Supporting information

Binding of polarity sensitive, hydrophobic ligands to erythroid and non-erythroid spectrin: Fluorescence and molecular modeling studies

Malay Patra,^a Madhurima Mitra,^b Abhijit Chakrabarti^b & Chaitali Mukhopadhyay^a

^aChemistry Department, University of Calcutta, Kolkata 700009, ^bStructural Genomics Division, Saha Institute of Nuclear Physics, Kolkata 700064, India

Corresponding Author

Prof. Chaitali Mukhopadhyay, PhD

E-mail: chaitalicu@yahoo.com

Tel.: 0091-33-2351 -8386; Fax: 0091 33 2351 9755.

Prof. Abhijit Chakrabarti, PhD

E-mail: abhijit.chakrabarti@saha.ac.in

Tel.: 0091-33-2337-5345-49 (Extn.-4626); Fax: 0091-33-2337-4637

Supplementary Figures :

Figure S1: Pyrene binding to erythroid and nonerythroid spectrin: Fluorescence emission spectra of 0.2 μ M pyrene at 25°C in (1) 10 nM (2) 300 nM (3) 800 nM erythroid spectrin (A) and (1) 5 nM (2) 50 nM (3) 300 nM non-erythroid spectrin (B). **SDS-Polyacrylamide Gel Electrophoresis of nonerythroid spectrin on 8% gel.** Lane 1 shows the bands corresponding to molecular weight standard (BioRad pre-stained, broad range markers) with molecular weights of 250 kDa, 150 kDa, 100 kDa, 75 kDa, 50 kDa & 37 kDa are seen from the top. Lanes 2, 3 are the protein before Sepharose 4B column chromatography and lane 4, 5, 6 are the non-erythroid spectrin after gel filtration on Sepharose 4B (C).

Figure S2: Plot of intensity ratio (I_1/I_3) of pyrene fluorescence as a function of increasing protein concentrations: (A) for erythroid spectrin (B) for non-erythroid spectrin. The pyrene concentrations are kept $0.2 \mu\text{M}$. Inset show Scatchard plot of pyrene binding to erythroid and non-erythroid spectrin respectively.

Figure S3 : Fits to the fluorescence intensity decay profiles of (A) prodan (0.2 μM) in 0.40 μM erythroid spectrin; (B) prodan in 0.40 μM erythroid spectrin; (C) ANS (25 μM) in 0.40 μM erythroid spectrin; (D) ANS in 0.40 μM non-erythroid spectrin and (E) shows the lamp profile of the spectrometer. The lower plot shows the weighted residuals of the decay profiles.

Figure S4: Energy minimized complexes of Prodan and pyrene with SH3 domain of erythroid and non-erythroid spectrin. (A) Prodan and SH3 domain of erythroid spectrin; (B) Prodan and SH3 domain of non-erythroid spectrin; (C) Pyrene and SH3 domain of erythroid spectrin and (D) Pyrene and SH3 domain of non-erythroid spectrin

Figure –S5 : The best docked result of prodan, pyrene with α -N-terminal of erythroid and non-erythroid spectrin:(A) Prodan and α -N-terminal of erythroid spectrin (left), Prodan and α -N-terminal of non-erythroid spectrin.(B) Pyrene and α -N-terminal of spectrin (left). Pyrene and α -N-terminal of non-erythroid spectrin (right).

Table - S1: Fluorescence polarization value of erythroid and non-erythroid spectrin tryptophan, Prodan, ANS bound erythroid and non-erythroid spectrin

	Trp	Prodan	ANS
Erythroid spectrin	0.19	0.29 (0.03)	0.28(0.02)
Non-erythroid spectrin	0.20	0.30 (0.03)	0.29(0.02)

The values in the brackets indicate those in the presence of aqueous buffer.

Table – S2: Structural domain of erythroid, non-erythroid spectrin and their respective template structure.

Model / structural domain	Template Structure
Erythroid spectrin self-association domain	Self-association domain erythroid spectrin (3LBX.pdb)
Non-erythroid spectrin self-association domain	Self-association domain erythroid spectrin (3LBX.pdb) [70% sequence homology]
Erythroid spectrin SH3 domain	SH3 domain of nonerythroid spectrin (1NEG.pdb) 93% sequence homology]
Non-erythroid spectrin SH3 domain	SH3 domain of nonerythroid spectrin(1NEG.pdb)
α-N terminal region of erythroid spectrin	α -N terminal region of erythroid spectrin (1OWA.pdb)
α-N terminal region of non-erythroid spectrin	α -N terminal region of non-erythroid spectrin (3F31.pdb)
Ankyrin binding domain of erythroid spectrin	Crystal structure of human erythroid beta spectrin repeats 14 and 15(3f57A.pdb)
Ankyrin binding domain of non-erythroid spectrin	Crystal structure of the repeats of 14-16 of beta2-spectrin(3edvA.pdb)