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Letter

Kanamycin Aptamer SELEX Coming to a Full Loop

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Abstract: A DNA aptamer for kanamycin originally reported in 2012 was unexpectedly found to belong to the same sequence family as a newly selected aptamer following binding assay-guided truncation and sequence analysis. This observation highlights how aptamers with apparently unrelated full-length sequences can converge to a common functional motif after removal of nonessential regions. The resulting aptamer, with a K_d below 100 nM under physiological conditions, is expected to be useful for monitoring kanamycin, an important aminoglycoside antibiotic with a narrow therapeutic window.

Keywords: kanamycin; aptamers; SELEX; binding assays; isothermal titration calorimetry

Aptamers are oligonucleotides capable of selectively binding target molecules [1]. A particularly powerful feature of these "chemical antibodies" is their binding-induced conformational change, a mechanism that researchers have exploited to develop sophisticated optical and electrochemical biosensors [2,3]. However, a foundational prerequisite for any successful biosensor is the availability of high-quality, well-characterized aptamers [4].

The landscape of aptamer discovery sometimes resembles an expansive, branching tree. While different laboratories occasionally report identical sequences [5], it is also common for them to uncover unique sequences that appear, at first glance, to be independent solutions to the same molecular puzzle. Yet, recent developments in the characterization of kanamycin-binding aptamers suggest these disparate branches are finally converging. By revisiting and refining sequences through systematic truncation and structural analysis, a full loop moment was reached: the realization that independent SELEX efforts have been orbiting the same optimal molecular motif for over a decade.

Kanamycin is a critical target for biosensing due to its narrow therapeutic window; precise monitoring is a clinical necessity to prevent irreversible ototoxicity and nephrotoxicity. Although the first DNA aptamer for kanamycin was reported in 2011 via column-based immobilization [6], the field subsequently struggled with reproducibility. The widely cited Ky2 aptamer, for instance, failed verification by isothermal titration calorimetry (ITC, also see Table S1 for a summary of K_d determination of various kanamycin aptamers) [7,8], casting a shadow over early discoveries.

In 2013, the Plaxco group converted an RNA aptamer for kanamycin to a DNA sequence for continuous in vivo monitoring [9], reporting a sub-mM K_d based on electrochemical measurements. Later, the Heemstra group employed a restriction-enzyme-based selection but again obtained sequences in the similar sub-mM range [10]. Despite multiple reported aptamers, the community lacked a short, high-affinity sequence that functioned reliably under physiological conditions.

The journey toward the optimal motif began in earnest with the 2012 report by Stoltenburg and coworkers, who introduced the term "capture-SELEX" to the literature [11]. Building on a strategy originally devised by Nutiu and Li in 2005 [12], the authors immobilized their library via a middle section through hybridization to a capture strand. This method bypassed the need to covalently modify the target molecule. Their efforts yielded many aptamer candidates. Among them, the 13_82 aptamer, a 98-nt sequence with a reported K_d of 5.1 μM was determined to be an optimal aptamer. In a follow-up work, the same group removed some terminal nucleotides

yielding a 62-nt sequence, which has retained the same binding affinity [13]. However, a clear secondary structure model is still lacking, making further truncation and thus rational sequence or biosensor design challenging.

In 2025, our lab revisited this target with two strategic pivots in new SELEX experiments. First, recognizing that kanamycin's four primary amines have pK_a values near 7 [13], we conducted selections at both pH 6 and pH 8 to account for distinct protonation states. Second, we utilized a modified capture-SELEX design, where immobilization occurred via a terminal primer-binding region supported by a designed duplex. This "structural foresight", a strategy pioneered by the Stojanovic group [14], made truncation to a 42-nt core structurally intuitive [7].

While our pH 6 selection yielded sub-micromolar binders at pH 6, their affinity plummeted under physiological conditions. Curiously, our pH 8 selection appeared poorly enriched; the top sequence, KAN8-1, had only 21 copies. We later discovered this "scarcity" was a paradox of success: the aptamer formed such a stable internal structure that it resisted hybridization to the capture strand during SELEX, leading to poor capture efficiency despite its exceptional binding performance [15].

The catalyst for unifying these narratives arrived via Dzantiev and coworkers. Using isothermal titration calorimetry (ITC), the authors re-evaluated previously reported kanamycin aptamers, discovering that the original 98-nt 13_82 aptamer was actually a sub-micromolar binder. Encouraged, they performed a series of rational truncations down to a 42-nt core, achieving a significantly improved K_d of approximately 100 nM. While many of the previously cited K_d values were obtained under differing solution conditions and by different methods (ITC, fluorescence, colorimetry, MST), the series of truncation work by the Dzantiev group was performed under the same condition allowing quantitative and meaningful comparison [8].

When we compared KAN8-1 to the truncated 13_82, the pieces fell into place. Despite different immobilization strategies and over a decade of separation, highly conserved regions were observed (Figure 1). We had rediscovered the same functional family. Our terminal immobilization strategy simply provided the structural clarity required to identify the short version of the aptamer that the original middle-immobilization strategy had obscured.

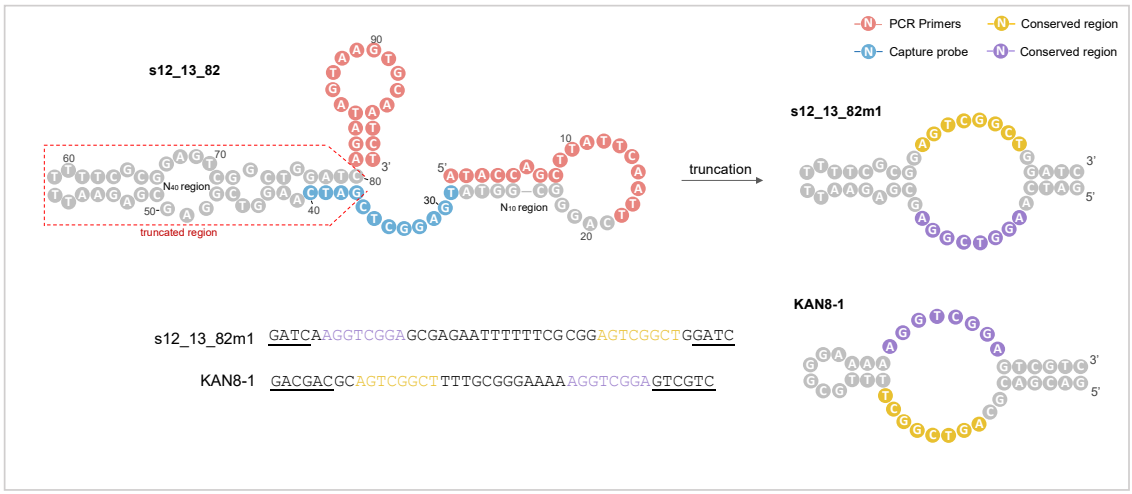

Figure 1. Truncation of the 13_82 aptamer and comparison with the KAN8-1 aptamer. The full-length s12_13_82 aptamer was redrawn based on Ref. [8] (CC BY 4.0 license).

This convergence highlights an important observation: length is the enemy of utility. Long aptamers, despite high affinity, often deter researchers due to a lack of structural control. The transition from a 98-nt "wild" sequence to a 42-nt "refined" motif provides the confidence required for integration into complex platforms like in vivo electrochemical sensors.

A similar challenge exists for cortisol aptamers. While the motifs differ, the difficulty of truncating sequences derived from internal-capture strategies remains a significant bottleneck in the field [16]. The discovery of the kanamycin motif suggests that this phenomenon is likely not an isolated incident. There are almost certainly other examples within the literature of seemingly distinct aptamers that, upon closer inspection, belong to the same functional family. However, identifying these hidden identities requires systematic truncation and rigorous binding assays such as ITC to strip away the noise of non-essential nucleotides. Only by uncovering the minimal functional core can we map the true convergence of independent SELEX experiments.

The unification of kanamycin SELEX results is a critical advancement in the understanding of kanamycin aptamers. We now possess a validated, 42-nt motif that: exhibits superior affinity K_d 50–100 nM under physiological conditions; offers a substantial improvement in affinity over sequences currently used in in vivo

studies; and unifies a decade of research. With this promising tool in hand, the next chapter of kanamycin monitoring moving from the bench to real-time in vivo diagnostics can finally begin with greater confidence.

Supplementary Materials

The additional data and information can be downloaded at: https://media.sciltp.com/articles/others/260724100 2394698/BAB-26050234-SI.pdf. Table S1: Literature reported kanamycin DNA aptamers and their K_d determination.

Author Contributions

Y.C. visualization, investigation, writing; J.L. conceptualization, supervision, writing, reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Use of AI and AI-Assisted Technologies

During the preparation of this work, the authors used ChatGPT to correct grammar mistakes. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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