**Abstract**

PEGylation is a routine strategy for enhancing protein pharmacokinetic properties, but it is challenging to predict sites where PEG will provide optimal pharmacokinetic benefits without diminishing biological activity. We hypothesize that PEG-based increases to protein conformational stability are a defining feature of optimal PEGylation sites; however, the interplay between PEGylation and conformational stability is incompletely understood. We have explored the impact of PEGylation on the stability of the WW domain of Pin 1.¹ We find that PEG-based stabilization of WW is associated with enhanced resistance to proteolysis, is entropic in origin, and likely involves disruption of the WW hydration shell. We have also formulated structure-based tools for predicting which sites in WW are most likely to experience PEG-based stabilization; we validated these tools by correctly predicting a stabilizing PEGylation site within the Src SH3 domain. We have made similar advances in an α-helical peptide model system.² Our results highlight the possibility of generating enhanced PEGylated proteins, in which PEG is installed only at locations that provide optimal PEG-based increases to conformational and proteolytic stability.

**References**
