Supporting Information

Antibody Microarrays Utilizing Site-Specific Antibody-Oligonucleotide Conjugates

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Materials and Methods

General procedure for the antibody-oligonucleotide conjugate immobilization onto the DNA array: Hybridization of the antibody conjugates onto the DNA arrays was accomplished via the direct addition of 100 uL of 10 ug anti-Her2 S202pAcF Fab-oligonucleotide conjugate onto the desired array(s) and was allowed to hybridize for 15 minutes at room temperature in a humidified chamber (to prevent evaporation). Following the 15 min of hybridization, the slide was washed thoroughly in PBS before use in either cell or protein binding experiments.

General procedure for the immobilization of Her2+ SK-BR-3 cells onto the DNA-directed antibody array: Following the antibody-oligonucleotide conjugate immobilization, the cells or cell mixture was applied directly onto the desired array(s) and allowed to localize for 30-45 min at room temperature. The slide was then washed by insertion into a 50 mL conical centrifuge tube (that has been filled with PBS). The tube was sealed and rotated 180º so that the top face of the slide was facing downwards and the slide was fully submerged. The slide was then allowed to sit in this position for 30 minutes while the unbound cells settled to the bottom of the centrifuge tube. The slide was removed and can either be imaged wet on an EF reader, or be dried via centrifugation and imaged on a slide reader.

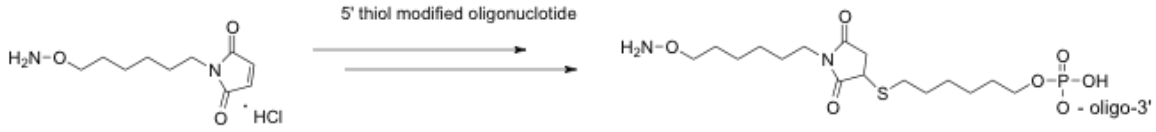
Expression of anti-Her2 S202pAcF Fab: pAcF was site-specifically introduced into the anti-Her2 Fab in response to the amber nonsense codon TAG with an orthogonal amber suppressor aminoacyl-tRNA synthetase/tRNA pair derived from *Methanococcus jannaschii*[1]. Each mutant was expressed either in shake flasks or fermented in *Escherichia coli* achieving yields upwards of 200 mg/L with fermentation. The Fabs were purified via Protein G chromatography. Mass spectrometry and SDS-PAGE gel showed >95% purity[2,3].

DNA Sequence Notation	DNA Sequence 5' to 3'
A	ATCCTGGAGCTAAGTCCGTA
B	GCCTCATTGAATCATGCCTA
C	GCACTCGTCT**CAGCTG**ACTATCGCTA
D	ATGGTCGAGA**CCATGG**TGTCAGAGTA
E	ATGTGAAGTGGCAGTATCTA
F	ATCAGGTAAGGTTCACGGTA
A'	TACGGACTTAGCTCCAGGAT
B'	TAGGCATGATTCAATGAGGC
C'	TAGCGATAGT**CAGCTG**AGACGAGTGC
D'	TACTCTGACA**CCATGG**TCTCGACCAT
E'	TAGATACTGCCACTTCACAT
F'	TACCGTGAACCTTACCTGAT

[a] The orthogonal DNA sequences were found in Bailey et al.[4]

Table S1. Orthogonal DNA sequences A-F and their respective complements A'-F' where the bold bases indicate a restriction site: Pvu II in C and C' and Nco I in D and D'.

Preparation of the aminooxy functionalized DNA: The aminooxymaleimide linker was synthesized following a previously reported method.[3,5] NMR and mass spectrometry were consistent with reported values (231.1 [M+H])[6]. Following reduction of the 5′ thiol oligonucleotide (IDT Technologies, 100 μM) with 5 mM tris(2-carboxyethyl) phosphine (TCEP) for 2 h at room temperature in PBS (pH 7.5), 10 mM maleimide linker was added. After 1.5 h, the reaction was purified on an anion exchange column (Mono Q 5/50 GL, Buffer A: 20 mM Tris, 10 mM NaCl, pH 7.5; Buffer B: 20 mM Tris, 2 M NaCl, pH 7.5), desalted (PD10 column), and lyophilized. Yield, 78%.



***Scheme S1*:** Structure and synthesis of the DNA-Linker molecule.

Preparation of the Fab-DNA conjugates: As previously described in Kazane et. al, the antibody-oligonucleotide conjugate was synthesized by reacting 100 μM anti-Her2 Fab (K169pAcF or S202pAcF) at 37 °C with 3 mM aminooxy-ssDNA in the presence of 100 mM 4-methoxyaniline in 100 mM acetate buffer (pH 4.5). After 24 h, the reaction was buffer exchanged with PBS (pH 7.0) with Zeba desalting columns (Pierce), and the crude reaction was purified by anion exchange chromatography (Mono Q 5/50 G, Buffer A: 20 mM Tris, 10 mM NaCl, pH 7.5; Buffer B: 20 mM Tris, 2 M NaCl, pH 7.5) to remove excess DNA and unreacted protein.[3,5]

Synthesis of anti-HER2-Alexa Fluor 488 conjugate: The previously described S202-pAcPhe anti-HER2 Fab (100 μM) was combined at 37 °C with 1 mM Alexa Fluor® 488 C_5-Aminooxyacetamide (Life Technologies) in the presence of 100 mM 4-methoxyaniline in 100 mM acetate buffer (pH 4.5)[2]. After 16 h, the product was purified by Zeba Spin desalting column (to remove excess dye and 4-methoxyaniline) before being analyzed by SDS-PAGE and mass spectrometry (data not shown).

Staining of the SK-BR-3 (HER2+) and MDA-MB-231 (HER2-) breast cancer cells lines: The cells were stained using CellTracker™ Orange (Life Technologies) by following the manufacturer's protocol.

Preparation of WBCs for blood spiking experiment: Blood was collected from The Scripps Research Institute Normal Blood Donor Service under Institutional Review Board (IRB) approved protocols. WBC count was determined with a HemoCue (HemoCue). Red blood cells (RBCs) were lysed (150 mM NH_4Cl, 10 mM $KHCO_3$, 130 nM EDTA), pelleted, and the RBCs in the supernatant were aspirated. The remaining WBCs were resuspended in 1× PBS.

Supplementary Figures

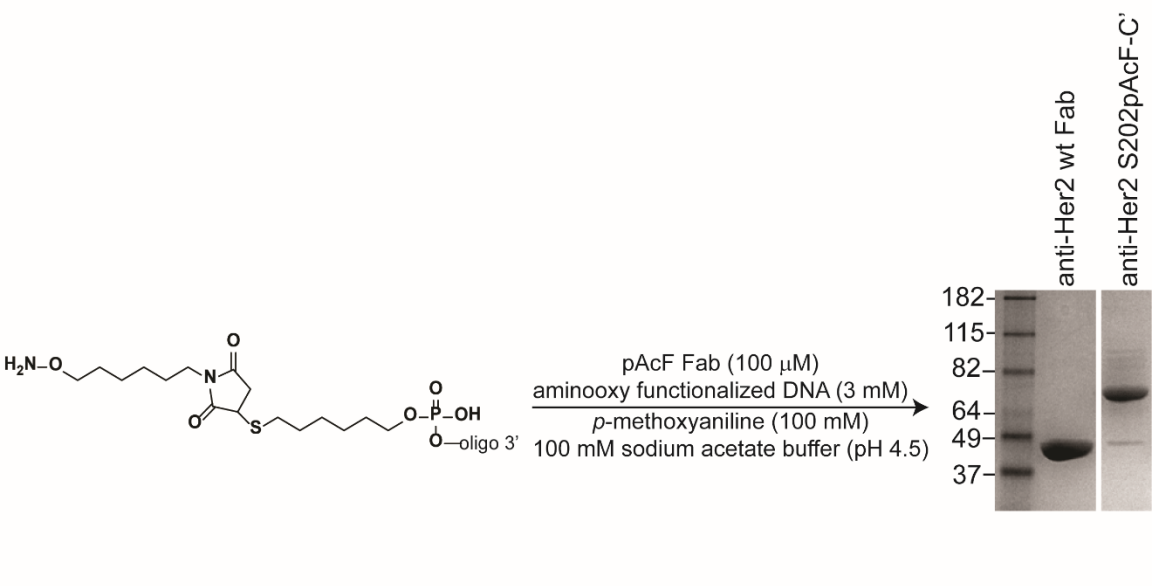


***Figure S1*:** Linker-DNA structure and reaction conditions for the synthesis of the antibody-DNA conjugate (left). SDS-page gel analysis of the reaction mixture prior to purification of the antibody-oligonucleotide conjugation reaction (right). The conjugation efficiency is >90% as determined by gel densitometry.

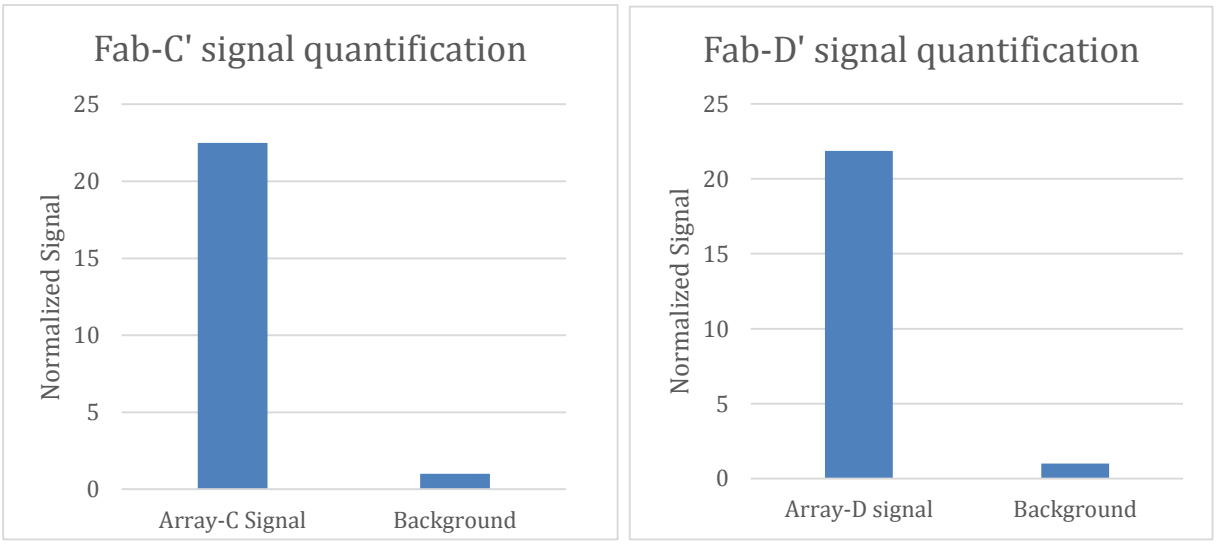


Figure S2: Signal quantification for Figure 2B, of the main text. Signal-to-noise comparison of anti-Her2 S202pAcF-oligonucleotide conjugates bound to C and D subarrays (detected with anti-kappa-R-PE). Quantification was performed using Image Studio Lite software. The values shown are the total signal (sum of the red, green, and blue channel signal values) normalized to background signal.

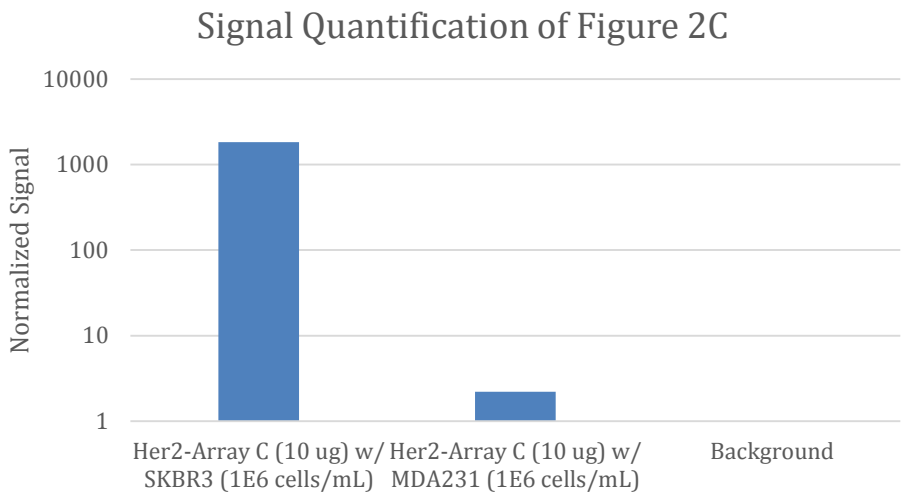


Figure S3: Signal quantification for Figure 2C, of the main text. This figure shows a comparison of signal between a HER2+ (SKBR3) cell line and a HER2- (MDA-MB-231) cell line when undergoing the cell binding assay described. Quantification was performed using Image Studio Lite software. The values shown are the total signal (sum of the red, green, and blue channel signal values) normalized to background signal.

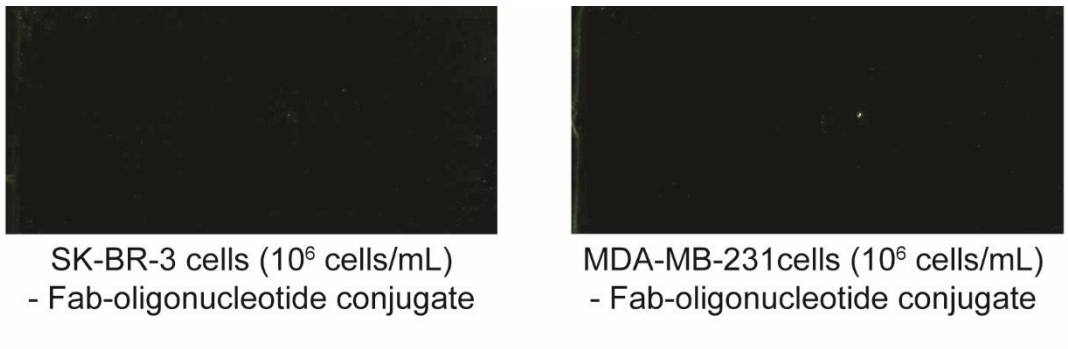


Figure S4: SK-BR-3 (Her2 +) or MDA-MB-231 (Her2 -) were stained with CellTracker™ orange and added to the array slides. An evanescence field reader was used to image the slides at a 555 nm excitation. No fluorescence was observed indicating the cells do no non-specifically stick to the array slides.

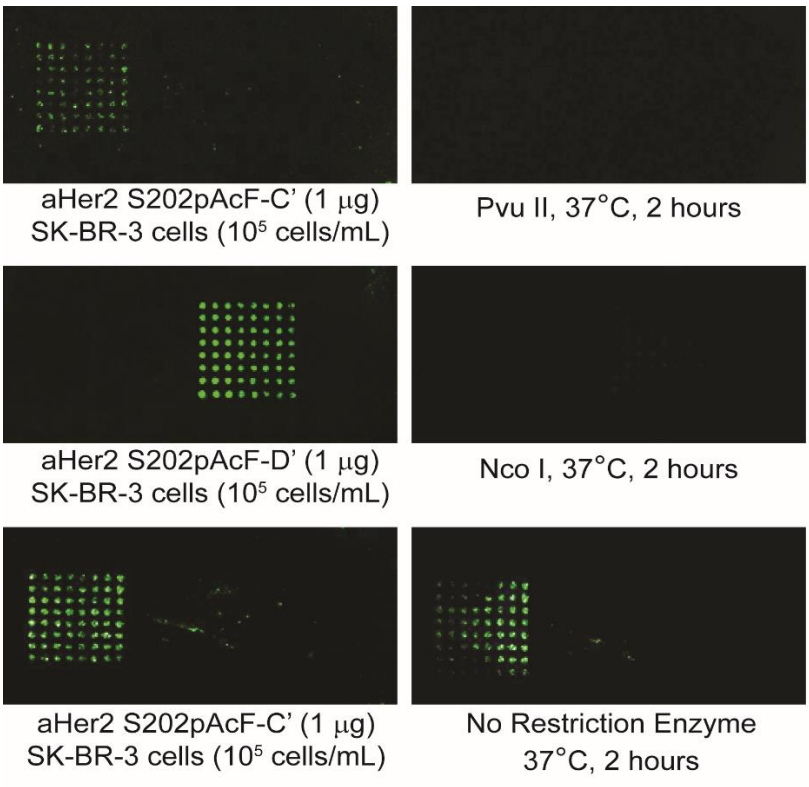
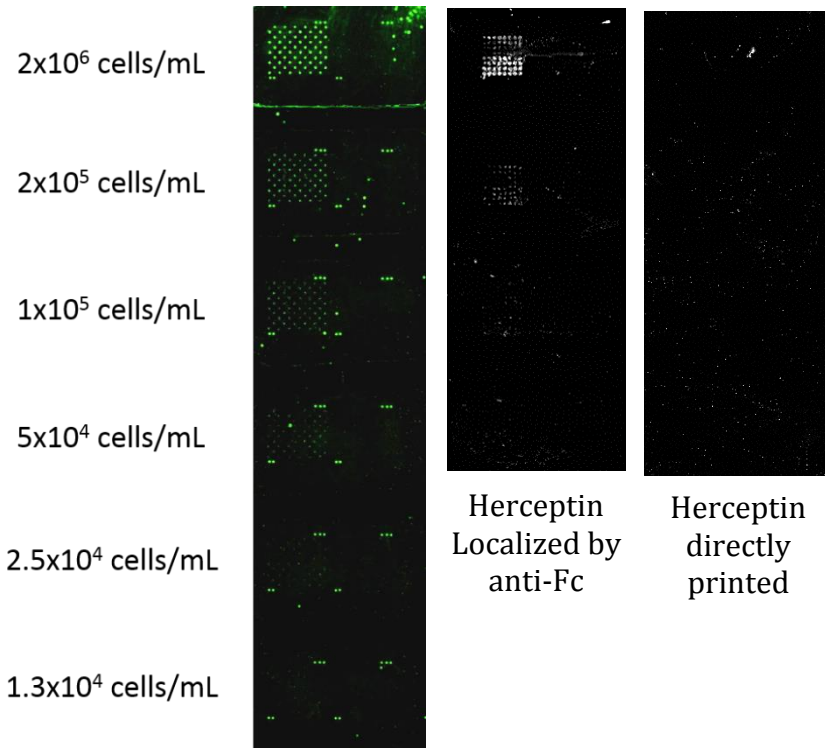


Figure S5: The general procedure for cell immobilization was followed using the indicated quantity of antibody conjugate and SKBR3 cells. 1 μL of the indicated restriction enzyme in 89 μL of water and 10 μL of NEB buffer 4 was placed on the array and incubated at 37 ºC for 2 hours before washing and imaging.

A



B

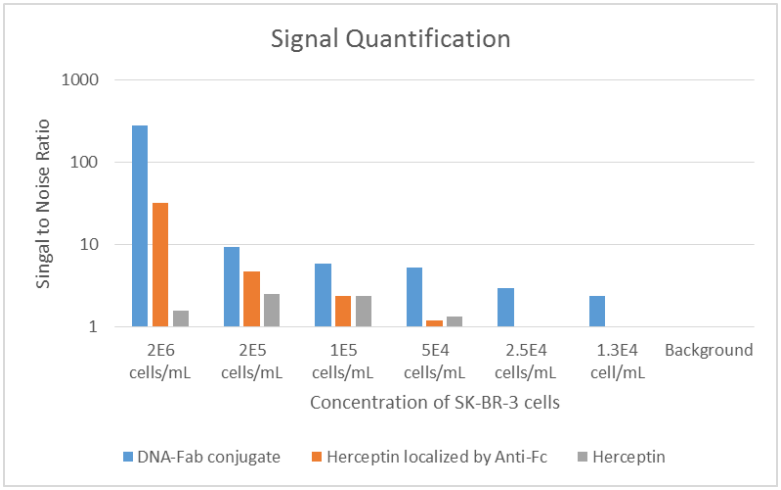


Figure S6: *A)* Titration of SK-BR-3 cells. Fab-Oligonucleotide slide was prepared with a constant quantity of 1 ug of anti-Her2 S202pAcF-C' (in 100 uL PBS) pre-hybridized to the slide before addition of cells in PBS. "Herceptin localized by anti-Fc" slide was prepared by printing anti- human Fc mAb at a concentration of 1 mg/mL onto the slide and then immediately prior to the cell localization experiment, 10 ug of Herceptin (in 100 uL PBS) was added and allowed to localize for 20 minutes. "Herceptin directly printed" slide was prepared by printing Herceptin onto the slide at a concentration of 1 mg/mL, this was followed by the cell immobilization experiement. Different concentrations of SK-BR-3 cells (stained with CellTracker™ Orange) in 100uL were added to each well of the slide. Slides were imaged at a 555 nm excitation. *B)* Quantification was performed using Image Studio Lite software. The values shown are the total signal (sum of the red, green, and blue channel signal values) to noise ratio.

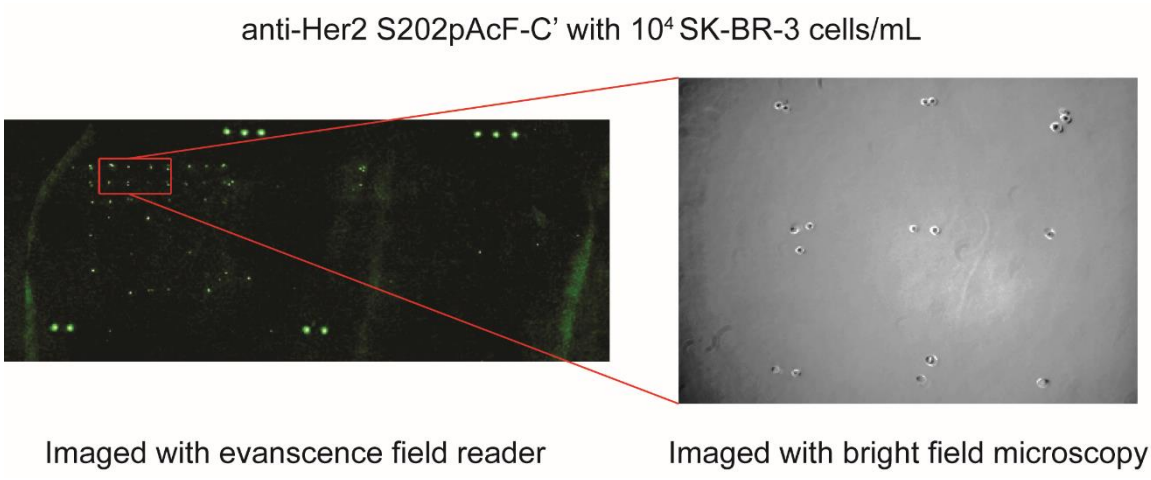


Figure S7: 1 μg anti-Her2 S202pAcF-C' pre-hybridized to the array followed by the addition of 10^4 SKBR3 cells/mL following the general procedure for cell immobilization. Imaged with both an evanescence field reader as well as with standard bright field microscopy. Bright green dots on the top and bottom of the array are used for reference.

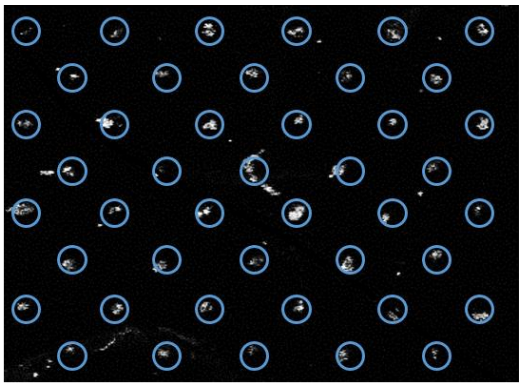


Figure S8: Array spot locations highlighted in cell binding assays. The array spot locations are highlighted (blue) to show the defined locations that the cells bind to when the array is saturated with the target cell type. These "highlights" were then transposed to show that the fluorescent spots fall within the array spot locations (Figure 4C).

Supplemental References:

(1) Wang, L., Zhang, Z., Brock, A., and Schultz, P. G. (2003) Addition of the keto functional group to the genetic code of Escherichia coli. *Proc. Natl. Acad. Sci. U. S. A. 100*, 56-61.

(2) Hutchins, B. M., Kazane, S. A., Staflin, K., Forsyth, J. S., Felding-Habermann, B., Schultz, P. G., and Smider, V. V. (2011) Site-specific coupling and sterically controlled formation of multimeric antibody fab fragments with unnatural amino acids. *J. Mol. Biol. 406*, 595-603.

(3) Kazane, S. A., Sok, D., Cho, E. H., Uson, M. L., Kuhn, P., Schultz, P. G., and Smider, V. V. (2012) Site-specific DNA-antibody conjugates for specific and sensitive immuno-PCR. *Proc. Natl. Acad. Sci. U. S. A. 109*, 3731-3736.

(4) Bailey, R. C., Kwong, G. A., Radu, C. G., Witte, O. N., and Heath, J. R. (2007) DNA-encoded antibody libraries: a unified platform for multiplexed cell sorting and detection of genes and proteins. *J. Am. Chem. Soc. 129*, 1959-1967.

(5) Hutchins, B. M., Kazane, S. A., Staflin, K., Forsyth, J. S., Felding-Habermann, B., Smider, V. V., and Schultz, P. G. (2011) Selective formation of covalent protein heterodimers with an unnatural amino acid. *Chem. Biol. 18*, 299-303.

(6) Berndt, M., Pietzsch, J., and Wuest, F. (2007) Labeling of low-density lipoproteins using the 18F-labeled thiol-reactive reagent N-[6-(4-[18F]fluorobenzylidene)aminooxyhexyl]maleimide. *Nucl. Med. Biol. 34*, 5-15.