Microbeam MAD Beamline for Challenging Protein Crystallography in TPS

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Abstract. The TPS-05A beamline is the first X-ray beamline at NSRRC built for micro protein crystallography experiment as well as one of the seven ID beamlines in phase I at the TPS synchrotron facility. A 2-meter in-vacuum undulator (IU22) serves as the photon source from which the harmonics #3 to #9 will provide brilliance of $10^{18}$–$10^{20}$ photons s$^{-1}$ mrad$^{-2}$ mm$^{-2}$ (0.1% bandwidth)$^{-1}$ and photon flux of $10^{13}$–$10^{14}$ photons s$^{-1}$ (0.1% bandwidth)$^{-1}$ in the required energy range of 5.7–20 keV (2.175–0.620 Å) to cover MAD phasing experiments at 1 Å and SAD phasing experiments at 2 Å. The beamline optics consists of a cryo-cooled double crystal monochromator (DCM) and a pair of focusing K-B mirrors. Requirements from the user group include a target focus size of 50 μm × 50 μm (H × V) at the sample position, photon flux greater than 2 × $10^{12}$ photons s$^{-1}$ at Se K-edge (0.9795 Å), pinholes for adjusting the beam size down to 5 μm. Calculation of heat load for the first optical element, i.e. the first crystal of DCM, is included in this paper.

1. Introduction
Membrane proteins and protein complexes represent the most challenging areas in terms of producing crystals of reasonable size (50–100 microns) [1, 2]. For large protein complexes, the major practical difficulty remains to obtain a large single crystal even with extensive trials. The ability to collect diffraction data from small crystals is therefore often crucial to the success of this type of project. There are also many examples of other systems where only microcrystals can be obtained in spite of extensive crystallization experiments. The common needle-like crystals with a long dimension of 100 microns (or more) but less than 10 microns in width also requires a micro X-ray beamline, as they can be translated (parallel to the long dimension) during data collection to minimize the effects of radiation damage [3–5]. The use of a very small, brilliant beam (2–20 microns) allows selection of a small volume of the crystal that shows much lower mosaicity than the whole crystal, leading to a significant improvement in the quality of the diffraction data [4, 6, 7]. Achieving these objectives requires very high signal-to-noise ratio of diffraction spots from a sample crystal. The rate at which crystals are characterized for optimization of crystallization conditions as well as structures that can be determined will be greatly facilitated. This, in turn, will facilitate the high throughput structural biological technology and biotechnological applications in NSRRC.
2. Photon source
Taking into consideration of the initial operating parameters of TPS and the required energy range of the beamline, a feasible vertical gap of 7 mm with period of 22 mm is chosen. However, the gap would be reduced to 5 mm in the future. The in-vacuum undulator IU22, named after its period length, will be placed in the short section of the storage ring to provide the required hard X-ray for the protein crystallography beamline. The undulator parameters are shown in table 1.

| Table 1. IU22 Source Parameters at TPS |
|---------------------------------------|
| In-vacuum undulator                   |
| Period length, $\lambda$             | IU22 |
| Number of period, $N_{\text{period}}$ | 95   |
| Peak field                            | 0.72 T |
| Deflection parameter, $K_{y_{\text{max}}}$ | 1.48 |
| Total magnetic length                 | 2.134 m |
| Minimum magnet gap$^a$                | 7 mm |
| Photon beam size and divergence $\sigma_{rx}(\sigma_{ry})$, $\sigma'_{rx}$ ($\sigma'_{ry}$) @12.4 keV | 120(5.3) $\mu$m, 18.6(8.3) $\mu$rad |
| Total power @ 500 mA                  | 3 kW |
| Power density @ 500 mA                | 29 kW mrad$^2$ |

$^a$ The magnetic gap of IU22 for initial stage is 7 mm, but might be changed to 5 mm in the future.

Due to low emittance of the TPS ring, at 500 mA storage ring current and a magnetic gap of 7 mm, the brilliance and the photon flux using harmonics #3 to #9, is greater than $3 \times 10^{18}$ photons s$^{-1}$ mrad$^{-2}$ mm$^{-2}$ (0.1% bandwidth)$^1$ and $1 \times 10^{13}$ photons s$^{-1}$ (0.1% bandwidth)$^1$, respectively, calculated by SPECTRA$^8$ in the range 5.7–20 keV.

3. Beamline design
Although this beamline is primarily designed for Se-MAD phasing experiments, it is still viable for S-SAD experiments. After several reviews, the new beamline for protein crystallography should meet the following requirements as well as flux density should be larger than $2 \times 10^{12}$ photons/s through a 50 $\mu$m $\times$ 50 $\mu$m slits and small beam divergence of 500 $\mu$rad and 100 $\mu$rad in horizontal and vertical directions, respectively, at sample position for Se $K$-edge (0.9795 Å); energy reproducibility is better than 0.25 eV at 12.65 keV; and accurate X-ray energy tenability in energy range of 5.7–20 keV; high positional stability (better than 1 $\mu$m).

According to the parameters of the source, the optical arrangement of this beamline consists of a DCM and a downstream K-B mirror set. The double crystal monochromator, which is positioned at 24.5 m employs a pair of plane Si(111) crystals for diffraction. Since the first crystal of DCM is the first optic from the upstream diamond windows, it absorbs the largest heat load per unit area. We adopt liquid nitrogen cooling system for both the crystals in order to maintain optimal performance.

The design target of the beam size at focus position is 50 $\mu$m, with tuning by a set of pinholes down to 5 $\mu$m. This method has the following advantages, (1) beam position drift from the source will not affect the sample position, (2) a well-defined beam shape with little tailing at the edges, (3) decrease the beam divergence, and (4) convenient change from 5 to 50 $\mu$m without adjusting the mirror. The drawbacks are, (1) beam size can only go down to 5 $\mu$m, and (2) loss of flux density when decreasing beam size.

To achieve the requirement of beam sizes, the horizontal focusing mirror (HFM) placed at 29.85 m is mainly determined by the size of 50 $\mu$m and required divergence of 500 $\mu$rad at focus point of 35 m to collect horizontal fan of 83 $\mu$rad. With the demand of equal beam size in two directions and gradual
divergence in vertical direction at focus, the vertical focusing mirror (VFM) is placed at 27 m for compromising on the minimization of angular error from mirror to collect vertical fan of 25 μrad. Thus, we determine to use cylindrical mirrors with demagnification ratio smaller than 6. For obtaining better focus at the sample position, both mirrors are bendable with a slope error of 1 μrad. The layout of the protein crystallography beamline TPS-05A is shown in figure 1.

**Figure 1.** Layout of the protein crystallography beamline TPS-05A.

The beamline scientists request the mirror coating and the incident angle to be optimized for energy range of 5.7–15.5 keV in order to simplify beamline operation and to provide a beam with less than 0.1 % higher harmonic contamination. Therefore, the surface of VFM is divided into two stripes parallel to the beam direction. One stripe is a Rh layer coated a thickness of 600 Å for the energy range 5.7–15.5 keV and the other stripe is a 50 Å Rh layer on top of a 250 Å Pt layer for extending the energy range to 20 keV. The purpose of Rh/Pt coating is to smooth out the L-edge structures of Pt in the 11–13 keV range. The two stripes are operated at identical grazing angle of 4.2 mrad. Moreover, the coated stripes and grazing angle of HFM are of the same settings as VFM.

4. Heat load analysis

The high flux density and the accompanying high heat load from undulator IU22 necessitate a comprehensive analysis of the beamline optics and effective mechanical and cooling design to ensure stable performance. Although the TPS ring will run at 500 mA and the gap of IU22 is 7 mm, we adopted higher beam current (600 mA) and a 5 mm gap for heat load analysis. The total power and power density in this case is 5.6 kW and 42.7 kW mrad⁻², respectively.

Most of the unwanted power is absorbed by a water-cooled aperture with opening of 83 × 25 μrad (H × V) at 18 m in the front end, such that the water-cooled Slits 1 set outside the shielding wall receives much less heat load. The power of photon beam after Slits 1 set is 87.3 W. The power of unwanted energies in the defined beam is further filtered out by a combination of diamond filter and beryllium window.

Si(111) is used for the double crystal monochromator and the first crystal has to withstand the intense white light beam. The flux density is even higher for high incidence angles. Side-cooling by liquid nitrogen is thus used for the crystals to maintain performance. The power after the Be window is 47.6 W absorbed by the first crystal of the DCM within a footprint of 1.33 × 2.0 mm² when tuning to 5 mm gap with a maximum temperature of 114 K. The resulting slope error is up to 3.13 μrad when the first crystal is cooled by liquid nitrogen cooling system. Since the downstream K-B mirror set only focuses monochromatic light and thus cooling is not needed.

5. Expected Performance

The total flux as a function of photon energy using harmonics #3 to #9 at the focus with 500 mA ring current is calculated by XOP[9] as shown in figure 2 (harmonic #1 is not shown). The flux through a 50 μm slits positioned 100 mm before the focus is above $10^{12}$ photons s⁻¹ in the energy range 5.7–15.5 keV, and above $10^{10}$ photons s⁻¹ through a 5 μm pinhole.
As discussed earlier in beamline design, the flux in the higher energy range up to 20 keV is extended by the Rh/Pt coating of K-B mirrors. With the same settings of grazing angle of mirrors, the flux through different pinhole at maximum energy is decreased by a factor of 100 compared to 1Å wavelength. Nevertheless, this is the trade-off on enhancing the flux in optimized energy range (5.7–15.5 keV). The flux in the energy range 11–13 keV falls in the harmonic #5 and is characterized by a monotonically decreasing curve with relatively small variation in intensity, which is very important to the energy scanning experiments to be performed by the end station users.

Figure 2. The calculated flux as a function of photon energy.

6. References
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