Degradation of fluorescent proteins for time depending gene expression analysis in Pichia pastoris

Andrea Mellitzer[A], Franz Hartner[A], Manuel Peter[A] and Anton Glieder[A]

[A]*Department of Molecular Biotechnology, Graz University of Technology, 8010 Graz, Austria;*
Phone: 0043-316-873-4072, E-mail: andrea.mellitzer@tugraz.at

Fluorescent proteins (FPs) are becoming increasingly important tools in biological sciences. Due to their usefulness there is a broad range of FP variants available, which offer distinct physical and biological characteristics [1, 2]. In applications where rapid reporter turnover is required, the extraordinary stability of fluorescent proteins is a clear drawback. In addition, if several genes have to be expressed at different time points, the employed fluorescent reporters can interfere with each other and therefore tamper the results. Hence alternatives or less stable variants can be favourable to avoid accumulation of reporter proteins.

The aim of our study was to prepare a set of fluorescent proteins, which are distinctly destabilised adjusted to different promoter activities. The strategies were either based on introduction of PEST sequences [3] or by employing the N-end rule [4]. In our experiments we expressed three distinct destabilised fluorescent proteins under the tight control of the AOX1 promoter in Pichia pastoris. Our results suggest a correlation between degradation rate and the maturation time of the fluorescent reporter. Longer maturation times of the fluorescent protein clearly influence the degradation rate of the fluorescence protein and if the maturation time of the employed reporter was more than 1 hour no decrease in fluorescence could be observed.

[1] Chattoraj M. *et al.* (1996). Biophysics Vol. **93** : 8362-8367
[2] Shaner N. *et al.* (2004). Letters Nature Biotechnology **22** : 1567 - 1572
[3] Li X., *et al.* (1998). J Biol Chem, **273**(52): 34970-34975
[4] Varshavsky A. (1997), Genes Cells, **2**(1): 13-28