Biacore s200 manual

Biacore s200 evaluation software manual.

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The system contains all the key functionalities neededfor day-to-day molecular interaction research with the purpose ofunderstanding protein function and biological mechanisms.Biacore systems, used extensively in academic researchworldwide, are cited in almost 10 000 peer-reviewed scientiicpublications.Real-time monitoring o binding events gives a deep understanding o thedynamics o molecular interactions, including valuable kinetic data. Biacoreanalysis allows or straightorward and rapid data generation, leading todependable conclusions and enabling the design o new innovative studies.Biacore X100 can be used or a broad range o applications, including structure-unction studies, pathway analysis, drug target identiication and validation,and assay development.Versatility and lexibility, in combination with unparalleled support, make iteasy to generate top-quality data or your publication. With Biacore X100 onyour bench, take your experiments to the next level and boost your proteininteraction research.8/14/2019 28-9617-44 AA Biacore X100 Brochure 4/1448/14/2019 28-9617-44 AA Biacore X100 Brochure 5/145-50510152025303540-500 0 500 1000 1500 2000 2500 3000Response(RU)Time (s)1 nM5 nM10 nM20 nM30 nMRun successul assays rom day oneNo regeneration required Aibody molecules allowedto partially dissociate romimmobilized HER2 betweeninjections (arrows)Sequential injection oincreasing concentrationso sample in onecontinuous analysis cycleBiacore X100 uses worklow-orientedsotware that provides a supportiveramework or assay developmentand data interpretation, building yourexpertise as you work.Researchers at Uppsala University usedBiacore X100 to determine the bindingcharacteristics o various Aibodymolecules to HER2 with regardsto speciicity, ainity, and kineticassociation- and dissociation-rates.Speciicity was quickly establishedby comparing binding levels othe dierent Aibody molecules toimmobilized HER2 and a reerenceprotein. As a next step, the kinetics ohigh-ainity binders was studied.Aibody molecules turned out tobe very diicult to regenerate. Thisproblem was overcome with single-cycle kinetics which eliminatesthe need or inding regenerationconditions.Acknowledgement:Thuy Tran and Proessor JrgenCarlsson, Uppsala University, SwedenThe sotware wizards were easy to use, and the help unction was extremely useul to have.I got good at this very quickly! Thuy Tran, PhD student, Uppsala University, SwedenGuided worklows and sotware wizards enable generation o reliable data and rapid development o your expertise.Single-cycle kinetics sensorgram.8/14/2019 28-9617-44 AA Biacore X100 Brochure 6/146IdeSIdeSIdeSIdeScystatinC+??+IgG-10000100020003000400050006000Response(RU)0 100 200 300 400 Time (s)Elucidate complex molecular mechanismsIdeS is a papain-like cysteineprotease rom the human pathogenStreptococcus pyogenesthatspeciically degrades IgG. The enzymehas been studied by Dr. Ulrich vonPawel-Rammingens group at UmeUniversity in Sweden. They discoveredthat human cystatin C has anunexpected stimulatory eect on theIgG-endopeptidase activity o IdeS.Evidence o an interaction betweenthe two proteins was irst ound usinga combination o gel iltration andWestern blotting. The mechanismso the interactions between IdeS,cystatin C, and IgG were urtherinvestigated using Biacore X100.Their studies showed that cystatin Cbinds to IdeS, that IdeS can ormhomodimers, and that IdeS bindingto IgG was not aected by cystatin C.The irst research paper includingdata rom Biacore X100 was publishedonly ive months ater the system wasinstalled.Having a Biacore X100 instrument in our own lab is o great value or our research andallows us to employ many new types o studies. Especially the possibility to run SPRexperiments according to our own research agenda is a valuable tool in increasing theunderstanding o this sophisticated molecular interaction network.Dr. Ulrich von Pawel-Rammingen, Ume University, SwedenVincents, B. et al. The human proteaseinhibitor cystatin C is an activating coactoror the streptococcal cysteine protease IdeS.Chemistry & Biology15, 960-968 (2008).Acknowledgement:Dr. Ulrich von Pawel-Rammingen,Ume University, SwedenActivity assay or IdeS cleavage o IgG. IgG1binding toimmobilized Protein A is measured. Upper curve showsa sensogram o uncleaved IgG1, while the lower curveshows a sensorgram or IgG1cleaved by addition o IdeS.The precise mechanisms o this complexnetwork o interactions are now beingurther investigated. To aid in this study,a standardized enzyme activity assayor IdeS cleavage o IgG is employed,which is run on Biacore X100.A schematic diagram showing thepossible interactions between IdeS,cystatin C, and IgG.Red lines indicate a stimulatoryeect on the interaction,illustrated by an arrow.8/14/2019 28-9617-44 AA Biacore X100 Brochure 7/1470210-5410-5610-5810-5110-41.210-41.410-41.610-4Wild type Cys3Ser/His75GlyCys3Ser/Leu73GlyCys3Ser/Tyr97AlaCys3SerConcentration(M)Concentration from A280measurementConcentration from CFCAll l lWild type Cys3Ser Cys3Ser/His75GlyCys3Ser/Tyr97AlaCys3Ser/Leu73Gly105106107ka(M-1s-1)kaWild type Cys3Ser Cys3Ser/His75GlyCys3Ser/Tyr97AlaCys3Ser/Leu73Glyll l lkd(s-1)kd KDKD(M)10-410-310-210-110-1010-910-810-7Increase the reliability o kinetic analysisCystatin B is an inhibitor o papain-like cysteine proteases. Four mutantswere produced in order to study theimportance o three dierent aminoacids at the C-terminal and secondbinding loop or its binding to papain.Since no standard was available,Calibration-ree concentration analysis(CFCA) was used to assess proteinconcentrations. CFCA is an innovativetool to measure protein concentrations,based on speciic binding activity,without using a standard curve.The combination o concentration, kinetics, and ainity analysis in a single study enabledthe right interpretation o the interaction mechanism.Mutants Cys3Ser/His75Gly,Cys3Ser/Tyr97Ala, and Cys3Sershowed a good agreement betweenconcentration values obtainedwith A280and CFCA, while mutantCys3Ser/Leu73Gly had a very lowconcentration as measured byCFCA. Introduction o mutationscan destabilize protein olding andprolonged storage or reeze/thawprocedures may also decrease theamount o protein capable o binding.Kinetic analysis based on the A280concentration measurement wouldlead to the conclusion that leucine 73is important or the association rate,giving about ten times slower bindingthan the other mutants. In contrast,CFCA allowed or correct assessmento association rate (ka) and ainity (KD),allowing an appropriate interpretationo the interaction mechanism. Thedecreased ainities or all mutantswere due to an increased dissociationrate (kd), while the association rate (ka)remained relatively constant.8/14/2019 28-9617-44 AA Biacore X100 Brochure 8/148-10-200 300 800 1300 Time (s)1030507090Response(RU)H2NOO--OOONHHONNNa+Na+OSSOOSO-200 300 800 1300 Time (s)Response(RU)-5051015202530H2NNH2SO O-200 300 800 1300 Time (s)Response(RU)-50510152025 30HNNH2SOOHClOOODevelop and run assays involving smallmolecule interactionsBiacore X100 provides thesensitivity required or challengingapplications. Low molecular weight(LMW) compounds represent onechallenging application, becausea small molecular size reducesthe signal levels. In addition, LMWcompounds oten require organicsolvents or solubility reasons. Theoptional Biacore X100 Plus PaPage 3

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