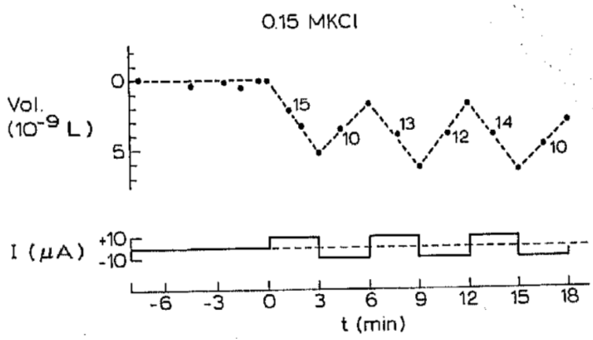
Questions: Biophysics II (Stand 22.6.2017)

1. How is the helix dipole generated? Explain its importance for the function of potassium channels, aquaporins, and the chloride cotransporter!
2. Derive Ohm's law for electrolytes from the Nernst-Planck electrodiffusion equation.
3. Explain the relationships between the conductivity of an individual ion, its diffusion coefficient, and its mechanical mobility.
4. Calculate the maximal unitary channel conductance if the solution resistivity, channel length, and channel radius are equal to 80 Ωcm, 5 Å, and 3 Å, respectively.
5. How does the unitary conductance of a membrane pore relate to channel radius? Which physical phenomena may confound the analysis? Are there other means to determine the radius of channels that are reconstituted in lipid bilayers?
6. The access resistance is known to increase channel resistance. Explain its origin and derive an expression to calculate the access resistance.
7. The streaming potential amounts to ~2.3 mV of a K^+- channel containing membrane that is exposed to an osmotic gradient of 1.9 M urea. Calculate the number of water molecules that are transported per K^+ ion!
8. The bi-ionic potential amounts to 53 mV of a cation channel containing membrane that separates a 100 mM KCl solution from a 100 mM NaCl solution. Calculate the ratio of K^+ to Na^+ permeabilities.
9. Calculate the streaming potential that develops upon unilateral addition of 1 M glucose to a cation selective membrane if protons are the only permeable cations in the system. Their concentration is equal to 200 mM at both sides of the membrane. Outline the transport processes that are observable in such a system.
10. You apply an external potential to a cation selective membrane. The pores are so narrow that transport occurs in a single file fashion. The iso-osmotic aqueous solutions at both sides of the membrane contain 100 mM KCl. You measure the water flux J_v and the current I as depicted in the figure:



Calculate the number of water molecules that are transported per ion!

11. Explain Grotthuss' mechanism of proton conductivity. Outline an experiment that proves the existence of the Grotthuss mechanism!
12. How do you account for unstirred layer effects when measuring the streaming potential across a membrane containing potassium channels? Use a scheme for your explanation!
13. Derive an expression for the electrostatic self-energy of ions!
14. The water filled cavity of the K^+- channel diminishes the energetic costs ΔG for moving a K^+- ion through the membrane by ΔΔG. Estimate ΔG and ΔΔG assuming that (i) the radius of K^+ is 1.5 A°, (ii) the cavity contains 50 water molecules, and (iii) the water densities in the cavity and in the bulk are equal to each other.
15. Which mechanisms contribute to the stabilization of K^+ ions in a K^+ selective channel?
16. Explain the role of the hydration energy in the selectivity of cation channels.
17. How does the selectivity filter of a potassium channel work? How does the channel exclude anions and how does it discriminate between potassium ions and sodium ions?
18. How do the open and closed structures of K^+-channels differ from each other? Which experiments led to the model of the open structure?
19. How does the pH sensor of KcsA work? Support your answer with a scheme!
20. Describe the experiments which revealed the presence of a time-dependent inactivation process in KcsA! Does the inactivation also occur in other K^+ channels?
21. Give a mechanistic interpretation of KcsA inactivation! Which structural features are responsible for the slow inactivation? Use a scheme to illustrate your reply!
22. How does the affinity of an ion to the channel determine the turnover number? What would be the maximal transport rate for a K^+ channel that binds K^+ with an equilibrium affinity of 29 μM?
23. Explain the knock-on mechanism in ion permeation through K^+ channels. How does it differ from the hard knock on mechanism found by molecular dynamics simulations?
24. How is ion affinity to a channel determined experimentally?

25. Outline how stopped flow experiments can be used for the determination of vesicular water membrane permeability! Start with a scheme of the experimental setup!
26. How do you experimentally determine the energetic cost for inserting a certain amino acid into the membrane?
27. What is the difference between the biological and the physico-chemical hydrophobicity scales of amino acids? How do they correlate with each other?
28. Draw a schematic model to explain the voltage dependence of KvAP! Explain how the movements of the voltage sensor results in pore opening!
29. The paddle model has been supported by numerous functional experiments. Explain the experimental approaches!
30. Fluorescence quenching by dipicrylamine has been used to falsify the paddle model of voltage sensing. Explain the experiment by using a schematic representation of the molecular movements of both quencher and voltage sensor.
31. Outline the experiments in support of the notion that the KvAP voltage sensor crosses rather short distances!
32. What experimental evidence supports the notion that the voltage sensor of potassium channels is exposed to a focused electrical field? Explain the experiment!
33. Assume (i) a 10% open probability of potassium channels at a membrane potential of -60 mV and (ii) a half maximal conductance of a K^+ channel containing membrane at -50 mV. Calculate the gating charge of the incorporated K^+ channels!
34. Explain the luminescence resonance energy transfer technique and its pros and contras as compared to FRET!
35. How does the open probability of a channel depend on membrane voltage and the gating charge? Use the Boltzmann theory of voltage dependence for your explanation!
36. Which experimental possibilities exist for the determination of gating charges? Explain the relationship between the observed parameter and the gating charge!
37. Several models have been developed to explain how ion channels may distinguish between sodium and potassium ions. Compare the rigid cage model with the induced fit model!
38. Several models have been developed to explain how ion channels may distinguish between sodium and potassium ions. Compare the kinetic model of selectivity with the coordination model!
39. How does the diffusion coefficient depend on the crystal radius of ions?
40. Compare the architecture of K^+- and Na^+- channels!

41. How did the analysis of Na^+, K^+, and Li^+ binding sites within the KcsA pore contribute to the understanding of the selectivity mechanism?
42. Compare the selectivity filter structures of K^+ and Na^+ channels! Which features are responsible for Na^+-selectivity?
43. Explain the ball and chain model of Na^+ channel inactivation and outline the experimental evidence.
44. Outline the key experiments leading to the discovery of aquaporins!
45. Aquaporin dysfunction is associated with diseases. Explain two examples!
46. Which structural features are responsible for the exclusion of anions and metal ions by aquaporins?
47. Explain the molecular mechanisms of proton exclusion from aquaporins!
48. Compare the selectivity filter structures of orthodox aquaporins and aquaglyceroproteins! Which features are responsible for glycerol permeation?
49. Derive van‘t Hoff's expression for osmotic pressure!
50. How can you use osmosis to determine the molecular weight of an unknown substance?
51. How are hydraulic and osmotic water permeabilities defined?
52. An aquaporin expressing oocyte increases its volume by 10% within 100 seconds after the osmolarity in the external solution has been dropped from 300 mOsm to 200 mOsm. Calculate the osmotic water permeability of the oocyte!
53. What determines the water permeabilities of aquaporins and other narrow membrane channels?
54. Why do the membrane permeabilities differ for positive and negative ions of the same size?
55. The presence of unstirred layers in membrane vicinity may result in an underestimation of experimental water permeability. Explain the phenomenon!
56. Assume that the bulk osmolyte concentrations at the two sides of an epithelium in an Ussing chamber are equal to 300 and 800 mM. Estimate the transepithelial concentration difference if the water permeability, the diffusion coefficient and the thickness of the unstirred layers are 50 µm/s, 10^{-6} cm^2/s, and 100 µm, respectively.
57. Assume a unitary water permeability $p_f = 10^{-14}$ cm^3/s of a membrane channel that is permeated by a single file of water molecules. Estimate water mobility within the channel! How does it compare to bulk water mobility?
58. What evidence determines that the protein ClC - EC1 does not function as a channel but as a carrier? Describe the experiment!

59. A membrane with the reconstituted ClC-EC1 transporter shows a reversal potential of 30 mV. Calculate the stoichiometry of the Cl-H exchange if the aqueous compartments at both sides of the membrane have a pH of five and the respective KCl concentrations are equal to 300 and 45!
60. Describe the experimental approach to show secondary active transport by the ClC-EC1 transporter: Cl^- driven proton transport and proton driven chloride transport.