Electronic Supplementary Material (ESI) for Chemical Communications.


# Supplementary Information

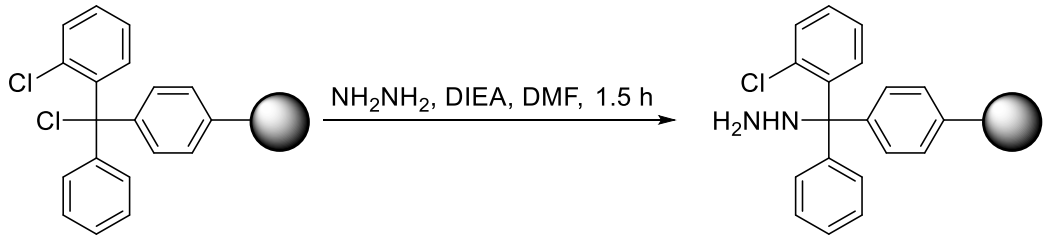
**Contents:**

## Materials

All solvents, chemicals, and reagents were purchased from commercial sources and used without further purification. Fmoc-α-*L*-amino acids and resins for solid-phase peptide synthesis were purchased from Aapptec, Bachem or Iris Biotech, diisopropylethylamine (DIEA) and piperidine from Sigma-Aldrich, and trifluoroacetic acid (TFA, Biograde) from Halocarbon. Fmoc-protected α-methylated *L*-amino acids were purchased from OKeanos Tech. Co. Ltd. (China). The coupling reagents O-(7-azabenzotriazol-1-yl)-N,N,N',N′-tetramethyluronium hexafluorophosphate (HATU) and O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) were obtained from Aapptec. All other chemicals were purchased from Sigma-Aldrich. Reactions involving compounds that are sensitive to oxidation or hydrolysis were performed under an argon atmosphere (thioesterification, ligation-desulfurization). For reactions that were heated or cooled the temperature of the bath is given.

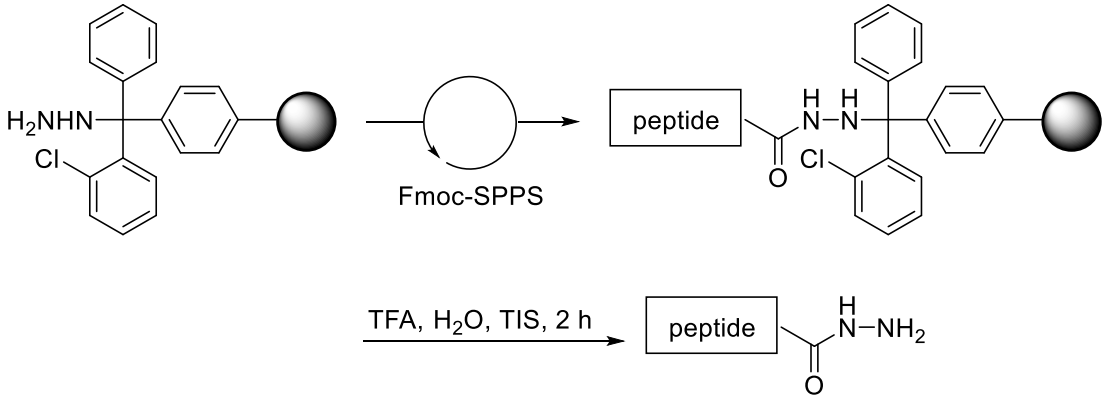
## Peptide synthesis

Synthesis of Cl-Trt-$NHNH_2$ resin[1]:




2-Chlorotrityl chloride resin (4.0 g, 1.6 mmol/g active groups) was swollen in DMF (12 mL) for 15 min. The suspension was cooled to 0 °C and a mixture of DIEA/hydrazine (64% in water)/DMF (2.66 mL/932 µL/8 mL) was added. The reaction mixture was warmed to room temperature and allowed to stir for 1.5 h. The reaction was quenched by the addition of MeOH (200 µL) and stirred for another 20 min. The obtained yellow resin was washed by DMF, $H_2O$, DMF, MeOH, $Et_2O$ and dried in vacuo. The product was obtained as yellow granules (3.76 g, resin load 0.4 mmol/g). In the reaction described above, only 30% of the active Cl has been converted to hydrazine due to concurrent hydrolysis.[2]

Fmoc-SPPS of peptides and peptide-$^{\alpha}$hydrazides[3]:




The synthesis of peptides was performed either manually or with the use of a robot (Syro I from Biotage). Initially, the resin was swollen for 20 min, thereafter the synthesis was accomplished by iterative coupling and deprotection reactions. For the synthesis of peptide-$^{\alpha}$hydrazides a 2-chlorotrityl hydrazide resin was used, whereas other peptides were synthesized on a Wang resin (preloaded with Fmoc-*L*-Asn(Trt)). Most of the amino acids were coupled according to the standard coupling procedure. However, in some cases different approaches were required to avoid side reactions. For Fmoc-α-methyl-Asp(O*t*Bu) and the subsequent amino acid in the respective sequence, a base-free coupling procedure was chosen.

In order to facilitate solid-phase synthesis of peptide-$^{\alpha}$hydrazide fragments of ACTR and NCBD proteins pseudoproline building blocks were incorporated into the respective sequences during SPPS. In the synthesis of the [1040-1069] ACTR fragment, a Fmoc-Leu-Ser(ψ$^{Me,Me}$pro)-OH building block was introduced at the positions 1056-1057 of the sequence. In the synthesis of the [2065-2098] NCBD fragment, two Fmoc-Lys(Boc)-Ser(ψ$^{Me,Me}$pro)-OH building blocks were introduced at the positions 2075-2076 and 2090-2091.

Standard coupling procedure (0.2 mmol scale): The respective amino acid (0.8 mmol, 0.4 M, 4 eq. relative to resin loading) was dissolved in a solution of HBTU (303 mg, 0.8 mmol, 0.4 M, 4 eq.) in DMF. Neat DIEA (200 µL, 6 eq.) was added and the solution was mixed thoroughly. After 2 min of pre-activation, the resulting solution was poured onto the resin. The suspension was stirred periodically and left reacting for 30 min. The solution was removed from the resin by vacuum filtration and the resin was washed with DMF four times. The completeness of the coupling reaction was verified by Kaiser test or chloranil test. In case of a positive test, the coupling reaction was repeated or the remaining free amino groups were capped by acetylation.

Base-free coupling procedure for Fmoc-α-methyl-Asp(O*t*Bu) and subsequent amino acid (0.1 mmol scale): The respective amino acid (0.3 mmol, 0.36 M, 3 eq. relative to resin loading) and HOBt (40 mg, 0.3 mmol, 0.36 M, 3 eq.) were dissolved in DMF. DIC (46 µL, 0.3 mmol, 0.36 M) was added to the solution and the solution was well stirred. After 3 min of pre-activation the resulting solution was poured onto the resin. The suspension was stirred periodically and left reacting for 30 min. The solution was removed from the resin by vacuum filtration and the resin was washed with DMF four times. The completeness of the coupling reaction was verified by Kaiser test or chloranil test. In case of a positive test, the coupling reaction was repeated or the remaining free amino groups were capped by acetylation.

Deprotection: To the resin 20% (v/v) piperidine solution in DMF was added. The suspension was stirred periodically and kept reacting for 10 min. The piperidine solution was removed by vacuum filtration and the resin was washed four times with DMF.

Acetylation: A mixture of DMF/DIEA/$Ac_2O$ (1.4 mL/200 µL/200 µL for 0.1 mmol scale synthesis) was added to the resin. The mixture was stirred periodically and left reacting for 6 min. The liquid was removed by vacuum filtration and the resin was washed several times with DMF.

Machine-assisted peptide synthesis was performed on a Syro I (Biotage) synthesizer. Usually, syntheses were performed on a 0.1 mmol scale. The coupling reactions were performed by adding amino acids dissolved in DMF (1.0 mL, 0.5 M), the coupling reagent HATU in DMF (1.0 mL, 0.5 M) and the activator (2.5 M solution of DIEA in *N*-methyl-2-pyrrolidone) to the respective resin and shaking the suspension for 40 min. To deprotect Fmoc-group the beads were shaken for 3 min in 40% (v/v) piperidine solution in DMF (1.2 mL) and a second time for 12 min in 20% (v/v) piperidine solution in DMF (1.2 mL).

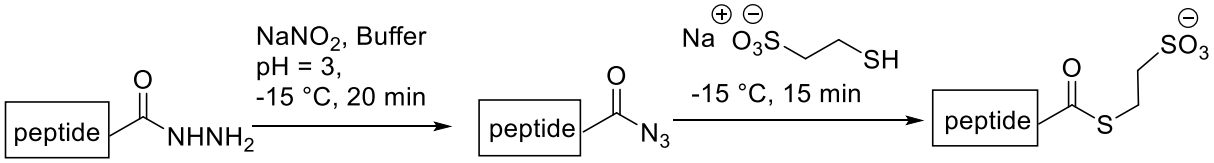
Monitoring of peptide synthesis: *Kaiser test*: Resin beads were taken and washed with ethanol two times. To the beads 100 µL of 0.2 mM KCN in $H_2O$/pyridine (1:50) and 100 µL of 0.3 M ninhydrine in ethanol were added. The suspension was heated for 3 min at 115 °C. A blue color indicated the presence of free primary amino groups.

*Chloranil test:* Resin beads were transferred to a glass tube and 50 µL of a 0.35 mM solution of acetaldehyde in DMF and 50 µL of a 80 µM solution of *p*-chloranil in DMF were added. The mixture was kept at room temperature for 15 min. A greenish blue color of the beads indicated the presence of free amino groups.

Cleavage and workup: After the final Fmoc-deprotection the resin was washed with DMF (2 x) and DCM (4 x) and was dried under an airflow for 15 min. The dry resin was transferred to a round bottom flask and a mixture of water/triisopropylsilane/TFA (5/5/95, 1 mL of cocktail for 100 mg resin) was added. The suspension was stirred for 2 h and then poured into chilled (-20 °C) diethylether (2 mL of reaction mixture were poured into 40 mL of $Et_2O$). The resulting suspension was centrifuged (4500 rpm, 3 min) and kept for 1 h at -20 °C, whereupon it was centrifuged again. The diethylether part was decanted and the pellet was dried by a gentle air stream for several minutes. Afterwards, it was dissolved in a mixture of water and acetonitrile (1:1) and filtered to remove the resin. The final filtrate was frozen in liquid nitrogen and lyophilized.

**Peptide ligations**[4]

Activation of peptide-$^{\alpha}$hydrazides and thioesterification:




The following reaction steps were performed under an argon atmosphere. Only thoroughly degassed buffers were used. The crude peptide-$^{\alpha}$hydrazide was dissolved in buffer (200 mM $Na_2HPO_4$, 6 M Gn.HCl, pH 3) at 2-4 mM concentration. The temperature of the solution was adjusted to -15 °C and $NaNO_2$ (5 eq., dissolved in water, *c* 0.2 M) was added dropwise. The solution was stirred for 15 min. The peptide-$^{\alpha}$azides were detectable by LC-MS.

To the solution of peptide-$^{\alpha}$azide, a 0.2 M solution of sodium mercaptoethanesulfonate (MESNA, 50 eq.) in buffer (200 mM $Na_2HPO_4$, 6 M Gn·HCl, pH 7) was added dropwise. The solution was stirred at -15 °C for 15 min. Under such conditions the thioesterification was complete after a few minutes (judged by LC-MS). Subsequently, the reaction was allowed to warm up to room temperature and directly injected into a preparative HPLC. For the purification of the peptide-$^{\alpha}$thioesters, a Kinetex XB-C18 column (Phenomenex, paricle size 5 µm, pore size 100 Å, column dimensions 250 x 21.2 mm) was used. After lyophilization, the pure thioesters were obtained as a white powder (6-9% yield based on resin loading of 2-chlorotrityl hydrazide resin).

Ligation-desulfurization of peptides:

These reactions were usually performed on a 2-3 µmol (peptide-$^{\alpha}$thioester) scale. Separate solutions of both peptides (peptide-$^{\alpha}$thioester: *c* 10 mM, 1 eq.; Cys-peptide: *c* 15 mM, 1.5 eq.) in aqueous buffer (0.2 M $Na_2HPO_4$, 6 M Gn.HCl, pH 7) were prepared. The resulting solutions were mixed, vortexed and the pH was adjusted carefully to 6.9-7.0 with 1 M NaOH. The solution was purged with argon and stirred for 4-6 h at 40 °C. The reaction was monitored by analytical HPLC.

The subsequent desulfurization was performed in a one pot manner. TCEP was dissolved in aqueous buffer (0.2 M $Na_2HPO_4$, 6 M Gn.HCl, pH 7) at *c* 0.53 M. The pH was adjusted to 6.8-6.9 with 6 M NaOH. Subsequently, the solution was purged thoroughly with argon and added to the reaction mixture of the ligating peptides resulting in a TCEP concentration of 200 mM (increasing the reaction mixture volume by factor 1.7). Subsequently, the radical initiator VAA-044 (20 eq.) and MESNA (40 eq.) were added as solids. The solution was vortexed and stirred for 12 h at 40 °C. The final product was 2-fold diluted with $H_2O$/MeCN (9:1) and subjected to preparative HPLC purification. For the purification a Kinetex XB-C8 column (Phenomenex, paricle size 5 µm, pore size 100 Å, column dimensions 100 x 21.2 mm) was used. After lyophilization the product was obtained as a white powder (yields were in the range 39-55% based on peptide-$^{\alpha}$thioester).

**Table S1.** Characterization of synthesized proteins by mass-spectrometry:

| Protein | Measured mass [Da] | Calculated mass [Da] |
|---|---|---|
| wild type ACTR | 5099.9 | 5099.6 |
| [1047Aib]ACTR | 5113.8 | 5113.6 |
| [1050MeAsp]ACTR | 5114.6 | 5113.6 |
| [1061Aib]ACTR | 5113.8 | 5113.6 |
| [1071MeLeu]ACTR | 5113.3 | 5113.6 |
| [1076MeLeu]ACTR | 5113.8 | 5113.6 |
| [1083MeLeu]ACTR | 5113.8 | 5113.6 |
| [1085MePro]ACTR | 5113.7 | 5113.6 |
| [1047Aib;1071MeLeu]ACTR | 5127.1 | 5127.6 |
| [1047Aib;1076MeLeu]ACTR | 5127.9 | 5127.6 |
| [1047Aib;1083MeLeu]ACTR | 5128.7 | 5127.6 |
| [1050MeAsp;1083MeLeu]ACTR | 5127.9 | 5127.6 |
| [1061Aib;1076MeLeu]ACTR | 5128.7 | 5127.6 |
| [1061Aib;1083MeLeu]ACTR | 5128.3 | 5127.6 |
| NCBD | 5250.9 | 5250.1 |
| NCBD-GGGK(Biotin) | 5977.9 | 5977.4 |

## Analytical methods

Mass-spectrometry:

Mass spectra of proteins were recorded on a Thermo Scientific LTQ XL linear ion trap mass spectrometer. Samples of proteins were prepared in 1:1 (v/v) $H_2O$/MeCN (containing 0.1% formic acid) and injected directly into the system. The mass spectra were recorded in the positive ionization mode. Obtained raw data were deconvoluted using MagTran (ver. 1.03) software.

HPLC:

Analytical reversed-phase HPLC of all peptides and proteins was performed on either a Dionex Ultimate 3000 (Thermo Fisher) equipped with a UV detector, a column heater set to 40 °C and an autosampler, or a NexeraXR (Shimadzu) HPLC instrument equipped with a CBM-20A communication module, a SPD-M20A photodiode array detector, a column heater set to 40 °C, LC-20AD pumps and a SIL-20AC autosampler. Analyses of all proteins were performed on a Kinetex EVO C18 column (Phenomenex, particle size 2.6 µm, pore size 100 Å, dimensions 50 x 2.1 mm) or a Kinetex XB-C18 column (Phenomenex, particle size 2.6 µm, pore size 100 Å, dimensions 50 x 2.1 mm) at a flow rate of 1 mL/min and a gradient of 2-50% of eluent B (0.08% TFA in acetonitrile) in eluent A (0.1% TFA in $H_2O$) within 4 min.

Preparative reversed-phase HPLC was performed on a Shimadzu instrument equipped with a CBM-20A communication module, a SPD-M20A UV detector, a SIL-10AP autosampler and a FRC-10A fraction collector. Peptides were purified on a Kinetex XB-C18 column (Phenomenex, particle size 5 µm, pore size 100 Å, dimensions 250 x 21.2 mm) at a flow rate of 10 mL/min and a gradient of 15-50% of eluent B (0.08%TFA in acetonitrile) in eluent A (0.1% TFA in $H_2O$) within 35 min. ACTR and NCBD proteins were purified on a Kinetex C8 column (Phenomenex, particle size 5 µm, pore size 100 Å, dimensions 100 x 21.2 mm) at a flow rate of 10 mL/min and a gradient of 20-50% of eluent B (0.08%TFA in acetonitrile) in eluent A (0.1% TFA in $H_2O$) within 37 min.

NMR:

NMR-spectra of ACTR, NCBD and their complex were recorded on a Bruker Avance III (600 MHz). The $^1$H-NOESY spectra (mixing time 300 ms) were acquired with the spectral width set to 9615.385 Hz, where 32 transients of 2048 complex points per transient were obtained for each of the 512 $t_1$ increments. The relaxation time of NMR experiments was 2.5 s. Samples were prepared in an aqueous buffer

(20 mM $Na_2HPO_4$, 10% $D_2O$, 0.05 wt% $NaN_3$, pH 6.5). The water signal was suppressed by excitation sculpting. Only few signals were observed for the disordered individual proteins ACTR and NCBD, whereas for the ACTR/NCBD complex a spectrum rich in signals was obtained (Fig. S1).

Measurement of protein concentrations:

After synthesis and purification proteins were obtained as TFA salts. In order to obtain precise concentrations of the samples used in biophysical measurements (CD, ITC and SPR) the following procedure was applied: the nitrogen content of two ACTR variants and two NCBD analogues was determined by elemental analysis. This allowed to exactly calculating the mass portion of protein in the purified product (~80%). These analogues were used to calibrate the analytical HPLC system. The derived calibrations were used to determine the concentrations of other ACTR and NCBD samples.

Circular dichroism (CD):

CD spectra were recorded on a J-1500 (Jasco) spectrophotometer. All samples were prepared in quartz cuvettes with a thickness of 1 mm and a volume of 300 µL. For every measurement proteins were dissolved in a buffered solution (20 mM phosphate, pH 7.4) at a concentration range of 14 µM to 25 µM. In addition, for each protein CD spectra of samples containing 10% or 20% (v/v) 2-trifluoroethanol (TFE) were recorded. For all scans the following parameters were set: scan range 280-180 nm, band width 1.00 nm, scanning speed 20 nm/min, data pitch 0.1 nm. Every CD curve was obtained by averaging 5 scans and subtracting the background signal. The obtained ellipticities were transformed to mean residue molar ellipticities using the following equation:

$$[\theta][deg \cdot cm^2 \cdot dmol^{-1}] = \frac{\theta\ [mdeg] \cdot 10^6}{d\ [mm] \cdot c\ [\mu M] \cdot (n-1)}$$

$[\theta]$: residue molar ellipticity

$\theta$: ellipticity

$d$ = 1 mm (pathlength or thickness of cuvette)

$c$: protein concentration

$n$: amount of amino acids in the protein sequence

Recorded CD spectra are shown in Figs. S3-S16.

Isothermal titration calorimetry (ITC):

ITC experiments were performed on a ITC 200 microcalorimeter (Malvern Instruments) equipped with a semi-automated syringe assembly, a measurement cell (V = 300 µL) and a reference cell (V = 300 µL). All titrations were performed at 31 °C in a buffered solution (50 mM NaCl, 10 mM Tris.HCl, pH 6.9). Solutions of ACTR analogues were used as titrants (concentration range of 25 µM to 120 µM) and solutions of NCBD protein were used as titrators (concentration range 250 µM to 1200 µM). For all analogs at least two titration curves were recorded. For each titration curve, 20 injections were performed with an initial delay of 60 s. The volume of the first injection was 0.2 µL, whereas the volume of the 19 subsequent injections was 2 µL with injection duration of 0.4 s and a spacing between the injections of 120 s. Peak intensities of up to ~1.2 µcal/s were obtained and the reference power was set to 6 µcal/s. The filter period was set to 5 s. To analyze the data SEDPHAT/ITCsy, NITPIC and GUSSI software were used.[5-7] For every curve a background subtraction and a correction of concentration deviations were performed. For each analogue the two recorded curves were analyzed together to obtain a global fit. Recorded curves are depicted in Figs. S17-S30. The maximal deviations for $K_d$ and $\Delta H$ values were ±0.13 µM and ±1.34 kcal/mol, respectively.

Surface plasmon resonance (SPR):

The BIACORE 3000 system, sensor chip CM5, surfactant P20, amine coupling kit containing *N*-hydroxysuccinimide (NHS) and *N*-ethyl-*N'*-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from BIACORE (Uppsala, Sweden). All biosensor assays were performed with HEPES-buffered saline (HBS-P) as running buffer (10 mM Hepes, 150 mM sodium acetate, 3 mM magnesium acetate, 0.005% surfactant P20, pH 7.4). The other compounds were dissolved in the running buffer. Immobilization of streptavidin onto the surface activated by EDC/NHS was performed by injecting into a sensor chip CM5, 40 µL of streptavidin (100 µg/mL in formate buffer, pH 4.3), which gave a signal of approximately 5000 response units (RU), followed by 20 µL of ethanolamine hydrochloride, pH 8.5 to saturate the free activated sites of the matrix. Biotinylated NCBD analogue (20 µM in formate buffer, pH 4.3) was injected to bind to streptavidin until a response of 4000 RU was obtained.

All binding experiments were carried out at 25 °C with a constant flow rate of 30 µL/min. Different concentrations of ACTR analogues were injected to monitor binding for 3 min, followed by a dissociation phase of 3 min.

The sensor chip surface was regenerated after each experiment by injection of 20 µL of a solution of 1 M NaCl, 50 mM NaOH. The kinetic parameters were calculated using the BIAevaluation 4.1 software. Analysis was performed using the simple Langmuir binding model to derive $k_{on}$ and $k_{off}$. The specific binding profiles were obtained after subtracting the response signal from the channel control (ethanolamine) and from blank buffer injection. The fitting was judged by the minimized chi-square values.

Recorded curves are shown in Figs. S31-S41.

ACTR/NCBD complex:

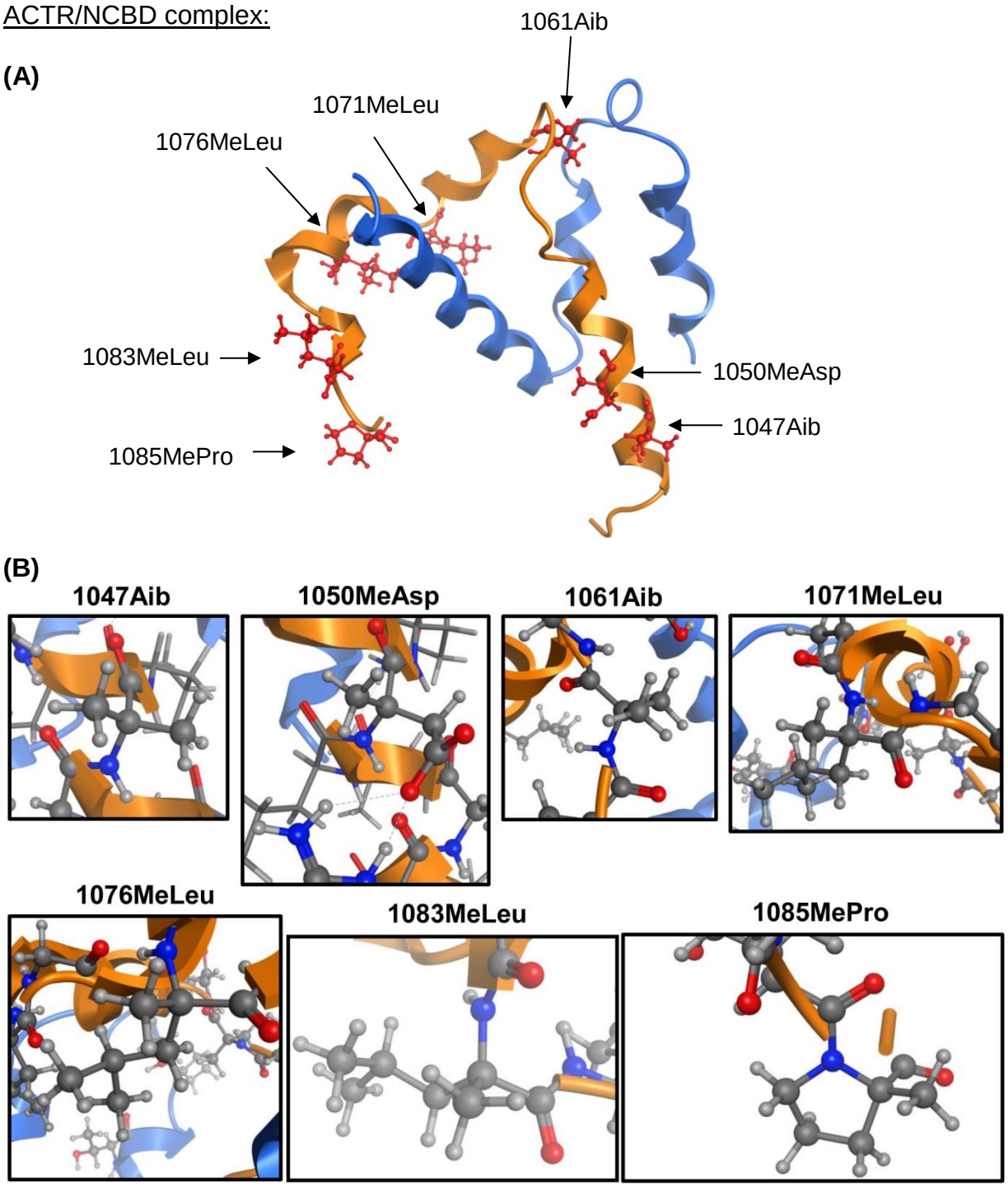



**Fig. S1.** (A) Depiction of α-methyl amino acids (in red) that were incorporated in ACTR/NCBD complex. (B) Close-up view of environment near each substituted α-methyl amino acid (shown in ball-and-stick representation). Molecular modelling was performed using MOE software.[8] After adding α-methyl groups, residues in close proximity were minimized by using force field AMBER10:EHT. No steric clashes were observed for α-methyl groups of the corresponding α-methyl amino acids. For residues 1047Aib and 1050MeAsp, the added methyl groups and the carbonyl oxygens of the same amino acid are in an eclipsed conformation, which might explain less favorable enthalpies compared to wild type (see Table 1 in main text) for analogues that contain such substitutions. Residues 1061Aib, 1083MeLeu and 1085MePro are in less structured parts of the complex, therefore, incorporation of α-methyl groups may stabilize secondary structures and this may explain more favorable enthalpies but less favorable entropies observed experimentally.

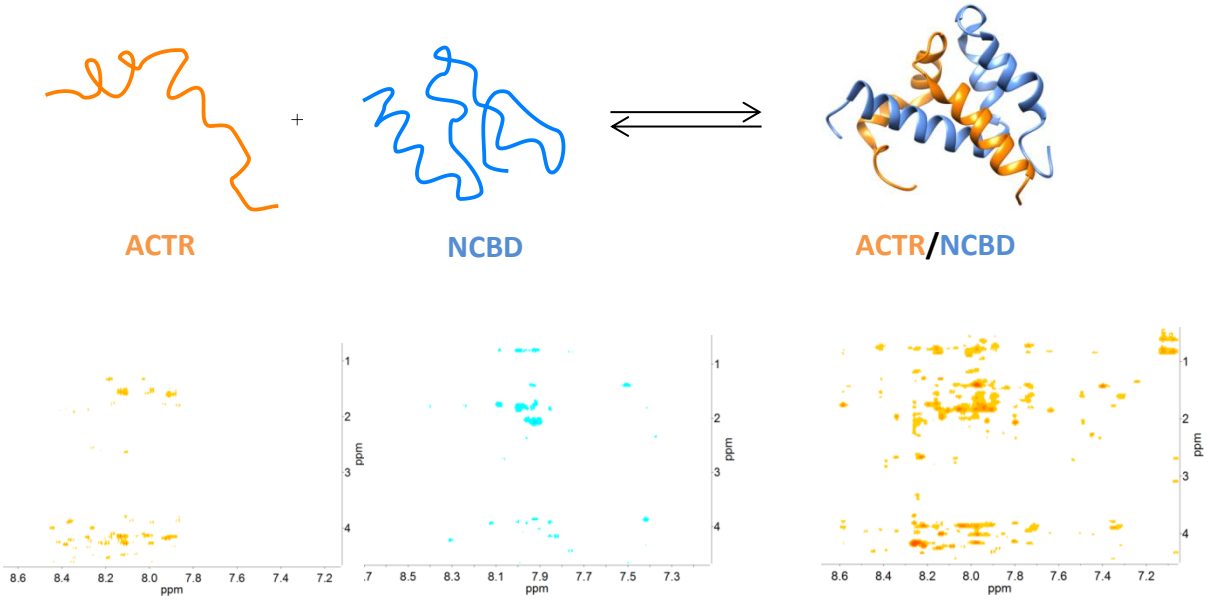



**Fig. S2.** Monitoring complex formation between ACTR and NCBD by $^{1}$H-NOESY.

CD spectra of ACTR variants

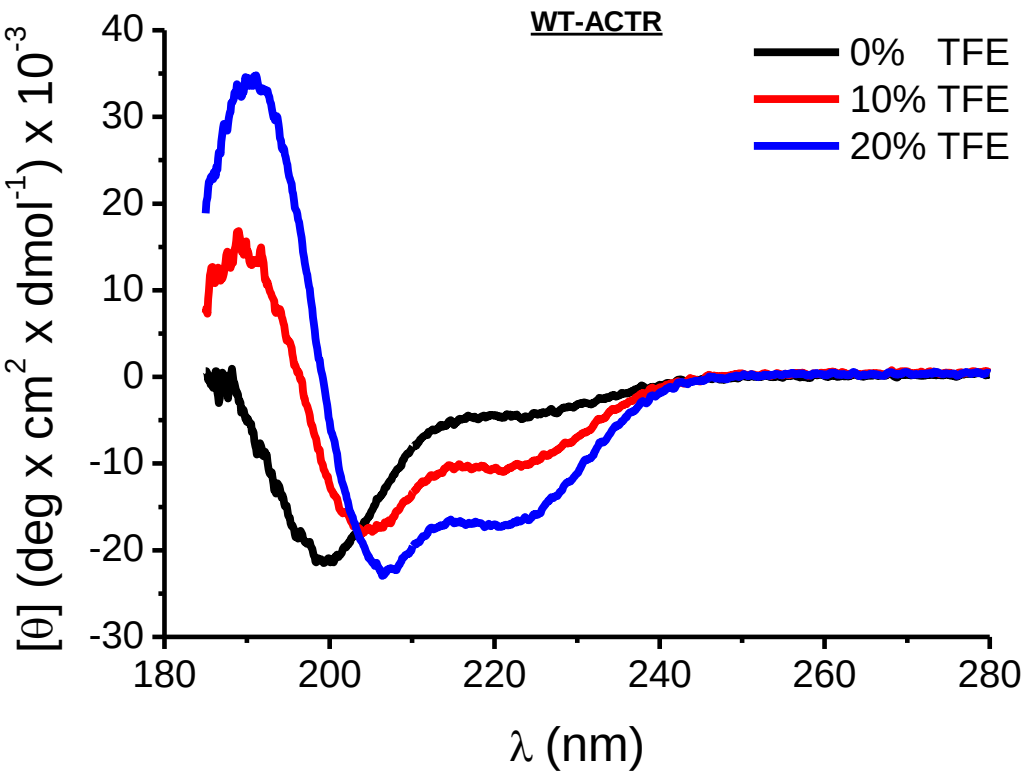



**Fig. S3.** CD data for wild type ACTR

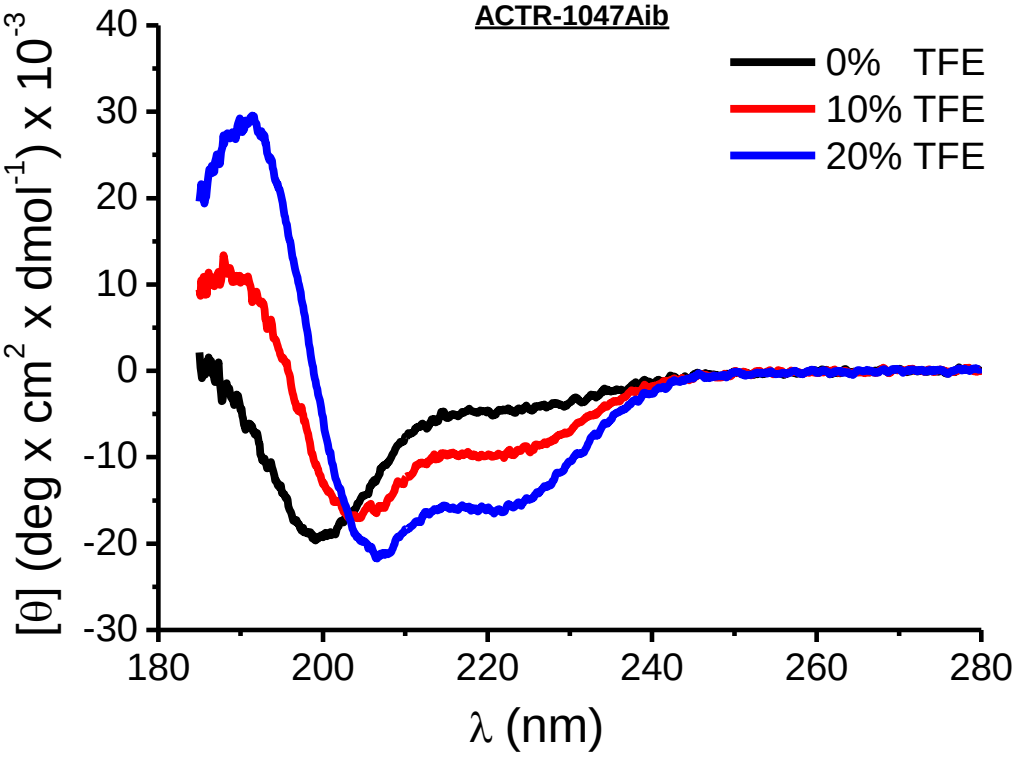



**Fig. S4.** CD data for [1047Aib]ACTR

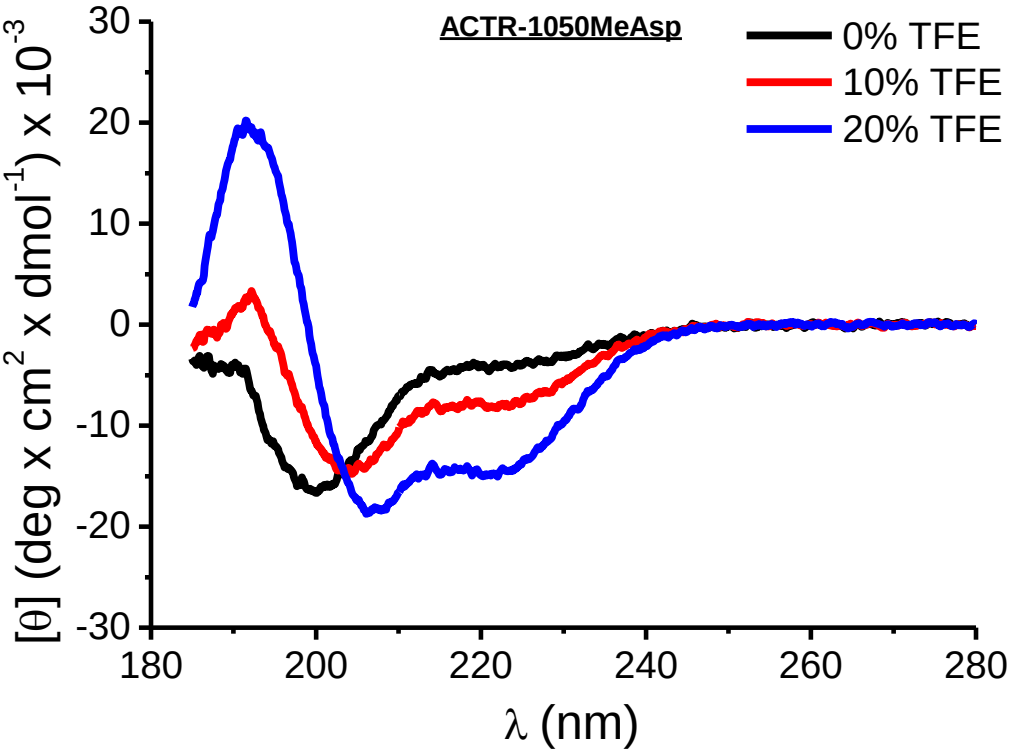



**Fig. S5.** CD data for [1050MeAsp]ACTR

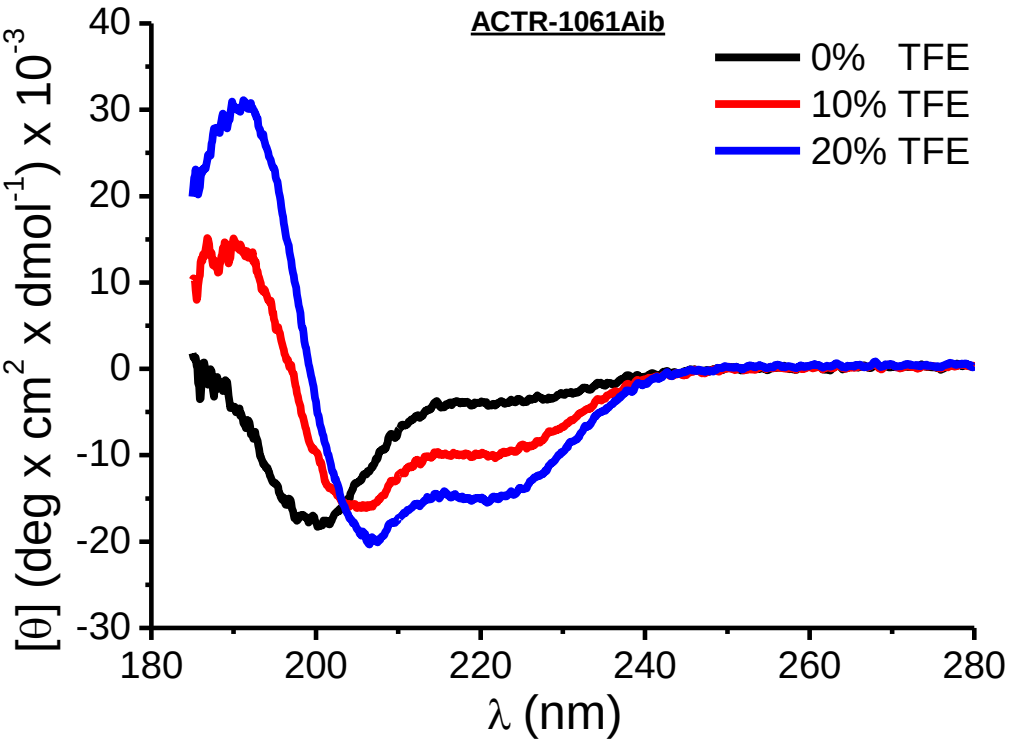



**Fig. S6.** CD data for [1061Aib]ACTR

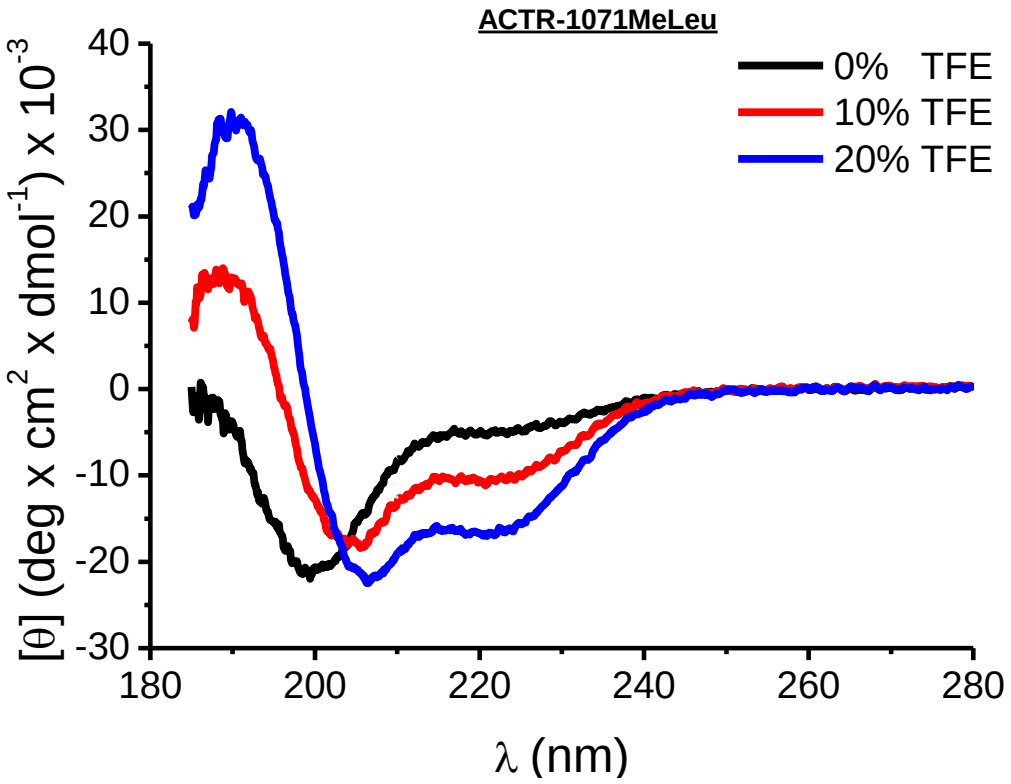



**Fig. S7.** CD data for [1071MeLeu]ACTR

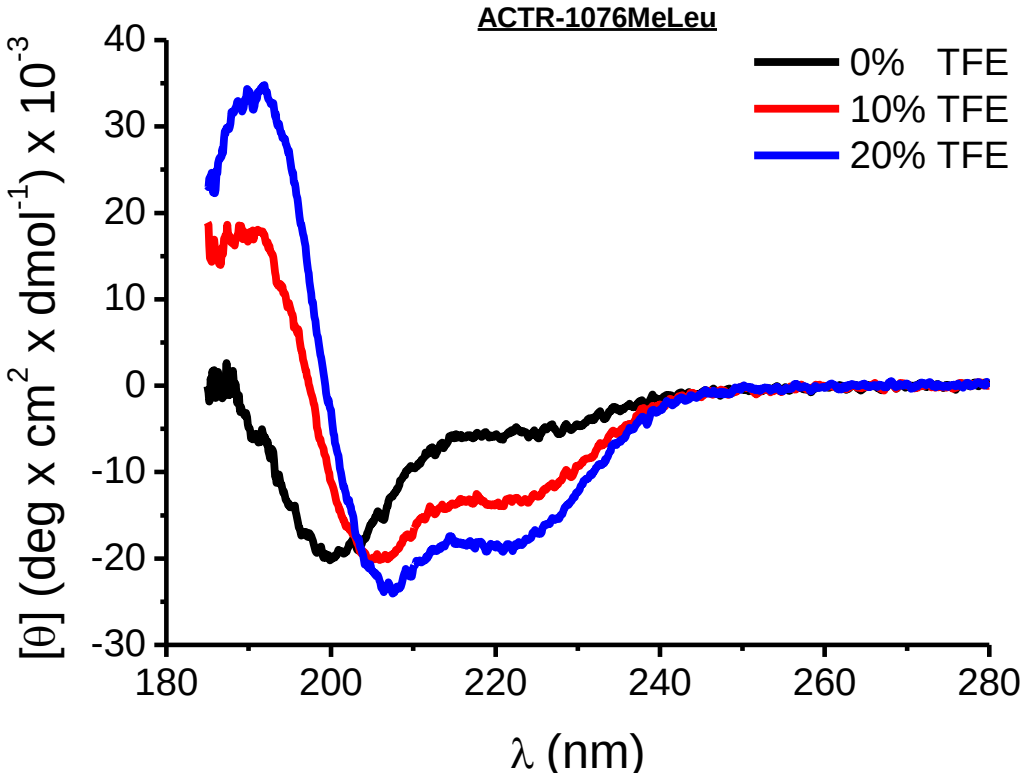



**Fig. S8.** CD data for [1076MeLeu]ACTR

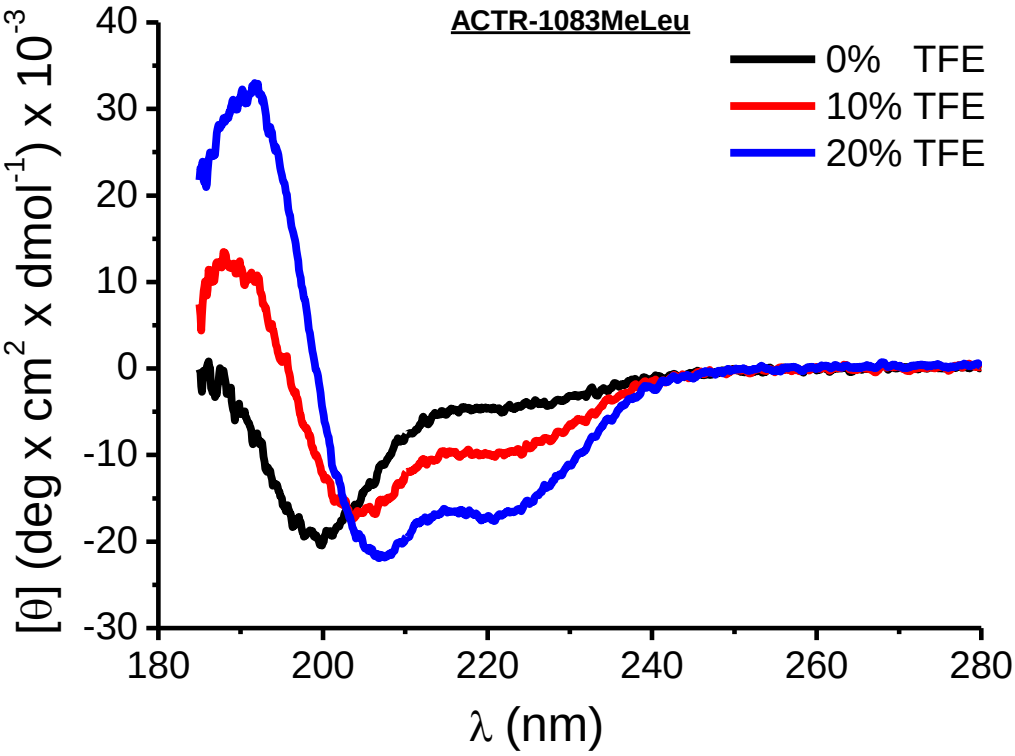



**Fig. S9.** CD data for [1083MeLeu]ACTR

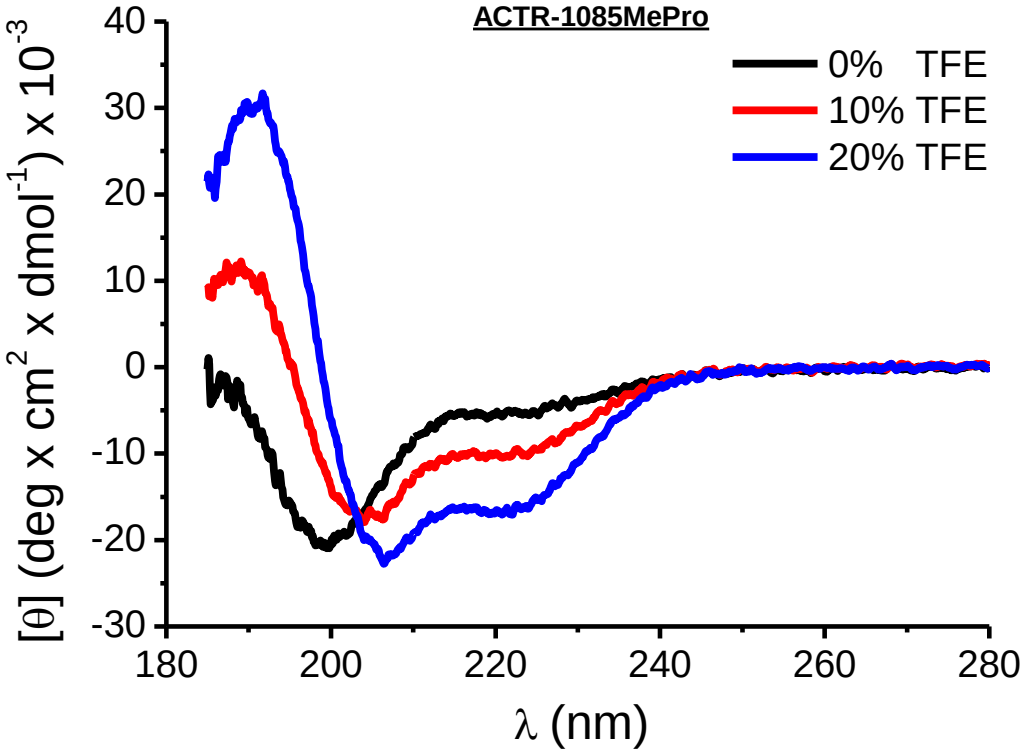



**Fig. S10.** CD data for [1085MePro]ACTR

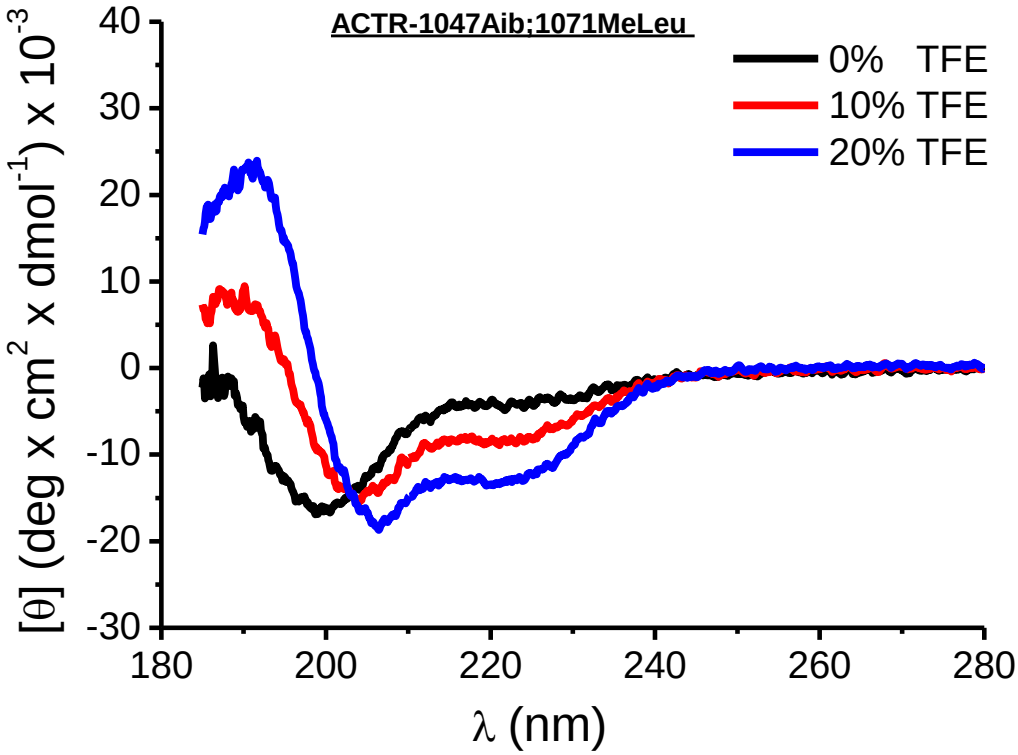



**Fig. S11.** CD data for [1047Aib;1071MeLeu]ACTR

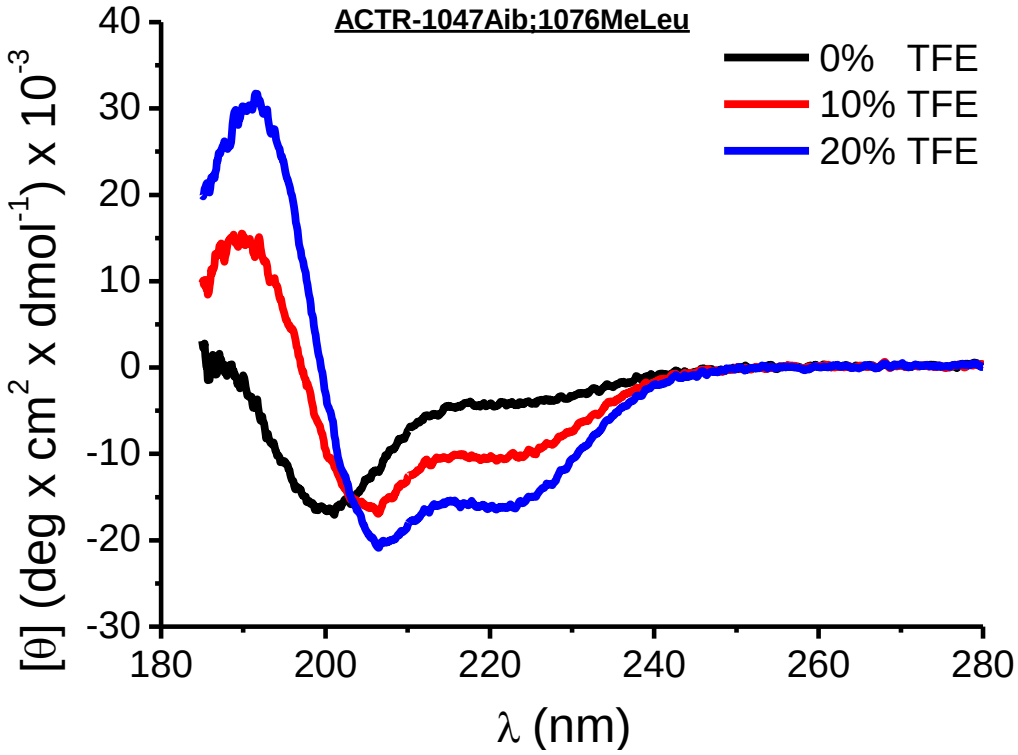



**Fig. S12.** CD data for [1047Aib;1076MeLeu]ACTR

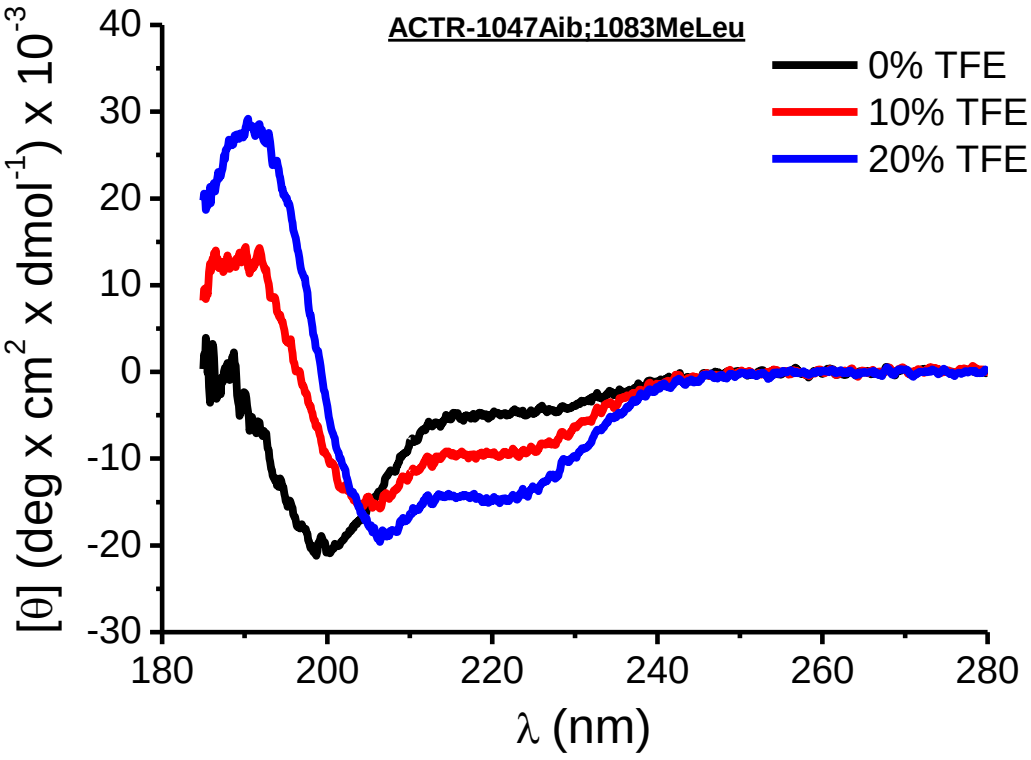



**Fig. S13.** CD data for [1047Aib;1083MeLeu]ACTR

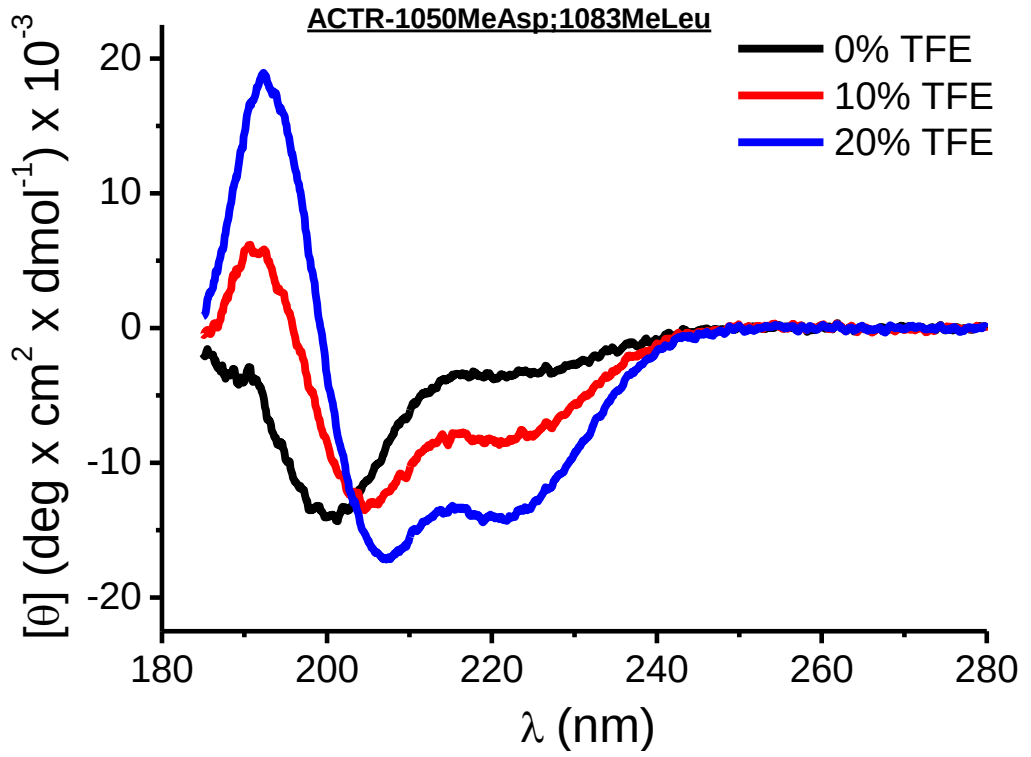



**Fig. S14.** CD data for [1050Asp;1083MeLeu]ACTR

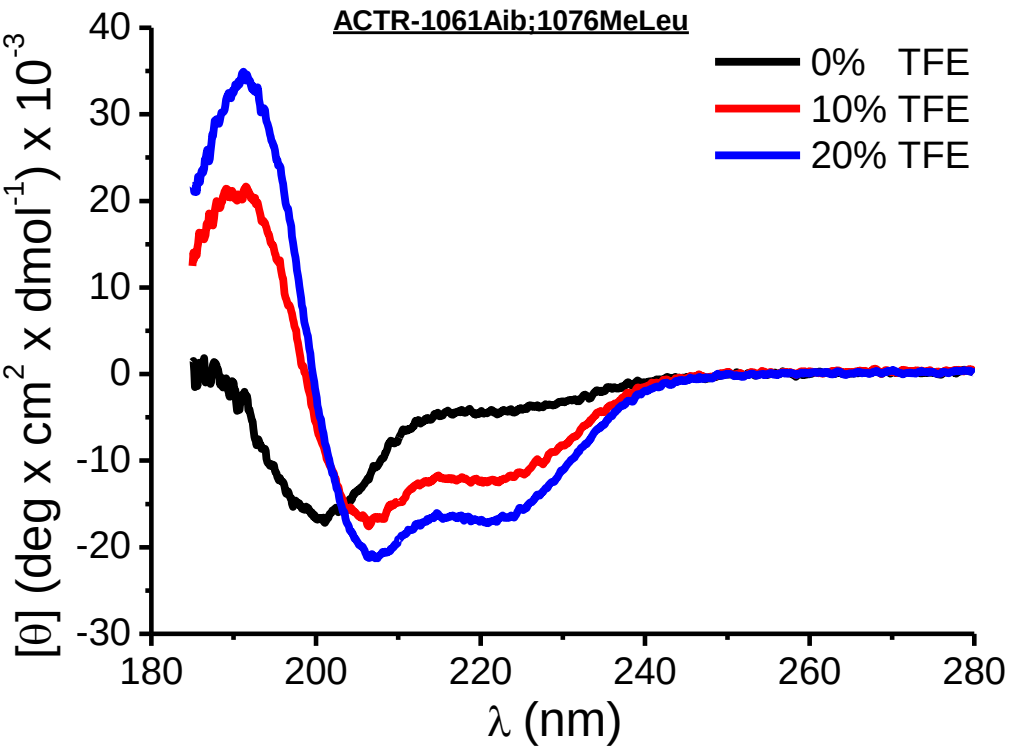



**Fig. S15.** CD data for [1061Aib;1076MeLeu]ACTR

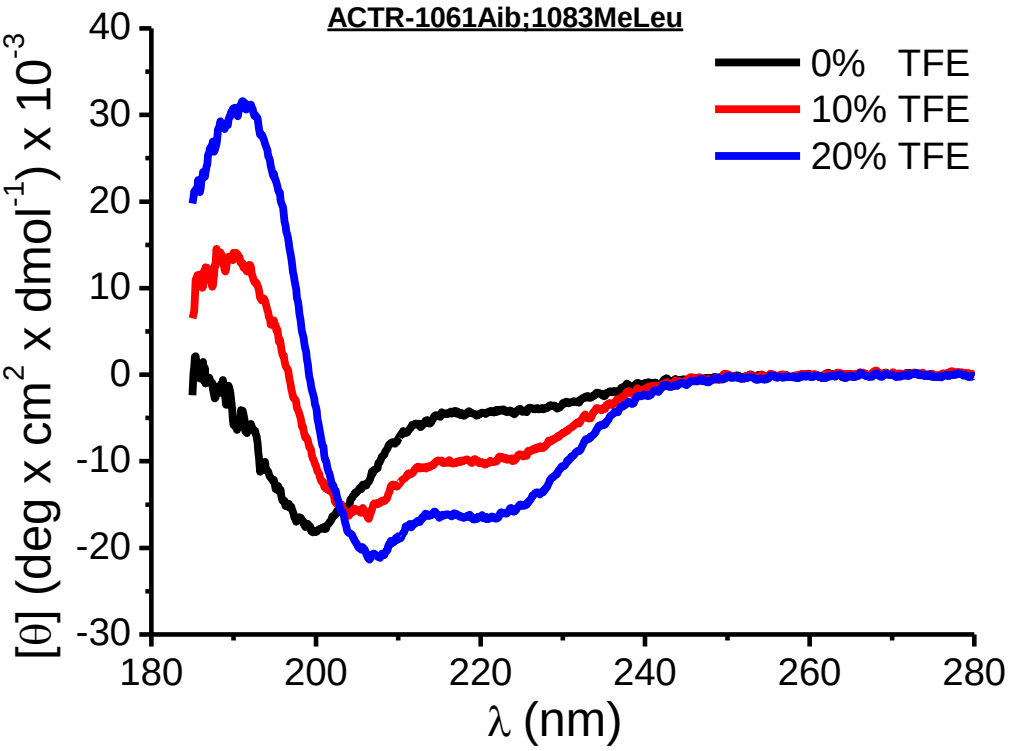



**Fig. S16.** CD data for [1047Aib;1083MeLeu]ACTR

ITC diagrams

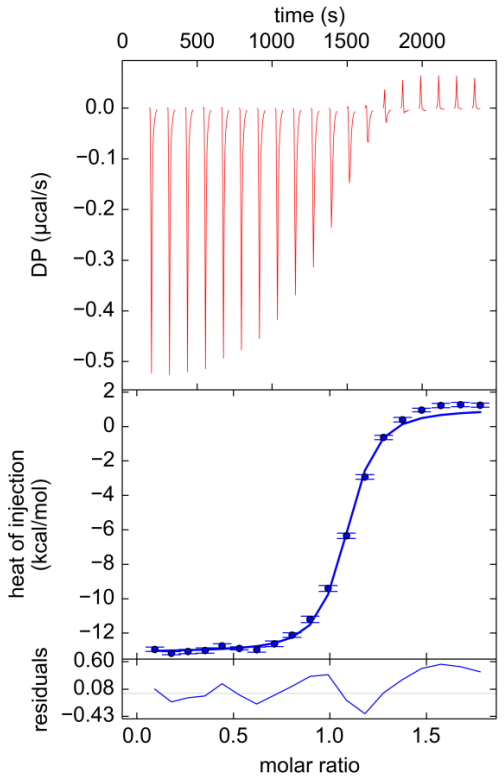



**Fig. S17.** Wild type ACTR

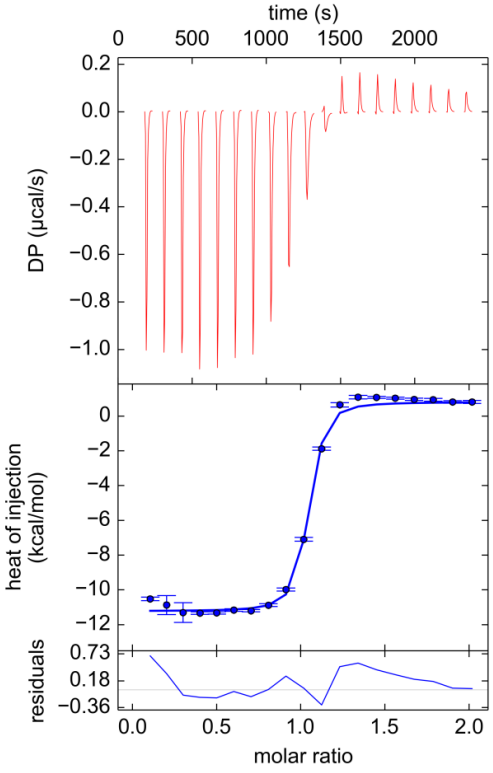



**Fig. S18.** [1047Aib]ACTR

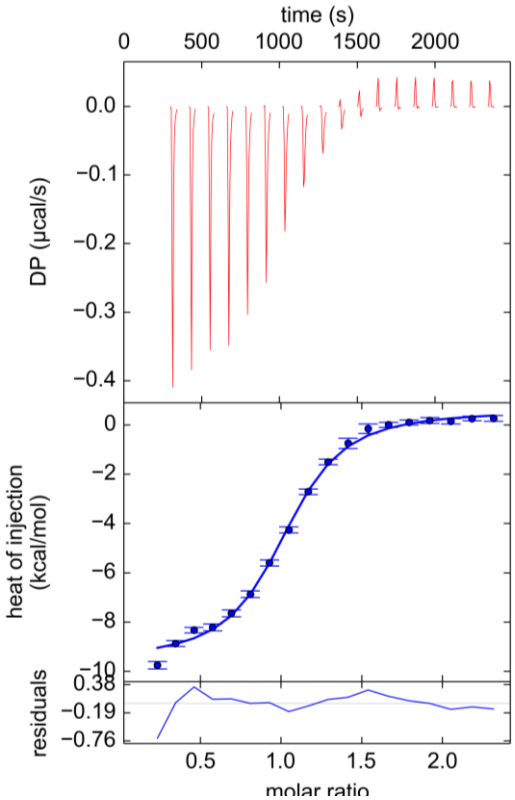



**Fig. S19.** [1050MeAsp]ACTR

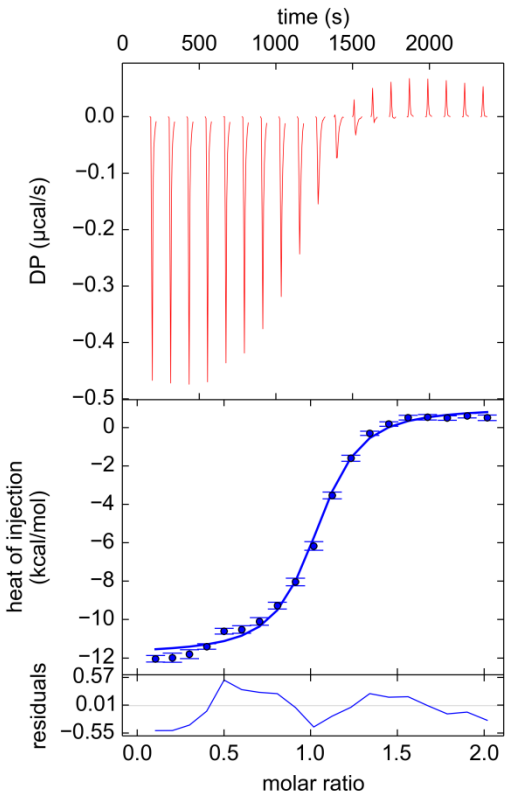



**Fig. S20.** [1061Aib]ACTR

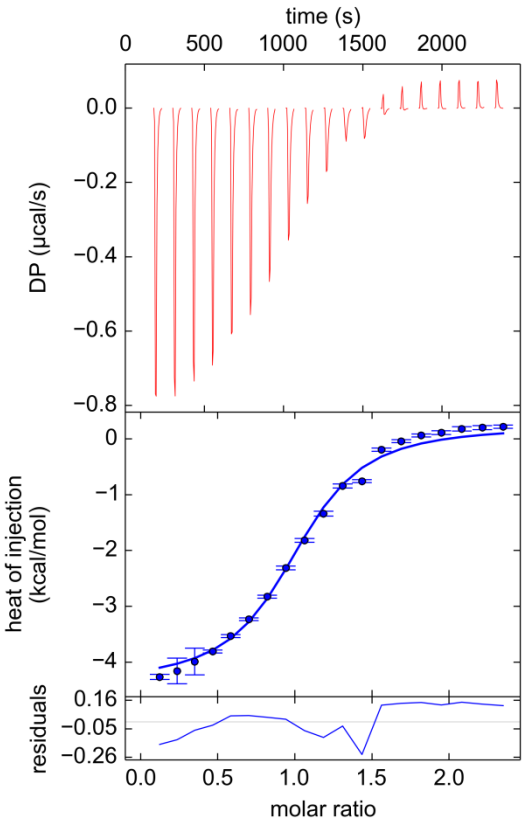



**Fig. S21.** [1071MeLeu]ACTR

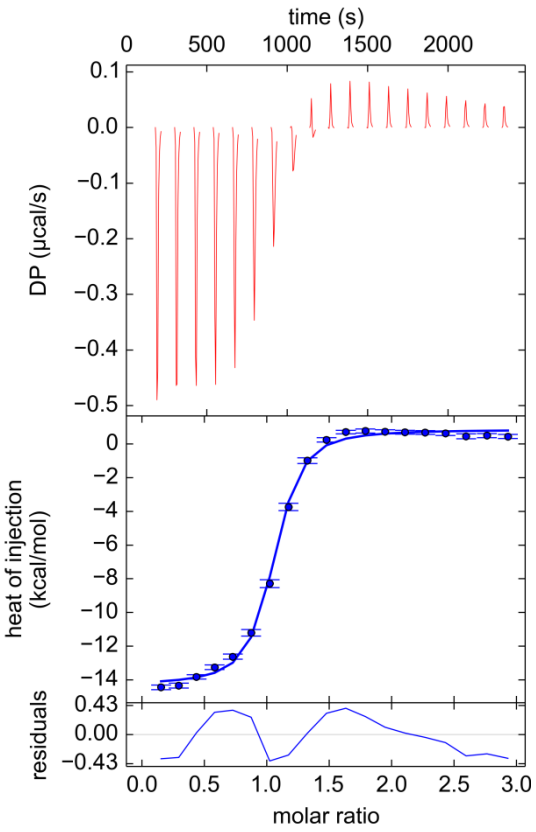



**Fig. S22.** [1076MeLeu]ACTR

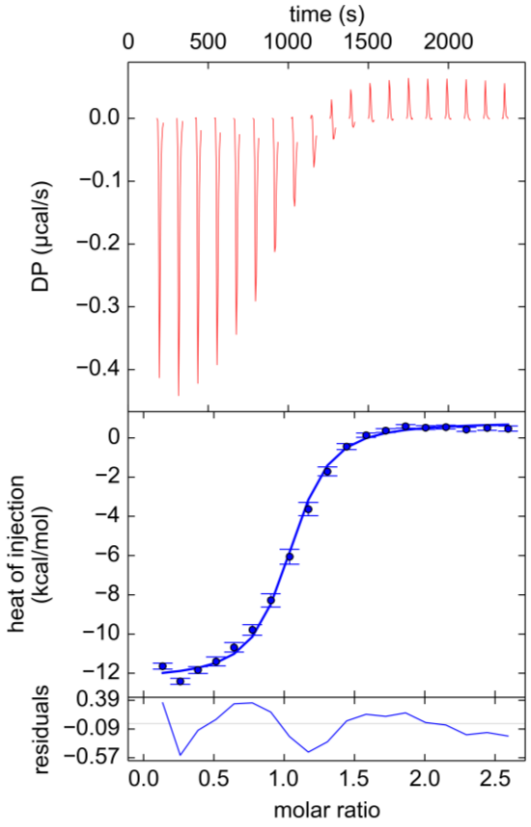



**Fig. S23.** [1083MeLeu]ACTR

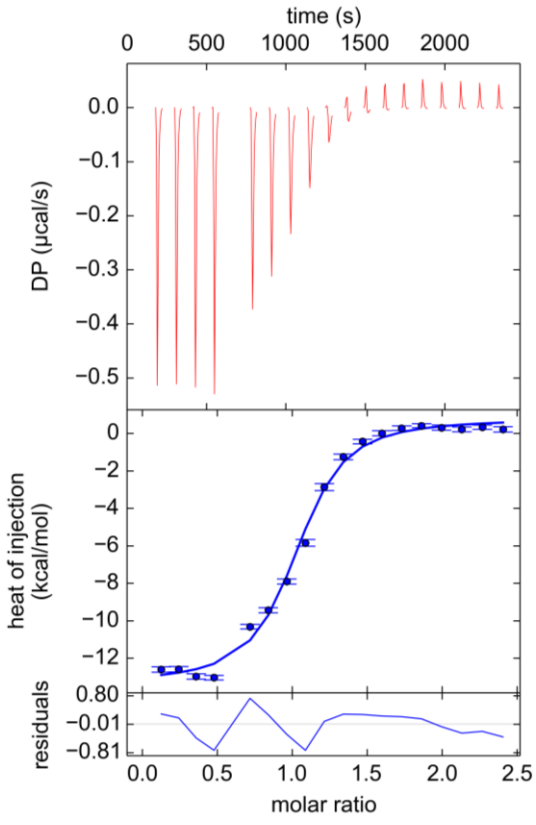



**Fig. S24.** [1085MePro]ACTR

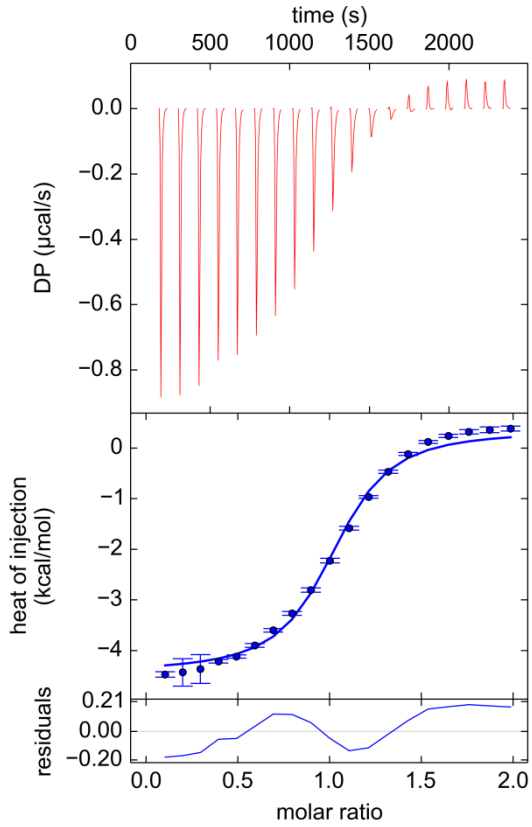



**Fig. S25.** [1047Aib;1071MeLeu]ACTR

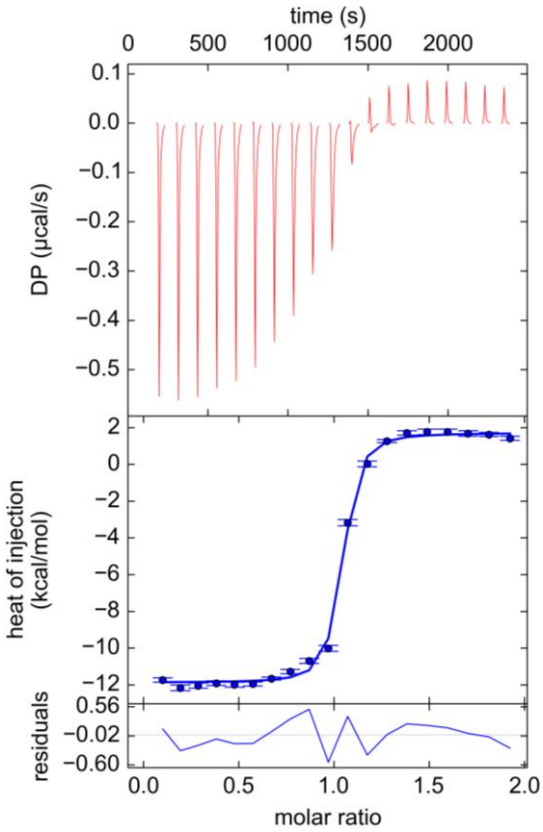



**Fig. S26.** [1047Aib;1076MeLeu]ACTR

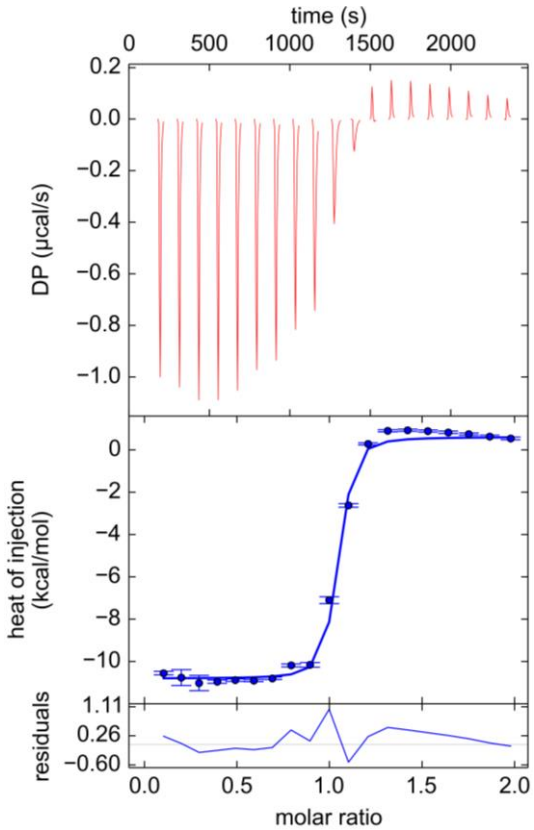



**Fig. S27.** [1047Aib;1083MeLeu]ACTR

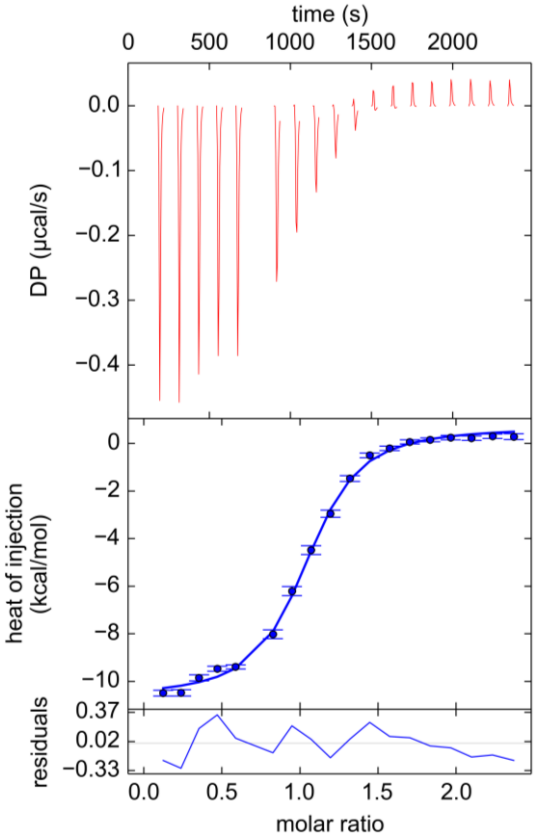



**Fig. S28.** [1050MeAsp;1083MeLeu]ACTR

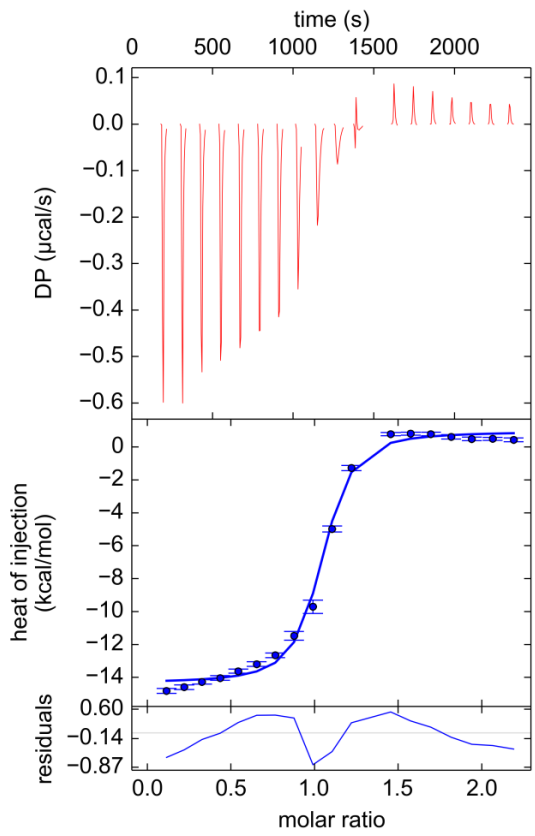



**Fig. S29.** [1061Aib;1076MeLeu]ACTR

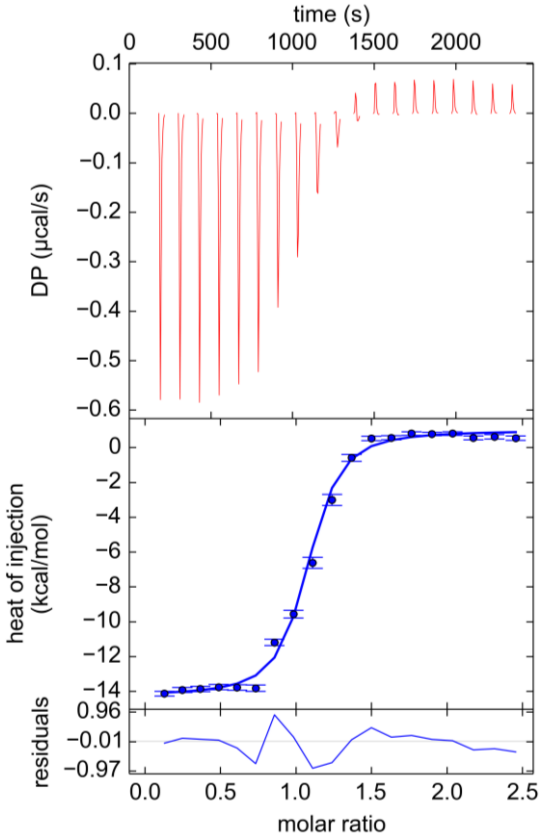



**Fig. S30.** [1061Aib;1083MeLeu]ACTR

SPR data

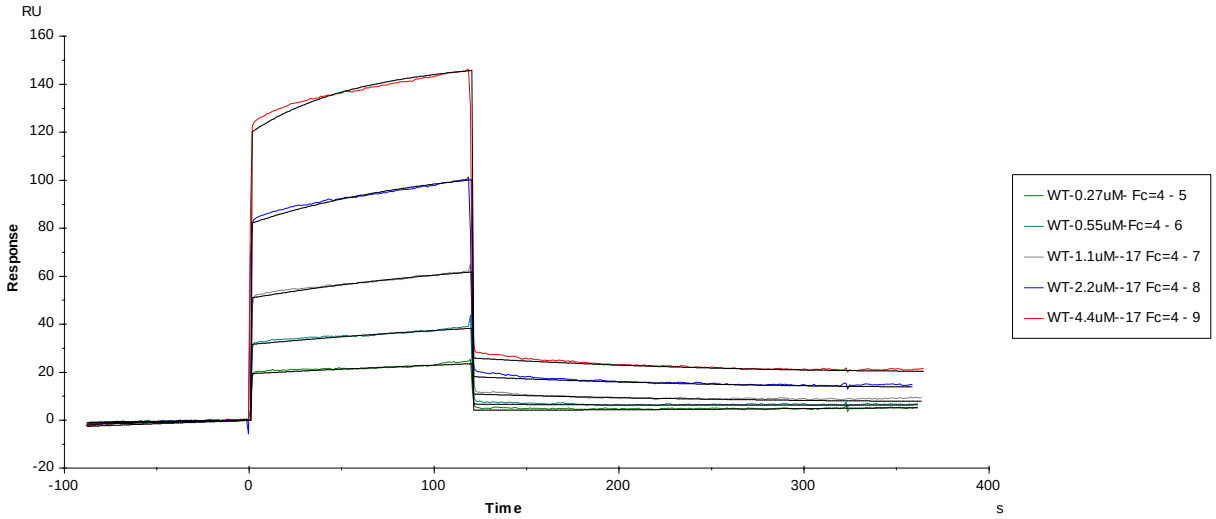



**Fig. S31.** SPR sensogram of wild type ACTR / NCBD(biotin) binding

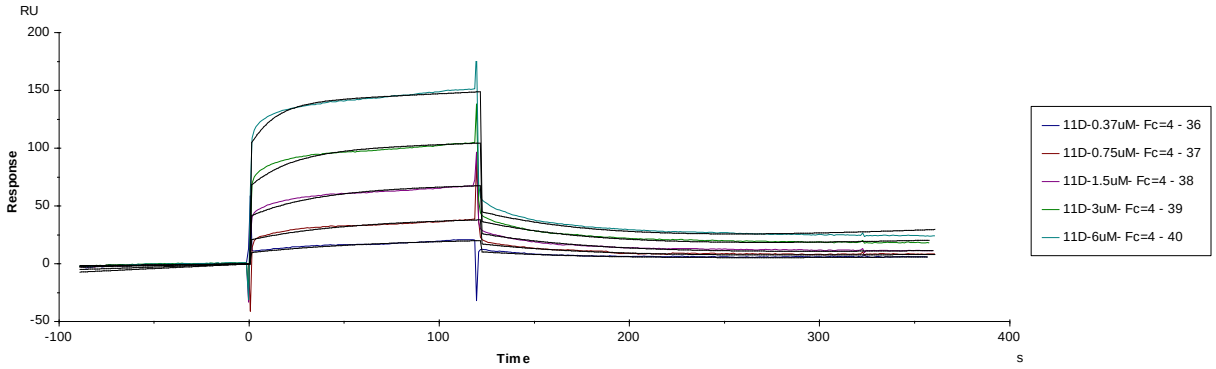



**Fig. S32.** SPR sensogram of [1050MeAsp]ACTR / NCBD(biotin) binding

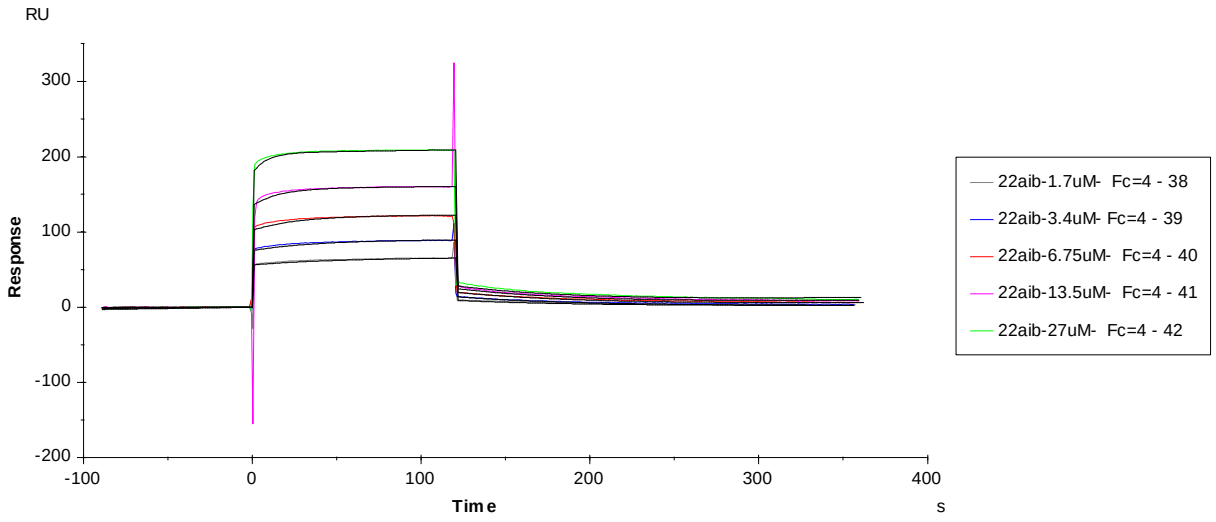



**Fig. S33.** SPR sensogram of [1061Aib]ACTR / NCBD(biotin) binding

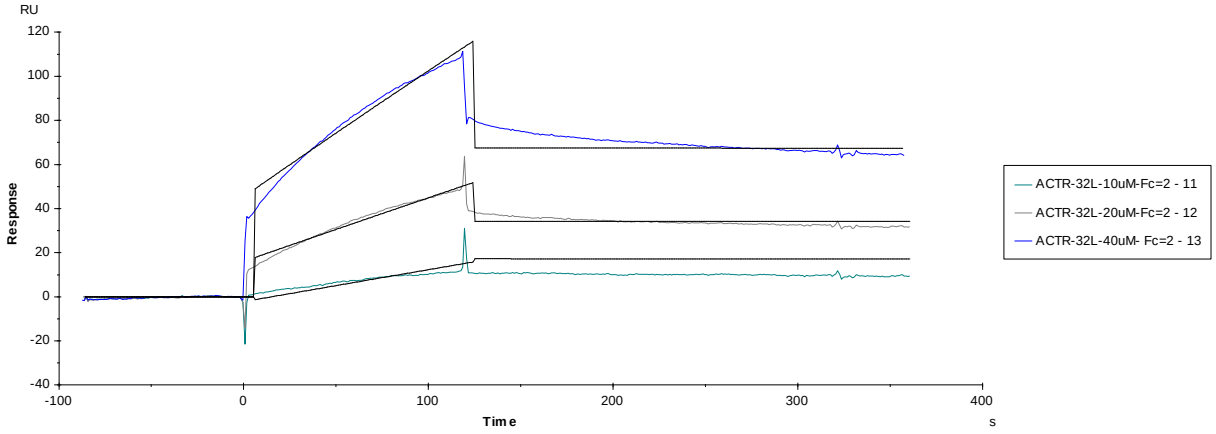



**Fig. S34.** SPR sensogram of [1071MeLeu]ACTR / NCBD(biotin) binding

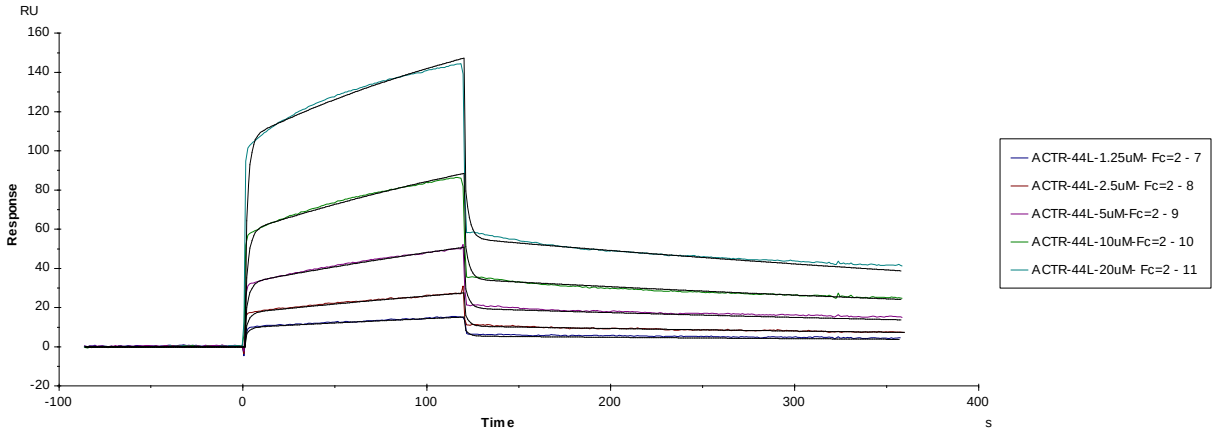



**Fig. S35.** SPR sensogram of [1083MeLeu]ACTR / NCBD(biotin) binding

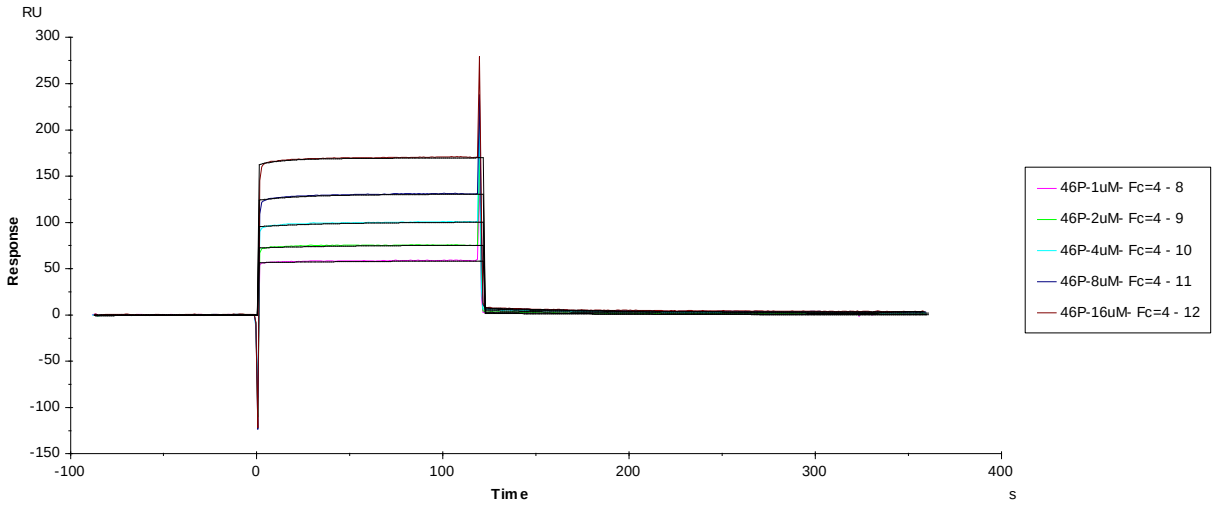



**Fig. S36.** SPR sensogram of [1085MePro]ACTR / NCBD(biotin) binding

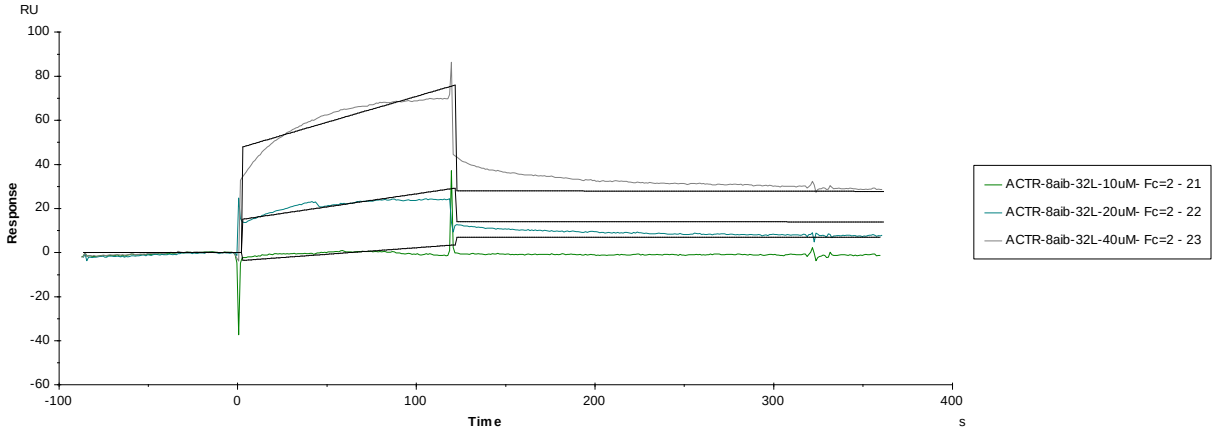



**Fig. S37.** SPR sensogram of [1047Aib;1071MeLeu]ACTR / NCBD(biotin) binding

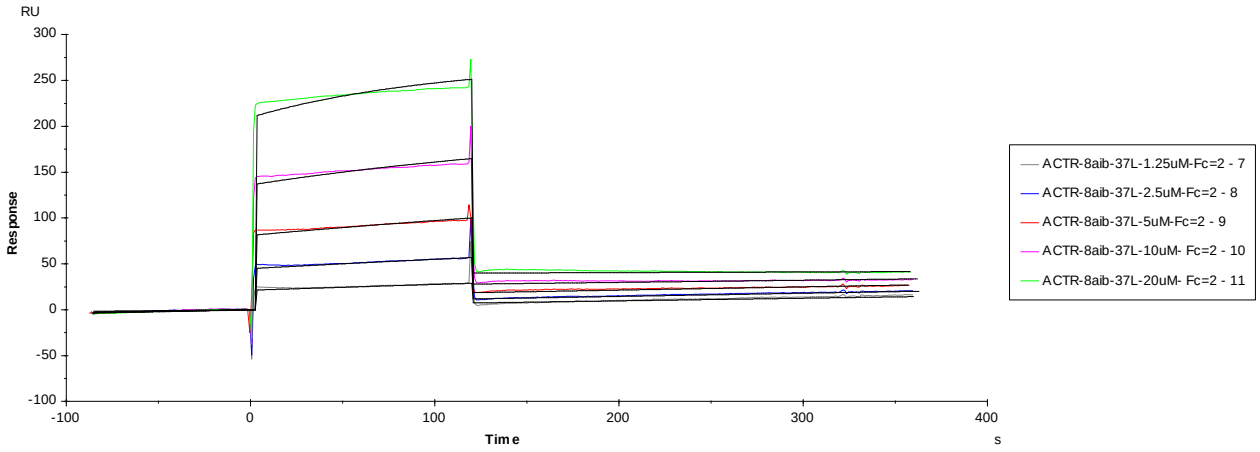



**Fig. S38.** SPR sensogram of [1047Aib;1076MeLeu]ACTR / NCBD(biotin) binding

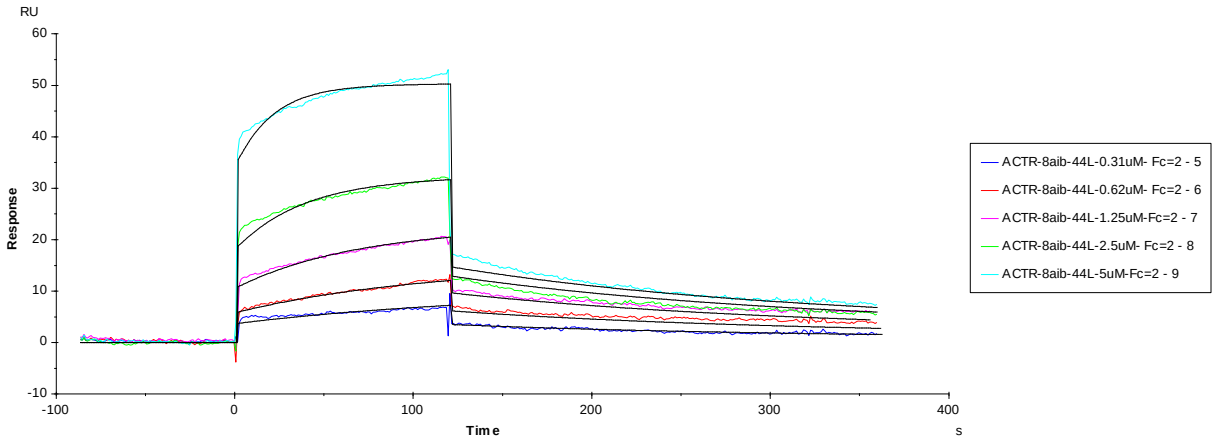



**Fig. S39.** SPR sensogram of [1047Aib;1083MeLeu]ACTR / NCBD(biotin) binding

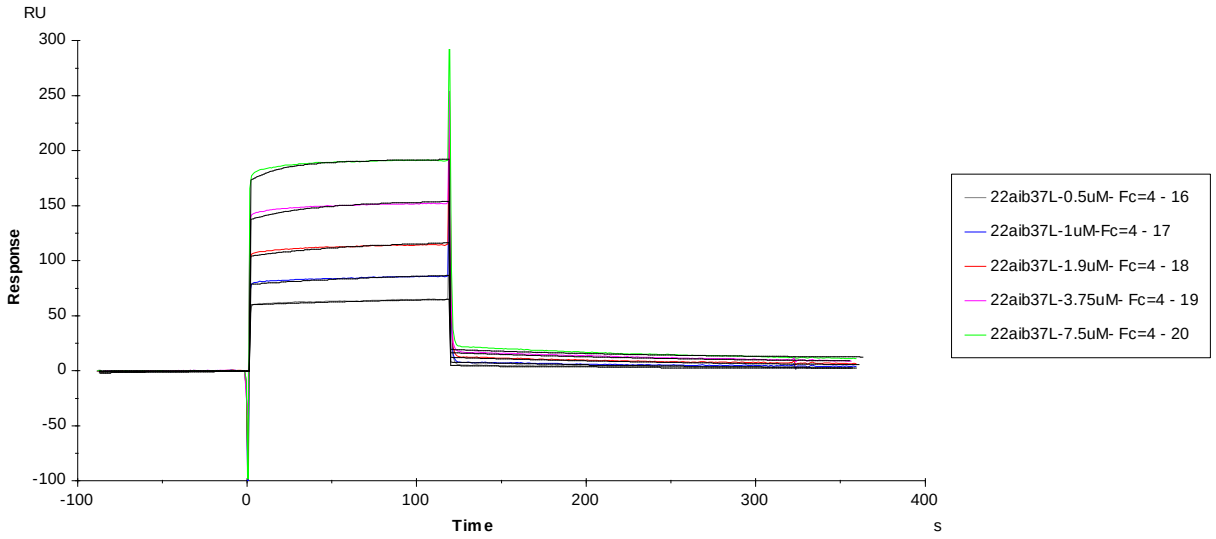



**Fig. S40.** SPR sensogram of [1061Aib;1076MeLeu]ACTR / NCBD(biotin) binding

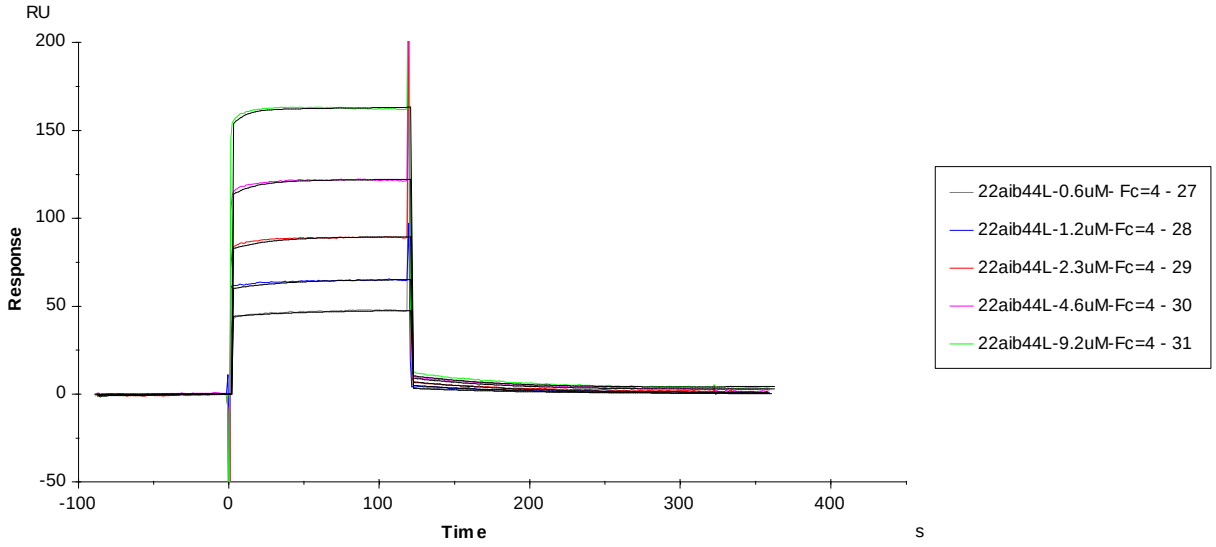



**Fig. S41.** SPR sensogram of [1061Aib;1083MeLeu]ACTR / NCBD(biotin) binding

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