1. Calculations

This document contains a number of formulas used with surface plasmon resonance and biomolecular interaction analysis. For the convenience of the reader, an Excel spreadsheet (BiaCalculations.xlsm) is available on www.sprpages.nl that facilitates the use of these formulas.

The abbreviations used in the formulas are:

Name	Meaning
Da	Dalton
RU	response units
M	Molar
Mr	molecular mass in Da
R	response in RU
Req	equilibrium response in RU
Rmax	maximal response in RU
k_a	association rate constant in $M^{-1}s^{-1}$
k_d	dissociation rate constant in s^{-1}
K_D	equilibrium constant in M
D	diffusion constant in m^2s^{-1}

The units used in the formulas are:

Symbol	Name	SI units
N	Newton	kg m s^{-2}
Pa	Pascal	N m^{-2}
J	Joule	N m
η	Liquid viscosity	Poise = Pa s

The constants used in the formulas are:

Symbol	Name	SI units
N_A	constant of Avogadro	6.0220 10^{23} mol^{-1}
R	Gas constant	8.3144 J mol^{-1} K^{-1}
k	constant of Boltzmann	1.3807 10^{-23} J K^{-1}
h	constant of Planck	6.6262 10^{-34} J s
c	speed of light (vacuum)	2.9979 10^{8} m s^{-1}

Each worksheet is divided in two parts:
(yellow) input of data
(orange) output of calculations

Please note that in order to obtain useful results, the input values should be in the correct units (as indicated per input cell).

2. Conversions

2.1. Response to concentration conversion

The surface concentration Gamma (G), for a protein can be calculated by the following formula (3). One Response Unit (RU) in the Biacore 2000/3000 machine corresponds to a surface coverage of 10^{-6} g m^{-2} for a typical protein. A 100 kDa protein generating a response of 1000 RU, corresponds to a surface coverage of 10^{-8} moles m^{-2}.

Formulas

$$Gamma = \frac{Response * 10^{-6}}{Mr} \text{ (mole.m}^{-2}\text{)} \quad (1)$$

$$surface\ density = \frac{response}{1000000} \quad (g\ m^{-2}) \quad (2)$$

$$binding\ sites = \frac{surface\ density}{molecular\ mass} \quad (mol\ m^{-2}) \quad (3)$$

$$concentration = \frac{response}{100 * molecular\ mass} \quad (mol\ L^{-1}) \quad (4)$$

Input		
Response	500	RU
Molecular mass	60000	Da
Calculated		
Binding sites	0.0083	pmol mm^{-2}
Binding sites	8.3E-09	mol m^{-2}
Surface density	0.5	ng mm^{-2}
Surface density	0.0005	g m^{-2}
Concentration	8.3E-05	mol L^{-1}
Concentration	5	mg ml^{-1}

2.2. Concentration calculation

Calculate molar concentrations based on mg ml^{-1} and molecular mass and calculate dilutions.

	Stock concentration			
Protein	**mg ml^{-1}**	**Da**	**M**	**µM**
A	2.7	150000	1.80E-05	18.00
B	4.66	150000	3.11E-05	31.07

	Pre dilutions			
	stock	**volume**	**end vol.**	**conc**
	µM	**µl**	**µl**	**nM**
A	18.00	10	720.0	250
B	31.07	10	310.7	1000

Use this table to calculate the concentration of small compounds when dissolving small quantities in small volumes.

compound	**amount**	**Mw**	**amount**	**conc**	**volume**	**conc**
	gram	**Da**	**Mol**	**M**	**ml**	**M**
T101	0.005	248.77	2.01E-05	2.01E-05	0.5	4.02E-02

2.3. Calculating immobilization

The table calculates the amount of ligand to immobilize to obtain a theoretical analyte response. In general, a R_{max} of 50 – 100 RU when the analyte is injected is sufficient (6). Immobilization of too much ligand can lead to mass transfer limitation and non-Langmuirian kinetics due to steric hindrance (4, 8).

Formula

$$R_{ligand} = \frac{Mr_{ligand} \cdot R_{max}}{Mr_{analyte} \cdot Valency_{ligand}} (RU) \qquad (5)$$

Ligand		
Molecular mass	60000	Da
Valency	1	
Activity	100	%
Analyte		
Molecular mass	2000	Da
Desired Rmax	100	RU
Calculations		
Ligand immobilization	3000	RU
Ligand sites	0.05000	pmol mm^{-2}

2.4. Calculations after immobilization

The table calculates some parameters after immobilization of the ligand and injection of the analyte. The theoretical R_{max} is calculated based on the molecular mass and amount of immobilized ligand and the molecular mass of the analyte. The same is done for the ligand sites and concentration. The amount of functional ligand (the ligand that is binding the analyte) is calculated on the basis of the measured Rmax (2).

Formulas

$$Rmax_{analyte} = \frac{Mr_{analyte} \cdot R_{ligand} \cdot Valency_{ligand}}{Mr_{ligand}} \quad (RU) \quad (6)$$

$$Ligand_{sites} = \frac{Response_{ligand}}{Mr_{ligand} \cdot Valency_{ligand}} \quad (pmol.mm^{-2}) \quad (7)$$

$$Ligand\ concentration = \frac{R_{ligand}}{100 \cdot Mr_{ligand}} \quad (mol.l^{-1}) \quad (8)$$

$$Ligand_{functional} = \frac{Rmax}{R_{ligand}} \cdot \frac{Mr_{ligand}}{Mr_{analyte}} \cdot 100 \quad (\%) \quad (9)$$

Ligand		
Molecular mass	40000	Da
Immobilization	111	RU
Valency	1	
Analyte		
Molecular mass	92000	Da
Rmax	49	Da
Calculations		
Rmax analyte	255	RU
Ligand sites	0.002775	pmol/mm^2
Ligand concentration	2.78E-05	mol/L
Ligand activity	19.2	%

3. Flow and Flow cell

3.1. Flow rate

The table converts the flow rate (µl/min) in different units.

Formulas

$$flowrate_{(l/s)} = \frac{\left(\frac{flow\ rate_{(ul/min)}}{60}\right)}{10000} \quad (l.s^{-1}) \quad (10)$$

$$flowrate_{(m3/s)} = \frac{\left(\frac{flow\ rate_{(ul/min)}}{60}\right)}{10000000} \quad (m^3.s^{-1}) \quad (11)$$

Flow rate	f	25	µl.min^{-1}
Flow rate		4.17E-07	l.s^{-1}
Flow rate		4.17E-10	m^3 s^{-1}

3.2. Flow cell

The table calculates the volume of the flow cell and the refresh rate.

Formulas

$$flowcell\ volume = l \cdot b \cdot h \quad (m^3) \quad (12)$$

$$refresh\ rate = \frac{flow\ rate}{flowcell\ volume} \quad (s^{-1}) \quad (13)$$

Flow rate	f	3	µl min^{-1}	5.00E-11	m^{3}s^{-1}
Height	h	0.05	mm	5.00E-05	m
Width	w	0.3	mm	3.00E-04	m
Length	l	0.8	mm	8.00E-04	m
Volume	v	1.20E-02	mm^3	1.20E-11	m^3
Refresh rate		250	times per min^{-1}	4.2	times per s^{-1}

3.3. Thickness of diffusion layer

The table calculates the thickness of the diffusion layer on basis of the diffusion coefficient (see below), flow cell dimensions and flow rate (5).

Formula

$$d = \sqrt[3]{\frac{(D \cdot h^2 \cdot w \cdot l)}{f}} \quad \text{(m)} \quad (14)$$

Diffusion coefficient	D	5.00E-11	m^2s^{-1}	5.00E-11	m^2s^{-1}
Flow rate	f	3	$\mu l\ min^{-1}$	5.00E-11	m^3s^{-1}
Height	h	0.05	mm	5.00E-05	m
Width	w	0.3	mm	3.00E-04	m
Length	l	0.8	mm	8.00E-04	m
Thickness	d	8.4	µm	8.43E-06	m

3.4. Time shift between flow cells

The table calculates the time delay between flow cells of the Biacore 1000, 2000 and 3000 machines.

Formula

$$t_s = \frac{0.3}{\text{flow rate}} 60 \quad \text{(s)} \quad (15)$$

Flow rate	f	10	$\mu l\ min^{-1}$
Flow cell volume	v	0.3	$\mu l\ min^{-1}$
Shift		1.8	s

3.5. Wall shear

The table calculates wall shear based on the flow rate and a constant.

Formula

$$q = jw \cdot 0.0121 \quad \text{(ul.min}^{-1}\text{)} \quad (16)$$

Flow rate	q	25	$\mu l\ min^{-1}$
Wall shear	jw	2066	s-1
Constant	f	0.0121	

4. Diffusion

4.1. Diffusion factor for globular proteins

The diffusion coefficient (D) for a globular protein can be approximated by the formula below (3). The f/fo is the friction factor and can be set to 1.2 if unknown. For proteins with an increasing degree of asymmetry (less globular), this value will become larger. The v/vo is the viscosity of the solution relative to water of 20°C. For water, the value is 0.89.

Formula

$$D = \frac{1.10^{-11} \cdot 324.3 \cdot Mr^{-(1/3)}}{\left(f/_{fo} \cdot v/_{vo}\right)} \quad (m^2.s^{-1}) \qquad (17)$$

Molecular mass	Mr	50000	Da
Friction factor	f/fo	1.2	
Solution viscosity	v/vo	0.89	
Factor 1		1.00E-11	
Factor 2		324.3	
Diffusion factor	D	8.27E-11	m^2s^{-1}

4.2. Mass transport coefficient (km)

$$k_m = \sqrt[3]{\frac{D^2 \cdot f}{0.3 \cdot h^2 \cdot w \cdot l}} \quad (m.s^{-1}) \qquad (18)$$

Diffusion factor	D	6.94E-11	$m^2\ s^{-1}$
flow rate	f	5.00E-11	m/s
flow cell height	h	5.00E-05	m
flow cell width	w	3.00E-04	m
flow cell length	l	8.00E-04	m
factor 1		0.3	
factor 2		0.98	
k_m		1.0803E-05	$m\ s^{-1}$

4.3. Mass transport coefficient (kt)

$$k_t = k_m \cdot Mr \cdot 10^9 \quad (RU.M^{-1}.s^{-1}) \tag{19}$$

k_m		1.08E-05	m s^{-1}
Molecular weight	Mr	66500	Da
Factor		1.00E+09	
k_t		7.18E+08	RU M^{-1} s^{-1}

4.4. Dynamic viscosity of water

$$v = A * 10^{\left(\frac{B}{(T-C)}\right)} \quad (Pa.s) \tag{20}$$

	A	2.41E-05	Pa.s
	B	247.8	K
	C	140	K
	T	298	K
	v	8.93E-04	Pa.s

The dynamic viscosity of water is 8.90 × 10−4 Pa·s or 8.90 × 10−3 dyn·s/cm2 or 0.890 cP at about 25 °C.
Water has a viscosity of 0.0091 poise at 25 °C, or 1 centipoise at 20 °C.
As a function of temperature T (K): (Pa·s) = A × 10B/(T−C)
where A=2.414 × 10−5 Pa·s ; B = 247.8 K ; and C = 140 K.[citation needed]

5. Dilution

Use the dilution table to calculate minimal injection volumes and dilution steps.
Start with the flow rate, injection time and replicates.
Then transport the total volume value to the final volume (you can adjust the final volume).
Adjust the dilution step, highest desired analyte concentration and stock concentration.
In the dilution scheme, the dilutions are given. In addition, some parameters are calculated from the input of the k_a, k_d and glycerol values. The column under Req (%) lists the Req value in percentage of Rmax which can be reached when the injection time is long enough. The values are green when the fall in the range of 10 – 90% Rmax.

	Biacore 2000/3000	T100/200	
Inject command	KInject	Inject	
Flow rate	30	30	ul/min
Inject time	2	2	min
Replicates	2	2	x
Extra volume	40	40	ul
Total volume	200	200	ul

Protein		
Dilution step	2	x
Final volume	200	µl
Highest conc.	1000	nM
Concentration	8642	nM

Vial	Stock (nM)	Stock (µl)	buffer (µl)	mix (µl)	Final solution (nM)	Final solution (µl)	K_D (x)	Req (%)	Glycerol (%)
1	8642.00	**46.29**	**353.7**	400.0	**1000.00**	**200.0**	10.00	91	11.571
2	1000.00	**200.0**	**200.0**	400.0	**500.00**	**200.0**	5.00	83	5.786
3	500.00	**200.0**	**200.0**	400.0	**250.00**	**200.0**	2.50	71	2.893
4	250.00	**200.0**	**200.0**	400.0	**125.00**	**200.0**	1.25	56	1.446
5	125.00	**200.0**	**200.0**	400.0	**62.50**	**200.0**	0.63	38	0.723
6	62.50	**200.0**	**200.0**	400.0	**31.25**	**200.0**	0.31	24	0.362
7	31.25	**200.0**	**200.0**	400.0	**15.63**	**200.0**	0.16	14	0.181
8	15.63	**200.0**	**200.0**	400.0	**7.81**	**200.0**	0.08	7	0.090
9	7.81	**200.0**	**200.0**	400.0	**3.91**	**200.0**	0.04	4	0.045
10	3.91	**200.0**	**200.0**	400.0	**1.95**	**400.0**	0.02	2	0.023

6. Equilibrium

6.1. Theoretical Req

The table calculates the theoretical Req on basis of the association and dissociation constants and the analyte concentration. Input of (calculated) values from the first analyte injection can provide estimates of the range of concentrations needed for the future experiments. The Rmax is included to visualize the actual data. The column with 'x KD' shows the relation between K_D and the analyte concentration.
The analyte concentrations come from the dilution page.

Formulas

$$R_{eq} = \frac{k_a \cdot C}{k_a \cdot C + k_d} R_{\max} \quad (RU) \qquad (21)$$

$$R_{eq} = \frac{R_{eq}}{R_{max}} \cdot 100\% \quad (\%) \tag{22}$$

$$xK_D = \frac{C}{K_D} \tag{23}$$

k_a	1.00E+06	$M^{-1}s^{-1}$
k_d	1.00E-02	s^{-1}
Rmax	100	RU
Rmax	95	%

C	C	KD	Rmax	Rmax
(M)	(nM)	(x)	(RU)	(%)
1.00E-06	1000.00	100.000	99	99
5.00E-07	500.00	50.000	98	98
2.50E-07	250.00	25.000	96	96
1.25E-07	125.00	12.500	93	93
6.25E-08	62.50	6.250	86	86
3.13E-08	31.25	3.125	76	76
1.56E-08	15.63	1.563	61	61
7.81E-09	7.81	0.781	44	44
3.91E-09	3.91	0.391	28	28
1.95E-09	1.95	0.195	16	16

6.2. Time to equilibrium

The table calculates the time to reach equilibrium based on the association and dissociation constants and the analyte concentration. The percentage of Req to reach is set to 95% of the Rmax. This can be adjusted at the kinetics input.

Formulas

$$t_\Theta = \frac{-\ln(1-\Theta)}{k_a \cdot C + k_d} \quad \text{(s)} \quad (24)$$

k_a	1.00E+06	$M^{-1}s^{-1}$
k_d	1.00E-02	s^{-1}
Rmax	100	RU
Rmax	95	%

C (M)	Time (s)	Time (min)	Time (hour)
1.00E-06	3	0	0.0
5.00E-07	6	0	0.0
2.50E-07	12	0	0.0
1.25E-07	22	0	0.0
6.25E-08	41	1	0.0
3.13E-08	73	1	0.0
1.56E-08	117	2	0.0
7.81E-09	168	3	0.0
3.91E-09	215	4	0.1
1.95E-09	251	4	0.1

6.3. Complex half-life

The table calculates the time to dissociate to half the starting value. The complex half-life depends only on the dissociation constant (1). As a rule of thumb, the dissociation curve should decrease at least 5% before analysis can be attempted (7).

Formula

$$t_{1/2} = \frac{\ln 2}{k_d} \quad (s^{-1}) \qquad (25)$$

kd	t1/2	t1/2	t1/2
(s-1)	(s)	(min)	(hour)
1.00E-01	7	0.1	0.0
1.00E-02	69	1.2	0.0
1.00E-03	693	11.6	0.2
1.00E-04	6931	115.5	1.9
1.00E-05	69315	1155.2	19.3
1.00E-06	693147	11552.5	192.5

6.4. Time to 5% dissociation

Formula

$$t_{1/2} = \frac{\ln(100-95)}{k_d} \quad (s^{-1}) \qquad (26)$$

kd	t	t	t
(s-1)	(s)	(min)	(hour)
1.00E-01	1	0.0	0.0
1.00E-02	5	0.1	0.0
1.00E-03	51	0.9	0.0
1.00E-04	513	8.5	0.1
1.00E-05	5129	85.5	1.4
1.00E-06	51290	854.8	14.2

6.5. Time to 95% dissociation

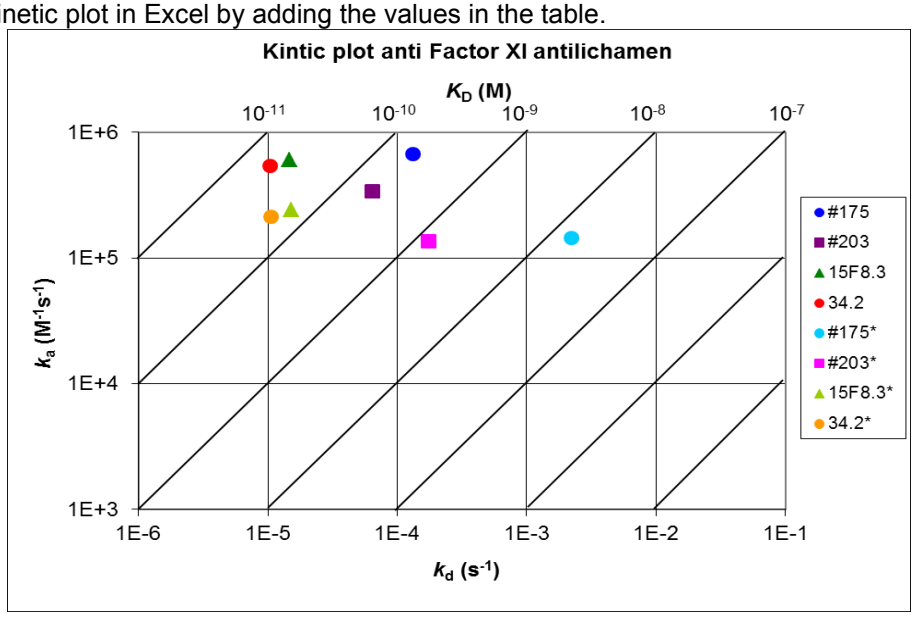
Formula

$$t_{1/2} = \frac{\ln(100-5)}{k_d} \quad (s^{-1}) \qquad (27)$$

kd (s-1)	Time (min)	Time (hour)
1.00E-01	0.5	0.0
1.00E-02	5.0	0.1
1.00E-03	49.9	0.8
1.00E-04	499.3	8.3
1.00E-05	4992.9	83.2
1.00E-06	49928.9	832.1

7. Kinetic plot

Create a kinetic plot in Excel by adding the values in the table.



8. Report points

Reorder the report points from a Biacore 2000/3000 report point table.

9. Kinetic converter

Convert the Excel notation to the ‘power off’ notation (sort of).

10. Simulation

Simulate the association and dissociation in Excel.

11. References

1. **BIACORE AB**; Kinetic and affinity analysis using BIA - Level 1. 1997.
2. **BIACORE AB**; BIACORE Technology Handbook; 1998.
3. **BIACORE AB**; BiaSimulation. 1998.
4. **Edwards, P. R. and Leatherbarrow, R. J.**; Determination of association rate constants by an optical biosensor using initial rate analysis. *Anal.Biochem.* **(246)**: 1-6; 1997.
5. **Glaser, R. W.**; Antigen-antibody binding and mass transport by convection and diffusion to a surface: a two-dimensional computer model of binding and dissociation kinetics. *Anal.Biochem.* **(213)**: 152-161; 1993.
6. **Karlsson, R., Michaelson, A, and Mattson, L**; Kinetic analysis of monoclonal antibody-antigen interactions with a new biosensor based analytical system. *J.Immunol.Methods* 229-240; 1991.
7. **Katsamba, P. S. et al**; Kinetic analysis of a high-affinity antibody/antigen interaction performed by multiple Biacore users. *Anal.Biochem.* (352): 208-221; 2006.
8. O'Shannessy, D. J. and Winzor, D. J.; Interpretation of deviations from pseudo-first-order kinetic behavior in the characterization of ligand binding by biosensor technology. Anal.Biochem. (236): 275-283; 1996.

12. Disclaimer

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