Structure of protein-surfactant nanoconjugates in deep eutectic solvents

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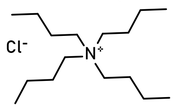
INTRODUCTION

Deep Eutectic Solvents (DES)

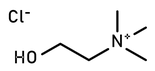
New class of ionic solvents which present a lower melting point than any of its individual components. The formation of a liquid compound at room temperature is due to the formation of hydrogen bonds between a hydrogen bond donor (HBD), and a hydrogen bond acceptor (HBA).

HBAs

Tetrabutylammonium Chloride (TC)

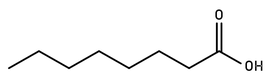


Choline Chloride (CC)

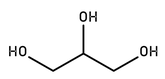


HBDs

Octanoic Acid (O)



Glycerol (G)



Kamlet-Taft Parameters

Used to measure the acidity (α), basicity (β) and polarity (Π*) of DES.

Measured using solvatochromic dyes: 4-nitroaniline, N,N-diethyl-4-nitroaniline and nile red.

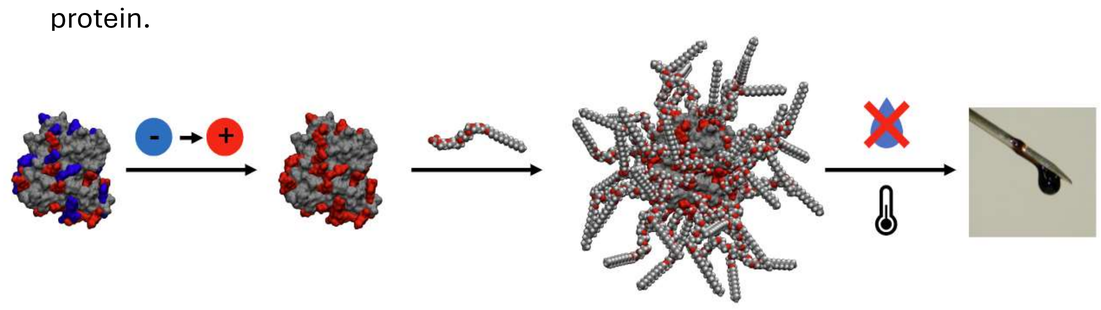
DES	α	β	Π*
CC:G	0.91	0.53	1.12
TC:G	0.86	0.65	1.12
TC:O	0.50	0.91	0.84

Why use DES?

- ✓ High thermal stability
- ✓ Tuneable polarity
- ✓ Ease of preparation
- ✓ Low volatility
- ✓ Low toxicity
- ✓ Easy availability from inexpensive components
- ✓ Nonflammability
- ✓ Low vapour pressure

Chemically modified Myoglobin

To ensure solubilisation in DES and at high temperatures. Steps involve cationisation of the protein surface, nanoconjugation via electrostatic complexation of anionic surfactants and lypophilisation into solvent-free liquid protein.

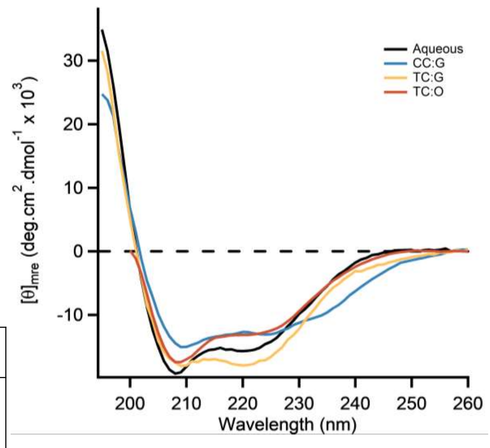


SECONDARY STRUCTURE

Far-UV Synchrotron Radiation Circular Dichroism (srCD)

Used to study the secondary structure of myoglobin in DES.

Results suggested that the effect of DES composition was not a straightforward correlation to either polarity or hydrogen bonding capabilities.



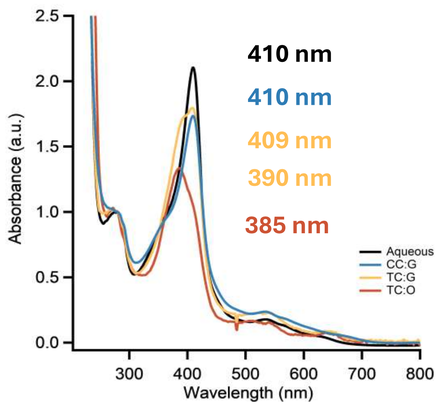
DES	α-helix	β-sheet	Turn	Other	NRMSD
CC:G	40.5	13.6	26.4	19.5	0.063
TC:G	47.2	8.6	12	32.2	0.02
TC:O	52.5	14.9	17.2	15.3	0.017
Water	49.9	3.3	11.9	34.9	0.0051

Deconvolution values from BestSel

TERTIARY STRUCTURE

UV-vis

The intensity and position of the **Soret band** gives information on the heme binding to the protein.

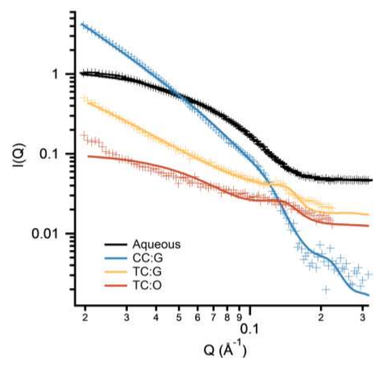


Bound heme at 409 nm and unbound heme at 385 nm

Small Angle Neutron Scattering (SANS)

Used to study the size distribution of the nanoconjugate in DES.

Results from **CC:G** and **TC:G** suggest a stabilisation mediated by hydrophobic and electrostatic interactions. **TC:O** indicated a near total loss of native tertiary structure but preserved secondary structure.



DES	Radius (Å)
CC:G	31.6
TC:G	24.7
TC:O	18.7
Water	24.1

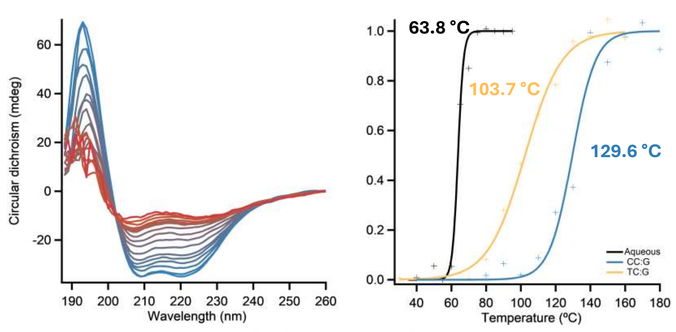
TEMPERATURE-DEPENDENT STUDIES

Thermal stability

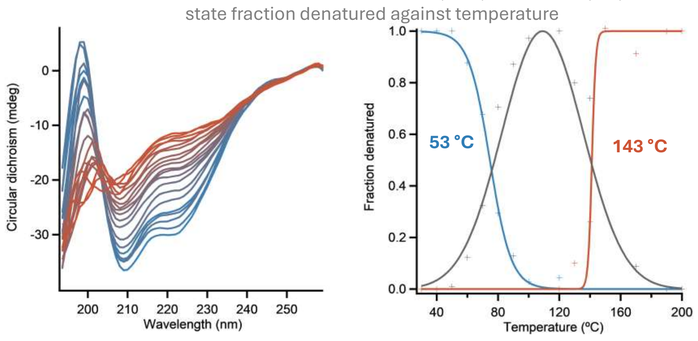
Temperature dependent-srCD showed that the protein denatures over a much-extended temperature range that in **aqueous** environments.

Overall, the stability of the protein in **TC:G** was lower in **CC:G**, confirming that TC destabilised the secondary structure of the protein.

In **TC:O**, an initial breakdown of supramolecular structure followed by a loss in secondary structure are observed.



Thermo-denaturation of **TC:G** between 25 °C (blue) and 200 °C (red) and 2-state fraction denatured against temperature

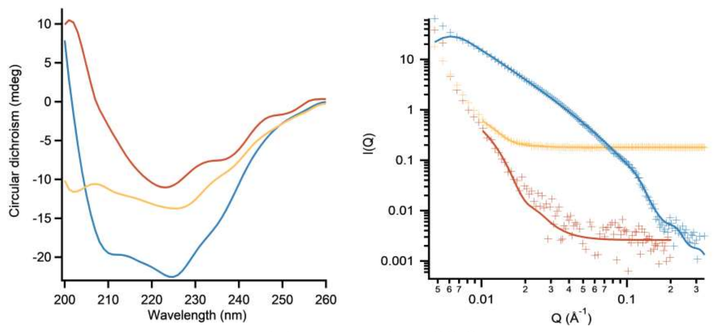


Thermo-denaturation of **TC:O** between 25 °C (blue) and 200 °C (red) and 3-state fraction denatured against temperature

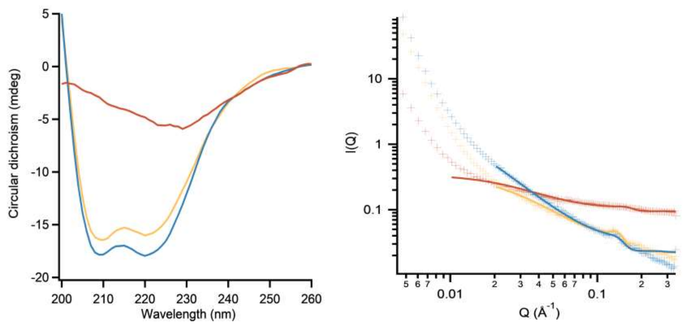
Protein refolding

The destabilisation of the folded state of the protein was attributed to the difference in acidity and basicity of **CC:G** and **TC:G**.

Significant refolding suggests destabilisation of the denatured state, which provides a thermodynamic basis for refolding as seen in **TC:O**.



Refolding data from **CC:G**



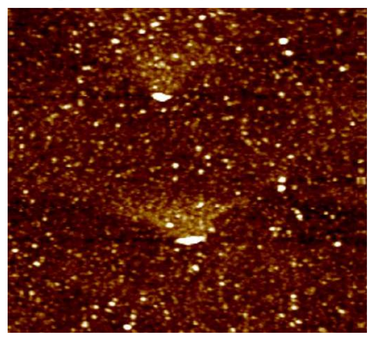
Refolding data from **TC:G**

DES	Radius (Å) at 25 °C	Radius (Å) at 125 °C	Radius (Å) at 25 °C return
CC:G	31.6	211.0	235.5
TC:G	24.7	19.4	24.9
TC:O	18.7	17.6	18.3
Water	24.1	-	-

FUTURE WORK

Atomic Force Microscopes (AFM)

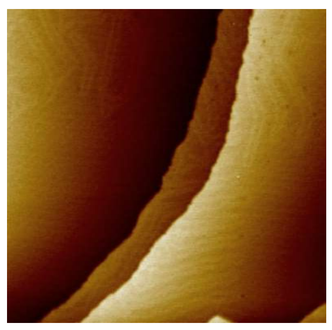
Size measurements of the immobilised protein on an Au (111) single crystal to support SANS data.



AFM image (200 nm) of native Myoglobin on gold, deconvoluted particle diameter of 3.15 nm.

Scanning Tunnelling Microscopes (STM)

STM can be used to compare how the different DES affect the electron transport through the protein.



EC-STM image (125 nm) of **CC:G** on Au(111) electrode with Pt tip and Pt wire held at a -1.2 V potential.

CONCLUSIONS

- ❖ The secondary and tertiary structures of the myoglobin nanoconstruct in different DES were studied using srCD, UV-vis and SANS.
- ❖ Overall, it was found that the stability of the protein increased with respect to aqueous conditions.
- ❖ The components of DES have different effects on the protein, and it is not always a straightforward correlation.

REFERENCES

1. M. Moniruzzaman, K. Nakashima, N. Kamiya, M. Goto; *Biochemical Engineering Journal*, **2010**, 48 (3)
2. C. Florindo, A. J. S. McIntosh, T. Welton, L. C. Branco, I. M. Marrucho, *Physical Chemistry Chemical Physics*, **2018**, 1
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