

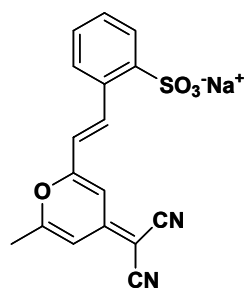
Novel Fluorescent Probe for Highly Sensitive Detection  
of Proteins and Application to Rapid Staining of SDS-  
PAGE

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It is of much importance for proteomic research to detect proteins. Therefore, biochemists always investigate novel protein detection methods that offer the advantages of easy handling, rapid monitoring, high sensitivity, and good binding linearity to facilitate both the qualitative and quantitative analyses of proteins. Fluorescence spectrometry is a conventional and highly sensitive analytical method, and fluorescent probes that exhibit a spectral response upon binding ions or neutral organic or inorganic molecules have enabled researchers to investigate the changes in the free guest ions or in the concentrations of molecules by fluorescence microscopy, flow cytometry, and fluorescence spectroscopy. Several fluorescent reagents that target proteins have been developed for the detection of proteins; these include fluorescamine, cyanine dyes, and NanoOrange that can detect proteins in solution and SYPRO Ruby that detects proteins in sodium dodecyl sulfate-polyacrylamide gels. Here we considered several requirements while designing fluorescent probes for proteins: (i) efficient excitation with most laser-based instrumentation systems; (ii) reduced interference from foreign substances; (iii) a higher molar extinction coefficient and quantum yield, which may enable the use of low dye concentrations; (iv) non-covalent interactions, such as hydrophobic or electrostatic interactions; and (v) elimination of the need for washing steps in the staining protocol to remove SDS and excess dyes.

In this study, a novel fluorescent molecular probe possessing a styryl, sulfonyl, and cyanopyranyl moieties termed compound **1** was designed and synthesized to detect proteins via non-covalent bonding. Compound **1** showed no fluorescence emission in the absence of proteins. However, its fluorescence spectrum demonstrated a dramatic increase in fluorescence intensity and a strong light emission of orange color after the addition of bovine serum albumin. This was caused by intramolecular charge transfer. The fluorescence intensities of compound **1** were plotted as a function of the protein concentrations. A good linear relationship was observed up to 325  $\mu\text{g/mL}$  of protein, and the lower detection limit was 70  $\text{ng/mL}$  under the given assay conditions, which was higher than that of previously reported compounds. To demonstrate the application of compound **1**, some proteins in SDS-PAGE gel were stained with compound **1** and were successfully imaged with higher sensitivity and shorter staining operation time than that of silver staining method and SYPRO Ruby staining method. Thus, easy and highly sensitive protein detection can be achieved with this fluorescent probe, and is ideally suited for proteomic application.



**Compound 1**