Supplementary Information

May 29, 2012

1 Details on the Anti-Settling Device

The rotation of the anti-settling device is powered by a 24V-DC motor (Conrad, DOGA DC-Motor 24 V, 1000 rpm, 0.75 Nm or Mattke 48 V Motor, 3000 rpm, 0.38 Nm) in conjunction with a gearbox with ratio 1:400 (Type MPG 80, 3 levels, Mattke AG, Freiburg Germany). The rotation platform parameters can be controlled via an intuitive user interface which - along with all other electronics except for the power supplies - is included in one 19" rack-mount circuit box. Depending on experimental requirements, the speed of rotation can be varied (0-1.5 rpm) and the waiting times at the 12 and 6 o'clock positions can be adjusted (0-10 min) via 3 continuously variable 10 turn potentiometers. Additionally, the user interface includes a start and a stop button, a switch for control of direction of the initial rotation and two emergency stop buttons (one on the interface board directly and one movable button on a 3 meter long cord).

The electronics controlling the speed of rotation and waiting times are based on a processing-logic-unit that consists of a commercial micro-controller board (Arduino, http://www.arduino.cc/) and a custom-made board. The Arduino board processes the input signals (user-originated and automatic). Its logic is programmable via USB in a C-like programming language. The board has digital I/Os for the start/stop buttons, direction and position switches as well as analog inputs with a resolution of 10 bits (corresponding to 1024 levels). The analog inputs are used for controlling the waiting time by two potentiometers (with a resolution of 586 ms for a total adjustable range of 0 to 10 minutes). The custom-made board acts as a support structure for the Arduino board and as an interface to the motor-control-unit.

The speed of rotation is controlled by the motor-control-unit via pulse width modulation (PWM) and can be adjusted by the user with the third 10-turn potentiometer which delivers a signal ranging from 0 - 5 V to the analog input of the motor-control-unit. The direction of rotation is given by the polarity of the input signal. Three position switches (at 5°, 180°, and 355°, automatically triggered by a pin protruding from the passing rack) provide information on the rotational position and direction of the rack. The desired direction is determined by the logic of the Arduino-board which switches a relay on the custom-made board to apply 5 V of the correct polarity to the 10-turn potentiometer.

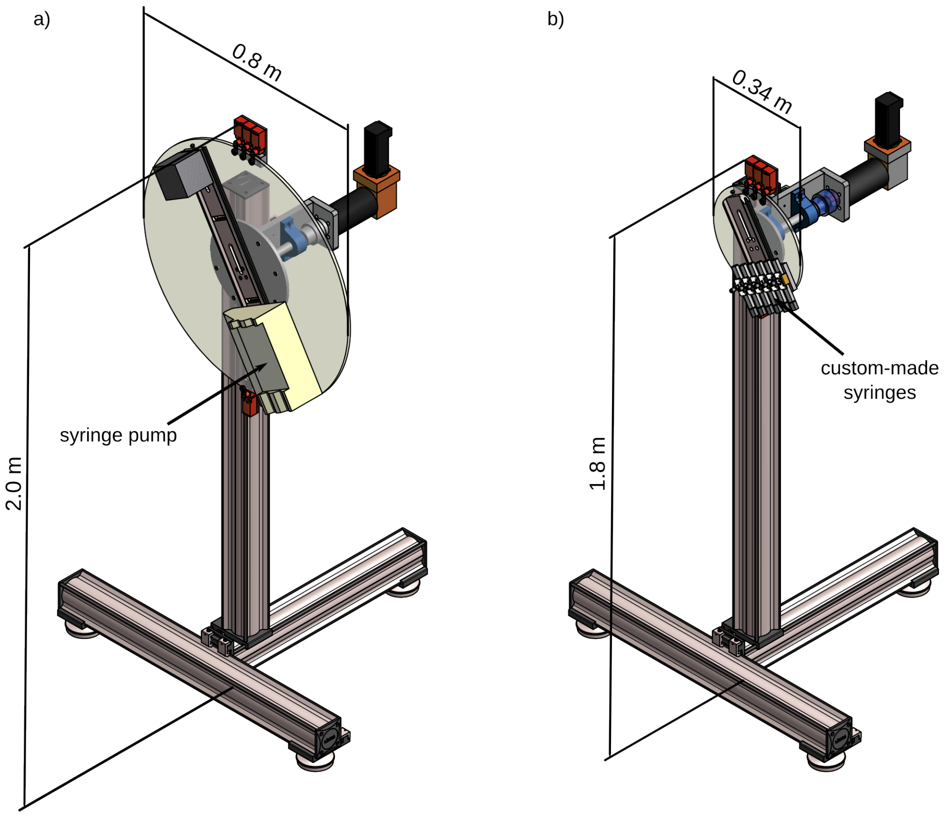

Figure 1: The anti-settling sample delivery device. The low-flow-rate version (a) is based on a commercial syringe pump. The size of high-pressure version (b) was significantly reduced to conform with the limited space often available in the experimental environment.

The on/off state of the motor is also controlled by the motor-control-unit based on an activation input signal coming from the Arduino board (The custom-made board transforms the 5 V coming from the Arduino board to 24 V which is the required voltage level for the activation input signal of the motor-control-unit). Power is supplied by two identical external fixed-voltage 24 V power supplies. Two power supplies are required as the PWM of the motor-control-unit causes voltage spikes in the system. These spikes are disruptive to the processing-logic-unit. Engaging one of the emergency stop buttons will cut power to the motor which stops the movement of the rack (this is sufficient because the high reduction ratio of the gearbox effectively provides a self-locking mechanism). Another safety device is a fourth position switch mounted at 360°. During normal alternating clock-wise and counter-clock-wise operation, this switch will not be triggered. If it is triggered, it indicates a failure in the logic of the micro-controller or some other catastrophic failure, and as a result, the motor is turned off.

1.1 Details on the high-pressure version

The HPLC system used to pressurize the system was purchased from Shimadzu Biotech (Duisburg, Germany). It consists of the standard Prominence HPLC components: 2 Prominence LC-20AD pumps (with automatic rinsing system, 4 position valves for solvent selection, solvent degassers and reagent bottle holders), 2 option box S with two FCV-14AH, 7 port, 6 position and two FCV-12AH, 6 port, 2 position, high pressure valves, Prominence CBM-20A interface/system controller and a notebook PC running LabSolution software. Other components that might be useful for additional applications include the Prominence SPD-20A UV-VIS 2 channel detector with high pressure cell and the Prominence SIL-20ACHT autoinjector with large sample loop. The standard optical cables to connect the modular components to the CBM controller were replaced with optical cables up to 30 m long (cable with HFBR 4506 connectors, purchased from http://www.fiber-shop.de/) to permit operation of components remote to the controller. Unlike conventional HPLC plumbing, the majority of the tubing connecting the components had large ID to reduce back pressure. Most rigid connections were made with 0.4 mm ID, 1/16" outer diameter (OD) stainless steel tubing (Besta Technik, Wilhelmsfeld, Germany) and flexible connections were with 0.03" ID PEEK tubing (Upchurch Scientific; purchased from Besta Technik). Back pressure regulators were inserted at various locations in the system to maintain the desired pressures when valves switch flow from one path to another. These back pressure regulators and other HPLC components (fittings, tubing, etc.) were also from Upchurch Scientific (Besta Technik). A Bronkhorst miniCoriFlow mass flow meter (Wagner Mess- und Regeltechnik, Offenbach, Germany) was installed just upstream of the syringe to monitor the liquid flow rate. All of these components are industrial quality and are capable of running continuously for weeks.

Working in the high pressure regime requires consideration of modes of failure to guarantee safety. In the worst case of a rapid pressure buildup, the sample syringe bodies have a burst pressure limit of 34 times the maximum pressure (for the 8 mm inner diameter bores) that can be delivered by the HPLC system. The most likely failure mode in everyday use would be the axial elongation of the threaded end caps causing a leak, which would occur at a lower limit (for 0.2 % elongation) of 12 times the maximum pressure of the HPLC system. These calculations suggest that the syringes are sufficiently over-dimensioned for safe use.

1.2 Dead volumes in the two systems

For the low-flow-rate version, the volume of sample remaining in the syringe when its plunger is fully inserted is on the order of 150 μl. The assembled syringe used in the high-pressure version has a dead volume of approximately 25 μl due to the shape of the plunger and the internal volume of the sample end cap. The amount of sample that cannot be pumped into the X-ray beam is not lost, but rather is fully recoverable upon removal and disassembly of the syringe.

2 Data Analysis

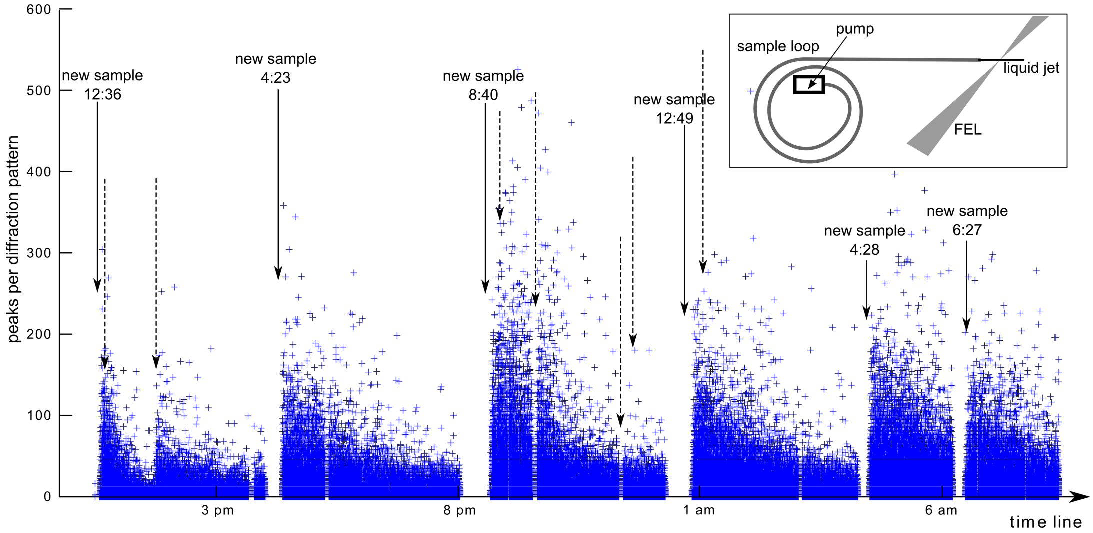

Figure 2: Number of Bragg peaks per diffraction pattern from PSI crystals [1]. The anti-settling device was not present during these measurements. Instead, a long loop of HPLC tubing was used as a sample reservoir as indicated by the schematic drawing in the inset. Arrows indicate either adjustments of the liquid jet parameters (dashed lines) or supply of new sample (continuous lines). The analysis also reveals the size dependence of the settling problem. Big crystals are most likely to show stronger diffraction signal. Thus, weak Bragg peaks will be more easily detected and result in diffraction patterns which contain more Bragg peaks. The bigger the crystals the faster they settled, leading to a reduction in the number of Bragg peaks per diffraction pattern observed with time. An inverse result was observed when analyzing the number of empty diffraction patterns. The number was smallest directly after sample reload and increased from then on in the course of the measurement.

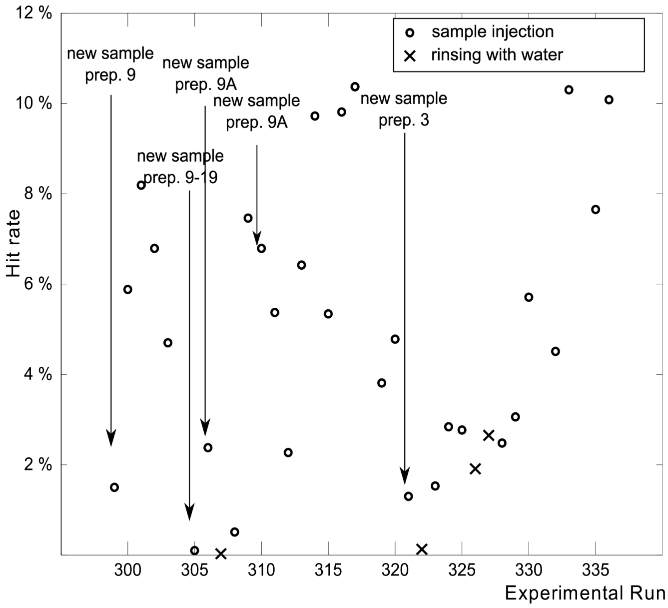

Figure 3: Hit rates, as determined with the CHEETAH software package [2] of lysozyme crystals. Collected diffraction patterns are stored in files containing the data of 5 to 35 minutes of data collection, called experimental runs. The total time period for the measurements presented was 15 hours. Continuous arrows indicate the time points when a new syringe containing the crystal suspension was mounted in the anti settling device. Crosses indicate runs in which the system was rinsed with water to prevent clogging. The first run displayed (#299) is the one where the device was not turned on.

References

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[2] A. Barty. Cheetah software. https://github.com/antonbarty/cheetah, 2011.