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Bioinspired Catalysis: Self-Assembly of a Protein and DNA as a Catalyst for the Aldol Reaction in Aqueous Media

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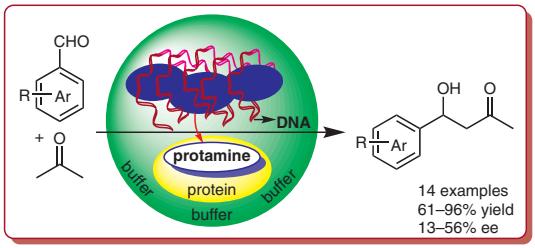

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Abstract An interesting bioinspired catalyst formed from readily available DNA and a protein through electrostatic interaction in situ proved to be efficient in catalyzing aldol reactions under mild conditions in water. By using a self-assembling catalytic system formed from protamine and DNA, aldol adducts were obtained with high yields and moderate enantioselectivities. Preliminary experiments demonstrated that the chirality of the DNA could be effectively transferred to the reaction product through the bound molecules or proteins.

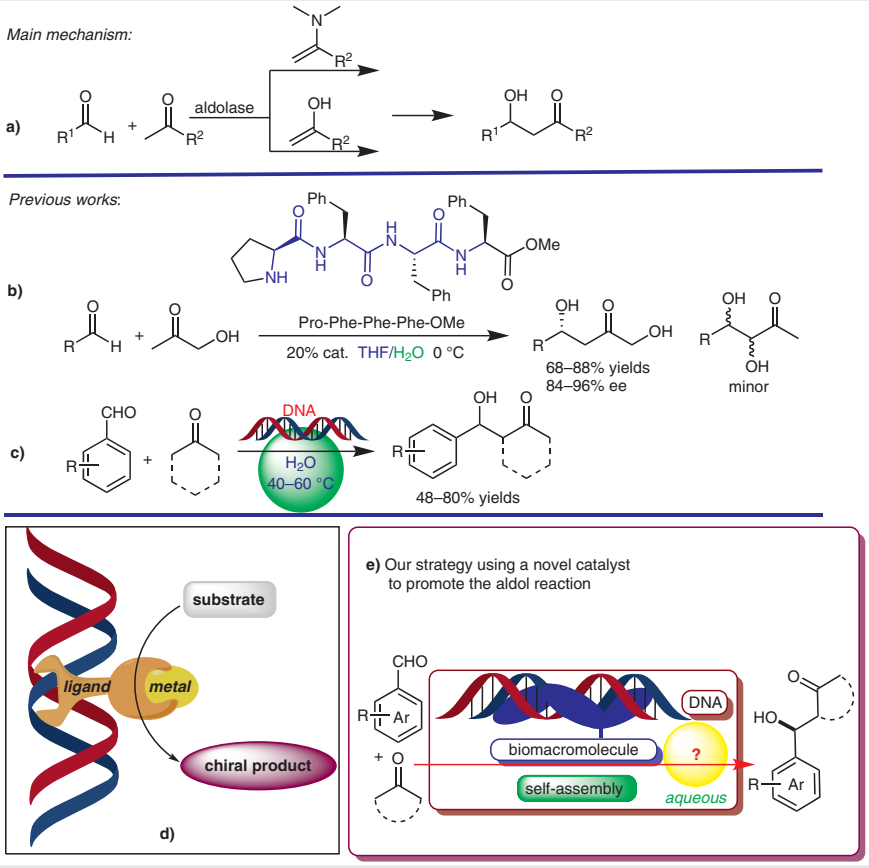
Key words self-assembly, protamine, DNA, electrostatic interaction, bioinspired catalysis, aldol reaction

The key principles and concepts of biological systems have always inspired chemists to develop new chemical reactions and catalytic processes[1–3] in so-called 'biomimetic chemistry'.[4–9] For example, the aldol reaction, which is frequently induced by aldolases in biological systems,[10–13] has also been developed as an important organic reaction in organic synthesis. The aldolase-catalyzed mechanism via an enamine or enol transition state (Scheme 1a)[13] has become more and more popular, and has encouraged numerous successes in antibody catalysis and organocatalysis.[14] Despite these achievements, mimicking the behavior of enzymes by employing other 'biomolecules' is still an attractive target, but has rarely been studied. In this field, small peptides have been successfully used as catalysts in aqueous media by Gong and co-workers (Scheme 1b).[15] Furthermore, peptides have also been reported to serve as catalysts in some other transformations.[16–21] However, stable and easily prepared DNA has rarely been employed in organic transformations. Since the report in 2005 by Feringa's group, DNA-based asymmetric catalysis has been developed (Scheme 1d).[22] By embedding transition-metal complexes into a chiral DNA scaffold, a variety of asymmetric catalytic reactions have been developed, and have achieved good enantioselectivities.[23–28] In contrast, and to the best of our knowledge, no metal-free DNA complex has been successfully employed in promoting asymmetric transformations. Inspired by the simple DNA-catalyzed racemic aldol reaction in aqueous conditions (Scheme 1c),[29] we became interested in exploring novel metal-free DNA complexes as catalysts for asymmetric reactions.

Here, we report our preliminary results on the DNA–protamine complex catalyzed asymmetric aldol reaction under aqueous conditions to give the desired products in high yields and moderate enantioselectivities (Scheme 1e). Importantly, the present results indicated the catalytic potential of the self-assembly of DNA with a protein in asymmetric transformations.

Because the sugar phosphate ester backbone of DNA is negatively charged in aqueous phases, we speculated that a positively charged moiety might bind to DNA through electrostatic interactions in aqueous solution to form an interesting catalytic system. For example, basic amino acids, such as L-lysine or L-arginine,[30–34] are positively charged through protonation in aqueous solution under neutral or acidic pH conditions, which might enable them to bind to DNA molecules through electrostatic interactions. Importantly, the free amino group of the DNA complex can activate a ketone donor, thereby acting as an aldolase mimic.

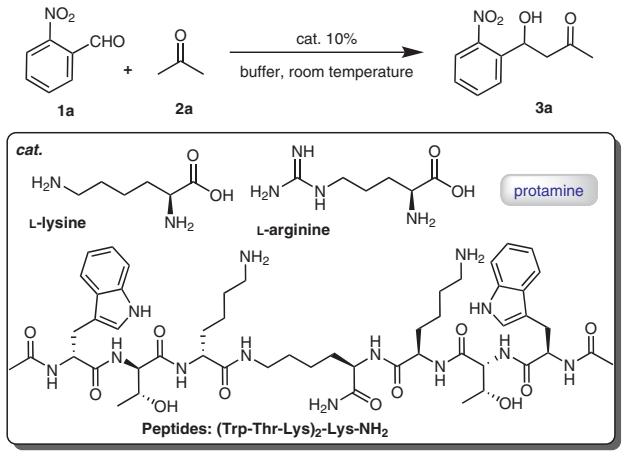
To verify our hypothesis, commercial DNA, derived from salmon sperm, and various amino acids were tested in a model aldol reaction of 2-nitrobenzaldehyde (**1a**) with acetone (**2a**) in a phosphate buffer (pH = 7.5) (Table 1). DNA alone afforded only a trace amount of the product (Table 1, entry 1), whereas L-lysine alone provided product **3a** in 45% yield, but with extremely low enantioselectivity (3% ee; entry 2). However, when a DNA–L-lysine complex was em-



Scheme 1 The development of the aldol reaction and our strategy

ployed as a catalyst, a slightly increased stereoselectivity was observed (8% ee; entry 3). This increase in enantioselectivity also occurred when a DNA–L-arginine complex was used in the same reaction (12% ee; entry 5), indicating a transfer of chirality from DNA to the aldol product. Next, we used a synthetic peptide containing two Trp-Thr-Lys tripeptide units as the catalyst. This peptide has been shown to bind efficiently with DNA.[35] Unfortunately, the catalytic effect was very poor (entries 6 and 7). When protamine was screened, to our delight the hybrid catalytic system efficiently catalyzed the aldol reaction with better enantioselectivity (24%, entry 9) than that produced by protamine alone (14% ee; entry 8). Our remaining preliminary investigations and optimization studies focused on screening various buffers. The buffer species was found to affect the enantioselectivity of the hybrid catalyst catalyzed reaction (entries 9–12), and 2-[1-(2-hydroxyethyl)piperazin-1-ium-4-yl]ethanesulfonate (Hepes) buffer was identified as the best choice (entry 12). Furthermore, we found that the pH had a significant influence on the yield and enantioselectivity (entries 12–16). The enantioselectivity reached 33% ee at a pH of 6.5 (entry 15). The pH plays an important role in the catalytic role of protamine; a lower pH value led to an obvious decrease in the yield of the product, indicating that a decrease in the basicity of protamine results in a reduction in its reactivity. However, changing the pH had little effect on the enantioselectivity of the product (entries 17–19). In an attempt to improve the yield, we examined the effect of the ratio of protamine to DNA (see Supporting Information), and we found that a 1:1 mass ratio was the best choice (entry 15; 46% yield, 33% ee). Finally, the best results, a 71% yield and 32% ee, were achieved by increasing the amount of acetone to 0.3 mL and extending the reaction time to two days at room temperature (entry 22).

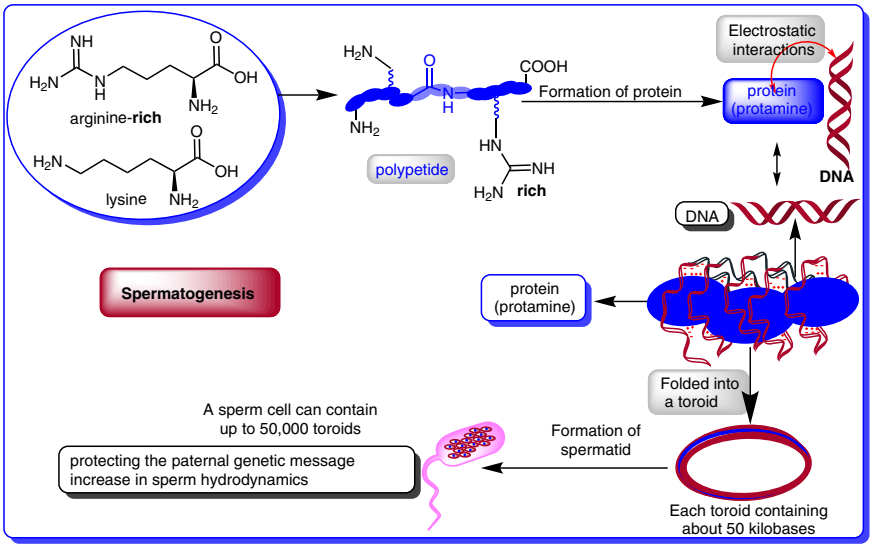
Table 1 Screening of the Effects of Reaction Conditions[a]



Entry	Catalyst	Buffer	pH	Yield[b] (%)	ee[c] (%)
1	DNA	PB[d]	7.5	trace	–
2	L-lysine	PB	7.5	45	3
3	DNA + L-lysine	PB	7.5	17	8
4	L-arginine	PB	7.5	94	4
5	DNA + L-arginine	PB	7.5	85	12
6	Peptide[e]	PB	7.5	37	0
7	DNA+ peptides	PB	7.5	10	3
8	protamine	PB	7.5	89	14
9	DNA + protamine	PB	7.5	76	24
10	DNA + protamine	MOPS[f]	7.5	66	27
11	DNA + protamine	Tris[g]	7.5	54	19
12	DNA + protamine	Hepes	7.5	65	28
13	DNA + protamine	Hepes	8.0	85	22
14	DNA + protamine	Hepes	7.0	55	30
15	DNA + protamine	Hepes	6.5	46	33
16	DNA + protamine	Hepes	6.0	26	31
17	protamine	Hepes	7.5	74	16
18	protamine	Hepes	7.0	68	16
19	protamine	Hepes	6.5	58	17
20[h]	DNA + protamine	Hepes	6.5	20	28
21[i]	DNA + protamine	Hepes	6.5	60	25
22[j]	DNA + protamine	Hepes	6.5	71	32

[a] Reaction conditions: **1** (0.1 mmol), acetone (**2**; 0.2 mL), catalyst (10 wt% of **1**), 20mM buffer (2 mL), r.t., 1 d.
[b] Isolated yield.
[c] Determined by chiral HPLC.
[d] Phosphate buffer
[e] Synthetic peptide containing two Trp-Thr-Lys tripeptide units.
[f] 3-(*N*-Morpholino)propanesulfonic acid
[g] $H_2NC(CH_2OH)_3$
[h] Protamine/DNA =1:1.5 (by mass).
[i] Protamine/DNA =1:0.5 (by mass).
[j] Acetone (0.3 mL) for 2 d at r.t.

The hybrid catalytic system of DNA and protamine efficiently catalyzed the reaction with better enantioselectivities. Protamine is an extremely important substance in medicine and genetics, and it contains an abundance of positively charged amino acids, particularly arginine. Consequently, it binds efficiently with DNA. In fact, the DNA that we used is folded into thousands of toroid-shaped structures in the sperm cell during spermatogenesis, and each toroid contains about 50,000 bases (Scheme 2).[36–39]



Scheme 2 The role of protamine in spermatogenesis

To verify the formation of a self-assembled catalyst through electrostatic interaction between protamine and DNA, we conducted gel-mobility-shift assay experiments through agarose gel electrophoresis to investigate the negatively charged DNA molecules (Figure 1A: for details, see Supporting Information). A DNA band was clearly detected on the gel (Lane 1; Figure 1A). Once mixed with protamine, the DNA molecules remained in the loading slot, confirming that a DNA–protein complex had formed, thereby markedly decreasing the mobility of DNA on the gel (Lane 2, Figure 1A). In comparison, when bovine serum albumin (BSA), which does not interact with DNA, was used in the same experiment, the DNA moved freely on the gel. Moreover, the fluorescence intensity of DNA stained by ethidium bromide (EB) showed an obvious decrease when protamine was added, whereas no fluorescence change occurred on addition of BSA (Figure 1B). Those results confirm the presence of a strong interaction between DNA and protamine, which is consistent with the conclusions of other research groups.[39–40]

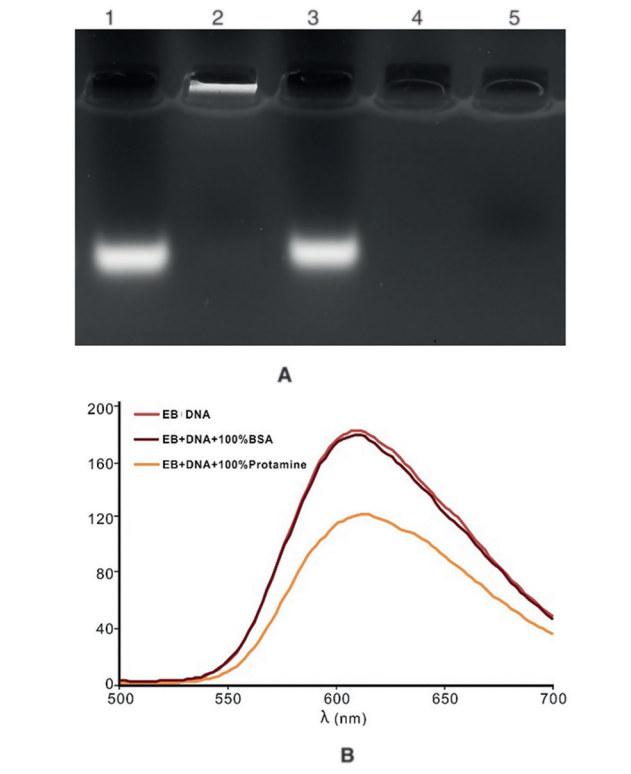


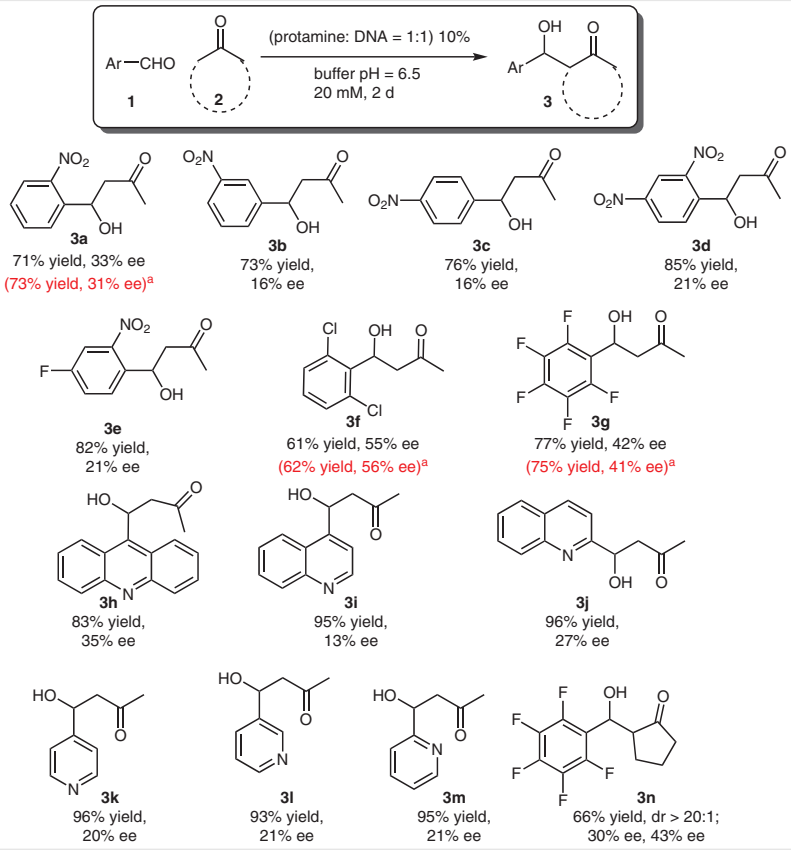
Figure 1 (A) Gel-mobility-shift assay. Lane 1: DNA only; Lane 2: DNA + protamine; Lane 3: DNA + BSA; Lane 4: protamine; Lane 5: BSA. (B) Fluorescence spectra (excited at 366 nm) of various DNA samples.

These primary researches proved that a novel hybrid self-assembled catalytic system consisting of DNA and protamine was formed through electrostatic interactions between the negatively charged DNA and positively charged protamine.

After elucidating the composition and catalytic mechanism of the self-assembled catalyst, we set out to explore the substrate generality of this reaction under the optimized reaction conditions. Various aromatic aldehydes gave the desired products in good yields and with moderate enantioselectivities (Scheme 3). The position of substituents on the aromatic ring of the aldehyde affected the enantioselectivity; for example, a nitro group in the *ortho*-position of **3a** led to a higher enantioselectivity (33% ee) than did the corresponding group in the *meta*- (**3b**, 16% ee) or the *para*-position (**3c**, 16% ee). In addition, polysubstituted aromatic aldehydes gave products with better enantioselectivity, especially substrates with substituents in both the 2- and 6-positions (**3f**, 55% ee; **3g**, 42%). We then evaluated the use of various N-heterocyclic aromatic aldehydes as substrates. Most of the reactions were completed within two days under the standard reaction conditions and gave products in excellent yields (83–96%) and moderate enantioselectivities (**3h–m**; 13–35% ee). For example, acridine-9-carbaldehyde reacted efficiently to afford the corresponding product **3h** in 83% yield with 35% ee. Pyridinecarbaldehydes and quinolinecarbaldehydes also reacted efficiently to afford the desired product **3i–m** in excellent yields (93–96%) and 13–27% ee. We also carried out the reactions of aldehydes **1a**, **1f**, and **1g** on a 0.5 mmol scale and, to our delight, we obtained products **3a**, **3f**, and **3g** in 62–75% yield and 31–56% ee.

Encouraged by these results, we attempted to expand the generality of the reaction with regard to the ketone. However, the desired products were not obtained in the presence of straight-chain aliphatic ketones (2-butanone or 2-heptanone) or aromatic ketones (acetophenone) with various aromatic aldehydes under the standard conditions. Next, we examined the reactions of cyclohexanone and cyclopentanone under the optimized conditions. By screening various aldehydes, we found that cyclopentanone underwent an aldol reaction with pentafluorobenzaldehyde when the hybrid self-assembled system was employed as the catalyst, and gave the corresponding product **3n** with relatively moderate enantioselectivity (43% ee) and high diastereoselectivity (dr > 20:1).

In summary, an electrostatic interaction was successfully employed in the construction of new DNA-based catalysts, endowing the DNA molecule with new and improved catalytic ability. With the self-assembled catalytic system formed from protamine and DNA, aldol adducts were obtained in high yields and moderate enantioselectivities in aqueous medium under mild conditions.[41] Preliminary experiments demonstrated that the chirality of the DNA could be effectively transferred to the reaction product through the bound protein. We believe that the present study considerably advances the concept of DNA-based catalysis and provides valuable information for researchers in this field.



Scheme 3 Substrate scope of various aldehydes and ketones. *Reaction conditions*: **1** (0.1 mmol), **2** (0.3 mL), protamine–DNA catalyst (10 mass% of **1**), Hepes buffer (2 mL), r.t., 2 d. Isolated yield of products are reported. The enantioselectivity was determined by chiral HPLC. [a] Reactions carried out on a 0.5 mmol scale.

Work to develop other self-assembled DNA catalysts through this strategy and to employ them in new reactions is underway in our laboratory.

Funding Information

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Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0036-1591854.

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(41) **4-Aryl-4-hydroxybutan-2-ones 3a–g; General Procedure**
A mixture of protamine (1.5 mg) and DNA (1.5 mg) in 20 mM Hepes buffer (2 mL) at r.t. was stirred with a magnetic stirrer for 1 h. Aldehyde **1** (0.1 mmol) and acetone (0.3 ml) were added, and the resulting mixture was stirred for 2 d at r.t. until the reaction was complete (TLC). The mixture was extracted with EtOAc (3 × 2 mL), and then the combined organic extracts were washed with brine (5 mL), dried (Na_2SO_4), and filtered. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography [silica gel, PE–EtOAc (5:1 to 3:1)].
4-Hydroxy-4-(2-nitrophenyl)butan-2-one (3a)
faint yellow solid; yield: 14.8 mg (71%; 33% ee); mp 58–60 °C (Lit.[42] 59–61 °C); HPLC: Chiralpak AS-H (hexane–*i*-PrOH (80:20); flow rate: 1 mL/min, λ = 254 nm): t_1 = 8.9 min; t_2 = 11.3 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.95 (dd, *J* = 8.2, 1.0, 1 H), 7.89 (d, *J* = 7.5, 1 H), 7.71–7.62 (m, 1 H), 7.50–7.36 (m, 1 H), 5.67 (dd, *J* = 9.4, 1.9, 1 H), 3.74 (s, 1 H), 3.12 (dd, *J* = 17.8, 2.0, 1 H), 2.72 (dd, *J* = 17.8, 9.5, 1 H), 2.23 (s, 3 H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 208.95, 147.22, 138.52, 133.95, 128.40, 128.29, 124.56, 65.73, 51.18, 30.57.
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