# Supplementary Information:

# Simplified Purification of Glycoprotein-modified Ferritin Nanoparticles for Vaccine Development

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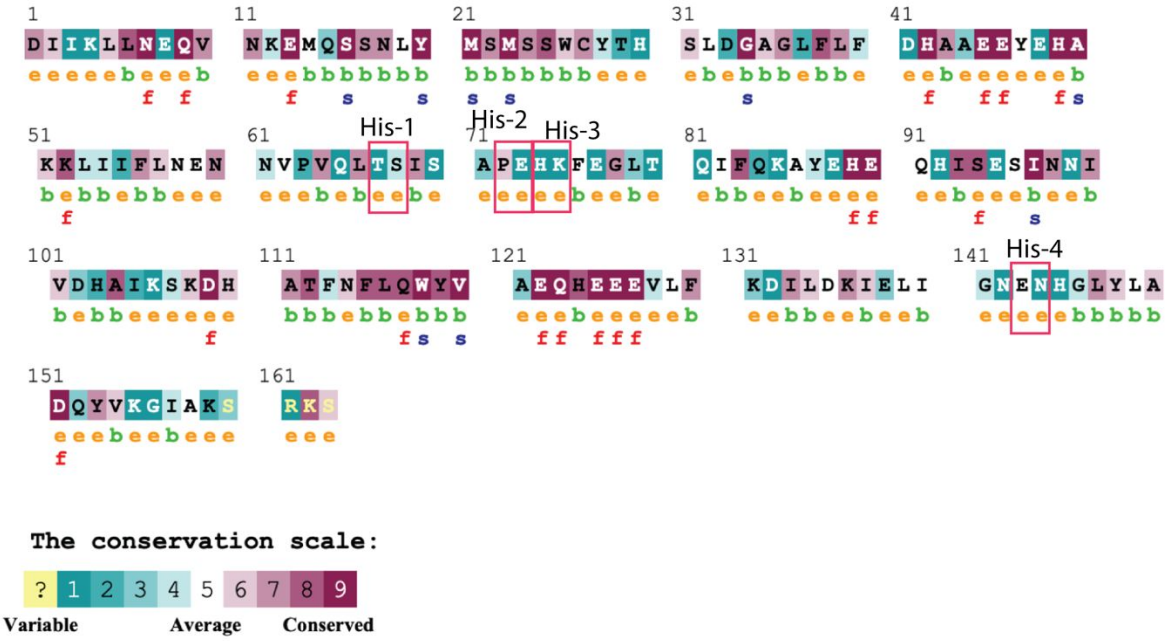
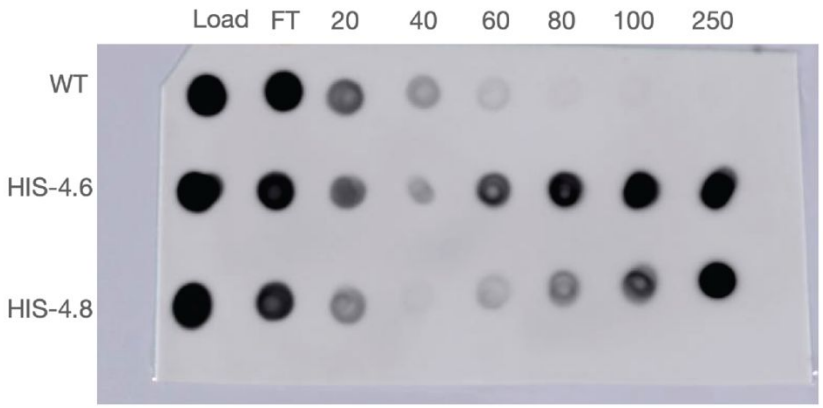
Supplemental Information

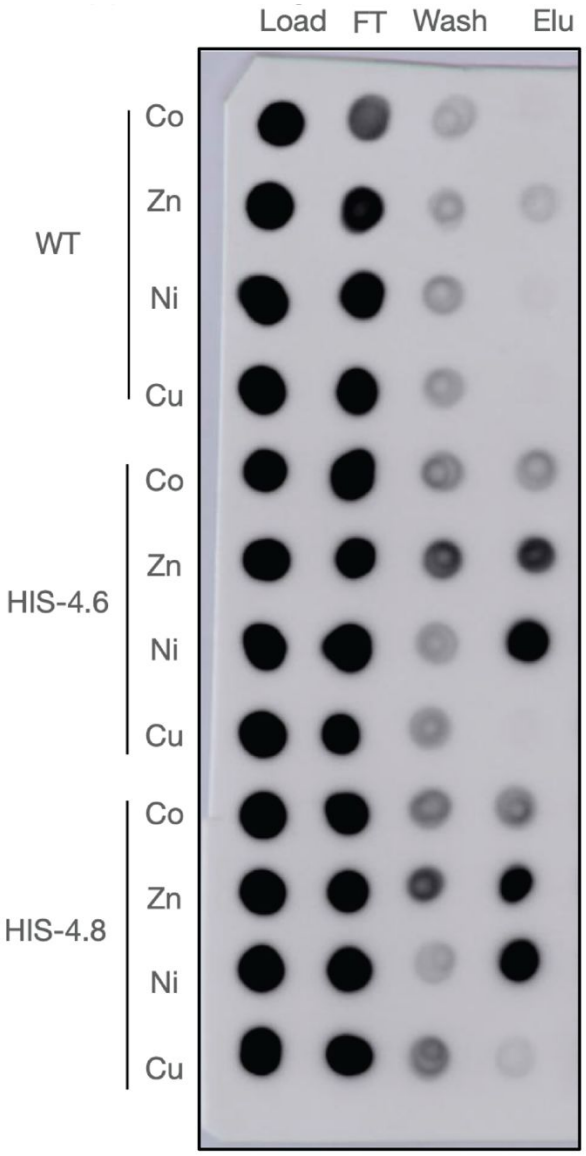
| Supplemental Table 1 | | |
|---|---|---|
| Plasmid description | Plasmid name (HA – based on accession ID KF356052) | Plasmid name (spike – based on accession ID QJE39038) |
| His-1 – GGSHHHHHHGGS was inserted into *H. Pylori* ferritin sequence between pos. 71 and 72 (QLT–SIS). N-terminus of ferritin starts at position 5 (DIIKL…) as previously described[1]. | HA-His-1-Fer | spike-His-1-Fer |
| His-2 – As with His-1 except the insertion is between pos. 77 and 78 (APE–HKF). | HA-His-2-Fer | spike-His-2-Fer |
| His-3 – As with His-1 except the insertion is between pos. 78 and 79 (PEH–KFE). | HA-His-3-Fer | spike-His-3-Fer |
| His-4 – As with His-1 except the insertion is between pos. 147 and 148 (GNE–NHG). | HA-His-4-Fer Also HA-His-4.6-Fer | spike-His-4-Fer Also spike-His-4.6-Fer |
| His-4.4 – As with His-4 except the insertion is GGSHHHHGGS | HA-His-4.4-Fer | HA-His-4.4-Fer |
| His-4.5 – As with His-4 except the insertion is GGSHHHHHGGS | HA-His-4.5-Fer | spike-His-4.5-Fer |
| His-4.7 – As with His-4 except the insertion is GGSHHHHHHHGGS | HA-His-4.7-Fer | spike-His-4.7-Fer |
| His-4.8 – As with His-4 except the insertion is GGSHHHHHHHHGGS | HA-His-4.8-Fer | spike-His-4.8-Fer |



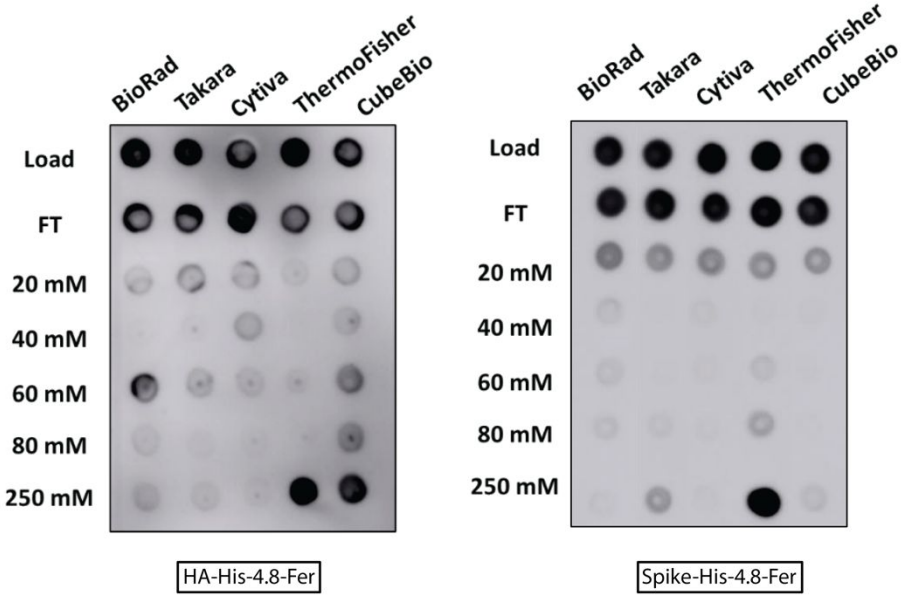

Supplementary Figure 1 – A conservation analysis of *H. pylori* ferritin generated using the ConSurf server with related counterparts identifies residues that are highly variable. Boxes indicate sites between which the polyhistidine insertions were made. Selections were made based on the surface exposure, flexibility, conservation, and computational modeling (Figure 1). Exposed (e), buried (b), predicted functional (f), predicted structurally important (s).



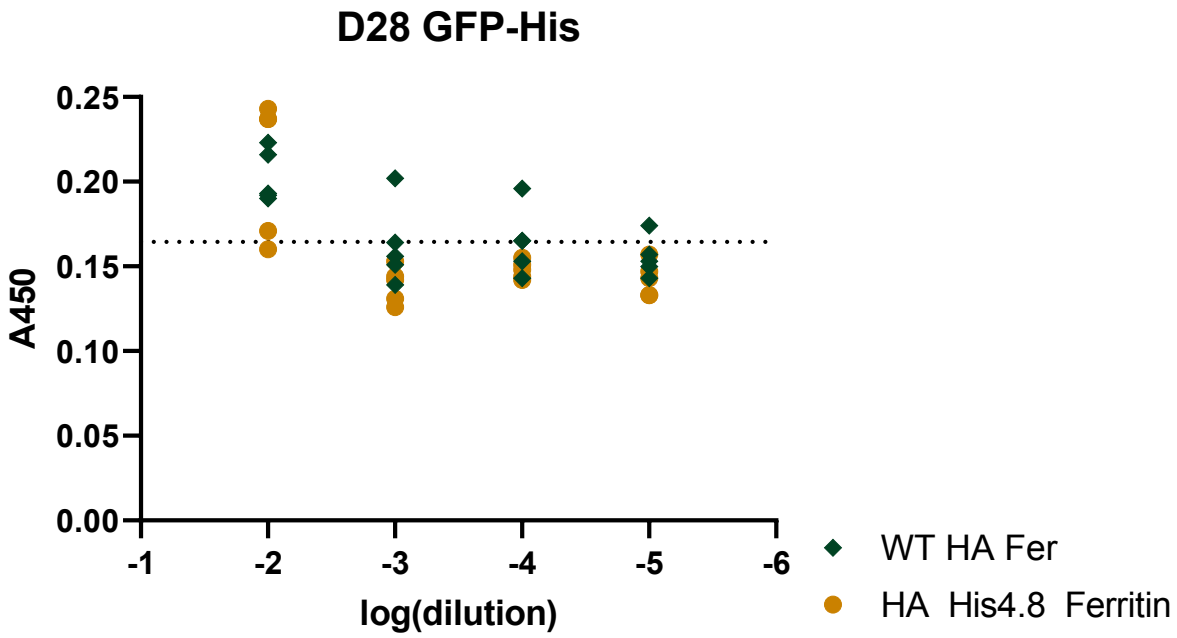

Supplementary Figure 2 – A dot blot demonstrating that an octahistidine insertion shows superior wash stability to a hexahistidine insertion for spike-His-Fer. Columns demonstrate the Load, flow through (FT), wash with 20mM, 40mM, 60mM, 80mM, 100mM, or elution with 250mM imidazole. Less protein dissociated from the column during the higher wash concentrations for the spike-His4.8-Fer (row 3) compared to spike-His4.6-Fer (row 2).




Supplementary Figure 3 – A dot blot demonstrating that $Ni^{2+}$-coordinated resin facilitated optimal purification compared to other divalent cations. Columns demonstrate the Load, flow through (FT), wash (Wash), and eluate (Elu) from cube-bio resins containing either $Co^{2+}$, $Zn^{2+}$, $Ni^{2+}$, or $Cu^{2+}$. Purifications were conducted with either the WT-spike-Fer (showing no elution) or His-4.6 or His-4.8. $Ni^{2+}$-coordinated resin facilitated maximal purification.




Supplementary Figure 4 – A dot blot demonstrating that ThermoFisher His-Pur resin was the optimal $Ni^{2+}$-coordinated resin among competitors. Columns show purifications from $Ni^{2+}$-coordinated resins from a range of vendors. Rows depict the Load, flow through (FT), wash with 20mM, 40mM, 60mM, 80mM imidazole, and elution (250nM). HA-His-4.8-Fer is shown in the left panel and spike-His4.8-Fer is shown in the right panel. Both nanoparticles were purified best using the ThermoFisher resin. Some resins could not accommodate nanoparticle purification.




Supplementary Figure 5 – ELISA binding against His-tagged sfGFP protein for serum from mice immunized with WT-HA-Fer (green) and HA-His-4.8-Fer (yellow) on day 28. Dotted line indicates the average signal from preimmunization serum from all animals (negative control). Incubation with mouse IgG1 anti-His Ab served as the positive control and resulted in an A450 signal of 2.6 (not shown). The binding results demonstrate that the two immune responses were similar, and that the HA-His-4.8-Ferritin protein does not elicit a significant anti-His-Tag response.

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P.furiosus      --GLSERMLKALNDQLNRELYSAYLYFAMAAYFEDLGLEGFANWMKAQAEEEIGHALRFY   58
A.fulgidus      MASISEKMVEALNRQINAEIYSAYLYLSMASYFDSIGLKGFSNWMRVQWQEELMHAMKMF   60
H.pylori        ------DIIKLLNEQVNKEMQSSNLYMSMSSWCYTHSLDGAGLFLFDHAAEEYEHAKKLI   54
E.coli          --MLKPEMIEKLNEQMNLELYSSLLYQQMSAWCSYHTFEGAAAFLRRHAQEEMTHMQRLF   58
                       ::: ** *:* *: *: **  *:::     :.* . ::  :  **  *  ::
                      His-1 His-2 His-3
P.furiosus      NYIYDKNGRVELDEIPKPPKEWESPLKAFEAAYEHEKFISKSIYELAALAEEEKDYSTRA   118
A.fulgidus      DFVSERGGRVKLYAVEEPPSEWDSPLAAFEHVYEHEVNVTKRIHELVEMAMQEKDFATYN   120
H.pylori        IFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKSKDHATFN   114
E.coli          DYLTDTGNLPRINTVESPFAEYSSLDELFQETYKHEQLITQKINELAHAAMTNQDYPTFN   118
                 :: : .   .:  :  *  ::..    *: .*:**  ::: * ::.  *  .:*. *
                                  His-4
P.furiosus      FLEWFINEQVEEEASVKKILDKLKFAKDSPQILFMLDKELSARAPKLPGLLMQGGE       174
A.fulgidus      FLQWYVAEQVEEEASALDIVEKLRLIGEDAAALLFLDKELSLRQFTPPAEEEK---       173
H.pylori        FLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS-------       163
E.coli          FLQWYVSEQHEEEKLFKSIIDKLSLAGKSGEGLYFIDKELSTLDTQN---------       165
                **:*:: ** ***    .*:::*: :  ..   * : *: :.
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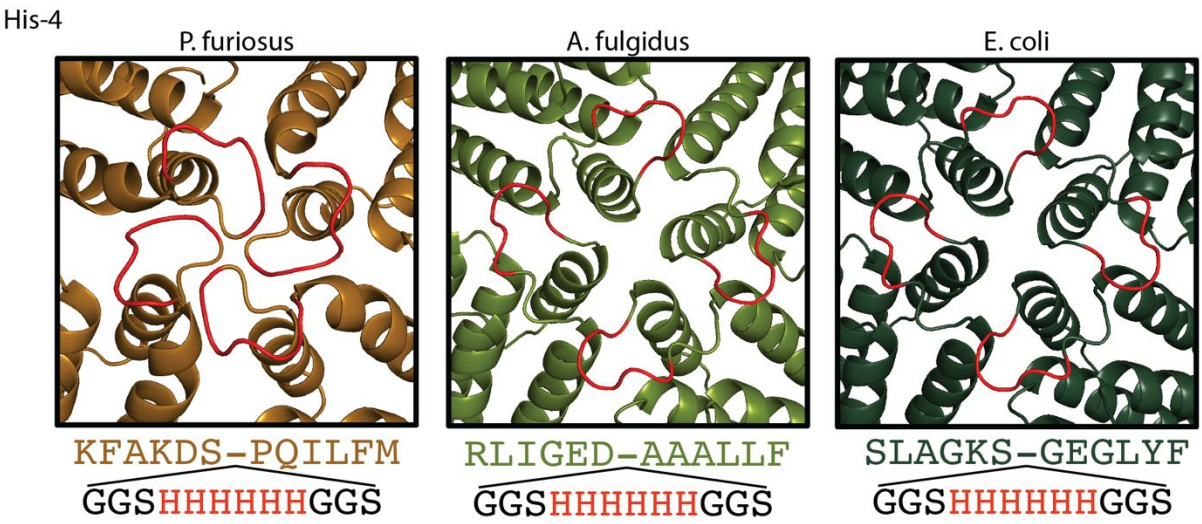


Supplementary Figure 6 – Sequence alignment of ferritin from other bacteria identifies His.4 as a homologous potential insertion site in other ferritins. (Top) A sequence alignment produced using Clustal Omega[2] for ferritin from P. furiosus, A. fulgidus, E. Coli. and H. pylori. H. pylori starts at position 5, the position of the glycoprotein fusion. (Bottom) The ferritin monomers for each of the bacterial ferritins was modeled using using RosettaRemodel (PDB ID: P. furiosus – 2JD6, A. fulgidus – 3KX9, E. Coli – 1EUM). Polyhistidine insertions of length 6 (6xHis) were made at each of the sequence-derived loop sites including the three flanking residues on either side to facilitate a mobile backbone. Ten structures were generated for each site, the mutated monomers were then multimerized and relaxed in Rosetta. Modeling demonstrates site His-4 has high homology between bacterial ferritins and may work for installation into other bacterial ferritins.

*Sequences*

**spike-Ferritin protein**

MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTW
FHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKV
CEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREF
VFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGW
TAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQP
TESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKL
NDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYN
YLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVL
SFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVR
DPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSN
VFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPGSASSVASQSIIAYTMSLGAENSVA
YSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAV
EQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQY
GDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQM
AYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQL
SSNFGAISSVLNDILSRLDPPEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSEC
VLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGV
FVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSGGDIIKLLNEQV
NKEMQSSNLYMSMSSWCYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEH
KFEGLTQIFQKAYEHEQHISESINNIVDHAIKSKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIG
NENHGLYLADQYVKGIAKSRKS

**HA-Ferritin protein**

MYRMQLLSCIALSLALVTNSDTICIGYHANNSTDTVDTVLEKNVTVTHSVNLLEDSHNGKLCLLK
GIAPLQLGNCSVAGWILGNPECELLISKESWSYIVETPNPENGTCFPGYFADYEELREQLSSVS
SFERFEIFPKESSWPNHTVTGVSASCSHNGKSSFYRNLLWLTGKNGLYPNLSKSYVNNKEKEV
LVLWGVHHPPNIGNQRALYHTENAYVSVVSSHYSRRFTPEIAKRPKVRDQEGRINYYWTLLEP
GDTIIFEANGNLIAPWYAFALSRGFGSGIITSNAPMDECDAKCQTPQGAINSSLPFQNVHPVTIG
ECPKYVRSAKLRMVTGLRNIPQRETGGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQGSGYA
ADQKSTQNAINGITNKVNSVIEKMNTQFTAVGKEFNKLERRMENLNKKVDDGFLDIWTYNAELL
VLLENERTLDFHDSNVKNLYEKVKSQLKNNAKEIGNGCFEFYHKCNNECMESVKNGTYDYPKY

SEESKLNREKIDGSSGGDIIKLLNEQVNKEMQSSNLYMSMSSWCYTHSLDGAGLFLFDHAAEE
YEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKSKDHATF
NFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS

**Site 1 His-Ferritin**

DIIKLLNEQVNKEMQSSNLYMSMSSWCYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPV
QLT**GGSHHHHHHGGS**SISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKSKDHATFNFLQ
WYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS

**Site 2 His-Ferritin**

DIIKLLNEQVNKEMQSSNLYMSMSSWCYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPV
QLTSISAPE**GGSHHHHHHGGS**HKFEGLTQIFQKAYEHEQHISESINNIVDHAIKSKDHATFNFLQ
WYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS

**Site 3 His-Ferritin**

DIIKLLNEQVNKEMQSSNLYMSMSSWCYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPV
QLTSISAPEH**GGSHHHHHHGGS**KFEGLTQIFQKAYEHEQHISESINNIVDHAIKSKDHATFNFLQ
WYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS

**Site 4 His-Ferritin**

DIIKLLNEQVNKEMQSSNLYMSMSSWCYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPV
QLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKSKDHATFNFLQWYVAEQHEEEVLF
KDILDKIELIGNE**GGSHHHHHHGGS**NHGLYLADQYVKGIAKSRKS

References


1. Powell, A. E. *et al.* A single immunization with spike-functionalized ferritin vaccines elicits neutralizing antibody responses against SARS-CoV-2 in mice. *ACS Cent. Sci.* **7**, 183–199 (2021).
2. Sievers, F. *et al.* Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.* **7**, 539 (2011).