Study of Complex of AEDG Tetrapeptide with KRH Dendrimer at Two Different pH by Computer Simulation

SOFIA E. MIKHTANIUK[1], EMIL I. FATULLAEV[1],
IGOR M. NEELOV[1,2,3,4], OLEG V. SHAVYKIN[1,2,5]
[1]Center of Chemical Engineering, ITMO University
[2]Department of Physics, St.Petersburg State University
[3]Peter the Great St.Petersburg Polytechnic University
[4]Department of Physics, Institute of Macromolecular Compounds
[5]Physics Department, Tver State University
St.Petersburg, 195277, Grazhdankii pr. 77-1
RUSSIA

Abstract: - In previous papers, we studied the behavior of lysine (Lys or K) based dendrimers of the second generation with repeating units KKK and KRR (i.e., with branched neutral lysine and charged double lysine or arginine (KK, RR) spacers). We also studied KLL, KAA, and KGG dendrimers with hydrophobic double leucine, alanine, and glycine (LL, AA, GG) spacers and pH-dependent KHH dendrimers with double histidine (HH) spacers. Their complexes with molecules of several medicinal peptides (including AEDG) were studied as well. It was shown that lysine dendrimers with charged spacers are suitable for the delivery of oppositely charged oligopeptides and genetic material, while dendrimers with hydrophobic internal spacers are good for the delivery of hydrophobic oligopeptides and fullerenes. In the present paper, we study complexes of molecules of AEDG peptide with KRH dendrimer containing arginine-histidine (RH) spacers. In this case, the amino acid residues in the spacer (R and H) of dendrimer are different, and the charge of the H residue depends on *pH*. We performed molecular dynamics simulations of the complexation of 16 AEDG molecules with a dendrimer at two different *pH*s: a) KRH at *pH*>7 with fully uncharged histidines (H) and b) KRHp at *pH*<5 with fully protonated (Hp) histidines in aqueous solution with explicit counterions. It was found that the dendrimer with protonated histidines forms a more compact complex. KRHp dendrimer can also carry more AEDG tetrapeptide molecules than KRH.

Key-Words: - Peptide dendrimers, medicinal oligopeptides, complexes, molecular dynamics simulation

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1 Introduction

Dendrimers are macromolecules with a regular almost spherical tree-like structure originating from a single root (core) and containing several spherical layers [1]. The number of these layers between core and terminal segments determines the number of generations G. The uniqueness of dendrimers lies in the fact that: (i) molecules of a given dendrimer with a given number of generations G, synthesized under the same conditions are practically monodisperse; (ii) the number of branching points and terminal groups of these molecules available for functionalization increases very quickly with increasing generation number G of the dendrimer, [2]. Dendrimers can also have hydrophobic core or carry a large electrical charge distributed throughout the entire volume of the dendrimer or only over its surface.

These properties determine the practical interest in the use of dendrimers in industry [3] and biomedicine [4]. Dendrimers can be used as nanocontainers for the delivery of drugs [5] and genetic material [6]. Dendrimers were applied in medicine primarily for increase the solubility of hydrophobic drugs, protect them during delivery, and protect healthy cells from exposure to toxic drugs. Application of poly(amidoamine) (PAMAM) and polypropylene imine (PPI) for delivery of genetic material was described in [7], for delivery DNA in [8], and for delivery of siRNA using PAMAM in [9] and using PAMAM and PPI in [10]. Recent study of new dendrimers of various structure and their applications were described in [11], synthesis, strucrture and functions of new dendrimers with internal functionalization in [12] and overview of different types of dendrimers in [13]. Review of history and recent development of

dendrimers for drug delivery in general were discussed in [14], and in particular for drug delivery to brain in [15]. Application of dendrimers for general anticancer diagnostics and therapy was described in [16], and specific of brain tumors diagnostics and treatment in [17]. Dendrimers as vectors for gene-directed enzyme prodrug therapy was discussed in [18]. Synthesis and evaluation of carbosilane dendrimers for siRNA delivery were described in [19] and silencing of SARS-CoV-2 with siRNA-peptide dendrimer formulation in [20].

Peptide dendrimers based on natural monomers, such as amino acid residues, are a good alternative to synthetic dendrimers. The simplest ones are lysine dendrimers consisting of only lysine monomers [21]. The repeating unit of such a dendrimer is a single branched lysine residue (Lys).

Experimental studies of the sizes of lysine dendrimers of generation G=1-10 were carried out in dimethylformamide [22] and in aqueous solutions for G=3, [23] and for G=1-5, [24]. Dendrimers were examined using molecular dynamics computer simulations. The dependences of the sizes and other structural and dynamic characteristics of these dendrimers on the number of generations (G) were studied for G=2-5, [25], G=1-6, [26], G=2, 4, [27], G=1-5,[28] and G=1-5, [29]. In the last paper, primary attention was paid to comparing lysine and PAMAM dendrimers of the same generations. More complicated peptide dendrimers containing lysine octa-branched core and linear peptides consisting of 9-16 amino acids attached to their ends were synthesized first in 1988 [30] for application as multiple antigen peptides (MAPs). Synthesis of similar dendrimers with 24-residue peptides attached to dendrimer ends as described in [31]. Lysine dendrimers with one additional amino acid between branching points were described in [24] for G=1-5 and [32] for G=2. Dendrimers were also described as having one or more other amino acid residues attached to their ends. In particular, dendrimers with arginine and histidine were studied for applications in gene delivery and as antibacterial, antiviral, and antiamiloid agents. In particular, PAMAM dendrimers of generation G=4 with terminal lysine or terminal arginine were studied in [33]. Arginine functionalized peptide dendrimers of generations G=5 and G=6 were investigated in [34]. Arginine-, lysine- and leucine-bearing polyethyleneimine (PEI) dendrimers were studied in [35]. Lysine dendrimers of generation G=6 with lysine, arginine, and histidine ends were used in [36]. PAMAM of generation G=4 with arginine and histidine ends was studied in, [37]. Dendritic polyglycerolamine with arginine and histidine end groups was investigated in [38]. Properties of different dendrimers with aminoacid residues were compared in [39]. However, there was practically no work on lysine dendrimers in which other amino acid residues were inserted in the internal generations of the dendrimer (except papers, [24], for G=5 and [32], for G=2).

Recently, new types of lysine-based dendrimers in which the internal part of the dendrimer was functionalized. These dendrimers were successfully synthesized and studied by the NMR method for dendrimers with double glycine and double lysine spacers, [40], double arginine spacers, [41] and double histidine (with protonated and non-protonated imidazole) spacers, [42]. The dendrimers were also tested as a carrier for delivery of genetic material with double glycine and double lysine spacers [43] and double lysine, arginine, and histidine spacers [44]. In this case, additional linear spacers were inserted into the dendrimers between all adjacent branch points. These spacers consisted of double amino acid residues of lysine or glycine, [40] and [43], arginine, [41] and [44], and protonated histidine, [42] and [44]. The repeating units in these dendrimers were Lys2Lys/Lys2Gly, Lys2Arg, and Lys2His, correspondingly.

Peptide dendrimers based on second-generation lysine dendrimers with spacers (2Lys or 2Gly, [45], 2Arg, [46], and 2His, [47]) inserted between their branch points have also been studied using computer modeling methods. However, there were almost no studies of peptide dendrimers with spacers containing two different aminoacids in the internal generations of the dendrimer. In this article, we close this gap. The insertion of such groups should increase the capabilities of peptide dendrimers for the transport of both hydrophobic and hydrophilic drugs.

It is well known that many dendrimers are amphiphilic. The inner region of the dendrimer is usually more hydrophobic and can be used to transport hydrophobic drug molecules. Improved solubility of the dendrimer, as well as its complex with hydrophobic drugs, is provided by hydrophilic monomers, which are located at the periphery of the dendrimer and are often positively charged. In our recent papers, we also studied dendrimers with double histidine spacers [48] and with histidine-arginine/arginine-histidine spacers [49] which could change their hydrophobicity with *pH.*

2 Problem Formulation

2.1 Formulation of the Problem

Previously, we used lysine dendrimers with two identical amino acid residues in each spacer between dendrimer branch points. In this work, the arginine residues (R) and histidine residue (H) are different as in [49]. One of them (R) is charged at all pH, the other (H) is uncharged at *pH* >7 and positively charged at *pH*<5. It means that the dendrimer as a whole is more charged as the pH decreases.

2.2 Model and Method

In this work, we used an all-atom model of a peptide dendrimer consisting of Lys-ArgHis (KRH) repeat elements including Lys branch points and ArgHis (RH) spacers between them. One KRH dendrimer with neutral histidines (H) in spacers or one KRHp dendrimer with protonated histidines (Hp) in spacers and 16 molecules of the medical tetrapeptide Ala-Glu-Asp-Gly (AEDG) were placed in a 9 nm periodic cubic cell filled with water and counterions. The simulation was carried out using the molecular dynamics (MD) method with the AMBER99SB-ILDN force field and the TIP3P water model in the Gromacs package [50]. Before the start of the simulation, the energy of individual subsystems was minimized. Further minimization of the entire system's energy in an aqueous solution was done before MD simulation. Electrostatic interactions were calculated using the PME method. The calculation of the main MD trajectory was carried out in the NPT ensemble. The constant temperature was ensured using a Nose-Hoover thermostat, and constant pressure was maintained using a Parrinello-Raman barostat. MD simulation was carried out for 500 ns.

3 Problem Solution

3.1 Process of Complex Formation

At the beginning of the MD simulation, the dendrimer was located at the center of the periodic cell, and 16 tetrapeptide molecules were located at the periphery of this cell). Therefore, at the beginning of the simulation, the distances between the dendrimer and the peptide molecules were large. To quantitatively describe the process of bringing the dendrimer and peptides closer together due to electrostatic interactions, the root-mean-square distances d between the center of the dendrimer and each of the 16 tetrapeptide molecules were calculated (see Fig. 1). From this graph it is clear that initial distance is between 4 and 5 nm. Then, it constantly decreases during the first 40-50 ns of MD simulation and reaches a plateau. This behavior indicates the establishment of dynamic equilibrium in the system, in which the root mean square distance *d* between the dendrimer and peptide molecules fluctuates but remains practically unchanged.

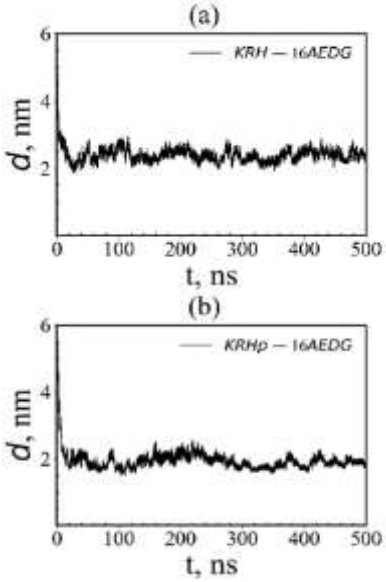

Fig. 1 – Time dependences of distances d(t) between the centers of the dendrimer and peptides in the system for dendrimer with: a) neutral histidine residues (H) in spacers, b) protonated histidine residues (Hp) in spacers.

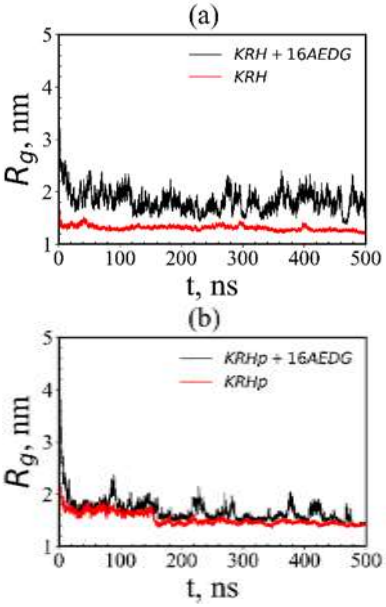

Fig. 2 - Dependences of the gyration radius *Rg* of dendrimer and of subsystem containing dendrimer and all peptides on time *t* for dendrimer with: a) neutral histidine residues (H) in spacers, b) protonated histidine residues (Hp) in spacers

Another characteristic demonstrating the formation of the complex is the radius of inertia of the subsystem consisting of a dendrimer and oppositely

charged tetrapeptides (see Fig.2). A decrease in the size of the subsystem consisting of dendrimer and all peptides confirms the formation of a complex of the dendrimer with peptides for both dendrimers with uncharged (a) and with charged (b) histidines. At the same time, the difference in the radii of the complex and the dendrimer in Fig. 2a means that not all peptides land on this dendrimer. In contrast, the coincidence of Rg in Fig. 2b means that a more charged dendrimer can hold almost all 16 tetrapeptides.

The number of hydrogen bonds between the dendrimer and the peptides indicates how tightly the peptides are bound to the dendrimer. From Fig. 3 it follows that the average number of hydrogen bonds at the beginning (before the peptides approach the dendrimer) is zero. It quickly increases within 40-50 ns after the first contact of the dendrimer with the peptide molecules. This value fluctuates significantly over time, but its average value practically does not change. The average number of hydrogen bonds for a dendrimer with uncharged histidines (Fig. 3a) is close to 62, and for a dendrimer with protonated histidines (Fig. 3b)), fluctuates around 76, which confirms a more compact arrangement of the dendrimer and peptides in the complex in the latter case.

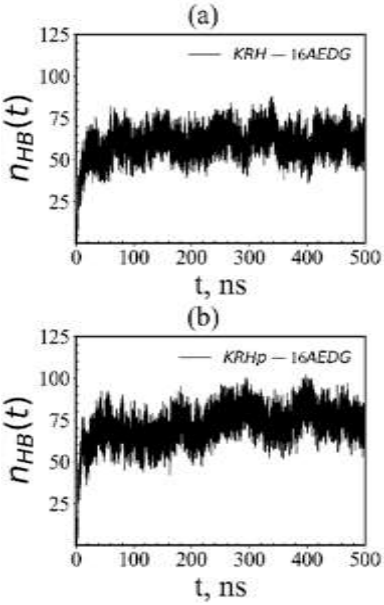

Fig. 3. Dependences of hydrogen bonds number n_{HB} *(t)* between dendrimer and tetrapeptides on time t for dendrimer with: a) neutral histidine residues (H) in spacers, b) protonated histidine residues (Hp) in spacers

To characterize the formation of the complex, we used the radius of gyration of the subsystem consisting of the dendrimer and all 16 peptides (see Fig. 2). However, this value well reflects the process of complex formation only if the dendrimer is capable of retaining all the tetrapeptides present in the system. A more accurate characteristic of the instantaneous size of the complex is the radius of gyration of the subsystem containing the dendrimer and only those tetrapeptides associated with it at a given time. The dependence of this value on time is shown on Fig. 4.

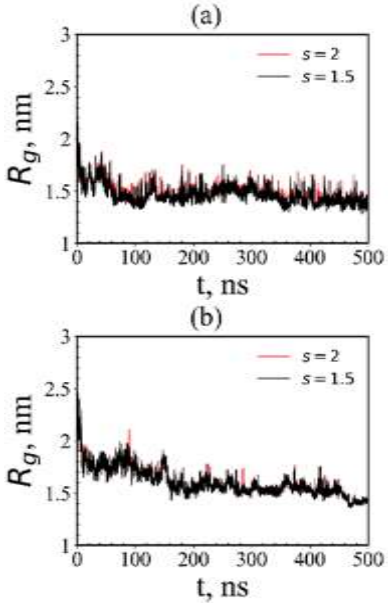

Fig.4. Time dependences of the gyration radius *Rg(t)* of complexes for dendrimer with: a) neutral histidine residues (H) in spacers, b) protonated histidine residues (Hp) in spacers

The most interesting value for a dendrimer-peptide complex is the time dependence of the instantaneous number of tetrapeptides *n* in the complex on time *t* and its average value at long simulation times (after reaching a plateau). This value is shown in Fig. 5.

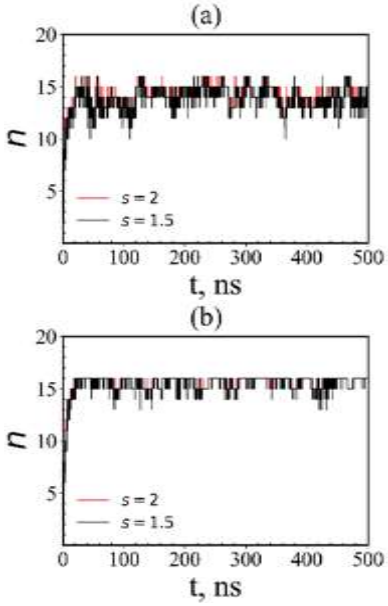

Fig.5. Time dependences of number of peptides *n(t)* in complexes: for dendrimer with: a) neutral

histidine residues (H) in spacers, b) protonated histidine residues (Hp) in spacers

The number of peptides in the complex is zero until the first contact of one of the peptides with the dendrimer. Then, it quickly grows to a maximum value in the first 20 ns. After that, it reaches a plateau and fluctuates around its average value of approximately n=14 (Fig. 5a) for a dendrimer with uncharged histidines and close to n=16 (Fig. 5b) for a dendrimer with protonated histidines.
The results can be illustrated by snapshots of the systems at the beginning and at the end of the simulation (see Fig. 6). It is easy to see that two tetrapeptides are out of complex for KRH dendrimer at the end of simulation (Fig.6b) while for KRHp dendrimer (Fig.6d) all 16 tetrapetides are in complex at the end of trajectory.

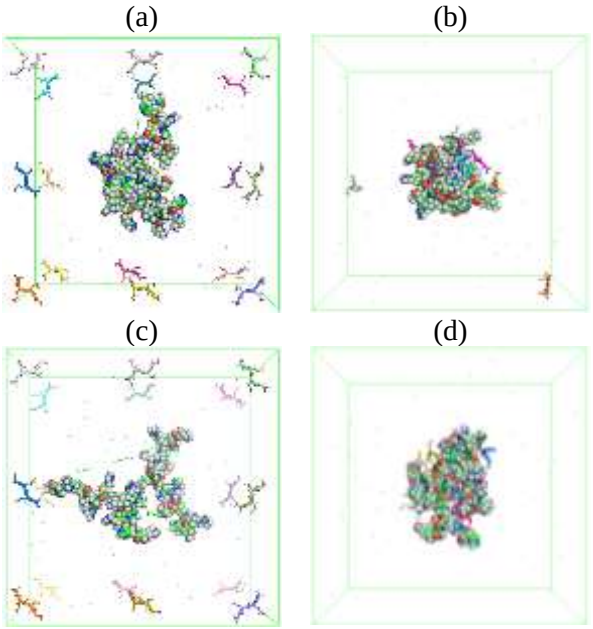

Fig.6. The snapshots of the systems KRH+16AEDG (a,b) and KRHp+16AEDG (c,d) at the start of simulation, at time t = 0 ns (a, c) and at the end of it, at t = 500 ns (b,d). Dendrimer atoms are shown as spheres with a diameter equal to their van der Waals radii while for tetrapeptides only main chains and valence bonds of side chains are shown

3.2 Equilibrium Properties of the Complexes

After all the characteristics of both complexes reach a plateau (approximately in the first 250 ns), any equilibrium characteristics can be calculated by averaging them over the second half (second 250 ns) of the trajectory.
Fig. 7 shows the equilibrium distribution functions of the distances between the center of mass of the dendrimer and tetrapeptide molecules. It clearly shows that complexes of tetrapeptides and a dendrimer with uncharged histidines (*KRH*) are less compact (the peak of the distribution *g(d)* for *KRH* is located at larger distances *d* than for a dendrimer with protonated histidines (*KRHp*)), and the width of the first distribution is also larger than for *KRHp*.

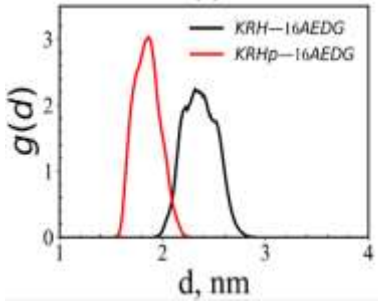

Fig.7. Distribution function *g(d)* of distances *d* between the centers of the dendrimer and peptides.

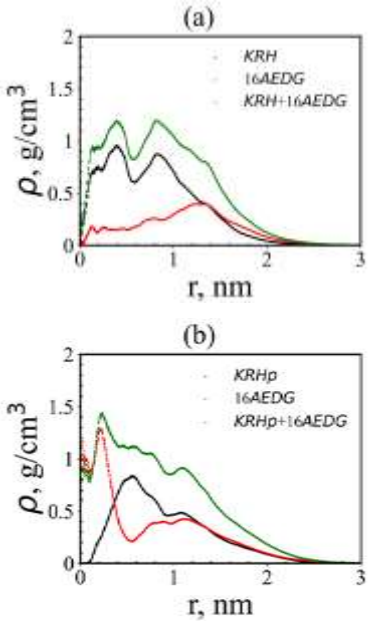

Fig. 8. The radial distribution function of dendrimer atoms, atoms of peptides and for all atoms of complex relatively center of mass of dendrimer for dendrimer with: a) neutral histidine residues (H) in spacers, b) protonated histidine residues (Hp) in spacers

The radial distribution of the number of atoms of the dendrimer, tetrapeptides, and all atoms in the system relative to the center of mass of the dendrimer provides the most complete information about the internal structure of the complex. For a dendrimer with uncharged histidines (Fig. 8a), the dendrimer atoms make up the majority near its center of inertia. Peptide atoms can penetrate the center of the dendrimer, but their density there is small compared to the density of the dendrimer in this place. The

density of tetrapeptide atoms has maximum, located at a distance of about 1.2 nm from the center of mass of the dendrimer. For a dendrimer with protonated histidines (Fig. 8b), tetrapeptide atoms, on the contrary, penetrate deeply into the dendrimer and make up the majority near the center of mass (at *r=0*). In contrast, dendrimer atoms are displaced from the center and have a maximum density at a distance of about *r* = 0.6 nm from the center of mass of the protonated dendrimer.

Average values obtained during second part of trajectory are presented in Table 1. The avegage distances <d> between the centers of dendrimers and peptides and the size of complex are smaller for dendrimer with protonated dendrimer (*KRHp*), The number of hydrogen bonds between dendrimers and peptides and the number of peptides in complexes are greater for dendrimer with protonated histidines (KRHp).

Table 1. The average values: average distances $<d>$ between dendrimer center and peptide molecule, number of hydrogen bonds $<N_{Hb}>$ between them, number of peptide molecules in the complex $<N_{lc}>$.

System	$<d>$	$<n_{Hb}>$	**<Nlc>**
KRH+16AEDG	2,36	61,7	14,0
KRHp+16AEDG	1,85	76,6	15,6

These average values are in good agreement with results obtained in parts 3.1 and 3.2 of the paper.

4 Conclusion

In the present paper, we studied the complexes of molecules of AEDG peptide with dendrimers containing arginine-histidine (*RH*) spacers with charge of histidines depending on *pH* value. We performed molecular dynamics simulations of the complexation of 16 AEDG molecules with a dendrimer at two different *pH*s: a) *pH*>7 with fully uncharged histidines (H) and b) *pH*<5 with fully protonated (Hp) histidines in aqueous solution with explicit counterions. It was found that the dendrimer with protonated histidines forms a more compact complex containing a larger number of AEDG tetrapeptide molecules.

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, 1 ed.; Wiley: England, 2001. doi:10.1002/0470845821.

[3] Patel, H.N.; Patel, P.M. Dendrimer applications - a review. Int J Pharm Bio Sci, 2013, 4, 454–463.

[4] Abbasi, E.; Aval, S.; Akbarzadeh, A.; et al. Dendrimers: synthesis, applications, and properties. Nanoscale Research Letters, 2014, 9, 247.

[5] Madaan, K.; Kumar, S.; Poonia, N.; et al. Dendrimers in drug delivery and targeting: Drug-dendrimer interactions and toxicity issues. J Pharm Bioallied Sci. 2014, 6, 139–150..

[6] Sherje, A.P.; Jadhav, M.; Dravyakar, B.R.; et al. Dendrimers: A versatile nanocarrier for drug delivery and targeting. Int J Pharm. 2018, 548, 707–720.

[7] Rabiee, N.; Ahmadvand, S.; Ahmadi, S.; et al. Carbosilane Dendrimers: Drug and Gene Delivery Applications. Journal of Drug Delivery Science and Technology 2020, p. 101879.

[8] Dufes, C.; Uchegbu, I.; Schatzlein, A.G. Dendrimers in gene delivery. Adv. Drug Delivery Rev. 2005, 57, 2177–2202.

[9] Zhou, J.; Wu, J.; Hafdi, N.; et al. PAMAM dendrimers for efficient siRNA delivery and potent gene silencing. Chem. Commun. 2006, 22, 2362–2364.

[10] Biswas, S.; Torchilin, V. Dendrimers for siRNA delivery. Pharmaceuticals 2013, 6, 161–183.

[11] Pérez-Ferreiro, M.; Abelairas, A.; Criado, A.; et al. Dendrimers: Exploring Their Wide Structural Variety and Applications. Polymers 2023, 15, 4369.

[12]Smith R.J., Gorman C., Menegatti S. Synthesis, structure, and function of internally functionalized dendrimers, J Polym Sci. 2021, 59:10–28.

[13] Sarode R.J., Mahajan H.S. Dendrimers for drug delivery: An overview of its classes, synthesis, and applications, 2024, 98, 105896

[14] Wang, J., Li, B., Qiu, L. et al. Dendrimer-based drug delivery systems: history, challenges, and latest developments. J. Biol. Eng., 2022, 16, 18.

[15] Zhu Y., Liu C., Pang Z. Dendrimer-Based Drug Delivery Systems for Brain Targeting, Biomolecules 2019, 9, 790.

[16] Bober, Z.;Bartusik-Aebisher, D.; Aebisher, D. Application of Dendrimers in Anticancer Diagnostics and Therapy. Molecules, 2022, 27, 3237.

[17] Kaurav M., Ruhi S., Al-Goshae H.A. et al Dendrimer: An update on recent developments and future opportunities for the brain tumors diagnosis treatment, Front. Pharmacol., 2023, 14

[18] Chis, A.A.; Dobrea, C.M.; Rus, L.-L. et al Dendrimers as Non-Viral Vectors in Gene-Directed

Enzyme Prodrug Therapy. Molecules 2021, 26, 5976.
[19] Zawadzki S., Martín‑Serrano A., Okła E. et al, Synthesis and biophysical evaluation of carbosilane dendrimers as therapeutic siRNA Carriers, Scientifc Reports, 2024, 14, 1615.
[20] Khaitov M., Nikonova A., Shilovskiy I. et al. Silencing of SARS-CoV-2 with modified siRNA-peptide dendrimer formulation, Allergy. 2021; 76, 2840-2854.
[21] Denkewalter, R.G.; Kolc, J.; Lukasavage, W.J. Macromolecular Highly Branched Homogeneous Compound Based on Lysine Units. United States Patent, US4289872A, 1981.
[22] Aharoni, S.M.; Crosby III, C.R.; Walsh, E.K. Size and solution properties of globular tert-butyloxycarbonyl-poly(α,ϵ-L-lysine).
Macromolecules 1982, 15, 1093–1098.
[23] Vlasov, G. et al. Lysine Dendrimers and Their Starburst Polymer Derivatives: Possible Application for DNA Compaction and in vitro Delivery of Genetic Constructs. Russian Journal of Bioorganic Chemistry 2004, 30, 15–24.
[24] Vlasov, G.; at al Dendrimers Based on a-Amino Acids: Synthesis and Hydrodynamic Characteristics. Doklady Physical Chemistry 2004, 399, 366–368.
[25] Markelov, D.A.; Falkovich, S.G. Ilyash, at al. Molecular dynamics simulation of spin–lattice NMR relaxation in poly-l-lysine dendrimers: a manifestation of the semiflexibility effect. Phys. Chem. Chem. Phys. 2015, 17, 3214–3226.
[26] Roberts, B.P.; Scanlon, M.J.; Krippner, G.Y.; Chalmers, D.K. Molecular Dynamics of Poly(l-lysine) Dendrimers with Naphthalene Disulfonate Caps. Macromolecules 2009, 42, 2775–2783.
[27] Neelov, I.; Markelov, D.; Falkovich et al S.; Mathematical simulation of lysine dendrimers. Temperature dependencies. Polymer Science, Ser. C 2013, 55, 154–161.
[28] Neelov, I.; Falkovich, S.; Markelov, D.; et al Dendrimer in Biological Applications, London, RSC, 2013, 99–114.
[29] Darinskii A.A., Gotlib, Y.Y., Lyulin, A.V. et al. Vysokomolek. Soed., Computer simulation of local dynamics of a polymer chain in the orienting field of the LC type, 1991, Vysokomolekularnye Soedineniya. Seriya A 33, 1211-1220.
[30] Tam, J. Synthetic peptide vaccine design: Synthesis and properties of a high-density multiple antigenic peptide system. Proc. Natl.Acad. Sci. USA 1988, 85, 5409–5413.
[31] Rao, C.; Tam, J. Synthesis of peptide dendrimer. J. Am. Chem. Soc. 1994, 116, 6975–6976
[32] Darbre, T.; Reymond, J.L. Peptide dendrimers as artificial enzymes, receptors, and drug-delivery agents. Acc. Chem. Res. 2006, 39, 925–934.
[33] Choi, J.S.; Nam, K.; Park, J.y.; Kim, J.B.; Lee, J.K.; Park, J.S. Enhanced Transfection Efficiency of PAMAM Dendrimer by Surface Modification with L-Arginine. J. Control. Release 2004, 99, 445-456.
[34] Luo, K.; Li, C.; Li, L.; She, W.; Wang, G.; Gu, Z. Arginine functionalized peptide dendrimers as potential gene delivery vehicles. Biomaterials 2012, 33, 4917-4927.
[35] Aldawsari, H.; Raj, B.S.; Edrada-Ebel, R.; Blatchford, D.R.; Tate, R.J.; Tetley, L.; Dufès, C. Enhanced gene expression in tumors after intravenous administration of arginine-, lysine- and leucine-bearing polyethylenimine polyplex. Nanomed. Nanotechnol. Biol. Med. 2011, 7, 615–623.
[36] Okuda, T.; Sugiyama, A.; Niidome, T.; Aoyagi, H. Characters of dendritic poly(L-Arhlysine) analogs with the terminal lysines replaced with arginines and histidines as gene carriers in vitro. Biomaterials 2004,49925, 537–544.
[37] Lee, H.; Choi, J.S. Larson R.G. Molecular Dynamics Studies of the Size and Internal Structure of the PAMAM Dendrimer Grafted with Arginine and Histidine. Macromolecules 2011, 44, 8681–8686
[38] Sheikhi Mehrabadi, F.; Zeng, H.; Johnson, M.; Schlesener, C.; Guan, Z.; Haag, R. Multivalent dendritic polyglycerolamine with arginine and histidine end groups for efficient siRNA transfection. Beilstein J. Org. Chem. 2015, 11, 763–772.
[39] Santos, A.; Veiga, F.; Figueiras, A. Dendrimers as Pharmaceutical Excipients: Synthesis, Properties, Toxicity, and Biomedical Applications. Materials 2020, 13, 65.
[40] Sheveleva, N.N.; Markelov, D.A.; Vovk, M.A.; et al. NMR studies of excluded volume interactions in peptide dendrimers. Scientific Reports 2018, 8, 8916.
[41] Sheveleva, N.N.; Markelov, D.A.; Vovk, M.A. et al. Lysine-based dendrimer with double arginine residues. RSC Advances 2019, 9, 18018–18026.
[42] Sheveleva, N.N.; Markelov, D.A.; Vovk et al Stable Deuterium Labeling of Histidine-Rich Lysine-Based Dendrimers. Molecules 2019, 24, 2481
[43] Gorzkiewicz, M.; Konopka, M.; Janaszewska, at al. Application of new lysine-based peptide dendrimers D3K2 and D3G2 for gene delivery: Specific cytotoxicity to cancer cells and transfection in vitro. Bioorg. Chem. 2020, 95, 103504.

[44] Gorzkiewicz, M.; Kopec, O.; Janaszewska, et al. Poly(lysine) Dendrimers Form Complexes with siRNA and Provide Its Efficient Uptake by Myeloid Cells: Model Studies for Therapeutic Nucleic Acid Delivery. Int. J. Mol. Sci. 2020, 21, 3138.
[45] Mikhtaniuk, S.E.; Bezrodnyi, V.V.; Shavykin O.V. et al. Comparison of Structure and Local Dynamics of Two Peptide Dendrimers with the Same Backbone but with Different Side Groups in Their Spacers. Polymers 2020, 12, 1657.
[46] Bezrodnyi, V.V.; Shavykin, O.V.; Mikhtaniuk, S.E. et al. 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.
[47] Bezrodnyi, V.V.; Mikhtaniuk, S. E.; Shavykin, O. V.; et al. Size and Structure of Empty and Filled Nanocontainer Based on Peptide Dendrimer with Histidine Spacers at Different pH, Molecules 2021, 26, 6552.
[48] Sheveleva et al. Local Orientational Mobility of Collapsed Dendrimers, Macromolecules, 2021, 54, 11083-11092.
[49] Sheveleva, N.N.; Tarasenko, I.I.; Vovk, M.A.; et al. NMR Studies of Two Lysine Based Dendrimers with Insertion of Similar Histidine-Arginine and Arginine-Histidine Spacers Having Different Properties for Application in Drug Delivery. Int. J. Mol. Sci. 2023, 24, 19.
[50] Abraham M.J., Murtola T., Schulz R. et al. GROMACS: High Performance Molecular Simulations through Multi-Level Parallelism from Laptops to Supercomputers. SoftwareX, 2015, 1–2, 19–25.

Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

Sofia Mikhtaniuk, Igor Neelov and Oleg Shavykin formulated the problem for modeling. Sofia Mikhtaniuk, Emil Fatullaev prepared initial conformations of molecules for simulation. and carried out the simulation. Sofia Mikhtaniuk and Oleg Shavykin prepared plots, and wrote intials text. Sofia Mikhtaniuk, Igor Neelov and Oleg Shavykin prepared final manuscript and formulated conclusion.

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Conflict of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.