

## **The Influence of Radiation Damage on MAD Data Collection and the Effectiveness of the Trichromatic Concept.**

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Most protein crystals are radiation damaged by incident X-ray beams. Although cryocrystallographic techniques are effective in slowing down the degradation, in some cases, such as for long exposures using highly brilliant synchrotron radiation, they are not enough. The MAD method is widely used as an experimental phasing method in modern protein crystallography.

We have previously proposed the trichromatic concept at SPring-8 RIKEN Beamline I (BL45XU), in order to try and optimize MAD experiments [1]. In the trichromatic concept, all the reflections for three different incident wavelengths are recorded successively in a short time, so that the condition of the sample at the three wavelengths should be the same, and there is thus negligible difference in signal caused by radiation damage.

A comparison between the conventional MAD data collection method and the trichromatic data collection method was carried out for some anomalous compounds. Each data set was independently processed and scaled to the independent MAD data. The dispersive Patterson maps and the phasing statistics using the trichromatic concept show a clear advantage over conventional MAD data collection techniques. We have collected MAD data sets from over 20 samples, including Bovine Rhodopsin [2] and Bacterial Flagellin [3], and have successfully achieved straightforward structure determination by MAD phasing. We summarize here the concept and our experimental results.

[1] Yamamoto, M. et al. (1998). *J. Synchrotron Rad.* 5, 222-226.

[2] Palczewski, K., et al. (2000). *Science.* 289, 739-745.

[3] Samatey, F.A., et al. (2001). *Nature* 410, 331-337.