Supporting Information

A novel high-throughput assay enables the direct identification of acyltransferases

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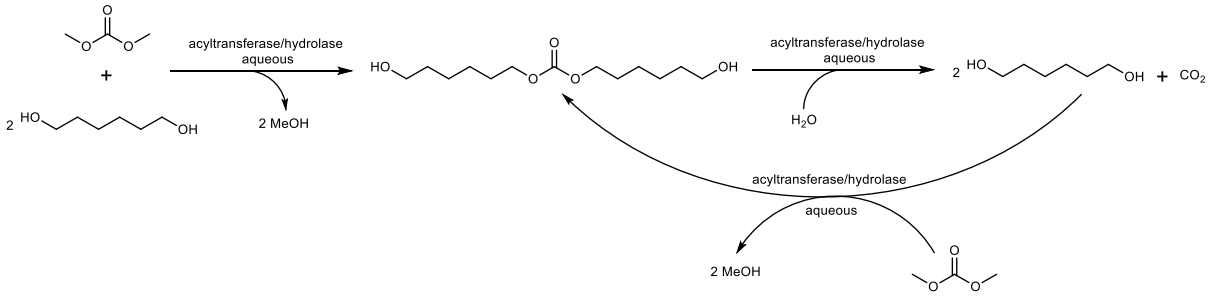

Scheme S1. Acyltransferase/hydrolase activity within a carbonate system. The highly water-soluble monomers dimethyl carbonate and 1,6-hexanediol are transesterified by an acyltransferase in aqueous medium to form oligocarbonates. When undesired hydrolysis of the carbonates formed takes place, CO_2 is released, and both alcohols are formed. With an additional DMC molecule, the alcohols can be connected again by a subsequent enzymatic transesterification step.

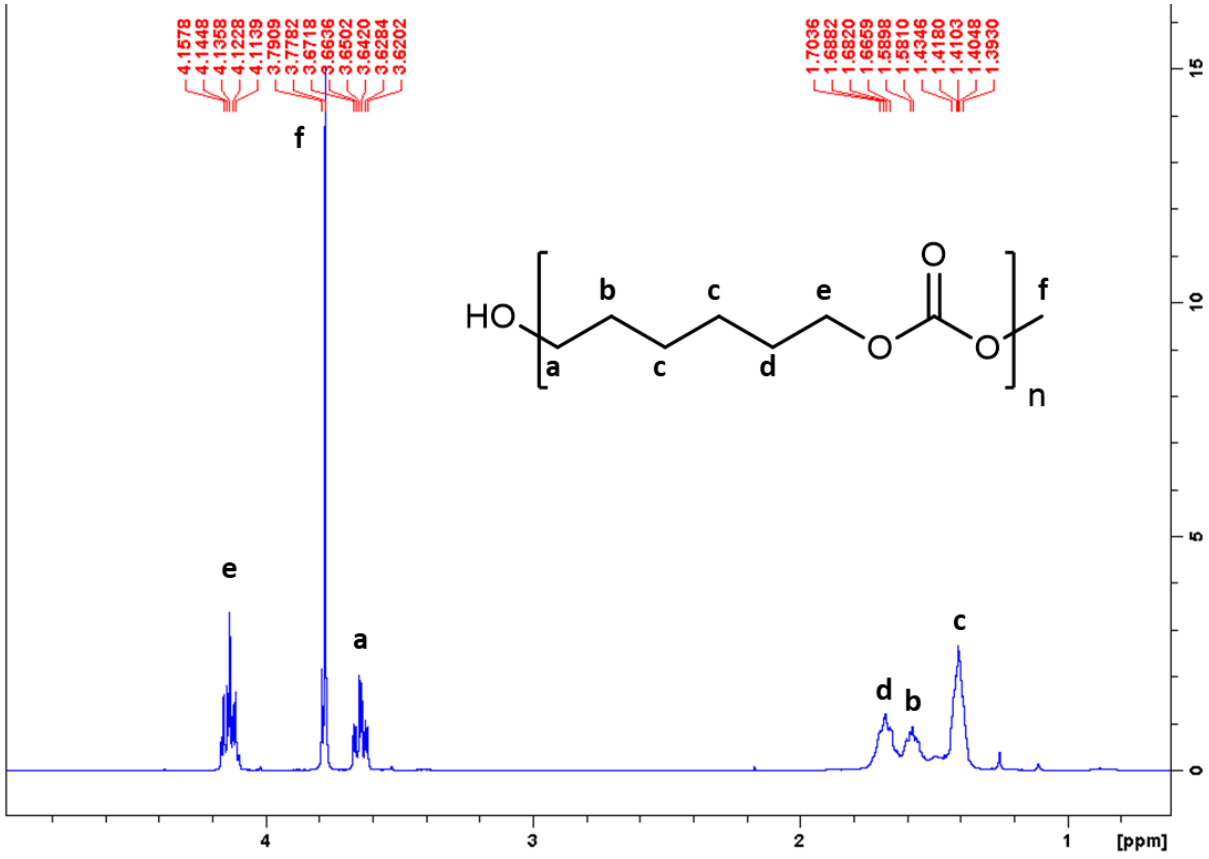

Figure S1. ^{1}H-NMR of the isolated oligocarbonate. The NMR was recorded in $CDCl_3$ and the shifts are in agreement with similar compounds reported in the literature. The methylene group, that is directly attached to the carbonate moiety (e) shows additional signals. This is likely caused by the different substitutions on the other side of the carbonate (methyl or hexyl) which are present in the product mixture and influence the chemical shifts.

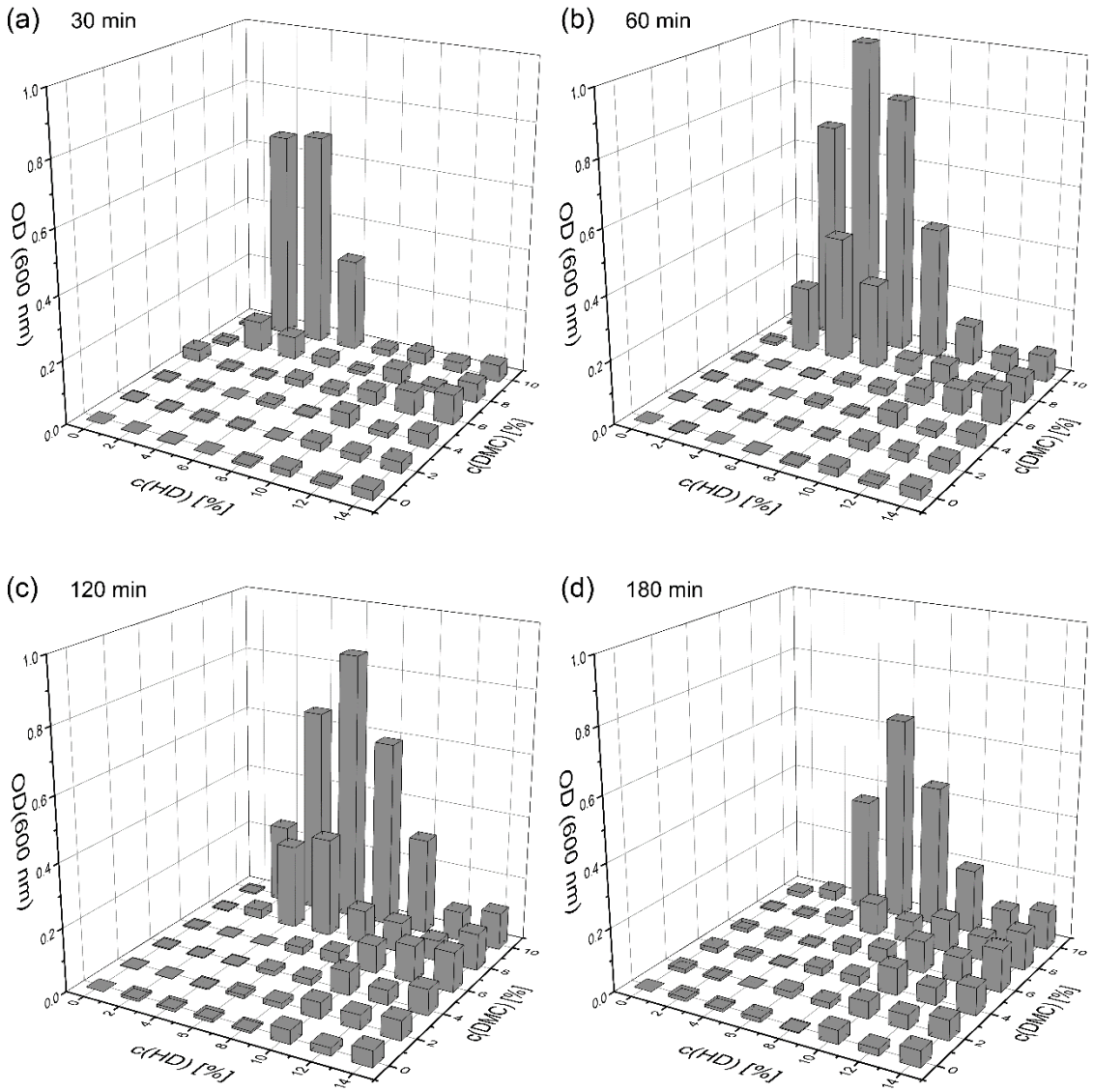

Figure S2. Optimization of the substrate concentrations. The absorbance at 600 nm was monitored over time to detect the formation of insoluble oligocarbonates. DMC was varied from 0 – 10% (v/v) while HD was varied from 0 – 14% (w/v) in 50 mM NaPi, pH 7.4 with 1 g L^{-1} Est8. Measurements were performed in duplicate and the average is shown after 30 min (**a**), 60 min (**b**), 120 min (**c**) and 180 min (**d**).

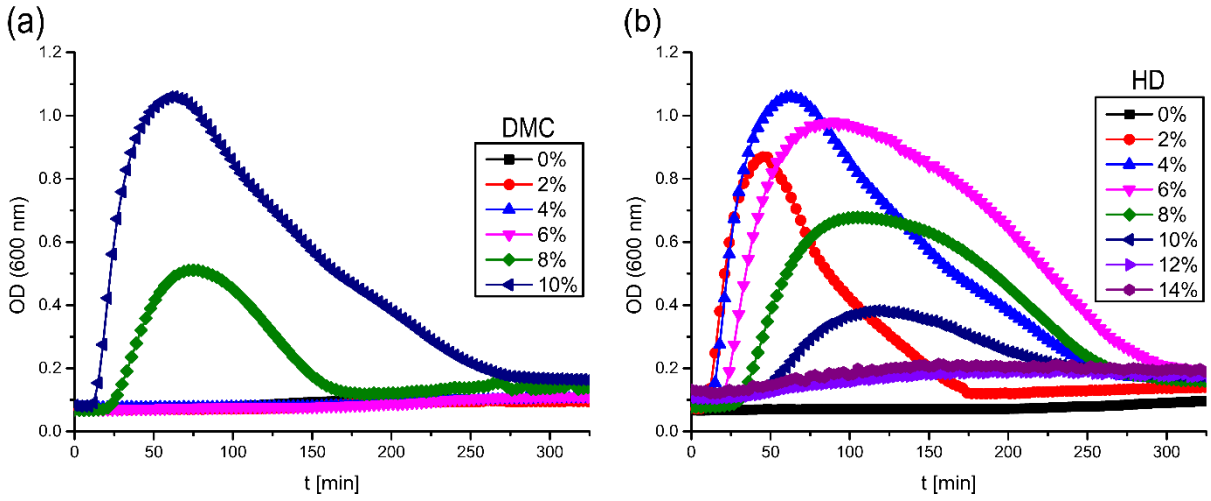

Figure S3. Time course of the formation of oligocarbonate at optimal substrate concentrations. The absorbance at 600 nm was measured over time to detect the formation of insoluble oligocarbonate. DMC was varied from 0 – 10% (v/v) while HD was varied from 0 – 14% (w/v) in 50 mM NaPi, pH 7.4 with 1 g L^{-1} Est8. The time courses of the variation of the DMC concentration at the optimal HD concentration (4% (w/v)) is shown in (**a**) while the variation of the HD concentration at optimal DMC concentration (10% (v/v)) is shown in (**b**). Measurements were performed in duplicate.

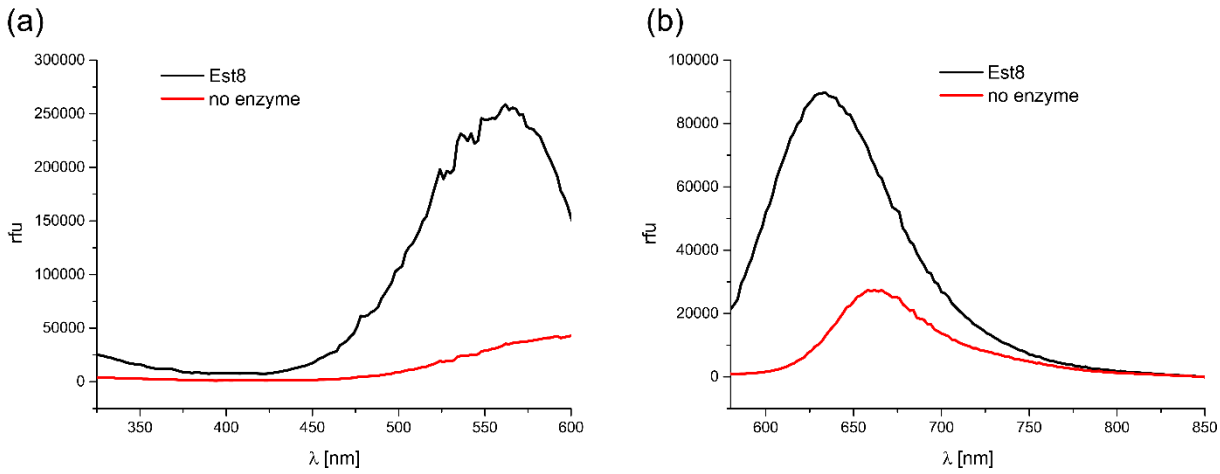

Figure S4. Determination of optimal excitation and emission wavelengths for Nile Red bound to oligocarbonate particles. Oligocarbonate particles formed with Est8 were diluted 1:2 in reaction buffer and stained with 2 µg mL^{-1} Nile Red. The reaction mixture without enzyme was used as a control. The optimal excitation wavelength (560 nm) was determined by a scan with fluorescence measurement at 630 nm (**a**) while the optimal emission wavelength (630 nm) was determined with excitation at 550 nm (**b**). Optimal wavelengths were chosen to distinguish free and oligocarbonate-bound Nile Red.