

Supplementary Material

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Figure S2. Validation of three-dimensional models of NG06 and NG19 using the Z-Score and local quality values.

Figure S3. Ramachandran plots for validation of the *de novo* predicted structures for the NG06 and NG19 proteins.

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Table S1. Primer sequence employed in the present study.

Table S2. Primary structure and physicochemical properties of vaccine antigen candidates

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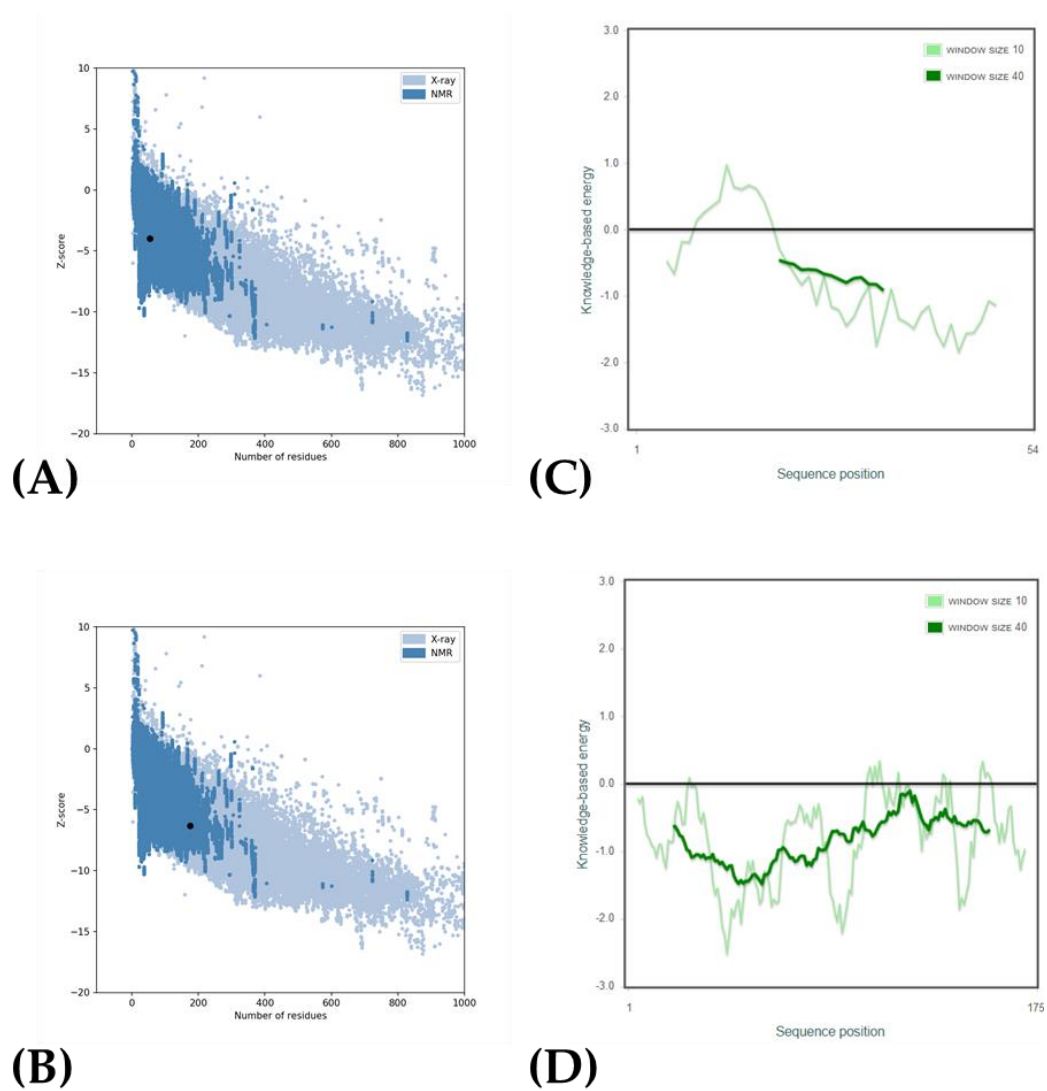


Figure S2. Validation of three-dimensional models of NG06 (A) and NG19 (B) using the Z-Score and local quality values for NG06 (C) and NG19 (D)

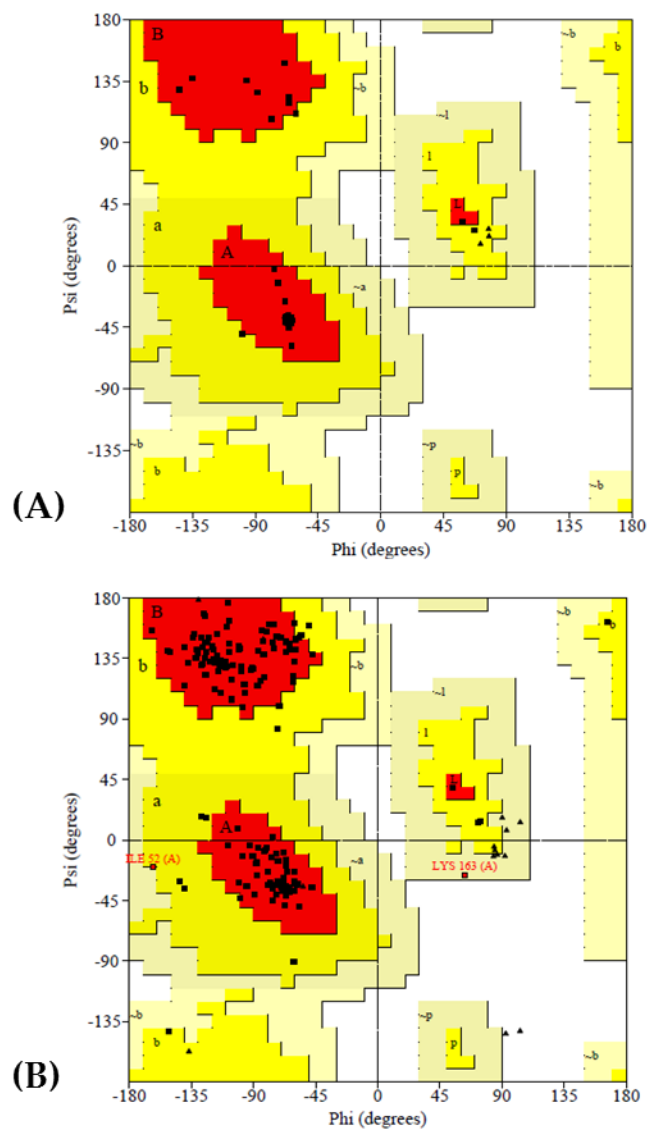


Figure S3. Ramachandran plots for validation of the de novo predicted structures for the NG06 (A) and NG19 (B) proteins

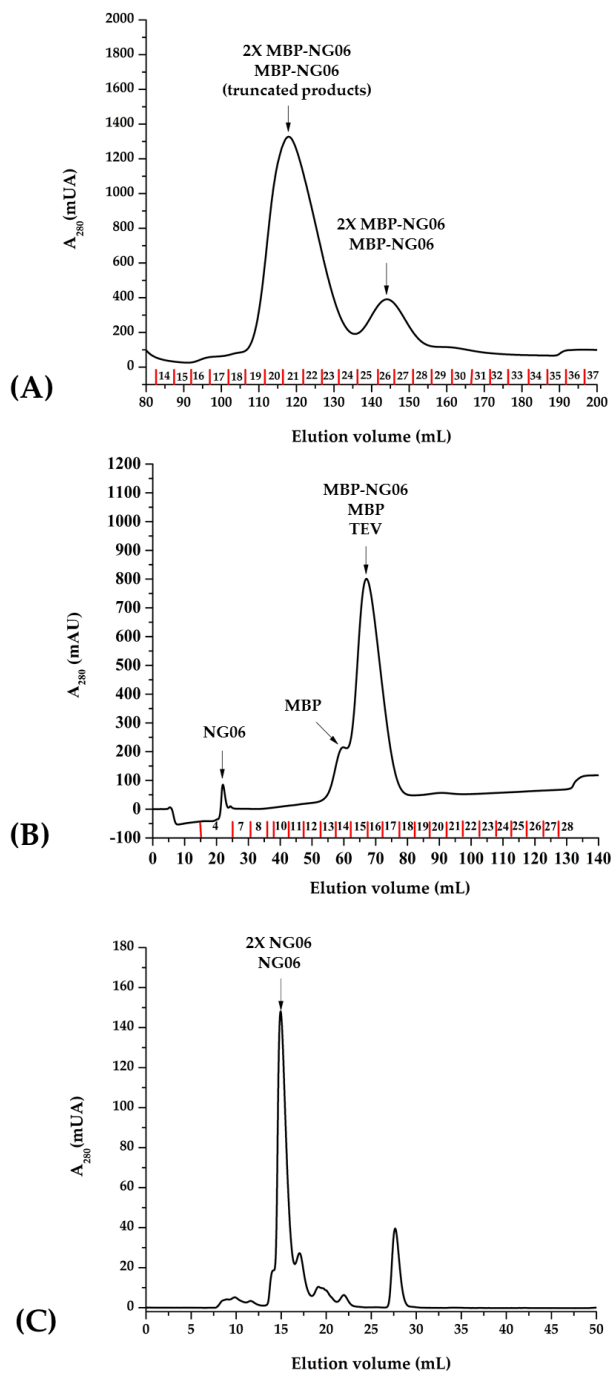


Figure S4. Chromatographic steps through FLPC for NG06 purification. A) Initial IMAC Chromatogram for NG06. B) Buffer exchange chromatogram for NG06 C) After TEV cleavage IMAC chromatogram for NG06. D) Final buffer exchange for NG06 purification

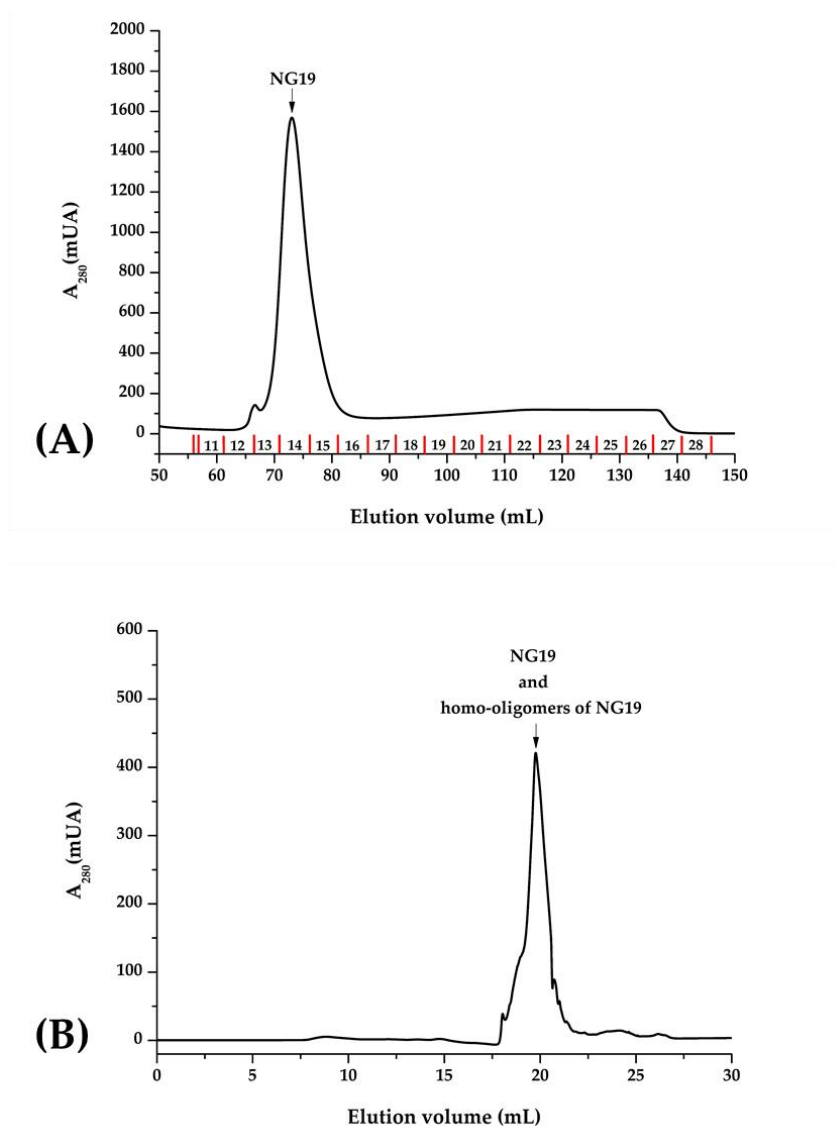


Figure S5. Chromatographic steps through FLPC for NG19 purification. A) Initial IMAC Chromatogram for NG19. B) SEC-Refolding chromatogram for NG19

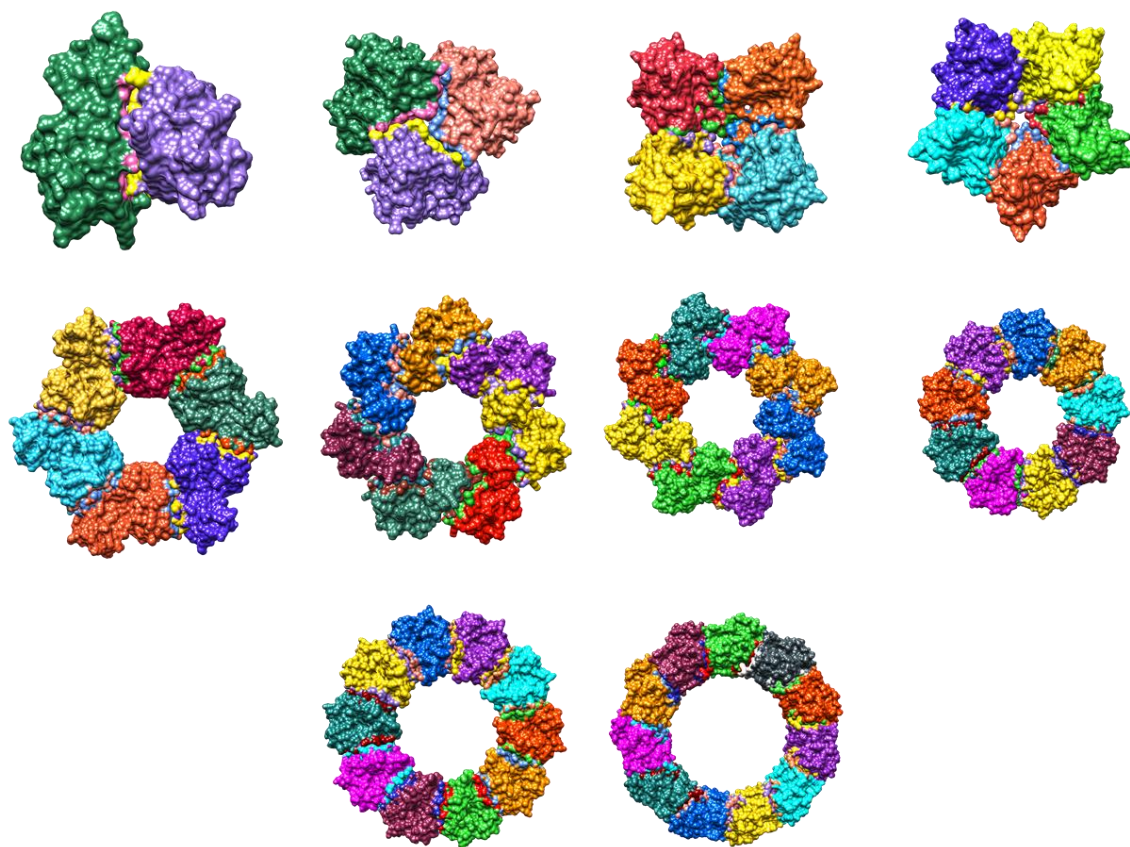


Figure S6: *In silico* oligomerization of NG19

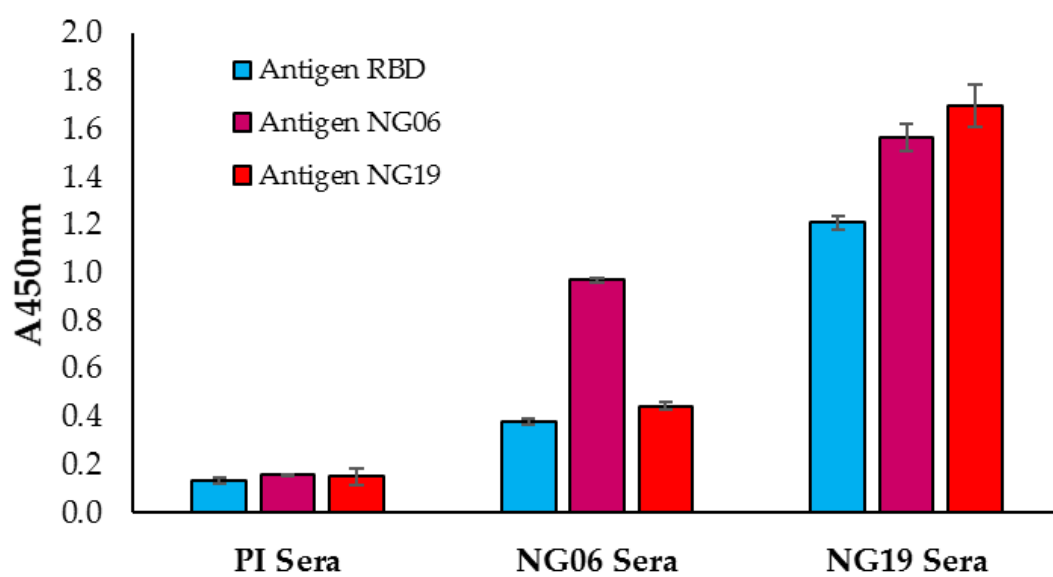


Figure S7: Comparison of antigen affinity for NG recombinant proteins and native RBD.

Table S1. Primer sequence employed in the present study

Primer Name	Sequence ('5→3')
eNG06-F	AGCAACAACCTGGATAGCAAAGTCGGCGG
eNG06-STOP-R	TTAGTTAAAACCTTCAACGCCGTTACACG
eNG19-F	AGCGCGAGCTTCTCCACCTTCAAATGCTATG
eNG19-KWCEC-STOP-R	TTAGCATTCGCACCATTTGAAGTTGACGCATT
eNG06-STOP-R	TTAGAAGTTGACGCATTTGTTTTTGACCAGGT
NcoI-eNG06-F	CATGCCATGGCCAGCAACAACCTGGATAGCAAAG
HindIII-eNG06-R	CCCAAGCTTTTAGTTAAAACCTTCAACGCCGTTAC
NcoI-eNG19-F	CATGCCATGGCCAGCGCGAGCTTCTCCACCTTC
HindIII-eNG19-R	CCCAAGCTTTTAGAAGTTGACGCATTTGTTTTTG

* Restriction sites for NcoI and HindIII are shown in bold.

Table S2. Primary structure and physicochemical properties of vaccine antigen candidates

	NG06	NG19
Length (aa)	54	175
Molecular Weight (kDa)	6.0	19.4
Positive Charged Residues	6 (11.1%)	17 (9.7%)
Negative Charged Residues	5 (9.3%)	13 (7.4%)
Isoelectric Point	8.10	8.64
Instability Index	12.08 (stable)	16.64 (stable)

Table S3: Hematological values of the immunized rabbits at day 35

		NG06	NG19	Reference Values
Hemogram	Hematocrit (L/L)	0.45	0.41	0.34-0.50
	Hemoglobin (g/L)	144.00	123.00	98.00-158.00
	Erythrocytes ($\times 10^{12}/L$)	6.50	6.30	5.10-6.50
	MCV (fL)	69.00	65.00	57.80-80.30
	MCHC (g/L)	320.00	300.00	287.00-357.00
	Platelets ($\times 10^9/L$)	201.00	512.00	-
	Proteins (g/L)	60.00	70.00	60.00-75.00
	Reticulocytes (%)	1.70	2.10	0.00-3.00
Diferential	Total Leukocytes ($\times 10^9/L$)	6.60	19.80	5.00-12.00
	Total Neutrophils (%)	56.10	77.98	18.80-46.40
	Band Neutrophils ($\times 10^9/L$)	0.00	0.00	0.00-0.30
	Metamyelocytes ($\times 10^9/L$)	0.00	0.00	0.00-0.00
	Myelocytes ($\times 10^9/L$)	0.00	0.00	0.00-0.00
	Lymphocytes (%)	38.03	11.01	44.60-77.80
	Monocytes (%)	1.97	10.00	0.00-13.00
	Eosinophils (%)	0.00	0.00	0.00-2.40
	Basophils (%)	5.00	1.01	0.00-6.20
	Nucleated eosinophils (/100 Leucocytes)	0.00	0.00	0.00-0.00
	Toxic neutrophils	Negative	Negative	Negative
	Atypical lymphocytes	Negative	Negative	Negative
	Erythrocyte morphology	Normal	Normal	Normal
	Anisocytosis	Positive	Positive	Positive/Negative
	Polychromasia	Positive	Positive	Positive
	Hemoparasites	Negative	Negative	Negative
	Dirofilaria	Negative	Negative	Negative
	Inclusion bodies	Negative	Negative	Negative
	Clumped platelets	Positive	Negative	Negative