Published in final edited form as:

J Phys Chem B. 2016 July 07; 120(26): 5896–5906. doi:10.1021/acs.jpcb.6b01023.

Virus-like Surface Plasmon Strain Sensors

Maryam Zahedian[†], Xinlei Huang[†], Irina B. Tsvetkova[†], Vincent M. Rotello[‡], William L. Schaich[¶], and Bogdan Dragnea[†]

Department of Chemistry, Indiana University, Bloomington, USA, Department of Chemistry, University of Massachusetts, Amherst, USA, and Department of Physics, Indiana University, Bloomington, USA

Abstract

The strong configuration dependence of collective surface plasmon resonances in an array of metal nanoparticles provides an opportunity to develop a bioinspired tool for sensing mechanical deformations in soft matter at the nanoscale. We study the feasibility of a strain sensor based on an icosahedral array of nanoparticles encapsulated by a virus capsid. When the system undergoes deformation, the optical scattering and absorption cross-section spectra, as well as the induced electric field profile change. By numerical simulations we examine how these changes depend on the symmetry and extent of the deformation and on both the propagation direction and polarization of the incident radiation. Such a sensor could prove useful in studies of the mechanisms of nanoparticle or virus translocation in the confines of a host cell.

Keywords

surface plasmon; virus; mechanical properties; deformation

Introduction

The optical response of a metallic nanoparticle (NP) depends on its size, shape, and surroundings.^{1,2} In addition collective properties of NP clusters often lead to enhancements in sensitivity and make them suitable for sensing purposes.^{3,4}

We examine here the possible utility of the fact that 2 and 3 dimensional (D) clusters of closely-spaced NPs exhibit optical properties sensitive to changes in the cluster geometry. Our concept is to use optical scattering or absorption to sense configuration changes of a 3D cluster due to interaction with its environment. Towards this end, we suggest making the system controllably malleable and optically addressable by encapsulating multiple NPs inside a protein shell in the form of a regular array with appropriate interparticle distances, Fig. 1(a). We call such a structure a multicore shell (MCS).

Correspondence to: William L. Schaich; Bogdan Dragnea.

[†]Department of Chemistry, Indiana University

[‡]Department of Chemistry, University of Massachusetts

[¶]Department of Physics, Indiana University

Previous work aimed at expanding optical metamaterials research from structures fabricated in 2D planar geometries to bottom-up assembled 3D structures, has shown that clusters of gold NPs can be encapsulated in an amphiphilic block copolymer shell.⁵ However, coordination and number of metal particles per cluster varied significantly. This heterogeneity would make it challenging to form deterministic hyper structures and materials. Moreover, while the previous work discussed various contributions to optical scattering spectra of static MCS structures, it did not examine how spectral properties might change under mechanical deformation. Roller *et al.* recently reported on spectral changes occurring in 2D particle clusters supported by a DNA framework due to drying, with the main dipole resonance shift being the result of shrinkage of the cluster during drying.⁶ Using AuNPs mono-conjugated to three-dimensional DNA “rung” structures, both discrete and extended linear assemblies were controllably prepared via addition of various templating backbone strands.⁷ While exquisite structural control was demonstrated, the relationship between deformation and spectral properties was not pursued.

Structural DNA assembly is very powerful in terms of architectural control, however, it is also known for slow kinetics, and stringent assembly conditions. On the other hand, the efficiency and speed of virus assembly, even in vitro, brings to mind Levinthal’s paradox.⁸ Thus, it is beneficial to explore other venues and seek inspiration from biological systems which have evolved under selective pressures to have precise stoichiometry and assemble over much faster time scales.

In this article, we take a bioinspired approach to address the issues of cluster stoichiometry control and of the relationship between optical properties and mechanical deformation. More specifically, we show for the first time that the remarkably precise stoichiometric control characterizing a small icosahedral virus assembly can be recapitulated by the assembly process of a MCS formed from 12 metal nanoparticles and the coat protein of a plant virus. In addition, we examine by numerical simulations the optical characteristics of an icosahedral particle cluster as a function of the geometry of deformation.

An example of potential application of such mechanical deformation sensors concerns the mechanism of NP entry in cells. Here, studies of microparticles’ pharmacokinetics in relation with drug delivery applications have already suggested that malleability may be an important factor determining blood circulation time.⁹ However, for nanoparticles, no systematic experiments have been performed yet to test the relationship between deformation and cellular entry efficiency, although it was suggested theoretically that nanoparticle stiffness may play a role in membrane adhesion and endocytosis.¹⁰ The malleability idea is directly relevant to nature’s most ubiquitous delivery vectors, viruses, which in certain cases may have to deform during interactions with host cell organelles, e.g. during translocation across membranes, nuclear pore complexes, or plasmodesmata.

The conventional view is that a virus initially attaches to a cell receptor and then the cell membrane distorts to engulf the virus and pull it into the cell (with the help of curvature generating molecules), via a variety of mechanisms.¹¹ However, the virus itself is not thought to change shape, or at least deformability has not been discussed as a part of viral translocation processes. This is somewhat surprising, since upon comparison of the bending

modulus of the plasma membrane¹² ($\sim 32k_B T$) and that of the virus shell¹³ ($\sim 10k_B T$) one would expect that the virus may also deform during the process. Could deformability be an evolutionary virus feature?

Moreover, many small icosahedral viruses are capable of large global structural transitions which allows them to gain function in response to environmental cues.^{14,15} How do these transitions occur?

A clarification of these problems by systematic experimentation with virus-like probes of the type proposed here is important, not only for understanding virus translocation and transformation, but also for the new knowledge it could provide for further development of therapeutic delivery technologies.

The remainder of this article is organized as follows. In the next section we present experimental data demonstrating that the phase diagram for in vitro assembled virus-like particles can be used to generate MCS complexes regular enough for electron microscopy image reconstruction. Then we briefly describe our simulation model and methods, followed by an examination of the physical origins of the optical response of the MCS system, working first with a pair of NPs and then with 12 NPs closely spaced at the vertices of an icosahedron. Finally, we provide a summary of findings and their consequences.

Experimental Studies

In recent years it has been found that, with certain solvent conditions of pH and ionic strength, many virus shells will assemble spontaneously in vitro from coat proteins in solution, on functionalized NP cores.^{16,17} The result of this process are virus-like particles (VLPs) which share many of the structural and physical characteristics of the native virus shell, but encapsulate a NP instead of a nucleic acid genome.¹⁸ Up to now, these VLPs have contained only a single NP, commensurate with the virus shell lumen. Here we show that multiple NPs, smaller in diameter than the native capsid lumen, can be co-encapsulated by self-assembly, thus leading to the formation of an MCS of regular structure determined by the virus shell scaffold and its interactions with NPs.

For encapsulation, we have utilized the coat protein (CP) of the brome mosaic virus (BMV), which has a capsid formed of 180 identical CPs with a ~ 28 nm outer diameter and ~ 10 nm inner diameter lumen. It is known that the BMV CPs will readily form capsids on NPs with structures that map hexagonal close packing onto an icosahedron according to the quasi-equivalence theory of Caspar and Klug.¹⁹ This efficient solution to the problem of packing identical subunits into an isometric cage requires 12 pentamers and $(T-1) \times 10$ hexamers, where T is an integer generated by the progression $T = h^2 + hk + k^2$ and h, k positive integers. The native BMV has a $T = 3$ cage.

However, ~ 6 nm diameter NPs, significantly smaller than the native capsid lumen diameter, will lead to the formation of $T = 1$ VLPs, which have capsids solely formed of pentamers.²⁰ At the same time, $T = 3$ empty capsids will form spontaneously, provided the electrostatic repulsive interactions between the charges uncompensated in absence of an anionic cargo are screened by salt, and the CP concentration is above a critical threshold for the formation of

empty shells.²¹ Therefore, hexamers (and pentamers) will form at high ionic strength, regardless the presence of a template, but only pentamers will form on the surface of smallest NPs, at low ionic strength. These observations led to a strategy for MCS assembly.

We employed 4.6 nm diameter FePt NPs functionalized with a layer of glutamic acid- TEG ligand. This choice of material was due to its particularly narrow size distribution and exceptional stability in solution²² with respect to other metal NPs. For these particles, high curvature and insufficient charge do not allow the growth of a full $T=1$ cage around a NP. Instead, at high CP concentrations, $T=3$ particles grow, which encapsulate 12 NP cores on average. As shown in Fig. 2b many of these NPs trapped inside the shell occupy regular locations. For efficient encapsulation to occur, the CP concentration has to be above ~ 0.5 mg/ml and the CP:NP molar ratio has to be above $\sim 60:1$. The average diameter of the MCSs measured from a set of more than 150 was 28.8 nm with 5% relative standard deviation. Thus the size of the MCS particles is similar to that of wtBMV and consistent with a $T=3$ shell organization.

To further explore the MCS structural details single particle reconstruction of negative-stain TEM images was performed on 4000 particles,²³ Fig. 2c. MCS structure reconstructions are low resolution, but enough structural detail is present to distinguish the presence of hexamers and pentamers. The measured outer diameter and the presence of these oligomers suggest formation of a $T=3$ capsid. Low shell resolution is a drawback of the intrusive nature and indirect contrast of negative stain TEM. However, intrinsic heterogeneity in the particle sample is another possible reason. Nevertheless, it is clear that the fraction of particles exhibiting core array symmetry consistent with icosahedral is much greater than expected from completely random (noncooperative) encapsulation. Furthermore, in most projection classes showing 5-fold symmetry, the observed gaps between NPs are greater than the thickness of their ligand coat, which suggests that NP positioning is mainly due to NP-CP interaction and not to NP-NP interaction. NP gap, abundance of projection images with consistent symmetry, and the correlation of NP position with pentamer position in reconstructed images, all suggest that the NP arrangement is mainly due to the protein scaffold and in a lesser measure to packing constraints in a spherical space (as in the case of spherical packings of colloids in droplets, which also favor icosahedral symmetry).

Ligand coverage and variations in particle surface charge are likely reasons why similar attempts with Au or Ag NPs have resulted in lower yields of icosahedral MCS particles (data not shown). We believe further optimization towards more efficient icosahedral core MCS assembly will be afforded by a systematic study of solution conditions and NP surface chemistry. In the following we will focus on whether the optical properties of icosahedral protein/Au-NP MCSs warrant pursuing such studies.

Numerical simulations

Our basic MCS structure consists of 12 identical, spherical metallic NPs, each of radius a , whose centers are located at the vertices of an icosahedron, which is circumscribed by a sphere of radius R . The icosahedral symmetry group I_h has 3 rotational symmetry elements: C_2 , C_3 , and C_5 . The deformations that we consider are produced by compressions along one

of these axes of rotational symmetry such that the circumscribing sphere becomes a volume-conserving, oblate spheroid. This is a type of symmetry-lowering transformation suggested by compression experiments using atomic force microscopy and by interactions with surfaces which are likely to flatten the virus. Of course, other phenomena may require a reconsideration of the geometry of the transformed state, but for illustration purposes we limit ourselves here to considering the final state as an oblate polyhedron derived from an icosahedron. The NPs are assumed to move along with the circumscribing surface as it stretches. Such a deformation preserves the rotational symmetry of the array around the axis of compression, but interparticle separations will change.

The optical properties of the system are described with dielectric functions, ϵ , that generally depend on frequency, but not on wave vector. For the media outside the NPs we use $\epsilon = n^2$, where $n = 1.33$ is the index of water, approximated as a real-valued constant. By assuming that the outside medium is homogeneous, we ignore optical contrast due to the CP shell, and the NP ligand layer properties. For gold NPs we use the interpolated ϵ from the data of Johnson and Christy.²⁴

Our use of spatially local dielectric functions and sharp boundaries between the NPs and the outside medium limits how close we can allow any 2 NPs to approach each other during compression. This local dielectric function approach is consistent with the existence of steric interactions coming from the ligand layer, which preclude the metal surfaces from touching. In order to account for the presence of a ligand layer imposing a dielectric gap and to avoid the emergence of significant quantum corrections to the response,^{25,26} we have kept NP surfaces at least 1 nm apart.

To calculate the optical response of an MCS we use commercially available software (Lumerical, Inc.) which solves Maxwell's equations on a rectangular grid within the finite-difference time-domain (FDTD) scheme.²⁷ The MCS is subjected to an incident plane wave described by a propagation direction $\hat{k}$, and a polarization direction, $\hat{e}$. The software produces the scattering or the absorption cross-section of the MCS as a function of the incident wavelength, along with the spatial distribution of the total electromagnetic field in and around the MCS.

For the simulations, we have chosen gold NPs of radius $a = 10$ nm. Since we find that the strongest dependence on configuration occurs when pairs of NPs closely approach each other, we begin with a brief study of only 2 NPs in a medium (water), Fig. 3. In this case, we consider that the surfaces of the 2 gold NPs are separated by a gap d and that the unit vector connecting their centers is $\hat{d}$. In Fig. 3 the geometry of illumination is such that $\hat{k}$ is perpendicular to the plane of the page, while $\hat{d}$ lies in the plane. There is a smooth evolution of the scattering cross-section spectra as the NP separation is varied, but it is remarkably different for the cases of the light polarization being parallel or perpendicular to the interparticle axis. This is expected and qualitatively consistent with previous work.²⁸ It is convenient to describe the relative orientation of $\hat{e}$ and $\hat{d}$ by the angle ϕ , defined by $\cos(\phi) = \hat{e} \cdot \hat{d}$. In Fig. 3a, $\phi = 0^\circ$ and the scattering cross-section red shifts and grows in magnitude as d decreases. For the closest separation, the red shift reaches $\lambda = 58$ nm and a second peak has begun to emerge at shorter wavelengths. Both peaks are associated with a strong field

enhancement in the small gap between the two NPs, which implies a significant contribution from higher multipoles.

In contrast, when $\phi = 90^\circ$, the scattering cross-section blue shifts and weakens as d decreases, Fig. 3b. There is only one peak with a maximum blue shift of $\lambda = 3.2$ nm and the field profile on resonance has a simple dipolar appearance on both NPs. The magnitude of the field enhancement is much less in Fig. 3e (perpendicular polarization) than in Fig. 3c, d (parallel polarization).

A qualitative understanding of these results is possible from the viewpoint of a dipole model. This model replaces each NP sphere by a point, dipole-polarizable entity with isotropic polarizability, α :

$$\alpha = n^2 a^3 \frac{\epsilon - n^2}{\epsilon + 2n^2} \quad (1)$$

where ϵ is the bulk dielectric function of the metal.

Consider two such entities placed a distance $r = d + 2a$ apart (i.e. at the centers of the physical NPs) and subjected to an incident beam moving in the plane perpendicular to the line joining the two. This configuration produces a system with an effective, anisotropic polarizability for each entity in the presence of the other. Thus:

$$\frac{\alpha_{\parallel}}{\alpha} = \left[1 - \frac{2\alpha}{n^2 r^3} (1 - inkr) e^{inkr} \right]^{-1}, \quad (2a)$$

$$\frac{\alpha_{\perp}}{\alpha} = \left[1 + \frac{2\alpha}{n^2 r^3} (1 - inkr) e^{inkr} - \frac{\alpha k^2}{r} e^{inkr} \right]^{-1} \quad (2b)$$

where $\alpha_{\parallel}$ and $\alpha_{\perp}$ are polarizabilities per particle when $\phi = 0^\circ$ and $\phi = 90^\circ$, respectively, and $k = 2\pi/\lambda$ with λ the wavelength of light in vacuum. Eqs. 2 a,b in turn lead to scattering cross-sections for the pair of dipole-polarizable entities:

$$\sigma_{\parallel, \perp} = 8\pi k^4 |\alpha_{\parallel, \perp}|^2 \cdot F_{\parallel, \perp} / 3 \quad (3)$$

where the F coefficients are dimensionless interference factors which both reduce to 2 or 4 for $nk r \gg 1$ or $nk r \ll 1$, respectively. This dipole model predicts red (blue) shifts in the scattering cross-section maxima for parallel (perpendicular) orientations. However due to the simplicity of the model, the shifts are not quantitatively accurate, especially for the parallel case. Still there is one more feature that does appear relevant to the full calculation. It can be shown, within this dipole model, that for a general orientation angle, ϕ :

$$\sigma(\phi) = \sigma_{\parallel} \cos^2(\phi) + \sigma_{\perp} \sin^2(\phi) \quad (4)$$

Later on, we shall see that this relation, although not exact beyond the dipole model, works fairly well and proves useful in understanding the optical scattering behavior of regular or deformed icosahedral arrays. In the nonretarded limit, $nkr \ll 1$, this relation also holds for any direction $\hat{k}$ of the incident beam.

To summarize the findings for dimers to this point: computations provide a feeling for the d/a ratio at which the coupling becomes significant. Furthermore, the calculations suggest that especially strong and red shifted scattering will occur from those dimers in an array whose d is small and whose orientation angle with respect to the light polarization is close to zero.

Next we turn our attention to the full icosahedral array. Fig. 4 shows the differences in scattering cross-sections for an undeformed icosahedral MCS as one switches the field polarization direction between different rotational symmetry axes. As one may expect, the optical response is nearly isotropic.

Here, as in the rest of this section, we have chosen the radius of the circumscribing sphere to be $R = 26$ nm. Combined with a gold NP radius of $a = 10$ nm, the nearest neighbor gap between NP surfaces is $d_{nn} = 7.3$ nm. Rotating $\hat{k}$ within a plane perpendicular to the symmetry axis leads to changes comparable to the small differences in Fig. 4. This nearly isotropic response will cease once deformations are allowed, which we now consider.

For each axis of distinct rotational symmetry, we first place the z-axis of our Cartesian coordinate system along the chosen symmetry axis and put the origin of coordinates at the center of the icosahedron. Then we mathematically compress the circumscribing sphere, as described earlier, until two or more NPs have their surfaces within 1 nm.

The first case to discuss is compression along the 5-fold axis with $\hat{a} \parallel \hat{z}$ and $\hat{k} \parallel \hat{x}$, Fig. 5.

The result of deformation on the optical response spectrum is significant. Clearly, in this case, the evolution of the spectrum could be used to measure compression e.g. by a ratiometric time-course measurement of scattering (or absorption) at two wavelengths (say, 600 nm/530 nm).

The drastic changes in spectral response are the result of a global redistribution of the electric field within the MCS structure. For the undeformed initial icosahedron, a cross-sectional map in the equatorial plane perpendicular to the compression axis shows a ring of enhanced electric field due to fields between NPs in the upper and lower 5-member rings of the icosahedron. There are ten nearest neighbor pairs whose gap is bisected by the $z = 0$ plane. It is remarkable that their combined fields exhibit little evidence of discrete sources. In the deformed state, the strongest field enhancement structure for each of the two resonances observed in the spectrum is at the center. It arises from the dimer located on the z-axis. This dimer has $d = 1$ nm and $\phi = 0^\circ$, so it corresponds to the parallel case of Fig. 3a.

Its resonance dominates at the long wavelength peak, which is shifted by ~ 59 nm, but is weaker at the short wavelength peak. Note that such a deformation would practically correspond to a complete flattening of the virus MCS. Nevertheless, this is not unreasonable since we know that, at least for BMV, virus particles will completely flatten on AFM substrates *in air*. Once rehydrated in the right buffer, at least a fraction of the virus particles are able to regain their spherical morphology.

One last feature to note in the field maps of Fig 5 is a slight asymmetry of the results at $+x$ and $-x$. We attribute this to a retardation effect since the incident field propagates along $\hat{x}$, and its wavelength compared to distances in the MCS is large, but not infinite.

Next, we rotate the incident beam so that $\hat{e} \parallel \hat{y}$ and $\hat{k} \parallel \hat{z}$. The spectral cross-section for the deformed MCS shown in Fig. 6 exhibits a modest red shift and increase in strength, but no extra peak.

The dimer responsible for the red shift and the hotspots in Fig. 5 now has $\phi = 90^\circ$ and produces little field enhancement between the two NPs with the smallest separation. However, significant enhancements do appear between NPs in the two 5-member rings perpendicular to the z -axis. Although there are ten such pairs (each with $d = 4.5$ nm), only those with ϕ close to zero produce strong fields. This is evident in Figs. 6 for the icosahedron and deformed array, respectively.

We next examine the 2-fold axis case. For this orientation it is helpful to realize that the 12 NPs of the icosahedron are on the vertices of three identical, but mutually perpendicular, rectangles. We choose the y -axis perpendicular to the tallest rectangle and the x -axis perpendicular to the other vertical (intermediate) rectangle. The horizontal rectangle lies in the $z = 0$ (mirror) plane. For the icosahedron, all three of these mutually perpendicular rectangles are the same size: 27.3 nm by 44.2 nm. They remain rectangles in the deformed array but each develop different dimensions. The compression is stopped by the NPs in the intermediate rectangle, when its short side has dropped from 27.3 nm to 21 nm. There are then two vertical dimers each with $d = 1$ nm.

The spectra and intensity maps for $\hat{e} \parallel \hat{z}$ and $\hat{k} \parallel \hat{x}$ are presented in Fig. 7. The deformed MCS again has a decreased σ and two peaks separated by ~ 64 nm.

Maps of the relative electric field in the $z = 0$ plane are also provided for the wavelengths corresponding to maxima in the spectral scattering cross-section. The $z = 0$ plane cuts through the center of four NPs but does not intersect any other NP. In all three field plots the pairs of hot spots are each associated with a dimer whose $\phi = 0^\circ$. The induced fields around other NPs appear hardly enhanced, but this is an artifact of our choice of only plotting across the $z = 0$ plane. There are strong fields just above and below the four NPs in the $z = 0$ plane and the four NPs at the top and bottom levels. Although their contribution to the total cross-section is significant, their resonance location scarcely shifts under deformation of the structure.

When the incident beam is rotated so that $\hat{e} \parallel \hat{y}$ and $\hat{k} \parallel \hat{z}$ the scattering cross-section for the deformed MCS shows a small increase in strength and a slight red shift of its single peak,

Fig. 8. The two dimers responsible for the hot spots in Fig. 7 now have $\phi = 90^\circ$ and, as in Fig. 3e, produce little field enhancement between the pairs of NPs with the smallest separation. Significant field enhancements appear instead between NPs in the $z=0$ plane, specifically those two pairs whose $\hat{d}$ is parallel to the incident polarization. The enhancement is greater for the icosahedron, where the relevant separation is 7.3 nm. In the deformed array the relevant d has increased to 11.2 nm, which reduces the enhancement.

Finally we consider the 3-fold axis case. Unlike the previous cases, there are no dimers connecting different NP layers that have $\phi = 0^\circ$. There are 4 parallel horizontal planes each containing three NPs. The three NPs in each layer form an equilateral triangle and the orientation of these triangles in adjacent layers is rotated by 180° . With these constraints, the compression of the structure stops when NPs in the first and third, or equivalently the second and fourth layers, reach the $d = 1$ nm limit. Since the nearest neighbor NP pairs most closely aligned with the z -axis connect the same pairs of layers, in order for the field profile plots to be in a plane that cuts through the middle of the dimer gaps with both the smallest d and the smallest ϕ ($= 34.3^\circ$), we plotted the relative field magnitude in a plane parallel to xOy passing midway between the first and third layer of triplets. This plane always cuts through the top part of the NPs in the second layer, while missing (nicking) the NPs in the first and third layers for the icosahedron (deformed MCS), respectively.

Results are shown in Fig. 9 for $\hat{e} \parallel \hat{z}$ and $\hat{k} \parallel \hat{x}$. As in previous cases, for the deformed MCS two resonances are visible with peaks now separated by $\lambda \sim 60$ nm.

When the incident beam is rotated so that $\hat{e} \parallel \hat{y}$ and $\hat{k} \parallel \hat{z}$, the cross-section for the deformed MCS still shows in Fig. 10 two distinct peaks, separated by ~ 69 nm. This behavior is in contrast to that for the previous compression axes, where the 90° rotation of the incident electric field polarization changed ϕ for the dimers with the smallest d between 0° and 90° . The special dimers of the MCS compressed along the 3-fold axis have instead $\phi = 34.3^\circ$ for $\hat{e} \parallel \hat{z}$ and at best $\phi = 55.7^\circ$ for $\hat{e} \parallel \hat{y}$. The smaller changes in ϕ lead to the similar MCS field profiles in Figs. 9,10, consistent with Eq. 4.

The field profiles at the single resonance of the icosahedron are in Fig. 10 due the 3 NPs in the second layer polarizing independently, while the fields in the dimer gaps between NPs in the first and third layer have considerably weakened compared to Fig. 9. This occurs since the angle ϕ for these dimers has changed from 20.9° for $\hat{e} \parallel \hat{z}$ to at best 69.1° for $\hat{e} \parallel \hat{y}$.

Discussion

The idea of exploiting the inherent symmetry arising from biomolecular interactions to organize abiotic nanoscopic materials is not new. For instance, directly relevant to our problem, Wang *et al.* have demonstrated installation of chemical structures, in particular 1.4 nm Au NPs, by covalent bonding at designated positions on the icosahedral protein template.²⁹ However, such NPs are much too small and the distance between them on the template much too great to exhibit optical coupling. More recently, covalently attached Au NPs which displayed surface plasmon resonance on the surface of a virus capsid were investigated.^{30,31} However precision of covalent bonding is decreased for larger NPs, like

the ones considered here, due to the larger surface areas involved. By contrast we have seen that, under certain conditions of CP concentration, pH and ionic strength, self-assembled MCS particles assemble frequently enough and reproducibly enough to provide material for single particle reconstruction of the icosahedral MCS structure. We have also seen that certain NP surface properties must be critical since attempts of Au NPs of the same nominal size as FePt NPs and coated with the same ligand, have not led to the same assembly quality. At this point, the surface properties that play a role in differences in assembly are not known. It is unclear whether they involve physical properties or are more related to the ligand surface chemistry. However, assembly efficiency could be optimized by constructing a phase diagram for VLPs similar to the one constructed by Lavelle *et al.* for virus capsids,³² which will help shed some light on these issues. Such a phase diagram may identify pathways in the parameter space from small, single-core VLPs to $T=3$ empty capsids, which presumably could lead more effectively to MCS structures.

Interparticle distances and correlations between NP position and that of CP oligomers suggests that the NPs prefer pentameric locations. This may be because the contact area between the pentamer and the NP is higher than anywhere else, or because of the assembly pathway. The latter requires an assembly-line scenario wherein complexes formed from proto-pentamers and NPs can assemble together with free protein, Fig. 1c. At this point, it is difficult to determine which of these mechanisms or any other ones may be responsible for this assembly result, but such structures are worth further study since they could harbor the interesting collective properties that the present computational study of optical characteristics suggests.

Specifically, the development of an extra, red shifted peak in the scattering cross-section as the MCS deforms from an icosahedral into an oblate, spheroidal structure is a general feature. Polarization-controlled experiments should allow determining the direction of strain. If one can experimentally determine and hold fixed a symmetry axis, then the evolving red shifted peak could provide a quantitative estimate of deformation extent. In this regard, viruses often bind to multiple cell membrane receptors via chemical moieties arranged in a symmetric pattern on the virus surface, hence via a well-defined orientation to the surface.

The calculations show that the scheme should work for either a 2-, 3-, or 5-fold axis of rotational symmetry. Although we have calculated only total scattering cross-sections, differential cross-sections integrated over a limited solid angle should also develop an additional peak in their wavelength dependence as deformation proceeds.

As a final note, our numerical analysis has focused on identifying the few dimers in a structure whose optical response is primarily responsible for the extra, red shifted peak of the deformed MCS. This approach is well supported by the field profile plots. It has a fair quantitative accuracy in accounting for the size of red shifts. The multiple-dimer picture provides a rapid, qualitative estimate of the magnitude of cross-sectional changes. For different classes of deformation – like uniform shrinking or squeezing into a prolate spheroid – it may not work as well.

Conclusion

In conclusion, virus-like particles encapsulating multiple nanoparticle cores having icosahedral symmetry and obeying the Caspar-Klug classification have been obtained by self-assembly of 4.6 nm anionic metal nanoparticles and bromo mosaic virus coat protein. The metal nanoparticles are bound to the inner surface of the capsid and their locations correlate with the capsid architecture, the most frequent binding site being the coat protein pentamer. Such viral capsids encapsulating multi-core nanoparticles illustrate the principle of using biomolecular interactions inherent to virus self-organization for the synthesis of materials whose properties emerge from their organized structure as much as from their composition.

The optical properties of Au nanoparticle icosahedral arrays modeled after the virus-like particle complex were for the first time considered with emphasis on their sensitivity to mechanical deformation of the structure. It was found that the coupling of nanoparticle resonances leads to sensitivity to deformation reflected in significant spectral changes, which make the virus-like particle approach a promising candidate for nanoscopic strain sensors. Moreover, for the specific deformation patterns considered here, the optical properties were dominated in general by the optical dipole response of dimers of particles. However, certain geometries were not completely describable in terms of dimer couplings, pointing to the importance of collective effects.

Acknowledgments

The authors acknowledge partial support by the U.S. Department of Energy (DOE), Office of Science, Basic Energy Sciences (BES), under Award # DE-SC0010507 (to B.D. for nanoparticle virus-like particle assembly), and by the U.S. Army Research Office under Award # W911NF-13-1-0490 (to B.D. for electromagnetic computations work). V. R. acknowledges support from the National Institutes of Health (NIH GM077173) and the National Science Foundation (NSF CHE- 1307021) (for nanoparticle synthesis and functionalization).

References

1. Kelly KL, Coronado E, Zhao LL, Schatz GC. The Optical Properties of Metal Nanoparticles: The Influence of Size, Shape, and Dielectric Environment. *J Phys Chem B*. 2003; 107:668–677.
2. Tam F, Chen AL, Kundu J, Wang H, Halas NJ. Mesoscopic nanoshells: geometry-dependent plasmon resonances beyond the quasistatic limit. *J Chem Phys*. 2007; 127:204703. [PubMed: 18052442]
3. Haes AJ, Van Duyne RP. A unified view of propagating and localized surface plasmon resonance biosensors. *Anal Bioanal Chem*. 2004; 379:920–30. [PubMed: 15338088]
4. Fan JA, Wu C, Bao K, Bao J, Bardhan R, Halas NJ, Manoharan VN, Nordlander P, Shvets G, Capasso F. Self-assembled plasmonic nanoparticle clusters. *Science*. 2010; 328:1135–8. [PubMed: 20508125]
5. Urban AS, Shen X, Wang Y, Large N, Wang H, Knight MW, Nordlander P, Chen H, Halas NJ. Three-dimensional plasmonic nanoclusters. *Nano Lett*. 2013; 13:4399–403. [PubMed: 23977943]
6. Roller EM, Khorashad LK, Fedoruk M, Schreiber R, Govorov AO, Liedl T. DNA-assembled nanoparticle rings exhibit electric and magnetic resonances at visible frequencies. *Nano Lett*. 2015; 15:1368–73. [PubMed: 25611357]
7. Lau KL, Hamblin GD, Sleiman HF. Gold nanoparticle 3D-DNA building blocks: high purity preparation and use for modular access to nanoparticle assemblies. *Small*. 2014; 10:660–6. [PubMed: 24115591]

8. Dykeman EC, Stockley PG, Twarock R. Solving a Levinthal's paradox for virus assembly identifies a unique antiviral strategy. *Proc Nat Acad Sci US A*. 2014; 111:5361–6.
9. Petros RA, DeSimone JM. Strategies in the design of nanoparticles for therapeutic applications. *Nat Rev Drug discovery*. 2010; 9:615–27. [PubMed: 20616808]
10. Yuan H, Li J, Bao G, Zhang S. Variable Nanoparticle-Cell Adhesion Strength Regulates Cellular Uptake. *Phys Rev Lett*. 2010; 105:138101. [PubMed: 21230813]
11. Marsh M, Helenius A. Virus entry: Open sesame. *Cell*. 2006; 124:729–740. [PubMed: 16497584]
12. Boal, D. Mechanics of the cell. Cambridge University Press; Cambridge UK; New York: 2002.
13. Nguyen TT, Bruinsma RF, Gelbart WM. Elasticity theory and shape transitions of viral shells. *Phys Rev E*. 2005; 72:51923.
14. Speir JA, MUNSHI S, Wang GJ, Baker TS, Johnson JE. Structures of the Native and Swollen Forms of Cowpea Chlorotic Mottle Virus Determined by X-ray Crystallography and Cryoelectron Microscopy. *Structure*. 1995; 3:63–78. [PubMed: 7743132]
15. Lata R, Conway JF, Cheng N, Duda RL, Hendrix RW, Wikoff WR, Johnson JE, Tsuruta H, Steven AC. Maturation Dynamics of a Viral Capsid. *Cell*. 2000; 100:253–263. [PubMed: 10660048]
16. Chen C, Kwak ES, Stein B, Kao CC, Dragnea B. Packaging of gold particles in viral capsids. *J Nanosci Nanotech*. 2005; 5:2029–2033.
17. Loo L, Guenther RH, Lommel SA, Franzen S. Encapsulation of nanoparticles by Red Clover Necrotic Mosaic Virus. *J Am Chem Soc*. 2007; 129:11111–11117. [PubMed: 17705477]
18. Aniahyei SE, DuFort C, Kao CC, Dragnea B. Self-assembly approaches to nanomaterial encapsulation in viral protein cages. *J Mater Chem*. 2008; 18:3763–3774. [PubMed: 19809586]
19. Caspar DLD, Klug A. Physical Principles in Construction of Regular Viruses. *Cold Spring Harbor Symp Quant Biol*. 1962; 27:1. [PubMed: 14019094]
20. Sun J, DuFort C, Daniel MC, Murali A, Chen C, Gopinath K, Stein B, De M, Rotello VM, Holzenburg A, et al. Core-controlled polymorphism in virus-like particles. *Proc Nat Acad Sci U S A*. 2007; 104:1354–1359.
21. Hagan MF. Modeling Viral Capsid Assembly. *Adv Chem Phys*. 2013; 155:1–67.
22. Srivastava S, Samanta B, Arumugam P, Han G, Rotello VM. DNA-mediated assembly of iron platinum (FePt) nanoparticles. *J Mater Chem*. 2007; 17:52–55.
23. Ludtke SJ, Baldwin PR, Chiu W. EMAN: Semiautomated software for high-resolution single-particle reconstructions. *J Struct Biol*. 1999; 128:82–97. [PubMed: 10600563]
24. Johnson PB, Christy RW. Optical Constants of the Noble Metals. *Phys Rev B*. 1972; 6:4370–4379.
25. David C, García de Abajo FJ. Spatial Nonlocality in the Optical Response of Metal Nanoparticles. *J Phys Chem C*. 2011; 115:19470–19475.
26. Marinica DC, Kazansky AK, Nordlander P, Aizpurua J, Borisov AG. Quantum plasmonics: nonlinear effects in the field enhancement of a plasmonic nanoparticle dimer. *Nano Lett*. 2012; 12:1333–9. [PubMed: 22320125]
27. Taflove, AC., Hagness, S. Computational electrodynamics: the finite-difference time-domain method. 3. Artech House; 2005. p. 1006
28. Romero I, Aizpurua J, Bryant GW, de Abajo FJ, García De Abajo FJ. Plasmons in nearly touching metallic nanoparticles: singular response in the limit of touching dimers. *Opt Express*. 2006; 14:9988–9999. [PubMed: 19529393]
29. Wang Q, Lin TW, Tang L, Johnson JE, Finn MG. Icosahedral virus particles as addressable nanoscale building blocks. *Angew Chemie-International Ed*. 2002; 41:459–462.
30. Fontana J, Dressick WJ, Phelps J, Johnson JE, Rendell RW, Sampson T, Ratna BR, Soto CM. Virus-templated plasmonic nanoclusters with icosahedral symmetry via directed self-assembly. *Small*. 2014; 10:3058–63. [PubMed: 24733721]
31. Lebedev N, Griva I, Dressick WJ, Phelps J, Johnson JE, Meshcheriakova Y, Lomonosoff GP, Soto CM. A virus-based nanoplasmonic structure as a surface-enhanced Raman biosensor. *Biosens Bioelectron*. 2016; 77:306–14. [PubMed: 26432193]
32. Lavelle L, Gingery M, Phillips M, Gelbart WM, Knobler CM, Cadena- Nava RD, Vega-Acosta JR, Pinedo-Torres LA, Ruiz-Garcia J. Phase Diagram of Self-assembled Viral Capsid Protein Polymorphs. *J Phys Chem B*. 2009; 113:3813–3819. [PubMed: 19673134]

Figure 1.

a) Schematic of a cluster of 12 nanoparticles placed at the vertices of a regular icosahedron.

b) Cartoon of virus-like particle assembly of coat proteins into a polyhedral cage encapsulating one NP of diameter commensurate with the lumen of the cage. Here, local curvature has been depicted as different at pentamer (light green) and hexamer (dark green) locations, with pentamers providing all the curvature required for shell closure. c)

Hypothetical pathway of MCS assembly in the case when pentamers accommodate curvature more readily than hexamers. This situation would correspond to the formation of a NP cluster like the one depicted in 3D in a).

Figure 2.

a) Low magnification transmission electron microscopy of MCS particles of uniform 28 nm diameter. b) Classes of high magnification frames with single MCSs with internal structure showing symmetry. c) Result of single particle reconstruction model of the MCS structure. NP locations are on the 5-fold symmetry axes of the coat protein shell.

Figure 3.

a) FDTD calculation of the optical scattering cross-section, σ_2 , as a function of wavelength for two Au NPs for different gaps in the case of incident field polarization parallel to the dimer axis, $\hat{d}$. b) Same for incident field polarization perpendicular to $\hat{d}$. c) and d) Maps of $\ln|E/E_0|$ for $z=0$ at the two wavelengths corresponding to local maxima in σ_2 with $d=1$ nm and incident polarization parallel to $\hat{d}$. $\vec{E}$ and $E_0 \cdot \hat{e}$ are the total and incident electric field amplitude, respectively. e) Map of $\ln|E/E_0|$ for $z=0$, at the peak wavelength of σ_2 with $d=1$ nm and incident field polarization perpendicular to $\hat{d}$. The dotted lines in (a), (b) show what the scattering cross-section would be if the particles did not feel each other's polarization field and their scattered waves combined with perfect constructive interference.

Figure 4. Scattering cross-sections of a 12 gold NP icosahedral MCS model with $a = 10$ nm and $R = 26$ nm. The incident electric field is aligned with the 2-fold (red), 3-fold (dashed blue) or 5-fold (green) rotational symmetry axis.

Figure 5.

Compression and incident polarization are both along the 5-fold axis. Scattering cross-sections as a function of wavelength for the initial (icosahedral) and the final (compressed) MCS model and maps of $|E/E_0|^2$ in the mirror symmetry (equatorial) plane perpendicular to the compression axis. Wavelengths and MCS states for each of the maps are identified with symbols on the spectral cross-section graph.

Figure 6.

Scattering cross-sections and relative field maps with the same symmetry axis as in Fig. 5. Compression is along the 5-fold axis but the incident electric field is perpendicular to this, while its propagation direction is along it. The field maps are in the equatorial plane and plot $|E/E_0|$ on a linear scale.

Figure 7.

Compression and incident polarization are both along the 2-fold axis. Scattering cross-sections as a function of wavelength for the initial (icosahedral) and the final (compressed) MCS model and maps of $\ln|\vec{E}/E_0|$ in the mirror symmetry (equatorial) plane perpendicular to the compression axis. Wavelengths and MCS states for each of the maps are identified with symbols on the spectral cross-section graph.

Figure 8.

Scattering cross-sections and relative field maps with the same symmetry axis as in Fig. 7. Compression is along the 2-fold axis but the incident electric field is perpendicular to this, while the propagation direction is along it. The field maps are in the equatorial plane and plot $|\vec{E}/E_0|$ on a linear scale.

Figure 9.

Compression and incident polarization are both along the 3-fold axis. Scattering cross-sections as a function of wavelength for the initial (icosahedral) and the final (compressed) MCS model and maps of $|E/E_0|^2$ in a plane perpendicular to the compression axis and midway between the first and third layer of NP triplets. Wavelengths and MCS states for each of the maps are identified with symbols on the spectral cross-section graph.

Figure 10.

Scattering cross-sections and maps of relative field $|\vec{E}/E_0|$ with the same symmetry axis as in Fig.9. Compression is along the 3-fold axis but the incident electric field is perpendicular to this, while the propagation direction is along to it. The field maps are across the same plane as in Fig. 9.