Exercises on protein-protein interactions

1) We wish to evaluate the influence of several amino acid residues in the stability of a protein-protein complex, by analysing the interaction energy change after mutations to Ala. The following table summarizes calculated changes in interaction and solvation energies due to the modification:

Mutation	ΔΔGint (Kcal/mol)	ΔΔGsolv (Kcal/mol)
Glu 300 Ala	10.5	-3.0
Trp 260 Ala	1.1	3.2
Val 310 Ala	0.1	0.3
Gln 302 Ala	1.2	-1.1

a) Order the analysed residues from less to more importance in the complex stability

b) Suggest for each residue the reason behind the observed energy values

c) Knowing that the experimental dissociation constant for the unmutated complex is 2.4 nM, evaluate the expected dissociation constants for the mutants.

2) We wish to evaluate the influence of several amino acid residues in the stability of a protein-protein complex (R-L). We have access to some mutagenesis experiment results where residues of R protein have been mutated to Ala, and the dissociation constant of the complex has been evaluated. The dissociation constant of the unmutated R-L complex es 2.4 nM.

At the same time, we have estimated theoretically the changes in interaction energy (Electrostatic + VdW). Results are summarized in the following table:

Mutation	Complex K_D	ΔΔGint (Kcal/mol)
Glu 300 Ala	0.76 mM	10.5
Trp 360 Ala	3.4 µM	1.1
Val 310 Ala	4.7 nM	0.1
Gln 302 Ala	2.8 nM	1.2

a) Evaluate the global ΔΔGwt→A and the contribution of the solvation to this value.

b) Indicate whether the following sentences are True or False, justifying briefly (with reference to the energy values obtained).

c) Indicate briefly how ΔΔGint can be evaluated.

d) Evaluate the degree of complex formation of wild-type and each mutant at a concentration of free L of 2.4 nM

3) We wish to evaluate the influence of several amino acid residues in the stability of a protein-protein complex (R-L), by analysing the interaction energy change after mutations to Ala. The following table summarizes calculated changes in interaction and solvation energies due to the modification:

Mutation	ΔΔGelec.int (Kcal mol^{-1})	ΔΔGvdw.int (Kcal mol^{-1})	ΔΔGsolv (Kcal mol^{-1})
Glu 300 Ala	10.0	0.5	-3.0
Trp 260 Ala	-0.1	1.2	3.2
Val 310 Ala	0.0	0.1	0.3
Gln 302 Ala	0.7	0.5	-1.1

a) Order these residues from less to more important in the stability of the complex.

b) Suggest explanations for the observed energy changes. Consider that Glu is a negative charged residue while Gln es polar but neutral. Trp and Val are both hydrophobic but Trp is much larger than Val.

c) Knowing that the experimental dissociation constant for the unmutated complex is 2.4 nM, evaluate

1. the expected dissociation constants for the mutants

2. the degree of complex formation at a concentration of L necessary for getting a 50% of the wild-type complex.

4) The analysis using FOLDX of some sequence variants on SARS-Cov-2 Spike protein from the Omicron strain regarding to the binding energy between RBD domain and ACE2 has given the following results. (Note: ΔΔG corresponds to ΔGMut – ΔGWt):

Variant	ΔΔGvdw (kcal/mol)	ΔΔGelec (kcal/mol)	ΔΔGsolv (kcal/mol)
Lys417Asn	0.56	2.23	-1.71
Glu484Ala	0	0	-0.23
Gln493Lys	-0.44	0.84	-0.20
Gly496Ser	-0.12	-0.85	0.35
Gln498Arg	-1.63	1.75	-0.24
Asn501Tyr	4.94	0.61	-2.08
Tyr505His	0.14	1.05	1.02

a) From the data provided, which would be the order of stability of the complex ACE2-RBD

b) Calculate the ratio of binding constants with respect to the complex made with WT Spike

c) For each variant choose from the following options, reasons that help to explain the change

a. Vdw collision due to increased sidechain

b. Loss of an electrostatic interaction/Hbond

c. Increased hydrophobic effect

d. Improved sidechain packing

e. Decreased hydrophobic effect

d) Considering that Omicron Spike is efficient in infection through the binding to ACE2, what can be said about the results obtained?

e) Indicate (considering the energy values) whether the following statements regarding the above variants are True of False (justify briefly)

a. Gln493Lys shows a Vdw collision due to increased sidechain

b. Gln493Lys, Gly496Ser, and Gln498Arg show an improved side-chain packing

c. Asn501Tyr shows an increase in the hydrophobic component of binding.

d. Lys417Asn shows an increase in the hydrophobic component of binding.

e. Tyr505His shows loss of electrostatic interaction

Exercises on binding

1) A series of drugs (A100-A102) have been designed to bind to an internal cavity of a given protease. Drugs act as competitive inhibitors with the following inhibition constants:

Compound	K_I (μM)
S (K_M)	2100
A100	156.8
A101	4.82
A102	353.3

The compounds have a common chemical group analogous to an Arg side chain, mimicking the natural ligand (S), and variable hydrophobic groups.

All compounds retain the original Arg-like positive charge and bind to the protein at the same site and conformation. In these conditions it can be assumed that the differences in binding are due only to differences in solvation.

Substrate (S) binding is in equilibrium; hence KM corresponds to a true dissociation constant. Consider T=298 K

a) Order the drugs regarding their inhibitory power

b) Determine the degree of inhibition (see Equations) caused by 5 μM of the drug at a concentration of S of 1 mM

c) Evaluate the difference of hydrophobic solvation energy between S and the inhibitors

Degree of inhibition for a competitive inhibitor (I)

$$i(I) = \frac{v(I=0) - v(I)}{v(I=0)} = \frac{K_M \, {}^{I}/_{K_I}}{K_M \left(1 + {}^{I}/_{K_I}\right) + S}$$

I: inhibition degree (no units); S: Substrate concentration; I: Inhibitor concentration; K_M: Michaelis constant; K_I: Inhibition constant. Units to match: S and K_M, I and K_I