Exercise 1

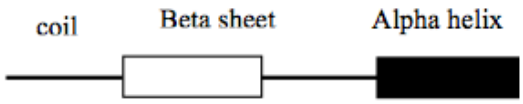
1. Below you will find an amino acid sequence:

   RPDHFMASYRKGAVLLKIKQYKLALPVLEAVVREKPED

   A. Which amino acids are expected to be exposed to the solvent and which are expected to be buried within the protein core?

   B.

   i) Manually predict the secondary structure of the above sequence by using the scheme below.



   *(The pattern above is a **general schematic** and **not a literal scheme** for the sequence; that is, depict the sequence using a straight line for a coil, an empty box for a beta sheet and a solid box for an alpha helix.)*

   ii) Afterwards, run the sequence through two different secondary structure prediction servers and describe and explain the differences between the outputs and your prediction.

2. You need to prepare a 1 L buffer at a concentration of 1 M and a pH value of 6.3.
   A. Which buffer will you use? Write down its name and explain why you chose that specific buffer.

   B. Please give details of how you plan to prepare the above buffer using the *mixing* technique (not via titration).

Table A1-2 Properties of Good Buffers

Acronym	Chemical Name	FW	pK_a	Useful Range (in pH units)
MES	2-(*N*-morpholino)ethanesulfonic acid	195.2	6.1	5.5–6.7
Bis-*Tris*	*bis*(2-hydroxyethyl)imino*tris*(hydroxymethyl)methane	209.2	6.5	5.8–7.2
ADA	*N*-(2-acetamido)-2-iminodiacetic acid	190.2	6.6	6.0–7.2
ACES	2-[(2-amino-2-oxoethyl)amino]ethanesulfonic acid	182.2	6.8	6.1-7.5
PIPES	piperazine-*N*,*N*′-*bis*(2-ethanesulfonic acid)	302.4	6.8	6.1–7.5
MOPSO	3-(*N*-morpholino)-2-hydroxypropanesulfonic acid	225.3	6.9	6.2–7.6
Bis-*Tris* Propane	1,3-*bis*[*tris*(hydroxymethyl)methylamino]propane	282.3	6.8[a]	6.3–9.5
BES	*N*,*N*-*bis*(2-hydroxyethyl)-2-aminoethanesulfonic acid	213.2	7.1	6.4–7.8
MOPS	3-(*N*-morpholino)propanesulfonic acid	209.3	7.2	6.5–7.9
HEPES	*N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid)	238.3	7.5	6.8–8.2
TES	*N*-*tris*(hydroxymethyl)methyl-2-aminoethanesulfonic acid	229.2	7.4	6.8–8.2
DIPSO	3-[*N*,*N*-*bis*(2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid	243.3	7.6	7.0–8.2
TAPSO	3-[*N*-*tris*(hydroxymethyl)methylamino]-2-hydroxypropanesulfonic acid	259.3	7.6	7.0–8.2
TRIZMA	*tris*(hydroxymethyl)aminomethane	121.1	8.1	7.0-9.1
HEPPSO	*N*-(2-hydroxyethyl)piperazine-*N*′-(2-hydroxypropanesulfonic acid)	268.3	7.8	7.1–8.5
POPSO	piperazine-*N*,*N*′-*bis*(2-hydroxypropanesulfonic acid)	362.4	7.8	7.2-8.5
EPPS	*N*-(2-hydroxyethyl)piperazine-*N*′-(3-propanesulfonic acid)	252.3	8.0	7.3–8.7
TEA	triethanolamine	149.2	7.8	7.3–8.3
Tricine	*N*-*tris*(hydroxymethyl)methylglycine	179.2	8.1	7.4–8.8
Bicine	*N*,*N*-*bis*(2-hydroxyethyl)glycine	163.2	8.3	7.6–9.0
TAPS	*N*-*tris*(hydroxymethyl)methyl-3-aminopropanesulfonic acid	243.3	8.4	7.7–9.1
AMPSO	3-[(1,1-dimethyl-2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid	227.3	9.0	8.3–9.7
CHES	2-(*N*-cyclohexylamino)ethanesulfonic acid	207.3	9.3	8.6–10.0
CAPSO	3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid	237.3	9.6	8.9–10.3
AMP	2-amino-2-methyl-1-propanol	89.1	9.7	9.0–10.5
CAPS	3-(cyclohexylamino)-1-propanesulfonic acid	221.3	10.4	9.7–11.1

Data compiled from various sources, including *Biochemical and Reagents for Life Science Research* 1994 (Sigma-Aldrich) and references therein.
[a] pK_a = 9.0 for the second dissociation stage.

Source: Maniatis, T. *Molecular Cloning: A Laboratory Manual*