## Decoding the Molecular Dynamics of ASH1L in Cancer Using NMR for Novel KMTase Inhibitor

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Cancer therapeutics currently have the lowest clinical trial success rate of all major diseases. Partly from the paucity of successful anti-cancer drugs, cancer continues to be a leading cause of mortality. Genome-wide studies identified histone and chromatin regulators as one of the most frequently dysregulated functional classes in cancer. Specifically, H3K36methyltransferases (KMTases) are especially promising as they are key drivers and markers of almost all cancers. However, no selective and cell-active small-molecule inhibitors of H3K36-specfic KMTases have been reported to date, underlining the challenges associated with this target class. This project studies the molecular dynamics of ASH1L as it is one of the most highly overexpressed H3K36-KMTase in various types of cancers. ASH1L purification was conducted in 3 conditional experiments using TRIS, MOPS, and bio-express buffers with varying pH for optimization. This was followed by titration of nucleosomes and different constructs of MRG15 cofactor. The samples then performed two NMR measurements: standard 1D 1H and 2D 1H-15N TROSY. After successfully optimizing ASH1L at 25.5kDa, I assigned the base NMR spectra for unbound ASH1LSET which revealed 65 newly discovered amino acids, validating the structural base consisting of 220 amino acids. TROSY measurements revealed a double autoinhibitory loop releasing activation mechanism for ASH1L: (1) MRG15 binding at ASH1L-N-terminal (2) MRG15MRG domain binding at ASH1L-N-terminal with nucleosomes at ASH1L-C-terminal. These findings serve not only as a correction but a fixed base for a novel field of cancer inhibitors that may allow for early cancer diagnosis and treatment.