MS1-P12 Development of serial crystallography at ESRF, ID13

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Development of serial diffraction methods, which are complementary to efforts currently taken at hard x-ray free electron laser(XFEL) facilities have an extremely high impact on structural biology. Where single molecule and nano-crystal diffraction is out of reach at conventional synchrotron sources, fast room temperature experiments on large ensembles of micron sized crystals could benefit from certain advantages over the XFEL approach.

First approach to develop serial crystallography of membrane proteins is to perform lipidic cubic phase(LCP) microjet-based serial millisecond crystallography at synchrotrons[1], similar to XFELs[2].The main advantages of this method are: crystal injection using LCP-jet combined with a micro-beam allows diffraction data to be collected at room temperature, without crystal freezing and difficult crystal handling steps; thousands of crystals can be screened in a short time with less than a milligram of protein and the method is well suited for time-resolved diffraction studies. The LCP-jet method has been recently demonstrated by solving a structure of the bacteriorhodopsin at a resolution of 2.4 Å[3].

Second approach is based on scanning micro-crystals, which are deposited on solid supports. First successful results have already been obtained using lysozyme micro-crystals[4]. This approach, relying rather on controlled spatial distribution and subsequent scanning, allows to overcome severe limitations of available sample volumes(in particular for membrane proteins) and also it has further opportunities for optimization.

The other approach is to use special crystallization plates[5]. This system is based on a new crystallization plate that allows growing crystals on very thin films that can then be excised with a laser beam to recover the crystalline material. Due to their design, plates allow to collect diffraction data in-situ with very low background and to reduce mechanical stress for the crystals.

References

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MS1-P13 Akt-mediated phosphorylation increases the binding affinity of hTERT for importin α to promote efficient nuclear translocation

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hTERT is a catalytic component of telomerase that can extend the telomere end of genomic DNA. This protein has been shown to highly express in tumor cell. Residue S227 of hTERT is phosphorylated by Akt kinase [1] and hTERT is strongly localized to the nucleus. hTERT has a nuclear localization signal (NLS) from G220 to A242 containing two basic regions which might interact with hImportin α with bipartite binding mode. We used isothermal titration calorimetry (ITC) to determine the specificity of hImportin α5 comfirming the effect of phosphorylation on binding affinity. As a result, Phosphorylated hTERT S227 is higher affinity than un-phosphorylated hTERT. To see the molecular mechanism in detail, the complex structure of hTERT_NLS peptide and hImportin α5 has been solved at a resolution at 2.4Å. As might be expected, hTERT_NLS is shown to interact with hImportin α5 with bipartite binding mode. Phosphorylated S227 of hTERT interacts with R395 of hImportin α5 by hydrogen bond, which explains increased affinity by phosphorylation resulting in more efficient nuclear localization of telomerase. This result suggests that phosphorylation of TERT is a regulation strategy of localization for telomerase activity control.

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