

# 1 Sample Preparation

## 1.1 Molecular Weight of Tat

The Tat peptide sequence used in x-ray experiments and MD simulations is YGRKKRRQRRR, where one letter notation of amino acids is used. The molecular weight of this sequence is  $181.2 + 75.1 + 146.1 + 2 \times 146.2 + 6 \times 174.2 - 10 \times 18 = 1560$ . Peptides are normally synthesized in trifluoroacetic acid, which has the chemical formula  $\text{CF}_3\text{CO}_2\text{H}$ , 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) ( $\text{C}_2\text{F}_3\text{O}_2$ ). Since Tat has six arginines and two lysines, it comes with eight trifluoroacetates. This complex has molecular weight of  $1560 + 113 \times 8 = 2464$ . 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	$\text{C}_6\text{H}_{14}\text{N}_2\text{O}_2$	146.2
R	Arginine	$\text{C}_6\text{H}_{14}\text{N}_4\text{O}_2$	174.2
G	Glycine	$\text{C}_2\text{H}_5\text{NO}_2$	75.1
Y	Tyrosine	$\text{C}_9\text{H}_{11}\text{NO}_3$	181.2
Q	Glutamine	$\text{C}_5\text{H}_{10}\text{N}_2\text{O}_3$	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  $\text{g}/\text{cm}^3$ , 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,

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

where  $\rho_{sol}$  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

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

where  $W_c$  is the molecular weight of the complex,  $N_A$  is the Avogadro's number, and  $\rho_w$  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 counterions, 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  $2964 \text{ \AA}^3 \times 1560/2464 = 1876 \text{ \AA}^3$ . 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.

$\rho_{sol}$	0.994180 g/cm <sup>3</sup>
$\rho_w$	0.993325 g/cm <sup>3</sup>
$m_w$	1212.6 mg
$m_T$	3.73 mg

Table 3: Measured Quantities in