PDB File: 4TQL

Question: How many and which residues can we put fluorescent tags on to maximize fluorescent readout and minimize quenching?

Parameters

1. How far apart (distance) do tags need to be to avoid self-quenching?
2. At what angle do tags need to be adjacent from each other to avoid self-quenching?

Optimization

1. Maximum number of cysteines that can attach fluorescent cargo
2. Minimum quenching

Other Vars

1. Rigid backbone

Constraints

1. Mutations must maintain functionality of 3HB
2. Avoid forming disulfide bonds – will not occur in the current variants bc rigid backbone – no intracellular disulfides but perhaps intercellularly may have some

Preliminary Data to Guide Computational Design:

1. Experimental incorporated cysteine residue mutants that worked and fluorescent readout for each
   1. 4a-b, 6a-b, 8a-b
2. Experimental incorporated cysteine residue mutants that self-quenched and fluorescent readout for each
   1. ?
3. Theoretical distance for quenching
4. Theoretical angle that quenches

Guide for how to approach the problem

1. Create PDB files for each of the mutants (introduced cysteines)
2. Data input of successful mutants
   1. Use R to tabulate individual distances and angles of cysteines from each other (reading in the PDB files) (e.g. 1-2, 1-3, 1-4, 2-3, 2-4, 3-4…) for cys introductions 1-4
   2. Use R to tabulate an average distance and angles of cys from each other against fluorescent readout (take avg of 2)
3. Data input of unsuccessful mutants
   1. Redo 2-3 tabulation but for mutants that quenched
4. Optimization strategy in R
   1. Get the Bio3D Package in R (reads structures)