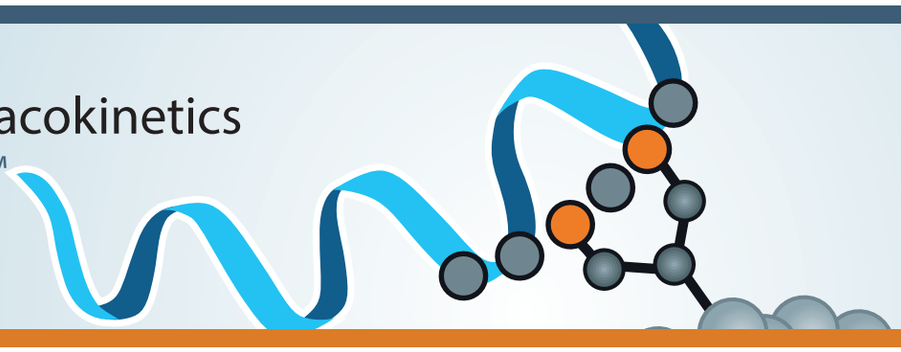
ABZENA

Enabling better biopharmaceuticals

# Optimisation of Pharmacokinetics

## TheraPEG™, HiPEG™, CyPEG™ & PolyPEG™



**Abzena provides a range of site-specific conjugation technologies to optimise the pharmacokinetics (PK) and pharmacodynamics (PD) of therapeutic peptides and proteins, including antibody fragments and other protein scaffolds.**

- Conjugated products are more stable and homogeneous than other well-established conjugation technologies
- Conjugation processes are efficient, reducing cost of manufacture
- Abzena also provides a low viscosity polymer, PolyPEG™, to enable easier injection of conjugated proteins at high concentration.

### Background

Therapeutic peptides and proteins are inherently chemically unstable and can be quickly cleared from circulation or generate unwanted immune responses. It has been established that linking polyethylene glycol (PEG) to proteins and peptides can increase stability, circulation time and may also reduce immunogenicity. These properties are derived through a combination of increased hydrodynamic volumes and masking of epitopes (in the case of immunogenicity) or sequences prone to enzymatic degradation (in the case of stability and circulation time). However, general PEGylation technologies are uncontrolled and lead to homogeneous products that are difficult to develop.

### Optimisation of Pharmacokinetics

Abzena proprietary PEGylation technologies enables PEG or other polymers to be attached to specified sites depending on the nature of the protein. TheraPEG™ conjugates PEG at disulfide bonds, HiPEG™ conjugates PEG to poly-histidine motifs and CyPEG™ conjugates PEG to a thiol on a free cysteine. These technologies are used to conjugate both linear and branched PEG to therapeutic proteins and have been successfully used for a broad range of protein types as shown in table 1.

These technologies reduce the heterogeneity of the conjugate, produce a stable product and, because the conjugation process is efficient and predictable, minimises reagent use.

| Category | Protein | Activity |
|---|---|---|
| Enzymes | Asparaginase | • |
| Cytokines | Interferon α-2a and α-2b<br>Interferon β | • |
| Hormones | Leptin<br>Erythropoietin | • |
| Peptides | Octreotide | • |
| Blood proteins | Coagulation factors (VIIa, VIII & IX) | • |
| Antibodies | Anti-CD4 Fab, Anti-TNF α domain<br>Novel formats, Scaffolds | • |

Table 1. Types of proteins that Abzena has optimised with retained activity.

## Novel comb-shaped polymer

### PolyPEG™ - modular polymer

PolyPEG™ is a novel low viscosity polymer comprising a polymethacrylate backbone with short PEG side chains that form a comb-like structure. PolyPEG™ can be conjugated to therapeutic proteins to extend their in vivo half-life. PolyPEG™ is available in a range of molecular weights enabling the pharmacokinetics and pharmacodynamics of the conjugated protein to be optimised.

- Alternative to linear and branched polymers where viscosity is an issue for manufacture or administration
- Modular architecture and range of conjugation chemistries available for optimal application of the technology

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## TheraPEG™, HiPEG™, CyPEG™ & PolyPEG™

## Conjugation technologies

### TheraPEG™ - disulfide conjugation

TheraPEG™ is a technology for attaching polymers, such as PEG, to proteins through site-specific conjugation at disulfide bonds. TheraPEG™ has the flexibility to conjugate a range of PEG formats, including linear and branched PEG and Abzena' low viscosity polymer, PolyPEG™

### CyPEG™ - cysteine conjugation

CyPEG™ is a conjugation technology for site-specific conjugation of polymers at a thiol side-chain on a free cysteine. The thiol residue on a cysteine readily undergoes selective and efficient conjugation. Proteins or peptides that do not have a cysteine can be engineered to provide a specific site for conjugation using CyPEG™

### HiPEG™ - conjugation at polyhistidine

HiPEG™ is a conjugation technology for site-specific conjugation of polymers at histidine sequences expressed within or at the N or C terminal ends of a protein. Conjugation at the terminus of the protein can have the advantage of reducing the shielding effect of the PEG on the functional part of the protein thereby minimising the effect on its activity. A histidine tag added to the terminus of a protein to facilitate purification can be used as the conjugation site without negative impact on purification methods utilising the poly-histidine tag.

## Case study: TheraPEG™ has been successfully used to PEGylate interferon (IFN) α-2b

Aim: To create a long-acting IFNα by efficiently conjugating TheraPEG™ to the accessible disulfides and confirm that the PEGylated IFNα had activity in vitro and an extended half-life in vivo.

The two accessible disulfides in IFNα were reduced under mild conditions. TheraPEG™ reagents were used in a low PEG : protein molar ratio to PEGylate the IFNα with a 20 kDa linear PEG (either mono- or di-PEGylated) or with a 2 x 20 kDa branched PEG:

- MonoPEGylated IFNα was the predominant species in the reaction mixture
- Pure monoPEGylated IFNα was prepared following a one-step purification process in exchange chromatography

### Half life extended

The half-life of 20 kDa TheraPEG™ IFNα was 18 times longer than the unPEGylated protein.

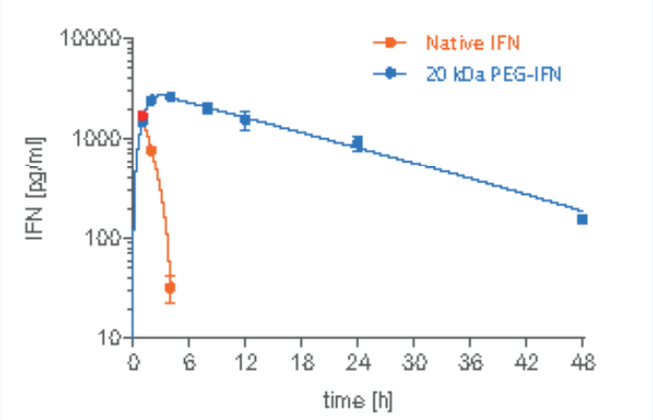


Figure 1. Half-life of 20 kDa TheraPEG™ IFNα and unPEGylated IFNα in rat serum

### Activity retained

TheraPEG™ IFNα compounds retained their activity in the antiviral assay.

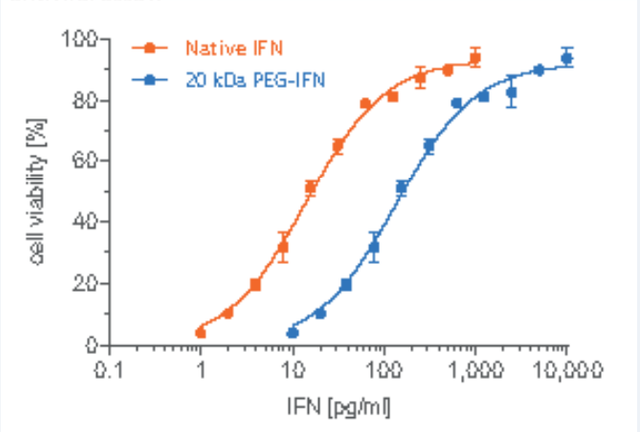


Figure 2. Following purification an in vitro antiviral assay was performed and a preclinical pharmacokinetics study was conducted with 20 kDa TheraPEG™ IFNα and native IFNα.

## Working with Abzena

We can PEGylate your protein or peptide in our laboratories or provide you with our conjugation reagents so you can undertake the work yourself. Our reagents are available in a range of PEG molecular weights and formats, including linear and branched PEGs.