**Protein Crystal Screening Service**

**Brief description of the screening service**

**The need for the crystal:** X-rays interact with proteins and macromolecules in general weakly and therefore diffraction from a single protein molecule will be difficult detect. However, a three dimensional crystal with tens of thousands of ordered protein molecules will enhance the signal many times thereby making it possible to record diffraction pattern. **The need for screening:** Despite the enormous progress structural biologists have made in solving protein structures, obtaining protein crystal is still hard and knowing *a priori* the condition in which a protein will crystallize is still not easy. That is why we need to screen several conditions to find one that will work. **The new service:** In order to expand the Institute of Molecular Biophysics (IMB) X-Ray Facility’s user-base to non-traditional users of the Facility and to seek out general interest for a future acquisition of an automated crystal screening service, IMB XRF is proposing a new protein crystal screening service (PCSS). This service will be open to current and new users and the fee structure will be based on recouping base cost of the materials. PCSS will essentially be a manual set-up of one batch of 96 individual crystallization screen conditions (selected either by the user or the Facility) for a target protein at room temperature. The screens will then be visually inspected at pre-set times, scored and the results summarized for the user. Optionally, the user can request a Pre-Crystallization Test (PCT) be performed before beginning screening service to determine whether concentration of the protein being provided for screening is at optimal concentration required for screening. **Preliminary data:** Based on the screen results, the user can then select to further optimize the conditions for growing a crystal at the Facility or in his/her own lab. The user can also select to collect preliminary data at the Facility or another location. The preliminary data will tell the user the crystal’s space group, solvent content, and number of molecules in the unit cell essential parameters in solving of the crystal structure. **Advantage of 3-d structure:** A functional protein’s 3d structure will show how its active site or binding site will interact with the substrate(s). A structural protein’s 3d structure can show how it interacts with other components. High resolution 3d structure of enzyme can show hydrogen bonding interactions with substrates that can lead the mechanism of action. Even though complete structure solution for a protein is not yet envisioned to be part of this service, but could be considered at a later time. The user fees will be based on what all services are requested (screening-only, crystal optimization, preliminary data collection, etc.).

**Detailed description of screening service**

**Pre-Crystallization Test (Optional)**

A pre-crystallization test (PTC) can be performed to check whether the concentration of the protein being provided for screening is ideally suited for crystallization.

**Material & Time Required:**

- 5-10 μL of the protein in low ionic strength buffer
- 4 hours

**Screen Set-up**

X-Ray Facility’s Protein Crystal Screening Service (PCSS) proposes to manually set-up one batch of 96 individual crystal screen conditions using hanging-drop crystallization method on four VDX pre-greased plates using 22 mm circular siliconized glass cover slips. The protein: precipitant volume ratio will be 1:1 and the plates will be set-up at room temperature *(Day 0)*.

The following screens will be available:

1. Hampton Research Crystal Screen 1 and 2 (96 conditions)
2. Hampton Research PEG & Ion Screen (96 conditions)
3. Emerald Biosystems Wizard I and II (96 conditions)
4. Emerald Biosystems Cryo I and II (96 conditions)

**Observation and scoring**

Each drop in the four trays will be visually inspected on Day 1, Day 3, Day 7, Day 14, Day 21, and Day 28 and scored for presence of crystal, micro-crystals, needles, precipitates, and phase-separation. Conditions that look promising may be photographed for further analysis. The scoring sheet will look similar to the one seen in Hampton Research Catalog Crystal Growth 101 (see the attachment at end of this proposal).

**Screen Summary**

Successful crystallization conditions (that show needles, micro crystals, plates, and crystals) will be repeated after Day 28 to ensure reproducibility and summarized for the user.

**Materials Required:**

- 200 to 1000 μL of the protein in low ionic strength buffer
- 15-20 mg/mL concentration protein (or different if PTC test suggests different concentration)
- Extinction coefficient at 280nm and gel picture showing the purity of the protein
- Molecular weight and sequence of the protein
- Light scattering results showing mono disperse conditions (optional)

**Pricing**

We propose to price the service at cost of the materials and will be $125/screen for FSU users and $250/screen for non-FSU academic users (commercial user price is not yet available). The price scheme will be revised once a year.

**Preliminary diffraction data**

**Preliminary information**

Successful conditions from the screening results will be selected and each condition will be further optimized for getting better and larger crystals. A preliminary data collection of the crystals will be carried out. The results from this preliminary data collection will yield the following information about the crystal(s).

- Diffraction resolution in Ångstroms (higher resolution better information)
- Unit cell dimension in Ångstroms
- Space group of the crystal
- Solvent content of the crystal
- Number of molecules in the unit cell
- Cryo conditions and ability to collect data at cryo conditions

As mentioned earlier the preliminary diffraction data collection can be carried out at the Facility or elsewhere. But we have the resources and expertise to carry them out locally and without much wait times. Complete data set for the native and heavy-atom derivatives can also be collected at home. If Se-Met data set is desired, Se-Met incorporated crystals can be sent for data collection at APS synchrotron source.
Structure solution and local expertise

Full data collection at home or synchrotron

Following successful preliminary diffraction data collection from the crystal the user can then select to collect the data set and solve the structure locally or carry it out elsewhere. The following lists the advantages of collecting full data set at home (or using our synchrotron account):

- Availability of Image Plate and CCD detectors
- Hands-on training, if needed, to collect and process data
- Synchrotron beam-time for in-person or remote data collection
- Sample storage and transport

Local expertise

Following successful diffraction data set collection, the user can then select to solve the complete structure at home. The following are advantages of solving the structure at home:

- Three faculty to collaborate in solving the structure
  - Prof. Michael Blaber, College of Medicine
  - Dr. Hong Li, IMB
  - Dr. M. Elizabeth Stroupe, IMB
- One full-time State funded staff scientist
- Computer hardware and software needed to solve structure.
## Crystal Screen Request Form

### User information (Required)

<table>
<thead>
<tr>
<th>User Name &amp; E-mail</th>
<th></th>
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<tbody>
<tr>
<td>PI &amp; Phone Number</td>
<td></td>
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<tr>
<td>Department</td>
<td></td>
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<tr>
<td>Budget Number</td>
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### Protein information

<table>
<thead>
<tr>
<th>Protein Name</th>
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<th>Molecular Weight (Da)</th>
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<tbody>
<tr>
<td>Extinction Coeff. at 280 Å</td>
<td></td>
<td>Molecular Weight (Da)</td>
</tr>
<tr>
<td>Concentration (mg/mL)</td>
<td></td>
<td>Volume (μL)</td>
</tr>
<tr>
<td>Buffer and pH</td>
<td>(Low ionic strength preferred; avoid phosphate buffer)</td>
<td></td>
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<tr>
<td>Sequence</td>
<td>(Provide it separately as one letter code)</td>
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<tr>
<td>Purity</td>
<td>(Provide a gel)</td>
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## IMB | X-Ray Facility | PCSS | Scoring Sheet

<table>
<thead>
<tr>
<th>User Name:</th>
<th>User PI:</th>
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<tbody>
<tr>
<td>Sample Name:</td>
<td>Buffer Detail:</td>
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<tr>
<td>Drop:</td>
<td>Protein:</td>
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<tr>
<td>Days Since:</td>
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</tr>
</tbody>
</table>

| A1 | A2 | A3 | A4 | A5 | A6 |
| B1 | B2 | B3 | B4 | B5 | B6 |
| C1 | C2 | C3 | C4 | C5 | C6 |
| D1 | D2 | D3 | D4 | D5 | D6 |