nature COMMUNICATIONS

ARTICLE

DOI: 10.1038/s41467-018-03492-6 OPEN

利用双通道微流控液滴筛选平台产生对应选择性酶的有效分子进化

Efficient molecular evolution to generate enantioselective enzymes using a dual-channel microfluidic droplet screening platform

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Directed evolution has long been a key strategy to generate enzymes with desired properties like high selectivity, but experimental barriers and analytical costs of screening enormous mutant libraries have limited such efforts. Here, we describe an ultrahigh-throughput dual-channel microfluidic droplet screening system that can be used to screen up to ~10^7 enzyme variants per day. As an example case, we use the system to engineer the enantioselectivity of an esterase to preferentially produce desired enantiomers of profens, an important class of anti-inflammatory drugs. Using two types of screening working modes over the course of five rounds of directed evolution, we identify (from among 5 million mutants) a variant with 700-fold improved enantioselectivity for the desired (*S*)-profens. We thus demonstrate that this screening platform can be used to rapidly generate enzymes with desired enzymatic properties like enantiospecificity, chemospecificity, and regiospecificity.

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Results

Screening of enzyme selectivity with the DMDS platform.

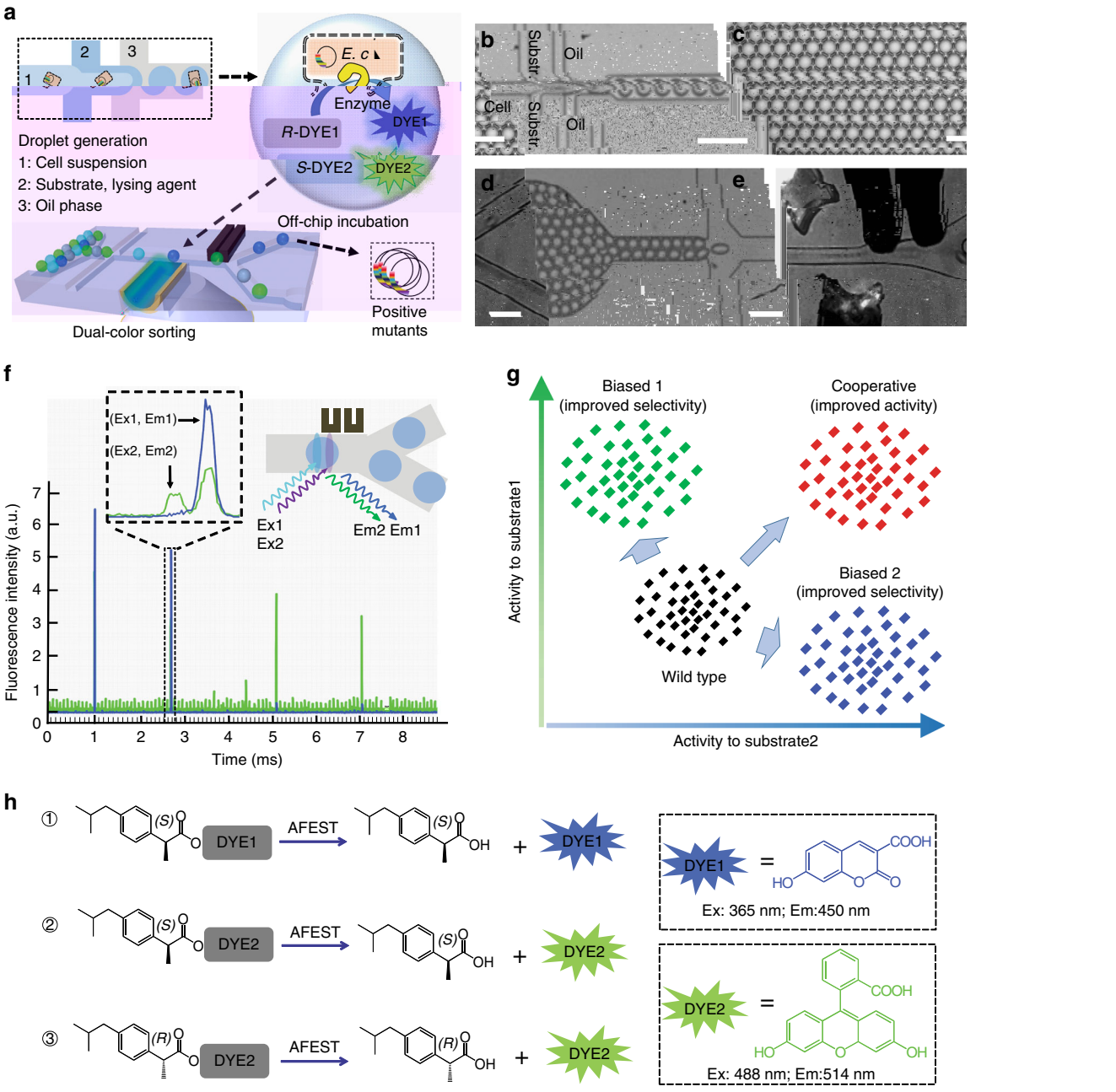

Fig. 1 DMDS platform for screening enzymatic enantioselectivity. **a** Schematic of DMDS operation. Mutant enzyme-expressing single cells are encapsulated in water-in-oil droplets with two fluorogenic substrates and lysis buffer. After the droplets are incubated for a specified time, those droplets containing the desired mutants are enriched via fluorescence-activated droplet sorting. Optical images of DMDS processes: **b** droplet generation; **c** off-chip incubation; **d** droplet reinjection; **e** fluorescence-activated droplet sorting. **f** To avoid crosstalk of two fluorescence signals, the droplets are excited by two spatially separated lasers, which generates two temporally separated emissions. **g** Sorting different populations in a mutation library with the DMDS platform is achieved via two screening modes: a cooperative mode and biased modes. **h** Three fluorogenic substrate designs and their enzymatic reactions yielding two different fluorescence signals. Scale bars: 100 μm

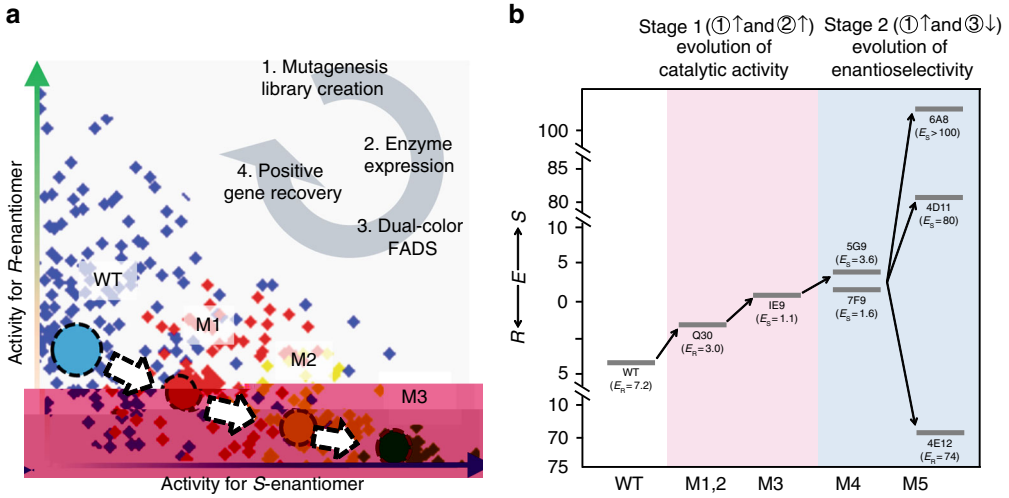

Fig. 2 Directed evolution of the enantioselectivity of AFEST. **a** Conceptual progression of enzymatic enantioselectivity enhancement by iterative rounds of mutagenesis and use of the DMDS process. **b** Cumulative improvement in the enantioselectivity of AFEST resulting from the various directed evolution steps of the present study

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Structural basis for improved enantioselectivity. A 6A8, 4D11, 4E12 89, x (D) *p*- (S)-i (R)-i AFE , : (S)-i (R)-i (Fi . 4). D 89C (R)- (Fi . 4), (S)- 89 (R)- 6A8 4D11 (S)- E , 89 ifi (S)- (Fi . 4) (R)- , x ifi 4E12 (R)- 6A8 4E12 x 4D11 6A8 *rac*-**2**, *rac*-**3**, *rac*-**4**, *rac*-**5**. D 4D11 6A8 , 4D11 6A8. F

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Discussion

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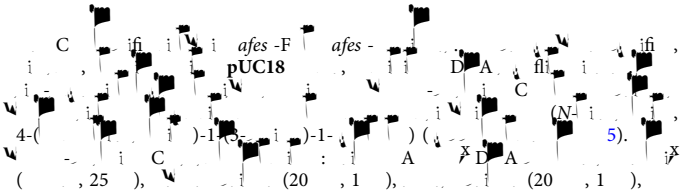

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Acknowledgements

i i F i C (21627812, 31670791, 31470788), i F i (CBE 1263889), F C i - F i F D A i i C F i i i E i D (2 1 000433). D A i C i i .

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Additional information

Supplementary Information i i :// i /10.1038/ 41467-018-03492-6.

Competing interests: i

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