Catching a virus in a molecular net

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A metal–organic molecular net composed of tannic acid (TA) and iron(III) was constructed around the bromo mosaic virus (BMV) particle to determine whether the added net could act as a transport barrier for water, and if the net could stabilize the virus in physically or chemically challenging environments. This new virus engineering strategy is expected to provide benefits both in the study and technological applications of viruses. For instance, a virus wrapped in a thin molecular layer could be extracted from solution either in air or vacuum, and its structure, composition and even internal dynamics could be interrogated by methods not compatible with a liquid environment. Atomic force microscopy (AFM) studies of Fe(III)–TA coated BMV in liquid and in air supported a marked resistance to dehydration when compared to wtBMV. Native charge detection mass spectrometry (CDMS), was employed to estimate the number of molecules in the molecular net which wrapped the virus. The CDMS data suggested that less than one molecular monolayer wrapped the virus. Additionally, it was found, that this very thin molecular coat was sufficient to render the coated viruses resistant to storage conditions that typically lead to virus disassembly over time. A temporary coat imparting increased resistance to disassembly could be useful in adding time delay control or alleviate required storage conditions of engineered viruses for therapeutic purposes.

Introduction

Viruses are minimalistic self-replicating biological entities, yet they are intimately involved in some of the most complex biological systems in nature: from the human gut microbiota, to the ebb and flow of geochemical cycles, and even in the evolution of entire species. Viruses have been studied from diverse perspectives, both as a threat and an opportunity.

Progress in understanding the underlying mechanisms of virus replication, and control of these mechanisms, has correlated closely with improvements in measurement techniques. Advances in X-ray crystallography and cryo-electron microscopy (cryo-EM) combined with molecular modeling, have allowed for structural features of viruses as large as 150 MDa to be determined at near atomic resolution. Virus dynamics have become accessible in real time via single particle fluorescence microscopy techniques, as well as live-cell atomic force microscopy (AFM). Finally, improvements in native mass spectrometry of macromolecular complexes have opened new avenues for virus metrology, promising to unveil the nature of intermediates along the assembly pathway, and to quantify the composition and stoichiometric variations in heterogeneous viruses.

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Here we will first discuss how these virus characterization approaches may further benefit from a method of wrapping viruses in a thin molecular layer acting as a barrier against dehydration.

AFM is compatible with a liquid operation environment and can probe both the surface morphology as well as the virus particle response to mechanical uniaxial compression. However, the soft probes that are required for biological materials imaging by dynamic mode AFM (which is the least intrusive of the AFM imaging modes), have lower resolution in liquid than in air. This is mainly due to damping of probe oscillations by the liquid environment. High spatial resolution in air or vacuum is more straightforward to achieve. Unfortunately, few viruses and, more generally, few non-covalently assembled complexes will maintain their structural integrity in air or under vacuum. It would therefore be useful to have a method of coating the surface of a virus with a molecular layer that is both thin enough to follow the underlying morphology, and which would prolong the lifetime of the native hydration state out of a liquid environment.

To overcome the challenge of incompatibility between biological matter and vacuum, studies of the native structures of macromolecular complexes by EM are done by freezing the sample in a thin (~100 nm) glassy ice matrix. The tradeoffs in this case are loss of dynamics access and low contrast between the surrounding ice and the biological material. Thus, to expand the reach of electron microscopy imaging, it would be
beneficial if the virus was coated in a thin molecular layer which would not perturb its structure, but would allow it to be placed in vacuum at room temperature while maintaining a native hydration state long enough to take a series of micrographs. Because of lower background and higher contrast, the required electron dose would be less than that for cryo-EM. Moreover, due to greater contrast between proteins and vacuum than proteins and water ice, defocusing to obtain enough phase contrast (at the expense of resolution), as is currently done in cryo-EM, would be less of a requirement. The prospect of large scale internal structural changes becoming possible to observe, similar to the beautiful real-time experiments of colloidal particle growth in droplets entrapped between graphene layers, is truly exciting.

Finally, in native mass spectrometry, another powerful method which is experiencing an increased presence in physical virology, the sample is dehydrated adiabatically subsequent to being electrosprayed into a low vacuum chamber. While some aspects of proteins are retained, protein complexes containing a central cavity have been observed to collapse by ion mobility mass spectrometry, yielding ions of low charge state with more compact structures than native ones. It is fundamentally interesting to determine how much water is carried by a virus; as hydration of viral nucleic acid is a topic of current debate, and central to the mechanisms of packaging and delivery. One could imagine comparative MS experiments between hydrated and dehydrated viruses that would help to directly investigate such fundamental issues.

Motivated by these possibilities, we have explored wrapping a virus in a thin, biocompatible metal–organic layer, following the approach of Ejima et al. who achieved reversible assembly of an iron(Fe(III))-tannic acid (TA) coat on various planar and particulate templates. Metal chelation by phenols and particularly TA is well known and formation of TA micro-capsules was also studied for range of metal ions. Metal–ligand coordination bond was exploited with respect to the direct packing of guest molecules on the interior interface of a virus-like particle. The general protein surface binding affinity of TA has been described in the literature. Like many tannins, TA interacts readily with proteins and, in certain cases, can cause their precipitation. At alkaline pH, however, tannins are unable to precipitate proteins, as the nonspecific interactions with proteins are being disrupted. It has been suggested that protein–TA interactions are primarily due to hydrogen bonding, although hydrophobic interactions also play a role in tannin adsorption at protein surfaces.

We have used these ideas to modify the original Ejima protocols for the coating of spherical virus templates. Specific adjustments sought the realization of a tight net that, ideally, would be thin enough to follow the virus topography, yet dense enough to entrap water and ions for extended periods of time.

A critical deviation from the original Ejima approach was that coordination is achieved within the net via iron ions electrostatically pre-associated with the virus surface. The association of the iron ions is completed prior to the addition of TA. Washing iron excess before TA addition decreased the chances of coordination occurring anywhere else in solution and ensured that only the monolayer of virus-adsorbed ions was available for coordination, Fig. 1.

From a functional point of view, we show that the thin metal–organic net acts as an efficient barrier to dehydration. This allowed taking the virus out of solution without discernable loss of structural integrity for further characterization. Moreover, we have observed that the molecular net imparts additional characteristics, which could be useful in relaxing storage conditions requirements and potentially, for timed drug delivery.

Materials and methods

All solutions were prepared with ultrapure water (18 MΩ cm at 25 °C) and analytical grade reagents. All salts and reagents, unless specified, were purchased from Sigma-Aldrich (St Louis, MO) and used as received. Tannic acid was purchased from Electron Microscopy Sciences, Inc. and used as received.

BMV purification

BMV is a well-characterized, small icosahedral, plant virus composed of 180 identical coat proteins encapsulating 1–2 molecules of single-stranded RNA cargo. In this work, BMV was expressed in *Nicotiana benthamiana* via *Agrobacterium*-mediated gene delivery. Seven days after infection, *N. benthamiana* leaves were homogenized in virus buffer [250 mM NaOAc, 10 mM MgCl₂ (pH 4.5)] and then centrifuged at 5000 rpm for 25 minutes using an Eppendorf F-35-6-30 rotor. The supernatant was layered on a 10% sucrose cushion in virus buffer and centrifuged at 26 000 rpm for 3 hours using a Beckman SW 32 rotor. The pellets were resuspended in 38.5% CsCl (w/v, virus buffer) and centrifuged at 45 000 rpm for 24 hours on a Beckman 65 TY rotor. The resulting virus band was collected and dialyzed against SAMA [50 mM NaOAc, 8 mM Mg(OAc)₂ (pH 4.6)] buffer for 24 hours, with three changes. Virus concentration was measured by UV-Vis absorption spectrometry; at 260 nm: ε₄₁₀(0.1%) = 5.15.

**Empty capsid assembly**

To obtain empty BMV capsids, purified virions were dialyzed against disassembly buffer [0.5 M CaCl₂ (pH 7.4)] for 48 hours,
with one change of buffer to precipitate RNA. Solution was centrifuged for 45 min at 40,000 rpm using a Beckman 70 Ti rotor. The supernatant with free protein dimers was dialyzed against Tris [10 mM Tris (pH 7.4)] for desalting and then against TKM [1 M KCl, 0.005 M MgCl2, 0.01 M Tris-HCl (pH 7.4)], for storage. Empty capsids were formed by dialysis of the protein dimers (0.5 mg ml⁻¹) against empty capsid reassembly buffer [50 mM NaOAc, 5 mM MgCl2, 1 M KCl (pH 4.7)] for 24 hours with one change of buffer. Protein concentration was measured by UV-Vis absorption spectrometry: at 280 nm, ε₉₅(1%) = 8.20.

Fe(III)-TA coating of BMV

The protocol for wrapping the virus in a Fe(III)-TA layer was modified from previously described.²⁶ The modification included scaling reaction mixtures accompanied by changes in concentrations aimed at reducing aggregation and layer thickness. Typically, 2 µl of water solution of FeCl₃·6H₂O (10 mg ml⁻¹) was added to 198 µl of 0.32 mg ml⁻¹ BMV (in SAMA buffer) and vortex mixed for ten seconds. This solution was placed in dialysis tubing (14–16 kDa cutoff) and stirred in SAMA buffer overnight at 4 °C.

The following day, the BMV–Fe(III) solution was diluted with 4.8 ml of SAMA buffer to total volume of 5 ml and vortex mixed. 2 µl of TA solution (40 mg ml⁻¹ in water) were added and mixed for ~10 seconds. The sample was concentrated to roughly 200 µl by placing it in sealed dialysis membrane tubing (6–8 kDa cutoff) in a 20% Ficoll (w/v, SAMA buffer) for 24 hours. Empty capsid coating was achieved following a similar procedure, with empty capsid buffer used at each step instead of SAMA buffer.

Transmission electron microscopy (TEM)

Samples were prepared for TEM imaging by placing 10 µl of solution on a carbon-coated copper grid. After ten minutes, excess solution was removed by blotting the edge of the grid with filter paper. The grid was immediately stained with 10 µl of 2% uranyl acetate and excess solution was removed after ten minutes by blotting with filter paper. Images were acquired at an accelerating voltage of 80 kV on a JEOL JEM1010 transmission electron microscope and a 4k × 4k CCD Gatan camera. Images were processed using ImageJ software to estimate virus particle diameters and obtain cross-sectional plot profiles of the individual virions.

Atomic force microscopy (AFM)

All AFM experiments were carried out at room temperature with a Cypher AFM (Asylum Research, Santa Barbara, USA). For in liquid imaging, a droplet of 50 µl virus stock solution was deposited on a freshly cleaved highly ordered pyrolytic graphite (HOPG) substrate. BioLever Mini tips (Olympus, Tokyo, Japan) were prewetted with 50 µl SAMA buffer and used for AC mode imaging. A droplet cantilever holder was utilized to reduce evaporation and thus maintain stable buffer conditions.

To prepare samples for in air imaging, a droplet of 50 µl stock solution was first deposited on a newly cleaved HOPG surface and incubated for 15 min. The droplet was then blotted with a piece of filter paper. Residual liquid was removed by drying under gentle air flow. The sample was imaged in air with AC160TS tips (Olympus, Tokyo, Japan) in AC mode.

Topographic images in both liquid and air were then used to produce particle height histograms. To measure the maximum height of a single particle, a cross section was taken across the highest point on the particle to generate a height profile. The height difference between the substrate and the highest point on the particle was then recorded. Over 100 particles were measured for each sample and summarized in height histograms.

Charge detection mass spectrometry (CDMS)

CDMS is a single particle technique where the masses of individual ions are determined from simultaneous measurement of their m/z and charge. CDMS can be used to measure masses for large objects and heterogeneous samples that are a challenge to analyze by conventional MS. Mass spectra of both coated and bare BMV were measured using a home-built CDMS instrument described in detail elsewhere.²⁵ Prior to CDMS analysis, samples were buffer exchanged into 100 mM ammonium acetate buffer. A trapping time of 95 ms was employed. Single virus particles were trapped for 95 ms and the masses were calculated by multiplying the simultaneous measurement of the charge and m/z of each ion. The resulting masses were binned in order to obtain the mass spectra. Note that the samples were stored for ~1 month before the buffer exchange and CDMS analysis.

Dynamic light scattering (DLS)

A Zetasizer Nano-S (Malvern Instruments) was used to carry out DLS measurements of the hydrodynamic radius of particles. The duration of the measurement was automatically determined and the data were averaged from three runs. Intensity and volume distributions were recorded for each sample.

Absorption spectroscopy

Absorbance spectra were recorded with a Varian Cary 100 Bio UV-Visible Spectrophotometer, from 250 to 700 nm.

Gel electrophoresis

For gel electrophoresis experiments, 8 µl of sample were loaded on 0.6% agarose gel together with 2 µl of pure glycerol and run in 1× Tris-acetate-EDTA buffer (TAE, 40 mM Tris, 1 mM EDTA, pH 7.4) for 1 h. Gels were stained immediately after completion with Coomassie blue protein stain. Gel images were recorded using a digital camera.
Results and discussion

The protocol for the formation of Fe(III)–TA films proposed by Ejima et al.26 was originally optimized for particulate substrates with diameters on the micrometer scale and formation of relatively thick films. Following exactly the original protocol’s ratio of substrate surface area to amounts of Fe(III) and TA resulted in heavily cross-linked extended networks which connected many virus particles together. In a few hours after preparation, these aggregates precipitated from solution. In order to obtain individually coated BMV virus particles additional protocol adjustments were required because, for our purposes, the desired thickness was no more than 1–2 nm, while previously reported thicknesses were 10 nm on average.26,36 Since the concentration of Fe(III) has been shown to control the thickness of the coating,26 in order to keep coating thickness at a minimum, we added a dialysis step and removed the majority of free Fe(III) ions from solution, keeping only the virus-adsorbed ones. To reduce aggregation, a dilution step was introduced before addition of TA and cross-linking. Thus, we reasoned that, because polar or charged amino acid residues on the surface of the capsid will interact with metal ions,37 some Fe(III) will adsorb at the capsid surface while solution excess could be removed by a subsequent dialysis step, Fig. 1(a). After the dialysis step, the sample was diluted twenty-five times with respect to the original protocol, and then TA was added. Washing iron excess before TA addition decreased the chances of coordination occurring anywhere else in solution and ensured that only the monolayer of adsorbed ions was available for coordination.

Presence of the molecular net

Occurrence of a thin molecular metal–organic network was experimentally supported by UV-Vis spectral analysis, Fig. 2. The color change reported previously26 was not directly observable due to the dilute sample. However, after completion of the coating procedure, the coated BMV sample shows a small, broad absorption peak at 547 nm, which can be detected in the spectrum of the coordinated complex (Fe(III)–TA), but not in wtBMV or TA alone. The BMV–Fe(III)–TA spectrum also has a pronounced shoulder at 335 nm. This feature is not present in wtBMV and TA alone. It is therefore associated with the presence of Fe, presumably bound to the capsid surface, following dialysis. In absence of BMV, Fe concentration was below the spectroscopic detection limit. These results suggest that virus-adsorbed Fe coordinate with TA.

The presence of the Fe(III)–TA complex on the surface of the virus was further confirmed by electrophoretic mobility changes, Fig. 3. BMV has a pI of 6.438 and the pKₐ of tannic acid is ∼10. At pH 8.2, wtBMV moved towards the positive electrode, while at pH 4.6, wtBMV moved towards the negative electrode. The wrapped virus moved in the direction of the negative electrode at both high and low pH.

Negative-stain transmission electron microscopy (TEM) images of wrapped virions suggest that Fe(III)–TA BMV is virtually identical in size with wtBMV, Fig. 4, i.e. the Fe(III)–TA coating is too thin (less than 1 nm) to affect the size distribution by TEM in any measurable way. Average diameters of wtBMV and Fe(III)–TA BMV were found to be 28.78 ± 0.99 nm.

Fig. 2 UV-Vis absorption spectra of the BMV@Fe(III)–TA and controls.

Fig. 3 Gel electrophoresis of wtBMV and coated BMV (cBMV) in TAE buffer (A) and SAMA buffer (B).

Fig. 4 TEM images of wtBMV (A), and Fe(III)–TA coated BMV (B), and histograms of diameters for BMV, (C), and Fe(III)–TA coated BMV, (D). Insets: Magnified single particles. Note: capsomers were observed in both cases.
and 28.98 ± 0.85 nm, respectively. Note that in all previously reported work on other particles and cells, encapsulated substrates had a much thicker shell of Fe(III)–TA easily observable by TEM, with and without staining.²⁶

The molecular mass of Fe(III)–TA wrapped BMV

To further interrogate the presence of a Fe(III)–TA coating on the virus surface, we have performed charge detection mass spectrometry comparisons between wrapped and bare virions. CDMS measurement of wtBMV yielded a mass peak centered at 4.6 MDa, which corresponds to the expected average mass of 180 coat proteins with a molecular weight of 20 300 Da plus the mass of a RNA cargo of ∼1 MDa. The wrapped virus yielded a mass peak centered at ∼5.31 MDa. Thus the mass shift after wrapping, with respect to the wtBMV control, was ∼650 kDa, Fig. 5. This mass shift accounts for entrapped water, salt, and the metal–organic coat. The molecular weight of TA spans between 1000 and 1500 Da. Assuming there is less than 1 Fe ion per TA,²⁶ the mass contribution from Fe is negligible when compared to the mass contribution of the molecular coat. From the chemical structure, the approximate average area per TA molecule is ∼4 nm². Dividing the virus area (∼2500 nm²) by this number, we obtain that one full monolayer of TA will consist of about 600 molecules. Therefore, the mass of one TA monolayer coat is expected to be between 0.6–0.9 MDa. Since the entire mass shift is 0.65 MDa, these results suggest that less than one monolayer of TA may be responsible for the observed properties of wrapped virus particles. We also note that this mass shift may contain a contribution from solvent of presently unknown weight. Further studies are currently being conducted with the aim of separating the two contributions via isotopic labeling, with the exciting prospect of learning how much water actually hydrates a virus.

Stability of Fe(III)–TA wrapped viruses and empty capsids

It is well known that wtBMV disassembles rapidly at high pH and ionic strength.⁴⁰,⁴¹ This is caused by ionic repulsion of deprotonated carboxylate groups at the three-fold and five-fold axes of the virus and shielding of the stabilizing electrostatic interactions between RNA and the positively charged terminal tails of the capsid proteins.¹² When put in disassembly buffer at room temperature, intact virions disappear completely in less than 48 h, being replaced by large (>500 nm) nucleo-protein aggregates.

Note that, if a molecular net is present, as spectroscopic data in the previous section suggested, and if its constitutive interactions are strong enough to counteract the tendency of BMV to disassemble, the expectation would be for the coated BMV to last longer than wtBMV when suspended in disassembly buffer. Indeed, inspection by TEM of a coated BMV sample held in disassembly buffer for one week indicated that Fe(III)–TA wrapped viruses remained fully intact, with an average diameter of 28.7 nm, Fig. 6.

DLS data confirmed that the sample was composed of virus-like particles with an average hydrodynamic diameter of 35 nm, and that no aggregates were present, Fig. 6.

A stability test applied to similarly-prepared empty capsids instead of viruses yielded similar results. Fe(III)–TA wrapped empty capsids dialyzed against disassembly buffer maintained their structural integrity for at least 48 h at conditions in which empty capsids would completely disassemble in seconds, Fig. 7. These significant changes in stability after wrapping suggest that constitutive interactions in the Fe(III)–TA net are strong enough for the net to act as an external scaffold and preserve the structural integrity of the protein shell even in absence of stabilizing RNA. Moreover, not only is the virus particle stabilized by the metal–organic net, but the molecular

![Fig. 5](https://www.nanoscale.org/content/8/16/16221_Fig5.png) **Fig. 5** CDMS spectrum of Fe(III)–TA wrapped BMV particles (red) and wtBMV (black). The broad peaks which appear below ≥24 MDa in the case of BMV–Fe(III)–TA is presumably due to TA aggregates which have not been filtered out from solution. The high mass tail on the peak attributed to the Fe(III)–TA wrapped BMV particles may be due to a distribution in the composition of the net or a distribution in the contents of the wrapped virus.

![Fig. 6](https://www.nanoscale.org/content/8/16/16221_Fig6.png) **Fig. 6** Disassembly buffer test of wrapped BMV stability. (A) TEM of wtBMV after 48 h in disassembly buffer. (B) TEM of Fe(III)–TA wrapped BMV after 1 week in disassembly buffer. (C) Hydrodynamic diameter distributions by DLS for wtBMV and Fe(III)–TA wrapped BMV in SAMA and disassembly buffers.
net also appears to be stabilized by its interactions with the coat proteins. Thus, the pH-responsiveness of the coating was discussed in earlier works\textsuperscript{26,39,43} having been presented as a promising feature of metal–organic framework films. Previous experiments showed gradual film decomposition below pH 5 over the course of ten days. This was due to the protonation of the galloyl groups and led to prompt disassembly of the TA framework.

By contrast, Fe(III)−TA wrapped BMV and empty capsid were stable for weeks at low pH and low salt concentration. Additionally, gel-electrophoresis in TAE buffer was used to test the stability of the glazing coat. Fe(\textit{u})−TA BMV samples were stored at pH 4.6 for up to 4 weeks and ran through a gel weekly. There were no visible differences in electrophoretic mobility, from one week to another.

We hypothesize that the added stability of the molecular net at low pH with respect to the stand-alone framework could be attributed to interactions between TA and coat proteins which either lower the pK\textsubscript{a} of TA or act to stabilize the Fe(\textit{u})−TA net by anchoring it to the surface via interactions that are insensitive to pH change.

Interestingly, the contrast of empty capsids in TEM pictures shows remarkable differences when compared to the contrast observed in wrapped empty particles, Fig. 7. Empty capsids have a dark interior, which suggests uranyl acetate penetration across the protein coat and subsequent accumulation inside the capsid lumen, Fig. 7(A). Wrapped empty capsids, however, have a brighter interior, Fig. 7(B). The bright interior suggests that the molecular net may act as a transport barrier against uranyl acetate diffusion. In this respect, previous work with Fe(\textit{u})−TA coated polystyrene microspheres has shown that ethanol is capable of passing through the polyphenol coating, dissolving the polystyrene template inside. This means that despite being thinner than the TEM spatial resolution, the coating is surprisingly dense.

**Structural integrity of wrapped particles is maintained upon exposure to air and during long storage**

Is it possible that the dense coat protein – Fe(\textit{u})−TA interface would act as a transport barrier for water, too? To test this possibility, wtBMV and coated BMV were imaged by AFM in both liquid and in air. The AFM images generated histograms for the maximum height of imaged particles. The rationale was that a hydrated particle will have a greater measured height over a substrate than a dehydrated (and collapsed) particle.

Representative AFM images of wrapped BMV particles and wtBMV in SAMA buffer are shown in Fig. 8. The Fe(\textit{u})−TA wrapped BMV sample displayed remarkable homogeneity as

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**Fig. 7** TEM micrographs of: empty capsids (A), coated empty capsids (B), and coated empty capsids after incubation in disassembly buffer (C).

**Fig. 8** AFM images and height histograms for Fe(\textit{u})−TA wrapped BMV in liquid (A and E, respectively) and in air (B and F, respectively), and for wtBMV in liquid (C and G) and air (D and H). Scale bars are 100 nm.
shown in the height histogram, Fig. 8. The height of wrapped and wtBMV particles in SAMA buffer was 28.4 ± 1.3 nm and 24.9 ± 2.6 nm, respectively. A possible explanation for the small difference between these two heights could be the added thickness from the Fe(III)–TA layer. However, a 2 nm thickness change should have been detectable by TEM. A different cause, which at this point seems more likely is a possible lesser propensity of the wrapped virus to deform under substrate adhesion forces, which tend to squat the particle.

After drying, uncoated wtBMV particles exhibited much smaller heights (~11 nm) than in liquid, although the sample remained monodisperse in character, Fig. 8. The 11 nm height roughly corresponds to twice the protein coat thickness: i.e. the virus was completely collapsed onto the substrate. In contrast, Fe(III)–TA wrapped BMV particles were 23.8 ± 0.8 nm in height.

The difference in height between wtBMV and coated BMV in liquid is much smaller than the significant height difference observed in the dried samples. Thus, we conclude that the coated BMV is indeed much more resilient to drying, presumably due to a combination of factors. The first being extra support may be provided by the metal–organic net against capillary forces between substrate and particle. The other is the possible preservation of water confined inside the virus by an unstretchable and semi-permeable molecular net.

In addition, we have found that the coating is still present on the virus surface after one year of storage at 4 °C. wtBMV undergoes disassembly and aggregation after few weeks in the same conditions. TEM and AFM of coated sample after one year shows fully intact spherical particles with same size and morphology as wtBMV (Fig. 9). After drying, old sample presented higher deformation (~19 nm) compared to freshly coated sample but still significantly more stable than wtBMV. This could be indication of partial uncoating of virus particles. Furthermore, in liquid, AFM imaging of partial coated viruses resolved presence of capsomers on virus surface alike to capsomers observable on wtBMV (inset Fig. 8(C)) confirming preservation of the structural integrity. This extended virus stability provided by a straightforward coating protocol could be useful when prolonged, but less strict storage conditions are required for biomedical applications and potentially, for timed drug delivery. Increased stability of the shell is also desirable for possible applications in biomaterials.44,45

Conclusions

A sub-monolayer iron–tannate coordination network was constructed around a small icosahedral virus. Wrapping the virus in a metal–organic molecular net significantly stabilized it against conditions that would normally lead to disassembly. Wrapped virus particles and capsids could be transferred from the native aqueous environment to air or vacuum without substantial changes in the capsid’s structural integrity. Negative stain TEM, and height measurements by AFM, were consistent with the molecular network acting as an effective transport barrier preventing water evaporation upon drying, as well as preventing stain diffusion prior to TEM imaging. Thus, once wrapped in the molecular net, the virus could be removed from solution to air or vacuum, in a hydrated state. Destabilizing the virus coat protein interactions by increasing the pH led to starkly different outcomes between intact and wrapped viruses. Thus, disassembly was arrested for the coated virus but not for the wild-type virus, which suggests that, despite being very thin, the molecular network does prevent dissociation of protein dimers from the capsid. These results suggest that a metastable virus structure might become stable enough to be studied by structural approaches that require conditions which are normally incompatible with hydrated biological samples.

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