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Expanding Reactivity in DNA-Encoded Library Synthesis via Reversible Binding of DNA to an Inert Quaternary Ammonium Support

Dillon T. Flood[1,⊥], **Shota Asai**[1,⊥], **Xuejing Zhang**[1], **Jie Wang**[1], **Leonard Yoon**[1], **Zoë C. Adams**[1], **Blythe C. Dillingham**[1], **Brittany B. Sanchez**[§], **Julien C. Vantourout**[1], **Mark E. Flanagan**[2], **David W. Piotrowski**[2], **Paul Richardson**[3], **Samantha A. Green**[1], **Ryan A. Shenvi**[1], **Jason S. Chen**[§], **Phil S. Baran**[1,*], **Philip E. Dawson**[1,*]

[1]Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, United States

[2]Pfizer Medicinal Chemistry, Eastern Point Road, Groton, CT 06340, United States

[3]Pfizer Medicinal Chemistry, 10578 Science Center Drive, San Diego, CA 92121, United States

[§]Automated Synthesis Facility, Scripps Research, 10550 North Torrey Pines Road, La Jolla, CA 92037, United States

Abstract

DNA Encoded Libraries have proven immensely powerful tools for lead identification. The ability to screen billions of compounds at once has spurred increasing interest in DEL development and utilization. Although DEL provides access to libraries of unprecedented size and diversity, the idiosyncratic and hydrophilic nature of the DNA tag severely limits the scope of applicable chemistries. It is known that biomacromolecules can be reversibly, non-covalently adsorbed and eluted from solid supports, and this phenomenon has been utilized to perform synthetic modification of biomolecules in a strategy we have described as reversible adsorption to solid support (RASS). Herein, we present the adaptation of RASS for a DEL setting, which allows reactions to be performed in organic solvents at near anhydrous conditions opening previously inaccessible chemical reactivities to DEL. The RASS approach enabled the rapid development of $C(sp^2)$-$C(sp^3)$ decarboxylative cross-couplings with broad substrate scope, an electrochemical amination (the first electrochemical synthetic transformation performed in a DEL context), and improved reductive amination conditions. The utility of these reactions was demonstrated through a DEL-rehearsal in which all newly developed chemistries were orchestrated to afford a compound rich in diverse skeletal linkages. We believe that RASS will offer expedient access to new DEL reactivities, expanded chemical space, and ultimately more drug-like libraries.

*Corresponding Author: pbaran@scripps.edu, dawson@scripps.edu.

⊥AUTHOR CONTRIBUTIONS

These authors contributed equally.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Detailed experimental procedures and data (PDF)

Introduction

Sydney Brenner and Richard Lerner's seminal 1992 report established a profound, new type of combinatorial chemistry.[1] They postulated that individual chemical transformations could be encoded in DNA, resulting in libraries of unprecedented size and chemical diversity.[1] Since their proposal, many groups and pharmaceutical companies have invested heavily into DEL research and technology.[2–4] Modern, industrialized DEL libraries routinely contain billions of compounds that are screened for biological activity, all at once.[2–4] Although many success stories have resulted from DEL-based discovery campaigns, including multiple therapeutic candidates in clinical trials, the synthetic pathways employed during DEL construction lag severely behind the unconstrained methodologies of modern organic and medicinal chemistry.[2,5–7] This glaring disparity can be attributed to the idiosyncratic reaction requirements of the encoding molecule, DNA, which manifests in three confounding ways (Figure 1A): (1) as DNA is insoluble in most organic solvents, reactions need to be conducted in the presence of water, (2) highly diluted conditions are required (<1 mM, due to solubility considerations) making bimolecular reactions sluggish, and (3) a high degree of chemoselectivity is required so as not to disturb the functional-group rich nucleotide backbone.[2,5,6,8] The pragmatic result of these factors is that most modern DEL libraries, while exhibiting broad diversity from the monomers employed, are comprised of a severely limited set of skeletal linkages.[2,9] To be sure, these are mostly comprised of amides, biaryls, and C–N linkages through 1,3,5-triazine "hubs" which create planar libraries lacking significant 3D shape.[2,9] Thus, although great numbers of compounds can be generated, often, drug likeness and implicit diversity suffer.[9] Even with those caveats, such libraries have shown some success for lead identification, fueling a resounding call for the development of more interesting DEL compatible chemistries and ultimately more drug-like libraries.[11]

Numerous labs have chosen to directly address this challenge by adapting reaction conditions to fit the unusually demanding requirements of DEL synthesis.[2,12–14] Although this approach has encountered some success, it has often proven to be a time consuming and laborious endeavor. For instance, the recently developed DNA-compatible Giese reaction developed by our lab (PSB) for use in the construction of high value sp^3-sp^3 linkages in DEL required a unique method for kinetic evaluation and optimization involving hundreds of optimization experiments.[13] Clearly, adapting organic reactions for use in dilute water presents many difficulties as many interesting bond forming reactions invoke water-incompatible reagents or intermediates. Thus, the dominant paradigm in this field is to bring organic reactions into water, whereas the simplest approach might just be to bring DNA into organic solvents.

An Organic Approach to DEL-Chemistry

Recent work in our lab (PED) exploited peptide and protein immobilization as a tool for synthetic chemistry. The Reversible Adsorption to Solid Support (RASS) approach, leverages the multivalent binding kinetics of biomacromolecules to selectively bind to and elute from an *inert* solid support, such as reversed phased silica.[16,17] Critically, the differences in binding kinetics of biomacromolecules and small molecules allow for

adsorbed biomolecules to react with small molecule reagents with the same logic as employed in conventional (covalently bound) solid phase organic synthesis strategies.[16,17] Thus, excess small molecule reagents are simply washed away while the polyvalent macromolecule remains adsorbed to the solid support.[16,17] This allows for the use of organic solvents and reaction conditions that would be otherwise incompatible for the biomolecule.[16,17]

Applied to DEL, the RASS strategy could dramatically expand the toolkit of available organic reactions for library construction by bringing DNA into organic solvents and protecting the backbone from reagent-induced degradation. To identify an appropriate solid support, we screened a series of commercially available inert resins/solid supports for their ability to selectively bind and elute a small DNA-fragment which mimics that typically employed in DEL (Figure 1). An important additional requirement was that the resin itself should not interject its own reactivity profile (eg. acids, bases or nucleophiles). Hydrophobic resins such as reversed phase silica could competently bind DNA, but unfortunately, the weak binding led to premature elution in organic media (Figure 1B). Weak anion exchange resins were ruled out as well, because most bear basic, nucleophilic, or otherwise reactive moieties (Figure 1B).[15] The potential of DNA immobilization is supported by the work of Harbury, who demonstrated peptide and peptoid synthesis on DNA using a weak anion exchange resin (DEAE Sepharose).[18,19] However, the presence of abundant hydroxylated functionality and tertiary amines has limited the scope of employable reactions (*vide infra*).

In contrast, a mixed mode polystyrene strong anion exchange resin, (Phenomenex, *Strata-XA*) which contains a butyl quaternary ammonium moiety, proved to be an excellent platform for RASS on DNA (Figure 1B). Such resins incorporate both hydrophobic interactions (polystyrene, butyl substituents) and electrostatic interactions (quaternary amine) and effectively anchor the DNA in a polyvalent manner. When model DEL headpiece with a pendent free amine, SI-1, in PBS (100 μM DNA), was added to the resin, it efficiently adsorbs to the surface, as indicated by the lack of DNA detected in the binding supernatant (Figure 1C). The bound DNA SI-1 could then be eluted into a high salt elution buffer, an approach that is widely applied in molecular biology.[20] Importantly, the *Strata-XA* resin allows the DNA-resin complex to be transferred from aqueous solution into neat organic solvents. Following removal of the solvent from the resin, the DNA SI-1 can be washed with aqueous buffer, eluted from the resin with salt and isolated through ethanol precipitation. Failures encountered with numerous other resins that were tested can be attributed to a lack of strong initial DNA binding or lack of retention in organic solvents. This represents a robust platform for the controlled, reversible binding and elution of DNA fragments to facilitate manipulation in organic media in ways that are not possible using conventional aqueous DEL techniques. As an initial proof of concept, a simple amide bond formation reaction SI-2 was performed on a model DEL headpiece SI-1 with organic solvents (CH_2Cl_2, THF, DMF, Dioxane, DMSO, and MeCN, see SI) commonly used in small molecule chemistry (>75% yield). Interestingly the observed yields were consistent with solvent depenedencies for small molecue carbodiimide couplings.[21]

Expanding the Reaction Space of DEL

To demonstrate the advantage of utilizing an inert support via RASS, a reaction that was previously recalcitrant in a DEL-setting was enabled: forging highly desirable sp^2-sp^3 linkages from ubiquitous building blocks and reagents. Specifically, the cross coupling between abundant (het)aryl halides and redox active esters (RAEs) derived from simple, ubiquitous, carboxylic acids was pursued. Numerous attempts at this reaction built off the success of the Giese reaction, by enlisting aryl iodides and RAEs in aqueous solution using Zn as a reductant under Ni-catalysis (SI).[13] Recent findings from the Molander group demonstrated the feasibility of such a bond formation, albeit only in the case of carboxylic acids bearing a radical stabilizing α-heteroatom.[22] Aiming for a transformation without this inherent limitation, 4-iodobenzoic acid was coupled to a common DEL head piece[5,8] resulting in DNA-Ar-I **1** (Figure 2A), setting the stage for a coupling with unstabilized RAE **2**. Conventional DEL-based conditions were interrogated (see SI for details) resulting in a maximum conversion of *ca.* 5% to **3** (entry 2). It was reasoned that a more soluble reductant capable of reducing both the RAE and the Ni^{II} precatalyst to its active low valent Ni state would be superior. Reports from the Weix[23] group and ours[24] demonstrated that low valent Ni is competent to reduce RAEs and form transient alkyl radicals while reports from the Shenvi group show that the combination of various silanes and base can reduce Ni^{II} to low valent Ni.[25–27] "RubenSilane" (isopropoxy(phenyl)silane, RS), developed by the Shenvi group, was therefore employed but unfortunately did not improve the conversion (entry 3). Numerous other attempts were pursued changing various reagent concentrations, solvent systems and surfactants to no avail.

As reported previously, the protocols used to improve the yield of a DEL-based reaction are quite different due to the kinetics of highly diluted conditions in water.[13] In stark contrast, the RASS-based approach enabled a more traditional optimization strategy that ultimately led to success. Thus, although application of Zn-based conditions to this system resulted in a heterogeneous dual surface reaction that could not be readily optimized (see SI), the homogeneous solution using RS as a soluble reductant proved immediately fruitful (entry 6) providing trace product **3** (Figure 3B, entry 6). From this initial hit, the reaction was optimized to yield **3** in 84% after 18-24 h (entry 8) by increasing the concentration of Ni catalyst and reductant (see SI for further details). The reaction time could be dramatically reduced by simply subjecting the substrate to two cycles of reagent addition (82% yield, 3 h total reaction time, entry 9). In striking contrast, extremely limited DNA recovery was observed (<5%) and only trace product was detected when utilizing sepharose or polystyrene-based weak anion exchange systems, with the optimized reaction conditions. Most likely, the basic nature of the reaction led to deprotonation of the tertiary amino groups of the DEAE sepharose, which are critical to DNA retention, and ultimately led to premature DNA release. This trend held true with a variety of other weakly binding (weak anion exchangers and reversed phase) resins (Entry 10, See SI).

With optimized conditions in hand, the utility of the reaction was demonstrated with >40 different relevant carboxylic acids. Excellent to satisfactory yields were obtained by utilizing either isolated RAEs or RAEs generated *in-situ* without altering conditions (Figure 2C). Multiple different (het)aryl iodides (>5), substituted at various positions, are also competent

coupling partners affording similar yields. Finally, the scope could be further expanded to aryl bromides which are more widely available. DNA-bound aryl bromide could be employed simply upon addition of 250 mM NaI forming coupled products, albeit in slightly attenuated yields. In accord with standard practice in DEL synthesis, yields are determined by integration of the total UV absorbance at 260 nm via HPLC/MS of the crude reaction mixture.[13,28,29] This protocol provides an accurate measure of relative yield on nanomole scale as DNA is the dominant chromophore.[28,29] To confirm the robustness of our measurements, all of experiments using our preferred Protocol B (Figure 2B, Entry 9) were conducted in triplicate.

Within the known realm of DNA-compatible chemistry, oxidative reactions are rare. This is because chemical oxidants lack chemoselectivity, indiscriminately attacking the sensitive functional groups found on the purine portion of the DNA backbone leading to degradation.[30–34] Such an event in the context of DEL is deleterious and irreversible as critical encoding information can be permanently lost.[31] Synthetic organic electrochemistry offers an opportunity to precisely control redox-potentials and provide superior selectivity but has never been applied in such a context.[35,36] This is likely due to the charged nature of DNA that, upon exposure to an electrode surface, will irreversibly be adsorbed. RASS offers a unique opportunity to site-isolate DNA thus rendering it amenable to redox transformations accessible electrochemically that might otherwise destroy it.[37] To test this hypothesis we turned to our recently reported Ni-catalyzed electrochemical aryl amination due to its highly modular nature and potential to improve upon the harsh conditions that are currently used in analogous Pd- and Cu-based reactions (Figure 2A).[38–40] Indeed, Ullmann-Buchwald-Hartwig type aminations are workhorse reactions to generate diversity in medicinal chemistry and drug discovery yet robust applications in the context of DEL are lacking.[9,41] The DEL-compatible variants require exotic ligand systems, scavengers and high temperatures due to the dilute aqueous conditions employed.[40] The Ni-catalyzed system was therefore investigated to probe both the DEL-compatibility of electrochemistry and to explore potential synergies with the RASS approach.

As predicted, attempts to employ electrochemical amination with a traditional aqueous system led to no recoverable DNA (Figure 3B, entry 1). In striking contrast, the site-isolation garnered by the RASS approach led to promising results on the first attempt. Thus, adapting the published conditions employing a $Ni(bpy)_3Br_2$ precatalyst (50 mM), DBU (300 mM), and 4 mA of current for 3 hours, furnished 37% of the alkyl-aryl amine **45** from aryl iodide **1** (entry 2).[38] Although this initial result was encouraging, the major side product was the corresponding phenol, likely a result of hydroxide formation *in situ*. Removing DBU reduced the hydroxylated side product at the expense of slightly attenuated yields of **45** (34%, entry 3). The addition of 4 Å molecular sieves to the base-free protocol reduced the hydroxylated side product and moderately boosted conversion (50%, entry 5). Finally, increasing concentration of the Ni precatalyst (100 mM) brought yields to acceptable levels (74%, entry 6). With optimized conditions in hand, various alkyl, and heteroaryl amines as well as one amide proved competent coupling partners with yields ranging from good, to useable (Figure 3C). Interestingly, aniline and most of the piperazines tested proved to be incompetent coupling partners (See SI). The observed yields are in line (20-70%) with those

previously seen using the harsh conditions mentioned above.[40] More importantly, this study provides a proof of concept for the use of electrochemical methodologies in the demanding context of DEL.

The RASS-based DEL platform presented above not only enables access to new reaction manifolds, it also can improve the scope of known DEL-compatible reactions. Towards this end, reductive amination, listed as among the top-three reactions used in medicinal, is often employed in DEL-library synthesis despite the fundamental limitation that the amine partner must be used in excess.[2,9,29,31] Specifically, performing reductive aminations between carbonyl-containing compounds and an amine partner loaded on-DNA has proven difficult and inefficient. For example, within Pfizer's DEL-based reductive amination screen using 218 different carbonyl compounds, less than 50 provided yields above 50%. Acyclic ketones, benzylic carbonyls, and sterically hindered carbonyls were all challenging substrates. Noticing this limitation in current DEL technology conditions were developed to overcome this challenge. Reductive aminations are typically performed with the carbonyl on-DNA and a vast excess of amine in solution.[31,42] These conditions force the equilibrium of the reducible imine species and is a common tactic in borohydride mediated strategies.[43] In classical organic chemistry conditions the use of excess carbonyl is generally avoided, as dialkylated products can result.[43] After significant optimization, conditions were identified to couple 4-heptanone **68** to DNA-headpiece **66,** a commercially available scaffold that is widely employed in DEL studies, to deliver **71** in 75% yield (Figure 4B, entry 8). Multiple ketones and aldehydes proved viable in this transformation with yields ranging from excellent to satisfactory. The conditions identified also allow for the use of other on-DNA primary and secondary amine building blocks in good yield. The enabling effect of boric acid could also be enlisted in a purely aqueous DEL system (Entry 9). Unlike the cross-coupling and e-amination described above, reductive amination is not strictly enabled using a RASS approach. Releasing the shackles of conventional DEL reaction development, a classical organic approach inadvertently led us to a new set of $B(OH)_3$-mediated conditions that can function with or without RASS. As with prior examples, the reactions reported in Figure 4C were conducted in triplicate.

RASS-Enabled DEL: Toward Creating Libraries

In order for a new strategies to be adopted for use in DEL library construction, individual diversity generating steps must be sufficiently chemoselective and DNA recovery sufficiently efficient for at least 30% of the DNA to be recovered.[31] While developing the above sp^2-sp^3 cross coupling, it became apparent that the reaction would result in sub-optimal DNA recovery (~20%). Fortunately, this problem was addressed by optimizing the EtOH precipitation procedure. Increasing the ratio of EtOH:elute buffer ratio from 5:1 to 10:1 resulted in satisfactory DNA recovery of ~30%. The observed low DNA recovery is likely a result of reaction workup and precipitation conditions, as opposed to actual reactivity based degradation. Fortunately, we did not observe any DNA recovery problems in any of the other reactions outlined above.

To further establish compatibility with library construction, the viability of the product DNA was confirmed. After a completion of a full RASS cycle (bind, reaction, elute, EtOH

precipitate) **3** was ligated to a 20-mer dsDNA primer, commonly used in DEL builds. The ligation efficientcy was identical to **1,** DNA that had not undergone a RASS cycle (SI).[14] This suggests that a DEL library member can be enzymatically encoded after an organic reaction in the RASS cycle.

As a DEL library is assembled, the encoding DNA tag increases in length. The applicability of the RASS approach with an elongated molecule, was demonstrated by selectively binding and eluting a double stranded 40 base pair oligo from the same solid support used in reaction development (Srata-XA) as well as a related solid support optimized for molecules larger than 10 kDa (Srata-XAL). This larger DNA, representative of DNA after the first encoding cycle, bound efficiently and eluted from the solid support with the same properties as the smaller DNA headpiece. Figure 5 illustrates the multistep process of DEL-rehearsal utilizing the three reactions reported herein. This three-cycle synthesis was devised using cross-coupling/deprotection, reductive amination, and finally electrochemical amination. A mock DEL build was performed using three cycles of chemistry, resulting in a skeleton, rich in linkage diversity (Figure 5). The DNA headpiece-small molecule intermediates were eluted from resin after each cycle, as would be requisite in a DEL build. Starting with **1** a decarboxylative sp^2-sp^3 cross coupling with Fmoc-proline was performed and the resulting product was deprotected. A reductive amination was performed on the resultant free amine, which allowed for the introduction of another aryl iodide moiety. The aryl-iodide product was then utilized in an electrochemical amination to produce the final compound **90** in 9% yield over 4 steps. This provides an example that these diversity generating chemistries can be coupled in series, through multiple RASS cycles, to yield on-DNA products that are rich in therapeutically relevant functionality.

Structural Insights

In addition to facilitating reactions in neat organic solvents, the RASS-DEL approach is distinguished by a significant resistance to DNA damage. This effect that was observed during the above studies could be a result of the DNA maintaining significant double helix structure while adsorbed to the support. To investigate the conformation of the imobnilized DNA we used fluorescence microscopy to observe the dsDNA specific interaction of a DNA intercalator (Figure 6). A 40-base pair dsDNA was designed to mimic the encoding molecule in a growing DEL library. As a control, two non-hydridizing 40-mers of single stranded DNA were used. Each DNA was adsorbed to Phenomenex Strata-XAL resin, and then treated with SYBR Green which increases in fluorescence when intercalated into dsDNA. The resins were washed in acetonitrile (5x) and suspended in ethanol for microscopy. The relative fluorescence observed by. confocal fluorescence microscopy was consistent with the dsDNA construct remaining in the duplex form (strong relative fluorescence).[44–46] All experiments were normalized to the total DNA content.

As shown in Figure 6, the average fluorescence intensity of the double stranded was much greater than that of the single DNA and the stained resin. The increase in fluorescence between the double stranded and single stranded samples was statistically significant (p <0.0001) while the difference in fluorescence between the single stranded DNA and resin (without any DNA) was insignificant (p >0.05). If the DNA was predominantly denatured

upon adsorbing to the resin, a reduced fluorescence output would have been observed, similar to that of the single stranded control. The observed difference in average fluorescence intensity supports the notion that DNA is double stranded while adsorbed to the resin.[44,45]

Conclusion

As the use of DEL in drug discovery increases, there is a growing need to expand the tool box of organic transformations available to practitioners. In this study we have outlined how strong-anion exchange resins based on quaternary ammonium moieties (RASS) can be used to facilitaite reactions in orgainic solvents. Reactions previously outside the realm of DEL including, complex sp^2-sp^3 cross couplings and electrochemical aminations have been demonstrated. The realization of a $B(OH)_3$-mediated reductive amination with expanded generality in both aqueous and RASS contexts was also discovered. Application of these three reactions in a DEL-rehearsal suggests that this technology is applicable to real-world library construction. Finally, structural studies have confirmed that DNA is still double-stranded while bound to the resin, a necessary proviso to protect the encoding molecule. As such, it is anticipated that this approach to DEL construction will find widespread utility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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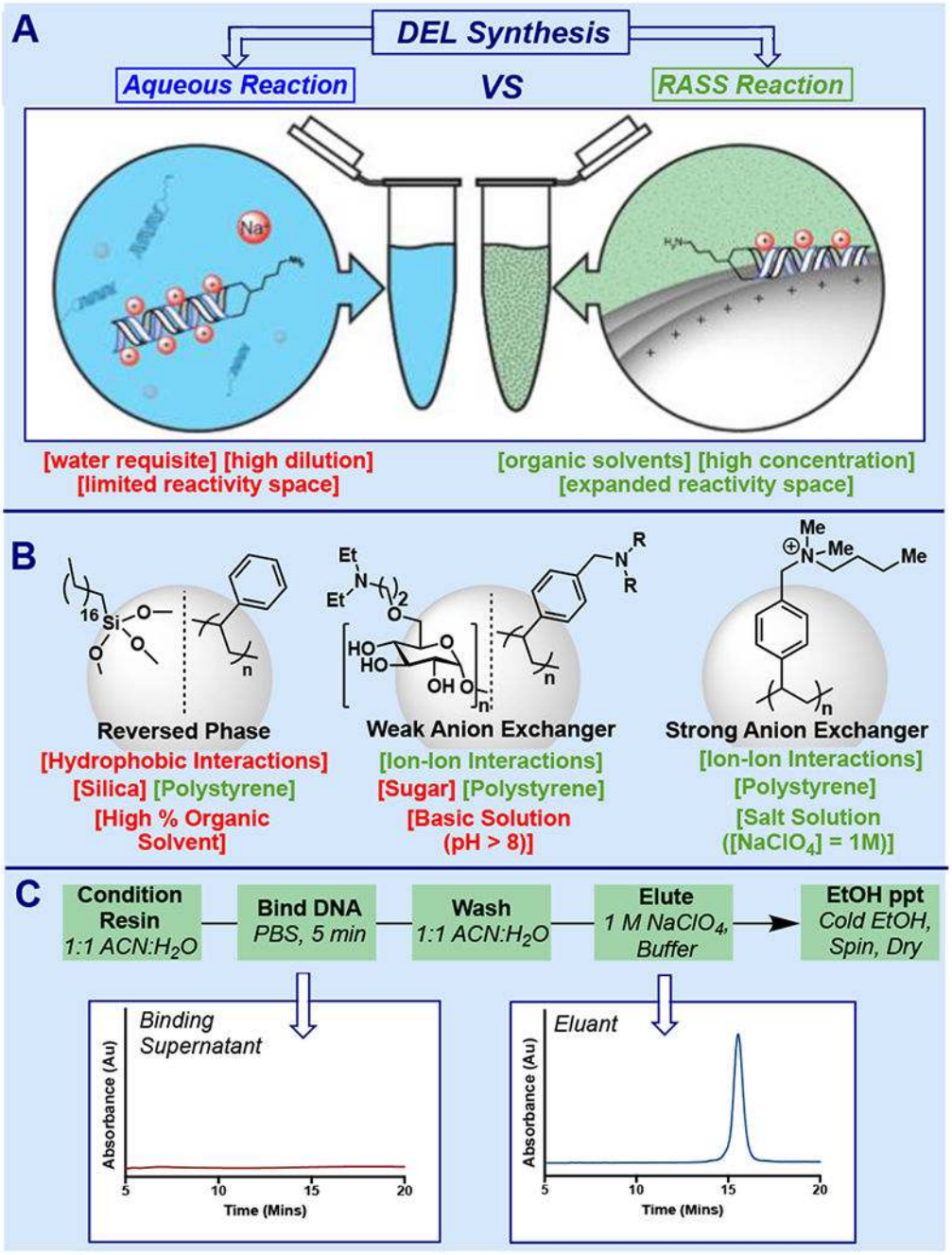


Figure 1.

DEL Synthesis *via* RASS. (A) Aqueous vs RASS reactions for DEL. (B) Resins selection considerations. (C) Basic DEL RASS workflow. DNA binding and elution of DNA by HPLC

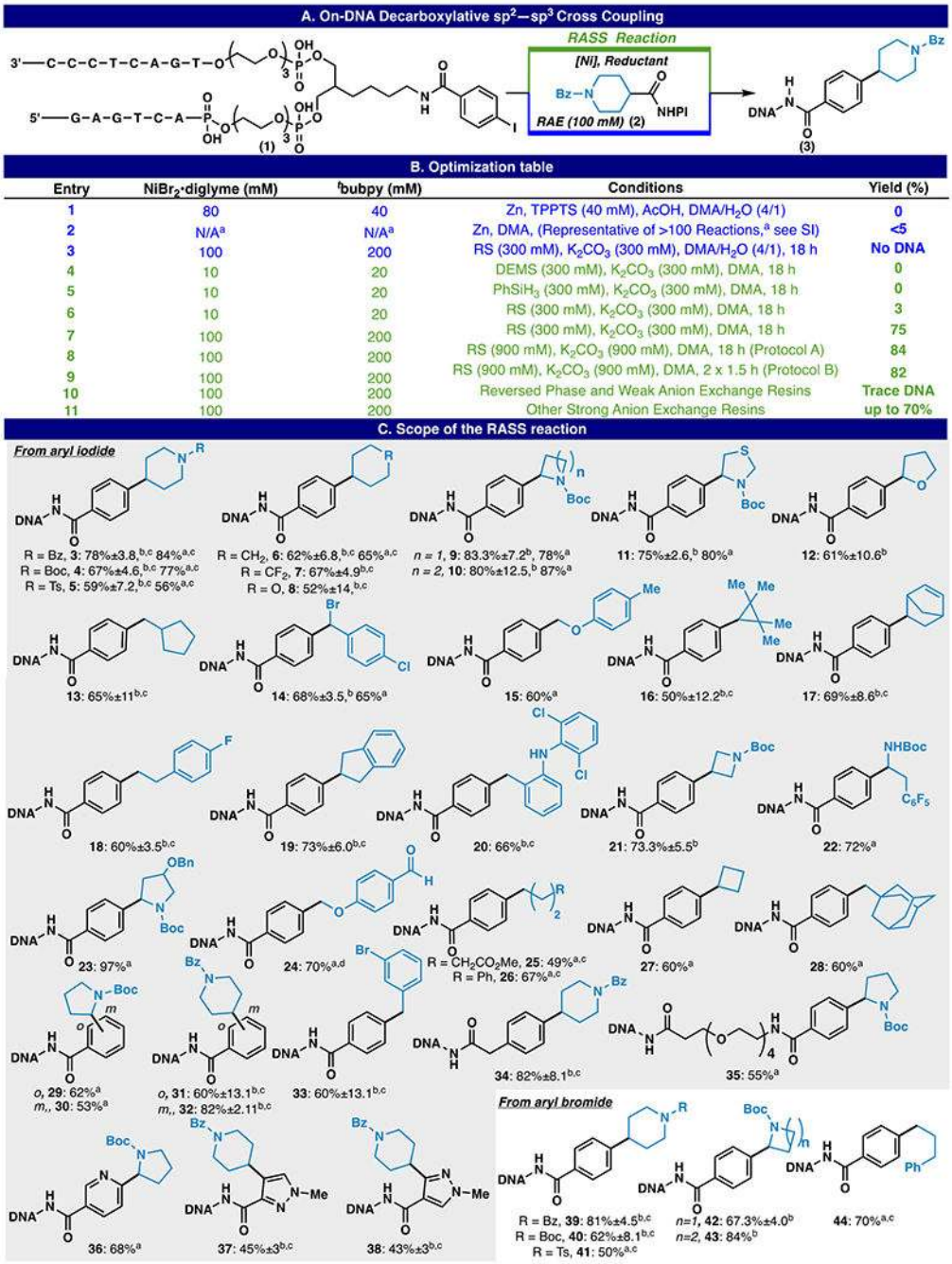
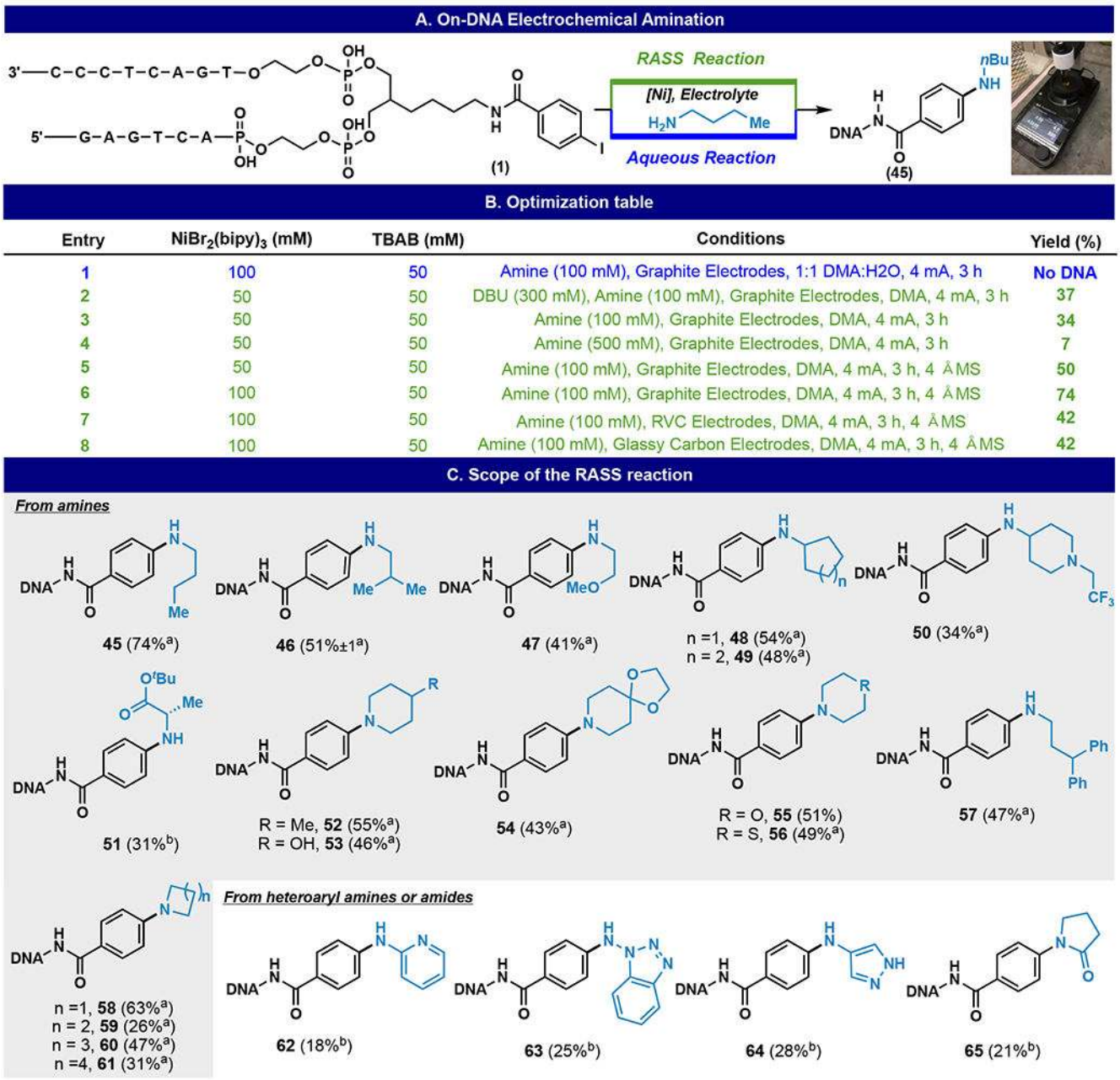


Figure 2.

On-DNA Decarboxylative sp^2-sp^3 Cross-Coupling. (A) Reaction Scheme. (B) Optimization Table; [a] Reactions include small molecule reactions under DEL conditions and on-DNA DEL reactions (C) Scope Table; [a] Protocol A (18 hr), [b] Protocol B (2 × 3 hr), [c] Isolated RAE, [d] 1:1 desired rroduct:reduced product.



Entry	$NiBr_2(bipy)_3$ (mM)	TBAB (mM)	Conditions	Yield (%)
1	100	50	Amine (100 mM), Graphite Electrodes, 1:1 DMA:H2O, 4 mA, 3 h	No DNA
2	50	50	DBU (300 mM), Amine (100 mM), Graphite Electrodes, DMA, 4 mA, 3 h	37
3	50	50	Amine (100 mM), Graphite Electrodes, DMA, 4 mA, 3 h	34
4	50	50	Amine (500 mM), Graphite Electrodes, DMA, 4 mA, 3 h	7
5	50	50	Amine (100 mM), Graphite Electrodes, DMA, 4 mA, 3 h, 4 ÅMS	50
6	100	50	Amine (100 mM), Graphite Electrodes, DMA, 4 mA, 3 h, 4 ÅMS	74
7	100	50	Amine (100 mM), RVC Electrodes, DMA, 4 mA, 3 h, 4 ÅMS	42
8	100	50	Amine (100 mM), Glassy Carbon Electrodes, DMA, 4 mA, 3 h, 4 ÅMS	42

Figure 3.

On-DNA Electrochemical Amination. (A) Reaction Scheme. (B) Optimization Table (C) Scope Table, [a] Conditions from Entry 6, [b] Conditions from Entry 6 + DBU (100 mM).

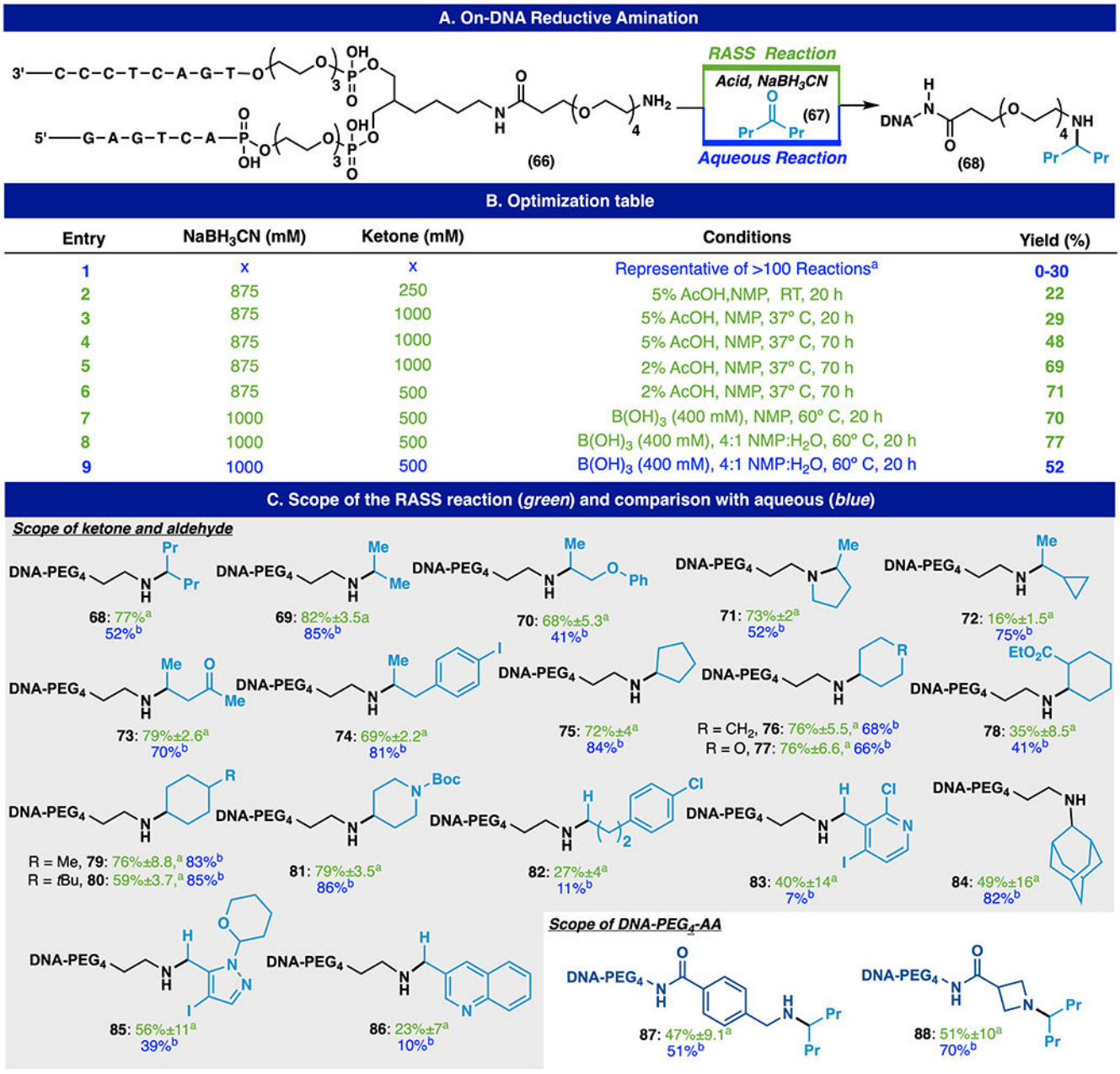


Figure 4.

On-DNA Reductive Amination. (A) Reaction Scheme. (B) Optimization Table, [a] Reactions conducted by Pfizer and results communicated through personal communications (C) Scope Table, [a] On Resin, [b] Aqueous Reaction.

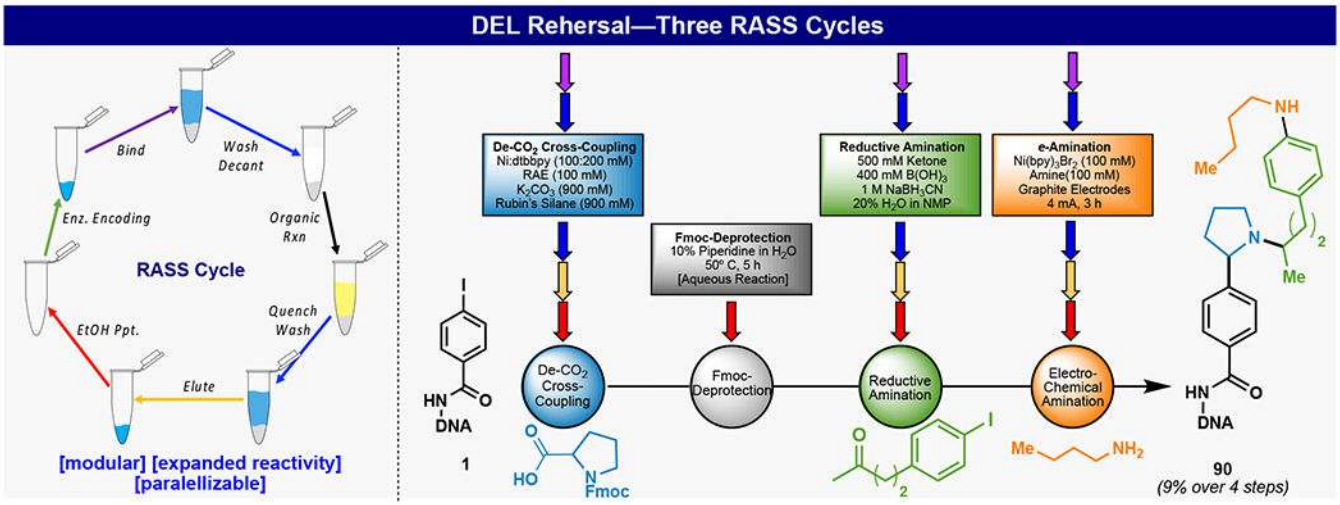


Figure 5.
DEL—Rehearsal, Graphical Workflow Representation and Synthesis of **90**.

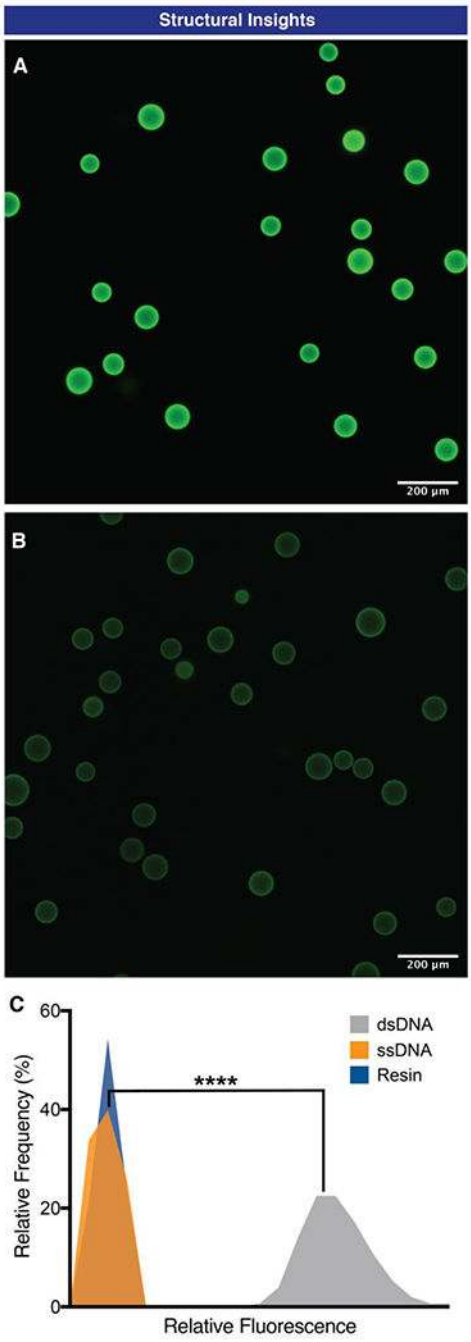


Figure 6.

Structural Insights. Confocal Microscopy Image of Resin with (A) Double Stranded DNA (B) Single Stranded DNA Adsorbed and Stained with SYBR Green. (C) Quantification of Resin Fluorescence.