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Smart engineering of various enzymes for asymmetric synthesis of chiral molecules on industrial scale

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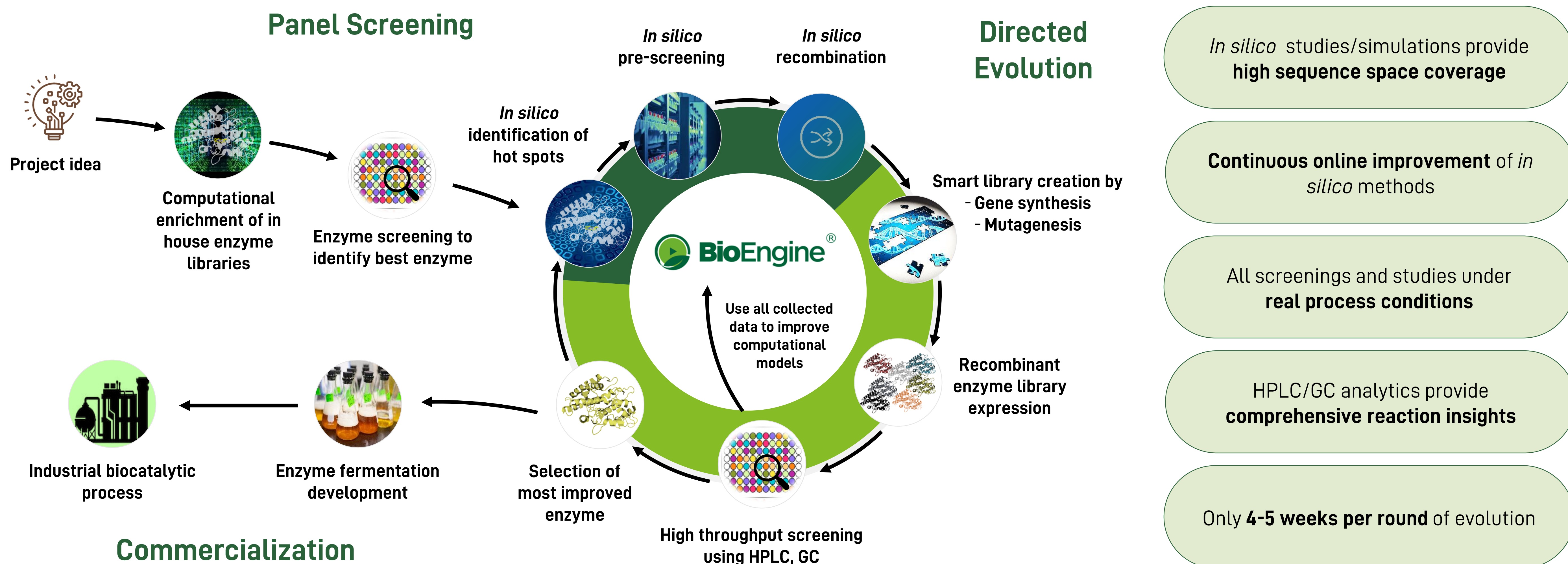
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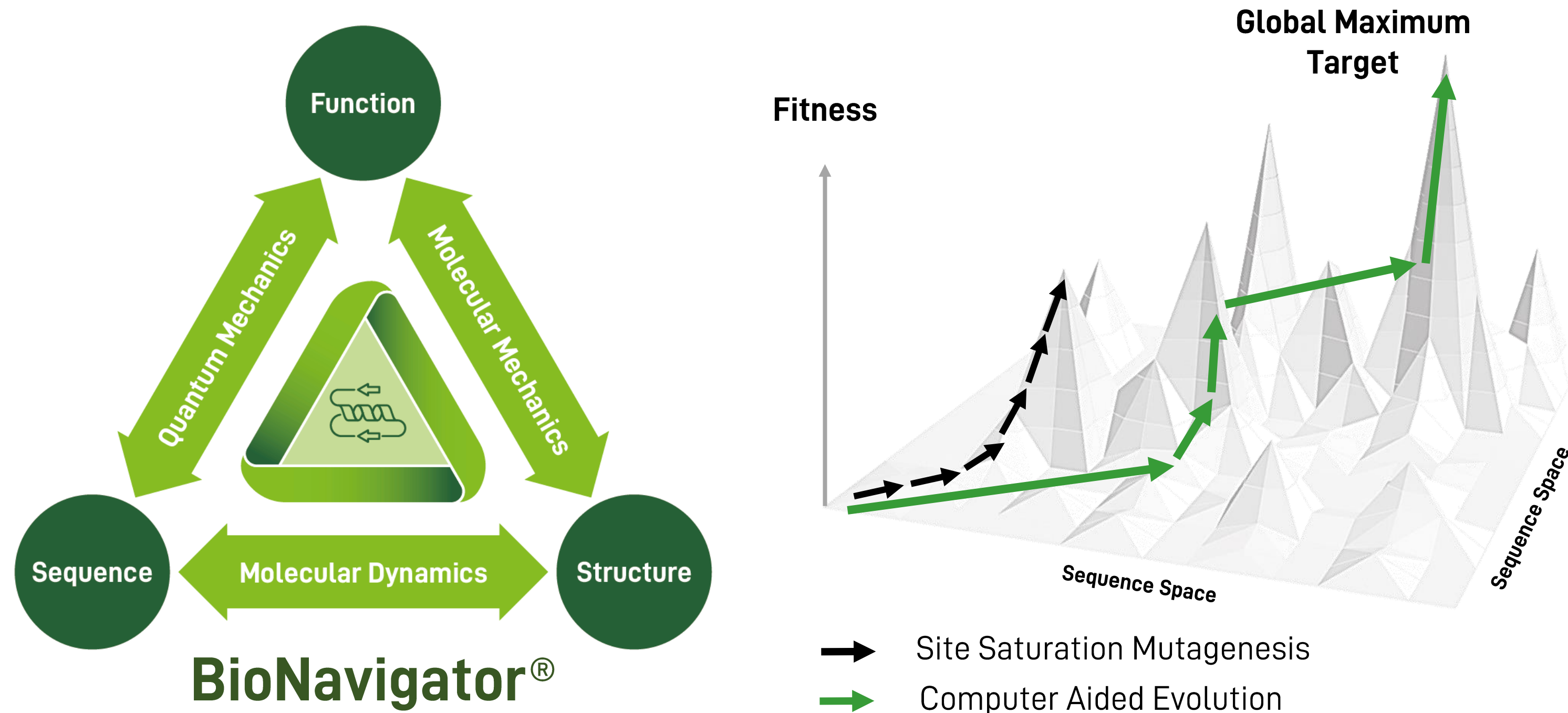
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BioEngine® is an integrated directed evolution platform that offers the full-spectrum solution from enzyme discovery, enzyme engineering, process development all the way to qualified product manufacturing. Contrary to traditional directed evolution approaches it is based on bioinformatics guided semi rational library design, taking the relationship between protein sequence, structure and function into account and enables **fast and efficient enzyme optimization** at Enzymaster.



BioNavigator® is our proprietary computational enzyme engineering toolbox for **hot spot identification**, ***in silico* enzyme library screening**, and ***in silico* recombination**. It allows us to shift most of the screening efforts from the lab into the computer and provides high sequence space coverage, while accounting for the real chemical process conditions.

Based on these results, we construct “**smart**” and **efficient libraries** of about 2000 enzyme variants, which can be conveniently screened on HPLC or GC to characterize a comprehensive range of desired enzyme properties.

Three industrial examples are shown below.

(R)-Phenylethylamine	(R)-1,3-butanediol	L-Tyrosine
<p>Transaminase</p>	<p>Ketoreductase</p>	<p>Tyrosine Phenol-Lyase</p>
<p>Wildtype Enzyme:</p> <ul style="list-style-type: none"> Inhibited at 5 g/L product Inactivated at 2M IPM Product titer: 50 g/L 	<p>Wildtype Enzyme:</p> <ul style="list-style-type: none"> Not thermally stable enough for heat treatment <50% conv. 200 g/L substrate 30% (v/v) loading of enzyme 	<p>Wildtype Enzyme:</p> <ul style="list-style-type: none"> Inactivated with 3 g/L phenol Enzyme loaded as whole cell Product titer: 80 g/L
<p>10 rounds of evolution</p>	<p>3 rounds of evolution</p>	<p>5 rounds of evolution</p>
<p>Industrial Enzyme:</p> <ul style="list-style-type: none"> Tolerates > 300 g/L product Stable at 2M IPM for 180 h Product titer: 160 g/L <p>1000 MT/a</p>	<p>Industrial Enzyme:</p> <ul style="list-style-type: none"> Stable at 85°C for 2h 99% conv. 400 g/L substrate 8% (v/v) loading of enzyme <p>50 MT/a</p>	<p>Industrial Enzyme:</p> <ul style="list-style-type: none"> Stable at 10 g/L phenol for 24 h Enzyme loaded as clear lysate Product titer: 200 g/L <p>Multi-Ton Scale</p>

References:

[1] WO 2019 128894 Engineered Transaminase Polypeptides and uses thereof

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