The efficacy of a commercially available tether molecule in creating a self-assembling monolayer.

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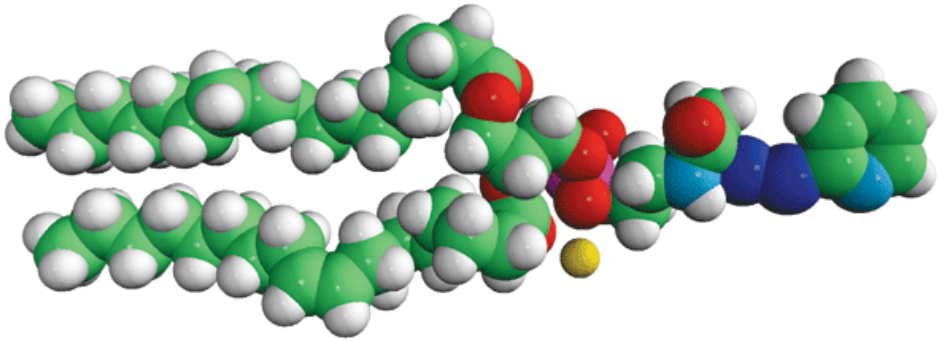
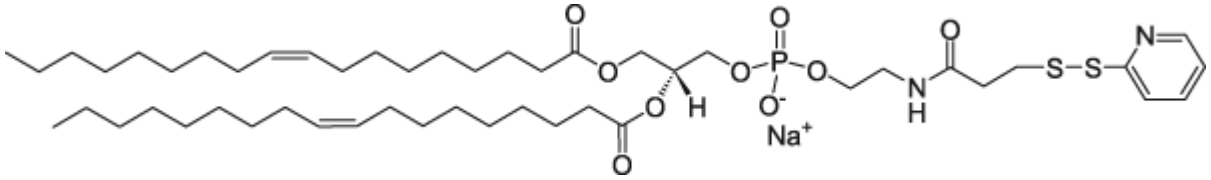
Project goal

- Test whether the tether molecule, **PDP PE**, can be used to create a self-assembling monolayer (SAM) on a gold substrate. Subsequently, a layer of lipids is deposited on top of the SAM to create a bilayer.





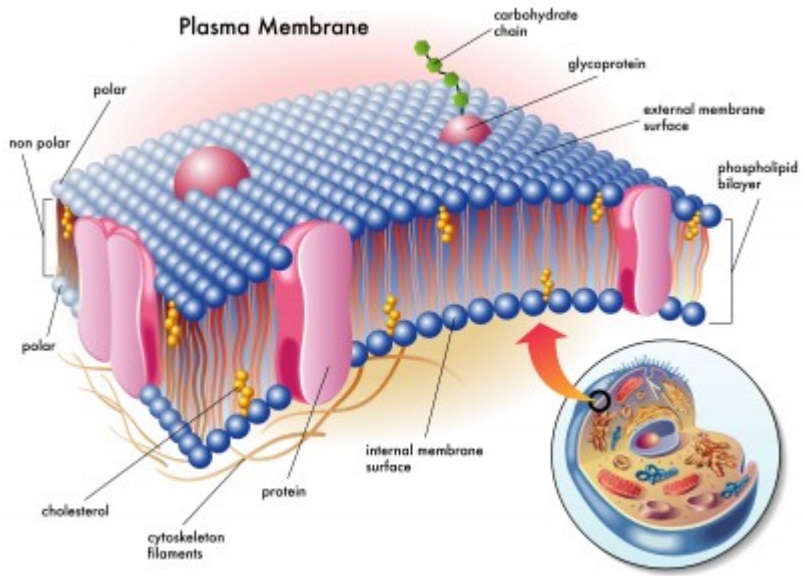
<u>PDP PE</u> (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio)propionate] (sodium salt))

Source: https://avantilipids.com/product/870202/

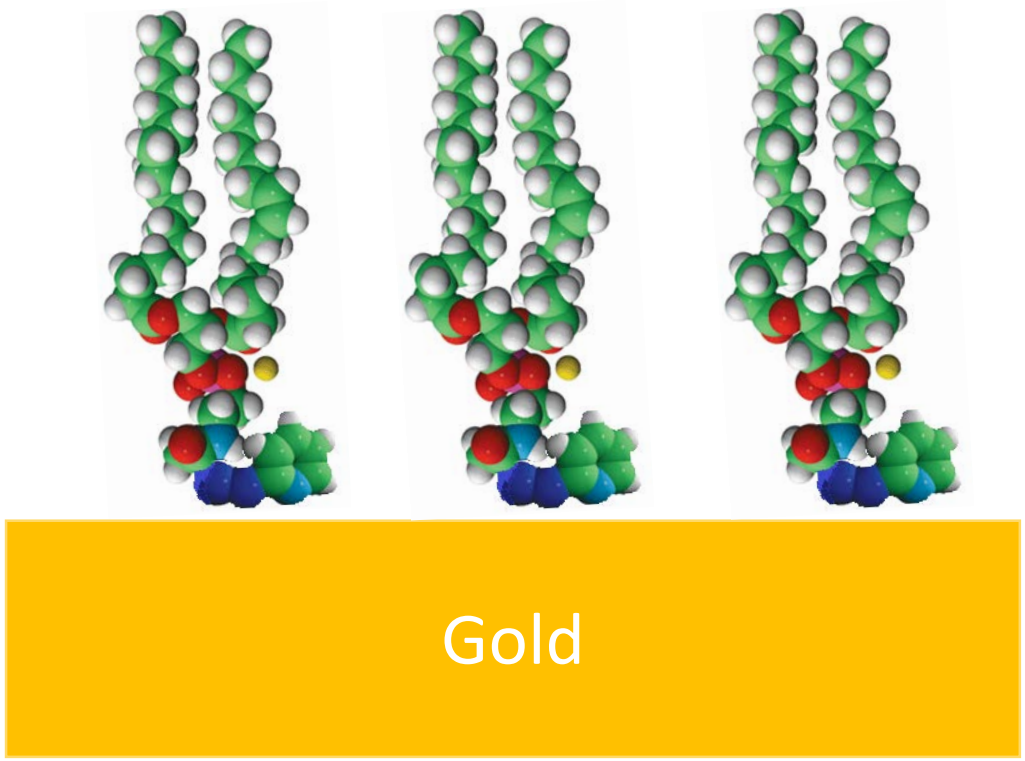
Rationale

- In organisms, lipids are a main component of biological membranes.

- Therefore, scientists can study characteristics of cell membranes if they can successfully create lipid bilayers in the lab.

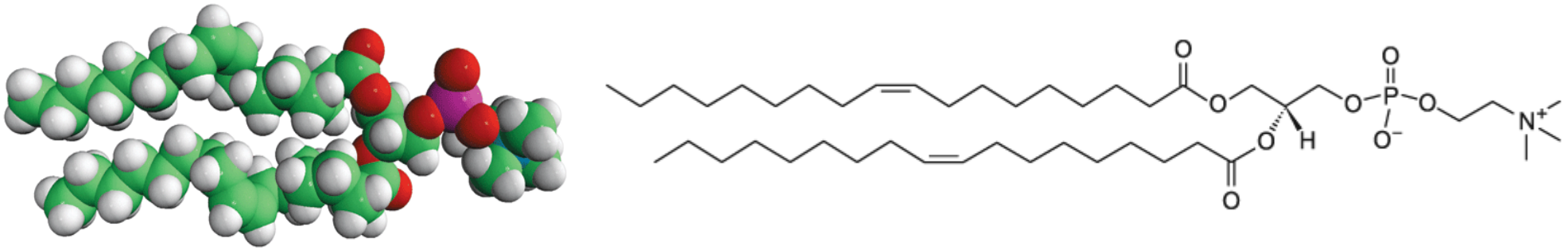


A **tether molecule**, like PDP PE, is one that attaches to a substrate, which in this case is gold. Tethering a membrane can increase its stability and allow for multiple scans to be completed.



Experiment #1 (cont.)

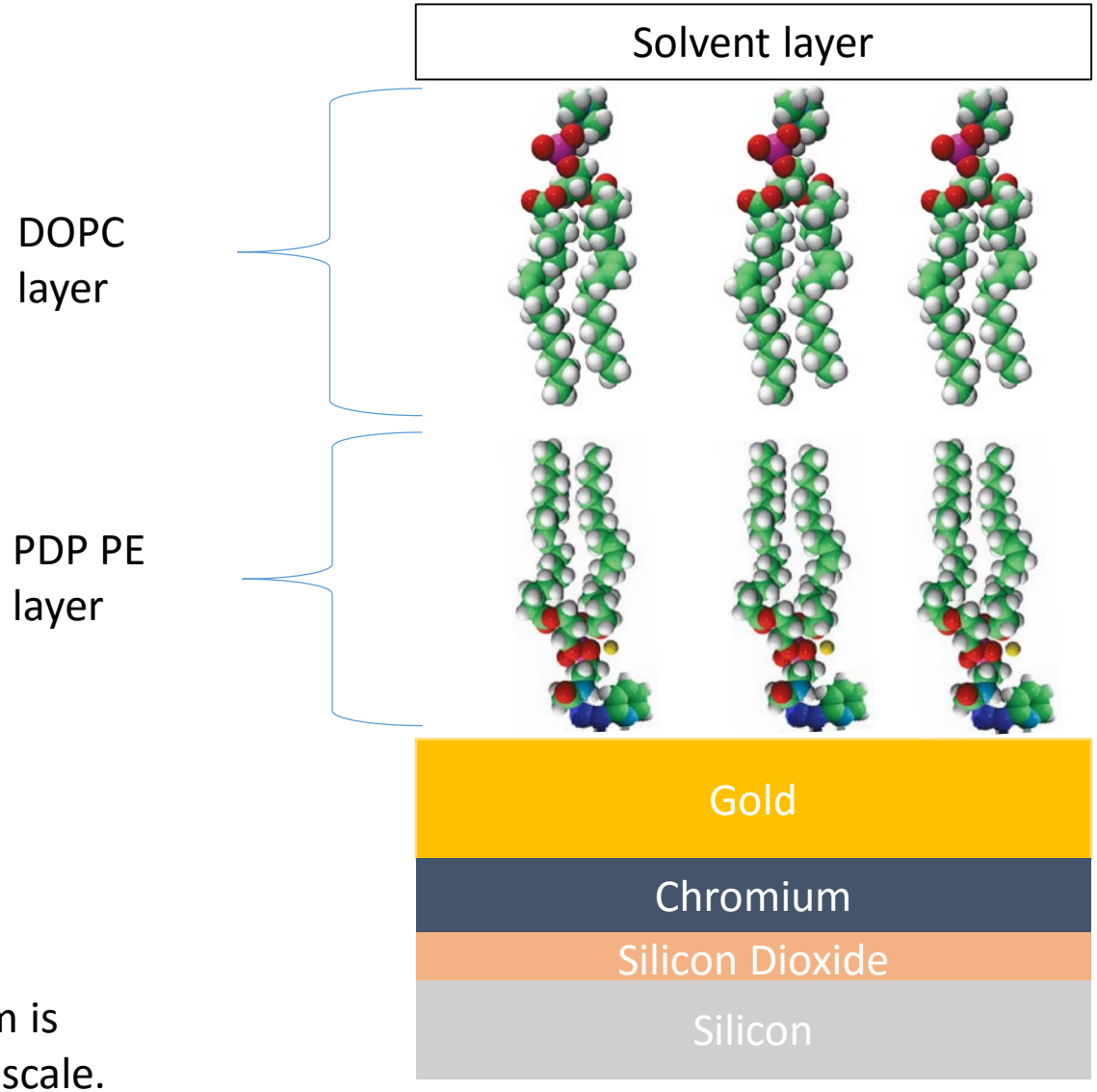
DOPC was used to complete the bilayer.



DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine)

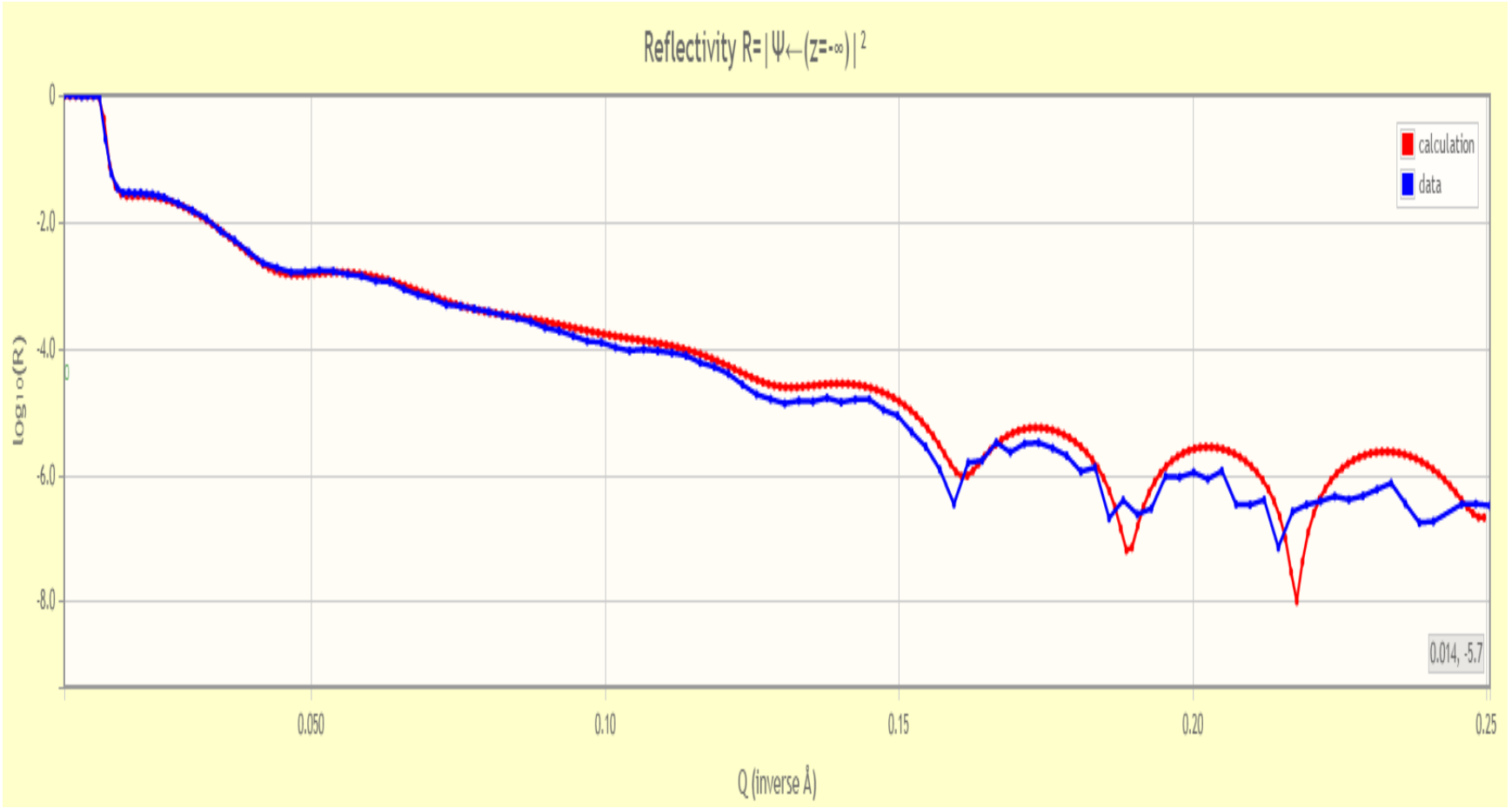
Image Source: https://avantilipids.com/product/850375/

Presumed Bilayer Composition for Experiment #1



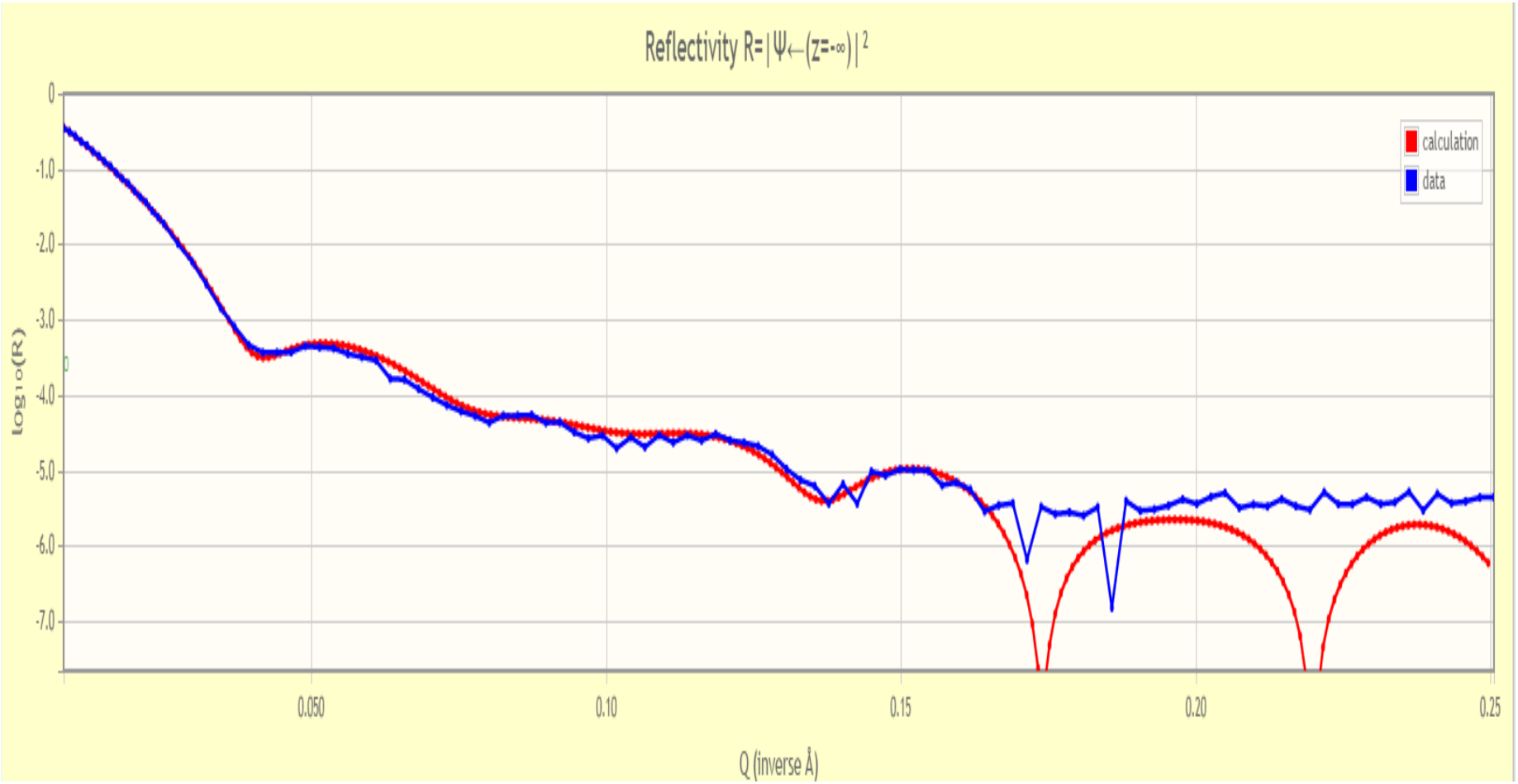
Note: Diagram is **not** drawn to scale.

Fitting of Data: Scan of Bilayer in D_20 Solvent



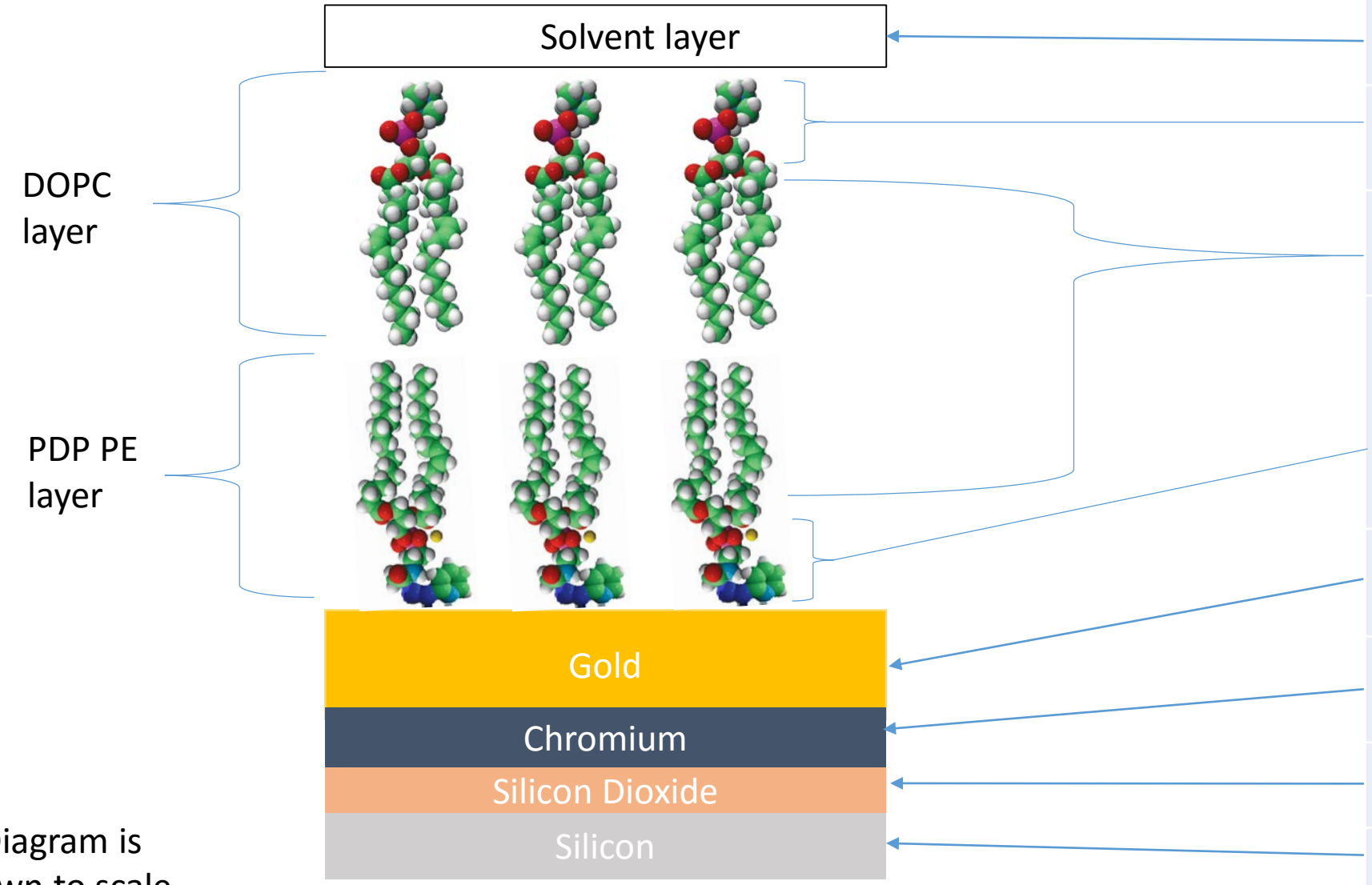
SLD ($x1e^{-6}$)	thickness (Å)
6.340	100.0
4.500	8.5
-0.300	28.5
3.000	11.0
4.400	142.5
3.030	22.0
3.510	8.5
2.070	100.0

Fitting of Data: Scan of Bilayer in H_20 Solvent



SLD ($x1e^{-6}$)	thickness (Å)
-0.560	100.0
0.500	8.5
-0.350	28.5
0.900	11.0
4.400	142.5
3.030	22.0
3.510	8.5
2.070	100.0

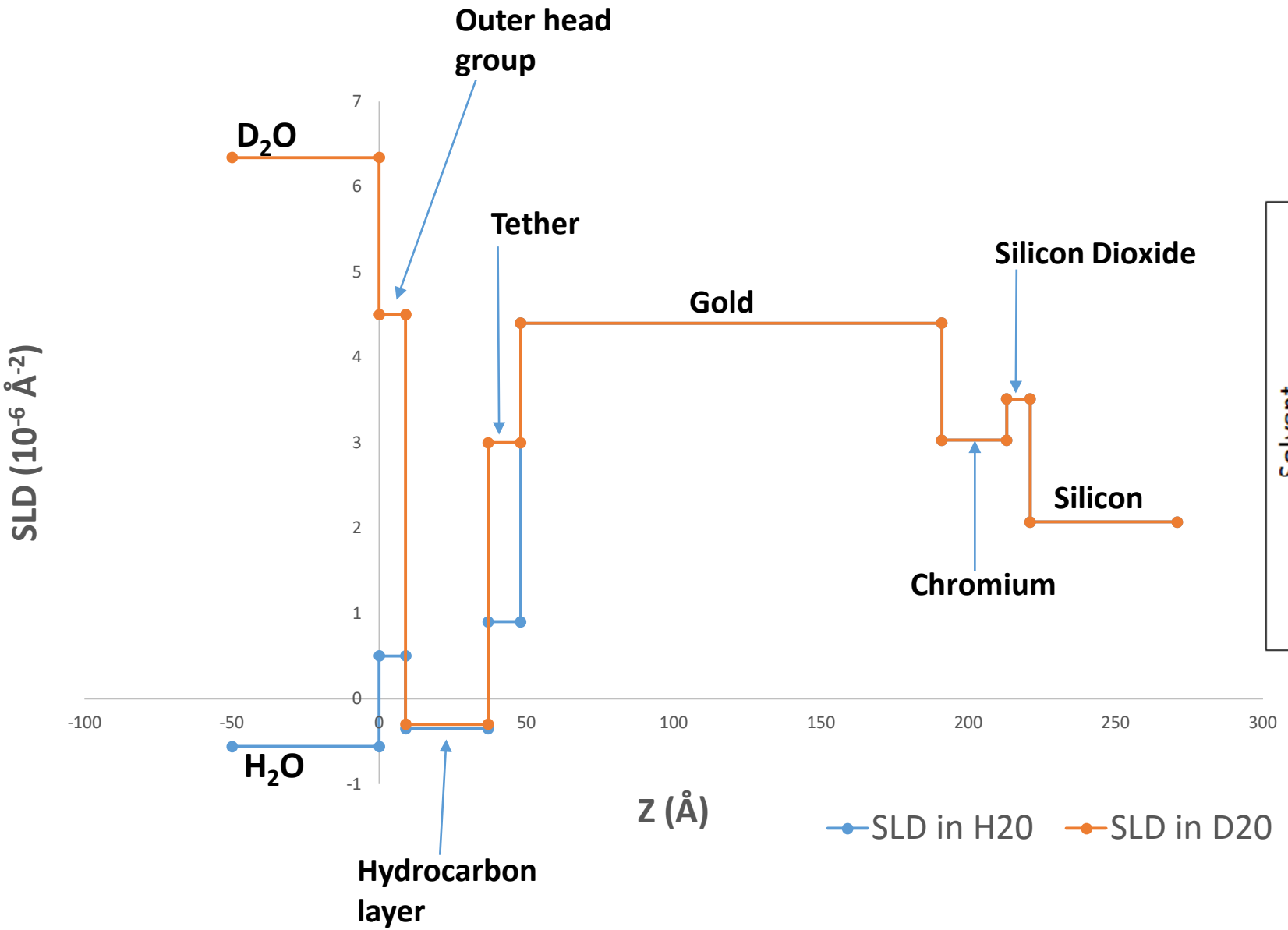
Conclusion of Bilayer Composition in Experiment #1

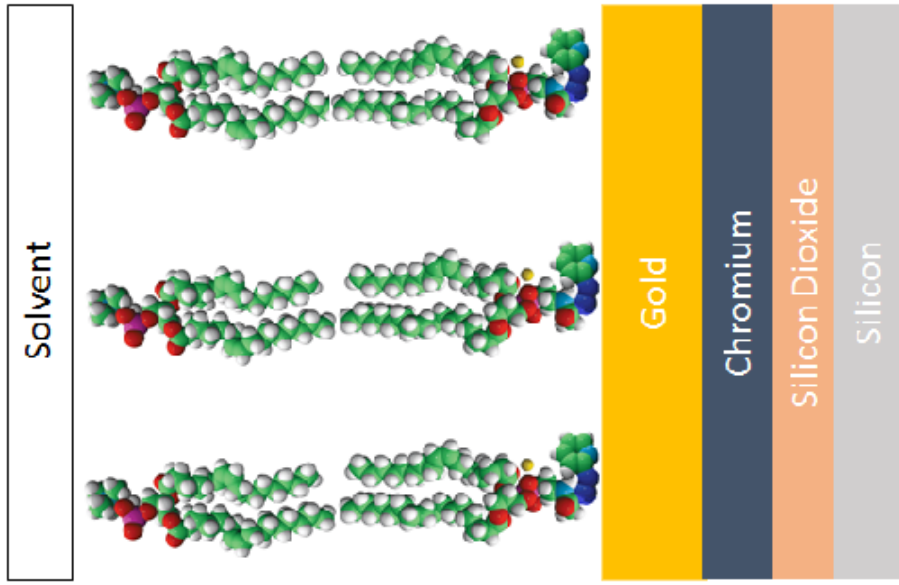


nSLD (x $1e^{-6}$)	Thickness (Å)
6.34 (D_2O) or -0.56 (H_2O)	NA
1.95	8.5
-0.6	28.5
1.57	11
4.4	142.5
3.03	22
3.51	8.5
2.07	NA

Note: Diagram is **not** drawn to scale.

Profile Comparison of Bilayer in D_20 and H_20





Comparing data from both fits to determine layer thicknesses

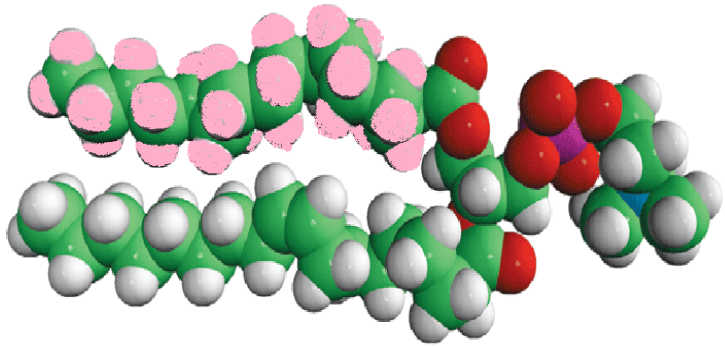
- Used the following equations that relate the volume fractions of materials in each layer to the SLD of the materials in each layer:

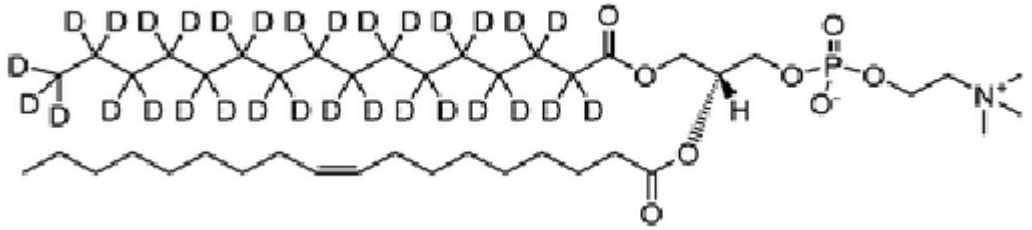
$$nSLD_{H2O} = f_m \cdot nSLD_m + (1-f_m) \cdot (-.56)$$
$$nSLD_{D2O} = f_m \cdot nSLD_m + (1-f_m) \cdot (6.34)$$

- It was calculated that the tether layer was 70% PDP PE and 30% solvent.

Experiment #2

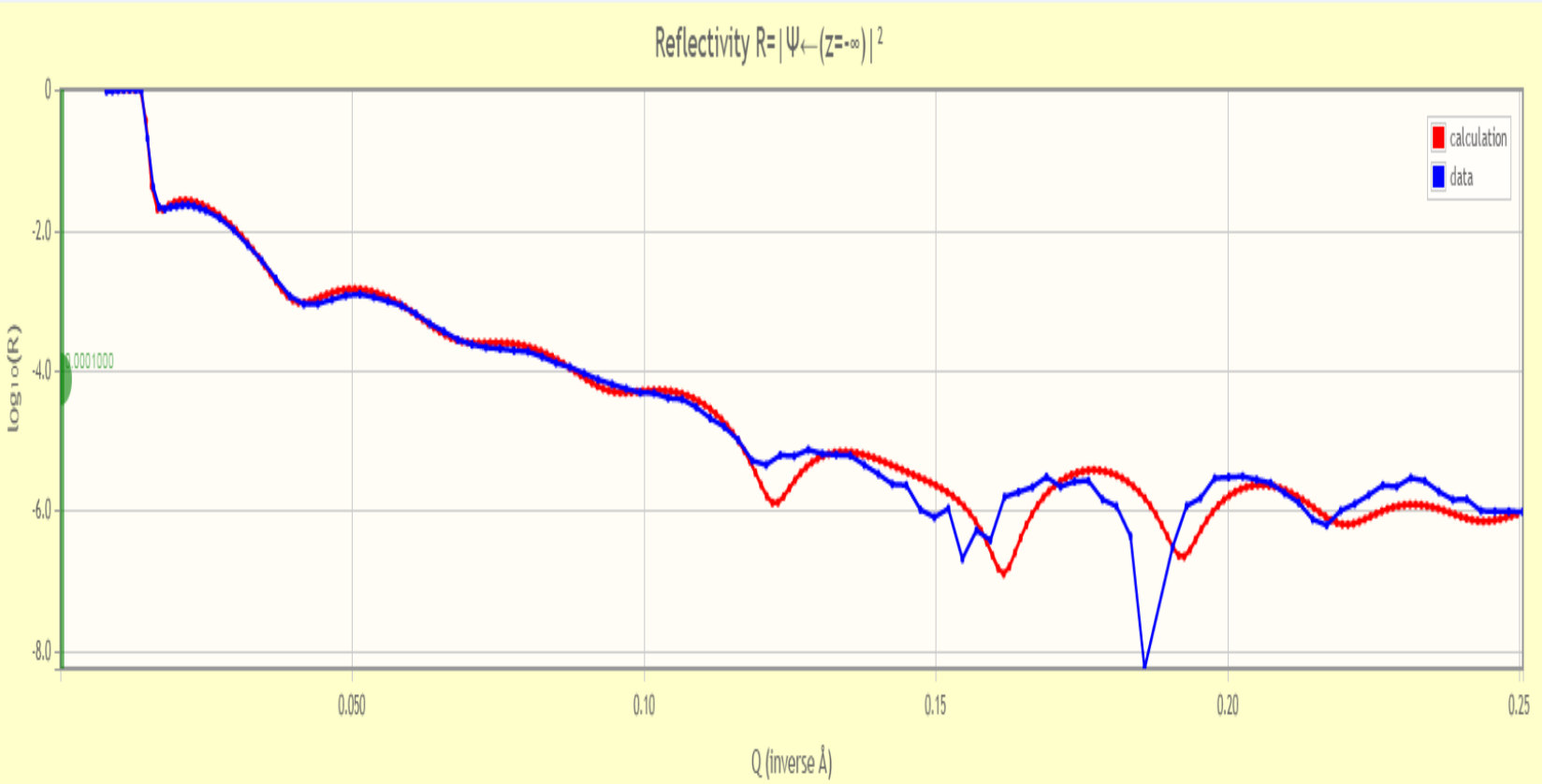
- The same **PDP PE lipid** was used for the tethered lower layer of the membrane and **tail-deuterated POPC** for the upper layer of the membrane. Using a deuterated lipid allows for its detection in the tether region.





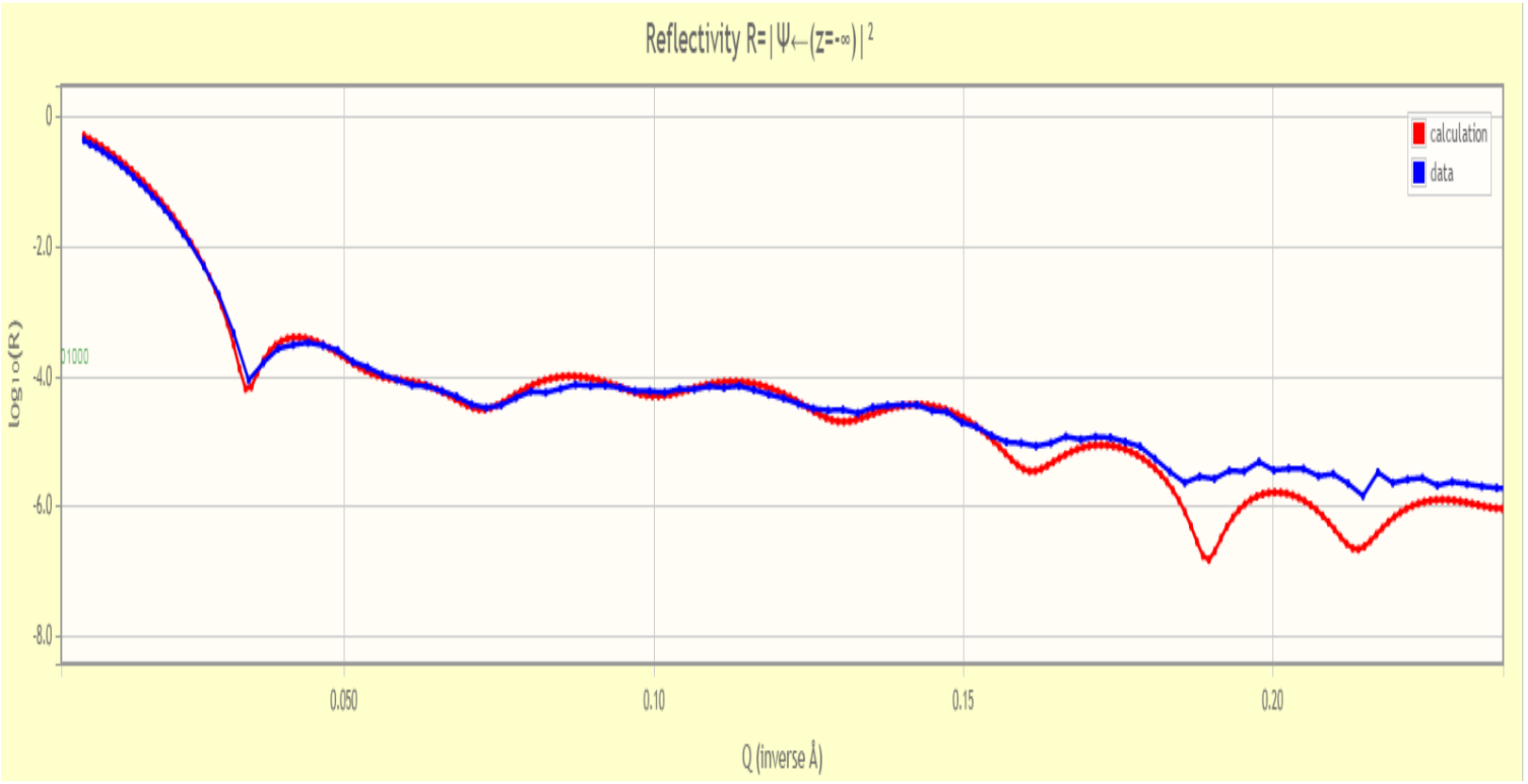
D31-POPC

Fitting of Data: Scan of Bilayer with Deuterated Lipid in D_20 Solvent



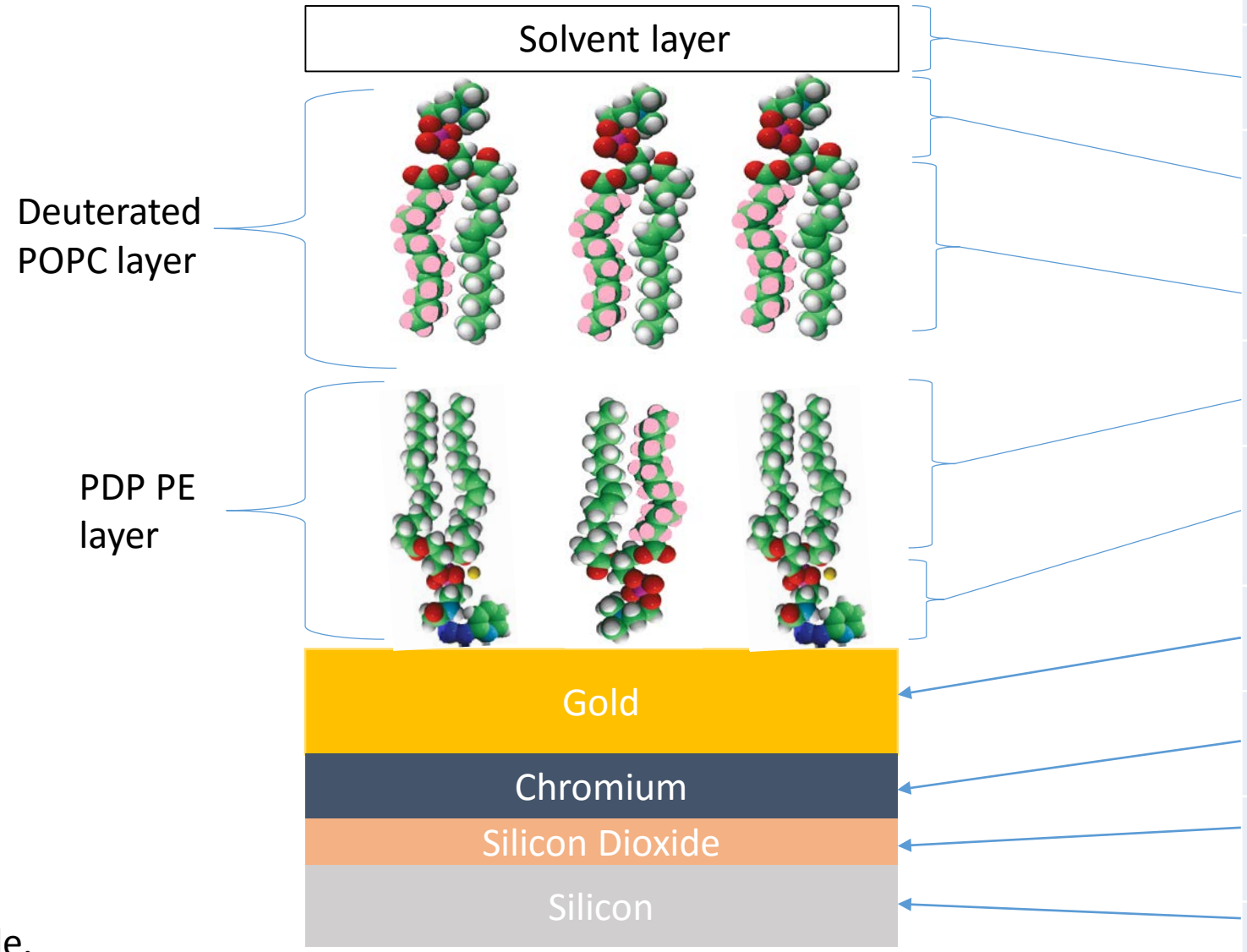
SLD ($x1e^{-6}$)	thickness (Å)
6.340	100.0
4.000	9.5
2.800	15.0
1.000	17.0
3.400	14.0
4.700	150.0
3.800	30.0
3.550	10.0
2.070	100.0

Fitting of Data: Scan of Bilayer with Deuterated Lipid in H_20 Solvent



SLD (x1e⁻⁶)	thickness (Å)
-0.460	100.0
0.600	9.5
3.200	15.0
0.800	17.0
1.600	14.0
4.700	150.0
3.800	30.0
3.550	10.0
2.070	100.0

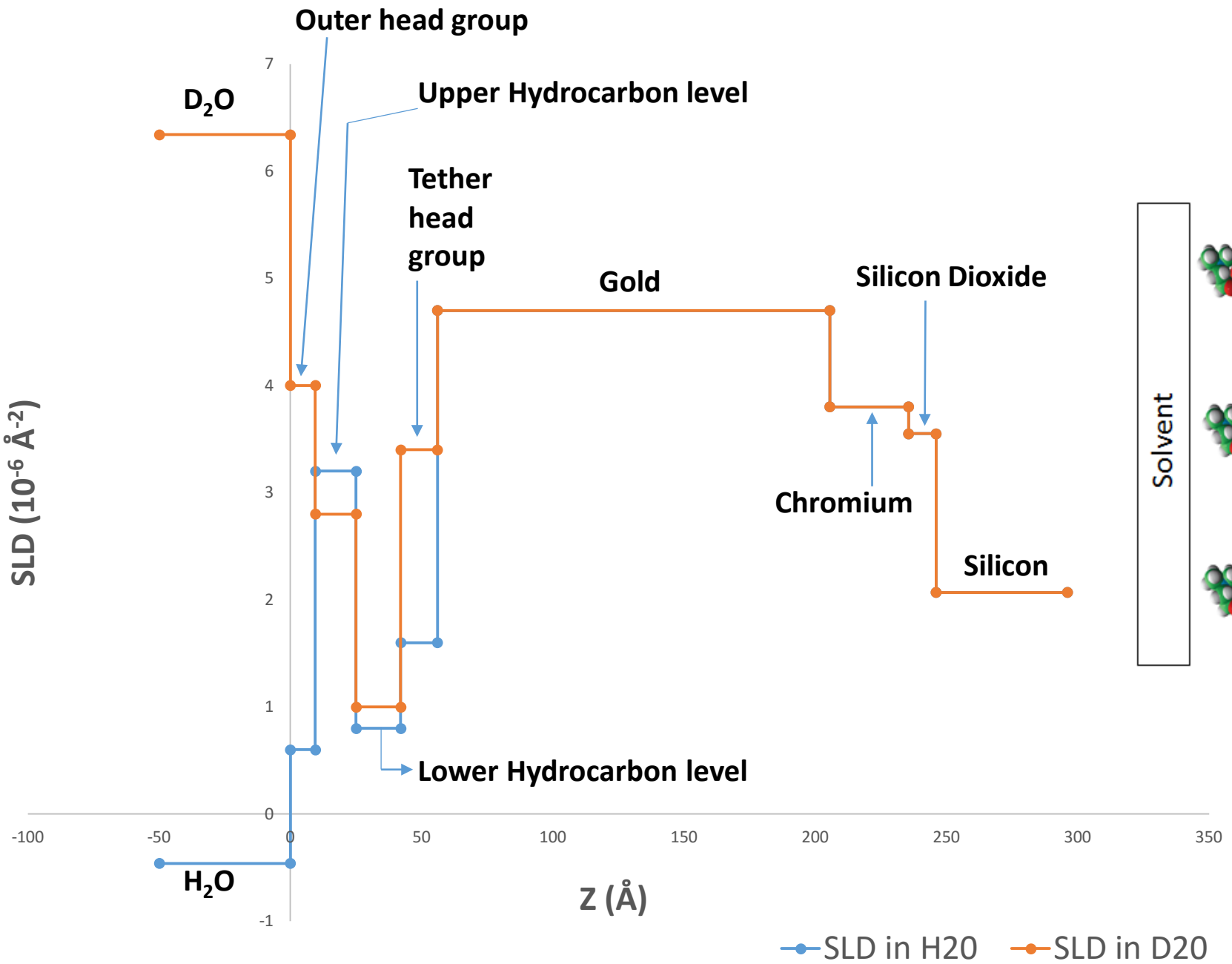
Conclusion of Bilayer composition for Experiment #2

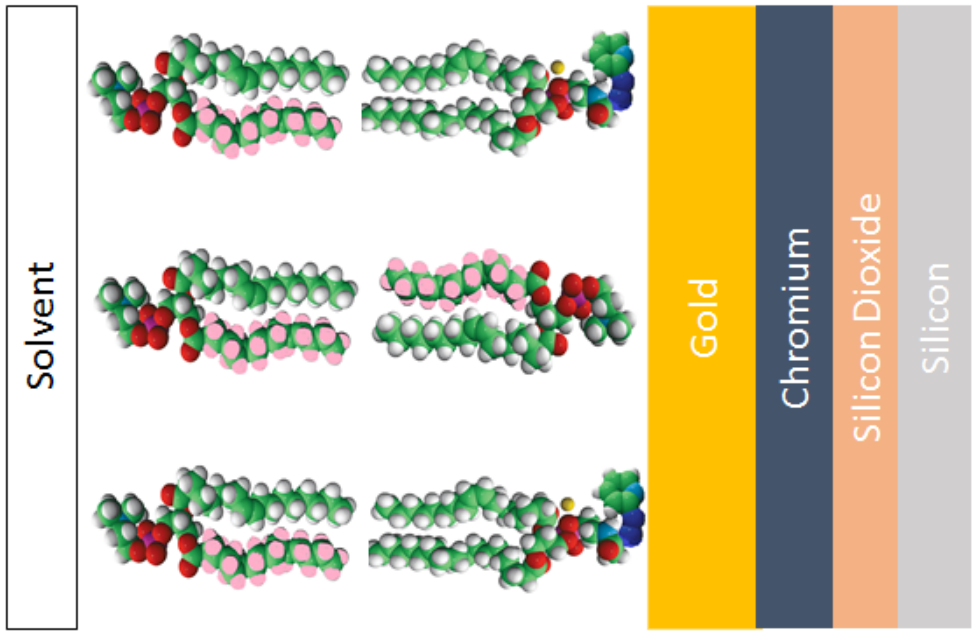


nSLD (x $1e^{-6}$)	Thickness (Å)
6.34 (D_2O) or -0.56 (H_2O)	NA
1.75	9.5
2.994	15
0.84	17
2.36	14
4.7	150
3.8	30
3.55	10
2.07	NA

Note: Diagram is **not** drawn to scale.

Profile Comparison of Bilayer with Deuterated Lipid in D_20 and H_20





Conclusions

- In both experiments, PDP PE formed a tethered SAM on the gold substrate.
- The thickness of the tethered SAMs was 11 and 14 Angstroms, for experiments 1 and 2 respectively, which is representative of tethered SAMs created in other experiments (Yap et al. 2014)
- The tethered SAM allowed for DOPC and deuterated POPC to arrange themselves to make a bilayer.

Conclusions

- Based on calculations comparing the SLDs of the layers in the second experiment, it was determined that there is 40% deuterated free lipid in the tether layer.
- This gives us an idea of how spaced out the tether molecules are on the gold substrate.
- This amount of free lipid in a tethered layer has been observed before when a solution of 25% tether and 75% competing molecule was used (Yap et al. 2014)

Back to the classroom: Takeaways

- I have new **experience** and knowledge of using neutron scattering tools that are used to confirm, refine, and expand what we know about biological membranes, proteins, and many other concepts in Science.
- I hadn't known before about this way in which Physics is used to learn about Biology. I will use this as yet another example of **creative thinking** in Science.
- I have learned just how much Chemistry, Biology, and Physics are required to work effectively as a Scientist at NCNR. It reinforces the **interdisciplinary nature of Science**.

Back to the classroom: Takeaways

- This helps me in instructing students about how **Science is integrated** and how they should look for patterns of overlap.
- This experience helps instruct about **tools used in verifying modeling** in Science that students can learn about.
- Students already learn about how the modeling of the cell membrane changed due to **improvements in technology** (electron microscopy). This continues the discussion of modeling the cell membrane even further.

Back to the classroom: Takeaways

- Students need to experience and understand more authentically the "**intellectual struggle**" inherent in Science. Students need to be able to fail and retry an experiment.
- Many times, lab experiences are set up to succeed, as the curriculum doesn't usually have time built in for repeated attempts. The **reflection** on, and repeat (or tweaking) of, an experiment is a excellent learning opportunity.
- **Scientists collaborate** and students work together also. I need to make sure that students collaborate and bring their own ideas together in meaning discourse, and not just for information sharing.

Back to the classroom: Takeaways

- The quality to the Scientific conclusion is directly proportional to the **quality of the experimentation** conducted (precision of measurements and proper handling of materials, methodology used, etc.).
- **Conducting scientific research is challenging**, but, with patience, can be very rewarding.

Thank you!

- Projects completed with the patient support of David Hoogerheide and Frank Heinrich.
- Thank you also to Yamali, Brian J., and Mikala.

Citation

Thai Leong Yap, Zhiping Jiang, Frank Heinrich, James M. Gruschus, Candace M. Pfefferkorn, Marilia Barros, Joseph E. Curtis, Ellen Sidransky, and Jennifer C. Lee. (2014). Structural Features of Membrane-bound Glucocerebrosidase and α-Synuclein Probed by Neutron Reflectometry and Fluorescence Spectroscopy: J Biol Chem, 290(2), 744–754.
doi: 10.1074/jbc.M114.610584