Diamond-II
Proposal for flagship project –
X-rays for Soft-Condensed Matter (X4SCM)

Science Group: Life Sciences

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X-rays for Soft-Condensed Matter (X4SCM)

1. Summary/Impact statement

Soft-condensed matter (SCM) science is the science of every-day life that provides understanding and improvements to foods, polymeric materials, additive manufacturing, electronics, life and life-style products. SCM is the science of self-assembly where molecules in seemingly random orientations, are partially ordered at length scales much greater than molecular dimension. Due to the partially ordered to disordered nature of SCM materials, investigations rely heavily on small-angle X-ray scattering (SAXS), infrared spectroscopy and imaging, and circular dichroism instrumentations. SAXS is the de-facto technique for providing structural insights on SCM systems. Here, we propose the X-ray source for nano-focused **X**-ray investigations **f**or **S**oft-**C**ondensed **M**atter (X4SCM) beamline. The beamline will provide monochromatic and high-flux, "pink" X-ray modes for a multi-purpose X-ray beamline enabling time-resolved studies that will simultaneously cover the USAXS/SAXS/WAXS range. *X4SCM will operate two end-station modalities*: HIERARCHY for structural investigations of partially disordered systems and SNAPSHOT for time-resolved studies using nano-focused, high-flux X-rays. Diamond has operated a single, phase I beamline, I22, since 2007 that has been the sole synchrotron X-ray instrument for UK SCM science. X4SCM will not only alleviate the over-subscription on I22 but also introduce new synchrotron techniques such as Diffracted X-ray Tracking (DXT), X-ray Foot-printing Mass Spectrometry (XFMS) and pink-beam SAXS experiments to our user community. The beamline will be designed to operate as a 38-meter camera with four strategically placed integrating detectors to cover the USAXS to WAXS range. We propose an undulator source for the lowest possible divergence to provide variable beam sizes between nano-focused (500 nm) and micro-focused (10 um) beam.

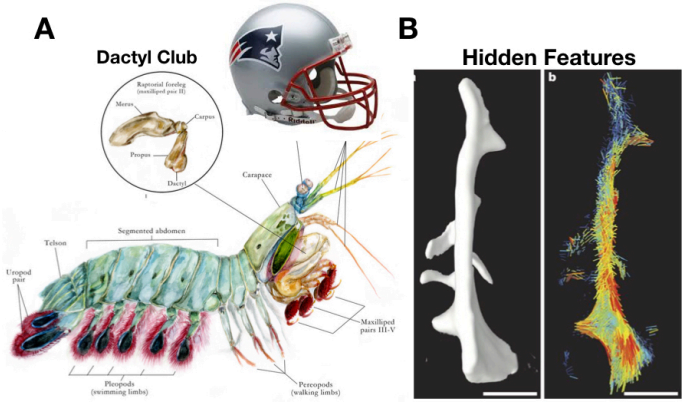

Figure 1: A) The mantis shrimp's dactyl foreleg punches at ~50 mph with enough force to break ¼-inch glass. The dactyl club and body armour, mainly composed of chitin, has evolved a unique structure that can withstand blows encountered during mating competition. Can replicating the arrangement of the nanostructured chitin lead to better helmets? B) Trabecular bone image (left) shows a seemingly homogenous material. SAXS tensor tomography reveals hidden structures illustrating localized arrangement of fibril bundles. Adapted *from Liebl, M. et al., Nature (2015) 527, 349-352*.

2. Scientific Case

SCM materials involve a wide-range of physical states that include liquid, semi-solid, waxes, glasses and aerosols. These materials can be derived from natural sources but SCM science's overarching goal is to design and engineer bespoke materials that often mimic natural materials[1]. Natural materials contain a complex arrangement of an underlying nano-structured building block (Figure 1). It is generally accepted that this complex arrangement confers the desired structure-function relationship.

The SCM group operates two SAXS beamlines namely I22 and B21. B21 is a phase III Diamond beamline that focuses on high-throughput solution-state samples. B21 users are mainly structural biologists which has allowed B21 to pursue a mail-in service strategy that optimally increases the number of experimental sessions offered per year (see Figure 4). In contrast, the prototypical SCM experiment is extensive where setup times and measurements take days and are aimed at mapping condition space, as well as phase space that tests samples under a variety of

temperatures, pressures and strains. This fundamentally limits the available sessions per year and necessitates an additional beamline for SCM SAXS experiments. In addition, SAXS tensor tomography (STT)[2-4], a new technique that maps structural features in 3-D (Figure 1), currently takes 1.5 days for a 2x1x1 mm^3 sample. STT is a flux hungry technique and the X4SCM beamline would map the same sample in ~30 minutes.

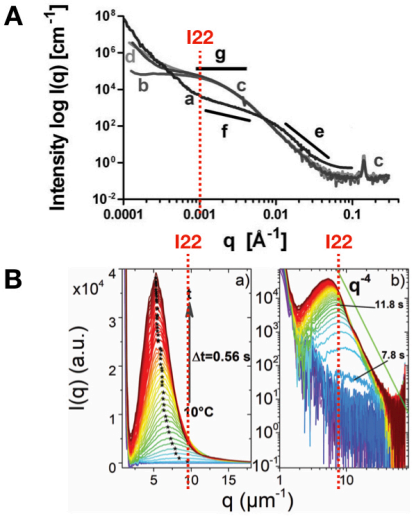

Figure 2 USAXS capabilities at Spring-8 (A, *from Journal of Applied Physics 122, 224304 (2017)*) and APS (B, *from Soft Matter, 2017, 13, 8756-8765*). Red-dashed lines highlight minimum scattering vector available on I22. Data to left of line shows significant information exists in USAXS regime.

HIERARCHY

I22 provides camera lengths between 1.9 and 9.9 meters. At full extension, the camera is suitable for measuring features at the 100's-of-nanometer length scales. This length scale is ideal for the building blocks of many soft materials such as diblock copolymers and micelle-like structures. However, for some materials assembled from large (>500 nm) nano-structured particles, the evolution of mesoscopic features will not be viewable on I22 (Figure 2). USAXS experiments at the Advanced Photon Source (APS, Chicago, IL, USA) [5] and Spring-8 (Hyogo Prefecture, Japan) [6] have demonstrated significant changes in mesoscopic features can occur as materials are pushed out-of-equilibrium. USAXS measurements not only complement canonical SAXS studies but also complete the observable length scales starting from the molecular level.

How nanoscale features correlate with a desired material property remains the goal of SCM science. Recent progress in large scale production of nano-structured materials [7] suggests that smart, designed materials may soon be a reality but a beamline that provides simultaneous USAXS/SAXS/WAXS studies is required to allow users to follow structural correlations over the requisite length scales [8-11].

SNAPSHOT

X4SCM will provide 10^{13} photons per second of monochromatic beam and nearly 10^{15} photons per second in pink beam. These photon fluxes open new opportunities for time-resolved studies at Diamond. We propose two new user techniques, Diffracted X-ray Tracking (DXT) [12] and X-ray Foot-printing Mass Spectrometry (XFMS) [13, 14] for studies in the micro- to milli-second. In addition, the high-frame rate integrating detectors will allow reliable measurements at slower time scales relevant to most SCM materials.

DXT is a single molecule technique that tracks the motions of gold nanocrystal-labeled materials. An individually labelled protein that is transiently fixed to a surface will likely produce a single diffracted (1,1,1) spot with a polychromatic X-ray beam. As the gold nanocrystal wobbles or rotates (a motion directly correlated to the internal motions of the protein), the diffracted spot will also move (Figure 3). DXT has been developed at Spring-8 by Prof. Yuji Sasaski [15] and applied to channel gating [16], peptide binding by MHC-II complex [17], chaperonins [18, 19] and *in vivo* studies of antifreeze proteins in *C. elegans* [20] and GPCR proteins expressed in HEK293 cells.

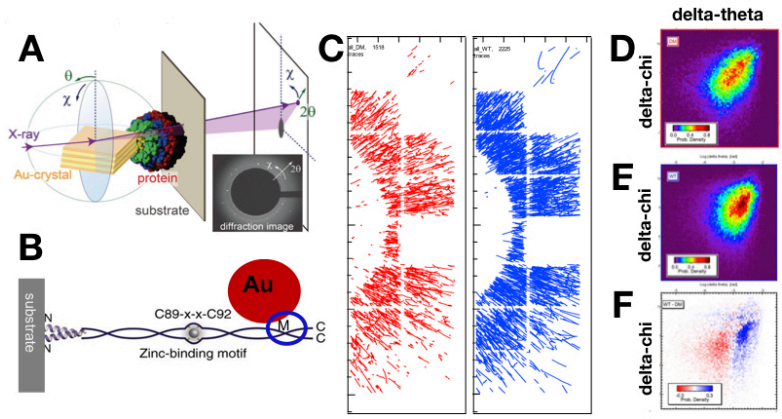

Figure 3 DXT experiment performed at B16 with prototype Tristan detector. A. Motions of gold-nanocrystal labelled protein produce changes in theta and chi angles relative to X-ray direction. B. Coil-coiled protein sample where zinc-binding motif was mutated. C. Motions of individual diffracted spots forms tracks (red WT, blue mutant). Changes in chi and theta produce distribution maps (D,E) where a difference map (F) illustrates changes in preferred motions between WT and mutant.

In 2019, we started developing DXT at Diamond with Prof. Sasaki utilizing the prototype Tristan detector which time-stamps photon events at 200 nanosecond resolution. We were able to show that this detector is ideal for DXT. Recording single-molecule diffraction events will be subject to high-background scattering due to air, Kapton windows and cross-sectional area of the illuminating X-ray beam. Our initial experiments on B16 used a 300 micron-squared beam, X4SCM will provide a nano-focused pink beam to optimally reduce background (by a factor of $>10^5$).

DXT provides a direct assessment of the protein's degrees-of-freedom. This information is highly complementary to bioSAXS. How a protein moves in solution is inferred through extensive molecular dynamics simulations and compared to bioSAXS results. While DXT offers a direct test of dynamics, **XFMS** uses a highly intense X-ray beam to probe dynamics at the sequence level. XFMS examines the rate of radiation-induced hydroxyl radical damage at microsecond time scales. High accuracy mass spectrometry allows residues to be specifically identified and mapped within a primary sequence, identifying solvent-accessibility. XFMS can uncover structural waters, cavities and complement structure-function studies [14, 21-23]. XFMS is a covalent modification of the residue through radiolysis. Unlike hydrogen-deuterium (HD) exchange where side-chain exchange rates are too fast for detection, solvent accessibility of residues by XFMS will not go undetected. XFMS is a productive technique with a notable publication detailing GPCR receptor G-protein complex assembly [24].

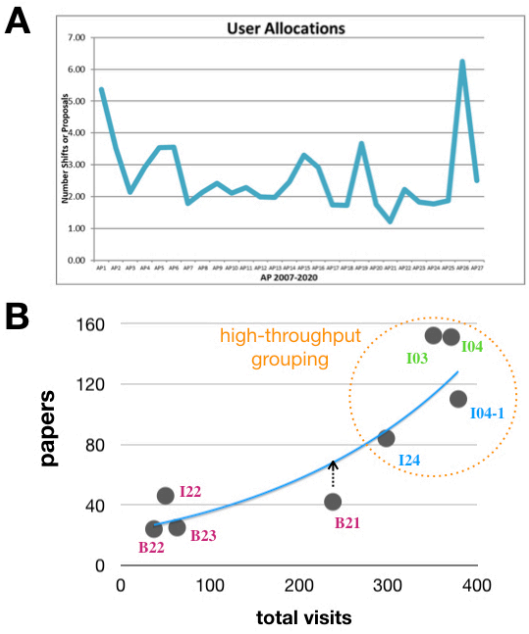

Figure 4 A) Historical oversubscription levels for I22 since 2007. Rate never falls below 1 and reaches a peak of 6. B) Highly rote experiments eanble high automation as achieved on MX beamlines; delivering 300 to 400 sessions per year. In contrast, SCM experiments with significant set-up times greatly limit accessibility.

3. Benefit to the Diamond research community

The X4SCM beamline addresses major needs in accessibility and experimental capabilities. Historically, I22 has been greater than 2-times oversubscribed (Figure 4). The oversubscription was not alleviated by the phase III beamline B21 because B21 largely caters for a non-overlapping user community. In fact, while B21 delivered its highest number of visits, the I22 oversubscription rate increased in the last 3 APs. This strongly suggests the demand for SAXS is high amongst

the SCM community and X4SCM would provide additional capacity as a USAXS/SAXS/WAXS instrument. More importantly, X4SCM will be a unique beamline providing exceptionally intense, nano-focused, X-rays for time-resolved and SAXS experiments on SCM materials and life sciences samples. The NSLS-II (Brookhaven, NY, USA) and ALS (Berkeley, CA USA) both offer dedicated beamlines for XFMS. X4SCM would be world leading in time-resolved single-particle tracking experiments by providing a dedicated user program for both DXT and XFMS. These techniques naturally link to the cryo-EM and MX structural biology community at Diamond.

4. Outline Specification

There is ~75 meters between the source point of an existing Diamond insertion device and outer wall of the synchrotron. Optimally, a 38-meter SAXS camera could be built within this space, exceeding the 31-meter ID02 camera at the ESRF. A 38-meter camera would allow the lowest scattering vector of 0.00056 nm^{-1} to be observed at 6 keV.

Source. X4SCM should provide the highest possible polychromatic flux for DXT and cover a suitable range of 5 to 30 keV for XFMS. The X4SCM beam line requires a hybrid permanent magnet undulator (HPMU). While the science described above could be achieved with a 1.5-meter device, we have a preferred choice of the longer, 2-meter, HPMU to maximize photon flux.

Device	Length (m)	Period	Gap (mm)	B(T)	K	Emin1	Emin1	keV range
HPMU	1.5	17.6	4	1.31	2.15	2.0	6.0	5 – 30

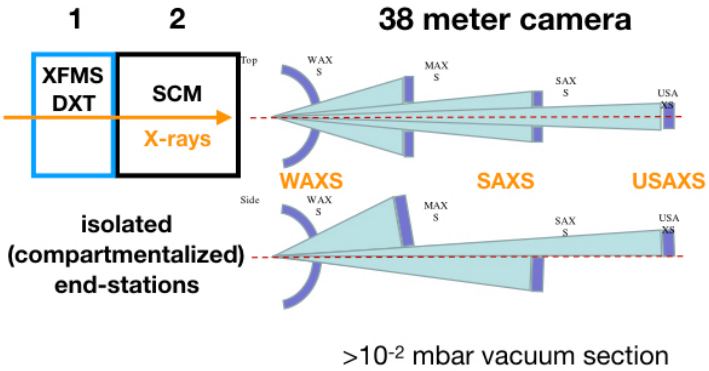

Figure 5 Schematic of dual end-station for SNAPSHOT (1) and HIERARCHY (2). Camera configuration for HIERARCHY requires 4 fixed detectors.

Optics. The beamline will require multiple focusing optics. To provide the most parallel, collimated beam, we desire a white-beam mirror upstream of the monochromator. Near the sample position we would further require the latest CRL technology or KB mirrors to provide variable focus or collimation configurations. Likewise, a single toroidal focusing mirror or single horizontal focusing element could be used to provide focused beam for DXT and XFMS experiments. Stability is essential where we will require nano-positioning to 10 nm. Beam stability at this level of precision is essential where the temperature environment of both storage ring and beamline needs to at least as good as ±0.1 degree. The beamline will use a double-crystal monochromator, a bypass design will be sought to allow direct beam from the undulator for SNAPSHOT experiments.

Experimental Hutch. X4SCM should have two compartmentalized, experimental hutches, one dedicated to HIERARCHY and the other to SNAPSHOT (Figure 5). Experiments in SNAPSHOT will be relatively quick, we envisage 2 to 8 hours per user visit. Separate experimental hutches will allow us to optimally interleave experiments between the two end-stations. DXT and XFMS experiments will be highly automated.

Detectors. HIERARCHY requires four Eiger-9M or equivalent integrating detectors strategically placed to provide seamless USAXS/SAXS/WAXS measurements. These detectors will operate in a vacuum at a frame rate > 500 Hz. SNAPSHOT will utilize a 4M TRISTAN detector for DXT measurements and an off-line mass spectrometer for XFMS experiments.

5. State of the art benchmark

ID02 (ESRF, Grenoble, FR), the APS USAXS facility (APS, Illinois, USA), and BL03XU (Spring-8, JP) currently provide USAXS measurements for SCM investigations. The APS facility can provide USAXS/SAXS/WAXS measurements within 5 minutes, but measurement is not simultaneous. ID02 offers a 31-meter camera length with a minimum beamsize of 20 microns-squared (8.0 – 20.0 keV). The USAXS/SAXS/WAXS range is achieved by a variable camera length with the detectors in vacuum. Again, the entire possible range of length scales is not made from a single measurement but a series of measurements at specified camera lengths. ID02 is the most competitive instrument within the EU that will be associated with a 4th generation synchrotron.

17-BM at the NSLS-II is the only dedicated XFMS beamline in the world. Beamline 3.2.1 (ALS, Berkeley, CA) as a dedicated XFMS source is currently under-development. 17-BM provides focused, 5-16 keV pink beam (no monochromator) for XFMS studies with beam sizes between 120x450 um to 2.6x2.6 mm.

6. Community engagement

The SCM community is large, and multidisciplinary which will create a bifurcated user community for X4SCM. Users for DXT and XFMS will largely be associated with structural biology, similar to B21. Nevertheless, SNAPSHOT will also have users from the general SCM community as DXT can be readily applied to study dynamics within porous or solid materials. In fact, our preliminary experiment on B16 examined synthetic rubber samples. We will arrange a workshop inviting both the SCM and structural biology community to investigate the sample environments that would best exploit the capabilities of X4SCM. A user working group would then be set up to assist in prioritizing the requirements.

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