Mechanism of Protein Stabilization by PEGylation

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PEGylation is widely used to improve the performance of biologics by protecting proteins from degradation and clearance in complex physiological environments. Despite its importance, the molecular mechanism by which PEGylation enhances protein stability remains incompletely understood. Because solvation is a central factor in protein folding and stability, this study examined how PEGylation changes solvent organization and protein–solvent interactions in the carbohydrate recognition domain of human Galectin-3.

This team of chemists and biophysicists investigated PEGylated Galectin-3 conjugates using two-dimensional infrared spectroscopy, multidimensional NMR spectroscopy, and molecular dynamics simulations. By comparing proteins modified with short and long PEG chains, they found that the longer PEG chain forms a more extensive, shroud-like interaction with the protein surface and significantly slows local solvent dynamics near the protein. Their results further show that this slower solvent reorganization correlates with increased thermal stability in the conjugated protein.

This work provides new mechanistic insight into how PEGylation stabilizes proteins and challenges the common assumption that stabilization occurs through surface dehydration. Instead, the findings support a model in which longer PEG chains reorganize and stabilize the surrounding solvation shell. These results could help guide the design of protein–polymer conjugates with more predictable stability and performance in biologic and therapeutic applications.

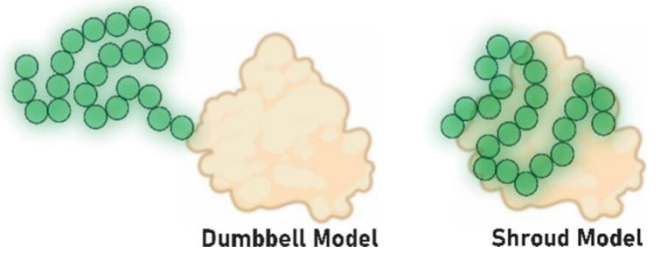

Figure 1: Model for how PEG length controls protein stabilization. A schematic comparing the dumbbell and shroud models of PEGylated Gal3C. The short PEG chain behaves more like a flexible appendage, while the longer PEG chain remains closely associated with the protein surface.

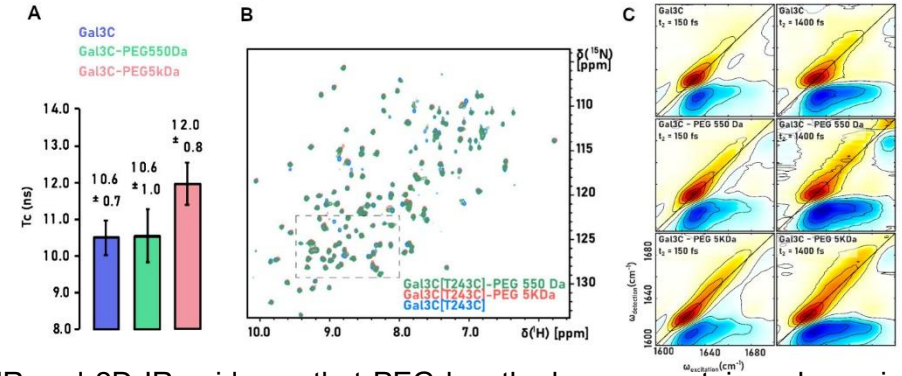

Figure 2: NMR and 2D IR evidence that PEG length changes protein–polymer interactions and solvent dynamics near the protein surface. (A) Rotational correlation times from ^{15}N relaxation data show that Gal3C conjugated with a longer chain PEG tumbles more slowly, supporting a shroud-like conformation of PEG. (B) Superimposed 2D ^{15}N-HSQC spectra show larger effects of PEG5k on the Gal3C protein backbone. (C) 2D IR spectra of the Gal3C amide I band show that longer PEG induces more pronounced changes in solvent interactions.

Facilities and instrumentation used: AMRIS Facility, University of Florida, 800 MHz NMR spectrometer with a cryogenic probe

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