

# UNIVERSITY OF MIAMI MILLER SCHOOL OF MEDICINE

## DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

### PROTEIN CRYSTALLIZATION AND CRYSTAL CHARACTERIZATION SERVICE

Protein crystallization is currently the rate-limiting step in the determination of new protein structures by X-ray crystallography. The availability of crystals suitable for X-ray crystal structure determination can play a very significant role in any research proposal. Knowledge of the X-ray crystal structure of any protein can open the way to many new ideas for further research into the function of that protein. We offer services that can facilitate that crucial first step in protein crystal structure determination, namely establishing conditions for the growth of suitable crystals.

#### **REQUIREMENTS:**

The client should be able to provide evidence that the protein sample is pure, homogeneous and correctly folded. SDS gel patterns, CD spectra and assays of biological activity may be submitted.

The protein should be dissolved in low concentration (eg 20 mM) buffer at a specified pH and in the absence of any other ions unless absolutely required.

The protein concentration should be as high as possible, but certainly no less than 5 mg ml<sup>-1</sup>

For an initial 96 well screen, 120 µl of protein solution will be required.

In the case of intrinsic membrane proteins the detergent used for isolation must be completely replaced by one of the following: LDAO, an alkyl-glucoside or an alkyl-maltoside at a concentration below the cmc.

## SCHEDULE OF SERVICES

CSF001 An initial consultation with a protein crystallographer is recommended to discuss the feasibility of the proposed crystallization project.

CSF002 Dynamic light scattering analysis of protein sample to determine potential homogeneity and/or aggregation problems.

CSF003 A preliminary experiment will be carried out to estimate the solubility of the protein in both ammonium sulfate and polyethylene glycols to establish a suitable protein concentration for the subsequent screening. A 96 well screen (Hampton Research) will then be performed using 1  $\mu$ l of protein solution per droplet. This can be carried out either at room temperature or at cold room temperature.

CSF004 Protein crystals can be distinguished from salt crystals, that frequently form, by testing with a protein sensitive dye. Commonly known as the "IZIT" test.

CSF005 One additional unit of 40 droplets for optimization of the crystal size of any micro-crystals obtained in screening.

CSF006 Protein will be recovered from droplets that fail to yield crystals. This protein will be pooled and re-purified by HPLC ion exchange chromatography. Typically a maximal recovery of 50% may be expected.

CSF007 Any X-ray diffractive analysis of crystals will require payment of a fee towards the maintenance of the X-ray diffraction equipment.

CSF008 A suitably sized crystal ( $> 0.2$  mm) will be mounted in a glass capillary tube and a single picture diffraction pattern recorded to give an estimate of the resolution of the diffraction pattern.

CSF009 As for CSF007 but crystal will be treated with a suitable cryo-protectant and flash frozen at  $-180^{\circ}$  c. The higher fee reflects the cost of a tank of liquid nitrogen .

CSF010 A complete set of native protein diffraction data will be recorded at  $-180^{\circ}$  c. Depending upon how strongly the crystals diffract, this typically takes 3 to 4 days and requires 3 tanks of liquid nitrogen. The client will receive a set of "raw images" on a suitable storage device.

CSF011 Raw images will be processed to yield a file of HKL, intensities.

CSF012 A paper describing the crystallization and crystal characteristics will be written and submitted to Acta Crystallographica part F.

CSF012 Contribution to the methods section of a grant proposal in which further X-ray structural studies are proposed.

### SCHEDULE OF FEES

<b>Service code</b>	<b>Description</b>	<b>Charge</b>
CSF001	Initial consultation (per hour)	\$ 50.00
CSF002	Dynamic light scattering	\$ 100.00
CSF003	Standard 96 well screen	\$ 200.00
CSF004	IZIT test for protein crystal	\$ 50.00
CSF005	40 well optimization	\$ 100.00
CSF006	Protein recovery and re-purification	\$ 250.00
CSF007	X-ray maintenance fee (1 month)	\$ 500.00
CSF008	Crystal diffraction test room temp.	\$ 150.00
CSF009	Crystal diffraction test (-180° c)	\$ 250.00
CSF010	Native data set (-180° c)	\$ 600.00
CSF011	Native data processing	\$ 300.00
CSF012	Paper for Acta Cryst F publication	\$ 400.00
CSF013	Methods page for grant proposal	\$ 100.00

N.B. The above fees apply to water soluble proteins only. For proteins that require detergent for solubility the fees are double for services CSF001 through CSF005, with the exception of CSF006 which is currently unavailable for detergent solubilised proteins.