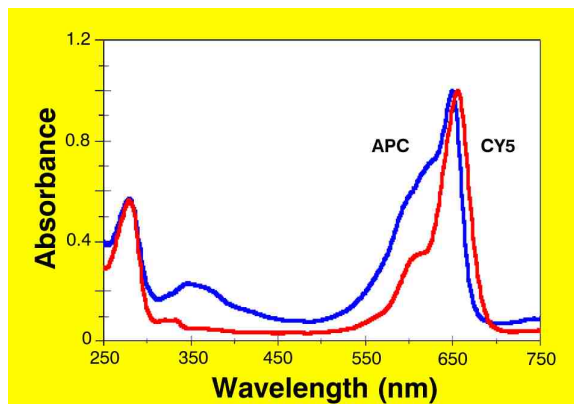


# SUITABILITY OF FLUORESCENT MOLECULES IN FRET ASSAYS

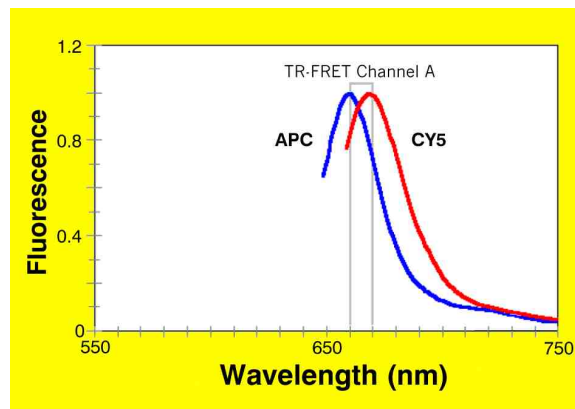
*The cyanine dye Cy5 has similar spectral characteristics to APC but is a weaker fluorescence acceptor. APC-streptavidin gave six times the FRET counts and twice the signal:noise as Cy5-streptavidin in a tyrosine kinase TR-FRET assay.*

Recently Cy5 (CyDye™) has been proposed as an acceptor molecule for the popular TR-FRET donor, Europium (Eu). Cy5 has similar spectral characteristics (Figures 1 and 2) to APC, which has become established as an acceptor partnered with Eu in TR-FRET assays. However, it is very different in its structure (Table 1). APC is a multi-subunit protein extracted from algae; there are 6 fluorophores per APC molecule which results in a high molar absorbance. Furthermore, as a result of its evolution as a light-gathering antenna, APC has a high quantum yield. Cy5 is a synthetic small molecule containing a single fluorophore.



**Figure 1 - Absorbance Spectra of SA Conjugates of Allophycocyanin and Cy5**

We report here the results of a comparison between streptavidin (SA) conjugates of Cy5 and APC in a model TR-FRET assay.



**Figure 2 - Emission Spectra of SA Conjugates of Allophycocyanin and Cy5**

**Table 1 - Fluorochrome Characteristics**

Fluorochrome	APC	Cy5
Absorption Max	650 nm	649 nm
Fluorescence Max	665 nm	670 nm
Extinction Coeff ( $M^{-1}cm^{-1}$ )	760,000	250,000
Mol Wt (kda)	104	~1

## METHODS

SA conjugates of APC and Cy5 were tested for performance in a model TR- FRET assay with  $\alpha$ -phosphoTyrosine-Europium chelate (PY20-Eu) acting as the fluorescence donor.

To each of the test wells containing:

- PY20-Eu - 300 ng/ml (2 nM) [PerkinElmer #Eu-W1024, Lance™ AD0066, Lot 728170]
- Peptide - 4.9 ng/ml (2 nM) biotinylated phosphopeptide [Pierce Chemical, 29934]

was added one of:

- SA-APC - 137 ng/ml (1 nM) [ProZyme PhycoLink® PJ25S, Lot 896 070]
- SA-Cy5 - 55 ng/ml (1 nM) [Amersham Biosciences FluoroLink™ PA45001, Lot 198626]

For each assay, 12 negative and 12 positive control wells, each containing 100 µl of assay mixture in 96-well black Costar plates were mixed, incubated for 24 hours and read in a Wallac Victor<sup>2</sup> 1420 Multilabel Counter (PerkinElmer) with the Lance 665/615 protocol, which excites the Eu donor at 340 nm and reads fluorescence at 615 nm (direct Eu fluorescence) and 665 nm (FRET to the acceptor). Calculations were performed as described in TechNote TNPJ100.04 *FRET Calculations*.

## RESULTS

Assays with APC conjugates had 6x the Net FRET counts, twice the sensitivity (S/N), and exhibited 5x the S/B, primarily due to the higher counting levels (Table 1). Thus, APC is a superior acceptor in this assay.

## TECHNICAL SERVICE

This and other TechNotes are available on ProZyme's webpage located at:

<http://www.prozyme.com/technical/index.html#technotes>

ProZyme customers are an important source of information regarding advanced or specialized uses of our products. We encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

PhycoLink® is a registered trademark of ProZyme, Inc. CyDye™ and FluoroLink™ are trademarks of Amersham Biosciences, Inc

**Table 2 - Comparison of APC vs. Cy5 Conjugates in a TR-FRET Assay**

	Value	STD (%)	S/N	Z'	S/B
A/B					
SA-APC	0.17196	2.1	47	0.93	40.27
SA-Cy5	0.03199	3.7	23	0.84	7.77
Net FRET					
SA-APC	2393	2.3	44	0.93	
SA-Cy5	389	4.4	22	0.83	



1933 Davis Street, Suite 207  
San Leandro, CA 94577-1258

TOLL FREE (800) 457-9444  
PHONE (510) 638-6900  
FAX (510) 638-6919

E-MAIL [info@prozyme.com](mailto:info@prozyme.com)  
WEB [www.prozyme.com](http://www.prozyme.com)

