

11th International Conference on Advanced Materials

Rio de Janeiro Brazil September 20 - 25

Preparation of PEGylated Green Fluorescent Protein for Bio-Luminescent Imaging

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PEGylation, covalent coupling of activated PEG to the protein surface, is by far the most successful approach for protein delivery (\$4 billion sale for the marketed products) [1]. Green Fluorescent Protein (GFP), a cylindrical 27kDa fluorescent protein isolated from the jellyfish Aequorea Victoria, is a novel fluorophore with no photoxicity or fluorescence quenching in aqueous media [2]. We aimed to synthesize PEGylated GFP for in-vivo bioimaging. PEG-Mal 5000Da was conjugated by nucelophilic addition to 2-iminothiolane modified primary amine of lysine and N-terminus GFP. PEGylated GFP was purified and characterized by SEC-HPLC and SDS-PAGE, respectively. Yield of modification and bioconjugation was determined by Ellman assay and fluorimetry. PEGylation has no sig. effect on GFP bioactivity. It is expected PEGylation leads to stabilize against proteolysis and RES uptake, to diminish the foreign protein immunogenicity, to promote plasma residence and lymph localization and possible tumor accumulation by EPR effect [3]. This could be applied for dual bioimaging and drug delivery if linked to bifunctionalized PEG.

References

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- [2] D. Filpula and H. Zhao. ADDR 60 (2008) 29-49.
- [3] P. Caliceti and F.M. Veronese. ADDR 55 (2003) 1261-1277.