Synthetic c-Myc1-84 library allows correlation of PTMs and PTM-crosstalk with binding interactions

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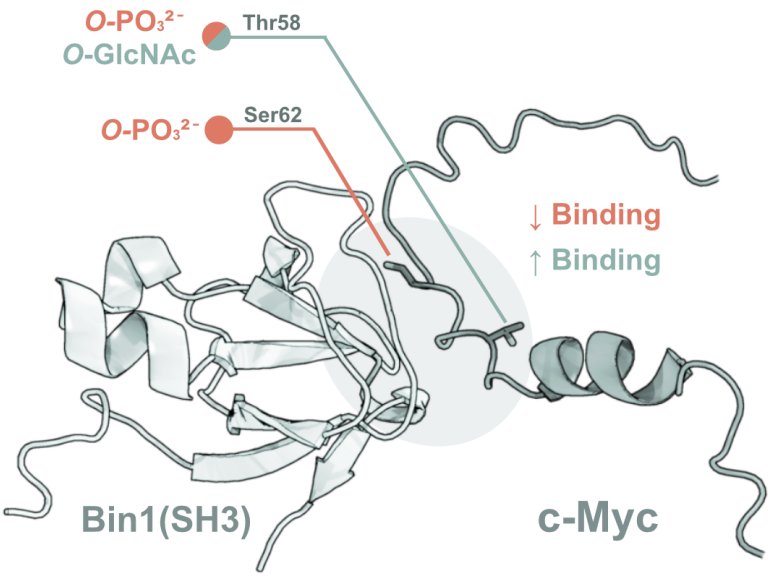
To date, the post-translational modifications (PTMs) of c-Myc and their effect on protein-protein interactions (PPIs) are yet to be systematically studied due to difficulties in obtaining PTM-variants of c-Myc by recombinant expression. To address this, we used Automated Fast-flow Protein Synthesis (AFPS)[1] to prepare a series of PTM-variants of the c-Myc N-terminus (amino acids 1–84) covering phosphorylation at T58, S62, and glycosylation at T58, as well as combinations thereof. All six c-Myc variants were produced in multi-milligram scale with >92% purity (after HPLC) using AFPS. We then looked at the impact these PTMs had on the interaction of c-Myc1-84 with Bin1, a tumor-suppressing protein,[2] using NMR. The same experiments were also performed with an analogous series of short c-Myc peptides (amino acids 55–68). We found that mono- or double-phosphorylation at c-Myc(T58/S62) decreased Bin1 binding affinity, yet to a much greater extent for c-Myc55-68 than c-Myc1-84, supporting literature hypotheses of additional Bin1 binding site(s) in c-Myc1-84.[3] Conversely, *O*-GlcNAcylation of c-Myc(T58) was found to strengthen the interaction of Bin1 with both c-Myc1-84 and c-Myc55-68, suggesting a phosphorylation/glycosylation switch.[4] The ease of PTM incorporation and the rapid synthesis of each protein (e.g. c-Myc1-84, *ca.* 3.5 hr synthesis time) showcases the potential of AFPS for further investigation of c-Myc PTMs and how they regulate c-Myc PPIs.



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