Chromobody® based High Content cell cycle analysis in live cells

Ulrich Rothbauer and Kouros Zolghadr - LMU Biozentrum, Department Biologie D-82152 Martinsried
Simone Schicktanz and Hartwig Preckel, PerkinElmer Cellular Technologies Germany GmbH, D-Hamburg

1 Introduction
In this study we describe how single live cells can be followed through the entire cell cycle using a specific cell cycle protein binding cameloid antibody (Chromobody®) tagged with GFP (green fluorescent protein). Multiple samplings can be analyzed in parallel on the Opera™ High Content Screening platform. It is equipped with environmental control and Nipkow spinning disc confocal fluorescence imaging technology low in photobleaching and phototoxicity particularly designed for imaging live cells over long periods of time.

2 Material & Methods
Experiments were performed in HeLa cells stably expressing the cell cycle protein specific Chromobody® fused to GFP.

4 Cell cycle analysis using Acapella™ texture features on Chromobody® signal
The fluorescence signal of the cell cycle protein specific Chromobody® used in this experiment appears evenly distributed throughout the nucleus and cytoplasm of the cells, when not bound to its target. As soon as the target protein is expressed the fluorescence of the nucleus gets granulated followed by the appearance of brightly fluorescent spots. The granularity disappears again and finally the cell divides (Fig. 3).

This transition which reflects Chromobody® binding can be analyzed very fast and efficiently using the new texture analysis features of Acapella. Of all the texture parameters available the "SER Saddle" (Spots – Edges – Ridges, Saddle) parameter is best suited for the Chromobody™ signal.

3 Chromobody® technology

Chromobodies® are a new class of fluorescent antibodies which are characterized by an extremely small size, high stability and excellent reproducibility. Most importantly, Chromobodies® can be used in living cells to target and trace their endogenous antigens in different subcellular compartments, which is not possible with conventional antibodies. Unlike classical GFP-fusion proteins, Chromobodies® have the potential e.g. to detect and visualize non-protein components or specific posttranslational modifications of target proteins. These characteristics render them valuable tools as direct live cell biomarkers on High Content Screening platforms. In addition to cellular assays, Chromobodies® are ideal reagents for a multitude of analytical and preparative applications such as purification of fusion proteins, antigen detection or selection of transfectants.

Key Features
- detection of endogenous proteins in living cells
- real-time visualization of posttranslational modifications
- no over-expression artifacts
- full flexibility of use in live cell or end-point assays

Fig. 2 | Schematic outline Camelidae-derived nanobody (V_h)H) and its functional Chromobody® derivates.

Cell cycle parameters of Chromobody® fusion proteins were determined by cell cycle analysis using Acapella. According to cell cycle parameters Chromobody® is suitable for use on HeLa cells from the 16th hour onwards.

5 Summary
We could show that fluorescently labeled Chromobodies® can be used for cell cycle analysis in live cells employing the Opera High Content Screening platform.
Cells in S and M phase can be distinguished based on Chromobody® signal and cell size, a nuclear dye can be added for distinguishing G1 and G2 based on DNA content. This method has potential for identifying the influence of compounds on the cell cycle of live cells in a High Content Screening scenario.