Published in final edited form as:

Phys Chem Chem Phys. 2013 January 28; 15(4): 1052–1064. doi:10.1039/c2cp42969a.

Visualization of Clusters in Polymer Electrolyte Membranes by Electron Microscopy

Sergey Yakovlev^a and Kenneth H. Downing^b

Sergey Yakovlev: syakovlev@lbl.gov; Kenneth H. Downing: khdowning@lbl.gov

^aMaterials Sciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, CA, U.S.A.

^bLife Sciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, CA, U.S.A, Fax: 510-486-6488; Tel: 510-486-5941

Abstract

The morphology of ionic clusters that form in polyelectrolyte membranes has a strong effect on transport and electrical properties. In spite of considerable research efforts the link between morphology and properties has not been clearly established, mainly due to difficulties in assessing nanoscale morphology. Electron microscopy (EM) has the potential to visualize morphology. However success in visualization has so far been moderate. In this review we focus on the potential of EM techniques to characterize the ionic domains. We use both experimental data and models to compare the capabilities of several EM techniques: BF TEM, HAADF, core-loss EELS, and low-loss EELS in projection imaging and STEM modes. The main problems common for all these EM modes are radiation damage and overlap of features in projection. Our models show that core loss EELS with exposures that are below the typical damage threshold is incapable of resolving 2 nm diameter sulfur-rich clusters in PEMs. While low loss EELS requires lower exposure the insight it can provide is quite limited. HAADF and BF TEM present the most effective modes for imaging the sulfur clusters in PEMs. While BF TEM uses scattered electrons more efficiently, HAADF using slightly higher doses can provide unique information due to in-focus imaging and transparent interpretation of the images. Fortunately, in at least some interesting cases the clusters themselves are much more radiation resistant than the polymer and can be studied at exposures high enough to obtain clear images. Our simulations also show that tomographic 3D reconstruction provides the best approach for solving the overlap problem. In spite of the abilities of electron tomography, data obtained from all EM techniques improves if thin sections are studied. We briefly discuss methods for obtaining such sections.

Introduction: PEM Morphology for Fuel Cell Applications

Polymer electrolyte membranes (PEMs) have been a focus of scientific studies for several decades. Recent interest in these materials is due to the ability to achieve high proton conductivity, which enables their use in hydrogen fuel cells. Other applications for PEMs include removal of salt and other ionic species from water. In spite of their industrial

importance, many questions remain unanswered regarding the interplay between nanoscale morphology within the membrane and transport through it. The major obstacle to answering these questions is the difficulty of directly visualizing the membrane morphology. Electron microscopy (EM), one of the most powerful tools for visualization and analysis of nanostructure, has been used to study the structure of PEMs since their discovery. In spite of considerable efforts, success in obtaining functional insights has been modest. The purpose of this perspective is to provide an overview of the EM techniques used in PEM studies and of the observations made by these techniques, as well as to discuss and quantify the techniques' limitations, in order to provide insights on how the field may develop in the future.

PEMs are composed of copolymers containing ionic and neutral monomers. Their properties arise from having both types of monomers in a single molecule. Ionic and neutral species phase separate, creating a system with competing properties within the bulk material: it is hydrophilic and conducts protons but also resists dissolution in water. Typically the balance between these properties is achieved by careful control over the ratio of ionic and neutral monomers. Polymers with too many ionic groups either dissolve when exposed to water or have poor mechanical properties due to excessive swelling. Polymers with too few ionic groups exhibit low proton conductivity.

The type of ions attached to the polymer molecule is also important. Most PEMs investigated contain sulfonic acid groups at the ionic sites. PEMs containing carboxylic acid groups generally have lower conductivity than sulfonic acid-based PEMs¹. Recently there have been a few reports of phosphonic and/or phosphinic acid-containing polymer membranes^{2–3}. These materials may offer high stability, but they also have lower ion conductance than sulfonic acid-based systems. Because of their better conductive properties we limit the scope of this perspective to sulfur-containing PEMs.

In the simplest case, the ionic species are randomly distributed, attached either to a hydrophobic backbone or to side chains grafted to the backbone. Block copolymers with an ion-containing block and a nonionic block are also used as PEMs, and offer possibilities to control the membrane mechanical properties enhanced by self organization of the blocks. In this case the ion containing part of the molecule still has to be resistant to dissolution. In all studies thus far, the ion-containing block is a random copolymer of ionic and nonionic monomers.

While we discuss data obtained from a wide variety of systems, we focus on two materials. One, the random copolymer Nafion, is currently the most widely used PEM. The ionic group in Nafion is situated on the end of an ether sidechain randomly attached to a hydrophobic tetrafluoroethylene backbone that provides excellent mechanical properties. The second material is the block copolymer poly(styrene sulfonate)-*block*-poly(methyl butylene) (PSS-PMB). The PSS block is a random copolymer of unsulfonated and sulfonated polystyrene monomers. While the conductivity of PSS by itself is reasonably good, it is generally considered to be too brittle, which is balanced by addition of the very soft PMB block.

Although the potential of EM in PEM studies has long been recognized, most of our understanding of the morphology of PEMs is actually based on small angle x-ray scattering (SAXS). An advantage of the scattering experiments is that they provide information averaged over macroscopic samples, while achieving nanometer-scale resolution. The major disadvantage is ambiguity of the results. While scattering results in a three-dimensional Fourier transform of the electron density distribution within the sample, the phase of the complex Fourier transform cannot be recorded. This prevents unambiguous determination of the electron density distribution (or equivalently, sample morphology). There are a few specific cases in which this problem can be easily solved such as crystalline materials with long-range periodicity and infinitely dilute solutions of monodisperse particles, but those cases are rarely relevant to the study of PEMs. There has been considerable effort to restore the real-space information directly from diffraction patterns, and while this approach has had some interesting successes⁴ it seems generally not definitive in the context of PEMs⁵⁻⁶. The algorithms used in this iterative approach are dependent on starting conditions, and, with PEM scattering data, convergence to the correct morphology is not guaranteed.

SAXS experiments on PEM reveal broad peaks with positions corresponding to 2–6 nm⁷⁻⁸. It is generally agreed that the position of the maximum corresponds to the average distance between scattering centers, but the meaning of the peak width and shape is a subject of controversy. Because the insight that scattering experiments provide on the size and geometry of the phases is limited, numerous theoretical simulations have been performed in attempts to deduce the true morphology. Various models for the ion-containing phase in PEMs have been suggested, including connected spheres⁹, core-shell structures¹⁰, hard spheres¹¹, bundles of rods¹², parallel cylindrical channels¹³, and lamellar channels¹⁴. Each of these models is consistent with the experimental scattering profiles, but the scattering data do not support selection of one model over the others. Important aspects of the conductive phase such as connectivity (branched or linear) and cross-sectional area (circular or lamellar) remain ambiguous.

The nanometer size scale of PEM morphology is ideally suited for study by electron microscopy. Today EM has been used to solve a great variety of materials characterization problems, using a wide range of modes available for conducting experiments such as direct imaging, diffraction, microanalysis, and three-dimensional (3D) tomography. Imaging and analysis modes include bright field transmission electron microscopy (BF TEM), scanning transmission electron microscopy (STEM), high angle annular dark field electron microscopy (HAADF), and electron energy loss spectroscopy (EELS). While the major problems related to analysis of PEMs are common for all these methods, the quantitative limits are different, as we discuss below.

It is important to recognize that EM is not simply an alternative to the scattering experiments. An intrinsic disadvantage of electron microscopy is that it generally examines only a small portion of a sample. Thus the information obtained by EM is prone to artifacts, such as nonuniformities in the sample due to poor sample preparation. The real value of electron microscopy is that it complements the scattering experiments and allows one to build robust morphological models which eliminate the ambiguity in the interpretation of the scattering experiments. In practice, though, clear visualization of morphology is not easy to

achieve. While numerous images of PEMs have been published, their interpretation remains a major challenge. For example, high resolution, phase contrast images of PEMs often resemble images of amorphous carbon films and provide little insight on morphological details. Improving the quality of the images is difficult due to three limitations: low contrast, high radiation sensitivity, and the small size and random character of the conducting domains. In projection, these factors result in weakly defined, noisy images with overlapping features.

While the interpretation of the SAXS results is subject of controversy, most of the published EM data point to a single morphology model: a network of ionic clusters distributed in a relatively uniform matrix^{15–16}. However the sizes, shapes and distributions of the clusters remain to be determined. Here we discuss the limitations that need to be overcome to perform the necessary analysis and their effects on the microscopy investigation of PEM.

The Two Major Limitations in EM of PEM: Radiation Sensitivity and Low Contrast

Radiation sensitivity is one of the most serious challenges for PEM imaging by electron microscopy. In the last several years, the field of electron microscopy has made significant progress, due in large part to improvements in instrumentation. With better mechanical and electrical stability and the development of aberration correctors microscope resolution has improved significantly. However, PEMs belong to the “soft matter” family of materials, a family that has not yet been significantly affected by these improvement. The resolution in soft materials imaging is typically limited not by the microscope resolution but rather by radiation sensitivity. Irradiation of soft matter with high energy electrons damages the sample, and may completely destroy the original structure. In order to avoid excessive damage, the exposure has to be limited, but this, in turn, results in low counting statistics and a high level of noise. It is often challenging to find the proper tradeoff between noise and damage when the length scale of the structures of interest approaches the nanometer range.

Qualitative aspects of the mechanism of radiation damage in polymers are well understood, but detailed studies have been done on only a few materials. High-energy electron irradiation causes a cascade of processes in the material including ionization, polymer chain scission, radiation-induced diffusion of atoms, and beam-induced sputtering of the material. Manifestation of damage in the electron microscope depends on the material composition. For example, the chemical structure of polystyrene rapidly changes under an exposure of 10^4 e/nm², evidenced by changes in the low loss EELS spectrum^{17–18}. In spite of this, polystyrene does not exhibit significant mass loss under such exposures, and observations in bright field or dark field TEM or STEM do not reveal significant changes. In contrast, polytetrafluoroethylene, the major component of Nafion, exhibits substantial mass loss and instability under irradiation¹⁹, which affects bright field and dark field TEM before the chemical changes can be detected by EELS²⁰. Chemical changes can be detected in EELS spectra of Nafion at higher exposures²¹. It is reasonable to expect that all polymeric materials will undergo some kind of chemical change under the electron beam. However if these changes are not associated with rapid mass loss, they do not necessarily pose a

problem for imaging of specimen morphology. Moreover, even mass loss may be tolerated to some degree, if the loss is confined to the surface and one is interested primarily in the bulk morphology.

In the context of PEM morphology imaging, the most serious problem caused by irradiation is electron beam-induced mass transfer and diffusion which would distort the appearance of the nanoscale structures. The amount of atom movement inside the sample depends on the material properties and atomic composition. Light atoms such as hydrogen possess high mobility which can cause problems such as bubbling^{22–23} and shrinkage. In contrast, heavier atomic species such as sulfur are more difficult to move, and their distribution at the nanometer scale can be preserved. For the study of PEMs, limited atomic mobility is very important, because it may enable the high exposures necessary for high-resolution imaging and compositional mapping. For many PEMs low mobility of the ionic species may present the only opportunity for obtaining high resolution data. In spite of the importance of irradiation-induced diffusion, this issue has generally been ignored in studies of PEMs. Here we present an experiment which illustrates the stability of the sulfur distribution to irradiation even when the chemical composition and density of the material change noticeably.

To illustrate the low mobility of sulfur in PEMs under irradiation, we examined a PSS-PMB block copolymer with molecular weights of the blocks 3.2 and 8.1 kg/mol respectively. The degree of sulfonation for the PSS was 40%. (All of the data that we discuss for the PSS-PMB system was obtained from this material.) Figures 1a and 1b show BF TEM images of a solvent-cast sample about 50 nm thick. The image in Figure 1a was collected using a low exposure of 10^3 e/nm², comparable to what is used for high resolution studies of proteins. Thus we can be confident that the image reflects the undamaged structure. One can see that the sample is separated into a brighter PMB phase and darker PSS phase which has a branched appearance. Contrast between in PSS and PMB microphases arises due to the fact that PMB is less dense than PSS. However the contrast between the phases is relatively low, and substantial defocusing was needed to obtain Figure 1a. After an exposure of 50,000 e/nm² and using the same defocus value, most of this contrast disappears as shown in Figure 1b. Further irradiation does not significantly change the appearance of the sample. The dependence of the image on exposure is not difficult to explain, as both phases are predominantly hydrocarbons. Exposure to ionizing radiation causes rapid loss of hydrogen^{19, 22, 24}. As the exposure accumulates, both phases are transformed into a saturated state with low hydrogen content. At this point the phases can no longer be described as PSS and PMB, but as radiation-stable hydrocarbons with a high concentration of double bonds. These phases have similar densities and therefore show no contrast in EM images. An interesting observation can, however, be made in these figures. While the outline of the PSS phase has disappeared, numerous dark spots seen in Figure 1a are also seen in Figure 1b. In fact, all of the dark spots seen in the damaged material in Figure 1b can be traced to the undamaged material in Figure 1a. We have marked five of the spots with circles on both images to illustrate this. We suggest that the spots are in fact ionic clusters with high sulfur content, as predicted several decades ago¹⁵. A high-resolution HAADF image collected with an exposure of 10,000 e/nm² from the same material (Figure 1c) supports this interpretation, showing bright spots that most probably contain the heavy sulfur

atoms. It is evident that the sulfur-containing clusters remain trapped in their original location in spite of irradiation-induced damage.

While having sulfur in PSS-PMB enables detection of the morphology of the hydrophilic phase, in many cases this may not be sufficient. In Nafion, for example, sulfur atoms are attached to an amorphous side chain with low density while the majority of the hydrophobic phase is a crystalline, Teflon-like phase with higher density⁶. The presence of S atoms in the low density phase reduces the overall contrast between ionic clusters and the matrix. One thus expects greater difficulty in determining the morphology of sulfur-containing clusters in Nafion.

One approach for increasing the contrast is to mark the hydrophilic phase with heavier atomic species using well-established staining techniques. Results obtained using this approach can sometimes be easily interpreted but may also be plagued by artifact. An example of such work has been presented by Xue *et al.*²⁵. In this work, BF TEM images of untreated Nafion clearly show uniformly distributed spots which could possibly be ionic clusters. However, the resolution of the images is not sufficient to interpret them with certainty. Even determination of the number of different phases is challenging. After staining with ruthenium tetroxide, RuO₄, not only is the contrast much more pronounced but the sizes of the clusters are larger than those in the unstained sample. It is likely that the clusters seen are not just the sulfur-rich areas but the entire hydrophilic domains including the side chains. The authors use infrared spectroscopy to provide convincing evidence that ruthenium oxide does not interact chemically with the constituents of Nafion, and that the main reaction during the staining is the oxidation of water, some amount of which is always present in Nafion. After staining, three different phases can be distinguished in the images, but whether or not all of these phases were present in the unstained sample is not clear. This work demonstrates that, while staining may provide additional insight into morphology, it does have the disadvantage that the stain can affect untargeted portions of the sample. An additional difficulty with staining is that proper stains are not always available for all materials. For example, identifying the sulfur-rich clusters in PSS by staining with ruthenium tetroxide would not be useful, because ruthenium would attack the aromatic ring of styrene and stain the entire PSS phase.

Another contrast-enhancing approach available for PEMs is ion exchange. This approach is preferable to staining because it enables more targeted contrast enhancement. In this technique, the sample is soaked in a solution of a heavy element salt and then carefully rinsed. As a result, ions of the heavy element are attached to the ionic sites in the material, improving visualization of the ionic sites. A large number of observations has been reported using a variety of heavy ions including Pb, Cs, Fe, Zn, and K. It was found, however, that the observed morphology may depend on the type of ion that was used in the ion-exchange step. For example, Li *et al.*²⁶ studied samples of acidic PSS as well as PSS neutralized with nickel, zinc and cesium. The acidic samples and those doped with cesium did not show any sign of microphase separated ionic domains, while samples doped with zinc and nickel clearly revealed the presence of ionic domains. More examples are discussed below that show advantages and disadvantage of contrast enhancement. Since PEMs are generally used in the acid form, it is desirable to image material without heavy ion exchange.

Microscopy Techniques for PEM Imaging: Main Limitations, Comparison and Suggestions

Electron microscopy encompasses a range of techniques that can be used for PEM imaging. The most important imaging modes are bright field TEM (BF TEM) and HAADF imaging in the STEM mode. Historically most of the EM data on PEMs have been obtained using BF TEM, since it has the simplest instrumental requirements. The main advantage of BF TEM is that it uses all of the scattered electrons except those cut off by the objective aperture. The disadvantages of this technique are the need for defocused imaging for contrast enhancement, and the relatively low sensitivity to atomic number of the light elements typically found in PEMs. HAADF imaging is more sensitive to atomic number, but in this mode only high angle scattering is registered; the rest of the scattered electrons are simply ignored. The difference between the efficiencies of BF TEM and HAADF is demonstrated in Fig. 2, which shows the relative numbers of electrons detected in the two modes. Figure 2a shows the experimentally measured intensity of electron scattering from a PSS sample as a function of scattering angle. This plot was produced by collecting a series of diffraction patterns at various camera lengths, azimuthally integrating the intensity of the diffraction patterns to obtain the integrated scattering intensity, I_n , as a function of the angle, θ , and then splicing individual data sets to obtain a single curve covering a wide angular range. As expected, I_n decreases rapidly with increasing θ . To compare the number of electrons recorded in BF TEM and HAADF modes, Figure 2b shows the integral of $I_n(\theta)$ from the lowest angle where measurements were possible to the angle of interest. The total signal recorded in the 50–100 mrad range, as might be used for HAADF, is only 10^9 counts, which is a factor of 10 lower than the signal recorded in the range 5–10 mrad which would contribute to bright field. If we assume that noise in the recorded signal is predominantly Poisson noise, then with the same exposure the 10-fold reduction in signal in HAADF relative to BF TEM will produce a signal-to-noise ratio in the HAADF image lower than that of the BF TEM image by a factor of 3.2 ($= 10$). In most HAADF experiments even larger scattering angles are used to enhance the atomic number dependence, yielding an even weaker signal.

To some extent, the loss in signal strength of HAADF is offset by higher scattering at large angles from heavier atoms. The difference in noise levels may be directly compared with this difference in scattering contrast. In Figure 2c we plot the scattering cross-sections of sulfur and carbon as a function of θ ²⁷. Figure 2d shows the ratio of the cross-sections as a function of θ . While this ratio increases with increasing θ , the factor by which it increases is much less than 3 in the accessible range of θ . We conclude that, for images of sulfur clusters collected in a dose-limited mode, the signal-to-noise ratio in HAADF is likely to be less than that of BF TEM. While this conclusion seems unfavorable for HAADF imaging, the technique does have other important advantages. It requires no defocus for contrast enhancement, and, most importantly, allows easier interpretation of the images, due to a linear dependence between image intensity and material scattering cross-section. While in most cases these advantages combine with a need for using a higher exposure, HAADF imaging frequently provides unique information important for understanding the function of PEMs.

We next review some of the observations that have been made in imaging of PEMs. The number of reports describing the visualization of ionic clusters is surprisingly high. Unfortunately most of these papers did not report the irradiation exposure that was used, which makes comparison of the results difficult. In most cases the applied exposure probably was sufficiently high for the material to be transformed into a damaged and stable state.

The morphology of a copolymer with PSS chains grafted onto either poly(trifluorochloroethylene) or polyethylene was investigated by Jozef²⁸. Samples were loaded with lead by immersion in a solution of $\text{Pb}(\text{NO}_3)_2$ and studied by BF TEM. Both samples revealed heterogeneous structure, and dark spots with sizes about 20–30 and 40–60 nm, respectively, were observed. These sizes are about an order of magnitude larger than cluster sizes estimated from SAXS. In the same work, a Nafion membrane was loaded with lead and features with sizes in the 3–6 nm range were reported. Because images were collected at relatively low magnification, more details about the morphology could not be gleaned. Images obtained from Nafion without lead ion treatment revealed no dark features. The author therefore concluded that the observed dark features reflect the location of higher concentrations of ionic groups.

Handlin *et al.*²⁹ investigated the morphology of a variety of sulfonated polymers after neutralizing the acidic groups with Cs counterions. The authors reported that they observed no nanoscale features in any of the samples without ion exchange treatment. However, immersion of a PSS film into CsOH resulted in formation of domains with diameters in the 6–10 nm range. Careful examination of these domains revealed that they were crystals of CsOH deposited onto the surface of the film. The authors concluded that ionic domains in their samples were smaller than 2 or 3 nm and therefore could not be detected.

In work by Fujimura *et al.*¹⁰, TEM images were obtained from sections of Nafion loaded with cesium ions. Ionic domains with sizes similar to those observed by Józef²⁸ were reported. However, the size of the domains increased under irradiation, making it difficult to characterize the native structure of the ionic clusters in Nafion. In more recent work by Peron *et al.*³⁰ unstained Nafion NR-211 membrane was investigated. While a different method of sample preparation was used (solvent casting, which we will discuss later), images obtained by TEM were similar to the images of Nafion 117 obtained earlier²⁵, with scattered dark spots.

Dalmas *et al.*³¹ prepared Zn-neutralized samples of PSS and obtained BF TEM images showing a highly nonuniform distribution of thin, curved features with various lengths approaching several hundred nanometers, which the authors interpreted as ionic aggregates. High resolution images presented in this paper also show a network of nanometer sized spots uniformly distributed in the material. While the resolution of these images is not sufficient to measure the sizes, the contrast of the spots is high enough to conclude that they contain heavy Zn ions.

One of the earliest studies of PEMs using STEM was conducted by Williams *et al.* in 1989³², who studied polystyrene with dicarboxylic acid functional groups that were

neutralized by Ba. The morphological features observed in the images varied widely and are thus unlikely to be the clusters that one expects in PEMs. STEM Images showing a more uniform distribution of the ionic sites were not presented until 1998 when a sample of Zn-neutralized poly(ethylene-co-methacrylic acid) copolymer was imaged in HAADF and bright-field STEM modes³³. In both modes, micrographs showed spots representing an electron-dense phase, with diameters of 2.8 ± 0.5 nm. In a subsequent publication³⁴, a similar sample was imaged in the HAADF mode with the sample held at two different tilt angles. This tilting enabled determination of the size of the features in different directions. The aggregates analyzed did not show significant anisotropy, as expected. However it was shown that the number density of aggregates depends on the extent of neutralization. This last observation, along with the other artifacts mentioned, illustrates the importance of imaging of membrane in the unmodified, acidic state.

Microanalysis methods (EELS)

One of the results of the rapid development in electron microscopy equipment is progress in microanalysis techniques that allow not only collecting images of samples but also extracting spatially resolved chemical information. Application of these techniques to imaging PEMs can potentially identify the composition of ionic clusters, and ultimately reveal the geometry of the conductive channels. Energy-dispersive X-ray spectroscopy (EDX) and EELS are two microanalysis methods routinely used to analyze samples in the electron microscope. EDX is a powerful method for identifying the sample composition, but due to its low collection efficiency it generally requires a very high exposure. Considering the irradiation sensitivity of PEMs, it is not surprising that visualization of nanometer-sized regions using EDX has never been reported.

While EELS has a higher collection efficiency than EDX, identification of atomic composition with nanometer-scale resolution is still challenging. Several reports have been published in attempts to answer this challenge. For example, Porat *et al.*³⁵ obtained a sulfur map from a sample of Nafion. The map revealed some variations in the sulfur composition, but the spatial resolution was not sufficient to clearly identify ionic clusters. Another report³⁶ presented a fluorine map of Nafion in a fuel cell assembly that allowed identification of the catalyst material at the membrane surface. Ionic clusters could not be distinguished in the published images, presumably due to the low magnification. So far, to our knowledge, observation by EELS of the increased local concentration of sulfur in ionic clusters has never been reported. This fact leads us to question the very possibility of mapping of the sulfur distribution. While atomic resolution EELS has been realized for hard materials, for soft materials the accuracy of EELS imaging is limited by the exposure that the sample is able to withstand³⁷. Limited exposure causes a high level of noise in the spectra and this noise compromises the accuracy of the analysis. The small size of the clusters may lead to a very weak signal from individual clusters that can easily be buried in the noise. In this work we attempt to assess the possibility of cluster analysis in PEMs using EELS. For core-loss imaging we perform computer simulations that predict the signal-to-noise ratio as a function of cluster size and composition. For low-loss EELS imaging we present experimental data that compare it with HAADF imaging.

Core-Loss EELS: Sulfur mapping simulations

We begin by considering a polymer that contains some amount of sulfur clustered in small volumes. We add a realistic amount of noise to spectra calculated for model clusters and then use standard methods to estimate the sulfur content. Because we do not know the real composition inside the clusters, we leave it as an adjustable parameter. The nature of the polymer used for the calculation is not important, since quantitative core loss elemental analysis ignores the difference in the low-loss part of the spectrum. Thus we can assume that spectra collected from the sulfur-rich and sulfur-depleted areas are similar in the low-loss region. We assume that the only difference is the sulfur edge, absent from spectra collected outside of the cluster but present in the spectra collected from the cluster. Thus we use two reference spectra to compute the model: the spectrum experimentally collected from a polystyrene film, and the polystyrene spectrum with the sulfur edge added in the corresponding energy region. The sulfur edge is taken from the Gatan EELS Atlas³⁸ and scaled in such a way that the pre-edge region of the sulfur spectrum is identical to the polystyrene spectrum. Both spectra are shown in Figure 3a. To determine the sulfur content from the simulated spectra, we use multiple least squares (MLS) fitting³⁹ rather than the three-window method because it has been shown that the MLS fitting may give superior results⁴⁰.

We perform simulations in order to find the relationship between the size of the features of interest and the uncertainty in the chemical analysis, which is expressed as the standard deviation of the resultant compositional values. Because the exposure per unit area of the soft material must be limited in order to avoid damage, decreasing the size of the irradiated area leads to fewer electrons producing the spectrum. As has been shown earlier²¹, the number of recorded electrons also depends on the thickness of the sample, as expressed by the equation

$$N = N_c \cdot d^2 \cdot \frac{t}{\lambda} \exp\left(-\frac{t}{\lambda}\right)$$

where N is the number of singly scattered electrons, N_c is the number of incident electrons, d is the cluster size, and t/λ is the relative thickness in units of mean free path.

To simulate the spectrum we build a linear combination of the references and normalize the area under the spectrum so that the integral is equal to N . We digitally add shot noise to the spectrum and perform EELS analysis on the simulated spectrum. Only the spectral features above the sulfur edge at 165 eV are used for the fitting the simulated spectrum to a linear combination of the references. Setting the upper limit for the fitting range is not straightforward. A higher limit will improve the fit but may introduce artifacts related to overlap with edges of other elements. In order to consider the best-case scenario, we set the upper limit to 273 eV, which corresponds to the carbon K -edge.

For a range of sulfur particle sizes and an exposure corresponding to 10,000 e/nm², 5000 spectra were simulated, and then subjected to EELS analysis. The mean and standard deviation of the resulting composition values were calculated. Not surprisingly, the mean

was close to the value used to construct the linear combination. The standard deviation depended on the size of the features, as shown in Figure 3b. It is noteworthy that the standard deviation did not exhibit a noticeable dependence on the composition. To confirm this, we also simulated clusters containing 0, 25, 50, 75, and 100 % sulphur and saw no difference in standard deviation.

In order to conclude whether the sulfur in clusters may be detected we need a criterion of visibility. The criterion that is widely accepted in the microscopy community is the Rose criterion⁴¹ which requires that the contrast in the feature of interest must be 5 times higher than the standard deviation of the noise. For the best-case scenario we may assume that all sulfur is concentrated in the clusters with none in the matrix. However, the apparent composition of the features observed in projection may be different, since they overlap with the sulfur-free matrix. We assume that the features we observe have a height similar to their lateral size. Thus, for a sample with thickness of 50 nm, the contrast in projection images of a cluster of size d containing 100% sulfur is equal to $C = d/50$ (for a sulfur particle with size $d = 50$ nm, contrast $C = 1$). To use the Rose criterion here, we multiply this contrast by 5 and plot it against the cluster size (see Figure 3c). Also plotted in Figure 3c is the dependence of composition uncertainty on cluster size. The crossing of the two lines gives the smallest feature size that may be distinguished by core-loss EELS. The analysis was repeated for clusters that contain 50, 25, and 12.5 % sulfur (see Figure 3c).

In Figure 3d we plot the minimum detectable cluster size using an exposure of 10,000 e/nm², as a function of the sulfur content. This limit should be relevant to most PEMs. For example, in dry Nafion the size of clusters has been estimated to be about 2 nm⁴². The results in Figure 3d indicate that clusters of this size can only be distinguished from the matrix if they contain 100% sulfur. Since the clusters must contain numerous other atoms besides sulfur, detection at this exposure would not be possible. The simulations may be easily extended to other exposure levels by recognizing that the compositional uncertainty is inversely proportional to the square root of the exposure level²¹. This dependence is shown in a 3 dimensional plot where the minimum detectable cluster size is plotted as a function of sulfur content and exposure level (Figure 3e). From Figure 3e it is clear that analysis of nanometer-size clusters with low sulfur content requires exposures that are beyond the value that causes terminal damage in most PEMs.

EELS: Low Loss mapping

The low-energy loss part of the EELS spectrum contains information about the chemical bonding in the material and thus may be useful to differentiate phases in PEMs. The signal in the low loss spectrum is considerably higher than in the core loss region. In fact, the low loss region contains almost all of the inelastic scattering. Thus using the low loss region will significantly reduce the noise in the spectrum, providing the potential for resolution improvement.

An important advantage of using the low loss part of the spectra is the ability to calculate the sample thickness based on the ratio of inelastic and total scattering intensity. Because the signal is integrated over a broad energy range, the solution obtained contains less noise and

the exposure required for this type of analysis is significantly less than that required for composition analysis.

However, low loss analysis introduces three problems that were not critical to core loss analysis: 1) The low-loss spectrum is more difficult to quantify, as the spectral features arise from interactions with valence electrons in the sample. These interactions typically do not produce sharp peaks; 2) Spatial resolution is low because delocalization of the electron interaction is much higher for the low-loss region; 3) Damage is manifested more strongly in the low loss region because the spectral features arise not only from the atomic species but also electrons participating in chemical bonding. Calculations of the resolution limit in low-loss EELS studies of soft matter have recently been presented²¹. Here we present experimental work that compares the abilities of low-loss EELS and HAADF imaging.

We applied low-loss EELS to study the PSS-PMB block copolymer discussed earlier. The sample was examined in the STEM mode and signals were recorded simultaneously with both EELS and HAADF detectors. Since the higher level of the HAADF signal allows substantially better resolution, we apply sub-pixel scanning and record an 8×8 pixel HAADF image within each pixel of the spectrum image (this technique is frequently called mosaic imaging). Simultaneous collection of EELS and HAADF signals allowed us to select the spectra that corresponded to the strongest and weakest signal on the HAADF detector and use those spectra as the references for MLS fitting of the spectrum image. The relative thickness map was directly calculated using the equation $I_0 = I_t \exp(-t/\lambda)$ where I_0 is the sum of zero-loss peak counts and I_t is the sum of total recorded counts (zero-loss peak plus whole loss part). Figure 4a shows a low magnification image acquired in STEM mode, and the low-loss reference spectra obtained by MLS are shown in figure 4b. The composition map, thickness map and mosaic HAADF image are shown in Figure 4c, 4d, 4e. The clusters of the heavier element, sulfur, are clearly visible in the HAADF image (Figure 4e). The composition map shows that regions rich with clusters are the ones that consist of one phase while the darker parts consist of a different phase. However the exact compositions corresponding to the references are unknown, and we only have qualitative information about the composition map. The uniformity of the thickness map (Figure 4d) indicates that the clusters we observe in the HAADF image are due to the presence of a strong high-angle scattering element (sulfur), and not due to thickness variations. Thus we conclude that for typical hydrocarbon PEM HAADF imaging may be more informative than low-loss EELS composition analysis. However thickness analysis may help to better interpret features observed in HAADF. Since the collection of data for all techniques is simultaneous and does not require additional irradiation, this method may prove to be useful for irradiation-sensitive PEM systems.

Interpretation of PEM Images: The Feature Overlap Problem

Due to possible overlap of the phases in projection, PEM images are often difficult to interpret. The thickness of TEM samples is typically more than 50 nm, while the average distance between the clusters is often an order of magnitude smaller. For example, for Nafion this distance is in the range 3–5 nm^{42–43} and for PSS it is 3–4 nm⁴⁴. It is clear that imaging of any PEM sample with a thickness of 50 nm will result in substantial overlapping

of the clusters. What is not clear, however, is the extent to which this affects interpretation of the PEM images. Digital simulations of the morphology of PEMs can help to estimate the effect of this overlap. Such simulations have been carried out earlier^{45–46}. In these simulations, the volume of the simulated specimen was filled with quasi-randomly arranged spheres so as to produce a predetermined average distance between the spheres. The authors showed that, within certain limits, the size of the spheres may be inferred from a Fourier transform of the projection image and that the inferred size does not depend on the extent of overlap. However, this inference requires postulating a spherical shape for the ionic clusters, which defeats much of the purpose of EM imaging of PEM. Generally the main goal for microscopy investigation of PEM is to determine the shape and size of the phases by direct observation. The ability to do so is affected not only by overlap but also by the contrast and level of noise always present in EM images. Keeping this goal in mind, we have extended our simulations to explore the effect of noise in low-exposure images on the ability to determine both size and shape of the clusters.

Modeling of clusters in PEM

We define the specimen and imaging conditions so as to model the best possible image of a Nafion film under the constraint of limited electron exposure. Nafion was chosen as it has one of the largest clusters among the PEMs. We assume that the ionic clusters are spheres randomly situated in a matrix at an average distance of 5 nm. We do not include any connections between the clusters. The size of the dry clusters was based on the assumptions that all of the sulfur atoms are located in the clusters and that the volume occupied by each atom in the PEM is the same. While these assumptions are unrealistic, they are close enough to provide a useful estimate of the maximum expected contrast. Another assumption we make, that scattering is proportional to the square of atomic number, will slightly overestimate contrast as well. We filled the volume of the sample with 2 nm diameter spheres. For each sphere, we chose the lateral position randomly. Then we moved it down along the z-axis in order to put it 5 nm from the bottom surface or closest of the spheres already set. This procedure allows us to achieve a packing density of 53 vol. %. The model thus obtained is shown in Figure 5a. The scattering from the spheres and matrix was set based on atomic composition. For each sulfur atom Nafion has on average 67 carbon, fluorine and oxygen atoms⁴⁷ which we assume are uniformly distributed.

In contrast to the previous work^{45–46} we include noise in the simulations due to the Poisson distribution of the electron irradiation. To simulate this noise we need to know the number of electrons contributing to image formation. We have shown earlier that this number is different for different imaging modes. In this simulation we ignore this complication and assume that all of the elastically scattered electrons are counted to produce the dark field image. Because PEMs consist of low atomic number elements, the total amount of scattering from various PEMs is similar and close to the scattering from carbon. The amount of elastic scattering may be measured experimentally, but it is actually easier to estimate it based on the inelastic scattering, for which reliable measurements have been made. The inelastic mean free path for most PEMs with 200 keV electrons is slightly over 200 nm, and elastic scattering from carbon is about a factor of 3 lower than inelastic scattering^{48–49}. For a 50 nm thick sample only about 7 % of the incident electrons will contribute to image formation.

Figures 5b, 5c, 5d, show projection images corresponding to a 50 nm thick PEM. Figure 5b is a noiseless projection, while Figure 5c has noise added corresponding to an exposure of 15,000 e/nm². Figure 5d corresponds to an exposure of 2,000,000 e/nm². In the noiseless image we can clearly differentiate the morphology, although strong overlapping of clusters is obvious. It is possible to identify several circular shapes which one can interpret as the cluster shape, but it is clear that these features arise from multiple clusters closely aligned in projection. In contrast, the noise level associated with an exposure of 15,000 e/nm² makes analysis of the morphology very difficult. We can see that the morphology is not uniform, but analysis of the cluster shape appears to be impossible. With the exposure increased to 2,000,000 e/nm², visibility improves and more detail can be distinguished. As in the noise-free image, only those clusters overlapping more than twice may be seen clearly. For the majority of the clusters the shape cannot be deduced; most aggregates have a wormlike appearance.

Reducing the thickness of the sample to a monolayer of spheres dramatically changes the situation, since there is no overlap possible. Even at an exposures of 15,000 e/nm² (Figure 5e) the shape of all clusters appears clear. The interpretability of these images is improved by both elimination of overlap and reduced scattering from the matrix, which decreases the background and results in a significant reduction of the noise. At 2,000,000 e/nm² (Figure 5f) clarity of image leaves no ambiguity in cluster size and shape. Our simulations clearly demonstrate the importance of working with thin sections for successful imaging of PEM morphology. In imaging real PEMs, where clusters have different sizes and shapes, having a monolayer of clusters is even more desirable.

3D imaging to address the overlap problem

In spite of the importance of working with very thin samples, no general technique for preparation of such samples has yet been presented. A film thin enough to constrain clusters in a monolayer, around 5 nm, may not have the necessary mechanical stability to be self supporting, so studies may have to be done on films that have many overlapping clusters in projection images. One approach to address the overlap problem is to visualize the morphology in 3D. There are relatively few publications taking advantage of this approach. One attempt to resolve the 3D structure was made by Pineri *et al.*⁵⁰. They collected two images of the same area with tilt angles +3° and -3° and then used a stereoscope to establish that the observed clusters were inside the specimen.

A more general approach is the application of electron tomography. In principle, tomography is capable of resolving the 3D morphology of a material, even if the original projections suffer from overlap. As has been discussed previously⁵¹⁻⁵², using the same total irradiation dose to collect data for a 3D reconstruction and for a projection image should result in the same quality in terms of feature visibility, but without ambiguity related to overlapping of the clusters. We recently presented a 3D tomographic reconstruction of a PSS-PMB sample using a tilt series of HAADF images⁵³. In that work, individual acidic clusters were clearly visible in parts of the projection images because cluster-rich monolayers formed spontaneously on the surface of the specimen. Even in regions of the PSS domain measured to be 35 nm thick clusters were individually resolved.

We performed further simulations using our hard sphere morphology model (Fig. 5a) to demonstrate the capability of tomographic 3D reconstructions at relatively low exposures. We simulated a series of projection images in the tilt range -70° to $+70^\circ$ in one-degree steps. We used an exposure of $15,000 \text{ e/nm}^2$ per image so that the total dose would be similar to the dose applied to obtain Figures 5d. We added Poisson noise to each of the projection images and performed the reconstruction of the 3D volume, using TOMO3D software⁵⁴. Projection images (Figure 5c is the zero degree projection) have too much noise to differentiate any morphology. However, a slice of the 3D reconstruction (Figure 5g show a single slice and Figure 5h shows the average of twenty slices, comparable to the thickness of the clusters) clearly shows the clusters. The quality of the images is sufficient to distinguish the cluster shape, and it can be seen that the aspect ratio of the clusters is close to 1:1. From these results it is clear that tomography may provide an avenue for solving the cluster overlap problem in microscopic studies of PEM.

Sample preparation is critical to the quality of images

While tomographic reconstruction allows analysis of the 3D distribution of the heavier phase, it is still clear that increasing sample thickness reduces the quality of the images and the signal-to-noise ratio. All modes of EM analysis benefit from the availability of thin specimens. There are two major established techniques for preparing electron-transparent films of PEMs: solvent casting and sectioning. For both of these techniques preparation of an extremely thin film is challenging, but it is particularly difficult with sectioning. We will start by discussing sectioning.

PEMs are typically sectioned at cryo-temperatures because they are too soft to cut at room temperature. It is widely believed that sectioning produces samples that are a good representation of the bulk morphology^{55–56}. There are many reasons to question this belief. The use of even the sharpest knife for sectioning results in pressure build-up on the edge of the blade that can deform morphologies and induce phase transitions. It has been reported that sectioning can transform crystalline ice into high-density amorphous ice⁵⁷. Based on this observation, the pressure at the edge of the knife was estimated to be about 200 MPa. Higher pressure may be expected when sectioning polymers because friction between long polymer chains is stronger than between small water molecules. Sectioning of some diblock copolymers may be especially difficult as the knife pressure may cause lamellar sheets to slide past each other.

Even when the sectioning-induced deformations are minimized, cryo-preservation may be necessary to retain bulk morphology. It has been established that the glass transition temperature of thin films may be less than that of the bulk sample. This may allow for structural relaxation at unexpectedly low temperatures. Most of the published EM studies of PEMs have been conducted either at room temperature or at low temperature but after warming the section to room temperature. To illustrate the effects of sectioning with a sharp diamond knife and the need for cryo-preservation, we present images collected from a cryo-sectioned PSS-PMB sample. Figure 6a shows a BF TEM image of a section about 150 nm thick that was cryo-preserved after sectioning and then imaged at low temperature. Low temperature restricts the movement of atoms and reorganization of the sample morphology.

Thus the imaged sample to a large degree reflects the state of the sample immediately after the cryo-sectioning. Randomly oriented lamellae, consistent with SAXS characterization⁵⁸, are seen in Figure 6a. Also seen in Figure 6a are larger features with a high level of contrast which are most probably related to the compression and crumbling of the section and/or thickness variations. Figures 6b and 6c show the effect of warming sections to room temperature. Thin sections (about 100 nm thick) develop holes, and the lamellae reorganize so that they lie parallel to the film surface (Figure 6b). Thicker sections (over 300 nm thick) rearrange so that the lamellae are arranged perpendicular to the film surface (Figure 6c). The former rearrangement completely obscures the lamellar morphology of the sample, while the latter rearrangement gives a very clear image, but the orientations of the lamellae are far from random. It is clear that neither Figure 6b nor 6c represents the bulk morphology. The minimum thickness obtained by sectioning is limited by the mechanical properties of the material, and is generally in the range of 50–150 nm (for the PSS-PMB copolymer the minimum thickness was above 100 nm). Avoiding the overlap of random, nanometer sized features in sectioned samples is thus extremely challenging.

Solvent-casting allows better control over the thickness of the film relative to cryo-sectioning. Nearly all PEMs are synthesized and/or processed in solution; however the morphology obtained from different casting processes may differ. Stresses generated during drying may affect the morphology, and rapid drying may not provide enough time to achieve equilibrium. Casting can be done on solid or liquid substrates followed by transfer onto a thin electron-transparent substrate such as lacey carbon. Another approach consists of casting the film directly on the lacey carbon substrate. The latter technique is convenient because it can give an extremely thin, free-standing film reliably mounted on the TEM sample grid. Unfortunately in many cases the stresses generated during film formation can break the substrate. Because the thin films tends to dry extremely fast, formation of trapped non-equilibrated morphologies is common. This problem may be reduced by slowing down the drying process. One approach is to place the sample in an atmosphere containing a high concentration of the solvent vapor. We present examples of a sample of PSS-PMB cast from THF solution onto a lacey carbon grid in open air (Figure 6d) and inside of a closed jar containing only a one mm diameter opening for the vapor to escape (Figure 6e). The rapidly-cast sample shows a trapped disordered morphology consistent with the presence of spherical micelles in solution. Slow drying gives a lamellar morphology (Figure 6e) similar to the thick cryo-sectioned sample (Figure 6c). Specimens of PSS-PMB as thin as 20 nm can be produced by casting.

In the context of producing the thinnest films to avoid overlapping features in images, solvent casting of a copolymer may be the ideal technique. Recently, we demonstrated a protocol for preparing extremely thin layers of the cluster-containing phase of a PSS-PMB copolymer⁵³ overlapping in projection with the cluster-free phase. The PMB phase serves as an electron transparent substrate providing mechanical stability to the film. As we noted, the PSS and PMB phases tend to separate. With properly chosen casting condition, free-standing films were obtained with thicknesses down to 20 nm. Lamellar sheets of PSS in such thin films are oriented parallel to the surface, resulting in extremely thin layers of the ionomer sandwiching a PMB layer. In thicker parts of the samples the arrangement of the PSS and PMB phases creates a honeycomb structure. Figure 7 shows a HAADF image of a

film that varies in thickness with a single lamella, parallel to the surface, at the top of the image and the honeycomb structure at the bottom. The honeycomb walls are formed because the distance between the phases cannot be larger than the length of the polymer molecule. The PMB in both regions is covered with a thin layer of PSS on both surfaces. These thin layers are ideal for obtaining images of the clusters with minimal overlap. Thus employing the self-organization phenomenon allowed us to achieve the thinnest cluster-containing PEM sample reported to date, which in turn led to characterization of the cluster size distribution. It is reasonable to expect that in the future various types of self-organization will be used for forming thin films and will significantly improve the quality of PEM images obtained by EM.

Conclusions

The occurrence and morphology of clusters that form from the ionic species of PEMs have a profound effect on charge transport, yet many questions regarding their structure remain unanswered. Electron microscopy has been used in many studies to characterize the ionic domains, but progress has been limited by practical problems in direct visualization of the clusters. The main problems with EM of PEMs are radiation damage and overlap of clusters in projection. We have used both experimental data and models to compare the abilities of different EM techniques to deal with these problems.

Considering the exposure limitations that arise from PEM radiation sensitivity, we compared the potential of different imaging modes for studies of PEM. We conclude that BF TEM uses electrons most efficiently and can provide the best images at the lowest exposure, but that these images can be difficult to interpret because of the requirement for defocusing to produce contrast. If the sample is capable of withstanding slightly higher exposures HAADF may be advantageous, since in-focus imaging produces sharper images with pixel brightness directly related to scattering power. An important advantage of HAADF imaging is its strong dependence on the atomic number, which allows visualizing clusters of heavy elements in PEMs. In the last decade HAADF imaging of PEM has become the preferred mode compared to BF TEM which had previously been used for most PEM imaging. Core loss EELS analysis has the potential to identify the composition of ionic clusters, but the irradiation required for analysis is so high that this approach is not practical for most PEMs. Low loss EELS analysis requires somewhat less exposure than core loss, but compositional analysis still requires exposures high enough to damage the polymer. Even at the exposures used to obtain clear images of the clusters by HAADF the resolution of low loss EELS is not sufficient for cluster imaging. However, collecting low loss spectra simultaneously with HAADF imaging may help to interpret the HAADF images by providing an easy way to calculate the mass thickness of the sample.

In any imaging mode, overlap of the small ionic aggregates presents problem for morphology analysis. Our simulations show that at low exposure little information can be obtain from projection images of a sample even as thin as 50 nm. While increasing exposure improves the clarity of the images, identifying the shape of the phases still may be difficult. Electron tomography can resolve the 3D structure of material and avoid overlap problem. In

practice, the signal to noise ratio in a 3D reconstruction is lower than in a projection image obtained with the same exposure.

Data obtained from all EM techniques are better if thin samples are used, and methods for obtaining such sections are discussed. Due to limitations of cryosectioning, obtaining sufficiently thin sections for cluster visualization is extremely difficult. In contrast, solvent casting allows better control over the thickness. Taking advantage of the tendency of diblock copolymers to phase separate may allow formation of extremely thin layers of polyelectrolyte which significantly reduce the overlap problem as well as improve signal to noise ratio.

Acknowledgments

Funding for this work was provided by the U.S. Department of Energy under Contract No. DE-AC02-05CH11231 through the Electron Microscopy of Soft Matter Program (electrolyte imaging) supported by the Office of Basic Energy Sciences, Materials Sciences and Engineering Division. Part of this work was performed at National Center for Electron Microscopy and the Molecular Foundry at Lawrence Berkeley National Lab, which are supported by the Office of Science, Office of Basic Energy Sciences of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

Notes and references

1. Çelik SÜ, Bozkurt A, Hosseini SS. *Progress in Polymer Science*. 2012; 37:1265–1291.
2. Hickner MA, Ghassemi H, Kim YS, Einsla BR, McGrath JE. *Chem Rev*. 2004; 104:4587–4611. [PubMed: 15669163]
3. Bock T, Möhwald H, Mülhaupt R. *Macromolecular Chemistry and Physics*. 2007; 208:1324–1340.
4. Jiang H, Song C, Chen C-C, Xu R, Raines KS, Fahimian BP, Lu C-H, Lee T-K, Nakashima A, Urano J, Ishikawa T, Tamanoi F, Miao J. *Proceedings of the National Academy of Sciences*. 2010; 107:11234–11239.
5. Elliott JA, Hanna S, Elliott AMS, Cooley GE. *Macromolecules*. 2000; 33:4161–4171.
6. Elliott JA, Wu D, Paddison SJ, Moore RB. *Soft Matter*. 2011; 7:6820–6827.
7. Gierke, DT.; Hsu, YW. *Perfluorinated Ionomer Membranes*. Vol. 180. American Chemical Society; 1982. p. 283-307.
8. Williams CE, Russell TP, Jerome R, Horrion J. *Macromolecules*. 1986; 19:2877–2884.
9. Hsu WY, Gierke TD. *Journal of Membrane Science*. 1983; 13:307–326.
10. Fujimura M, Hashimoto T, Kawai H. *Macromolecules*. 1982; 15:136–144.
11. Yarusso DJ, Cooper SL. *Macromolecules*. 1983; 16:1871–1880.
12. Loppinet B, Gebel G. *Langmuir*. 1998; 14:1977–1983.
13. Schmidt-Rohr K, Chen Q. *Nat Mater*. 2008; 7:75–83. [PubMed: 18066069]
14. Litt M. *Polymer Preprint*. 1997; 38:80.
15. Eisenberg A. *Macromolecules*. 1970; 3:147–154.
16. Eisenberg A, Hird B, Moore RB. *Macromolecules*. 1990; 23:4098–4107.
17. Hunt JA, Disko MM, Behal SK, Leapman RD. *Ultramicroscopy*. 1995; 58:55–64.
18. Varlot K, Martin JM, Quet C. *Journal of Microscopy*. 1998; 191:187–194. [PubMed: 9767482]
19. Bahr GF, Johnson FB, Zeitler E. *Laboratory Investigation*. 1965; 14:1115. [PubMed: 14317025]
20. Yakovlev S, Libera M. *Microscopy and Microanalysis*. 2006; 12:996–997.
21. Yakovlev S, Balsara NP, Downing KH. *Ultramicroscopy*. 2012; 116:39–46.
22. Leapman RD, Sun SQ. *Ultramicroscopy*. 1995; 59:71–79. [PubMed: 7571121]
23. Yakovlev S, Misra M, Shi S, Libera M. *Journal of Microscopy*. 2009; 236:174–179. [PubMed: 19941557]
24. Patel GN. *Journal of Polymer Science: Polymer Physics Edition*. 1975; 13:351–359.

25. Xue T, Trent JS, Osseo-Asare K. *Journal of Membrane Science*. 1989; 45:261–271.
26. Li C, Register RA, Cooper SL. *Polymer*. 1989; 30:1227–1233.
27. Jablonski, A.; Salvat, F.; Powell, CJ. NIST Electron Elastic-Scattering Cross-Section Database - Version 3.2. National Institute of Standards and Technology; Gaithersburg, MD: 2010.
28. Józef C. *Polymer*. 1978; 19:73–76.
29. Handlin DL, MacKnight WJ, Thomas EL. *Macromolecules*. 1981; 14:795–801.
30. Peron J, Mani A, Zhao X, Edwards D, Adachi M, Soboleva T, Shi Z, Xie Z, Navessin T, Holdcroft S. *Journal of Membrane Science*. 2010; 356:44–51.
31. Dalmás F, Vonnet J-F, Randriamahefa S, Gaillet C. *Macromolecular Chemistry and Physics*. 2010; 211:1765–1774.
32. Williams, CE.; Colliex, C.; Horron, J.; Jérôme, R. *Multiphase Polymers: Blends and Ionomers*. Vol. 395. American Chemical Society; 1989. p. 439-444.
33. Laurer JH, Winey KI. *Macromolecules*. 1998; 31:9106–9108.
34. Winey KI, Laurer JH, Kirkmeyer BP. *Macromolecules*. 1999; 33:507–513.
35. Porat Z, Fryer JR, Huxham M, Rubinstein I. *The Journal of Physical Chemistry*. 1995; 99:4667–4671.
36. Scheiba F, Benker N, Kunz U, Roth C, Fuess H. *Journal of Power Sources*. 2008; 177:273–280.
37. Yakovlev S, Libera M. *Micron*. 2008; 39:734–740. [PubMed: 18096395]
38. Ahn, CC.; Krivanek, OL. *EELS Atlas: A Reference Collection of Electron Energy Loss Spectra Covering All Stable Elements*. Gatan; 1983.
39. Shuman H, Somlyo AP. *Ultramicroscopy*. 1987; 21:23–32. [PubMed: 3824681]
40. Leapman RD, Swyt CR. *Ultramicroscopy*. 1988; 26:393–403. [PubMed: 3238813]
41. Rose, A. *Vision: Human and Electronic*. Plenum Press; New York: 1973.
42. Gierke TD, Munn GE, Wilson FC. *Journal of Polymer Science: Polymer Physics Edition*. 1981; 19:1687–1704.
43. Haubold HG, Vad T, Jungbluth H, Hiller P. *Electrochimica Acta*. 2001; 46:1559–1563.
44. Peiffer DG, Weiss RA, Lundberg RD. *Journal of Polymer Science: Polymer Physics Edition*. 1982; 20:1503–1509.
45. Benetatos NM, Chan CD, Winey KI. *Macromolecules*. 2007; 40:1081–1088.
46. Chan CD, Seitz ME, Winey KI. *Microscopy and Microanalysis*. 2011; 17:872–878. [PubMed: 22053943]
47. Mauritz KA, Moore RB. *Chem Rev*. 2004; 104:4535–4585. [PubMed: 15669162]
48. Egerton, RF. *Electron energy-loss spectroscopy in the electron microscope*. Plenum Press; New York: 1996.
49. Malis T, Cheng SC, Egerton RF. *Journal of Electron Microscopy Technique*. 1988; 8:193–200. [PubMed: 3246607]
50. Pineri M, Meyer C, Bourret A. *Journal of Polymer Science: Polymer Physics Edition*. 1975; 13:1881–1891.
51. McEwen BF, Downing KH, Glaeser RM. *Ultramicroscopy*. 1995; 60:357–373. [PubMed: 8525549]
52. Hoppe W, Hegerl R. *Ultramicroscopy*. 1981; 6:205–206.
53. Yakovlev S, Wang X, Ercius P, Balsara NP, Downing KH. *Journal of the American Chemical Society*. 2011; 133:20700–20703. [PubMed: 22107080]
54. Agulleiro JI, Fernandez JJ. *Bioinformatics*. 2011; 27:582–583. [PubMed: 21172911]
55. Andrews EH, Bennett MW, Markham A. *Journal of Polymer Science Part A-2: Polymer Physics*. 1967; 5:1235–1247.
56. Michler, GH. *Electron Microscopy of Polymers*. Springer; 2008.
57. Al-Amoudi A, Dubochet J, Studer D. *Journal of Microscopy*. 2002; 207:146–153. [PubMed: 12180960]
58. Wang X, Yakovlev S, Beers KM, Park MJ, Mullin SA, Downing KH, Balsara NP. *Macromolecules*. 2010; 43:5306–5314.

Figure 1.

Bright field TEM images of cast PSS-PMB membrane. a) undamaged material irradiated with 1000 e/nm^2 for image collection; b) damaged material pre-irradiated with $50,000 \text{ e/nm}^2$, and imaged with 1000 e/nm^2 . While the contrast between the PSS and PMB phases fades most of the dark spots remain in the same positions; c) HAADF image of a similar specimen collected with $10,000 \text{ e/nm}^2$.

Figure 2.

a) Azimuthally integrated scattering intensity, I_n , as a function of scattering angle, θ . b) Integral of scattering intensity from 5 mrad to specific scattering angle. c) Elastic scattering cross-section for carbon and sulfur. d) Ratio of scattering from sulfur to scattering from carbon.

Figure 3.

Simulated energy loss spectra from sulfur and sulfur free part of the sample. b) Composition uncertainty as a function of cluster size, with specimen exposure limited to 10,000 e/nm². c) Composition uncertainty and contrast multiplied by five for different cluster compositions: 100, 50, 25, 12.5 % of sulfur. d) Minimum detectable cluster size as a function of the sulfur content of the clusters. e) Resolution of EELS imaging as a function of the sulfur content of the clusters for various exposures.

Figure 4.

Low loss EELS analysis of PSS-PMB film. a) Low magnification image showing location of area for data collection. b) EELS spectra of two components in image. c) Composition map. d) Thickness map. e) High resolution mosaic HAADF image.

Figure 5.

Simulations of imaging a 3D array of random spheres: a) model; b) noiseless projection of 50 nm layer; c) projection image corresponding to $15,000 \text{ e/nm}^2$; d) projection image corresponding to $2,000,000 \text{ e/nm}^2$; e) projection image of monolayer corresponding to $15,000 \text{ e/nm}^2$; f) projection image of monolayer corresponding to $2,000,000 \text{ e/nm}^2$; g) slice of a tomographic reconstruction of 3D volume; h) average of 20 slices of the of 3D reconstruction.

Figure 6.

Cryo-section of PSS-PMB cryo-preserved and imaged at low temperature $-180\text{ }^{\circ}\text{C}$; b) thin cryo-section after warming to room temperature; c) thick cryo-section after warming; d,e) thin PSS-PMB film cast on lacey support film and rapidly dried (d) or slowly dried (e).

Figure 7.
PSS-PMB with thickness gradient showing single lamellae in the thin part (top) and honeycomb in the thick part (bottom)