Conjugation to nickel-chelating nanolipoprotein particles increases the potency and efficacy of subunit vaccines to prevent West Nile encephalitis

Nicholas O. Fischer,[†] Ernesto Infante,[‡] Tomohiro Ishikawa,[‡,‖] Craig D. Blanchette,[†] Nigel Bourne,[‡] Paul D. Hoeprich,[*,†] and Peter W. Mason[*,‡,§,⊥]

[†] Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, California 94551, [‡] Department of Pathology and § Microbiology & Immunology, University of Texas Medical Branch, Galveston, Texas 77555
[‖] Current address: Department of International Health, Kobe University Graduate School of Health Sciences, Kobe, 654-0142 Japan
[⊥] Current address: Novartis Vaccines and Diagnostics, Cambridge, Massachusetts 02139

E-mail: hoeprich2@llnl.gov, peter.mason@novartis.com

Supporting Information

Experimental Details

Materials

1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and the nickel salt of 1,2-dioleoyl-sn-glycero-3-{[N(5-amino-1-carboxypentyl)iminodiacetic acid] succinyl} (DOGS-NTA-Ni) were purchased from Avanti Polar Lipids (Alabaster, AL). All other reagents were ordered from Sigma-Aldrich (St. Louis, MO). The scaffold protein apoE422K was expressed and purified as previously reported (*1*).

Viruses

The snowy owl isolate of WNV NY99 (*2*) (R.B. Tesh, UTMB) was used for all animal studies. Mice were challenged with 1000 ffu of virus (corresponding to 10 LD_{50} in 8-week-old mice).

Preparation of truncated E (trE-His) protein

WNV E antigen was produced by a cell line expressing a persistently replicating Venezuelan equine encephalitis virus replicon (VEErep/WNprMtrE/Pac) encoding WNV prM followed by the first 421 codons of the E gene (80 % of full-length E) fused to a 6X histidine tag (*3*). Supernatant harvested from cells grown in serum free media was used for ELISA assays (see below). For vaccine preparations, trE-His was incubated with Ni-NTA agarose resin (Invitrogen, Carlsbad, CA) in HBS (20mM HEPES, 150mM NaCl, pH 7.4) containing 5 mM imidazole for 1 hour at 4°C. Resin was transferred to a disposable column, washed with 8 column volumes HBS with 5 mM imidazole, and the trE-His was eluted with 2.5 column volumes

of HBS with 100 mM imidazole. The trE-His elution fractions were assessed by SDS-PAGE, and those fractions containing trE-His were pooled. Purified trE-His was then dialyzed overnight at 4°C against HBS and concentrated using a 5 kDa molecular weight cut-off (MWCO) Vivaspin centrifugal concentrator (Sartorius Stedim, Aubagne, France). The trE-His was quantified relative to an ovalbumin standard by densitometry of a SDS-PAGE gel following SyproRuby (BioRad, Hercules, CA) staining. Gels were imaged using the Typhoon 9410 Variable Mode Imager (GE Healthcare, Piscataway, NJ) and analyzed using ImageQuant software package (GE Healthcare).

Preparation of NiNLPs

NiNLPs were assembled as previously described (*4*). Briefly, a mixture of DMPC and DOGS-NTA-Ni (9:1 molar ratio) in chloroform was aliquoted into a glass vial. Using a stream of N_2, chloroform was removed to form a thin lipid film on the inside of the glass vial. Lipids were further dried in vacuo for at least 2 hours. Lipids were subsequently solubilized in TBS buffer (10 mM Tris, 150 mM NaCl, pH 7.4) using 20 mM sodium cholate. The apoE422K scaffold protein was added and the mixture incubated at room temperature for at least 4 hours prior to an overnight dialysis against TBS buffer to remove cholate. Assemblies were run on SEC (Superdex 200, 10/300 GL column, GE Healthcare) in TBS buffer (0.5 mL/min flow rate, λ=280 nm). Fractions corresponding to NiNLPs were collected (Supporting Figure 1A). These fractions were run on 4-20 % Tris-glycine polyacrylamide gels (Invitrogen) to determine purity. All gels were stained with SyproRuby (BioRad) and imaged using the Typhoon 9410 Variable Mode Imager (GE Healthcare). NiNLP SEC fractions with a single dominant band were then pooled (Supporting Figure 1A). Purified NiNLPs were then dialyzed against HBS and stored at 4°C until needed.

Immobilization of trE-His on NiNLPs

To immobilize trE-His on NiNLPs, NiNLPs were incubated with trE-His (1:2 molar ratio) at room temperature for 45 minutes. A portion from each sample was subsequently filtered through a 100 kDa MWCO Microcon spin concentrator (Millipore, Burlington, MA) and washed three times with 200 µL HBS. For control experiments, NiNLPs were pre-incubated in buffer containing 4 mM ethylenediaminetetraacetic acid (EDTA) for 45 minutes at room temperature. For these samples, the wash buffer also contained 4mM EDTA. Total and retentate samples were then analyzed by denaturing gel electrophoresis on 16 % Tris-tricine polyacrylamide gels (Invitrogen). Gels were stained and imaged as indicated above. ImageQuant (GE Healthcare) was used for densitometry measurements. Previously published experimental and modeling data were used to extrapolate NiNLP concentration assuming that NiNLPs contain four apoE422K lipoproteins per NiNLP (*5*).

Atomic Force Microscopy

AFM was used to measure NiNLP dimensions and to verify immobilization of trE-His on the NiNLPs. AFM analysis of NiNLPs was conducted as previously described (*4, 5*).

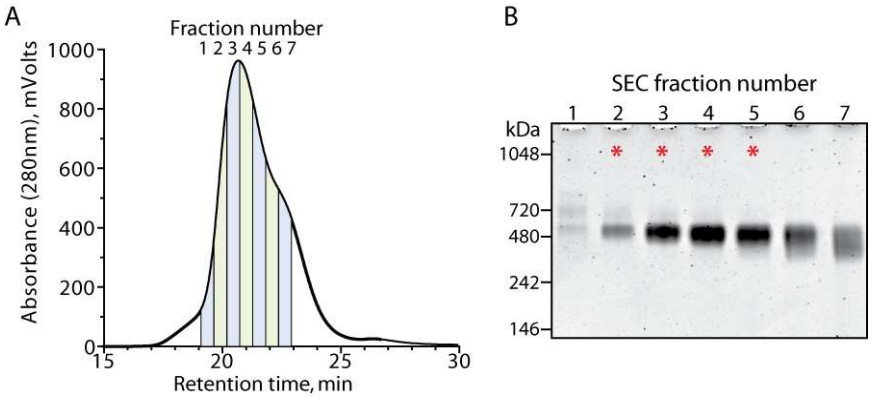
NiNLP-trE-His animal studies

In the pilot experiment, groups of five 6-week-old female Swiss Webster mice (Harlan Sprague Dawley, Indianapolis, IN) were immunized intraperitoneally (i.p.) with NiNLP alone, trE-His alone, NiNLP-trE-His, or diluent alone (mock). Antigen-containing inocula consisted of 2.5 µg trE-His. All inocula were delivered in 100 µl of L-15 medium containing 10 mM HEPES and 0.5 % FBS. Animals were monitored for vaccine-induced side effects including lethargy and hind-limb paralysis. At 21 days post-vaccination serum was collected from all of the animals by retro-orbital bleed. Fourteen days later animals were challenged i.p. with 10 LD_{50} of WNV NY99 and monitored for changes in weight and health for 21 days. Animals scored moribund were humanely euthanized in compliance with UTMB Animal Care and Use requirements and scored as "dead" the following day. In the second experiment, groups of ten 5-week-old female Swiss Webster mice were immunized i.p. with trE-His alone, NiNLP-trE-His, or diluents alone. All other experimental parameters were identical, except animals were challenged seven days after serum collection.

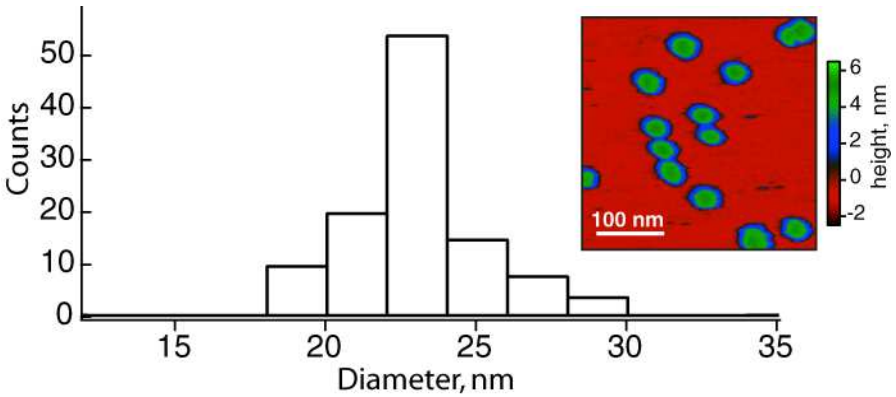
ELISAs

Serum antibody titers to WNV E were measured using an enzyme-linked immunosorbent assay (ELISA). Immulon 2HB microtiter plates (Thermo Labsystems, Franklin, MA) were sensitized with trE-His protein, and then incubated with individual sera (diluted 1:100) for 1 hour. Goat anti-mouse IgG HRP-conjugated antibody (KPL, Gaithersburg, MD) was added to the plates for 1 hour, and the bound HRP was detected by incubation with TMB (Sigma), prior to quenching with 1 M HCl. The reaction product was quantitated spectrophotometrically at 450 nm, and values were corrected for background activity detected from wells that received diluent in place of sera.

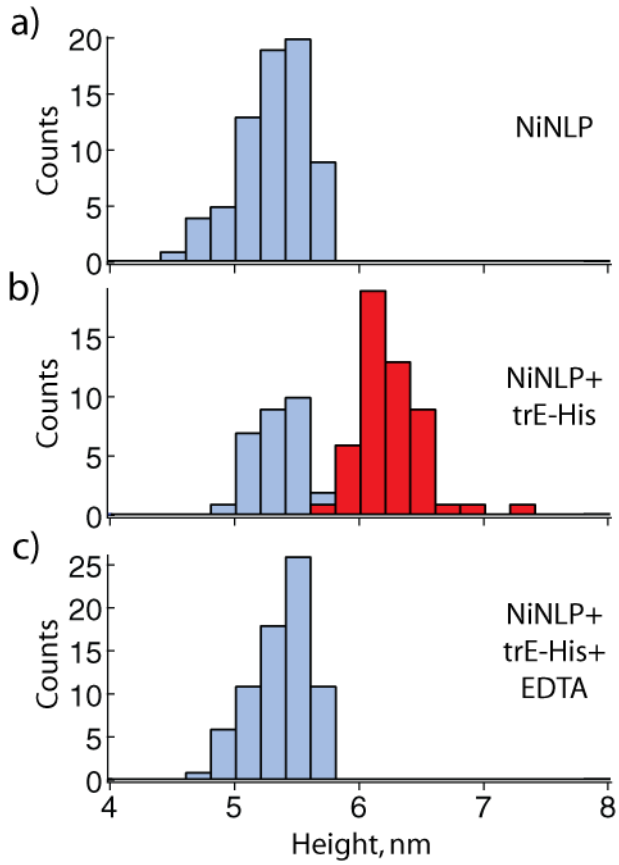
Supporting Figures



Supporting Figure 1. Purification of NiNLPs. (a) NiNLP assembly reactions were run on SEC after dialysis. Fractions were collected across the NiNLP peak. (b). SEC fractions were analyzed by NDGGE to determine sample purity. Only fractions displaying a single major band (astericks) were deemed homogeneous and subsequently pooled for downstream applications.

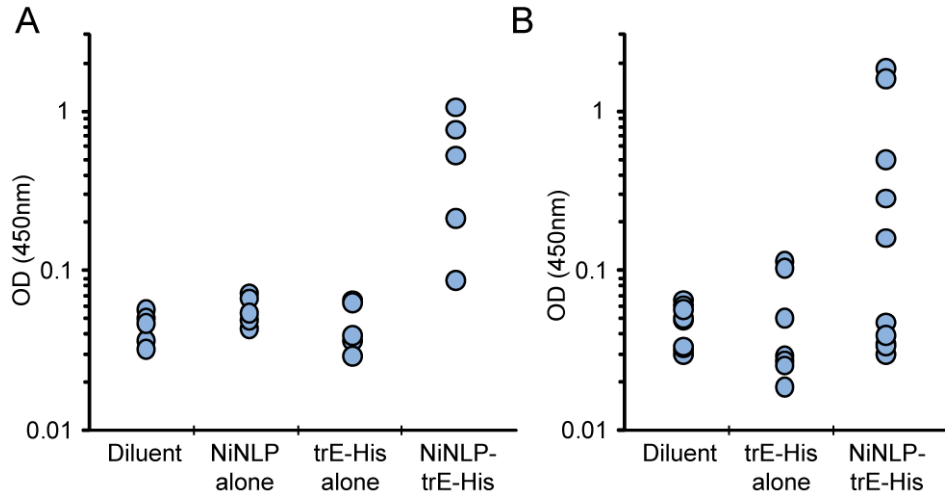


Supporting Figure 2. AFM diameter analysis of representative NiNLPs (n=110). The average diameter of the NiNLPs was 23.2 ± 2.3 nm, based on measurements of 110 individual NiNLPs. (Inset) Representative AFM micrograph. Scale bar is 100 nm.



Supporting Figure 3. AFM height analysis of NiNLP and trE-His immobilization. Height profiles of NiNLP samples immobilized on a mica surface were analyzed by AFM. Blue bars represent NiNLPs scored as not

containing immobilized trE-His. Red bars represent NiNLPs scored as containing immobilized trE-His. (a) NiNLPs alone displayed heights of ~5.5 nm (n=70). (b) When incubated with trE-His, approximately 60% of the NiNLPs on the mica surface displayed heights of ~6.2 nm (red bars) (n=76). (c) No NiNLPs of higher height are observed upon addition of EDTA to NiNLPs during incubation with trE-His (n=73).



Supporting Figure 4. Serology results from individual mice in (a) the pilot experiment (n=5) and (b) the second experiment (n=10). Variability in antibody response is due to high and low responders within each group. ELISAs were performed at least in duplicate with identical results.

(1) Chromy, B. A., Arroyo, E., Blanchette, C. D., Bench, G., Benner, H., Cappuccio, J. A., Coleman, M. A., Henderson, P. T., Hinz, A. K., Kuhn, E. A., Pesavento, J. B., Segelke, B. W., Sulchek, T. A., Tarasow, T., Walsworth, V. L., and Hoeprich, P. D. (2007) Different apolipoproteins impact nanolipoprotein particle formation. *J. Am. Chem. Soc. 129*, 14348-14354.

(2) Xiao, S. Y., Guzman, H., Zhang, H., da Rosa, A., and Tesh, R. B. (2001) West Nile Virus infection in the golden hamster (Mesocricetus auratus): A model for West Nile encephalitis. *Emerg. Infect. Dis 7*, 714-721.

(3) Widman, D. G., Ishikawa, T., Fayzulin, R., Bourne, N., and Mason, P. W. (2008) Construction and characterization of a second-generation pseudoinfectious West Nile virus vaccine propagated using a new cultivation system. *Vaccine 26*, 2762-2771.

(4) Fischer, N. O., Blanchette, C. D., Chromy, B. A., Kuhn, E. A., Segelke, B. W., Corzett, M., Bench, G., Mason, P. W., and Hoeprich, P. D. (2009) Immobilization of His-tagged proteins on nickel-chelating nanolipoprotein particles. *Bioconjugate Chem. 20*, 460-465.

(5) Blanchette, C. D., Law, R., Benner, W. H., Pesavento, J. B., Cappuccio, J. A., Walsworth, V., Kuhn, E. A., Corzett, M., Chromy, B. A., Segelke, B. W., Coleman, M. A., Bench, G., Hoeprich, P. D., and Sulchek, T. A. (2008) Quantifying size distributions of nanolipoprotein particles with single-particle analysis and molecular dynamic simulations. *J. Lipid Res. 49*, 1420-1430.