Supporting Information for

Glutathione limits aquacopper(I) to sub-femtomolar concentrations through cooperative assembly of a tetranuclear cluster

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Table of Contents

Derivation of Equation (4)

The concentrations of the tetranuclear complex $[Cu_4GS_6]$, deprotonated glutathione [GS], and uncomplexed $[Cu(I)_{aq}]$ are related by the complex stability constant β_{46} according to equations (S1) and (S2):

$$\beta_{46} = \frac{[Cu_4(GS)_6]}{[Cu(I)_{aq}]^4[GS]^6} \quad \text{(S1)}$$

$$\log\beta_{46} = \log[Cu_4(GS)_6] - 4\log[Cu(I)aq] - 6\log[GS] \quad \text{(S2)}$$

Solving for $pCu = -\log[Cu(I)_{aq}]$, yields equation (S3):

$$pCu = 1.5\log[GS] - 0.25\log[Cu_4GS_6] + 0.25\log\beta_{46} \quad \text{(S3)}$$

Treating glutathione as a monoprotic acid within the pH range investigated, [GS] is related to the total concentration of unbound glutathione $[GSH]_u$, the pH, and its pK_a according to equation (S4):

$$[GS] = \frac{[GSH]_u}{10^{pKa - pH} + 1} \quad \text{(S4)}$$

The total concentration of unbound glutathione is $[GSH]_u = [GSH] - [Cu_4(GS)_6]/6$. Since $[Cu(I)_{aq}]$ represents a negligible fraction of total Cu(I), $[Cu_4(GS)_6] \approx [Cu(I)]/4$ and $[GSH]_u \approx [GSH] - 1.5[Cu(I)]$. Substituting these approximations and equation (S4) into equation (S3) yields equation (S5):

$$pCu = 1.5\log\left(\frac{[GSH] - 1.5[Cu(I)]}{10^{pKa-pH} + 1}\right) - 0.25\log\left(\frac{[Cu(I)]}{4}\right) + 0.25\log\beta_{46} \quad \text{(S5)}$$

Due to the predominance of $Cu_4(GS)_6$ over the other copper-glutathione complexes, we found that formula (S5) is accurate to within 0.02 log units of the full three-cluster model above pH 6. Furthermore, when GSH is in large excess over total Cu(I) and its thiol-group is largely undissociated, the expression can be further simplified by the approximations $[GSH]_u \approx [GSH]$ and $\log[GS] \approx \log[GSH] + pH - pK_a$, yielding equation (S6):

$$pCu \approx 1.5\log[GSH] + 1.5pH - 1.5pK_a - 0.25\log[Cu(I)] + 0.25\log4 + 0.25\log\beta_{46} \quad \text{(S6)}$$

Equation (4) in the main text is then obtained after substituting the combined constant terms in equation (S6), $0.25\log\beta_{46} + 0.25\log4 - 1.5pK_a$ for the approximate value of 8.256.

Table S1: Ratio of stoichiometric coefficients (B/A) calculated for clusters $\{Cu_xS_y\}$ with stoichiometric indices x and y varying between 1 and 12 for equilibrium (1) (see main text for details). Crystallographically characterized structures are marked in blue (synthetic compounds) and green (metalloprotein).

		Stoichiometric index of Cu (x)											
	$\{Cu_xS_y\}$	1	2	3	4	5	6	7	8	9	10	11	12
Stoichiometric index of thiolate (y)	1	**0.5**	2	3.5	5	6.5	8	9.5	11	12.5	14	15.5	17
	2	-0.5 [a]	1	2.5	4	5.5	7	8.5	10	11.5	13	14.5	16
	3	-1.5 [b]	0	1.5 [c]	3	4.5	6	7.5	9	10.5	12	13.5	15
	4	-2.5	-1 [d]	**0.5**	2 [e]	3.5	5	6.5	8	9.5	11	12.5	14
	5	-3.5	-2	-0.5	1	2.5	4	5.5	7	8.5	10	11.5	13
	6	-4.5	-3	-1.5 [f]	0 [g]	1.5 [h]	3 [i]	4.5	6	7.5	9	10.5	12
	7	-5.5	-4	-2.5	-1	**0.5** [j]	2	3.5	5	6.5	8	9.5	11
	8	-6.5	-5	-3.5	-2	-0.5	1	2.5	4 [k]	5.5	7	8.5	10
	9	-7.5	-6	-4.5	-3	-1.5	0	1.5 [l]	3	4.5	6	7.5	9
	10	-8.5	-7	-5.5	-4	-2.5	-1	**0.5**	2 [m]	3.5	5	6.5	8
	11	-9.5	-8	-6.5	-5	-3.5	-2	-0.5	1	2.5	4	5.5	7
	12	-10.5	-9	-7.5	-6	-4.5	-3	-1.5	0	1.5	3	4.5	6 [n]

References: [a] Groysman, S.; Majumdar, A.; Zheng, S. L.; Holm, R. H., *Inorg. Chem.* **2010,** *49*, 1082-1089; Koch, S. A.; Fikar, R.; Millar, M.; Osullivan, T., *Inorg. Chem.* **1984,** *23*, 121-122; Sampanthar, J. T.; Vittal, J. J.; Dean, P. A. W., *J. Chem. Soc. Dalton Trans.* **1999**, 3153-3156. [a,g] Kohner-Kerten, A.; Tshuva, E. Y., *J. Organomet. Chem.* **2008,** *693*, 2065-2068. [a,h] Fujisawa, K.; Imai, S.; Kitajima, N.; Moro-oka, Y., *Inorg. Chem.* **1998,** *37*, 168-169. [b] Garner, C. D.; Nicholson, J. R.; Clegg, W., *Inorg. Chem.* **1984,** *23*, 2148-2150. [b,g] Coucouvanis, D.; Murphy, C. N.; Kanodia, S. K., *Inorg. Chem.* **1980,** *19*, 2993-2998; Fujisawa, K.; Imai, S.; Suzuki, S.; Moro-oka, Y.; Miyashita, Y.; Yamada, Y.; Okamoto, K., *J. Inorg. Biochem.* **2000,** *82*, 229-238. [c] Rungthanaphatsophon, P.; Barnes, C. L.; Walensky, J. R., *Dalton Trans.* **2016,** *45*, 14265-14276. [a,b,d,g,h] Zeevi, S.; Tshuva, E. Y., *Eur. J. Inorg. Chem.* **2007**, 5369-5376. [e,k] Schröter-Schmid, I.; Strähle, J., *Z. Naturforsch., B: Chem. Sci.* **1990,** *45*, 1537-1542. [e,n] Block, E.; Kang, H.; Oforiokai, G.; Zubieta, J., *Inorg. Chim. Acta* **1990,** *167*, 147-148. [f] Rao, C. P.; Dorfman, J. R.; Holm, R. H., *Inorg. Chem.* **1986,** *25*, 428-439. [g] Baumgartner, M.; Schmalle, H.; Dubler, E., *Polyhedron* **1990,** *9*, 1155-1164; Dance, I. G.; Bowmaker, G. A.; Clark, G. R.; Seadon, J. K., *Polyhedron* **1983,** *2*, 1031-1043; Dance, I. G.; Calabrese, J. C., *Inorg. Chim. Acta* **1976,** *19*, L41-L42. [g,j] Baumgartner, M.; Schmalle, H.; Baerlocher, C., *J. Solid State Chem.* **1993,** *107*, 63-75. [h] Dance, I. G., *J. Chem. Soc. Chem. Commun.* **1976**, 68-69; Bowmaker, G. A.; Clark, G. R.; Seadon, J. K.; Dance, I. G., *Polyhedron* **1984,** *3*, 535-544. [a,i] Ferrara, S. J.; Mague, J. T.; Donahue, J. P., *Inorg. Chem.* **2012,** *51*, 6567-6576. [j] Dance, I. G., *J. Chem. Soc. Chem. Commun.* **1976**, 103-104; Dance, I. G., *Aust. J. Chem.* **1978,** *31*, 2195-2206. [k] Yang, Q. C.; Tang, K. L.; Liao, H.; Han, Y. H.; Chen, Z. G.; Tang, Y. Q., *J. Chem. Soc. Chem. Commun.* **1987**, 1076-1077. [l] Dubler, E.; Baumgartner, M., *Solid State Ionics* **1990,** *43*, 193-202. [m] Calderone, V.; Dolderer, B.; Hartmann, H. J.; Echner, H.; Luchinat, C.; Del Bianco, C.; Mangani, S.; Weser, U., *Proc. Natl. Acad. Sci. USA* **2005,** *102*, 51-56. [n] Block, E.; Gernon, M.; Kang, H. K.; Oforiokai, G.; Zubieta, J., *Inorg. Chem.* **1989,** *28*, 1263-1271.

Table S2: Calculated fold increase in relative abundance of the indicated species versus $Cu_4(GS)_6$ upon acidification from pH 7 to pH 6 assuming an initial relative abundance of 10% at 100 µM Cu(I) and 1 mM GSH. Species marked in blue correspond to authenticated Cu(I)-thiolate cluster structures with B/A = 0-2. Additional hypothetical species with B/A = 0.5 are marked in red.

Species	Fold increase [a]	log β_{xy} ($Cu_x(GS)_y$) [b]
$Cu_3(GS)_3$	41	54.30
$Cu_4(GS)_4$	88	74.27
$Cu_5(GS)_6$	22	99.08
$Cu_5(GS)_7$	3.0	103.90
$Cu_7(GS)_9$	14	143.88
$Cu_8(GS)_{10}$	21	163.88
Cu(GS)	3.3	14.36
$Cu_3(GS)_4$	3.3	59.12
$Cu_7(GS)_{10}$	2.7	148.71

[a] Abundance relative to $Cu_4(GS)_6$; [b] Formation constant used for simulation (see text for details).

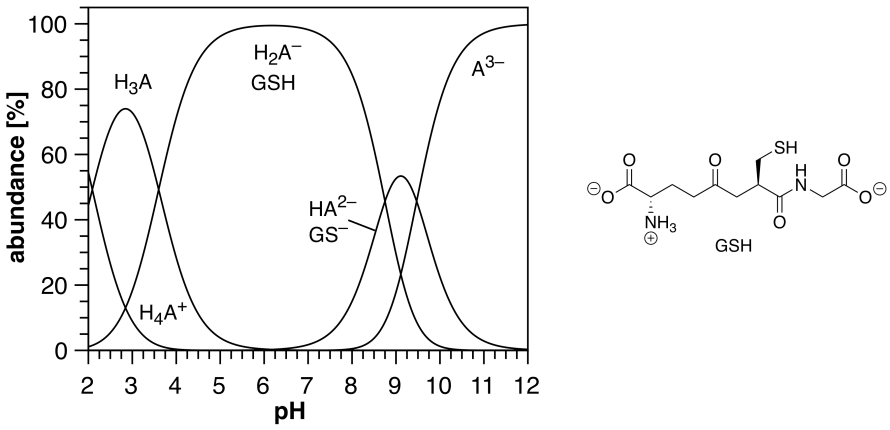

Figure S1: Species distribution diagram for the stepwise protonation of glutathione as a function of pH. Fully deprotonated glutathione carries a –3 net charge, denoted as A^{3-} in the diagram. At physiological pH, the di-protonated monoanion H_2A^- represents the major species, which is referred to as GSH in the main text. The corresponding line drawing of GSH is shown to the right. The diagram was computed using Hyss software (30) based on the reported macroscopic pK_a values of 1.98, 3.49, 8.64, and 9.36 (20), which were corrected upward by 0.11 logarithmic units to account for the proton activity at ionic strength 0.1 (17).

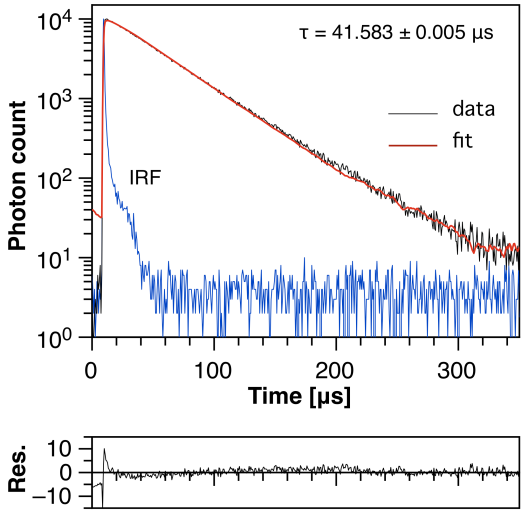

Figure S2: Time-dependent luminescence decay profile for a solution composed of 100 µM Cu(I) (supplied by addition of $CuSO_4$) and 4 mM GSH in vitrified solution (10 mM PIPES, 100 mM KCl + 33% propylene glycol) at 77 K. IRF = instrument response function (blue trace); the decay data are displayed in black, and a non-linear least-squares fit of the luminescence decay to a mono-exponential decay model is shown as a red trace.

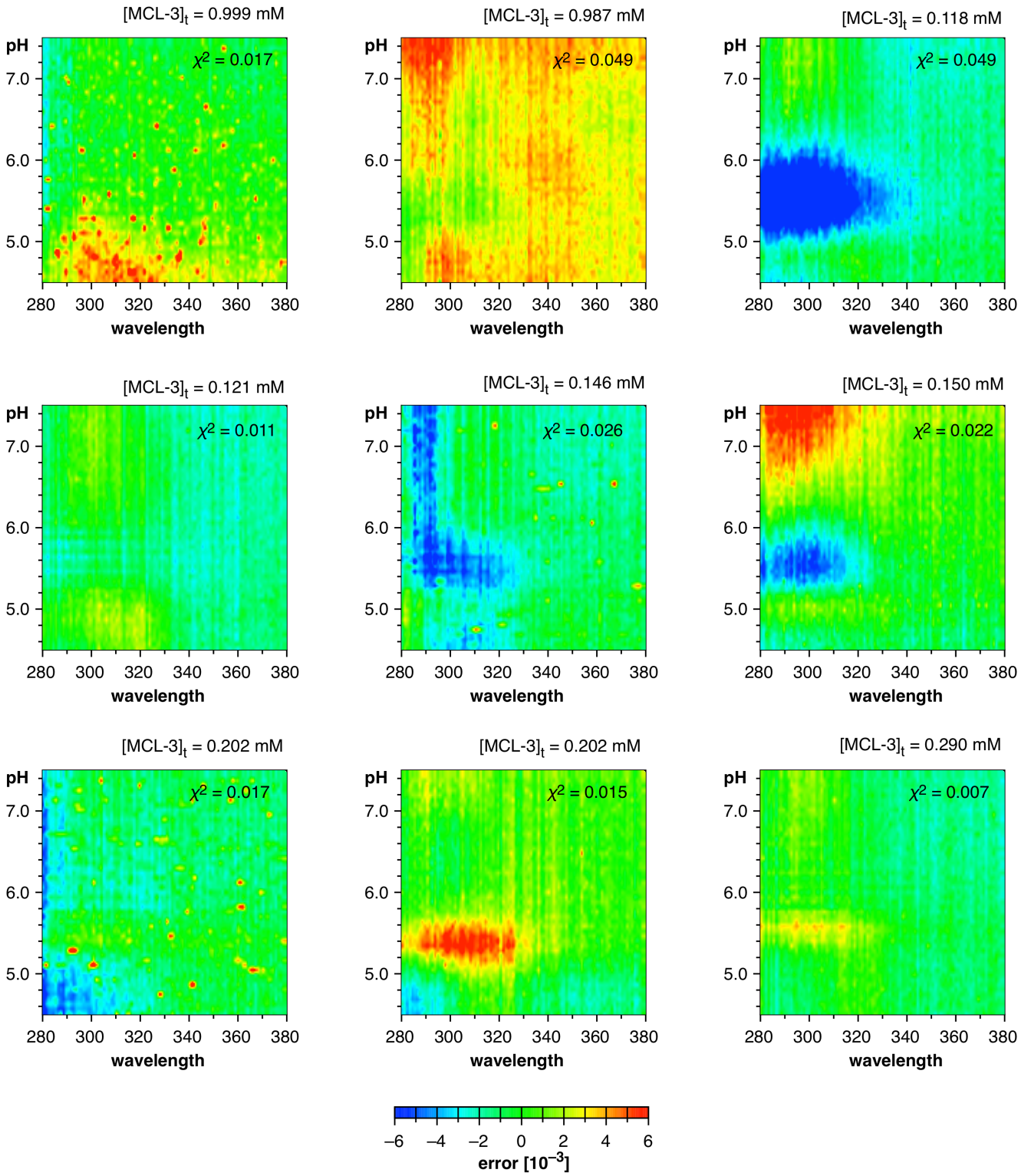

Figure S3: Contour plots for the residuals from global non-linear least squares fitting of spectrophotometric pH titration data for the Cu(I)-glutathione equilibrium system using MCL-3 as competitor ligand (100 µM Cu(I), 4 mM GSH, universal buffer, 0.1 M KCl, 25°C). Global non-linear least squares fitting was performed based on a data set composed of 18 independent pH titrations, each of which was conducted at a different MCL-3 concentration ranging between 0 to 1.7 mM (see main text and Experimental Procedures for details).

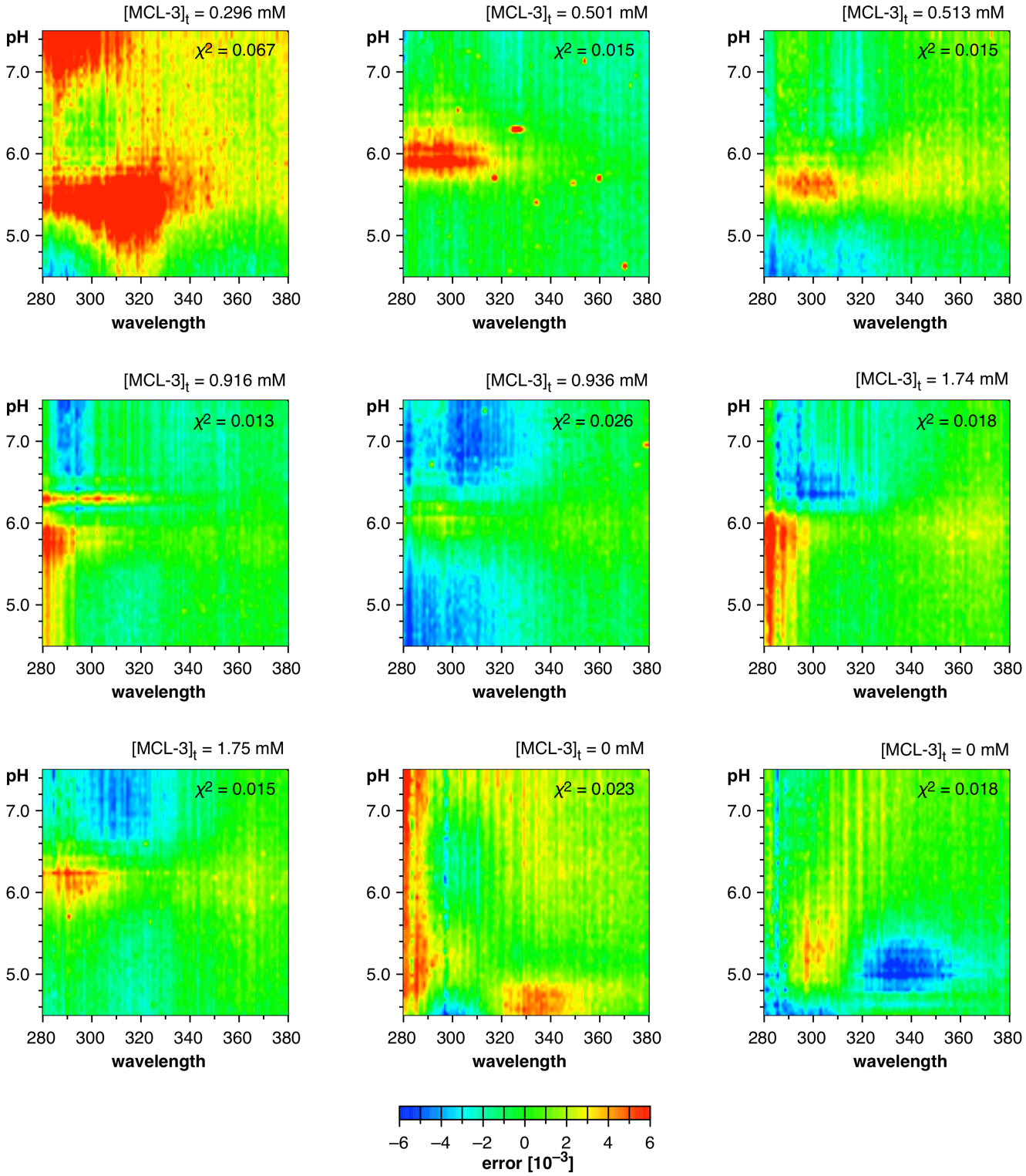

Figure S3 (continued)