



Quantitative Structural Characterization of CRISPR-Cas Protein–Nucleic Acid Interactions Through Image-Based Analysis

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ABSTRACT

Electrophoretic mobility shift assays (EMSAs) provide a valuable approach for investigating protein–nucleic acid interactions and characterizing molecular complexes associated with CRISPR–Cas systems. The electrophoretic band distributions under control, mutant, mutant with Cas11, wild-type (WT) and crRNA conditions were quantified and evaluated in this study. Five sets of EMSA gel images, each containing 50 electrophoretic lanes with 100 band intensities were examined. The analytical method used was lane identification, background correction, pre-integration of the intensity of the bands, and normalization within the lane to obtain relative signal distributions. In the control condition, two band regions (22.34%, 77.66%) of normalized intensity were observed while in the mutant (Cas11 condition), two band regions (43.93%, 56.07%) of normalized intensity were observed. In the mutant condition, there was only one band region exhibited and in the WT there were three band regions exhibited with 7.87%, 32.26% and 59.87% respectively. The condition of the crRNA exhibited two band areas, 31.53% and 68.47%, with the difference in electrophoretic signal distribution. Comparative evaluation showed the distinct pattern of band dominance, electrophoretic heterogeneity and variability in the experimentation conditions in the lanes. The findings in this study illustrate the utility of systematic characterization of CRISPR-associated electrophoretic profiles using quantitative EMSA densitometry as a foundation for further studies of molecular recognition, protein–nucleic acid interactions, and protein organization of the CRISPR-associated complexes.

Keywords: CRISPR–Cas systems; Electrophoretic mobility shift assay; Protein–nucleic acid interactions; Band densitometry; Molecular recognition.

1. Introduction

CRISPR and CRISPR-associated (Cas) proteins form a type of adaptive immune system which allows bacteria and archaea to identify and react to the invading genetic elements. Their nucleic acid recognition capabilities, which can be programmed, have revolutionized molecular biology in the fields of genome editing, molecular diagnostics and the study of protein–nucleic acid interactions. A coordinated interaction of guide RNAs, effector proteins, and complementary nucleic acid substrates is essential for CRISPR-associated molecular functions. These interactions include conformational changes that affect target recognition, molecular stability, and subsequent biochemical activity. Structure studies have revealed a lot of diversity in the architecture of the CRISPR–Cas complexes, and this diversity could explain the functional specificity that protein organization and nucleic acid recognition provides (Wang et al., 2022A). The knowledge of these mechanisms serves as a critical starting point to study electrophoretic variations in wild-type proteins, mutate variants and CRISPR-associated components.

The molecular structure of CRISPR systems differs significantly among evolutionary groups and by their functions. Structural studies of the CRISPR associated transposon complexes have shown that DNA recognition occurs in tandem, with both specialized protein domains and guide-directed interactions coordinating the DNA recognition process (Halpin-Healy et al., 2020). In a similar vein, the studies of type I-C Cascade complexes have also led to the identification of the contribution of noncanonical small subunits in molecular assembly and structural organization (O'Brien et al., 2020). The results suggest that the individual protein components may affect the assembly and structure of the CRISPR-associated complexes. Further, the diversity of these molecular platforms has been expanded by RNA-targeting CRISPR systems. The specificity of RNA recognition by the single protein Cas7-11 effector highlights RNA recognition mechanisms used by CRISPR-associated proteins (Özcan et al., 2021). The molecular diversity makes it important to study experimentally interactions between guide RNAs and various protein structures.

CRISPR associated molecular recognition has specificity that extends beyond that of nucleic acid sequence complementarity. The recognition process involves structural rearrangements, conformational changes of the substrate and protein-based stabilization. The interrogation of sequences by CRISPR–Cas9 has been shown to involve twisting and bending of DNA, highlighting the role of sequence recognition in the geometry of nucleic acids (Cofsky et al., 2022). Regulatory proteins can also affect the CRISPR-associated activity by modifying molecular recognition or disrupting the functions of effectors. Several regulatory mechanisms have been discovered in anti-CRISPR proteins that can alter the activity of CRISPR systems during structural analysis of anti-CRISPR proteins (Jia & Patel, 2021). From these observations, it can be concluded that detectable differences in electrophoretic migration patterns can be observed after the alteration of protein composition and/or molecular organization. But these differences should be interpreted carefully, for they cannot be used to determine the structural mechanism.

The diversity of the other functions of CRISPR-associated complexes is also evident in their ability to organize nucleic acids and to program genetic integration. Higher-order genome organisation has been linked to the molecular processes of integration into the CRISPR arrays (Santiago-Frangos et al., 2023). The power of using structurally informed molecular design for targeted genetic insertion has also been exemplified by advances in CRISPR-associated transposon engineering (Lampe et al., 2025). Programmable transposon systems for the integration of therapeutic genetic material in the human genome have been explored in related investigations (Liu et al., 2025). Further structural characterization has shown that there are specialized molecular architectures linked to type VII CRISPR–Cas activity (Yang et al., 2024). As another striking example of the diversity of mechanisms used by CRISPR systems, differences in horizontal transfer mechanisms between the type I and type V CRISPR-associated transposons are evident (Hu et al., 2024). All these advances highlight the need for understanding molecular interactions in various experimental conditions.

For exploring the interactions between proteins and nucleic acids by exploiting their differential mobility in electrophoretic movement, the electrophoretic mobility shift assay (EMSA) method is a practical option. Association may affect migration characteristics, resulting in distinct band regions which can be studied qualitatively and quantitatively. Controlled experimental conditions have been established using the established EMSA methodologies for detection and characterization of protein–nucleic acid interactions (Mansouri-Noori & Bayfield, 2024). Electrophoretic measurements have also been shown to be useful for studying the interactions between nucleotide and protein in the context of nonradioactive competitive EMSA (Wang et al., 2022B). Quantitative densitometry goes beyond visual interpretation by quantifying the intensity of the bands, normalizing for background variation, and calculating relative signal contributions. However, the proper interpretation of the binding requires verified molecular identity, good controls, and concentration-dependent measurements.

Even with this methodological progress, comparative characterization of the electrophoretic profile related to CRISPR is still difficult in the presence of several band regions and different experimental conditions. The present study was thus based on quantitative image analysis of the various EMSA band distributions under the four conditions: Controls, Mutant with Cas11, Mutant and wild-type. The present study was thus based on the quantifiable image analysis of the various EMSA band distributions under different conditions: under Controls condition, under Mutant with Cas11 condition, under Mutant condition and under wild-type condition, and under crRNA condition. A reproducible identification of bands, normalisation of band intensities and descriptive comparison were stressed, with no assumption being made that electrophoretic redistribution was a direct indication of binding affinity differences. The objectives of this study are:

- To characterize electrophoretic band distributions across control, mutant, mutant with Cas11, wild-type, and crRNA conditions.
- To quantify relative band intensities and evaluate lane-wise variations using background-corrected densitometric measurements.
- To compare electrophoretic signal patterns and assess their potential relevance to CRISPR-associated molecular interactions.

2. Materials and Methods

2.1. Study Design and Electrophoretic Mobility Shift Assay Analysis

The study used a quantitative image-based analytical design to examine electrophoretic band distributions of the molecular components related to CRISPR. Images of the electrophoretic mobility shift assay (EMSA) experiments were analyzed for five experimental conditions: Controls, Mutant with Cas11, Mutant, wild-type (WT), and CRISPR RNA (crRNA) to characterize electrophoretic migration differences and relative band intensities (McBride et al., 2020). The five TIFF images of gels were analyzed with image-processing, statistical tools and computational densitometry in Python. The analytical framework included lane identification, electrophoretic band localization, background correction, signal integration and band intensities normalization. Each of the five experimental conditions was tested in 50 electrophoretic lanes. The investigation was not aimed at the direct measurement of molecular binding affinities, but rather on the quantification of observable electrophoretic signal distributions. The same analytical procedures were used throughout to ensure methodological uniformity to all images.

2.2. Image Preprocessing and Lane Identification

Individual lanes were computed to identify in the electrophoretic images and characterize the spatial variations of the signal intensity. Pixel-intensity distributions were measured horizontally and vertically within the electrophoretic lanes and background regions, to distinguish electrophoretic lanes from background regions. The initial lane positions were determined based on characteristics of the intensity-profile, while the refinement of the lane positions was done by analysing the patterns of the spatial signal based on periodicity. The positions of the candidate lanes were then confirmed, and the horizontal coordinates of the center of the lanes were stored to evaluate quantitatively. Nine lanes were used in Controls, ten lanes in Mutant with Cas11, ten lanes in Mutant, ten lanes in WT, and eleven lanes in crRNA. Vertical intensity profiles were plotted for each lane, which are used to look at the distributions of electrophoretic signals along the migration direction. This process allowed for uniform positioning of the candidate band areas and minimized positioning variance due to lane positioning differences. The lane coordinates were later checked and verified during subsequent densitometric calculations to ensure the correct correspondence of the electrophoretic features detected with the image source coordinates.

2.3. Electrophoretic Band Detection and Background Correction

The lane-specific vertical intensity profiles were analysed for the identification of the regions of electrophoretic bands. At the local level, the intensity of electrophoretic features was explored in an attempt to distinguish features of interest from background fluctuations. The vertical signal distributions of the candidates were used to further define the candidate band positions, and boundaries around the identified regions were created based on the integration. Background correction was carried out separately for every lane by estimating row-wise from the 85th percentile of the intensity of each lane over its width. The background-corrected signal was determined as the positive difference between the estimated background and the observed pixel intensity (with negative differences given a value of zero). High frequency fluctuations were minimized by smoothing with a Gaussian filter (2-pixel standard deviation) in corrected intensity profiles. The intensities of each band were then integrated within the specified range of each band. Analysis revealed two selected band regions in Controls, two in Mutant with Cas11, one in Mutant, three in WT and two in crRNA. These areas were not automatically assigned to free nucleic acids or bound molecular complexes, but instead were areas with detectable electrophoretic signal features.

2.4. Integrated Band Intensity Quantification and Normalization

Quantitative densitometry was performed by integrating background-corrected electrophoretic signals within each selected band region. Integrated intensity represented the cumulative corrected pixel signal associated with a defined electrophoretic feature. The 100 band measurements were conducted on 50 accurate lanes, and were collected across the five experimental images. Each lane was treated independently when normalizing the intensities of the integrated signal in the selected band, to compensate for the difference in total selected-band signal between lanes. The normalized contribution of a specific band was done by taking the integrated intensity of a band and dividing it by the sum of the intensities of all the selected bands in the same lane in a corresponding scan, and then multiplying by 100:

$$P_{ij} = \frac{I_{ij}}{\sum_{k=1}^{m_i} I_{ik}} \times 100$$

where P_{ij} represented the normalized intensity percentage of band j in lane i , I_{ij} denoted the integrated intensity of the corresponding band, and m_i represented the total number of selected band regions within that lane. This normalization provided a standardized measure of relative electrophoretic signal distribution. For lanes containing only one selected band, the normalized percentage was necessarily 100%, irrespective of the underlying molecular composition. Therefore, normalized values were interpreted as relative contributions of detected band regions rather than direct estimates of binding efficiency.

2.5. Comparative Densitometric Analysis and Statistical Evaluation

The variations in electrophoretic band distributions were compared by using comparative densitometric analysis which was done both within and between the five experimental conditions. Python libraries such as NumPy, pandas, SciPy, and Matplotlib were used for data processing and numerical calculations. Descriptive statistical parameters such as mean integrated intensity, mean normalized band percentage and standard deviation were calculated for each selected band region. Lane-wise intensity distributions were analysed to observe the variations of electrophoretic features in terms of relative contribution. The distribution of the selected signals within individual lanes were characterized using stacked band-percentage profiles, and comparative graphical representations summarized band-intensity patterns across experimental images. Provisional classification of potential mobility-shifted species was based on electrophoretic shift positions, but definitive classification was only possible if band identities were confirmed independently. Standard deviations described variation among electrophoretic lanes and were not interpreted as estimates of biological replicate variability. Because independent replicate assignments, experimentally verified free and shifted bands, and lane-specific protein concentrations were unavailable, inferential statistical comparisons and equilibrium dissociation constant (K_d) estimation were not undertaken. The analytical interpretation was consequently limited to descriptive differences in electrophoretic signal intensity and band-distribution characteristics.

3. Results

3.1. Quantitative Characterization of EMSA Band Profiles

Five electrophoretic mobility shift assay (EMSA) images were acquired under different conditions (Controls, Mutant with Cas11/ Mutant/ wild-type (WT)/ CRISPR RNA (crRNA)). In all, there were 50 electrophoretic lanes, and 100 integrated band-intensity measurements. The number of lanes analyzed was nine in Controls and eleven in crRNA, with ten lanes in the other conditions. Two regions were selected in Controls, two in Mutant with Cas11, one in Mutant, three in WT and two regions were selected in crRNA. The relative abundance of the electrophoretic signal detectable in the images varied between them, resulting in a normalized intensity distribution that was different for each image. Band 2 of Controls and crRNA showed the 2nd and 3rd highest mean normalized contribution, respectively, followed by the single selected band in Mutant (100%). These measurements were used to give a quantitative description of the electrophoretic distributions of signals but did not make any assumptions about molecular identities of the signal bands.

Table 1. Quantitative distribution of normalized EMSA band intensities across experimental conditions

Experimental condition	Band	Lanes (n)	Mean intensity (%)	SD (%)
Controls	1	9	22.34	15.71
Controls	2	9	77.66	15.71
Mutant with Cas11	1	10	43.93	39.69
Mutant with Cas11	2	10	56.07	39.69

Mutant	1	10	100.00	0.00
WT	1	10	7.87	2.09
WT	2	10	32.26	28.93
WT	3	10	59.87	30.67
crRNA	1	11	31.53	20.81
crRNA	2	11	68.47	20.81

3.2. Electrophoretic Signal Distribution in Control and Mutant Conditions

The control EMSA image had two separate electrophoretic band regions, where the relative contribution to the normalized signal was not equal. Band 2 accounted for $77.66 \pm 15.71\%$ of the selected intensity, whereas Band 1 contributed $22.34 \pm 15.71\%$. The mean integrated intensities for the two were 271,370.71 and 61,080.34 arbitrary units (a.u.) respectively. For the Mutant with Cas11 condition, the two detected regions exhibited an even mean distribution with Band 1 $43.93 \pm 39.69\%$ and Band 2 $56.07 \pm 39.69\%$. The relatively high standard deviations revealed a significant difference in band distribution between the 10 lanes. A single band region was observed for the Mutant image with the mean integrated intensity 164,281.61 a.u. It thus normalized to 100% for each lane, considering normalization of only one band, not full molecular binding. The differences in the electrophoretic profiles observed in these images confirmed that there were differences in the profile detectable in the three images.

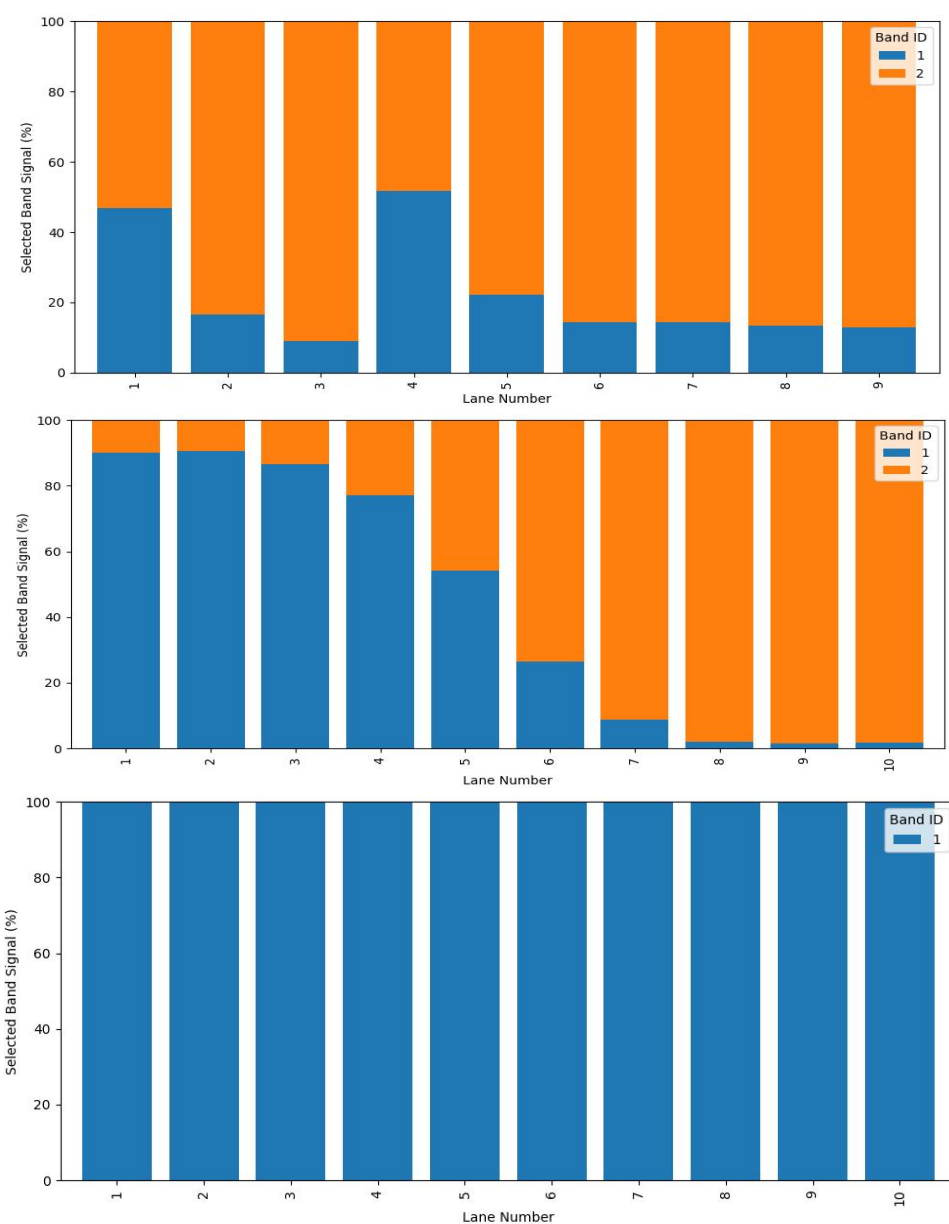


Figure 1. Lane-wise normalized band-intensity distributions in Controls(a), Mutant with Cas11 (b), and Mutant EMSA images (c).

3.3. WT Electrophoretic Band Heterogeneity

The image from the WT EMSA comprised three selected electrophoretic band regions, which was the most complex band profile detected of the five images. Band 3 contributed the greatest mean normalized intensity ($59.87 \pm 30.67\%$), followed by Band 2 ($32.26 \pm 28.93\%$) and Band 1 ($7.87 \pm 2.09\%$). Band 2 and 3 showed significant lane-to-lane variation while Band 1 had a fairly low and steady contribution. The lane-wise profiles analysed showed a lane shift of signal from Band 2 to Band 3 for the WT lanes. Three electrophoretically distinct regions, but not conclusively assigned to individual molecular species, were found. The observed redistribution was therefore considered as a relative change in band-signal abundance, and not as evidence of changed binding affinity.

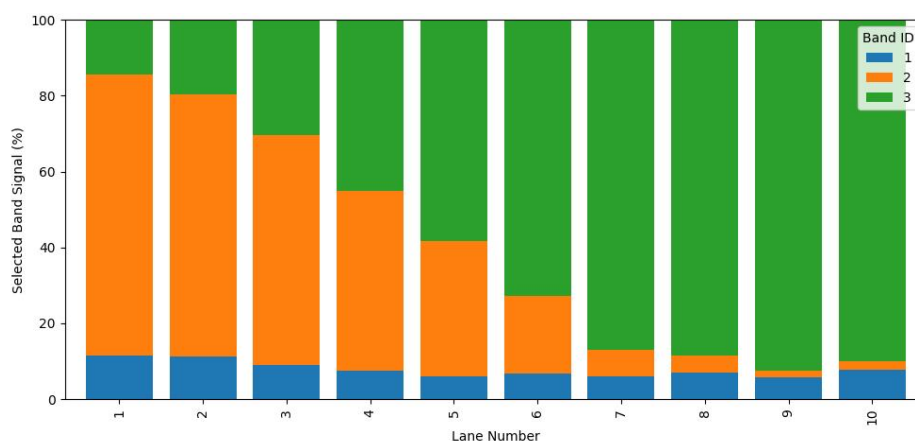


Figure 2. Lane-wise distribution of normalized electrophoretic band intensities in the WT EMSA image.

3.4. crRNA Band Distribution and Intensity Variation

The crRNA EMSA image had 11 electrophoretic lanes and 2 lanes selected for the band regions. Band 2 contributed $68.47 \pm 20.81\%$ of the mean normalized signal, whereas Band 1 accounted for $31.53 \pm 20.81\%$. Mean integrated intensities were 768,389.19 a.u. for Band 2 and 214,102.22 a.u. for Band 1. The lane-wise profiles showed significant differences in the amount of both regions, suggesting non-uniform electrophoretic signal distributions. Band 2 also had a relatively wide vertical integration area which may include diffuse signal as well as a localized electrophoretic feature. Thus, the higher integrated intensity would not have been attributed entirely to one molecular complex. In spite of this, the quantitative results showed that the signal of the selected image in the crRNA image was mainly placed in Band 2.

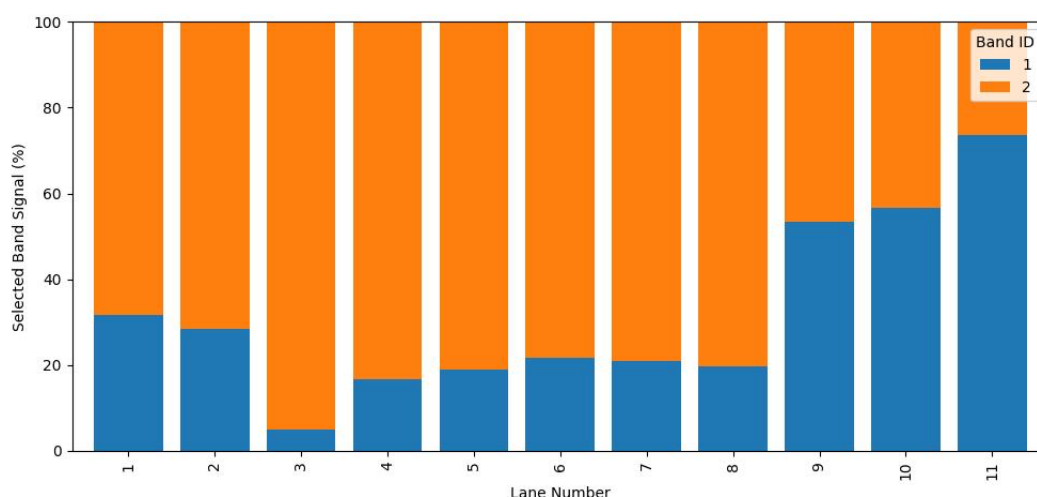


Figure 3. Lane-wise normalized electrophoretic band distribution in the crRNA EMSA image.

3.5. Comparative Densitometric Characteristics Across Experimental Conditions

The comparative analysis showed that the electrophoretic signal distribution was different for the five experimental images. While Band 2 showed the greatest contribution for the two conditions, the Mutant

with Cas11 image had a relatively even split between its two selected bands. Three regions were selected in the WT image and Band 3 had the highest amount of normalized signal. The Mutant image had no other selected regions, and its redistribution could not be compared to those of other bands. The highest mean integrated intensity was observed in crRNA (768,389.19 a.u.) and the second highest mean integrated intensity was observed in Controls (271,370.71 a.u.). No direct differences in molecular abundance or binding efficiency were assumed, however, since the absolute intensities of the images differed.

Table 2. Comparative quantitative characteristics of dominant EMSA band regions

Experimental condition	Lanes (n)	Selected bands	Dominant band	Mean integrated intensity (a.u.)
Controls	9	2	2	271370.71
Mutant with Cas11	10	2	2	63240.28
Mutant	10	1	1	164281.61
WT	10	3	3	90890.01
crRNA	11	2	2	768389.19

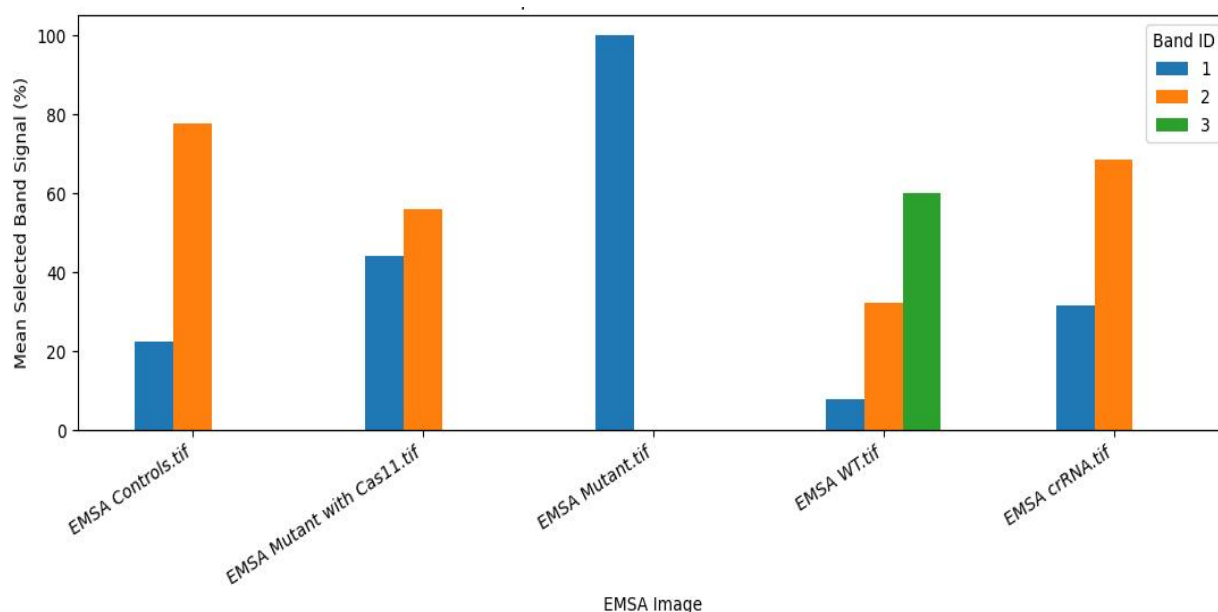


Figure 4. Comparative mean normalized EMSA band-intensity distributions across the five experimental conditions.

3.6. Preliminary Evaluation of Mobility-Shifted Signal Contributions

A preliminary analysis was carried out on electrophoretic migration positions in order to check the possibility of slower migrating areas contributing to the overall result. Assuming that the upper electrophoretic fractions are candidate shifted species, their mean normalized proportions were: 22.34% for Controls, 43.93% for Mutant with Cas11, 40.13% for WT and 31.53% for crRNA. These values were obtained from the intensity distributions selected in the band and were only preliminary estimates of the binding fraction based on mobility rather than experiment rather. Only one band region was found in the Mutant condition, so it was not possible to evaluate in the same way. The results showed measurable differences in electrophoretic band profiles, however, the lack of confirmed dissociation constants, concentration-dependent lane assignment, and independent biological replicate information did not enable the accurate estimation of dissociation constants or statistical significance. Overall, the analysis described clear patterns of electrophoretic signal distribution in the five images, paving the way for quantitative experiments to validate molecular interactions at the CRISPR locus.

Table 3. Provisional Mobility-Shifted Band Contributions Across EMSA Conditions

Experimental condition	Candidate shifted bands	Mean shifted signal (%)	Mean remaining signal (%)
Controls	Band 1	22.34	77.66
Mutant with Cas11	Band 1	43.93	56.07

Mutant	Unassigned	—	—
WT	Bands 1 and 2	40.13	59.87
crRNA	Band 1	31.53	68.47

4. Discussion

In this study, electrophoretic mobility shift assay (EMSA) band distributions were quantitatively characterized for the control, mutant, mutant with Cas11, wild type (WT), and crRNA conditions. Differences in the number of bands, relative signal intensity and variability between lanes within the same gel were identified in 50 electrophoretic lanes from 5 gel images. Results demonstrate the use of background-corrected densitometry to describe electrophoretic heterogeneity that is related to experimental conditions using a CRISPR approach. The distribution of the signals observed are quantitative evidence of the differences in electrophoretic profiles, and are used to compare differences between the molecular interaction patterns among the conditions studied.

Two band regions were selected in the control condition, 22.34% and 77.66% of the mean normalized intensities, respectively. This non-uniform distribution suggests that the majority of the electrophoretic signal quantified was from the second selected region. The distribution of the mutant with Cas11 condition was more balanced, 43.93% and 56.07% for the two mutants, respectively. However there was a significant difference in this condition from lane to lane, indicating that there was some variation in electrophoretic behaviour from lane to lane. The differences are due to differences in the distribution of molecular species and complex organization that is electrophoretically distinguishable. Changes in the molecular interactions of CRISPR surveillance complexes have been observed during their comparative electrophoretic characterization, associated with the structural and functional organization of these complexes, suggesting the biological relevance of these changes (Kupcinskaite et al., 2022).

Under the mutant condition, the electrophoretic band of the selected gene showed only a single band, accounting for 100% of the normalized signal in each lane analyzed. This discovery suggests a focused distribution of signals in the region measured by electrophoresis. The electrophoretic characteristics of the WT condition, on the other hand, were more complex with three bands accounting respectively for around 7.87%, 32.26% and 59.87% of the normalized intensity. The third band dominates and there is a significant variation in the second and third regions, indicative of redistribution between electrophoretically separable signals. The results indicate that there are band heterogeneity differences between mutant and WT. CRISPR-associated complexes recognize the molecular targets through structural and kinetic changes such as the formation and stabilization of RNA–DNA hybrid structures. In addition to the above, formation of R-loops offers a mechanistic framework to describe multiple conformational states of molecular recognition (Kauert et al., 2023).

For the crRNA condition, two band regions were selected for which 31.53% and 68.47% of the normalized intensity was measured. The second area dominated the area overall but the relative contribution of the first area became greater in later analysed lanes. In this pattern the distribution of the electrophoretic signal shows measurable variation from one condition (crRNA) to the other. In the case of CRISPR target searching, target recognition and complex stabilization are coordinated molecular processes that have been dynamically investigated (Aldag et al., 2023). Furthermore, quantitative research of the Cascade-mediated recognition has shown that the kinetic behaviour of interacting molecular components influences the target identification and rejection (Rutkauskas et al., 2022). These observations offer molecular context to the electrophoretic heterogeneities found in the present analysis.

Further differences were found in the background-corrected integrated intensities of experimental conditions when compared. The mean integrated intensities of the dominant selected band were 271,371 in the controls, 63,240 in the mutant with Cas11, and 164,282 in the mutant, 90,890 in the WT, and 768,389 in the crRNA. These are measurements of the electrophoretic signal captured in the image of the gels; normalized within-lane percentages are measurements of the relative distribution of selected bands. A more detailed evaluation of electrophoretic characteristics was possible than just visually, by employing the combination of integrated intensity measurements and normalized signal fractions. The molecular structure is also important in the substrate recognition and processing of the CRISPR effector complexes, underscoring the need to study the electrophoretic behavior of these components in concert with the known molecular mechanisms (Schwartz et al., 2024).

The comparative results also give insight into the electrophoretic properties which are correlated with the experimental set-up of the Cas. Different distributions of bands were detected for the differences between mutant and mutant with Cas11 conditions, which suggested that different electrophoretic profile were generated by different experimental conditions. The observation that the multiple selection of band regions

observed in the mutant under the condition of the Cas11 is not observed in the mutant is important as a descriptive observation. Previous research on RNA-guided transposition has revealed that protein recruitment of the targeted sequence is a crucial determinant of targeting selectivity and specificity (Hoffmann et al., 2022). Thus, the present results are part of the ongoing study of the relationship between the known molecular interaction patterns and components of CRISPR.

The analytical approach was introduced and gave a consistent framework for the assessment of electrophoretic band distribution – identification of lanes, background correction, integrated intensity measurement and within lane normalization. This resulted in measurements that showed differences in band dominance, signal heterogeneity, and lane-wise redistribution for the five experimental conditions. All these results together give the quantitative foundation for the comparison of electrophoretic profiles of CRISPR-associated molecules and discovery of experimentally relevant patterns of molecular interactions. This can be extended further by biochemical characterization which correlates the electrophoretic signal distributions with the molecular state and/or function. In general, the nature of this study highlights the usefulness of quantitative EMSA densitometry in exploring variation in molecular interactions involved in CRISPR and offers a systematic approach to future molecular investigations.

5. Conclusion

The efficacy of a quantitative electrophoretic mobility shift assay (EMSA) densitometry for understanding electrophoretic band distribution under control, mutant, mutant with Cas11, wild-type (WT), and crRNA experimental conditions. The electrophoretic pattern of 50 electrophoretic lanes in five gel images showed different lane-wise variability, different band intensity distribution and different band dominance indicating differences in electrophoretic properties between the investigated conditions. The normalized intensity of the two major band regions was 77.66% for the second band in the control condition, while in the mutant condition with the Cas11 condition, the normalized intensity of the second band was 56.07% and that of the first band was 43.93%. The electrophoretic pattern of the mutant condition had only one band-region, whereas the WT condition had three band-regions, suggesting higher electrophoretic heterogeneity. Likewise, the crRNA condition had two band regions, with relative contributions of 31.53% and 68.47%, which showed that there was variation in the distribution of electrophoretic signals. These results underscore the need for quantitative characterization of bands to detect variations in the CRISPR associated molecular interaction profiles. Background correction, band intensity measurement, and within lane normalization gave a systematic framework for analyzing electrophoretic patterns. The differences observed are not binding in themselves, but provide useful descriptive evidence of the molecular heterogeneity between experiments under the given conditions. In summary, this investigation is a quantitative characterization of CRISPR-associated electrophoretic interactions and provides a foundation for further biochemical investigations of nucleic acid-protein recognition, molecular complex organization and the functional roles of the CRISPR-associated components in nucleic acid-protein interactions.

References

1. Aldag, P., Rutkauskas, M., Madariaga-Marcos, J., Songailiene, I., Sinkunas, T., Kemmerich, F., ... & Seidel, R. (2023). Dynamic interplay between target search and recognition for a Type I CRISPR-Cas system. *Nature Communications*, 14(1), 3654.
2. Cofsky, J. C., Soczek, K. M., Knott, G. J., Nogales, E., & Doudna, J. A. (2022). CRISPR–Cas9 bends and twists DNA to read its sequence. *Nature structural & molecular biology*, 29(4), 395-402.
3. Halpin-Healy, T. S., Klompe, S. E., Sternberg, S. H., & Fernández, I. S. (2020). Structural basis of DNA targeting by a transposon-encoded CRISPR–Cas system. *Nature*, 577(7789), 271-274.
4. Hoffmann, F. T., Kim, M., Beh, L. Y., Wang, J., Vo, P. L. H., Gelsinger, D. R., ... & Sternberg, S. H. (2022). Selective TnsC recruitment enhances the fidelity of RNA-guided transposition. *Nature*, 609(7926), 384-393.
5. Hu, K., Chou, C. W., Wilke, C. O., & Finkelstein, I. J. (2024). Distinct horizontal transfer mechanisms for type I and type V CRISPR-associated transposons. *Nature communications*, 15(1), 6653.
6. Jia, N., & Patel, D. J. (2021). Structure-based functional mechanisms and biotechnology applications of anti-CRISPR proteins. *Nature Reviews Molecular Cell Biology*, 22(8), 563-579.
7. Kauert, D. J., Madariaga-Marcos, J., Rutkauskas, M., Wulfken, A., Songailiene, I., Sinkunas, T., ... & Seidel, R. (2023). The energy landscape for R-loop formation by the CRISPR–Cas Cascade complex. *Nature structural & molecular biology*, 30(7), 1040-1047.
8. Kupcinskaite, E., Tutkus, M., Kopūstas, A., Ašmontas, S., Jankunec, M., Zaremba, M., ... & Sinkunas,

- T. (2022). Disarming of type IF CRISPR-Cas surveillance complex by anti-CRISPR proteins AcrIF6 and AcrIF9. *Scientific Reports*, 12(1), 15548.
9. Lampe, G. D., Liang, A. R., Zhang, D. J., Fernández, I. S., & Sternberg, S. H. (2025). Structure-guided engineering of type IF CASTs for targeted gene insertion in human cells. *Nature Communications*, 16(1), 7891.
 10. Liu, J., Aliaga Goltsman, D. S., Alexander, L. M., Khayi, K. K., Hong, J. H., Dunham, D. T., ... & Thomas, B. C. (2025). Integration of therapeutic cargo into the human genome with programmable type VK CAST. *Nature communications*, 16(1), 2427.
 11. Mansouri-Noori, F., & Bayfield, M. A. (2024). Electrophoretic mobility shift assays (EMSAs) for in vitro detection of protein-nucleic acid interactions. *STAR protocols*, 5(2).
 12. McBride, T., Schwartz, E., Kumar, A., Taylor, D., Fineran, P., & Fagerlund, R. (2020). Diverse CRISPR-Cas complexes require independent translation of small and large subunits from a single gene [Data set]. Mendeley Data. <https://doi.org/10.17632/pbkft648ww.2>
 13. O'Brien, R. E., Santos, I. C., Wrapp, D., Bravo, J. P., Schwartz, E. A., Brodbelt, J. S., & Taylor, D. W. (2020). Structural basis for assembly of non-canonical small subunits into type IC Cascade. *Nature communications*, 11(1), 5931.
 14. Özcan, A., Krajcski, R., Ioannidi, E., Lee, B., Gardner, A., Makarova, K. S., ... & Gootenberg, J. S. (2021). Programmable RNA targeting with the single-protein CRISPR effector Cas7-11. *Nature*, 597(7878), 720-725.
 15. Rutkauskas, M., Songailiene, I., Irmisch, P., Kemmerich, F. E., Sinkunas, T., Siksnys, V., & Seidel, R. (2022). A quantitative model for the dynamics of target recognition and off-target rejection by the CRISPR-Cas Cascade complex. *Nature communications*, 13(1), 7460.
 16. Santiago-Frangos, A., Henriques, W. S., Wiegand, T., Gauvin, C. C., Buyukyoruk, M., Graham, A. B., ... & Wiedenheft, B. (2023). Structure reveals why genome folding is necessary for site-specific integration of foreign DNA into CRISPR arrays. *Nature structural & molecular biology*, 30(11), 1675-1685.
 17. Schwartz, E. A., Bravo, J. P., Ahsan, M., Macias, L. A., McCafferty, C. L., Dangerfield, T. L., ... & Taylor, D. W. (2024). RNA targeting and cleavage by the type III-Dv CRISPR effector complex. *Nature communications*, 15(1), 3324.
 18. Wang, F., Yao, T., Yang, W., Wu, P., Liu, Y., & Yang, B. (2022B). Protocol to detect nucleotide-protein interaction in vitro using a non-radioactive competitive electrophoretic mobility shift assay. *STAR protocols*, 3(4).
 19. Wang, J. Y., Pausch, P., & Doudna, J. A. (2022A). Structural biology of CRISPR–Cas immunity and genome editing enzymes. *Nature Reviews Microbiology*, 20(11), 641-656.
 20. Yang, J., Li, X., He, Q., Wang, X., Tang, J., Wang, T., ... & Zhang, H. (2024). Structural basis for the activity of the type VII CRISPR–Cas system. *Nature*, 633(8029), 465-472.