| | | Ancestral protein structure | | | | | | |
| | | Change | | No change | | Variation ratio | | |
PDB code	CASE	Boundary	Non boundary	Boundary	Non boundary	RB$^+$	RB$^-$	R=(RB$^+$/RB$^-$)
4CSV	Kinase	111	16	660	843	0.1440	0.0186	7.7293
4UEU	Kinase	173	35	653	770	0.2094	0.0435	4.8172
4ZV1	Enzyme	70	23	430	524	0.1400	0.0420	3.3296
4ZV2	Enzyme	69	26	422	521	0.1405	0.0475	2.9565
5CBX	DNA-binding	51	10	278	341	0.1550	0.0285	5.4410
5CBY	DNA-binding	41	13	284	338	0.1262	0.0370	3.4062
5CBZ	DNA-binding	50	10	279	341	0.1520	0.0285	5.3343

Table 1. Secondary structure changes between modern and actual reconstructed ancestral protein structures. RB^+ is frequency of mutation at boundaries, i.e., changes at boundary divided by total boundary sites. RB^- is frequency of mutation at non-boundaries, i.e., changes at non-boundaries divided by total non-boundary sites.

| | | Ancestral protein structure | | | | | | |
| | | Change | | No change | | Variation ratio | | |
Inference	CASE	Boundary	Non boundary	Boundary	Non boundary	RB$^+$	RB$^-$	R=(RB$^+$/RB$^-$)
Branch73 (4CSV, 4UEU)	Kinase	178	24	765	869	0.1888	0.0269	7.0234
Branch326 (4ZV1, 4ZV2)	Enzyme	137	30	389	538	0.2605	0.0528	4.9313
Branch83 (5CBX)	DNA-binding	62	10	284	343	0.1792	0.0283	6.3254
Branch82 (5CBY)	DNA-binding	62	10	284	343	0.1792	0.0283	6.3254
Branch103 (5CBZ)	DNA-binding	62	10	284	343	0.1792	0.0283	6.3254

Table 2. Secondary structure changes between modern protein and ancestral secondary structure inferences based on DSO-sec. DSO-sec infers ancestral secondary structure for the internal nodes of the phylogenetic trees, in which the inferences can be compared with corresponding ancestral structures. RB^+ is frequency of mutation at boundaries, i.e., changes at boundary divided by total boundary sites. RB^- is frequency of mutation at non-boundaries, i.e., changes at non-boundaries divided by total non-boundary sites.

| | | Pfam phylogenetic tree | | | | | | |
| | | Change | | No change | | Variation ratio | | |
#Proteins (length)	Pfam	Boundary	Non boundary	Boundary	Non boundary	RB$^+$	RB$^-$	R=(RB$^+$/RB$^-$)
32 (49)	PF00514	48	7	889	1418	0.0512	0.0049	10.4284
23 (99)	PF07654	70	8	1353	1547	0.0492	0.0051	9.5617
37 (29)	PF00036	53	11	720	1125	0.0686	0.0097	7.0808
21 (410)	PF02518	107	12	1529	965	0.0654	0.0123	5.3249
26 (564)	PF00702	349	89	2301	2354	0.1317	0.0364	3.6150
37 (177)	PF00061	225	37	3355	2984	0.0628	0.0122	5.1315
26 (101)	PF01423	68	10	1272	1633	0.0507	0.0061	8.3376
34 (510)	PF13378	175	33	4501	2728	0.0374	0.0120	3.1312
22 (246)	PF00293	161	22	1291	1284	0.1109	0.0168	6.5823
25 (481)	PF07714	267	42	5232	4552	0.0486	0.0091	5.3109
23 (252)	PF00227	181	12	2838	2804	0.0600	0.0043	14.0691
23 (37)	PF00806	45	14	561	779	0.0743	0.0177	4.2062
24 (261)	PF00073	168	30	2609	2280	0.0605	0.0130	4.6583
25 (79)	PF00076	84	11	1381	1191	0.0573	0.0092	6.2655
24 (348)	PF00089	343	60	3489	1659	0.0895	0.0349	2.5644
58 (130)	PF00400	76	4	1559	501	0.0465	0.0079	5.8685

Table 3. Secondary structure changes between modern protein and ancestral secondary structure inferences based on DSO-sec within nineteen Pfam domains. RB^+ is frequency of mutation at boundaries, i.e., changes at boundary divided by total boundary sites. RB^- is frequency of mutation at non-boundaries, i.e., changes at non-boundaries divided by total non-boundary sites.