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

The global ΔΔG for the mutations is the combination of interaction and solvation so:

Mutation	ΔΔGint (Kcal/mol)	ΔΔGsolv (Kcal/mol)	ΔΔG (Kcal/mol)
Glu 300 Ala	10.5	-3.0	7.5
Trp 260 Ala	1.1	3.2	4.3
Val 310 Ala	0.1	0.3	0.4
Gln 302 Ala	1.2	-1.1	0.1

All ΔΔG are positive, so all mutations are distabilizing, and the order is:

Gln302 < Val310 < Trp260 < Glu300

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

Glu 300: A large change is in the interaction energy. Since Glu is a negative amino acid, this suggest that the mutation may break a electrostatic interaction in the complex. Solvation change is negative as the solvation of the Glu side chain is not lost, what confirms that Glu is buried in the interface.

Trp 260: Low change in the interaction energy, that can be attributed to less Vdw interaction (Ala is a lot smaller than Trp). Most of the change comes from solvation, what is compatible with the larger surface of Trp, what in turn indicates that Trp is also buried in the interface.

Val 310: Same explanations as in the case of Trp, but the change from Val to Ala is much smaller.

Gln 302: Gln is polar but neutral, it may lose hydrogen bonds. Solvation effect is positive what indicates the Gln is in the interface (there is a surface change). In any case these effects are small, so the implication of this residue is not relevant.

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

$$\Delta\Delta G_{WT\to A}=\Delta G_{Ala}-\Delta G_{WT}=RT\ln K_D^{Ala}-RT\ln K_D^{WT}=RT\ln\frac{K_D^{Ala}}{K_D^{WT}}$$

$$K_D^{Ala}=K_D^{WT}e^{\frac{\Delta\Delta G_{WT\to A}}{RT}}$$

$$K_D^{WT}=2.4nM\ R=1.987\frac{cal}{molK},T=298\,K$$

Mutation	**ΔΔG (Kcal/mol)**	**K_D**
Glu 300 Ala	7.5	0.76 mM ($7.6\ 10^{-4}$ M)
Trp 260 Ala	4.3	3.4 µM ($3.4\ 10^{-6}$ M)
Val 310 Ala	0.4	4.7 nM ($4.7\ 10^{-9}$ M)
Gln 302 Ala	0.1	2.8 nM ($2.8\ 10^{-9}$ M)

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.

Using $\Delta G = RTLnK_D$ we can obtain the $\Delta G_{binding}$ for each protein, including WT. Then the differences with WT give $\Delta\Delta G_{WT\rightarrow A}$. This can be done as follows.

$$\Delta\Delta G_{WT\rightarrow A} = \Delta G_A - \Delta G_{WT} = RTlnK_{D(A)} - RTlnK_{D(WT)} = RTln\frac{K_{D(A)}}{K_{D(WT)}}$$

NOTE that both K_D's should be expressed in the same UNITS!!

$\Delta\Delta G_{WT\rightarrow A}$ is the sum of the interaction component and the solvation component, then the solvation component is just the difference.

$$\Delta\Delta G^{solv}_{WT\rightarrow A} = \Delta\Delta G_{WT\rightarrow A} - \Delta\Delta G^{int}_{WT\rightarrow A}$$

Protein	K_D (M)	$\Delta G_{binding}$ (Kcal/mol)	$\Delta\Delta G_{WT\rightarrow A}$ (Kcal/mol)	$\Delta\Delta G^{solv}_{WT\rightarrow A}$ (Kcal/mol)
WT	2,40E-09	-11,75		
Glu - Ala	7,60E-04	-4,25	7,50	-3,00
Trp - Ala	3,42E-06	-7,45	4,30	3,20
Val - Ala	4,72E-09	-11,35	0,40	0,30
Gln - Ala	2,84E-09	-11,65	0,10	-1,10

WARNING: Energy units depend on which value of the R constant is used, anyone is fine but ALWAYS indicate the UNITS, and DO NOT MIX them!!!! Note that int energies were expressed in Kcal/mol.

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

a. **Glu 300 is involved in an electrostatic interaction in the interface**
   True. Glu 330 Ala show a large ΔΔGWT→A that comes mostly from ΔΔGint. Since Glu is charged is should be the loss of an electrostatic interaction.

b. **Trp 360 is a central residue in the protein-protein interface**
   True. Most of the change appears as a solvation contribution. Since Trp is a large hydrophobic, Trp should be buried in the interface after binding (ASA loss)

c. **Val 310 is a central residue in the protein-protein interface**
   False. Val is also hydrophobic, but the solvation energy contribution is much lower, so Val is not buried, as that change in surface is not much relevant.

d. **Gln 302 is involved in an electrostatic interaction in the interface**
   False. Gln is neutral, so it cannot be involved in an electrostatic interaction.

e. **Solvation energies are only relevant for polar residues**
   False. Solvation is important for all residues.

c) Indicate briefly how ΔΔGint can be evaluated.

Interaction energies can be obtained using the standard electrostatic and vdw equations, evaluated between all pairs of atoms in the two components of the complex. The WT→A comes either from the difference of those values when removing the contribution the appropriate atoms of the side chain or repeating the calculation after replacing the residue.

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

The degree of complex formation corresponds to the "saturation degree" Y = RL/Rtot
WARNING: Again UNITS are relevant, KD and L should be expressed in same units. Reulst should show that higher KD correspond to less affinity, end then less saturation.

	K_D (nM)	Y
WT	2.40	0.50
Glu - Ala	7.60E+05	3.16E-06
Trp - Ala	3.42E+03	7.01E-04
Val - Ala	4.72	0.34
Gln - Ala	2.84	0.46

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.

The importance of each residues is obtained from the global change in binding energy after mutation.

ΔΔGBinding is the sum of all contributions.

Mutation	ΔΔGbind (Kcal mol^{-1})
Glu 300 Ala	7.5
Trp 260 Ala	4.3
Val 310 Ala	0.4
Gln 302 Ala	0.1

Gln302 < Val 310 < Trp 260 < Glu 300

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.

NOTE: the question was not about explaining the importance of the residues, but about explaining the energies obtained -All mutants. Changes in Vdw energies a related to the change in the relative size of the side chains.

Glu 300. It is a charged residue, a large change in electrostatic energy indicates a loss of an electrostatic interaction that was important for the complex so it should be buried in the interface. Change in solvation indicates an improvement since we move from a charged residue (better solvated in the unbound form) to a mildly hydrophobic (better solvated in the complex)

Trp 260. Most change come from the solvation energy, which is compatible with a large change in ASA on binding. Trp 260 is probably buried in the complex interface.

Val 310. Similar reasoning as Trp 260, but since the energies are almost negligible, there is no change in ASA on binding, so Val 310 is not buried in the interface.

Gln 302. is polar. Change in solvation energy is negative (as it was in Glu300) but much smalled, hence indicating the Gln 302 is close but not in the interface. Since it is neutral electrostatic contribution is small, probably due to the loss of some hydrogen bonds.

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

1. the expected dissociation constants for the mutants

$$\Delta G = RTlnK_D$$

$$\Delta\Delta Gwt \rightarrow A = \Delta G_A - \Delta G_{wt} = RTln\frac{K_D^A}{K_{D^{wt}}}$$

$$K_D^A = K_D^{wt} e^{\frac{\Delta\Delta Gwt \rightarrow A}{RT}}$$

UNITS:

$\Delta\Delta Gwt \rightarrow A \wedge Rshouldusethesameenergyunits$!!!

$K_D^A \wedge K_D^{wt}$ K_D use the same units.

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

Saturation degree comes from $Y = \frac{L}{K_D + L}$

Value of L corresponds to the necessary to get a 50% of formation of the WT complex. By definition the concentration of L that gives a 50% saturation is K_D = 2.4 nM = L. then the saturation degree for each mutant comes from the corresponding K_D value.

Mutation	ΔΔGbind (Kcal mol^{-1})	K_D (M)	Y
Glu 300 Ala	7.5	$7.6\ 10^{-4}$ (0.76 mM)	$3.26.10^{-6}$
Trp 260 Ala	4.3	$3.42.10^{-6}$ (3.42 μM)	$7.01.10^{-4}$
Val 310 Ala	0.4	$4.72.10^{-9}$ (4.72 nM)	0.34
Gln 302 Ala	0.1	$2.84.10^{-9}$(2.84 nM)	0.46

NOTE to check results: All ΔΔGbind are positive, so all KD's should be larger (less binding) than WT, much larger as larger is ΔΔGbind (there a logarithmic relationship), and all saturation degrees should be smaller than 0.5 (the WT one)

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

Total ΔΔGbind for each individual mutant is the sum of all energy components.components. The Ratio between binding constants comes from

$$\Delta\Delta G_{bind} = \Delta G_{mut} - \Delta G_{wt} = -RT \ln K_{Bind}^{Mut} + RT \ln K_{Bind}^{WT} = -RT \ln \frac{K_{Bind}^{Mut}}{K_{Bind}^{WT}} = +RT \ln \frac{K_D^{Mut}}{K_D^{WT}}$$

Note that the ratios deduced from K_{bind} are the inverse from ratios deduced from K_D. Both were correct as long there were correctly indicated.

Variant	$\Delta\Delta G_{vdw}$ (Kcal/mol)	$\Delta\Delta G_{elec}$ (Kcal/mol)	$\Delta\Delta G_{solv}$ (Kcal/mol)	$\Delta\Delta Gbind_v$ (Kcal/mol)	$\frac{K_{Bind}^{Mut}}{K_{Bind}^{WT}}$	$\frac{K_D^{Mut}}{K_D^{WT}}$	Reasons
Lys417Asn	0.56	2.23	-1.71	1.08	0.16	6.20	b,c
Glu484Ala	0	0	-0.23	-0.23	1.47	0.68	c
Gln493Lys	-0.44	0.84	-0.20	0.2	0.71	1.40	b,d
Gly496Ser	-0.12	-0.85	0.35	-0.62	2.85	0.35	d
Gln498Arg	-1.63	1.75	-0.24	-0.12	1.22	0.82	b,c,d
Asn501Tyr	4.94	0.61	-2.08	3.47	$2.85\ 10^{-3}$	350.8	a,b,c
Tyr505His	0.14	1.05	1.02	2.21	$2.39\ 10^{-2}$	41.78	b,e

The order of COMPLEX STABILITY from most to least stable is

Gly486Ser > Glu484Ala > Gln498Arg > Gln493Lys > Lys417Asn > Tyr505His > Asn501Tyr

Note: Always use the correct value of the R constant considering the units. Be aware that R = 1.987 cal/K.mol or 0.001987 Kcal/K.mol. R can be also used in J/K.mol but in that case energies should be converted to KJ/mol

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

a. Vdw collision due to increased sidechain

Can be recognized by a high, and positive ΔΔGvdw . In this case only Asn501Tyr.

b. Loss of an electrostatic interaction/Hbond

Recognized by a positive ΔΔGelec. Appears in Lys417Asn (loss of electrostatic interaction) and also in less extent (compatible with Hbond pattern change) in Gln493Lys, Gln408Arg, Asn501Tyr, Tyr505His

c. Increased hydrophobic effect

Recognized by a negative (favourable) ΔΔGsolv but only where the major component of the solvation is hydrophobic (the mutant involved apolar amino-acids): Glu484Ala, Asn501Tyr. Other favourable solvations are probably due to the electrostatic component.

d. Improved sidechain packing

Recognized by a favourable (negative) ΔΔGvdw.. Gln493Lys, Gly496Ser, Gln498Arg

e. Decreased hydrophobic effect

Recognized by a positive (unfavourable) ΔΔGsolv but only where the major component of the solvation is hydrophobic: Tyr505His.

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

Considering that Omicron strain contains ALL the mutations shown in the table, and assuming that the effects are additive, the total ΔΔG would be +5.99 Kcal/mol, indicating that the complex ACE2-RBD would ~24,000 fold LESS stable with respect to the WT case. Since the experimental result is that the complex is formed, the only conclusion is that the FOLDX energy values are overestimated, and the theoretical analysis should be re-evaluated.

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

False. ΔΔGvdw is negative. A collision should give a positive values

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

True, all them have negative ΔΔGvdw indicating that side chains are better packed

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

True. ΔΔGsolv is negative indicating an increased unfavourable solvation energy before binding, as expected from the amino acid change.

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

False. ΔΔGsolv is negative but none of the involved amino acids is hydrophobic, so the change should correspond to a decrease in the electrostatic solvation (as can be expected from a change to a charged residue to a neutral one)

e. Tyr505His shows loss of electrostatic interaction

True. ΔΔGelec is positive. As none of the amino acids is charged the value should correspond to differences on hydrogen bond patterns.

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