



## Peptoid-based macrodiscs of variable lipid composition for structural studies of membrane proteins by oriented-sample solid-state NMR

Azamat R. Galiakhmetov, Adit A. Shah, Addison Lane, Carolyn M. Davern, Caroline Proulx, Alexander A. Nevzorov\*

Department of Chemistry, North Carolina State University, 2620 Yarbrough Drive, Raleigh, NC 27695-8204, United States

### ARTICLE INFO

Edited by Bauke W. Dijkstra

#### Keywords:

Solid-state NMR  
Magnetically aligned bilayers  
Lipids  
Peptoids  
Membrane proteins  
Spectroscopic resolution

### ABSTRACT

Solid-state Nuclear Magnetic Resonance (NMR) in combination with magnetically aligned discoidal lipid mimics allows for studying the conformations of membrane proteins in planar, lipid-rich bilayer environments and at the physiological temperature. We have recently demonstrated the general applicability of macrodiscs composed of DMPC lipids and peptoid belts, which yield magnetic alignment and NMR spectroscopic resolution comparable or superior to detergent-containing bicelles. Here we report on a considerable improvement in the magnetic alignment and NMR resolution of peptoid-based macrodiscs consisting of a mixture of the zwitterionic and negatively charged lipids (DMPC/DMPG at the 85% to 15% molar ratio). The resulting linewidths are about 30% sharper due to the higher orientational order parameter likely arising from the stabilizing electrostatic repulsion between the discs. Moreover, highly aligned, detergent-free macrodiscs can be formed with a longer-chain lipid, DPPC. Interestingly, the spectra of Pf1 in the two lipid mimetics are almost indistinguishable, which would mean that the overall transmembrane helix tilt might be governed not only by the hydrophobic matching but also possibly by the interactions of the flanking lysine and arginine residues at the membrane interface.

### Introduction

Magnetically oriented lipid bilayers such as anisotropic bicelles (Durr et al., 2012; Gayen et al., 2013; Glover et al., 2001; Prosser et al., 1998; Sanders and Prestegard, 1990; Sanders and Schwonek, 1992; Verardi et al., 2011; Vold and Prosser, 1996) have been widely utilized for structure determination of membrane proteins by oriented-sample (OS) NMR spectroscopy. Bicelles are typically aligned with their membrane plane parallel to the field of the NMR magnet, due to the negative susceptibility anisotropy of the lipid hydrophobic alkyl chains (Scholz et al., 1984). In OS NMR, the positions of the spectral peaks are highly sensitive to the changes in the local structure and environment since they arise from angular-dependent observables such as chemical shift anisotropy and dipolar interactions. Thus, this technique can be effectively used for structural and dynamic studies of membrane proteins in their native-like, fully hydrated planar lipid membrane environments. Bicelles are usually prepared by the mixing of long-chain lipids (mainly DMPC) with either short-chain lipids [usually DHPC (De Angelis et al., 2004)] or detergents [TX-100 (Park and Opella, 2010) or CHAPSO (Sanders and Prestegard, 1990)] at higher molar ratios  $q$  of DMPC to the

detergent molecules, typically at  $q = 3-5$ . Moreover, in order to be able to study lipid-induced protein conformations and activity, it is important to create lipid mimetics closely resembling the composition of biological cells. The bilayer milieu is now believed to be composed of heterogeneous lipid domains, called lipid rafts (Simons and Toomre, 2000), which give rise to different bulk bilayer properties such as viscosity and elasticity. Recently, DMPC-based bicelles rich in cholesterol (De Angelis et al., 2022) and cholesterol and sphingomyelin (Hutchison et al., 2020) have been introduced for structure-function studies of membrane proteins by solid-state and solution NMR. Despite their utility for protein structure determination, however, the presence of detergents may influence the structure and function of the embedded membrane proteins. To address this important issue, recently magnetically alignable lipodiscoidal mimetics have been actively explored including peptide-belt stabilized macrodiscs (Park et al., 2011) and discs encircled by styrene-maleic acid (SMA) co-polymer chains (Park et al., 2020; Radoicic et al., 2018; Ravula et al., 2017). The peptide belts are derived from the naturally occurring lipoproteins, whose overall length and folding stabilizes the disc and determines its size. This constraint may make the lipodiscs less stable when loaded with higher amounts of the

\* Corresponding author.

E-mail address: [alex\\_nevzorov@ncsu.edu](mailto:alex_nevzorov@ncsu.edu) (A.A. Nevzorov).

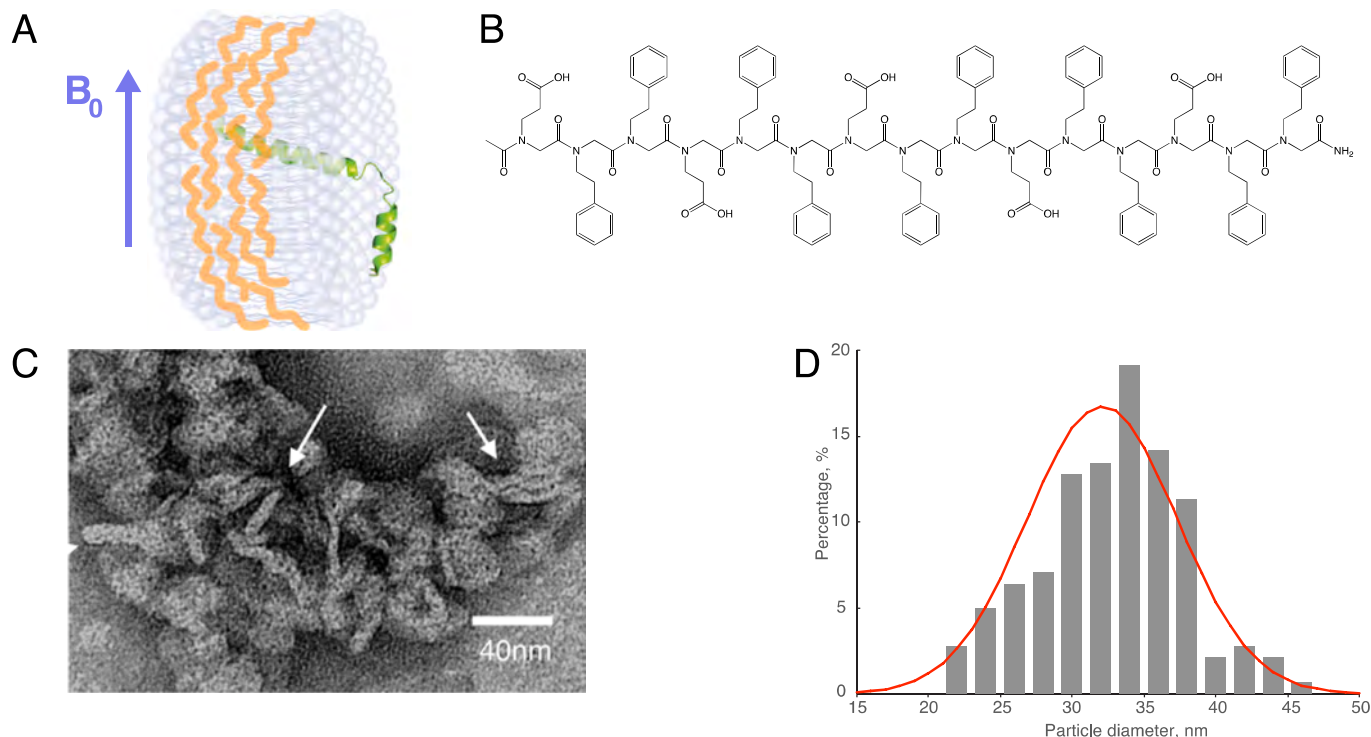
lipids and the membrane protein of interest. Moreover, the main disadvantage of commercially available SMA copolymers is a high degree of variation in the molecular length and concentration from batch to batch. Typically, broad bimodal size distributions are observed, which are governed by an abundance of short oligomers as indicated by ca. two-fold difference between the average molecular weight and the number average molecular weight for the polymer. Such broad distributions can give rise to highly polydisperse species and, therefore, lower orientational order parameters, thereby yielding broader NMR lines. By contrast, utilizing amphipathic belts having both high flexibility and uniformity in length would make the disc size and their magnetic alignment more homogeneous. This, in turn, would undoubtedly lead to a considerable improvement in NMR resolution for oriented membrane proteins.

In our recent work (Galiakhmetov et al., 2022) we reported on novel highly aligned macrodiscs formed by amphipathic 15-mer peptoid belts, cf. Fig. 1A. Peptoids (Zuckermann et al., 1992) consist of oligomers of *N*-substituted glycines having side-chains attached to the nitrogen atoms rather than the  $\alpha$ -carbons. They are readily synthesized by submonomer solid-phase synthesis using commercially available reagents (Zuckermann et al., 1992; Sun and Zuckermann, 2013). Due to the lack of the main chain hydrogen-bond donor (i.e. the  $H_N$  proton) and tertiary amide bond (Sun and Zuckermann, 2013; Zuckermann and Kodadek, 2009), in the absence of structure-inducing side chains peptoids exhibit high structural flexibility. Thus, they represent an attractive class of peptidomimetics that maintain a peptide-like atomic backbone arrangement, with their conformations likely dominated by sidechain interactions with the surrounding lipids and water, which assist in the formation of macrodiscs. Due to the high biocompatibility and proteolytic resistance of peptoids, they have also been used for the delivery of genetic materials and therapeutics (Fowler and Blackwell, 2009) and as antimicrobial agents (Mojsoska et al., 2015; Molchanova et al., 2017). An important prerequisite for making stable peptoid-based macrodiscs is the ability to

control the peptoid-lipid interactions and the disc size. Similarly to SMA copolymers and lipoproteins, peptoid constructs having periodic and amphipathic side-chain arrangements can self-assemble around a lipid membrane forming peptoid-encapsulated lipid fragments (Zhang et al., 2017; Zhang et al., 2019). The interaction between the amphipathic peptoid and the lipid membrane is driven by the hydrophobicity of the side-chain on the one side, hydrophilicity on the other, and was found to be dependent on the length of the amphipathic peptoid polymer (Jing et al., 2012; Andreev et al., 1860; Landry et al., 2019) and the overall peptoid-to-lipid molar ratio. The side chains and their relative arrangement can be chosen to mimic SMA co-polymers with the styrene and maleic acid monomers substituted by *N*-(2-phenylethyl)glycine (Npe) and *N*-(2-carboxyethyl)glycine (Nce) residues, respectively, incorporated at the 2:1 molar ratio. Unlike SMA, however, the synthesized peptoids have a highly controllable and regular length, which has greatly improved the stability and alignment of the macrodiscs as compared to bicelles and peptide-belt macrodiscs (Galiakhmetov et al., 2022).

## Materials and methods

A peptoid sequence composed of fifteen (15) alternating hydrophobic and hydrophilic side chains, patterned at a pre-defined molar ratio of 2:1 (Fig. 1B), has been synthesized from submonomers of phenylethyl amine and 3-aminopropanoic acid *tert*-butyl ester ( $\beta$ -Alanine *tert*-butyl ester) as previously described (Galiakhmetov et al., 2022). Briefly, the peptoid was synthesized on 200 mg Rink amide resin with a loading of 0.698 mmol/g as a solid support. The solid phase submonomer cycle starts with acylation by addition of 0.6 M bromoacetic acid in dimethylformamide (DMF) and 86  $\mu$ L of *N,N'*-diisopropylcarbodiimide. The acylation stage is followed by addition of a 1.5 M solution of primary amine in DMF for 1 h for the displacement step. The cycle is repeated until completion of the 15-mer peptoid, making sure to drain the



**Fig. 1.** A. A pictorial representation of a peptoid-stabilized lipid macrodisc with the incorporated Pf1 coat protein (green helix in a ribbon representation; pdb ID 2KSJ) aligned in the presence of magnetic field; the peptoid chains are sketched in orange color. B. The chemical structure for the 15-mer peptoid belt with periodically alternating hydrophilic and hydrophobic side chains (chemical formula  $C_{127}H_{150}N_{16}O_{26}$ , MW = 2.3 kDa). C. Negative-stain TEM micrograph of peptoid-stabilized DMPC macrodisc with incorporated Pf1 coat-protein. D. Particle size distribution histogram with a normalization curve obtained from TEM images collected for the DMPC macrodisc with a lipid-to-peptoid ratio of 23:1.

reagents and wash the resin between each step. The 15-mer is acetylated using a 10 % acetic anhydride solution in DMF. The peptoid is then cleaved from the resin with simultaneous side chain deprotection using a solution of 95 % trifluoroacetic acid and 5 % water. The crude peptoid is purified by HPLC and the purity and structure of the peptoid is confirmed by LC-MS analysis. (Galiakhmetov et al., 2022) About 10–15 mg of pure peptoid material can be easily obtained using an automated synthesizer. The size and uniformity of the formed macrodiscs are evaluated by TEM (Fig. 1C and 1D).

In the present study, the following samples were prepared: DMPC macrodiscs having a lipid-to-peptoid ratio of 27:1 with the incorporated  $^{15}\text{N}$ -labeled Pf1 coat protein; DPPC macrodiscs with a lipid-to-peptoid ratio of 25:1 (with  $^{15}\text{N}$ -labeled Pf1 membrane protein) and 22:1 (empty macrodiscs). DMPC/DMPG 85/15 macrodiscs had a lipid-to-peptoid ratio of 25:1 (with  $^{15}\text{N}$ -labeled Pf1 membrane protein). The lipid macrodisc preparation has been performed as described in ref. (Galiakhmetov et al., 2022). Briefly, to form lipid macrodiscs, the desired lipid mixture is dissolved in chloroform and dried under a nitrogen gas flow to form a thin film followed by overnight lyophilization. For macrodiscs with the incorporated Pf1 coat protein, a TFE solution of Pf1 protein is added to the thin film and subjected to another cycle of drying/lyophilization. Afterwards, the film is suspended in HEPES buffer pH 8.0 and subjected to 5 freeze–thaw cycles to obtain an MLV (multi lamellar vesicle) solution. The peptoid dissolved in the same buffer is added in 3–5 small aliquots of 70–100  $\mu\text{L}$  to the lipid mixture and subjected to 5 freeze–thaw cycles per aliquot addition. The resulting lipid macrodisc solution is subsequently concentrated using Amicon Ultra-4 centrifuge filters with MWCO 10 kDa and additional five (5) freeze–thaw cycles are performed. The spectrum of Pf1 in 14-mer peptide discs were taken from ref. (Tesch et al., 2018) for comparison, having a 10 % w/v lipid concentration and a peptide-to-lipid ratio of 1:13.3.

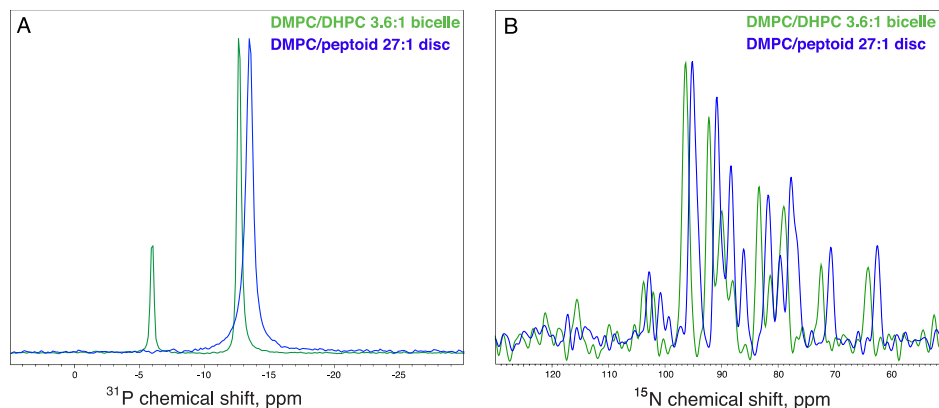
All NMR spectra have been measured on a commercial 500 MHz  $^1\text{H}$  frequency Bruker (Bruker Biospin, Billerica, MA) Avance II spectrometer with a low-E probe (custom-built by Black Fox, LLC, Tallahassee, FL) developed at the National High Magnetic Field Laboratory (Tallahassee, FL) (Gor'kov et al., 2007; Gor'kov et al., 2010). The probe has a 5.35 mm inner coil for the X-Y channels and is equipped with switchable tuning cards for  $^1\text{H}/^{31}\text{P}$  and  $^1\text{H}/^{13}\text{C}/^{15}\text{N}$  nuclei. The total sample volume is 124  $\mu\text{L}$ . 2D NMR spectra of Pf1 coat protein have been measured using the ROULETTE two-dimensional (2D) separated local field (SLF) sequence correlating the amide  $^{15}\text{N}$  chemical shifts with their  $^1\text{H}$ - $^{15}\text{N}$  dipolar couplings (Lapin and Nevzorov, 2020).

## Results

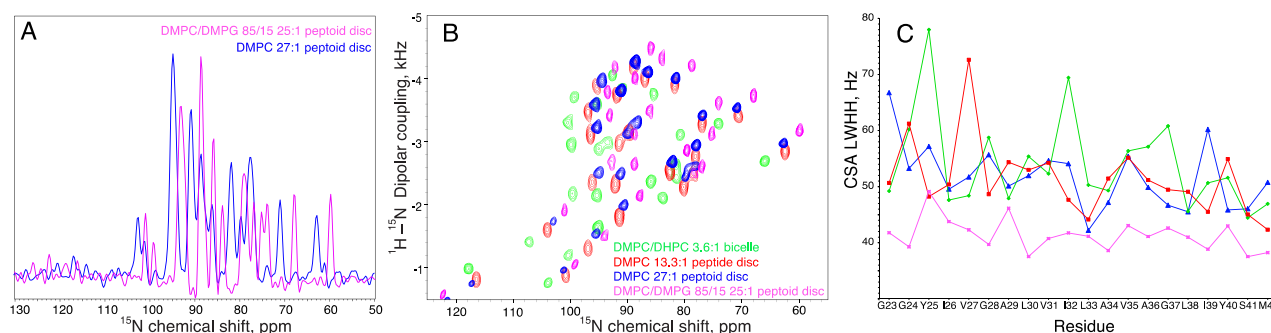
The degree of magnetic alignment of peptoid macrodiscs around the gel-to-liquid crystalline phase lipid transition temperature has been investigated and quantified using  $^{31}\text{P}$  NMR. The  $^{31}\text{P}$  NMR spectrum shown in Fig. 2A exhibits a single anisotropic phosphate group peak having a chemical shift of  $-13.8$  ppm, which demonstrates that the DMPC bilayer is oriented with its normal being perpendicular to the direction of the applied magnetic field above the gel-to-liquid crystalline phase temperature. By contrast, the bicelle sample exhibits two resonances originating from two lipid constituents: DHPC ( $-5.9$  ppm) and DMPC ( $-12.6$  ppm). The average DMPC  $^{31}\text{P}$  NMR peak linewidths were measured to be about 80–100 Hz (0.4 ppm) within a broad temperature range of 32–43  $^{\circ}\text{C}$  and at the lipid to peptoid ratios varying from 23:1 to 27:1. While their linewidths are comparable to those observed for  $q = 3.6$  DMPC/DHPC bicelles (ca. 60–80 Hz), the  $^{31}\text{P}$  NMR peak in the macrodiscs is shifted to the right by 2 ppm. Thus, the greater values of the anisotropic  $^{31}\text{P}$  NMR chemical shift indicate a higher degree of alignment and order parameter of the peptoid-based macrodiscs in comparison with detergent-based bicelles.

Fig. 2B shows a one-dimensional (1D)  $^{15}\text{N}$  chemical shift spectrum of uniformly  $^{15}\text{N}$ -labeled Pf1 coat protein reconstituted in the peptoid-based macrodiscs vs. the same spectrum obtained in magnetically aligned bicelles. The spectrum shows well-resolved resonances arising mainly from the amide  $^{15}\text{N}$  nitrogens in the transmembrane helix of Pf1 (60 ppm–100 ppm). As can be seen from Fig. 2B,  $^{15}\text{N}$  spectroscopic resolution in detergent-free peptoid macrodiscs is somewhat better than in the bicelle spectrum. However, the increase in the magnetic alignment is clearly manifested by concomitant displacements of both the  $^{31}\text{P}$  and  $^{15}\text{N}$  NMR spectra to the right, i.e. away from their corresponding isotropic chemical shift values.

The spectroscopic resolution for both  $^{31}\text{P}$  (lipids) and  $^{15}\text{N}$  (protein) NMR can be dramatically improved by adding ca. 15 % of the negatively charged DMPG lipid to the DMPC bulk lipid (with the overall 25:1 lipid-to-peptoid ratio) as shown in Fig. 3A. One can attribute this increase in the alignment by additional disc stabilization via electrostatic surface repulsion between the discs (Krishnarjuna et al., 2023). Fig. 3B shows two-dimensional SLF spectra of uniformly  $^{15}\text{N}$  labeled Pf1 reconstituted in various aligned lipid mimics (color coded for clarity) showing a clear advantage of peptoid-based macrodiscs for high-resolution OS NMR of membrane proteins. More than 20 peaks corresponding to the Pf1 transmembrane domain are well resolved in Fig. 3B. The arrangement of the NMR peaks in each spectrum exhibits a circular wheel-like pattern characteristic a transmembrane  $\alpha$ -helix tilted at ca. 25 $^{\circ}$  with respect to the bilayer normal (Park et al., 2010).



**Fig. 2.** A.  $^{31}\text{P}$  chemical shift NMR spectrum of Pf1 reconstituted in DMPC peptoid-macrodisc with 27:1 lipid-to-peptoid ratio and 55:1 lipid-to-Pf1 ratio (blue) and conventional DHPC/DMPC bicelle with a ratio 1:3.6 (green) and 72:1 lipid-to-Pf1 ratio, at the sample temperature 37  $^{\circ}\text{C}$ . B.  $^{15}\text{N}$  chemical shift NMR spectrum of Pf1 reconstituted in DMPC peptoid-macrodisc with a 27:1 lipid-to-peptoid ratio (blue) and conventional DHPC/DMPC bicelle with a ratio 1:3.6 (green), at the sample temperature 37  $^{\circ}\text{C}$ .



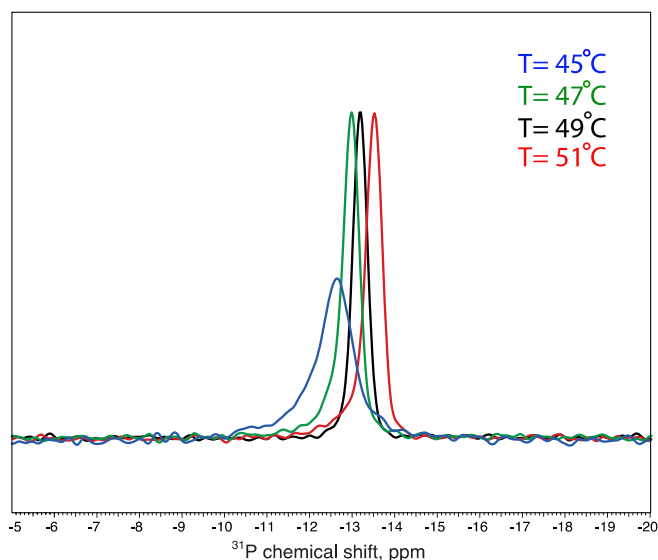
**Fig. 3.** A. A dramatic improvement in spectroscopic resolution and the orientational order parameter obtained by adding charged DMPG lipids (magenta line) to peptoid/DMPC (blue) macrodiscs as evidenced by 1D  $^{15}\text{N}$  spectra of Pf1 coat protein. B. Systematic increase in the orientational ordering demonstrating the advantage of peptoid-based magnetically aligned macrodiscs vs. detergent-containing DHPC/DMPC bicelles and peptide-based macrodiscs. Superimposed are 2D SLF NMR spectra of Pf1 coat protein reconstituted in different membrane mimics including:  $q = 3.2$  DMPC/DHPC bicelles (green contours), 14-mer belt peptide-DMPC macrodiscs (red), 15-mer peptoid-DMPC macrodisc (blue); mixture of DMPG/DMPC macrodiscs at a 15%/85% molar ratio (magenta). C.  $^{15}\text{N}$  CSA linewidths plotted for the resolved residues in the spectra showing uniform, sub-ppm linewidths for DMPC/DMPG peptoid macrodiscs.

As can be seen from Fig. 3B, 2D SLF NMR spectra show a systematic increase in the degree of magnetic ordering for the aligned macrodiscs vs. DMPC/DHPC bicelles as given by an increased range for the maximum observed dipolar couplings ( $>4$  kHz). Such an improvement in the order parameter is clearly seen by comparing 14-mer belt peptide (Park et al., 2011; Tesch et al., 2018) macrodiscs (red contours) with DMPC/DHPC bicelles (green contours) ( $S_0 = 0.83$  vs. 0.80), which increases to 0.85 in the case of DMPC-only 15-mer peptoid-based macrodiscs (blue peaks), and even further for the sample containing 15% of DMPG ( $S_0 = 0.9$ ; magenta peaks). Fig. 3C shows experimental  $^{15}\text{N}$  CSA linewidths plotted as a function of the residue number. It is clear that DMPG-containing peptoid macrodiscs exhibit uniform, sub-ppm resolution as opposed to all other alignment media, especially in comparison with bicelles and peptide-based macrodiscs where the linewidths are broader and non-uniform.

As a next step towards creating oriented lipid environment of variable composition, Fig. 4 demonstrates that highly oriented peptoid-based macrodiscs can also be formed from the longer DPPC lipids (Fig. 4) having 16-member alkyl chains, which play an important role in the activation of pulmonary surfactant cells (Han and Mallampalli,

2015).

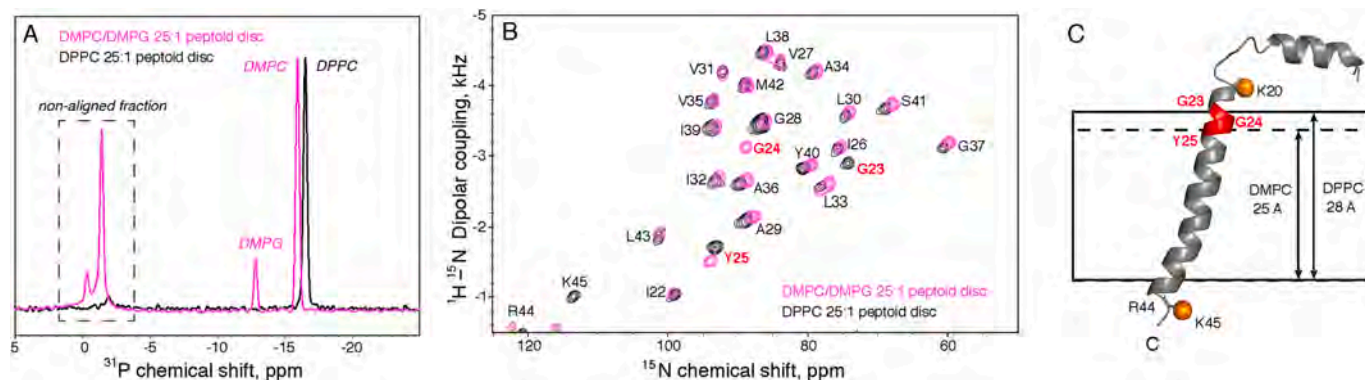
The spectrum of Fig. 4 illustrates the ability of peptoid-based macrodiscs to incorporate lipids of larger hydrophobic thickness (as compared to the model DMPC lipid) over a broad (albeit ca.  $10^\circ\text{C}$  higher than for DMPC) temperature range that can be employed to study lipid-induced conformational changes of membrane proteins, for example. Surprisingly, however, the incorporation of  $^{15}\text{N}$ -labeled Pf1 coat protein into DPPC-based macrodiscs did not reveal any substantial conformational changes in DPPC vs. DMPC bilayers that could have been expected due to an increased hydrophobic thickness of DPPC vs. DMPC or DMPG lipids. The overall degree of magnetic alignment for the two macrodisc constructs is similar as evidenced by the close  $^{31}\text{P}$  chemical shifts for the PC fractions in the spectra of Fig. 5A, which are also somewhat higher than for the 22:1 pure DPPC macrodiscs, cf. Fig. 4. Fig. 5B shows an overlay of  $^1\text{H}$ - $^{15}\text{N}$  ROULETTE (Lapin and Nevzorov, 2020) spectra of Pf1 measured in DPPC macrodiscs (black contours) with the previous spectra from Fig. 3A measured for DMPC/DMPG peptoid macrodiscs. The NMR assignment was taken from ref. (Park et al., 2010). While the Pf1 resonances in pure DMPC lipids are systematically shifted to the left due to the lower orientational order parameter,  $S_0 = 0.85$  (cf. Fig. 3A), the peaks corresponding to DPPC and DMPC/DMPG ( $S_0 = 0.9$ ) bilayers overlay nearly perfectly except for the resonances G23, G24, and Y25 showing the most pronounced deviations. These residues are located at the beginning of the Pf1 transmembrane helix, which would imply that the protein adjusts to the change in the bilayer thickness asymmetrically, cf. Fig. 5C. The preservation of the overall Pf1 structure and its overall orientation within the membrane could be dominated by electrostatic interactions between the lipid head groups and the flanking Lysine residue, K20, on the N-terminal side, and Arginine R44 and K45 residues on the C-terminal side. In addition, two positively charged R44 and K45 sidechains may interact more strongly with the negatively charged lipid phosphate groups on the C-terminus side, thereby leading to an asymmetric response to a change in the lipid bilayer environment. Thus, the overall helix tilt of the Pf1 transmembrane helix is not entirely governed by the hydrophobic match, and even a local increase in the bilayer thickness may occur. Similar conclusions have been made in ref. (Gleason et al., 2013) and also by comparing the spectra of Pf1 in nanotubular DMPC arrays vs. unsaturated DOPC/DOPG lipids (Marek et al., 2015). This interesting observation deserves a more thorough investigation.



**Fig. 4.**  $^{31}\text{P}$  NMR spectra of peptoid macrodiscs (without any protein) formed at the 22:1 DPPC to peptoid molar ratio showing the degree of alignment at various temperatures (45 °C, blue; 47 °C, green; 49 °C, black; 51 °C, red). The sharpest linewidth of 90 Hz is achieved at 49 °C, thus indicating an optimal alignment temperature for DPPC lipid discs.

## Conclusions

We have demonstrated unprecedented magnetic ordering and enhanced NMR spectroscopic resolution in oriented peptoid-based macrodiscs containing DMPC/DMPG mixture of lipids vs. bicelles and



**Fig. 5.** A. Overlay of  $^{31}\text{P}$  NMR spectra of Pf1-containing DMPC/DMPG (magenta) and DPPC peptoid macrodiscs (prepared at 24:1 M ratio; with ca. 3 mg of the Pf1 protein). The chemical shift values for the PC groups are close for DMPC and DMPG, thus indicating a highly similar degree of macroscopic alignment. B. Overlay of 2D spectra of Pf1 coat protein showing unchanged or similar (black labels) and perturbed (red labels) NMR assignments in the two lipid environments. For the most part,  $^{15}\text{N}$  NMR frequencies in the spectrum indicate little change in the overall helix conformation and tilt in the thicker DPPC vs. DMPC/DMPG bilayers. C. Schematic locations of the residues within Pf1 coat protein (pdb ID 2KSJ) which experience the most pronounced spectral perturbations (red labels). Hydrophobic core layers for DMPC (2.5 nm) and DPPC (2.8 nm) (Kucerka et al., 1808) are drawn to scale with respect to the protein. The flanking residues K20 and K45 are shown as orange spheres.

macrodiscs based on peptide belts. The addition of the negatively charged DMPG lipids dramatically increases the magnetic ordering due to electrostatic repulsions between the discs, thereby yielding sub-ppm linewidths and higher order parameters in OS NMR spectra. Peptoid-based macrodiscs possess the ability to incorporate lipids of different thickness. However, no substantial conformational change has been revealed for Pf1 coat protein reconstituted in DMPC- vs. DPPC-containing macrodiscs, which may indicate that the positively charged lysine and arginine side chains may be responsible for maintaining the overall transmembrane helix tilt by strongly interacting with the negatively charged lipid phosphate groups.

These findings illustrate the utility of peptoid-based macrodiscs as a new and very promising direction towards radically improving resolution in OS NMR for structural studies of membrane proteins in planar, detergent-free bilayers and their interactions with the lipid environment.

#### CRedit authorship contribution statement

**Azamat R. Galiakhmetov:** Conceptualization, Methodology. **Adit A. Shah:** Investigation, Validation. **Addison Lane:** Validation, Data curation. **Carolynn M. Davern:** Investigation, Supervision. **Caroline Proulx:** Supervision, Methodology. **Alexander A. Nevzorov:** Funding acquisition, Supervision, Writing – review & editing.

#### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Alexander A. Nevzorov reports financial support was provided by National Science Foundation. Alexander A. Nevzorov reports financial support was provided by US Army Research Office.

#### Data availability

Data will be made available on request.

#### Acknowledgements

This material is based upon work supported by the National Science Foundation under Grant No. MCB 1818240 and by U.S. Army Research Office under contract number W911NF1810363 to A.A.N. TEM was performed at the Analytical Instrumentation Facility (AIF) at North Carolina State University, which is supported by the State of North

Carolina and the National Science Foundation (award number ECCS-2025064). The AIF is a member of the North Carolina Research Triangle Nanotechnology Network (RTNN), a site in the National Nanotechnology Coordinated Infrastructure (NNCI).

#### References

- Andreev, K., Martynowycz, M.W., Huang, M.L., Kuzmenko, I., Bu, W., Kirshenbaum, K., Gidalevitz, D., 1860. Hydrophobic interactions modulate antimicrobial peptoid selectivity towards anionic lipid membranes. *BBA-Biomembr.* 2018, 1414–1423.
- De Angelis, A.A., Nevzorov, A.A., Park, S.H., Howell, S.C., Mrse, A.A., Opella, S.J., 2004. High-resolution NMR spectroscopy of membrane proteins in “unflipped” bicelles. *J. Am. Chem. Soc.* 126, 15340–15341.
- De Angelis, A., Park, S.H., Opella, S.J., 2022. Magnetically aligned lipid bilayers with high cholesterol for solid-state NMR of membrane proteins. *Biochemistry* 61, 1561–1571.
- Durr, U.H.N., Goldenberg, M., Ramamoorthy, A., 2012. The magic of bicelles lights up membrane protein structure. *Chem. Rev.* 112, 6054–6074.
- Fowler, S.A., Blackwell, H.E., 2009. Structure-function relationships in peptoids: Recent advances toward deciphering the structural requirements for biological function. *Org. Biomol. Chem.* 7, 1508–1524.
- Galiakhmetov, A.R., Davern, C.M., Esteves, R.J.A., Awosanya, E.O., Guthrie, Q.A.E., Proulx, C., Nevzorov, A.A., 2022. Aligned peptoid-based macrodiscs for structural studies of membrane proteins by oriented-sample NMR. *Biophys. J.* 121, 3263–3270.
- Gayen, A., Banigan, J.R., Traaseth, N.J., 2013. Ligand-Induced conformational changes of the multidrug resistance transporter emrA probed by oriented solid-state NMR spectroscopy. *Angew. Chem. Int. Ed.* 52, 10321–10324.
- Gleason, N.J., Vostrikov, V.V., Greathouse, D.V., Koeppe, R.E., 2013. Buried lysine, but not arginine, titrates and alters transmembrane helix tilt. *PNAS* 110, 1692–1695.
- Glover, K.J., Whiles, J.A., Wu, G., Yu, N.-J., Deems, R., Struppe, J.O., Stark, R.E., Komives, E.A., Vold, R.R., 2001. Structural evaluation of phospholipid bicelles for solution-state studies of membrane-associated biomolecules. *Biophys. J.* 81, 2163–2171.
- Gor'kov, P.L., Chekmenev, E.Y., Li, C.G., Cotten, M., Buffy, J.J., Traaseth, N.J., Veglia, G., Brey, W.W., 2007. Using low-E resonators to reduce RF heating in biological samples for static solid-state NMR up to 900 MHz. *J. Magn. Reson.* 185, 77–93.
- Gor'kov, P.L., Brey, W.W., Long, J.R., 2010. Probe development for biosolids NMR spectroscopy. *eMagRes.*
- Han, S., Mallampalli, R.K., 2015. The role of surfactant in lung disease and host defense against pulmonary infections. *Ann. Am. Thorac. Soc.* 12, 765–774.
- Hutchison, J.M., Shih, K.C., Scheidt, H.A., Fantin, S.M., Parson, K.F., Pantelopulos, G.A., Harrington, H.R., Mittendorf, K.F., Qian, S., Stein, R.A., Collier, S.E., Chambers, M.G., Katsaras, J., Voehler, M.W., Ruotolo, B.T., Huster, D., McFeeters, R.L., Straub, J.E., Nieh, M.P., Sanders, C.R., 2020. Bicelles rich in both sphingolipids and cholesterol and their use in studies of membrane proteins. *J. Am. Chem. Soc.* 142, 12715–12729.
- Jing, X.N., Kasimova, M.R., Simonsen, A.H., Jorgensen, L., Malmsten, M., Franzyk, H., Foged, C., Nielsen, H.M., 2012. Interaction of peptidomimetics with bilayer membranes: biophysical characterization and cellular uptake. *Langmuir* 28, 5167–5175.
- Krishnarajuna, B., Marte, J., Ravula, T., Ramamoorthy, A., 2023. Enhancing the stability and homogeneity of non-ionic polymer nanodiscs by tuning. *J. Colloid Interface Sci.* 634, 887–896.

- Kucerka, N., Nieh, M.P., Katsaras, J., 1808. Fluid phase lipid areas and bilayer thicknesses of commonly used phosphatidylcholines as a function of temperature. *BBA* 2011, 2761–2771.
- Landry, M.R., Rangel, J.L., Dao, V.P., MacKenzie, M.A., Gutierrez, F.L., Dowell, K.M., Calkins, A.L., Fuller, A.A., Stokes, G.Y., 2019. Length and charge of water-soluble peptoids impact binding to phospholipid membranes. *J. Phys. Chem. B* 123, 5822–5831.
- Lapin, J., Nevzorov, A.A., 2020. De novo NMR pulse sequence design using Monte-Carlo optimization techniques. *J. Magn. Reson.* 310, 106641.
- Marek, A., Tang, W.X., Milikisijants, S., Nevzorov, A.A., Smirnov, A.I., 2015. Nanotube array method for studying lipid-induced conformational changes of a membrane protein by solid-state NMR. *Biophys. J.* 108, 5–9.
- Mojsoska, B., Zuckermann, R.N., Jenssen, H., 2015. Structure-activity relationship study of novel peptoids that mimic the structure of antimicrobial peptides. *Antimicrob. Agents Chemother.* 59, 4112–4120.
- Molchanova, N., Hansen, P.R., Franzyk, H., 2017. Advances in development of antimicrobial peptidomimetics as potential drugs. *Molecules* 22.
- Park, S.H., Berkamp, S., Cook, G.A., Chan, M.K., Viadiu, H., Opella, S.J., 2011. Nanodiscs versus macrodiscs for NMR of membrane proteins. *Biochemistry* 50, 8983–8985.
- Park, S.H., Opella, S.J., 2010. Triton X-100 as the “Short-Chain Lipid” improves the magnetic alignment and stability of membrane proteins in phosphatidylcholine bilayers for oriented-sample solid-state NMR spectroscopy. *J. Am. Chem. Soc.* 132, 12552–12553.
- Park, S.H., Marassi, F.M., Black, D., Opella, S.J., 2010. Structure and dynamics of the membrane-bound form of Pfl Coat protein: implications of structural rearrangement for virus assembly. *Biophys. J.* 99, 1465–1474.
- Park, S.H., Wu, J., Yao, Y., Singh, C., Tian, Y., Marassi, F.M., Opella, S.J., 2020. Membrane proteins in magnetically aligned phospholipid polymer discs for solid-state NMR spectroscopy. *Biochim. Biophys. Acta Biomembr.* 1862, 183333.
- Prosser, R.S., Hwang, J.S., Vold, R.R., 1998. Magnetically aligned phospholipid bilayers with positive ordering: A new model membrane system. *Biophys. J.* 74, 2405–2418.
- Radoicic, J., Park, S.H., Opella, S.J., 2018. Macrodiscs comprising SMALPs for oriented sample solid-state NMR spectroscopy of membrane proteins. *Biophys. J.* 115, 22–25.
- Ravula, T., Ramadugu, S.K., Di Mauro, G., Ramamoorthy, A., 2017. Bioinspired, size-tunable self-assembly of polymer-lipid bilayer nanodiscs. *Angew. Chem. Int. Edit.* 56, 11466–11470.
- Sanders, C.R., Prestegard, J.H., 1990. Magnetically orientable phospholipid bilayers containing small amounts of a bile salt analogue, CHAPSO. *Biophys. J.* 58, 447–460.
- Sanders, C.R., Schwonek, J.P., 1992. Characterization of magnetically orientable bilayers in mixtures of DHPC and DMPC by solid state NMR. *Biochem.* 31, 8898–8905.
- Scholz, F., Boroske, E., Helfrich, W., 1984. Magnetic-anisotropy of lecithin membranes - a new anisotropy susceptometer. *Biophys. J.* 45, 589–592.
- Simons, K., Toomre, D., 2000. Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell Biol.* 1, 31–39.
- Sun, J., Zuckermann, R.N., 2013. Peptoid polymers: A highly designable bioinspired material. *ACS Nano* 7, 4715–4732.
- Tesch, D.M., Pourmoazzen, Z., Awosanya, E.O., Nevzorov, A.A., 2018. Uniaxial diffusional narrowing of NMR lineshapes for membrane proteins reconstituted in magnetically aligned bicelles and macrodiscs. *Appl. Magn. Reson.* 49, 1335–1353.
- Verardi, R., Traaseth, N.J., Shi, L., Porcelli, F., Monfregola, L., De Luca, S., Amodio, P., Veglia, G., Scaloni, A., 2011. Probing membrane topology of the antimicrobial peptide distinctin by solid-state NMR spectroscopy in zwitterionic and charged lipid bilayers. *BBA-Biomembr.* 1808, 34–40.
- Vold, R.R., Prosser, R.S., 1996. Magnetically oriented phospholipid bilayered micelles for structural studies of polypeptides. Does the ideal bicelle exist? *J. Magn. Reson. B* 113, 267–271.
- Zhang, Y.H., Xuan, S.T., Owoseni, O., Omarova, M., Li, X., Saito, M.E., He, J.B., McPherson, G.L., Raghavan, S.R., Zhang, D.H., John, V.T., 2017. Amphiphilic polypeptides serve as the connective glue to transform liposomes into multilamellar structures with closely spaced bilayers. *Langmuir* 33, 2780–2789.
- Zhang, Y.H., Heidari, Z., Su, Y., Yu, T.Y., Xuan, S.T., Omarova, M., Aydin, Y., Dash, S., Zhang, D.H., John, V., 2019. Amphiphilic polypeptides rupture vesicle bilayers to form peptoid-lipid fragments effective in enhancing hydrophobic drug delivery. *Langmuir* 35, 15335–15343.
- Zuckermann, R.N., Kodadek, T., 2009. Peptoids as potential therapeutics. *Curr. Opin. Mol. Ther.* 11, 299–307.
- Zuckermann, R.N., Kerr, J.M., Kent, S.B.H., Moos, W.H., 1992. Efficient method for the preparation of peptoids oligo(n-substituted glycines) by submonomer solid-phase synthesis. *J. Am. Chem. Soc.* 114, 10646–10647.