Computer-Aided Design of Diubiquitin-binding Foldamers

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Abstract

With the computational tools developed in our lab for accurate prediction of structures and solution dynamics of aromatic foldamers, we now target protein-protein interaction (PPI) by appending proteinogenic side chains on the external surfaces of the helical aromatic foldamers. Particularly, the activation of a regulatory protein NF-κB, a key player in the inflammatory response and cell proliferation, involves binding of NEMO (NF-κB Essential Modulator) with linear diubiquitin. In this study, we designed various sequences based on established protocols and investigated, by utilizing molecular dynamics (MD) simulations, their potential binding to diubiquitin, specifically targeting the NEMO binding interface. This study complements experimental works related to synthesis, characterization, and crystallization of aromatic foldamers. Here, we present structural analysis of MD trajectories from simulations of 21 aromatic foldamers/diubiquitin systems with explicit solvent (aqueous) to gain insight into the binding interaction.

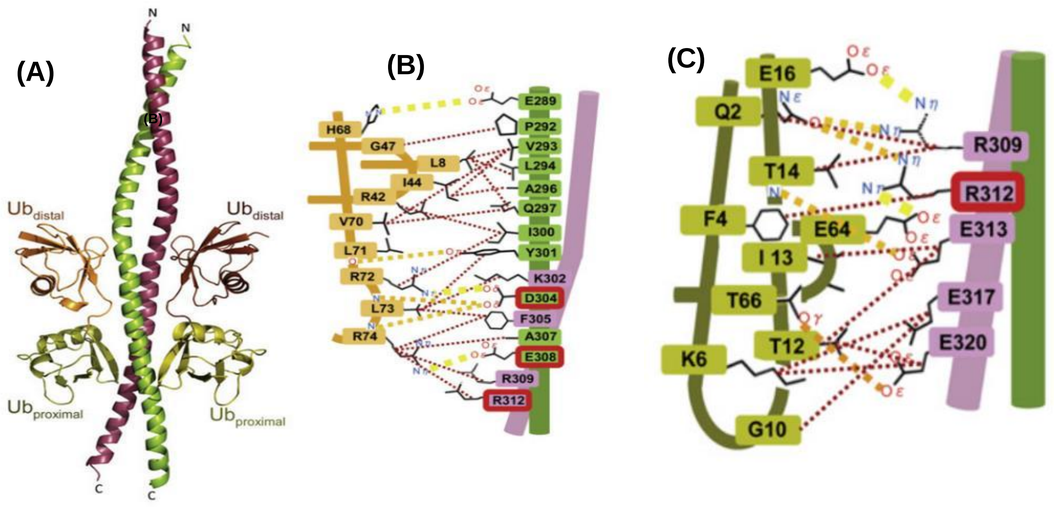

Figure 1. **(a)** Structure of the NEMO-diubiquitin complex and schematic diagrams of the interactions of **(b)** Ub-distal, and **(c)** Ub-proximal with NEMO.

Methods

Quinoline based aromatic foldamer residues (Figure 3) with various side chains were created by following previously developed protocols. Multi-conformational RESP charge fitting was used. Torsional parameters for rotations around arylamide bonds were minimized and optimized to ensure accuracy of the simulation. Force fields used are: GAFF2 with modified aryl-amide torsional parameters, the ff19SB version of AMBER force field for proteins, and OPC isometric water model. All MD simulations were performed using the AMBER22 Package.

Initial structure is created by aligning each helical foldamer along the protein interacting surface. Each system is solvated with explicit water in a periodic box (~80Å each side). Equilibration of the system includes minimization of solvents, heating, and NPT simulation to achieve the right density of system. Each system was then simulated at 300K in an NVT ensemble for 500 to 800 ns for analysis.

A disulfide linkage was established between residue 1 of the foldamer and residue 68 of the protein. Our research involves targeting ubiquitin and subsequently linear diubiquitin by mimicking the α-helical surface of NEMO using functionalized AOFs. During analysis, the central focus was on hydrogen bond, salt bridge, and hydrophobic interaction between foldamer side chains and the diubiquitin protein surface.

Foldamer Design Principle for Foldamer/Protein Interaction

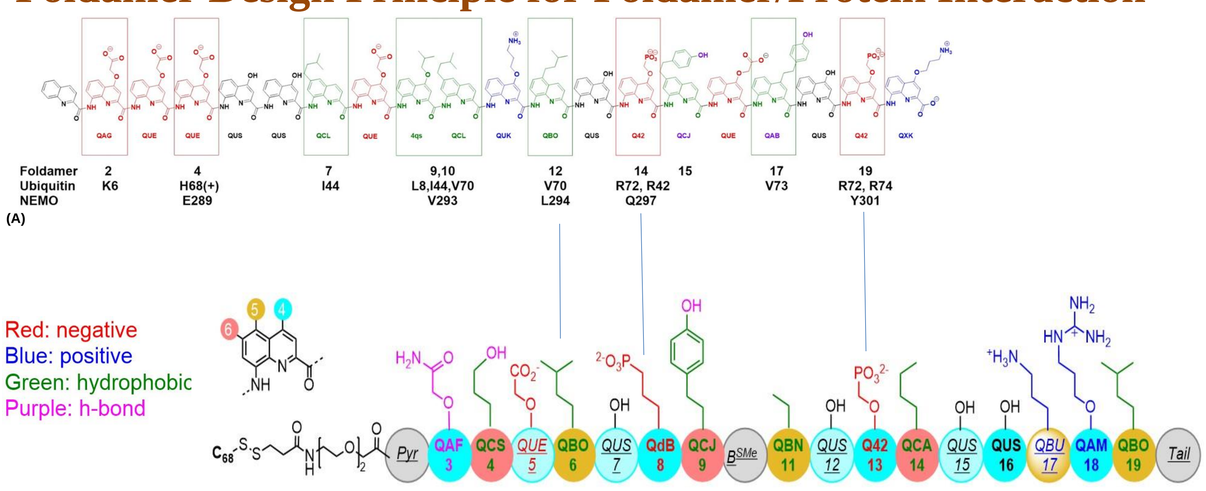

Figure 2. **(a)** Sequence of previously designed diubiquitin-binding aromatic foldamers. Residues in boxes are on the interacting side of the structure. The colors Red, Blue, Green and Purple indicate negative, positive, hydrophobic and hydrogen-bonding side chains. **(b)** Sequence of the new foldamer, showing the position of the side chain on the quinoline structure

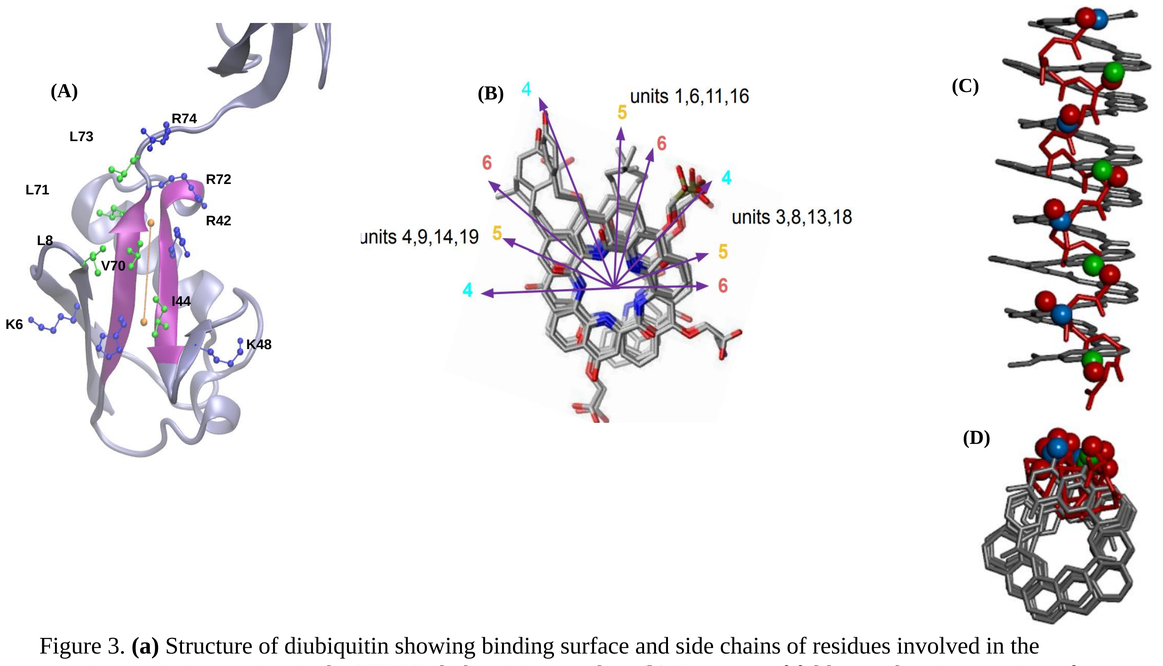

Figure 3. **(a)** Structure of diubiquitin showing binding surface and side chains of residues involved in the protein-protein interaction in the NEMO-diubiquitin complex. **(b)** Structure of foldamer showing topview of the side chains **(c)** Aromatic foldamers as α-helix surface mimetics **(d)** Overlay of the side chains of aromatic foldamer and an ideal 3.66_{13} α-helix with an RMSD of 1.50 Å.

Results and Discussion

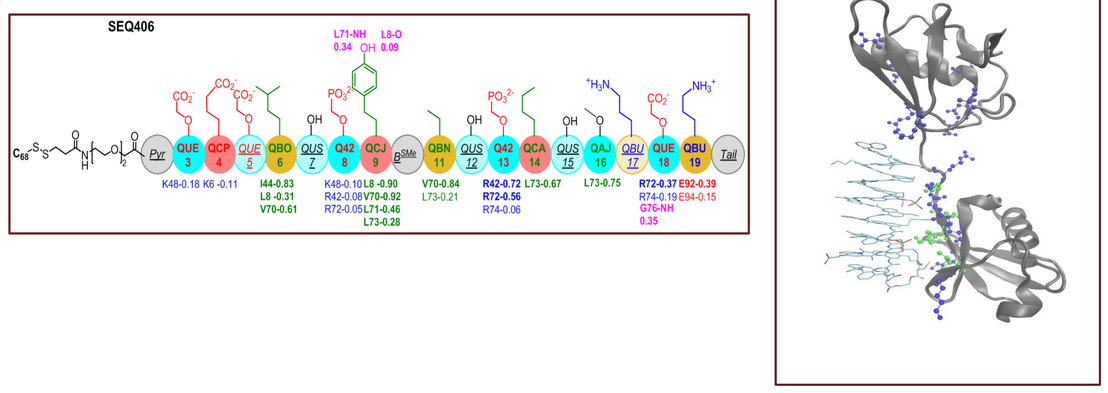

Figure 4a. Left: Snapshot of foldamer sequence 406 interacting with diubiquitin.
Right: Structure of sequence 406 showing Salt bridges (Green), hydrophilic (Blue), and hydrogen bond (Purple) interaction between the foldamer side chains and the amino acid residues of the protein.

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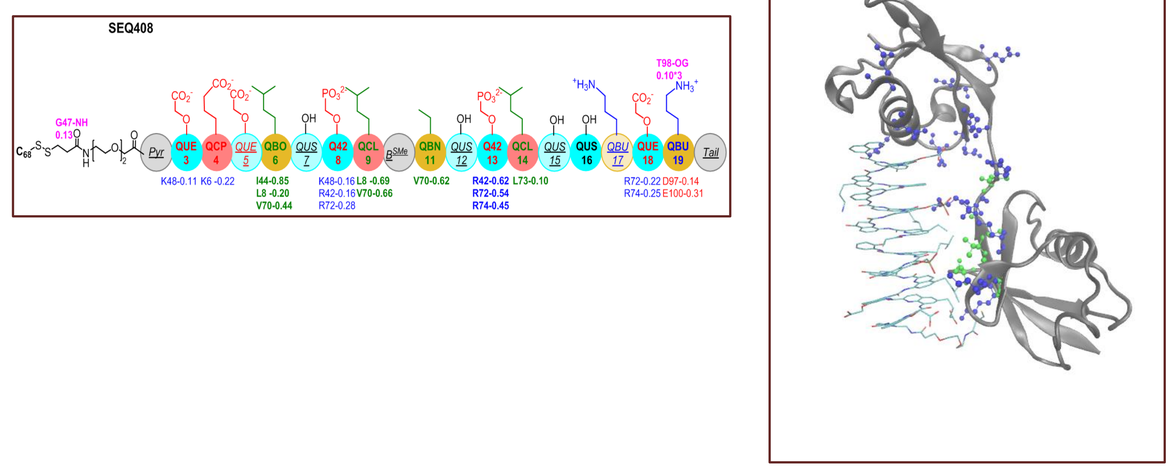

Figure 4b. Left: Snapshot of foldamer sequence 408 interacting with diubiquitin.
Right: Structure of sequence 408 showing Salt bridges (Green), hydrophilic (Blue), and hydrogen bond (Purple) interaction between the foldamer side chains and the amino acid residues of the protein.

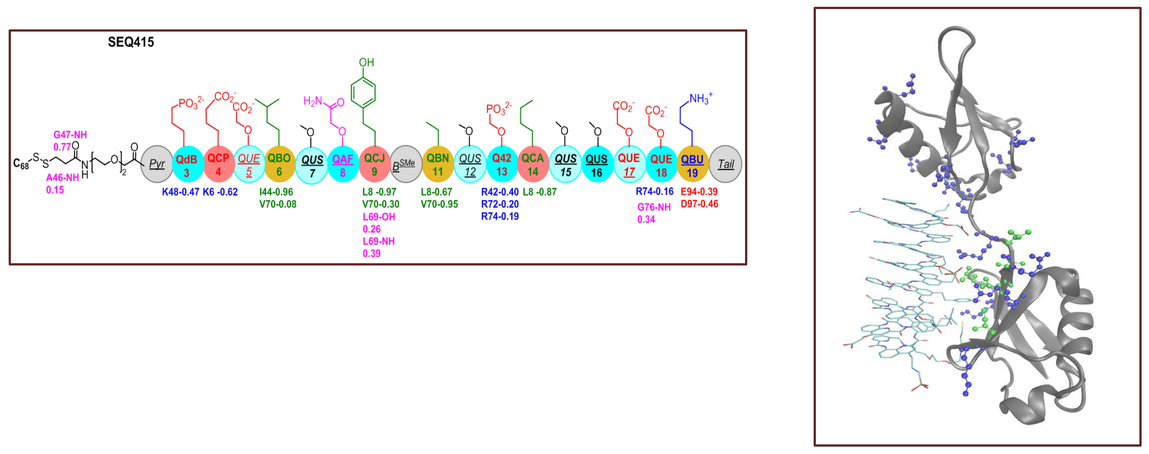

Figure 4c. Left: Snapshot of foldamer sequence 415 interacting with diubiquitin.
Right: Structure of sequence 415 showing Salt bridges (Green), hydrophilic (Blue), and hydrogen bond (Purple) interaction between the foldamer side chains and the amino acid residues of the protein.

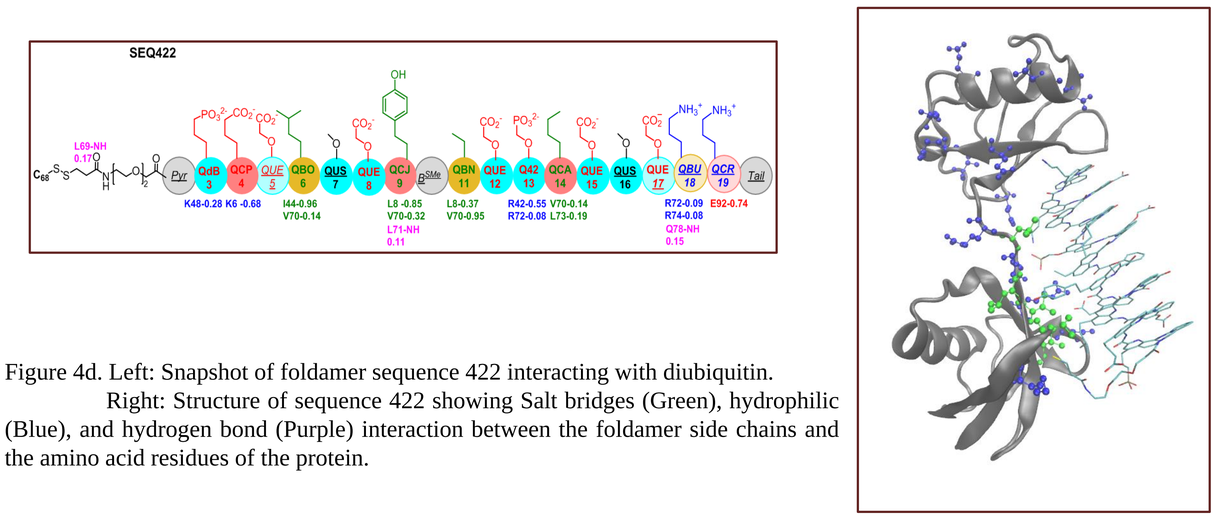

Figure 4d. Left: Snapshot of foldamer sequence 422 interacting with diubiquitin.
Right: Structure of sequence 422 showing Salt bridges (Green), hydrophilic (Blue), and hydrogen bond (Purple) interaction between the foldamer side chains and the amino acid residues of the protein.

Conclusion – Work in Progress

Sequences 406, 408, 415, and 422 showed the best interaction with the diubiquitin out of the 21 sequences designed. The disulfide linkage that was established between resid 68 of the protein and resid 1 of the foldamer, and other modifications to the foldamer sequence allowed for better protein-foldamer interaction compared to the previously designed foldamer sequences. These sequences helped us gain more insight into the interaction between the protein and the foldamer, and also provide a very base for further optimization of the sequence.

From this analysis, we will continue to optimize the design and find more foldamer sequences that are able to form salt-bridge, hydrophobic interactions, as well as hydrogen bonds.

Reference

(1) Rahighi, S.; Ikeda, F.; Kawasaki, M.; Akutsu, M.; Suzuki, N.; Kato, R.; Kensche, T.; Uejima, T.; Bloor, S.; Komander, D.; et al. Specific recognition of linear ubiquitin chains by NEMO is important for NF-kappaB activation. *Cell* **2009**, *136* (6), 1098-1109. DOI: 10.1016/j.cell.2009.03.007.