SPR 분석서비스 결과

Core-Lab

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분석 개요

❖ 분석 내용 및 샘플 정보

Experiment	Ligand	Analyte
단백질과 화합물의 결합 확인 및 kinetic evaluation	Carbonic Anhydrase II	Furosemide

❖ 분석 정보

- Date: 2023-07-24 ~ 2023-07-25
- Instrument: iMSPR-ProX

1-1. Methods_Immobilization

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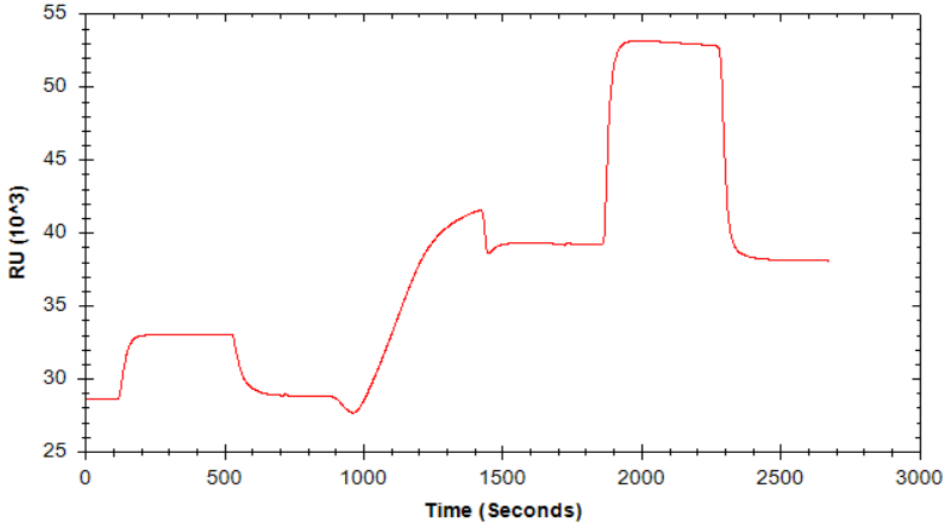
❖ Immobilization

- Amine coupling
- Sensor chip: HC1000M
- Running buffer: 10 mM Phosphate, 137 mM NaCl, 2.7 mM KCl, pH 7.4
- Flow rate: 10 µl/min

Immobilization		
Ch 1 (Ligand)	**Activation**	200 mM EDC+100 mM NHS, 7-min injection
	Ligand injection	50 µg/ml Carbonic Anhydrase II in 5 mM acetate buffer (pH 5.0), Inject time: 9-min
	Blocking	1 M Ethanolamine*HCl, 7-min injection
Ch 2 (blank)	**Activation**	200 mM EDC+100 mM NHS, 7-min injection
	Blocking	1 M Ethanolamine*HCl, 7-min injection

❖ Immobilization Results



Immobilization level: 9257.8 RU

1-2. Methods_Binding Analysis

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❖ Binding Analysis

- Running buffer: 10 mM Phosphate, 137 mM NaCl, 2.7 mM KCl, 1% DMSO, pH 7.4
- Flow rate: 50 µl/min
- Analyte concentration: 10 µM

Binding Analysis	
Ch 1-2	**Association time** 2 min
	Dissociation time 2.5 min

❖ Regeneration

- Remove the analyte
- Regeneration solution: 3 M NaCl
- Flow rate: 50 µl/min

Regeneration	
Ch 1-2	**Injection time** 1 min
	Stabilization time 5 min

1-3. Results_Binding Analysis

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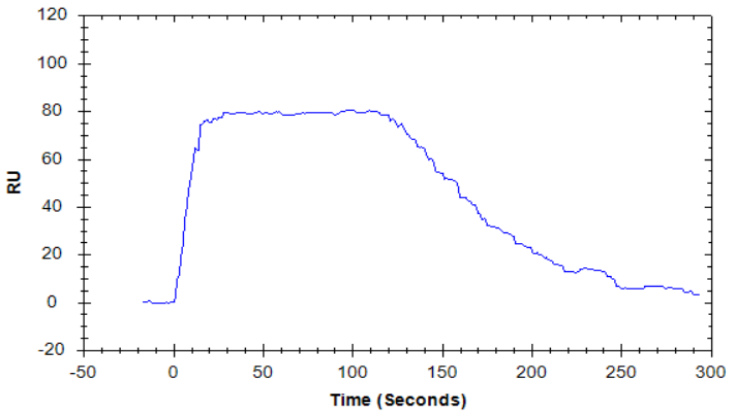
❖ Furosemide_10 μM



→ Kinetic 분석 기준 충족

❖ 분석 기준

Affinity 분석 기준
: RU(association)>10RU

Kinetic 분석 기준
: RU(association)>10RU
RU(dissociation)>5RU

1-4. Methods_Kinetic evaluation

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❖ Binding Analysis

- Running buffer: 10 mM Phosphate, 137 mM NaCl, 2.7 mM KCl, 1% DMSO, pH 7.4
- Flow rate: 50 µl/min
- Analyte concentration: 0.156, 0.313, 0.625, 1.25, 2.5, 5, 10, 0 µM

Binding Analysis		
Ch 1-2	**Association time** 2 min	
	Dissociation time 6 min	

❖ Regeneration

- Remove the analyte
- Regeneration solution: 1 M NaCl
- Flow rate: 50 µl/min

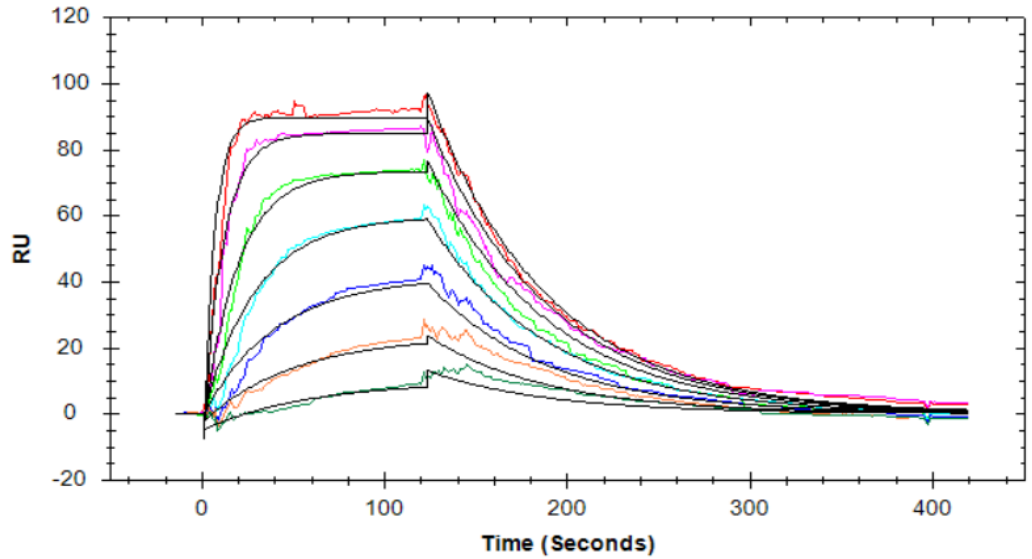
Regeneration		
Ch 1-2	**Injection time** 1 min	
	Stabilization time 5 min	

❖ Evaluation

- Evaluation S/W: TraceDrawer

1-5. Results_Kinetic evaluation

❖ Furosemide



	k_a (1/Ms)	k_d (1/s)	R_{max} (RU)	RI (RU)	K_A (1/M)	K_D (M)	Chi^2
	1.54E+04	1.52E-02	106.61		1.01E+06	9.87E-07	6.72
0.156 μM				-4.87			
0.313 μM				-22.3			
0.625 μM				0.07			
1.25 μM				0.11			
2.5 μM				-3.15			
5 μM				-4.26			
10 μM				-7.41			