# Combining UV Absorbance and Diagnostic CID Fragment Ions to Identify and Distinguish Isobaric Chromophores on Phycobiliproteins

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**Novel-Aspect**

The exact chromophore in the biliproteins has not been confirmed in-vivo by any other method.

**Introduction**

Certain cyanobacteria change the tetrapyrrole pigments attached to phycoerythrin in response to the color of light available. These tetrapyrrole pigments consist of phycoerythrobilin (PEB) and phycourobilin (PUB) which are structural isomers PEB and PUB differ in the position of one double bond and are therefore isobaric but have different UV-absorbance spectra. Each phycobiliprotein may have several bilin pigments attached to various cysteine residues. Researchers purify microgram amounts of protein to perform HPLC-UV-VIS experiments to identify the pigments utilized by the organism. The pigments attached to phycoerythrins produced in green vs blue light in Synechococcus RS 9916 have not been determined. This work utilizes the CID fragmentation patterns of different bilipeptides in conjunction with UV-absorbance to facilitate pigment attachment site identifications.

**Methods**

Phycobiliproteins were obtained by ammonium sulfate fractionation of phycobilisomes. Two microgram samples were digested with trypsin at 37 ºC overnight in ammonium bicarbonate buffer. 20- µL of each sample was then injected onto the LC-UV-MS/MS system composed of a Dionex 3000 Ultimate LC interfaced to an Agilent 1200 DAD and an LTQ-Orbitrap mass spectrometer with a Michrom ESI source. The LC column was a Zorbax C18 (75 mm length, 0.3mm ID, 3.5 µm particle size) operated at 4 µL/minute flow rate. The MS was operated in an automated data-dependent mode alternating between an FTMS scan and 3 collision-induced dissociation (CID) scans in positive ion mode.

**Preliminary-Data**

Samples of phycobiliproteins from the cyanobacterium Synechococcus RS 9916 having multiple attachments of PEB, PUB, or a combination of both depending on the light conditions under which cells were grown, were obtained. The LC chromatograms of trypsin digested phycobiliproteins revealed that the bilin groups render the peptides to which they are attached more hydrophobic and therefore, they elute later in the LC run using reversed-phase conditions. PEB bilin has its maximum UV absorbance at 550 nm (green light) whereas PUB bilin absorbs at 490 nm (blue light). The LTQ-CID spectra of chromopeptides having PEB or PUB attachments revealed several diagnostic fragment ions. Bilin fragmentation was found to be favored over peptide fragmentation. Fragment ions corresponding to a free tetrapyrrole and peptide, to a free tripyrrole and a peptide attached to the remaining pyrrole unit, and to a dipyrrole were all found.

Combining the retention time delay of bilipeptides, their UV absorbance wavelength and CID diagnostic ions, resulted in the identification of all the sites and types of isobaric bilin attachments to phycobiliproteins produced in green or blue light from Synechococcus RS 9916.