# Application Note #6

## C-Dex105: Expanding from proteins to small molecules

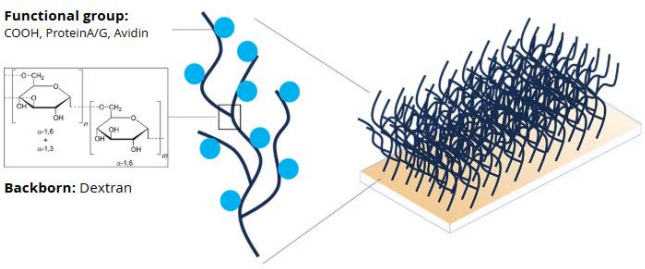
### Introduction

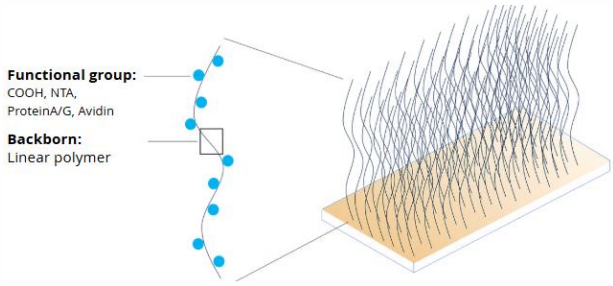
#### C-Dex105: Upgraded from C-Dex100

C-Dex100 sensor chip has a dextran matrix-based surface structure (Figure 1) and has been widely used for protein and antibody analyses. However, its relatively low immobilization capacity, typically around 5,000 RU, limits its performance in small-molecule application.

To resolve the limitations of C-Dex100, iCLUEBIO enhanced the production process of the sensor surface and developed a new sensor chip: C-Dex105, capable of supporting a wider scope of analyses from large molecules to small molecules.

This application note evaluated the performance of C-Dex105 by observing ionic and covalent immobilization signals of a standard immobilization target, BSA (Bovine Serum Albumin). We also performed kinetic analyses using a standard protein–protein interaction (Protein A–IgG) and a standard protein–small molecule interaction (CAII–Furosemide), and compared the results with those obtained from existing sensor chips (C-Dex100 and HC1000)




[Figure 2. Surface properties of the HC1000 linear polymer sensor chip]

### Materials and methods

#### Materials

- Instrument : iMSPR-Pro2X [CAT#INPX2000]
- Sensor chip : C-Dex105 [CAT#CDDH1105]
- Amine coupling kit [CAT#IMAM1000]
- Running buffer : HBST [CAT#RBHT1010-500]
- Ligand : BSA / Protein A / CAII
- Analyte : IgG / Furosemide
- Regeneration solution :
  Glycine-HCl, pH 1.5 [CAT#RGGH1015]

| Product | Lot number |
|---|---|
| C-Dex100 | CD1002508001 |
| C-Dex105 | CD1052509001 |
| HC1000 | 0423.a |

# Application Note #6

iCLUEBIO

### Preparation

1. Load the prism holder equipped with the C-Dex105 chip onto the iMSPR-Pro2X, connect 1X HBST, and proceed with Prime.
2. Perform Precondition by injecting 50 mM NaOH for 3 cycles, 1 minute each, at a flow rate of 30 µl/min.

### BSA

### Preconcentration

1. Preconcentration: Inject 100 µg/ml BSA in acetate buffer (pH 4.0) into Pro2X at a flow rate of 30 µl/min for 3 minutes.
2. Inject 50 mM NaOH at 30 µl/min for 1 minute three times to verify whether the sensor chip surface returns to baseline.

### Immobilization

1. Activation: Inject a mixed solution of 200 mM EDC and 100 mM NHS into the ligand and reference channels in continuous mode for 7 minutes at 20 µl/min.
2. Amine coupling: Prepare BSA at a concentration of 100 µg/ml by dilution in acetate buffer (pH 4.0). Inject it into the ligand channel in individual mode for 15 minutes at 10 µl/min.
3. Blocking: Switch back to continuous mode and inject 1M ethanolamine-HCl for 7 minutes at 20 µl/min to block the remaining COOH functional groups.

### Protein A-IgG

### Immobilization: Protein A

1. Activation: Inject a mixed solution of 200 mM EDC and 100 mM NHS into channels 3 and 4 of the Pro2X in continuous mode for 7 minutes at 20 µl/min.
2. Amine coupling: Prepare Protein A at a concentration of 10 µg/ml by dilution in acetate buffer (pH 4.0). Inject it into the ligand channel in individual mode for 10 minutes at 10 µl/min.
3. Blocking: Switch back to continuous mode and inject 1M ethanolamine-HCl for 7 minutes at 20 µl/min to block the remaining COOH functional groups.

### Kinetic analysis

1. Prepare the IgG analyte by 2-fold serial dilution in running buffer, as concentration of 100 nM, 50 nM, 25 nM, 12.5 nM, 6.25 nM, 3.125 nM, and 1.5625 nM, with 300 µl for each dilution.
2. Association: Inject the prepared IgG solution for 3 minutes at 50 µl/min.
3. Dissociation: Observe dissociation for an additional 8 minutes while the running buffer flows.
4. Regeneration: After observing dissociation, inject Glycine-HCl (pH 1.5) for 1 minute at 50 µl/min, followed by stabilization with 1xHBST for 3 minutes.
5. Repeat step 4 for each concentration solution.

### CAII-Furosemide

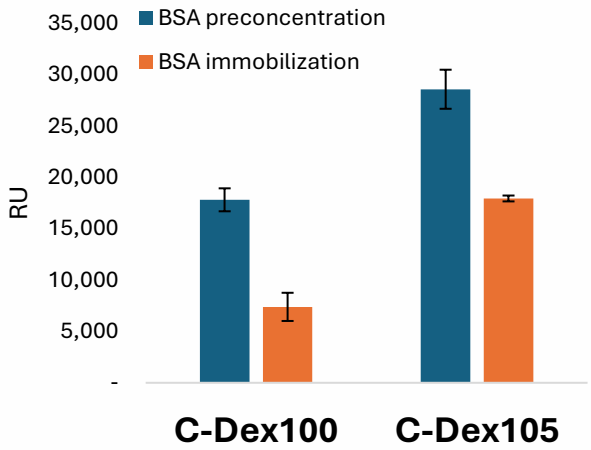
### Immobilization: CAII

1. Activation: Inject a mixed solution of 200 mM EDC and 100 mM NHS into channels 1 and 2 of the Pro2X in continuous mode for 7 minutes at 20 µl/min.
2. Amine coupling: Prepare CAII at a concentration of 50 µg/ml by dilution in acetate buffer (pH 5.0). Inject it into the ligand channel in individual mode for 9 minutes at 10 µl/min.
3. Blocking: Switch back to continuous mode and inject 1M ethanolamine-HCl for 7 minutes at 20 µl/min to block the remaining COOH functional groups.

### Kinetic analysis

1. Prepare the Furosemide analyte by 2-fold serial dilution in running buffer, as concentration of 10,000 nM, 5,000 nM, 2,500 nM, 1,250 nM, 625 nM, 313 nM, and 156 nM, with 300 µl for each dilution.
2. Association: Inject the prepared Furosemide solution for 2 minutes at 50 µl/min.
3. Dissociation: Observe dissociation for an additional 6 minutes while the running buffer flows.
4. Repeat steps 3 for each concentration solution.

## Results and Discussion

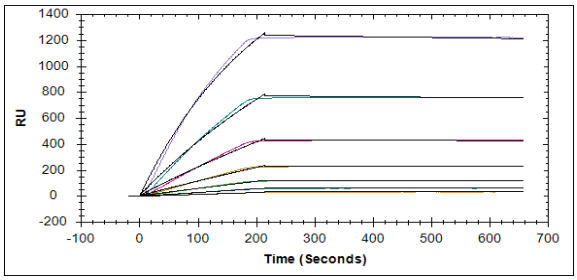



[Figure 3. Preconcentration and immobilization level of BSA on C-Dex100 and C-Dex105]

The newly developed C-Dex105 sensor chip demonstrated a substantial improvement in immobilization performance compared with the previous C-Dex100, as shown in Figure 3. In the comparison of ionic and covalent immobilization signals using BSA, C-Dex105 exhibited more efficient ligand immobilization, indicating that the surface carboxyl group density was effectively increased. As a results, C-Dex105 showed approximately 1.6-fold higher ionic interaction signals and about 2.5-fold higher covalent coupling signals relative to C-Dex100.

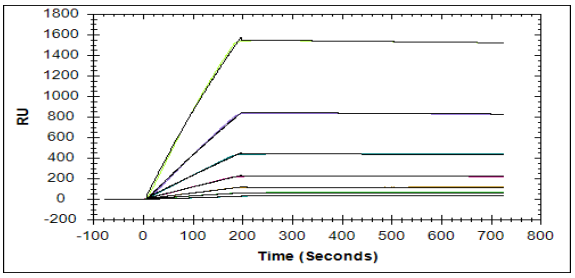
To assess whether the enhanced immobilization properties of C-Dex105 translate into practical binding performance, we evaluated its kinetic analysis capability using two representative binding models: a protein–protein interaction and a protein–small molecule interaction. In the protein–protein binding model (Protein A–IgG,

(1) C-Dex100




| $k_{on}$ ($M^{-1}s^{-1}$) | $k_{off}$ ($s^{-1}$) | $K_D$ (M) |
|---|---|---|
| $4.73 \times 10^4$ | $4.82 \times 10^{-5}$ | $1.02 \times 10^{-9}$ |

(2) C-Dex105



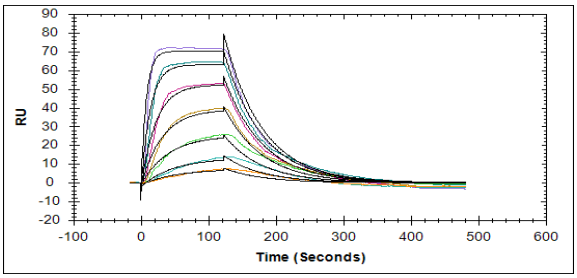

| $k_{on}$ ($M^{-1}s^{-1}$) | $k_{off}$ ($s^{-1}$) | $K_D$ (M) |
|---|---|---|
| $1.94 \times 10^4$ | $1.97 \times 10^{-5}$ | $1.02 \times 10^{-9}$ |

[Figure 4. Kinetic analysis results for Protein A-IgG on C-Dex100 and C-Dex105]

Figure 4), C-Dex105 exhibited no significant differences in $k_{on}$ and $k_{off}$ values compared with C-Dex100, and both chips produced similar $K_D$ values in the $10^{-9}$ M range. These results indicate that, despite the substantial increase in surface carboxyl group density enabled by optimized manufacturing, the C-Dex105 surface does not compromise protein structural stability or binding accessibility.

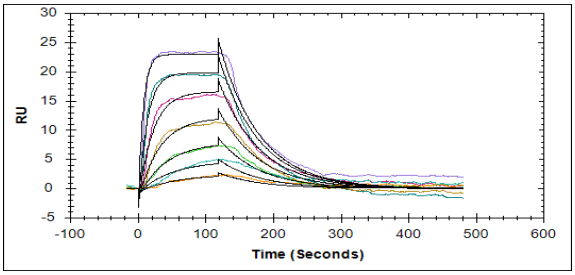
We further examined whether the increased binding capacity of C-Dex105 is also advantageous for small-molecule analysis by

(1) C-Dex105




| $k_{on}$ ($M^{-1}s^{-1}$) | $k_{off}$ ($s^{-1}$) | $K_D$ (M) |
|---|---|---|
| 1.16 x $10^4$ | 1.77 x $10^{-2}$ | 1.53 x $10^{-6}$ |

(2) HC1000




| $k_{on}$ ($M^{-1}s^{-1}$) | $k_{off}$ ($s^{-1}$) | $K_D$ (M) |
|---|---|---|
| 1.25 x $10^4$ | 1.68 x $10^{-2}$ | 1.35 x $10^{-6}$ |

[Figure 5. Kinetic analysis results for CAII-Furosemide on C-Dex105 and HC1000]

performing comparative measurements with HC1000, a sensor chip optimized for low–molecular-weight analytes. In the protein–small molecule model (CAII–Furosemide, Figure 5), C-Dex105 and HC1000 both yielded comparable $K_D$ values in the $10^{-6}$ M range, with stable and well-defined sensorgrams observed for both sensor chips.

Taken together, C-Dex105 demonstrated robust and reproducible performance across a wide molecular weight range, from protein–protein interactions to protein–small molecule binding analyses. These characteristics suggest that C-Dex105 can serve as a versatile standard sensor chip within the iMSPR platform, particularly in research environments requiring broad analytical capability or preliminary condition screening. Although the applicability of C-Dex105 has expanded significantly through this study, it should be interpreted in a complementary context relative to HC1000, as the two chips address different analytical needs. HC1000, characterized by its linear polycarboxylate surface, offers inherently high surface accessibility and reduced non-specific binding. Therefore, in experiments involving complex samples, conditions prone to nonspecific binding, or precise kinetic analyses of ultra–low–molecular-weight analytes near the detection limit of SPR, HC1000 may represent the more suitable option. By selecting between the two chips according to experimental objectives and sample characteristics, researchers can establish optimized and reliable analytical strategies.