分子間相互作用解析 - 質量分析法（MIK-MS）による創薬ターゲット化合物スクリーニングシステムの開発と諸課題

Development of Drug Discovery Screening System by Molecular Interaction & Kinetics Mass Spectrometry (MIK-MS)

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Introduction

At the annual conference in 2015, we reported new method of intermolecular interaction analysis integraded of mass spectrometry analysis using the principle of Nano Pore Optical Interferometry (nPOI)(Fig. 1), aimed at high-throughput screening (HTS) of drug discovery candidate compounds. Molecular Interaction Kinetics - Mass Spectrometry (MIK-MS) would be a powerful tool to screen drug candidates by integrating LC-MS with nano Pore Optical Interferometry (nPOI). nPOI can measure the association and dissociation behavior of biomolecules without labels such as Surface Plasmon Resonance[1]. Since nPOI has much larger ligand binding capacity, all analytes eluted can be directly detected by nano ESI LC-MS integrated, thereby confirming the binding behavior and identification of analytes. The interaction strength on association - dissociation between the protein, carbonic anhydrase II (CAII), and compounds with the low molecular weights, 172 to 341 Da, were evaluated as Molecular Interaction Kinetics - Mass Spectrometry (MIK-MS)

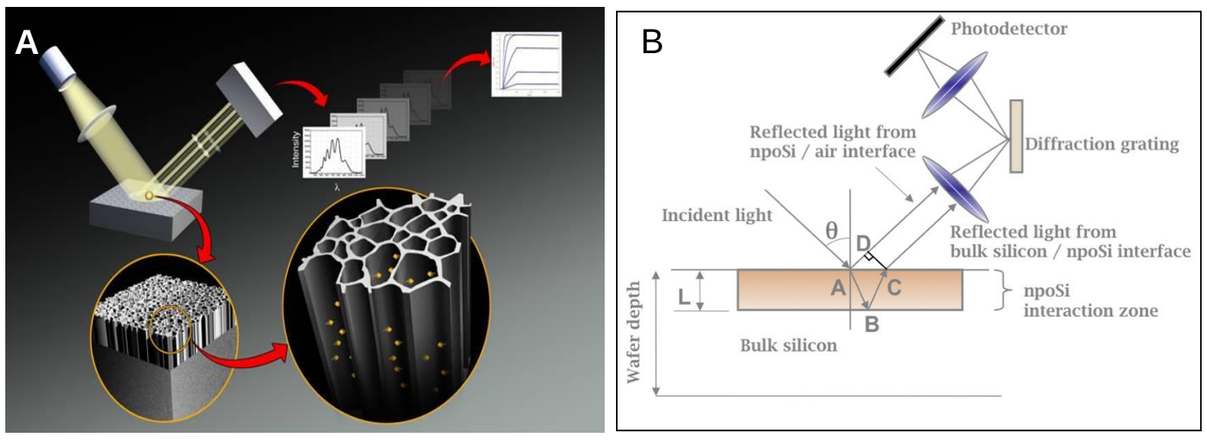
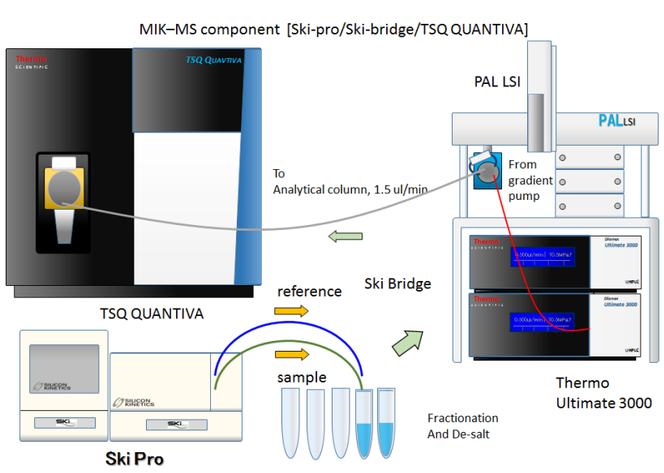


Figure 1. A: nano-porous biosensor principle. B: Overview of optical path and physics of signal acquisition [2].



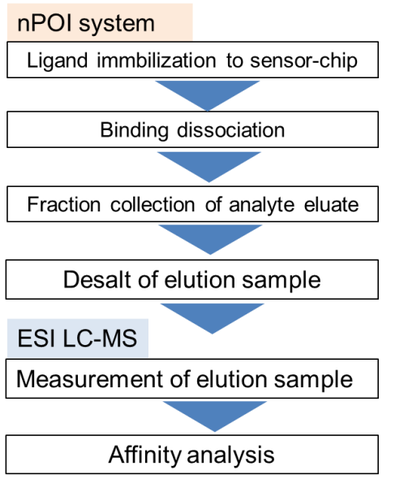
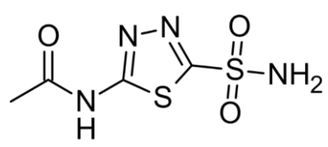


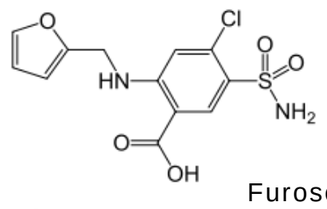
Figure 2. Diagram of the component of MIK-MS system(left) and experimental flow of MIK-MS analysis(right)

Materials and Methods

CAII was immobilized onto the COOH sensor chip (Silicon Kinetics Inc. San Diego, CA) using standard amine coupling. Briefly, the surface was activated using 200 mM EDC and 50 mM sNHS solution, and 500 µg/mL CAII was exposed 7 minutes to the activated surface for 7 minutes at pH 4.5. The surface was then blocked with 1 M ethanolamine, pH 8.5 for 7 minutes and equilibrated into 0.1 % DMSO in a with PBS buffer. A reference surface was prepared by activating and subsequently blocking the surface under otherwise identical conditions. Associations and dissociations were performed in PBS containing 0.1 % DMSO at a flow rate of 20 µL/min. Furosemide, Sulpiride, DNSA, Acetazolamide, 4-CBSA and Sulfanilamide were dissolved in 0.1 % DMSO with PBS buffer as 16 µM stocks(Fig. 3,4, Table 1). The 50 µL of mix solution was injected over the reference and CAII flow cells for 90 seconds(Fig.5). Then the elution after throw sensor chip are collected from waste line of SKi-Pro System every 30 seconds. Reconstructed elution samples were injected into the LC-MS/MS system. (Fig. 2).

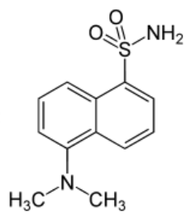


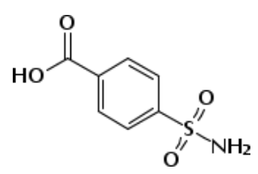
Acetazolamide



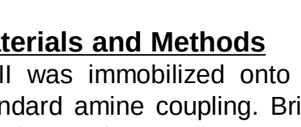
Furosemide

Dansylamide
(DNSA)

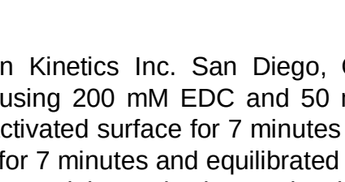




4-Carboxybenzene-sulfonamide
(4-CBSA)



Sulfanilamide



Sulpiride

Figure 3. Chemical structural formula of small molecules, applied on MIK-MS evaluation

Table 1. Summary of results for small molecule to CAII binding. Data on the right represents published results obtained by SPR [3].

Compound	M.W. Da	nPOI Data					SPR Data				
		k_{on}, M^{-1} s^{-1}	k_{off}, s^{-1}	K_D, µM (kinetics fit)	Maximum Response, nm	Ranking	k_{on}, M^{-1} s^{-1}	k_{off}, s^{-1}	K_D, µM (kinetics fit)	Maximum Response, nm	Ranking
Acetazolamide	222.2	3.76E+05	2.74E-02	0.073(2)	0.21	1	2.93E+06	5.58E-02	0.019(1)	24	1
Furosemide	330.7	1.24E+04	1.21E-02	0.976(6)	0.59	2	9.66E+04	4.96E-02	0.513(1)	37	2
Dansylamide	250.3	5.50E+04	1.50E-01	2.73(2)	0.36	4	2.22E+05	1.68E-01	0.756(3)	32	3
4-CBSA	201.2	1.14E+04	1.14E-02	1.24(2)	0.37	3	4.13E+04	3.69E-02	0.893(3)	22	4
Sulfanilamide	172.2	9.70E+03	4.20E-02	4.33(3)	0.18	5	2.27E+04	1.33E-01	5.86(6)	22	5
Sulpiride	341.4	3.95E+02	1.43E-01	362(6)	0.6	6	3.44E+03	6.41E-01	186(5)	35	6

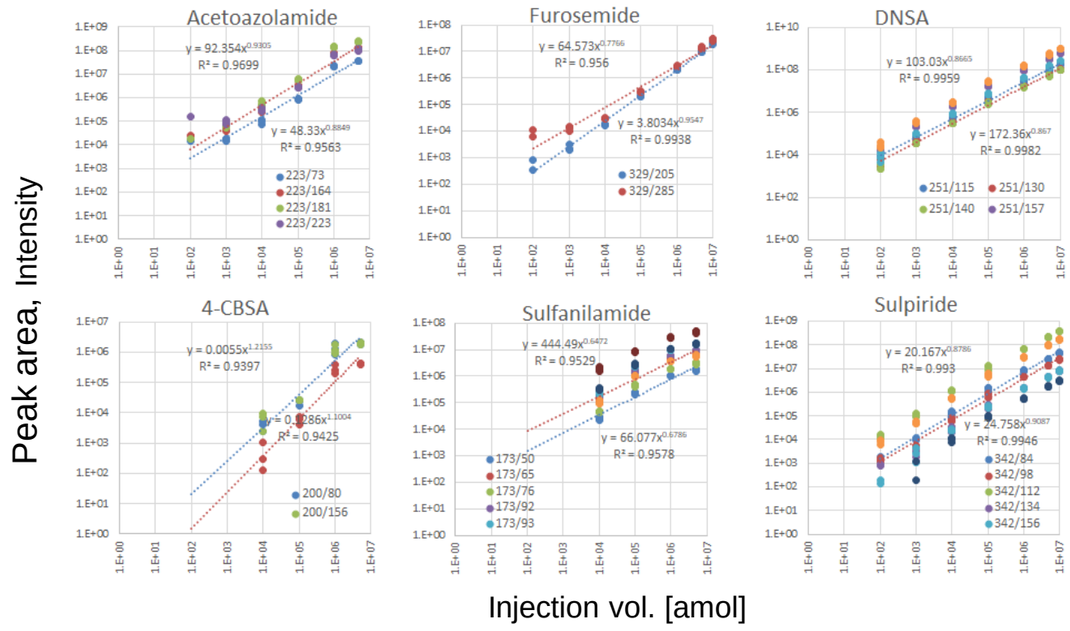


Fig. 4 Calibration curve of six small compounds, 100 amol – 10 pmol

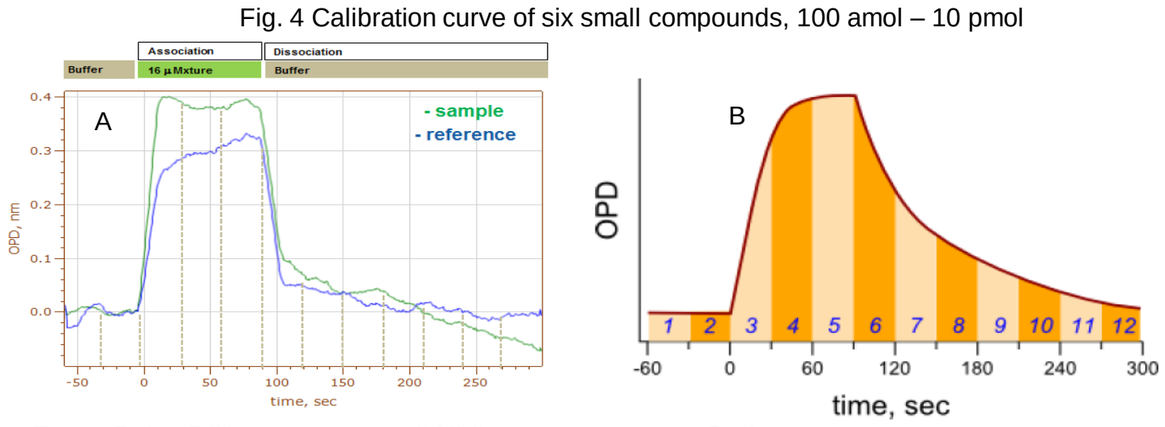


Figure 5: A: nPOI sensorgram of CAII/6 small compounds. B: Scheme for collecting fractions in every 30sec in the mass spec sensorgram experiment.

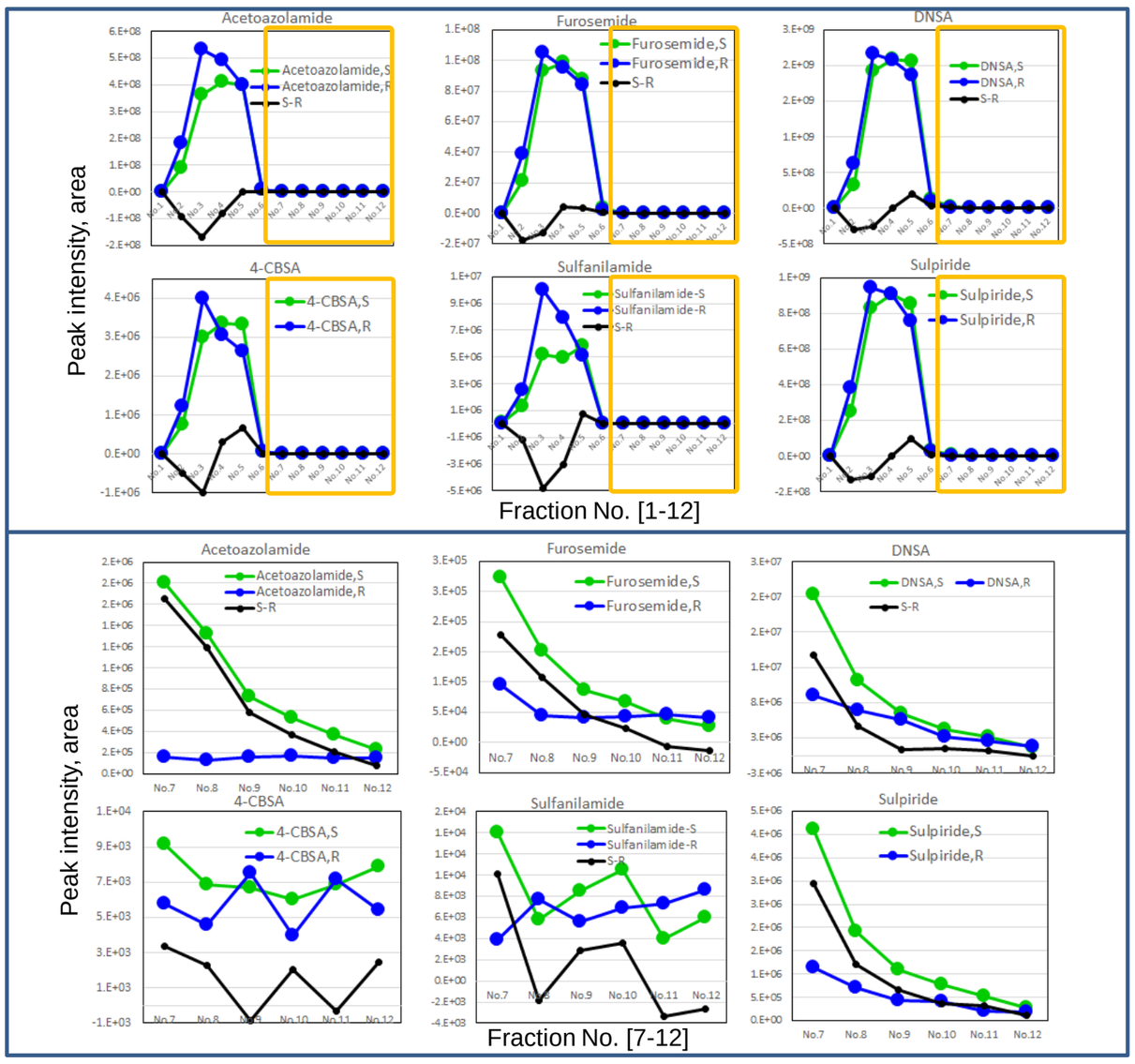


Figure 6: MS sensorgram of CAII / 6 mixture compounds by MIK-MS.
Upper: Franction No.1-12, Lower: Fraction No. 7-12

Table 2. SRM transition of six small molecules and affinity ranking by MIK-MS

Compound	MW, Da	+/-*	Precursor Ion m/z	Product Ions m/z				MIK-MS k_{off}
Acetazolamide	222.25	+	223.00	73.1	181.1	223.2	-	0.019
Furosemide	330.75	-	329.00	78.1	126.0	205.0	285.0	0.022
Dansylamide	250.32	+	251.09	115.1	157.2	171.2	236.1	0.025
4-CBSA	201.20	+	202.02	121.1	-	-	-	-
Sulfanilamide	172.21	+	173.04	92.2	93.2	156.1	173.1	-
Sulpiride	341.43	+	342.15	98.2	112.2	214.1	285.0	0.021

*The +/- column indicates the polarity used.

CONCLUSIONS

-The calibration curves of six small compounds were obtained on the Triple-Q MS in SRM-mode(Fig.4).

-Binding dissociation behavior between Ligand and Analyte can be measured by using MIK-MS system.

-The good results were found for acetozoamide, DNSA and furosemide and sulpiride(Fig. 6, Table 2).

-The results to date suggest that MIK-MS can determine the Association-dissociation curve of a mixed sample in one-time analysis.

References

1) Nakayama N, Bando Y, Fukuda T, Kawamura T, et. al., Drug metabolism and pharmacokinetics, (2015) 1-9, Volume 31, Issue 1, Pages 3-11
2) Martin Latterich et al, Proteome Science, 2008. 6: 31
3) Application note 11. Charactorization of Small molecules to Protein Binding. www.siliconkinetics.com