Appendix I: Thermodynamic model for the calcium-dependent interaction of a subunit of S100B with a target protein.

The complete description of the thermodynamic equilibria describing the calcium-dependent interaction of a subunit of S100B with a target protein is shown (Figure 1S) with the 13 possible dissociation constants defined as follows:

$K_I = [A][M]/[AM_I]$
$K_{II} = [A][M]/[AM_{II}]$
$K_{III} = [AM_I][M]/[AM_{I,II}]$
$K_{IV} = [AM_{II}][M]/[AM_{I,II}]$
$K_V = [AM_I][S]/[AM_IS]$
$K_{VI} = [AM_IS][M]/[AM_{I,II}S]$
$K_{VII} = [AM_{II}][S]/[AM_{II}S]$
$K_{VIII} = [AM_{I,II}][S]/[AM_{I,II}S]$
$K_{IX} = [AM_{II}S][M]/[AM_{I,II}S]$
$K_X = [BM_{II}][S]/[BM_{II}S]$
$K_{XI} = [BM_{II}][M]/[BM_{I,II}]$
$K_{XII} = [BM_{I,II}][S]/[BM_{I,II}S]$
$K_{XIII} = [BM_{II}S][M]/[BM_{I,II}S]$

where A=S100B prior to the 90° re-orientation of helix three of S100B (*1*, *2*); B = S100B after 90° re-orientation of helix three (*1*, *2*); M_I = a Ca^{2+} ion bound to EF Hand I (pseudo EF-hand); M_{II} = a Ca^{2+} ion bound to EF Hand II (typical EF-hand); $M_{I,II}$ = Ca^{2+} ions bound to EF Hand I and EF Hand II; S = free target protein (i.e. p53); L_I to L_{IV} represent the four possible conformational changes; and M = free Ca^{2+}.

The pseudo EF-hand binds calcium with lower affinity such that K_I and $K_{IV} >> K_{II}$. Therefore, K_I, K_{III}, K_{IV}, K_V, K_{VI}, K_{VIII}, L_{II} and L_{III} can be ignored. Likewise, K_{IX}, K_{XI}, K_{XII}, and K_{XIII} are not significantly occupied at low calcium concentrations due to the weak calcium-binding affinity of the pseudo EF-hand. This assumption provides a simplified model (Figure 2S; illustrated in bold) with four predominant states (A, AM_{II}, $AM_{II}S$, BM_{II}, and $BM_{II}S$), three equilibria (K_{II}, K_{VII}, and K_X), and two conformational changes L_I, L_{IV} where $K_{VII} \cdot L_{IV} = L_I \cdot K_X$.

References

(1) Drohat, A. C., Baldisseri, D. M., Rustandi, R. R., and Weber, D. J. (1998) Solution structure of calcium-bound rat S100B(ßß) as determined by NMR spectroscopy. *Biochemistry 37*, 2729-2740.

(2) Weber, D. J., Rustandi, R. R., Carrier, F., and Zimmer, D. B. (2000) *Interaction of dimeric S100B(ββ) with the tumor suppressor protein: A model for Ca-dependent S100-target protein interactions*, Kluwer Academic Publishers, Dordrecht, The Netherlands.

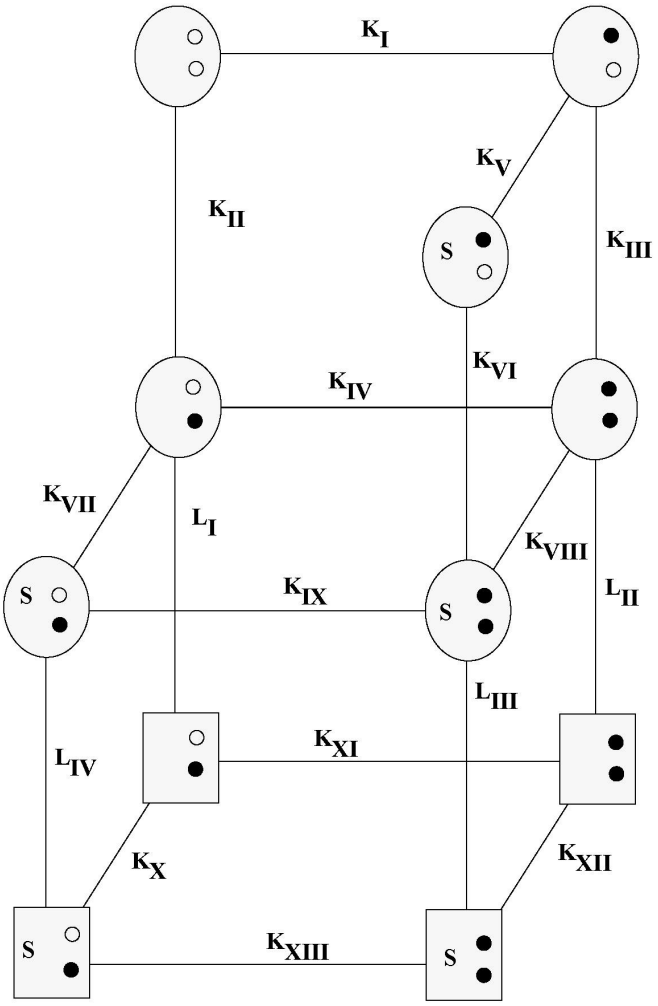

Figure 1S: Thermodynamic model for the calcium-dependent interaction of S100B with a target protein such as p53. Ovals represent S100B prior to the large reorientation of helix 3. After the conformational change (L), S100B is represented as a square. Binding of calcium to the pseudo EF-hand is represented by a filled circle (top) and binding of calcium to the typical EF-hand is represented by a filled circle (bottom). Open circles represent the same two calcium binding sites in the free state, respectively. A target protein, such as p53, is represented by the letter S. Overall, the calcium-dependent interaction of S100B with target proteins involves 13 equilibrium constants (K_I to K_{XIII}), 11 states, and 4 conformational changes (L_I-L_{IV}).

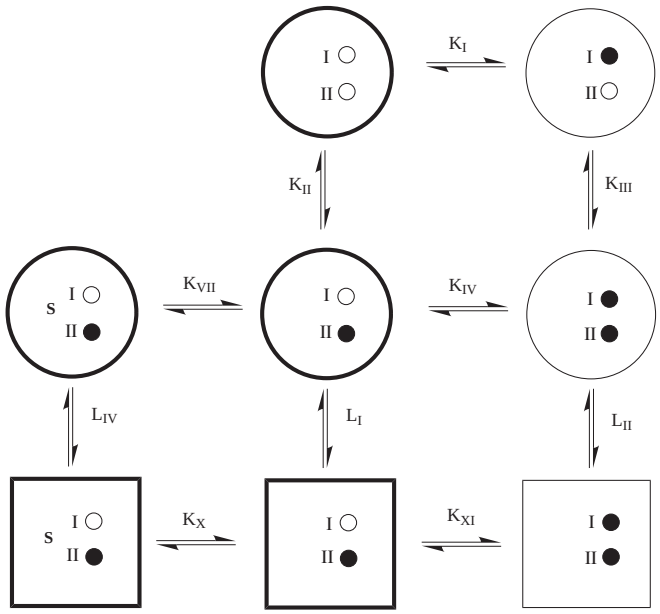

Figure 2S: A simplified model for the calcium-dependent interaction of wild-type S100B with a target protein. The pathway in bold shows five states (A, AM_{II}, $AM_{II}S$, BM_{II}, and $BM_{II}S$), three equilibria (K_{II}, K_{VII}, and K_X), and two conformational changes L_I, L_{IV} that are predominant at low calcium concentrations. The minor pathways and lower occupied states (i.e. those not in bold) are illustrated for comparison to other calcium-binding proteins (Figure 8).