UNIVERSITÀ DEGLI STUDI FIRENZE

PeptLab
INTERDEPARTMENTAL RESEARCH UNIT OF PEPTIDE AND PROTEIN CHEMISTRY AND BIOLOGY

iCCOM Istituto di Chimica dei Composti Organometallici
Consiglio Nazionale delle Ricerche

Design, synthesis, and biological activity of tumor necrosis factor-alpha peptides for adalimumab recognition

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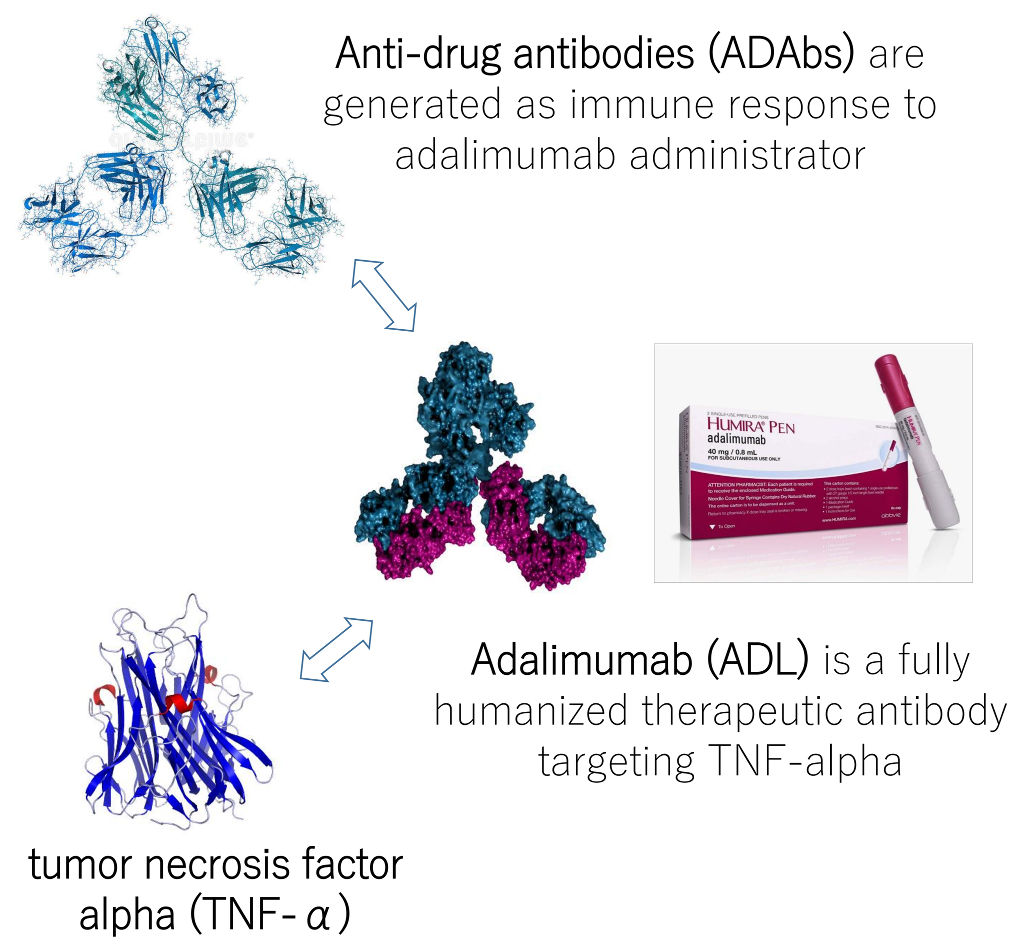
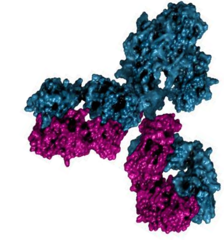
Biologics targeting the **tumor necrosis factor alpha (TNF-α)** have revolutionized treatments in a wide variety of diseases, including juvenile idiopathic arthritis (JIA). Unfortunately, a significant proportion of patients fail to reach any therapeutic response owing to drug ineffectiveness (primary failure) and/or acquired therapeutic resistance eliciting anti-drug antibodies (ADAbs) as immunoresponse (secondary failure) [1]. Despite immunogenicity drawbacks have been addressed by using fully human antibodies as **adalimumab (ADL)**, this is still an open issue. Monitoring both ADL and anti-drug antibodies is fundamental in the follow-up of patients.



TNF-α sequence uniprot code P01375 (TNFA_HUMAN)

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFL
IVAGATTLFCLLHFGVIGPQREFPRDLSLISPLAQAVRSSS
RTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVEL
RDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRI
AVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGG
VFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL

Aim of the project:
Set up a new method to monitor ADL using TNF-α peptides

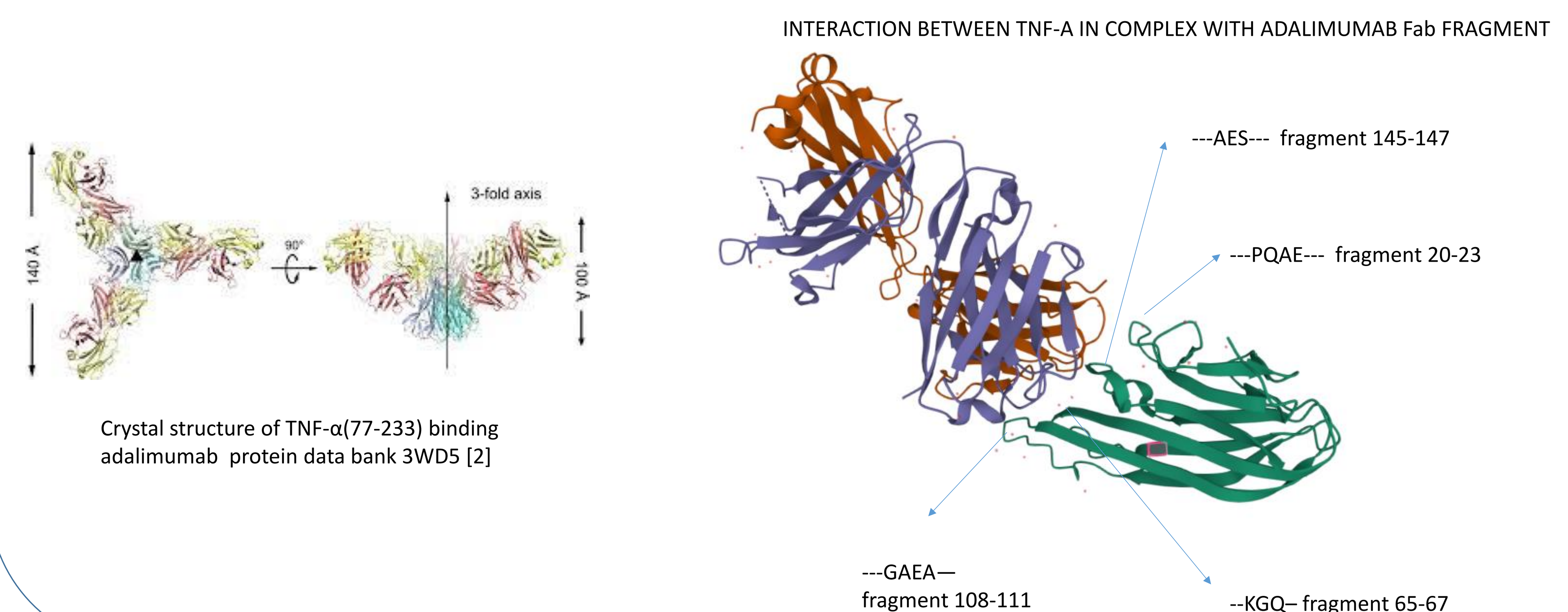


During ADL treatment, the symptoms of the autoimmune disease can re-emerge in a percentage of patients due to two possible reasons: anti-drug antibodies formation and decreasing drug concentration in the blood. In this context, adalimumab concentration quantification becomes necessary during treatment monitored together with ADAbs presence. Up to now ADL quantification is performed using TNF-α or monoclonal antibodies against ADL as antigens. The use of peptides mimicking the appropriate epitopes of the TNF-α could display enhanced ADL recognition specificity eliminating or minimizing potential cross-reactivity between structurally homologous or non relevant protein epitopes improving detection. With all these considerations in mind, the aim of the project is the design and synthesis of TNF-α peptides to be used as antigens in solid-phase ELISA and surface plasmon resonance for ADL quantification.

TNF-α and adalimumab interaction

The interaction between TNF-α and the light and heavy chains of the Adalimumab is stable and strong because of the hidrogen bonds. Hu et al. previously described the interaction identificating Adalimumab conformational epitope on TNF-α, the binding sites are composed of four discontinuous segments [2].

INTERACTION BETWEEN TNF-A IN COMPLEX WITH ADALIMUMAB Fab FRAGMENT



Crystal structure of TNF-α(77-233) binding adalimumab protein data bank 3WD5 [2]

Peptides design and synthesis

1.- Chimeric strategy

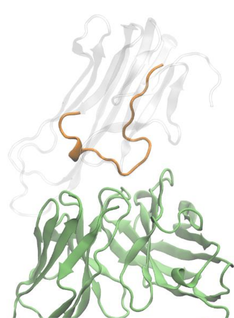
A chimera with the fragments 20-23 and 145-147 maintaining the distances between the two residues of the TNF-α has been designed. Both peptides were selected using Chembio3D Ultra by calculating the number of amino acids between the proline (P,20) of the fragment 20-23 and the serine (S, 23) of the fragment 145-147 to maintain the real distance in the adalimumab-TNF-α complex of 9,1 Å. Finally, it was decided to lengthen the sequence of the fragments as necessary, resulting two different peptides:

TNF-α-(17-26)-(142-150) → VANPQAEGQLLDFAESGQV

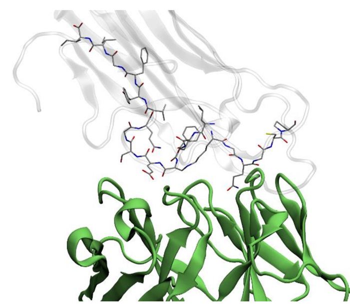
TNF-α-(15-28)-(140-150) → HVVANPQAEGQLQDYLDFAESGQV

2.- Molecular modelling strategy

A linear peptide on TNF-α C-terminal targeting the H chain of adalimumab has been designed (PeptC).



A new peptide trying to maximize the binding interation site has been designed, targeting both the L and H chains of ADL (FUSpept)



PeptC → INRPDYLDFAESGQVYFGIIAL

FUSpept → KGQGCPLNFAESGQVYFGIIAL

Synthesis of peptides were performed by using solid phase peptide synthesis in manual combined with microwave assisted synthesis following the Fmoc/tBu strategy, starting from 200 mg of Wang resin.

Analytical characterization of peptides

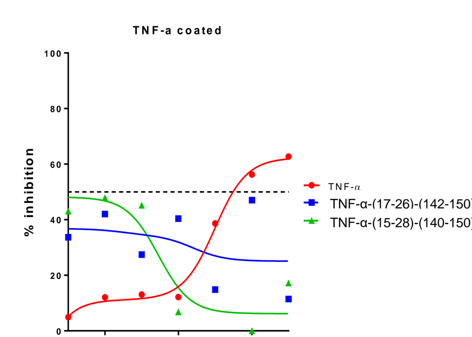
Peptide	Retention time (min)	HPLC gradient B (%)	ESI-MS (m/z) $[M+2H]^{2+}$ found (calc.)	Molecular weight (g/mol)
TNF-α-(17-26)-(142-150)	1,48	30-90	987,57 (987,54)	1791.96
TNF-α-(15-28)-(140-150)	3,43	20-80	1308,91 (1309,27)	2615.20
PeptC	2.92	35-55	1252 (1251.13)	2501.78
FUSpept	3.05	35-60	1157 (1156.59)	2312.64

Peptides were purified by Reversed-Phase Flash Liquid Chromatography on a Biotage® IsoleraTM (Biotage, Uppsala, Sweden) and Semi-preparative RP-HPLC on a Waters 600 chromatograph with solvent system A (0.1% TFA in H_2O) and B (0.1% TFA in CH_3CN). The purity of the peptides was analyzed by analytical HPLC using a Waters HPLC coupled to a single quadrupole ESI-MS (Waters ZQ Mass Detector)

Biological experiments

All peptides were tested in solid phase ELISA as antigens for adalimumab identification. Unfortunately, no significance signals were observed. We strongly hypothesized that binding sites of peptides were also involved in the coating to the ELISA plate. For this reason, inhibition ELISA experiments and surface plasmon resonance binding experiments will be proposed.

First inhibition experiments with peptides TNF-α-(17-26)-(142-150) and TNF-α-(15-28)-(140-150) showed no inhibitory activity for both peptides, whereas TNF-α was able to inhibit adalimumab interaction in solution.



Conclusions

- A series of peptides reproducing binding sites of TNF-α have been design and proposed for adalimumab identification.
- Peptides have been successfully synthesized and purified by solid phase peptide synthesis following the Fmoc/tBu strategy.
- Peptides were not able to identify adalimumab in solid phase ELISA used as antigens. A second round of molecular modelling calculations will be performed.
- You can find more details of adalimumab quantification using surface plasmon resonance visiting the poster number P2.134.

References

[1] Real-Fernández, F. et al. *Analytical and bioanalytical chemistry* (2015):407.24, 7477-7485 [2] *Hu, S., Liang, S.Y., Guo, Y.J., Lou, Z.Y. (2013) J Biol Chem 288: 27059-27067*

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