High-Throughput Screening of One-Bead-One-Compound Libraries: Identification of Cyclic Peptidyl Inhibitors against Calcineurin/NFAT Interaction

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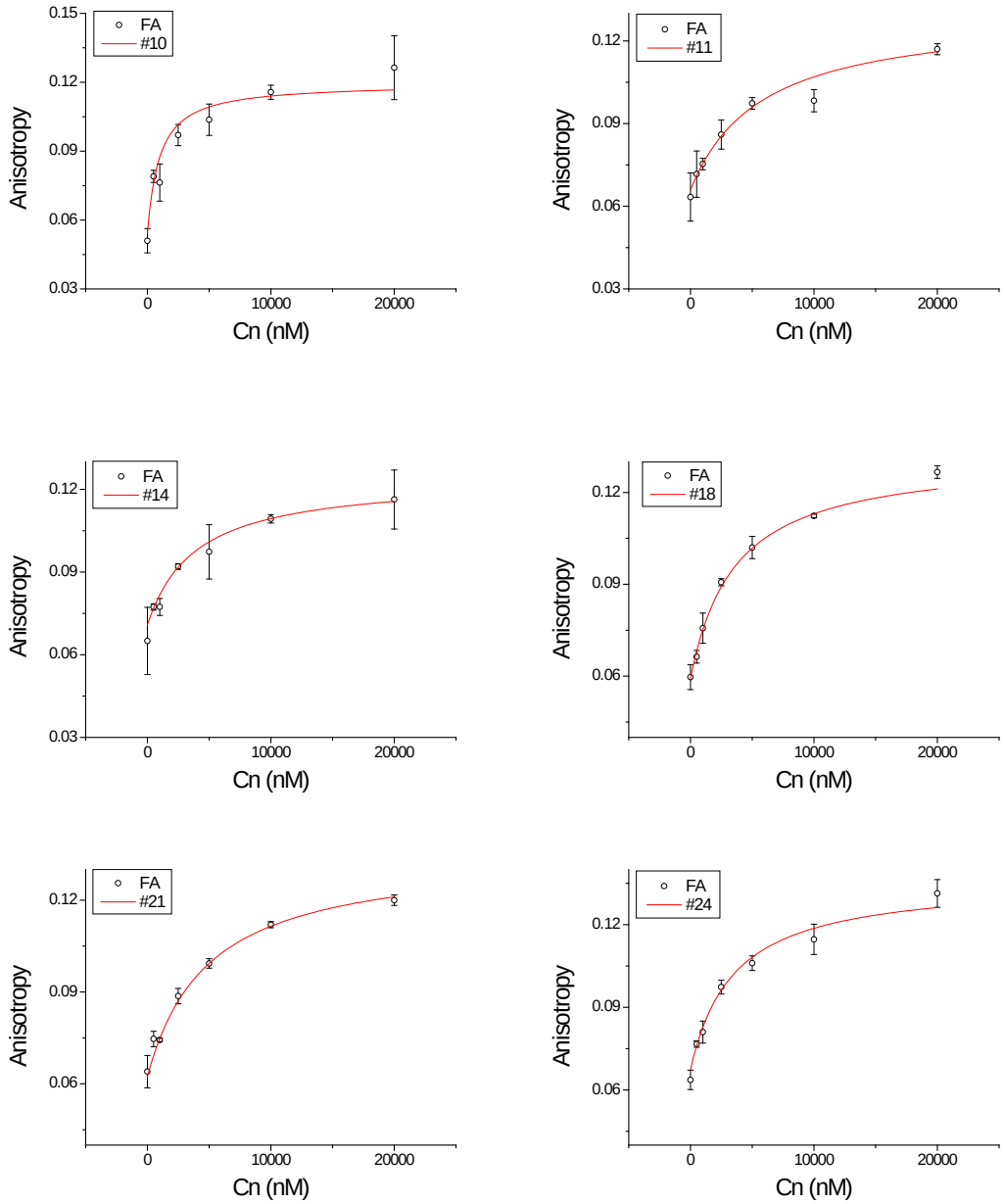
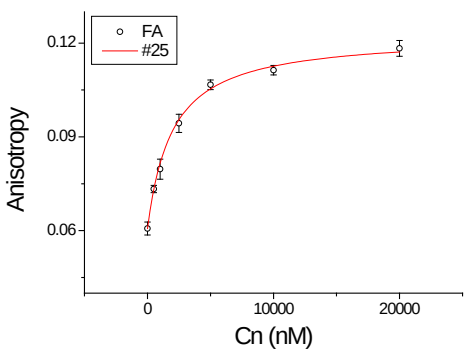
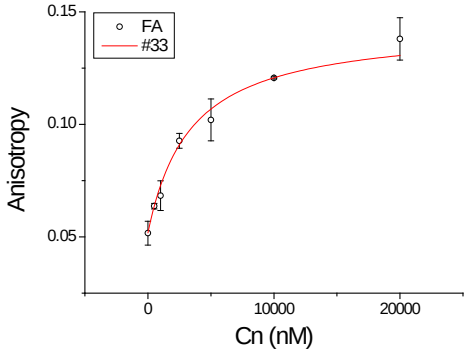
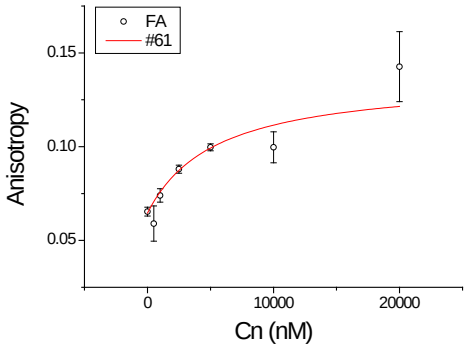
Supporting Information

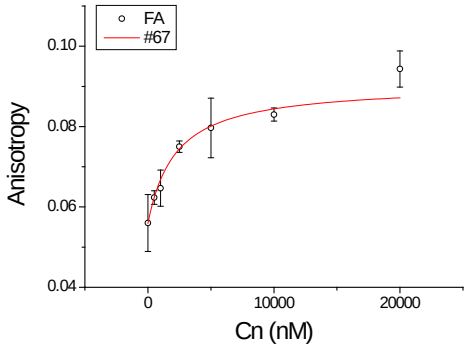
Figure S1. FA titration curves showing the binding interaction between Cn (0-20000 nM) and cyclic peptides released from individual hit beads (50 nM). All experiments were carried out in triplicates and the error bars indicate the standard deviation from the three measurements. The sequences of the 19 cyclic peptides are listed in Table 1.

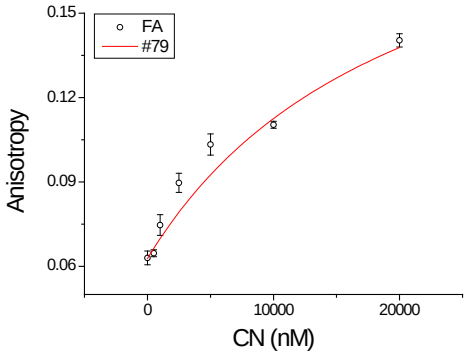


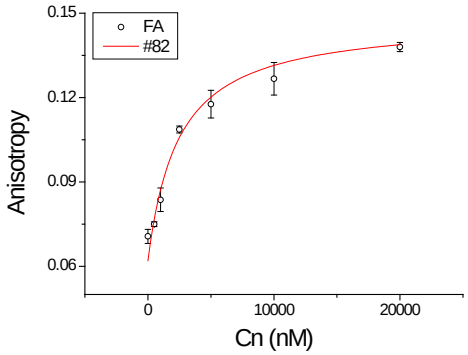

FA
#25
Anisotropy
0.12
0.09
0.06
0
10000
20000
Cn (nM)

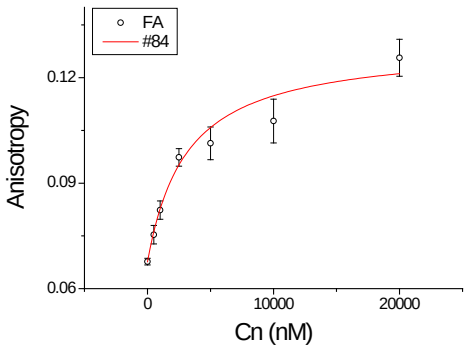

FA
#33
Anisotropy
0.15
0.10
0.05
0
10000
20000
Cn (nM)

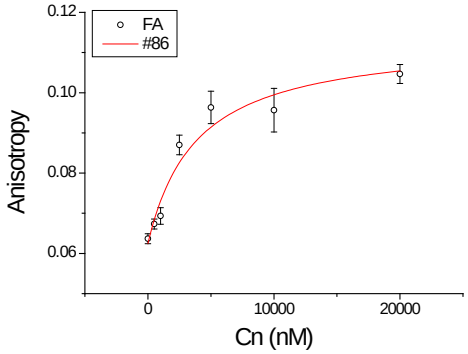

FA
#61
Anisotropy
0.15
0.10
0.05
0
10000
20000
Cn (nM)

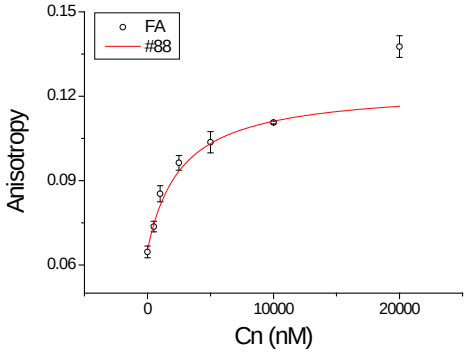

FA
#67
Anisotropy
0.10
0.08
0.06
0.04
0
10000
20000
Cn (nM)

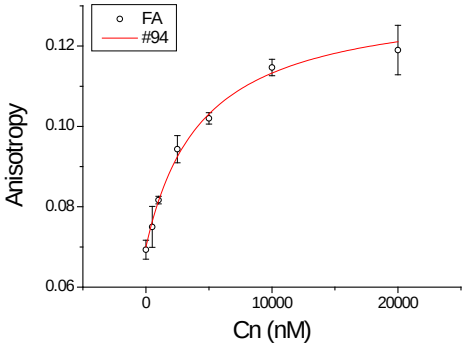

FA
#79
Anisotropy
0.15
0.12
0.09
0.06
0
10000
20000
CN (nM)

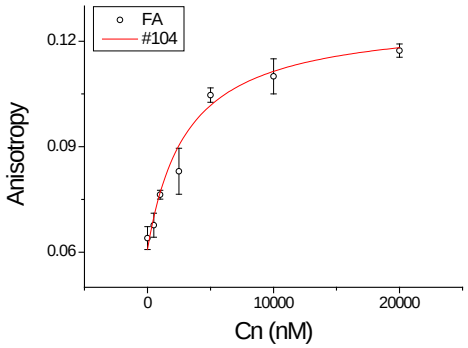

FA
#82
Anisotropy
0.15
0.12
0.09
0.06
0
10000
20000
Cn (nM)

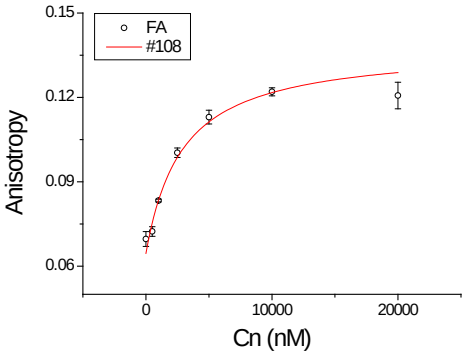

FA
#84
Anisotropy
0.12
0.09
0.06
0
10000
20000
Cn (nM)


FA
#86
Anisotropy
0.12
0.10
0.08
0.06
0
10000
20000
Cn (nM)


FA
#88
Anisotropy
Cn (nM)
0.15
0.12
0.09
0.06
0
10000
20000


FA
#94
Anisotropy
Cn (nM)
0.12
0.10
0.08
0.06
0
10000
20000


FA
#104
Anisotropy
Cn (nM)
0.12
0.09
0.06
0
10000
20000


FA
#108
Anisotropy
Cn (nM)
0.15
0.12
0.09
0.06
0
10000
20000

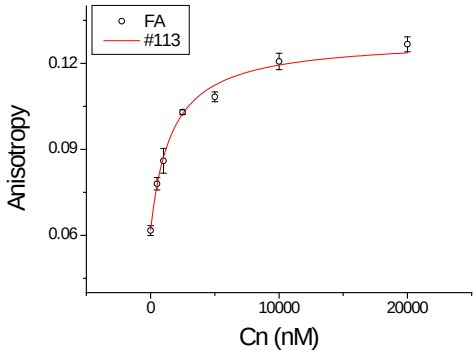
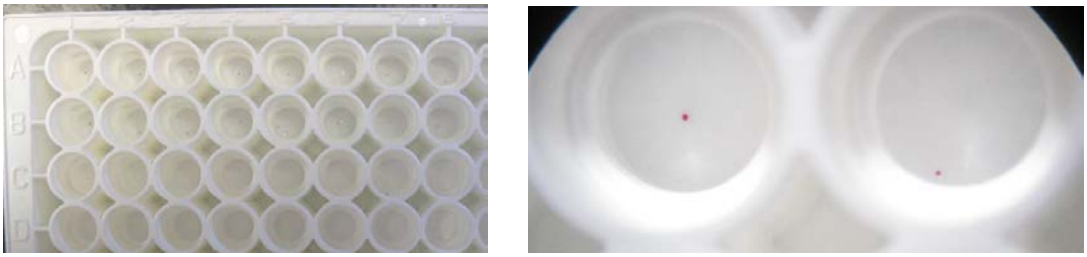
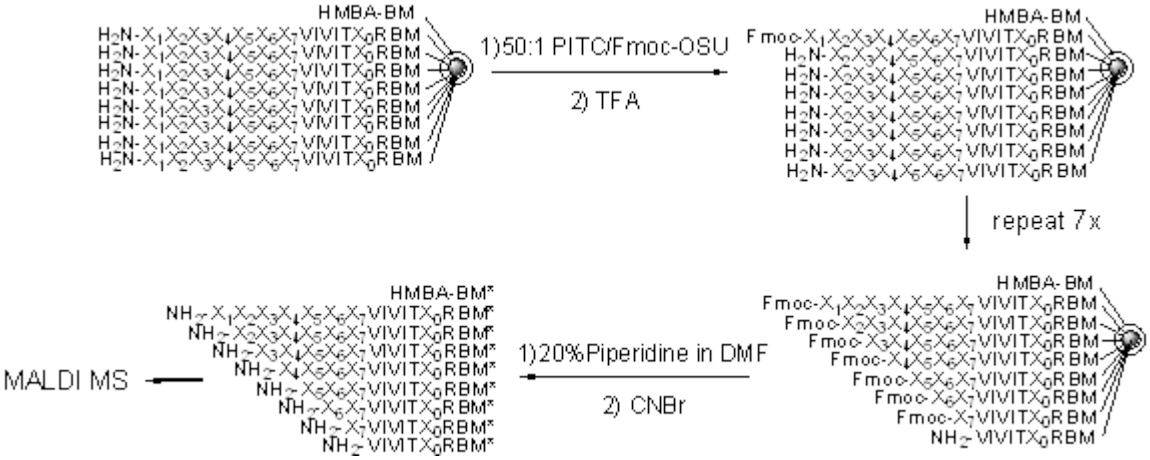

FA
#113
Anisotropy
Cn (nM)
0.12
0.09
0.06
0
10000
20000

Figure S2. Peptide sequencing by PED/MS. A) A photograph of the AcroPrep 96-well filter plate (Pall Corporation, PN5030) used for the PED reaction. B) Reactions and conditions of PED. C) A representative MALDI/TOF MS spectrum, derived from a bead containing the sequence of cyclo(Ser-Pip-Gln-Asp-Tyr-D-Ala-Pro-Val-Ile-Val-Ile-Thr-Ala-Ala-Ala-Glu).

A



B



C

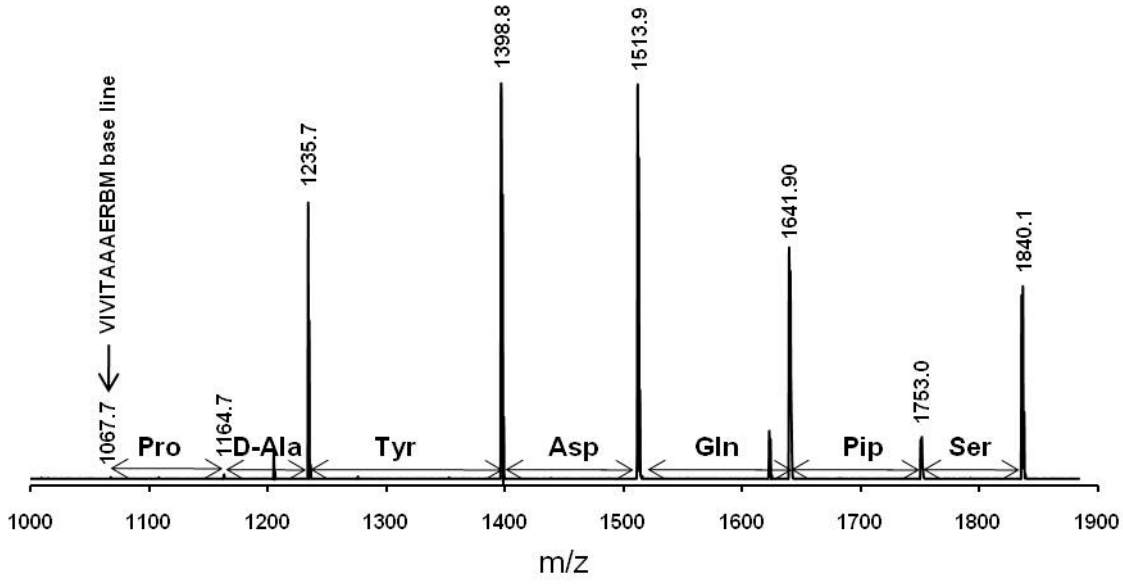


Figure S3. FA titration curves showing the binding interaction between Cn (0-20000 nM) and resynthesized and HPLC purified cyclic peptides (100 nM). All experiments were carried out in triplicates and the error bars indicate the standard deviation from the three measurements. The sequences of the 6 cyclic peptides are listed in Table 1.

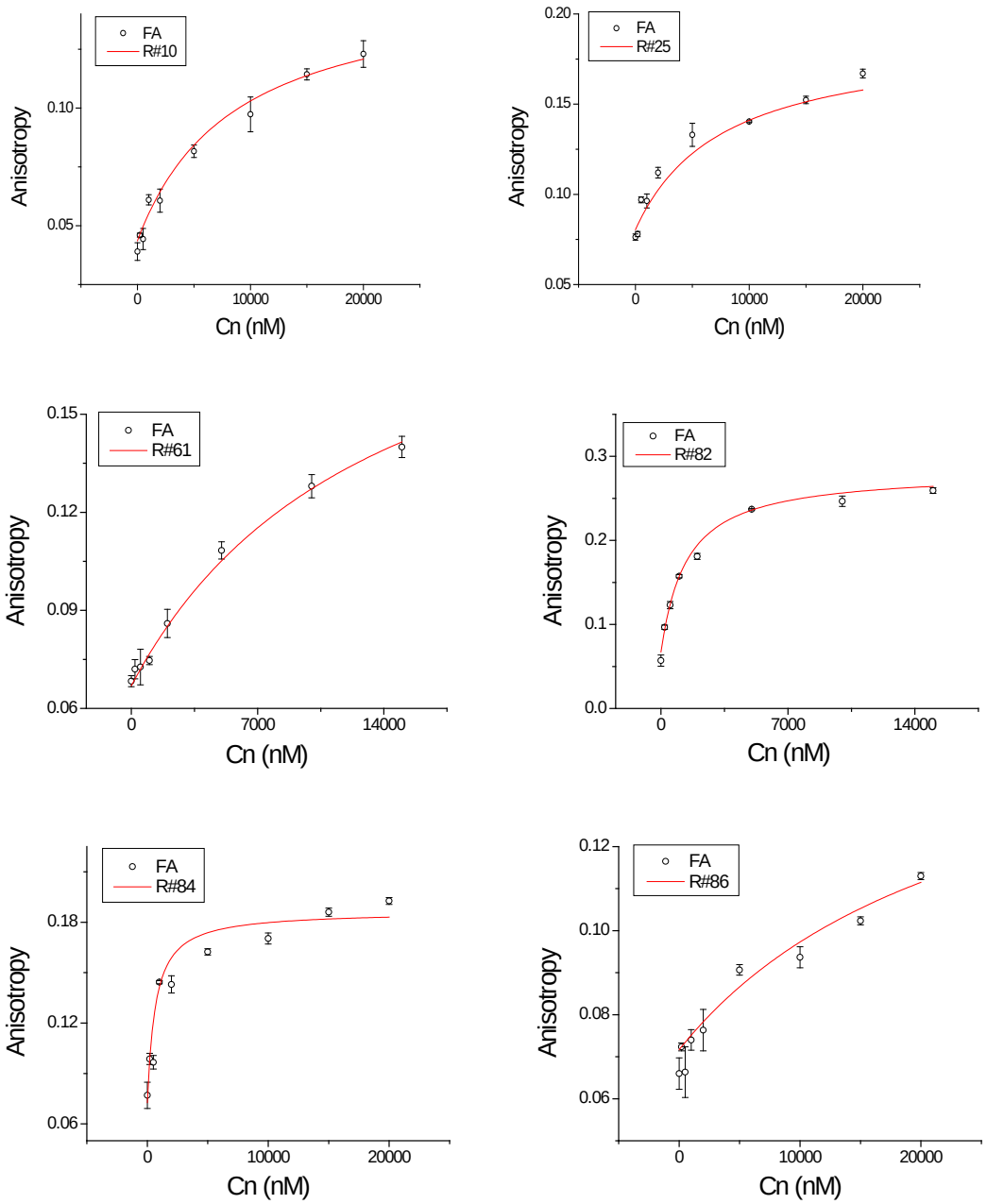
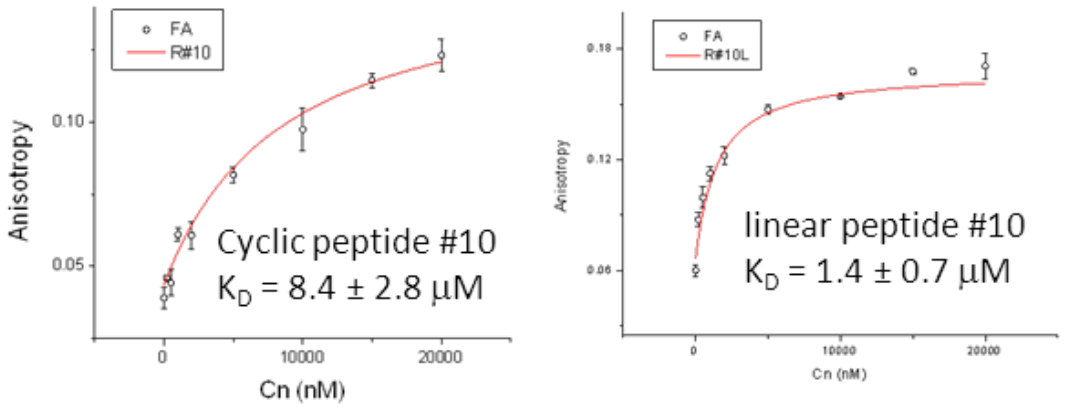
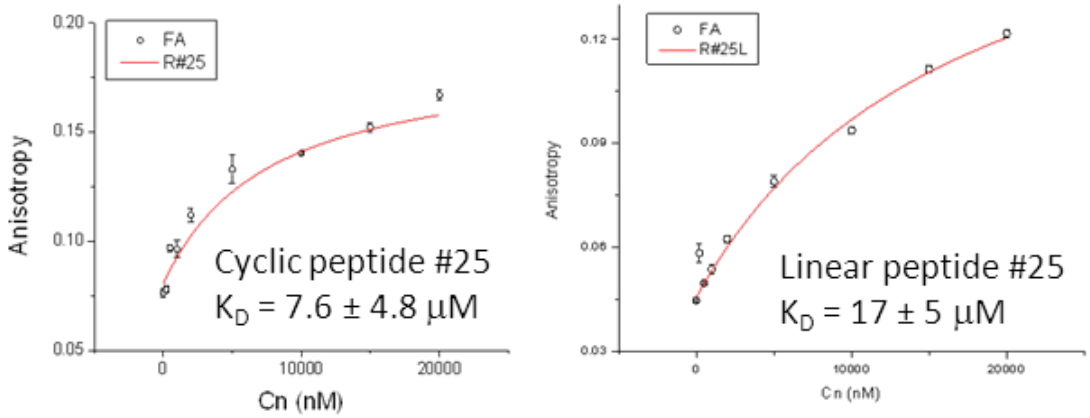


Figure S4. Comparison of cyclic vs linear peptides for their binding affinity to Cn. It was found that for some sequences (e.g. #10), the linear peptides had higher affinity than the corresponding cyclic peptides, while for others (e.g., #25) the trend was opposite.

A) Binding of cyclic and linear peptide #10 to Cn



B) Binding of cyclic and linear peptide #25 to Cn



C) Binding of peptide GPHPVIVIT to Cn