Supporting Information Accompanying

Discovery and Characterization of Peptide Inhibitors for CIB1

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General Information

Solvents and Alexa Fluor 647 C2 Maleimide were purchased through Fisher Scientific and used as received unless otherwise noted. All SPPS reagents (Amino acids and their protected derivatives, activating agents and *N*,*N*-diisopropylethyl amine; DIPEA) were purchased from Chem-Impex Int'l. Inc.

LCMS measurements were recorded using an Agilent 6520 Accurate-Mass Q-TOF ESI positive in high-resolution mode, 350 °C temperature and 250 V fragmentor, with a 100 x 2.10 mm Kinetex® 2.6 A C8 100 Å column or a 50 x 2.1 mm Kinetex® 2.6 A C18 100 Å column; both were purchased from Phenomenex. MS-MS was performed with collision energy of 45 V or 60 V. Predicted masses were extracted to $\pm$ 5 ppm. Two methods were utilized:

Method A:

Time	**% Solvent B**
0 - 2 min	2%
2 - 5 min	2 - 45%
5 – 22 min	45 - 60%
22 - 23 min	60 – 95%
23 - 24 min	95%
24 – 25 min	95 - 2%

Method B:

Time	**% Solvent B**
0 -2 min	2%
2- 13 min	2 - 98%
13– 15 min	98%

Preparatory HPLC was performed in a Shimadzu UFLC CBM-20A with a dual channel wavelength detector at 220 nm and 254 nm with a LUNA 10® C18(2) 100 Å, AXIA (Phenomenex) semi-preparatory column with a 15 mL/min flow rate. Purifications were carried out with a two-solvent system (solvent A = 0.1% trifluoroacetic acid in water; solvent B = 0.1% trifluoroacetic acid in acetonitrile). Analytical HPLC was performed with a dual channel wavelength detector at 254 and 220 or 350 nm on a Kinetex 10® C18 100 Å, (Phenomenex) column with a 0.5 mL/min flow rate.

Method C:

Time	**% Solvent B**
0 -2 min	5%
2- 8 min	5 - 35%
8 – 23 min	35-55%
23- 25 min	55 – 100%
25 – 28.5 min	100%
28.5 – 30 min	100 – 5%
30 – 32 min	5%

Method D:

Time	% Solvent B
0 -2 min	5%
2- 15 min	5 - 95%
15 - 18 min	95%
18 - 19 min	95 – 5%
19 – 20 min	5%

Solid Phase Peptide Synthesis

All peptides were synthesized by standard Fmoc-SPPS on a MiniBlock with Rink Amide ChemMatrix resin following the general procedures below:

Resin Swelling: Resin (20-100 mg) was initially swollen in DMF (1.5-6.0 mLs) for 30 min at room temperature.

Deprotections: Excess (1-2 mLs) 20% piperidine in DMF was added to the reaction vessel and allowed to incubate for 10 min while shaking at room temperature. The reaction vessel was then drained and the resin was washed 4x with 1 mL of DMF.

Couplings: Fmoc-AA-OH (5.0 equiv.; 0.5 M in DMF), HCTU (5.0 equiv.; 0.5 M in DMF), and DIPEA (10.0 equiv.; 1.0 M in DMF) were added to the reaction vessel in that order. The resulting suspension was shaken for 15 min at room temperature. The reaction vessel was then drained and the resin was washed 4x with 1 mL of DMF.

Acetyl Capping: Acetic anhydride (10.0 equiv.; 5.0 M in DMF) followed by and DIPEA (10.0 equiv.; 0.5 M in DMF) were added to the reaction vessel in that order. The resulting suspension was shaken for 10 min at room temperature. The reaction vessel was then drained and the resin was washed 4x with 1 mL of DMF.

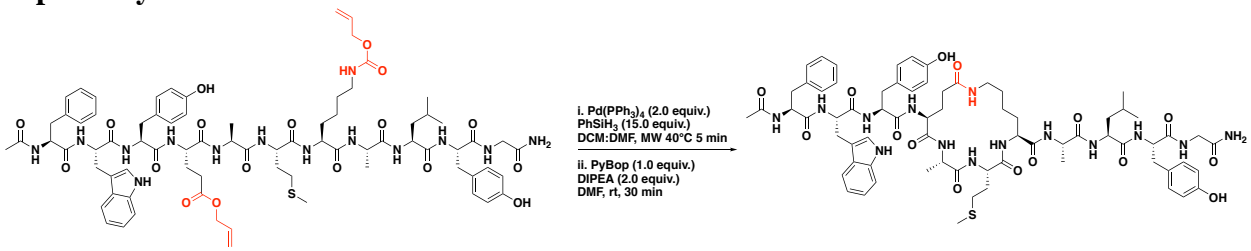
Peptide Cleavage: The reaction vessel was then drained and the resin was washed 4x with 1 mL of DMF and then 4x with 1 mL of DCM. The resin was dried and cleaved using a cleavage cocktail (TFA/TIPS/H_2O; 95:2.5:2.5) at 37°C for 1 h. The resin was then filtered from the cleavage cocktail solution. The solution was dried by vigorously blowing N_2 over the top of the solution. Cooled diethyl ether (at least 10 times the volume of the residual cleavage cocktail) was added to crash out the crude peptide. The crude peptide was pelleted by centrifugation (10 min at 15,000 rom at 4°C) and then decanted. The crude peptide was dissolved in 1:1 acetonitrile:H_2O and purified by reverse phase Prep HPLC using method C. Fractions containing peptide of interest were analyzed by LCMS to verify their identities and lyophilized.

Alexa Fluor 647 Labeling Procedure

UNC10245092 was synthesized with an N-terminal cysteine and labeled with Alexa Fluor 647 C2 Maleimide following the Invitrogen thiol-reactive probes user manual with slight modification. In short, the cysteine containing peptide was dissolved in 10 mM PBS buffer (pH 7.5) to a final concentration of 100 μM and reduced with 10 equiv. of TCEP (introduced as a solid) at room temperature for 30 min. A 1 mM stock of Alexa Fluor C2 Maleimide in DMF was made immediately prior to its use. 1 equiv. of Alexa Fluor C2 Maleimide was added to the reaction

vessel. The solution stirred at room temperature for 2 h and then immediately purified by reverse Prep HPLC using method C. Fractions containing peptide of interest were analyzed by LCMS to verify their identities and lyophilized.

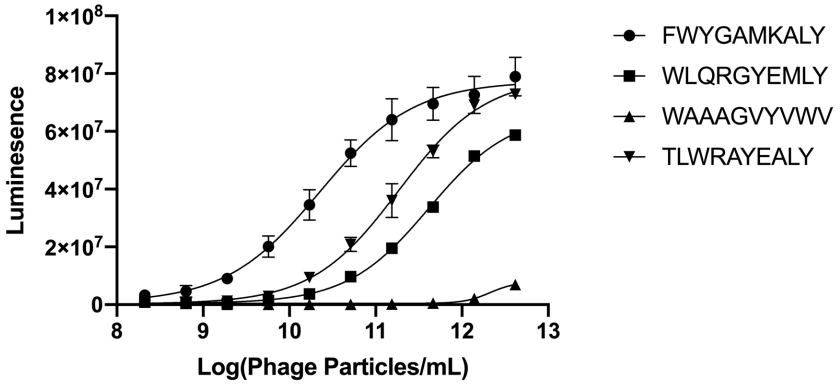
Peptide Cyclization Procedure



Alloc Deprotection: Both alloc groups on the linear, resin-bound peptide were deprotected simultaneously using the Biotage Initiator + Alstra™ Microwave. Resin was swollen in DMF (1.5-6.0 mLs) for 30 min at room temperature, then the reaction vessel was drained. The resin was then suspended in a 1:1 solution of DCM:DMF and 2.0 equiv. of $Pd(PPh_3)_4$ and 15.0 equiv. of phenylsilane were added in that order. The reaction was heated by microwave irradiation to 40°C for 5 min while stirring. The resin was then washed 4x with 1 mL of 1:1 DCM:DMF or until the light brown discoloration was removed from the resin. The reaction and wash steps were repeated one more time to ensure full deprotection was achieved.

Macrocyclization: Immediately after the deprotection procedure was completed PyBOP (1.0 equiv.; 0.5 M in DMF) and DIPEA (2.0 equiv.; 0.5 M in DMF) were added to the reaction vessel in that order. The resulting suspension was shaken for 30 min at room temperature. The reaction vessel was then drained, and the resin was washed 4x with 1 mL of DMF. The crude peptide was cleaved and purified following the general protocols previously described.

Representative Phage ELISAs for CIB-selected Peptide Clones



Sequence	IC50 (Phage Particles/mL)	# occurences
FWYGAMKALY	**2.24 E 10**	**12**
TLWRAYEALY	**1.85 E 11**	**2**
WLQRGYEMLY	**4.14 E 11**	**1**
WAAAGVYVWV	**n/a**	**1**

Figure S1. Phage ELISA binding curves for peptide phage binding to immoblilized CIB1 using 16 randomly picked round three and round four CIB1-selected peptide clones. Line shows fit using four parameter analysis.

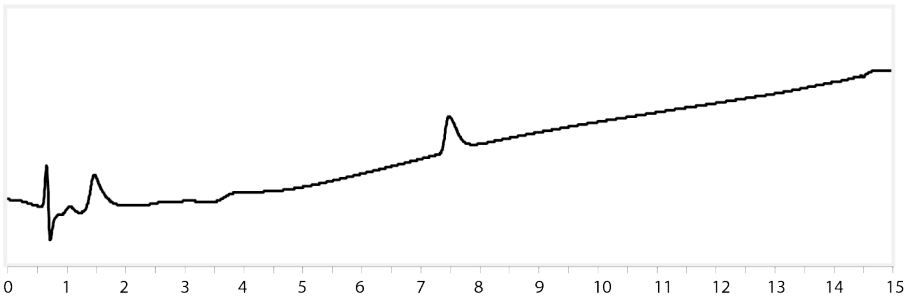
Proof of Purity for Synthetic Peptides

Name	Sequence	Chemical Formula	Expected Masses	Observed Masses	Purity
UNC10245092	EDGGSFWYGAMKALYG	$C_{81}H_{112}N_{19}O_{23}S^+$	1750.7894 $[M+H]^{+1}$, 875.4000 $[M+2H]^{+2}$	1750.7909 $[M+H]^{+1}$, 875.4070 $[M+2H]^{+2}$	>98%
UNC10245092 F1A	**A**WYGAMKALY	$C_{61}H_{88}N_{14}O_{14}S^+$	1271.6241 $[M+H]^{+1}$, 636.3157 $[M+2H]^{+2}$	1271.6252 $[M+H]^{+1}$, 636.3216 $[M+2H]^{+2}$	95%
UNC10245092 W2A	F**A**YGAMKALY	$C_{59}H_{86}N_{13}O_{14}S^+$	1232.6132 $[M+H]^{+1}$, 616.8103 $[M+2H]^{+2}$	1232.6285 $[M+H]^{+1}$, 616.8048 $[M+2H]^{+2}$	95%
UNC10245092 Y3A	FW**A**GAMKALY	$C_{61}H_{87}N_{14}O_{13}S^+$	1255.6292 $[M+H]^{+1}$, 628.3183 $[M+2H]^{+2}$	1255.6403 $[M+H]^{+1}$, 628.3202 $[M+2H]^{+2}$	>98%

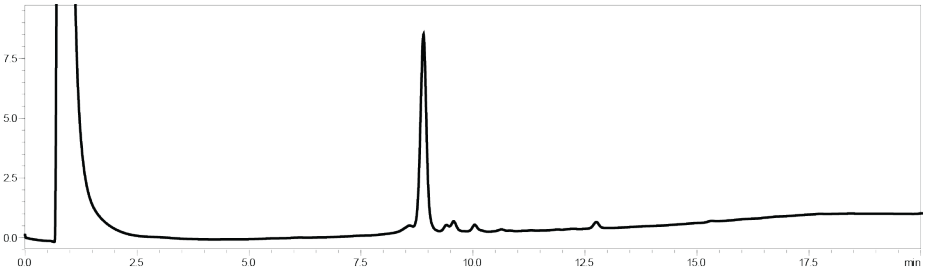
UNC10245092 G4A	FWY**A**AMKALY	$C_{68}H_{93}N_{14}O_{14}S^+$	1361.6711 $[M+H]^{+1}$, 681.3392 $[M+2H]^{+2}$	1361.6497 $[M+H]^{+1}$, 681.3349 $[M+2H]^{+2}$	>98%
UNC10245092 A5G	FWYG**G**MKALY	$C_{66}H_{89}N_{14}O_{14}S^+$	1333.6398 $[M+H]^{+1}$, 667.3235 $[M+2H]^{+2}$	1333.6313 $[M+H]^{+1}$, 667.3164 $[M+2H]^{+2}$	95%
UNC10245092 M6A	FWYGA**A**KALY	$C_{65}H_{87}N_{14}O_{14}^+$	1287.6521 $[M+H]^{+1}$, 644.3297 $[M+2H]^{+2}$	1287.5458 $[M+H]^{+1}$, 644.2725 $[M+2H]^{+2}$	>98%
UNC10245092 K7A	FWYGAM**A**ALY	$C_{64}H_{84}N_{13}O_{14}S^+$	1290.5976 $[M+H]^{+1}$, 1312.5795 $[M+Na]^+$	1290.6265 $[M+H]^{+1}$, 1312.6069 $[M+Na]^{+1}$	95%
UNC10245092 A8G	FWYGAMK**G**LY	$C_{66}H_{89}N_{14}O_{14}S^+$	1333.6398 $[M+H]^{+1}$, 667.3235 $[M+2H]^{+2}$	1333.6571 $[M+H]^{+1}$, 667.3297 $[M+2H]^{+2}$	90%
UNC10245092 L9A	FWYGAMKA**A**Y	$C_{64}H_{86}N_{14}O_{14}S^+$	1305.6085 $[M+H]^{+1}$, 653.3079 $[M+2H]^{+2}$	1305.6250 $[M+H]^{+1}$, 653.3125 $[M+2H]^{+2}$	80%
UNC10245092 Y10A	FWYGAMKAL**A**	$C_{61}H_{87}N_{14}O_{13}S^+$	1255.6292 $[M+H]^{+1}$, 628.3183 $[M+2H]^+$	1255.6378 $[M+H]^{+1}$, 628.3175 $[M+2H]^{+2}$	>98%
UNC10245121	FWYGAMKALYG	$C_{64}H_{84}N_{13}O_{14}S^+$	1401.6660 $[M+H]^{+1}$, 1423.6480 $[M+Na]^{+1}$	1401.6793 $[M+H]^{+1}$, 1423.6670 $[M+Na]^{+1}$	>98%
UNC10245350	GRKKRRQRRRPQEDGGS FWYGAMKALYG	$C_{146}H_{234}N_{53}O_{37}S^+$	1677.8940 $[M+2H]^{+2}$, 1118.9318 $[M+3H]^{+3}$, 839.4506 $[M+4H]^{+4}$, 671.7620 $[M+5H]^{+5}$	1678.3879 $[M+2H]^{+2}$, 1119.2613 $[M+3H]^{+3}$, 839.6978 $[M+4H]^{+4}$, 671.7637 $[M+5H]^{+5}$	95%
UNC10245351	GRKKRRQRRRPQEDGGS F**A**YGAMKAL**A**G	$C_{132}H_{225}N_{52}O_{36}S^+$	1574.3598 $[M+2H]^{+2}$, 1049.9089 $[M+3H]^{+3}$, 787.6835 $[M+4H]^{+4}$, 630.3483 $[M+5H]^{+5}$	1574.8634 $[M+2H]^{+2}$, 1049.9107 $[M+3H]^{+3}$, 787.6835 $[M+4H]^{+4}$, 630.3470 $[M+5H]^{+5}$	95%

Alexa647-UNC10245092	Alexa647-EDGGSFWYGAMKALYG	$C_{126}H_{169}N_{24}O_{39}S_6^+$	2834.0298 $[M+H]^{+1}$, 1418.0202 $[M+2H]^{+2}$, 945.6826 $[M+3H]^{+3}$	2834.0375 $[M+H]^{+1}$, 1418.0210 $[M+2H]^{+2}$, 945.6892 $[M+3H]^{+3}$	90%

UNC10245092



UNC10245092 F1A



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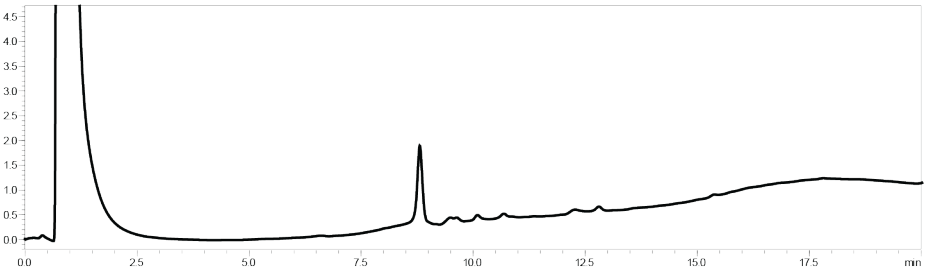

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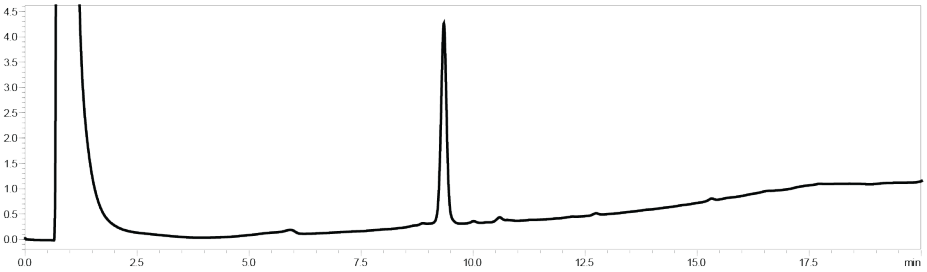

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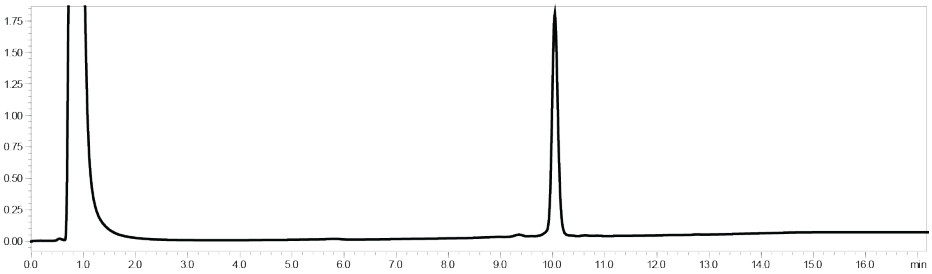

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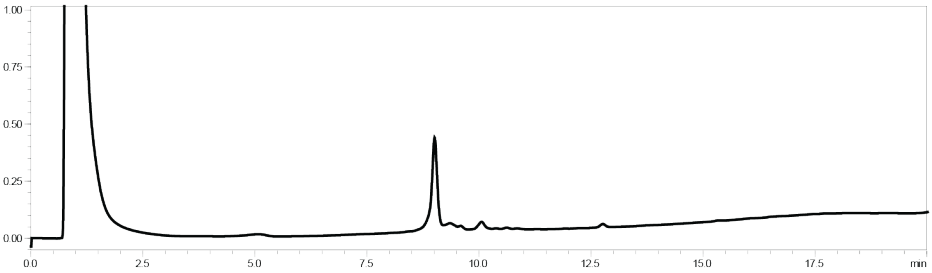

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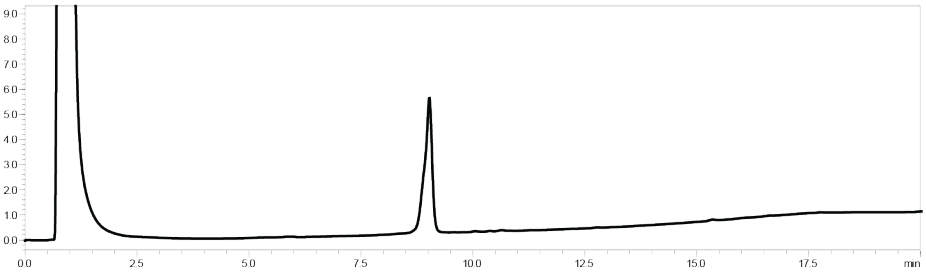

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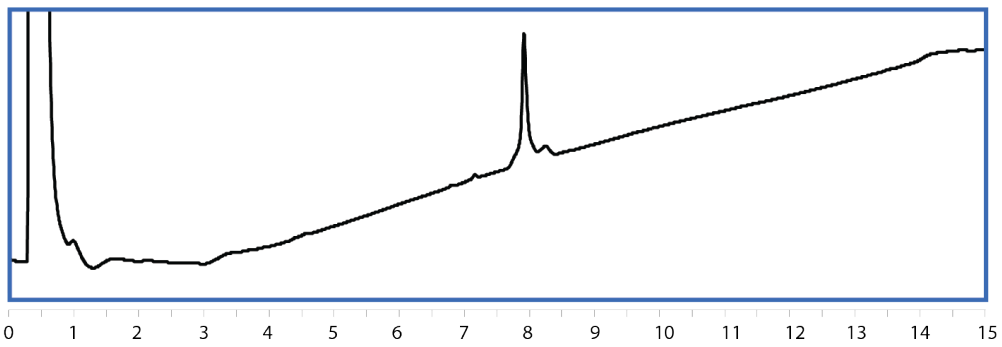

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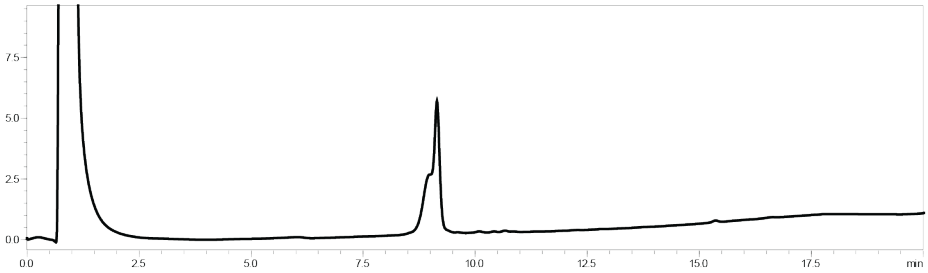

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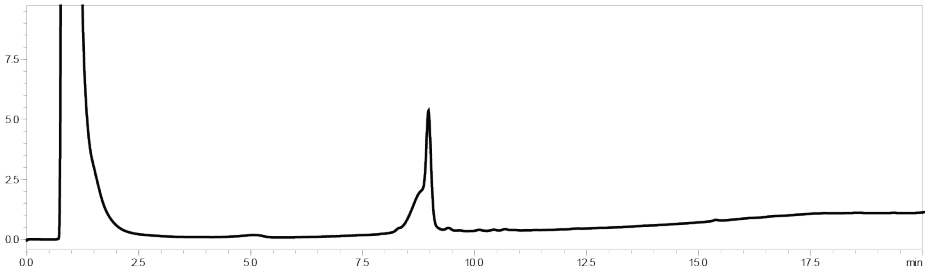

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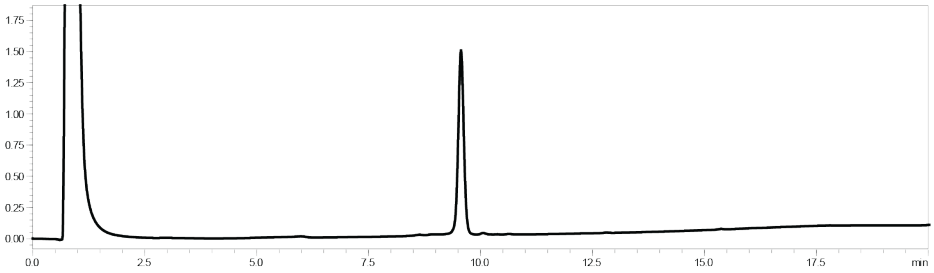

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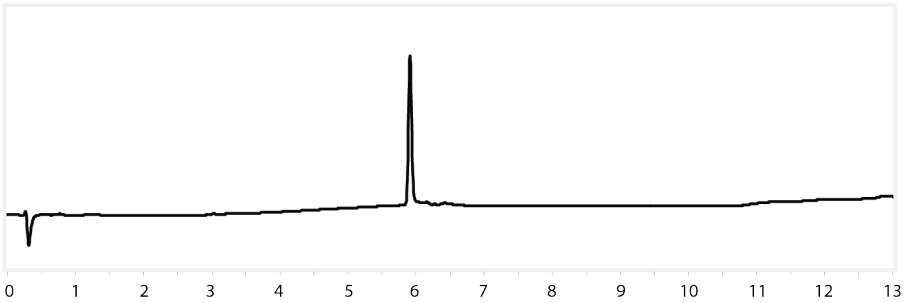

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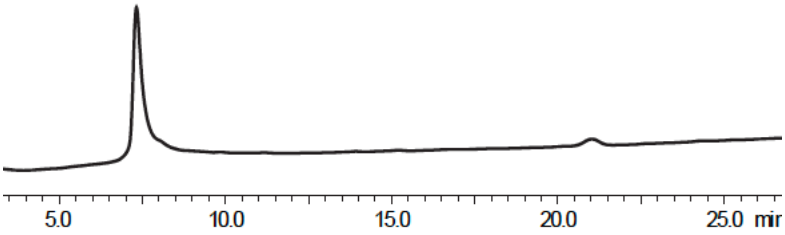

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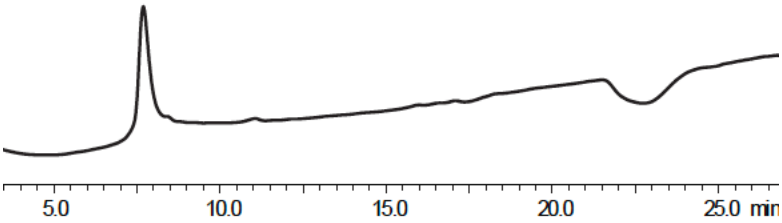
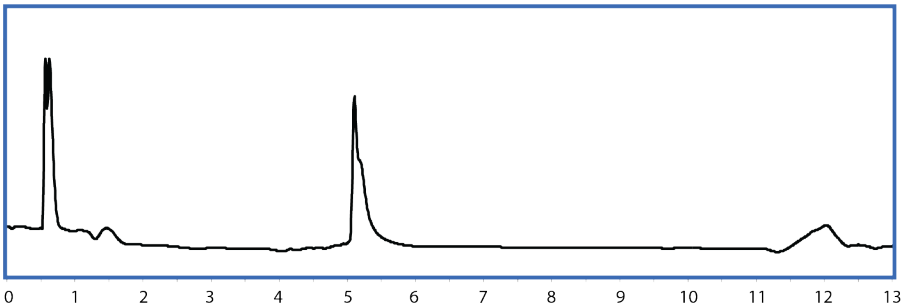
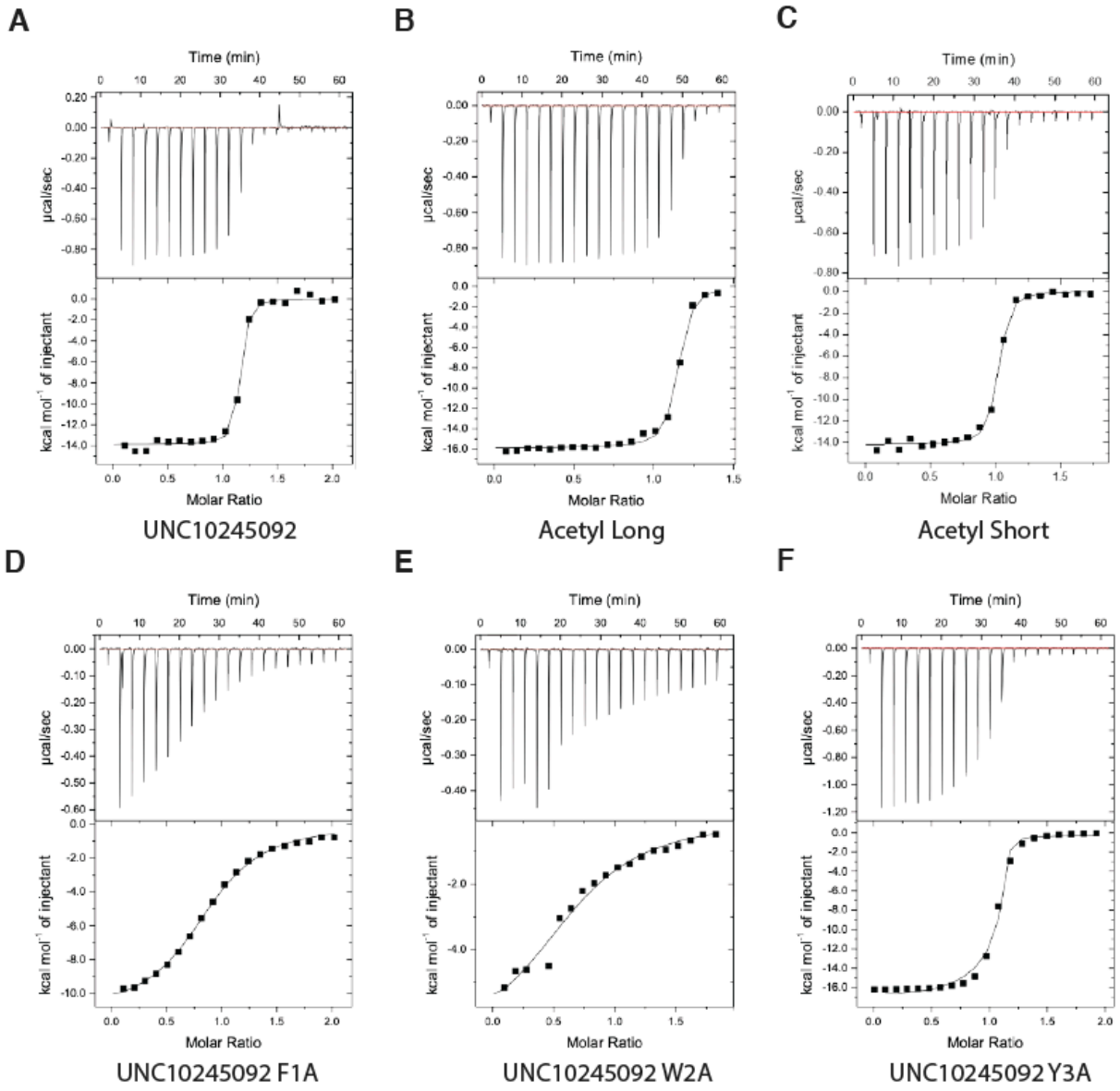


Alexa647 Labeled UNC10245092




A
Time (min)
µcal/sec
kcal mol⁻¹ of injectant
Molar Ratio
UNC10245092
B
Time (min)
µcal/sec
kcal mol⁻¹ of injectant
Molar Ratio
Acetyl Long
C
Time (min)
µcal/sec
kcal mol⁻¹ of injectant
Molar Ratio
Acetyl Short
D
Time (min)
µcal/sec
kcal mol⁻¹ of injectant
Molar Ratio
UNC10245092 F1A
E
Time (min)
µcal/sec
kcal mol⁻¹ of injectant
Molar Ratio
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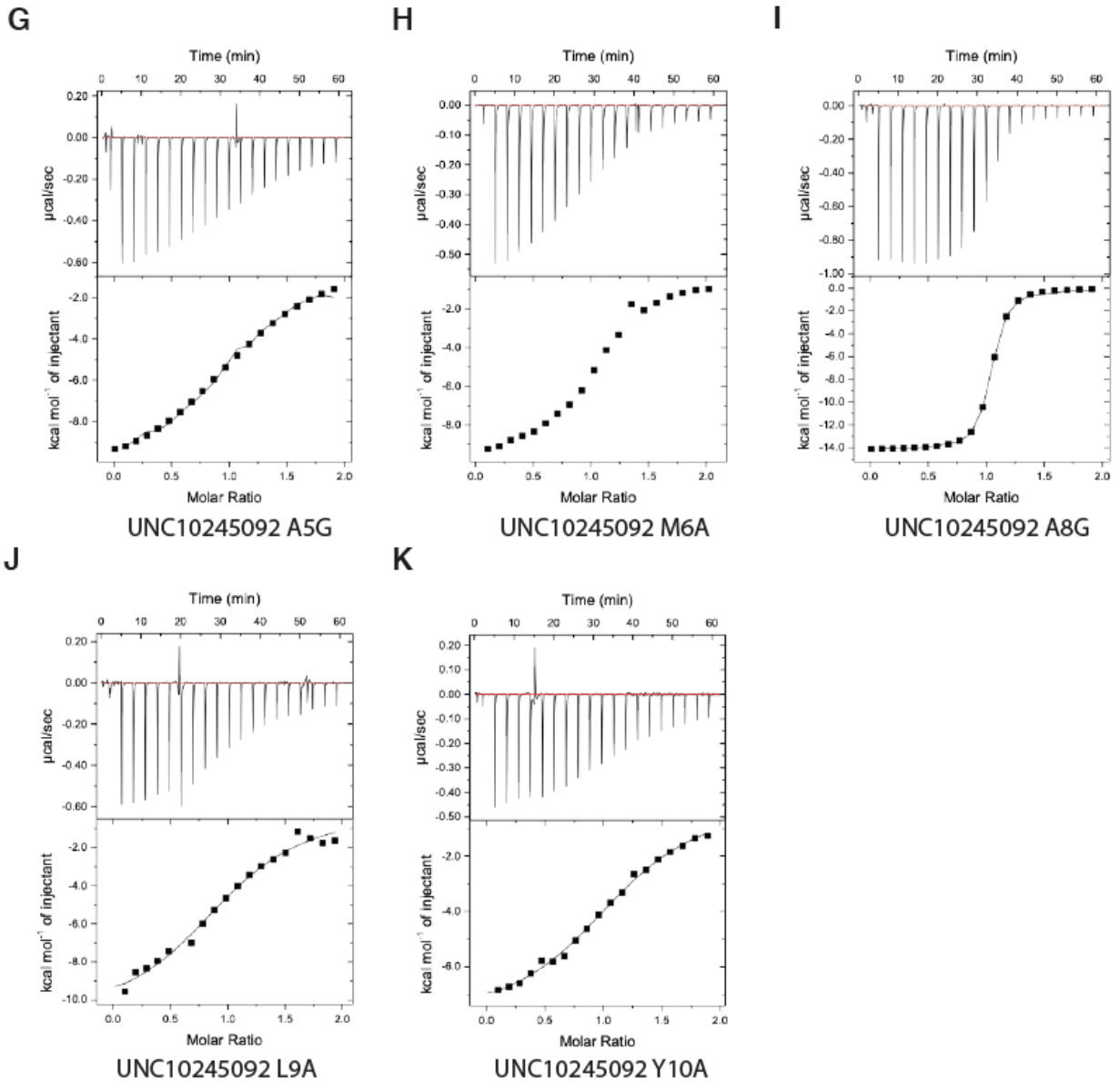

Figure S2. ITC curves of compounds found in Table 1. Curves are representative of a single run in ITC.

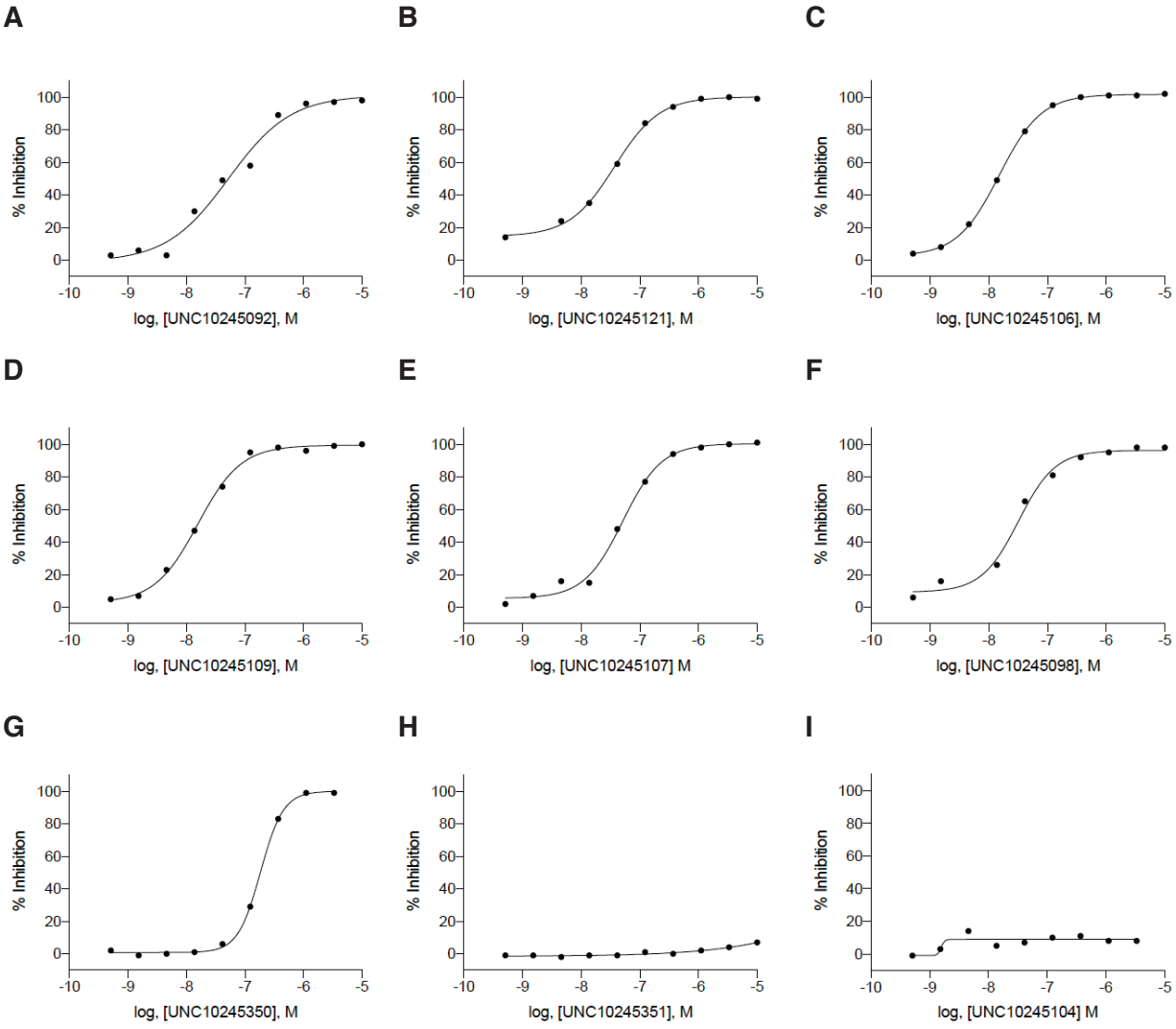

Figure S3. IC_{50} curves of compounds listed in Table 2. Curves are representative of a single run in TR-FRET.

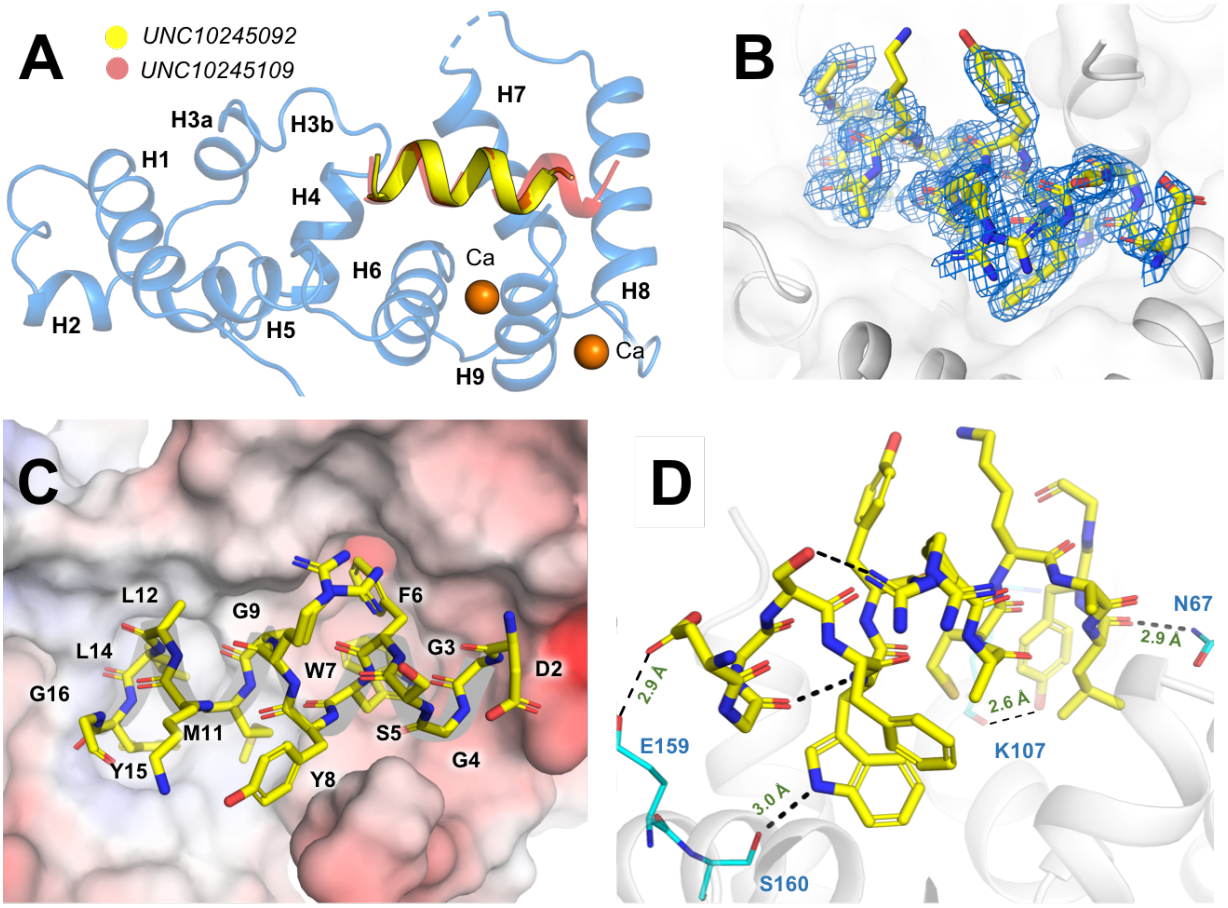

Figure S4. A) Crystal model of CIB1 (blue) in complex with peptides UNC10245092 (yellow) and UNC10245109 (red). Calcium atoms are depicted as orange spheres. B) 2Fobs-Fcalc electron density map of peptide UUNC10245109. (sigma of 1.0). C) Surface view of peptide UNC10245109 bound to CIB1. Surface is colored according with protein electrostatic potential calculated in APBS. D) Contact details of peptide UNC10245109 (yellow) and CIB1 (blue).

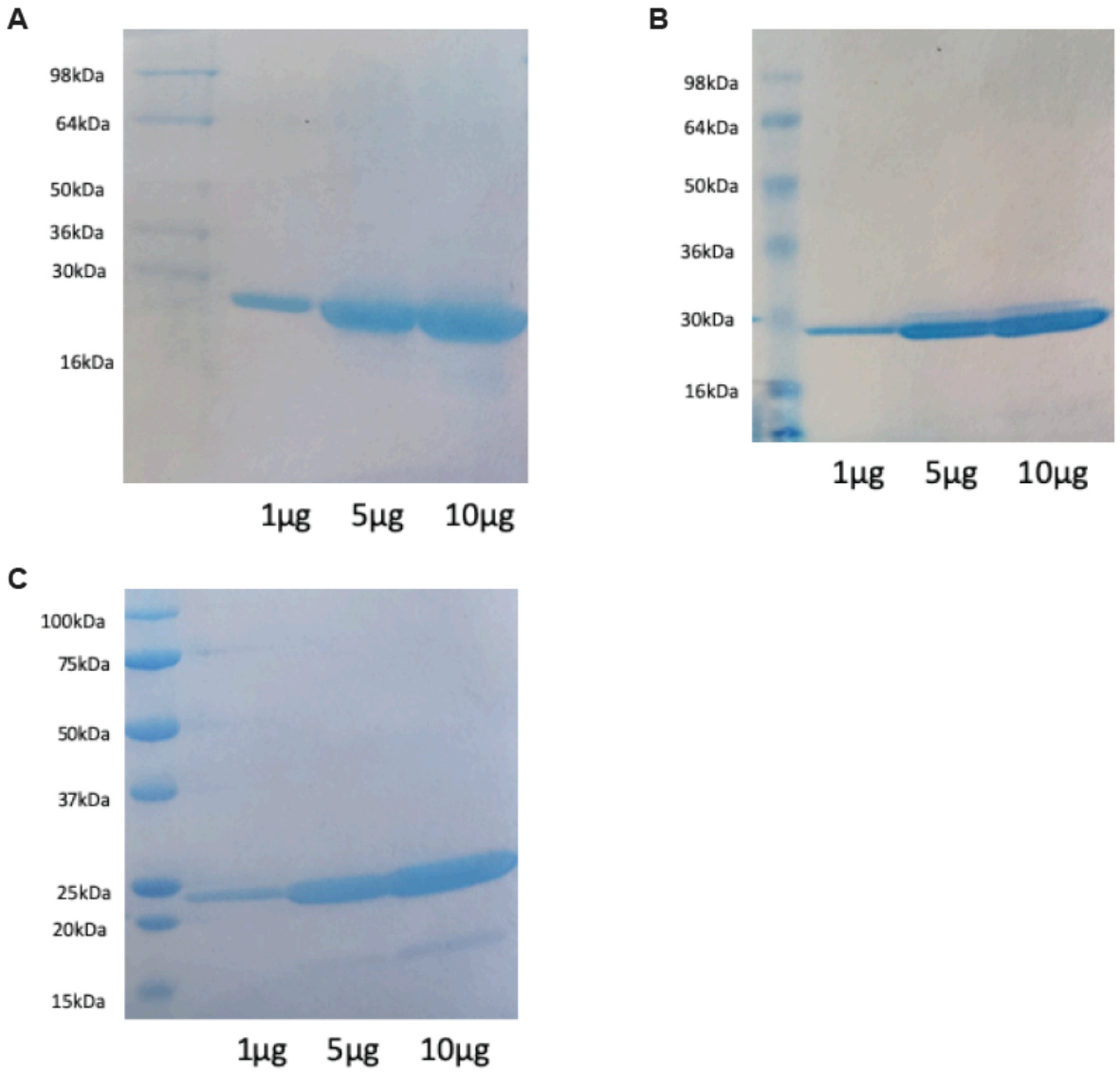

Figure S5. SDS-PAGE Gels of CIB1 proteins. All gels are 4-20% SDS. A) SDS-PAGE Coomassie stained gel of increasing concentrations of purified full length CIB1. Predicted molecular weight 25.4kDa. B) SDS-PAGE Coomassie stained gel of increasing concentrations of purified CIB1Δ1-8. Predicted molecular weight 21kDa. C) SDS-PAGE Coomassie stained gel of increasing concentrations of purified, biotinylated full length CIB1. Predicted molecular weight 25.6kDa.

Table S1. Data collection and refinement statistics (value in parentheses are for the outer shell)

	UNC10245092	UNC10245109
Space Group	$P2_1$	$P2_1$
Cell dimensions		
a,b,c (Å)	34.86, 85.97, 67.29	75.86, 33.03, 162.40
α,β,γ (°)	90, 91.91, 90	90, 99.85,90
Resolution (Å)	34.83-2.15 (2.25-2.15)	27.11-1.9 (1.97-1.9)
$R_{sym,}$ *(%)*	0.049 (0.644)	0.066 (0.637)
Mean I/σ*(I)*	8.3 (3.3)	5.57 (1.57)
$CC_{1/2}$	0.99 (0.63)	0.995 (0.609)
Completeness (%)	94.20 (74.99)	99.49 (99.68)
Redundancy	3.5 (2.5)	6.1 (6.0)
Refinement		
No of reflections	20253 (1580)	63567 (6305)
Molecules in a.s.u	2	4
R_{work}/R_{free} (%)	18.7/22.9	21.2/24.6
No. of atoms		
Protein	2684	5405
Water	192	696
Calcium	6	8
Average *B*-factor (Å^2)		
Protein	42.62	37.01
Water	49.91	31.62
Calcium	53.17	48.45
R.m.s deviations		
Bond lengths (Å^2)	0.012	0.013
Bond angles (^{O})	1.39	1.51
Ramachandran plot (%)		
Favored	100	99.85
Outliers	0	0
PDB code	6OD0	6OCX