Supporting Materials and Methods

Virus-Like Particle (VLP) Purification. Biosynthesis, *in vitro* assembly, and purification of VLPs was as described in ref. 1. Briefly, EcoB-type *Escherichia coli* was transformed with plasmid pALVP1-tac expressing polyomavirus VP1 protein and grown at 37°C in 6 liters under ampicillin (100 μg/ml) selection to an optical density of OD_{600} = 25, using a biostat fermenter and batch-feeding fermentation. The expression of recombinant VP1 was induced by addition of 0.5 mM isopropyl-beta-D-thiogalactopyranoside at 15°C for 15 h.

For protein purification, *E. coli* was pelleted and resuspended in 10 ml/g in buffer Ao (50 mM Tris, pH 7.4/200 mM NaCl/5% glycerol/1 mM EDTA/4 mM DTT). After lysis by French press in the presence of EDTA-free complete protease inhibitor mixture (Roche Diagnostics), the cell lysate was adjusted to 2 mM $MgCl_2$ with the addition of Benzonase (Merck) for DNA digestion for 30 min at 4°C. EDTA was then added to a final concentration of 4 mM, and the lysate was clarified by centrifugation yielding a supernatant containing the soluble VP1. Solid ammonium sulfate was added for 30 min to a 20% final concentration (wt/vol), and insoluble material was removed by centrifugation. Clarified lysate was added to ammonium sulfate to 25% final concentration, and, after an incubation of 30 min at 4°C, the insoluble fraction containing the VP1 protein was pelleted. The VP1-containing pellet was resuspended in 100 ml of buffer Ao in the presence of protease inhibitors (Roche Diagnostics) and DTT (2 mM) and dialyzed overnight against 10 volumes of buffer Ao containing DTT (2 mM) and EDTA (2 mM). The dialyzed lysate was concentrated by using Tangential Flow Filtration (Sartorius, Geottingen, Germany) to a final concentration of 0.5 mg/ml. To obtain purified pentamers of VP1, gel filtration was preformed with a Superdex 200 prep grade (hiLoad 16/60) chromatography column (120-ml volume, 5 ml/min flow rate; Amersham Biosciences) equilibrated in buffer Ao.

For *in vitro* reassembly of pentamers into VLPs, fractions containing VP1 pentamers were pooled and dialyzed for 24 h at 4°C against buffer Aca (50 mM Tris, pH 7.4/200 mM NaCl/5% glycerol/0.4% NH_4SO_4/2 mM $CaCl_2$), with a redox shuffle system (4.5 mM glutathione disulfide and 0.5 mM glutathione). Assembled particles were purified by gel filtration using a Superdex 200 prep grade (hiLoad 16/60) chromatography column equilibrated in buffer Aca. The purified particles were stored at –80°C.

Fluorescence Labeling of VLPs. Before labeling, VLP particles were centrifuged (2.5 h, 160,000 × *g*, 4°C, Beckmann SW41Ti rotor) through a 5-20% (wt/vol) linear sucrose gradient with a 0.5 ml 60% sucrose cushion in 10 mM Hepes, pH 7.4/1 mM $CaCl_2$. After fractionation, the 0.5-μl fractions were analyzed by transmission electron microscopy after negative staining, and fractions with homogenous particle populations were pooled and used for covalent coupling of fluorescent dyes, which was performed according to the manufacturers directions. Specifically, Alexa Fluor 568 (AF568)- and FITC-succimidyl ester derivatives (Molecular Probes) were coupled at a 10-fold molar excess of dye to VP1 protein, resulting in an average of 182 fluorophores per VLP as determined according to the manufacturer's instructions by spectrophotometry. Unbound dye was removed by chromatography with a Nap-5 column (Amersham Biosciences).

Microscopy Setup. With the exception of fluorescence recovery after photobleaching, all microscopy experiments were performed on a custom modified Olympus IX71 inverted microscope. Modifications included a heated incubation chamber that surrounded the microscope stage set to 37°C, an objective-type total internal reflection fluorescence microscopy setup from TILL Photonics (Grafeling, Germany), and a monochromator for epi-fluorescence excitation with a controller allowing hardware-controlled fast switching between total internal reflection fluorescence and epi-fluorescence excitation and acquisition (TILL Photonics). The 488- and 568-nm line of a Spectra Physics laser as well as monochromator light were coupled into the microscope condensor (TILL Photonics) through optical fibers. Images were acquired using a TILL Image QE charge-coupled device camera and TILLVISION software (both from TILL Photonics). The total internal reflection angle was manually adjusted for every experiment.

Fluorescence Recovery After Photobleaching. Fluorescence recovery after photobleaching was performed using a confocal laser-scanning microscope (Zeiss LSM 510 meta). Fluorescence emission from the 488-nm excitation of fluorescein-dipalmitoylphosphatidylethanolamine was detected using the filter sets supplied by the manufacturer. All experiments were performed at room temperature using a 100 × 1.4 NA Plan-Apochromat objective (Carl Zeiss Microimaging) with an open pinhole. Fluorescein-dipalmitoylphosphatidylethanolamine was photobleached by the 488-nm laser line at full power in a circular region of interest 45 μm in diameter. The size of the region was chosen to yield a satisfying time resolution of recovery. Prebleach and postbleach images were acquired at low laser intensity. Fluorescence recovery in the bleached region and overall photobleaching were quantified in IMAGE J (National Institutes of Health) after export as 8-bit TIFF files from Zeiss file formats (.lsm). Diffusion coefficients were calculated with $D = 0.88 \cdot r^2/4t_{1/2}$. The recovery halftime was estimated by linear interpolation. Calculations were performed in Microsoft EXCEL.

Image Analysis for Single-Particle Tracking. Particle positions were iteratively refined by using the intensity centroid for subpixel interpolation. Trajectories were computationally extracted from the recorded movie sequences using the feature-point tracking algorithm described in ref. 2. Only trajectories longer than 100 frames were considered for analysis.

Trajectory Analysis. The analysis of the particle motion was based on calculating the moments of displacement as follows. Let $x_l(n)$ the position vector on trajectory l at time $n\Delta t$ for $n = 0,1,2,\ldots,M_l - 1$, where M_l is the total number of points in trajectory l, i.e., its length, and Δt is the real-time difference between two subsequent frames. The moment of order ν for a specific frame shift Δn, corresponding to a time shift $\delta t = \Delta n \Delta t$, is defined as

$$\mu_{\nu,l}(\Delta n) = \frac{1}{M_l - \Delta n} \sum_{n=0}^{M_l - \Delta n - 1} \left| x_l(n + \Delta n) - x_l(n) \right|^{\nu} \qquad [1]$$

where |.| denotes the 2-norm (Euclidean norm). The special case of $\nu = 2$ is called Mean Square Displacement (MSD).

To quantify the particle motion (3), these moments were calculated for $\nu = 0,1,2,\ldots,6$ and $\Delta n = 1,\ldots,M_l/3$ and were drawn versus δt in a double logarithmic plot. Assuming that each moment depends on the time shift in a power law $m_n(\delta t) \propto \delta t^{\gamma\nu}$ (2), all scaling coefficients γ^ν were determined by a linear least-squares regression to $\log(m_n)$ versus $\log(\delta t)$. In addition, the generalized two-dimensional diffusion coefficients of all orders were obtained from the y-axis intercepts y_0 as: $D_\nu = 4^{-1}e^{y0}$. D_2 corresponds to the regular diffusion constant in the case of strongly self-similar, pure diffusion. The plot of γ^ν vs. ν is termed moment scaling spectrum (MSS) according to Ferrari *et al.* (3). For all strongly self-similar processes, the MSS shows a straight line through the origin as γ_0 is always equal to 0. The slope of this line is an excellent measure for the type of observed motion. Finding this slope using a linear least-squares fit is a very robust procedure because of the almost perfect linearity of the MSS for strongly self-similar processes. Moreover, the MSS slope has good and uniform sensitivity to detect different modes of motion within the same trajectory. For normal (free) and strongly self-similar diffusion, the MSS slope is 1/2. A slope of 1 indicates ballistic, i.e., uniform and directed, motion. A slope of 0 characterizes a stationary object. The region between 0 and 1/2 is the subdiffusive regime (e.g., confined diffusion) and between 1/2 and 1 is superdiffusion (e.g., diffusion with deterministic drift, Lévy flights). Every strongly self-similar process will yield scaling coefficients γ^ν that linearly depend on ν. A curved or kinked plot is indicative of a weakly self-similar process (3).

The 1-σ intervals for the diffusion coefficient, the slope of the MSS, and the parameter α (γ^2) were computed numerically from the standard deviation in the moments of displacement. The latter were calculated according to ref. 4, generalizing $(1/K)(4D_{2\Delta}n\Delta t)$ to $(1/K)(2\nu D\nu\ \Delta n\Delta t)$ for the higher moments. The factor $K = 3\Delta n(M_l - \Delta n + 1)/(2\Delta n^2 + 1)$,

according to ref. 4, corrects for the fact that the individual samples in Eq. **1** are statistically dependent because of overlapping windows.

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