Supporting Information *for*

Developing a Biosensing Prototype Utilising 7CB Liquid Crystal for Human Insulin Detection

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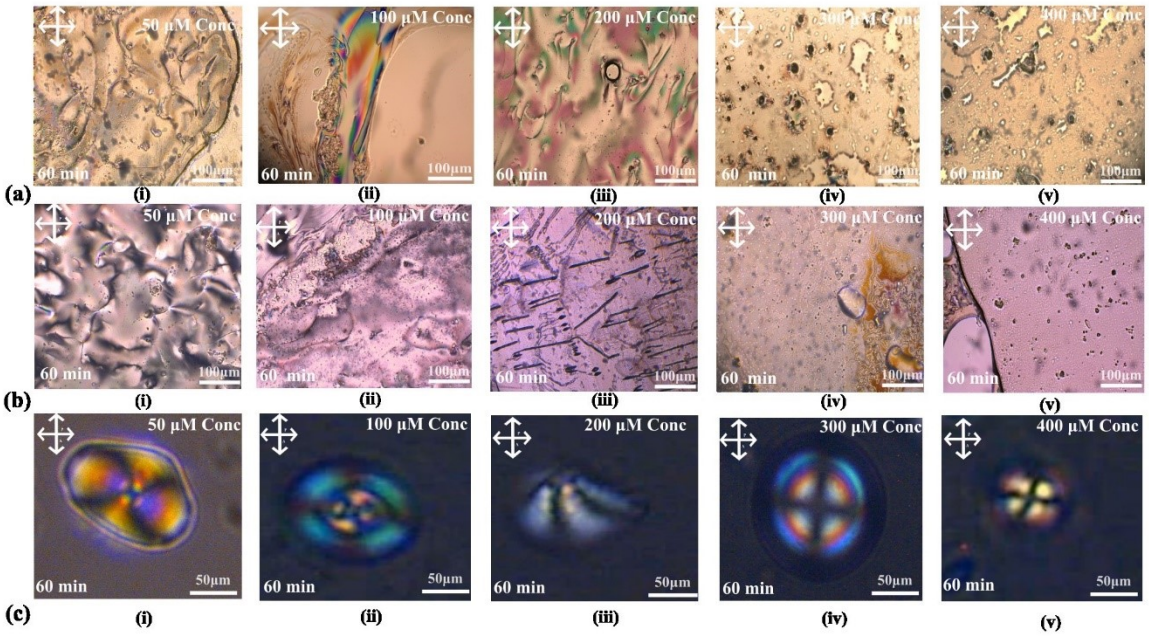


Supplementary Fig.1. POM images of 7CB-LC interacting with proteins of concentration ranging from 50 µM to 400 µM such as (a) 7CB-Ascorbic acid, (b) 7CB-HSA and 7CB-protein mixture (Ascorbic acid, HSA and insulin). *(All the concentrations were studied at a time period of 60 minutes and 0.5 µL volume for GI analysis).*

Supplementary Section 1:

Auto dock tool (ADT 1.5.7) employs the Lamarckian genetic algorithm (LGA), which is a variant of the traditional genetic algorithms. The binding affinities between the 7CB ligand and human insulin protein were evaluated using a semi-empirical free energy scoring function. This semi-empirical free-energy scoring function accounts for various other terms, such as van der Waals (vdW), electrostatics, desolvation, hydrogen bonding, and entropy. ADT 1.5.7 generates a 3D grid around the protein's binding site to analyse the interaction energies for various atom types, allowing for faster posture evaluations via energy interpolation. This method accelerates the scoring function by avoiding multiple energy calculations. ADT 1.5.7 allows for thorough docking data analysis, including RMSD (root mean square deviation) clustering, binding energy estimate, and interaction pose visualisation.

The DLG file generated in the case of 7CB-insulin containing the RMSD data file is shown below (**Supplementary Table 1**). From the RMSD data, the docking position with the minimum binding energy site (-6.62 Kcal/mol) was chosen due to its high binding affinity.

Supplementary Table 1. RMSD Table

Rank	Sub Rank	Run	Binding Energy (Kcal/mol)	Cluster RMSD	Reference RMSD
1	1	5	-6.62	0.00	20.16
2	1	6	-6.35	0.00	19.68

2	2	2	-6.03	0.51	19.71
3	1	7	-6.34	0.00	16.65
3	2	10	-6.29	0.84	17.16
3	3	1	-6.14	0.89	17.21
3	4	3	-6.13	0.51	16.93
3	5	8	-6.08	0.52	16.79
3	6	4	-5.96	0.80	16.91
4	1	9	-5.62	0.00	11.2

The semi-empirical free energy scoring function details for the RUN 5 having the minimum binding energy location is given below:

(1) Final Intermolecular Energy = -8.71 kcal/mol

vdW + H bond + Desolvation Energy = -8.69 kcal/mol

Electrostatic Energy = -0.02 kcal/mol

(2) Final Total Internal Energy = -0.74 kcal/mol

(3) Torsional Free Energy = +2.09 kcal/mol

(4) Unbound System's Energy [= (2)] = -0.74 kcal/mol

- Estimated Free Energy of Binding = -6.62 kcal/mol [= (1)+(2)+(3)-(4)]
- Estimated Inhibition Constant, Ki = 14.01 uM (micromolar) [Temperature = 298.15 K]

INFORMATION ENTROPY ANALYSIS FOR THIS CLUSTERING

Information entropy for this clustering = 0.47 (rmstol = 2.00 Angstrom)

STATISTICAL MECHANICAL ANALYSIS

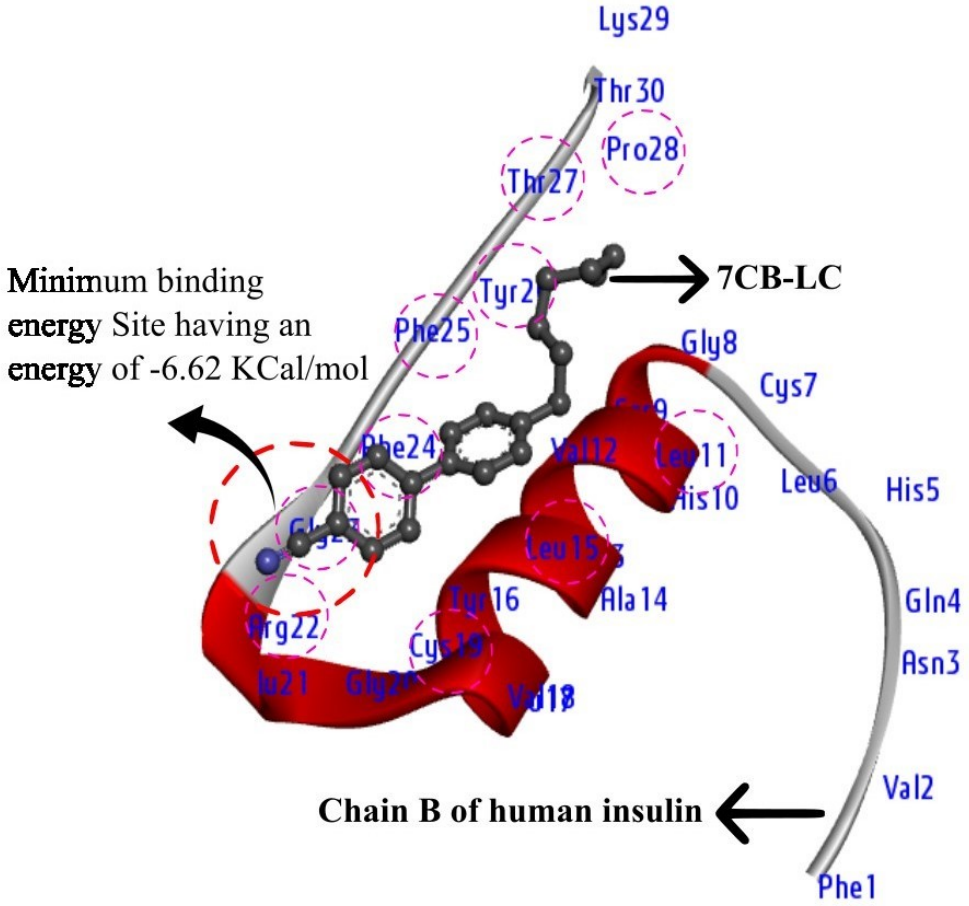
Partition function = Q = 10.10 at Temperature, T = 298.15 K

Free energy = A ~ -1370.39 kcal/mol at Temperature, T = 298.15 K

Internal energy = U = -6.16 kcal/mol at Temperature, T = 298.15 K

Entropy = S = 4.58 kcal/mol/K at Temperature, T = 298.15 K

Based on the DLG file generated, 7CB-LC docked at the minimum binding energy site of Chain B is given below (**supplementary Fig.2**).



Supplementary Fig.2. 7CB-LC docked at the minimum binding energy site of Chain B of human insulin (minimum binding energy site shown in red-dot circle) and the corresponding amino acid residues of human insulin, which makes significant interactions with 7CB-LC (shown in magenta-dot circles).

 # Supplementary Section 2:

Raman spectroscopy works in LC-protein complexes by measuring the inelastic scattering of monochromatic light from a laser when it interacts with molecules' vibrational modes. The approach detects variations in the energy of scattered photons, yielding molecular fingerprints that disclose structural and conformational information about both the LC and the protein in the combination.

The interaction between 7CB-LC and human insulin induces various forces, including electrostatic interactions, hydrogen bonding, and van der Waals interactions. These forces can modify the electronic environment of both the LC and the protein complexes. LCs, due to their inherent properties, have specific molecular arrangements in their nematic phase. When insulin interacts with 7CB in its nematic state, this can disrupt the molecular arrangement, leading to changes in vibrational modes detectable through Raman spectroscopy. Proteins exhibit characteristic vibrational modes related to their amide bonds, known as Amide I, Amide II, and Amide III vibrations.

When LCs bind to proteins, they can alter the proteins secondary and tertiary structures. These structural modifications can lead to conformational changes, such as the transformation from helix to sheet formations. Such changes are reflected in the Raman spectra as shifts in the Amide bands, observed as peak shifts as shown in **Fig.12**.