Table S1. Summary of the experimental characterization of designs.

Design name	Expressed	Soluble	Oligomeric state†	CD spectra (25 °C)	T_m(°C)	HSQC quality*
BH_1	Y	Y	A			
BH_2	Y	Y	A			
BH_3	N					
BH_4	Y	Y	A			
BH_5	Y	Y	D			
BH_6	Y	Y	D			
BH_7	Low	Y				
BH_8	Y	Y				
BH_9	Low	Y	A			
BH_10	Y	Y	M	β	> 95°C	High
BH_11	Y	Y	M	β	> 95°C	High
BH_12	Y	Y	A			
BH_6_ss1	Y	Y	D			
BH_6_ss2	Y	Y	D			
BH_6_ss3	Y	Y	A			
BH_12_ss1	Y	Y	A			
BH_12_ss2	Y	Y	A			
BH_12_ss3	Y	Y	A			
BH_13	Y	Y	A			

† Oligomeric state of the dominant species based on SEC-MALS ('M', monomer; 'D', dimer). 'A' denotes dominant aggregate or high-molecular weight species.

* Well-dispersed HSQC spectra with a number of peaks closely matching the number of amide resonances were considered of high quality and promising for NMR structure determination.

Table S2. Designed protein sequences. The lowest E-value obtained from BLAST searches (against the NCBI nr database of non-redundant protein sequences) is shown.

Design name	Amino acid sequence	E-value
BH_1	PETKTYRFTPGEEREYEFNTDVEVEVNHDMEITVNGQTQRYT PGTSVRVPPGSRVRIRVNDDVKVNWHER	3.2
BH_2	QHTRTYRLTPGEEQEFKYNTPMTMHVEVNTDVEIEYNGKEQR YPPGTEVEIEVRPGTKVRIKVNTDVRVEIREN	2.8
BH_3	PETRTYRFTPGEEREFEFDTNVEFRFDSDVEVTVNGQTTRVP PGSSVEVPPGSRIRIRVNTDLQVEVRRR	1.1
BH_4	PETRTYRFTPGEEREFEHDTNVKWKFNTDVEIERNGERTRFT PGEEVEVPPGTRVRIRVNTDVQFTLERN	3.6
BH_5	PERREIRLSPGERYTFTVDTDVQFRVEKPVRVRHDGTETEYK PGTHLRLPPGTSVTFEVDTDVRFEIQRN	0.016
BH_6	PERREIRLSPGERYTFTVDTPVQFRVEKPVRVRYDGTETELKP GSHLRLPPGTSITFEVDTPVRFEIQRN	0.011
BH_7	SRYEITGNPGTRVELRENPGSRVKSNAPGRSERNGEHRTWN PGESRTSNRPSTMEVESDGPISIEIRE	0.8
BH_8	ESKKITVNAGERMTLHLNAGTEVRSEGPGREHSNGQTQQWP PGSTIRSDQPTTTTFESDRPLTLEVRQ	3.5
BH_9	KTKTYTVNPGEKVTITMNPGDEMTAEGPVTSRARGQEQTVNP GETVRVNEPGTFTLESDRPVTVKIQH	>10
BH_10	TRETKVTVNPGEEYEVKVNPGTRVEIQAKGPAEFEGGGTRTR LNPGESYKFENLTSQPLRIRLRNLSDTPIEFRIREE	6.3
BH_11	SERREYEVNPGERMEFTINKGERFEFKTNRPMTVRVELDGRE ERYTATPGESISVQNNSDNPARVEIQNDSDEPVRVEVRRH	4.5
BH_12	PIDVRIRMPPGSTFRVTIKTDVEVQVNKPVRVEHDGTRTEYKP GTHLRIPPGSEVRFEVDTDVEFRFKVTDPETVKEMEEHAREH GLEYETRSD	1.0
BH_6_ss1	PERRCIRLSPGERYTFTVDTPVCFRVEKPVRVRYDGTETELKP GSHLCLPPGTSITFEVDTPVRFCIQRN	0.013
BH_6_ss2	PERREICLSPGERYTFTVDTPVQFRVEKPVRVRYDGTETELKP GSHLRLPPGTSITFEVDTPVCFEIQRN	0.003
BH_6_ss3	PERREIRLSPGERYTFTVDTPVQFCVEKPVRVRYDGTETELKP GSCLRLPPGTSITFEVDTPVRFEIQRN	0.012
BH_12_ss1	PIDVRICMPPGSTFRVTIKTPVEVQVNKPVRVEYDGTRTELKP GSHLRIPPGSEIRFEVDTPVCFRFKVTDPETVKEMEEHAREHG LEYETRSD	0.98
BH_12_ss2	PIDCRIRMPPGSTFRVTIKTPVEVQVNKPVRVEYDGTRTELKP GSHLRIPPGSEIRFEVDTPVEFRFKVTDPETVKEMEEHAREHG LEYECRSD	6.8
BH_12_ss3	PIDCRIRMPPGSTFRVTIKTPVEVQVNKPVRVEYDGTRTELKP GSHLRIPPGSEIRFEVDTPVEFRFKVTDPETVKECEEHAREHG LEYETRSD	7.7
BH_13	NCDVRVRVPPGSEVRLTFKTDVRIEVKNPMEVRHDGTETRYT PGTHLRIPPGSQVDFRVNTDVEFHLEMDNPETAKEVEEQARR QGVEVEVRCQ	1.9

Table S3. Computed scores for experimentally characterized designs. Hydrogen bonding (hbond_bb_sc, hbond_lr_bb and hbond_sr_bb) and backbone torsional (rama) energy scores were averaged over all loop residue positions, and the fa_atr score term accounting for attractive Van der Waals interactions was averaged over all residues. Designs in the table were ranked based on "hbond_sum" (sum of the three hydrogen bonding average scores), rama and "loops w/Pro" (number of loops containing at least one proline). Designs with substantially higher fa_atr scores (indicative of underpacked hydrophobic cores) were placed at the end of the ranking (BH_7, BH_9 and BH_8). Only those designs without disulfide bonds or extra helical domains were considered in this analysis. The two monomeric designs with well-ordered structures (BH_10 and BH_11) are those with the best compromise of the three scores considered.

Design name	hbond_ sum	hbond_ bb_sc	hbond_ lr_bb	hbond_ sr_bb	rama	loops w/Pro	fa_atr
BH_11	-0.651	-0.220	-0.328	-0.103	-0.161	5	-4.510
BH_10	-0.556	-0.109	-0.348	-0.099	-0.142	6	-4.334
BH_2	-0.557	-0.120	-0.391	-0.046	-0.138	4	-4.557
BH_1	-0.545	-0.086	-0.406	-0.053	-0.150	3	-4.609
BH_3	-0.547	-0.076	-0.429	-0.042	-0.113	3	-4.367
BH_6	-0.531	-0.073	-0.396	-0.061	-0.085	6	-4.478
BH_4	-0.515	-0.055	-0.337	-0.124	-0.137	3	-4.679
BH_5	-0.535	-0.077	-0.395	-0.063	-0.088	4	-4.575
BH_7	-0.617	-0.162	-0.333	-0.122	-0.125	6	-3.691
BH_9	-0.557	-0.094	-0.344	-0.120	-0.104	6	-3.652
BH_8	-0.622	-0.150	-0.338	-0.134	-0.087	4	-3.842

Table S4. Contact order of *de novo* designed all-α protein domains with experimentally solved structures from previous studies. We have considered as domains those designs with more than or equal to 30 amino acids.

PDB id	Size (aa)	Contact order	PDB id	Size (aa)	Contact order
6B85	215	9.4	5CWP	244	16.7
6B87	100	10.9	5CWQ	240	18.8
5TGY	109	9.9	5CWN	217	14.0
5UOI	43	7.9	5CWO	219	17.2
2ND2	44	7.5	2LSE	101	11.5
5KWZ	26	7.8	4TQL	229	28.5
5J0L	130	17.2	4UOT	35	3.6
5IZS	77	10.0	4UOS	193	20.1
5J0H	72	10.5	4YXX	208	16.5
5J0I	75	8.5	4YXZ	278	20.2
5J0J	70	10.6	5BYO	372	21.3
5J0K	78	10.3	4YY2	99	13.5
5J10	70	8.7	4N9G	35	5.8
5J2L	76	10.9	4OYD	159	24.4
5J73	75	6.5	5VMR	140	12.7
5E6G	113	14.7	5VID	131	12.7
2N8W	104	12.8	5V2O	62	8.7
2N8I	100	11.2	5WOD	38	11.2
5CWB	230	20.5	4PN8	30	3.5
5CWC	238	17.5	4PN9	29	3.5
5CWD	172	18.8	4PNA	31	3.6
5CWF	198	16.7	4PNB	30	3.5
5CWG	250	19.5	4PND	31	3.5
5CWH	179	9.2	3S0R	30	3.5
5CWI	260	24.1	5EHB	27	3.4
5CWJ	190	15.1	2A3D	73	10.0
5CWK	175	11.5	1RH4	30	3.3
5CWL	195	16.5	4DAC	26	3.5
5CWM	228	13.9			

Table S5. Contact order of *de novo* designed αβ-protein domains with experimentally solved structures from previous studies. We have considered as domains those designs with more than or equal to 30 amino acids.

PDB id	Size (aa)	Contact order	PDB id	Size (aa)	Contact order
5L33	109	20.2	2LV8	110	21.7
5TPH	120	20.3	2LVB	112	27.8
5TPJ	122	20.0	2MTL	88	23.1
5TRV	118	19.9	2LR0	134	24.3
5TS4	101	17.5	2KPO	110	21.9
5U35	130	22.4	2LRH	134	23.2
5KPH	85	11.9	2LTA	110	21.4
5KPE	120	16.9	2LN3	83	18.2
4R80	76	13.9	2LND	112	29.5
5UP1	64	14.8	2LCI	134	25.3
5UP5	40	14.2	2L82	162	30.2
5UYO	45	9.2	2L69	134	22.7
5JG9	47	15.7	2KL8	85	24.2
5JHI	35	14.7	4RJV	83	24.3
5JI4	37	14.0	2MR5	136	26.5
2ND3	38	10.0	2MRA	117	22.7
2N75	99	17.4	2MR6	136	26.4
2N76	112	29.9	2MBM	120	21.2
2N2T	84	25.4	2MQ8	112	30.5
2N2U	77	21.2	5GAJ	134	25.7
2N3Z	99	17.4	5BVL	180	23.7
5VLI	39	14.3	2KI0	36	12.8
1QYS	91	18.2			