SUPPORTING INFORMATION

Supplemental Figures



Supplemental Figure S1. Φ/Ψ angle RMSD versus normalized C α RMSD for alpha helical peptides, Related to Figure 2. A. Φ (red) and Ψ (blue) angle RMSD plotted against the C α RMSD per residue for each alpha helical membrane peptide (AH MP) in our benchmark set. B. Φ (red) and Ψ (blue) angle RMSD plotted against the C α RMSD per residue for each alpha helical soluble (AH SL) peptide in our benchmark set. Φ/Ψ RMSD and C α RMSD was calculated pairwise for all 5 AF2 models compared to each model in the NMR ensemble. The minimum RMSD among pairwise comparisons was plotted.



Supplemental Figure S2. Φ/Ψ angle RMSD versus normalized C α RMSD for mixed secondary structure peptides, Related to Figure 3. A. Φ (red) and Ψ (blue) angle RMSD plotted against the C α RMSD per residue for each mixed secondary structure membrane peptide (MIX MP) in our benchmark set. B. Φ (red) and Ψ (blue) angle RMSD plotted against the C α RMSD per residue for each mixed secondary structure soluble (MIX SL) peptide in our benchmark set. Φ/Ψ RMSD and C α RMSD was calculated pairwise for all 5 AF2 models compared to each model in the NMR ensemble. The minimum RMSD among pairwise comparisons was plotted.



Supplemental Figure S3. Φ/Ψ angle RMSD versus normalized C α RMSD for β -hairpin and disulfide rich peptides, Related to Figure 4. A. Φ (red) and Ψ (blue) angle RMSD plotted against the C α RMSD per residue for each β -hairpin peptide (BHPIN) in our benchmark set. B. Φ (red) and Ψ (blue) angle RMSD plotted against the C α RMSD per residue for each disulfide rich peptide (DSRP) in our benchmark set. Φ/Ψ RMSD and C α RMSD was calculated pairwise for all 5 AF2 models compared to each model in the NMR ensemble. The minimum RMSD among pairwise comparisons was plotted.



Supplemental Figure S4. X-ray models versus AlphaFold2 compared to NMR models versus AlphaFold2, Related to Star Methods. There was not a statistically significant difference between AF2's ability to predict NMR vs. Xray crystal structures. Surprisingly, NMR/X-ray RMSD comparisons failed to demonstrate lower RMSD than AF2 vs. either method, demonstrating the draw backs of both experimental and computational techniques.



Supplemental Figure S5. Number of lowest RMSD model pairs by pLDDT ranking, Related to Star Methods. AF2 ranks structures by backbone pLDDT. We enumerated the number of rank 1, rank 2 etc. by pLDDT AF2 models for each lowest RMSD NMR model/AF2 model pairwise comparison in our benchmark set. This bar graph showed rank 1-3 models are more likely to predict NMR models than rank 4 and 5 AF2 models.



Supplementary Figure S6. AlphaFold2 predicted short peptides with higher accuracy than long peptides, Related to Figure 5. A. Scatter plot of Cα RMSD versus peptide length demonstrates a positive trend, which increasing Cα RMSD for longer peptides. Point color coded by peptide type with DSRPs in red, BHPINs in orange, AH MP in blue, AH SL in green, MIX MP in yellow, and MIX SL in purple. Note, DSRPs showed the least correlation and make up most long length peptides. These peptides also have more contacts and thus AF2 was better at folding them because MSAs provided structural information, unlike for the more unstructured peptide classed considered in this study. **B.** The same scatter plot with the y axis adjusted from 1.0 to 3.0 Å for clear viewing of low Cα RMSD range.

Supplemental Tables

Supplemental Table 2, in relation to Figure 4: List of all the DSRP disulfide connectivity patterns as identified in experimental structures versus AF2-predicted structures, along with information on whether the particular peptide was identified as an RMSD outlier.

NMR d bond p	isulfide battern	AF2 di bond p	RMSD outliers?				
		1EWS					
3	29	3	28				
5	12	5	19	n			
9	28	9	29				
		1G26					
4	16	4	26				
10	26	10	16	- n			
		1IM1					
2	18	3	12				
3	12	_	_	- n			
		1K0P					
2	28	7	28				
7	25	-	-	n			
		1M4E					
2	18	2	18				
5	17	5	8				
6	14	6	14				
8	9	9	17				
		1M4F					
7	23	7	23				
10	22	10	12				
11	19	11	19	У			
13	14	13	22				
1N5G							
4	30	4	27				
9	27	9	30	Γ Υ			
1S6W							
2	19	2	19				
5	18	5	8				
6	15	6	15				
8	9	9	18				

1ТҮК								
2	17	2	16					
9	23	9	9	n				
16	30	17	30					
2B5B								
4	30	4	29					
8	29	8	24] у				
12	24	12	30					
		2BBG						
5	35	5	18					
11	26	11	28					
18	28	19	39	У				
19	39	-	-					
		2DDL						
3	23	3	23					
8	28	8	28	n				
12	30	12	30	11				
18	33	-	-					
2L1J								
1	16	1	16					
8	15	8	22	n				
24	31	15	33					
		24	31					
	2MIX							
4	20	4	21					
5	21	7	16	У				
7	16	-	-					
2MM5								
1	16	1	16					
8	15	8	21	n				
		15	29					
2MSF								
4	12	4	21					
7	28	7	26	v				
11	21	11	28	1				
16	26	-	-					
3BBG								
5	35	5	11	v				
11	26	18	26	7				

18	28	19	39					
19	39							
	6NUG							
1	13	1	13					
7	23	7	23	n				
14	24	-	-					
		6NW8						
2	23	12	23					
5	18			У				
12	25							
7ELY								
2	25							
12	26	12	28	У				
14	20							
7L7A								
2	9	-	-	V				
3	11	-	-	У				

Supplemental Table 3, in relation to Star Methods: The PDB IDs, range of the structured regions, length and sequence information, and the description of the peptides used for the comparison of NMR and X-ray structures that have identical ordered regions.

N M P D B ID	Xr ay P D B IB	Ty p e	Struc ture d Regi on for RMS D calcu latio n	NMR FASTA Sequence	Xray FASTA sequence	Le ngt h	Description
							High Resolution
							Solid-state NMR
20	30	DS		GVEINVKCSGSPQCLKPCKDA	GVEINVKCSGSPQCLKPCKDA		structure of
VS	DV	RP	1-38	GMRFGKCMNRKCHCTPK	GMRFGKCMNRKCHCTPK	38	Kaliotoxin
							solution structure of the
1E	1F	DS		PVTCLKSGAICHPVFCPRRYK	GIGDPVTCLKSGAICHPVFCP		human defensin
4Q	D3	RP	28-64	QIGTCGLPGTKCCKKP	RRYKQIGTCGLPGTKCCKKP	37	hBD-2
							Solution
4.5	411	D C					structure of the
	TI TI	DS RP	33-68			36	human defensin hBD-1
1E	6E	DS		DACEQAAIQCVESACESLCTE	DACEQAAIQCVESACESLCTE		THE NMR SOLUTION STRUCTURE OF THE PHEROMONE ER- 1 FROM THE CILIATED PROTOZOAN EUPLOTES
RC	60	RP	1-40	GEDRTGCYMYIYSNCPPYV	GEDRTGCYMYIYSNCPPYV	40	RAIKOVI
1Q 2K	3E 8Y	DS RP	1-31	AACYSSDCRVKCVAMGFSSG KCINSKCKCYK	AACYSSDCRVKCVAMGFSSG KCINSKCKCYK	31	Solution structure of BmBKTx1 a new potassium channel blocker from the Chinese Scorpion Buthus martensi Karsch

1RIRIRIRIRSTRUCTURE OF04LDSRSCIDTIPKSRCTAFQCKHSMRSCIDTIPKSRCTAFQCKHSMSHK TOXIN, NMR,0FQRP3-35KYRLSFCRKTCGTC3520 STRUCTURES1IRIRIRIRIRIRSCORPION TOXIN1IRIRIRIRIRIRSCORPION TOXIN1T6ADSVVIGQRCYRSPDCYSACKKLVGSVVIGQRCYRSPDCYSACKKLGSVVIGQRCYRSPDCYSACKKL1TIRIRIRIRIRIR11IRIRIRIRIRIR11IRIRIRIRIRIR11IRIRIRIRIRIR11IRIRIRIRIRIR11IRIRIRIRIRIR11IRIRIRIRIRIR11IRIRIRIRIRIR12IRIRIRIRIRIR13IRIRIRIRIRIR14IRIRIRIRIRIR15IRIRIRIRIRIR16IRIRIRIRIRIR17IRIRIRIRIRIR18IRIRIRIRIRIR19IRIRIRIRIRIR </th <th></th> <th></th> <th></th> <th></th> <th></th> <th></th> <th></th> <th>NMR SOLUTION</th>								NMR SOLUTION
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1T 6A DS VVIGQRCYRSPDCYSACKKLV GSVVIGQRCYRSPDCYSACKKL GSVVIGQRCYRSPDCYSACKKL 1T 6A DS VVIGQRCYRSPDCYSACKKLV GSVVIGQRCYRSPDCYSACKKL CONDUCTANCE SK TL RP 1-35 GKATGKCTNGRCDC GSVVIGQRCYRSPDCYSACKKLV 30 STRUCTURES Solution Structure of Endothelin-1 Structure of Structure of	0	FQ	RP	3-35	KYRLSFCRKTCGTC	KYRLSFCRKTCGTC	35	20 STRUCTURES
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SK TL RP 1-35 GKATGKCTNGRCDC LVGKATGKCTNGRCDC 35 30 STRUCTURES Image: SK TL Solution Solution Image: SK TL Solution Image: SK TL Solution Image: SK TL Solution Image: SK TL Solution Image: SK TL Solution Image: SK TL Solution Image: SK TL Solution Image: SK TL Solution Image: SK TL Image: SK TL Image: SK TL Image: SK TL Solut Solut <td>1T</td> <td>6A</td> <td>DS</td> <td></td> <td>VVIGQRCYRSPDCYSACKKLV</td> <td>GSVVIGQRCYRSPDCYSACKK</td> <td></td> <td>CHANNEL, NMR,</td>	1T	6A	DS		VVIGQRCYRSPDCYSACKKLV	GSVVIGQRCYRSPDCYSACKK		CHANNEL, NMR,
Solution Structure of Endothelin-1	SK	TL	RP	1-35	GKATGKCTNGRCDC	LVGKATGKCTNGRCDC	35	30 STRUCTURES
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Endothelin-1								Structure of
								Endothelin-1
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Plectasin:A								Plectasin:A
peptide								peptide
antibiotic with								antibiotic with
therapeutic								therapeutic
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COMPLETE								COMPLETE
KALIOTOXIN								KALIOTOXIN
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TX DV RP 3-38 GMRFGKCMNRKCHCTPK GMRFGKCMNRKCHCTPK 38 STRUCTURES	ТΧ	DV	RP	3-38	GMRFGKCMNRKCHCTPK	GMRFGKCMNRKCHCTPK	38	STRUCTURES
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1Z Antimicrobial		1Z						Antimicrobial
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							(wR1) peptide from Wrightia
							religiosa
							NMR solution
2N	50	DS		YCQKWMWTCDSERKCCEG	YCQKWMWTCDSERKCCEG		structure of
9Т	0U	RP	1-28	MVCRLWCKKKLW	MVCRLWCKKKLW	30	ProTx-II
							3D STRUCTURE
							OF THE M8L
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2V	1L	DS		RVCPRILLECKKDSDCLAECVC	RVCPRILLECKKDSDCLAECVC		SQUASH TRYPSIN
1V	U0	RP	2-29	LEHGYCG	LEHGYCG	29	INHIBITOR CMTI-I
							Heteronuclear
	6A						Solution
5L	Т	DS		MCMPCFTTDHQMARKCDD	GSMCMPCFTTDHQMARKC		Structure of
1C	W	RP	1-36	CCGGKGRGKCYGPQCLCR	DDCCGGKGRGKCYGPQCLCR	36	Chlorotoxin