**Actin Chromobody: a Novel Intracellular Biosensor of Actin**

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Molecular tools enabling non-invasive visualization of actin organization and dynamics in living cells are essential for understanding fundamental cell biology in health and disease.

Here we present a Chromobody that enables visualization of the actin cytoskeleton in mammalian cells by fluorescence microscopy. The Chromobody technology is a unique approach to detect endogenous structures and dynamic processes in living cells. The key elements of this technology are Camelidae heavy chain antibodies, which antigen-binding domains (VHVs) are build of single polypeptide chains. By fusing fluorescent proteins to these antigen-binding domains we generate small and stable Chromobodies, which can be efficiently introduced into cultured cells as plasmid DNA by means of simple transfection or viral transduction. Within living cells Chromobodies label endogenous antigens and function as intracellular biosensors.

This new tool for live actin detection, the Actin Chromobody, was isolated from a Camelidae VHH library. We show that it enables real time monitoring of reorganization of the actin cytoskeleton. We could visualize the dynamic disorganization and reassembly of microfilaments in various cell types including HeLa, PC3 and LNCaP upon treatments with Cytochalasin D and Jasplakinolide (a representative time lapse series is shown). In contrast to the traditional GFP-actin overexpression, the Chromobody does not alter the cellular actin concentration. The Actin Chromobody can be used as a marker of cellular morphology and as a probe for cellular motility, as it does not affect cell viability or migration (demonstrated with several mammalian cell lines).

In summary, the Actin Chromobody offers a new non-invasive method of actin cytoskeleton visualization in living cells.

### Advantages of Actin Chromobody

- Overexpression of Actin-RFP affects actin concentration in the cells, which leads to artifacts, such as appearance of the rod-shaped structures during recovery from Cytochalasin D treatment (upper left image).
- Expression of the Actin Chromobody does not induce this artifact (upper right image). This was also confirmed by the actin antibody staining.

### Transient binding of Actin Chromobody to actin cytoskeleton

- FRAP experiments reveal a more profound recovery of the Actin Chromobody fluorescence in comparison to Actin-RFP, suggesting a transient interaction between the Actin Chromobody and the actin protein.
- Experiments were performed with transfected HeLa cells. After bleaching a microfilament within a 5x5 µm region, recovery was monitored for 3 minutes. Normalized intensity values are plotted (data are means±SD, N=12-14).

### Conclusion

Actin Chromobody is a new tool for live visualization of actin cytoskeleton in mammalian cells.