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 bC3a and the des-Arg-bC3a (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-Arg³⁴-bC5a dimer.
Figure S3. (a) Conformational clustering over 50 ns of MD illustrates the high degree of conformational heterogeneity in hC5a, compared to (b) mC5a. (c) The Ca distance plot between the terminal residues of hC5a and mC5a indicates higher conformational rigidity in mC5a.

Figure S4. Ramachandran plot demonstrating the contrasting conformational access of the L1 region residues, respectively in hC5a (Lys14 and His15) and mC5a (Lys19 and His20).
Figure S5. The contrasting hydrogen bond interaction observed between the side chains of the L$_1$ region residues, respectively in (a) $^b$C5a and in (b) $^m$C5a, 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-Arg$^{74}$-$^b$C5a dimer. (b) The central structure of the major microstate representing the Chain B of des-Arg$^{74}$-$^b$C5a dimer. (c) The C$\alpha$ distance plot between the terminal residues indicates higher conformational flexibility in the isolated Chain B, compared to the intact Chain B in des-Arg$^{74}$-$^b$C5a 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 Lys$^{14}$ and His$^{15}$ residues in the L$_1$ region, over 50 ns of MD. Chain A in (a) and (b) represents the isolated monomer of the des-Arg$^{74}$-hC5a dimer. Des-Arg$^{74}$-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 Arg$^{74}$ sidechain with the backbone carbonyls of (a) Leu$^{2}$ (helix 1) and (b) Leu$^{72}$ on the C-terminus of hC5a.
**Figure S9.** (a) Comparison of the root mean square fluctuation of Tyr^{23} (helix 2), respectively in the \(^{b}C5a\), isolated Chain A and B of \(\text{des-Arg}^{74} C5a\), including the corresponding Tyr^{28} in \(^{m}C5a\). (b) Monitoring the hydrogen bonding interaction between the Tyr^{28} (helix 2) with Glu^{12} (helix 1) in \(^{m}C5a\).

**Figure S10.** Monitoring the hydrophobic interactions between the interacting side chains of the helix 1 and helix 4 residues, respectively in \(^{b}C5a\) and \(^{m}C5a\). 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 \(^\text{hC5a}\). The helix-coil transitions observed in the helix 3 region are presented, respectively for the (a) \(^\text{hC5a}\), (b) Chain A of \(^\text{des-Arg}^{74}\text{hC5a}\), (c) Chain B of \(^\text{des-Arg}^{74}\text{hC5a}\) and (d) \(^\text{mC5a}\).

Figure S12. The helix-coil transitions observed for the helix 3 region are respectively, presented for the (a) Arg\(^{37}\)/Ala, (b) Arg\(^{37}\)/Lys and (c) Arg\(^{37}\)/Asp mutants of \(^\text{hC5a}\).
Figure S13. The Arg$^{74}$-Tyr$^{23}$ “cation-π” distance (mean ± SD), respectively calculated for the native and mutants $^h$C5a, over 50 ns of MD. The dotted line indicates the cut-off distance for the “cation-π” interactions.

Figure S14. (a) Monitoring the Arg$^{42}$-Phe$^{56}$ “cation-π” distance in $^m$C5a, over 50 ns of MD. The angle (mean ± 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 Arg$^{42}$-Phe$^{56}$ “cation-π” interaction observed in the crystal structure of $^m$C5a.
| Species       | Sequence                                                                 |
|--------------|--------------------------------------------------------------------------|
| Human        | ---TLQKIEIAAKYKHSVVKCCGACVNDTECQRAARISLGPPIKAPTECCVVASQLRANISHKDMQLGR |
| Chimpanzee   | ---LQKIGEAIAKYKHSVVKCCGACVNDTECQRAARISLGLRCVKAITECCVVASQLRANISHKDMQLGR   |
| Gorilla      | ---TLQKIAIEIAAAYKHSVVKCCGACVNDTECQRAARISGLRCVKAITECCVVASQLRANISHKDMQLGR   |
| Orangutan    | ---TLQKIEIEIAAKYKHSVVKCCGARVNDTECQRAARGISGRCVKAITECCVVASQLRANSHKDMQLGR   |
| Monkey       | ---TLQKIEIEIAAAYKHLVKKCCGARDINDECTRAARISVGPRCAITCECCVVASQLRANSKHDMQLGR   |
| Horse        | ---LQKRIEVEVAKYKHAKIKRCYDAFRRNDECTRAARITIGPRCVQVKDCCAIAEQLRANESHKHIQLGR   |
| Camel        | ---LQKIEIEIAAAYKHMVKCCGADHNNDECECRAARTITPRCKIAKDCCAIAQHRFYESYKMNQLGR    |
| Bovine       | ---MLKKIIEIEAAKYARNWVKCCGAGRNNDECECRAARIAIGPECIAKDCCAIAQHRFYESYKMNQLGR   |
| Pig          | ---MLKKIIEIEAAKYAMLKKCCGADYRNDECECRAARISKPRCKYKDCCYIANQVRAEQSHEKNIQLGR   |
| Mouse        | NLHLLRQKIEIEIAQAAKYKHSVVKCCGARVNYETCQRAVARVITGPLCIRAOCCTIANKIRKESFHHPVQLGR |
| Rat          | DLQNLHQQKEIEQAAKYHRVPKCCGAREVENYETCQRAVARVITGPHCIRAOCCTIADKIRKESHHKMLGR   |

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