

S008 Bridging the Gap for PEGylated Proteins.

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The increasing use of protein-based medicines by clinicians means that the issues relating to their effective delivery are now very important because proteins are expensive, rapidly cleared and pharmaceutically labile. The covalent conjugation of poly (ethylene glycol) (PEG) to proteins (PEGylation) is now delivering remarkable healthcare benefits such as the cure of millions of patients with Hepatitis C infection. This is in addition to improving protein pharmacokinetics and pharmaceutical properties, and reducing toxicity. However, at present, PEGylated proteins are mixtures with the PEG conjugated at multiple sites on the protein. We have developed a PEGylation strategy that exploits the thiol selective chemistry of *both* of the sulfur atoms that are derived from the ubiquitous and accessible native disulfides of proteins. This avoids the need to recombinantly engineer a therapeutic protein for site-specific PEGylation. Our approach involves (1) protein disulfide reduction to free the two sulfur atoms, and (2) reaction with a *bis*-thiol specific PEG reagent. Conjugation occurs by an interactive mechanism of addition-elimination reactions which is chemically efficient. A stable 3-carbon bridge connects the two sulfur atoms of the original native disulfide (with PEG bound) in a site-specific manner to the middle of the bridge. Our approach has been validated for the intra-chain disulfides of interferon and the inter-chain disulfides of antibody fragments. Tertiary structure and biological activity are maintained. This simple and cost-effective approach is applicable to cytokines, enzymes and cyclic peptides.