

Interactions of the antiviral drug glycyrrhizin and coronavirus E-protein with membrane mimetics by solution NMR studies

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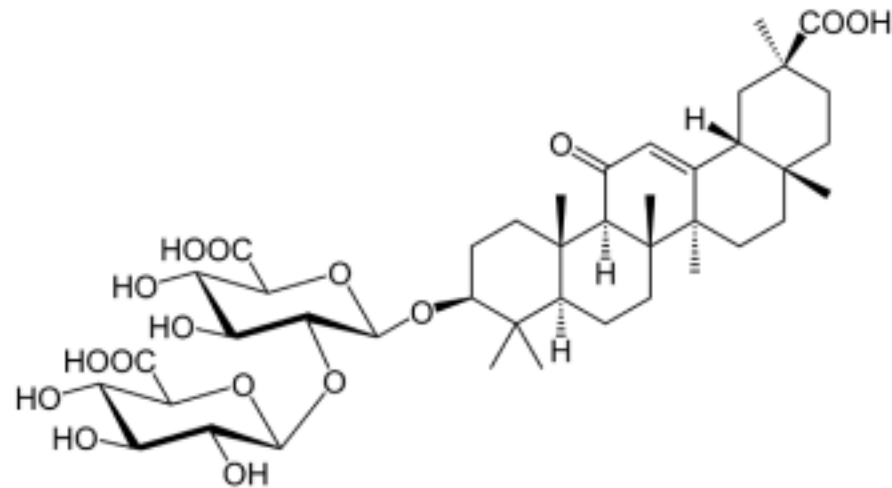
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Glycyrrhizin

- main active component in licorice root (*Glycyrrhiza glabra*)
- popular medicinal herb with nutritional and therapeutic values
- have antiviral activity, including activity against SARS-coronavirus



The mechanism of its antiviral action - unclear.

Possible mechanism: prevention of fusion of the virus envelope with the plasma membrane of the host cell.



Glycyrrhiza glabra



E-protein coronavirus

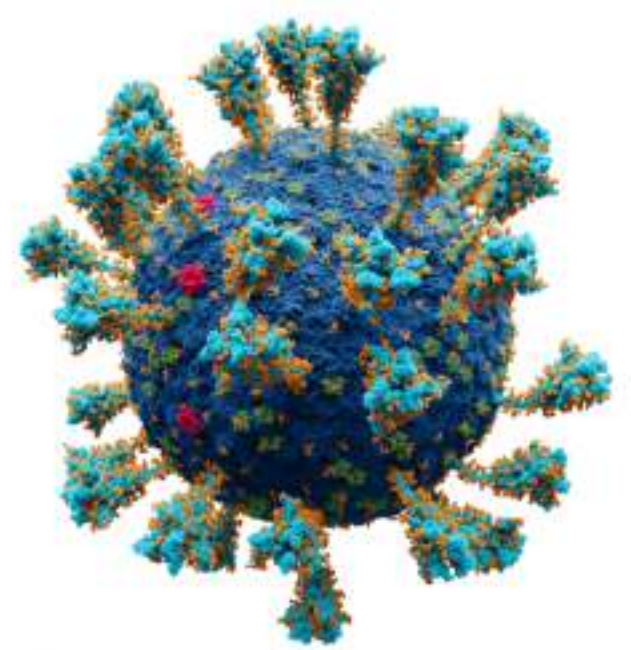
- potential antiviral target
- inhibition reduces viral pathogenicity
- contains one transmembrane α -helical domain (ETM), which is responsible for the observed ion channel activity

We used transmembrane domain:

ETGTLIVNSVLLFLAFVVFLVTLAILTALR (96%, Pepmic)

31 aminoacids; Mw=3361.38Da

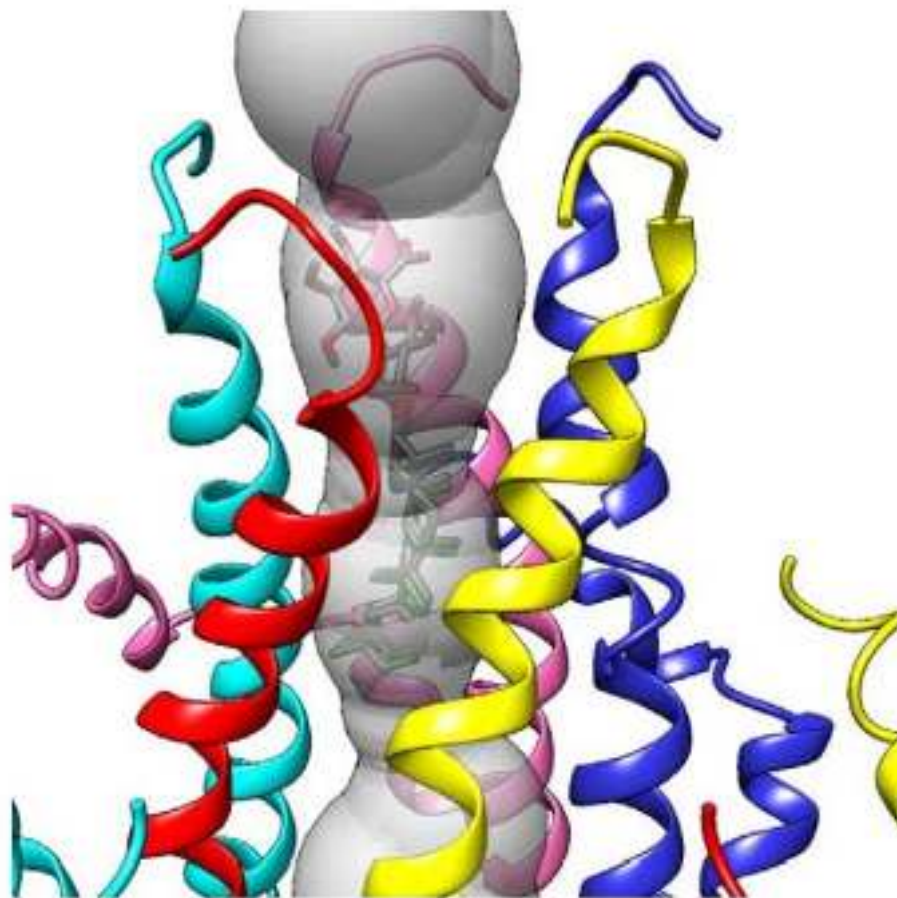
GluThrGlyThrLeulleValAsnSerValLeuLeuPheLeuAlaPheValValPheLeuLeuValThrLeuAlalleLeuThrAlaLeuArg



- Turquoise: [spike glycoprotein \(S\)](#)
- Red: [envelope proteins \(E\)](#)
- Green: [membrane proteins \(M\)](#)
- Orange: [glycan](#)

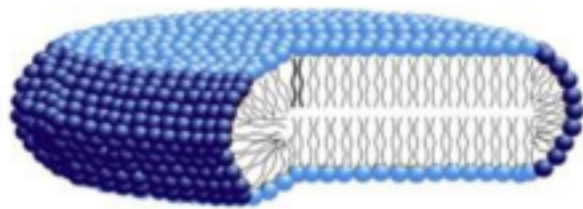
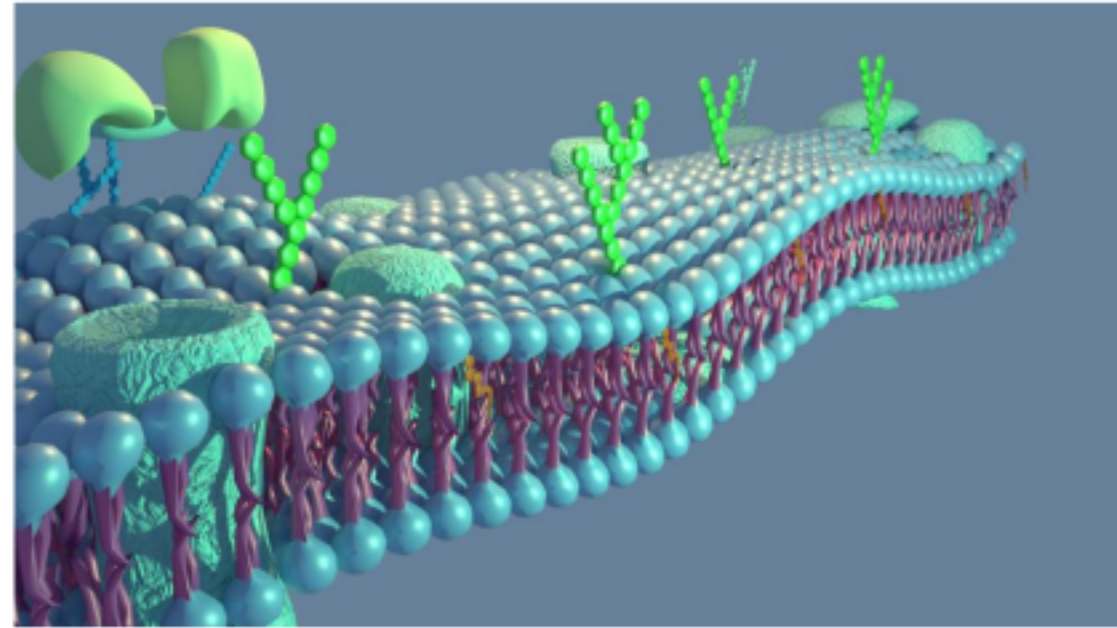
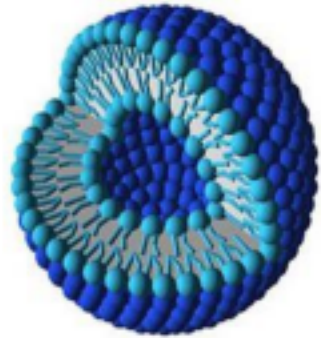


The glycyrrhizic acid inside the E channel pore



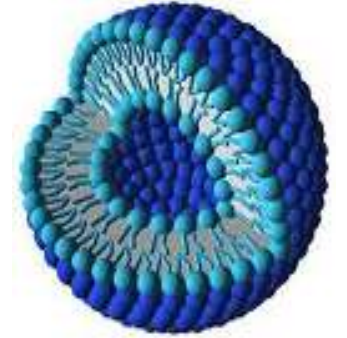
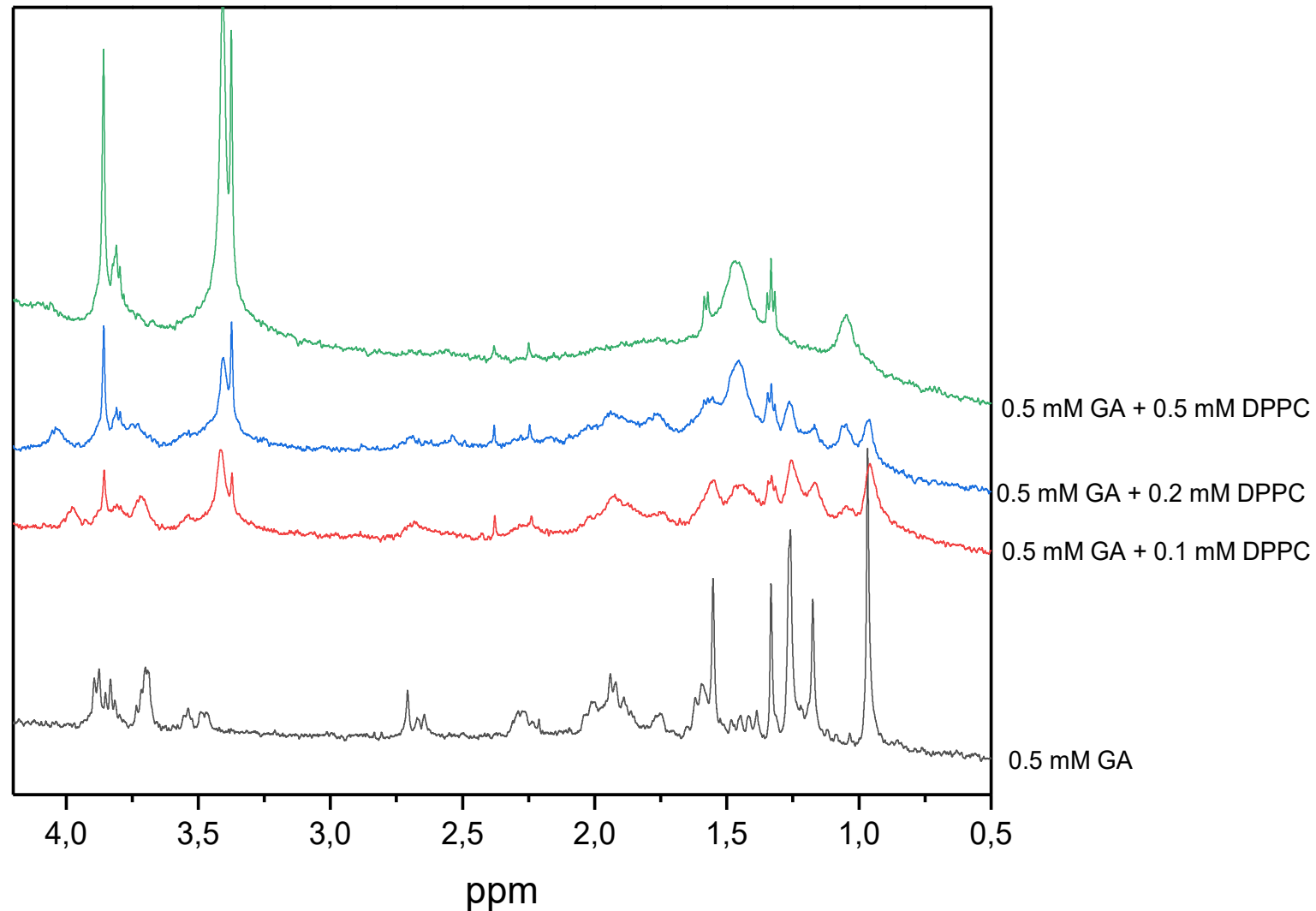
Membrane model

Liposomes

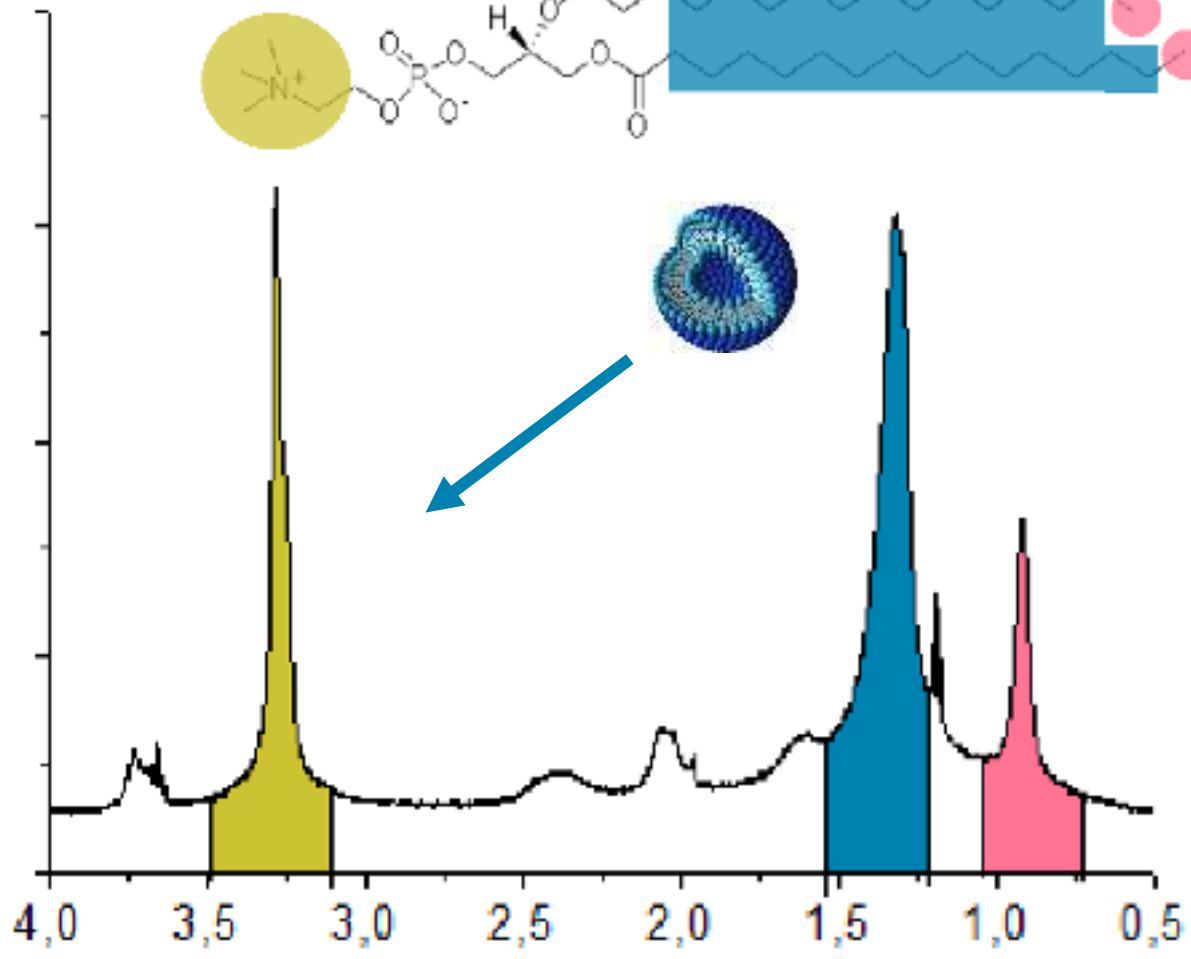
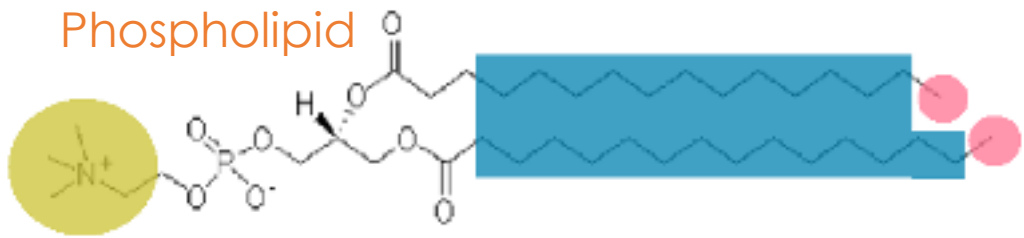


Bicelles DMPC/DHPC (molar ratio 1:2, $q=0.5$)

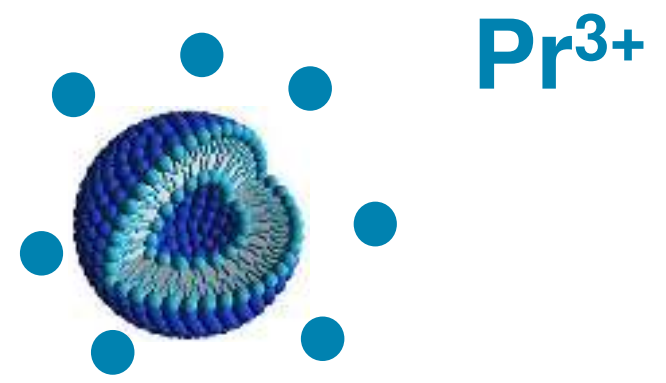
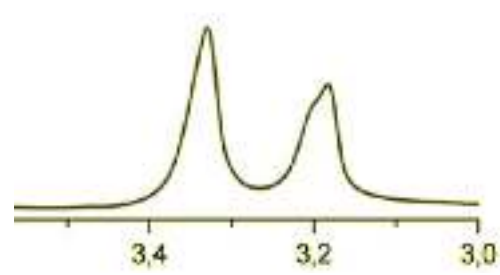
The addition of lipid to GA led to the **disappearance** of the GA signal, which indicates its incorporation into the lipid bilayer.



Phospholipid

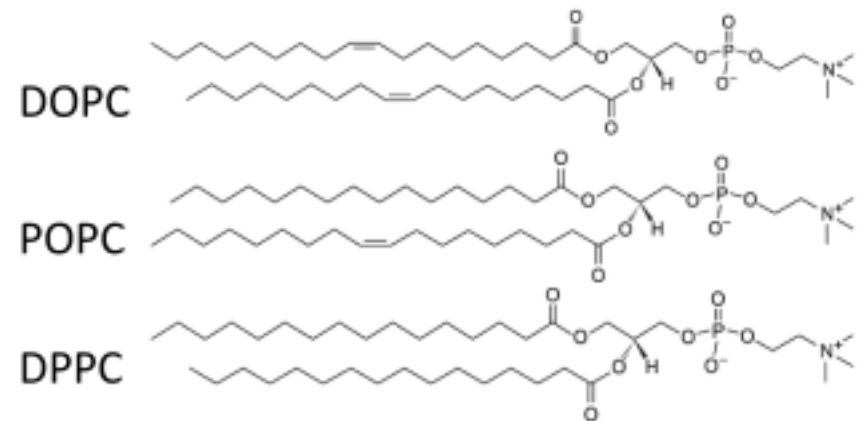


ppm



GA incorporates into the membrane

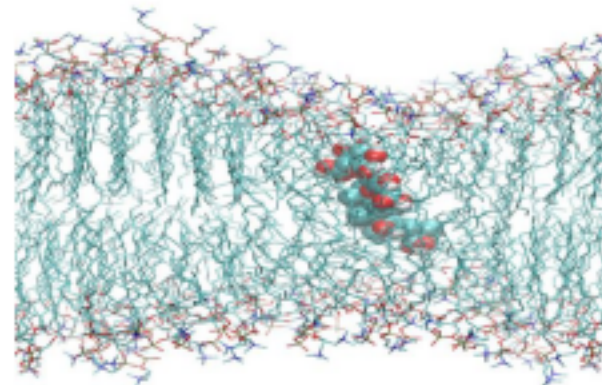
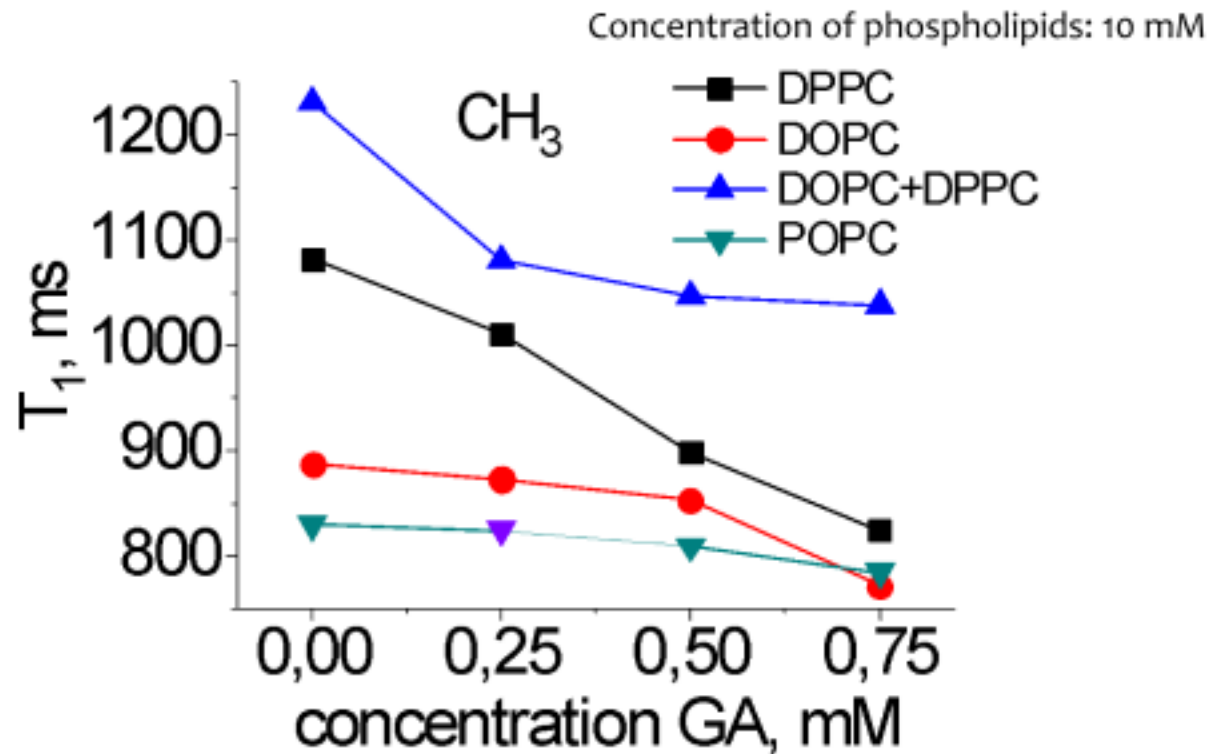
Reduced T_1 relaxation for all types of phospholipids



The presence of GA molecules in the membrane bilayer “freezes” neighboring phospholipids.

Mobility decreases.

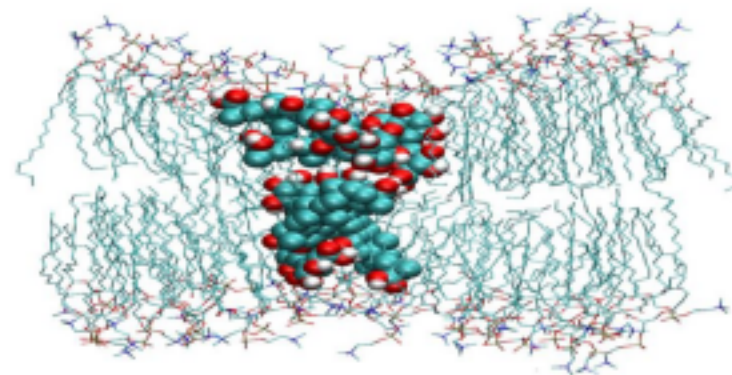
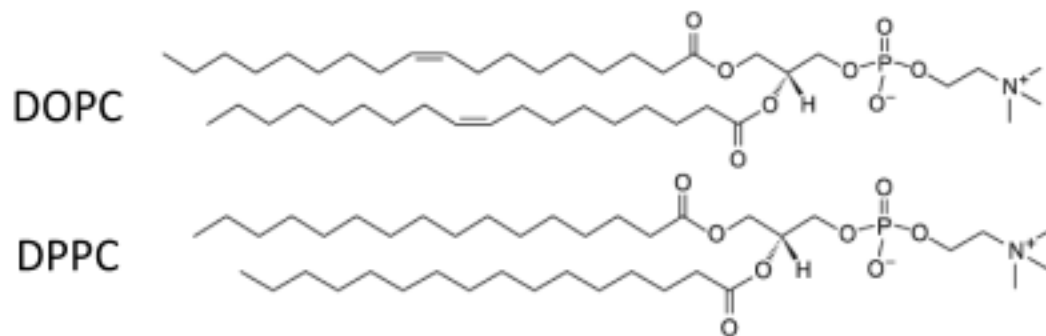
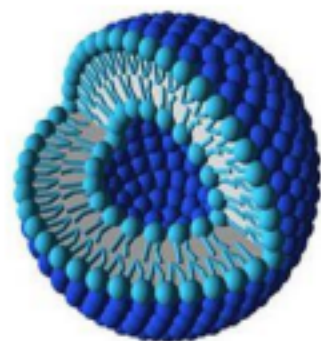
Spin-lattice relaxation times (T_1) are decreasing.



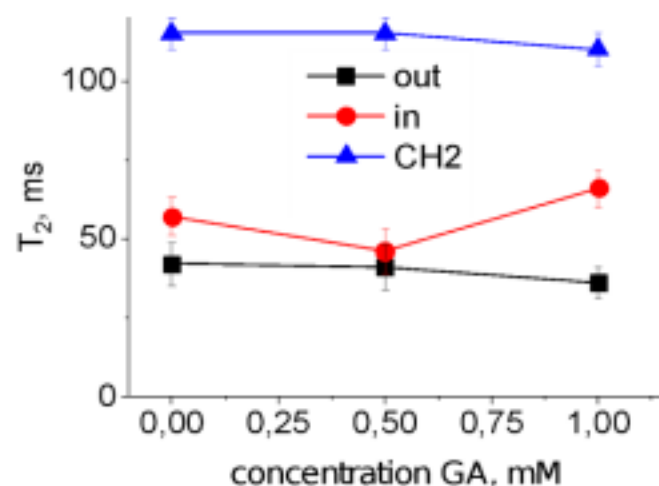
GA interaction with DPPC bilayer (Molecular dynamics)

GA tends to form associates in more ordered membranes

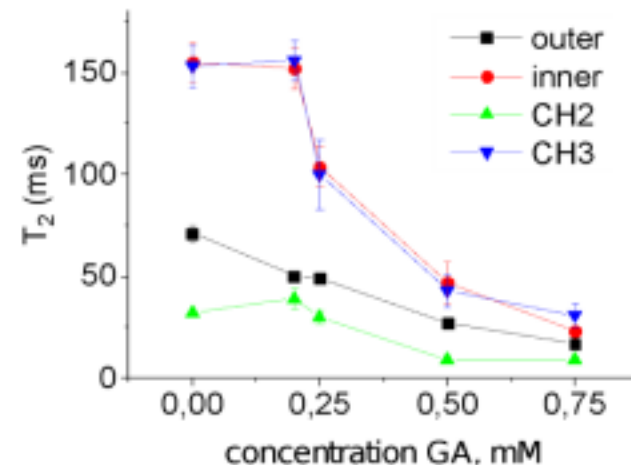
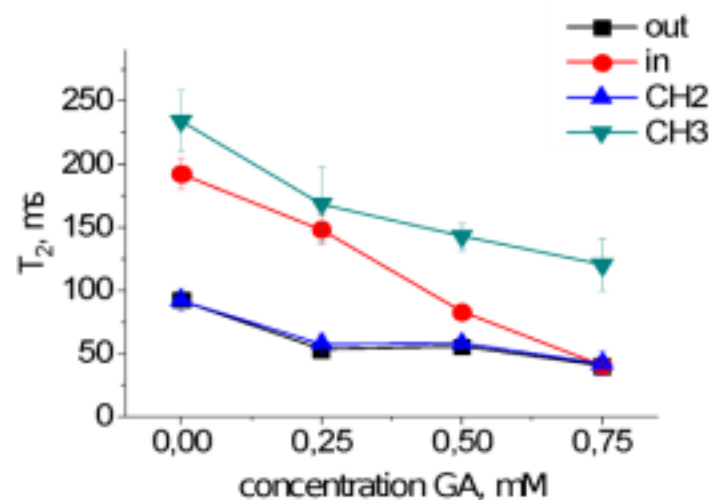
GA interaction with DPPC bilayer (Molecular dynamics)



DOPC: no dependence



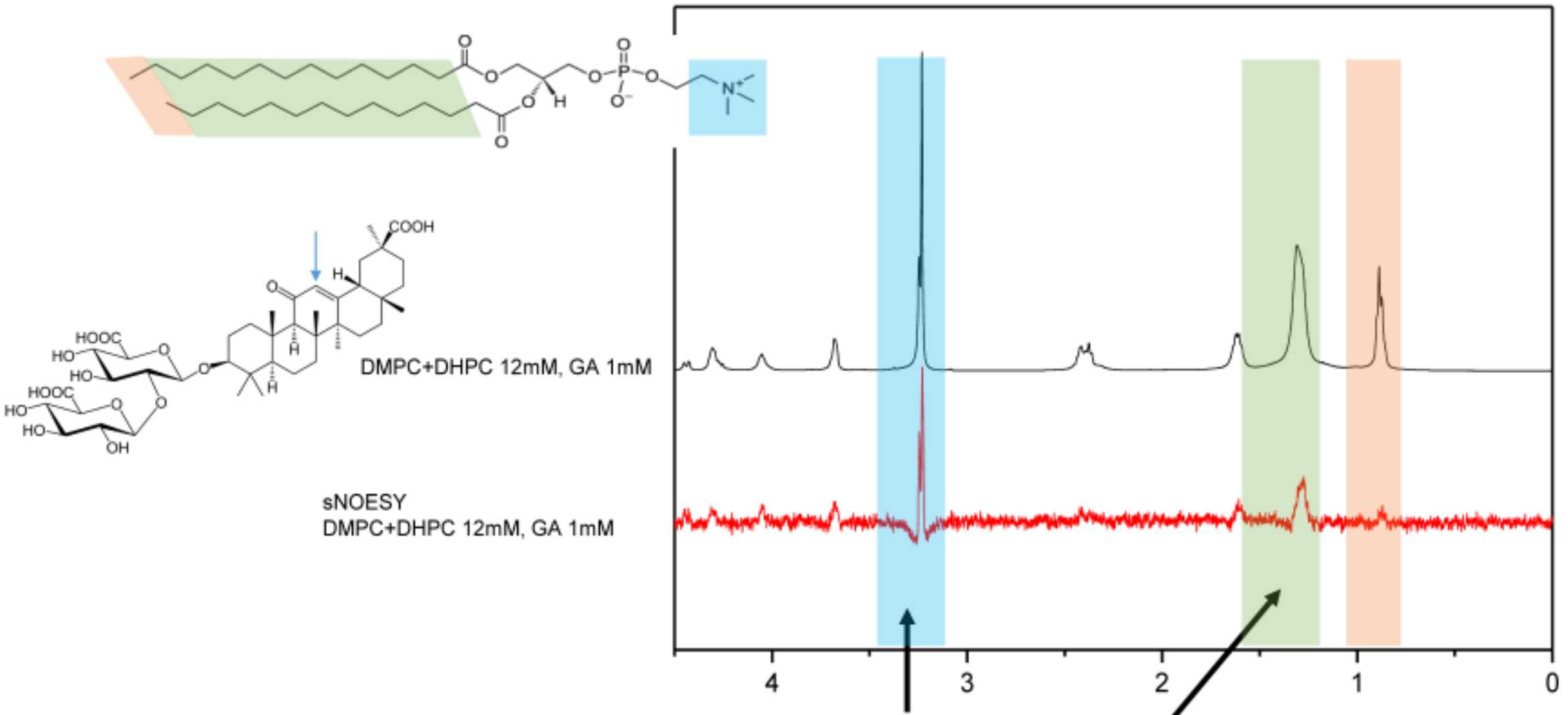
DOPC+DPPC, DPPC: reduced T_2 relaxation times



Concentration of phospholipids: 10 mM



GA is incorporated into bicelles



There are cross peaks in the NOESY spectrum

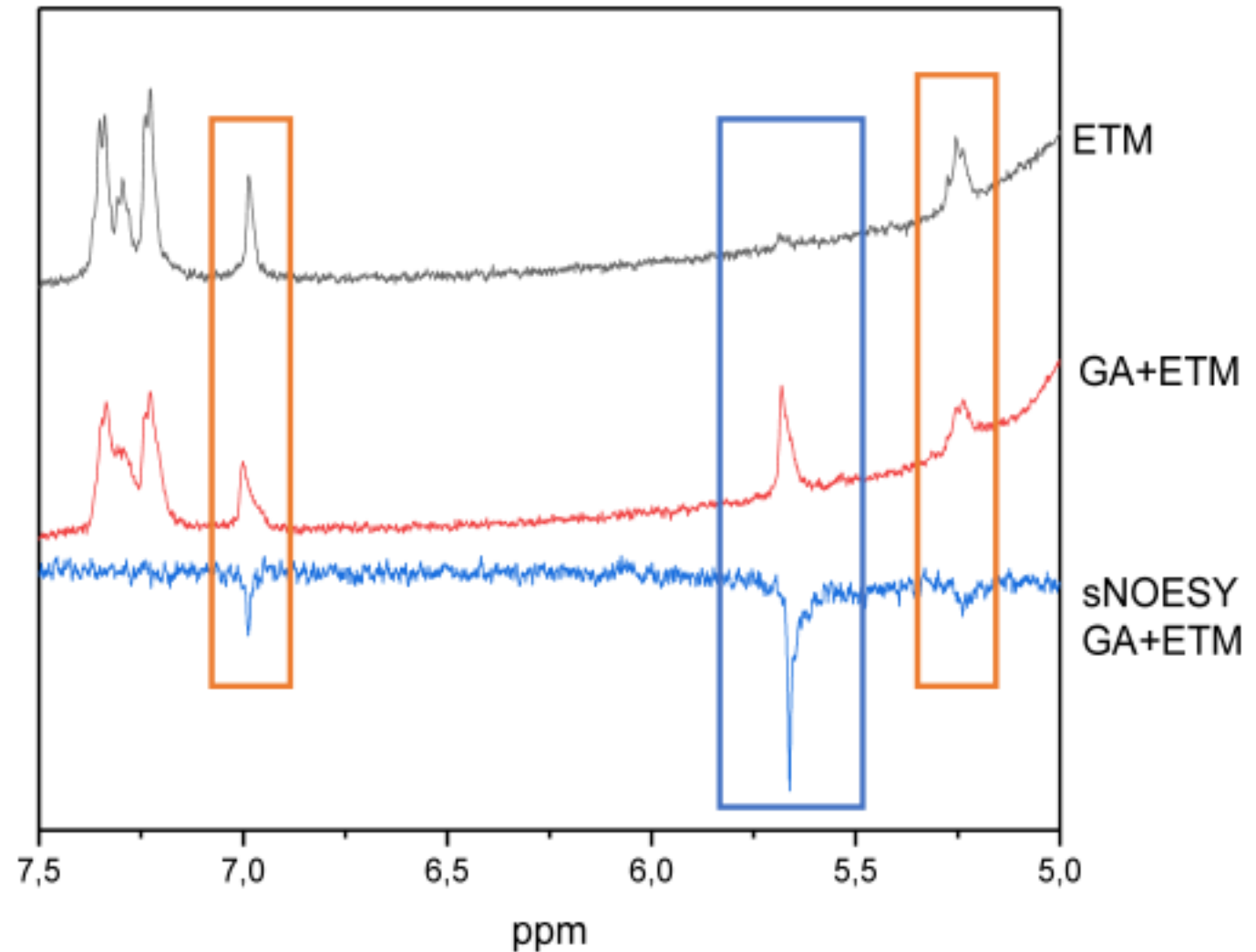
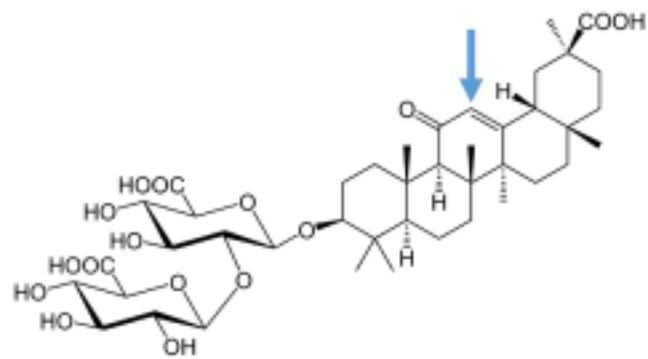
ppm

Conclusion



GA is embedded in the bilayer

GA and ETM interact with each other (in water)

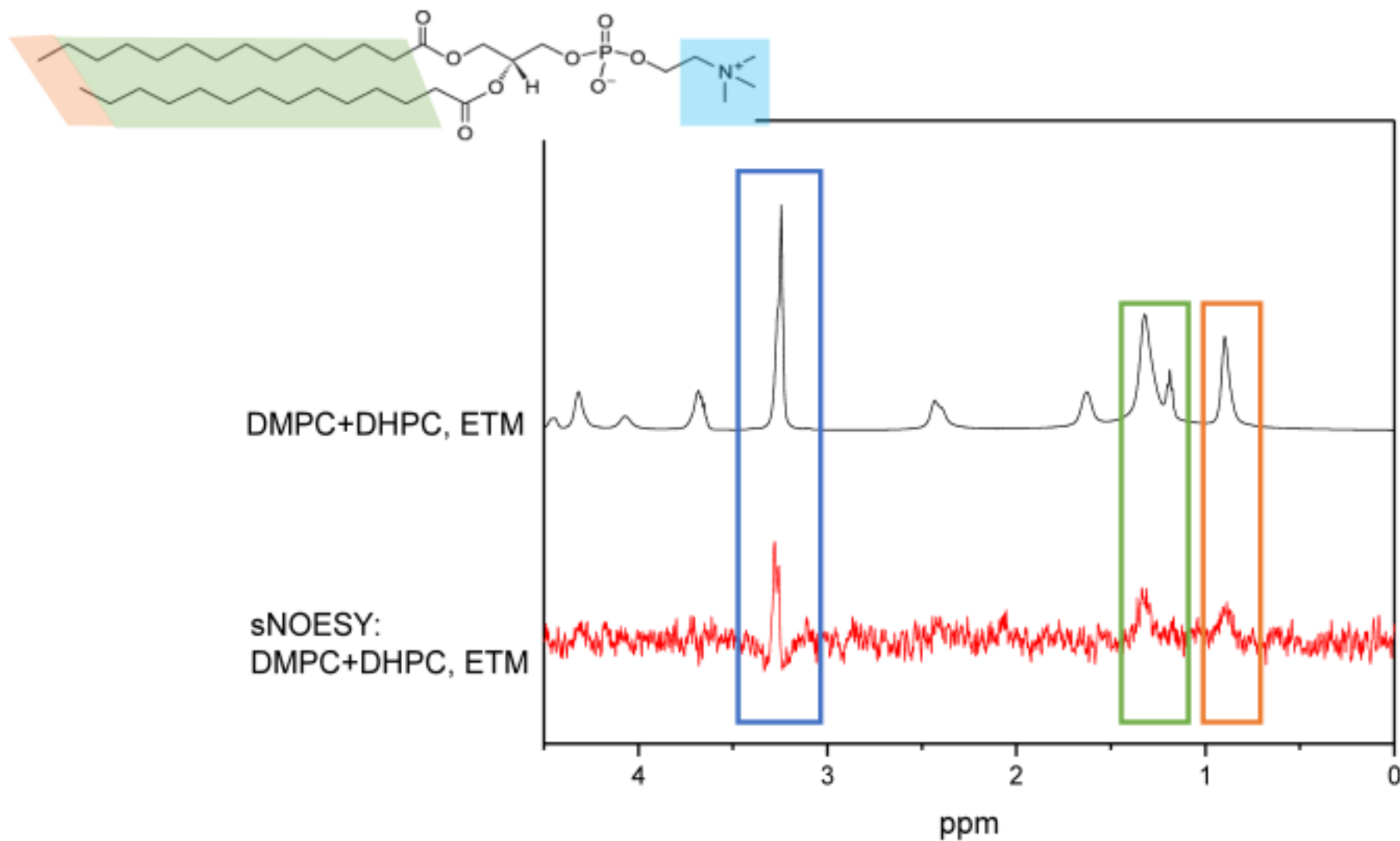


Conclusion

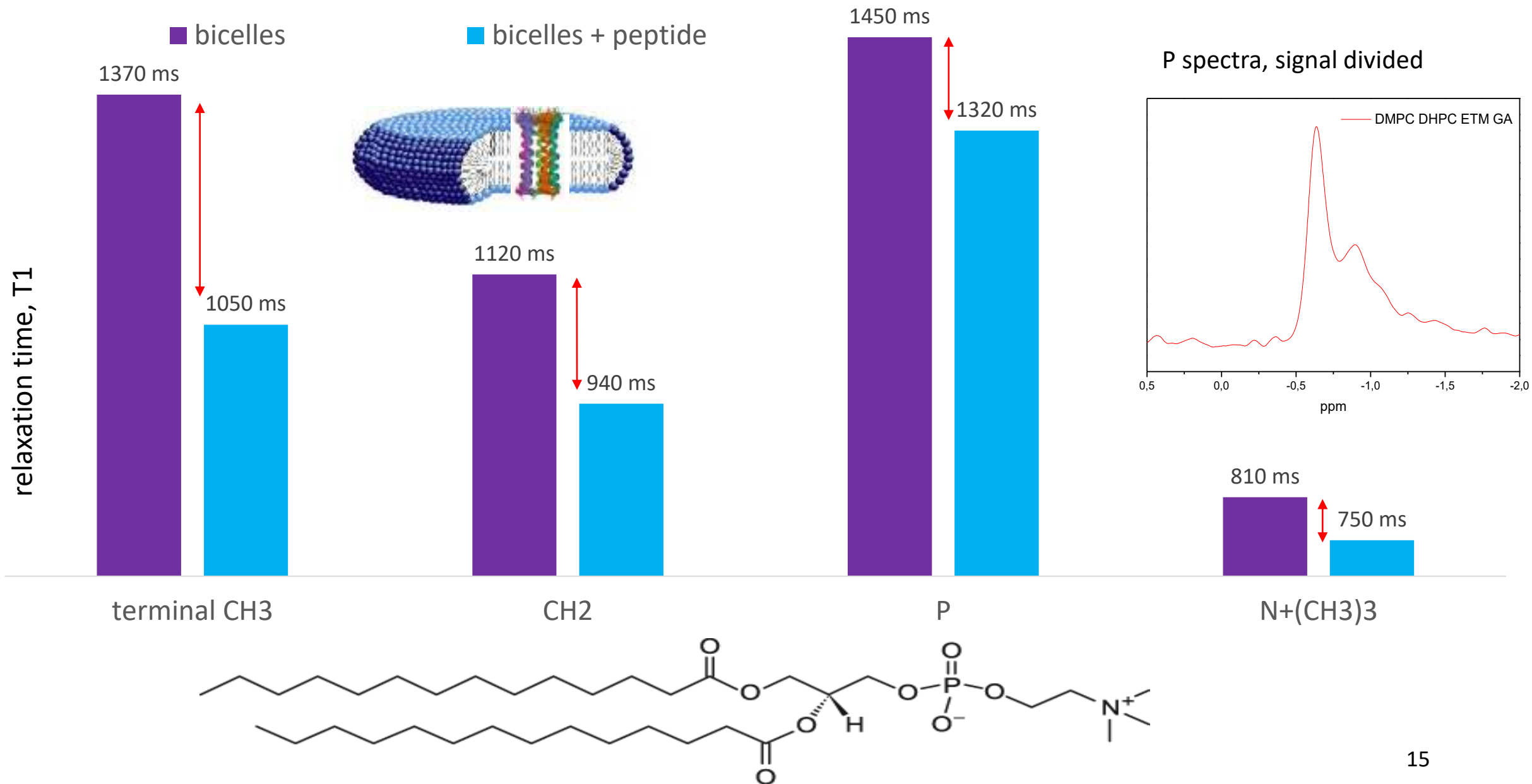


GA and ETM interact with each other in water

ETM is incorporated into bicelles



Peptide pierces the entire bilayer, and **affects** all lipid groups

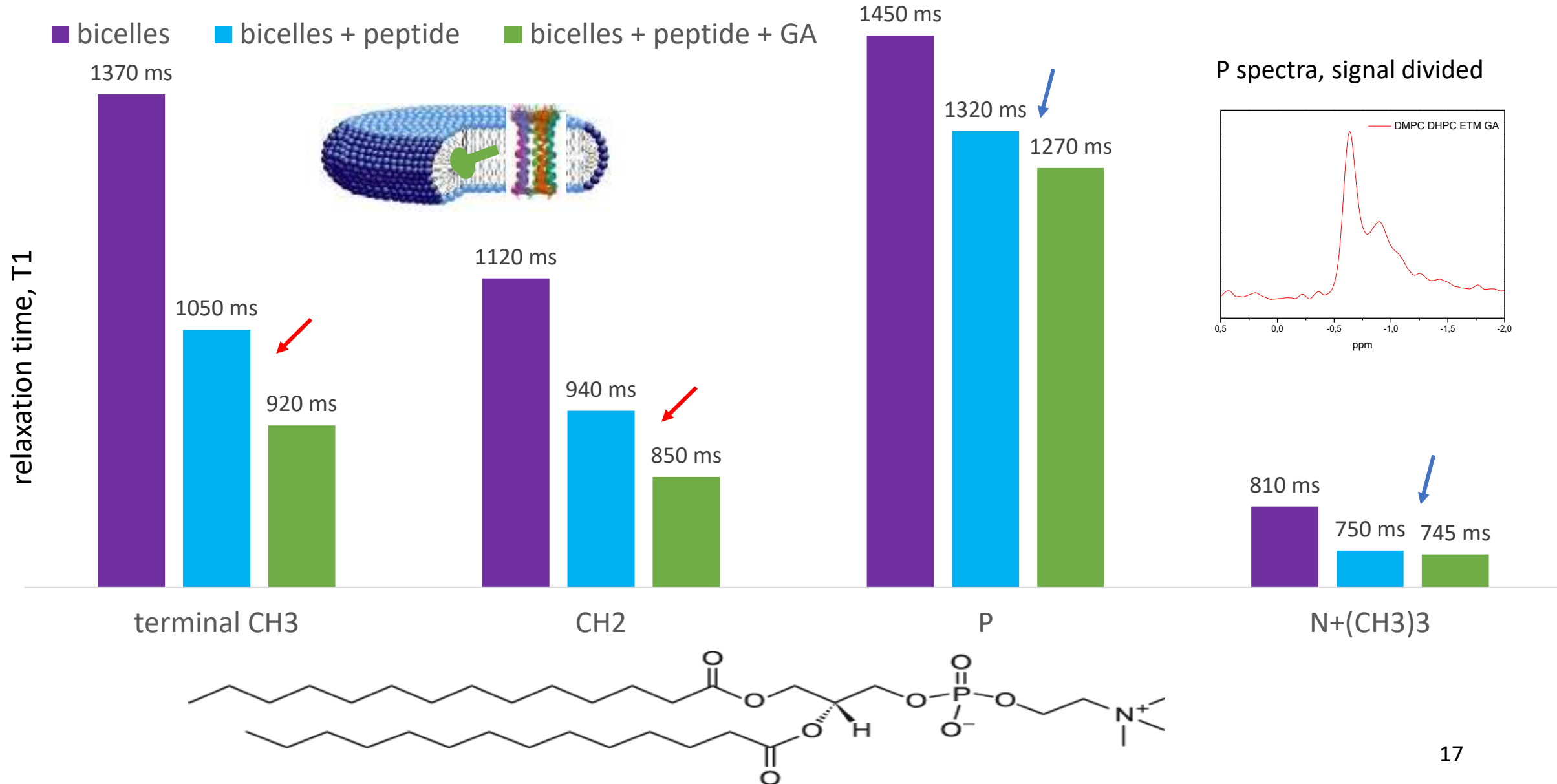


Conclusion

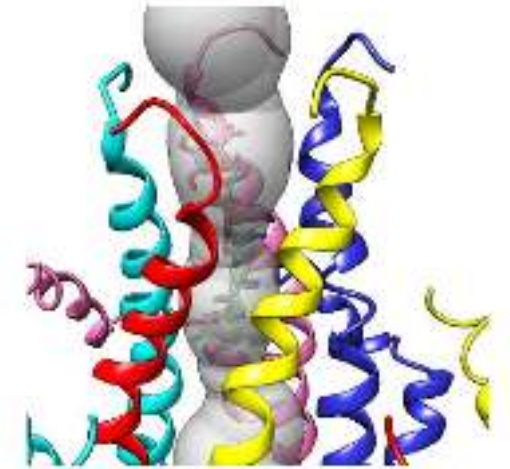
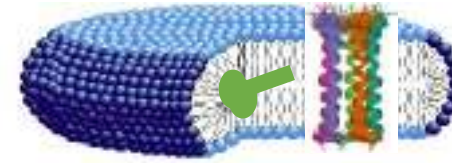


ETM is embedded in the bilayer

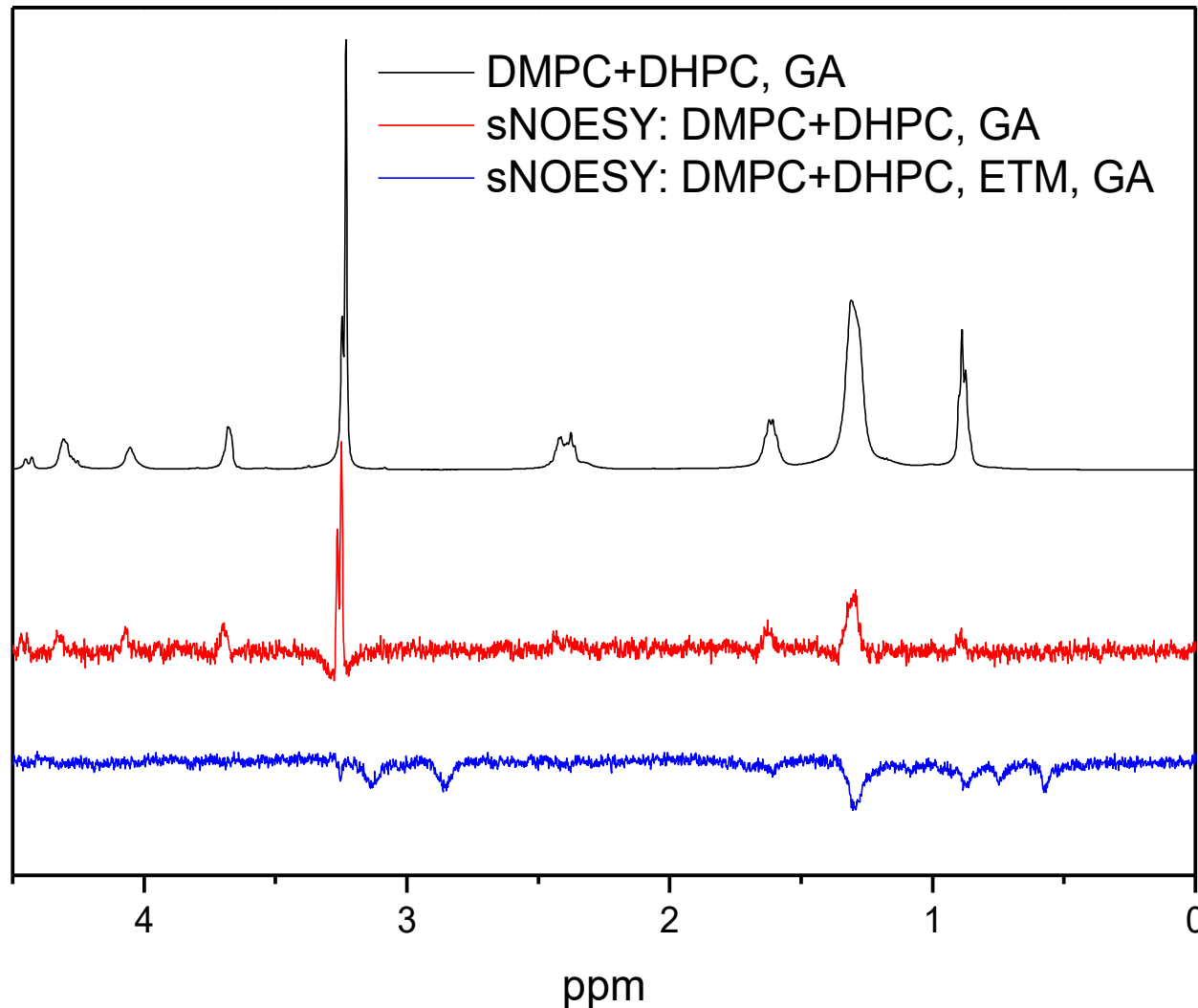
The addition of **glycyrrhizin** affects the relaxation time of “tails”, but does not affect the “head”



There is an interaction of the ETM and GA with the bicelle, but there is no influence on each other



Pharmaceutical Targeting the Envelope Protein of SARS-CoV-2: the Screening for Inhibitors in Approved Drugs Anatoly Chernyshev XR Pharmaceuticals Ltd., Cambridge, New Zealand



Peptide relaxation times significantly changed in the presence of GA

bicelles + peptide	585 ± 45 ms
bicelles + peptide + GA	230 ± 60 ms

ETGTLIVNSVLLFLAFVVFLLVTLAILTALR

Conclusion



GA affects ETM indirectly via lipids

Conclusions



GA is embedded in the bilayer



GA and ETM interact with each other in water



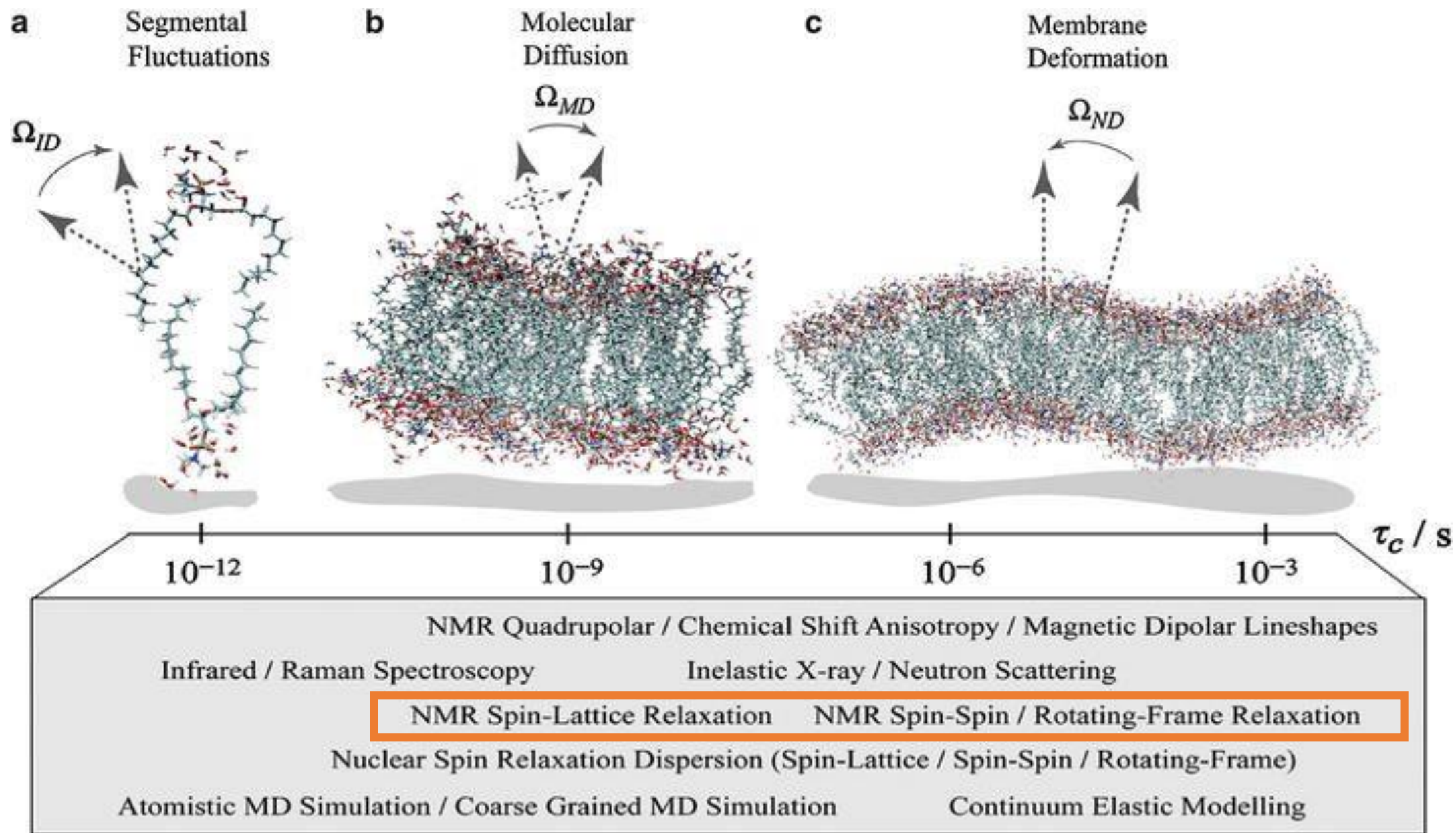
ETM is embedded in the bilayer



GA affects ETM indirectly via lipids

Thanks

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ETM is incorporated into **bicelles**

