SUPPORTING INFORMATION (SI)

Analysis of a common cold virus and its subviral particles by gas-phase electrophoretic mobility molecular analysis and native mass spectrometry

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This SI file contains additional information on chemicals and reagents, instrumentation and sample preparation. Additionally, GEMMA spectra obtained with various GEMMA device generations using comparable samples are shown (Figure S1). Data as employed for setup of a M_r / EM diameter correlation ($corr_{new}$) as presented in Figure 6 is given (Figure S3).

Additional information on materials and methods

Chemicals and reagents. Boric acid (analytical grade, *pro analysis*, *pa*) was obtained from Fluka (Steinheim, Germany). Dimethylsulfoxide (DMSO, ≥ 99.9 %), benzoic (≥ 99.9 %), trifluoroacetic (TFA, ≥ 99.5 %) and sinapic acid (*pa*) were purchased from Fluka (Buchs, Switzerland). Ammonium acetate (≥ 99.99 %) and ammonium hydroxide (28.2 % ammonia in water) were from Sigma Aldrich (St. Louis, MO, USA). Sodium hydroxide (≥ 99 %) and acetonitrile (ACN, ACS reagent) were purchased from Merck (Darmstadt, Germany). Sodium dodecyl sulfate (SDS, ≥ 95 %) was obtained from J. T. Baker (Avantor Performance Materials, Center Valley, PA, USA). Water was of Millipore grade (18.2 MΩcm resistivity at 25°C). Ovalbumin (grade VI, approx. 99%), catalase (bovine liver, aqueous solution) and bovine serum albumin (BSA, ≥ 96%) were from Sigma Aldrich and thyroglobulin (from bovine thyroid glands ≥ 90 %) from Calbiochem (Darmstadt, Germany). Several derivatives of keyhole limpet hemocyanin (KLH, Stellar Biotechnologies, Port Hueneme, CA, USA) (approx. 10 mg/mL in sodium phosphate, 10 to 20 mM, pH 6.7 to 7.2) were used.

Instrumentation. CE was on an Agilent 3D CE instrument (Agilent Technologies, Waldbronn, Germany) equipped with a 50 µm inner / 375 µm outer diameter fused silica capillary (L_{tot} / L_{eff} = 60.0 / 51.5 cm from Polymicro, Phoenix, AZ, USA) at 20 °C. 25 kV separation voltage (42 kV/m field strength) were applied. Sample injection was for 9 s at 50 mbar, detection at 200 nm and 260 nm by UV absorbance, respectively. The capillary was pre- / post-conditioned via flushing with BGE / sodium hydroxide solution (1 M) and Millipore grade water. TEM measurements were on a FEI Morgagni 268D electron microscope (FEI Tecnai, Eindhoven, The Netherlands). GEMMA measurements were on a commercial available TSI Inc (Shoreview, MN, USA) instrument consisting of (i) a nano ES aerosol generator (model 3480) including a ^{210}Po charge reduction device, (ii) an electrostatic

classifier (series 3080) with a nano DMA and (iii) an ultrafine, n-butanol driven CPC (model 3025 A). Alternatively, a custom-built nano DMA with 50 Lpm sheath flow (Tapcon & Analysensysteme, Salzburg, Austria) and a water driven CPC (TSI Inc, model 3786) were employed [11]. We used 25 µm as well as 40 µm cone-tipped fused silica capillaries (TSI Inc) for analysis. Other settings are described in supplementary Figure S-2. GEMMA spectra were calculated as median value from at least n = 7 individual measurements. For data evaluation, single Gauss peaks were fitted to corresponding spectra via OriginPro (8 SR0, v8.0724, OriginLab Corporation, Northampton, MA, USA) and the peak apex taken as EM diameter value. EM diameter values of protein mono- and dimers were taken for setup of a M_r / EM diameter correlation.

In native mass spectrometry experiments the capillary and cone voltages were kept constant at 1.25 kV and 150 V, respectively. For native MS analysis the voltage before the collision cell (collision energy) was 150 V for intact HRV-A2 and 180 V for empty HRV-A2 capsids. Ions were introduced into the source at a pressure of 10 mbar. Collision-induced dissociation (CID, tandem MS) analysis was carried out without precursor ion isolation and the collision energy varied from 0-400 V with CID spectra recorded at incremental increases of 50 V. The signal intensity of the released VP subunits was measured relative to the signal intensity of the undissociated virus to allow for estimation of the relative intensities of the dissociated subunits. ESI tips were prepared in-house from borosilicate glass tubes of 1.2 mm (outer diameter) and inner diameter of 0.68 mm (World Precision Instruments, Sarasota, FL, USA) using a P-97 micropipette puller (Sutter Instruments, Novato, CA, USA). The ESI tips were gold coated using a Scancoat six Pirani 501 sputter coater (Edwards Laboratories, Milpitas, CA, USA).

Sample preparation. For experiments targeting the lipase sensitivity of the virus contaminant, samples included 1.8 nM desalted HRV-A2 (obtained by the conventional preparation protocol) and 0.01 µM thyroglobulin (as internal standard) in 50 mM NH_4OAc (pH 8.4). The sample was subjected to lipase digestion by overnight incubation at 28°C and gentle mixing at 650 rpm in an Eppendorf Thermomixer, at a final enzyme concentration of 0.03 U/µL prior to addition of thyroglobulin. For comparison of the HRV-A2 preparation methods, GEMMA samples were adjusted to 0.5 nM HRV-A2 in 50 mM NH_4OAc (pH 8.4); an internal standard was omitted. For measurements with a custom-built instrument, the samples included 2.4 nM HRV-A2 and 0.03 µM thyroglobulin in 10 mM NH_4OAc at pH 9.5. Virus conversion into empty capsids was triggered by heating to 56°C (9 and 15 min, respectively, to also obtain various amounts of A-particles) in an Eppendorf Thermomixer. The KLH derivates (in phosphate buffer) were diluted with NH_4OAc (25 mM, pH 7.4) to result in final phosphate concentrations of 33 µM, 20 µM, 14 µM to assess an impact of insoluble buffer components on the EM diameter determination via GEMMA. BSA, ovalbumin, catalase and thyroglobulin (all at ~1 µM) were dissolved in NH_4OAc (25 mM, pH 7.4).

For CE analysis, the corresponding HRV-A2 samples were diluted 1:20 (v/v) in BGE. Samples included DMSO diluted 1:4,000 (v/v) as marker for the electroosmotic flow and 0.15 mM benzoic acid as internal standard. Because of SDS, heating for 10 min to 56°C lead to dissociation of the virus into its components.

Samples for TEM were prepared as the samples for the respective GEMMA measurements but without thyroglobulin.

Native MS analysis was carried out with HRV-A2 as obtained after desalting (16 nM virus in 10 mM NH_4OAc, pH 9.5). For analysis of empty capsids, an aliquot was incubated at 56°C for 10 min (final concentration 8 nM B-particles in 10 mM NH_4OAc, pH 9.5).

For MALDI MS measurements, samples were diluted 1:3 and 1:10 (v/v) in aqueous 0.1 % TFA, respectively. Subsequently, 3 µL of each diluted sample was mixed with 3 µL of matrix and 1 µL of the respective mixture was applied to the MALDI target and dried at RT.

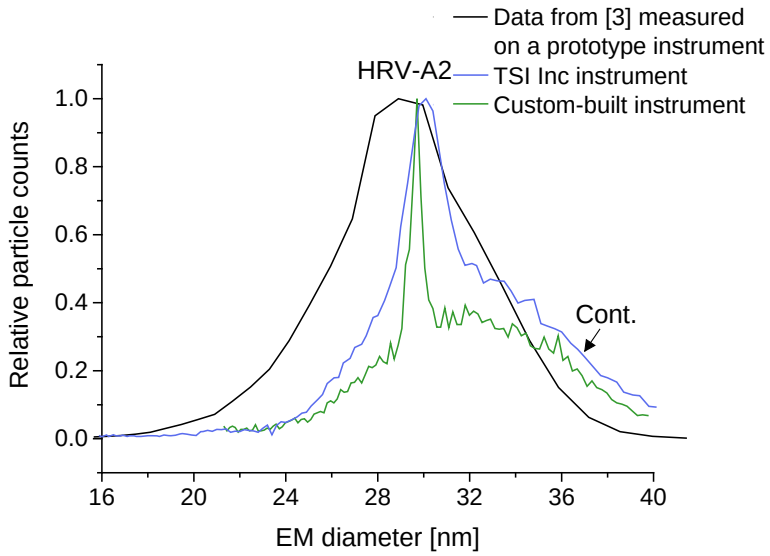

Figure S-1: Comparison of GEMMA signals obtained for a sample of native HRV-A2 on instruments of various generations. It is of note that the increasing resolution between first measurements and measurements carried out later on a conventional instrument allowed for discrimination of peaks corresponding to intact virions and a contamination of HRV-A2 preparations. The resolution (in terms of peak broadness) is substantially improved on a custom-built instrument.

	TSI Inc instrument	Custom-built instrument
Voltage for sample injection	2 kV	-2.0 kV
Current for sample injection	-400 nA	200 nA
Pressure difference for sample injection	0.30 bar	0.25 bar
Sheath flow at the capillary tip	1 Lpm	1.1 Lpm
Sheath flow in the DMA	15 Lpm	50 Lpm

Figure S-2: Instrumental settings for GEMMA analyses. Approximate values are given.

Analyte	EM diameter [nm], average	EM diameter [nm], STDEV	Number of measurements	Reference for EM diameter determination	M_r [kDa], average	M_r [kDa], STDEV	Number of measurements	Reference for M_r determination
Fab 3120, monomer	6.319	0.030	n = 6	-	47.964	0.09	n = 5	[22]
Fab 3120, dimer	7.916	0.029	n = 4	-	-	-	-	-
Ovalbumin, monomer	6.117	0.062	n = 4	-	44.287	-	-	A2512 (Sigma)
Ovalbumin, dimer	7.734	0.051	n = 4	-	-	-	-	-
Catalase, monomer	10.572	0.048	n = 4	-	~ 250	-	-	C3155 (Sigma)
Catalase, dimer	13.602	0.040	n = 4	-	-	-	-	-
Thyroglobulin, monomer	15.037	0.023	n = 4	-	~ 660	-	-	609310 (Calbiochem)
Thyroglobulin, dimer	18.572	0.040	n = 4	-	-	-	-	-
BSA, monomer	7.005	0.019	n = 4	-	~ 66	-	-	A8022 (Sigma)
BSA, dimer	8.884	0.045	n = 4	-	-	-	-	-
KLH derivate 1, monomer	13.228	0.149	n = 3	-	448.9	1.7	n = 4	-
KLH derivate 1, dimer	16.456	0.137	n = 3	-	-	-	-	-
KLH derivate 2, monomer	13.536	0.261	n = 3	-	464.8	1.5	n = 5	-
KLH derivate 2, dimer	17.065	0.211	n = 3	-	-	-	-	-
KLH derivate 3, monomer	13.226	0.141	n = 3	-	419.7	0.8	n = 6	-
KLH derivate 3, dimer	16.371	0.154	n = 3	-	-	-	-	-
KLH derivate 4, monomer	12.747	0.151	n = 3	-	403.6	7.6	n = 5	-
KLH derivate 4, dimer	16.221	0.192	n = 3	-	-	-	-	-
HRV-A2 150S	29.865	0.416	n = 6	-	na	na	na	na
MS2	23.931	0.651	n = 8	[10, 11, 44, 45, 47 - 50]	3600	-	-	[46]
RYMV	28.250	0.354	n = 2	[10, 47]	6500	-	-	[51]

Figure S-3: Data employed for setup of a M_r / EM diameter correlation ($corr_{new}$) as presented in Figure 6. The M_rs of KLH derivates were obtained via MALDI linear time-of-flight MS.