Supporting Information

Binding and Inhibitory effect of the food colorants Sunset Yellow and Ponceau 4R on amyloid fibrillation of Lysozyme

Sabera Millan[a], Lakkoji Satish[a], Krishnendu Bera[b], and Harekrushna Sahoo[a,*]

[a] Department of Chemistry, National Institute of Technology (NIT), Rourkela, Odisha, India

[b] CEITEC MU, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic

*Corresponding Author:

Dr. Harekrushna Sahoo
Assistant Professor
Department of Chemistry
NIT Rourkela

Rourkela-769008
Sundergarh, INDIA

Email: sahooh@nitrkl.ac.in

Phone: +91 6612462665

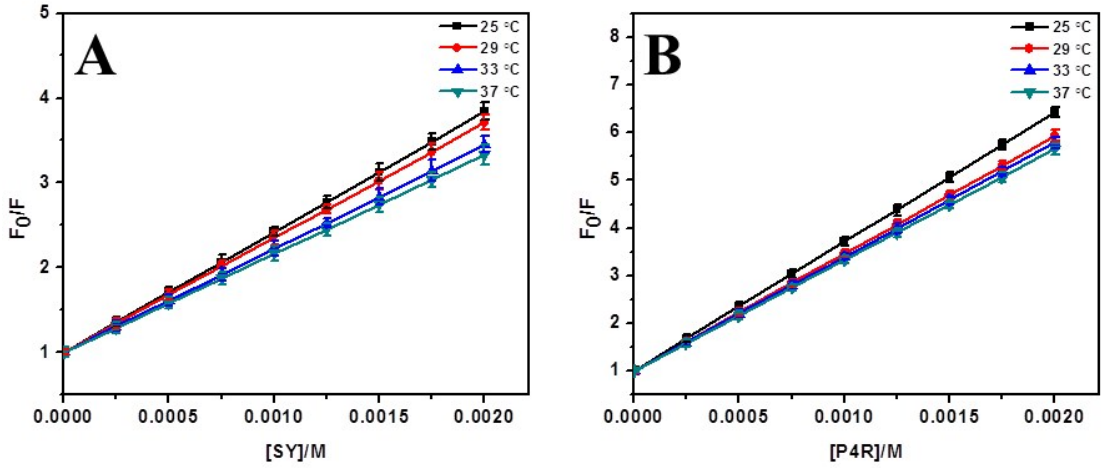

Figure S1: Stern-Volmer (SV) Plot of Lyz as a function of concentration of SY (A) and P4R (B) at different temperatures.

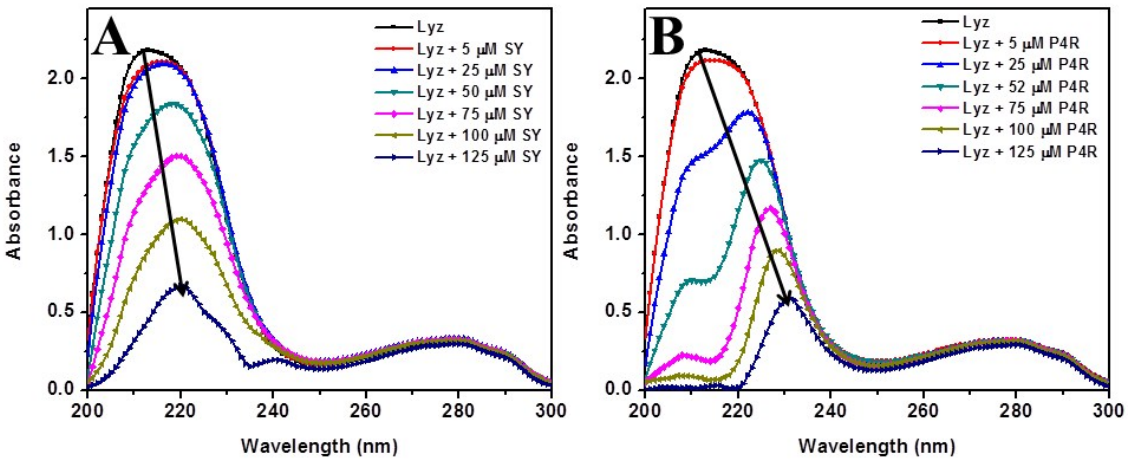

Figure S2: UV–vis absorption spectra of Lysozyme-SY system (A) and Lysozyme-P4R system (B). [Lysozyme] = 12 μM; [SY/P4R] = 0, 5, 25, 50, 75, 100 and 125 μM; pH = 7.4, T = 298 K. Each absorption spectrum was obtained by subtracting the appropriate background (without protein) from the experimental protein spectrum.

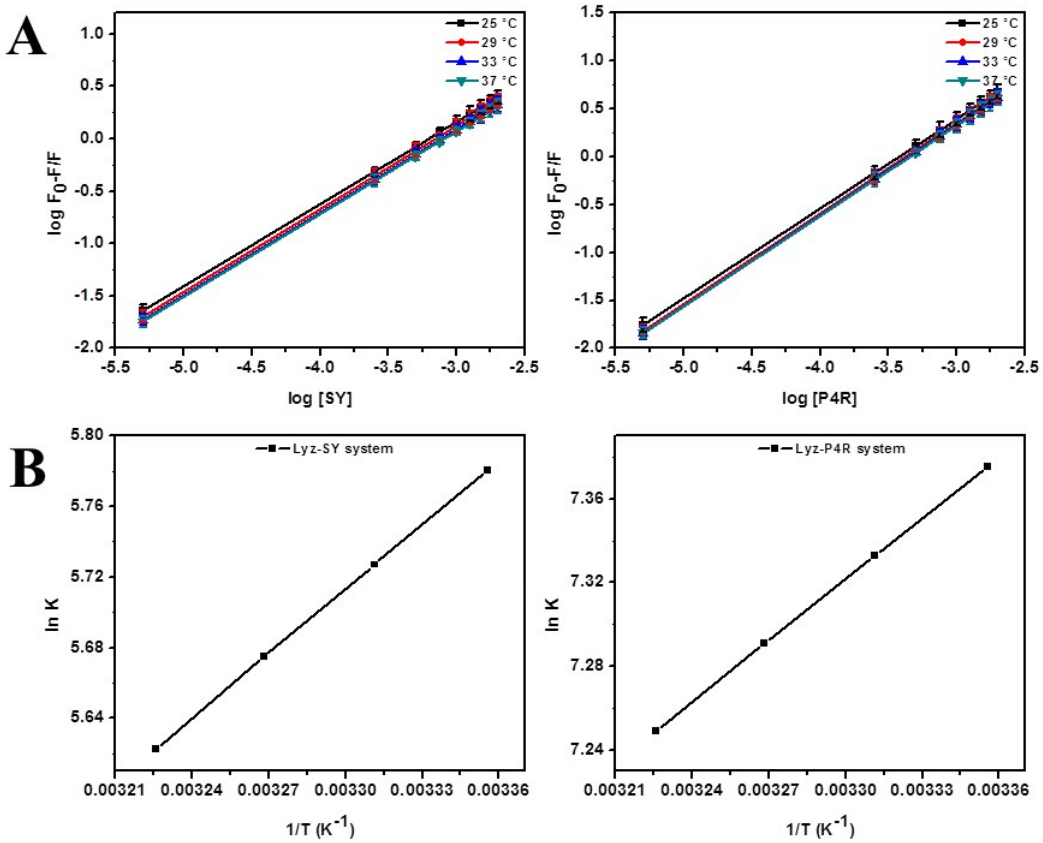

Figure S3: (A) Binding plot (log (F_0-F)/F vs. log [Q]) of the Lyz-SY and Lyz-P4R complex, (B) van't Hoff plots for Lyz−SY and Lyz−P4R system at different temperatures.

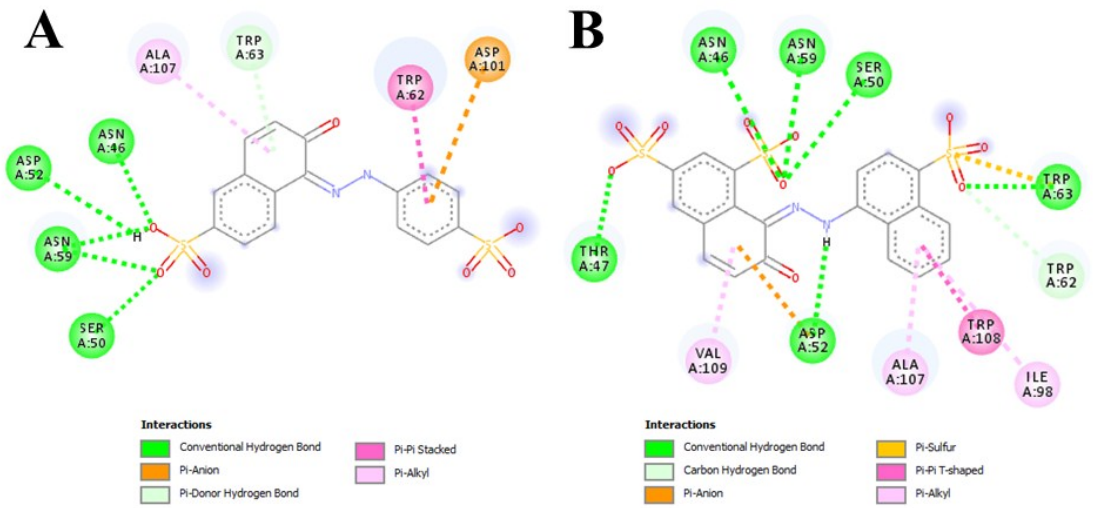

Figure S4: The 2D detailed view shows the interaction between SY (A) and P4R (B) with binding sites residues of Lyz. (For interpretation of the mode of binding interaction in these figures, the reader is referred to the web version of this article.)

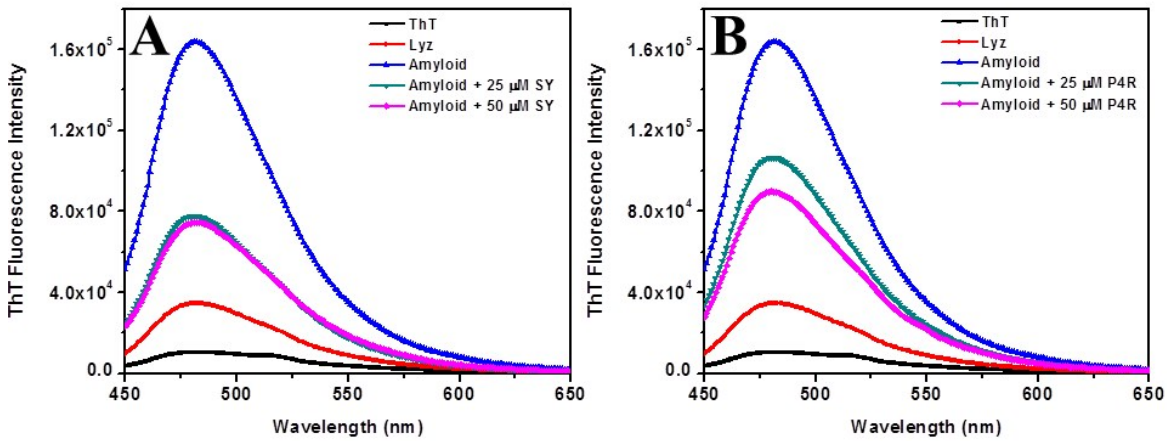

Figure S5: ThT Fluorescence spectra of Lysozyme in the absence and presence of SY (A) and P4R (B) after incubation for 6 h at 65 ºC and pH 2.0. (λ_{ex} = 440 nm, λ_{em} = 450-650 nm)

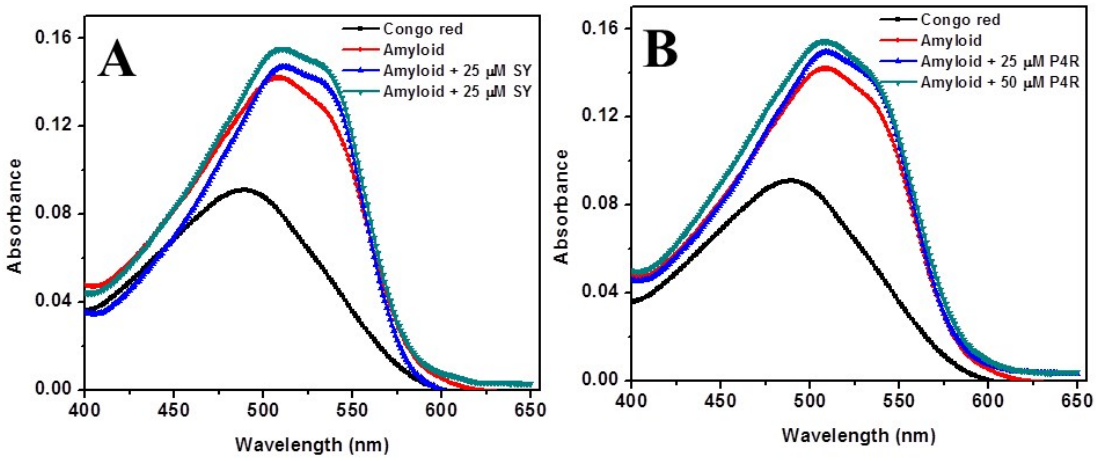

Figure S6: Congo red absorption spectra of Lysozyme samples at pH 2.0 and 65 ºC in absence and presence of SY (A) and P4R (B). Fibrillar sample with Congo red was subtracted from fibrillar sample without Congo red.

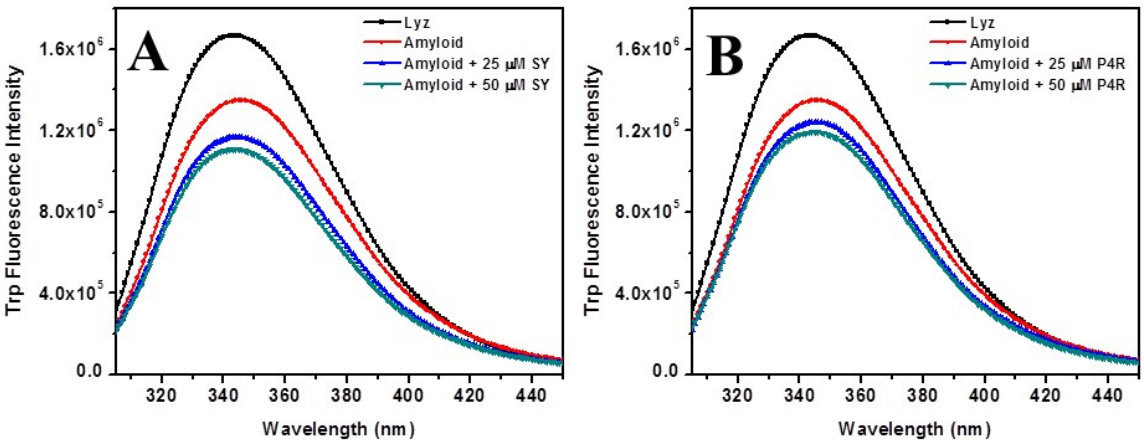

Figure S7: Tryptophan Fluorescence spectra of Lysozyme samples in the absence and presence of SY (A) and P4R (B) incubated in the amyloidogenic condition. [SY/P4R] = 25, 50 μM.

Table S1: Binding energy of ligands (SY and P4R) with Lysozyme, and their interaction with amino acid residues in docked pose.

Ligands	Binding Affinity (kcal/mol)	Hydrogen Bond
P4R	–9.2	Asn 46, Asn 59, Ser 50, Thr 47, Asp 52 and Trp 63
SY	–8.4	Asn 46, Asp 52, Asn 59 and Ser 50

Table S2: % Occupancy of H-bonds formed over the course of simulation.

Complex	Donor-Acceptor	Atom Number	Occupancy (%)
Lyz-P4R	62TRP(HE1) - 130LP(OAD)	651 - 1343	98.2
	48ASP(H) - 130LP(OAR)	505 - 1337	41.4
	48ASP(H) - 130LP(OAS)	505 - 1335	40.9
	47THR(HG1) - 130LP(OAR)	500 - 1337	45.7
	47THR(HG1) - 130LP(OAS)	500 - 1335	50.0
	47THR(H) - 130LP(OAR)	496 - 1337	19.7
	47THR(H) - 130LP(OAS)	496 - 1335	26.4
Lyz-SY	103ASN(D21) - 130LIG(OAY)	1033 - 1357	35.6
	103ASN(D21) - 130LIG(OAZ)	1033 - 1356	26.3
	48ASP(H) - 130LIG(OAB)	505 - 1337	84.6
	47THR(HG1) - 130LIG(OAB)	500 - 1337	92.1
	47THR(H) - 130LIG(OAB)	496 - 1337	48.7