Electronic Supplementary Material (ESI) for RSC Advances.

Supporting information

Malachite Green interacts with membrane skeletal protein, spectrin

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Fig S1: SDS-Polyacrylamide Gel Electrophoresis of erythroid and non-erythroid spectrin on 7.5% gel.

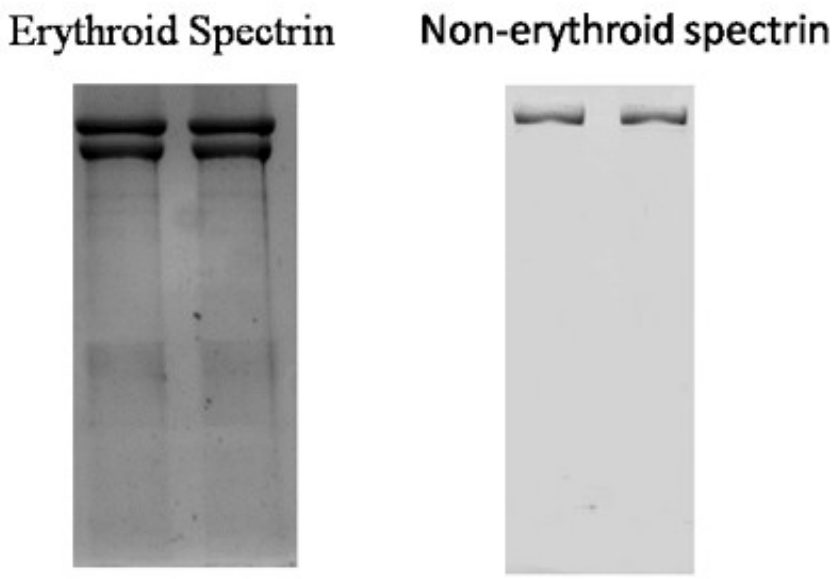

Fig S2: Energy minimized complexes of the malachite green with the N terminal domain , self associating domain , and ankyrin binding of erythroid spectrin (upper panel) and non-erythroid spectrin (lower panel).

N-terminal of αsp-spectrin

Ankyrin binding domain

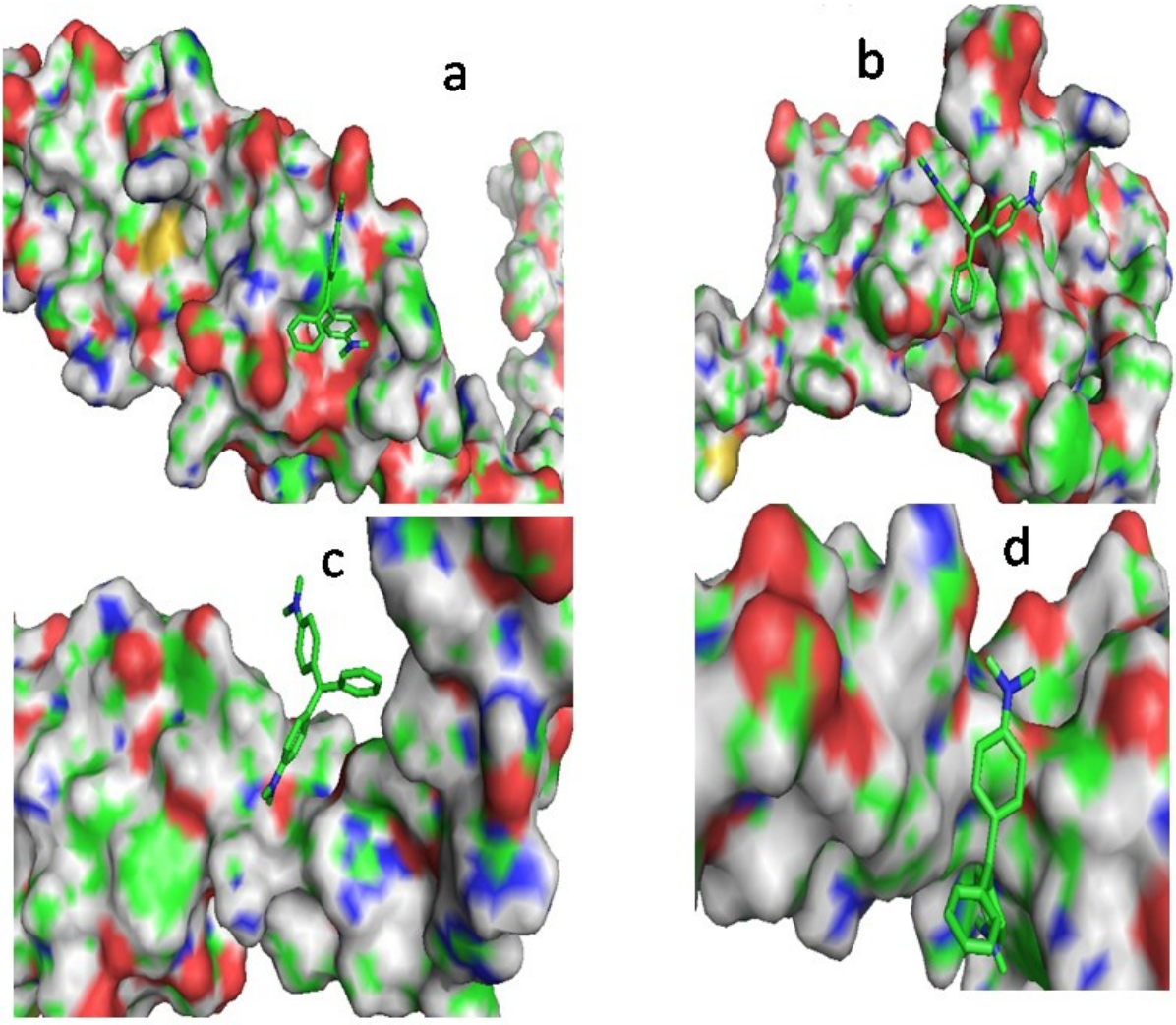

Table S1: Stern–Volmer (K_{SV}) and bimolecular quenching constants (k_q) for the molecular recognition of spectrin with malachite green.

Protein	Temperature (K)	K_{sv} ($\times 10^4$ M^{-1})	k_q ($\times 10^{13}$ $M^{-1}S^{-1}$)
Erythroid spectrin	298	5.723	1.506
	303	5.173	1.361
	308	4.621	1.216
Non-erythroid spectrin	298	7.0727	1.849
	303	6.420	1.689
	308	6.222	1.636

Table S2: Effect of ionic strength on the binding of erythroid and non-erythroid spectrin.

Protein	**Salt**	**K_d (μM)**
Erythroid Spectrin	0.02M NaCl 1M NaCl 2M NaCl	34±4 11±3 5±2
Non-erythroid Spectrin	0.2 M NaCl 1M NaCl 2M NaCl	25±5 13±2 9±2

Table S3: Fluorescence lifetime components of erythroid and nonerythroid spectrin in the presence and absence malachite green.

Sample	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)	A_1	A_2	A_3	τ (ns)	χ^2
Spectrin	**1.26**	**4.32**	**0.143**	**0.2188**	**0.7821**	**0.029**	**4.03**	**0.99**
Spectrin+ Malachite-Green	**1.08**	**4.0783**	**0.114**	**0.2296**	**0.6869**	**.0835**	**3.822**	**1.04**
Fodrin	**1.27**	**4.08**	**0.230**	**0.2720**	**0.6760**	**.0570**	**3.80**	**1.06**
Fodrin+ Malachite-green	**1.07**	**3.8559**	**0.12678**	**0.2269**	**0.7214**	**.0517**	**3.62**	**1.00**

Table S4: Residues of spectrin involved in the binding of malachite green.

Complex	Residues involved in the bonding
SH3 domain of erythroid spectrin-Malachite green	Leu (10), Lys (37), Asp (38), Trp (40), Ala (53), Ala (54), Lys (56), Asp (60).
SH3 domain of non-erythroid spectrin-Malachite green	Ala (12), Arg (13), Ser(14), Pro (15), Val (18), Thr(19), His (44), Glu (45).
Self associating domain of erythroid spectrin- Malachite green	Ser (36),Val (37), Arg (115).
Self associating domain of non-erythroid spectrin- Malachite green	Glu (88), Glu (92), Lys (91), Asp (95).