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Detection of IP-10 protein marker in undiluted blood serum via an electrochemical E-DNA scaffold sensor

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Abstract

We describe an electrochemical analog of fluorescence polarization that supports the quantitative measurement of a specific protein, the chemokine IP-10, directly in undiluted blood serum. The sensor is label-free, wash-free, and electronic, suggesting it could support point-of-care detection of diagnostic proteins in largely unprocessed clinical samples.

1. Introduction

A fundamental axiom of medicine is that faster diagnosis permits earlier intervention, ultimately improving outcomes. The vast majority of current molecular diagnostics, however, are slow, cumbersome, laboratory-bound processes that require hours or even days to return clinically actionably information. The development of effective point-of-care (POC) diagnostics that instead return an answer during the 15 minutes of a typical patient/provider interaction would thus accrue significant benefits, such as decreasing the lag between diagnosis and treatment, improve on-going monitoring, and strengthen screening efforts[1–3].

As many important biomarkers are proteins, the above arguments would suggest that quantitative, point-of-care protein detection would be of significant value. Despite this need, however, the only quantitative protein detection technology to have achieved significant point-of-care penetration is fluorescence polarization (FP)[4, 5]. These assays, which report on the presence of a target antigen via binding-induced changes in the tumbling of a fluorophore-modified antibody, offer a number of advantages in POC applications as they do not require repetitive wash steps to remove unbound reagents and their signal is independent of the concentration of the fluorescent reagent or the absolute fluorescence of the sample. Despite these attributes, FP remains limited to only the most valuable, time-sensitive analytes for several reasons[6–8, 1, 2]. First, FP requires careful background subtraction and signal averaging, as the intensity difference between the two polarizations is only ~15% for a typical antibody-antigen complex, and must be measured against background polarizations typically ranging from 5 to 10% [e.g., refs[9–12]]. As well, FP requires venous blood draws for sufficient sample volume and does not easily support

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multiplexing. Most importantly, due to absorbance and scattering, FP fails entirely when challenged with whole blood, and even serum must be diluted significantly (typically 1:100) prior to measurement[12, 10].

In response to the limitations of FP, we have developed an electrochemical analog that retains the approach's positive attributes whilst avoiding its weaknesses. Our strategy uses a versatile electrochemical platform, termed E-DNA[13] (electrochemical, DNA), that couples binding-induced changes in the conformation, flexibility or steric bulk of an electrode-bound DNA probe with a concomitant change in electron transfer from an attached redox reporter[14]. The platform is reagentless, single-step and selective enough to deploy in crude clinical samples, such as blood serum and saliva[15]. It is also easily multiplexed (even for small sample volumes), driven by inexpensive, hand-held electronics[16], reusable (in most implementations)[17–19], and readily adaptable to microfluidic and multiplexed platforms[20], suggesting it is well suited for point-of-care applications.

We have previously shown that, by linking an antigenic peptide epitope or small-molecule hapten to the DNA probe, the E-DNA platform supports the relatively straightforward detection of antibodies[21–23]. In this application, antibody binding is signalled by a large change in redox current, presumably because the efficiency with which the attached redox reporter approaches the electrode is reduced by the high molecular weight (~150 kDa) and significant steric bulk of the target[24]. Further exploring this signalling mechanism[25–27], we extend it here to the reagentless, electrochemical detection of a much lower molecular weight, *non-antibody* target. Specifically, we have fabricated a sensor against the 10 kDa chemokine IP-10, a secreted chemo-attractant that is a biomarker for the diagnosis of inflammation[28]. For example, IP-10 (Interferon-γ inducible Protein-10 kDa; also known as CXCL10) has been shown to be a sensitive and specific biomarker for kidney allograft rejection, with blood levels that increase up to 30-fold during acute rejection episodes[29–32]. Of note, while kidney allograft rejection rates have decreased in recent decades, acute rejection episodes are still observed in 10-30% of first kidney transplants[33] and are essentially asymptomatic until extensive kidney damage has occurred. Given this difficulty, serum creatinine levels, which are indicative of renal function, have been used as a non-invasive indicator of rejection episodes but by the time immune mediated graft rejection leads to elevated creatinine levels, the graft injury is extensive[34]. Because of this, biopsy is considered the gold standard diagnostic for rejection, but this highly invasive process carries a risk of graft injury, bleeding and even graft loss. The ability to monitor IP-10 levels at the point of care could thus provide a non-invasive yet highly accurate means for the timely detection of graft injury before it otherwise clinically manifests.

2. Materials and Methods

2.1. Sensor Preparation

Sensors were prepared on gold disk electrodes (1 mm^2 surface area, CH Instruments), which were physically polished and electrochemically cleaned as previously described[35]. Our biosensor consists of this gold electrode surface coated with a self-assembled monolayer of thiol-gold bound DNA anchor strands (sequence 5'- thiol – GCA GTA ACA AGA ATA AAA CGC CAC TGC - methylene blue -3', Biosearch) and backfilled 1-mercapto-6-hexanol (Sigma Aldrich)[35]. After preparation, the modified electrodes were immersed for 1 hour in hybridization buffer (10 mM potassium phosphate, 1 M sodium chloride, pH 7) containing 100 nM of the peptide-PNA chimera (see below). After hybridization, the sensors were briefly washed with hybridization buffer, then placed into buffer or serum for >30 min before titration with increasing amounts of IP-10. For experiments in buffer, 1x phosphate buffered saline (10 mM phosphate buffer, 0.154 M NaCl, pH 7.4) was used as received (Sigma Aldrich). For testing in blood serum, undiluted fetal calf serum was used as received

(Sigma Aldrich). In either media, unlabeled recombinant IP-10 (Cell Science) was titrated into the solution at final concentrations from 1 nM to 2 μM. Centrifugal filtration to concentrate supplied IP-10 for higher concentration trials resulted in significant insolubility and was not further pursued. At each step the sensor was equilibrated followed by electrochemical measurement.

2.2. Peptide-chimera generation

The peptide-PNA chimera was based on a peptide fragment known to interact with IP-10, is derived from amino acid residues 22-42 of human CXCR3 isoform A (with cysteine residues at positions 37 and 38 replaced with serine[31]) and was prepared by automated chemical synthesis of the peptide and PNA segments individually (Panagene), followed by crosslinking using Succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate ("linker") to yield the final sequence: NFS SSY DYG ENE SDS SST SP C-(linker)-cag tgg cgt ttt att ctt gtt act g –$CONH_2$; where upper-case letters denote the peptide amino acid sequence and lower-case letters denote PNA nucleobases.

2.3. Electrochemistry parameters

Electrochemical scanning was performed using square wave voltammetry on a CH Instruments 650C potentiostat, with a platinum wire counter electrode and a standard Ag/AgCl reference electrode (CH Instruments). The square wave voltammetry parameters were amplitude 50 mV, frequency 60 Hz, scanning from 0 V to -0.5 V at a rate of 1 mV/s. Methylene blue redox electron transfer yields a response current peak at ~ −0.25 V, and differences in this peak current from background measurement of the equilibrated sensor were used to calculate signal change.

3. Results and Discussion

To detect IP-10 we have employed a 21-residue polypeptide binding element derived from CXCR3, one of the chemokine's naturally occurring receptors[31], as a recognition element. This was grafted onto a peptide-nucleic-acid strand that, in turn, is hybridized to a methylene-blue-modified DNA strand attached to a gold electrode via a terminal thiol group. The binding of IP-10 to this recognition element reduces current from the attached methylene blue, resulting in a reduction in current transfer that is readily observed using square wave voltammetry as a reduction in peak current (Figure 1).

The IP-10 sensor rapidly and reproducibly achieves the specific detection of its target protein at low nanomolar concentrations (Figure 2 and a time-course of repeated scans in Supplemental Data). Although this limit of detection falls short of the ~60 pM IP-10 levels observed during episodes of acute rejection[30], it is nevertheless well below the ~200 nM dissociation constant of this peptide for the chemokine, suggesting that the platform is limited by the affinity of its recognition element. The signal change we observe at the highest IP-10 concentrations we have employed is approximately 30%. The fact that the signal change does not reach 100% at saturating target concentrations presumably arises due to one or both of two effects: 1) non-trivial electron transfer from even the bound probe (which is perhaps unavoidable, due to the relatively small size of IP-10) and 2) a population of scaffolds that are inaccessible for binding (e.g., due to anchor strands adherent on the surface)[36].

The sensor is specific and selective. For example, the IP-10 sensor does not respond when challenged with an antibody directed against the FLAG epitope even when challenged at 500 nM (Figure 3). A control sensor displaying the FLAG epitope[21], in contrast, readily detects its target (the anti-FLAG antibody) while, similarly, not responding to 500 nM

IP-10. The sensor is also highly selective and works well even when deployed directly in undiluted blood serum. Under these conditions, however, the magnitude of observed signal change is half that seen in buffer. This decrease in signalling efficiency has been noted in prior E-DNA sensors when similarly challenged, and we theorize that this difference may arise because of non-specific serum component binding to the sensor surface as well as reduced probe-target affinity under these conditions[37–39]. Unfortunately, however, IP-10 precipitates at concentrations much above 2 μM, rendering it difficult for us to test this hypothesis.

4. Conclusions

Previously we have demonstrated the utility of the E-DNA "scaffold" approach for the detection of multiple specific antibodies[21]. Antibodies, however, are quite high molecular weight and have very high affinity interactions with epitopes, rendering them relatively easy to detect with approaches, such as this, that rely on binding-induced changes in tumbling, diffusion or other dynamical or mass-based properties. Here, however, we have shown that the scaffold approach meaningfully supports the sensitive detection of a protein less than $1/10^{th}$ the size of an antibody. This work thus illustrates that, like fluorescence polarization, the E-DNA platform is versatile and broadly applicable to the detection of a variety of protein targets. Moreover, the unlike fluorescence polarization E-DNA sensors function well even in unprocessed, undiluted blood serum, are electronic and thus can be easily incorporated into multiplexed systems and point-of-care platforms. Additionally, recent work has enabled robust strategies for the generation of peptides directed at arbitrary targets[40, 41]. Thus, the general approach introduced here, of finding a protein-protein interaction (such as that between IP-10 and it's receptor CXCR3) and using that as the sensor element of an E-DNA scaffold, represents a powerful means to readily generate biosensors suitable for diagnostic purposes against a variety of targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Notes and references

1. Wang J. Biosensors and Bioelectronics. 2006; 21:1887–1892. [PubMed: 16330202]
2. Soper SA, Brown K, Ellington A, Frazier B, Garcia-Manero G, Gau V, Gutman SI, Hayes DF, Korte B, Landers JL, Larson D, Ligler F, Majumdar A, Mascini M, Nolte D, Rosenzweig Z, Wang J, Wilson D. Biosensors and Bioelectronics. 2006; 21:1932–1942. [PubMed: 16473506]
3. Urdea M, Penny LA, Olmsted SS, Giovanni MY, Kaspar P, Shepherd A, Wilson P, Dahl CA, Buchsbaum S, Moeller G, Hay Burgess DC. Nature. 2006; 444(Suppl):73–79. [PubMed: 17159896]
4. Melanson SE. Point of Care. 2005; 4:123–127.
5. Von Lode P. Clinical biochemistry. 2005; 38:591–606. [PubMed: 16009140]
6. Pechard A, Besson AS, Mialon A, Berny C, Manchon M. Annales de Biologie Clinique. 1999; 57:525–537. [PubMed: 10518054]
7. Huckle D. Expert Review of Medical Devices. 2006; 3:421–426. [PubMed: 16866639]
8. Estacio PL. Academic Emergency Medicine. 2006; 13:1135–1137. [PubMed: 17085739]
9. Dandliker WB, Kelly RJ, Dandliker J, Farquhar J, Levin J. Immunochemistry. 1973; 10:219–227. [PubMed: 4580370]

10. Tencza SB, Islam KR, Kalia V, Nasir MS, Jolley ME, Montelaro RC. J. Clin. Microbiol. 2000; 38:1854–1859. [PubMed: 10790112]
11. Lucero NE. Journal of Medical Microbiology. 2003; 52:883–887. [PubMed: 12972582]
12. Furzer GS, Veldhuis L, Hall JC. Journal of agricultural and food chemistry. 2006; 54:688–693. [PubMed: 16448169]
13. Xiao Y, Lai RY, Plaxco KW. Nat Protoc. 2007; 2:2875–2880. [PubMed: 18007622]
14. Xiao Y, Uzawa T, White RJ, Demartini D, Plaxco KW. Electroanalysis. 2009; 21:1267–1271. [PubMed: 20436787]
15. Cash K, Ricci F, Plaxco K. Journal of the American Chemical. 2009; 131:6955–6957.
16. Rowe AA, Bonham AJ, White RJ, Zimmer MP, Yadgar RJ, Hobza TM, Honea JW, Ben-Yaacov I, Plaxco KW. PloS one. 2011; 6:e23783. [PubMed: 21931613]
17. Lubin AA, Lai RY, Baker BR, Heeger AJ, Plaxco KW. Anal Chem. 2006; 78:5671–5677. ST – Sequence–specific, electronic detecti. [PubMed: 16906710]
18. Ricci F, Plaxco KW. Microchimica Acta. 2008; 163:149–155.
19. Cash KJ, Ricci F, Plaxco KW. J Am Chem Soc. 2009; 131:6955–6957. [PubMed: 19413316]
20. Swensen JS, Xiao Y, Ferguson BS, Lubin AA, Lai RY, Heeger AJ, Plaxco KW, Soh HT. J Am Chem Soc. 2009; 131:4262–4266. [PubMed: 19271708]
21. White RJ, Kallewaard HM, Hsieh K, Patterson AS, Kasehagen JB, Cash KJ, Uzawa T, Soh HT, Plaxco KW. Analytical chemistry. 2011; 84:1098–1103. [PubMed: 22145706]
22. Cash KJ, Ricci F, Plaxco KW. Chem Commun (Camb). 2009:6222–6224. ST – A general electrochemical method for. [PubMed: 19826675]
23. Cash KJ, Ricci F, Plaxco KW. J Am Chem Soc. 2009; 131:6955–6957. [PubMed: 19413316]
24. Uzawa T, Cheng RR, White RJ, Makarov DE, Plaxco KW. Journal of the American Chemical Society. 2010; 132:16120–16126. [PubMed: 20964337]
25. Xiao Y, Uzawa T, White RJ, Demartini D, Plaxco KW. Electroanalysis. 2009; 21:1267–1271. [PubMed: 20436787]
26. Ricci F, Bonham AJ, Mason AC, Reich NO, Plaxco KW. Anal Chem. 2009; 81:1608–1614. ST – Reagentless, electrochemical approac. [PubMed: 19199570]
27. White RJ, Phares N, Lubin AA, Xiao Y, Plaxco KW. Langmuir. 2008:10513–10518. ST – Optimization of electrochemical. [PubMed: 18690727]
28. El Sawy T, Fahmy NM, Fairchild RL. Current Opinion in Immunology. 2002; 14:562–568. [PubMed: 12183154]
29. Kanmaz T, Feng P, Torrealba J, Kwun J, Fechner JH, Schultz JM, Dong Y, Kim HT, Dar W, Hamawy MM, Knechtle SJ, Hu H. Transplantation. 2004; 78:1002–1007. [PubMed: 15480165]
30. Hu H, Brian DA, Alice P, Jeanine AB, Majed MH, Stuart JK. American Journal of Transplantation. 2004; 4:432–437. [PubMed: 14961998]
31. Booth V, Keizer DW, Kamphuis MB, Clark-Lewis I, Sykes BD. Biochemistry. 2002; 41:10418–10425. [PubMed: 12173928]
32. Melter M, Exeni A, Reinders MEJ, Fang JC, McMahon G, Ganz P, Hancock WW, Briscoe DM. Circulation. 2001; 104:2558–2564. [PubMed: 11714650]
33. Bodziak, KA.; Hricik, DE. Kidney Transplantation. Hricik, DE.; Remedica, editors. 2003. p. 1-12.
34. Rush D, Somorjai R, Deslauriers R, Shaw A, Jeffery J, Nickerson P. Annals of transplantation. 2000; 5:44–49. [PubMed: 11217206]
35. Rowe AA, White RJ, Bonham AJ, Plaxco KW. Journal of Visualized Experiments. 2011; 52:e2922.
36. Biagiotti V, Porchetta A, Desiderati S, Plaxco KW, Palleschi G, Ricci F. Analytical and Bioanalytical Chemistry. 2012; 402:413–421. [PubMed: 21928081]
37. Baker BR, Lai RY, Wood MS, Doctor EH, Heeger AJ, Plaxco KW. Journal of the American Chemical Society. 2006; 128:3138–3139. [PubMed: 16522082]
38. Xiao Y, Rowe AA, Plaxco KW. Journal of the American Chemical Society. 2006; 129:262–263. ST – Electrochemical Detection of Parts–P. [PubMed: 17212391]

39. Xiao Y, Lubin AA, Heeger AJ, Plaxco KW. Angew Chem Int Ed Engl. 2005; 44:5456–5459. ST – Label–free electronic detection of th. [PubMed: 16044476]

40. Little LE, Dane KY, Daugherty PS, Healy KE, Schaffer DV. Biomaterials. 32:1484–1494. [PubMed: 21129772]

41. Dane KY, Gottstein C, Daugherty PS. Molecular cancer therapeutics. 2009; 8:1312–1318. [PubMed: 19417151]

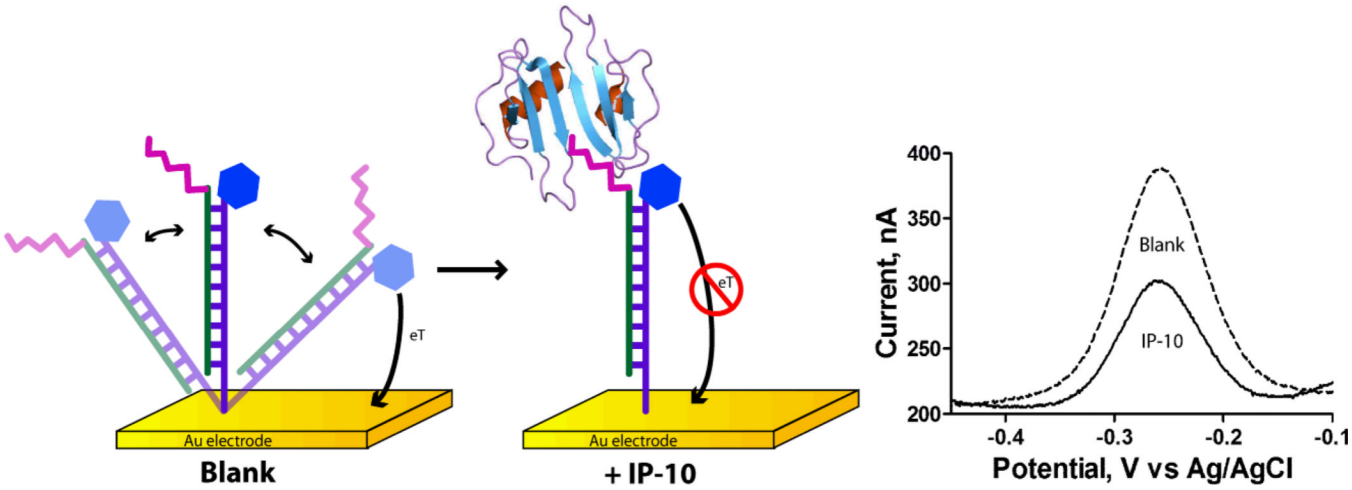

Fig. 1. An E-DNA scaffold electrochemical sensor for the detection of the chemokine IP-10

A DNA anchor strand (purple) with a distal methylene blue redox reporter (blue hexagon) is attached via thiol-on-gold self-assembled monolayer chemistry to an interrogating electrode and then hybridized to a PNA recognition strand (green) covalently linked to an IP-10 binding peptide motif (pink). In the absence of IP-10 a significant current is observed, presumably because the methylene blue is free to collide with the electrode, resulting in efficient electron transfer. Upon IP-10 binding this current is reduced, presumably because the steric bulk of the target protein reduces the efficiency with which the methylene blue can approach the electrode.

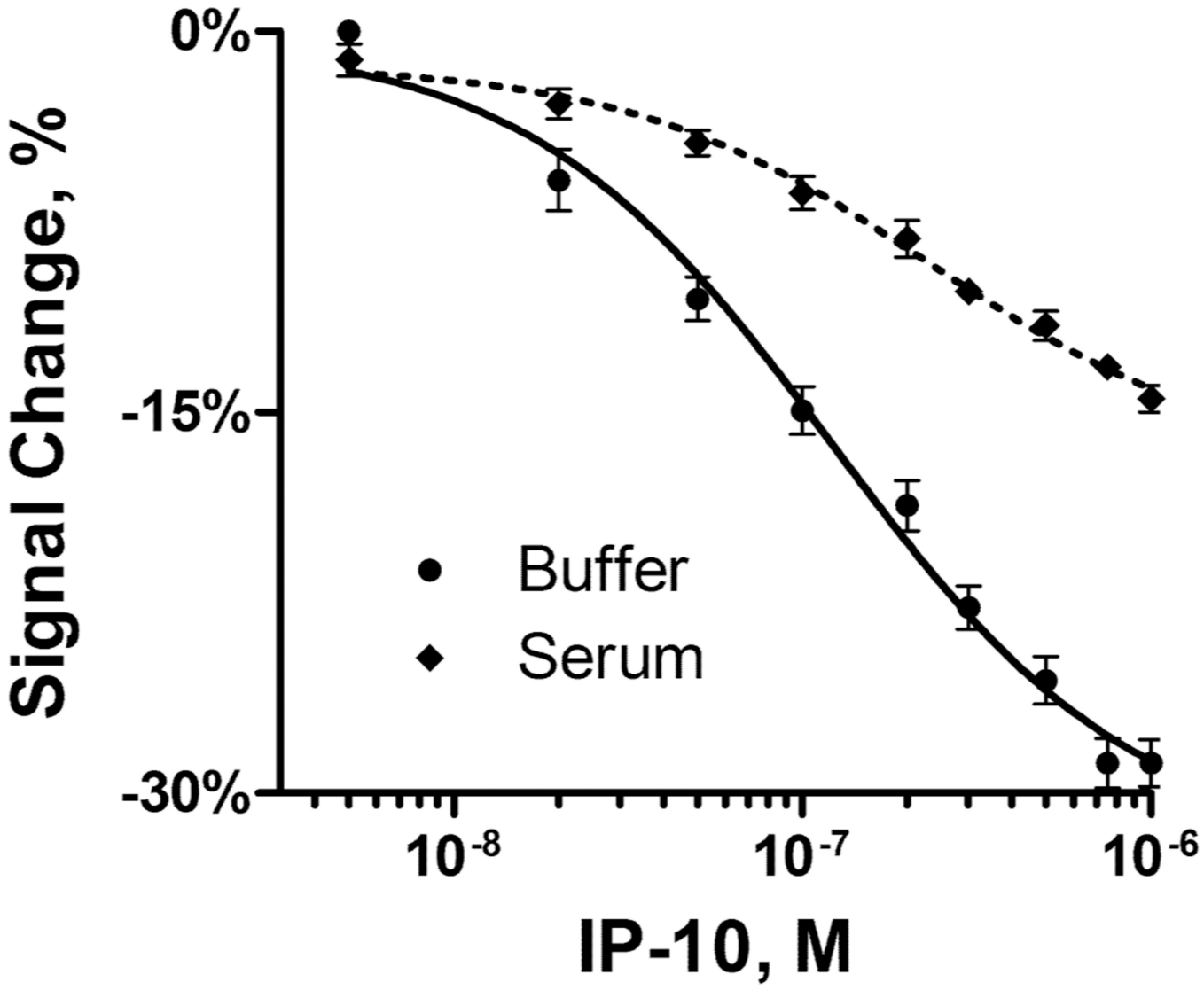

Fig. 2.
Addition of IP-10 gives a robust, quantitative response both in buffer and in undiluted blood serum to the highest target concentration we can achieve under our experimental conditions. Error shown is standard deviation from n > 4 independent trials.

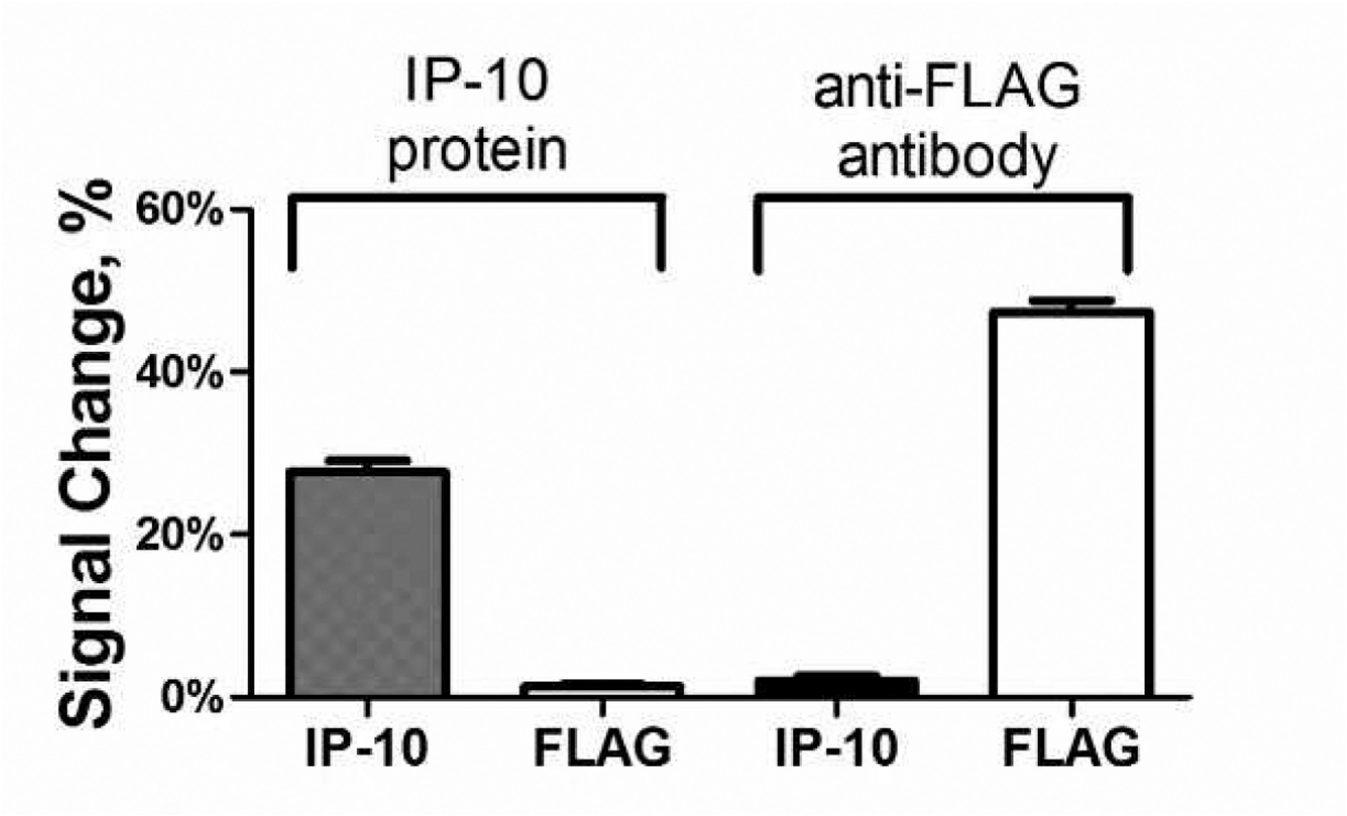

Fig. 3.
Scaffold sensors directed against IP-10 and the FLAG antibody display selective signal response in buffer against cross-target interactions, even when challenged at 500 nM target.