Chemical synthesis of c-Myc transactivation domain using a synthesis/solubility tag

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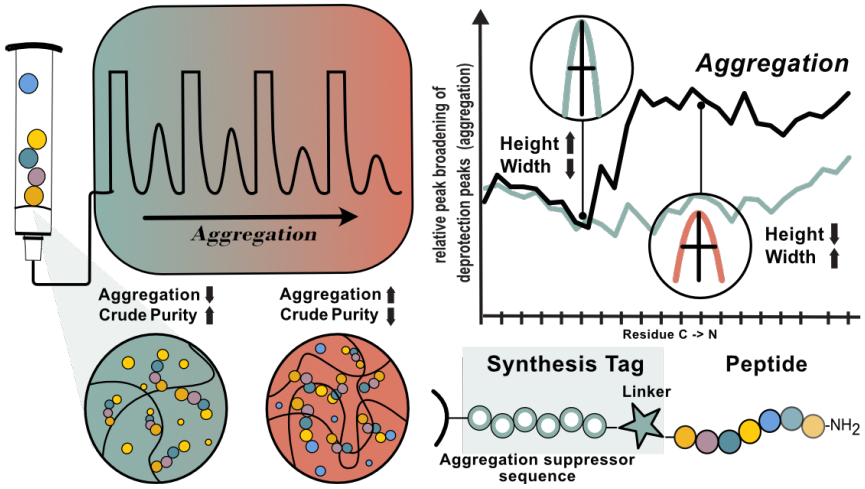
Chemical protein synthesis enabled by solid-phase peptide synthesis (SPPS) provides peptide and protein samples with a virtually unlimited chemical space (including PTMs) through incorporation of non-canonical amino acids and backbone modifications. Decades of improvement and optimization have increased the length of synthesized peptide chains of up to 50 amino acids.[1] Over this limit, Native Chemical Ligation (NCL) has been developed to join synthesized fragments, ultimately leading to the production of larger proteins.[2] Yet, generating fragments by SPPS in good yield and purity requires extensive synthesis efforts. A particular problem during the synthesis itself is aggregation, which is highly sequence dependent. While several solutions have been developed to address the aggregation problem, identifying and suppressing its cause is still very challenging. A deeper understanding of aggregation, as well as a more general solution to this problem are therefore urgently needed. Amino acid closest to the resin, and more specifically aromatic residues, have been described previously for being aggregation-inducing.[3] We hypothesized that the cleavable linkers used in SPPS, which are mostly large, electron-rich aromatic moieties, might also play an important role, an effect that has never been investigated. Our flow-based fast peptide synthesizer (AFPS) with in-line UV-Vis, has the capacity to monitor aggregation during synthesis. Upon aggregation, the Fmoc groups are less accessible for deprotection resulting in a peak broadening effect as the deprotection efficiency decreases. Using this platform, we screened linkers commonly used in SPPS on different aggregating peptides in collaboration. Importantly, we could observe that almost all linkers which are most commonly used in SPPS heavily promote aggregation, the noteworthy exception being the Dawson linker, which is usually used in NCL protocols.[2] Next, we screened short sequences for their capacity of suppressing aggregation. By combining those two approaches, we were able to develop a versatile “synthesis tag”. The tag helped both to reduce aggregation on different “difficult peptide”, converting into drastically improved crude purities as well as enhanced solubility, overall facilitating peptide handling. We then used our “synthesis tag” for the chemical synthesis of the heavily aggregating c-Myc[86-143] fragment belonging to the transactivation domain (TAD) of the intrinsically disordered transcription regulator c-Myc.[4] With this approach, we were able to synthesize an otherwise unobtainable fragment in sufficient quantities for ligation protocols, allowing us to retrieve the full c-Myc TAD.



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