Published in final edited form as:

Anal Chem. 2019 February 19; 91(4): 2695–2700. doi:10.1021/acs.analchem.8b04153.

DNA-Barcoded pMHC Tetramers for Detection of Single Antigen-Specific T Cells by Digital PCR

Shreyas N. Dahotre[†], Yun Min Chang^{†,‡}, Anna M. Romanov[†], and Gabriel A. Kwong^{*,†,§,⊥,||,▽}

[†]Department of Biomedical Engineering, Wallace H. Coulter, Georgia Tech and Emory School of Medicine, Atlanta, GA 30332, United States

[‡]Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322, United States

[§]Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Tech, Atlanta, GA 30332, United States

[⊥]Institute for Electronics and Nanotechnology, Georgia Tech, Atlanta, GA, 30332, United States

^{||}Integrated Cancer Research Center, Georgia Tech, Atlanta, GA, 30332, United States

[▽]Georgia ImmunoEngineering Consortium, Georgia Tech and Emory University, Atlanta, GA 30332, United States

Abstract

Antigen-specific T cells are found at low frequencies in circulation but carry important diagnostic information as liquid biomarkers in numerous biomedical settings, such as monitoring the efficacy of vaccines and cancer immunotherapies. To enable detection of antigen-specific T cells with high sensitivity, we develop peptide-MHC (pMHC) tetramers labeled with DNA barcodes to detect single T cells by droplet digital PCR (ddPCR). We show that site-specific conjugation of DNA via photocleavable linkers allows barcoded tetramers to stain T cells with similar avidity compared to conventional fluorescent tetramers, and efficient recovery of barcodes by light with no loss in cell viability. We design an orthogonal panel of DNA-barcoded tetramers to simultaneously detect multiple antigen-specific T cell populations, including from a mouse model of viral infection, and discriminate single cancer-specific T cells with high diagnostic sensitivity and specificity. This approach of DNA-barcoding can be broadened to encompass additional rare cells for monitoring immunological health at the single cell level.

Graphical abstract

*To whom correspondence should be addressed., Address: 345 Ferst Dr, Atlanta, GA 30332, Phone: 404-385-3746, gkwong@gatech.edu, ORCID:0000-0002-6255-6755.

Supporting Information

Supplementary materials and methods, table of DNA barcodes, gating scheme for cell enrichment, comparison of barcodes on different transgenic T cells, sensitivity of free barcode detection by ddPCR, detection of low frequency cells by ddPCR

Rare cells circulating at low frequencies in blood (e.g., 1–10 cells in 1 ml blood)¹, such as stem cells^{2,3}, circulating tumors cells^{4,5}, and antigen-specific immune cells^{6,7}, have important applications in basic research and translation^{8,9}, including use as liquid biomarkers for monitoring disease. Low frequency T cell subsets are particularly important and carry a wealth of information; for example, the number of naïve T cells predicts the strength of an immune response^{10,11}, the dynamics of T cell expansion and contraction correlate with pathogen infection and clearance^{12–16}, and the quantity of circulating antigen-specific T cells is a key biomarker of efficacy for cancer immunotherapies including engineered T cells and checkpoint blockade^{17–21}. Despite their importance, it remains technically challenging to detect antigen-specific T cells, requiring expression of engineered peptide MHC (pMHC) multimers²², and complex analytical platforms capable of multiplexed analysis such as multi-color combinatorial flow cytometry^{23,24}, next generation sequencing²⁵, or cytometry by time of flight (CyTOF)²⁶. Although powerful, these approaches require deep technical expertise and target cells cannot be recovered after analysis, precluding routine use for biomedical diagnostics.

Here we develop pMHC tetramers labeled with photo-labile DNA barcodes to allow detection of rare viral-specific and cancer-specific T cells by droplet digital PCR (ddPCR). Analytical platforms based on DNA leverage the unique properties of nucleic acids, such as base-pairing, to achieve ultrasensitive or highly multiplexed detection. These approaches include the use of protein-DNA conjugates for self-assembled cellular arrays^{27,28} and engineered tissues²⁹, and dynamic DNA gates for multiplexed cell sorting³⁰. DNA barcodes have also been incorporated into lipid nanoparticle libraries for drug delivery screens *in vivo*³¹, and used for ultrasensitive detection of clinical biomarkers with nanoparticles^{32,33}, immunoPCR^{34–36}, and proximity ligation^{37,38}. By labeling pMHC tetramers with photo-labile DNA barcodes, our approach allows staining of antigen-specific T cells in a complex sample, harvest of barcodes by light while preserving cell viability, and ultrasensitive detection of single T cells by droplet digital PCR.

Materials and Methods

DNA Barcode Conjugation to StvC

DNA barcodes containing a 5' NH₂, an internal Cy5, and an internal photocleavable linker (see Table S1) were purchased from Integrated DNA Technologies with HPLC purification and reconstituted to 100μM in 1X PBS. StvC was produced as described in Supporting Information. For bioconjugation, StvC was reduced with 10mM TCEP and then reacted with 50-fold excess MHPH (Solulink), and amine-terminated DNA was reacted with 25-fold

excess S-4FB (Solulink) for 4 hours. Excess linker was removed by buffer exchanging into citrate buffer (50mM sodium citrate, 150mM NaCl, pH 6) using Amicon spin filters (Millipore). Functionalized DNA and StvC were combined at a 1:1 ratio and reacted overnight before purifying on a Superdex 200 Increase 10/300 GL column using an AKTA Pure FPLC (GE Healthcare). Conjugation was verified by SDS-PAGE followed by Coomassie staining.

To compare binding avidity of DNA-barcoded to conventional fluorophore-labeled pMHC tetramers, tetramers were synthesized by reacting biotinylated Db-GP100_{25–33} pMHC with StvC conjugated with either Cy5 or DNA barcode-Cy5 at a 4:1 molar ratio. Splenocytes isolated from pmel TCR transgenic mice (Jackson Labs) were co-stained with anti-mouse CD8 (Clone: 53–6.7, BD Biosciences) and tetramer at various concentrations, and Cy5 intensity was analyzed by flow cytometry using a BD Accuri C6. Mean Cy5 intensity in the tetramer positive population was used as a measure of tetramer binding, and binding data was fit to a one site model using GraphPad Prism 6.0.

Barcode Release and Cell Viability

pMHC tetramers were generated as described above using PE-conjugated streptavidin (Invitrogen) or StvC conjugated with DNA barcode containing an internal photocleavable linker and a 3' FAM (see Table S1). Splenocytes were co-stained with anti-mouse CD8 (Clone: 53–6.7, BD Biosciences), tetramer-PE, and tetramer-DNA-FAM, and then tetramer positive cells were enriched using anti-PE microbeads (Miltenyi Biotec) and LS columns (Miltenyi Biotec) according to the manufacturer's instructions. Eluted cells were resuspended in 100µl PBS + 1% BSA in 1.5ml Eppendorf tubes, placed on ice, and irradiated for various durations in a UVP CL-1000L Crosslinker (365nm). FAM and PE signal was measured by flow cytometry using a BD Accuri C6. To measure cell viability after UV irradiation, unstained splenocytes were irradiated under UV light for various durations, stained using a Annexin V Apoptosis Detection Kit (BD Biosciences) according to the manufacturer's instructions, and then analyzed using a BD Accuri C6.

DNA Barcode Analysis by ddPCR

To check for orthogonality between multiple barcodes and primer sets, free barcodes were serially diluted 1:10 in nuclease-free water to 10–13 M followed by a single 1:5 dilution to 2×10–14 M. A QX200 EvaGreen Supermix master mix (Bio-Rad) was created containing 125nM primers, and 5µl of each diluted barcode was combined with 20µl of each primer master mix. 20µl of this reaction was used to create droplets using a QX200 Droplet Generator (Bio-Rad) according to the manufacturer's instructions. Droplets were thermal cycled in a Eppendorf Mastercycler using the following conditions: 95°C for 5 min, 40 cycles of 95°C for 30 sec and 60°C for 1 min, 4°C for 5 min, 90°C for 5 min, and hold at 4°C. Droplets were then analyzed on a QX200 Droplet Reader (Bio-Rad).

For cell studies, splenocytes from OT1, P14, and/or pmel TCR transgenic mice (Jackson Labs) or from mice infected with LCMV Armstrong were stained with DNA barcoded tetramers. Barcodes were isolated by irradiating stained cells in a UVP CL-1000L Crosslinker (365nm) on ice for 30 min. Cells were spun down at 1000xg for 5 min and the

supernatant containing barcode was analyzed by ddPCR as described above. See Supporting Information for more detailed experimental conditions.

Results and Discussion

Conventional pMHC tetramers are produced by combining biotinylated pMHC monomers with streptavidin to produce a four-fold valent construct. To label tetramers with DNA barcodes, we expressed recombinant streptavidin engineered with C-terminal cysteines (StvC)³⁹ to introduce free thiol groups for site-specific conjugation. We reacted DNA barcodes containing a 5' amine and a 3' Cy5 fluorescent reporter (Table S1) with StvC using heterobifunctional linkers (MHPH (maleimide-to-hydrazine) and S-4FB (NHS ester-to-aldehyde)) to form a stable bis-arylhydrazone bond as described previously²⁸ (Figure 1A). The resulting StvC-DNA conjugates were purified by size-exclusion chromatography (Figure 1B) and the coupling efficiency was quantified by gel mobility shift assays (~1.4 DNA barcodes per StvC molecule; Figure 1C). To analyze tetramer binding avidity, we compared the ability of DNA-barcoded tetramers to stain cancer-specific T cells isolated from transgenic pmel mice, which recognize the melanoma antigen gp100 (Db-GP100₂₅₋₃₃), to control fluorescent tetramers made by reacting StvC with Cy5 maleimide (Figure 1D). By quantifying the mean fluorescent intensities at tetramer staining concentrations ranging from 0–600 nM, we observed nearly identical binding curves and calculated apparent dissociation constants ($K_{d,app}$)⁴⁰ of 32 nM and 12 nM for DNA-barcoded and fluorescent tetramers respectively (Figure 1E). Together these results demonstrated that DNA-barcoded tetramers, made by site-specific coupling to StvC, stain antigen-specific T cells with binding similar to standard fluorescent tetramers.

We next sought to incorporate photo-cleavable linkers to allow harvest of DNA barcodes from the surface of target T cells without affecting cell viability. We synthesized DNA barcodes containing a photocleavable 1-(2-nitrophenyl) ethyl linker, which is sensitive to cleavage by long-wavelength UV light (~365nm). To quantify cleavage efficiency on the surface of T cells, we co-stained pmel splenocytes with photocleavable tetramers labeled with FAM, and conventional tetramers labeled with PE as a control (Figure 2A). We found that co-stained T cells initially exhibited fluorescence in both FAM and PE channels as expected (0 minute sample), but after 30 minutes of UV-light irradiation, we detected barcode cleavage by loss of FAM fluorescence (Figure 2B). By contrast, the intensity of PE fluorescence remained at identical levels after photo-cleavage, indicating that the ability of tetramers to bind to T cells was not affected by exposure to UV-light. We further stained splenocytes with Annexin V and propidium iodide to quantify the percentage of apoptotic and necrotic cells, respectively, following photo-cleavage. We observed no increase in the frequency of Annexin V+/PI– or Annexin V+/PI+ cells post-irradiation, indicating that our barcode isolation workflow did not affect cell viability (Figure 2C). Taken together, these results showed that DNA barcodes can be efficiently recovered from the surface of target cells by UV light while preserving cell viability.

We next designed a multiplex set of DNA barcodes (A–C) containing orthogonal primer binding regions to minimize potential amplification biases during barcode quantification by ddPCR (Table S1); barcodes contained 50% G/C content with primer melting temperatures

between 63–64°C and were screened for minimal secondary structure *in silico* using NUPACK⁴¹. ddPCR uses a water-in-oil emulsion to dilute target DNA until a single molecule is isolated within a nanoliter droplet. Only droplets containing a strand of target DNA are amplified during thermal cycling, and the final frequency of amplified droplets allows the target DNA concentration to be calculated by Poisson statistics. In samples containing a matched barcode-primer set, target counts were amplified to greater than 103 copies per μl compared to unmatched controls (<5 copies per μl), and the coefficient of variance of the barcode-primer sets was less than 3% (Figure 3A). These results were within the range of values reported by previous studies designing orthogonal primers for unbiased multiplexed PCR⁴². We synthesized a panel of three pMHC tetramers to simultaneously detect T cells specific for the model immunogen ovalbumin (Kb-OVA_{257–264}; Barcode A), the viral antigen LCMV glycoprotein (Db-LCMV GP_{33–41}; Barcode B), and the human melanoma antigen gp100 (Db-GP100_{25–33}; Barcode C). In samples containing a mixture of different frequencies of transgenic T cells specific for these antigens (OT1, P14, and pmel T cells respectively), we stained T cells *en masse*, and quantified barcodes released by UV-light cleavage by ddPCR (Figure 3B,C). For each antigen-specific T cell population, barcode counts were highly correlated ($R^2 = 0.95–0.99$) with T cell frequency within the population as measured by flow cytometry (Figure 3D, Figure S1). Importantly, we found that detection of each strain was barcode sequence independent as switching the barcode associated with each tetramer resulted in similar counts by ddPCR (Figure S2). Because TCR transgenic mice express high affinity, monoclonal TCRs, we then tested if this approach could be used to monitor T cell dynamics during an endogenous polyclonal immune response, where T cells may have a broad range of avidities to different antigens derived from a single pathogen⁴³. We infected mice with the Armstrong strain of lymphocytic choriomeningitis virus (LCMV), isolated splenocytes 0, 3, 8, and 148 days post-infection, and performed barcode analysis to detect T cells specific for the LCMV antigens GP33, GP276, and NP396 (Figure 4A). We found that barcode counts correlated well with frequencies as measured by flow cytometry (Figure 4B), which validated this approach for multiplexed analysis of T cells.

To determine whether barcoded tetramers could resolve single T cells, we first quantified the sensitivity of ddPCR by assaying samples containing free DNA barcodes at a copy number range of 7.5×10^1 – 1.5×10^5 (or 5×10^{-14} – 2.5×10^{-17} M in 5 μl), which we selected to correspond to the average number of surface TCRs per cell⁴⁴. Under these conditions, barcode counts were linearly quantified across the entire range of concentrations ($R^2 = 0.99$) down to 0.025 fM (Figure S3), demonstrating the potential of ddPCR to detect free barcodes at levels expected from single cells. We verified this observation using a ladder of P14 T cells sorted by fluorescence-activated cell sorting (FACS) and stained with barcoded tetramer and similarly found a linear relationship between cell number and barcode concentration ($R^2=0.99$), even for low cell counts (Figure 5A). Sensitivity was not reduced in the presence of high background, as we could detect P14 T cells spiked into control B6 splenocytes at frequencies as low as 5 P14 T cells in 1 million B6 splenocytes ($\sim 0.01\%$ when gated on CD8 positive cells) (Figure S4). To determine the diagnostic sensitivity and specificity of single T cell detection, we quantified barcode counts from pmel splenocytes co-stained with barcoded GP100 and control GP33 tetramers, and found that single cell

barcode counts for the pMHC tetramer were significantly elevated compared to control P14 tetramers (Figure 5B). By receiver operating characteristic (ROC) analysis – which is used to determine the sensitivity (true positive rate) and specificity (true negative rate) of a diagnostic classifier – DNA-barcoded tetramers discriminated single antigen-specific T cells with an area under the curve (AUC) accuracy of 0.91, which is significantly higher than a random classifier (AUC = 0.5, perfect classifier AUC = 1.0) (Figure 5C). These results show that staining by DNA-barcoded tetramers followed by ddPCR analysis can be used to measure the frequency of multiple antigen-specific T cell populations from biological samples with single-cell sensitivity.

Here we develop DNA barcoded pMHC tetramers for analysis of antigen-specific T cells by ddPCR. We show that barcoded tetramers stain T cells equivalent to their fluorescent counterparts and demonstrate that barcodes are non-destructively isolated from the surface of cells by UV irradiation. Using ddPCR, we show multiplexed detection of T cells against three different antigens from an LCMV-infected mouse and achieve single cell sensitivity. Importantly, this barcoding approach is compatible with advances in tetramer staining protocols⁴⁵ and the development of higher order multimers⁴⁶ for detection of T cells expressing low affinity TCRs. In the future, the library of DNA barcodes can be expanded to screen peptides for TCR reactivity⁴⁷ and to monitor the dynamics of T cells through naïve, effector, and memory states during the course of disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank D. Arafat for assistance with ddPCR. This work was funded by an NIH Director's New Innovator Award (Award No. DP2HD091793) and by the National Center for Advancing Translational Sciences of the NIH (Award No. UL1TR000454). S.N.D. is supported by the NSF Graduate Research Fellowships Program (Grant No. DGE-1650044) and the NSF Integrative Graduate Education and Research Traineeship (Grant No. DGE-0965945). Y.M.C. is supported by the National Institute of General Medical Sciences of the NIH (Award No. T32GM8169–30). G.A.K. holds a Career Award at the Scientific Interface from the Burroughs Wellcome Fund. This work was performed in part at the Georgia Tech Institute for Electronics and Nanotechnology, a member of the National Nanotechnology Coordinated Infrastructure, which is supported by the NSF (Grant No. ECCS-1542174).

References

- (1). Brinkmann F; Hirtz M; Haller A; Gorges TM; Vellekoop MJ; Riethdorf S; Muller V; Pantel K; Fuchs H *Sci Rep* 2015, 5, 15342. [PubMed: 26493176]
- (2). Massberg S; Schaerli P; Knezevic-Maramica I; Kollnberger M; Tubo N; Moseman EA; Huff IV; Junt T; Wagers AJ; Mazo IB; von Andrian UH *Cell* 2007, 131, 994–1008. [PubMed: 18045540]
- (3). Xu L; Li G *Journal of Orthopaedic Translation* 2014, 2, 1–7.
- (4). Hodgkinson CL; Morrow CJ; Li Y; Metcalf RL; Rothwell DG; Trapani F; Polanski R; Burt DJ; Simpson KL; Morris K; Pepper SD; Nonaka D; Greystoke A; Kelly P; Bola B; Krebs MG; Antonello J; Ayub M; Faulkner S; Priest L, et al. *Nat Med* 2014, 20, 897–903. [PubMed: 24880617]
- (5). Miller MC; Doyle GV; Terstappen LW J *Oncol* 2010, 2010, 617421. [PubMed: 20016752]
- (6). Lee PP; Yee C; Savage PA; Fong L; Brockstedt D; Weber JS; Johnson D; Swetter S; Thompson J; Greenberg PD; Roederer M; Davis MM *Nat Med* 1999, 5, 677–685. [PubMed: 10371507]

- (7). Laban S; Suwandi JS; van Unen V; Pool J; Wesselius J; Holtt T; Pezzotti N; Vilanova A; Lelieveldt BPF; Roep BO PLoS One 2018, 13, e0200818. [PubMed: 30089176]
- (8). Rosenberg SA; Restifo NP Science 2015, 348, 62–68. [PubMed: 25838374]
- (9). Miller IC; Castro MG; Maenza J; Weis JP; Kwong GA Acs Synthetic Biology 2018, 7, 1167–1173. [PubMed: 29579381]
- (10). Moon JJ; Chu HH; Pepper M; McSorley SJ; Jameson SC; Kedl RM; Jenkins MK Immunity 2007, 27, 203–213. [PubMed: 17707129]
- (11). Schmidt J; Neumann-Haefelin C; Altay T; Gostick E; Price DA; Lohmann V; Blum HE; Thimme R J Virol 2011, 85, 5232–5236. [PubMed: 21367907]
- (12). Doherty PC; Christensen JP Annu Rev Immunol 2000, 18, 561–592. [PubMed: 10837069]
- (13). Whitmire JK Virology 2011, 411, 216–228. [PubMed: 21236461]
- (14). DeWitt WS; Emerson RO; Lindau P; Vignali M; Snyder TM; Desmarais C; Sanders C; Utsugi H; Warren EH; McElrath J; Makar KW; Wald A; Robins HS J Virol 2015, 89, 4517–4526. [PubMed: 25653453]
- (15). Ravindran R; McSorley SJ Immunology 2005, 114, 450–458. [PubMed: 15804280]
- (16). Ewer K; Millington KA; Deeks JJ; Alvarez L; Bryant G; Lalvani A Am J Respir Crit Care Med 2006, 174, 831–839. [PubMed: 16799072]
- (17). Gros A; Parkhurst MR; Tran E; Pasetto A; Robbins PF; Ilyas S; Prickett TD; Gartner JJ; Crystal JS; Roberts IM; Trebska-McGowan K; Wunderlich JR; Yang JC; Rosenberg SA Nat Med 2016, 22, 433–438. [PubMed: 26901407]
- (18). Akyuz N; Brandt A; Stein A; Schliffke S; Mahrle T; Quidde J; Goekkurt E; Loges S; Haalck T; Ford CT; Asemisen AM; Thiele B; Radloff J; Thenhausen T; Krohn-Grimberghe A; Bokemeyer C; Binder M Int J Cancer 2017, 140, 2535–2544. [PubMed: 27925177]
- (19). Butler MO; Friedlander P; Milstein MI; Mooney MM; Metzler G; Murray AP; Tanaka M; Berezovskaya A; Imataki O; Drury L; Brennan L; Flavin M; Neuberg D; Stevenson K; Lawrence D; Hodi FS; Velazquez EF; Jaklitsch MT; Russell SE; Mihm M, et al. Sci Transl Med 2011, 3.
- (20). Jacquelot N; Roberti MP; Enot DP; Rusakiewicz S; Ternes N; Jegou S; Woods DM; Sodre AL; Hansen M; Meirow Y; Sade-Feldman M; Burra A; Kwek SS; Flament C; Messaoudene M; Duong CPM; Chen L; Kwon BS; Anderson AC; Kuchroo VK, et al. Nature Communications 2017, 8.
- (21). Hong X; Sullivan RJ; Kalinich M; Kwan TT; Giobbie-Hurder A; Pan SW; LiCausi JA; Milner JD; Nieman LT; Wittner B; Ho U; Chen TQ; Kapur R; Lawrence DP; Flaherty KT; Sequist LV; Ramaswamy S; Miyamoto DT; Lawrence M; Toner M, et al. P Natl Acad Sci USA 2018, 115, 2467–2472.
- (22). Altman JD; Moss PA; Goulder PJ; Barouch DH; McHeyzer-Williams MG; Bell JI; McMichael AJ; Davis MM Science 1996, 274, 94–96. [PubMed: 8810254]
- (23). Hadrup SR; Bakker AH; Shu CJ; Andersen RS; van Veluw J; Hombrink P; Castermans E; Thor Straten P; Blank C; Haanen JB; Heemskerk MH; Schumacher TN Nat Methods 2009, 6, 520–526. [PubMed: 19543285]
- (24). Newell EW; Klein LO; Yu W; Davis MM Nat Methods 2009, 6, 497–499. [PubMed: 19543286]
- (25). Bentzen AK; Marquard AM; Lyngaa R; Saini SK; Ramskov S; Donia M; Such L; Furness AJS; McGranahan N; Rosenthal R; Straten PT; Szallasi Z; Svane IM; Swanton C; Quezada SA; Jakobsen SN; Eklund AC; Hadrup SR Nature Biotechnology 2016, 34, 1037–1045.
- (26). Newell EW; Sigal N; Nair N; Kidd BA; Greenberg HB; Davis MM Nat Biotechnol 2013, 31, 623–629. [PubMed: 23748502]
- (27). Bailey RC; Kwong GA; Radu CG; Witte ON; Heath JR J Am Chem Soc 2007, 129, 1959–1967. [PubMed: 17260987]
- (28). Kwong GA; Radu CG; Hwang K; Shu CJ; Ma C; Koya RC; Comin-Anduix B; Hadrup SR; Bailey RC; Witte ON; Schumacher TN; Ribas A; Heath JR J Am Chem Soc 2009, 131, 9695–9703. [PubMed: 19552409]
- (29). Gartner ZI; Bertozzi CR Proc Natl Acad Sci U S A 2009, 106, 4606–4610. [PubMed: 19273855]
- (30). Dahotre SN; Chang YM; Wieland A; Stammen SR; Kwong GA Proc Natl Acad Sci U S A 2018, 115, 4357–4362. [PubMed: 29632190]

- (31). Dahlman JE; Kauffman KJ; Xing Y; Shaw TE; Mir FF; Dlott CC; Langer R; Anderson DG; Wang ET *Proc Natl Acad Sci U S A* 2017, 114, 2060–2065. [PubMed: 28167778]
- (32). Halo TL; McMahon KM; Angeloni NL; Xu Y; Wang W; Chinen AB; Malin D; Strekalova E; Cryns VL; Cheng C; Mirkin CA; Thaxton CS *Proc Natl Acad Sci U S A* 2014, 111, 17104–17109. [PubMed: 25404304]
- (33). Kim D; Daniel WL; Mirkin CA *Anal Chem* 2009, 81, 9183–9187. [PubMed: 19874062]
- (34). Sano T; Smith CL; Cantor CR *Science* 1992, 258, 120–122. [PubMed: 1439758]
- (35). Niemeyer CM; Adler M; Wacker R *Trends Biotechnol* 2005, 23, 208–216. [PubMed: 15780713]
- (36). Niemeyer CM; Adler M; Wacker R *Nature Protocols* 2007, 2, 1918–1930. [PubMed: 17703203]
- (37). Gullberg M; Gustafsdottir SM; Schallmeiner E; Jarvius J; Bjarnegard M; Betsholtz C; Landegren U; Fredriksson S *Proc Natl Acad Sci U S A* 2004, 101, 8420–8424. [PubMed: 15155907]
- (38). Fredriksson S; Dixon W; Ji H; Koong AC; Mindrinos M; Davis RW *Nat Methods* 2007, 4, 327–329. [PubMed: 17369836]
- (39). Reznik GO; Vajda S; Cantor CR; Sano T *Bioconjug Chem* 2001, 12, 1000–1004. [PubMed: 11716692]
- (40). Stein RA; Wilkinson JC; Guyer CA; Staros JV *Biochemistry* 2001, 40, 6142–6154. [PubMed: 11352752]
- (41). Zadeh JN; Steenberg CD; Bois JS; Wolfe BR; Pierce MB; Khan AR; Dirks RM; Pierce NA *J Comput Chem* 2011, 32, 170–173. [PubMed: 20645303]
- (42). Carlson CS; Emerson RO; Sherwood AM; Desmarais C; Chung MW; Parsons JM; Steen MS; LaMadrid-Herrmannsfeldt MA; Williamson DW; Livingston RJ; Wu D; Wood BL; Rieder MJ; Robins H *Nature Communications* 2013, 4.
- (43). Zehn D; Lee SY; Bevan MJ *Nature* 2009, 458, 211–214. [PubMed: 19182777]
- (44). Schodin BA; Tsomides TJ; Kranz DM *Immunity* 1996, 5, 137–146. [PubMed: 8769477]
- (45). Rius C; Attaf M; Tungatt K; Bianchi V; Legut M; Bovay A; Donia M; Straten PT; Peakman M; Svane IM; Ott S; Connor T; Szomolay B; Dolton G; Sewell AK *Journal of Immunology* 2018, 200, 2263–2279.
- (46). Huang J; Zeng X; Sigal N; Lund PJ; Su LF; Huang H; Chien YH; Davis MM *P Natl Acad Sci USA* 2016, 113, E1890–E1897.
- (47). Birnbaum ME; Mendoza JL; Sethi DK; Dong S; Glanville J; Dobbins J; Ozkan E; Davis MM; Wucherpfennig KW; Garcia KC *Cell* 2014, 157, 1073–1087. [PubMed: 24855945]

Figure 1.

DNA-barcoded pMHC tetramers stain similarly to conventional fluorophore tagged pMHC tetramers. (A) Schematic of DNA-barcoded tetramer. (B) FPLC chromatogram of StvC-DNA reaction. The first peak in the StvC-DNA trace is collected as the conjugated product. (C) SDS-PAGE gel of purified StvC-DNA. (D) pmel splenocytes stained with control tetramer-Cy5 or tetramer-DNA-Cy5 show equivalent staining by flow cytometry. (E) Binding curve of pmel splenocytes stained with different concentrations of tetramer-Cy5 or tetramer-DNA-Cy5. $K_{d,app}$ was determined by fitting the data to model of receptor-ligand binding.

Figure 2.

DNA barcodes are efficiently released from the surface of cells by exposure to long-wave UV light. (A) Splenocytes from pmel mice were co-stained with tetramer-DNA-FAM and tetramer-PE followed by irradiation with UV light to cleave barcodes. (B) Cells exposed to UV light for 30 minutes lose FAM but not PE signal, indicating that barcodes are released. (C) Cells remain viable after irradiation with long-wave UV light.

Figure 3.

ddPCR analysis of DNA barcodes allows for multiplex detection of single antigen-specific T cells. (A) A panel of DNA barcodes do not exhibit crosstalk and amplify similarly, demonstrating ddPCR analysis is not affected by primer bias. (B) A mixture of different antigen-specific T cells can be stained with barcoded tetramers *en masse*. Barcodes are isolated by irradiation with UV light and analyzed by ddPCR. (C) Samples containing cells from OT1, P14, and pmel TCR transgenic mice were mixed at different ratios and stained with a pool of DNA-barcoded tetramers. After isolating barcodes by UV irradiation,

barcodes were analyzed by ddPCR to determine the relative frequency of individual target cell populations. (D) Barcode concentrations from ddPCR correlate strongly with cell frequencies determined by flow cytometry.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 4.

DNA-barcoded pMHC tetramers can monitor polyclonal T cell responses during infection. (A) Mice were infected with LCMV Armstrong and splenocytes were harvested at various timepoints for barcoded tetramer staining and ddPCR analysis. (B) The dynamics of T cells specific for three different LCMV derived antigens was monitored during viral infection and clearance. Data shown as mean $\pm$ s.d. $n=2$ for days 0, 3, 8; $n=1$ for day 148.

Figure 5.

ddPCR resolves single-antigen specific T cells with high diagnostic sensitivity and specificity. (A) Barcode count is proportional to number of antigen-specific T cells and can detect single cells. Data shown as mean $\pm$ s.d, $n=4$, $**p<0.01$ by two-tailed t-test (B) Single CD8⁺ T cells from pmel mice have significantly higher barcode counts from cognate pmel DNA-barcoded tetramers compared to irrelevant control P14 DNA-barcoded tetramers. $*p<0.05$ by two-tailed t-test (C) Barcode analysis by ddPCR correctly classifies single cells by antigen-specificity with high sensitivity and specificity.