

## Time constant reaction measurement in M-line of myofibrils by Fluorescence Loss In Photobleaching

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Creatine kinase 2.7.3.2. (CK) is a buffer-balance enzyme of muscular metabolism where it continuously and efficiently replenishes ATP utilized during muscle contraction. Myofibrillar bound CK isoenzyme is localized at the sarcomeric M-band, where it is functionally coupled to the myosin ATP-ase [1]. The unique interaction involves two parts, namely the molecular origin for MM-CK binding to the myofibrillar M-line and the exchange of MM-CK between the M-line and its surroundings [2], neither of which is well understood. In order to evaluate the rate of the latter process, *in situ* exchange assays with iodoacetamidofluorescein (IAF) labelled MM-CK have been studied by confocal microscopy [3].

The real exchange time of IAF-MM-CK was determined in longitudinal two-dimensional sections, using Fluorescence Loss in Photobleaching (FLIP) method [4]. The bleaching region has been defined as surroundings of the myofibrillar M-lines to isolate them, from IAF-MM-CK able to fluoresce (figure 1). The mutual motion of the MM-CK in the media was not changed and we could observe the exchange of MM-CK between the M-line and its surroundings by evaluation of the loss of fluorescence signal. The loss in the fluorescence signal was due to the fact that the new IAF-MM-CK, before reaching the M-line sites of reaction, have to pass the bleaching region. Therefore we observed the exchange between the fluorescent and the bleached IAF-MM-CK molecules. The time-lapse sequence showed the decay of the fluorescence intensity in the M-line. We applied fitting with a single exponential decay function because of the nature of single molecule reaction. The time constant of the reaction was measured to be  $20.4 \pm 5.3$  s.



Figure 1: (Field of view 28,5 x 28,2 [ $\mu\text{m}$ ]). The bleaching region in white, the M-line of the myofibril is marked by an arrow.

### References

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