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Reversible regulation of metallo-base-pair interactions for DNA dehybridization by ultrasound†

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Mechanical force applied by ultrasound in solution leads to the dissociation of DNA metallo-base-pair interactions when these motifs are functionalized with oligodeoxynucleotide sequences of sufficient length. The annealing and force-induced denaturing process is followed by the attachment of distance-sensitive fluorescent probes and is found to be reversible.

In the field of polymer mechanochemistry, the macroscopic force transmitted through a polymer architecture is used to induce productive conformational changes or bond scission events within force-sensitive molecules (mechanophores) mostly with the aim to activate latent functionality.^{1,2} Therefore, force is applied in various ways ranging from compression and extension, over freezing, to shearing in solution.^{2,3} For the latter, ultrasound (US) that generates inertial cavitation can be regarded as the most prevalent, technically straightforward method.^{4,5} Concomitantly, its non-invasive character and broad clinical applications⁶ hold the promise to eventually permit the application of the polymer mechanochemical principle in a biomedical context. However, a delicate balancing of mechanochemical sensitivity and thermal stability of the mechanophore is required to lead this endeavour to success. Recently, we made first efforts in this direction by the US-induced activation of both latent drugs^{7–10} and proteins^{11,12} within their (bio)macromolecular frameworks.

Tailoring the force-sensitivity of a mechanophore can be achieved by relying on supramolecular interactions, among

which the polymer mechanochemical activation of various metal–ligand bonds is well-investigated. For example, Pd and Pt pincer complexes,¹³ Pd coordination cages,¹⁰ metallocenes;^{14–16} Zn, Cu, Ni, Rh, and Ru acetylacetonates;^{17,18} N-heterocyclic carbene complexes with Ag, Ru,^{19–23} and Cu;^{24–27} as well as Pd phosphanes²⁸ were successfully activated by force. However, all of the above examples use synthetic polymers and mechanophores, while the use of biomacromolecular units remains unexplored in this context. Yet, metal complexes of biomacromolecules are widely investigated in *e.g.* nucleic acid research.²⁹ There, the metal-mediated formation of base-pairs (metallo-base-pairs), such as C–Ag⁺–C, T–Hg²⁺–T, or U–Hg²⁺–U (C: cytosine, T: thymine, U: uracil), allows the construction of DNA superstructures and nanomachines.³⁰ Thereby, the hydrogen bonds of Watson–Crick base-pairing are replaced by metal-base interactions. The selective regulation of these interactions is essential for their functional and structural application, which is why external control over this process is desirable.^{31,32}

Here we show the regulation of metallo-base-pair interactions by US on C–Ag⁺–C base-pairs allowing the reversible US-induced metal-base bond dissociation (Fig. 1). For this, we append long and stretchable multi-T DNA domains to the Ag⁺-paired motifs. By the introduction of a fluorescent dye and

Fig. 1 Mechanochemical regulation of C–Ag⁺–C base-pairing in DNA structures.

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† Electronic supplementary information (ESI) available: ODN sequences, fluorescence spectroscopy as well as sonication procedures. See DOI: 10.1039/d1cc02402g

a quencher as a distance-sensitive probe, we visually follow the reversible dissociation process.

We designed a series of Ag^+ -base-paired oligodeoxynucleotide (ODN) sequences (Table S1, ESI[†]) and labelled them with fluorescein (F) and its quencher dabcyI (Q), respectively (Fig. 1). In the Ag^+ base-paired state, both sequences hybridize to each other resulting in Förster resonance energy transfer (FRET) between F and Q. This was verified by the incremental addition of Ag^+ to unsubstituted and multi-T tail-bearing ODNs gradually reducing the fluorescence intensity of the solution (Fig. 2). Addition of 500 nM Ag^+ led to *ca.* 34% of the initial fluorescence intensity for unsubstituted ODNs (Fig. 2A). Increasing the length of the multi-T tail on the ODN weakened the Ag^+ -mediated hybridization (Fig. 2) as visible through a residual fluorescence intensity of 40% when using a 50T tail. This was expected as the C bases were progressively screened by the hydrodynamic random coil with increasing tail length and were thus decreasingly able to hybridize efficiently. Moreover, the additional negative charges on the polyT tails decreased the interstrand association due to the presence of additional repulsive electrostatic forces. Yet, Ag^+ promoted the hybridization for each combination rendering the designed ODNs suitable to investigate the mechanochemical response of the metal-base interaction.

We hypothesized that mechanical force would cleave the Ag^+ -base-pair interactions leading to fluorescence recovery. To investigate this process, we hybridized each ODN mixture with Ag^+ before subjecting it to US generated by an immersion probe setup at 20 kHz for 20 min using a 1 s on, 1 s off pulse sequence (Fig. 3A). Control experiments, where ODN-F and ODN-F alongside ODN-Q without the addition of Ag^+ were sonicated, showed no significant fluorescence alterations despite some minor degradation behaviour (Fig. 3, red and black lines).

Fig. 2 Fluorescence spectra ($\lambda_{\text{exc}} = 490$ nm) of Ag^+ -base-paired fluorescein- and quencher-labeled ODNs ($c = 10$ nM) upon the addition of 0, 50, 100, 200, and 500 nM Ag^+ . (A) Unsubstituted ODNs, (B) ODNs with 10T tail, (C) ODNs with 30T tail, and (D) ODNs with 50T tail.

Fig. 3 Normalized fluorescence intensity of Ag^+ -base-paired ODNs ($c = 10$ nM) over the course of their sonication. (A) Timeline for the experimental sequences leading to fluorescence intensity ($\lambda_{\text{em}} = 520$ nm) alterations of (B) ODNs without tail, (C) ODNs with 10T tail, (D) ODNs with 30T tail, and (E) ODNs with 50T tail. Mean values $\pm$ SD from the mean, $N = 3$ independent experiments.

When using no (Fig. 3B) or a short 10T tail (Fig. 3C), only negligible fluorescence recovery was recorded. We reasoned that the necessary contour length for polymer mechanochemical activation was not reached in these structures.^{33,34} In contrast, we observed a considerable fluorescence recovery when longer tails with 30T (Fig. 3D) and 50T (Fig. 3E) were used. With a 50T tail, the fluorescence recovered from *ca.* 55% to *ca.* 80% after US irradiation indicating the successful force-induced dissociation of the ODNs at the metal-base bond. The US-induced dehybridization process was further investigated by gel electrophoresis (Fig. S1, ESI[†]).

Hereafter, we investigated the reversibility of this process over three ultrasonication and re-hybridization cycles. Therefore, we hybridized ODNs bearing a 50T tail for 30 min with Ag^+ before the initial US treatment (Fig. 4A). The reversibility of the process was expressed in the US-induced recovery of the fluorescence while subsequent resting of the solution led to rebinding and thus again to fluorescence quenching (Fig. 4B). This reversibility is comparable to the thermally induced annealing and denaturation process commonly employed (Fig. S2, ESI[†]).³⁵ We attributed the apparent fatigue of the system to non-specific chain scission along the ODN backbone and possibly degradation of Q over time likely led to diminished hybridization capability after prolonged US irradiation as well. The reversibility of the presented system could potentially be enhanced by the incorporation of the C- Ag^+ -C-base-pairs in a looped structure. Then, the bond scission and rebinding would be an intramolecular process analogous to unfolding single-chain nanoparticles as recently demonstrated by Diesendruck and co-workers.¹⁸

Fig. 4 Normalized fluorescence intensity of Ag⁺-base-paired ODNs with 50T tail ($c = 10$ nM) over 3 sonication and resting cycles. (A) Timeline for the experimental sequences leading to (B) fluorescence intensity ($\lambda_{\text{em}} = 520$ nm) alterations. Mean values $\pm$ SD from the mean, $N = 3$ independent experiments.

We here presented a biomacromolecular system to control the metal-mediated base-pair dehybridization of DNA by mechanical force induced by US in solution. We directly observed the dehybridization process by distance-sensitive fluorescent probes. Importantly, the regulation of this process was found to be reversible. We expect that US as an external stimulus will be useful in the future to regulate DNA hybridization and dehybridization processes in many functional systems where remote control over DNA assembly is desirable.

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Conflicts of interest

There are no conflicts to declare.

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