Supplementary Material

Allosterism in human Complement Component 5a (C5a): A Damper of C5a Receptor (C5aR) Signaling

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Figure S1. (a) Backbone RMSD (~ 0.36 Å) comparison reveals the incredible structural similarity between the ^mC5a (red) and the *des*-Arg⁷⁹-^mC5a (light blue). (b) Absence of conformational heterogeneity is also noted between ^hC3a and the *des*-Arg-^hC3a (backbone RMSD ~ 0.26 Å).

Figure S2. Ramachandran plot demonstrating the contrasting conformational access of the Lys¹⁴ and His¹⁵ residues in the L₁ region of the Chain A and Chain B in the *des*-Arg⁷⁴-^hC5a dimer.

Figure S3. (a) Conformational clustering over 50 ns of MD illustrates the high degree of conformational heterogeneity in $^h\text{C5a}$, compared to (b) $^m\text{C5a}$. (c) The Cα distance plot between the terminal residues of $^h\text{C5a}$ and $^m\text{C5a}$ indicates higher conformational rigidity in $^m\text{C5a}$.

Figure S4. Ramachandran plot demonstrating the contrasting conformational access of the L_1 region residues, respectively in $^h\text{C5a}$ (Lys¹⁴ and His¹⁵) and $^m\text{C5a}$ (Lys¹⁹ and His²⁰).

Figure S5. The contrasting hydrogen bond interaction observed between the side chains of the L₁ region residues, respectively in (a) ^hC5a and in (b) ^mC5a, over 50 ns of MD.

Figure S6. (a) Conformational clustering over 50 ns of MD illustrating the high degree of conformational flexibility in the isolated Chain B of *des*-Arg⁷⁴-^hC5a dimer. (b) The central structure of the major microstate representing the Chain B of *des*-Arg⁷⁴-^hC5a dimer. (c) The Cα distance plot between the terminal residues indicates higher conformational flexibility in the isolated Chain B, compared to the intact Chain B in *des*-Arg⁷⁴-^hC5a dimer (^{Di}Chain B). (d) Comparison of the backbone RMSDs between the isolated Chain B and the ^{Di}Chain B over 50 ns of MD.

Figure S7. The ramachandran plot illustrating the conformational access of the Lys¹⁴ and His¹⁵ residues in the L₁ region, over 50 ns of MD. Chain A in (a) and (b) represents the isolated monomer of the *des*-Arg⁷⁴-^hC5a dimer. *Des*-Arg⁷⁴-^hC5a in (c) and (d) represents the structural mutant generated from the central structure of the major microstate in ^hC5a (Figure 6a) that has been subjected to 50 ns of MD at 300K.

Figure S8. Monitoring the plausible hydrogen bond interaction of Arg⁷⁴ sidechain with the backbone carbonyls of (a) Leu² (helix 1) and (b) Leu⁷² on the C-terminus of ^hC5a.

Figure S9. (a) Comparison of the root mean square fluctuation of Tyr²³ (helix 2), respectively in the ^hC5a, isolated Chain A and B of *des*-Arg⁷⁴-^hC5a, including the corresponding Tyr²⁸ in ^mC5a. (b) Monitoring the hydrogen bonding interaction between the Tyr²⁸ (helix 2) with Glu¹² (helix 1) in ^mC5a.

Figure S10. Monitoring the hydrophobic interactions between the interacting side chains of the helix 1 and helix 4 residues, respectively in ^hC5a and ^mC5a. The solid lines indicate the cut-off distance for the hydrophobic interactions.

Figure S11. Comparison of the conformational flexibility observed over 50 ns of MD in the helix 3 region of $^h\text{C5a}$. The helix-coil transitions observed in the helix 3 region are presented, respectively for the (a) $^h\text{C5a}$, (b) Chain A of $\text{des-Arg}^{74}\text{-}^h\text{C5a}$, (c) Chain B of $\text{des-Arg}^{74}\text{-}^h\text{C5a}$ and (d) $^m\text{C5a}$.

Figure S12. The helix-coil transitions observed for the helix 3 region are respectively, presented for the (a) $\text{Arg}^{37}/\text{Ala}$, (b) $\text{Arg}^{37}/\text{Lys}$ and (c) $\text{Arg}^{37}/\text{Asp}$ mutants of $^h\text{C5a}$.

Figure S13. The Arg⁷⁴-Tyr²³ “cation- π ” distance (mean $\pm$ SD), respectively calculated for the native and mutants ^hC5a, over 50 ns of MD. The dotted line indicates the cut-off distance for the “cation- π ” interactions.

Figure S14. (a) Monitoring the Arg⁴²-Phe⁵⁶ “cation- π ” distance in ^mC5a, over 50 ns of MD. The angle (mean $\pm$ SD) between the “cation- π ” interactions is also provided. The dotted line indicates the cut-off distance for the “cation- π ” interaction. (b) Schematic illustration of the Arg⁴²-Phe⁵⁶ “cation- π ” interaction observed in the crystal structure of ^mC5a.

Human	---TLQKKIEEIAAKYKHSVVKCCYDGACVNND ETCEQ <u>RA</u> ARISLGPRCIKA <u>F</u> TECCVVASQLRANISHKDMQL <u>GR</u>
Chimpanzee	----LQKKIGEIAAKYKHSVVKCCYDGACVNND ETCEQ <u>RA</u> ARISLGLRCVKA <u>F</u> TECCVVASQLRANISHKDMQL <u>GR</u>
Gorilla	---TLQKAIEEIAAKYKHSVVKCCYDGACVNND ETCEQ <u>RA</u> ARISIGPRCVKA <u>F</u> TECCVVASQLRANNSHKDMQL <u>GR</u>
Orangutan	---TLQKKIEEIAAKYKHSVVKCCYDGARVNND ETCEQ <u>RA</u> ARISIGPRCVKA <u>F</u> TECCVVASQLRANNSHKDMQL <u>GR</u>
Monkey	---TLQKKIEEIAAKYKHLVVKCCYDGARINR DETCEQ <u>RA</u> ARISVGPRCIKA <u>F</u> TECCVVASQLRANDSLKDLHL <u>GR</u>
Horse	----LQKRIEEVAKYKHAKIKRCCYDGAFRND LETCEQ <u>RA</u> ARITIGPRCVQV <u>F</u> KDCCAIAEQLRANESHKHIQL <u>GR</u>
Camel	----LQKKIEEEAAKYKHMVVKCCYDGAHRND DESCEQ <u>RA</u> SRITIGPRCIKA <u>F</u> KDCCAIAQQHRFNESYKNMQL <u>GR</u>
Bovine	---MLKKKIEEEAAKYRNAWVKCCYDGAHRND DETCEE <u>RA</u> ARIAIGPECIKA <u>F</u> KSCCAIASQFRADEHHKNMQL <u>GR</u>
Pig	---MLQKKIEEEAAKYKYAMLKKCCYDGAYRND DETCEE <u>RA</u> ARIKIGPKCVKA <u>F</u> KDCCYIANQVRAEQSHKNIQL <u>GR</u>
Mouse	NLHLLRQKIEEQAAKYKHSVPKKCCYDGARVNFY ETCEE <u>R</u> VARVTIGPLCIRA <u>F</u> NECCTIANKIRKESPHKPVQL <u>GR</u>
Rat	DLQLLHQKVEEQAAKYKHRVPKKCCYDGARENKY ETCEQ <u>R</u> VARVTIGPHCIRA <u>F</u> NECCTIADKIRKESHKGMMLL <u>GR</u>

Figure S15. Multiple sequence alignment of the C5a highlighting the conserved allosteric “cation- π ” pair residues across the species.