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Opportunities for Rapid, High-quality Space-grown and Earth-grown Protein Crystal Growth and Structural Determination with ExtremoZyme, Inc.

ExtremoZyme, Inc. (http://www.extremozyme.com) performs high-throughput protein crystal growth, diffraction analysis, and three-dimensional structure determination useful for a wide variety of pharmaceuticals. We have developed a process stream that enables rapid crystal growth and structure determination of hundreds of protein solutions at a time, each with tailored saturation and crystallization parameters, followed by x-ray and neutron diffraction analysis of selected crystals.

ExtremoZyme specializes in developing protein structure knowledge through precision protein crystallization by capillary counterdiffusion on earth and in microgravity, and by special access to synchrotron x-ray beams. We provide customers with

- High throughput, rapid response protein crystallization;
- Either normal gravity or microgravity (in space) crystallization of dozens of crystals in rapid timeframe - we are prepared to fly selected protein solutions to the International Space Station within six months for a small fraction of historical prices, and to potentially repeat the process several times per year;
- X-ray and neutron diffraction maps; and/or
- Structures down to atomic resolution.

In its own research, ExtremoZyme is committed to developing recombinant gene products for useful biocatalysis based on extremophile microorganisms. The company also offers its gene-to-structure pipeline capabilities to support the work of other organizations.

The crystallization process that is ExtremoZyme's specialty is capillary counterdiffusion. Using this technique, hundreds of x-ray capillary tubes are arranged in a small cassette. Each tube is typically 0.2 to 1.0 mm in diameter, with a length of 60 mm. Within each tube, the volumes of the precipitating agent and protein solution are arranged in juxtaposition to one another. The two solutions are set to diffuse against each other, resulting in a spatial-temporal gradient of supersaturation along the length of the capillary. Rather than specifically choosing unique conditions, chemical reagents, and protein concentrations to examine one condition at a time for maximum crystal growth, the counterdiffusion crystallization procedure is able to compose a continuous supersaturation gradient, promoting crystal growth by the progression of a nucleation front resulting from the nonlinear interplay among mass transport, protein crystal nucleation, and growth. This reduces crystal mosaicity, while microgravity crystal

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growth promotes convection-free crystal growth by maintaining slight supersaturation without convection at the crystallization front.

ExtremoZyme has routine access to an x-ray beamline in the Advanced Photon Source at the Argonne National Laboratory for structural determination, and is developing ready access to collimated neutron sources for neutron diffraction analysis as well. This allows us to rapidly perform diffraction analyses of crystals shortly after they are grown.

Through a proprietary agreement with Richard Garriott, ExtremoZyme has special access to the microgravity environment of space during Garriott's private space mission for crystallization in the absence of sedimentation, convective forces and gravitationally induced stresses on the crystal during solidification. A strictly comparative crystallographic analysis has revealed that crystals grown in space diffract X-rays more intensely and have a lower mosaicity compared to control crystals prepared in otherwise identical conditions on Earth.

This commercial mission (see http://72.29.31.40/index.cfm?fuseaction=orbital.Clients) provides a unique opportunity to access space unencumbered by traditional schedule and procedural bottlenecks associated with past programs. Launch of the two to three week mission to the International Space Station is scheduled on a Russian Soyuz rocket in October 2008. Because of the unique nature of this mission the price of access is very affordable for microgravity protein crystal growth. Additional flights are expected to be available for continuing research and development.

For further information on ExtremoZyme crystal growth and structural determination capabilities – on earth and in space, please contact:

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