Supplementary Material to:

Unexpected outcome of surface-engineering an integral membrane protein: improved crystallization of cytochrome ba_3 from *Thermus thermophilus*

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Including:

Tables S1 through S4 (output of CONTACT analyses)

Table S1 (Supplemental)

Output from the CCP4 program, CONTACT [native cytochrome ba_3 oxidase from *Thermus thermophilus* (1EHK.pdb); Space group: $P4_32_12$]; atom-atom search will be done within the limits of 0.00 to 3.10 Å

```
   1: -Y+1/2,   X+1/2,  Z+3/4                -K,  H,  L
   2: -X,      -Y,      Z+1/2                -H, -K,  L
   3:  Y+1/2,  -X+1/2,  Z+1/4                 K, -H,  L
   4:  X+1/2,  -Y+1/2, -Z+1/4                 H, -K, -L
   5:  Y,       X,     -Z                     K,  H, -L
   6: -X+1/2,   Y+1/2, -Z+3/4                -H,  K, -L
   7: -Y,      -X,     -Z+1/2                -K, -H, -L
   8:  X,       Y,      Z
```

LIST OF CONTACTS:

source atoms	target atoms	distance	angle	symmetry operation
Tyr 15A N	Glu 168B OXT	3.05	***	3: Y+1/2, -X+1/2, Z+1/4
Ala 172A O	Arg 560A N	3.04	***	4: X+1/2, -Y+1/2, -Z+1/4
Glu 510A OE1	Glu 510A OE1	2.50	***	5: Y, X, -Z
Arg 560A N	Ala 172A O	3.04	***	4: X+1/2, -Y+1/2, -Z+1/4
Glu 168B OXT	Tyr 15A N	3.05	***	1:-Y+1/2, X+1/2, Z+3/4

5 contacts indicating a strong hydrogen bond at < 3.1 Å

Table S2 (Supplemental)

Output from the CCP4 program, CONTACT [recombinant cytochrome ba_3 oxidase from *Thermus thermophilus* (1XME.pdb); Space group: $P4_32_12$]; atom-atom search will be done within the limits of 0.00 to 3.10 Å

```
   1: -Y+1/2,   X+1/2,  Z+3/4                -K,  H,  L
   2: -X,       -Y,      Z+1/2                -H, -K,  L
   3:  Y+1/2,  -X+1/2,  Z+1/4                 K, -H,  L
   4:  X+1/2,  -Y+1/2, -Z+1/4                 H, -K, -L
   5:  Y,       X,     -Z                     K,  H, -L
   6: -X+1/2,   Y+1/2, -Z+3/4                -H,  K, -L
   7: -Y,      -X,     -Z+1/2                -K, -H, -L
   8:  X,       Y,      Z
```

LIST OF CONTACTS:

source atoms	target atoms	distance	angle	symmetry operation
Ser 6A N	Glu 96B OE2	2.79	***	3: Y+1/2, -X+1/2, Z+1/4
Ile 8A O	Glu 168B N	2.43	***	3: Y+1/2, -X+1/2, Z+1/4
Arg 10A NE	Glu 168B OXT	3.00	***	3: Y+1/2, -X+1/2, Z+1/4
Arg 10A NH1	Glu 168B O	2.78	***	3: Y+1/2, -X+1/2, Z+1/4
	Lys 167B O	3.07	***	3: Y+1/2, -X+1/2, Z+1/4
Ala 172A O	Arg 560A N	2.97	***	4: X+1/2, -Y+1/2,-Z+1/4
Lys 258A NZ	Glu 510A OE2	2.61	***	5: Y, X, -Z
Glu 510A OE2	Lys 258A NZ	2.61	***	5: Y, X, -Z
Arg 560A N	Ala 172A O	2.97	***	4: X+1/2, -Y+1/2,-Z+1/4
Glu 96B OE2	Ser 6A N	2.79	***	1:-Y+1/2, X+1/2, Z+3/4
Lys 167B O	Arg 10A NH1	3.07	***	1:-Y+1/2, X+1/2, Z+3/4
Glu 168B N	Ile 8A O	2.43	***	1:-Y+1/2, X+1/2, Z+3/4
Glu 168B O	Arg 10A NH1	2.78	***	1:-Y+1/2, X+1/2, Z+3/4
Glu 168B OXT	Arg 10A NE	3.00	***	1:-Y+1/2, X+1/2, Z+3/4

14 contacts indicating a strong hydrogen bond at < 3.1 Å

Table S3 (Supplemental)

Output from the CCP4 program, CONTACT [I-K258R recombinant cytochrome *ba*$_3$ oxidase from *Thermus thermophilus* (2qpd); Space group: $P4_12_12$]; atom-atom search will be done within the limits of 0.00 to 3.10 Å

```
   1: -Y+1/2,   X+1/2,  Z+1/4                -K,  H,  L
   2: -X,      -Y,      Z+1/2                -H, -K,  L
   3:  Y+1/2,  -X+1/2,  Z+3/4                 K, -H,  L
   4:  X+1/2,  -Y+1/2, -Z+3/4                 H, -K, -L
   5:  Y,       X,     -Z                     K,  H, -L
   6: -X+1/2,   Y+1/2, -Z+1/4                -H,  K, -L
   7: -Y,      -X,     -Z+1/2                -K, -H, -L
   8:  X,       Y,      Z
```

LIST OF CONTACTS:

source atoms			target atoms			distance	angle	symmetry operation
Ser 6A	OG	Ala 65B	O	...	2.56	***	6:-X+1/2, Y+1/2, -Z+1/4
Ser 6A	O	Arg 59B	O	...	2.93	***	6:-X+1/2, Y+1/2, -Z+1/4
Glu 7A	OE1	Gln 60B	O	...	2.96	***	6:-X+1/2, Y+1/2 -Z+1/4
Arg 10A	N	Glu 500A	O	...	2.72	***	5: Y, X, -Z
Arg 10A	NE	Leu 501A	O	...	2.99	***	5: Y, X, -Z
Arg 10A	NH1	Trp 403A	O	...	2.72	***	5: Y, X, -Z
		Asn 407A	N	...	2.74	***	5: Y, X, -Z
Arg 10A	O	Glu 503A	O	...	2.70	***	5: Y, X, -Z
Tyr 23A	OH	Glu 61B	OE1	...	2.98	***	6:-X+1/2, Y+1/2, -Z+1/4
Asn 98A	OD1	Arg 100A	NH1	...	2.71	***	5: Y, X, -Z
Asn 98A	ND2	Arg 100A	NH1	...	3.06	***	5: Y, X, -Z
		Arg 100A	NH2	...	3.01	***	5: Y, X, -Z
Arg 100A	NH1	Asn 98A	OD1	...	2.71	***	5: Y, X, -Z
		Asn 98A	ND2	...	3.06	***	5: Y, X, -Z
Arg 100A	NH2	Asn 98A	ND2	...	3.01	***	5: Y, X, -Z
Leu 326A	O	Thr 163B	OG1	...	2.85	***	3: Y+1/2,-X+1/2, Z+3/4
Arg 327A	NH1	Phe 88B	O	...	2.74	***	3: Y+1/2,-X+1/2, Z+3/4
Trp 403A	O	Arg 10A	NH1	...	2.72	***	5: Y, X, -Z
Asn 407A	N	Arg 10A	NH1	...	2.74	***	5: Y, X, -Z
Pro 499A	O	Pro 499A	O	...	2.84	***	5: Y, X, -Z
Glu 500A	O	Arg 10A	N	...	2.72	***	5: Y, X, -Z
Leu 501A	O	Arg 10A	NE	...	2.99	***	5: Y, X, -Z
Glu 503A	O	Arg 10A	O	...	2.70	***	5: Y, X, -Z
Asp 3B	O	Glu 168B	OE2	...	3.04	***	4: X+1/2,-Y+1/2, -Z+3/4
Arg 59B	O	Ser 6A	O	...	2.93	***	6:-X+1/2, Y+1/2, -Z+1/4
Gln 60B	O	Glu 7A	OE1	...	2.96	***	6:-X+1/2, Y+1/2, -Z+1/4
Glu 61B	OE1	Tyr 23A	OH	...	2.98	***	6:-X+1/2, Y+1/2, -Z+1/4
Ala 65B	O	Ser 6A	OG	...	2.56	***	6:-X+1/2, Y+1/2, -Z+1/4
Gln 73B	NE2	Pro 76B	O	...	2.68	***	5: Y, X, -Z
Pro 76B	O	Gln 73B	NE2	...	2.68	***	5: Y, X, -Z
Phe 88B	O	Arg 327A	NH1	...	2.74	***	1:-Y+1/2, X+1/2, Z+1/4
Thr 163B	OG1	Leu 326A	O	...	2.85	***	1:-Y+1/2, X+1/2, Z+1/4
Glu 168B	OE2	Asp 3B	O	...	3.04	***	4: X+1/2, -Y+1/2,-Z+3/4
Lys 4C	NZ	Lys 4C	NZ	...	2.84	***	7:-Y, -X, -Z+1/2

34 contacts indicating a strong hydrogen bond at < 3.1 Å

Table S4 (Supplemental)

Output from the CCP4 program, CONTACT [I-K258R/II-E4Q recombinant cytochrome ba_3 oxidase from *Thermus thermophilus* (2qpe); Space group: $P4_12_12$]; atom-atom search will be done within the limits of 0.00 to 3.10 Å

```
   1: -Y+1/2,   X+1/2,  Z+1/4                -K,  H,  L
   2: -X,      -Y,      Z+1/2                -H, -K,  L
   3:  Y+1/2,  -X+1/2,  Z+3/4                 K, -H,  L
   4:  X+1/2,  -Y+1/2, -Z+3/4                 H, -K, -L
   5:  Y,       X,     -Z                     K,  H, -L
   6: -X+1/2,   Y+1/2, -Z+1/4                -H,  K, -L
   7: -Y,      -X,     -Z+1/2                -K, -H, -L
   8:  X,       Y,      Z
```

LIST OF CONTACTS:

source atoms	target atoms	distance	angle	symmetry operation
Ser 6A OG	Ala 65B O	2.35	***	6:-X+1/2, Y+1/2, -Z+1/4
Ser 6A O	Arg 59B O	3.03	***	6:-X+1/2, Y+1/2, -Z+1/4
Glu 7A OE2	Gln 60B O	2.96	***	6:-X+1/2, Y+1/2, -Z+1/4
Arg 10A NH1	Leu 501A O	2.82	***	5: Y, X, -Z
Ala 14A O	Asn 98A OD1	3.03	***	5: Y, X, -Z
Asn 98A OD1	Ala 14A O	3.03	***	5: Y, X, -Z
	Arg 100A NH1	2.64	***	5: Y, X, -Z
Arg 100A NH1	Asn 98A OD1	2.64	***	5: Y, X, -Z
Arg 258A NH1	Asn 93B O	2.97	***	3: Y+1/2,-X+1/2, Z+3/4
Arg 258A NH2	Asn 93B O	2.97	***	3: Y+1/2,-X+1/2, Z+3/4
Leu 326A O	Thr 163B OG1	2.78	***	3: Y+1/2,-X+1/2, Z+3/4
Arg 327A NH1	Phe 88B O	2.43	***	3: Y+1/2,-X+1/2, Z+3/4
Pro 499A O	Pro 499A O	3.02	***	5: Y, X, -Z
Leu 501A O	Arg 10A NH1	2.82	***	5: Y, X, -Z
Asp 3B OD1	Glu 144B OE2	3.01	***	3: Y+1/2,-X+1/2, Z+3/4
Gln 4B NE2	Glu 96B OE2	2.66	***	3: Y+1/2,-X+1/2, Z+3/4
Arg 59B O	Ser 6A O	3.03	***	6:-X+1/2, Y+1/2, -Z+1/4
Gln 60B O	Glu 7A OE2	2.96	***	6:-X+1/2, Y+1/2, -Z+1/4
Ala 65B O	Ser 6A OG	2.35	***	6:-X+1/2, Y+1/2, -Z+1/4
Gln 73B NE2	Pro 76B O	2.98	***	5: Y, X, -Z
Pro 76B O	Gln 73B NE2	2.98	***	5: Y, X, -Z
Phe 88B O	Arg 327A NH1	2.43	***	1:-Y+1/2, X+1/2, Z+1/4
Asn 93B O	Arg 258A NH1	2.97	***	1:-Y+1/2, X+1/2, Z+1/4
	Arg 258A NH2	2.97	***	1:-Y+1/2, X+1/2, Z+1/4
Glu 96B OE2	Gln 4B NE2	2.66	***	1:-Y+1/2, X+1/2, Z+1/4
Glu 144B OE2	Asp 3B OD1	3.01	***	1:-Y+1/2, X+1/2, Z+1/4
Thr 163B OG1	Leu 326A O	2.78	***	1:-Y+1/2, X+1/2, Z+1/4

27 contacts indicating a strong hydrogen bond at < 3.1 Å