Na/K-ATPase as a target for anticancer metal based drugs: insights into the molecular interactions with selected gold(III) complexes

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1. Tryptophan distribution in Na, K-ATPase

Na/K ATPase is an enzyme rich in Trp residues.[1] There is a total of 16 tryptophan residues (Trp), 12 of them being situated in α-subunit and 4 in β-subunit; no Trp residues are in the γ-subunit. Most of Trp residues from the α-subunit (10 Trp) are located in trans-membrane domain (Trp82, Trp98, Trp310, Trp883, Trp887, Trp899, Trp924, Trp980, Trp981, and Trp1009), and only two residues (Trp385 and Trp411) are located in the intracellular domain (N domain). Three tryptophan residues from *β*-subunit are located in trans-membrane domain (Trp12, Trp17, and Trp32), and one residue (Trp155) is located in extracellular part of *β*-subunit. The distribution of tryptophan residues is illustrated in **Fig. S1**.

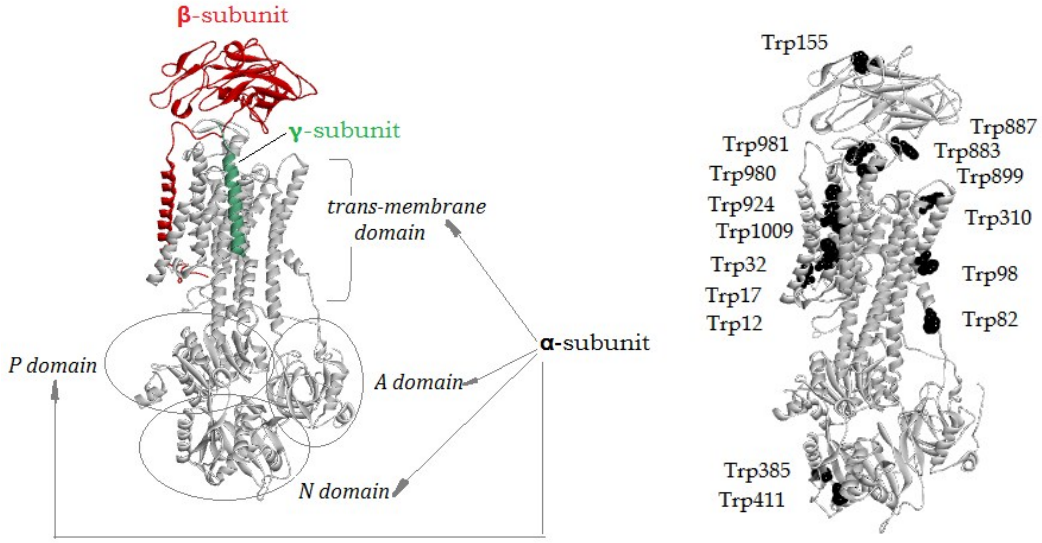


Fig. S1 The structure of Na/K ATPase and distribution of Trp residues in the Na/K ATPase in E1 state.

2. Influence of gold (III) complexes on Na,K-ATPase fluorescence spectra

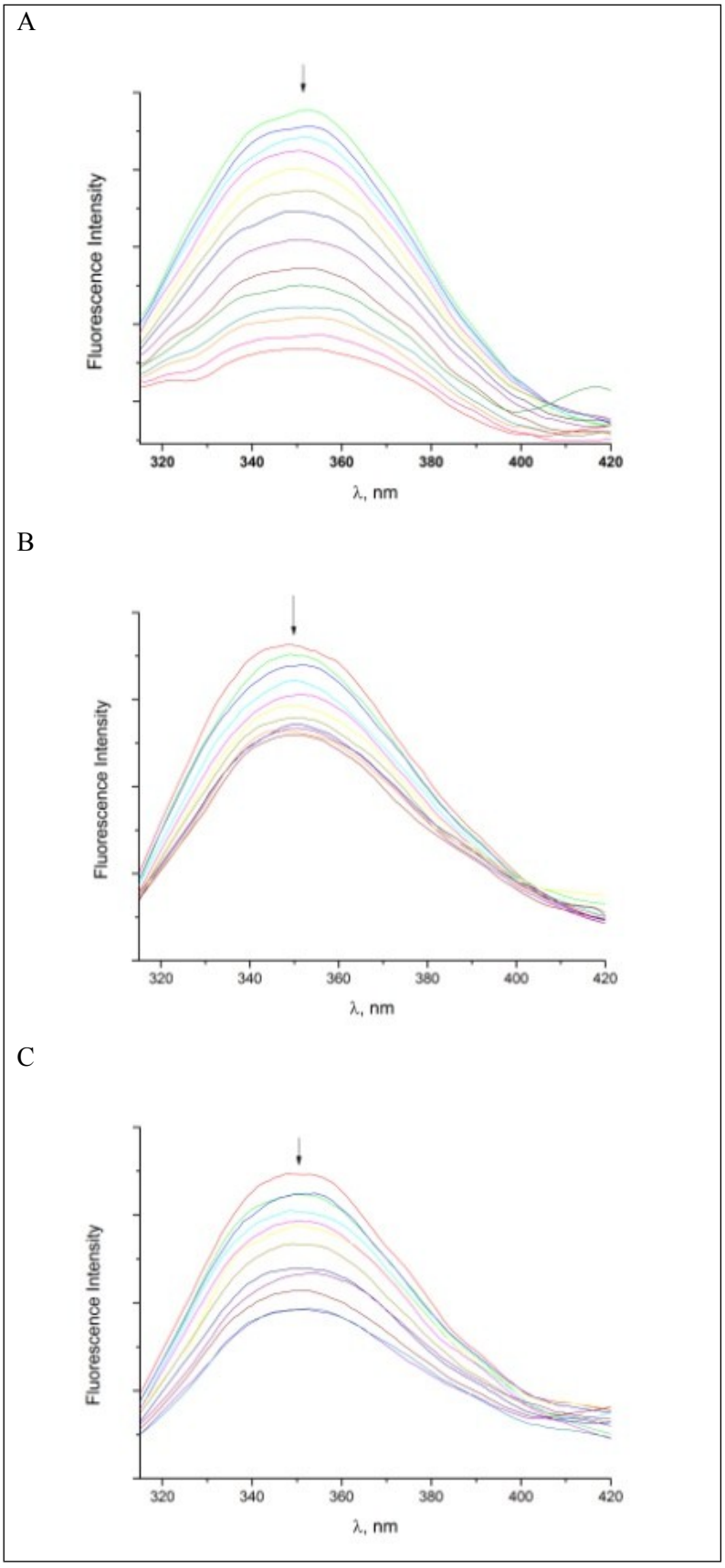


*Fig. S2 Fluorescence spectra of the Na/K-ATPase – gold complex assembly. A) Complex **3** in concentration range from 1 × 10^{-8} to 1.25 × 10^{-5} M, the concentration of Na/K-ATPase is 1 × 10^{-7}M. B) Complex **1** in concentration range from 1 × 10^{-8} to 6.5 × 10^{-6} M, the concentration of Na/K-ATPase is 5 ×10^{-8}M. C) Complex **2** in concentration range from 1 × 10^{-8} to 5.5 × 10^{-6} M, the concentration of Na/K -ATPase is 5 ×10^{-8}M. The solution contains 100 mM NaCl, pH of solution is 7.4. The enzyme is in $E_1(Na^+)_3$ conformation. The fluorescence intensity decreased with the increasing of gold complex concentration in the direction of arrow.*

3. Stern Volmer plot

Stern Volmer plot for fluorescence quenching is desribed by **Eq. S1**:

$$\frac{I_0}{I} = 1 + K_{SV}[Au] = k_q\tau_0[Au] \quad \ldots (\mathbf{S1})$$

where I_0 and I denote the fluorescence intensities in absence and presence of quencher (gold complex) respectively, K_{sv} is the Stern Volmer quenching constant and [Au] is the concentration of the added gold complex. The results presented in **Fig.S3** clearly indicate that the Stern - Volmer plot for all three gold complexes showed downward curves toward the x-axes suggesting in the simplest case, the existence of two distinct fluorophore populations, one of them not being accessible to the quencher.

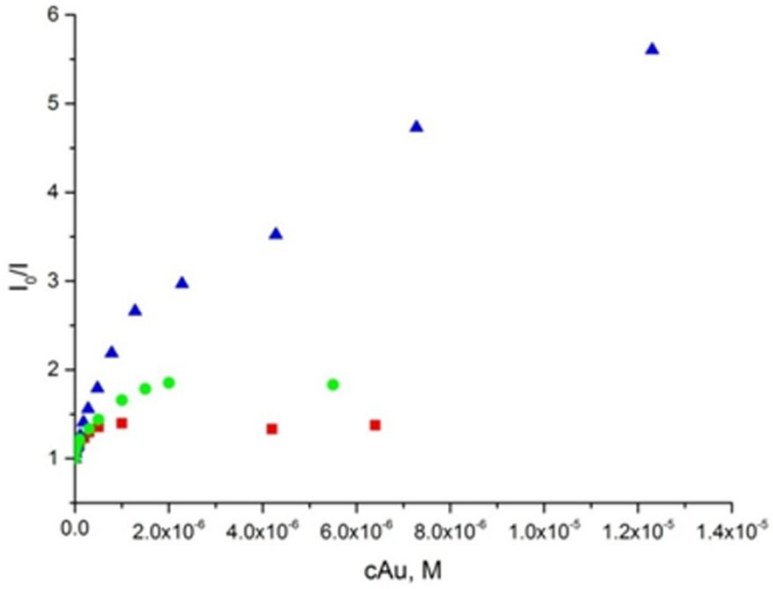


*Fig. S3 Quenching curves of Na/K ATPase in the presence of gold complexes **1**,**2** and **3**.*

4. Determination of free gold(III) complexes concentration from fluorescent measurements

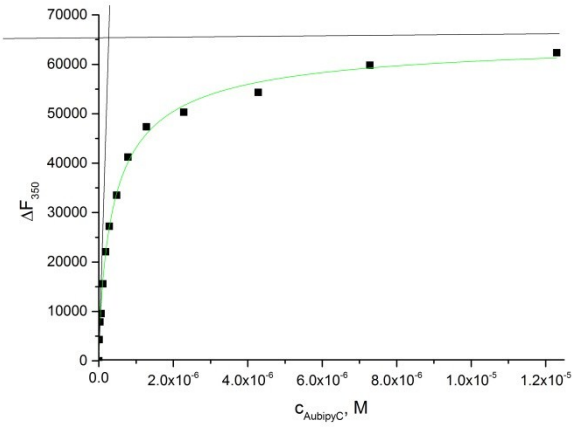


*Fig. S4 Changes ΔF_{350} vs. total added concentration of complex **3***

Concentration of free gold complex in the solution is determined from differences the concentration of total added gold and the concentration of bound gold complex obtained from plot $\Delta F_{350}=fc_{totAu}$

5. CD spectra of Na/K ATPase after addition of gold complexes

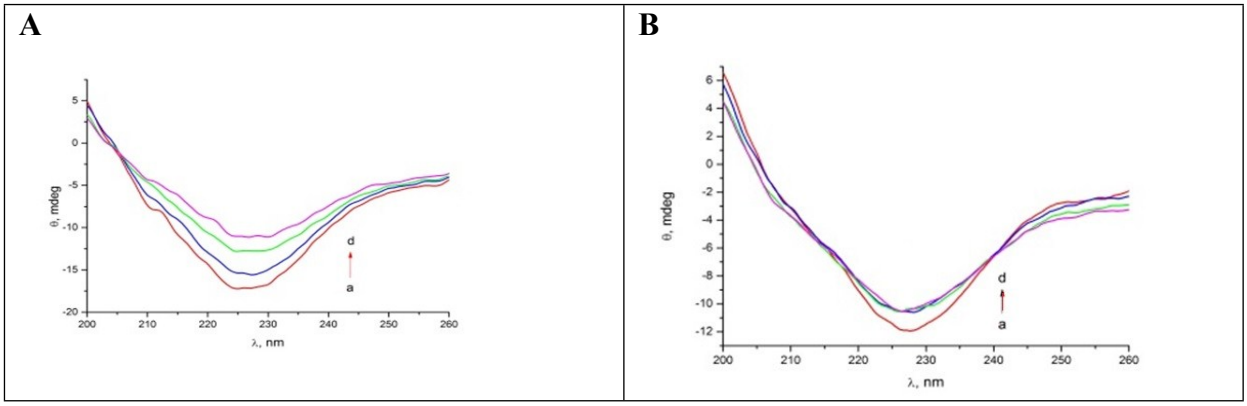
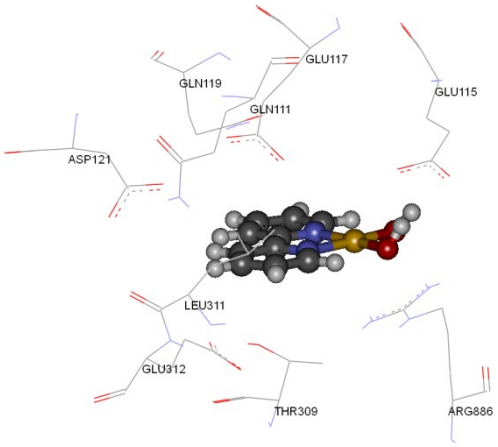
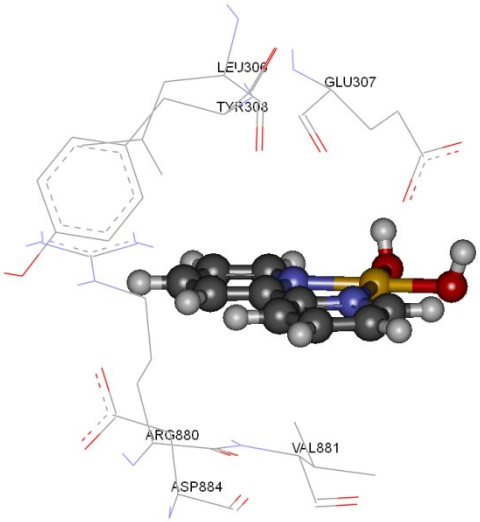


*Fig.S5 CD spectra of 2 × 10^{-6} M Na/K ATPase in absence (a) and presence of increasing concentration of complex **1** (A) and complex **2** (B). The concentration of complexes was 2 ×10^{-6} - 1×10^{-5} M.*

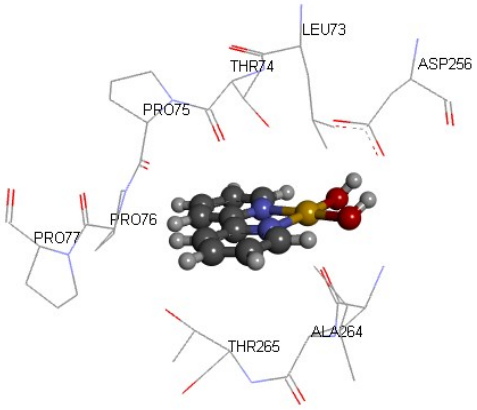
6. Docking studies



Binding site 1

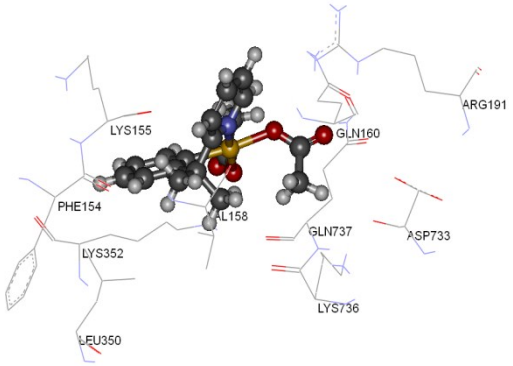


Binding site 2

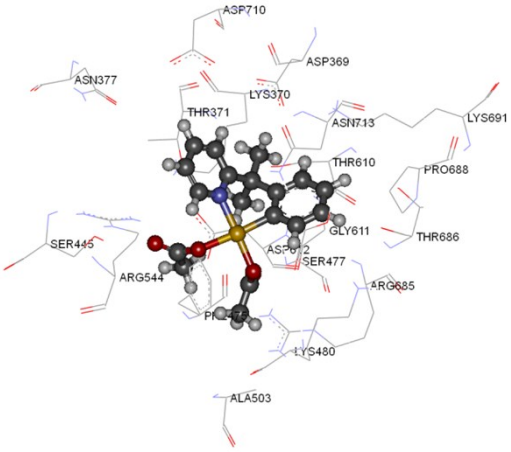


Binding site 3

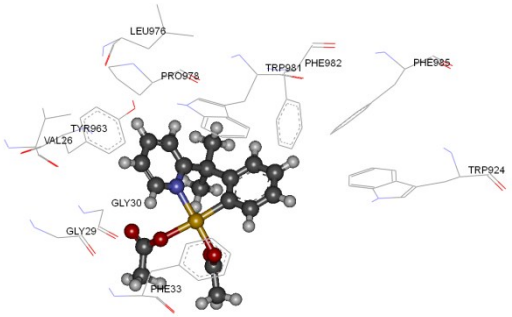
Figure S6. *The illustration of interactions between complex **1** (displayed by ball and stick style) and amino acid residues at binding sites on Na/K-ATPase.*



Binding site 1

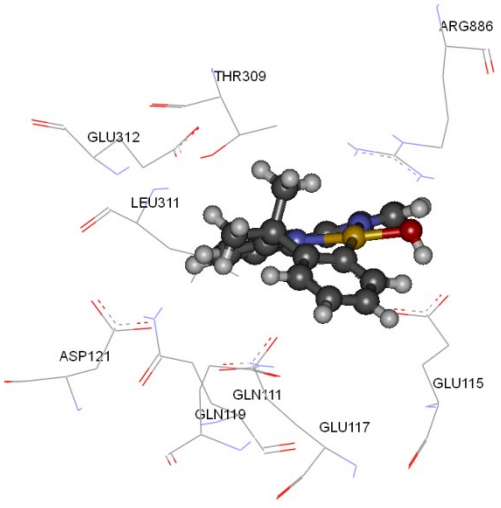


Binding site 2

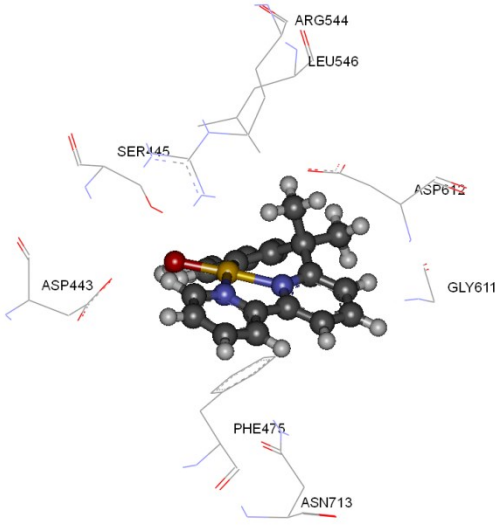


Binding site 3

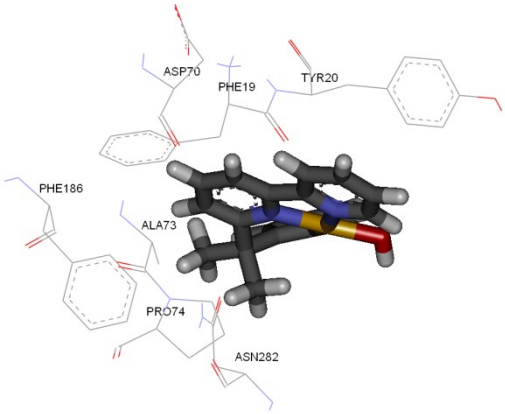
Figure S7. *The illustration of interactions between complex* ***2*** *(displayed by ball and stick style) and amino acid residues at binding sites on Na/K-ATPase.*



Binding site 1



Binding site 2



Binding site 3

Figure S8*. The illustration of interactions between complex* ***3*** *(displayed by ball and stick style) and amino acid residues at binding sites on Na/K-ATPase.*

Table S1. *The distribution of triptophane residues in environment of individual metal complex binding sites (with value of binding energy) defined by distance up to 20 Å.*

Amino acid		**Aubipy(OH)$_2^+$**			**Aupy(OAc)$_2$**			**AubipyC$^+$**			**Σ**
		1	2	3	1	2	3	1	2	3	
	ΔE	-4.80	-4.20	-4.15	-4.60	-3.99	-3.98	-5.75	-5.66	-5.42	
Trp82 (α)		-	-	***	***	-	-	-	-	-	1
Trp98 (α)		***	-	-	-	-	-	***	-	-	2
Trp310 (α)		***	***	-	-	-	-	***	-	-	3
Trp385 (α)		-	-	-	-	***	-	-	***	-	2
Trp411 (α)		-	-	-	-	***	-	-	***	-	2
Trp883 (α)		***	***	-	-	-	***	***	-	***	5
Trp887 (α)		***	***	-	-	-	***	***	-	***	5
Trp899 (α)		***	***	-	-	-	***	***	-	***	5
Trp924 (α)		-	-	-	-	-	-	-	-	-	0
Trp980 (α)		-	-	-	-	-	***	-	-	***	2
Trp981 (α)		-	-	-	-	-	***	-	-	***	2
Trp1009 (α)		-	-	-	-	-	-	-	-	-	0
Trp12 (β)		-	-	-	-	-	-	-	-	-	0
Trp17 (β)		-	-	-	-	-	-	-	-	-	0
Trp32 (β)		-	-	-	-	-	-	-	-	-	0
Trp155 (β)		-	-	-	-	-	***	-	-	***	2
Σ		5	4	1	1	2	6	5	2	6	
Na			**10**			**9**			**13**		
Nb			**6**			**9**			**10**		

To describe an environment of tryptophan residues the program BIOVIA Discovery Studio Visualizerwas used [2], in which there is option to select the all species in area around individual amino acids. We analysed only gold(III) complexes located at a distance shorter than 20 Å in respect to individual tryptophan residue. If the metal complex is located in environment of tryptophan residue the label (***) is used, if not then we used the label (-).

Na is total number of tryptophan residues (with repeating of residues) obtained in environments of the same type Au complex, defined by distance up to 20 Å. **Nb** is total number of tryptophan residues (without repeating of residues) obtained in environments of the same type Au complex, defined by distance up to 20 Å.

References

1. Nyblom, M., et al., *Crystal structure of Na+, K(+)-ATPase in the Na(+)-bound state.* Science, 2013. **342**(6154): p. 123-127.
2. Dassault Systèmes BIOVIA, Discovery Studio Modeling Environment, Release 2017, San Diego: Dassault Systèmes, 2016.