

Supplementary Materials

Enabling Efficient Folding and High-Resolution Crystallographic Analysis of Bracelet Cyclotides

Yen-Hua Huang ^{1,2,†}, Qingdan Du ^{1,2,†}, Zhihao Jiang ^{1,2}, Gordon J. King ³, Brett M. Collins ¹, Conan K. Wang ^{1,2} and David J. Craik ^{1,2,*}

¹ Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD 4072, Australia; y.huang@imb.uq.edu.au (Y.-H.H.); qingdan.du@imb.uq.edu.au (Q.D.); zhihao.jiang@uq.net.au (Z.J.); b.collins@imb.uq.edu.au (B.M.C.); c.wang@imb.uq.edu.au (C.K.W.)

² Australian Research Council Centre of Excellence for Innovations in Peptide and Protein Science, The University of Queensland, Brisbane, QLD 4072, Australia

³ The Centre for Microscopy and Microanalysis, The University of Queensland, Brisbane, QLD 4072, Australia; g.king@uq.edu.au

* Correspondence: d.craik@imb.uq.edu.au; Tel.: +61-7-3346-2019

† These authors contributed equally to this work.

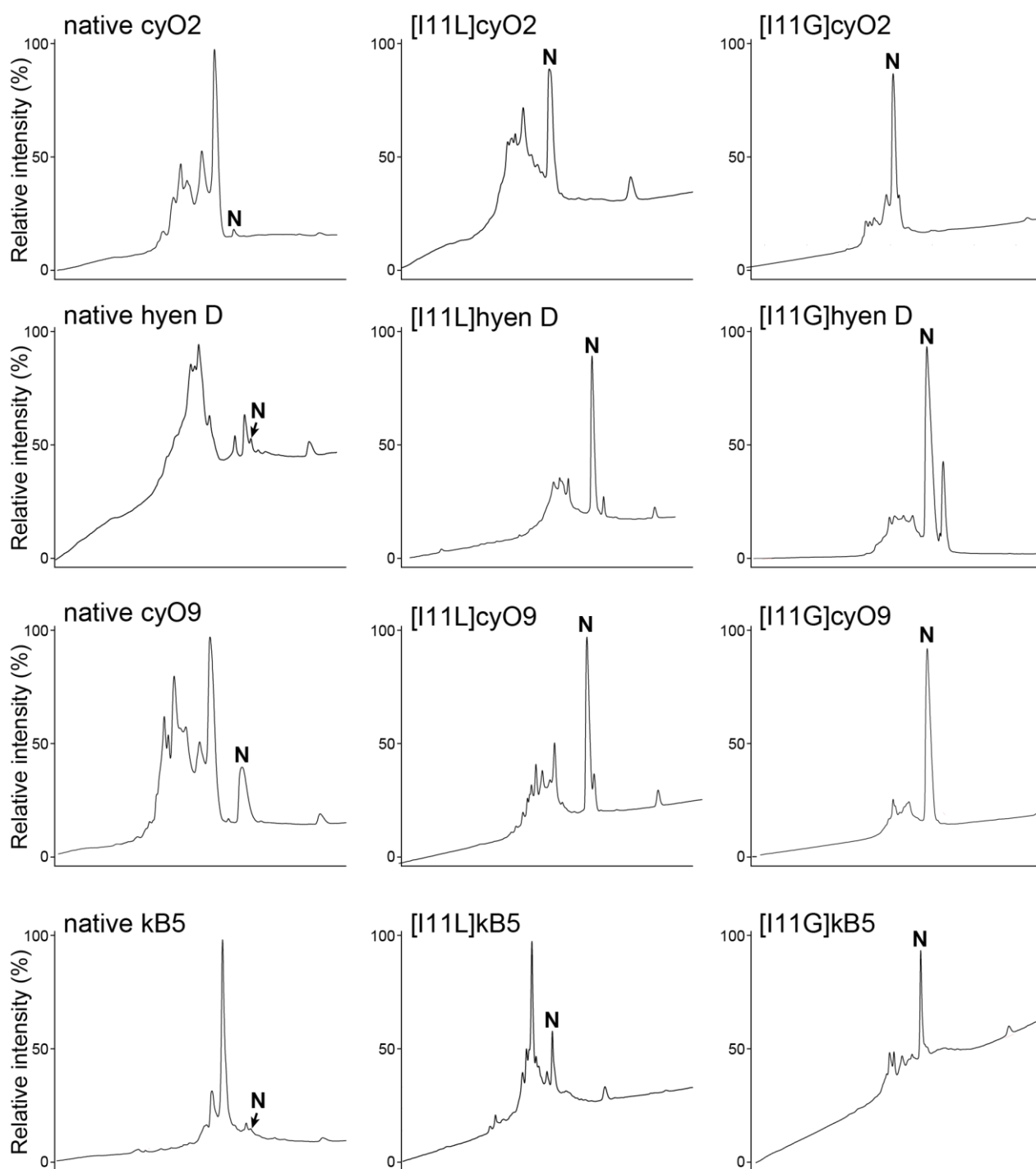


Figure S1. Oxidative folding traces of native bracelet cyclotides and their I11L and I11G mutants. 'N' represents peptide peaks with the native-like conformation.

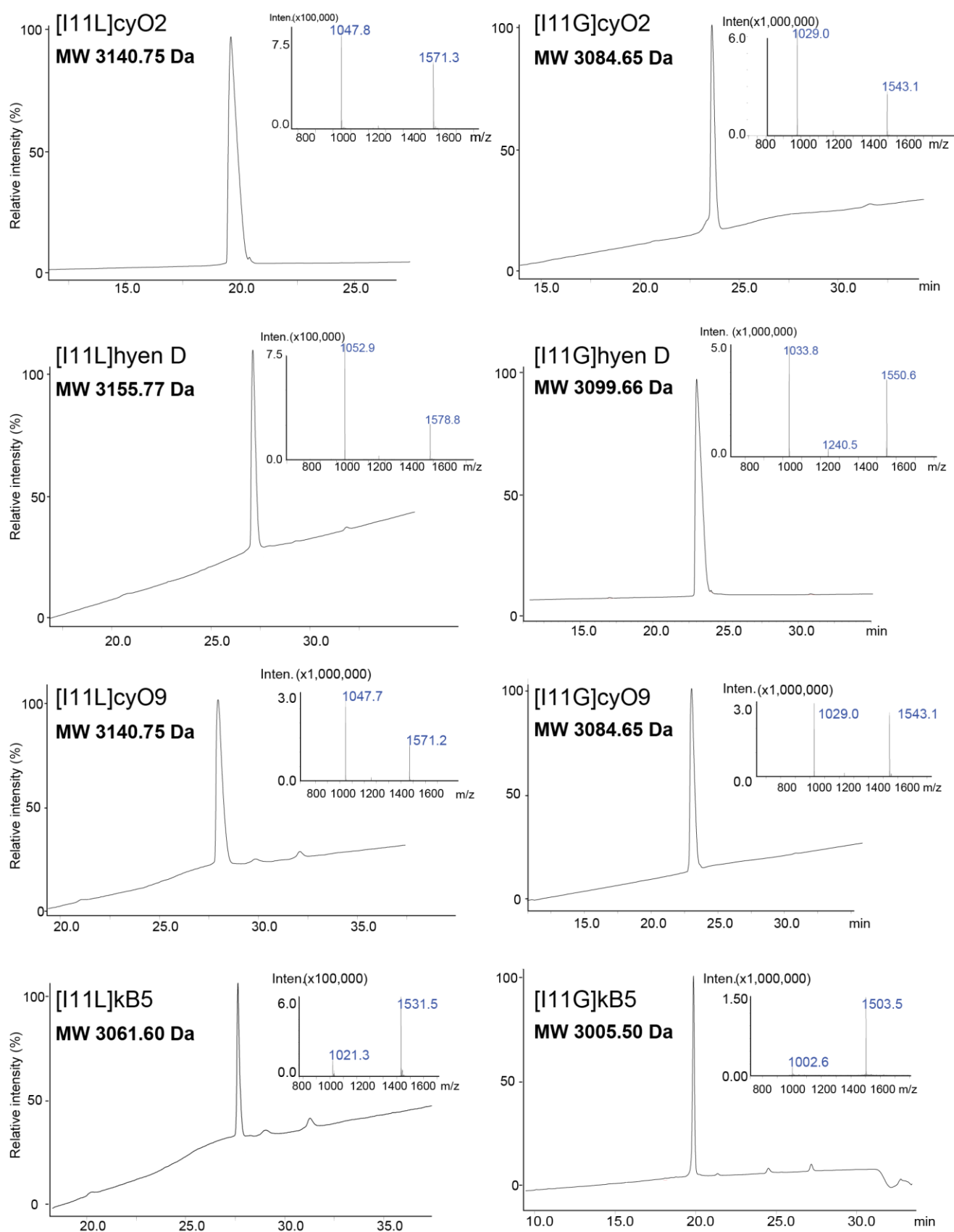


Figure S2. The purity and mass spectra of synthetic peptides analyzed using LC/ESI-MS.

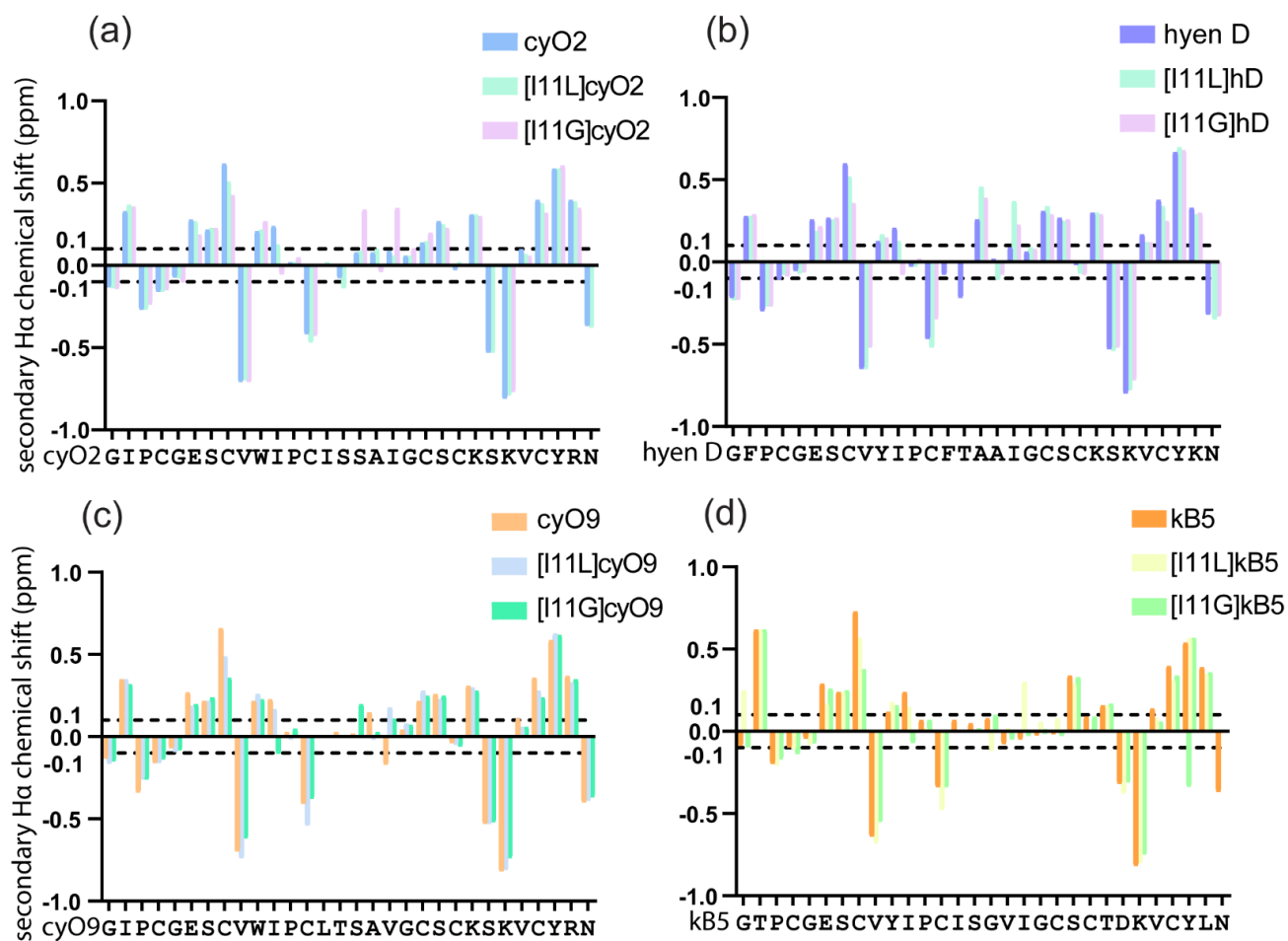


Figure S3. Secondary H α chemical shift comparison of Ile-11 mutants to their native bracelet cyclotides hyen D (a), cyO2 (b), cyO9 (c), or kB5 (d) individually. Dashed lines indicate 0.1 and -0.1 ppm.

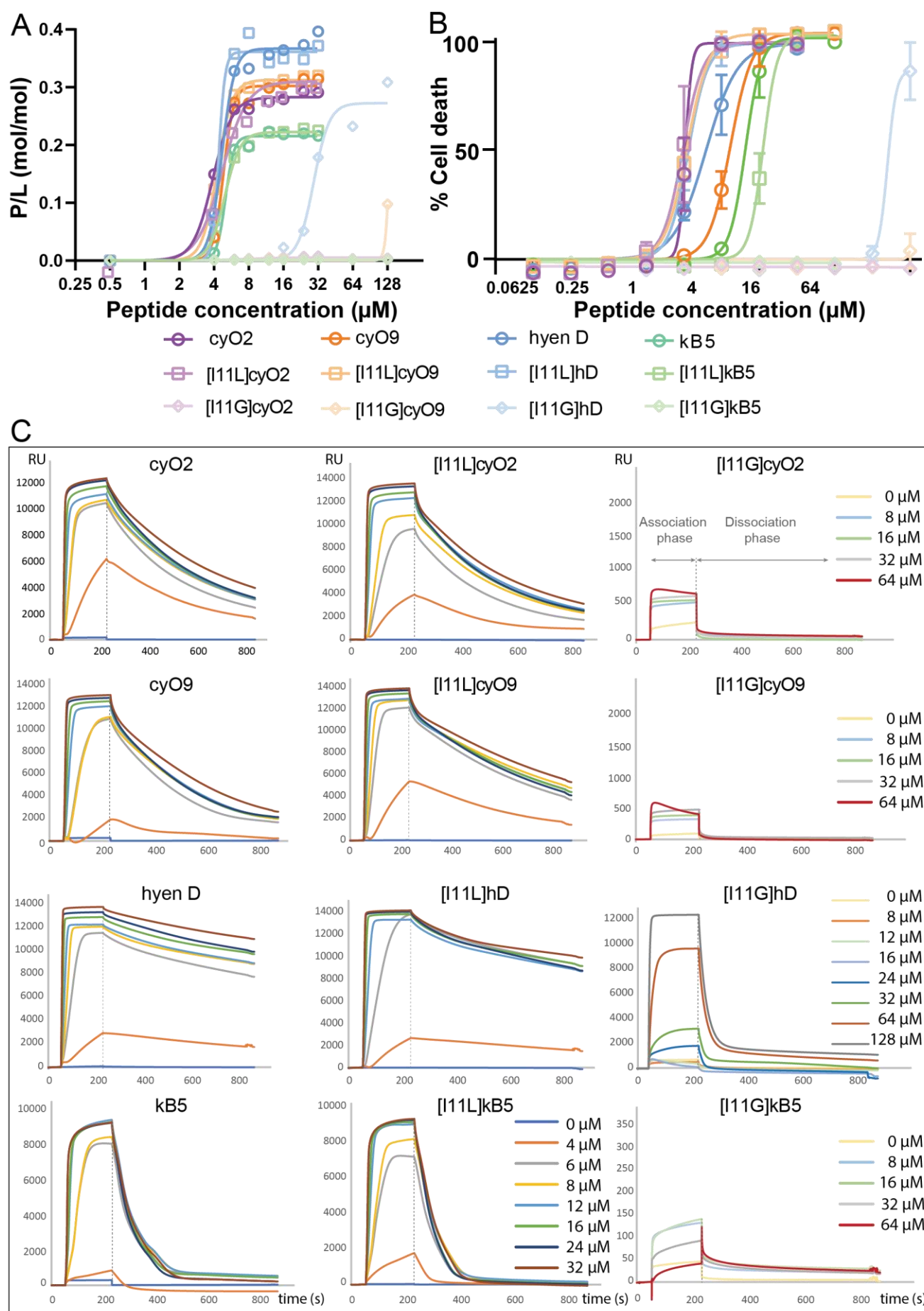


Figure S4. Membrane binding and cytotoxicity of bracelet cyclotides and their mutants. a) Concentration-response curves of cyO2, cyO9, hyen D, kB5 and their variants against the model membrane (POPC/POPE, 80/20) evaluated using SPR. b) The cytotoxicity of cyO2, cyO9, hyen D, kB5 and their variants evaluated against HeLa cells. c) Representative sensorgrams of cyclotides and their mutants obtained from serial diluted analyte concentrations. The gray dashed line marks the end of analyte injection/the beginning of dissociation phase.

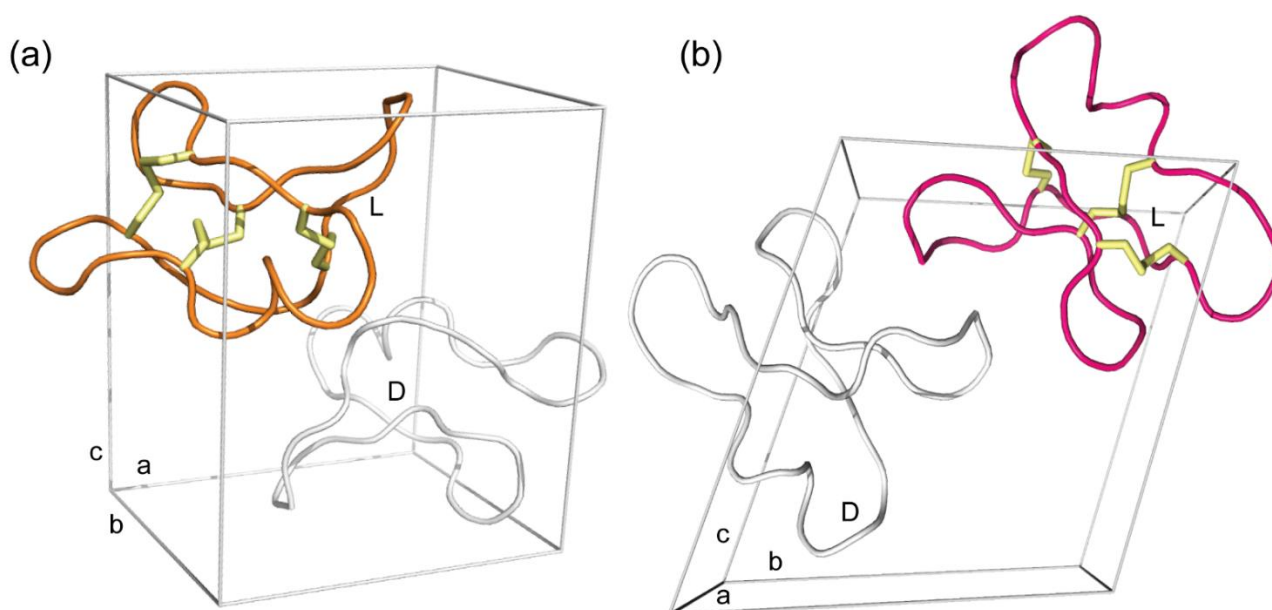


Figure S6. Unit cells of [I11L]hD and [I11G]hD. a) Unit cell of the true racemate of [I11L]hD in space group Pi with ribbon representations of the cyclic peptide molecules. The L-enantiomer is shown in orange and the D-enantiomers in white. The disulfide bonds are shown as yellow sticks. b) Unit cell of quasi-racemate of [I11G]hD in space group $P1$. [I11G]hD is shown in hot pink and D-[I11L]hD in white.

Table S1. Summary of crystallographic data and refinement statistics*.

	cyO2 quasi-racemate	[I11L]cyO2 racemate	[I11G]cyO2 quasi-racemate	Hyen D quasi-racemate	[I11L]hD racemate	[I11G]hD quasi-racemate
Data Collection						
Space group	P 1 2 ₁ 1	P 1 2 ₁ 1	P 1 2 ₁ 1	P 1 2 ₁ 1	P $\bar{1}$	P 1
Unit cell dimensions (a,b,c) (Å)	28.00	27.10	27.29	26.038	18.755	22.655
	24.74	24.76	25.11	24.731	21.153	23.85
	29.91	30.24	29.93	34.729	26.296	24.246
Unit cell angles (α,β,γ) (°)	90.00	90.00	90.00	90.00	87.25	65.582
	113.9	115.2	115.7	102.467	82.94	66.173
	90.00	90.00	90.00	90.00	89.13	76.034
Wavelength (Å)	0.95370	0.95372	0.96410	0.95366	0.95372	0.97626
Resolution range (Å)	27.35-1.17 (1.21-1.17)	24.51-1.04 (1.08-1.04)	24.11-1.10 (1.14-1.10)	18.51-1.35 (1.40-1.35)	21.13-1.22 (1.264-1.22)	20.73-1.30 (1.35-1.30)
Total reflections	25265 (2431)	33274 (3303)	29798 (2939)	30296 (2790)	38367 (3922)	17843 (1790)
Unique reflections	12686 (1232)	17453 (1715)	14958 (1474)	9116 (883)	11130 (1091)	9743 (954)
Multiplicity	2.0 (2.0)	1.9 (1.9)	2.0 (2.0)	3.3 (3.2)	3.4 (3.6)	1.8 (1.9)
Completeness (%)	98.82	98.98	99.6	93.70	93.26	93.85
	(96.77)	(97.89)	(99.9)	(91.04)	(90.46)	(89.39)
Mean I/ σ (I)	20.46 (7.73)	6.47 (2.08)	19.0 (9.80)	13.11 (4.60)	5.09 (2.14)	17.04 (10.71)
R-merge	0.013	0.040	0.016	0.050	0.129	0.029
	(0.057)	(0.225)	(0.038)	(0.239)	(0.440)	(0.053)
R-meas	0.018	0.056	0.023	0.060	0.154	0.042
	(0.080)	(0.318)	(0.054)	(0.287)	(0.521)	(0.076)
R-pim	0.013	0.040	0.016	0.033	0.0827	0.029
	(0.057)	(0.225)	(0.038)	(0.157)	(0.276)	(0.053)
CC(1/2)	1.000	0.998	0.999	0.998	0.989	0.998
	(0.995)	(0.912)	(0.996)	(0.975)	(0.889)	(0.994)
Refinement						
R-work	0.2117 (0.2298)	0.1783 (0.2173)	0.153 (0.131)	0.181 (0.247)	0.205 (0.315)	0.146 (0.158)
R-free ^a	0.2317 (0.2582)	0.1980 (0.2565)	0.176 (0.158)	0.215 (0.297)	0.223 (0.361)	0.163 (0.210)
Number of non-hydrogen atoms	470	476	481	543	260	509
Number macromolecule atoms	430	430	426	437	223	430
Number water atoms	34	40	46	80	32	67
RMS ^b bonds (Å)	0.009	0.014	0.018	0.007	0.008	0.008
RMS angles (°)	1.40	1.59	1.78	1.15	1.18	1.42
Ramachandran favored (%)	85.71	92.86	92.86	92.86	89.29	96.43
Ramachandran allowed (%)	14.29	7.14	7.14	7.14	10.71	3.57
Ramachandran outliers (%)	0.00	0.00	0.00	0.00	0.00	0.00
Clashscore	2.40	0.00	4.87	0.00	0.00	0.00
Molprobtity score	1.15	0.95	1.47	0.95	1.07	0.74

Average B-factor	8.30	8.37	8.20	12.93	9.17	8.21
-------------------------	------	------	------	-------	------	------

*Statistics for the highest-resolution shell are shown in parentheses

^aR-free, R-work with 10% of the experimental observations sequestered before refinement

^bRMS, Root mean square