Molecular Dynamics & NMR of Peptide Dendrimers with Dipeptide Spacers

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The goal of this work is to understand the difference in 3d structure and especially in local mobility of peptide dendrimers with Lys2LArg and Lys2Lys repeating units

Abstract: Dendrimers are macromolecules regularly branched from central core. Their repeating units have a fork-like structure usually with two (rarely with three or more) prongs. Due to this reason, the number of atoms in them doubled (or tripled) in each generation. They have spherical shape, precise size and many terminal groups capable to interact with other biological molecules (PCCP, 2016, 18, 24307; Polymer, 2017, 125, 292; Polymer, 2018, 146, 256; Pharmaceutics, 2018, 10, 129; Macromolecules, 2020, 53, 7298). Lysine dendrimers (Dendrimers in Biomedical Applications, RSC, 2013, 99; Polym.Sci, Ser. C., 2013, 55, 154; PCCP 2015, 17, 3214; Langmuir, 2018, 34, 1613) and peptide dendrimers (Sci. Rep. 2018, 8, 8916; Polymers, 2020,12(8), 1657) were used as carriers for drug and gene delivery (Int. J. Mol. Sci. 2020, 21, 3138; Bioorg.Chem., 2020, 95, 103504) as well as for many other biomedical applications. We performed synthesis, characterization and computer simulation of novel peptide dendrimers with the same AlaLys core, Lys2Arg, Lys2Lys and Lys2His repeating units and charged terminal Lys aminoacid residues. Temperature dependences of equilibrium characteristics and orientational relaxation times of terminal, main chain and side CH2 groups in the temperature interval T=283K-343 K were studied. It was demonstrated that size and density distribution of these dendrimers is near the same and do not depend on temperature. Temperature dependences of relaxation times and spin-lattice relaxation time T1H of CH2 groups of all dendrimers were calculated and it was shown that they are very close to experimental results obtained for these dendrimers by NMR (Sci. Rep. 2018, 8, 8916; RSC Adv. 2019, 9, 18018; Molecules, 24, 2019, 2481).

Model and Method

The molecular dynamics method consists in numerical solution of the classical Newton equations of motion for all atoms of the all molecules in the system. In present paper simulation was performed for systems consisting of one peptide dendrimer with repeating units Lys2Arg, Lys2Lys, Lys2His (i.e. with charged linear 2Arg, 2Lys&2His spacers between branching points) and explicit CL- counterions in water.

In all simulation GROMACS software package and AMBER_99SB-ildn force fields were used. The potential energy of this force field includes energies of valence bonds and angles, dihedral angles, van-der-Waals and electrostatic interactions. The procedure of molecular dynamics simulation for lysine and peptide dendrimers and for other linear or branched polyelectrolytes has been described earlier. In all calculations the temperature was varied from T=283K to 343K.

Results

In **Fig.1** the chemical structures of dendrimers are presented. Core of denrimers are shown in green color, backbone - in black, side groups of spacers are marked by violet and terminal Lys residues – by red color.

Fig.2 the instant sizes Rg and fluctuations of all lysine based peptide dendrimers with similarly positively charged 2Arg, 2Lys (and 2His, not shown)) spacers are similar

Fig.3 demonstrates that overall rotation $P1_{rot}(t)$ and breathing $C_{Rg}(t)$ correlation functions of all dendrimers decrease similarly and corresponding characteristic times are significantly smaller than total simulation time equal to 1000 ns

Fig.4 shows that size of Lys2Arg dendrimer (as well as Lys2Lys and Lys2His (not shown)) doesn't depend on temperature T (a) and the ratio Rh/Rg of hydrodynamic and gyration radia is close to 1 and doesn't depend on T also

Fig.5 demonstrates that radial densities in Lys2Arg, Lys2Lys (and Lys2His, not shown) dendrimers are similar.

Fig.6 shows that (a) distribution function of number of hydrogen bonds n between Lys2Arg dendrimer and water molecules is rather wide and that (b) its average number decreases with temperature

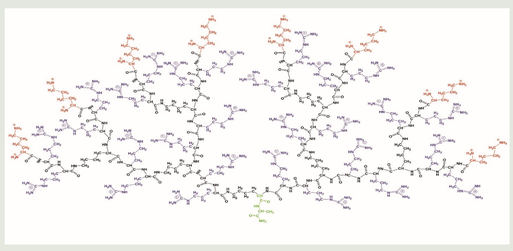
Fig.7 demonstrates that pair correlation function of positively charged groups of similarly charged dendrimers with Cl- counerions are very similar

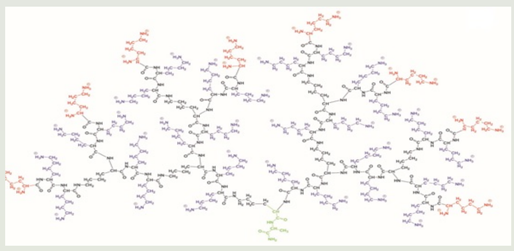
Fig.8 shows that radial distribution of charges (a) and T dependence of relative charge ratio in both dendrimers (b) are very similar

Fig.9 demonstrates that orientational relaxation of CH2 groups in side chains of 2Arg spasers in Lys2Arg dendrimer (a) occurs essentially slower than in 2Lys spacers of Lys2Lys dendrimer (b) despite their similar structures&charge distribution

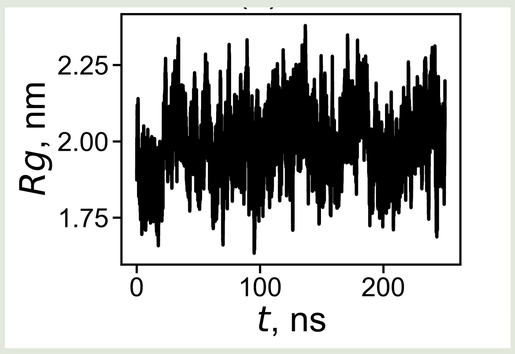
Fig 10 shows that (a) temperature (T) dependences of MD simulated spine-lattice $1/T_{1H}$ relaxation times (filled symbols) of inner (squares), spacers side (circles) and terminal (triangles) groups are in good agreement with experimental NMR data (open symbols); (b) the corresponding times of CH2 groups of 2Arg spacers (filled circles) in Lys2Arg and in 2Lys spacers (open circles) of Lys2Lys dendrimer are very different. We demonstrated that this difference is not due to Arg-Arg pairing effect as it was suggested earlier but due to longer contour length from the end of side 2Arg chains of spacers to NMR active CH2 group in these spacers in comparison with 2Lys spacers in Lys2Lys dendrimer

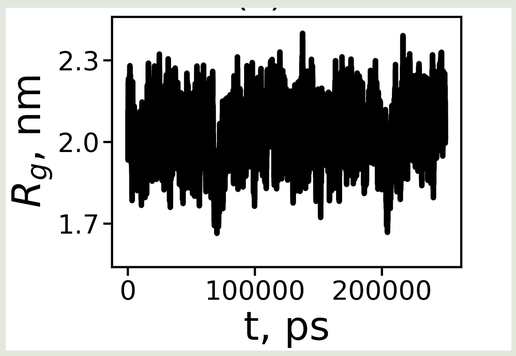
1. Lys2Arg dendrimer of 2nd generation **Lys2Lys dendrimer of 2nd generation**



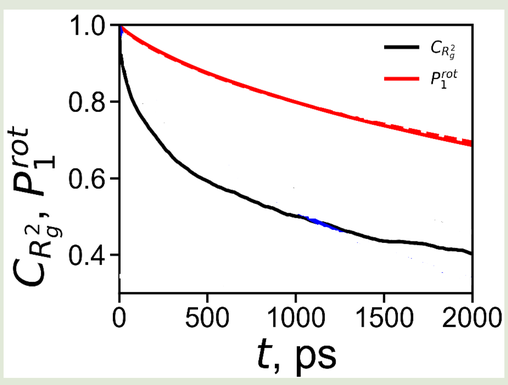


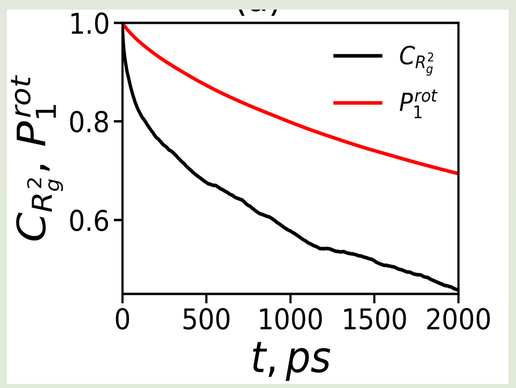
2. The size of Lys2Arg and Lys2Lys dendrimers



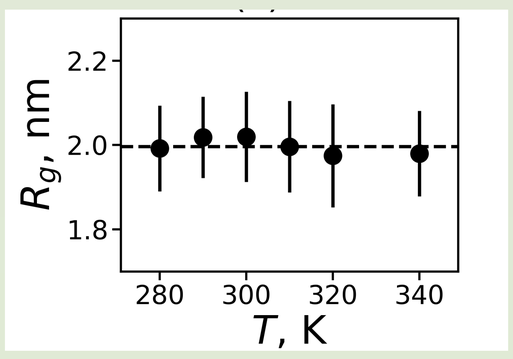


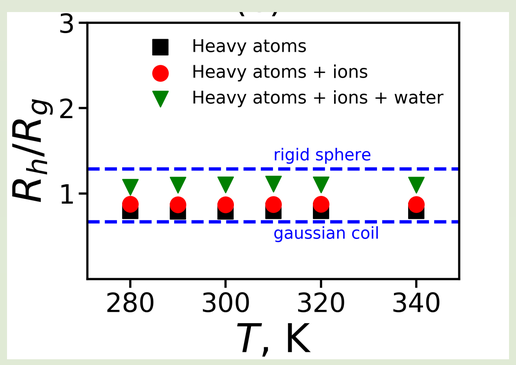
3. Time ACF for Lys2Arg (a) and Lys2Lys dendrimers (b)



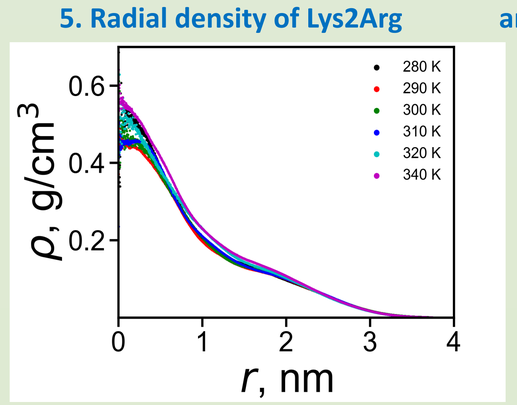


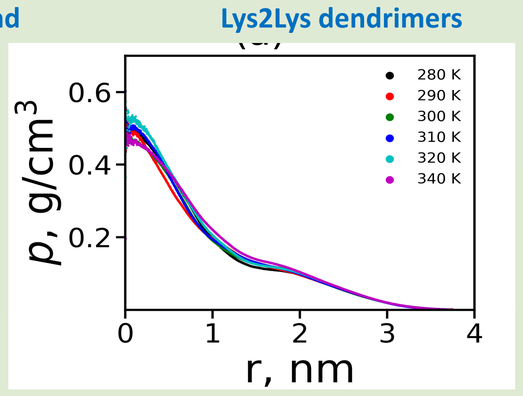
4. Rg vs T (a) and Rh/Rg vs T (b) for Lys2Arg dendrimer



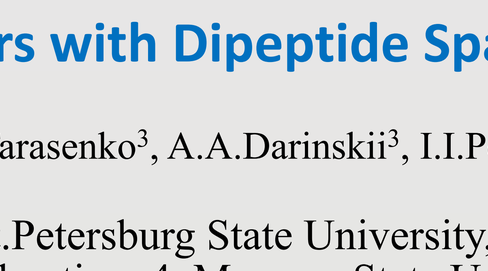


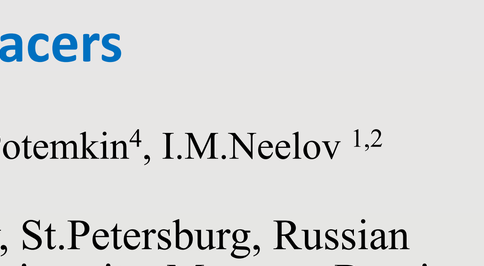
5. Radial density of Lys2Arg and Lys2Lys dendrimers



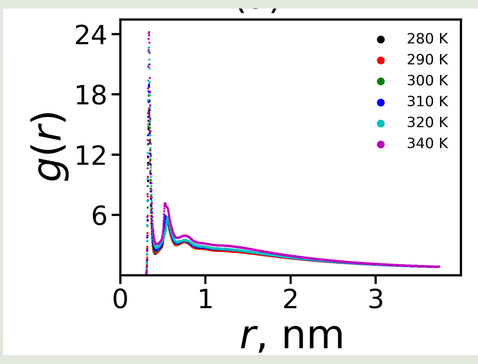


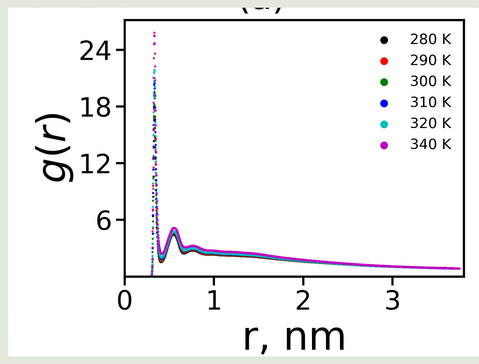
6. Distribution of hydrogen bonds (a) and its average number vs T (b) in Lys2Arg



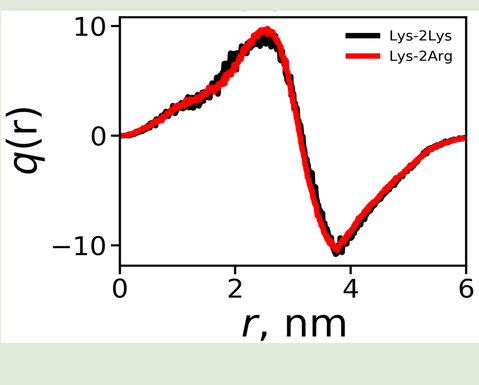


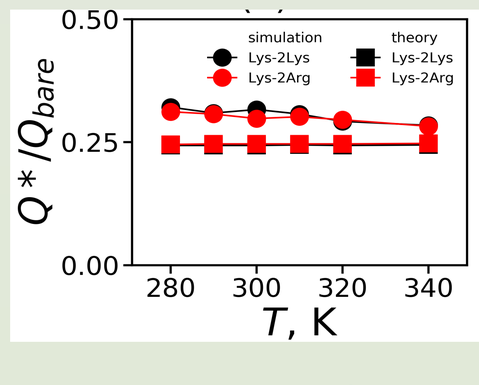
7. Pair correlations in Lys2Arg and Lys2Lys (of CH3+ and Cl- counterions)



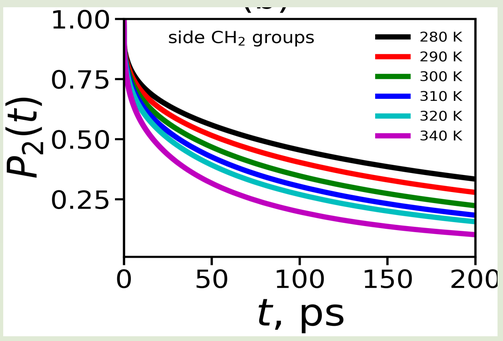


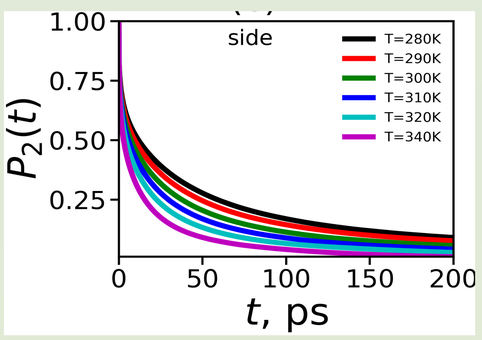
8. Radial distribution of charge (a) and relative charge vs T in both dendrimers



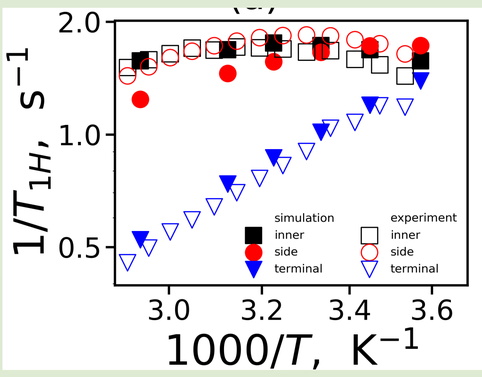


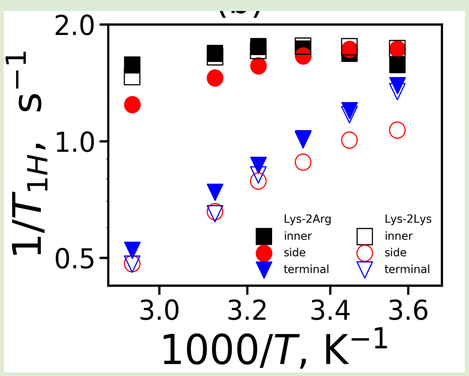
9.Local rotational mobility in Arg2Lys and Lys2Lys dendrimers





10.Spine-lattice relaxation times Lys2Arg and Lys2Arg/Lys2Lys dendrimers





Conclusions

The size (Rg) and internal structure of lysine based peptide dendrimer with Lys2Arg repeating units are close to that of other Lys based peptide dendrimers (Lys2Lys or Lys2His) with similar positively charged (2Lys or 2His) spacers. These characteristics of the dendrimers practically does not depend on temperature. The charge distributions for all these dendrimers are also similar. Thus all structural and electrical properties of these dendrimers are similar.

The local orientational relaxation properties of NMR active CH2-N groups of Lys 2Arg dendrimer (in main chain, in side chains of spacers and in terminal groups) calculated from MD simulations are very close to corresponding groups of this molecule obtained from NMR experiment earlier. Relaxation times of main chain CH2 groups of Lys2Arg dendrimer were close to that of Lys2Lys dendrimers. Similar result was obtained for terminal CH2 groups of both dendrimers. However relaxation times of CH2 groups in side chains of spacers of Lys2Arg dendrimer were close to relaxation times of main chain CH2 groups while in Lys2Lys dendrimers they were close to corresponding times of terminal groups. We demonstrated that this difference is not due to Arg-Arg pairing effect as it was suggested earlier but due to longer contour length from the end of side 2Arg chains of spacers to NMR active CH2 group in this dendrimer in comparison with 2Lys spacers in Lys2Lys dendrimer.

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References

1. Buhleier, E.; Wehner, E.; Vogtle, F. "Cascade" and "nonskid-chain-like" syntheses of molecular cavity topologies. Synthesis **1978,** 78, 155–158.
2. Frechet, M.J.; Tomalia, D.A. (Eds.) Dendrimers and Other Dendritic Polymer, 1st ed.; Wiley: London, UK, **2001**.
3.Neelov, I.; Falkovich, S.; Markelov, D.; Paci, E.; Darinskii, A.; Tenhu, H. Molecular Dynamics of Lysine Dendrimers. Computer Simulation and NMR. In Dendrimers in Biomedical Applications; Royal Society of Chemistry: London, UK, **2013**; pp. 99–114.
4. Falkovich, S.; Markelov, D.; Neelov, I.; Darinskii, A. Are structural properties of dendrimers sensitive to the symmetry of branching? Computer simulation of lysine dendrimers. J. Chem. Phys. **2013,** 139, 064903.
5. Neelov, I.;Markelov, D.; Falkovich, S.; Ilyash.,M.; Okrugin, B.; Darinskii, A. Mathematical simulation of lysine dendrimers. Temperature dependencies. Polym. Sci. Ser. C **2013,** 55, 154–161.
6. Markelov, D.A.; Falkovich, S.G.; Neelov, I.M.; Ilyash, M.Y.; Matveev, V.V.; Lahderanta, E.; Ingman, P.; Darinskii, A.A. Molecular dynamics simulation of spin–lattice NMR relaxation in poly-L-lysine dendrimers: manifestation of the semiflexibility effect. Phys. Chem. Chem. Phys. **2015**, 17, 3214.
7.O.V. Shavykin, I. M. Neelov, A. A. Darinskii. Is the manifestation of the local dynamics in the spin–lattice NMR relaxation in dendrimers sensitive to excluded volume interactions? // Phys. Chem. Chem. Phys., 2016,18, 24307-24317.
8. Shavykin, O.; Mikhailov, I.; Darinskii, A.; Neelov, I.; Leermakers, F. Effect of an asymmetry of branching on structural characteristics of dendrimers revealed by Brownian dynamics simulations. Polymer **2018,** 146, 256–266.
9. Shavykin,O.V.; Leermakers, F.A.;Neelov, I.M.;Darinskii,A.A. Self-Assembly of Lysine-Based Dendritic Surfactants Modeled by the Self-Consistent Field Approach. Langmuir **2018,** 34, 1613–1626.
10. Sheveleva, N.N.; Markelov, D.A.; Vovk, M.A.; Mikhailova, M.E.; Tarasenko, I.I.; Neelov, I.M.; Lahderanta, E. NMR studies of excluded volume interactions in peptide dendrimers. Sci. Rep. **2018,** 8, 8916.
11. Sheveleva, N.N.; Markelov, D.A.; Vovk, M.A.; Mikhailova, M.E.; Tarasenko, I.I.; Tolstoy, P.M.; Neelov, I.M.; Lähderanta, E. Lysine-based dendrimer with double arginine residues. RSC Adv. **2019**, 9, 18018–18026.
12. Sheveleva, N.N.; Markelov, D.A.; Vovk, M.A.; Tarasenko, I.I.; Mikhailova, M.E.; Ilyash, M.Y.; Neelov, I.M.; Lahderanta, E. Stable Deuterium Labeling of Histidine-Rich Lysine-Based Dendrimers. Molecules **2019,** 24, 2481.
13. Gorzkiewicz, M.; Konopka, M.; Janaszewska, A.; Tarasenko, I.I.; Sheveleva, N.N.; Neelov, I.M.; Klajnert-Maculewicz, B. Application of new lysine-based peptide dendrimers D3K2 and D3G2 for gene delivery: Specific cytotoxicity to cancer cells & transfection. Bioorg.Chem.**2020**, 95, 103504.
14. M.Gorzkiewicz,O. Kopeć, A.Janaszewska, M.Konopka, … I.I.Tarasenko,V.V.Bezrodnyi, I.M.Neelov, B. Klajnert-Maculewicz Poly(lysine) Dendrimers (with charged 2Arg, 2Lys and 2His spacers) Form Complexes with siRNA and Provide Its Efficient Uptake by Myeloid Cells, IJMS, **2020**, 21(9), 3138
15. V.V.Bezrodnyi, O.V.Shavykin, S.E.Mikhtaniuk, I.M.Neelov, N.N. Sheveleva, D.A.Markelov Why the Orientational Mobility in Arginine and Lysine Spacers of Peptide Dendrimers Designed for Gene Delivery Is Different? Int. J. Mol. Sci. **2020,** 21, 9749;