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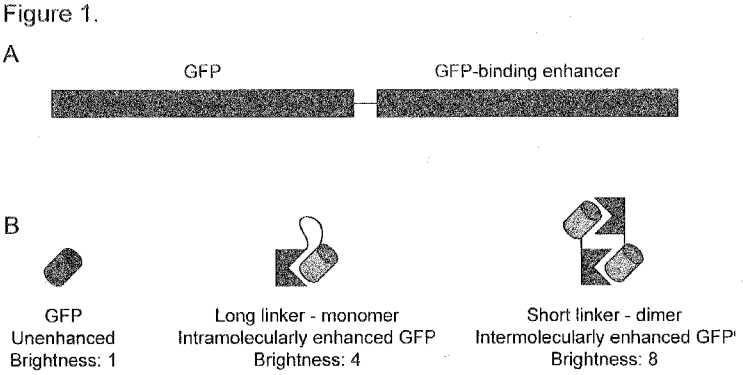
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(54) Title: NANOBODY-FLUORESCENT PROTEIN FUSION

Figure 1.



(57) Abstract: We describe the rational structure-based design of monomeric and dimeric forms of a nanobody-enhanced GFP (termed vsfGFP) that demonstrates ~1.3-fold higher brightness than sfGFP in a monomeric form and ~2.5-fold higher brightness in a dimeric form. These new vsfGFP variants demonstrate high stability and brightness in both bacterial and eukaryotic cells and are thus ideal for in vivo imaging applications. The combination of higher brightness, facile folding, stable expression, and tunable dimerization makes them ideal partners in essentially all in vitro applications already described for fluorescent proteins, including antibody fusion-based molecular probes, for which the higher brightness and tunable dimerization provide distinct advantages. Furthermore, the vsfGFP variants retain folding properties of sfGFP that enable bright fluorescence in oxidizing environments such as the bacterial periplasm. In particular, periplasmic expression enables the general construction of functional, fluorescent single domain antibody fusions, markedly enhancing the breadth and utility and reducing the cost of these molecular probes. Generalization of the vGFP strategy should provide similar improvements to other fluorescent proteins and may be extendable to higher order (and thus higher brightness) complexes.

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