

1           **A tool for computation of changes in Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup> channels and**  
2           **transporters due to apoptosis by data on cell ion and water content**  
3           **alteration**

4  
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10 **Running title:** MONOVALENT IONS IN APOPTOSIS

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13 apoptotic volume decrease  
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## 32 **Abstract**

33

34 The study aims to know how the apoptotic alteration of cell ionic balance follows from the  
35 quantitatively characterised time dependent decrease in the sodium pump rate constant and changes  
36 in permeability coefficients of  $\text{Cl}^-$ ,  $\text{K}^+$ , and  $\text{Na}^+$  channels. New experimental data on changes in cell  
37  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Cl}^-$ , water contents, and the  $\text{Na}^+/\text{K}^+$ -ATPase-mediated  $\text{K}^+$  influx during the first 4 h of the  
38 staurosporine (STS) induced apoptosis are used as a basis for quantitative characterisation of  
39 channels and transporters responsible for apoptotic cell ion balance alteration. New computational  
40 tool is developed. It is found that the dynamics of alteration of ion and water balance in the studied  
41 U937 cells were associated with the decrease in the  $\text{Na}^+/\text{K}^+$ -ATPase rate coefficient by 2.2 times for  
42 4 h, and a time-dependent increase in potassium channel permeability, and a decrease in the  
43 sodium channel permeability, whereas the early decrease in  $[\text{Cl}^-]_i$  and cell volume were associated  
44 with an approximately 5-fold increase in the chloride channel permeability. The developed  
45 approach and the provided executable file can be used to identify the channels and transporters  
46 responsible for alterations of cell ion and water balance not only during apoptosis but in other  
47 physiological scenarios.

48

## 49 **Introduction**

50

51 A characteristic feature of apoptosis, one of the basic genetically encoded cell death mechanisms, in  
52 contrast to accidental death, is that it is not associated with cell swelling or plasma membrane  
53 rupture (Galluzzi et al. 2018). The apoptotic volume decrease (AVD) in cells is a common although  
54 unnecessary hallmark of apoptosis (Maeno et al. 2000, 2006; Okada et al. 2001; Yurinskaya et al.  
55 2005*a, b*; Bortner & Cidlowski, 2007). Cell swelling in apoptosis is prevented by the specific  
56 alteration of the monovalent ion balance in apoptotic cells, as the monovalent ions are major cell  
57 water regulators. It is believed that monovalent ions play an important role in apoptosis (Lang et al.  
58 2005; Lang and Hoffman, 2012; Bortner and Cidlowski, 2014; Hoffmann et al. 2015; Kondratsky et  
59 al. 2015; Jentsch, 2016; Wanitchakool et al. 2016). However, this opinion is based mostly on the  
60 fact that ion channels and transporters are altered somehow during apoptosis and that their  
61 pharmacological or genetic modification has an effect on apoptosis. The mechanism of specific  
62 apoptotic alteration of cell ion and water balance has gotten much less attention than the molecular  
63 identity of channels and transporters involved in apoptosis. The mechanistic studies are hampered  
64 by the interdependence between ion fluxes via the numerous channels and transporters in the  
65 plasma membrane. This difficulty can be overcome by the computational analysis of whole-cell ion  
66 flux balance, which has been developed for normal cells (Jakobsson, 1980; Lew and Bookchin,  
67 1986; Lew et al. 1991; Terashima et al. 2006; Vereninov et al. 2014, 2016). However, no successful  
68 analyses have been done on apoptotic cells. We have studied the relationships between alterations  
69 of the sodium pump or  $\text{K}^+$ ,  $\text{Na}^+$ , and  $\text{Cl}^-$  channels and transporters and the apoptotic alteration of the  
70 entire cell water and ion balance in U937 cells treated with staurosporine (STS) and etoposide  
71 (Yurinskaya et al. 2005*a, b*; Yurinskaya et al. 2011). However, we lacked the necessary  
72 experimental data and a proper programme code for computation of transient processes in cell ion  
73 homeostasis and analysed only apoptotic cells at a single time point, 4 h. Here, we studied ionic  
74 events during apoptosis development from 30 min to 4 h. The background data included  $\text{K}^+$ ,  $\text{Na}^+$ ,  
75  $\text{Cl}^-$ , and water contents and ouabain-sensitive and -resistant  $\text{Rb}^+$  influx in U937 cells that were  
76 induced to undergo apoptosis by STS. An original algorithm of the numerical solution of the cell  
77 monovalent ion flux balance equations and the programme code were developed, which allowed us  
78 to account for the continuous changes in the sodium pump activity. To our knowledge, this is the  
79 first attempt to study the dynamics of the alteration of  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Cl}^-$ , and water contents during  
80 apoptosis. The approach developed to study STS-induced apoptosis in U937 cells may be  
81 recommended for identification of channels and transporters responsible for alteration of cell ion  
82 and water balance in various situations.

83

## 84 **Methods**

85

### 86 **Reagents**

87 RPMI 1640 medium and foetal bovine serum (FBS, HyClone Standard) were purchased from Biolut  
88 (Russia). STS and ouabain were from Sigma-Aldrich (Germany), Percoll was purchased from  
89 Pharmacia (Sweden). The isotope  $^{36}\text{Cl}^-$  was from "Isotope" (Russia). Salts were of analytical grade  
90 and were from Reaches (Russia).

91

### 92 **Cell cultures**

93 U937 human histiocytic lymphoma cells were obtained from the Russian Cell Culture Collection  
94 (Institute of Cytology, Russian Academy of Sciences, cat. number 160B2). The cells were cultured in  
95 RPMI 1640 medium supplemented with 10% FBS at 37 °C and 5% CO<sub>2</sub>. For the induction of  
96 apoptosis, the cells, at a density of  $1 \times 10^6$  cells per ml, were exposed to STS for 0.5-4 h. All the  
97 incubations were done at 37 °C.

98

### 99 **Determination of cell ion and water contents**

100 The experimental methods used in this work have been described in detail earlier (Yurinskaya et al.  
101 2005a, b, 2011; Vereninov et al. 2007, 2008). In summary, the cells were pelleted in RPMI  
102 medium, washed five times with MgCl<sub>2</sub> solution (96 mM) and treated with 5% trichloroacetic acid  
103 (TCA). TCA extracts were analysed for ion content. Intracellular K<sup>+</sup>, Na<sup>+</sup> and Rb<sup>+</sup> contents were  
104 determined by flame emission on a Perkin-Elmer AA 306 spectrophotometer. To determine the  
105 intracellular Cl<sup>-</sup>, the cells were cultured for 90 min or more at 37 °C in RPMI medium containing  
106  $^{36}\text{Cl}^-$  (0.12 μCi ml<sup>-1</sup>). The radioactivity of  $^{36}\text{Cl}^-$  in TCA extracts was measured using a liquid  
107 scintillation counter (Beckman LS 6500). The intracellular Cl<sup>-</sup> content was calculated, taking into  
108 account the specific activity of  $^{36}\text{Cl}^-$  (~2 counts min<sup>-1</sup> μmol<sup>-1</sup>). The TCA precipitates were dissolved  
109 in 0.1 N NaOH and analysed for protein by the Lowry procedure, with serum bovine albumin as a  
110 standard. The cell ion content was calculated in micromoles per gram of protein.

111 Cell water content was determined by measurements of the buoyant density of the cells in  
112 continuous Percoll gradient. Percoll solution was prepared according to the manufacturer's  
113 instructions, and a thick cell suspension (0.1-0.2 ml, ~  $3 \times 10^6$  cells) was placed on the solution surface  
114 and centrifuged for 10 min at  $400 \times g$  (MPW-340 centrifuge, Poland). The buoyant density of the  
115 cells was estimated using density marker beads (Sigma-Aldrich, Germany). The water content per  
116 gram of protein,  $v_{\text{prot}}$ , was calculated as  $v_{\text{prot}} = (1 - r/r_{\text{dry}})/[0.72(r-1)]$ , where  $r$  is the measured buoyant  
117 density of the cells and  $r_{\text{dry}}$  is the cell dry mass density, which was 1.38 g ml<sup>-1</sup>. The proportion of  
118 protein in dry mass was 72%.

119

### 120 **The sodium pump rate coefficient determination**

121 The sodium pump rate coefficient was determined based on the assay of the ouabain-sensitive Rb<sup>+</sup>  
122 influx and cell Na<sup>+</sup> content. The cells were incubated in medium with 2.5 mM RbCl and with or  
123 without 0.1 mM ouabain for 10 min. The rate coefficient of the sodium pump (*beta*) was calculated  
124 as the ratio of the Na<sup>+</sup> pump efflux to the cell Na<sup>+</sup> content given the assumption of the simple linear  
125 dependence of Na<sup>+</sup> efflux on cell Na<sup>+</sup> in the studied range of concentrations. The pump Na<sup>+</sup> efflux  
126 was calculated from ouabain-sensitive (OS) Rb<sup>+</sup> influx assuming proportions of [Rb]<sub>o</sub> and [K]<sub>o</sub> of  
127 2.5 and 5.8 mM, respectively, and Na/K pump flux stoichiometry of 3:2.

128

### 129 **Calculation of the monovalent ion flux balance**

130 The mathematical model of cell ion homeostasis and the algorithm of the numerical solution of the  
131 flux balance were described in detail earlier (Vereninov et al. 2014; 2016). The reader can  
132 reproduce all presented computed data and perform new calculations for various parameters by  
133 using the execution file to programme code BEZ01B (How to use programme code BEZ01B.zip).

134 This software differs from the previous BEZ01 by the additional parameter  $kb$ , which characterizes  
135 a decrease in the pump rate coefficient  $\beta$  with time. Symbols and definitions used are shown in  
136 **Table 1**. The input data used in calculation as file DataB.txt (see BEZ01B.zip) are the following:  
137 extracellular and intracellular concentrations ( $na0$ ,  $k0$ ,  $cl0$  and  $B0$ ;  $na$ ,  $k$  and  $cl$ );  $kv$ ; the pump rate  
138 coefficient ( $\beta$ ); the pump Na/K stoichiometric coefficient ( $\gamma$ ); parameter  $kb$ ; channel permeability  
139 coefficients ( $pna$ ,  $pk$ ,  $pcl$ ); and the rate coefficients for the NC, KC and NKCC cotransporters ( $inc$ ,  
140  $ikc$ ,  $inkcc$ ). The results of our computations appear in the file RESB.txt (**Table 2**) after running the  
141 executable file.

142 The flux equations were:

$$143 \frac{d([Na]_i V)}{dt} = V \{ (p_{Na} u ([Na]_i \exp(u) - [Na]_o) / g - b[Na]_i + J_{NC} + J_{NKCC} ) \}$$

$$144 \frac{d([K]_i V)}{dt} = V \{ (p_K u ([K]_i \exp(u) - [K]_o) / g + \beta[Na]_i / \gamma + J_{NKCC} + J_{KC} ) \}$$

$$145 \frac{d([Cl]_i V)}{dt} = V \{ (p_{Cl} u ([Cl]_o \exp(u) - [Cl]_i) / g + J_{NC} + J_{KC} + 2J_{NKCC} ) \}$$

146 The left-hand sides of these three equations represent the rates of change of cell ion content. The  
147 right-hand sides express fluxes, where  $u$  is the dimensionless membrane potential related to the  
148 absolute values of membrane potential  $U$  (mV), as  $U = uRT/F = 26.7u$  for 37 °C and  $g = 1 - \exp(u)$ .  
149 Transmembrane electrochemical potential differences for  $Na^+$ ,  $K^+$ , and  $Cl^-$  were calculated as:  $\Delta\mu_{Na}$   
150  $= 26.7 \cdot \ln(na/na0) + U$ ,  $\Delta\mu_K = 26.7 \cdot \ln(k/k0) + U$ , and  $\Delta\mu_{Cl} = 26.7 \cdot \ln(cl/cl0) - U$ , respectively. The values of  
151 electrochemical potential differences for  $Na^+$ ,  $K^+$  and  $Cl^-$ , denoted also as  $mun$ ,  $muk$  and  $mucl$ , are  
152 important because they show the driving force and the direction of ion movement via channels and  
153 transporters under the indicated conditions. It is the changes in  $mun$ ,  $muk$  and  $mucl$  that are  
154 responsible for the possible fast effects of the membrane potential (MP) on ion fluxes via  
155 “electroneutral” transporters.

## 156 **Statistical analysis**

157 Data are presented as the mean  $\pm$  SEM.  $P < 0.05$  (Student’s  $t$  test) was considered statistically  
158 significant.  
159

160 **Table 1 Symbols and definitions.**

Definitions	In text and Figures	In files DATA.txt, RES.txt	Units
Ion species	$\text{Na}^+, \text{K}^+, \text{Cl}^-, \text{Rb}^+$	Na, K, Cl	
Types of cotransport		NC, NKCC, KC	
Concentration of ions in cell water or external medium	$[\text{Na}]_i, [\text{K}]_i, [\text{Cl}]_i, [\text{Na}]_o, [\text{K}]_o, [\text{Cl}]_o$	<i>na, k, cl, na0, k0, cl0</i>	mM
External concentrations of membrane-impermeant non-electrolytes such as mannitol introduced in artificial media		<i>B0</i>	mM
Intracellular content of membrane-impermeant osmolytes		<i>A</i>	mmol, may be related to g cell protein or cell number, etc.
Cell water volume		<i>V</i>	ml, may be related to g cell protein or cell number, etc.
Membrane-impermeant osmolyte concentration in cell water		<i>A/V*1000</i>	mM
Cell water content per unit of A		<i>V/A</i>	$\text{ml} \cdot \text{mmol}^{-1}$
Mean valence of membrane-impermeant osmolytes, A	<i>z</i>	<i>z</i>	dimensionless
Permeability coefficients	<i>pNa, pK, pCl</i>	<i>pna, pk, pcl</i>	$\text{min}^{-1}$
Pump rate coefficient	<i><math>\beta</math></i>	<i>beta</i>	$\text{min}^{-1}$
Na/K pump flux stoichiometry	<i><math>\gamma</math></i>	<i>gamma</i>	dimensionless
Membrane potential, MP		<i>U</i>	mV
Dimensionless membrane potential $U = uRT/F$		<i>u</i>	dimensionless
Net fluxes mediated by cotransport	$J_{\text{NC}}, J_{\text{NKCC}}, J_{\text{KC}}$	<i>NC, KC, NKCC</i>	$\mu\text{mol} \cdot \text{min}^{-1} \cdot (\text{ml cell water})^{-1}$
Na efflux via the pump	$-\beta[\text{Na}]_i$	<i>PUMP</i>	$\mu\text{mol} \cdot \text{min}^{-1} \cdot (\text{ml cell water})^{-1}$
K influx via the pump	$\beta[\text{Na}]_i/\gamma$	<i>PUMP</i>	$\mu\text{mol} \cdot \text{min}^{-1} \cdot (\text{ml cell water})^{-1}$
Net fluxes mediated by channels		<i>Channel</i>	$\mu\text{mol} \cdot \text{min}^{-1} \cdot (\text{ml cell water})^{-1}$
Unidirectional influxes of Na, K or Cl via channels or cotransport		<i>IChannel, INC, IKC, INKCC</i>	$\mu\text{mol} \cdot \text{min}^{-1} \cdot (\text{ml cell water})^{-1}$
Unidirectional effluxes of Na, K, or Cl via channels, or cotransport		<i>EChannel, ENC, EKC, ENKCC,</i>	$\mu\text{mol} \cdot \text{min}^{-1} \cdot (\text{ml cell water})^{-1}$
Time derivatives of concentrations		<i>prna, prk, prcl</i>	$\text{mM} \cdot \text{min}^{-1}$
Cotransport rate coefficients		<i>inc, ikc inkcc</i>	$\text{ml} \cdot \mu\text{mol}^{-1} \cdot \text{min}^{-1}$ $\text{ml}^3 \cdot \mu\text{mol}^{-3} \cdot \text{min}^{-1}$
Ratio of “new” to “old” media osmolarity when the external osmolarity is changed		<i>kv</i>	dimensionless
Number of time points between output of results		<i>hp</i>	dimensionless
Transmembrane electrochemical potential difference for $\text{Na}^+, \text{K}^+$ , or $\text{Cl}^-$	$\Delta\mu_{\text{Na}}, \Delta\mu_{\text{K}}, \Delta\mu_{\text{Cl}}$	<i>mun, muk, mucl</i>	mV
Ratio of ouabain-sensitive to ouabain-resistant $\text{Rb}^+$ ( $\text{K}^+$ ) influx	OSOR	<i>OSOR</i>	dimensionless
Parameter $\beta$ decreases linearly with time with coefficient		<i>kb</i>	$\text{min}^{-1}$

161 **Table 2. Results of computation. Transition of the system to the balanced state as displayed in**  
 162 **the file RESB.txt.** The values similar to those for U937 cells with a rather high  $U$  and  $mucl$  were  
 163 chosen for this example of a transition to the balanced state. The displayed values of fluxes as well  
 164 as  $OSOR$  correspond to the latest time point. The values of fluxes for other moments can be  
 165 obtained by setting the necessary time interval with the  $hp$  value. The presented flux data do not  
 166 include the fluxes involved in one-for-one exchange because they have no effect on cell ion or  
 167 water content or MP and can be ignored here. The flux data clearly demonstrate how the net fluxes  
 168 via different channels and transporters compensate for each other and come finally, under  
 169 appropriate conditions, to a fully balanced ion distribution when the balance of influx and efflux is  
 170 achieved for all ion species and  $prna$ ,  $prk$ ,  $prcl$  tend to zero.

171 (a) Time course of variables.

$t$	$U$	$na$	$k$	$cl$	$V/A$	$mun$	$muk$	$mucl$	$prna$	$prk$	$prcl$
0	-44.3	33.0	152.0	45.0	12.50	-82.8	42.9	19.0	0.00000	0.00000	0.00000
24	-44.5	36.1	148.9	45.2	12.52	-80.7	42.2	19.3	0.07612	-0.07696	0.00308
**											
216	-44.7	38.0	147.0	45.1	12.51	-79.5	41.7	19.4	0.00007	0.00004	-0.00040
240	-44.7	38.0	147.0	45.1	12.51	-79.5	41.7	19.4	0.00004	0.00005	-0.00032

172 \*\* Time points not shown.

173

174 (b) Parameter values (copy of the file DATAB.txt).

$na0$	$k0$	$cl0$	$B0$	$kv$	$beta$	$gamma$	$z$	$kb$
140	5.8	116	48.2	1.0	0.039	1.5	-1.75	0
$pna$	$pk$	$pl$	$inc$	$ikc$	$inkcc$	$A/V*1000$	$hp$	
0.00382	0.022	0.0091	3E-5	0	0	80.0	240	

175

176 (c) Flux balance under the balanced state.

177

Net flux	PUMP	Channel	NC	KC	NKCC
Na	-1.4811	1.0451	0.4359	0.0000	0.0000
K	0.9874	-0.9878	0.0000	0.0000	0.0000
Cl	0.0000	-0.4363	0.4359	0.0000	0.0000
Influx	PUMP	IChannel	INC	IKC	INKCC
Na	0.0000	1.1012	0.4872	0.0000	0.0000
K	0.9874	0.2627	0.0000	0.0000	0.0000
Cl	0.0000	0.4082	0.4872	0.0000	0.0000
Efflux	PUMP	EChannel	ENC	EKC	ENKCC
Na	-1.4811	-0.0561	-0.0513	0.0000	0.0000
K	0.0000	-1.2505	0.0000	0.0000	0.0000
Cl	0.0000	-0.8445	-0.0513	0.0000	0.0000
OSOR	3.76				



## 178 **Results**

179

### 180 **Computational approach to solution of the problem how the entire cell ion and water balance** 181 **depends on the state of various channels and transporters**

182 The first of the two main aims of the present study is the demonstration of the computational  
183 approach to solution of the problem how the entire cell ion and water balance depends on the  
184 parameters of various channels and transporters. The second aim is the analysis of the ion and water  
185 balance changes during apoptosis in real U937 cells. This aim is an example of using the developed  
186 approach. Some background points should be considered first. The basic mathematical model used  
187 in our approach is similar to the known model developed by pioneers for analysis of ion  
188 homeostasis in normal cells (Jakobsson, 1980; Lew & Bookchin, 1986; Lew et al. 1991). Our  
189 algorithm of the numerical solution of the flux equations and basic software were published earlier  
190 (Vereninov et al. 2014; 2016). Some minor differences in mathematical models used by previous  
191 authors consist in the number of transporters included in the calculations. Only the sodium pump  
192 and electroconductive channels were considered in the early computational studies of cell ion  
193 balance. Lew and colleagues were the first who found that the sodium pump and electroconductive  
194 channels cannot explain monovalent ion flux balance in human reticulocytes because they cannot  
195 explain the nonequilibrium  $\text{Cl}^-$  distribution under the balanced state without NC (Lew et al. 1991).  
196 Cotransporters NC and KC were investigated by Hernández and Cristina (1998). The NKCC  
197 cotransport was included in ion balance modelling in cardiomyocytes (Terashima et al. 2006). Our  
198 software accounts for  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  channels, the sodium pump and the NC, KC and NKCC  
199 cotransporters. We found that NC is necessary as a rule in the calculation of the resting monovalent  
200 ion flux balance in U937 cells, while NKCC and KC are not. Nevertheless, the parameters  
201 characterizing these two transporters are present in our code, and fluxes via transporters can be  
202 accounted for if these parameters differ from zero.

203 Two points may worry experimentalists. First, the sodium pump activity is characterized by a  
204 single rate coefficient. However, a set of ion binding sites are known in the pump, and its operation  
205 kinetics in biochemical studies is described commonly by more than one parameter. The single rate  
206 coefficient is used because the evaluation of the properties of all the ion binding sites of the pump in  
207 experiments in whole cells is infeasible and because it appears to be quite sufficient for the  
208 calculation of entire-cell ion homeostasis. This idea was demonstrated by the quantitative prediction  
209 of the dynamics of monovalent ion redistribution after stopping the sodium pump (Vereninov et al.  
210 2014, 2016). Single rate coefficients for characterizing the ion carriage kinetics via transporters are  
211 commonly used for the same reason. The second point causing disapproval might be that an integral  
212 permeability coefficient is used in the calculation of the flux balance for all  $\text{Na}^+$  or  $\text{K}^+$  or  $\text{Cl}^-$   
213 channels, whereas a great variety of channels for each ion species is located in the plasma  
214 membrane. The single permeability coefficients are commonly used in analysis of the entire-cell  
215 flux balance because in an analysis of such a complex system with many channels and transporters,  
216 the matter of primary importance is to understand whether ion flux changes due to alteration of the  
217 force driving the ions or by properties of the channel per se.

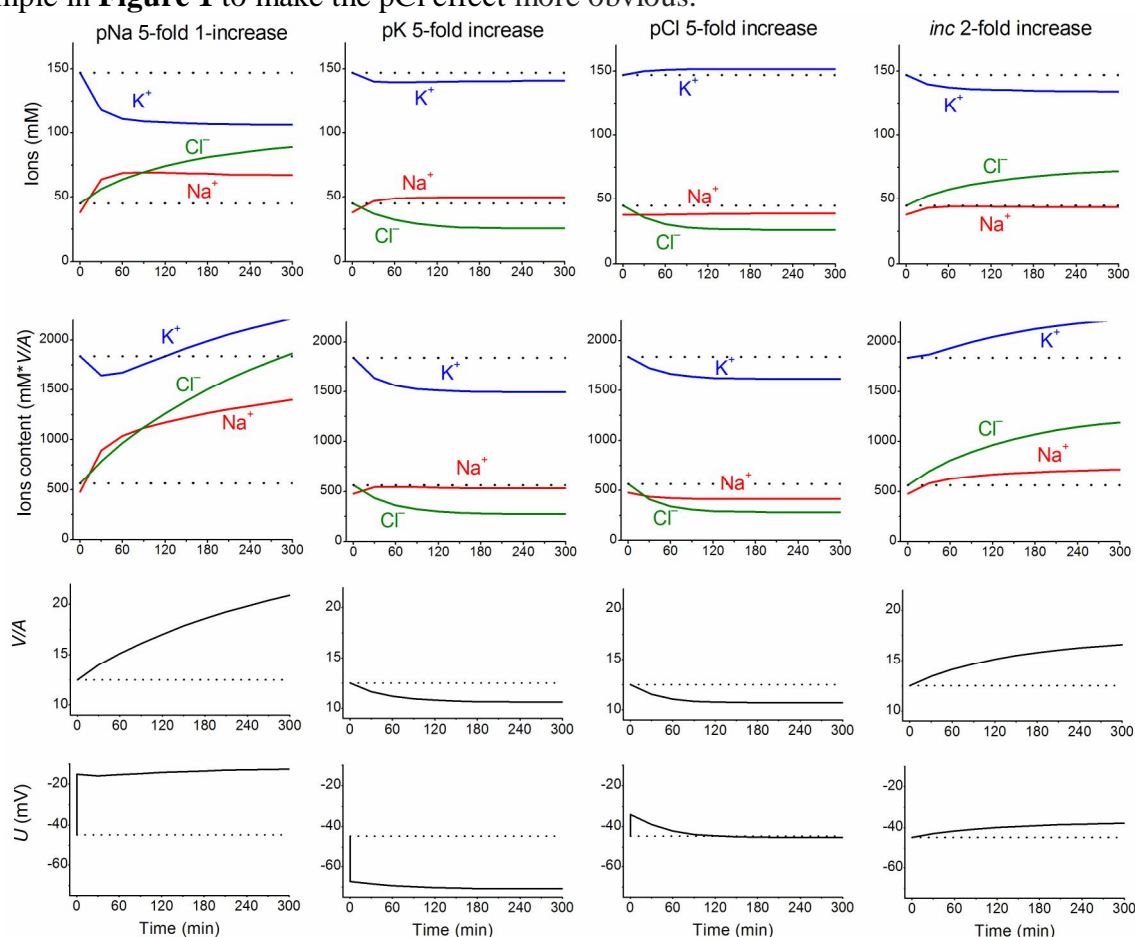
218

### 219 **Computation of ion flux balance in cells similar to U937 cells**

220 Parameters in absolute units are used in our calculations. Their initial, “standard”, values are  
221 obtained from the calculation based on the distribution of monovalent ions and ouabain-sensitive  
222  $\text{Rb}^+(\text{K}^+)$  influx measured in cells under normal physiological conditions and the cell balanced state.  
223 The parameters varied until the tested values give a calculated entire ion and water homeostasis  
224 similar to that in real cells. The “standard” parameters can vary in real cells depending on the cell  
225 physiological state, the age of the culture, the conditions of cell cultivation, etc.

226 Nevertheless, these parameters can remain invariant under a varying environment. We found  
227 that the kinetics of the disturbance of cell ion and water balance caused by blocking the sodium  
228 pump when the intracellular  $\text{K}^+/\text{Na}^+$  ratio is highly changed and even reversed can be predicted

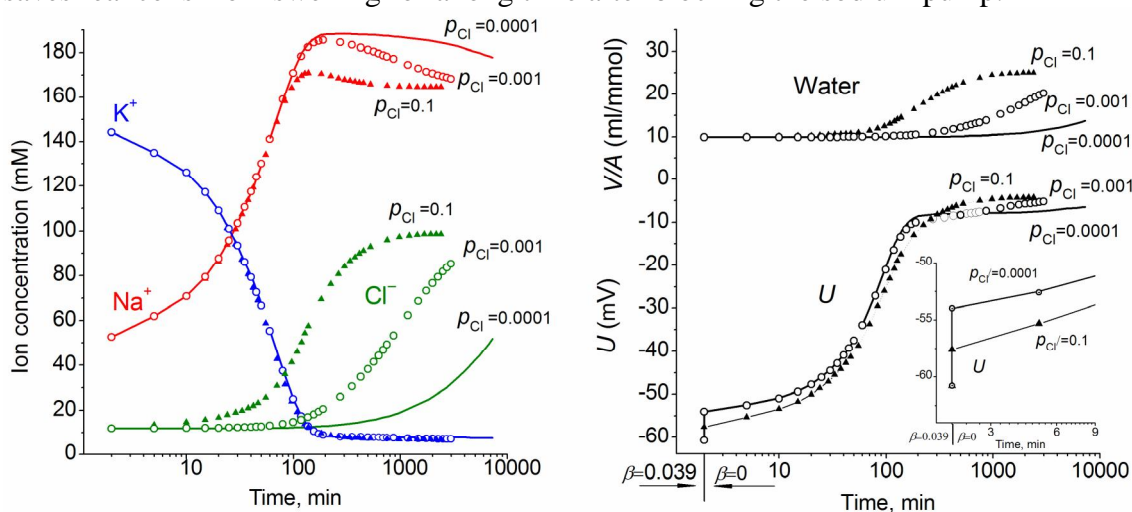
229 sufficiently well by calculation with the invariant parameters (Vereninov et al. 2014; 2016). A set of  
 230 examples is presented in **Figure 1** to show how changes in a single channel or transporter species  
 231 (one permeability coefficient or rate constant) can alter the intracellular concentrations of all major  
 232 ions, cell water content and the MP. Unlike pNa and *inc*, changes in pK or pCl lead, over the course  
 233 of 60-100 min, to a new balanced state. Even this small set of examples demonstrates that some  
 234 effects seem to be unexpected at first sight. Intracellular  $K^+$  concentration decreases monotonically  
 235 with the pNa increase, while the intracellular  $K^+$  content decreases initially and increases further  
 236 due to superposition of the initial drop MP and the slow increase in cell water-volume. An increase  
 237 in the coupled equivalent transport  $Na^+$  and  $Cl^-$  (*inc*) causes a decrease in cell  $K^+$  concentration,  
 238  $[K^+]$ , and, in contrast, an increase in cell  $K^+$  content because of changes in cell water volume and in  
 239 MP. The  $[K^+]$  and MP are shifted in this case in opposite directions. It should be stressed that the  
 240 effects of parameter variation are highly dependent on the cell species. Our previous paper  
 241 presented the typical dependences for the cells with high MP and high intracellular K/Na ratio, for  
 242 the cells with low MP and high K/Na ratio (high potassium erythrocytes) and for the low-MP and  
 243 low-K/Na-ratio cells (low-potassium erythrocytes of some carnivores and ruminants) (Vereninov et  
 244 al., 2014). Cells such as U937 and their variant with relatively high *mucl* (19.4 mV) are chosen as  
 245 an example in **Figure 1** to make the pCl effect more obvious.



246 **Figure 1. The calculated effects of an abrupt increase in the permeability coefficients of  $K^+$ ,**  
 247  **$Na^+$ ,  $Cl^-$  channels, or the NC cotransport rate coefficient on cell  $K^+$ ,  $Na^+$ , and  $Cl^-$  content and**  
 248 **concentrations, water-volume ( $V/A$ ) and MP ( $U$ ). The data were calculated by using the software**  
 249 **BEZ01B. The initial parameters were  $na0$  140,  $k0$  5.8,  $cl0$  116,  $B0$  48.2,  $kv$  1,  $beta$  0.039,  $gamma$**   
 250 **1.5,  $pna$ , 0.00382,  $pk$  0.022,  $pcl$  0.0091,  $inc$  0.00003,  $ikc = inkcc = 0$ ,  $kb$  0, and  $hp$  300, i.e., much**  
 251 **like U937 cells; the changed parameters are shown on the plots.**  
 252  
 253  
 254



255 The ion and water redistribution caused in U937 cells by stopping the sodium pump was  
 256 studied earlier in silico and in an experiment (Vereninov et al. 2014; 2016). Here, it is interesting to  
 257 demonstrate this case as an example of asynchrony in changes of  $K^+$ ,  $Na^+$  and  $Cl^-$  after blocking the  
 258 pump (**Figure 2**). In the earlier stage, the electroneutrality of the net ion fluxes is achieved mainly  
 259 by the balance of fluxes  $K^+$  outward and  $Na^+$  inward via channels, whereas, later, the  $Cl^-$  influx  
 260 becomes significant. There is no alteration of total intracellular osmolytes during the equal  $K^+/Na^+$   
 261 exchange, and it is for this reason that no swelling occurs after blocking the pump for a rather long  
 262 time. It should be stressed that no specific carrier is responsible for the balanced  $K^+/Na^+$  exchange.  
 263 This result is realized via electroconductive channels only due to the dependence of fluxes on MP.  
 264 The long-term balanced state in monovalent ions and water distribution after stopping the pump is  
 265 unattainable, and cell swelling will go on infinitely. However, the kinetics of the entire process may  
 266 be different in dependence on the  $p_{Cl}$  level. It should be noted that cell water content and  
 267 intracellular concentration are changing synchronously. It is the low  $Cl^-$  channel permeability that  
 268 saves real cells from swelling for a long time after blocking the sodium pump.



269 **Figure 2. The effect of  $p_{Cl}$  on the time course of the ion and water balance disturbance caused**  
 270 **by turning off the pump.** The data were calculated by using the software BEZ01B with the  
 271 following parameters:  $na0$  140,  $k0$  5.8,  $cl0$  116,  $B0$  48.2,  $kv$  1,  $na$  52.6,  $k$  144.2,  $cl$  11.9,  $beta$  0 (at  
 272 the initial balanced value of 0.039),  $gamma$  1.5,  $pna$  0.006,  $pk$  0.06,  $p_{Cl}$  0.1 (triangles) or 0.001  
 273 (circles) or 0.0001 (solid lines),  $inc = ikc = inkcc = 0$ ,  $kb$  0.

### 274 275 276 277 **Changes in $K^+$ , $Na^+$ , $Cl^-$ and water contents during early apoptosis in U937 cells induced by** 278 **STS**

279 Most data on the redistribution of monovalent ions during apoptosis relates to the 4-5 h stage (see  
 280 references in Arrebola et al. 2005a). Our simultaneous determination of  $K^+$ ,  $Na^+$ ,  $Cl^-$  and water  
 281 contents in U937 cells treated with STS for 4 h was published earlier (Yurinskaya et al. 2011). The  
 282 data related to this apoptosis stage confirmed the osmotic mechanism of AVD, i.e., they showed  
 283 that the water loss was caused mostly by the loss of the total monovalent ion content and much less  
 284 by a decrease in content of the “impermeant intracellular anions”,  $A^-$ . The initial changes in all  
 285 major monovalent ions and water content during apoptosis have been studied much less, although  
 286 the early cell shrinkage is supposed to be crucial for triggering apoptosis. Our current data on the  
 287 changes in ion and water content during STS apoptosis in U937 cells with the earliest time point 30  
 288 min are presented in **Table 3**. The values of the independently determined ion content and water  
 289 content correspond to the osmotic mechanism of AVD at the early stages as well as at the 4 h stage  
 290 studied before. A decrease in cell water fits a decrease in the total content of intracellular osmolytes.  
 291 The data on water content in Table 3 were obtained by the best method, i.e., by cell buoyant  
 292 density. These data agree well with the data obtained by using a Coulter counter and flow cytometer

293 (Yurinskaya et al. 2017). Calculation of the changes in  $K^+$ ,  $Na^+$ , and  $Cl^-$  net fluxes underlying the  
 294 changes in cell ion and water content shows that for the first hour, the  $K^+$  loss is electrically  
 295 balanced predominantly by the  $Cl^-$  loss, whereas later it is mostly balanced by the  $Na^+$  gain (Table  
 296 | 4, last columns).

297

298 **Table 3. Changes in  $K^+$ ,  $Na^+$ ,  $Cl^-$  and water contents during the early stages of STS apoptosis**  
 299 **in U937 cells.** Means  $\pm$  SEM from 3 independent experiments with duplicate determinations.

300

Time	$K^+$	$Na^+$	$Cl^-$	$A^-$	Water
min	$\mu\text{Eqv}\cdot(\text{g prot.})^{-1}$				ml/g
0	$712 \pm 22$	$192 \pm 8$	$246 \pm 11$	658	$6.08 \pm 0.08$
30	$615 \pm 12$	$175 \pm 10$	$133 \pm 4$	657	$5.37 \pm 0.21$
120	$595 \pm 13$	$179 \pm 4$	$109 \pm 5$	665	$4.70 \pm 0.05$
240	$493 \pm 21$	$261 \pm 5$	$117 \pm 4$	637	$4.85 \pm 0.08$

301

302 The apoptotic changes in ion content obtained in our study by flame photometry and  
 303 radiotracer assay are very close to the data obtained by the X-ray microanalysis in U937 cells during  
 304 several types of apoptosis, including early STS apoptosis (Arrebola et al. 2005b, 2006). Unlike that  
 305 X-ray microanalysis study, we could more easily validate changes in cell water content during  
 306 apoptosis and therefore better estimate ion concentrations per cell water volume. This approach  
 307 enabled us to calculate the entire cell electrochemical model and, in this way, to identify channels  
 308 and transporters critical for AVD.

309

310

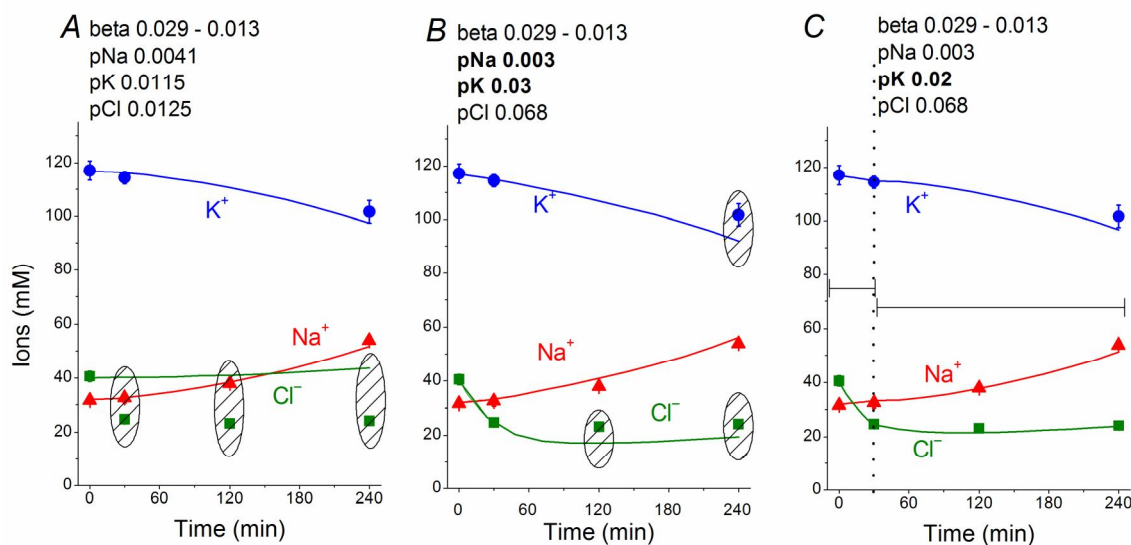
### 311 **Matching the real and calculated changes in cell $K^+$ , $Na^+$ and $Cl^-$ concentrations during** 312 **apoptosis**

313 The real changes in  $Na^+$ ,  $K^+$ ,  $Cl^-$  and water contents during STS apoptosis in U937 cells differ from  
 314 the calculated example presented in **Figure 1**. Evidently, other changes in rate parameters during  
 315 the transient process can occur in real cells. Indeed, a decrease in the sodium pump activity is a  
 316 peculiar feature of apoptosis and has been revealed without any computation, particularly in U937  
 317 cells treated with STS (Arrebola et al. 2005a, b; Vereninov et al. 2008; Yurinskaya et al. 2010,  
 318 2011). The rate coefficient of the sodium pump can be validated by OS  $Rb^+$  influx and intracellular  
 319  $Na^+$  content (Vereninov et al. 2014, 2016). We found that the OS  $Rb^+$  influx for the first 4 h of STS  
 320 apoptosis in U937 cells decreased mostly linearly (Yurinskaya et al. 2010).

321

322 The linear decrease in the sodium pump rate coefficient with time was accounted for in the  
 323 current programme code BEZ01B. **Figure 3A** shows the transient process during STS apoptosis in  
 324 U937 cells calculated under the assumption that the sodium pump rate coefficient decreases linearly  
 325 due to the decrease in the coefficient  $kb$ , as found by OS- $Rb^+$  influx assay in experiment. The values  
 326 calculated for this simplest model (lines) correspond approximately to the real ion concentrations  
 327 (symbols) for  $K^+$  (circles) and  $Na^+$  (triangles) but differ significantly for  $Cl^-$  (squares). The  
 328 additional assumption that triggering apoptosis is accompanied by stepwise increases in  $pcl$  and  $pk$   
 329 and by a slight decrease in  $pna$  improves the agreement between calculated and real values for  $Cl^-$   
 330 in the first 30-40 min, but not later (**Figure 3B**). A change in  $pCl$  alone is ineffective because of the  
 331 small  $\Delta\mu_{Cl}$ . The agreement may be obtained for the whole 4 h time interval by assuming that  $pk$   
 332 further decreases (**Figure 3C**). How unique is the found fitting? By trial and error, we found that a  
 333  $pna$  decrease and a  $pk$  increase alone without a  $pcl$  increase could be sufficient to get agreement

333 between real and calculated chloride concentrations for the first 30 min. However, this case should  
 334 be rejected because the value OSOR becomes unacceptably low.



335  
 336 **Figure 3. Time course of [K<sup>+</sup>], [Na<sup>+</sup>] and [Cl<sup>-</sup>] in real U937 cells treated with 1 μM STS**  
 337 **(symbols) and calculated (lines) for different parameter datasets.** Symbols – experimental data,  
 338 means ± SEM from 3 independent experiments with duplicate determinations. Small SEM values  
 339 are masked by symbols. Lines – calculated data obtained for the parameters indicated on the graphs.  
 340 The initial parameters were *na0* 140, *k0* 5.8, *cl0* 116, *B0* 48.2, *kv* 1, *na* 32, *k* 117, *cl* 40, *beta* 0.029,  
 341 *gamma* 1.5, *pna* 0.0041, *pk* 0.0115, *pcl* 0.0125, *inc* 0.000003, *ikc* = *inkcc* = 0, and *kb* 0.000068. The  
 342 changed parameters are shown in the layers head. (A) Linear decrease in *beta* only. (B) Decrease  
 343 in *beta* and changes in *pna*, *pk*, and *pcl*. (C) Additional decrease in *pk*. Shaded regions show  
 344 significant disagreement of experimental and predicted values. The calculated data were obtained  
 345 by using code BEZ01B.

346  
 347 The joint effect of pK, pNa and pCl shift is interesting. The cells shown in **Figure 3** had  
 348 initially a rather low *U* (−29.9 mV) and a *mucl* of approximately 1.5 mV under the normal state  
 349 (**Table 4**). The variation of pCl alone at so small a *mucl* has no significant effect. A decrease in pNa  
 350 hyperpolarizes cells promptly, and an increase in pK alone hyperpolarizes cells as well (**Table 5**).  
 351 As a result, the pCl increase becomes effective and sufficient to get both the necessary agreement  
 352 between real and calculated chloride concentrations for the initial 30-40 min and the necessary  
 353 OSOR.

354 The *inc* and pCl parameters change cell water and [Cl]<sub>i</sub> in opposite directions (**Figure 1**).  
 355 However, we could not replace the pCl increase with the *inc* decrease in our fitting procedure, as  
 356 the [Cl]<sub>i</sub> decrease in the latter case is too small. We came to conclusion that an increase in pCl is a  
 357 critical factor for the complex water and ion rearrangement at the initial stage of STS apoptosis in  
 358 U937 cells, whereas its role becomes less significant or even non-significant later.

359 We conclude that the redistribution of K<sup>+</sup>, Na<sup>+</sup>, and Cl<sup>-</sup> underlying AVD in the studied U937  
 360 cells treated with STS is caused (1) by a progressive linear decrease in the sodium pump rate  
 361 coefficient from the initial 0.029 to 0.013 at 4 h, (2) by a significant increase in pCl (0.0125 to  
 362 0.068) and changes in pK (0.0115 to 0.03 and later 0.02), and (3) by a moderate decrease in pNa  
 363 (0.0041 to 0.003). The most critical factors for changes in cell K<sup>+</sup> and Na<sup>+</sup> are suppression of the  
 364 pump, an increase in pK and a decrease in pNa, whereas the early decreases in Cl<sup>-</sup> and water  
 365 content (early AVD) are associated primarily with an increase in pCl by approximately 5 times and  
 366 an increase in pK by approximately 2.6 times.  
 367

368 **Table 4. Changes in cell  $[K^+]$ ,  $[Na^+]$ ,  $[Cl^-]$ , MP (U) and net fluxes calculated upon a linear**  
 369 **decrease in the pump rate coefficient and stepwise changes in the  $K^+$ ,  $Na^+$  and  $Cl^-$  channel**  
 370 **permeability corresponding to the experimental data on apoptotic alteration of  $K^+$ ,  $Na^+$ , and**  
 371  **$Cl^-$  concentrations and pump fluxes.** The initial parameters were  $na0$  140,  $k0$  5.8,  $cl0$  116,  $B0$   
 372 48.2,  $kv$  1,  $na$  32,  $k$  117,  $cl$  40,  $gamma$  1.5,  $inc$  0.000003, and  $kb$  0.000068. The changed parameters,  
 373 including  $beta$ ,  $pna$ ,  $pk$ , and  $pcl$ , are shown in the Table. \*\* Time points not shown. The data were  
 374 obtained by using the code BEZ01B. The time of channel alteration is indicated by horizontal lines.  
 375 Outward net fluxes are defined as negative.  
 376

Time, min	$beta$	$pk$	$pna$	$pcl$	$U$	$na$	$k$	$cl$	$V/A$	$mun$	$muk$	$mucl$	Net fluxes		
													$Na^+$	$K^+$	$Cl^-$
Before	0.029	0.0115	0.0041	0.0125	-29.9	32	117	40	8.26	-69.3	50.3	1.5	0	0	0
0	0.029	0.03	0.0041	0.0125	-34.6	32.0	117	40.0	8.26	-74.0	45.6	6.2	0	0	0
10	0.028				-38.5	32.3	116.4	33.5	7.82	-77.7	41.6	5.3	-0.119	-0.622	-0.733
20	0.028				-42.0	32.7	115.7	28.4	7.51	-80.8	37.9	4.4	-0.065	-0.481	-0.540
30	0.027				-45.0	33.3	114.9	24.6	7.29	-83.3	34.8	3.5	-0.020	-0.366	-0.381
60	0.025	0.02	0.003	0.068	-44.8	33.8	114.3	22.3	7.16	-82.8	34.8	0.8	0.031	-0.079	-0.048
120	0.021				-45.1	37.7	110.3	21.7	7.13	-80.1	33.5	0.3	0.086	-0.078	0.008
**															
210	0.015				-43.2	47.2	100.9	23.2	7.21	-72.2	33.1	0.1	0.139	-0.110	0.029
240	0.013				-42.2	51.4	96.7	24.0	7.25	-68.9	33.0	0.1	0.163	-0.127	0.036

377

378 The calculated MP in the considered model of apoptosis was slightly hyperpolarizing (by 12  
 379 mV). Our preliminary results from flow cytometry using DiBAC<sub>4</sub>(3) did not show a significant  
 380 change in MP under STS-induced apoptosis (unpublished data). These results differ from previous  
 381 reports of MP depolarization during apoptosis, e.g., in the Fas-L induced apoptosis of Jurkat cells  
 382 (Franco et al. 2006). Further studies are required to determine whether MP changes are highly  
 383 dependent on the apoptosis inducer and/or on the cell species or whether cell depolarization occurs  
 384 in more severe apoptosis.

385 **Table 5. The effects of pK, pNa and pCl shifts at the initial stage of apoptosis on *U*, *mucl* and**  
 386 **ion concentrations.** The initial parameters were as follows: *na0* 140, *k0* 5.8, *cl0* 116, *B0* 48.2, *kv* 1,  
 387 *beta* 0.029, *na* 32, *k* 117, *cl* 40, *gamma* 1.5, *inc* 0.000003, *kb* 0.  
 388

<i>t</i>	<i>pk</i>	<i>pna</i>	<i>pcl</i>	<i>U</i>	<i>na</i>	<i>k</i>	<i>cl</i>	V/A	<i>mun</i>	<i>muk</i>	<i>mucl</i>
<b>pK shift</b>											
initial	0.0115	0.0041	0.0125	-29.9	32.0	117.0	40.0	8.26	-69.3	50.3	1.5
0				<b>-41.1</b>	32.0	117.0	40.0	8.26	-80.6	39.1	<b>12.7</b>
15				-42.1	35.1	113.7	36.6	8.03	-79.0	37.4	11.3
30	<b>0.03</b>	0.0041	0.0125	-42.9	37.2	111.5	33.8	7.83	-78.3	36.0	10.0
45				-43.6	38.6	110.0	31.5	7.69	-78.0	34.9	8.8
60				-44.2	39.2	109.0	29.5	7.57	-78.0	34.1	7.7
<b>pNa shift</b>											
initial	0.0115	0.0041	0.0125	-29.9	32.0	117.0	40.0	8.26	-69.3	50.3	1.5
0				<b>-36.6</b>	32.0	117.0	40.0	8.26	-76.0	43.6	<b>8.2</b>
15				-37.2	30.6	118.3	38.0	8.12	-77.8	43.3	7.4
30	0.0115	<b>0.003</b>	0.0125	-37.8	29.7	119.1	36.2	8.00	-79.2	42.8	6.7
45				-38.4	29.2	119.5	34.6	7.90	-80.2	42.4	6.1
60				-38.9	28.9	119.7	33.3	7.81	-81.0	41.9	5.6
<b>pK and pNa shift</b>											
initial	0.0115	0.0041	0.0125	-29.9	32.0	117.0	40.0	8.26	-69.3	50.3	1.5
0				<b>-44.8</b>	32.0	117.0	40.0	8.26	-84.2	35.4	<b>16.4</b>
15				-46.3	32.5	116.3	35.6	7.96	-85.3	33.8	14.7
30	<b>0.03</b>	<b>0.003</b>	0.0125	-47.6	32.8	115.8	31.9	7.72	-86.4	32.3	13.1
45				-48.8	33.1	115.4	28.8	7.53	-87.3	31.1	11.5
60				-49.7	33.3	115.0	26.2	7.38	-88.1	30.0	10.0
<b>pK, pNa and pCl shift</b>											
initial	0.0115	0.0041	0.0125	-29.9	32.0	117.0	40.0	8.26	-69.3	50.3	1.5
0				-34.6	32.0	117.0	40.0	8.26	-74.0	45.6	<b>6.2</b>
15				-40.4	32.3	116.2	30.7	7.65	-79.6	39.7	4.9
30	<b>0.03</b>	<b>0.003</b>	<b>0.068</b>	-45.2	32.6	115.6	24.4	7.28	-84.2	34.7	3.6
45				-48.7	32.9	115.1	20.5	7.07	-87.4	31.1	2.5
60				-50.9	33.2	114.7	18.3	6.95	-89.4	28.8	1.6

389

## 390 Discussion

391

392 Monovalent ion channels and transporters are involved in apoptosis (Burg et al. 2006; Lang et al.  
 393 2007; Dezaki et al. 2012; Orlov et al. 2013; Hoffman et al. 2009, 2015; Kondratskyi et al. 2015;  
 394 Wanitchakool et al. 2016; Lang & Hoffmann 2012; Pedersen et al. 2016; Jentsch, 2016). However,  
 395 this phenomenon may be caused by two reasons: because the monovalent ions are the major cell  
 396 volume regulators and should be responsible for AVD only for this reason, or because they also  
 397 play important roles in cell signalling by affecting MP. It is not easy to distinguish these two causes  
 398 at present. We aimed to answer the question how alteration of distinct channels and transporters  
 399 affects the balance of monovalent ion fluxes across the cell membrane, cell water content and MP at  
 400 apoptosis. We studied the time course of the monovalent ion balance redistribution during the first 4  
 401 h development of apoptosis induced in U937 cells treated with STS as the established model of  
 402 apoptosis with significant AVD. Apoptosis in U937 cells is accompanied by rapid changes in light  
 403 scattering and cell water (volume) balance, whereas the positive annexin test and intensive  
 404 generation of apoptotic bodies are revealed, starting at 3-4 h (Yurinskaya et al. 2017). The  
 405 identification of channels and transporters responsible for the observed changes in monovalent ion



406 distribution, water balance and the sodium pump fluxes was based on the computational modelling  
407 of these changes. Such an approach was applied here to study apoptosis for the first time, although  
408 the monovalent flux balance under the normal physiological state and during redistribution of ions  
409 due to stopping the sodium pump has been calculated successfully before (see ref. in: Vereninov et  
410 al. 2014, 2016). Our previous code was modified currently to account for a continuous decrease in  
411 the sodium pump rate coefficient.

412 One of the most detailed studies of the kinetics of the monovalent ion balance  
413 rearrangement during apoptosis was performed by X-ray microanalysis and in U937 cells treated  
414 with STS in particular (Arrebola et al. 2005*a, b*). The experimental data obtained by flame emission  
415 and radiotracer assays in our study agree very well with the data obtained by this quite different  
416 method. Unfortunately, the accurate cell water content evaluation is hard to combine with the X-ray  
417 elemental microanalysis. Therefore, the complete mathematical model of the monovalent ion flux  
418 balance could not be developed using only those data.

419 Earlier we tried to relate the changes in ion and water contents to the monovalent fluxes in the  
420 sodium pump,  $K^+$ ,  $Na^+$ , and  $Cl^-$  channels and certain cotransporters in U937 cells after 4 h of STS-  
421 induced apoptosis (Yurinskaya et al. 2011). We came to the conclusion that the sodium pump  
422 suppression accompanied by a decrease in  $Na^+$  channel permeability might be responsible for AVD  
423 under the considered conditions. However, our current computational tool had not been developed  
424 at that time, the experimental data were limited to single time point 4 h, and the assumption was  
425 used that the balanced monovalent ion distribution is reached at 4 h of STS-induced apoptosis in  
426 U937 cells. More complete current data show that the cells at the 4 h time point are far from the  
427 balanced state (Table 3). There is significant  $Na^+$  gain (0.163) that is by  $\frac{3}{4}$  balanced by  $K^+$  leak  
428 (0.127) and  $\frac{1}{4}$  by the gain of  $Cl^-$  (0.036).

429 Currently, we substantially revised and developed our previous conception of the participation  
430 of the major channels and transporters in AVD during the STS-caused apoptosis of U937 cells. It  
431 remains true that a slow decrease in the sodium pump activity is a primary factor responsible for  
432 AVD at the late (4 h) stage of apoptosis. Recalculation of the data published earlier (Yurinskaya et  
433 al. 2011) with use of the current programme code and without intricate hypotheses confirmed a  
434 decrease in pNa at 4 h. The current data show that the pNa decrease at 4 h is significant indeed. The  
435 most interesting and important phenomenon is a more than 5-fold increase in the  $Cl^-$  channel  
436 permeability, which is much more important at the early stage. It is remarkable that the effect of the  
437 pCl increase disappears further because of a decrease in intracellular  $Cl^-$  concentration and  
438 associated decrease in chloride electrochemical potential difference,  $\Delta\mu_{Cl}$  (*mucl* in Table 4). The  
439 effects of the early increase in pK and a decrease in pNa on  $U$  are significant because they lead to  
440 an increase in  $\Delta\mu_{Cl}$  that drives chloride outward. A large body of electrophysiological evidence  
441 published recently indicates that the state of the chloride channels can change upon initiation  
442 apoptosis (Hoffmann et al. 2015; Kondratskyi et al. 2015; Wanitchakool et al. 2016; Pedersen et al.  
443 2016; Jentsch, 2016). However, there were no attempts to use these data for the quantitative  
444 description of early AVD.

445 The current computations show that changes in not a single type of channel but in  $K^+$ ,  $Na^+$ , and  
446  $Cl^-$  channels and in the sodium pump are responsible for the apoptotic ion balance alteration and  
447 that the effect of various channels and transporters on ion balance may be different at different  
448 stages of apoptosis. Certainly, the question arises how many parameters can provide an accordance  
449 between the calculated and real data? The computation enables us to answer this question, although  
450 certain time may be needed. In the case of STS-induced apoptosis in U937 cells in our experiments,  
451 we can exclude alternative variants by taking into account additionally the value of OSOR, which  
452 appeared to be different in different parameter setups, giving sufficiently good accordance between  
453 the real and calculated data. In other cases, the problem could be solved probably not by using  
454 OSOR but by some other way. The computation shows also how the real behaviour of cells should  
455 depend on the initial state of cells. Certainly, as soon as basic experimental data vary, the obtained  
456 numerical values of parameters will vary also. We can see from our long experience studying ion



457 balance in cultured cells that the variability of the cell physiological state rather than the inaccuracy  
458 of assays hamper the quantitative description of cell ion and water balance.

459 A skeptical view is spread among the experimentalists on the using calculations in analysis of the  
460 ion flux balance in cells. There is also a great deal of sometimes convoluted discussion about the  
461 merits and validity of certain assumptions that need to be made for the models and real data to be  
462 reconciled. As believed it is very difficult to accurately assess the value of the models and their  
463 conclusions. In this regard, we should note the following. If a required set of experimental data is a  
464 unique solution appears independently on any hypotheses on the number and types of channels and  
465 transporters which could present in cell membrane. Our system of the flux equations accounts all  
466 currently known types of ion transfer across membrane characterized only by the ion driving forces:  
467 electrochemical potential difference for movement of single ion species (electrodiffusion through  
468 electroconductive channels), the sum of electrochemical potential differences for the linked  
469 movement of several species of ions (cotransport, countertransport), and a combination of the  
470 electrochemical and chemical potential differences in case of the Na,K-ATPase pump. Computer  
471 decides what number of transporting units of each type should be for implementation of two  
472 physically mandatory demands and which ion pathways do not play a role under given ion  
473 conditions. The mandatory demands are electroneutrality of the any macroscopic ion redistribution  
474 and osmotic balance between distensible animal cell and the medium. Any hypothesis on the  
475 mechanism regulating cell water and ion content or membrane potential must be checked for these  
476 demands implementation. This cannot be done without computation in system with a numerous  
477 species of ions and a numerous ion pathways. Experimentalists avoid calculation and prefer using  
478 inhibitors and genetic cell modification simply because there is no sufficiently suitable tool for  
479 computation. We attempted to reduce computational tool deficiency.

480

## 481 **Conclusions**

482

483 1. The experimental data on the time course of  $K^+$ ,  $Na^+$ , and  $Cl^-$  concentrations and ouabain-  
484 sensitive and -resistant  $Rb^+$  influx in U937 cells treated with STS for 0.5-4 h enabled us to evaluate  
485 the changes in the pump rate coefficient and to compute alterations of the  $K^+$ ,  $Na^+$ , and  $Cl^-$  channel  
486 permeability coefficients associated with the initial stages of apoptosis and AVD.

487 2. The redistribution of  $K^+$ ,  $Na^+$ , and  $Cl^-$  underlying AVD in U937 cells is caused (1) by a  
488 progressive decrease in the sodium pump rate coefficient from an initial 0.029 to 0.013 at 4 h, (2)  
489 by a significant increase in  $pCl$  (0.013 to 0.068) and increases in  $pK$  (0.012 to 0.03, later 0.02), and  
490 (3) by a moderate decrease in  $pNa$  (0.004 to 0.003). The most critical factors for changes in cell  $K^+$   
491 and  $Na^+$  are the suppression of the pump, an increase in  $pK$  and a decrease in  $pNa$ , whereas the  
492 early decrease in  $Cl^-$  and water content (early AVD) are associated primarily with an increase in  
493  $pCl$  by approximately 5 times and an increase in  $pK$  by approximately 2.6 times.

494 3. Our approach demonstrates how to calculate the dependence of cell ion and water balance  
495 on the states of channels and transporters in the plasma membrane.

496

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500

## 501 **Author contributions**

502 All authors contributed to the design of the experiments, performed the experiments, and analysed  
503 the data. A.V. wrote the manuscript with input from all authors. All authors have approved the final  
504 version of the manuscript and agree to be accountable for all aspects of the work. All persons  
505 designated as authors qualify for authorship, and all those who qualify for authorship are listed.

506

## 507 **Conflict of Interest Statement**

508 The authors declare that the research was conducted in the absence of any commercial or financial  
509 relationships.

510

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614 **Supporting information**

615 Executional file to the programme code BEZ01B and Instruction: How to use programme code  
616 | BEZ01B.zip. This file is attached to the article electronic version.