Biacore 8K Sample Submission Guideline

I. Sample Submission

1. Apply an account on the BCF reservation system using your official email address, which will be used to identify your affiliations. BCF will not accept service requests from public e-mail domains, such as gmail, outlook or yahoo, etc.
2. File the sample submission form in "Service Request" tab under instrument "Biacore 8K". Please note that there is a setup fee associated with your first sample. Additional samples, applying **the same experimental setting** as the first one, may be applicable for discount rates. Samples (ligands or analytes) require pH scouting, buffer selection and/or regeneration buffers selection tests will be treated as "first sample".
3. After confirming the charges and experiment time through reservation system, samples and buffers may be submitted to Miss Jin-Hsuan Yu (Tel: 27855696 x4024, e-mail: bcf@gate.sinica.edu.tw). If BCF does not receive your samples before the scheduled time listed in the service request application form, the facility will add an extra charge for the delay, unless early notification is sent by emails more than two days in advance
4. Samples submitted to BCF should be non-hazardous, non-toxic and nonpathogenic. No radioactive or microbial samples are allowed.
5. Data analysis is the responsibility of users. However, preliminary analysis is provided in Excel format.
6. BCF will not compensate for your sample loss or data loss under any circumstances (hardware or software failure, operator error, or others). All experimental results are for research only. Without written permission from Academia Sinica, the user shall not claim, announce, or mislead the public into interpreting that the results of this testing is in any way related to the commercial development of the user. In addition, the user shall not in any form (including but not restricted to commercial marketing, for example advertisements, either online or offline, product packaging, catalogs, investment information etc,) use the title, logo, name, trademark or symbols that are that of Academia Sinica or similar to that of the facility, that gives the false impression of a commercial collaboration.

II. Sample preparation

Samples should be >95% pure by SDS-PAGE or SEC and no visual precipitation when spun at 12,000 x g for 10 minutes. Please provide SDS-PAGE or SEC results by e-mail. Additives with primary amines, such as Tris, amino acids, gentamycin and BSA, are prohibited in the buffer for ligand if amine coupling is used for immobilization. For less background bulk interference, high refractive index materials, such as glycerol and imidazole, should be avoided in the analytes' buffers.

Ligand:

Two aliquots of 100uL of 1-10uM ligand for assay development and experiments.

Analyte:

Three aliquots of 100uL analyte with a concentration of "**100*20 * estimate K_D**", preferably dissolved in the running buffer. If no estimate K_D can be referred, try 10nM for antibodies, 5uM for proteins, 50uM for small molecules and 500uM for polysaccharides and fragments. Direct-immobilization instead of capturing method is recommended when analytes are small molecules and fragments.

* Please pick up unused samples and sensor chips within one week when completed.

III. Experimental setting:

1. ***Immobilization***:
   Direct-immobilization: amine coupling and SA (***sensor chips provided by user***).
   Capturing: NTA, anti-mouse antibodies, anti-human IgG (Fc) antibody, anti-GST antibodies, anti-histidine antibody, protein A (***sensor chips provided by the facility***).

2. ***Single Cycle Kinetics (SCK)***:

   Temperature (℃): 25

   Association time (s): 150
   Dissociation time (s): 600
   Concentration ranges: depending on the highest concentration of analyte provided and estimate K_D
   Regeneration condition: according to manufacturer's suggestion or running buffer
   Cycle numbers (6): 3 startup cycles + 2 blanks + 1 cycle of 5 concentrations

3. ***Multiple Cycle Kinetics (MCK)***:

   Temperature (℃): 25

   Association time (s): 150 or according to previous SCK results
   Dissociation time (s): 300 or according to previous SCK results
   Concentration range: according to previous SCK results
   Regeneration condition: according to manufacturer's suggestion or scouting results.
   Cycle numbers (11): 3 startup cycles + 2 blanks + 5 concentrations + 1 concentration duplicate

4. The experiment will be repeated 3 times by either SCK or MCK. **Please note that SCK and MCK may be performed repeatedly only if regeneration is feasible. Cycle numbers (SCK or MCK) and replicates depend on the amount of samples provided by user.**

IV. Data Analysis:

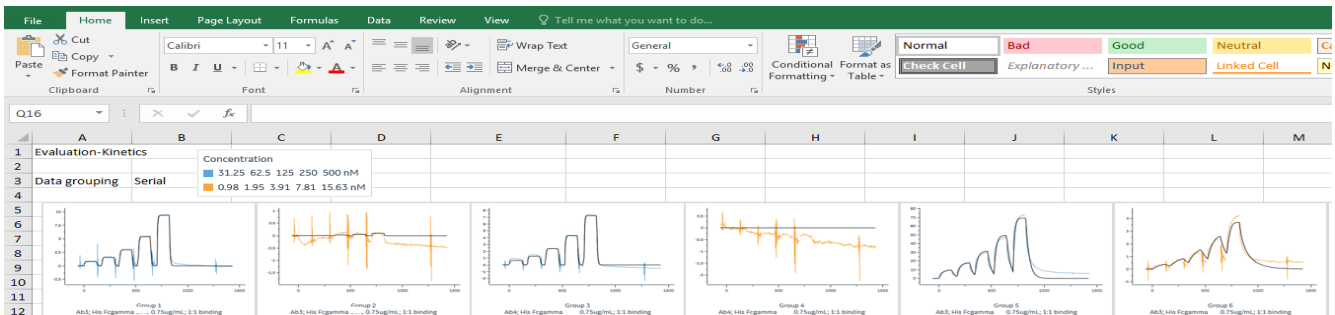
1. Raw data and preliminary analysis can be downloaded through the BCF reservation system.
2. Double reference is performed by subtracting reference channel and the preceding sensorgram.
3. The dissociation equilibrium constant (K_D), association rate constant (k_{on} or k_a) and dissociation rate constant (k_{off} or k_d) are presented as is fitted using kinetics "1:1 binding" model in files of Excel format.
4. Please check Biacore8K System Handbook for more fitting models, for example, "Two state reaction", "Bivalent analyte" and "Heterogeneous ligand" models.
5. Biacore Insight Evaluation software is available in the laboratory for data processing.

V. Acknowledgement

Please acknowledge us if research supported and/or data generated by this instrument results in publications. For example,

"We acknowledge Biacore 8K data collected by [operator] in the Biophysics Core Facility funded by Academia Sinica Core Facility and Innovative Instrument Project (AS-CFII-111-201)."

VI. Example of kinetics results of SCK during method development.



		General			Injection variables		Quality	1:1 binding				
	Group	Kinetics model	Channel	Accepted	Capture 1 Solution	SCK Solution	Kinetics Chi² (RU²)	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	tc
16	1	1:1 binding	1		His FcgammaRIIIa XX	Ab3	6.53E-02	1.42E+05	1.78E-01	1.25E-06	32.8	4.41E+17
17	2	1:1 binding	2	Rejected	His FcgammaRIIIa XX	Ab3	7.48E-02	2.61E+05	5.84E-01	2.24E-06	14.8	1.22E+10
18	3	1:1 binding	3		His FcgammaRIIIa XX	Ab4	6.95E-02	1.67E+05	1.68E-01	1.01E-06	21.8	6.37E+12
19	4	1:1 binding	4	Rejected	His FcgammaRIIIa XX	Ab4	2.49E-01	2.04E+04	1.09E-02	5.33E-07	0.0	1.01E+08
20	5	1:1 binding	5		His Fcgamma RIIa OO	Ab3	2.87E+01	9.10E+04	3.04E-02	3.34E-07	115.7	3.90E+13
21	6	1:1 binding	6	Rejected	His Fcgamma RIIa OO	Ab3	1.44E-01	1.43E+10	1.33E+02	9.30E-09	5.9	1.49E+06
22	7	1:1 binding	7		His Fcgamma RIIa OO	Ab4	1.08E+01	9.08E+04	3.71E-02	4.08E-07	114.5	7.96E+11
23	8	1:1 binding	8	Rejected	His Fcgamma RIIa OO	Ab4	1.01E-01	2.17E+09	3.66E+01	1.69E-08	7.4	1.71E+06

2022/01/01 Chris Jao