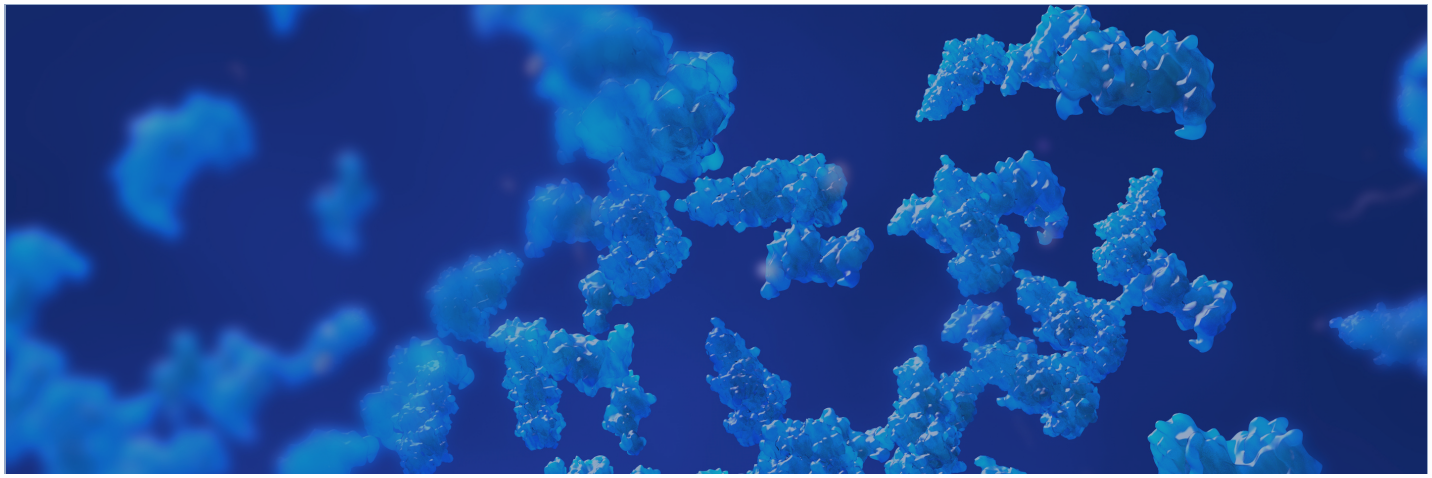
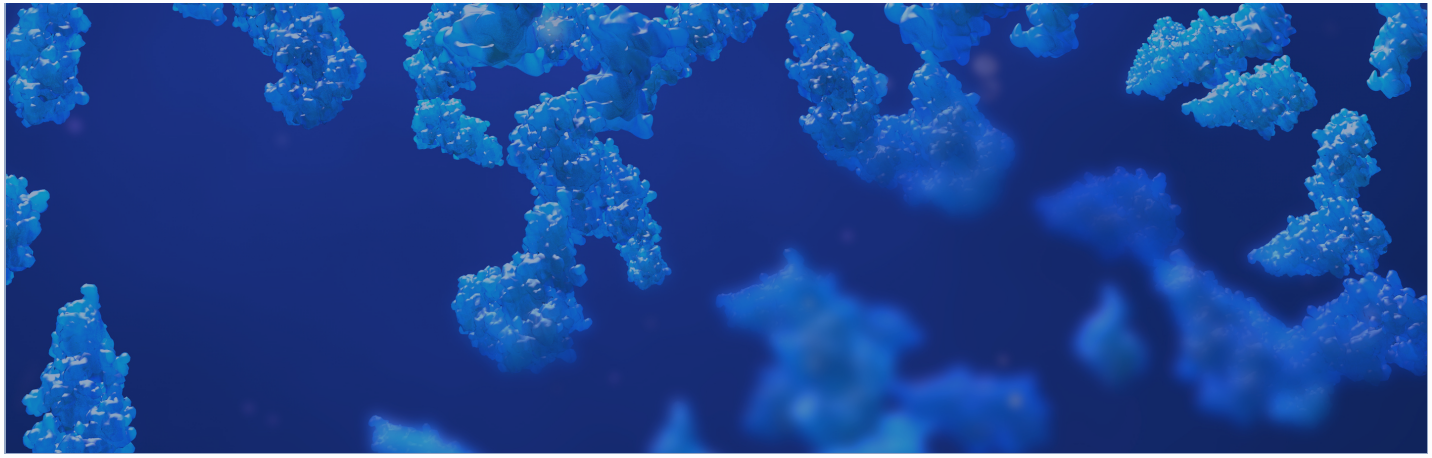


Case Study

IFN α-2a

X'PROCHEM



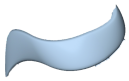
Context

Interferon Alpha-2a (IFN α-2a) is a type 1 interferon composed of 165 amino acid residues, a medium-sized therapeutic protein traditionally produced by recombinant technology. This cytokine is widely used for its antiviral and antineoplastic properties and requires high quality and purity production.

Objectif : We wanted to produce pure and perfectly structured IFN α-2a in a 10 mg scale for a client.

Synthesis Strategy

1



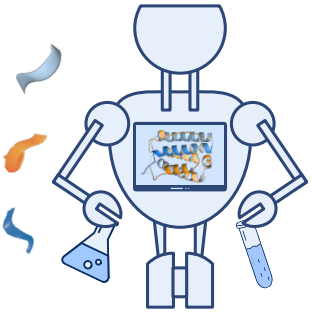
IFN fragment 1

IFN fragment 2

IFN fragment 3

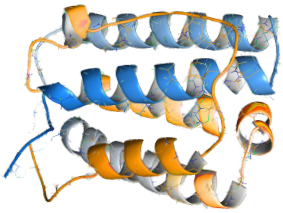
Design the desired INF α-2a and synthesise 3 short fragments by classical SPPS with our proprietary resin.

2



Assembly the 3 peptides fragments by our proprietary technology in a one-shot reaction to produce a linear IFN α-2a. Purification by RP-HPLC, IEX, or Size Exclusion Chromatography

3

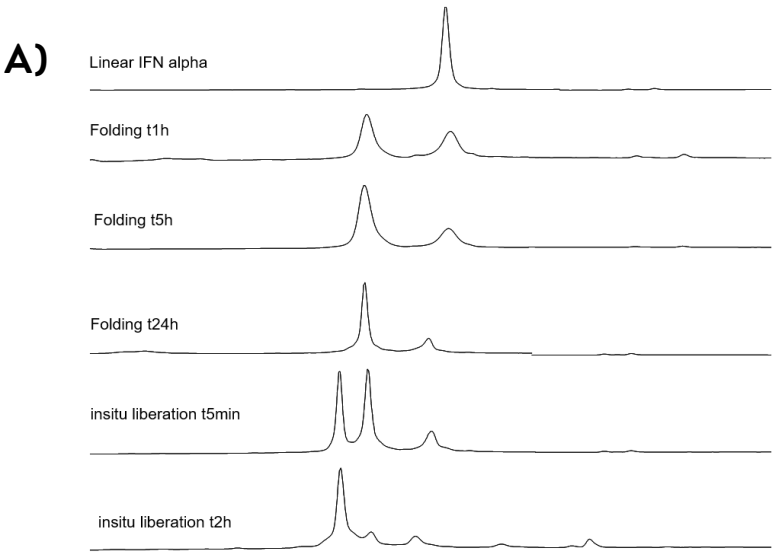


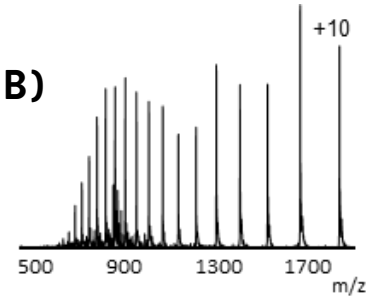
IFN α-2a (165 AA)

Folding step to make the protein biologically active.

Semi-controlled Folding

Using sequence modification of the protein, we have achieved highly efficient folding in solution, leading to high yields and biological activity. After completion of the folding step, the release of the native folded protein was performed by in-situ processing.





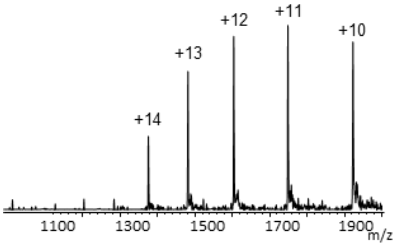
Semi-controlled folding of IFN α-2a

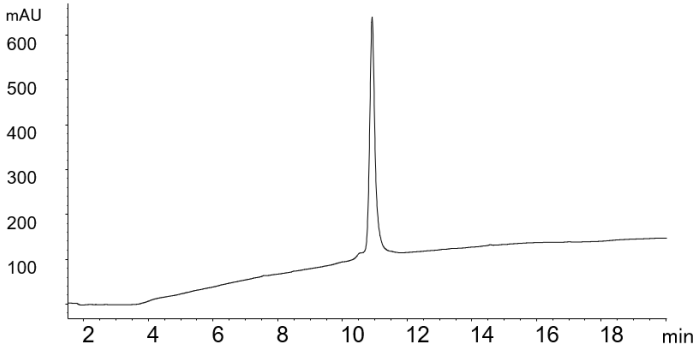
A) MS Spectra of linear protein, Calculated [M+H]+ = 19237.17; Observed [M+H]+ = 19237.15.

B) UPLC analysis at 215 nm

Caracterization

We analysed the protein to ensure optimal purity and the correct structure of IFN α-2a. A QA/QC was performed to ensure that the protein was more than 95% pure.





Purified IFN α-2a analysis

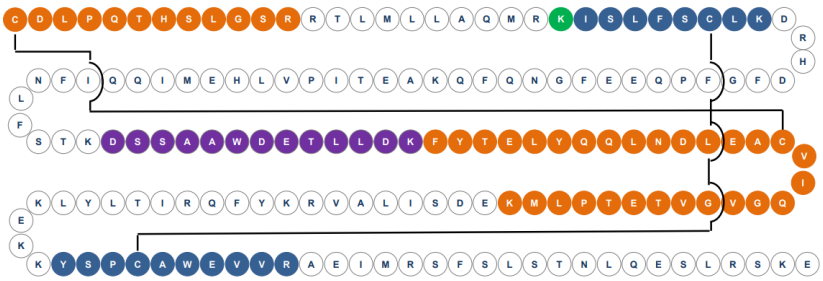
C) MS spectra, Calculated [M+H]+ = 19237.17; Observed [M+H]+ = 19237.15

D) UPLC analysis at 215 nm

Disuflide bridge elucidation

IFN α-2a is known to have 2 disulfide bridges: Cys1-Cys98 and Cys29-Cys138. We demonstrated the correct cysteines by enzymatic digestion (trypsin) and UHPLC/mass spectrometry analysis of the resulting fragments.

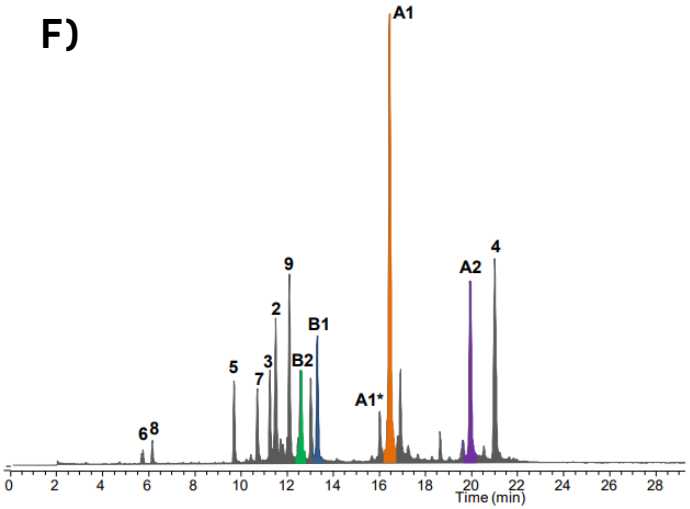
E)



IFN α-2a structure

E) Disulfide bridges localization.
- 2 disulfides bridges
- 168 AA

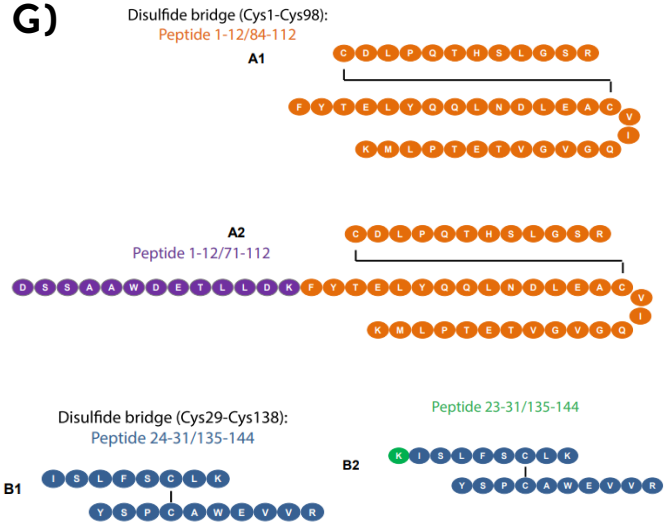
F)



Analysis of IFN α-2a by LC-MS

F) UHPLC analysis of an enzymatic digestion of IFN α-2a. Peaks 1 to 9 represent linear non linked fragments. Peaks A1,A2,B1 and B2 represent branched liked fragments

G)



Linked peptide fragments identification

IFN α-2a was enzymatically cleaved. The fragments was separated and identified by UHPLC/Mass. Identity of fragments was determined and correct disulfides bridges confirmed.