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A biomolecule-compatible visible-light-induced azide reduction from a DNA-encoded reaction-discovery system

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Using a system that accelerates the serendipitous discovery of new reactions by evaluating hundreds of DNA-encoded substrate combinations in a single experiment, we explored a broad range of reaction conditions for new bond-forming reactions. We discovered reactivity that led to a biomolecule-compatible, Ru(II)-catalysed azide-reduction reaction induced by visible light. In contrast to current azide-reduction methods, this reaction is highly chemoselective and is compatible with alcohols, phenols, acids, alkenes, alkynes, aldehydes, alkyl halides, alkyl mesylates and disulfides. The remarkable functional group compatibility and mild conditions of the reaction enabled the azide reduction of nucleic acid and oligosaccharide substrates, with no detectable occurrence of side reactions. The reaction was also performed in the presence of a protein enzyme without the loss of enzymatic activity, in contrast to two commonly used azide-reduction methods. The visible-light dependence of this reaction provides a means of photouncaging functional groups, such as amines and carboxylates, on biological macromolecules without using ultraviolet irradiation.

Reaction discovery provides new tools for chemical synthesis, illuminates new modes of reactivity and can accelerate the discovery of molecules with properties that are desired in many fields of science. Traditional approaches to reaction discovery focus on one transformation at a time and typically search different reaction conditions for their ability to effect the needed transformation, starting with one set of substrates. Recently, several research groups took a complementary approach to reaction discovery that searches many substrate combinations for new reactivity under various reaction conditions without focusing on any particular type of transformation[1–3]. Our group previously developed a DNA-templated reaction-discovery system using (deoxy)ribonucleic acid (DNA) hybridization to organize substrate pairs in a single solution such that bond formation transfers a biotin group to the DNA template that encodes the identities of the reactive substrates[1]. *In vitro* selection, polymerase chain reaction (PCR) amplification and DNA microarray analysis subsequently reveals the identities of reactive substrate pairs. A second-generation, DNA-encoded reaction-discovery system expanded the set of compatible reaction conditions to include those that do not support DNA hybridization, including organic solvents and elevated temperatures[3]. Here we report results from the application of this second-generation system to explore regions of transition-metal and reaction-condition space, and describe the development of a resulting Ru(II)-catalysed azide reduction induced by visible light and compatible with a wide variety of functional groups, including those present on biological macromolecules.

The reduction of azides to give amines is an important transformation in organic synthesis. Readily prepared in regio- and stereoselective processes, azides are used widely as amine precursors[4]. Current methods for this reduction include metal-hydride reduction[4], catalytic hydrogenation[5], treatment with thiols[6] and the phosphine-based Staudinger reduction[7], as well as other methods that require harsher conditions[4]. Although widely used, these methods have significant limitations. In general, azide-reduction reactions that involve metal hydrides or catalytic hydrogenation cannot be used on substrates with acidic groups, such as alcohols and acids, or with unsaturated groups, such as alkenes, alkynes and carbonyls[5]. Similarly, the Staudinger reduction is not compatible with disulfides and alkyl halides[8,9] and can require elevated temperatures and lengthy reaction times[7]. The limitations of the existing azide-reduction methods may explain the near absence of examples of azide reduction in biological macromolecules[10], despite the common use of both azides[11–14] and amines[15–17] as chemical handles in proteins, nucleic acids and oligosaccharides. In addition to being highly tolerant of a variety of acidic and basic functional groups, a reaction compatible with biological macromolecules must also operate efficiently in neutral aqueous environments[18]. The azide reduction discovered and developed in this work exhibits these key features of a reaction compatible with biological macromolecules.

Results and discussion

Choice of substrates and reaction conditions. The second-generation DNA-encoded reaction-discovery system[3] requires a pool of $n \times m$ DNA-linked substrate pairs assembled from two separate subpools of n and m substrates (Fig. 1a). We selected 30 substrates (two groups of 15) that comprised 28 distinct functional groups based on two criteria: (1) substrates with high latent energy that also exhibited kinetic stability in unactivated form and (2) substrates anticipated to be compatible with DNA and pool-assembly conditions (Fig. 1b). Collectively, these substrates represent 225 (15×15) potentially reactive substrate pairs. We assembled the system using a modified version of our protocol reported previously, starting with 5 nmol of DNA-linked substrates[3]. After enzymatic ligation and primer extension steps, we obtained ~1 nmol of the fully assembled pool of 15×15 pairwise substrate combinations. Each reaction-discovery experiment required only 0.5 pmol of the substrate pool, so this quantity of material was sufficient to assess the reactivity of these

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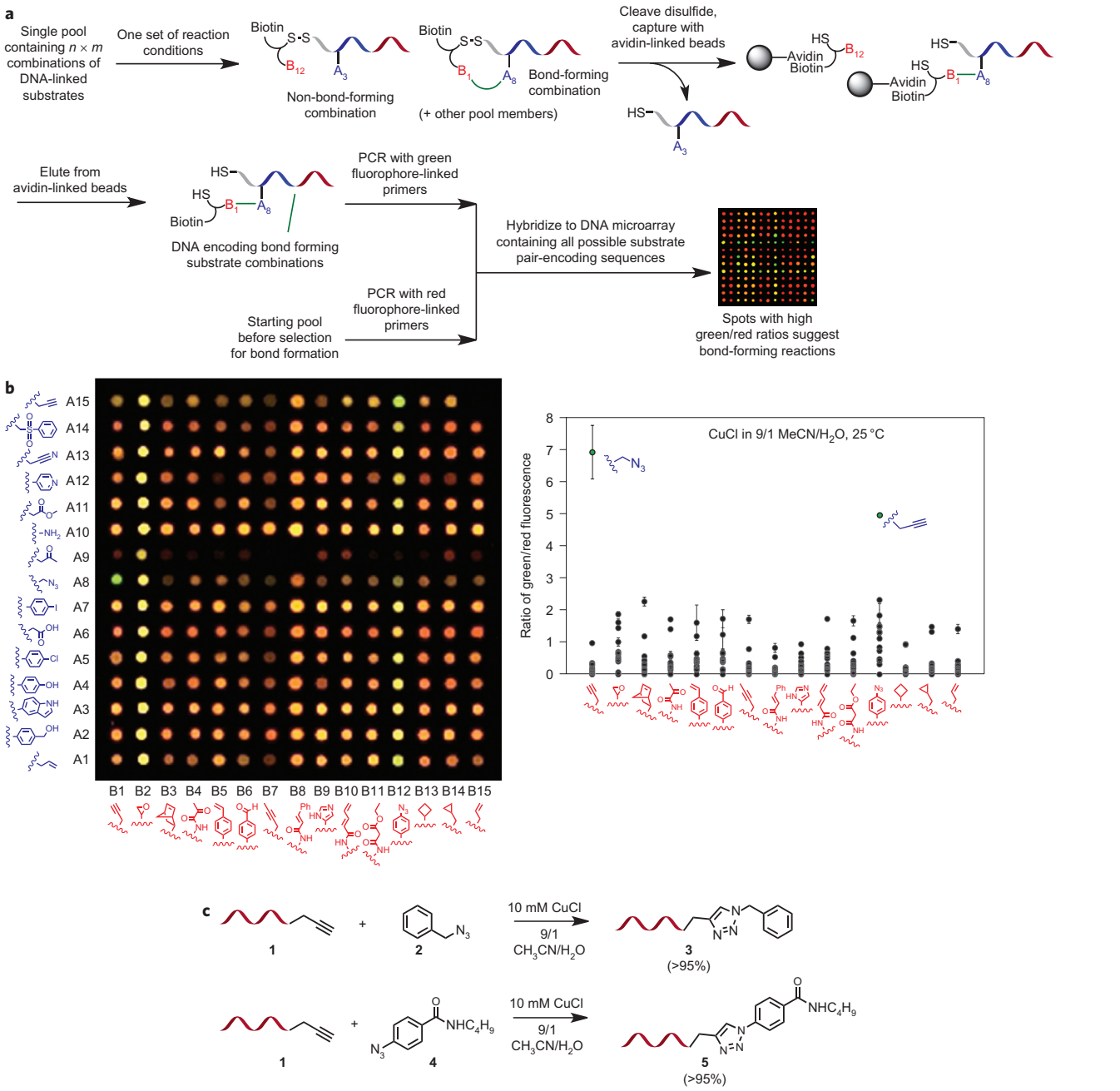

Figure 1 | DNA-encoded reaction-discovery system and validation experiments. a, Selection and analysis method for the detection of bond-forming reactions between DNA-linked small-molecule substrates. **b**, Detection of the known Cu(I)-catalysed cycloaddition[19] between alkynes and azides. Conditions: 10 mM CuCl in 9/1 CH_3CN/H_2O, 25 °C, 10 minutes. The green/red ratio of each spot on the DNA microarray (left) is plotted on the right. Error bars represent the standard deviation of signals from three microarray replicates. **c**, The reaction products of DNA-linked alkynes and non-DNA-linked azides are consistent with the anticipated triazole products. Conversion efficiencies, estimated by LC-MS analysis, are shown in parentheses.

substrate combinations under ~2,000 different reaction conditions, which collectively represent the evaluation of ~450,000 potential reactions. See the Supplementary Information for full details of the procedures used to assemble and characterize the substrate pool.

We performed control experiments to validate the system using the known Cu(I)-catalysed cycloaddition between alkynes and azides[19]. We confirmed the ability of the system to detect alkyne–azide coupling under these conditions in the DNA-encoded library format and verified the formation of the expected triazole products using DNA-linked substrates (Fig. 1b,c).

Next we determined regions of reaction-condition space compatible with the reaction-discovery system. As the second-generation system does not rely on DNA hybridization to organize substrates into pairwise combinations, compatible reaction conditions include any that do not degrade nucleic acids rapidly. Reaction conditions that led to the chemical modification or partial degradation of DNA were not necessarily excluded, given the high sensitivity of PCR amplification and the tolerance of PCR to some types of chemical modifications; indeed, conditions observed to degrade DNA were shown to be compatible with our earlier reaction-discovery system[1,3].

Conditions that preserved the ability of the remaining DNA to be detected readily after 25 cycles of PCR were considered potentially compatible reaction conditions. These conditions included the presence of transition metals (Fe(II), Fe(III), Co(II), Ni(0), Ni(II), Cu(I), Cu(II), Ru(0), Ru(II), Ru(III), Rh(I), Rh(II), Pd(0), Pd(II), La(III) and

Au(III)), common metal ligands (anions, phosphines, dienes, carbon monoxide and nitrogen-based ligands), organic catalysts (amines and thiazolium salts), common oxidants (benzoquinones and peroxides) and reagents for single-electron transfer (hypervalent iodine, SmI_2, $Mn(OAc)_3$ and cerium ammonium nitrate (CAN)). A detailed list of potentially compatible reaction conditions is provided in Supplementary Fig. S2.1.

***In vitro* selection for bond formation and DNA microarray analysis.** We treated 0.5 pmol of the substrate pool with a variety of compatible reaction conditions in 200 μl of aqueous or organic solvent (Figs 1b and 2; details are provided in the Methods section)[3]. After exposure to the reaction conditions, the disulfide bonds in the substrate-pool members were cleaved with tris(2-carboxyethyl)phosphine (TCEP) such that only DNA templates linked to (and encoding) substrates that had undergone bond formation remained covalently attached to biotin. These biotinylated DNA templates were captured with streptavidin-linked magnetic particles, washed and eluted by heating in deionized water at 70 °C (ref. 20). The eluted DNA was amplified by PCR using fluorophore-linked primers (Cy3 for the samples subjected to reaction conditions and *in vitro* selections and Cy5 for the initial substrate pools) and then hybridized in triplicate to DNA microarrays that contained all 225 possible DNA sequences complementary to each pool member (Fig. 1a).

We observed a number of spots on the DNA microarrays with reproducibly high Cy3/Cy5 (green/red) fluorescence ratios suggestive of potential bond-forming reactions between two substrates (Fig. 2). We grouped these initial positives into several categories. Some positives corresponded to substrate combinations known to undergo bond formation under the reaction conditions tested (green circles in Fig. 2a). Although these positives did not represent new reactions, they provided additional validation that the second-generation reaction-discovery system can detect bond-forming reactivity. For example, we observed phenol A4 + malonic ester B11 and alkene A1 + malonic ester B11 when the system was treated with CAN in methanol (Fig. 2a), consistent with previous reports that CAN mediates oxidative coupling reactions between malonic esters and electron-rich π systems (see Fig. 1b for the structures of substrates A1–A15 and B1–B15)[21,22].

A second group of positives represented reactions that are potentially new, but consistent with known reaction mechanisms. For example, copper (II) chloride resulted in a positive signal that corresponded to coupling between indole A3 and pyruvamide B4 (green circle in Fig. 2b). In the flask, treatment of indole **12** and ethyl pyruvate **13** in the presence of copper chloride ($CuCl_2$) or copper triflate led to the formation of a Friedel–Crafts type of coupling product (**14**) together with the bisindole adduct (**15**) in aqueous solvents, and in organic solvents bisindole formation was observed exclusively (Fig. 2b)[23]. We also observed substrate combinations that could be facilitated by a variety of Lewis acids or transition metals (orange circles in Fig. 2a–d). For instance, coupling between indole A3 and aldehyde B6, consistent with a reaction of the Friedel–Crafts type (orange circles in Fig. 2b–d), and coupling between azide A8 and norbornene B3, consistent with a cycloaddition (orange circles in Fig. 2a,b,d), were observed under several tested reaction conditions that involved transition metals, including Fe(III), Co(II), Ni(II), Cu(II) and Ru(II)[24,25].

A third category of positives corresponded to potential coupling reactions that were reproducible in the DNA-encoded system, but when tested in an intermolecular format did not result in the formation of a coupling product. For example, hypervalent iodine[26] resulted in robust positive signals that corresponded to the coupling of indole A3 with imidazole B9, as well as the coupling of benzyl alcohol A4 and imidazole B9 (green circles in Fig. 2c). When the corresponding reactions were attempted in flasks using small-molecule substrates, however, only the oxidation by-products 3-oxoindole **18** and benzaldehyde **20** were observed (Fig. 2c). We speculate that for this category of positives, bond-forming reactivity in the DNA-linked reaction-discovery system is dependent, in part, on the highly dilute, intramolecular format of the system, or on interactions with groups in DNA that are absent when these reactions are explored in the flask.

A fourth category of positives corresponded to bond-forming reactions not reported previously and not easily predicted based on known reactivity that we observed to take place in an intermolecular format with either DNA-linked or non-DNA-linked substrates. For example, when the system was treated with tris(bipyridine)ruthenium(II) dichloride ($Ru(bpy)_3Cl_2$) in the presence of a visible-light source (a 26 W compact fluorescent lamp (CFL) placed ~20 cm from a tube that contained the substrate library), we observed positives that corresponded to the coupling of aryl azide B12 and several functional groups (alkene A1, sulfone A14, phenol A4 and nitrile A13; green circles in Fig. 2d). Although $Ru(bpy)_3Cl_2$ as a visible-light photoredox catalyst is well-studied in inorganic chemistry[27,28], its exploration in synthetic organic chemistry is more recent[29–32]. Given the emerging importance of organic transformations mediated by visible light and the lack of previous reports of azide reactivity under these conditions, we chose to explore these reactions in greater detail.

Azide-coupling and reduction reactions induced by visible light. We first studied the reaction between DNA-linked aryl azides and nitriles. Under conditions similar to those used in the DNA-encoded system (5 mM $Ru(bpy)_3Cl_2$ in 200 μl of water/acetonitrile (1/9) with 30 mM Na_2CO_3 placed 20 cm from a 26 W CFL bulb), DNA-linked aryl azide **21** reacted with acetonitrile to generate a product consistent, by liquid chromatography–mass spectroscopy (LC-MS) analysis, with the imidate adduct **22** (Fig. 2d). No significant product formation was observed in the absence of the ruthenium complex or in the absence of a CFL light source.

As bond formation between aryl azides and nitriles induced by visible-light catalysis was unprecedented, we developed a mechanistic hypothesis to explain the above observations (Fig. 3a). Ground-state $[Ru(bpy)_3]^{2+}$ (**23**) is known to absorb visible light ($\lambda_{max} = 452$ nm) to generate the excited triplet $[Ru(bpy)_3]^{2+\star}$ (**24**)[27,28]. When an electron donor is present, **24** can be quenched reductively to give $[Ru(bpy)_3]^{+}$ (**25**)[30,31,33]. We speculated that **25** may reduce azide **26** through a one-electron transfer to generate the azide radical anion **27**. After extrusion by nitrogen gas and protonation, the resulting aminyl radical **28** may undergo a radical addition to give the nitrile[34,35].

With this mechanistic hypothesis in mind, we studied these reactions further in a non-DNA-linked format on a 0.1 mmol scale (Table 1). Although, for reasons unknown, the conditions that generated the coupling product of the DNA-linked azide and nitrile did not lead to significant coupling product formation with azide **4** in the flask, the addition of the commonly used sacrificial electron donor diisopropylethylamine[30,31] to these reaction conditions resulted in moderate yields (~60%) of the azide-reduction product **31** after 96 hours (Table 1, entry 1). The amine can form through formal hydrogen abstraction by the proposed aminyl radical intermediate (Fig. 3a)[35,36], although future studies are needed to probe these and other mechanistic possibilities. We speculated that this reaction may offer significant advantages over existing azide-reduction methods and sought to further develop the transformation.

Optimization of an azide-reduction reaction induced by visible light. Although the initial azide-reduction reaction proceeded slowly and in moderate yields, the addition of formic acid or the Hantzsch

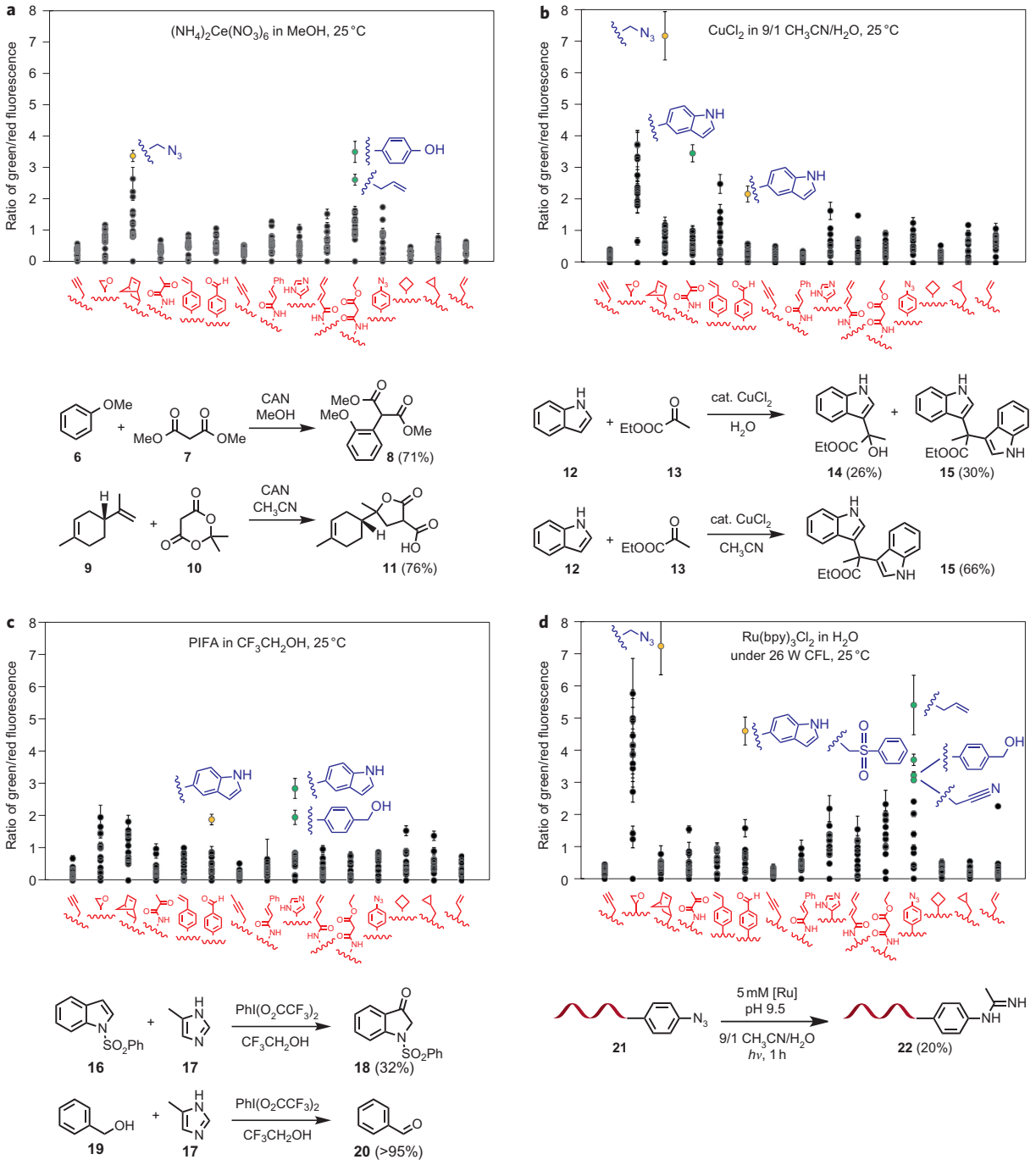

Figure 2 | Selection results from four reaction conditions. a–d, The scatter plots show green/red fluorescence ratios of substrate combinations in the DNA-encoded library. The schemes show the outcomes of reactions in a flask. **a**, In the presence of CAN, coupling was observed between malonic esters and electron-rich π-systems[21,22]. Conditions: 10 mM CAN in MeOH, 25 °C, 2 h. **b**, $CuCl_2$-induced coupling of indoles and pyruvates, consistent with coupling of the Friedel–Crafts type. Conditions: 1 mM $CuCl_2$ in 9/1 CH_3CN/H_2O, 25 °C, 16 h. Et = ethyl, cat. = catalytic. **c**, Phenyliodine bis(trifluoroacetate) (PIFA)-generated positives consistent with imidazole–indole and imidazole–benzyl alcohol coupling, but in a non-DNA-linked format only substrate oxidation was observed. Conditions: 10 mM PIFA in CF_3CH_2OH, 25 °C, 2 h. **d**, $Ru(bpy)_3Cl_2$ under visible-light irradiation resulted in several positives including azide–nitrile coupling. Conditions: 10 mM $Ru(bpy)_3Cl_2$ in 100 mM aqueous sodium carbonate, pH 9.5, 20 cm from a 26 W CFL bulb, 25 °C, 1 h. For (**a**), (**b**) and (**c**), isolated yields are shown in parentheses; for (**d**), the conversion efficiency by LC-MS analysis is shown in parentheses. Error bars represent the standard deviation of signals from three microarray replicates.

ester (diethyl 1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate) as a hydrogen donor to supplement or replace tertiary amines as hydrogen donors[31] decreased the reaction time to 12 hours and increased yields to ~90% (Table 1, entries 2 and 3). Without the ruthenium complex or the CFL light source, no azide reduction was observed under these conditions (Table 1, entries 4 and 5). Further optimization revealed that the tertiary ammonium salt provided a neutral reaction media and, when combined with the Hantzsch ester, improved the reaction efficiency to 92–96% yields in 2 hours in a variety of solvents (dimethylformamide (DMF), acetonitrile or dichloromethane; Table 1, entries 6–8). The use of acetate rather than formate as

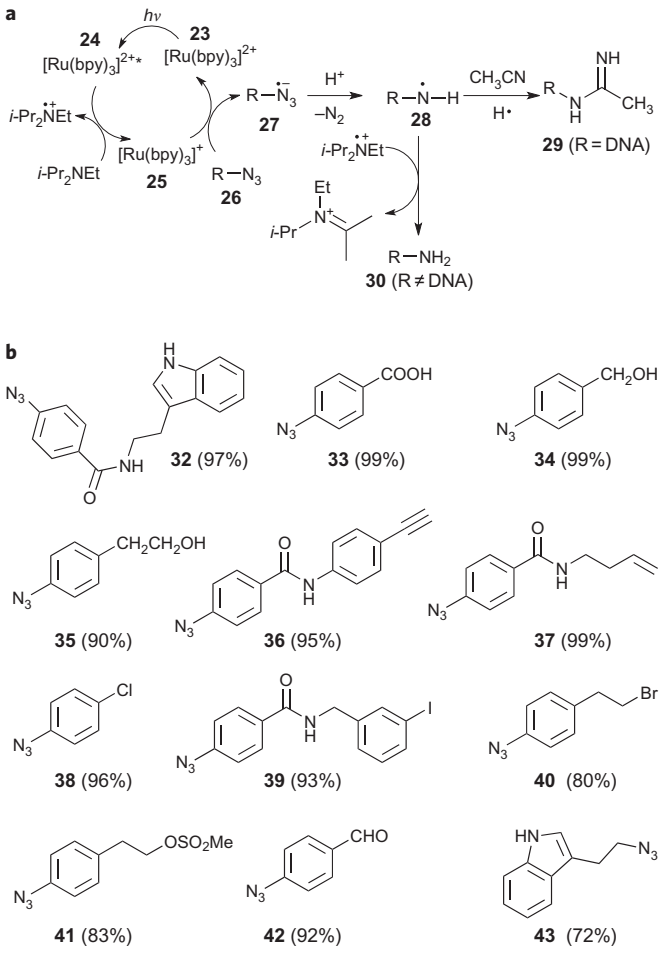

Figure 3 | Development of the $Ru(bpy)_3Cl_2$-mediated azide-reduction reaction induced by visible light. a, Proposed mechanism for the azide–nitrile coupling reaction and the azide-reduction reaction. **b**, Substrate scope of the azide-reduction reaction under the conditions shown in Table 1, entry 8 (except for substrate **43**, which was reduced with *i*-Pr_2NEt and Hantzsch ester in 24 hours using conditions described in the Methods section). Isolated yields are shown in parentheses.

the ammonium counterion generated similar excellent product yields in 2 hours (Table 1, entry 9). As little as 1 mol% $Ru(bpy)_3Cl_2$ was sufficient to afford the reduction product in 95% yield in 2 hours (Table 1, entry 10).

Next we evaluated the functional group compatibility of the optimized azide reduction under the conditions shown in Table 1, entry 8 (5 mol% $Ru(bpy)_3Cl_2$, 10 equiv. *i*-Pr_2NEt/HCOOH, 1.5 equiv. Hantzsch ester in CH_2Cl_2, *hν*, 25 °C). We observed that a variety of substrates that contained protic functional groups, including free indoles, acids and alcohols, were compatible with the reaction (Fig. 3b, substrates **32–35**). In addition, functional groups sensitive to hydrogenation, including alkenes, alkynes and aryl halides, were not affected by these reaction conditions (Fig. 3b, substrates **36–39**). Similarly, functional groups sensitive to nucleophiles, including alkyl halides, alkyl mesylates and aldehydes, also emerged intact under the reaction conditions (Fig. 3b, substrates **40–42**). An alkyl azide was also reduced in 72% yield in 24 hours under modified conditions (Fig. 3b, substrate **43**).

Encouraged by the remarkable chemoselectivity of this reaction, we sought to develop an aqueous version to explore the possibility that this azide reduction might be applicable to biological molecules. Under the optimized conditions above (Table 1, entry 8), but in CH_3CN/H_2O (1/1), the efficiency of the reaction was reduced drastically (Table 1, entry 11). Efficient product formation was restored with the removal of formic acid, which presumably increased the availability of an unprotonated tertiary amine as a competent electron donor (Table 1, entry 12). When the water-soluble reducing agent nicotinamide adenine dinucleotide (NADH) was used instead of the Hantzsch ester there was only a modest reduction in reaction efficiency (Table 1, entry 13). Although effective, we speculated that the basic conditions above may compromise the reaction's compatibility with biological macromolecules. We discovered, however, that sodium ascorbate can act as both an electron donor and a hydrogen donor[37] without significantly lowering yields or lengthening reaction times (Table 1, entry 14). In the presence of sodium ascorbate the reduction proceeded efficiently in buffers of different acidities (pH 4.0, pH 7.4 and pH 9.5; see Supplementary Fig. S3.1).

Compatibility of the azide-reduction reaction with DNA, oligosaccharide and protein. Previous reports demonstrated the use of $Ru(bpy)_3Cl_2$ to mediate reactions on protein substrates in crude cell extracts[38], and related complexes were used as cell-permeable agents to image DNA in living eukaryotic and prokaryotic cells[39]. These observations, coupled with the excellent

Table 1 | Optimization of the azide-reduction reaction.

Entry	Reaction conditions	[Ru] (%)	Solvent	Time (h)	Yield (%)*
1	2 equiv. *i*-Pr_2NEt, *hν*	5	DMF	96	60
2	10 equiv. *i*-Pr_2NEt/HCOOH, *hν*	5	CH_3CN	12	90
3	2 equiv. *i*-Pr_2NEt, 1.5 equiv. Hantzsch ester, *hν*	5	CH_3CN	12	89
4	10 equiv. *i*-Pr_2NEt/HCOOH, no light	5	CH_3CN	48	<5
5	10 equiv. *i*-Pr_2NEt/HCOOH, *hν*	0	CH_3CN	1,680	<5
6	10 equiv. *i*-Pr_2NEt/HCOOH, 1.5 equiv. Hantzsch ester, *hν*	5	CH_3CN	2	92
7	10 equiv. *i*-Pr_2NEt/HCOOH, 1.5 equiv. Hantzsch ester, *hν*	5	DMF	2	92
8	10 equiv. *i*-Pr_2NEt/HCOOH, 1.5 equiv. Hantzsch ester, *hν*	5	CH_2Cl_2	2	96
9	10 equiv. *i*-Pr_2NEt/CH_3COOH, 1.5 equiv. Hantzsch ester, *hν*	5	CH_2Cl_2	2	96
10	10 equiv. *i*-Pr_2NEt/HCOOH, 1.5 equiv. Hantzsch ester, *hν*	1	CH_2Cl_2	2	95
11	10 equiv. *i*-Pr_2NEt/HCOOH, 1.5 equiv. Hantzsch ester, *hν*	5	1/1 CH_3CN/H_2O	108	10
12	2 equiv. *i*-Pr_2NEt, 1.5 equiv. Hantzsch ester, *hν*	5	1/1 CH_3CN/H_2O	48	91
13	2 equiv. *i*-Pr_2NEt, 1.5 equiv. NADH, *hν*	5	1/1 CH_3CN/H_2O	60	64
14	10 equiv. sodium ascorbate, *hν*	5	1/1 DMF/H_2O	48	90

hν denotes light from a 26 W CFL bulb at 20 cm. Reactions were performed at 25 °C.
*Reported yields above 5% are isolated yields.

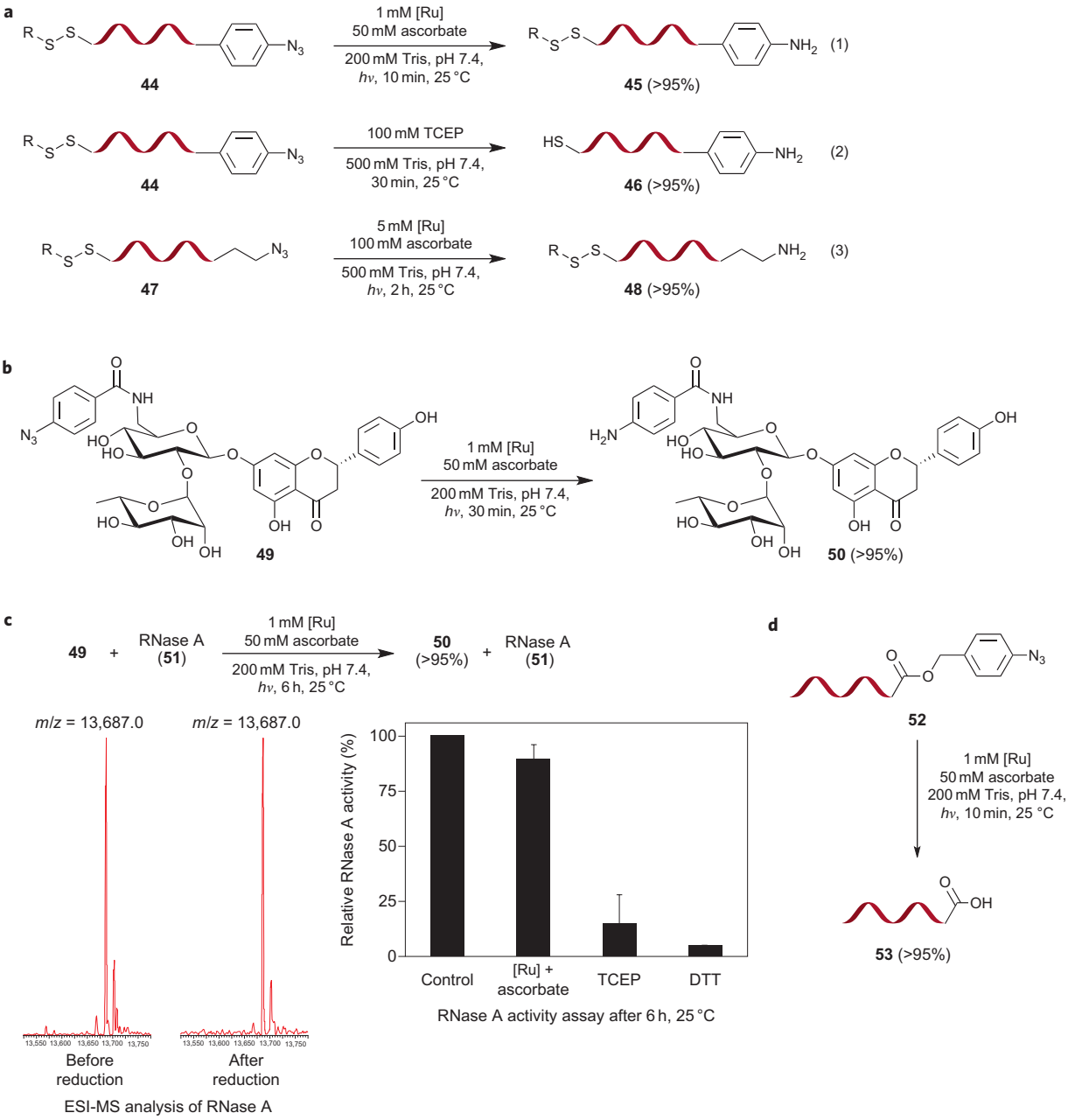

Figure 4 | Compatibility of the azide-reduction reaction with biological molecules. a, Azide- and disulfide-linked DNA oligonucleotides (0.5 μM each) were reduced to the corresponding amines under the aqueous reaction conditions shown without inducing side reactions, such as disulfide reduction. In contrast, TCEP reduced both the azide and disulfide groups. Reaction conversions were calculated by LC-MS analysis. Conversion efficiencies are shown in parentheses. **b**, A functional group-rich azide analogue of the disaccharide naringin (100 μM) was reduced cleanly to give the corresponding amine. Conversion was calculated by HPLC analysis. **c**, $Ru(bpy)_3Cl_2$-mediated reduction, induced by visible light, of the naringin azide analogue (100 μM) in the presence of the protein enzyme RNase A (10 μM in the reaction, 0.83 μM when assayed) did not alter the enzyme's covalent structure or activity. In contrast, thiol- or phosphine-induced azide reduction both resulted in loss of RNase A activity. Conditions are as follows. Control: 200 mM Tris, pH 7.4, 20 cm from 26 W CFL, 6 h, 25 °C. [Ru] + ascorbate: 1 mM [Ru], 50 mM ascorbate, 200 mM Tris, pH 7.4, 20 cm from 26 W CFL, 6 h, 25 °C. TCEP: 100 mM TCEP in 1 M phosphate, pH 8.0, 6 h, 25 °C. DTT: 500 mM DTT in 1 M phosphate, pH 8.0, 6 h, 25 °C. Error bars represent the standard deviation of results from three independent activity assays. **d**, Photouncaging, triggered by visible light, of the carboxylic acid functional group in oligonucleotide substrates. Conversion was calculated by LC-MS analysis.

functional group tolerance and mild reaction conditions of the azide reduction, raised the possibility that this reaction could be performed on biomolecules rich in functional groups without inducing any side reactions. We tested this possibility by performing the reduction on, or in the presence of, nucleic acids, a protein and an oligosaccharide and characterizing these macromolecules after each reaction.

We prepared DNA oligonucleotide **44**, which contains both an aryl azide and a disulfide, and treated 0.5 μM of this substrate with 1 mM $Ru(bpy)_3Cl_2$ and 50 mM of sodium ascorbate in aqueous 200 mM tris(hydroxymethyl)aminomethane (Tris), pH 7.4, positioned 20 cm from a 26 W CFL bulb (standard aqueous reduction conditions). Azide reduction proceeded cleanly in 10 minutes without disulfide-bond reduction (equation (1) in Fig. 4a).

In the absence of light, no azide reduction was observed after 15 hours, and exposure to light after 15 hours of darkness resulted in complete azide reduction in 10 minutes. In comparison, TCEP, a common Staudinger reaction agent, reduced both the azide and disulfide non-selectively (equation (2) in Fig. 4a). Likewise, DNA oligonucleotide **47**, which contains an alkyl azide and a disulfide, underwent selective azide reduction in 2 hours when treated with 5 mM $Ru(bpy)_3Cl_2$ and 100 mM of sodium ascorbate (equation (3) in Fig. 4a).

To test the compatibility of the azide-reduction reaction with oligosaccharide substrates we prepared an azide-containing variant of naringin, a flavanone disaccharide (**49**) (Fig. 4b). When 100 μM of **49** was treated with standard aqueous reduction conditions, we observed complete reduction in 30 minutes with no other transformations detected by high-performance liquid chromatography (HPLC), ^{1}H NMR spectroscopy or MS (Fig. 4b).

Finally, we performed the azide reduction in the presence of a protein enzyme. We treated a solution that contained 100 μM of naringin azide (**49**) and 10 μM of RNase A with standard aqueous reduction conditions. After 6 hours, azide **49** was reduced completely, as detected by HPLC (Fig. 4c). We isolated the RNase A from the completed reaction by size-exclusion chromatography. No new species consistent with the covalent modification of RNase A were observed by electron spray ionization (ESI)-MS of the macromolecular fraction. Likewise, enzymatic assay of RNase A activity, which requires intact disulfide bonds[40], revealed a ≤10% loss of catalytic activity from the azide-reduction reaction developed in this work, although thiol-based reduction and the Staudinger azide reduction both resulted in the loss of 85–100% of enzyme activity (Fig. 4c).

Photouncaging of carboxylic acids triggered by visible light. We hypothesized that the reaction described above may also enable azide-protected groups beyond amines to be uncaged efficiently on functional group-rich biomolecules under mild conditions with visible light. Current photouncaging[41,42] reactions typically require ultraviolet light that can induce undesired side reactions, including protein degradation, photobleaching, inhibition of photosynthesis and nucleic acid damage[43].

We prepared a DNA oligonucleotide-linked carboxylic acid caged as a 4-azidobenzyl ester[10,44] (**52**) and treated it with standard aqueous reduction conditions. Photouncaging of the free carboxylic acid through azide reduction and 1,6-elimination was complete within 10 minutes without any detected by-products by LC-MS (Fig. 4d). As a deprotection strategy, this reaction proceeded rapidly under very mild conditions and is orthogonal to most carboxyl-deprotection strategies used currently, such as acid or base hydrolysis[45].

Conclusion

Using a second-generation, DNA-encoded reaction-discovery system, we explored a broad range of transition metals and reaction conditions to identify combinations of substrates and reaction conditions that result in bond formation between substrates. From the reactivity revealed by the resulting hits we developed a Ru(II)-catalysed azide-reduction reaction induced by visible light that is efficient and can be conducted in organic or aqueous solvents, open to the air, at room temperature and under neutral conditions. This reaction exhibited remarkable chemoselectivity, in contrast to existing azide-reduction methods, and is compatible with alcohols, phenols, acids, alkenes, alkynes, aldehydes, alkyl halides, mesylates and disulfides. The unusual functional group compatibility and mild conditions required for the reaction enabled azide reduction to be performed on oligonucleotide and oligosaccharide substrates, and in the presence of a protein enzyme, without compromising the structure or the enzymatic activity of the biomolecules. This reaction can also be used to photouncage groups, such as amines and carboxylates, on biomolecules without the need of ultraviolet light.

Methods

See the Supplementary Information for additional experimental details.

General reaction-discovery procedure. Each reaction-discovery experiment was performed in 200 μl total volume that contained 0.5 pmol of the total substrate pool, which corresponded to 2.2 fmol of each unique substrate combination. After exposure to the reaction conditions shown in Figs 1b and 2, each solution was precipitated with ethanol to recover DNA-linked species. The pellet was dissolved in 30 μl of water, and 150 μl of 0.1 M TCEP in 1.0 M aqueous sodium phosphate, pH 8.0, was added to effect disulfide cleavage. After 30 minutes at 25 °C, this solution was combined with streptavidin-linked magnetic particles (14 μl, which corresponded to a 20 pmol biotin-binding capacity, Roche Biosciences) suspended in 300 μl of 10 mM Tris-Cl, 0.1 M NaCl, 1 mM EDTA, pH 7.5. After incubation for 15 minutes at 25 °C the supernatant was removed and the particles captured by a magnetic separator, then rinsed once with 200 μl of 10 mM Tris-Cl, 1 M NaCl, 1 mM EDTA, pH 7.5, and once with 200 μl H_2O. The particles were suspended in 40 μl deionized water and incubated at 70 °C for 5 minutes to elute the captured DNA[20]. The supernatant was collected and the elution step repeated once. The combined supernatants were used directly in PCR reactions for microarray analysis, as described previously[3] and detailed in the Supplementary Information.

Representative small-molecule azide reductions. For aryl azides in organic solvent, to a solution of $Ru(bpy)_3Cl_2$ (7.5 mg, 0.01 mmol, 0.05 equiv.), Hantzsch ester (76 mg, 0.3 mmol, 1.5 equiv.), *i*-Pr_2NEt (350 μl, 2.0 mmol, 10 equiv.) and HCOOH (75 μl, 2.0 mmol, 10 equiv.) in 1 ml CH_2Cl_2 was added azide **4** (43.6 mg, 0.2 mmol, 1.0 equiv.). The dark-orange solution was stirred at 25 °C at a distance of 20 cm from a 26 W CFL bulb until thin-layer chromatography (TLC) indicated the complete consumption of azide **4** (usually ≤2 hours). The reaction mixture was subjected directly to silica-gel chromatography. Isolated yields for other aryl azides are shown in Fig. 3b.

For aryl azides in mixed aqueous–organic solvent, to a solution of $Ru(bpy)_3Cl_2$ (3.8 mg, 0.005 mmol, 0.05 equiv.) and azide **4** (21.8 mg, 0.1 mmol, 1.0 equiv.) in 1 ml DMF/H_2O (1/1 ratio) was added sodium ascorbate (198 mg, 1.0 mmol, 10 equiv.) in two equal portions (the second portion was added after 24 hours). The orange solution was stirred at 25 °C at a distance of 20 cm from a 26 W CFL bulb until TLC indicated the complete consumption of azide **4** (~48 hours). The resulting reaction mixture was diluted with ethyl acetate, washed with water and brine, dried over Na_2SO_4, concentrated *in vacuo* and subjected to silica-gel chromatography.

For alkyl azides, to a solution of $Ru(bpy)_3Cl_2$ (15 mg, 0.02 mmol), Hantzsch ester (102 mg, 0.4 mmol) and *i*-Pr_2NEt (105 μl, 0.6 mmol) in 2 ml DMF/MeOH (3/1 ratio) was added alkyl azide **43** (37.2 mg, 0.2 mmol). The dark-orange suspension was stirred at 25 °C at a distance of 20 cm from a 26 W CFL bulb until TLC indicated the complete consumption of azide **43** (~24 hours). To the reaction mixture was added di-*t*-butyl dicarbonate (87.3 mg, 0.4 mmol) and triethylamine (56 μl, 0.4 mmol) and this mixture was stirred for 12 hours. The resulting solution was diluted with ethyl acetate, washed with water and brine, dried over Na_2SO_4, concentrated *in vacuo* and subjected to silica-gel chromatography.

Representative DNA-linked azide reduction. To a solution of 1 mM $Ru(bpy)_3Cl_2$ and 50 mM sodium ascorbate in 200 μl of aqueous 200 mM Tris-Cl, pH 7.4, was added DNA-linked azide to give a final concentration of 0.5 μM. After incubation at 25 °C at a distance of 20 cm from a 26 W CFL bulb for 10 minutes, the reaction was quenched by precipitation with ethanol. The pellet containing DNA-linked small molecules was dissolved in 20 μl of 10 mM phosphate buffer, pH 8, and subjected to LC-MS analysis.

Naringin-azide reduction in the presence of RNase A. To a solution of 1 mM $Ru(bpy)_3Cl_2$ and 50 mM sodium ascorbate in 200 μl of aqueous 200 mM Tris-Cl, pH 7.4, was added naringin azide and RNase A to final concentrations of 100 μM and 10 μM, respectively. After incubation at 25 °C at a distance of 20 cm from a 26 W CFL bulb for 6 hours, RNase A was collected through size-exclusion chromatography (Centri-Sep column, Princeton Separations) and characterized by MS. After sample dilution 12-fold, RNase A enzyme activity was assayed by following cytidine 2′,3′-cyclic phosphate hydrolysis spectrophotometrically, as described previously[46]. The small-molecule fraction from polyethersulfone membrane ultrafiltration (Corning Life Sciences) was analysed directly by reverse-phase HPLC to assess the naringin-azide reduction.

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Author contributions

Y.C., A.S.K., J.B.S. and D.R.L. designed the research, analysed the data and co-wrote the manuscript, Y.C., A.S.K. and J.B.S. performed the experiments.

Additional information

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