Binding efficacy of different polyphenolic phytochemicals with β-Lactoglobulin and Human Serum Albumin: Implication for therapeutics against neurodegenerative diseases

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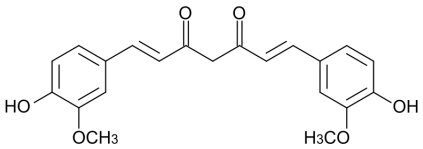
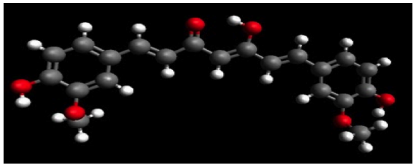
1. Introduction

- Nitrosative stress has recently been demonstrated as a crucial causal factor in the pathogenesis of Parkinson's (PD) and Alzheimer's (AD) diseases.
- Specifically, increased levels of NO disrupt the redox activity of protein-disulfide isomerase, a key endoplasmic reticulum-resident chaperone by S-nitroso modification of its redox-active cysteines.
- This leads to aggregation of misfolded proteins in AD and PD.

2. Curcumin

- Derivative of turmeric (Indian spice).
- Small ligand molecule.
- Well known free radical scavenger.
- Low bioavailability.

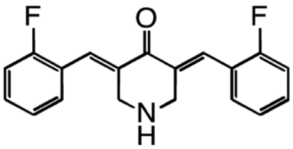




(1E,6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione
(Curcumin).

3. EF-24

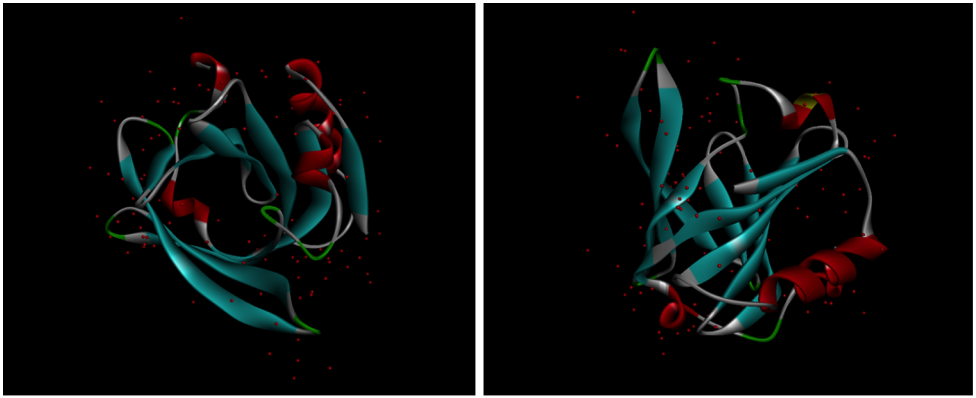
- Curcumin analog.
- Small ligand molecule.
- High bioavailability.
- Potent nitrosative stress scavenger.



EF24

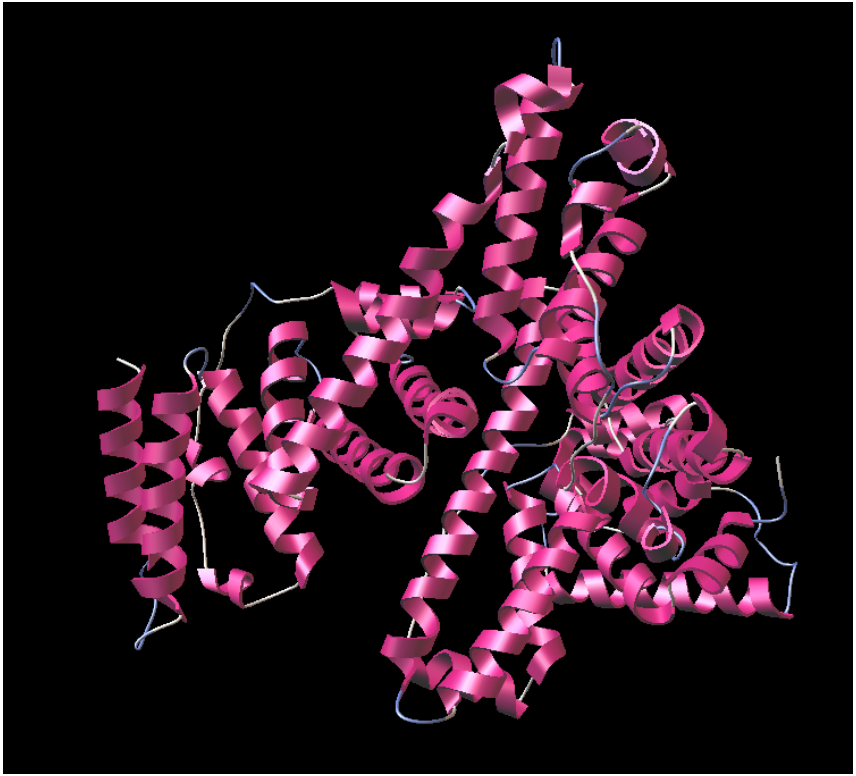
3, 5-bis (2-flurobenzylidene) piperidin-4-one (EF-24).

4. β-Lactoglobulin

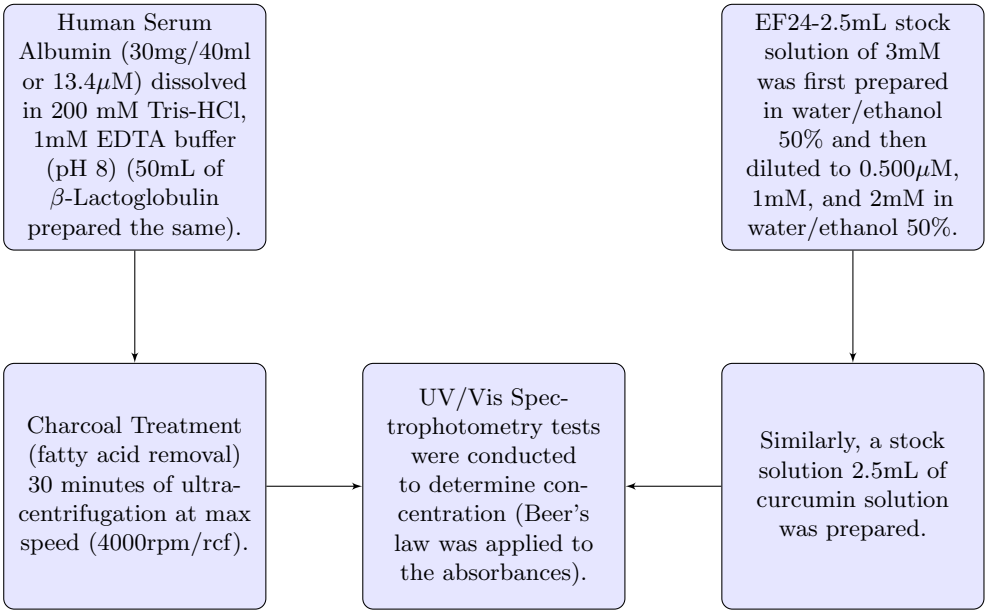


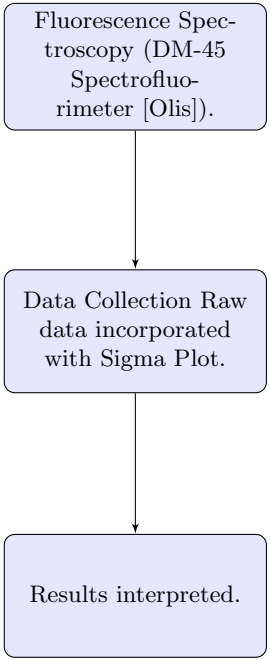
β Lactoglobulin structure (PDB: 1B8E).

5. Human Serum Albumin



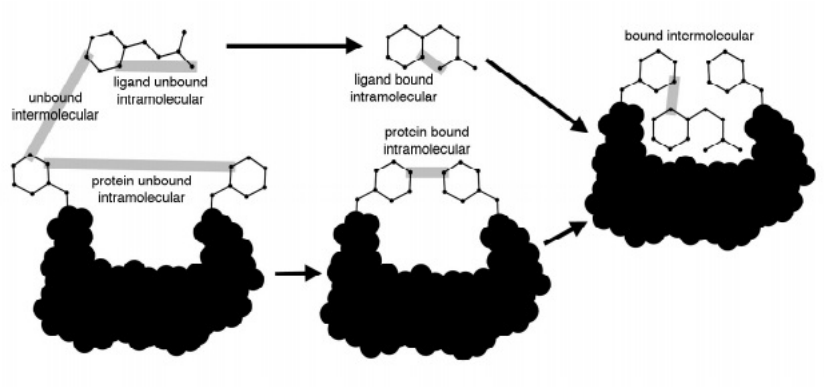
6. Methodology





7. Docking with Auto-Dock

- Energy scoring function



$$\Delta G = (V_{bound}^{L-L} - V_{unbound}^{L-L}) + (V_{bound}^{P-P} - V_{unbound}^{P-P}) + (V_{bound}^{L-P} - V_{unbound}^{L-P} + \Delta S_{conf}).$$

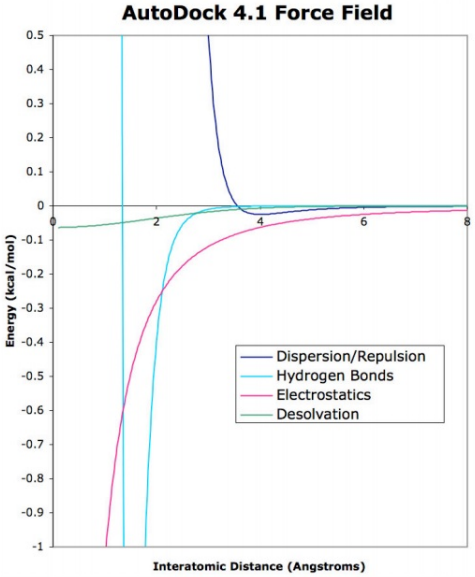
– Semi-empirical force field

$$V = W_{vdw} \sum_{i,j} \left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}} \right)$$

$$+W_{hbond} \sum_{i,j} E(t) \left(\frac{C_{ij}}{r_{ij}^{12}} - \frac{C_{ij}}{r_{ij}^{10}} \right)$$

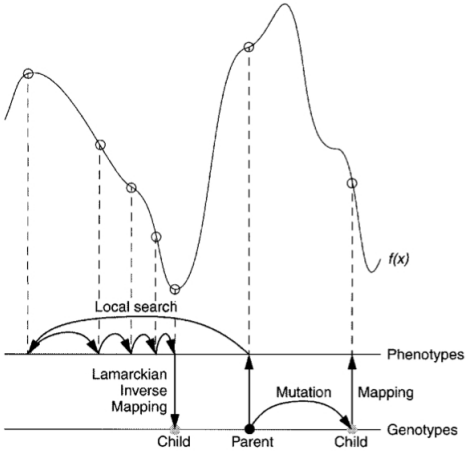
$$+W_{elec} \sum_{i,j} \frac{q_i q_j}{\epsilon(r_{ij}) r_{ij}}$$

$$+W_{sol} \sum_{i,j} (S_i V_j + S_j V_i) e^{-r_{ij}^2/2\sigma^2}.$$



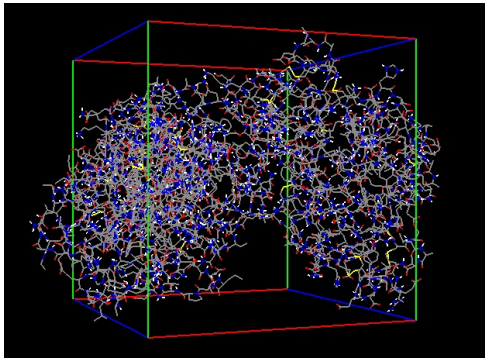
8. Auto-Dock Algorithms

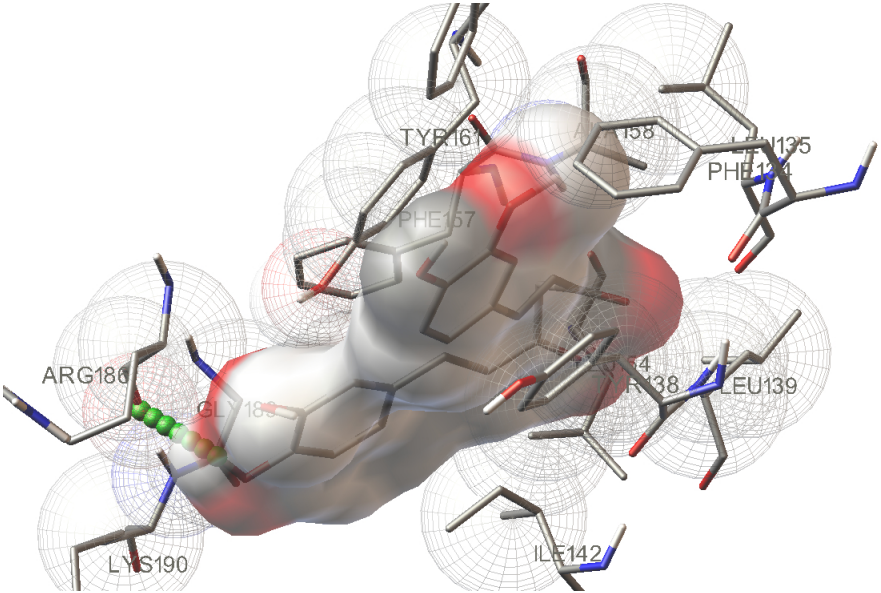
- Algorithms
  - Simulated annealing
  - Local search
  - Genetic algorithm
  - Lamarckian algorithm.



9. Auto-Dock Docking Procedure

- Obtaining PDB files for protein and ligand.
- Preparing protein file
  - Deleting waters and extra atoms
  - Adding hydrogens.
- Preparing ligand
  - Specifying rotatable bonds.
- Specifying flexible resides (if known).
- Grid parameters.
- Docking parameters.





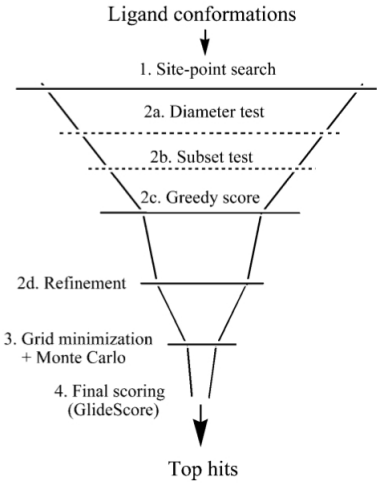
10. Docking with Glide

- Energy scoring function (Chemscore)
  - Empirically based

$$\Delta G_{bind} = C_0 + C_{lipo} \sum f(r_{lr})$$

$$+C_{hbond} \sum g(\Delta r)h(\Delta\alpha) + C_{metal} \sum f(r_{lm}) + C_{rotb} H_{rot}.$$

- Docking algorithm
  - Conformation generation
  - Initial screening of ligand poses
  - Energy minimization using molecular mechanics scoring function.



11. Glide docking procedure

- Importing PDB file.
- Preparing protein (Protein preparation wizard).
- Preparing ligands (LigPrep).
- Grid parameters.
- Docking parameters.

12. Results to Date

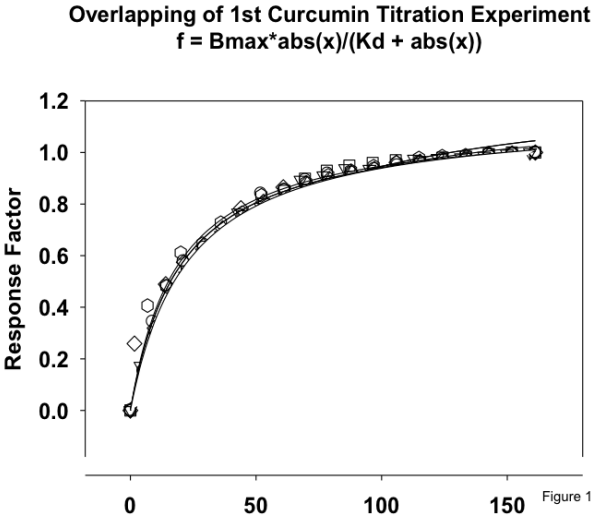


Figure 1

Table 1

	Bmax	Kd Constant
▽ Titration 1: L Free vs Response Factor	1.1998	24.5021
□ Titration 2: L Free vs Response Factor	1.2651	33.8088
○ Titration 3: L Free vs Response Factor	1.1526	22.7309
⬡ Titration 4: L Free vs Response Factor	1.1261	18.8798
◇ Titration 5: L Free vs Response Factor	1.1284	20.0892
—— Fit Curve		

Compounds	Binding Sites	Kd Constant Average and Stdev
B-Lactoglobulin +Curcumin	1	23.1484 ± 5.6825

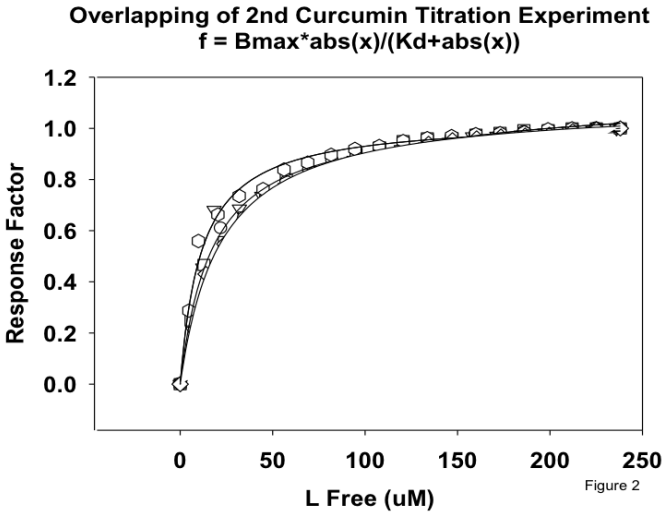


Figure 2

Table 2

	Bmax	Kd Constant
▽ Titration 1: L Free vs Response Factor	1.0558	17.2569
□ Titration 2: L Free vs Response Factor	1.1008	21.6957
○ Titration 3: L Free vs Response Factor	1.1358	27.0992
⬡ Titration 4: L Free vs Response Factor	1.0375	11.5490
◇ Titration 5: L Free vs Response Factor	1.1181	28.0947
—— Fit Curve		

Compounds	Binding Sites	Kd Constant Average and Stdev
B-lactoglobulin + Curcumin	1	21.1391 ± 6.9147

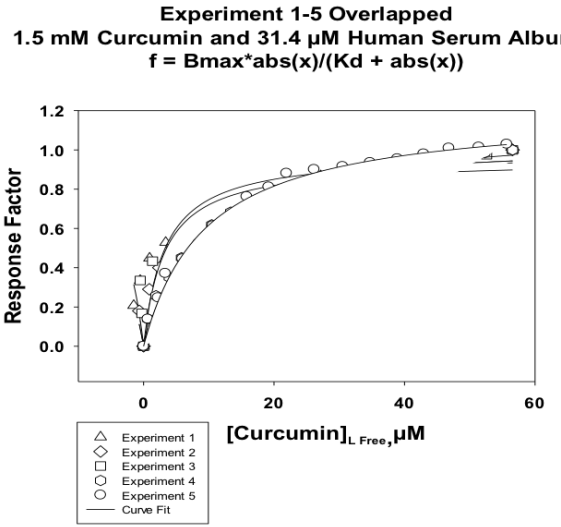


Table 1. *Dissociation constant and summary of binding stoichiometries for the interaction of EF 24 and Human Serum Albumin.*

Interaction of Curcumin with	Binding Sites	Kd (µM)	Stoichiometry
Human Serum Albumin	9		
Experiment 1		3.2275	0.9873
Experiment 2		5.2960	1.0322
Experiment 3		3.1613	0.9480
Experiment 4		9.1486	1.1307
Experiment 5		9.4717	1.2018

[a]Conditions were 200 mM Tris-HCl, pH 8, 25°C

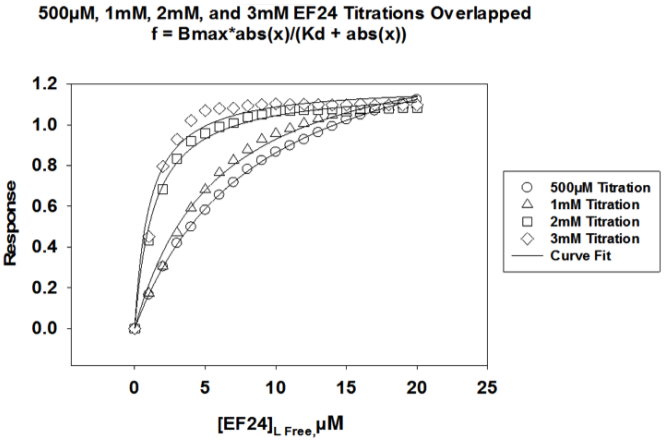


Table 1. *Dissociation constant and summary of binding stoichiometries for the interaction of EF 24 and Human Serum Albumin.*

Interaction of EF 24 with	Binding Sites	Kd (µM)	Stoichiometry
Human Serum Albumin	9		
500µM		1.27 ± 0.63	1.67 ± 0.21
1mM		1.37 ± 0.45	1.47 ± 0.14
2mM		6.26 ± 0.14	1.18 ± 0.03
3mM		7.48 ± 0.49	1.19 ± 0.07

[a] Conditions were 200 mM Tris-HCl, pH 8, 25°C

13. Conclusions

- By conducting extensive spectrofluorimetry experiments, we have determined the binding efficacy of curcumin to β-Lactoglobulin and curcumin to human serum albumin, which is formidable.
- Similarly, the same spectrofluorimeter experiments were carried out and we have determined that the binding efficacy of this EF-24 to β-Lactoglobulin and human serum albumin is exceptional.
- Using docking results on binding affinities we will be able to reproduce a binding curve and validate by comparing to experimental results.

14. References

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