Task 5 for obtaining credits for B130P16E
(Practical basics of scientific work)

Task:

Write a short manuscript of the scientific paper on the **"The role of polar auxin transport in the phototropism".**

Structure of the manuscript:

First page:

Title, authors, affiliations – use your imagination

Abstract:

Try to briefly summarize the introduction into the topic, description of methods and results achieved and summarization of the relevance of the message that could be taken form this report (2-4 sentences).

Key words:

Find the most relevant terms according to which readers will find our article in the database

Introduction:

Put the reader into the true picture of these days knowledge in the field of auxin research. Mention briefly what is auxin and how important is its transport. The information could be find in the attached file (info_auxin_for_task5.ppt).

Material and methods:

Describe the design of the experiments, you can find technical details below, do it briefly.

Results:

Assemble a consistent text describing results of experiments 1 and 2, make the reference to the respective image.

Discussion:

Discuss your results in the context of general knowledge and also try to make your own interpretations. Suggest some additional experiments, if needed.

Acknowledgements:

Here you can specify grant support as well as mentioning colleagues or donators of experimental material.

Reference list:

Make the reference list for the all papers that are mentioned in your article, they could be found in the file (info_auxin_for_task5.ppt). Use Pubmed, WOS, or Scopus and EndNoteWeb.

Figure captions:

Concise description of chat is presented in the respective image. It should be self-explaining and easy to understand.

Figures: Always 1 figure per one page.

Experiment 1:

Two synthetic auxins 2,4-D a NAA enter the plant cell through the plasma membrane by two different mechanisms. The first is active utilizing specific membrane carrier (energy-dependent), the second is passive utilizing the diffusion of auxin across plasma membrane.

Using radioactively labelled auxins [3H]2,4-D and [3H]NAA their accumulation inside tobacco cells was measured. Tobacco cell line BY-2 (Bright Yellow) was harvested after two days of cultivation and transferred to the buffer of pH 5.8. After 2h of the equilibration in this buffer, radioactively labelled auxin ([3H]2,4-D or [3H]NAA) was added and during subsequent 30 min period samples for the determination of the radioactivity were harvested. Control cells and cells treated with the inhibitor of the active transport inside cells 1-NOA (1-naphtoxyacetic acid) were used. Inhibitor was added after 1 minute.

Following values of radioactivity inside cells were obtained.

For 2,4-D:

Time (min)	Sample number	[^{3}H]2,4-D (dpm)		[^{3}H]2,4-D plus 1-NOA (dpm)
	1	4063		
	2	4635		
	3	5008		
0	4	5309		
	5	13830	29	9881
	6	14303	30	10046
	7	12719	31	9792
3	8	14703	32	9715
	9	16039	33	9175
	10	15901	34	9389
	11	15757	35	8727
5	12	16555	36	10175
	13	16112	37	8722
	14	16750	38	8486
	15	16574	39	8787
11	16	16799	40	8834
	17	14524	41	9106
	18	15943	42	10500
	19	16290	43	8674
17	20	16694	44	8843
	21	15952	45	8128
	22	15438	46	8509
	23	15519	47	8444
25	24	16738	48	8744
	25	15688	49	8357
	26	15763	50	8789
	27	15593	51	8281
32	28	16029	52	8278

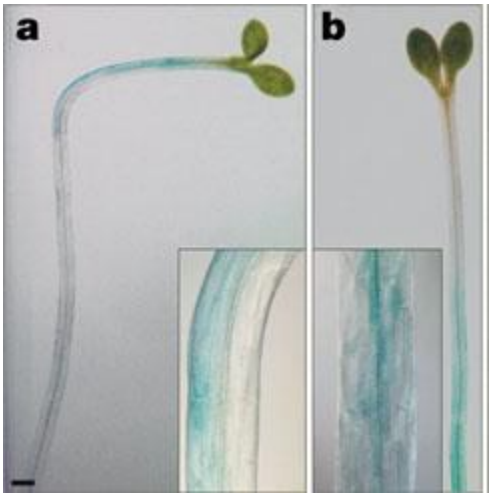
For NAA:

Time (min)	Sample number	[^{3}H]NAA (dpm)		[^{3}H]NAA plus 1-NOA
	1	4827		
	2	5363		
	3	5586		
0	4	5883		
	5	7360	29	7591
	6	8076	30	8064
	7	7898	31	8211
3	8	8269	32	8348
	9	7164	33	7904
	10	7446	34	7994
	11	7822	35	8380
5	12	7260	36	8461
	13	8187	37	8304
	14	7279	38	8505
	15	7682	39	8113
11	16	7734	40	7987
	17	7308	41	8040
	18	7432	42	8134
	19	8279	43	8446
17	20	7765	44	8168
	21	7669	45	8192
	22	7516	46	8198
	23	7416	47	8376
25	24	7812	48	8588
	25	7710	49	8316
	26	8581	50	8610
	27	8301	51	8860
32	28	8064	52	8931

Make plots from these values and based on the data determine which of these two auxins enter the cell passively and which actively. Results of this experiment also clearly show that at least one of these two synthetic auxins enters plant cells by active carrier-mediated transport across plasma membrane.

Experiment 2:

In the first experiment we have followed the entrance of auxins inside cells; here we will follow their efflux out of the cell. We will be interested what would be the consequence of the inhibition of active auxin efflux carrier for the reaction of plants to the light (phototropism).



From this image (take it as the result of your work for the purpose of this trial manuscript) it is obvious that the inhibitor of auxin efflux carrier NPA (1-N-naphtylphtalamic acid) disturbed the reaction of *Arabidopsis thaliana* seedlings stems on the directional illumination from the right side. Under standard conditions **(a)** the stem is bending towards light. After NPA treatment **(b)** the stem is not bending at all. This image is also demonstrating how the auxin is distributed in this plant. Blue colour indicates sites, where the gene expression provoked by auxin takes place. Clearly, it is enhanced at the shaded side of the stem that is elongating more than the illuminated side. After the application of NPA this gradient can not be formed. We are asking the question what it means for the elongation of cells at both sides of the stem.

For the purpose of this experiment these plants were transformed with the gene coding for ß-glucuronidase enzyme under the promoter sensitive to auxin. The expression of this enzyme takes place only when there is sufficient amount of auxin around. Its activity subsequently creates blue precipitate after the addition of chromogenic substrate to the fixed plants, X-gluc (5-bromo-4-chloro-3-indolyl ß – D glucuronide).

From this experiment, you can conclude (at least indirectly) what is the role of active auxin transport during the bending of the stem of A*rabidopsis thaliana* seedling. Describe the experiment with your words.