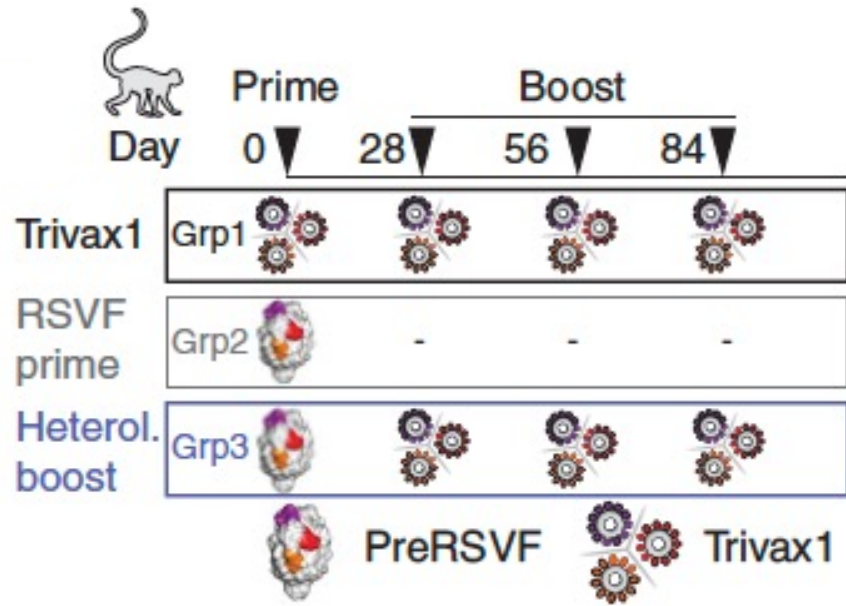


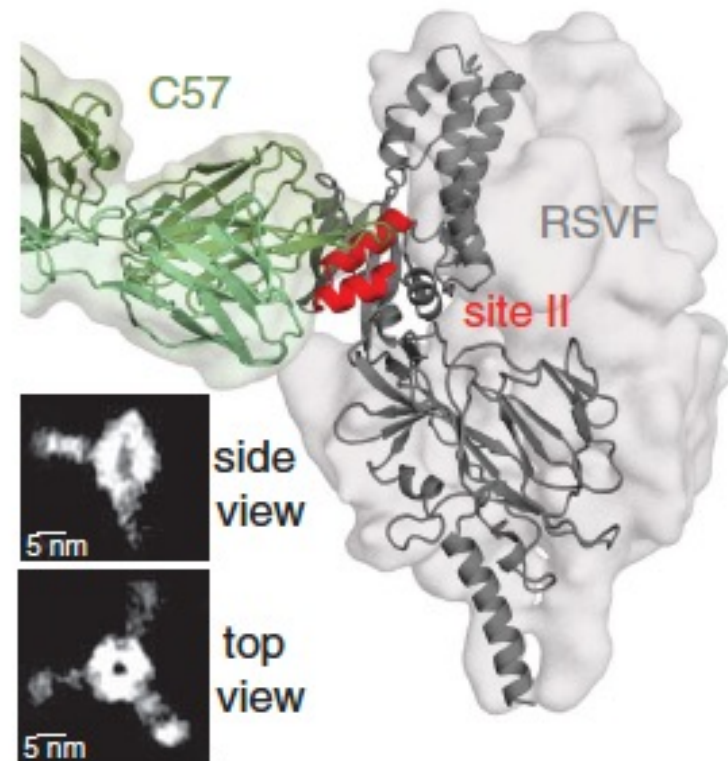
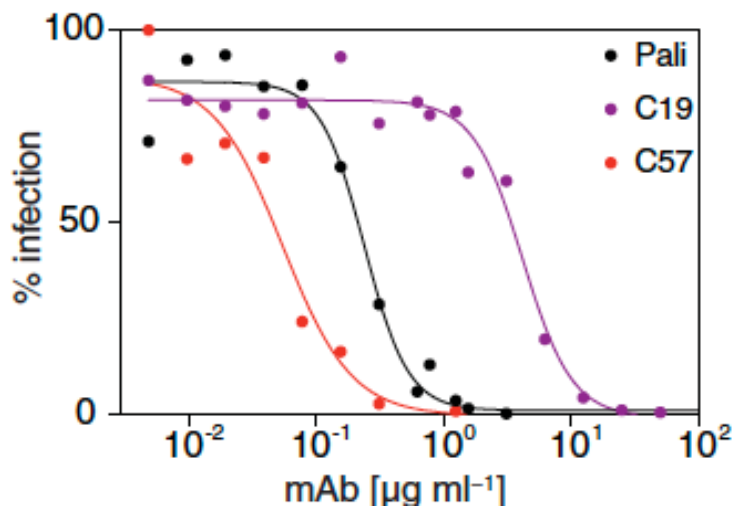
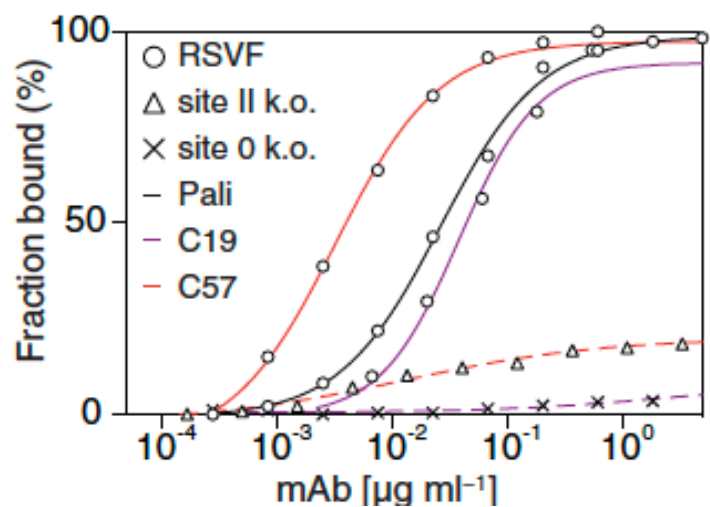
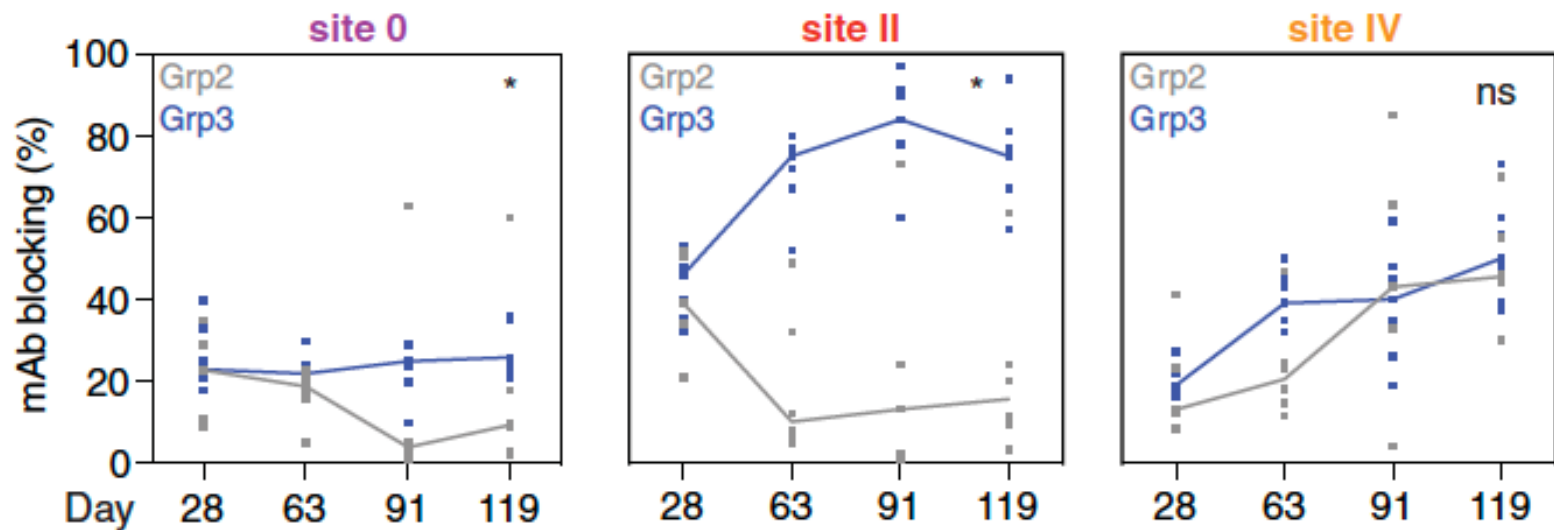
The top-scored designs highly resembles the structure of the original protein from RSVF: **what is the point for this design?**

➤ Pre-AlphaFold *de novo* design



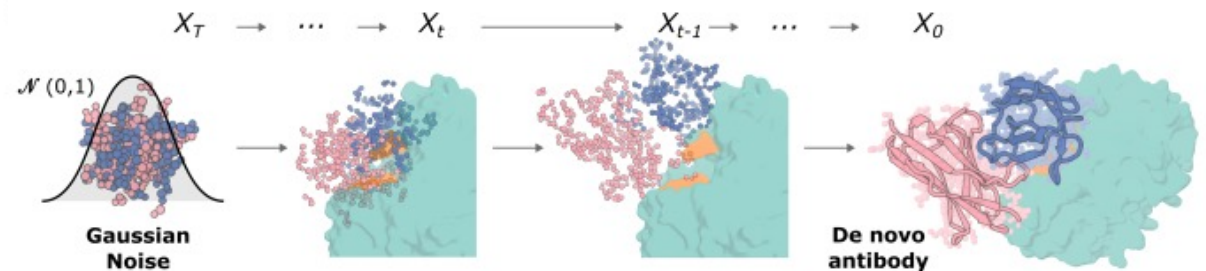
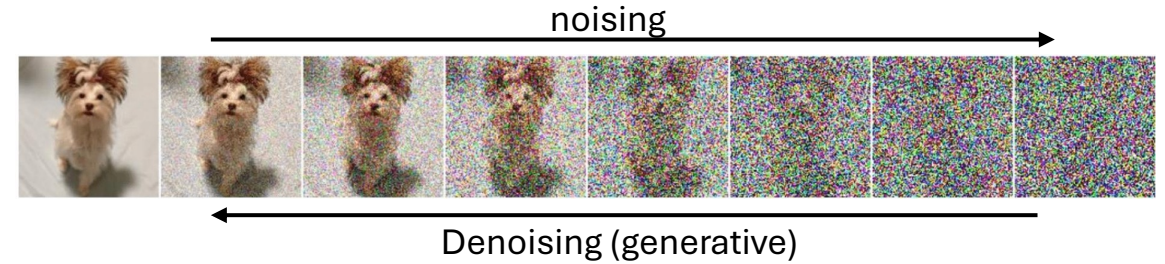
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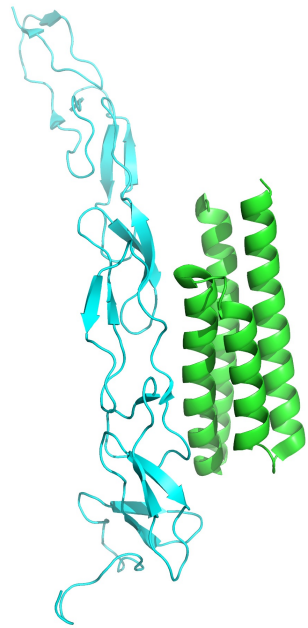


Atomically accurate *de novo* design of antibodies with RFdiffusion

Nathaniel R. Bennett^{†1,2,3}, Joseph L. Watson^{*†1,2}, Robert J. Ragotte^{*†1,2}, Andrew J. Borst^{†1,2}, DéJenaé L. See^{†1,2,4}, Connor Weidle^{†1,2}, Riti Biswas^{†1,2,3}, Yutong Yu^{†5,6,7}, Ellen L. Shrock^{†1,2}, Russell Ault^{†8,9}, Philip J. Y. Leung^{†1,2,3}, Buwei Huang^{†1,2,4}, Inna Goreschnik^{†1,2,10}, John Tam^{†11}, Kenneth D. Carr^{†1,2}, Benedikt Singer^{†1,2}, Cameron Criswell^{†1,2}, Basile I. M. Wicky^{†1,2}, Dionne Vafeados^{†2}, Mariana Garcia Sanchez^{†2}, Ho Min Kim^{†12,13}, Susana Vázquez Torres^{†1,2,14}, Sidney Chan^{†2}, Shirley M. Sun^{†16,17}, Timothy Spear^{†15}, Yi Sun^{†16,17}, Keelan O'Reilly^{†15}, John M. Maris^{†9,15}, Nikolaos G. Sgourakis^{†16,17}, Roman A. Melnyk^{†11,18}, Chang C. Liu^{†5,6,19,20}, David Baker^{*†1,2,10}

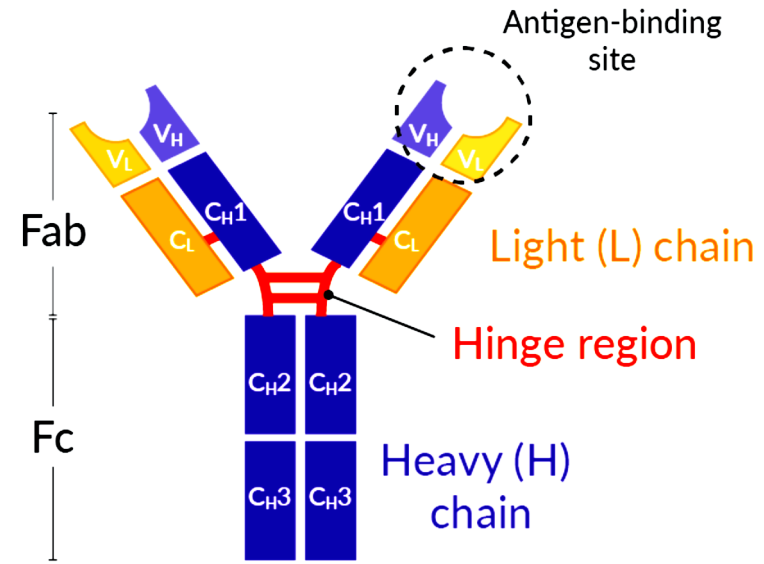


How does antibody design differ from normal protein binder design?



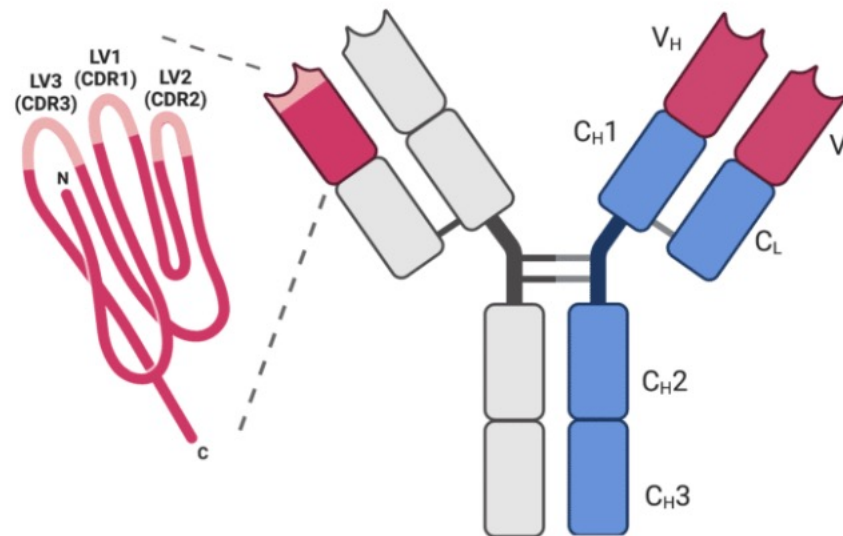
**TNFR2
Binder mb1**

PDB ID: 9cu8



Fab: Fragment antigen-binding region
Fc: Fragment crystallizable region

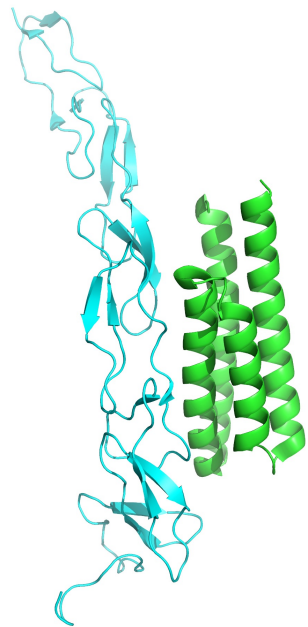
V_{LH}: variable region
C_{LH}: constant region



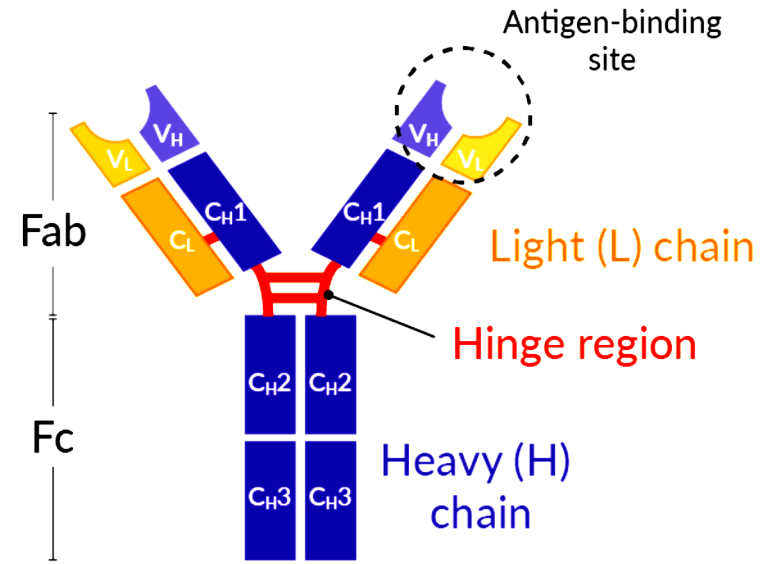
LEGEND

- Complementarity Determining Regions (CDRs)
- Framework region
- Antigen binding region
- Variable region
- Constant region

Why not just Binder-Fc Fusion Proteins?

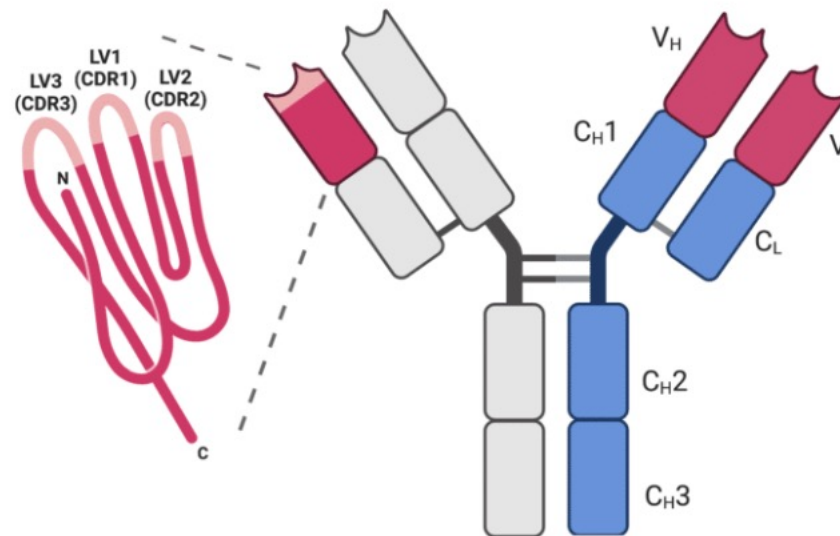


**TNFR2
Binder mb1**
PDB ID: 9cu8



Fab: Fragment antigen-binding region
Fc: Fragment crystallizable region

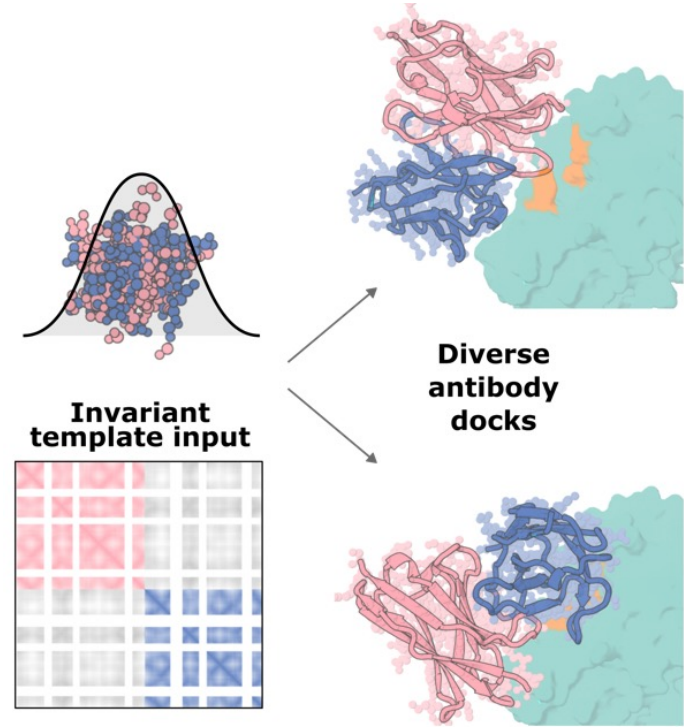
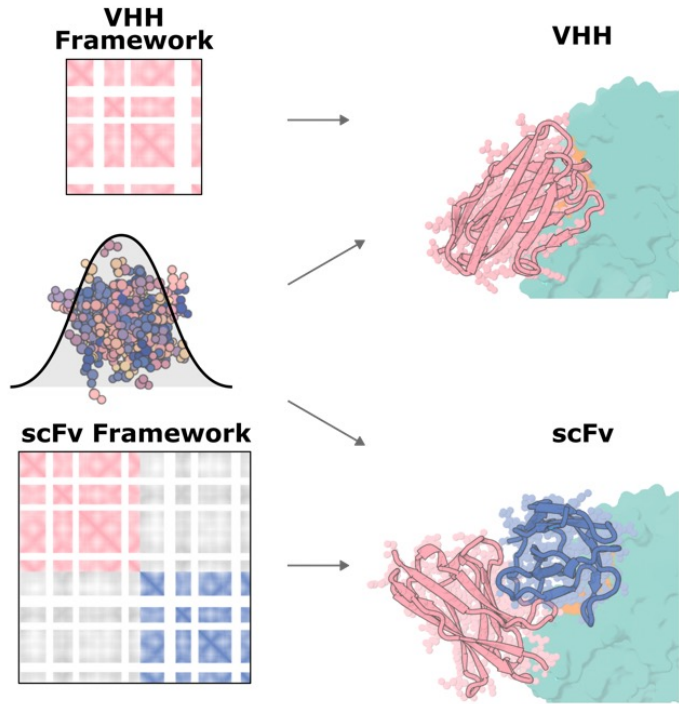
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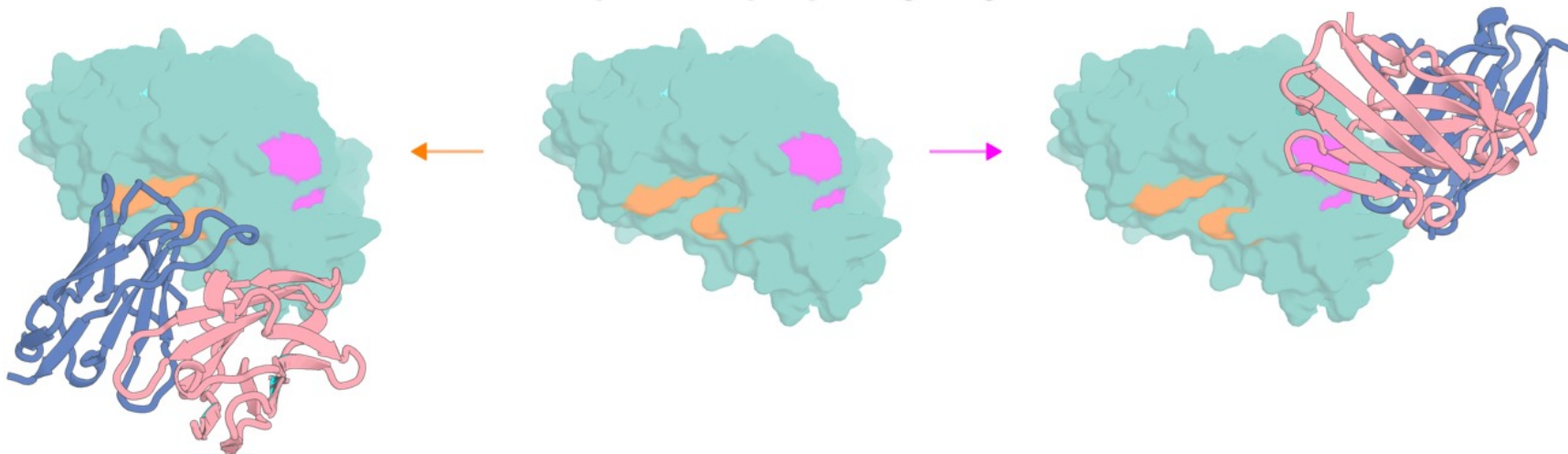
LEGEND

- Complementarity Determining Regions (CDRs)
- Framework region
- Antigen binding region
- Variable region
- Constant region

➤ Post-AlphaFold *de novo* design

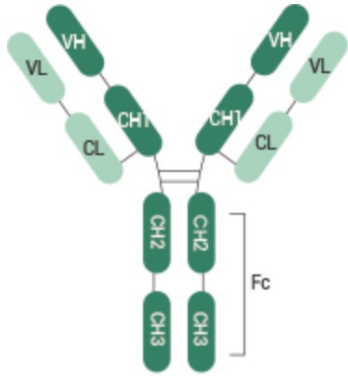


User-specified epitope targeting

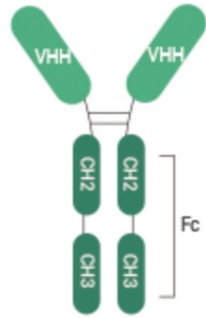


➤ Post-AlphaFold *de novo* design

VHH design



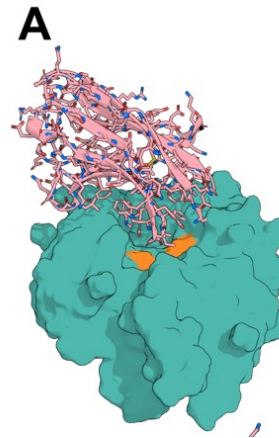
a. Conventional antibodies



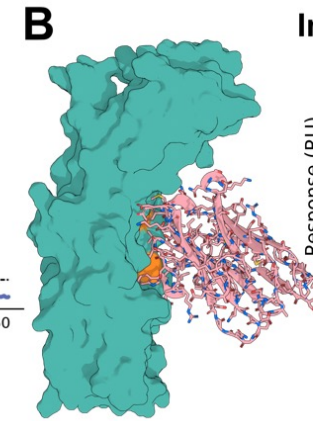
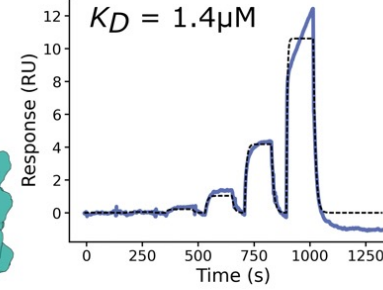
b. Heavy-chain antibodies



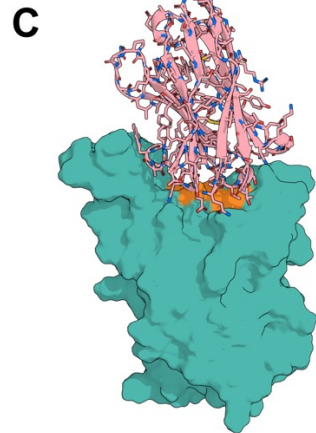
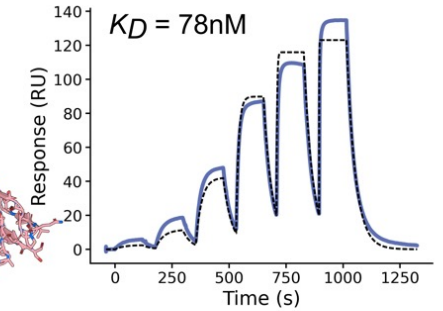
c. VHH



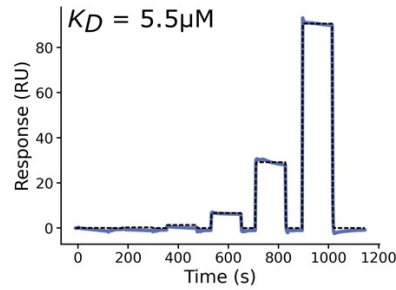
RSV Site III



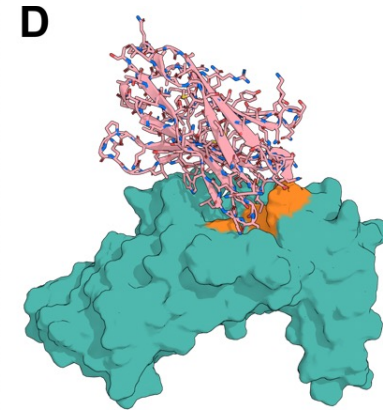
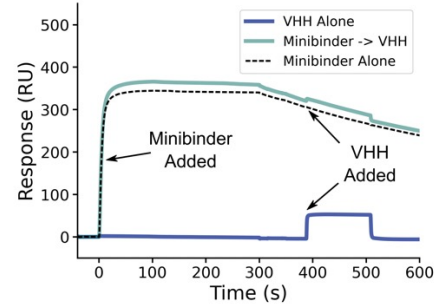
Influenza HA



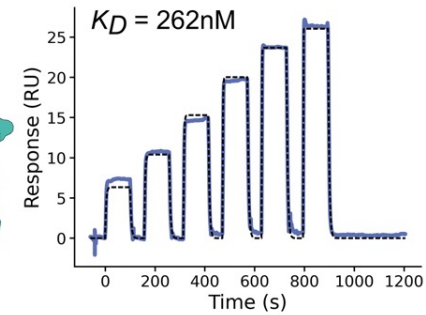
SARS-CoV2-RBD



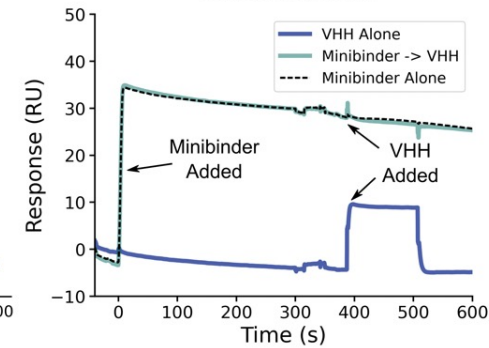
Competition with RBD Minibinder



TcdB

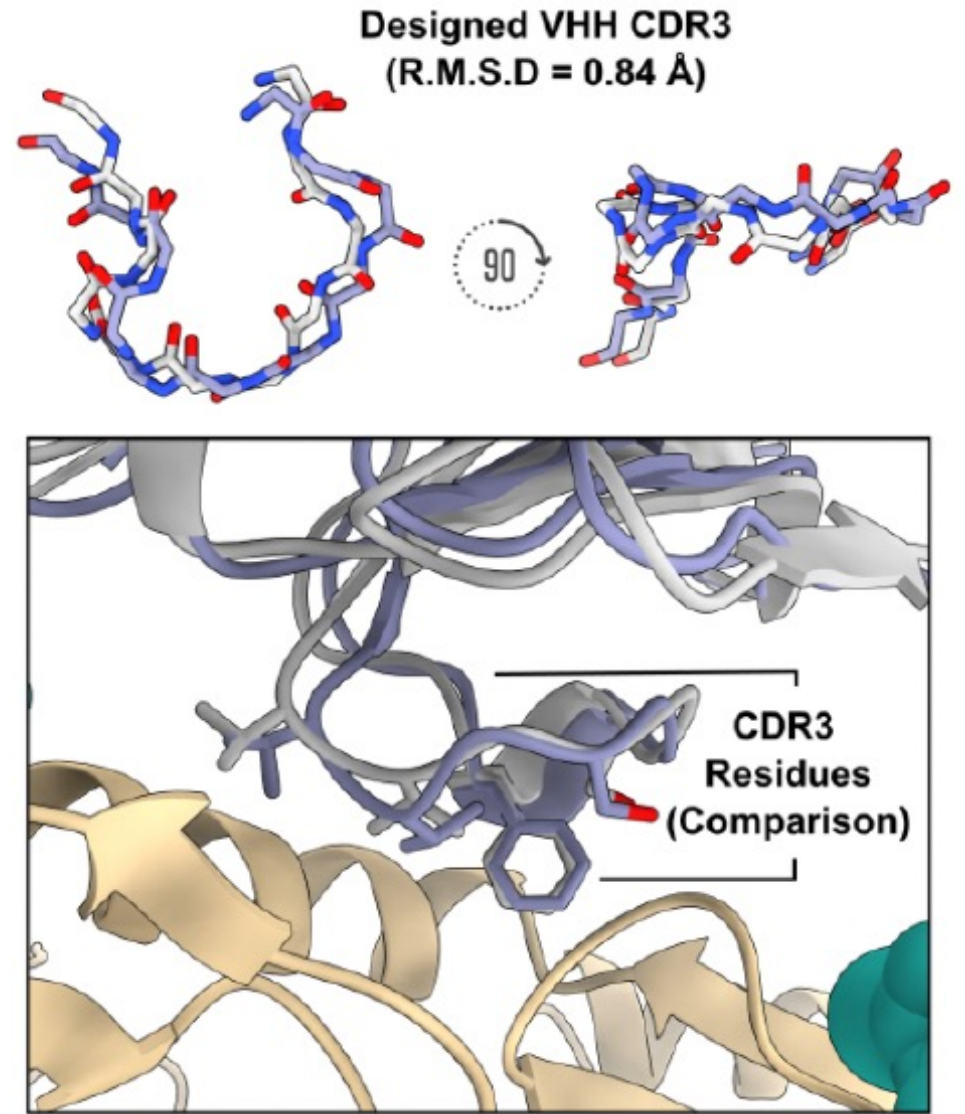
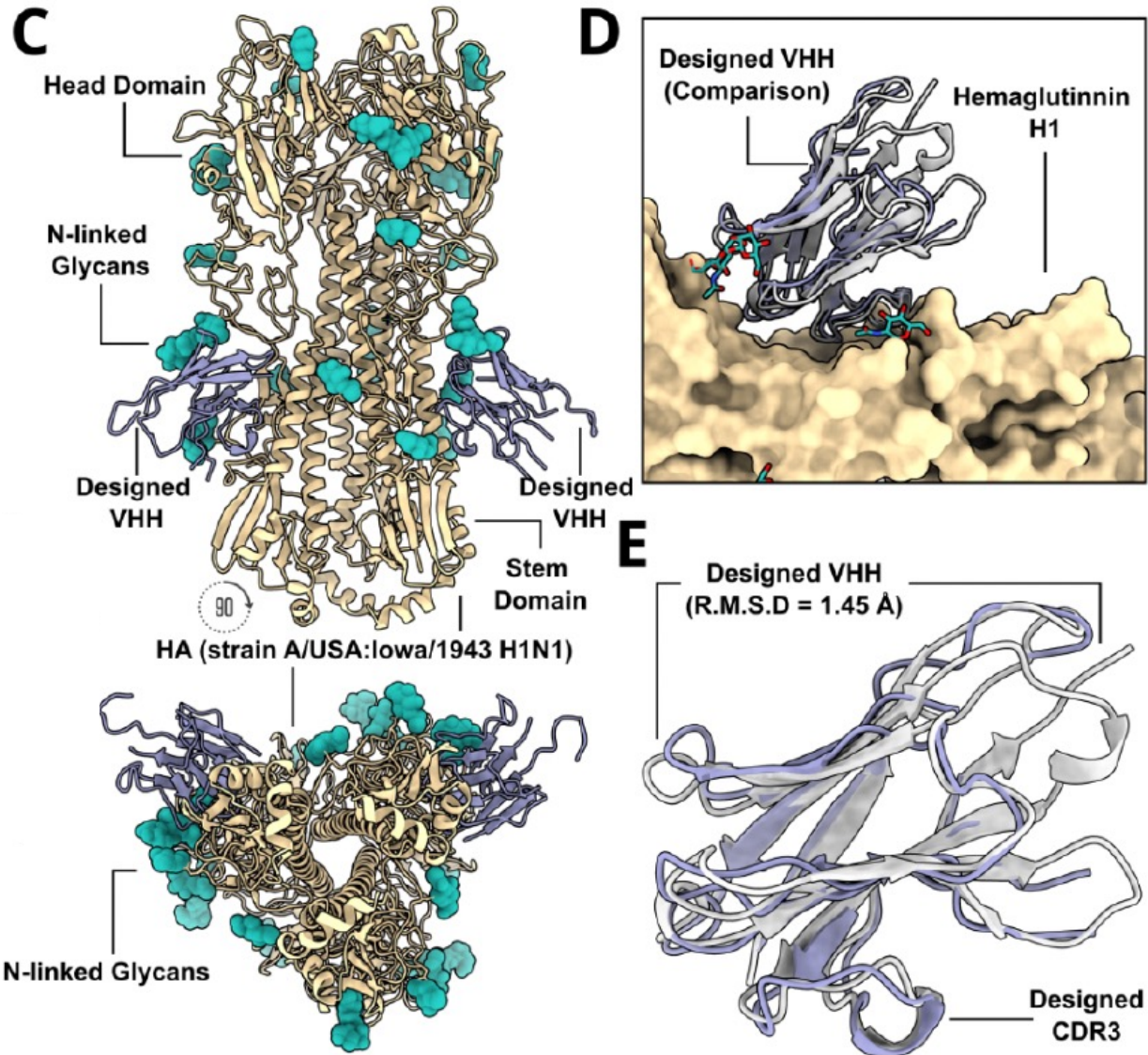


Competition with TcdB Minibinder



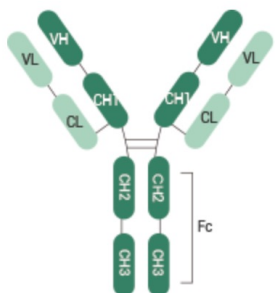
What can be seen from the SPR competition experiments?

➤ Post-AlphaFold *de novo* design

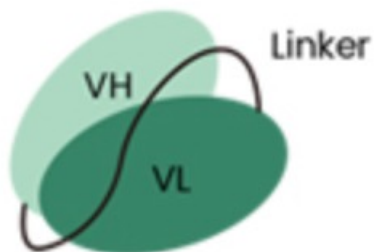


➤ Post-AlphaFold *de novo* design

scFv design

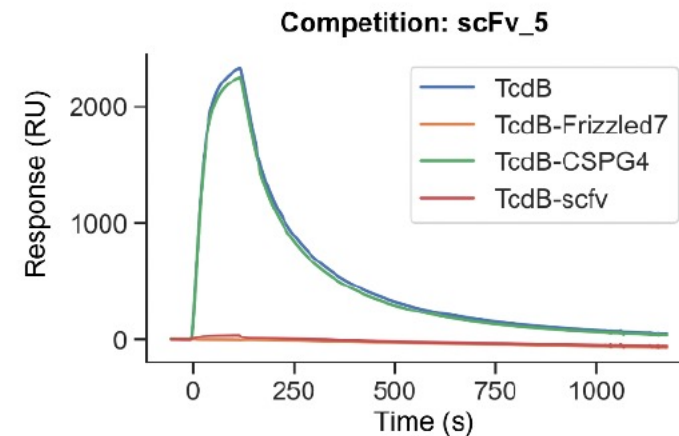
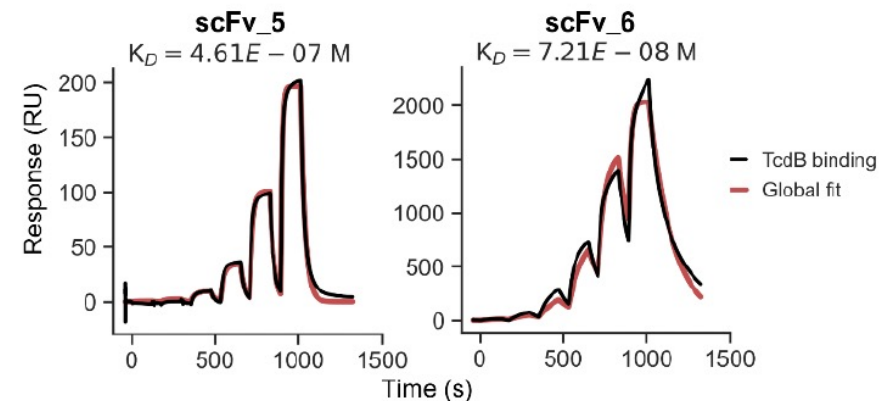
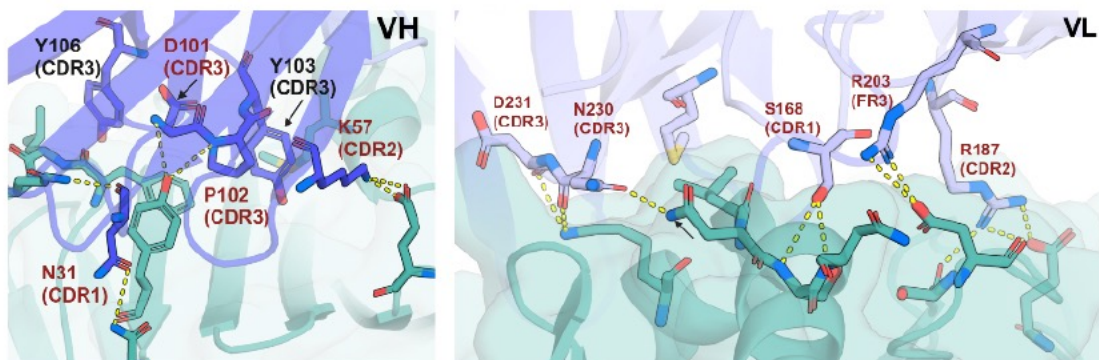
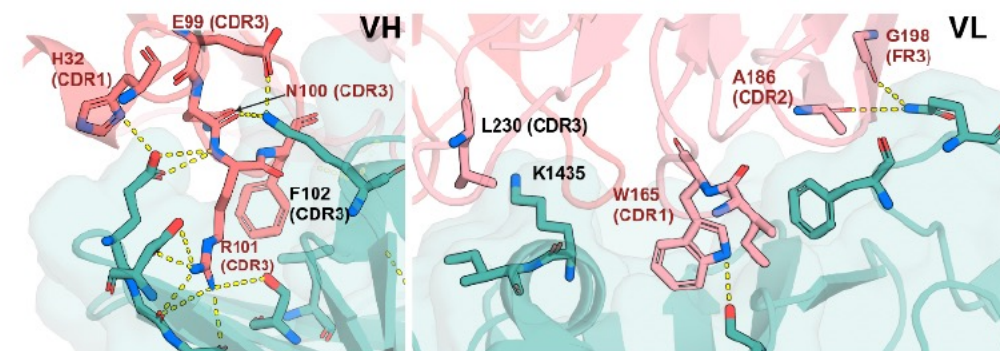
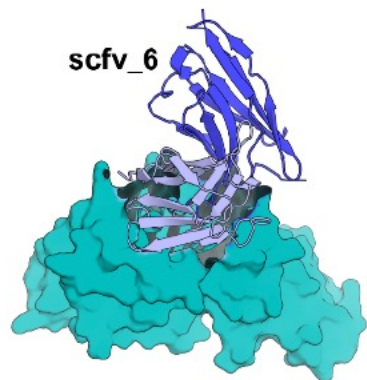
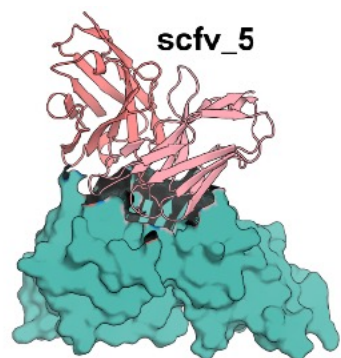


a. Conventional antibodies

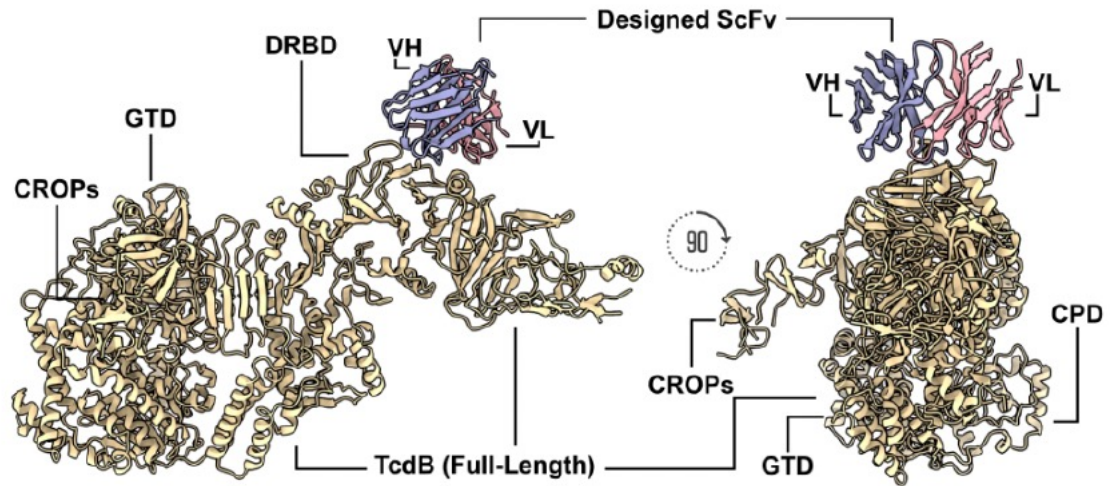
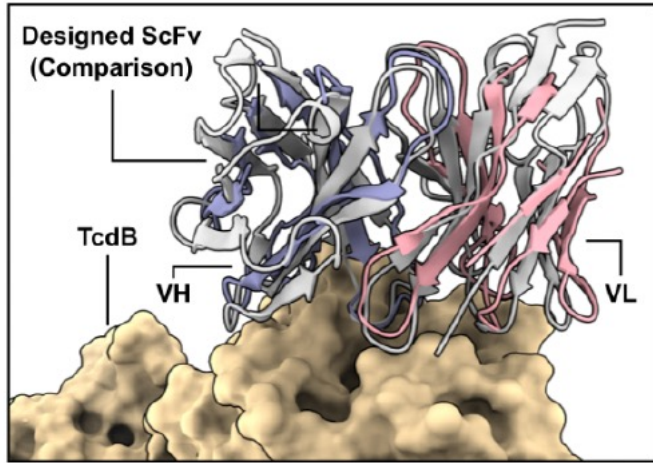


scfv_1	DLSEH	NPKTGS	EN---RFGALTV
scfv_4	DLKEK	NVKTGS	EN---RLGALTV
scfv_3	NLSEK	HVGTGS	EN---RLGALTV
scfv_2	DLSEH	NPKTGS	EN---RFGSLTV
scfv_5	NLKEH	NPSTGS	EN---RFGALTV
scfv_6	SIKNT	WPANGK	QLDPYNLYGNDV
	CDRH1	CDRH2	CDRH3

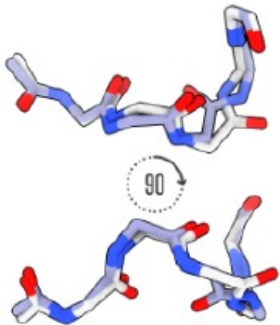
KTSRESKYIL-DIS	AAVVRAP	KSESQ-D-NVV
TTSSYVP--M-LVS	SSVIRAP	SVLQK-DGNLV
KTSSYVS--M-LVS	SSVVRAP	AVLDK-NGNLV
KTSE-LN--L-FVS	AASVRAP	SVLNK-NGNLV
KTSEKELP--IWSVS	ASAVRAP	AALKDENLTLV
KTSSS---YVN	RNSFRAP	STMNN-DGNLV
CDRL1	CDRL2	CDRL3



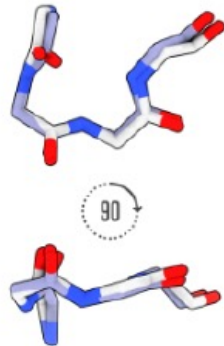
➤ Post-AlphaFold *de novo* design



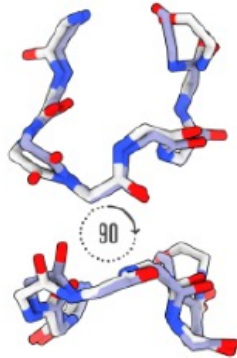
CDRH1
(R.M.S.D = 0.4 Å)



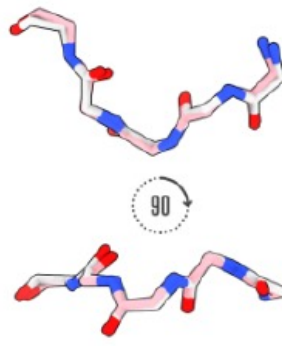
CDRH2
(R.M.S.D = 0.3 Å)



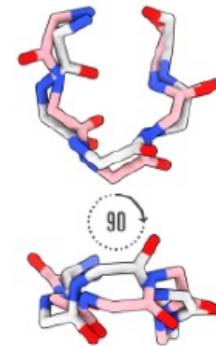
CDRH3
(R.M.S.D = 0.7 Å)



CDRL1
(R.M.S.D = 0.2 Å)



CDRL2
(R.M.S.D = 1.1 Å)



CDRL3
(R.M.S.D = 0.2 Å)

