ERC TeleSeminar Series

Carbon-based nanoparticle ESH : dispersibility and aggregation in lipid membranes

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Outline

1. Experiments on “real” systems (cell or animal studies)
2. Idea of a model system
3. Computer (molecular) models

one outcome: appreciation for the complexity of the problem

Arch Toxicol (2012) 86:1809–1827, Trpkovic et al.
DOI 10.1007/s00204-012-0859-6

REVIEW ARTICLE

Toxicity of pristine versus functionalized fullerenes: mechanisms of cell damage and the role of oxidative stress

C_{60} is able to generate highly reactive oxygen species (ROS = free radicals) after excitation by visible or UV light. Also some C_{60} preparations are able to kill cells even in the absence of light.

But C_{60} can also have protective antioxidant effects.

It seems safe to conclude that the differences in cytotoxic potency and underlying mechanisms displayed by various fullerene preparations are mainly due to some physico-chemical characteristics, such as particle size (surface/volume ratio), surface charge, and aggregation properties.

It is presently unrealistic to make definite conclusions about their toxicological behavior.

It appears that most of the pristine and functionalized fullerene preparations are not overtly toxic unless photo-excited or used at very high concentrations that are unlikely to be encountered environmentally.

Advanced Drug Delivery Reviews 64 (2012) 1694–1699, Boczkowski et al.

Respiratory toxicities of nanomaterials — A focus on carbon nanotubes

review on lung toxicity of CNT

CNT, when in suspension in the air, form an aerosol that can be deposited throughout the lungs.

Respiratory exposure to CNT is often followed by
- formation of multifocal granulomas
- development of pulmonary fibrosis.

Oxidative stress (more oxidant production than antioxidants defense) is proposed to be a major mechanism underlying CNT's biological effects.

Importantly, biodegraded nanotubes did not generate an inflammatory response when aspirated into the lungs of mice, suggesting that degradation by peroxidase attenuated CNT toxicity.

From a limited number of studies, CNT can be broken down and eliminated from the lung before translocating to other organs (liver, heart, spleen, etc).

Advanced Drug Delivery Reviews 64 (2012) 1694–1699, Boczkowski et al.

Respiratory toxicities of nanomaterials — A focus on carbon nanotubes

review on lung toxicity of CNT

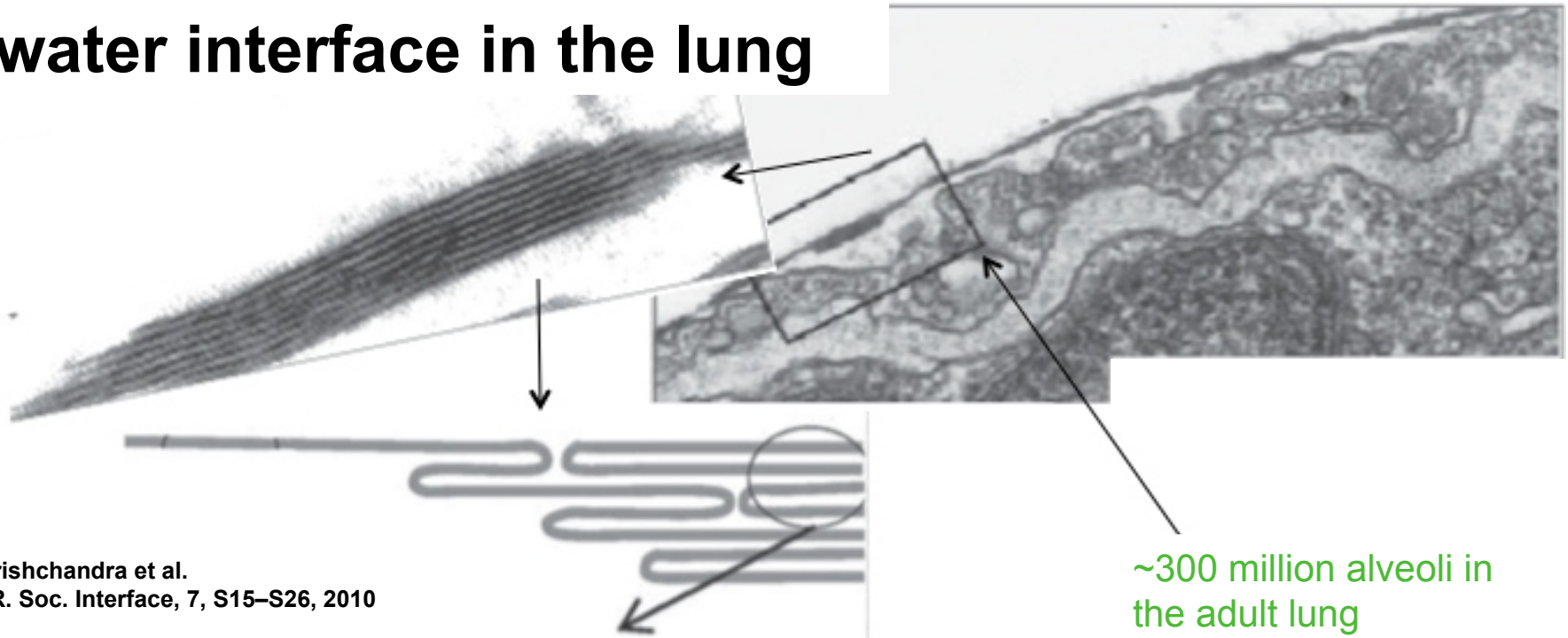
Overall, protein corona of CNT appears as a key mechanism for modulating, in both advantageous and deleterious way, their biological effects.

Several physico-chemical factors (length, surface properties, etc.) of CNT are major determinants of their subsequent biological effects.

The question of the dispersion and subsequent potency for CNT to form aggregates in solution is often proposed as an important determinant of their biological effects. What is currently believed is that well dispersed CNT preferentially induce the development of fibrosis, whereas less dispersed CNT lead to the formation of granuloma.

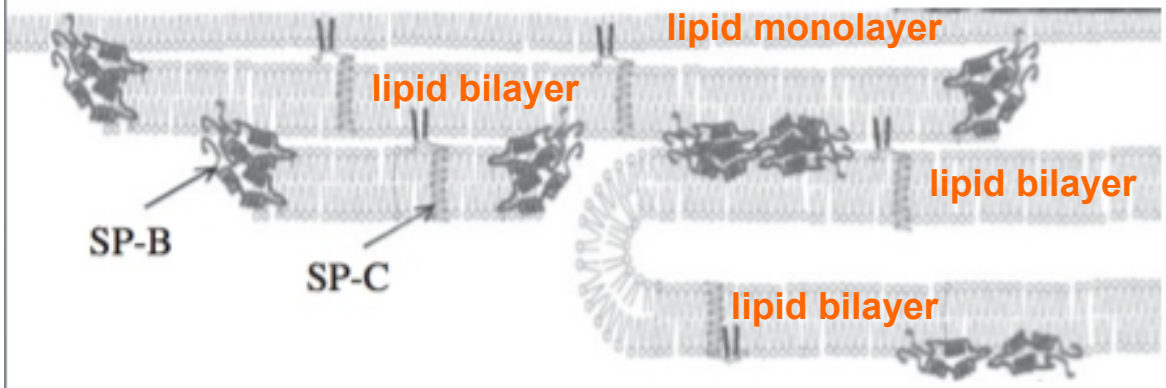
One common thread that emerges: toxicity *in vivo* is modulated by the aggregation of the nanomaterial.

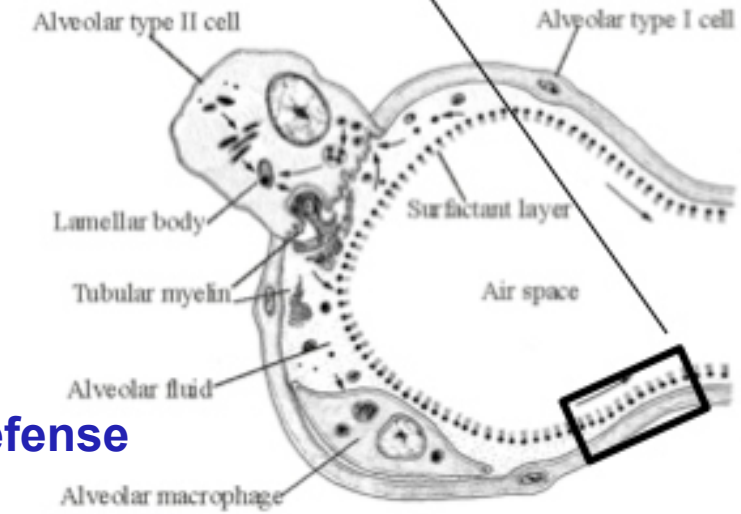
Air/water interface in the lung



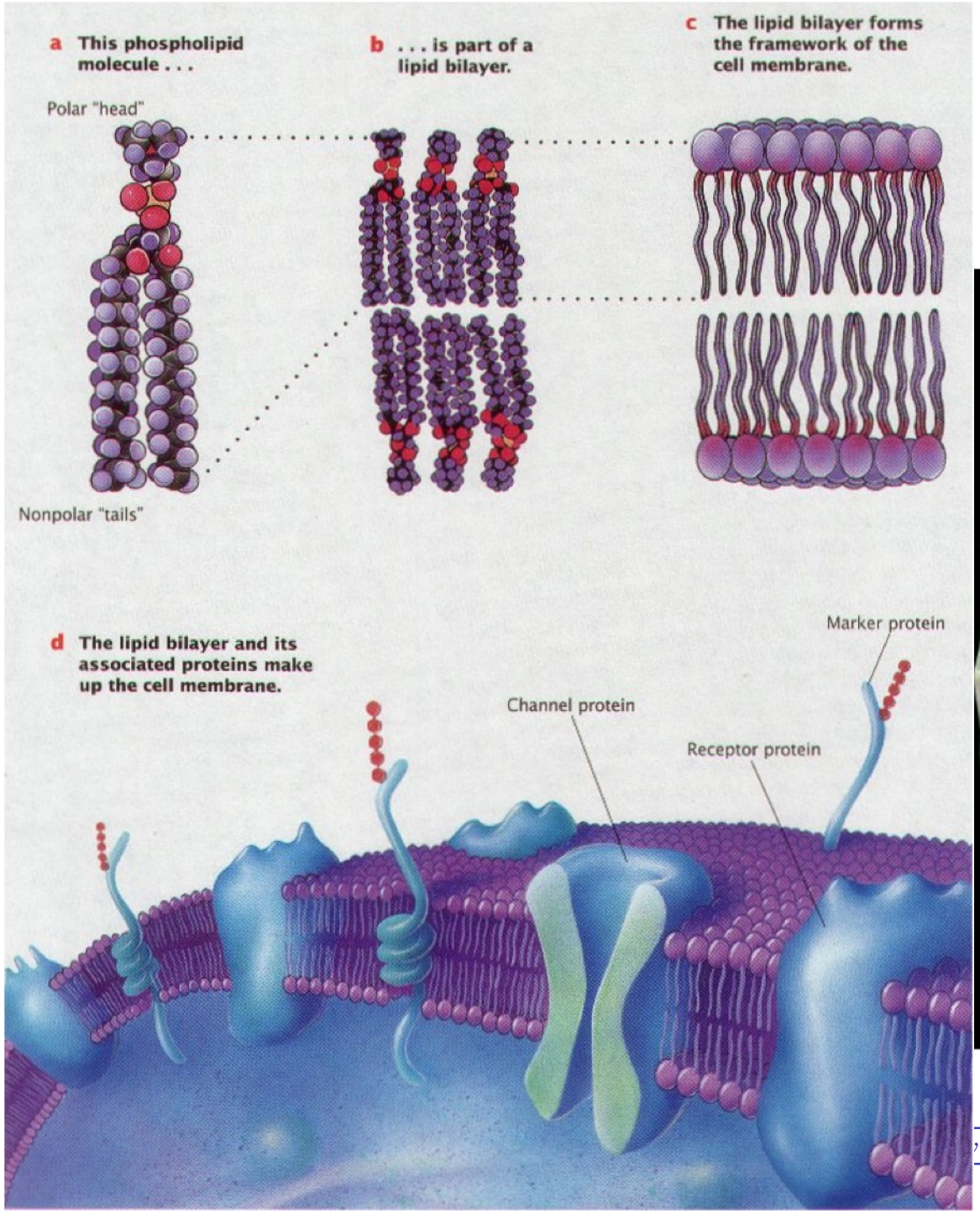
Harishchandra et al.
J. R. Soc. Interface, 7, S15–S26, 2010

~300 million alveoli in the adult lung





One of the most important functions of the lung surfactant monolayer is to form the first line of defense against inhaled aerosols such as nanoparticles.



Lipid monolayer or bilayer membranes surround each cell and organelle.

Idea of a model system

Choose a key concept, which we will take as:

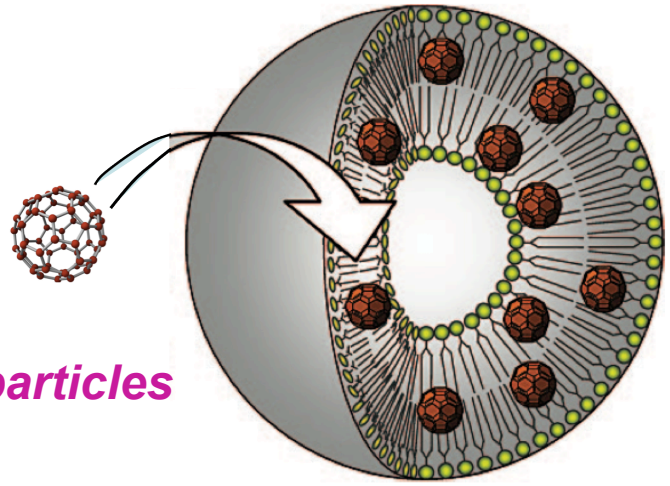
> **One common thread that emerges: toxicity *in vivo* is modulated by the aggregation of the nanomaterial.**

and use a model system to investigate this concept more thoroughly.

A model system only contains a few selected components in order to study a phenomena at its most basic level.

Focus on carbon-based nanoparticle behavior (dispersibility and aggregation) in lipid membranes

components: lipids and nanoparticles



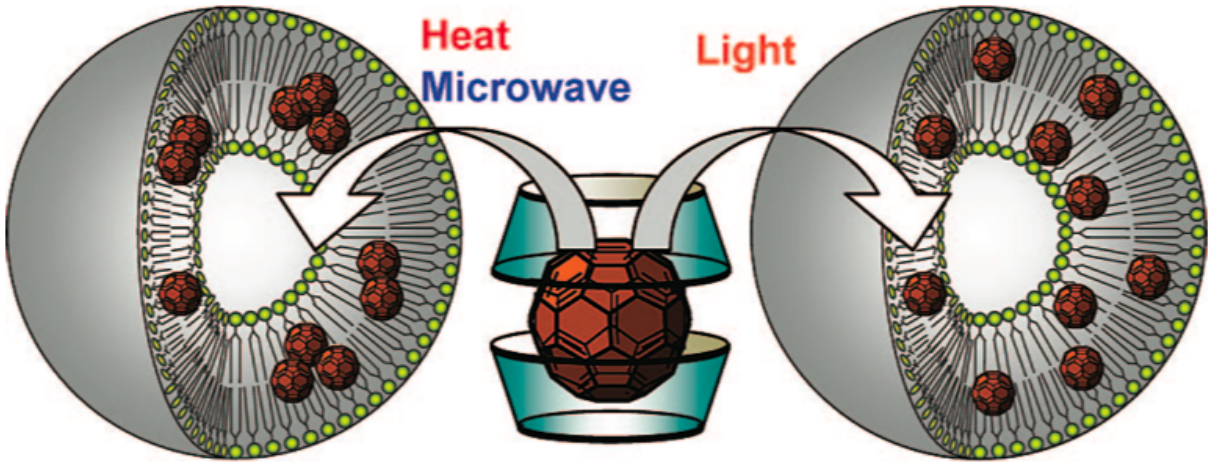
Simplest(?) question:

Do carbon-based nanoparticles aggregate in lipid membranes? Under what conditions?

One of the most careful series of studies is being conducted by Prof. Atsushi Ikeda.

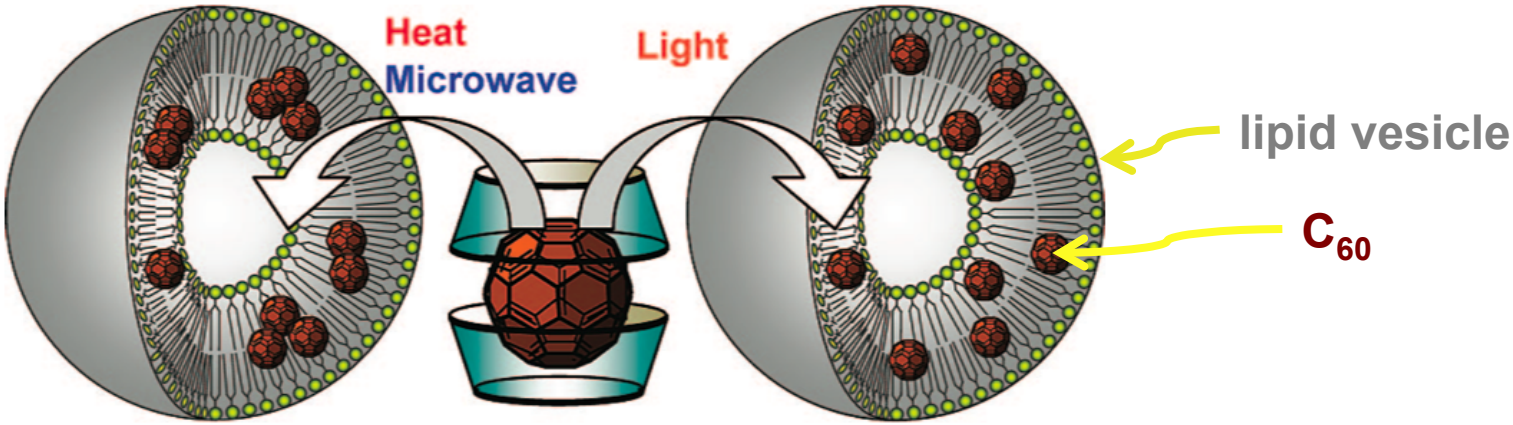
His motivation is a little different. He is interested in the ability of C_{60} to generate highly reactive oxygen species for potential use as photosensitisers for photodynamic therapy.

For this application, aggregation is undesirable because of self-quenching.



Simplest(?) question:

Do carbon-based nanoparticles aggregate in lipid membranes?



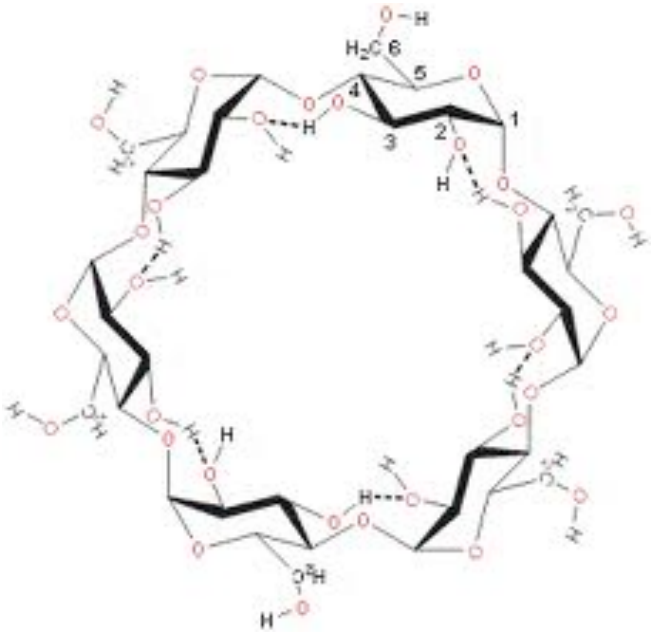
Answer: seems to depend on the preparation procedure.

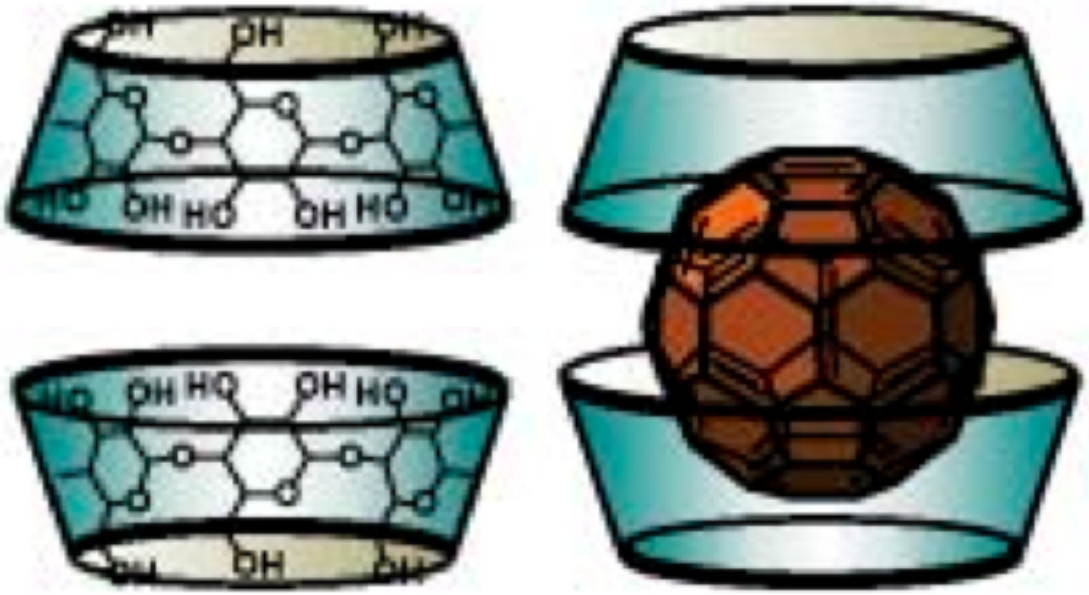
This preparation method has two steps:

1. **assembly of lipid bilayer vesicles**
2. **transfer of C_{60} into the bilayer of the vesicles.**

The transfer is done by using a molecular exchange reaction from a water-soluble host molecule (cyclodextrin)

Cyclodextrin host molecule makes an unstable host-guest complex (cyclodextrin-bicapped fullerene)

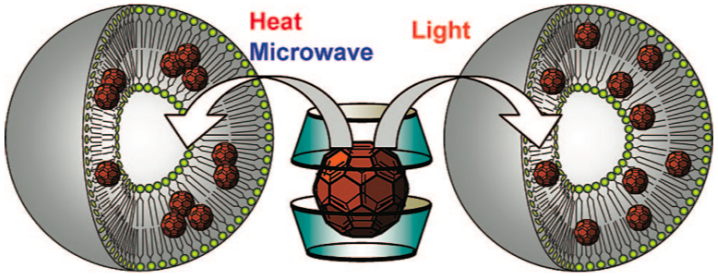




Upon a trigger event, the unstable complex dissociates and the C_{60} can be transferred to a more stable location, namely inside the lipid bilayer.

The trigger can be:

1. **a photo-trigger**
2. **heat**
3. **microwave irradiation**



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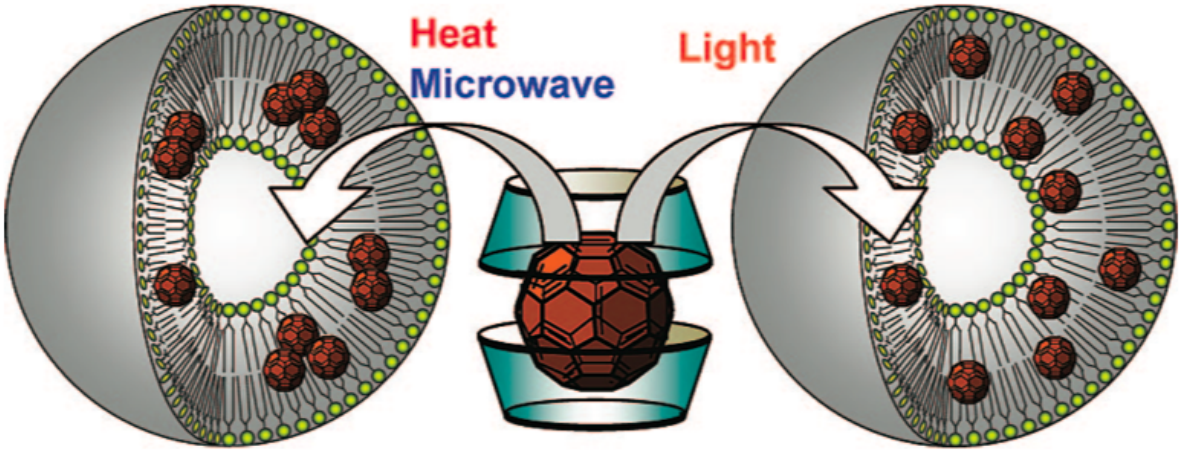
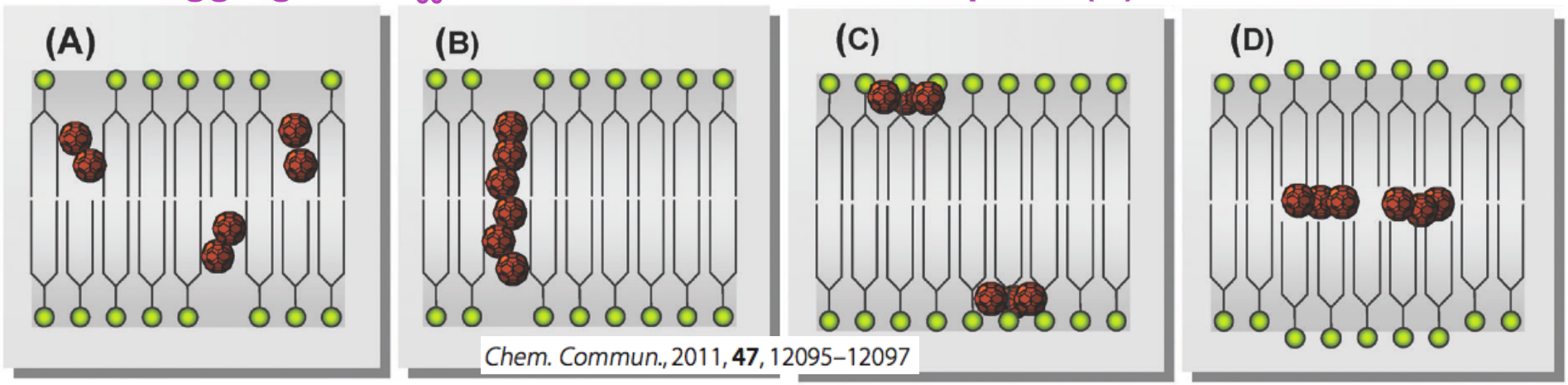


Outcome:

- **photo-induced transfer results in dispersed C_{60}**
- **heating or microwave-induced transfer results in aggregated C_{60}**
- **heating the photo-induced dispersion results in aggregated C_{60}**

ORGANIC LETTERS
2008
Vol. 10, No. 18
4077–4080

For the aggregated C_{60}, it is found consistent with panel (D) based on NMR data



Chem. Commun., 2011, **47**, 12095–12097

In panel (D) C_{60} contacts the lipid alkyl chains to the least degree

Computer (molecular) models: can study the model system in its "pure" form

no need for a host (cyclodextrin),
no possible contaminants (organic solvent),
no need to use a trigger, …

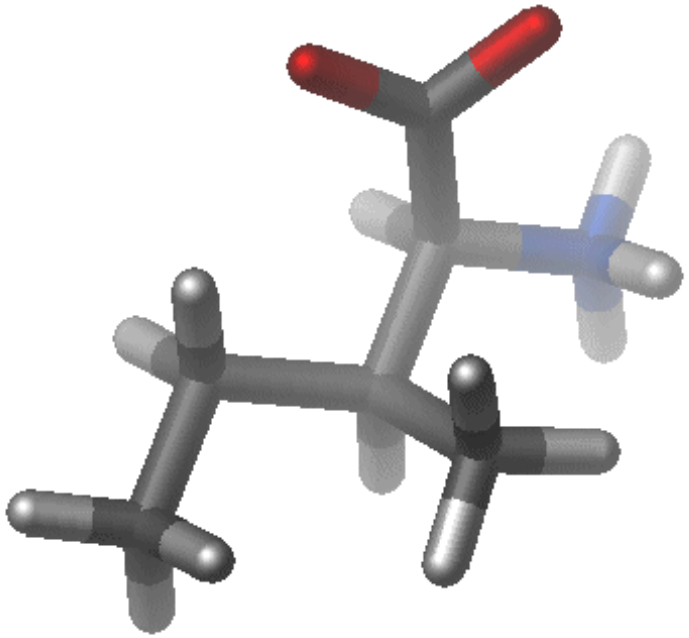
In computer modeling, the fundamental ingredients are:

1. **a mapping that assigns an energy to each chemical molecular structure**
2. **an algorithm for updating the chemical structure as times evolves**

With computer modeling, we can simulate the behavior of chemical systems, specifically nanoparticle behavior in lipid membranes.

The Force Field

The structure-energy relationship is encoded in a Force Field. The energy has contributions from different components.



$$V(r) = V_{bonded} + V_{non\text{-}bonded}$$

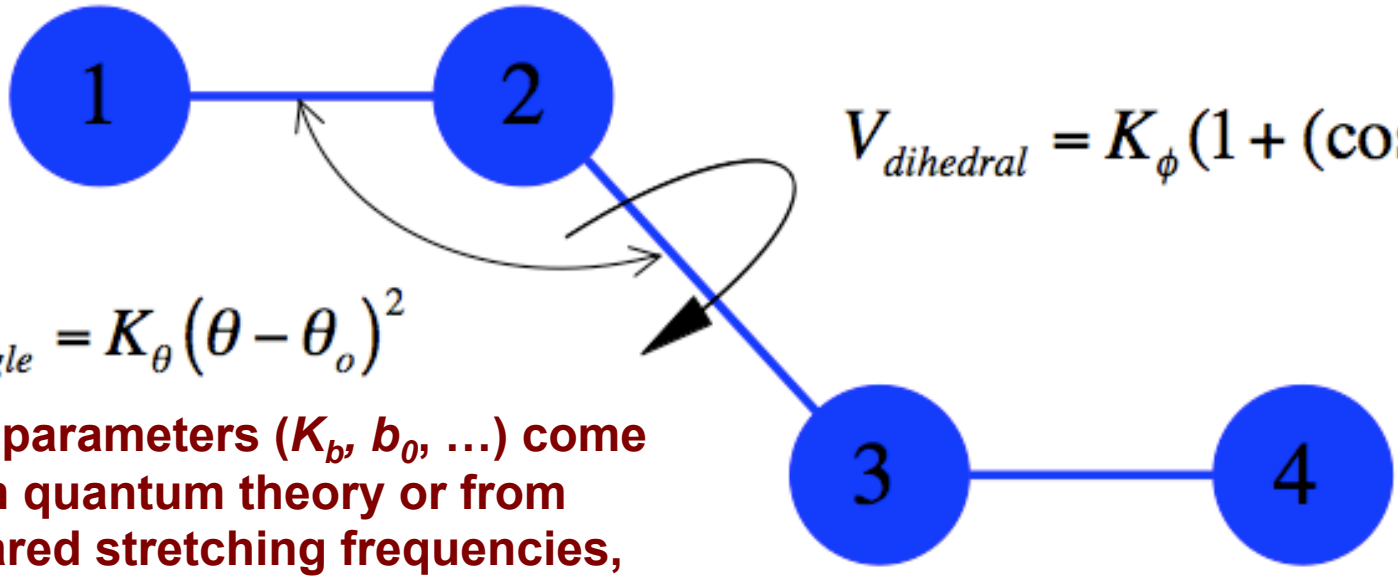
$$V_{bonded} = V_{bond\text{-}stretch} + V_{angle\text{-}bend} + V_{dihedral}$$

Diagram of bonded energy terms

$$V_{bond} = K_b(b - b_o)^2$$

K_b = bond strength

b_0 = bond length



$$V_{dihedral} = K_\phi(1 + (\cos n\phi - \delta))$$

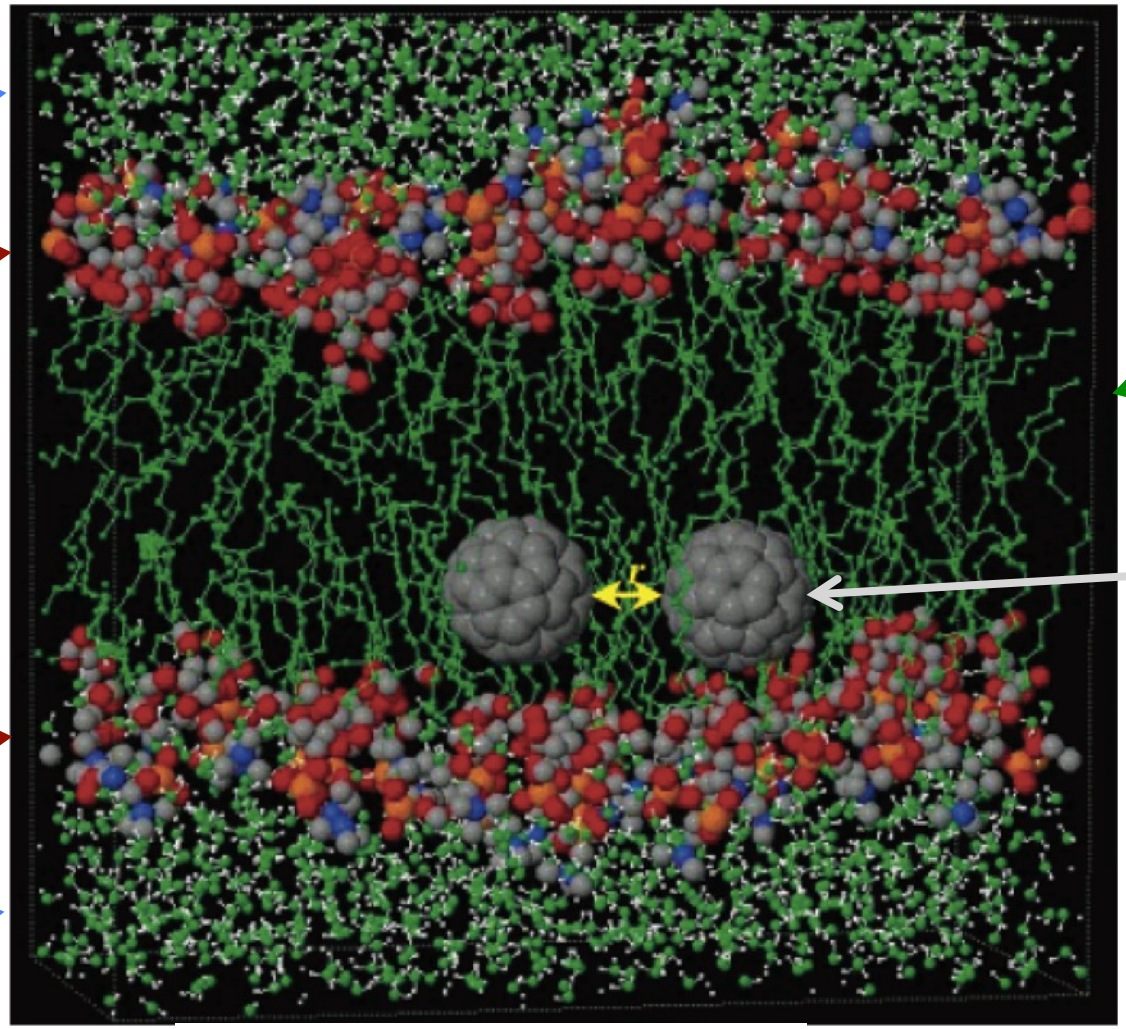
$$V_{angle} = K_\theta(\theta - \theta_o)^2$$

The parameters (K_b, b_0, …) come from quantum theory or from infrared stretching frequencies, crystal structures, microwave data, NMR data, …

There are different Force Fields for different applications

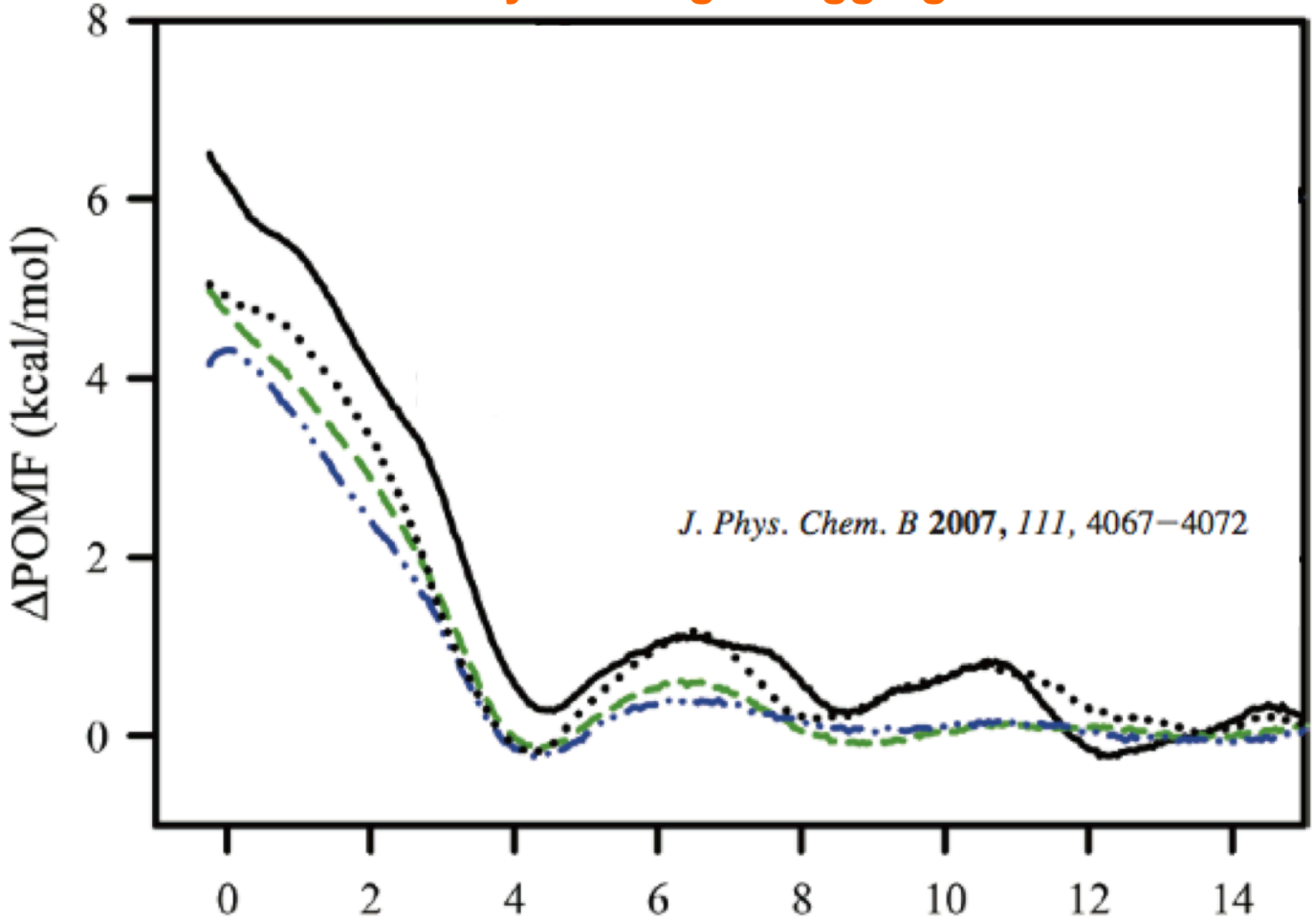
One of the most successful ones is CHARMM. It is designed to study proteins, nucleic acids, lipids, carbohydrates, and drug-like molecules.

One of the first attempts to look at C_{60} behavior in a lipid bilayer membrane was done by Prof. G. Smith of the University of Utah.



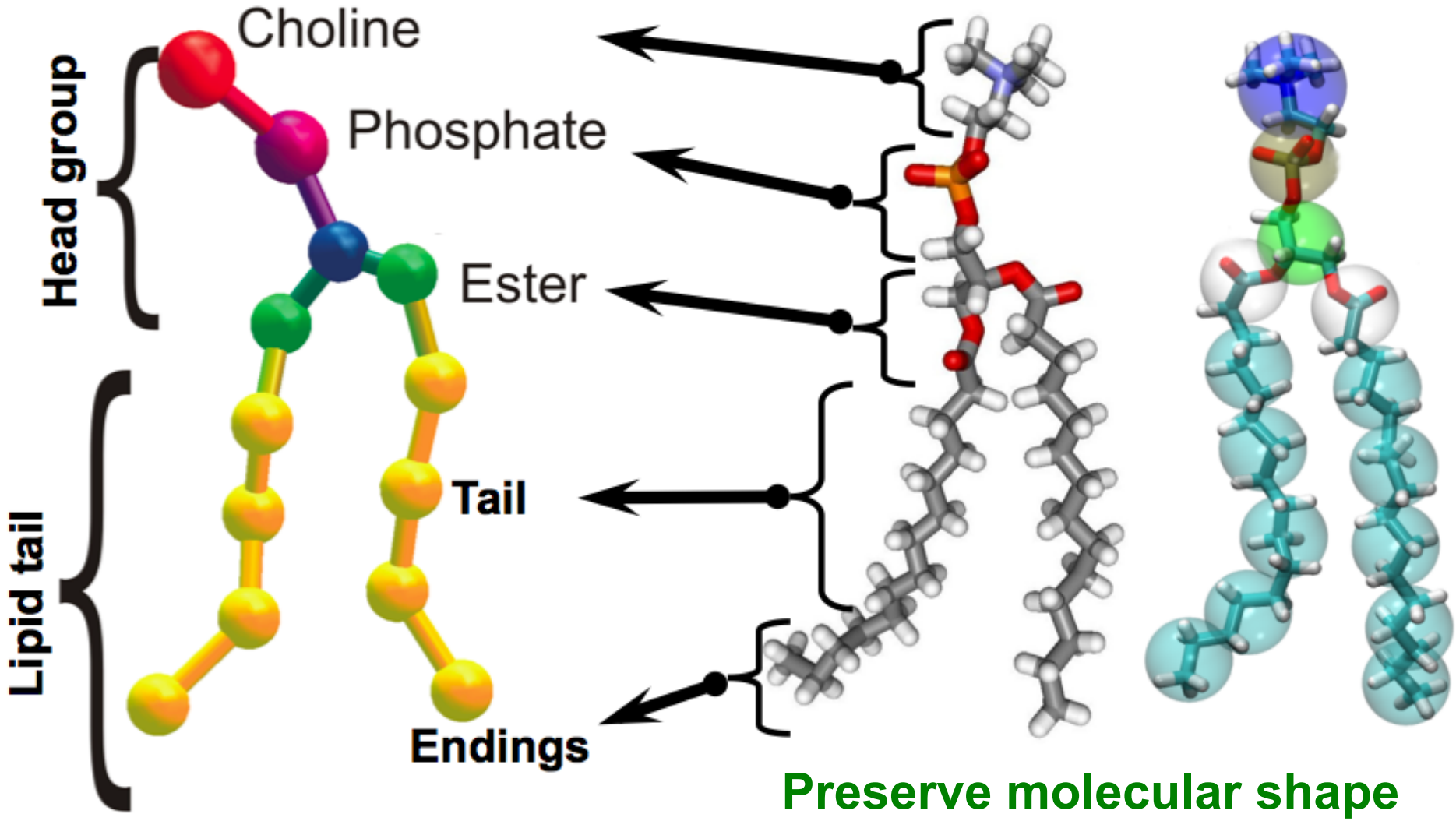
J. Phys. Chem. B **2007**, *111*, 4067−4072

Smith found that the dimerization energy of two C_{60} molecules in a lipid bilayer is ~ 0 (solid line). Therefore no compelling evidence for aggregation.
But he couldn't simulate many C_{60}'s with the available computer resources and hence could not directly investigate aggregation.



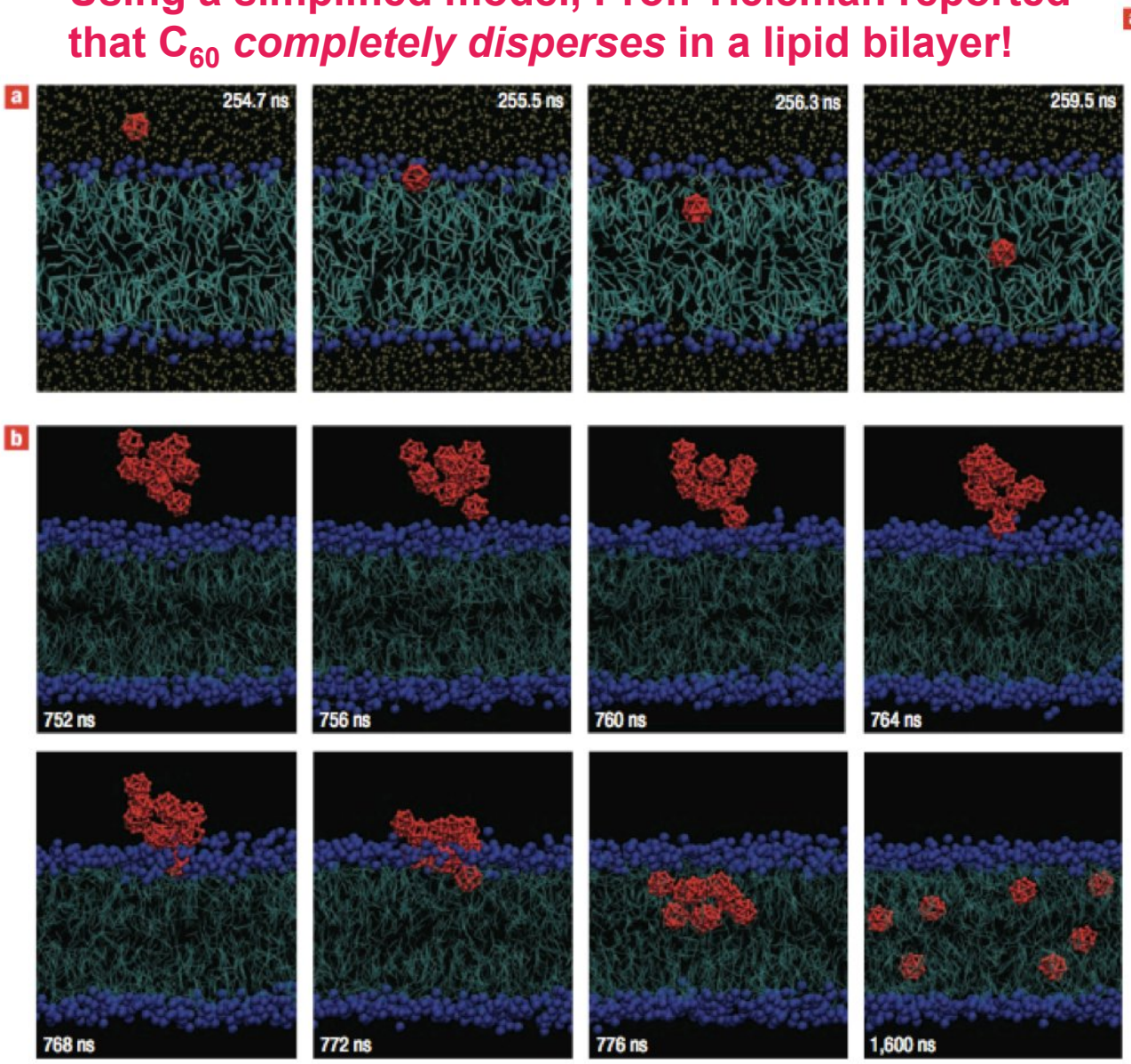
r (Å) = distance between C_{60} molecules

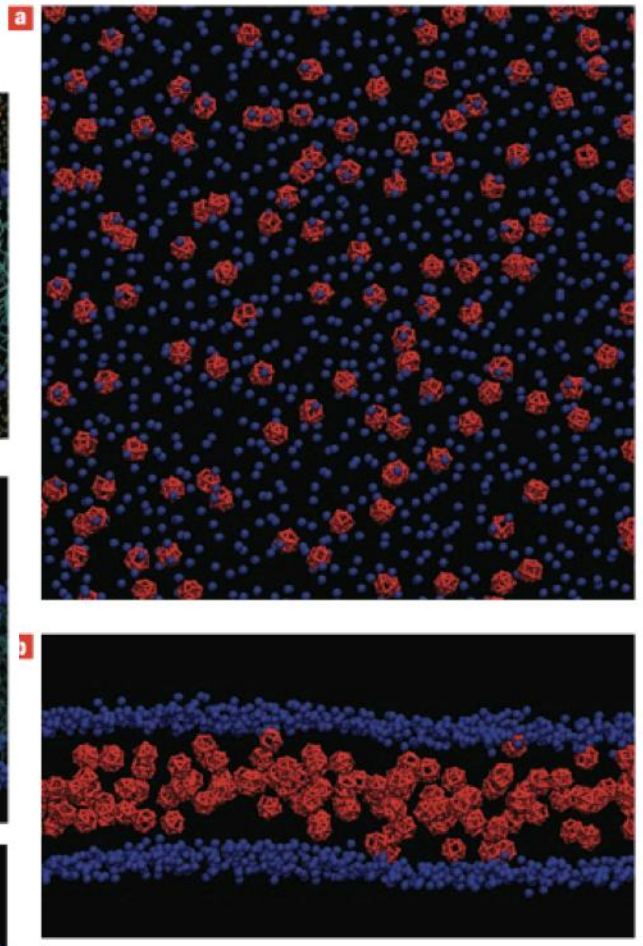
To directly investigate aggregation, simplified molecular models were developed by many research groups.



Preserve molecular shape

Using a simplified model, Prof. Tieleman reported that C_{60} *completely disperses* in a lipid bilayer!

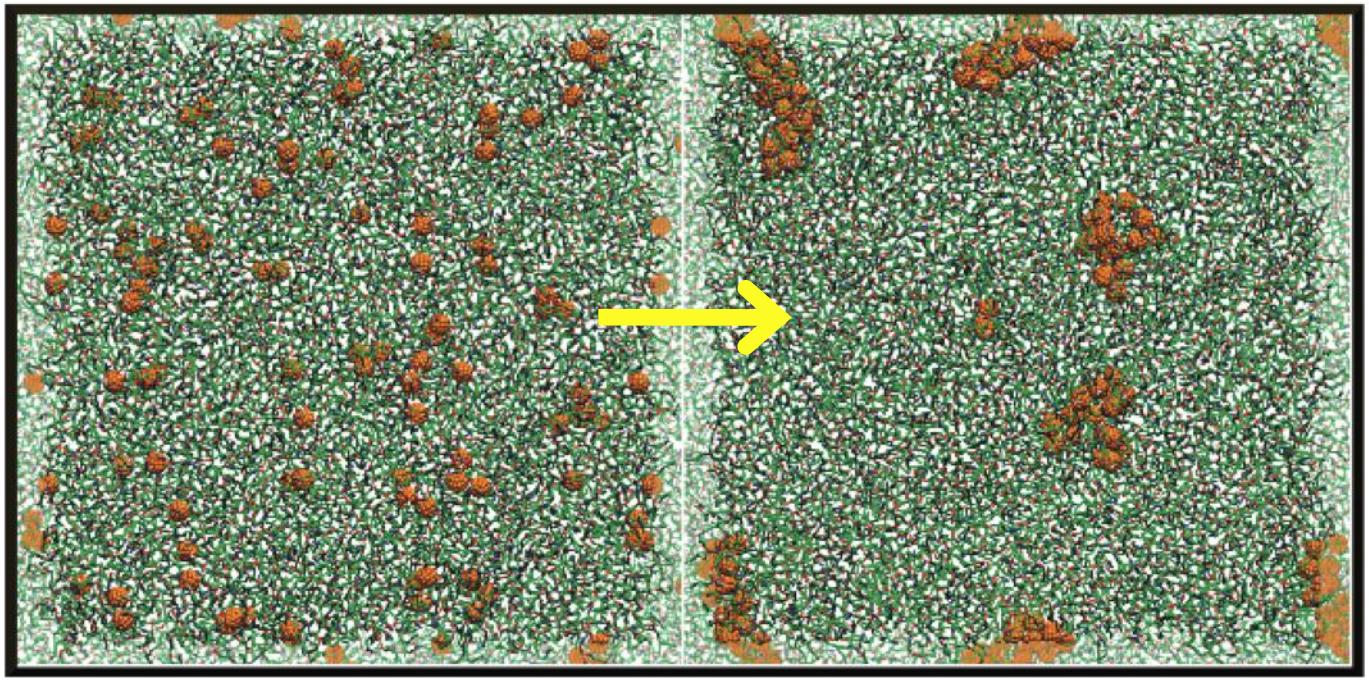




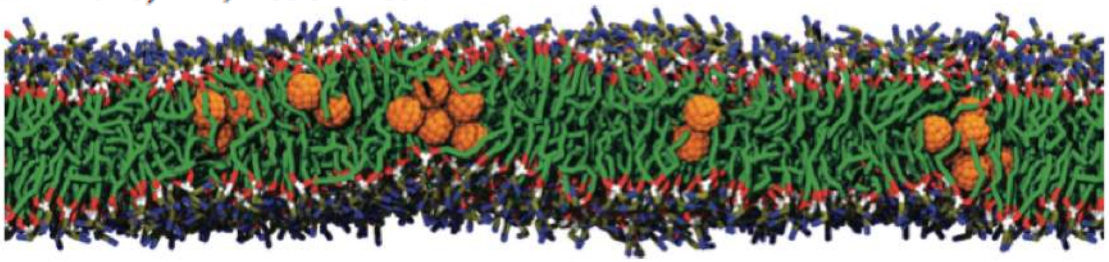
nature nanotechnology | VOL 3 | JUNE 2008 | 363-368

Using another simplified model, my colleagues and I reported that C_{60} *aggregates* in a lipid bilayer.

system prepared with C60 initially dispersed **system at a later time**



J. Phys. Chem. B **2010,** *114,* 16364–16372

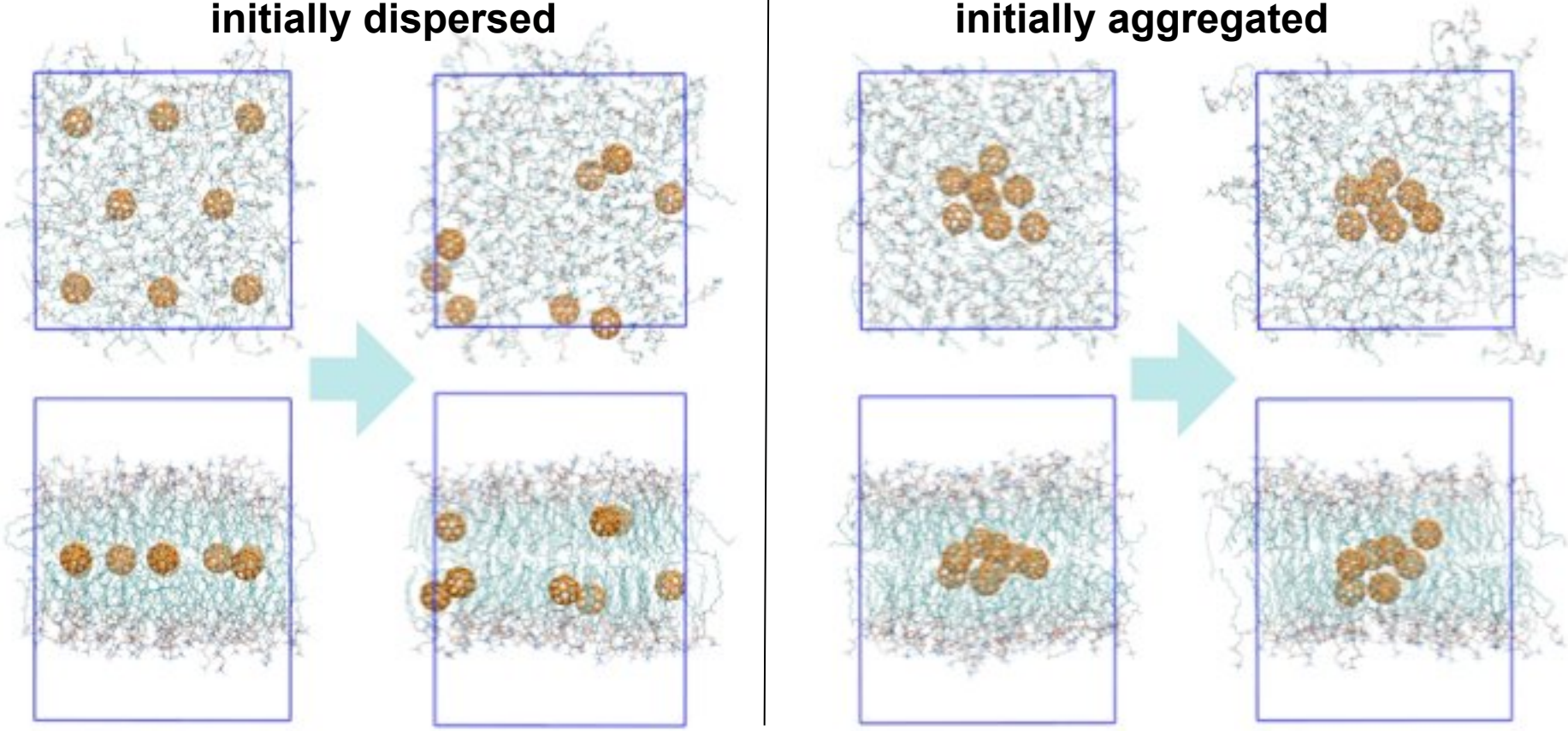


How do we proceed?

Since computers continually become more powerful, we can try the fully atomistic CHARMM force field again instead of using a simplified model.

system prepared with C_{60} initially dispersed

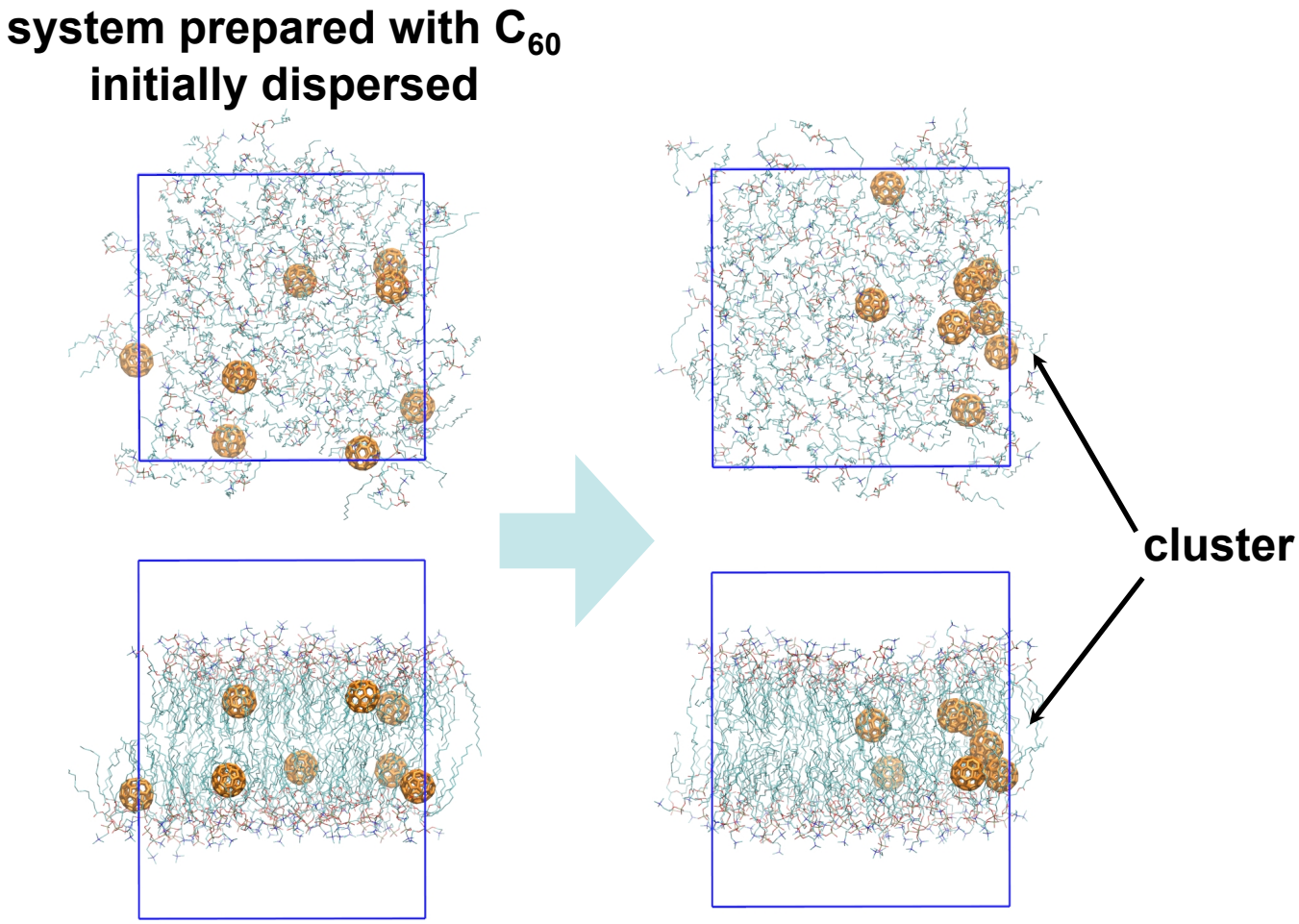
system prepared with C_{60} initially aggregated



body temperature (310 K)

W. Shinoda, unpublished data

Now do what Prof. Ikeda did: Raise the temperature



Conclusions

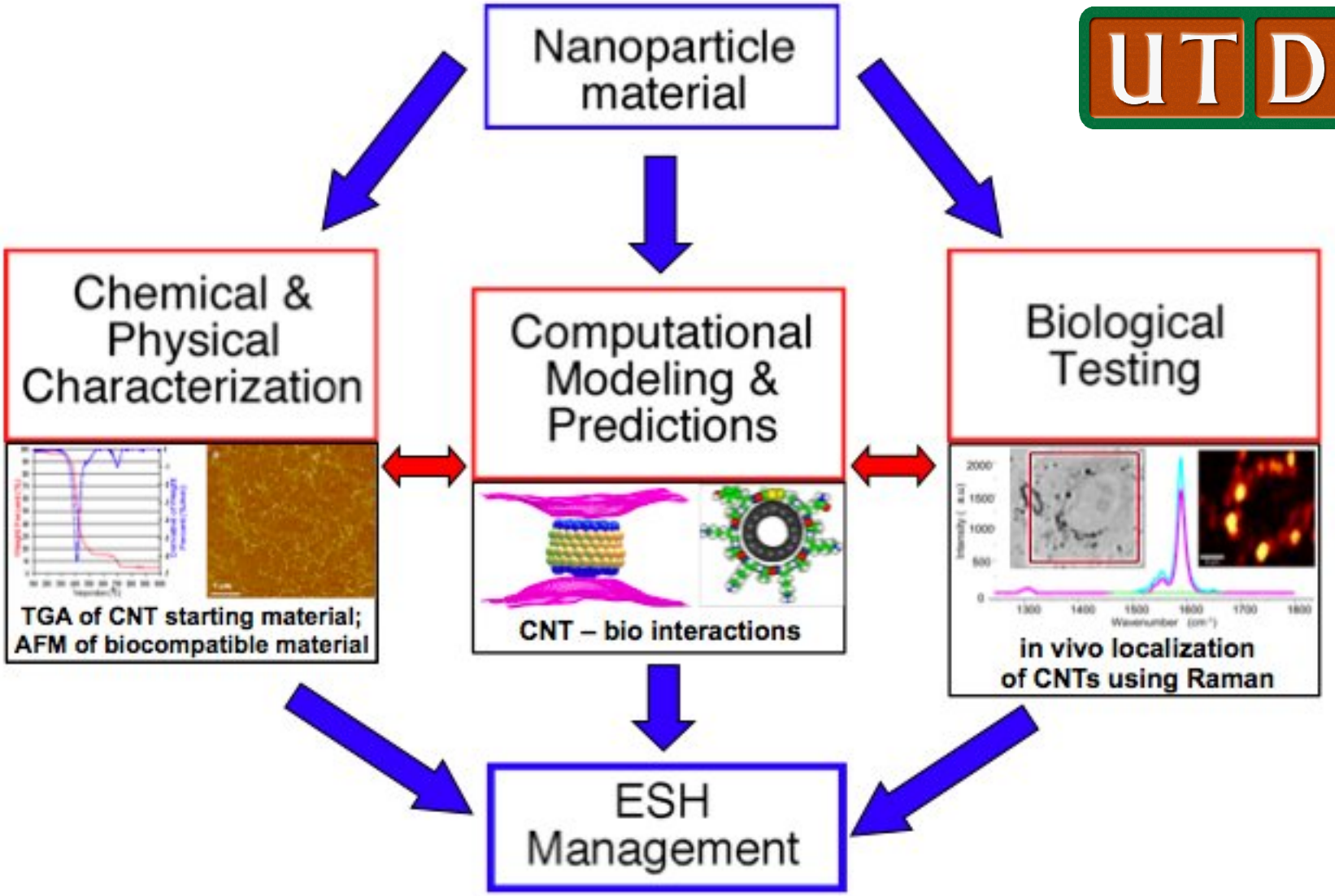
Computer modeling and experiments using model systems are showing encouraging agreement.

Further studies using model systems will increase our understanding of the factors that control the aggregation of nanoparticles in lipid membranes.

Model systems can also be used to study other factors (nanoparticle size, surface charge, etc.) and their impact on lipid membrane properties.

The results from these studies will be invaluable to help the interpretation of ESH data on "real" systems.

Dispersion, Bioaccumulation, and Mechanisms of Nanoparticle Toxicity (SRC Task 425.042)



Acknowledgements

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