Biomimetic Antigenic Nanoparticles Elicit Controlled Protective Immune Response to Influenza

Dustin P. Patterson[1,2,#], Agnieszka Rynda-Apple[3,#], Ann L. Harmsen[3], Allen G. Harmsen[3*], Trevor Douglas[1,2]*

[1]Department of Chemistry and Biochemistry, [2]Center for Bio-Inspired Nanomaterials, Montana State University, Bozeman, MT 59717, USA
[3]Department of Immunology and Infectious Diseases, Montana State University, Bozeman, MT 59717, USA

[#] These authors contributed equally to this work.

* To whom correspondence should be addressed,
tdouglas@chemistry.montana.edu
phone (406) 994-6566
or
aharmsen@montana.edu
phone (406) 994-7626

Supporting Information

DNA Sequences

Nucleoprotein (as received in pTrcHis)

ATGGCGCTCGAGGCGAGCCAGGGCACCAAACGTAGCTATGAGCAGATGGAAACCGATGG
CGAACGTCAGAACGCGACCGAAATTCGTGCGAGCGTGGGCAAAATGATTGGCGGCATTG
GCCGTTTTTATATTCAGATGTGCACCGAACTGAAACTGAGCGATTATGAAGGCCGTCTG
ATTCAGAACAGCCTGACCATTGAACGTATGGTTCTGAGCGCGTTTGATGAACGTCGTAA
CAAATACCTGGAAGAACATCCGAGCGCGGGCAAAGATCCGAAAAAAACCGGCGGTCCGA
TTTATCGTCGTGTGAACGGCAAATGGATGCGTGAACTGATCCTGTACGATAAAGAAGAA
ATTCGTCGTATTTGGCGTCAGGCGAACAATGGCGATGATGCGACCGCCGGTCTGACCCA
TATGATGATTTGGCATAGCAACCTGAACGATGCGACCTATCAGCGTACCCGTGCGCTGG
TGCGTACCGGCATGGATCCGCGTATGTGCAGCCTGATGCAGGGTAGCACCCTGCCGCGT
CGTAGCGGTGCGGCGGGTGCGGCCGTGAAAGGTGTGGGCACGATGGTGATGGAACTGGT
GCGTATGATTAAACGTGGCATCAACGATCGTAACTTTTGGCGTGGCGAAAACGGCCGTA
AAACCCGTATTGCGTATGAACGTATGTGCAACATCCTGAAAGGCAAATTTCAGACCGCG
GCGCAGAAAGCGATGATGGATCAGGTGCGTGAAAGCCGTAACCCGGGCAACGCGGAATT
TGAAGATCTGACCTTTCTGGCGCGTAGCGCGCTGATTCTGCGTGGCAGCGTGGCGCATA
AAAGCTGCCTGCCGGCGTGCGTTTATGGTCCGGCGGTGGCGAGCGGCTATGATTTTGAA
CGTGAAGGCTACAGCCTGGTGGGCATTGATCCGTTTCGTCTGCTGCAGAACAGCCAGGT
GTATAGCCTGATTCGTCCGAACGAAAACCCGGCGCACAAAAGCCAGCTGGTGTGGATGG
CGTGTCATAGCGCGGCGTTCGAAGATCTGCGTGTTCTGAGCTTTATTAAAGGCACCAAA
GTGCTGCCGCGTGGCAAACTGAGCACCCGTGGCGTGCAGATTGCGAGCAACGAAAACAT
GGAAACGATGGAAAGCAGCACCCTGGAACTGCGTAGCCGTTATTGGGCGATTCGTACCC
GTAGCGGCGGCAACACCAACCAGCAGCGTGCGAGCGCGGGTCAGATTAGCATTCAGCCG
ACCTTTAGCGTGCAGCGTAACCTGCCGTTTGATCGTACCACCATTATGGCGGCGTTTAA
CGGCAACACCGAAGGCCGTACCAGCGATATGCGTACCGAAATTATCCGTATGATGGAAA
GCGCGCGTCCGGAAGATGTGAGCTTTCAGGGCCGTGGCGTGTTTGAACTGAGCGATGAA
AAAGCGGCGAGCCCGATTGTGCCGAGCTTTGATATGAGCAACGAAGGCAGCTATTTTTT
TGGCGATAACGCGGAAGAATACGATAACGTCGAC

*Base highlighted in yellow required modification to remove BamHI site for insertion into pETDuet-1 vector with P22 scaffold protein (see sequence below).

NP_{163}-SP Fusion

```
ATGGCGCTCGAGGCGAGCCAGGGCACCAAACGTAGCTATGAGCAGATGGAAACCGATGG
CGAACGTCAGAACGCGACCGAAATTCGTGCGAGCGTGGGCAAAATGATTGGCGGCATTG
GCCGTTTTTATATTCAGATGTGCACCGAACTGAAACTGAGCGATTATGAAGGCCGTCTG
ATTCAGAACAGCCTGACCATTGAACGTATGGTTCTGAGCGCGTTTGATGAACGTCGTAA
CAAATACCTGGAAGAACATCCGAGCGCGGGCAAAGATCCGAAAAAAACCGGCGGTCCGA
TTTATCGTCGTGTGAACGGCAAATGGATGCGTGAACTGATCCTGTACGATAAAGAAGAA
ATTCGTCGTATTTGGCGTCAGGCGAACAATGGCGATGATGCGACCGCCGGTCTGACCCA
TATGATGATTTGGCATAGCAACCTGAACGATGCGACCTATCAGCGTACCCGTGCGCTGG
TGCGTACCGGCATGGATCCCCTGGTGCCGCGCGGCAGCTGTCGCAGCAATGCCGTAGCA
GAACAGGGCCGCAAGACTCAGGAGTTTACCCAGCAATCAGCGCAATACGTCGAAGCTGC
CCGCAAACACTATGACGCGGCGGAAAAGCTCAACATCCCTGACTATCAGGAGAAAGAAG
ACGCATTTATGCAACTGGTTCCGCCTGCGGTTGGGGCCGACATTATGCGCCTGTTCCCG
GAAAAGTCCGCCGCGCTCATGTATCACCTGGGGGCAAACCCGGAGAAAGCCCGCCAGTT
ACTGGCGATGGATGGGCAGTCCGCGCTGATTGAACTCACTCGACTATCCGAACGCTTAA
CTCTCAAGCCTCGCGGTAAACAAATCTCTTCCGCTCCCCATGCTGACCAGCCTATTACC
GGTGATGTCAGCGCAGCAAATAAAGATGCCATTCGTAAACAAATGGATGCTGCTGCGAG
CAAGGGAGATGTGGAAACCTACCGCAAGCTAAAGGCAAAACTTAAAGGAATCCGATAA
```

*Base highlighted in green resulted from a random base insertion, which subsequently maintained the desired reading frame for the SP fusion.

NP-SP Fusion

```
ATGGCGCTCGAGGCGAGCCAGGGCACCAAACGTAGCTATGAGCAGATGGAAACCGATGG
CGAACGTCAGAACGCGACCGAAATTCGTGCGAGCGTGGGCAAAATGATTGGCGGCATTG
GCCGTTTTTATATTCAGATGTGCACCGAACTGAAACTGAGCGATTATGAAGGCCGTCTG
ATTCAGAACAGCCTGACCATTGAACGTATGGTTCTGAGCGCGTTTGATGAACGTCGTAA
CAAATACCTGGAAGAACATCCGAGCGCGGGCAAAGATCCGAAAAAAACCGGCGGTCCGA
TTTATCGTCGTGTGAACGGCAAATGGATGCGTGAACTGATCCTGTACGATAAAGAAGAA
ATTCGTCGTATTTGGCGTCAGGCGAACAATGGCGATGATGCGACCGCCGGTCTGACCCA
TATGATGATTTGGCATAGCAACCTGAACGATGCGACCTATCAGCGTACCCGTGCGCTGG
TGCGTACCGGCATGGACCCGCGTATGTGCAGCCTGATGCAGGGTAGCACCCTGCCGCGT
CGTAGCGGTGCGGCGGGTGCGGCCGTGAAAGGTGTGGGCACGATGGTGATGGAACTGGT
GCGTATGATTAAACGTGGCATCAACGATCGTAACTTTTGGCGTGGCGAAAACGGCCGTA
AAACCCGTATTGCGTATGAACGTATGTGCAACATCCTGAAAGGCAAATTTCAGACCGCG
GCGCAGAAAGCGATGATGGATCAGGTGCGTGAAAGCCGTAACCCGGGCAACGCGGAATT
TGAAGATCTGACCTTTCTGGCGCGTAGCGCGCTGATTCTGCGTGGCAGCGTGGCGCATA
AAAGCTGCCTGCCGGCGTGCGTTTATGGTCCGGCGGTGGCGAGCGGCTATGATTTTGAA
CGTGAAGGCTACAGCCTGGTGGGCATTGATCCGTTTCGTCTGCTGCAGAACAGCCAGGT
GTATAGCCTGATTCGTCCGAACGAAAACCCGGCGCACAAAAGCCAGCTGGTGTGGATGG
CGTGTCATAGCGCGGCGTTCGAAGATCTGCGTGTTCTGAGCTTTATTAAAGGCACCAAA
GTGCTGCCGCGTGGCAAACTGAGCACCCGTGGCGTGCAGATTGCGAGCAACGAAAACAT
GGAAACGATGGAAAGCAGCACCCTGGAACTGCGTAGCCGTTATTGGGCGATTCGTACCC
GTAGCGGCGGCAACACCAACCAGCAGCGTGCGAGCGCGGGTCAGATTAGCATTCAGCCG
ACCTTTAGCGTGCAGCGTAACCTGCCGTTTGATCGTACCACCATTATGGCGGCGTTTAA
CGGCAACACCGAAGGCCGTACCAGCGATATGCGTACCGAAATTATCCGTATGATGGAAA
GCGCGCGTCCGGAAGATGTGAGCTTTCAGGGCCGTGGCGTGTTTGAACTGAGCGATGAA
AAAGCGGCGAGCCCGATTGTGCCGAGCTTTGATATGAGCAACGAAGGCAGCTATTTTTT
TGGCGATAACGCGGAAGAATACGATAACGTCGACGGATCCCTGGTGCCGCGCGGCAGCT
GTCGCAGCAATGCCGTAGCAGAACAGGGCCGCAAGACTCAGGAGTTTACCCAGCAATCA
GCGCAATACGTCGAAGCTGCCCGCAAACACTATGACGCGGCGGAAAAGCTCAACATCCC
TGACTATCAGGAGAAAGAAGACGCATTTATGCAACTGGTTCCGCCTGCGGTTGGGGCCG
ACATTATGCGCCTGTTCCCGGAAAAGTCCGCCGCGCTCATGTATCACCTGGGGGCAAAC
CCGGAGAAAGCCCGCCAGTTACTGGCGATGGATGGGCAGTCCGCGCTGATTGAACTCAC
TCGACTATCCGAACGCTTAACTCTCAAGCCTCGCGGTAAACAAATCTCTTCCGCTCCCC
ATGCTGACCAGCCTATTACCGGTGATGTCAGCGCAGCAAATAAAGATGCCATTCGTAAA
CAAATGGATGCTGCTGCGAGCAAGGGAGATGTGGAAACCTACCGCAAGCTAAAGGCAAA
ACTTAAAGGAATCCGATAA
```

*Base highlighted in yellow was modified from the original pTrcHis vector to remove BamHI site for insertion into pETDuet-1 vector.

P22 Coat Protein

ATGGCTTTGAACGAAGGTCAAATTGTTACACTGGCGGTAGATGAAATCATCGAAACCAT
CTCCGCAATCACTCCAATGGCGCAGAAAGCCAAGAAATACACCCCGCCTGCTGCTTCTA
TGCAGCGCTCCAGCAATACCATCTGGATGCCTGTAGAGCAAGAGTCACCCACTCAGGAG
GGCTGGGATTTAACTGATAAAGCGACAGGGTTACTGGAACTTAACGTCGCGGTAAACAT
GGGAGAGCCGGATAACGACTTCTTCCAGTTGCGTGCTGATGACTTGCGAGACGAAACTG
CGTATCGTCGCCGCATCCAGTCTGCCGCTCGCAAGCTGGCGAACAACGTTGAGTTGAAA
GTCGCAAACATGGCCGCCGAGATGGGTTCGCTGGTTATCACCTCCCCTGATGCCATCGG
CACTAATACCGCAGACGCCTGGAACTTTGTGGCCGACGCAGAAGAAATCATGTTCTCCC
GCGAACTTAACCGCGACATGGGGACATCGTACTTCTTCAACCCTCAGGACTACAAAAAA
GCGGGTTACGACCTGACCAAGCGTGACATCTTCGGGCGTATTCCTGAAGAAGCATACCG
AGATGGCACCATTCAGCGTCAGGTCGCTGGCTTCGATGATGTCCTGCGCTCTCCGAAAC
TTCCTGTGCTGACCAAATCCACCGCAACTGGCATCACTGTATCCGGTGCGCAGTCCTTC
AAGCCTGTCGCATGGCAACTGGATAACGATGGCAACAAAGTTAACGTTGATAACCGTTT
TGCTACCGTCACCCTGTCTGCAACTACCGGCATGAAACGCGGCGACAAAATTTCGTTTG
CTGGCGTTAAGTTCCTTGGTCAGATGGCTAAGAACGTACTGGCTCAGGATGCGACTTTC
TCCGTAGTCCGCGTTGTTGACGGTACTCATGTTGAAATCACGCCGAAGCCGGTAGCGCT
GGATGATGTTTCCCTGTCTCCGGAGCAGCGTGCCTACGCCAACGTTAACACCTCGCTGG
CTGATGCAATGGCAGTGAACATTCTGAACGTTAAAGACGCTCGCACTAATGTGTTCTGG
GCTGACGATGCTATTCGTATCGTGTCTCAGCCGATTCCGGCTAACCATGAACTTTTTGC
AGGTATGAAAACTACCTCATTCAGCATCCCTGATGTTGGCCTGAACGGTATCTTCGCTA
CGCAGGGTGATATTTCCACCCTGTCCGGCCTGTGCCGTATTGCGCTGTGGTACGGCGTA
AACGCGACACGACCGGAGGCAATCGGTGTTGGCCTGCCTGGTCAGACTGCGTAATAG

Amino Acid Sequences

NP

MALEASQGTKRSYEQMETDGERQNATEIRASVGKMIGGIGRFYIQMCTELKLSDYEGRL
IQNSLTIERMVLSAFDERRNKYLEEHPSAGKDPKKTGGPIYRRVNGKWMRELILYDKEE
IRRIWRQANNGDDATAGLTHMMIWHSNLNDATYQRTRALVRTGMDPRMCSLMQGSTLPR
RSGAAGAAVKGVGTMVMELVRMIKRGINDRNFWRGENGRKTRIAYERMCNILKGKFQTA
AQKAMMDQVRESRNPGNAEFEDLTFLARSALILRGSVAHKSCLPACVYGPAVASGYDFE
REGYSLVGIDPFRLLQNSQVYSLIRPNENPAHKSQLVWMACHSAAFEDLRVLSFIKGTK
VLPRGKLSTRGVQIASNENMETMESSTLELRSRYWAIRTRSGGNTNQQRASAGQISIQP
TFSVQRNLPFDRTTIMAAFNGNTEGRTSDMRTEIIRMMESARPEDVSFQGRGVFELSDE
KAASPIVPSFDMSNEGSYFFGDNAEEYDNVD

NP$_{163}$-SP

MALEASQGTKRSYEQMETDGERQNATEIRASVGKMIGGIGRFYIQMCTELKLSDYEGRL
IQNSLTIERMVLSAFDERRNKYLEEHPSAGKDPKKTGGPIYRRVNGKWMRELILYDKEE
IRRIWRQANNGDDATAGLTHMMIWHSNLNDATYQRTRALVRTGMDPLVPRGSCRSNAVA
EQGRKTQEFTQQSAQYVEAARKHYDAAEKLNIPDYQEKEDAFMQLVPPAVGADIMRLFP
EKSAALMYHLGANPEKARQLLAMDGQSALIELTRLSERLTLKPRGKQISSAPHADQPIT
GDVSAANKDAIRKQMDAAASKGDVETYRKLKAKLKGIR

NP-SP

MALEASQGTKRSYEQMETDGERQNATEIRASVGKMIGGIGRFYIQMCTELKLSDYEGRL
IQNSLTIERMVLSAFDERRNKYLEEHPSAGKDPKKTGGPIYRRVNGKWMRELILYDKEE
IRRIWRQANNGDDATAGLTHMMIWHSNLNDATYQRTRALVRTGMDPRMCSLMQGSTLPR
RSGAAGAAVKGVGTMVMELVRMIKRGINDRNFWRGENGRKTRIAYERMCNILKGKFQTA
AQKAMMDQVRESRNPGNAEFEDLTFLARSALILRGSVAHKSCLPACVYGPAVASGYDFE
REGYSLVGIDPFRLLQNSQVYSLIRPNENPAHKSQLVWMACHSAAFEDLRVLSFIKGTK
VLPRGKLSTRGVQIASNENMETMESSTLELRSRYWAIRTRSGGNTNQQRASAGQISIQP
TFSVQRNLPFDRTTIMAAFNGNTEGRTSDMRTEIIRMMESARPEDVSFQGRGVFELSDE
KAASPIVPSFDMSNEGSYFFGDNAEEYDNVDGSLVPRGSCRSNAVAEQGRKTQEFTQQS
AQYVEAARKHYDAAEKLNIPDYQEKEDAFMQLVPPAVGADIMRLFPEKSAALMYHLGAN
PEKARQLLAMDGQSALIELTRLSERLTLKPRGKQISSAPHADQPITGDVSAANKDAIRK
QMDAAASKGDVETYRKLKAKLKGIR

P22 Coat Protein

MALNEGQIVTLAVDEIIETISAITPMAQKAKKYTPPAASMQRSSNTIWMPVEQESPTQE
GWDLTDKATGLLELNVAVNMGEPDNDFFQLRADDLRDETAYRRRIQSAARKLANNVELK
VANMAAEMGSLVITSPDAIGTNTADAWNFVADAEEIMFSRELNRDMGTSYFFNPQDYKK
AGYDLTKRDIFGRIPEEAYRDGTIQRQVAGFDDVLRSPKLPVLTKSTATGITVSGAQSF
KPVAWQLDNDGNKVNVDNRFATVTLSATTGMKRGDKISFAGVKFLGQMAKNVLAQDATF
SVVRVVDGTHVEITPKPVALDDVSLSPEQRAYANVNTSLADAMAVNILNVKDARTNVFW
ADDAIRIVSQPIPANHELFAGMKTTSFSIPDVGLNGIFATQGDISTLSGLCRIALWYGV
NATRPEAIGVGLPGQTA

NP Sequence Homology Alignment

```
....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                 5         15         25         35         45         55
H3N2         MASQGTKRSY EQMETDGERQ NATEIRASVG KMIGGIGRFY IQMCTELKLS DDYEGRLIQN
H1N1         MASQGTKRSY EQMETDGERQ NATEIRASVG KMIGGIGRFY IQMCTELKLS DDYEGRLIQN
H5N1         MASQGTKRSY EQMETDGERQ NATEIRASVG KMIGGIGRFY IQMCTELKLS DDYEGRLIQN
H2N2         MASQGTKRSY EQMETDGERQ NATEIRASVG KMIDGIGRFY IQMCTELKLS DDYEGRLIQN
H1N2         MASQGTKRSY EQMETDGERQ NATEIRASVG KMIDGIGRFY IQMCTELKLS DDYEGRLIQN
H5N2         MASQGTKRSY EQMETGGERQ NATEIRASVG RMVGGIGRFY IQMCTELKLS DDYEGRLIQN

             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                65         75         85         95        105        115
H3N2         NLTIERMVLS AFDERRNKYL EEHPSAGKDP KKTGGPIYRR VNGKWMRELI LYDKEEIRRI
H1N1         SLTTERMVLS AFDERRNKYL EEHPSAGKDP KKTGGPIYRR VNGKWMRELI LYDKEEIRRI
H5N1         SLTIERMVLS AFDERRNKYL EEHPSAGKDP KKTGGPIYRR VNGKWMRELI LYDKEEIRRI
H2N2         SLTIERMVLS AFDERRNKYL EEHPSAGKDP KKTGGPIYKR VNGKWMRELV LYDKEEIRRI
H1N2         SLTIERMVLS AFDERRNKYL EEHPSAGKDP KKTGGPIYKR VGGKWMRELV LYDKEEIRRI
H5N2         SITIERMVLS AFDERRNKYL EEHPSAGKDP KKTGGPIYRR RDGKWMRELI LYDKEEIRRI

             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
               125        135        145        155        165        175
H3N2         WRQANNGDDA TAGLTHMMIW HSNLNDATYQ RTRALVRTGM DPRMCSLMQG STLPRRSGAA
H1N1         WRQANNGDDA TAGLTHMMIW HSNLNDATYQ RTRALVRTGM DPRMCSLMQG STLPRRSGAA
H5N1         WRQANNGDDA TAGLTHMMIW HSNLNDATYQ RTRALVRTGM DPRMCSLMQG STLPRRSGAA
H2N2         WRQANNGDDA TAGLTHMMIW HSNLNDTTYQ RTRALVRTGM DPRMCSLMQG STLPRRSGAA
H1N2         WRQANNGDDA TAGLTHMMIW HSNLNDTTYQ RTRALVRTGM DPRMCSLMQG STLPRRSGAA
H5N2         WRQANNGEDA TAGLTHMMIW HSNLNDATYQ RTRALVRTGM DPRMCSLMQG STLPRRSGAA

             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
               185        195        205        215        225        235
H3N2         GAAVKGVGTM VMELVRMIKR GINDRNFWRG ENGRKTRIAY ERMCNILKGK FQTAAQKAMM
H1N1         GAAVKGVGTM VMELVRMIKR GINDRNFWRG ENGRKTRIAY ERMCNILKGK FQTAAQKAMM
H5N1         GAAVKGVGTM VMELVRMIKR GINDRNFWRG ENGRKTRIAY ERMCNILKGK FQTAAQKAMM
H2N2         GAAVKGVGTM VMELIRMIKR GINDRNFWRG ENGRKTRIAY ERMCNILKGK FQTAAQRAMM
H1N2         GAAVKGVGTM VMELIRMIKR GINDRNFWRG ENGRKTRIAY ERMCNILKGK FQTAAQRAMM
H5N2         GAAVKGVGTM VMELIRMIKR GINDRNFWRG ENGRRTRIAY ERMCNILKGK FQTAAQRAMM

             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
               245        255        265        275        285        295
H3N2         DQVRESRNPG NAEFEDLTFL ARSALILRGS VAHKSCLPAC VYGPAVASGY DFEREGYSLV
H1N1         DQVRESRNPG NAEFEDLTFL ARSALILRGS VAHKSCLPAC VYGPAVASGY DFEREGYSLV
H5N1         DQVRESRNPG NAEFEDLTFL ARSALILRGS VAHKSCLPAC VYGPAVASGY DFEREGYSLV
H2N2         DQVRESRNPG NAEIEDLIFL ARSALILRGS VAHKSCLPAC VYGPAVASGY DFEKEGYSLV
H1N2         DQVRESRNPG NAEIEDLIFL ARSALILRGS VAHKSCLPAC VYGPAVASGY DFEKEGYSLV
H5N2         DQVRESRNPG NAEIEDLTFL ARSALILRGS VAHKSCLPAC VYGLAVASGY DFEREGYSLV

             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
               305        315        325        335        345        355
H3N2         GIDPFRLLQN SQVYSLIRPN ENPAHKSQLV WMACHSAAFE DLRVLSFIKG TKVLPRGKLS
H1N1         GIDPFRLLQN SQVYSLIRPN ENPAHKSQLV WMACHSAAFE DLRVLSFIKG TKVLPRGKLS
H5N1         GIDPFRLLQN SQVYSLIRPN ENPAHKSQLV WMACHSAAFE DLRVLSFIKG TKVVPRGKLS
H2N2         GIDPFKLLQN SQVYSLIRPN ENPAHKSQLV WMACNSAAFE DLRVSSFIRG TKVIPRGKLS
H1N2         GIDPFKLLQN SQVYSLIRPN ENPAHKSQLV WMACNSAAFE DLRVSSFIRG TKVIPRGKLS
H5N2         GIDPFRLLQN SQVFSLIRPN ENPAHKSQLV WMACHSAAFE DLRVSSFIRG TRVVPRGQLS

             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
               365        375        385        395        405        415
H3N2         TRGVQIASNE NMETMESSTL ELRSRYWAIR TRSGGNTNQQ RASAGQISIQ PTFSVQRNLP
H1N1         TRGVQIASNE NMETMESSTL ELRSRYWAIR TRSGGNTNQQ RASAGQISIQ PTFSVQRNLP
H5N1         TRGVQIASNE NMETMESSTL ELRSRYWAIR TRSGGNTNQQ RASAGQISIQ PTFSVQRNLP
H2N2         TRGVQIASNV NMDTMESSTL ELRSRYWAIR TRSGGNTNQQ RASAGQISVQ PTFSVQRNLP
H1N2         TRGVQIASNE NMDTMESSTL ELRSRYWAIR TRSGGNTNQQ RASAGQISVQ PTFSVQRNLP
H5N2         TRGVQIASNE NMETMDSSTL ELRSRYWAIR TRSGGNTNQQ RASAGQISVQ PTFSVQRNLP

             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
               425        435        445        455        465        475
H3N2         FDRTTIMAAF NGNTEGRTSD MRTEIIRMME SARPEDVSFQ GRGVFELSDE KAASPIVPSF
H1N1         FDRTTIMAAF NGNTEGRTSD MRTEIIRMME SARPEDVSFQ GRGVFELSDE KAASPIVPSF
H5N1         FDRTTVMAAF TGNTEGRTSD MRTEIIRMME SARPEDVSFQ GRGVFELSDE KAASPIVPSF
H2N2         FDKTTIMAAF TGNVEGRTSD MRAEIIRMME GAKPEEVSFQ GRGVFELSDE KATNPIVPSF
H1N2         FDKPTIMAAF TGNAEGRTSD MRAEIIRMME GAKPEEVSFQ GRGVFELSDE KATNPIVPSF
H5N2         FERATIMAAF TGNTEGRTSD MRTEIIRMME NARPEDVSFQ GRGVFELSDE KATNPIVPSF

             ....|....| ....|....
               485        495
H3N2         DMSNEGSYFF GDNAEEYDN
H1N1         DMSNEGSYFF GDNAEEYDN
H5N1         DMSNEGSYFF GDNAEEY--
H2N2         DMSNEGSYFF GDNAEEYDN
H1N2         DMSNEGSYFF GDNAEEYDN
H5N2         DMSNEGSYFF GDNAEEYDN
```

Purification by Linear Cesium Chloride Gradient

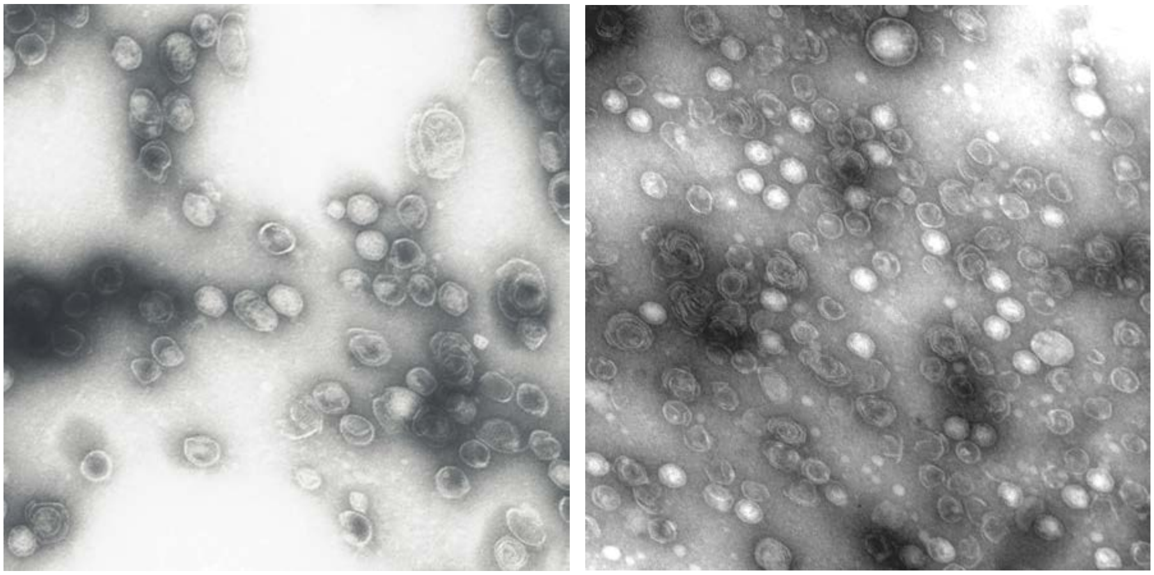


Figure S1. TEM images of initial purifications of NP_{163}-P22 (left) and NP-P22 (right) using ultracentrifugation through a sucrose cushion. Many empty and misformed structures are observed in addition to correctly formed, filled P22 VLPs.

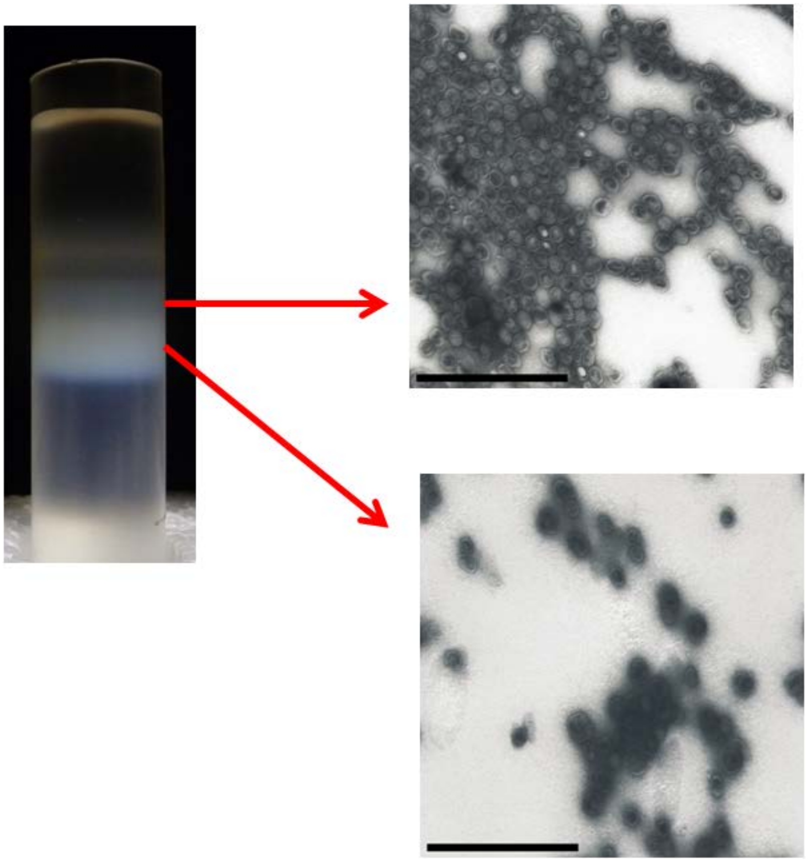


Figure S2. Purification of NP_{163}-P22 by cesium chloride gradient ultracentrifugation. The figure shows the separation of distinct bands on a linear cesium chloride gradient (left) and analysis of the band fractions by TEM. The upper band showed homogeneous particles with expected diameters for the P22 VLP and dense packing on the interior (right top image), whereas the lower band showed misformed aggregated structures with little or no density on the interior of the particles. Scale bars are 1 μm in both images.

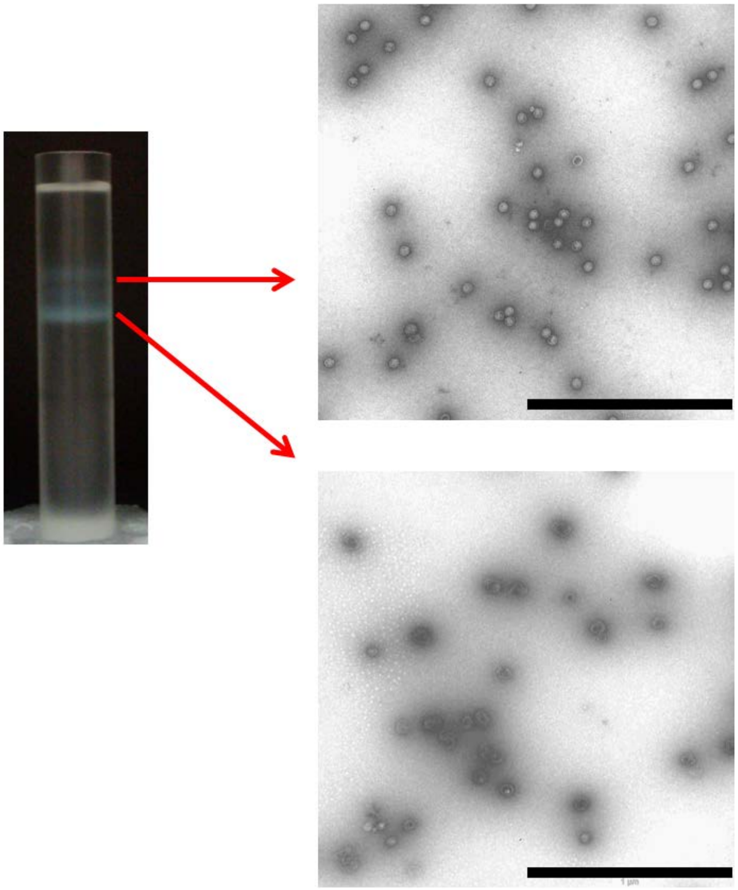
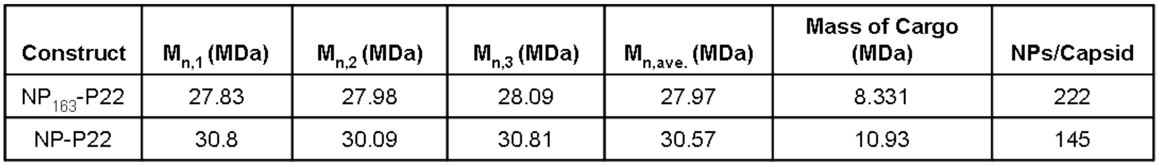


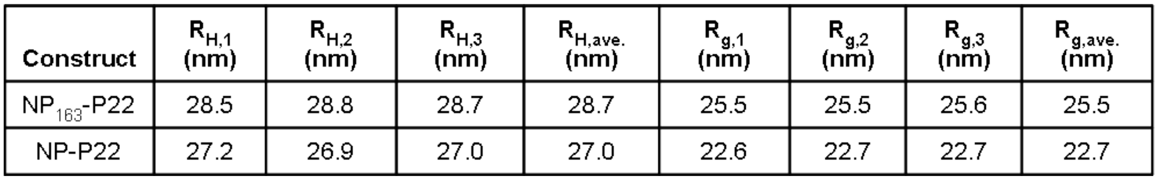
Figure S3. Purification of NP-P22 by cesium chloride gradient ultracentrifugation. The figure shows the separation of distinct bands on a linear cesium chloride gradient (left) and analysis of the band fractions by TEM. The upper band showed homogeneous particles with expected diameters for the P22 VLP and dense packing on the interior (right top image), whereas the lower band showed misformed aggregated structures with little or no density on the interior of the particles. Scale bars are 1 μm in both images.

Characterization by size exclusion chromatography (MALS/QELS/RI detection)



Construct	$M_{n,1}$ (MDa)	$M_{n,2}$ (MDa)	$M_{n,3}$ (MDa)	$M_{n,ave.}$ (MDa)	Mass of Cargo (MDa)	NPs/Capsid
NP_{163}-P22	27.83	27.98	28.09	27.97	8.331	222
NP-P22	30.8	30.09	30.81	30.57	10.93	145

Table S1. Size exclusion chromatography molar mass values for NP-P22 constructs run in triplicate and corresponding capsid NP (either NP_{163} or NP) loading values.



Construct	$R_{H,1}$ (nm)	$R_{H,2}$ (nm)	$R_{H,3}$ (nm)	$R_{H,ave.}$ (nm)	$R_{g,1}$ (nm)	$R_{g,2}$ (nm)	$R_{g,3}$ (nm)	$R_{g,ave.}$ (nm)
NP_{163}-P22	28.5	28.8	28.7	28.7	25.5	25.5	25.6	25.5
NP-P22	27.2	26.9	27.0	27.0	22.6	22.7	22.7	22.7

Table S2. Size exclusion chromatography MALS and QELS detected radii for NP-P22 constructs run in triplicate.

In vivo evaluation of NP-P22

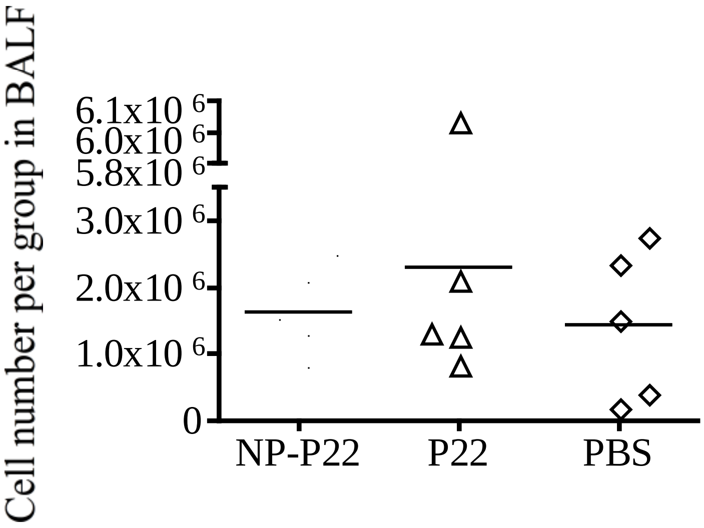


Figure S4. Plot showing the total number of cells extracted from BALF and used for flow cytometry.

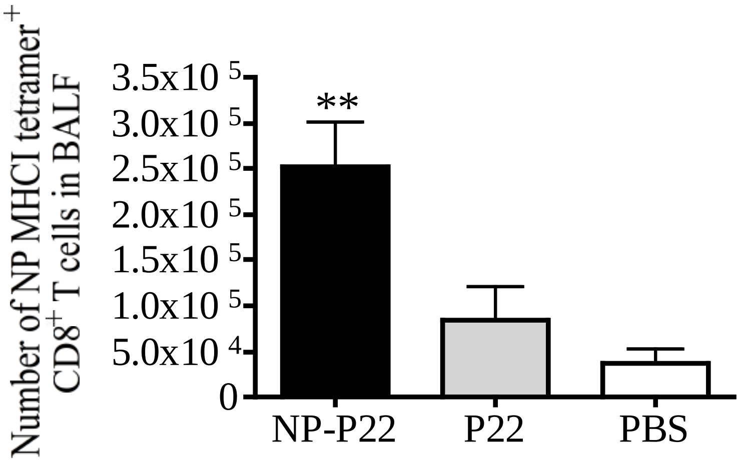


Figure S5. Quantification of NP specific CD8$^+$ T cells from BALF from mice. Plot comparing the total number of CD8$^+$ T cells from mice receiving different immunization treatments.