Still, the use of ***firefly luciferase*** (De Wet et al. 1985) (*luc*) predominates the field, and the number of applications described outperforms those of many other reporter proteins with enzymatic activities known.[11,12,13] Luciferase has been cloned in 1986 already and generates light with an emission peak at 562 nm by catalysing the conversion of luciferin to oxyluciferin in an ATP-dependent manner. Today, improved assay reagents are available, which prolong the duration of the luminescent signals from initially 1 to 5 min to hours so that quite a number of microplates can be processed in parallel without loss in signal intensity. In addition, the availability of different luciferase reporters (i.e. firefly and *Renilla* luciferase (Parsons et al. 2000)) can be used to set up dual luciferase reporter systems in which the second reporter notifies of unspecific, unrelated cellular processes, which likewise interfere with reporter protein expression, production and activity. The *Renilla* enzyme uses another substrate, i.e. coelenterazine instead of luciferin, with differing kinetics and spectral properties.

A similar well internal referencing capability comes with the combination of ***ß-lactamase*** (Moore et al. 1997) (*BLA*) as a reporter protein and a cell-permeable, artificial substrate that is composed of two dyes with different spectral properties linked together via a beta-lactam ring. Upon cell loading, this substrate becomes trapped intracellularly at physiological pH. Excitation at 409 nm allows for fluorescence resonance energy transfer (FRET) between a donor (coumarin) and an acceptor dye (fluorescein) leading to a green emission signal at 518 nm. Once expressed, BLA enzyme activity separates both FRET partners and abolishes signal generation as the resonance energy transfer is highly sensitive to smallest changes in the distance between the donor and the acceptor partners. Nevertheless, a blue emission signal at 447 nm can be observed upon excitation at 409 nm instead. Thus, the calculation of the ratio between the 447 nm and the 518 nm emission signals can be employed to eradicate well-to-well cell variations and to detect fluorescence interference events. Although this procedure is leaving the cells intact for subsequent studies, it should be noted that the BLA reporter currently used is a genetically engineered version devoid of any signal peptide and resides inside the cell, whereas the prototype enzyme, encoding the bacterial ampicillin resistance marker, belongs to the class of secreted reporter proteins which can be utilised to quantify reporter gene activity in supernatants. In our lab, both luc ad BLA reporter systems are used in an orthogonal manner to either confirm screening hits or exclude artefacts resulting from transcriptional or translational processes as well as fluorescence interference.

Any over-amplification of weak primary signals by luciferase (Hill et al. 2001) or BLA enzyme activity has to be considered seriously, as the magnification of weak, basal responses may either mask partial activity effects or lead to an

[11] For chloramphenicol acetyltransferase (CAT), see Devinoy et al. (1991).

[12] For secreted alkaline phosphatase (SEAP), see: Cullen and Malim (1992).

[13] For secreted urokinase, see Langer et al. (1995).