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Amyloid Beta (A β 40) Aggregation**

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**Design of Heparin Oligosaccharide Based Molecules for Inhibition of Alzheimer
Amyloid Beta (A β ₄₀) Aggregation**

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Abstract. In this computational study, we have combined molecular docking and molecular dynamics (MD) simulations techniques to explore interactions of monomeric and aggregated forms of Alzheimer's amyloid beta ($A\beta_{40}$) with seven chemically distinct heparin derived glycoaminoglycans (GAGs) referred to as ADC, SDC, DC, V1, V2, V3 and V4. The docking procedure proposed two major binding sites, i.e one present at the top of the fibril (site A), and the other located in the hairpin region (site B). Due to its position, site B offers an interesting target to design molecules with anti-aggregation properties. Our results predicted that out of seven GAGs, only three (ADC, SDC and DC) binds to site B. The identification of these molecules can advance our efforts to develop therapeutic interventions for this deadly disease.

Keywords: glycoaminoglycans, heparin disaccharide, amyloid fibril, amyloid beta

I. Introduction.

Alzheimer's disease (AD) is a neurological disorder that affects more than 5 million Americans and is characterized by the accumulation of soluble and insoluble amyloid oligomers in the brain.¹⁻² The increasing number of people developing AD is placing a great strain on the healthcare system.¹⁻⁵ The major components of amyloid oligomers are individual amyloid beta (A β) peptides consisting of 40-42 amino acids produced from the cleavage of the amyloid precursor protein (APP).⁶⁻⁷ This process is catalyzed by two specialized enzymes, β -site APP-cleaving enzyme-1 (BACE1) which cleaves the C-terminus fragment and γ -secretase which subsequently cleaves the fragment produced by BACE1.⁸⁻¹⁰ After cleavage, the A β 40-42 peptides tend to aggregate forming fibrils and later plaques within the brain.¹¹ Therefore, one main area of interest in treating AD is the development of therapeutic approaches; such as small molecule inhibition¹²⁻¹⁵, that can decrease the A β aggregation in the brain. This task could be accomplished by employing low molecular weight (LMW) glycoaminoglycans (GAGs) such as enoxaparin, which has been shown to reduce A β load within the brain.^{8, 16-18} GAGs (examples include hyaluronic acid, heparin, dermatan sulfate, heparan sulfate, and keratan sulfate), are linear hetero-polysaccharides consisting of a hexosamine (e.g. hexuronic acid, L-iduronic acid, galactose), disaccharide units, and sulfate substituents.¹⁹ The natural form of heparin, a linear sulfated polysaccharide chain, can potentially disrupt the production of A β peptides.^{11, 20} GAGs may regulate APP processing by β -secretase, but also might prevent the persistence of toxic forms of A β oligomers by blocking the cell surface so repeating units cannot further oligomerize.^{11, 17}

Within A β 40-42 peptides, charged amino acid residues between region 1-11 are critical to A β pro-inflammatory response.^{11, 21-23} Therefore, inhibiting the activity within this region

could be useful in slowing the progress of AD. In binding to this site, heparin can prevent more than 70% of A β peptide binding to heparan sulfateproteoglycans (HSPG) and may also block cell surface adhesion of A β monomer units.^{11, 21, 24-26} Both effects could protect neurons and vascular endothelial cells against the toxic effects of A β aggregation.^{11, 21, 25-26} It has also been demonstrated that GAGs significantly attenuate the toxic effect of A β on neuronal PC12 cells.²⁷⁻²⁹ In addition, GAG association with A β may prevent further aggregation or induce a different kind of aggregation altogether.^{11, 21, 27} Furthermore, GAGs might cover the aggregate form of A β so that it cannot interact efficiently within cells to produce a toxic response.^{11, 21, 27-30} Heparin and its sulfated forms are candidates for such a therapeutic activation.^{11, 21, 24-29}

The development of heparin oligosaccharide analogs which can be used in the treatment of AD will require several criteria to be met, such as; (1) the heparin oligosaccharide analogs must be able to cross the blood-brain barrier,^{11, 31-32} (2) possess high binding affinity for A β peptides,⁸ (3) and provide specificity for binding to only certain proteins within a cell line.^{8, 31-32} In order to achieve this, structural information about the exact binding sites on either monomer and/or fibril forms of A β 40-42 peptides must be elucidated. It has been reported that the specificity of haparan sulfate (HS) for binding to fibroblast growth factor receptors is controlled primarily through its sulfation pattern.³³⁻³⁵ Furthermore, it is not well understood how the number of sulfated groups present on the GAGs will affect their binding; therefore, fine tuning the number of sulfated groups present could further provide specificity for binding.^{11, 35} Additionally, heparin derived hexa-sulfated disaccharide has been shown to inhibit allergic inflammatory responses in the sheep model of allergic asthma for which a di-sulfated disaccharide was ineffective, further supporting the role of increased sulfation.³⁵ Given the wide variety of GAGs which possess different molecular weights, charge densities, degree of

sulfation, and types of saccharide units, the amount of possible motifs are enormous.¹¹ However, structures of A β -GAGs complexes and information regarding the nature of interactions between them are not available. In this study, we have combined molecular docking and molecular simulations (MD) techniques to study interactions of both monomeric and fibrillar forms of A β ₄₀ with a variety of chemically distinct GAG molecules (Figure 1). Our results will help to develop novel LMW GAG compounds that could be used as a potential therapeutic option for the treatment of AD.

II. Computational Details.

IIa. Computational models. All GAGs were modeled using the Gaussian 09 program package³⁶ and optimized without any geometrical constraint. The A β ₄₀ fibril structures were generously provided by Robert Tycko using solid-state nuclear magnetic resonance (NMR) method (PDB ID: 2LMN).³⁷ The starting structure of the A β ₄₀ monomer was extracted from the NMR structures determined in aqueous SDS micelles at pH 5.1 (model 2, PDB ID: 1BA4).³⁸ Both the A β ₄₀ monomer and fibril structures were relaxed separately for 100 and 5 ns, respectively. Here, the former was only partially relaxed by keeping every eighth alpha carbon frozen to retain its β -sheet rich structure. In the next step, to include flexibility into the docking procedure, 100 snapshots for the A β ₄₀ monomer and 5 for fibrils were taken at 1 ns intervals throughout the simulations. These snapshots were used for the rigid docking of ADC (amide disaccharide), SDC (super-sulfated disaccharide), and DC (heparin disaccharide) molecules using the YASARA³⁹ software. This software employs the Vina scoring function and AutodockLGA (hybrid local search genetic algorithm) that are also implemented in the Autodock Vina.⁴⁰ Both Autodock Vina and YASARA software have been used for docking in this study and were found to provide

exactly the same results. In the docking procedure, the receptor was kept fixed, but the ligand was allowed to change its conformation. Antechamber⁴¹⁻⁴² was used in order to parameterize the ligands [ADC, SDC, DC, and its four variants (V1-V4)]. This step was performed in order to accurately represent the sulfate groups during the docking and MD simulations.

IIb. Molecular docking. Molecular docking procedures were performed using YASARA³⁹ software to investigate the binding of the different forms of heparin to the A β ₄₀ fibril and monomer. The simulation cell chosen surrounded the whole ligand–protein/peptide complex, and the spacing was kept to 1.00 Å. Further docking was performed with the simulation cell focused on sites of interest (side binding sites) once they had been identified. Each docking trial produced 20 poses implementing an exhaustiveness value equal to 20.

IIc. MD simulations. The best 10 poses provided by the previous docking simulations were further equilibrated for 10-20 ns to confirm the stability of binding sites. A cluster analysis of these short simulations provided starting structures for the subsequent long range MD simulations. The all-atom simulations of ADC, SDC, DC, and all four variants with the A β ₄₀ fibril were performed using GROMACS⁴³ using the AMBER03⁴² force field. Furthermore, MD simulations were also performed on the ADC and SDC in complex with the A β ₄₀ monomer. For all simulations, the starting structures were placed in a cubic box with dimensions of 80 × 70 × 60 Å for fibril and 40 × 40 × 40 Å for the monomer simulations. The size of the box used in these simulations was adequate and visualization of MD trajectories using the Visual Molecular Dynamics (VMD)⁴⁴ program did not show any interaction of the fibril and monomer with the walls of the box. This eliminated unwanted effects that may have arisen from the applied

periodic boundary conditions (PBC).⁴⁵ The box was filled with single point charge (SPC) water molecules.⁴⁶ Some water molecules were replaced by sodium and chloride ions in order to neutralize the system and simulate standard physiological conditions in mass percent of 0.9%. These structures were energy-minimized with a steepest descent method over 3000 steps. The results of these minimizations produced the starting structures for the next round of MD simulations. During both the energy minimization and MD simulations, every eighth alpha carbon was frozen in order to maintain the structure of the fibril. Without these restraints, the fibril structure became highly irregular and distorted. Each MD simulation was run for 100 nanoseconds. The simulations were carried out with a constant number of particles (N), pressure (P), and temperature (T) (NPT ensemble). The long-range electrostatic interactions were calculated by the Particle-Mesh Ewald (PME) method.⁴⁷ A constant pressure of 1 bar was applied with a coupling constant of 1.0 ps; peptide, water molecules, and ions were coupled separately to a bath at 298 K with a coupling constant of 0.1 ps. The equation of motion was integrated at each 2 fs time steps. The tools available in the VMD⁴⁴ program were utilized to analyze the MD trajectories. The most representative structures were identified for structural elucidation. The most representative structures were derived from a cluster analysis, where the trajectories were analyzed by grouping structurally similar frames [root-mean-square deviation (rmsd) cutoff of 0.30 nm], while the frame with the largest number of neighbors was denoted as a middle structure that represents that particular cluster. The computed root-mean-square-deviations (rmsd) confirmed that all simulations attained equilibration within 50 or 100 ns for monomers and fibrils, respectively (Figure S1-S3). The YASARA program³⁹ was used for molecular visualization throughout the study and for the preparation of structural diagrams.

IId. Binding energy Calculations. The binding energy was computed using the following equation:

$$\Delta G_{sol} = \Delta G_{Electrostatic\ binding} + \Delta G_{Nonpolar\ binding}$$

Where $\Delta G_{Electrostatic\ binding}$ and $\Delta G_{Nonpolar\ binding}$ represents the electrostatic and nonpolar contributions to the solvation energy, respectively. A continuum electrostatic calculation was performed to compute the electrostatic interaction between the GAGs (A) and the A β_{40} fibrils/monomers (B) utilizing the Adaptive Poisson-Boltzmann Solver (APBS) software. The relative electrostatic binding energies were calculated using the following equation:

$$\Delta G_{Electrostatic\ binding} = \Delta G_{desol\ A}^{elec} + \Delta G_{desol\ B}^{elec} + E_{A-B}^{elec}$$

Where E_{A-B}^{elec} is the electrostatic interaction energy between the GAGs and the A β_{40} fibrils/monomers when they were bound in solution. E_{A-B}^{elec} was computed using the electrostatic potential (ψ_i) generated by A at the position of the atomic charges (q_i) of B by solving the following equation:

$$E_{A-B}^{elec} = \sum_i^N \varphi_i q_i$$

$\Delta G_{desol\ A}^{elec}$ and $\Delta G_{desol\ B}^{elec}$ represent the electrostatic desolvation free energies of A (GAGs) and B (fibrils/monomers), respectively, which are defined as the loss of the electrostatic interaction between the A and B upon binding. The following two step approach was used to calculate this energy: (1) calculation of the interaction energy of GAGs and the surrounding solvent in the absence of the A β_{40} fibrils/monomers and (2) calculation of the electrostatic energy of GAGs with the surround solvent in the presence of the A β_{40} fibrils/monomers. The differences in energies calculated in these two steps provided the electrostatic desolvation energies $\Delta G_{desol\ A}^{elec}$ or $\Delta G_{desol\ B}^{elec}$.

The complexation between the GAGs and A β ₄₀ fibrils/monomers was computed by solving the Poisson-Boltzmann equation. These calculations were performed at room temperature using two dielectric constants, 2.0 and 78.0 that represent protein and water environments, respectively. A probe sphere of 1.5 Å was used for the calculation of the solute surface, and grid spacing was kept to 0.35 Å. The dielectric boundary was defined as the van der Waals surface and a salt concentration of 55 mM was used. For all these calculations, all clustered structures in the PDB format were converted to the PQR format using the PDB2PQR server. The charges of GAGs were calculated based on initial resp charges calculated using quantum mechanical methods (Gaussian 09). The nonpolar contribution to the free energy was computed from the burial of solvent accessible surface area (SASA) of the GAGs and the A β ₄₀ fibrils/monomers upon binding using the following equation.

$$\Delta G_{Nonpolar\ binding} = \gamma \times SASA$$

Where SASA values of the individual components GAGs and A β ₄₀ fibrils/monomers, as well as, complexations were calculated utilizing the YASARA program. The microscopic surface tension coefficient ($\gamma = 0.0054$ kcal/mol Å²) connects the solvent accessible surface area to the free energy of transferring a molecule from alkane to water.

III. Results and Discussion.

IIIa. Docking of ADC, SDC, and DC to A β ₄₀. Docking of ADC, SDC, and DC to the A β ₄₀ fibril showed two major binding sites (A and B in Figure 2). The sites A and B are located in two distinct H14-K16 and E22-K28 regions, respectively. The site A runs longitudinally to the fibril axis and its number would increase with the growth of fibril. It is formed by residues H14, Q15, and K16 that are located on the top of the fibril. This binding site was generated by the

interactions between the negatively charged sulfates attached to ADC, SDC, and DC and the positively charged amino acid residues on the fibril's surface. For instance, the carboxylate and sulfate groups of both ADC and SDC formed hydrogen bonds with H14 and K16 residues of the A β ₄₀ fibril. DC showed similar hydrogen bonding interactions with three K16 residues of the fibril. On the other hand, the second site (site B) located at the terminal of the A β fibrils within the hairpin region is scarce considering the direction of the fibril elongation. It is generated by residues E22, D23, V24, G25, S26, N27, and K28 in the E22-K28 region of the fibril. Due to its location, binding to this site has the potential to inhibit the aggregation of A β fibrils; therefore, this binding site has been specifically focused on in our studies.¹³ The ADC, SDC, and DC molecules were also found to bind to the site B. Furthermore, ADC, SDC, and DC were docked to the A β ₄₀ monomer in order to elucidate the possible binding interactions between the earliest stage of A β ₄₀ aggregation and our heparin analogues. All three analogues studied bind to the N-terminus region of the peptide and strongly interact with polar amino acid R5, as well as, neutral residue H6.

IIIb. A β ₄₀ – ADC interactions. The ADC molecule shows binding to the N-terminus region of the A β ₄₀ monomer (Figure 3), between residues A1 and H6. Strong electrostatic interactions between the sulfated group present on ADC and polar residues D1 and R5 at distances of 2.13 and 1.97 Å respectively were observed. Furthermore, H6 shows a hydrogen bonding interaction with the terminal amine of ADC at a distance of 1.77 Å. The binding energy computed for this complex was -18.4 kJ/mol (Table 1).

MD simulations were also run with ADC binding to both A and B sites of the A β ₄₀ fibril (Figure 4). Site A was stabilized by many hydrogen bonds originating from polar and charged

amino acid residues H14 and K16, respectively. Moreover, ADC interacts through a host of hydrogen bonding interactions with polar residues located at site B (E22-K28) of the A β ₄₀ fibril. The amine present on ADC displays strong hydrogen bonding with a negatively charged oxygen of E22 at a distance of 2.23 Å. Additionally, ADC interacts with multiple K28 residues on separate monomers within the fibril through hydrogen bonding at distances shorter than 2.5 Å. The binding energy calculated for this complex was -22.4 kJ/mol (Table 1), which is more negative when compared to the binding of ADC to the peptide form.

IIIc. A β ₄₀ – SDC interactions. The supersulfated form (SDC) of the heparin disaccharide interacts with the A β ₄₀ monomer through its N-terminus region. The SDC is stabilized within this pocket by three electrostatic interactions with the N-terminus of residue D1 at distances of 2.06, 2.35, 3.32 Å (Figure 5). Positive residue R5 also shows two strong hydrogen bonds with a sulfate group of SDC at distances of 2.30 and 2.44 Å. These strong interactions kept SDC in place over the course of the MD simulation. The binding energy calculated for this complex indicates stable binding energies within this site (-20.2 kJ/mol, Table 1). This is slightly better than the previous case with ADC. However, this trend was expected due to an increase in negative charge for the SDC with compared with the ADC (more sulfate groups were added). This increase in negative charge would increase the attraction between SDC and the positively charged residues present on the A β ₄₀ aggregate's surface. SDC also interacted strongly with both site A and B of the fibril (Figure 6). MD simulations of site A reveal a host of hydrogen bonding interactions originating from polar and charged amino acid residues H14 and K16, respectively. All hydrogen bonds are within 3.0 Å, which indicates strong hydrogen bonding between the fibril's surface and the sulfate groups present on SDC. Interestingly, only three of the six sulfate groups interact with the

fibrils surface, most likely due to steric clashes or strain and electrostatic repulsion that would arise from the charged groups being too close together. For site B, again there were multiple hydrogen bonding interactions that were responsible for the binding of the ligand to the fibril. One sulfate group exhibited hydrogen bonding with N27 and K28. Another sulfate group interacted with V24 and the K28 of the neighboring monomer at distances of 1.84 and 2.01, respectively. The carboxylate group present on SDC also interacted with K28 at a distance of 2.12 Å. The binding energy calculated for this complex indicates stable binding energies within this site (-28.8 kJ/mol, Table 1). Since inhibition of the generation of higher order A β ₄₀ oligomers has been proposed as one of the promising strategies to prevent AD, the interactions of only the fibril form have been investigated with the remaining GAG molecules.

IIId. A β ₄₀ – DC interactions. At site A, the side chain of K16 of A β ₄₀ interacted with the negatively charged oxygen located within a sulfate group of DC. Only one sulfate group present on DC was not interacting with the fibril. The binding pocket was further stabilized by H14, showing a strong hydrogen bond with a sulfate group at a distance of 1.958 Å. Furthermore, down the fibril, K16 associated with a hydroxyl present on the ring of the heparin analogue. In many of our simulations, binding to different sections of the fibril caused the DC molecule to move to the top binding site. However, the association with site B of the fibril remained intact throughout the simulation time. At this site, DC exhibited several hydrogen bonding interactions as detailed in Figure 7. One of the sulfate groups of DC interacted with V24 and S26 residues in the hairpin region at distances of 1.80 and 2.00 Å, respectively. Additional interactions included hydrogen bonding between the carboxylate group of DC and a K28 residue and between a hydroxyl group of DC and another K28 residue. These strong hydrogen bonding interactions

were sufficient to keep DC bound to the fibril as seen over the course of a MD simulation. DC shows the weakest binding energy when comparing the three molecules studied, but was expected. With increasing negative charge present within the heparin analogues the binding energy also increases, this is not surprising as the negative charges of the heparin analogues would be attracted to the positively charged residues on the aggregates surface, whether on the side or top of the fibril. However, binding to the side of the fibrils surface would block the addition of more peptide units thereby controlling the aggregation of these critical peptides.

IIIe. A β ₄₀ – DC variants (V1, V2, V3 and V4) interactions. In addition to the three models described ADC, SDC, and DC, four more analogues were studied to see the effect of modifying the functional group present within our standard DC molecule. These four analogues are shown in Figure 1. In the first variant studied (V1), both the hydroxyl and carboxyl groups were deprotonated. The main reasons for the changes were to allow for more hydrogen bonding acceptors on the surface of V1 to increase the interaction with site B of the A β ₄₀ fibril. Furthermore, the deprotonated carboxylic acid could function as a molecular hook allowing for hydrogen bonding interactions at either end of their functional groups. However, V1 only interacted with site A and would not bind to site B of the fibril. In the second variant (V2), one carboxylic acid group was substituted for an aromatic benzene ring to see if the addition of a hydrophobic group would help stabilize site B. This site could be stabilized by interacting with some of the hydrophobic residues hidden within the stacks of the A β ₄₀ fibrils. However, no binding to this site of the fibrils was observed, mostly due to the steric bulkiness of the benzene group present on V2. In all of the simulations, V2 failed to stay bound to site B but did bind to a new pocket. Interestingly, V2 shifts to a more hydrophobic site on the top of the fibril closer to

hydrophobic residues V18 and F19. In the third variant (V3), the aromatic benzene was kept and a carboxylic acid group substituted for an amide group. Again, due to the bulkiness of the benzene ring, V3 binds only to the hydrophobic site formed by residues K16-F19. In the last Variant studied V4, methoxy groups were added to the DC fame work to gain the hydrophobic binding benefit but lose the steric bulkiness of having a benzene ring. Again, as seen for V3, V4 binds to the hydrophobic site formed between residues K16-F19 and did not bind to site B of the A β ₄₀ fibril.

IV. Conclusions. In this study, we have employed molecular docking and MD simulations techniques to investigate interactions of both monomeric and aggregated forms of A β ₄₀ with a wide range of chemically diverse forms of GAGs referred to as ADC, SDC, DC, V1, V2, V3 and V4 (Figure 1). GAGs such as heparin have been experimentally proposed to inhibit the generation of neurotoxic forms of amyloid oligomers.^{18, 48-49} Our docking results predicted two major binding sites (A and B) on A β ₄₀ fibrils (Figure 2). Site A is located at the top of the fibril, while site B is present in the hairpin region. Since the latter lies along the axis of fibrils, it is a novel target to design molecules to inhibit A β aggregation.¹³

The binding of ADC and SDC was investigated with both monomeric and fibrillar forms of A β ₄₀, while the interactions of the remaining GAGs were studied only with the former. Both ADC and SDC interacted with N-terminus, D1-H6 region, of the A β ₄₀ monomer (Figure 3 and 5). On the other hand, ADC, SDC, and DC associated with both A and B sites of the A β ₄₀ fibril in the H14-K16 and E22-K28 regions, respectively (Figure 4, 6 and 7). Among the four analogues of DC, V1 interacted only with site A and V2, V3 and V4 bound to neither site (Table

2). These results suggested that ADC, SDC and DC can inhibit amyloid aggregation and lead to the development of molecules for the prevention of AD.

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VI. Supporting Information Material (SI). Figure S1-S3: The figures showing the computed root-mean-square-deviations (rmsd).

VI. References.

1. Gaugler, J.; James, B.; Johnson, T.; Scholz, K.; Weuve, J., *Alzheimer's and Dementia* **2014**, *10*.
2. Wahlster, P.; Niederländer, C.; Kriza, C.; Schaller, S.; Kolominsky-Rabas, P. L., *Dement. Geriatr. Cogn. Disord.* **2013**, *36*, 263.
3. Ryan, S. M.; Kelly, Á. M., *Ageing Res. Rev.* **2016**, *27*, 77. doi: 10.1016/j.arr.2016.03.007.
4. Neumann, U.; Rueeger, H.; Machauer, R.; Veenstra, S. J.; Lueoend, R. M.; Tintelnot-Blomley, M.; Laue, G.; Beltz, K.; Vogg, B.; Schmid, P.; Frieauff, W.; Shimshek, D. R.; Staufenbiel, M.; Jacobson, L. H., *Mol. Neurodegener.* **2015**, *10*, 44. doi: 10.1186/s13024-015-0033-8.
5. Santana-Blank, L.; Rodríguez-Santana, E.; Santana-Rodríguez, K. E.; Reyes, H., *Photomed. Laser Surg.* **2016**, *34*, 93. doi: 10.1089/pho.2015.4015.
6. Masters, C.; Simms, G.; Weinman, N., *Proc. Natl. Acad. Sci. U.S.A.* **1985**, 4245.
7. Glenner, G.; Wong, C., *Biochem. Biophys. Res. Commun.* **1984**, *120*. doi: 10.1016/S0006-291X(84)80190-4.
8. Cui, H.; Hung, A.; Freeman, C.; Small, D., *J. Neurochem.* **2012**, 447. doi: 10.1111/j.1471-4159.2012.07929.x.
9. Grimm, M. O. W.; Mett, J.; Stahlmann, C. P.; Grösgen, S.; Haupenthal, V. J.; Blümel, T.; Hundsdörfer, B.; Zimmer, V. C.; Mylonas, N. T.; Tanila, H.; Müller, U.; Grimm, H. S.; Hartmann, T., *Front. Aging Neurosci.* **2015**, *7*, 77. doi: 10.3389/fnagi.2015.00077.
10. Cappai, R., *J. Neurochem.* **2016**. doi: 10.1111/jnc.13546.
11. Bergamaschini, L.; Rossi, E.; Vergani, C.; Simoni, M. D., *Scientific World J.* **2009**, 891.

- 355 12. Derrick, J. S.; Kerr, R. A.; Nam, Y.; Oh, S. B.; Lee, H. J.; Earnest, K. G.; Suh, N.; Peck, K.
356 L.; Ozbil, M.; Korshavn, K. J.; Ramamoorthy, A.; Prabhakar, R.; Merino, E. J.; Shearer, J.; Lee,
357 J.-Y.; Ruotolo, B. T.; Lim, M. H., *J. Am. Chem. Soc.* **2015**, *137*, 14785. doi:
358 10.1021/jacs.5b10043.
- 359 13. Cook, N. P.; Ozbil, M.; Katsampes, C.; Prabhakar, R.; Martí, A. A., *J. Am. Chem. Soc.* **2013**,
360 *135*, 10810. doi: 10.1021/ja404850u.
- 361 14. Prabhakar, R., *Future Med. Chem.* **2009**, *1*, 119. doi: 10.4155/fmc.09.10.
- 362 15. Echeverria, V.; Zeitlin, R.; Burgess, S.; Patel, S.; Barman, A.; Thakur, G.; Mamcarz, M.;
363 Wang, L.; Sattelle, D. B.; Kirschner, D. A.; Mori, T.; Leblanc, R. M.; Prabhakar, R.; Arendash,
364 G. W., *J. Alzheimers Dis.* **2011**, *24*, 817. doi: 10.3233/JAD-2011-102136.
- 365 16. Cui, H.; Hung, A. C.; Klaver, D. W.; Suzuki, T.; Freeman, C.; Narkowicz, C.; Jacobson, G.
366 A.; Small, D. H., *PLoS ONE* **2011**, *6*, e23007. doi: 10.1371/journal.pone.0023007.
- 367 17. Timmer, N. M.; van Dijk, L.; der Zee, C. E. E. M. v.; Kiliaan, A.; de Waal, R. M. W.;
368 Verbeek, M. M., *Neurobiol. Dis.* **2010**, *40*, 340. doi: 10.1016/j.nbd.2010.06.008.
- 369 18. Bergamaschini, L.; Rossi, E.; Storini, C.; Pizzimenti, S.; Distaso, M.; Perego, C.; De Luigi,
370 A.; Vergani, C.; Grazia De Simoni, M., *J. Neurosci.* **2004**, *24*, 4181. doi:
371 10.1523/jneurosci.0550-04.2004.
- 372 19. Hanin, I.; Dudas, B.; Mervis, R. F.; Cornelli, U.; Lee, J. M.; Lorens, S. A.; Fareed, J. In
373 *Mapping the Progress of Alzheimer's and Parkinson's Disease*; Mizuno, Y.; Fisher, A.; Hanin,
374 I., Eds. Springer US: Boston, MA, 2002; 171.
- 375 20. Sain, M.; Kovacic, V.; Radic, J.; Ljutic, D.; Jelacic, I., *Drug & Aging* **2012**, *29*, 1. doi:
376 10.2165/11592870-000000000-00000.

- 377 21. Bergamaschini, L.; Donarini, C.; Gobbo, G.; Parnetti, G., *Mech. Ageing Dev.* **2001**, 1971.
378 doi: 10.1016/S0047-6374(01)00311-6.
- 379 22. Velazquez, P.; Cribbs, D. H.; Poulos, T. L.; Tenner, A. J., *Nat. Med.* **1997**, 3, 077. doi:
380 10.1038/nm0197-77.
- 381 23. Webster, S.; Bonnell, B.; Rogers, J., *Am. J. Pathol.* **1997**, 1531.
- 382 24. Ma, Q.; Cornelli, U.; Hanin, I.; Jeske, W.; Linhardt, R., *Curr. Pharm. Des.* **2007**, 1607.
- 383 25. Tyrrell, D.; Horne, A.; Preuss, K.; Page, C., *Adv. Pharmacol.* **1999**, 151.
- 384 26. Watson, D.; Lander, A.; Selkoe, D., *J. Biol. Chem.* **1997**, 31617. doi:
385 10.1074/jbc.272.50.31617.
- 386 27. Bergamaschini, L.; Donarini, C.; Rossi, E.; Luigi, A. D.; Vergani, C., *Neurobiol. Aging.*
387 **2002**, 531. doi: 10.1016/S0197-4580(02)00003-9.
- 388 28. Pollack, S.; Sanders, I.; Hawtin, S., *Neurosci. Lett.* **1995**, 211. doi: 10.1016/0304-
389 3940(95)11939-T.
- 390 29. Pollack, S.; Sanders, I.; Hawtin, S., *Neurosci. Lett.* **1995**, 113. doi: 10.1016/0304-
391 3940(94)11182-I.
- 392 30. Woods, A.; Cribbs, D.; Whittemore, E.; Cotman, C., *Brain Res.* **1995**, 53. doi:
393 10.1016/0006-8993(95)00775-L.
- 394 31. Leveugle, B.; Ding, W.; Laurence, F.; Dehouck, M.; Scanameo, A.; Cecchelli, R.; Fillit, H.,
395 *J. Neurochem.* **1998**, 736. doi: 10.1046/j.1471-4159.1998.70020736.x.
- 396 32. Leveugle, B.; Ding, W.; Durkin, J.; Mistretta, S.; Eisle, J.; Matic, M.; Siman, R.; Greenberg,
397 B.; Fillit, H., *Neurochem. Int.* **1997**, 543. doi: 10.1016/S0197-0186(96)00103-9.
- 398 33. Nurcombe, V.; Ford, M.; Wildschut, J.; Bartlett, P., *Science* **1993**, 260, 103. doi:
399 10.1126/science.7682010.

- 400 34. Zhu, H.; Yu, J.; Kindy, M. S., *Mol. Med.* **2001**, *7*, 517.
- 401 35. Ahmed, T.; Smith, G.; Abraham, W. M., *Pulm. Pharmacol. Ther.* **2014**, *28*, 77. doi:
402 10.1016/j.pupt.2013.12.001.
- 403 36. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J.
404 R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li,
405 X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara,
406 M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.;
407 Nakai, H.; Vreven, T.; Montgomery Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd,
408 J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari,
409 K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.;
410 Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.;
411 Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R.
412 L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich,
413 S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian*
414 *09*, Gaussian, Inc.: Wallingford, CT, USA, 2009.
- 415 37. Petkova, A. T.; Yau, W. M.; Tycko, R., *Biochemistry* **2006**, *45*, 498. doi:
416 10.1021/bi051952q.
- 417 38. Coles, M.; Bicknell, W.; Watson, A. A.; Fairlie, D. P.; Craik, D. J., *Biochemistry* **1998**, *37*,
418 11064. doi: 10.1021/bi972979f.
- 419 39. Krieger, E.; Vriend, G., *Bioinformatics* **2002**, *18*, 315.
- 420 40. Trott, O.; Olson, A. J., *J. Comput. Chem.* **2010**, *31*, 455. doi: 10.1002/jcc.21334.
- 421 41. Wang, J.; Wang, W.; Kollman, P. A.; Case, D. A., *J. Mol. Graph. Model.* **2006**, *25*, 247. doi:
422 10.1016/j.jmgm.2005.12.005.

- 423 42. Wang, J.; Wolf, R. M.; Caldwell, J. W.; Kollman, P. A.; Case, D. A., *J. Comput. Chem.*
424 **2004**, *25*, 1157. doi: 10.1002/jcc.20035.
- 425 43. Lindahl, E.; Hess, B.; van der Spoel, D., *J. Mol. Model.* **2001**, *7*, 306. doi:
426 10.1007/s008940100045.
- 427 44. Humphrey, W.; Dalke, A.; Schulten, K., *J. Mol. Graph.* **1996**, *14*, 33. doi: 10.1016/0263-
428 7855(96)00018-5.
- 429 45. Makov, G.; Payne, M. C., *Phys. Rev. B.* **1995**, *51*, 4014. doi: 10.1103/PhysRevB.51.4014.
- 430 46. Berendsen, H. J. C.; Postma, J. P. M.; van Gunsteren, W. F.; Hermans, J. In *Intermolecular*
431 *Forces: Proceedings of the Fourteenth Jerusalem Symposium on Quantum Chemistry and*
432 *Biochemistry Held in Jerusalem, Israel, April 13–16, 1981*; Pullman, B., Ed. Springer
433 Netherlands: Dordrecht, 1981; 331.
- 434 47. Darden, T.; York, D.; Pedersen, L., *J. Chem. Phys* **1993**, *98*, 10089. doi:
435 doi:10.1063/1.464397.
- 436 48. Leveugle, B.; Ding, W.; Laurence, F.; Dehouck, M.-P.; Scanameo, A.; Cecchelli, R.; Fillit,
437 H., *J. Neurochem.* **1998**, *70*, 736. doi: 10.1046/j.1471-4159.1998.70020736.x.
- 438 49. Walzer, M.; Lorens, S.; Hejna, M.; Fareed, J.; Hanin, I.; Cornelli, U.; Lee, J. M., *Eur. J.*
439 *Pharmacol.* **2002**, *445*, 211. doi: 10.1016/S0014-2999(02)01759-4.

Figure 1. Atomic representation of heparin analogues used within this study. ADC (amide disaccharide), SDC (super-sulfated disaccharide), DC (heparin disaccharide), and structural models of DC variants (V1, V2, V3 and V4) are shown. Functional group substitutions are shown in red compared to DC.

Figure 2. A β_{40} fibril structure. Binding site A is formed between residues H14 and K16. Binding site B is formed between residues E22 and K28.

Figure 3. ADC (amide disaccharide) binding to the monomer form of the Aβ₄₀ peptide. (A) zoomed out version showing the monomer binding pocket in magenta. (B) zoomed in view with interacting residues and distances labeled.

Figure 4. ADC (amide disaccharide) binding to the Aβ₄₀ fibril. (A) zoomed out version showing the fibril binding pocket shown in magenta. (B) zoomed in view with interacting residues and distances labeled.

Figure 5. SDC (super-sulfated disaccharide) binding to the monomer form of the Aβ₄₀ peptide. (A) zoomed out version showing the monomer binding pocket. (B) zoomed in view with interacting residues and distances labeled.

Figure 6. SDC (super-sulfated disaccharide) binding to the Aβ₄₀ fibril. (A) zoomed out version showing the fibril binding pocket shown in magenta. (B) zoomed in view with interacting residues and distances labeled.

Figure 7. DC (heparin disaccharide) binding to the Aβ₄₀ fibril. (A) zoomed out version showing the fibril binding pocket shown in magenta. (B) zoomed in view with interacting residues and distances labeled.

System	E_{elec}	$\Delta G_{\text{desol_A}}$	$\Delta G_{\text{desol_B}}$	ΔG_{elec}	$\Delta G_{\text{nonpolar}}$	$\Delta G_{\text{binding}}$
ADC+monomer	-48.2	12.4	25.3	-10.5	-7.9	-18.4
ADC+Fibril	-81.0	34.8	39.7	-6.5	-15.9	-22.4
SDC+monomer	-74.2	36.4	26.0	-11.8	-8.4	-20.2
SDC+Fibril	-103.7	43.6	40.2	-19.9	-8.9	-28.8
DC+Fibril	-60.2	36.8	22.6	-1.0	-15.9	-16.9

Table 1. Shows the relative binding energies calculated with separate energy contributions determined. All energy values are shown in kJ/mol.

Heparin Analogues	Site A	Site B
ADC	X	X
SDC	X	X
DC	X	X
V1	X	O
V2	O	O
V3	O	O
V4	O	O

Table 2. List of successful heparin analogue binding to the $A\beta_{40}$ fibril. The “X” represents binding and the “O” represents no binding to that site.