Supporting Online Material for

Creating Protein Affinity Reagents by Combining Peptide Ligands on Synthetic DNA Scaffolds

Berea A. R. Williams, Chris W. Diehnelt, Paul Belcher, Matthew Greving, Neal W. Woodbury, Stephen A. Johnston*, John C. Chaput*

*To whom correspondence should be addressed:
Stephen A. Johnston: Telephone: (480) 727-0792, E-mail: stephen.johnston@asu.edu
John C. Chaput: Telephone: (480) 727-0392, E-mail: john.chaput@asu.edu

This PDF file includes

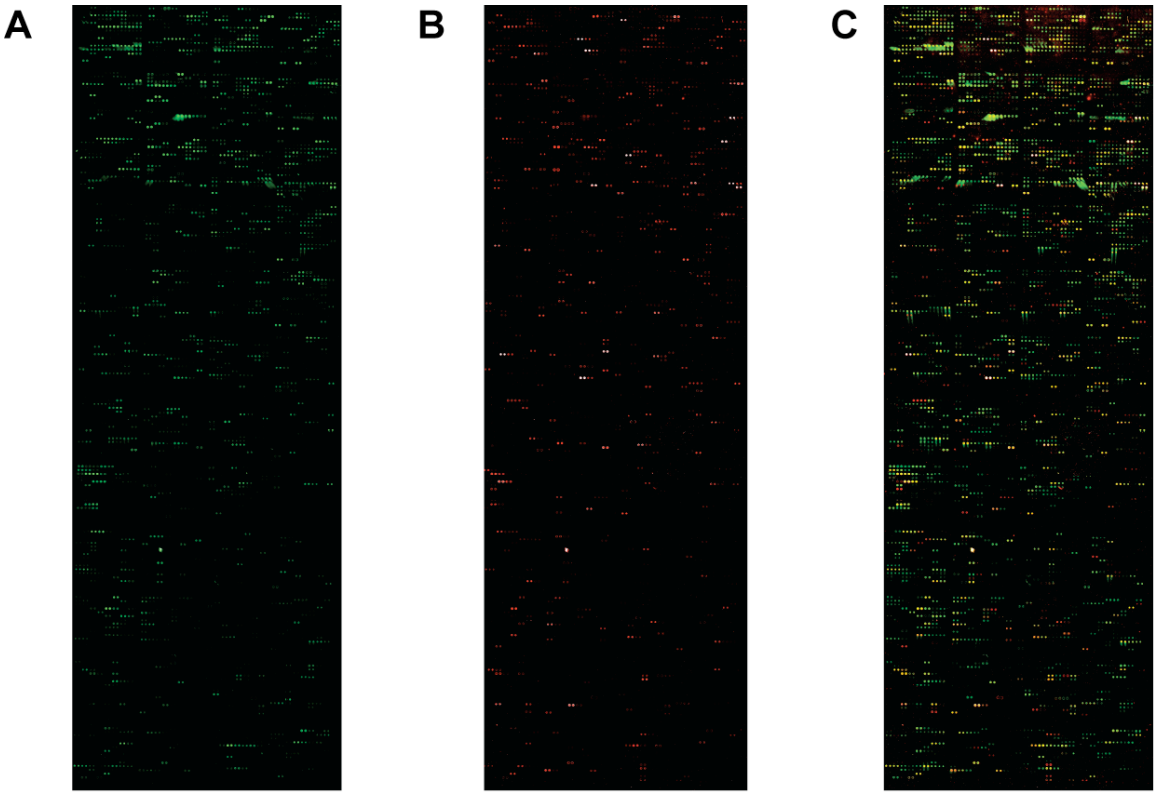


Figure S1. Transferrin ligand discovery using peptide microarrays. Custom peptide microarrays containing 10,000 unique 20-mer peptides were used to identify transferrin ligands. Alexa-555 labeled transferrin and Alexa-647 labeled *E. coli* lysate were mixed and incubated with the microarray. The slide was scanned for fluorescence at (A) 565 and (B) 665 nm, respectively. (C) The overlay of fluorescence at 565 and 665 nm was used to identify peptides with affinity to human transferrin in the presence of competing total *E. coli* lysate.

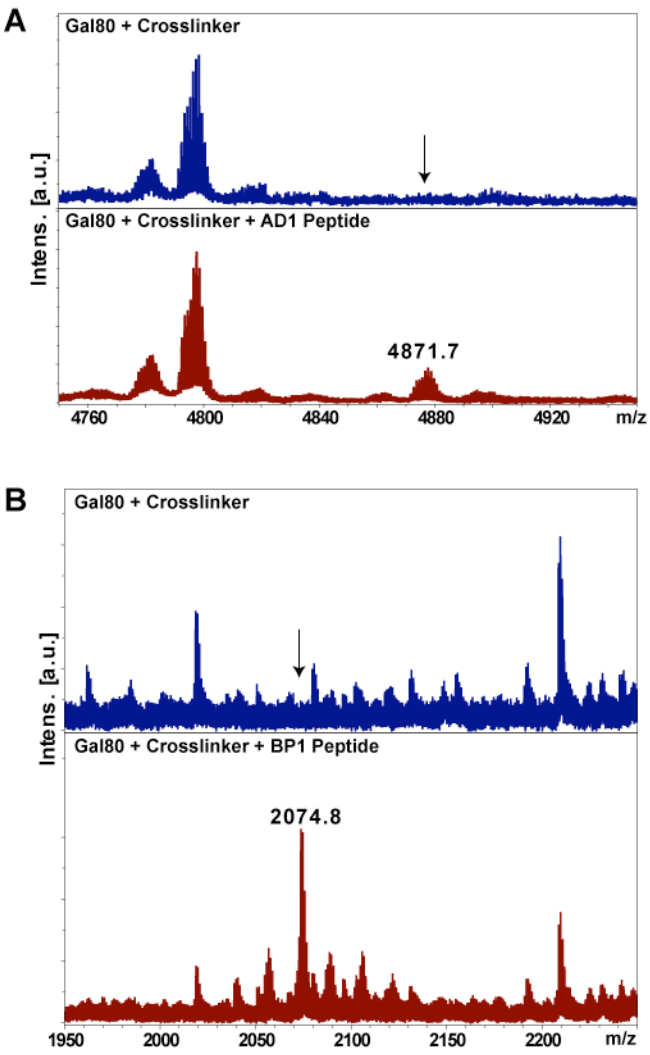


Figure S2. MALDI-TOF analysis of Gal80 crosslinked to each peptide ligand after trypsin digestion. (A) Mass spectrum of digested Gal80 protein with crosslinker (top) and Gal80 protein crosslinked to AD1 peptide (bottom). The peak that appears in the bottom but not the top spectra (indicated by arrow) corresponds to amino acid residues 1-4 of AD1 crosslinked to residues 384-420 of Gal80 (expected = 4871.5, observed = 4871.7). (B) Mass spectrum of Gal80 protein with crosslinker (top) and Gal80 protein crosslinked to BP1 peptide (bottom). The peak that appears in the bottom but not the top spectra (indicated by arrow) corresponds to amino acid residues 1-8 of BP1 crosslinked to residues 9-12 of Gal80 (expected = 2074.9, observed = 2074.8).

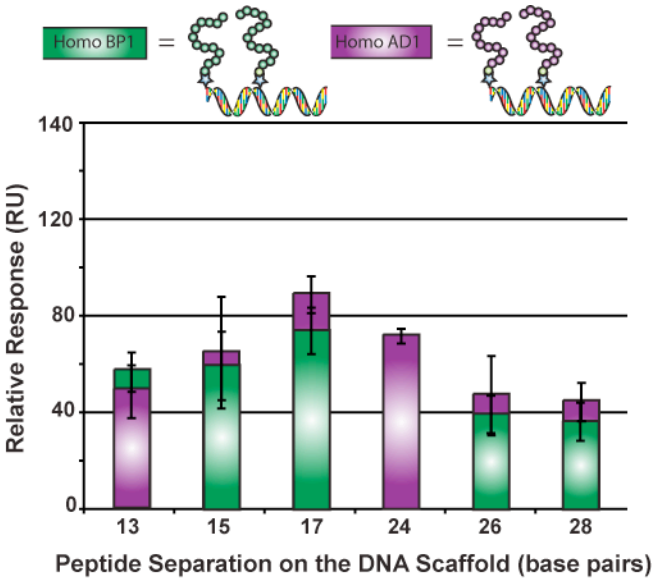


Figure S3. Relative binding affinity for homo-peptide Gal80 synbody combinations. Synbodies with homo-peptide pairs were spaced at different distances on the DNA scaffold and assayed for affinity to Gal80. Homo-BP1 synbody distance 24 was not assayed.

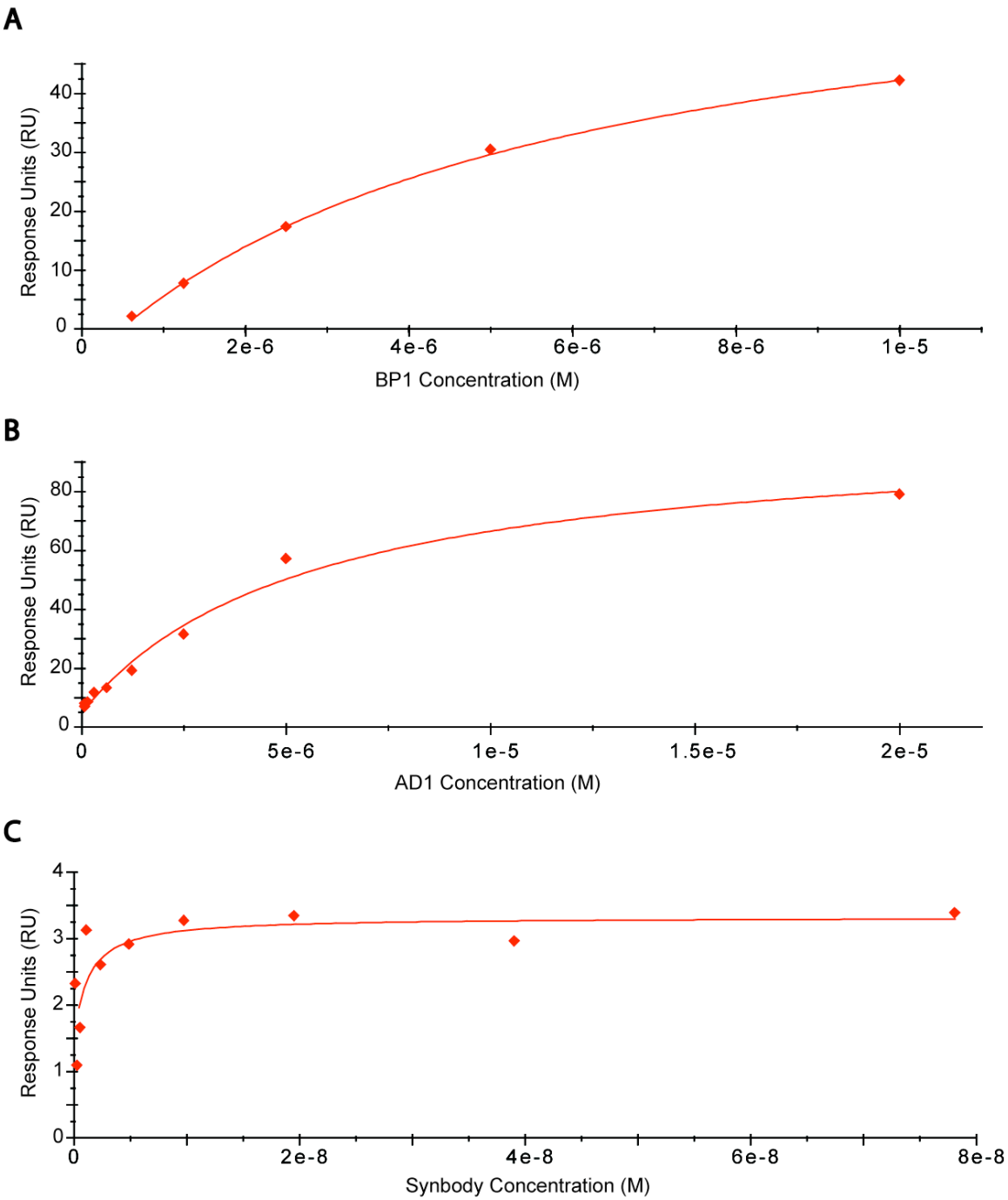


Figure S4. SPR analysis of Gal80 peptides and synbody construct 13. (A-C) Representative affinity plots for BP1, AD1, and SC-13 taken from multiple SPR assays, respectively. Affinity plots were created using the 1:1 binding model with the Biacore evaluation software.

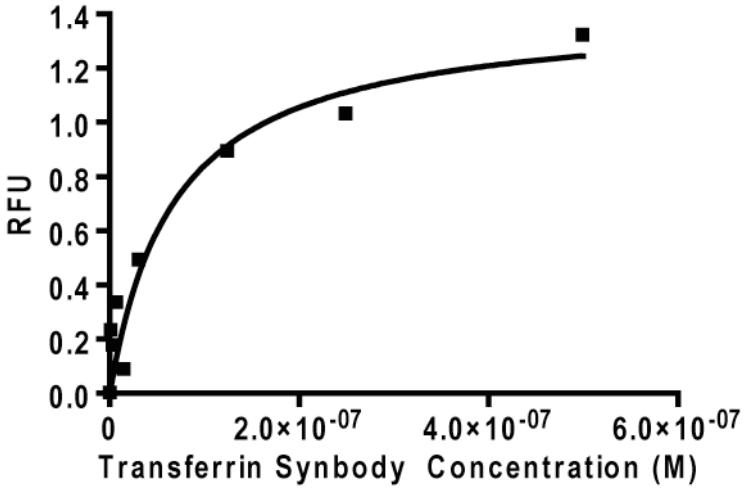


Figure S5. Binding affinity of the transferrin synbody. The binding affinity of SC-6 to transferrin was determined to be 68.4 ± 28.1 nM using an ELISA-type assay.

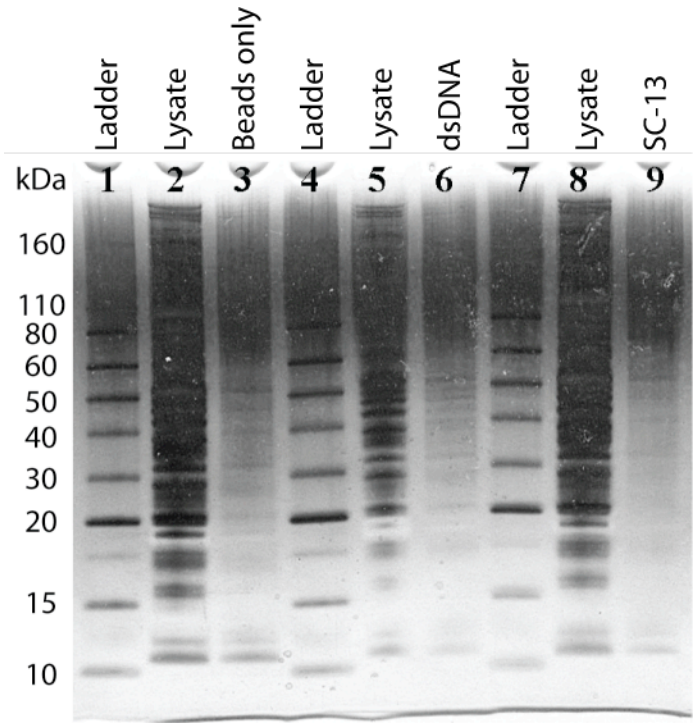
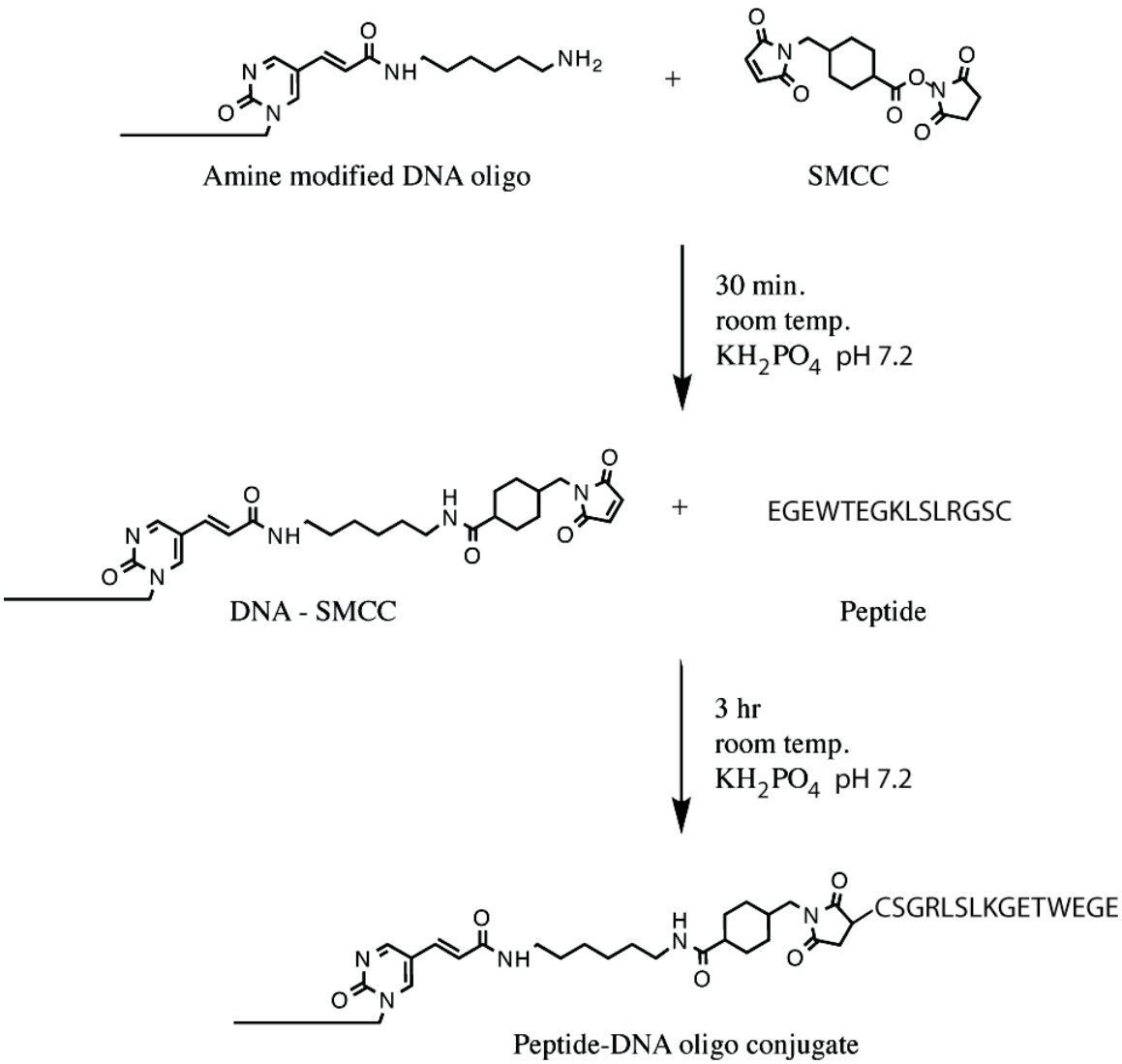


Figure S6. Control pulldown assay for non-specific binding to endogenous *E. coli* proteins. Background binding was analyzed using streptavidin coated magnetic (SA) beads (lane 3), SA beads containing the dsDNA scaffold (lane 6), and SC-13 (lane 9). Lanes 1, 4, and 7 contain the protein ladder; lanes 2, 5, and 8 contain crude *E. coli* lysate; lanes 3, 6, and 9 contain SDS elution from the beads. Each elution contained 100-fold greater volume than the crude lysate lane.



Scheme S1. Peptide conjugation to single-stranded DNA. The amine modified DNA oligonucleotide is conjugated to each peptide by first reacting the primary amine on the DNA with the NHS ester of the SMCC reagent, and then coupling a C-terminal cysteine residue on the peptide with the maleimide moiety of the DNA-SMCC intermediate.

Table S1. Gal80 Peptide Fluorescent Intensity Values[1]

Name	Sequence (N-C)	Gal80	Gal80 Blocked	Gal80/Gal80-Blocked	AAT	Transferrin
BP1	EGEWTEGKLSLR	236.55	11.86	19.95	0.75	1.09
BP2	WSKSRLLSGLSR	125.49	14.21	8.83	0.77	1.33
BP3	SERWESTSELRR	186.77	10.35	18.05	0.63	1.20
BP4	LWLETREGSLTR	180.19	10.22	17.63	0.58	1.52
BP5	GWSERGGSRTRL	143.39	17.81	8.05	0.69	1.32
BP6	WLESSGSTSLRR	141.74	16.50	8.59	1.08	1.03
AD1	GTEKGTSGWLKT	106.85	1.64	65.04	1.05	2.24
AD2	SGWGSSELRKER	21.11	0.68	30.85	0.50	0.72
AD3	TLWSETRTKRER	82.73	1.94	42.65	0.58	0.86
AD4	KKWSKLTEKLRT	56.28	1.94	28.99	0.77	1.78

[1]Fluorescent values are the average of Cy3 and Cy5 fluorescent intensities per peptide spot divided by the background fluorescent intensity of the array.

Table S2. Transferrin Peptide Fluorescent Intensity Values

Name	Sequence (N-C)	Transferrin	Transferrin/*E. coli*
TRF19	KEDNPGYSSEQDYNKLDGSC	19954.5	7.6
TRF20	GQTQFAMHRFQQWYKIKGSC	16295.8	7.3
TRF21	QYHHFMNLKRQGRAQAYGSC	15007.3	7.0
TRF22	HAYKGPGDMRRFNHSGMGSC	6012.8	6.2
TRF23	FRGWAHIFFGPHVIYRGGSC	12277.8	5.7
TRF24	SVKPWRPLITGNRWLNSGSC	10104.3	5.7
TRF25	APYAPQQIHYWSTLGFKGSC	7701.0	5.6
TRF26	AHKVVPQRQIRHAYNRYGSC	22763.0	5.6
TRF27	LDPLFNTSIMVNWHRWMGSC	16298.8	5.4
TRF28	RFQLTQHYAQFWGHYTWGSC	7502.0	5.2

[1]Fluorescent values are for Alexa-555 labeled transferrin and Alexa-657 labeled *E. coli* lysate per peptide spot.

Table S3. Transferrin-Peptide Crosslinking Analysis.

Peptide	Observed Mass (Da)	Transferrin Fragment	Amino Acid Fragment
TRF23	1798.9	KCSTSSLLEACTFRRP	664-679
TRF23	2003.9	APNHAVVTRKDKEACVHK	582-599
TRF23	2015.9	SDNCEDTPEAGYFAVAVVK	415-433
TRF26	1435.7	SASDLTWDNLKGK	435-447
TRF26	2518.3	KSCHTAVGRTAGWNIPMGLLYNK	448-470

Table S4. Gal80 Synbody Oligonucleotide Sequences.

Name	Sequence (5'-3')	Notes
Template	5CCGAAACAACCGCGAGAGGCACGCGCGTAGC	5= amino-modifier C6 dC
Fluorescein -Template	5CCGAAACAACCGCGAGAGGCACGCGCGTAGC3	5= amino-modifier C6 dC 3= 6-Fluorescein
Variable Construct 13	GCTACGCGCGTGCCTCTCG5GGTTGTTTCGGG	5= amino-modifier C6 dC
Variable Construct 15	GCTACGCGCGTGCCTCT5GCGGTTGTTTCGGG	5= amino-modifier C6 dC
Variable Construct 17	GCTACGCGCGTGCCT5TCGCGGTTGTTTCGGG	5= amino-modifier C6 dC
Variable Construct 24	GCTACGCG5GTGCCTCTCGCGGTTGTTTCGGG	5= amino-modifier C6 dC
Variable Construct 26	GCTACG5GCGTGCCTCTCGCGGTTGTTTCGGG	5= amino-modifier C6 dC
Variable Construct 28	GCTA5GCGCGTGCCTCTCGCGGTTGTTTCGGG	5= amino-modifier C6 dC
Crosslinking Template	5CCGAAACAACCGCGAGAGGCACGCGCGTAGCC GTCACCGGCTAT	5= amino-modifier C6 dC
Crosslinker-thiol	4TAGCCGGTGTGAAGTTTCTGCTAGTAATG6	4= psoralen 6= thiol modifier C3
Crosslinker-biotin	4TAGCCGGTGTGAAGTTTCTGCTAGTAATG6	4= psoralen 6= biotin

Table S5. Transferrin Synbody Oligonucleotide Sequences.

Name	Sequence (5'-3')	Notes
Template	5CCGAAACAACCGCGAGAGGCACGCGCGTAGC	5= amino-modifier C6 dC
Fluorescein -Template	5CCGAAACAACCGCGAGAGGCACGCGCGTAGC3	5= amino-modifier C6 dC 3= 6-Fluorescein
Variable Construct 3	GCTACGCGCGTGCCTCTCGCGGTTGTTTC5GG	5= amino-modifier C6 dG
Variable Construct 6	GCTACGCGCGTGCCTCTCGCGGTTGT5TCGGG	5= amino-modifier C6 dT
Variable Construct 9	GCTACGCGCGTGCCTCTCGCGGT5GTTTCGGG	5= amino-modifier C6 dT
Variable Construct 12	GCTACGCGCGTGCCTCTCGC5GTTGTTTCGGG	5= amino-modifier C6 dG
Variable Construct 15	GCTACGCGCGTGCCTCT5GCGGTTGTTTCGGG	5= amino-modifier C6 dC
Variable Construct 18	GCTACGCGCGTGCC5CTCGCGGTTGTTTCGGG	5= amino-modifier C6 dT
Variable Construct 21	GCTACGCGCGT5CCTCTCGCGGTTGTTTCGGG	5= amino-modifier C6 dG
Variable Construct 24	GCTACGCG5GTGCCTCTCGCGGTTGTTTCGGG	5= amino-modifier C6 dC
Variable Construct 27	GCTAC5CGCGTGCCTCTCGCGGTTGTTTCGGG	5= amino-modifier C6 dG

Complete reference from main text;

(6) Taussig, M. J.; Stoevesandt, O.; Borrebaeck, C.; Bradbury, A.; Cahill, D.; Cambillau, C.; de Daruvar, A.; Dubel, S.; Eichler, J.; Frank, R.; Gibson, T.; Gloriam, D.; Gold, L.; Herberg, F.; Hermjakob, H.; Hoheisel, J.; Joos, T.; Kallioniemi, O.; Koegll, M.; Konthur, Z.; Korn, B.; Kremmer, E.; Krobitsch, S.; Landegren, U.; van der Maarel, S.; McCafferty, J.; Muyldermans, S.; Nygren, P.; Palcy, S.; Pluckthun, A.; Polic, B.; Przybylski, M.; Saviranta, P.; Sawyer, A.; Sherman, D.; Skerra, A.; Templin, M.; Ueffing, M.; Uhlen, M. *Nat. Methods* **2007**, *4*, 13-17.