

Supplementary information

Engineering and exploiting synthetic allostery of NanoLuciferase

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Supplementary note 1: Analysis of cpNanoLuc tacrolimus biosensor specificity, activation rate and stability

To assess the suitability of the developed biosensors for diagnostic applications, we tested the effect of rapamycin and cyclosporine A on the performance of the tacrolimus biosensor. We titrated 1nM of tacrolimus biosensor either in buffer or in the presence of 200 molar excess of rapamycin (200nM) or 1000 molar excess of cyclosporine A (1 μ M). The data shown in the supplementary figure 1D shows that while neither rapamycin nor cyclosporin A led to activation of the tacrolimus biosensor both drugs interfered with its activation. This is not surprising as all biosensors share one common binding domain–FKBP between rapamycin and tacrolimus biosensors and Calcineurin A/ Calcineurin B complex between tacrolimus and cyclosporine A biosensors. Given the fact that even at 1000X drug excess the biosensor retained 7-10% of its dynamic range it is likely that one can use the developed biosensors to measure tacrolimus levels in the clinical samples containing both drugs. Further, it is possible that use of biosensor combination may allow even more accurate measurement of drug concentrations in such patients. Additional experiments are needed to ascertain this assumption.

To further characterize the performance of the developed Tacrolimus biosensor we tested the time dependence of its response to the ligand. We carried out the activity measurements following different incubation periods with the ligand. As shown in *supplementary figure 2A* the amplitude of response increased with increasing incubation time coming to saturation at 20 minutes. The full activity of the biosensor was determined to be about 20% compared to wt NanoLuc (Supplementary figure 2B).

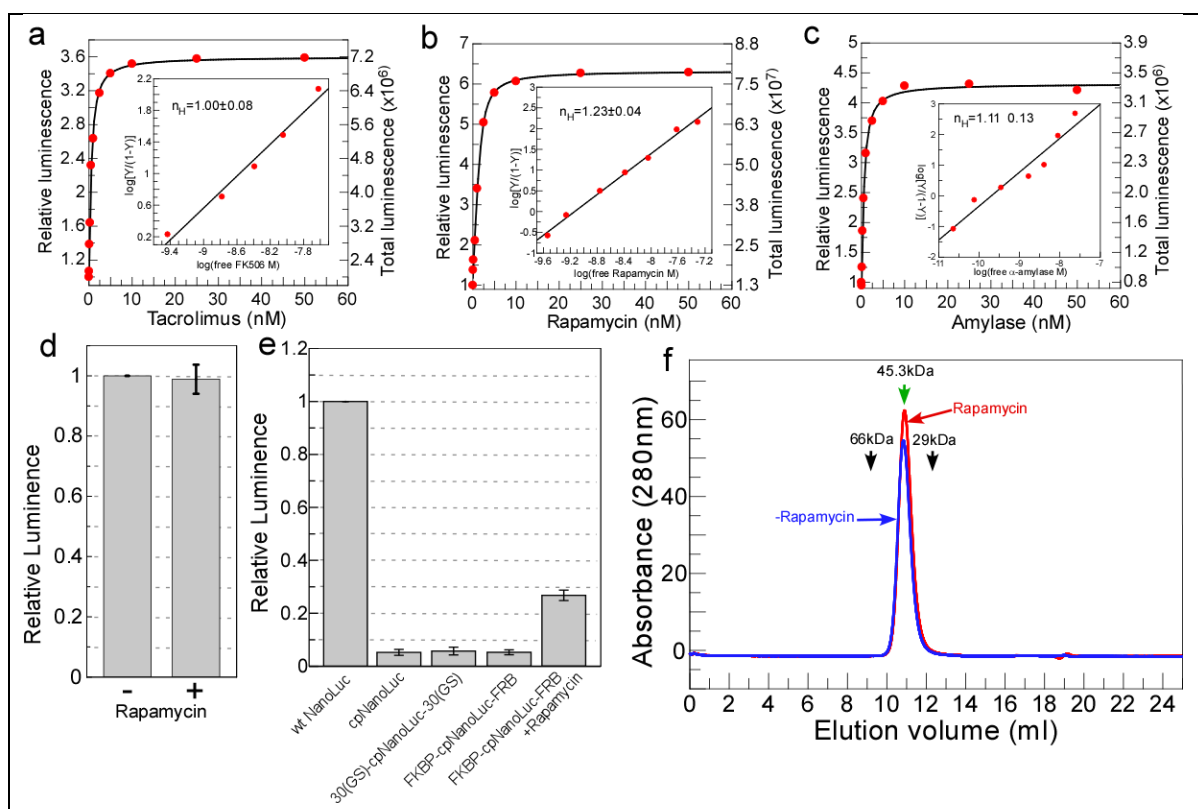
We subsequently quantified the luminescent yield of the wild type NanoLuc, CaM-NanoLuc and the tacrolimus biosensor. As can be seen in supplementary figure 2B the CaM NanoLuc retained 70% of the luminescent yield of the parental enzyme while the tacrolimus biosensor retained 20% of the luminescent yield. This can be related to incomplete enzyme

reconstitution upon ligand binding or alternatively to changes in the K_m values of NanoLuc resulting from mutagenesis. We observed that with increase of the furimazine concentration the signal increased linearly till it was too strong for the available instruments.

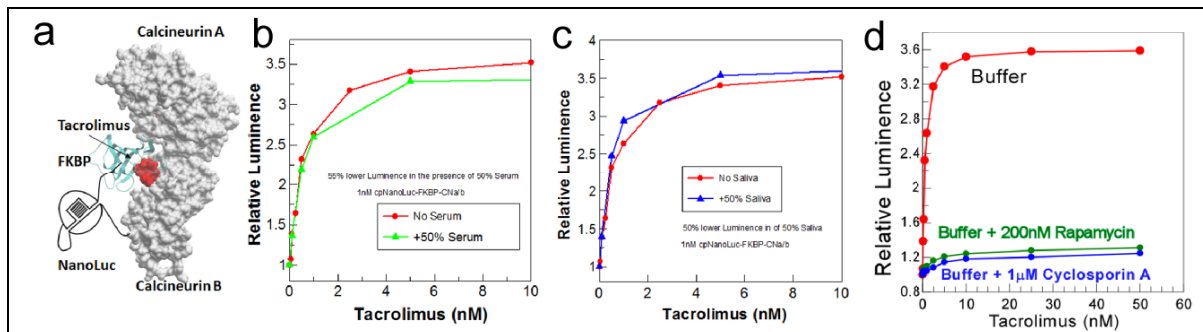
Next, we tested the stability of the tacrolimus biosensor under drying conditions. Supplementary figure 2C shows that the biosensor can be dried and rehydrated in functional form without the need of any optimization of the conditions. We subsequently tested the compatibility of the biosensor with lysed and whole blood. We observed that 50% hypotonically lysed blood significantly diminished the biosensor's signal (Supplementary figure 2D). This is not surprising as the emission wavelength of NanoLuc overlaps with the absorption wavelength of hemoglobin.

Emission absorption is a commonly encounter problem of Luciferase-based biosensors in biological systems. One of the ways of overcoming this is to employ a luminescent converter that absorbs light in the blue part of the spectrum and emits it at a higher wavelength in a phenomenon termed Bioluminescence Resonance Energy Transfer (BRET). We chose to test it using a green fluorescent protein that has matching excitation wavelength. In the context of the developed architecture introduction of such domain is not trivial as both N- and C-termini are occupied by large binding domains. Therefore, we decided to test this idea by using green fluorescent protein as a linker between the domains of the rapamycin biosensor.

Supplementary figure 3C shows the emission scan of such rapamycin biosensor in the absence and presence of rapamycin. It can be seen that the emission of the biosensor is shifted to the maximum of 510nm as expected and corresponds to the emission of EGFP. The results of titration of the red shifted biosensor with rapamycin are shown in supplementary figure 3D and demonstrate that despite the integration of the wavelength "extender" domain the biosensor faithfully produced enhanced light emission with the maximum at 510nm. The signal change of the biosensors in the presence of rapamycin could be fitted to a K_d of 0.5nM that demonstrates that despite introduction of the BRET-forming domain the function of the biosensor remained largely unchanged.

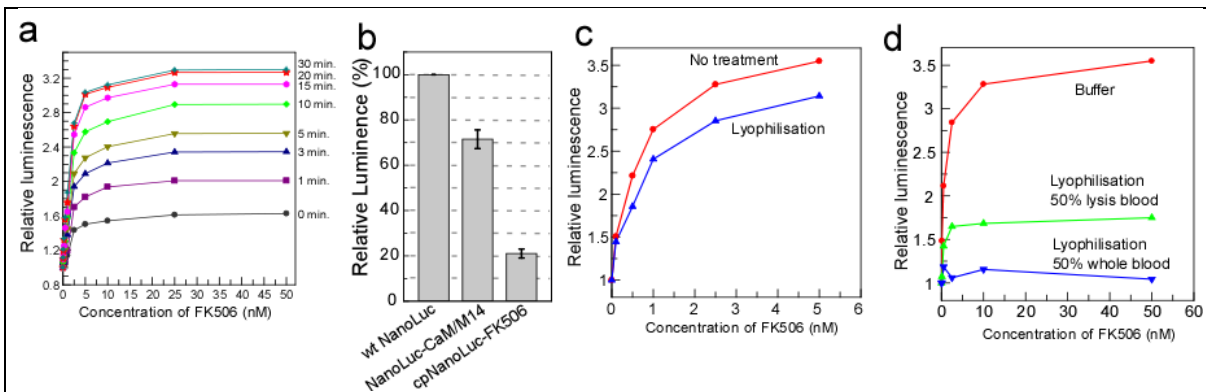


Supplementary figure S1 Analysis of cpNanoLuc biosensors of macrocyclic compounds and α -amylase. (A) Fit of the titration data for tacrolimus to a quadratic equation and the Hill coefficient (insert) (B) As in A but using rapamycin biosensor and rapamycin as titrant. (C) As in A but using the α -amylase biosensor and α -amylase as the titrant. The titrations were performed as described in Figure 1. (D) Activity analysis of 1nM solution of FRB-wtNanoLuc-FKBP fusion in 200 μ l buffer containing 20mM Tris pH7.4, 100mM NaCl and 0.1 μ l NanoLuc substrate in the presence and absence of 50nM of Rapamycin. (E) Activity analysis of 1nM solutions of wild type NanoLuc, cpNanoLuc, cpNanoLuc with bearing 30 glycine, serine (GS) amino acid repeats on both N and C termini, FRB-cpNanoLuc-FKBP and FRB-wtNanoLuc-FKBP in the presence of 50nM rapamycin. The assay was performed as in (D), (F) Elution profile of Superdex 75 10/300 GL column loaded with 250 μ l of 1mg/ml solution cpNanoLuc of Rapamycin biosensor in the absence (blue graph) or presence (red graph) rapamycin. The black arrow heads indicate the positions of the gel filtration markers while green arrowhead indicates the calculated molecular weight of cpNanoLuc. In all plots the bars represent values of average of three independent measurements performed in the same experiment. The error bars denote positive and negative boundaries of the standard error of mean.



Supplementary figure 2 Structure and performance of cpNanoLuc tacrolimus biosensor (A)

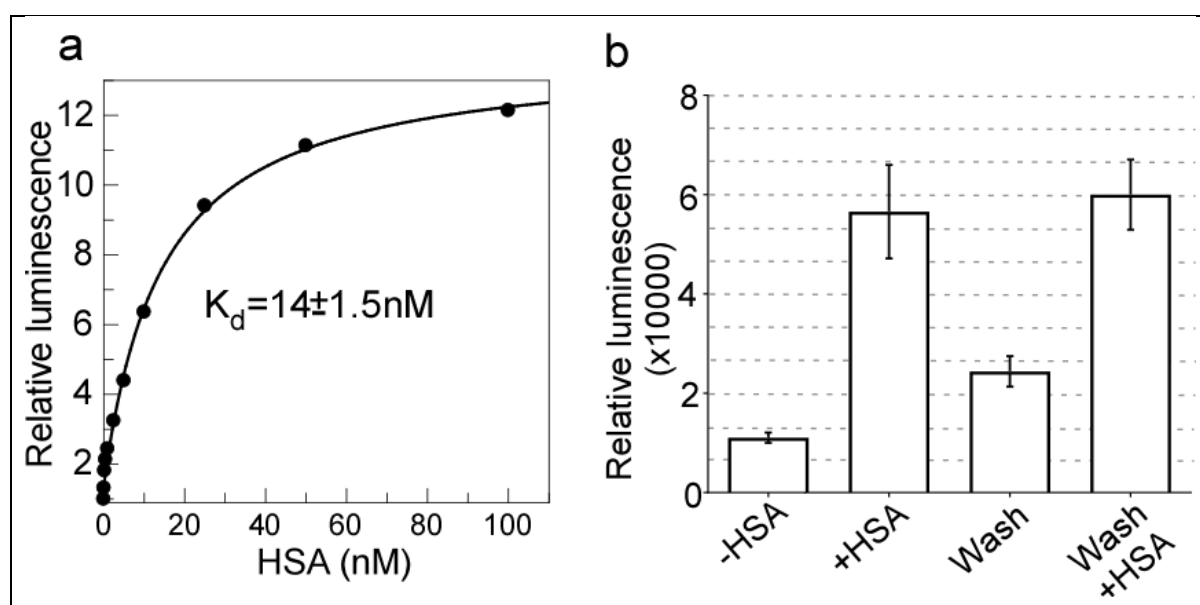
A model of a single component tacrolimus biosensor composed of circular permuted NanoLuc flanked with FKBP (displayed as ribbon) and a fusion of Calcineurin A and Calcineurin B proteins (displayed as a molecular surface). (B) Titration of 200μl solution of 1nM tacrolimus biosensor supplemented with 0.25μl furimazine stock solution in buffer containing 20mM Tris-HCl pH 7.2, 20mM NaCl with increasing concentrations of the drug in the presence or absence of 50% serum. The data was fitted to a K_d value of 0.4nM. (C) same as in (B) but in the presence or absence of 50% saliva. The data was fitted to a K_d of 0.4nM. (D) Analysis of the sensitivity of the tacrolimus biosensor to rapamycin. In the experiment 1nM solution of tacrolimus biosensor either alone or with 200nM rapamycin or 1mM cyclosporine A was titrated with increasing concentrations of tacrolimus.



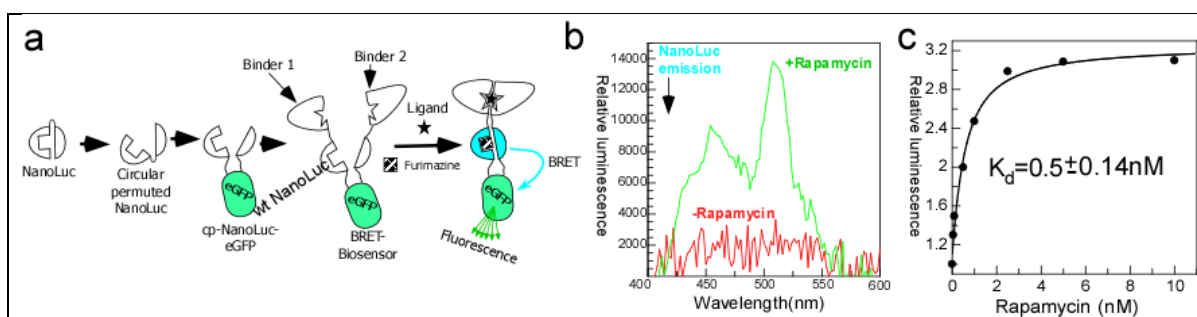
Supplementary figure 3 Assessing the performance of cpNanoLuc tacrolimus biosensor (A)

Assessing the time dependence of biosensor activation. The stock reaction containing 1nM tacrolimus biosensor supplemented with 0.25μl/200μl furimazine stock solution were mixed with the indicated concentrations of tacrolimus and incubated for indicated periods of time in the 96 well plate. The luminescence of the samples was then measured and the data was plotted against the concentration of tacrolimus. (B) The comparison of the luminescence yield of 1nM solution of the wild type recombinant NanoLuc, CaM-NanoLuc in the presence

of 100nM of CaM-BP and NanoLuc-based tacrolimus biosensor in the presence of 50nM of tacrolimus. In the plot the bars represent values of average of three independent measurements performed in the same experiment. The error bars denote positive and negative boundaries of the standard error of mean. (C) Assessment of the ability of tacrolimus biosensor to withstand de- and rehydration. In the experiment 5 μ l of 40nM NanoLuc-based tacrolimus biosensor in assay buffer was dried in a 96 well plate and then re-suspended in 200 μ l of reaction buffer. The reactions were initiated by the addition of 0.25 μ l furimazine and indicated concentrations of tacrolimus and the samples were measured after 20 minutes incubation. In the control reactions the stock solution of the biosensors was diluted to the final concentration of 1nM with the reaction buffer, furimazine and the indicated concentrations of tacrolimus. (D) Assessment of tacrolimus biosensor performance in whole and lysed blood. The experiments were performed using as in C but in the presence of 50% of whole or hypotonically lysed blood.

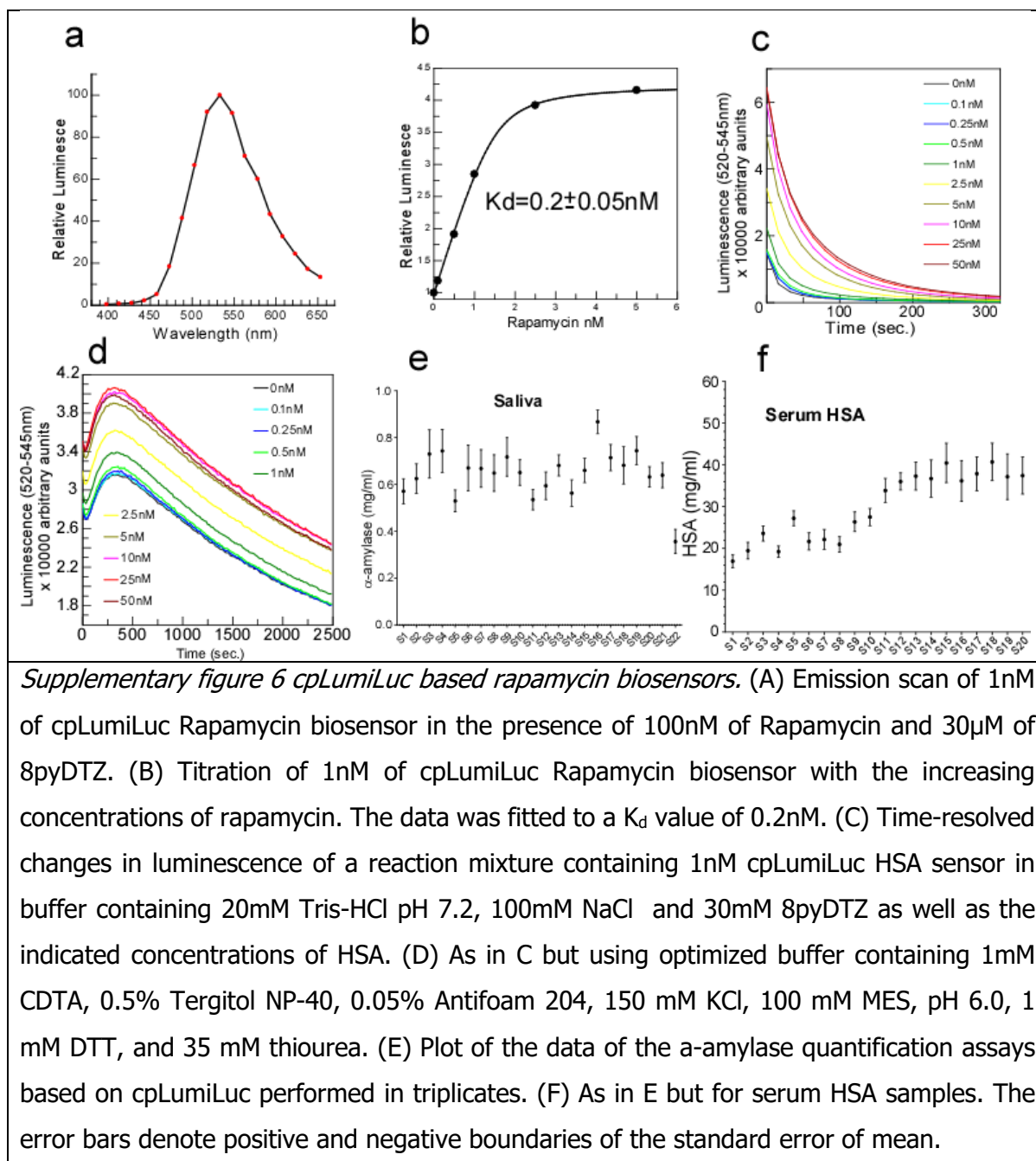


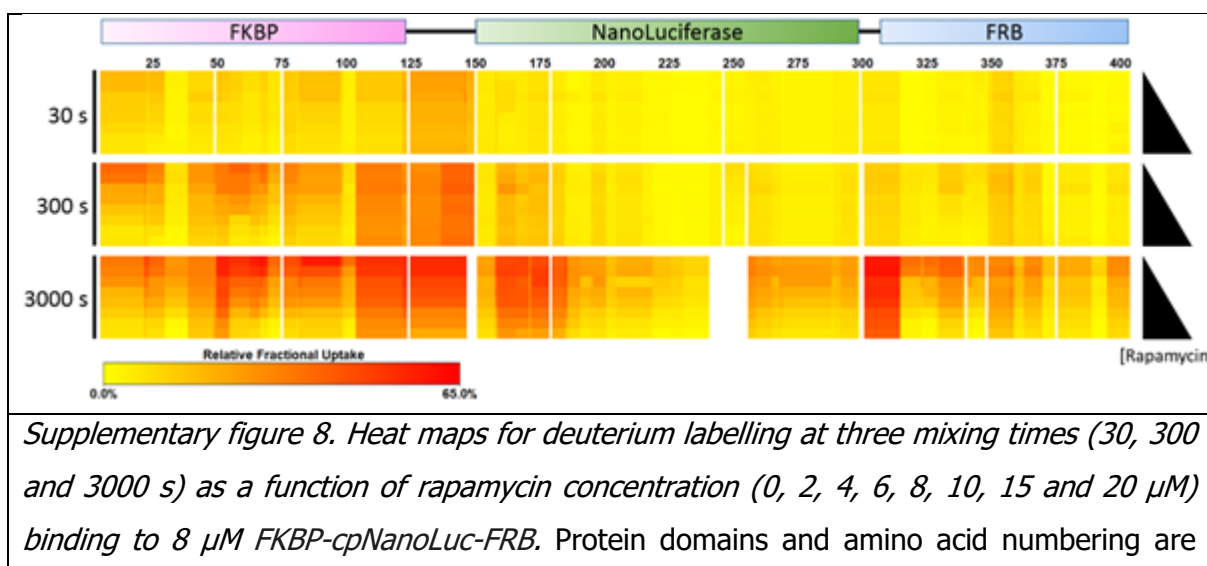
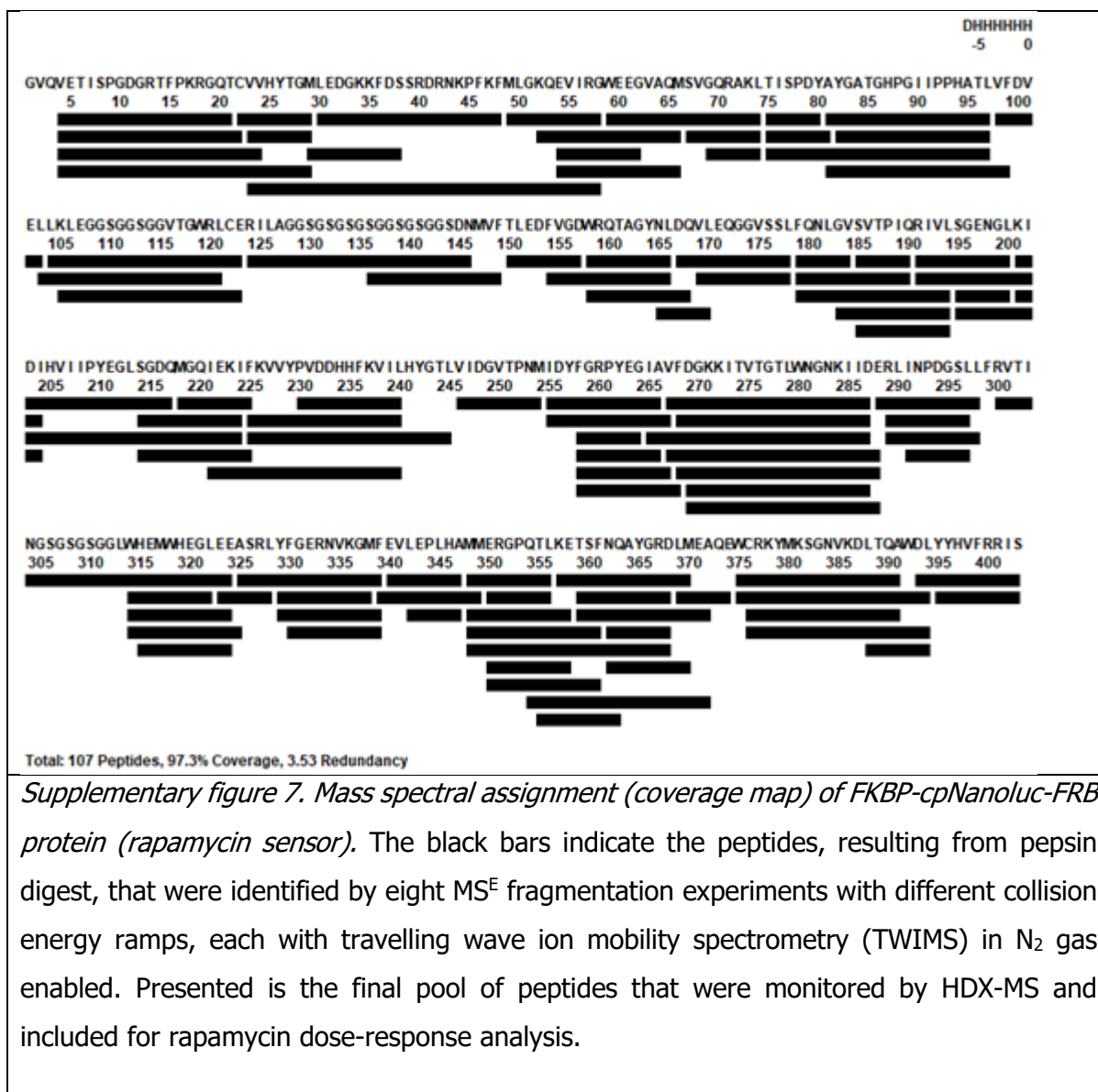
Supplementary figure 4. Construction of cpNanoLuc human serum albumin biosensor (HSA) and its interaction with the ligand. (A) Ligand titration experiment where 1nM solution of the biosensor was titrated with increasing concentrations of HSA. The relative luminescence was plotted against the concentration of the ligand and fitted to a K_d value of 14nM. (B) Activity analysis of cpNanoLuc HSA biosensor immobilized on the polystyrene plates and subjected to the repeated cycles of ligand binding and removal. In the plot the bars represent values of average of three independent measurements performed in the same experiment. The error bars denote positive and negative boundaries of the standard error of mean.



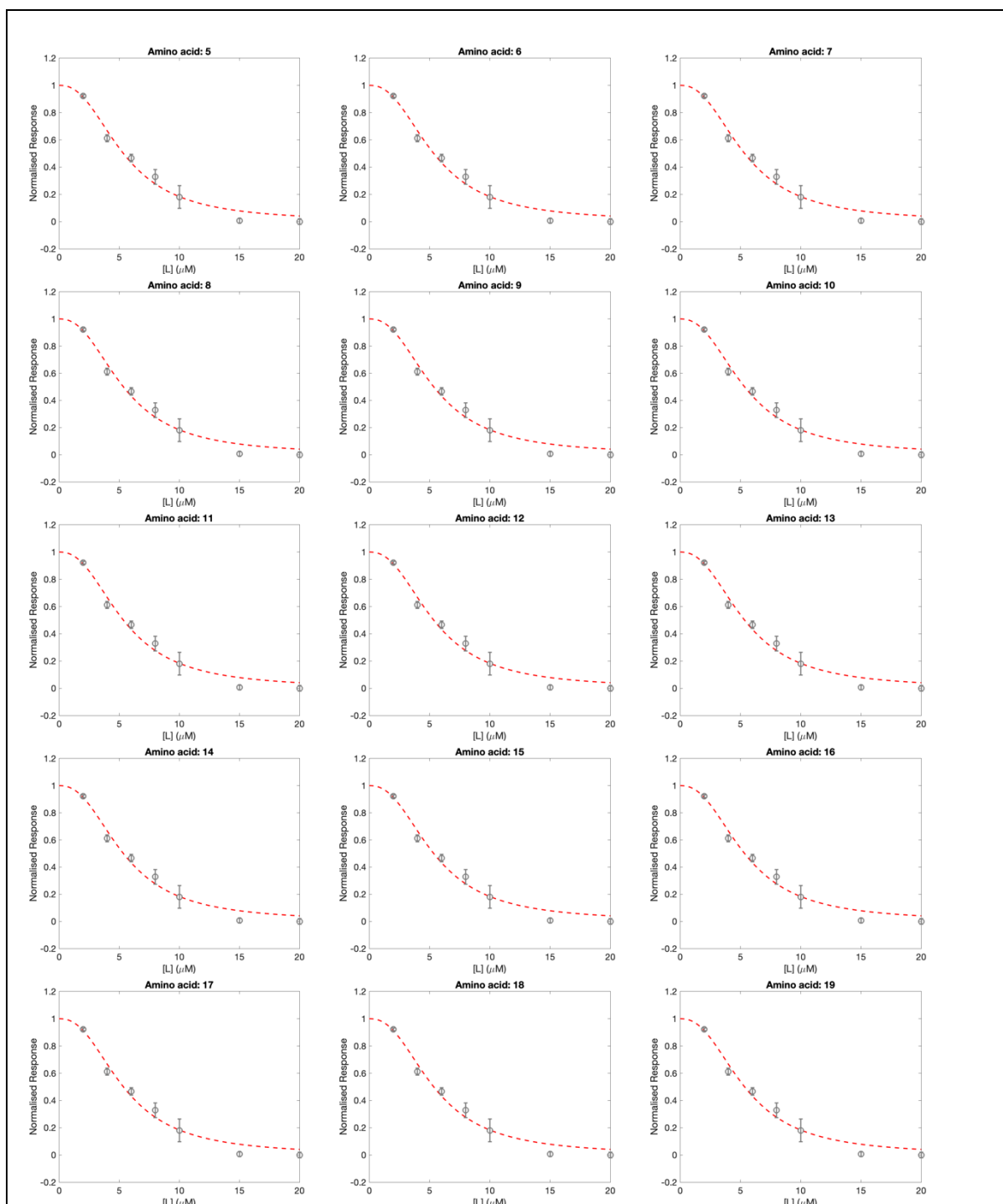
Supplementary figure 5 Construction of NanoLuc biosensors with red-shifted emission (A)

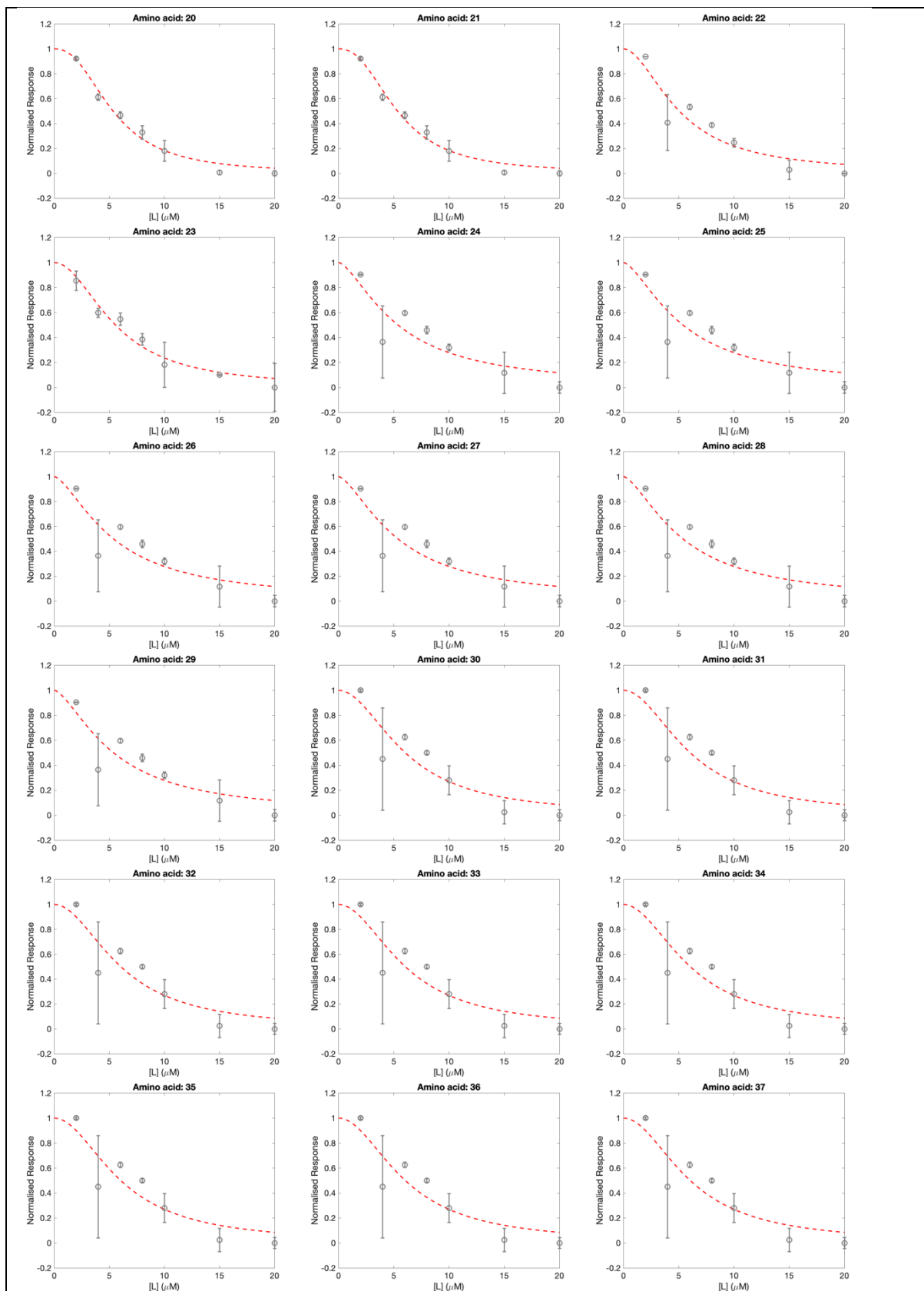
The design sequence of converting wild type NanoLuc into a BRET sensor. The circular permutation of NanoLuc followed by the insertion of a fluorescent protein domain (in this case EGFP) between *de novo* created N- and C- terminus. The subsequent addition of the binding domains creates a biosensor that when activated by a ligand results in photon emission at 460nm that leads to fluorescent excitation of the fused EGFP that subsequently emits light with the emission maximum of 510nm. (B) A fluorescent scan of 1nM solution of rapamycin biosensor constructed as shown in (A). The red trace represents the emission of the biosensor in the absence of rapamycin while the green trace represents emission of the biosensor solution supplemented with 20nM of rapamycin. The maximum emission of NanoLuc is indicated by an arrow. (C) Titration of 1nM solution of rapamycin biosensor from A and B with increasing concentration of rapamycin. The luminescence over 480nm was recorded and the determined values were plotted against the concentrations of rapamycin. The data was fitted to the quadratic equation leading to a K_d value of 0.5nM which is very close to the values obtained for the parental biosensor.

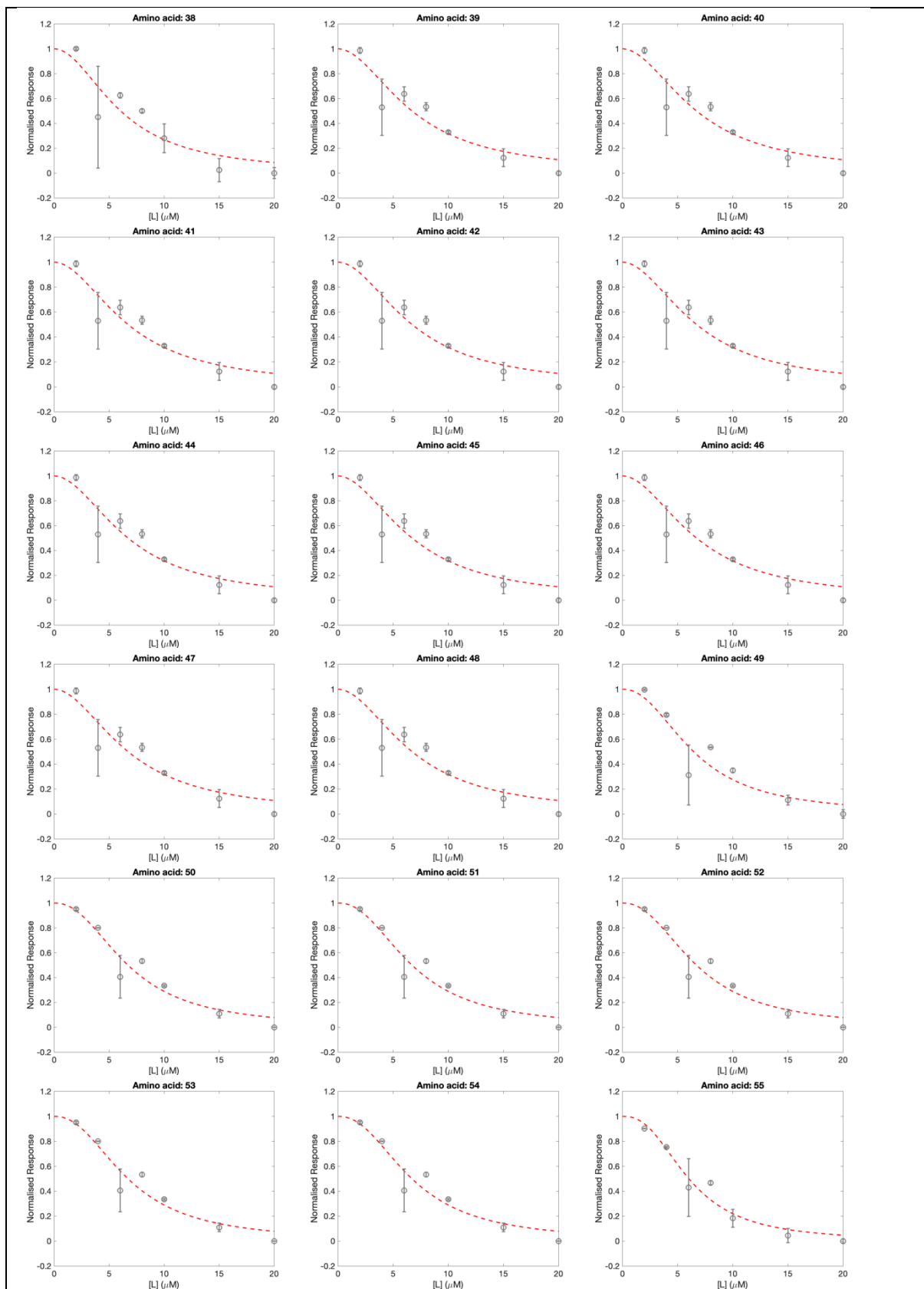


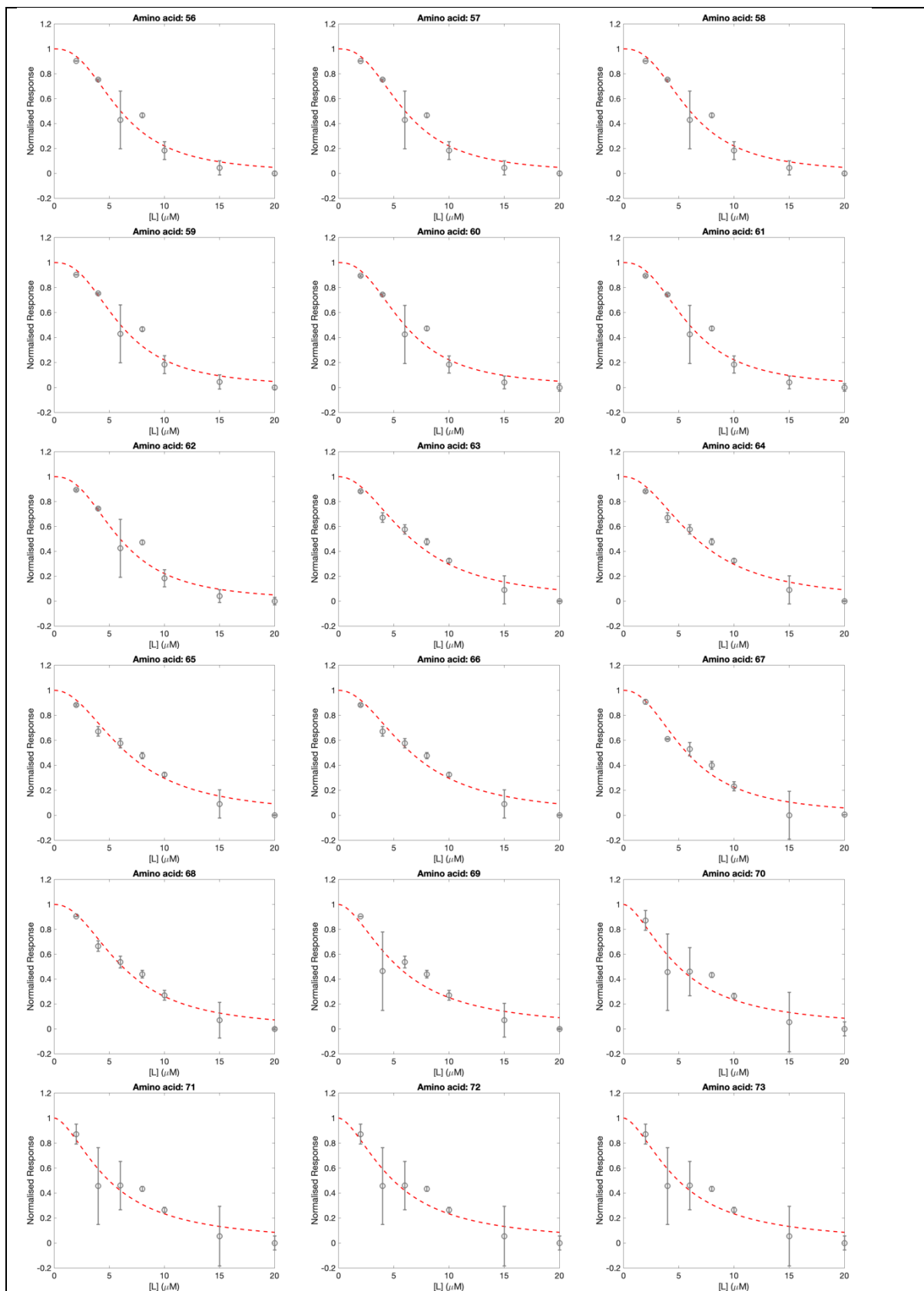


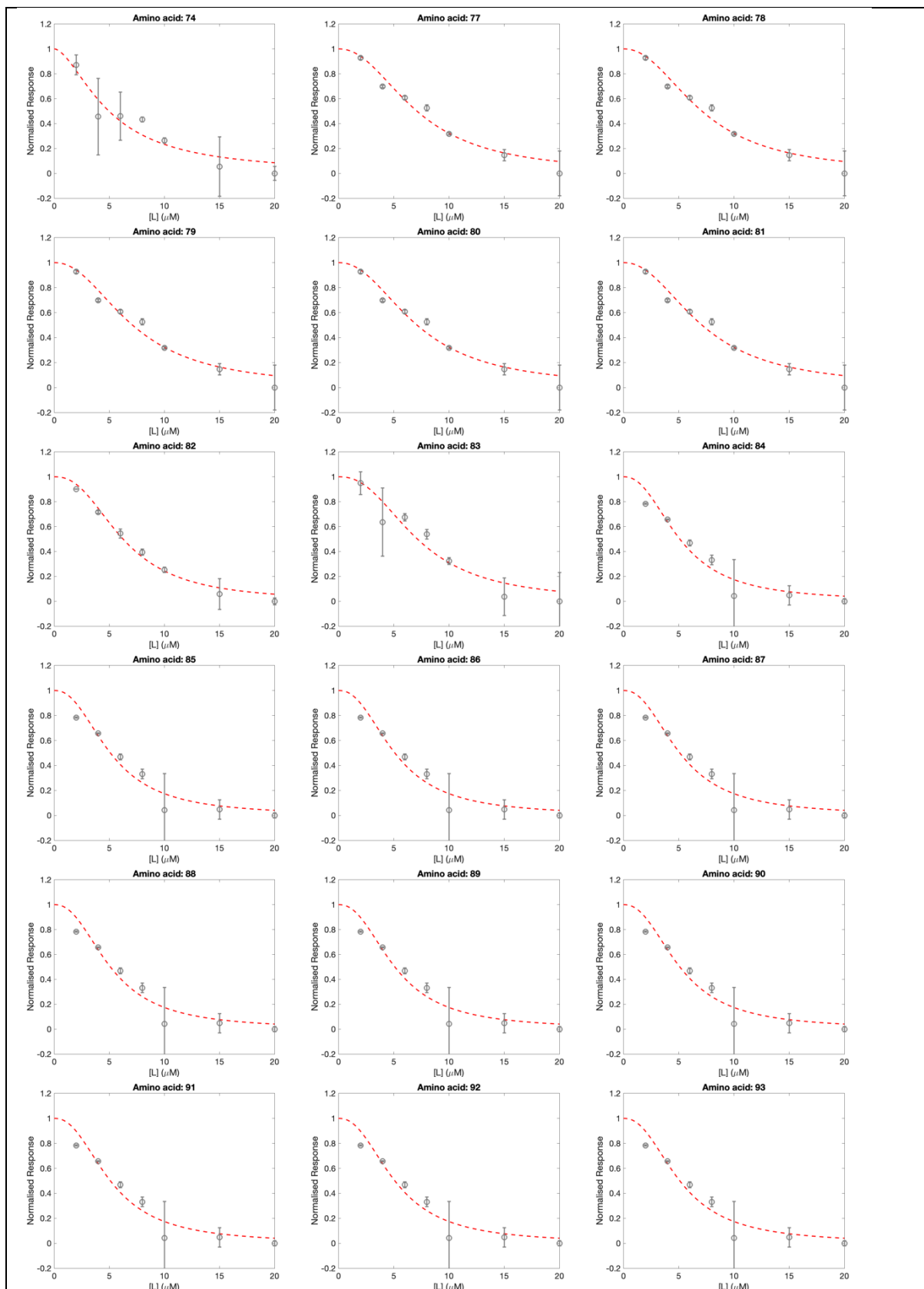
shown above. The deuterium labelling is rendered at the single residue level as the mean of all overlapping peptides at each amino acid with linear weighting of the uptake across all exchangeable sites within those peptides. Created in DynamX 3.0 (Waters).

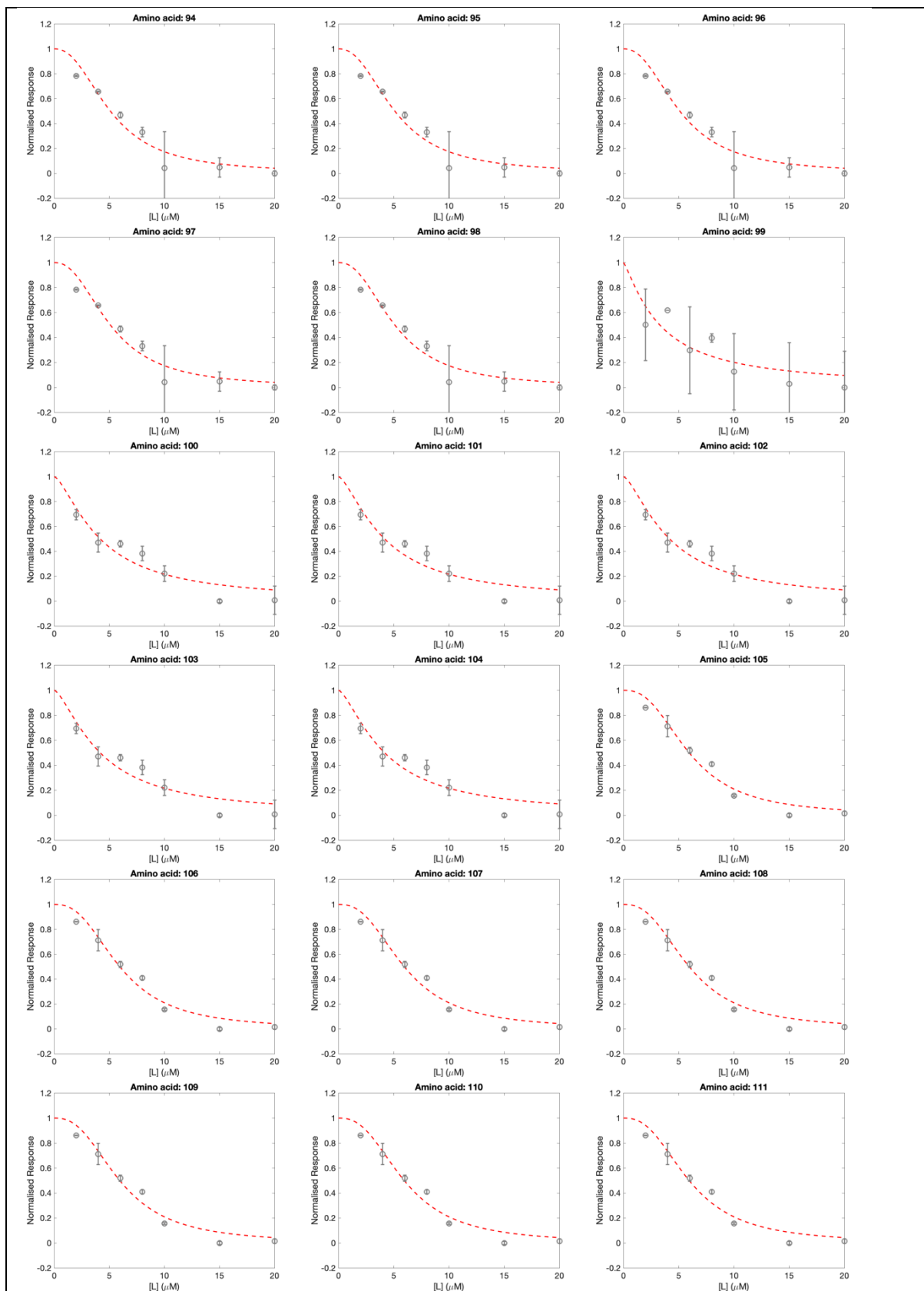


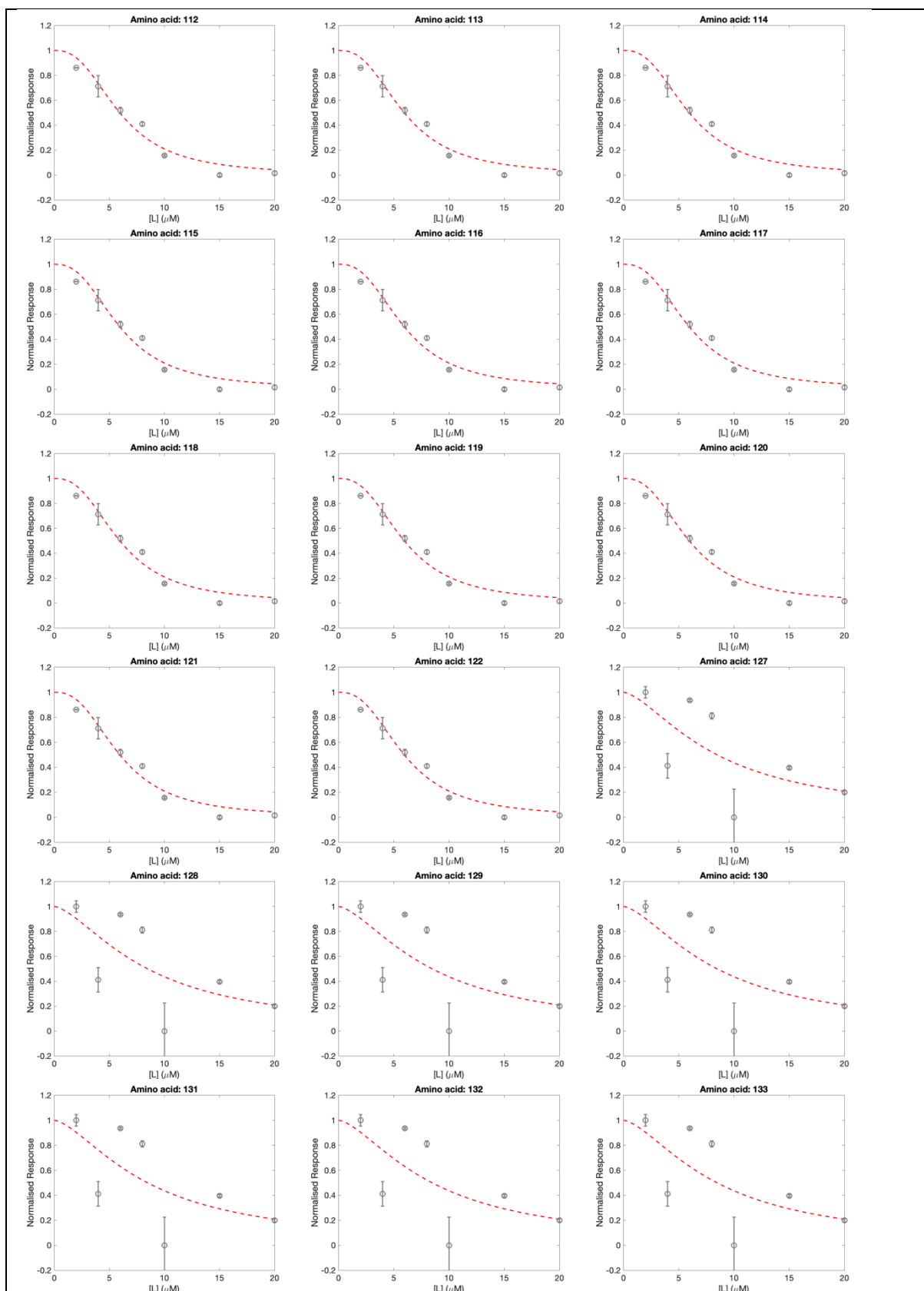


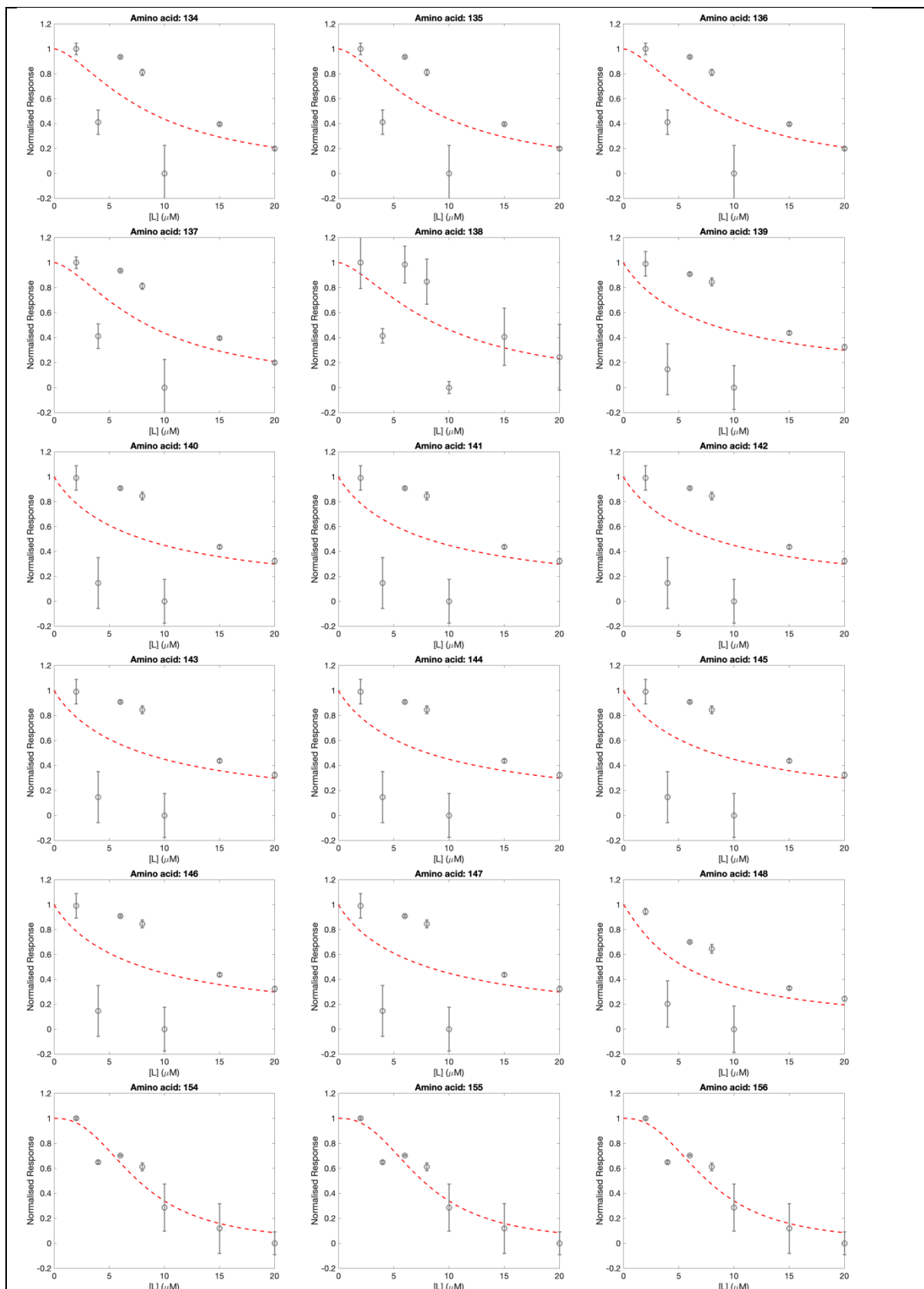


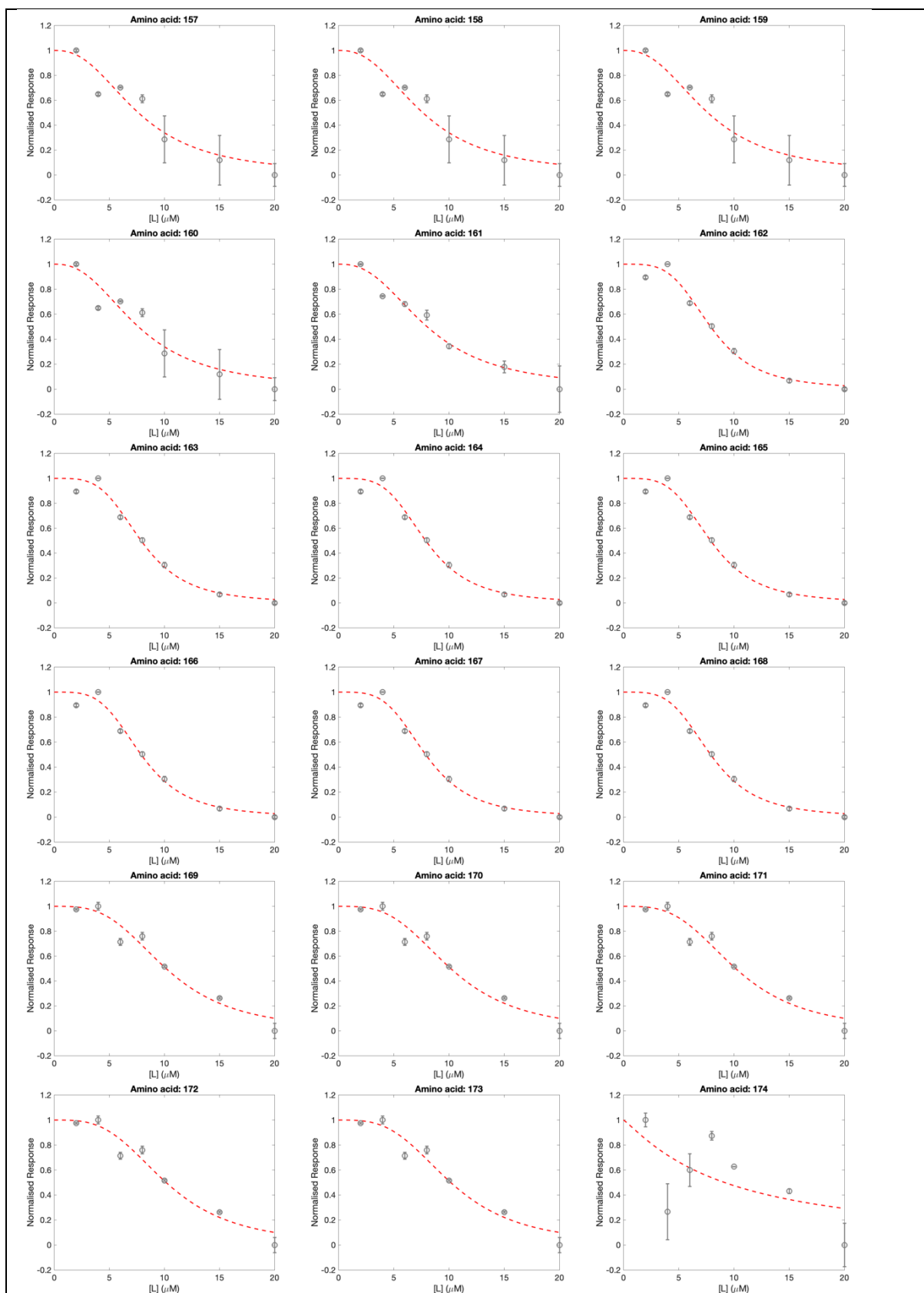


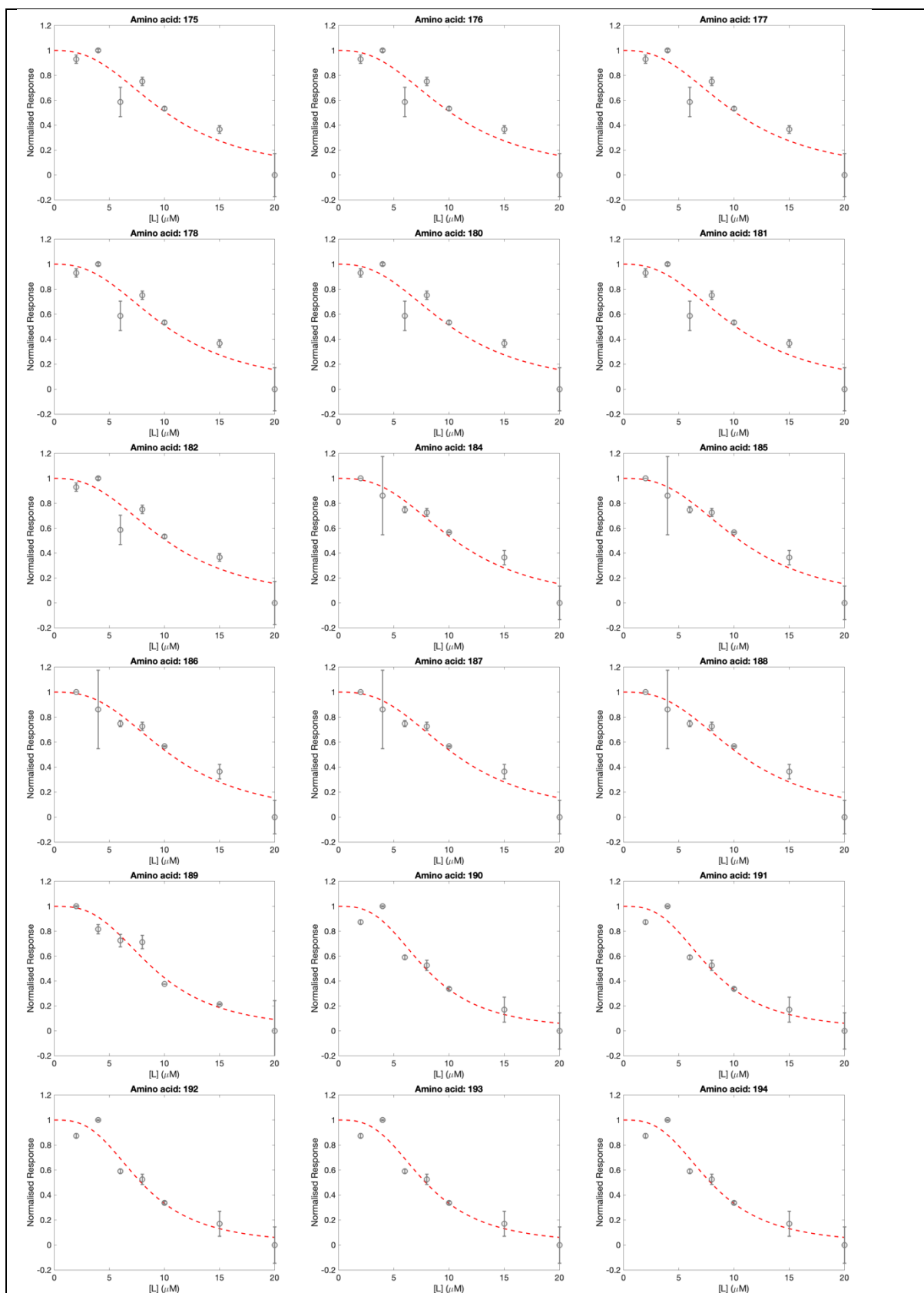


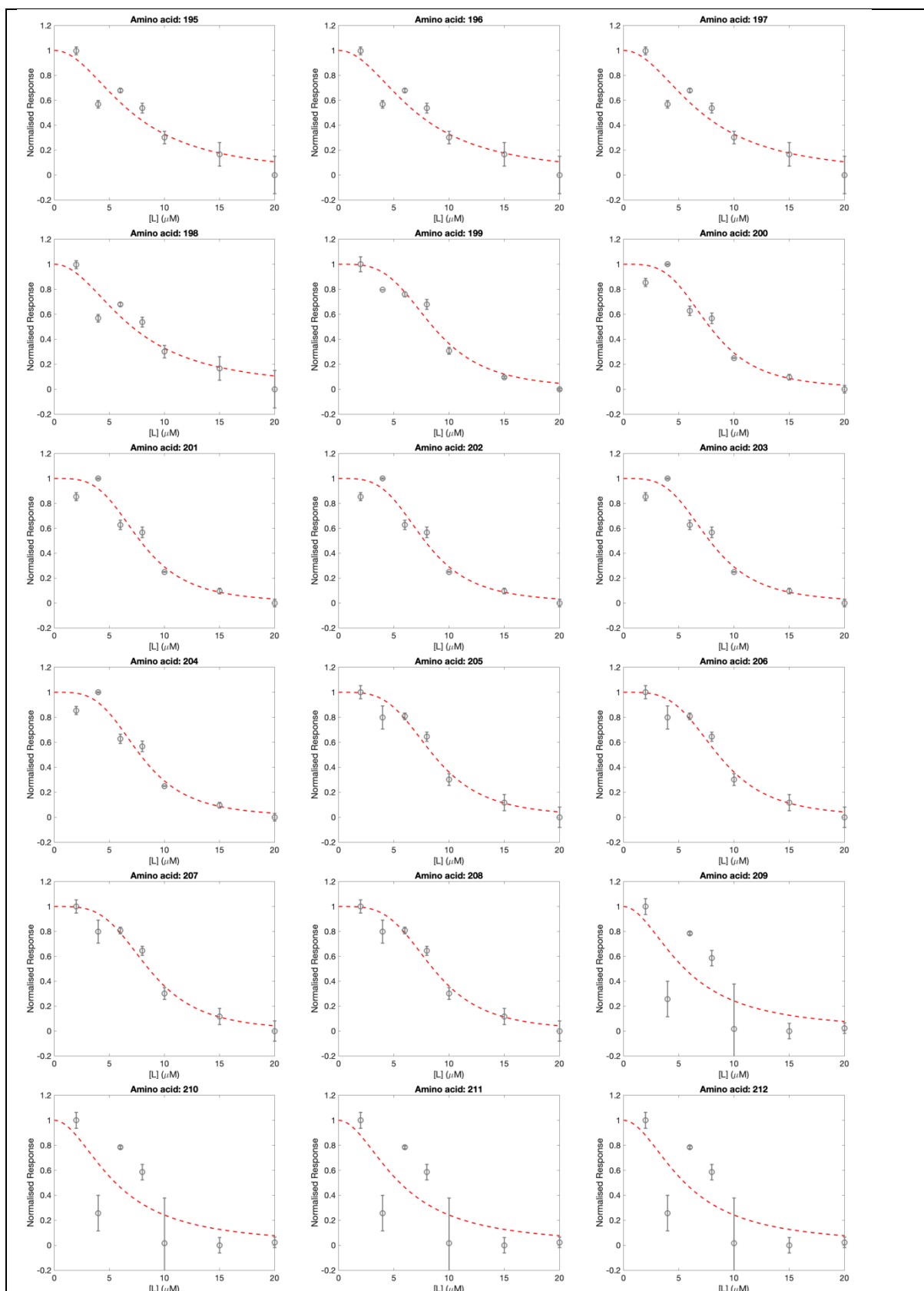


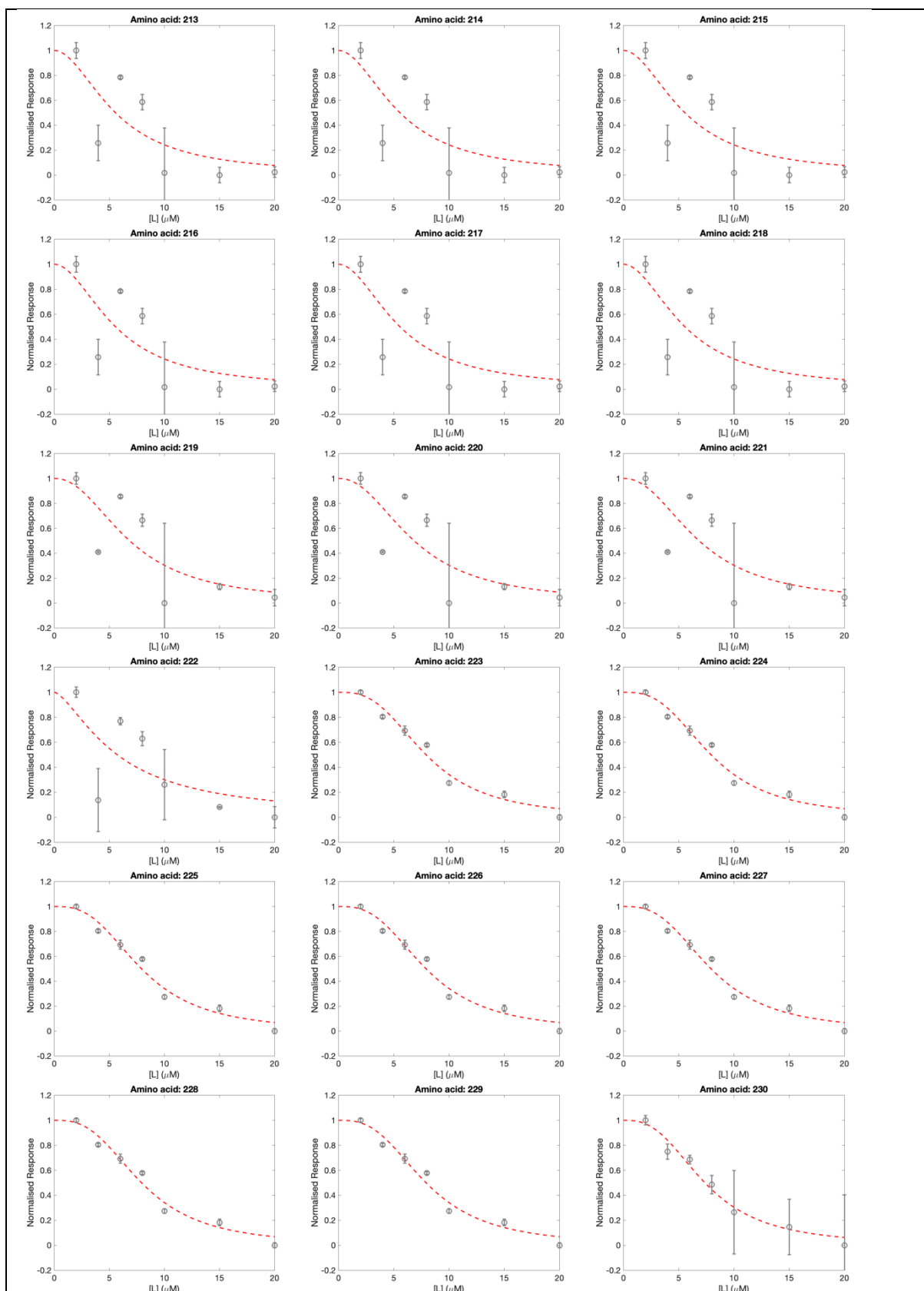


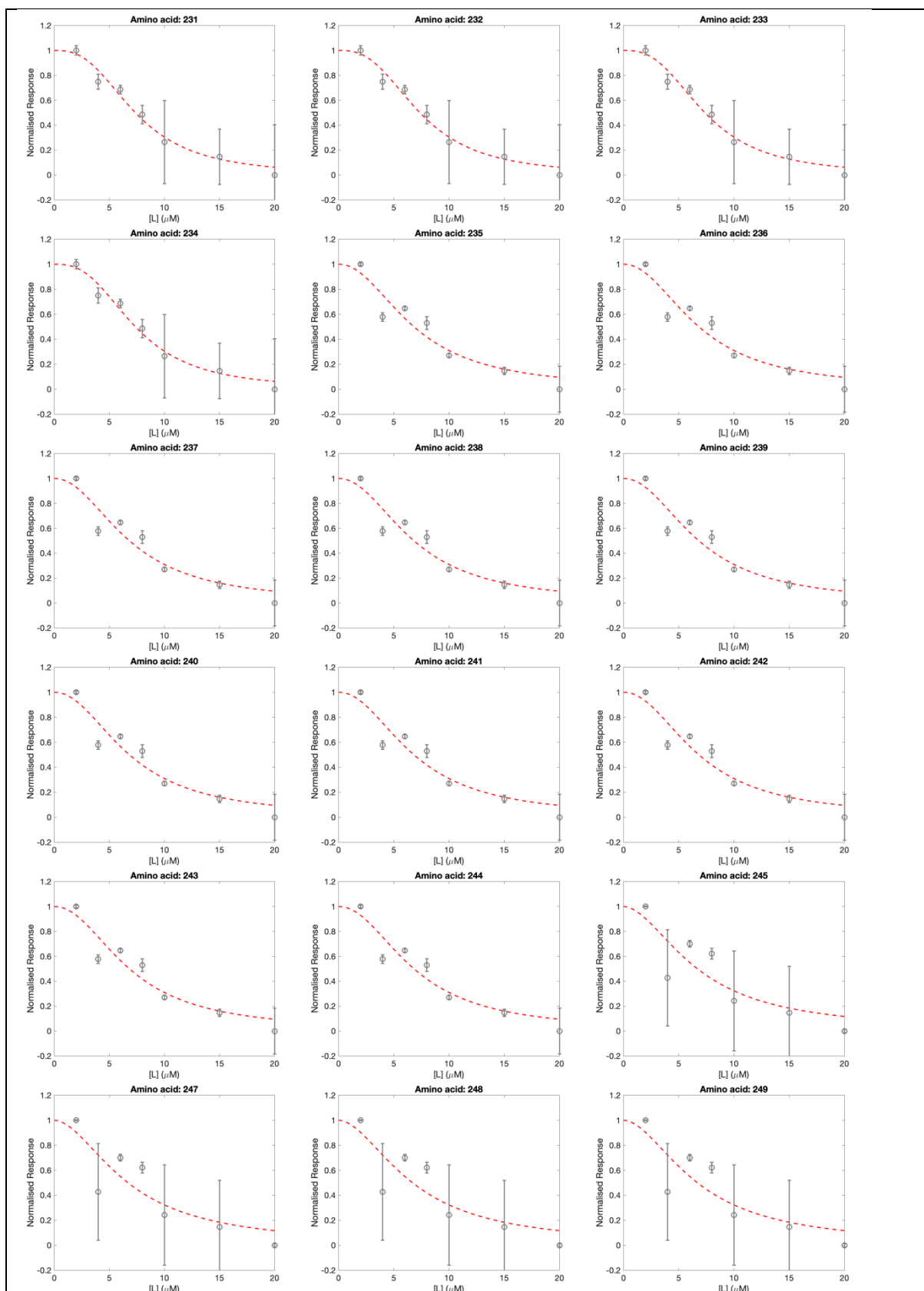


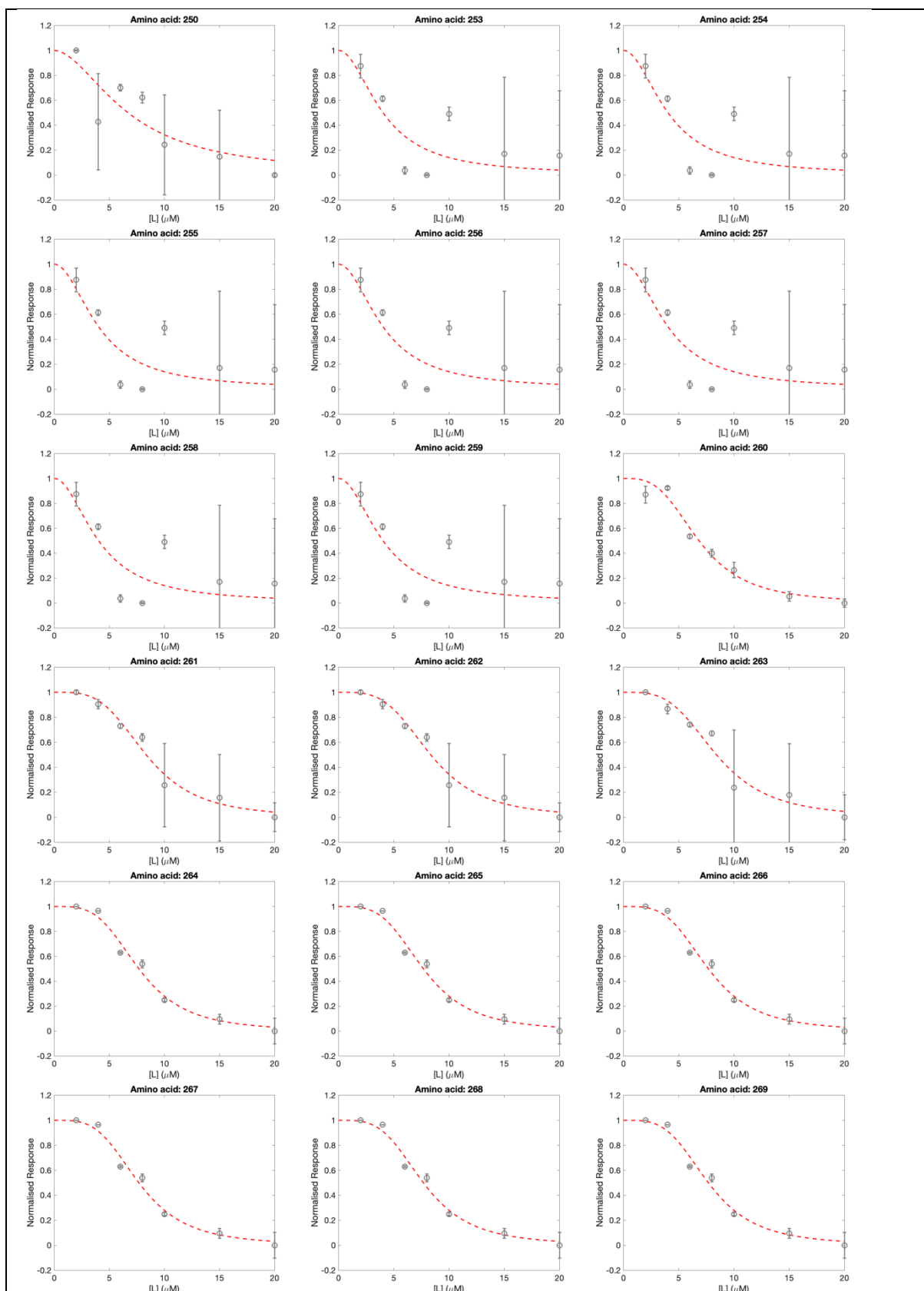


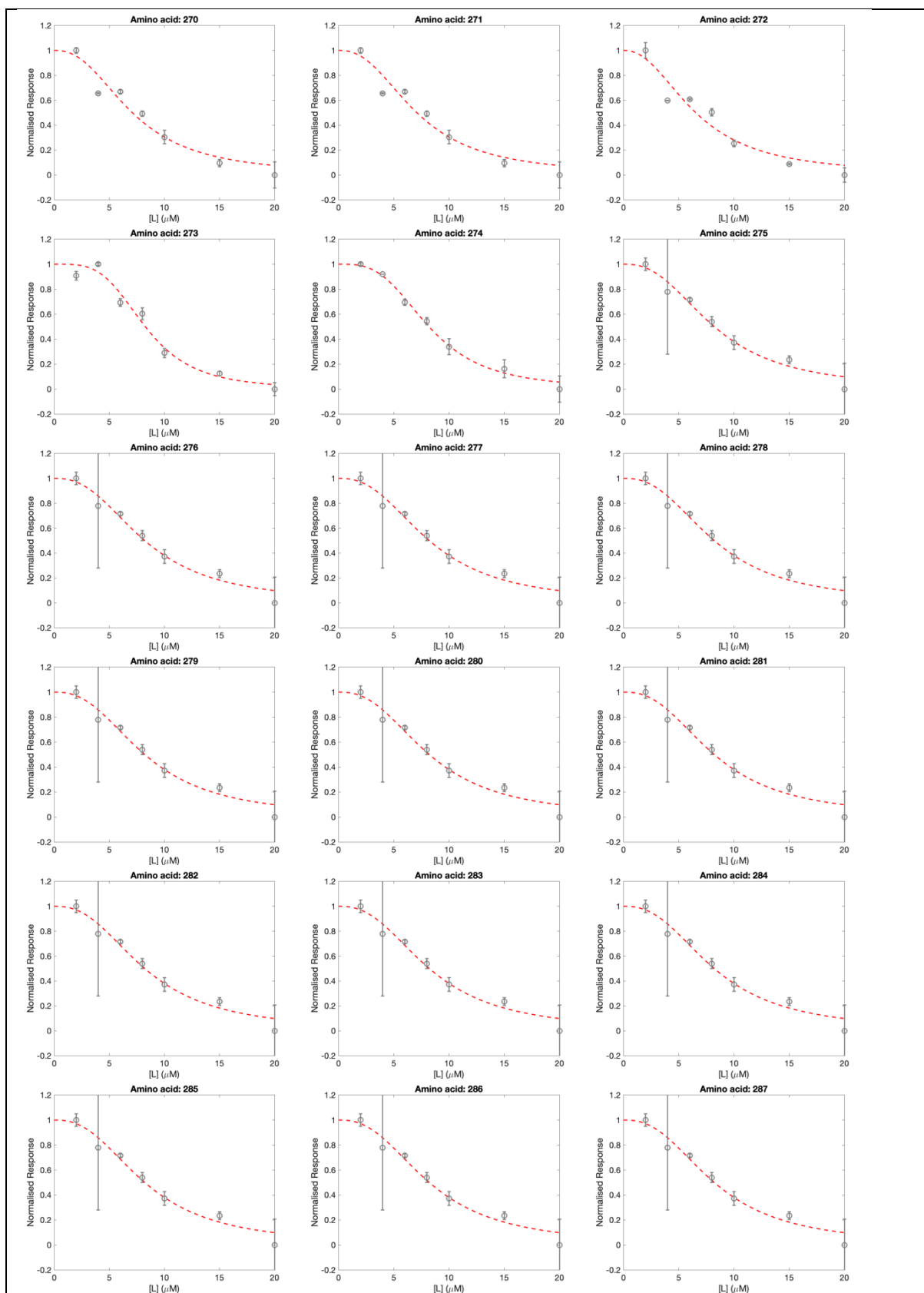


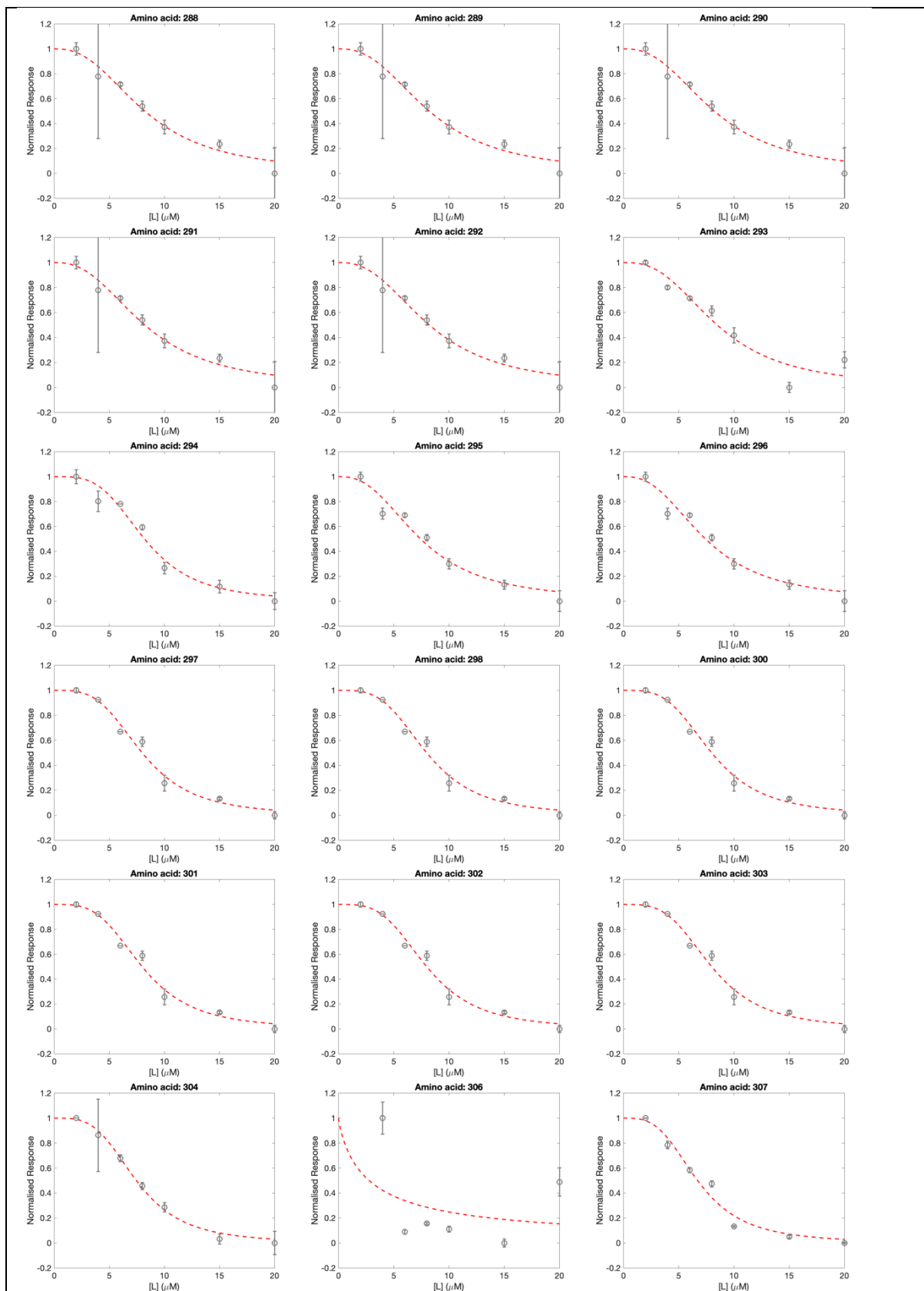


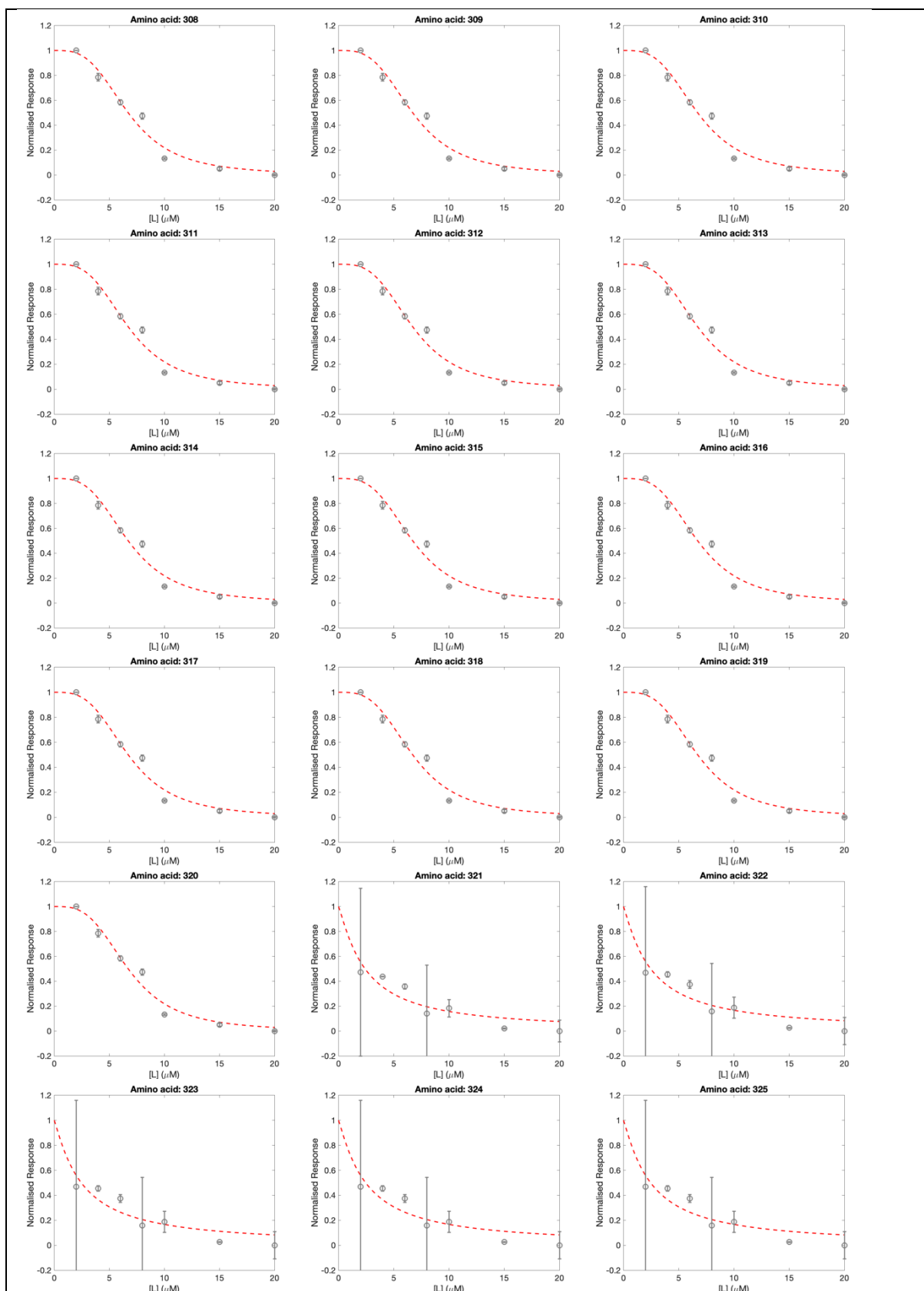


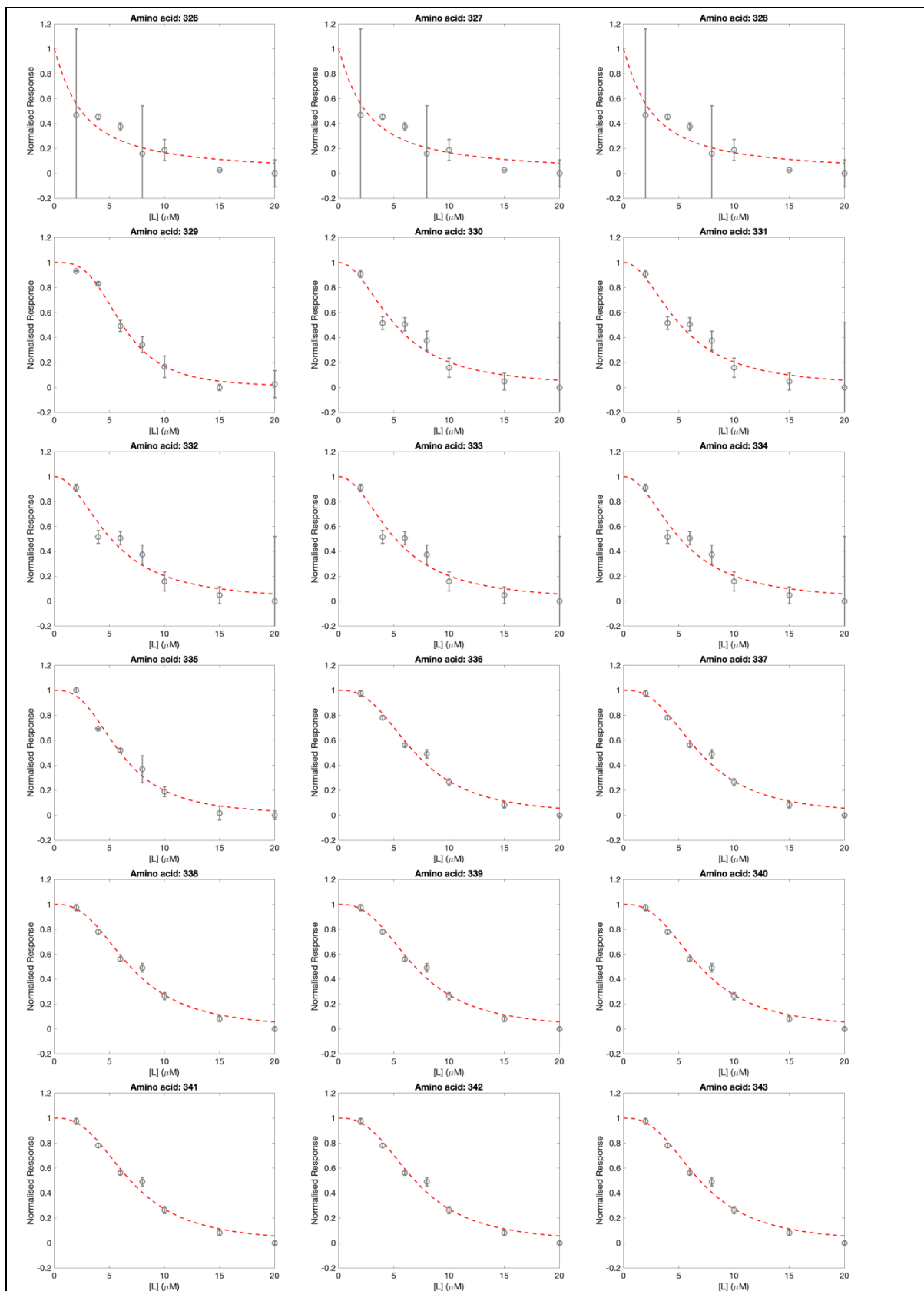


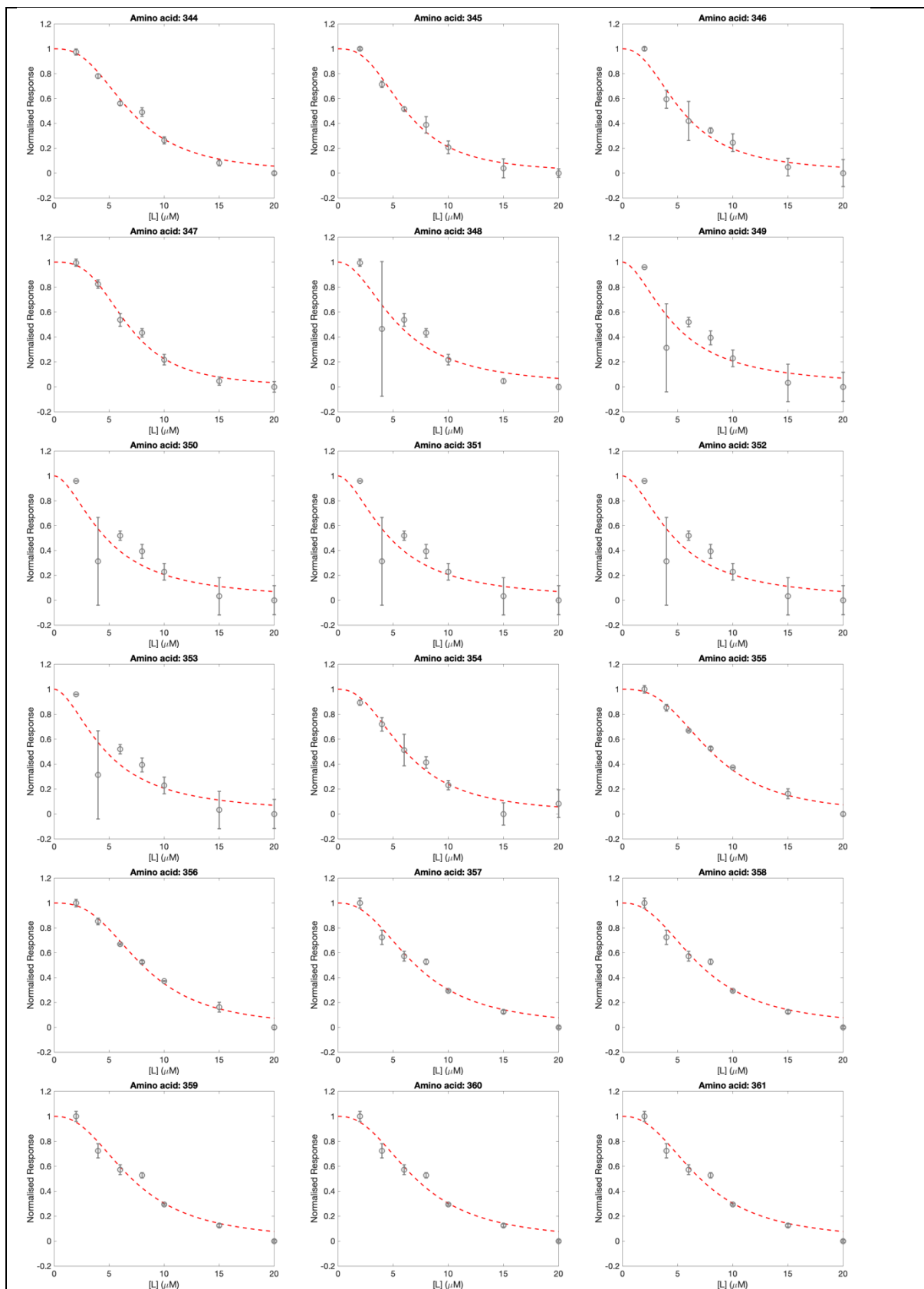


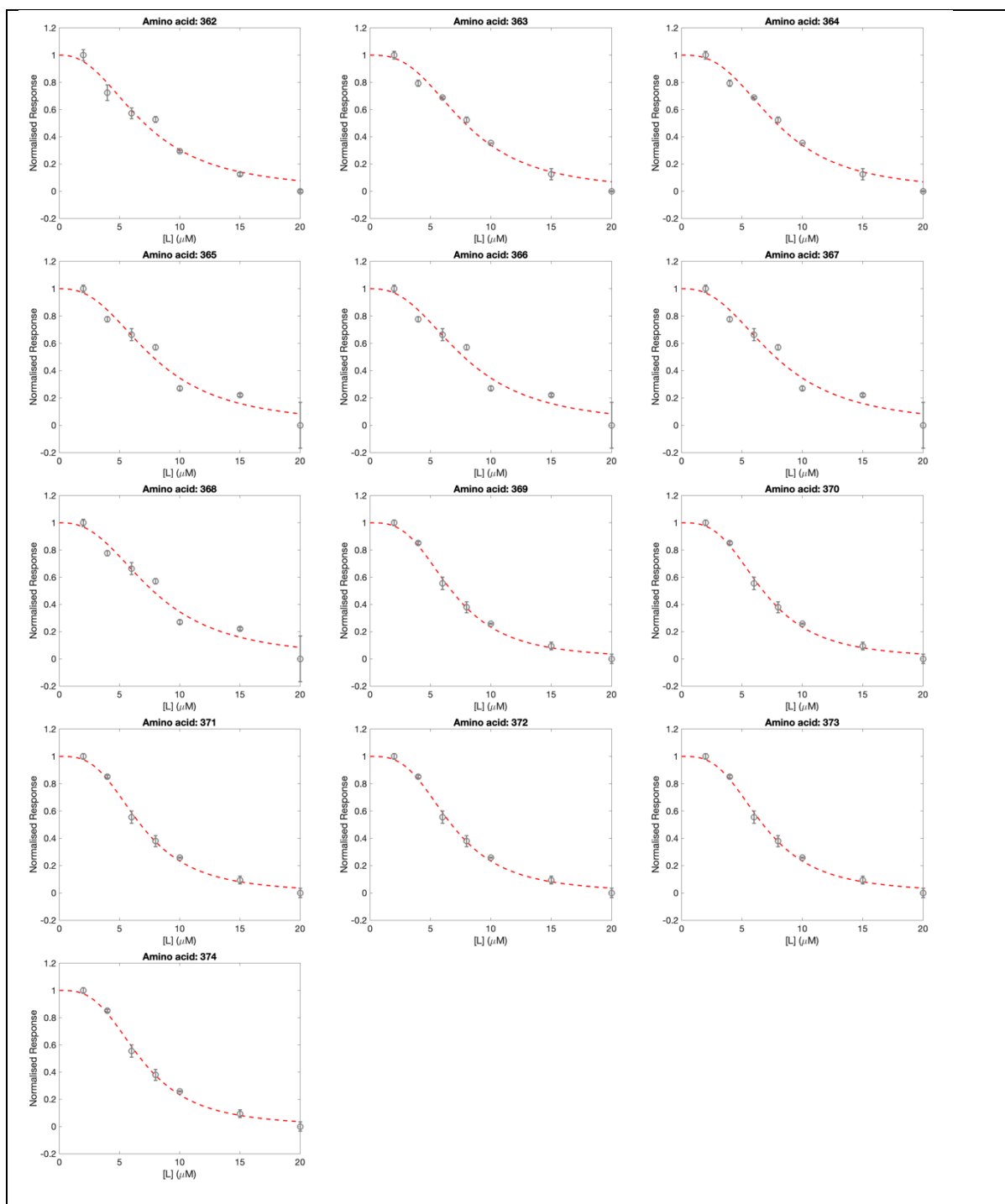






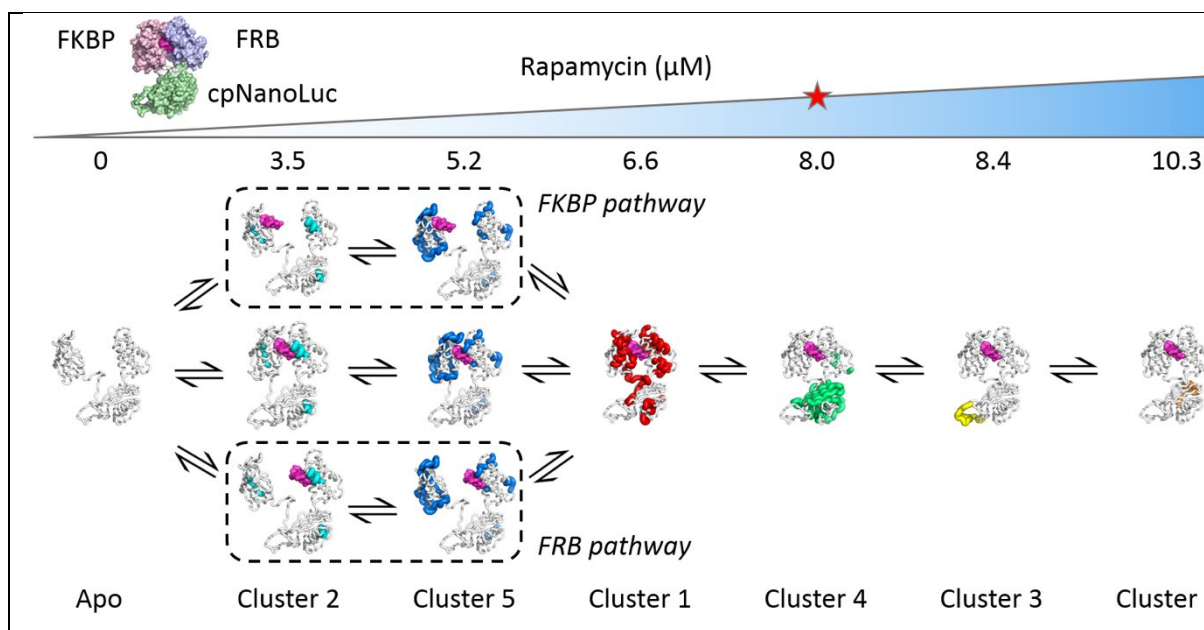






Supplementary figure 9. Normalized relative deuterium incorporation curves as a function of rapamycin concentration for all of the FKBP-cpNanoluc-FRB pepsin digest fragments (peptides) identified in supplementary figure 6 under conditions stated in Table S2. HDX measurements for FKBP-cpNanoluc-FRB peptides at different deuterium labelling times across three orders of magnitude (0, 30, 300 and 3000 s) were summed and linearly weighted by maximal uptake using the heatmap function in DynamX (Waters) to obtain total deuterium uptake resolved per amino acid. These were then normalized per amino acid to the maximum uptake observed over the rapamycin titration. Nonlinear regression to

a normalized Hill dose-response curve (SI equation 2) was done with lsqcurvefit() in Matlab (Mathworks). Data (open circles) were the mean of all assigned charge states for $n=2$ independent experiments with different protein stocks, summed over three time points. Error bars (± 1 s.d.). Fit to Hill equation (red dashed line).



Supplementary figure 10. Clusters of amino acids with rapamycin dose-response, as determined by hydrogen-exchange, mapped onto structural models of FKBP-cpNanoLuc-FRB to reveal the structural equilibrium pathway of activation. Rapamycin (purple) binds to the FKBP (pink) and FRB (blue) domains and stabilizes a 'closed' form of the central cpNanoLuc (green) domain in which the β -10 strand has intercalated into the β -sheet. The centroid of each cluster has its midpoint at the given concentrations. Parallel pathways exist for ligand binding, though the central pathway (simultaneous FKBP/FRB binding to rapamycin) is improbable. Few structural changes beyond 1:1 stoichiometry of binding (8 μ M) where the biosensor is 96% bound to rapamycin.

Supplementary Table 1 Sequences of the open reading frames encoding recombinant proteins used in this study

Construct Name	Sequence
CaM-NanoLuc-FKBP	GSDNMVFTLEDFVGDWRQTAGYNLDQVLEQGGVSSLFQNLGVSVTPIQRIVLSGE NGLKIDIHVIIPYEGLSGDQMGQIEKIFKVVPVDDHHFKVILHYGTLVIDGVTNMI

(Rapmycin/FK506 sensor component 1)	DYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLINPDGSLLFRVTINGSGGTEEQIA EFKEAFSLFDKDGDTITTKELGTVMRSLGQNPTAEALQDMINEVDADGNGTIDFP EFLTMMARKMKDSTDSEEEIREAFRVFDKDGNGYISAAELRHVMTNLGEKLTDEEV DEMIREADIDGDGQVNYEEFVQMMTAGGSGGVTGWRLCERILAGGSGSGGGVQ VETISPGDGRTPFKRGQTCVVHYTGMLEDGKKFDSSRDNRNPKFKMLGKQEVIRG WEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLEKLAAALEH HHHHH
FRB-CaM-BP (Rapmycin sensor component 2)	AHHHHHHSSGTRVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMME RGPQTLKETSFNQAYGRDLMEAEQWCRKYMKSGNVKDLTQAWDLYYHVFRISG GSGSGSGSGSGSGGKRRWKKNFIAVASASA
Calcineurin A- Calcineurin B- CaM-BP (FK506 sensor component 2)	HHHHHHSSGTSEPKAIDPKLSTTDRVVKAVFPFPPSHRLTAKEVFDNDGKPRVDILKA HLMKEGRLEESVALRIITEGASILRQEKNLDDIDAPVTCGDIHGQFFDLMKLFEVGG SPANTRYLFLGDYVDRGYFSIECVLYLWALKILYPKTLFLLRGNHECRHLEYFTFKQE CKIKYSERVYDACMDAFDCLPLAALMNQQFLCVHGGLSPEINTLDDIRKLDRFKEPP AYGPMCDILWSDPLEDFGNEKTQEHFTHNTVRGCSYFYSYPAVCEFLQHNNLSILR AHEAQDAGYRMYRKSQTTGFPSLITIFSAPNYLDVYNNKAAVLKYENNVMNIRQFN CSPHPYWLPNFMDVFTWSLFPVGEKVTEMLVNLNICSDDDELGSEEDGSGSGSGG GNEASYPLEMCSHFDADAIEKRLGKRFKKLDLNSGSLSVEEFMSLPQLQNPVQVRV IDIFDTDGNGEVDKFIEGVSQFSVKGDKEQKLRFARIYDMDKDGYISNGELFQVL KMMVGNNLKDTQLQQIVDKTIINADKDGGRISFEFCVVGGLDIHKKMVVDVG GSGSGSGSGSGSGGKRRWKKNFIAVASASA
FKBP-cpNanoLuc-FRB (Rapmycin sensor)	DHHHHHHGVQVETISPGDGRTPFKRGQTCVVHYTGMLEDGKKFDSSRDNRNPKFKF MLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELL KLEGGSGSGSGGVTGWRLCERILAGGSGSGSGSGSGSGSDNMVFTLEDFVGDW RQTAGYNLDQVLEQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDHVIIPYEGLSGD QMGQIEKIFKVVPVDDHHFKVILHYGTLVIDGVTNPMIDYFGRPYEGIAVFDGKKI TVTGTWNGNKIIDERLINPDGSLLFRVTINGSGSGSGGLWHEMWHEGLEEASRLY FGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEQWCRKYMKS GNVKDLTQAWDLYYHVFRIS
Calcineurin A- Calcineurin B - cpNanoLuc-FKBP (FK506 sensor)	DHHHHHHSSGTSEPKAIDPKLSTTDRVVKAVFPFPPSHRLTAKEVFDNDGKPRVDILKAHL MKEGRLEESVALRIITEGASILRQEKNLDDIDAPVTCGDIHGQFFDLMKLFEVGGSPANT RYLFLGDYVDRGYFSIECVLYLWALKILYPKTLFLLRGNHECRHLEYFTFKQECKIKYSERV YDACMDAFDCLPLAALMNQQFLCVHGGLSPEINTLDDIRKLDRFKEPPAYGPMCDILW SDPLEDFGNEKTQEHFTHNTVRGCSYFYSYPAVCEFLQHNNLSILRAHEAQDAGYRMY RKSQTTGFPSLITIFSAPNYLDVYNNKAAVLKYENNVMNIRQFNCSPHPYWLPNFMDVF TWSLFPVGEKVTEMLVNLNICSDDDELGSEEDGSGSGSGGGNEASYPLEMCSHFDADAIE KRLGKRFKKLDLNSGSLSVEEFMSLPQLQNPVQVRVIDIFDTDGNGEVDKFIEGVS QFSVKGDKEQKLRFARIYDMDKDGYISNGELFQVLKMMVGNNLKDTQLQQIVDKTII NADKDGGRISFEFCVVGGLDIHKKMVVDVGSGSGSGSGGVTGWRLCERILAGGSG SGSGSGSGSGSDNMVFTLEDFVGDWRQTAGYNLDQVLEQGGVSSLFQNLGVSVT PIQRIVLSGENGLKIDHVIIPYEGLSGDQMGQIEKIFKVVPVDDHHFKVILHYGTLVIDG VTPNMIDYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLINPDGSLLFRVTINGSGSSG SGGKVQVETISPGDGRTPFKRGQTCVVHYTGMLEDGKKFDSSRDNRNPKFKMLGKQE VIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLE
VHH1 -cpNanoLuc- VHH2 (amylase sensor)	DHHHHHHDDTVSEAPSCVTLYQSWRYSQADNGCAETVTVKVYEDDTEGLCYAV APGQITTVGDGYIGSHGHARYLARCLGGSGSGSGVTGWRLCERILAGGSGSGSGS GGSGSGSDNMVFTLEDFVGDWRQTAGYNLDQVLEQGGVSSLFQNLGVSVTPIQ RIVLSGENGLKIDHVIIPYEGLSGDQMGQIEKIFKVVPVDDHHFKVILHYGTLVIDG VTPNMIDYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLINPDGSLLFRVTINGSG

	SGSGGQVQLVESGGGTVPAGGSLRLSCAASGNTLCTYDMSWYRRAPGKGRDFVS GIDNDGTTTTYVDSVAGRFTISQGNAKNTAYLQMDSLKPDdTAMYYCKPSLRYGLPG CPIIPWGQGTQVTVSS
VHH1 -cpNanoLuc- VHH2 (HSA sensor)	QVQLQESGGGLVQAGGSLRLSCAASGYISDAYYMGWYRQAPGKEREFVATITHGT NTYYADSVKGRFTISRDNNAKNTVYLQMNSLKPEDTAVYYCAVLETRSYSFYWGQG TQVTVSSGGSGSGSSDSGSGSGSDGSSGSGSSGSDSSGSGDGSGGGVTGWRLCERIL AGGSGSGSGSGSGSGSGSDNMVFTLEDVFGDWRQTAGYNLDQVLEQGGVSSLF QNLGVSVTPIQRIVLSGENGLKIDIHVIIPYEGLSGDQMGQIEKIFKVVPVDDHHFK VILHYGTLVIDGVTNPMIDYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLINPDG SLLFRVTINGGGSGSGSSDSGSGSDGSGSDGSGSGSGSGSGGGPGGGAVDANSLA EAKVLANRELDKYGVSDFYKRLINKAKTVEGVEALKHLAALPKLAAALEHHHHHH
FKBP-cpNanoLuc— eGFP-FRB (Rapamycin sensor)	DHHHHHHGVQVETISPGDGRFTFPKRGQTCVVHYTGMLDGGKFDSSRDNRNPKPKF MLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIIPPHATLVFDVEL LKLEGGSGSGSGVTGWRLCERILAGGSGSGGQLVSKGEEELFTGVVPILEVELDGDV NGHKFSVSGEGEDATYGKLTCLKFICTTGKLPVWPVTLVTTLYGVQCFSRYPDH MKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAIEVKFEEDTLVNRIELKIDFKE DGNILGHKLEYNYNHNHYIMADKQKNGIKVNFKIRHNIEDGSQLADHYQQNT IGDGPVLLPDNHYLSTQSALS KDPNEKRDMVLEFVTAAGITLGMDELYKGGGS GGSDNMVFTLEDVFGDWRQTAGYNLDQVLEQGGVSSLFQNLGVSVTPIQRIVLSG ENGLKIDIHVIIPYEGLSGDQMGQIEKIFKVVPVDDHHFKVILHYGTLVIDGVT PNMIDYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLINPDGSLLFRVTINGSG SGSGGGLWHEMWHEGLEEASRLYFGERNVKGMEFEVLEPLHAMMERGPQTLKETSF NQAYGRDLMEAEQEWCRKYMKSGNVKDLTQAWDLYYHVFRIS
FKBP-cpLumiLuc-FRB (Rapamycin sensor)	DHHHHHHGVQVETISPGDGRFTFPKRGQTCVVHYTGMLDGGKFDSSRDNRNPKPKF MLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIIPPHATLVFDVEL LKLEGGSGSGSGVTGWRLHERILAGGSGSGSGSGSGSGSGSDNMVFTLGDVFGDW RQTAGYNQAQVLEQGGTSLFQNLGVSVTPIQRIVLSGENGLKIDIHVIIPYEGL SCDQMAQIEKIFKVVPVDDHHFKAILHYGTLVIDGVTNPMIDYFGQPYEGIAKF DGKKITVTGTLWNGNTIIDERLINPDGSLLFRVTINGSGSGSGGLWHEMWHEGLE EASRLYFGERNVKGMEFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEQEWCRK YMKSGNVKDLTQAWDLYYHVFRIS
FKBP-cpLumiLuc- FRB-mScarlet (Rapamycin sensor)	DHHHHHHGVQVETISPGDGRFTFPKRGQTCVVHYTGMLDGGKFDSSRDNRNPKPKF MLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIIPPHATLVFDVEL LKLEGGSGSGSGVTGWRLHERILAGGSGSGSGSGSGSGSGSDNMVFTLGDVFGDW RQTAGYNQAQVLEQGGTSLFQNLGVSVTPIQRIVLSGENGLKIDIHVIIPYEGL SCDQMAQIEKIFKVVPVDDHHFKAILHYGTLVIDGVTNPMIDYFGQPYEGIAKF DGKKITVTGTLWNGNTIIDERLINPDGSLLFRVTINGSGSGSGGLWHEMWHEGLE EASRLYFGERNVKGMEFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEQEWCRK YMKSGNVKDLTQAWDLYYHVFRISGGMVSKEAVIKEFMRFKVHMEGSMNGHEF EIEGEGEGRPYEGTQTAKLKVTGGPLPFSWDILSPQFMYGSRAFIKHPADIPDY YKQSFPEGFKWERVMNFEDGGAVTQDTSLEDGTLIYKVKLRGTNFPDPGPVMQ KKTMGWEASTERLYPEDGVLKGDIKMALRLKDGGRYLADFKTITYKAKKPVQMPGA YNVDRKLDITSHNEDYTVEQYERSEGRHSTG
FKBP-cpLumiLuc- mScarlet-FRB (Rapamycin sensor)	DHHHHHHGVQVETISPGDGRFTFPKRGQTCVVHYTGMLDGGKFDSSRDNRNPKPKF MLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIIPPHATLVFDVEL LKLEGGSGSGSGVTGWRLHERILAGGSGSGSGMVSKEAVIKEFMRFKVHMEGSM NGHEFEIEGEGEGRPYEGTQTAKLKVTGGPLPFSWDILSPQFMYGSRAFIKHPA DIPDYKQSFPEGFKWERVMNFEDGGAVTQDTSLEDGTLIYKVKLRGTNFPDP GPVMQKKTMGWEASTERLYPEDGVLKGDIKMALRLKDGGRYLADFKTITYKAKKPV QMPGAYNVDRKLDITSHNEDYTVEQYERSEGRHSTGSGSGSGSGSDNMVFTLGD FVGDWRQTAGYNQAQVLEQGGTSLFQNLGVSVTPIQRIVLSGENGLKIDIHVIIP YEGLSCDQMAQIEKIFKVVPVDDHHFKAILHYGTLVIDGVTNPMIDYFGQPYE GIAKFDGKKITVTGTLWNGNTIIDERLINPDGSLLFRVTINGSGSGSGGLWHEMW HEGLEEASRLYFGERNVKGMEFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQ EWCRKYMKSGNVKDLTQAWDLYYHVFRIS

