Comparison of In Silico Signal Sequence-Phospholipid Results with Described In Vitro and In Vivo Protein Translocation Studies Seems to Underscore the Significance of Phospholipids

Rob C. A. Keller

Section Chemistry, Charlemagne College, Nijmegen, The Netherlands;
rcakeller@kpnmail.nl

Supplementary materials

INTRODUCTION

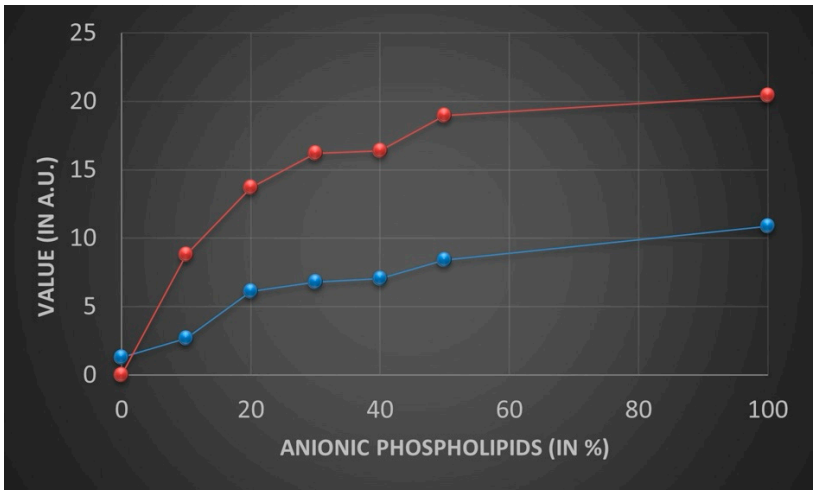
This supplement contains additional information and data as indicated in the main text of the paper. Details can be found regarding:

- Figure S1 Details prePhoE SP
- Comparison prePhoE signal peptide with and without additional amino acids.
- Additional information Figure 1.
- Data ER peptides.
- Details related to the type II signal-anchor protein and Preprolactin signal peptide.
- The MCPep results of the b5 transmembrane sequence and additional amino acids and helical wheel plot of indicated lipid binding region as obtained by Heliquest.
- Remarks when it comes to using MCPep and potential alternatives.

RESULTS

Figure S1 Details prePhoE SP.

In Figure S1 is the anionic phospholipid dependency depicted for the prePhoE SP. In red the change in distance from the membrane midplane of the peptide (in Å) is depicted and in blue the effect of the total free energy difference (in kT). Both show a dependency of anionic phospholipid content.



At 0% PG the depth towards the midplane (red) is set to zero and the differences are plotted subsequently in Figure S1. The values of the free energy difference (blue) are changed from negative to positive in order to make the graph comparable.

Comparison wild-type and mutated prePhoE signal peptide with and without additional amino acids.

In Table S1 the differences are depicted as obtained by using MCPep.

Table S1: Comparison WT and mutated prePhoE SP

Peptide/Configuration	ΔG_{total}	ΔG_{conf}	ΔG_{SIL}	ΔG_{coul}	ΔG_{def}
PhoE SP WT/Surface	-3.7 ± 0.3	0.1 ± 0.3	-1.1 ± 0.2	-2.9 ± 0.0	0.3 ± 0.0
PhoE SP WT/TM	-5.9 ± 0.2	1.7 ± 0.0	-3,6 ± 0.1	-4.8 ± 0.3	0.8 ± 0.0
PhoE WT+7/Surface	-1.6 ± 0.4	1.7 ± 0.4	-1.5 ± 0.1	-2.1 ± 0.0	0.2 ± 0.0
PhoE WT+7/TM	-9.2 ± 0.2	0.2 ± 0.2	-7.3 ± 0.0	-2.7 ± 0.0	0.7 ± 0.0
PhoE SP G(-10)L/Surface	-10.7 ± 0.3	2.0 ± 0.2	-9.3 ± 0.1	-3,7 ± 0.0	0.3 ± 0.0

PhoE SP G(-10)L/TM	-9.7 ± 0.2	2.2 ± 0.2	-8.0 ± 0.1	-4,7 ± 0.0	0.8 ± 0.0
PhoE G(-10)L+7/Surface	-6.5 ± 0.4	1.4 ± 0.4	-5.9 ± 0.1	-2.2 ± 0.0	0.3 ± 0.0
PhoE G(-10)L+7/TM	-13.3 ± 0.2	0.2 ± 0.2	-11.4 ± 0.1	-2.7 ± 0.2	0.7 ± 0.0

Additional information Figure 1.

In addition to what is already discussed in Figure 1, the Table S2 gives the data as discussed in the main text. Data collected using MCPep.

Table S2: Additional info Figure 1

Name	Translocated mature	ΔG_{total}	Predict. in vivo	In vivo activity	PG content
2K9V	+2AA (Surf.)	-1	13	-	5%PG
2K9V	+5AA (Surf.)	-2	33	-	10%PG
2K9V	+9AA (TM)	-1	60	-	15%PG
2K9V	+15AA (TM)	-1	100	-	20%PG
2K10V	+17AA (TM)	-3	100	-	5%PG
2K10V	+17AA (TM)	-6	100	-	12,5%P
2K10V	+17AA (TM)	-9	100	-	20%PG
2K8L	+20 AA (TM)	-15	100	30	5%PG
2K8L	+19 AA (TM)	-16	100	100	20%PG
2K9L	+18AA (TM)	-19	100	90	5%PG
2K9L	+18AA (TM)	-21	100	90	20%PG

Data related to ER peptides.

Details related to the type II signal-anchor protein and Preprolactin signal peptide.

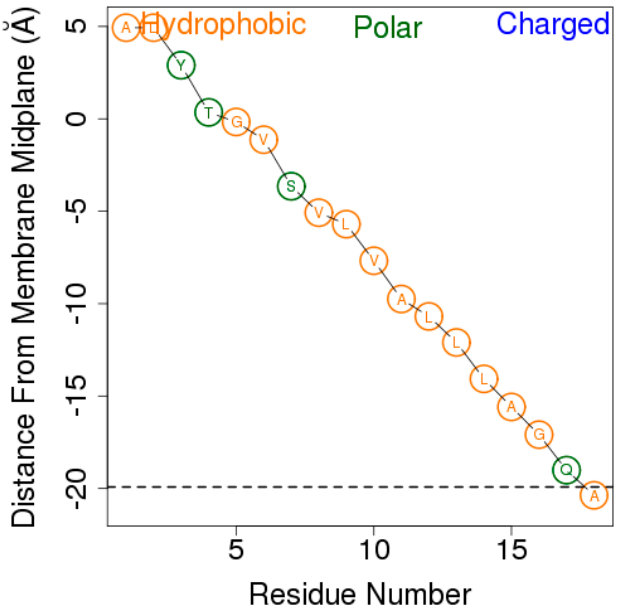
Results of Heliquest for Signal anchor peptide of the type II membrane protein invariant chain (LiTAG41) (30-58) type II signal-anchor protein (Ii)

>sp|P04441|30-55

GALYTGVSVLVALLLAGQATTAYFLY

2**ALYTGVSVLVALLLAGQA**19

H=0.798 µH=0.201z=0



And MCPep:

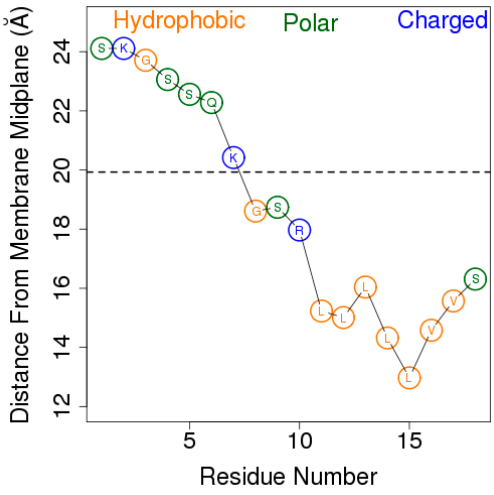
SAtype.pdb

Heliquest result of Preprolactin signal peptide (13-25 hydrophobic)

MDSKGSSQKGSRLLLLLVVSNLLLCQGVVS

3**SKGSSQKGSRLLLLLVVS**20

H=0.418 µH=0.130 z=3



and MCPep:

preprolactin.pdb

MCPep results of b5 transmembrane domain and additional amino acids.

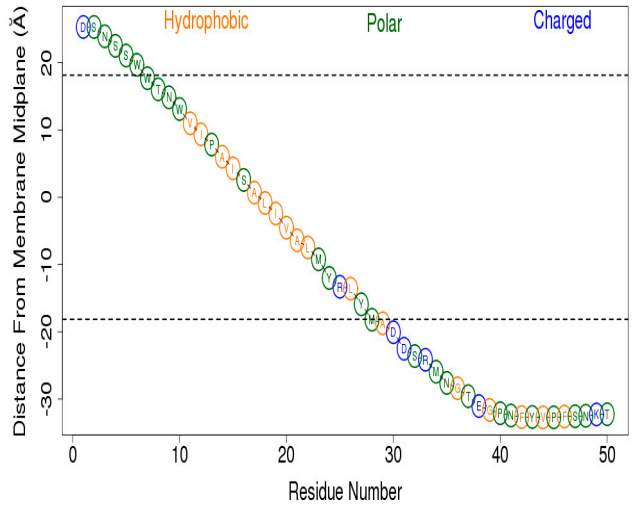
As described in the RESULTS section, the additional information belonging to the MCPep results are given below. For the MCPep results the following sequence b5-ops-28 is used as input:

D**SNSSWWTNWVIPAISALIVALMY**RLYMADDSRMNGTEGPNFYVPFSNKT(VD)

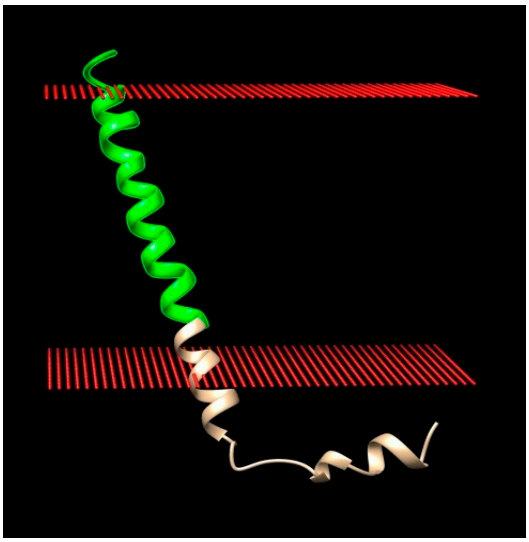
The transmembrane domain (TMD) region is indicated in bold. Due to the limitation of the MCPep program (no more than 50 AA) the last two amino acids (indicated between brackets) are not included. In Figure S1 the results of a significant clusters as found by the MCPep program is depicted. In Figure S1A the graphic presentation and in Figure S1B the model of the membrane configuration are shown.

Figure S2: MCPep results of b5 TMD and additional amino acids. Both the graphic presentation (A) as well as the model presentation (B) of the membrane configuration are depicted.

A:

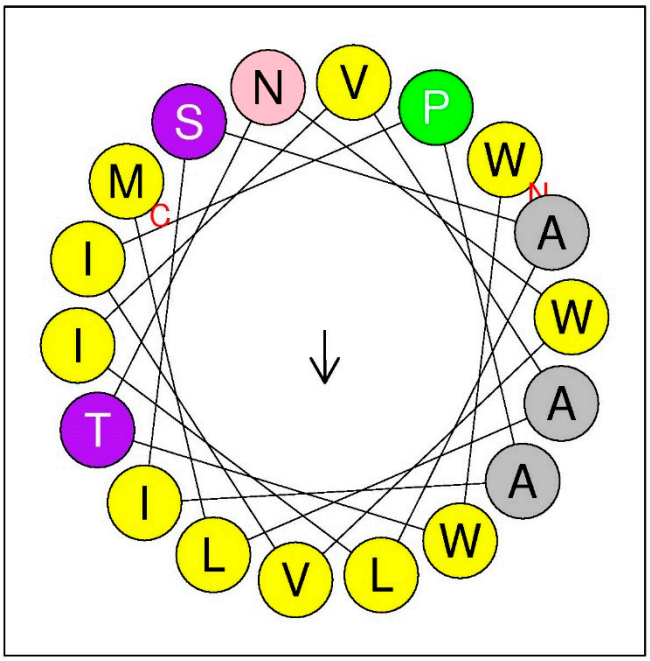


B:



The b5 TMD region is according to Heliquest indeed a typical transmembrane region with a mean hydrophobicity of 1.138, which is way above the threshold value for a transmembrane region of 0.75. In Figure S2 the corresponding helical wheel plot is shown of region AA 5-22.

Figure S3: Helical wheel plot of region b5 AA 5-22 according to the Heliquest program. Depicted is the <μH> vector (hydrophobic moment) as arrow. Hydrophobic residues are shown in yellow, serine and threonine in purple, asparagine and glutamine in pink, alanine and glycine in grey and proline in green (Basic residues in dark blue, acidic residues in red).



Remarks when it comes to using MCPep and potential alternatives.

During my years of working with MCPep I noticed that sometimes the program faces some issues. Besides normal and apparently unavoidable issues like lack of speed, delay in receiving the results etc., the most important matter is that sometimes the software doesn't find significant clusters. The only good reason for this is that there are no significant clusters to be found in that particular case. However sometimes significant clusters should be found (since I/you found them a year earlier for example). Asking the people behind the program it appeared that for example something in the transfer of the corresponding pdb files went wrong due to (recent) changes made by the IT department. It can be fixed and after that the program is reliable again.

Hint: Check one or two known examples found in literature when you don't trust the output.

There are at present a number of programs/servers that (in part) are able to perform similar jobs as MCPep:

The **FMAP server** (https://membranome.org/fmap) was used to check the prePhoE signal sequence plus 17 AA of the mature part. The result is shown in Figure A.

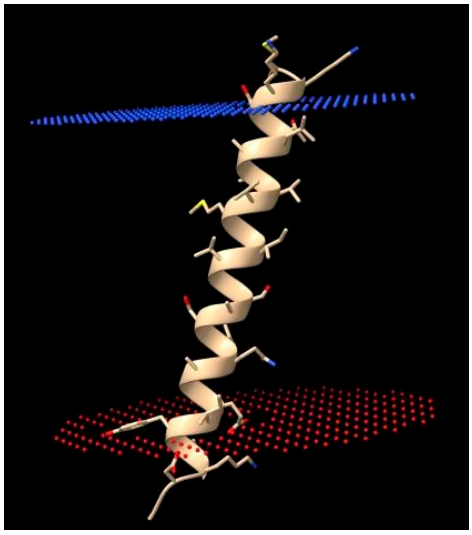


Figure A. Result of MKKSTLALVVMGIVASASVQAAEIYNKDGNKLDVYGKV using FMAP server and the membrane used had a lipid composition that mimics the Gram-negative membrane. The visualization of the pdb file is made with Chimera X (https://www.rbvi.ucsf.edu/chimerax/).

The **PPM server** (https://opm.phar.umich.edu/ppm_server3_cgopm) was used to check the prePhoE signal sequence plus 17 AA of the mature part. The input for the PPM server was made by running the signal peptide sequence first through Robetta since the server needs a pdb file as input (https://robetta.bakerlab.org). The end result is shown in Figure B.

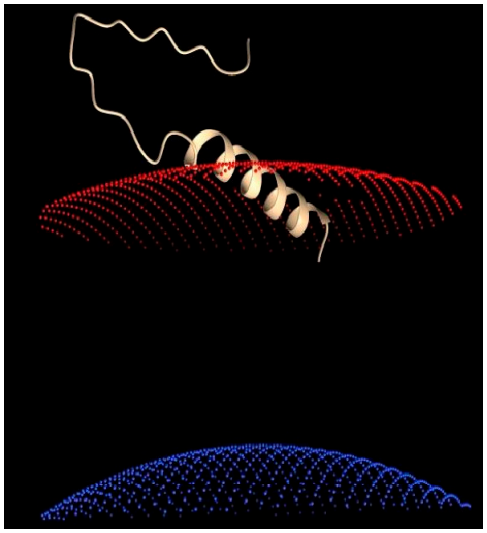


Figure B. Result of MKKSTLALVVMGIVASASVQAAEIYNKDGNKLDVYGKV using the PPM server. The visualization of the pdb file is made with Chimera X (https://www.rbvi.ucsf.edu/chimerax/).

PMI-Pred (https://pmipred.fkt.physik.tu-dortmund.de/curvature-sensing/) classified the SP of prePhoE to the class of "sensors" (data not shown). The same pdb file obtained from Robetta was used as input.

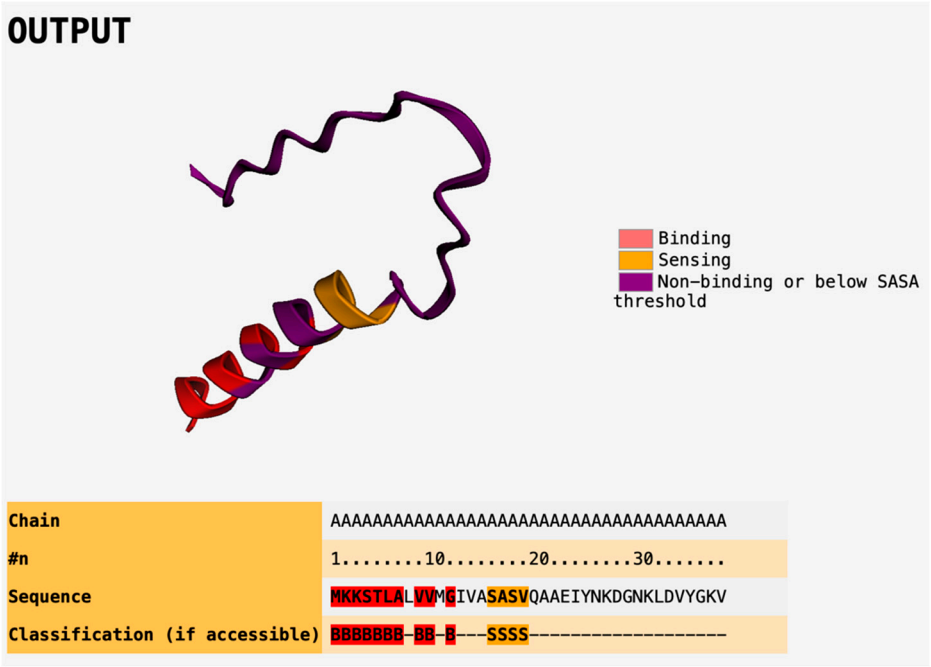


Figure C. Result of MKKSTLALVVMGIVASASVQAAEIYNKDGNKLDVYGKV using the PMI-Pred server and choosing negatively charged membrane. The visualization of the pdb file is made with Chimera X (https://www.rbvi.ucsf.edu/chimerax/