



SOMAscan[®] Proteomic Assay
Technical White Paper

Introduction

The SOMAscan[®] assay is a highly multiplexed, sensitive, quantitative, and reproducible proteomic tool for discovering previously undetected biomarkers for drug discovery, pre-clinical and clinical drug development, and clinical diagnostics, across a wide range of important diseases and conditions. The SOMAscan assay measures more than 1,300 protein analytes in only 150 μ L of serum, plasma or cerebrospinal fluid, or equally small amounts of a variety of other biological matrices. The assay offers exceptional dynamic range, quantifying proteins that span over 8 logs in abundance (from femtomolar to micromolar), with excellent reproducibility (4.6 median %CV for plasma).

The SOMAscan proteomic assay is enabled by a new generation of protein-capture SOMAmer[®] (Slow Off-rate Modified Aptamer) reagents. SOMAmer reagents are constructed with chemically modified nucleotides that greatly expand the physicochemical diversity of the large randomized nucleic acid libraries from which the SOMAmer reagents are selected. The SOMAscan assay measures native proteins in complex matrices by transforming each individual protein concentration into a corresponding SOMAmer reagent concentration, which is then quantified by standard DNA techniques such as microarrays or qPCR. The assay takes advantage of SOMAmer reagents' dual nature as both protein affinity-binding reagents with defined three-dimensional structures, and unique nucleotide sequences recognizable by specific DNA hybridization probes. The assay is performed under Good Laboratory Practice (GLP)-like quality systems, and can be run under GLP when necessary. To date, the SOMAscan assay has been applied successfully to biomarker discovery and validation in many pharmaceutical research and development projects, diagnostics discovery and development projects, and academic research projects.

SOMAmer Reagents are Discovered Using Robust SELEX Technology with Proprietary Chemical Modification

SOMAmer reagents are single stranded DNA-based protein affinity reagents that benefit from aptamer technology developed over the past 20 years (Ellington and Szostak, 1990) (Tuerk and Gold, 1990). The more recent proprietary innovation incorporates chemically modified nucleotides (Fig. 1) that mimic amino acid side chains, expanding the chemical diversity of standard aptamers and enhancing the specificity and affinity of protein-nucleic acid interactions (Gold et al., 2010). These modified nucleotides are incorporated into nucleic acid libraries used for the iterative selection and amplification process called SELEX (Systematic Evolution of Ligands by EXponential enrichment) from which SOMAmer reagents are selected (Vaught et al., 2010) (Eaton, 1997) (Davies et al., 2012). Repeatedly, by using a novel, proprietary SELEX process, SomaLogic has generated SOMAmer reagents to proteins that had been resistant to selection with unmodified nucleic acids (ACTG traditional aptamers) (Gold et al., 2010). A key advantage of this artificial selection process is that conditions can be tailored to select for the desirable properties of specificity and slow off-rate as well as to mimic the assay conditions under which the reagents will be used.

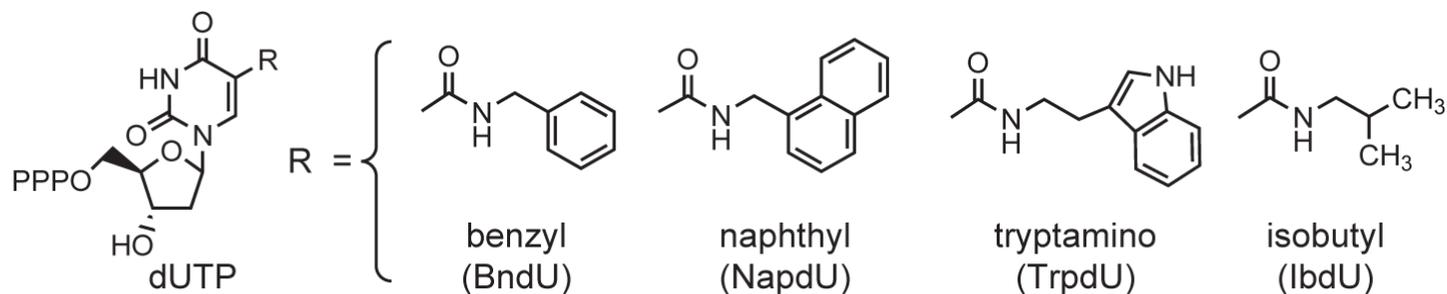


Figure 1. Modified nucleotides. Nucleotide triphosphate analogs modified at the 5-position (R) of uridine (dUTP); 5-benzylaminocarbonyl-dU (BndU); 5-naphthylmethylaminocarbonyl-dU (NapdU); 5-tryptaminocarbonyl-dU (TrpdU); and 5-isobutylaminocarbonyl-dU (lbdU).

SOMAmer reagents are selected against proteins in their native folded conformations and are therefore generally found to require an intact, tertiary protein structure for binding. As such, unfolded and denatured – and therefore presumably inactive proteins – are not detected by SOMAmer reagents. Three co-crystal structures have been solved for SOMAmer reagents bound to their cognate protein target by X-ray crystallography and indicate that the modified nucleotides contribute extensively to intra-molecular folding of the SOMAmer reagent and to inter-molecular binding to cognate targets (Davies et al., 2012). In Figure 2, a model derived from the X-ray crystal structure of PDGF-BB SOMAmer reagent binding to PDGF-BB demonstrates that the interactions between the SOMAmer reagent and its cognate protein are mainly mediated via the modified nucleotides. These structures show very specific interactions between SOMAmer reagent and target with binding site dimensions of 1100-1200 Å² similar to antibody-antigen interactions (Ramaraj et al., 2012).

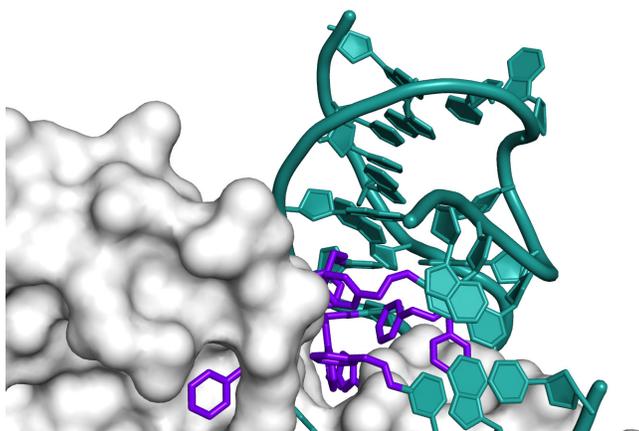


Figure 2. X-ray crystal structure of SOMAmer reagents binding to PDGF-BB. Modifications to the bases are shown in purple, and the DNA backbone and unmodified bases are in teal.

The dissociation kinetics of a large subset of SOMAmer reagents binding to their respective targets were determined using a solution-phase radiolabeled binding assay (Gold et al., 2010), and a subset confirmed using the Biacore Flexchip surface plasmon resonance biosensor. Association and dissociation rate constants and calculated equilibrium dissociation constants (K_D) for ten SOMAmer-protein pairs measured on the Biacore Flexchip are summarized in Table 1. Biosensor results confirm slow dissociation off-rates, ranging from 10^{-4} to 10^{-5} s⁻¹ that correlate well with dissociation rate constants measured by solution-phase filter binding assays. Lead SOMAmer reagents are required

to have a K_D of 1nM or better unless they are against highly abundant proteins. In total, SOMAmer reagents are analogous to high-quality antibodies that recognize intact tertiary protein structures, with the notable exception that they are made out of nucleic acids, leading to several advantages over antibodies such as tailored in vitro selection conditions, chemical synthesis, storage stability, and detection using sensitive and advanced DNA detection methods.

Protein Target	Biacore Flexchip ¹			Filter Assay ²
	k_a , $M^{-1}s^{-1}$	k_d , s^{-1}	K_D , nM	K_D , nM
4-1BB ligand	5.5×10^5	1.6×10^{-4}	0.3	1.00
TNF sR-I	4.9×10^5	4.3×10^{-5}	0.09	0.07
Rab GDP dissociation inhibitor beta	4.7×10^6	1.7×10^{-5}	0.004	0.01
Thrombin	1.4×10^6	2.2×10^{-4}	0.2	0.75
VEGF	5.4×10^5	4.9×10^{-5}	0.09	0.09
IgE	4.0×10^6	5.0×10^{-4}	0.1	2.5
sL-Selectin	3.1×10^5	5.9×10^{-4}	1.9	0.3
4-1BB	1.7×10^5	8.1×10^{-5}	0.5	0.9
Cystatin C	5.0×10^4	1.5×10^{-4}	3	2.7
Transferrin	1.2×10^4	6.5×10^{-5}	5	18

Table 1. Kinetic measurements for SOMAmer binding to cognate protein by Biacore Flexchip and radiolabel filter binding assay.

¹ SOMAmer-target association and dissociation kinetics were measured using SOMAmer reagents immobilized onto neutravidin-coated biosensor chips.

² Equilibrium binding constants were determined using radiolabeled SOMAmer reagents equilibrated with increasing amounts of target protein. Bound and free SOMAmer reagents were separated by filtration and captured SOMAmer reagents quantified by phosphorimaging (Gold et al., 2010).

SOMAmer Reagents to Over 1,300 Proteins

SOMAmer reagents have been created for over 1,300 protein targets that cover a diverse set of molecular functions, including cancer, inflammation and cardiovascular, to name a few. Targets to date extensively cover major gene families including receptors, kinases, growth factors and hormones, and span a diverse collection of secreted, intracellular and extracellular proteins or domains.

Lead SOMAmer Reagents are Chemically Synthesized, Stable and Rigorously Analyzed

After identification using SELEX technology, the SOMAmer reagents are chemically synthesized, purified and analyzed by methods including ultra-high performance liquid chromatography (UPLC), capillary gel electrophoresis (CGE) and mass spectrometry (MS). Extensive functional analysis ensures consistent high performance of the SOMAmer reagents as quantitative affinity reagents. Nearly half of the SOMAmer reagents used in the current SOMAscan assay have been evaluated for cross reactivity to related proteins with 50% similarity or better. Two examples demonstrating SOMAmer reagent specificity are in Appendix C and several more examples and details can be found in Gold et al., 2010.

The Assay Steps

The SOMAscan assay quantitatively transforms the proteins present in a biological sample into a specific SOMAmer-based DNA signal (Fig. 3). A SOMAmer-protein binding step is followed by a series of partitioning and wash steps that converts relative protein concentrations into measurable nucleic acid signals that are quantified using DNA detection technology, which for the SOMAscan assay with over 1,300 SOMAmer reagents is by hybridization to custom DNA microarrays. Assays with smaller numbers of SOMAmer reagents (i.e., 1 – 100, called “SOMAmer panels”), have been quantified by either qPCR or Luminex beads using sequences complementary to the SOMAmer reagent sequences. Assay details are provided in Appendix A and in Gold et al., 2010. Whichever detection method is used, the readout in relative fluorescent units (RFU) is directly proportional to the amount of target protein in the initial sample, as informed by a standard curve generated for each protein-SOMAmer pair.

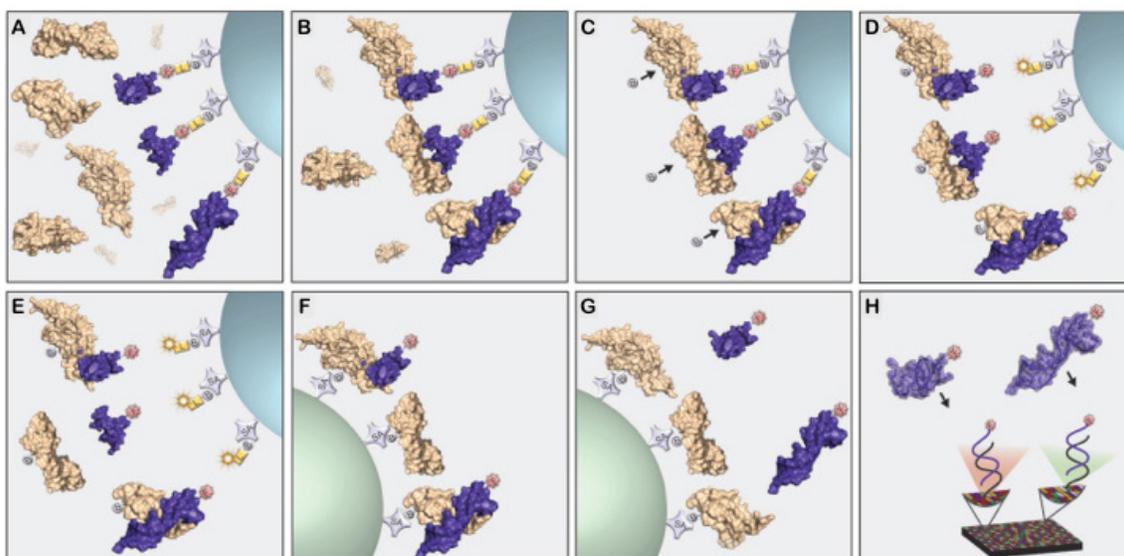


Figure 3. Multiplexed SOMAmer affinity assay. (A) SOMAmer reagents labeled with a 5' fluorophore, photocleavable linker, and biotin are immobilized on streptavidin (SA)-coated beads and incubated with samples containing a complex mixture of proteins. (B) Cognate (top and bottom) and noncognate (middle) SOMAmer-target protein complexes form on the beads. (C) The beads are washed, removing the unbound proteins and the proteins are tagged with biotin. (D) SOMAmer-protein complexes are released from the beads by photocleavage of the linker with UV light. (E) Incubation in a buffer containing a polyanionic competitor selectively disrupts nonspecific interactions. (F) SOMAmer-protein complexes are recaptured on a second set of streptavidin-coated beads through biotin-tagged proteins followed by additional washing steps that facilitate further removal of nonspecifically bound SOMAmer reagents. (G) SOMAmer reagents are released from the beads in a denaturing buffer. (H) SOMAmer reagents are hybridized to complementary sequences on a microarray chip and quantified by fluorescence. Fluorescence intensity is related to protein amount in the original sample. (Adapted from Rohloff et al., 2014.)

Achieving the 10⁸ Dynamic Range: SOMAmer Reagent Mixes

The large dynamic range of SOMAscan results from the detection range of each SOMAmer reagent in combination with three serial dilutions of the sample (Fig. 4). The least concentrated sample is designed to detect the most abundant proteins (~ μM concentration in 100% sample), and the most concentrated solution is designed to detect the least abundant proteins (fM to pM concentration in 100% sample).

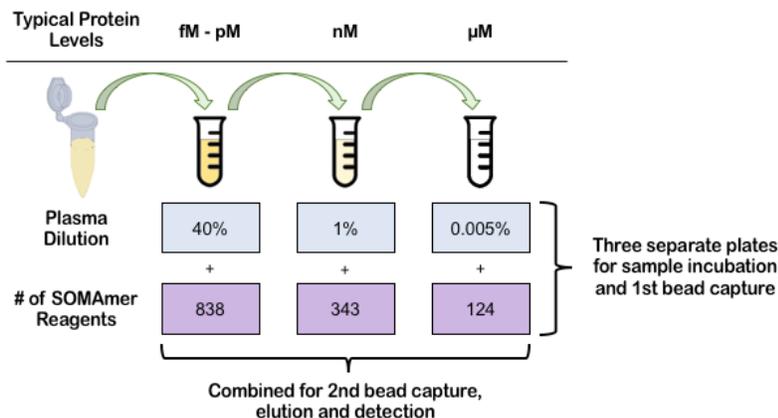


Figure 4. SOMAscan assay dynamic range. Custom SOMAmer reagent mixes are prepared for plasma, serum, or other matrices to achieve optimal detection in mixtures with a large range of concentrations. Shown here is the dilution distribution of SOMAmer reagents for SOMAscan assay with plasma.

SOMAscan Assay Characterization

The SOMAscan assay has been characterized for reproducibility, a summary of which is in Table 2, and experimental details in Appendix A. The SOMAscan assay has excellent reproducibility, with half of the SOMAmer reagents having a median %CV of 4.6 or lower and only 5% of the SOMAmer reagents having a median %CV of 10.5 or higher in plasma. All values were determined in the multiplex assay, profiling more than 1,300 analytes simultaneously.

Percentile of SOMAmers	%CV (total in plasma)
5%	≤ 2.7%
25%	≤ 3.7%
50%	≤ 4.6%
75%	≤ 6.0%
95%	≤ 10.5%

Table 2. Summary of the total (intra- plus inter-run) %CV in plasma. (Note: Values for the SOMAmer reagents were determined as described in Appendix B.)

Sample Types

The SOMAscan platform is amenable to proteomic profiling of numerous matrices, allowing for discovery of biomarkers that may translate across all phases of drug discovery and basic research, and target organ compartments that may store the most disease-relevant biomarker information. Currently, human plasma (EDTA, citrate, heparin or ACD), serum, and cerebrospinal fluid (CSF) are the most well characterized matrices, and only 150 μL is required to run them in the SOMAscan assay. Other biological matrices such as cell culture supernatant, cell and tissue lysates, synovial fluid, and bronchoalveolar and nasal lavage have been used successfully (Table 3). Additionally, the SOMAscan assay has been used to detect differential expression in drug-treated preclinical xenograft models. SOMAmer reagents generated to pure human proteins have varying degrees of cross reactivity to non-human orthologs and therefore the assays can be used to identify differential expression of some analytes in non-human samples, including non-human primates and rodents. We are developing optimized protocols for many of the “non-standard” matrices, and are open to discussing additional sample types with collaborators. Specific protocols for various sample preparations are available on request.

Tier	Tier Name	Matrices	
		Species	Sample Types
1	"Qualified" - For intended purpose in biomarker discovery. Performance in SOMAscan extensively evaluated with assay parameters developed for matrix specific optimization.	Human	EDTA-Plasma, Serum, CSF, Cell lysate ^A , Heparin plasma, Conditioned media
2	"Developed" - Performance in SOMAscan using existing parameters has been characterized.	Human	Lymphocytes, ACD/Citrate plasma
		Dog	EDTA Plasma
3	"Research" - Performance in SOMAscan using existing parameters has been minimally characterized or not at all. These matrices are run as standalone experiments requiring internal control.	Human	Nasal lavage, Wound fluid ^B , Urine ^B , BAL ^A , Sputum (Hargreave with/without DTT) ^B , Synovial fluid ^B , Exosomes ^A , Tears in buffer, Tissue homogenate (tumor, muscle, mucosa) ^A
		Non-Human Primate	Plasma, Serum, CSF, Aqueous Humor
		Rat	EDTA Plasma ^B , Serum ^B , CSF ^B , Muscle ^A , BAL ^A , Citrate Plasma
		Mouse	EDTA Plasma ^B , Serum ^B , Cell lysate ^A , Conditioned media, BAL ^A , Tissue homogenate (xenograft, muscle) ^B
		Dog	Serum, Synovial fluid ^B , Urine ^B
		Cat	Plasma, Serum
4	"Not tested" - Matrix not tested on platform.	All	Everything not mentioned

Table 3. SOMAscan sample matrices.

^A This sample type requires a total protein quantification prior to assay.

^B Matrices that require additional pre-assay sample preparation.

SomaLogic Quality Systems

The SOMAscan assay is run under the SomaLogic Quality System (QS) and SomaLogic is prepared to run samples under GLP when required. The assay is performed in a facility that contains both access and environmental control. Equipment within the facility is maintained, calibrated, and operated in compliance with controlling Standard Operating Procedures (SOPs). Equipment and associated software is validated for its intended use in support of the SOMAscan assay. Method validation has been completed for processes that could impact the performance of the SOMAscan assay. Standard Operating Procedures cover the incoming receipt, inspection, and release of raw materials to assure

that the materials used in the production of assay reagents or directly in the assay maintain the performance requirements established during the development of the SOMAscan assay. Reagent expiration dating, including SOMAmer reagents, calibrators, controls, and Master Mix retest dating, is set with all available background information and extended following review of data collected in the Stability Program.

Custom SOMAmer Panels

SomaLogic can develop custom SOMAmer panels (Kraemer et al., 2011) for specific research needs (e.g., particular pathways, classes of proteins). The streamlined SOMAmer panel format allows for a seamless transition from SOMAscan assay to a smaller custom multi-analyte assay.

Summary

The SOMAscan assay is a powerful, highly multiplexed platform for discovering novel biomarkers during drug discovery, pre-clinical and clinical drug development, and for the development of clinical diagnostics, across a wide range of clinically important diseases (Table 4 summarizes the SOMAscan assay metrics). SomaLogic's SOMAscan technology provides significant advantages in sample size, cost, time, multiplexing capability, dynamic range, and flexibility of readout over many alternate protein biomarker platforms. It has been used successfully for many sample types and matrices (including non-human primates, mouse, rat, dog, and cat) and is optimized for human analytes. It scales easily to progress from biomarker discovery to focused products without the need for new assay development. This technology can be readily employed as a valuable tool from basic research to drug discovery to diagnostic development.

Metric	Condition	Current Version of SOMAscan Assay
Sensitivity in buffer	Median LOD	*38 fM or 1.6 pg/mL
Dynamic Range in buffer	Median range per SOMAmer	*4.2 logs
	Custom SOMAmer reagent mixes	*10 ⁸
Precision	Median Total %CV in plasma	4.6%
Sample Volume	Human serum, plasma or CSF (per sample)	150 µL
Multiplex Size	Current number of proteins per sample	>1,300**

Table 4. Summary of SOMAscan assay metrics unique human targets.

*Indicates characterized on the SOMAscan Assay 1.1k.

**Please see SOMAscan Assay 1.3k Content (SomaLogic SSM-045).

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Appendix A

Details of the SOMAscan Assay

The first step of the SOMAscan assay is the dilution of a biological sample of interest. The sample dilutions are incubated with the respective SOMAmer reagent mixes pre-immobilized onto streptavidin (SA)-coated beads. The beads are washed to remove all non-specifically associated proteins and other matrix constituents. Proteins that remain specifically bound to their cognate SOMAmer reagents are tagged using an NHS-biotin reagent. After the labeling reaction, the beads are exposed to an anionic competitor solution that prevents non-specific interactions from reforming after they are disrupted (Gold et al., 2010). Essentially pure cognate-SOMAmer complexes and unbound (free) SOMAmer reagents are released from the SA beads using ultraviolet light that cleaves a photo-cleavable linker. The photo-cleavage eluate, which contains all SOMAmer reagents (some bound to a biotin-labeled protein and some free), is separated from the beads and then incubated with a second streptavidin-coated bead that binds the biotin-labeled proteins and the biotin-labeled protein-SOMAmer complexes. The free SOMAmer reagents are then removed during subsequent washing steps. In the final elution step, protein-bound SOMAmer reagents are released from their cognate proteins using denaturing conditions. These SOMAmer reagents can then be quantified by standard DNA quantification techniques, which for the SOMAscan assay with more than 1,300 SOMAmer reagents is by hybridization to custom DNA microarrays. The cyanine 3 signal from the SOMAmer reagent is detected on microarrays, phycoerythrin for Luminex[®] bead formats and SYBR[®] Green is detected in qPCR.

Data Analysis

Normalization procedures have been developed to assure data consistency. In the simplest form, normalization is performed using a set of hybridization control sequences introduced into the assay eluate prior to hybridization and measured independently for each sample array, which corrects for any systematic effects on the data introduced during the hybridization step.

A more robust normalization scheme uses all the SOMAmer reagent signals on a given array to allow for comparison of samples across a plate, within similar groups. It corrects for variation that may be introduced in the course of the SOMAscan assay, including natural variation in initial sample concentration that may occur. Each normalization method computes a single scale factor for each sample that is subsequently applied to the signal on all features within an array. Calibration is performed to allow for sample measurements across runs. Every plate contains replicates of a calibrator sample that is chosen to match the matrix type of the samples in the study (e.g., serum, EDTA-plasma). The median value is calculated across all the calibrator samples within the study for each SOMAmer reagent. These median values are compared to a previously established reference calibration value that generates a calibration scale factor that is then applied to all measurements for that SOMAmer reagent within the set of samples in the study. Figure A1 demonstrates the effect of calibration for one SOMAmer reagent. Data are reported in relative fluorescent units (RFU) after normalization and calibration.

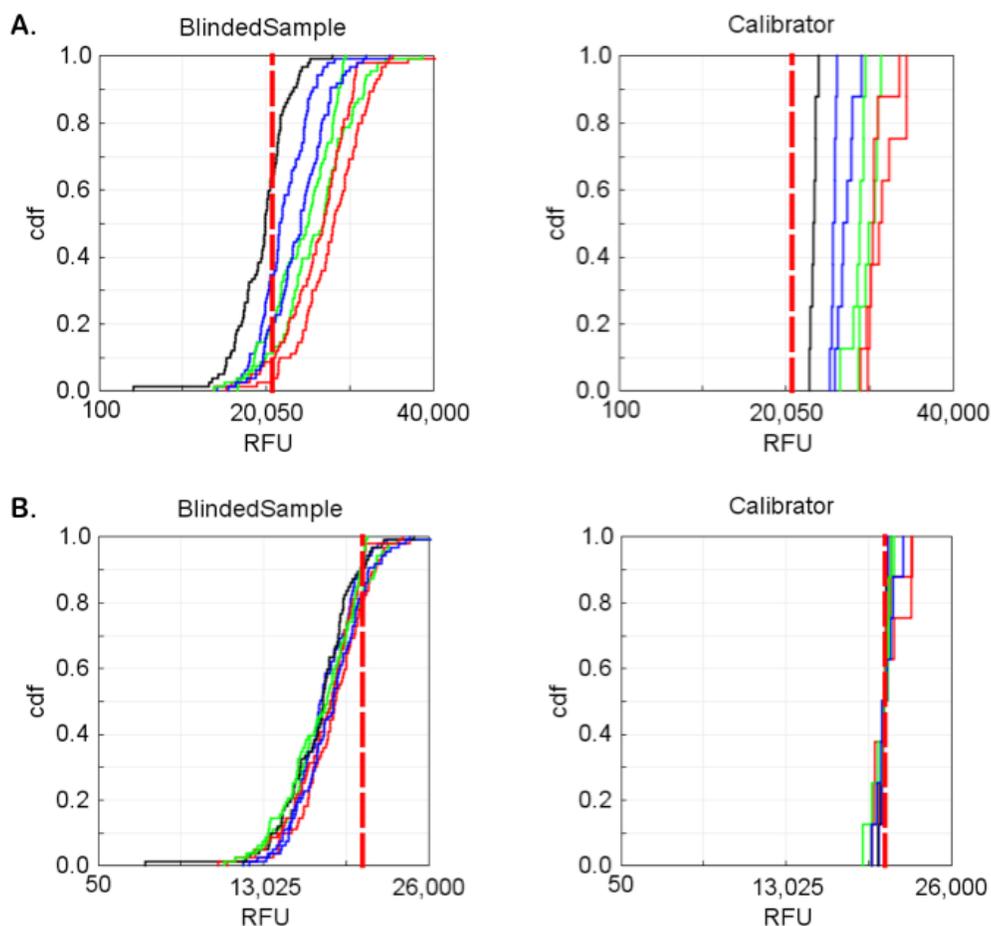


Figure A1. Illustration of using calibration to remove systematic bias between assay runs.

(A) Cumulative distribution functions (cdf) for a single SOMAmer reagent for a set of samples (left plot) were generated for each of seven independent assay runs, color coded by run. The cdfs for the eight replicate calibrator sample measurements for that SOMAmer reagent are displayed on the right for each run, color coded as on the left. The vertical red bar is the global calibrator reference obtained from a separate independent set of calibrator runs and is the target calibration RFU for this SOMAmer reagent. Note the correlation of shifts between the cdfs for the samples and the calibrator. (B) Cumulative distribution functions (cdf) for the set of samples and calibrators in (A) after calibration. Note the collapse of the sample distributions to essentially a single distribution after calibration.

Appendix B

Experiments to Characterize the SOMAscan Assay

Reproducibility

Reproducibility was assessed by running 14 different serum samples and 14 different plasma samples of three independent assay runs. Three samples were run in triplicate, with seven replicates of the associated calibrator sample, two Quality Control samples, and three buffer control wells on each runs. The separate runs utilized different operators to simulate typical run-to-run variability. The total %CV for each SOMAmer reagent was computed across the nine replicates over the three plate runs for serum or plasma samples after normalization and calibration. The overall median %CVs for intra-assay measurements are generally less than those for total %CV as expected (not shown) and are less than 5% for both serum and plasma. The distribution of the total %CV for all SOMAmer reagents in plasma is in Table 2 of the main text.

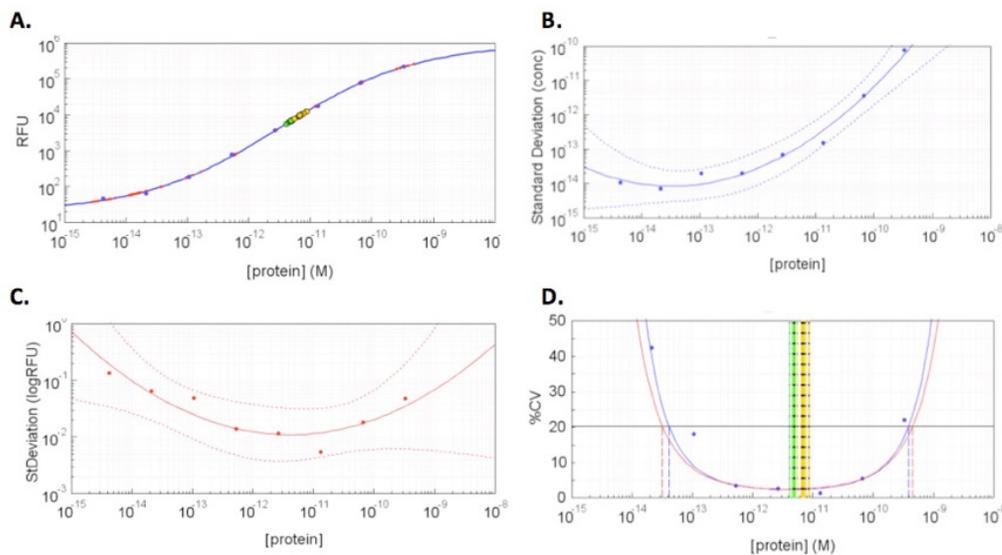


Figure B1. Representative plots from the quantification assessment. (A) The average RFU from the VEGF sR2 SOMAmer reagent at each concentration of input VEGF sR2 protein is denoted by the blue markers and the 12 individual measurements used to compute the average are denoted by the red markers plotted on the four parameter logistic curve fit (solid blue line). The eight replicate serum (orange markers) and plasma (green markers) measurements are denoted on the standard curve. (B) The standard deviation for computed concentration is denoted by the blue markers. The quadratic fit is displayed as a solid blue line and the 95% confidence bands for the fit are displayed as blue dashed lines. (C) The standard deviations for $\log(\text{RFU})$ are denoted by the red markers. The quadratic fit is displayed as a solid red line and the 95% confidence bands for the fit are displayed as red dashed lines. (D) Precision profiles are displayed for the direct computation of the standard deviation of the computed concentrations from (B) (solid blue line) or using the standard deviations of $\log(\text{RFU})$ from (C) transformed with the logistic curve fit to standard deviations in concentration (solid red line). Both methods give equivalent results here. The computed %CVs obtained directly from the data are displayed as solid blue markers and the sets of vertical dashed lines correspond to the limits of quantification at 20% CV for the two sets of precision profiles.

Appendix C

SOMAmer Specificity

Over 400 SOMAmer reagents used in the SOMAscan assay have been evaluated for cross reactivity to related proteins using an affinity capture technique very similar to immuno-precipitation. The SOMAmer reagent under evaluation is incubated with either its target protein or with any commercially available protein related to the target by 50% sequence similarity or better. The protein is first incubated with the SOMAmer reagent bound to streptavidin beads. The complex is carried through a series of washes that mimic the first chromatographic step of the SOMAscan assay, with an additional protein labeling step using an Alexa fluorophore. The eluted protein is run on an SDS-PAGE gel and compared to the input protein. Results for affinity capture using SOMAmer reagents to EGFR and ENA-78 are shown in Figures C1 and C2 to demonstrate typical outcomes.

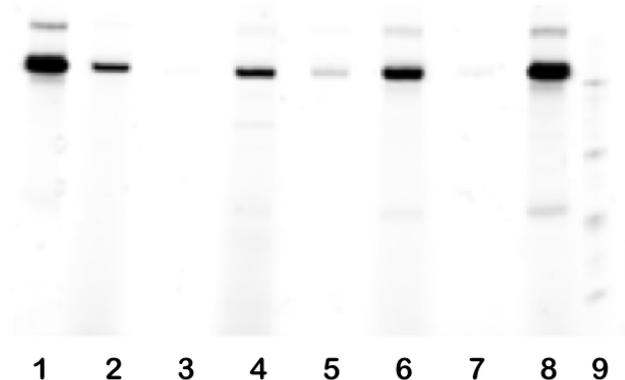
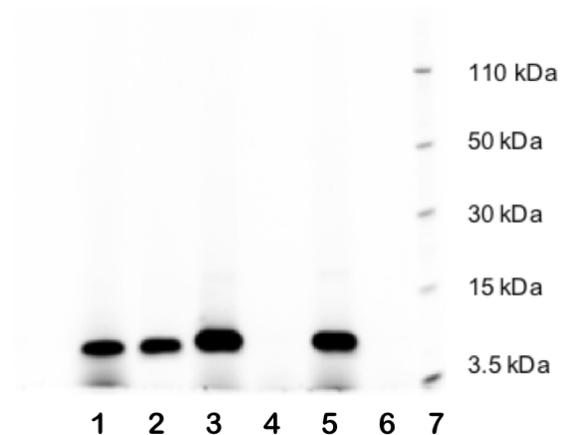


Figure C1. SDS-PAGE results of affinity capture assay using SOMAmer reagent to EGFR and purified EGFR and related proteins in buffer. Lanes: (1) EGFR-SOMAmer capture of EGFR, (2) EGFR, (3) EGFR-SOMAmer capture of HER2, (4) HER2, (5) EGFR-SOMAmer capture of HER3, (6) HER3, (7) EGFR-SOMAmer capture of HER4, (8) HER4, and (9) MW standards.

Figure C2. SDS-PAGE results of affinity capture assay with SOMAmer reagent to ENA-78 using purified ENA-78 and related proteins. Lanes: (1) ENA-78, (2) ENA-78-SOMAmer capture of ENA-78, (3) GCP-2, (4) ENA-78-SOMAmer capture of GCP-2, (5) Gro- α (6) ENA-78-SOMAmer capture of Gro- α , (7) MW standards.



Additional examples of specificity of SOMAmer reagents are provided in Gold et al., 2010. In these experiments, proteins were spiked into plasma except for the high abundant proteins LBP and TIG-2, which were captured directly out of plasma, and additional chromatographic steps were employed that mimicked subsequent steps of the assay. In these examples, the resulting eluates are expected to be more representative of the selectivity of SOMAmer reagents to proteins in complex matrices in the context of the assay.

SOMAscan and SOMAmer Related Patents

For a list of our patents, please see the SomaLogic website (www.somallogic.com).

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