

full description of the vast array of FRET assays is well beyond the scope of this text. In general, if it is possible to create a donor/acceptor pair that can assemble and disassemble as the result of a biological process, then a FRET-based assay can be designed to monitor the process.

There are, however, some important limitations and issues that should be kept in mind when reviewing data from a FRET assay. Interference with the signal can occur in a variety of forms, leading to false positives and false negatives. Test compounds that fluoresce, quench fluorescence, or are colored can interfere with detection methods. In addition, background fluorescence of the assay matrix, proteins, cellular material, and even the plastic of the assay plate can create interference that may limit the sensitivity of a FRET-based assay.⁴⁸

Time-Resolved Fluorescence Resonance Energy Transfer (TRFRET)

As mentioned above, a significant limitation of FRET technology is the presence of background fluorescence that, irrespective of the source, can create misleading data and decrease the sensitivity of a FRET-based assay. In the vast majority of cases, background fluorescence emits energy in the same short-lived time frame as the donor/acceptor pairs employed in FRET experiments. The key to eliminating background fluorescence is the application of lanthanide metals such as Europium (Eu), Terbium (Tb), and other lanthanide (rare earth) elements (Figure 4.19).⁴⁹ On their own, the lanthanides are poor

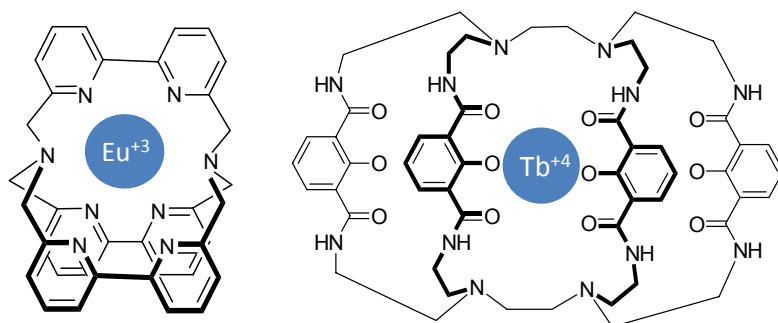


FIGURE 4.19 Organic scaffolds employed in TRFRET systems provide a suitable micro-environment that supports increased fluorescent lifetime of lanthanide-based fluorophores. Fluorescent signals from caged lanthanides outlast background fluorescence, providing a measurement window free of interference.

fluorophores, and cannot be used for FRET experiments. When captured in an appropriate organic scaffold, however, the lanthanides provide fluorescent species that can not only be employed as part of a donor/acceptor FRET pair, but also have fluorescent emission profiles that last substantially