Supporting Information

Colloidal Transformations in MS2 Virus Particles: Driven by pH, Influenced by Natural Organic Matter

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Material and Methods

Broth preparation:

The broth was prepared by adding 10 g/l of tryptone (Fluka Analytics, St. Louie MO, USA), 1 g/l of select yeast extract (Sigma life science, St. Louie MO, USA), 8g/l of NaCl (≥ 99.5% purity, Sigma-Aldrich Chemie GmbH, Steinheim, Germany), 0.3 g/l of $CaCl_2$ (97% purity, Fluka Analytics, Buchs, Switzerland), 1 g/l Dextrose(Biotechnology grade, Amresco, Solon OH, USA) and 2 mg/l Streptomycin (Fluka Chemie GmbH, Buchs, Switzerland) in ultra-pure water.

Virus dilution buffer:

The virus dilution buffer was prepared by adding 0.78 g/l $NaH_2PO_4 \cdot 2H_2O$ (≥ 98.0% purity, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and 0.58 g NaCl (≥ 99.5% purity, Sigma-Aldrich Chemie GmbH, Steinheim, Germany). The pH was equilibrated to 7.0 using NaOH (≥ 99% purity, Carl Roth GmbH, Karlsruhe, Germany) and HCl (ACS reagent grade, Sigma-Aldrich, Buchs, Switzerland).

SDS-PAGE

SDS-Polyacrylamide gel electrophoresis was done as previously described[1]. In short, 3µl of purified MS2 bacteriophage solution were mixed with 9 µl of buffer composed of 125 mM Tris-HCl, 6% SDS (99% purity, Merck, Darmstadt, Gremany) 30%v/v Glycerol (Fluka, Buchs, Swizerland), 10% 2-Mercaptoethanol (99% purity, Fluka, Buchs, Swizerland), 0.1% w/v Bromphenol blue (Sigma-Aldrich, Steinheim, Germany), and 6 µl H_2O and separated on a 13% acrylamide gel. The 10 ml gel was composed of 32.5% v/v of 40% acrylamide solution (Sigma-Aldrich, Steinheim, Germany), 25% v/v 1.5 M Tris-HCl buffer pH 8.8, 41.5% H_2O, 1% v/v of 10% solution of SDS (99% purity, Merck, Darmstadt, Germany) to which 5µl TEMED (99% purity, Sigma-Aldrich, Steinheim, Germany) and

50 µl of 10% Ammoniumpersulfate (98% purity, Sigma-Aldrich, Steinheim, Germany) were added. The voltage was set to 200V. The gel was stained with Coomasie blue composed of 2.5 g Brilliant Blue (Sigma-Aldrich, Steinheim, Germany), 450 ml methanol (purity 99.8%, Sigma-Aldrich, Steinheim, Germany), and 100 ml acetic acid (99% purity, Fluka, Buchs, Switzerland) completed to 1l with H_2O. The ladder was a PageRuler prestained protein ladder plus (#sm1811, Fermentas, Waltham MA, USA).

Core-shell form factor model

The core-shell model was calculated as follows [2]:

$$P(q) = \frac{scale}{V} F^2(q) + background \quad (Eq.S1)$$

Where $scale$ allows to scale to the source intensity, V is the sample volume, $background$ is the background signal and $F(q)$ is defined as follow:

$$F(q) = \frac{3}{V_s}\left[V_c(\rho_c - \rho_s)\frac{sin(qr_c) - qr_c cos(qr_c)}{(qr_c)^3} + V_s(\rho_s - \rho_{solv})\frac{sin(qr_s) - qr_s cos(qr_s)}{(qr_s)^3}\right] \quad (Eq.S2)$$

where V_s is the volume of the whole particle, V_c is the volume of the core, $r_s = r_c + shell\ thickness$ is the radius of the particle, r_c is the radius of the core, ρ_c is the scattering length density of the core, ρ_s is the scattering length density of the shell, ρ_{solv} is the scattering length density of the solvent.

The polydispersity was accounted for using a Gaussian distribution defined as:

$$f(x) = \frac{1}{Norm} e^{-\frac{(x - x_{mean})^2}{2\sigma^2}} \quad (Eq.S3)$$

Where x_{mean} is the mean distribution and $Norm$ is the normalization factor.

The polydispersity is defined then by

$$PD = \frac{\sigma}{x_{mean}} \qquad (Eq.S4)$$

The shell electron density was set to about 130% of the core electron density as shown by deconvolution (figure 1C in the main manuscript) and the polydispersity value taken from the dynamic light scattering (DLS) and set to be the same in the core and shell.

The fitting was done using SASview software that can be found at www.sasview.org.

Supporting Figures

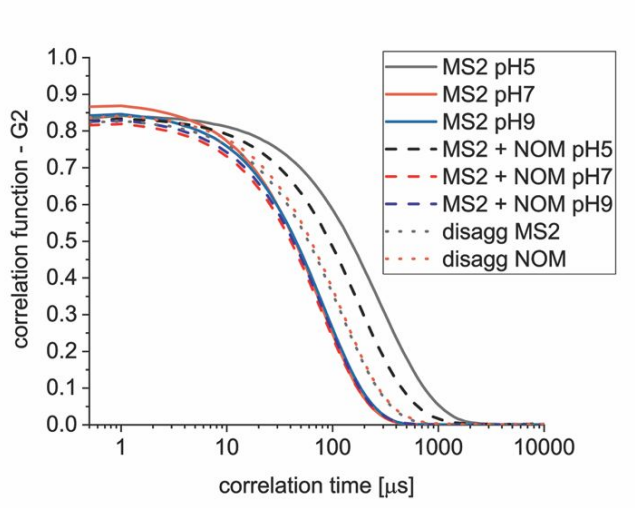

Figure S1: DLS intensity correlation functions of samples reported in this study.

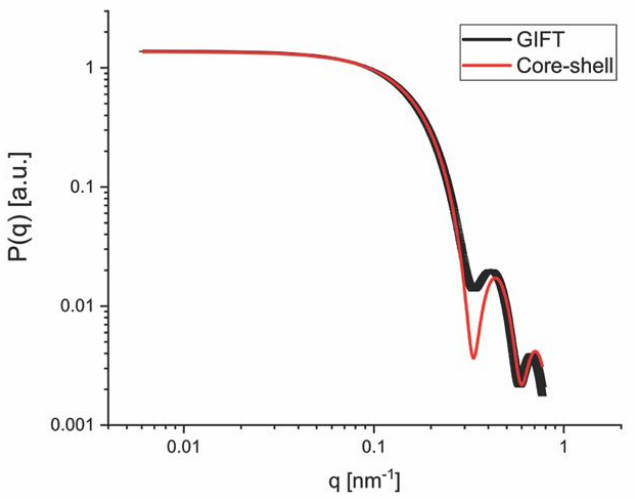

Figure S2: Form factor scattering calculated using the generalized indirect Fourier transformation (GIFT) method (black line) with the corresponding fit calculated with core-shell form factor model of a spherical core-shell particle using equation S1 (red line). The corresponding parameters are summarized in Table S2.

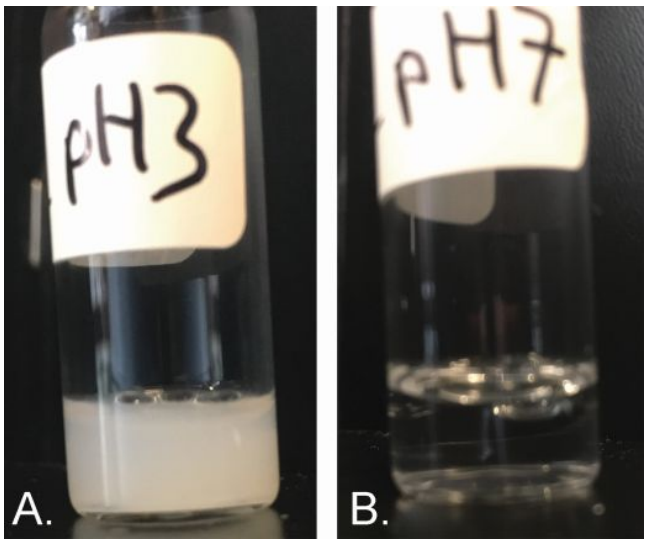

Figure S3: A: MS2 aggregation at pH = 3.0 led to a turbid appearance of the sample due to multiple scattering effects at large, micrometer sized particles. B: MS2 resuspended spontaneously after pH increased from 3.0 to 7.0 leading to a transparent sample due to the small virus nanoparticles.

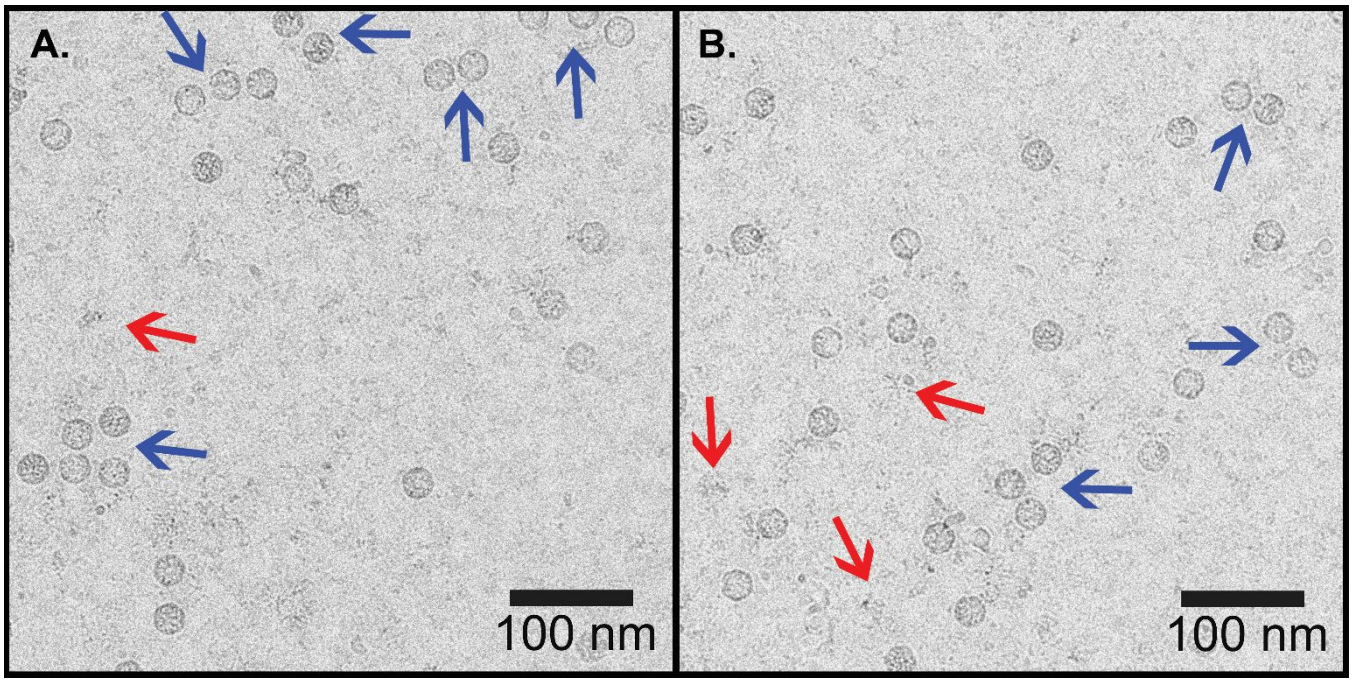

Figure S4: Additional cryo-TEM images of disaggregated MS2 at pH = 9.0. Individual viruses together with virus aggregates (representative ones indicated with blue arrows) and debris (selected ones indicated with red arrows) can be observed.

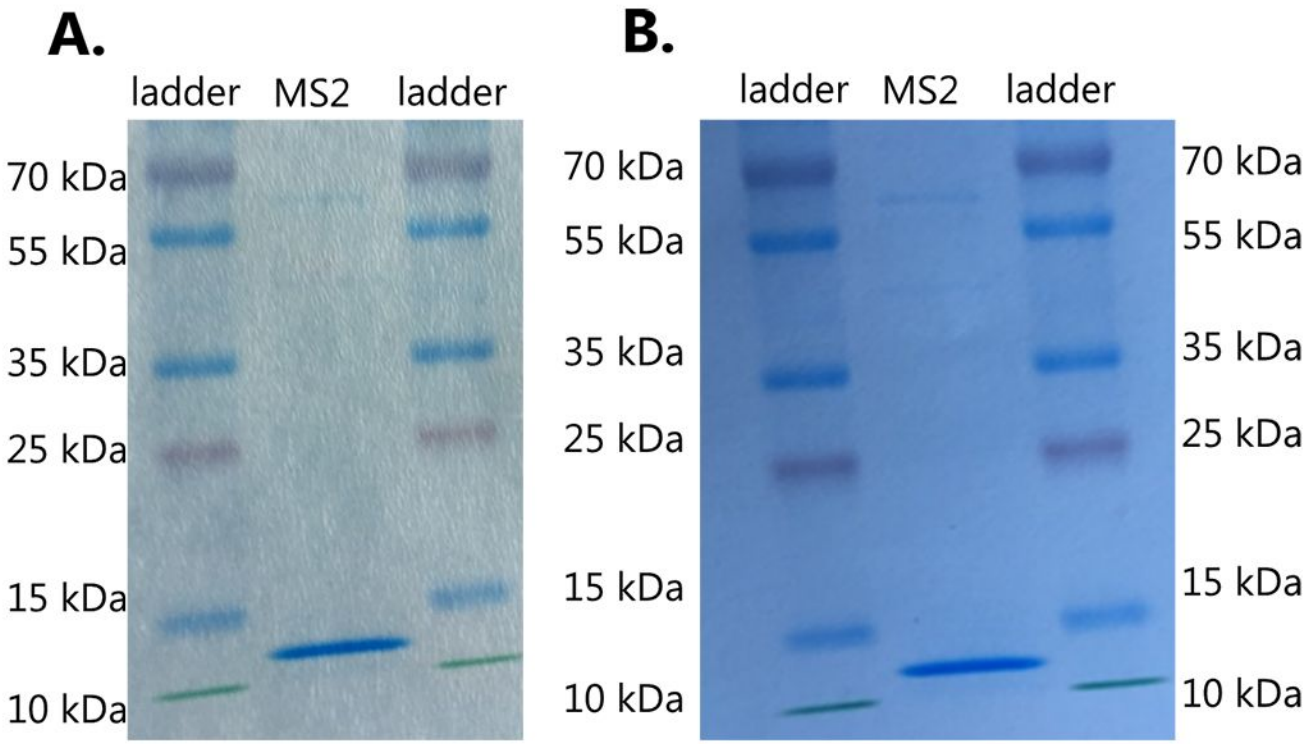

Figure S5: A: SDS-PAGE gel of 1st batch of purified MS2. B: SDS-PAGE gel of 2nd batch of purified MS2. The gels show a single protein band between 10 and 15 kDa corresponding to the size of the coat protein of MS2[3], hence the MS2 bacteriophage solutions shows no detectable contamination from other proteins.

Supporting Tables

Table S1: Structure factor parameters for the MS2 particles. The effective charge is taken from ζ-potential measurements, salt concentration is the ionic strength of the virus dilution buffer, the dielectric constant for water at 25°C[4].

	pH 7.0	*pH 9.0*	*Disaggregated*	*pH 7.0 with NOM*	*pH 9.0 with NOM*	*Disaggregated with NOM*
Volume faction	0.0275± 0.0043	0.0463± 0.0102	0.00606± 0.0521	0.0693± 0.0117	0.0765± 0.00582	0.0445± 0.0164
Apparent interaction radius [nm]	18.5 ± 4.9	18.5 ± 1.3	20.2 ± 10.1	21.5± 1.4	22.5± 0.5	25.5± 2.1
Effective charge [mV] (constant)	25	29	10	15	17	15
Salt [mM] (constant)	35	35	35	35	35	35
Dielectric constant (constant)	78.30	78.30	78.30	78.30	78.30	78.30
Temperature [K] (constant)	298	298	298	298	298	298

Table S2: The parameters for the spherical core-shell form factor model. The values for radius and shell thickness of the core-shell particle were optimized to obtain the best possible fit to the form factor scattering obtained from GIFT. The shell electron density is set to 1.3 times the core electron density as estimated from the model-free fitting method by deconvolution of the *p(r)* function. Polydispersity is estimated from DLS measurements.

Parameter	*Value*
Radius	9.0 ± 0.01 nm
Thickness	3.5 ± 0.01nm
Core electron density	0.684 a.u.
Shell electron density	0.889 a.u.
Electron density solvent	0.333 a.u.
Polydispersity core	0.08
Polydispersity shell	0.08
Background	$0.0014 \pm 3.6\ 10^{-18}$ a.u.
Scale	$6.96 \pm 6.7\ 10^{-17}$ a.u.

References

1. Laemmli, U. K., Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. *Nature* **1970**, *227*, 680-685.

2. Guinier, A.; Fournet, G., *Small-Angle Scattering of X-Rays*. John Wiley and Sons: New York, 1955.

3. Kuzmanovic, D. A.; Elashvili, I.; Wick, C.; O'Connell, C.; Krueger, S., Bacteriophage MS2: Molecular Weight and Spatial Distribution of the Protein and RNA Components by Small-Angle Neutron Scattering and Virus Counting. *Structure* **2003**, *11*, 1339-1348.

4. Malmberg, C. G.; Maryott, A. A., Dielectric Constant of Water from 0°C to 100°C. *J. Res. Natl. Bur. Stand.* **1956**, *56*, 1-8.