

# TOTAL CHEMICAL SYNTHESIS OF NANOBODIES FOR TARGETED CANCER THERAPY

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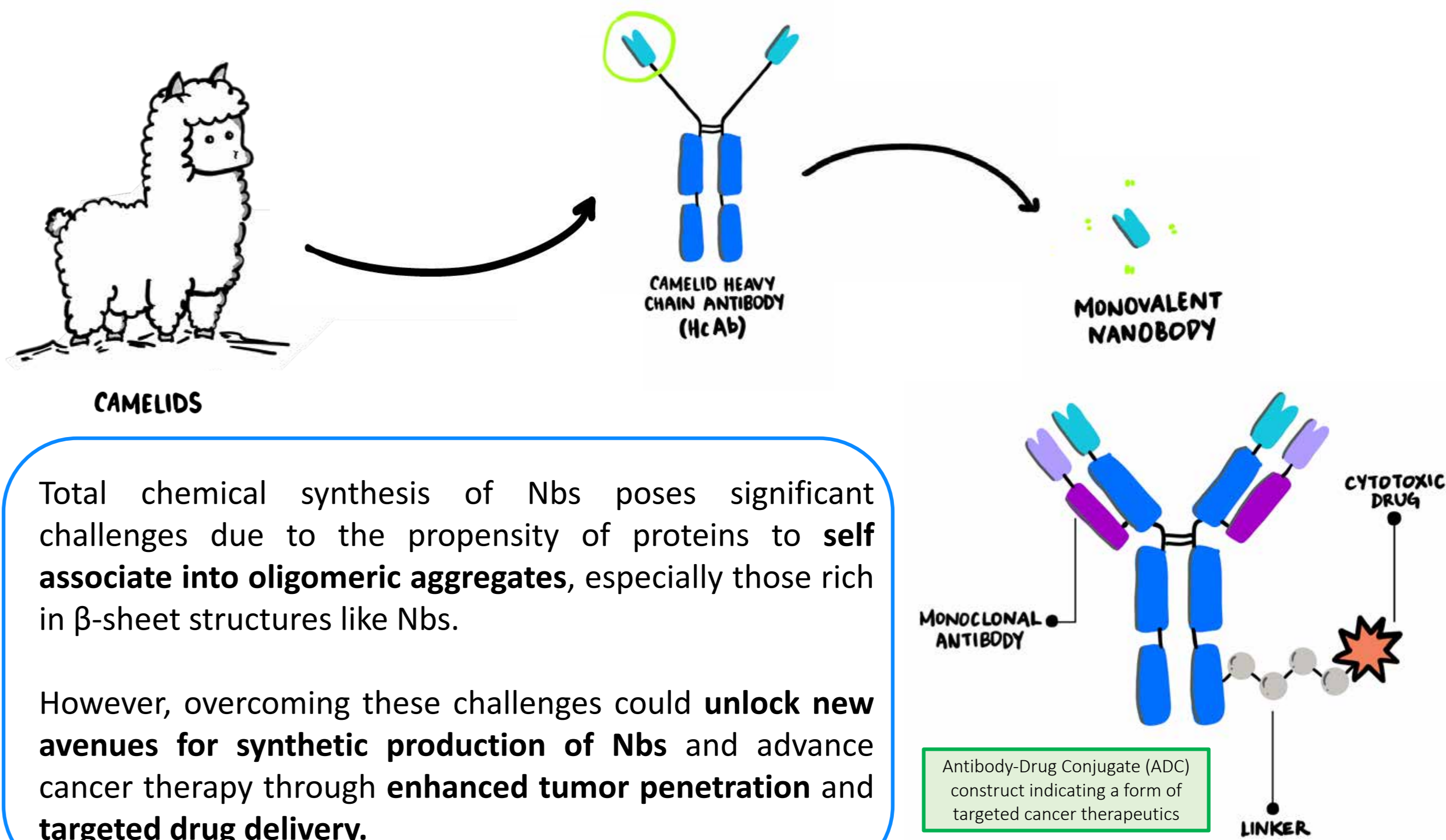
<https://doi.org/10.17952/37EPS.2024.P2014>

## Introduction:

Nanobodies (Nbs) are fragments isolated from heavy-chain-only antibodies (HcAbs) in the sera of the Camelidae family.

Nbs encompass superior characteristics when compared to full-size antibodies including, **low immunogenicity**, **exquisite antigen-binding affinity** (0.1 – 10 nM), and **small size** (MW 15 kDa; dimensions: 4 nm × 2.5 nm × 3 nm)

Their implementation has been hindered by the limited flexibility of existing production methods (e.g., challenges in incorporating non-natural amino acids) and manufacturing/purification challenges (e.g., removing bacterial endotoxins).

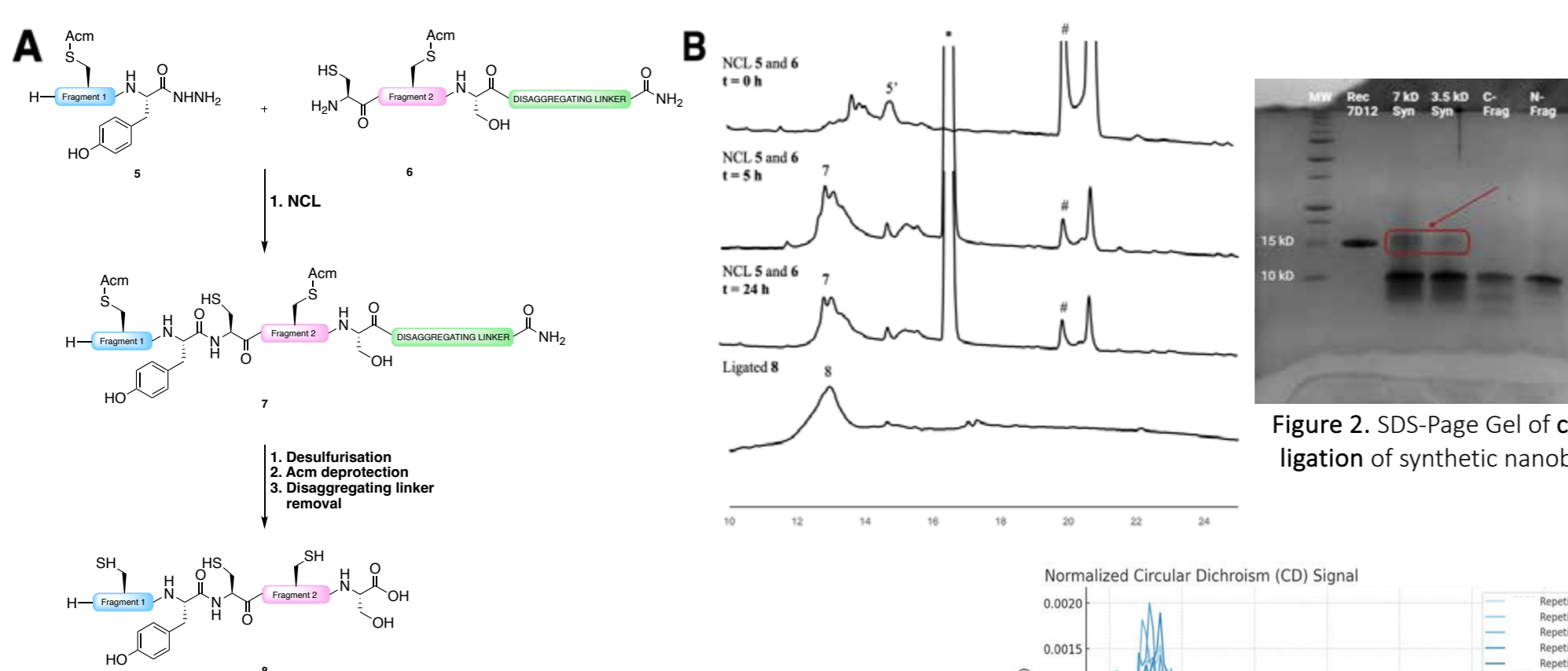


Total chemical synthesis of Nbs poses significant challenges due to the propensity of proteins to **self associate into oligomeric aggregates**, especially those rich in  $\beta$ -sheet structures like Nbs.

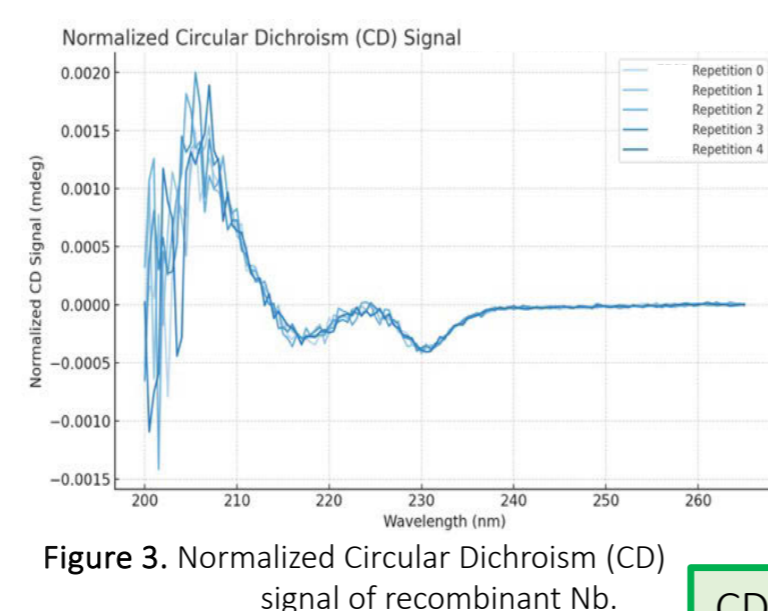
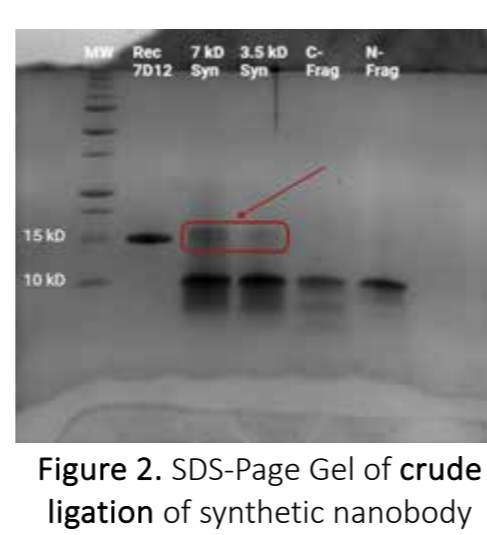
However, overcoming these challenges could **unlock new avenues for synthetic production of Nbs** and advance cancer therapy through **enhanced tumor penetration and targeted drug delivery**.

## Results:

### Two-fragment Native Chemical Ligation

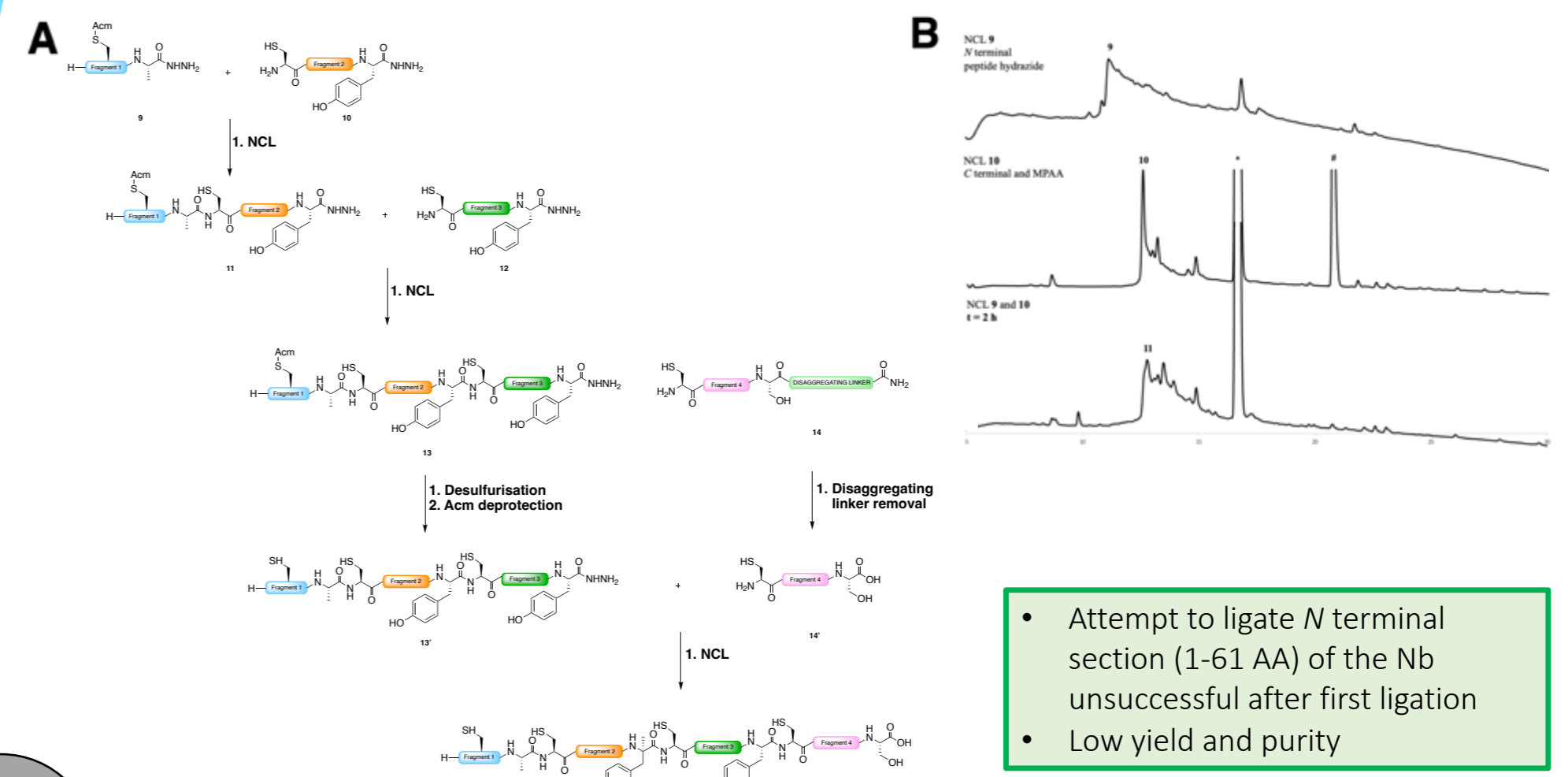


### Biological Results



1-24 amino acid residues of highly  $\beta$ -sheet containing Nb fragment without disaggregating linker

### Four-fragment Native Chemical Ligation



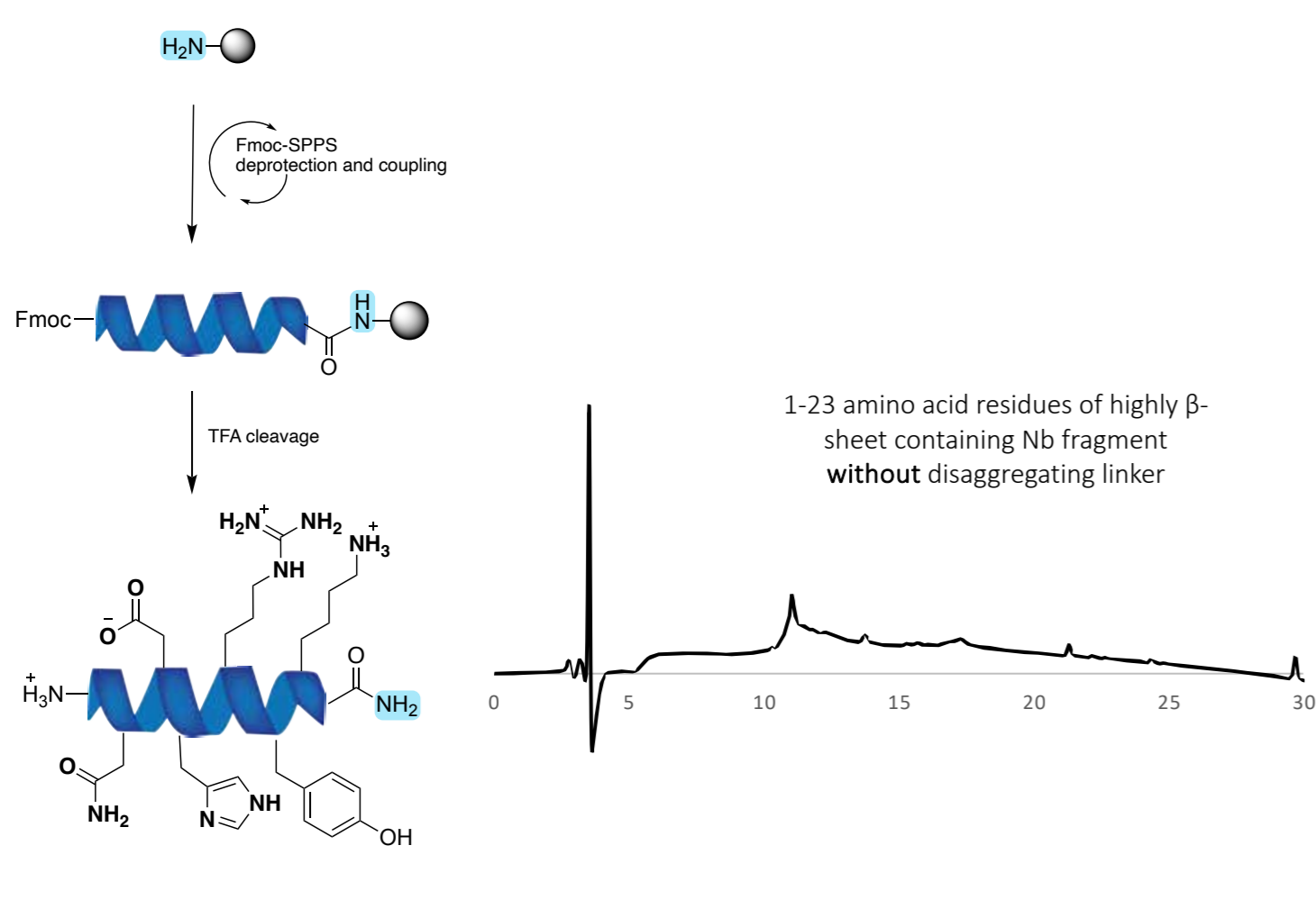
- Attempt to ligate N terminal section (1-61 AA) of the Nb unsuccessful after first ligation
- Low yield and purity

CD Data to confirm the  $\beta$ -sheet nature of the recombinant Nb for comparison with the synthetically produced Nb

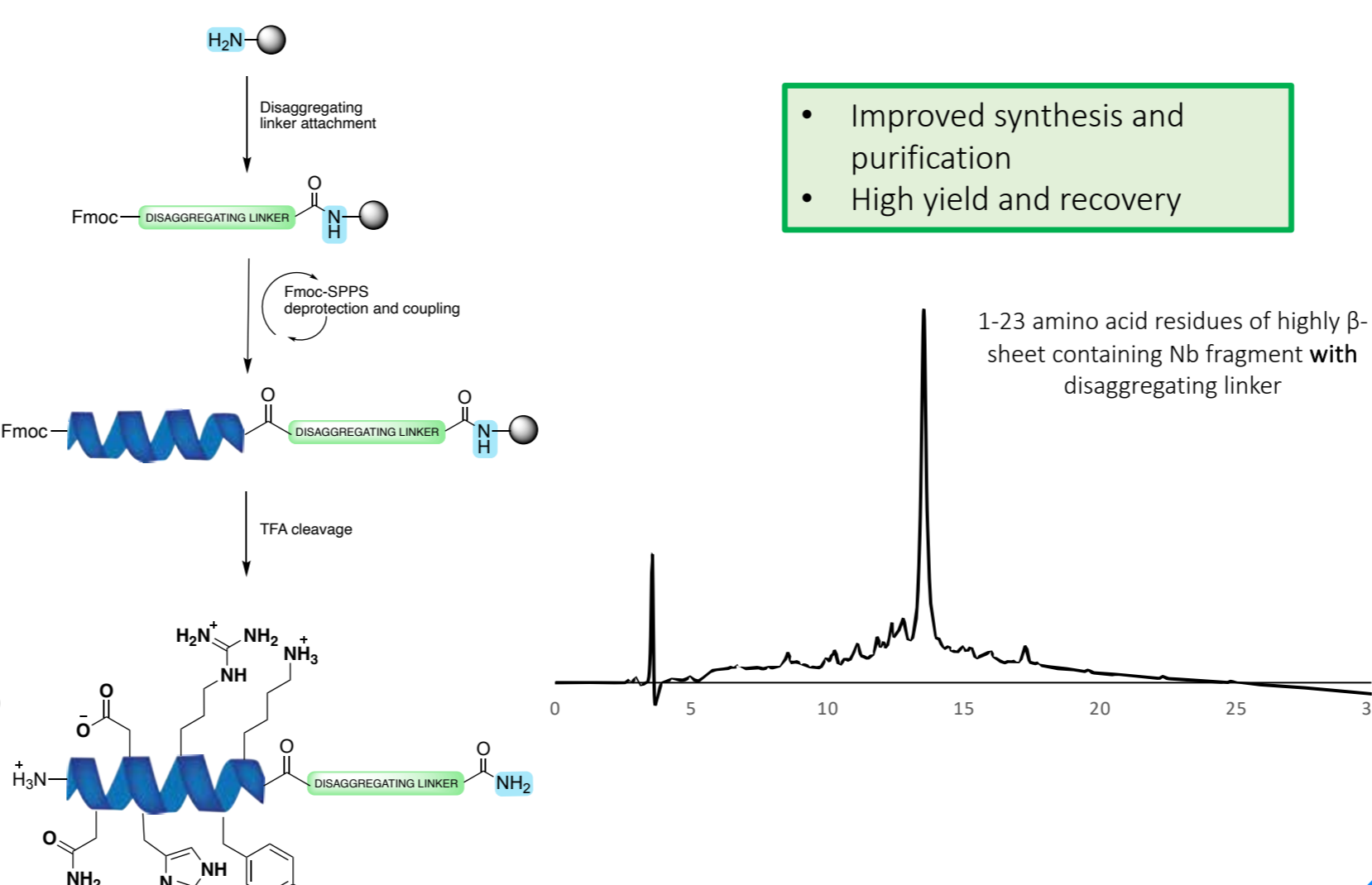
- Challenging synthesis and purification
- Low yield and recovery
- Inability to track via analytical HPLC and ESI-MS

## Future:

### Standard SPSS



### Our methodology



## Conclusion:

In summary, synthetic access to Nbs holds major significance in the field of cancer therapeutics and diagnostics.

Our work shows contribution to the field thus towards the total chemical synthesis of Nbs.

In future, we hope to develop a novel and robust synthetic route to produce nanobodies.

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