Assembly of Helical Chimeras with Natural Peptides: Simulating Molecular Interaction Surfaces

J. Mortier[1]*, E. K. Nyakatura[1], R. R. Araghi[1], V.S. Radtke[1], S. Wieczorek[1], C. Baldauf [2], G. Wolber[1], B. Koksch[1]

[1] *Department of Biology, Chemistry and Pharmacy, Freie Universität Berlin, Germany* - [2] *Fritz-Haber-Institute der MPG, Berlin, Germany* - * *jeremie.mortier@fu-berlin.de*

Motivation

Foldamers with specific biological activities as modulators of protein-protein interactions

The α-helix is an abundant structural motif found at the interface of important intracellular and pathogenic protein-protein interactions. Foldamers that closely resemble both protein folding and function can be designed using β- and γ-amino acids that can mimic the self-assembly of native peptides (**Fig. 1**). [1] However the precise mimicry of side chain topology and recognition properties of natural α-helical sequences remains challenging. Understanding interactions created by such residues with natural amino acids in the context of native protein folds may yield important information that can facilitate the prediction of the properties of novel non-natural peptide therapeutics or biomaterials.

Background

Empirical search for high-affinity binders to an αβγ-chimeric sequence

The designed Acid-pp/Base-pp tetramer tolerates the substitution of the central heptad in Base-pp by a pentad of alternating β- and γ-amino acids (**Fig. 2**), [2] although a loss in thermal stability is observed when compared to that of its parental system. Ideal core packing with matching side-chain composition and geometry between a βγ-foldameric binding groove and α-peptides were searched. Using phage display, the four central heptad positions of Acid-pp that directly interact with the βγ-segment of the chimeric B3β2γ peptide were randomized (**Fig. 3**), with parallel and anti-parallel orientation.

Aim

Understanding of the intermolecular interactions stabilizing the new systems

The aim of this study was to investigate the selected peptides that assembled with the chimeric sequence using MD. These simulations were conducted for up to 300 ns using the Gromos 53a6 force field implemented within the Gromacs Suite.

Base-pp L S A L K E K L A S L K E K L S A L K E K L A S L K E K L S A L K E K

B3β2γ L S A L K E K L A S L K E K βL γD βL γK βK L A S L K E K L S A L K E K

H_2N- a b c d e f g a b c d e f g a b c d e f g a b c d e f g a b c d e f g -OH

Residue number 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 23 22 23 24 25 26 27 28 29 30 31 32 33 34 35

H_2N- a' b' c' d' e' f' g' a' b' c' d' e' f' g' a' b' c' d' e' f' g' a' b' c' d' e' f' g' -OH

Acid-pp L S A L E K E L A S L E K E L S A L E K E L A S L E K E L S A L E K E

↑ **Fig.3**: Sequences with substituted heptad of Base-pp (grey) and randomized sites in Acid-pp (black)

Results

Unusual interactions stabilizes the hetero-assembly of αβγ-chimeras with natural peptides

Parallel phage display experiment led to the selection of a Acid-pp variant comprising one cysteine that significantly improves coiled-coil core packing through the formation of an interhelical H-bond with a backbone carbonyl of the αβγ-chimera (**Fig. 4**). [3]

parallel orientation

Acid-ICEF L S A L E K E L A S L E K E I S A C E K F L A S L E K E L S A L E K E

Four sequences evolved from anti-parallel phage display experiments (**Fig. 5**). [4] In two of these peptides (Acid-LLLE and Acid-LFYE), the hydrophobic core was extended to positions that are usually designated for charged residues (**Fig. 6**). The remaining two sequences harbor charged amino acids in positions that were originally designated for hydrophobic residues. MD simulations of selected bundles revealed that the extension of the hydrophobic core can significantly contribute to the stability of tetrameric systems, while additional polar or ionic interactions might facilitate the formation of dimeric coiled-coil bundles that contain alternating β- and γ-amino acids.

anti-parallel orientation

Acid- LLLL L S A L E K E L A S L E K E L S A L L K L L A S L E K E L S A L E K E

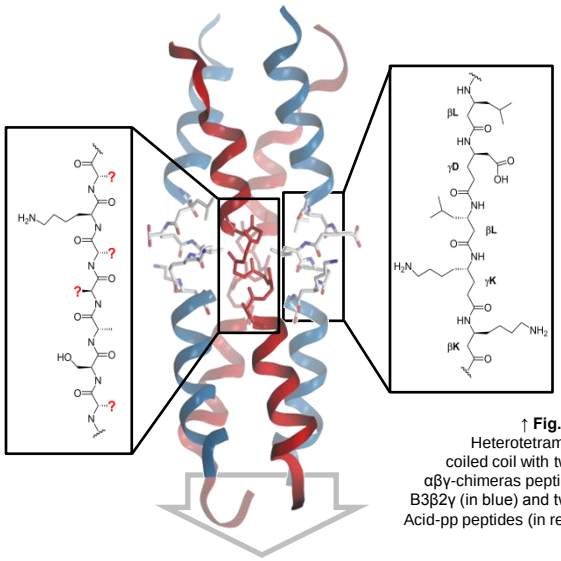
Acid-MTER L S A L E K E L A S L E K E M S A T E K R L A S L E K E L S A L E K E

Acid-HCAN L S A L E K E L A S L E K E H S A C A K N L A S L E K E L S A L E K E

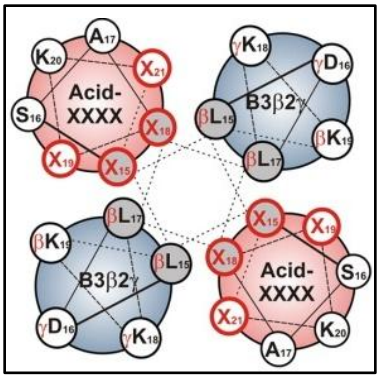
Acid-LFYL L S A L E K E L A S L E K E L S A F Y K L L A S L E K E L S A L E K E

↑ **Fig. 5**: Acid-pp sequences selected by phage display with highlighted randomized positions (black)

→ **Fig. 6**: Intermolecular interactions with the side chain of 19Y from Acid-LFYL and the B3β2γ residues 18γK (π-cation, in blue) and 20L (H-bond, in orange)

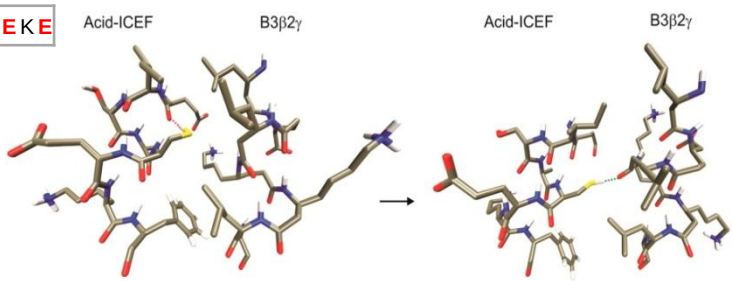


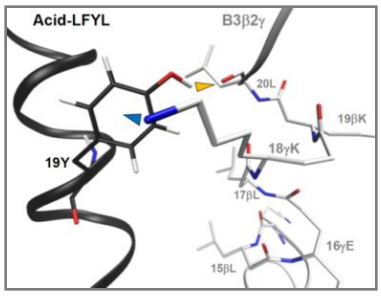
↑ **Fig. 1**: Heterotetramer coiled coil with two αβγ-chimeras peptide B3β2γ (in blue) and two Acid-pp peptides (in red)



← **Fig. 2**: Helical view of a tetramer with βγ-pentads (red) and interacting heptads of two Acid-pp (blue), in which randomized positions are marked as X's.

↓ **Fig. 4**: Snapshots showing one Acid-ICEF and one B3β2γ strand after 5 ns (left) and 65 ns (right) of simulation - red dotted line depicts intrahelical SH Cys18–CO Glu14 H-bond and green dotted line depicts interhelical SH Cys18–CO βL17 H-bond





Conclusion

We undertook a broad survey for peptides that specifically match an αβγ-chimera in a coiled-coil assembly. Some bound the chimeric sequence with high thermal stability. MD showed that the presence of the Cys side chain can significantly influence the core-packing of chimeric coiled coils through interhelical H-bond with the αβγ-chimera.

Our results also indicate that a modification of the classical interaction features with polar side chains allows for the fine-tuning of affinities and specificities of coiled-coil bundles. Thus, this systematic study provides a knowledge base for the rational design of peptide based pharmaceuticals targeting helical protein interfaces.

References

1. Baldauf, C; Gunther, R; Hofmann, HJ, *J Org Chem* **2006**, *71* (3), 1200.
2. Rezaei Araghi, R, Jäckel, C, Cölfen, H, *et al.* ChemBioChem **2010**, 11, 335.
3. Nyakatura, EK; Rezaei Araghi, R; Mortier, J *et al., ACS Chem Biol* **2014**, 9 (3), 613
4. Nyakatura, EK; Mortier, J; Radtke VS *et al., manuscript in preparation.*