

Simultaneous FRAP and FRET reveal compartmentalisation of androgen receptor protein-protein interactions in living cells

Martin E. van Royen¹, Sónia M. Cunha¹, Maartje C. Brink², Karin A. Mattern¹, Alex Nigg¹, Pernette J. Verschure, Hendrikus J. Dubbink¹, Jan Trapman¹ and Adriaan B. Houtsmuller¹

¹Department of Pathology; Josephine Nefkens Institute; Erasmus MC Rotterdam; The Netherlands ²Swammerdam Institute for Life Sciences; University of Amsterdam; The Netherlands

E-mail: a.houtsmuller@erasmusmc.nl

Keywords: FRAP, FRET, protein-protein interactions, transcription regulation, live cell imaging

Steroid receptors (SRs) activate gene transcription by binding specific DNA sequences in the enhancers of the genes they regulate. Steroid activated SRs also interact through their ligand binding domain (LBD) with coregulators containing LxxLL or FxxLF motifs. The androgen receptor (AR) is regulated at an extra level by interaction of an FQQLF motif in its N-terminal domain (NTD) with its C-terminal LBD (N/C-interaction). Although it is generally recognised that these protein-protein interactions are essential for proper regulation of gene transcription by SRs, it is largely unknown where and when they are required.

To investigate the spatio-temporal organisation of AR-coregulator and AR N/C-interactions, we have developed a simultaneous fluorescence resonance energy transfer (FRET) and fluorescence recovery after photobleaching (FRAP) assay which allows to differentiate between the mobility (and immobilisation) of interacting and non-interacting molecules [1]. Briefly, upon photobleaching of the FRET-acceptor (YFP) the redistribution of increased FRET-donor (CFP) fluorescence reflects the mobility (and immobilisation) of interacting molecules only. Since DNA-interacting molecules typically are immobilised when they bind to their target sites in the DNA, combined FRET and FRAP analysis provides a strong tool to investigate the kinetics of protein-protein interactions essential for proper regulation of the various DNA-metabolising machineries responsible for transcription, replication and DNA-repair.

Combined FRET and FRAP analysis in living cells expressing ARs double tagged with the YFP and CFP at the N- and C-terminus respectively provided evidence that AR N/C-interactions occur predominantly when ARs are freely mobile in the nucleus, possibly to prevent unfavourable or untimely cofactor interactions. In contrast, FRET and simultaneous FRET/FRAP experiments suggests that AR-coregulator interactions occur preferentially when ARs are immobilized by DNA-binding. In addition, FRET-ratio-imaging suggested that the transiently immobilised ARs reside inside numerous 'speckles'. Moreover, BrUTP pulse labeling experiments showed that AR-speckles partly (but not completely) overlap with sites of active transcription.

[1] M.E. van Royen, S.M. Cunha, M.C. Brink, K.A. Mattern, A.L. Nigg, H.J. Dubbink, P.J. Verschure, J. Trapman, A.B. Houtsmuller. Compartmentalization of androgen receptor protein-protein interactions in living cells. *J Cell Biol*, **177**, 63-72 (2007).