

1 **SUPPORTING INFORMATION**

2 **Study of oxygen transfer across milk proteins at an**
3 **air-water interface with scanning electrochemical**
4 **microscopy**

5 *Outi Toikkanen^{†,*}, Maija Lähteenmäki[†], Timo Moisio[†], Pirkko Forssell[†], Riitta Partanen[†], Lasse*
6 *Murtomäki[‡]*

7 [†]VTT Technical Research Centre of Finland, P.O. Box 1000, FI-02044 VTT, Finland,

8 [‡]Aalto University, Department of Chemistry, P.O. Box 16100, FI-00076 Aalto, Finland.

9 **FLUORESCENCE EXPERIMENTS**

10 **Materials and methods**

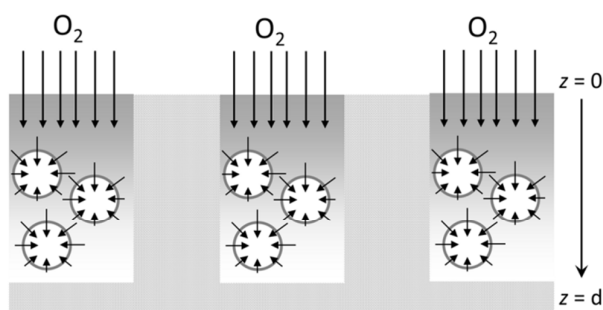
11 The emulsions were prepared using rapeseed oil (Raisio, Finland) and various emulsifiers: sodium
12 caseinate (Kaslink Foods, Finland), cross-linked sodium caseinate, whey (BiPro, Davisco Foods Int,
13 USA), pectin (Danisco, Denmark), gum arabic (Sigma, Steinheim, Germany), SDS (Sigma-Aldrich,
14 Steinheim, Germany), Tween80 (Fluka Chemie, Germany), modified starch (HiCap 100, National
15 Starch & Chemical, USA) and hydrophobin (HFB II, VTT, Finland). The fluorescent probe tris(4,7-
16 diphenyl-1,10-phennathroline) ruthenium(II) bis(hexafluorophosphate) complex was purchased
17 from Santa Cruz Biotechnology (USA) and was dissolved in chloroform (Rathburn Chemicals Ltd.,
18 Germany).

19 The fluorescence experiments were performed following the protocol from Tikekar et al.^{S1} using a
20 multilabel reader (Victor², Wallac, Finland). Excitation wavelength was 485 nm and emission
21 wavelength 615 nm. The emulsions were purged with wet nitrogen for 1 hour. 200 µl was pipetted

22 onto the well in a black microplate with transparent bottom (Corning 3603, USA) and the
 23 fluorescence quenching was followed by measuring from the bottom for 15 mins.

24 **Results and discussion**

25 Emulsion solution from which oxygen was removed with nitrogen purging was injected in the well-
 26 plates and thus exposed to atmospheric oxygen. An outline of the well-plate used for fluorescence
 27 experiments is depicted in Figure S1.



28

29 Figure S1. Scheme of a diffusion experiment in a well-plate; well depth = d .

30 When oxygen diffuses into the solution, it transfers consequently into the oil droplets, causing
 31 fluorescence quenching. Mathematically, the process inside the droplet of the radius a is
 32 described as follows:

33
$$\begin{cases} \frac{dc}{dt} = -k_{ox} c_o c + K_p (c_w - c) \frac{A}{V} \\ \frac{dc_o}{dt} = -k_{ox} c_o c \end{cases} \quad (S1)$$

34 In Eq. (S1), c is the oxygen concentration in the oil droplet, c_o is the concentration of unoxidized
 35 oil, c_w the oxygen concentration in the aqueous phase (outside the droplet), k_{ox} the oxidation
 36 reaction rate constant, K_p the permeability coefficient of oxygen in the protecting layer (D/h); A is
 37 the surface area and V the volume of the droplet ($A/V = 3/a$). In our experiments, the time scale of

38 the oxidation reaction is several orders of magnitude larger than that of diffusion, and the reaction
 39 can thus be neglected in the model. Since the fluorescence measurement detects the sum of
 40 intensities along the optical path, the local aqueous oxygen concentration can be replaced with
 41 the average value in the well.

42 The oxygen concentration at the water-air interface is given by Henry's law: $c^0 = p(\text{O}_2)/K_H$ (0.273
 43 mM at $T= 298$ K and $p = 1$ atm) where K_H is the Henry constant. The concentration profile of
 44 oxygen is given as:^{S2}

$$45 \quad c_w(z,t) = c^0 \operatorname{erfc}\left(\frac{z}{2\sqrt{D_w t}}\right) \quad (\text{S2})$$

46 where z is the distance from the air-water interface as depicted in Figure S1 and D_w is the diffusion
 47 coefficient of oxygen in water. The average value in the well is thus:

$$48 \quad \bar{c}_w = \frac{1}{d} \int_0^d c_w(z,t) dz = \frac{1}{d} \int_0^\infty c_w(z,t) dz = c^0 \frac{2\sqrt{D_w t}}{d\sqrt{\pi}} \quad (\text{S3})$$

49 The replacement of the upper limit of the integral is allowed because within the time scale of the
 50 experiment oxygen does not reach the bottom of the well. Hence, the differential equation for
 51 oxygen in the dimensionless form is:

$$52 \quad \frac{dC}{dt} = \Lambda X \sqrt{t} - \Lambda C \quad ; \quad \Lambda = \frac{3}{a} K_p \quad ; \quad X = \frac{2}{d} \sqrt{\frac{D_w}{\pi}} \quad (\text{S4})$$

53 where $C = c/c^0$. Matlab was used to integrate Eq. (S4) numerically and simultaneously fit the
 54 parameters Λ and X , putting C into Eq. (2) in the manuscript in the place of $[\text{O}_2]/[\text{O}_2]_\infty$. The
 55 fluorescence data did not fit to the model, and forcing the first 5 minutes of data into the fit gave
 56 extremely low values of K_p , or as converted into the diffusion coefficient, values of the order of

57 10^{-14} cm²/s. But if the data was fitted into a biexponential equation $F = ae^{-\lambda_1 t} + be^{-\lambda_2 t}$, the fit was
58 almost perfect ($R^2 = 0.9971$). In chemical kinetics this kind of behaviour implies for two consecutive
59 processes of separable time constants λ_1 and λ_2 . Therefore, it was decided to simplify the problem
60 by using a more straightforward, well-defined set-up.

61 **References**

- 62 S1. Tikekar, R. V.; Johnson, A.; Nitin, N. Real-time measurement of oxygen transport across an oil–
63 water emulsion interface. *J. Food Eng.* **2011**, *103*, 14-20.
- 64 S2. Crank, J. *The Mathematics of Diffusion*, 1st Ed.; Oxford University Press: Oxford, Great Britain,
65 1956; p.30.