NOVEL BENZANTHRONE DYE FOR MEMBRANE STUDIES AND AMYLOID FIBRIL DETECTION

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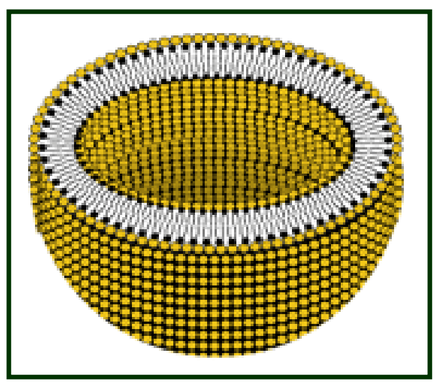
INTRODUCTION

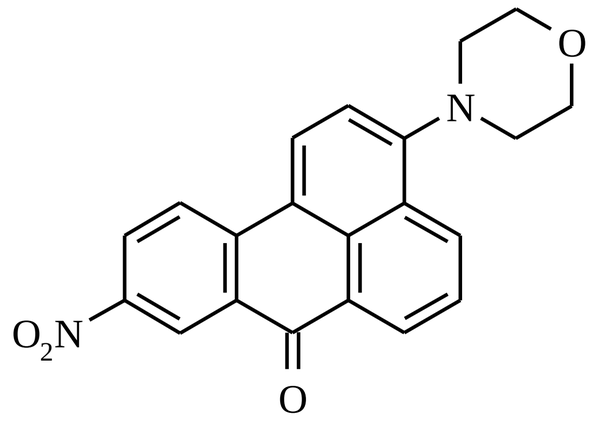
Benzanthrone dyes have a wide variety of applications in biomedical research due to their high photostability, large Stokes shifts and extinction coefficients. In particular, benzanthrone derivatives were employed in DNA, protein and membrane studies. Furthermore, these dyes displayed pronounced sensitivity to the changes in immune status of a human organism at different pathologies. This study was aimed at testing the potential of the novel benzanthrone compound, referred to here as MN2, for its ability to monitor the changes in physicochemical properties of the model lipid membranes, as well as to detect the disease-related protein aggregates, amyloid fibrils

MATERIALS AND METHODS



Liposome

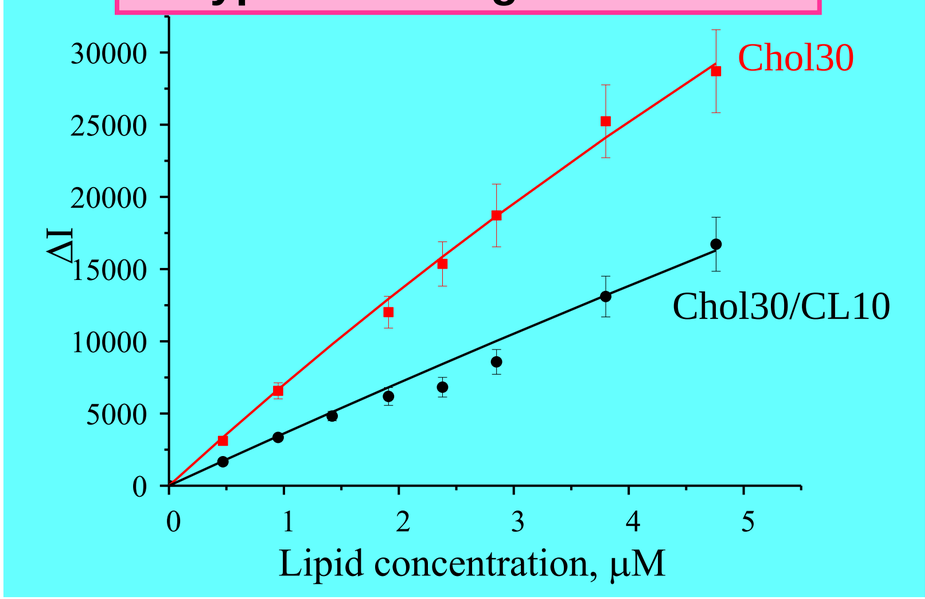
Liposomes composed of phosphatidylcholine (PC) and its mixture with cardiolipin (CL5, CL10 and CL20) and cholesterol (Chol30) or both lipids (CL10 /Chol30) were used as model membranes. Lipid vesicles were prepared by the extrusion method. Amyloid fibrils of insulin were obtained at 50 °C under continuous orbital rotation (155 rpm) for 18 hours. Benzanthrone dye MN2 was synthesized at the Department of Applied Chemistry of Daugavpils University .

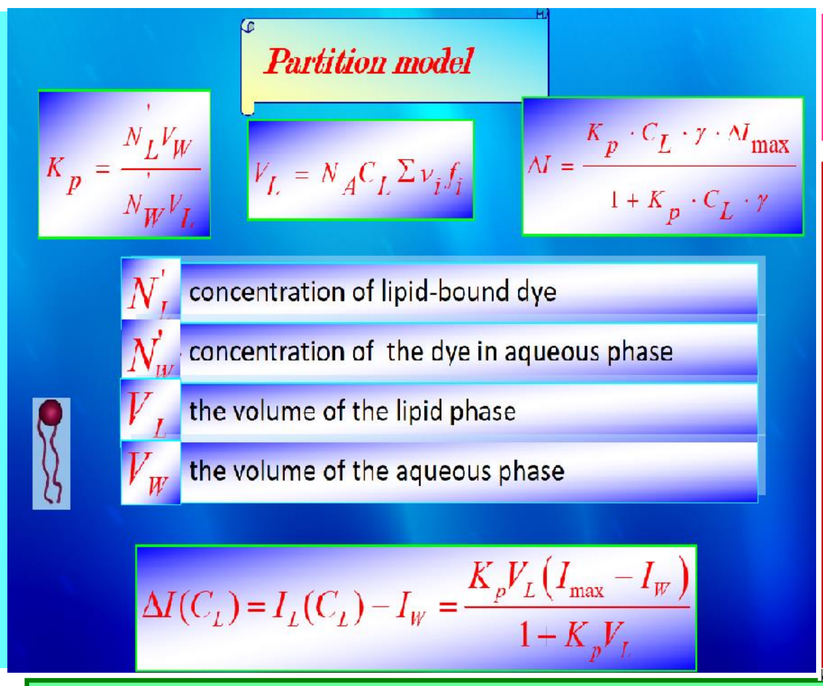


Structural formula of MN2

RESULTS AND DISCUSSION

Typical binding isotherms

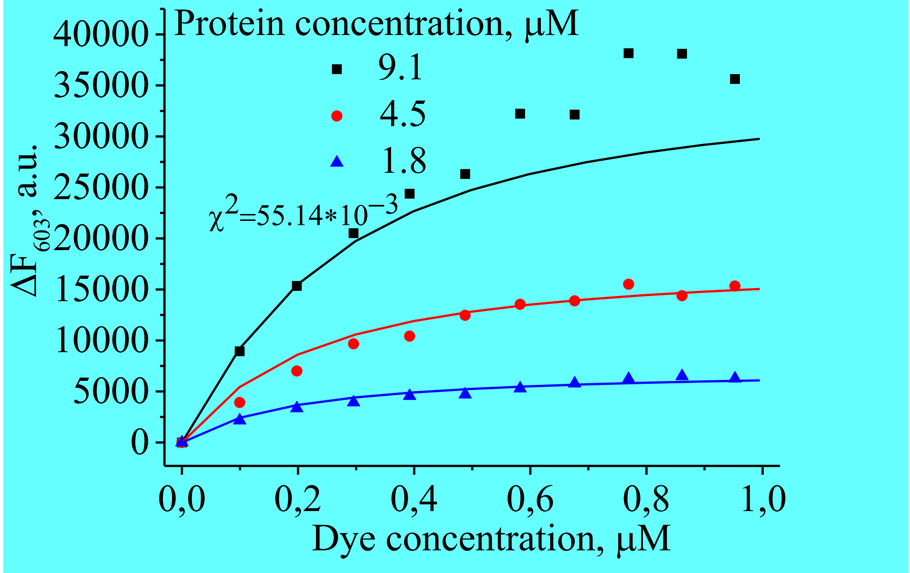




Parameters of the benzanthrone dye partitioning into lipid systems

Liposome¤	$K_p \times 10^4$¤	$\Delta I_{max} \times 10^5$¤
PC¤	1.96±0.5¤	1.48±0.3¤
CL5¤	0.6±0.1¤	4.7±0.7¤
CL10¤	0.43±0.09¤	5.9±0.9¤
CL20¤	0.37±0.08¤	1.88±0.4¤
Chol30¤	3.8±0.5¤	2.76±0.5¤
Chol30/CL10¤	1.5±0.6¤	3.5±0.8¤

The isotherms of MN2 binding to amyloid fibrils



Global fit using Langmuir absorption model

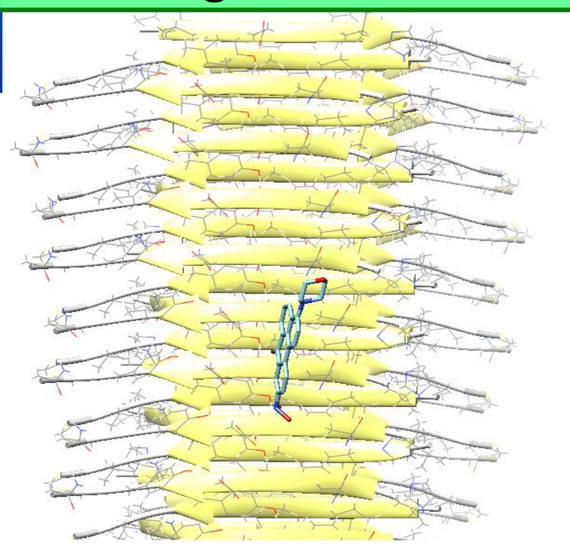
$$\Delta I = 0.5a\left[Z_0 + nC_P + 1/K_a - \sqrt{(Z_0 + nC_P + 1/K_a)^2 - 4nC_P Z_0}\right]$$

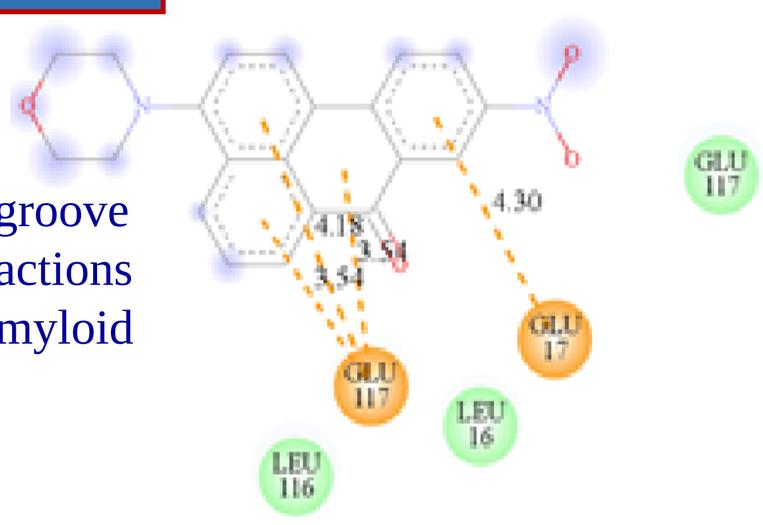
Z_0 – total dye concentration, K_a – association constant, n – binding stoichiometry, C_p – protein concentration, a – molar fluorescence of the bound dye

Binding characteristics of MN2 and ThT

Dye¤	Ka,μM^{-1}¤	n¤	Fmol,·μM^{-1}¤	χ^2¤
MN2/InsF¤	5.9¤	0.017¤	2.4*10^5¤	0.05514¤
ThT/InsF¤	12.2¤	0.017¤	6.2*10^5¤	0.04955¤
MN2/InsN¤	1.6¤	0.086¤	2.1*10^4¤	0.0114¤

Molecular docking studies using SwissDock





- MN2 possesses a strong lipid-associating ability
- the MN2 partitioning ability depends on lipid bilayer composition
- MN2 possesses a strong selectivity to the fibrillar protein aggregates
- the potential fibril binding site for MN2 was represented by the Q15A_E17A surface groove
- the MN2-protein complex was stabilized by the dye Pi-anion and van-der-Waals interactions with E17A and L16A, E17A residues of the β-strands constituting the model insulin amyloid fibril

CONCLUSIONS

. Overall, our findings suggest that MN2 can be used for probing the membrane state and amyloid fibril detection and characterization