Supplementary Data

for the manuscript entitled

Spectrin: An alternate target for cytoskeletal drugs

Sansa Dutta[1,2]*, Dipayan Bose[1, 3], Semanti Ghosh[1] & Abhijit Chakrabarti [1, 3 *]

1Crystallography & Molecular Biology Division, Saha Institute of Nuclear

Physics, Kolkata 700064, 2 Department of Chemistry, Indian Institute of Technology Kharagpur 721302 and 3 Homi Bhabha National

Institute, Mumbai 400094, India

∗Corresponding author: Sansa Dutta, Abhijit Chakrabarti,

Crystallography & Molecular Biology Division,

Saha Institute of Nuclear Physics, 1/AF Bidhannagar, Kolkata 700064, India.

Department of Chemistry, Indian Institute of Technology Kharagpur: 721302

E-mail: sansaind@gmail.com, abhijit.chakrabarti@saha.ac.in; abhijit1960@gmail.com

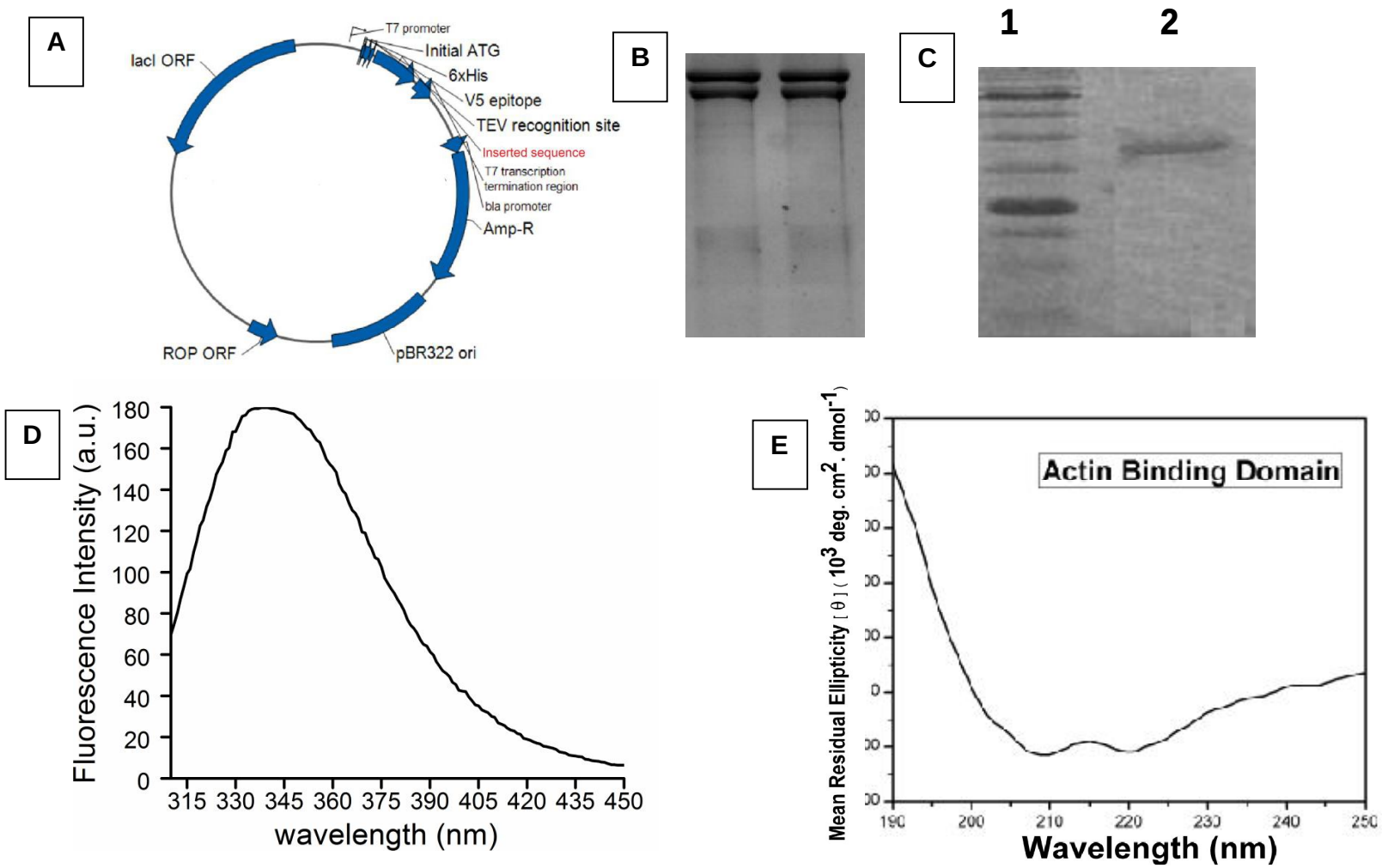

Figure S1: (A) The representative plasmid map of the ABD construct purchased from Invitrogen with labeled location of the different functional sequences. (B) SDS-PAGE (7.5%) of erythroid spectrin. (C) The SDS-PAGE of Ni-NTA resin purified ABD. The lane 1 denotes the ladder (The Prism Ultra prestained protein ladder was used and the two most prominent bands show 24 kDa and 70 kDa respectively. (D) Fluorescence spectra of purified Actin Binding Domain of β-spectrin (E) The CD spectra of purified the acin binding domain

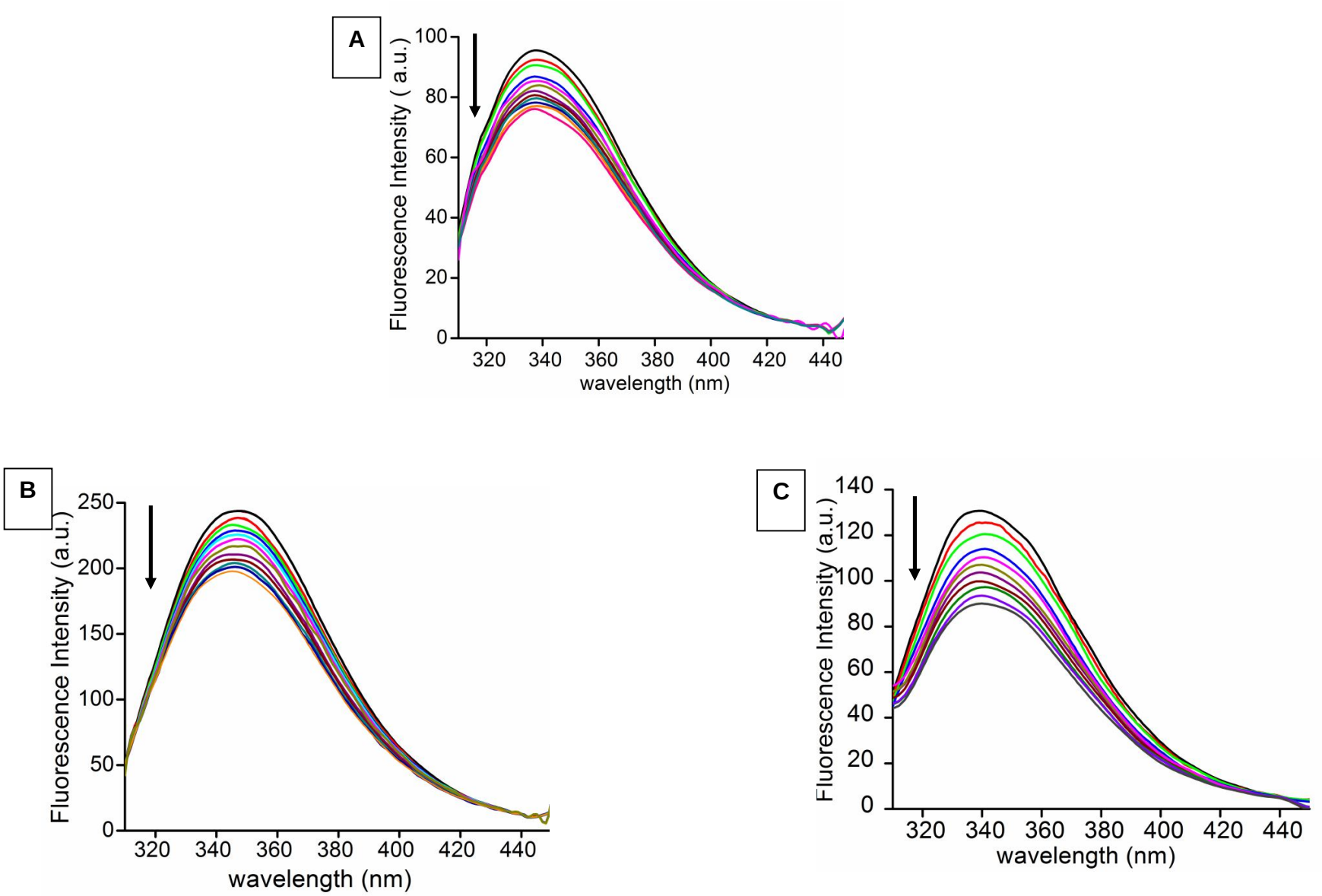

Figure S2: Fluorescence spectra of erythroid spectrin upon gradual addition of (A) Cytochalasin B (2-60 μM) (B) Latrunculin B (2.5- 40.5 μM) and (C) Taxol (2.3 – 53.6 μM). The erythroid spectrin concentration is maintained at 0.2 μM, 0.45 μM and 0.3 μM for A, B and C respectively.

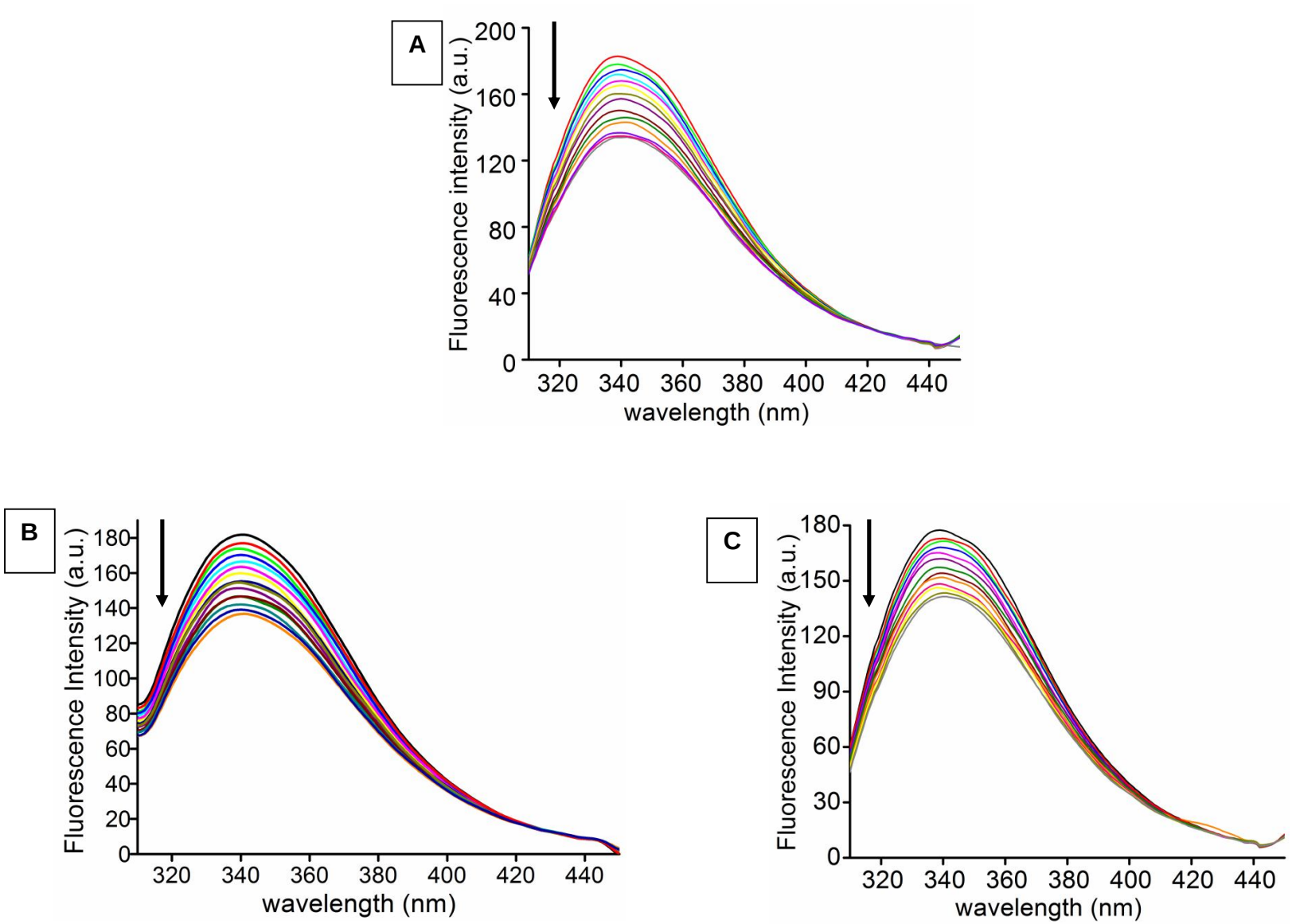

Figure S3: Fluorescence spectra of actin binding domain upon gradual addition of (A) Cytochalasin B (2- 60 µM) (B) Latrunculin B (2.5- 40.5 µM) and (C) Taxol (2.3 – 53.6 µM). The concentration of ABD used is 4.79 µM

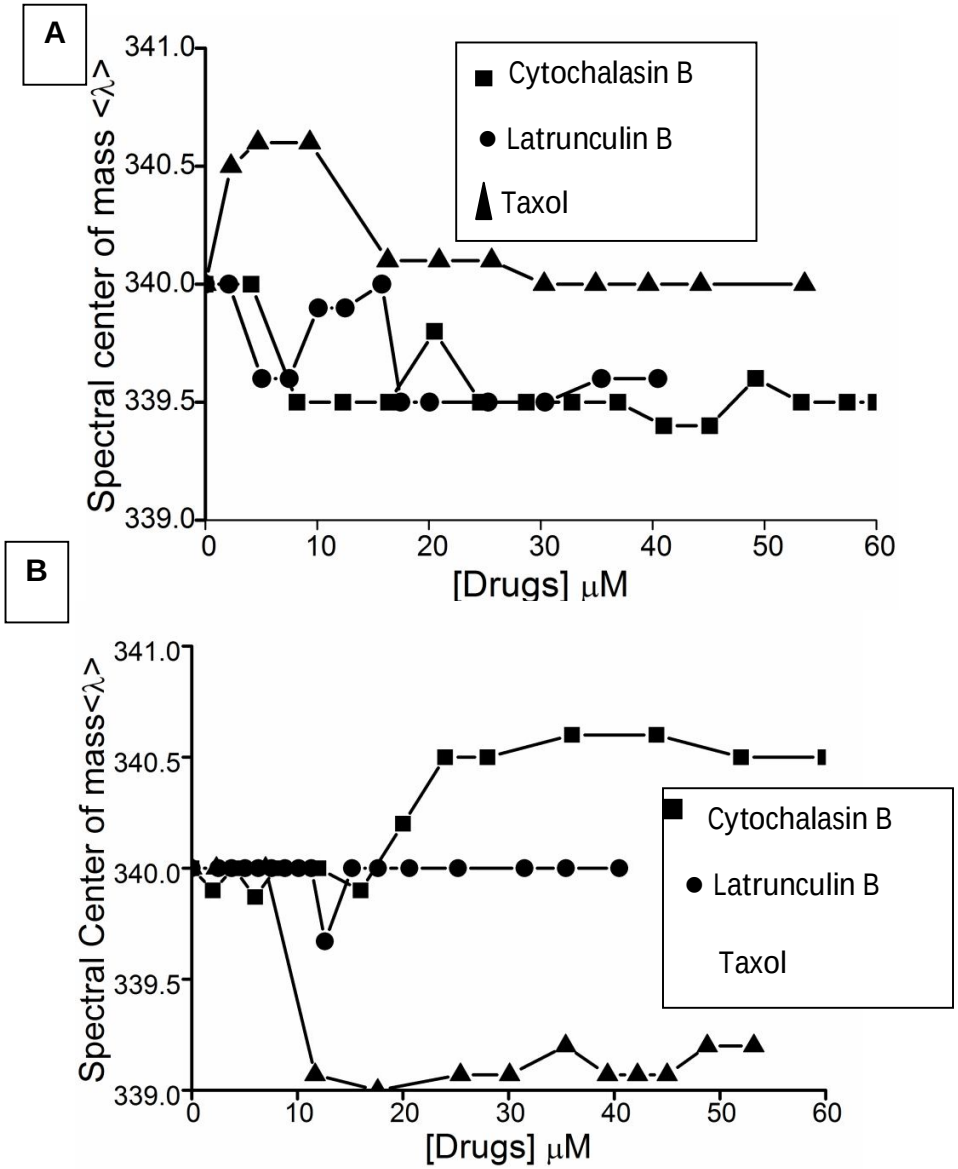

Figure S4: The spectral center of mass (<λ>) plot vs. concentration plot (A) Intact Spectrin and (B) Actin Binding Domain (ABD)

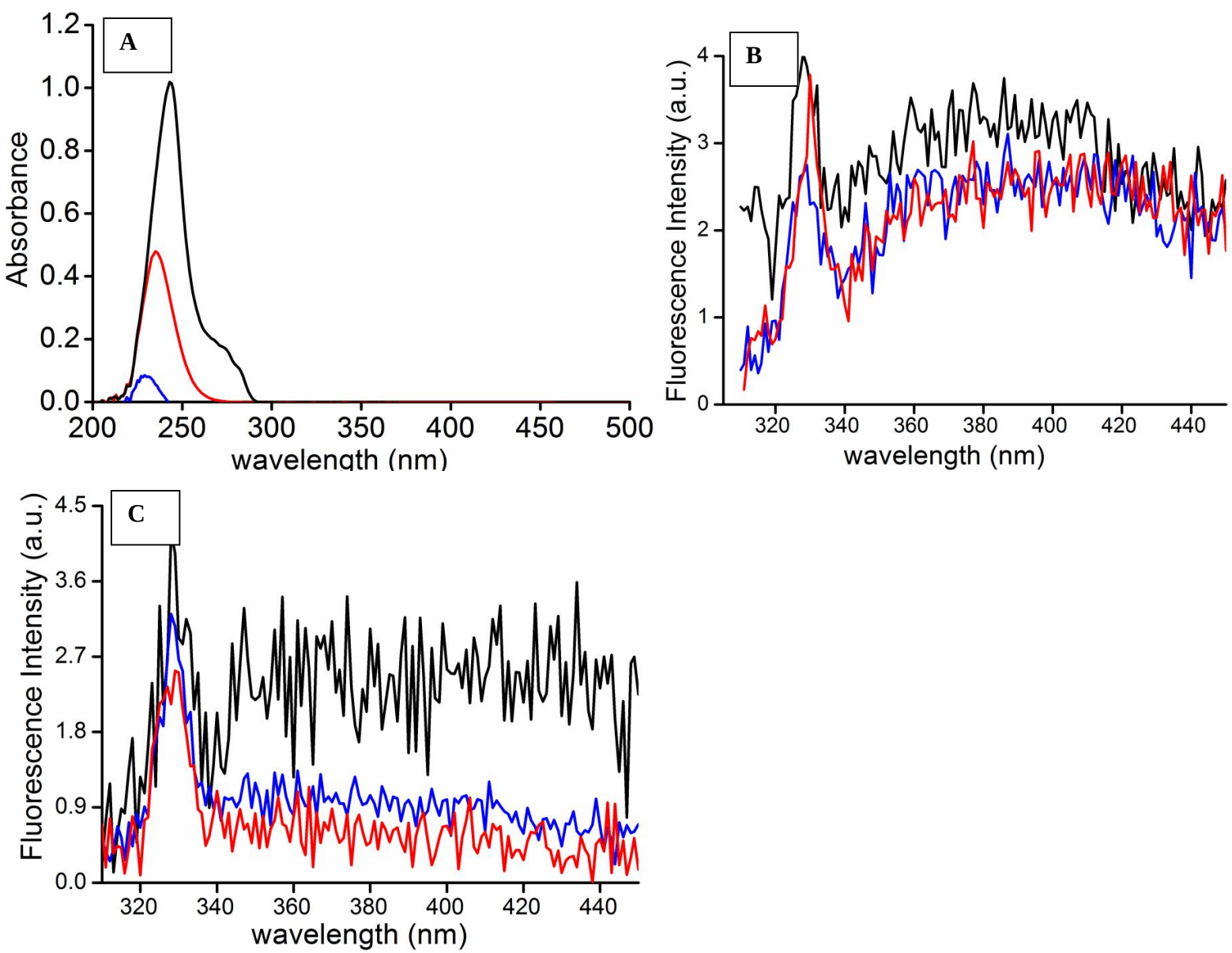

Figure S5: (A) The overlaid absorbance spectra of the drugs over the range starting from 200nm to 500nm; Cytochalasin (60 µM, Blue), Latrunculin (40.5 µM, Red) and Taxol (53.6 µM, Black) (B) The baseline reference for fluorometric titration of ABD with Cytochalasin (60 µM, Blue), Latrunculin (40.5 µM, Red) and Taxol (53.6 µM, Black) (C) The baseline reference for fluorometric titration of spectrin with Cytochalasin (60 µM, Blue), Latrunculin (40.5 µM, Red) and Taxol (53.6 µM, Black)

Table S1: The Ksv values obtained from the Stern Volmer Plot (A) and modified Stern Volmer Plot (B) upon titration of spectrin and ABD with increasing concentration of the drugs at variable temperatures.

A

	K_{sv} values (M^{-1}) at 25°C	K_{sv} values (M^{-1}) at 30°C	K_{sv} values (M^{-1}) at 35°C
Erythroid Spectrin + Cytochalasin B	3.0×10^3	3.2×10^3	3.5×10^3
ABD + Cytochalasin B	2.0×10^4	2.2×10^4	2.4×10^4
Erythroid Spectrin + Latrunculin B	5×10^3	5.1×10^3	5.2×10^3
ABD + Latrunculin B	2.5×10^4	2.7×10^4	2.8×10^4
Erythroid Spectrin + Taxol	2.9×10^4	3.2×10^4	3.3×10^4
ABD + Taxol	1.9×10^4	2.2×10^4	2.5×10^4

B

	K_{sv} values (M^{-1}) at 25°C	K_{sv} values (M^{-1}) at 30°C	K_{sv} values (M^{-1}) at 35°C
Erythroid Spectrin + Cytochalasin B	1.20×10^3	1.23×10^3	1.27×10^3
ABD + Cytochalasin B	2.5×10^4	2.8×10^4	3×10^4
Erythroid Spectrin + Latrunculin B	5.4×10^3	5.5×10^3	5.6×10^3
ABD + Latrunculin B	2.7×10^4	2.8×10^4	3×10^4
Erythroid Spectrin + Taxol	3×10^4	3.2×10^4	3.3×10^4
ABD + Taxol	2×10^4	2.1×10^4	2.4×10^4

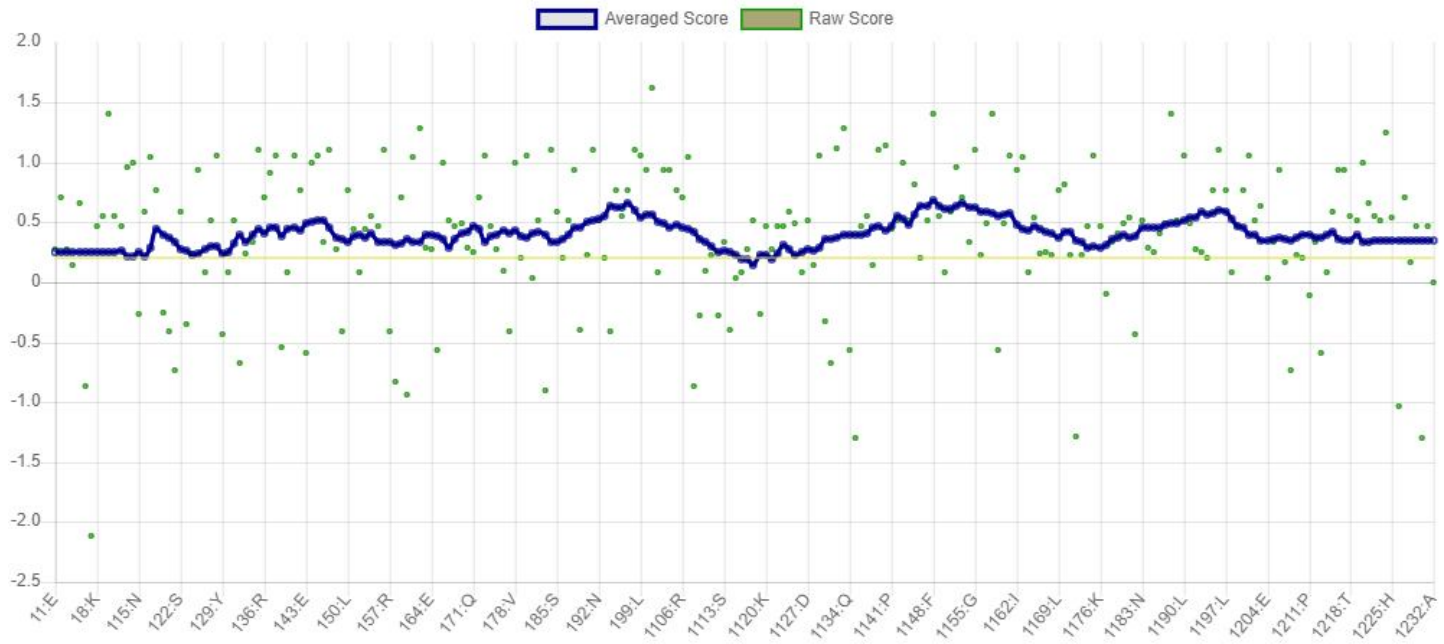

Figure S6: Structure validation using VERIFY 3D program in the SAVES server version 6. 98.28% of the residues have averaged 3D-1D score >= 0.2

Table S2A: The binding energy value of the most stable conformer in the clustering Histogram obtained from Autodock.

Drugs	**Cytochalasin B**	**Latrunculin B**	**Taxol**
Binding Energry (kcal/mol)	-7.54	-4.78	-5.43

Table S2B: Change in Accessible Surface Area (ΔASA) in Å2 of the interacting residues of ABD and its complexes with the drugs. The superscript a and b indicates the docked structures and the structures obtained after simulations

Interacting residues	Cytochalasin D	Latrunculin B	Taxol
Lys 57			18.9[a], 33.21[b]
Lys 58			30.5[a], 17.47[b]
Thr 61			31.75[a], 2.95[b]
Lys 62			89.49[a], 40.83[b]
Trp 63	34.77[a], 11.05[b]	34.36[a], 12.71[b]	
Asn 65			26.36[a], 32.66[b]
Ser 66		17.62[a], 29.54[b]	
His 67	15.43[a], 29.7[b]	21.3[a], 26.4[b]	
Ala 69			0[a], 5.46[b]
Arg 70	55.84[a], 139.42[b]	57.39[a], 88.36[b]	
Val 71	0[a], 84.61[b]		
Ile 75			62.08[a], 75.16[b]
Thr 76			4.94[a], 77.98[b]

Asp 77			22.24[a], 5.58[b]
Leu 78			10.1[a], 10.9[b]
Tyr 79			52.84[a], 1.92[b]
Leu 91	0[a], 3.57[b]		
Leu 95	17.9[a], 14.74[b]	18.99[a], 11.26[b]	
Ser 96	0[a], 45.1[b]		
Arg 109		0[a], 12.72[b]	
Ile 110		0[a], 36.84[b]	
Leu113		0[a], 22.96[b]	
Glu 114		0[a], 10.65[b]	
Ser 135		0[a], 9.86[b]	
His 136		0[a], 16.49[b]	
Val 139		38.12[a], 15.17[b]	
Ile 153	0[a], 0.24[b]		
Arg 156		4.9[a], 83.86[b]	
Phe 157	50.66[a], 8.78[b]	47.33[a], 17.21[b]	
Gln 158	7.53[a], 1.06[b]	19.15[a], 28.46[b]	
Gly 246	14.82[a], 24.58[b]		

Leu 247	13.35[a], 23.2[b]		
Thr 248		20.7[a], 63.79[b]	
Val 271	5.61[a], 9.1[b]		
Thr 272	39.8[a], 9.91[b]	38.91[a], 4.94[b]	
Tyr 274	0[a], 77.12[b]		
His 275	4.85[a], 36.28[b]		
Tyr 276	4.64[a], 175.33[b]		
Phe 277	39.58[a], 37.87[b]		

Table S3: Relative accessibilities of the tryptophan residues (all atoms) present in the actin binding domain (ABD)

Trp 23	**Trp 63**	**Trp 151**	**Trp 182**	**Trp 202**
0.8	38.40	33.68	25.56	0.28

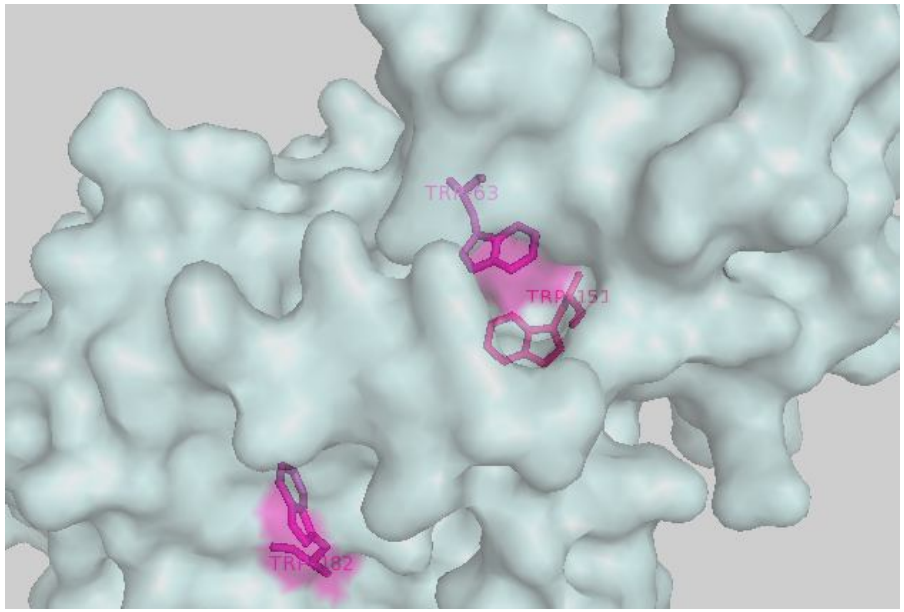

Figure S7: Pictorial representation of the solvent accessible Trp residues shown in pink

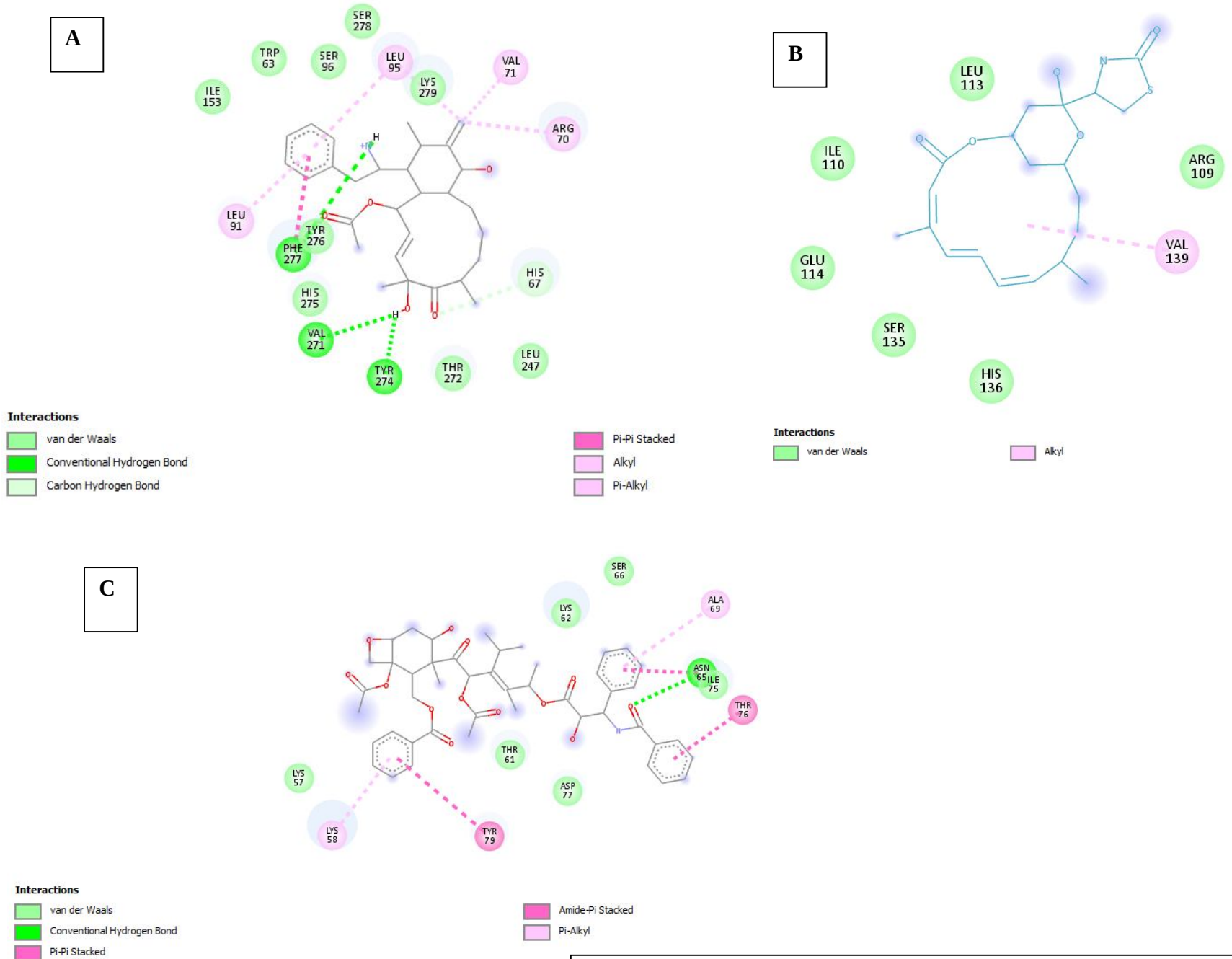

Figure S8: The actin binding domain drug interaction on a 2D diagram (A) Cytochalasin B (B) Latrunculin B and (C) Taxol