An electrochemical biosensor exploiting binding-induced changes in electron transfer of electrode-attached DNA origami to detect hundred nanometer-scale targets†‡

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The specific detection in clinical samples of analytes with dimensions in the tens to hundreds of nanometers, such as viruses and large proteins, would improve disease diagnosis. Detection of these “mesoscale” analytes (as opposed to their nanoscale components), however, is challenging as it requires the simultaneous binding of multiple recognition sites often spaced over tens of nanometers. In response, we have adapted DNA origami, with its unparalleled customizability to precisely display multiple target-binding sites over the relevant length scale, to an electrochemical biosensor platform. Our proof-of-concept employs triangular origami covalently attached to a gold electrode and functionalized with redox reporters. Electrochemical interrogation of this platform successfully monitors mesoscale, target-binding-induced changes in electron transfer in a manner consistent with coarse-grained molecular dynamics simulations. Our approach enables the specific detection of analytes displaying recognition sites that are separated by ~40 nm, a spacing significantly greater than that achieved in similar sensor architectures employing either antibodies or aptamers.

DNA origami1 – the Watson–Crick controlled folding of a long DNA scaffold strand using hundreds of short, rationally designed oligonucleotide “staples” – enables the construction of nanoscale objects, including devices capable of computation, molecular recognition, and response to specific molecular stimuli.2 Modification of origami with recognition elements for specific small molecules, nucleic acids or proteins enables their use in biosensing3 often using binding-induced changes to produce a fluorescent or plasmonic output.4,5 Optical approaches fail, however, at analyte detection in biological fluids, an ability necessary for the direct sensing of analytes in clinical settings.

In contrast to optical approaches, electrochemical sensors that rely on structure-switching signal transduction successfully achieve molecular detection in biological fluids.6 In this the binding-induced conformational change of an electrode-bound redox-reporter-modified receptor produces a measurable change in electrochemical output.7 Given the specificity of this signal transduction mechanism, such sensors have proven particularly well suited for performing prolonged measurements in whole blood.8,9

Origami is capable of supporting binding-induced electrochemical outputs but this has yet to be demonstrated. Instead, non-electrophoretic experiments involving the exposure of origami to electric fields have been limited to origami pore conductivity measurements10–12 control of origami fluorescent switches,13 electromechanical actuation of origami levers14 and fluorescent readout of voltage-controlled origami movement.15 Recently, electrochemical analysis of surface-bound origami has been used to detect ss-miRNA16 and to probe spatial determinants of redox-active enzyme activity.17 However, none of these approaches appear to be generalizable in a manner that could exploit binding-induced changes in electrochemical detection as a means of signal transduction. This mode of detection could enable the measurement of clinically relevant, mesoscale analytes such as large proteins and whole viruses (rather than their subcomponents), a feat that is undemonstrated with current DNA-based platforms.
Motivated by these arguments, we report here the first electrochemical biosensors employing full-size (∼5 MDa) origami, which we use to sense the binding of mesoscale analytes with multiple target-binding sites separated by ∼40 nm.

To synthesize the “receptor” component of our sensor, we modified a literature triangle design‡ with twelve signal-generating, DNA duplexes functionalized with Methylene Blue that project these redox reporters laterally from its outer edges, six thiol-modified “surface anchors” projecting down from the inner edge’s “bottom” face, and fifteen target-binding A₄₀ strands projecting up from the centers of its “top” face (Fig. 1A and B). We covalently attached these receptors to a 1,6-hexanediol self-assembled monolayer (SAM) formed on the surface of smooth gold electrodes. We employed the SAM for the purposes of (1) passivating the gold surface, preventing non-desired electrochemical reactions and (2) to provide strong anchoring points for the receptors. After testing various surface preparations (Fig. S1+) and SAM-forming reagents (Fig. S2 and S3‡) we ultimately chose 1,6-hexanediol on electrochemically cleaned, e-beam deposited gold because it forms reproducible, smooth, densely packed and highly oriented SAMs (Fig. S2 and S3‡) with large receptor-originated faradaic currents (Fig. S2 and S3‡).

We interrogated the origami-based sensor using square-wave voltammetry. As an aid in maximizing sensor response, we determined the dependence of the reduction current on square-wave frequency‡ (Fig. S5‡); a frequency of 60 Hz produces voltammograms with the largest signal-to-noise ratio, giving 20–80 nA peak currents for a 3.15 mm² electrode (Fig. 2A).

To show that these currents originate specifically from the electrochemical reduction of the origami’s methylene blue-modified strands (“MB-strands”), we equipped these with 7-base single-stranded “toeholds”, allowing their displacement (Fig. S19‡) from the triangle edges via the introduction of fully complementary “anti-strands”.‡ When challenged with 1 μM of the anti-strand, peak current fell by 78% (Fig. 2B); when pushed to 2 μM the current fell by 90%, indicating displacement of the MB-strands from the surface-bound origami (Fig. S6 and S7‡).

The origami-based sensor selectively responds to its polyvalent target. To demonstrate this we designed three target origami triangles as follows (Fig. 3A–C): (1) a non-binding target, K₁ NC, containing five non-complementary A₄₀ DNA strands on each of its three sides; (2) a single-sided target, K₁, decorated with five T₄₀ strands on one of its three sides; and (3) the polyvalent triple-sided, rigid target, K₃, decorated with five T₄₀ strands on each of its three sides and rigidified by the addition of staple linkers between its internal trapezoidal edges. We challenged our sensor with each of these targets while continuously monitoring their square-wave voltammograms every 15 s (Fig. 3D).

Challenging with target K₃ produces a monotonic decrease in signaling current equilibrating at −28% after 50 min, serving as proof-of-concept to demonstrate electrochemical sensing of polyvalent targets across distances of ∼40 nm. That is, we posit that the observed decrease is driven by the polyvalent binding of K₃, which, by having three sets of target-binding sites and extra rigidity via linker staples on its internal trapezoidal edges, binds to R (Fig. SA‡), causing it to undergo a structural change. This change, in turn, results in the redox reporters on R undergoing fewer interactions with the surface, thus decreasing the observed current (Fig. S5‡). Challenging the sensor with K₁, in contrast, produces only a small decrease in peak current, equilibrating after ∼50 min at −7%. This decrease is not statistically different from the intrinsic drift (−6%) of the system. We posit that this occurs because, although K₁ does bind to R (Fig. SA‡), the binding does not deform R in a manner that significantly changes electron
transfer. This might be expected as K1 displays complementary binding sites on only one side of the triangle, suggesting that it will bind to only a single side of R. Finally, as expected the addition of the non-complementary, non-binding (Fig. SA†) control KNC to the sensor produced only a small (~2%), instantaneous increase in peak current that returned to baseline levels after 30 min (Fig. 3). Gel-shift analysis of all three target-receptor complexes when free in bulk solution confirms that strand displacement does not occur upon target binding (Fig. SA†), and AFM analyses of all triangles indicates they fold to the expected dimensions (Fig. SB†).

The specific decrease in signaling current generated by K3 binding is caused by a reduction in the structural flexibility of R. We demonstrate this by employing a previously described computational workflow,24 featuring the OxDNA molecular dynamics package,25–27 to simulate fluctuations in the spatial positioning of redox reporters (Fig. 4) in the systems R, R + K1 and R + K3. These simulations indicate that, upon binding K3, R undergoes a change in structural rigidity, which dampens the ability of the reporters to approach the electrode closely enough to transfer electrons.

The results presented here demonstrate the engineering of origami-based electrochemical biosensors for the detection of mesoscale targets, a target length scale not accessible to any other rationally customizable sensing platform reported to date that can perform in complex biological matrices. The closest analogous self-assembled nanostructure that is utilized in electrochemical biosensing are 10 nm-scale DNA tetrahedra. These tetrahedra are mostly exploited as rigidifying spacers modified with a single recognition element (e.g., an aptamer); the tetrahedron itself is not directly involved in binding-induced signal transduction.3 Although tetrahedra have been used to compare through-space and through-duplex electron charge transfer,28 and to monitor low nanometer-scale binding events by displacing a DNA strand using a single ferrocene-labeled tetrahedron29 by virtue of their size and simplicity they cannot perform recognition over length scales accessible to origami, nor radically improve signaling capability compared to their appended recognition element.

DNA origami-based electrochemical sensing offers the ideal approach to the measurement of clinically relevant mesoscale analytes. This is because (1) origami offers molecular surfaces...
that can accommodate mesoscale arrays of target binding sites and (2) origami can be rationally designed to produce target binding-induced signaling across larger distances than conventional DNA-based sensors. For example, single-layer origami could achieve the specific detection of viruses by displaying arrays of viral-capsid-binding aptamers\textsuperscript{30} with an inter-aptamer resolution of $\sim$2 nm, displaying tens of aptamers across mesoscale distances. If greater spatial resolution in the placement of target binding sites, anchors or redox reporters is required, multilayer origami can be designed to display these functional groups with sub-ångström precision.\textsuperscript{31}

Beyond offering unprecedented structural customizability for target recognition, origami-based sensors could also overcome inherent limitations of electrochemical biosensors that rely on single or double stranded nucleic acids for detection. For example, origami can be selectively modified with hundreds of fluorescent\textsuperscript{32} or redox reporters, as opposed to just a few reporters in single-stranded, double-stranded or DNA tetrahedra. This would improve the detection of single analytes by increasing the signal-to-noise ratio of electrochemical signaling. Moreover, redox reporters and target binding sites are locatable on separate strands in the origami; thus their position and flexibility can be tuned at will. This should enable the signal gain of an origami-based sensor to be maximized by design, instead of being limited by the length or flexibility of a single or double strand, as is the case of conventional DNA sensors.

Other powerful features of the proposed electrochemical origami-based sensing approach are the possibility of improving its resistance to degradation in harsh environments by using crosslinking\textsuperscript{13,34} or other strategies,\textsuperscript{35} and the fact that mesoscale target detection has historically been hard to achieve but is readily supported by origami.\textsuperscript{18} Moreover, the simplicity of placing origami on lithographically patterned surfaces\textsuperscript{16} could enable the multiplexed detection of many of these large biological targets on electronic sensor arrays, with the potential ability to create biomolecular profiles of disease states, a feat not achievable with current technologies. And because electrochemistry-based sensing readily supports measurements in biological fluids,\textsuperscript{9,37} origami-based electrochemical detection could support these measurements in whole blood \textit{in vitro} and \textit{in vivo}. Overall, the approach described here offers an unprecedented opportunity to realize truly customized, direct detection of specific, clinically-relevant mesoscale targets.

**Author contributions**

This project was conceived by PSL with formative and substantive conceptual input from NA and KWP. NA designed, optimized and ran electrochemical experiments with PSL. MS, AKN, YF, NW, TA and PSL designed, synthesized and characterized the origami and conducted AFM analyses. CMH designed and ran simulations under the supervision of HJS and CEC. NA and RCEA synthesized and analyzed the gold substrates. NA, CEC, KWP and PSL wrote the paper.

**Conflicts of interest**

The Authors declare no conflicts of interest.

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**Notes and references**


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