Rapid Identification of an Extraction System for the Purification of Enzymes

Identifying tailor-made aqueous two-phase system for industrially relevant biocatalysts

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Industrial relevant enzymes have reached industrial competitiveness over classical chemical catalysts due to gentle operating conditions and high specificity for the substrate. Within their production, the purification of the enzyme using Aqueous Two-Phase Extraction (ATPE) has been shown to be a promising alternative to time- and cost-intensive chromatographic separation. Within this work, we applied an optimized method to identify tailor-made Aqueous Two-Phase Systems (ATPS) to be used in ATPE. It was shown, that for an industrially relevant enzyme (that is Chimera [supplied by Georgia Tech, Bommarius Lab]) it was possible to identify a suitable ATPS as well as a process window for the ATPE in less than three weeks. With achievable yields above 78%, the possibility to influence the partitioning behavior of Chimera, this system outclasses the previously randomly selected ATPS (Trial-and-Error High Throughput Screening) by a factor of four, at the same time keeping precipitation of the enzyme below 17% (previously 70%).

The optimization of the downstream processing of biomolecules (this work: enzymes) has often been neglected. Thus, up to 50-60% of the total production costs attributed to the downstream processing. ATPE using an ATPS offers a valuable alternative to classical “standard” chromatography. ATPS consist of two phase formers dissolved in water above a critical concentration, generating two (immiscible) liquid phases. However, ATPE has not yet made it to industrial application, because selection of an appropriate ATPS is often based on time and cost-intensive high-throughput screening. To solve this challenge, we developed a method combining thermodynamic modeling and a small set of experiments to select phase formers and process conditions that create a tailor-made ATPS. Within this work, the method was applied for the purification of a previously unknown enzyme (Chimera) considering sodium citrate (Na_3Cit), sodium glutamate (NaGlu), polyethylene glycol 2000 (PEG2000) and polypropylene glycol (PPG400) as phase formers.

In a first step, thermodynamic modeling and investigations on the conformational biomolecule stability in the presence of the respective phase formers (using the unfolding temperature $T^{Unfolding}$) used to pre-select suitable phase formers.

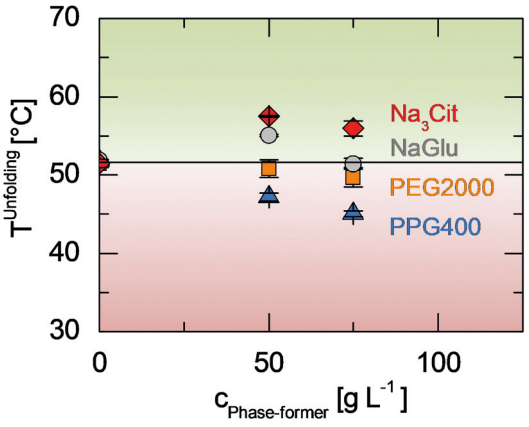

Figure 1: $T^{Unfolding}$ of Chimera in aqueous solutions with different concentrations of the phase formers PPG400, PEG2000, NaGlu and Na_3Cit at pH 7 and 1 bar.

The biomolecule stability is decreased, if a phase former drastically decrease $T^{Unfolding}$ upon increasing phase former concentration as seen for PPG400 in Figure 1. This excludes the phase former from being a potential phase former candidate. Next, the colloidal stability of the biomolecule is investigated by measuring biomolecule-biomolecule interactions estimating the aggregation propensity in presence of the phase formers. Therefore, the diffusion-interaction parameter (k_D) was measured (Figure 2) using dynamic light scattering.

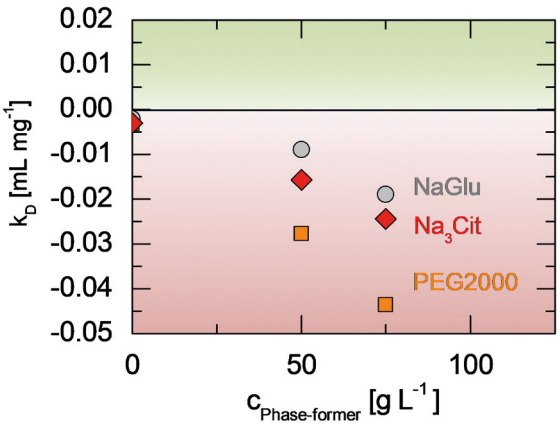

Figure 2: k_D of Chimera in aqueous solutions with different concentrations of the phase formers PEG2000, NaGlu and Na_3Cit at pH 7, 298.15 K and 1 bar.

Positive (negative) values indicate repulsive (attractive) interactions between biomolecules. Attractive interactions cause aggregation and thus strongly negative values are undesirable. With respect to the results shown in Figure 2, NaGlu is thus favored over Na_3Cit. In the subsequent ATPE, a Chimera yield of 78.7% was achieved being a four-fold improvement to the previously randomly selected ATPS (ammonium citrate - PEG4000 ATPS). Prospectively, this method will support the integration of ATPE in downstream processing of biomolecules by reducing the effort for ATPS development.

Publications:
M. Wessner, G. Schembecker, C. Brandenbusch, Identifying a Process Tailored Extraction System, Talk at AIChE 2019, Orlando (FL).

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