Fluorescence quenched glycans enable visualisation and quantification of microbial carbon cycling

Conor J. CRAWFORD [1], Greta REINTJES [2], Vipul SOLANKI [2], Jan-Hendrik HEHEMANN [2], Peter H. SEEBERGER [1]

[1] Max Planck Institute of Colloids and Interfaces, [2] Max Planck Institute for Marine Microbiology

conor.crawford@mpikg.mpg.de

Marine microalgae sequester as much CO_2 into carbohydrates as terrestrial plants. Bacteria are major players in the cycling of this carbon as they breakdown photosynthetic glycans. Identifying the rates and specificities of microbial carbon degraders on a molecular level remains challenging. Förster resonance energy transfer (FRET) is a powerful, simple and robust phenom that can be exploited to allow super-resolved optical measurements and provide real-time information about intra- and intermolecular distances on the nanometer level. Due to this it has widespread utility biomolecular research.[1] However, access and utility of fluorescence quenched glycan probes is not yet routine and a key bottleneck is the complexity and expertise required for glycan synthesis.[2–4] A toolbox of fluorescence quenched glycans would allow high-throughput, sensitive and quantitative means to discover microbes with differing abilities and feeding strategies to break down glycans. For these reasons, we have developed a general strategy to access these tools using automated solid-phase synthesis, enabling fast and reproducible access to these tools. Key in this method is the use of a terminal 6-amino 6-deoxy monosaccharide building block which allows assembly of bi-functional oligosaccharides, that once cleaved from the solid support can be derivatized into FRET probes. We then use these glycan-FRET probes as tools to visualize and quantify heterotrophic microbes that digest glycans in the ocean. Example one is an alpha-mannan fluorescence quenched probe, which is used to characterise *Salegentibacter sp* Hel_I_6, a microbe that possesses the rare ability to break down marine fungal glycans.[5] The second example details a beta-glucan based fluorescence quenched probe which we use to visualise laminarin digestion, a major molecule in the global carbon cycle.[6] These fluorescence quenched glycan tools allowed us to kinetically characterise microbial carbon digestion at the level of individual enzymes and image whole live cells. These tools offer a complementary approach to genomic based approaches with the advantage of being sensitive to changes in proteome expression.

Bibliographic references:

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