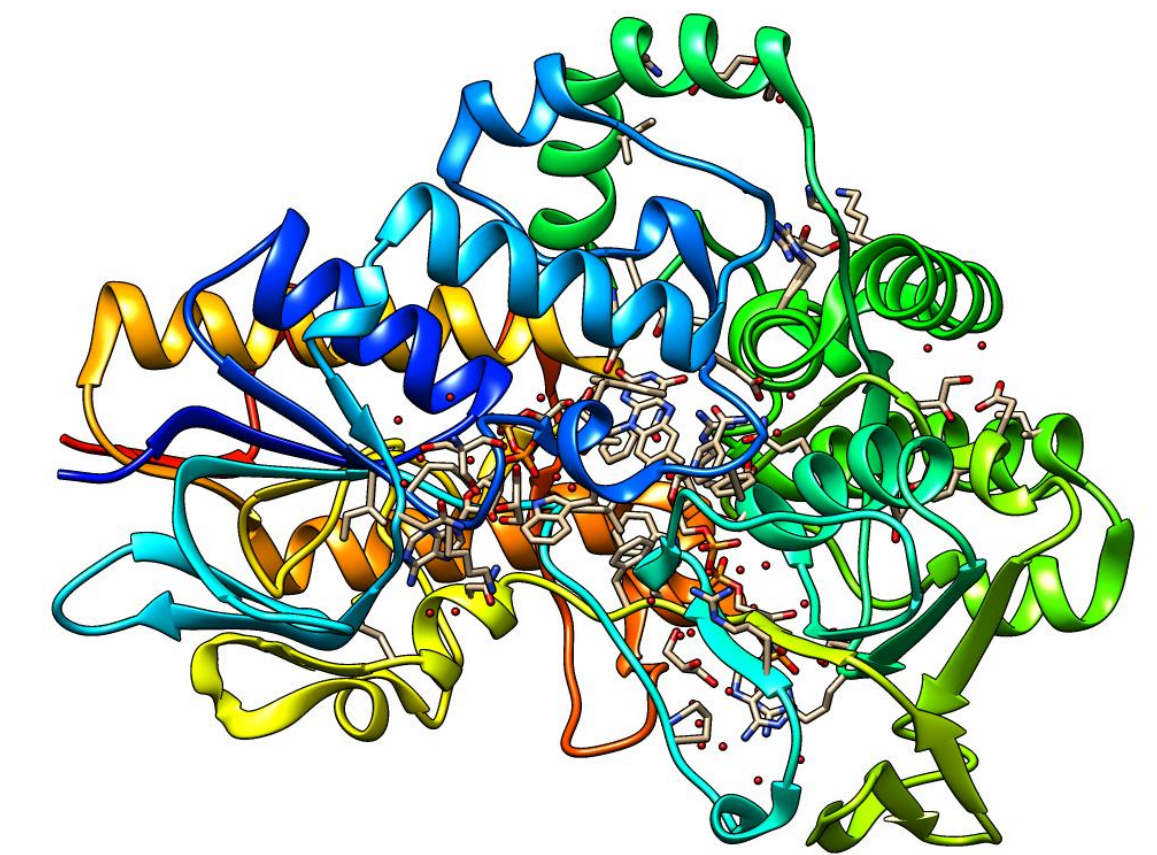


What can oxidative biocatalysis do for polymeric materials?

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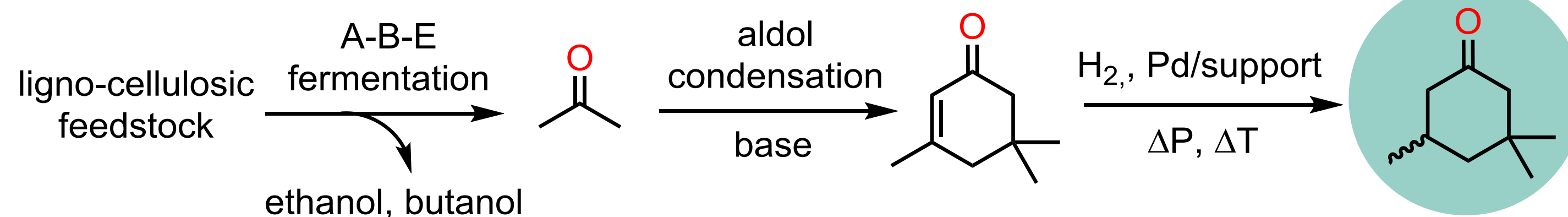
Introduction

- Baeyer-Villiger monooxygenases** are enzymes which catalyze the oxidation of (cyclic) ketones to lactones using O_2 as oxidant. They have for example been applied to the synthesis of chiral compounds for the pharmaceutical industry.
- Poor thermostability and solvent resistance of BVMOs hinders their application at industrial scale.
- In this work: **thermostable cyclohexanone monooxygenase from *Thermocrispum municipale*** (TmCHMO) as biocatalyst for monomer synthesis for polymeric materials.
- TmCHMO: broad substrate scope towards branched cyclic ketones^{1,2} and good retention of activity in the presence of several organic solvents when applied to the **oxidation of 3,3,5-trimethylcyclohexanone**.^{1,3}
- Goal:** optimize the TmCHMO-catalyzed oxidation of 3,3,5-trimethylcyclohexanone for the synthesis of the branched lactones β,δ,δ -trimethyl- ϵ -caprolactone and β,β,δ -trimethyl- ϵ -caprolactone (TMCL) as **monomers for polyesters** by ring opening polymerization.
 - Targets: **high product concentration** and **high space-time yield** by investigation of **substrate supply strategies**.
 - These lactones are then polymerized either as homopolymer or as copolymer.



TmCHMO: *Thermocrispum municipale* cyclohexanone monooxygenase²
 $T_m = 48^\circ C^2$
Very good resistance to organic solvents^{1,2,3}

(Biobased) branched cyclic ketone as substrate



Applied biocatalysis: strategies for substrate supply

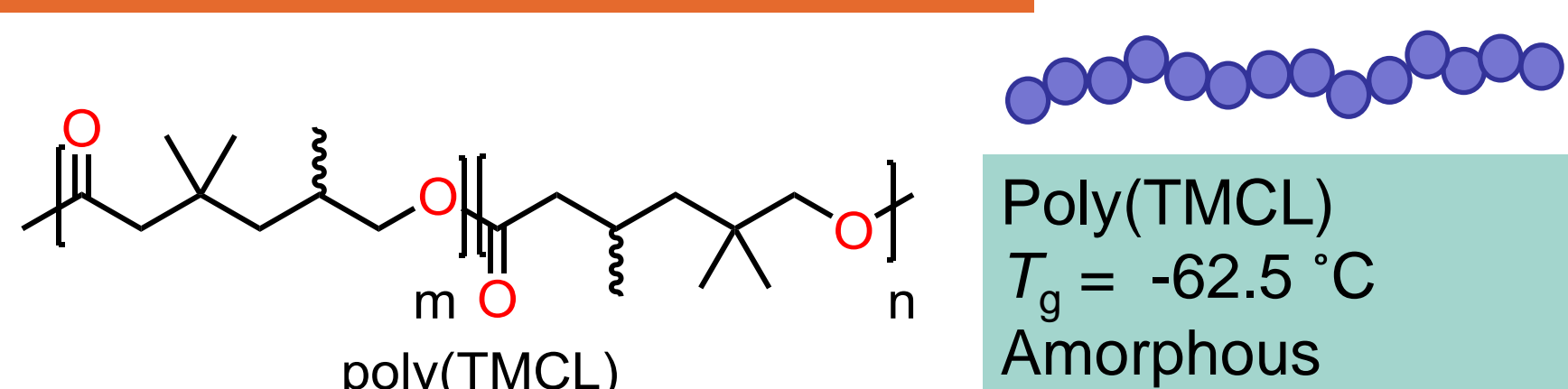
Requirements for this reaction: NADP⁺ co-factor regenerated with a glucose dehydrogenase (GDH), pH control (auto-dosing with base), temperature and oxygen flow control (oxidant)

Substrate supply strategies to overcome substrate inhibition & increase product concentration

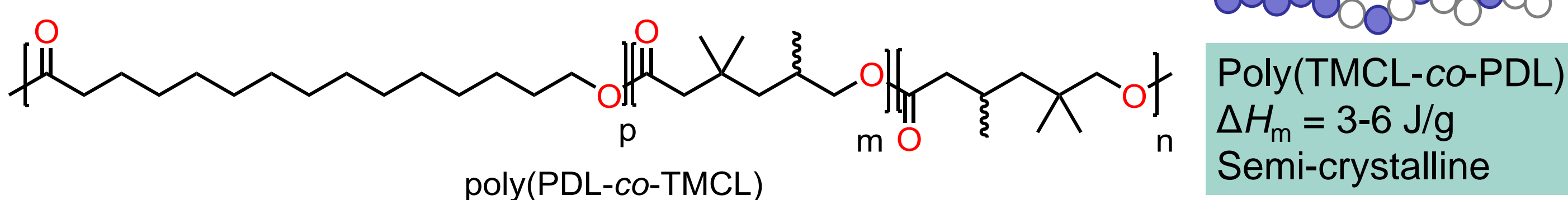
- Continuous substrate feeding** (concentration of substrate and product below inhibitory level): feed rate & total substrate fed
- Biphasic system** with water-immiscible organic solvents (*n*-butyl acetate or toluene): substrate and product mainly in organic phase

Branched polyesters from TMCL⁴

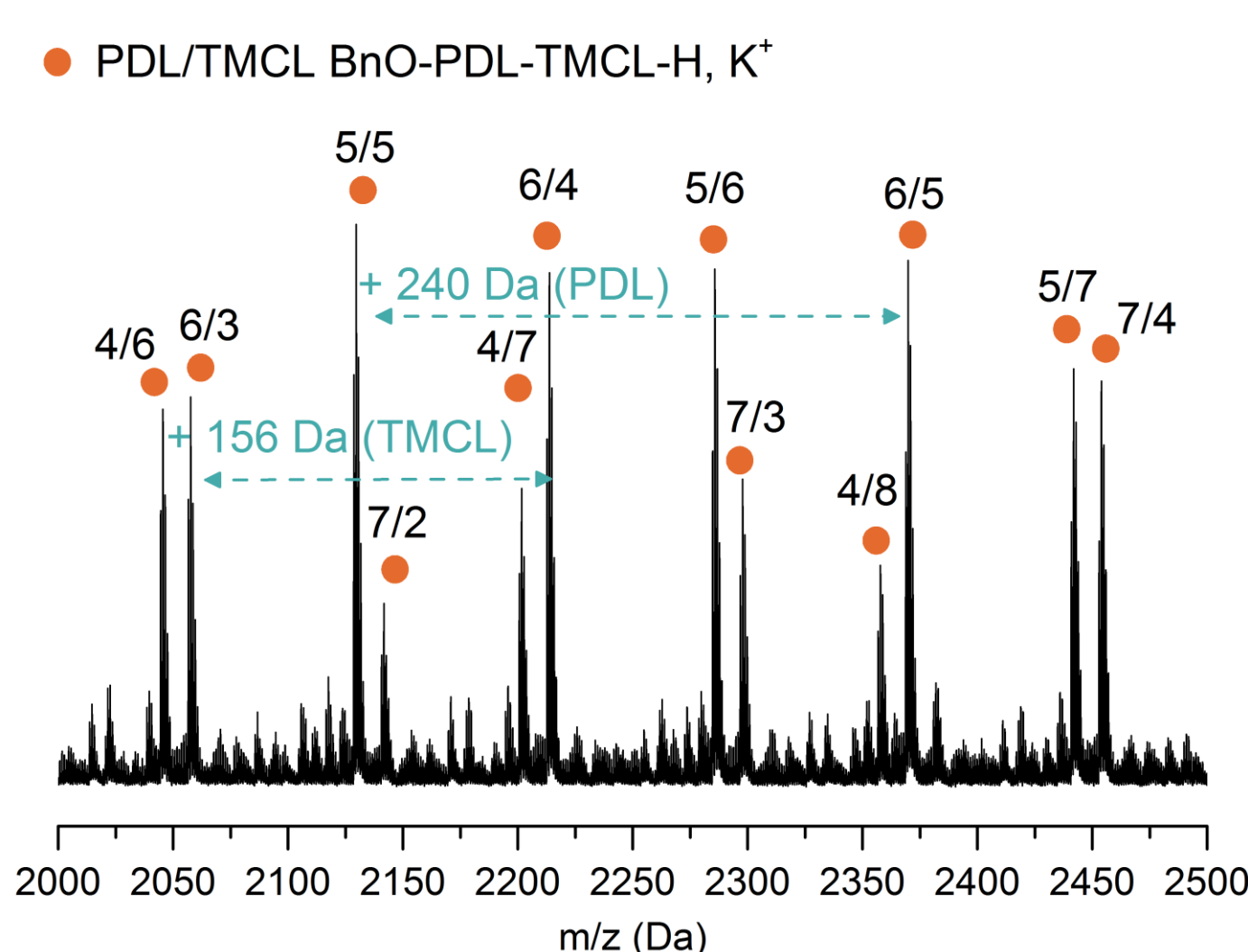
Homopolymer



Copolymer with ω -pentadecalactone (PDL)

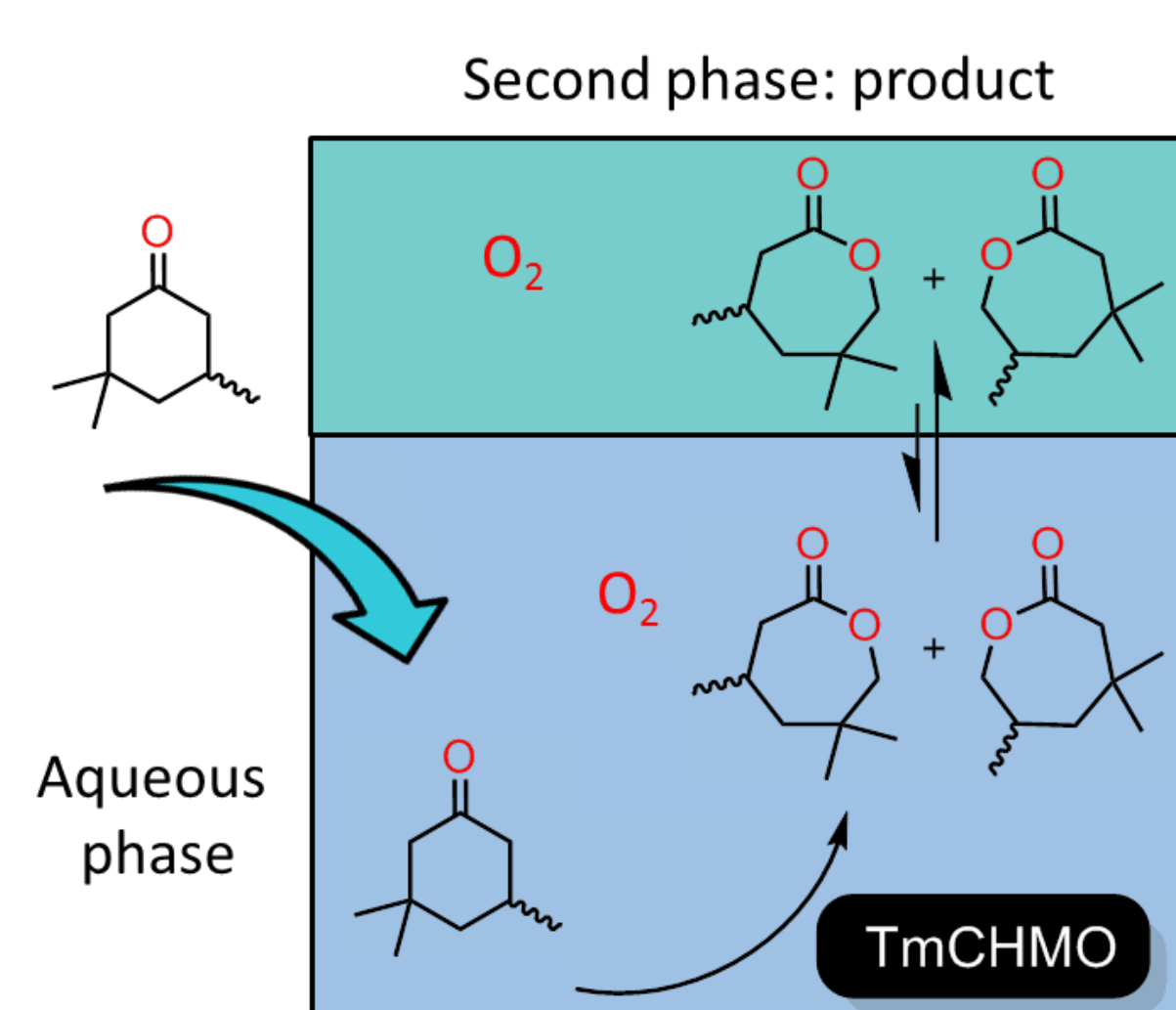


MALDI-ToF-MS of poly(TMCL-co-PDL)

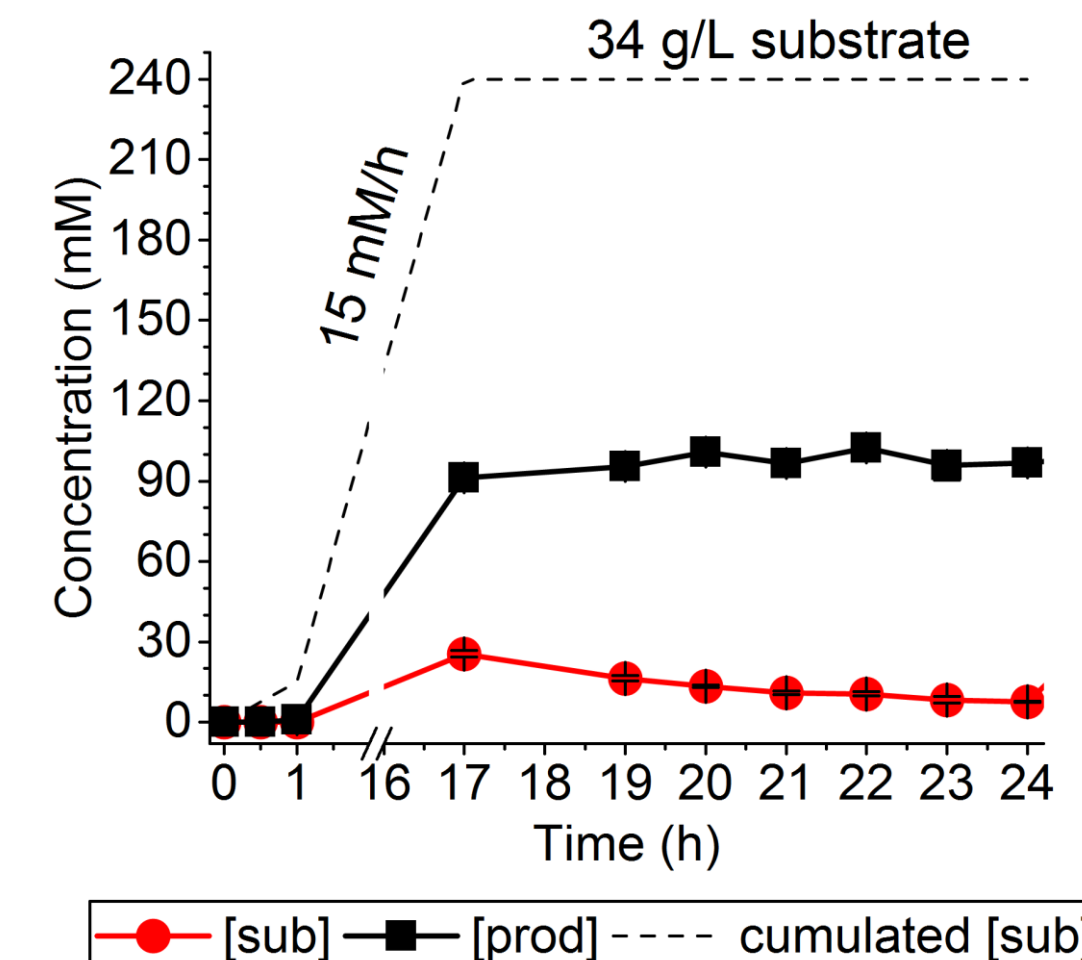


- Microstructure: block-like copolymers (¹³C NMR)
- Crystallinity reduced by 95 % by co-polymerization (compared to poly(PDL) homopolymer)
- Increased solubility (from chloroform only for poly(PDL) to non-polar and borderline aprotic solvents such as THF for the poly(TMCL-co-PDL) copolymers)

1 Continuous substrate feeding

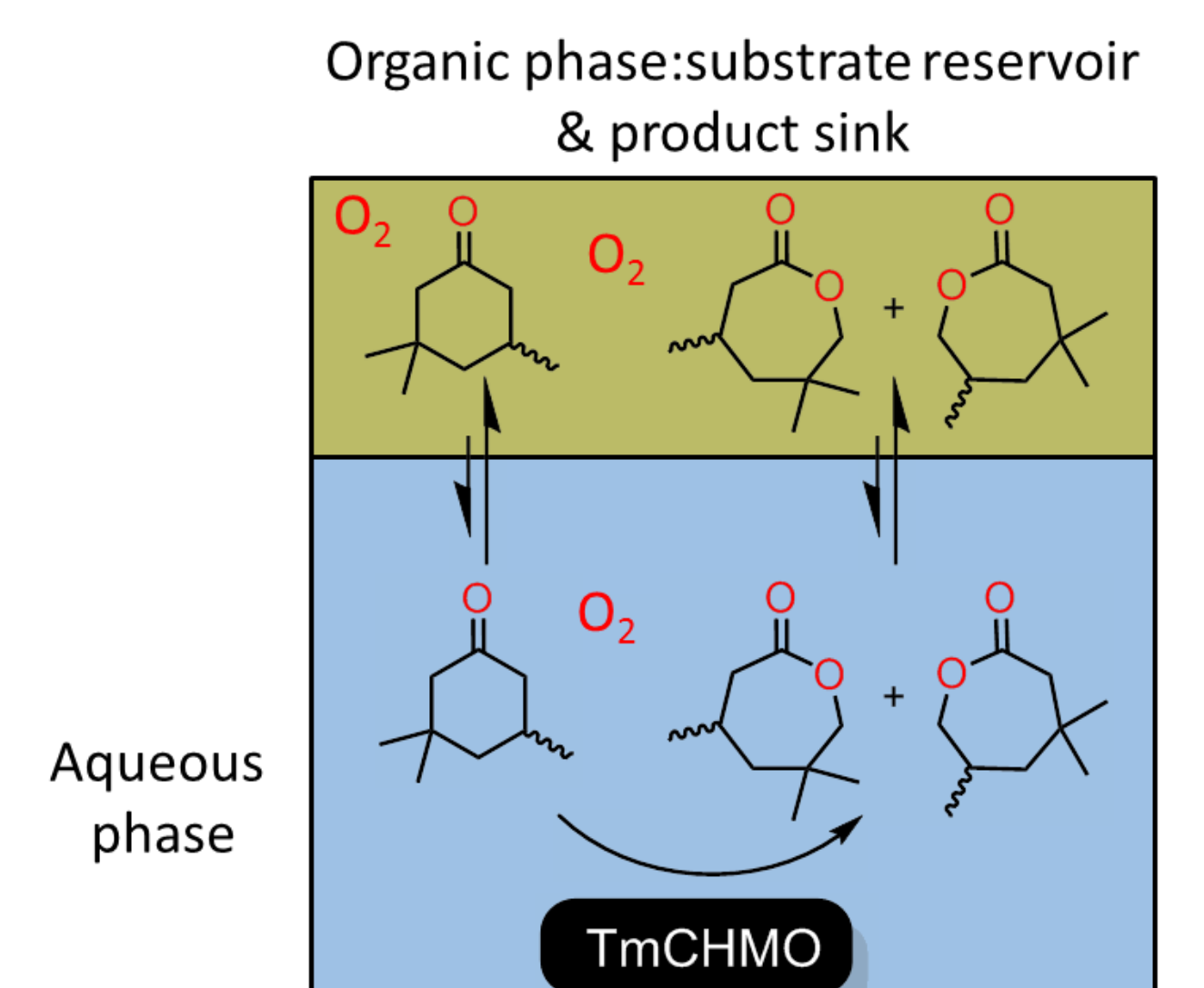


Kinetics of reaction (30 mL volume)

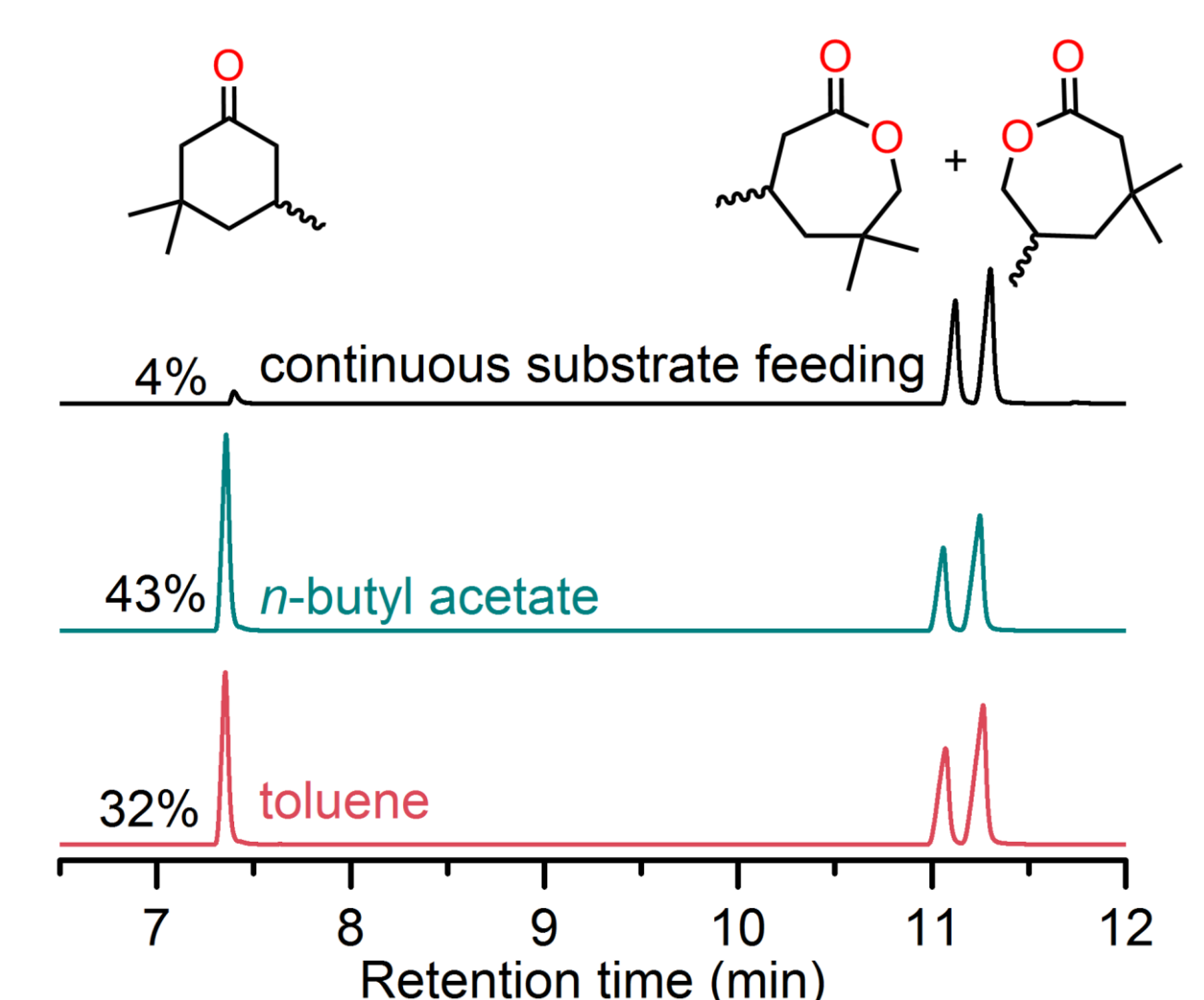


Gram scale synthesis of TMCL (500 mL-scale reaction):
Substrate conversion > 99%
[product] = 38 g/L
Space-time yield = 1.35 g/L/h

2 Biphasic system



Composition of purified products



Biphasic systems with *n*-butyl acetate or toluene (33 % v/v): unreacted substrate due to increased protein denaturation (organic/aqueous phase emulsification)

References

- Delgove, M. A. F. *et al.* *ChemBioChem* **2018**, 19, 354.
 - Romero, E. *et al.* *Angew. Chem. Int. Ed.* **2016**, 55, 15852.
 - Delgove, M. A. F. *et al.* *Journal of Chemical Technology & Biotechnology* **2018**.
 - Delgove, M. A. F. *et al.* *Polym. Chem.* **2017**, 8, 4696.
- TmCHMO crystal structure: 5m10

Acknowledgements

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