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Wide-Ranged Fluorescent Molecular Weight Size Markers for Electrophoresis

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A molecular weight size marker (MWSM) is commonly used in gel electrophoresis for protein and nucleic acid analyses.[1] In electrophoresis, biomolecules are detected by a wide variety of labels such as direct tagging, antibody, and silver stain, often with a set of protein or DNA standards for molecular weight based visual identification of analytes.[2,3] Development of enhanced MWSMs has been made to ease the size indications, and visible and fluorescent rainbow MWSMs have been well-acknowledged for the convenience of color-based size indications to overcome limitations and difficulties in use of identically stained markers (GE healthcare and Invitrogen, US).[4,5] However, increasing use of fluorescent molecules from a wide range of emission (em) wavelengths (400 to 700 nm) needs MWSM with a broader fluorescence spectrum.[6,7] Currently only a few numbers of MWSMs with limited wavelengths are known and commercially available to date.[8] For example, GE Healthcare's latest MWSM of dual indication with visible and fluorescent dyes is useful for proteomics analysis, but the use of Cy3 and Cy5 dyes limits it detection range.[4,5,9]

Here in, we report our effort to develop a wide ranged fluorescent MWSM, which covers upto near-infrared wavelengths, consisted of sequentially arranged cyanine dyes enabling proteomic analysis without post-stain for size indication. The development of new MWSM system that can cover all range of the fluorescent wavelength is an imperative task. Previously reported cyanine dyes equipped with vinyl sulfone linker (FPG-456, FPR-553 and FPR-648) were utilized in this work due to their optimal stability and robustness for protein conjugation.[10] The absorption (abs) wavelength of FPG-456 is 495 nm and its em wavelength is 522 nm, and those of FPR-553 and FPR-648 are 551 nm/570 nm and 648 nm/672 nm (abs/em), respectively. Combination of three dyes can create a range of emission spectra from 450 to 700 nm. Commonly used six standard proteins,[11] phosphorylase b (97.6 kDa), albumin (66 kDa), glyceraldehyde-3-phophate dehydrogenase (35 kDa), carbonic anhydrase (29 kDa), trypsin inhibitor (20 kDa), and lysozyme (14 kDa) were used as size indicating markers (Aldrich, US). The conjugation between a protein and a dye was precisely followed previously reported protocol.[10] In brief, 1.0 eq of given protein and 1.0 eq of vinyl sulfone dye were incubated in aqueous pH 8.0 phosphate or carbonate buffer (0.1 M) at 36 ºC for 4 h to afford corresponding protein-dye complex which was stored and used in the analyses without purification. From the product solution, the dye-conjugated protein solution (1 μL) was diluted to 200 folds in PBS.

Optical properties of products were measured with a fluorescence plate reader with a monochromator (EnSpire, Perkin-Elmer, Germany). Figure 1(a) shows abs spectra of six FPG-456 dye stained protein complexes (left), comparison of both abs (dot, middle) and em intensities (bar, middle) and SDS-PAGE bands (right) under irradiation of maximum abs wavelength. Figures 1(b) and 1(c) are those of FPR-553

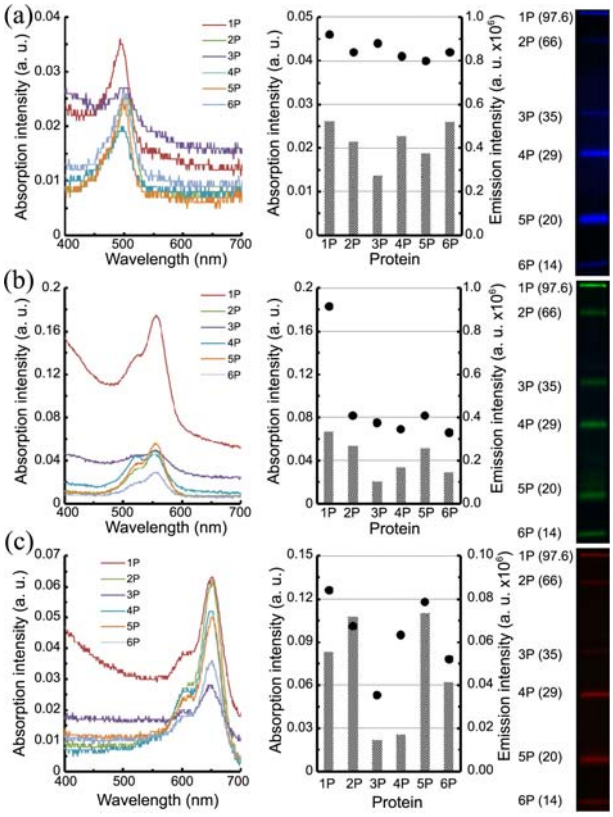


Figure 1. Optical property of dye-protein complexes. [phosphorylase b (**1P**), albumin (**2P**), glyceraldehyde-3-phophate dehydrogenase (**3P**), carbonic anhydrase (**4P**), trypsin inhibitor (**5P**), lysozyme (**6P**)]. (a) (left) Abs spectra of 6 FPG-456 labeled proteins, (middle) comparison of em intensity (abs 495 nm, dot; em 522 nm, bar) and abs intensity, (right) results 6 compounds' SDS-PAGE (excitation (ex) 495 nm, FITC filter); (b) Results from FPG-553 (ex 551 nm, RITC filter); (c) Results from FPR-648 (ex 648 nm, Cy5 filter).

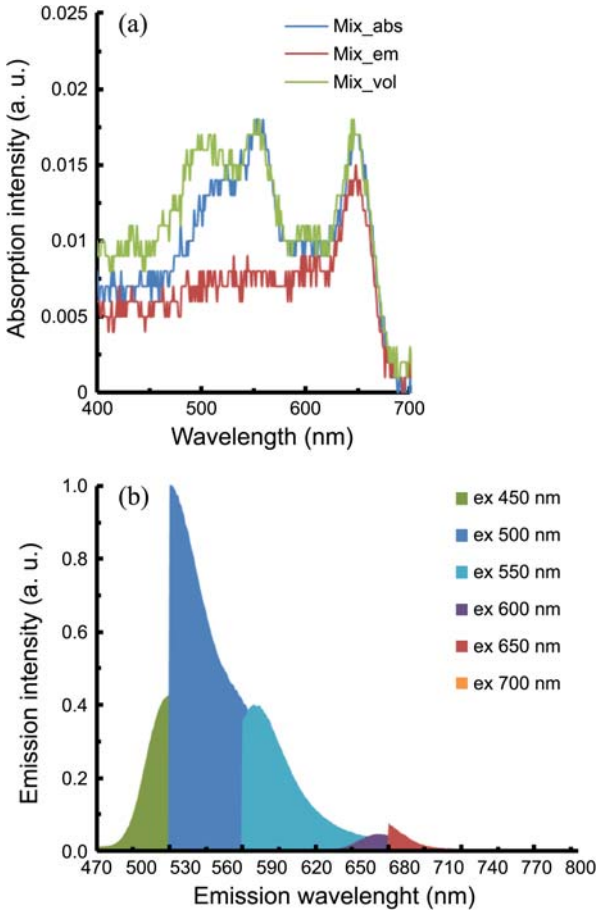


Figure 2. Abs and em spectra of the mixture of all MWSMs. (a) Abs spectra of varing mixing (Mix_abs: adjusting amount of the marker to exhibit same abs intensity, Mix_em: varying amount of the marker to exhibit same em intensity, Mix_vol: mixing with same amount of marker); (b) Fluorescent spectra of Mix_em.

and FPR-648 respectively. The labeling yield of a protein by a dye depends on several factors such as numbers of nucleophilic or electrophilic amino acid residues, and the three-dimensional structure of protein, hydrophilicity. Thus, the concentration of each protein-dye complex for SDS-PAGE was varied to produce similar fluorescent intensity by repeating gel electrophoresis with changed concentrations. Finally, concentrations of six complexes were determined as phosphorylase b (5.0 pmol), albumin (0.63 pmol), dlycer-aldehyde-3-phophate dehydrogenase (1.25 pmol), carbonic anhydrase (2.5 pmol), trypsin inhibitor (2.5 pmol), and lysozyme (2.5 pmol). With these optimal concentrations, all of 18 complexes provided closely matched em intensities in gel electrophoresis (Graphic abstract).

Given the fact that the optical properties of a protein marker were closely match with those of labeling dye, the initial blending ratio of prepared MWSMs was based on dye's optical properties and fluorescence intensity and fine-tuned with experimental adjustment. All MWSMs were calibrated into three categories: (1) same abs intensity (Mix_abs), (2) same em intensity (Mix_em), (3) identical amount of three dye-markers (Mix_vol). Since FPR-648 exhibited the lowest quantum yield, FPR-648 labeled protein of each MWSM was set as internal standard. Three groups of six stained MWSMs were tested their optical properties in the range from 450 to 700 nm with the interval of 50 nm (Figure 2).

In summary, we constructed a wide-ranged fluorescent MWSM system consisted of commonly used standard proteins and three cyanine dyes with vinyl sulfone linker. The goal of this work is construction of a wide range MWSM that can respond to any excitation wavelength in the visible region. Our MWSM had a broad emi spectrum at 400 to 700 nm region, and it has a potential be improved to the full range MWSM by addition of a dye with abs max at 600 nm.

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