Sensitivity and Specificity of Photoaptamer Probes*

Drew Smith‡§, Brian D. Collins‡, James Heil‡, and Tad H. Koch¶

The potential of photoaptamers as proteomic probes was investigated. Photoaptamers are defined as aptamers that bear photocross-linking functionality, in this report, 5-bromo-2′-deoxyuridine. A key question regarding the use of photoaptamer probes is the specificity of the cross-linking reaction. The specificity of three photoaptamers was explored by comparing their reactions with target proteins and non-target proteins. The range of target/non-target specificity varies from 100- to >10⁴-fold with most values >10⁴-fold. The contributions of the initial binding step and the photocross-linking step were evaluated for each reaction. Photocross-linking never degraded specificity and significantly increased aptamer specificity in some cases. The application of photoaptamer technology to proteomics was investigated in microarray format. Immobilized anti-human immunodeficiency virus-gp120 aptamer was able to detect subnanomolar concentrations of target protein in 5% human serum. The levels of sensitivity and specificity displayed by photoaptamers, combined with other advantageous properties of aptamers, should facilitate development of protein chip technology. Molecular & Cellular Proteomics 2:11–18, 2003.

Proteomics, the study of protein expression at the scale of cell, tissue, or organism (1, 2), has been defined by a single technology: two-dimensional gel separation followed by mass spectrometric analysis (3, 4). Although this technology is mature, powerful, and wonderfully sophisticated, it suffers from evident limitations in speed and sensitivity. Several days are required to process a single sample, and only ~1000 of the most abundant proteins can be detected (5). The ideal proteomic technology would process samples in minutes or hours and be able to quantify even the most weakly expressed proteins.

Two-dimensional gels and chromatographic methods separate and identify proteins on the basis of their physical characteristics. An alternative approach is to identify proteins by specific recognition. The potential advantage of this approach is that proteins that have similar size and charge but which differ in sequence and conformation can be resolved and assayed independently with minimal cross-talk. Various strategies for high density arraying and multiplexing of oligonucleotides are in advanced stages of development; hence, a protein chip, analogous to a gene chip, is a logical step in proteomics.

The demands on a successful protein probe technology are considerable. First, probes must be generated very rapidly, thousands or tens of thousands in a few years. Second, probes for different proteins must function under similar assay conditions. Third, the manufacture and arraying of the probes must be standardized. Fourth, the probes must demonstrate high levels of sensitivity and specificity toward their targets. The requirements for specificity will be especially rigorous: the limits of detection of existing instrumentation are not set by inherent machine sensitivity but by assay background and noise. For example, the confocal scanners used as gene chip readers can detect a few hundred fluorophores on a 100-μm feature, but probe detection limits are thousands-fold higher due to nonspecific binding to both probe and substrate. Nucleic acids can be selectively amplified to overcome these limitations, but proteins cannot.

Monoclonal antibodies have many of the desirable features for protein probes and have the benefit of decades of technological development (6). Aptamer technology is considerably less mature but has features that may be of advantage in the development of protein probe technology (7–9). Among the principal advantages of aptamers are the facts that they are synthetic molecules and are identified entirely in vitro by the SELEX process (10, 11). The former feature will facilitate manufacture and arraying, while the latter has facilitated automation for high throughput probe generation.

The synthetic nature of aptamers bestows another potentially critical advantage: the ability to introduce desired chemical functions into libraries and select probes that have novel and compatible activities. We have argued that photocross-linkable cross-linking is a desirable function for a protein probe (7–9) because it allows proteins to be covalently captured onto an array surface in a controllable manner. This capture allows washing, labeling, and reading steps to be performed.

From ‡SomaLogic, Inc., Boulder, Colorado 80301 and the ¶Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado 80309-0215

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The abbreviations used are: SELEX, systematic evolution of ligands by exponential enrichment; A, aptamer; aFGF, acidic fibroblast growth factor; bFGF, basic fibroblast growth factor; PDGF, platelet-derived growth factor; T, target protein; HIV, human immunodeficiency virus.

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under the harshest and most stringent conditions necessary to reduce background and improve signal. What is not established is the effect of photocross-linking on the specificity of the capture step.

We set out to characterize, systematically and quantitatively, a set of photocross-linking aptamers, photoaptamers, with regard to their sensitivity and specificity. The photoreactive unit incorporated into our photoaptamers is 5-bromodeoxyuridine (BrdUrd), used for decades in protein-nucleic acid cross-linking studies. Rather than use short wave (254 or 266 nm) UV light for cross-linking, however, we irradiate at 308 nm using a XeCl excimer laser. This technique was developed by Koch and colleagues (12–16) and has been shown to result in specific and high yield cross-linking reactions. Light at 308 nm induces photoelectron transfer from a nearby electron donor to the bromouracil base via either excitation of the BrdUrd, excitation of the electron donor, or excitation of a BrdUrd-electron donor charge transfer state (17, 18). Amino acid residues that can serve as electron donors in BrdUrd photocross-linking include Tyr, Trp, His, Phe, Cys, Cys-Cys, and Met of which only Tyr and Trp are excited at 308 nm (16). The specificity and high yield cross-linking reactions. Light at 308 nm induces photoelectron transfer from a nearby electron donor to the bromouracil base via either excitation of the BrdUrd, excitation of the electron donor, or excitation of a BrdUrd-electron donor charge transfer state (17, 18). Amino acid residues that can serve as electron donors in BrdUrd photocross-linking include Tyr, Trp, His, Phe, Cys, Cys-Cys, and Met of which only Tyr and Trp are excited at 308 nm (16–20). Cross-linking results from subsequent reaction of the resulting radical ion pair. In the absence of an electron donor the BrdUrd efficiently relaxes back to ground state (17).

We hypothesized that photocross-linking via photoelectron transfer would actually enhance the specificity of the aptamer-protein capture reaction: although a protein might bind an aptamer nonspecifically, the probability that an appropriate amino acid would be positioned to cross-link with a BrdUrd residue would be low. Some evidence for this view has been presented by Golden and co-workers (9), who showed that basic fibroblast growth factor (bFGF) photoaptamers could cross-link picomolar concentrations of target in the presence of serum with very little nonspecific cross-linking.

Using these bFGF photoaptamers and a new photoaptamer raised against the HIV coat protein gp120, we evaluated both the equilibrium binding constant and the relative rate of cross-linking to target proteins. We then compared these values to the values for a set of non-target proteins. These non-target proteins were chosen to provide an exacting test of specificity: 1) aFGF and gp120; 2) platelet-derived growth factor (PDGF) is a highly basic heparin-binding growth factor that is notorious for its nonspecific DNA binding; and 3) thrombin is another heparin-binding protein.

These experiments confirm the specificity of the photocross-linking reaction in the solution phase. We extend these results to microarray format by measuring cross-linking of immobilized photoaptamers to target protein. We find that the sensitivity and specificity of photocross-linking are maintained in this format: target proteins can be detected at subnanomolar concentrations in buffer and at nanomolar concentrations when spiked into serum.

### EXPERIMENTAL PROCEDURES

**Photoaptamers**—Photoaptamers 0615 and 0650 were discovered by Golden and co-workers (21). Their sequences are shown as follows with X representing BrdUrds. 0615: GGG AGG AGC ATG CGG GGG AGG CAC CGA GXX CAX AGX CCA CAG ACG AGC GGG A; 0650: GGG AGG AGC ATG CGG XGA CGX AAX AGX GXX AXC GAX GCA GCC XGC XGG CAG ACG ACG AGC GGG A.

Photoaptamer 0518 was discovered using a procedure analogous to that described by Golden and co-workers (9, 21), and its sequence is as follows, again with X representing BrdUrds. 0518: GGAG AGG AGT CGG AAX CCG GCA GCC XCC GAA AAG GAA AXX ACG CAG AGC ACG ACG GGG A.

Photoaptamers were synthesized enzymatically by replication of a complementary synthetic DNA sequence using the Klenow fragment of *Escherichia coli* DNA polymerase I and a synthetic DNA primer. The reaction mix included BrdUTP (TriLink) in place of TTP with all NTPs radiolabeled with 32P at their 5’ ends. The gels were stained with SYBR green, washed, eluted from the gel by standard methods. To avoid exposing the BrdUrds-containing DNA to UV light, the gels were stained with SYBR Green I (Molecular Probes) and visualized using a blue light Dark Reader transilluminator (Clare Chemical Research, Denver, CO).

**Equilibrium Dissociation Constant Measurements**—Aptamers were radiolabeled with 32P at their 5’ end with polynucleotide kinase to a specific activity of 2–5 × 106 dpm/pmol. Aptamers were mixed with excess protein in PDB buffer and allowed to equilibrate at room temperature for 10 min. The mixtures were irradiated in 0.6-ml disposable cuvettes. The laser used was a TuiLaser S-200 XeCl excimer laser that emits 308 nm light. The beam size measured 6 mm, and the cuvette position was adjusted so that the entire sample was within the beam. The standard settings produced 2.0 mJ/pulse with a repetition rate of 200 Hz. As discussed in a separate paper2 the cross-linking extent was governed strictly by the total light dose; neither the peak power nor the pulse frequency affected cross-linking. A set of photocross-linking aptamers, photoaptamers, could cross-link picomolar concentrations of target protein. We find that the specificity of photocross-linking is maintained in this format: target proteins can be detected at subnanomolar concentrations in buffer and at nanomolar concentrations when spiked into serum.

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\[ A + T \xrightleftharpoons[k_{\text{off}}]{k_{\text{on}}} A:T \]

\[ K_d = \frac{[A][T]}{[A:T]} = \frac{k_{\text{off}}}{k_{\text{on}}} \]

\[ A:T + h\nu \xrightarrow{k_{\text{dl}}} X \]

\[ A + h\nu \xrightarrow{k_i} X \]

Fig. 1. A kinetic scheme showing the reaction pathways resulting from irradiation of mixtures of A and T. A:T is the complex between A and T, A-T represents A cross-linked to T, X is photoinactivated A that is no longer capable of binding to T, and X:T represents the transient complex from photoinactivation of the A:T complex (X:T dissociates to X and T). The rate constants, \( k_{\text{dl}} \) and \( k_i \), for cross-linking and inactivation are the respective composite rate constants equal to the rate constants for absorption times the respective cross-linking and inactivation are the respective composite rate constants. The relationship between \( f_x \) and \( K_d \) and \( K_{\text{st}} \) is shown in Equation 3.

\[ f_x = \frac{f_x^{\text{max}} [T]}{(1 - f_x^{\text{max}}) K_d + [T]} = \frac{k_{\text{on}} [T]}{K_d + 1} \]  

(Eq. 3)

The quotient \( f_x^{\text{max}}/K_d \) is then a second measure that relates cross-linking activity to protein concentration. The parameter \( f_x^{\text{max}} \) will be linearly dependent upon protein concentration for \([T] \ll K_d\). Under these conditions, \( f_x^{\text{max}}/K_d \) should be a good approximation of \( f_x^{\text{max}}/K_{\text{st}} \); we use this approximation in evaluating cross-linking to non-target proteins where determination of \( f_x^{\text{max}} \) is impractical. A derivation of Equation 3 will be included in a separate paper.2

**Detection of Target Protein with Photoaptamer on Slides—** Photoaptamers 0518, 0615, and 0650 were synthesized with a 5′-C6-amino substituent using standard DNA synthesis methods. The DNA was immobilized on N-hydroxysuccinimide-activated slides (Surmodics) by spotting 1 nl (Gene Machines) of a 20 μM solution in 150 mM phosphate, pH 8.5, 20 μM polyethylene glycol-NH2 (Mw = 2000). Feature diameter is 100 μm. After an overnight coupling reaction, unreacted amines were blocked with 0.1 M Tris, 50 mM ethanolamine, pH 9.0 at 50 °C for 15 min. The slides were washed two times with water, then washed with 4X SSC, 0.1% SDS at 50 °C for 15 min, and then rinsed two times with water. Residual amines, which might react with NHS-Cy3 dye (below), were capped by reaction with 0.1 mg/ml sulfo-NHS-acetate in 100 mM NaHCO3, pH 8.4 at room temperature for 30 min and were then rinsed three times with SELEX buffer (40 mM HEPES, pH 7.5, 111 mM NaCl, 5 mM KCl, 1 mM CaCl2, 1 mM MgCl2, 0.05% Tween 20).

Protein assays were performed in 50-μl-volume perfusion chambers (Grace BioLabs), eight chambers/slide. The slides were blocked for 30 min in 1× SELEX buffer, 0.05% Tween 20. 200 μg/ml λ carrageenan (Sigma), 200 μg/ml Ficol Type 400 (Amersham Biosciences), and 1 mg/ml diethylamine casein (Sigma). This buffer was removed, and fresh buffer containing test proteins and/or human defibrinated serum (SeraCare) was added and incubated for 1 h at room temperature. The protein solutions were then removed, and the slide was washed three times with SELEX buffer. Photocross-linking was performed with 308 nm excimer laser light at a total dose of 5 J/cm². Uncross-linked protein was removed by washing in 500 ml of SDS wash buffer (20 mM sodium phosphate, pH 7.4, 1 mM EDTA, 150 mM NaCl, 0.1% SDS) for 20 min with stirring and then with water for 5 min. Bound proteins were then stained by addition of 0.1 mg/ml Cy3-NHS (Amersham Biosciences) in 0.1 M Na2HCO3, pH 8.4 for 30 min at room temperature. Free dye was removed by repeating the SDS wash followed by washes in methanol and 20 mM NaOH. Salt residues were removed by rinsing with water, and the slides were dried under a stream of N2. Image data were collected in an Array-Worx scanner (Applied Precision) in the Cy3 channel at 0.2-s exposure, 5-μm pixel resolution.

**RESULTS**

**Equilibrium Binding**—To begin our analysis of photoaptamer specificity, we determined equilibrium binding constants for target and non-target proteins. To provide a stringent test of binding specificity, we chose a set of non-target proteins for which the sequence similarity is greater than 30% with the target sequence. The observed equilibrium constant for each target/non-target pair was determined by fitting binding isotherms to the Langmuir model. The dissociation constant for each target protein was determined from the intercept of the linear regression analysis of the binding isotherms. The binding affinities for each non-target protein were determined by fitting binding isotherms to the Langmuir model, and the dissociation constant for each non-target protein was determined from the intercept of the linear regression analysis of the binding isotherms.
proteins likely to cross-react with the aptamers. Using a BLAST search, we identified the proteins most similar to the target proteins. The most closely related protein to bFGF is aFGF, which shares 55% identity. The closest relative to gp120_{MN} is HIV coat protein from the SF2 strain, gp120_{SF2}, which is 81% identical. To these proteins we added two proteins known to have very high nonspecific affinity for nucleic acids, PDGF and thrombin. Both contain polyanion (that is, heparin) binding sites. In addition, PDGF is highly basic, having a predicted pI of ~9.5, and the AB dimer is expected to have a net charge at neutral pH of +20.

Using the nitrocellulose filter binding method, affinities of the photoaptamers for target and non-target proteins were determined (Fig. 2). With the exception of PDGF, the affinities of the photoaptamers for the non-cognate proteins are so weak that detection of binding is difficult. $K_d$ values are compared in Table I.

Measurement of Cross-linking to Potentially Competing Proteins—Aptamer specificity can also be gauged by contrasting the extent of cross-linking to the target protein with that to related target proteins. The measure we use is $f_{x1}^{\text{max}}/K_{x1}$. This term is useful because, when multiplied by protein concentration, the product is the fraction aptamer cross-linked at saturating light dose for protein concentrations significantly less than $K_{x1}$ (see Equation 3). Except when the $f_{x1}^{\text{max}}$ is high, $K_{x1}$ is typically close to the equilibrium binding constant $K_d$ (Table I). For cross-linking of each photoaptamer to its target protein, we performed light dose titrations over a full series of protein concentrations. The maximal fraction cross-linked at a given protein concentration ($f_{x1}^{\text{max}}$) was evaluated using Equation 2. Parameters $f_{x1}^{\text{max}}$ and $K_{x1}$ were determined by fitting Equation 3 to a plot of $f_{x1}$ as a function of protein concentration. For non-target proteins, we quickly recognized that the use of saturating protein concentrations to determine $f_{x1}^{\text{max}}$ or $K_{x1}$ individually was impractical. Instead we determined $f_{x1}$ at protein concentrations less than $K_{x1}$, assuming that these protein concentrations would also be less than $K_{x1}$. Under these conditions $f_{x1}^{\text{max}}/K_{x1}$ should approximate $f_{x1}^{\text{max}}/K_{x1}$. Fig. 3 shows plots of $f_{x1}^{\text{max}}/K_{x1}$ versus protein concentration. The values for $f_{x1}^{\text{max}}/K_{x1}$ estimated from these data are reported in Table I. Non-target proteins (the open symbols) are displaced far down and to the right as compared with target proteins. As with the filter binding experiments, detection of non-target-cross-linked complexes is quite difficult, requiring high protein concentrations and light doses (Fig. 4). The lower ranges of these values are probably no more accurate than 2–5-fold.

Activity of Immobilized Photoaptamers—Our goal is to use photoaptamers as probes in microarray format. Having evaluated their activity and specificity in solution phase, we extended our analysis to the solid phase. The photoaptamers 0518, 0615, and 0650 were synthesized with a 5’ C6-amino linker and immobilized by spotting on NHS-activated slides (see “Experimental Procedures”). The aptamers were then assayed for photocross-linking activity by dose response to their targets. Protein dilutions were applied to subarrays in perfusion chambers for 1 h at room temperature, rinsed briefly, and then cross-linked with 5 J/cm² 308 nm laser light. Most protein not covalently bound was removed by successive washes in 0.1% SDS and 20 mM NaOH. Captured proteins were stained via their primary amines by NHS-Cy3 reactive dye. Excess dye was removed by repeating the denaturing washes.

After scanning in a slide reader, the capture reactions were
evaluated as a function of feature intensity minus local background (Imagene). Fig. 5 shows plots of fluorescence intensity versus protein concentration. As both bFGF and gp120 aptamers were present on the arrays, cross-reactivity could also be evaluated. These results show that the aptamers are as well behaved on surfaces as they are in solution: subnanomolar sensitivity with little or no cross-reactivity.

A previous study (9) demonstrated specific cross-linking of the bFGF aptamers in serum. We extended these results by measuring the dose response for gp120 in serum. There should be no endogenous gp120 in normal serum, which might confound the interpretation of results. gp120 was diluted into 5% defibrinated delipidated human serum (total protein, $110\,\text{mg/ml}$), and the response was assayed as described under “Experimental Procedures.” Fig. 6 shows a definite dose response to gp120 protein from its cognate aptamer and none from the non-cognate aptamer. The sensitivity of the response is less than in buffer, presumably due to interference with serum components.

**DISCUSSION**

Three photoaptamers bearing between five and seven Brd-Urd nucleotides in place of T nucleotides have recently been identified through the photoSELEX methodology: two, 0650 and 0615, show specific affinity and cross-linking to bFGF, and one, 0518, shows specific affinity and cross-linking to HIV gp120MN.

We have proposed photoaptamers as probes for highly multiplexed protein assays (7, 8), an application which requires an extremely high degree of specificity. Previous studies of aptamer equilibrium binding have shown good specificity of binding (e.g. Refs. 23–25; see Ref. 26 for a counter example). Our studies of the photocross-linking mechanism indicate the requirement that cross-linking occurs within a complex of aptamer and protein. This requirement suggests that photocross-linking should enhance probe specificity; our work tests and quantifies this hypothesis.

Nucleic acid-protein photocross-linking reactions generally occur through formation of a highly reactive species, independent of prior nucleic acid-protein complexation (18). In contrast, photocross-linking with a BrdUrd photoaptamer to its cognate protein is initiated predominantly via photoelectron transfer between the bromouracil chromophore of the nucleic acid and an electron-donating chromophore of the protein, frequently the phenolic group of a Tyr residue. In the

**TABLE I**

| Aptamer | Protein      | $K_d$  | $f_{\text{max}}$ | $K_{\text{xl}}$ | $f_{\text{max}}/K_{\text{xl}}$ |
|---------|--------------|-------|-----------------|-----------------|-------------------------------|
|         |              | $M^{-1}$ | $M^{-1}$        | $M^{-1}$        | $M^{-1}$                       |
| 0518    | gp120$_{\text{MN}}$ | $8.3 \times 10^{-8}$ | 0.30            | $9.7 \times 10^{-8}$ | $3.1 \times 10^8$             |
|         | gp120$_{\text{SF2}}$  | $>1 \times 10^{-4}$   |            | $1 \times 10^3$       |                               |
|         | PDGF         | $5.7 \times 10^{-7}$  |                | $7 \times 10^4$       |                               |
|         | Thrombin     | $>1 \times 10^{-4}$   |                | $<1 \times 10^3$      |                               |
| 0615    | bFGF         | $7.8 \times 10^{-11}$ | 0.22           | $6.6 \times 10^{-11}$ | $3.3 \times 10^9$             |
|         | aFGF         | $>1 \times 10^{-4}$   |                | $3 \times 10^4$       |                               |
|         | PDGF         | $2.0 \times 10^{-7}$  |                | $6 \times 10^5$       |                               |
|         | Thrombin     | $1 \times 10^{-4}$    |                | $3 \times 10^4$       |                               |
| 0650    | bFGF         | $4.5 \times 10^{-10}$ | 0.81           | $1.4 \times 10^{-10}$ | $5.8 \times 10^9$             |
|         | aFGF         | $>1 \times 10^{-4}$   |                | $4 \times 10^4$       |                               |
|         | PDGF         | $2.2 \times 10^{-7}$  |                | $3 \times 10^5$       |                               |
|         | Thrombin     | $>1 \times 10^{-4}$   |                | $2 \times 10^4$       |                               |
absence of an electron donor, excited bromouracil decays back to the electronic ground state. Hence, BrdUrd phototapamers exhibit covalent molecular recognition. This mechanism should not only maintain but increase the specificity of target capture by an aptamer: binding of a non-target protein is less likely to result in the appropriate geometry for photoelectron transfer-initiated cross-linking. We tested this model by measuring the specificity of binding and cross-linking reactions.

Specificity of photoaptamers was measured by determining Kd values and cross-linking activities for a few proteins that might interfere with detection of the cognate protein (Table I). These included proteins with high sequence homology to the cognate proteins (aFGF and gp120SF2) and proteins that have high nonspecific affinity for nucleic acids because of their polyanionic binding sites (PDGF and thrombin). Although aFGF has 55% sequence homology with bFGF, it shows 5–6 orders of magnitude lower affinity for the bFGF aptamers 0650 and 0615. Similarly, gp120SF2 has 81% sequence homology with gp120MN but shows 3 orders of magnitude lower affinity for aptamer 0518. PDGF, which has a very high positive charge, shows much higher affinity for the aptamers (Kd 200–500 nM) than do any of the other non-cognate proteins. Despite the presence of a heparin binding site, thrombin bound very weakly to all aptamers.

Cross-linking activities, defined as fmax/Kx, were determined for non-cognate proteins, and these are also reported in Table I. These activities are substantially smaller for non-cognate proteins than for cognate proteins. The ratios for aFGF with 0650 and 0615 are smaller by 5 orders of magnitude relative to the ratios for the cognate protein bFGF. Similarly, the ratio for gp120SF2 is 3 orders of magnitude smaller relative to the ratio for the cognate protein gp120MN. PDGF shows the greatest nonspecific cross-linking activity with all three aptamers. A comparison of the specificity of cross-linking activity with that of binding affinity is instructive. PDGF binding is 7–, 2500–, and 500-fold weaker than cognate protein binding for aptamers 0518, 0615, and 0650, respectively (Table I). Correspondingly, cross-linking activity of PDGF is
40-, 5000-, and 20,000-fold lower than cross-linking activity of cognate proteins. The cross-linking reaction, therefore, imparts 5-40-fold greater specificity over affinity binding alone in all three cases.

A similar quantitative comparison of affinity and cross-linking specificity for the other non-target proteins (aFGF, gp120SF2, and thrombin) is problematic because the measurements made for these reactions are near the limits of detection for the assays used. Qualitatively, however, it is clear that there is no apparent loss of specificity in the cross-linking reactions as compared with the affinity binding reactions; both are extremely specific.

Previous studies have shown that immobilized aptamers are active in affinity purification on a porous support (24) and in protein assays on beads (22). We extended this work by showing that photoaptamer-based assays are feasible in microarray format. All three aptamers responded to target protein in a near linear fashion over 3 orders of magnitude of protein concentration with subnanomolar limits of detection (Fig. 6). In the presence of 5% serum (~3 mg/ml protein) we were able to detect ≤5 nm gp120 (~3 × 10⁻⁴ mg/ml), a sensitivity and specificity >1/10⁶. We expect this sensitivity to increase as our assay methods are further developed. It is worth emphasizing that these results were obtained without the use of a secondary reagent. We have argued (7) that covalent capture by photoaptamers would allow the use of reactive dyes in protein detection, and these results confirm this expectation.

CONCLUDING REMARKS

One of the goals of our study was to assess the suitability of photoaptamers as probes for highly multiplexed protein assays. A chief concern is the specificity of the photocross-linking reaction: although aptamer binding is generally quite specific, photocross-linking might be unselective. Our work shows that, in the three examples explored here, the cross-linking reaction significantly enhanced probe specificity in the case where affinity specificity was weakest and had a small but positive effect when affinity specificity was strongest. It thus seems plausible that a single photoaptamer can be used as a capture agent. The current standard in protein measurement is the sandwich assay, which requires matching pairs of antibodies with compatible affinities and non-overlapping epitopes. This process is burdensome when developing single protein assays and small panels; it will become a major challenge when the goal is to measure hundreds or thousands of protein levels simultaneously. We have shown that arrayed photoaptamers can function as sensitive and specific single detection reagents. The sensitivity and specificity of photoaptamers, combined with the ability to automate and scale up their selection and the ability to use them on solid surfaces, indicate that they could become an important factor in the development of proteomic technology.

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§ To whom correspondence may be addressed. Fax: 303-545-2525; E-mail: drew.smith@somalogic.com.

∥ Supported by the Council for Tobacco Research and recipient of a faculty fellowship from the University of Colorado Council on Research and Creative Work. To whom correspondence may be addressed: Dept. of Chemistry and Biochemistry, 215UCB, University of Colorado, Boulder, CO 80309-0215. Fax: 303-492-5894; E-mail: tand.koch@colorado.edu.
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