Unraveling the chemical composition of modern, resistant, organic-walled dinoflagellate cysts via FTIR spectroscopy

Meyvisch Pjotr[1], Mertens Kenneth[2], Gurdebeke Pieter[1], Pospelova Vera[3], Vrielinck Henk[4] and Louwye Stephen[1]

[1] Geology Department, Ghent University, Krijgslaan 281, 9000 Gent, Belgium (Campus Sterre, Building S8) E-mail: pjotr.meyvisch@ugent.be

[2] Ifremer, LITTORAL, Quai de la Croix, 29900 Concarneau, France

[3] Earth and Environmental Sciences Department, University of Minnesota, 116 Church St SE, Minneapolis, MN 55455, United States

[4] Solid-State Sciences Department, Ghent University, Krijgslaan 281, 9000 Gent, Belgium (Campus Sterre, Building S1)

Dinoflagellates are unicellular protists that are – similar to other aquatic microalgal groups – capable of forming organic-walled cysts which allow them to survive periods with unfavorable environmental conditions. These dinocysts are often very resistant to physical and chemical degradation resulting in the occurrence of both modern and fossil forms which are commonly used in (paleo)ecological, (paleo)climatological, and (paleo)environmental studies.

The chemical nature of dinocysts is highly understudied and the suite of resistant macromolecules (i.e. 'dinosporin') present in their walls is incompletely characterized. Past studies used microscopic Fourier transform infrared (micro-FTIR) spectroscopy to determine that dinosporin is different from other resistant biomolecules like sporopollenin (pollen and spores) and algaenan (green algae); that it is a strongly cross-linked carbohydrate-like polymer (somewhat similar to cellulose; Versteegh *et al.* 2012); that its composition slightly varies with the nutritional strategy of associated motile cells (heterotrophic cysts contain N, autotrophs do not; Bogus *et al.* 2014). Using a more robust spectral data collection method based on attenuated total reflectance micro-FTIR spectroscopy (Meyvisch *et al.* 2022), this study further explores the compositional diversity of dinosporin in modern dinocysts from surface sediments, characterizes it in more detail, and re-evaluates the previously established dietary relationship. A large dataset of 216 spectra (10 families, 25 genera, 51 species) from 17 locations across the Northern hemisphere reveals that dinosporin is more variable than previously thought, leading to the erection of four spectrochemical groups, some with striking similarities to sporopollenin and algaenan. It is also shown that pigments significantly contribute to the spectral composition of colored dinocysts, and that eumelanin is likely present in these forms acting as a sunscreen against harmful ultraviolet radiation. Finally, detailed spectral analyses suggest that N is present in all observed dinosporin types and that it originates from sunscreen pigments rather than through heterotrophic prey accumulation. Therefore, the previously established dietary relationship by Bogus *et al.* (2014) is evaluated to be ambiguous.

This study provides a reference framework for a more systematic investigation of resistant biomolecules in dinocysts and other resting stages, as well as their associated ecological roles. Furthermore, the spectral dataset can be used for comparisons with fossil dinocysts and other microorganisms/-fossils with unknown affinities (i.e. acritarchs) in the light of chemotaxonomic studies.

References

- Bogus, K., Mertens, K.N., Lauwaert, J., Harding, I.C., Vrielinck, H., Zonneveld, K.A.F. & Versteegh, G.J.M. 2014. Differences in the chemical composition of organic-walled dinoflagellate resting cysts from phototrophic and heterotrophic dinoflagellates. *J Phycol*. 50:254–66.
- Meyvisch, P., Gurdebeke, P.R., Vrielinck, H., Mertens, K.N., Versteegh, G. & Louwye, S. 2022. Attenuated Total Reflection (ATR) Micro-Fourier Transform Infrared (Micro-FT- IR) Spectroscopy to Enhance Repeatability and Reproducibility of Spectra Derived from Single Specimen Organic-Walled Dinoflagellate Cysts. *Appl Spectrosc*. 76.
- Versteegh, G.J.M., Blokker, P., Bogus, K., Harding, I.C., Lewis, J., Oltmanns, S., Rochon, A. *et al.* 2012. Infra red spectroscopy, flash pyrolysis, thermally assisted hydrolysis and methylation (THM) in the presence of tetramethylammonium hydroxide (TMAH) of cultured and sediment-derived *Lingulodinium polyedrum* (Dinoflagellata) cyst walls. *Org Geochem*. 43:92–102.

Keywords
Microalgae; Dinoflagellate Cysts; ATR Micro-FTIR Spectroscopy; Molecular Composition; Resistant Biomolecules; Pigments; Nutritional Strategy