The revelation of interactions in model myelin with FTIR spectroscopy

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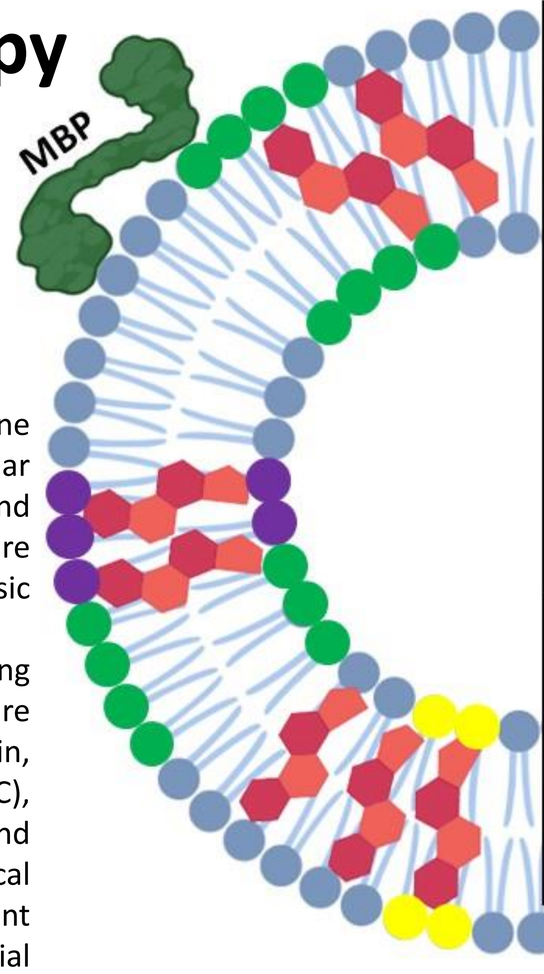
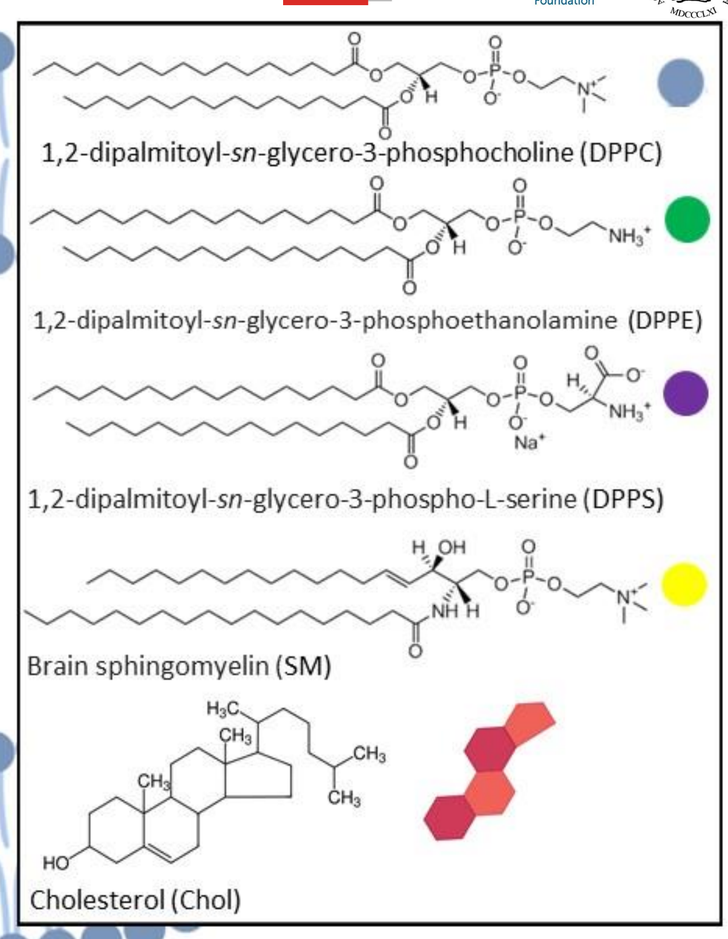
INTRODUCTION

The myelin sheath in the central nervous system is a compact multilamellar membrane system that enables rapid transmission of nerve impulses. [1] Unlike the cellular membranes, myelin has a very high lipid-to-protein mass ratio (roughly 80 %-to-20 %), and high proportions of glycosphingolipids and cholesterol.[2] Along with lipids, which are dominant in myelin composition, the complex structure is maintained by myelin basic protein (MBP), which is presumably crucial for myelin disruption in multiple sclerosis. [1]
Many biophysical studies showed that MBP interacts with lipid membranes employing electrostatic and hydrophobic interactions to assemble the proper multilamellar structure of myelin sheath. To gain an insight into the interactions of MBP with model myelin, multilamellar liposomes (MLVs) were constituted from phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), sphingomyelin (SM) and cholesterol at molar ratios in order to mimic myelin at physiological and pathological conditions, respectively. [2] MLVs were studied using calorimetric and different spectroscopic techniques [3], emphasizing FTIR spectroscopy that provided crucial information on the engagement of MBP cationic amino acids in association with model myelin. In this work, special attention is paid to the influence of cholesterol on the interactions of myelin components.



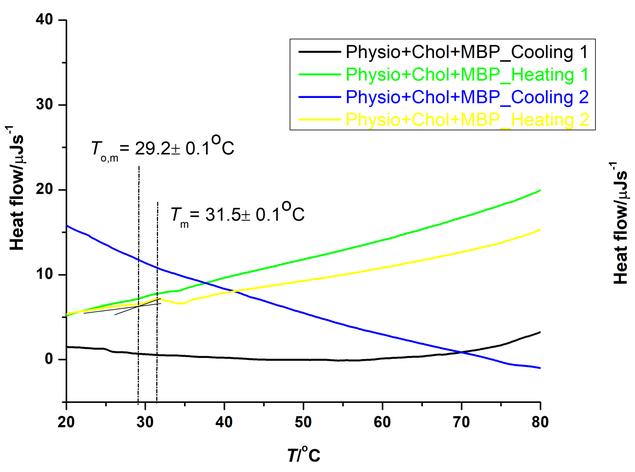


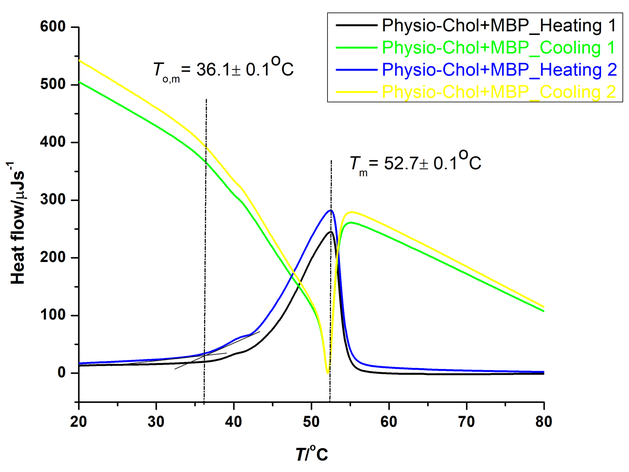
	PHYSIOLOGICAL	PATHOLOGICAL
	mol.%	mol. %
DPPC	21	15
DPPE	16	17
DPPS	3	7
SM	5	2
Chol	33	38

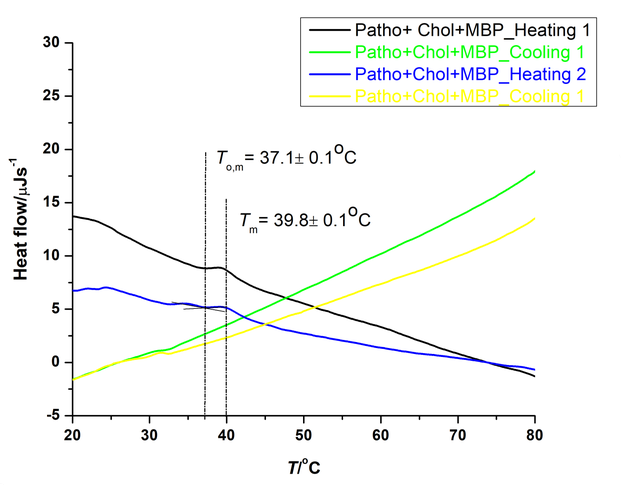
SAMPLE PREPARATION AND ANALYSIS

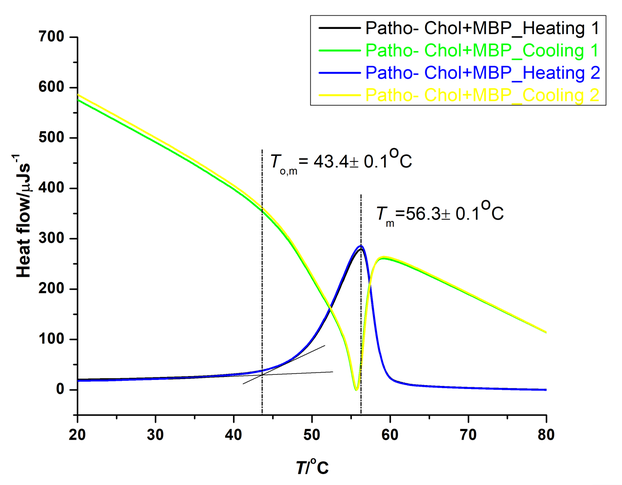
- MLVs liposome suspensions for FTIR ATR and DSC were prepared at γ= 5 mg ml^{-1} in physiological and pathological ratios ± cholesterol (shown in Table)[2] with MBP protein that the total protein to lipid molar ratio is 1: 200[3]
- FTIR ATR spectra were performed at two temperatures (30 and 60 °C) for gel (L_β) and liquid (L_α) phase
- DSC experiments were carried at a scan rate of 1°C min^{-1} in the temperature range 10—90 °C in two heating and cooling cycles

DSC

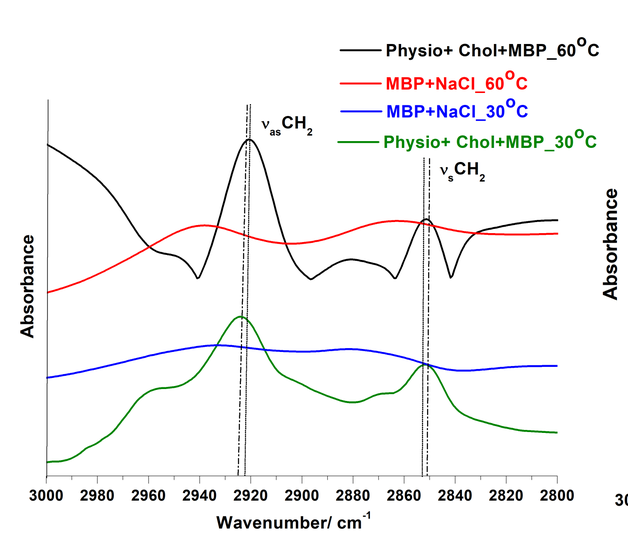


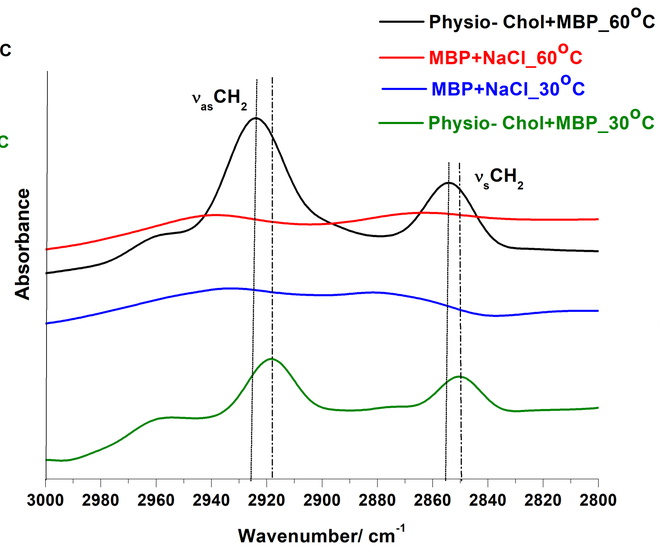


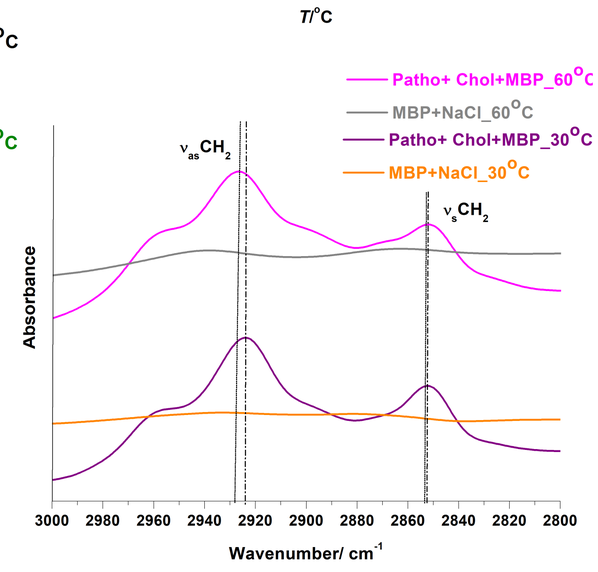


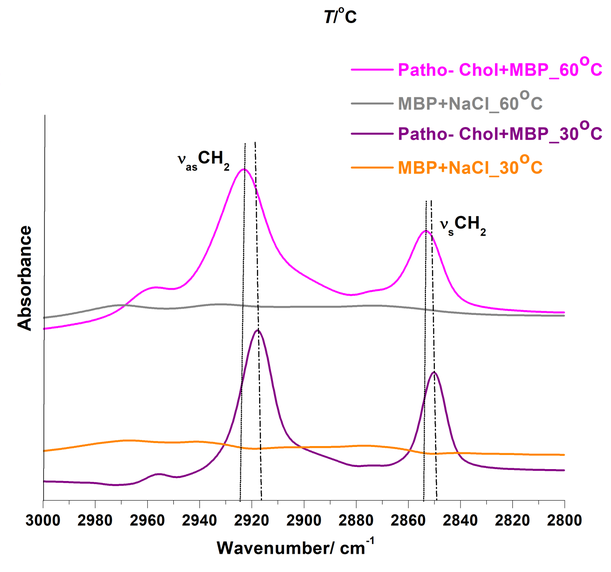


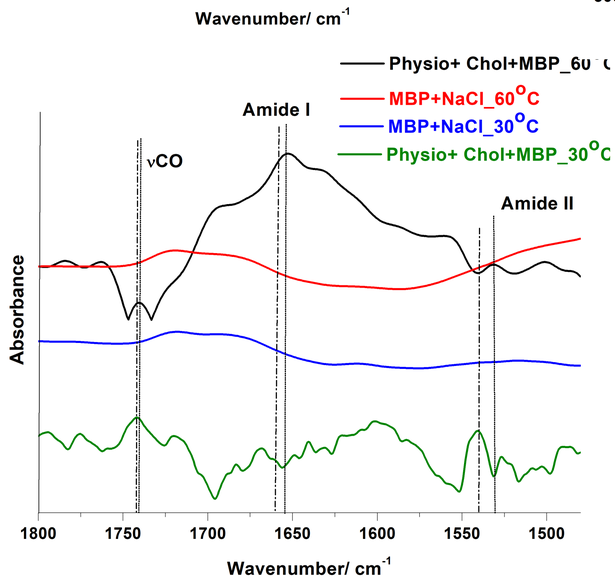
FTIR

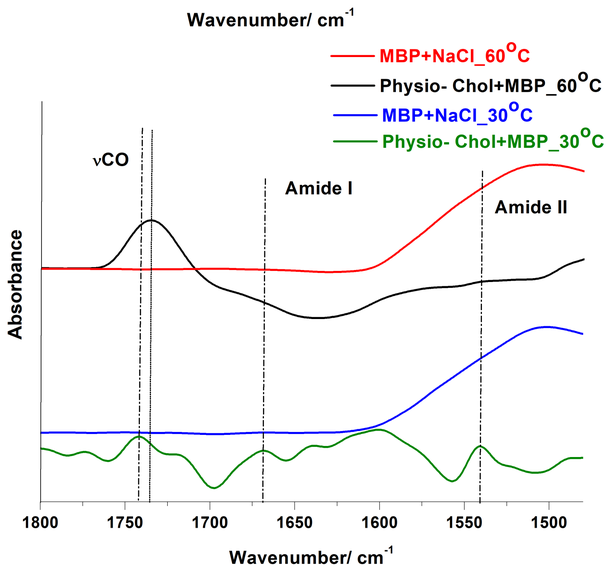


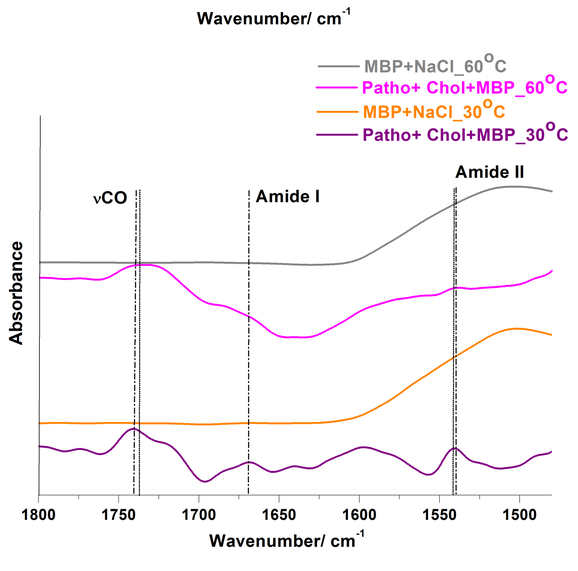


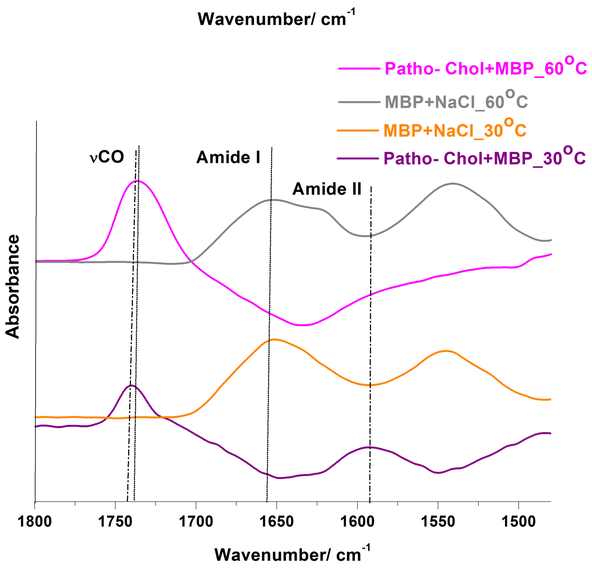












CONCLUSIONS

- The analyzed DSC curves show that cholesterol diminishes gel (L_β) → liquid (L_α) phase transition temperature
- The pathological MLVs show larger T_m than physiological, due to the increase of ratios of DPPS and DPPE
- Cholesterol increases the rigidity of the lipid bilayers (in both cases)
- The amide bands I/II remain the same through the lipid phase change in cholesterol-free samples

REFERENCES

1. G. Harauz, V. Ladizhansky, J. M. Boggs, *Biochem. 48,* **2009.**,8094– 8104.
2. Y. Min, K. Kristiansen, J. M. Boggs et. al, *PNAS 10,* **2009.**, 3154– 3159.
3. S.Schmitt, L.C. Castelvetri, M. Simons, *BBA - Mol. Cell Biol. Lipids 1851*, **2015.**, 999– 1005.
4. P. Maleš, Z. Brkljača, I. Crnolatac, D. Bakarić, *Colloids Surf. B 201*, **2021.**, 111645.
5. R. N. A. H. Lewis, R. N. McElhaney, *Biochim. Biophys. Acta - Biomembr. 1828,* **2013.**, 2347– 2358.

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