Supporting Information

Assessing the structural heterogeneity of isomeric homo and copolymers: an approach combining ion mobility mass spectrometry and molecular dynamics simulations

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Materials and methods: Synthesis of the polymer samples

Preparation of the α-methyl, ω-hydroxy-PLA sample (M_n ~ 1500 g mol^{-1}) : In a glovebox under nitrogen pressure (O_2 < 5 ppm, H_2O < 1 ppm), a vial was charged with L-lactide (L-LA) (1.00 g, 6.9 mmol). CH_2Cl_2 (10.0 g) was added, followed by the addition of methanol (30 µL, 0.74 mmol) and DBU (112 µL, 0.74 mmol). After 1 min under stirring, benzoic acid (100 mg, 0.8 mmol) was added. The as-obtained DBU/benzoic acid salt and residual L-LA were removed by precipitation into cold methanol to give α-methyl, ω-hydroxy-PLA.

Preparation of the α-methyl, ω-hydroxy-PPL sample (M_n ~ 1000 g mol^{-1}) : In a glovebox under nitrogen pressure (O_2 < 5 ppm, H_2O < 1 ppm), a vial was charged with PL (187 mg, 2.9 mmol), CH_3OH (5 µl, 0.12 mmol) and 3.5 g of $CHCl_3$. Out of the box, the medium is kept at 50 °C for 10 minutes before addition of a solution of TfOH in $CHCl_3$ (11 µl of TfOH (0.12 mmol) in 0.49 g of $CHCl_3$). The polymerization is conducted at 50 °C for 5 minutes, cooled down and precipitated twice in cold heptane.

Preparation of the α-methyl, ω-hydroxy-(PPL-b-PLA) sample (M_n ~ 1500 g mol^{-1}) : In a glovebox under nitrogen pressure (O_2 < 5 ppm, H_2O < 1 ppm), a vial was charged with PL (95 mg, 1.3 mmol), CH_3OH (5 µl, 0.12 mmol), TfOH (11 µl, 0.12 mmol) and 1.2 g of toluene. Out of the box, the medium is kept at 50 °C for 2.5 hours before the addition of L-LA (90 mg, 0.63 mmol) and extra TfOH (11 µl, 0.12 mmol). After 20 hours at 50 °C, the polymerization medium is cooled down and precipitated in cold heptane. This procedure was adapted from the protocol reported by Nakayama *et al.* (See Reference 48 of the main text)

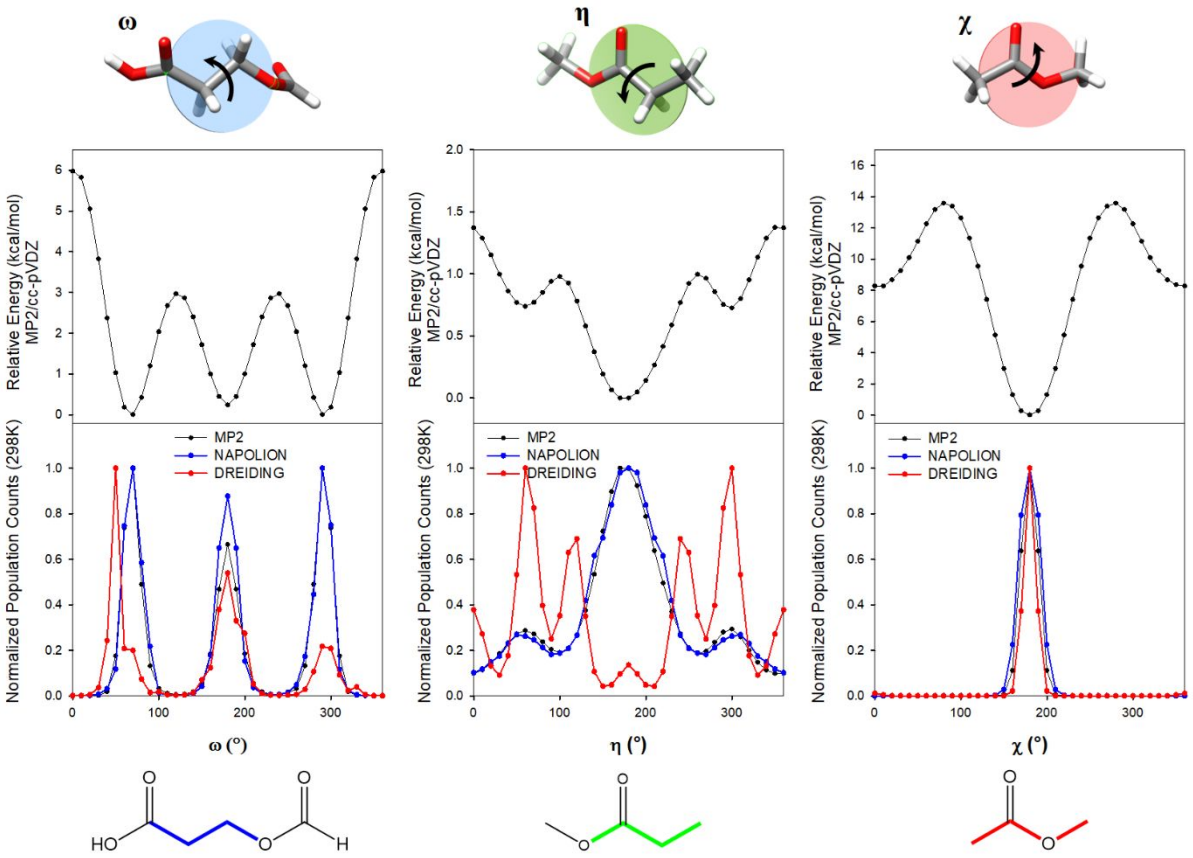

Figure S1. Torsion energy profiles generated at the MP2/cc-pVDZ level (top) for each dihedral angle of interest in the PPL backbone, denoted **ω**, **η** and **χ**. All geometrical parameters are optimized except for the dihedral of interest when building each torsional profile. These energy profiles are then converted into normalized population distributions (bottom) according to the Boltzmann equation at 298K:

$$\text{Normalized population fraction} = \frac{e^{\frac{-E_I}{kT}}}{\sum_i e^{\frac{-E_I}{kT}}}$$

The same procedure has been carried out at the molecular mechanics level with DREIDING and with our reparametrized force field, NAPOLION. An excellent agreement is found between NAPOLION and quantum-chemical calculations, with a RMSD < 5% obtained for each population profile.

Additional details regarding the parametrization of the Lennard-Jones terms in the force field can be found in the reference **Hoyas, S. et al. (2018), PEPDROID: Development of a Generic DREIDING-Based Force Field for the Assessment of Peptoid Secondary Structures. Adv. Theory Simul., 1: 1800089.** In summary, we adjusted the equilibrium distance and well depth involving H atoms to 2.83 Å and 0.0152 kcal mol^{-1}, respectively.

Dihedral parameters for the PLA backbone were already adjusted in the reference **Duez, Q. et al. (2020), Effects of electrospray mechanisms and structural relaxation on polylactide ion conformations in the gas phase: insights from ion mobility spectrometry and molecular dynamics simulations. Phys. Chem. Chem. Phys., 22 (7), 4193-4024.**

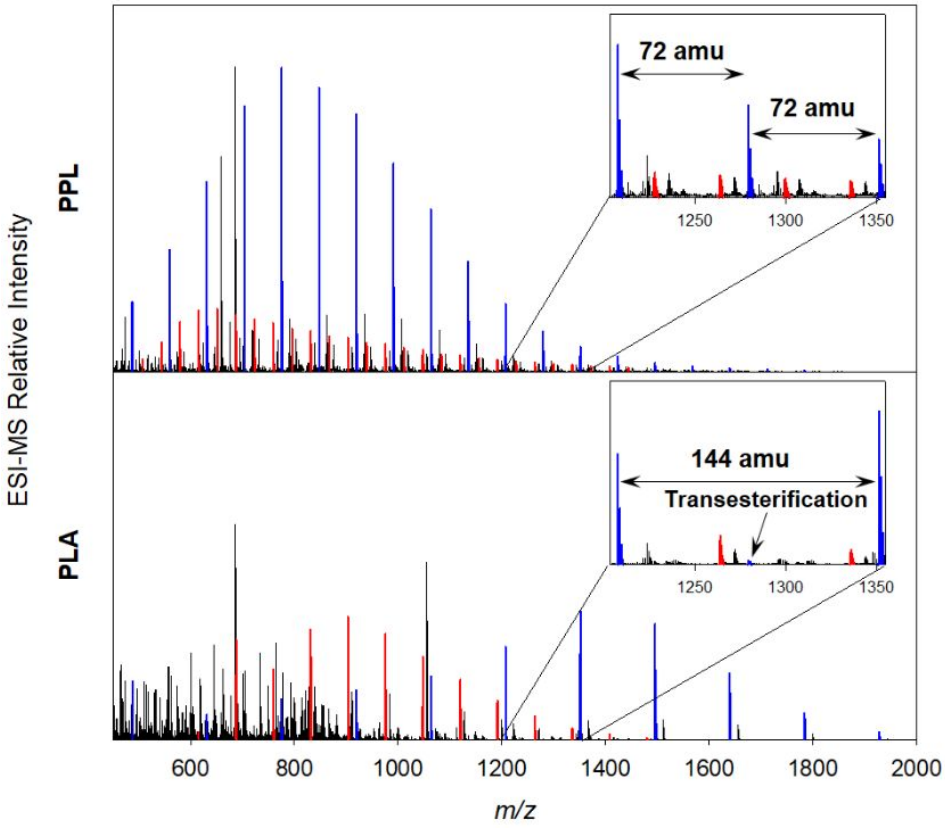

Figure S2. ESI-MS analysis of PLA and PPL samples with respective M_n ~ 1500 and ~ 1000 g mol^{-1}. Each detected charge state is highlighted in a distinct color (Blue - 1+, Red - 2+).

Upon Electrospray Ionization (ESI) of PLA (M_n ~ 1500 g mol^{-1}) and PPL (M_n ~ 1000 g mol^{-1}) samples, singly charged $[P + Na]^+$ and doubly charged $[P + 2\ Na]^{2+}$ polymer ions are mass detected (**Figure S2**). Higher mass PLA (M_n ~ 4000 g mol^{-1}) and PPL (M_n ~ 3000 g mol^{-1}) samples were also analyzed and were shown to produce $[P + 2\ Na]^{2+}$ and $[P + 3\ Na]^{3+}$ ions. As shown in the insets of **Figure S2**, the *m/z* difference between singly charged PPL signals amounts to 72 u and to 144 u for PLA.

This is because the two polymers are synthesized by methanol-initiated ring-opening polymerization (ROP) from different lactone reactants (*β*-propiolactone for PPL and L-lactide for PLA - **Figure S3A**).[53] Therefore, a lactidyl unit (*i.e.*, an opened lactide) is isomeric to two PPL units. In ROP of lactones, parasite transesterification reactions are also susceptible to take place. In such reactions, an intermolecular esterification occurs between two growing chains, ultimately cleaving ester bonds. Since two ester bonds are present in each lactidyl repeating unit, transesterification can result in the production of half-monomer units (*i.e.*, lactoyl units) (**Figure S3B**). Transesterification reactions have usually a low probability to occur in controlled polymerization,[53] as shown by the low intensity signals detected at *m/z* 1279 in the inset of **Figure S2**. Such side reactions can also take place for PPL, but do not result in additional mass signals. Since lactoyl units are susceptible to appear in our experiments, for the sake of clarity, a monomer unit will be considered as a lactoyl unit for PLA and as a propionidyl unit for PPL. In other words, for both PLA and PPL, a monomer unit contains **one** carbonyl group (see **Scheme 1**).

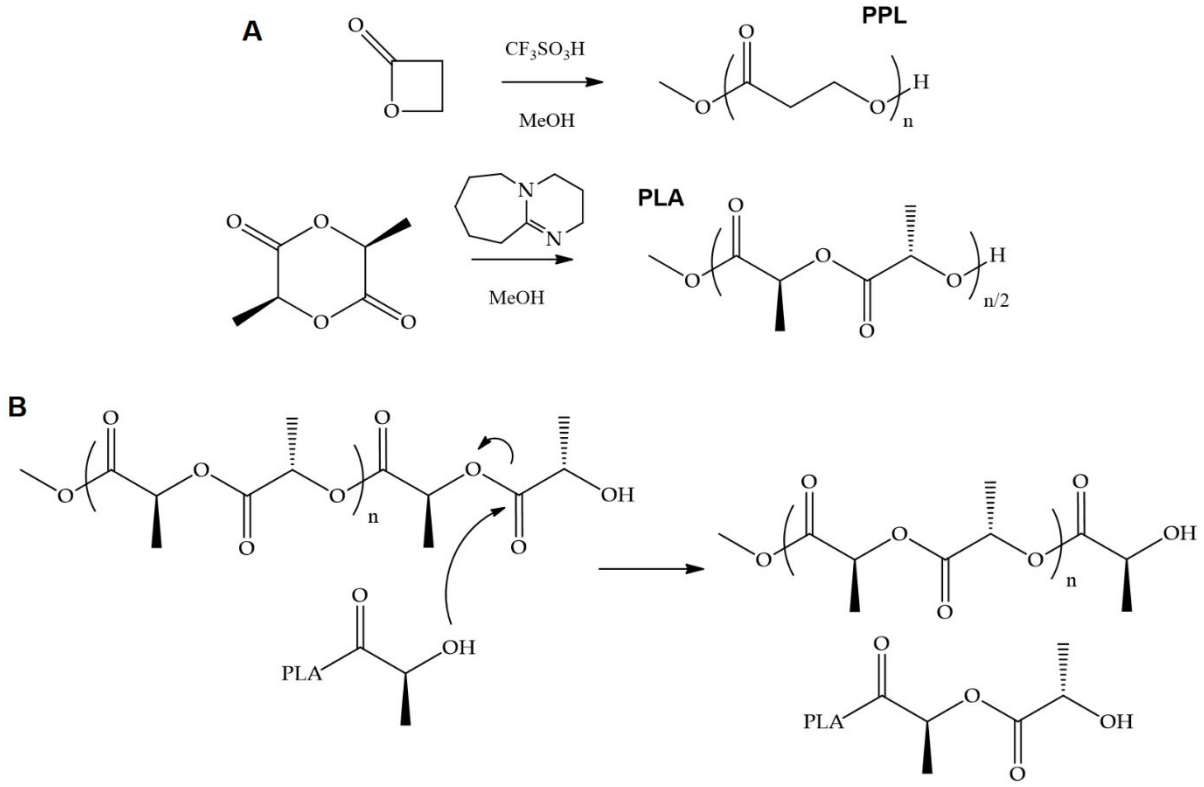

Figure S3. (A) Synthetic routes leading to PPL and PLA. **(B)** Example of a transesterification reaction between two PLA chains.

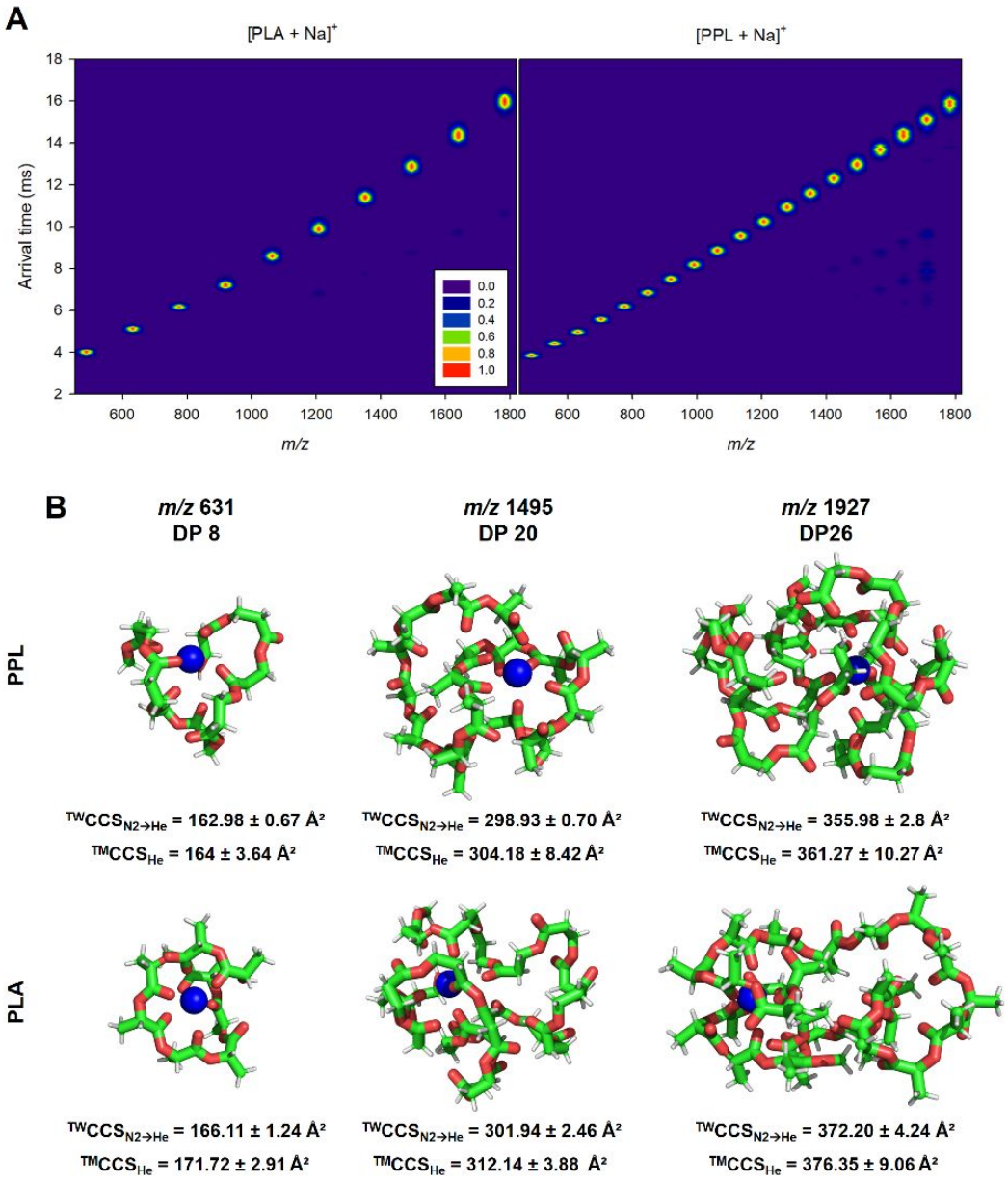

Figure S4. (A) Contour plots including normalized ATDs recorded for all detected $[PLA + Na]^+$ and $[PPL + Na]^+$ ions. **(B)** Representative snapshots obtained from MD simulations. Corresponding experimental $^{TW}CCS_{N2\rightarrow He}$ and theoretical $^{TM}CCS_{He}$ are also shown. Experimental CCSs are averaged over three separate analyses, with errors corresponding to standard deviations. Computed CCSs are averaged over 100 candidate structures, with errors corresponding to standard deviations. Carbon atoms are shown in green, oxygen in red, hydrogen in white. Na^+ are shown in blue.

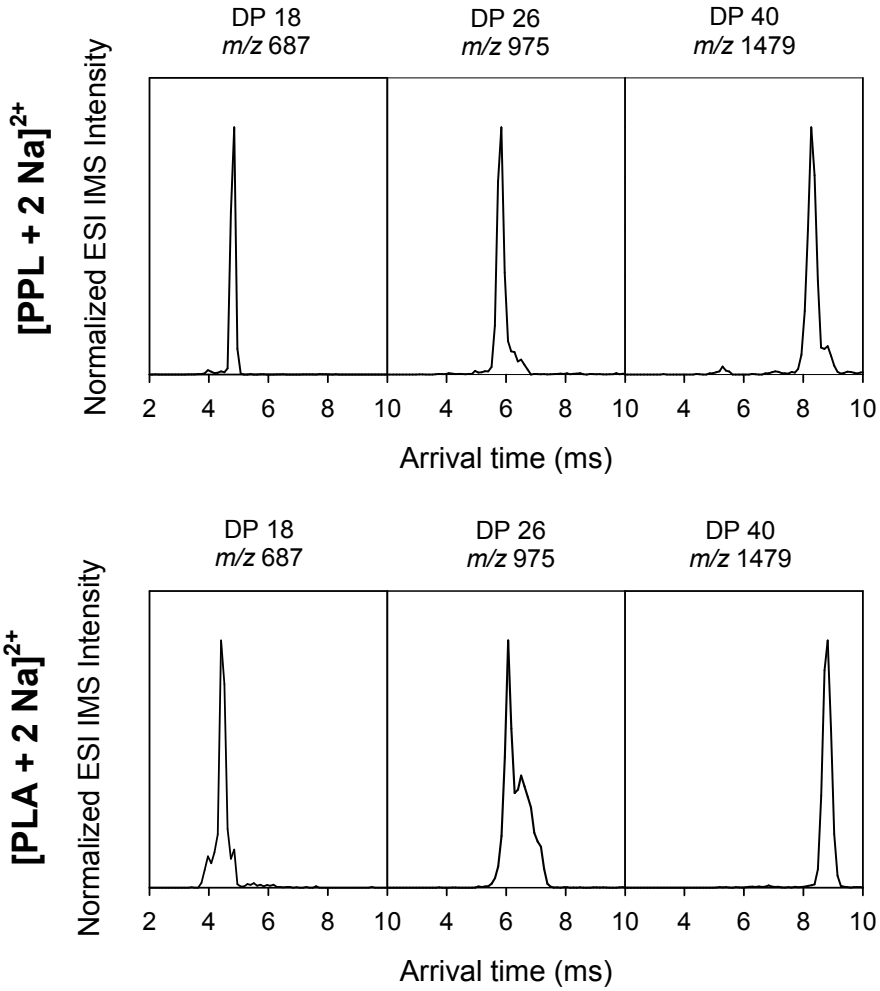

Figure S5. Selected ATDs corresponding to the contour plot shown in **Figure 1** of the main text.

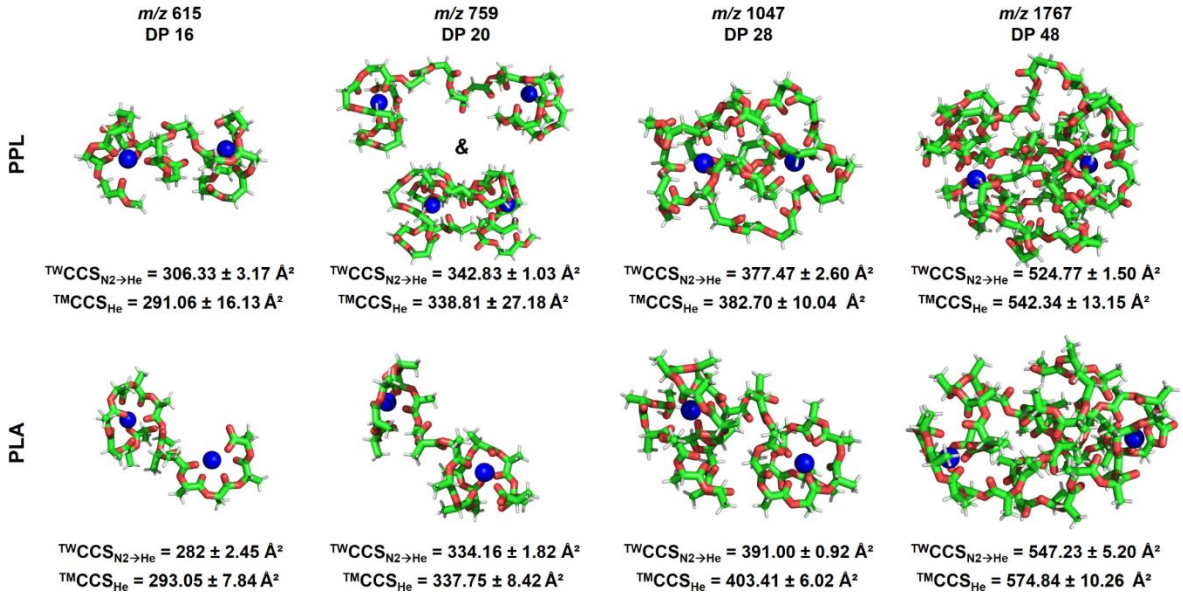

Figure S6. Representative snapshots obtained from MD simulations of 2+ PLA and PPL ions. Corresponding experimental $^{TW}CCS_{N2\rightarrow He}$ and theoretical $^{TM}CCS_{He}$ are also shown.

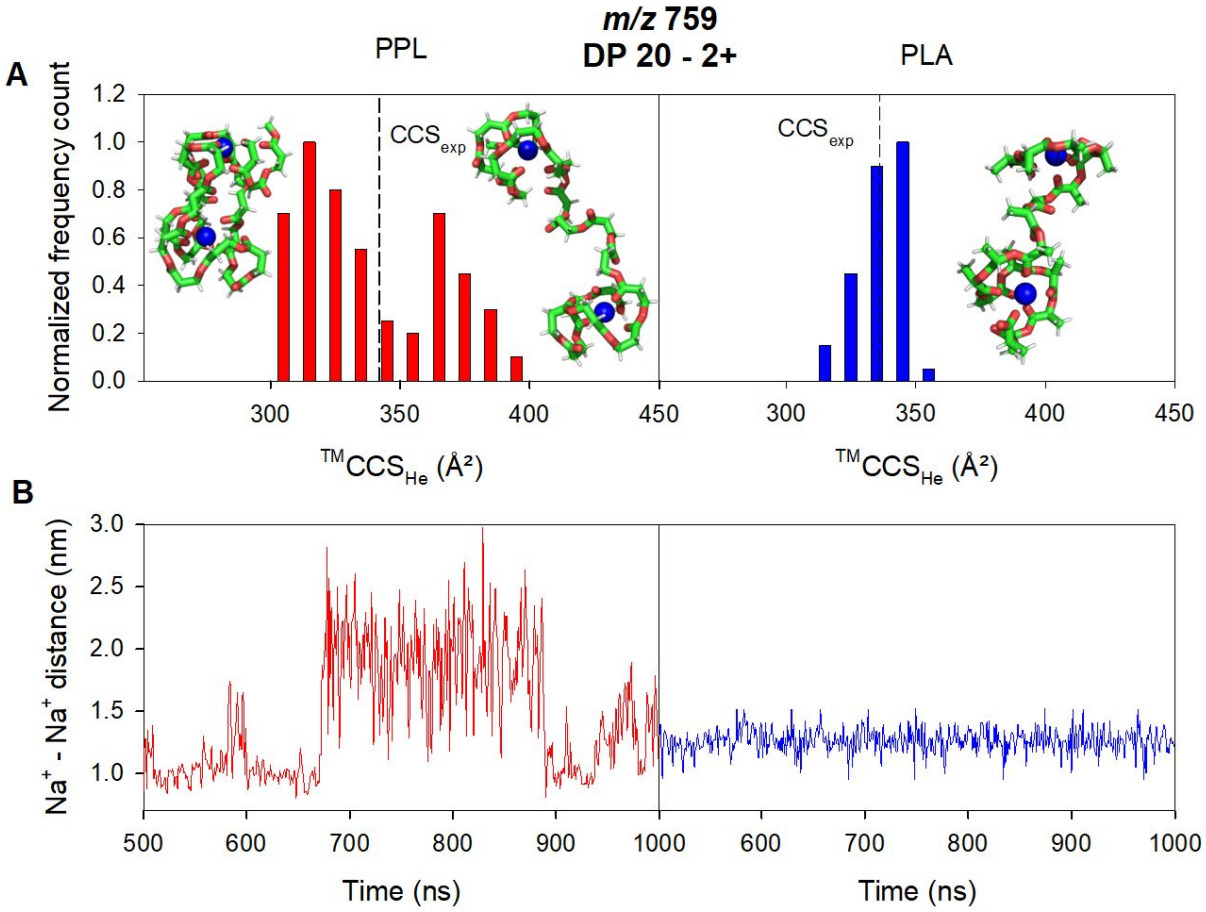

Figure S7. MD data for 2+ PPL and PLA ions with DP20. **(A)** $^{TM}CCS_{He}$ distributions of MD-generated conformations. Corresponding experimental CCSs are highlighted by vertical dashed lines. Representative structures are shown. **(B)** Time evolution of the Na^+-Na^+ distance during the final 500 ns of the simulations.

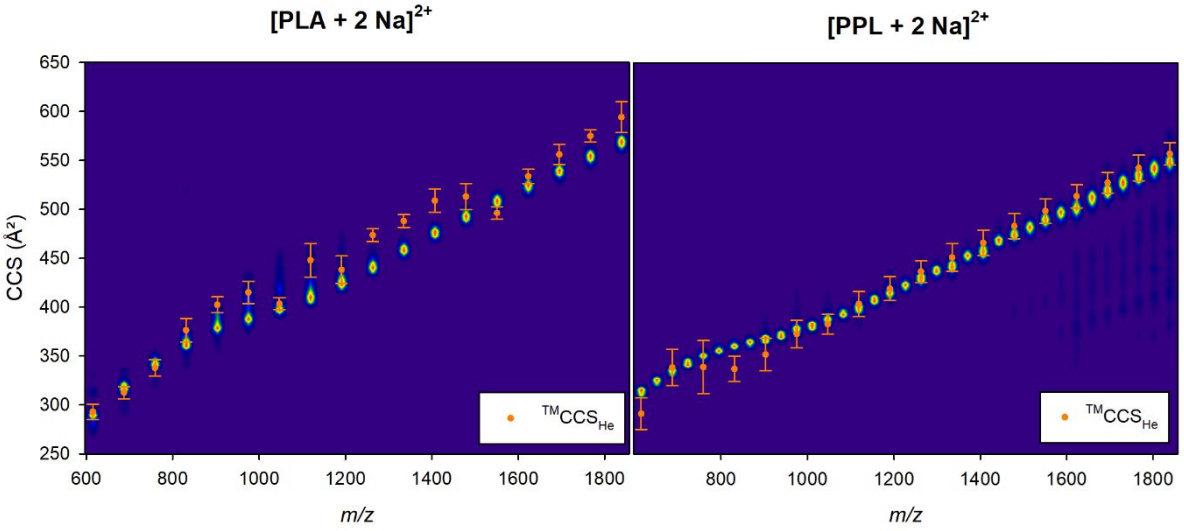

Figure S8. Comparison between $^{TM}CCS_{He}$ computed on MD-generated structures and experimental values. $^{TM}CCS_{He}$ are averaged over 100 candidate structures, with error bars corresponding to standard deviations. The experimental values correspond to the values extracted from the contour plot shown in Figure 3, and further converted into $^{TW}CCS_{N2\rightarrow He}$.

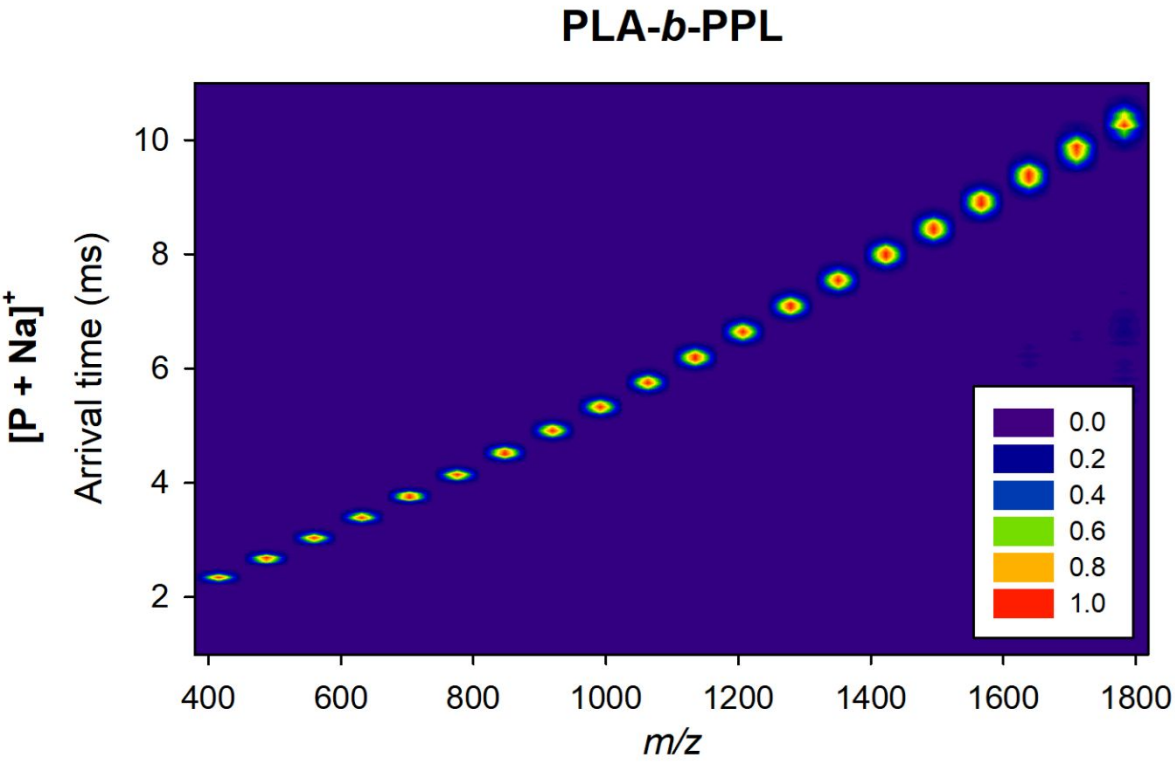

Figure S9. Contour plot including the normalized ATDs recorded for all detected [PPL-*b*-PLA + Na]$^+$ ions. ATDs were recorded without UPLC separation.

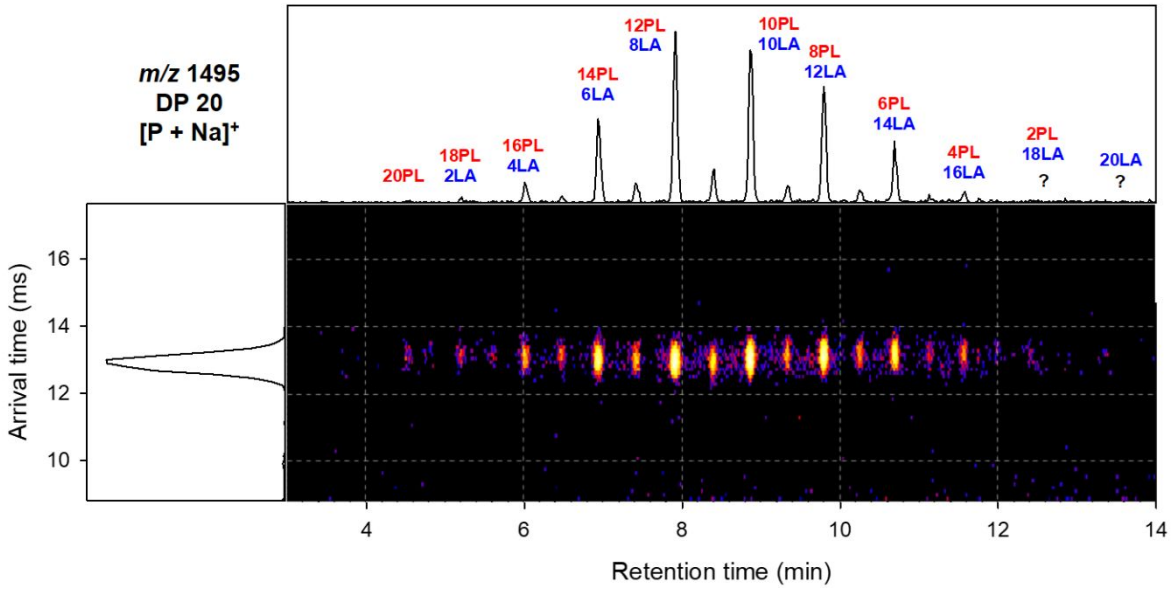

Figure S10. Contour plot including the UPLC chromatogram and IMS arrival time distribution recorded for [PPL-*b*-PLA + Na]$^+$ ions with DP 20.

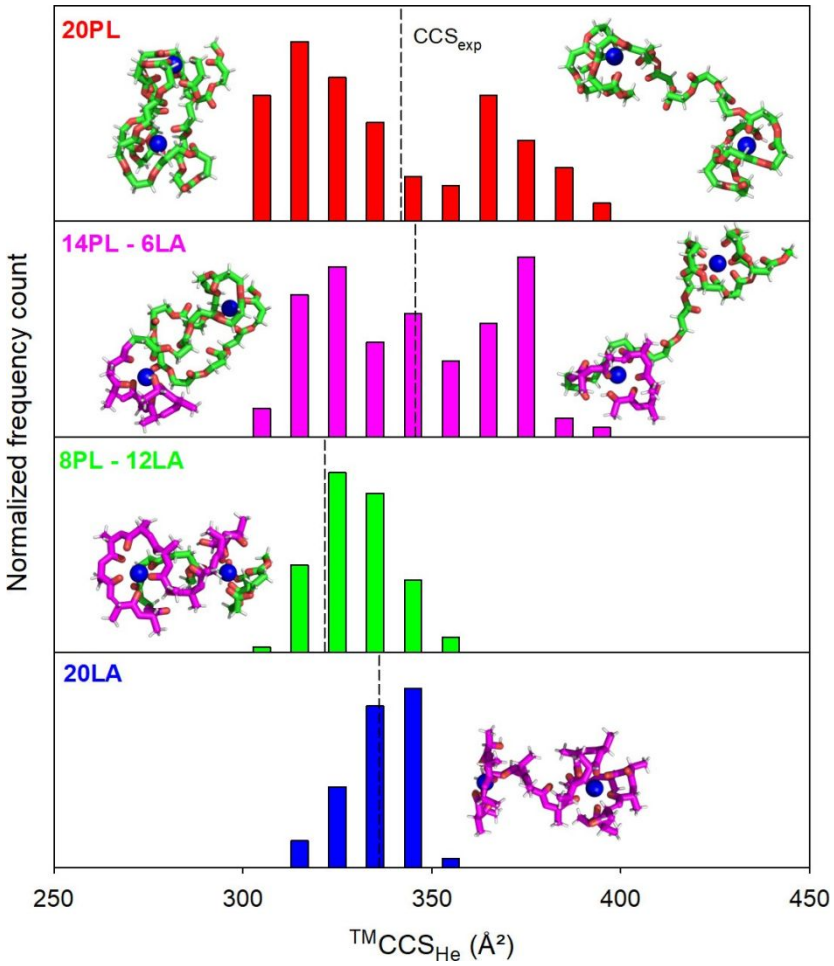

Figure S11. $^{TM}CCS_{He}$ distributions of MD-generated conformations for doubly charged PLA and PPL with DP 20 and doubly charged 14PL-6LA and 8PL-12LA. Corresponding experimental CCSs are highlighted by vertical dashed lines. Representative structures are shown, in which the PLA moiety is highlighted in magenta. All other atoms follow the color code described above.

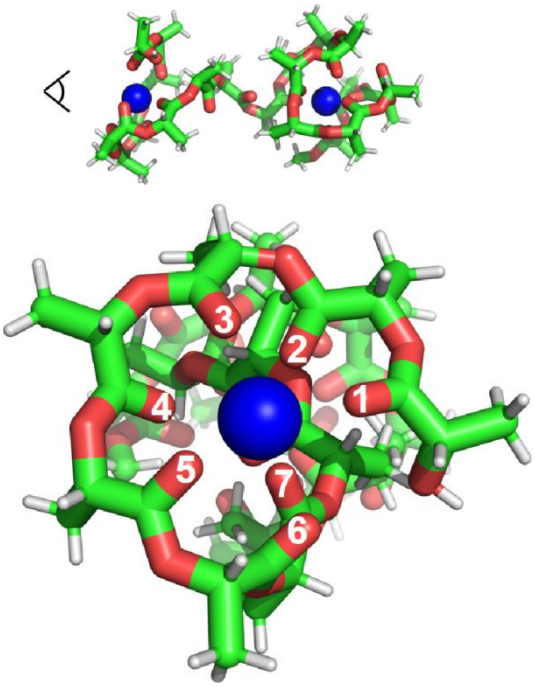

Figure S12. Side view of a representative MD-generated structure for $[20LA + 2\ Na]^{2+}$. Seven carbonyl groups, highlighted by numbers, are complexing one of the Na^{+}.

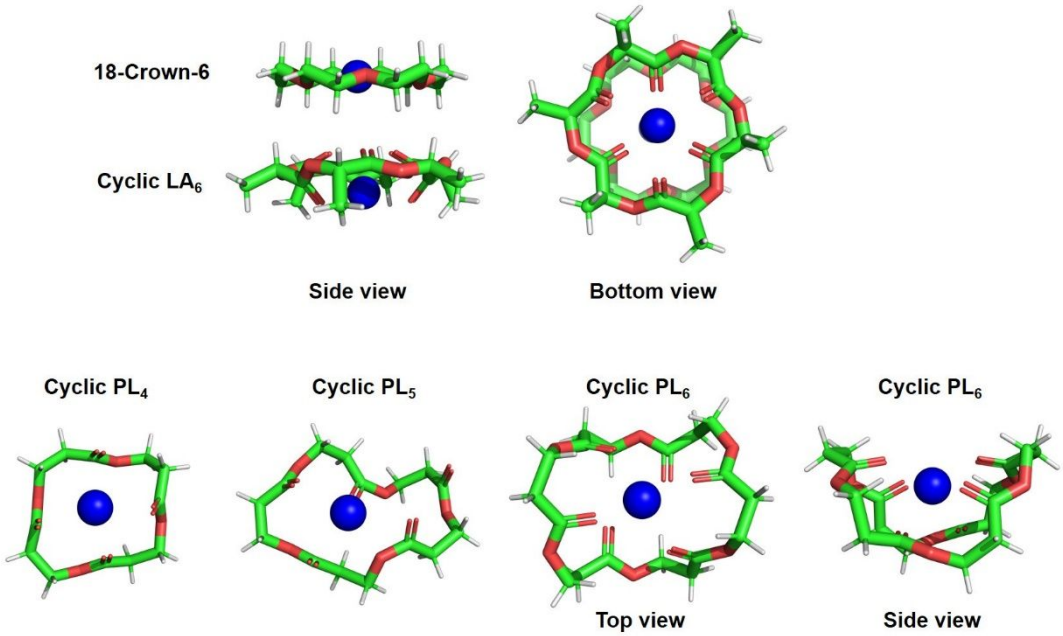

Figure S13. Structures of a cyclic PLA bearing 6 monomer units and of an 18-crown-6 crown ether, containing each 18 backbone atoms. Bottom: Structures of cyclic PPL bearing 4, 5 and 6 monomer units, *i.e.* with 16, 20 and 24 backbone atoms. All structures are energy minimized at the classical level with the NAPOLION force field.