Electronic Supplementary Information

Conformations and membrane-driven self-organization of rodlike fd virus particles on freestanding lipid membranes

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Contents

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S1. Detailed description of experimental procedures

S1.1. Materials

The lipids 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) were purchased as chloroform solutions from Avanti Polar Lipids (Alabaster, AL, USA). The lipid analog dye 1,1´-dioctadecyl-3,3,3´,3´-tetramethylindodicarbocyanine (DiD) was obtained from Life Technologies (Darmstadt).

A sample of fd virus grown in a JM101 host *E. Coli* strain was kindly provided by Dr. M. P. Lettinga (Forschungszentrum Jülich). Virus particles in the sample were already fluorescently labeled with Alexa 488 dye according to a previously described procedure [S1]. This procedure results in homogeneously labeled virus particles carrying about 300 dye molecules per unit virus length. Samples of fd virus particles were stored in 20 mM phosphate buffer, pH 7.5, at 4 ºC.

Degassed deionized ELGA purified water (dd-H_2O) was used for preparation of samples.

S1.2 Cationic supergiant unilamellar vesicles, lipid nanotubes, and supported bilayers

Cationic supergiant unilamellar vesicles (SGUVs) with diameters >100 μm constituting a perfect model system to mimic freestanding lipid membranes were prepared by electroformation as previously described [S2]. Electroformation was carried out on indium-tin-oxide (ITO) coated glass coverslips (#1.5, Mentzel Gläser, Braunschweig, Germany; ITO coating by GeSiM, Grosserkmannsdorf, Germany), which were mildly annealed in air — see ref. [S2] for details. A lipid solution in chloroform with a total lipid concentration of 10 mg/ml was prepared using a mixture of DOPC and DOTAP lipids with a content of the cationic DOTAP lipid ranging from 1 to 3 mol%, depending on the experiment. A volume of 0.7 μl of this lipid solution was spread in a single snake-like pattern on one of the ITO-coated coverslips. After removal of the traces of the organic solvent by placing the coverslip under vacuum for at least 30 min, the electroformation chamber was assembled. The chamber consisted of two ITO-coated coverslips (one of which with the deposited lipid layer) with the ITO-coated surfaces facing each other which were separated and sealed with a 3-mm thick silicon rubber spacer with inlet and outlet channels. The assembled chamber was slowly filled with 300 μl of dd-H_2O at a flow rate of 5–10 μl/min using a syringe pump

(neMESYS, CETONI, Korbussen, Germany). A sinusoidal AC electric field (10 Hz, 1.2 V (rms)) was applied to the ITO electrodes for approximately 2 h to form dome-shaped cationic SGUVs residing on the ITO surface at a density of about 5 mm^{-2}.

Some SGUV samples produced at moderate concentrations of the cationic DOTAP lipid (≥ 2 mol%), additionally contained lipid nanotubes, which were spontaneously formed during sample preparation. These lipid nanotubes were suspended above the coverslip at distances of a few tens of micrometers and typically stretched through the whole observation chamber, thus featuring a straight-line geometry with typical lengths reaching up to an order of ~1 cm. The diameter of a lipid nanotube is expected to be of order of 20–200 nm (see refs. S3-S4). Our experimental resolution did not allow for quantitative determination of the nanotube diameter. Based on the microscopy images it was only possible to conclude that the diameter of the nanotubes did not exceed the optical resolution of our microscopy setup.

Under the conditions of our experiments, lipid bilayers with the above compositions are in the fluid state and are thus characterized by high lipid mobility and bending flexibility.

Supported cationic lipid bilayers were prepared on freshly cleaved mica as described elsewhere [S5].

To facilitate fluorescence microscopy observations, 0.1 mol% DiD was added to all lipid mixtures.

S1.3. Samples of fd virus particles

The stock sample of fd virus in 20 mM phosphate buffer, pH 7.5, predominantly contained monomeric virus particles. The sample also contained a small fraction of polyphage particles fd_m with lengths $L_m = mL_1$, where $m = 2, 3, \ldots$ is the multimerization degree. The amount of polyphage particles fd_m with m ranging from 2 to 11 that could be found in the sample dropped sharply with the multimerization degree m. This agrees with the understanding that polyphages are produced by linear sequential assembly at the cell membrane, as a result of which the probability of forming progressively longer polyphage particles fd_m is expected to drop roughly exponentially with m.

For experiments aimed at determination of the contour length and flexibility of phage particles adsorbed on weakly charged cationic lipid membranes (cationic lipid fraction of 1 mol%, Section 3), virus samples with the enriched content of polyphages

were produced using an ultracentrifugation-based approach. To this end, 1.5 ml of the stock solution of fd virus was ultracentrifuged for 30 minutes at 100,000*g*, after which 1.45 ml of supernatant containing almost exclusively monomeric virus particles was removed, and 1.45 ml of dd-H_2O was added to the remaining pellet, which was then redissolved by gentle vortexing. By repeating these steps three times, a solution of fd virus particles in dd-H_2O with a concentration of ~10^{-12}–10^{-13} M was produced. The ionic strength of this solution is I ~10^{-7} M, which corresponds to the Debye screening length of ~1 μm. The fraction of polyphage particles in this solution was still low, but already at the level that allowed for their experimental observation, which nevertheless required a great amount of patience from an experimenter, especially in case of long polyphage particles ($m \geq 6$).

The same low ionic strength solution of virus particles was used to study interaction of fd virus with moderately charged cationic lipid bilayers (cationic lipid fraction ≥ 2 mol%, Section 4) .

For experiments addressing the effects of membrane-driven self-organization of virus particles adsorbed on weakly charged cationic bilayers (cationic lipid fraction of 1 mol%, Section 5), two solutions with higher ionic strengths I~10^{-4} M and I~10^{-3} M and corresponding Debye screening lengths of ~30 nm and ~10 nm, were prepared based on supernatant solution of the second ultracentrifugation step. These solutions predominantly contained monomer virus at a concentration of ~10^{-12} M; the fraction of polyphage particles in these samples was negligibly low.

S1.4. Adsorption of fd virus particles on cationic lipid membranes

To study interaction of fd virus particles with cationic SGUVs, 300 μl of the fd virus solution was slowly injected into the SGUV chamber at a flow rate of 10 μl/min, which was controlled by a syringe pump. After injection, the sample was left to incubate for one hour which allowed virus particles to reach the surface of SGUVs by Brownian motion.

Experiments were carried out at the typical surface density of membrane-bound virus particles in the range of ~10^{-3}–10^{-2} monomer virus particles per μm^2.

S1.5. Fluorescence microscopy

Experiments were carried out using an epifluorescence setup based on an Axiovert 200 inverted microscope (Zeiss, Germany) with a home-built laser coupling. In

experiments addressing the dimensions and persistence length of fd virus particles, excitation at the green and red fluorophores was carried out using the 488 nm and 647 nm lines of an Innova 70C Spectrum Ar/Kr ion gas laser (Coherent, Germany); the appropriate sets of emission filters and a W-View Optics A8509 beam-splitter (Hamamatsu, Germany) to separate fluorescence emission in the green and red spectral channels. In other experiments, the diode-pumped solid-state lasers Cobolt Calypso 04-01 (Cobolt, Solna, Sweden) and LuxX 642-140 (Omicron-Laserage, Rodgau-Dudenhofen, Germany) were used to provide excitation at 491 nm and 642 nm, respectively; the green and red fluorescence emission was separated using a set of appropriate filters and an OptoSplit II beam splitter (Cairn Research, Faversham, UK). Simultaneous imaging of fd virus particles and lipid membranes in the green and red spectral channels was carried out using an Andor iXon+ 897 EMCCD camera (Andor Technology, Belfast, UK). The camera controlled an acousto-optical tunable filter in the excitation laser pathway allowing for fluorescence excitation only during the acquisition of an image. To prevent photobleaching and photodamage of the samples, degassed water with a reduced concentration of dissolved oxygen was used for sample preparation. The excitation power density at the sample was kept low, at a level of a few hundred $mW \cdot cm^{-2}$. To minimize the number of unknown interactions in the samples, no oxygen scavengers or other similar chemical agents were employed. All experiments were carried out at the temperature of 23±1 ºC.

S1.6. Image acquisition and analysis

When the upper pole of an SGUV with a radius larger than 50 μm is imaged by means of wide-field fluorescence microscopy (Fig. 3 in the main text), a region of an essentially flat freestanding membrane with an area exceeding 460 μm^2 is found in focus of the microscope (assuming a focal depth of ~1.5 μm), which allows one to study conformations and motion of macromolecules and colloidal particles bound to the flat freestanding membrane. We imaged and tracked individual membrane-bound virus particles using fluorescence video-microscopy and determined the position, orientation, and shape of each filamentous particle.

To study the interaction of fd virus particles with freestanding lipid membranes, membrane-bound virus particles were imaged on the upper pole of SGUVs (see Fig. 3 in the main text) using the standard wide-field fluorescence microscopy. To this end, a Zeiss LD C-Apochromat (40x, 1.1 NA, water immersion) long-distance objective was

used, which provided an image resolution of 389 nm/pixel. When necessary, an additional lens was placed in the detection pathway to achieve a higher pixel resolution of the images, which was equal to 75 nm/pixel in experiments addressing the dimensions and conformations of individual fd virus particles (Section 3) and 243 nm/pixel in experiments described in Sections 4 and 5 of the main text.

In the experiments addressing the contour length and flexibility of membrane-bound fd virus particles (Section 3), movies were acquired at a frame rate of 31.25 fps and exposure time of 30 ms. A number of image sequences with the lengths ranging from 200 to 2000 frames were obtained for virus monomers and polyphage particles.

To determine and characterize the size and shape of individual virus particles, their contours were reconstructed from the corresponding fluorescence microscopy images using a procedure based on a filament refinement routine [S6]. For each of the images in a sequence, the reconstructed particle contour was analyzed to determine the contour length, squared end-to-end distance, and squared radius of gyration of the virus particle. By averaging over all the frames of the image sequence, the mean values of these quantities and their mean-square errors were determined. The values of the contour length of the membrane-bound virus particles were subsequently used to classify the observed particles into monomers and polyphage fd_m with different values of the multimerization degree m (see Section 3 of the main text). In particular, for virus particles with m = 1, 2, 3, 4, 5, 6, 7, 9, 10, and 11, the number of recorded movies was 49, 26, 16, 4, 15, 4, 3, 9, and 1, respectively. Finally, the parameters determined from individual movies along with their corresponding mean-square errors were used to determine the weighted mean values of the contour length L, mean-square end-to-end distance $\left\langle R_{\mathrm{E}}^2 \right\rangle$, and mean-square radius of gyration $\left\langle R_{\mathrm{G}}^2 \right\rangle$ for particles with a given multimerization degree m, as well as the statistical errors of these values.

In experiments described in Sections 4 and 5 of the main text, movies were acquired at a frame rate of 18.32 fps and exposure time of 30 ms.

S2. Previous studies of fd virus contour length

The direct, microscopy-based, approaches included electron microscopy (EM) [S7-S10], scanning transmission electron microscopy (STEM) [S11, S12], and atomic force microscopy (AFM) [S13].

The indirect approaches were based on application of hydrodynamics-based models to experimental data obtained using light scattering (LS) [S14] and a combination of transient electric birefringence (TEB) and dynamic light scattering (DLS) techniques [S15].

The following values of the mean contour length L_1 of the fd virus monomer have been reported: 872 ± 15 nm (EM) [S7], 866 ± 37 nm (EM) [S8], 886 ± 29 nm (EM) [S9], 888 ± 12 nm (EM) [S10], 883 ± 11 nm (STEM) [S11], 886 ± 19 nm (STEM) [S12], 883 ± 33 nm (AFM in air) [S13], 883 ± 72 nm (AFM in water) [S13], 940 ± 50 nm (LS) [S14], and 895 ± 20 nm (TEB + DLS) [S15]. Combining these results gives the following weighted-average estimate of the contour length of the fd virus monomer: $L_1 = 884 \pm 6$ nm.

S3. Previous studies of fd virus persistence length

We are aware of only three previous studies of the persistence length of fd virus [S16-S18]. Three more experimental works addressed the persistence length of the closely related M13 phage [S19-S21]. These two phages have very close genomes, and their major coat proteins differ only by a single amino acid [S12, S22] (the negatively charged aspartate asp_{12} in fd is replaced by the neutral asparagine asn_{12} in M13). As a result, close to the neutral pH, where fd has the linear charge density of 10 e^- nm^{-1}, M13 is characterized by a ~30% lower surface charge of 7 e^- nm^{-1} (see refs. [S23] and [S24]). When the ionic strength of the environment is not too low, this is not expected to lead to any noticeable difference in the persistence length of these two viruses [S24], although, to the best of our knowledge, the persistence lengths of fd and M13 phages have never been compared directly under identical conditions.

The experimental techniques used to assess the persistence length of fd and M13 phage included EM [S16, S17, S19], DLS [S16, S20], optical tweezers [S21], and fluorescence microscopy [S18]. With the exception of the most recent work [S18], all these studies have been carried out on monomer virus particles.

The main challenge of the studies of the persistence length of the monomer virus particle stems from its high rigidity ($L/l_p \sim 0.4$), as a result of which the measured quantities depend very weakly on the persistence length. Therefore, a very high accuracy of measurements is required to obtain l_p with a reasonable error margin for such a rigid particle. For example, the EM-based determination of the persistence

length is based on its relation with the mean-square end-to-end distance Eq. (1). It is easy to show that, in order to estimate the persistence length of an fd monomer with the accuracy of ~10%, the mean-square end-to-end distance should be measured with the accuracy of ~0.3%. The same pertains to other types of experimental measurements on these weakly bending filaments.

The EM-based study [S16] of monomer fd virus satisfies the above-stated criteria. The samples were prepared using a variant of the Kleinschmidt technique (see ref. [S25] and refs. therein) where the objects (in this case, fd virus) are embedded in a flat monolayer of denatured cytochrome *c*. This sample preparation approach allows one to successfully obtain unperturbed 2D conformations of much softer DNA molecules and to successfully determine their persistence length [S26-S29]. As a result, one should expect that the mean-square end-to-end distances of the fd virus monomer obtained under these conditions indeed reflects its true bending rigidity. The very good statistics of the data collected by the authors under these conditions supports reliability of the estimate $l_p = 2.0 \pm 0.2$ μm.

In the EM-based study [S19] of monomer M13, estimates of the persistence length were obtained for bacteriophage adsorbed on mica using two different techniques. Although reasonable statistics of the data on the phage end-to-end distance was collected, the authors, using an unconventional data analysis approach, obtained the persistence length ranges for two sample preparation techniques of 0.4–1.0 and 1.2–4.2 μm. Re-evaluation of their results based on Fig. 2 of ref. [S19] gives 0.4 and 1.4 μm, respectively. As discussed by the authors, both preparation techniques are not free from artifacts, which may explain why their results strongly depend on the preparation method and are different from those reported previously [S16].

An estimate of the persistence length of the fd virus of $l_p = 1.0 \pm 0.06$ μm has been obtained [S17] based on an analysis of the internal mean-square end-to-end distance of the virus monomer measured from EM images. This work, however, does not disclose the EM sample preparation details, which can crucially affect the observed conformations of the surface-adsorbed virus [S19]. Furthermore, the statistics of the experimental data presented in the paper does not seem to be sufficient to extract a reliable l_p value for such a rigid filament.

Two DLS experiments [S16, S20] gave close estimates of the persistence length of fd and M13 phage monomers in solution: 2.0 ± 0.2 μm and 2.2 ± 0.2 μm, respectively. Although the high rigidity of the monomer virus complicates both the

measurements and data analysis, the excellent agreement of the DLS-based results with the EM experiment [S20] adds credibility to these values.

The estimate of the persistence length of monomer M13 phage $l_p = 1.3 \pm 0.2$ μm was obtained from force–extension curves in an optical tweezers experiment [S21] using monomer phage particles tethered to the coverslip and a polystyrene bead by their distal and proximal ends, respectively. Evaluation of results of such an experiment for a weakly bending filament with the contour length of order of the persistence length is a non-trivial task. In this case, the model should properly account for finite-length effects and the boundary conditions [S30, S31], as well as the rotational motion of the tethered bead in the optical trap [S31]. However, the model used in ref. [S21] to analyze the experimental data implied a somewhat different geometry than the one of the experimental setup; additionally, it did not account for the rotational motion of the bead. As a result, the value reported in ref. [S21] may underestimate the persistence length of the phage.

In contrast to the above studies, the most recent work [S18] employed fd polyphage particles. The study is based on fluorescence microscopy imaging of fd polyphage mildly adsorbed to a glass surface by depletion interactions, and the persistence length is obtained from an analysis of fluctuating conformations of filaments in terms of Fourier modes. Compared to the above-discussed studies, this approach [S18] is not affected by the properties of the substrate and requires very few assumptions of the analysis. The estimate obtained by this method is $l_p = 2.8 \pm 0.7$ μm.

To summarize, the most credible estimates of the persistence length of filamentous virus particles are 2.0 ± 0.2 μm (fd) [S16], 2.8 ± 0.7 μm (fd) [S18], and 2.2 ± 0.2 μm (M13) [S20].

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