ACS SyntheticBiology
Research Article
pubs.acs.org/synthbio

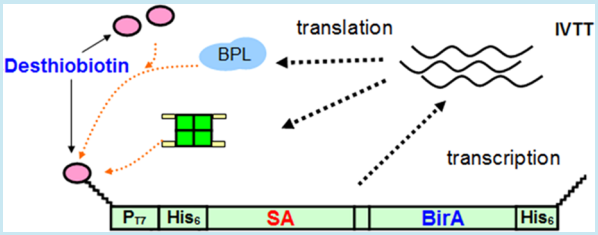
Design and Selection of a Synthetic Operon

Wei-Cheng Lu[†] and Andrew D. Ellington*[,†,‡]

[†]Institute of Cellular and Molecular Biology University of Texas at Austin, Austin, Texas, United States
[‡]Center for Systems and Synthetic Biology University of Texas at Austin, Austin, Texas, United States

Supporting Information

ABSTRACT: Cell-free systems are showing increasing promise for biosynthesis of both proteins and small molecules. However, *in vitro* transcription and translation reactions have so far primarily been used for the production of single proteins. In order to demonstrate the possibilities for coupled reactions, we designed synthetic operons that included different combinations of wild-type or evolved biotin ligases and streptavidins and demonstrated a mechanism for self-selection of operons following expression *in vitro*. Peptide substrates for biotin ligase were conjugated to the DNA operons and could be modified by a biotin ligase specific for either biotin or desthiobiotin and subsequently captured via a streptavidin specific for either biotin or desthiobiotin.



KEYWORDS: *synthetic operon, genetic circuit, biotin ligase, streptavidin, in vitro transcription and translation*

Metabolic pathways in living organisms can be extremely complex and are maintained by homeostasis and optimized by evolution. Even simple pathways in bacteria are often coexpressed as operons.

In contrast, while metabolic pathways can be constructed *ex vivo* using isolated enzymes, there are few examples of pathways that can construct themselves via gene expression and *in vitro* transcription and translation (IVTT) reactions. For the most part, IVTT has been used for the production, sometimes at scale, of individual enzymes. There are also some examples of more complex IVTT reactions, such as multistep biocatalytic transformations. For example, a multienzyme (10-step), cell-free reaction has been developed for efficient dihydroxyacetone phosphate (DHAP) production from glucose.[1] The flux into product was further enhanced 2.5-fold by constructing a synthetic operon that expressed hexokinase, glyceraldehyde-3-phosphate dehydrogenase, fructose biphosphate aldolase, and lactate dehydrogenase in the cell-free extract.[2] The whole of glycolysis has also been reconstituted and optimized in extracts, with about 30% of glucose being converted to pyruvate.[3] Similar multistep biocatalytic reactions have been developed for *in vitro* hydrogen production.[4] In addition to synthetic pathways, genetic regulatory circuits have also been shown to function in cell-free systems. The Libchaber group has developed a three-gene circuit in a cell-free system that acted as an amplifier to control (drive or block) the expression of a reporter.[5]

In contrast, the emulsification of IVTT and the concomitant expression of genes and production of proteins has proven possible, and has now been extensively used for the evolution of enzymes.[6−8] For example, streptavidin has previously been optimized for its ability to bind an alternative substrate, desthiobiotin,[9,10] and similar methods have been applied to altering the substrate specificity of biotin ligase (see also Figure 1).

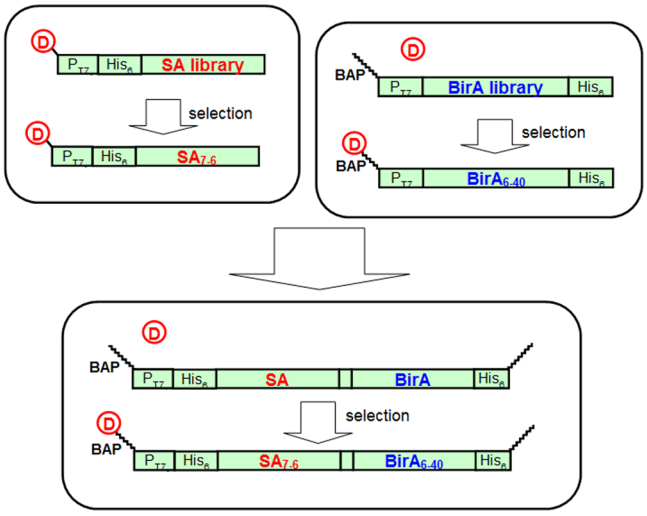


Figure 1. Synthetic operons with streptavidin and biotin ligase variants. A previously evolved a streptavidin variant (SA_{7-6}) shows improved binding to desthiobiotin. We have evolved an *E. coli* biotin ligase ($BirA_{6-40}$) that demonstrates an improved use of desthiobiotin as a substrate. By combining the streptavidin and biotin ligase variants, we generated a synthetic operon where the two proteins should cooperate in the use of desthiobiotin.

It would be useful to combine the ability to self-produce functional pathways *in vitro* with the ability to evolve individual enzymes *in vitro*. To this end, we have created an artificial operon

Special Issue: Cell-Free Synthetic Biology

Received: October 7, 2013
Published: March 13, 2014

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that combines streptavidin with biotin ligase, have coexpressed the proteins in a way that leads to the self-capture of the operon by the very proteins it produces, and have taken steps toward the evolution of this pathway. These experiments open the way to the *in vitro* co-optimization of parts for genetic circuits.

RESULTS AND DISCUSSION

Operon Design and Function. In order to demonstrate the possibilities for metabolic pathway optimization in the context of IVTT systems, we created a synthetic operon encompassing biotin ligase and streptavidin such that both genes were necessary for the capture of the operon DNA. We had previously conjugated biotin ligase genes with a peptide substrate (BAP)[11] such that expression of the ligase *in vitro* resulted in biotinylation of the peptide and subsequent capture of the gene via externally added streptavidin. This scheme was now extended by coexpressing streptavidin with the biotin ligase.

The synthetic operon contained a wild-type streptavidin gene (SA_{wt}) and a wild-type biotin ligase gene ($BirA_{wt}$), each with their own ribosomal binding sites, 6xHistidine tags (His-tags), and one unique restriction site (Figure 2A). Overall transcription was driven by a single T7 RNA polymerase promoter. The operon was first tested for expression and activity in *E. coli* lysates (Figure 2B). Either the operon [SA_{wt}-$BirA_{wt}$], the biotin acceptor peptide fused to a chloramphenicol acetyltransferase gene (CAT-BAP), or both were introduced into IVTT reactions and incubated for 1.5 h at 30 °C. Biotin ligase expression was confirmed by Western blot analysis stained with alkaline phosphatase conjugated anti-His antibodies (anti-His Ab-AP; Figure 2B, lane 1) and ligase activity was confirmed via staining the biotinylated CAT-BAP substrate with a streptavidin/alkaline phosphatase conjugate (SA-AP; Figure 2B, lane 6). The cotranslation of streptavidin was also found to shift the biotinylated CAT-BAP band (Figure 2B, lane 3).

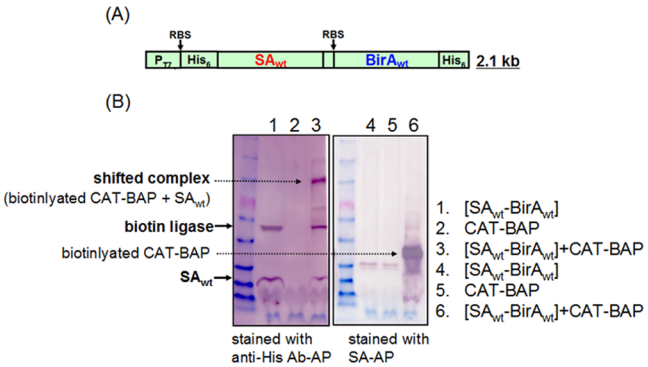

Figure 2. Wild-type operon demonstrates both expression and function. (A) The operon was composed of wild-type streptavidin and wild-type *E. coli* biotin ligase, that is, [SA_{wt}-$BirA_{wt}$]. With a length of ~2.1 kb pairs, the operon contained a T7 promoter and terminator as well as two ribosomal binding sites (RBS) upstream of streptavidin and biotin ligase, respectively. (B) The wild-type operon (lanes 1 and 4), CAT-BAP (lanes 2 and 5), and the two combined (lanes 3 and 6) were incubated with IVTT reagents at 30 °C for 1.5 h and then analyzed with alkaline phosphatase conjugated anti-His antibodies (left Figure 2B) or alkaline phosphatase conjugated streptavidin (right Figure 2B) via Western blot analysis. Translated His-tagged biotin ligase, His-tagged streptavidin (lanes 1 and 3), biotinylated CAT-BAP (lane 6), and shifted complex (lane 3) could be detected in the Western blot analysis.

Moreover, we introduced two different versions of both the biotin ligase and streptavidin into operons. The wild-type enzymes were optimized for biotin, while the mutant variants—termed biotin ligase 6−40 ($BirA_{6-40}$) and streptavidin 7−6 (SA_{7-6})—had previously been selected to function with desthiobiotin.[9,10,12] Using the two different types of genes (biotin-utilizing and desthiobiotin-utilizing) we created four different operons: [SA_{wt}-$BirA_{wt}$], [SA_{wt}-$BirA_{6-40}$], [SA_{7-6}-$BirA_{wt}$], and [SA_{7-6}-$BirA_{6-40}$]. All four operons were driven by the same T7 RNA polymerase promoter but contained different restriction sites within the intergenic region in order to facilitate identification (Figure 3). As before, the operons were initially assayed *in vitro* to determine if proteins were produced and whether the appended peptides were biotinylated; high concentrations (20 μM) of biotin were used to ensure that even the desthiobiotin-utilizing gene products could be detected. The four operons were separately incubated in an IVTT reaction at 30 °C for 1.5 h and then divided into two sets for gel electrophoresis and Western blot analysis. One set of samples was boiled and the other set of samples was not boiled prior to gel electrophoresis in order to avoid disrupting the shifted complex (Figure 4). Lanes 1 to 4 and lanes a to d were [SA_{wt}-$BirA_{wt}$], [SA_{wt}-$BirA_{6-40}$], [SA_{7-6}-$BirA_{wt}$], and [SA_{7-6}-$BirA_{6-40}$], respectively, and lanes 5 to 8 and lanes e to h were the same operons in the presence of the CAT-BAP gene. The Western blot was again stained with anti-His Ab-AP (left side of Figure 4) to detect synthesized His-tagged biotin ligases and streptavidins, and then further stained with SA-AP to detect biotinylated CAT-BAP (right side of Figure 4). The unboiled sample again showed a gel shift of the biotinylated CAT-BAP gene by cotranslated streptavidin (Figure 4, left side, lanes e to h). All four synthetic operons expressed and functioned well in the IVTT reaction.

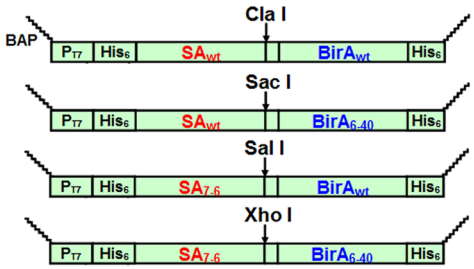

Figure 3. Pool of synthetic operons. Wild-type and variant streptavidins (SA_{wt} and SA_{7-6}), and wild-type and variant biotin ligases ($BirA_{wt}$ and $BirA_{6-40}$) were used to generate a representative pool of four synthetic operons: [SA_{wt}-$BirA_{wt}$], [SA_{wt}-$BirA_{6-40}$], [SA_{7-6}-$BirA_{wt}$], and [SA_{7-6}-$BirA_{6-40}$]. Each operon has a unique intergenic restriction site.

A selection scheme was designed to allow these four synthetic operons to compete either in the presence of biotin or desthiobiotin (Figure 5). Individual operon/peptide conjugates were emulsified with an IVTT reaction such that less than one conjugate was present per compartment.[9,11,13−15] Each emulsion vesicle also contained all the necessary components for IVTT. The operon design was such that (i) translated biotin ligases should differentially recognize whatever substrate (biotin or desthiobiotin) was present and conjugate it to the peptides at the ends of the operon, after which (ii) translated streptavidins in turn should recognize and bind to the derivatized peptides, forming a "complex." The streptavidin variants were fused to His-tags, and after breaking the emulsions, the complexes were recovered using anti-His antibodies immobilized on agarose beads. To the extent that proteins did function preferentially, we anticipated that in the presence of biotin the fully wild-type

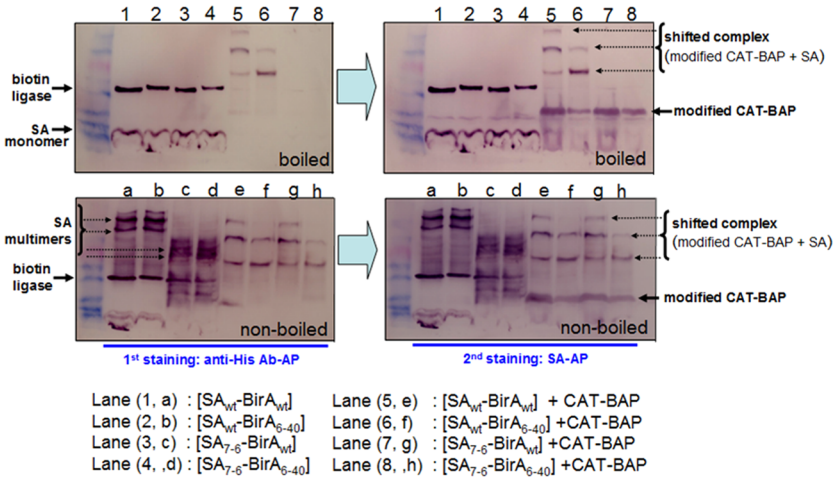

Figure 4. Synthetic operons demonstrate both expression and function. The four synthetic operon pairs were separately incubated in an IVTT reaction at 30 °C for 1.5 h. Lanes 1 to 4 and lanes a to d show reactions containing the four operons. Lanes 5 to 8 and lanes e to h show reactions with the four operons and CAT-BAP. After IVTT, the reactions were divided into two sets. One set (top half) was boiled while the second set (bottom half) was not. A Western blot was carried out and first stained with anti-His Ab-AP (left side) and then with SA-AP (right side). Translated His-tagged biotin ligase and streptavidin (lanes 1 to 4 and lanes a to d), biotinylated CAT-BAP (lanes 5 to 8 and lanes e to h), and shifted complexes (lanes 5 to 6 and lanes e to h) were detected in the Western blot analysis.

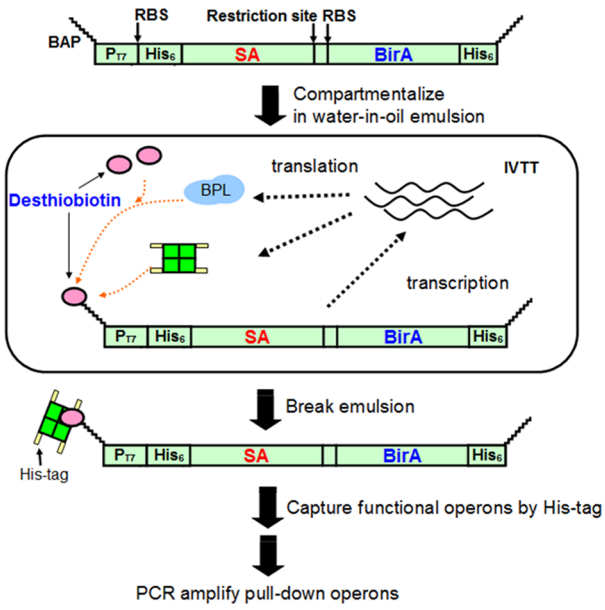

Figure 5. Selection scheme for synthetic operons via *in vitro* compartmentalization. First, the synthetic operons are conjugated with BAP. The BAP/operon conjugates and IVTT reagents are then mixed with oil/surfactant to generate compartments. Conjugates that are functional generate biotin ligases, which attach the desthiobiotin substrate to BAP. Streptavidin binds to desthiobiotin and streptavidin-bound conjugates are then bound by anti-His antibody agarose beads. Finally, streptavidin-bound conjugates are amplified by PCR.

operon would be preferentially captured, while in the presence of desthiobiotin the fully mutant operon would predominate. Following capture, any selected operons could be further amplified by PCR and the relative concentrations of any of the four products could be simply determined by restriction digest.

Mock Operon Selection. Before we tested whether the operons could preferentially compete in the presence of either biotin or desthiobiotin, we first carried out a mock selection. A functional operon containing a conjugated BAP tag was mixed with progressively larger amounts (0:1, 1:9, and 1:99) of nonfunctional operons lacking a BAP tag and the recovery of the BAP-tagged operons was determined after emulsion (Figure 6). In the absence of a BAP tag (0:1), no operons were recovered. When the BAP tagged constructs were present at 1 part in 10 (1:9) the results demonstrated enrichment as indicated by a restriction digest specific for the operon (Xho I; Figure 6). However, there was no significant enrichment observed for BAP-tagged operons from the 1:99 pool. These results implied that in a selection, the enrichment per round would likely be on the order of 10-fold.

To further ensure that the selection observed was due to BirA and SA function, we included an additional negative control. A round of selection was carried out with the BAP/operon conjugate ([SA_{wt}-$BirA_{wt}$]) but in the absence of biotin. As expected, only a weak signal was observed in comparison to selection with a BAP/operon conjugate ([SA_{wt}-$BirA_{wt}$]) and biotin in the mixture (data not shown).

Ligand-Specific Operon Selection. Building on the methods developed for the mock selection, the four operons were allowed to compete in the presence of 50 μM desthiobiotin. After emulsification such that there was roughly one operon per compartment, operons that could desthiobiotinylate and capture themselves were recovered, and the relative representations of the four different operons were determined using the previously embedded operon-specific restriction sites (Cla I, Sac I, *Sal*I, or Xho I; Figure 7). By directly comparing the signal intensities of the digested fragments, it should be possible to determine which of the four different configurations was most active in the presence of desthiobiotin.

Unexpectedly, there was no observed enrichment of any particular operon, implying either that the conditions chosen did not favor discrimination or that some technical aspect of the complex set of reactions and recoveries necessary for selection *in vitro* had failed to reflect an underlying preference. We had previously found that higher concentrations of desthiobiotin during a selection for BirA function tended to favor the recovery of enzyme variants with improved k_{cat} values that functioned well under almost single turnover conditions (only two BAP per operon per emulsion bubble). However, $BirA_{6-40}$ should

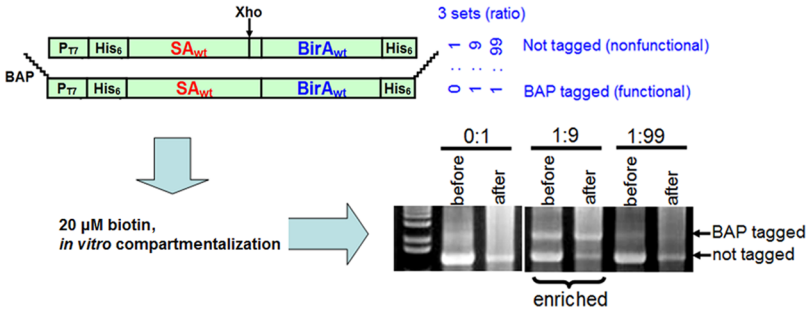

Figure 6. Mock selection using the wild-type operon. Functional wild-type operons and nonfunctional operons lacking a BAP-tag were mixed in three different ratios (0:1, 1:9, and 1:99) with 20 μL biotin for mock selection. Nonfunctional operons were designed with an intergenic Xho I restriction site in order to differentiate them from functional operons. Recovered operons were identified by restriction digest.

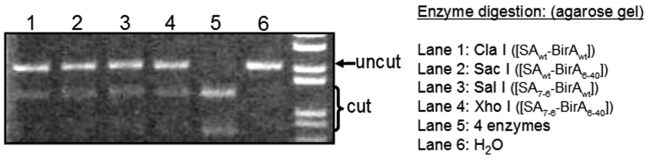

Figure 7. Competition between wild-type and variant synthetic operons. The four operon conjugates with their unique restriction sites were mixed in equivalent ratios and emulsified in the presence of 50 μM desthiobiotin. After one round of selection the samples underwent restriction digestion to identify which operons were in the recovered pool. Lane 1: Cla I([SA_{wt}-$BirA_{wt}$]). Lane 2: SacI([SA_{wt}-$BirA_{6-40}$]). Lane 3: *Sal*I([SA_{7-6}-$BirA_{wt}$]). Lane 4: Xho I([SA_{7-6}-$BirA_{6-40}$]). Lane 5 was cleavage with all four enzymes in parallel. Lane 6 was a negative control in which only water was added, rather than restriction enzymes.

nonetheless have been a much better enzyme with desthiobiotin than $BirA_{wt}$, while the k_{off} of SA_{7-6} should have been 50-fold higher than SA_{wt}. Thus, as long as the wild-type enzyme and streptavidin were not somehow saturated with desthiobiotin in the context of the emulsion in a way that was different than their previous performance in bulk, some combination of desthiobiotin-favoring enzymes and streptavidin variants should have been preferred.

To better determine whether technical issues were preventing the recovery of operons that favored the use of desthiobiotin, we dissected the previous selection scheme into two steps (Figure 8A), ligation of biotin and capture by streptavidin. Both steps have to work well to enrich the desthiobiotin-favoring operon in the selection. To test the first step, we attempted to determine whether [SA_{wt}-$BirA_{6-40}$] and [SA_{7-6}-$BirA_{6-40}$] could modify themselves to larger extents than [SA_{wt}-$BirA_{wt}$] and [SA_{7-6}-$BirA_{wt}$]. To test the second step, we attempted to determine whether SA_{7-6} in fact bound more tightly to modified BAP/operon conjugates than SA_{wt}. In order to test the first step,

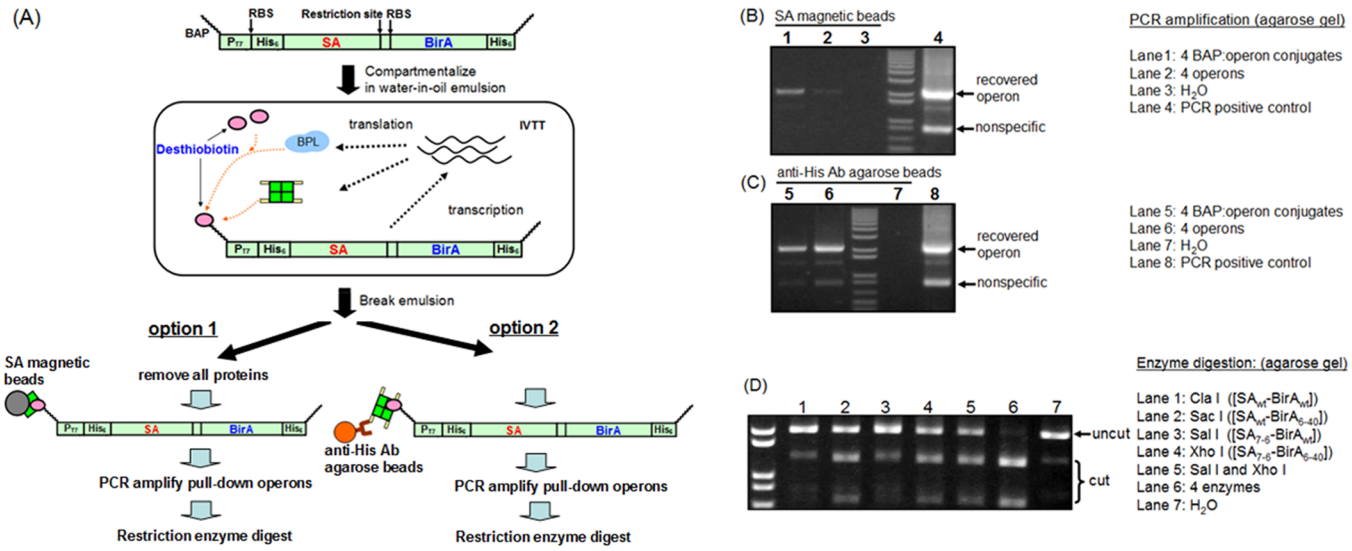

Figure 8. Evaluation of the selection scheme. (A) In comparison to the initial scheme (represented by option 2), all translated proteins were removed after the emulsions were broken and streptavidin magnetic beads were added to pull down the desthiobiotinylated conjugates (option 1). After either option, the pull-down operons were recovered by PCR amplification and identified by restriction digest. (B) PCR amplification of the pull-down operons after BAP-tagged operons (lane 1), nonfunctional operons (lane 2, lacking BAP tags), and water (lane 3) underwent selection with option 1. Lane 4 is amplification of the initial pool (4 BAP/operon conjugates) prior to selection and serves as a positive control. (C) PCR amplification of the operons selected via option 2: BAP-tagged operons (lane 1), nonfunctional operons lacking BAP tags (lane 2), and water (lane 3) underwent selection with option 2. Lane 4 is an amplification reaction of the input pool (4 BAP/operon conjugates and a positive control for amplification. (D) Restriction digests of the recovered conjugates from (B). Lane 1: Cla I([SA_{wt}-$BirA_{wt}$]). Lane 2: SacI([SA_{wt}-$BirA_{6-40}$]). Lane 3: *Sal*I([SA_{7-6}-$BirA_{wt}$]). Lane 4: Xho I([SA_{7-6}-$BirA_{6-40}$]). Additionally, lane 6 and lane 7 were restriction digests of the recovered pool with all 4 enzymes or water, respectively. The two nonspecific bands in parts B to D are side-products from the assembly of the T7 promoter and T7 terminator fragments with the large operon coding sequence.[9,17] These side products did not interfere with correct assembly, as judged by sequencing.

operons that were emulsified and allowed to translate and react in the presence of desthiobiotin were stripped of all proteins and then directly captured on streptavidin beads (rather than attempting their capture with cotranslated streptavidin). Following release, the operons were amplified by PCR. Those operons that had best desthiobiotinylated themselves should predominate and in fact there was a lower background signal (operon pool without BAP tags) than when BAP was present (Figure 8B, lanes 1 and 2). More importantly, the [SA_{wt}-$BirA_{6-40}$] and [SA_{7-6}-$BirA_{6-40}$] operons had clearly modified themselves more than the [SA_{wt}-$BirA_{wt}$] and [SA_{7-6}-$BirA_{wt}$] operons (Figure 8D, lanes 2 and 4). Therefore, the first step of the selection was not apparently the issue. While we have previously used a similar selection to identify $BirA_{6-40}$,[16] we have noted that the size of the DNA construct can restrict capture, and thus it was important to prove that such a selection would still work in the context of the operon and cotranslation. Similar experiments were then carried out using anti-His antibody agarose beads, which would once again rely on cotranslation of functional streptavidins. However, when we performed PCR amplification to recover operons we found that the background signal from untagged operons was consistently higher than that from BAP-tagged operons (Figure 8C, lanes 5 and 6).

Taken together, these results suggested that nonspecific background binding of operons to anti-His antibody agarose beads was large enough to obscure any desthiobiotin-specific signal, and that improved capture of desthiobiotin-bearing operons was required. To reduce this background we assessed different wash buffers and capture approaches. The original wash buffer was TBS with Tween 20 (TBST)[9]). To potentially reduce nonspecific DNA binding, we increased the salt concentration (150 mM, 300 mM, 600 mM, and 1 M NaCl) but did not see significant decrease in nonspecific DNA binding by the anti-His antibody agarose beads. Similarly, longer wash times, larger wash volumes, and more sequential washes did not yield improvements. On the other hand, blocking nonspecific binding sites on anti-His antibody agarose beads with salmon testes DNA (Sigma) seemed to improve signal capture somewhat (Figure 9, lanes 4 and 5). Ultimately, even with preblocked beads and a modified wash protocol (100 mM Tri-HCl, pH 7.4, 150 mM NaCl, 0.1% Tween 20; 7 consecutive washes) a selection performed with 50 μM desthiobiotin still did not reveal any significant enrichment.

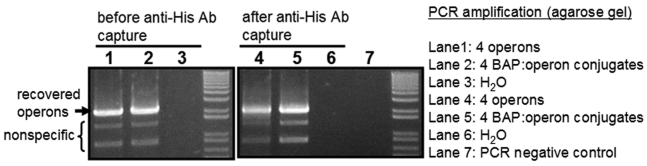

Figure 9. Improved wash buffer reduces nonspecific DNA binding. The anti-His antibody agarose beads were preblocked with salmon testes DNA to reduce the nonspecific binding of BAP/operon DNA and then incubated with the selection reaction after breaking the emulsions. Subsequently, the recovered BAP/operon conjugates (lane 2, before capture; lane 5, after capture), unconjugated operons (lane 1, before capture; lane 4, after capture), and water (lane 3, before capture; lane 6, after capture) were then amplified by PCR. Lane 7 was a PCR control that had water as an input rather than recovered DNA.

Besides the original anti-His Ab agarose beads (Sigma-Aldrich; St. Louis, MO), two additional materials, anti-His Ab resin (R&D Systems; Minneapolis, MN) and cobalt-immobilized metal affinity chromatography (IMAC) magnetic beads (Life Technologies; Carlsbad, CA) were tested in order to see if they might reduce nonspecific binding and enhance signal recovery. However, the latter two proved to have such poor recovery compared to the original anti-His Ab agarose beads that signal enhancement was impossible to obtain (data not shown).

One of the great advantages of cell-free biology is the ability to rationally understand and engineer individual molecules and pathways. By generating a synthetic operon that was capable of its own modification following *in vitro* transcription and translation, we were able to show that the substrate specificities of enzymes and binding proteins could be exploited to guide reaction pathways. Moreover, by coupling the simultaneous expression of different proteins with genetic capture, a basal framework for the directed evolution of wholly *in vitro* systems was developed.

■ METHODS

Synthesis of Operons and Conjugates. *Operon Synthesis.* All oligonucleotides were purchased from Integrated DNA Technologies (IDT; Coralville, IA). A wild-type synthetic operon (2.1 kb) was generated by inserting a wild-type BirA coding sequence (with a C-terminal His-tag), a ribosomal binding site, and a restriction enzyme site into the linear construct used in Levy's experiment[9] (also in the manual of RTS 100 *E.coli* LinTempGenSet His-tag from 5 Prime, Inc.; Gaitherburg, MD). Alternative synthetic operons were generated by replacing the wild-type streptavidin or biotin ligase with an evolved streptavidin (SA_{7-6})[9] or an evolved biotin ligase ($BirA_{6-40}$). $BirA_{6-40}$ was selected from a randomly mutagenized BirA library by the authors and has several substitutions in the wild-type BirA coding sequence (I11V, P85S, M157T, K163E, and I224T) . A complete wild-type operon sequences used in this study can be found in the Supporting Information.

Cross-linking BAP with Operon DNA. The BAP used in these experiments was a custom-made, 23 amino acid peptide (CGGGSGGGSGLNDIFEAQKIEWH) purchased from Bio-Synthesis, Inc. (Lewisville, TX). Operon DNA was first PCR amplified with an oligonucleotide pair containing a 5′-amino group. Amplified operon DNA (2 μg) was then mixed with 500 μL of a 10 mM amine-to-sulfhydryl cross-linker (Sulfo-SMCC, Pierce; Rockford, IL) at room temperature for 1 h. The activated operon DNA was purified with a QIAquick PCR purification kit (QIAGEN; Valencia, CA) and incubated with BAP overnight at room temperature. The resulting BAP/operon conjugates were purified once more with a QIAquick PCR purification kit (QIAGEN).

***In Vitro* Transcription and Translation (IVTT).** CAT-BAP plasmids were taken from the RTS AviTag Biotinylation Kit (Roche; Indianapolis, IN) whereas IVTT reactions refer to the RTS 100 kit (Roche). All other reagents were purchased as indicated. First, either 180 ng operon DNA, 1350 ng CAT-BAP, or both were incubated with 50 μL of an IVTT reaction, 20 μM biotin (Sigma-Aldrich; St. Louis, MO), and 4 mM ATP (Roche) at 30 °C for 1.5 h. The identity of the operon(s) used in each reaction is described in the text. Following incubation, each reaction was mixed with 2× SDS sample-loading buffer. Some reactions were boiled at 95 °C for 10 min before electrophoresis and others were not, as denoted in the text. Western blots were stained with either anti-Histidine antibody alkaline phosphatase (Sigma-Aldrich) or streptavidin alkaline phosphatase (Promega; Madison, WI).

***In Vitro* Compartmentalization Selection Scheme.** *In vitro* compartmentalization selections were carried out as previously described[9,14] with slight modifications. The IVTT reaction used in the selections was biotin-depleted by streptavidin magnetic beads (Dynabeads M-270 Streptavidin, Life Technologies; Carlsbad, CA). BAP/operon conjugates (2 ng) were added to the biotin-depleted IVTT reaction containing 0.5% sodium deoxycholate, 50 μM desthiobiotin (Sigma-Aldrich), and 4 mM ATP. The reactions were emulsified by addition to 500 μL of an oil/surfactant mixture containing mineral oil, 4.5% Span 80, 0.5% Tween 80, and 0.1% Triton X-100. The mixtures were stirred for 4 min on ice, after which the emulsions were transferred to 2.0 mL Eppendorf tubes and incubated at 30 °C for 2 h.

Emulsions were broken by 3 × 900 μL extractions with water-saturated ether in the presence of 500 μL Tris-buffered saline (TBS; 100 mM Tris-HCl, pH 7.4, 150 mM NaCl). Following extraction, excess ether was removed by vacuum centrifugation for 10 min at room temperature. The remaining aqueous phase was added to 500 μL TBS with Tween 20 (TBST; 100 mM Tri-HCl, pH 7.4, 150 mM NaCl, 0.1% Tween 20) containing 100 μL anti-His antibody agarose beads. After 1 h incubation at room temperature on a tube rotator, the beads were washed with 7 × 1 mL TBST. The captured conjugates were released from the beads using a QIAquick PCR purification kit and were recovered via PCR amplification. Recovered operon DNA was incubated with restriction enzymes Cla I, Sac I, *Sal*I, or Xho, respectively (New England BioLab, Inc.; Ipswich, MA), and was identified via electrophoresis.

In some cases (Figure 8, option 1), the BAP/operon conjugates were purified after the emulsions were broken; in this additional step, all translated proteins were removed using a QIAquick PCR purification kit before the conjugates were incubated with 100 μL streptavidin magnetic beads. In other experiments (Figure 9), the anti-His antibody agarose beads were blocked with 20 μg salmon testes DNA (Sigma-Aldrich) prior to incubation with BAP/operon conjugates.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Wild-type operon sequences used in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +1-512-471-6445. Fax: +1-512-471-7014. E-mail: andy.ellington@mail.utexas.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Welch Foundation (F-1654), the Defense Advanced Research Projects Agency (HR-0011-12-C-0066), and the Department of Defense, Air Force Office of Science Research (FA9550-10-1-0169).

■ ABBREVIATIONS

IVTT, *in vitro* transcription and translation; DHAP, dihydroxyacetone phosphate; BAP, biotin acceptor peptide; SA, streptavidin; BirA, biotin ligase gene; His-tag, 6xHistidine tags; CAT-BAP, chloramphenicol acetyltransferase fused to biotin acceptor peptide

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