NANUQ™: Automated Ultra-Fast Cryocooling For Optimized Biomolecular Structure Characterization

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Challenges: Biomolecular CryoCrystallography

- Cooling **damages crystals**, especially when ice forms.
- **Ice forms** in solvent **inside** and outside **crystals**.
- **Ice** accumulates in LN_2 and **contaminates crystals**.
- **20% of PDB deposited data sets** - and a much larger fraction of all data sets - **show structure factor errors due to ice.**
- High-value targets **with large solvent contents** (e.g., membrane proteins) and **large solvent cavities** (e.g., large complexes) are the most challenging to cool successfully.
- **Cryoprotectants** soaks reduce ice, but can **damage** or **dissolve** crystals.
- **Cryoprotectants** can **displace** or be mistaken for **ligands** and **modify protein conformation.**
- Cooling **damage** depends on **cooling rate** and **cryoprotectant concentration**.
- **Cooling by hand** plunging in LN_2 is **highly irreproducible**.
- **Cooling rates** are **too small** to **kinetically capture** the biological temperature **structural ensemble**.
- **Crystals** are **lost** during hand plunging and loading into pucks; a significant fraction of **loops** sent to synchrotrons have **no crystals**.
- Crystals often **dehydrate** during manual handling before plunging.

NANUQ™: Features and Patented Technology

High speed sample translation stage:

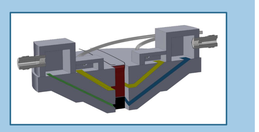
- Maximizes **cooling rates** using LN_2.
- **Programmable sample plunge speeds** from 2 m/s (for fastest cooling of small crystals) down to < 0.01 m/s (for optimized cooling of large crystals and for quasi-equilibrium cooling to explore, e.g., cold denaturation).
- Controlled decelerations **prevent sample loss**.

Automated, computer controlled operation:

- Time from crystal harvest to fully cryocooled **< 2 seconds**; time between samples < 5 seconds.

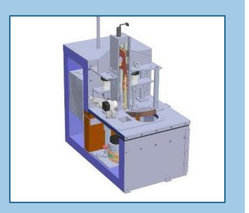
Plunge manifold:

- Controls plunge environment to remove or define cold gas layers above the LN_2 and provide a controlled temperature plunge path.
- Eliminates cold gas above LN_2 and waves on the LN_2 surface for the fastest cooling
- Can trap cold gas for variable rate cooling.
- Computer controlled dry gas flows, vacuum, and heaters eliminate frosting and minimize LN_2 consumption.



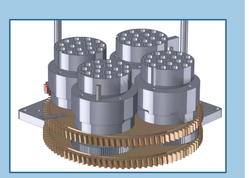
High capacity LN_2 storage and precision level control:

- High efficiency insulation and large LN_2 capacity.
- Allows continuous operation in high-throughput applications without risk of frosting.



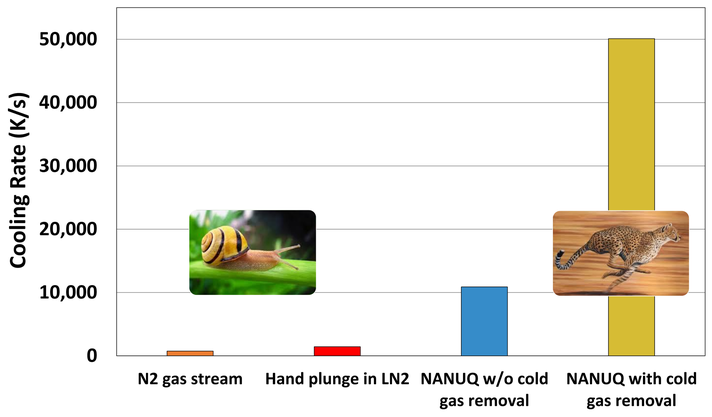
Automated Sample Puck Loading:

- Carousel holds 4 Uni-Pucks and 64 samples in LN_2.
- Positions pucks in a programmable sequence to capture and organize samples for optimal synchrotron data collection.
- Easy access allows loaded pucks to be removed and transferred to a dry shipper or storage Dewar, and empty pucks to be loaded.
- All manual placement of cold samples is eliminated.



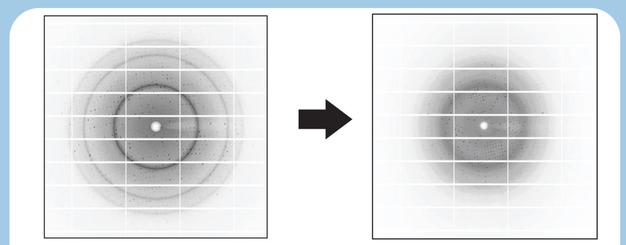
NANUQ™: Automated Hyperquenching Cryocooler

Maximize cooling rates using LN_2:



- **Cooling times < 2 ms** → **better kinetic capture** of room temperature **structure** & **ligand binding** configuration
- **Highly reproducible cooling** → science-based **optimization** of **soaks, cooling,** and **diffraction**
- **Improve diffraction quality** and **reduce mosaicity**

Eliminate internal and external ice:



Hand plunged (LN2)
Sample: CA IX-mimic Crystal
Cryprotectant: 20% sucrose
Result: Significant ice rings

Cryocooled on NANUQ™ (LN2)
Sample: CA IX-mimic Crystal
Cryprotectant: none
Result: No ice rings

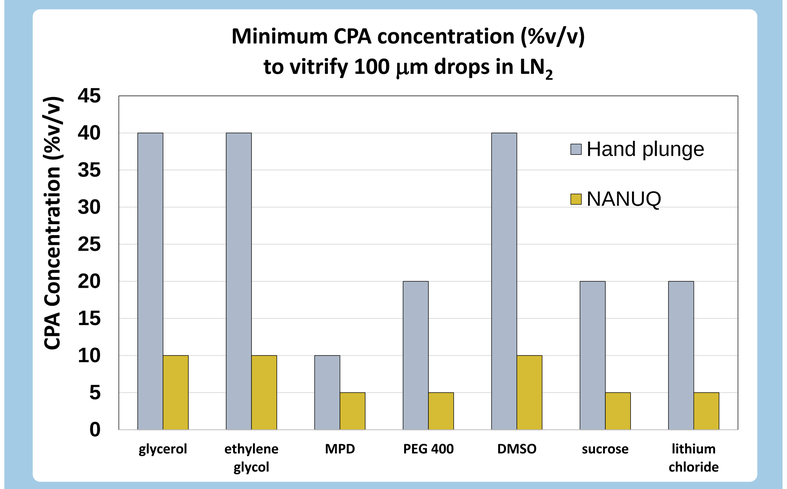
- **Prevents ice** formation **inside** crystals
- **Prevents ice contamination** from frost in LN_2
- **Prevents ice** from **frosting** during puck loading

Make efficient use of lab and beam time:

- Get **everyone** in your lab using the **same** reliable **protocols**
- Harvest and cool **64 crystals / hour** with **complete confidence**
- **No dehydrated crystals** because of delays between harvest and plunge
- **Prevent lost crystals** due to manual plunging / loading accidents
- **Prevent ice** from cooling, LN_2 or frosting during puck loading
- Easily **sequence and track** samples in pucks for optimized data collection.

Incredible Advantages

Dramatically reduce cryoprotectant needs:



- **No penetrating cryoprotectants required** to prevent internal ice. Only add for contraction matching.
- **No cryo soaks necessary** - just quick swipes through a dilute cryo solution / oil to protect / remove external solvent
- **No ligand displacement** and no crystal damage or dissolving by cryoprotectants

Take control of every plunge:

- Gas-layer management and plunge speed control gives controllable cooling rates, enabling **new experiments in crystallography**

Access the Technology:

NANUQ™ Facility - Open to Users

- Bring your samples and cool them, or send them to us for cooling.
- Send your solutions and trays and we will crystallize, cool, and ship.

- Available equipment: Torrey Pines vibration-free crystallization incubator, HC35 cryogenic refrigerator, manual plate set up equipment, V8 & V20 Carl Zeiss microscopes and imaging cameras.