



A widely used CRP aptamer does not bind CRP, revealing a general route to surface-driven pseudoaffinity in biosensors

Quan Duc Le^{a,b}, An Thi Hoai Le^{a,b}, Tong Ye Wang^{a,b}, Svetlana M. Krylova^{a,b}, Noah A. Clark^c, David Seghetti-Hebble^c, Ken-Ichiro Matsunaga^d, Michiko Kimoto^d, Ichiro Hirao^d, Sergey N. Krylov^{a,b,*}

^a Department of Chemistry, York University, 4700 Keele Street, Toronto, Ontario, M3J 1P3, Canada

^b Centre for Research on Biomolecular Interactions, York University, 4700 Keele Street, Toronto, Ontario, M3J 1P3, Canada

^c Illumina, Inc., 2945 Wilderness Pl, Boulder, CO, 80301, USA

^d Xenolis Pte. Ltd., 85 Science Park Drive, #02-08 the Cavendish, Singapore, Republic of Singapore

ARTICLE INFO

Keywords:

Aptamers
Biosensors
Surface-driven artifacts
Solution-phase binding
Surface plasmon resonance
C-Reactive protein (CRP)

ABSTRACT

Aptamer sequences are often reused in biosensors and diagnostics without independent confirmation of binding activity, even though rigorous validation requires quantitative solution-phase testing by orthogonal methods. Here, we re-examined a DNA sequence reported in 2010 to bind C-reactive protein (CRP) with a K_d of 3.5 nM by surface plasmon resonance (SPR) and subsequently adopted in many CRP aptasensor studies. We tested this sequence by two orthogonal solution-phase methods and by SPR. In solution-phase assays, we found no detectable binding of the reported aptamer to native CRP. In SPR, we found that CRP binds strongly to streptavidin-containing sensor surfaces even in the absence of immobilized DNA, whereas the reported sequence produced no measurable ligand-specific SPR response under lower-background capture conditions. Thus, the originally reported affinity is best explained by surface-driven pseudoaffinity rather than true aptamer-CRP recognition. More broadly, this case does not merely add another example of aptamer nonbinding, but reveals a general route by which nonbinding aptamers can acquire apparent affinity through surface-driven pseudoaffinity and then be propagated through the biosensor literature as validated molecular recognition reagents. This study underscores the need for orthogonal solution-phase validation before aptamers are reused in biosensor development and related analytical applications.

1. Introduction

Aptamers are short oligonucleotides selected in vitro to recognize specific molecular targets through noncovalent interactions (Ellington and Szostak, 1990; Tuerk and Gold, 1990). Their stability, chemical simplicity, and ease of modification have made them attractive molecular recognition elements for biosensors and related diagnostic assays (McKeague and DeRosa, 2012; Song et al., 2012). Aptamers are often viewed as synthetic analogues of biologically produced antibodies (Keefe et al., 2010). Once an aptamer sequence is published, it becomes easy for other researchers to obtain the same oligonucleotide and incorporate it into diverse assay formats such as lateral-flow tests, nanoparticle-based colorimetric assays, electrochemical sensors, and

microarrays (Tombelli et al., 2005). This portability of sequence, combined with the perceived analogy to antibodies, has created a culture in which published aptamers are frequently reused without independent confirmation that they bind the intended target under the new assay conditions (McKeague and DeRosa, 2012; Phung et al., 2020; Villalonga et al., 2022). As a result, qualitative claims of binding and even specific reported equilibrium dissociation constants (K_d) can persist and propagate through the literature long after the original report without independent verification of the underlying molecular interaction (McKeague et al., 2015). Systematic re-examinations in recent years have revealed that several widely cited aptamers for small molecules, metal ions, and proteins show no detectable binding or drastically weaker affinity when tested in carefully controlled homogeneous assays (Bottari et al., 2020;

* Corresponding author. Department of Chemistry, York University, 4700 Keele Street, Toronto, Ontario, M3J 1P3, Canada.

E-mail addresses: quan1504@yorku.ca (Q.D. Le), anhoai@yorku.ca (A.T.H. Le), wty1986@yorku.ca (T.Y. Wang), krylova@yorku.ca (S.M. Krylova), nclark1@illumina.com (N.A. Clark), dseghetti@illumina.com (D. Seghetti-Hebble), ken.ichiro@xenolis.com (K.-I. Matsunaga), michiko.kimoto@xenolis.com (M. Kimoto), ichiro.hirao@xenolis.com (I. Hirao), skrylov@yorku.ca (S.N. Krylov).

<https://doi.org/10.1016/j.bios.2026.118875>

Received 20 April 2026; Received in revised form 17 May 2026; Accepted 27 May 2026

Available online 28 May 2026

0956-5663/© 2026 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Ruscito and DeRosa, 2016).

One recurring theme in these failed validations is that the original evidence for binding was obtained from surface-based or nanomaterial-based readouts rather than from quantitative solution assays (Zara et al., 2021). In many diagnostic formats, aptamers are immobilized on functionalized supports, for example, thiol-modified gold films, streptavidin-coated substrates, dextran-based surface plasmon resonance chips, or nanoparticle surfaces, while the analyte (target) in solution interacts with this composite interface of DNA, linker chemistry, and substrate material (Balamurugan et al., 2008; Pichon et al., 2015). Under these conditions, the apparent signal can arise from nonspecific adsorption of the target to the surface, to linker molecules, or to the nanomaterial, from electrostatic interactions with highly charged proteins, from aggregation of the target, or from buffer components and cofactors that interact with the interface (Lichtenberg et al., 2019; Frutiger et al., 2021). Multiple studies have revisited canonical aptamers that showed strong responses in such formats, including sequences for antibiotics, pesticides, environmental toxins, metabolites, and metal ions, and have found that the observed sensor signals were dominated by target adsorption or matrix effects rather than true aptamer-target binding (Ruscito and DeRosa, 2016; Shi et al., 2023; Wu et al., 2023; Zhang and Liu, 2021; Lopez and Liu, 2021; Tao et al., 2020; Zong and Liu, 2019; Stangherlin et al., 2025). Because these surface-driven artifacts are dictated by common chemistries and assay geometries, they can be reproducible across laboratories, allowing nonbinding or weakly binding sequences to acquire the appearance of genuine affinity and become entrenched in the biosensor literature.

Most of these re-examinations concluded that binding validation requires at least two orthogonal solution-based methods that rely on different physical principles and can independently confirm complex formation and determine K_d values equal within the defined error limits (Shi et al., 2023; Tao et al., 2020; Kim et al., 2009; Catasti et al., 1997). Because K_d values are not measured directly but inferred from assay-specific observables in the absence of reference standards, orthogonal confirmation is essential for defensible K_d determination. We recently introduced a roadmap that outlines how to select and combine suitable quantitative solution-based assays depending on the nature of the target and the number of sequences that require validation, from a few in reassessment of published aptamers to dozens or more in new selection campaigns (Le et al., 2026).

Reflection on the need for rigorous solution-based validation prompted us to examine how frequently developers of aptasensors, one of the most widespread applications of aptamers in biosensing and diagnostics, verify binding of the sequences they adopt. We conducted six case studies involving aptasensors reported for three protein targets and three small-molecule targets and found that none of 209 publications reported independent auditable quantitative solution-phase verification of binding for the aptamer construct used under homogeneous conditions prior to device construction (Krylov et al., 2026). Moreover, nearly all reports also lacked characterization by standard surface-based methods such as surface plasmon resonance (SPR) or biolayer interferometry (BLI) and instead relied solely on device output as evidence of recognition. Among these case studies, aptasensors intended to detect C-reactive protein (CRP) attracted particular attention because a large fraction of reported aptasensors relied on the same published aptamer sequence without independent verification of binding by any method (Phung et al., 2020; Villalonga et al., 2022; Liu et al., 2019; Huang et al., 2010; Whitehouse et al., 2024; Sinha et al., 2019; Chen et al., 2025; Zhang et al., 2025; Chang et al., 2025).

CRP is a Ca^{2+} -dependent pentameric acute-phase protein that binds phosphocholine on microbial and damaged cell membranes and serves as a major biomarker of systemic inflammation in clinical diagnostics (Volanakis, 2001; Salazar et al., 2014; Thompson et al., 1999). A widely cited DNA aptamer for CRP emerged from a 2010 *in vitro* selection report in which CRP was immobilized, and binding was characterized by SPR in a surface format that ultimately yielded an apparent K_d of

approximately 3.5 nM (Huang et al., 2010). The originating report did not provide raw sensorgrams, replicate traces, or fitting parameters, and it did not include an EDTA kill control to confirm Ca^{2+} dependence. It also did not include a scrambled-sequence negative control. The ostensibly high affinity, together with the sequence's short length, ease of biotinylation, and compatibility with streptavidin-based immobilization, led to its rapid adoption as a generic CRP recognition element across multiple sensing platforms. It is now routinely used as the default CRP-binding ligand in lateral-flow strips, electrochemical sensors, fluorescence assays, microarrays, and nanoparticle-based devices, often in complex matrices such as serum or plasma (Phung et al., 2020; Villalonga et al., 2022; Liu et al., 2019; Huang et al., 2010; Whitehouse et al., 2024; Sinha et al., 2019; Chen et al., 2025; Zhang et al., 2025; Chang et al., 2025). In most of these reports, analytical performance is inferred from sensor calibration curves or signal changes, while the underlying aptamer-CRP interaction is assumed rather than verified. As a result, the sequence has acquired the reputation of a validated affinity reagent and underpins a substantial and expanding body of CRP aptasensor literature, despite the absence of independent confirmation of its binding in homogeneous solution.

To address this question, we designed an experimental strategy that applies rigorous, orthogonal, solution-based evaluation to the canonical CRP aptamer. Guided by the methodological criteria established in our solution-phase assay roadmap (Le et al., 2026), we assessed binding using two independent homogeneous techniques: nonequilibrium capillary electrophoresis of equilibrium mixtures and fluorescence anisotropy, each implemented in both direct and competitive formats. A validated CRP-binding SOMAmer was used as an internal positive control for binding competence and buffer compatibility and also served as a tracer in the competitive assays (Le et al., 2025). In parallel, we reproduced the original SPR protocol used to report the 3.5 nM K_d , allowing us to distinguish genuine aptamer recognition from surface- or streptavidin-mediated pseudoaffinity. Thus, the aim of this work was not simply to show that a published aptamer fails validation, but to determine how a widely reused CRP aptamer acquired an apparently convincing affinity in a biosensor-relevant surface format and was subsequently propagated as a validated recognition element. This integrated approach was designed to determine whether this aptamer forms a specific complex with native CRP and whether the originally reported SPR affinity reflects ligand-specific recognition or surface-driven pseudoaffinity.

Using this integrated strategy, we found no evidence that the canonical CRP aptamer binds native pentameric CRP in homogeneous solution. Across all NECEEM and FA assays, the aptamer produced no measurable complex formation under any tested conditions, consistent with a K_d far above 1 μM . In competitive formats, the unlabeled aptamer did not displace the CRP-binding SOMAmer tracer, providing no indication of specific competition for CRP. Replication of the original SPR protocol independently in two laboratories revealed that the previously reported K_d of approximately 3.5 nM was dominated by nonspecific adsorption of CRP to the streptavidin-modified surface; when surface conditions were adjusted to suppress this adsorption, the aptamer produced only baseline-level responses. Together, these observations indicate that the reported CRP aptamer does not exhibit detectable ligand-specific recognition of native CRP and that the originally reported affinity is best explained by a surface-driven pseudoaffinity rather than genuine CRP-aptamer binding.

The implications of this finding extend well beyond a single aptamer-target pair. The CRP case reveals a general route by which nonbinding sequences can acquire apparent affinity through surface-driven pseudoaffinity and then be propagated through reuse and citation as validated recognition reagents. Preventing such propagation requires systematic validation of aptamer-target interactions under homogeneous conditions, the use of multiple orthogonal solution-based methods where feasible, and transparent reporting of the evidence supporting any claimed affinity. Establishing and following such standards is

essential to ensure that diagnostic technologies relying on aptamers are grounded in genuine molecular recognition rather than reproducible artifacts.

2. Results and discussion

2.1. NECEEM direct binding assay

To establish notation, we refer to the validated CRP-binding SOMAmer as S49; all fluorescently labeled ligand derivatives, whether SOMAmer or aptamer (APT), are denoted with an asterisk (e.g., S49*, APT*). Our prior work confirmed robust CRP recognition by S49* in the SOMAmer buffer (40 mM HEPES, 1 mM CaCl₂, 1 mM MgCl₂, pH 7.5) (Le et al., 2025). In the present study, we re-evaluated this interaction in the published CRP aptamer buffer (10 mM Tris-HCl, 50 mM NaCl, 2 mM CaCl₂, pH 7.5) using the same NECEEM assay format (Huang et al., 2010).

We conducted the NECEEM experiment with 10 nM S49* alone and with 300 nM CRP to directly assess complex formation in both SOMAmer and aptamer buffers (Fig. 1A and B). Consistent with our previously published results for this same CRP-S49* binding pair in the SOMAmer buffer, addition of CRP caused a pronounced loss of the free S49* peak and the appearance of a slower-migrating species corresponding to the CRP-S49* complex. The selective formation of this single 1:1 complex in the aptamer buffer confirms that CRP recognition by S49* is preserved across buffer systems and that NECEEM workflow reliably detects this binding pair in free solution. These observations remain fully consistent with the independently validated nanomolar affinity of CRP-binding SOMAMers and provide a rigorous benchmark for comparison with the published CRP aptamer. In our prior NECEEM study of the CRP-S49 interaction, the first two stepwise dissociation constants were determined as $K_{d1} = 3.0\text{--}7.8$ nM and $K_{d2} = 41\text{--}160$ nM, reported as 95% accuracy confidence intervals (Le et al., 2025). In the present study, the fixed-concentration NECEEM experiment with S49* was used as a positive control for CRP binding competence and buffer compatibility, not as a de novo NECEEM-based determination of K_d for S49*.

In contrast, the fluorescently labeled published CRP aptamer (APT*) showed no evidence of complex formation with CRP at the same concentrations (Fig. 1C and D). APT* consistently produced only the single peak corresponding to the free aptamer, and its position, shape, and intensity remained unchanged upon the addition of 300 nM CRP. No slower-migrating species or loss of free-aptamer signal was observed. These data indicate that the aptamer does not form a stable complex with CRP in solution at concentrations where a genuine high-affinity interaction would be easily detectable. If binding occurs at all, it must be too weak or transient to produce any observable peak shift within the NECEEM measurement time.

To directly test whether CRP–ligand recognition requires Ca²⁺, we

performed an EDTA kill–rescue experiment using the validated S49 SOMAmer tracer (Section S1). In Ca²⁺-containing SOMAmer buffer, S49* formed a clear CRP complex peak as indicated by NECEEM. Addition of 5 mM EDTA eliminated this complex and returned nearly all signal to the free S49* peak, consistent with Ca²⁺ chelation disrupting binding. Reintroducing Ca²⁺ (10 mM) fully restored the complex peak. This reversible loss and recovery of binding confirms that CRP–ligand interactions are strictly Ca²⁺ dependent. Some published aptasensor applications using this aptamer employed binding or measurement buffers that lacked Ca²⁺ and, in some cases, included EDTA, conditions under which native CRP would not be expected to form specific ligand complexes.

2.2. NECEEM competitive binding assay

To test whether the published CRP aptamer could compete with a known CRP binder, we used a NECEEM-based competitive format with fluorescent SOMAmer S49* as a tracer. As a reference competitor, we employed a second CRP-binding SOMAmer, S50 (unlabeled), which is known to bind CRP at a site overlapping that of S49*. In this assay, 10 nM S49* tracer was pre-equilibrated with 300 nM CRP, after which the unlabeled competitor (S50 or APT) was introduced across a range of concentrations to assess its ability to displace the tracer.

In the SOMAmer buffer (Fig. 2A), the S49*–CRP complex peak progressively decreased in intensity as the concentration of S50 was increased from 0.15 to 1500 nM, accompanied by a corresponding increase in the free S49* peak; a similar trend was observed in the aptamer buffer (Fig. 2B), and at the highest S50 concentration the free S49* signal was restored to the level observed in the absence of CRP, confirming efficient displacement of S49* and validating the NECEEM competitive assay design.

Under identical conditions, we then evaluated unlabeled APT as a competitor. In the SOMAmer buffer (Fig. 2C), increasing APT concentration (50–5000 nM) produced only minimal changes in the electropherograms: the S49*–CRP complex peak remained essentially unchanged, with only a slight increase in the free S49* peak at the highest APT concentrations. A similar pattern was observed in the aptamer buffer (Fig. 2D), where the complex and free ligand signals were largely invariant and only a modest increase in free S49* was detected at elevated APT levels, likely reflecting weak or nonspecific oligo-oligo interactions rather than genuine competition for CRP (Section S2).

Taken together, these results show that while S50 efficiently displaces S49* from CRP and thus confirms the feasibility and sensitivity of the NECEEM-based competitive assay, the unmodified aptamer APT does not measurably compete with S49* for CRP binding under either SOMAmer or aptamer buffer. This competitive NECEEM experiment was intended to validate the assay design and provide a functional positive-control benchmark, not to determine new NECEEM-based K_d values for

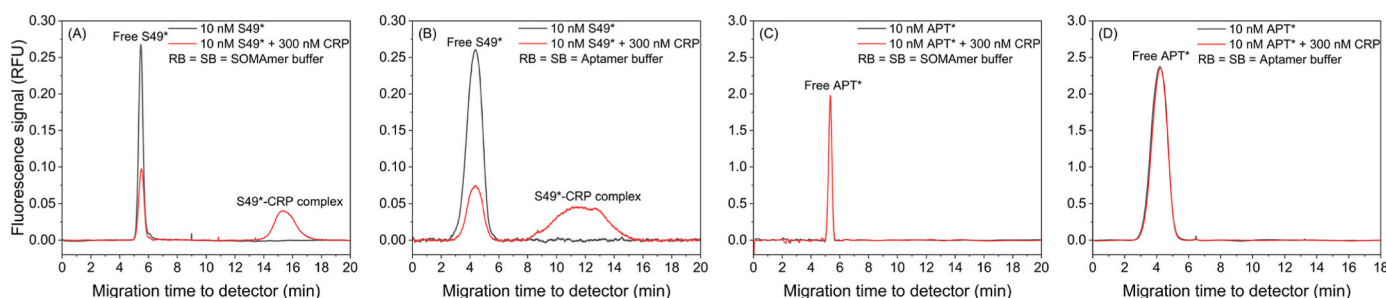


Fig. 1. Direct NECEEM analysis of S49* and APT* binding to CRP. Electropherograms show 10 nM fluorescent ligand alone (black) or with 300 nM CRP (red); running buffer (RB) = sample buffer (SB). (A) S49*–CRP binding assay in SOMAmer buffer; (B) S49*–CRP binding assay in aptamer buffer; (C) APT*–CRP binding assay in SOMAmer buffer; (D) APT*–CRP binding assay in aptamer buffer. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

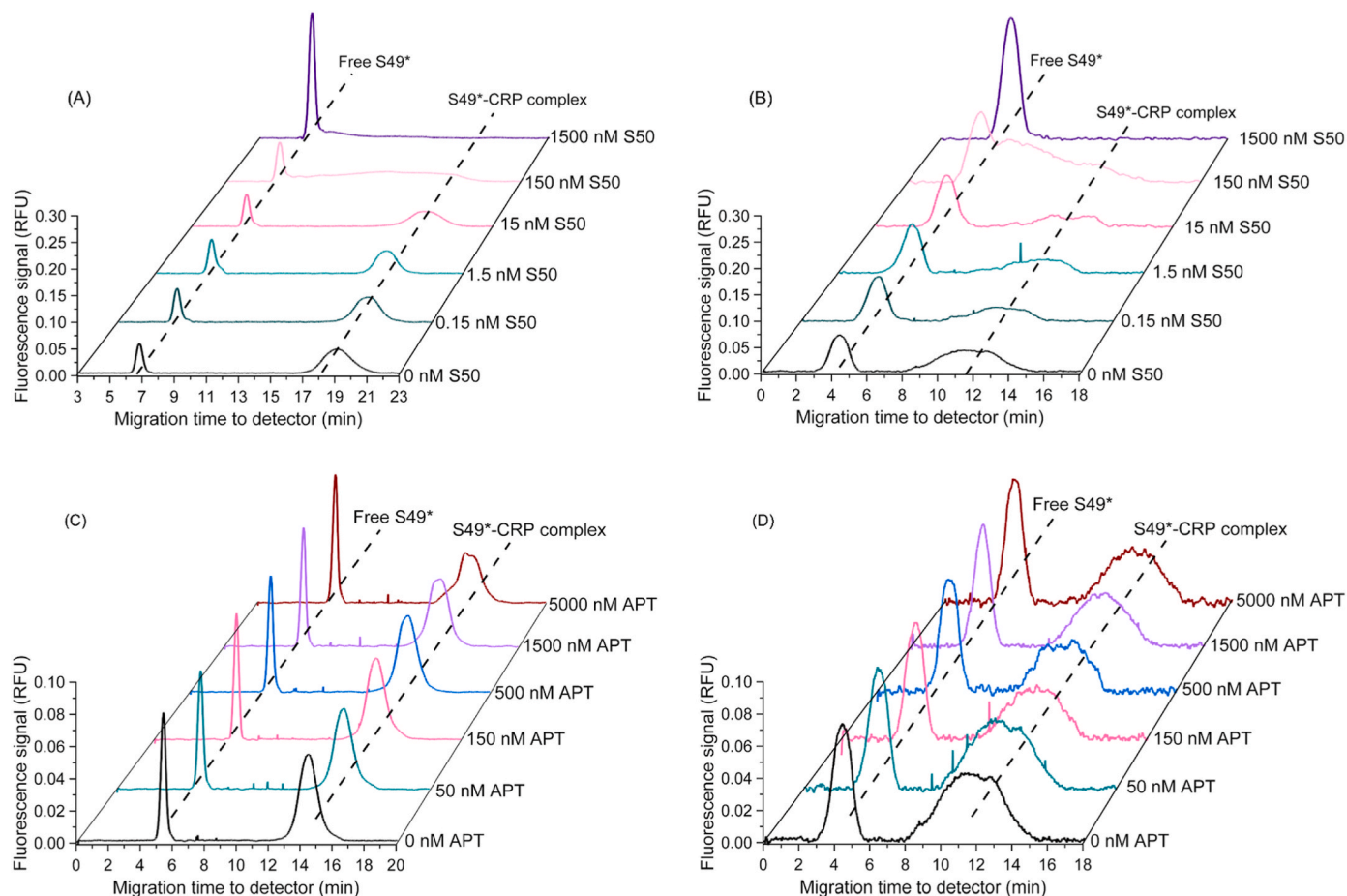


Fig. 2. NECEEM-based competitive binding assay for displacement of S49* from CRP by S50 or APT. Electropherograms show 10 nM S49* pre-equilibrated with 300 nM CRP and titrated with increasing concentrations of unlabeled competitor. (A, B) Unlabeled SOMAmer S50 in SOMAmer buffer (A) or aptamer buffer (B). (C, D) Unlabeled APT in SOMAmer buffer (C) or aptamer buffer (D).

S49 or S50.

2.3. Fluorescence anisotropy binding assay

To validate our findings with an orthogonal solution-phase method, we assessed CRP binding by fluorescence anisotropy (FA) in both direct and competitive formats.

In the direct FA assay (Fig. 3A and B), 10 nM S49* or APT* was incubated with CRP (0, 100, or 300 nM) in either SOMAmer or aptamer buffer. In both buffers, the addition of CRP produced a clear or increase in the FA signal for S49* (black trace), consistent with the formation of a higher-molecular-weight S49*-CRP complex, whereas the FA of APT* remained unchanged up to 300 nM CRP, indicating no detectable binding (red trace).

In the competitive FA assay (Fig. 3C and D), 10 nM S49* was pre-equilibrated with 300 nM CRP, and the unlabeled competitor oligonucleotide (S50 or APT) was titrated in both buffers. Increasing S50 concentration produced a concentration-dependent decrease in FA toward the free S49* baseline (black trace), consistent with displacement of S49* from CRP and a corresponding shift of the fluorophore from a slower tumbling CRP-S49* complex to a faster-tumbling free S49* species. In contrast, the addition of the unlabeled APT up to 500 nM did not measurably alter the FA signal (red trace). To address the possibility that this negative result reflected a DNA synthesis error, we repeated the competitive FA assay using the same aptamer sequence synthesized by an independent vendor (Section S3); the FA profiles were indistinguishable, further arguing against detectable CRP binding by the reported aptamer.

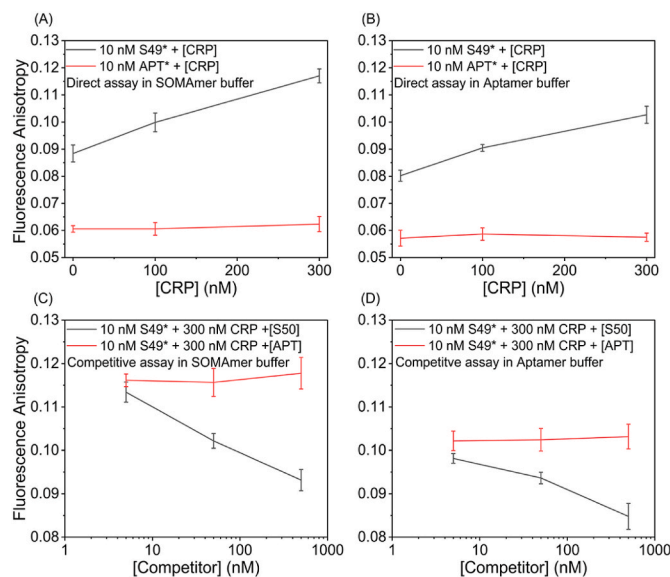


Fig. 3. Fluorescence anisotropy (FA) analysis of S49* and APT* binding to CRP. Direct binding assays (A, B) were performed with 10 nM S49* or APT* incubated with 0–300 nM CRP in SOMAmer buffer (A) and aptamer buffer (B). Competitive assays (C, D) were performed with 10 nM S49* + 300 nM CRP while titrating 5–500 nM unlabeled competitor (S50 or APT) in SOMAmer buffer (C) and aptamer buffer (D).

Taken together, these FA data corroborate the NECEEM results and demonstrate that, although the S49/S50 pair engages specific CRP binding in solution, the reported CRP aptamer does not bind detectably to CRP or compete with S49* under any tested condition. The direct and competitive FA experiments with S49/S50 were likewise intended to validate the assay logic and provide a positive-control benchmark for comparison with the reported aptamer, rather than to establish new FA-based K_d values for the SOMAMers.

2.4. Surface plasma resonance binding assay

To reassess the binding behavior originally reported for the CRP aptamer, we conducted surface plasmon resonance (SPR) measurements using the same assay configuration and buffer formulation as described in the initial publication (Huang et al., 2010). In that work, biotinylated APT was immobilized on a streptavidin-coated CM5 chip and CRP was injected as the analyte. To test whether the observed responses truly reflected CRP–APT binding, we repeated the experiment but used a streptavidin-coated CM5 sensor chip without aptamer immobilization. When CRP was injected at concentrations from 0 to 40 nM over this streptavidin surface, we observed large, concentration-dependent responses (Fig. 4), with maximal signals approaching ~2300 RU and a clear association and dissociation phase. Kinetic fitting of the sensorgrams using a 1:1 interaction model gave an apparent K_d of ~0.74 nM, demonstrating that CRP itself exhibits strong non-specific binding to surface-bound streptavidin. Additional injections over a blank CM5 chip confirmed that CRP also binds non-specifically to the carboxymethyl dextran matrix. Thus, the large SPR signals obtained in the original configuration can be largely attributed to non-specific interactions of CRP with dextran and streptavidin, rather than to specific recognition of CRP by the aptamer. This comparison helps explain the disagreement with Huang et al. (2010): although the original study reported an apparent K_d of ~3.5 nM in the same overall SPR configuration, our controls show that CRP can generate strong nanomolar-range apparent binding in that format even without immobilized aptamer, indicating that the reported affinity was most likely dominated by surface-driven pseudoaffinity. A cautionary control evaluating CRP non-specific adsorption to a streptavidin planar chip is presented in Section S4, where the resulting ligand-independent apparent kinetics and affinity are reported.

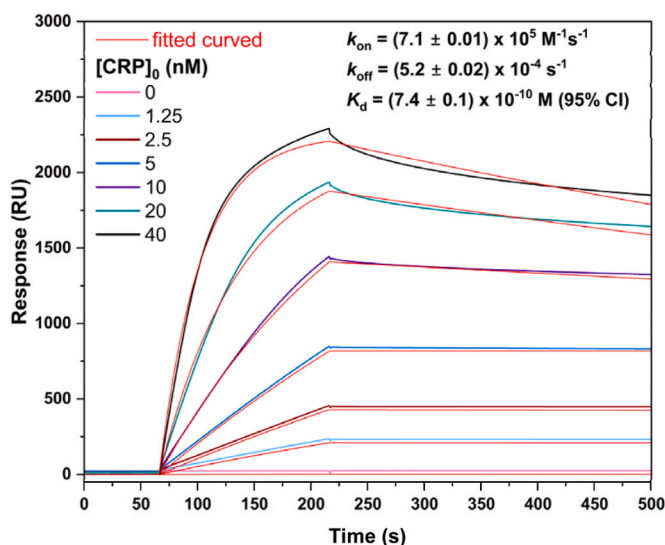


Fig. 4. Apparent high-affinity binding of CRP to a streptavidin surface in the absence of immobilized aptamer. Sensorgrams show injections of CRP (0–40 nM) over a streptavidin-coated CM5 chip lacking APT, with large, concentration-dependent responses arising from non-specific CRP–streptavidin/dextran interactions.

To minimize the contribution of non-specific CRP–surface interactions observed on streptavidin/dextran, we reconfigured the SPR assay using a neutravidin-coated chip. As an alternative to streptavidin-coated surfaces, neutravidin-based capture layers (e.g., Sensor Chip NA) are widely used because deglycosylated, acidic avidin derivatives such as neutravidin/neutralite avidin retain high biotin affinity but exhibit markedly reduced non-specific binding, and are often recommended as lower-background alternatives to streptavidin in immobilization and affinity-capture formats (Meyer et al., 2006; Marttila et al., 2000). Biotinylated S49 and the reported CRP aptamer were immobilized in quadruplicate on separate regions of interest (ROIs) on a neutravidin-functionalized planar carboxymethyl dextran chip. CRP was injected at 1, 3.16, 10, 31.6 and 100 nM in the same buffer system used in the original aptamer paper, supplemented with 800 nM C-5 modified oligonucleotide polyanionic competitor to further suppress non-specific binding (Gold et al., 2010). Under these conditions, S49 exhibited robust, concentration-dependent binding with clean association and dissociation phases (Fig. 5A) and global 1:1 Langmuir fits yielded a mean K_d of 2.0 ± 0.03 nM (95% CI), consistent with high-affinity, specific recognition of CRP. In contrast, the cited aptamer produced only minimal responses that were statistically indistinguishable from baseline, even at 100 nM CRP (Fig. 5B), precluding meaningful kinetic analysis.

Together with the NECEEM and FA measurements, these SPR data provide convergent evidence that the reported CRP aptamer sequence does not form a detectable, specific complex with CRP under any of the conditions we tested. Consequently, the originally reported K_d values are most plausibly explained by surface-driven pseudoaffinity arising from nonspecific CRP–surface interactions rather than a genuine high-affinity CRP–aptamer interaction.

More broadly, these findings illustrate how surface chemistry can generate apparent affinity for a nonbinding aptamer and thereby promote the propagation of surface-driven pseudoaffinity in the literature. Because streptavidin is widely used to immobilize biotinylated aptamers, and streptavidin-coated surfaces are prone to nonspecific adsorption of abundant serum proteins, many streptavidin-based aptasensors may be susceptible to surface-driven artifacts when operated in complex clinical matrices. Taken together, our SPR experiments show that neutravidin-coated sensors provide a substantially cleaner background for CRP than conventional streptavidin/dextran surfaces while still supporting high-affinity capture of biotinylated ligands. We therefore recommend that future SPR studies aimed at determining K_d values for CRP–aptamer interactions employ neutravidin-based capture layers whenever possible, particularly in experiments using serum-like matrices. Such surfaces should help minimize background-driven artifacts and improve confidence that the extracted kinetic and equilibrium parameters reflect ligand-specific recognition rather than nonspecific CRP–surface interactions.

3. Conclusion

In this work, we systematically re-examined a widely cited DNA sequence reported to bind C-reactive protein with low-nanomolar affinity. Using two orthogonal solution-phase methods, NECEEM and fluorescence anisotropy, each in direct and competitive formats with a validated CRP-binding SOMAmer tracer, we observed no detectable binding of the reported aptamer to native CRP under any condition tested. Replication of the original SPR assay further revealed strong, low-nanomolar apparent binding of CRP to streptavidin-containing sensor surfaces in the absence of any immobilized ligand, whereas the aptamer itself produced only baseline-level responses under lower-background neutravidin capture conditions. Taken together, these data show that the originally reported 3.5 nM “aptamer–CRP” K_d is best explained by surface-driven pseudoaffinity rather than genuine molecular recognition. Although additional orthogonal methods such as ITC could provide further confirmatory support, the present conclusion rests

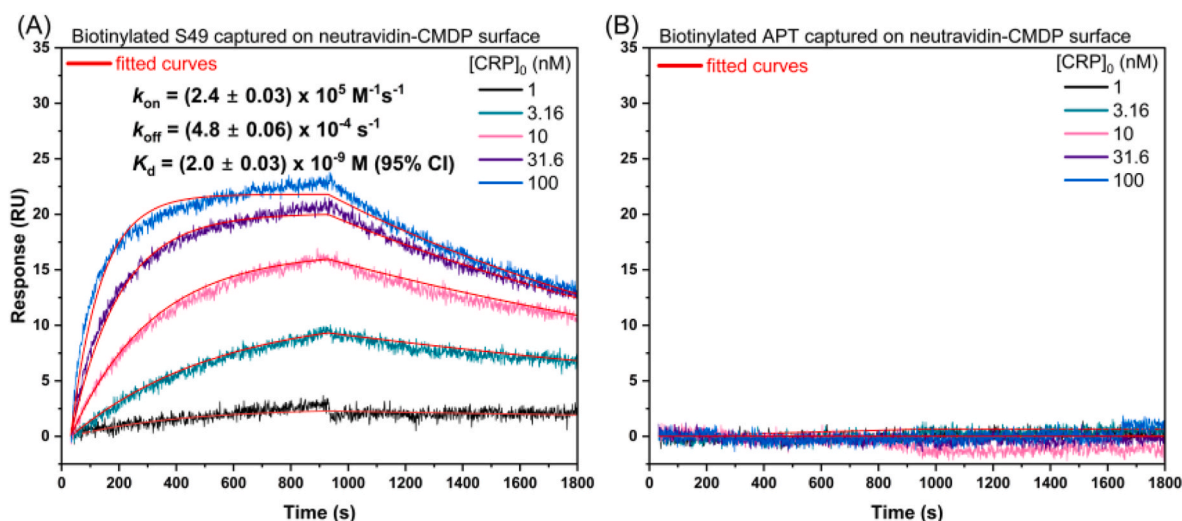


Fig. 5. SPR analysis of CRP binding to immobilized oligonucleotides on a neutravidin-CMDP surface. Biotinylated ligands (A) S49; (B) APT were captured on the surface and CRP was injected at concentrations of 1, 3.16, 10, 31.6 and 100 nM in the original aptamer buffer supplemented with a polyanionic competitor to further suppress non-specific binding. Errors shown for k_{on} , k_{off} , and K_d values are standard errors of fitting and do not include other sources of uncertainty.

on concordant evidence from multiple complementary solution-phase and surface-based assays rather than on any single experimental format.

Beyond this specific case, our findings show how a nonbinding sequence can acquire the status of a canonical reagent through surface-driven pseudoaffinity and citation cascades, thereby revealing a concrete mechanism by which erroneous affinity claims can persist and spread in the biosensor literature. By clarifying this failure mode in a widely reused CRP aptamer lineage, the present work may also help prevent further misallocation of effort and resources toward CRP aptasensor development based on a nonbinding recognition sequence. Our SPR results further indicate that lower-background neutravidin capture conditions provide a cleaner platform for evaluating CRP-ligand interactions than streptavidin-based surfaces, and we therefore recommend their use in future SPR studies of CRP-aptamer interactions where feasible.

More broadly, this work reinforces the need to treat quantitative solution-phase validation, ideally using multiple orthogonal methods and appropriate negative controls, as the primary standard for establishing aptamer-target interactions intended for analytical or diagnostic applications. Implementing such validation as routine practice will help prevent the propagation of ghost affinities, improve the reliability of aptamer reagents, and ensure that emerging diagnostic technologies are grounded in bona fide molecular recognition rather than reproducible artifacts.

Notes

The authors declare no competing financial interest.

Funding

This work was supported by an NSERC Discovery grant number RGPIN-2022-04563 to S.N.K.

CRediT authorship contribution statement

Quan Duc Le: Data curation, Investigation, Writing – review & editing. **An Thi Hoai Le:** Methodology, Validation, Writing – review & editing. **Tong Ye Wang:** Formal analysis, Methodology. **Svetlana M. Krylova:** Conceptualization, Methodology, Supervision, Writing – original draft. **Noah A. Clark:** Investigation, Writing – review & editing. **David Seghetti-Hebble:** Investigation. **Ken-Ichiro Matsunaga:**

Investigation. **Michiko Kimoto:** Supervision, Writing – review & editing. **Ichiro Hirao:** Supervision, Writing – review & editing. **Sergey N. Krylov:** Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known financial, professional, or personal conflicts of interest that could have influenced the work presented in this manuscript. No competing interests, such as financial relationships, consulting engagements, intellectual property, or other affiliations, have impacted the design, execution, or interpretation of this study.

Acknowledgements

The authors thank Dr. Dan Schneider and Dr. Brian Hicke of SomaLogic (now Illumina) for their support, insightful discussions, and valuable comments on the manuscript, which helped improve its clarity.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bios.2026.118875>.

Data availability

The raw and processed data has been published on FigShare: <https://doi.org/10.6084/m9.figshare.31029670>

References

- Balamurugan, S., Obubuafo, A., Soper, S.A., et al., 2008. Anal. Bioanal. Chem. 390, 1009–1021. <https://doi.org/10.1007/s00216-007-1587-2>.
- Bottari, F., Daems, E., De Vries, A.-M., et al., 2020. J. Am. Chem. Soc. 142, 19622–19630. <https://doi.org/10.1021/jacs.0c08691>.
- Catasti, P., Chen, X., Deaven, L.L., et al., 1997. J. Mol. Biol. 272, 369–382. <https://doi.org/10.1006/jmbi.1997.1248>.
- Chang, S.-C., Wang, C.-H., Cheng, K.-H., et al., 2025. Sens. Actuators, B Chem. 440, 137949. <https://doi.org/10.1016/j.snb.2025.137949>.
- Chen, T.-W., Wang, C.-H., Kuo, F.-C., et al., 2025. Sens. Actuators, B Chem. 424, 136932. <https://doi.org/10.1016/j.snb.2024.136932>.
- Ellington, A.D., Szostak, J.W., 1990. Nature 346, 818–822. <https://doi.org/10.1038/346818a0>.
- Frutiger, A., Tanno, A., Hwu, S., et al., 2021. Chem. Rev. 121, 8095–8160. <https://doi.org/10.1021/acs.chemrev.1c00044>.

- Gold, L., Ayers, D., Bertino, J., et al., 2010. PLoS One 5, e15004. <https://doi.org/10.1371/journal.pone.0015004>.
- Huang, C.-J., Lin, H.-I., Shiesh, S.-C., et al., 2010. Biosens. Bioelectron. 25, 1761–1766. <https://doi.org/10.1016/j.bios.2009.12.029>.
- Keefe, A.D., Pai, S., Ellington, A., 2010. Nat. Rev. Drug Discov. 9, 537–550. <https://doi.org/10.1038/nrd3141>.
- Kim, M., Um, H.-J., Bang, S., et al., 2009. Environ. Sci. Technol. 43, 9335–9340. <https://doi.org/10.1021/es902407g>.
- Krylov, S.N., Krylova, S.M., Le, A.T.H., et al., 2026. Biosens. Bioelectron. XX. <https://doi.org/10.1016/j.bios.2026.118807>. XXXX-XXXX.
- Le, A.T.H., Krylova, S.M., Krylov, S.N., 2026. ACS Sens. 11, 779–791. <https://doi.org/10.1021/acssensors.6c00130>.
- Le, Q.D., Le, A.T.H., Wang, T.Y., et al., 2025. Anal. Chem. 97, 18847–18853. <https://doi.org/10.1021/acs.analchem.5c03911>.
- Lichtenberg, J.Y., Ling, Y., Kim, S., 2019. Sensors 19, 2488. <https://doi.org/10.3390/s19112488>.
- Liu, Z., Luo, D., Ren, F., et al., 2019. RSC Adv. 9, 11960–11967. <https://doi.org/10.1039/C9RA01352K>.
- Lopez, A., Liu, J., 2021. Anal. Chem. 93, 3018–3025. <https://doi.org/10.1021/acs.analchem.0c05062>.
- Marttila, A.T., Laitinen, O.H., Airenne, K.J., et al., 2000. FEBS Lett. 467, 31–36. [https://doi.org/10.1016/S0014-5793\(00\)01119-4](https://doi.org/10.1016/S0014-5793(00)01119-4).
- McKeague, M., De Girolamo, A., Valenzano, S., et al., 2015. Anal. Chem. 87, 8608–8612. <https://doi.org/10.1021/acs.analchem.5b02102>.
- McKeague, M., DeRosa, M.C., 2012. J. Nucleic Acids 2012, 748913. <https://doi.org/10.1155/2012/748913>.
- Meyer, S.C., Gaj, T., Ghosh, I., 2006. Chem. Biol. Drug Des. 68, 3–10. <https://doi.org/10.1111/j.1747-0285.2006.00401.x>.
- Phung, N.L., Walter, J.G., Jonczyk, R., et al., 2020. ACS Comb. Sci. 22, 617–629. <https://doi.org/10.1021/acscmbosci.0c00080>.
- Pichon, V., Brothier, F., Combès, A., 2015. Anal. Bioanal. Chem. 407, 681–698. <https://doi.org/10.1007/s00216-014-8129-5>.
- Ruscito, A., DeRosa, M.C., 2016. Front. Chem. 4, 14. <https://doi.org/10.3389/fchem.2016.00014>.
- Salazar, J., Martínez, M.S., Chávez-Castillo, M., et al., 2014. Int. Sch. Res. Not. 2014, 653045. <https://doi.org/10.1155/2014/653045>.
- Shi, L., Jin, Y., Liu, J., 2023. Langmuir 39, 14774–14781. <https://doi.org/10.1021/acs.langmuir.3c02208>.
- Sinha, A., Tai, T.-Y., Li, K.-H., et al., 2019. Biosens. Bioelectron. 129, 155–163. <https://doi.org/10.1016/j.bios.2019.01.001>.
- Song, K.-M., Lee, S., Ban, C., 2012. Sensors 12, 612–631. <https://doi.org/10.3390/s120100612>.
- Stangherlin, S., Malloch, T., Clarke, A.J., et al., 2025. Angew. Chem. Int. Ed. 64, e202510518. <https://doi.org/10.1002/anie.202510518>.
- Tao, X., He, F., Liu, X., et al., 2020. Microchim. Acta 187, 668. <https://doi.org/10.1007/s00604-020-04644-6>.
- Thompson, D., Pepys, M.B., Wood, S.P., 1999. Structure 7, 169–177. [https://doi.org/10.1016/S0969-2126\(99\)80023-9](https://doi.org/10.1016/S0969-2126(99)80023-9).
- Tombelli, S., Minunni, M., Mascini, M., 2005. Biosens. Bioelectron. 20, 2424–2434. <https://doi.org/10.1016/j.bios.2004.11.006>.
- Tuerk, C., Gold, L., 1990. Science 249, 505–510. <https://doi.org/10.1126/science.2200121>.
- Villalonga, A., Sánchez, A., Vilela, D., et al., 2022. Microchim. Acta 189, 309. <https://doi.org/10.1007/s00604-022-05420-4>.
- Volanakis, J., 2001. Mol. Immunol. 38, 189–197. [https://doi.org/10.1016/S0161-5890\(01\)00042-6](https://doi.org/10.1016/S0161-5890(01)00042-6).
- Whitehouse, W.L., Lo, L.H.Y., Kinghorn, A.B., et al., 2024. ACS Appl. Bio Mater. 7, 3721–3730. <https://doi.org/10.1021/acsabm.4c00061>.
- Wu, N., Zandieh, M., Yang, T., et al., 2023. Anal. Chem. 95, 8948–8955. <https://doi.org/10.1021/acs.analchem.3c00954>.
- Zara, L., Achilli, S., Chovelon, B., et al., 2021. Anal. Chim. Acta 1159, 338382. <https://doi.org/10.1016/j.aca.2021.338382>.
- Zhang, R., Shao, H., Hu, X., et al., 2025. Sens. Actuators, B Chem. 431, 137408. <https://doi.org/10.1016/j.snb.2025.137408>.
- Zhang, F., Liu, J., 2021. Langmuir 37, 11939–11947. <https://doi.org/10.1021/acs.langmuir.1c02239>.
- Zong, C., Liu, J., 2019. Anal. Chem. 91, 10887–10893. <https://doi.org/10.1021/acs.analchem.9b02789>.

SUPPORTING INFORMATION

A Widely Used CRP Aptamer Does Not Bind CRP, Revealing a General Route to Surface-Driven Pseudoaffinity in Biosensors

Quan Duc Le,^{a,b} An Thi Hoai Le,^{a,b} Tong Ye Wang,^{a,b} Svetlana M. Krylova,^{a,b} Noah A. Clark,^c David Seghetti-Hebble,^c Ken-Ichiro Matsunaga,^d Michiko Kimoto,^d Ichiro Hirao,^d and Sergey N. Krylov^{a,b,*}

^aDepartment of Chemistry, York University, 4700 Keele Street, Toronto, Ontario M3J 1P3, Canada

^bCentre for Research on Biomolecular Interactions, York University, 4700 Keele Street, Toronto, Ontario M3J 1P3, Canada

^cIllumina, Inc., 2945 Wilderness Pl, Boulder, CO 80301, USA

^dXenolis Pte. Ltd., 85 Science Park Drive, #02-08 The Cavendish, Singapore, Republic of Singapore

*Corresponding author's email address: skrylov@yorku.ca

Table of Contents

Section S1. Calcium dependence of the SOMAmer-CRP complex demonstrated by EDTA “kill’ and Ca²⁺ “rescue” experiments.....	S2
Section S2. Oligo-Oligo Control Study and potential complementary regions between S49 and APT	S3
Section S3. Additional FA competitive assay with independently synthesized aptamer	S5
Section S4. Cautionary control: streptavidin planar chips report similar CRP kinetics/affinity regardless of immobilized oligonucleotide.....	S6
Section S5. Experimental section.....	S7
Supporting References.....	S8

Additional supplementary files

The following supplementary files can be found on Figshare: <https://doi.org/10.6084/m9.figshare.31029670>

File name	Description/Experiment
FA raw data.zip	Raw data from FA experiments and Origin files for data visualization in the main text and SI
NECEEM raw data.zip	Raw data from NECEEM experiments and Origin files for data visualization in the main text and SI
SPR raw data.zip	Raw data from SPR experiments and Origin files for data visualization in the main text and SI
Figure.zip	Figures in the main text and SI

Section S1. Calcium dependence of the SOMAmer-CRP complex demonstrated by EDTA “kill” and Ca^{2+} “rescue” experiments.

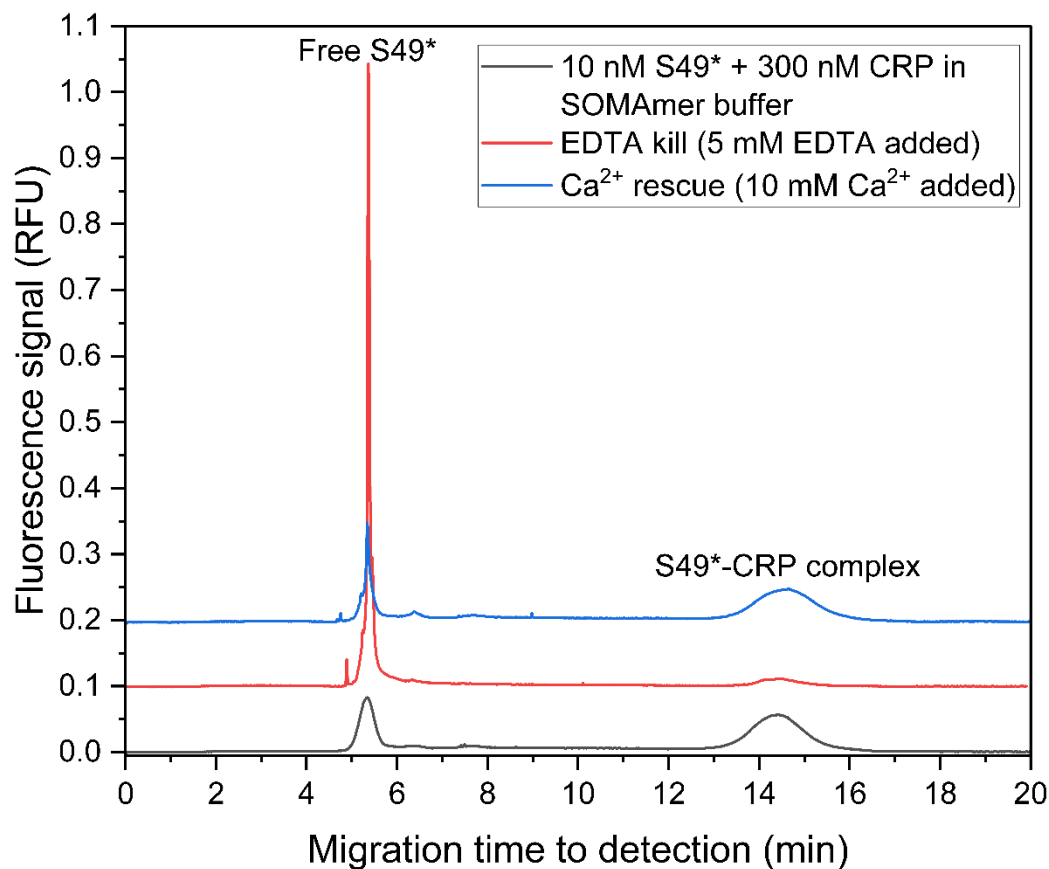


Figure S1. Calcium dependence of the SOMAmer–CRP complex demonstrated by EDTA “kill” and Ca^{2+} “rescue” experiments.

To confirm the calcium dependency of the SOMAmer–CRP interaction, a calcium chelation and rescue experiment was performed (Figure S1). Upon addition of 5 mM EDTA to the sample buffer, the S49*–CRP complex peak (red trace) decreased markedly, accompanied by a corresponding increase in the free S49* peak, indicating dissociation of the complex due to calcium removal. Subsequent addition of 10 mM Ca^{2+} successfully restored the S49*–CRP complex peak and reduced the free S49* signal back to baseline levels (blue trace). These results clearly demonstrate that the formation and stability of the SOMAmer–CRP complex are calcium-dependent, consistent with the known calcium requirement for CRP’s structural integrity and ligand-binding activity.

Section S2. Oligo-Oligo Control Study and potential complementary regions between S49 and APT

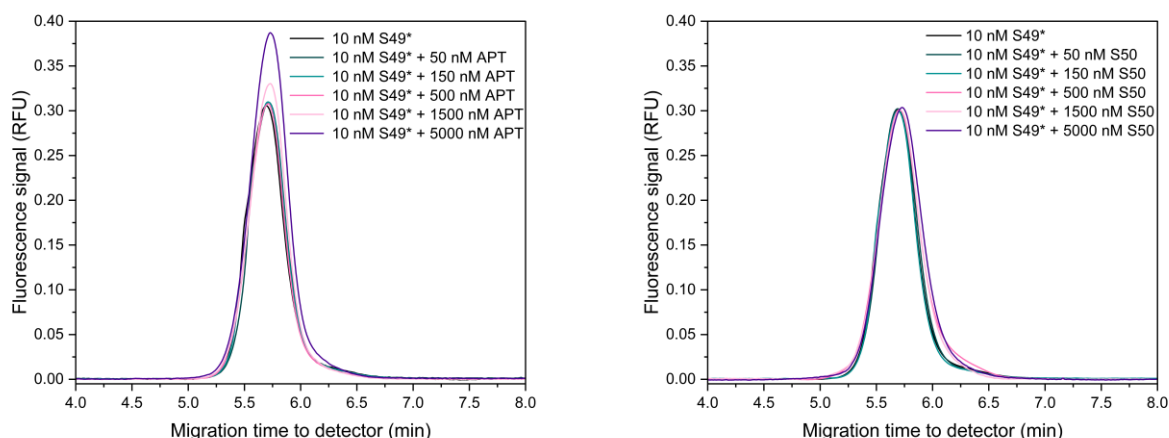


Figure S2. Oligo–oligo control for potential nonspecific interactions between SOMAmer and aptamer sequences.

To evaluate potential nonspecific hybridization between oligonucleotides, control experiments were conducted by mixing the fluorescently labeled SOMAmer (S49*) with increasing concentrations of either unlabeled aptamer (APT) or unlabeled SOMAmer (S50) in the absence of CRP (Figure S2). In the presence of 0–500 nM APT, the electropherograms showed that the S49* peak remained largely unchanged. However, at higher APT concentrations (1500 and 5000 nM), a slight increase in the free S49* peak was observed, consistent with the behavior seen in the competition assays. This increase likely results from transient or weak nonspecific interactions between S49* and APT. During electrophoretic separation, partial dissociation of transient S49*–APT complexes could release free S49*, which migrates within the free ligand zone, leading to an apparent increase in signal intensity despite a constant total S49* concentration. In contrast, increasing concentrations of unlabeled SOMAmer (S50) had no measurable effect on the S49* signal, indicating minimal or no duplex formation between the two SOMAMers under the tested conditions. We therefore view S49–APT heterodimer formation as a minor side reaction that can slightly perturb tracer behavior under extreme competitor conditions.

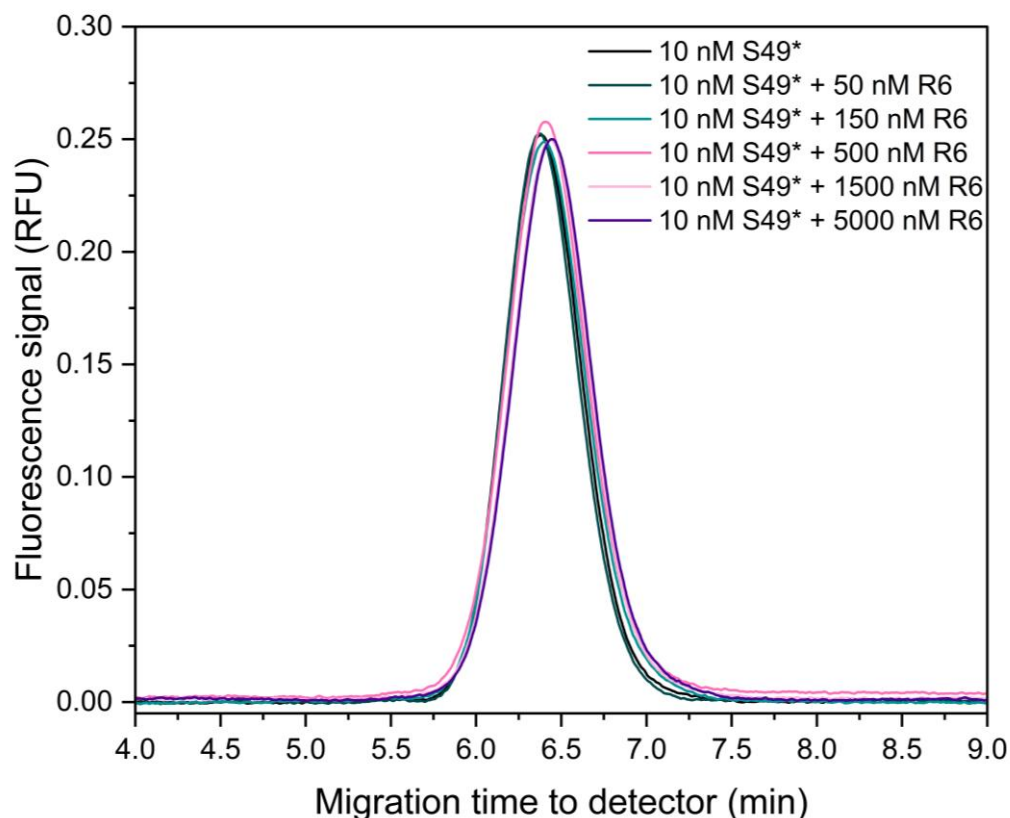


Figure S3. Oligo–oligo control with nonbinding aptamer (R6) to assess nonspecific interaction.

To further assess the specificity of oligonucleotide interactions, an additional control experiment was conducted using the R6 aptamer, a sequence of approximately the same length as the anti-CRP aptamer (APT) but lacking CRP-binding capability (Figure S3). The fluorescently labeled SOMAmer (S49*) was incubated with increasing concentrations of unlabeled R6 (50–5000 nM) in the absence of CRP. Across the entire concentration range, the electropherograms revealed no detectable change in the migration time or intensity of the S49* peak, indicating that the R6 aptamer did not interact with or perturb the labeled SOMAmer under the tested conditions. This result confirms that the minor signal changes observed in the S49*–APT series arise from sequence-specific or transient oligo–oligo interactions rather than nonspecific hybridization between unrelated DNA sequences.

Section S3. Additional FA competitive assay with independently synthesized aptamer

To test whether the absence of competition by the reported CRP aptamer could reflect a synthesis error or batch-specific impurity, we repeated the competitive FA assay using two independently synthesized preparations of the aptamer. The original sequence was ordered from Integrated DNA Technologies (APT1) and from Bio Basic (APT2), and each was titrated as an unlabeled competitor against the fluorescent S49*–CRP complex in both SOMAmer and aptamer buffers. In both buffers, S50 produced the expected concentration-dependent decrease in anisotropy, consistent with efficient displacement of S49* from CRP. In contrast, neither APT1 nor APT2 measurably reduced the FA signal even at 500 nM competitor (Figure S4). The small upward drift in FA observed at the highest APT concentrations remained within the experimental variability and did not approach the free S49* baseline. These data suggest that the lack of competition is not due to a synthesis error in a single vendor lot, and further support the conclusion that the reported CRP aptamer does not bind CRP detectably under our solution conditions.

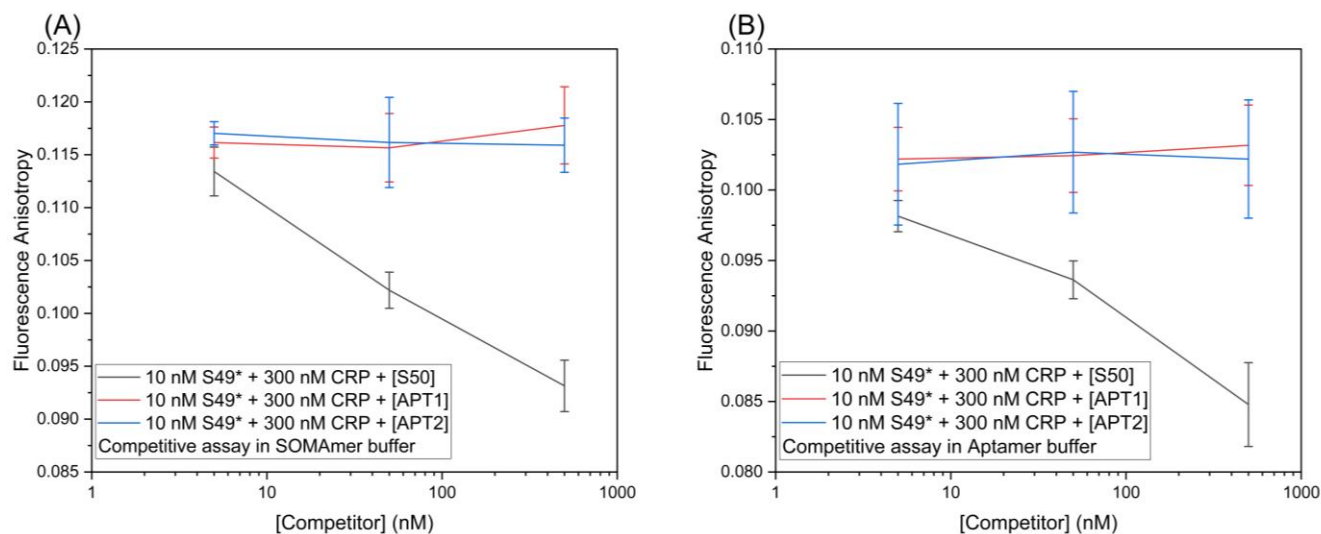


Figure S4. Competitive fluorescence anisotropy assay with independently synthesized CRP aptamer. Competitive assays were performed with 10 nM S49* + 300 nM CRP while titrating unlabeled competitor (S50, APT1 and APT2) in SOMAmer buffer (A) or aptamer buffer (B).

Section S4. Cautionary control: streptavidin planar chips report similar CRP kinetics/affinity regardless of immobilized oligonucleotide

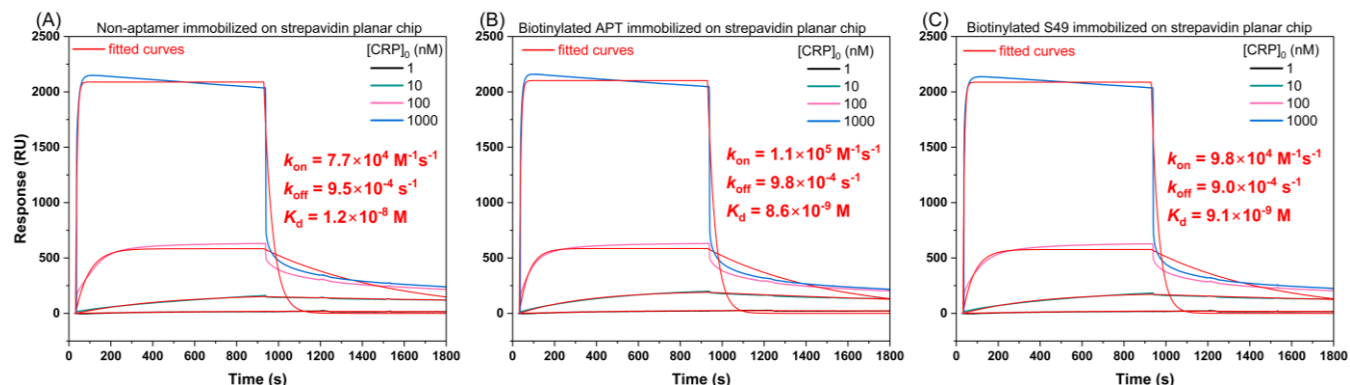


Figure S5. SPR sensorgrams for CRP injected at 1, 10, 100, and 1000 nM over (A) a non-aptamer control surface, (B) biotinylated APT, and (C) biotinylated S49 captured on a streptavidin planar chip. Similar responses and kinetics across all surfaces indicate strong non-specific binding of CRP to the streptavidin/dextran matrix.

To determine whether the SPR responses observed on streptavidin planar chips reflect specific oligonucleotide–CRP interactions or adsorption to the chip matrix, we compared CRP sensorgrams collected on (i) a non-aptamer control surface, (ii) biotinylated APT, and (iii) biotinylated S49 immobilized via streptavidin on the same planar chip format (Figure S5). CRP was injected over each surface at 1, 10, 100, and 1000 nM.

Across all three surfaces, CRP produced large, concentration-dependent binding signals with similar association and dissociation behavior, including substantial responses even on the non-aptamer control surface (Figure S5A). Notably, the response magnitude at 1000 nM CRP approached the same high level (~2,000 RU) on the control, APT-, and S49-functionalized surfaces (Figure S5A–C). The close similarity of the sensorgrams indicates that the dominant contribution to the signal arises from non-specific interactions between CRP and the streptavidin/dextran chip matrix rather than sequence-specific recognition by the immobilized oligonucleotide.

Consistent with this interpretation, global fitting of the sensorgrams to a 1:1 Langmuir model yielded comparable apparent kinetic parameters for the control surface and for both immobilized oligonucleotides (Figure S5, red text). Because a strong response is already present in the absence of an immobilized binder, these fitted parameters should be regarded as effective (matrix-dominated) adsorption/desorption kinetics rather than true molecular binding constants for APT–CRP or S49–CRP. Collectively, these experiments demonstrate that the streptavidin planar (dextran-containing) chip format supports substantial non-specific CRP binding, motivating the use of alternative surface chemistries with reduced matrix interactions for quantitative SPR validation.

Section S5. Experimental section

Chemicals and Materials. All chemicals were purchased from Sigma-Aldrich (Oakville, ON, Canada) unless otherwise stated. Fused-silica capillaries with inner and outer diameters of 75 and 360 μm , respectively, were obtained from Molex Polymicro (Phoenix, AZ, USA). Human C-reactive protein (CRP), consisting of five identical non-glycosylated subunits of 206 amino acids each (M.W. $\approx$ 23 kDa per subunit), was purchased from Sigma-Aldrich (Oakville, ON, Canada). A 5'-Cy3-labeled SOMAmer S49* (SL1124; cyanine-3-4337-49-2) and an unlabeled SOMAmer S50 (SL1127 AB-H-4337-50-1) targeting CRP were provided by SomaLogic (Boulder, CO, USA). The nucleotide sequences and chemical modifications of these SOMAmer reagents are proprietary and therefore cannot be disclosed here; to ensure traceability, the exact SomaLogic reagent identifiers and labeling/modification codes are provided.

The specific unlabeled anti-CRP aptamer (APT) and an Alexa Fluor 488-labeled (APT*) were synthesized by Integrated DNA Technologies (Coralville, IA, USA). The aptamer sequence was 5'-GGC AGG AAG ACA AAC ACG ATG GGG GGG TAT GAT TTG ATG TGG TTG TTG CAT GAT CGT GGT CTG TGG TGC TGT-3' (Huang et al., 2010). To minimize the probability of synthesis error, the same sequence was also ordered from another vendor, BioBasic (Markham, ON, Canada). All fluorescence-labeled ligands in this study are denoted by an asterisk (e.g., S49*, APT*).

Before dilution and preparation of equilibrium mixtures, all SOMAmer and aptamer stock solutions were thermally treated by melting and reannealing to ensure proper folding. Specifically, oligonucleotide solutions were heated at 90 $^{\circ}\text{C}$ for 2 min and then cooled to 20 $^{\circ}\text{C}$ at a rate of 0.5 $^{\circ}\text{C s}^{-1}$. The binding assay was performed in two different buffer systems: the SOMAmer buffer (40 mM HEPES, 1 mM CaCl_2 , 1 mM MgCl_2 , pH 7.5) and the aptamer buffer (10 mM Tris-HCl, 50 mM NaCl, 2 mM CaCl_2 , pH 7.5) (Huang et al., 2010; Le et al., 2025). For the CE experiments, the sample buffer was always identical to the running buffer to prevent artifacts arising from buffer mismatch. Accordingly, all dilutions of sample components were made using the same running buffer employed for CE separation. Under these buffer conditions, C-reactive protein (CRP) remains predominantly in its native pentameric form, consistent with previous reports demonstrating its structural stability in calcium-containing near-physiological buffers.

Capillary Electrophoresis experiments. All CE experiments were performed using a P/ACE MDQ system (SCIEX, Concord, ON, Canada) equipped with a laser-induced fluorescence (LIF) detection module. Fluorescence excitation was achieved using a 488 nm solid-state laser, and emission was detected at 595 nm for Cy3-labeled SOMAmer S49 and at 520 nm for Alexa-labeled aptamer, using spectrally optimized filter sets. Poly (vinyl alcohol) (PVA)-coated fused-silica capillaries were employed throughout this work. For experiments in SOMAmer buffer, capillaries of 50 cm total length (40 cm effective length) were used, while those in the aptamer buffer employed shorter capillaries of 30 cm total length (20 cm effective length) to minimize analysis time due to longer migration times in high-salt conditions. The coating procedure for the PVA capillaries has been described previously (De Jong and Krylov, 2012).

Prior to each run, the capillary was rinsed with the running buffer at 20 psi (138 kPa) for 4 min. Separations were performed at an electric field strength of 500 V/cm (25 kV across 50 cm or 15 kV across 30 cm), with the negative electrode positioned at the injection end. Equilibrium mixtures containing CRP and SOMAmer were first incubated for 30 min at room temperature to allow complex formation, followed by the addition of the competitive ligand (i.e., S50 or APT) and an additional 30 min incubation prior to injection. Samples were introduced hydrodynamically by applying a 0.5 psi ($\sim$ 3.5 kPa) pressure pulse for 10 s, corresponding to a 10 mm sample plug. This was followed by the injection of a 5.7 cm buffer plug using a 0.9 psi ($\sim$ 6.2 kPa) pressure pulse for 30 s to advance the sample through the uncooled inlet section. The capillary temperature was maintained at 15 $^{\circ}\text{C}$ throughout the experiments.

Fluorescence Anisotropy experiments. Assays were conducted in low-binding, black, flat-bottom 384-well microplates (Corning® 384 Well Microplate, Millipore Sigma, Toronto, ON, Canada) to minimize background signal and sample adsorption. Plates were sealed with optically clear film during measurements to reduce evaporation and photobleaching, and fluorescence anisotropy signals were recorded using a Synergy H4 Hybrid Microplate Reader (BioTek, Winooski, VT, USA) equipped with polarized optics. Cy3-labeled probes were excited at 485/20 nm and emission was collected at 590/35 nm, with instrument gain auto-optimized before each run. All measurements were performed at 25 $^{\circ}\text{C}$.

Surface Plasma Resonance experiments. All SPR experiments were performed at the same conditions as provided in the original aptamer-selection paper (Huang et al., 2010). A subset of measurements was carried out at Xenolis using a Biacore T200 instrument (Cytiva, Uppsala, Sweden). For nonspecific binding control assays, a CM5 sensor chip was functionalized with streptavidin via standard amine-coupling chemistry; no aptamer or SOMAmer ligand was immobilized on these surfaces. Similar streptavidin-derived assays were also prepared at Illumina (Boulder, CO, USA) using a Carterra LSA^{XT} HT-SPR instrument (Carterra, Salt Lake City, UT, USA) with a SAP sensor chip and the same ligand-free format.

To avoid any non-specific streptavidin-mediated interactions during ligand-dependent binding studies, Illumina additionally prepared a CMDP sensor chip with NeutrAvidin via standard amine coupling chemistry. In detail, a 5 mm planar CMDP chip (Carterra) was functionalized with NeutrAvidin (Pierce) using the lawn surface preparation wizard. The carboxymethyl dextran surface was activated using freshly prepared 100 mM EDC and 25 mM s-NHS in 100 mM MES, pH 5.5, followed by coupling of 50 $\mu\text{g/mL}$ NeutrAvidin in 10 mM sodium acetate, pH 4.5 (Carterra). Uncoupled groups were quenched with ethanolamine. The average coupled NeutrAvidin level was approximately 1800 RU per region of interest (ROI). An array of 192 ligands, mostly SOMAmer reagents along with “APT” and chip controls, was captured in duplicate to occupy all 384 ROIs using the Capture Kinetics wizard (Carterra). S49* and APT were immobilized for 15 minutes in quadruplicate at each of three concentrations (2.5 nM, 1.0 nM, and 0.5 nM) to ensure a condition with ideal loading density. In this format, biotinylated aptamers or SOMAmer reagents were captured at low surface density, and CRP binding was measured using the same running

buffer conditions, plus 800 nM Z-Block, a proprietary modified oligonucleotide used as a polyanionic competitor, allowing evaluation of ligand-mediated binding with reduced nonspecific interference (Gold et al., 2010).

Supporting References

- De Jong, S., Krylov, S.N., 2012. *Anal. Chem.* 84, 453-458. <https://doi.org/10.1021/ac2030333>
- Gold, L., Ayers, D., Bertino, J., et al., 2010. *PLoS ONE* 5, e15004. <https://doi.org/10.1371/journal.pone.0015004>
- Huang, C.-J., Lin, H.-I., Shiesh, S.-C., et al., 2010. *Biosens. Bioelectron.* 25, 1761-1766. <https://doi.org/10.1016/j.bios.2009.12.029>
- Le, Q.D., Le, A.T.H., Wang, T.Y., Krylova, S.M., Krylov, S.N., 2025. *Anal. Chem.* 97, 18847-18853. <https://doi.org/10.1021/acs.analchem.5c03911>