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Mapping Structurally Significant Areas of G-CSF during Thermal Degradation with NMR

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Abstract : Proteins are capable of exploring vast mutational spaces. This makes it difficult for protein engineers to devise rational methods to improve stability and function via mutagenesis. Deciding which residues to mutate requires knowledge of the characteristics they elicit. We probed the characteristics of residues in granulocyte-colony stimulating factor (G-CSF) using a thermal melt (from 295K to 323K) to denature it in a 700 MHz Bruker spectrometer. These characteristics included dynamics, micro-environmental changes experienced/ induced during denaturing and structure-function relationships. 15N-1H HSQC experiments were performed at 2K increments along with this thermal melt. We observed that dynamic residues that also undergo a lot of change in their microenvironment were predominantly in unstructured regions. Moreover, we were able to identify four residues (G4, A6, T133 and Q134) that we class as high priority targets for mutagenesis, given that they all appear in both the top 10% of measures for environmental changes and dynamics (∑Δ and ΔPI). We were also able to probe these NMR observables and combine them with molecular dynamics (MD) to elucidate what appears to be an opening motion of G-CSFs binding site III. V48 appears to be pivotal to this opening motion, which also seemingly distorts the loop region between helices A and B. This observation is in agreement with previous findings that the conformation of this loop region becomes altered in an aggregation-prone state of G-CSF. Hence, we present here an approach to profile the characteristics of residues in order to highlight their potential as rational mutagenesis targets and their roles in important conformational changes. These findings present not only an opportunity to effectively make biobetters, but also open up the possibility to further understand epistasis and machine learn residue behaviours.

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