

Form B: IGEM-HERC Full Proposal Cover Sheet

Idaho State Board of Education

PROPOSAL NUMBER: (to be assigned by HERC)	TOTAL AMOUNT REQUESTED: \$145,000		
Proposal Track (select one): • Proof of Concept			
NSF I-Corps participation: Have you participated in, or plan to participate in the NSF I-Corps Program? Yes If so, when did you or will you? Fall 2025			
TITLE OF PROPOSED PROJECT: Formulation Development Using Soluble Ice-Nucleating Proteins			
SPECIFIC PROJECT FOCUS: This project focuses on pilot-scale production and purification of soluble fungal ice-nucleating proteins from the Mortierellaceae family in collaboration with Hyacinth Proteins. In addition, liquid and carrier-based formulations will be developed and validated under food, cryopreservation, and atmospheric-relevant conditions to support reliable, scalable, and commercially deployable ice nucleation products.			
PROJECT START DATE: 7/1/2026		PROJECT END DATE: 6/30/2027	
NAME OF INSTITUTION: Boise State University		DEPARTMENT: Chemistry and Biochemistry	
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NAME:		TITLE:	SIGNATURE:
PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR	Konrad Meister	Professor	
CO-PRINCIPAL INVESTIGATOR			
NAME OF PARTNERING COMPANY: Hyacinth Proteins		COMPANY REPRESENTATIVE NAME: Christopher Hubb	
SIGNATURE: 			
Authorized Organizational Representative	NAME: Ella Christiansen Assistant Director, Pre-Award	SIGNATURE: 	

IGEM-HERC Full Proposal
Principal Investigator: Dr. Konrad Meister
Institution: Boise State University
Project Title: Formulation Development Using Ice-Nucleating Proteins
Total Amount: \$145,000

Key Personnel

Konrad Meister, PhD, Principal Investigator, Boise State University

Konrad Meister is an Assistant Professor of Chemistry at Boise State University with expertise in protein biophysics and biomolecular characterization, including structure–function relationships of ice-nucleating proteins. He will lead the overall project and will be responsible for coordinating experimental design, data interpretation and coordination with Hyacinth Proteins. His group will focus on the expression, purification, and functional analysis of the fungal ice-nucleating proteins, including assays to assess ice nucleation activity and protein stability.

Ronan Lanam, Ph.D. Graduate Student, Boise State University

Ronan Lanam is a Ph.D. student in the Biomolecular Sciences program at Boise State University and a member of the Meister lab. He will produce and test ice-nucleating proteins as part of his dissertation research and he will lead the development of the ice-nucleating protein-carrier formulations and generate stability and activity data after frying, storage, and rehydration to support commercial evaluation for cloud seeding.

Jayden Brandt, Laboratory Technician, Boise State University

Jayden Brandt is a full-time technician in the Meister lab with several years of research experience at Boise State. She is trained in protein purification and the ice nucleation assays, and will perform the standardized production, purification, and droplet freezing-based activity measurements required for reproducible laboratory-scale benchmarking and for comparison to pilot-scale material.

Christopher Hlubb, Collaborator, Hyacinth Proteins

Christopher Hlubb is the chief executive officer of Hyacinth Proteins, an Idaho-based company that specializes in the purification of naturally-occurring, bioactive proteins without affecting their native structure. Hyacinth Proteins is part of the Agrilogics group (Lactea Therapeutics; Hyacinth Proteins; Agrilogics Animal Health), specialized in developing innovative healthcare solutions. For this project, he will oversight the pilot-scale fermentation and purification run at Hyacinth Proteins and provide process performance data required to select a scalable production route, including yield (mg/L) and costs. His team brings expertise in scale-up, process development, and quality control, and will provide access to a bioreactor and a small-scale prototype purification system to support early-stage process testing.

Significance

The freezing of liquid water into crystalline ice is one of the most important phase transitions on earth, with profound implications across disciplines as diverse as cryobiology, geology, industrial processes, and atmospheric science¹. At ambient conditions, ice formation becomes thermodynamically favorable below 0 °C, yet the crystallization process is kinetically hindered, allowing pure water to remain in a supercooled liquid state to temperatures as low as -40 °C, below which homogenous ice nucleation occurs². In real-world scenarios, freezing almost always occurs via heterogeneous ice nucleation, where foreign particles or interfaces lower the nucleation barrier and trigger ice formation at warmer subzero temperatures. Since many different materials can act as heterogeneous ice nucleators (INs), the temperature at which freezing occurs varies is inherently stochastic. For practical applications, this causes significant challenges. First, cooling water-based materials to low temperatures is energetically costly, and second, stochastic nucleation leads to heterogeneity in freezing temperatures and ice crystal growth, which is a major uncontrolled variable in the cryostorage of any aqueous material³. These limitations have motivated the search for potent soluble ice-nucleating agents that reliably induce freezing at high subzero temperatures, thereby reducing energy requirements while enabling consistent and predictable freezing behavior. In nature, ice formation is facilitated by both biogenic or abiotic INs. Biological INs are found across all kingdoms of life, with

those from bacteria and fungi being particularly efficient, enabling ice formation at temperatures as high as $-2\text{ }^{\circ}\text{C}^{4,5}$. In contrast, most abiotic INs like mineral dust or organic crystals, freeze water below $-15\text{ }^{\circ}\text{C}^6$, while silver iodide, the widely used commercial cloud seeding agent initiates ice formation around $-6\text{ }^{\circ}\text{C}^7$. The exceptional ice nucleation activity of bacteria originates from specialized ice-nucleating proteins (INPs) that are embedded in the bacterial outer membrane⁴. These membrane-bound INPs form functional assemblies and align water molecules into an ice-like lattice, thereby promoting ice crystal formation with remarkable efficiency⁸. Their potent activity has already been exploited commercially in the form of Snomax™, an inactivated whole-cell bacterial product used for artificial snowmaking⁹. Beyond snowmaking, bacterial INPs have also been explored in frozen food processing, advanced freeze-drying, and cryopreservation research^{9,10}. However, these applications remained at the proof-of-concept stage and have not translated into commercial products. The limited utility of bacterial INPs arises from their strict dependence on membranes for full activity^{8,11,12}, their inability to be isolated as soluble proteins, and their availability only as whole-cell preparations. This strict membrane-dependence reduces stability^{8,13}, limits reproducibility, prevents clean formulation, and introduces regulatory barriers that constrain use in food and biomedical applications. Following bacteria, fungi have been identified as equally potent biological INs, but the molecular origin of their ice-nucleating abilities has long remained unknown^{14,15}. Recent advances in the Meister Laboratory at Boise State University have identified a new class of fungal INPs from the fungal *Mortierellaceae* family (*Mo*INPs) that overcome the bacterial limitations¹⁶. The fungal INPs represent only the second known class of genetically encoded INPs and importantly, they are water-soluble and membrane-independent and retain activity across physicochemical stresses. Figure 1 shows the predicted structures of different fungal *Mo*INPs and a bacterial INP. The INPs share similar β -solenoid structures with repetitive threonine-rich motifs that form the ordered ice-templating surfaces. However, in contrast to bacterial INPs, the *Mo*INPs lack membrane-anchoring domains, the C-terminal capping motif, and rather have cysteine-rich capping motifs that stabilize the solenoid and prevent unfolding in solution. As a result, *Mo*INPs are more stable, active at much lower concentrations, and retain full ice-nucleating activity under conditions that reduce bacterial ice nucleation activity^{14,16}. Figure 2 highlights that in the presence of surfactants like SDS or at elevated temperatures, the bacterial INPs lose activity, whereas the *Mo*INPs are not affected. Importantly, the Meister lab has also established the heterologous expression of *Mo*INPs in other organisms such as the yeasts *Saccharomyces cerevisiae* and *Pichia pastoris*, providing a scalable route for recombinant production, modification and purification^{16,17}.

These advances establish *Mo*INPs as the first stable, soluble, and manufacturable INPs and provide a pathway to produce defined INP formulations rather than unstable whole-cell suspensions, a product that is not currently available on the market.

Soluble INP formulations will enable controlling ice formation in a variety of industrial applications. In cryopreservation, initiating ice formation at warmer subzero temperatures reduces intracellular ice formation, limits osmotic stress, and improves post-thaw cell and tissue viability^{3,18}. For precipitation enhancement applications, soluble INPs provide a more potent traceable and environmentally benign alternative to silver iodide, which is directly relevant to Idaho's investments in weather-modification programs that support snowpack and water availability. In food processing and agricultural storage, controlled ice nucleation enables faster

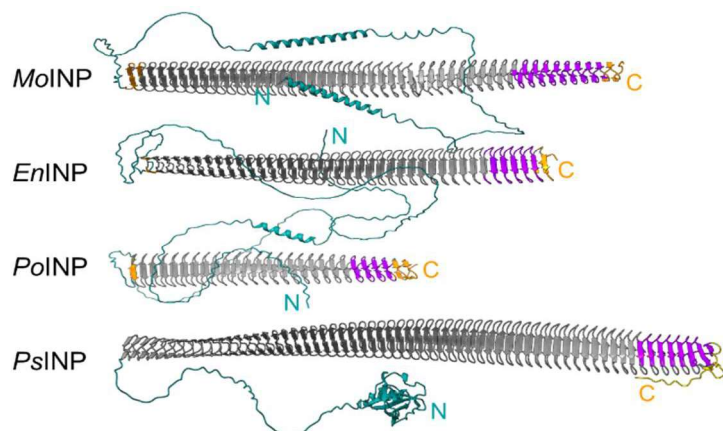


Figure 1. Predicted structures of membrane-independent fungal *Mo*INPs in comparison to a bacterial INP (*Ps*INP) structure, which requires membrane attachment for full ice nucleation activity.

and more uniform freezing, limits ice crystal growth, preserves texture, and improves freeze-drying efficiency, all of which are directly relevant to freezing, storage, and processing practices used across Idaho's agricultural industries¹⁰. The primary translational barrier for *MoINPs* is no longer discovery, but formulation, scale-up, and deployment readiness. Insights from NSF I-Corps interviews with potential end users confirmed that a variety of industries are extremely interested in soluble INP solutions, but that the different applications have distinct requirements for formulations, functionalities under application-specific stresses, delivery formats, and acceptable unit costs. Biomedical cryopreservation requires highly controlled, ultrapure, and low-concentration formulations compatible with buffers, cryoprotectants³, and different cells, while food processing demands solutions that remain active in concentrated matrices at substantially lower cost. Successful atmospheric deployment of the *MoINP* has additional requirements related to dispersibility and compatibility with carrier materials and drone-based deployment platforms. Yet, across all applications, the most important requirement is reliable access to large quantities of pure, soluble, and potent INPs at application-appropriate costs.

Research Goal

The overarching goal of this one-year Proof-of-Concept HERC project is to establish reproducible production, purification, and formulation strategies for *MoINPs* that support application-driven evaluation. The proposed work focuses on translational readiness by reducing technical risks associated with *MoINP* production, formulation stability, and deployment. To achieve this goal, we will pursue three objectives: (1) establish pilot-scale production and purification of *MoINPs* in collaboration with Hyacinth Proteins; (2) functionally validate *MoINP* formulations under application-specific cryopreservation and food processing stresses; and (3) evaluate mineral-carrier formulations of *MoINPs* to support drone-based field delivery for cloud seeding. The long-term goal is to establish high-purity, scalable, and Idaho-produced *MoINP* products suitable for industrial deployment. The project develops an Idaho-based capability for pilot-scale production and formulation of soluble INPs, enabling Idaho to supply defined biologic ice nucleators to global weather-modification programs and for commercial partners evaluating cryopreservation and food freezing technologies.

Objective 1: Establish Scalable Production and Purification of *MoINPs*

Rationale. Environmental and physiological factors such as growth temperature, nutrient availability, and growth phase are known to influence bacterial ice nucleation activity, resulting in reductions in activity or variability between cultures^{19, 20}. In contrast, the ice nucleation activity of the fungus *Fusarium* remained relatively stable throughout the growth cycle, with consistently higher activity in cell-free culture supernatants compared to hyphal pellets. Moreover, nutrient starvation and cold exposure, which enhanced INA in bacteria, did not lead to increased activity in *Fusarium*²¹. The extent to which these environmental and physiological variables influence the ice nucleation activity and INP production in the *Mortierellaceae* family is currently unknown. Resolving this question is an important prerequisite for reproducible production of *MoINPs*.

Approach. Within this objective we will establish reproducible and scalable methods to produce *MoINPs* in defined, soluble form, suitable for downstream formulation and application testing. At laboratory scale, we will determine the influence of growth temperature (4 °C, 20 °C, 35 °C) and growth medium composition (nutrient-rich vs nutrient-limited) on *MoINP* activity and yield. We will quantify the ice nucleation activity using the Twin-plate Ice Nucleation Assay (TINA), which enables high-throughput and quantitative measurements across full dilution series^{4, 22}. The fungal biomass and culture supernatants will be harvested for protein extraction, and we will purify the *MoINPs* using our established size-exclusion chromatography protocols to compare yields, and activity^{16, 23}. In parallel with the laboratory-scale optimization, we will evaluate production routes compatible with pilot-scale operation. We will evaluate two production routes at pilot scale using 10 - 50 L bioreactor vessels in collaboration with Hyacinth Proteins. We will grow the native *Mortierella Alpina* strain to assess *MoINP* production directly from a natural host, and we will use an optimized *Pichia pastoris* strain that expressed *MoINPs* and that was developed through a strategic partnership with Ginkgo Bioworks to optimize *MoINP* production. The pilot-

scale production and purification will be carried out at the facilities of Hyacinth Proteins, and both production routes will be evaluated for ice-nucleation activity, yield, and suitability for downstream purification. Fermentation material will be transferred from Hyacinth Proteins to Boise State, where the samples will be tested for ice nucleation activity. Upon confirmation of ice nucleation activity of the products, we will translate the existing laboratory-scale purification workflow of the Meister lab to Hyacinth's modular chromatography platform, which will be adapted to the physicochemical properties of *MoINPs*, including molecular size, charge state, and solubility. Process parameters such as buffer composition, loading conditions, and elution strategies will be refined to preserve activity and improve throughput. The purified *MoINPs* from both production routes will be characterized at Boise State University to confirm structural integrity, and activity. Ice nucleation activity will be assessed using TINA, while protein integrity will be verified by gel electrophoresis and Circular Dichroism spectroscopy²⁴. These analyses will establish whether *MoINPs* produced via native fungal cultivation or recombinant yeast expression retain equivalent activity and stability after scale-up.

Outcomes. This objective will define conditions that maximize *MoINP* yield and reproducibility, establish pilot-scale purification workflows compatible with native production and recombinant expression, and generate quantitative benchmarks for yield (mg/L), activity, reproducibility, and cost. *Key deliverables will include the physical transfer of fermentation results from Hyacinth Proteins to Boise State and performance results of the crude and purified product.* This data will guide selection of production routes (natural organism vs yeast) for potential commercialization efforts.

Objective 2: Evaluate *MoINP* Functionality Under Cryopreservation and Food-Relevant Conditions

Rationale. In technical applications, the *MoINPs* encounter non-ideal conditions, including exposure to a wide range of pH values, cosolutes, temperature stress, and chemically complex sample environments. Bacterial INPs are known to be sensitive to many of these variables and the ice nucleation activity is reduced by changes in the ionic strength, pH, solvents, and temperature (Figure 2)^{8, 12, 13, 25, 26}. In contrast, our data shown in Figure 2 suggests that *MoINPs* maintain activity under chemically stressful conditions. For translational deployment, *MoINPs* must remain potent ice nucleation activity under the application-specific chemical and physical stresses encountered in cryopreservation and food processing. In cryopreservation, *MoINPs* would be used at minimal concentrations to reduce contamination risk, reduce downstream removal requirements, and avoid adverse biological effects, while also remaining active in the presence of cells and high concentrations of cryoprotective agents (e.g. DMSO, glycerol). In food processing, *MoINPs* must retain activity in chemically complex and highly crowded environments created by high solute concentrations, emulsions, and heterogeneous matrices.

Approach. We will first determine universally relevant functional parameters of *MoINPs* for applied use, including the minimum *MoINP* concentration at which potent ice nucleation activity is retained, the influence of ionic environments, pH stability across a broad range (pH 2–12), temperature sensitivity, and resistance to repeated freeze–thaw cycles. At the lowest concentration, we will examine *MoINP* functionality in buffered solutions that contain common cryoprotectants like DMSO and glycerol, at concentrations representative of standard cryopreservation protocols. Ice nucleation activity will be quantified using TINA, which will provide complete freezing spectra as a function of temperature and dilution. The resulting freezing

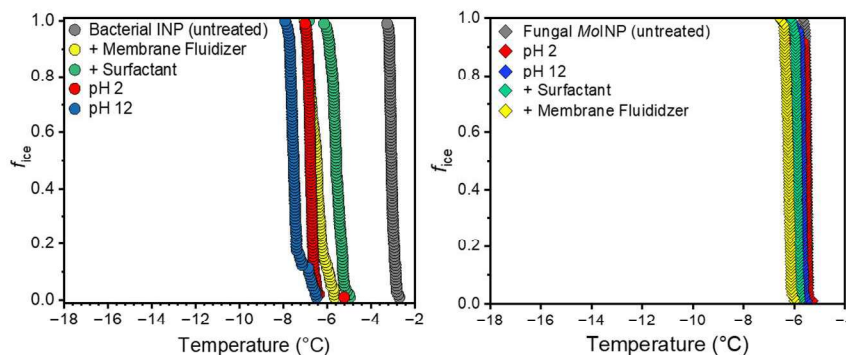


Figure 2. Freezing experiments of fungal and bacterial INPs show that their ice nucleation activity is differently affected by cosolutes. Bacterial INPs are more instable and affected by several stressors.

spectra data will be analyzed using the HUB-method, which identifies IN subpopulations and enables quantitative assessment of how environmental changes affect ice nucleation activity²⁷. *Mo*INP formulations will also be evaluated in cellular suspensions, including red blood cells and mammalian cell lines. Cell survival and viability will be assessed after freezing and thawing to relate freezing behavior to biological outcome. For food-relevant evaluations, *Mo*INPs will be tested under conditions of molecular crowding (Ficoll) and high solute content typical of frozen food matrices. These tests will include aqueous sugar solutions spanning 10–40% (w/v), orange juice, and dairy-based samples¹⁰. Ice nucleation activity will be measured before and after freeze–thaw cycling to assess reproducibility of activity during storage and handling.

Outcomes. This objective will define general formulation and concentration boundaries for *Mo*INPs for use in cryopreservation and food applications, establish minimum effective doses, and generate quantitative datasets that support up-scaling decisions. *Key deliverables will be performance datasets of MoINPs in application-relevant conditions.*

Objective 3: Evaluate Carrier-Based *Mo*INP Formulations for Solid Deployment

Rationale. Previous studies have shown that fungal macromolecules can retain ice nucleation activity even after detachment from their original cellular structures, remaining stable across freeze-thaw cycles and over broad pH ranges^{14, 28}. These macromolecules have been shown to readily adsorb to mineral surfaces, a process that may enhance ice-nucleating efficiency facilitate atmospheric transport²⁹. While *Mo*INPs provide a biologically derived and environmentally benign potent alternative to silver iodide for precipitation enhancement, their commercial use in cloud seeding will depend on whether they can be dispersed in the atmosphere without loss of activity. Rainmaker Technology, a California-based cloud seeding company, currently holds a one-year option agreement with Boise State University to evaluate *Mo*INPs for precipitation enhancement. Emerging cloud-seeding strategies, particularly drone-based deployment, impose specific requirements on *Mo*INP formulation and delivery, favoring materials that can be readily aerosolized, remain stable during dispersal, and retain activity under atmospheric dilution and acidic conditions. Whether *Mo*INPs meet these requirements in soluble form, or instead require association with particulate carrier materials to enable effective aerosolization and deployment, remains unknown. Addressing this uncertainty is essential for advancing *Mo*INPs toward operational atmospheric applications.

Approach. We will evaluate the potential of *Mo*INPs combined with inexpensive, non-toxic carrier materials relevant to atmospheric deployment. Mineral carriers, including feldspar and quartz^{6, 30}, as well as lignin-based nanoparticles³¹, will be examined. High-resolution freezing spectra will be acquired for the carrier materials alone and in the presence of varying *Mo*INP concentrations to quantify changes in ice nucleation activity. These measurements will enable us to determine the minimum *Mo*INP concentration required to enhance the carrier's ice nucleation activity. The stability of *Mo*INP–carrier formulations will be assessed through consecutive freeze–thaw cycles and controlled aging experiments conducted in both aqueous and dried form. Freezing experiments will be performed using TINA, and the data will be analyzed using the HUB method and associated Python codes to quantify changes in the IN subpopulations²⁷. Figure 3 shows that the ice-nucleation activity of feldspar can substantially be enhanced by low concentrations of *Mo*INPs. Combined, these experiments will determine whether carrier

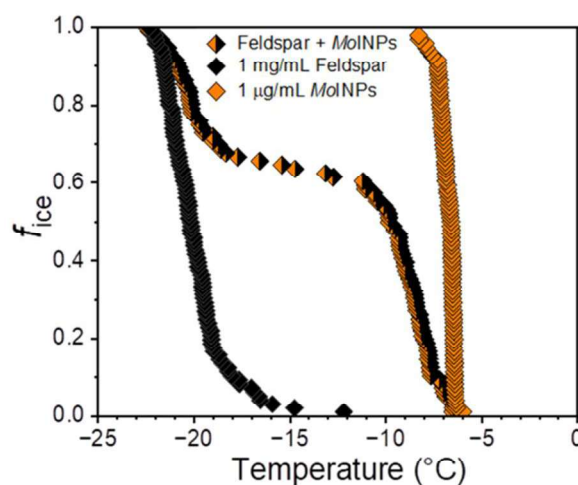


Figure 3. Ice nucleation activity of a feldspar suspension increases significantly upon addition of *Mo*INPs.

association preserves or enhances *MoINP* activity and will identify formulation conditions that support efficient dispersal. Emphasis will be placed on formulations compatible with drone-based deployment and suitable for future field testing with Rainmaker Technology.

Outcomes. This objective will determine whether *MoINPs* retain ice nucleation activity when incorporated into carrier-based formulations suitable for solid atmospheric deployment. *Key deliverables will be the performance results of carrier-based MoINP formulations.* The results will identify carrier materials and conditions that support activity retention and will provide a basis for selecting *MoINP* formulations for cloud seeding and field evaluation.

Project Timeline

The *MoINP* technology currently resides at Technology Readiness Level (TRL) 4, supported by proof-of-concept demonstration of functional ice nucleation activity in bacteria and yeast. *MoINPs* have been identified, produced, and validated at laboratory scale using droplet freezing assays in the Meister laboratory, and the activity has been independently confirmed by Rainmaker Technology using their application-relevant experimental setups. The discovery of *MoINPs* was enabled through current National Science Foundation (NSF) and Department of Defense (DoD) awards to Dr. Meister, and a current NSF Translate Seed grant is supporting evaluation of *MoINP*. By the conclusion of this one-year proof-of-concept HERC project, the technology is expected to advance to TRL 6, defined by pilot-scale production and validated performance in formulation environments relevant to cryopreservation, food matrices, and cloud seeding. TRL 5 will be achieved by demonstrating consistent pilot-scale production in 10 – 50 L vessels and a reproducible mg/L values with verified activity retention. TRL 6 will be achieved by validating *MoINP* performance in application-relevant formulation environments, including cryoprotectant-containing solutions, food matrices, and at least one carrier-based formulation evaluated by an external industry partner (Rainmaker). The project will extend prior NSF-supported work by advancing pilot-scale purification with Hyacinth Proteins, optimizing liquid and lyophilized formulations, quantifying performance under application-relevant conditions, and establishing yield and costs models to support selection of production routes and partner testing decision. The project is organized into three objectives that address translational barriers in advancing *MoINPs* from laboratory discovery to application-ready materials, with the project timeline in Table 1 providing a breakdown of aims and milestones for each objective. All experimental capabilities required to perform the project are available from the beginning, including native fungal strains, recombinant yeast expression systems, droplet freezing assays, and laboratory-scale purification workflows. *MoINPs* are already available at laboratory scale, enabling testing to begin immediately and to proceed in parallel with production optimization and scale-up. The project will begin in August. During the first quarter we will optimize laboratory-scale growth and production parameters for *MoINPs* using *M. Alpina* cultures and the optimized recombinant yeast strain developed by Ginkgo Bioworks. Growth temperature, medium composition, and cultivation conditions will be varied, and ice nucleation activity and protein yield will be quantified using established protocols. These measurements will be performed by Jayden Brandt, the dedicated laboratory technician trained in these protocols. *Milestone 1 is completion of reproducible laboratory-scale production for both routes with quantified yield and activity and selection of conditions to transfer to pilot-scale runs.* Existing *MoINPs* samples will be evaluated under physical and chemical stressors relevant to applications. These studies will establish key stability parameters, including dilution series to determine minimum effective concentrations, activity as a function of pH, freeze-thaw cycles, temperature, and sensitivity to common ionic cosolutes. *Milestone 2 are quantitative datasets that define concentration- and formulation-dependent MoINP activity boundaries that can be shared with industry representatives.* During the second and third quarter, Hyacinth Proteins will perform pilot-scale growth experiments in 10 - 50 L bioreactors using both native fungal strains and optimized yeast strains under conditions defined at laboratory scale. Hyacinth will use a pilot scale setup of their purification workflows to attempt purification of *MoINPs*, and ice nucleation activity and protein yield will be quantified at Boise State University. *Milestone 3 is completion of pilot-scale production and purification for both routes with quantitative comparison of yield (mg/L), activity retention,*

and estimated unit cost, enabling selection of the preferred scale-up route. During the later project phase, formulation testing will be continued in cryopreservation-relevant formulations containing cryoprotectants and cell lines, as well as food-relevant matrices characterized by high solute content and molecular crowding. We will also start preparing *MoINPs* combined with carrier materials such as feldspar and lignin-based particles and perform activity and stability studies following drying, rehydration, dilution, and storage to simulate handling and dispersal. These measurements will be performed by Ph.D. student Ronan Lanam. *Milestone 4 is identification of at least one MoINP-carrier formulation that retains potent activity after drying and rehydration and is suitable for transfer to Rainmaker Technology for external evaluation.* Within the final quarter, datasets on production scalability, formulation stability, and deployment-relevant performance will be consolidated, and reproducibility will be confirmed. *Milestone 5 is an integrated dataset package covering production yield, purification recovery, formulation stability boundaries, and carrier formulation performance, suitable for industrial partner evaluation and for supporting licensing and additional external testing agreements.*

Project Management

Dr. Meister will oversee project execution, data analysis, milestone tracking, and reporting. He will hold biweekly meetings with Jayden Brandt and Ronan Lanam to review experimental results, evaluate progress toward milestones, and plan subsequent characterization experiments. These meetings will focus on laboratory-scale workflows, *MoINP* activity datasets, and carrier-based stability testing as outlined in the project timeline in Table 1. Coordination with Hyacinth Proteins will occur through scheduled bi/tri-monthly meetings with Christopher Hlubb, which are already established. These meetings will review updates on *MoINP* pilot-scale production, purification performance, yield (mg/L), and estimated cost metrics, and will address technical challenges associated with scale-up. During active pilot-scale production, these meetings will transition to monthly virtual meetings and will include members of Chris Hlubbs team participating in the production efforts to support real-time decision making and production route evaluation. In addition, four in-person visits to the Hyacinth Proteins facility in Twin Falls, Idaho are scheduled during the project period. These visits will evaluate small scale purification prototypes and will support pilot-scale fermentation and purification runs, direct evaluation of process parameters, and coordination of sample transfer and analytical validation at Boise State University. Milestone completion, as defined in Tables 1, 2, are decision points for progression to subsequent stages, including selection of the preferred production route, transfer of validated *MoINP* formulations to Rainmaker for external evaluation, and, if appropriate, engagement with the Boise State University Office of Technology Transfer to initiate outreach to industrial partners in food science and cryopreservation applications.

Table 1. Project timeline of the proposed work with milestones (MS) highlighted.

#	PROJECT OBJECTIVES	Year 1			
		Q1	Q2	Q3	Q4
1	Purify <i>MoINPs</i> from fungal and yeast cultures Milestones: Optimize <i>MoINP</i> expression, Determine yield (mg/L) and costs of <i>MoINP</i> production at Pilot-Scale with Hyacinth Proteins		MS		
2	Determine <i>MoINPs</i> Stability for Cryopreservation/Food Applications Milestones: Quantitative dataset of concentration-dependent <i>MoINP</i> activity across application-relevant physical and chemical stressors.			MS	
3	Evaluate <i>MoINPs</i> -Carrier Formulations for Solid Dispersal Milestones: Datasets of stability and activities of carrier-based <i>MoINP</i> formulations for solid dispersal				MS

Feasibility, Risk Mitigation & Contingency

Our preliminary results, extensive experience with ice-nucleating proteins^{8, 16, 32}, and a patent-pending novel *MoINP* class provide a strong foundation for successful execution of the proposed work. We have access

to the fungal cultures, recombinant yeast strains, and plasmid vectors, as well as established droplet freezing assays in the Meister laboratory⁴ and professionally maintained protein purification infrastructure in the Biomolecular Research Center at Boise State University. These resources reduce the experimental risks at the laboratory scale. The primary anticipated challenge is production scalability. Although *Mo*INPs have been successfully produced and purified at laboratory scale^{16, 17}, scale-up may result in reduced yields or less activity. We aim to mitigate this risk by pursuing production strategies for both recombinant yeast and a native fungal strain. If native fungal production underperforms, alternative *Mo*INP-producing fungal strains that are available in the Meister laboratory will be evaluated. If recombinant yeast expression is unsuccessful, we will discuss with Ginkgo Bioworks, with whom a cooperation agreement is in place, to redesign and optimize *Mo*INP variants for improved production yield using their strain engineering and biomanufacturing expertise. A second anticipated challenge is retention of ice nucleation activity across diverse formulation environments. Our data demonstrates that *Mo*INPs retain activity across extreme solution and cosolutes chemistries (Figure 2)²³. However, it is expected that certain combinations of pH, crowding, or cosolutes will reduce or eliminate activity. This risk is addressed by stepwise testing across defined physical and chemical parameters prior to evaluation. Identification of non-compatible conditions is an informative outcome that will guide formulation selection rather than a project failure. Unanticipated research challenges will be managed through parallelization and milestone-driven decision making. The availability of multiple production routes, alternative sources, established analytical workflows, and pre-existing collaborations with experienced industrial partners enables rapid redirection of effort if a given approach proves ineffective. The long-term relevance of the proposed work is underscored by the widespread need to control ice formation across biological, industrial, and atmospheric disciplines. Environmentally benign freezing technologies are particularly important since the U.S. increasingly pursues activities in the Arctic, where ice has the potential to be a logistical burden or an operational enabler. In fact, DARPA launched a massive Ice Control for Cold Environments program aimed to develop new biologically inspired strategies for protecting personnel and enabling sustained military operations in cold environments. In cryopreservation, uncontrolled ice formation has been identified as the single most important variable, directly causing variability in cell recovery, viability, and function between samples. In cloud seeding, there is growing need for traceable environmental benign ice nucleators.

Potential Economic Impact

Over the past decade, the use of protein-based therapeutics, biologics, and stem cell-derived therapies has expanded significantly, increasing demand for technologies that enable controlled freezing and storage. Ice-binding proteins, including antifreeze proteins (AFPs) and INPs, represent an emerging class of such enabling biomolecules. The global market for AFPs was ~\$10 million in 2023 and is projected to reach \$50 million by 2028, reflecting growing adoption in food, cosmetics, and specialty biotechnology applications³³. In contrast purified and soluble INPs are not commercially available, despite their advantages for applications that require controlled ice formation at high subzero temperatures. Current commercial use of biological ice nucleation technology is limited to Snomax®, a freeze-dried preparation derived from bacteria that is used for artificial snowmaking. The absence of purified, scalable INP formulations represents a clear commercialization gap. This gap is especially relevant to cloud seeding and precipitation enhancement, a global market estimated at \$400 million and projected to approach \$1 billion within the next decade, driven by increasing water scarcity, agricultural demand, and climate changes³⁴. Existing operations rely on silver iodide and related chemical agents. Stable, soluble, and manufacturable *Mo*INPs provides a biologically derived traceable alternative. By establishing pilot-scale production at Hyacinth Proteins and producing defined formulations at Boise State, this project creates a pathway for Idaho-based manufacturing services, future supply agreements for formulated *Mo*INPs, and licensing revenue tied to *Mo*INP use in cloud seeding and related freezing applications. Near-term economic outcomes will be measured by converting the current one-year option agreement with Rainmaker into a license agreement and by execution of external evaluation partnerships or option agreements in additional commercial sectors.

Success Criteria

Success of this project will be measured using objective and quantifiable outcomes. By the end of the first project quarter, success will be demonstrated by completion of a laboratory-scale growth runs for *MoINPs* produced from a native *Mortierella* culture and recombinant yeast, with the protein yield and ice nucleation activity quantified using TINA. Results of these experiments will demonstrate functional activity and reproducibility at laboratory scale. The mid-project, success will be measured by completion of pilot-scale growth and purification of 10 - 50 L cultures at Hyacinth Proteins. Pilot-scale success criteria include measured ice nucleation activity of purified material, a measured protein yield (mg/L), and estimated production costs calculated from measured yield and process inputs. Selection of a preferred production route based on these measured parameters will constitute a defined project milestone.

Formulation-related success can directly be measured by the generation of quantitative datasets describing *MoINP* performance across relevant physical and chemical conditions, including minimum effective concentration, pH, temperature, dilution, freeze–thaw cycling, and ionic cosolutes. Successful performance will be demonstrated by measurable and reproducible ice nucleation activity in cryoprotectant-containing buffers, cellular cryopreservation samples, and food-relevant matrices. For deployment-relevant applications, success will be measured by demonstration that *MoINPs* retain ice nucleation activity following combination with carrier materials such as feldspar and lignin-based particles and after drying, rehydration, dilution, and storage. Completion of these measured outcomes will demonstrate advancement from TRL 4 at project start to TRL 6 by project completion, defined by pilot-scale producibility and validated performance in application-relevant formulation environments. The resulting datasets on production, formulation performance, and cost will provide the basis for industry evaluation, follow-on funding, and progression to the next HERC development stage.

An additional objective success parameter will be a license agreement with Rainmaker Technology, replacing the current one-year option agreement for *MoINP* use in cloud seeding. Success will also be measured by signing one or more new option agreements with industrial partners for testing and evaluation of *MoINPs* in applications.

Table 2. Success criteria and deliverables of the proposed work

Objective	Success Criteria	Timeline	Personnel	Deliverable
1. Scalable <i>MoINP</i> Production & Purification	Lab-scale production for native and yeast routes with quantified yield and TINA activity. Completion of 50 L pilot-scale production with measured yield (mg/L), activity retention, and estimated unit cost, enabling selection of a preferred route	Q1 (lab), Q2–Q3 (pilot)	Jayden Brandt (lab scale), Matt Bender (pilot-scale), Konrad Meister (oversight & analysis)	Production report including yields, TINA spectra, and production cost per mg INP
2. Formulation Validation (Cryo & Food)	Minimum effective concentration of <i>MoINPs</i> and stability boundaries across pH, freeze–thaw cycles, cryoprotectants, and food matrices, with reproducible TINA datasets.	Q2–Q3	Jayden Brandt (TINA measurements), Konrad Meister (oversight & analysis)	TINA activity and stability datasets
3. Carrier-Based Deployment Readiness	Identification of at least one carrier-based <i>MoINP</i> formulation that retains activity after drying and rehydration and is delivered to Rainmaker Technology for evaluation.	Q3–Q4	Ronan Lanam, Konrad Meister (formulations & analysis), Jayden Brandt (TINA, activity)	TINA activity of <i>MoINP</i> -Carrier formulations
Project-Level Outcome	Advancement from TRL 4 to TRL 6 through pilot-scale production and validated application or carrier performance.	Q4	Konrad Meister	Consolidated technical dataset and TRL summary

Budget Justification

We request funds for Jayden Brandt, a full-time laboratory technician in the Meister laboratory at Boise State University who will be responsible for performing the characterization and purification experiments, including ice nucleation activity measurements, SDS-PAGE, and size-exclusion chromatography. Ms. Brandt is already trained in the analytical workflows required for this project and has been working with Dr. Meister for two years. Support is requested for 12 months of salary in the amount of \$54,010 plus fringe benefits at 46.61%, for a total personnel cost of \$79,200.

We also request funds for the purchase of a console freeze dryer, which is essential for reducing the volume of the fermentation samples after *MoINP* growth and for purifications in Objective 1, as well as for freeze-drying stability experiments required in Objectives 2 and 3. Although a freeze dryer is available at Boise State University, that instrument is low throughput, heavily scheduled, and located in a laboratory in another department, making it impractical for the sustained, high-volume usage required for this project. The requested freeze dryer can support simultaneous processing of multiple large-volume samples, enabling analysis of dozens of *MoINP* preparations. After conclusion of the project, the Freeze-dryer will be placed

in a shared laboratory of the Chemistry department at Boise State, and will be made available to other researchers across Boise State University or the College of Western Idaho free of charge. The total cost of the console freeze dryer, including the required accessories, is \$34,400.

We request \$10,000 for supplies and materials to support fungal and yeast growth, protein purification and characterization, and ice nucleation measurements. These funds will be used for media components, reagents, purification consumables, and analytical supplies required for protein analysis. Most of the supplies budget will support ice nucleation measurements using the TINA platform, especially consumables for full concentration-series experiments that rely on an automated Eppendorf liquid handling station. Additional supplies include osmolytes, cryoprotectants, cell lines, and formulation components required for stability testing under application-relevant conditions.

Additional funds are requested to support domestic travel for meetings and experimental work at the Hyacinth Proteins facilities in Burley, Idaho. Travel will include two one-day trips for project coordination and two two-day trips to support pilot-scale protein production and purification experiments on site. The estimated travel costs for these activities are \$1,800. Finally, we request funds to support Ronan Lanam, a PhD graduate student in Biomolecular Science at Boise State University and a member of the Meister laboratory. His doctoral research focuses on *Mo*INP modification, formulation development, and application-relevant performance testing. He has experience evaluating *Mo*INP functionality under environmental stressors and will lead the carrier–*Mo*INP formulation and characterization experiments and associated stability and activity measurements. Support in the amount of \$19,600 is requested for his graduate student salary and fringe for nine months of the project period. Table 3 provides an overview of all the requested funds and justifications. Importantly, all costs associated with pilot-scale production and purification of *Mo*INPs at the Hyacinth Proteins facilities will be covered by Hyacinth Proteins as part of the collaboration.

Table 3. Overview of the budget of the proposed work.

Item Request	Justification	Total Request
Personnel	Support for lab tech who performs characterization measurements & GRA who will develop and characterize <i>Mo</i> INP-carrier formulations.	\$98,800
Equipment	Console freeze dryer, which is essential for reducing the volume of the samples after <i>Mo</i> INP growth and purifications in and for freeze-drying stability experiments.	\$34,400
Consumables	Consumables for ice nucleation measurements and fungal/yeast growth and <i>Mo</i> INP purification.	\$10,000
Travel	Funds for domestic travel for several meetings and experimental work at the Hyacinth Proteins facilities in Burley, Idaho	\$1,800
Total		\$145,000

Future Funding

To advance beyond the one-year Proof-of-Concept scope, federal funding will be pursued to advance *Mo*INP structure–function relationships and translational development. As a current NSF CAREER award recipient, Dr. Meister is eligible to apply to the NSF Translation to Practice (TTP) program, and the Explore track is well aligned with early-stage translational development of *Mo*INP-based technologies. Given the relevance of controlled ice formation to cryopreservation and biomanufacturing, we will also target funding opportunities from the National Institutes of Health (NIH) to advance *Mo*INP-based technologies for cryopreservation-relevant applications. Appropriate mechanisms include the NIH R21 program for exploratory translational studies and the R33 mechanism for technology development and validation in cell-based systems. Given the strong initial results and an existing option agreement with Rainmaker, the NSF Transform program at Boise State, which supports translational activities and currently funds Dr. Meister’s work on the *Mo*INPs translation through a seed grant, offers scale-up funding and represents an additional pathway to advance the project to the next stage of development and implementation.

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Appendix A: Facilities, Equipment & Other Resources

The following describes the resources, external and internal, including facilities, equipment, personnel and additional resources the project team will provide in support of the HERC proposal *Formulation Development Using Ice-Nucleating Proteins*.

FACILITIES

LABORATORY

The Meister lab is located on the 3rd floor of the Environmental Research Building, in the Department of Chemistry at Boise State University. The main lab is approximately 1400 square feet, capable of accommodating 6-10 researchers. The laboratory includes nine large benches for chemical and physicochemical experiments, two hoods for synthetic chemistry, and a sterile workbench. Minor equipment useful for the project include but are not limited to Confocal Raman microscope, Nanodrop UV-VIS spectrophotometer; a pH meter; analytical and preparative balances, several chillers, a preparative Beckman tabletop centrifuge; a water purification system; a grinder, and several freezer and fridges.

COMPUTER

Dr. Meister has a Dell laptop and desktop. Numerous laboratory-owned computers are available and equipped with appropriate software for data processing, literature access, and manuscript/figure preparation. The BSU Library provides free access to a wide range of search engines, scientific literature, and books, as well as the ability to obtain any missing resources at no cost to the Principal Investigator. BSU provides electronic storage for faculty at no cost, which can be used for electronic data storage. The research group further has access to the BSU Chemistry Computer lab, a shared facility with >50 desk spaces.

OFFICE

Dr. Meister has an office adjacent to the lab with dedicated furniture and computer. The space is around 150 square feet, large enough for one-on-one discussions with students. Group meetings and presentations are held in nearby conference rooms. In addition, a breakout room equipped with a small kitchen and coffee machine near the lab is great for informal scientific discussions.

MAJOR EQUIPMENT

Ice-Specific Assays, several low-throughput ice nucleation and antifreeze assays as well as high-precision temperature-controlled stages are readily available. These include a Clifton Nanoliter Cryoscope and a Nanoliter Osmometer, which allow for precise control of sample temperatures (<0.001°C), enabling observation of ice nucleation, growth, and melting.

Optical Microscopy, the Meister lab is equipped with a Zeiss Axioscope 5 fluorescence microscope and a Zeiss dissection microscope equipped with digital cameras and temperature-controlled Linkham cooling stages.

Twin Plate Ice Nucleation Assay (TINA), the Meister lab is equipped with a state-of-the-art home-build high-throughput droplet freezing assay. TINA enables the simultaneous measurement of a complete dilution series with each series composed of hundreds of droplets of a few microliters with high statistics, enabling the analysis and characterization of the efficiency of particles with unmatched accuracy.

Liquid Handling Station, the Meister lab is equipped with an automated liquid handling station (epMotion ep5073, Eppendorf, Hamburg, Germany) which enables rapid sample preparations in the 384-well plates used for TINA measurements.

Dynamic Light Scattering, the Meister lab is equipped with a temperature-controlled -15 to 110°C ($\pm$ 0.1°C) BeNano 180 Pro Instrument to measure particle size, molecular weight, & microrheology of particles and molecules.

Sterile Bench and Incubators, the Meister lab is equipped with a sterile Cabinet for cell culture and two temperature-controlled Corning incubators for cell growth.

OTHER RESOURCES

CORE FACILITIES

The Meister lab has access to all core facilities on campus, with all of them having a significantly discounted price for users on campus. The facilities likely being used in the proposed work are mostly located at the Biomolecular Research Cluster and the Boise State Center for Materials Characterization.

BIOMOLECULAR RESEARCH CENTER

The BRC at Boise State is equipped with all state-of-the-art experimental setups for the purification of *MoINPs*. Facilities include but are not limited AKTAs, protein expression and purification services, a Bruker Daltonics maXis Quadrupole Time-of-Flight,

DEPARTMENTAL AND INSTITUTIONAL SUPPORT

The department offers junior faculties, including Dr. Meister, a mentoring program, which helps young researchers to get started and provides additional support. The chemistry department chair has bi-weekly lunch meeting with all tenure track faculty to provide guidance and mentorship. In addition, the department and institution have dedicated administrative support for grant submission, grant management, and general departmental administrative duties.

IDENTIFYING INFORMATION:

NAME: Meister, Konrad

ORCID iD: <https://orcid.org/0000-0002-6853-6325>

POSITION TITLE: Assistant Professor

PRIMARY ORGANIZATION AND LOCATION: Boise State University, Chemistry, Boise, Idaho, United States**Professional Preparation:**

ORGANIZATION AND LOCATION	DEGREE (if applicable)	RECEIPT DATE	FIELD OF STUDY
Ruhr University Bochum, Bochum, Nordrhein-Westfalen, DE	Physical Chemistry / Ph.D	10/2009 - 09/2013	Chemistry
Ruhr University Bochum, Bochum, Nordrhein-Westfalen, DE	Biochemistry/Mas ter of Science	10/2007 - 09/2009	Chemistry
Ruhr University Bochum, Bochum, Nordrhein-Westfalen, DE	Biochemistry/Bac helor of Science	10/2004 - 09/2007	Chemistry

Appointments and Positions

2023 - present Assistant Professor, Boise State University, Chemistry, Boise, Idaho, United States

2018 - present Affiliated Group Leader, Max Planck Institute for Polymer Reseach, Mainz, Not Applicable, N/A, Germany

2020 - 2022 Assistant Professor, University of Alaska Southeast, Juneau, Alaska, United States

Products**Products Most Closely Related to the Proposed Project**

1. Schwidetzky R, de Almeida Ribeiro I, Bothen N, Backes A, DeVries A, Bonn M, Fröhlich-Nowoisky J, Molinero V, Meister K. Functional aggregation of cell-free proteins enables fungal ice nucleation. Proceedings of the National Academy of Sciences. 2023 November 09; 120(46):- . Available from: <https://pnas.org/doi/10.1073/pnas.2303243120> DOI: 10.1073/pnas.2303243120
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3. Galit Renzer, Ingrid de Almeida Ribeiro, Hao-Bo Guo, Janine Fröhlich-Nowoisky, Rajiv J. Berry, Mischa Bonn, Valeria Molinero, Konrad Meister. Hierarchical assembly and environmental enhancement of bacterial ice nucleators. Proceedings of the National Academy of Sciences. 2024 October. DOI: 10.1073/pnas.2409283121
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Understanding Bacterial Ice Nucleation. The Journal of Physical Chemistry B. 2022 January. Available from: <https://doi.org/10.1021%2Facs.jpcc.1c09342> DOI: 10.1021/acs.jpcc.1c09342

5. Max Lukas, Ralph Schwidetzky, Anna T. Kunert, Ellen H.G. Backus, Ulrich Pöschl, Janine Fröhlich-Nowoisky, Mischa Bonn, Konrad Meister. Interfacial Water Ordering Is Insufficient to Explain Ice-Nucleating Protein Activity. The Journal of Physical Chemistry Letters. 2021 January; 12(1):218--223. Available from: <https://doi.org/10.1021%2Facs.jpclett.0c03163> DOI: 10.1021/acs.jpclett.0c03163

Other Significant Products, Whether or Not Related to the Proposed Project

1. de Almeida Ribeiro I, Meister K, Molinero V. HUB: a method to model and extract the distribution of ice nucleation temperatures from drop-freezing experiments. Atmospheric Chemistry and Physics. 2023 May 22; 23(10):5623-5639. Available from: <https://acp.copernicus.org/articles/23/5623/2023/> DOI: 10.5194/acp-23-5623-2023
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4. Galit Renzer, Rosemary J. Eufemio, Ralph Schwidetzky, Janine Fröhlich-Nowoisky, Mischa Bonn, Konrad Meister. Polyol-Induced 100-Fold Enhancement of Bacterial Ice Nucleation Efficiency. The Journal of Physical Chemistry C. 2024 December. DOI: 10.1021/acs.jpcc.4c07422
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Certification:

I certify that the information provided is current, accurate, and complete. This includes, but is not limited to, information related to current, pending, and other support (both foreign and domestic) as defined in 42 U.S.C. § 6605.

In accordance with Section 10632 of the CHIPS and Science Act of 2022 (42 U.S.C. § 19232), each individual identified as a senior/key person must certify that they are not a party to a malign foreign talent recruitment program.

Research Security Training Requirement for Federal Award Personnel: In accordance with Section 10634 of the CHIPS and Science Act of 2022 (42 U.S.C. § 19234), each individual identified as a senior/key person must certify that they have completed the requisite research security training that meets the requirements specified in Item 2 of Important Notice No. 149 within 12 months prior to

proposal submission.

Certified by Meister, Konrad in SciENcv on 2026-02-14 14:46:03

Appendix C: Senior Personnel

SENIOR PERSONNEL

DR. KONRAD MEISTER, PI, BOISE STATE UNIVERSITY

Dr. Meister is an Assistant Professor of Chemistry at Boise State University with expertise in protein biophysics and biomolecular characterization, including structure–function relationships of ice-nucleating proteins. Dr. Meister will lead and direct the proposed work including managing the intellectual, personnel, and reporting aspects of the project. Bi-weekly group meetings will be supplemented by weekly meetings with the lab manager and student involved in the project. He will lead the overall project and will be responsible for coordinating experimental design, data analysis and coordination with Hyacinth Proteins.

JAYDEN BRANDT, LABORATORY TECHNICIAN, BOISE STATE UNIVERSITY

The project will support the salary of a full-time laboratory technician for one year. Jayden Brandt has served as a technician in the Meister laboratory for two years and has additional research experience at Boise State University. She is fully trained in protein purification and in biophysical and ice nucleation characterization assays. She will carry out the standardized experiments required for this project, including expression, extraction, purification, and functional testing of *Mo*INPs from both native fungal and recombinant systems, as well as testing of application-relevant formulations.

RONAN LANAM, PH.D GRADUATE STUDENT, BOISE STATE UNIVERSITY

Ronan Lanam is a first-year PhD graduate student in Biomolecular Science at Boise State University working in the Meister laboratory and will be supported on this project for twelve months. His work focuses on *Mo*INP formulations and application-relevant performance. He has experience purifying *Mo*INP from yeast, and evaluating *Mo*INP functionality under environmental stressors. He will lead the carrier–*Mo*INP formulation experiments and analysis of *Mo*INP activity under complex, multi-stress conditions relevant to deployment and application environments and will coordinate with Rainmaker Technology for field testing.

CHRISTOPHER HLUBB, INDUSTRIAL COLLABORATOR, HYACINTH PROTEINS

Christopher Hlubb is the chief executive officer of Hyacinth Proteins, an Idaho-based company that specializes in the purification of naturally-occurring, bioactive proteins without affecting their native structure. Hyacinth Proteins is part of the Agrilogics group (Lactea Therapeutics; Hyacinth Proteins; Agrilogics Animal Health), specialized in developing innovative healthcare solutions. For this project, he will oversight the pilot-scale fermentation and purification run at Hyacinth Proteins and provide process performance data required to select a scalable production route, including yield (mg/L) and costs. His team brings expertise in scale-up, process development, and quality control, and will provide access to a bioreactor and a small-scale prototype purification system to support early-stage process testing.



16 February 2027

HERC-IGEM Proposal Review Committee

Dear Committee,

On behalf of Hyacinth Proteins, I am writing to express our strong support for Dr. Konrad Meister's HERC-IGEM proposal entitled "*Formulation Development Using Ice-Nucleating Proteins*". We are enthusiastic about the proposed research and committed to contributing to its success through collaborative efforts and technical expertise.

Hyacinth Proteins has had a productive and ongoing collaboration with Dr. Meister and his laboratory at Boise State University since 2022. This partnership has focused on the biochemical analysis and functional characterization of bioactive proteins, culminating in upcoming peer-reviewed publications. Beyond this work, we have also been actively involved with Dr. Meister's team in the exploration of purification strategies for antifreeze and ice-nucleating proteins directly from natural resources, including efforts to produce these biomolecules at scale.

We believe that this line of research not only advances scientific understanding of protein function but also opens exciting possibilities for real-world applications in cold-chain logistics, cryopreservation, and precipitation enhancement. Hyacinth Proteins is eager to continue supporting this work, both through access to technical infrastructure and through ongoing research collaboration.

Please do not hesitate to contact us for any additional information regarding our support of Dr. Meister's proposal.

Christopher Hlubb

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