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Entropy-Driven Stabilization of Highly Charged Peptoid Nanosheets

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ABSTRACT

Free-standing two-dimensional (2D) peptoid nanosheets are promising for many aqueous applications, yet how the ionic environment governs their structure and thermal stability remains poorly understood. Here we design an ultrathin (~ 2 nm) crystalline peptoid nanosheet with an extremely small ionizable (ethylammonium) polar domain as a structurally well-defined, highly charged model system, whose molecular packing is directly resolved by cryogenic electron microscopy. Solution-state differential scanning calorimetry reveals that salt-induced stabilization is dominated by entropy rather than enthalpy. Our Molecular Dynamics (MD) simulations attribute this to the release of surface-condensed counterions upon melting, which is set by both the surface's counterion-condensing capacity and the bulk ion concentration, with our computed release entropies tracking the measured melting entropies. With sodium halide salts, the melting temperatures follow a Hofmeister-like ordering ($F^- > Cl^- > Br^-$), reflecting F^- 's higher propensity for contact ion pairing with the *N*-terminal ammonium groups, as demonstrated by our MD simulations. Bulky organic cations such as tetrabutylammonium add enthalpic stabilization, which we ascribed to the direct dispersion contacts with hydrophobic surface groups between their alkyl chains of tetrabutylammonium and hydrophobic surface-exposed side chains, whereas Na^+ does not. By resolving how ion identity partitions stabilization between entropic and enthalpic interfacial pathways, this work establishes electrolyte composition as a key design parameter for tuning free-standing, highly charged 2D soft materials.

1. INTRODUCTION

Polypeptoids, or poly(*N*-substituted glycines), are a highly tailorable, bio-inspired class of sequence-defined polymers capable of encoding diverse molecular interactions within their monomers and sequences.¹⁻³ Through the precision engineering of their chemical sequence and termini, peptoids have been used to self-assemble into diverse nanostructures, such as nanotubes,⁴ nanoribbons,⁵ nanobrushes,⁶ nanostars,⁷ and nanosheets.^{5, 8-10} Among these assemblies, free-standing two-dimensional (2D) nanosheets are typically formed through the self-assembly of amphiphilic diblock copolypeptoids that contain one to six hydrophilic blocks composed of charged or polar monomers.^{9, 11-13} These hydrophilic blocks form an amorphous hydrophilic corona on the peptoid nanosheet surface.^{8, 9, 14-16} 2D peptoid nanosheets have emerged as particularly compelling systems because they unite molecularly defined crystallinity with ultrathin thickness, large surface-to-volume ratios, and chemical displays in aqueous environments,^{17, 18} enabling applications spanning enzyme^{19, 20} and antibody mimetics,²¹ sensing platforms,^{22, 23} and soft photonic materials.²⁴

Recent studies have demonstrated that the nanosheet structure can be maintained even when the hydrophilic block is reduced in size to a single charged moiety by incorporating one cationic (6-aminohexanoic acid, Ahx) or anionic (diglycolic acid) monomer at the terminus.^{11, 12} This simplified design reduces the structural complexity associated with the amorphous hydrophilic shell, thereby facilitating more precise control and molecular-level interpretation of nanosheet properties. Interestingly, in many self-assembled polymeric systems,²⁵⁻²⁸ increasing charge density between building blocks can hinder condensed or crystalline phase formation because strong electrostatic repulsion opposes the close molecular packing required for ordered assembly. In contrast, peptoid chains in reported nanosheet lattices are close packed with intermolecular spacings of ~ 5 Å,^{9, 14, 29, 30} placing charged groups in close proximity,

potentially resulting in electrostatic repulsion within the nanosheet lattice. How these highly charged, ultrathin nanosheets can still sustain ordered 2D assembly therefore remains an open question. Understanding how ions affect this stability may provide important insight into the underlying design principles and advance the applications of these novel nanomaterials.

We designed a peptoid, β -alanine-[*N*-(2-phenylethyl)glycine]₆ (β -Ala-*N*pe₆) (Figure 1A), by replacing the amorphous polar *N*te block from the previously reported nanosheet-forming *N*te₄-*N*pe₆ system with a single β -alanine (β -Ala) residue at its *N*-terminus (Figure S1).⁹ This design was intended to produce an ultrathin peptoid nanosheet, approximately 2.0 nm in thickness, with a high surface charge density, potentially representing one of the thinnest peptoid nanosheet systems reported to date (Figure 1B). This structurally well-defined system provides an ideal platform for directly interrogating how ions influence the thermodynamic stability of crystalline peptoid nanosheets.

Herein, we demonstrate that the physical and chemical properties of ions influence the thermal stability of the peptoid nanosheets in aqueous solution. We employed solution-state differential scanning calorimetry (nanoDSC) to measure their melting transition temperature changes to investigate the thermal stability of these nanosheets in various ionic aqueous environments. We found that all ions studied stabilize the sheets relative to pure water systems, but that some ions are more stabilizing than others. Interestingly, entropic driving forces were found to be the primary determinant of the thermal stability provided by added salts. By integrating structural characterization, thermal stability measurements, and molecular dynamics (MD) simulations, we show that solution ions are not simply background electrolytes for electrostatic screening but instead provide design parameters that can directly influence nanosheet stability, originating from the strong counterion condensation enabled by the exposed, highly charged surface of these nanosheets. Our MD results further suggest that

different ions may stabilize the charged peptoid surface through distinct interfacial mechanisms: fluoride (F^-) shows a higher propensity to form contact ion pairs with the *N*-terminal ammonium groups on peptoid nanosheets, whereas bromide (Br^-), showing the least stabilization effect, is more frequently associated with more diffuse solvent-mediated interactions. More broadly, these findings suggest that understanding ion-specific interfacial interactions with molecularly defined systems will be important for controlling their stability and function in application-relevant environments.

2. MATERIALS AND METHODS

2.1 Peptoid synthesis

Peptoids were synthesized by the solid-phase submonomer method using the Symphony X peptide synthesizer on 100 mg Rink amide resin (0.64 mmol/g, Novabiochem) following a previous report.⁹ To incorporate a short aminoalkyl moiety at the *N*-terminus, Fmoc- β -Ala-OH (or Fmoc-Gly-OH) (20 equiv) and DIC (20 equiv) in DMF (5 mL) were added to the reaction cartridge containing resin-bound *N*pe₆, and the mixture was stirred for 45 minutes at room temperature. Fmoc deprotection was performed using 20% 4-methylpiperidine in DMF (5 mL, v/v) for 15 minutes at room temperature. The crude β -Ala-*N*pe₆ was cleaved from the resins using 95% TFA cleavage cocktail (TFA:TIPS:H₂O = 95: 2.5: 2.5, v/v/v) over 20 min. The collected solutions were dried using a Biotage V-10 evaporator. A preparative HPLC system, the Waters PrepLC system (Waters Corp.), was employed for purifying the crude peptoids. The system included a Waters 2489 UV/visible detector, Waters fraction collector III, and a C18 column (Xbridge™ BEH300 Prep C18, 19 mm x 100 mm, 300 Å, 5 μ m, Waters Corporation, MA, USA). The flow rate was set at 17 mL/min. The mobile phases employed were H₂O (A, with 0.1% TFA) and CH₃CN (B, with 0.1% TFA). Prior to sample

injection, the column was conditioned with a 50% B solvent/50% A solvent composition. Following injection, a gradient elution from 50% to 95% B over 10 minutes was applied. The purity of the resulting fractions was assessed using the Waters UPLC-MS system (Figures S2 and S3). Fractions containing product of greater than 95% purity were collected, lyophilized, and stored at -80 °C.

2.2 Peptoid nanosheet self-assembly

The purified polypeptoid powder (4 mg) was dissolved in THF (1 mL) at a concentration of 4 mg/mL. An equal volume of ultrapure water (MilliQ water, 1 mL) was added to obtain an assembly solution containing peptoids at an initial concentration of 2 mg/mL in a 1:1 THF/water mixture (v/v). The assembly vials were loosely capped, allowing THF to evaporate slowly at 4 °C over a period of up to 14 days. The evaporation period was chosen to match the established assembly conditions for the *Nte*₄-*Npe*₆ nanosheet systems, allowing direct comparison of nanosheets with different hydrophilic blocks under the same protocol while ensuring complete self-assembly.^{9, 31} After evaporation, the final concentration of peptoids was adjusted to 4 mg/mL (3.4 mM for β -Ala-*Npe*₆) by compensating for the volume loss with either H₂O or a concentrated salt solution. The initial concentration of peptoids was also varied from 1.25 to 5 mM, and well-defined nanosheet formation was observed across the entire concentration range (Figures S4 and S5). The resulting solutions were used for further analysis.

2.3 Scanning electron microscopy (SEM)

The pristine nanosheet solutions after the evaporation step were diluted 10 times (ca. 0.4 mg/mL) with H₂O. Then the aliquot of the diluted nanosheet solution (5 μ L) was deposited

on the pre-cut 4-inch silicon wafers (5×7 mm chips, Ted Pella, Inc.), which had been plasma-cleaned using an Ar/O₂ (80/20, v/v) mixture in a Harrick plasma cleaner. The drop was dried rapidly with a stream of nitrogen. After drying overnight in a vacuum chamber, the prepared samples were analyzed using InLense mode on a Zeiss Gemini ultra-55 analytical field emission (FE)-SEM. The results are shown in Figure S4.

2.4 Atomic force microscopy (AFM)

The dried peptoid nanostructures were imaged using an Asylum Jupiter XR atomic force microscope (Oxford Instruments) operating in tapping mode. The peptoid assembly solution was diluted 20-fold with ultrapure water to a final concentration of 0.2 mg/mL. A 5 μ L aliquot of the diluted solution was deposited onto pre-cut 4-inch silicon wafers (5×7 mm chips, Ted Pella, Inc.), which had been plasma-cleaned using an Ar/O₂ (80/20, v/v) mixture in a Harrick plasma cleaner. The drop was dried rapidly with a stream of nitrogen. Imaging was performed using TAP 150 AL-G tips with a resonant frequency of 150 kHz and a force constant of 5 N/m. The acquired AFM images were processed and analyzed using Gwyddion software. The results are shown in Figures S6 and S7. The dilution did not substantially affect the nanosheet morphology or thickness, as shown in Figure S8.

2.5 Wide angle X-ray scattering (WAXS)

Solution WAXS data were obtained at the ALS beamline 7.3.3 in Lawrence Berkeley National Laboratory. A total of 40 μ L of the nanosheet assembly product (4 mg/mL) was loaded into a quartz capillary (O.D. = 1.5 mm). The sample was exposed to a 10 keV (1.24 Å) X-ray beam. The scattering signal was collected by a Pilatus 2M detector and processed using Igor 8 software combined with Nika package. The results are shown in Figure S9.

2.6 Cryo-TEM analysis

A 3 μL droplet of the nanosheet suspension was applied to a C-flat holey carbon grid (Protochips, Inc.) and blotted with filter paper prior to plunge-freezing in a Vitrobot (Thermo Fisher Scientific). The vitrified grid was transferred to an FEI Titan Krios G3 (Thermo Fisher Scientific) equipped with a K3 direct electron detector and an energy filter (Gatan, Inc.) for low-dose imaging. Dose-fractionated movies were collected with a total accumulated dose of approximately $20\text{ e}/\text{\AA}^2$ and aligned using MotionCorr2.³² The motion-corrected micrographs were processed using the electron crystallography software package Focus for contrast transfer function estimation, lattice indexing, and lattice unbending.³³ Small sections of 400×400 pixels were extracted from the unit cell locations identified by Focus in multiple micrographs, with a 50% overlap between adjacent sections. These sections were sorted and averaged in RELION using 2D classification.³⁴ The identification of only one lattice structure during 2D classification suggests the presence of homogeneous molecular packing within the nanosheets.

2.7 Apparent pKa measurement.

The apparent pKa of the β -Ala-Npe₆ nanosheets was determined by potentiometric acid-base titration using an Accumet AB15 pH meter equipped with a LabSen® 241-3 micro pH electrode, following modified literature procedures for assembled polymeric materials.^{10, 35, 36} The nanosheet solution (2 mM) in 0.1 M NaCl(aq) was first adjusted to pH 10.6 with 0.1 M NaOH(aq) and then titrated with 2 μL aliquots of 0.02 M HCl(aq) until the pH reached 2.9. To determine the onset of protonation of the nanosheet ammonium groups, the titration curve was compared with that of a salt-only reference (0.1 M NaCl(aq) without peptoid) starting at the same initial pH. The two curves began to diverge at pH 9.99, which was taken as the onset of nanosheet protonation. The completion of protonation was determined from the intersection of

two linear fits to the nanosheet titration curve (linear fits 1 and 2 in Figure S10). The apparent pK_a was calculated as the midpoint between the onset and completion pH values, yielding a value of approximately 8.4.

2.8 Powder X-ray diffraction (PXRD)

The nanosheet suspensions were dispensed onto a MiTeGen micromesh and subsequently dried under vacuum conditions. This procedure was iterated multiple times to ensure a sufficient accumulation of nanosheets on the micromesh. PXRD measurements were performed at ALS beamline 8.3.1, utilizing both multiple wavelength anomalous diffraction and monochromatic macromolecular crystallography. The beamline is equipped with a super bend source covering an energy spectrum of 5-17 keV. Data acquisition was conducted with the detector positioned 350 mm away from the specimen. The data were analyzed using Fit2D software.

2.9 Differential scanning calorimetry (NanoDSC)

The melting transitions of the assembled peptoid nanostructures were analyzed in solution using a NanoDSC (CSC Model 6100, TA Instruments). The assembly products were examined in aqueous solutions (4 mg/mL), and the solution background was degassed using a degassing station (Model 6326, TA Instruments) at 80 mbar for 20 minutes. Concentrated salt solutions (1.0–5.0 M) were prepared and subsequently diluted with the peptoid solution to achieve the desired salt concentration. A total of 650 μ L of the sample and background solutions were loaded into the sample and reference capillaries, respectively. The solutions were equilibrated at 5 °C for 15 minutes before being heated at a rate of 1 °C/min from 5 to

100 °C under a pressure of 3.0 atm. The collected data were exported after baseline subtraction using NanoAnalyze software and plotted using Origin software (Figure S11).

2.10 Variable temperature small angle X-ray scattering

Solution SAXS data were obtained at the Advanced Light Source (ALS) beamline 7.3.3 at Lawrence Berkeley National Laboratory. A 40 μ L aliquot of nanosheet solution (3.4 mM) was loaded into a quartz capillary (O.D. = 1.5 mm). Samples were mounted on a temperature-controlled stages and heated from 50 to 90 °C in 2 °C increments with a ramp rate of 2 °C/min and a 30 s equilibration time at each temperature. The samples were exposed to a 10 keV (λ = 1.24 Å) X-ray beam with a sample-to-detector distance of 1804.35 mm. The scattering signal was collected by a Pilatus 2M detector and processed using Igor 8 software combined with the Nika package (Figure S12).

2.11 MD Simulations

The initial coordinates for the MD simulations were constructed to be consistent with the experimentally determined crystalline lattice using cryo-TEM and PXRD analysis, following modeling rationale established for analogous peptoid nanosheets.^{9, 13, 37} Specifically, the cryo-TEM images and their power spectra (Figures 2C and 2D) directly revealed the characteristic parallel V-shaped molecular packing, where the bright central oval feature corresponds to the peptoid backbone and the two arms extended from this center correspond to the side *N*pe side chains. The model incorporated the *a* and *c* spacings from PXRD (4.5 Å and 16.0 Å, respectively; Figure 2B), which are consistent with the cryo-TEM values (Figure 2C). Because these data constrain the projected in-plane packing but do not, by themselves, specify the full three-dimensional atomic arrangement, the through-thickness stacking, the all-*cis*

backbone conformation,³⁸⁻⁴¹ and the side chain packing were built by analogy to the previously reported *Nte4-Npe6* nanosheet structure^{9, 31} and were subsequently refined by energy minimization and MD relaxation. The resulting model comprised six stacks, each containing 12 β -Ala-*Npe6* molecules arranged in a parallel packing configuration with *cis*-amide backbone conformations. The model incorporated the *a* and *c* spacings from PXRD, (4.5 Å and 15.9 Å, respectively) and was prepared as an infinite nanosheet, with the two pairs of side surfaces contacting each other across the periodic boundaries. The experiment-inspired structures were fully solvated with TIP3P water.

For the peptoid residues, we employed the validated CGenFF-based force field framework from our prior studies,^{14, 30, 42, 43} which combines the backbone parameters of Weiser and Santiso⁴⁴ with the side-chain parameters from the standard CGenFF.⁴⁵ For the β -alanine residue, we integrated the CHARMM-compatible β -amino-acid force field of Wachar et al.^{46, 47} The partial charges for the peptoid nanosheet were assigned by these force field frameworks accordingly. Tetrabutylammonium cation was parameterized using CGenFF, with all assigned penalty scores below 1. Parameters for trifluoroacetate anion were adopted from our previous work,^{30, 42} and its partial charges were obtained using the RESP algorithm from density functional theory calculations. The monatomic halide counterions (F^- , Cl^- , Br^-) each carry a formal charge of -1 , and their parameters were taken from the CHARMM-compatible halide-ion parameter set of Orabi et al.⁴⁸ Formal charges, counterions, and background salts for all six simulated systems are summarized in Table S1. All peptoid molecules were modeled with their *N*-terminal groups in the protonated ($+1$) state, consistent with the assembly pH being well below the apparent pK_a of the assembled nanosheets (see Section 3.2).

All MD simulations were performed using GROMACS 2023.3.⁴⁹ The system temperature was maintained at 300 K using the Bussi thermostat,⁵⁰ and pressure was controlled sequentially using the Berendsen and Parrinello–Rahman barostats (Figure S13–S16).^{51, 52} All bonds involving hydrogen atoms were fixed in length using the LINCS algorithm, enabling a 2 fs integration time step.⁵³ Simulations were run for at least 50 ns to obtain the relaxed structure for β -Ala-Npe₆ nanosheets. All interaction energies are nonbonded energies (short-range Coulomb + Lennard-Jones) computed with GROMACS from the production trajectories using the CHARMM36 force field, with PME electrostatics ($r_{\text{coulomb}} = 1.2$ nm) and a force-switched van der Waals cutoff ($r_{\text{vdw}} = 1.2$ nm, switch at 1.0 nm). Each value was converted to kcal/mol, normalized per peptoid molecule ($N = 72$), and reported as the mean $\pm$ standard deviation (SD) over the final 10 ns across five independent replicas ($n = 5$). We note that these nonbonded energies describe only the assembled states prior to melting. Because the molecular conformations of the peptoids in the disassembled states after melting are not well understood, we do not interpret the MD-derived nonbonded energies as absolute melting enthalpies associated with disruption of the crystalline lattice. Instead, we use these energies as a comparative measure of ion-specific interfacial stabilization across different ionic conditions. The potential energy was tracked until it stabilized, and the trajectory was recorded for subsequent analysis. Five independent MD replicas were performed for each condition. Across all conditions, condensed-ion counts, ion-density profiles, and structural metrics, including out-of-plane corrugation, in-plane area, and nearest-neighbor spacing, reached stable plateaus over the analysis window (the final 20% of each trajectory), indicating convergence of the simulations (Figures S17–S23). We analyzed the simulation data and trajectory files using a custom Python analysis script based on the MDtraj Python library.⁵⁴

3. RESULTS AND DISCUSSIONS

3.1 Peptoid design and self-assembly

Nte4-Npe6 was used as a reference peptoid sequence since its nanosheet structure was atomically resolved and previously reported by Xuan et al. (Figure S1).⁹ We modified the model sequence of *Nte4-Npe6* by removing all four *Nte* residues and introducing a single 3-aminopropanoic acid (β -Ala) at the *N*-terminus, affording β -Ala-*Npe6* (Figure 1A). This new construct has a shorter linker to the ionic head group than previously employed Ahx monomers and glycolic acids,^{11, 12} which were designed to minimize the hydrophobicity of the *N*-terminus. This system was designed to minimize rotational degrees of freedom by positioning the charged *N*-terminal moiety closer to the crystalline *Npe6* block within the nanosheet crystalline core (Figure 1B). These peptoids were synthesized using solid-phase peptoid synthesis and purified via reverse-phase high-performance liquid chromatography (RP-HPLC) to achieve a purity exceeding 95% (see Section 2.1 for details on peptoid synthesis). The obtained peptoids, isolated as the TFA salt form owing to their basic *N*-terminal amines, were directly used in the self-assembly procedure without additional desalting or ion-exchange steps.

The two peptoids, β -Ala-*Npe6* and *Nte4-Npe6*, were self-assembled by the evaporation of a 50% THF/H₂O (v/v) peptoid solution over 14 days (see Section 2.2 for details on peptoid nanosheet self-assembly). Nanosheets were deposited on silicon substrates for obtaining morphology and high profiles using SEM and AFM (Figures S4 and S6). Although, both peptoids formed thin nanosheet structures, β -Ala-*Npe6* produced wider nanosheet with relatively lower aspect ratios, suggesting that removal of the *Nte* block would influence the growth kinetics that determine nanosheet aspect ratio (Figure S4). In AFM analysis, the absence of the PEG-like *Nte* block results in a thinner nanosheet (2.0 ± 0.2 nm for β -Ala-*Npe6* and 3.2 ± 0.2 nm for the *Nte4-Npe6* nanosheets, Figures 2A and S6). It is noteworthy that the

obtained β -Ala-*Npe*₆ nanosheet is one of thinnest 2D peptoid materials reported, at 2.0 nm thickness. In nanosheet-forming peptoids featuring an *Npe*₆ hydrophobic domain, the crystalline core consists of chains in an extended all-*cis* amide backbone Sigma-strand conformation, which is approximately 1.6 nm in length.⁴¹ Thus, the measured 2.0 nm thickness of the β -Ala-*Npe*₆ nanosheets suggests that these peptoids share a similarly extended all-*cis* backbone conformation within the unit cell.

3.2. Molecular packing in ultrathin peptoid nanosheets

To investigate nanosheet crystallinity, we conducted powder X-ray diffraction (PXRD) and wide-angle X-ray scattering (WAXS) analyses (Figures 2B and S9). Consistent with a previous study,⁹ our *Nte*₄-*Npe*₆ nanosheets exhibited a (100) reflection at 4.5 Å and a (002) reflection at 16.0 Å in their PXRD patterns, corresponding to the *a*-spacing and *c*-spacing of the peptoid lattice, respectively (black line, Figure 2B). The *a*-spacing was assigned to the distance between two peptoid backbones stacked in a row, and the *c*-spacing was assigned to the distance between two peptoid rows (Figure S1B). Similarly, β -Ala-*Npe*₆ nanosheets displayed nearly identical lattice parameters in their PXRD patterns (red line, Figure 2B), with *a*- and *c*-spacings of 4.5 Å and 15.9 Å, respectively, suggesting a highly comparable crystalline structure (Figure 1B). However, the broadened full width at half-maximum observed for both the (100) and (002) reflections in the β -Ala-*Npe*₆ nanosheets (FWHM at (100) = 0.23 Å⁻¹ and FWHM at (002) = 0.15 Å⁻¹, Figure 2B) indicates that the obtained nanosheets are less ordered than previous *Nte*₄-*Npe*₆ nanosheets. WAXS analysis confirmed that β -Ala-*Npe*₆ nanosheets maintain a crystalline lattice with an *a*-spacing of 4.5 Å and a *c*-spacing of 16.0 Å in aqueous conditions (Figure S9). These spacings in WAXS patterns, which are highly similar to those observed in the crystalline lattice of *Nte*₄-*Npe*₆ nanosheets both in solution and dry states,⁹

suggests that despite a significant shortening of the polar domain, the crystalline cores share a common lattice structure.

Cryo-TEM analysis was performed to reveal the molecular packing within β -Ala-*Npe*₆ nanosheet crystals. Using the vitrified β -Ala-*Npe*₆ nanosheets in 0.1 M tetrabutylammonium chloride (*n*-Bu₄NCl)_(aq), the molecular packing of β -Ala-*Npe*₆ was visualized directly, as shown in Figure 2C. These cryo-TEM images of β -Ala-*Npe*₆ nanosheets represent the molecular-level visualization revealing the actual packing of the crystalline domains in β -Ala-*Npe*₆ nanosheets, designed with a minimal charged motif as the end group of peptoids.^{11, 12} This nanosheet presented parallel V-shaped packing in the molecular lattice, as previously reported for *Nte*₄-*Npe*₆.⁹ The bright center spot of the V-shaped conformation corresponds to backbone, and the two arms represent the side chains. The lattice dimensions are indicated by white arrows in the cryo-TEM image: $a = 4.5 \text{ \AA}$ (100) and $c = 16.2 \text{ \AA}$ (002), which were also confirmed by the power spectrum (Figure 2D). The a and c spacings observed in cryo-TEM analysis are in good agreement with the PXRD results (Figure 2B), indicating that the cryo-TEM lattice of β -Ala-*Npe*₆ accurately reflects the overall nanosheet structure (Figure 1B).

The reported pK_a values of β -alaninamide and 2-[2-(2-methoxyethoxy)ethoxy] are 9.2 (experimental value)⁵⁵ and 9.5 (calculated value),⁵⁶ respectively. Given that the measured pH of the nanosheet solutions after self-assembly is in the range of 4.0–4.6 (pH = 4.0 for β -Ala-*Npe*₆ nanosheets, pH = 4.6 for *Nte*₄-*Npe*₆ nanosheets), both peptoids' *N*-termini are expected to be largely protonated and carry a significant positive charge density on the nanosheet surface, with different tethering lengths to the crystalline domains. Because the pK_a of charged residues can vary dramatically upon assembly compared to soluble small molecules, we performed a pH titration of assembled β -Ala-*Npe*₆ nanosheets, which yielded an apparent pK_a of 8.4 (see Section 2.7 and Figure S10). This indicates that, across most of the pH conditions examined in

this study (pH 4.2–6.9), at least 96% of the *N*-termini are protonated and positively charged. Thus, the nanosheet surface remains highly protonated with an estimated charge density of $+0.7 \text{ e/nm}^2$, comparable to the charge densities of biomolecular systems known to promote counterion condensation, such as anionic lipid bilayers ($0.8\text{--}1.5 \text{ e/nm}^2$)⁵⁷ and dsDNA ($\sim 1 \text{ e/nm}^2$).⁵⁸

To more clearly elucidate the charge state on the nanosheet surface, where it is posited that nearly all densely displayed *N*-termini can carry charges, we constructed a molecular model of the β -Ala-*Npe*₆ nanosheet and carried out MD simulations (see Section 2.11 for details on molecular simulations). A full-atomistic model of the β -Ala-*Npe*₆ nanosheet with all-*cis* backbones was constructed based on cryo-TEM, XRD, and WAXS data, together with the established *Nte*₄-*Npe*₆ nanosheet structure.⁹ The initial model of the β -Ala-*Npe*₆ nanosheet was prepared with all the *N*-terminal groups set to a positively charged state (Figure S13). This lattice structure was simulated over 50 ns, and the nanosheets maintained their structural integrity (Figure 2E). This result indicates that noncovalent interactions among the *Npe*₆ blocks are sufficient to maintain the lattice and support a highly charged surface in an ultrathin crystalline nanosheet, even when the *N*-termini are fully protonated. The measured *a*- and *c*-spacings (4.9 Å and 16.2 Å, respectively) are in good agreement with the experimental observations (4.5 Å and 16.2 Å, respectively) from the cryo-TEM analysis (Figures 2C and 2D), indicating that the simulated atomic models reasonably represent the experimental results.

From the relaxed MD models of the peptoid nanosheets, we found that the intercharge distance on the surface of the β -Ala-*Npe*₆ nanosheets is $5.44 \pm 0.01 \text{ Å}$ (Figure 2E), which is shorter than the Bjerrum length (0.7 nm at 25 °C) in water,^{58, 59} indicating that the electrostatic interactions between charges on the β -Ala-*Npe*₆ nanosheets may not be negligible when constructing the lattice against thermal fluctuations ($k_{\text{B}}T = \sim 0.6 \text{ kcal/mol}$ at 25 °C). However,

whether this subtle electrostatic repulsion leads to a meaningful impact on lattice thermal stability remains unclear, despite the presence of increased lattice disorder indicated by the broadened PXRD patterns (Figure 2B).

3.3 Thermal stability of nanosheets

The thermal stability of the nanosheets was analyzed using NanoDSC by measuring the melting temperature (T_m) in various aqueous solutions (Figure 3A). The T_m of the β -Ala-*N*pe₆ nanosheets is 69.4 °C, whereas that of the *N*te₄-*N*pe₆ nanosheets is 87.3 °C in water,³¹ indicating that the absence of the PEG-like amorphous block, *N*te, reduced the thermal stability of the nanosheets. This reduced thermal stability may be attributed to increased local structural disorder in the β -Ala-*N*pe₆ sheets, as shown by the broadened diffraction peaks (increased FWHM) observed in the PXRD analysis (Figure 2B), which may in turn arise in part from electrostatic repulsion between the densely localized surface charges.

To evaluate the extent to which electrostatic repulsion contributes to lattice stability, the electrostatic repulsion between terminal groups was calculated from Coulomb's law (eq1), where $q_1=q_2=e^+$ (1.602×10^{-19} C), r is the intercharge distance, ϵ_0 is the vacuum permittivity (8.85×10^{-12} Fm⁻¹), and ϵ_r is the relative dielectric constant of water (78.5).

$$U_{(r)} = \frac{1}{4\pi\epsilon_0\epsilon_r} \frac{q_1q_2}{r} \times \frac{N_A}{4184} \text{ (kcal/mol)} \quad [eq1]$$

The surface charges on the β -Ala-*N*pe₆ nanosheets with a calculated intercharge distance of 5.44 Å would destabilize the nanosheet structure by 0.78 kcal/mol per pair of adjacent charges in water. This intercharge distance differs from the crystallographic a -spacing determined by WAXS because the charged *N*-terminal ammonium groups are not fixed at the backbone lattice positions: each ammonium is tethered to the crystalline core through two

methylene units ($-\text{CH}_2-\text{CH}_2-$), whose conformational freedom allows the charges to become off-aligned from the a -axis. Given an intercharge distance of $6.2 \pm 0.24 \text{ \AA}$ in the $N\text{te}_4\text{-Npe}_6$ nanosheets (Figure S14), the electrostatic repulsion between adjacent N -terminal charges was estimated to be 0.68 kcal/mol in water. The difference in electrostatic repulsion of surface charges between $\beta\text{-Ala-Npe}_6$ and $N\text{te}_4\text{-Npe}_6$ is therefore only 0.10 kcal/mol.

To further investigate the origin of this difference in thermal stability, we measured the enthalpy of the melting transitions for both nanosheets. The reported enthalpy for the melting transitions of the $N\text{te}_4\text{-Npe}_6$ nanosheets is approximately 20 kcal/mol.³⁷ Our measured enthalpy for the $\beta\text{-Ala-Npe}_6$ sheets was found to be $19.6 \pm 1.6 \text{ kcal/mol}$, indicating that the melting enthalpy is not the primary determinant of the change in nanosheet thermal stability. The non-covalent interactions that stabilize the nanosheet lattice, including $\text{CH}\cdots\text{O}=\text{C}$ hydrogen bonds between amide backbones, $\pi-\pi$ interactions among aromatic side chains, and hydrophobic contacts, appear to be maintained in both peptoid nanosheets and to remain strong enough to offset the electrostatic repulsion between adjacent surface charges,³⁷ even at the shorter intercharge distance of the $\beta\text{-Ala-Npe}_6$ nanosheet. Accordingly, the increased lattice disorder indicated by the broadened PXRD peaks of the $\beta\text{-Ala-Npe}_6$ nanosheets is not large enough to alter the melting enthalpy, and the intermolecular interactions within the lattice sufficiently accommodate the inter-charge repulsion.

Consistent with this interpretation, nanosheet formation was also observed upon removing an additional methylene group from the charged N -terminus by the incorporation of glycine (Gly-Npe_6) (Figure S7), which is expected to further reduce the terminal charge separation to $5.07 \pm 0.02 \text{ \AA}$, corresponding to an electrostatic repulsion potential of 0.84 kcal/mol in water, as indicated in the MD simulations (Figure S15). This result indicates that

electrostatic repulsion does not play a dominant role in determining lattice stability in nanosheets with short hydrophilic terminal groups.

Given the comparable melting enthalpies of the two systems, the lower melting temperature of the β -Ala-*N*pe₆ nanosheets relative to *N*te₄-*N*pe₆ suggests a dominant entropic contribution. To investigate this entropic origin, we performed MD simulations of each nanosheet, in which every peptoid was assigned a single TFA[−] anion at its *N*-terminus as the counterion, introduced stoichiometrically during RP-HPLC purification. The last 10 ns of each trajectory were used to examine the TFA[−] counterion distribution, which differs markedly between the two surfaces. In the β -Ala-*N*pe₆ sheet, the *N*-terminal ammonium groups associate strongly with the TFA[−] oxygens, producing a sharp first peak in the $N_{peptoid}^+ \cdots O_{TFA}^-$ radial distribution function (RDF) at 2.75 Å ($g(r) \approx 67$; Figure S24A): on average ~34 of the 72 TFA[−] counterions reside within 5.5 Å of an *N*-terminal ammonium (first coordination shell), with ~48% of the *N*-termini forming direct contact ion pairs (CIPs; $N_{peptoid}^+ \cdots O_{TFA}^- < 3.5$ Å) and a further ~23% forming solvent-separated ion pairs (SSIPs; $N_{peptoid}^+ \cdots O_{TFA}^- = 3.5\text{--}5.5$ Å), so that ~71% of the *N*-termini retain a counterion within the first shell (Figure S24B).

In contrast, the *N*te₄-*N*pe₆ sheet shows only weak association ($N_{peptoid}^+ \cdots O_{TFA}^-$ RDF's first peak at 2.75 Å, $g(r) \approx 7$, Figure S24A): on average ~5 of the 72 TFA[−] counterions lie within 5.5 Å of an *N*-terminal ammonium, with only ~6% of the *N*-termini forming CIPs and ~7% forming SSIPs (Figure S24B). This suppression arises because the flexible ethylene-glycol (*N*te) side chains fold back toward the *N*-terminal ammonium, allowing ether oxygens within the same molecule side chains to coordinate the secondary ammonium directly (Figure S14B). This intramolecular solvation partially shields the charged terminus and competes with both TFA[−] ion pairing and hydration by water, thereby reducing counterion condensation at the

Nte4-Npe6 nanosheet surface. Consequently, the densely charged ammonium groups of β -Ala, positioned directly above the crystalline core, sequester a substantially larger condensed counterion population. Upon melting (disassembly), this population is released, liberating a correspondingly greater translational entropy from the condensed counterions. Approximating this contribution as $\Delta S_{\text{release}} = n_{\text{condensed}} \times R \times \ln(c_{\text{local}} / c_{\text{bulk}})$ with $n_{\text{condensed}}$ and c_{local} from our MD trajectory analysis and c_{bulk} set to the experimental bulk TFA⁻ concentrations (3.4 and 2.1 mM, respectively), yields ~ 5.4 cal/molK for β -Ala-*Npe6* versus ~ 0.7 cal/molK for *Nte4-Npe6*, corresponding to an approximately eightfold lower contribution for *Nte4-Npe6* nanosheets (Table S2). The distinct counterion condensation imposed by the different hydrophilic-block designs of β -Ala-*Npe6* and *Nte4-Npe6* therefore introduces an additional entropic contribution to melting, which helps explain the lower T_m of β -Ala-*Npe6*.

3.4 Thermal stability with additional salt

Having established that entropy plays a key role in governing the T_m of added salt-free β -Ala-*Npe6* nanosheets, we next examined how added salts modulate the thermal stability of these highly charged nanosheets and whether a similar entropic mechanism operates. We evaluated the melting transitions in the presence of various salts at a concentration of 0.1 M (Figures 3A and S11). We used Na⁺ as a common cation with three different halide anions (F⁻, Cl⁻, and Br⁻) in solution, and the melting enthalpy and entropy were determined by solution-state DSC (entry 2-4, Figure 3C). In the presence of 0.1 M NaF, the highest T_m was observed at 80.9 ± 0.29 °C, followed by 0.1 M NaCl ($T_m = 79.8$ °C $\pm$ 0.98 °C), and 0.1 M NaBr ($T_m = 73.8$ °C $\pm$ 0.03 °C). We confirmed that the observed T_m represents the melting of the lattice itself using variable temperature SAXS analysis by tracing the q value corresponding to the c -spacing (Figures 3B and S12).

Although the addition of salt leads to an increase in T_m values compared to those measured in the absence of added salt, indicating enhanced thermal stability, the corresponding enthalpy changes measured in the presence of salt are smaller (ca. 11–15 kcal/mol) than those obtained without added salt (19.6 kcal/mol, Figure 3C). Interestingly, this observation demonstrates that the enhanced thermal stability of β -Ala-Npe₆ nanosheets does not originate from enthalpic contributions, such as charge screening.

Based on the two-state model for melting transitions, we calculated the associated entropy changes (Figure 3C). All measured entropy changes for the melting transition were lower than that the values calculated without any additional salts (57 ± 5 cal/molK), indicating that the entropy is the main contributor to the increased thermal stability in the presence of dissolved salts.⁶⁰

To elucidate the entropic origin of the enhanced thermal stability reflected in the increased T_m , we performed MD simulations of the positively charged nanosheets in the presence of 0.1 M NaX (X = F, Cl, and Br) to match the experimental concentration, in a solvent box of dimensions $60 \times 100 \times 98$ Å ($a \times b \times c$). Because the box-imposed concentration of the one TFA[−] paired with each peptoid in the preceding simulations did not adequately reflect the experimental TFA[−]/salt ratio (3.4 mM TFA[−] vs 100 mM NaX), this TFA[−] was replaced by X[−], so that the positive charge of the *N*-termini was compensated by X[−] consistent with the added salt, thereby reflecting the experimental conditions as closely as possible in the simulations (Figures 4A and S16). After 50 ns of simulation, the peptoid lattice remained structurally stable in the presence of added salts, and counteranions were observed to interact with the ammonium groups on the peptoid surface (Figure 4A).

From the RDF plotted with the *N*-terminal ammonium nitrogen and the halide ions ($N_{peptoid}^+ \cdots X^-$) (Figure S25), computed over the final 10 ns of trajectory to approximate the charge condensation, we find that very strong counterion condensation occurs for F^- , with a sharp contact peak at 2.65 Å reaching $g(r) \approx 301$. The condensation weakens markedly for the larger, more weakly hydrated halides: Cl^- peaks at 3.15 Å with $g(r) \approx 59$, and Br^- at 3.25 Å with $g(r) \approx 26$. Consistent with this trend, the number of halide ions condensed within the first shell decreases from 58 of 107 for F^- to 32 for Cl^- and 27 for Br^- (Table S2).

Using these counts to estimate the entropy released upon melting, $\Delta S_{\text{release}} = n_{\text{condensed}} \times R \times \ln(c_{\text{local}} / c_{\text{bulk}})$, with c_{bulk} set to the experimental bulk concentration (0.1 M), gives 4.74, 1.85, and 1.38 cal/molK for F^- , Cl^- , and Br^- , respectively (Table S2). These values track the measured melting entropies trends ($\Delta S_{\text{melt}} = 42, 35$, and 32 cal/molK; Figure 3C), reproducing the experimental ordering and correlating linearly across all four ionic conditions, including the salt-free TFA reference (Pearson $r = 0.90$, slope ≈ 5.0 ; Figure S26). Both quantities are expressed per peptoid, yet the theoretical values are smaller in absolute experimental terms because $\Delta S_{\text{release}}$ isolates a single contribution, the translational entropy recovered when condensed counterions return to bulk, whereas the calorimetric ΔS_{melt} is the total melting entropy, which additionally includes the conformational entropy gained as the peptoid chains disorder, the reorganization of hydration water, and the disassembly of the 2D crystalline lattice. The counterion-release term therefore captures one ion-specific component that modulates ΔS_{melt} rather than its absolute magnitude. The near-constant intercept of the correlation (≈ 24.9 cal/molK, Figure S26) may reflect these counterion-independent contributions, which are approximately constant across the series.

Notably, all three NaX conditions in MD simulations give smaller $\Delta S_{\text{release}}$ values than the nanosheet with one TFA⁻ per peptoid ($\Delta S_{\text{release}} = 5.4 \text{ cal/molK}$), because the added 0.1 M NaX raises the bulk free-ion concentration ~ 30 -fold ($3.4 \rightarrow 100 \text{ mM}$), shrinking the $\ln(c_{\text{local}}/c_{\text{bulk}})$ term (Table S2). This effect dominates even for F⁻, although F⁻ condenses more counterions than TFA⁻ ($n_{\text{condensed}} = 58$ for F⁻ vs. 34 for TFA⁻), its $\Delta S_{\text{release}}$ is still lower because the elevated bulk concentration outweighs the larger condensation.

In addition, this entropic penalty is possibly further amplified for strongly hydrated (kosmotropic) anions such as F⁻ because, in the condensed state, an ammonium-bound F⁻ carries on average 4.5 first-shell water molecules, whereas a free F⁻ in bulk is coordinated by 6.6 waters (Table S3). Thus, upon release, each F⁻ recruits ~ 2 additional water molecules into its tight hydration shell, ordering bulk water and partially offsetting its own translational entropy gain, further lowering the net entropy available on melting. Taken together, these results provide a molecular-level entropic explanation for the salt-induced increase in T_m . By increasing the bulk free-ion concentration, added salt reduces the counterion-release entropy changes ($\Delta S_{\text{release}}$) that intrinsic surface condensation would otherwise contribute during melting in added-salt free conditions.

At the same time, the residual release entropy still follows the strength of counterion condensation, with $\text{F}^- > \text{Cl}^- > \text{Br}^-$, thereby reproducing the experimental ΔS_{melt} trend (Figure 3C). However, the T_m values are mostly dominated by the enthalpic term rather than entropic terms across NaX salt added conditions. The highest T_m ($80.9 \pm 0.29 \text{ }^\circ\text{C}$) was observed with F⁻, which has a greater electronegativity, and a lower polarizability with smaller size, compared to the other halide anions, aligning with the Hofmeister ion series for counteranions.⁶¹

We found that the nonbonded energies between the peptoids and the cations or anions in MD simulations were correlated with the melting enthalpy (ΔH_{melt}) observed experimentally (Table S4). Overall, the stabilization associated with the total nonbonded energies was greatest for NaF, followed by NaCl and NaBr (Table S4). These results are in good agreement with the nanoDSC experiments (entries 2–4, Figure 3C), in which F^- produced the strongest stabilizing effect on the nanosheets among the halide anions. We find the correlation between the specific nonbonded energy between peptoid-anion would dominantly affect the total nonbonded energy (Figure S27B). When we decomposed the peptoid–anion nonbonded energy into Coulombic (electrostatic) and Lennard–Jones (van der Waals) contributions. The Coulombic interaction is strongly attractive and follows the same $\text{F}^- > \text{Cl}^- > \text{Br}^-$ trend as the measured ΔH_{melt} (Table S5). In contrast, the Lennard-Jones contribution is repulsive between peptoid-anion pairs and is most unfavorable for F^- . However, this repulsion is much weaker than the attractive Coulombic interaction. Thus, the anion-dependent stability of the nanosheets is primarily governed by electrostatic interactions between the charged nanosheet surface and the condensed counteranions.

In addition, the previous RDF analysis demonstrated that the effective electrostatic interaction with the highest density at the closest distance from the nanosheet was observed for F^- , followed by Cl^- and Br^- (Figure S25). When the population density was investigated for the distance between *N*-terminal ammonium and X^- (Figure 4C), F^- ions showed the highest population at 2.67 Å, corresponding to CIPs (Figure 4B), while Br^- ions presented the lowest population in this regime (Figure 4C).^{62, 63} A second distinct population emerged at ~5.0–6.0 Å for all three halides, consistent with SSIPs in which a water molecule bridges the ion and the ammonium group. Classifying each *N*-terminus by its nearest $\text{N}^+ \cdots \text{X}^-$ distance, 83% of termini form contact ion pairs (CIP) with F^- and a further 10% are solvent-shared ion pairs (SSIP),

whereas only 40%/24% (Cl^-) and 26%/29% (Br^-) are CIP/SSIP, respectively (Figure 4D). The CIP-to-SSIP population ratio decreased progressively from F^- to Cl^- to Br^- (Figure 4D), indicating that the smaller, less polarizable F^- preferentially forms direct contacts with the *N*-terminal ammonium groups, whereas the larger, more polarizable Br^- favors solvent-mediated interactions.⁶¹

These results suggest that the molecular origin of the specific ion effect on T_m , which follows the Hofmeister ordering, may arise from counteranion-dependent charge condensation on the nanosheets. These findings highlight the importance of understanding the intrinsic properties of counteranions and their interactions with the surface charges of peptoid nanosheets to elucidate their thermal behavior at the molecular level.

Consistent with this ion-specific, condensation-based picture, T_m showed only a weak and non-monotonic dependence on NaCl concentration over the ~ 100 -fold range from 0.01 to 1.0 M (77.1–79.8 °C; entries 3, 5, and 6; Figure 3C), despite a nearly ten-fold decrease in the Debye screening length from 30.4 to 3.0 Å across the intercharge distance of adjacent surface ammonium groups (5.44 Å). This lack of correlation indicates that electrostatic charge screening alone does not account for nanosheet stabilization.

To investigate cation effects, we examined the melting transitions in the presence of NaCl and *n*-Bu₄NCl at a concentration of 0.1 M, both of which share the same anion (Cl^-). The influence of countercations is generally considered less dominant than that of counteranions on protein self-aggregation and on highly positively charged surfaces.⁶¹ As expected, the β -Ala-*N*pe₆ nanosheets in 0.1 M *n*-Bu₄NCl_(aq) exhibited the T_m of 78.8 ± 0.48 °C (entry 7, Figure 3C), which is comparable to that observed within 0.1 M NaCl_(aq) ($T_m = 79.8 \pm 0.96$ °C, entry 3, Figure 3C). Consistent with their comparable T_m values, the total nonbonded energies

calculated for the NaCl and *n*-Bu₄NCl systems were nearly identical (-367.17 ± 0.68 and -367.39 ± 0.31 kcal/mol; Table S4), indicating that the overall ion-mediated stabilization is dominated by the shared Cl⁻ counterion. However, despite the similar T_m values, the associated enthalpy changes differed subtly, with a ΔH of 15.1 ± 0.03 kcal/mol in 0.1 M *n*-Bu₄NCl_(aq), and a ΔH of 12.2 ± 1.6 kcal/mol in 0.1 M NaCl_(aq). Given that β -Ala-*N*pe₆ nanosheets possess a high positive surface charge density and exhibit strong counteranion effects, it was not expected that solution cations would provide an additional enthalpic contribution to nanosheet stabilization.

The *n*-Bu₄N⁺ is a relatively lipophilic and bulky cation capable of engaging in dispersion interactions with other hydrophobic *N*pe side chains (Figure 5A).⁶⁴ We therefore examined whether *n*-Bu₄N⁺ interacts with the solvent-exposed *N*pe side-chain blocks. In MD simulations of β -Ala-*N*pe₆ nanosheets in 0.1 M *n*-Bu₄NCl, the ion-density profile along the *b*-axis shows that the *n*-Bu₄N⁺ cations are electrostatically excluded from the immediate vicinity of the positively charged ammonium surface, where instead the Cl⁻ counterions condense (Figure S28). Despite this electrostatic exclusion, the flexible butyl chains of *n*-Bu₄N⁺ make direct van der Waals contacts with the exposed hydrophobic *N*pe side chains. On average, ~ 1.8 of the 35 *n*-Bu₄N⁺ cations are in direct contact (< 4.5 Å) with *N*pe carbons at any point in the MD, and their closest-approach distribution shows a distinct contact population at 3.5–4.5 Å that is absent for Na⁺ (Figure 5B). Quantitatively, *n*-Bu₄N⁺ contacts the hydrophobic side chains ~ 50 -fold more often than Na⁺ (5.3 % vs. 0.1% of cations within 4.5 Å; Figure S29), so that these dispersion contacts may explain why *n*-Bu₄N⁺ exhibit a negative (favorable) peptoid–cation nonbonded energy, of -0.15 kcal/mol (Table S4). In contrast, the small, strongly solvated Na⁺ retains its hydration shell and makes negligible direct contact with the hydrophobic side chains (Figure 5B). Accordingly, it shows a positive peptoid–cation nonbonded energy across the NaF, NaCl, and NaBr simulations, indicating a negligible or even

destabilizing interaction between peptoid and cations (Table S4). This result suggests that the chemical identity of the cation can introduce an enthalpic contribution to nanosheet stabilization, even for a positively charged surface dominated by anion interactions.

4. CONCLUSION

In this study, we developed ultrathin peptoid nanosheets with a minimal charged motif as a structurally well-defined model system for examining ion-dependent thermal stability in highly charged 2D soft materials. Structural analyses showed that β -Ala-*Npe*₆ homopolypeptoids forms crystalline nanosheets with a molecular packing closely related to that of the *Nte*₄-*Npe*₆ diblock copolypeptoids, while presenting a simplified and highly charged crystalline surface. Looking ahead, tuning the length of the hydrophobic *Npe* block offers a complementary strategy for controlling nanosheet thickness. However, shortening the *Npe* block may require additional side chain engineering, such as incorporating halogenated aryl or biphenyl groups,^{9, 13} to strengthen inter-sidechain interactions and maintain robust crystalline assembly with shorter crystallizing blocks.

Solution-state DSC and structural analyses revealed that the thermal stability of these nanosheets depends strongly on the aqueous ionic environment. The β -Ala-*Npe*₆ nanosheets exhibited a lower melting temperature than *Nte*₄-*Npe*₆ nanosheets, yet the two systems showed nearly identical melting enthalpies, indicating that this difference in thermal stability is governed by an entropic term. MD simulations showed that *Nte*₄-*Npe*₆ nanosheets have a diminished capacity for counterion condensation relative to β -Ala-*Npe*₆ nanosheets because the hydrophilic *Nte* blocks hydrate and form a solvating pocket around the ammonium termini. On this basis we interpret the lower stability of β -Ala-*Npe*₆ nanosheets as arising from the larger

translational entropy ($\Delta S_{\text{release}}$) gained upon melting, since its more strongly condensed counterions are released.

The addition of NaX salts to β -Ala-Npe₆ nanosheets increased their thermal stability (i.e., raised T_m). Because the magnitude of ΔH instead decreased relative to the salt-free condition, this change in thermal stability was likewise governed by an entropic term. The counterion-release effect is attenuated upon adding salt since the elevated bulk free-ion concentration reduces the entropy ($\Delta S_{\text{release}}$) liberated upon melting. Using MD simulations to reproduce the condensation behavior of each added salt, we showed that the resulting release entropies correlate with the melting entropies determined experimentally.

The halide series showed a clear ion-specific trend, with F⁻ producing the strongest enthalpic stabilization, followed by Cl⁻ and Br⁻. In particular, F⁻ exhibited a higher propensity for CIP with the *N*-terminal ammonium groups on the surface, whereas MD simulations for Br⁻ found more solvent-mediated interactions. Together, these results indicate that ion-specific stabilization in this system is likely linked to differences in the interfacial ion-binding mode rather than to charge screening alone.

Overall, this work demonstrates that salt-induced stabilization of highly charged peptoid nanosheets is governed primarily by the entropy associated with the release of condensed counterions upon melting, while solute ion identity provides an additional, enthalpic contribution to stability through distinct interfacial binding modes. By resolving how individual ions partition between entropic and enthalpic stabilization at a molecularly defined charged interface, the combined calorimetry, electron microscopy, and MD simulations introduced here provides a transferable strategy for tuning the stability and function of these materials through their ionic environment.

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SUPPORTING INFORMATION

The Supporting Information includes supporting figures and tables (Figures S1–S20 and Table S1–S3) (PDF). The initial frames and their corresponding MD trajectories are available on Zenodo (DOI: 10.5281/zenodo.22689333).

MD models are included:

PDB file showing the initial MD model of β -Ala-Npe₆ nanosheets (PDB)
PDB file showing the final MD model of β -Ala-Npe₆ nanosheets (PDB)
PDB file showing the initial MD model of Nte₄-Npe₆ nanosheets (PDB)
PDB file showing the final MD model of Nte₄-Npe₆ nanosheets (PDB)
PDB file showing the initial MD model of Gly-Npe₆ nanosheets (PDB)

PDB file showing the final MD model of Gly-*N*pe₆ nanosheets (PDB)
PDB file showing the initial MD model of β -Ala-*N*pe₆ nanosheets with NaF salts (PDB)
PDB file showing the final MD model of β -Ala-*N*pe₆ nanosheets with NaF slats (PDB)
PDB file showing the initial MD model of β -Ala-*N*pe₆ nanosheets with NaCl salts (PDB)
PDB file showing the final MD model of β -Ala-*N*pe₆ nanosheets with NaCl slats (PDB)
PDB file showing the initial MD model of β -Ala-*N*pe₆ nanosheets with NaBr salts (PDB)
PDB file showing the final MD model of β -Ala-*N*pe₆ nanosheets with NaBr slats (PDB)
PDB file showing the initial MD model of β -Ala-*N*pe₆ nanosheets with *n*-Bu₄NCl salts (PDB)
PDB file showing the final MD model of β -Ala-*N*pe₆ nanosheets with *n*-Bu₄NCl slats (PDB)

NOTES

The authors declare no competing financial interest.

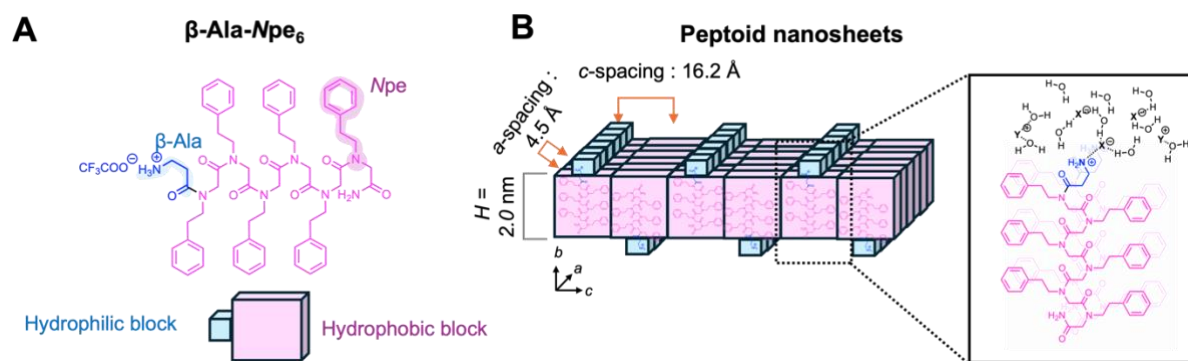


Figure 1. (A) Chemical structures of β -Ala-Npe₆. (B) Schematic representation of monolayered peptoid nanosheets obtained from crystallization driven self-assembly.

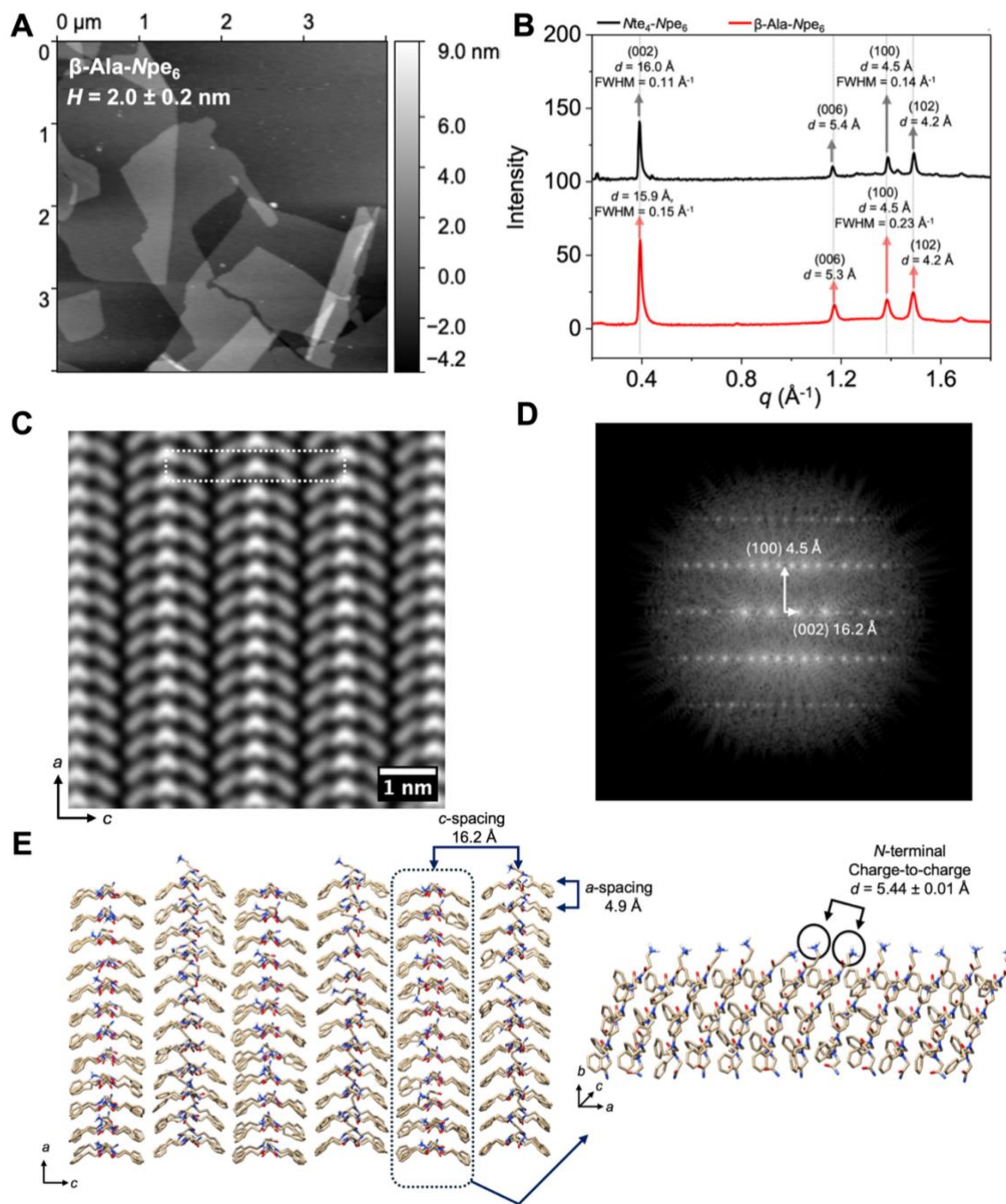


Figure 2. (A) AFM micrographs of $\beta\text{-Ala-Npe}_6$ nanosheets. The height average (H) and standard error ranges were calculated by averaging independent height measurements ($N=11$). The representative height measurements of nanosheets are provided in Figure S6. (B) PXRD patterns of dried peptoid nanosheets. (C) Cryo-TEM images of $\beta\text{-Ala-Npe}_6$ nanosheets in the presence of 0.1 M $n\text{-Bu}_4\text{NCl}_{(\text{aq})}$. The white dotted box indicates the unit cell of peptoid lattice.

(D) The power spectrum of the cryo-TEM image on panel B. (E) All atomistic model of β -Ala-*Npe*₆ nanosheet, built with positively charged *N*-terminal groups with TFA anions, after 50 ns simulation.

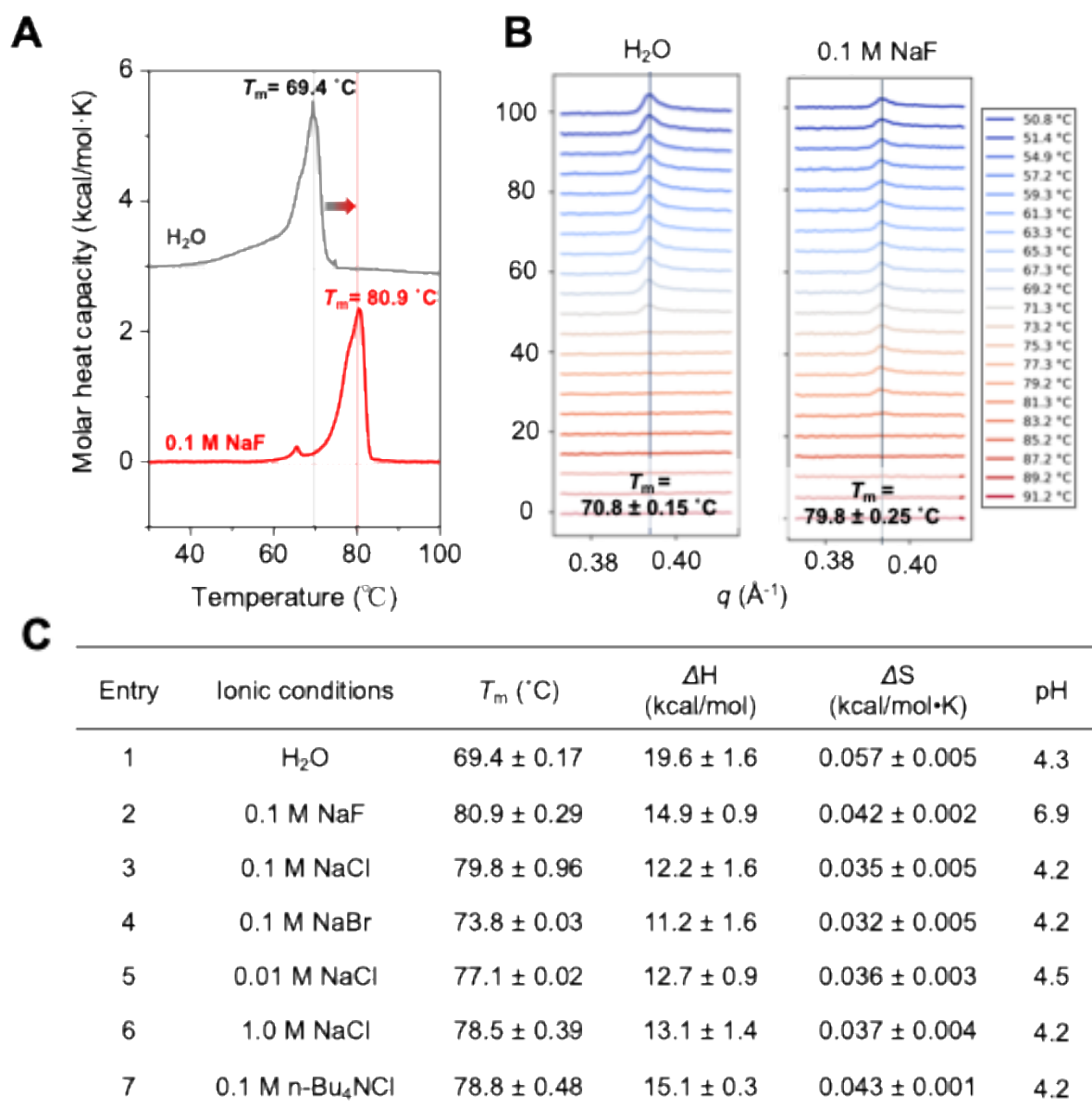


Figure 3. (A) NanoDSC trace of β -Ala-*Npe*₆ nanosheets with and without 0.1 M added NaF salt. (B) Variable temperature-SAXS traces at $q = \sim 0.39 \text{ \AA}^{-1}$ corresponding to *c*-spacing of peptoid lattices with and without added 0.1 M NaF salt. (C) Summarized thermal stability and

parameters of β -Ala-Npe₆ nanosheets in different ionic conditions measured by NanoDSC (Figure S11). ΔS was calculated based on the two-state model for melting transitions, in which $\Delta G = 0$ at T_m by definition.

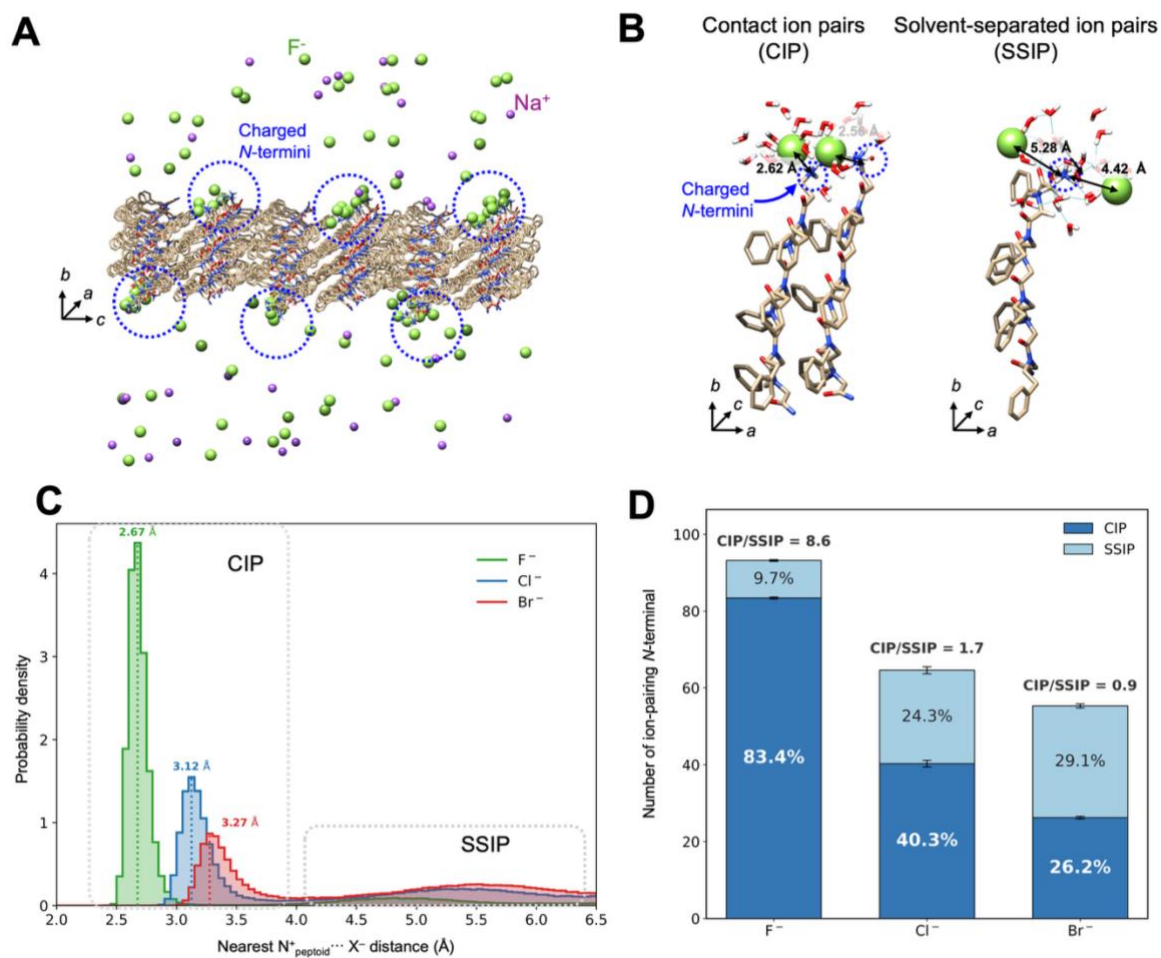


Figure 4. Interactions between counter anions and surface charges on peptoid nanosheets revealed by MD simulations. (A) Final frame of the relaxed MD model of β -Ala-Npe₆ nanosheets in the presence of NaF after 50 ns. (B) Representative contact ion pairs (CIP) and solvent-separated ion pairs (SSIP) observed in the relaxed MD model of β -Ala-Npe₆ nanosheets in the presence of 0.1 M NaF after 50 ns. (C) Histogram of the distances between the N atoms of the N-terminal ammonium groups of the peptoids and the counter anions over the last 10 ns of the MD simulations. The bin size is 0.05 Å. (D) CIP vs SSIP classification of

the 72 N-termini by nearest $N^+ \cdots X^-$ distance (final 10 ns), averaged over five independent replicas. CIP/SSIP cutoffs were set for F^- (CIP < 3.5 Å; SSIP 3.5–5.5 Å) and shifted for the heavier halides by the difference in ionic radius relative to F^- (Pauling radii F^- 1.33, Cl^- 1.81, Br^- 1.96 Å), giving CIP < 3.98 Å / SSIP 3.98–5.98 Å for Cl^- and CIP < 4.13 Å / SSIP 4.13–6.13 Å for Br^- .

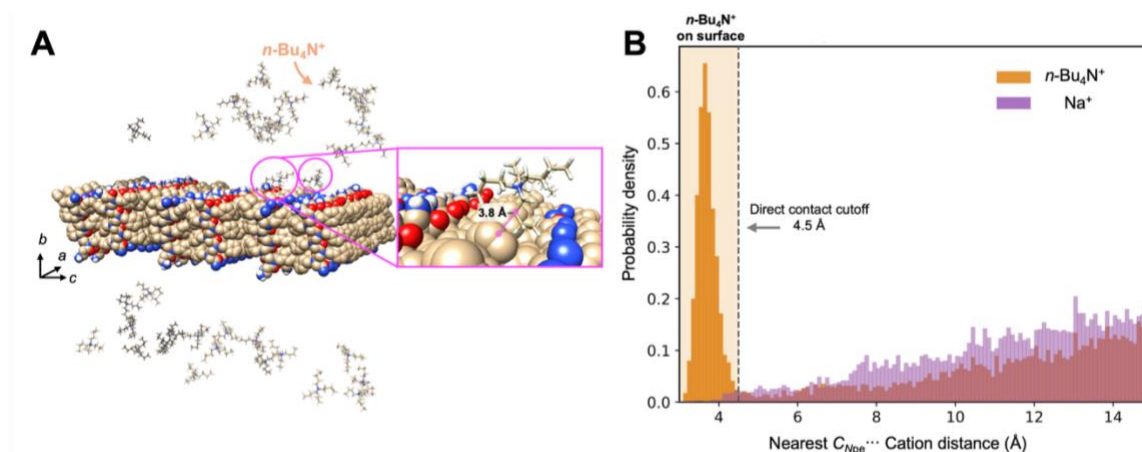


Figure 5. The interaction between cations in solution and peptoid nanosheet surface. (A) The last frame of MD simulation of $\beta\text{-Ala-Npe}_6$ nanosheets with $n\text{-Bu}_4\text{NCl}$. (B) Direct contact between the Npe side chains and the solution cations, from MD simulations of $\beta\text{-Ala-Npe}_6$ nanosheets in 0.1 M $n\text{-Bu}_4\text{NCl}$ (orange) and 0.1 M $NaCl$ (purple), over the final 10 ns, averaged over five independent replicas. The cation–surface distance is defined as the minimum heavy-atom separation between the cation and any Npe side-chain carbon (over the 16 butyl carbons for $n\text{-Bu}_4\text{N}^+$, excluding the central N; the single ion for Na^+). The bin size is 0.1 Å.

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GRAPHICAL ABSTRACT

Highly charged peptoid nanosheets

