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MIRG Survey 2011: Snapshot of Rapidly Evolving Label-Free Technologies Used for Characterizing Molecular Interactions

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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 10 years. The most widely used analytical technologies for characterizing biomolecular interactions are surface plasmon resonance (SPR), isothermal titration calorimetry (ITC), biolayer interferometry (BLI), and analytical ultracentrifugation (AUC). Measuring interaction parameters accurately and quantitatively is challenging, as it requires specialized expertise, training, and instrumentation. The Molecular Interaction Research Group (MIRG) conducted an online survey designed to capture the current profile of label-free technologies, including ITC, SPR, and other biosensors used in academia and the pharmaceutical industry sector. The main goal of the survey was to take a snapshot of laboratory, instrumentation, applications for measuring various biophysical parameters, confidence in data interpretation, data validation and acceptability, and limitations of using various technologies. Through this survey, we anticipate that the participating laboratories will be able to gauge their own capabilities and gain insights into the relative success of the different technologies that they use for characterizing molecular interactions.

KEY WORDS: biomolecular, surface plasmon resonance, biolayer interferometry, isothermal titration calorimetry

INTRODUCTION

Label-free technologies have proven to be powerful for characterizing biomolecular interactions to define a complete picture of biochemical intricacies of cellular systems. From a biochemical and biophysical point of view, the formation of a complex when two molecules interact may be quite complicated and largely depends on the type of noncovalent bonds defined by kinetic and thermodynamic parameters. Many types of optical biosensors and isothermal titration calorimeters are now used routinely to quantitatively determine the binding affinities, kinetics, and other thermodynamic parameters of molecular interactions in real time without use of a molecular label. These tools allow us to define protein-protein, protein-nucleic acid, antigen-antibody, and protein-small molecule interactions to high accuracy. These technologies have also taken a center stage for high-throughput screening of antibodies and small molecules in drug development. The aims of this MIRG survey were to assemble a profile of label-free technologies used in molecular interaction analysis, compare their current status with the survey results that were recorded previously by the MIRG in 2007,[1] and perhaps provide some insight as to where this technology is going. Currently, two entirely different technologies dominate the field, namely, isothermal titration calorimetry (ITC) and biosensors, such as surface plasmon resonance (SPR), biolayer interferometry (BLI), and quartz crystal microbalance (QCM). In addition, it is unknown how accurate these techniques are in practice across multiple laboratories having varied levels of expertise. The MIRG began looking at some of these variables in this year's study.

The MIRG of the Association of Biomolecular Resource Facilities (ABRF) was established with a mission to educate member laboratories on advances in these technologies. The MIRG has launched surveys in the past on several technologies used to quantitatively measure molecular interactions.[1] The main goal of this particular survey was to take a snapshot of the rapidly

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In no case did the ABRF or the MIRG recommend or endorse particular instruments and equipment mentioned in this manuscript from the survey conducted in 2011.

doi: 10.7171/jbt.12-2303-002

evolving field of label-free biosensors and ITC to evaluate the following aspects:

- type of laboratories that use biosensors, ITC and other technologies, i.e., academic, industry, nonprofit, or commercial
- type of instrumentation used
- what kind of biophysical parameters are measured
- confidence in data interpretation
- data validation and acceptability
- limitations of using biosensors and ITC

From the results of this survey, participating laboratories and vendors will be able to assess their own capabilities.

Users will gain insight into the relative confidence in using various instruments and data interpretation.

METHODOLOGY

A web-based general survey using Survey Monkey on biosensors and ITC was conducted to gauge the current profiles of academic, industry, nonprofit research institutions, and commercial laboratories who use these biophysical technologies. For analyzing protein structure and function, clearly, there is no one-size-fits-all approach. The survey consisted of questions related to the type of laboratory, type of label-free technology used in the laboratory, and application of the technology. The survey also had specific questions on SPR technology, BLI, ITC, and other label-free biosensor technologies. The survey questions were posted on the website www.surveymonkey.com. The launch of the survey announcement was e-mailed to all ABRF members and to other laboratories that use label-free technologies to measure molecular interactions. The survey was launched on January 14, 2011, and the data received until February 11, 2011. The participating laboratories were asked to answer 20 questions about various aspects of these technologies; a final question, 21, was optional: What new or improved capability would be most valuable to your laboratory in studying biomolecular interactions? The question was meant to get feedback from users about making improvements in the technology that currently exists in the marketplace or innovation that does not currently exist in the marketplace but would be useful. In total, 82 laboratories that use various label-free technologies responded.

RESULTS

A summary of observations made from the survey responses is displayed in Table 1.

TABLE 1

Summary of Observations Made from the MIRG Survey 2011

Q1. What type of biomolecular interaction analysis laboratory do you have? (check one)

Answer options	Response percent	Response count
Academic	17.1%	14
Industry (i.e., pharmaceutical company, biotech, etc.)	69.5%	57
Commercial (i.e., your facility is a business)	3.7%	3
Research institution (outside of academia)	9.8%	8
	answered question	82

Q2. What technologies do you use for quantitative analysis of biomolecular interactions? (check all that apply)

Answer options	Response percent	Response count
Surface plasmon resonance	79.2%	61
Biolayer interferometry	44.2%	34
Isothermal titration calorimetry	31.2%	24
Differential scanning calorimetry	16.9%	13
Analytical ultracentrifugation	13.0%	10
Nuclear magnetic resonance	10.4%	8
Other (MS, FACS, ELISA, FRET, fluorescence, etc.)	22.07%	17
	answered question	77

Q3. For what applications do you use the instruments in your lab? (check all that apply)

Answer options	Response percent	Response count
Protein-protein interaction analysis	92.6%	75
Protein-nucleic acid interaction analysis	29.6%	24
Protein-small molecule	53.1%	43
Protein-carbohydrate interaction analysis	16.0%	13
Protein-lipid interaction analysis	13.6%	11
Antigen-antibody interaction characterization	72.8%	59
DNA-small molecule	8.6%	7
Other	6.2%	5
	answered question	81

Q4. What type of molecular parameters do you find most valuable to know from the above technologies?[a]

Answer options	Highest importance	Average importance	Lowest importance	Response count
Binding affinity	97.6%	2.4%	0	82
Association and dissociation kinetics	79.5%	15.4%	5.1%	78
Thermodynamics (enthalpy, entropy, heat capacity)	28.9%	38.2%	32.9%	76
Stoichiometry	47.3%	35.1%	17.6%	74
Concentration analysis	46.7%	24%	29.3%	75

[a]Rating of importance on a scale of 1–5, with 1 being of Highest importance (Highest importance=rating 1+2; Average importance=rating 3+4; Lowest importance=rating 5).

Q5. What range of K_d values are normally measured in your laboratory? (check all that apply)

Answer options	Response percent	Response count
Weak (micromolar to millimolar)	45.7%	37
Medium (nanomolar to micromolar)	91.4%	74
Tight (subnanomolar)	58.0%	47
	answered question	81

Q6. How confident are you that affinity values determined by the following methods are accurate?

Answer options	High confidence	Medium confidence	Low confidence[#]	Total responses
Surface plasmon resonance	73.90%	21.74%	4.30%	69
Biolayer interferometry	43.20%	40.50%	15.40%	37
Isothermal titration calorimetry	51.30%	43.60%	5.10%	39
Differential scanning calorimetry	21.70%	47.80%	30.40%	23
Analytical ultracentrifugation	36.40%	40.90%	22.70%	22

[#]until validated by an orthogonal technique.

Q7. How important is it for your work to use more than one technology for determining quantitative biomolecular interaction parameters?

Answer options	Response percent	Response count
One technology is sufficient for my studies.	20.7%	17
I sometimes cross-validate my results against an orthogonal technology.	56.1%	46
I always validate or troubleshoot my results with an orthogonal technology.	23.2%	19
	answered question	82

Q8. What type of instruments do you have in your laboratory? (please indicate the number of instruments available)

Answer options	Number of instrument in the lab				
Type of instrument	*1*	*2*	*3*	*4*	Total responded
Attana	2	0	0	0	2
Biacore 3000	21	8	1	0	30
Biacore 2000	9	3	2	0	5
Biacore 1000	4	1	0	0	5
Biacore S51	4	0	0	0	4
Biacore C	0	1	3	0	4
Biacore X	1	1	0	0	2
Biacore X100	1	0	0	0	1
Biacore T100	19	10	2	1	32
Biacore A100	4	1	0	0	5
Biacore Flexchip	0	0	0	0	0
ProteoOn XPR36 (Bio-Rad)	10	2	0	0	12
Sensi Q	3	0	0	0	3
IAsys	0	0	0	0	0
ForteBio's Octet system	24	5	2	1	32
Microcal ITC 200	16	0	0	0	16
Microcal VP ITC	9	1	2	0	12
Microcal auto ITC	3	0	0	0	3
Microcal MCS ITC	2	0	0	0	2
CSC Nano ITC	0	0	0	0	0
Microcal VP DSC	7	1	0	0	8
Microcal CapDSC(auto)	3	1	0	0	4
CSC DSC	2	0	0	0	2
Beckman XL-A	7	1	0	0	8
Beckman XL-I	6	1	0	0	7
Others					11

Q9. What type of measurements do you routinely carry out using biosensor technologies? (please check all that apply)

Answer options	Response percent	Response count
Yes/no binding	77.5%	62
Full kinetic measurements (association rate, dissociation rate, affinity)	82.5%	66
Affinity only	51.3%	41
Concentration analysis	50.0%	40
Ranking dissociation rates	57.5%	46
Other	12.5%	10
	answered question	80

Q10. What analyte size range(s) do you study using biosensor technologies? (please check all that apply)

Answer options	Response percent	Response count
<500 Da	33.8%	27
500–1500 Da	40.0%	32
1500–5000 Da	42.5%	34
5000–25,000 Da	71.3%	57
25,000+ Da	80.0%	64
	answered question	80

Q11. What method do you use to determine binding affinity using biosensor technologies? (please check all that apply)

Answer options	Response percent	Response count
Global kinetic fit	84.4%	65
Steady-state (equilibrium)	68.8%	53
"Affinity in solution"	27.3%	21
"Single cycle kinetics" (or equivalent)	33.8%	26
Other	2.6%	2
	answered question	77

Q12. What are the main limitations in using biosensor interaction technologies?

Answer options	Rating from 1–5[a]					Response count
	1 (Most important)	2	3	4	5 (Least important)	
Cost of instrumentation	31.4%	29.1%	24.2%	8.9%	6.3%	79
Training for instrumentation operation	20.8%	23.4%	19.5%	24.7%	11.7%	77
Assay development time	20.5%	33.3%	29.5%	11.5%	5.1%	78
Difficulty in regeneration step	21.9%	17.8%	31.5%	19.2%	10.9%	73
Difficulty obtaining enough reagents	9.1%	18.2%	20.8%	24.7%	27.3%	77
Data analysis software	23.1%	26.9%	23.1%	12.8%	14.1%	78
Data interpretation	37.7%	15.6%	20.1%	18.2%	6.5%	77

[a]Rating of importance on a scale of 1–5, with 1 being of highest importance (Most important) and 5 being Least important.

Q13. If set up properly, most biosensor instruments can detect small molecule/protein interactions down to, and even below, 150 Da to targets larger than 50 KDa

Answer options	Response percent	Response count
True	65.8%	48
False	35.6%	26
	answered question	74

Q14. If the biosensor data do not fit a simple 1:1 binding model, what is the most important thing to do next?

Answer options	Response percent	Response count
Fit with a conformational change model.	8.6%	7
Fit with a heterogenous ligand or heterogenous analyte model.	9.9%	8
Reverse the immobilzation of analyte as ligand for the interaction.	21.0%	17
Verify the purity, homogeneity, and activity of my reagents.	46.9%	38
I don't fit biosensor data.	13.6%	11
	answered question	81

Q15. When fitting equilibrium biosensor 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

Answer options	Response percent	Response count
True	62.5%	50
False	16.3%	13
I don't fit biosensor data.	21.3%	17
	answered question	80

Q16. When your Biosensor data has observable kinetics, do you fit for kinetics or only do equilibrium fitting?

Answer options	Response percent	Response count
Fit kinetics	40.0%	32
Only analyze equilibrium values	0.0%	0
Both	45.0%	36
I don't fit biosensor data.	15.0%	12
	answered question	80

Q17. What type of measurements do you routinely carry out by ITC? (please check all that apply)

Answer options	Response percent	Response count
Simple equilibrium binding to determine affinity and stoichiometry	30.8%	24
Equilibrium binding to determine enthalpy only	6.4%	5
Full thermodynamic profiles involving multiple buffer conditions and experimental temperatures	11.5%	9
Studies of systems involving more than one binding site	6.4%	5
Displacement studies to determine very high or very low affinities	7.7%	6
Steady-state enzyme kinetics using enzymatic amounts of protein	3.8%	3
I don't do ITC.	67.9%	53
	answered question	78

Q18. What are the main limitations in using calorimetry in your lab?

	Rating from 1–5					
Answer options	1 (Most important)	2	3	4	5 (Least important)	Response count
Cost of instrumentation	17.6%	26.5%	26.5%	5.9%	23.5%	34
Training for instrumentation operation	14.3%	25.7%	17.1%	25.7%	17.1%	35
Assay development time	14.7%	23.5%	29.4%	14.7%	17.6%	34
Stability of the reagents	22.9%	20.0%	25.7%	11.4%	20.0%	35
Difficulty obtaining enough reagents	67.6%	8.8%	14.7%	5.9%	2.9%	34
Data analysis software	11.8%	23.5%	26.5%	11.8%	26.5%	34
Data interpretation	24.2%	18.2%	30.3%	6.1%	21.2%	33

Q19. Do you account for linkage of coupled equilibria (e.g., proton uptake or release) when interpreting thermodynamic data obtained by ITC?

Answer options	Response percent	Response count
Yes	2.5%	2
No	13.6%	11
Sometimes	14.8%	12
I don't fit ITC data.	69.1%	56
	answered question	81

Q20. How do you use change in enthalpy and change in heat-capacity data?

Answer options	Response percent	Response count
Use for quantitative, rational drug design	8.1%	6
Use to distinguish molecular binding mechanisms for different ligands or drugs	13.5%	10
Use to test if the binding model is a simple, noncooperative, single-binding site case or not	5.4%	4
Use as a strategy for measuring K_d at one temperature and then calculating K_d as a function of temperature	5.4%	4
No current need to use enthalpy change or heat capacity change	78.4%	58
	answered question	74

Q21 (optional). What new or improved capability would be most valuable to your laboratory in studying biomolecular interactions? (answer can be something that currently exists in the marketplace or a technology innovation that does not currently exist in the marketplace)

Answer options	Response count
answered question	24
skipped question	59

DISCUSSION

In the 2011 MIRG survey, over two-thirds (69.5%) of the respondents who use label-free technologies were from industry (Pharma or Biotech), 17.1% were from academia. This is a shift compared to the previous 2007 survey where 50% of respondents were from academia and only 31.3% were from industry.

The most widely used label free technology was SPR (79.2%), followed by BLI (44.2%), then ITC (31.2%). This is also a change from the 2007 survey results, where ITC was the most widely used technique (68.5%), closely followed by SPR (56.2%). BLI was not included as a specific option in the 2007 survey. It will be interesting to follow how widespread newer technologies become in future surveys.

The label-free technologies are used for various purposes, yet most respondents measured binding affinities (97.6%), closely followed by binding kinetics (79.5%). Stoichiometry and concentration analysis were approximately equal (47.3% and 46.7% respectively), while thermodynamic parameters were the least commonly measured (28.9%). In the 2007 survey the trend was similar with 79% of respondents recording binding affinity as the highest importance. However, stoichiometry was previously the next most desired parameter (47.0%) followed by association and dissociation kinetics (40.0%).

Of the type of interactions measured in 2011, protein-protein and antigen-antibody were by far the most common (92.6% and 72.8% respectively), followed by protein-small molecule (53.1%). In 2007 protein-protein interactions were also the major interaction sub-class (88.0%). However, protein-small molecule were the next (76.0%) followed by antigen-antibody (42.7%).

In the present survey more users of SPR had high confidence in the measured affinity values (73.9%), followed next by ITC (51.3%) and BLI (43.2%). This is a significant shift from 2007, where most users of ITC (77.0%) had high confidence in affinity values, followed by AUC (44%) and SPR (33%). In 2011 the majority of users occasionally cross validated results and only 23.3% always used an orthogonal technique. This finding was similar to 2007, where 57.8% of uses cross validated results sometimes and 34.7% of users cross validated all the time.

For users of SPR and calorimetry (ITC & DSC) the limitations remained approximately unchanged from in 2011 compared to the previous 2007 study. In 2011 respondents listed data interpretation, cost of instrumentation (37.7% and 31.4% respectively) as the most important limitation of SPR. Cost of instrumentation (31.0%) followed by data interpretation (35.0%) were most common responses in 2007. For calorimetry, difficulty in obtaining enough reagent remained the most important limitation in 2011 (67.6%) as in 2007 (53.0%).

In response to the optional question 21, users were asked what new or improved capability would be of most value. Of the 24 respondents who answered this question suggestions included: higher throughput calorimeters and biosensors, greater sensitivity, lower cost of instrumentation, and reduced artifacts associated with small molecular screening.

ACKNOWLEDGMENTS

The MIRG acknowledges ABRF for providing the necessary tools and funds for the survey.

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