

## CyPEG™ PEGylation Technology

### The need to extend half-life. Why is PEGylation important?

Protein and peptide-based drugs are generally cleared from the body very quickly. Their rapid clearance results in decreased efficacy and the only clinical solution is to increase their frequency of dosing. This increases the risk of immunogenicity and the frequency of side effects. During preclinical studies it is difficult to conduct dose optimisation studies with a rapidly cleared molecule. Of all the technologies being developed to extend half-life, PEGylation is clinically proven to be safe and is the most widely used technology for increasing the duration of action of many different proteins.

### What are the limitations of PEGylation ?

As a technology, there is a continued need (i) to improve the site-specificity of PEGylation to achieve better product homogeneity and (ii) to improve the efficiency of PEGylation so that more economic medicines can be produced. PolyTherics' site-specific technologies (HiPEG™, TheraPEG™ and CyPEG™) provide for highly efficient, stable and precise addition of PEG to any protein or peptide.

### When is PEGylation at a free cysteine thiol appropriate?

Researchers have long tried to address the need for site-specificity by engineering a free cysteine into a protein because the thiol residue on cysteine readily undergoes selective and efficient PEGylation compared to the residues from other amino acids. Novel proteins and peptides can be developed with a free cysteine that can be readily exploited for site specific PEGylation.

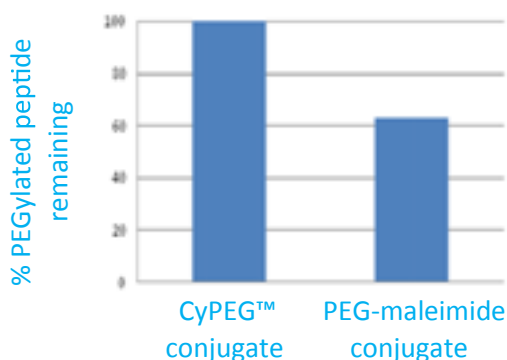
### Why has CyPEG™ been developed?

PolyTherics has developed CyPEG™ specifically to attach PEG to the thiol side chain on a free cysteine because the currently used reagents are not stable. PEG-maleimide derivatives are typically used to conjugate PEG site-specifically to a free cysteine thiol. The instability of PEG maleimide conjugates is well-known and leads to the formation of acidic by-products from ring-opening of the maleimide group and also to de-PEGylation, which results in conjugate fragmentation. The instability of PEG-maleimide conjugates means that these products must be lyophilised.

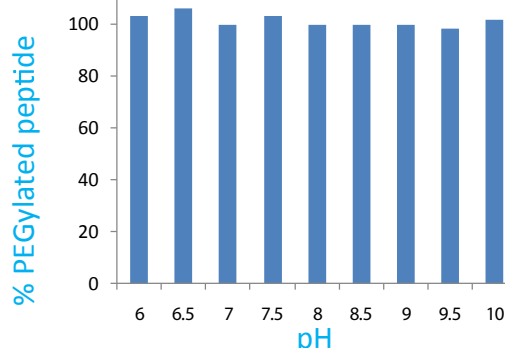
### What are the key advantages of CyPEG™ ?

CyPEG™ PEGylation generates conjugates that are more stable than those produced using PEG maleimide reagents (Figure 1). A CyPEG™ PEGylated peptide was stable for 12 days whereas the same peptide PEGylated using maleimide conjugation chemistry was shown to degrade by 37% over the same time period. CyPEG™ reagents are hydrolytically stable and unlike other PEG reagents, conjugates can be 'chemically locked' to prevent de-PEGylation. CyPEG™ can be used over a wide pH range with quantitative conversion (Figure 2). As with PolyTherics' other technologies, CyPEG™ PEGylation is site-specific. This aids interpretation of preclinical results in early screening studies, simplifies product purification, increases overall yield and is consistent with the regulatory requirement to produce a product of consistent high quality.

**Figure 1: Stability of CyPEG™ PEGylated peptide**



**Figure 2: Quantitative reaction over a wide pH range**



### Contact

Ajay Mistry, Business Development Manager

Email: [ajay.mistry@polytherics.co.uk](mailto:ajay.mistry@polytherics.co.uk)

Office: +44 (0)207 691 2061

Mobile: +44 (0)7881 811 739

Website: [www.polytherics.co.uk](http://www.polytherics.co.uk)