

## Résumé

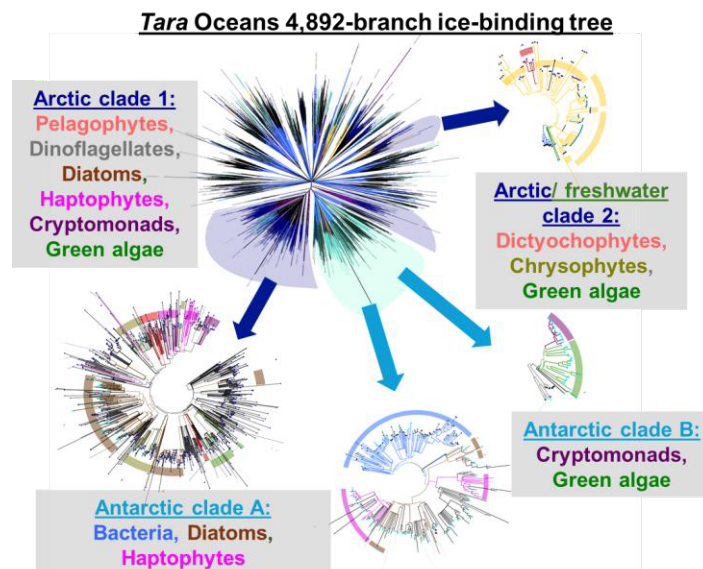
Les écosystèmes cryogéniques, tels que les océans polaires, les glaciers et la neige de l'étage nival, regorgent de vie malgré leur environnement hostile. Ces écosystèmes sont soutenus par une collection taxonomiquement diversifiée d'algues (diatomées, algues vertes, algues dorées...) des glaces et des neiges, qui utilisent des protéines de liaison à la glace (ice binding proteins, IBP) pour moduler de manière adaptative leurs réponses aux cristaux de glace cellulaires et environnementaux. Les IBP ont une histoire évolutive complexe, caractérisée par des transferts génétiques horizontaux répétés entre des taxons éloignés dans des environnements cryogéniques spécifiques. Elles ont des rôles fonctionnels divers, qui comprennent à l'intérieur et à l'extérieur des cellules, permettant aux algues de survivre au stress hypo-osmotique ou aux saumures hypersalines, et ayant des activités cinétiques fortes ou faibles d'une manière imprévisible d'un point de vue phylogénétique.

Mission-GLACE va établir un bilan computationnel et expérimental complémentaire sur l'évolution et le fonctionnement des IBP dans la cryosphère algale. Tout d'abord, nous allons générer un arbre phylogénétique des IBP, incluant plusieurs nouveaux génomes algaux séquencés dans l'océan Arctique et dans la neige des montagnes par le directeur et le co-directeur de thèse, et l'annoter avec les localisations protéiques prédites, leurs réponses à la salinité grâce aux données transcriptomiques, et l'architecture des domaines. Nous chercherons à développer un outil prédictif permettant d'identifier les activités cinétiques probables de diverses protéines IBP, à partir de données expérimentales issues d'IBP exprimées caractérisées *in vitro*. Enfin, en nous appuyant sur de nouvelles données de séquençage environnementaux du projet scientifique participatif Living Snow, nous essayerons de démêler les fonctions physiologiques probables des IBP dans les communautés algales naturelles, en tenant compte des impacts de la température et de la salinité, pour mieux comprendre la fragilité et la résilience de ces communautés face au changement climatique anthropique.

## Mission-GLACE : biochimie, évolution et outils predictiveS pour des protéines de liaison à la GLACE

**Context:** Cryogenic ecosystems, including polar oceans, snow and continental glaciers, are characterized by year-round near-freezing temperatures, and persistent presence of ice with salinities ranging from near-freshwater to hypersaline brines<sup>1</sup>. Despite their harsh conditions, these ecosystems are teeming with life, with primary production supported by a diverse range of snow and ice algae<sup>2-4</sup>. Pioneering studies of polar marine algae have revealed widespread presence of ice-binding proteins, a family unified via a conserved PFAM domain (PF11999/ DUF3494)<sup>3,5,6</sup>. This consists of six repeated threonine-rich motifs arranged in a  $\beta$ -solenoid, that can bind to ice crystals<sup>6</sup>. This domain is not seen in other proteins, except for an independently evolved PFAM (PF20597) associated with diatom adhesive trails<sup>5,7</sup>.

Despite their conserved structure, IBPs are functionally diverse, with some having roles in regulating ice crystal expansion inside the cell, and others secreted for ice adhesion<sup>6</sup>. Many are involved in salt tolerance, relevant to the cryosphere due to its impacts on water freezing points, and the wide salinity ranges found in sea-ice<sup>1</sup>, but some respond to hyposmotic and others to hyperosmotic stresses<sup>8</sup>. The activity of individual IBPs (measured as “thermal hysteresis”, i.e. their capacity to modify the freezing temperature of water<sup>6</sup>) is variable, with comparatively little known about what residues determine IBP kinetics. The functional diversity of IBPs is complemented with evolutionary diversity, with different families associated with Arctic, Antarctic and freshwater algae, independent of species taxonomy, and probably transferred between species by within-habitat horizontal gene transfers (HGT)<sup>3,6</sup>.



**Fig. 1.** Topology of a 5,000 tip tree of IBP diversity, shaded by taxonomic affiliation and biogeographical origin<sup>3</sup>. Algal sequences typically sort by biogeography (i.e., into Arctic, Antarctic clades), independent of taxonomy. Arctic clade 2 groups marine and freshwater snow algae together.

**Scientific objectives:** Mission-GLACE will build on a seminal study of the thesis director, Richard Dorrell (*RD*) revealing Arctic and Antarctic-specific IBPs<sup>3</sup> (**Fig. 1**). *RD*'s group has since assembled genomes of thirty further Arctic species from different lineages, habitats (water, sea-ice) and salinity and temperature niches<sup>9</sup>. The co-director (Eric Maréchal, *EM*) and co-supervisor (Robin Kodner, *RK*) have performed primary censuses of algae in mountain snow, providing a unique genetic

resource to connect the “third pole” of continental ecosystems to Arctic and Antarctic biomes<sup>2,10</sup>.

Three interlocking objectives are planned:

**Objective 1.** Build a comprehensive tree of algal IBP diversity and connect this to protein function. An updated IBP phylogeny<sup>3</sup>, enriched with new genomes from *RD*, *EM* and *RK* will be annotated for taxonomy and biogeography to identify environment-specific (marine, freshwater, sea-ice, snow) IBP subfamilies. Sequences will be annotated with *in silico* localization using pipelines from *RD*<sup>11</sup>; expression from transcriptomes of representative species across salinity gradients of 0% (freshwater) to 7% (twice

seawater)<sup>8,9</sup>; and with IBP classification tools trained on non-algal sequences<sup>6</sup>. The deliverable (D1) will be a state-of-the-art evaluation of IBP taxonomic and functional diversity across the algal tree of life.

Objective 2. Construct a new tool for predicting IBP thermal hysteresis from sequence data. Up to 20 IBPs selected from taxonomically and environmentally diverse cryogenic algae<sup>9</sup>, will be cloned for expression in *E. coli* using a dual-tag (HA, TwinStrep) system used by RD and EM's groups<sup>12</sup>. IBPs will be expressed in inducible *E. coli* (Rosetta) strains, purified and shipped on dry ice to EM for thermal hysteresis characterization<sup>6</sup>. Alternative expression in the diatom model *Phaeodactylum* used routinely in EM's lab will be used in case of low *E. coli* expression<sup>12</sup>. Measured activities, alongside published ones, will be used to train a new, publicly-available tool (D2) for classifying IBP kinetics from sequence alone, using protein language-model tools (ProfileView, GEMME) with Alessandra Carbone (CQSB)<sup>13,14</sup>.

Objective 3. Assess IBP taxonomic and functional diversity in wild snow and ice algae. Global meta-genome sequences, e.g. Tara Oceans and EBI Magnify, will be assessed for IBPs<sup>3,5,12</sup>, and completed with new data, only available to Mission-GLACE, from citizen science Living Snow Project samples (RK)<sup>15</sup> and on transects from freshwater snow to marine oceans<sup>9</sup>. Environmental IBPs will be classified by taxonomy, localization, and kinetics, based on cultured species data, and related to environmental variables (temperature, salinity) at each site. These data will be used to build a predictive model for IBP physiological roles (ice-adhesion, hyper and hypo-osmolarity tolerance) in the wild, and to identify marker IBPs for stresses (cold, heat, melting) in cryogenic habitats exposed to climate change (D3)<sup>9</sup>.

**Justification of scientific approach:** Mission-GLACE seeks to explore complementary questions of major biological and environmental interest. Microalgae, separated from model organisms (yeast, plants) by hundreds of millions of years of evolution, contain large numbers of genes of unknown function<sup>11,16</sup>. Even the localization or kinetics of algal proteins may confer different functions to homologues from model organisms<sup>12,17</sup>. Developing new functional classification tools for IBP sequences (D1)<sup>13,14</sup>, particularly if trained on experimental data (D2)<sup>6,8</sup>, may open up gateways for understanding other "dark matter" proteins in algal genomes, e.g. PF20597<sup>7</sup>. At an environmental level, cryogenic ecosystems are under focus for sensitivity to climate change, which has complex impacts on temperature, salinity, nutrients and light<sup>1,9</sup>. Little is known about how polar algae mitigate these environmental fluctuations, and IBPs may enable ecosystem restructuring that allow them to survive transient salinity stresses, or confer a competitive advantage over invasive species during winter<sup>3</sup>. Integrating data from controlled lab experiments<sup>9</sup> with more chaotic field conditions (D3)<sup>2,15</sup>, we aim to develop holistic tools for evaluating the real-world IBP functions, in current and in future cryogenic environments.

Mission-GLACE will be performed over three years, each objective forming one year. All work will be performed at the CQSB except T2B, on a secondment at the LPCV (CEA, Grenoble). Planned tasks: T1A- IBP homologue recovery, phylogeny (months 1-4); T1B- localization, structural, and salinity annotation (M5-8); T1C- writing primary paper (target *mBio*) for D1 (M9-12). T2A- IBP selection, amplification, cloning (M13-18); T2B- IBP expression, thermal hysteresis calculation (M19-20); T2C- tool development for IBP kinetic classification (M21-24). T3A- IBP meta-gene retrieval and phylogeny (M25-27); T3B- habitat modelling of wild IBP salinity and thermal niche (M28-30); T3C- thesis writeup, including a second paper (target *ISME J*) on D2 and D3 (M31-36). The student will be supported by an M1 intern for T2A, and present their work at one international conference (e.g., GRC Polar Marine Science) in Y3.

**Interdisciplinary nature of the project:** Mission-GLACE unifies **comparative genomics, phylogenomics, biochemistry, ecology** and **predictive modelling**. The co-directors and co-supervisor bring complementary expertise necessary to building a project that goes beyond their individual domains.

Richard Dorrell (RD, CQSB, UMR7238) is an evolutionary phycologist, specialized in understanding HGT processes across algal life. In the past five years, RD has published on the frequency, dynamic and cellular functions of HGT in eukaryotic algae, based on high-throughput phylogenetics and mathematical reconciliation<sup>11,16</sup>; including a seminal study on Arctic genomes, demonstrating the presence of Arctic-specific IBPs shared by within-oceanic HGT<sup>3</sup>; and has assembled the genomes of thirty new Arctic species which will form the basis of Mission-GLACE<sup>9</sup>. The CQSB counts extensive expertise in classifying protein fitness landscapes and functions from sequence alone, and collaborations are in place with the groups of Alessandra Carbone<sup>13</sup> and Elodie Laine<sup>14</sup> to apply this to algae. RD will lead phylogenetic, computational (localization, kinetics, salinity) and tool development (D1, D2) in Mission-GLACE.

Eric Maréchal (EM, LPCV, UMR5168) is a plant and algal biochemist, specialized in functional innovations associated with algal lipid metabolism, including snow and ice algae. In the past five years, EM has characterized algal carbon and lipid metabolism pathways associated with cryogenic habitats, including one study coauthored with RD<sup>12,17,18</sup>; and has established a new project, “AlpAlga”, to perform the first characterization of red snow algae from Alpine ecosystems<sup>2,4</sup>. The LPCV contains all equipment required to profile algal physiology and protein biochemistry, including a state-of-the-art lipidomics platform, LIPANG <https://irig.cea.fr/drf/irig/Pages/Infrastructures/Lipang.aspx>. EM will lead experiments for expression and functional characterization (e.g., thermal hysteresis) of selected IBPs for D2.

Robin Kodner (RK) is a snow ecologist at Western Washington University, attached to Université Grenoble-Alpes until end 2027. RK is director of the Living Snow Project, a citizen science-led mission to profile snow algae (observations, samples, meta-genomics) on a global scale<sup>10,15</sup>. RK will lead IBP meta-genomics in freshwater snow, and environmental modelling for D3 (salinity, temperature, altitude).

**Funding** for experimental work in Mission-GLACE will be supplied from an ERC StG (“ChloroMosaic”) awarded to RD 2023-2027 and an HFSP (“Trapped in Ice”) to EM 2024-2027. Neither project can cover the full length of the PhD contract (2026-2029). Additional funding is currently being sought from the Paris Biofoundry (RD as coordinator) and the ANR (PRC, “PoleToPole”, RD and EM as coordinators).

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