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October 12, 2022

VIA EMAIL

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Dear Ms. Lawrence-Patel,

RE: Request for Accommodation (COVID Vaccine Requirement) – Josh Jacob

I write as counsel for Josh Jacob, a first-year student at Western. The purpose of this letter is to formally request Creed-based accommodation for Mr. Jacob regarding Western's COVID vaccine requirement pursuant to section 1.0(i)(b) of Policy 3.1.1, effective September 6, 2022. Mr. Jacob requires in-person access to campus, yet section 1.0(i)(a) of the Policy excludes students who do not prove receipt of the first two doses of the COVID vaccines. Unless, that is, they have been granted a human rights accommodation.

Mr. Jacob requested accommodation on the basis of his religious beliefs at the start of the school year, but received the same brief, unexplained, anonymous denial of his request as many other Christian Western students.

Mr. Jacob is unable to receive any COVID vaccines due to his Christian religious beliefs. So as to confirm that he holds sincere religious beliefs that have a nexus with religion (Christianity), and that would be interfered with in a manner that is more than trivial or insubstantial,[1] enclosed is a statement of Mr. Jacob's relevant religious beliefs. These are the beliefs he will attest to

[1] See *Syndicat Northcrest v Amselem*, 2004 SCC 47, at paragraphs 39-59. The *Amselem* test for demonstrating a protected religious belief has been adopted by the Ontario Human Rights Tribunal.

should accommodation be denied and he is compelled to submit an application to the Ontario Human Rights Tribunal.

Any assertions on the part of Western that Mr. Jacob's religious beliefs are not sincerely held or do not have a nexus with Christianity are disingenuous, including suggestions that Mr. Jacob's beliefs are somehow not "personal" enough because they are shared by other Christian students. As you know, Ms. Lawrence-Patel, I am in receipt of your letters of September 28 in which you communicated Western's refusal to reverse its denial of accommodation to other Christian Western students who cannot receive the COVID vaccines due to their sincere Christian religious beliefs. Contrary to your bizarre conclusion in said letters that a Christian student's beliefs are not protected by law if they are similar to those of other students, such shared, similar beliefs ***reinforces*** the nexus to religion, it does not sever it. So much is obvious to those not determined to find any excuse to defend their discriminatory actions.

The specificity of Mr. Jacob's appended beliefs plainly establishes the required nexus with a religion (Christianity). Further, any ***reasonable*** person, upon reviewing the highly-detailed beliefs he herein provides, would conclude that receiving the COVID vaccines would interfere with Mr. Jacob's religious beliefs in a manner that is far more than trivial or insubstantial.

I need not remind you your client may not discriminate against Christians by denying reasonable accommodation.

COVID vaccine mandates may assist Western in establishing its *bona fides* as a "woke" institution, but the law—and dare I say ethics—cares about whether such discriminatory policies are scientific. Notwithstanding the ideological and optical motivation for Western's COVID vaccine mandates, Mr. Jacob reminds your client that, scientifically, both the COVID vaccines themselves and vaccine mandates are unnecessary and ineffective, like so many other undesirable control measures that only serve to abrogate rights and undermine human dignity. If indeed the vaccines are effective, unvaccinated persons pose no concern whatsoever. If the COVID vaccines are ineffective, which real-world data repeatedly demonstrates, then discriminating against, stigmatizing, and excluding those who are unable to receive the vaccines due to protected characteristics is not only unlawful, it is nothing short of asinine and cruel. Providing reasonable accommodation to Mr. Jacob jeopardizes the "health and safety" of no one on campus.

It would seem Western concedes the foregoing, as neither you nor Western have suggested otherwise, preferring instead to arbitrarily claim the duty to accommodate has not been triggered because the beliefs of Christians who cannot receive the COVID vaccines are somehow not good enough. The contempt your client has shown for the Christian students it has discriminated against by disingenuously claiming their beliefs are not protected at law is disgraceful.

Mr. Jacob submits he is entitled at law to be accommodated such that he is able to effectively complete all his in-person academic requirements, notwithstanding his inability to receive the COVID vaccines due to his religious beliefs.

He requests a substantive response to his request herein no later than October 26, 2022. Should your client persist in rebelling against its legal obligation to accommodate Mr. Jacob, he will be compelled to take all legal action necessary to enforce his rights and receive compensation for any resulting delay or loss to his education.

Regards,

James S.M. Kitchen
Barrister & Solicitor
Counsel for Josh Jacob

cc John Doerksen, Vice-Provost, Students (doerksen@uwo.ca)
vaxinfo@uwo.ca

Enclosures

STATEMENT OF SINCERE RELIGIOUS BELIEFS OF JOSH JACOB

Introduction

1. I, Josh Jacob, am a first-year undergraduate student at Western University. I have not received any of the COVID vaccines.

2. This statement of my sincere religious beliefs I make for the purposes of requesting human rights accommodation on the basis of religious belief, or "Creed" as it is referred to in the Ontario *Human Rights Code*.

3. I am a Christian; a follower and disciple of the Lord Jesus Christ, and I believe the entirety of the Holy Bible is true.

4. I understand, as part of my request for accommodation, I am obligated to demonstrate I hold sincere religious beliefs that have a nexus with religion (Christianity) and would be interfered with in a manner that is more than trivial or insubstantial. Reproduced herein is the full articulation of my relevant sincere Christian religious beliefs, including references to the Bible, regarding the COVID vaccines.

5. I sincerely hold to Christian religious beliefs that compel me to not receive into my body the COVID vaccines due to the known and unknown contents of the vaccines, the manner in which they were produced, the deception that surrounds them, and the coercion involved in the mandatory imposition of them upon myself and those around me.

6. If I received any of the COVID vaccines, it would be an act of disobedience against the Lord Jesus Christ. It would be sin. A sin which, if I committed, would grieve me deeply and cause me spiritual and moral distress.

Foundational Beliefs of General Application to All of My Life, Including the Issue of the COVID Vaccines and Mandates to Receive Them

7. I steadfastly believe that "all scripture is God-breathed" (II Timothy 3:16-17), and it is through the Scriptures that God communicates His instructions and expectations, which are perfect and true for my life (Psalms 18:30). God desires and values obedience to His

word, stemming from our love for Him, more than any religious ritual or sacrifice (1 Samuel 15:22; Hosea 6:6; Mark 12:32-33). I am duty-bound out of love for my God and King to abide by all the commands in Scripture to the best of my ability. As a Bible-believing follower of Christ, my first loyalty is to Christ and His Word (the Bible), not to any particular Christian leader or their teachings. For this reason, my beliefs are exclusively grounded in scripture, which is the Word of God, and wholly independent of any philosophies, beliefs or doctrines of *any* congregational associations I may have. Thus, for example, it is religiously irrelevant to me that some Christian teachers or leaders, even very important ones such as the Pope, have endorsed the COVID vaccines.

8. Out of love and obedience to God, I strive to live by the scriptures and "live a life worthy of the calling to which [I] have been called" (Ephesians 4:1), that is, a godly life reflective of the salvation gifted to me at Calvary.

9. Jesus Christ is the supreme King of all, and I am subject to His final authority alone in all matters of conscience, what I do with my body, and how I respond to societal injustice and coercive efforts to control my behaviour, which is a form of temptation (Ephesians 4:4-6; Colossians 1:16-18). Christians recognize that they will one day stand before God to give an account for their lives and therefore seek to exercise wisdom and discernment in all their decisions, and to live to please the Lord (Romans 12:2; II Corinthians 5:10).

10. God commands me to "take pains to have a clear conscious towards both God and man" (Acts 24:16), "desiring to act honourably in all things" (Hebrews 13:18) and warns me not to reject my conscience or otherwise shipwreck my faith (1 Timothy 1:18-19). James, the brother of Christ, states it clearly: "any person who knows what is right to do but does not do it, to him it is sin" (James 4:17). Thus, I will not blindly conform to the rules, regulations or mandates of any country, state, principality, or institution but will first test everything to ensure it is within the will of God, and only when those rules align with God's Law will I comply (Romans 12:2; 1 Corinthians 8:1-13).

Specific Beliefs Which Preclude Me from Receiving any COVID Vaccines

The known and unknown contents of the vaccines, and the manner in which they were produced

11. Fundamental to the Christian faith is the belief that all human persons bear the image of God (Genesis 1:27, 9:6). This has two applications relevant to the COVID vaccines.

12. Christians are instructed by Scripture to view their bodies as temples of the Holy Spirit and to steward their bodies as creatures ultimately accountable to God (Romans 12:1-2; I Corinthians 3:16-17, 6:19-20). For me, this extends to not injecting my body with substances such as those contained in the COVID mRNA vaccines. Some of these substances are not fully known, potentially dangerous, and have the effect of interfering with or manipulating my natural cell composition and cell mechanisms. The mRNA and the toxic "spike protein" this mRNA codes for are examples. I am compelled to maintain both the physical and spiritual integrity of my body, including my divinely-designed cell mechanisms, and to not take unnecessary risks with my body. To do would be an expression of a lack of faith and a manifestation of putting my trust in man before God, when I am called to do the opposite. It is for this reason that I do not take hard drugs, drink alcohol to excess, engage in high-risk thrill-seeking behaviour, permit my body to be used for medical experimentation, or engage in high-risk sexual behaviour.

13. I believe I am fearfully and wonderfully made by God and must trust my health first to God, to His design of my immune system over a man-made intervention may which alter the function of my immune system and is necessarily inferior to God's design. I further trust the sources of natural health and healing that God has provided (Genesis 1; Exodus 23:25; Psalms 91:5-7 and 139:14; I Corinthians 3:19; Jeremiah 17:5).

14. Indeed, when I recently contracted COVID, I trusted in both God's design of my immune system and His healing power and I came through just fine, acquiring natural immunity in the process.

15. How products for human consumption are obtained and what they are derived from is important for me, just like it was for King David and the prophet Daniel (2 Samuel 23:15

-17 and Daniel 1:8-14). Christians are required to fully, diligently, and resolutely honour the sanctity of human life, which I believe manifests at conception and includes pre-natal life (Genesis 1:27, 4:1 and 17, 9:6; Jeremiah 1:5; Psalms 22:10-11, 106:35-38, 113:7-9, 127:3, 139:13-16; Matthew 18:1-4, 19:13-15). "Whoever sheds human blood, by humans shall their blood be shed; for in the image of God has God made mankind" (Genesis 9:6). For me, this necessarily extends to protecting, through my choices, unborn children from medical experimentation in the ***testing, development, or production*** of vaccines, and not participating ***directly or indirectly*** in any vaccine that was derived ***directly or indirectly*** from aborted human fetal cell lines (Exodus 20:13, Leviticus 18:21, 20:2-5, Deuteronomy 12:30-32, 18:10, 2 Kings 16:3, Psalm 16:38).

16. Receiving any of the COVID vaccines available in Canada would be to violate this belief and commit a sin before God (James 4:17; Romans 14:23; Leviticus 20:1-5), as they were all developed from and/or researched or produced in a way that was dependent on human fetal cell lines. It would implicate me in endorsing an act that intrinsically asserts that human life is neither sacred nor valuable.

17. I understand and am aware that a new COVID vaccine has recently become available which Western purports is not implicated by the sin of Abortion (Novavax). The fact is, Novavax ***is*** implicated by abortion. The cell line HEK293, notoriously derived from an aborted fetus, was used in the ***testing*** of the Novavax COVID vaccine. Enclosed with my statement is a peer-reviewed scientific paper published in October 2020 and an article from Liberty Counsel which discusses this.

The deception surrounding the COVID vaccines

18. I sincerely believe the COVID vaccines are covered in deception. This is not merely or primarily a "political" or "personal" belief. It arises out of my Biblically-informed views and Holy Spirit-guided discernment. It is first and foremost a religious belief and my religious belief.

19. The Holy Spirit is the third person of the Trinity (consisting of God the Father, the Son Jesus Christ, and the Holy Spirit). As Jesus said, he gave Christians the Holy Spirit, the

"Spirit of Truth", to guide and teach us (John 14:17, 26; 16:13). Part of being a Bible-following Christian is having, to the degree possible, the mind of Christ and being able to discern between what *appears* to be right and what *is actually* right (I Corinthians 2:14; I John 4:1; Colossians 2:8; Romans 12:2; Proverbs 9:10). The Bible says, and I believe, I must not be conformed to the ways of the world, but be transformed by the renewing of my mind based on God's ways, which are above the ways of the world and the ways of men, so that I "will be able to test and approve what God's will is—his good, pleasing and perfect will (Romans 12:2; Isaiah 55: 8-9). As I believer, I am to test everything (1 Thessalonians 5:21).

20. Based on the discernment described above, I believe it is a deception that the COVID vaccines are as safe as Western and other institutions and authorities propagate them to be. I further believe it is a deception that the COVID vaccines are effective at preventing the spread and infection of the virus that causes COVID. These deceptions form the purported rationale for COVID vaccine mandates.

21. As a Christian, I am called to live according to truth and not be complicit in deceit (John 4:23; I John 3:18 and 4:1; John 8:32; I Peter 3:10; Psalms 5:6 and 101:7; Proverbs 9:10, 12:17, 14:8, 19:9, and 20:17; II Timothy 3:13; and Colossians 2:8 and 3:9). Walking in the light and truth of Jesus and shunning all lies and deceit is fundamental to my walk with the Lord. I cannot receive the COVID vaccines and thereby knowingly participate in deceit, which would be to walk in darkness.

22. Further, in all circumstances Christians seek to live in freedom, without fear, and openly before God and other people because "it is for freedom that Christ set us free" (Galatians 5:1). If I received the COVID vaccines, it would be for no other reason than because someone in a position of power over me told me to. To do that would be to act out of fear. I am commanded by the Bible to fear God, not other people (Matthew 32-33; Galatians 1:10; Proverbs 1:7, 29:25). If I received the COVID vaccinations, I would be violating my conscience and committing a sin (James 4:17). "Do not be afraid of those who kill the body but cannot kill the soul. Rather, be afraid of the One who can destroy both soul and body in hell. … Whoever acknowledges me before others, I will also

acknowledge before my Father in heaven. But whoever disowns me before others, I will disown before my Father in heaven" (Mathew 10:28).

The injustice and immorality of coercion

23. My Lord and Saviour, Jesus Christ is a God of justice who hates injustice, oppression, and tyranny (Deuteronomy 10:17–20; Isaiah 1:15–17; Ecclesiastes 3:16–17; 4:1; 5:8; Psalms 7:11; 9:7–9). He has appointed government to do good and to avenge evil (Romans 13:3–4; 1 Peter 2:13–14). Jesus' concern for justice is my concern, as a follower of Jesus Christ. Out of love for neighbor and obedience to Christ, I am called to resist oppression and vitiation of consent, which necessarily includes coercive vaccination programs, whether at the hands of government or private entities (Ephesians 5:11).

24. If I were to submit to a COVID vaccine mandate such as Western's, I would be an endorser of oppression and injustice, acting in hate and spite of God, my neighbours, and even myself. Once discriminatory and oppressive policies are normalised in peoples' hearts and minds, freedom ends, mass atrocities begin, and evil reigns. I must abstain from all evil (I Thessalonians 5:22-23).

25. I believe, as a Christian, it is morally wrong and sinful to take away the ***free*** choice of something as important as what people put into their bodies. I care deeply about matters of free will and consent. I believe all human beings have the divine right to exercise free will, as established by God long before the creation of the State (Deuteronomy 30:19; Joshua 24:15; Revelation 3:20). Jesus gives people free will and lets them choose whether they will follow Him in love, or live for themselves. Conversely, Satan uses coercive, extortionary, forceful, manipulative, and deceitful tactics to make people comply with his directives (Genesis 2; Matthew 4:1-11; Job 1:6-22).

26. By their very nature, COVID vaccine mandates are irrefutably coercive and extortionary in nature. The entire impetus for such mandates is to withhold something important from someone until that person does something they will not voluntarily do and would not otherwise do. These tactics reflect the darkness and bondage of Satan, not the light and freedom of Jesus. They are the tactics of those who are controlled by fear and therefore

seek to control the actions of others so as to selfishly create for themselves the illusion of the elimination of all risk to themselves from those around them.

27. Obtaining an advanced education is no mere luxury or leisure activity. It is often required for full participation in society and the economy, and is certainly required to fulfil dreams to enter a particular vocation. Some may call it a "choice" to "choose" between a post-secondary education and injecting unwanted substances into one's body, but my definition of choice is not so ethically and morally bankrupt. I concur with the 1992 comments of former Justice La Forest of the Supreme Court of Canada:

> "A man cannot be said to be 'willing' unless he is in a position to choose freely; and ***freedom of choice predicates the absence from his mind of any feeling of constraint interfering with the freedom of his will***." A "feeling of constraint" so as to "interfere with the freedom of a person's will" can arise in a number of situations ***not*** involving force, threats of force, fraud or incapacity.
> …
> Professor Klippert in his book *Unjust Enrichment* (1983) refers to the doctrines of duress, undue influence, and unconscionability as "justice factors." He lumps these together under the general term "***coercion***" and states…, "In essence the common thread is ***an illegitimate use of power or unlawful pressure which vitiates a person's freedom of choice***."[1]

The mark of the beast

28. I am deeply concerned that the COVID vaccine and associated vaccine passports and mandates are a precursor to or may become the "mark of the beast" referred to in the Bible (Revelation 13:16-17). I am compelled to exercise wisdom regarding this matter (Revelation 13:18), as to receive the mark is to commit a sin and to betray Christ, while the rewards of remaining loyal to Christ are eternal life (Revelation 14:11, 16:2, 19:20, 20:4). I must avoid anything that could be associated with the mark of the beast.

Conclusion

29. I have prayed about the above beliefs as they apply to the COVID vaccine and associated mandates and I have received confirmation from the Holy Spirit that it is the Lord's Will

[1] *Norberg v Wynrib*, [1992] 2 S.C.R. 226, at paragraphs 27-28 [emphasis added].

for me to act in accordance with these beliefs in rejecting the COVID vaccines. I sincerely believe that if I received the COVID vaccines, I would commit a sin before God and that God's will for me is to not receive the COVID vaccines.

30. I affirm the above statements are true.

Josh Jacob

Date: October 10, 2022

Novavax COVID Shot Associated With Aborted Fetal Cells

Jun 9, 2022

Despite erroneous claims that Novavax's COVID-19 injection (NVX-CoV2373) does not have any connection to abortion-derived cell lines, evidence from Novavax's own published study shows otherwise.

While Novavax claims that no human fetal-derived cell lines or tissue, including HEK293 cells, were used in the development, manufacture, or production of the Novavax COVID-19 shots, it is not true that there is no association with abortion because *an aborted fetal cell line was used in the testing phase of Novavax*.

Novavax's published study, "Structural Analysis of Full-Length SARS-CoV-2 Spike Protein From an Advanced Vaccine Candidate," shows that the HEK293 aborted fetal cell line was used in the testing phase. The HEK293 cell line was originally harvested from the kidney of an aborted baby girl in 1973.

In a letter obtained by the Charlotte Lozier Institute, Novavax admits that HEK293 cells were indeed used as part of the testing phase for NVX-CoV2373:

"Testing was conducted to compare the structural integrity of the SARS-CoV-2 spike protein produced in the Sf9 insect cells versus the spike protein produced in the mammalian human embryonic kidney HEK 293F cells. The comparison determined the Sf9 cell technology produced spike proteins were comparable in structural integrity as the spike proteins produced in the HEK 293F cell."

Novavax's COVID-19 shot has now been recommended for emergency use authorization (EUA) in the U.S. for adults 18 and older by the Food and Drug Administration's Vaccines and Related Biological Products Advisory Committee. This came after they voted 21 to 0 with one abstention when asked if the benefits of the two-dose "vaccine" series outweigh the risks for U.S. adults "based on the totality of scientific evidence available," according to the live-streamed committee meeting.

The Novavax shot, like mRNA COVID injections, has also demonstrated increased risk for heart inflammation. Several cases of myocarditis and pericarditis were observed in the trial participants within two weeks of injection.

Similar to Moderna and BioNTech, Novavax has never successfully brought a product to market in its 33-year history. The company has zero track record of success, safety, and/or efficacious products.

Novavax and the FDA panel have already stated that two doses of the Novavax shot will not be enough to "protect" people from the virus and repeated boosting will be necessary. The shot was developed over two years ago, but there is no trial data on the Omicron variant.

The FDA said: "Relevant data to assess effectiveness of NVX-CoV2373 (Novavax shot) against the Omicron variant and sublineages, including observational data from use in other countries where the vaccine has been deployed, are currently unavailable."

Liberty Counsel Founder and Chairman Mat Staver said, "Novavax used aborted fetal cell lines in the testing phase, and it is already known that Novavax increases the risk of myocarditis. But the FDA continues to rubber stamp pharmaceutical companies with no history of success so long as the product is associated with COVID-19. The FDA is supposed to be the watchdog to protect public health, but, sadly, the FDA has become the lapdog of Big Pharma."

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REPORT

Structural analysis of full-length SARS-CoV-2 spike protein from an advanced vaccine candidate

SANDHYA BANGARU, GABRIEL OZOROWSKI, HANNAH L. TURNER, ALEKSANDAR ANTANASIJEVIC, DELI HUANG, XIAONING WANG, JONATHAN L. TORRES, JOLENE K. DIEDRICH, JING-HUI TIAN, [...] ANDREW B. WARD +9 authors Authors Info & Affiliations

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Structure of a vaccine candidate

Much effort is being targeted at developing vaccines that will provide protection against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). A trimeric spike protein that decorates the virus is a primary target of the host immune system and the focus of vaccine development. Bangaru *et al.* present the structure of a leading vaccine candidate: a full-length spike protein with some modifications aimed at enhancing stability that is formulated in polysorbate 80 detergent. The study confirms that the full-length immunogen is in a stable prefusion conformation and provides a basis for understanding immune responses to the vaccine.

Science, this issue p. 1089

Abstract

Vaccine efforts to combat the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is responsible for the current coronavirus disease 2019 (COVID-19) pandemic, are focused on SARS-CoV-2 spike glycoprotein, the primary target for neutralizing antibodies. We performed cryo–election microscopy and site-specific glycan analysis of one of the leading subunit vaccine candidates from Novavax, which is based on a full-length spike protein formulated in polysorbate 80 detergent. Our studies reveal a stable prefusion conformation of the spike immunogen with slight differences in the S1 subunit compared with published spike ectodomain structures. We also observed interactions between the spike trimers, allowing formation of higher-order spike complexes. This study confirms the structural integrity of the full-length spike protein immunogen and provides a basis for interpreting immune responses to this multivalent nanoparticle immunogen.

Severe acute respiratory syndrome coronavirus (SARS-CoV) caused a global outbreak from 2002 to 2003 (*1*). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), from the same lineage of the β-CoV genus as SARS-CoV, recently emerged in China and spread rapidly, infecting more than 28 million people worldwide by September 2020 (*2*). Coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2, was declared a pandemic by the World Health Organization (WHO). In response, several SARS-CoV-2 vaccine candidates are being developed and tested at various stages of clinical trials (*3*–*5*). The SARS-CoV-2 spike (S) trimeric glycoprotein is a focus of vaccine development because it is the primary target of host immune defenses (*5*, *6*).

Like other type 1 fusion proteins, the SARS-CoV-2 S prefusion trimer is metastable and undergoes structural rearrangement from a prefusion to a postfusion conformation upon S-protein receptor binding and cleavage (*7*, *8*).

The structure of the stabilized SARS-CoV-2 spike ectodomain has been solved in its prefusion conformation and resembles the SARS-CoV spike (*9*–*11*). Here, we describe the structure of a leading SARS-CoV-2 S vaccine candidate (NVAX-CoV2373) based on a full-length (FL) S, residues 1 to 1273, which includes the transmembrane (TM) and the cytoplasmic tail (CT) (Fig. 1A). The final construct, SARS-CoV-2-3Q-2P, was also modified at the S1/S2 polybasic cleavage site from RRAR to QQAQ to render it protease resistant, along with two proline substitutions at residues K986 and V987 in the S2 fusion machinery core for enhanced stability (Fig. 1A). The FL spikes, expressed and purified from insect cells, were formulated in 0.01% (v/v) polysorbate 80 (PS 80) detergent. To characterize the structural integrity of the 3Q-2P-FL immunogen, we performed negative-stain electron microscopy of the FL spike constituted in PS 80 in the presence of Matrix-M adjuvant, recapitulating the vaccine formulation being tested in humans. Imaging revealed trimeric spike proteins present as free trimers or as multitrimer rosettes, containing as many as 14 trimers with their TM domains enclosed in micellar cores of PS 80 detergent (Fig. 1B). Tight clustering of the spikes in the NVAX-CoV2373 nanoparticle formulation may lead to stronger immune responses over soluble trimers alone, similar to other viral glycoprotein immunogens (hemagglutinin and respiratory syncytial virus F) (*12*, *13*).

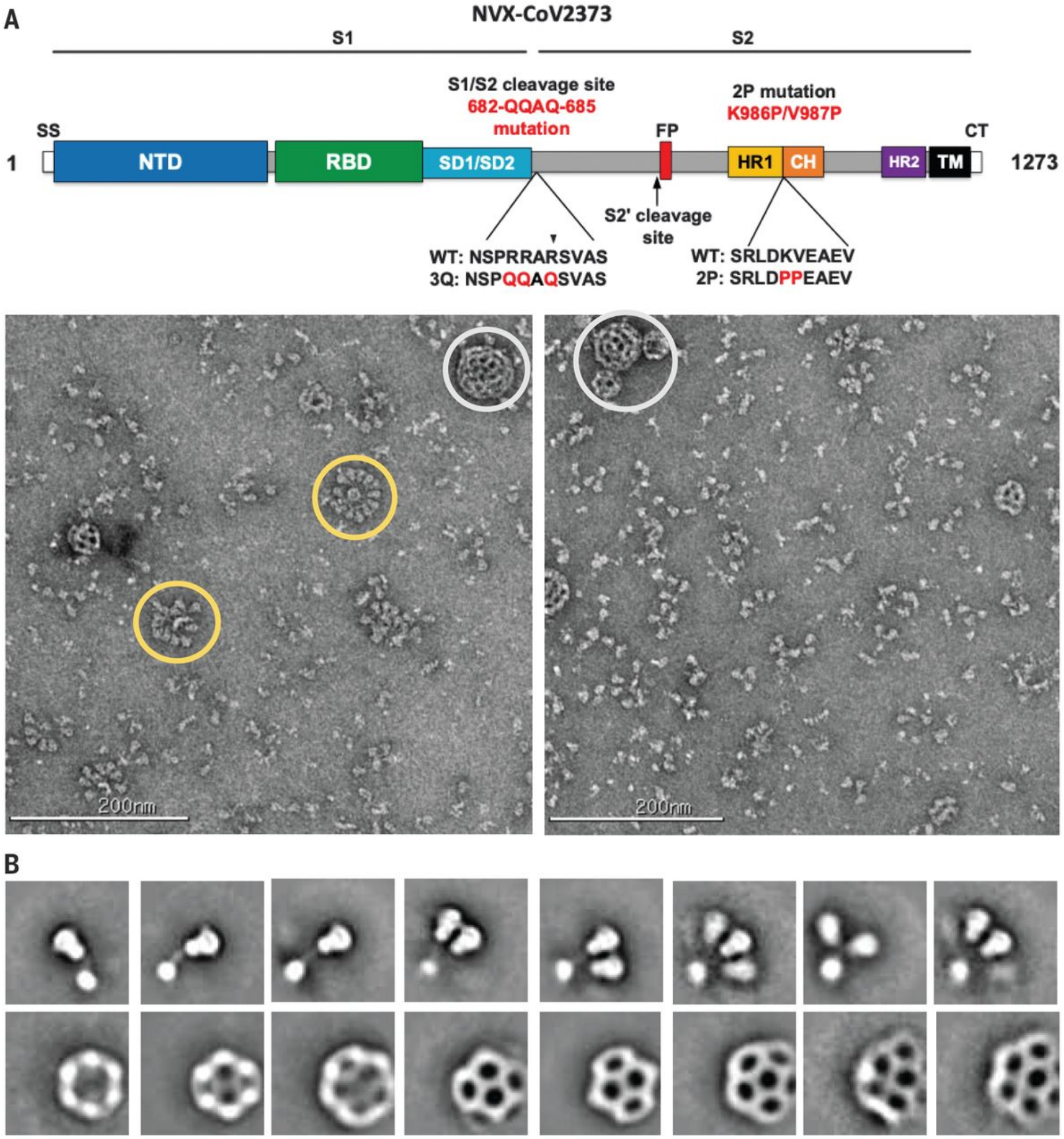

Fig. 1 Evaluation of SARS-CoV-2 3Q-2P-FL spike glycoprotein.

(**A**) Linear diagram of the sequence and structure elements of the FL SARS-CoV-2 spike protein showing the S1 and S2 ectodomain. Structural elements include a cleavable signal sequence (SS, white), NTD (blue), RBD (green), SD1 and SD2 (light blue), protease cleavage site 2' (S2', arrow), fusion peptide (FP, red), heptad repeat 1 (HR1, yellow), central helix (CH, brown), heptad repeat 2 (HR2, purple), TM domain (black), and

CT (white). The native furin cleavage site was mutated (RRAR→QQAQ) to be protease resistant and stabilized by introducing two proline (2P) substitutions at positions K986P and V987P to produce SARS-CoV-2 3Q-2P-FL spike. A, Ala; D, Asp; E, Glu; K, Lys; L, Leu; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; V, Val. (**B**) Representative negative-stain EM images and 2D classes of SARS-CoV-2 3Q-2P-FL, formulated in PS 80 detergent in the presence of Matrix-M adjuvant. In the raw micrograph, spike rosettes are circled in yellow and Matrix-M adjuvant cages are circled in white. 2D classes showing individual spikes, higher-order spike nanoparticles, and Matrix-M cages of different sizes. Matrix-M does not appear to interact with the spike nanoparticles.

We next performed single-particle cryo–electron microscopy (cryo-EM) on the spike formulated in PS 80 detergent (Fig. 2A). Initial two-dimensional (2D) classification revealed the presence of two distinct classes: free spike trimers and dimers of trimers (Fig. 2A). The threefold symmetric (C3) reconstruction of the free spike trimer resulted in a 3.6 Å–resolution map, whereas the asymmetric reconstruction (C1) was refined to 3.8-Å resolution (Fig. 2B and fig. S1, A and B). In previous structures, receptor binding domains (RBDs) exist in either a closed (RBD-down) or an open (RBD-up) conformation that can engage in ACE2 binding (*9*, *10*, *14*). By contrast, we observed that all three RBDs on the 3Q-2P-FL spike trimer were in the closed conformation in our reconstructions (Fig. 2B and fig. S1C). Despite the RBD-down conformation, binding analysis of the 3Q-2P-FL immunogen to ACE2 by both biolayer interferometry and enzyme-linked immunosorbent assay clearly shows binding to ACE2, indicating that the RBD is dynamic and the receptor binding site accessible (*15*). Another study on the prefusion structure of an FL spike protein reported similar findings with RBDs clamped down as a consequence of potential clashes between S2 residues 828 to 853 and subdomain 1 (SD1) when RBD is in open conformation (*16*). Recent reports by Henderson *et al*. have revealed that introducing mutations and removing N-linked glycosylation at certain positions can alter the propensity toward “up” and “down” states of the RBD (*17*, *18*).

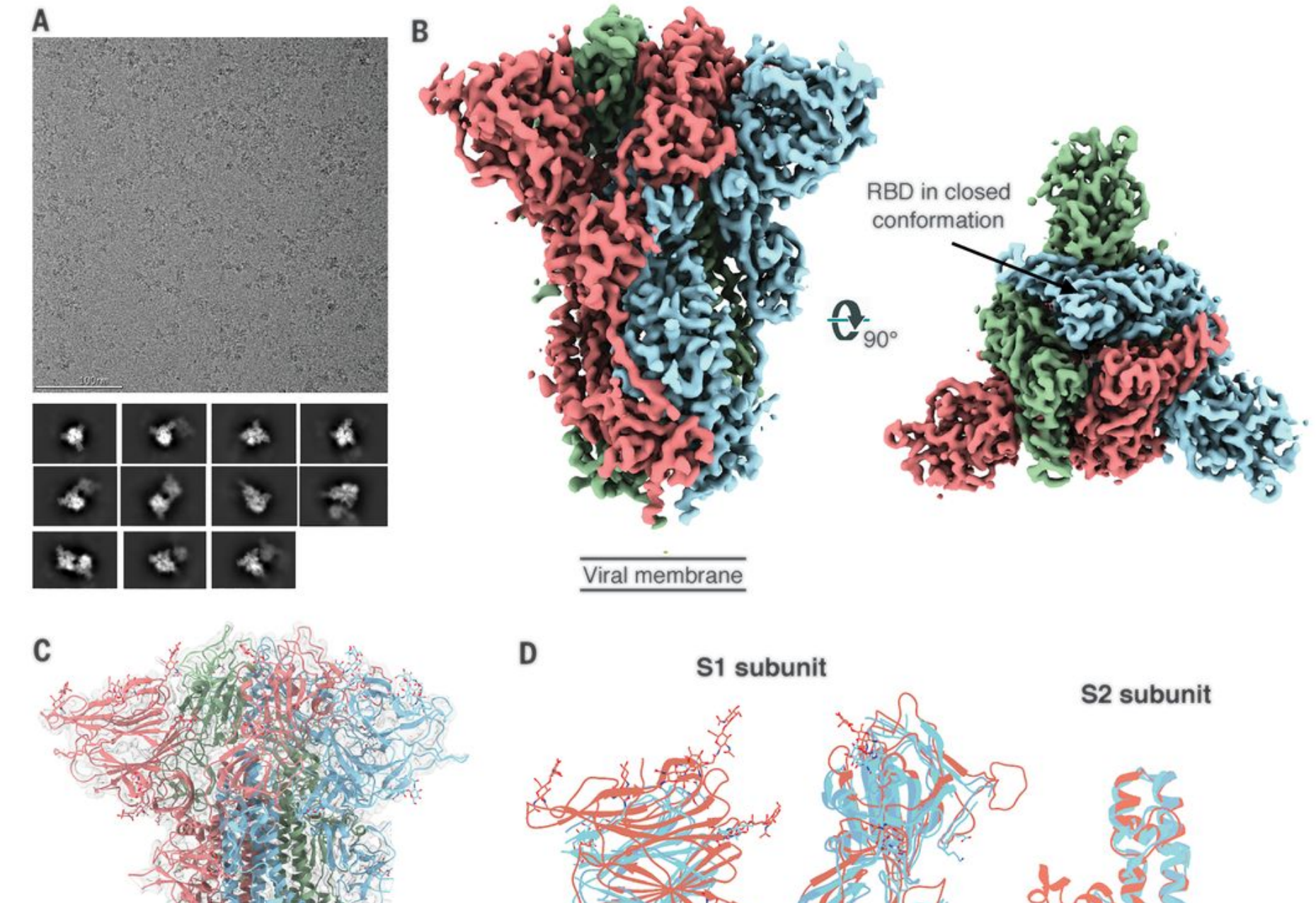

Fig. 2 Cryo-EM analysis of SARS-CoV-2 3Q-2P-FL spikes.

(**A**) Representative electron micrograph and 2D class averages of 3Q-2P-FL spikes showing free trimers and complexes of trimers. (**B**) Side and top views of the B factor–sharpened cryo-EM map of 3Q-2P-FL free trimers showing the spike in prefusion state, with the RBDs in closed conformation. The protomers are colored in blue, green, and coral for clarity. (**C**) Side and top view of the atomic model of free trimer represented as a ribbon diagram fit into the map density. The protomers are colored in blue, green, and coral, and the map is shown as a transparent gray density. (**D**) Comparison of 3Q-2P-FL spike with published structures (PDB IDs 6VXX and 6VSB) on a subunit level. PDB 6VXX is shown in cyan, PDB 6VSB in blue, and 3Q-2P-FL spike in coral.

Overall, our cryo-EM map was well resolved in both S1 and S2 subunits (fig. S1D), enabling us to model the full S1 N-terminal domain (NTD) and C-terminal domain (CTD) that were less resolved in previous structures (*9*, *10*). Our final atomic model contains residues 14 to 1146 with breaks only in the flexible loop (619 to 631) and the cleavage site (678 to 688) (Fig. 2C). Superimposition of the coordinate models of 3Q-2P-FL spike with published spike structures [Protein Data Bank (PDB) IDs: 6VXX and 6VSB] revealed substantial domain rearrangements in the S1 subunit of 3Q-2P-FL spike (Fig. 2D). The S1 NTD rotated ~14° relative to published models, whereas the CTD and sub-

domains showed minor local rearrangements (Fig. 2D). Another recent study also observed differences in NTD conformations at lower pH, although our cryo-EM studies were carried out at neutral pH (*19*). In our 3Q-2P-FL structure, we observed a shift in residues flanking the 615 to 635 loop, resulting in a salt bridge between residue D614 on one protomer and K854 on a neighboring protomer (Fig. 3A). This observation is particularly notable given the increased prevalence of D614→G (D614G) mutation in the emerging SARS-CoV-2 strains and its potential role in viral transmission and pathogenesis (*20*). The 615 to 635 loop that is generally disordered in spike trimer structures, including ours, was recently modeled as a helix (PDB ID: 6X6P) (Fig. 3B), although the cryo-EM density (EMD-22078) does not support this assignment (fig. S1E) (*11*).

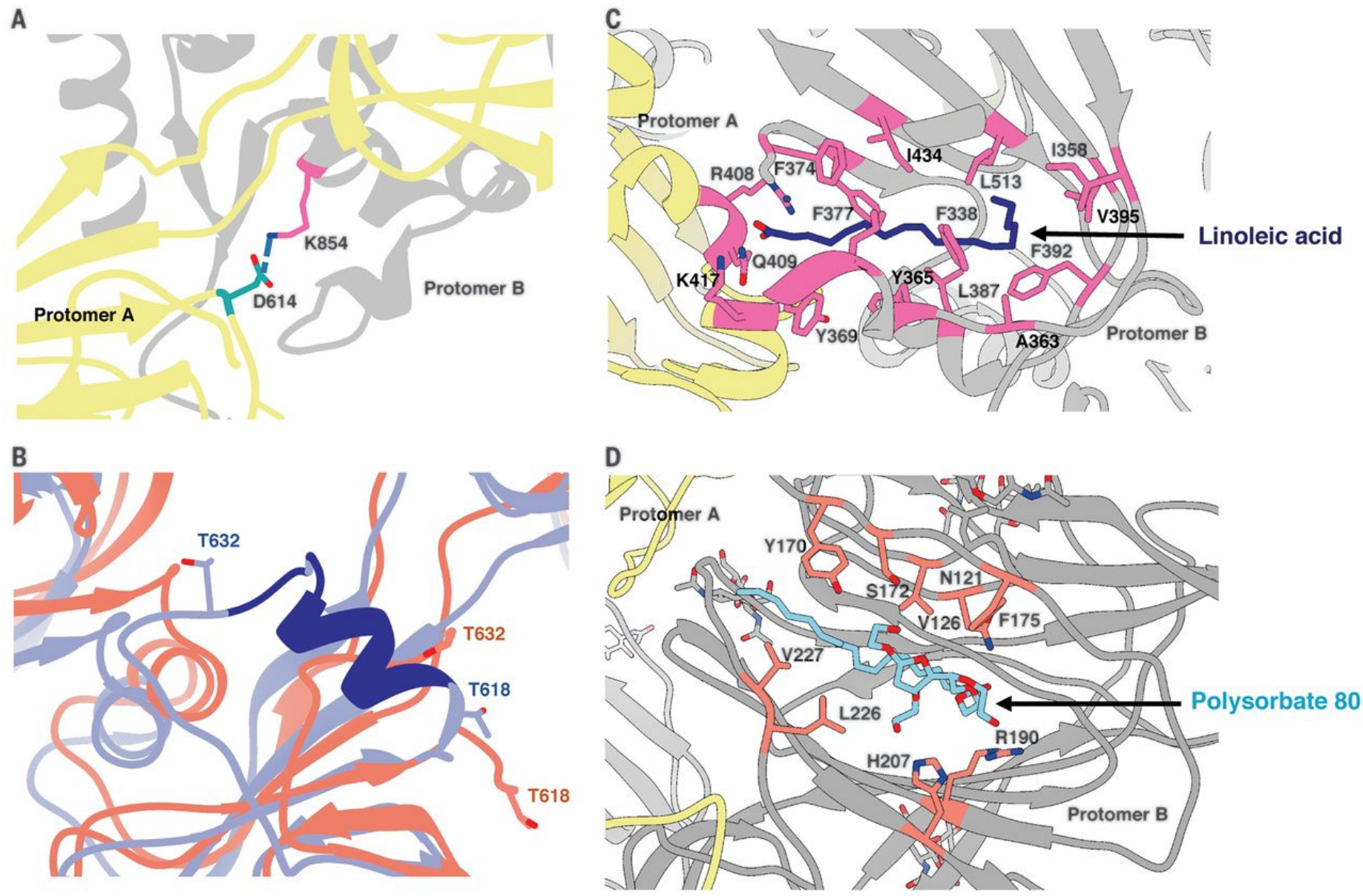

Fig. 3 Structural features of the SARS-CoV-2 3Q-2P-FL spike trimer.

(**A**) Interprotomeric salt-bridge interaction between D614 and K854 in 3Q-2P-FL spike trimer. (**B**) Comparison of the 615 to 635 loop between 3Q-2P-FL spike shown in coral and PDB 6X6P shown in blue. The residues that were built in 6X6P model but not in our model are shown in dark blue. Threonines at positions 618 and 632 flanking the gap in the 3Q-2P-FL trimer model are shown on both models to highlight their relative positions. T, Thr. (**C**) Linoleic acid (dark blue) binding within a hydrophobic pocket of one RBD where the fatty acid head group reaches out to interact with the closed RBD of the adjacent protomer. The interacting residues are shown in pink. F, Phe; I, Ile; Y, Tyr. (**D**) PS 80 detergent (blue) binding within the NTD with potential hydrogen bonding with R190 and H207. The interacting residues are shown in orange. Adjacent protomers are shown in yellow and gray in (A), (C), and (D). H, His.

We observed two additional densities in the S1 subunit that did not correspond to peptide or glycans within the spike (fig. S2A). The first density was buried within a hydrophobic pocket of the CTD (Fig. 3C). We have previously showed palmitoleic acid occupying a similar pocket in the structure of porcine epidemic diarrhea virus (*21*). This density in SARS-CoV-2 S corresponded to linoleic acid, a polyunsaturated fatty acid; the presence of this ligand was confirmed by mass spectrometry of 3Q-2P-FL spike (fig. S2, B and C). The main chain carboxyl group of linoleic acid interacts with the R408 and Q409 residues of the RBD from the adjacent protomer, potentially stabilizing the observed RBD-down state (Fig. 3C) and consistent with a recent report (*22*). The second unassigned density, present in the NTD, was larger and more surface exposed than the first (Fig. 3D and fig. S2D). The aliphatic tail of PS 80 fit well into this hydrophobic pocket, whereas the carbonyl and hydroxyl groups were in proximity to residues R190 and H207 with potential for multiple hydrogen bonds between them (Fig. 3D and fig. S2D). The location of the PS 80 ligand provides a possible explanation for the S1 shift seen in our FL trimer density. PS 80 is specific to the formulation of the Novavax 3Q-2P-FL immunogen, but other ligands may also bind this pocket and provide a potential target for drug design against SARS-CoV-2.

Classification of multimeric spike trimer particles yielded two separate classes: a dimer-of-trimers class that reconstructed to a final resolution of 4.5 Å with twofold symmetry and a trimer-of-trimers class that was resolved to

8.0-Å resolution (Fig. 4, A and B, and fig. S3A). In both reconstructions, the interaction between each pair of trimers involved the SD2 of one protomer from each trimer engaging with the NTD of the adjacent trimer (Fig. 4C), with trimer axes tilted 44.5° relative to each other. The dimer-of-trimer interaction was mainly coordinated by the 615 to 635 loop, which, in contrast to the free-trimer structure, was now fully resolved (Fig. 4D). The loop reaches into and induces subtle changes to a pocket on the adjacent NTD compared with the free-trimer model (Fig. 4D). Residues Y145 and H146 in the binding pocket appear to switch positions in the loop-bound state, resulting in a salt-bridge interaction between H146 and D627 and potential stacking between W152 and H146 (Fig. 4E). We also observed minor displacement of residues 68 to 75 and 248 to 250 surrounding the pocket. In the dimer-of-trimers, we also observed N282 glycans at the dimer interface (fig. S3B). As a control, we also performed cryo-EM studies of the SARS-CoV-2-3Q-FL (without 2P). Notably, the structures of the trimers were identical, and we also observed dimers of trimers (fig. S3, C to E)

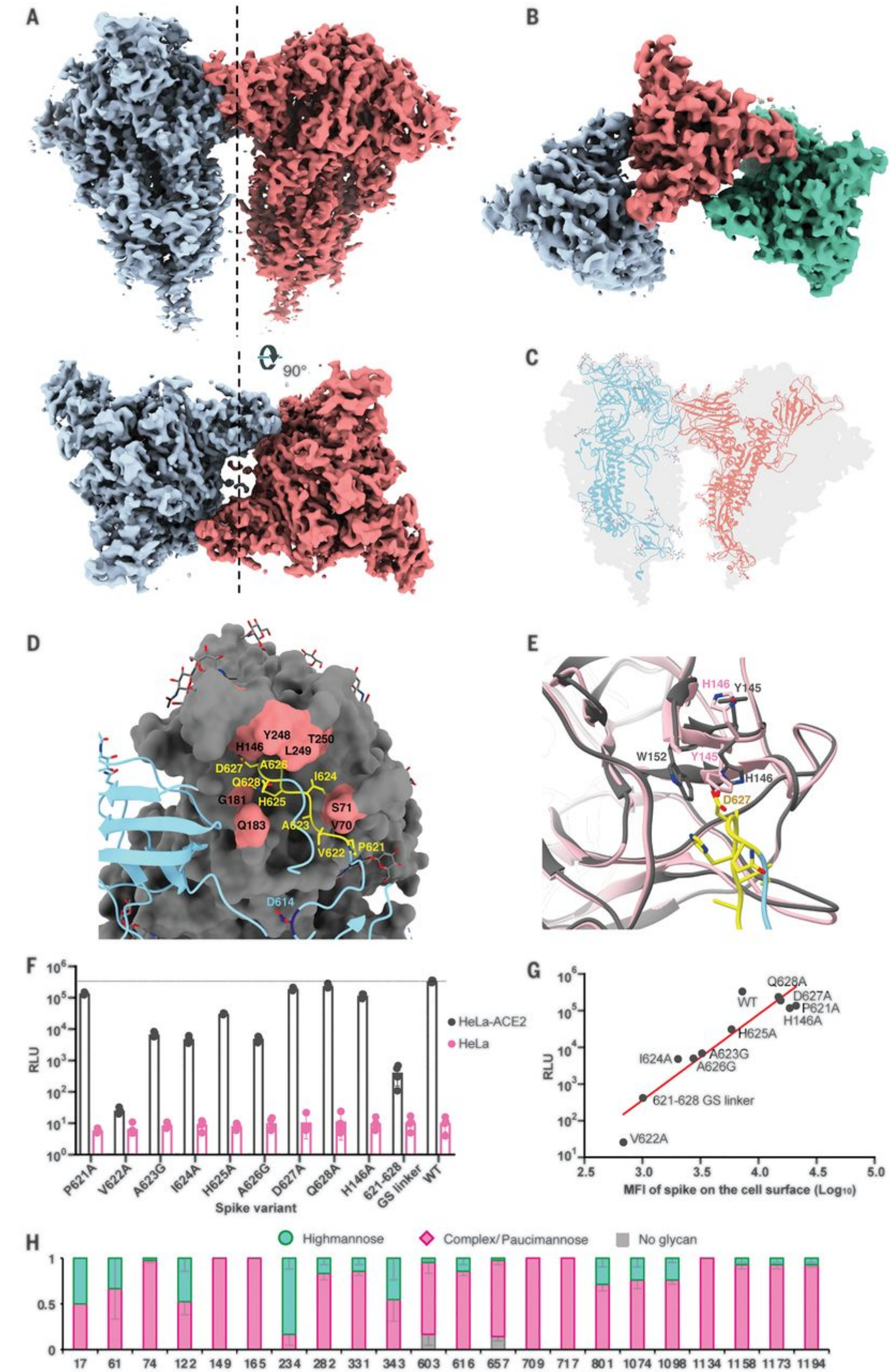

Fig. 4 **Trimer-trimer interactions and glycan analysis.**

Expand for more

Sequence alignment of residues in the 615 to 635 loop and corresponding NTD binding pocket across representative CoV strains belonging to lineage B of betacoronaviruses revealed residues 621-PVAIHADQ-628 are well conserved, but there are notable differences in the binding pocket residues (fig. S4A). Substantial gaps in the interacting NTD loops along with the absence of H146 at the corresponding site on SARS-CoV make it unlikely that SARS-CoV participates in similar intertrimeric interactions. Although the residues in the NTD pocket were almost identical between SARS-CoV-2 and its closely related bat strain Bat-SL-RatG13, we observed some residue differences and one to three amino acid deletions in the loops comprising the NTD binding pocket of representative strains Bat-SL-CoVZC45, BetaCoV/pangolin/Guangdong/1/2019, and BetaCoV/pangolin/Guangxi/P4L/2007 (fig. S4A).

Some human CoVs, including OC43, exclusively use NTD–sialic acid (SA) interactions as their receptor engagement, whereas others such as Middle East respiratory syndrome (MERS) CoV that use the CTD-RBD for primary receptor binding have also been reported to bind SA receptors through their NTD to aid initial attachment to the host cells (*23*–*25*). Structural comparisons of the SARS-CoV-2 NTD dimerization pocket with that of the SA binding site on MERS spike revealed that they did not coincide with each other (PDB ID: 6Q04) (*25*) (fig. S4B). Computational and structural studies have proposed residues on SARS-CoV-2 spike that may be involved in SA binding (*26*, *27*). Structural comparison of this putative glycan binding site to the dimerization site revealed them situated adjacent to one another with residues in loop 70 contributing to both the binding pockets (fig. S4C).

We next performed cell surface expression and pseudovirus replication assays with SARS-CoV-2 wild-type (WT) spike and spikes containing mutations in the 615 to 635 loop and NTD pocket. Each residue in the loop 621-PVAIHADQ-628 and residue H146 in the binding pocket were individually mutated to either alanine or glycine. Additionally, we made a spike construct with all eight residues 621-PVAIHADQ-628 replaced with a glycine-serine (GS) linker to completely abrogate binding. Compared with the WT, the mutants generally exhibited lower levels of infectivity (Fig. 4F). Cell surface expression of these mutants in 293T cells revealed that these mutations also disrupted surface expression of the spike protein, with linear correlation between surface expression and pseudovirus replication (Fig. 4G).

Glycans on viral glycoproteins play a wide role in protein folding, stability, and immune recognition and also in facilitating immune evasion. We therefore conducted site-specific glycosylation analysis of the SARS-CoV-2 prefusion spike protein produced in Sf9 insect cells as previously described (*28*) to assess the extent of glycosylation and the degree of glycan processing from high-mannose or hybrid type to complex type. The analysis detected glycosylation at all 22 N-linked glycan sequons present on SARS-CoV-2 spike (Fig. 4H). Overall, there was high glycan occupancy of >98%, with only two sites (603 and 657) >5% unoccupied. We did not see clear glycan density at either 603 or 657 in the cryo-EM reconstruction of the 3Q-2P-FL spike. Most sites showed extensive glycan processing to complex or paucimannose-type glycans, with only four sites exhibiting ≥40% oligomannose. The glycan analysis also confirmed the presence of glycans at sites 1158, 1173, and 1194 present in the membrane-proximal region of the spike not resolved by cryo-EM. By comparison with site-specific glycan processing of the spike protein produced in mammalian human embryonic kidney (HEK) 293F cells, both mammalian cells and insect cells exhibit extensive processing at most sites. In general, however processing of glycans on the 2019 CoV prefusion spike protein from insect cells was somewhat greater, particularly at sites 709 and 717, which were predominately oligomannose in spike from HEK293 cells but exclusively complex or paucimannose in spike from Sf9 cells (*29*).

Our structural work is consistent with the burgeoning body of spike structures, albeit with notable differences in the rearrangement of S1 domains and formation of intertrimer interactions (*9*, *10*). Both these findings were seen in the FL spike immunogens assembled into compact and dense nanoparticles. Cryo–electron tomographic reconstructions of intact SARS-CoV-2 virions showed a relatively dispersed distribution of spike protein trimers on the viral surface and no evidence of higher-order aggregates (*30*). However, another study showed that the D614G mutation present in close proximity to the dimerization loop results in a several-fold increase of spike numbers on the viral surface, resulting in higher spike protein density and a more infectious virion (*20*). The greater density may be aided by the ability to form such higher-order multimers. Alternatively, the loop that mediates interspike interactions may play a role in viral viability, consistent with our loop mutant data.

Analysis of safety and immunogenicity of the Novavax SARS-CoV-2-3Q-2P-FL immunogen in mice and baboons revealed strong B and T cell responses to the vaccine with no evidence of vaccine-associated enhanced respiratory disease (*15*). Phase 1 and 2 clinical trial results showed that the vaccine induced immune responses exceeding lev-

els seen in COVID-19 patients (*31*). Overall, we found that NVAX-CoV2372 is stable, homogeneous, and locked in the antigenically preferred prefusion conformation. With structural, biophysical, and antigenic characterization now complete, ongoing evaluation in humans will provide the true proof-of-principle for this vaccine concept.

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Supplementary Material

Summary

MDAR Reproducibility Checklist

Resources

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