This model was simulated at the computer in form of a multi-layer stochastic cellular automaton (see fig. 5). Here, the lower layer of automaton cells models the proton channel with the states H=0 or H=1 standing for the proton occupation at the sites in the channel. The middle layer accounts for the magnitude of the $pK_a$ value at the channel sites: state S=1 is the normal state with high $pK_a$ (strong bonding of the proton) and S=0 is the energized state with low $pK_a$ (weak bonding of the proton). Consequently the S-states of two neighboring sites are responsible for the hopping-probability (per unit time) of a proton to a neighboring (empty) site:

$p(i \dashrightarrow i+1) = p_0 * \exp(-\alpha S(i)) * \exp(-dE)$

$p(i+1 \dashrightarrow i) = p_0 * \exp(-\alpha S(i+1)) * \exp(+dE)$ , $dE = U/(2n)$ ,

where $p_0$ is a normalized constant probability and $\alpha$ an amplifying factor. The second exponent describes the effect of the superposed membrane field (U is the electrical potential difference between inside and outside of the membrane and n the number of sites in the proton channel. For simplicity only steps perpendicular to the membrane and parallel to the electric field are assumed.) . Finally, the upper layer (in Fig. 5) consists of only one automaton-cell accounting for the global protein - property of photon sensitivity: A=1 means that a photon can energize the molecule (in a kind described later), whereas A=0 describes the state of refractarity, in which a photon is not able to energize the molecule.

The following rules of the stochastic automaton were defined:

(1) In the normal state the H-layer is filled (all H=1) and the $pK_a$-layer is not energized (all S=1) but the system is sensitive for photon activation (A=1).

(2) After absorption of a photon of suitable energy the state of the $pK_a$-layer changes in all automaton-cells to S=0 (energized) and simultaneously an extrusion of the proton near the Schiff base (at the site no. i=n) to the outside of the membrane will become very probable. This probability is so defined, that it slows down with increasing proton concentration (number of protons per unit volume, $N_{out}$/Volume) in the outer electrolyte medium:
$p(n \dashrightarrow out) = p_0 * \exp(-\alpha S(n))/(1+N_{out}/\text{Volume})$ .

(3) If an proton-site i becomes empty, the $pK_a$-state of the corresponding automaton-cell becomes non-energized: S(i)=0 --> S(i)=1 . This rule implements the positive feedback as mentioned above.

(4) The hopping probabilities will be again calculated using the new values of S(i) and after then the hopping of each proton, which has a neighboring empty site, will be probed using a pseudo-random number generator (generating equal distributed real numbers in the interval [0,1]) in the following way:
If $p_{random}(i) < p(i \rightarrow i+1)$ the proton will jump to the site i+1, otherwise the proton will remain unmoved. This probing will be done in both directions with a randomly defined order to exclude the preference of one direction.

(5) The steps (3) and (4) will be repeated until all $pK_a$-states will be disactivated or until a characteristic time (which corresponds to the relaxation time of the energized protein) will be exceeded.

(6) After that the molecule is again sensitive to photon excitation and the process continues with step (2).

This rules are sufficient to simulate a photon activated proton pumping along a relatively long distance and against a remarkable electrostatic membrane field if only the condition ( $\alpha$-2dE) > 0 is fulfilled, i.e. the established gradient in the chemical potential exceeds the local electric field. One program run with typical parameters is shown in fig. 7. Each element shows the time sequence of proton vacancy migration trough the assumed array of proton sites as the consequence of absorption of one photon. The following details are seen:

- proton vacancies are indeed migrating through the assumed reprotonation channel against the existing field upon illumination. (1) This means protons are pumped in the opposite direction;
- the process is statistical and results in irregular transport patterns (2);
- one to three proton vacancies may be present in the reprotonation channel at the same time (3);
- occasionally photon absorption does not let to proton pumping (4).

Altogether it can be seen that there is no stoichiometric correlation between absorbed photons and protons pumped.

The pH-dependence of the proton release probability at the Schiff-base leads, for suitable parameter values, to a saturation effect of the proton pumping due to the increased proton concentration in the outer medium. Fig. 8 compares the cumulative

transport of protons of a deterministic linear model (straight line) with the calculated stochastic model, which shows a potential dependent saturation of proton transport.

The experimentally observed oscillations in the proton pumping process can be understood, if one postulates that with increasing difference of the outer and inner (inside the vesicle or liposome) proton concentration and the corresponding increase of the membrane potential the protein looses its ability to be energized by the absorption of a photon if a critical value $U_{up}$ of the membrane potential will be exceeded. Conversely it is reasonable to assume, that with the decrease of the membrane potential a hysteresis occurs in the sense that the critical value $U_{lo}$ at which the sensitivity of the protein will be recovered is lower: $U_{up} > U_{lo}$ (see fig. 9). For calculation of the entire cycle a parallel process of slow normal diffusion driven by the concentration gradient through chemically inert parts of the membrane must be considered at each time step.

Thus the automaton rules must be completed:

(7) After each cycle of steps (2) to (6) the difference of the outer and inner proton concentrations will be computed. If it exceeds the upper critical value $U_{up}$, the state of the sensitivity parameter A changes from 1 to 0.

(8) If A=0 the protein cannot pump protons despite of illumination. The normal diffusion through the membrane will now slowly equalize the inner and outer concentration of protons. If the difference in proton concentrations falls short of the lower critical value $U_{lo}$, the state of the sensitivity parameter A again changes from 0 to 1 and the process repeats with the step (2).

The implementation of the full set of automaton rules (1) to (8) indeed is able to simulate oscillations of the proton pumping activity of one single bacteriorhodopsin molecule located in a natural or artificial membrane (fig. 10). But, due to the stochastic nature of the pumping process, a set of (initially synchronized) isolated oscillating molecules will desyncronize after several oscillation cycles. Accordingly the measured outer concentration will be damped gradually out (fig. 11).

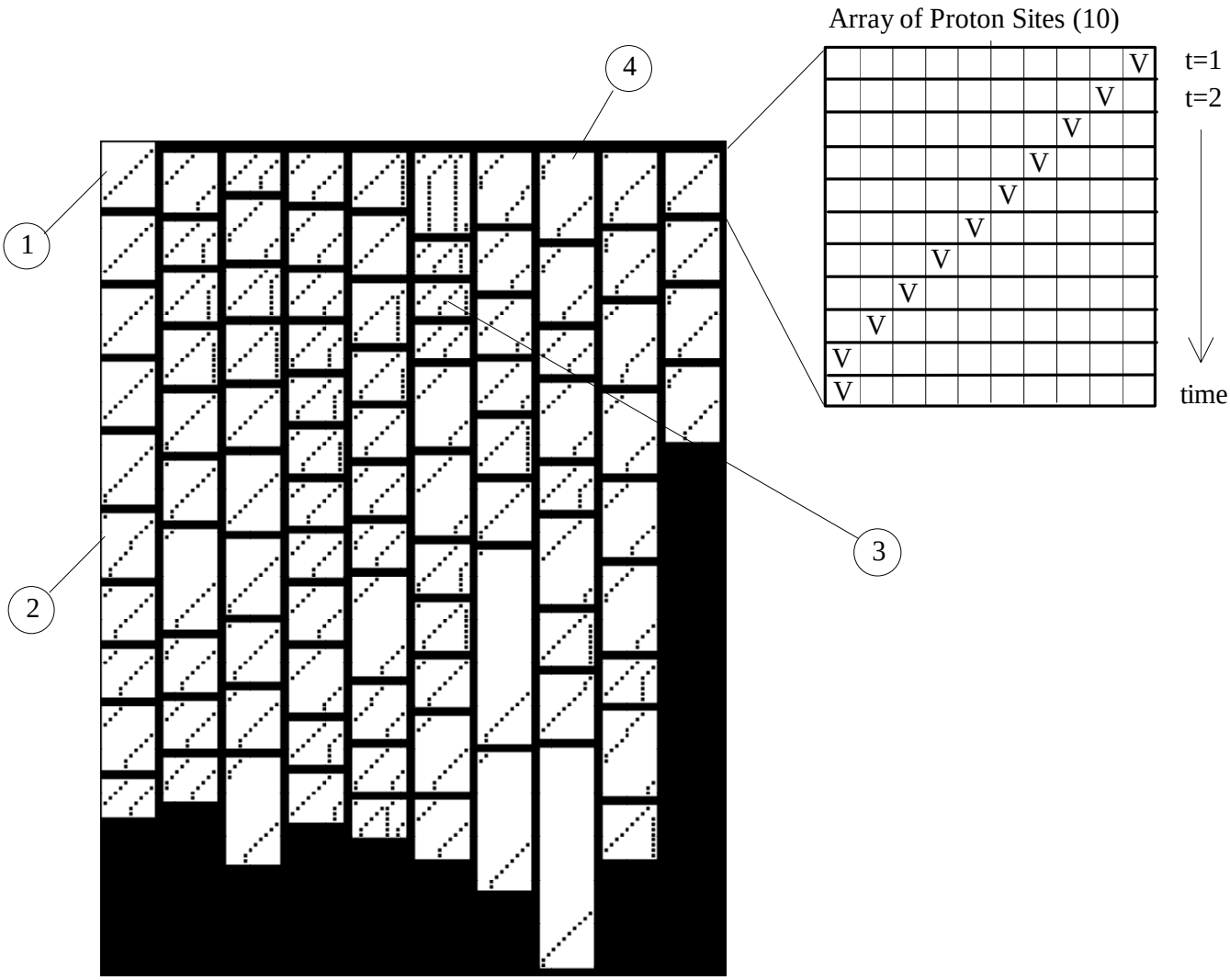
Fig.: 7. A typical simulation run of the automaton model. Here, each element (explanation on the right side) in the columns shows the array of occupation states of the „proton layer“ (white is occupied, black is a vacancy). The process starts at the upper left element (t = 0) and proceeds along the columns. Each element in the column represents one photon activated cycle of the pumping process. The simulation parameters are: Chain length: 10, characteristic time=100 steps, p0=0.99, $\alpha$=6.0, dE=0, volume=10.0 . Inserted numbers: (1) regular pumping, (2) irregular transport, (3) three simultaneously occuring vacancies, (4) unsuccessful pumping operation.

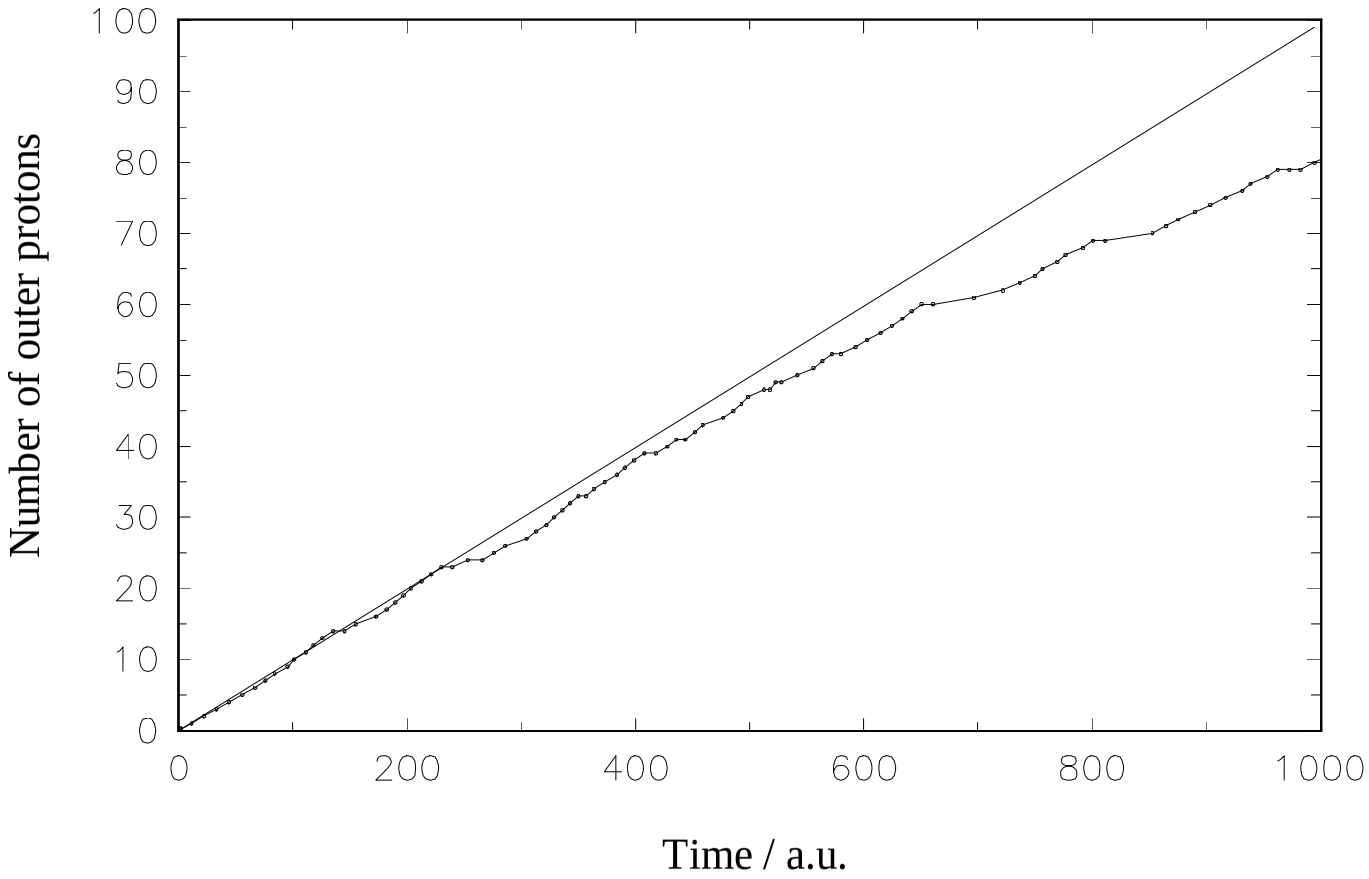
Fig.: 8. The increase of the number of protons in the outer medium corresponding to the program run in Fig. 7. The stochastic output is compared with a deterministic automaton model without the saturation effect due to increasing proton concentration (straight line).

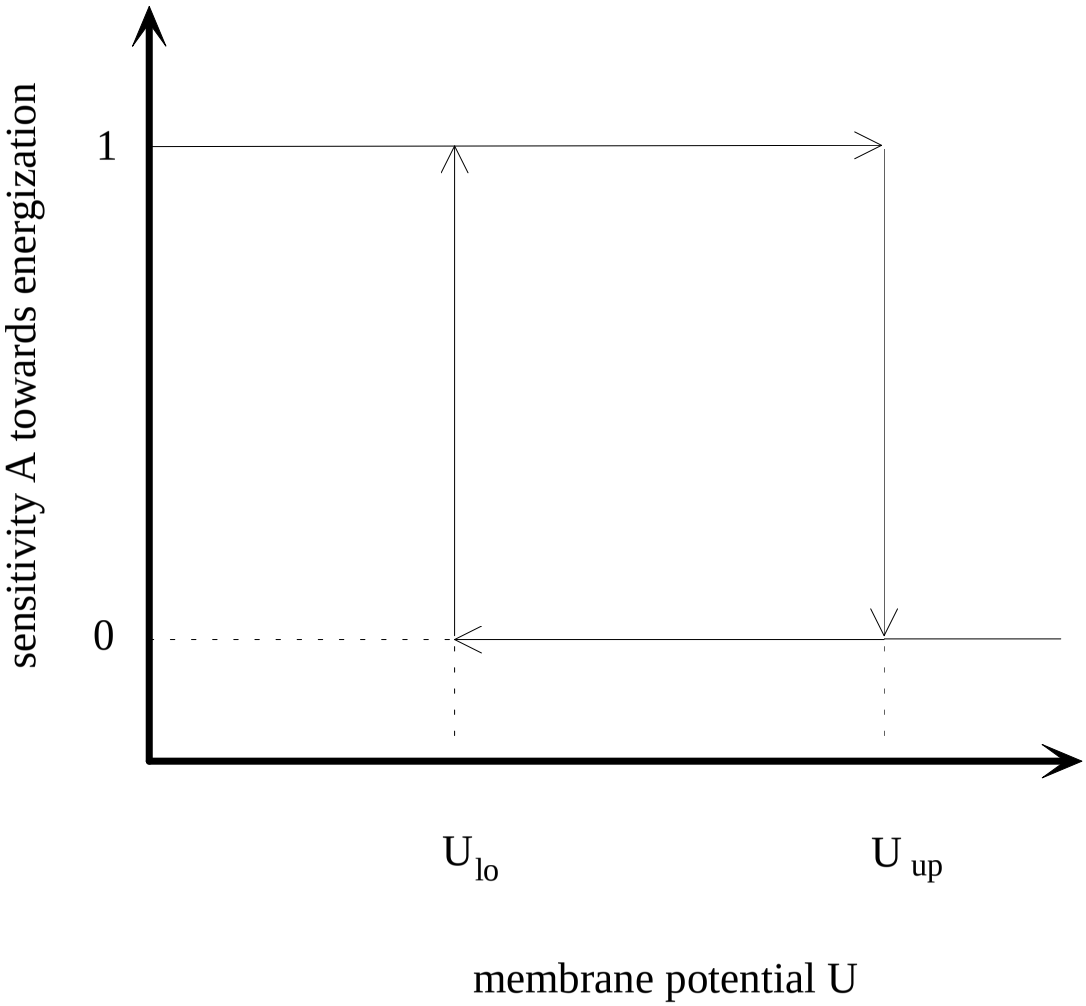
Fig.: 9. Schematic representation of the hysteresis effect in the potential dependent sensitivity A of the automaton model.

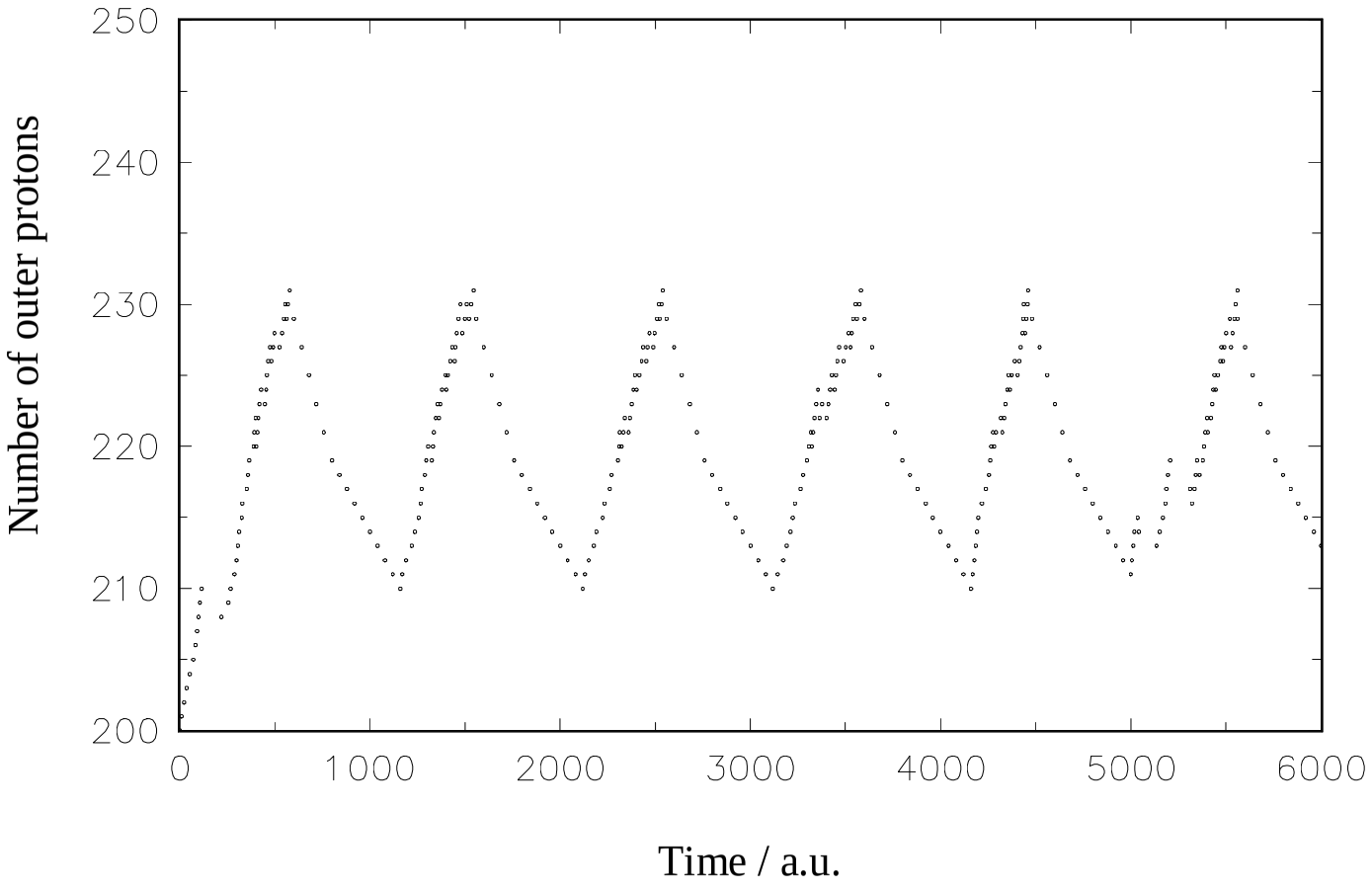
Fig.: 10. Calculated oscillations in the outer proton concentration due to the pumping of the automaton. (parameters: volume=100.0, diffusion coefficient D=0.004 per time step, upper critical potential $U_{up}$=0.15, lower critical potential $U_{lo}$=0.05, remaining parameters as in Fig. 7)

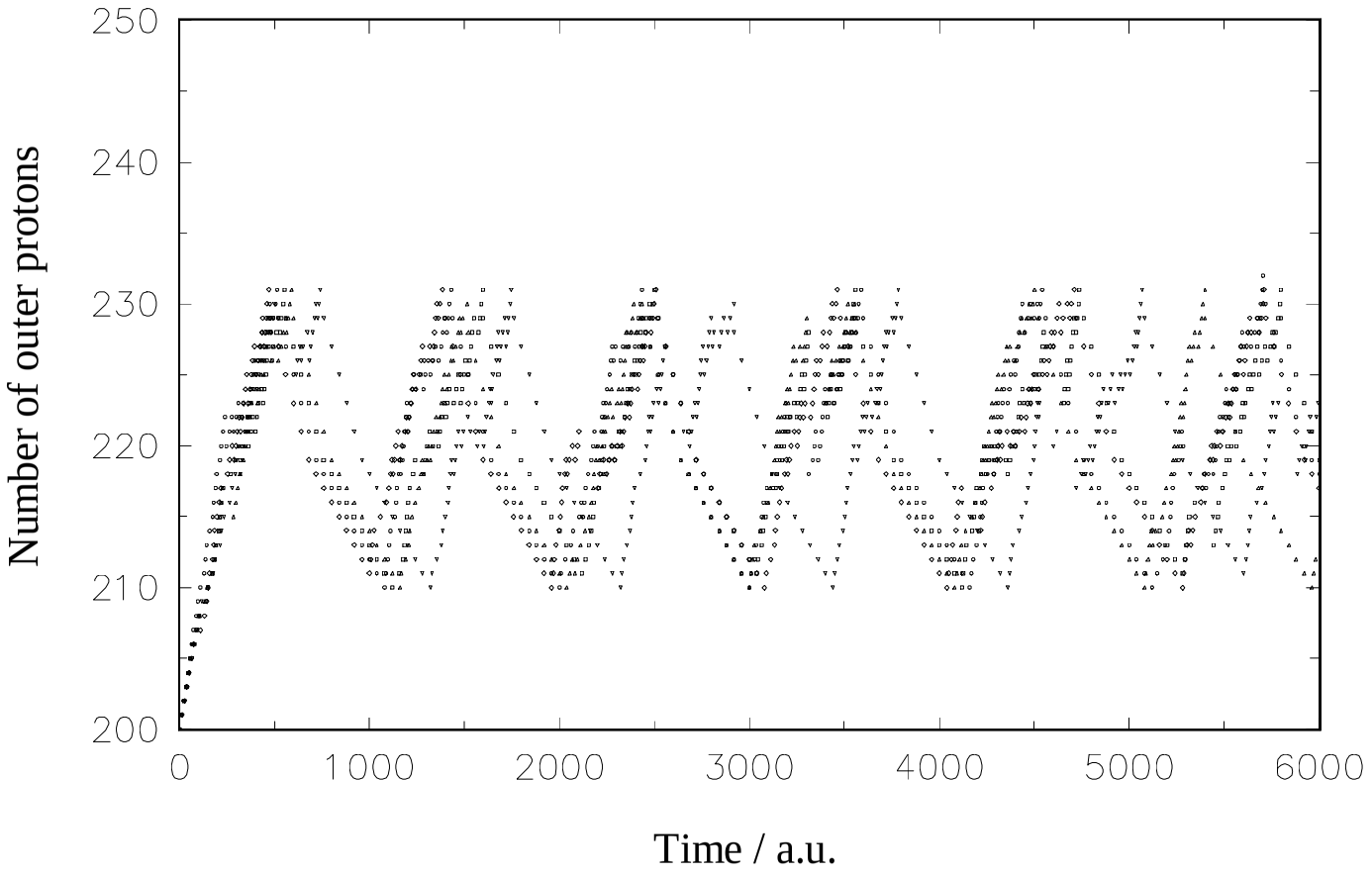
Fig.: 11. Several simulation runs with unchanged parameter values (see fig. 10) showing the increasing desynchronisation of individual protein molecules due to the stochastic nature of the pumping process (fig. 11a). The mean value of all runs (standing for the measurable concentration in the outer solution) shows a damped oscillation (fig. 11b).

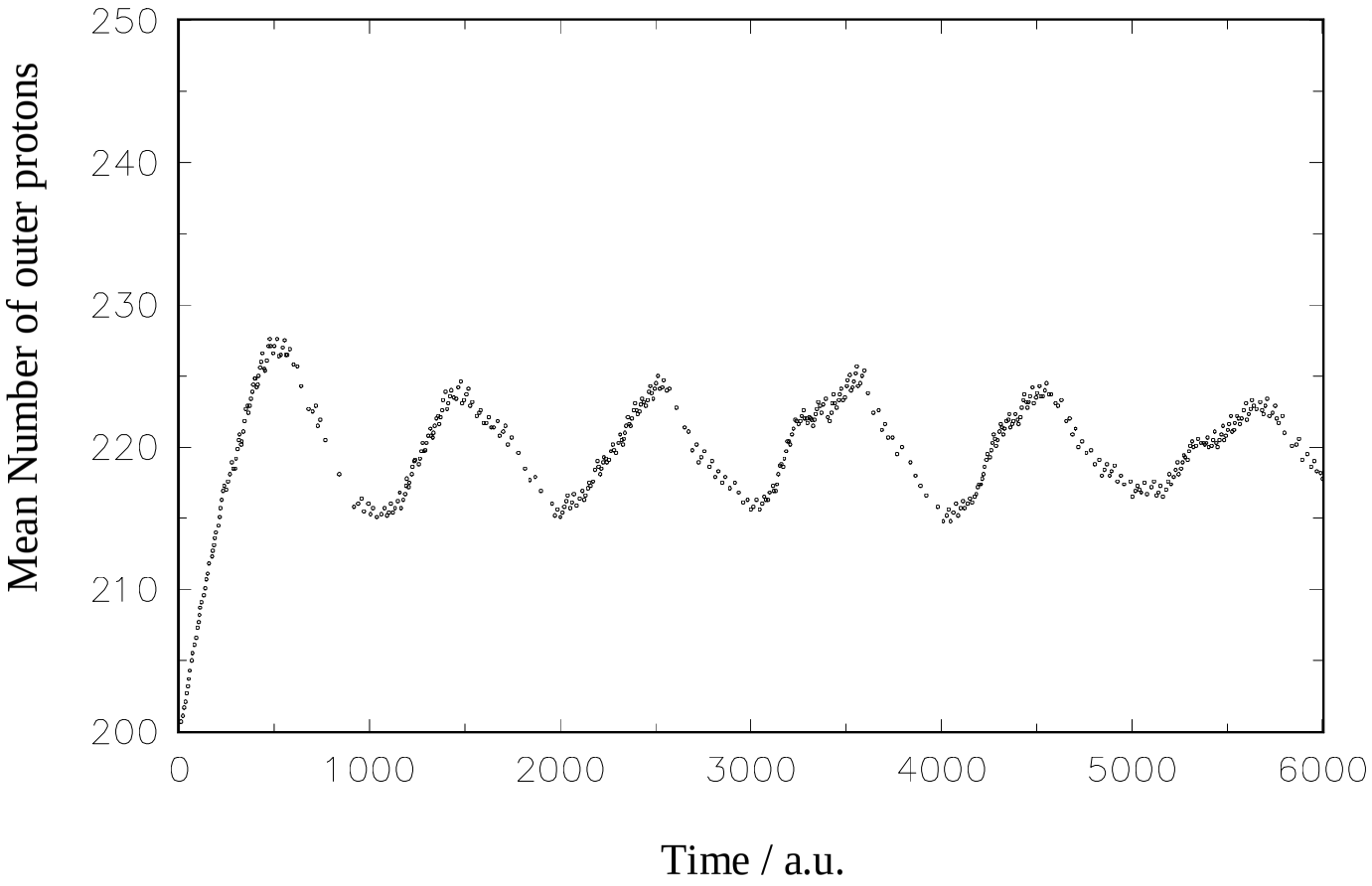

Array of Proton Sites (10)
t=1
t=2
time
1
2
3
4

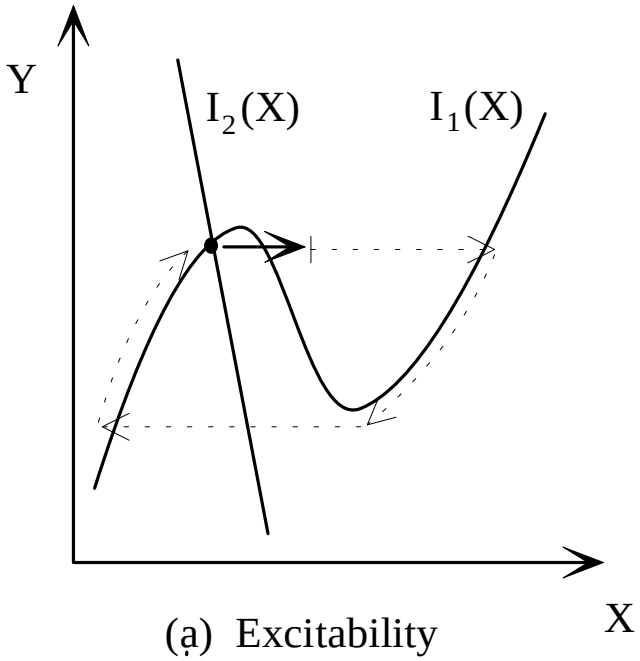

Number of outer protons
100
90
80
70
60
50
40
30
20
10
0
0
200
400
600
800
1000
Time / a.u.


sensitivity A towards energization
1
0
U lo
U up
membrane potential U

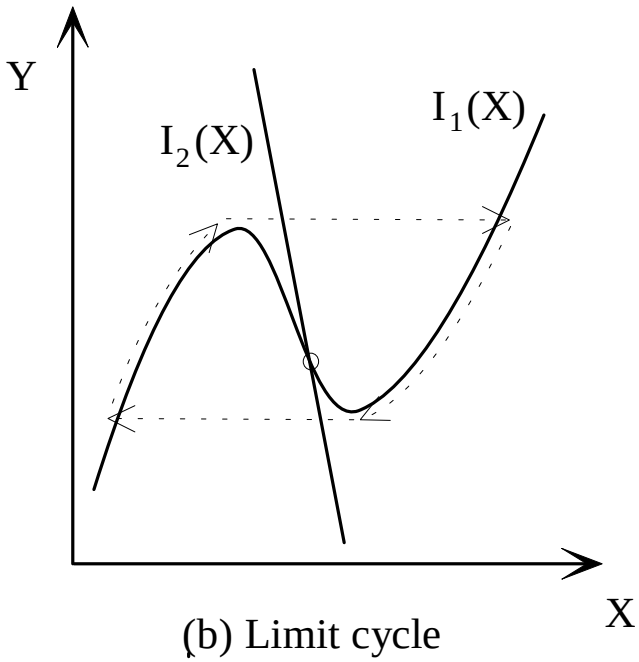

Number of outer protons
250
240
230
220
210
200
0
1000
2000
3000
4000
5000
6000
Time / a.u.


Number of outer protons
250
240
230
220
210
200
0
1000
2000
3000
4000
5000
6000
Time / a.u.

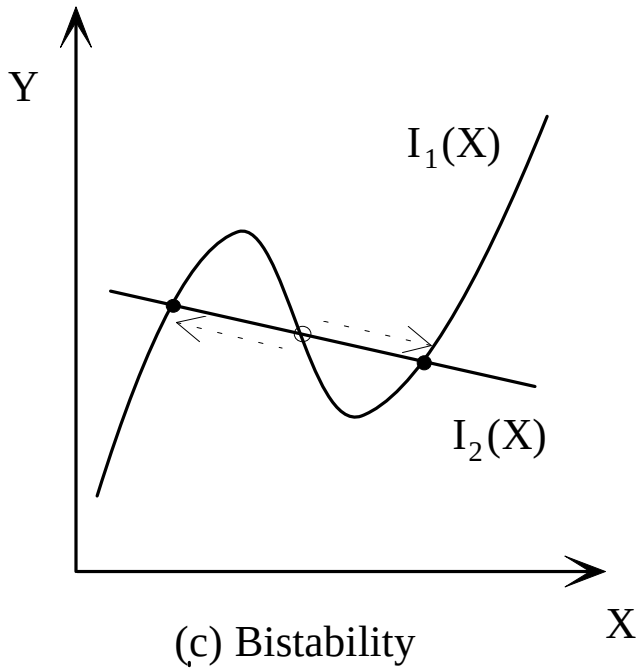

Mean Number of outer protons
250
240
230
220
210
200
0
1000
2000
3000
4000
5000
6000
Time / a.u.



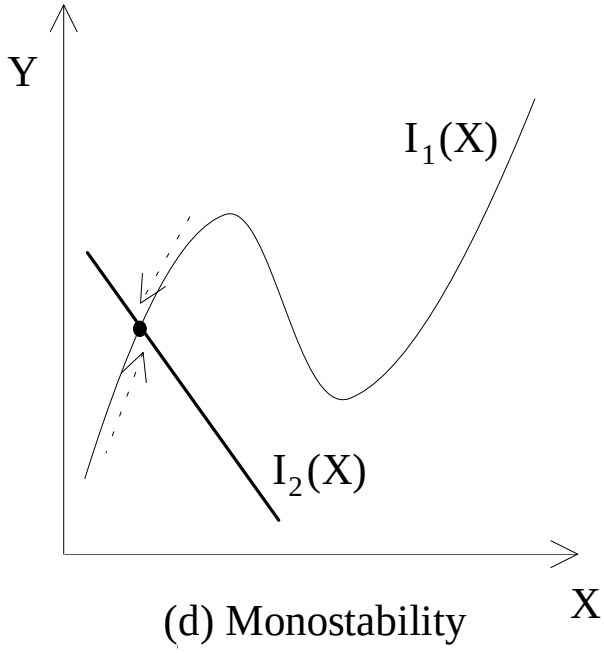

(a) Excitability
(one loop after excitation)



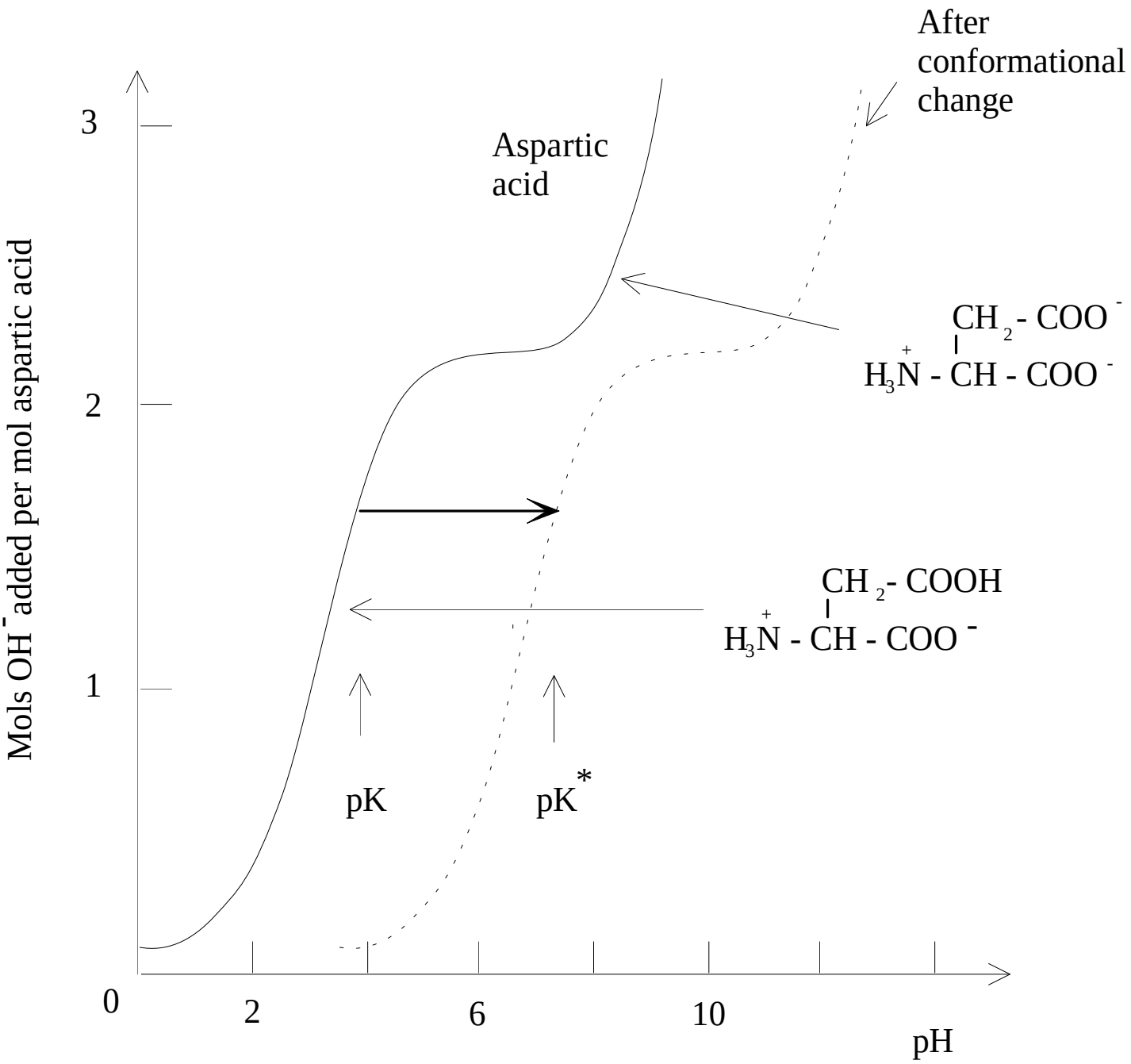

(b) Limit cycle
(sustained oscillations)




(c) Bistability




(d) Monostability


After conformational change
Aspartic acid
3
2
1
0
2
6
10
pH
Mols OH- added per mol aspartic acid
CH2 - COO-
H3N+ - CH - COO-
CH2 - COOH
H3N+ - CH - COO-
pK
pK*