SUPPLEMENTARY INFORMATION

Hen egg Lysozyme fibrils act as amyloid nucleating agents for β-lactoglobulin and Insulin

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Table S1. List of research reports describing fluctuation of lysozyme level in the body and the associated complications

Peptide	Lysozyme level	Associated Disease
Lysozyme	High level(in serum)	Systemic granulomatous disease[1]
Lysozyme	High level(in serum)	Crohn's disease and ulcerative colitis[2]
Lysozyme	High level(in serum)	Sarcoidosis[3]
Lysozyme	High level(in serum)	Pulmonary tuberculosis[4]
Lysozyme	High level(in serum)	Ulcerative alimentary disease[5]
Lysozyme	High level(in serum)	Rheumatic diseases[6]
Lysozyme	High level(in serum)	Hodgkin's disease[7]
Lysozyme	High level(in serum)	Clonal monocytosis[8]
Lysozyme	High level(in blood)	Dry Eye Disease[9]
Lysozyme	Low level(in blood)	Giant papillary conjunctivitis and vernal conjunctivitis[10]

Table S2. List of recent research reports describing the cross-seeding effect of Lysozyme amyloid aggregates with different proteins.

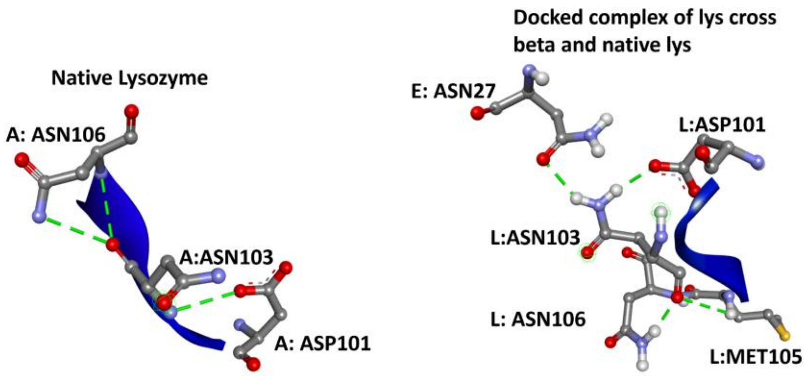
Protein	Work	Type
BSA (by lysozyme seed)	In vitro	Cross seeding[11]
lysozyme, bovine serum albumin, insulin, and cytochrome c	In vitro	coaggregation and cross-seeding[12]
α-synuclein aggregation (by Lysozyme seed)	In vitro	cross-seeding[13]
Soy protein isolate and lysozyme	In vitro	Coaggregation[14]
Phenylalanine fibrils (myoglobin, Lys, BSA, Cyt2 c, Ins)	In vitro/ In vivo	Cross seeding[15]
α-synuclein aggregation (by β-lac seed)	In vitro	Cross seeding[13]
Aβ and α-synuclein	In vitro/ In silico / In vivo	Cross seeding[16]
BSA, Insulin, and Lysozyme	In vitro	Cross seeding[12]
Tau aggregation (α-synuclein seed)	In vitro/ In vivo	Cross seeding[17]

Table S3. Analysis of the interactions of native structures of proteins with the cross-β structure of Lysozyme.

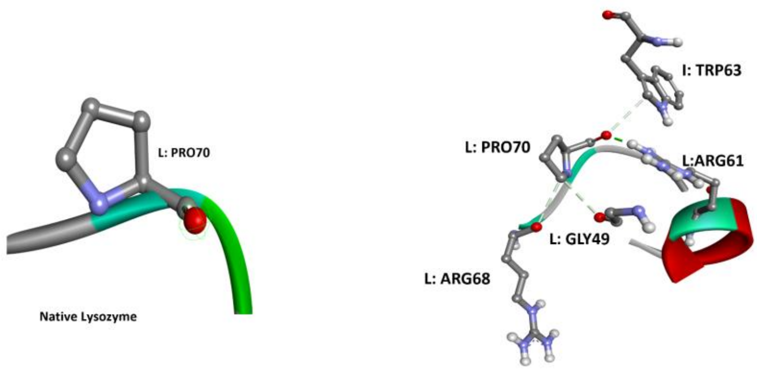
Protein –Protein interaction	**No. of H-Bond**	**No. of Electrostatic Bond**	**No. of Hydrophobic bond**	**ΔG (kJ.mol^{-1})**	**Kd (M)**
Lys **β fibril with native lys**	14	5	3	-48.53	3.3e-09
Lys **β fibril with native β lac**	8	7	3	-48.11	3.5e-09
Lys **β fibril with native ins**	3	1	5	-51.88	7.6e-10

Table S4. Details of the experimental conditions used for the seeding and cross-seeding experiments which are conducted in this study

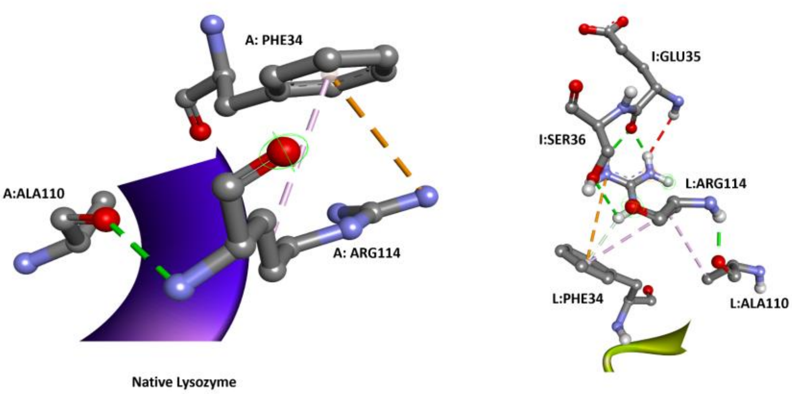
Protein	Concentration	Buffer (pH)	Temp (℃)	Condition Type
Lysozyme	5mg/ml	GnHCL (2.0)	58	Self-assembly
Lys + Lys seed	10 µM + 20% w/w	PBS (7.4)	65	Self-seeding
Ins + Lys seed	10 µM + 20% w/w	PBS (7.4)	65	Cross-seeding
β-lac + Lys seed	10 µM + 20% w/w	PBS (7.4)	65	Cross-seeding
Lys + Lys seed	70 µM + 15% w/w	PBS (7.4)	37	Self-seeding
Ins + Lys seed	172 µM + 15 % w/w	PBS (7.4)	37	Cros-seeding
β-lac + Lys seed	54 µM + 15% w/w	PBS (7.4)	37	Cross-seeding
Ins, Lys, β-lac	10 µM each	PBS (7.4)	65	Coaggregation



Interacting residue	Interaction within lysozyme	Interaction with lysozyme cross-β-structure
ASN103	H-bond(A:ASN103:N - A:ASP101:OD1)	H-bond(L:ASN103:HD21 - L:ASP101:OD2)
	H-bond(A:ASN106:N - A:ASN103:O)	H-bond(L:ASN103:HD22 - E:ASN27:OD1)
	H-bond(A:ASN106:ND2 - A:ASN103:O)	H-bond(L:MET105:HN - L:ASN103:O)
		H-bond(L:ASN106:HN - L:ASN103:O)
		H-bond(L:ASN106:HD21 - L:ASN103:O)

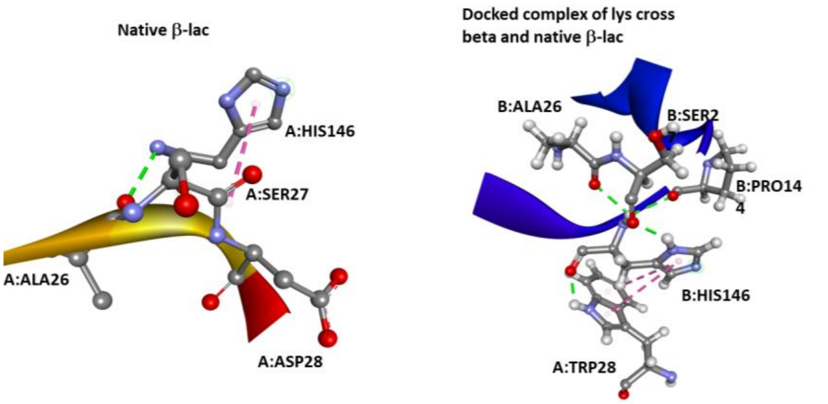


Interacting residue	Interaction within lysozyme	Interaction with lysozyme cross-β-structure
PRO70		H-bond(L:ARG61:HH11 - L:PRO70:O)
		H-bond(I:TRP63:CD1 - L:PRO70:O)
		H-bond(L:PRO70:CD - L:GLY49:O)
		H-bond(L:PRO70:CD - L:ARG68:O)

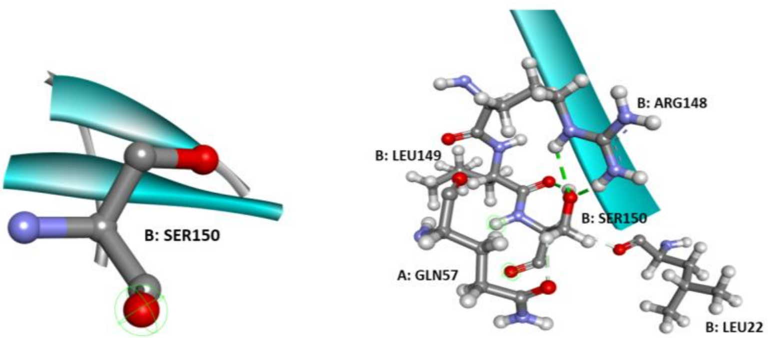


Interacting residue	Interaction within lysozyme	Interaction with lysozyme cross-β-structure
ARG114	H-bond(A:ARG114:N - A:ALA110:O)	H-bond(L:ARG114:HN - L:ALA110:O)
	Electrostatic(A:ARG114 :NH2 - A:PHE34)	H-bond(L:ARG114:HE - I:SER36:OG)
	Hydrophobic(A:PHE34 - A:ARG114)	H-bond(L:ARG114:HH11 - I:GLU35:O)
		H-bond(L:ARG114:HH21 - I:GLU35:O)
		H-bond(L:ARG114:HH22 - I:SER36:OG)
		Electrostatic(L:ARG114:NH2 - L:PHE34)
		H-bond(L:ARG114:HE - L:PHE34)
		Hydrophobic(L:ALA110 - L:ARG114)
		Hydrophobic(L:PHE34 - L:ARG114)

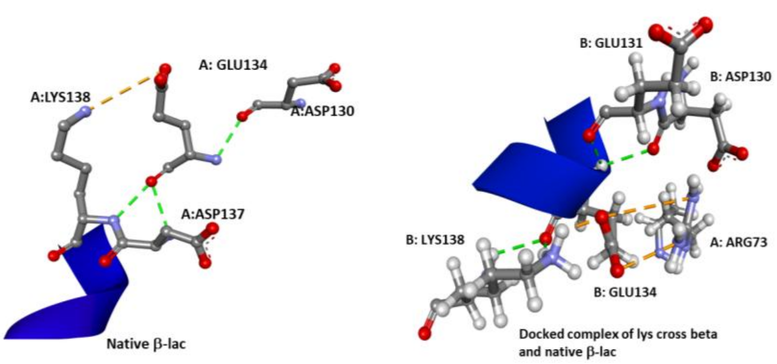
***Figure S1.* Analysis of the Molecular docking studies on the interaction of the cross-β structure of Lysozyme with the native structures of Lysozyme.** The interactions of *PRO70 ASN 103, ARG 114,* of Lysozyme before and after its interaction with cross-β structure of lysozyme.



Interacting residue	Interaction within β-lac	Interaction with lysozyme cross-β-structure
HIS146	Hydrophobic(A:SER27:C,O; ASP28:N - A:HIS146)	H-bond(A:TRP28:HE1 - B:HIS146:O)
	H-Bond(A:HIS146:N - A:ALA26:O)	H-bond(B:HIS146:HN - B:ALA26:O)
		H-Bond(B:HIS146:HN - B:SER27:O)
		H-bond(B:HIS146:HN - B:PRO144:O)
		H-bond(B:HIS146:HD1 - B:SER27:O)
		Hydrophobic(A:TRP28 - B:HIS146)
		Hydrophobic(B:HIS146 - A:TRP28)

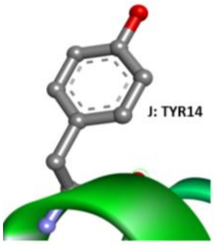


Interacting residue	Interaction within β-lac	Interaction with lysozyme cross-β-structure
SER150		H-Bond(B:ARG148:HE - B:SER150:OG)
		H-Bond(B:ARG148:HH22 - B:SER150:OG)
		H-Bond(B:SER150:HG - B:LEU149:O)
		H-Bond(B:SER150:HA - B:LEU22:O)
		H-Bond(B:SER150:HB1 - A:GLN57:OE1)

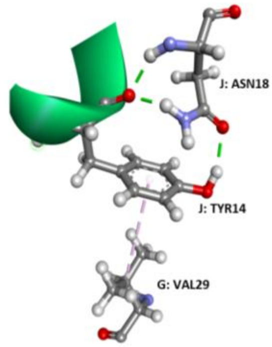


Interacting residue	Interaction within β-lac	Interaction with lysozyme cross-β-structure
GLU134	Electrostatic(A:LYS138:NZ - A:GLU134:OE2)	H-Bond;Electrostatic(B:LYS138:HZ3 - B:GLU134:OE2)
	H-Bond(A:GLU134:N - A:ASP130:O)	Electrostatic(A:ARG73:NH1 - B:GLU134:OE2)
	H-bond(A:ASP137:N - A:GLU134:O)	Electrostatic(A:ARG73:NH2 - B:GLU134:OE1)
	H-bond(A:LYS138:N - A:GLU134:O)	H-bond(B:GLU134:HN - B:ASP130:O)
		H-Bond(B:GLU134:HN - B:GLU131:O)
		H-bond(B:LYS138:HN - B:GLU134:O)
		H-bond(B:LYS138:HZ2 - B:GLU134:O)

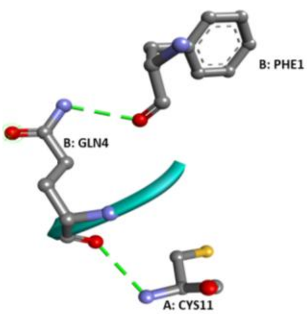
***Figure S2*. Analysis of the Molecular docking studies on the interaction of the cross-β structure of Lysozyme with the native structures of β-lactoglobulin.** The interactions of *HIS 146, SER 150* and *GLU 134* of β-lactoglobulin before and after its interaction with cross-β structure of lysozyme.



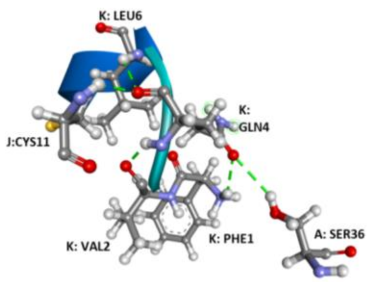
Native Insulin



Interacting residue	Interaction within insulin	Interaction with lysozyme cross β complex
TYR14		H-bond(J:TYR14:HH - J:ASN18:OD1)
		H-bond(J:ASN18:HN - J:TYR14:O)
		H-bond(J:ASN18:HD21 - J:TYR14:O)
		Hydrophobic(J:TYR14 - G:VAL29)



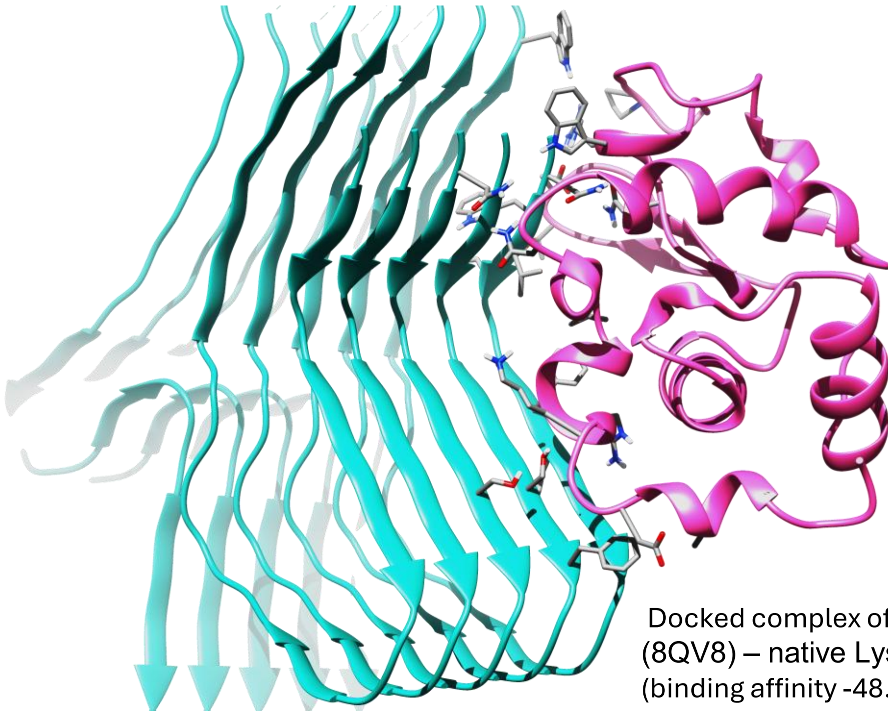
Insulin Before docking



After Docking

Interacting residue	Interaction within insulin	Interaction with lysozyme cross b complex
GLN4	H-bond(A:CYS11:N - B:GLN4:O)	H-bond(A:SER36:HG - K:GLN4:OE1)
	H-bond(B:GLN4:NE2 - B:PHE1:O)	H-bond(J:CYS11:HN - K:GLN4:O)
		H-bond(K:PHE1:HT3 - K:GLN4:OE1)
		H-bond(K:GLN4:HN - K:VAL2:O)
		H-bond(K:LEU6:HN - K:GLN4:O)

***Figure S3*. Analysis of the Molecular docking studies on the interaction of the cross-β structure of Lysozyme with the native structures of Insulin.** The interactions of *TYR 14* and *GLN4* of Insulin before and after its interaction with cross-β structure of lysozyme.



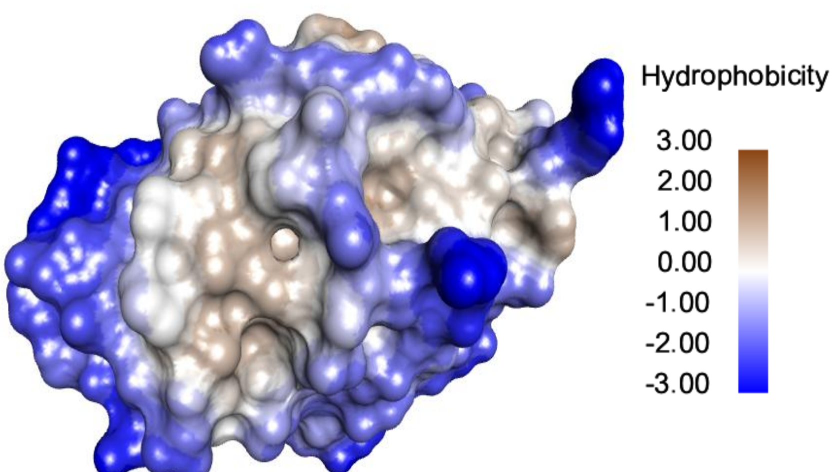
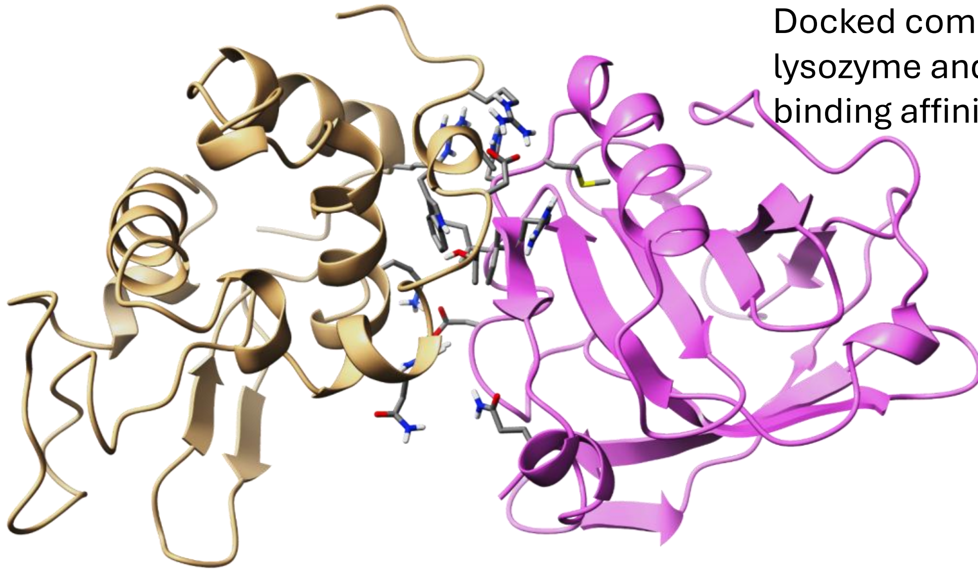


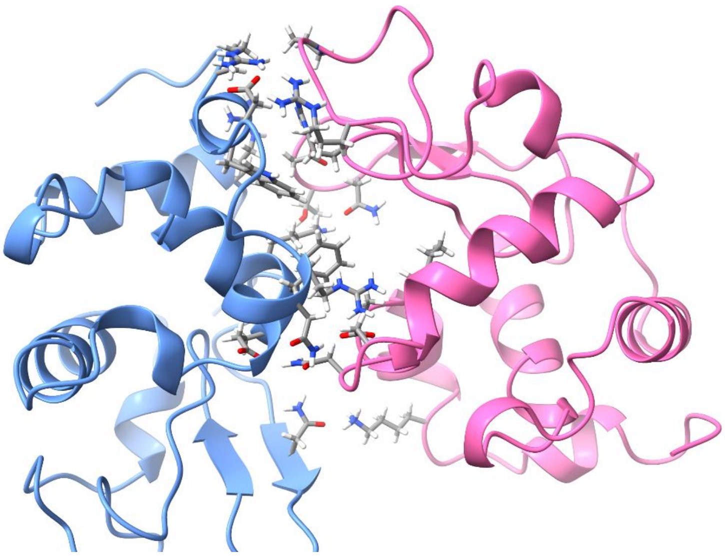
Figure S4. A, Molecular docking studies on the interaction of native Lysozyme (PDB ID: 5WRA) with Lysozyme's cross-β (PDB ID: 8QV8). Inset shows type of interactions. ***B,*** Display of surface hydrophobicity of lysozyme.



Docked complex of Native lysozyme and native β-lac binding affinity -34.1 kJ/mol

Interacting residues	Bond length	Types of bond
A:ARG114:HH21 - B:ASP33:OD2	1.95	Hydrogen Bond;Electrostatic
A:LYS33:NZ - B:ASP33:OD1	2.73	Electrostatic
A:ARG114:NH1 - B:ASP33:OD1	5.14	Electrostatic
B:ARG148:NH1 - A:ASP119:OD2	5.18	Electrostatic
A:ARG5:HH22 - B:HIS146:NE2	2.69	Hydrogen Bond
A:ARG114:HH22 - B:ASP33:O	1.79	Hydrogen Bond
B:HIS146:HD1 - A:ALA122:O	2.05	Hydrogen Bond
B:GLN155:HE22 - A:ASN113:O	2.86	Hydrogen Bond
A:THR118:CB - B:ARG148:O	3.11	Hydrogen Bond
A:ARG125:CD - B:MET145:O	3.65	Hydrogen Bond
B:HIS146:CA - A:ALA122:O	2.98	Hydrogen Bond
B:HIS146:CE1 - A:ARG125:O	3.11	Hydrogen Bond
A:ARG5:NH2 - B:HIS146	3.20	Electrostatic
A:LYS33 - B:ILE29	4.51	Hydrophobic
A:TRP123 - B:ILE29	4.75	Hydrophobic
A:TRP123 - B:ILE147	4.72	Hydrophobic

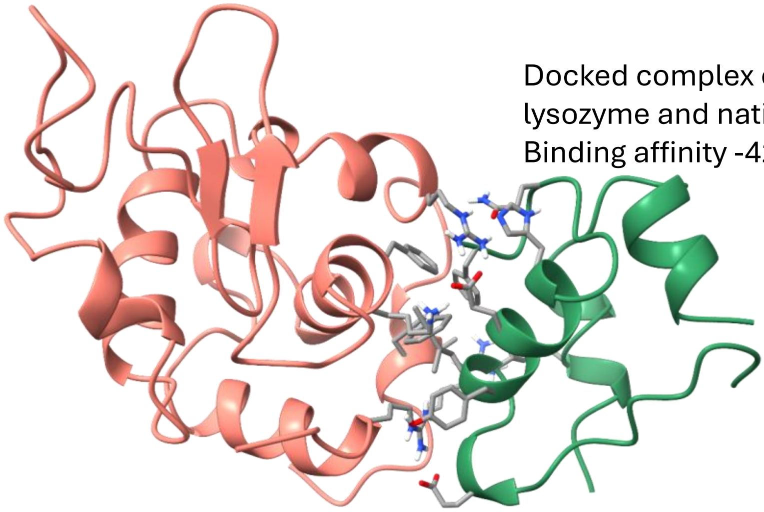
Figure S5. A, Molecular docking studies on the interaction of native Lysozyme (PDB ID: 5WRA) with native β-lactoglobulin (PDB ID: 5IO5), heteromeric interaction. Inset below shows details of the type of interactions.



Docked complex of Native lysozyme and native lysozyme Binding affinity -45.18 kJ/mol

Interacting residues (5WRA_5WRA)	Bond length	Type of bond
A:ARG114:HH11 - B:ASP101:OD2	2.13	Hydrogen Bond;Electrostatic
A:ARG114:HH12 - B:ASP101:OD1	1.90	Hydrogen Bond;Electrostatic
A:LYS33:NZ - B:ASP48:OD2	5.46	Electrostatic
A:ARG114:NH2 - B:ASP101:OD1	5.04	Electrostatic
B:ARG73:NH2 - A:ASP119:OD2	4.01	Electrostatic
A:ARG5:HN - B:THR47:O	2.84	Hydrogen Bond
A:ARG5:HE - B:ASP48:O	1.91	Hydrogen Bond
A:ARG5:HH22 - B:ASP48:O	2.68	Hydrogen Bond
A:LYS33:HZ1 - B:ASN46:OD1	1.64	Hydrogen Bond
A:LYS33:HZ3 - B:THR47:OG1	1.67	Hydrogen Bond
A:ASN113:HD21 - B:ASP101:OD1	2.22	Hydrogen Bond
A:ARG114:HH11 - B:ILE98:O	1.98	Hydrogen Bond
A:ARG114:HH21 - B:ILE98:O	1.85	Hydrogen Bond
A:ARG125:HH12 - B:PRO70:O	1.91	Hydrogen Bond
B:ASN103:HD22 - A:GLU35:OE1	2.11	Hydrogen Bond
B:LYS116:HZ1 - A:ASN44:OD1	1.69	Hydrogen Bond
A:ARG114:HD1 - B:ASP101:OD1	**2.74**	**Hydrogen Bond**
A:ARG125:HD1 - B:PRO70:O	2.46	Hydrogen Bond
B:ASP48:OD1 - A:TRP123	4.69	Electrostatic
A:PHE34 - B:ALA107	4.827676	Hydrophobic
A:TRP123 - B:ARG61	4.938858	Hydrophobic
A:TRP123 - B:ARG61	4.751226	Hydrophobic

Figure S6. A, Molecular docking studies on the interaction of native Lysozyme (PDB ID: 5WRA) with native Lysozyme (PDB ID 5WRA), homomeric interaction. Inset below shows details of the type of interactions.



Interacting residues	Bond length	Types of bond
C:ARG5:HH21 - B:GLU21:OE2	2.14	Hydrogen Bond;Electrostatic
C:ARG5:HH22 - B:GLU21:OE1	1.81	Hydrogen Bond;Electrostatic
C:LYS33:HZ3 - B:GLU13:OE1	1.73	Hydrogen Bond;Electrostatic
C:ARG114:HH11 - B:GLU13:OE2	1.83	Hydrogen Bond;Electrostatic
C:ARG114:HH21 - B:GLU13:OE2	1.86	Hydrogen Bond;Electrostatic
C:ARG125:HH21 - A:GLU17:OE1	1.85	Hydrogen Bond;Electrostatic
C:ARG5:NH1 - B:GLU21:OE1	4.96	Electrostatic
C:ARG114:NH2 - B:GLU13:OE1	4.40	Electrostatic
C:ARG125:NH1 - A:GLU17:OE1	2.74	Electrostatic
C:ARG125:NH2 - A:GLU17:OE2	3.91	Electrostatic
C:ARG5:HN - B:TYR16:OH	2.35	Hydrogen Bond
C:ARG5:HH12 - B:LEU17:O	2.99	Hydrogen Bond
C:ARG114:HE - B:GLN4:OE1	1.95	Hydrogen Bond
C:ARG114:HH22 - B:GLN4:OE1	1.89	Hydrogen Bond
C:ARG125:HH12 - B:VAL18:O	2.16	Hydrogen Bond
B:PHE1:HT1 - C:ARG114:O	1.76	Hydrogen Bond
C:ARG125:CD - B:VAL18:O	3.67	Hydrogen Bond
C:ARG114:NH2 - B:HIS10	3.46	Electrostatic
C:TRP123:HE1 - B:PHE1	2.01	Hydrogen Bond
B:LEU17:CD1 - C:PHE34	3.99	Hydrophobic
C:TRP123 - B:PHE1	4.23	Hydrophobic
C:TRP123 - B:PHE1	5.61	Hydrophobic
C:ALA122 - B:VAL18	4.69	Hydrophobic
C:TRP123 - B:LEU17	2.69	Hydrophobic
C:TRP123 - B:LEU17	1.37	Hydrophobic
B:PHE1 - C:ALA122	4.74	Hydrophobic
B:TYR16 - C:ARG5	4.93	Hydrophobic

Figure S7. A, Molecular docking studies on the interaction of native Lysozyme (PDB ID: 5WRA) with native Insulin (PDB ID 3I3Z), heteromeric interaction. Inset below shows details of the type of interactions.

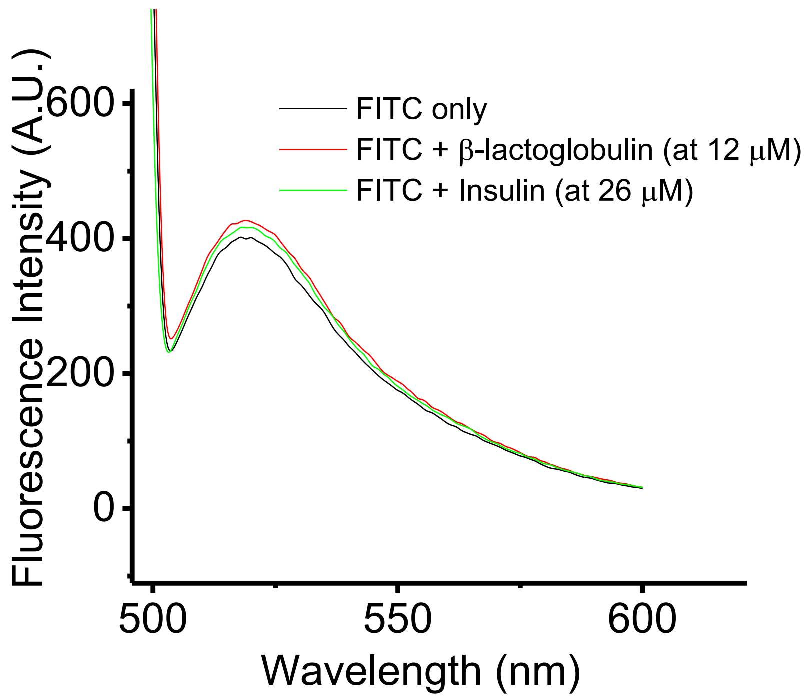


Figure S8. Fluorescence quenching data of control FITC with Insulin and β-lactoglobulin. FITC concentration was fixed at 2.5 μM; and the quenching was performed in the presence of Insulin and β-lactoglobulin at 26 μM and 12 μM respectively.

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