Supporting Information

Shape matters: the diffusion rates of TMV rods and CPMV icosahedrons in a spheroid model of extracellular matrix are distinct

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Experimental Section

CPMV and TMV propagation: Previously published procedures were used to propagate CPMV and TMV in *V. unguiculata* and *N. benthamiana*. A combination of chloroform:butanol extraction, PEG precipitation, and ultracentrifugation over sucrose gradients was used to extract CPMV and TMV from infected leaf materials.[1, 2] Virus was resuspended and kept in 0.1 M potassium phosphate buffer pH 7.0; virus concentration was determined by UV/visible spectroscopy using known extinction coefficients at 260 nm (ε_{CPMV}=8.1 mLmg^{-1}cm^{-1} and ε_{TMV}=3.0 mLmg^{-1}cm^{-1}).

Bioconjugate chemistry to modify CPMV and TMV with A555 and O488: CPMV (in 0.1 M potassium phosphate buffer pH 7.0) was reacted with NHS-A555 (Invitrogen). Reagents were added in a 10% (v/v) final concentration of DMSO and incubated overnight at room temperature, with agitation. NHS-A555 was added using a molar excess of 2,000 per CPMV. CPMV has a molar mass of 5.6x10^6 gmol^{-1}. Purification was performed through extensive dialysis. To add alkynes to the interior carboxylic acids, TMV (in 0.1 M potassium phosphate buffer pH 7.0) was first reacted with propargylamine, along with the addition of ethylene carbodiimide (EDC) coupling to activate the carboxylic acids. Propargylamine and EDC were added using an excess of 25 and 15 equivalents (eq), respectively. To decorate the exterior TMV surface, the phenol ring of tyrosine underwent an electrophilic substitution (pH=9, 30 min.) with the diazonium salt generated from 3-ethynylaniline (25 molar eq) to incorporate a terminal alkyne. TMV has a molar mass of 39.4x10^{6}gmol^{-1}. The reaction was incubated overnight at room temperature. TMV-interior alkyne (TMV-iAlk) and TMV-exterior alkyne (TMV-eAlk) were centrifuged at 42,000 rpm (Beckman 50.2 Ti) for 2.5 hours at 4°C. The pellet was resuspended overnight in 1 mL of 0.1 M potassium phosphate buffer (pH 7.0). TMV-iAlk/eAlk was reacted with O488-azide (Invitrogen) using Cu(I)-catalyzed azide-alkyne 1,3-dipolar cycloaddition (CuAAC) reaction. O488-azide was added using a molar excess of 2 eq. The reaction was incubated on ice for 5 minutes, followed by a 25 minute incubation at room temperature. TMV-O488 was purified at 27,000 rpm (Beckman 50.2 Ti) over a 10-40% sucrose gradient for 2 hours. The TMV protein band was collected and subsequently ultracentrifuged at 42,000 rpm (Beckman 50.2 Ti) for 2.5 hours, and the resulting pellet was resuspended in 1 mL 0.1 M potassium phosphate buffer (pH 7.0) overnight. We found no differences studying TMV labeled with O488 at the interior *versus* exterior sites.

UV/visible spectroscopy: UV/visible spectra were recorded using a NanoDrop 2000 spectrophotometer.

Denaturing gel electrophoresis*:* 10 μg protein samples were analyzed on 4-12% NuPage gels (Life Technologies) in 1x MOPS SDS running buffer. Protein bands were visualized under UV light before staining and under white light after staining the gels with Coomassie blue (0.25% w/v).

Size exclusion chromatography (SEC): Size exclusion chromatography was performed using a Superose 6 column on the ÄKTA Explorer chromatography system (GE Healthcare). CPMV-A555 nanoparticles and TMV-O488 nanorods (100 μg in 200 μL) were analyzed at a flow rate of 0.5 mL min^{-1} in 0.1 M potassium phosphate buffer (pH 7.0).

Transmission electron microscopy (TEM): CPMV-A555 nanoparticles and TMV-O488 nanorods (20 μL. 0.1 mg mL^{-1}) were negatively stained with 2% (w/v) uranyl acetate for 5 min on a carbon-coated copper grid. Samples were analyzed using a Zeiss Libra 200FE transmission electron microscope operated at 200 kV.

Dynamic light scattering (DLS) and zeta potential measurements: DLS and zeta potential measurements were carried out using a 90 Plus zeta potential analyzer (Brookhaven Instruments Co., USA). Four measurements for CPMV-A555 nanoparticles and TMV-O488 nanorods (1.5 mL of 0.1mg mL^{-1} solutions) were taken, each comprising eight runs.

Preparation of the spheroids: Spheroids were prepared from 1% (w/v) agarose type VII (2-hydroxyethylagarose type VII, low gelling temperate, Sigma-Aldrich) in PBS at pH 7.4. To dissolve the agarose, the mixture was heated at 50°C for 30 min with magnetic stirring. Upon uniform mixing, the agarose reservoir was then used to generate spheroids in an untreated 24-well dish. In brief, 0.25 – 0.75 μL of liquid agarose was dropped on the bottom of the well where it gelled instantaneously as a half-spheroid. The spheroid hemispheres were kept between 200-800 μm in diameter. After placement of the spheroids, 75 μL of PBS were added to keep them hydrated. Spheroids were prepared fresh for all experiments.

Confocal imaging: An Olympus FV1000 laser scanning confocal microscope was used for imaging. The spheroid plate was placed on the confocal stage, and the optical scope was used to find a spheroid of appropriate size and shape that was without defects. Imaging was performed using 10x magnification. The imaging plane was kept constant during the entire study. First blank images were taken to correct for autofluorescence. Then, VNPs were added at 0.032 mg/mL protein concentration in PBS (total volume was 200 μL). Image files were saved as .raw files and analyzed using ImageJ software. Data were processed in MatLab.

MatLab data analysis: (RAW (grey scale) images were imported into MatLab, converted into DOUBLE data types, divided by 255, and stored as pixel intensities (ranging from 0-1) in CELL arrays. Within these arrays, all images were normalized according to the same scheme. Specifically, for each image, the average intensity of the surrounding solution (outside the spheroid) was determined. Each image was then multiplied individually by the inverse of its own solution intensity, resulting in a new normalized solution intensity of 100% for all images. The images were then rescaled to populate the entire plot range. All normalization procedures were performed symmetrically between VNP data, such that TMV and CPMV diffusion properties could be compared. To generate the figures, four radially oriented pixel sections were selected from each image, spanning outwards from the center of a spheroid to 50 μm past its edge (into the surrounding solution). All pixel sections were equivalent in size (20 pixels wide by 250 pixels long, ~ 25 μm x 325 μm). Each section was adjusted such that all four sections were aligned in the same direction, and horizontal averaging of sections was performed, resulting in a single average intensity vector with greatly improved signal-to-noise ratio. Using this intensity vector, plots were generated depicting the average intensity *versus* distance, at multiple time points. Additionally, plots depicting the average intensity *versus* time, at 100 μm (from the edge of the a spheroid toward its center), were also generated. The rate of intensity change *versus* time, at 100 μm, was also calculated from the data.

References:

[1] Foster GD, Taylor S. Plant Virology Protocols: From Virus Isolation to Transgenic Resistance. Humana Press; 1998.

[2] Steinmetz NF, Cho CF, Ablack A, Lewis JD, Manchester M. Cowpea mosaic virus nanoparticles target surface vimentin on cancer cells. Nanomedicine. 2011;6:351-64.

Supporting Figures

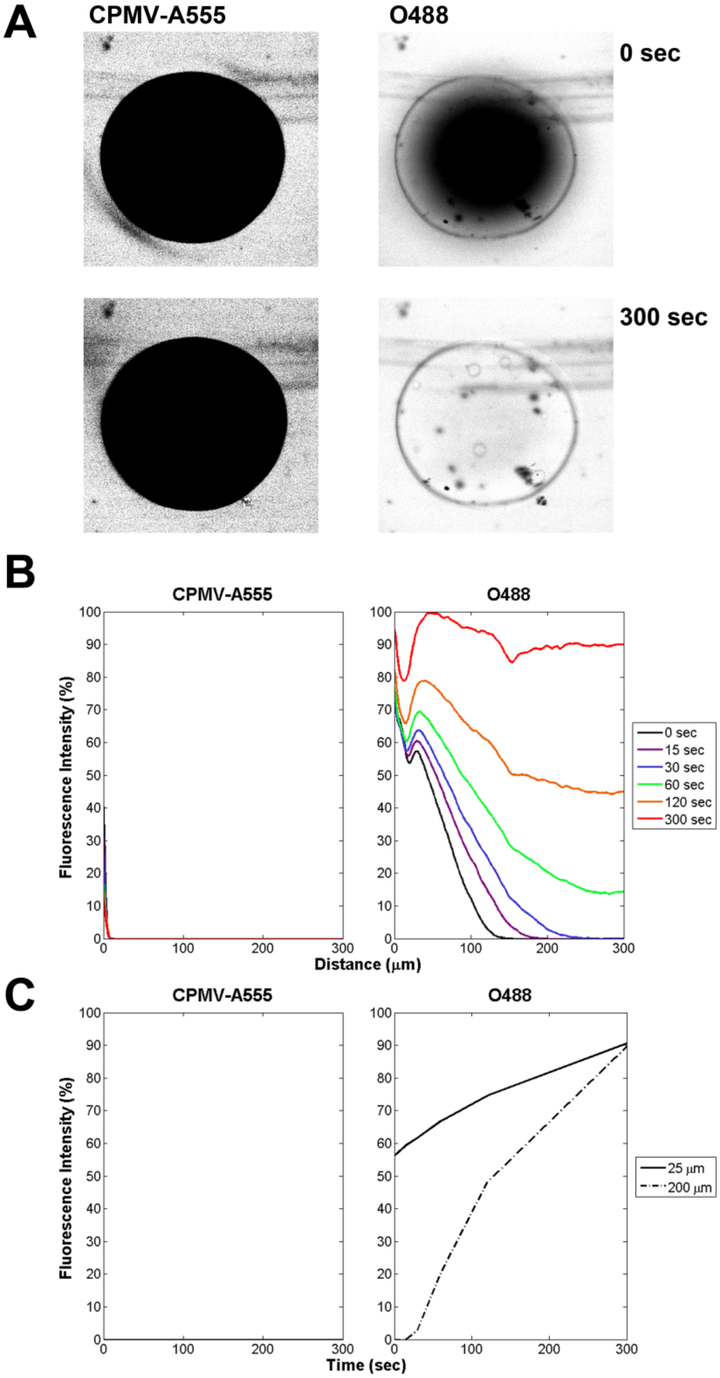

Figure S1: Diffusion rates of CPMV sphere versus free O448 dye into agarose half-spheroids. A: *Snapshots of confocal images showing distinct diffusion of CPMV-A555* versus *O488 dye. Time point 0 sec does not represent 0 sec exactly: this is due to the delay between adding the samples into the reservoir and acquiring the first image. It is apparent from the data that dye diffusion occurs rapidly and the spheroid is saturated within minutes.* ***B:*** *Imaging data were analyzed using ImageJ and MatLab software. The fluorescence intensity normalized against reservoir fluorescence intensity (saturated with VNPs or dyes) is plotted over distance, with 0 μm being the edge of the spheroid structure. Fluorescence intensity versus distance is plotted over time.* ***C)*** *Fluorescence intensity over time is plotted measured at 25 and 200 μm distance within the spheroid.*

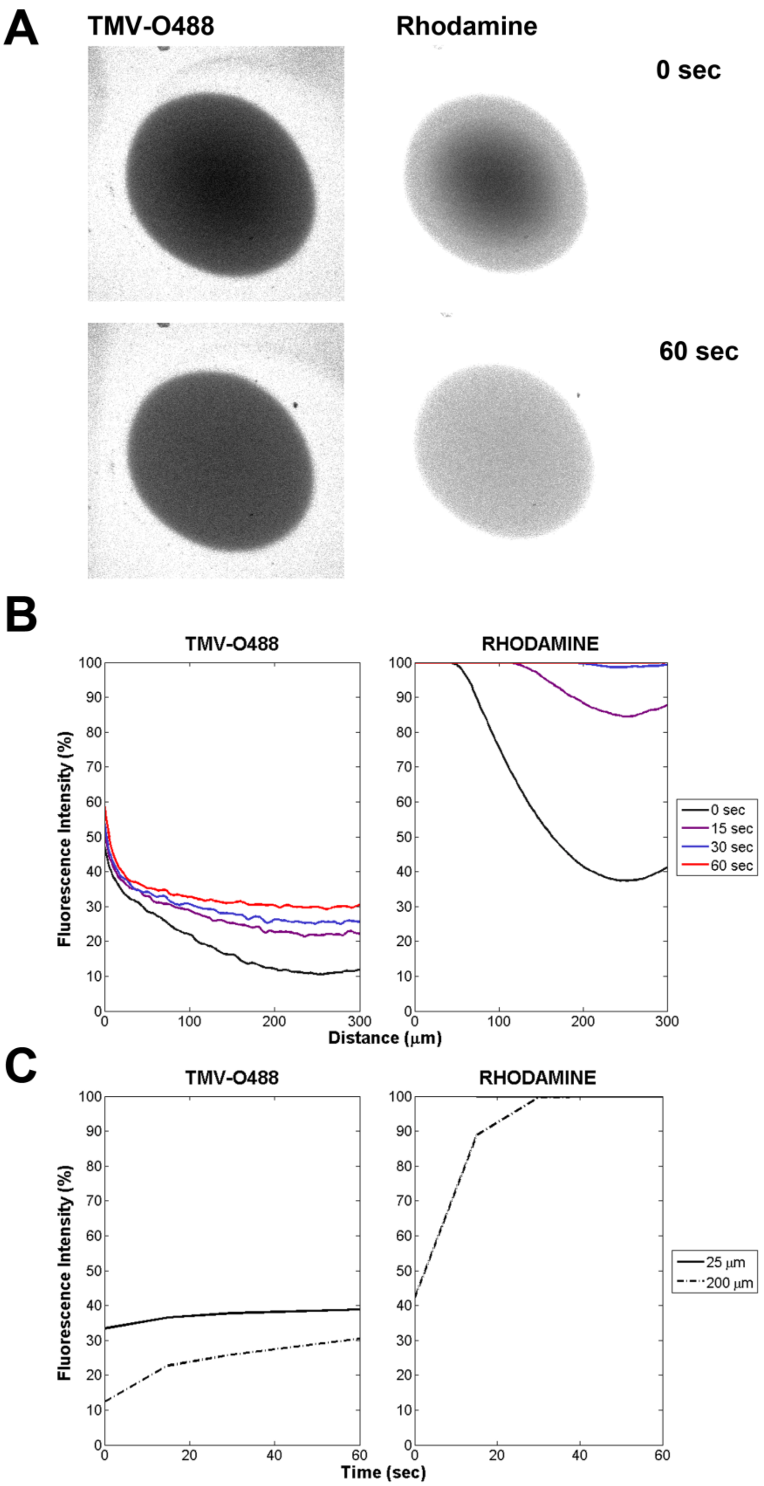

Figure S2: Diffusion rates of TMV rod versus free Rhodamine red dye into agarose half-spheroids. A: *Snapshots of confocal images showing distinct diffusion of TMV-O488* versus *Rhodamine red dye. Time point 0 sec does not represent 0 sec exactly: this is due to the delay between adding the samples into the reservoir and acquiring the first image. It is apparent from the data that dye diffusion occurs rapidly and the spheroid is saturated within minutes.* ***B:*** *Imaging data were analyzed using ImageJ and MatLab software. The fluorescence intensity normalized against reservoir fluorescence intensity (saturated with VNPs or dyes) is plotted over distance, with 0 μm being the edge of the spheroid structure. Fluorescence intensity versus distance is plotted over time.* ***C:*** *Fluorescence intensity over time is plotted measured at 25 and 200 μm distance within the spheroid.*