



Article

Amyloid β Peptide Modifies Membrane Architecture and Surface Electrostatic Properties of Human Red Blood Cells

Galya Staneva ^{1,2} , Vesela Yordanova ^{1,2} , Avgustina Danailova ¹, Ana-Maria Marinovska ¹, András Dér ³ and Stefka G. Taneva ^{1,*}

¹ Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, “Acad. G. Bonchev” Str., Block 21, 1113 Sofia, Bulgaria; gstaneva@bio21.bas.bg (G.S.); vyordanova@biophys.bas.bg (V.Y.); avgustina_danailova@abv.bg (A.D.); anamariya.walter@gmail.com (A.-M.M.)

² Department of Endocrinology, Faculty of Medicine, Medical University—Sofia, “Acad. Ivan Geshov” Blvd. No. 15, 1000 Sofia, Bulgaria

³ Institute of Biophysics, HUN-REN Biological Research Centre, H-6701 Szeged, Hungary; derandra123@gmail.com

* Correspondence: sgtaneva@gmail.com

Abstract

Abnormal accumulation of amyloid-beta ($A\beta$) peptides in the brain is a hallmark of Alzheimer’s disease (AD). Importantly, the peripheral blood cells are also exposed to the effects of pathological peptides that accumulate in AD. Herein, the interaction of $A\beta$ 42 oligomers ($A\beta$ 42) with human red blood cells (RBCs) and erythrocyte ghosts as in vitro models for AD is studied combining fluorescence spectroscopy, fluorescence microscopy, and electrokinetics. The binding of $A\beta$ 42 to RBCs was evidenced by the use of a fluorescent-labeled peptide. The membrane lipid order increased with the increase in both the $A\beta$ 42 concentration and the incubation time, creating a lipid–protein microenvironment characterized by higher molecular order and reduced heterogeneity in RBC membranes compared to control conditions. Notably, the increase in lipid order was less pronounced in erythrocyte ghosts than in intact RBCs. Furthermore, the ζ -potential measurements revealed $A\beta$ 42 induced alteration of the surface potential of RBCs in a concentration- and time-dependent manner, with freshly isolated RBCs exhibiting a highly negative potential that became increasingly negative at higher $A\beta$ 42 concentrations. These findings suggest that $A\beta$ 42 not only impacts neuronal function but also significantly alters the physical properties of RBCs that might compromise their function, potentially contributing to the systemic effects observed in AD.

Keywords: red blood cells; amyloid beta; membrane lipid order; ζ -potential; confocal microscopy; Alzheimer’s disease



Academic Editor: Claudia Ricci

Received: 30 October 2025

Revised: 14 November 2025

Accepted: 20 November 2025

Published: 24 November 2025

Citation: Staneva, G.; Yordanova, V.; Danailova, A.; Marinovska, A.-M.; Dér, A.; Taneva, S.G. Amyloid β Peptide Modifies Membrane Architecture and Surface Electrostatic Properties of Human Red Blood Cells. *Int. J. Mol. Sci.* **2025**, *26*, 11361. <https://doi.org/10.3390/ijms262311361>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Neurodegenerative diseases are characterized by abnormal accumulation and misfolding of specific proteins, such as amyloid-beta ($A\beta$) peptide, tau (τ) protein, and α -synuclein (α -syn), in the brain and in peripheral tissues. Alzheimer’s disease (AD) is particularly associated with progressive accumulation of extracellular amyloid plaques of $A\beta$ peptide (40 ($A\beta$ 40) to 42 ($A\beta$ 42) amino acids long) in the human brain [1–10], characterized by extracellular deposition of amyloid β ($A\beta$) peptides, predominantly $A\beta$ 40 and $A\beta$ 42, and intracellular neurofibrillary tangles composed of hyperphosphorylated τ -protein [11–16]

that can cross the blood–brain barrier and reach the blood plasma [5,17]. Hence, the increased A β production in the brain may also be associated with higher A β concentrations in the blood of patients than in healthy subjects [18–20]. It should be noted here that the production of A β in healthy individuals represents a risk of AD development [21]. Thus, A β can affect not only neurons but also peripheral body fluids (plasma) and peripheral blood cells (erythrocytes (red blood cells, RBCs), platelets, and leukocytes) [22–25]. The A β peptide can be sequestered by a number of plasma proteins (albumin, fibrinogen, α 2-macroglobulin, α 1-antichymotrypsin, complement proteins, apolipoproteins, etc.) as well as by blood cells [22–29].

Different hypotheses have been proposed for the origin of AD. The most popular “amyloid cascade hypothesis” suggests a major role of amyloid plaques in AD [30–36] and is further modified to the “oligomeric amyloid hypothesis” that considers soluble A β oligomers as a trigger of the AD development [34,37–42]. Importantly, A β accumulation is detected not only in the brain of adults but also in young individuals [37,39,43], in patients with other pathologies [1,44–49] or brain injury [50,51], as well as in individuals without health problems [37,47,52,53]. All these studies suggest more complex pathophysiological mechanisms of AD [5,54]. Evidence for the unspecificity of A β plaques and soluble A β oligomers that contradict the “amyloid cascade hypothesis” are discussed in detail in [55]. On the other hand, generation of reactive oxygen species by A β that could induce oxidative damage of RBC membrane and in turn reduction of oxygen delivery from RBCs to the brain tissue has been reported [23,56]. The impaired oxygen release from RBCs to the brain and the related abnormal cellular metabolism have led to the erythrocytic hypothesis of the development of AD [55,57,58].

In addition, the involvement of intracellular A β peptides in mitochondrial dysfunction is the basis for the “mitochondrial cascade hypothesis” that considers the accumulation of individual mitochondrial dysfunctions along the process of cell aging, which affects A β peptide homeostasis and aggregation [59,60]. Nevertheless, there is uncertainty whether A β interaction with mitochondrial proteins and accumulation in the mitochondrial matrix contribute to mitochondrial dysfunction in early stages of the disease or whether multiple pathological events can cause mitochondrial disorders [61–63]. Important information for this pathology is gained by *in vitro* studies of the A β interaction with membrane proteins [64], lipids [65,66], model membranes [67,68], and cells, including RBCs [69–73]. Molecular dynamics (MD) simulations and time-lapse atomic force microscopy (AFM) imaging revealed aggregation of A β 42 on bilayer surfaces and dramatic transformation of A β 42 monomers to misfolded, aggregation-prone conformations [74]. The conformational changes in A β are assumed to be strongly related to the membrane lipid composition [75,76]. Details of the initial stages of A β monomer–membrane interaction are provided by investigation on model lipid monolayers and bilayers containing negatively charged phospholipids in both the liquid-disordered (L_d) and the liquid-ordered (L_o) state [67,77,78]. The interaction is shown to be weak in the absence and stronger in the presence of negatively charged phospholipids (phosphatidic acid or cardiolipin) using a number of techniques and confirmed by MD simulations. MD has also shown that fibril formation on the membrane surface depends on the bilayer composition [77]. The binding of A β 42 monomers to gangliosides containing sphingomyelin/cholesterol bilayers is shown to facilitate the aggregation of A β 42, giving rise to more extended β -sheets [78]. Hence, lipid rafts, i.e., membrane domains enriched in sphingomyelin (SM) and cholesterol (Chol), are proposed to play a key role in the A β peptide aggregation at neuronal membranes and formation of toxic oligomers, and the high Chol content of cellular membrane lipid rafts favor the amyloidogenic pathway [68,79–81].

Furthermore, AFM-based imaging of blood reveals alterations in RBC size, shape, and morphology related to neurocognitive disorder and presence of fibrils assembled in crystals on the surface of RBCs from older AD patients [71]. The Girasole research group [82] demonstrates A β -induced changes in the membrane cytoskeleton and the membrane integrity, and accelerated morphological alterations and aging of human RBCs. In line with these studies, we have recently reported similar alterations in the morphology and nanomechanics of RBCs derived from patients with AD [83]. Using flow cytometry, Jayakumar et al. [69] reported that A β 40 amyloid fibrils have high binding affinity for RBCs. The alterations in the RBC membrane architecture in AD subjects have been attributed to A β interactions with RBCs and/or to changes in the RBC membrane proteome [84].

In this work, we report on the interaction of A β 42 oligomers (A β 42) with freshly isolated human RBCs and hemoglobin-free human erythrocytes, so-called erythrocyte ghosts (RBC ghosts) as in vitro AD models, using a number of experimental approaches (fluorescence spectroscopy, fluorescence microscopy, and electrokinetics). Our findings demonstrate that A β 42 interaction with both RBCs and erythrocyte ghosts is associated with changes in the membrane lipid order and surface charge. Fluorescence microscopy shows localization of A β 42 on the cell surface.

2. Results

2.1. RBC Aging Monitored by Laurdan GP

The effect of A β 42 on RBCs was studied at different oligomer concentrations (1–60 μ M) and different incubation time (from 1 to 48 h). Peripheral plasma A β levels are typically in the pico- to nanomolar range [85,86]. The lowest concentration used here (1 μ M) therefore seems to be supraphysiological for bulk blood but serves as a mechanistic probe of membrane interactions and may approximate local effective concentrations at microvascular interfaces or on RBC-bound aggregates. However, the bulk concentration is not the most relevant parameter for our experimental context. What is physiologically important is the effective ratio between red blood cell membrane lipids and A β molecules. At 20% hematocrit (Hct), the total concentration of RBC membrane lipids is approximately 2 mM, meaning that in our experiments, there are roughly 1000–2000 lipid molecules per amyloid molecule. This ratio does not represent an excessive A β load and supports the use of micromolar A β concentrations as mechanistic probes of lipid–protein interactions.

We first performed experiments with untreated RBCs at the same incubation time as for A β 42-treated cells.

The dataset in Figure 1 illustrates the typical time-dependent changes in Laurdan generalized polarization (GP) values that occur during RBC aging. Only two samples were presented in the RBC aging graphs, but all nine studied samples were analyzed both for aging alone and for aging in the presence of A β 42. The graph demonstrates the direction and magnitude of the aging effect in terms of membrane lipid order, rather than to perform statistical analysis of aging across different samples. The statistics shown in the figure reflect the significance of GP differences between fresh and aged RBCs for each individual sample. Aging of RBCs is related to GP increase (Figure 1) that corresponds to an increase in membrane dehydration/membrane lipid order.

As shown in Table 1, the temporal changes in GP values vary between individuals (data presented for two representative volunteers), with the most significant changes occurring within the first 24 h. Additionally, the rate of RBC aging was greater in membranes with lower initial lipid order (e.g., sample 2) compared to those with higher initial lipid order (e.g., sample 1). In other words, the more ordered the membrane at baseline, the smaller the relative GP increase over time. Although Figure 1 and Table 1 present data from two representative donors, the same inverse relationship between initial lipid or-

der and the magnitude of the GP change was consistently observed across all nine RBC samples analyzed.

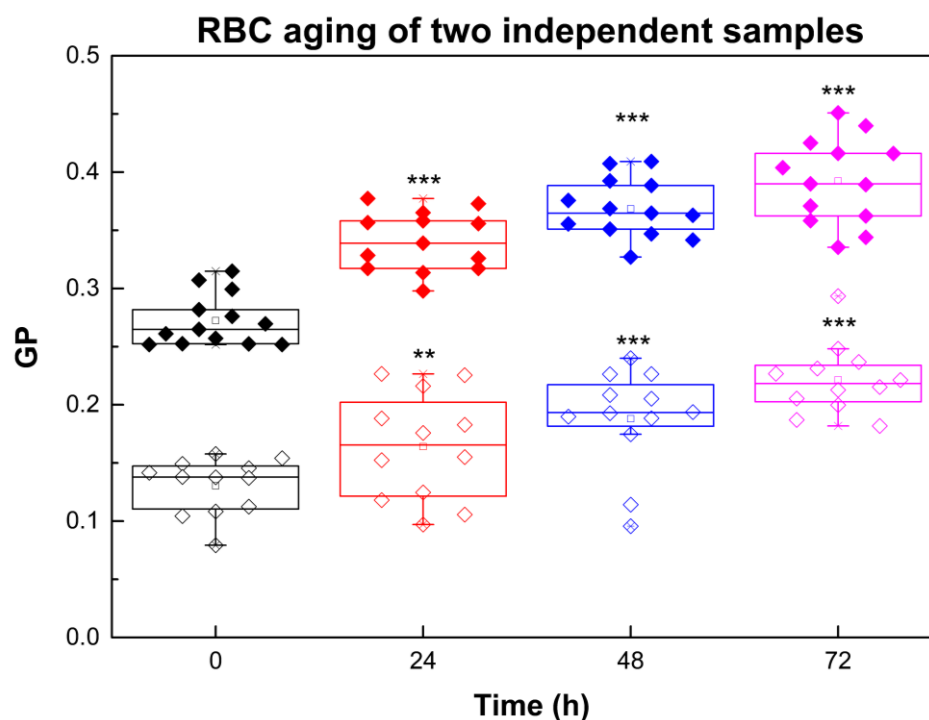


Figure 1. Laurdan GP of RBCs taken from two independent donors (sample 1 (filled symbols) and sample 2 (empty symbols)) as a function of time: black, 0 h; red, 24 h; blue, 48 h; purple, 72 h. For measurements of Laurdan fluorescence RBC suspensions with 20% Hct were diluted 200 times. Error bars correspond to the standard deviations (SDs). SD of 12 measurements per sample with 0.1% Hct at 37 °C at each studied time is shown. Nonparametric Mann–Whitney test was applied to determine p between control (0 h) and 24, 48 and 72 h: $p < 0.01$ (**); $p < 0.001$ (***).

Table 1. Relative change in GP (in % of GP for fresh RBCs, GP_0) as a function of time calculated at $t_i = 24, 48$ and 72 h.

Sample	ΔGP at 24 h	$\Delta GP/GP$ at 24 h (%)	ΔGP at 48 h	$\Delta GP/GP$ at 48 h (%)	ΔGP at 72 h	$\Delta GP/GP$ at 72 h (%)
Sample 1	0.07 ± 0.03	25	0.09 ± 0.03	35	0.12 ± 0.03	44
Sample 2	0.03 ± 0.02	26	0.06 ± 0.05	44	0.09 ± 0.03	69

$$\Delta GP/GP (\%) = ((GP_{t_i} - GP_0)/GP_0) \times 100.$$

2.2. Interaction of A β 42 Oligomers with RBC

Likewise, a noticeable increase in GP is observed with the increase in both the A β 42 concentration and the duration of RBC incubation with A β 42 (Figures 2 and 3). A 1 h incubation results in a 10% increase in membrane lipid order at 1 μ M A β 42, a 20% increase at 5–40 μ M A β 42, and a 30% increase at 50–60 μ M A β 42. 30% increase in the membrane lipid order can also be achieved for 24 h incubation of RBCs with 1 μ M A β 42. Further increase in the membrane lipid order to 60% is reached for 48 h incubation time.

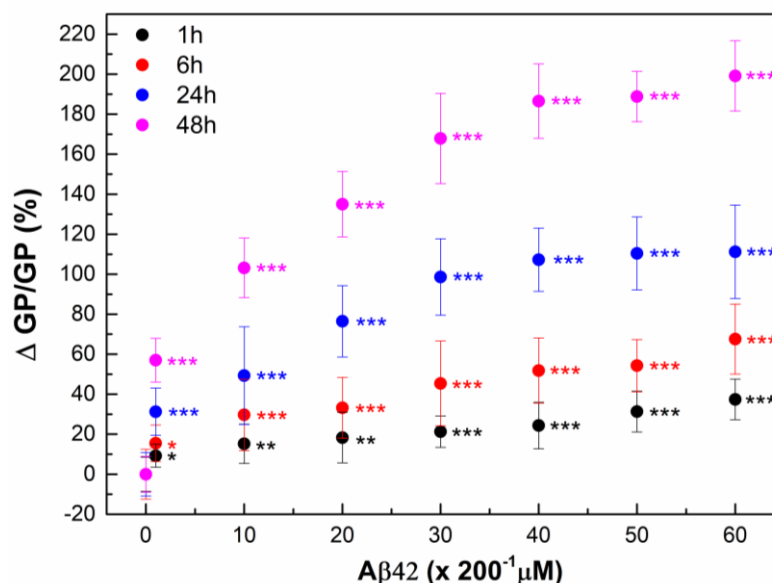


Figure 2. The relative change in Laurdan GP, $\Delta GP/GP$ (%), of RBCs was calculated as $((GP_{Ci} - GP_0)/GP_0) \times 100$ of 9 independent samples (individuals) as a function of time. RBC suspensions with 20% Hct were incubated with A β 42 oligomers in concentrations from 1 to 60 μM ($C_i = 1, 10, 20, 30, 40, 50$ and $60 \mu M$) for 1 (black)-, 6 (red)-, 24 (dark blue)- and 48 (pink)-hours ($t_i = 1, 6, 24$ and 48 h) at $37^\circ C$. GP_0 indicates GP value of non-treated RBCs ($0 \mu M$ A β 42). For measurements of Laurdan fluorescence the non-treated and A β 42-treated RBC suspensions were diluted 200 times. The data represented 6 measurements of each RBC suspension with 0.1% Hct at $37^\circ C$. SD of 54 measurements per point is shown. Nonparametric Mann–Whitney test was applied to determine p between control non-treated and A β 42-treated RBCs: $p < 0.05$ (*); < 0.01 (**); < 0.001 (***)

2.3. Monitoring RBC Aging with and Without A β 42 Using GP

The data presented in Figure 3 highlight two parallel processes influencing membrane lipid order: the concentration-dependent effect of A β 42 oligomers and the gradual aging of RBCs, both of which manifested as an increase in GP. The pronounced changes observed within the first day indicate that the structural perturbation of the RBC membrane is driven mainly by the direct interaction of A β 42 with the membrane. Once the non-bound peptide is removed, ΔGP remains stable, suggesting that A β 42 binding induces a relatively fixed alteration in membrane organization rather than a progressive effect. In addition, the continued increase in GP over time reflects intrinsic RBC aging process. Consequently, while the absolute GP values rise with time, the relative contribution of A β 42 ($\Delta GP/GP$) diminishes as RBCs age (Table 2). Both A β 42 interaction and cellular aging promote higher membrane lipid order; however, the extent of this ordering differs. Aging of intact RBCs over 72 h leads to an approximately 70% increase in membrane lipid order, whereas A β 42 alone induces about a 50% increase at the higher concentration, and aging of A β 42-treated RBCs results in an approximately 120% increase. These findings suggest that A β 42 binding modifies the membrane structure in a way that accelerates the aging trajectory in terms of lipid order, consistent with AFM data from Girasole's group [87], showing that A β 42 significantly accelerates morphological and nanomechanical alterations relative to normal aging.

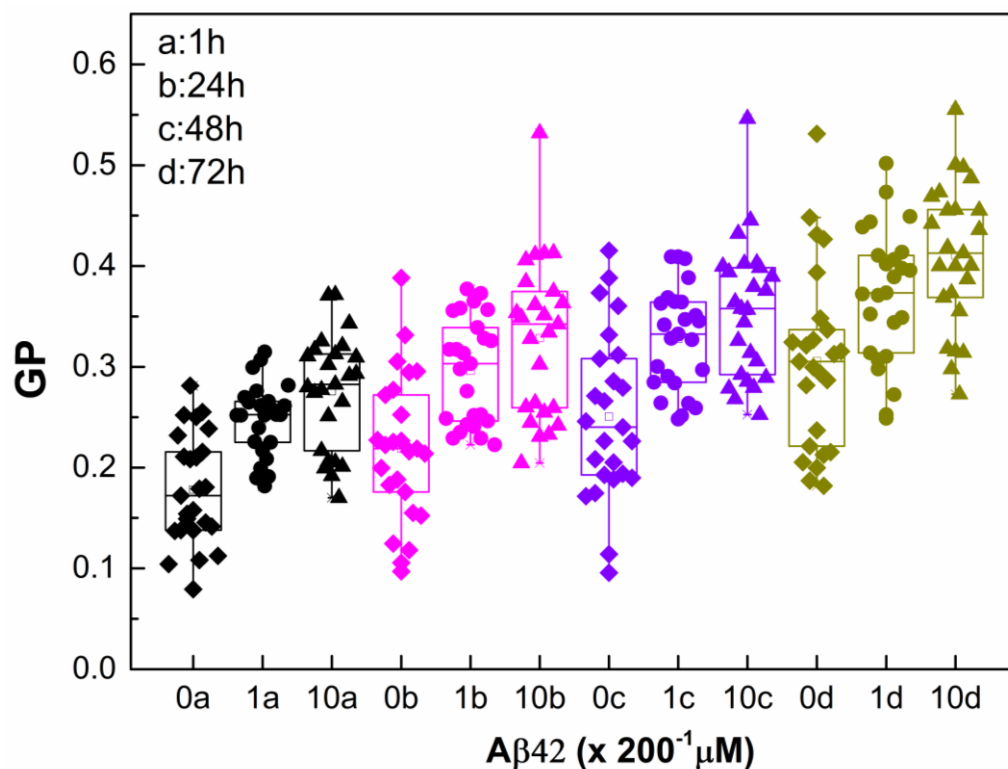


Figure 3. Laurdan GP of non-treated and A β 42-treated RBCs taken from 2 independent samples as a function of time. The RBC suspensions with 20% Hct were treated with A β 42 oligomers (0 μ M (diamonds), 1 μ M (circles), and 10 μ M (triangles)), incubated for 1 h, washed and measured immediately (1 h (black)), and after 24 h incubation with A β 42 oligomers at 37 $^{\circ}$ C, the samples were washed and measured at 24 (pink), 48 (purple), and 72 h (olive). For measurements of Laurdan fluorescence, the non-treated and A β 42-treated RBC suspensions were diluted 200 times. SD of 12 measurements per sample with 0.1% Hct at 37 $^{\circ}$ C at each studied time is shown. Nonparametric Mann–Whitney test is applied to determine p between control non-treated and A β 42-treated RBCs. Each studied concentration is significantly different from its control with $p < 0.001$.

Table 2. Relative change in GP (%) of non-treated RBCs ($GP_{t0\mu M}$) and cells treated with A β 42 oligomers (1 and 10 μ M) for 24 h. The unbound A β 42 was washed and $\Delta GP/GP$ was calculated for time $t_i = 1, 24, 48$, and 72 h.

Time (h)	A β 42 (μ M)	GP	ΔGP	$\Delta GP/GP$ (%)
1	0	0.1780		
	1	0.2479	0.0699	39
	10	0.2756	0.0976	55
24	0	0.2187		
	1	0.2961	0.0774	35
	10	0.3285	0.1098	50
48	0	0.2505		
	1	0.3276	0.0771	31
	10	0.3552	0.1047	42
72	0	0.3057		
	1	0.3714	0.0657	21
	10	0.4105	0.1048	34

$$\Delta GP/GP (\%) = ((GP_{t_i} - GP_{t0\mu M})/GP_{t0\mu M}) \times 100.$$

2.4. Visualization of A β 42-RBCs Interaction

Visualization of fluorescent A β 42 proves A β binding to RBCs; higher A β 42 concentration leads to the formation of larger A β 42 aggregates on the RBC membranes

(Figure 4). Some of these aggregates appear to be associated with the formation of RBC membrane spikes.

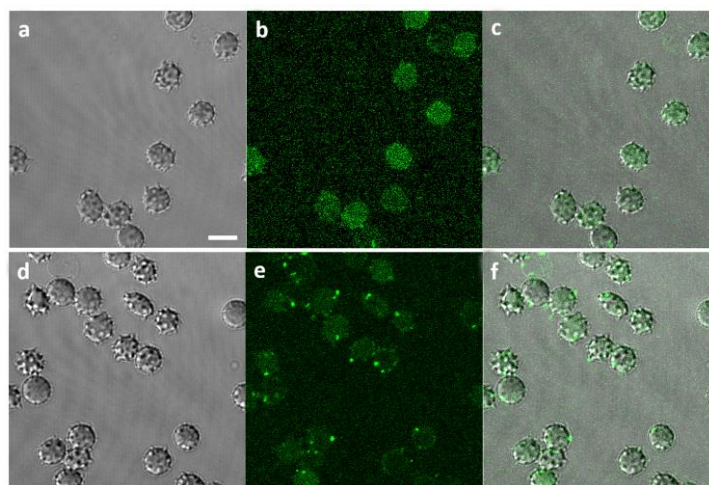


Figure 4. Visualization of A β 42 binding to RBCs: (a) Phase contrast of RBCs incubated with 1 μ M fluorescent A β (1–42) HiLyte™ Fluor 488 (A β *) for 48 h; (b) Green channel visualizing 1 μ M A β 42* binding to RBCs; (c) Merged channels at 1 μ M A β 42*/RBCs; (d) Phase contrast of RBCs incubated with 10 μ M A β 42* for 48 h; (e) Green channel visualizing 10 μ M A β 42* binding to RBCs; (f) Merged channels at 10 μ M A β 42*/RBCs. Scale bar: 5 μ m.

2.5. Plasma Membrane Lipid Order Visualization by Di-4-ANEPPDHQ Fluorescence Microscopy

GP profiles of intact not-treated RBCs and cells treated with A β 42 oligomers were monitored after 48 h incubation. The GP profiles shift to higher GP values in A β 42 concentration-dependent way (Figure 5), and the higher the GP value, the higher the membrane lipid order.

Figure 6 demonstrates that at least two distinct GP profiles (microenvironments) exist in control untreated RBCs.

One profile is centered at -0.25 , corresponding to membranes in the liquid-disordered phase (L_d), while the other is centered at 0.38 , representing membranes in the liquid-ordered phase (L_o). Incubation of RBCs with 1 μ M A β 42 oligomers for 48 h increases the GP values of both profiles and alters the relative ratio between the two coexisting GP distributions, as well as their full width at half maximum (FWHM). Increasing A β 42 concentrations up to 20 μ M do not significantly affect the membrane lipid order of the distributions but do shift the proportions between the coexisting microenvironments. The FWHM quantifies the heterogeneity of the two coexisting microenvironments, revealing a clear difference in heterogeneity between A β 42-treated and untreated RBCs. A decrease in FWHM describes a reduction in heterogeneity. Therefore, A β 42 appears to induce a microenvironment with higher lipid order and a substantially lower degree of heterogeneity compared to the control conditions.

2.6. Interaction of A β 42 Oligomers with RBC Ghosts

We used hemoglobin-free erythrocytes (RBC ghosts) for two main reasons. The first is technical—to avoid spectral overlap caused by hemoglobin absorption and fluorescence, as well as the potential quenching effect of hemoglobin on Laurdan emission [88]. The second is mechanistic—to distinguish the effects of A β 42 interaction with membrane components from possible interactions with intracellular hemoglobin [89,90]. In addition, this approach allowed us to specifically assess the interactions of A β 42 oligomers with the erythrocyte membrane, independent of intracellular components.

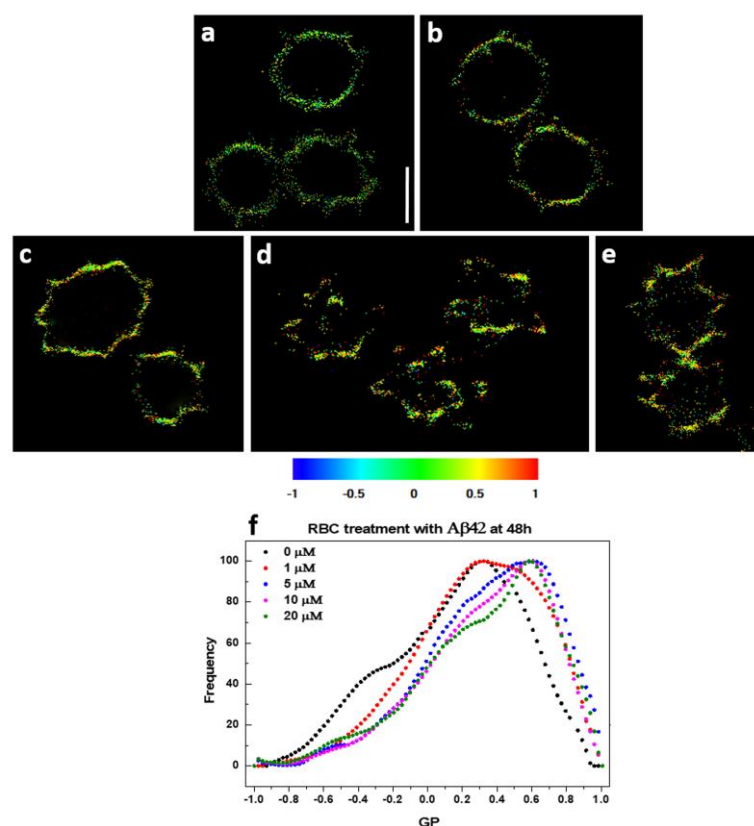


Figure 5. Calculated GP micrographs of control and Aβ42-treated RBCs stained with Di-4-ANEPPDHQ. (a) Untreated RBCs and RBCs treated with (b) 1 μM, (c) 5 μM, (d) 10 μM, and (e) 20 μM Aβ42 oligomers. Regions of high (red, GP = 1) and low (blue, GP = −1) membrane lipid order are visualized. Scale bar = 5 μm. (f) GP profiles of control and Aβ42-treated RBCs. Black curve corresponds to untreated RBCs; whereas red (1 μM), blue (5 μM), pink (10 μM) and green (20 μM) represent Aβ42-treated RBCs.

Aβ42 induced a smaller increase in membrane lipid order in RBC ghosts compared to intact RBC membranes (Figures 2 and 7). At incubation with 1 μM Aβ42, the change in membrane lipid order in RBC ghosts was two-fold lower than that observed in intact RBC membranes. Increasing Aβ42 concentration and incubation time progressively enhanced the difference in Aβ42-induced changes in membrane lipid order between RBC ghosts and intact RBC membranes.

2.7. ζ-Potential of Human RBCs After Interaction with Aβ42 Oligomers

To further validate the binding of Aβ42 to RBCs, we evaluate the ζ-potential of RBCs before and after interaction with Aβ42 oligomers. The ζ-potential, i.e., the electrostatic potential at the shear plane that separates the compact layer of strongly associated ions from the diffuse layer, is a measure of accessible surface charges on the cell surface [91,92]. ζ-potential measurements showed changes in the RBC potential in the presence of Aβ42 in a concentration- and incubation time-dependent manner (Figure 8a). The potential of fresh non-treated RBCs was -18.64 ± 0.27 mV; its absolute value became increasingly negative with increasing the Aβ42 concentration and was more negative for 48 h than for 1 and 6 h of RBC incubation with Aβ42. Importantly, significantly lower Aβ42 concentration of 20 μM induced the same change in ζ-potential at 48 h incubation time as 40 μM for 1 and 6 h of incubation of RBCs with Aβ42 (Figure 8a).

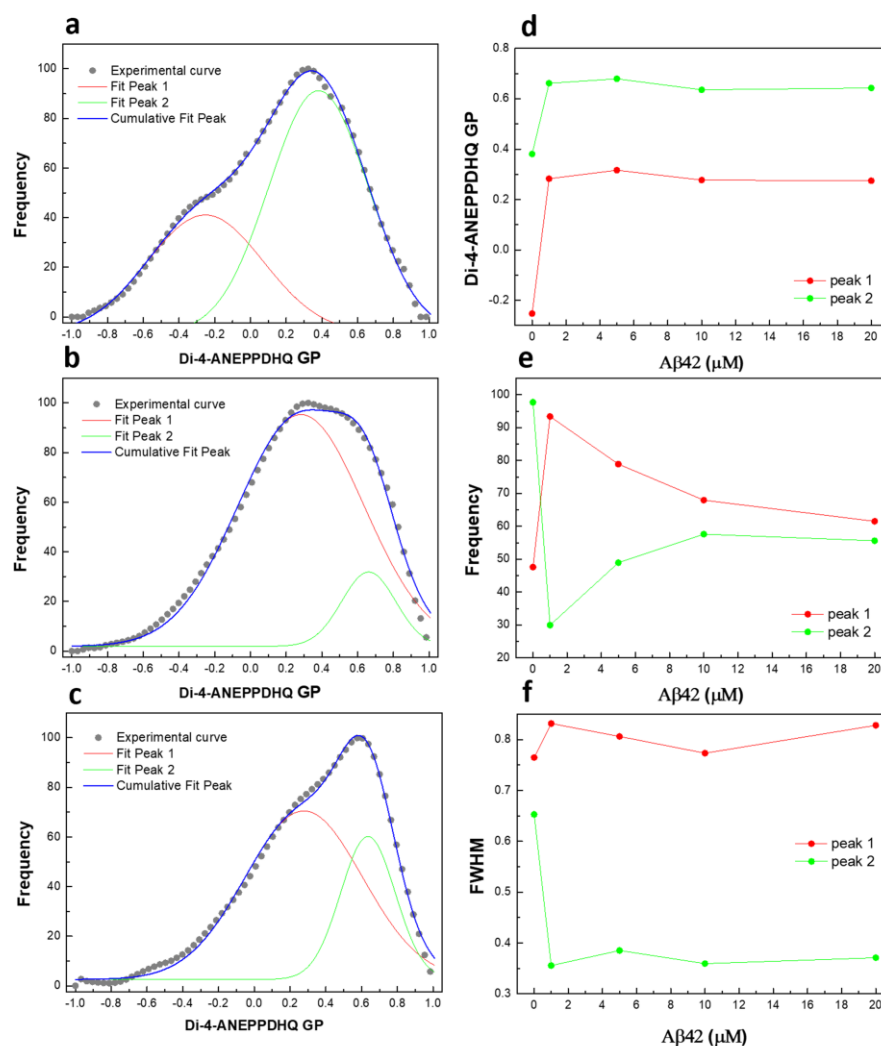


Figure 6. Deconvolution of GP profiles by two peaks: (a) untreated RBCs and cells treated with (b) 1 μM and (c) 10 μM Aβ42 oligomers at 48 h; (d) GP value at max frequency of each peak as a function of Aβ42 concentration; (e) frequency as a function of Aβ42 concentration; (f) full width at half maximum (FWHM) of each peak as a function of Aβ42 concentration.

In Figure 8b, the difference between the ζ -potential of fresh non-treated RBCs ($\zeta_{\text{RBC}t=0}$) ($t = 0$ h) and cells treated with Aβ oligomers (RBCs/Aβ42 complexes) ($\zeta_{\text{RBC}ci}$) incubated for 1, 6 and 48 h ($\Delta\zeta = \zeta_{\text{RBC}t=0} - \zeta_{\text{RBC}c=0}$ or $\zeta_{\text{RBC}ci}$) is presented. The highest $\Delta\zeta$ values of ca. 1 mV were estimated for the longest interaction time (48 h) at Aβ42 concentrations of 30 μM and above, while rather close values of $\Delta\zeta$ were evaluated for 1 and 6 h incubation time. At the highest Aβ42 concentrations of 50–60 μM, $\Delta\zeta$ reached a value of 1 mV after only 1 h incubation time.

The ζ -potential of RBC ghosts was -33.28 ± 0.71 mV, a value significantly more negative than that determined for RBCs, which can be attributed to the different ionic strength of the buffers RBCs and ghosts were prepared. As with the case of RBCs, Aβ42 induced an increase in the negative ζ -potential of erythrocyte ghosts in a concentration-dependent way (Figure 9). The ζ -potential changes observed in Aβ42-treated ghosts were larger than those measured in Aβ42-treated intact RBCs at both incubation times (1 h and 48 h) (Figures 8b and 9), suggesting a higher propensity of the ghosts to interact with Aβ42. This is likely due to the purification of the cell membranes, which removes not only hemoglobin but also other intracellular and membrane-associated components that could otherwise modulate the electrostatic interactions between the cells and Aβ42.

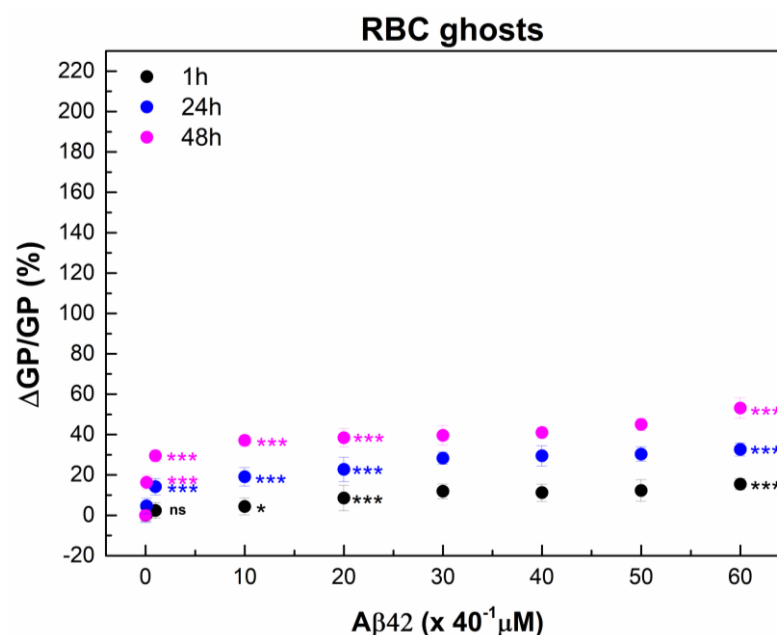


Figure 7. The relative change in Laurdan GP, $\Delta GP/GP$ (%), of RBC ghosts calculated as $((GP_{Ci} - GP_0)/GP_0) \times 100$ of 3 independent samples (individuals) as a function of time. RBC ghost suspensions with final total protein (TP) concentration of 2 mg/mL were incubated with A β 42 oligomers in concentrations from 0.1 to 60 μM ($C_i = 0.1, 1, 10, 20, 30, 40, 50$, and 60 μM) for 1 (black), 24 (dark blue) and 48 (pink) hours ($t_i = 1, 24$ and 48 h) at 37 °C. GP_0 indicates GP value of non-treated RBC ghosts (0 μM A β 42). For Laurdan spectra measurements, non-treated and A β 42-treated RBC ghost suspensions were diluted 40 times. The data represented 6 measurements of each RBC ghost suspension with 50 $\mu g/mL$ TP at 37 °C. SD of 18 measurements per point is shown. Nonparametric Mann–Whitney test was applied to determine p between control non-treated and A β 42-treated RBCs: $p < 0.05$ (*); < 0.001 (***), and no statistically significant differences were denoted by ns.

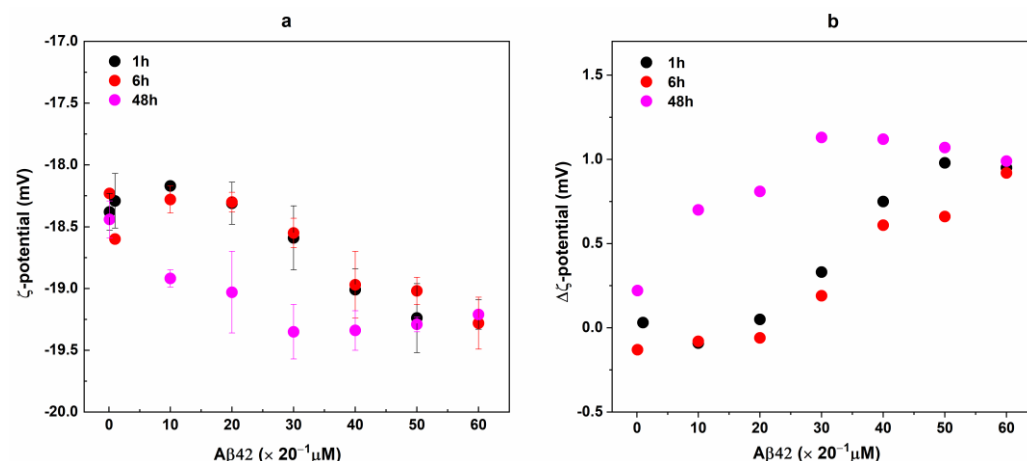


Figure 8. ζ -potential of A β 42-treated RBCs taken from 3 independent samples each measured in 3 replicates (a). RBC suspensions with 20% Hct were incubated with A β 42 oligomers in concentrations from 0.1 to 60 μM for 1 (black), 6 (red), and 48 (pink) hours at 37 °C. For ζ -potential measurements non-treated and A β 42-treated RBC suspensions were diluted 20 times. The data represented 9 measurements of RBC suspensions with 1% Hct at 25 °C. Error bars corresponded to the standard deviations (SDs). The difference, $\Delta \zeta$, between the mean values of ζ -potential of non-treated ($RBC_{C=0}$) and A β 42-treated RBCs (RBC_{Ci}) at $t = 1, 6$, and 48 h (b).

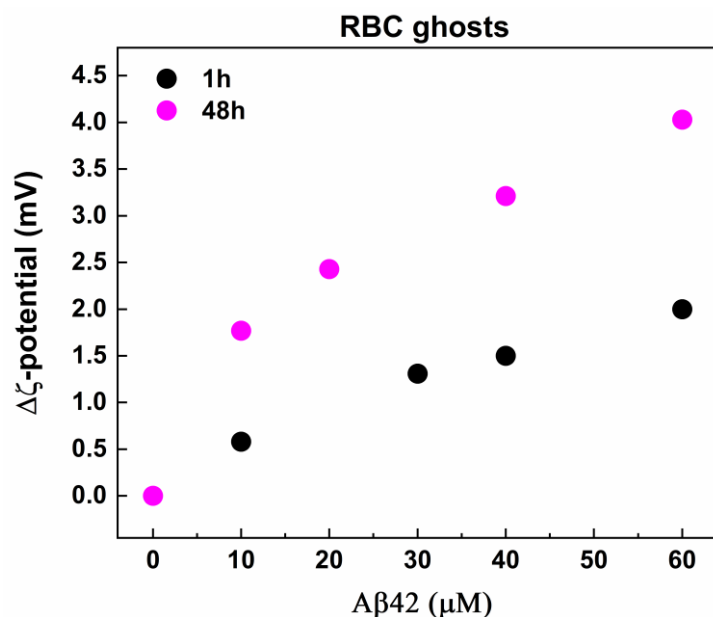


Figure 9. The difference, $\Delta\zeta$, between the mean value of ζ -potential of fresh non-treated RBC ghosts and the mean values of ζ -potential of non-treated and A β 42-treated RBC ghosts with final TP concentration of 2 mg/mL incubated for 1 (black) and 48 (pink) hours at 37 °C. The ghost suspensions were treated with A β 42 oligomers in concentrations from 0.1 to 60 μ M and were measured without dilution of samples at 25 °C.

3. Discussion

Herein, we report on the interaction of A β 42 oligomers, considered the most fibrillogenic and neurotoxic A β isoform, with RBCs and its effect on the cells' physical features. The amphiphilic peptide is known to have a tendency to aggregate at hydrophilic–hydrophobic interfaces and at cell membrane surfaces [93–95], as well as affect RBC morphology and deformability [96]. Depending on the concentration, the peptide exhibits neurotrophic and neurotoxic properties [82,97].

Our data suggest that the RBC membrane architecture and surface properties are highly sensitive to the presence of A β 42. We confirmed that A β 42 binds to RBCs and forms large aggregates on the surface of the cell membrane. This is consistent with AFM data, which show development of crenatures, proto-spicules, and peculiar nanoscale features on the cell membrane upon A β 42/RBC interaction, accompanied by acceleration of both the weakening of the cell cytoskeleton contacts and the age-related morphological and biochemical modifications of RBCs [82]. AFM imaging and computational modeling revealed aggregation of A β 42 on the membrane surfaces and changes in the conformation of A β 42 monomers [74]. In addition, AFM of smears of blood from patients with cognitive deficits display different types of protein aggregates (oligomers, protofibrils, and fibrils) on RBCs depending on patient age and the severity of the disorder, as well as crystals of fibrils for AD patients over 80 years old [71]. While previous RBC studies reported by Girasole et al. [82] emphasized nanotopography and mechanics (AFM morphology/roughness), our approach combines Laurdan GP (lipid packing) with ζ -potential (surface electrostatics) and uses hemoglobin-free ghosts to isolate membrane-intrinsic effects of A β 42 oligomers, thereby providing complementary mechanistic insight.

Concomitant with formation of large A β 42 aggregates on the cell membrane surface, we observed progressive increase in the membrane lipid order upon RBC interaction with higher A β 42 concentration. It is worth mentioning that the fluorescence GP parameter shows that the lipid order also increases along the aging process. Moreover, the rate of cell

aging depends on the membrane order, the less the membrane order the higher the aging rate. These results suggest an interaction of A β 42 with RBC membrane lipids.

Binding of A β 42 to membrane lipids has been studied extensively in model systems and damage to the bilayer structure has been reported [65,66,98]. It has also been found that as a consequence of the interaction with cell membrane lipids, the conformation of the peptide is changed [65,99] depending on the lipid composition [75,76,98]. It was suggested that the lipid rafts, e.g., nanodomains of cell membrane enriched in SM and Chol [67,100], are the place where oligomers and aggregates of exogenous A β 42 are formed [80]. Furthermore, the interaction with SM is supposed to occur on the surface of cell membrane and that with Chol within the lipid bilayer, which might lead to fibrillization of the peptide and formation of aggregates, respectively [101]. A recent in vitro study has shown that gangliosides increased the A β 42 peptide amount bound to the SM/Chol bilayer and facilitated peptide aggregation, but as the authors have discussed, the role of gangliosides at the nanoscale cannot be predicted [78].

Specialized submicrometric structures, enriched in Chol and sphingolipids, have been recently described in RBC membrane [102–105]. The RBC lipid bilayer, composed of phospholipids, a large proportion of Chol, as well as sphingolipids, is localized between the external glycocalyx layer and the membrane cytoskeleton, e.g., a network of proteins on the inner layer of the lipid bilayer [105]. The submicrometric domains are detected in the outer monolayer of RBC membrane, the Chol-enriched domains are preferentially linked to the edges, and the SM-enriched ones to the center of the membrane. Furthermore, the domains enriched in Chol are assembled at high curvature areas and those enriched in SM at low curvature areas of the membrane [101]. Using specific, sensitive, and quantitative probe (His-mCherry-theta-D4 (theta *)) capable of detecting Chol Carquin et al. [106] have shown clustering of lipids in two submicrometric domains at RBC membranes containing well-organized and less-organized Chol pools.

In concert with the above results, we found that at least two microenvironments exist in fresh RBC membranes (in the absence of A β 42). This is consistent with X-ray diffraction data of oriented, multi-lamellar stacks of RBC membranes. The authors provide evidence of a patchy structure of RBC membranes composed of liquid-ordered (L_o) and liquid-disordered (L_d) lipid domains, as well as integral coiled-coil peptides domains [107].

Apparently, A β 42 is able to change the lipid order of the two microenvironments and the proportion between the coexisting environments depending on the A β 42 concentration. Thus, A β 42 induced a microenvironment with higher lipid order and much lower degree of heterogeneity compared to untreated cells. Taking into account the earlier findings [101,106], we hypothesize that the peptide preferentially incorporates into the SM-enriched domains, contributing to the increase in the proportion of the ordered lipid phase. The glycosylated sphingolipids (e.g., GM1), together with the glycoproteins (e.g., Glycophorins A-D, or Band 3 protein) [108] form the hydrophilic glycocalyx layer [109], to which the hydrophilic N-terminus, which contains charged amino acid residues, is supposed to bind, while the hydrophobic C-terminus might anchor the bilayer. This is in line with the recent results of Yagi-Utsumi et al. [110] based on solid-state nuclear magnetic resonance studies, who have established that a unique antiparallel assemblage of A β 42 is formed at the amphiphilic interface of the GM1-rich glycocalyx layer and the hydrophobic cell membrane, which then functions as a template for amyloid fibril formation.

We also found that the changes in RBC membrane architecture are accompanied by alteration of the cell surface electrochemical properties. The measured zeta potential, which reflects the surface electrical charge at the RBC membrane, increases with the increase in A β 42 concentration and the time of treatment. Indeed, such an effect is expected when the peptide, possessing an overall negative charge, is associated with the membrane–

glycocalyx interphase. Our results agree with the main conclusion of Chen et al. [111], according to which the decrease in the membrane elasticity results from the decrease in the surface charge of the cell. On the other hand, our results clearly demonstrate that an increased negative surface charge density can accompany a more rigid membrane structure in A β 42-treated RBCs. Interestingly, the effect of A β 42 on membrane lipid order was more pronounced in intact RBCs than in erythrocyte ghosts, whereas the ζ -potential changes showed the opposite trend—being larger in ghosts. This apparent discrepancy can be rationalized by considering the structural and compositional differences between intact RBCs and ghosts. In intact cells, A β 42 interacts not only with the lipid bilayer but also with the glycocalyx, transmembrane proteins (such as Band 3), and the underlying spectrin–actin cytoskeleton [23,82,84]. The coupling between the bilayer and cytoskeleton allows A β 42-induced changes in local lipid order to propagate and amplify throughout the membrane, leading to a measurable increase in overall membrane rigidity. In contrast, in ghosts, the absence of cytoplasmic components and partial loss of membrane-associated proteins reduce this cooperative coupling, so the same amount of A β 42 produces smaller structural rearrangements in the lipid matrix. At the same time, ζ -potential reflects the net surface charge and the distribution of ions at the membrane–solution interface. The stronger ζ -potential changes observed in ghosts likely result from their exposed membrane surface and reduced shielding by extracellular proteins and glycocalyx components. In intact RBCs, the negatively charged sialic acid residues of the glycocalyx and adsorbed plasma proteins partially mask or buffer the electrostatic contribution of A β 42 binding, whereas in ghosts, A β 42 molecules can interact more directly with the phospholipid headgroups, enhancing the overall surface charge density. Thus, A β 42 may affect different structural levels in intact RBCs (lipid–protein–cytoskeleton coupling) and in ghosts (lipid–electrostatic layer), explaining the opposing trends in lipid order and ζ -potential changes.

Beyond these membrane-related factors, intracellular components may also influence the electrostatic properties of intact RBCs. The ζ -potential of erythrocytes mainly arises from negatively charged sialylated glycoproteins, whereas intracellular iron is confined within hemoglobin and does not directly contribute to surface charge. Nevertheless, hemoglobin's heme iron can participate in redox reactions that modify membrane components and local electrostatics. The amyloid β peptide family have been shown to bind hemoglobin through the heme moiety [89,90] and modulate iron redox states associated with oxidative stress [112]. Consequently, A β 42 oligomers interacting with and partially internalizing into RBCs could affect hemoglobin's redox balance, indirectly influencing the electrokinetic properties of intact cells. Although some residual hemoglobin remains in erythrocyte ghosts, as it has been detected by two photon excitation fluorescence microscopy [113], the presence of very low level of hemoglobin-bound iron and its associated redox effects may be related to the more negative ζ -potential.

These findings emphasize that A β 42–membrane interactions are governed not only by lipid composition but also by the presence of protein and carbohydrate structures that modulate the mechanical and electrostatic properties of the cell. Therefore, intact RBCs represent a more physiologically relevant but also more complex system, where A β 42 binding induces cooperative stiffening of the membrane, while the simpler ghost membranes reveal the direct electrostatic contribution of the peptide. Moreover, the use of RBC ghosts ensured that the observed alterations in membrane lipid order and electrostatic potential induced by A β oligomers reflected direct A β 42–membrane interactions rather than any effects mediated by its interaction with intracellular hemoglobin.

Such membrane-level effects are particularly significant in the broader context of Alzheimer's disease, where peripheral and neurovascular mechanisms are increasingly recognized. Beyond neuronal toxicity, RBCs can bind misfolded proteins, and RBC-associated

aggregates have been proposed as disease-relevant readouts, with the 120-day RBC lifespan enabling temporal integration. Alterations in RBC membrane packing and surface electrostatics are expected to affect deformability and microvascular flow, with potential consequences for oxygen delivery and endothelial interactions in cerebral microvessels. Our findings therefore provide a biophysical link between A β 42 oligomer exposure and RBC membrane properties relevant to neurovascular contributions in AD.

4. Materials and Methods

4.1. Preparation of RBC and Erythrocyte Ghost Suspensions and Treatment with A β 42 Oligomers

Blood samples were derived from 10 healthy volunteers by venipuncture into EDTA (ethylenediaminetetraacetic acid) vacutainers (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). The erythrocytes were isolated by centrifugation of the blood samples at 3000 rpm for 15 min at 4 °C and washed three times with PBS buffer (10 mM sodium phosphate, pH 7.2, 140 mM NaCl, and 1 mM EDTA) according to Girasole et al. [87].

To prepare erythrocyte ghosts, we followed the methodology described in [114]. Washed RBCs were hemolyzed in 5 mM Tris, pH 8.0 by centrifugation at 15,000 rpm and 4 °C for 30 min. This procedure was repeated until whitish ghost membranes were produced.

The hemoglobin (Hb) concentration of RBCs and total protein (TP) concentration of ghosts were determined spectrophotometrically (Specord 50 Plus, Analytik Jena, Jena, Germany). For TP concentration, the Biuret method was used [115]. RBCs with final Hb concentration of 67 mg/mL (20% hematocrit, Hct) and ghosts with final TP concentration of 2 mg/mL were incubated with the desired concentrations of A β 42 in PBS and Tris buffer, respectively, at 37 °C for 1, 6, 24, 48 and 72 h under continuous gentle shaking. The unbound peptide was removed by centrifugation at 3000 rpm for 15 min and the suspensions were immediately used for the experiments after appropriate dilutions according to the requirements of the methods used, while maintaining the ratios of RBCs/A β 42 and ghosts/A β 42.

4.2. Preparation of A β 42 Solutions

A β 42 and fluorescent A β 42 (HiLyte™ Fluor 488) peptides were purchased from Eurogentec (Kaneka Eurogentec S.A., LIEGE Science Park, Seraing, Belgium). Nucleation of amyloid proteins leads to rapid aggregation and occurs quickly in a concentrated solution, so dissociating any preformed A β 42 seeds is necessary to prevent aggregation. Briefly, to prepare monomeric aliquots, the lyophilized peptide was dissolved in pure HFIP (1,1,1,3,3,3-Hexafluoro-2-propanol > 99%, Sigma-Aldrich (St. Louis, MO, USA) to a concentration of 0.57 mg/mL, sonicated for 1 h, aliquoted, dried under argon, and held under vacuum for 2 h. The resulting dried A β 42 aliquots were stored at −22 °C. Before each experiment, A β 42 was dissolved in PBS followed by 2 min of vortexing. The A β 42 solution was then filtered using a syringe filter with 0.45 μ m and 0.22 μ m pore size hydrophilic PVDF (polyvinylidene difluoride) membranes (Millipore, Burlington, MA, USA; MILLEX-GV). No dimethyl sulfoxide (DMSO) was used for A β 42 solubilization in our protocols. A β 42 oligomers were prepared according to our previously published protocols [116,117]. Briefly, monomeric A β 42 was dissolved in PBS (pH 7.4) to a concentration of 20–200 μ M and incubated for 1 h at 37 °C, yielding predominantly oligomers, as verified previously by DLS, ThT fluorescence assays, and TEM. Depending on the desired final concentration of oligomers to be applied to RBCs or ghosts, aliquots of the oligomer stock solution were taken and added to the samples. Comparable 1 h oligomer formation in PBS has also been reported by other groups using independent biophysical techniques [118–121]. The physicochemical characterization of the oligomers has been published in [122]. The same holds for the preparation of fluorescent A β 42 (A β 42*) in PBS solutions. No mature fibrils

were used in the present experiments. The choice of oligomers reflects their widely reported higher membrane activity and toxicity relative to monomers or fibrils [123].

For the imaging experiments, A β 42 and (HiLyte™ Fluor 488)-labeled A β 42 peptides were mixed in HFIP at 1:1 molar ratio.

4.3. Laurdan Fluorescence Spectroscopy of RBCs and Ghosts

The membrane lipid order was assessed by measuring the generalized polarization (GP) of Laurdan (a fluorescent probe localized in the membrane bilayer at the level of the phospholipid glycerol backbone). Laurdan GP provides information about the degree of hydration/lipid order in the polar head region near the glycerol backbone. Alterations in membrane water content cause changes in the excitation and emission spectra of the probe. Lower GP values (−1) correspond to a more disordered lipid membrane (L_d) and higher values (+1) to a more ordered membrane (L_o or L_β). Emission spectra were recorded from 390 to 600 nm. Samples were excited at 355 nm. GP is calculated using fixed emission wavelengths 440 nm and 490 nm ($GP = (I_{440} - I_{490}) / (I_{440} + I_{490})$), where I_{440} and I_{490} are the emission intensities at a characteristic wavelength of ordered phase (440 nm) and of disordered phase (490 nm).

Laurdan, dissolved in DMSO in stock concentration of 0.25 mg/mL, was added to the RBCs (8 μ L Laurdan) and ghosts (1 μ L Laurdan) and incubated at 37 °C for 1 h. Measurements were performed by means of FP-8300 spectrofluorometer (Jasco Inc., Easton, MD, USA). The desired temperature (37 °C) was maintained using a circulating water bath (Julabo, Seelbach, Germany). For analysis of the steady-state spectra, OriginPro 9.0 software was used.

To verify that the observed changes in Laurdan GP values originated from direct interactions of A β 42 oligomers with the erythrocyte membrane rather than from nonspecific effects of the surrounding medium, several control experiments were conducted. After incubation with A β 42, RBCs were thoroughly washed to remove unbound oligomers. A GP shift was observed only when RBCs were incubated with A β 42 oligomers, whereas the GP value of the corresponding supernatant was close to that of A β 42 oligomers alone in PBS. Additional control experiments were performed in which Laurdan (dissolved in DMSO) was added to (i) buffer alone and (ii) A β 42 oligomers dispersed in the same buffer. Both samples exhibited identical GP values, markedly different from those of RBC membranes. These results confirm that neither A β 42 oligomers in solution nor components of the medium affect Laurdan fluorescence, indicating that the observed GP changes result from direct oligomer–membrane interactions.

4.4. Di-4-ANEPPDHQ Microscopy Measurements

Di-4-ANEPPDHQ staining was visualized using a laser scanning confocal microscopy (Carl Zeiss Microscopy GmbH, Jena, Germany) with a 100 $\times$ immersion objective. GP calculations were based on the eq. $GP = (I_{500-580} - GI_{620-750}) / (I_{500-580} + GI_{620-750})$, as previously described [124]. Pseudo-colored GP images were merged with mean fluorescence intensity images to preserve structural information. We used a plugin to calculate GP and redeveloped for the CellTool software package by Georgi Danovski [125]. Thresholding of the images were performed before calculation of GP values in order to optimize the signal-to-noise ratio. The photomultiplier tube (PMT) gains were adjusted in such a way that both channels did not contain saturated pixels and have approximately the same intensity. As the assessment of the membrane order is a ratiometric measurement between the two channels, the PMT gain of each channel was kept constant between two sets of experiments, treated and non-treated with A β 42 RBCs. The G factor for sensitivity between the two channels was calculated, and then GP values were assessed from raw fluorescent images

and averaged from a minimum of 90 RBCs for each experiment performed at least three times. Data with $p < 0.05$ were considered significant when nonparametric Mann–Whitney test was applied.

4.5. Electrokinetic Measurements of RBCs and Ghosts

Measurements of ζ -potential were performed with Zetasizer (Nano zeta, Malvern Instruments, Malvern, UK) at 25 °C using disposable folded capillary cell cuvettes DTS1070 (Malvern, UK). The ζ -potential of RBCs and ghosts was determined in the absence and presence of A β 42 at concentrations 0.1–60 μ M, dissolved in buffer, after 1, 6, and 48 h of incubation at 37 °C. A minimum of three ζ -potential measurements were performed on each sample using the monomodal analysis model.

The electrophoretic mobility, u , was used for evaluation of the ζ -potential using the Smoluchowski equation [126]: $\zeta = 4\pi\eta u / \epsilon$, where η is the viscosity and ϵ is the dielectric permittivity of the solvent.

4.6. Statistical Analysis

All statistical calculations were performed with OriginPro 9.0 software. Data were analyzed with nonparametric Mann–Whitney test.

5. Conclusions

Our findings demonstrate that A β 42 oligomers interact with RBC membranes in a concentration- and time-dependent manner, inducing pronounced alterations in both membrane structure and surface charge. The A β 42 oligomers reorganize the lipid phase separation within RBC membranes and exert a stronger effect on membrane lipid order in intact cells than in erythrocyte ghosts. This difference reflects the cooperative roles of the spectrin–actin cytoskeleton and the glycocalyx in amplifying or modulating the peptide’s impact.

In parallel, A β 42 enhances the negative ζ -potential of both RBCs and ghosts, with larger changes observed in the latter due to the absence of glycocalyx shielding and the more direct exposure of charged lipid headgroups. Together, these observations reveal that A β 42 perturbs two interconnected levels of membrane organization: the lipid–protein–cytoskeleton continuum governing mechanical rigidity, and the electrostatic double layer controlling surface charge and ionic interactions.

Such dual modulation of RBC membrane properties may have functional consequences for blood rheology and oxygen transport, providing a plausible link between peripheral A β 42 accumulation and microcirculatory impairment in Alzheimer’s disease. The present results support the emerging view that erythrocyte membranes are active participants in the systemic pathophysiology of neurodegeneration and may serve as accessible peripheral biomarkers for early detection of A β 42-related disorders.

Future studies should aim to quantify the specific contributions of individual lipid species and cytoskeletal proteins to A β 42 binding, as well as to explore how oxidative modifications or plasma components modulate these interactions under physiological and pathological conditions.

Author Contributions: Conceptualization, S.G.T. and G.S.; methodology, G.S. and S.G.T.; investigation, V.Y., A.D. (Avgustina Danailova) and A.-M.M.; data curation, V.Y., G.S. and S.G.T.; writing—S.G.T.; writing and editing—G.S., A.D. (András Dér) and V.Y.; supervision, S.G.T. and G.S.; project administration, G.S.; funding acquisition, G.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by “National Center for Biomedical Photonics” D01-101/2025 (Zetasizer Nano ZS analyzer) as part of the Bulgarian National Roadmap for Scientific Infrastructures 2020–2027.

Institutional Review Board Statement: This study was approved by the Ethics Committee of the Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia (consent number № 58 ND/17.01.2025) in agreement with the principles of the Declaration of Helsinki.

Informed Consent Statement: Informed consent was obtained from all healthy donors involved in the study.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available upon obtaining the permission of the corresponding author for its use.

Acknowledgments: The authors thank the Bulgarian Ministry of Education and Science for the support provided via the “Scientific Infrastructure on Cell Technologies in Biomedicine (SICTB)” D01-361/2023 (Spectrofluorometric measurements), part of the Bulgarian National Roadmap for Scientific Infrastructures 2020–2027 and Project № BG-RRP-2.004-0004-C01/29.12.2022 “STRATEGIC RESEARCH AND INNOVATION PROGRAMME FOR DEVELOPMENT OF MEDICAL UNIVERSITY—SOFIA” financed by the European Union through the National Plan for Recovery and Sustainability of the Republic of Bulgaria. (András Dér) A.D. was supported by the NKFI-1 ADVANCED 150958 grant of the National Research Development and Innovation Office, a Hungarian granting agency.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Glenner, G.G.; Wong, C.W. Alzheimer’s Disease and Down’s Syndrome: Sharing of a Unique Cerebrovascular Amyloid Fibril Protein. *Biochem. Biophys. Res. Commun.* **1984**, *122*, 1131–1135. [\[CrossRef\]](#)
2. Seubert, P.; Vigo-Pelfrey, C.; Esch, F.; Lee, M.; Dovey, H.; Davis, D.; Sinha, S.; Schioosmacher, M.; Whaley, J.; Swindlehurst, C.; et al. Isolation and Quantification of Soluble Alzheimer’s B-Peptide from Biological Fluids. *Nature* **1992**, *359*, 325–327. [\[CrossRef\]](#)
3. Selkoe, D.J.J. Normal and Abnormal Biology of the Beta-Amyloid Precursor Protein. *Annu. Rev. Neurosci.* **1994**, *17*, 489–517. [\[CrossRef\]](#)
4. Sperling, R.A.; Aisen, P.S.; Beckett, L.A.; Bennett, D.A.; Craft, S.; Fagan, A.M.; Iwatsubo, T.; Jack, C.R., Jr.; Kaye, J.; Montine, T.J.; et al. Toward Defining the Preclinical Stages of Alzheimer’s Disease: Recommendations from the National Institute on Aging-Alzheimer’s Association Workgroups on Diagnostic Guidelines for Alzheimer’s Disease. *Alzheimer’s Dement.* **2011**, *7*, 280–292. [\[CrossRef\]](#)
5. Bloom, G.S. Amyloid-B and Tau: The Trigger and Bullet in Alzheimer Disease Pathogenesis. *JAMA Neurol.* **2014**, *71*, 505–508. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Ashe, K.H. The Biogenesis and Biology of Amyloid B Oligomers in the Brain. *Alzheimer’s Dement.* **2020**, *16*, 1561–1567; Erratum in *Alzheimer’s Dement.* **2023**, *2*, 1104–1104. [\[CrossRef\]](#)
7. Liu, N.; Haziyyihan, A.; Zhao, W.; Chen, Y.; Chao, H. Trajectory of Brain-Derived Amyloid Beta in Alzheimer’s Disease: Where Is It Coming from and Where Is It Going? *Transl. Neurodegener.* **2024**, *13*, 42. [\[CrossRef\]](#)
8. Johnson, J.T.; Awosimilala, F.W.; Anumudu, C.K. Exploring Protein Misfolding and Aggregate Pathology in Neurodegenerative Diseases: From Molecular Mechanisms to Clinical Interventions. *Appl. Sci.* **2025**, *15*, 10285. [\[CrossRef\]](#)
9. Sweeney, P.; Park, H.; Baumann, M.; Dunlop, J.; Frydman, J.; Kopito, R.; McCampbell, A.; Leblanc, G.; Venkateswaran, A.; Nurmi, A.; et al. Protein Misfolding in Neurodegenerative Diseases: Implications and Strategies. *Transl. Neurodegener.* **2017**, *6*, 6. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Sidoryk-Węgrzynowicz, M.; Adamiak, K.; Strużyńska, L. Targeting Protein Misfolding and Aggregation as a Therapeutic Perspective in Neurodegenerative Disorders. *Int. J. Mol. Sci.* **2024**, *25*, 12448. [\[CrossRef\]](#)
11. Kosik, K.S.; Joachim, C.L.; Selkoe, D.J. Microtubule-Associated Protein Tau (Tau) Is a Major Antigenic Component of Paired Helical Filaments in Alzheimer Disease. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 4044–4048. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Ballatore, C.; Lee, V.M.-Y.; Trojanowski, J.Q. Tau-Mediated Neurodegeneration in Alzheimer’s Disease and Related Disorders. *Nat. Rev. Neurosci.* **2007**, *8*, 663–672. [\[CrossRef\]](#)
13. Hanger, D.P.; Lau, D.H.W.; Phillips, E.C.; Bondulich, M.K.; Guo, T.; Woodward, B.W.; Pooler, A.M.; Noble, W. Intracellular and Extracellular Roles for Tau in Neurodegenerative Disease. *J. Alzheimer’s Dis.* **2014**, *40* (Suppl. S1), S37–S45. [\[CrossRef\]](#)
14. Wu, J.-R.; Zhou, B.; Huang, Y.-P.; Sun, Z.-D.; Hu, J.-X. Toxicities of Amyloid-Beta and Tau Protein Are Reciprocally Enhanced in the Drosophila Model. *Neural Regen. Res.* **2022**, *17*, 2286–2292. [\[CrossRef\]](#)

15. Lacosta, A.M.; Insua, D.; Badi, H.; Pesini, P.; Sarasa, M. Neurofibrillary Tangles of A β x-40 in Alzheimer's Disease Brains. *J. Alzheimer's Dis.* **2017**, *58*, 661–667. [[CrossRef](#)]
16. Moda, F.; Ciullini, A.; Dellarole, I.; Lombardo, A.; Campanella, N.; Bufano, G.; Cazzaniga, F.; Giaccone, G. Secondary Protein Aggregates in Neurodegenerative Diseases: Almost the Rule Rather Than the Exception. *FBL* **2023**, *28*, 255. [[CrossRef](#)]
17. Zenaro, E.; Piacentino, G.; Constantin, G. The Blood-Brain Barrier in Alzheimer's Disease. *Neurobiol. Dis.* **2017**, *107*, 41–56. [[CrossRef](#)]
18. Yang, Y.H.; Huang, L.C.; Hsieh, S.W.; Huang, L.J. Dynamic Blood Concentrations of A β 1–40 and A β 1–42 in Alzheimer's Disease. *Front. Cell Dev. Biol.* **2020**, *8*, 768. [[CrossRef](#)]
19. Blömeke, L.; Rehn, F.; Pils, M.; Kraemer-Schulien, V.; Cousin, A.; Kutzsche, J.; Bujnicki, T.; Freiesleben, S.D.; Schneider, L.-S.; Preis, L.; et al. Blood-Based Quantification of A β Oligomers Indicates Impaired Clearance from Brain in Apoe E4 Positive Subjects. *Commun. Med.* **2024**, *4*, 262. [[CrossRef](#)] [[PubMed](#)]
20. Matsubara, E.; Ghiso, J.; Frangione, B.; Amari, M.; Tomidokoro, Y.; Ikeda, Y.; Harigaya, Y.; Okamoto, K.; Shoji, M. Lipoprotein-Free Amyloidogenic Peptides in Plasma Are Elevated in Patients with Sporadic Alzheimer's Disease and Down's Syndrome. *Ann. Neurol.* **1999**, *45*, 537–541. [[CrossRef](#)] [[PubMed](#)]
21. Reiman, E.M.; Chen, K.; Liu, X.; Bandy, D.; Yu, M.; Lee, W.; Ayutyanont, N.; Keppler, J.; Reeder, S.A.; Langbaum, J.B.S.; et al. Fibrillar Amyloid-B Burden in Cognitively Normal People at 3 Levels of Genetic Risk for Alzheimer's Disease. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 6820–6825. [[CrossRef](#)] [[PubMed](#)]
22. Blass, J.P.; Hanin, I.; Barclay, L.; Kopp, U.; Reding, M.J. Red Blood Cell Abnormalities in Alzheimer Disease. *J. Am. Geriatr. Soc.* **1985**, *33*, 401–405. [[CrossRef](#)]
23. Kiko, T.; Nakagawa, K.; Satoh, A.; Tsuduki, T.; Furukawa, K.; Arai, H.; Miyazawa, T. Amyloid B Levels in Human Red Blood Cells. *PLoS ONE* **2012**, *7*, e49620. [[CrossRef](#)] [[PubMed](#)]
24. Li, Q.X.; Whyte, S.; E Tanner, J.; Evin, G.; Beyreuther, K.; Masters, C.L. Secretion of Alzheimer's Disease Abeta Amyloid Peptide by Activated Human Platelets. *Lab Invest.* **1998**, *78*, 461–469.
25. E Gibson, G.; Huang, H.-M. Oxidative Processes in the Brain and Non-Neuronal Tissues as Biomarkers of Alzheimer's Disease. *Front. Biosci. A J. Virtual Libr.* **2002**, *7*, d1007–d1015. [[CrossRef](#)]
26. Kuo, Y.-M.; Kokjohn, T.A.; Kalback, W.; Luehrs, D.; Galasko, D.R.; Chevallier, N.; Koo, E.H.; Emmerling, M.R.; Roher, A.E. Amyloid-B Peptides Interact with Plasma Proteins and Erythrocytes: Implications for Their Quantitation in Plasma. *Biochem. Biophys. Res. Commun.* **2000**, *268*, 750–756. [[CrossRef](#)]
27. Zamolodchikov, D.; Berk-Rauch, H.E.; Oren, D.A.; Stor, D.S.; Singh, P.K.; Kawasaki, M.; Aso, K.; Strickland, S.; Ahn, H.J. Biochemical and Structural Analysis of the Interaction between B-Amyloid and Fibrinogen. *Blood* **2016**, *128*, 1144–1151. [[CrossRef](#)]
28. Ahn, H.J.; Chen, Z.L.; Zamolodchikov, D.; Norris, E.H.; Strickland, S. Interactions of B-Amyloid Peptide with Fibrinogen and Coagulation Factor Xii May Contribute to Alzheimer's Disease. *Curr. Opin. Hematol.* **2017**, *24*, 427–431. [[CrossRef](#)]
29. Shi, M.; Chu, F.; Zhu, F.; Zhu, J. Peripheral Blood Amyloid-B Involved in the Pathogenesis of Alzheimer's Disease Via Impacting on Peripheral Innate Immune Cells. *J. Neuroinflamm.* **2024**, *21*, 5. [[CrossRef](#)]
30. Hardy, J.A.; Higgins, G.A. Higgins. Alzheimer's Disease: The Amyloid Cascade Hypothesis. *Science* **1992**, *256*, 184–185. [[CrossRef](#)]
31. Strazdaite, S.; Roeters, S.J.; Sakalauskas, A.; Sneideris, T.; Kirschner, J.; Pedersen, K.B.; Schjøtt, B.; Jensen, F.; Weidner, T.; Smirnovas, V.; et al. Interaction of Amyloid-B-(1–42) Peptide and Its Aggregates with Lipid/Water Interfaces Probed by Vibrational Sum-Frequency Generation Spectroscopy. *J. Phys. Chem. B* **2021**, *125*, 11208–11218. [[CrossRef](#)] [[PubMed](#)]
32. Niu, Z.; Zhang, Z.; Zhao, W.; Yang, J. Interactions between Amyloid B Peptide and Lipid Membranes. *Biochim. Biophys. Acta (BBA)—Biomembr.* **2018**, *1860*, 1663–1669. [[CrossRef](#)]
33. Wiatrak, B.; Piasny, J.; Kuźniarski, A.; Gąsiorowski, K. Interactions of Amyloid-B with Membrane Proteins. *Int. J. Mol. Sci.* **2021**, *22*, 6075. [[CrossRef](#)]
34. Hardy, J.; Selkoe, D.J. The Amyloid Hypothesis of Alzheimer's Disease: Progress and Problems on the Road to Therapeutics. *Science* **2002**, *297*, 353–356. [[CrossRef](#)] [[PubMed](#)]
35. Musiek, E.S.; Holtzman, D.M. Three Dimensions of the Amyloid Hypothesis: Time, Space and 'Wingmen'. *Nat. Neurosci.* **2015**, *18*, 800–806. [[CrossRef](#)] [[PubMed](#)]
36. Castellani, R.J.; Jamshidi, P.; Plascencia-Villa, G.; Perry, G. The Amyloid Cascade Hypothesis: A Conclusion in Search of Support. *Am. J. Pathol.* **2025**, *195*, 1988–1997. [[CrossRef](#)]
37. Kuo, Y.-M.; Emmerling, M.R.; Vigo-Pelfrey, C.; Kasunic, T.C.; Kirkpatrick, J.B.; Murdoch, G.H.; Ball, M.J.; Roher, A.E. Water-Soluble A β (N-40, N-42) Oligomers in Normal and Alzheimer Disease Brains. *J. Biol. Chem.* **1996**, *271*, 4077–4081. [[CrossRef](#)]
38. McLean, C.A.; Cherny, R.A.; Fraser, F.W.; Fuller, S.J.; Smith, M.J.; Vbeyreuther, K.; Bush, A.I.; Masters, C.L. Soluble Pool of A β Amyloid as a Determinant of Severity of Neurodegeneration in Alzheimer's Disease. *Ann. Neurol.* **1999**, *46*, 860–866. [[CrossRef](#)]
39. Tabaton, M.; Piccini, A. Role of Water-Soluble Amyloid-B in the Pathogenesis of Alzheimer's Disease. *Int. J. Exp. Pathol.* **2005**, *86*, 139–145. [[CrossRef](#)]

40. Wirths, O.; Multhaup, G.; Bayer, T.A. A Modified B-Amyloid Hypothesis: Intraneuronal Accumulation of the B-Amyloid Peptide—The First Step of a Fatal Cascade. *J. Neurochem.* **2004**, *91*, 513–520. [\[CrossRef\]](#)
41. Cline, E.N.; Bicca, M.A.; Viola, K.L.; Klein, W.L. The Amyloid-B Oligomer Hypothesis: Beginning of the Third Decade. *J. Alzheimer's Dis.* **2018**, *64*, S567–S610. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Tang, H.; Andrikopoulos, N.; Li, Y.; Ke, S.; Sun, Y.; Ding, F.; Ke, P.C. Emerging Biophysical Origins and Pathogenic Implications of Amyloid Oligomers. *Nat. Commun.* **2025**, *16*, 2937. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Calderón-Garcidueñas, L.; Solt, A.C.; Henríquez-Roldán, C.; Torres-Jardón, R.; Nuse, B.; Herritt, L.; Villarreal-Calderón, R.; Osnaya, N.; Stone, I.; García, R.; et al. Long-Term Air Pollution Exposure Is Associated with Neuroinflammation, an Altered Innate Immune Response, Disruption of the Blood-Brain Barrier, Ultrafine Particulate Deposition, and Accumulation of Amyloid B-42 and A-Synuclein in Children and Young Adults. *Toxicol. Pathol.* **2008**, *36*, 289–310. [\[CrossRef\]](#)
44. DeKosky, S.T.; Abrahamson, E.E.; Ciallella, J.R.; Paljug, W.R.; Wisniewski, S.R.; Clark, R.S.B.; Ikonovic, M.D. Association of Increased Cortical Soluble A β 42 Levels with Diffuse Plaques after Severe Brain Injury in Humans. *Arch. Neurol.* **2007**, *64*, 541–544. [\[CrossRef\]](#)
45. de la Torre, J.C. Is Alzheimer's Disease a Neurodegenerative or a Vascular Disorder? Data, Dogma, and Dialectics. *Lancet Neurol.* **2004**, *3*, 184–190. [\[CrossRef\]](#)
46. Teller, J.K.; Russo, C.; Debusk, L.M.; Angelini, G.; Zaccheo, D.; Dagna-Bricarelli, F.; Scartezzini, P.; Bertolini, S.; Mann, D.M.; Tabaton, M.; et al. Presence of Soluble Amyloid B-Peptide Precedes Amyloid Plaque Formation in Down's Syndrome. *Nat. Med.* **1996**, *2*, 93–95. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Head, E.; Lott, I.T. Down Syndrome and Beta-Amyloid Deposition. *Curr. Opin. Neurol.* **2004**, *17*, 95–100. [\[CrossRef\]](#)
48. Head, E.; TLott, I.; MWilcock, D.; ALemere, C. Aging in Down Syndrome and the Development of Alzheimer's Disease Neuropathology. *Curr. Alzheimer Res.* **2016**, *13*, 18–29. [\[CrossRef\]](#)
49. Salehi, A.; Wesson Ashford, J.; Mufson, J.E. The Link between Alzheimer's Disease and Down Syndrome. A Historical Perspective. *Curr. Alzheimer Res.* **2016**, *13*, 2–6. [\[CrossRef\]](#)
50. Ramos-Cejudo, J.; Wisniewski, T.; Marmar, C.; Zetterberg, H.; Blennow, K.; de Leon, M.J.; Fossati, S. Traumatic Brain Injury and Alzheimer's Disease: The Cerebrovascular Link. *EBioMedicine* **2018**, *28*, 21–30. [\[CrossRef\]](#)
51. Mielke, M.M.; Ransom, J.E.; Mandrekar, J.; Turcano, P.; Savica, R.; Brown, A.W. Traumatic Brain Injury and Risk of Alzheimer's Disease and Related Dementias in the Population. *J. Alzheimer's Dis.* **2022**, *88*, 1049–1059. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Mann, D.M.A.; Jones, D.; South, P.W.; Snowden, J.S.; Neary, D. Deposition of Amyloid B Protein in Non-Alzheimer Dementias: Evidence for a Neuronal Origin of Parenchymal Deposits of B Protein in Neurodegenerative Disease. *Acta Neuropathol.* **1992**, *83*, 415–419. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Weller, R.O.; Massey, A.M.; Roher, A.E. Cerebral Amyloid Angiopathy: Accumulation of A β in Interstitial Fluid Drainage Pathways in Alzheimer's Disease. *Ann. N. Y. Acad. Sci.* **2000**, *903*, 110–117. [\[CrossRef\]](#)
54. Gallardo, G.; Holtzman, D.M. Amyloid-B and Tau at the crossroads of Alzheimer's Disease. In *Tau Biology*; Akihiko, T., Benjamin, W., Luc, B., Eds.; Springer: Singapore, 2019; pp. 187–203.
55. Kosenko, E.; Tikhonova, L.; Alilova, G.; Urios, A.; Montoliu, C. The Erythrocytic Hypothesis of Brain Energy Crisis in Sporadic Alzheimer Disease: Possible Consequences and Supporting Evidence. *J. Clin. Med.* **2020**, *9*, 206. [\[CrossRef\]](#) [\[PubMed\]](#)
56. Butterfield, D.A.; Lauderback, C.M. Lipid Peroxidation and Protein Oxidation in Alzheimer's Disease Brain: Potential Causes and Consequences Involving Amyloid B-Peptide-Associated Free Radical Oxidative Stress. *Free Radic. Biol. Med.* **2002**, *32*, 1050–1060. [\[CrossRef\]](#)
57. Kosenko, E.A.; Aliev, G.; Kaminsky, Y.G. Relationship between Chronic Disturbance of 2,3-Diphosphoglycerate Metabolism in Erythrocytes and Alzheimer Disease. *CNS Neurol. Disord. Drug Targets* **2016**, *15*, 113–123. [\[CrossRef\]](#)
58. Kosenko, E.A.; Tikhonova, L.A.; Montoliu, C.; Barreto, G.E.; Aliev, G.; Kaminsky, Y.G. Metabolic Abnormalities of Erythrocytes as a Risk Factor for Alzheimer's Disease. *Front. Neurosci.* **2018**, *11*, 728. [\[CrossRef\]](#)
59. Swerdlow, R.H.; Burns, J.M.; Khan, S.M. The Alzheimer's Disease Mitochondrial Cascade Hypothesis: Progress and Perspectives. *Biochim. Biophys. Acta (BBA) Mol. Basis Dis.* **2014**, *1842*, 1219–1231. [\[CrossRef\]](#)
60. Swerdlow, R.H. The Alzheimer's Disease Mitochondrial Cascade Hypothesis: A Current Overview. *J. Alzheimer's Dis.* **2023**, *92*, 751–768. [\[CrossRef\]](#)
61. Mossman, D.; Vögtle, F.N.; Taskin, A.A.; Teixeira, P.F.; Ring, J.; Burkhart, J.M.; Burger, N.; Pinho, C.M.; Tadic, J.; Loreth, D.; et al. Amyloid- β Peptide Induces Mitochondrial Dysfunction by Inhibition of Preprotein Maturation. *Cell Metab.* **2014**, *20*, 662–669. [\[CrossRef\]](#)
62. Cenini, G.; Rüb, C.; Bruderek, M.; Voos, W. Amyloid B-Peptides Interfere with Mitochondrial Preprotein Import Competence by a Coaggregation Process. *Mol. Biol. Cell* **2016**, *27*, 3257–3272. [\[CrossRef\]](#)
63. Hemmerová, E.; Špringer, T.; Křištofiková, Z.; Homola, J. Ionic Environment Affects Biomolecular Interactions of Amyloid?: Spr Biosensor Study. *Int. J. Mol. Sci.* **2020**, *21*, 9727. [\[CrossRef\]](#)

64. Banerjee, S.; Lyubchenko, Y.L. Interaction of Amyloidogenic Proteins with Membranes and Molecular Mechanism for the Development of Alzheimer's Disease. *Alzheimer's Res. Ther.* **2019**, *2*, 106.
65. Chang, C.-C.; Edwald, E.; Veatch, S.; Steel, D.G.; Gafni, A. Interactions of Amyloid-B Peptides on Lipid Bilayer Studied by Single Molecule Imaging and Tracking. *Biochim. Biophys. Acta (BBA) Biomembr.* **2018**, *1860*, 1616–1624. [[CrossRef](#)]
66. Meker, S.; Chin, H.; Sut, T.N.; Cho, N.J. Amyloid-B Peptide Triggers Membrane Remodeling in Supported Lipid Bilayers Depending on Their Hydrophobic Thickness. *Langmuir* **2018**, *34*, 9548–9560. [[CrossRef](#)] [[PubMed](#)]
67. Goñi, F.M. Rafts: A Nickname for Putative Transient Nanodomains. *Chem. Phys. Lipids* **2019**, *218*, 34–39. [[CrossRef](#)] [[PubMed](#)]
68. Staneva, G.; Puff, N.; Stanimirov, S.; Tochev, T.; Angelova, M.I.; Seigneuret, M. The Alzheimer's Disease Amyloid-B Peptide Affects Size-Dynamics of Raft-Mimicking Lo Domains in Gm1 Containing Lipid Bilayers. *Soft Matter* **2018**, *14*, 9609–9618. [[CrossRef](#)] [[PubMed](#)]
69. Jayakumar, R.; Kusiak, J.W.; Chrest, F.J.; Demehin, A.A.; Murali, J.; Wersto, R.P.; Nagababu, E.; Ravi, L.; Rifkind, J.M. Red Cell Perturbations by Amyloid B-Protein. *Biochim. Biophys. Acta (BBA) Gen. Subj.* **2003**, *1622*, 20–28. [[CrossRef](#)]
70. Dinarelli, S.; Girasole, M.; Misiti, F. Amyloid B Peptide Affects Erythrocyte Morphology: Role of Intracellular Signaling Pathways. *Clin. Hemorheol. Microcirc.* **2019**, *71*, 437–449. [[CrossRef](#)]
71. Nirmalraj, P.N.; Schneider, T.; Felbecker, A. Spatial Organization of Protein Aggregates on Red Blood Cells as Physical Biomarkers of Alzheimer's Disease Pathology. *Sci. Adv.* **2021**, *7*, eabj2137. [[CrossRef](#)]
72. Carelli-Alinovi, C.; Giardina, B.; Misiti, F. Amyloid Beta Peptide (1–42)-Mediated Antioxidant Imbalance Is Associated with Activation of Protein Kinase C in Red Blood Cells. *Cell Biochem. Funct.* **2015**, *33*, 196–201. [[CrossRef](#)] [[PubMed](#)]
73. Schneider, T.R.; Stöckli, L.; Felbecker, A.; Nirmalraj, P.N. Protein Fibril Aggregation on Red Blood Cells: A Potential Biomarker to Distinguish Neurodegenerative Diseases from Healthy Aging. *Brain Commun.* **2024**, *6*, fcae180. [[CrossRef](#)]
74. Banerjee, S.; Hashemi, M.; Zagorski, K.; Lyubchenko, Y.L. Interaction of A β 42 with Membranes Triggers the Self-Assembly into Oligomers. *Int. J. Mol. Sci.* **2020**, *21*, 1129. [[CrossRef](#)]
75. Stefani, M.; Rigacci, S. Protein Folding and Aggregation into Amyloid: The Interference by Natural Phenolic Compounds. *Int. J. Mol. Sci.* **2013**, *14*, 12411–12457. [[CrossRef](#)]
76. Drabik, D.; Chodaczek, G.; Kraszewski, S. Effect of Amyloid-Beta Monomers on Lipid Membrane Mechanical Parameters—Potential Implications for Mechanically Driven Neurodegeneration in Alzheimer's Disease. *Int. J. Mol. Sci.* **2021**, *22*, 18. [[CrossRef](#)]
77. Ahyayauch, H.; Raab, M.; Busto, J.V.; Andraka, N.; Arrondo, J.-L.R.; Masserini, M.; Tvaroska, I.; Goñi, F.M. Binding of B-Amyloid (1–42) Peptide to Negatively Charged Phospholipid Membranes in the Liquid-Ordered State: Modeling and Experimental Studies. *Biophys. J.* **2012**, *103*, 453–463. [[CrossRef](#)]
78. Ahyayauch, H.; De La Arada, I.; Masserini, M.E.; Arrondo, J.L.R.; Goñi, F.M.; Alonso, A. The Binding of A β 42 Peptide Monomers to Sphingomyelin/Cholesterol/Ganglioside Bilayers Assayed by Density Gradient Ultracentrifugation. *Int. J. Mol. Sci.* **2020**, *21*, 1674. [[CrossRef](#)] [[PubMed](#)]
79. Burns, M.P.; Igbavboa, U.; Wang, L.; Wood, W.G.; Duff, K. Cholesterol Distribution, Not Total Levels, Correlate with Altered Amyloid Precursor Protein Processing in Statin-Treated Mice. *NeuroMol. Med.* **2006**, *8*, 319–328. [[CrossRef](#)] [[PubMed](#)]
80. Devanathan, S.; Salamon, Z.; Lindblom, G.; Gröbner, G.; Tollin, G. Effects of Sphingomyelin, Cholesterol and Zinc Ions on the Binding, Insertion and Aggregation of the Amyloid A β 1–40 Peptide in Solid-Supported Lipid Bilayers. *FEBS J.* **2006**, *273*, 1389–1402. [[CrossRef](#)]
81. Watanabe, C.; Puff, N.; Staneva, G.; Seigneuret, M.; Angelova, M.I. Antagonism and Synergy of Single Chain Sphingolipids Sphingosine and Sphingosine-1-Phosphate toward Lipid Bilayer Properties. Consequences for Their Role as Cell Fate Regulators. *Langmuir* **2014**, *30*, 13956–13963. [[CrossRef](#)]
82. Carelli-Alinovi, C.; Dinarelli, S.; Sampaiolese, B.; Misiti, F.; Girasole, M. Morphological Changes Induced in Erythrocyte by Amyloid Beta Peptide and Glucose Depletion: A Combined Atomic Force Microscopy and Biochemical Study. *Biochim. Biophys. Acta (BBA) Biomembr.* **2019**, *1861*, 236–244. [[CrossRef](#)] [[PubMed](#)]
83. Strijkova-Kenderova, V.; Todinova, S.; Andreeva, T.; Bogdanova, D.; Langari, A.; Danailova, A.; Krumova, S.; Zlatareva, E.; Kalaydzhev, N.; Milanov, I.; et al. Morphometry and Stiffness of Red Blood Cells—Signatures of Neurodegenerative Diseases and Aging. *Int. J. Mol. Sci.* **2022**, *23*, 227. [[CrossRef](#)] [[PubMed](#)]
84. Mohanty, J.G.; Shukla, H.D.; Williamson, J.D.; Launer, L.J.; Saxena, S.; Rifkind, J.M. Alterations in the Red Blood Cell Membrane Proteome in Alzheimer's Subjects Reflect Disease-Related Changes and Provide Insight into Altered Cell Morphology. *Proteome Sci.* **2010**, *8*, 11. [[CrossRef](#)]
85. Lue, L.-F.; Kuo, Y.-M.; Sabbagh, M. Advance in Plasma Ad Core Biomarker Development: Current Findings from Immunomagnetic Reduction-Based Squid Technology. *Neurol. Ther.* **2019**, *8*, 95–111. [[CrossRef](#)] [[PubMed](#)]
86. Pais, M.V.; Forlenza, O.V.; Diniz, B.S. Plasma Biomarkers of Alzheimer's Disease: A Review of Available Assays, Recent Developments, and Implications for Clinical Practice. *J. Alzheimer's Dis. Rep.* **2023**, *7*, 355–380. [[CrossRef](#)]

87. Girasole, M.; Dinarelli, S.; Boumis, G. Structure and Function in Native and Pathological Erythrocytes: A Quantitative View from the Nanoscale. *Micron* **2012**, *43*, 1273–1286. [[CrossRef](#)]
88. Günther, G.; Herlax, V.; Lillo, M.P.; Sandoval-Altamirano, C.; Belmar, L.N.; Sánchez, S.A. Study of Rabbit Erythrocytes Membrane Solubilization by Sucrose Monomyristate Using Laurdan and Phasor Analysis. *Colloids Surf. B Biointerfaces* **2018**, *161*, 375–385. [[CrossRef](#)]
89. Chuang, J.-Y.; Lee, C.-W.; Shih, Y.-H.; Yang, T.; Yu, L.; Kuo, Y.-M. Interactions between Amyloid-B and Hemoglobin: Implications for Amyloid Plaque Formation in Alzheimer's Disease. *PLoS ONE* **2012**, *7*, e33120. [[CrossRef](#)]
90. Perry, R.T.; Gearhart, D.A.; Wiener, H.W.; Harrell, L.E.; Barton, J.C.; Kutlar, A.; Kutlar, F.; Ozcan, O.; Go, R.C.; Hill, W.D. Hemoglobin Binding to A β and Hb α 2 Snp Association Suggest a Role in Alzheimer's Disease. *Neurobiol. Aging* **2008**, *29*, 185–193. [[CrossRef](#)]
91. Tokumasu, F.; A Nardone, G.; Ostera, G.R.; Fairhurst, R.M.; Beaudry, S.D.; Hayakawa, E.; A Dvorak, J. Altered Membrane Structure and Surface Potential in Homozygous Hemoglobin C Erythrocytes. *PLoS ONE* **2009**, *4*, e5828. [[CrossRef](#)]
92. Fernandes, H.P.; Cesar, C.L.; Barjas-Castro, M.d.L. Electrical Properties of the Red Blood Cell Membrane and Immunohematological Investigation. *Rev. Bras. Hematol. Hemoter.* **2011**, *33*, 297–301. [[CrossRef](#)]
93. Yagi-Utsumi, M.; Kato, K.; Nishimura, K. Membrane-Induced Dichotomous Conformation of Amyloid B with the Disordered N-Terminal Segment Followed by the Stable C-Terminal B Structure. *PLoS ONE* **2016**, *11*, e0146405. [[CrossRef](#)] [[PubMed](#)]
94. Fantini, J.; Yahi, N. Molecular inside into Amyloid Regulation by Membrane Cholesterol and Sphingolipids: Common Mechanisms in Neurodegenerative Diseases. *Expert Rev. Mol. Med.* **2010**, *12*, e27. [[CrossRef](#)]
95. Itoh, S.G.; Okumura, H. Promotion and Inhibition of Amyloid-B Peptide Aggregation: Molecular Dynamics Studies. *Int. J. Mol. Sci.* **2021**, *22*, 1859. [[CrossRef](#)]
96. Mohanty, J.G.; Eckley, D.M.; Williamson, J.D.; Launer, L.J.; Rifkind, J.M. Do Red Blood Cell-B-Amyloid Interactions Alter Oxygen Delivery in Alzheimer's Disease? *Adv. Exp. Med. Biol.* **2008**, *614*, 29–35.
97. Villaflores, O.B.; Chen, Y.-J.; Chen, C.-P.; Yeh, J.-M.; Wu, T.-Y. Curcuminoids and Resveratrol as Anti-Alzheimer Agents. *Taiwan. J. Obstet. Gynecol.* **2012**, *51*, 515–525. [[CrossRef](#)]
98. Azouz, M.; Cullin, C.; Lecomte, S.; Lafleur, M. Membrane Domain Modulation of A β 1–42 Oligomer Interactions with Supported Lipid Bilayers: An Atomic Force Microscopy Investigation. *Nanoscale* **2019**, *11*, 20857–20867. [[CrossRef](#)]
99. Bokvist, M.; Lindström, F.; Watts, A.; Gröbner, G. Two Types of Alzheimer's B-Amyloid (1–40) Peptide Membrane Interactions: Aggregation Preventing Transmembrane Anchoring Versus Accelerated Surface Fibril Formation. *J. Mol. Biol.* **2004**, *335*, 1039–1049. [[CrossRef](#)]
100. Lai, A.Y.; McLaurin, J. Mechanisms of Amyloid-Beta Peptide Uptake by Neurons: The Role of Lipid Rafts and Lipid Raft-Associated Proteins. *Int. J. Alzheimer's Dis.* **2011**, *2011*, 548380. [[CrossRef](#)] [[PubMed](#)]
101. Leonard, C.; Conrard, L.; Guthmann, M.; Pollet, H.; Carquin, M.; Vermynen, C.; Gailly, P.; Van Der Smissen, P.; Mingeot-Leclercq, M.P.; Tyteca, D. Contribution of Plasma Membrane Lipid Domains to Red Blood Cell (Re)Shaping. *Sci. Rep.* **2017**, *7*, 4264. [[CrossRef](#)] [[PubMed](#)]
102. Carquin, M.; Pollet, H.; Veiga-Da-Cunha, M.; Cominelli, A.; Van Der Smissen, P.; N'Kuli, F.; Emonard, H.; Henriët, P.; Mizuno, H.; Courtoy, P.J.; et al. Endogenous Sphingomyelin Segregates into Submicrometric Domains in the Living Erythrocyte Membrane [S]. *J. Lipid Res.* **2014**, *55*, 1331–1342. [[CrossRef](#)]
103. Conrard, L.; Stommen, A.; Cloos, A.-S.; Steinkühler, J.; Dimova, R.; Pollet, H.; Tyteca, D. Spatial Relationship and Functional Relevance of Three Lipid Domain Populations at the Erythrocyte Surface. *Cell. Physiol. Biochem.* **2018**, *51*, 1544–1565. [[CrossRef](#)] [[PubMed](#)]
104. Leonard, C.; Pollet, H.; Vermynen, C.; Gov, N.; Tyteca, D.; Mingeot-Leclercq, M.-P. Tuning of Differential Lipid Order between Submicrometric Domains and Surrounding Membrane Upon Erythrocyte Reshaping. *Cell. Physiol. Biochem.* **2018**, *48*, 2563–2582. [[CrossRef](#)]
105. Li, H.; Lykotrafitis, G. Erythrocyte Membrane Model with Explicit Description of the Lipid Bilayer and the Spectrin Network. *Biophys. J.* **2014**, *107*, 642–653. [[CrossRef](#)]
106. Carquin, M.; Conrard, L.; Pollet, H.; Van Der Smissen, P.; Cominelli, A.; Veiga-Da-Cunha, M.; Courtoy, P.J.; Tyteca, D. Cholesterol Segregates into Submicrometric Domains at the Living Erythrocyte Membrane: Evidence and Regulation. *Cell. Mol. Life Sci.* **2015**, *72*, 4633–4651. [[CrossRef](#)]
107. Himbert, S.; Alsop, R.J.; Rose, M.; Hertz, L.; Dhaliwal, A.; Moran-Mirabal, J.M.; Verschoor, C.P.; Bowdish, D.M.E.; Kaestner, L.; Wagner, C.; et al. The Molecular Structure of Human Red Blood Cell Membranes from Highly Oriented, Solid Supported Multi-Lamellar Membranes. *Sci. Rep.* **2017**, *7*, 39661. [[CrossRef](#)] [[PubMed](#)]
108. Aoki, T. A Comprehensive Review of Our Current Understanding of Red Blood Cell (Rbc) Glycoproteins. *Membranes* **2017**, *7*, 56. [[CrossRef](#)] [[PubMed](#)]
109. Walter, F.R.; Santa-Maria, A.R.; Mészáros, M.; Veszelka, S.; Dér, A.; Deli, M.A. Surface Charge, Glycocalyx, and Blood-Brain Barrier Function. *Tissue Barriers* **2021**, *9*, 1904773. [[CrossRef](#)]

110. Yagi-Utsumi, M.; Itoh, S.G.; Okumura, H.; Yanagisawa, K.; Kato, K.; Nishimura, K. The Double-Layered Structure of Amyloid-B Assemblage on Gm1-Containing Membranes Catalytically Promotes Fibrillization. *ACS Chem. Neurosci.* **2023**, *14*, 2648–2657. [\[CrossRef\]](#)
111. Chen, X.-Y.; Huang, Y.-X.; Liu, W.-J.; Yuan, Z.-J. Membrane Surface Charge and Morphological and Mechanical Properties of Young and Old Erythrocytes. *Curr. Appl. Phys.* **2007**, *7*, e94–e96. [\[CrossRef\]](#)
112. Peters, D.G.; Connor, J.R.; Meadowcroft, M.D. The Relationship between Iron Dyshomeostasis and Amyloidogenesis in Alzheimer's Disease: Two Sides of the Same Coin. *Neurobiol. Dis.* **2015**, *81*, 49–65. [\[CrossRef\]](#)
113. Bukara, K.; Jovanić, S.; Drvenica, I.T.; Stančić, A.; Ilić, V.; Rabasović, M.D.; Pantelić, D.; Jelenković, B.; Bugarski, B.; Krmpot, A.J. Mapping of Hemoglobin in Erythrocytes and Erythrocyte Ghosts Using Two Photon Excitation Fluorescence Microscopy. *J. Biomed. Opt.* **2017**, *22*, 026003. [\[CrossRef\]](#)
114. Flores, V.G.; Martínez-Martínez, A.; Pérez, J.A.R.; Bezada, J.A.; Aguirre-Tostado, F.S.; Casillas, P.E.G. Biointeraction of Erythrocyte Ghost Membranes with Gold Nanoparticles Fluorescents. *Materials* **2021**, *14*, 6390. [\[CrossRef\]](#)
115. Itzhaki, R.F.; Gill, D. A Micro-Biuret Method for Estimating Proteins. *Anal. Biochem.* **1964**, *9*, 401–410. [\[CrossRef\]](#)
116. Watanabe, C.; Seigneuret, M.; Staneva, G.; Puff, N.; Angelova, M.I. On the Possible Structural Role of Single Chain Sphingolipids Sphingosine and Sphingosine 1-Phosphate in the Amyloid-B Peptide Interactions with Membranes. Consequences for Alzheimer's Disease Development. *Colloids Surf. A Physicochem. Eng. Asp.* **2016**, *510*, 317–327. [\[CrossRef\]](#)
117. Staneva, G.; Watanabe, C.; Puff, N.; Yordanova, V.; Seigneuret, M.; Angelova, M.I. Amyloid-B Interactions with Lipid Rafts in Biomimetic Systems: A Review of Laboratory Methods. In *Lipid Rafts: Methods and Protocols*; Erhard, B., Ed.; Springer: New York, NY, USA, 2021; pp. 47–86.
118. Numaguchi, Y.; Tsukakoshi, K.; Takeuchi, N.; Suzuki, Y.; Ikebukuro, K.; Kawano, R. Real-Time Monitoring of the Amyloid B1–42 Monomer-to-Oligomer Channel Transition Using a Lipid Bilayer System. *PNAS Nexus* **2024**, *3*, pgad437. [\[CrossRef\]](#) [\[PubMed\]](#)
119. Sant, V.; Som, M.; Karkisaval, A.G.; Carnahan, P.; Lal, R. Scavenging Amyloid Oligomers from Neurons with Silica Nanobowls: Implications for Amyloid Diseases. *Biophys. J.* **2021**, *120*, 3329–3340. [\[CrossRef\]](#) [\[PubMed\]](#)
120. Nirmalraj, P.N.; List, J.; Battacharya, S.; Howe, G.; Xu, L.; Thompson, D.; Mayer, M. Complete Aggregation Pathway of Amyloid B (1-40) and (1-42) Resolved on an Atomically Clean Interface. *Sci. Adv.* **2020**, *6*, eaaz6014. [\[CrossRef\]](#)
121. Tsai, Y.-C.; Luo, J.-C.; Liu, T.-I.; Lu, I.-L.; Shen, M.-Y.; Chuang, C.-Y.; Chern, C.-S.; Chiu, H.-C. Capturing Amyloid-B Oligomers by Stirring with Microscaled Iron Oxide Stir Bars into Magnetic Plaques to Reduce Cytotoxicity toward Neuronal Cells. *Nanomaterials* **2020**, *10*, 1284. [\[CrossRef\]](#)
122. Watson, D.; Castaño, E.; Kokjohn, T.A.; Kuo, Y.M.; Lyubchenko, Y.; Pinsky, D.; Connolly, E.S.; Esh, C.; Luehrs, D.C.; Stine, W.B.; et al. Physicochemical Characteristics of Soluble Oligomeric Aβ and Their Pathologic Role in Alzheimer's Disease. *Neurol. Res.* **2005**, *27*, 869–881. [\[CrossRef\]](#)
123. Sehlín, D.; Stina, S.; Bénédicte, B.; Henryk, B.; Gérard, N.B.; Delphine, B.; Hennig, B.; Karl, P.B.; Per, B.; Donna, C.; et al. Engineered Antibodies: New Possibilities for Brain Pet? *Eur. J. Nucl. Med. Mol. Imaging* **2019**, *46*, 2848–2858. [\[CrossRef\]](#) [\[PubMed\]](#)
124. Owen, D.M.; Rentero, C.; Magenau, A.; Abu-Siniyeh, A.; Gaus, K. Quantitative Imaging of Membrane Lipid Order in Cells and Organisms. *Nat. Protoc.* **2012**, *7*, 24–35. [\[CrossRef\]](#) [\[PubMed\]](#)
125. Danovski, G.; Dyankova-Danovska, T.; Stamatov, R.; Aleksandrov, R.; Kanev, P.-B.; Stoyanov, S. CellTool: An Open-Source Software Combining Bio-Image Analysis and Mathematical Modeling for the Study of DNA Repair Dynamics. *Int. J. Mol. Sci.* **2023**, *24*, 16784. [\[CrossRef\]](#) [\[PubMed\]](#)
126. Domingues, M.M.; Castanho, M.A.R.B.; Santos, N.C. Rbpi21 Promotes Lipopolysaccharide Aggregation and Exerts Its Antimicrobial Effects by (Hemi)Fusion of Pg-Containing Membranes. *PLoS ONE* **2009**, *4*, e8385. [\[CrossRef\]](#)

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.