Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences

Tome 79, No 11, 2026

BIOLOGY

Biophysics

PETASIN MODULATES THE STRUCTURAL ORGANIZATION OF LIPID BILAYERS MODELLING RESTING AND ACTIVATED MAST CELL MEMBRANES

Vesela Yordanova[1], Ralitsa Angelova[1]✉, Rusina Hazarosova[1], Aleksandrina Nesheva[1], Dayana Benkova[1], Tanya Tancheva[1], Ralitsa Veleva[2], Dilyana Doneva[3], Roumen Pankov[2], Albena Momchilova[1], Galya Staneva[1]✉

Received on September 18, 2026
Accepted on October 27, 2026

Abstract

Petasin, the major sesquiterpene ester isolated from *Petasites hybridus*, is known for its anti-inflammatory and antiallergic properties, but its direct effects on membrane biophysical properties remain poorly understood. In this study, the interaction of petasin with large unilamellar vesicles modelling resting and activated mast cell plasma membranes was investigated. Membrane order was evaluated using Laurdan generalized polarization (*GP*) and DPH steady-state fluorescence anisotropy, probing the membrane interface and hydrophobic core, respectively, while dynamic light scattering (DLS) was used to determine vesicle size and homogeneity. Petasin significantly increased Laurdan *GP* in both membrane models, indicating enhanced lipid order at the membrane interface. DPH anisotropy increased significantly only in activated membrane models, demonstrating increased acyl chain order within the bilayer core. DLS

This research was financially supported by the National Science Fund of Bulgaria under Grant No. KP-06-M81/3/04.12.2024. The authors thank the Bulgarian Ministry of Education and Science for the support provided via Scientific Infrastructure on Cell Technologies in Biomedicine (SICTB) D01-105/2025 and the National Center for Biomedical Photonics (NCBP) D01-101/2025, part of the Bulgarian National Roadmap for Scientific Infrastructures 2020–2027. The authors also acknowledge support from COST Action CA22153 EuroCurvoBioNet.

`https://doi.org/10.7546/CRABS.2026.11.06`

measurements revealed minor changes in vesicle diameter, whereas the polydispersity index remained low, confirming that petasin did not compromise vesicle integrity. These findings demonstrate that petasin directly modulates the structural organization of lipid bilayers, particularly in membranes modelling activated mast cells, and provides a biophysical perspective that complements its established anti-inflammatory and antiallergic activities.

Key words: petasin, mast cell model membranes, lipid order, Laurdan, DPH, vesicle size

Introduction. Allergic diseases are chronic inflammatory disorders resulting from dysregulated immune responses to otherwise harmless environmental antigens. Mast cells are among the principal effector cells involved in these conditions. Following activation, they rapidly release preformed mediators, including histamine and proteases, and subsequently produce cytokines and lipid mediators such as leukotrienes, thereby amplifying the inflammatory response. The key event initiating mast cell activation is the cross-linking of IgE-bound high-affinity receptors (FcεRI), which triggers intracellular signalling pathways leading to calcium influx, degranulation, and mediator synthesis [1, 2].

Accumulating evidence indicates that the plasma membrane actively participates in FcεRI signalling rather than serving as a passive structural barrier. The lipid composition and lateral organization of the membrane influence receptor mobility, clustering, and interactions with downstream signalling proteins. In particular, cholesterol- and sphingolipid-rich membrane domains (*rafts*) have been implicated in the assembly of signalling complexes required for efficient mast cell activation [3–5]. Consequently, simplified lipid bilayer models that mimic the composition of resting and activated mast cell membranes provide a useful experimental platform for investigating membrane-related aspects of mast cell signalling.

In addition to changes in membrane domain organization, mast cell activation is accompanied by alterations in membrane phospholipid asymmetry. Under resting conditions, phosphatidylserine (PS) is predominantly confined to the inner leaflet of the plasma membrane by ATP-dependent lipid transporters. Following FcεRI-mediated activation, transient redistribution and increased exposure of PS contribute to membrane remodelling and are associated with calcium-dependent signalling events. Beyond its role as a marker of cell activation, PS is an anionic phospholipid that determines membrane surface charge and promotes the membrane recruitment and spatial organization of numerous peripheral signalling proteins through electrostatic interactions. Consequently, incorporation of PS into model membranes provides a more physiologically relevant representation of the lipid environment associated with activated mast cells [6, 7].

Petasin, the major sesquiterpene ester isolated from *Petasites hybridus*, has been extensively studied because of its anti-inflammatory and antiallergic properties. It has been reported to suppress leukotriene production by inhibiting the

5-lipoxygenase pathway and to reduce calcium-dependent inflammatory responses in immune cells [8]. Despite these findings, little is known about the direct effects of petasin on the structural and biophysical properties of lipid membranes.

In the present study, the interactions of petasin with heterogeneous lipid bilayers modelling resting and activated mast cell plasma membranes were investigated. Membrane order was evaluated using Laurdan generalized polarization and DPH steady-state fluorescence anisotropy, which probe the membrane interface and the hydrophobic core, respectively [9,10]. Dynamic light scattering (DLS) was further employed to determine the hydrodynamic diameter and polydispersity index (PDI) of the liposomes, allowing assessment of vesicle size distribution and sample homogeneity [11]. These complementary biophysical techniques were used to characterize the effects of petasin on the structural organization of model mast cell membranes.

Materials and methods. ***Reagents.*** The lipids used in this study included egg phosphatidylcholine (EggPC), 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (PAPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (POPS), egg sphingomyelin (EggSM), bovine milk disialoganglioside GD3 (GD3), and cholesterol (Chol), all purchased from Avanti Research (Alabama, USA). 6-dodecanoyl-N, N-dimethyl-2-naphthylamine (Laurdan), 1,6-Diphenyl-1,3,5-hexatriene (DPH), and dimethyl sulphoxide (DMSO) were obtained from Sigma-Aldrich (Darmstadt, Germany). Dulbecco's phosphate-buffered saline (DPBS, without calcium and magnesium) was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Petasin (purity $\geq$95%) was supplied by Carl Roth (Karlsruhe, Germany).

Preparation of lipid model systems. Large unilamellar vesicles (LUVs) representing resting and activated mast cell membrane models were prepared by the thin-film hydration and extrusion technique. Lipid mixtures were prepared from stock solutions, dried under a nitrogen stream, and further desiccated under vacuum overnight to obtain uniform lipid films. The films were hydrated with DPBS (pH 7.4), followed by successive cycles of heating, vortex mixing, ultrasonication, and cooling. The resulting multilamellar vesicles were converted into homogeneous LUVs by extrusion through 800 nm and 100 nm polycarbonate membranes at 60 °C using an Avanti Mini-Extruder (Alabama, USA).

Petasin treatment. Petasin was initially dissolved in chloroform:methanol (1:1, v/v). Following solvent evaporation, the dried compound was reconstituted in DMSO:H_2O (9:1, v/v) to prepare the working solution. LUV suspensions were assigned to three experimental conditions: untreated LUVs, DMSO-containing controls, and petasin-treated LUVs. Petasin was added to achieve a final concentration of 20 µM (lipid:petasin molar ratio 10:1), whereas control samples received an equivalent volume of the DMSO:H_2O mixture. After treatment, all samples were incubated at 37 °C for 4 h before measurements.

Fluorescence measurements. Changes in membrane order induced by 20 μM petasin were assessed in LUVs modelling resting and activated mast cell membranes using two complementary fluorescence approaches. Laurdan was employed to evaluate lipid packing at the membrane interface, while DPH was used to probe the ordering of lipid acyl chains in the hydrophobic core [9, 12, 13]. Lipid:probe molar ratios were 200:1.

Laurdan fluorescence spectra were recorded on a Tecan Infinite 200 PRO microplate reader ($\lambda_{ex} = 355$ nm, $\lambda_{em} = 390 - 600$ nm), and the generalized polarization (GP) was calculated as [9]:

$$GP = \frac{I_{440} - I_{490}}{I_{440} + I_{490}},$$

where I_{440} and I_{490} are the emission fluorescence intensities at 440 and 490 nm. Higher GP values are associated with a more ordered membrane state [14].

DPH steady-state fluorescence anisotropy (r) was measured on a Jasco FP-8300 spectrofluorometer ($\lambda_{ex} = 358$ nm, $\lambda_{em} = 430$ nm) and calculated according to [13]:

$$r = \frac{I_{VV} - G \cdot I_{VH}}{I_{VV} + 2G \cdot I_{VH}},$$

where I_{VV}, I_{VH}, I_{HV}, and I_{HH} denote the fluorescence intensities measured with the corresponding excitation and emission polarizer orientations, while $G = I_{HV}/I_{HH}$ is the instrumental correction factor. Higher r values indicate increased lipid acyl chain order and reduced probe rotational mobility [10].

Dynamic light scattering. The hydrodynamic diameter and polydispersity index of LUVs were measured by dynamic light scattering using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). Prior to analysis, samples (200 μM total lipid) were equilibrated for 10 min at 37 °C. Particle size was calculated from the translational diffusion coefficient derived from Brownian motion using the Stokes–Einstein relationship, while PDI was used to assess the homogeneity of the vesicle preparations. Size distributions are reported as intensity-weighted values [11].

Results and discussion. ***Effect of petasin on membrane lipid order.*** To investigate whether petasin (Fig. 1) modifies the physicochemical properties

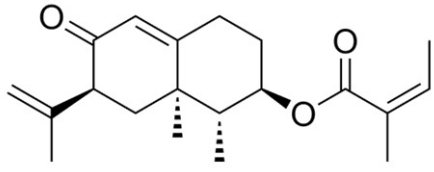

Fig. 1. 2D structure of petasin (CAS No.: 26577-85-5, MedChemExpress) [15]

of mast cell membranes, its effects were examined using LUVs reproducing the lipid composition of resting and activated mast cell plasma membranes. The resting membrane model consisted of EggPC:PAPC:EggSM:GD3:Chol (30:10:30:10:20 mol/mol), whereas the activated membrane model additionally contained 5 mol% phosphatidylserine (POPS) (EggPC:POPS:PAPC:EggSM:GD3:Chol, 25:5:10:30:10:20 mol/mol), reflecting the changes in membrane phospholipid composition accompanying mast cell activation.

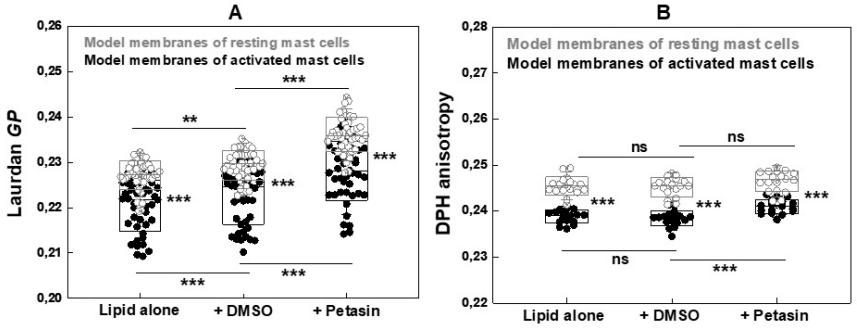

Fig. 2. Effect of petasin on the lipid order at the membrane interface (A) and in the hydrophobic core (B) of LUVs mimicking resting (gray open circles) and activated (black filled circles) mast cell membranes evaluated by Laurdan *GP* and DPH anisotropy, respectively. Measurements were performed in untreated vesicles (lipid alone), DMSO-containing controls, and petasin-treated samples. Data are shown as box-and-whisker plots with individual values. Statistical comparisons were performed using a Pair-Sample *t*-test. Significance levels are indicated as $p < 0.01$ (**), $p < 0.001$ (***), and ns (not significant)

To evaluate membrane organization, lipid order was assessed independently at the membrane interface using Laurdan *GP* and within the hydrophobic core using DPH steady-state fluorescence anisotropy (Fig. 2). As shown in Fig. 2A, untreated LUVs modelling activated mast cell membranes exhibited lower Laurdan *GP* values than the corresponding resting membrane models, indicating lower lipid order at the membrane-water interface. Similar differences were observed in the DMSO-containing controls, demonstrating that the solvent itself did not affect the characteristic differences between the two membrane models. These observations are consistent with the distinct lipid compositions of the models, particularly the incorporation of POPS into the activated membrane, which modifies intermolecular lipid interactions and membrane organization. Treatment with petasin significantly increased Laurdan *GP* in both resting and activated membrane models compared with their respective DMSO controls. Nevertheless, resting membrane models retained significantly higher *GP* values than activated membrane models after treatment, indicating that petasin increased membrane order without eliminating the intrinsic differences between the two lipid compositions. These observations demonstrate that petasin increases lipid order at the membrane in-

terface while preserving the characteristic physicochemical properties of resting and activated mast cell membranes.

The effect of petasin on membrane organization within the hydrophobic core was evaluated by DPH fluorescence anisotropy (Fig. 2B). Under all experimental conditions, resting membrane models exhibited significantly higher anisotropy values than activated membrane models, reflecting greater acyl chain order. In contrast to Laurdan *GP*, DPH anisotropy was not significantly affected by DMSO in either membrane model. Petasin treatment produced a significant increase in DPH anisotropy in activated membrane models, whereas no statistically significant change was detected in resting membrane models. Consequently, activated membrane models remained less ordered than resting membranes even after treatment. These results indicate that petasin exerts a more pronounced ordering effect on the hydrophobic core of activated membrane models, whereas its influence on the already highly ordered resting membranes is comparatively limited.

The complementary information obtained from Laurdan *GP* and DPH anisotropy indicates that petasin influences both the membrane interface and the hydrophobic core, although the magnitude of its effect depends on membrane composition. Together, the observed increases in *GP* and anisotropy indicate that petasin promotes membrane ordering, particularly in membrane models representing activated mast cells.

These observations are biologically relevant because membrane lipid organization is an important determinant of mast cell activation. The lipid environment regulates FcεRI receptor mobility, clustering, and interactions with signalling proteins, while incorporation of POPS into the activated membrane model modifies membrane surface charge and lipid organization [3, 6]. The present study demonstrates that petasin directly modifies the organization of heterogeneous lipid bilayers in the absence of membrane proteins, receptors, or intracellular signalling components. This finding complements previous reports showing that petasin inhibits leukotriene biosynthesis through suppression of the 5-lipoxygenase pathway and attenuates calcium-dependent inflammatory responses [8]. Although the present experiments do not examine FcεRI signalling or mediator release directly, they suggest that modulation of membrane biophysical properties may represent an additional mechanism contributing to the biological activity of petasin. Rather than replacing its established intracellular targets, membrane remodelling could modify the physicochemical environment in which receptor activation and early signalling events occur.

Importantly, petasin increased membrane order without causing extensive membrane rigidification or eliminating the characteristic differences between resting and activated membrane models. This observation suggests that petasin modulates membrane organization while preserving the dynamic structural properties required for normal membrane function. Such moderate changes in lipid order

are consistent with fine regulation of membrane-associated processes rather than nonspecific perturbation of membrane integrity.

Effect of petasin on vesicle size. To determine whether these effects on membrane organization were accompanied by changes in vesicle structure, the hydrodynamic diameter of LUVs was measured by DLS (Fig. 3). Untreated activated membrane models exhibited significantly larger hydrodynamic diameters than resting membrane models. Addition of DMSO resulted in a significant increase in vesicle diameter in both membrane models compared with untreated samples. In activated membrane models, petasin did not significantly alter vesicle diameter relative to the corresponding DMSO controls. In contrast, petasin significantly reduced the hydrodynamic diameter of resting membrane models compared with DMSO-containing vesicles, although the values remained slightly higher than those of untreated samples. The polydispersity index (PDI) remained consistently low in all experimental groups, ranging from 0.07 to 0.10, indicating narrow particle size distributions and excellent homogeneity of the liposome preparations. These values are characteristic of well-defined populations of extruded LUVs and demonstrate that neither DMSO nor petasin measurably affected sample uniformity.

Overall, the DLS results indicate that petasin does not induce vesicle aggregation, destabilization, or major structural rearrangements under the experimental conditions employed. Therefore, the fluorescence changes observed following petasin treatment are unlikely to arise from differences in vesicle size or heterogeneity and instead reflect genuine alterations in membrane organization.

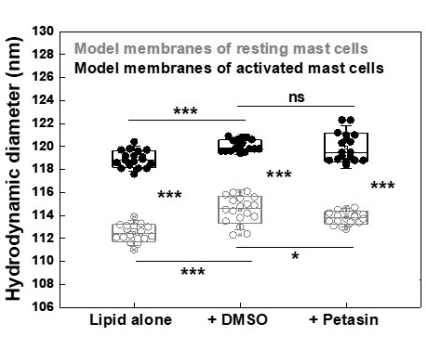

Fig. 3. Effect of petasin on the hydrodynamic diameter of LUVs mimicking resting (gray open circles) and activated (black filled circles) mast cell membranes determined by DLS. Measurements were performed in untreated vesicles (lipid alone), DMSO-containing controls, and petasin-treated samples. Data are shown as box-and-whisker plots with individual values. Statistical comparisons were performed using a Pair-Sample t-test. Significance levels are indicated as $p < 0.05$ (*), $p < 0.001$ (***), and ns (not significant)

Collectively, the present findings demonstrate that petasin directly modulates the biophysical properties of lipid bilayers modelling resting and activated mast cell plasma membranes. By increasing membrane order at both the membrane interface and, particularly, within the hydrophobic core of activated membrane models, petasin modifies the lipid environment in which membrane-associated signalling is initiated. Although the present study does not establish a direct effect on FcεRI activation, calcium signalling, or mast cell degranulation, it provides a biophysical framework that complements the known anti-inflammatory actions of petasin. Considering the central role of membrane orga-

nization in mast cell activation, these present observations support the hypothesis that membrane remodelling may contribute to the prophylactic and therapeutic potential of petasin in allergic diseases.

Conclusions. The present study identifies petasin as a modulator of membrane biophysical properties in lipid bilayers modelling mast cell plasma membranes. The observed changes in membrane organization, together with the preserved structural integrity of the vesicles, suggest that lipid membranes may represent an additional target underlying the biological activity of petasin. These findings expand the current understanding of petasin beyond its established effects on inflammatory mediators and provide a basis for future studies addressing the contribution of membrane remodelling to mast cell signalling and allergic inflammation.

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[1]*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Akad. Georgi Bonchev St, Bl. 21, 1113 Sofia, Bulgaria*
e-mails: vyordanova@biophys.bas.bg, https://orcid.org/0000-0003-0753-8084
ralitsa.angelova@biophys.bas.bg, https://orcid.org/0000-0002-3053-544X
rhazarosova@biophys.bas.bg
nescheva@gmail.com, https://orcid.org/0009-0002-4474-5277
dayanabenkova369@abv.bg, https://orcid.org/0009-0008-1770-8773
tania.t@biophys.bas.bg, https://orcid.org/0009-0005-9071-5564
albena_momchilova@abv.bg, https://orcid.org/0000-0001-6608-0881
g_staneva@yahoo.com, https://orcid.org/0000-0003-2089-3603

[2]*Faculty of Biology, Sofia University "St. Kliment Ohridski", 8 Dragan Tzankov Blvd, 1164 Sofia, Bulgaria*
e-mails: ralitsa_veleva@biofac.uni-sofia.bg,
https://orcid.org/0000-0003-2064-4869
rpankov@biofac.uni-sofia.bg, https://orcid.org/0000-0002-3157-3659

[3]*Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Akad. Georgi Bonchev St, Bl. 21, 1113 Sofia, Bulgaria*
e-mail: donevadiliana@gmail.com