Supporting information (SI)

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Comprehensive size-determination of whole virus vaccine particles using gas-phase electrophoretic mobility macromolecular analyzer (GEMMA), atomic force microscopy (AFM) and transmission electron microscopy (TEM)

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Experimental

Chemicals. Ammonium acetate, ammonia, hydrochloric acid, magnesium chloride hexahydrate, sodium chloride, sodium hydrogen carbonate, sodium hydroxide and tris(hydroxymethyl)aminoethane were purchased from Merck (Darmstadt, Germany). N,N-Dimethylformamide, anti-mouse IgG (whole molecule)−alkaline phosphatase antibody produced in goat and Tween 20 were obtained from Sigma-Aldrich (St. Louis, MO, USA). 5-Bromo-4-chloro-3-indolyl phosphate (BCIP), nitro blue tetrazolium (NBT) and a pure nitrocellulose membrane (pore size 0.45 µm) were purchased from Bio-Rad Laboratories (Hercules, CA, USA). The mouse-derived anti-virus antibody and the HSA-containing PBS buffer without TBE virions were kindly provided by Baxalta Innovations (Orth/Donau, Austria; previously Baxter Innovations). Uranyl acetate war purchased from Fluka (Buchs, Switzerland).

Reagents. Ammonium acetate buffers for size exclusion were prepared with a concentration of 20 mM, 50 mM and 100 mM, pH = 7.4; the pH-value was adjusted with ammonia. The TBS-buffer consisted of 0.9 % sodium chloride and 20 mM tris(hydroxymethyl)aminomethane. The pH-value was adjusted to 8.3 with sodium hydrochloride. For the so called “TBS-buffer with Tween 20”, 0.1% Tween 20 was added. The carbonate-buffer for the color development with the alkaline phosphatase consisted of 70 mM sodium hydrogen carbonate and 1 mM magnesium chloride hexahydrate. The pH-value was adjusted to 9.8 with sodium hydroxide. The BCIP and NBT solutions were prepared as described in the manuals of Bio-Rad. The sucrose concentration was determined with an analytical UV-test for sucrose/D-glucose purchased from R-Biopharm

(Darmstadt, Germany). For the negative staining of the samples for TEM 1 % uranyl acetate solution was prepared.

All buffers and solutions were prepared with water of ultra-high quality with a specific resistivity of 18.2 MΩ × cm at 25°C delivered by a Simplicity UV apparatus (Millipore, Billerica, MA, USA).

Instrumentation. The SEC-System (Pump P-500, Valve V-7 and Fraction Collector Frac-100, Pharmacia, Uppsala, Sweden) was equipped with a prepacked Superdex 200 10/300 GL size exclusion column (GE Healthcare Bio-Sciences, Uppsala, Sweden). The vacuum centrifuge Univapo 100H was purchased from UniEquip (Planegg, Germany). For calculating the sucrose concentration the adsorption was measured with an IMPLEN NanoPhotometer (Munich, Germany). The gas-phase electrophoretic mobility macromolecular analyzer (GEMMA) instrument consisted of an electrospray aerosol generator (Model 3480, TSI Inc, Shoreview, MN, USA), an electrostatic classifier control unit (Model 3080, TSI Inc, Shoreview, MN, USA) equipped with a nano differential mass analyzer (nano DMA, Model 3085, TSI Inc, Shoreview, MN, USA) and an ultrafine condensation particle counter (CPC, Model 3025A, TSI Inc, Shoreview, MN, USA) for detecting the analyte or alternatively an electrostatic nano particle sampler (ENPS, Model 3089, TSI Inc, Shoreview, MN, USA) for collecting the analyte on a selected substrate. A fused silica capillary with an ID of 25 µm (polyimide coated, OD: 150 µm, TSI Inc, Shoreview, MN, USA) and grounded at one end to a conical tip was used for the spray process. The AFM images were recorded on a NanoScope III Multimode SPM instrument (Veeco Instruments, Santa Barbara, CA, USA) using silicon cantilevers with integrated silicon tips (NanoWorld, Neuchâtel, Switzerland, Arrow type: NC) and mica platelets for AFM (Plano,

Wetzlar, Germany). Transmission electron microscopy was performed on a Tecnai G2 20 instrument (FEI, Hillsboro, OR, USA) on 300 mesh copper grids with carbon and formvar coating purchased from Plano (Wetzlar, Germany).

SEC conditions. Size-exclusion chromatography was used for buffer exchange, desalting and purification of the virions. For SEC the sample were loaded onto the column through a 500 µL sample loop and eluted with 50 mM ammonium acetate pH = 7.4 with a flow rate of 25 mL/h, unless otherwise indicated. Prior to fractionation either no solution or 5 µL of a Tween 20 solution (at a concentration of 0.01 % or 0.1 %) were provided in the collection tubes. Fractions of approximately 500 µL were collected every 1.20 min. The volume of the fractions was reduced in a vacuum centrifuge to ~ 50 µL prior to GEMMA measurements, unless otherwise indicated.

Sucrose concentration determination. For every SEC fraction the sucrose concentration was determined by an enzymatic treatment (hexokinase and glucose-6-phosphate dehydrogenase) with subsequent photometric analysis. The set of enzymatic reactions generates NADPH in a stoichiometric amount equal to the sucrose quantity present in the solution. The NADPH concentration can be measured photometrically at 340 nm wavelength. The test (product number 10139041035) was purchased from R-Biopharm (Darmstadt, Germany) and performed according to the instructions provided, but with a slight modification namely one tenth of the suggested volume was used per sample.

TEM operating conditions. For transmission electron microscopy samples were adsorbed on copper grids, coated with formvar (polyvinyl formal) and carbon, for 1 min or directly collected on the grids in the nanometer aerosol sampler. The samples were negatively stained with 1 % uranyl acetate for 1 min. Recordings were performed using an acceleration voltage of 80 kV at various magnifications. Particle diameters are measured in three different angles (0°, 45° and 90°) of every particle with the Photoshop CS 5 software 12.0x32 (Adobe Systems, San Jose, CA, USA).

AFM operating conditions. The AFM images were recorded in air in tapping, constant amplitude mode. 10-20 µL of the sample solutions were dropped onto freshly cleaved mica and after 5 min of deposition the mica surface was rinsed with double distilled water and blown dry with nitrogen. Mica platelets used in the nanometer aerosol sampler were measured directly after collection. Depending on the scanned area, scan rates of 1 Hz for areas of 5 µm² or 2 Hz for areas of 1 µm² were used. Plane correction of the AFM images were performed with the Research Nanoscope 7.30 software (Veeco Instruments, Santa Barbara, CA, USA) and evaluated with the particle and pore analysis tool of SPIP 5.1.6 (Image Metrology, Hørsholm, Denmark) software.

Dot Blot conditions. Nitrocellulose membranes mounted in the ENPS were removed from the instrument after collection and washed with TBS-buffer with Tween 20 for 30 min. The membrane was subsequently incubated with a mouse-derived anti-TBE virus antibody (dilution 1: 1000, V/V) in TBS-buffer with Tween 20 for 1 h. The unbound antibody was removed by three washing steps for 5 min with TBS-buffer with Tween 20. The membrane was incubated

with an anti-mouse IgG alkaline phosphatase conjugate antibody for 30 min. For removing the unbound antibody the membrane was washed again three times with the TBS-buffer with Tween 20 for 5 min each. A washing step with the plain TBS-buffer followed. BCIP and NBT solutions were used as a 1: 100 (V/V) dilution in the carbonate-buffer for color development. The color reaction was stopped by the addition of water after 5-10 min.

Figure 1-S and Table 1-S

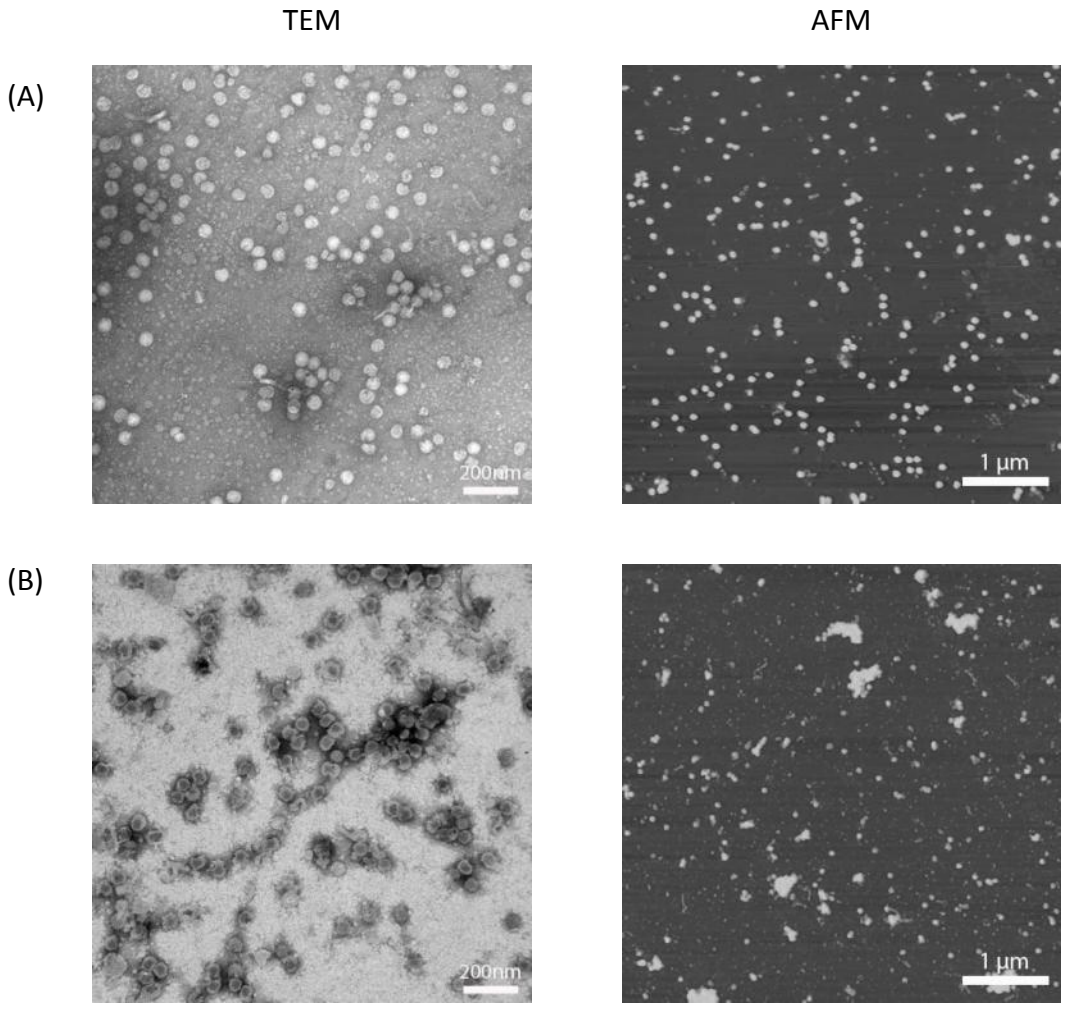

Figure 1-S. Microscopic AFM and TEM images of TBEV samples (A) before and (B) after SEC separation of fraction 17.

Applied method	Measured sample	Measured diameter [nm]	Standard deviation [nm]	Number of measurements
AFM	Without SEC separation	58.2	± 4.4	84 [1)]
AFM	After SEC separation fraction 17	60.4	± 8.5	125 [1)]
TEM	Without SEC separation	51.8	± 5.4	111 [2)]
TEM	After SEC separation fraction 17	53.5	± 5.3	99 [2)]
GEMMA	Without SEC separation	- [3)]	-[3)]	-[3)]
GEMMA	After SEC separation fraction 17	46.8	± 1.1	30

[1)] Software calculated values (SPIP 5.1.6)

[2)] 3 measurements per particle (0°, 45°, 90°)

[3)] No possible measurement due to non-volatile buffer system and high contaminants concentrations

Table 1-S. Influence of SEC separation on mean TBE vaccine particle diameter measured by AFM, TEM and GEMMA.