SOMAmer® Reagents: A New Class of Protein Binding Reagents

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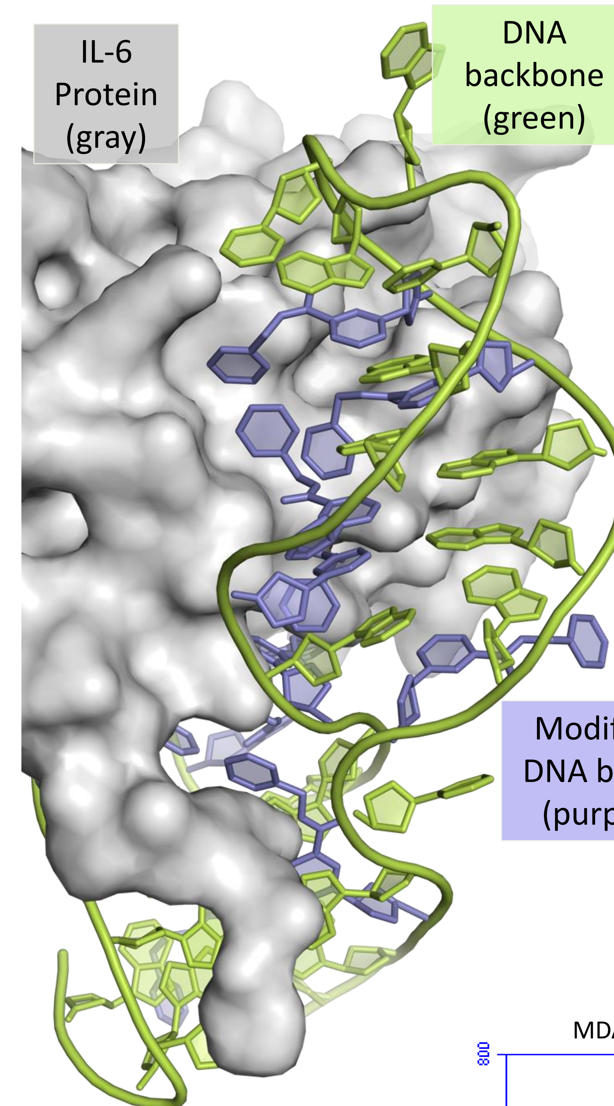
Abstract

SOMAmer® reagents (Slow Off-rate Modified Aptamers) are DNA-based high affinity (average K_d < 1 nM) protein binding reagents with proprietary chemical modifications that provide hydrophobic characteristics not present in natural DNA. These modifications enhance protein binding through direct hydrophobic contacts with the target protein, resulting in increased binding affinities and slower complex dissociation rates compared to unmodified aptamers. Due to their high specificity and strong affinity for protein targets, SOMAmer reagents can be used in many life science research applications that traditionally rely on antibodies. These applications include flow cytometry, ELISA, histochemical staining, fluorescent cell microscopy, and target enrichment for mass spectrometry. Since SOMAmer reagents are chemically-synthesized modified DNA, product consistency and stability are superior to antibodies.

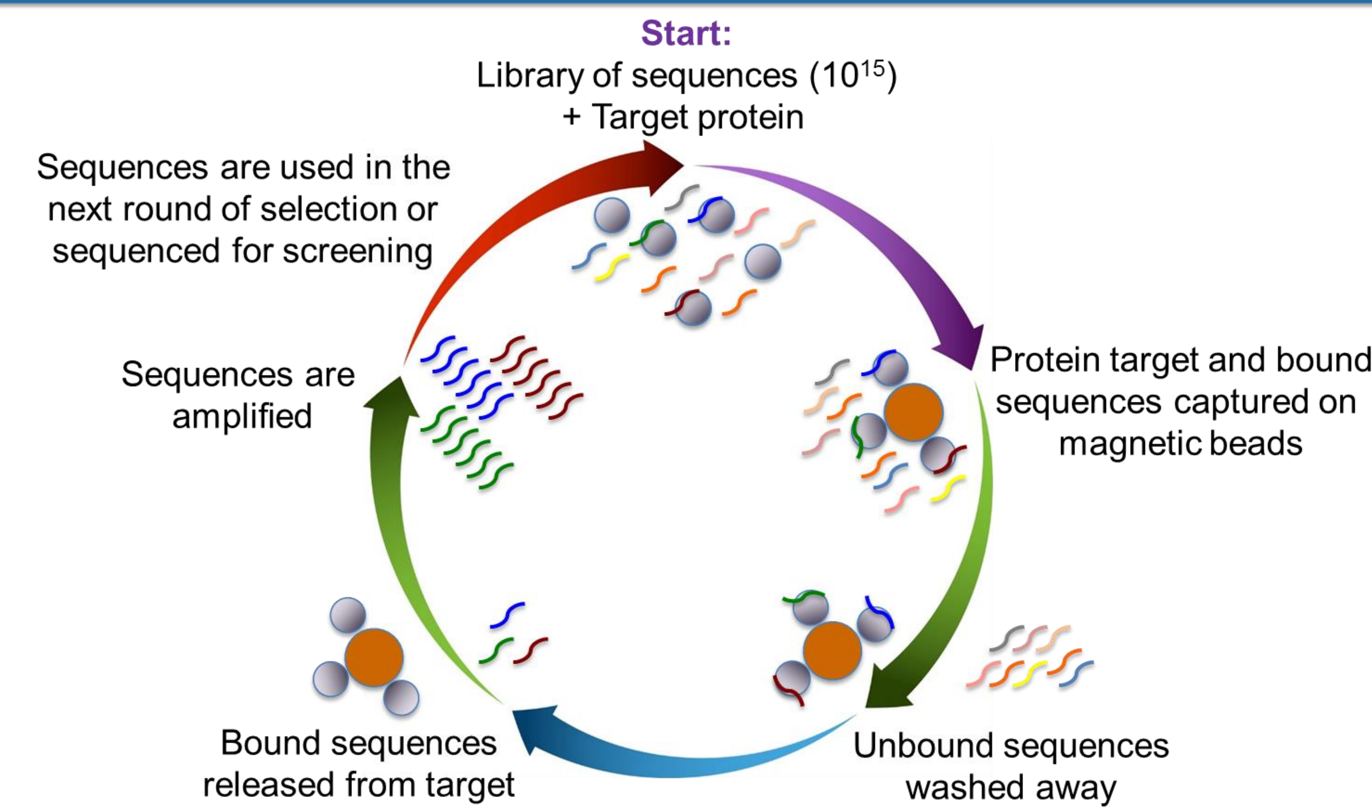
SOMAmer Reagents: Modified DNA Aptamers



- Chemically synthesized modified DNA
- DNA modifications result in:
  - Increased chemical diversity
  - High affinity (sub-nM)
  - Unique binding profiles enabled by hydrophobic interactions
- A variety of 5' functional groups are possible (e.g., fluorophores, biotin, amine, azide, PEG)
- Current public menu of 277 SOMAmer binding reagents to a wide variety of proteins
- Custom selections available for unique targets, desired binding properties
- Available only from SomaLogic

SELEX: SOMAmer Reagent Discovery Method

Systematic Evolution Of Ligands By Exponential Enrichment



Applications: Flow Cytometry

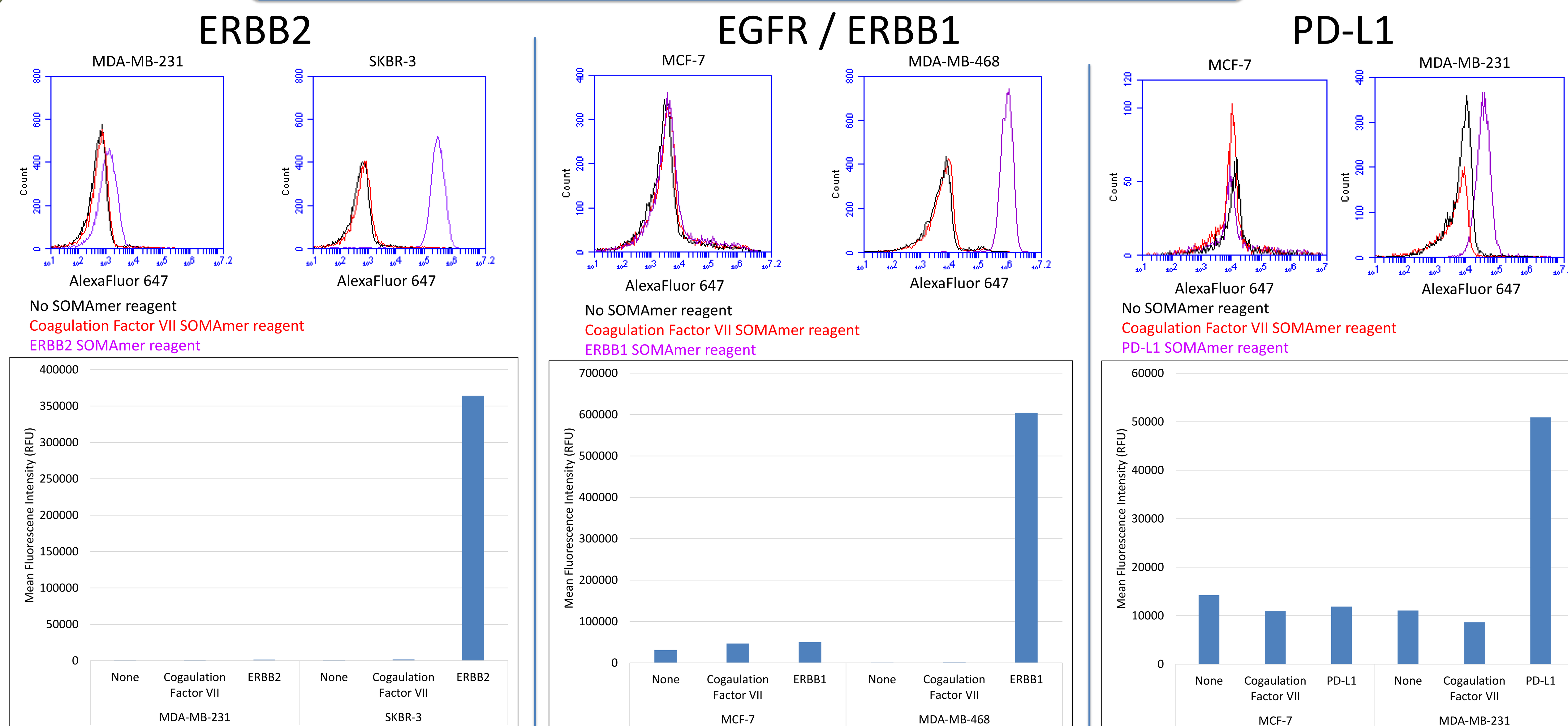


Figure 2. Detection of ERBB2, ERBB1 and PD-L1 using SOMAmer reagents in Flow Cytometry. Histogram plots of AlexaFluor 647 intensity illustrate a significant shift in population fluorescence for cells expressing high levels of ERBB2, ERBB1, or PD-L1 (SKBR-3, MDA-MB-468, and MDA-MB-231 respectively) when labeled with target-specific SOMAmer reagents compared to cells expressing low levels of these receptors (MDA-MB-231, MCF-7, and MCF-7 respectively). Bar graphs represent quantitated Mean Fluorescence Intensity for the cell populations labeled with target-specific and control SOMAmer reagents presented as corresponding histogram plots.

Characterizing SOMAmer Reagent Specificity

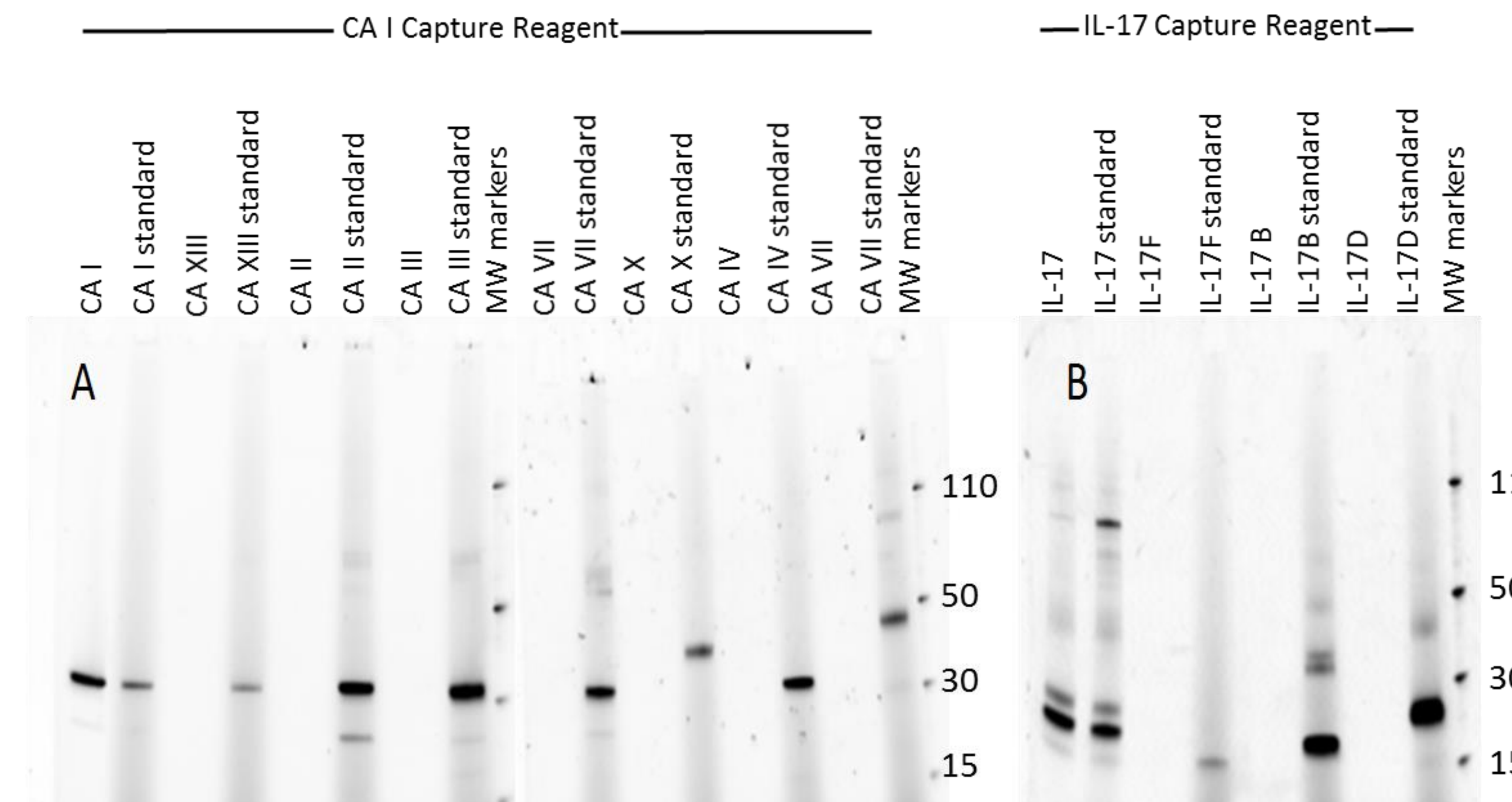


Figure 1. SDS-PAGE analysis of Alexa-647-labeled proteins captured by the A) CA I (Carbonic Anhydrase I) SOMAmer reagent or B) IL-17 SOMAmer reagent. For each set of experiments, there are pairs of samples – protein captured by the SOMAmer reagent (pulldown) followed by the protein standard (input). The SOMAmer reagents capture the cognate protein target used in SELEX but not the related proteins.

Applications: Fluorescent Cell Microscopy

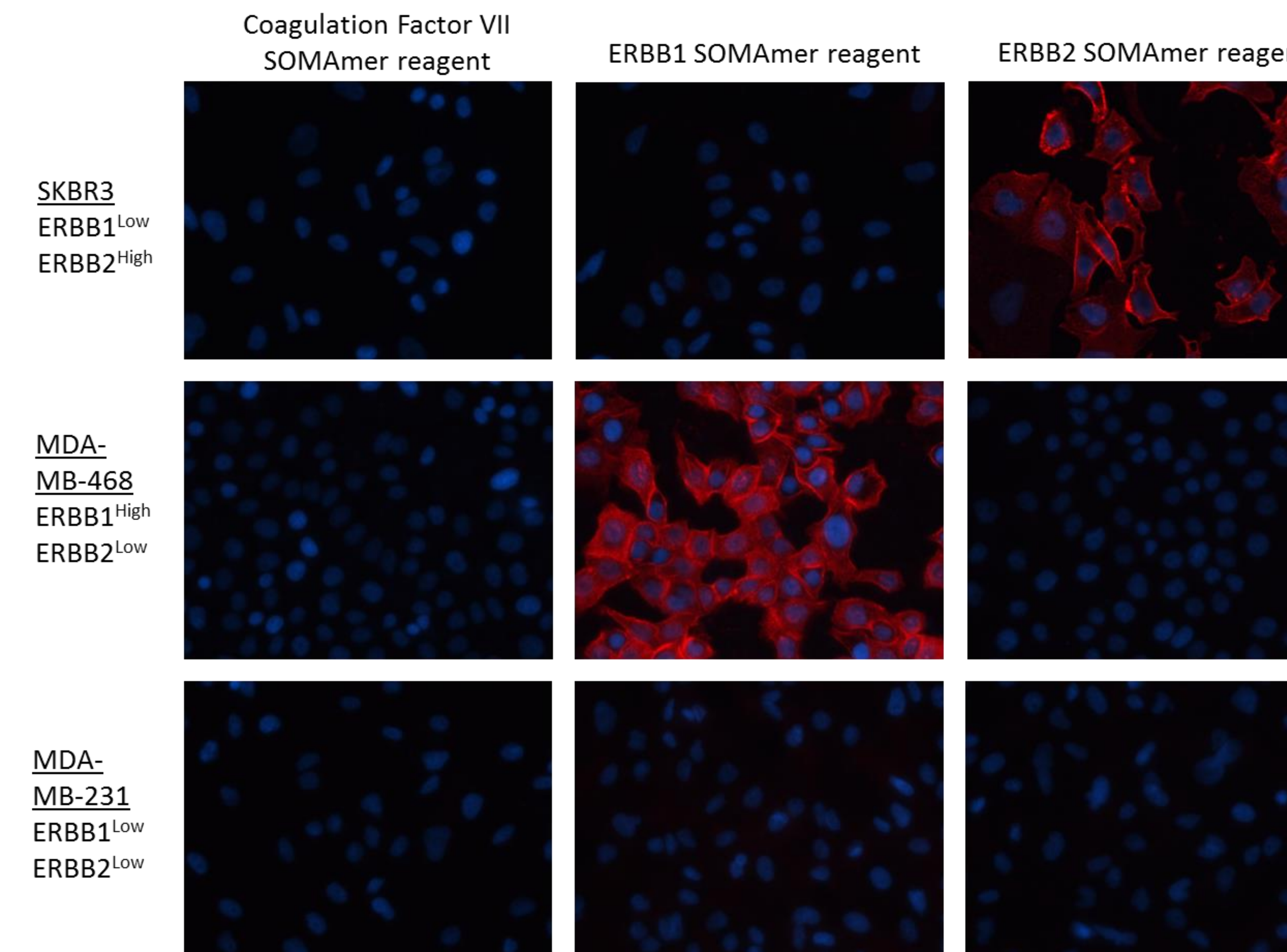


Figure 3. Fluorescent cell microscopy using SOMAmer reagents as primary detection reagents. ERBB1-specific SOMAmer reagent labeling is limited to the $ERBB1^{High}$ cell line MDA-MB-468. ERBB2-specific SOMAmer reagent labeling is limited to the $ERBB2^{High}$ cell line SKBR-3. The $ERBB1^{Low}$, $ERBB2^{Low}$ cell line MDA-MB-231 is not labeled by either SOMAmer reagent. The control SOMAmer reagent against Coagulation Factor VII does not label SKBR-3, MDA-MB-468, or MDA-MB-231 cell lines. Biotinylated SOMAmer Reagents were detected with Streptavidin-PI conjugate. Cell Nuclei were counterstained with DAPI.

Methods

SOMAmer Reagents: Biotinylated SOMAmer reagents were heated to 95° C for 3 minutes, then slowly cooled in a 25 °C water bath for 20 minutes prior to dilution in cell staining buffer. SOMAmer reagents were diluted to 1 µM in 5 mM HEPES, 1 mM EDTA, pH 8 for heat and cool procedure.

Protein Pulldowns: SOMAmer reagents were immobilized on Streptavidin-coated agarose beads and incubated with proteins diluted to 100 nM in protein binding buffer (40 mM HEPES, 120 mM NaCl, 5mM $MgCl_2$, 5 mM KCl, 0.05% Tween 20, pH 7.5) for 90 minutes at 37° C. SOMAmer reagent-protein complexes were washed with protein binding buffer and proteins labeled with NHS-AlexaFluor 647. Proteins were eluted with 10mM NaOH and electorphoretically separated by SDS-PAGE under denatured and reduced conditions.

Polyanionic Competitor: Inclusion of a competitor during or after cell labeling can be valuable in distinguishing between specific and non-specific binding in all SOMAmer reagent applications. While many applications do not involve high levels of proteins with high random DNA affinity, they often include very high concentrations of proteins with modest affinity (e.g., some highly abundant plasma proteins that can bind DNA). In these experiments, 10 µM SomaLogic Polyanionic Competitor and 2 mM Dextran Sulfate were used during incubation of cells with SOMAmer reagents.

Cell culture: Cell lines SKBR-3, MDA-MB-231, and MDA-MB-468 were obtained from the American Type Culture Collection (ATCC). All cell lines were cultured in DMEM supplemented with 10% Fetal Bovine Serum and 10mM L-Glutamine.

Cell labeling: Cells were briefly rinsed with PBS before fixation with 4% formaldehyde for 15 minutes at 25° C. Fixative was removed and cells were washed twice with PBS for 5 minutes at 25° C. SOMAmer reagents were diluted to 100 nM in cell labeling buffer (PBS with 5 mM $MgCl_2$, 1%BSA, 10 µM SomaLogic Polyanionic Competitor (SomaLogic, Inc., product #910-00001), and 2 mM Dextran Sulfate). Cells were incubated in the above SOMAmer labeling solution for 30 minutes at 25° C. Cell labeling solution was removed and cells were washed 3 times with PBS with 5 mM $MgCl_2$ for 10 minutes at 25° C. Streptavidin-fluorophore conjugate was added to cells at 0.5ug/mL in PBS with 5 mM $MgCl_2$ and incubated for 30 minutes at 25° C. Cell labeling solution was removed and cells were washed 3 times with PBS with 5 mM $MgCl_2$ for 10 minutes at 25° C. For fluorescent microscopy imaging, cell nuclei were stained with DAPI at 300 nM in PBS with 5 mM $MgCl_2$. Cells were washed with two times with PBS with 5 mM $MgCl_2$ for 5 minutes at 25° C. Labeled cells were stored in PBS at 4° C until imaging or analysis by flow cytometry.

Cell Imaging: Cells in PBS with 5 mM $MgCl_2$ were imaged on an EVOS Fl digital inverted fluorescence microscope (Advanced Microscopy Group, Thermo Fisher). Epifluorescence images were captured in the RFP and DAPI channels

Flow Cytometry: Data were acquired on a BD Accuri C6 flow cytometer by gating conservatively on the live cell population on the basis of forward and side light scatter. The mean fluorescence intensity (MFI) in the FL-4 channel, measuring AlexaFluor 647 fluorescence, was calculated for 10,000 events in the live cell gate. All data were analyzed using BD CSampler™ Software.

Conclusions

SomaLogic, Inc. has recently made 277 SOMAmer reagents, each targeting unique protein targets, available as Research Use Only (RUO) reagents for life science researchers. The target binding affinity and specificity are measured and reported for each SOMAmer reagent. In order to provide high-quality, well-characterized affinity reagents, any cross-reactive binding to highly-related proteins is tested, documented and reported to the end user. Here we present data demonstrating highly specific binding of SOMAmer reagents to the intended target protein, but not to related proteins. We also provide examples of SOMAmer reagents used successfully in affinity pulldown, flow cytometry and fluorescent cell microscopy applications.

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