

# Native Electrospray Ionization of Multi-Subunit Proteins via a Bead Ejection Mechanism

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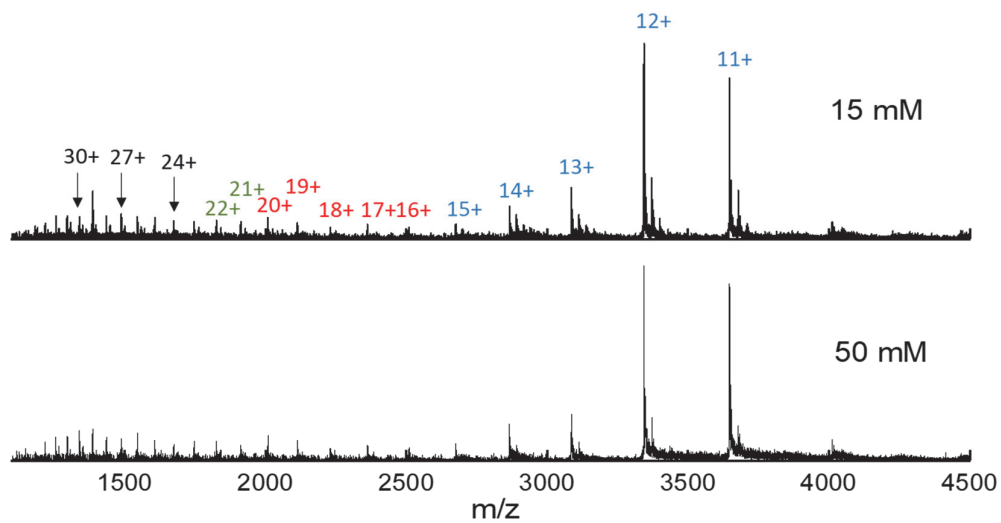
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<sup>c</sup> Merck Biodevelopment SAS / An Affiliate of Merck KGaA – Darmstadt, Germany / F-33650 Martillac, France

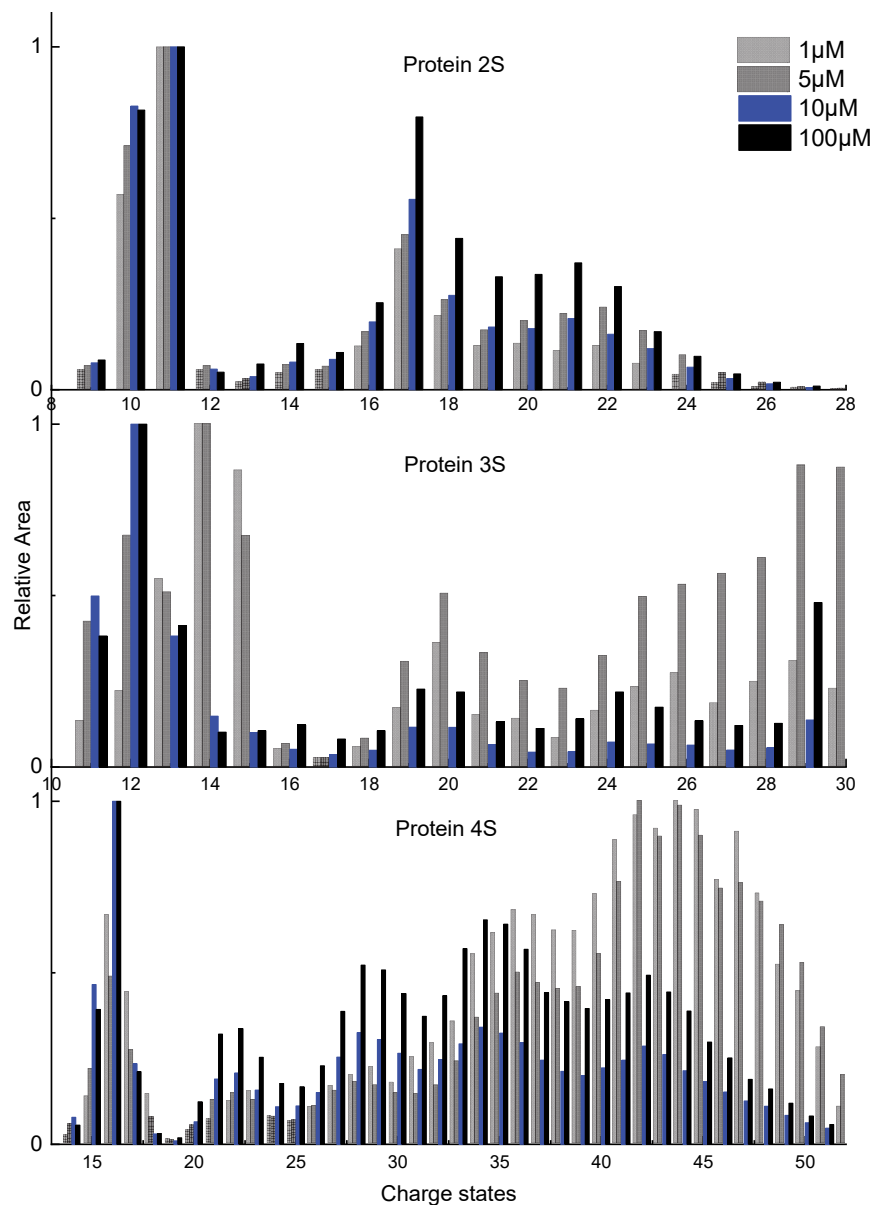
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## Supporting Information:

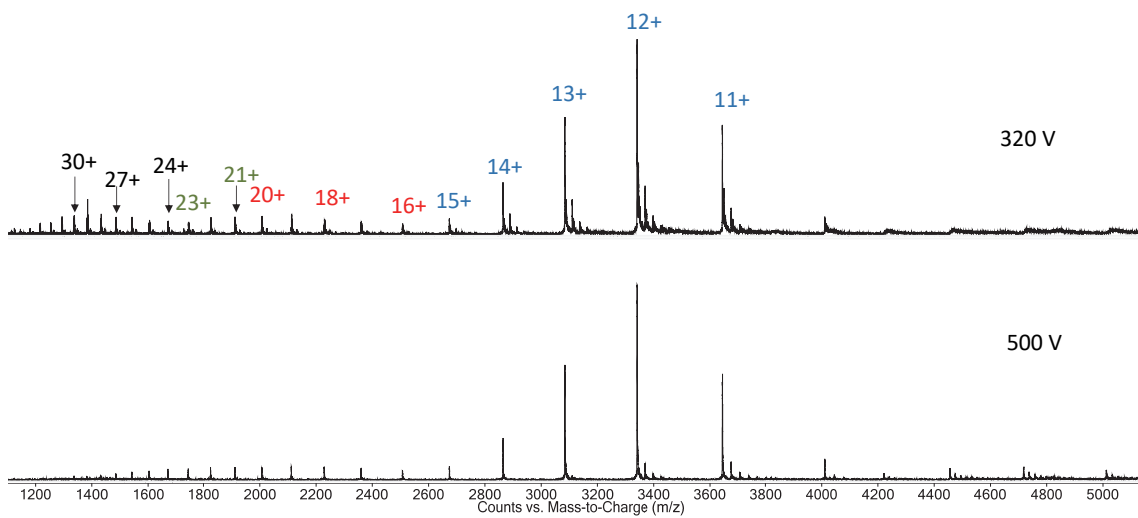
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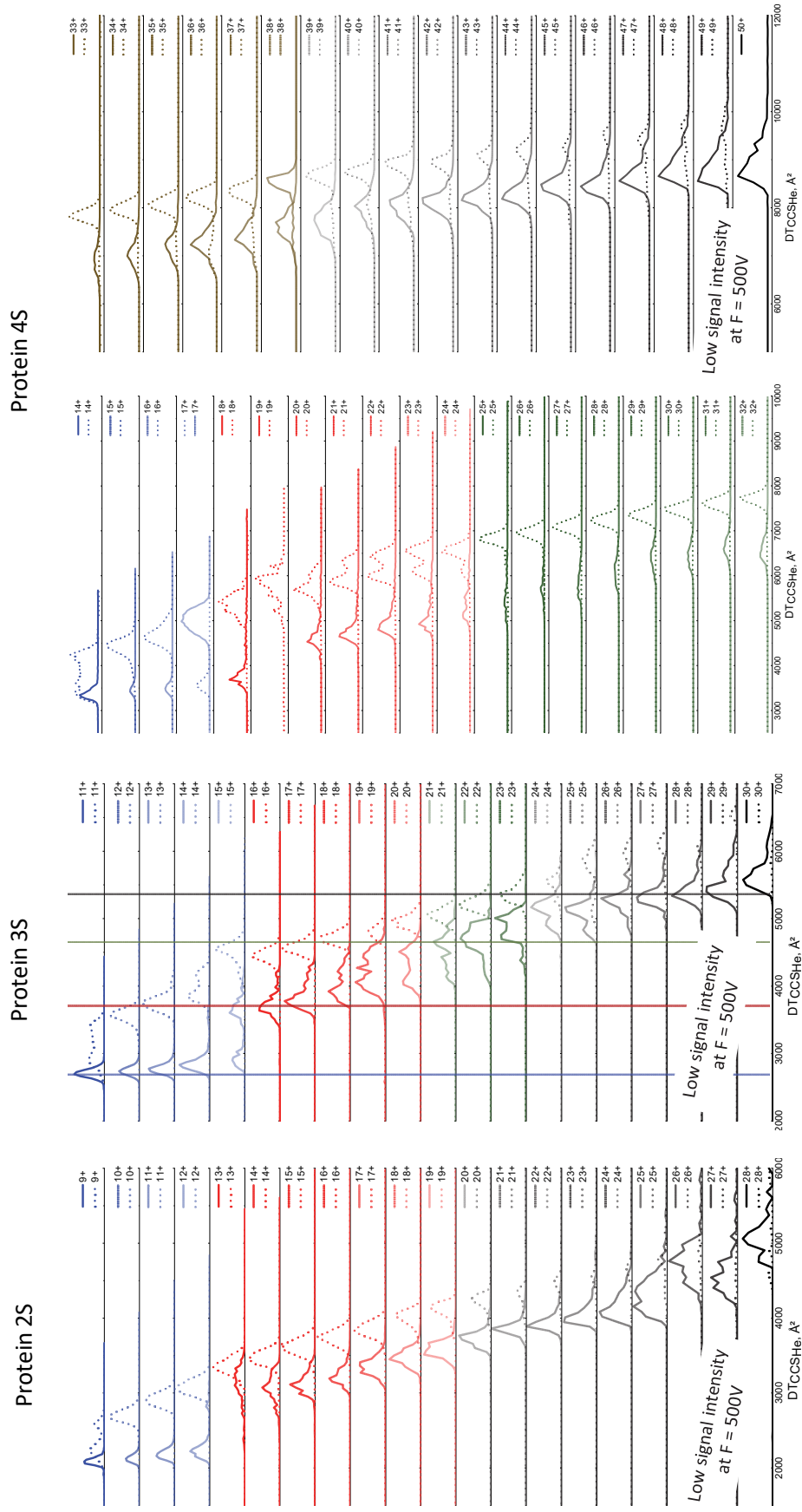
**Figure S1.** Mass spectra recorded for 10  $\mu$ M (flow rate 3.5  $\mu$ L/min) protein 3S in 15 mM and 50 mM NH<sub>4</sub>OAc show 3-4 charge state distributions (experiments with different [NH<sub>4</sub>OAc] performed the same day).



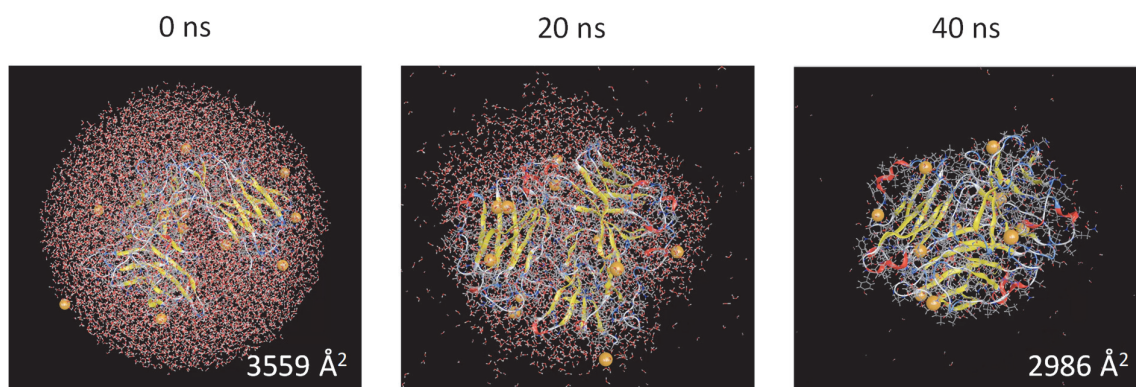
**Figure S2.** Relative peak area as a function of charge state for the multi-subunit proteins injected at different concentrations.



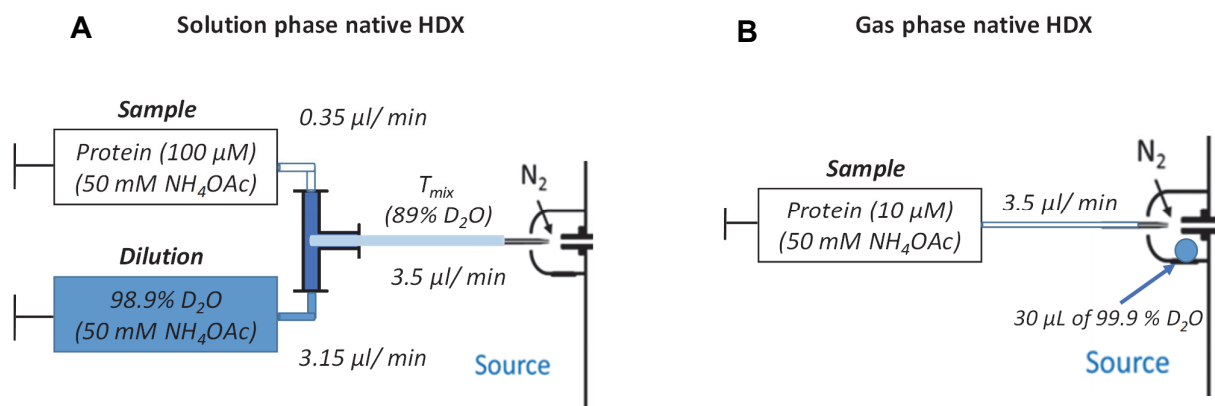
**Figure S3.** Mass spectra recorded for 10  $\mu\text{M}$  (flow rate 3.5  $\mu\text{L}/\text{min}$ ) protein 3S in 50 mM  $\text{NH}_4\text{OAc}$  with low and high fragmentor voltages (experiments were performed the same day).



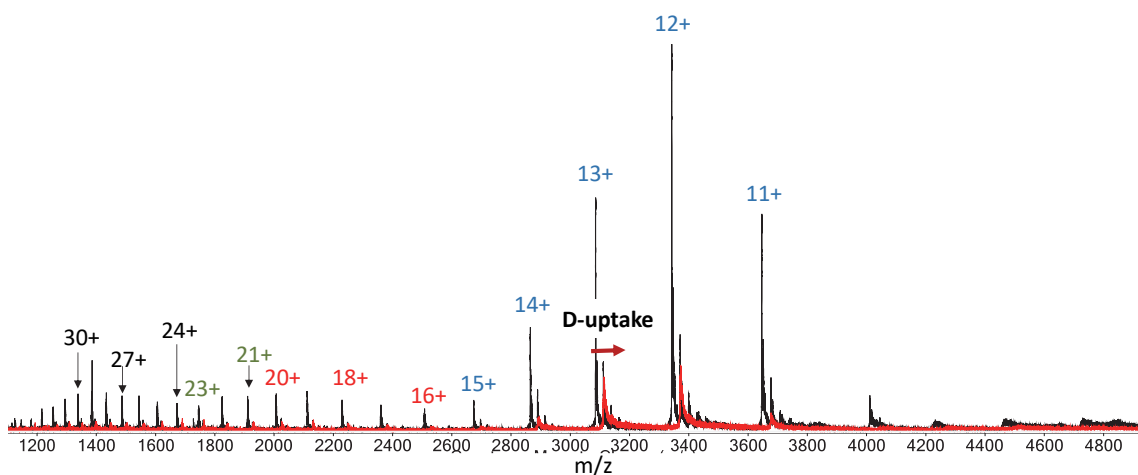
**Figure S4.** CCS distributions for different charge state ions of protein 2S, 3S and 4S in 50 mM NH<sub>4</sub>OAc without activation 320 V vs Fragmentor 500 V). activation dash lines (Fragmentor 320 V vs Fragmentor 500 V).



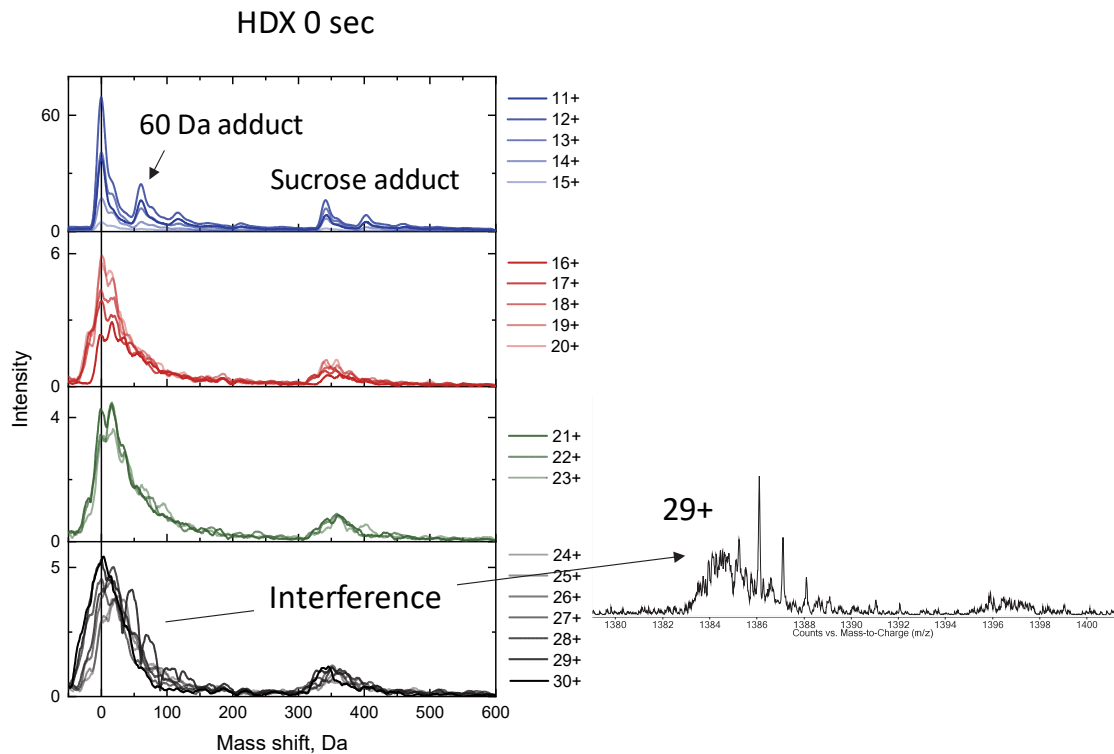
**Figure S5.** Snapshots from MD simulation of compact protein 3S structure in water droplet containing 11 Na<sup>+</sup> ( $3 \times 10^4$  atoms).



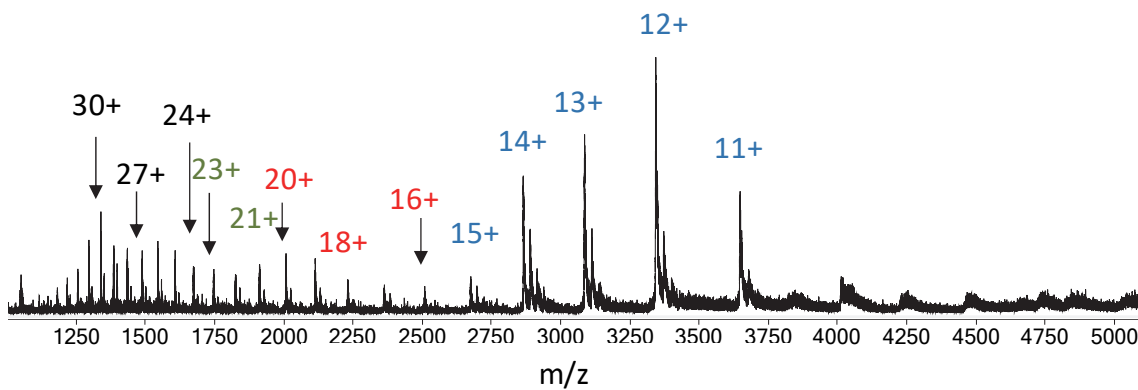
**Figure S6.** Schemes of experimental set-ups for (A) the in-solution continuous-flow HDX and (B) gas phase HDX in the ESI chamber.



**Figure S7.** Mass spectra recorded for 10  $\mu\text{M}$  (flow rate 3.5  $\mu\text{L}/\text{min}$ ) protein 3S in 50 mM  $\text{NH}_4\text{OAc}$  without (**black**) and with (**red**) continuous flow HDX.



**Figure S8.** Left: mass peak shifts without H/D exchange (0 sec time of exchange). The charge state grouping and color code is the one used for IM-MS data. The average values of the mass distribution maximum of ion groups are shown with vertical lines. **Right:** interference detected for the 29+ charge state.



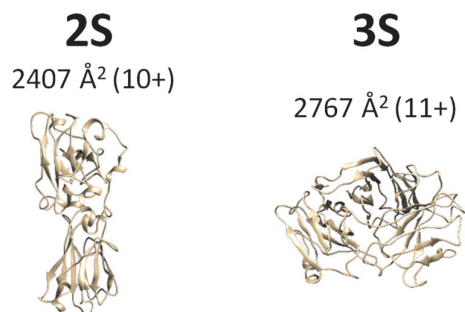
**Figure S9.** Mass spectrum recorded with gas phase HDX in the ESI chamber set-up for protein 10  $\mu$ M in 50 mM  $\text{NH}_4\text{OAc}$ .



## Section S10. Molecular modeling procedures (references are in the main text)

Steps used to generate the candidate protein structures with compact linkers.

1. Homology modelling of protein structures were performed using SWISS-MODEL.<sup>23</sup>
2. The linkers' length was varied using UCSF Chimera v. 1.14.<sup>24</sup>
3. The geometry was optimized using MOE 2018.01 software in AMBER99<sup>25</sup> force field (gradient 0.1 kcal/mol/Å<sup>2</sup>).
4. The 10 Na<sup>+</sup> and 11 Na<sup>+</sup> as charge carrier and water droplet with  $3.5 \times 10^4$  and  $10^4$  water molecules was added for 2S and 3S, respectively.
5. The geometry was optimized using MOE 2018.01 software in AMBER99<sup>25</sup> force field (gradient 0.1 kcal/mol/Å<sup>2</sup>).
6. MD simulation was carried with Amber99 force field at 450 K with 1 fs time step after slow heating 25 K/ 100 ps using NAMD 2.14<sup>28</sup> (Win64 multicore CUDA). Solvent molecules that have left the droplet was removed from the simulation after every ~5 ns simulation.<sup>29</sup> The NAMD calculations were carried out using graphics processing unit (GPU). MD simulations were stopped after 30 ns and 40 ns for 2S and 3S, respectively.
7. The semiempirical PM7<sup>30</sup> calculations were performed using the MOPAC2016 code<sup>31</sup>
8. The theoretical CCS in helium of the obtained structures with charges indicated in the figure were calculated using the trajectory method<sup>26</sup> implemented in Collidoscope.<sup>27</sup>



Steps used to generate the candidate protein structures with normal, extended and elongated linkers.

1. Homology modelling of protein structures were performed using SWISS-MODEL.<sup>23</sup>
2. The linkers' length was varied using UCSF Chimera v. 1.14.<sup>24</sup> In the structures with elongated linkers the subunits before their disulfide bridge staple were unfolded and extended, by adjusting torsions using UCSF Chimera v. 1.14 software.
3. The geometry was optimized using MOE 2018.01 software in AMBER99<sup>25</sup> force field (gradient 0.1 kcal/mol/Å<sup>2</sup>).
4. The theoretical CCS in helium of the obtained structures with charges indicated in the figure were calculated using the trajectory method<sup>26</sup> implemented in Collidoscope.<sup>27</sup>

