Electronic Supplementary Information

Controlling Enzymatic Activity and Kinetics in

Swollen Mesophases by Physical

Nano-Confinement

Wenjie Sun, Jijo J. Vallooran, Alexandru Zabara, Raffaele Mezzenga*

ETH Zurich, Food and Soft Materials Science, Institute of Food, Nutrition & Health,

Department of Health Science and Technology, Schmelzbergstrasse 9,

CH-8092 Zurich, Switzerland



Figure S1. *Visual appearance of the standard mesophase before a) and after b) the HRP enzymatic reaction.*



Figure S2. SAXS spectra of four Pn3m phases with increasing amount of SE: 0%, 10%, 15% and 20% (from bottom to up), just below maximum hydration. The Pn3m phase symmetry was identified via the specific spacing of the reflection peaks following the ratio $\sqrt{2}:\sqrt{3}:\sqrt{4}:\sqrt{6}:\sqrt{8}:\sqrt{9}$.



Figure S3. Absorbance as a function of ABTS concentration at a fixed wavelength of 740 nm and 37 °C.-This curve was taken as the calibration curve for determining the partition coefficient of ABTS in lipids. The absorbance of the ABTS containing dark green water phase was measured before and after mixing with lipid phase (linoleic acid was used as a representative lipid) and 48h incubation. By calculation, we found out that only 4% ABTS participated into lipids and its log P value equal -1.638. This indicates that in mesophases only a negligible amount of ABTS can enter the lipid bilayers.



Figure S4. Progress curve of the HRP catalytic reaction in pure water with enlarged time scale, under the same condition as in Figure 2a: [E] = 0.003 mg/mL, ABTS = 2 mM, $H_2O_2 = 20 \text{ mM}$.



Figure S5. Progress curves of the HRP enzymatic kinetics study conducted at various ABTS concentrations for: a) standard Pn3m mesophase, at a fixed [HRP] = 0.009 mg/mL in water; b) swollen mesophase, at a fixed [HRP] = 0.006 mg/mL in water.



Figure S6. Investigations of the influence of H_2O_2 on the hosting mesophases by SAXS: a) standard Pn3m (monolinolein alone with 33% water); b) swollen Pn3m (monolinolein:SE 80:20 with 52% water). Addition of 20 mM and 100 mM H_2O_2 did not change the lattice, indicating that up to 100 mM, H_2O_2 can safely be used for the in-meso enzymatic activity study.



Figure S7. Investigations of the influence of different concentrations of HRP on the hosting mesophases by SAXS: a) Standard Pn3m (monolinolein-33% water); b) Swollen Pn3m (monolinolein:SE 80:20 with 52% water). These results indicate the loaded enzymes have no influence on the lattices of both mesophases.



Figure S8. Initial velocity plotted as a function of H_2O_2 concentrations in 3 different environments: swollen Pn3m (red circle, a), standard Pn3m (blue triangle, a) and pure water solution (b). The HRP concentration is 0.006 mg/mL, 0.009 mg/mL, 0.0003 mg/mL, respectively.