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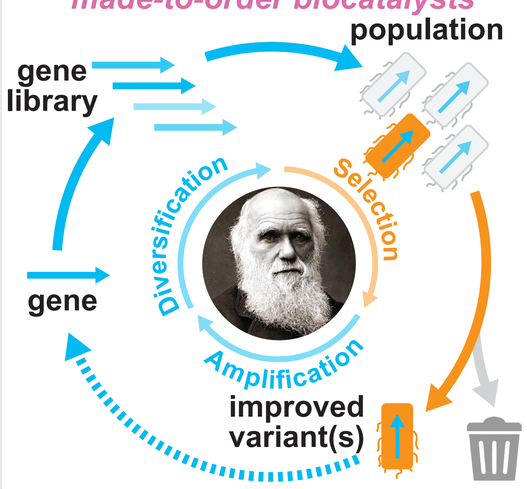
Phenotypic selections for the directed evolution of enzymes

Harnessing the power of evolution for enzyme engineering

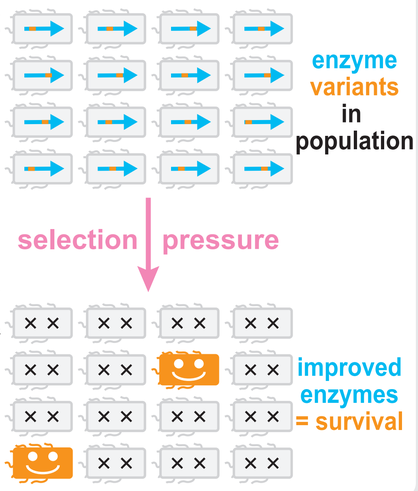
Directed evolution: made-to-order biocatalysts



Enzymes are impressive catalysts, with unmatched rates and selectivity that function in mild, environmentally friendly conditions[1,2]. However, poor stability or narrow substrate scope can limit their industrial applications. Luckily, nature's catalysts are evolvable and directed evolution can tailor enzymatic properties to fit a user's need. To do so, one mimics the Darwinian algorithm in the laboratory by performing iterative cycles of diversification, selection, and amplification.

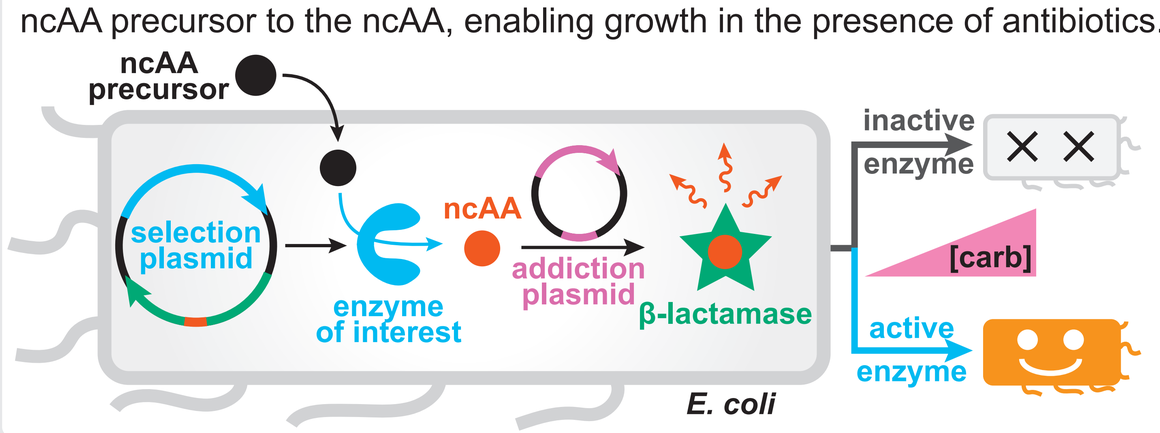
Assaying enzymatic activity is a persistent bottleneck in the directed evolution of enzymes. Laborious screening methods that analyze each variant one-by-one greatly impede throughput and are in stark contrast to nature's selection of improved variants from a diverse population. To mimic nature's survival of the fittest, we have established a link between enzyme performance and cellular fitness. A diverse population is subjected to selection pressure. Cells featuring improved enzymes have adapted the best and will therefore survive.

Survival of the fittest

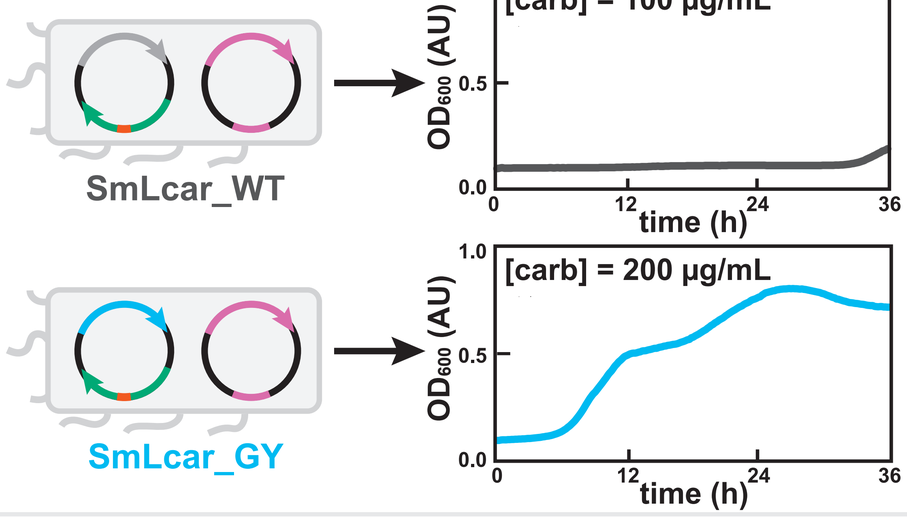


Linking enzyme activity to survival

Cells are addicted to a non-canonical amino acid (ncAA) through genetic code expansion[3,4]. The enzyme of interest is able to convert an appropriate ncAA precursor to the ncAA, enabling growth in the presence of antibiotics.

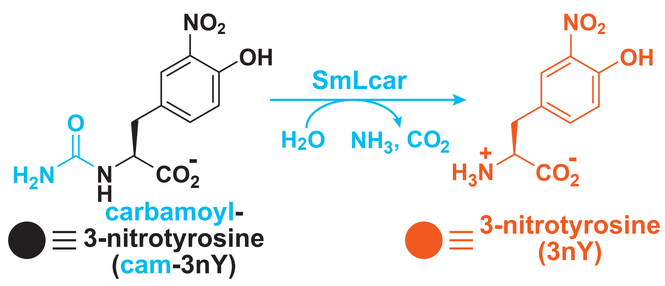


Cells featuring improved enzymes have higher fitness under selection pressure. They grow faster and under more stringent conditions.

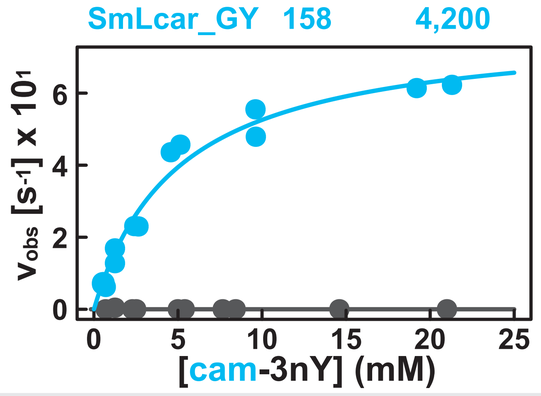


Carbamoylases as model biocatalysts

Carbamoylases hydrolyze L-*N*-carbamoyl amino acids.



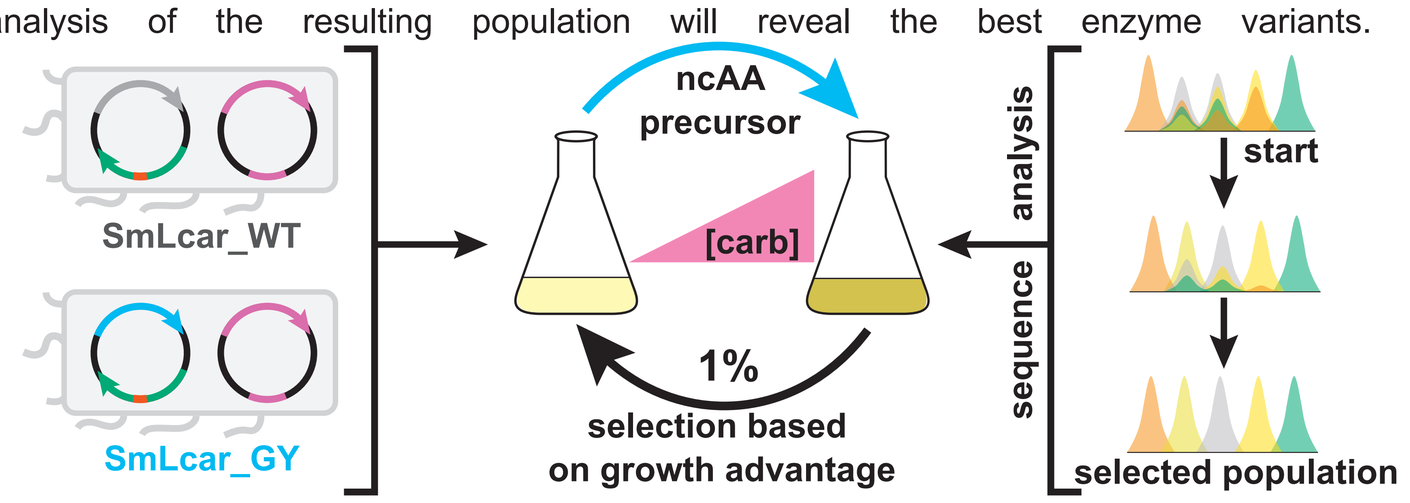
	k_{cat}/K_m ($M^{-1}s^{-1}$)	relative activity
SmLcar_WT	0.038	1
SmLcar_GY	158	4,200



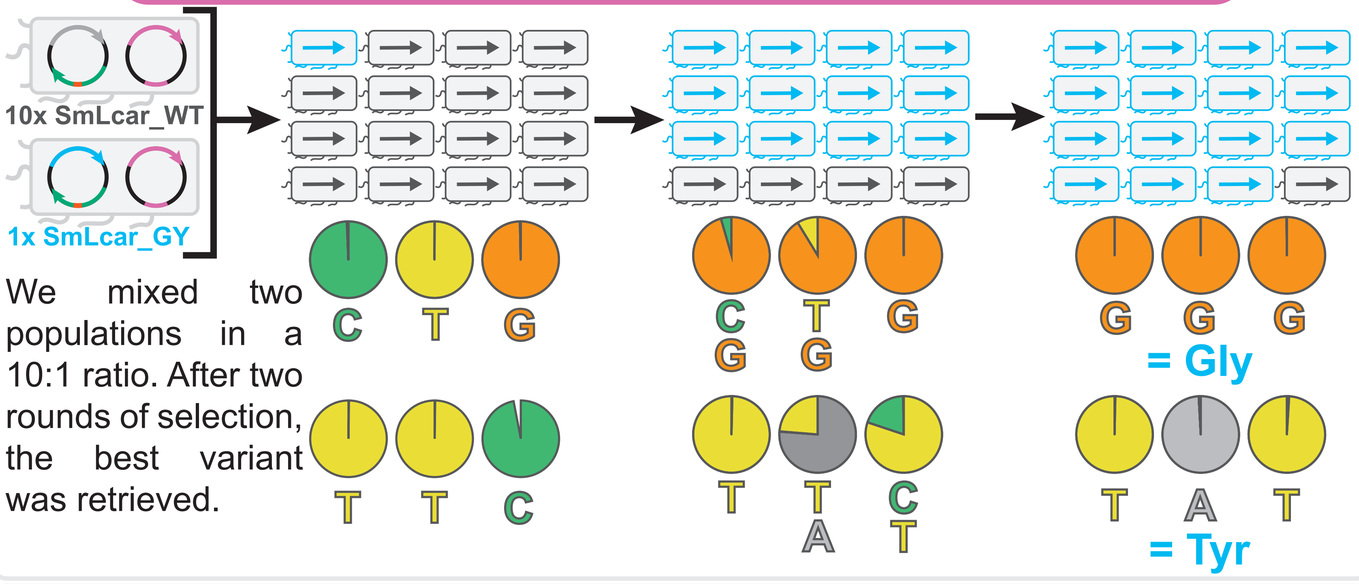
Two rounds of directed evolution on a carbamoylase, SmLcar, resulted in an enzyme variant with two substitutions which displayed over 4000-fold increased activity.

Improved enzymes provide a growth advantage: the basis for selection

We reasoned that we could use this growth advantage to select for improved enzymes. When a diverse population is mixed and subjected to continuous selection pressure, cells featuring better variants will outcompete the rest, causing their extinction. Sequence analysis of the resulting population will reveal the best enzyme variants.

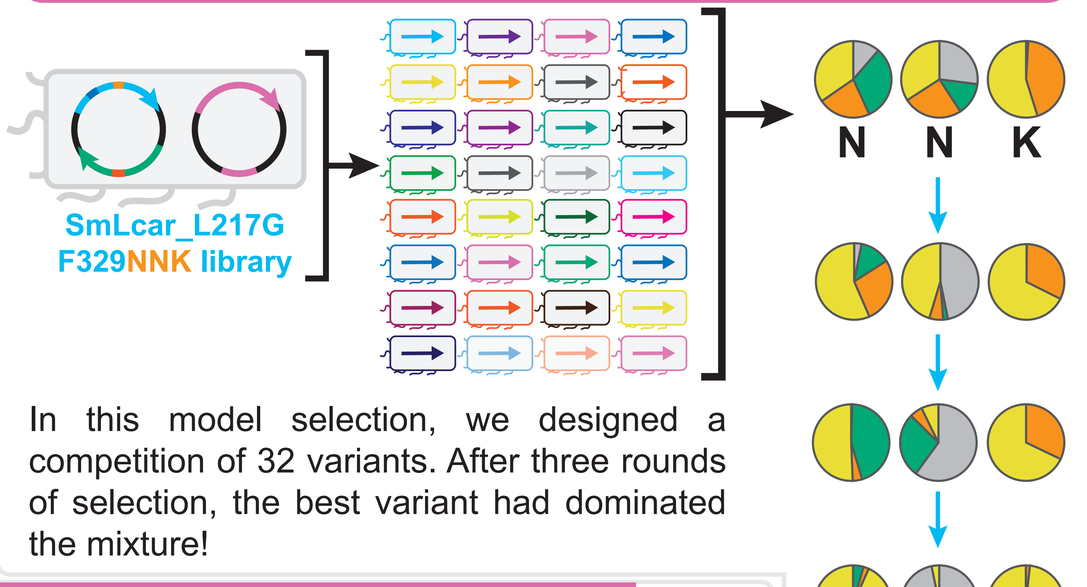


Mock selection: mixing two populations



We mixed two populations in a 10:1 ratio. After two rounds of selection, the best variant was retrieved.

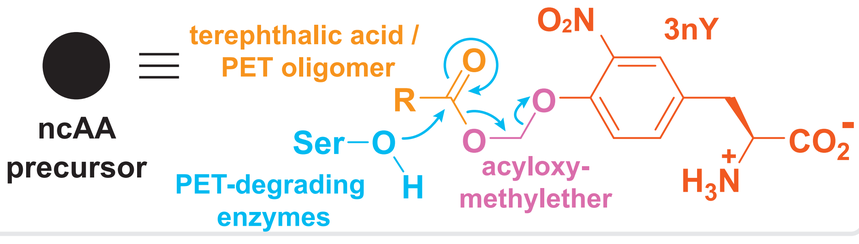
Model selection: 32 variants compete!



In this model selection, we designed a competition of 32 variants. After three rounds of selection, the best variant had dominated the mixture!

Outlook: toward diverse populations and plastic-degrading enzymes

To expand our continuous evolution platform, we will generate more diverse populations that simultanously assess millions of enzyme variants. We anticipate that many mechanistically-diverse biocatalysts can be engineered with our approach. Of particular interest is the directed evolution of polyethylene terephthalate (PET) degrading enzymes for more efficient PET recycling strategies.



References
1. Schmid *et al.* **2001**, *Nature* 409, 258; 2. Leemhuis *et al.* **2009**, *IUBMB Life* 61, 222; 3. Rubini & Mayer **2020**, *ACS Chem. Biol.* 15(12), 3093; 4. Young & Schultz **2018**, *ACS Chem. Biol.* 13(4), 854.