

MIRG 2007 Survey on Label-Free Biophysical Technologies Used in Characterizing Biomolecular Interactions

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ABSTRACT

The field of label-free biophysical technologies used to quantitatively characterize macromolecular interactions with each other and with small molecules has grown enormously in the last ten years. The most widely used analytical technologies for characterizing biomolecular interactions are surface plasmon resonance (SPR), isothermal titration calorimetry (ITC), and analytical ultracentrifugation (AUC). Measuring interaction parameters accurately and quantitatively is challenging because it requires specialized expertise, training and instrumentation. The Molecular Interaction Research Group (MIRG) conducted an on-line survey designed to capture the current profile of ITC, SPR and other technologies used in both academia and pharmaceutical industry sector. The main goal of the survey was to take a snap shot on the type of laboratory, instrumentation, type of applications for measuring various biophysical parameters, confidence of data interpretation, data validation and acceptability, and limitations of using various technologies. We anticipate that the participating laboratories will be able to gauge their own capabilities and gain insight into the relative success of the different technologies that they employ for characterizing molecular

1. What type of Biomolecular Interaction Analysis laboratory do you have (check one)?	Response Percent	Response Count
Academic	50.0%	40
Industry (i.e., pharmaceutical company, biotech, etc.)	31.3%	25
Research Institution (outside academia)	16.3%	13
Other	2.5%	2
answered question		79
skipped question		1

2. What technologies do you use for quantitative analysis of biomolecular interactions (check all that apply)?	Response Percent	Response Count
Surface plasmon resonance	56.2%	41
Isothermal titration calorimetry	68.5%	50
Differential scanning calorimetry	32.9%	24
Analytical ultracentrifugation	41.1%	30
Nuclear magnetic resonance	30.1%	22
Other	(24.66%)	18
answered question		73
skipped question		6

3. What applications do you use the instruments in your lab (check all that apply)?	Response Percent	Response Count
Protein-Protein interaction analysis	88.0%	66
Protein-Nucleic Acid interaction analysis	34.7%	26
Protein-small molecule	76.0%	57
Protein-Carbohydrate interaction analysis	22.7%	17
Protein-Lipid interaction analysis	24.0%	18
Antigen-Antibody interaction characterization	42.7%	32
DNA-small molecule	14.7%	11
Other	8%	6
answered question		75
skipped question		4

4. What type of molecular parameters do you find most valuable to know from the above technologies?	1 Highest Importance	2	3	4	5 Lowest Importance	Rating Average	Response Count
Binding affinity	79.0% (59)	19.0% (14)	3.0% (2)	0.0% (0)	0.0% (0)	1.24	75
Association and dissociation kinetics	40.0% (29)	32.0% (23)	15.0% (11)	6.0% (4)	7.0% (5)	2.10	72
Thermodynamics (enthalpy entropy, heat capacity)	29.0% (22)	24.0% (18)	32.0% (24)	7.0% (5)	8.0% (6)	2.40	75
Stoichiometry	47.0% (35)	27.0% (20)	21.0% (16)	5.0% (4)	0.0% (0)	1.87	75
Concentration analysis	18.0% (12)	22.0% (16)	19.0% (14)	23.0% (17)	18.0% (13)	3.01	73
answered question							76
skipped question							3

5. What range of KD values are normally measured in your laboratory (Check all that apply)?	Response Percent	Response Count
Weak (micromolar to millimolar)	53.3%	40
Medium (nanomolar to micromolar)	97.3%	73
Tight (subnanomolar)	40.0%	30
answered question		75
skipped question		4

6. How confident are you that affinity values determined by the following methods are accurate?	High confidence	Medium confidence	Low confidence until validated by an orthogonal technique	Rating Average	Response Count
Surface plasmon resonance	33.0% (19)	42.0% (24)	25.0% (14)	1.91	57
Isothermal titration calorimetry	77.0% (46)	20.0% (12)	3.0% (2)	1.29	60
Differential scanning calorimetry	25.0% (11)	43.0% (19)	32.0% (14)	2.07	44
Analytical ultracentrifugation	44.0% (18)	44.0% (18)	12.0% (5)	1.70	41
answered question					76
skipped question					3

7. How important is it for your work to use more than one technology for determining quantitative biomolecular interaction parameters?	Response Percent	Response Count
One technology is sufficient for my studies	14.7%	11
I sometimes cross validate my results against an orthogonal technology	50.78%	38
I always validate or troubleshoot my results with an orthogonal technology	34.7%	26
answered question		75
skipped question		4

8. What type of instruments do you have in your laboratory (Please indicate the number of instruments available)?	1	2	3	4	5	6	Response Count
Biacore 3000	78% (18)	9.0% (2)	9.0% (2)	4.0% (1)	0.0% (0)	0.0% (0)	23
Biacore 2000	86% (12)	7.0% (1)	7.0% (1)	0.0% (0)	0.0% (0)	0.0% (0)	14
Biacore 1000	100% (7)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	7
Biacore S51	100% (4)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	4
Biacore C	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0
Biacore X	100% (4)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	4
Biacore X100	100% (1)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	1
Biacore T100	86% (12)	14% (2)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	14
Biacore A100	50% (1)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	50% (1)	2
Biacore Flexchip	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0
ProteinOn XPR36 (BIORAD)	100% (6)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	6
Sensi Q	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0
iAsys	100% (1)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	1
ForteBio's Octet system	100% (1)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	1
Microcal ITC 200	100% (4)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	4
Microcal VP ITC	77% (33)	21% (9)	0.0% (0)	2% (1)	0.0% (0)	0.0% (0)	43
Microcal auto ITC	100% (6)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	6
Microcal MCS ITC	100% (6)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	6
CSC Nano ITC	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0
Microcal VP DSC	96% (23)	4% (1)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	24
Microcal CapDSC (auto)	100% (7)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	7
CSC DSC	100% (1)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	1
Beckman XL-A	81% (13)	13% (2)	6% (1)	0.0% (0)	0.0% (0)	0.0% (0)	16
Beckman XL-I	94% (15)	0.0% (0)	6% (1)	0.0% (0)	0.0% (0)	0.0% (0)	16
other							12
answered question							67
skipped question							12

9. What are the main limitations in using SPR interaction technologies?	1 Most Important	2	3	4	5 Least Important	Rating Average	Response Count
Cost of instrumentation	31.0% (16)	33.0% (17)	25.0% (13)	10.0% (5)	2.0% (1)	2.16	52
Training for instrumentation operation	16.0% (8)	29.0% (15)	24.0% (12)	20.0% (10)	12.0% (6)	2.77	51
Assay development time	27.0% (14)	31.0% (16)	29.0% (15)	10.0% (5)	4.0% (2)	2.29	52
Difficulty in regeneration step	12.0% (6)	29.0% (15)	38.0% (20)	12.0% (6)	10.0% (5)	2.84	52
Difficulty obtaining enough reagents	0.0% (0)	16.0% (8)	31% (16)	24.0% (12)	29.0% (15)	3.69	51
Data analysis software	21.0% (11)	10.0% (5)	33.0% (17)	15.0% (8)	21.0% (11)	3.08	52
Data interpretation	35.0% (18)	16.0% (8)	35.0% (18)	8.0% (4)	6.0% (3)	2.33	51
answered question							74
skipped question							5

10. If setup properly, most SPR instruments can detect small molecule/protein interactions down to, and even below, 150 Da to targets larger than 50 KDa?	Response Percent	Response Count
True	65.6%	40
False	34.4%	21
answered question		61
skipped question		18

11. If the SPR data do not fit a simple 1:1 binding model, what is the most important thing to do next?	Response Percent	Response Count
Fit with a conformational change model	5%	2
Fit with a heterogenous ligand or heterogenous analyte model	15%	6
Verify the purity, homogeneity and activity of my reagents	80%	32
answered question		40
I don't fit SPR data		29
skipped question		10

12. When fitting equilibrium SPR data, I always take my data points from the flat part of the association phase (dR/dt=0). If my sensorgrams aren't flat I rerun with longer association phases.	Response Percent	Response Count
True	71.43%	25
False	28.57%	10
answered question		35
I don't fit SPR data		31
skipped question		13

13. When your SPR data has observable kinetics do you fit for kinetics or only do equilibrium fitting?	Response Percent	Response Count
Fit kinetics	24.32%	9
Only analyze equilibrium values	5.41%	2
Both	70.27%	26
answered question		37
I don't fit SPR data		30
skipped question		12

14. What are the main limitations in using calorimetry in your lab?	1 Most Important	2	3	4	5 Least Important	Rating Average	Response Count
Cost of instrumentation	19.0% (11)	26.0% (15)	28.0% (16)	16.0% (9)	11.0% (6)	2.70	57
Training for instrumentation operation	0.0% (0)	21.0% (12)	29.0% (16)	27.0% (15)	23.0% (13)	3.50	56
Assay development time	7.0% (4)	21.0% (12)	25.0% (14)	27.0% (15)	20.0% (11)	3.29	56
Stability of the reagents	9.0% (5)	24.0% (13)	22.0% (12)	29.0% (16)	16.0% (9)	3.22	55
Difficulty obtaining enough reagents	53.0% (30)	23.0% (13)	14.0% (8)	7.0% (4)	4.0% (2)	1.89	57
Data analysis software	9.0% (5)	14.0% (8)	25.0% (14)	29.0% (16)	23.0% (13)	3.42	56
Data interpretation	14.0% (8)	25.0% (14)	14.0% (8)	21.0% (12)	3.04	56	
answered question							75
skipped question							4

15. Do you account for linkage of coupled equilibria (e.g. proton uptake or release) when interpreting thermodynamic data obtained by ITC?	Response Percent	Response Count
Yes	28.3%	15
No	18.87%	10
Sometimes	52.83%	28
answered question		53
I don't fit ITC data		18
skipped question		8

16. What type of measurements do you routinely carry out by ITC (Please Check all that apply)?	Response Percent	Response Count
Simple equilibrium binding to determine affinity and stoichiometry	98.0%	50
Equilibrium binding to determine enthalpy only	43.4%	23
Full thermodynamic profiles involving multiple buffer conditions and experimental temperatures	38.9%	28
Studies of systems involving more than one binding site	52.8%	26
Displacement studies to determine very high or very low affinities	39.6%	21
Steady state enzyme kinetics using enzymatic amounts of protein	9.4%	5
answered question		53
I don't fit ITC data		19
skipped question		7

CONCLUSION

- Half of the respondents who employ label-free technologies were from academia and about one third were from Pharmaceutical and Biotech companies.
- The most widely used label-free technologies for measuring biomolecular interactions were ITC (68.5%) and SPR (56.2%).
- Majority of respondents utilize these technologies for characterizing protein-protein (88%) and protein-small molecule (76.0%) interactions.
- Binding affinity was the most commonly measured parameter (79%) and almost all the respondents determine nanomolar to micromolar KD values.
- About 77% users of ITC were highly confident of their data, but only 33% users of SPR and 44% users of AUC were highly confident of their data and about a third of respondents always validated their data by orthogonal technology
- Based on the survey responses the main limitation of SPR based technologies were data interpretation (35%), cost of instrumentation (31%) and assay development time (27%).
- The main limitations of using ITC technology were difficulty in getting enough reagents (53%), cost of instrumentation (19%) and data interpretation (14%).