Fluorescent protein spectra

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The cloning of the green fluorescent protein (GFP) from the jellyfish Aequoria victoria and its expression in heterologous systems was a significant advance for optical microscopy of living cells (Chalfie et al., 1994). Mutagenesis of jellyfish GFP has yielded proteins that fluoresce from blue to yellowish green, and genetic manipulations have generated GFP variants that are better suited for fluorescence microscopy than wild-type GFP and have optimized codon usage (Tsien, R. Y., 1998). For example, a green color variant of Aequoria GFP (EGFP) has been extensively used as an in vivo reporter because of its high quantum yield and resistance to photobleaching. However, the useful cyan fluorescent variant (ECFP) has low absorption and low quantum yield, whereas the yellowish-green fluorescent protein (EYFP) has the highest absorption and quantum yield but is more susceptible to photobleaching than are most other mutants. The recent cloning of a gene that encodes a red fluorescent protein (dsRed) from the Indo-Pacific sea anemone Discosoma striata has provided yet another fluorescent protein that is further red-shifted (Matz, M. V. et al. 1999). The dsRed shares only ~25% sequence identity with Aequoria GFP; other usable GFPs are therefore likely to be discovered in the future. Several limitations to the use of dsRed have been identified, including slow protein maturation and a strong tendency to form tetramers (Baird, G. S., et al. 2000).

The poster shows excitation and emission spectra determined for each fluorescent protein (excitation spectra are shown in lighter shades). Methods for purification and characterization of fluorescent proteins are described in detail elsewhere (Piston et al. 1999). Briefly, the cDNA of each GFP was subcloned to produce an N-terminal His6 fusion protein. His6-tagged GFPs were expressed in E. coli grown at 37°C, and

(See poster insert)
purified on a Ni NTA agarose column. Protein concentrations were determined by BCA assay, and the purification efficiencies were determined by scanning densitometry of SDS gels. Only proteins that were purified to >95% homogeneity were used for experiments. Extinction coefficients (see Table 1) were determined by Beers Law and data from an absorption spectrophotometer. Fluorescence excitation and emission spectra were measured and quantum yields were determined by using either fluorescein (QY = 0.85) or 1-aminoanthracene (QY = 0.61) as a reference standard. Photobleaching and pH stability measurements were performed as described previously (Patterson et al., 1997).

The fluorescent images shown in each panel are were obtained from the following proteins: GFP-Pit-1 (localized to the nucleus); BFP-C/EBPβ deletion 1-243 (localized to subnuclear foci); CFP-C/EBPβ (localized to subnuclear foci); Ds-RED (throughout cell); GFP-GRIP-1 (subnuclear puncta), BFP-C/EBPβ deletion 1-243 (subnuclear foci), and PML-DsRed (nuclear dots). In each case, cells were transfected with expression plasmids by electroporation, plated on glass coverslips and viewed after approximately 24 h in culture. The fluorescence images were acquired using an Olympus IX-70 inverted microscope (Olympus America, Melville, NY) equipped with a 60x aqueous-immersion objective lens and 100 W mercury-xenon arc lamp excitation light source. The detector used was a Hamamatsu Orca II cooled CCD camera.

### REFERENCES


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### Table 1. Properties of fluorescent proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Residue changes</th>
<th>Extinction coefficient (M⁻¹ cm⁻¹)</th>
<th>Quantum yield (%)</th>
<th>Excitation peak (nm)</th>
<th>Emission peak (nm)</th>
<th>pH dependence (EC50)</th>
<th>Bleaching time (relative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBFP</td>
<td>F64L, Y66H, Y145F</td>
<td>31,000</td>
<td>25</td>
<td>383</td>
<td>445</td>
<td>5.8</td>
<td>3</td>
</tr>
<tr>
<td>ECFP</td>
<td>S65A, Y66W, S72A, N1461L, M153T, V163A</td>
<td>26,000</td>
<td>40</td>
<td>434</td>
<td>477</td>
<td>4.7</td>
<td>85</td>
</tr>
<tr>
<td>EGFP</td>
<td>F64L, S65T</td>
<td>55,000</td>
<td>60</td>
<td>489</td>
<td>508</td>
<td>5.9</td>
<td>100</td>
</tr>
<tr>
<td>EYFP</td>
<td>S65G, V68L, S72A, T203Y</td>
<td>84,000</td>
<td>61</td>
<td>514</td>
<td>527</td>
<td>6.5</td>
<td>35</td>
</tr>
<tr>
<td>dsRed</td>
<td>S65A, Y66W, S72A, N1461I, M153T, V163A</td>
<td>72,500</td>
<td>68</td>
<td>558</td>
<td>583</td>
<td>4.3</td>
<td>145</td>
</tr>
</tbody>
</table>

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