Aequorin-based measurements

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There are special proteins that do produce light by themselves (bioluminescence; no excitation light is necessary). An example is the aequorin-GFP-complex (GFP, green fluorescent protein) that naturally occurs in the jellyfish *Aequorea victoria*. Interestingly, the famous paper by Shimomura[53] that identified GFP as a protein only mentioned GFP in one sentence because the paper was primarily dealing with luminescent aequorin. Because the operating principles and measurement techniques are different from those of fluorescent probes, they are discussed in their own chapter.

Aequorin became a popular calcium sensor after its first use as such in 1968[54] and before the age of the current generation of small molecule dyes, which started in 1980[55] (cp. section 3.1 - Calcium sensors). This protein shares the advantages of the genetically encoded calcium sensors as outlined in section 3.1 and does not require a light source for illumination, thereby circumventing the autofluorescence of the cells. It can report calcium in a wide concentration range, between 0.1 μM and 100 μM[56,57]. However, when expressed in cells (cp. section 6.3 - Genetic manipulation), the transfected or transduced DNA codes for apoaequorin, which requires the small organic molecule coelenterazine to form aequorin. In the bioluminescent reaction, aequorin reacts with 3 calcium ions to produce apoaequorin coelenteramide, carbon-dioxide and light at 466 nm. For the reconstitution of aequorin, the availability of coelenterazine was shown to be the rate-limiting step[58].

In addition to the expression of apoaequorin, purified aequorin is commercially available (Molecular Probes, Eugene, USA) and can be injected into cells as a calcium sensor. Although the protocols for the use of aequorin are demanding, a number of applications, such as *in vivo* imaging of calcium[59] or calcium assays for high-throughput screens[60], benefit from the properties of aequorin. The use of aequorin to visualise calcium dynamics was recently reviewed.[61]

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