SUPPORTING INFORMATION

Directed Evolution's Influence on Rapid Density Fluctuations Illustrates How Protein Dynamics can become Coupled to Chemistry

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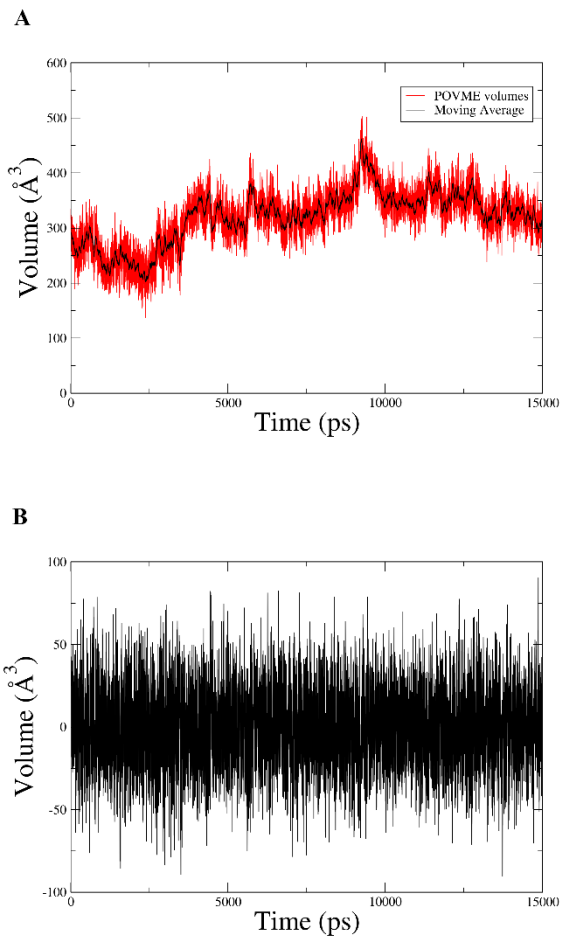

Figure S1: (A) Example of volume data obtained from POVME for a 1.5 ns trajectory with the moving average (B) Subtracting the moving average from the POVME data gives the fastest volume fluctuations.

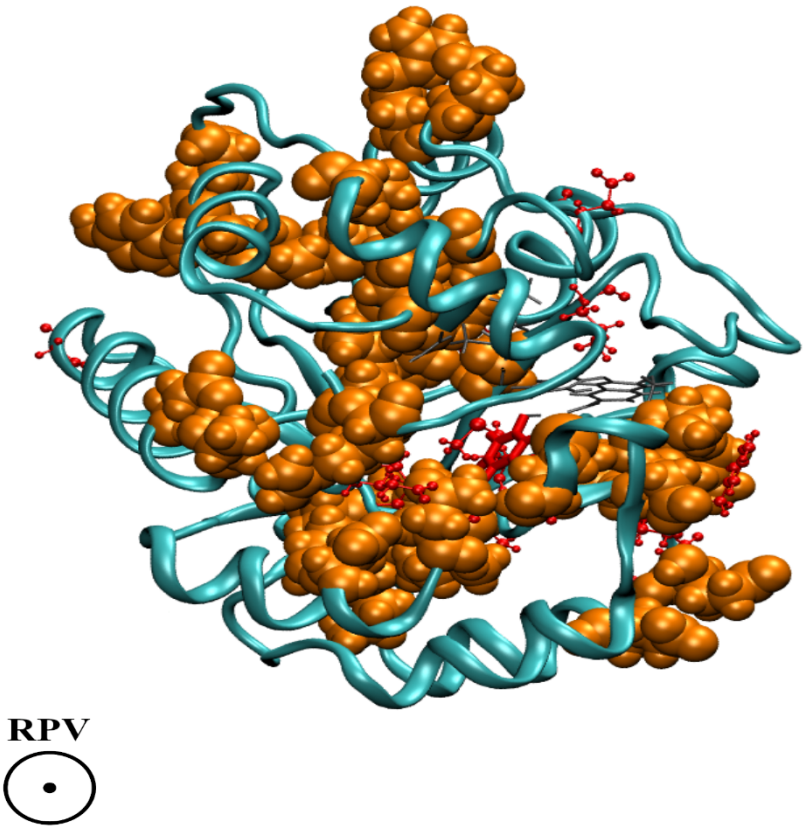

Figure S2: Variant V shown with all hydrogen bond decreases in orange and the thirteen mutations between variant IV and V shown in red.

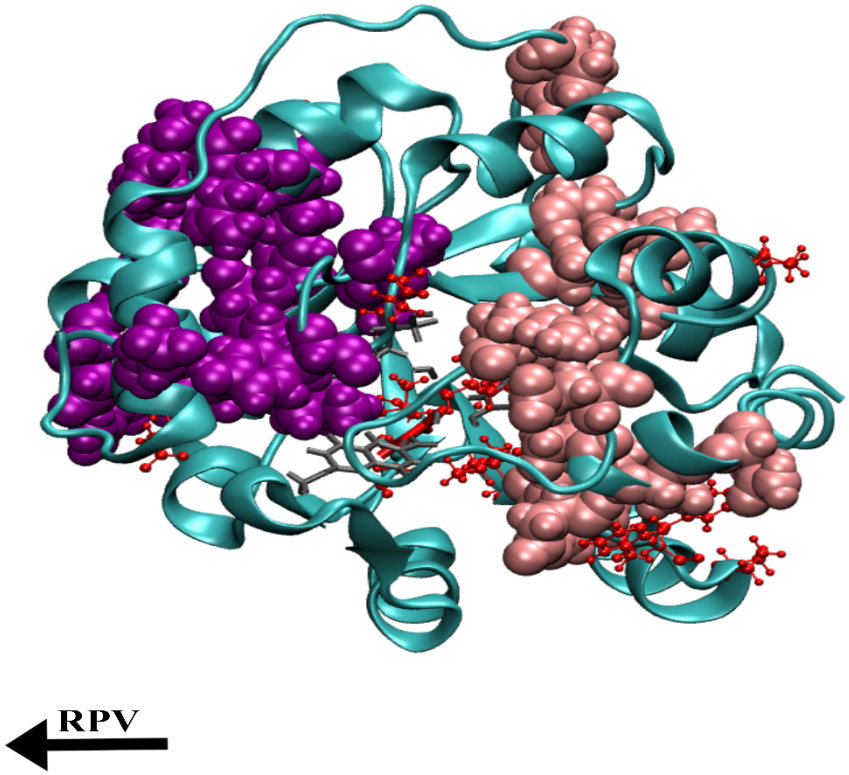

Figure S3: Variant V shown with all hydrogen bond increases shown in purple and pink and the thirteen mutations between variant IV and V shown in red.

Table S1: Pairs of residues which have significant decreases in hydrogen bonding going from variant IV to variant V. Reported is the change in percentage of frames which have a hydrogen bond.

Donor Residue		Acceptor Residue		Change in Percent
ARG	97	GLU	94	-58.59
SER	104	GLU	74	-56.4
ARG	19	THR	84	-48.21
LEU	108	LEU	80	-41.96
ASN	228	LYS	207	-41.14
ASN	161	ASP	165	-40.73
THR	138	GLU	141	-39.01
ARG	26	ALA	127	-36.2
THR	84	ASP	111	-35.54
LYS	242	GLU	245	-32.46
THR	138	GLU	141	-31.75
ALA	50	GLY	79	-31.45
LYS	207	ASN	228	-30.84
MET	182	ASN	161	-29.77
GLU	215	GLU	218	-28.51
SER	234	ASP	212	-26.84
SER	181	ASN	190	-25.39
ARG	3	ILE	136	-25.16
ILE	179	VAL	208	-24.52
ARG	26	TYR	123	-24.3
VAL	208	ILE	177	-24.02
ALA	100	LEU	96	-22.87
MET	237	SER	233	-22.82
SER	101	ARG	97	-21.9
ARG	175	GLU	155	-21.69
ARG	150	GLU	155	-21.42
ALA	127	ALA	122	-21.06
ASP	167	GLU	163	-20.57
LEU	170	LEU	166	-19.51
ARG	150	GLU	147	-19.27
ASN	193	SER	181	-19.09
ASN	193	ASN	190	-18.14
LYS	98	GLU	94	-17.66
LEU	197	ASN	193	-17.34
ALA	122	GLN	118	-17.04
VAL	227	LEU	222	-15.87
TYR	123	ILE	119	-15.86
GLY	91	GLU	85	-14.88
TYR	76	GLU	248	-14.83
SER	81	ALA	50	-14.64
TYR	72	PRO	240	-14.38
LYS	42	GLU	39	-14.28
LEU	157	VAL	130	-14.25
ASN	110	ILE	82	-13.9
TYR	88	GLU	85	-13.74
LYS	71	GLU	74	-13.69
LEU	222	GLU	218	-11.13
MET	73	TYR	69	-10.65
ILE	82	LEU	108	-10.08
PHE	230	ALA	47	-10.08
LYS	207	ASN	228	-10.05

Table S2: Pairs of residues which have significant increases in hydrogen bonding going from variant IV to variant V. Reported is the change in percentage of frames which have a hydrogen bond.

Donor Residue		Acceptor Residue		Change in Percent
ARG	150	GLY	173	49.31
SER	233	LEU	211	38.45
SER	235	ASP	212	33.91
GLU	147	GLU	143	33.67
GLN	118	PHE	112	30.43
ARG	54	GLU	63	29.89
LEU	80	PRO	106	28.4
ILE	49	PHE	230	27.95
THR	84	ASN	110	27.48
ARG	36	VAL	78	25.26
VAL	134	LEU	159	24.7
ARG	171	GLU	139	23.97
TYR	88	ASP	11	23.8
LYS	115	SER	117	23.24
ASN	239	SER	235	23.03
ARG	28	PRO	29	22.19
LYS	6	GLU	185	21.37
ILE	99	MET	95	21.33
ARG	223	VAL	227	20.97
ARG	140	GLU	147	19.99
ARG	54	ASP	65	19.99
ILE	37	LEU	33	19.08
THR	84	ASN	110	19.03
LYS	87	ASP	11	17.14
ASN	41	ASN	228	16.96
ILE	232	ILE	49	16.95
ARG	26	ARG	28	15.82
LYS	83	TYR	52	15.46
TYR	52	SER	81	13.82
ASN	41	ILE	37	13.62
TYR	148	SER	144	13.43
ARG	36	SER	32	13.03
ARG	28	ASP	128	12.86
SER	81	ALA	50	12.55
LYS	115	ARG	19	12.01
GLU	39	GLU	35	11.62
TYR	51	ILE	232	10.99
GLU	221	ASN	217	10.8
SER	102	LYS	98	10.67

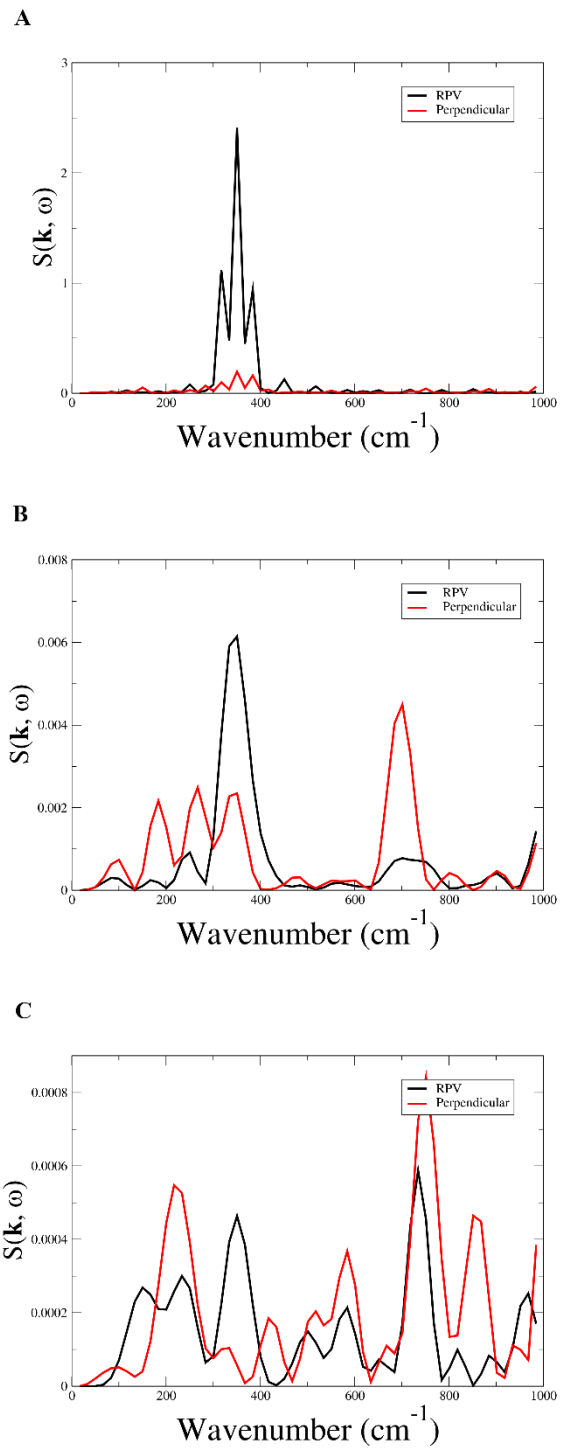

Figure S4: Coherent dynamical structure factor of variant IV. Plotted from are vectors corresponding to the entire protein, |**k**| = 0.130 (A), one-third the proteins length, |**k**| = 0.346 (B), and inter-residue distance, |**k**| = 1.038 (C).

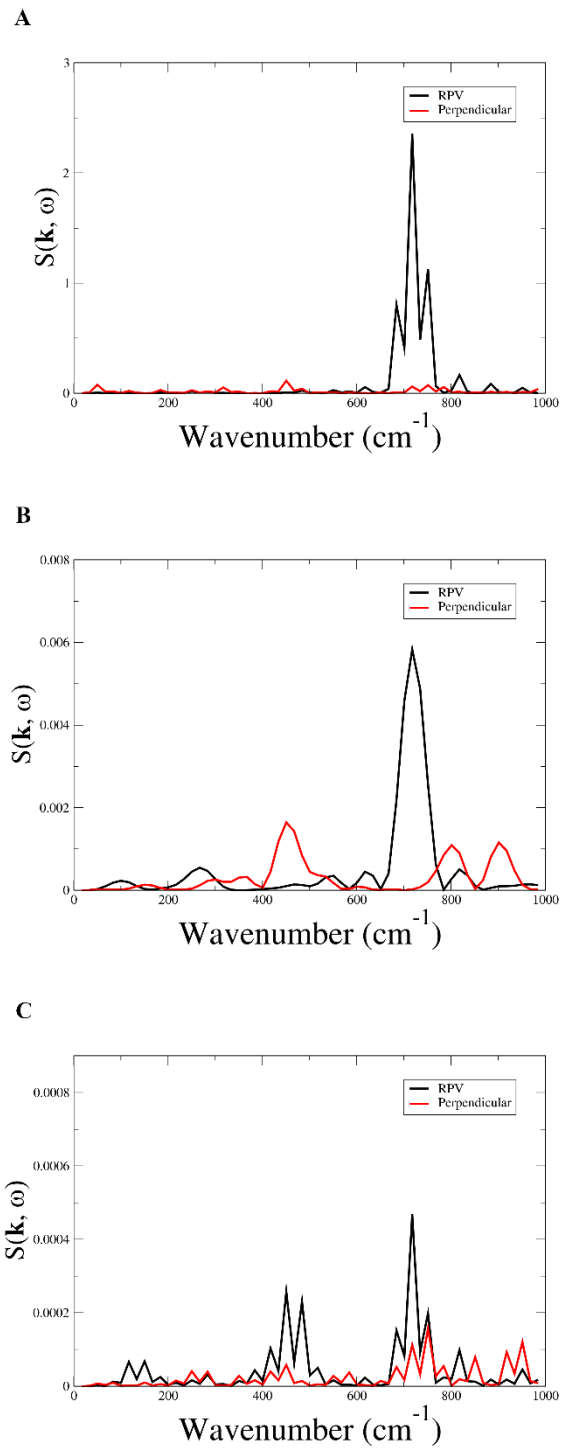

Figure S5: Coherent dynamical structure factor of variant V for proposed mechanism. Plotted from are vectors corresponding to the entire protein, |**k**| = 0.132 (A), one-third the proteins length, |**k**| = 0.365 (B), and inter-residue distance, |**k**| = 1.096 (C).

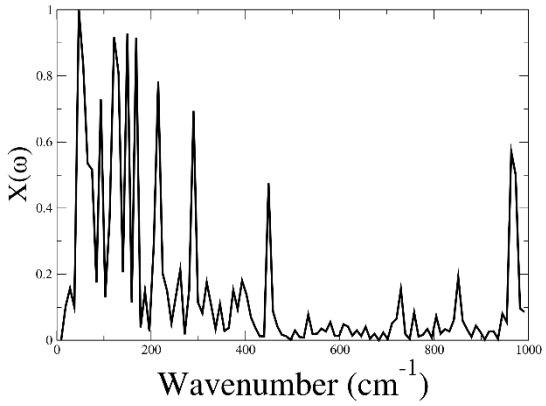

Figure S6: Fourier transform of the motion of the terminal carbon of Met182 to the closest ring of 6-methoxy-2-naphthaldehyde.