Exploring the potential of CXCR4 mimetic peptides to target cancer cells

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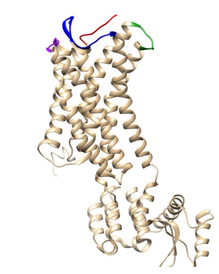
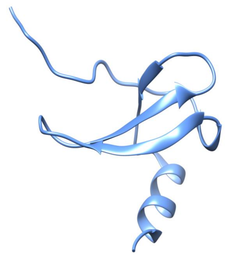
The chemokine receptor CXCR4 plays crucial roles in various physiological processes and is implicated in diseases such as HIV-1 infection and cancer[1]. In cancer, the interaction between CXCR4 and the chemokine CXCL12 has been shown to stimulate multiple oncogenic processes[2]. In HIV-1 infection, CXCR4 serves as a coreceptor for viral entry into the host cell[3]. Previously, we designed a peptide that presents the three extracellular loops (ECLs) of CXCR4, which was shown to inhibit both HIV-1 infection[4] and CXCR4 signaling[5]. We have now optimized the sequence of this peptide, CX4M1, to enhance its affinity for CXCL12 by truncation, among other modifications. The optimized CX4M1 variants bind to CXCL12 with higher affinity while being considerably shorter than the original peptide.

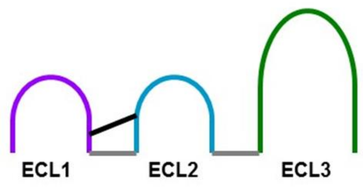
These peptides have been shown to interfere with the migration of CXCR4-dependent cancer cells and inhibit the binding of CXCL12 to CXCR4-expressing cancer cells.



CXCR4
PDB ID: 3ODU



CXCL12
PDB ID: H3P3



CX4M1

Results & Discussion

Design & Optimization

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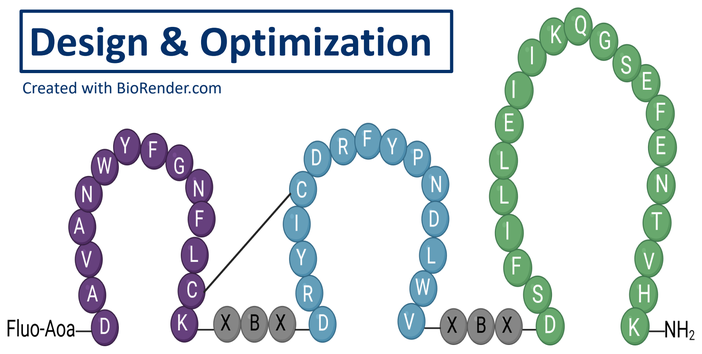
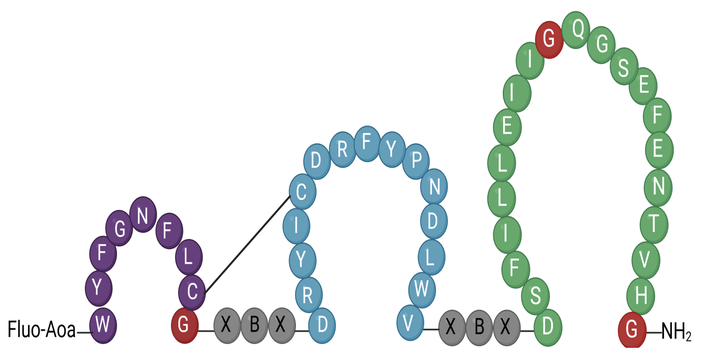
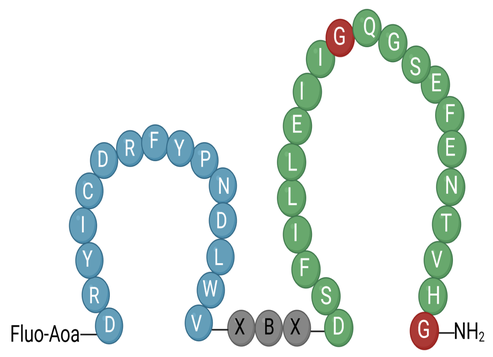


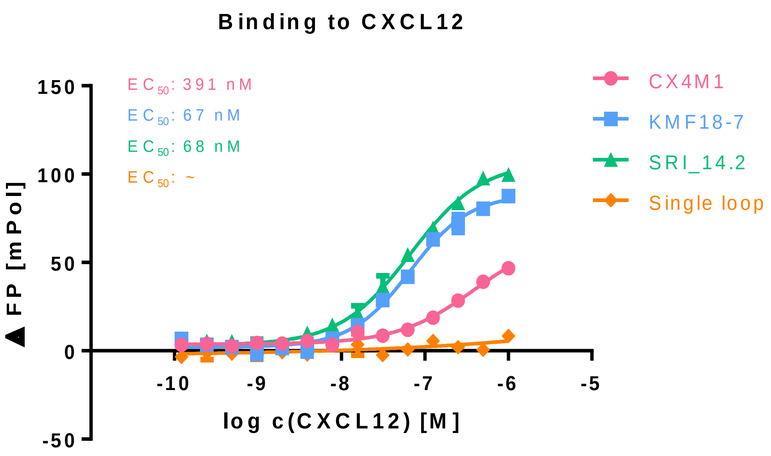
CX4M1



KMF18-7 (-5N)

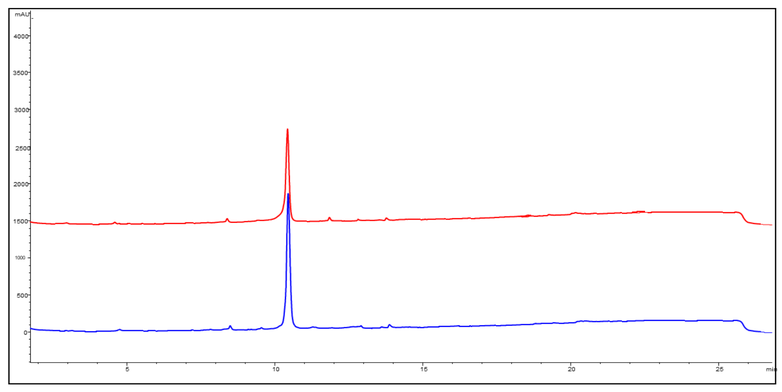


SRI_14.2 (-ECL1)



- Replacement of basic amino acids by glycine & N-terminal truncation up to 5 amino acids (**KMF18-7**)
- Truncation of ECL1 (**SRI_14.2**) binding affinity towards CXCL12 is preserved

Proteolytic stability of KMF18-7



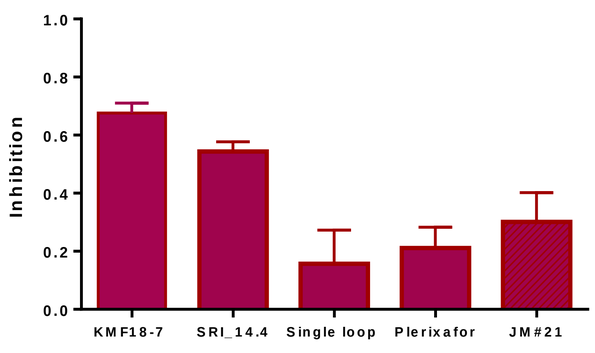
KMF18-7 after 0 h (blue) and 24 h(red) incubation with human serum

- KMF18-7 exhibited significant resistance to enzymatic degradation in human serum → can be tested in cell-based Assays

Investigation of Cancer Cell processes

Disruption of CXCL12-Binding to CXCR4

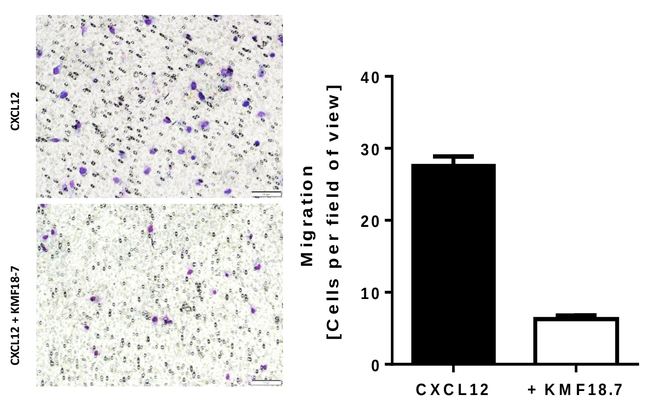
Via FACS



- CXCR4-expressing cells were incubated with CXCL12-Fc, CXCR4 mimetic peptides, Plerixafor[6]and JM#21[7] (each at 1 µM) and analyzed via FACS
- KMF18-7 and SRI_14.2 strongly disrupted binding of CXCL12 to CXCR4

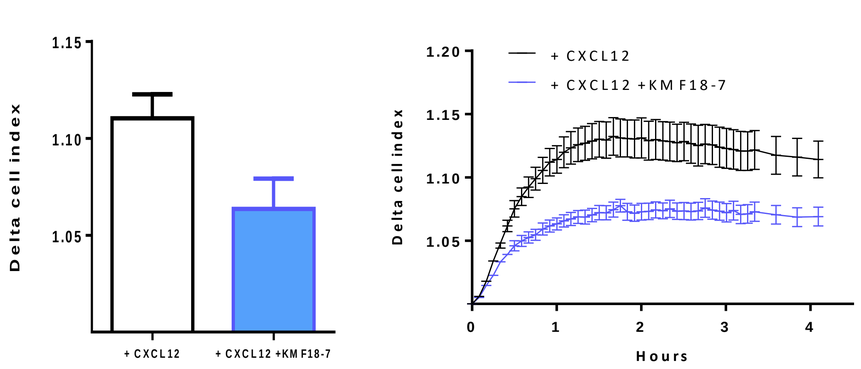
Inhibition of Migration

Via Boyden chambers



- Cancer cell migration was analyzed using Boyden chambers
- CXCR4-expressing cells were seeded in the upper chamber
- CXCL12 and a CXCR4 mimetic peptide were added to the lower chamber
- The migration duration was 4 hours

Via Real-Time Cell Analysis (RTCA)



- Spreading and attachment of cells were detected using RTCA and expressed as the cell index (CI) value
- CXCR4-expressing cells were stimulated with CXCL12, leading to increased cell spreading and attachment
- This response was reduced by the CXCR4 mimetic peptide

Outlook

- Further truncation to find minimal active peptide length
- Investigating the CXCR4 mimetic peptides in an *in vivo* model
- Introducing the N-terminal tail as a CXCR4 mimetic peptide → testing in cancer cell based assays

References

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