SSX and SFX probe the cyanobacterial FutA iron binding protein by X-ray induced photoreduction and reveal a switch for Fe(II) / Fe(III) binding

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Marine cyanobacteria are main contributors to carbon and nitrogen fixation, yet they are limited by iron availability. The most abundant and smallest photosynthetic organism on Earth is the cyanobacterium *Prochlorococcus* that can thrive in low nutrient waters [1]. Interestingly, *Prochlorococcus* has a single FutA iron binding protein, in contrast to cyanobacteria that possess two FutA proteins to bind Fe(II) and Fe(III). Using heterologous expression in the model cyanobacterium *Synechocystis*, we previously provided evidence for dual localisation and function of the single *Trichodesmium* FutA protein [2], but the mechanism that would allow FutA to bind two different iron charge states remained unknown. Here we used neutron and X-ray diffraction to characterise iron binding in different oxidation states in *Prochlorococcus* FutA at room temperature. Experiments required optimisation of crystallisation for serial experiments [3]. While neutron diffraction and SFX serial data characterised FutA in the oxidised iron binding state, home source diffraction data showed a reduced iron binding state [4]. To reveal the transition between oxidised Fe(III) and reduced Fe(II), we exploited X-ray induced photo-reduction of the iron centre, as revealed by spectroscopic characterisation [4]. A novel X-ray pump probe experiment was designed (Fig. 1, left) that used an attenuated X-fel pulse for photoreduction followed by a productive pulse to record diffraction data (Fig. 1, middle). The transition was also shown in a serial synchrotron X-ray dose series [4]. The experiments reveal an alternative positioning of the Arg203 side chain in the second coordination shell of iron to maintain an overall charge neutral binding site for Fe(II) and Fe(III) states. This molecular switch provides a plausible mechanism for iron binding promiscuity.

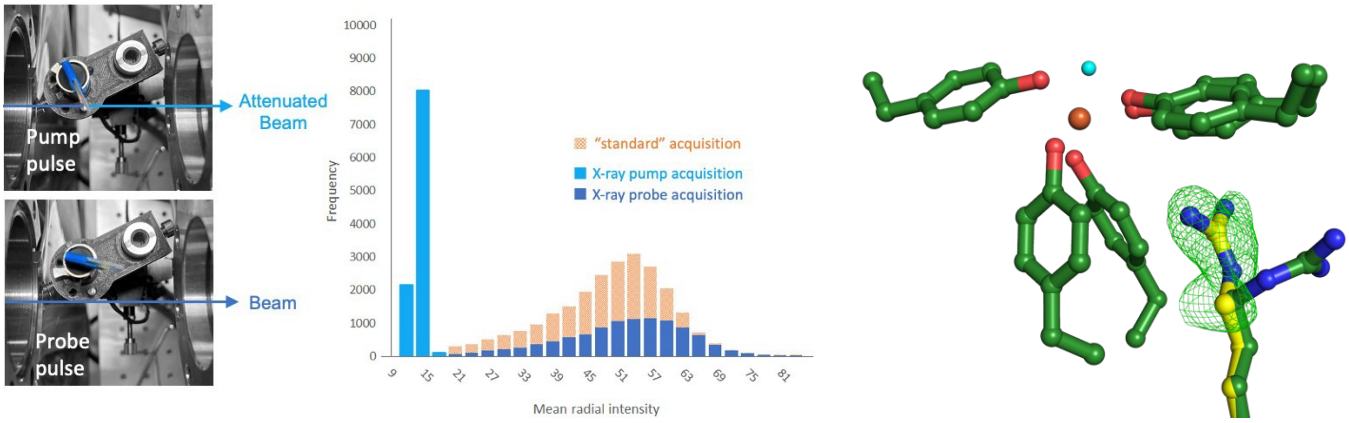

Figure 1. *Left:* a fast, self-restoring rotary shutter (sapphire wafer) mounted upstream of the sample was used to reduce the flux with alternating pulses; *middle:* with the crystal steady for two consecutive diffraction images, X-ray pump (light blue) and X-ray probe (dark blue) are separated based on average diffraction intensity, with data from a standard SFX experiment shown for comparison (salmon); *right:* the difference density (3ꙅ) reveals a repositioning of the Arg203 side chain in response to the X-ray pump pulse [4].

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