

# FACT SHEET

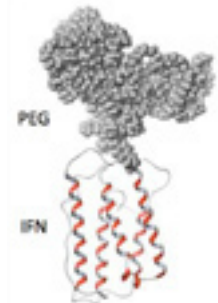
## TheraPEG™ PEGylation Technology



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.



Interferon (IFN) is one of many proteins amenable to TheraPEG™

### What is TheraPEG™?

TheraPEG™ has been developed by PolyTherics to specifically attach PEG to one or more of the accessible naturally occurring disulfides present in proteins. The technology has been used to conjugate branched and linear PEG molecules to a diverse range of therapeutic proteins and peptides, including cytokines, antibodies and enzymes. Patents for TheraPEG™ have been granted in the USA, Europe, China and India.



TheraPEG™ PEGylation can be accomplished during protein folding:

1. Reduction of the disulfide to expose the two cysteine thiols
2. Formation of a 3-carbon bridge
3. Covalent attachment of the PEG to the bridge

Selectivity of the TheraPEG™ reagent for the two cysteine thiols ensures only one PEG molecule is conjugated to each disulfide.

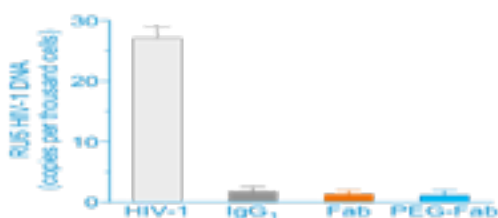
### What are the key advantages of TheraPEG™?

TheraPEG™ PEGylation is site specific and requires no prior structural modification of the protein. It has been successfully applied site-specifically to proteins with many disulfides (up to 13). TheraPEG™ results in fewer isomers than that produced by traditional PEGylation methods and consequently bioactivity and safety are more predictable. 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. TheraPEG™ PEGylation is very efficient so less PEG is needed than for traditional PEGylation methods, contributing to a lower cost of goods.

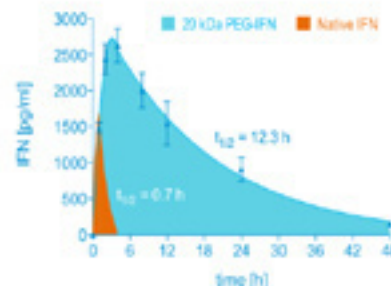
### What data supports the use of TheraPEG™?

TheraPEG™ has been used to extend the half-life of a wide range of proteins and peptides which have retained biological activity. Data for two examples, an antibody fragment (Fab) and IFN  $\alpha$ -2b are shown below.

#### Anti-CD4 Fab retains activity post-PEGylation



#### Half-life longer than non-PEGylated IFN $\alpha$ -2b



Blockade of CD4-entry of HIV-1 into cells in vitro is equivalent for PEGylated and non-PEGylated Fab.

Half-life is 18-fold longer for TheraPEG™ IFN  $\alpha$ -2b versus non-PEGylated protein (preclinical model).

### References

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