

Supplementary Information

Table S1. Validated set of GAS_{right} designs

Design ID	Sequence	Computational Score (kcal/mol)	GpA (%)	ΔG (kcal/mol)
G_001	LLL TALLVGLLGGLLFLL LILI	-24.58	0.29 ± 0.05	NA
G_002	LLL LFLLATLLGYLLSIL LILI	-31.69	0.42 ± 0.04	-2.49 ± 0.23
G_003	LLL FLLGTLLGALIAY LILI	-31.69	1.15 ± 0.16	-3.85 ± 0.54
G_004	LLL VTLFSLLGALI ASLILI	-17.19	0.48 ± 0.05	-2.74 ± 0.28
G_005	LLL YILLGLLGTLI ASLILI	-32.08	0.31 ± 0.04	-0.52 ± 0.07
G_006	LLL VLLTGLLFGLIYAL LILI	-26.51	0.31 ± 0.08	-0.06 ± 0.01
G_007	LLL FALLASILGILITLL LILI	-26.35	0.19 ± 0.04	NA
G_008	LLL TFLAVLLGLLGIL LILI	-21.45	0.65 ± 0.11	-3.18 ± 0.53
G_009	LLL SFLVLLGTLIAY LILI	-21.46	0.19 ± 0.07	NA
G_010	LLL YLLLGALLGTLISFL LILI	-32.9	0.53 ± 0.04	-2.9 ± 0.22
G_011	LLL TVLLGLLGILIGFL LILI	-32.92	0.54 ± 0.06	-2.93 ± 0.3
G_012	LLL YFLLGTLIGALI GLLILI	-33.02	0.81 ± 0.07	-3.44 ± 0.3
G_013	LLL AVLTSLLGGLLFLL LILI	-21.13	0.25 ± 0.04	NA
G_014	LLL VTLLLGLLFALLS TLLILI	-26.16	0.25 ± 0.05	NA
G_015	LLL TFLLGLLGYLLAS LILI	-33.27	1.23 ± 0.08	-3.93 ± 0.27
G_016	LLL YVLLGALLGILITLL LILI	-33.55	0.95 ± 0.1	-3.62 ± 0.39
G_017	LLL YFLGVVLGTLI GLLILI	-33.58	1.07 ± 0.03	-3.76 ± 0.11
G_018	LLL YALLALLGTLIGFL LILI	-16.12	0.19 ± 0.03	NA
G_019	LLL YLLLASILGILIAFL LILI	-34.17	0.15 ± 0.02	NA
G_020	LLL YLLLGALLGALITFL LILI	-34.32	1.12 ± 0.17	-3.82 ± 0.59
G_021	LLL TVLLGLLFGLLFAL I	-22.26	0.5 ± 0.05	-2.81 ± 0.3
G_022	LLL TVLLALLGFLLGS LILI	-9.61	0.78 ± 0.07	-3.39 ± 0.3
G_023	LLL YVLLTLLGALLGCF LILI	-22.36	0.26 ± 0.08	NA
G_024	LLL YVLLGLLGTLIAFL LILI	-34.97	0.32 ± 0.14	-1.25 ± 0.56
G_025	LLL YFLLATLLGLLGV LILI	-35.12	0.36 ± 0.02	-2.02 ± 0.14
G_026	LLL VGLLFALLGILITLL LILI	-33.23	0.36 ± 0.05	-2.08 ± 0.29
G_027	LLL TFLLAIIIGLLIGV LILI	-21.01	0.64 ± 0.05	-3.15 ± 0.26
G_028	LLL AALLALLGVLLGTL LILI	-17.8	0.39 ± 0.04	-2.33 ± 0.22
G_029	LLL AALFSLLGLLGTL LILI	-26.92	0.3 ± 0.09	NA
G_030	LLL YALLTALLGGLLFLL LILI	-19.63	0.22 ± 0.06	NA
G_031	LLL LTLLVGLLFALLYGL LILI	-27.85	0.25 ± 0.02	NA
G_032	LLL YLLGLLGTLIATLAF LILI	-28.2	0.25 ± 0.09	NA
G_033	LLL LVLLATLGGLLSIL LILI	-19.93	0.26 ± 0.05	NA
G_034	LLL YGULFALLGILITLL LILI	-28.27	0.28 ± 0.01	NA
G_035	LLL STLLGALLGLLAV LILI	-28.31	0.91 ± 0.09	-3.58 ± 0.36
G_036	LLL YVLLFTLLGALI GLLILI	-27.82	0.22 ± 0.03	NA
G_037	LLL YVLLATLLGLLIGFL LILI	-28.48	0.3 ± 0.06	NA
G_038	LLL YLLGTLLGILIAFL LILI	-19.35	0.43 ± 0.07	-2.55 ± 0.43
G_039	LLL YGULFLLGVLLATL LILI	-27.64	0.4 ± 0.04	-2.39 ± 0.22
G_040	LLL YFLLATLLGVLLG LILI	-19.14	0.34 ± 0.05	-1.8 ± 0.24
G_041	LLL YLLTLLAGLIFSL LILI	-20.09	0.17 ± 0.1	NA
G_042	LLL TFLLAIIIGYLGS LILI	-20.12	0.95 ± 0.14	-3.62 ± 0.52
G_043	LLL VLLLTLLGALLSAL I	-27.56	0.28 ± 0.03	NA
G_044	LLL LTLLVALLAGLIFSL LILI	-20.31	0.31 ± 0.03	NA
G_045	LLL YGULFLLGVLLAS LILI	-28.77	0.32 ± 0.06	-1.31 ± 0.23
G_046	LLL YVLLALLGTLIGFL LILI	-27.25	0.18 ± 0.03	NA
G_047	LLL TALLVGLLGLLGFL LILI	-18.68	0.27 ± 0.02	NA
G_048	LLL YLLGALLGVLLSTL LILI	-24.54	0.84 ± 0.22	-3.48 ± 0.9
G_049	LLL AALLFALLGILISTL LILI	-18.68	0.25 ± 0.12	NA
G_050	LLL YFLAVLLGLLGTL LILI	-29.4	0.28 ± 0.06	NA
G_051	LLL YGULFALLGTLISL LILI	-29.53	0.19 ± 0.01	NA
G_052	LLL VALLALLGTLISFL LILI	-27.01	0.28 ± 0.05	NA
G_053	LLL YLLGALLGILISTL LILI	-29.9	0.75 ± 0.08	-3.35 ± 0.34
G_054	LLL TFLAVLLGLLIGLS LILI	-27.01	0.6 ± 0.14	-3.08 ± 0.7
G_055	LLL YFLAVLLGTLISL LILI	-15.45	0.17 ± 0.05	NA
G_056	LLL YFLALLGTLIGLS LILI	-35.41	0.23 ± 0.02	NA
G_057	LLL TALLFLLGVLLAS LILI	-16.15	0.82 ± 0.11	-3.45 ± 0.45
G_058	LLL AGLIFLLGVLLATL LILI	-22.42	0.33 ± 0.01	-1.62 ± 0.03
G_059	LLL YVLLATLLGGLLF LILI	-23.03	0.21 ± 0.06	NA
G_060	LLL TALLALLFGLLIFSL LILI	-13.75	0.38 ± 0.03	-2.21 ± 0.2
G_061	LLL YVLTLLGGLLFAL I	-13.73	0.32 ± 0.04	-0.98 ± 0.12
G_062	LLL AYLLGTLIGLLGFL LILI	-45.74	1.14 ± 0.15	-3.83 ± 0.49
G_063	LLL VLLATLLGSLIGFL LILI	-13.7	0.31 ± 0.07	-0.17 ± 0.04
G_064	LLL ALLGLLGTLISFL LILI	-45.31	1.04 ± 0.05	-3.72 ± 0.18
G_065	LLL LVLLTTLLGGLLFAL I	-25.32	0.29 ± 0.04	NA
G_066	LLL AYLLGLLGFLIGTL LILI	-39.49	1.11 ± 0.11	-3.81 ± 0.37
G_067	LLL YTLIATLLGLLGFL LILI	-44.96	0.24 ± 0.03	NA
G_068	LLL TSLLVGLIAYLIGFL LILI	-11.56	0.41 ± 0.02	-2.45 ± 0.09
G_069	LLL AYLLGVLLGLLIAFL LILI	-35.55	1.12 ± 0.09	-3.81 ± 0.31
G_070	LLL YFLAVLLGLLGFLGS LILI	-23.94	0.25 ± 0.15	NA

G_071	LLL S YLLGVLL G LLL G T L L I	-44.36	0.95 ± 0.08	-3.62 ± 0.32
G_072	LLL A LL G VLL G F L L I S L I L I	-43.23	1.26 ± 0.1	-3.96 ± 0.31
G_073	LLL T ALL A LL G VLL G L I S V L I L I	-13.06	0.31 ± 0.02	-0.87 ± 0.05
G_074	LLL S YLLGVLL G LLI A T L I L I	-41.31	1.03 ± 0.11	-3.71 ± 0.41
G_075	LLL S YLLGVLL G LL G T L I G F L I L I	-42.81	0.79 ± 0.12	-3.4 ± 0.5
G_076	LLL S YLL G LL G T L I G F L I L I	-42.79	0.83 ± 0.06	-3.47 ± 0.24
G_077	LLL Y LL G ALL A FL I S T L I L I	-25.05	0.18 ± 0.01	NA
G_078	LLL T YLLGVLL G LL G F L I S T L I L I	-42.23	0.87 ± 0.19	-3.52 ± 0.76
G_079	LLL L VLL G ALL G F L I S F L I L I	-24.93	0.44 ± 0.07	-2.6 ± 0.39
G_080	LLL A YLLGVLL G LL G S L I L I	-41.95	1.11 ± 0.12	-3.81 ± 0.41
G_081	LLL A LL G VLL G T L I G F L I L I	-38.02	0.95 ± 0.12	-3.62 ± 0.44
G_082	LLL S YLL G LL G F L I S T L I L I	-38.42	1.09 ± 0.18	-3.78 ± 0.62
G_083	LLL Y LL G ALL G F L I S T L I L I	-48.02	1.11 ± 0.17	-3.8 ± 0.58
G_084	LLL Y LL F ALL G ALL T L I L I L I	-24.61	0.36 ± 0.04	-1.99 ± 0.22
G_085	LLL F VLL T IL G ALL S L I L I	-15.06	0.25 ± 0.05	NA
G_086	LLL Y LL V ALL A LL G L I L I L I	-22.53	0.33 ± 0.05	-1.44 ± 0.22
G_087	LLL Y SL A LL G T L I G F L I L I	-22.59	0.15 ± 0.05	NA
G_088	LLL A LL L T V LL G GL F S L I L I	-25.5	0.26 ± 0.04	NA
G_089	LLL T SL L LL G LL F ALL A Y L I L I	-24.73	0.22 ± 0.13	NA
G_090	LLL Y LL G IL G T L I A F L I L I	-36.83	0.53 ± 0.04	-2.9 ± 0.2
G_091	LLL V LL L GL F GL I F ALL L I L I	-22.79	0.83 ± 0.23	-3.47 ± 0.95
G_092	LLL Y ILL T LL G AL I A F L I L I	-6.71	0.29 ± 0.14	NA
G_093	LLL F YLL G V L G LL A S L I L I	-47.35	0.92 ± 0.1	-3.58 ± 0.39
G_094	LLL Y VLL F LL G AL I G T L I L I	-25.05	0.17 ± 0.02	NA
G_095	LLL T LL G LL G V L G S L I L I	-37.29	0.91 ± 0.21	-3.57 ± 0.83
G_096	LLL A LL G LL G V L G S L I L I	-37.44	1.04 ± 0.21	-3.73 ± 0.74
G_097	LLL T VL G LL G LL A F L I L I	-37.57	0.71 ± 0.06	-3.28 ± 0.25
G_098	LLL S LL F LL G V L G T L I L I	-6.06	0.3 ± 0.06	NA
G_099	LLL V LL T GL F ALL A Y L I L I L I	-14.18	0.25 ± 0.07	NA
G_100	LLL A V L F S LL G LL G T L I L I L I	-22.9	0.27 ± 0.04	NA
G_101	LLL T ALL F LL G V L G I L I L I	-13.97	0.72 ± 0.06	-3.29 ± 0.27
G_102	LLL A GL L F L LL G T L I S Y L I L I	-25.42	0.14 ± 0.02	NA
G_103	LLL Y VL G LL G F L I S T L I L I	-52.21	1.04 ± 0.05	-3.72 ± 0.19
G_104	LLL A FL L G T LL G V L G L I L I	-37.15	1.33 ± 0.24	-4.02 ± 0.74

Table S2. Validated set of Left designs

Design ID	Sequence	Computational Score (kcal/mol)	GpA (%)	ΔG (kcal/mol)
L_001	LLLLLLLLTALLFLVSLLILI	-24.86	0.23 ± 0.09	NA
L_002	LLLLYVLLIAALLTLFSLLILI	-19.91	0.28 ± 0.05	NA
L_003	LLIVALLLILITFLLLASLLILI	-19.85	0.35 ± 0.06	-1.97 ± 0.35
L_004	LLLLFVLTLLAALYSLLLILI	-27.78	0.19 ± 0.1	NA
L_005	LLLAYVLLAALLFLTSLLILI	-24.92	0.32 ± 0.07	-1.18 ± 0.27
L_006	LLLYAALLFVLTALLLSLLILI	-20.96	0.49 ± 0.08	-2.78 ± 0.45
L_007	LLLYYVLLAATLLASFLLLILI	-20.52	0.28 ± 0.08	NA
L_008	LLLLAATLILAVLFSLLILI	-25.76	0.61 ± 0.06	-3.09 ± 0.3
L_009	LLIVALLLILFLLTSLLLILI	-22.22	0.48 ± 0.23	-2.75 ± 1.31
L_010	LLLYFVLLAALVTLLSLLILI	-22.59	0.19 ± 0.11	NA
L_011	LLLLFVLLAALTTLLSLLILI	-19.85	0.15 ± 0.05	NA
L_012	LLLLAVLLFLATLISLLILI	-26.1	0.22 ± 0.05	NA
L_013	LLLLLLFLIATLAVLSTLILI	-26.11	0.16 ± 0.05	NA
L_014	LLLYYVLLAATLFLSLLILI	-21.98	0.41 ± 0.16	-2.44 ± 0.94
L_015	LLLLTLLVALLLFLISLLILI	-26.31	0.41 ± 0.26	-2.4 ± 1.54
L_016	LLLYFVLLAALAVTSLLILI	-21.69	0.27 ± 0.09	NA
L_017	LLLYLLLTVIALLLFSLLILI	-21.39	0.23 ± 0.06	NA
L_018	LLLTLLLAIALVLFSLLLILI	-21.35	0.47 ± 0.12	-2.7 ± 0.69
L_019	LLLLVLLTILFLLSALIL	-26.56	0.23 ± 0.04	NA
L_020	LLLYYLLFLIATLFSLLILI	-23.39	0.21 ± 0.09	NA
L_021	LLLLLFLLATLILLSLLILI	-21.08	0.25 ± 0.05	NA
L_022	LLLYYLLAVLITALLFSLLILI	-21.06	0.27 ± 0.09	NA
L_023	LLLYYVLLAALFTLISLLILI	-25.06	0.18 ± 0.12	NA
L_024	LLVTLLLAIALLFLSLLILI	-20.94	0.27 ± 0.06	NA
L_025	LLLYYALLFLIATLFSLLILI	-26.79	0.45 ± 0.14	-2.61 ± 0.83
L_026	LLLLLLFLFVLTATLFSLLILI	-26.85	0.23 ± 0.07	NA
L_027	LLLFSLVLLTALLYLLSLLILI	-20.71	0.23 ± 0.05	NA
L_028	LLLTILLVLLAATLFLSLLILI	-26.94	0.23 ± 0.19	NA
L_029	LLLFILLLAAILVLLSTLILI	-23.7	0.21 ± 0.05	NA
L_030	LLLYVLLAALFTLFSLLILI	-23.75	0.23 ± 0.07	NA
L_031	LLLVAVLFLIATLTSLLILI	-27.1	0.41 ± 0.08	-2.45 ± 0.5
L_032	LLIVALLTIIAALFLSLLILI	-27.98	0.41 ± 0.15	-2.45 ± 0.88
L_033	LLLVSSLILITALLFLSLLILI	-22.28	0.35 ± 0.11	-1.89 ± 0.59
L_034	LLLFALLTIIAALVLSLLILI	-28.01	0.36 ± 0.05	-2.09 ± 0.26
L_035	LLLVAVLTAIPLLFSLLILI	-36.08	0.21 ± 0.13	NA
L_036	LLLYYVLTAAFLLLSLLILI	-35.8	0.26 ± 0.1	NA
L_037	LLLVVLLTIIAFLFLSLLILI	-34.96	0.21 ± 0.09	NA
L_038	LLLYWYLVSLILITFLSLLILI	-34.36	0.48 ± 0.16	-2.75 ± 0.89
L_039	LLLVVSLLILITALLFLSLLILI	-34.32	0.41 ± 0.11	-2.42 ± 0.67
L_040	LLLYAYLTIVLSSIFLFLSLLILI	-16.04	0.38 ± 0.08	-2.25 ± 0.48
L_041	LLLAATLILALFLVLSLLILI	-34.1	0.3 ± 0.1	NA
L_042	LLLLFVLLLIAATLISLLILI	-36.31	0.27 ± 0.08	NA
L_043	LLYTLLAVLIAALLSLLILI	-16.38	0.44 ± 0.09	-2.56 ± 0.52
L_044	LLLLFILLAVLAVLTLFSLLILI	-32.64	0.22 ± 0.13	NA
L_045	LLIVALLFLSLLILYTLISLLILI	-32.59	0.27 ± 0.08	NA
L_046	LLLLVLLLAATLFLSLLILI	-32.33	0.29 ± 0.07	NA
L_047	LLIVALLFLITSLLYLISLLILI	-27.98	0.21 ± 0.07	NA
L_048	LLLLFLLAVLYLLSTLISLLILI	-32.17	0.32 ± 0.14	-1.14 ± 0.51
L_049	LLLSYLVAVLFTFLSLLILI	-32.13	0.34 ± 0.08	-1.82 ± 0.4
L_050	LLLFILLLAATVLLSLLILI	-32.09	0.33 ± 0.1	-1.46 ± 0.44
L_051	LLLYFLAVLITLILSLLILI	-33.12	0.18 ± 0.09	NA
L_052	LLLTYYLLAALFVLLLSLLILI	-32.09	0.25 ± 0.12	NA
L_053	LLIVLLLAATFLLLTSLLILI	-36.54	0.24 ± 0.09	NA
L_054	LLLYTLLAVLILLLASLLILI	-14.73	0.15 ± 0.05	NA
L_055	LLYALLTIIAFLFSLLILI	-8.65	0.55 ± 0.3	-2.94 ± 1.59
L_056	LLLLLLAVLITALLFLSLLILI	-44.72	0.28 ± 0.06	NA
L_057	LLLLVALLLITSLLFLSLLILI	-44.48	0.48 ± 0.05	-2.72 ± 0.26
L_058	LLYALLLVIITLILFSLLILI	-11.73	0.35 ± 0.12	-1.95 ± 0.64
L_059	LLLFALLLITALLYVLLSLLILI	-12.21	0.37 ± 0.12	-2.12 ± 0.69
L_060	LLLFYLTALLLILVSLLILI	-12.34	0.28 ± 0.15	NA
L_061	LLLLVALLLITAFLFSLLILI	-41.95	0.43 ± 0.16	-2.53 ± 0.93
L_062	LLLAATLAVLFLSTLISLLILI	-36.63	0.25 ± 0.11	NA
L_063	LLLLLAFLVTSLLYLISLLILI	-41.84	0.41 ± 0.04	-2.44 ± 0.24
L_064	LLLYVLLAALLLTSLLILI	-13.11	0.36 ± 0.29	-2.08 ± 1.65
L_065	LLLYWLLTILAFLFSLLILI	-40.46	0.4 ± 0.13	-2.34 ± 0.77
L_066	LLLYSITALLFVLLSLLILI	-39.3	0.27 ± 0.06	NA
L_067	LLLTTVLLAALFLSLLILI	-38.49	0.2 ± 0.12	NA
L_068	LLLVVALLLTSLLFLSLLILI	-37.95	0.27 ± 0.11	NA
L_069	LLLFVLLAIALYLLSTLISLLILI	-37.79	0.35 ± 0.08	-1.89 ± 0.41
L_070	LLLFALLLLVTLLYSLLILI	-36.99	0.29 ± 0.07	NA

L_071	LLLLFWLLAVLTALLYLILLI	-41.58	0.38 ± 0.06	-2.23 ± 0.37
L_072	LLLLAVLLTIALLISFLILLI	-32.06	0.26 ± 0.03	NA
L_073	LLLLVALLLILFLLSTLILLI	-32.21	0.28 ± 0.08	NA
L_074	LLLLSYLVALLTTLFLLSTLILLI	-31.72	0.37 ± 0.28	-2.18 ± 1.61
L_075	LLLLLVLLAILLFLSTLILLI	-29.41	0.24 ± 0.1	NA
L_076	LLLLYWLLTILVALLSFLILLI	-29.15	0.42 ± 0.06	-2.5 ± 0.34
L_077	LLLYYLLLAVITSLLFLLLILLI	-28.74	0.14 ± 0.1	NA
L_078	LLLALLLFITVLLSILILLI	-28.66	0.24 ± 0.07	NA
L_079	LLLLLFITALLYVLISLLILLI	-28.61	0.28 ± 0.27	NA
L_080	LLLLYVLLLAITLILFSLLILLI	-19.09	0.19 ± 0.11	NA
L_081	LLLLYLLLAVLSSLFLFTLILLI	-29.49	0.26 ± 0.08	NA
L_082	LLLYFLLAVLILTLFSLLILLI	-19.2	0.16 ± 0.09	NA
L_083	LLLLLALLLILVTLFSLLILLI	-28.35	0.32 ± 0.1	-1.29 ± 0.41
L_084	LLLLWTLIAILLLVLFSLILLI	-28.25	0.32 ± 0.08	-1.09 ± 0.29
L_085	LLLYALLFILTSLLFSLLILLI	-28.21	0.14 ± 0.08	NA
L_086	LLLLLLLAVLYTLLISFLILLI	-28.07	0.23 ± 0.04	NA
L_087	LLLYAALLLIVTLLFSLLILLI	-28.03	0.22 ± 0.08	NA
L_088	LLLYAALLFVULLLTSLLILLI	-19.75	0.17 ± 0.07	NA
L_089	LLLLAILLLLTIVLFSLLILLI	-28.43	0.18 ± 0.08	NA
L_090	LLLLYILLALLFVLILSTLILLI	-30	0.22 ± 0.02	NA
L_091	LLLYAALLFVULLLFSLLILLI	-18.68	0.14 ± 0.05	NA
L_092	LLLLAALLFILTSILYVLLILLI	-31.34	0.36 ± 0.04	-1.99 ± 0.22
L_093	LLLYWLLTVILFLLLASLILLI	-17.52	0.28 ± 0.03	NA
L_094	LLLLYFLILATLVALLLSLLILLI	-31.23	0.47 ± 0.13	-2.7 ± 0.73
L_095	LLLLILLIALLFVLTSLLILLI	-30.54	0.35 ± 0.14	-1.89 ± 0.76
L_096	LLLAAALLLIVTLLFSLLILLI	-17.48	0.53 ± 0.08	-2.89 ± 0.43
L_097	LLLLAVLLILITLILSFLILLI	-30.86	0.38 ± 0.2	-2.19 ± 1.14
L_098	LLLLTVLLAILFLLSLLILLI	-30.91	0.29 ± 0.09	NA
L_099	LLLYSLLLLITFLLAVLILLI	-31.39	0.2 ± 0.18	NA
L_100	LLLLAVLLAILLILISTLILLI	-31.53	0.2 ± 0.02	NA

Table S3. Validated set of Right designs

Design ID	Sequence	Computational Score (kcal/mol)	GpA (%)	ΔG (kcal/mol)
R_001	LLLYTLLVLLAFLLSLIL	-26.75	0.3 ± 0.07	NA
R_002	LLLYSLLTLLFVLLALLIL	-30.22	0.3 ± 0.13	NA
R_003	LLLYTLLIALFVLLSLLIL	-26.69	0.28 ± 0.04	NA
R_004	LLLYSLLVALFTLLFLLIL	-26.6	0.23 ± 0.1	NA
R_005	LLLSLLFALLIAVTLIL	-24.71	0.29 ± 0.03	NA
R_006	LLLYLLLFTLLAVVLSLIL	-8.79	0.3 ± 0.06	NA
R_007	LLYALLVALFTLLSLLIL	-24.88	0.26 ± 0.05	NA
R_008	LLTYLLFVLAILLSLIL	-24.89	0.29 ± 0.1	NA
R_009	LLLYTLLISLVALFLIL	-25.05	0.25 ± 0.1	NA
R_010	LLLYTLLVSLLALLAFLIL	-25.05	0.31 ± 0.07	0.43 ± 0.1
R_011	LLLYLYVALISLISLFTLIL	-25.89	0.39 ± 0.18	-2.33 ± 1.07
R_012	LLYSLLIALFVLLTILIL	-27.63	0.3 ± 0.06	NA
R_013	LLLVLLIALFTLLSYLIL	-25.24	0.33 ± 0.04	-1.62 ± 0.21
R_014	LLSYLLVLLAFLTTLIL	-25.39	0.35 ± 0.08	-1.9 ± 0.43
R_015	LLLYTLLISLFLVLLTIL	-26.81	0.31 ± 0.08	0.25 ± 0.07
R_016	LLYFLLVALTLLSLLIL	-26.06	0.27 ± 0.09	NA
R_017	LLSYLLFVLLALLSLLIL	-26.54	0.39 ± 0.07	-2.28 ± 0.4
R_018	LLSYLLTLLAFLSLLIL	-26.9	0.55 ± 0.08	-2.94 ± 0.45
R_019	LLWTLVLFVLLAILLSLIL	-29.79	0.29 ± 0.06	NA
R_020	LLSYLLFTLAILLTLLIL	-29.93	0.3 ± 0.09	NA
R_021	LLYSLLIALFVLLTILIL	-27.75	0.28 ± 0.1	NA
R_022	LLSYLLFTLALLIAVILIL	-27.41	0.38 ± 0.03	-2.26 ± 0.15
R_023	LLYALLTLLVALLSLLIL	-10.03	0.33 ± 0.05	-1.61 ± 0.26
R_024	LLYTLLIALVLLFSLIL	-24.33	0.31 ± 0.18	-0.41 ± 0.24
R_025	LLFYLLVLLALLSLLIL	-16.37	0.29 ± 0.14	NA
R_026	LLFYLLVALISLTLIL	-16.35	0.34 ± 0.05	-1.79 ± 0.24
R_027	LLYILLATLAFLLSLLIL	-16.29	0.27 ± 0.12	NA
R_028	LLFYLLVALTLLSLLIL	-16.26	0.4 ± 0.08	-2.36 ± 0.48
R_029	LLYALLIALFTLLVLLIL	-16.15	0.3 ± 0.03	NA
R_030	LLYALLTLLSVLLFLIL	-16.09	0.44 ± 0.07	-2.58 ± 0.43
R_031	LLTALLIALFVLLSLLIL	-16.08	0.33 ± 0.01	-1.57 ± 0.04
R_032	LLYSLLTLLVALLIFLIL	-15.9	0.24 ± 0.11	NA
R_033	LLYSLLALATLLVFLIL	-15.86	0.24 ± 0.07	NA
R_034	LLYVLLTLLALLSFLIL	-15.76	0.34 ± 0.13	-1.84 ± 0.72
R_035	LLYILLATLFLVLLSLLIL	-15.7	0.31 ± 0.03	-0.91 ± 0.1
R_036	LLYTLLIALAVVLSLIL	-15.58	0.37 ± 0.05	-2.1 ± 0.31
R_037	LLYALLVALITLLFLIL	-15.3	0.3 ± 0.07	NA
R_038	LLFYLLITLLVALLSLLIL	-15.25	0.34 ± 0.09	-1.79 ± 0.49
R_039	LLYILLATLALIIFSLLIL	-15.07	0.14 ± 0.06	NA
R_040	LLYILLATLAVLLSFLIL	-16.45	0.25 ± 0.07	NA
R_041	LLYSLLTLLATLIAFLIL	-16.53	0.21 ± 0.08	NA
R_042	LLVSLLLTLLFALLAYLIL	-16.54	0.29 ± 0.03	NA
R_043	LLTALLVALAYLLSFLIL	-16.55	0.5 ± 0.04	-2.81 ± 0.24
R_044	LLTYLLTLLAFLSLLIL	-17.5	0.41 ± 0.05	-2.44 ± 0.27
R_045	LLLLVLLITLLFALLSYLIL	-17.49	0.32 ± 0.06	-1.37 ± 0.27
R_046	LLYILLTTLLVALLSFLIL	-17.43	0.44 ± 0.05	-2.58 ± 0.31
R_047	LLYLLLVTLLAILLSFLIL	-17.35	0.17 ± 0.09	NA
R_048	LLYSLLVALATLLFLIL	-17.33	0.26 ± 0.07	NA
R_049	LLLAILLVTLFTLLSLLIL	-17.29	0.36 ± 0.09	-1.99 ± 0.48
R_050	LLYALLTALSVLLFLIL	-17.19	0.37 ± 0.03	-2.1 ± 0.18
R_051	LLYILLTALLVALLSLLIL	-15.06	0.31 ± 0.05	-0.94 ± 0.15
R_052	LLFSLLTLLVALLSLLIL	-17.12	0.26 ± 0.08	NA
R_053	LLVYLLTALLIALSLLIL	-16.69	0.37 ± 0.05	-2.14 ± 0.31
R_054	LLYSLLTLLVALLSFLIL	-16.68	0.29 ± 0.1	NA
R_055	LLYALLVALTALISLIL	-16.67	0.35 ± 0.08	-1.96 ± 0.42
R_056	LLTALLIALAVLLSFLIL	-16.65	0.4 ± 0.06	-2.35 ± 0.38
R_057	LLAVLLTLLFALLSYLIL	-16.62	0.26 ± 0.06	NA
R_058	LLYILLTLLAVLLSFLIL	-16.59	0.24 ± 0.04	NA
R_059	LLYFLLVALALLSLLIL	-16.58	0.32 ± 0.03	-1.1 ± 0.11
R_060	LLYILLFAITVLLSLLIL	-17.06	0.26 ± 0.06	NA
R_061	LLYVLLIALATLLSLLIL	-15.04	0.33 ± 0.06	-1.52 ± 0.29
R_062	LLYALLVALSLLFTLIL	-15.04	0.35 ± 0.03	-1.87 ± 0.14
R_063	LLYSLLTALLVALLFLIL	-14.78	0.29 ± 0.08	NA
R_064	LLTYLLVLLAIIISFLIL	-12.04	0.19 ± 0.12	NA
R_065	LLYVLLIALFTLLSLLIL	-11.97	0.26 ± 0.07	NA
R_066	LLYVLLTILLALLAFLIL	-11.94	0.32 ± 0.09	-1.38 ± 0.4
R_067	LLYLLLSTLLAFLIAILIL	-11.93	0.28 ± 0.04	NA
R_068	LLTYLLIVLLALLSFLIL	-11.69	0.41 ± 0.02	-2.4 ± 0.1
R_069	LLYLLLFTLLAALTSLLIL	-11.61	0.12 ± 0.06	NA
R_070	LLYALLFALLSLLVTLIL	-11.25	0.27 ± 0.02	NA

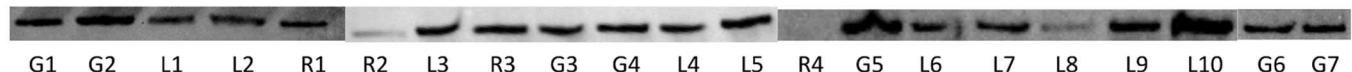
R_071	LLLTYLLAVLLAAILLFLLLIL	-12.11	0.32 ± 0.07	-0.98 ± 0.21
R_072	LLLYVLLTTLFALLSLLIL	-11.22	0.28 ± 0.08	NA
R_073	LLLAALLSLLFLLVFLILI	-10.66	0.46 ± 0.05	-2.65 ± 0.27
R_074	LLLYLLLATLAVLLSLLIL	-10.64	0.24 ± 0.07	NA
R_075	LLLYALLSLLTALLSVFLILI	-10.61	0.28 ± 0.09	NA
R_076	LLLTALLIALFLLSVLIL	-10.55	0.4 ± 0.04	-2.36 ± 0.25
R_077	LLLYVLLFALLTVLLSLLIL	-10.4	0.3 ± 0.02	NA
R_078	LLLYSLLFVLLAAILLTLIL	-10.33	0.25 ± 0.1	NA
R_079	LLLAYLLVLLATLLSFLLIL	-10.19	0.34 ± 0.07	-1.71 ± 0.38
R_080	LLLYVLLAAILTLLLSFLIL	-10.68	0.27 ± 0.1	NA
R_081	LLLYTLLATLVLVLLSFLIL	-17.69	0.25 ± 0.07	NA
R_082	LLLYVLLATLALLSFLIL	-12.44	0.26 ± 0.03	NA
R_083	LLLYALLLTLLVALLSFLIL	-12.82	0.25 ± 0.08	NA
R_084	LLLYAALLTLSLLVFLIL	-14.69	0.22 ± 0.04	NA
R_085	LLLYAALLLFSLLVTFLIL	-14.68	0.3 ± 0.1	NA
R_086	LLLYLLTALLIAILFVLIL	-14.55	0.27 ± 0.06	NA
R_087	LLLIILLSTLATLIVFLIL	-14.36	0.23 ± 0.05	NA
R_088	LLLYILLTLLVALLSFLIL	-14.33	0.28 ± 0.01	NA
R_089	LLLAALLFALLVSLLTWLIL	-14.2	0.3 ± 0.0	NA
R_090	LLLTVLLALLFALLSILIL	-14.11	0.36 ± 0.06	-2.01 ± 0.32
R_091	LLLYVLLTALLSFLIL	-12.66	0.33 ± 0.07	-1.51 ± 0.34
R_092	LLLYVLLFTLALLSLLIL	-13.95	0.28 ± 0.06	NA
R_093	LLLYVLLALTSLLVFLIL	-13.89	0.39 ± 0.03	-2.3 ± 0.16
R_094	LLLYSLLLILLAVLLTFIL	-13.86	0.24 ± 0.07	NA
R_095	LLLYTLLVFLALLSILIL	-13.66	0.34 ± 0.09	-1.68 ± 0.45
R_096	LLLYAALLTLLSVLLFIL	-13.65	0.3 ± 0.07	NA
R_097	LLLAALLVTLAAILLSFLIL	-13.32	0.17 ± 0.07	NA
R_098	LLLTVLLALLAFLLSILIL	-12.94	0.35 ± 0.06	-1.9 ± 0.33
R_099	LLLYTLLVALIAAILLFLIL	-12.88	0.37 ± 0.05	-2.12 ± 0.3
R_100	LLLYVLLTLLFALLSLLIL	-13.89	0.3 ± 0.11	NA
R_101	LLLYVLLVALISLLTLLIL	-24.44	0.32 ± 0.03	-1.09 ± 0.09
R_102	LLLYVLLVALATLSSFLIL	-17.7	0.22 ± 0.08	NA
R_103	LLLGYLLLTLLVALLSILIL	-17.85	0.48 ± 0.06	-2.74 ± 0.36
R_104	LLLYTLLFTLAVLLSLLIL	-22.18	0.36 ± 0.04	-2.01 ± 0.25
R_105	LLLYWLLTALLSLLFVLIL	-22.11	0.21 ± 0.05	NA
R_106	LLLYVLLTLLATLSSFLIL	-21.91	0.22 ± 0.12	NA
R_107	LLLYTLLVALAAILLSLLIL	-21.41	0.28 ± 0.02	NA
R_108	LLYSLLLTLLAVLLFSILIL	-21.39	0.21 ± 0.1	NA
R_109	LLLYLLITLLFSLLVFLIL	-21.29	0.3 ± 0.05	NA
R_110	LLYSLLALLFALLVTFLIL	-21.29	0.27 ± 0.05	NA
R_111	LLLYSLLVALFTLLFLIL	-21.29	0.28 ± 0.12	NA
R_112	LLLFSLLLTLLAALLSYLIL	-21.16	0.21 ± 0.07	NA
R_113	LLLYILLSTLALLIAFLIL	-21.11	0.21 ± 0.04	NA
R_114	LLYSLLITLLFVLLALIL	-20.93	0.25 ± 0.09	NA
R_115	LLLYTLLATLFLLSVLIL	-20.85	0.27 ± 0.11	NA
R_116	LLLYVLLFIALLLSTLIL	-20.83	0.22 ± 0.1	NA
R_117	LLLYILLSVLLALLTFIL	-20.82	0.19 ± 0.08	NA
R_118	LLLYTLLVALFALLSLLIL	-20.72	0.3 ± 0.07	NA
R_119	LLLYVLLIALFSLLTLLIL	-22.22	0.34 ± 0.04	-1.71 ± 0.23
R_120	LLLYALLTLLFTFLSLLIL	-22.25	0.28 ± 0.15	NA
R_121	LLLYALLTSLLVALLSFLIL	-22.5	0.34 ± 0.02	-1.73 ± 0.08
R_122	LLLYSLLALLFALLTIL	-22.52	0.45 ± 0.06	-2.6 ± 0.36
R_123	LLYTLLVSLLFALLLIL	-24.31	0.23 ± 0.06	NA
R_124	LLLYVLLTALLSLLFLIL	-24.25	0.37 ± 0.07	-2.17 ± 0.4
R_125	LLLYWLLVLLATLSSFLIL	-24.21	0.35 ± 0.07	-1.95 ± 0.39
R_126	LLLYSLLLTLLATLFLIL	-24.19	0.21 ± 0.08	NA
R_127	LLLYILLATLTLSSFLIL	-24.1	0.21 ± 0.11	NA
R_128	LLLFVLLATLAYLLSLLIL	-23.75	0.36 ± 0.03	-2.07 ± 0.14
R_129	LLLYSLLVALFALLTIL	-23.75	0.37 ± 0.07	-2.16 ± 0.4
R_130	LLLYLLAVLAPLLTSLLIL	-20.67	0.24 ± 0.09	NA
R_131	LLLSYLLVFLLATLSSLLIL	-23.6	0.39 ± 0.06	-2.32 ± 0.34
R_132	LLLYSLLLTLLAALLAFIL	-23.54	0.3 ± 0.08	NA
R_133	LLLYSLLLTLLFALLVIL	-23.43	0.36 ± 0.02	-2.01 ± 0.12
R_134	LLLYVLLATLAFLLSLLIL	-23.31	0.29 ± 0.06	NA
R_135	LLLYLLVTLLFALLSILIL	-23.2	0.37 ± 0.07	-2.15 ± 0.4
R_136	LLLATLISLLFTLLVLLIL	-23.06	0.43 ± 0.09	-2.51 ± 0.56
R_137	LLLYLLATLLFVLLSLLIL	-22.94	0.21 ± 0.12	NA
R_138	LLLYAALLLVSLLEFTLIL	-22.59	0.38 ± 0.06	-2.2 ± 0.38
R_139	LLLYVLLAALLTALLSILIL	-23.55	0.36 ± 0.11	-2.07 ± 0.63
R_140	LLLFYLLVALTALLSLLIL	-20.62	0.39 ± 0.03	-2.31 ± 0.17

R_141	LLLVYLLITLLFALLSLLLILI	-20.57	0.43 ± 0.04	-2.51 ± 0.25
R_142	LLLYIILLTVLLALLLSFLIL I	-20.38	0.26 ± 0.05	NA
R_143	LLLYTLLVALSTLLEFL LILI	-19.39	0.23 ± 0.09	NA
R_144	LLLTLTLLATLLAVLLSFL LILI	-19.38	0.37 ± 0.03	-2.09 ± 0.16
R_145	LLLVYLLLAFLTLSSLL LILI	-19.07	0.47 ± 0.06	-2.68 ± 0.36
R_146	LLLYVLLTAFLFALLSLL LILI	-19.06	0.32 ± 0.06	-0.98 ± 0.18
R_147	LLLLTLLIAVALLSFL LILI	-18.94	0.34 ± 0.07	-1.8 ± 0.37
R_148	LLLFVLLTTLAAILLSL LILI	-18.76	0.22 ± 0.02	NA
R_149	LLLYFLLAVALAVLLT LILI	-18.7	0.24 ± 0.06	NA
R_150	LLLTLTLLIAFLFVLLYS LILI	-19.44	0.43 ± 0.06	-2.52 ± 0.34
R_151	LLLVALLLALLFALLS TLLILI	-18.56	0.36 ± 0.04	-2.01 ± 0.23
R_152	LLVALLLTLLAAILLSFL LILI	-18.48	0.28 ± 0.05	NA
R_153	LLLYIILLVALLATLLT LILI	-18.48	0.34 ± 0.11	-1.84 ± 0.61
R_154	LLLYLILLTVLAFPLSIL LILI	-18.43	0.27 ± 0.05	NA
R_155	LLLTYLVALVALIAALSL LILI	-18.14	0.36 ± 0.02	-2.0 ± 0.09
R_156	LLLAVALVALSTLLIF LILI	-18.12	0.39 ± 0.06	-2.31 ± 0.37
R_157	LLTYLLIAALLAFLFV LILI	-18.08	0.4 ± 0.01	-2.35 ± 0.05
R_158	LLYSILLVALTLLLF LILI	-17.88	0.31 ± 0.13	-0.84 ± 0.34
R_159	LLLALLFALLTVLLS LILI	-18.52	0.44 ± 0.02	-2.59 ± 0.13
R_160	LLYSILLVALAVLLT LILI	-17.72	0.31 ± 0.22	0.32 ± 0.23
R_161	LLLAAILLTFLFVLLS LILI	-19.44	0.33 ± 0.06	-1.49 ± 0.26
R_162	LLTYLLVALTF LLSLLILI	-19.5	0.59 ± 0.25	-3.04 ± 1.29
R_163	LLAVLLTFLFTL SYLILI	-20.37	0.21 ± 0.11	NA
R_164	LLYSILLVALFLLAT LILI	-20.33	0.28 ± 0.1	NA
R_165	LLYSILLTLLAALLVF LILI	-20.13	0.23 ± 0.08	NA
R_166	LLLYLILLVALVALS TLLILI	-20.11	0.3 ± 0.05	NA
R_167	LLYTLLVALFLLS LILI	-20.05	0.27 ± 0.1	NA
R_168	LLLFYLLTALLIAALSL LILI	-20	0.38 ± 0.03	-2.23 ± 0.15
R_169	LLYWLLVALLLALLS TLLILI	-19.98	0.36 ± 0.01	-2.06 ± 0.04
R_170	LLLYIILLFTLLVALS LILI	-19.46	0.19 ± 0.06	NA
R_171	LLLFALLTILAYLLS LILI	-19.95	0.4 ± 0.06	-2.34 ± 0.34
R_172	LLLYAVALVALLTLLS WLILI	-19.84	0.27 ± 0.04	NA
R_173	LLYSILLFTLLAVALIAL LILI	-19.77	0.32 ± 0.1	-1.4 ± 0.42
R_174	LLLYWLLVALVALS TLLILI	-19.77	0.3 ± 0.04	NA
R_175	LLLALLIAALFVLLS TLLILI	-19.62	0.27 ± 0.05	NA
R_176	LLLYTLLVSLLAIIITL LILI	-19.61	0.33 ± 0.09	-1.58 ± 0.45
R_177	LLLFTYLLTALLITLLV LILI	-19.54	0.31 ± 0.09	NA
R_178	LLLSYLLVFLALLLAT LILI	-19.53	0.48 ± 0.07	-2.74 ± 0.39
R_179	LLLTYLLAVALVSLLF TLILI	-19.87	0.47 ± 0.09	-2.68 ± 0.54
R_180	LLLYVLLATLLTFLLS LILI	-9.81	0.29 ± 0.02	NA

Data for validated sets of designs where clashing mutations < 35% GpA

Interfacial designed positions of each sequence in bold

Designs where a positive ΔG was calculated are designated NA



Western ID	Sequence	Design ID
G1	LLL A VLL L T L GG L F A L I L I	
G2	LLL Y V L L G A L G I L T L I I	G_016
L1	LLL L LL F L A T L L I L S V L I I	L_021
L2	LLL F S LLL L LL V A L L T L L I I	
R1	LLL Y I L T A L L V A L S L I I	R_051
R2	LLL F Y L L V A L T A L S L I I	R_140
L3	LLL L LL A V L F L A L T S L I I	L_031
R3	LLL T V L L A L L F A L S I L I I	R_090
G3	LLL T A L L A L L F G L F S L I I	G_060
G4	LLL T A L L I G L F G L I V L I I	
L4	LLL L LL Y L L A V L T A L F S L I I	L_022
L5	LLL L LL L L L A L I F V L L S T L I I	
R4	LLL T S L L A L L F V L L I L I I	
G5	LLL V A L L L A L L G T L S F L I I	G_052
L6	LLL L LL F A L L A L L Y V L T S L I I	
L7	LLL L LL A I L A V L F T L S L I I	
L8	LLL Y A L L F V L T A L S L I I	L_006
L9	LLL L LL A V L F L L A L T S L I I	L_031
L10	LLL F A L L V L I T L L Y S L I I	
G6	LLL T V L L A L L G F L L G S L I I	G_022
G7	LLL V A L L L A L L G T L S F L I I	G_052

Figure S1. Immunoblotting of designed constructs. A subset of designed constructs were verified for their expression levels with western blots. While GAS_{right} and Left show similar levels of expression, Right designs often have fainter bands on western blots. Sequences without a Design ID are present in our sort-seq data but not part of our validated set (clashing mutants < 35% GpA)

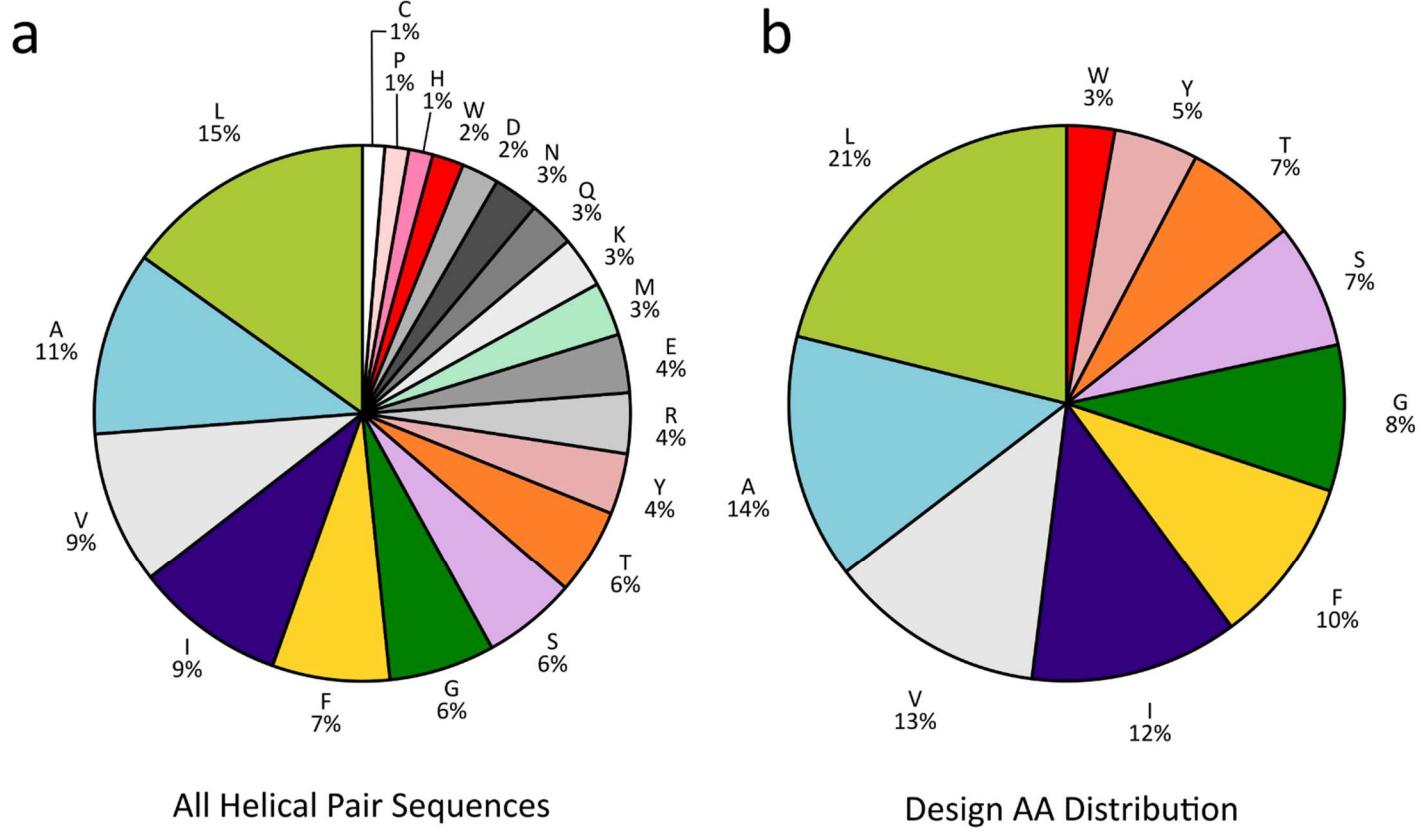


Figure S2. Amino acid composition. A) Frequency of amino acids from all transmembrane helical sequences extracted from OPM. B) The frequency of the design amino acids, adjusted to add up to 100% after removing the non-design amino acids. Sequences were designed with interfaces aiming to match the frequency of the design amino acid distribution (SEQUENCE_ENTROPY).

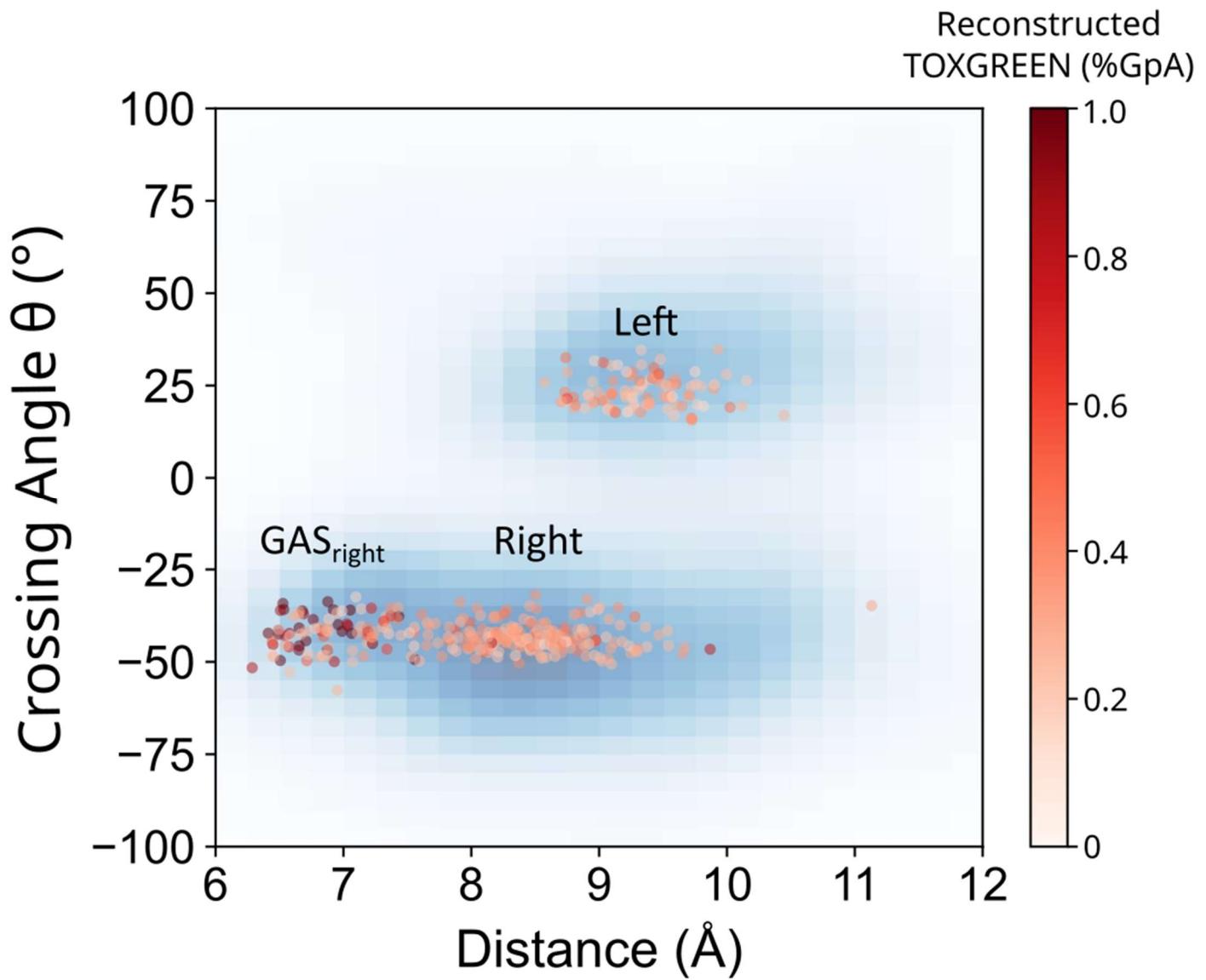


Figure S3. Output Geometry vs Dimerization Propensity. Distance and crossing angle of validated designed sequences plotted against %GpA in red. Sequences with highest dimerization propensity are dark red, most often found in GAS_{right}.

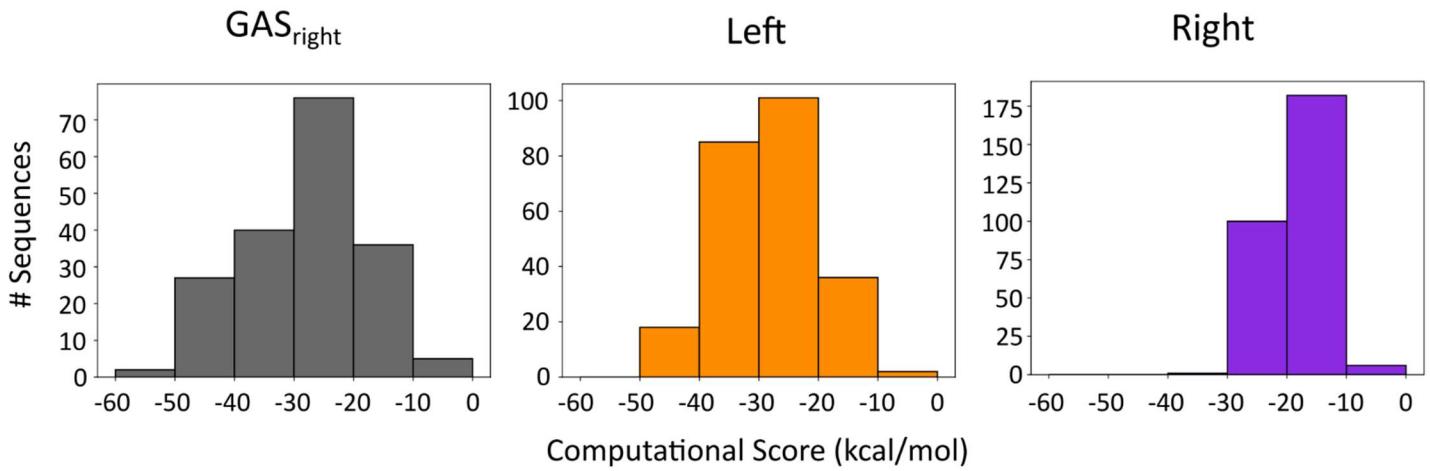


Figure S4. Frequency of sequences by computational score. Computational energy score of sequences present in sort-seq and the frequency for each design region.

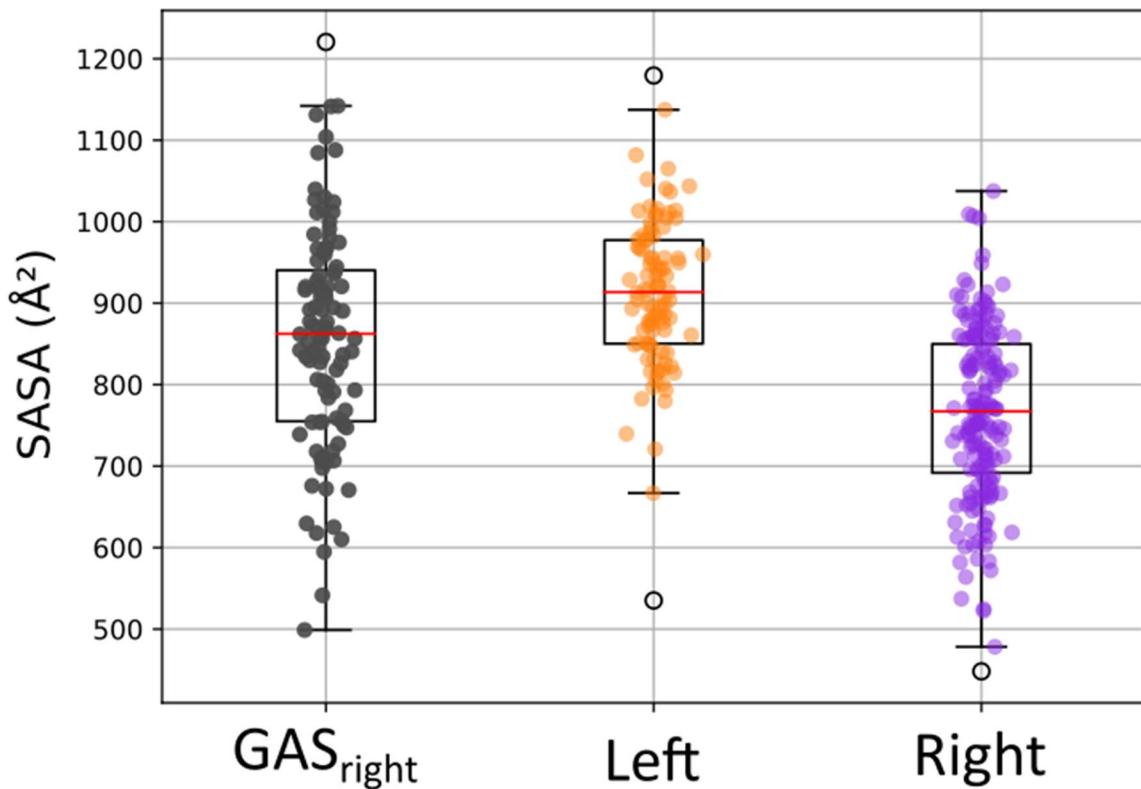


Figure S5. Interface SASA. Boxplots of calculated SASA at the interface of design structures of validated sequences. GAS_{right} and Left design interfaces are larger than Right, which may contribute to Right designs having a lower computational energy score.

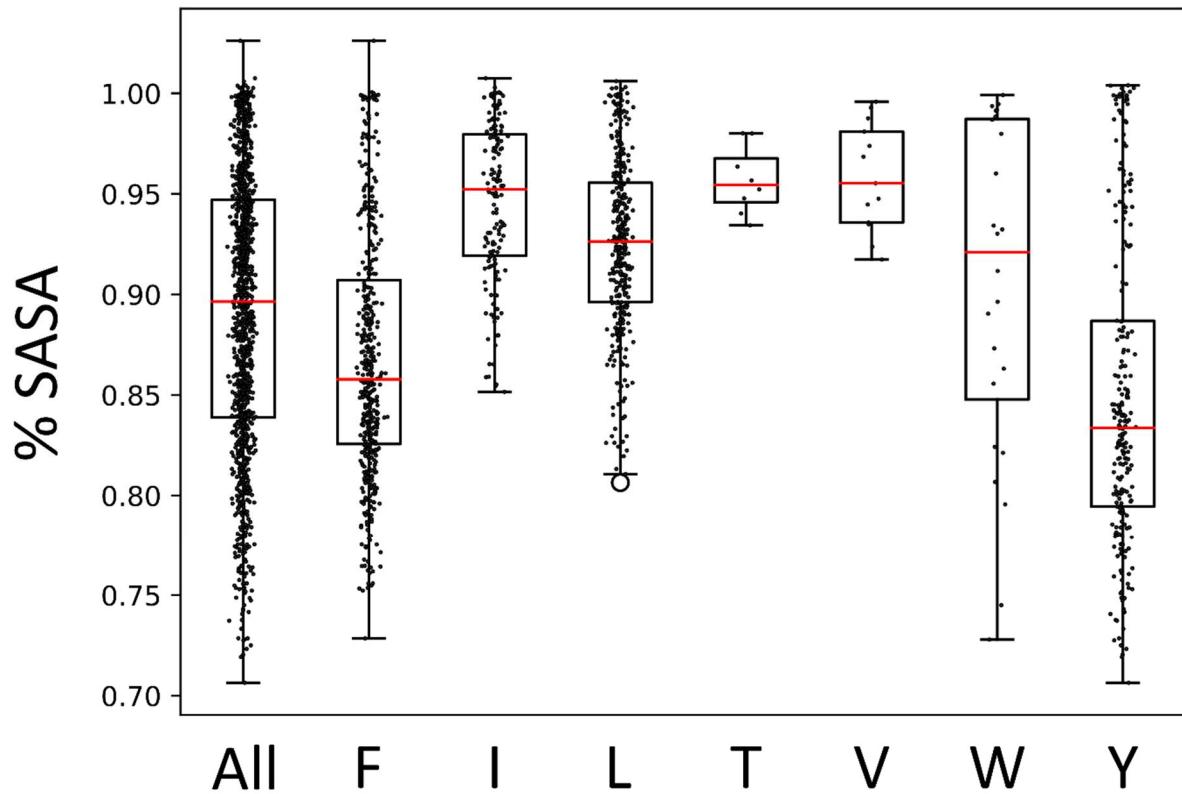


Figure S6. % SASA of Large→Ala. Single alanine point mutations were made on all interfacial positions designed structures. Mutants where the interface decreased the most, or the smallest % SASA (Design Interface SASA/Mutant Interface SASA x 100%), were selected.

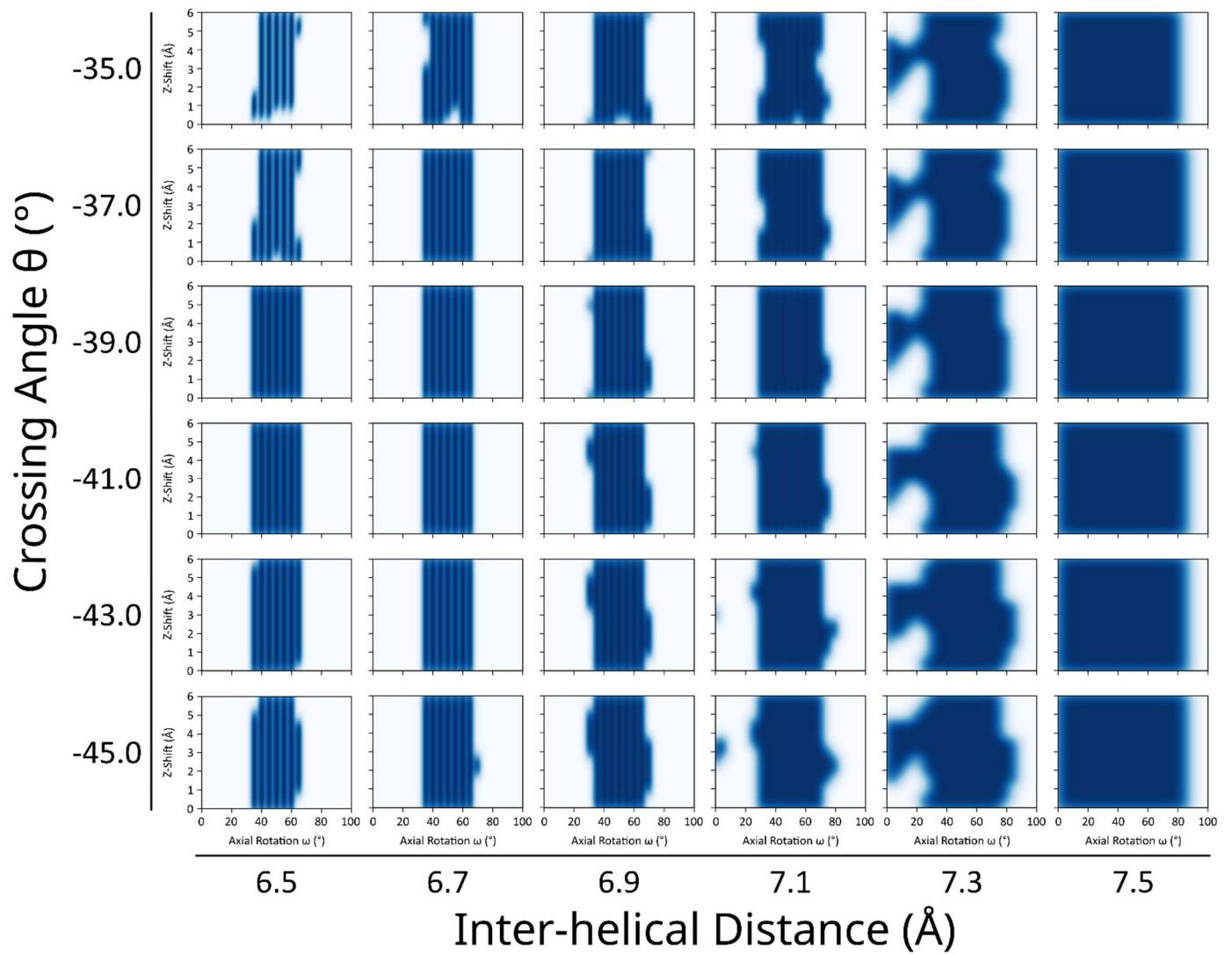


Figure S7. GAS_{right} axial rotations and z-shifts. A 4-dimensional grid of geometries were randomly generated to determine axial rotations and z-shifts for design. Poly-leucine helices with Ala at the design interface positions were placed at 3000 geometries and computational energy score measured. Geometries where energy score < 10 kcal/mol were plotted on a KDE plot of Z-Shift vs Axial Rotation. Values with KDE > 0.8 were randomly selected for design.

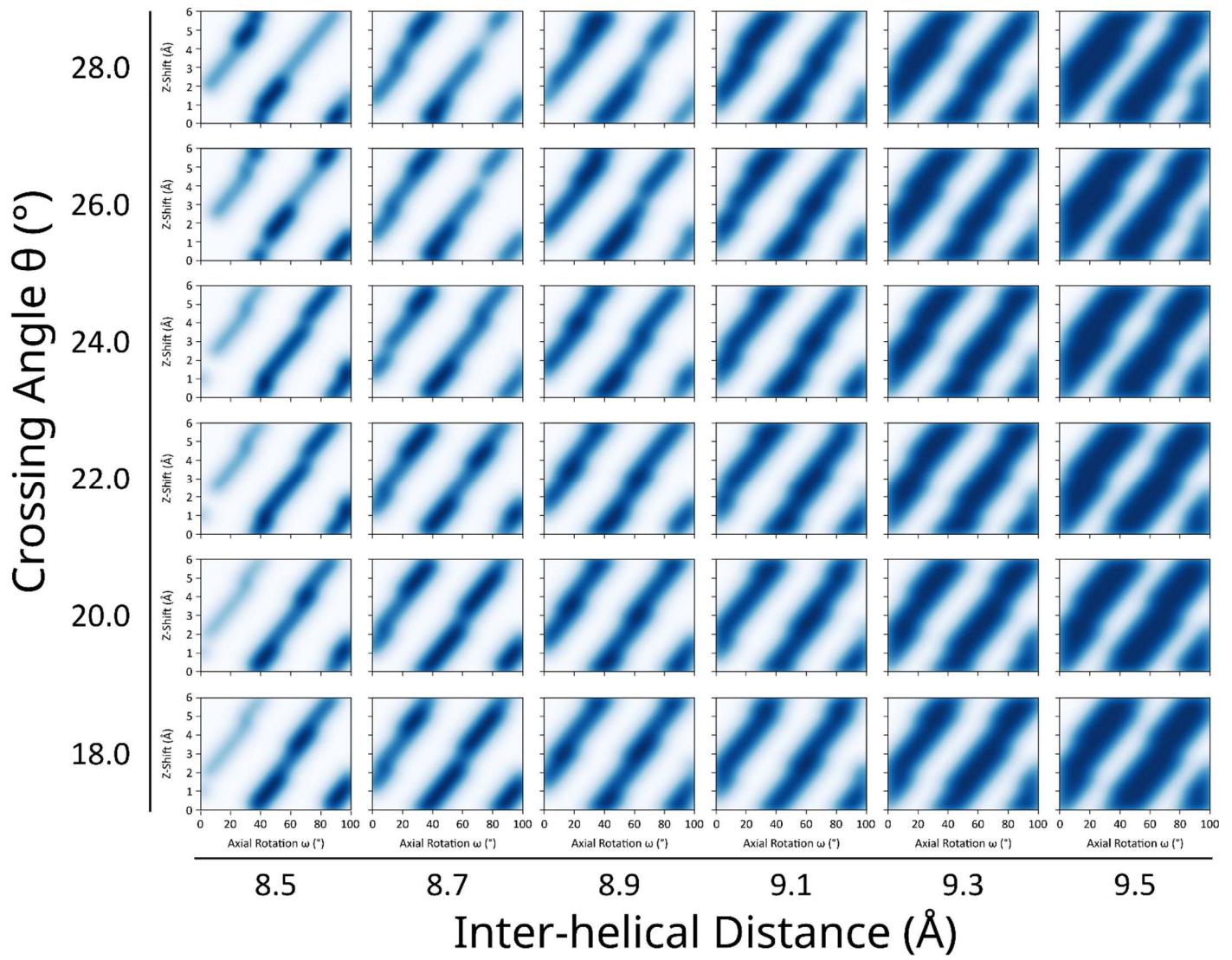


Figure S8. Left axial rotations and z-shifts. A 4-dimensional grid of geometries were randomly generated to determine axial rotations and z-shifts for design. Poly-leucine helices with Ala at the design interface positions were placed at 3000 geometries and computational energy score measured. Geometries where energy score < 10 kcal/mol were plotted on a KDE plot of Z-Shift vs Axial Rotation. Values with KDE > 0.8 were randomly selected for design.

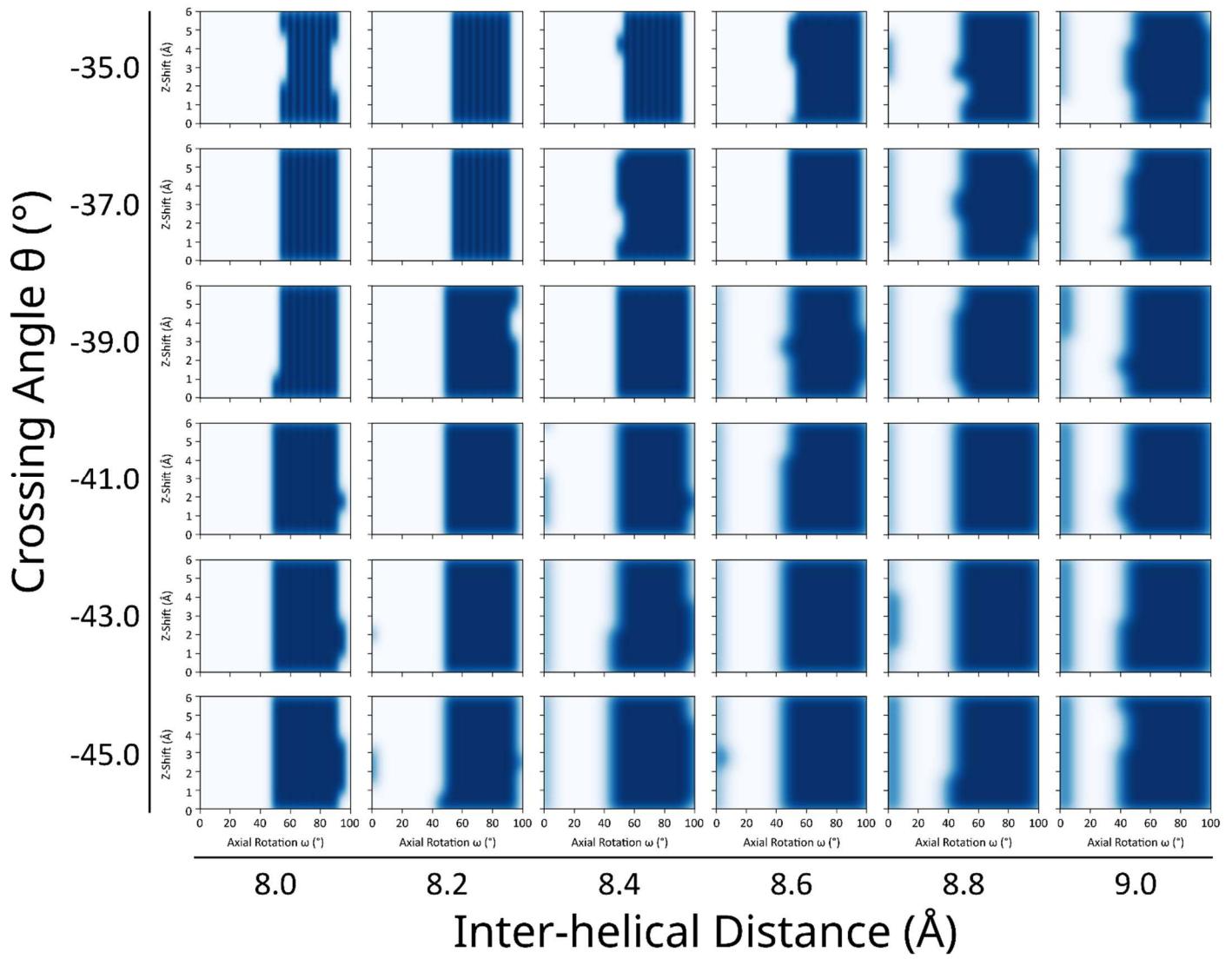


Figure S9. Right axial rotations and z-shifts. A 4-dimensional grid of geometries were randomly generated to determine axial rotations and z-shifts for design. Poly-leucine helices with Ala at the design interface positions were placed at 3000 geometries and computational energy score measured. Geometries where energy score < 10 kcal/mol were plotted on a KDE plot of Z-Shift vs Axial Rotation. Values with KDE > 0.8 were randomly selected for design.

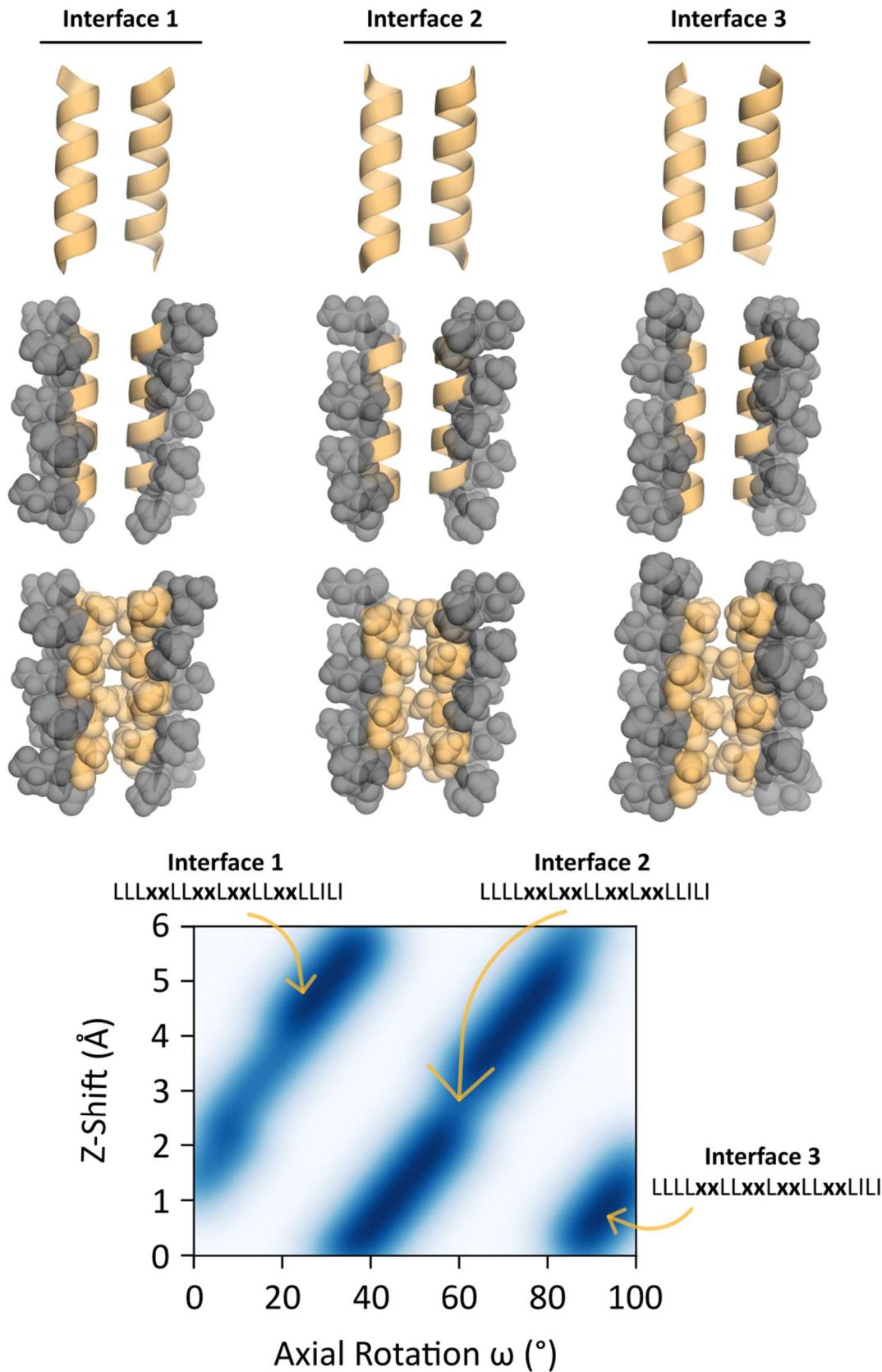


Figure S10. Left interfaces. Left-handed areas of high density were visually inspected to determine interfacial positions. Each design interface corresponds to a striped region of the axial rotation and z-shift density. Structures with Ala at corresponding interfacial positions are as visual representations.