

Interactive comment on “Review article: Potential application of surface methods for the monitoring of organic matter dynamics in marine systems” by Galja Pletikapić and Nadica Ivošević DeNardis

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Received and published: 11 September 2016

We would like to thank Dr. A. Podesta for his constructive and positive comments which help us to improve the manuscript. We have responded to his comments below. The new version of the manuscript is uploaded as separate pdf file.

1) According to what is written in Page 6, line 30, samples are imaged by AFM in dehydrated conditions, and this is found to be "the most adequate condition". A line of comment here would be useful, as the reader may not find obvious that such fragile samples (including vesicles) can be studied outside their native aqueous environment. Another question that may arise is to which extent dehydration preserves the native properties of the objects under study. This also marks an asymmetry with respect to

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the chronoamperometry investigation, where the measurement is carried on in water.

Answer: Typically, AFM measurements of marine organic samples are performed at room temperature and 50-60 % of relative humidity, which leaves samples with a small hydration layer adhering to the substrate, helping maintain the native structure (Balnois and Wilkinson, 2002). It was found that direct deposition of a drop of seawater (5 μ L) on freshly cleaved mica, followed by the rinsing of sea salts and evaporation of water excess, was the procedure that caused the least impact on the original structures of biopolymers and other assemblies in seawater (Mišić Radić, 2011; Svetličić et al., 2013). Such an approach could offer an alternative way to visualize fragile organic structures when dealing with a large number of seawater samples. While an aqueous environment would be preferable, direct AFM imaging of marine polymers, vesicles and organic droplets as very soft and deformable structures is often hampered by their weak interaction with the interface. While interpreting the data, one should consider possible surface and/or dehydration artefacts. On the other hand, electrochemical measurement of dispersed marine particles is based on adhesion, disruption and spreading and therefore formation of intimate contact with the electrode which causes double-layer charged displacement from the interface. AFM and electrochemical method of amperometry at the DME are complementary surface methods. For example, electrochemical detection of gel microparticles or increased concentration of submicron particles in the seawater is in line with AFM imaging of gel network formation at nanometric scale.

2) Page 8, the last sentence starting at line 20. A new (AFM-based ?) approach is mentioned (Shon et al, 2013...), but nothing is said about it. It is not clear what could be the advantage of this approach (it is not even clear which approach is C2 NHESSD Interactive comment Printer-friendly version Discussion paper this), with respect to the possibility of exploiting the full potential of AFM. This sentence should be expanded so to shortly present and discuss alternative and/or complementary (AFM-based) approaches, not only the one proposed by Shon et al, so to provide an overview of extra

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AFM capabilities and their potential to the study of the organic matter in marine systems. For example, one such approach could be the combination of topographic and mechanical characterization, which would widen the spectrum of information on the system under study, in particular on the structural properties of the network/aggregates of particles; another could be the combination of topographic and chemical/affinity mapping, by means of functionalized tips, etc... These approaches could be very effective also for the study of small vesicles. In summary, the authors describe mostly the topographic imaging capabilities of the AFM, but it would be very interesting to provide a short overview also of the (many) other interfacial characterization capabilities of the AFM technique.

Answer: Significant advances in the field are expected by integrating AFM into versatile hybrid devices that would combine two or three complementary techniques in one instrument, allowing a more detailed and comprehensive analysis of marine samples. While simultaneous AFM imaging and mechanical mapping (stiffness, friction, dissipation and/or adhesion) is already showing its potential by increasing the number of studies conducted on biological samples (Dufrene et al., 2013, and references therein), including also marine samples (Francius et al., 2008; Pletikapić et al., 2012), of particular interest for investigation of marine organic matter is coupling AFM with different optical, spectroscopic and/or interfacial techniques (Flores and Toca-Herreta, 2009). In line with the topic covered in this review, integration of mercury as a substrate in the AFM setup is drawing particular attention (Schon et al., 2013). An AFM cantilever has been developed using a mercury fountain pen probe allowing simultaneous probing of mechanical and electrical properties, for instance in biological membrane research. In addition, a hybrid AFM- optical (fluorescence) microscope (Kassies et al., 2005; Geisse et al., 2009) would be extremely useful for deepening the research on different classes of organic particles traditionally detected by staining and microscopic analysis. An additional step forward in the field is expected by combining AFM and IR to provide simultaneous correlation of topographical (organizational) and chemical data (Dazzi et al., 2012; Amenabar et al., 2013; Kulik et al., 2014). Alternatively, one could use chem-

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ically functionalized tips, and by simultaneously performing imaging and force curve acquisition, generate chemical maps (Blanchette et al., 2008). Such studies would allow relating molecular organization of marine organic matter with chemical information on the μm and nm scales, which would undoubtedly widen the spectrum of information about samples of interest.

3) The possibility of imaging the oil micro/nano droplet by AFM is intriguing. It would be useful if the authors could comment a bit more on which particular information can/could be extracted by a similar investigation. For instance, could AFM help characterizing biodegradation processes, i.e. the interaction of hydrocarbon-degrading bacteria and small oil droplets? And why and how would AFM be better than other conventional techniques (such as high-resolution optical microscopy etc.).

Answer: AFM could be of particular importance in imaging interactions between bacteria and oil droplets on the nanometeric scale, which would enable better understanding of the mechanism of biofilm formation and therefore provide an insight into the efficiency of the biodegradation process. In addition, force spectroscopy can be applied to probe the adhesion force between the bacterial cell and the droplet and to measure nanomechanical properties of both the bacterial cell and oil droplets. Moreover, AFM can provide an insight into the nanoscale surface morphology of biofilms or aggregates.

4) What is the role of inorganic particles in the processes relevant to the marine ecosystem? The attention of the authors is focused on the organic fraction only, and as far as I understand the e-chem method is sensitive to it, only. But what are we missing in the global picture, for instance in the two case-studies presented, by neglecting the inorganic component? A short comment on this would be useful in the manuscript, with some advices on how to address the study of the inorganic matter (whenever relevant).

Answer: Biogeochemical transformation of organic matter is a rather complex process where chemical composition of seawater plays an important role. The fate of marine organic matter depends on the inorganic constituents but as well on biota influencing

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its physicochemical properties, distribution, stability and bioavailability of metal-organic complexes. A recent review highlights the AFM potential in revealing new information about the interaction of inorganic nanoparticles with living and non-living organic matter (Ciglencečki and Svetličić, 2015). Thus, the main focus in this review is given on the potential of the surface methods for organic matter characterization in the seawater but not on the understanding of the global carbon cycle.

5) The chronoamperometric method is based on the study of the current vs time curves. Qualitative examples are provided of different curve shapes corresponding to different organic matter composition/structure as well as to different interfacial phenomena. For online monitoring purposes, the qualitative interpretation of these curves is probably the most effective approach. However, I wonder whether there exist also models of the e-chem interface that can be used to interpret more quantitatively the chronoamperometric output (in terms i.e. of equivalent circuits etc.)? A short comment on this would make the picture more complete.

Answer: In order to access quantitative information from the amperometric signal of an organic particle, the measurement has to be performed in a previously deaerated solution under nitrogen purging for a few minutes so as to remove redox reaction of dissolved oxygen. In this way, it is possible to retrieve information about: organic particle diameter, particle surface area at the interface, number of molecules in the monolayer, surface charge of the particle, critical interfacial tensions of adhesion, and kinetic parameters of the adhesion process through analysis of the amperometric signal using a reaction kinetics model of the first order and the corresponding methodology (Ivošević DeNardis et al., 2012; Ivošević DeNardis et al., 2015).

Technical corrections 1) Section 3.3 has been skipped. Check the section numbering. Thank you, it is corrected.

2) Page 4, line 22. The acronym DME should be introduced before in the text. Same for the acronym SAP (surface active particles). Thank you, it is introduced.

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3) Page 8, line 3-5. Sentence not clear, please rephrase. The sentence is modified and now reads: Recently, it was reported that membrane vesicles of 100 nm in diameter can be released by marine cyanobacteria and are frequently found in marine ecosystems in the concentration range 10⁵ to 10⁶ vesicles mL (Soler et al., 2015). Such vesicles may entrap nutrients, toxins and a variety of other organic molecular species present in seawater. These components may be concentrated and preserved from degradation in the inner cavity of the vesicle and/or attached to the membrane of the vesicle. This ability of vesicles to transport and deliver diverse compounds in discrete packages adds another layer of complexity to the flow of information, energy and biomolecules in marine microbial communities (Biller et al., 2014).

4) Page 5, line 15: delete the parenthesis before "as a measure...". 4b) Page 5, line 16: delete the extra parenthesis after "hydrophobicity". Done.

5) Page 5, line 1: explain →explaining The sentence is modified (see answer on comment No.5).

6) Increase figures with maps as much as possible, as characters are small. Done.

In general, re-read and check carefully the manuscript for typos and english. Done.

Please also note the supplement to this comment:

<http://www.nat-hazards-earth-syst-sci-discuss.net/nhess-2016-178/nhess-2016-178-AC1-supplement.pdf>

Interactive comment on Nat. Hazards Earth Syst. Sci. Discuss., doi:10.5194/nhess-2016-178, 2016.

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