Loading the dice: The orientation of virus-like particles adsorbed on titanate assisted organosilanized surfaces

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The organization of virus-like particles (VLPs) on surfaces is a relevant matter for both fundamental and biomedical sciences. In this work, the authors have tailored surfaces with different surface tension components aiming at finding a relationship with the affinity of the different geometric/surface features of icosahedral P22 VLPs. The surfaces have been prepared by titanate assisted organosilanization with glycidyl, amino, and perfluoro silanes. Vibrational and photoelectron spectroscopies have allowed identifying the different functional groups of the organosilanes on the surfaces. Atomic force microscopy (AFM) showed that, irrespective of the organosilane used, the final root mean square roughness remains below 1 nm. Contact angle analyses confirm the effective formation of a set of surface chemistries exhibiting different balance among surface tension components. The study of the adsorption of P22 VLPs has involved the analysis of the dynamics of virus immobilization by fluorescence microscopy and the interpretation of the final VLP orientation by AFM. These analyses give rise to statistical distributions pointing to a higher affinity of VLPs toward perfluorinated surfaces, with a dominant fivefold conformation on this hydrophobic surface, but threefold and twofold symmetries dominating on hydrophilic surfaces. These results can be explained in terms of a reinforced hydrophobic interaction between the perfluorinated surface and the dominating hydrophobic residues present at the P22 pentons. *Published by the AVS.* <https://doi.org/10.1116/1.5077010>

I. INTRODUCTION

The study of the adsorption of colloidal bioparticles at solid-liquid interfaces is of key significance not only for technological applications in materials, food, and biomedical sciences, but also for performing fundamental studies. Beyond virus–host interaction, the investigation of viral particle adsorption is decisive for understanding virus spreading through the environment. In fact, the attachment of viruses to surfaces greatly facilitates both air and waterborne transmissions.^{1,2} Virus adsorption studies are also crucial in many methods for determining virus concentrations in the environment.³ In addition, the advent of single molecule techniques, such as atomic force microscopy (AFM), that can study and manipulate individual viruses in liquid environments, require a high level of control over virus adsorption on surfaces for performing fundamental studies.^{4,5}

Virus-like particles (VLPs, also referred to in the literature as protein cages, protein shells, or virus capsids) are typically attached to substrates by exploiting physical interactions with the substrate, including polar and nonpolar interactions.⁶ There is a generalized agreement that hydrophobic surfaces are efficient for the physisorption of many types of virus structures, which suggests that some of their facets consist

dominantly of hydrophobic residues.¹ Each type of virus particle has individualized features, such as hydrophobic patches or local charge densities,⁶ that can be used for adsorption on different substrates; including glass, mica, and highly oriented pyrolytic graphite (HOPG). HOPG presents a nonpolar surface, and virus particles adsorb on it via hydrophobic interactions. In particular cases, this interaction is very strong and can destroy the samples.⁷ The hydrophobic interaction can also be induced when functionalizing glass surfaces with hexamethyldisilazane.⁸ In contrast, mica exhibits a negatively charged surface that can be changed to positive by using counterions.⁶ On these surfaces, it is expected that the attachment takes place through the negatively charged locations of the VLP. Thus, the virus adsorption mechanism depends strongly on the dominant proteins at the VLP surface. Since different viruses expose different residues to the environment,⁹ each one requires a particular adsorption strategy.

Looking at the virus-surface interaction from the adsorbent side, the possibility to engineer surfaces for precise biological interaction^{10,11} should lead to virus adsorption patterns showing differences in viral retention efficiency and final VLP orientation. Among these techniques, evaporation induced self-assembly allows forming surfaces with different types of nanostructures bearing molecular capping groups,¹² having a direct influence on the final surface tension.¹³ Relevantly, an evaporation induced condensation process involving colloidal titanates

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and organosilanes has led to amino-functional surfaces with low surface roughness, allowing the controlled adsorption of colloidal gold and DNA.¹⁴ The process is governed by a sol-gel transformation of tetraisopropyl-ortho-titanate (TIPT), which catalyzes the homogeneous surface condensation and segregation of a less reactive alkoxysilane.¹⁵

In this work, we aim at studying the adsorption of bacteriophage P22 VLPs on three differently organosilanized surfaces. P22 VLP presents an icosahedral structure and can be adsorbed in fivefold (S5), threefold (S3), and twofold (S2) symmetry axes.¹⁶ These viruses have a maximal outer diameter of 71.0 nm and an average outer diameter of 60.4 nm.¹⁷ We propose the formation of adsorbent titanate assisted organosilanized surfaces (TAOs) with two silane bearing groups inducing an acid-like and a base-like (polar, hydrophilic) surface tension and an additional perfluorosilane inducing a dispersive surface tension (hydrophobic and oleophobic). For the aims of the present study, the surfaces should present roughness values in the 1 nm scale to avoid artifact organization of VLPs of ca. 50 nm. We prepared and characterized the silanized surfaces and observed the differences in virus adsorption efficiency and symmetry orientation.

II. EXPERIMENT

A. Preparation of organosilanized surfaces

Organosilanized surfaces were prepared on $2 \times 2 \text{ cm}^2$ glass slides (soda lime, Fisher Scientific) after sequential trichloroethylene, acetone, and ethanol cleaning and a final step of drying in N_2 . The organosilanization was performed by assisting the condensation of the organosilanes with TIPT (98% Sigma Aldrich). A reactive sol was prepared by dissolving TIPT in ethanol (absolute, 99.8%, Sigma Aldrich) at 0.4M and fixing pH at 1.27 with HCl (37%, Sigma Aldrich) until a final TIPT/water molar ratio of 0.82.¹⁴ Organosilanes (3-aminopropyl) triethoxysilane (APTS, 98%), (3-glycidyloxypropyl) trimethoxysilane (GPTS, 98%) and 1H, 1H, 2H, 2H-Perfluorodecyltriethoxysilane (PFDS, 98%) (all from Sigma Aldrich) were diluted in ethanol at 0.004M and then mixed with the TIPT sol at 50 vol. %. Additional concentrations of GPTS at 0.002 and 0.008M were prepared for x-ray photoelectron spectroscopy (XPS) analysis. These solutions were then spin casted on the glass supports by dropping 50 μl of the reactive solutions while spinning at 2000 rpm under N_2 flow. In a few seconds, a sequence of interference fringes is observed denoting evaporation of the solvent and accomplishment of TAOs. Additional samples were prepared on intrinsic Si (100) wafers for Fourier transformed infrared spectroscopy (FTIR) characterization.

B. Characterization

To estimate the thickness and organic content of the layers, ellipsometry was done over the samples with an M2000 Spectroscopic Ellipsometer (JAWoollam, USA). The ellipsometry was performed in 250–1500 nm range at 70° with respect to the surface normal. To infer the values of thickness and organic content of the hybrid film from ellipsometric

data,¹⁸ an effective medium approximation model was used with TiO_2 and polycarbonate reference phases. FTIR was performed by using a Vector 22 (4 cm^{-1} resolution, Bruker, Germany) in the medium region ($500\text{--}3700 \text{ cm}^{-1}$). Samples for FTIR were spin casted over double side polished (100) Si ($800\text{--}1000 \Omega \text{ cm}$, ITME, Poland) to minimize substrate related signal.¹⁹

XPS was performed in a PHOIBOS 150 9MCD (SPECS, Germany) on three different spots of freshly prepared samples. Spectra were obtained after irradiation with a nonmonochromatic Al K α source acquiring at normal take off angles with a pass energy of 75 eV for survey spectra and 25 eV for core level spectra. The data were processed with CASA XPS software using Gaussian/Lorentzian fractions of 30, Shirley baseline and core level energies referred to the C1s line at 285.0 eV.

The topography of the samples was analyzed by AFM using a Dulcinea instrument (Nanotec Electronica, Spain). Si_3N_4 cantilevers from Olympus, OMCL-RC series with dimensions $L = 100 \mu\text{m}$ and $W = 20 \mu\text{m}$ with a nominal spring constant k of 0.3 N/m were used. Measurements of bare surfaces were performed in contact mode in air, obtaining $500 \times 500 \text{ nm}^2$ images and 128 points in each line. To process the images, wSXM 5.0 Software was used.²⁰ For AFM characterization of viruses, both high resolution images and height measurements were used to identify VLP adsorption orientation (i.e., the height values have been used to identify the symmetry of the virus when the topographic resolution does not allow resolving the VLP facet). Finally, the samples were also characterized by sessile drop contact angle measurements using a CAM100 (KSW, Finland) contact angle goniometer. Three liquids with different surface tension components were used to obtain the surface tension components and total surface tension through the Van Oss-Chaudhury-Good (VOCG) model.²¹ The employed liquids were deionized water (W), ethylene glycol (EG), and α -bromonaftalene (α -BrN). To determine the contact angles, five drops of each liquid were deposited over each type of organosilanized surface. The contact angles were calculated by using CAM 2008 software, and the calculus of the surface tension components was obtained by using SURFTEN 4.3.²²

C. Virus adsorption

Bacteriophage P22 VLPs were suspended in 100 mM phosphate buffered saline and 50 mM NaCl (ionic strength of 600 mM), with pH = 7 at a concentration leading to a protein equivalent of 2 mg/ml or 4.14×10^{13} VLPs/ml. The experiments were performed at 18°C . Rutinary control of pH after the experiment with pH paper stripes demonstrated no drastic pH drift during adsorption. To study the efficiency of virus adsorption on the silanized surfaces, total internal reflection fluorescence microscopy (TIRFM) was employed. This technique exploits the evanescent wave that propagates when the exciting laser beam undergoes total internal reflection. Since the evanescent wave only penetrates $\sim 100 \text{ nm}$ in the solution, the measured fluorescence avoids the background noise from the bulk, obtaining data only from the

adsorbed particles. Here, we work with a home-made system consisting of an Apochromat TIRF 100× oil immersion objective (Nikon ref. MRD01991), a 488 nm blue laser diode (Nichia NDS4116) working at ~0.5 mW, optical fibers, filters, mirrors, shutters, and lenses (which ensure a TIRFM regime) and a CCD detector (IDS camera ref. UI-2410SE-M-GL). P22 proheads were filled with green fluorescent protein by fusing with the virus scaffolding protein that remains bound to the lumen of the virus, providing a long retention time.²³ This protein emits visible light when is illuminated in the blue-ultraviolet range and possesses a photobleaching half-life of around 5 s. In TIRFM experiments, the viruses were diluted in a proportion 1:600 and incubated for 20 min in contact with the silanized surfaces. Then, the sample was excited with a laser beam of 488 nm and a TIRFM image was acquired. These images were analyzed with IMAGEJ (Ref. 24) to count the fluorescent proheads over the surface. Error for the counts was estimated from the standard deviation of three different fields obtained from two replica samples for each organosilane termination.

AFM measurements were obtained in jumping mode, using cantilevers from Olympus with $L = 200 \mu\text{m}$ and $W = 20 \mu\text{m}$. The constant force of these cantilevers is $k = 0.05 \text{ N/m}$. Since the maximum set point used was 50 mV, the maximum force applied during the measurements was ~90 pN. The P22 viruses in these experiments were empty expanded heads. The viruses were diluted in 1:400. After the 20 min incubation at 18 °C, the sample was rinsed five times with 25 ml of buffer solution, ensuring that an adsorption-desorption equilibrium had been reached and that most of the viruses that had not adsorbed were removed from the surface.⁴ Again, error in the counts of the different orientations was estimated from the standard deviation obtained from five scans performed on three different replica samples for each organosilane.

Finally, for the interpretation of the adsorption results, the hydrophobic and hydrophilic (charged and polar) residues were modeled using bacteriophage P22 mature virion capsid protein crystallographic data (PDB ID 5UU5)²⁵ in UCSF Chimera software.²⁶ The relative surface areas of the regions were established by collecting the mean pixel values of each color using IMAGEJ 1.51s.

III. RESULTS

A. Titanate assisted organosilanization

The intrinsic properties of the surfaces produced by spin casting the solutions of TIPT containing 0.004M APTS, GPTS, and PFDS were initially studied by ellipsometry. The functionalization layers presented optical quality (homogeneous interference layer) in all cases. The simulation of the resulting optical properties with an effective medium approximation allowed performing an estimation of the thickness of the TAO and the organic content in the film (two first columns, Table I).

The organosilanized films presented a thickness of ca. 70 nm in all cases, indicating that the film formation was dominated by the TIPT fraction of the films. However, the film

TABLE I. Properties of the TAOs as extracted from ellipsometry (thickness t and organic content $Org. \%$) and dispersive (Lifshitz-Van der Waals, LW), acid (+) and basic (−) components, and derived polar and total surface tensions (in mJ/m^2).

	t (nm)	Org. $\Delta t = 2$ nm	γ_{LW} $\Delta \gamma = 1$	γ_+ $\Delta \gamma = 1$	γ_- $\Delta \gamma = 1$	γ_{Polar} $\Delta \gamma = 1$	γ_{Total} $\Delta \gamma = 2$
APTS	62	36	40	29	0	4	44
GPTS	63	36	43	0	14	2	45
PFDS	65	39	22	0	6	3	25

containing PFDS, contained 3% more organic matter with respect to the APTS and GPTS films, which is in agreement with the longer perfluoroalkyl chain integrated in PFDS.

Fourier transformed infrared spectroscopy was used to characterize the products of the hybrid condensation of the organosilanes by TIPT activation. Figure 1 shows the transmission spectra of pure and organosilane loaded TIPT condensed films formed after spin casting. It was observed that the silanized substrates present common molecular features, with clear differences with respect to TIPT control sample, but also characteristic molecular bands. All TAOs present three common bands with different intensities. From right to left (Fig. 1), the first common band appears at 634 cm^{-1} in silanized substrates and at 613 cm^{-1} in TiO_2 . This band corresponds to Ti—O—Ti bonds, which are present in all the compounds, and could shift in the case of silanized samples due to Ti—O—Si substitutions.²⁷ Two additional bands at

FIG. 1. FTIR spectra in the medium region of the pure titanate (TIPT) and TAOs with APTS, GPTS, and PFDS. The characteristic bands of each organosilane group have been labeled.

732 and 879 cm^{-1} related to the Ti—O—Ti network are observed in the TIPT sample but are hidden in the silanized samples.

Another common band is found at 1617 cm^{-1} , which corresponds to vibrations of carboxylate groups (COO^-). Nevertheless, this band is more intense in the case of APTS since it overlaps with the δNH_2 deformation band and bands related to imine formation and oxidation of amine-dicarbonate salts.¹¹ A similar overlapping occurs with the band at 1110 cm^{-1} between TIPT and APTS. This band corresponds to vibrations of $\nu(\text{Ti—O—C})$ for TIPT, and to C—N bond (more intense) for APTS.²⁸ In GPTS and PFDS, this band is affected at its edge by a wider absorption band due to the development of an amorphous silica network (Si—O—Si). Additionally, in PFDS, this band is hidden on the counter wavenumbers by the band associated to CF_2 group at around 1200 cm^{-1} . The last common band is positioned at 3200 cm^{-1} and is associated with hydroxyl groups in alcohol and adsorbed water molecules. In APTS, this band appears more intensely because it overlaps with the asymmetric stretching band of the amino group $\nu_{\text{asy}}(\text{NH}_2)$.²⁸ A specific molecular band corresponding to the labeling and condensation of GPTS at 1409 cm^{-1} (CCO epoxide ring vibration) appears hidden by the other C—O absorption bands from adsorbed ethoxy, methoxy groups.²⁹

To further characterize the surface composition, XPS was used to monitor the elemental composition of the assembled organosilanes. XPS was initially used to monitor the incorporation of the silanes on the final surface by using different molar concentrations of GPTS as a model silane. The spectra corresponding to pure TIPT and TIPT doped with GPTS at concentrations of 0.002, 0.004, and 0.008M, are presented in Fig. 2(a). The spectra display the characteristic peaks of Ti, C, and O for the TIPT reference and an additional Si peak for GPTS doped samples. Furthermore, traces of Cl, Na, and N were also found on all the surfaces, presumably due to wet

process impurities (pH control). The analysis of the surfaces to estimate the atomic composition demonstrated that the surfaces presented correlative stoichiometry, with increasing composition of C and Si at increasing GPTS doping. Furthermore, all impurities were present at less than 1 at. %. In order to highlight the increase of the silane presence on the surface, the ratio of C to C + O and the ratio of Si to Si + Ti were calculated and appear plotted in Fig. 2(b). The trend of these two ratios is toward saturation, although the saturation appears to be reached for the C curve, but not for the Si one. By taking the GPTS molecule as a reference, which contains six carbon and five oxygen atoms in its condensed form, one could expect a saturation value at ca. 0.55 (note red dotted line at the bottom abscissa for reference). Since this value is exceeded by the smallest GPTS concentration considered, the results illustrate the presence of additional hydrocarbon adsorption, which enriches the surface in functional species. Nevertheless, for the GPTS concentration of 0.004M, a saturation value is identified at ca. 0.70.

By monitoring the Si to Ti + Si ratio, we were able to monitor the surface selectivity for the GPTS condensation with respect to TIPT. In this case, the system does not seem to have reached saturation for GPTS concentrations of 0.008M. Nevertheless, taking into account the solution stoichiometry ($[\text{GPTS}]/([\text{TIPT}] + [\text{GPTS}]) \sim [\text{GPTS}]/[\text{TIPT}] = 0.008/0.4 = 0.02$) the surface is drastically enriched in Si with ca. 40 times more surface Si than expected [see 0.7 value for $\text{Si}/(\text{Ti} + \text{Si})$ in Fig. 2(b)]. This result illustrates the efficiency of TIPT toward a condensation process favoring GPTS entrapment and migration toward the surface. In view of these results, the GPTS concentration of 0.004M was selected for the subsequent steps. This value was also used for comparative purposes with the other two organosilanes (APTS and PFDS). The survey spectra of these layers additionally contained N (1.9 at. %) or F (13.2 at. %) for APTS and PFDS, respectively.

FIG. 2. (a) Widescan XPS spectra of surfaces prepared by TAOs with GPTS, present in the solution at different concentrations: from bottom to top, pure TIPT, 0.002, 0.004, 0.008M GPTS. (b) Plot monitoring the progress of the GPTS silanization at the different atomic concentrations: total C normalized to total C + O (left axis) and total Si normalized to total Si + Ti (right axis) with corresponding exponential fitting. Horizontal dotted line represents the reference C/C + O value (0.55) for the precursor GPTS molecule.

FIG. 3. C1s core level spectra from the surfaces of TIPT activated condensation of the different organosilanes at 0.004M concentration: (a) pure TIPT, (b) GPTS, (c) APTS, and (d) PFDS. Note common horizontal and vertical axes.

The C1s XPS spectra for all the organosilanized surfaces are presented in Fig. 3 and compared with a TIPT reference spectrum. Room temperature condensation of TIPT leads to poorly densified materials with an abundant organic byproduct, which adds to the typical hydrocarbon contamination on the surface. This leads to a C1s spectrum [Fig. 3(a)] with three contributions at 285.0, 286.6, and 288.9 eV, which can be assigned to C—C—H, C—C—O, and C=C=O bonding, respectively. This backbone composition is used as a reference for the assignment of additional peaks on samples containing the organosilanes.

The addition of APTS shows relatively small changes [Fig. 3(b)], although the increase of the C=C=O contribution suggests the adsorption of atmospheric CO₂ on the highly reactive amine groups, and the presence of a C—N component of similar intensity, which is observed at 285.8 eV.^{11,30} The addition of GPTS does not involve any new component in the C1s spectrum [Fig. 3(c)], but the predominant increase of the C—C—O component, which is in fact the dominant molecular structure in the glycidoxy functional group.³¹ The presence of the perfluoro groups on the surface of PFDS functionalized samples [Fig. 3(d)] is clearly identified by the presence of a new C—C—F contribution at 291.8 eV.³² Thus, the data illustrate that the surface of TIPT condensate happens to be coherently modified with the groups of the binding organosilane.

B. Surface topography and wetting

The topography of the three different surfaces was analyzed by using AFM as shown in Fig. 4(a). To compute the

roughness of the surfaces, both estimations of RMS roughness and height distribution histograms are shown [Fig. 4(b)]. The images show that APTS and GPTS present a very similar topographic structure, with granular features of a few nanometers on the surface. On the other hand, PFDS presents smaller filamentary features. The RMS roughness estimations successfully show that the surfaces are homogeneous with typical values below 0.5 nm (ca. 0.5 nm for APTS, 0.4 nm for GPTS, and 0.3 nm for PFDS).

The influence of surface composition on the wetting properties was studied by estimating the surface tension components for each organosilanized surface. From Fig. 5, which reports the contact angle data used for the estimation of surface tension, it is observed that the surfaces can be classified into two groups in view of their interaction with water.

The contact angle between water and PFDS is $88^\circ \pm 2^\circ$ but for GPTS and APTS is $48^\circ \pm 1^\circ$ and $49^\circ \pm 3^\circ$, respectively. Thus, the PFDS surface can be categorized as close to hydrophobic and the GPTS and APTS surfaces can be considered as hydrophilic. Contact angles were additionally measured with EG and α -BrN. The interaction of PFDS with EG and α -BrN was similar in terms of contact angle and lower than on any of the other two organosilanized surfaces.

The surface components of the surface tension were estimated from contact angles by using the VOG model and are presented in Table I. With respect to the organosilanes exhibiting amino and epoxy terminal groups, respectively, APTS and GPTS exhibit inverse dominant polar components (APTS presents a remarkably higher acid component, and GPTS presents a mildly higher basic component). This goes in line with the tendency of APTS to induce positively

FIG. 4. (a) $500 \times 500 \text{ nm}^2$ AFM images of the TAOs with APTS, GPTS, and PFDS. (b) Associated roughness is quantified through the inserted RMS values and the height histograms fitted with a red Gaussian line and black dashed line locating the center at $2.4 \pm 0.5 \text{ nm}$ for APTS, $1.3 \pm 0.4 \text{ nm}$ for GPTS, and $1.3 \pm 0.3 \text{ nm}$ for PFDS.

charged surfaces³³ and of GPTS to induce negatively charged surfaces,³⁴ as estimated with Zeta potential measurements. Meanwhile, the perfluorinated surface presents only a significant basic component. The most relevant difference between PFDS and the other two surfaces is observed in the dispersive component, emerging from the long hydrophobic chain. This determines that APTS and GPTS exhibit higher total surface

FIG. 5. Results of sessile water, ethylene glycol, and α -bromo naphthalene contact angle measurements on the surfaces formed by TIPT assisted condensation of APTS, GPTS, and PFDS.

FIG. 6. TIRFM images of the substrates after 20 min of incubation.

tension than PFDS. The results are thus consistent with the presence of heterofunctional surfaces comprising the presence of protonated amines in APTS, a potential role of additional OH in GPTS, and a clear low surface tension behavior for PFDS surfaces. This heterogeneity in surface functionalities can control protein conformation upon adsorption³⁵ and should thus help controlling preferred adsorption of certain facets of VLPs.

C. Virus adsorption

TIRFM was used to study which type of organosilane surface was more effective for the adsorption of viruses. Figure 6 shows characteristic images of P22 virus adsorption on the different organosilane surfaces after 20 min of incubation. The TAOs presented a different affinity toward the P22 virus with 467 counts on APTS, 230 counts on GPTS, and 711 counts on PFDS. The relative efficiencies, taking the PFDS surface as 100% reference, are 66% and 32% for APTS and GPTS, respectively.

The final symmetry of P22 VLPs over the organosilane surfaces was determined by AFM [Fig. 7(a)]. A set of representative images for each of the symmetries is shown beside an ideal icosahedron in an equivalent orientation. Sometimes, the resolution at the top of the VLP allowed us to discern among a pentagonal pyramid for S5, a flat equilateral triangle for S3, and the edge between two triangular facets for S2. In some cases, it is difficult to discriminate between S3 and S2 symmetries.

By classifying the images of VLP population for each organosilane surface, we could observe that on APTS and GPTS there is a predominance for S3 and S2 symmetries (considered together), but the opposite happens for PFDS where orientation on S5 is dominant [Fig. 7(b)]. Modifications to these statistics can be considered taking into account the efficiency in adsorption determined previously by TIRFM (see associated red bars in the histogram). For this, the incidence of each of the VLP orientations was compared, taking the most efficient PFDS as 100% reference. The values of the histogram were thus multiplied by a factor of $467/711$ for APTS, $230/711$ for GPTS, and $711/711$ for PFDS. The red capping values in Fig. 7(b) thus reflect the higher natural affinity of P22 VLPs for PFDS surfaces.

These statistics can be also modified according to the incidence of each symmetry-axis of one icosahedron. To do so, we considered the 30 edges, 20 facets, and 12 vertexes present on each icosahedron and, assuming an equal opportunity of adsorption for each one, the probability of

FIG. 7. (a) AFM images of the three possible adsorption symmetries. From left to right: S5, S3, and S2. (b) Histogram presenting the number of virus with a characteristic adsorption symmetry at a particular TAO (APTS, GPTS, or PFDS). Symmetries S3 and S2 are grouped. Red lines indicate modification of the statistics with respect to general adsorption efficiency measured by fluorescence microscopy. Green lines indicate modification of the statistics according to the number of possible geometric features leading to such orientation (see text for details). (c) VLP height corresponding to the different symmetries for each functionalized surface. Red line on height axis indicates the VLP diameter along S5. Green line indicates mean radius diameter along S2 and S3.

adsorption results in only 19% for the S5 symmetry with respect to the S2 and S3 considered together (81%). The values of the histogram in Fig. 7(b) were thus multiplied by (100–81)% for S2 and S3 symmetries and by (100–19)% for S5 symmetry and renormalized to fit the 100% chart of Fig. 7(b). The corrected values are indicated in the histogram with a green trace, which illustrates that the real incidence of S5 symmetry, especially on PFDS, considerably exceeds what could be expected from a purely random adsorption and is clear evidence that some physical virus-surface interaction promotes this organization.

Additionally, the height of the different orientations could be used for identification of symmetry as illustrated in Fig. 7(c). This histogram served as a control for the identified symmetry, given that the height of S5 was significantly higher than that of S3/S2. Considering that the transverse distance through the S5 symmetry of the P22 is 1.26 times that of the S3 symmetry (and seemingly to the S2 symmetry), we could compare the effect that the adsorption induces on the VLPs. In APTS, GPTS, and PFDS, the ratios of the measured height in the two different orientations, $h(S5)/h(S23)$, were 1.4, 1.24, and 1.28, respectively. The values remain very close to the nominal values for GPTS and PFDS, but for APTS a strong deformation is induced, which tends to alter the original icosahedral structure.

IV. DISCUSSION

It has been shown that TAOs consist of homogeneous films with an effective medium comprising organic matter and a titanate phase. The functional groups integrated by each organosilane provide a different surface capping, which was evident from the vibrational and photoelectron spectra. The surface molecular groups determine the chemical contribution to the surface tension and, consequently, the differences in contact angle measurements. APTS exhibits a higher acid component, while GPTS has a higher basic component. On the other hand, the perfluoroalkyl terminal group of PFDS determines a very low polar interaction, with an overall surface tension close to half the value obtained for the other two surfaces. The analysis of the topography shows that all the surfaces are homogeneous in the nanometer scale, with RMS roughness values below 1 nm. This suggests that the surface topography has no significant influence in the wetting behavior of the organosilanized surfaces. These low values of roughness also support the application of these films for the study of P22 virus adsorption.

The adsorption of P22 viruses is most efficient on PFDS surface, while the other two adsorb significantly lower quantities of virus (Fig. 6). This fact suggests that the hydrophobic interactions govern the virus adsorption at pH = 7. Beyond the number of virus particles adsorbed on the different surfaces, AFM allows a characterization of the orientation of individual P22 particles. Taking viruses as icosahedra, the virus adsorption can take place through 12 vertices, 30 edges, and 12 facets to result in fivefold, twofold, and threefold symmetry axes, respectively. On a pure probabilistic approach, the adsorption through edges (if the number of possible geometric features is considered as probability determinant) or through facets (if the area associated to the geometric feature is considered as probability determinant) should be favored. However, it has been observed that symmetry S5 is favored on PFDS, while S3 and S2 symmetries have preference on the hydrophilic substrates.

The simultaneous effects on efficiency and privileged adsorption through pentons (S5) further support that hydrophobic interactions favored onto PFDS govern the virus adsorption. This fact can be explained by considering that P22 virus presents hydrophobic residues at the S5 pentons. In PFDS, these regions interact strongly with the surface in order to contact the least amount of water, causing the adsorption. In the case of the other hydrophilic organosilanized surfaces, the hydrophobic domains of the VLP are repelled (pointing to the low sorption observed) and inducing dominant adhesion according to S3 and S2 symmetries, which favor interaction with full VLP edges and facets where hydrophobic residues are not dominant.

Figure 7(c) informs about the conformation of P22 VLPs along the different symmetries of the icosahedron (pentons, facets, and ridges). In regular icosahedra, the coefficient relating the vertex-to-vertex diameter $h(S5)$ (fivefold symmetry) to the facet-to-facet diameter $h(S2)$ is $h(S5)/h(S2) = 1.26$. It is possible to estimate this ratio from the AFM measurements performed on viruses adsorbed on each surface to

FIG. 8. Protein residue maps at the different symmetries of the P22 virus. From left to right: S2 (facets), S3 (edges), and S5 (pentons). Maps are accompanied by the percentage of surface exposed features of each type (hydrophobic in green and polar in red), as well as the relative value (corner down right).

obtain 1.4, 1.24, and 1.28 for APTS, GPTS, and PFDS, respectively. From these values, we can conclude that APTS tends to deform the virus VLP as it is adsorbed through the facets or the ridges (S2/S3). However, no considerable deformation is observed on GPTS and PFDS. Relevantly, adsorption on the latter surface takes place dominantly through pentons. This small deformation can be related, in the case of P22, to a residual stress distribution, which makes the penton areas harder but also more fragile.³⁶

Interestingly, the overall virus adsorption mechanism can be understood in a qualitative way when considering the different distribution of protein residues on the P22 VLPs, as illustrated in Fig. 8. The figure indicates also the proportion of surface exposed hydrophobic versus hydrophilic features, and their ratio. The results clearly indicate that the hydrophobic character of exposed proteins is greatest at the S5 pentons. This can explain both the higher efficiency in virus retention through hydrophobic–hydrophobic interaction on PFDS and the preferred S5 orientation on this surface.

In parallel, for the S2/S3 symmetries, in which the hydrophobic and hydrophilic residue distribution is more even, the interaction with the TAOs is influenced by facet, distance, and deformation (among others). For the case of amino group rich surfaces, close analogues to APTS based TAOs of this work, positive surface charge densities of ca. 30 mC/m² can be reached.³⁷ The consequence is that a strong polar attraction takes place at neutral pH when VLPs are adsorbed through S2–S3, leading to the observed deformation pattern of the VLP height. The surface charge effect is somehow milder in the GPTS surfaces (although this effect can be exacerbated by UV irradiation of the epoxy group in the glycidylxypropyl chain), which agrees with the softer deformation induced to the capsids in whichever orientation of adsorption on GPTS.

The fact that dominant hydrophobic residue areas tend to be surrounded by uncharged polar residues has a strong influence in the VLP assembly³⁸ and can explain the evidenced weak interaction we have observed between pentons in P22 virus and the polar surfaces engineered in this work.

V. CONCLUSIONS

The TAOs employed in this work allowed a study of the effects of hydrophobic/hydrophilic surface motives on interaction with virus residues and the influence on their adsorption. The study of virus adsorption on surfaces with different

components of the surface tension allows explaining the induced adsorption symmetries over the different surfaces taking into account the dominant interactions expected at each surface termination. The observation that PFDS is the most efficient substrate reinforces the idea that hydrophobic interactions enhance the adsorption of P22 VLPs and agrees with the generalized use of alkyl surfaces (such as the ones of HOPG) to enhance this adsorption. Moreover, the use of a perfluorinated surface allows the establishment of a link between more adsorption and a precise S5 favored symmetry, which further supports that hydrophobic interactions govern the P22 adsorption process. The interpretation of the results provides some indirect chemical information about the organization of hydrophobic molecular residues at the vertices of the P22 VLP, which is confirmed by residue distributions analysis based on previous crystallographic studies.²⁵ For the sake of generalization of the results, the study of adsorption on hydrophobic organosilanized surfaces of additional VLP families with alternative distributions of charged/hydrophobic residues is in progress.

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