

Cisplatin, glutathione and the third wheel: copper-(1,10-phenthroline) complex modulates cisplatin-GSH interactions from antagonism to synergism in cancer cells resistant to cisplatin

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SUPPORTING INFORMATION

Table S1. Citotoxicity of CDDP and C0 against CCRF-CEM-wt and CCRF-CEM-res after 14, 26 and 52 passages in the presence of CDDP.

	CDDP	C0	Doxorubicin
CCRF-CEM-wt	$\pm 1.12 \mu\text{M}$	1.0 μM	0.02 μM
CCRF-CEM-res	2.6 μM	0.8 μM	0.02 μM
R.I.*= 2.32 (14th passage)			
CCRF-CEM-wt	$\pm 1.12 \mu\text{M}$	1.1 μM	0.02 μM
CCRF-CEM-res	2.52 μM	0.8 μM	0.02 μM
R.I.= 2.25 (26th passage)			
CCRF-CEM-wt	$\pm 1.12 \mu\text{M}$	1.0 μM	0.02 μM
CCRF-CEM-res	6.98 μM	0.74 μM	0.02 μM
R.I.= 6.23 (52th passage)			

* Resistance Index

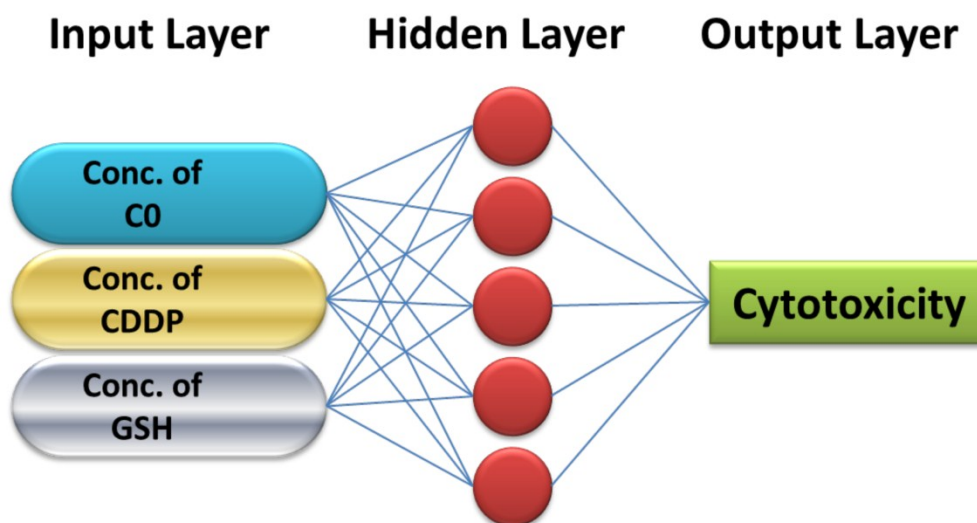


Figure S1 Architecture of the network used for the study of **C0-CDDP-GSH** ternary system.

The ANN architecture is formed by a series of logic units, called neurons, organized in layers, i.e. the input, hidden and output layers. The input layer contains variables such as, for example, concentrations, temperatures or pH, while the output layer contains the response functions, for example cytotoxicity, absorbance, conductivity. The information is passed from the first layer to the second one and then to the third. The more complex is the system, the greater is the number of hidden layers. As for the human brain, the ANN needs to be trained with a set of known input-response data, called training set. The objects of the set are presented to the net that estimates their real output, as a function of the input vector and of the weights, from the input to the hidden layer, and then from the hidden layer to the output one. The quality of the training is measured by the difference between calculated and real outputs and this entity is minimized by changing the weights. The principal feature of the ANN is its generalization ability that provides the possibility to predict a response for a new series of input whose outputs are unknown.

ANNs computation was performed using EasyNN-plus (Neural Planner Software Ltd, Cheadle Hulme, UK). All computations were performed on an Intel-based i7 PC computer with Microsoft Windows 10 as operating system. The standard back-propagation was used as training algorithm [1]. The optimal neural network architecture was searched for using the criteria of lowest Root Mean Square Error (RMSE), calculated according to Eq. 1, where \mathbf{N} , \mathbf{M} , \mathbf{O}_{pk} , and \mathbf{O}_{pk}^* , are the number of experiments used for training, the number of response variables, the estimated and the actual output value, respectively.

$$RMSE = \sqrt{\frac{\sum_{p=1}^N \sum_{k=1}^M (O_{pk} - O_{pk}^*)^2}{N \times M}}$$

Equation (1):

The available data set was divided into three subsets: (i) training, (ii) verification and (iii) the test set. The verification set was used during training to monitor the generalization ability of the network. Training was stopped when the minimum *RMSE* for the verification set has been reached. Once the network was trained and verified, it was validated by using the *test step*, i.e. by comparing

the estimated and experimental response for new input data, prepared on the basis of the results obtained in the previous steps.

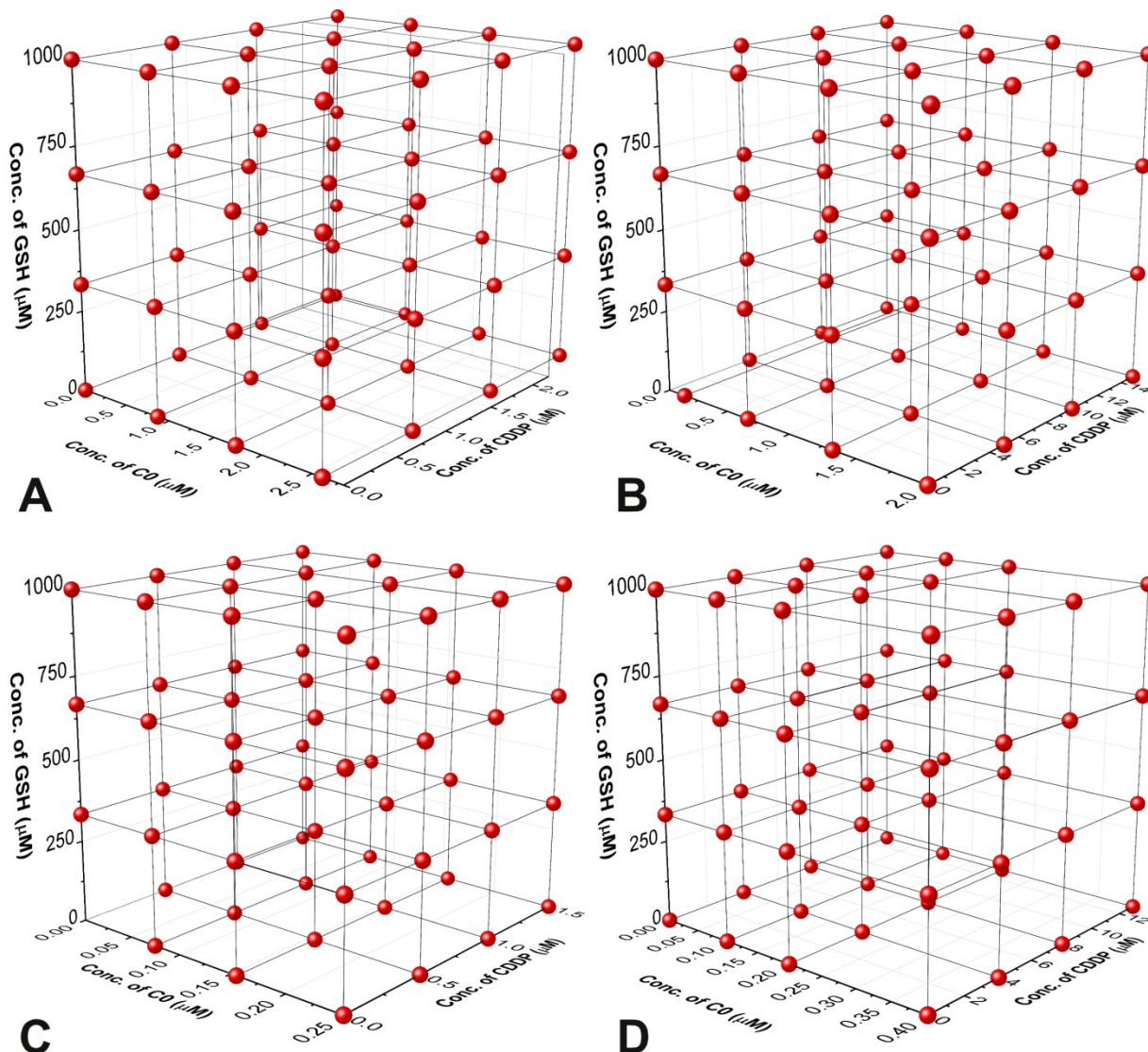


Figure S2. Experimental Designs followed in the study of drug cocktails against CCRF-CEM-wt (A), CCRF-CEM-res(B), A2780-wt (C) and A2780-res (D). C0 is $[\text{Cu}(\text{phen})_2(\text{H}_2\text{O})](\text{ClO}_4)_2$, CDDP is cisplatin, GSH is glutathione.

For CCRF-CEM-wt cells, the following systems were prepared and tested: *i*) 6 binary combinations of GSH and C0; *ii*) 6 binary combinations of GSH and CDDP; *iii*) 7 binary combinations of C0 and CDDP; *iv*) 52 ternary combinations of GSH, C0 and CDDP; *v*) 10 solutions of CDDP alone, *vi*) 9 solutions of GSH alone and *vii*) 11 solutions of C0 alone.

For CCRF-CEM-res *i*) 9 combinations of GSH and C0; *ii*) 9 combinations of GSH and CDDP; *iii*) 9 combinations of C0 and CDDP; *iv*) 27 combinations of GSH, C0 and CDDP; *v*) 3 solutions of CDDP alone, *vi*) 3 solutions of GSH alone and *vii*) 3 solutions of C0 alone.

For A2780-wt and A2780-res cells, the following systems were prepared and tested: *i*) 9 binary combinations of GSH and C0; *ii*) 9 binary combinations of GSH and CDDP; *iii*) 9 binary combinations of C0 and CDDP; *iv*) 27 ternary combinations of GSH, C0 and CDDP; *v*) 3 solutions of CDDP alone, *vi*) 3 solutions of GSH alone and *vii*) 4 solutions of C0 alone.

Solutions *i*), *vi*) and *vii*) were used to study GSH-C0 system; solutions *ii*), *v*) and *vi*) were used to study GSH-CDDP system; solutions *iii*), *v*) and *vii*) were used to study CDDP-C0 system; solutions *iv*), *v*), *vi*) and *vii*) were used to study the ternary system CDDP-GSH-C0.

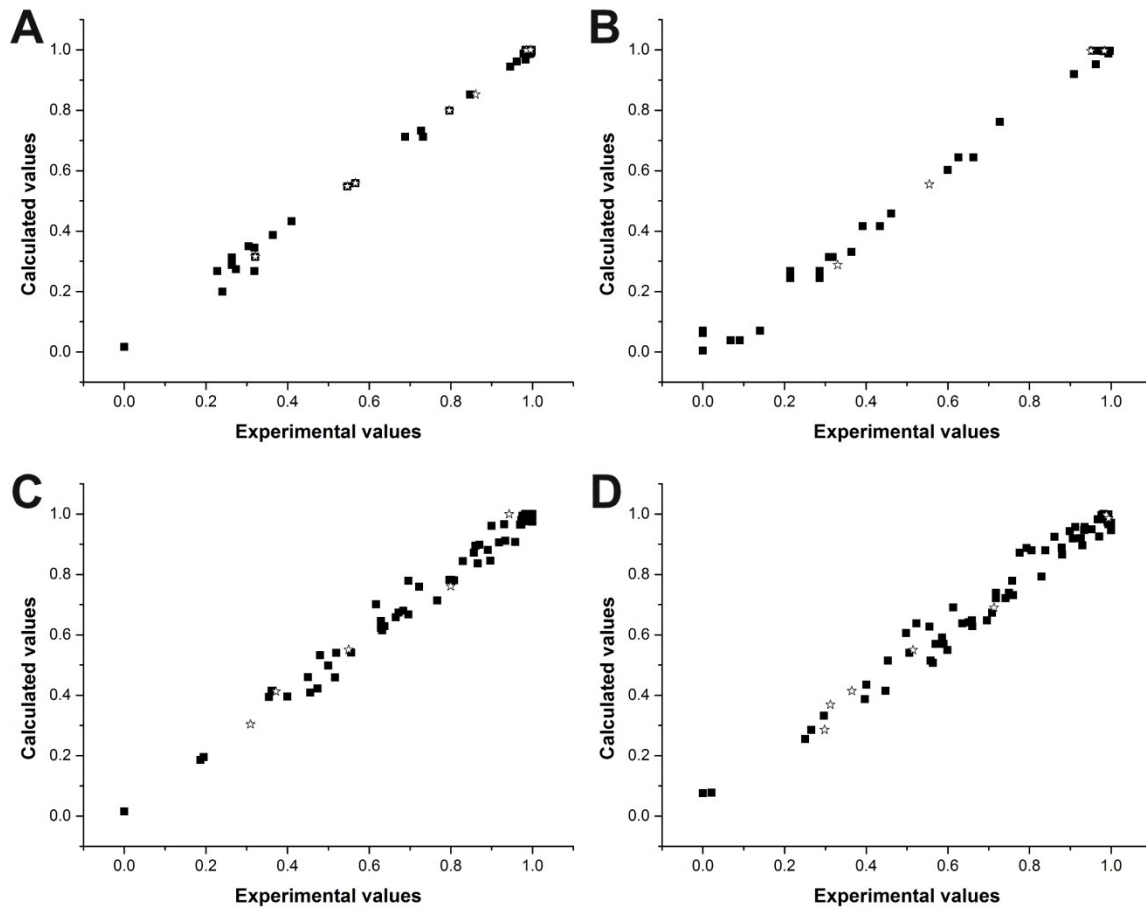


Figure S3. Comparison between experimental and calculated mortality values (■ training set, ☆ validation set) shown by ternary systems C0-CDDP-GSH against (A) CCRF-CEM-wt cell line (linear fitting parameters for the equation $y = mx + q$ are $m = 0.9903$, $q = 0.0138$ with $r = 0.9987$ for training set, $m = 1.0182$, $q = 0.0129$ with $r = 0.9997$ for validation set, $m = 0.9929$, $q = 0.0011$ with $r = 0.9987$ for all the data); (B) CCRF-CEM-res cell line (linear fitting parameters for the equation $y = mx + q$ are $m = 1.0085$, $q = -0.0002$ with $r = 0.9979$ for training set, $m = 1.1023$, $q = 0.0679$ with $r = 0.9988$ for validation set, $m = 1.0121$, $q = -0.0031$ with $r = 0.9978$ for all the data); (C) A2780-wt cell line (linear fitting parameters for the equation $y = mx + q$ are $m = 0.9905$, $q = 0.0092$ with $r = 0.9928$ for training set, $m = 1.0133$, $q = 0.0033$ with $r = 0.9906$ for validation set, $m = 0.9908$, $q = 0.0096$ with $r = 0.9928$ for all the data); (D) A2780-res cell line (linear fitting parameters for the equation $y = mx + q$ are $m = 0.9538$, $q = 0.0442$ with $r = 0.9848$ for training set, $m = 0.9465$, $q = 0.0472$ with $r = 0.9955$ for validation set, $m = 0.9531$, $q = 0.0446$ with $r = 0.9865$ for all the data).

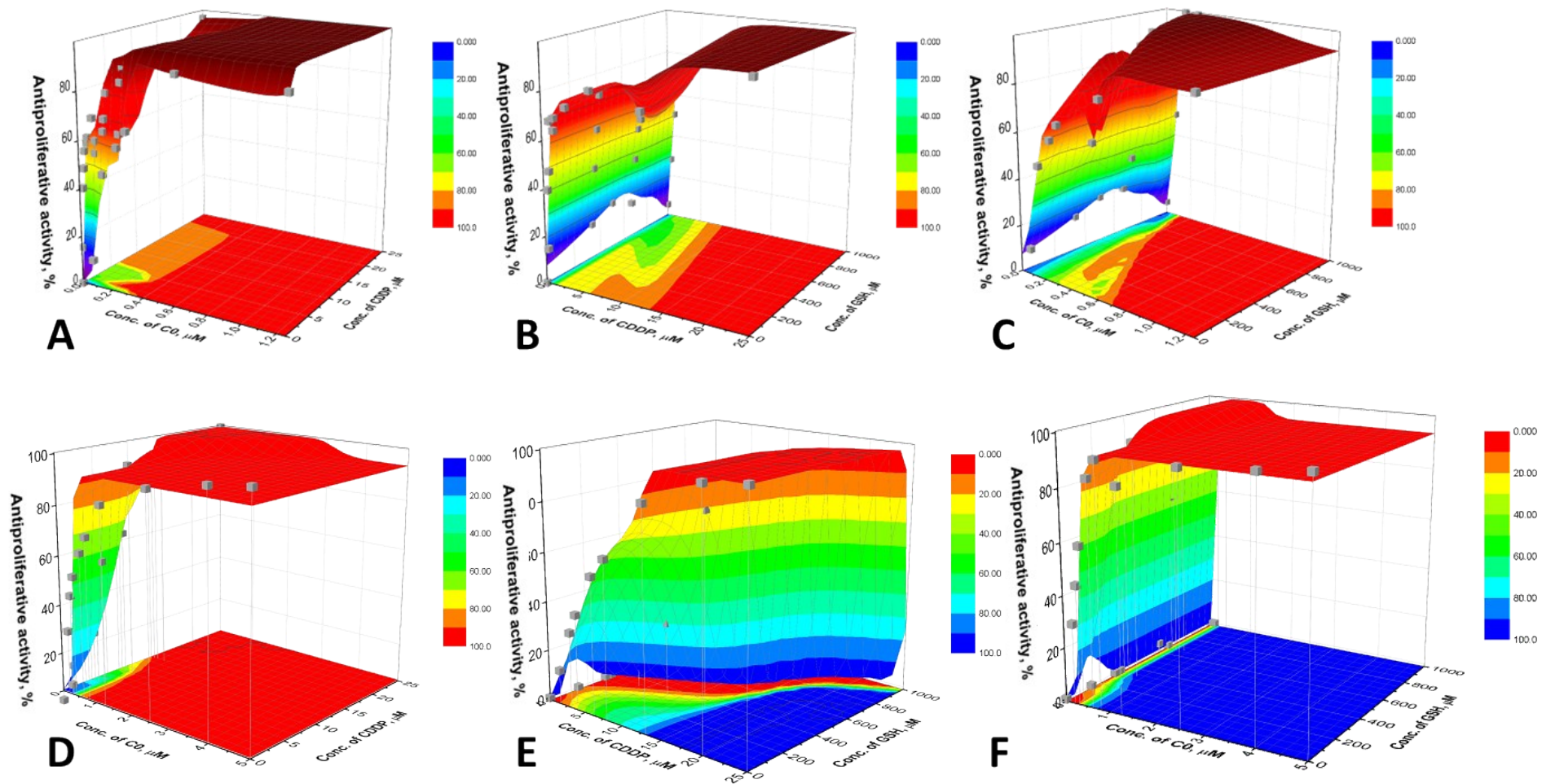


Figure S4. Calculated response surface for the systems C0-CDDP (A, D), CDDP-GSH (B, E) and C0-GSH (C, F) for A2780-wt (upper) and a2780-res (bottom) cell lines. The experimental points (■) are superimposed to the surfaces. The contour plots of cytotoxicity iso-values are also shown.

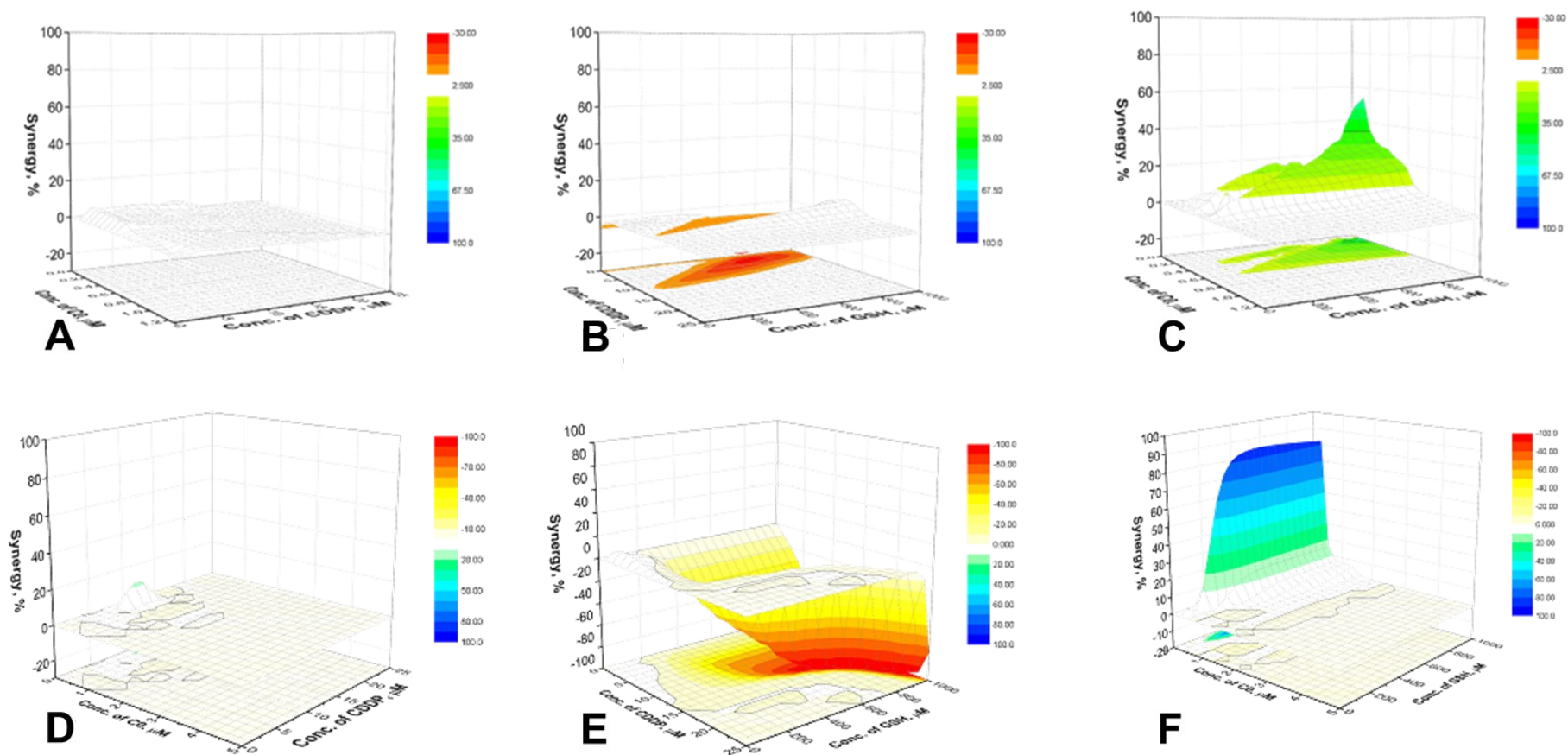


Figure S5. Synergistic surfaces for the binary systems C0-CDDP (A, D), CDDP-GSH (B, E) and C0 - GSH (C, F) for A2780-wt (upper) and A2780-res (bottom) cell lines.

- [1] D. Svozil, V. Kvasnicka, J. Pospichal, Introduction to multi-layer feed-forward neural networks, Chemom. Intell. Lab. Syst. 39 (1997) 43–62. doi:10.1016/S0169-7439(97)00061-0.