Peptide-DNA scaffold for the electrochemical detection of the chemokine IP-10 in undiluted blood serum

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We report a reagentless, electrochemical sensor for the detection of the diagnostic chemokine IP-10 (CXCL10) employing a short polypeptide as its recognition element. An electrode-tethered, redox-reporter-tagged DNA/PNA heteroduplex serves as a rigid scaffold presenting this peptide. Binding of the chemokine to the peptide reduces the efficiency with which the redox tag approaches the electrode, producing a readily measurable change in current and allowing the sensor to rapidly and selectively detect its target protein directly in unmodified, undiluted blood serum. This design is also reusable, label-free, and electronic, suggesting it could support the point-of-care detection of diagnostic proteins in largely unprocessed clinical samples.

Chemokines, small, secreted cytokines with chemoattractant and proinflammatory properties, have been identified as potentially promising biomarkers for the diagnosis of inflammation, such as that seen in acute transplant rejection[1]. The chemokine IP-10 (Interferon-γ inducible Protein-10 kDa) is a CXCL class chemokine (thus its alternate name: CXCL10) involved in the rejection of solid allografts, and 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[2–5]. 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[6] 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. In contrast, kidney biopsy is considered the gold standard diagnostic, but this highly invasive process carries a risk of graft injury, bleeding and, rarely, graft loss. Moreover, by the time immune mediated graft rejection leads to elevated creatinine levels the graft injury is extensive[7]. The ability to monitor chemokine levels at the point of care could thus enable the timely detection of graft injury before it otherwise clinically manifests.

Despite widespread clinical appreciation of the biomarker utility of chemokines, methods for their quantitative measurement in blood remain slow and cumbersome. One approach that offers excellent selectivity is based on biomolecular recognition, and this approach is well established in methodologies such as ELISAS and Western Blots. However, while these approaches readily achieve clinically relevant sensitivity and specificity they remain multi-step, resource-intensive batch processes that are ill suited for deployment as rapid, point-of-care diagnostics.

Indeed, while a handful of point-of-care methods for detecting unrelated targets have seen limited commercial development, these methods have significant drawbacks

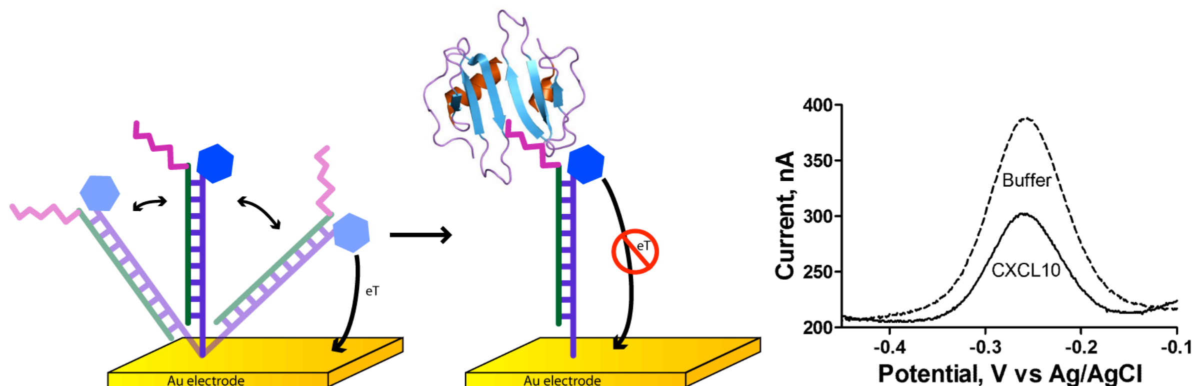

Figure 1. Peptide-DNA scaffold electrochemical sensor for the detection of the chemokine CXCL10. A thiolated DNA anchor strand (purple) with a distal methylene blue redox tag (blue hexagon) is hybridized with a PNA capture strand (green) that is covalently linked to a peptide recognition motif from CXCR3 (pink). This conformation freely collides with the gold surface, resulting in electron transfer in buffer. When CXCL10 binds the recognition peptide, collision rate is slowed by the steric bulk of protein, resulting in reduced electron transfer.

and thus the overall need for technologies for the rapid, quantitative detection of specific protein markers remains largely unmet[8–12]. In response to the largely unmet need for effective point-of-care molecular diagnostics we have developed a broadly applicable, electrochemical sensing platform, termed E-DNA[13] (electrochemical, DNA) sensors, that maintain the specificity of biomolecular recognition, but enables rapid, quantitative measurement through a direct response in signal output (electrical current) from changes in the dynamics (flexibility and folded state) of the probe molecule upon target binding[14]. This approach is reagentless, allowing multiple measurement cycles and a long shelf-life[15], and has proven readily adaptable to microfluidic and multiplexed platforms[16]. An additional, key advantage of the E-DNA approach is that it is highly selective, working well even when deployed directly in clinical samples. This selectivity occurs for two reasons. First, the signal-generating, binding-induced change in collisional dynamics is neither mimicked nor hindered by the non-specific binding of contaminants to the sensor surface. Second, the electrochemical readout avoids false positives as contaminants that undergo redox reactions at the same potential as the redox tag employed in our sensors are rare in clinical materials. For these reasons E-DNA sensors perform well when challenged with blood serum, urine and other complex clinical materials[17].

Building on the E-DNA platform, we present here an electrochemical strategy for monitoring chemokine levels in blood serum. Our approach is similar to a previously described E-DNA architecture for the detection of antibodies in which the binding of the target antibody to a recognition epitope appended to the E-DNA sensors leads to a significant change in E-DNA current[18]. Here we have replaced the recognition epitope with a 20-residue chemokine binding element derived from CXCR3, a naturally occurring IP-10 receptor[4]. Specifically, we have anchored a methylene-blue-modified DNA strand to a gold electrode using thiol-on-gold chemistry (see sensor preparation details in **Supporting Information**). Hybridization of a PNA-peptide chiera to this presents the receptor motif from CXCR3. The binding of IP10 to this peptide recognition element alters efficiency with which the methylene blue approaches the electrode, resulting in a reduction in current transfer (**Figure 1**). This response mechanism is similar to prior work with DNA-binding proteins that directly bind the DNA scaffold[19], and provides a selective (**Figure 2**), rapid measurement of IP10 concentration to a detection limit of 20 nM (**Figure 3**).

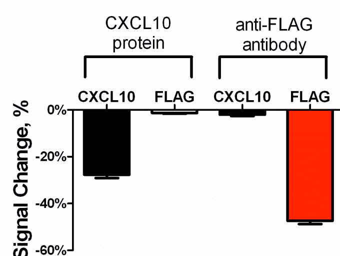

Figure 2. Scaffold sensors directed against CXCL10 and the FLAG antibody display selective signal response in buffer against cross-target interactions, even when challenged with 500 nM target.

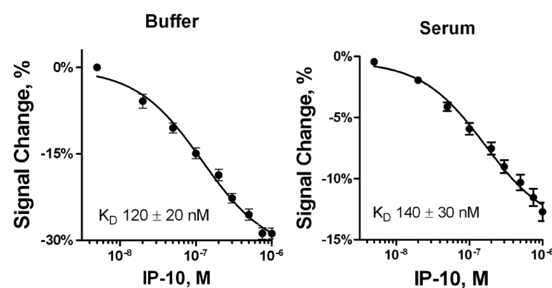

Figure 3. The sensor displays a sensitive, quantitative dose-response to IP10 in both buffer and undiluted blood serum. The affinity of the sensor is slightly reduced in serum, but is still capable of detecting clinically relevant concentrations of IP10, such as those seen during acute tissue rejection episodes.

experiments with IP10. For experiments in buffer, 1x PBS (10 mM phosphate buffer, 0.154 M NaCl, pH 7.4, Sigma Aldrich) was used as received. For serum studies, undiluted fetal calf serum (Sigma Aldrich) was used as received. In either media, IP10 recombinant protein (Cell Science) was titrated into the solution at final concentrations from 1 nM to 2 uM. At each step, the sensor was allowed to equilibrate for 10 minutes, followed by electrochemical measurement (electrochemical parameters in **Supporting Information**). 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.

The sensor displays a robust signal decrease in the peak current upon addition of target (**Figure 1**). Signal change can be expressed as the fraction of pre-target addition signal, and a maximum signal decrease of approximately 30% is seen at saturating concentrations of IP10. The inability to suppress signal beyond this point likely stems from two phenomena: infrequent collisions that result in electron transfer from the bound state and a population of scaffolds that are inaccessible for binding (e.g., due to the anchoring strand lying down on the surface). This signal suppression is quite selective when challenged with an antibody directed against the FLAG epitope, which is a large, non-target protein (**Figure 2**). Here, we use as a control a scaffold sensor where the CXCR3-derived capture peptide is replaced with the FLAG peptide, as the scaffold's recognition of the large antibody target has been previously reported[18]. We observe negligible cross-reactivity between the two classes of scaffolds, even when challenged with concentrations of up to 500 nM of their non-cognate target.

The scaffold sensor displays a quantitative response to IP10 in both buffer and undiluted blood serum (**Figure 3**), an essential capability for diagnostics that can distinguish normal levels of IP10 in blood samples from elevated levels during inflammation and rejection episodes. The sensor's affinity for target and thus, sensitivity, is reflected in the thermodynamic dissociation constant, K_D, and we find an affinity for IP10 of 120 ± 20 nM for the sensor in buffer. Presumably due to sensor blockage due to the large variety of other proteins in blood serum, the affinity in serum is only slightly reduced, at 140 ± 30 nM.

Despite this success, however, the sensor falls short as a diagnostic tool because IP10 levels fall below the limit of detection even during episodes of acute rejection[3]. While future efforts may enable a greater sensitivity, even in its current state this sensor design illustrates the potential versatility and broad applicability of E-DNA type sensors for a broader variety of protein targets. The sensor functions well in unprocessed blood serum and its electrochemical output is easily incorporated into microfluidic systems and point-of-care electronics, suggesting that sensors of this type may find great use in future medical diagnostics.

ASSOCIATED CONTENT

Sensor fabrication, preparation, and cleaning; electrochemistry parameters; and kinetics studies. This material is available free of charge via the Internet at http://pubs.acs.org

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ABBREVIATIONS

CXCL10, C-X-C motif chemokine 10; CXCR3, C-X-C motif receptor 3.

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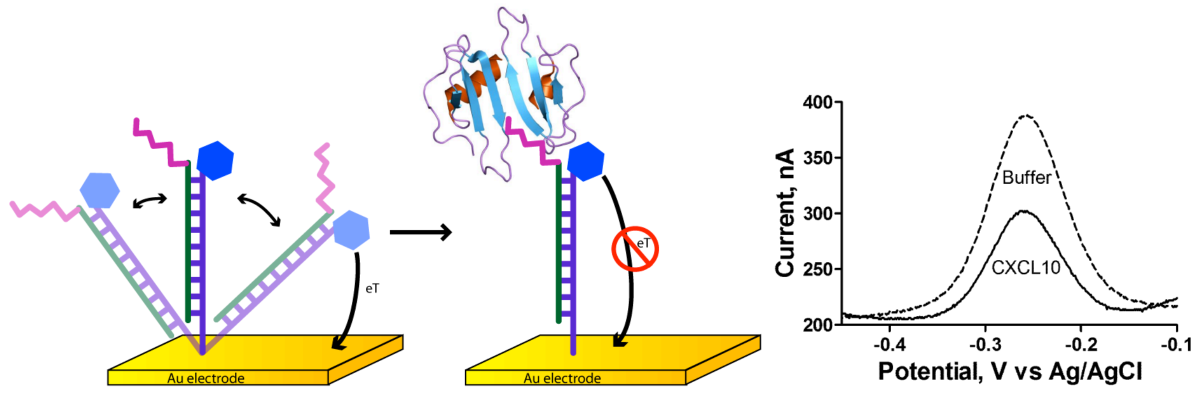
Au electrode
eT
Au electrode
eT
Current, nA
400
350
300
250
200
Buffer
CXCL10
-0.4
-0.3
-0.2
-0.1
Potential, V vs Ag/AgCl

Supporting Information for:

Peptide-DNA scaffold for the electrochemical detection of the chemokine CXCL10 in undiluted blood serum

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Methods

The bio-sensor consists of a gold electrode surface with a self-assembled monolayer of DNA anchor strands (sequence 5'- thiol – GCA GTA ACA AGA ATA AAA CGC CAC TGC - methylene blue -3', Biosearch) and 1-mercapto-6-hexanol (used for sensor passivation, Sigma Aldrich). Gold disk electrodes (1 mm^2 surface area, CH Instruments) were physically polished and electrochemically cleaned as previously described[20].

After preparation, these modified electrodes were immersed for 1 hour in hybridization buffer (10 mM potassium phosphate, 1 M sodium chloride, pH 7.0) containing a final concentration of 100 nM peptide-PNA chimeric molecules. The peptide-PNA was prepared by chemical synthesis of the peptide and PNA segments individually (Panagene), followed by crosslinking using Succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) to yield the final sequence NH_2- NFS SSY DYG ENE SDS SST SP Cys-SMCC-cag tgg cgt ttt att ctt gtt act g –$CONH_2$. The peptide sequence, known to interact with CXCL10, is derived from amino acid residues 22-42 of human CXCR3 isoform A, with the cysteine residues at positions 37 and 38 replaced with serine[4].

After hybridization, the sensors were briefly washed with hybridization buffer, then introduced to sample media and allowed to reach equilibrium for >30 minutes before titration

Electrochemistry

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/sec.