INTRODUCTION

The interrogation of proteomes in a highly multiplexed and efficient manner remains a coveted and challenging goal in biology and medicine. We introduce an entirely new way to bind proteins, the SOMAmer® reagent, as an evolutionary leap forward in protein recognition. These high affinity protein-binding reagents are very specific and allow for extremely high multiplexing (currently 1,129 and growing) of protein measurements in a high-throughput and reproducible manner with very small sample volume requirements. SOMAmer reagents form the backbone of our SOMAscan™ assay platform and represent a powerful new method for biomarker discovery.

DATA/RESULTS

SOMAmer reagents are discovered using robust SELEX technology with proprietary chemical modifications

SOMAmers are single stranded DNA-based protein affinity reagents that are discovered via an aptamer selection technology (SELEX) developed over the past 25 years (1, 2). Our most recent proprietary innovation incorporates chemically modified nucleotides (Fig. 1) that mimic amino acid side chains, thus expanding the chemical diversity of standard aptamers and enhancing the specificity and affinity of protein-nucleic acid interactions (3). The inclusion of these mostly hydrophobic and aromatic side groups to the uracil ring has resulted in the generation of higher affinity SOMAmer reagents to proteins that had been resistant to selection with unmodified nucleic acids (3). A key advantage of the 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.

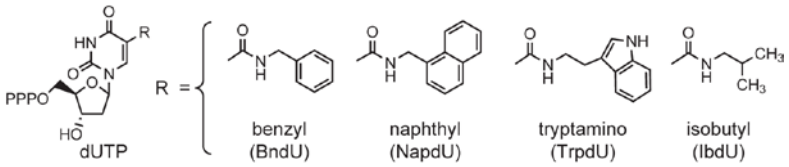

Figure 1. Modified nucleotides used in SOMAmer selection. Nucleotide triphosphate analogs modified at the 5-position (R) of uridine (dUTP); 5-benzylamino-carbonyl-dU (BndU); 5-naphthylmethylaminocarbonyl-dU (NapdU); 5-tryptaminocarbonyl-dU (TrpdU); and 5-isobutylaminocarbonyl-dU (iBudU).

Lead SOMAmer reagents are chemically synthesized, stable and rigorously analyzed

After identification using SELEX technology, the lead 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. Comparable to other DNA-based reagents for diagnostics and therapeutics, these reagents exhibit exceptional chemical stability: they can be frozen for a year or more, are amenable to denaturation/re-naturation with base or heat, and are produced with robust methods. Incorporation of these unique amino-acid like side chains is achieved with high purity, scalable modified nucleosides with optimized methods to ensure high lot-to-lot reproducibility and consistent performance.

Lead SOMAmers reagents have high affinity and slow dissociation rates

The majority of SOMAmer reagents selected as leads bind their target proteins with high affinity (K_d < 1 nM). This high affinity is due primarily to slow complex dissociation rates, on the order of 10^{-4} to 10^{-5} s^{-1} (or $t_{1/2}$ values of 2 to 20 hours). The dissociation kinetics of 72 SOMAmer reagents binding to their respective targets have been determined using a solution-phase radiolabeled binding assay (3), and a subset confirmed using surface plasmon resonance.

SOMAmer reagents are characterized for specificity

SOMAmer reagents identified for use in panels or as individual reagents, are evaluated for cross reactivity to related proteins using an affinity capture technique very similar to immunoprecipitation. The SOMAmer reagent is incubated with either its target protein or with commercially available proteins related to the target. The protein is first incubated with the SOMAmer bound to streptavidin beads. The complex is carried through a series of washes that mimic the first binding step of the SOMAscan assay, with an additional protein labeling step using an NHS-Alexa-647 Fluorescent dye. The eluted protein is run on an SDS-PAGE gel and compared to the input protein. Data for affinity capture using the SOMAmer reagent to EGFR shown in Figure 2 demonstrates typical outcomes. When the protein is at very high endogenous concentrations the target protein is pulled directly out of serum or plasma (3).To date, specificity experiments such as these have been run on about one-third of the 1,129 proteins on the menu with the remaining being tested on an ongoing basis. Details about specificity for each SOMAmer are available on request.

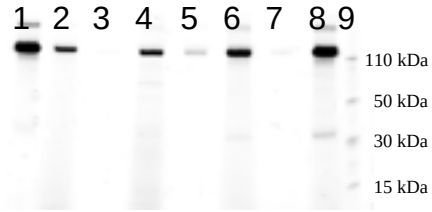

Figure 2. PAGE results of affinity capture assay using a SOMAmer reagent to EGFR and purified EGFR and related proteins in buffer without competitor. Lanes: (1) EGFR-SOMAmer capture of EGFR, (2) EGFR, (3) EGFR-SOMAmer capture of HER2, (4) HER2, (5)EGFR-SOMAmer capture of HER3, (6) HER3 , (7) EGFR-SOMAmer capture of HER4, (8) HER4, and (9) MW standards.

X-Ray crystal structure of SOMAmer reagent binding to its cognate protein

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-dimensional structures have been determined by X-ray crystallography for three SOMAmer reagents bound to their protein targets, and indicate that the modified nucleotides contribute extensively to intra-molecular folding of the SOMAmer reagent and to inter-molecular binding to its cognate target (4). In Figure 3, a model derived from the x-ray crystal structure of PDGF-BB SOMAmer binding to PDGF-BB demonstrates that the interactions between a 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 A^2 similar to antibody-antigen interactions (5). Additionally, the modified nucleotides confer higher structural stability as inferred from the significant increase in the melting temperature as compared to the corresponding non-modified sequences.

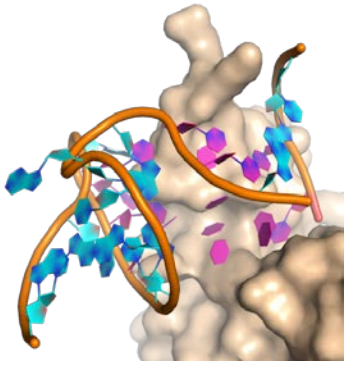

Figure 3. The crystal structure of the PDGF-BB SOMAmer reagent recognizing the 3-dimensional shape of its cognate protein PDGF-BB (4). The DNA backbone of the SOMAmer reagent is shown in gold, the blue and teal indicate natural bases and pink, the modified benzyl appendage on uracil, binding to PDGF-BB (brown).

SOMAmer affinity reagents to over 1100 proteins

SOMAmers have been created for over 1100 protein targets that comprise a diverse set of molecular functions, including many known disease and physiology associations. SOMAmer targets cover major gene families including receptors, kinases, growth factors and hormones, and span a diverse collection of secreted, intracellular and extracellular proteins or domains.

CONCLUSION

SOMAmer reagents are DNA-based molecular recognition reagents analogous to high-quality antibodies. SOMAmer reagents bind conformational epitopes with high affinity and specificity, and offer several advantages over antibodies. SOMAmer reagents are discovered with a tailored *in vitro* selection process that requires no animals, are manufactured chemically using cost-effective and highly reproducible processes, are stable to extremes of temperature and pH, and can be dessicated, rehydrated, denatured and renatured with no loss of activity. In addition, SOMAmer detection and quantification is possible using readily available advanced DNA detection technologies. These affinity reagents are enabling new breakthroughs in biology and medicine due to their suitability for use in highly multiplexed ligand-binding assays (6). For more information on SOMAmer reagents, or to learn what breakthrough discovery awaits you, please contact us at info@somalogic.com or visit www.somalogic.com.

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