

[Artificia](https://doi.org/10.1016/j.ailsci.2023.100073)l[IntelligenceintheLifeSciences3(2023)100073](https://doi.org/10.1016/j.ailsci.2023.100073)

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| ArtificialIntelligenceintheLifeSciences |
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ResearchArticle   
Designingmicroplatelayoutsusingartificialintelligence MaríaAndreínaFranciscoRodríguez∗,JordiCarrerasPuigvert,OlaSpjuth *DepartmentofPharmaceuticalBiosciencesandScienceforLifeLaboratory,UppsalaUniversity,Sweden*

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| article | info | abstract |
| *Keywords:*  Platedesign  Plateeffects  Qualitycontrol |  | Microplatesareindispensableinlarge-scalebiomedicalexperimentsbutthephysicallocationofsamplesand controlsonthemicroplatecansignificantlyaffecttheresultingdataandqualitymetricvalues.Weintroduce anewmethodbasedonconstraintprogrammingfordesigningmicroplatelayoutsthatreducesunwantedbias andlimitstheimpactofbatcheffectsaftererrorcorrectionandnormalisation.Wedemonstratethatourmethod appliedtodose-responseexperimentsleadstomoreaccurateregressioncurvesandlowererrorswhenestimating IC50/EC50,andfordrugscreeningleadstoincreasedprecision,whencomparedtorandomlayouts.Italsoreduces theriskofinflatedscoresfromcommonmicroplatequalityassessmentmetricssuchas*𝑍*′factorandSSMD.We makeourmethodavailableviaasuiteoftools(PLAID)includingareferenceconstraintmodel,awebapplication, andPythonnotebookstoevaluateandcomparedesignswhenplanningmicroplateexperiments. |

**1.Introduction**

Intheeraofdata-drivenlifescience,theamountsofdataproduced arecontinuouslyexpanding,andartificialintelligencetechniquessuch asmachinelearningalgorithmsareseeingadoptionformanyapplica-tionsinordertoconvertthedataintoactionableinsights[1–5].While inmanyapplicationstheprimaryfocushasbeentoobtainasmuchdata aspossible,theimportanceofhavingdataofhighqualitycannotbe understated[6–8].Forlarge-scalebiomedicalexperiments,manyissues relatedtodataqualitypertainingtohumanoperationscanbeeffectively reducedoreliminatedbyusingautomatedsetupsandrobotisedequip-ment[9].However,severalartefactsduetophysical,biological,and temporalconditionsstillremain,andeffortsgeneratinglargequantities ofdatacanbefruitlessifintheendconclusionscannotbedrawndueto data-qualityissues.Acommonapproachtoincreasetheconfidencein thedataistoperformmultipletechnicalandbiologicalreplicates,but thisisassociatedwithhighercostsandlongerexperiments,andoften leadstoatrade-off betweenthenumberofsamplesanalysedandthe numberofreplicatespersample.Anotherapproachisto*improvetheex-perimentaldesign*,withtheaimtocarryouttheexperimentinsuchaway thatitmaximisestheconclusionsthatcanbedrawnfromtheresulting data[10].

Microplates,ormicrowellplates,arestandardcomponentsinmany biomedicalexperiments.Theyareflatplateswithmultiplewellsused assmalltesttubes,organisedina2:3matrix.Theycomeinastandard physicaldimensiontoensurecompatibilitywithdifferentlabequip-ment,andtypicallycontain24,96,384,or1536wells.Experiments

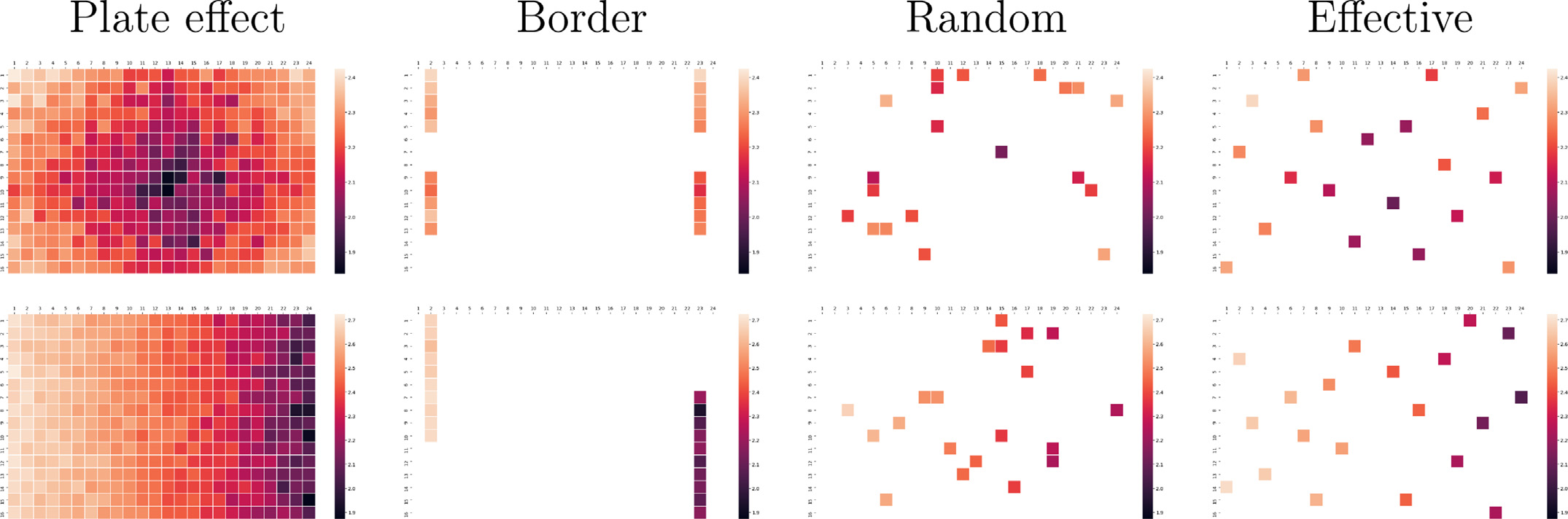
carriedoutusingmicroplatescommonlyexhibitplateeffects[11],also knownaspositionaleffects,whicharesystematicvariationsacrossthe geometryofamicroplate(within-plateeffects)oracrossdifferentplates (between-plateeffects)duetofactorssuchaswelllocation,tempera-tureandhumiditybeingunequallydistributed,andcanaffectthere-sultstothepointofrenderingtheexperimentunusable.Otherfactors thatcancontributetoexperimentalvariationarethelabequipment, suchasimprecisemanualpipetting,andinconsistentormalfunctioning liquidhandlinginstruments.Commonpatternsofwithin-plateeffects include:(i)linearroweffects;(ii)linearcolumneffects;(iii)linearrow andcolumneffects;and(iv)bowl-shapedspatialeffects[11];examples arevisualisedinFig.1.Identifyingandcorrectingforbothwithin-and between-plateeffectsisimportantinordertoadjustthedatasothatthe impactoftheerrorscanbereducedoravoided.Variousnormalisation techniqueshavebeendevelopedtothisend[12,13],butanappropriate microplatelayoutisofparticularimportanceforthenormalisationto beeffective[12,14].A*control*isasamplethathasbeensubjectedtoa knowntreatmentwiththegoalofaccountingfortheeffectsofvariables otherthanwhatisbeingtested,thusincreasingthereliabilityofthere-sults.Inparticular,a*negativecontrol*isasamplethathasbeensubjected toatreatmentthatinducesnoeffect,whilea*positivecontrol*isasam-plethathasbeensubjectedtoatreatmentwithanexpectedmaximal response[15].Inordertomitigateplateeffectsandgainthemostout ofusingcontrolsamplesanderrorcorrectionmethods,scientistshave beenadvocatingfortheuseofrandomisedplatelayouts[16,17]. Awidelyusedapproachtodayistodesignplatelayoutsmanually inordertosimplifyforhumaninteraction;e.g.placingcontrolsinthe

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<https://doi.org/10.1016/j.ailsci.2023.100073>  
[Received18January2023;Receivedinrevise](https://doi.org/10.1016/j.ailsci.2023.100073)dform8March2023;Accepted12April2023   
Availableonline14April2023   
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**Fig.1.**Visualisationofsystematicplateeffectsanddistributionsof20negativecontrolsusingborder,randomandeffectivelayouts.Thecoloursindicatetheintensity

measuredateachwell.Toprow:Datawithstrongsystematicerrorshavingabowl-shapedrelationshiptowellposition.Bottomrow:Datawithstrongsystematic

errorshavingalinearrelationshiptocolumnnumber.

outer-mostwells(borderlayout)anddistributingthesamplesfollowing patternsthatareeasytodesignandtopipettemanually[18,19].Indeed manyresearchersstilluseborderlayoutsastheyhelpreducehuman pipettingerrors,allowforstraight-forwardvisualisationofresultsby humans,forexampleintheformofheatmaps[12],andcanbeeasily designedusingpenandpaper[20].Yetborderlayoutscanonlybeused toeffectivelyidentifyandadjustforonlyafewplateeffects[13,14], suchaslinearrelationshipstorowsorcolumnsthataffectthewhole plate(Fig.1).

Forlarge-scaleexperimentswithmicroplateshaving384ormore wells,humanpipettingbecomesinfeasibleandrobotsforliquidhan-dlingarenecessary.Inrecentyears,pipettingrobotshavebecomecom-moninbiomedicallabsandtheyallowforfullyflexiblearrangementsof controlsandsamplesonplates,makingrandomisedlayoutsmoreacces-sible.However,purerandomisationcanstillproduceineffectivelayouts, forexamplelargeareasoftheplatemightendupnothavinganycon-trolsamples,makingitdifficultorevenimpossibletodetectandcorrect errorsinthoseareas[16,21,22].Further,replicatesplacedinadjacent wellsarethenlikelytobeaffectedbythesameplateeffects.Notonly isitaproblemthattheywillbesimilarlybiased,butithasalsobeen shownthatclustersofsimilarsamples,includingsimilardosesofthe samecompoundaswellastechnicalreplicates,canaffecttheresults ofadjacentwells[12].Consequently,platedesignsthatdistributeboth controlsandsamplesinaneffectivewayareneededinordertoreduce unwantedbias,aswellastoaidtodetectandcorrectplateeffects.We refertosuchdesignsas*effective*layouts.Figure1(toprow)displays examplesofmicroplateswithtwostrongsystematicplateeffects(bowl-shapedandlineargradient)andexamplesofhowcontrolscanbelocated usingborder,randomandeffectivelayouts.

Severalplatelayouteditorsareavailable,suchasBrunn[23], FlowJo[24],Labfolder[25],PlateDesigner[26],andPlateEditor[27]. Whilesomeareabletogeneraterandomisedlayouts,noneofthemhave capabilitiestogenerateeffectivelayouts.Thereis,ofcourse,thepossi-bilityofgeneratingseveralrandomlayoutsandthenevaluatethemin ordertoselectthebestone[28],butthatdoesnotguaranteethateffec-tiveplatelayoutshavebeenselected,regardlessofhowmanylayouts aregenerated.

Inthismanuscriptweintroduceanartificial-intelligencebased modelfordesigningeffectivemicroplatelayoutsthatcaneasilybe adaptedfordifferentexperimentalsettings,andevaluateitfordose-responseandscreeningapplications.Inordertosimplifyitsusage,we developedasuiteoftools(PLAID),includingaweb-appforeasilyde-signingeffectivemicroplatelayouts,togetherwithPythonnotebooksfor simulatingdifferentexperimentaldesignsandallowforplanningand designingeffectiveexperiments.

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detected,especiallywhentheerrorshavebeenintroducedconsistently inallplates,forexamplebyamalfunctioningdispensingequipment. Thesameconstraintscouldalsobeappliedtosamplereplicatesacross plates.

*1.2.Effectivelayoutswithconstraintprogramming*

Aboveweintroduceddesiredpropertiesofeffectiveplatelayoutsas asetofconstraints.Oneoptiontosatisfytheseconstraintswouldbe torandomlygeneratemicroplatelayoutsuntilonethatfitsthecriteria isfound.Whilethisinitselfconstitutesanon-trivialtask,findingsuch layoutcouldtakeanunreasonablylongtime,andifnolayoutfulfilling thecriteriaexists,thisprogramwouldneverfinish.Amoreefficientand naturalsolutionistoframeourcharacterisationofeffectivemicroplate layoutsasaconstraintsatisfactionproblem(CSP):wevieweachwell ofeachplateasavariablewhosevaluerepresentsitscontentanddesir-ablepropertiesofalayoutasconstraints.*Constraintprogramming*(CP) isasubareaofartificialintelligencethatoffersaflexibleframework forsolvingconstraintsatisfactionproblemsthathasseenlargeadoption invariousfields(seeMethodssection).ThegeneralideabehindCPis thataCSPcanbemodelledasaconjunctionofhigh-levelconstraints onvariablesrangingoverinitialdomains,andthensaidmodelisgiven toageneral-purposeconstraintsolverwhichperformsacombinationof intelligentreasoningandsystematicsearchinordertofindconstraint-satisfyingdomainvaluesforthevariables.Inthisprojectweimplement aconstraintmodelthatgenerateseffectiveplatelayoutsfortwodiffer-entapplications:dose-responseandscreeningexperiments.

**2.Effectivelayoutsleadtomoreaccurateresultsin dose-responseexperiments**

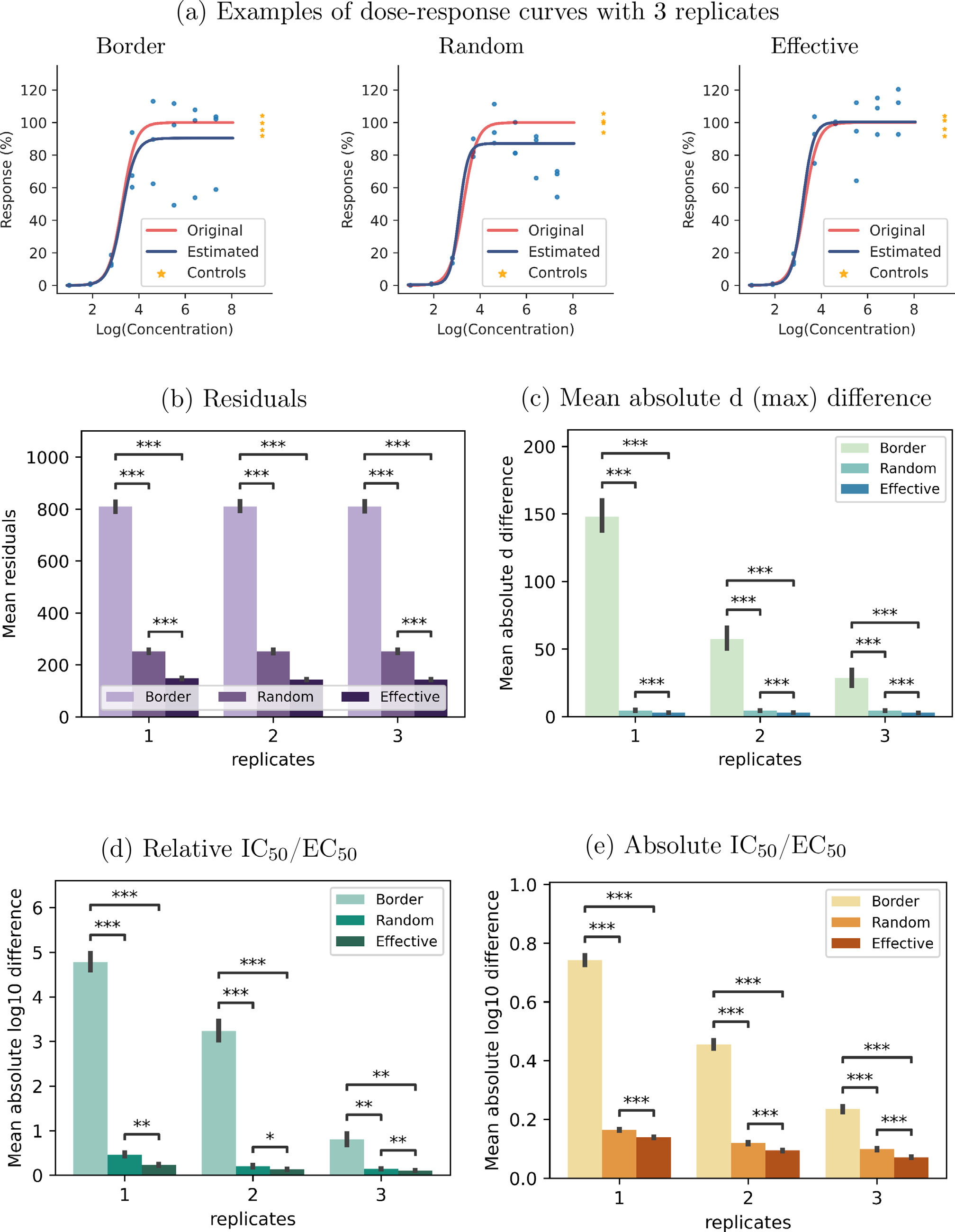
Dose-responseexperimentsattempttoevaluatetheeffectofasub-stanceinaspecificassayatincreasingconcentrations[29].Theeffect can,inmanycases,beestimatedbyfittingasigmoidcurvetothedata points,andisfrequentlysummarisedbydeterminingthehalfmaximal inhibitoryconcentration(IC50),orthehalfmaximaleffectiveconcen-tration(EC50).Inordertoevaluatetheimpactofdifferenttypesofmi-croplatelayoutsindose-responseexperiments,wesimulatedatotalof 43,200microplatesfordoseresponseexperimentswithborderlayouts, randomlayouts,andeffectivelayoutsgeneratedusingconstraintpro-grammingandtheconstraintsdefinedinSupplementaryListing1.The experimentsconsistedof20compoundsofvaryingpotencyin6,8,and 12doses,andfor1,2,and3replicates.Plateeffectsaddedhadarela-tionshiptocolumnnumberorwerebowl-shaped,bothinmediumand highstrength.Thedatawasnormalisedusinglinearregressioninthe caseofborderlayouts,andLOESSregressionforeffectiveandrandom layouts,andfour-parameterlog-logistic(LL4)curveswerefittedtothe resultingdata.ExamplesofthecurvesproducedcanbeseeninFig.2(a) andSupplementaryFig.1.Foracompletedescriptionoftheexperiment, seeMethodssection.

Figure2(b)andSupplementaryFig.12showthemeansquareder-ror(MSE)oftheresidualscalculatedwithrespecttothedose-response curvesusedtogeneratethedata.Itisevidentthat,aftererrorcorrection usingLOESSregressionandnormalisingtothemeanofthenegativecon-trols,effectivelayoutsleadtostatisticallysignificantsmallerMSEthan othertypeslayouts(*𝑝<*10−4forallpairwisecomparisons,*t*-test).That is,thedataobtainedusingoureffectivelayoutsismuchclosertotheir expectedvalues,thanthedataobtainedwhenusingeitherrandomand borderlayouts.

Itisstandardpracticetodiscarddose-responsecurvesthatarecon-sideredtohavelowquality,forexample,curveswheremorethan20%of thevariabilityisunexplainedbythecurvefit,thatis,with*𝑅*2*<*0*.*8[15]. Ingeneral,oureffectivelayoutsleadtoahigherpercentageofhigh-qualitycurves,ascanbeseeninSupplementaryFigs.13–15.Forex-ample,inthecaseofexperimentswith8dosesand3replicates,and strongplateeffectswithalinearrelationshiptocolumnnumberonthe

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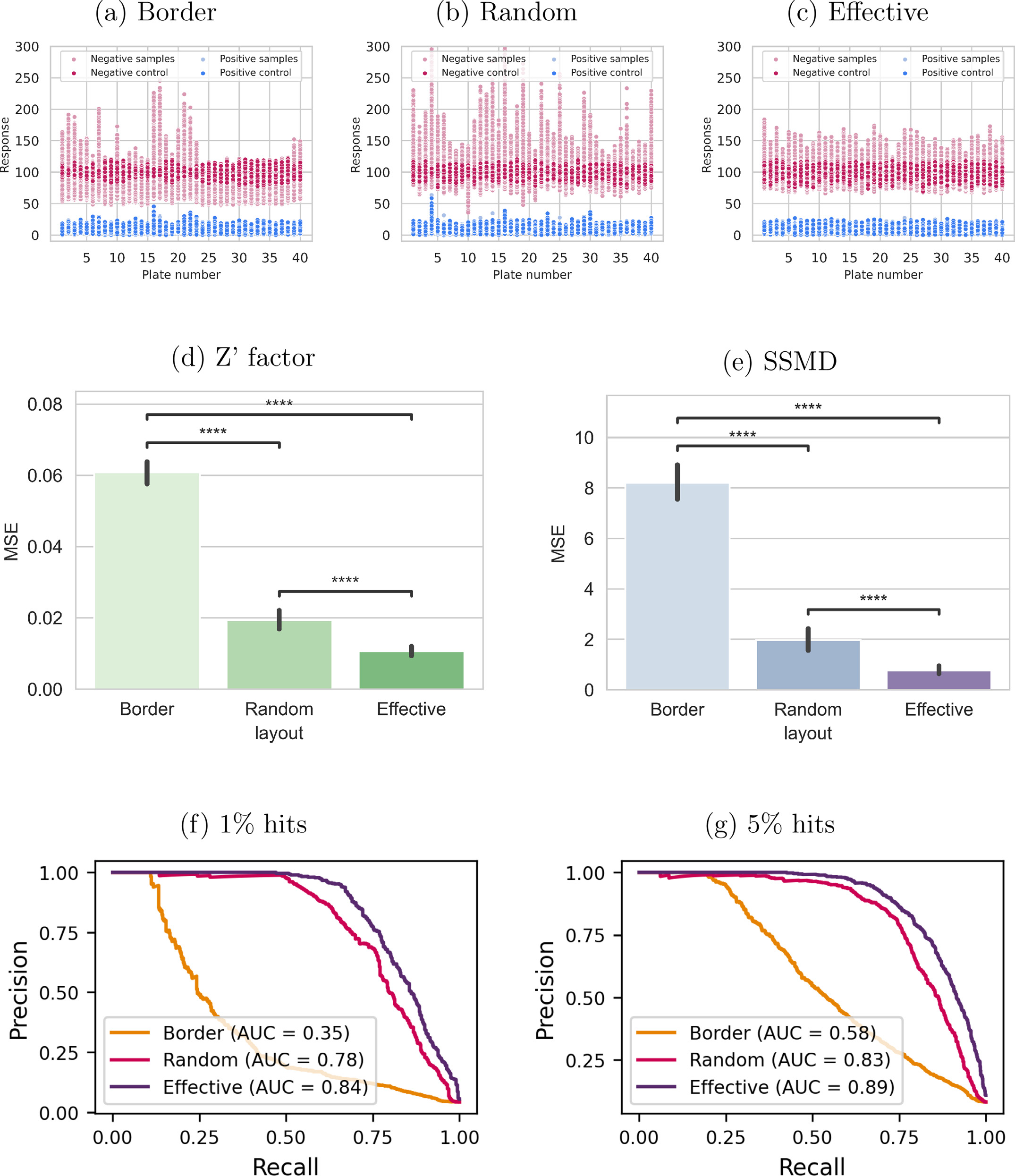


**Fig.2.**Comparisonbetweenexpectedandobtainedvaluesfordoseresponsecurveswith8doses,1,2,or3replicates,20negativecontrolson384-wellplate,and

strongplateeffectswithalinearrelationshiptocolumnnumberontherightsideoftheplate.∗indicates*𝑝<*10−4,∗∗indicates*𝑝<*10−12,∗∗∗indicates*𝑝<*10−43.

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**Fig.3.**Screeningexperimentsconsistingof40microplatesforeachtypeoflayout,eachwith10positiveand10negativecontrolsandonly1replicatepercompound. Hitsarerandomlydistributedoneachplate.(a)–(c):Simulatedresponsedatawith1%hitrateaftererrorcorrectionandnormalisationinthepresenceofmildbowl-shapedplateeffects.MSEbetweenexpectedandobtained(d)*𝑍*′factorand(e)SSMDinthepresenceofmildbowl-shapedplateeffects.Theexpectedvalueswere calculatedusingplatesrandomlyfilledwith50%positivecontrolsand50%negativecontrols,constitutingtheoptimalvaluesobtainablebythesemetrics.(f)and (g):PR(precisionrecall)curvesforexperimentswithvaryinghitratesinthepresenceofstrongbowl-shapedplateeffects.

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dicatedbylowmetricscores,butwherehighscoresarenotaguarantee forhigh-qualityresultsontheplate.Themainreasonforthisisthat both*𝑍*′factorandSSMDonlytakeintoaccountpositiveandnegatives controlsregardlessoftheirphysicallocationontheplate,andwitha sub-optimallayoutthesemetricsmightnotaccuratelycapturethereal plateeffects.Inordertoanalysetheeffectofdifferentlayoutsonqual-itymetrics,wecalculatedtheexpectedvaluesforboth*𝑍*′factorand SSMDusingwholeplatesfilledwith50%positivecontrolsand50% negativecontrols,constitutingtheoptimalqualityvaluesobtainableby thesemetrics.Wethencomparedtheresultingvaluesagainstthesame metricsbeingcalculatedusingonlyasubsetofthecontrolsontheplate accordingtoborder,random,andeffectivelayouts.Figure3(d)and(e) showthatforboththe*𝑍*′factorandSSMD,theestimatesobtainedus-ingeffectivelayoutsyieldaqualitymetricvaluethatisclosertothe expectedvaluewhencomparedtorandomandborderlayouts.Thisdif-ferenceisalwaysstatisticallysignificantaslongasthereissomedegree ofaplateeffect(SupplementaryFigs.23and24).

**4.ThePLAIDsoftwaresuite**

Inordertomakeourmethodeasilyaccessible,wedevelopedPLAID (PlateLayoutsusingArtificialIntelligenceDesign),asuiteoftoolsthat canbeusedtodesignandevaluatemicroplatelayoutsunderawide rangeofconditions.

*4.1.ThePLAIDreferenceconstraintmodel*

Weimplementedaconstraintmodelcomprisingtheconstraintsde-scribedhereusingMiniZinc[32].Advanceduserscaninteractwithand personalisethemodelbyaddingorremovingconstraints,whichcanbe ranusingtheMiniZincIDE,scripts,orcommandline.Itisalsopossi-bletoincorporatethemodelintoexistingworkflows,forexample,with thehelpoftheMiniZincPythonpackage.Instructionsonhowtorun ourMiniZincmodelusingthecommandlineortheMiniZincIDEare availableat<https://github.com/pharmbio/plaid>.

*4.2.ThePLAIDplatedesigntool*

InordertoeasetheuseofthePLAIDc[onstraintmodel,wedevel](https://plaid.pharmb.io/)oped aninteractivewebinterfaceavailableat<https://plaid.pharmb.io/>that allowsforspecifyingexperimentaldetail[sandgeneratinglayouts(](https://plaid.pharmb.io/)Sup-plementaryFig.25).Theexperimentaldesign(e.g.selectionofsam-ples,concentrations,etc.)canbedownloadedfromthewebinterface inaJSONformatthatcanbelateruploadedintothewebsiteinor-dertocreatemoreplatedesignsforthesameexperimentorasabase fornewexperiments.TheproducedlayoutsgeneratedbythePLAID constraintmodelcanbevisualisedwithinthewebinterface(Supple-mentaryFigs.26and27),anddownloadedinCSV,andJSONfile formats,aswellasanimage(SupplementaryFig.28).Producedlay-outsinJSONformatcanbere-uploadedintothewebsitetousethe visualisationfeatures(SupplementaryFigs.26and27).Examplesof bothexperimentalsettingsandlayouts,aswellasconveniencemeth-odsfortranslatinglayoutsintospecificformatstobedirectlyusedin EC[HOandI.DOTcompounddispensingrobot](https://www.github.com/pharmbio/plaid)s,areavailableonGitHub at<https://www.github.com/pharmbio/plaid>.

*4.3.ThePLAIDanalysisandvisualisationnotebooks*

Experimentdesignscansubstantiallyvary,andnoone-solution-fits-allexists.Differentassays,laboratoryconditions,equipment,etc,lead todifferenttypesandstrengthsofplateeffectsthataffectexperiments. WedevelopedinPythonalibraryofparametricplateeffects,alibrary ofplatenormalisationanderrorcorrectionfunctions,doseresponseand highthroughputscreeningsimulations,aswellasvisualisationfunction-ality.Thislibrarycanbeused,forexamplefromwithinPythonnote-books,toevaluatedifferentexperimentaldesigns,suchastoexplore

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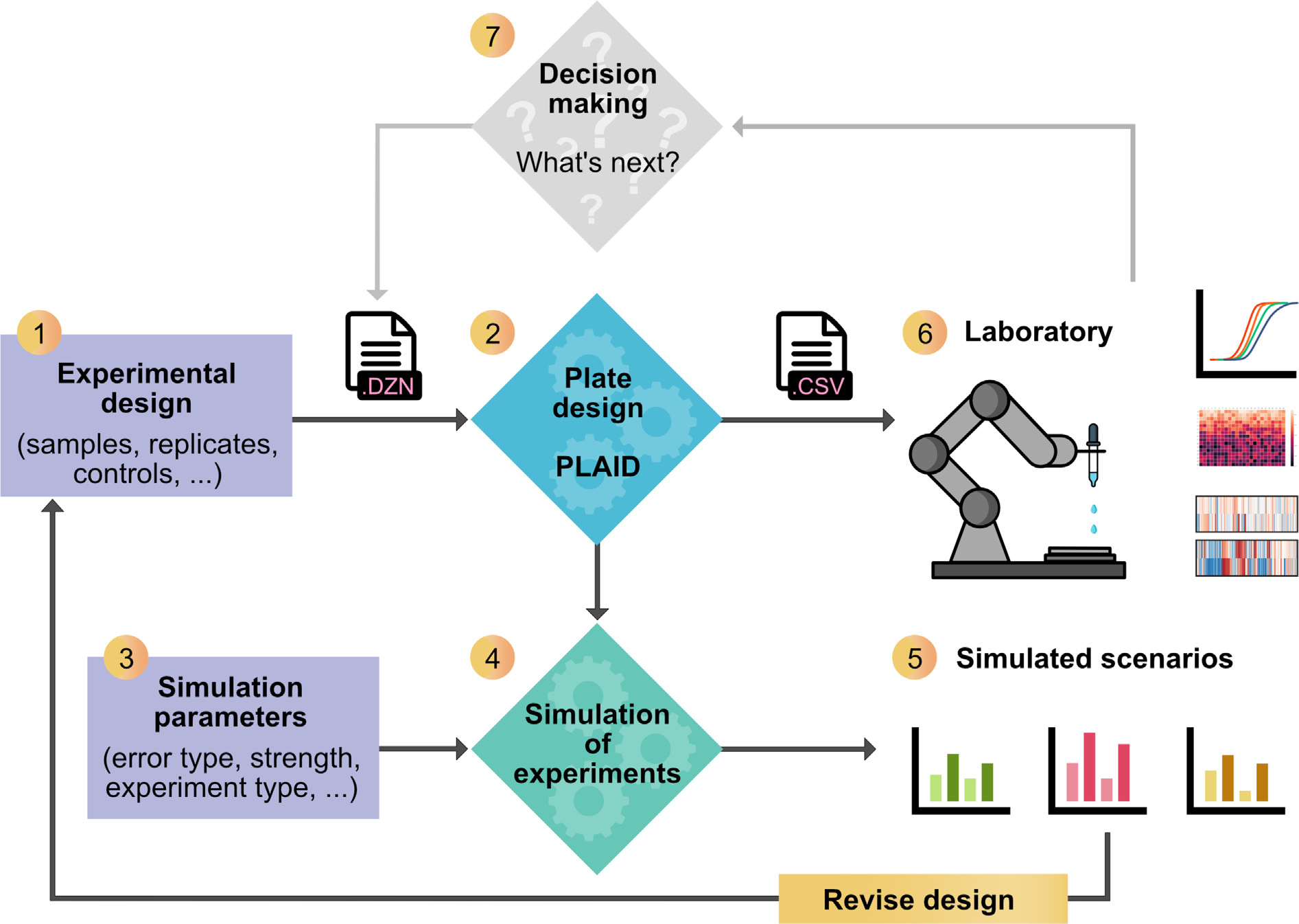
Thebenefitsandlimitationsofeffectivemicroplatelayoutsare tightlycoupledtotheuseandimpactofmethodsfordatanormalisa-tion,thatinturnaredependentontheuseof,andsufficientnumber of,controlsamples.Further,amulti-plateexperimentalsooffersmore opportunitiesforfindingeffectivelayouts.Inourworkwefocusedon LOESSnormalisation[34],whichisawidelyusednormalisationtech-niquethatisrobusttowardsdifferenttypesofplateandexperimental effects.Theerrormodelusedinthisworkisbasedontheoneproposed byZhangetal.[11],buttheintensityandtypeofplateeffectsobserved mightdifferdependingonfactorssuchasthetypeofexperiment,lab-oratoryfacilities,andtemperature,amongothers.Akeyadvantageof usingaconstraintmodelfordesigninglayoutsisthatsuchparameters canbeeasilyadjusted,duetothedeclarativenatureofthemodel,and evaluatedusingtheprovidedPLAIDPythonnotebooks.Wehaveput togetherasuiteofconstraintsthatarewidelyuseful,butthefinalselec-tionofconstraintsisuptothescientistsplanningexperimentsanditis easytoe.g.removeconstraintssuchas‘nosamplesinouterwells’ifthey arenotdesirable.Fromapracticalperspective,suchaswhenpipetting manually,itcanbebeneficialtousethesamesampleonlyononeplate. Forautomatedliquidhandlinginstruments,thenumberofplates(and hencesourcesamples)accessiblecanhaveanimpact.Thesescenarios arenotcoveredinthisstudy,butthereisnohindertoalsoimplement suchconstraintsintoaplatelayoutmodel.

*5.1.Iterativeexperimentation*

AImethodssuchasunsupervisedandsupervisedlearningarenowa-dayswidelyusedtoanalysetheresultsfromlarge-scaleexperiments usingmicroplates.Therearealsoemergingapproachestosequentially planaseriesofexperimentstosystematicallyimprovetheaccuracyof AImodels[35,36].Data-centricAIisaconceptthatproposestoshift fromthecurrentpracticeofhavingasetoffixeddataandthenspend-ingmuchtimeandefforttofine-tuneamachinelearningmodel,toin-steadfocusonaniterativeapproachtooptimisethedatausedtotrain themodel[37].Thismethodologyfundamentallybuildsonthepropo-sitionthathigh-qualitydataisbetterthanjustmoredata,something thatforalongtimehasbeenarguedintraditionalexperimentaldesign guidelines[33].Selectingthenextbatchofexperimentsishowevernon-trivial,andautonomousdecision-makingiscurrentlyanactiveresearch fieldwithautonomousvehiclesasabigdriver.Activemachinelearn-ingisoneapproachtoselectnewexperimentswhich,combinedwith robotics,hasthepotentialtoautomatescientificdiscoveries[38–41]. Fig.4showshowthePLAIDsuitesupportsiterativeexperimentation. Thefirststepconstitutesaninitialdecisiononsamples,replicates,con-trols,etc.,anditsdefinitioninadeclarativefileformatformicroplate experiments(SupplementarySection1).Thisexperiment-definitionfile istheninputtothePLAIDplatedesigntoolthatappliestheconstraint modeltogenerateeffectiveplatelayouts.Theproducedlayoutscanthen beevaluatedtowardssimulatedexperimentsdefinedbydifferenterror parameters,whichovertimecanbetailoredtoparticularexperimen-talandlaboratorysetups.Basedontheoutcomeofthesimulations,the experimentdesignmightberevisedandnewplatelayoutscanbegen-erated.Whenadecisionismadetoacceptthelayouts,thesecanbe translatedtocustomformatsthatcanbereadbylabinstruments.We providetranslationsfortwocommonchemicaldispensinginstruments (ECHOandI.DOT),butitisstraightforwardtocreatemoreadaptors forotherinstruments.Acceptingplatelayoutscanbedonemanually byhumans,orautonomouslyusinganalgorithm.Ifthedataacquisition andanalysisfromthephysicalexperimentscanbeautomated,thenonly thedecisionmakingregardingthenextroundofexperimentsremains. Giventhatautonomousdecisionmakinghasbeenimplementedinorder toselectthenextexperiment,itcanbedefinedinthePLAIDfileformat formicroplateexperiments,closingtheloopforthenextexperimentit-eration.Wespeculatethatsuchautomatedanditerativescientificexper-imentswillbeincreasinglycommoninthefuture,andthatPLAID,given itsflexibilityduetothedeclarativenatureofconstraintmodelling,its

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**Fig.4.**OverviewofthePLAIDecosystem.Experimentdesign(1)comprisestheselectionofsamples,replicates,concentrations,etc,andcanbedefinedinaconfig-urationfileorviathePLAIDPlateDesignwebinterface,andthenthePLAIDconstraintmodeliscalledtogeneratetheresultinglayouts(2).Inordertoevaluatethe design,simulationparameterssuchastypeandstrengthofexpectedplateeffectsneedtobedefined(3)andthenasimulatedexperimentusingthelayoutscanbe carriedoutinthePLAIDAnalysisandVisualisationNotebooks(4).Differentscenarioscanbecompared,suchasdifferentnumbersofconcentrationsandreplicates, anddesignsmaythenberevisedaccordingly.Whenanacceptabledesignhasbeengenerated,itcanbeusedtodriveliquidhandlinginstruments,suchasautomated pipetterobots(6).Aftertheexperimentisperformedandanalysed,adecisioncanbemadeonsubsequentexperimentse.g.confirmfindings,re-runfailedsamples, evaluatemoreconcentrations,etc.(7).ImplementingautomateddecisionmakingthatdefinesthenextexperimentinthePLAIDconfigurationfileformatenables autonomoussequentialexperimentation.



**Fig.5.**ExamplesofconstraintsinMiniZincsyntax.

Ontopofincludingallthedesirablepropertiesofeffectivemi-croplatelayouts,wehavechosentoincludeotherconstraintsthatare neededforpracticalmatters.Forexample,weenforcethatforeachsam-ple,allconcentrationlevelsofagivenreplicamustappearonthesame plate.Technicalreplicatesofasamplecanbechosentoappearonthe sameplate,onadifferentplate,oramixtureofboth.Wehavealso includedthedimensionsofthemicroplateasparametersintermsof numberofrowsandcolumns,allowingtheuseofanykindofplatesize. Finally,itisalsopossibletospecifyhowmanyrowsandcolumnsshould beleftemptyontheborderofeverymicroplateinordertomitigateedge effects.

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ifasufficientnumberofconcentrationsweretobeused,acomplete doseresponsecurvewouldbegenerated.Togeneratethesigmoidcurves correspondingtoeachcompoundtheonlyparameterremainingtobe specifiedistheEC50/IC50.WegeneratedcurveswithEC50/IC50values rangingfrom1to96tosimulatecompoundshavingallkindsofpotency. Thehighestconcentrationwasarbitrarilysetto100μM.Foreachtest concentration,thereplicatesweregeneratedbyaddingarandomvalue within±1%tothevaluesampledfromthecurveinordertorepresenta verysmallerrorinmeasurementbetweenwellshavingthesamecom-poundinthesameconcentration.

Borderlayoutsweregeneratedbydistributing20negativecontrolsin columns2and23,andplacingallothersampleshorizontallyfromtopto bottom.RandomlayoutsweregeneratedusingthePythonrandompack-age.Effectivemicroplatelayoutsweregeneratedusingourconstraint programmingmodelimplementedinMiniZinc[32].ThePythonfunc-tionsandMiniZincmodelusedtogeneratethelayoutsaswellasthere-sultinglayoutsareavailableat<https://github.com/pharmbio/plaid>.We thenappliedthesameplateeff[ecttoeverymicroplatehavingeithe](https://github.com/pharmbio/plaid)r:(i) abowl-shaperelationshiptowellposition,or(ii)alinearrelationshipto columnnumberontheright-handsideoftheplate.Strongplateeffects aredesignedaccordingtotheexamplesinZhang[11],whilemoderate plateeffectsaredesignedtobehalfwaybetweenno-effectandastrong plateeffect.Afterapplyingplateeffects,weadjustedthedatausinglin-earregressioninthecaseofborderlayouts,andLOESSregressionas implementedinCappellarietal.[55]fortherest,andnormalisedthe dataasapercentageofthemeanofthenegativecontrols.Finally,we estimatedtherelativeandabsoluteEC50/IC50usingthecurve\_fit functionofthescipyPythonlibrary,whichusestheTrustRegionRe-flectivealgorithm.Foreachdose-responsecurve,wecalculatedtheab-solutevalueofthedifferencebetweenthelog10ofthetrue(expected) andtheestimatedEC50/IC50values.Moreover,foreverymeasurement, wecalculatedthedifferencewithrespecttoboththeexpected(true) valueaswellaswithrespecttotheestimatedcurves.

*7.4.Screeningexperiments*

Wesimulatedscreeningexperimentsconsistingof40384-wellmi-croplates,eachofwhichcontainedeither(i)8positivecontrolsand 8negativecontrols,(ii)10positivecontrolsand10negativecontrols, or(iii)10positivecontrolsand20negativecontrols.Theremaining wellscontainedrandomsampleswithhit-ratesof1%,5%,10%,20%, 33%,and40%.Wethenappliedvariousstrengthsofbowl-shapedef-fectstoeverymicroplate.Strongplateeffectsaredesignedaccording totheexamplesinZhang[11],whilemoderateplateeffectsarede-signedtobehalfwaybetweenno-effectandastrongplateeffect.For errorcorrectionandnormalisationweusedlinearregressioninthecase ofborderlayouts,andLOESSregression(asimplementedinCappellari etal.[55])inthecaseofrandomandeffectivelayouts,andscaledthe dataasapercentageoftheaverageofthenegativecontrols.Bothlinear andLOESSregressionareperformedbasedonnegativecontrolsonly, withoutassumingalowhit-rate.Weusedtheerror-correcteddatato calculatethe*𝑍*′factorandtheSSMDofeachmicroplate.Finally,we usedthesklearnPythonlibrarytocalculatetheresultingprecision-recall(PR)andreceiveroperatingcharacteristic(ROC)curves,aswell astheircorrespondingareaunderthecurve(AUC)values.

**Dataavailability**

ThePythonlibrariesandnotebooksdevelopedfortheanalysis,the experimentalresults,togetherwiththespecificmicroplatelayoutstested areavailableat<https://github.com/pharmbio/plaid>.

**Codeavailability**

AllsourcecodeforPLAID,includingourconstraintmodel,libraries andPythonnotebooksforsimulating,evaluatingandvisualisingex-

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