Molecular Dynamics Study of Disulfide Bond Stability and Surface Modification in InterLeukin-4 (IL-4)

E. G. S. H. Gamage[1], B. L. A. Perera[2], K. M. N. de Silva[1]

1. Department of Chemistry, University of Colombo, Sri Lanka 2. Division of Genetic Medicine and Clinical Pharmacology, Department of Medicine,Vanderbilt University Medical Center, Nashville, TN, USA

Introduction

- Interleukin-4 (IL-4) is a key immunoregulatory cytokine involved in both adaptive and innate immune responses.
- IL-4 Topology: A regulatory cytokine with 129 residues featuring a compact, left-handed four-helix bundle arranged in an up-up-down-down topology.
- IL-4 has three distinct disulfide bonds:
  - Cys3–Cys127: link between N- and C-termini.
  - Cys24–Cys65: anchoring the AB and CD loops.
  - Cys46–Cys99: essential link for proper protein folding.
- Disulfide bonds play a critical role in protein folding and stability, yet the mechanisms that compensate for the loss of these covalent stabilizing interactions and whether alternative stabilization strategies can be engineered remain poorly understood.

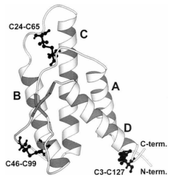


Figure 1. Structure of Interleukin-4[1]

Objectives

- Investigate the impact of disulfide bond removal through all-atom molecular dynamics simulations, by comparing Wild-Type IL-4 with Cys3–Cys127 and Cys24–Cys65 bond deleted mutants.
- Model surface functionalization by grafting charge-neutral initiators onto lysine residues to evaluate initiator clustering at high grafting densities

Methodology

1. START: PDB ID: 1RCB

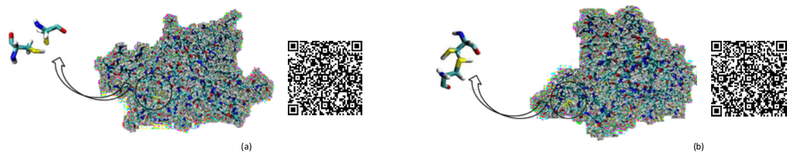
2.SOLVATION & IONIZATION
- Explicit TIP3P Water Box
- Neutralized system with NaCl counter-ions

3.SYSTEM PREPARATION
- Wild-Type Structure (WT)
- Cys3–Cys127 Deletion Mutant (Cys3 mutant)
- Cys24–Cys65 Deletion Mutant (Cys24 mutant)
- Initiator-protein complex

4.MD SIMULATION PROTOCOL
- Engine: NAMD
- Force Field: CHARMM36m

1. Minimization (Conjugate Gradient)
   Removes steric clashes & local strains
2. Heating (0 K to 300 K)
   Gradual velocity initialization
3. Equilibration (NVT)
   Stabilizes system
4. Production MD Run (NPT)
   Generates trajectory data for analysis

Simulation Trajectories



(a) (b)

Figure 2: Mutated IL-4 structural visualizations and trajectory QR codes. (a) Cys3 mutant with QR code to the 500 ns MD trajectory. (b) Cys24 mutant with QR code to the 200 ns MD trajectory

WT, Cys3, and Cys24 Mutants

Global Stability

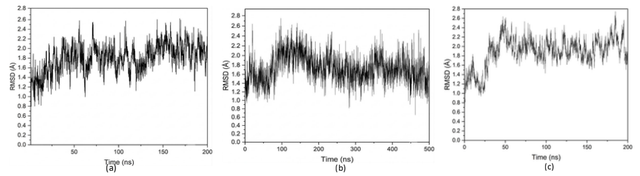


Figure 3: Global Structural Stability Analysis via Backbone RMSD throughout the simulation length. (a) Wild-Type (200 ns) (b) Cys3-Cys127 mutant (500 ns) (c) Cys24-Cys65 mutant (200 ns).Trajectories are aligned to respective equilibrated starting structures.

Cys24 mutant: The initial jump captures a backbone shift into an alternative stable state.

Equilibrium Plateau: All variants maintained stability without a significant global unfolding.

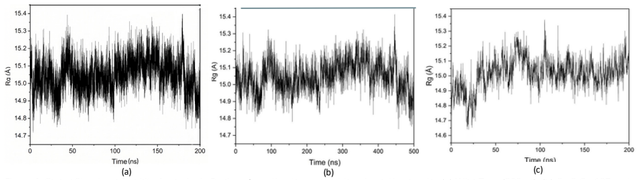


Figure 4: Global Structural Stability Analysis via Radius of gyration throughout the simulation length. (a) Wild-Type (200 ns) (b) Cys3-Cys127 mutant (500 ns) (c) Cys24-Cys65 mutant (200 ns). Trajectories are aligned to respective equilibrated starting structures.

All protein variants maintain a compact, globular shape throughout the trajectories

Cys3 mutant showed a Rg drop in its final 50 ns.

Local Stability

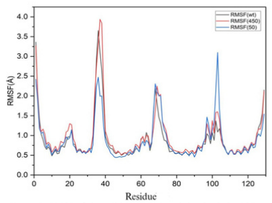


Figure 5: RMSF of the wild type IL-4 and the Cys3-Cys127 Mutant. Wild type :black, Cys3 Mutant first 450ns trajectory:red, Cys3 Mutant last 50ns trajectory:blue .

Terminal stabilization: The Cys3 mutant exhibits reduced terminal mobility due to increased compensatory inter-terminal non-covalent contacts

Peak around residues101-104 : During the final 50 ns, Ala104 takes over the loop-loop interface stabilization from Val101 / Glu103, rewiring contacts with the AB loop alongside an elevated RMSF peak.

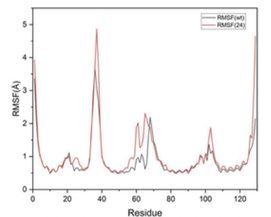
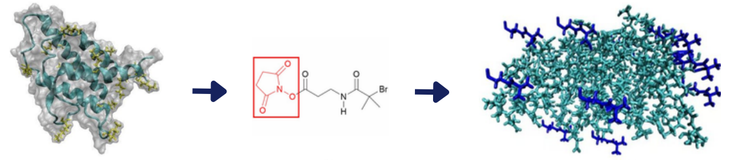


Figure 6: RMSF of the wild type IL-4 and the Cys24 mutant. Wild type: black, mutant :red.

The Cys24 mutant exhibits increased C-terminal flexibility due to disruption of an internal anchoring interaction, which eliminates native stabilizing contacts involving residues 127–129

The Cys24 mutant exhibits increased BC- and AB-loop flexibility following disulfide bond removal.

Protein-Initiator Conjugates



(a) (b) (c)

Figure 7: (a) Surface representation of the Cys3 mutant showing lysine residues (yellow). (b) Charge-neutral initiator, I(N), with the N-hydroxysuccinimide (NHS) leaving group highlighted in red. (c) Protein–initiator conjugate, with the protein shown in green and attached initiators (I(N)) shown in blue.

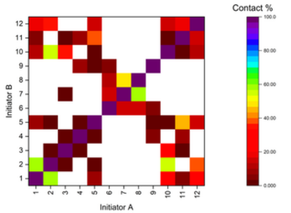


Figure 8: Contact map of surface-bound initiators of IL-4 mutants.

Stable Int1–Int2 and Int2–Int10 interactions (contact distance ≤ 6 Å for >50% of the trajectory) indicate a localized interaction cluster linking the N-terminus, Helix A, and C-terminus.

SDG Alignment

SDG 3 (Good Health & Well-Being) - Therapeutic Innovation: Uncovers the structural role of native disulfide bonds to establish an atomistic framework for stable, disulfide-independent cytokine scaffolds, optimizing IL-4 molecular resilience against biophysical stress to enable more reliable, accessible treatments.

SDG 9 (Industry, Innovation, and Infrastructure) - Supports innovative biomanufacturing by improving protein stability for scalable and cost-effective biotherapeutic production

References

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