An Effective Strategy for Stabilizing Minimal Coiled Coil Mimetics

Michael G. Wu, Andrew B. Mahon, and Paramjit S. Arora

Department of Chemistry, New York University, 100 Washington Square East, New York, NY 10003

e-mail: arora@nyu.edu

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| Sequence                          | \(\Delta\Delta G\) from Rosetta analysis in kcal/mol |
|----------------------------------|-----------------------------------------------------|
|                                 | E501 (1.41)                                         |
| S01 - EWKHLDHLLN                | H504 (1.81)                                         |
|                                 | L508 (1.40)                                         |
| S18 - KTRRSLTVLR                | V525 (1.05)                                         |
|                                 | S522 (H-bonding)                                    |
Fig S4. Helical wheel diagrams of (a) mono-crosslinked and (b) disulfide-stabilized double crosslinked NHR2 helix dimers. (c) CD spectra of designed CHD_NHR2 peptides. CD spectra were acquired in 50 mM aqueous KF, pH 7.4.
Supporting Methods

General. Research grade solvents and reagents were used without further purification. Fmoc amino acids and peptide synthesis reagents were purchased from Novabiochem and Chem-Impex International. Fmoc-azido amino acids were synthesized as previously described.\textsuperscript{3,4} Hoveyda-Grubbs (second generation) catalyst, and molecular biology grade salts and buffers were obtained from Sigma.

Solid-phase peptide synthesis

Figure S5. Solid phase synthesis of hydrogen-bond-surrogate coiled-coiled mimic AB-1
Synthesis of Hydrogen-Bond Surrogate Coiled Coil Mimic (AB-1)

HBS peptides were synthesized as previously described. Peptide sequences up to the i+3rd residue of the parent strand were synthesized on solid phase on a CEM® Liberty Peptide Synthesizer. A solution containing premixed o-nitrobenzenesulfonyl chloride (10 eq) and 2,4,6-collidine (10 eq) in DCM was added to resin containing Fmoc-deprotected peptide. Resin was washed sequentially with dichloromethane, dimethylformamide and diethyl ether (3x5 mL each). Resin was dried overnight under vacuum. Dried resin, PPh3, and Pd2(dba)3, were flushed under inert argon for 30 minutes. The resin with reactants was swelled in THF, and allyl methyl carbonate was added to the reaction vessel. The solution was agitated at room temperature for 3 to 5 hours under argon to afford allylated peptide. Resin was filtered and washed with DCM, DMF, 0.2 M sodium diethylcarbamate trihydrate in NMP, and diethyl ether (3x5 mL). The noryl protecting group was then removed by the addition of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 5 eq) and 2-mercaptoethanol (10 eq.) in DMF. Resin was washed with DMF, DCM, and diethyl ether (3x5 mL) and treated with the desired Fmoc amino acid (20 eq.), DIC (20 eq.) and HOAt (10 eq.) in DMF. The reaction was allowed to agitate at room temperature for 12 to 16 hours. Resin containing elongated peptide was washed, and coupled to the desired Fmoc amino acid residue (5 eq.) and 4-pentenoic acid (5 eq.) with HBTU (5 eq.) and DIEA (10 eq.) in DMF. Ring-closing metathesis of bis-olefin 9 was performed with HoveydaGrubbs II catalyst (20 mol%) in 1,2-dichloroethane under microwave irradiation at 120 ºC for 10 min as previously described. The ring-closing reaction was monitored by MALDI-TOF. Peptides were cleaved from the resin using 95% trifluoroacetic acid, 2.5% TIPS, and 2.5% H2O, and purified by reversed-phase HPLC (gradient 15-60 acetonitrile/water with 0.1% TFA over 60min) and characterized by MALDI-TOF.
**Figure S6.** Solid phase synthesis for macrocycle coiled-coiled mimic AB-2

**Synthesis of Macrocycle Coiled Coil Mimic (AB-2)**

PEG RAM resin 0.4 mmol/g was swelled in DMF and preloaded with Fmoc-Asp-OAllyl (1 equiv), HBTU (1.5 equiv), and diisopropylethylamine (1.5 equiv) for 1 hr. The resin was then N-acetyl capped with 0.5 M acetic anhydride (2x5 mL) and the loading was modified to ~0.2 mmol/g loading as assessed by % loading. Solid phase peptide synthesis was performed using standard Fmoc solid phase chemistry on a CEM® Liberty Peptide Synthesizer. The resin bearing the parent peptide was transferred to a fritted polypropylene SPE tube. Following N-terminal deprotection with 20% piperidine in NMP (2x5 mL) and washing with dichloromethane, methanol and dimethylformamide (3x5 mL each), allyl deprotection was performed using Pd(PPh₃)₄ (3 equiv) in a solution of chloroform: acetic acid: N-methylmorpholine (37:3:1). After 3 hours, the resin was washed again with dichloromethane, methanol and dimethylformamide (3x5 mL each). Addition of PyBOP (1.5 equiv) and DIPEA (1.5 equiv) yielded complete macrocyclization with no observed linear product. Peptides were cleaved from the resin using 95% trifluoroacetic acid, 2.5% TIPS, and 2.5% H₂O, and purified by reversed-phase HPLC (gradient 15-60 acetonitrile/water with 0.1% TFA over 60min) and characterized by MALDI-TOF.
Figure S7. Solid phase synthesis for disulfide coiled-coiled mimic AB-3

Synthesis of Disulfide Coiled Coil Mimic (AB-3)

Parent peptide (0.25 mmol) was synthesized on a CEM® Liberty Peptide Synthesizer using standard Fmoc solid phase chemistry with Knorr Amide MBHA resin, and N-acetyl capped with 0.5 M acetic anhydride in DMF (2x5 mL) resulting in resin-bound coiled coil mimic. The peptide was treated with a solution containing 95% trifluoroacetic acid, 2.5% TIPS, and 2.5% H$_2$O. After 3 hours, the reaction mixture was filter and concentrated in vacuo. The crude solid was precipitated with cold diethyl ether and dried under a stream of nitrogen gas. Peptides were purified by reversed-phase HPLC (gradient 15-60 acetonitrile/water with 0.1% TFA over 60 min), and after lyophilization yielded bisthiol as a white powder. The bisthiol was oxidized with 20% DMSO, 20% TFE in 0.8 M ammonium bicarbonate and 0.84 M acetic acid, pH 6.0 affording only intramolecular disulfide formation as monitored by MALDI-TOF.$^{8,9}$ 10-30 mg purified product recovered from 0.25 mmol scale.

Synthesis of Crosslinked Helix Dimer Coiled Coil Mimic (AB-4)

Parent peptide (0.25 mmol) was synthesized on a CEM® Liberty Peptide Synthesizer using standard Fmoc solid phase chemistry with Knorr Amide MBHA resin. The resin bearing the parent peptide was transferred to a fritted
polypropylene SPE tube, washed, and transferred to a microwave tube. The resin was subsequently swelled in 3 mL of NMP and the bisalkyne propargyl ether (257 μL, 2.5 mmol, 10 equiv) was added.

A solution of CuSO$_4$ (20 mg, 0.125 mmol, 0.5 equiv) dissolved in 500 μL of water was separately prepared. To this solution, Tris[[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (132 mg, 0.25 mmol, 1 equiv) dissolved in 1 mL of NMP was added. This mixture was added to a solution of sodium ascorbate (495 mg, 2.5 mmol, 10 equiv) prepared in 1.5 mL of water. The resulting mixture was pipetted into the microwave tube containing propargyl ether and peptide. A magnetic stir bar was added, and the reaction mixture was subjected to microwave irradiation at 85 °C for 45 min, after which the resin was transferred to a fritted polypropylene SPE tube and washed with a 20 mM solution of sodium diethyldithiocarbamate in water (3x15 mL) followed by NMP (3x15 mL). A microcleavage of resin (95% trifluoroacetic acid, 2.5% TIPS, and 2.5% H$_2$O) showed the starting material to be consumed after one reaction.

Following the initial CuAAC reaction, the mono-triazole peptide was transferred to another microwave tube containing CuSO$_4$ (20 mg, 0.125 mmol, 0.5 equiv), sodium ascorbate (149 mg, 0.75 mmol, 3 equiv), Fmoc-azidolysine-NH$_2$ (294 mg, 0.75 mmol, 3 equiv), and Tris[[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (132 mg, 0.25 mmol, 1 equiv) in prepared solution as described above. The resulting reaction mixture was subjected to microwave irradiation at 85 °C for 45 min, after which the resin was transferred to a fritted polypropylene SPE tube and washed as described above. A microcleavage of resin (95% trifluoroacetic acid, 2.5% TIPS, and 2.5% H$_2$O) showed the starting material to be consumed after one reaction.

The resulting on-resin peptide was added to the CEM Liberty microwave peptide synthesizer. The sequence was continued using standard FMOC solid phase chemistry and N-acetyl capped with 0.5 M acetic anhydride in DMF (2x5 mL) resulting in resin-bound coiled coil mimic. The peptide was treated with a solution containing 95% trifluoroacetic acid, 2.5% TIPS, and 2.5% H$_2$O. After 3 hours, the reaction mixture was filter and concentrated in vacuo. The crude solid was precipitated with cold diethyl ether and dried under a stream of nitrogen gas. HPLC purification (gradient 15-60 acetonitrile/water with 0.1% TFA over 60min) and lyophiliziation yielded peptide as a white powder characterized by MALDI-TOF. CHD-peptides yield, sequence dependently, 20-40 mg of peptide from a 0.25 mmol scale.
Synthesis of Crosslinked Helix Dimer\textsuperscript{18} Coiled Coil Mimics

Parent peptide (0.25 mmol) was synthesized as described above where upon elongation, consecutive CuAAC reactions were implemented, and further elongated using standard FMOC solid phase chemistry to afford the dithiol crosslinked helix dimer (CHD\textsuperscript{DT}). After N-acetyl capping with 0.5 M acetic anhydrided in DMF (2x5 mL), the CHD\textsuperscript{DT}-peptide was cleaved from resin using 94% trifluoroacetic acid, 2.5% 1,2-ethanedithiol, 2.5% H\textsubscript{2}O, and 1% triisopropylsilane. The resulting peptide was precipitated with cold diethyl ether and dried under a stream of nitrogen gas. Crude peptide was purified by reversed-phase HPLC (gradient 15-60 acetonitrile/water with 0.1% TFA over 60min), and after lyophilization yielded bisthiol as a white powder. The bisthiol was oxidized with 20% DMSO, 20% TFE in 0.8 M ammonium bicarbonate and 0.84 M acetic acid, pH 6.0 affording only intramolecular disulfide formation as monitored by MALDI-TOF.\textsuperscript{3,9}
**Analytical HPLC and Mass Spectrometry.** Analytical HPLC traces of peptides were obtained at 220 nm from a gradient of 10% B to 90% B over 10 min on an XTerra RP18 3.5 µm 2.1 x 150 mm column (Part No. 186000410); A: 0.1% aqueous TFA, B: acetonitrile; flow rate 400 µL/min. Exact masses were found using a Bruker Matrix-assisted laser desorption/ionization (MALDI-TOF) instrument. 10-30 mg purified product recovered from 0.25 mmol scale.

![Analytical HPLC traces of peptides](image)

**Figure S8.** Analytical HPLC traces of coiled-coil mimics
Figure S8 (cont’d). Analytical HPLC traces of coiled-coil mimics
Circular Dichroism Spectroscopy. CD spectra were recorded on an AVIV 202SF CD spectrometer equipped with a temperature controller using 1 mm length cells and a scan speed of 5 nm/min. The spectra were averaged over 8 scans with the background subtracted according to the analogous experimental conditions. Each sample was prepared in a 50 mM potassium fluoride solution in water (pH 7.4) with a final concentration of 20 µM. The concentrations of each peptide was determined by the UV absorption of tryptophan residues at 280 nm.

Table S2. Summary of circular dichroism spectroscopy data for antiparallel coiled coil mimics

| Compound | Modification | Minimum at 222/208 | $\theta_{222}$ |
|----------|--------------|--------------------|---------------|
| AB*      | N/A          | 0.58               | 9510          |
| AB-1     | N-terminal HBS constraint | 0.73               | 8475          |
| AB-2     | Two GGSSGG linkers at the | 0.62               | 8950          |
| AB-3     | Interhelical cysteine disulfide bridges at | 0.62               | 9460          |
| AB-4‡    | Bis-triazole azidolysine | 0.86               | 9935          |
| AB-5‡    | Bis-triazole azidohomoalnine | 0.71               | 11450         |
| AB-6‡    | Bis-triazole azidoalanine | 0.46               | 8505          |

*Exhibits random coil-like signature; ‡Click reaction performed with propargyl ether
NMR Spectroscopy. All experiments were carried out on a Bruker Avance 600 MHz spectrometer at 25 °C. A 500 μM solution of AB-4 was prepared in 400 μL of 10% d3-CH3CN in H2O with 0.1% trifluoroacetic acid pH =5. Proton NMR, TOCSY, and NOESY spectra were used to assign amide protons (Table S2, S3) Solvent supression was achieved with a 3919 Watergate pulse sequence. All 2D spectra were recorded by collecting 4092 complex data points in the t2 domain by averaging 64 scans and 128 increments in the t1 domain with the States-TPPI mode. TOCSY experiments were performed with a mixing time of 80 ms, while NOESY experiments were performed with a mixing time of 300 ms. All NMR data were processed and analyzed using the Bruker TOPSPIN program. The original free induction decays were zero-filled to give a final matrix of 2048 by 2048 real data points. A 90° sine-squared window function was applied in both dimensions. Nucleaver Overhauser effect (NOE) cross-peaks are listed in Table S3.

Figure S9. $^1$H NMR spectrum of AB-4
Fig S10. Fingerprint region of NOESY from AB-4
Table S3. $^1$H NMR assignments and chemical shifts ($\delta$, ppm) for AB-4 (298 K) in 10% d$_3$-CH$_3$CN in D$_2$O (pH 5), and calculated dihedral angles, $\Phi$, derived from $^3$J$_{NH\alpha H}$ coupling constants

| Residue | $\Phi^\circ$ | NH   | Ha   | H$\beta$ | H$\gamma$ | H$\delta$ | He   |
|---------|-------------|------|------|---------|---------|--------|------|
| E (A1)  | -53.86      | 7.824| 4.10 | N/A     | N/A     | N/A    | N/A  |
| L (A2)  | -52.67      | 8.217| 4.15 | 1.89    | N/A     | 0.756  | N/A  |
| A (A3)  | -52.67      | 8.06 | 4.03 | 1.54    | N/A     | N/A    | N/A  |
| E (A4)  | -55.60      | 7.907| 3.97 | 1.96    | 2.31    | N/A    | N/A  |
| L (A5)  | -57.84      | 7.75 | 4.03 | 1.58    | N/A     | N/A    | N/A  |
| Z (A6)  | -52.07      | 7.16 | 4.06 | 1.76    | 1.57    | N/A    | 3.08 |
| W (A7)  | -51.47      | 7.65 | 4.48 | 3.19    | N/A     | N/A    | N/A  |
| R (A8)  | -57.29      | 7.85 | 3.9  | 1.58    | 1.04    | N/A    | N/A  |
| L (A9)  | -52.67      | 7.818| 4.10 | 1.44    | 1.39    | 1.38$\delta$ | 1.29$\delta$ |
| L (B1)  | -55.60      | 7.76 | 3.80 | 2.05    | 1.41    | 1.04   | N/A  |
| W (B2)  | -55.60      | 7.91 | 4.38 | 3.19    | N/A     | N/A    | N/A  |
| E (B3)  | -56.73      | 8.18 | 3.98 | N/A     | N/A     | N/A    | N/A  |
| R (B4)  | -52.07      | 7.143| 4.04 | N/A     | N/A     | N/A    | N/A  |
| I (B5)  | -53.27      | 7.77 | 3.92 | 1.76    | N/A     | 0.74   | N/A  |
| A (B6)  | -55.60      | 8.03 | 3.97 | 1.24    | N/A     | N/A    | N/A  |
| R (B7)  | -55.60      | 8.2  | 4.12 | N/A     | N/A     | N/A    | N/A  |
| L (B8)  | -49.62      | 7.78 | 4.13 | 1.60    | N/A     | 0.76   | N/A  |
| Z (B9)  | -58.38      | 7.1  | 4.04 | 1.66    | 1.39    | N/A    | 3.5   |

$^3$J$_{NH\alpha H}$ coupling constants were obtained from TOCSY spectrum. $^9$ $\phi$ angles were calculated by applying the Pardi parameterized Karplus equation. $^{11}$
Table S3. Observed NOE crosspeaks from NOESY spectra of AB-4

| Residue       | Chemical shift, ppm | NOE intensity |
|---------------|---------------------|---------------|
| Atom 1        | Atom 2              |               |
| E(A1)-NH      | E(A1)-Ha            | 7.82          | 4.10          | strong          |
| E(A1)-Ha      | L(A5)-Hβ            | 4.10          | 1.58          | weak            |
| L(A2)-NH      | L(A2)-Ha            | 8.216         | 4.15          | strong          |
| L(A2)-Ha      | L(A2)-Hβ            | 4.15          | 1.89          | medium          |
| L(A2)-Ha      | A(A3)-Hβ            | 4.15          | 1.54          | weak            |
| L(A2)-Ha      | L(A2)-Hβ            | 4.15          | 1.898         | weak            |
| A(A3)-NH      | A(A3)-Ha            | 8.06          | 4.12          | strong          |
| A(A3)-NH      | Z(A6)-Ha            | 8.06          | 4.06          | weak            |
| A(A3)-NH      | E(A4)-Ha            | 8.06          | 4.06          | medium          |
| A(A3)-NH      | A(A3)-Hβ            | 8.06          | 1.54          | medium          |
| E(A4)-NH      | E(A4)-Ha            | 7.907         | 4.06          | strong          |
| E(A4)-NH      | E(A4)-Hγ            | 7.907         | 2.31          | weak            |
| E(A4)-NH      | E(A4)-Hβ            | 7.907         | 1.96          | strong          |
| E(A4)-Ha      | E(A4)-Hβ            | 4.06          | 1.96          | strong          |
| L(A5)-NH      | L(A4)-Ha            | 7.75          | 4.03          | strong          |
| L(A5)-Ha      | I(B5)-Hβ            | 4.03          | 1.76          | weak            |
| Z(A6)-NH      | Z(A6)-Ha            | 7.16          | 4.06          | strong          |
| Z(A6)-Ha      | Z(A6)-Hβ            | 4.06          | 1.758         | medium          |
| Z(A6)-Ha      | Z(A6)-Hε            | 4.06          | 3.08          | strong          |
| W(A7)-NH      | W(A7)-Ha            | 7.65          | 4.48          | strong          |
| W(A7)-NH      | R(A8)-Ha            | 7.65          | 3.9           | medium          |
| W(A7)-Ha      | W(A7)-Hβ            | 4.48          | 3.19          | strong          |
| R(A8)-NH      | R(A8)-Ha            | 7.85          | 4.48          | strong          |
| R(A8)-NH      | L(A9)-Ha            | 7.85          | 3.9           | medium          |
| R(A8)-NH      | R(A8)-Hγ            | 7.85          | 1.04          | medium          |
| R(A8)-NH      | R(A8)-Hβ            | 7.85          | 1.58          | medium          |
| L(A9)-NH      | L(A9)-Ha            | 7.82          | 4.10          | strong          |
| L(A9)-NH      | L(B1)-Ha            | 7.82          | 3.8           | weak            |
| L(A9)-NH      | L(A9)-Hβ            | 7.82          | 1.44          | medium          |
| L(A9)-NH      | L(A9)-Hγ            | 7.82          | 1.41          | strong          |
| L(A9)-NH      | L(A9)-Hδ            | 7.82          | 1.38          | medium          |
| L(A9)-Hδ      | L(B1)-Hγ            | 1.38          | 1.04          | weak            |
| L(A9)-Ha      | I(B5)-Hβ            | 4.10          | 1.758         | weak            |
| L(B1)-NH      | L(B1)-Ha            | 7.76          | 3.8           | strong          |
| L(B1)-NH      | W(B2)-Ha            | 7.76          | 3.19          | strong          |
| L(B1)-NH      | L(B1)-Hβ            | 7.76          | 2.05          | weak            |
| L(B1)-NH      | L(B1)-Hγ            | 7.76          | 1.41          | strong          |
| L(B1)-NH      | L(B1)-Hδ            | 7.76          | 1.04          | medium          |
| L(B1)-Ha      | I(B5)-Hβ            | 3.80          | 1.758         | weak            |
| L(B1)-Hγ      | L(B1)-Hδ            | 1.41          | 1.04          | medium          |
| W(B2)-NH      | W(B2)-Ha            | 7.91          | 4.38          | strong          |
| W(B2)-NH      | E(B3)-Ha            | 7.91          | 3.98          | medium          |
| W(B2)-NH      | W(B2)-Hβ            | 7.91          | 3.19          | strong          |
| E(B3)-NH      | E(B3)-Ha            | 8.18          | 3.98          | medium          |
NMR Structure Calculation. The solution NMR structure of AB-4 was computed using Monte Carlo conformational search in Macromodel 2015. The Merck Molecular Force Field (MMFF) was applied to the AB-4 peptide with water as an explicit solvent. A total of 70 conformers were obtained using 65 NOESY and 18 dihedral angle (Φ) constraints. The 20 lowest energy structures show minimal overall deviation from each other. Distance constraints were implemented in the structural model according to observed NOE crosspeaks: strong (2.5 Å), medium (4.0 Å), and weak (5.5 Å). The $^3$J$_{\text{NH}CH}$ coupling constants were used to calculate the Φ angles from the Karplus equation.

Protein expression and purification. GST-labeled NHR2 protein was expressed and purified as previously reported. The pGEX4T-3-NHR2 fusion vector was transformed into BL21 (DE3) competent E. coli (Novagen) in LB media. Protein production was induced with 1 mM IPTG at OD$_{600}$ of 0.75 for 4 hours at 25 °C. Production of the desired GST-NHR2 fusion product was verified by SDS-PAGE. Cells were harvested and resuspended in lysis buffer (50 mM Tris pH 7.5, 150 mM NaCl, 0.05% TritonX 100) with 10 mg/mL Leupeptin A, 1 mg/mL Pepstatin A, 500 μM PMSF, 1 mM DTT, and 0.5% glycerol (Sigma). Cell pellets were lysed via sonication and centrifuged at 4 °C at 5,000 rpm for 40 min. The bacterial supernatant was poured over pre-equilibrated glutathione Sepharose beads (G-Biosciences) and allowed to bind for 1 hr at 25 °C. Nonspecific binding proteins were removed from resin using washed buffer (100 mM Tris pH 8.0, 0.5% glycerol, 1 mM...
DTT), and the fusion protein GST-NHR2 was eluted with elution buffer ((100 mM Tris pH 8.0, 0.5% glycerol, 1 mM DTT, 10 mM glutathione). Purity was assessed using SDS-PAGE.

**Peptide binding assay.** The relative affinity of native GST-tagged NHR2 protein and CHD-NHR2 peptides was determined using a fluorescence polarization based direct binding assay with fluorescein-labeled N2B peptide, flu-N2B. The polarization experiments were performed with a DTX 880 Multimode Detector (Beckman) at 25 °C, with excitation and emission wavelengths at 485 and 525 nm, respectively. Each binding experiment was prepared in 96-well plates in assay buffer: 10 mM Tris 20 mM NaCl pH=7.4 with 0.1% pluronic F-68 (Sigma). The binding affinity (KD) values reported for each CHD peptide and GST-NHR2 are from experiments performed in triplicate. Raw values were fit to a sigmoidal dose-response nonlinear regression model in GraphPad Prism 6.0.

The affinity of flu-N2B for its native partner GST-NHR2 was first determined. Addition of serially diluted concentrations of GST-NHR2 from 2.35 mM to 2.5 μM into 100 nM of flu-N2b in assay buffer afforded the saturation binding curve in agreement with previously reported results. The affinity of flu-N2b for each CHD-NHR2 peptide was measured in the same manner; 100 nM flu-N2b was used with each peptide with the exception of CHDNS-NHR2-3. 10 nM flu-N2b was used for binding analysis with CHDNS-NHR2-3

\[ K_D = \frac{R_T \times (1 - F_{SB}) + L_{ST} \times F_{SB}^2}{F_{SB} - L_{ST}} \]

where,

- \( R_T \) = Total concentration of NHR2
- \( L_{ST} \) = Total concentration of fluorescent peptide
- \( F_{SB} \) = Fraction of bound fluorescent peptide

**Figure S11:** Structure of flu-N2B used in peptide-binding assay (top), and its analytical UV trace measured at 220 nm on an X Terra RP18 3.5 μm 2.1 x 150mm column (Part No. 186000410). 10% B to 90% B over 10 min; A: 0.1% aqueous TFA, B: acetonitrile; flow rate 400 μL/min. Exact mass calc’d [M+H]+ (m/z): 2056.0; found: 2055.9 m/z
Figure S12a: Normalized saturation binding of flu-N2B with designed CHD mimetics and GST-NHR2.²

Figure S12b: Saturation binding of NHR2_{482-551}, CHD-NHR2-2 and CHD_{DS}-NHR2-3 with flu-N2B (non-normalized curves).

Table S4. Summary of binding data

| Compound       | Sequenceᵃ | K_d (μM)ᵇ   |
|----------------|-----------|-------------|
| NHR2           | GST-NHR2(482-551) | 356 ± 90    |
| CHD-NHR2-1     | EWKHLZHLN/KTRRSSLTVLZ | >10,000    |
| CHD-NHR2-2     | ELWHLZELLR/ELWRSIRVLZ | 236 ± 90ᶜ  |
| CHD_{DS}-NHR2-3| ELWHLZELCR/ECWRSIRVLZ | 53 ± 20    |
| CHD-NHR2-6     | ALWHLZEA_LR/ELWRSIRVLZ | >3000     |
| CHD-NHR2-7     | ELWHLZELLR/ELWAIRALZ  | >3000      |

ᵃZ= azidolysine; alanine mutations are undelined. ᵇBinding affinity calculated using a fluorescence anisotropy assay with fluorescein-labeled N2B peptide. ᶜCHD-NHR2-2 is not fully soluble at >1 mM concentrations leading to a noisy upper bound and non-optimal curve fit.
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