Front. Med. 2025, 19(6): 1323−1327
https://doi.org/10.1007/s11684-025-1188-5

COMMENT

“Zero-distance” photocrosslinking: a paradigm shift in probing DNA-protein dynamics

Fengting Guo[1,2], Sisi Fan[3], Xueqian Ouyang[3], Lanfang Li (✉)[3], Lifang Li (✉)[2,1]

[1]Guangxi University of Chinese Medicine, Nanning 530022, China; [2]Department of Traditional Chinese Medicine, Shenzhen Second People’s Hospital, Shenzhen 518000, China; [3]Institute of Pharmacy and Pharmacology, University of South China, Hengyang 421001, China

Deoxyribonucleic acid (DNA) is a double-stranded macromolecule composed of two antiparallel polynucleotide chains. Each chain is formed by deoxyribose and phosphate as the backbone and forms complementary base pairs through hydrogen bonds. This structure ensures the stability of DNA and enables the precise replication of genetic information [1]. In biological processes, DNA collaborates with various biomolecules, including proteins, RNAs, small molecules, lipids, and carbohydrates [2]. DNA interacts with proteins, including histones and DNA polymerases, to regulate gene expression, transmit genetic information, and maintain chromatin structure [3]. DNA collaborates with RNAs, such as long non-coding RNAs and mRNAs, to facilitate transcription and translation [4]. Additionally, DNA interacts with small molecules (e.g., nucleotide analogs, intercalators, alkylating agents) that can modulate replication, repair, or epigenetic regulation [5]. Furthermore, DNA interactions with lipids help package viral genomes, while associations with carbohydrates can influence DNA–protein interactions or DNA stability [6]. In summary, DNA-biomolecule interactions are central to life processes. In human cells, DNA–protein interactions are particularly critical, directly regulating gene expression, cell differentiation, and development, and participating in aging and disease [7].

Various techniques are used to investigate direct DNA–protein interactions, such as electrophoretic mobility shift assay (EMSA), fluorescence imaging, chemical cross-linking mass spectrometry (XL-MS), chromatin immunoprecipitation followed by sequencing (ChIP-seq), and artificial intelligence (AI) modeling. EMSA detects specific DNA–protein interaction interactions by observing changes in the electrophoretic mobility of DNA–protein complexes. It is straightforward and useful for initial validation but lacks physiologic relevance and has limited sensitivity [8]. Fluorescence imaging enables real-time tracking in living cells via fluorescently labeled molecules. This approach supports live-cell imaging and dynamic monitoring, but labeling may perturb protein function, and photobleaching restricts the observation time [9]. XL-MS employs cross-linking reagents to covalently stabilize DNA–protein complexes, which are then characterized by mass spectrometry to identify interaction interfaces. While it can resolve the specific regions of DNA–protein binding, the method is technically demanding and often misses transient interactions [10]. ChIP-seq, a widely used genomic technique, maps genome-wide binding sites of transcription factors and chromatin-associated proteins through immunoprecipitation and high-throughput sequencing. Although it offers high-throughput *in vivo* binding data, it depends on high-quality antibodies and involves cross-linking that may compromise resolution [11]. AI modeling methods leverage known structures or binding preferences to predict DNA binding proteins and interaction patterns without experimental input, offering speed and resource efficiency. However, their accuracy relies heavily on training data quality, and they struggle to capture the complexity of biological systems [12,13]. In summary, current methods for studying direct DNA–protein interactions are often constrained by limited physiologic context, poor capture of transient events, low spatial resolution, antibody-related biases, and significant genetic perturbation. There is thus a critical need for novel technologies that can operate under native live-cell conditions, capture transient complexes with high spatiotemporal precision, and enable unbiased, genome-wide profiling of DNA–protein interactions.

Recently, Trendel *et al*. developed a “zero-distance”

Received July 19, 2025; accepted September 19, 2025
Correspondence: Lanfang Li, 2005001782@usc.edu.cn;
Lifang Li, zykllf@126.com

photocrosslinking strategy that significantly overcomes the limitations of conventional methods for studying DNA–protein interactions [14]. This approach combines 4-thiothymidine (4ST) metabolic labeling, enhanced UV activation in living cells (UVEN), crosslinked DNA extraction (XDNAX, a protocol to isolate crosslinked DNA–protein complexes), and liquid chromatography-mass spectrometry (LC-MS). Briefly, cells were cultured with 100 μmol/L 4ST for 3–5 days, enabling its incorporation into newly synthesized DNA. As a thiolated analog of thymine, 4ST replaces the oxygen atom at the C4 position with sulfur, conferring photosensitivity that allows covalent cross-linking to proximal proteins upon UV irradiation [15]. After labeling, cells were subjected to UVEN irradiation (365 nm) for 60 s at approximately 2000 mW/cm^2, delivering a total energy of 125 J/cm^2. This system provides approximately 100-fold faster photoactivation compared to conventional UV bulbs and ensures uniform irradiation over an area of approximately 175 cm^2. Subsequently, crosslinked complexes were purified using the XDNAX method. This process begins with TRIZOL extraction to remove the majority of non-crosslinked proteins, RNA, lipids, and other cellular debris. The resulting chromatin-containing interphase was then treated with RNase to digest residual RNA, followed by ultrasonication to shear DNA and boiling denaturation to fully denature proteins. Subsequent rigorous silica-based washing step was performed to thoroughly remove any remaining non-crosslinked contaminants, yielding purified protein–DNA crosslinks. Finally, photo-crosslinked proteins were released from DNA by nuclease treatment and digested into peptides with trypsin for subsequent LC-MS analysis (Fig. 1).

This combination of techniques enables precise, genome-wide mapping of DNA–protein interactions under physiologic conditions. Using the "zero-distance" photocrosslinking strategy, Trendel *et al*. constructed a comprehensive DNA–protein interaction map in human breast cancer MCF7 cells, precisely locating numerous interaction sites. The pathogenesis and progression of breast cancer are closely linked to genomic dysregulation, processes reliant on direct DNA- protein interactions. They identified 1805 DNA-interacting proteins. These proteins encompassed diverse functional categories, including transcription factors, DNA repair proteins, and chromatin remodelers. Notably, they precisely mapped 688 nucleotide-peptide cross-linking sites involving 379 proteins at single-amino-acid resolution —a 20-fold improvement over previous reports. Furthermore, applying multidimensional dynamic analyses—including drug stimulation, dose gradients, and time series—combined with "zero-distance" photocrosslinking strategy revealed the dynamics of transcription factors, DNA repair factors, and chromatin regulators in gene expression.

A key application of this technique is the sensitive detection of rapid changes in transcription factor binding DNA under cellular perturbations. Trendel *et al*. treated MCF7 cells with 12 estrogen concentrations (ranging from 30 fmol/L to 10 nmol/L) for 45 min, comparing DNA interactions between treated and control groups. Estrogen stimulation caused a marked 30-fold increase in

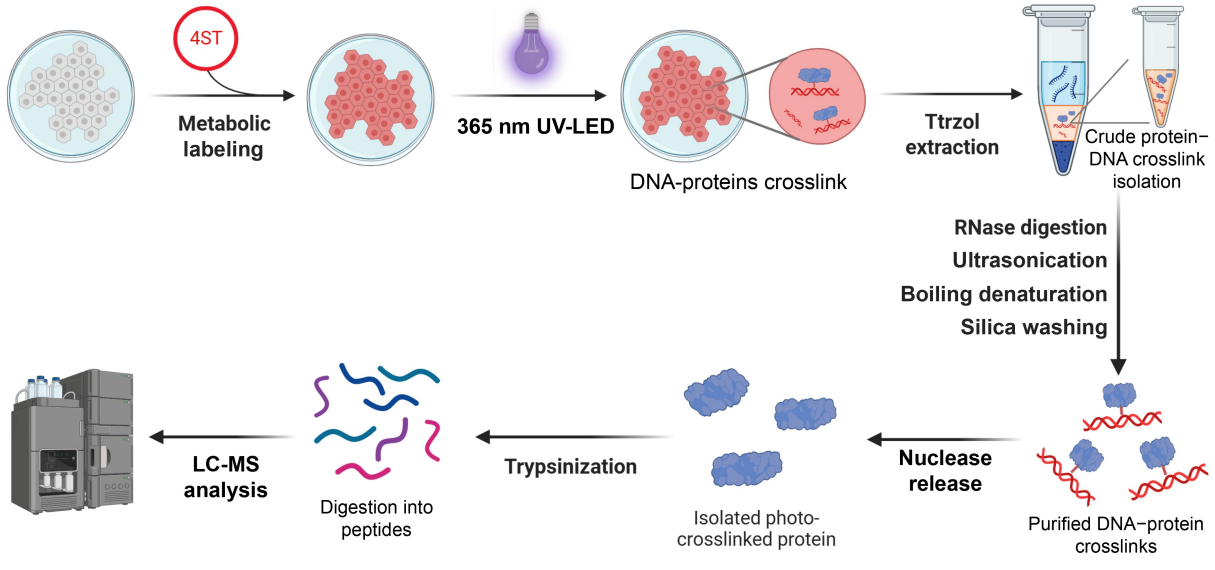

Fig. 1 Schematic diagram of the "zero-distance" photocrosslinking strategy. The procedure begins with metabolic incorporation of 4-thiothymidine (4ST) into DNA, followed by 365 nm UV-LED irradiation to crosslink proximal proteins. Crude complexes are isolated via TRIZOL extraction and purified through RNase digestion, ultrasonication, denaturation, and silica washing. Crosslinked proteins are released by nuclease treatment, digested into peptides by trypsinization, and analyzed by LC-MS for proteomic identification.

DNA-bound estrogen receptor α (ESR1) abundance, making it the most significantly altered protein ($P = 2.6 \times 10^{-42}$). Concurrently, DNA binding of known ESR1 co-factors POLR2A and SMARCA4 increased, while binding of inhibitory factors SET, CBX3, and CBX5 decreased significantly. Using the estrogen concentration gradient, they determined a half-effect concentration (EC_{50}) of 35 pmol/L with a high coefficient of determination ($R^2 = 0.94$). The proximity of this EC_{50} to the reported dissociation constant (Kd = 50 pmol/L) of ESR1 for its ligand in cell-free assays demonstrates the method's high precision for quantitative interaction analysis —its measurements align with well-established biophysical data. The technique was also successfully applied to primary mouse macrophages. After 3 h of lipopolysaccharide (LPS) stimulation (100 ng/mL LPS), DNA binding of key inflammatory transcription factors Cebpb, Cebpd, and Rela was significantly increased. This underscores the technique's broad applicability, as it can reliably detect transcription factor dynamics under physiologic perturbations beyond standard cell culture models.

The "zero-distance" photocrosslinking strategy also revealed how genotoxic drugs affect the direct interactions between DNA repair proteins and the genome. Trendel *et al*. treated MCF7 cells for 24 h with the genotoxic drugs etopside, cisplatin, and oxaliplatin (each at a final concentration of 100 μmol/L), comparing DNA interactions between drug-treated and the control group. All three drugs increased DNA binding of cell cycle regulator CDKN1A/p21 and core DNA repair proteins TP53BP1, SIRT7, and HINT1, while causing significant loss of the telomerase complex component NHP2 from DNA. Moreover, each drug induced distinct DNA repair pathway signatures. Etoposide primarily activated non-homologous end joining, base excision repair, and nucleotide excision repair pathways, accompanied by accumulation of topoisomerase II α/β proteins. Within 4 h of etoposide treatment, TOP2A showed a 6-fold accumulation on DNA and significant phosphorylation, alongside recruitment of the SUMO-ligase PIAS4 —this ability to capture early-stage protein modifications and co-factor recruitment reflects the method's high temporal resolution. Oxaliplatin induced significant loss of nucleolar proteins. Oxaliplatin induced significant loss of nucleolar proteins involved in ribosome biogenesis. Cisplatin significantly recruited proteins of the ATM-CHEK2 kinase pathway (ATM, CHEK2, PPP5C) and flap endonuclease 1 (FEN1). Knockdown of the enriched repair enzymes PPP5C and FEN1 under cisplatin treatment significantly increased apoptosis. These finding demonstrate the "zero-distance" photocrosslinking strategy's ability to precisely capture drug-specific effects on DNA repair protein-genome interactions, revealing differential cellular repair mechanisms and suggesting potential strategies for enhancing chemotherapy efficacy by targeting specific repair proteins.

Furthermore, the "zero-distance" photocrosslinking strategy captured dynamic changes in chromatin regulatory proteins upon inhibition of the chromatin remodeling complex BAF. BAF (BRG1/BRM-associated factor) is a key chromatin remodeling complex that regulates nucleosome positioning and chromatin structure. They treated MCF7 cells with the BAF inhibitor BRM014 and analyzed DNA–protein interactions at time points. They treated MCF7 cells with the BAF inhibitor BRM014 (1 μmol/L final concentration) and analyzed DNA–protein interactions at time points (0, 4, 8, 12, 16, 20 min) via label-free quantification (LFQ) and NBM testing. Interactions of chromatin organization proteins showed significant time-dependent changes. Within just 4 min of BAF inhibition, binding significantly increased for HMGB1 (which promotes DNA opening) and the histone chaperones ANP32A and ANP32E. This rapid response (detectable within 4 min) highlights the method's exceptional time resolution —unlike conventional formaldehyde crosslinking, which requires 10 min of fixation at 37 °C and cannot capture early dynamics, the technique's photocrosslinking occurs at 4 °C and takes only 60 s of irradiation, enabling capture of transient regulatory events. Overall, this time-dependent dynamic change clearly presents the compensatory-decompensatory process that cells undergo in response to chromatin remodeling complex dysfunction.

Notably, the "zero-distance" photocrosslinking strategy directly addresses major constraints of existing methods by operating under physiologic conditions *in vivo*, capturing transient complexes with high temporal resolution, achieving single-amino-acid binding precision, avoiding antibody dependence, and minimizing perturbation through sparse metabolic labeling. Together, these capabilities provide an unprecedented platform for genome-wide, high-resolution profiling of dynamic DNA–protein interactions within native cellular environments. Despite these transformative capabilities, the technique faces a significant limitation in the cytotoxicity associated with the 4ST label. While 4ST itself shows minimal toxicity after incorporation into DNA, its combination with UVA irradiation induces substantial DNA damage. The photoactivation of 4ST leads to the generation of reactive oxygen species (ROS) and causes direct DNA distortion, including the formation of DNA single-strand breaks and cross-links. These lesions severely disrupt DNA replication and repair, ultimately leading to significant cytotoxicity. Critically, this cytotoxicity introduces a risk of experimental artifacts. Cellular stress responses triggered by extensive DNA damage may provoke non-specific or stress-induced

DNA–protein interactions. These aberrant interactions could be mistakenly captured during photocrosslinking, leading to false positives in the resulting interactome and compromising the specificity of the technique. To mitigate these concerns, several strategies can be considered. First, optimizing labeling conditions, such as using lower concentrations of 4ST (e.g., ⩽ 50 μmol/L) and reducing UVA exposure, may help minimize DNA damage while retaining sufficient crosslinking efficiency. Second, the development of alternative, less toxic photosensitizers with red-shifted activation spectra could reduce genotoxicity and improve biocompatibility. Lastly, incorporating appropriate controls would help identify and subtract non-specific interactions, thereby enhancing the reliability of the identified DNA–protein binding profiles [16] (Fig. 2).

The "zero-distance" photocrosslinking strategy could be extended to the study of mitochondrial DNA (mtDNA)-protein interactions, revealing new mechanisms of mitochondrial genome regulation. Unlike the study by Trendel *et al*., which profiled genome-wide DNA protein interactions across the entire cellular chromatin, mtDNA presents a distinct biological context. mtDNA is a compact, circular genome located within mitochondria and encodes essential subunits of oxidative phosphorylation complexes. The transcription and replication of mtDNA are regulated by a specific set of mtDNA binding proteins, and their dysregulation can lead to alterations in mtDNA copy number or functional decline. Such defects are closely implicated in cancer, cardiovascular diseases, and neurodegenerative disorders [17]. However, methods with high spatiotemporal resolution for studying dynamic mtDNA–protein binding and functional coordination are still lacking. The "zero distance" photocrosslinking approach, with its capability for *in vivo* operation under physiologic conditions and minimal genetic perturbation, could be strategically adapted to target the mitochondrial compartment. This adaptation could enable precise mapping of the mtDNA–protein interactome, providing unprecedented insight into the real-time regulatory dynamics of the mitochondrial genome.

Furthermore, deep integration of the "zero-distance" photocrosslinking with AI could open new paradigms for DNA–protein interaction research. The large-scale interaction data generated could train graph neural network-based deep learning models to develop high-precision DNA–protein binding site prediction algorithms. This integration offers a dual advantage: it could overcome the spatiotemporal limitations of experimental techniques, enable genome-wide interaction prediction, and guide the detection of transient or weak interactions that are elusive to traditional methods. Integrating structural biology and epigenetic data, AI models can also predict the functional impact of mutations on DNA binding, providing new insights for disease mechanism research and precision therapy.

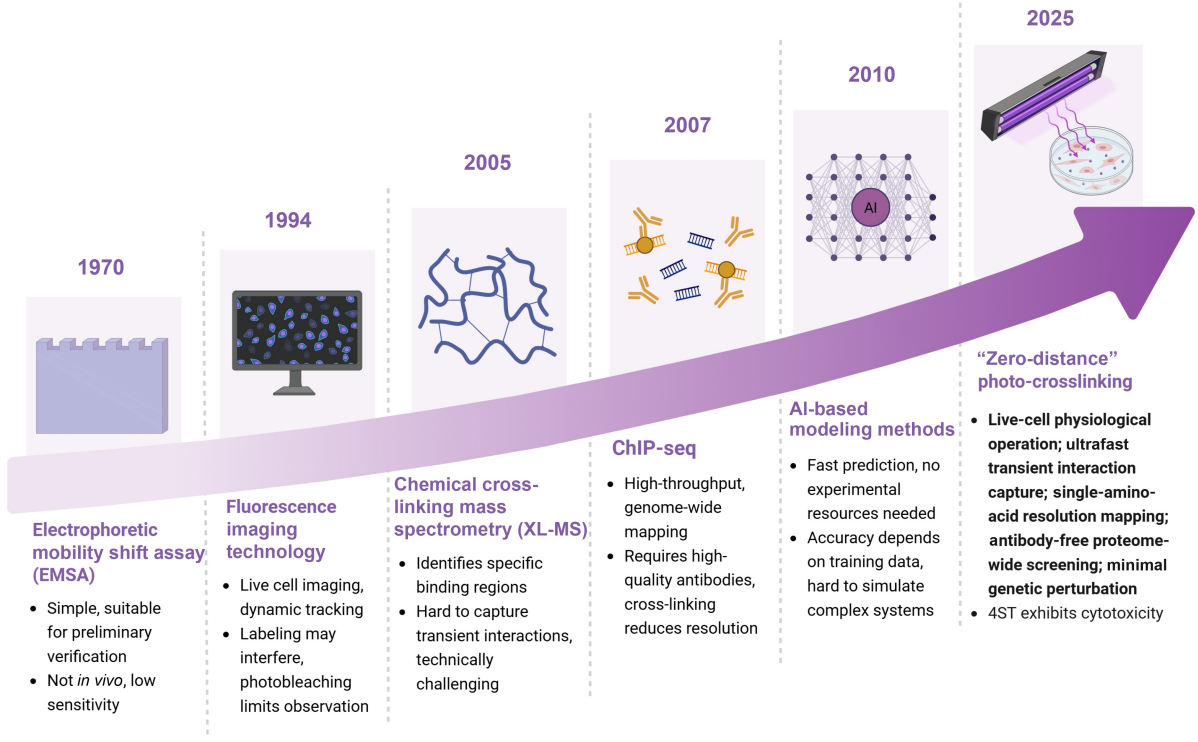

Fig. 2 Comparison of techniques for studying direct DNA–protein interactions.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 81970431), the Hunan Provincial Natural Science Foundation of China (Nos. 2024JJ9370 and 2025JJ50502), the Postgraduate Scientific Research Innovation Project of Hunan Province (Nos. CX20230992 and CX20240840), the Municipal Financial Subsidy for Shenzhen TCM Key Discipline Construction, and the Flagship Department Construction Project for Integrated Traditional Chinese and Western Medicine.

Compliance with ethics guidelines

Conflicts of interest Fengting Guo, Sisi Fan, Xueqian Ouyang, Lanfang Li, and Lifang Li have declared that no conflict of interest exists.

This manuscript is a comment article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

References

1. Zhang X, Spiegel J, Martínez Cuesta S, Adhikari S, Balasubramanian S. Chemical profiling of DNA G-quadruplex-interacting proteins in live cells. Nat Chem 2021; 13(7): 626–633
2. Chen X, Xu H, Shu X, Song CX. Mapping epigenetic modifications by sequencing technologies. Cell Death Differ 2025; 32(1): 56–65
3. Liu B, Dong X, Zheng C, Keener D, Chen Z, Cheng H, Watts JK, Xue W, Sontheimer EJ. Targeted genome editing with a DNA-dependent DNA polymerase and exogenous DNA-containing templates. Nat Biotechnol 2024; 42(7): 1039–1045
4. Statello L, Guo CJ, Chen LL, Huarte M. Gene regulation by long non-coding RNAs and its biological functions. Nat Rev Mol Cell Biol 2021; 22(2): 96–118
5. Barderas R, Srivastava S, LaBaer J. Protein microarray-based proteomics for disease analysis. Methods Mol Biol 2021; 2344: 3–6
6. Colombani T, Peuziat P, Dallet L, Haudebourg T, Mével M, Berchel M, Lambert O, Habrant D, Pitard B. Self-assembling complexes between binary mixtures of lipids with different linkers and nucleic acids promote universal mRNA, DNA and siRNA delivery. J Control Release 2017; 249: 131–142
7. Yan Y, Huang T. The Interactome of Protein, DNA, and RNA. Methods Mol Biol 2023; 2695: 89–110
8. Hellman LM, Fried MG. Electrophoretic mobility shift assay (EMSA) for detecting protein-nucleic acid interactions. Nat Protoc 2007; 2(8): 1849–1861
9. Corrêa IR Jr. Live-cell reporters for fluorescence imaging. Curr Opin Chem Biol 2014; 20: 36–45
10. Zhang Z, Zhao Q, Gong Z, Du R, Liu M, Zhang Y, Zhang L, Li C. Progress, challenges and opportunities of NMR and XL-MS for cellular structural biology. JACS Au 2024; 4(2): 369–383
11. Roh TY. ChIP-seq strategy to identify Z-DNA-forming sequences in the human genome. Methods Mol Biol 2023; 2651: 167–177
12. Gupta T, Sharma P, Malik S, Pant P. AIoptamer: artificial intelligence-driven aptamer optimization pipeline for targeted therapeutics in healthcare. Mol Pharm 2025; 22(7): 4076–4090
13. Cui F, Zhang Z, Cao C, Zou Q, Chen D, Su X. Protein-DNA/RNA interactions: machine intelligence tools and approaches in the era of artificial intelligence and big data. Proteomics 2022; 22(8): 2100197
14. Trendel J, Trendel S, Sha S, Greulich F, Goll S, Wudy SI, Kleigrewe K, Kubicek S, Uhlenhaut NH, Kuster B. The human proteome with direct physical access to DNA. Cell 2025; 188(16): 4424–4440.e17
15. Chopra A. Methyl-^{11}C-4′-thiothymidine. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US), 2004–2013
16. Reelfs O, Macpherson P, Ren X, Xu YZ, Karran P, Young AR. Identification of potentially cytotoxic lesions induced by UVA photoactivation of DNA 4-thiothymidine in human cells. Nucleic Acids Res 2011; 39(22): 9620–9632
17. Al Khatib I, Shutt TE. Advances towards therapeutic approaches for mtDNA disease. Adv Exp Med Biol 2019; 1158: 217–246