Engineered dsRNA ligases can efficiently scale siRNA manufacturing

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For large scale siRNA manufacturing, ligation can enhance the yield and purity of the final output. When a double-stranded RNA (dsRNA) ligase joins multiple short RNA fragments efficiently to form the desired dsRNA duplex, it can minimize the formation of unwanted by-products and increase final product yield. Even though ligation is being increasingly adopted as an approach, wild-type dsRNA ligases are often expensive and inefficient on challenging substrates. With a library of engineered ligases available today, Codexis helps shorten and de-risk the ligase optimization cycle so that you can realize the benefits of enzymatic approaches sooner.

In this joint poster, Codexis and Bachem compare the performance between wild-type dsRNA ligase and engineered ligases to demonstrate improvements in ligation efficiency and scalability. We then show how different reaction conditions affect the various substrate-ligase pairings, demonstrating the significance of ligation protocol optimization. Finally, we present the performance of engineered enzymes at high substrate loading, demonstrating scalability.

Engineered ligases achieve higher conversion faster than wild-type ligase

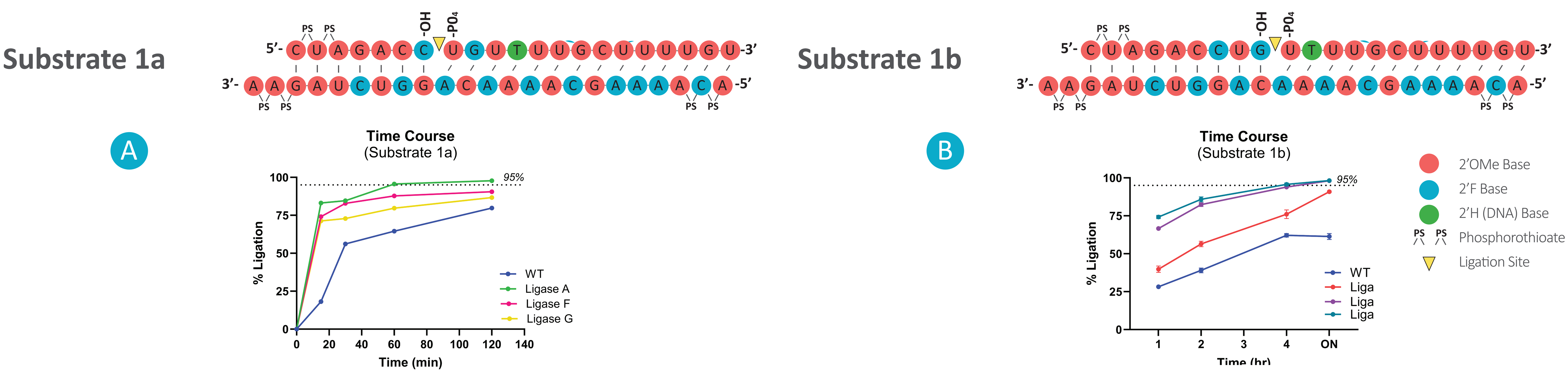

Figure 1: Engineered ligases outperform WT (T4 Rnl2) ligase **A)** For Substrate 1a - Ligases A and F exhibit optimal performance, achieving >95% conversion in 30 min; and >98% conversion in 2 hours. Reaction conditions: 20 g/L substrate, Tris pH 8.5, MgCl2, DTT, ATP, at 37 °C. **B)** For Substrate 1b - Multiple evolved variants (B, C and D) show optimal performance with >95% conversion within 4 hours. Reaction conditions: 20 g/L substrate, Tris pH 8.5, MgCl2, DTT, ATP, 33 °C incubation.

Engineered ligases enable scalability through higher substrate loading

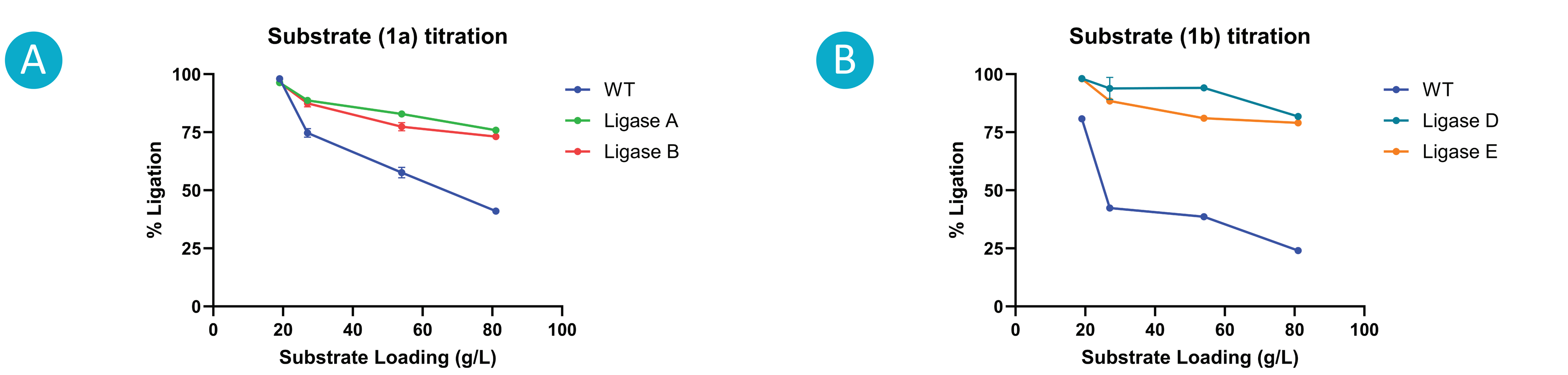

Figure 2: Engineered ligases outperform WT ligase at high substrate loading. **A)** For Substrate 1a - Ligases A and B exhibit optimal performance, achieving 76% conversion at 80 g/L substrate loading. Reaction conditions: 20-80 g/L substrate, Tris pH 8.5, MgCl2, DTT, ATP, 33 °C incubation for 4-6 hours. **B)** For Substrate 1b - Ligases D and E exhibit optimal performance, achieving 82% conversion at 80 g/L substrate loading. Reaction conditions: 20-80 g/L substrate, Tris pH 8.5, MgCl2, DTT, ATP, 33 °C incubation for 4-6 hours

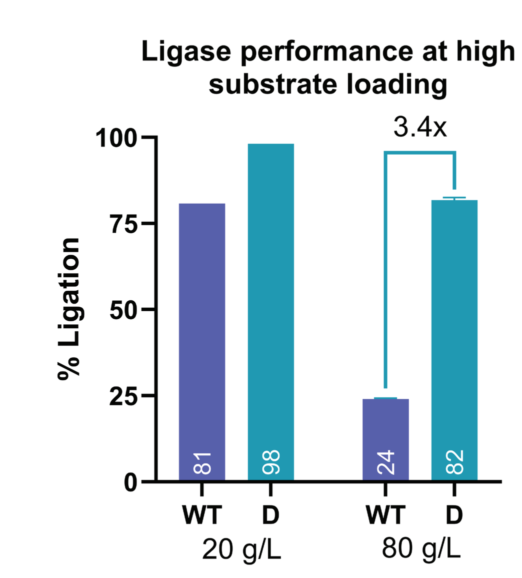

Figure 3: Engineered Ligase D outperforms WT ligase at both 20 g/L and 80 g/L substrate loading. Engineered ligases work across a wide (4-fold) range of substrate loading concentrations.

Ligation optimization to establish robust, process relevant conditions

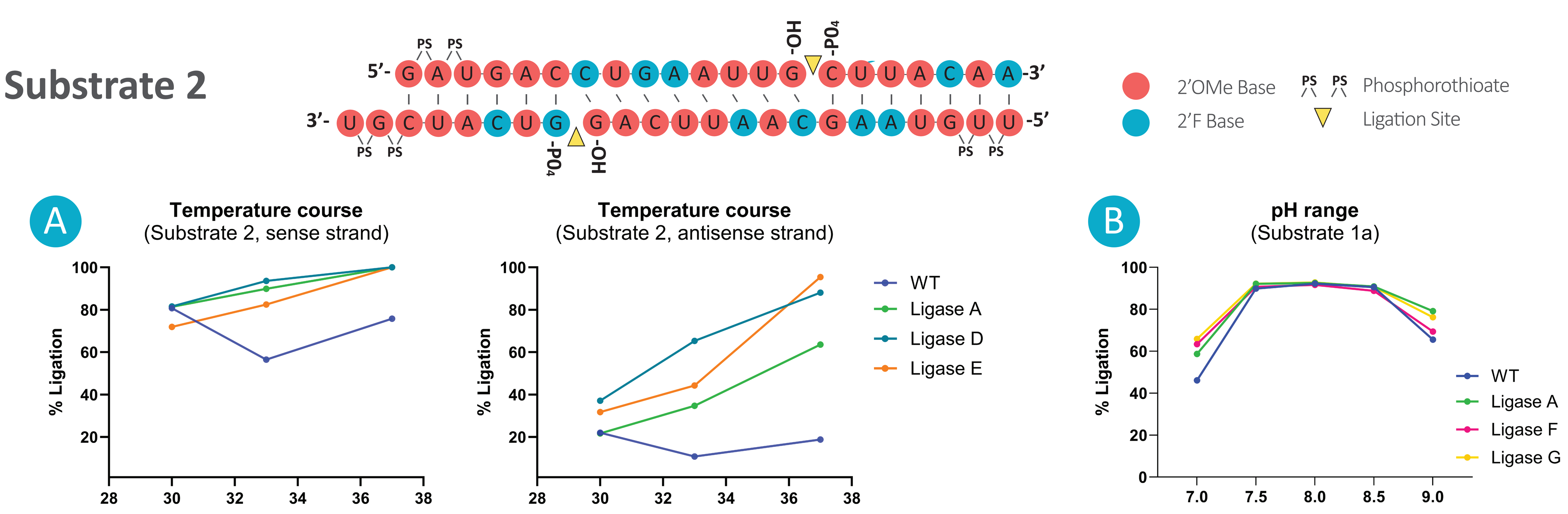

Figure 4: Engineered ligases exhibit strong correlation between temperature, pH and enzyme activity A) For substrate 2, WT ligase exhibits no significant change in activity when temperature varies, however Ligase variants D and E show enhanced performance with increasing temperature, indicating temperature-dependent activity improvements. Reaction conditions tested at 1 h incubation. B) For substrate 1a – all ligases tested show optimal performance between pH 7.5–8.5.

Conclusions:

1. Engineered ligases outperform WT ligase across multiple substrate designs.
2. Reaction condition optimization (pH, time, temperature, substrate design) is key for high substrate conversion.
3. Engineered ligases enable scalability through high conversion across a wide substrate loading range.

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