1 Sample Preparation

1.1 Molecular Weight of Tat

The Tat peptide sequence used in x-ray experiements and MD simulations is YGRKKRRQRRR, where one letter notation of amino acids is used. The molecular weight of this sequence is $181.2+75.1+146.1+2\times146.2+6\times174.2-10\times18 = 1560$. Peptides are normally synthetized in trifluoroacetic acid, which has the chemical formula CF_3CO_2H, and made into a powder form by the freeze-dry method (ref?). Therefore, each positively charged amino acid such as an arginine and lysine is counter-balanced by a trifluoroacetate (TFA) ($C_2F_3O_2$). Since Tat has six arginines and two lysines, it comes with eight trifluoroacetates. This complex has molecular weight of $1560+113\times8 = 2464$. When Tat peptides are weighed, one must use the molecular weight of the complex in order to calculate the molarity of a Tat in water solution correctly.

Molecule	Molecular Weight	Volume
Tat (YGRKKRRQRRR)	1560	1876
Tat + TFA	2464	2964

Table 1: Important Quantities for Tat Peptide

Code	Amino acid	Chemical Formula	Molecular weight (g/mol)
K	Lysine	$C_6H_{14}N_2O_2$	146.2
R	Arginine	$C_6H_{14}N_4O_2$	174.2
G	Glycine	$C_2H_5NO_2$	75.1
Y	Tyrosine	$C_9H_{11}NO_3$	181.2
Q	Glutamine	$C_5H_{10}N_2O_3$	146.1

Table 2: Amino Acids Data

1.2 Volume Measurement

The volume of Tat was measured using a densimeter, which measure the average density of a solution. First, the mass of Tat and water were measured to be 3.7 and 1212.6 mg via a digital balance. The density of water and Tat-water solution were measured to be 0.993325 and 0.99418 g/cm^3, respectively.

The Tat volume is calculated in the following way. Assuming that Tat molecules in water does not change the volume of water molecules, the density of Tat-water solution is equal to the mass of Tat-water solution divided by the sum of volumes of water and Tat,

$$\rho_{sol} = \frac{m_w + m_c}{V_w + V_c N_c}, \tag{1}$$

where ρ_{sol} is the density of the solution, m_w and m_c are the total mass of water and Tat-TFA complex, V_w is the total volume of water, V_c is the volume of a Tat-TFA complex, and N_c is the total number of the complex in the solution. Using $V_w = m_w/\rho_w$ and $N_c = N_A m_c/W_c$, after some simple algebra, we arrive at

$$V_c = \frac{W_c}{\rho_{sol} N_A}\left(1 + \frac{m_w}{m_c}\left(1 - \frac{\rho_{sol}}{\rho_w}\right)\right), \tag{2}$$

where W_c is the molecular weight of the complex, N_A is the Avogadro's number, and ρ_w is the density of water. The measured values of these quantities are shown in Table 3. As described in the previous section, Tat powder comes with couterions, so the volume measured here is that of a Tat-TFA complex. Assuming here for simplicity that the molecular volume scales with the molecular weight, the volume of Tat was measured to be 2964 $\text{Å}^3\times$ 1560/2464=1876 Å^3. This value is in quite a good agreement with the value from a peptide calculator website (ref?). As will be shown in a later chapter, MD simulations also predict a similar value.

ρ_{sol}	0.994180 g/cm^3
ρ_w	0.993325 g/cm^3
m_w	1212.6 mg
m_T	3.73 mg

Table 3: Measured Quantities in