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# Rheological properties of cysteine-modified ovalbumin at the air-water interface

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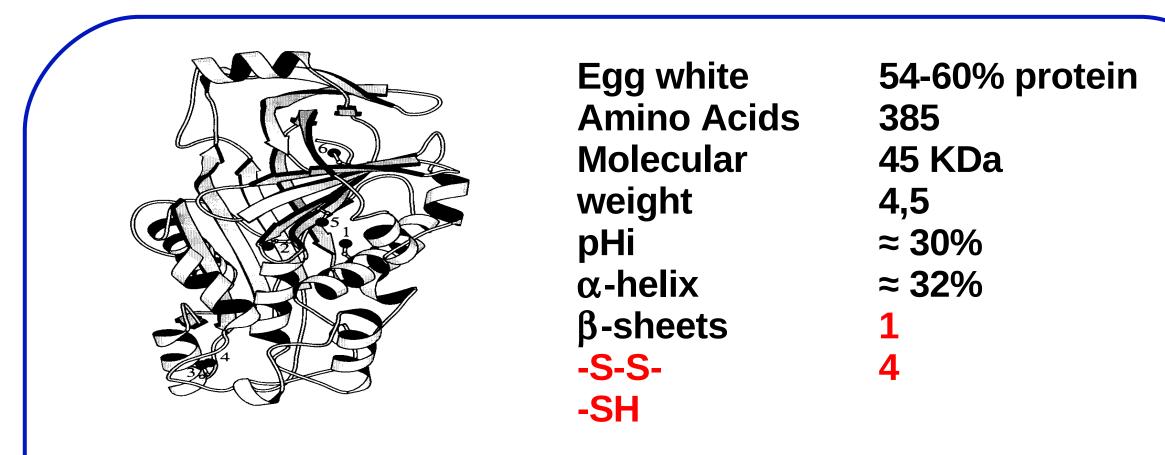
Air

### Introduction

Ovalbumin is a phosphorylated and glycosylated globular protein which contributes to a large part of the functionality of egg-white which it is widely used in the food industry as a foaming agent. However, the molecular bases of its interfacial behaviour are not fully understood.

### **Objective**

To investigate whether the sulfhydryldisulfide exchange influences the interfacial film rheology, we studied structural and interfacial properties of cysteine-modified ovalbumin.



#### F≈ F≈ water HS お S-S

### □ Three forms : A3: A2: A1 (Ser<sup>68</sup> et Ser<sup>344</sup>) 2: 13 : 85 (%) **OP: 1P : 2P (phosphate groups)** ++ glyco forms (Asn<sup>292</sup>) 2 N-acétylglucosamines et 4 mannoses

## **Experimental procedure**

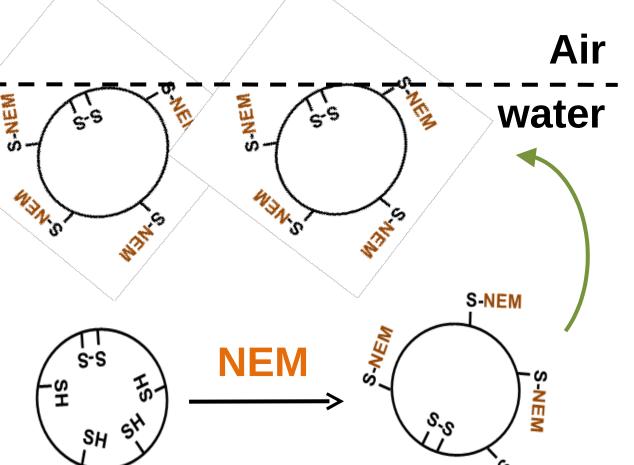
□ Native Ovalbumin (Native-OVA) was modified by the thiols blocking agent N-ethylmaleimide (NEM).

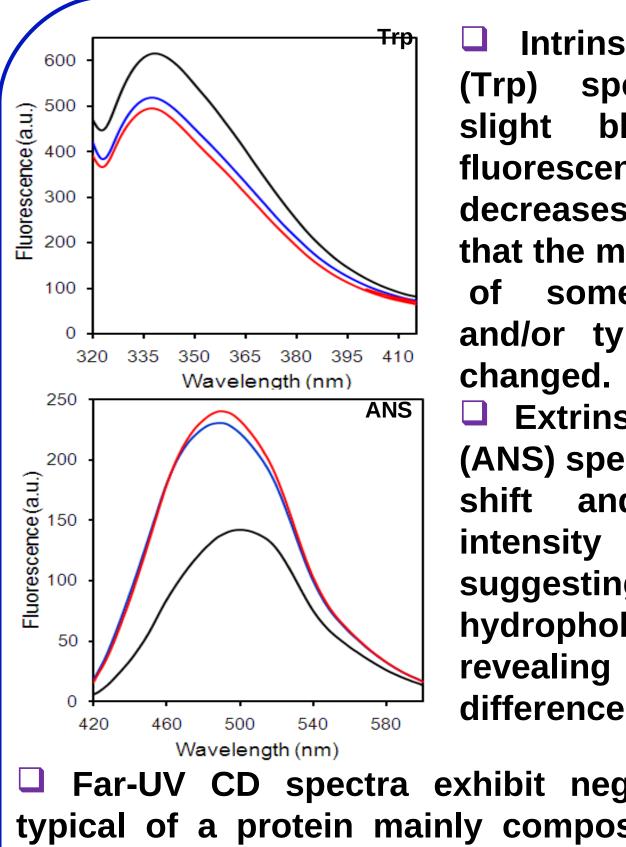
□ Native-OVA (0.225 mM) was incubated with a 40-fold molar excess of NEM in 100 mM sodium phosphate buffer (pH 7.2) at 20-21°C for 4 and 24 hours in the dark under stirring.

□ NEM in excess was removed by dialtrafiltration.

□ NEM/OVA ratio was estimated and the effect of the binding on the net charge and structure stability of the modified OVA forms was studied.

□ interfacial behavior of different OVA forms was studied at the airwater interface.

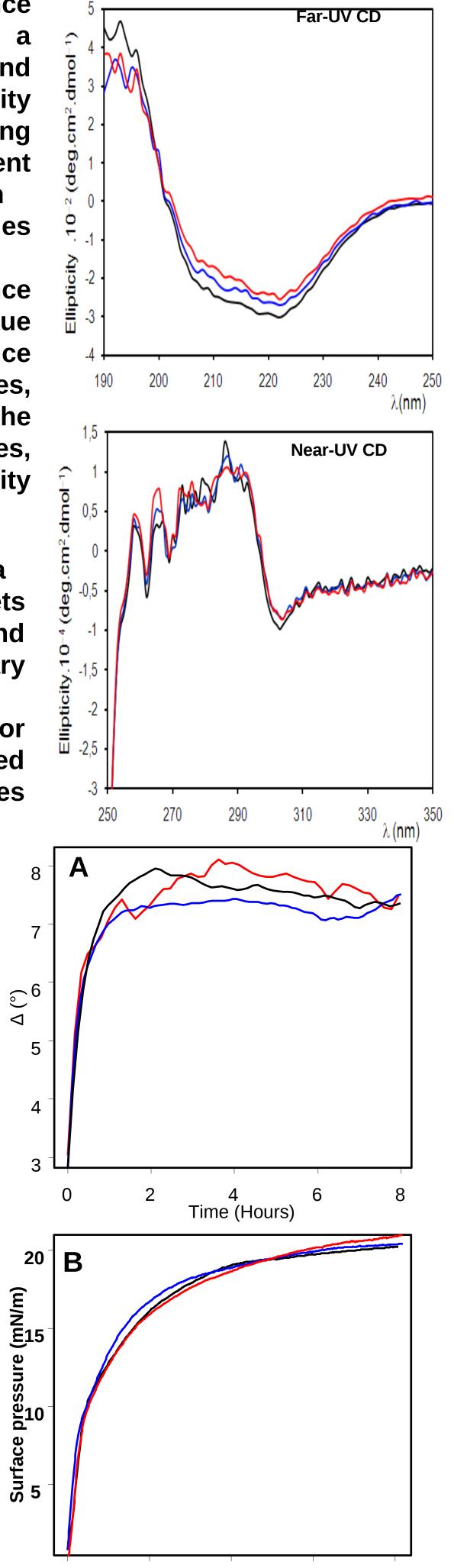




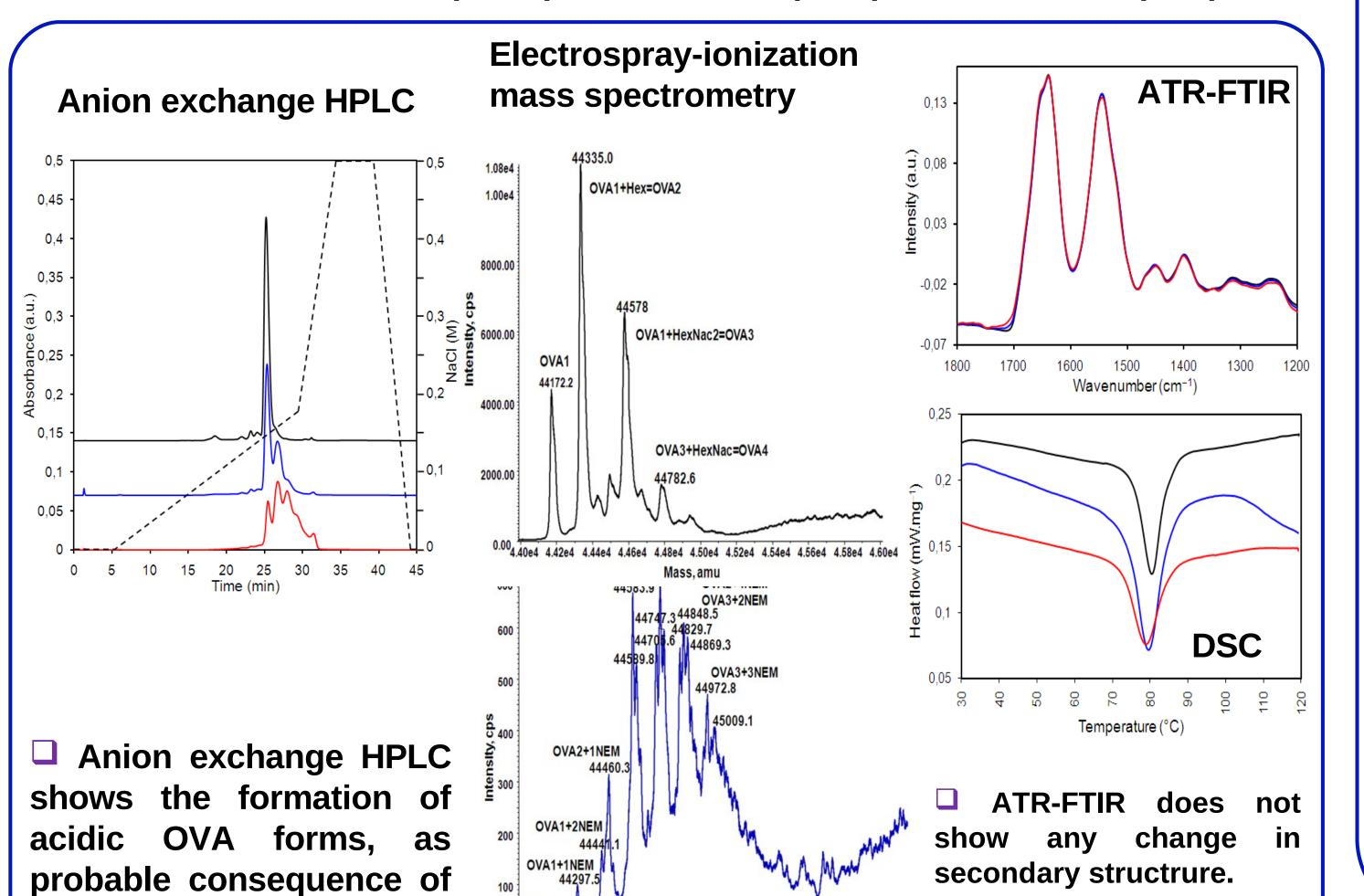
fluorescence Intrinsic spectra show a shift and slight blue fluorescence intensity decreases, suggesting that the microenvironment of some tryptophan and/or tyrosine residues

**Extrinsic** fluorescence (ANS) spectra show a blue shift and fluorescence increases, suggesting the hydrophobicity increases, flexibility differences.

Far-UV CD spectra exhibit negative maxima typical of a protein mainly composed of  $\beta$ -sheets and  $\alpha$ -helix with minor modifications at 193, 208 and 222 nm, characteristic of  $\alpha$ -helix secondary



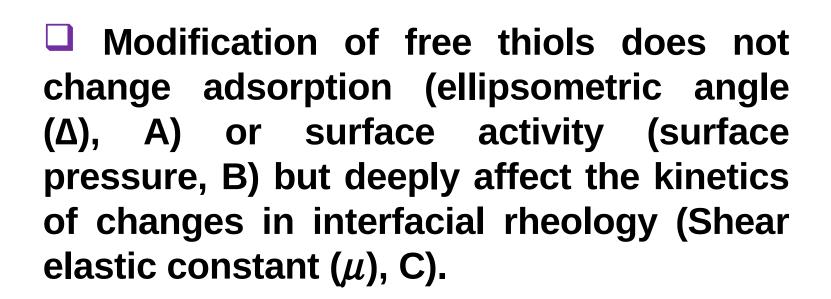
### Results

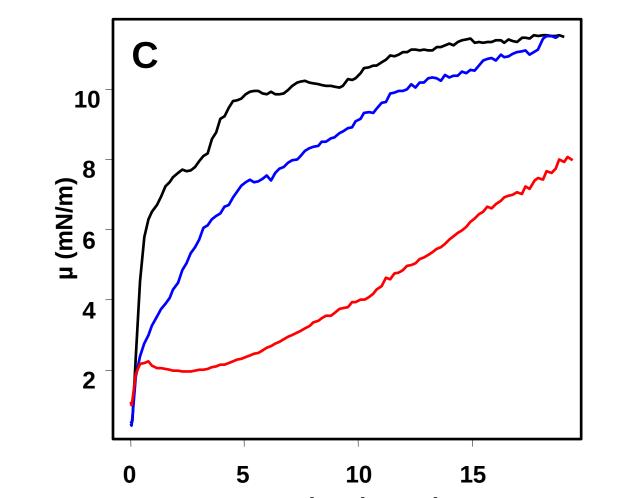


Color lines, native-OVA (black), OVA-NEM 4h (Blue), OVA-NEM 24h (Red)

structure

Near-UV CD spectra is characterized by minor modification at 262, 266, 286 and 303 nm attributed chiral environment of aromatic residues to confirming the minor changes in flexibility.





NEM hydrolysis after its fixation on thiols OVA groups.

**Electrospray-ionization** mass spectrometry (ESI-MS) shows the binding of up to four NEM molecules per ovalbumin.

much Differential Scanning 440e4 442e4 444e4 446e4 448e4 450e4 452e4 454e4 456e4 Calorimetry (DSC) shows OVA2+4NEM OVA3+2NEM 44872.2 44994.8 a maintained structural 45015.0 OVA3+4NEM OVA2+3NEM stability. 44726.4 denaturation temprerature  $(T_{D})$  of native-OVA and 44708.4 treated by NEM OVA during 4 and 24 hours OVA2+2NEM slightly (80.3°C, 79.4°C, 78.8°C, respectivelly). OVA1+2NEM 4.40e4 4.42e4 4.44e4 4.46e4 4.48e4 4.50e4 4.52e4 4.54e4 4.56e4 Mass, amu

Time (Hours)

Time (s)

### Conclusion

The

decreases

Kinetic changes in interfacial rheology can be attributed either to the blocking of thiol groups by the N-ethylmaleimide, or more probably to structural and net charge changes of ovalbumin. Further investigations using other pHs are needed to determine the impact of each factor on the surface rheology of ovalbumin films.





