This article has been accepted for publication in IEEE Transactions on NanoBioscience. This is the author's version which has not been fully edited and content may change prior to final publication. Citation information: DOI 10.1109/TNB.2026.3724260

Repurposing the *Escherichia coli* HU as a Universal dsDNA Amplicon Detection Tool

Ankur Ruhela, Monica Choudhary, Vasso Skouridou, and Lluis Masip

***Abstract*—DNA binding proteins interact with DNA in a sequence-specific manner or indiscriminately without sequence specificity. While these properties make them highly attractive for assay development, their use as DNA biorecognition elements has remained limited. In this work, we describe a new application using the non-sequence specific *Escherichia coli* histone-like HU protein for general DNA detection. We engineered a single-chain HU heterodimer (scHupBA) that exhibits high binding affinity for double-stranded DNA (≥50 bp) and considerably lower affinity for single-stranded DNA. By combining this protein with a scHupBA-horseradish peroxidase conjugate, we developed an assay capable of detecting PCR amplicons, either post-purification or directly within a PCR reaction, using microtiter plates and magnetic beads. This system efficiently detected DNA fragments >100 bp regardless of sequence, with detection signals increasing non-linearly with fragment length. To the best of our knowledge, this is the first report of the use of a non-sequence-specific double-stranded DNA binding protein for general DNA detection. The approach described herein is target-independent, it does not require any primer modification or labeling nor the use of expensive reagents. It is an overall cost-effective and efficient method for universal dsDNA amplicon detection, especially for rapid analysis of amplification reactions.**

***Index Terms*— Recombinant protein, PCR, amplicon, microplate, magnetic beads, HRP**

I. Introduction

MOLECULAR diagnostics have enabled rapid and accurate detection of diseases and genetic disorders and facilitate environmental and food safety surveillance. It is a rapidly evolving field that involves the detection and quantification of biological molecules, such as DNA, RNA, and proteins [1], [2]. Biosensing systems based on the detection of double-stranded DNA (dsDNA) specifically have found various applications in clinical diagnostics [3], environmental monitoring [4] and food safety [5]. DNA biosensors utilize the principles of molecular recognition and signal transduction to provide sensitive and specific detection of target DNA molecules, and they are typically used to detect DNA after amplification [6] but also non-amplified DNA [7].

Biosensing of DNA amplification products was based exclusively on the detection of polymerase chain reaction (PCR) amplicons since PCR was the gold standard in DNA amplification, however this changed in the early 2000's when alternative isothermal amplification techniques emerged [8]. While PCR requires a dedicated instrument (thermal cycler), equipped with a Peltier module to rapidly heat and cool at different temperatures in a repetitive manner, isothermal amplification can be performed at a constant temperature utilizing simple heating elements. Direct detection of non-amplified DNA is also attractive since it circumvents amplification and potentially labeling as well, thus lowering the overall cost and time-to-result. Indeed, detection of non-amplified genomic DNA has been documented, even though to a much lesser extent compared to amplified DNA, and novel nanomaterial and signal amplification strategies have been employed to achieve the required sensitivity without amplification [9], [10]. Hybridization chain reaction, an isothermal amplification-free method, can be alternatively used for highly sensitive detection of DNA. It employs two metastable hairpin DNA probes which hybridize between them only in the presence of the target sequence which acts as the initiator of a hybridization cascade reaction between the two probes. This results in the generation of nicked double stranded DNA molecule with many repeating units [11].

DNA amplicons and genomic DNA can be detected using UV-Vis spectroscopy and DNA stains but neither approach can provide information on specific DNA sequences. This is achieved using biosensors and bioanalytical assays which incorporate target-specific biorecognition elements. Many types of DNA biosensors have been developed for this purpose, and the most common ones are optical and electrochemical ones [12], [13], [14]. Nucleic acid lateral flow tests are another type of biosensors used for colorimetric or fluorescence detection of DNA and are considered especially suitable for point-of-care (POC) testing as they fulfil the REASSURED criteria for ideal POC tests [15], [16], [17].

DNA binding proteins are proteins which recognize and bind single stranded (ssDNA) [18] or dsDNA in a general, non-sequence specific or in a sequence-specific manner [19], and they play crucial roles in various cellular processes,

This work was supported financially by the CELLECTRA project (PID2020-112976RB-C22) funded by the Ministerio de Ciencia e Innovación (MICINN), the Agencia Estatal de Investigación (AEI) and the Fondo Europeo de Desarrollo Regional (FEDER). A.R. acknowledges the Ministerio de Ciencia e Innovación (MICINN), the Agencia Estatal de Investigación (AEI) and the Fondo Europeo de Desarrollo Regional (FEDER) for his Ph.D. scholarship.

The authors are with the Interfibio Research Group, Departament d'Enginyeria Química, Universitat Rovira i Virgili, 26 Països Catalans, 43007 Tarragona, Spain (e-mail: ankur.ruhela11@gmail.com; vasoula.skouridou@urv.cat; lluis.masip@urv.cat).

This article has supplementary downloadable material.

This work is licensed under a Creative Commons Attribution 4.0 License. For more information, see https://creativecommons.org/licenses/by/4.0/

This article has been accepted for publication in IEEE Transactions on NanoBioscience. This is the author's version which has not been fully edited and content may change prior to final publication. Citation information: DOI 10.1109/TNB.2026.3724260

including transcription, replication, repair, and recombination. Despite the existence of a variety of DNA binding proteins with different properties, only a handful have been exploited in DNA biosensors as biorecognition elements: the *Escherichia coli* single stranded DNA binding protein SSB for electrochemical [20] or fluorescent [21] detection of total DNA; the human sequence-specific dsDNA binding protein p53 for electrochemical miRNA detection [22], [23]; and the sequence-specific SpoIIID transcription factor from *Bacillus subtilis* for colorimetric detection of dsDNA amplification products from PCR [24] and loop-mediated isothermal amplification (LAMP) [25]. The DNA sequence-specific repressors from bacteriophage lambda Cro and from *E. coli* (LacI headpiece domain) have been engineered by our group as single chain variants (scCro and dHP, respectively) and have been used individually or simultaneously for the colorimetric detection of ssDNA and dsDNA in enzyme-linked oligonucleotide assays (ELONA), lateral flow assays (LFAs) or magnetic bead-based assays [26], [27], [28], [29], [30], [31]. To develop the assays, scCro and dHP were conjugated to the signal-generating enzymes horseradish peroxidase (HRP) and glucose oxidase (GOX), to carbon nanoparticles and to magnetic beads. The specific DNA binding sites for these proteins were incorporated in detection probes or in primers for ssDNA or PCR amplicon detection, respectively.

In this work, we demonstrate the use of a non-sequence-specific dsDNA binding protein for universal and label-free detection of DNA, which is expected to be compatible with amplicons generated with PCR or isothermal workflows. Specifically, we report the engineering of a single-chain heterodimeric variant of the histone-like HU protein from *E. coli* by fusing its α and β monomers. The resulting scHupBA protein was expressed in *E. coli* and purified, and its DNA binding properties were characterized by an enzyme-linked oligonucleotide assay (ELONA). Finally, scHupBA was immobilized on microtiter plates and magnetic beads and used in combination with a scHupBA-HRP conjugate in sandwich assays for target DNA detection (Fig. 1). To the best of our knowledge, this is the first work reporting the use of a non-sequence-specific DNA binding protein for general detection of DNA. With this study, we seek to highlight the great potential of these proteins as universal DNA bioreceptors for label-free and cost-effective detection of DNA fragments, with a specific focus on establishing a target-independent platform for the rapid analysis of amplification products >100 bp.

II. Materials and Methods

A. Material

Phosphate buffered saline (PBS; 10 mM phosphate, 137 mM NaCl, 2.7 mM KCl, pH 7.4), the TransformAid Bacterial Transformation Kit, the aLICator LIC Cloning and Expression Kit 3, the *E. coli* DH5a and BL21(DE3) cells, the GeneJET Plasmid Miniprep Kit, PD-10 desalting columns, PES protein concentrators 3K MWCO, Zeba Micro Spin desalting column 7K MWCO, the Micro BCA Protein Assay Kit, HisPure Ni-NTA agarose, SP Sepharose Fast Flow resin, Con A Sepharose 4B resin, horseradish peroxidase (HRP), maleimide activated HRP, skim milk powder, the magnetic beads (Dynabeads M-270 Epoxy and Dynabeads MyOne Streptavidin T1 (SA-MB)), DreamTaq DNA polymerase, dNTPs and N-succinimidyl S-acetylthioacetate (SATA) were from Fisher Scientific (Spain). Streptavidin-polyHRP80 conjugate (SA-pHRP) was from SDT (Germany), human genomic DNA from whole blood was obtained from Merck Life Science S.L.U. (Spain), Zymo Research DNA purification kits from Ecogen (Spain), the BioFX TMB Super Sensitive One Component HRP Microwell Substrate and BioFX TMB One Component HRP Membrane Substrate from Surmodics (USA). The DNA oligonucleotides were purchased from Biomers.net (Germany) and their sequences are shown in Tables S1 and S2. All other reagents and material were obtained from Fisher Scientific (Spain), Scharlab (Spain) and Merck (Spain).

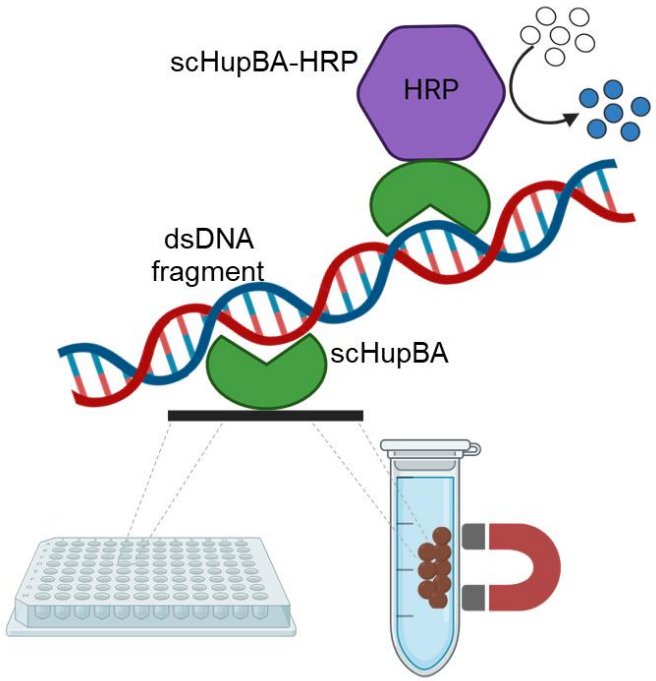


Fig. 1. Schematic representation of Enzyme Linked Oligonucleotide Assay (ELONA) using microtiter plates and magnetic beads for dsDNA fragment detection using scHupBA and scHupBA-HRP.

B. Cloning, expression and purification of scHupBA and conjugation to horseradish peroxidase (HRP)

The sequence of the scHupBA fusion gene was based on the UniProt accession IDs P0ACF0 (HU α monomer) and P0ACF4 (HU β monomer). The two genes were fused together, the final fusion gene was synthesized by Eurofins Genomics (Germany), and it was inserted into the pLATE31 vector as detailed in the Supplementary Material. The protein was expressed in *E. coli* BL21(DE3) cells and purified by immobilized metal affinity chromatography (IMAC) and ion

This work is licensed under a Creative Commons Attribution 4.0 License. For more information, see https://creativecommons.org/licenses/by/4.0/

This article has been accepted for publication in IEEE Transactions on NanoBioscience. This is the author's version which has not been fully edited and content may change prior to final publication. Citation information: DOI 10.1109/TNB.2026.3724260

exchange chromatography (IEC). The scHupBA was conjugated to HRP via SATA crosslinking and it was purified by IMAC and concanavalin A (ConA) affinity chromatography. All details regarding the expression and purification of the protein and the preparation of the scHupBA-HRP conjugate can be found in the Supplementary Material.

C. Characterization of the DNA binding properties of scHupBA

The optimum concentration of scHupBA required to coat the microtiter plate wells was evaluated using an ELISA. Specifically, the protein was used at 0.6–40 μg/mL (50 μL in 5 mM carbonate-bicarbonate buffer pH 9.4) and it was incubated on Nunc MaxiSorp immuno 96-well plates for 1 h at 22ºC. The wells were washed (3x200 μL of PBST), blocked with 200 μL of 5 % w/v skim milk/PBST for 30 min under mild agitation and the immobilized protein was detected with a mouse anti-His tag antibody (1 μg/mL in 1 % w/v skim milk/PBST) in combination with a rabbit anti-mouse-HRP conjugate (1 μg/mL in 1 % w/v skim milk/PBST) as described in section earlier for the bead-ELISA. To assess the DNA binding properties of scHupBA by Enzyme Linked Oligonucleotide Assay (ELONA), the protein was used at 10 μg/mL to coat the wells followed by washing and blocking as described above. Then, biotinylated dsDNA or ssDNA probes of different lengths (25, 47 or 100 bases; prepared as detailed in the Supplementary Material) were added to the wells (50 μL of 0–4000 nM in PBS) and incubated for 30 min under mild agitation. After incubation, the wells were washed again with PBST and 50 μL of SA-pHRP (1 μg/mL in PBST) were added for another 30-min incubation. Finally, the wells were washed, 50 μL of TMB microwell substrate were added and 5 min afterwards, the reaction was stopped with 50 μL of 1 M sulfuric acid and the absorbance was recorded at 450 nm. Duplicate values from three independent experiments were used for the calculation of the affinity dissociation constants (K_D values). Background-subtracted absorbance values were plotted against DNA concentration and the data was fitted to the "One site specific binding" model using GraphPad Prism for K_D determination.

D. Analysis of dsDNA and PCR reactions on microtiter plates using scHupBA and scHupBA-HRP

The scHupBA protein (50 μL of 10 μg/mL of the protein in 5 mM carbonate-bicarbonate buffer pH 9.4) was immobilized on Nunc MaxiSorp immuno 96-well plates for 1 h at 22ºC. The wells were blocked with 200 μL of 5 % w/v skim milk/PBST for 30 min and washed with PBST (3x200 μL). Then, dsDNA or ssDNA fragments of different lengths (47, 100 and 176 bases) was added to the wells (50 μL in PBS) and incubated for 30 min. The plate was washed again with PBST and 50 μL of 5 μg/mL scHupBA-HRP (in PBS) were added, followed by another 30 min incubation. The concentration of the conjugate was previously optimized using the 176 bp dsDNA fragment as the target. Finally, the plate was washed, 50 μL of TMB microwell substrate were added and signal was stopped after 15 min by adding 50 μL of 1 M sulfuric acid. Absorbance was recorded at 450 nm. When DNA fragments of different lengths and type (dsDNA or ssDNA) were used, the concentration of DNA was constant at 500 nM. The effects of buffer composition (water, 0.1x PBS and 1x PBS) and scHupBA-HRP concentration (0–20 μg/mL) on signal intensity were studied. To evaluate the sensitivity of the assay, the 176 bp model PCR amplicon was used at a concentration range of 0–1000 nM (0–115 ng/μL) in phosphate buffer containing 30–240 mM of NaCl. The data were fitted to a logarithmic model and the limit of detection (LOD) was interpolated as the blank plus three times its standard deviation. All steps were performed at 22ºC under mild agitation. Duplicate values from at least two independent experiments were used for these studies. In the case of direct analysis of unpurified PCR reactions, 10 μL of each PCR reaction performed using different starting amounts of target template (2.5 fg/mL to 25 pg/mL) were diluted with 40 μL of water and then added to the microplate wells containing scHupBA as detailed above.

E. Direct analysis of PCR reactions using scHupBA immobilized on magnetic beads (MB) and scHupBA-HRP

The immobilization of scHupBA on epoxy-activated magnetic beads is described in the Supplementary Material. For DNA detection, scHupBA-MB (1 μL of 30 mg/mL in PBS) were blocked with 100 μL of 5 % w/v skim milk/PBST for 30 min and then washed with PBST (3x100 μL). The beads were resuspended with diluted PCR reaction (5 μL of PCR reaction and 45 μL of water) performed using 2.5 fg/mL to 25 pg/mL of the HPV16 176 bp dsDNA as template and without prior purification, followed by incubation for 30 min. Subsequently, the beads were washed with PBST and scHupBA-HRP was added (50 μL of 5 μg/mL in PBS) for another 30 min incubation. Finally, the beads were washed and 50 μL of TMB microwell substrate were added. Signal generation was stopped after 5 min by the addition of 50 μL of 1 M sulfuric acid. The supernatants were separated from the magnetic beads and transferred to the wells of a 96-well plate to measure the absorbance at 450 nm. The effects of the type of blocking solution for the magnetic beads (5 % w/v skim milk or 1 % w/v BSA in PBST) and the buffer used for the incubations on signal intensity were evaluated. All incubation steps were performed at 22ºC under mild rotation. Duplicate values from at least two independent experiments were used for these studies.

III. RESULTS AND DISCUSSION

A. Recombinant expression of the scHupBA single-chain heterodimer

The *E. coli* HU is a heterodimer composed of the two

This work is licensed under a Creative Commons Attribution 4.0 License. For more information, see https://creativecommons.org/licenses/by/4.0/

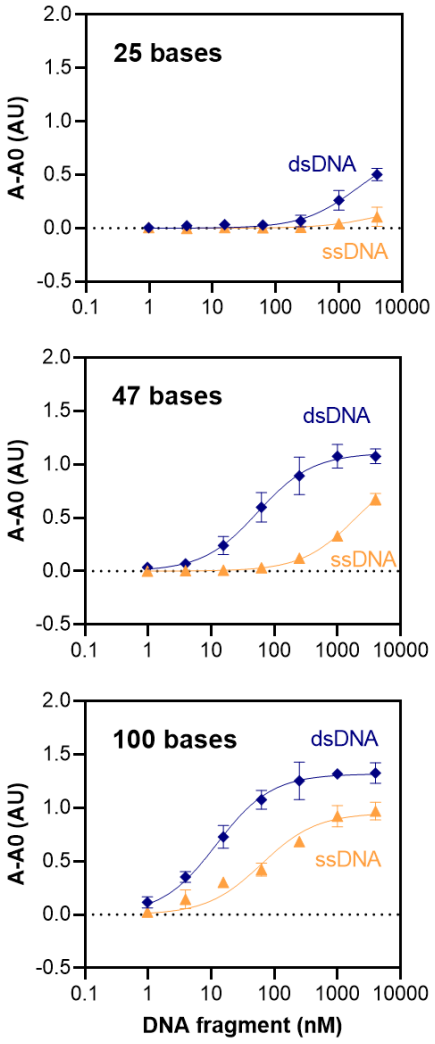
This article has been accepted for publication in IEEE Transactions on NanoBioscience. This is the author's version which has not been fully edited and content may change prior to final publication. Citation information: DOI 10.1109/TNB.2026.3724260

highly homologous subunits α and β of approximately 9.5 kDa each [32]. They share almost 70 % amino acid sequence identity and the HUαβ heterodimer is the predominant native form [33]. HU proteins bind to DNA fragments in a sequence-independent manner, exhibiting higher affinity in lower salt concentrations [34]. Each dimer binds to a fragment of approximately 9 base pairs (9 bp), causing slight bending to DNA once several HU dimers are bound [35]. The global DNA binding properties of these proteins make them particularly attractive as biorecognition molecules for universal DNA bioanalysis and in this work, we sought to engineer a recombinant HUαβ heterodimer for this purpose. To facilitate expression and purification, a single-chain format of the protein was designed by fusing the C-terminal of the β monomer to the N-terminal of the α monomer using a flexible glycine-rich 16 amino acid [36]. In the three-dimensional structure of the *E. coli* HU bound to a 9 bp DNA fragment [37], it was observed that these ends are further away from the protein-DNA interaction surface (Fig. S1a), hence less likely to interfere with the DNA binding properties of the protein. The fused gene was inserted in an *E. coli* expression vector for the expression of the protein with a C-terminal His-tag (Fig. S1b).

The expression of scHupBA protein in *E. coli* BL21(DE3) cells was highly controlled, since a band corresponding to scHupBA was only visible in induced cells (whole cell and soluble lysate), confirming the production of soluble protein (Fig. S2a). The protein was purified from the soluble fraction of lysed cells using first IMAC and cation exchange chromatography (IEC) (Fig. S2b). The final purified scHupBA had an apparent size of approximately 26 kDa and it exhibited high purity of > 95 % (Fig. S2c), whereas Western blot analysis of IEC fractions also verified the size of the protein (Fig. S2d). Protein concentration was determined using bicinchoninic acid (BCA) as the protein does not absorb at 280 nm due to the lack of tryptophan and tyrosine residues. The final yield of scHupBA was approximately 6 mg of purified protein per 1 L of bacterial culture.

B. DNA binding properties of scHupBA

HU histone-like proteins are ubiquitous in bacteria like *E. coli* and they bind various DNA structures to regulate chromosome compaction and transcription. While HU binds DNA in a sequence-independent manner, it has a structural preference for "distorted" or damaged DNA architectures including replication forks, DNA gaps, nicks and 3' and 5' overhangs [35]. Here, we assessed the DNA binding properties of the expressed scHupBA protein to simple synthetic dsDNA and ssDNA fragments on microtiter plates using an enzyme-linked oligonucleotide assay (ELONA). The optimum amount of scHupBA to be physically adsorbed on the plate wells was first determined by ELISA as 10 µg/mL (Fig. S3). A range of concentrations of biotinylated DNA probes of 25, 47 or 100 bases (Table S2), both dsDNA and ssDNA, were prepared as detailed in the Supplementary Material and were used for the binding experiments. Bound DNA was detected with a streptavidin-HRP conjugate and a chromogenic substrate.



DNA fragment	Probe	K_D (nM)	R^2
25 b	dsDNA	1937 ± 518	0.9350
	ssDNA	4160 ± 5705	0.5286
47 b	dsDNA	55 ± 7	0.9515
	ssDNA	2008 ± 202	0.9862
100 b	dsDNA	13 ± 1	0.9791
	ssDNA	67 ± 12	0.9370

Fig. 2. Binding of scHupBA to dsDNA and ssDNA fragments of 25, 47 and 100 bases. Error bars correspond to the standard deviation (n≥4).

Concentration-dependent binding curves were obtained for all three DNA fragments (Fig. 2), confirming that the engineered single-chain HU (scHupBA) maintains its DNA binding properties. For dsDNA, the dissociation constants (K_D) decreased as fragment length increased (1937 nM for 25 bp, 55 nM for 47 bp and 13 nM for 100 bp). This apparent length-dependent affinity suggests that longer fragments accommodate more protein molecules, considering that four HU dimers can bind per 42 bp [37]. The protein also bound

This work is licensed under a Creative Commons Attribution 4.0 License. For more information, see https://creativecommons.org/licenses/by/4.0/

This article has been accepted for publication in IEEE Transactions on NanoBioscience. This is the author's version which has not been fully edited and content may change prior to final publication. Citation information: DOI 10.1109/TNB.2026.3724260

ssDNA, though with markedly lower affinity (K_D of 4160, 2008, and 67 nM for 25, 47, and 100 bases). This weaker ssDNA binding, which may be partially driven by interactions with intramolecular stem-loop structures, is consistent with reports that HU dimers require 24 nt for initial ssDNA binding and 12 nt per subsequent dimer [38]. Nevertheless, the strong binding affinity of scHupBA for dsDNA fragments of ≥ 50 bp in combination with its evidently lower affinity for short ssDNA are highly advantageous for bioanalytical assays employed for dsDNA detection, especially amplicons in crude reactions where short ssDNA primers are also present.

C. Preparation of the scHupBA-HRP conjugate

The purified scHupBA was conjugated to HRP using SATA. The main conjugation product conjugate had an apparent size of 64 kDa (Fig. S4a, lane 3), consistent with a conjugate at a 1:1 stoichiometry. The unreacted proteins were removed from the conjugation reaction by first performing IMAC to eliminate HRP (Fig. S4b, lanes 6–10) and then ConA affinity chromatography to eliminate scHupBA (Fig. S4c, lanes 11–13). The final purified and concentrated conjugate was free of unreacted protein species (Fig. S4d), yet it was heterogeneous containing multiple conjugation products.

D. Microplate-based detection of dsDNA

To develop a scHupBA-based bioanalytical assay for dsDNA detection, the protein was physically absorbed on microtiter plates for DNA capture, whereas the scHupBA-HRP conjugate was used for detection (reporter), as illustrated in Fig.1. By using these two proteins in a sandwich format, dsDNA molecules can be detected regardless of their specific sequence. And if these DNA molecules are amplification products from PCR or an isothermal amplification method, the primers do not require any labeling or modification. The concentration of the scHupBA-HRP conjugate was previously optimized at 5 µg/mL (Fig. S5) using scHupBA at 10 µg/mL for DNA target capture (Fig. S3) to avoid full saturation of the DNA binding sites and allow simultaneous binding of the reporter since both interact with the same binding sites. Although the scHupBA-HRP conjugate was heterogeneous and susceptible to batch-to-batch variation, its use in excess as a reporter is expected to limit the impact of this variability on assay performance. Moreover, routine quality control and further optimization of the conjugation reaction to favor 1:1 stoichiometry should minimize any remaining effect on performance. Initially, the detection of three DNA fragments with varying length was evaluated, using both dsDNA and ssDNA prepared in PBS under physiological salt conditions (137 mM NaCl and 2.7 mM KCl). Only the 176 bp fragment was reliably detected, very low signal was observed for the 100 bp fragment, whereas the 47 bp dsDNA fragment could not be detected (Fig. 3a). These results indicate that since capture and detection molecules share the same binding sites, a minimum DNA length is required to allow both proteins to bind simultaneously and produce a measurable signal. On the other hand, ssDNA did not produce significant signals, suggesting that the proposed detection strategy preferentially detects dsDNA fragments >100 bp in length, consistent with the typical size of nucleic acid amplification products.

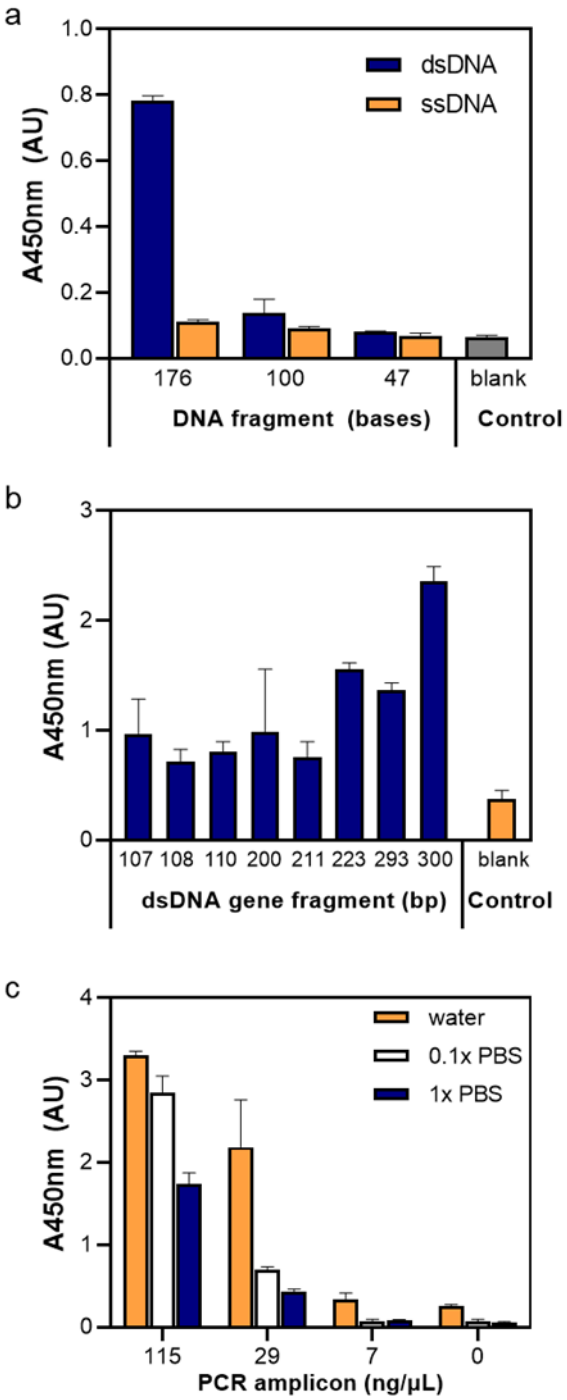


Fig. 3. Detection of DNA by microplate-ELONA, using. scHupBA for capture and scHupBA-HRP for detection. (a) Detection of dsDNA and ssDNA of varying length (47, 100, 176 bases). All fragments were used at 500 nM. (b) Effect of dsDNA fragment length on signal intensity. Each fragment was used at 100 nM. (c) Effect of the composition of buffer used for DNA binding on signal intensity for the detection of the 176 bp PCR amplicon. The data represents averages from duplicate measurements with at least two independent experiments, and the error bars their standard deviation.

This work is licensed under a Creative Commons Attribution 4.0 License. For more information, see https://creativecommons.org/licenses/by/4.0/

This article has been accepted for publication in IEEE Transactions on NanoBioscience. This is the author's version which has not been fully edited and content may change prior to final publication. Citation information: DOI 10.1109/TNB.2026.3724260

To confirm that the assay is sequence-independent and primarily driven by DNA length, various dsDNA gene fragments of 107 to 300 bp (39.8–52.7% GC) were analyzed. These fragments spanning 107–110 bp, 200–223 bp and 293–300 bp were prepared by PCR amplification of human or bacterial (*E. coli*) genomic DNA using specific primer pairs (Table S1), followed by purification (Fig. S6) prior to analysis with the microplate assay. Their expected sequences (Table S3) were predicted using Primer-BLAST. As can be seen in Fig. 3b, the detection signal showed an overall tendency to increase with increasing dsDNA fragment length, although the relationship was not linear. Specifically, while the shorter fragments under 211 bp showed a low and steady baseline signal, longer fragments showed a length-dependent jump in the detection signal, which peaked at 300 bp. In all cases, the signals were well above the blank control, confirming specific detection. The non-linear relationship between DNA length and signal may reflect a combination of increased availability of binding sites on longer DNA fragments as well as steric constraints due to the simultaneous binding of immobilized scHupBA and detector scHupBA-HRP molecules, rather than DNA length alone. Furthermore, since *E. coli* HU induces bending of the DNA backbone upon binding [35], this bending mechanism may be less favorable on shorter DNA fragments, where steric hindrance limits the number of proteins that can be accommodated, while longer fragments provide greater spatial flexibility, allowing multiple HU proteins to bind and bend the backbone more readily.

The interaction of native HupBA heterodimer with DNA is salt-dependent, with increased affinity at sub-physiological conditions (≤ 100 mM NaCl) and complete dissociation from DNA for > 300 mM NaCl [39]. To evaluate whether this salt-dependent DNA binding is maintained in the engineered scHupBA protein, different buffer conditions were employed for the detection of the model target 176 bp PCR amplicon. As can be observed in Fig. 3c, higher signals were measured in water followed by 0.1x PBS (13.7 mM NaCl and 0.27 mM KCl), while considerably lower ones were recorded in 1x PBS (137 mM NaCl and 2.7 mM KCl). However, when the assay was performed in water, higher background non-specific signals were observed, which were effectively eliminated in the presence of even low concentrations of salt (14 mM).

Based on these observations, we evaluated the assay sensitivity over a range of NaCl concentrations (30–240 mM) in phosphate buffer. As shown in Fig. S8, the limit of detection (LOD) remained unchanged (2.2–3.5 ng/µL PCR amplicon, corresponding to 19–31 nM), regardless of the salt concentration used. However, the assay sensitivity increased at lower NaCl concentrations, with the signal span at 30 mM NaCl being approximately 1.5-fold higher than at 240 mM (1.72 AU vs 1.12 AU). These results confirm the salt-dependent DNA binding properties of the engineered scHupBA, with the lower ionic strength promoting stronger DNA binding and improved assay performance.

E. Direct analysis of PCR reactions

Having established the use of scHupBA for detection of purified dsDNA on microtiter plates, the compatibility of the assays with direct analysis of PCR reactions without prior amplicon purification was finally tested using microtiter plates and magnetic beads. Magnetic beads (MB) are an extremely useful tool for bioanalysis because of their attractive properties, such as high binding capacity for diverse biomolecules, facile and quick magnetic separation as well as pre-concentration of dilute samples for improved sensitivity, especially for nucleic acid detection [40]. The scHupBA protein was immobilized on epoxy-activated magnetic beads and verified by bead-ELISA (Fig. S9a), whereas the type of blocking solution for MB-based assays was also optimized (Fig. S9b). PCR reactions were performed using different concentrations of the 176 bp HPV16 target (Fig. S7). For analysis using the scHupBA-based assays, the reactions were simply diluted five-fold and the amplicons were detected as detailed in the respective sections. The results obtained with the purified dsDNA amplicon on microtiter plates suggested better performance in the presence of low salt concentration, therefore water was chosen for this part as the diluent. All other conditions were identical to the ones used for the other assays detailed earlier.

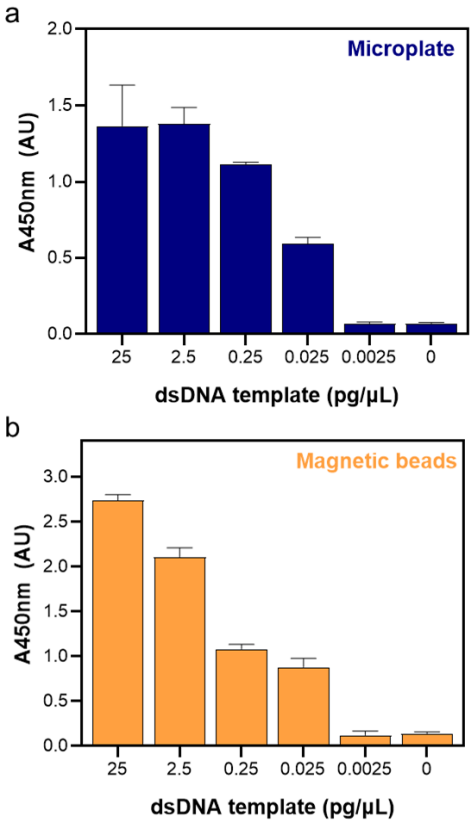


Fig. 4. Detection of PCR amplicons directly in the PCR reactions without prior purification by colorimetric ELONA using microtiter plates and magnetic beads. The PCR reactions were performed using different dsDNA template concentrations (0.0025–25 pg/µL). *n*=2, error bars=standard deviation.

As shown in Fig. 4, the dsDNA amplicon was successfully detected by microplate and magnetic beads ELONA directly in the PCR reactions without prior purification when 0.025–25 pg/µL (0.219–219 pM) of template dsDNA was used for

This work is licensed under a Creative Commons Attribution 4.0 License. For more information, see https://creativecommons.org/licenses/by/4.0/

This article has been accepted for publication in IEEE Transactions on NanoBioscience. This is the author's version which has not been fully edited and content may change prior to final publication. Citation information: DOI 10.1109/TNB.2026.3724260

amplification. Background-level signals were obtained when 0.0025 pg/µL were used. The LODs were calculated using a logarithmic model and they were very similar at 2.1 fg/µL (18.4 fM) and 3.5 fg/µL (30.6 fM) for the microplate and magnetic beads-based assays, respectively (Fig. S10). These results confirm the suitability of the scHupBA-based assays for detecting amplicons in amplification reactions without the requirement of purification. The absence of signals from the control PCR reactions with no target template but which contain the starting concentration of primers further confirms the very low binding affinity of scHupBA for small ssDNA fragments. This is very important since they do not interfere with the detection of the dsDNA amplicons, especially the ones with a length of more than 100 bp, and their removal by purification is not required.

Table S4 summarizes the previous works reported in the literature employing DNA binding proteins in assays and biosensors for DNA detection. The first report was 35 years ago which described the first use of the sequence-specific ssDNA binding protein SSB from *E. coli* for total DNA detection (LOD of 2 pg of dsDNA), an approach requiring different protein conjugates [20]. A second work employing the same protein was reported 22 years later, using a fluorophore-labelled SSB [21]. Sequence-specific dsDNA binding proteins have also been successfully used for highly sensitive detection of DNA. The human p53 protein was used for miRNA detection based on biotinylated capture probes and detection probes containing the specific DNA binding sequence for p53. The electrochemical biosensors described were highly sensitive (92 aM to 22 fM of miRNA) but the overall platform required two steps for magnetic capture and release prior to measurements [22], [23]. The *Bacillus subtilis* SpoIIID protein was used to detect dsDNA PCR [24] and LAMP [25] amplicons employing modified primers containing the SpoIIID binding sites in colorimetric microplate-based assays, with the LAMP-based assay able to detect 2–24 copies of genomic DNA from bovine mastitis pathogens. The bacteriophage lambda Cro DNA binding protein in an engineered single-chain format was used for the development of colorimetric assays exploiting microtiter plates, magnetic beads and lateral flow assays for the detection of ssDNA [26] and dsDNA PCR amplicons from amplification of synthetic DNA [31] and genomic DNA [26], [30], [31]. The same Cro protein was also combined with the *E. coli* repressor LacI headpiece domain, engineered by our group into a single-chain variant (dHP), for the detection of ssDNA [27] and a dsDNA PCR amplicon [29] on microtiter plate and a lateral flow assay, respectively. For all assays with the scCro and dHP proteins, modified probes or primers with the Cro and LacI binding sites were required and the LODs achieved varied depending on the specific application. In the work, we demonstrate the use of the HU DNA binding protein for the universal detection of dsDNA (as a purified PCR amplicon or as a template employed in a PCR reaction) without using any modified probes or primers since it is a sequence-independent approach. The sensitivities achieved were similar or improved compared to the use of sequence-specific DNA binding proteins in combination with modified probes and primers. Although in this work we focused on microplate and magnetic bead-based assays, the approach is adaptable and could be implemented, alone or in combination with the HRP conjugate, in a wide range of platforms, including lateral flow assays and electrochemical biosensors.

IV. CONCLUSION

This study explores the use of the *E. coli* histone-like HU protein as a generic dsDNA detection tool. A single-chain heterodimeric scHupBA protein was engineered by fusing the two HU subunits and expressed in *E. coli*. The DNA binding properties of scHupBA were evaluated using biotinylated probes, showing increased affinity with dsDNA length and substantially lower binding to ssDNA. To enhance the DNA detection capability of scHupBA-based assays, the protein was conjugated to HRP and applied in sandwich assay formats on microtiter plates and magnetic beads for detecting PCR amplicons. Using PCR-amplified dsDNA gene fragments from human and bacterial sources (107–300 bp), detection appeared to be sequence-independent. Although longer fragments produced higher signals, the non-linear relationship suggests that assay response might depend not only on DNA length but also on factors such as the availability of HU binding sites, as well as steric constraints associated with simultaneous binding of immobilized and detector HU molecules and HU-induced DNA bending. Assay performance improved at lower NaCl concentrations, consistent with stronger HU-DNA interactions at reduced ionic strength. The system enabled direct detection of amplicons in unpurified PCR reactions without interference from residual ssDNA primers. These results suggest that scHupBA functions as a sequence-independent and primer-insensitive dsDNA bioreceptor. Such assays could be applied to monitoring amplification in routine PCR or isothermal workflows without primer modification or labelling, thus reducing costs and complexity. They could also be integrated into lateral flow formats or other portable platforms for straightforward detection of amplification products, particularly in low-resource settings where simplified protocols and minimal sample processing are advantageous. Further work is focused on optimizing assay conditions and better understanding the factors affecting binding efficiency and signal generation.

REFERENCES

[1] Y. Hua, J. Ma, D. Li, and R. Wang, “DNA-based biosensors for the biochemical analysis: a review”, *Biosensors*, vol. 12, no. 3, Mar. 2022, Art. No. 183.

[2] Y. Su, and M. C. Hammond, “RNA-based fluorescent biosensors for live cell imaging of small molecules and RNAs”, *Curr. Opin. Biotechnol.*, vol. 63, pp. 157–166, Jun. 2020.

[3] M. Li, F. Yin, L. Song, X. Mao, F. Li, C. Fan, X. Zuo, and Q. Xia, “Nucleic acid tests for clinical translation”, *Chem. Rev.*, vol. 121, no. 17, pp. 10469–10558, Sep. 2021.

[4] I. Palchetti, and M. Mascini, “Nucleic acid biosensors for environmental pollution monitoring”, *Analyst*, vol. 133, no. 7, pp. 846–854, Jul. 2008.

[5] S. Ceuppens, D. Li, M. Uyttendaele, P. Renault, P. Ross, M. V. Ranst, L. Cocolin, and J. Donaghy, “Molecular methods in food safety microbiology: interpretation and implications of nucleic acid detection”, *Compr. Rev. Food Sci Food Saf.,* vol. 13, no. 4, pp. 551–577, Jul. 2014.

[6] D. Chang, K. Tram, B. Li, Q. Feng, Z. Shen, C. H. Lee, B. J. Selena, and Y. Li, “Detection of DNA amplicons of polymerase chain reaction using Litmus test”, *Sci. Rep.*, vol. 7, no. 1, Jun. 2017, Art. No. 3110.

[7] T. Kaewphinit, S. Santiwatanakul, C. Promptmas, and K. Chansiri, “Detection of non-amplified *Mycobacterium tuberculosis* genomic DNA

This work is licensed under a Creative Commons Attribution 4.0 License. For more information, see https://creativecommons.org/licenses/by/4.0/

This article has been accepted for publication in IEEE Transactions on NanoBioscience. This is the author's version which has not been fully edited and content may change prior to final publication. Citation information: DOI 10.1109/TNB.2026.3724260

using piezoelectric DNA-based biosensors", *Sensors*, vol. 10, no. 3, pp. 1846–1858, 2010.

[8] B. B. Oliveira, B. Veigas, and P. V. Baptista, "Isothermal amplification of nucleic acids: the race for the next "gold standard"", *Front. Sensors*, vol. 2, Sep. 2021, Art. No. 752600.

[9] D. Li, and C. Fan, "Optical detection of non-amplified genomic DNA", in *Detection of non-amplified genomic DNA*, G. Spoto, and R. Corradini, Eds. Dordrecht, The Netherlands: Springer, 2012; pp. 153–183.

[10] S. Marín, and A. Merkoçi, "Electrochemical detection of DNA using nanomaterials based sensors", in *Detection of non-amplified genomic DNA*, G. Spoto, and R. Corradini, Eds. Dordrecht, The Netherlands: Springer, 2012; pp. 185–201.

[11] J. Wu, J. Lv, X. Zheng, and Z. S. Wu, "Hybridization chain reaction and its applications in biosensing", *Talanta*, vol. 234, Nov. 2021, Art. No. 122637.

[12] V. S. A. Piriya, P. Joseph, S. C. G. K. Daniel, S. Lakshmanan, T. Kinoshita, and S. Muthusamy, "Colorimetric sensors for rapid detection of various analytes", *Mater. Sci. Eng. C Mater. Biol. Appl.*, vol. 78, pp. 1231–1245, Sep. 2017.

[13] Q. Wu, Y. Zhang, Q. Yang, N. Yuan, and W. Zhang, "Review of electrochemical DNA biosensors for detecting food borne pathogens", *Sensors*, vol. 19, no. 22, Nov. 2019, Art. No. 4916.

[14] S. Leonardo, A. Toldrà, and M. Campàs, "Biosensors based on isothermal DNA amplification for bacterial detection in food safety and environmental monitoring", *Sensors*, vol. 21, no. 2, Jan. 2021, Art. No. 602.

[15] C. Zheng, K. Wang, W. Zheng, Y. Cheng, T. Li, B. Cao, Q. Jin, and D. Cui, "Rapid developments in lateral flow immunoassay for nucleic acid detection", *Analyst*, vol. 146, no. 5, pp. 1514–1528, Mar. 2021.

[16] J. García-Bernalt Diego, P. Fernández-Soto, and A. Muro, "The future of point-of-care nucleic acid amplification diagnostics after COVID-19: time to walk the walk", *Int. J. Mol. Sci.*, vol. 23, no. 22, Nov. 2022, Art. No. 14110.

[17] J. Mussin, G. Giusiano, J. C. Porras, L. H. Corredor Sanguña, M. I. Pividori, "Carbon nanoparticle-based lateral flow assay for the detection of specific double-tagged DNA amplicons of *Paracoccidioides* spp.", *Mikrochim. Acta*, vol. 191, no. 5, Apr. 2024, Art. No. 287.

[18] R. L. Karpel, "Single-stranded nucleic acid binding proteins", *Semin. Cell Dev. Biol.*, vol. 86, pp. 89–91, Feb. 2019.

[19] A. A. Anashkina, "Protein-DNA recognition mechanisms and specificity", *Biophys. Rev.*, vol. 15, no. 5, pp. 1007–1014, Sep. 2023.

[20] V. T. Kung, P. R. Panfili, E. L. Sheldon, R. S. King, P. A. Nagainis, B. Jr. Gomez, D. A. Ross, J. Briggs, and R. F. Zuk, "Picogram quantitation of total DNA using DNA-binding proteins in a silicon sensor-based system", *Anal. Biochem.*, vol 187, no. 2, pp. 220–227, Jun. 1990.

[21] K. Hedgethorne, and M. R. Webb, "Fluorescent SSB as a reagentless biosensor for single-stranded DNA", in *Single-Stranded DNA Binding Proteins. Methods in Molecular Biology*, J. Keck, Ed. Totowa, NJ, USA: Humana Press, 2012, pp. 219–233.

[22] M. Umer, N. B. Aziz, R. G. Mahmudunnabi, Y. B. Shim, C. Salomon, and M. J. A. Shiddiky, "A novel DNA binding protein-based platform for electrochemical detection of miRNA", *Analyst*, vol. 146, no. 18, pp. 5496–5501, Sep. 2021.

[23] R. G. Mahmudunnabi, M. Umer, K. D. Seo, D. S. Park, J. H. Chung, M. J. A. Shiddiky, and Y. B. Shim, "Exosomal microRNAs array sensor with a bioconjugate composed of p53 protein and hydrazine for the specific lung cancer detection", *Biosens. Bioelectron.*, vol. 207, Jul. 2022, Art. No. 114149.

[24] Z. Miao, and J. De Buck, "Biosensor for PCR amplicons by combining split trehalase and DNA-binding proteins: a proof of concept study", *J. Microbiol. Methods*, vol. 211, Aug. 2023, Art. No. 106780.

[25] Z. Miao Z, and J. De Buck, "Discriminating bovine mastitis pathogens by combining loop-mediated isothermal amplification and amplicon-binding split trehalase assay", *Front. Vet. Sci.*, 11, Jun. 2024, Art. No. 1389184.

[26] G. B. Aktas, V. Skouridou, and L. Masip, "Novel signal amplification approach for HRP-based colorimetric genosensors using DNA binding protein tags", *Biosens. Bioelectron.*, vol. 74, pp. 1005–1010, Dec. 2015.

[27] G. B. Aktas, V. Skouridou, and L. Masip, "Nucleic acid sensing with enzyme-DNA binding protein conjugates cascade and simple DNA nanostructures", *Anal. Bioanal. Chem.*, vol. 409, no. 14, pp. 3623–3632, May 2017.

[28] G. B. Aktas, J. H. Wichers, V. Skouridou, A. van Amerongen, and L. Masip, "Nucleic acid lateral flow assays using a conjugate of a DNA binding protein and carbon nanoparticles", *Mikrochim. Acta*, vol. 186, no. 7, Jun. 2019, Art. No. 426.

[29] G. B. Aktas, A. Ribera, V. Skouridou, and L. Masip, "DNA immobilization and detection using DNA binding proteins", *Anal. Bioanal. Chem.*, vol. 413, no. 7, pp. 1929–1939, Mar. 2021.

[30] I. Ginés, G. Gaiani, A. Ruhela, V. Skouridou, M. Campàs, and L. Masip, "Nucleic acid lateral flow dipstick assay for the duplex detection of *Gambierdiscus australes* and *Gambierdiscus excentricus*", *Harmful Algae*, vol. 110, Dec. 2021, Art. No. 102135.

[31] A. Ruhela, V. Skouridou, and L. Masip, "Capture, detection and purification of dsDNA amplicons using a DNA binding protein on magnetic beads", *Anal. Biochem.*, vol. 658, Dec. 2022, Art. No. 114923.

[32] B. Laine, D. Kmiecik, P. Sautiere, G. Biserte, and M. Cohen-Solal, "Complete amino-acid sequences of DNA-binding proteins HU-1 and HU-2 from *Escherichia coli*", *Eur. J. Biochem.*, vol. 103, no. 3, pp. 447–461, Feb. 1980.

[33] V. L. Barlow, and Y. H. Tsai, "Acetylation at lysine 86 of *Escherichia coli* HUβ modulates the DNA-binding capability of the protein", *Front. Microbiol.*, vol. 12, Feb. 2021, Art. No. 809030.

[34] E. Bonnefoy, and J. Rouvière-Yaniv, "HU and IHF, two homologous histone-like proteins of *Escherichia coli*, form different protein-DNA complexes with short DNA fragments", *EMBO J.*, vol. 10, no. 3, pp. 687–696, Mar. 1991.

[35] D. Kamashev, and J. Rouvière-Yaniv, "The histone-like protein HU binds specifically to DNA recombination and repair intermediates", *EMBO J.*, vol. 19, no. 23, pp. 6527–6535, Dec. 2000.

[36] R. Jana, T. R. Hazbun, J. D. Fields, and M. C. Mossing, "Single-chain lambda Cro repressors confirm high intrinsic dimer-DNA affinity", *Biochemistry*, vol. 37, no. 18, pp. 6446–6455, May 1998.

[37] M. Hammel, D. Amlanjyoti, F. E. Reyes, J. H. Chen, R. Parpana, H. Y. Tang, C. A. Larabell, J. A. Tainer, and S. Adhya, "HU multimerization shift controls nucleoid compaction", *Sci. Adv.*, vol. 2, no. 7, Jul. 2016, Art. No. e1600650.

[38] D. Kamashev, A. Balandina, A. K. Mazur, P. B. Arimondo, and J. Rouviere-Yaniv, "HU binds and folds single-stranded DNA", *Nucleic Acids Res.*, vol. 36, no. 3, pp. 1026–1036, Febr. 2008.

[39] B. Xiao, R. C. Johnson, and J. F. Marko, "Modulation of HU–DNA interactions by salt concentration and applied force", *Nucleic Acids Res.*, vol. 38, no. 18, pp. 6176–6185, May 2010.

[40] C. Tang, Z. He, H. Liu, Y. Xu, H. Huang, G. Yang, Z. Xiao, S. Li, H. Liu, Y. Deng, Z. Chen, H. Chen, and N. He, "Application of magnetic nanoparticles in nucleic acid detection", *J. Nanobiotechnology*, vol. 18, no. 1, pp. 1–19, Apr. 2020.

This work is licensed under a Creative Commons Attribution 4.0 License. For more information, see https://creativecommons.org/licenses/by/4.0/