Sensor Chip NA for high-performance Biacore SPR analysis

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Introduction

Biotinylation is the process of attaching biotin to proteins and other macromolecules. The new Series S Sensor Chip NA has NeutrAvidin™ coupled to a carboxymethylated dextran matrix for high affinity capture of biotinylated molecules and is designed for LMW and fragment applications in Biacore™ systems. Series S Sensor Chip NA is ready-to-use and saves hours in routine coupling reagents preparation and up to days in optimization of coupling conditions.

Here, we present user cases of the new Series S Sensor Chip NA with advantages over existing options and approaches for LMW/fragment applications.

Characteristics: Sensor Chip NA

- Enables coupling of high pI targets
- High baseline stability
- RYD recognition sequence removed in NeutrAvidin and thus no known off-target binding domains like for streptavidin
- Versatile: immobilize biotinylated peptides, proteins, or nucleic acids for oriented capture
- Robust: reproducible high-affinity ligand capture and robust capacity for more than 200 cycles (Fig 3)
- Saves hours in preparation time compared to instrument immobilization

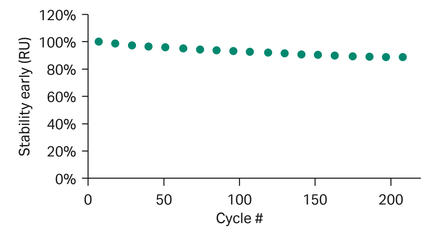
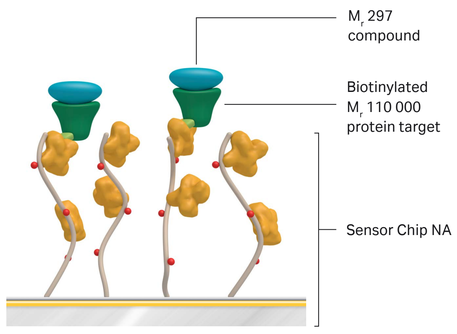


Fig 3. An M_r 500 compound injected regularly over an M_r 36 000 target protein. After 200 cycles, > 90% of the surface activity remained.

Assess LMW binding to high molecular weight protein targets

- Mol. weight (M_r) 297 compound binding to 110 000 protein (reagents provided by Novartis Institutes for BioMedical research, Cambridge, USA)
- 1500 RU of target protein coupled to Sensor Chip NA
- Injection of compound in two-fold dilution series
- Assessment of kinetics and affinity (Fig 1)
- Good correlation with previous data



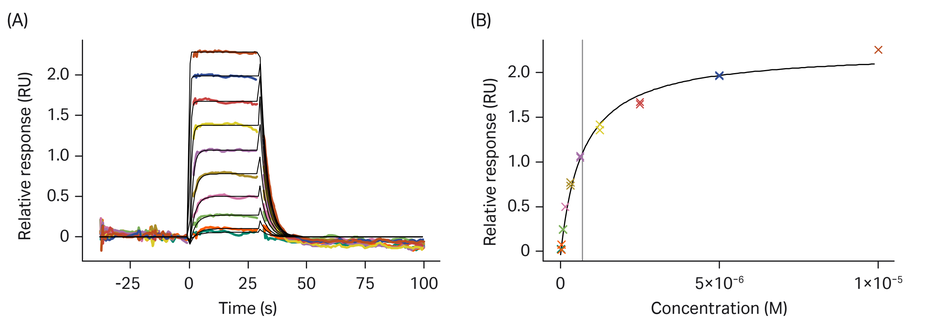


Fig 1. Assessment of kinetics (A) and affinity (B) of small molecule analyte binding to M_r 110 000 protein ligand captured on Sensor Chip NA. Data correlates well with known values of kinetics and affinity.

k_a (M^{-1} s^{-1})	k_d (s^{-1})	K_D (M)	R_{max} (RU)	Chi² (RU)
8.2×10^5	3.4×10^{-1}	4.2×10^{-7}	2.5	0.003

Eliminate problems with sticky fragments

The absence of the RYD amino acid sequence in NeutrAvidin reduces binding of sticky fragments to Sensor Chip NA. In a fragment clean screen run vs an M_r 30 000 protein coupled to Sensor Chip NA and Sensor Chip SA, more fragments with residual binding to the sensor surface were found for Sensor Chip SA compared to Sensor Chip NA (Fig 2).

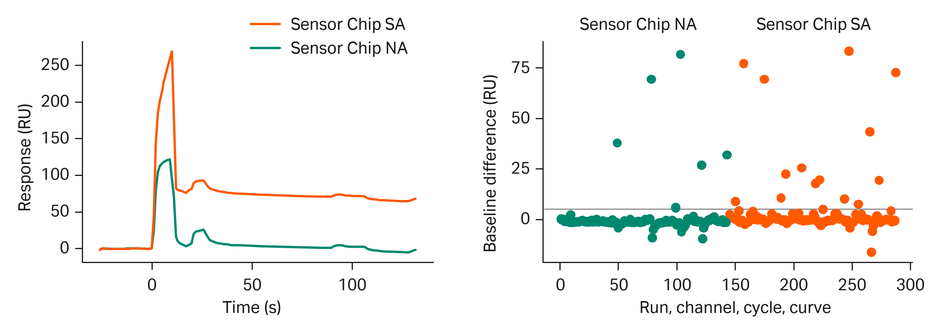


Fig 2. Clean screen of fragments vs an M_r 30 000 protein coupled to Sensor Chip NA and Sensor Chip SA, respectively.

Reduce the need for assay development

Until now, the only option to have NeutrAvidin on a sensor chip was to make it in house — a procedure which is time consuming and requires optimization. Sensor Chip NA is manufactured using a controlled and optimized process. All raw materials are closely monitored to ensure high quality and the finished product is tested with validated QC methods to ensure consistency in product quality and performance.

A positive control was injected over Avi-tagged protein captured on an instrument immobilized NeutrAvidin surface (Fig 4A). Poor data indicated need for further immobilization optimization. The assay was run also on Sensor Chip NA (Fig 4B) resulting in good data on first attempt without time-consuming reagent preparations and optimizations.

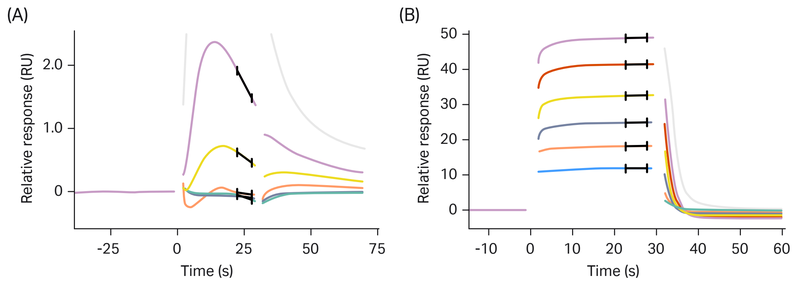


Fig 4. Positive control injected over 5000 RU Avi-tagged Protein A coupled to an instrument immobilized NeutrAvidin surface (A) and to Sensor Chip NA (B), respectively.

Immobilize high pI proteins

A high pI (~ 9.9) homodimeric protein target was found to bind nonspecifically to streptavidin on Sensor Chip SA (Fig 5A) and to single-stranded DNA on Sensor Chip CAP (Fig 5B). Sensor Chip NA (Fig 5C) eliminated the nonspecific binding and allowed specific capture of Avi-tagged target protein.

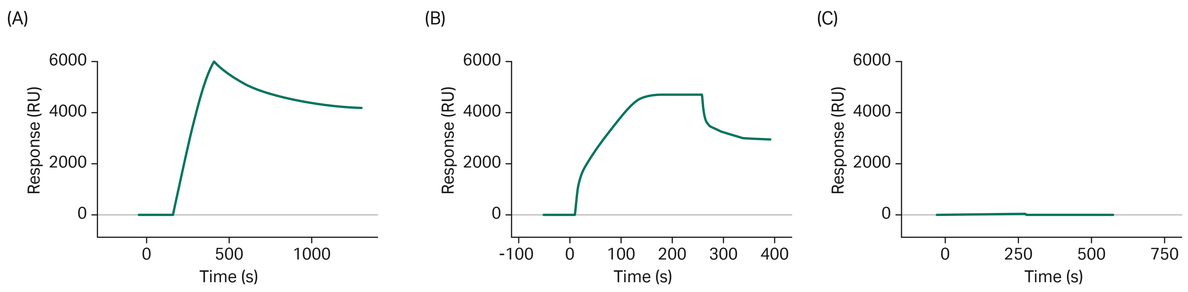


Fig 5. Non-biotinylated, high pI ligand binds nonspecifically to Sensor Chip SA (A) and to Sensor Chip CAP (B) but not to Sensor Chip NA (C).

After coupling the protein retained binding activity and bound to its small molecule binding partners with 2:2 stoichiometry (Fig 6). Data provided by Casma Therapeutics.

Fig 6. (A) Capture of biotinylated target protein to Sensor Chip NA. (B) binding of compound 1 and (C) binding of compound 2 to target protein.

Conclusions

Biacore Sensor Chip NA is a new capture surface for screening and characterization of small molecules and fragments that:

- Enables coupling and analysis of high pI proteins known to bind non-specifically to streptavidin and ssDNA.
- Reduces binding of sticky fragments to the sensor surface.
- Ensures consistency in product quality and performance while saving hours in preparation and optimization time compared to homemade surfaces.

Acknowledgement

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