Supporting Information

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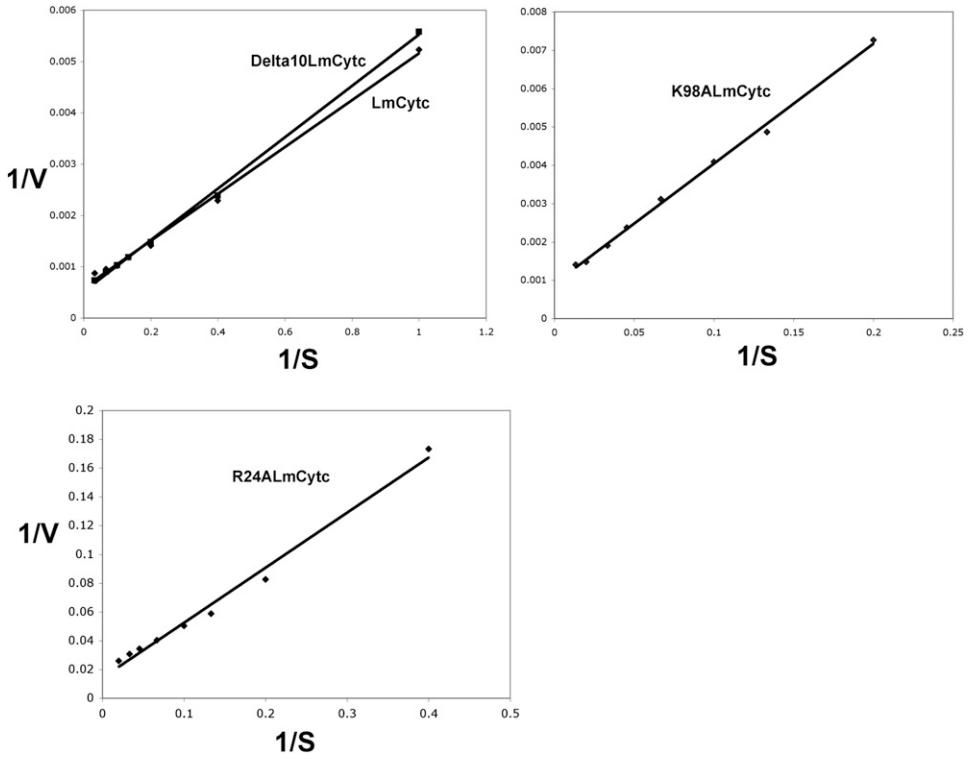

Fig. S1. Lineweaver–Burk plots for wild-type and mutant LmCytcs.

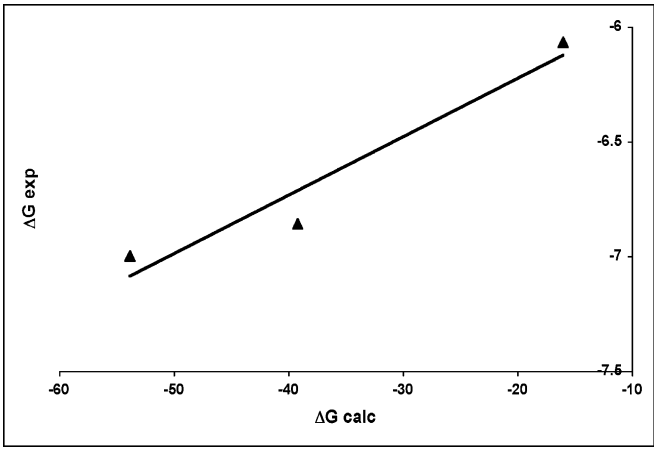

Fig. S2. The experimental ΔG (in kilocalories per mole) of forming the LmP–LmCytc complex was estimated by assuming $K_M = K_D$ and then using $\Delta G = -RT\ln(1/K_D)$. The computed ΔG was obtained using the MM-PBSA procedure described in the text. The computed ΔG does not include entropy, which is why the computed and calculated values are not on the same scale. We have used this procedure quite successfully in matching experimental and computed free energies for inhibitors of nitric oxide synthase, which works well for inhibitors of similar size and number of rotatable bonds. Thus, the errors introduced by ignoring the entropy term is negligible (1, 2).

1. Delker SL, et al. (2010) Unexpected binding modes of nitric oxide synthase inhibitors effective in the prevention of a cerebral palsy phenotype in an animal model. *J Am Chem Soc* 132 (15):5437–5442.
2. Xue F, et al. (2010) Potent, highly selective, and orally bioavailable gem-difluorinated monocationic inhibitors of neuronal nitric oxide synthase. *J Am Chem Soc* 132(40):14229–14238.

Table S1. Crystallographic data collection and refinement statistics

	Data set	
	LmP-Δ10LmCytc SAD	LmP-Δ10LmCytc HiRes
Space group	$P4_22_12$	$P4_22_12$
Unit cell dimensions, Å	a = 149.42, b = 149.42, c = 36.40	a = 149.74, b = 149.74, c = 36.42
Resolution range, Å	37.3–2.29	37.4–1.83
Radiation source	SSRL 12.2	SSRL 12.2
Wavelength, Å	1.73	0.97
Total observations	258,590	365,858
Unique reflections (highest shell)	19,056 (2,724)	36,729 (5,063)
Completeness, % (highest shell)	100.0 (100.0)	99.2 (95.8)
*$R_{p.i.m.}$ (highest shell)	0.031 (0.466)	0.033 (1.411)
Redundancy	7.3 (6.9)	10.0 (10.1)
$<I/\sigma>$ (highest shell)	17.8 (1.7)	12.4 (0.6)
Wilson B factor	54.5	42.3
Reflections used in refinement	18,965	35,729
Resolution range (Å) in refinement	23.31–2.30	31.99–1.84
*R_{work}	0.188	0.185
*R_{free}	0.216	0.197
$rmsd_{angles}$	0.95	0.91
$rmsd_{bonds}$	0.009	0.008

*$R_{p.i.m.}$ is the precision-indicating R factor, which is related to the traditional R_{merge} (R_{sym}) but provides a better estimate of data quality. See refs. 1 and 2.
†R factor = $\Sigma||Fo| - |Fc||/\Sigma|Fo|$, where Fo and Fc are the observed and calculated structure factors, respectively.
‡R_{free} was calculated with the 5% of reflections set aside randomly throughout the refinement. The values in parentheses were obtained in the outermost resolution shell.

1. Diederichs K, Karplus PA (1997) Improved R-factors for diffraction data analysis in macromolecular crystallography. *Nat Struct Biol* 4:269–275.
2. Weiss MS (2001) Global indicators of X-ray data quality. *J Appl Crystallogr* 34:130–135.