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Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET)

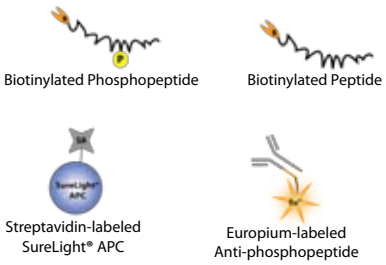
An Introduction

Background

Time-resolved fluorometry (TRF) combined with fluorescence resonance energy transfer (FRET) offers a powerful tool for drug discovery researchers. TR-FRET combines the low background aspect of TRF with the homogeneous assay format of FRET. The resulting assay provides an increase in flexibility, reliability and sensitivity in addition to higher throughput and fewer false positive/ false negative results.

FRET involves two fluorophores, a donor and an acceptor. Excitation of the donor by an energy source (e.g. flash lamp or laser) produces an energy transfer to the acceptor if they are within a given proximity to each other. The acceptor in turn emits light at its characteristic wavelength.

Because of this energy transfer, interactions between biomolecules can be assessed by coupling each partner with a fluorescent label and detecting the level of energy transfer. Acceptor emission as a measure of energy transfer can be detected without needing to separate bound from unbound assay compontents (e.g. a fitration or wash step). This homogeneous format reduces both assay time and costs.



FRET is driven by several factors including spectral overlap and the proximity of the fluorophores involved, wherein energy transfer occurs only when the distance between the donor and the acceptor is small enough. In practice, FRET systems are characterized by the Förster's radius (R_0): the distance between the fluorophores at which FRET efficiency is 50%. For many FRET parings, R_0 lies between 20 and 90 Å, depending on the acceptor used and the spatial arrangements of the fluorophores within the assay.

Advantages

Biological fluids or serum contain many compounds and proteins which are naturally fluorescent. Therefore the use of conventional, steady-state fluorescence presents serious limitations in assay sensitivity. Long-lived fluorophores, such as rare earth elements called lanthanides, combined with time-resolved detection (a delay between excitation and emission detection) minimizes prompt fluorescence interference.

Time-resolved fluorometry takes advantage of the unique properties of lanthanides. Commonly used lanthanides in assays are samarium (Sm), europium (Eu), terbium (Tb), and dysprosium (Dy). Lanthanide ion complexes are well suited for this appilcation due to their large Stoke's shifts and extremely long emission lifetimes (from µsec to msec) compared to more traditional fluorophores.

TR-FRET assays also offer advantages over fluorescent polarization (FP) assays. In FP assays, background fluorescence due to library compounds is normally depolarized and background signal due to scattered light (e.g. precipitated compounds) is normally polarized. Depending on the assay configuration, either case can lead to a false positive or false negative result. However, because the donor species used in a TR-FRET assay has a fluorescent lifetime that is many orders of magnitude longer than background fluorescence or scattered light, emission signal resulting from energy transfer can be measured after any interfering signal has completely decayed. TR-FRET assays can also be formatted to use limiting receptor and excess tracer concentrations (unlike FP assays), which can provide further cost savings.

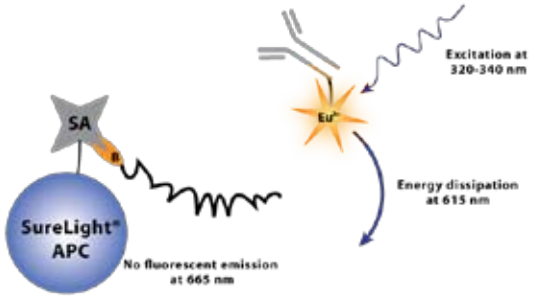


Figure 1 No FRET occurs when the two fluorescent species are not brought together through biochemical interactions.

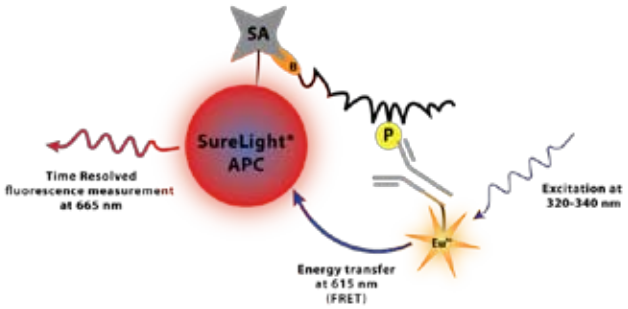


Figure 2 FRET occurs when the two fluorescent species are brought together through biochemical interactions.

Figure 3 shows the intersection of the emission from Europium with the excitation of allophycocyanin (APC) where energy transfer occurs when Europium and APC are brought into proximity via biomolecular interactions.

When these two fluorophores are brought together by a biomolecular interaction, a portion of the energy captured by the Europium during excitation is released through fluorescence emission at 620nm, while the remaining energy is transfered to the APC. This energy is then released by APC as specific fluorescence at 665 nm only via FRET with Europium.

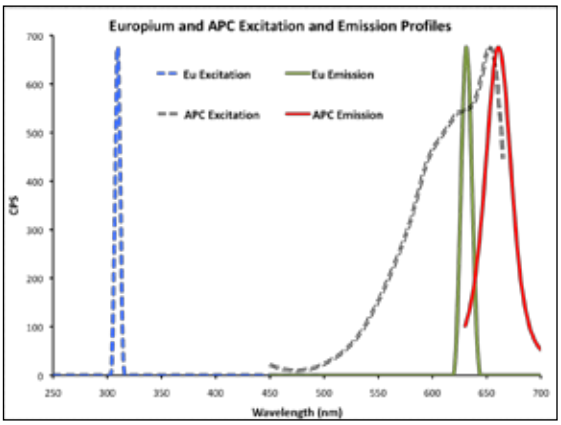
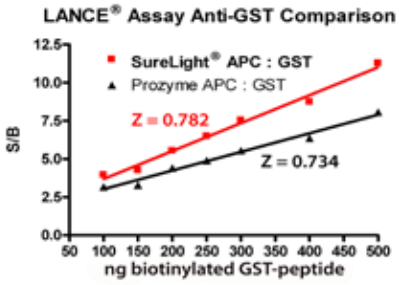
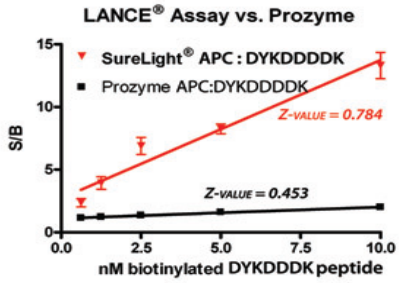
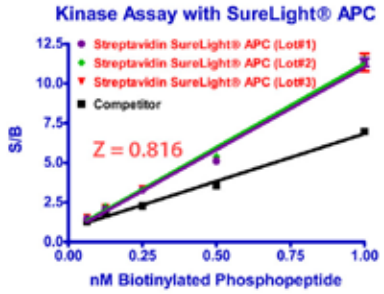


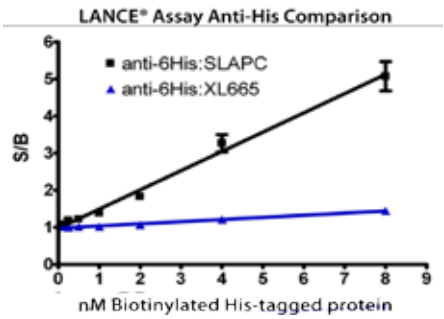
Figure 3 Overlapping excitation and emission spectra for Europium and allophycocyanin provide the basis for TR-FRET.

SureLight® APC Reagents for Use in TR-FRET Assays







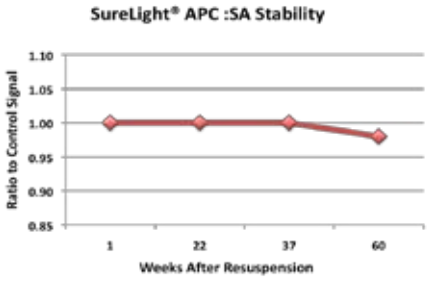


Performance

SureLight® APC reagents provide robust assay performance across a range of targets from protein:protein interactions to phosphorylation events. Inter-lot consistency provides for single digit coefficients of variance and Columbia Biosciences can produce lot sizes up to 1 gram to further ensure long-term assay comparability.

Stability

Columbia Biosciences' SureLight® APC reagents provide superior stability versus similar regents from competitors. Other suppliers recommend using their material within 2 weeks of reconstitution. SureLight® APC reagents have been shown to retain greater than 95% of the original signal after more than 1 year from reconstitution.



Performance of reconstituted SureLight® APC stored at 4 C over time versus same-day reconstituted SureLight® APC streptavidin in a model kinase assay.

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