Supplementary Information

Prestress strengthens the shell of Norwalk Virus nanoparticles

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Materials and Methods

Protein preparation

The full-length[1] and mutant[2] versions of the Norwalk virus capsid proteins were expressed in *Spodoptera frugiperda* (Sf9) cells using a baculovirus expression system, as described previously.[1,2] The Sf9 cells were harvested 5-7 days after the baculovirus infection and the particles were purified using centrifugation in a CsCl gradient. The assembled NVLPs where then stored in water while the CT303 particles were stored in a 200 mM sodium phosphate buffer (pH 7) with 250 mM NaCl. Both stock solutions were kept at 4 ^{o}C. The HBV particles were obtained as described in ref. 3.

Atomic force microscopy

Prior to AFM measurements, the NVLP sample was diluted to 14 µg/ml, and the CT303 sample to 1 µg/ml, both in buffer solution (200 mM sodium phosphate, 500 mM sodium chloride, pH 6.2). Subsequently, a droplet of 100 µL with either the NVLP or the CT303 particles was deposited on a hydrophobic glass cover slip (hexamethyldisilazane-treated)[4] and incubated for 20 min before the addition of 100 µL of buffer solution without particles. The particles were imaged[5,6] and nanoindented[7] with an AFM from Nanotec Electronica (Madrid, Spain), operated in jumping mode.[8] All experiments were performed in buffer solution. Rectangular Silicon nitride cantilevers (Olympus OMCL-RC800PSA) with nominal tip radii of 20 nm were used and calibrated using the Sader method yielding an average spring constant of 50.9 ± 0.2 pN/nm.[9]

The nanoindentation procedure is as follows: A series of zoomed-out images are performed on the sample to determine the particle distribution and density. Before indentation, an isolated particle is selected and zoomed-in. After a high-resolution image of the particle is completed, the AFM tip is positioned at the centre of the particle. When the tip is locked in position, it ceases scanning in x- and y-directions and starts nanoindentation by pushing on the particle in the z-direction at an average loading velocity of 50 nm/s and step size of 0.4 nm. Five successive indentations are performed and the approach/retraction force-indentation curves are recorded. Finally, the particle is imaged again providing visual inspection of the structural state of the particle after the indentation. Stated errors are, unless specified otherwise, Standard Error of the Mean (SEM).

Mass spectrometry and native gel electrophoresis

High-resolution and tandem mass spectra were recorded on a modified Q-ToF 1 instrument (Waters, Manchester, UK) in positive-ion mode.[10] Experiments on NVLPs (0.2 µM capsid concentration) and CT303 (0.1 µM capsid concentration) were performed in 250 mM ammonium acetate buffer (pH 7). To enhance the transmission of the large Virus Like Particle (VLP) ions Xenon was used in the collision cell at a pressure of 2 x 10^{-2} mbar.[11] The voltages and pressures were also optimized for large non-covalent protein complexes. Briefly, the capillary and cone voltages were held at 1.1 – 1.4 kV and 155-175 V, respectively. The voltage before the collision cell was varied from 10 to 400 V, but generally left at 50 V for the accumulation of native ESI mass spectra. Ions were introduced into the source under an elevated pressure of 10 mbar. Experiments on HBV, at 0.04 µM capsid concentration in 200 mM ammonium acetate buffer (pH 6.8), were performed as described.[12]

In addition to MS analysis, the VLPs were analysed by blue native polyacrylamide gel electrophoresis (BN-PAGE) to confirm their purity and integrity.[13] The VLPs were diluted into sample loading buffer, 50 mM bis-Tris (pH 7) and 5 % (w/v) Serva Blue G coomassie brilliant blue G250 (serva blue), and then 25 µg of each VLP was directly loaded onto the gel. The stacking and resolving gels consisted of 3% and 4% (w/v) acrylamide, respectively. Both gels were prepared in a 150 mM bis-Tris buffer (pH 7) with 600 mM aminohexanoic acid. The cathode buffer was prepared just prior to analysis and consisted of a 50 mM bis-Tris buffer (pH 7) with 0.02% (w/v) serva blue. The anode buffer was a 50 mM bis-Tris buffer (pH 7). Electrophoresis was carried out at 4ºC and 25 V for half an hour, then 150 V for a further 6 hours.

Figures

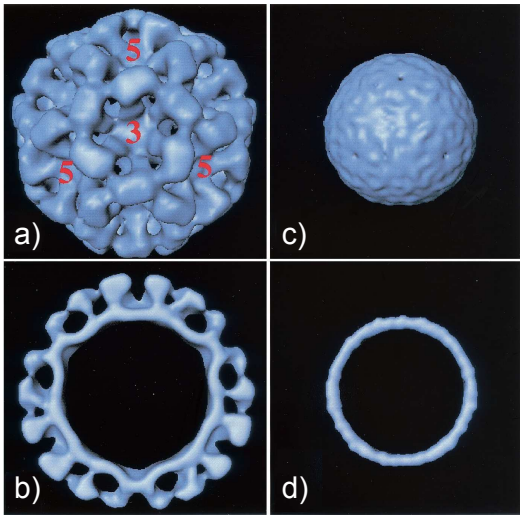

Figure S1. Electron microscopy reconstructions (surface and cross section) of NVLP (a & b) and CT303 (c & d) particle. Hollows at the 3- and 5-fold symmetry axes are depicted in a). Modified from ref. 2, reproduced with permission (copyright © 2002, American Society for Microbiology).

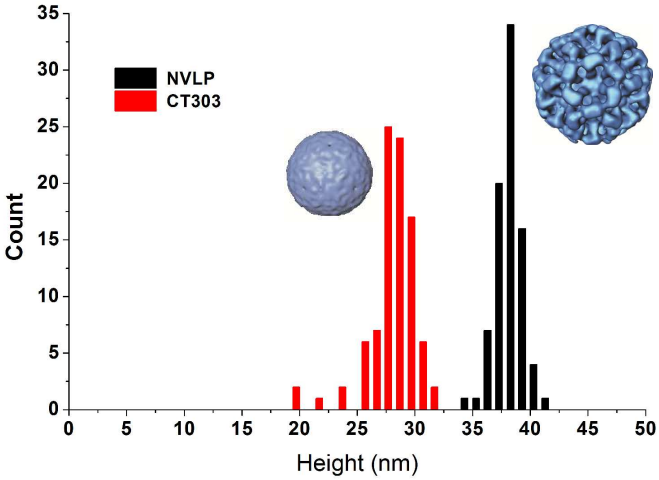

Figure S2. Particle size distribution of NVLP (84 particles in total) and CT303 (92 particles in total), determined using the height profiles of the AFM images. The number of particles is depicted on the y-axis.

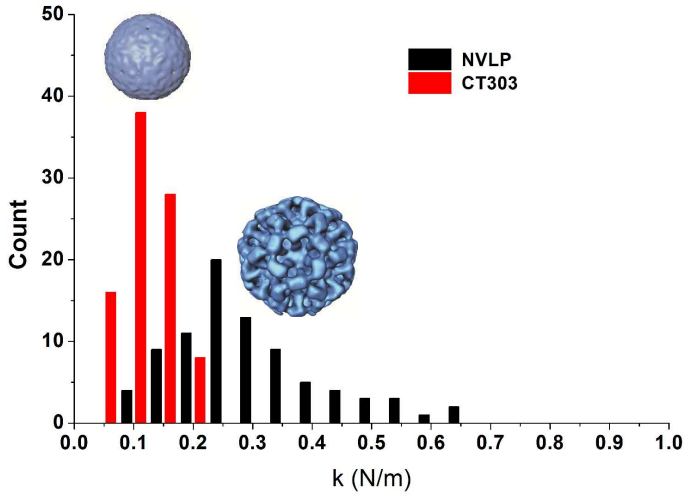

Figure S3. Spring constant distribution of NVLP and CT303 particles, as determined from the AFM nanoindentation curves. The number of particles is depicted on the y-axis.

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