EXPANDING THE REPERTOIRE OF SORTASES APPLICABLE FOR ADVANCED PROTEIN ENGINEERING

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Sortases are transpeptidases which are used to modify, ligate and immobilize peptides or proteins in a site-specific manner 1. To extend the portfolio of sortases used to engineer proteins in vitro, a new type of assay was developed, applicable as high throughput screening tool but also as very sensitive analytical method. With this so-called Reporter Immobilization Assay (REIA) it was possible to perform a biodiversity screening of a variety of wild type sortases found in different species as well as a directed evolution campaign on one of those wild type enzymes 2. The biodiversity screening revealed that the sortase A from Listeria monocytogenes (Lm-SrtA) holds a high reactivity to the unusual LPXTA sort motif. This newly described specificity of Lm-SrtA can be used for efficient orthogonal double-labeling approaches e.g. in combination with sortase A from S. aureus (Sa-SrtA) catalyzing conjugation reactions at the conventional LPXTG sort motif. Furthermore, the Lm-SrtA appears calcium-independent and for this reason is able to catalyze conjugation reactions in vivo. Based on this finding, the Lm-SrtA is suggested as intracellular bioengineering tool. Another conclusion of the biodiversity screening was that Sa-Srt appears as the most efficient wild type enzyme for conjugation at the LPXTG motif, as described also by previous studies performed by others 3. Based on its high starting efficiency, a directed evolution approach was performed with the REIA with the intent to further increase activity and affinity of Sa-SrtA. After four rounds of directed evolution, indeed a five-time mutant of Sa-SrtA was identified possessing a dramatically increased catalytic efficiency and even slightly increased thermo stability. Finally, molecular dynamics simulations gave first insights that two of these five beneficial mutations found in the engineered Sa-SrtA variant are stabilizing the catalytically active Arginine which is in charge of substrate binding and oxyanion stabilization.

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