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

**Task:** Try to write 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, main results and why is this contribution important (2-3 sentences).

**Key words**: try to 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:** try to describe the design of the experiments, you can find details below, do it briefly.

**Results:** try to write consistent text describing our results of experiments 1 and 2, make the reference to the respective image.

**Discussion:** discuss our 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:** try to 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 [$^3$H]2,4-D and [$^3$H]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 ([$^3$H]2,4-D or [$^3$H]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 |

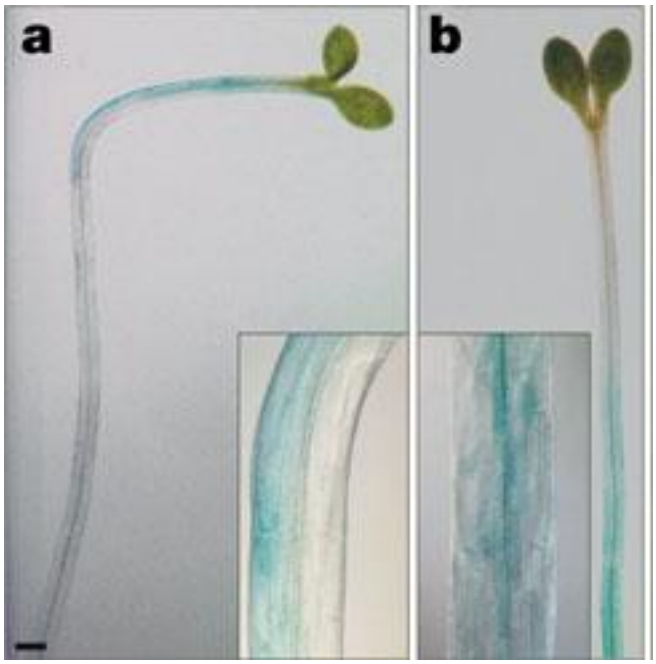
**For NAA:**

| 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.