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Chemical Synthesis and Intracellular Delivery of P53 Transactivation Domain (TAD) Constructs

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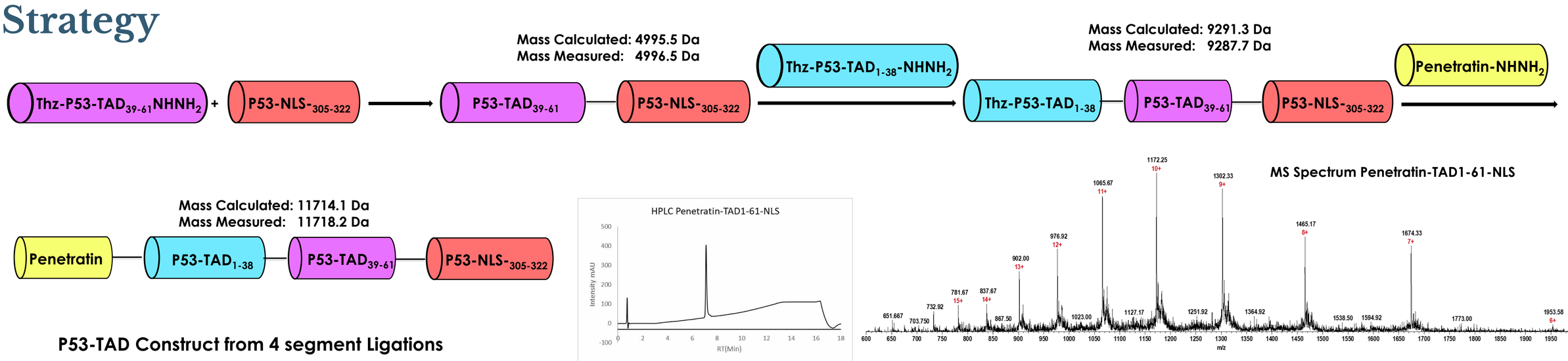
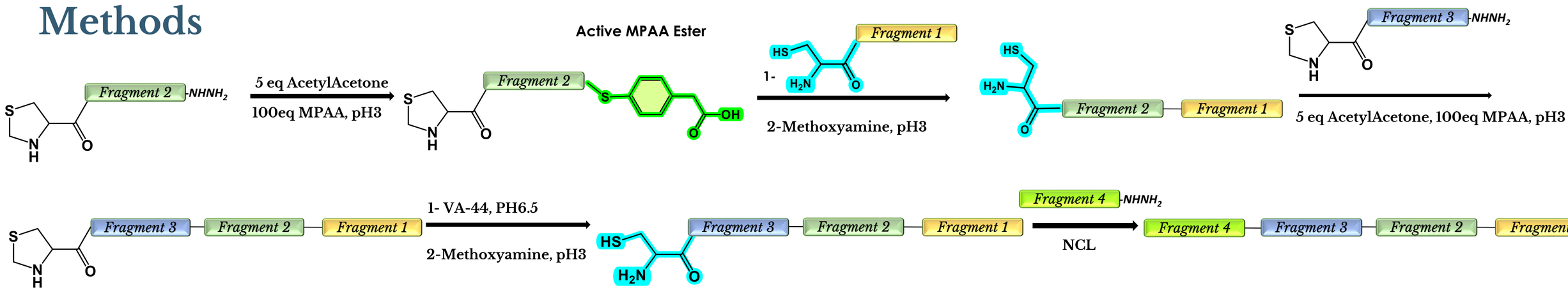
The tumor suppressor (P53) is a well-known oncoprotein, known for its involvement in majority of cancers. This protein consists of several domains, one of which is the Transactivation domain (TAD-P53). TAD-P53 is an intrinsically disordered region, that is responsible for the binding of P53 with numerous targets. Upon cellular stress, phosphorylation leads to the release of P53 from its negative regulators, HDM2 and CBP[1,2]. To understand the various interactions of TAD-P53, modifications can be effected such as α-methylation[3], or phosphorylation[4]. We have already demonstrated the chemical synthesis of TAD-P53 via three segments using various approaches[4,5]. To enable in vitro studies of TAD-P53, the intercellular delivery of chemically synthesized variants of TAD-53 is considered. Herein, we demonstrate the chemical synthesis of TAD-P53 analogues, conjugated to a cell penetrating peptide (CPP) and Nuclear localization signals (NLS) to aid the intracellular to delivery of TAD-P53 constructs. The inclusion of a CPP already proved to be an effective method, to enhance the delivery of various cargo, and in this project, Penetratin sequence was introduced at N-terminus. Even though TAD maybe delivered, it may not reach the nucleus. Therefore, to overcome this obstacle, the NLS of TAD-P53 (305-322) was attached at C-terminus. The chemical synthesis of the constructs was accessed via native chemical ligation, joining 4 segments to yield a 106 amino acids-long protein.

Strategy



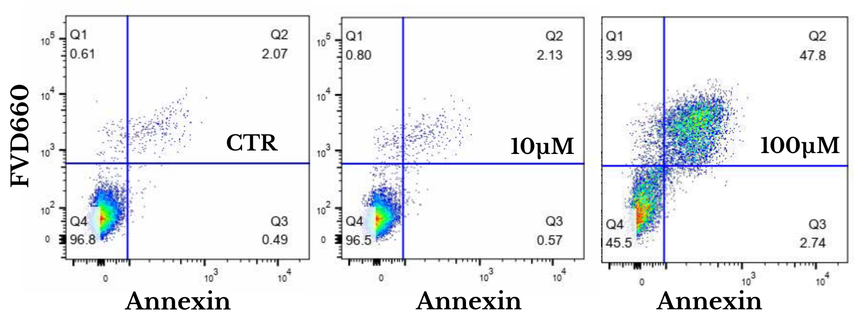
Methods



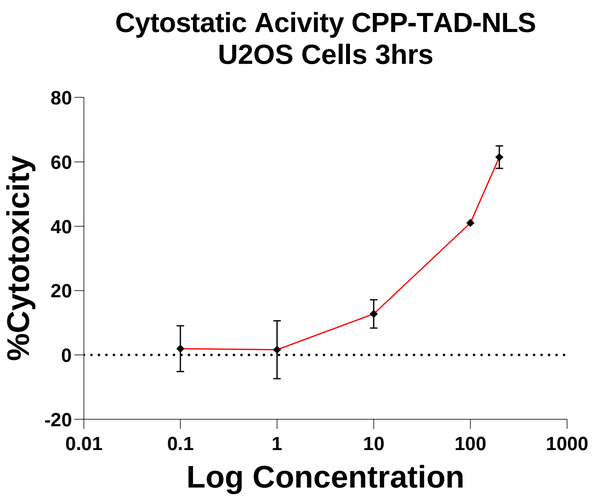
The use of NCL is the backbone of this work, allowing the chemical synthesis of the target product, from several peptide segments. Newer and more sophisticated methods, such as Knorr pyrazole[6] synthesis were adopted. The use of this method preserves the thiazolidine moiety (Thz) from any side reaction, as would take place in a classical thioester formation method with $NaNO_2$.

The resulting thioester from the Knorr Pyrazole method undergoes a ligation reaction, joining two segments (1+2). Thz can be subsequently deprotected to cysteine via methoxyamine treatment, and reacted with the next segment (1-2 +3). A subsequent desulfurization reaction, using VA-044 converts the Cys residue to an Ala residue. The final step involves a classical NCL reaction to yield the desired product.

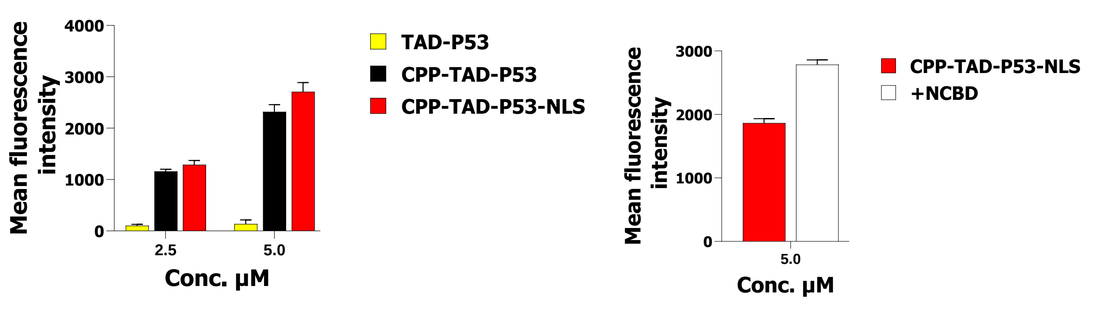
Results



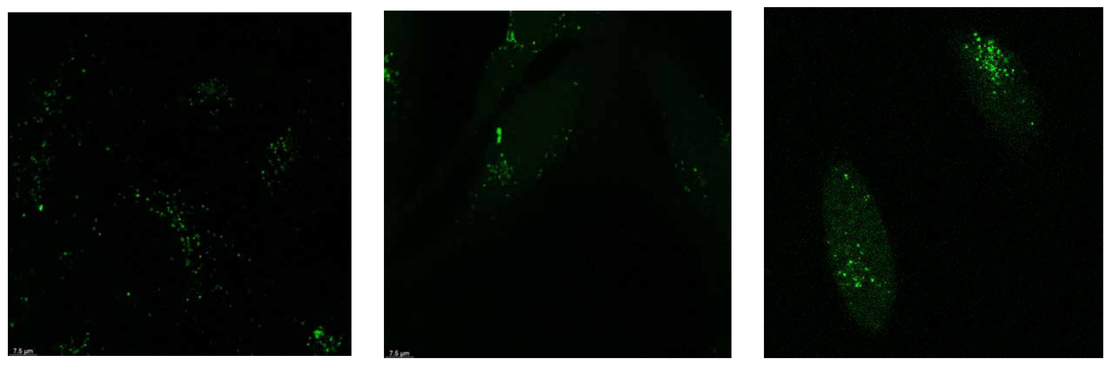
Apoptosis assay of CPP-TAD-NLS with Annexin PCP-eFluor 710. Late apoptosis is evident at 100µM



Effect of CPP-TAD-NLS on cell proliferation, cells were treated for 3Hrs and incubated for 3 days, preceding the MTT assay.



Flow Cytometry experiment showing the relative uptake of the constructs, second graph shows the increased uptake upon the introduction of NCBD protein in the cell media.



TAD_{1-61} $CPP\text{-}TAD_{1-61}$ $CPP\text{-}TAD_{1-61}\text{-}NLS_{305-322}$

Live Confocal Microscopy showing the increased uptake of fluorescent TAD-P53 $_{1-61}$ constructs when CPP is introduced. U2OS Cells are treated for 3Hrs at 37°C.

CONCLUSIONS

TAD of P53 was chemically synthesized using modern methods. This allows further modifications in the structure of TAD-P53 permitting the investigation of its interactions. The prepared constructs were successfully delivered, and their biological studies prove that our design permitted increased permeability. The biological activity of CPP-TAD-P53-NLS instigates that TAD can solely affect the cell survival.

References

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