Construction and Molecular Dynamics of a Nanodisc for Membrane Protein Simulation

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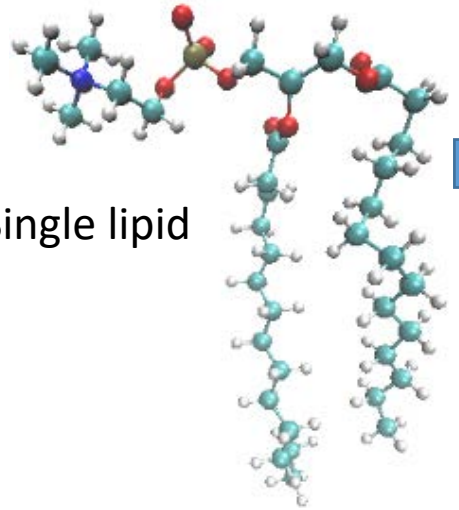
Mentored by Joseph Curtis

Overview

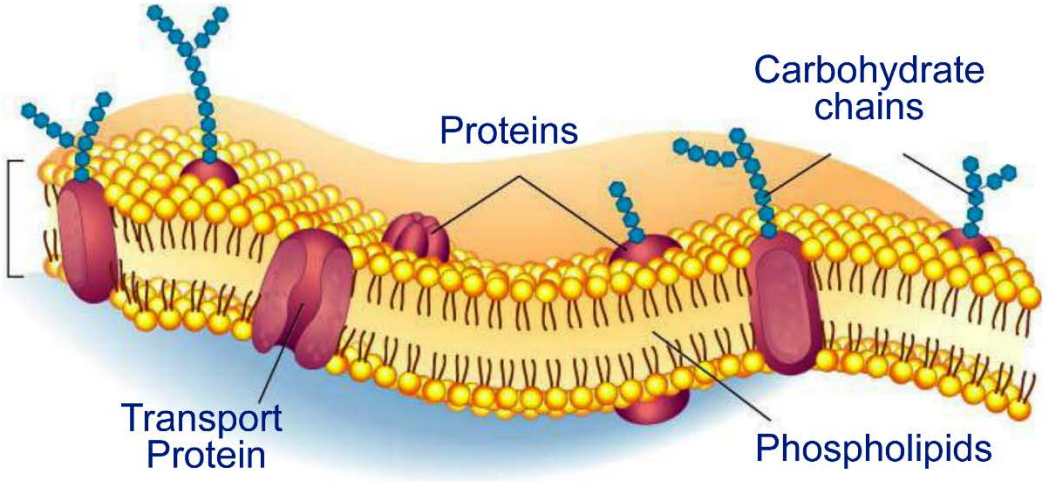
(-)



Single lipid

Lipid Bilayer

Figure 1. Cell membrane



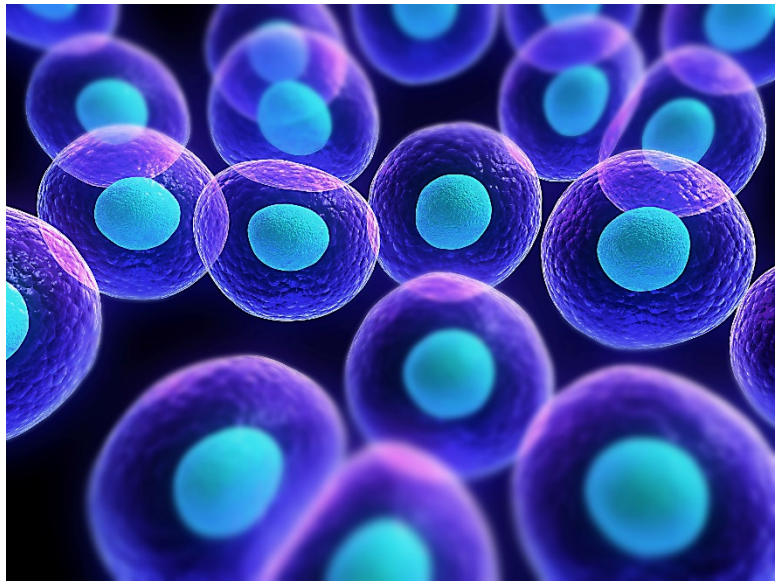


Figure 3. Biological cells

Figure 4.

(Figure 1) http://www.fitwit.com/blog/2014/03/19/guest-post-good-fats-healthy-cell-structure/
(Figure 3) http://biology.usf.edu/cmmb/undergrad/bio/
(Figure 4) http://www.silhouettegraphics.net/man-running-silhouette/

Prions

- Misfolded proteins can be toxic, or in some cases may behave like a disease
- These harmful misfolded proteins are prions
- Prions are associated with various diseases, such as mad cow disease (infectious and fatal neurodegenerative disease)

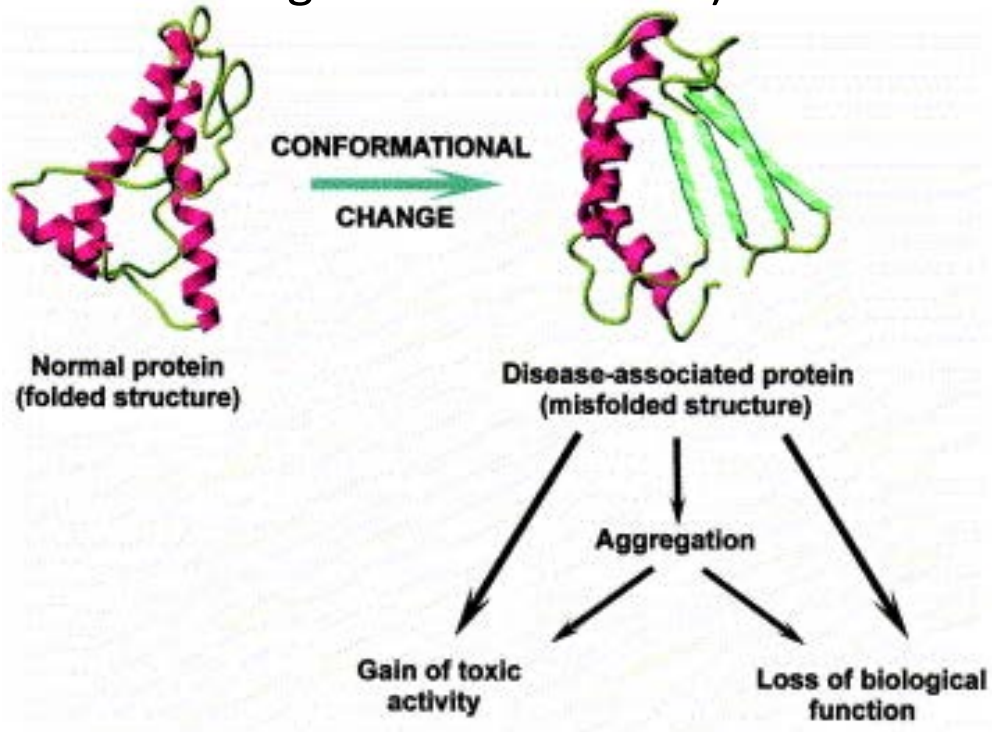


Figure 5. Misfolding of a protein

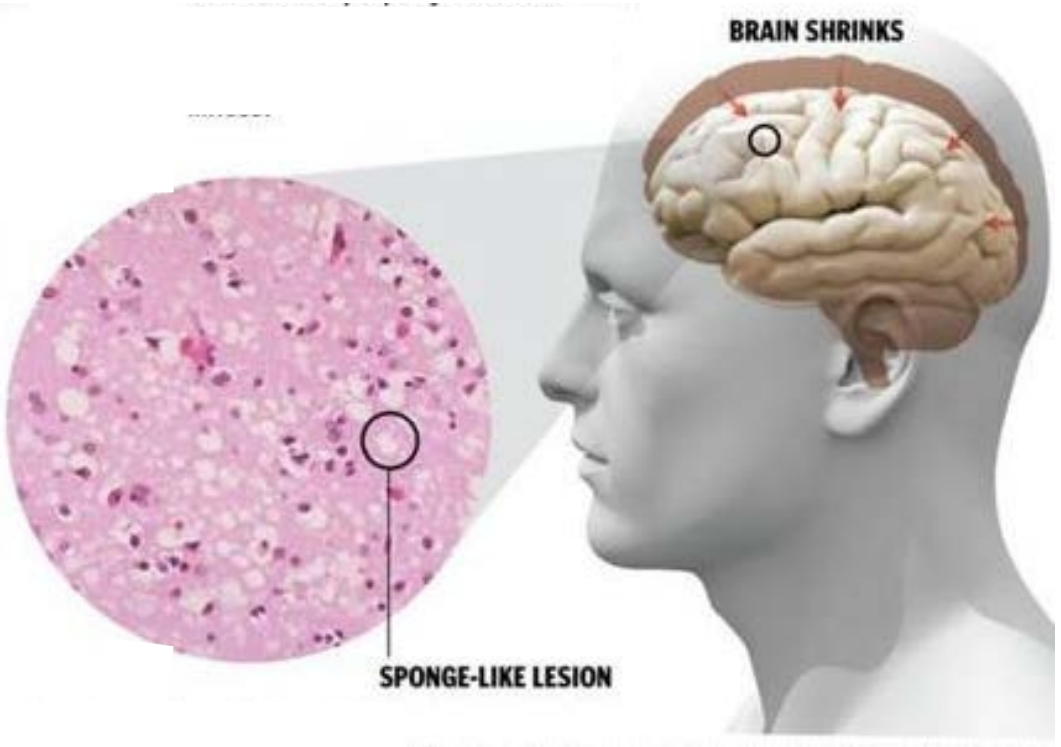
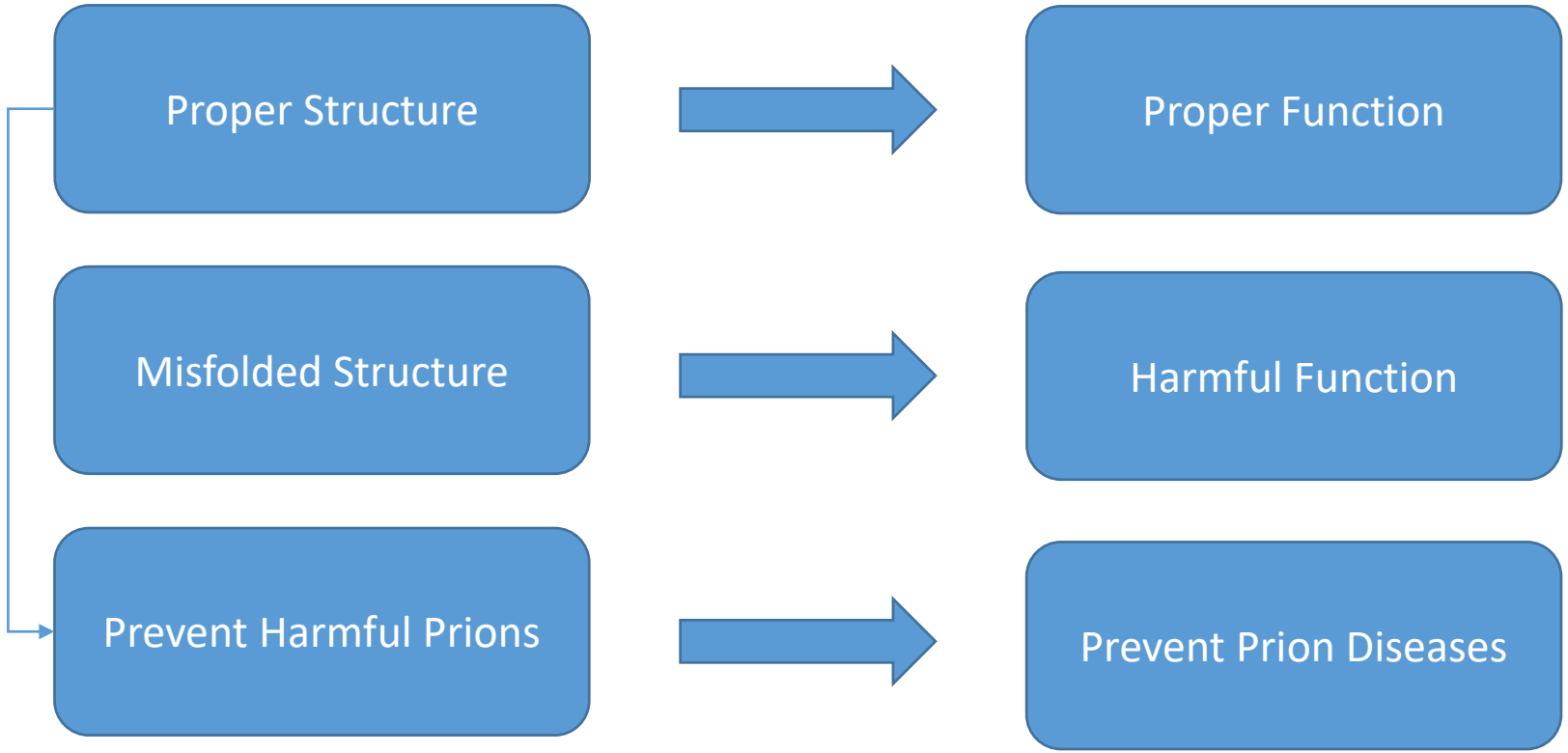


Figure 6. Mad cow disease in humans

Figure 5. http://srxawordonhealth.com/tag/misfolded-proteins/
Figure 6. http://truthfrequencyradio.com/neurosurgery-patients-exposed-to-human-mad-cow-disease-in-n-h/

Understanding Prions

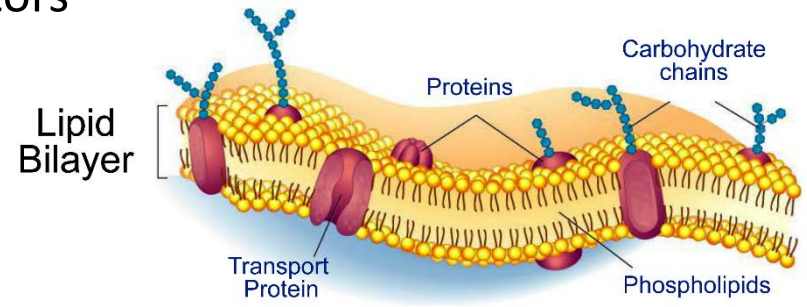
- To prevent and study these harmful prions, the structure of properly folded proteins must be determined



Overview

- Proteins are often insoluble in water, making crystallization and structure determination difficult
- Ongoing research to correlate structure to function
- Nanodiscs are used to stabilize several varieties of membrane proteins
  - Transmembrane proteins
  - G-protein coupled receptors
  - Cytochromes
  - Blood clotting cofactors

Figure 2. Cell membrane



(Figure 5) Timothy H. Bayburt, Stephen G. Sligar, Membrane protein assembly into Nanodiscs, FEBS Letters, Volume 584, Issue 9, 3 May 2010, Pages 1721-1727, ISSN 0014-5793

Two MSP1D1 nanodiscs on top. MSP belts are blue and orange

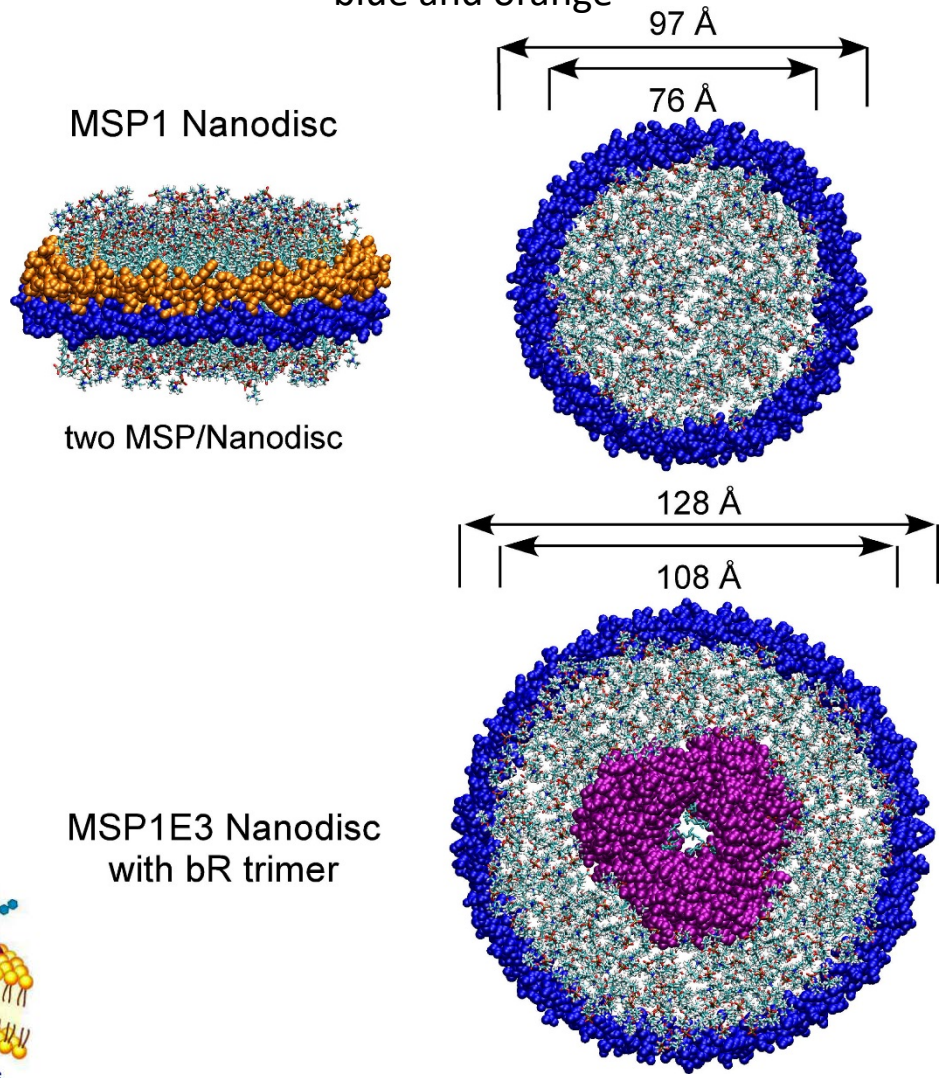


Figure 5. MSP1E3 Nanodisc with MSP and bR transmembrane protein

Overview

- Membrane proteins denature when removed from their native environment
- Proteins must retain structural integrity for experimental studies to have biological significance
- Small-angle neutron scattering (SANS) and contrast variation are used to determine shape/structure
- Computer-generated molecular dynamics simulations are used to validate experimental results

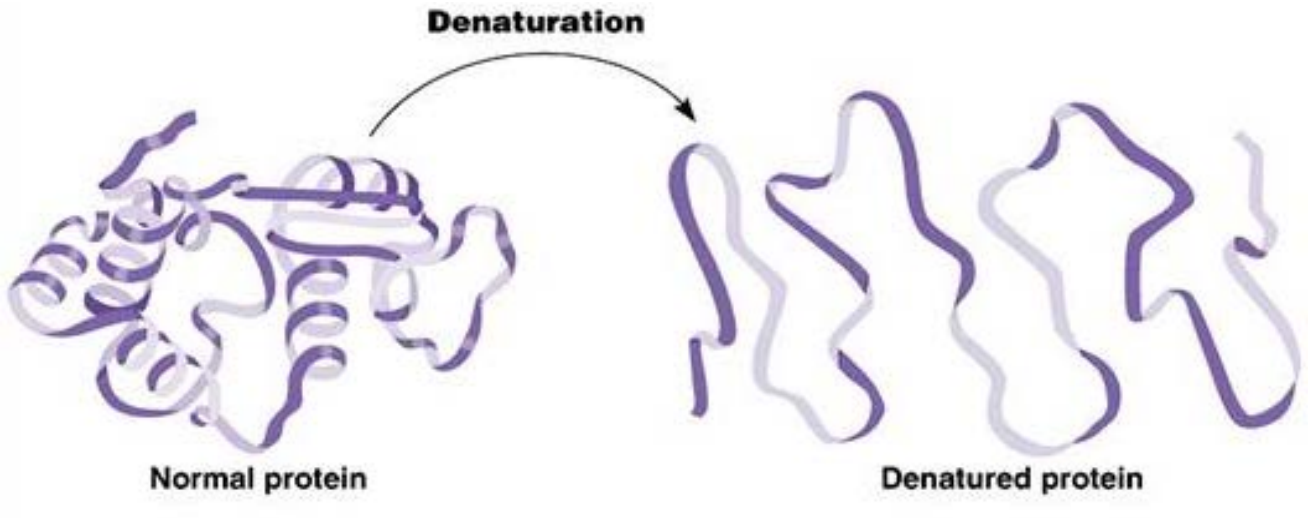
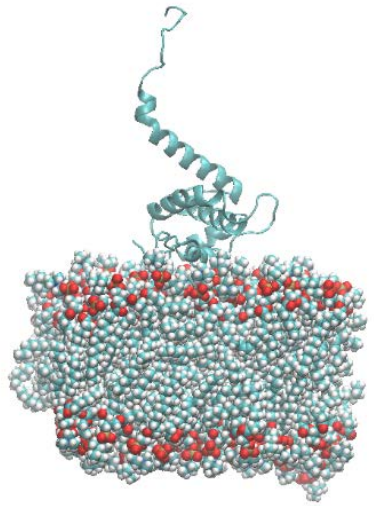


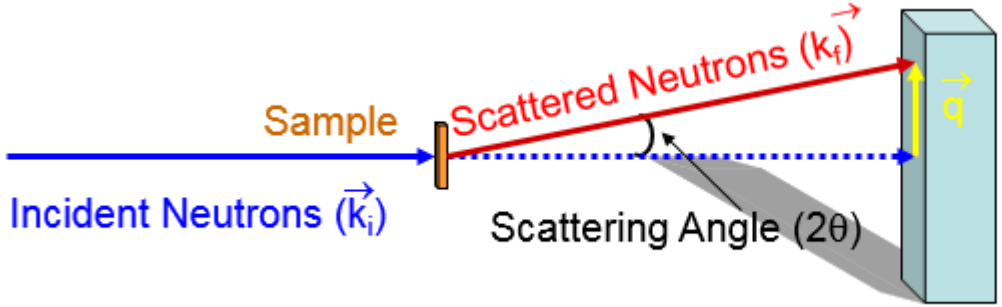
Figure 2.
http://chronopause.com/chronopause.com/index.php/2011/02/23/does-personal-identity-survive-cryopreservation/



example MD simulation

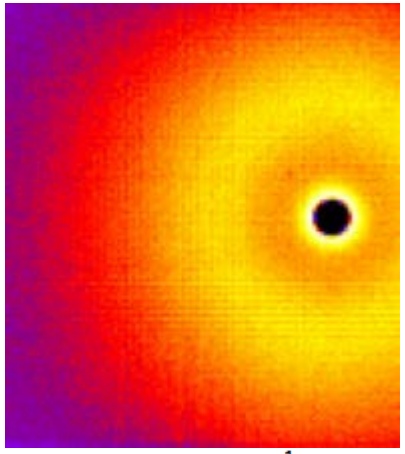
Basic SANS

Figure 3

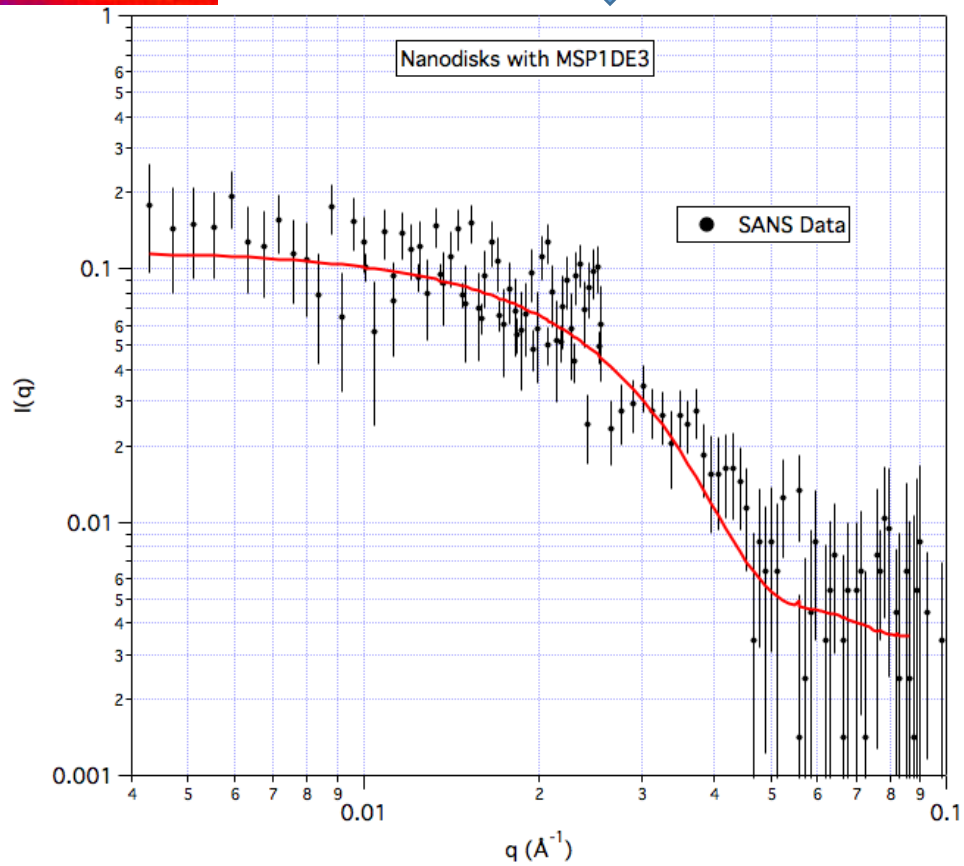


$$q = \frac{4\pi \sin(\theta)}{\lambda}$$

Wavelength



Determine scattering profile by radially averaging data



Structural conclusions based on the neutron scattering profile

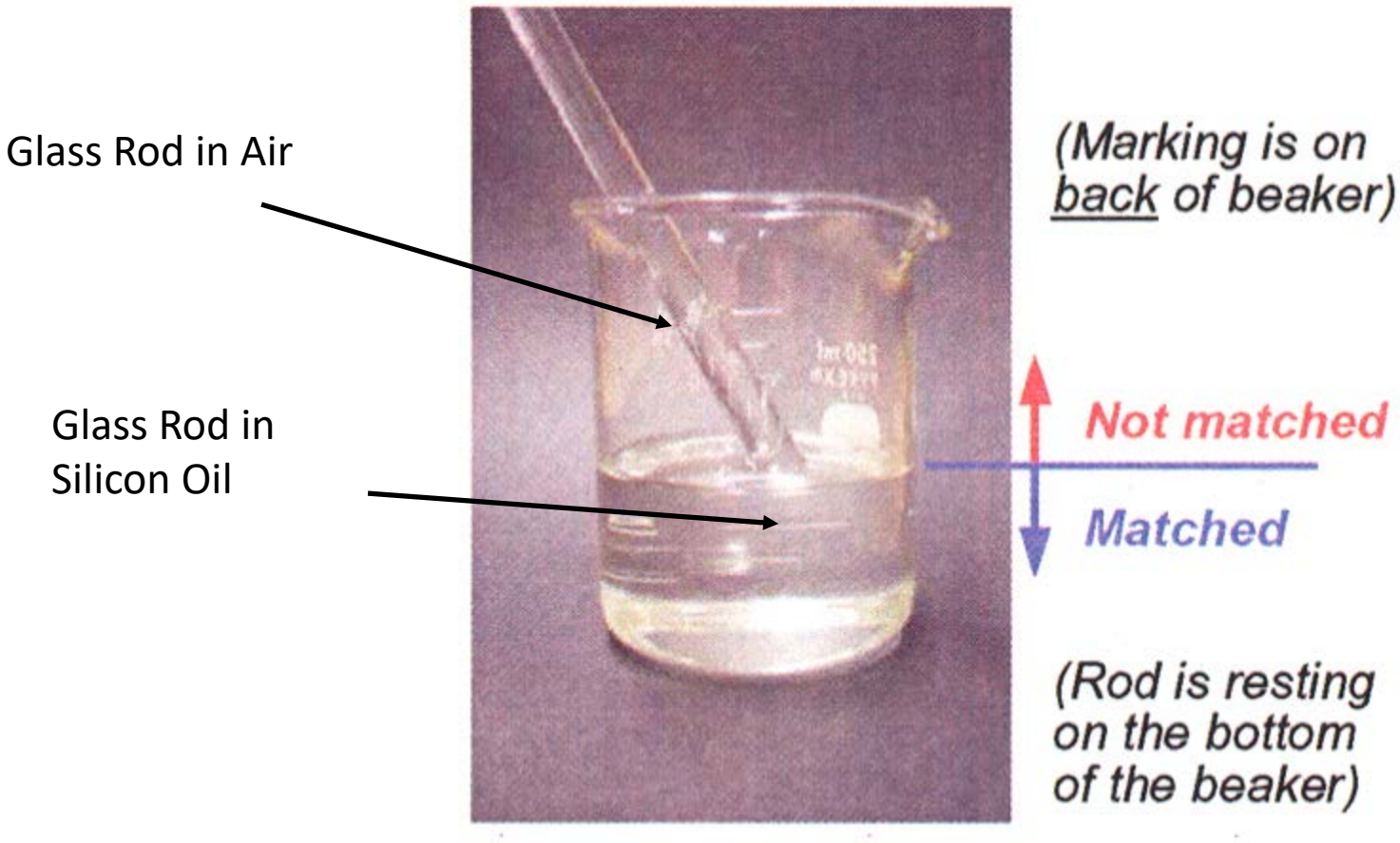
Inverse space, $q = \frac{2\pi}{r}$

(Figure 3) Small-Angle Neutron Scattering Study of Protein Crowding in Liquid and Solid Phases: Lysozyme in Aqueous Solution, Frozen Solution, and Carbohydrate Powders
Joseph E. Curtis, Hirsh Nanda, Sheila Khodadadi, Marcus Cicerone, Hyo Jin Lee, Arnold McAuley, and Susan Krueger
The Journal of Physical Chemistry B 2012 *116* (32), 9653-9667

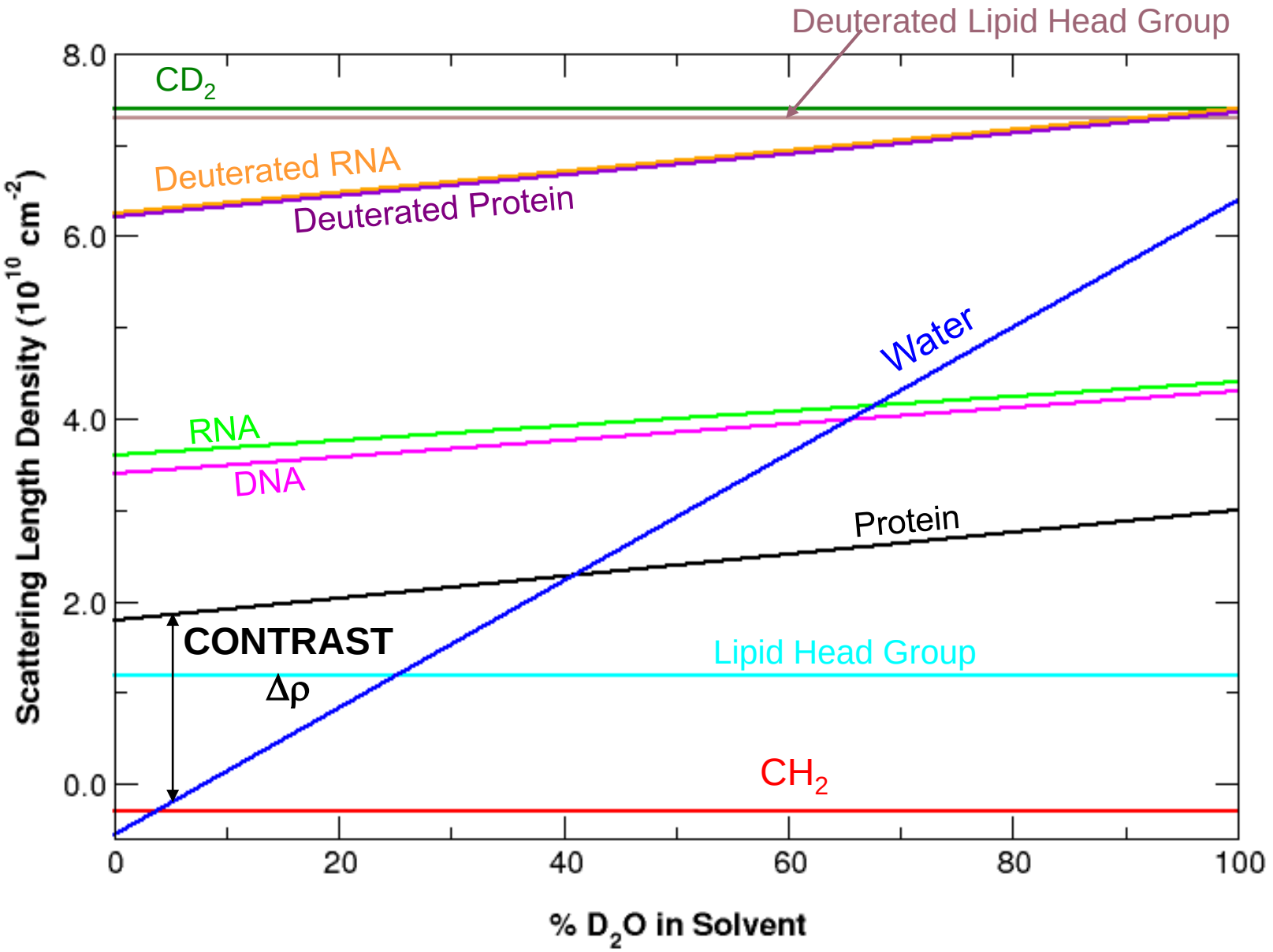
CHRNS

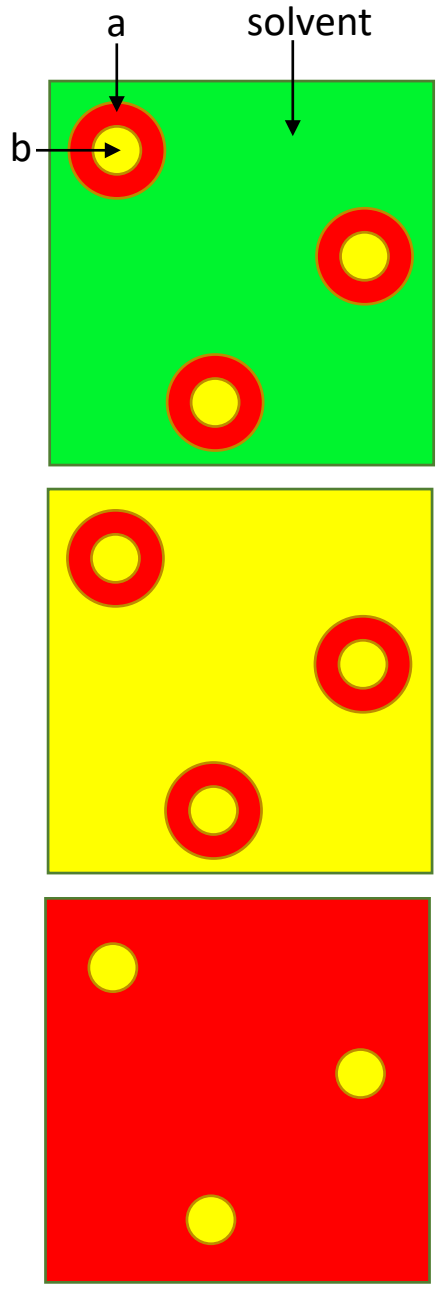
Contrast Variation

Example for Visible Light

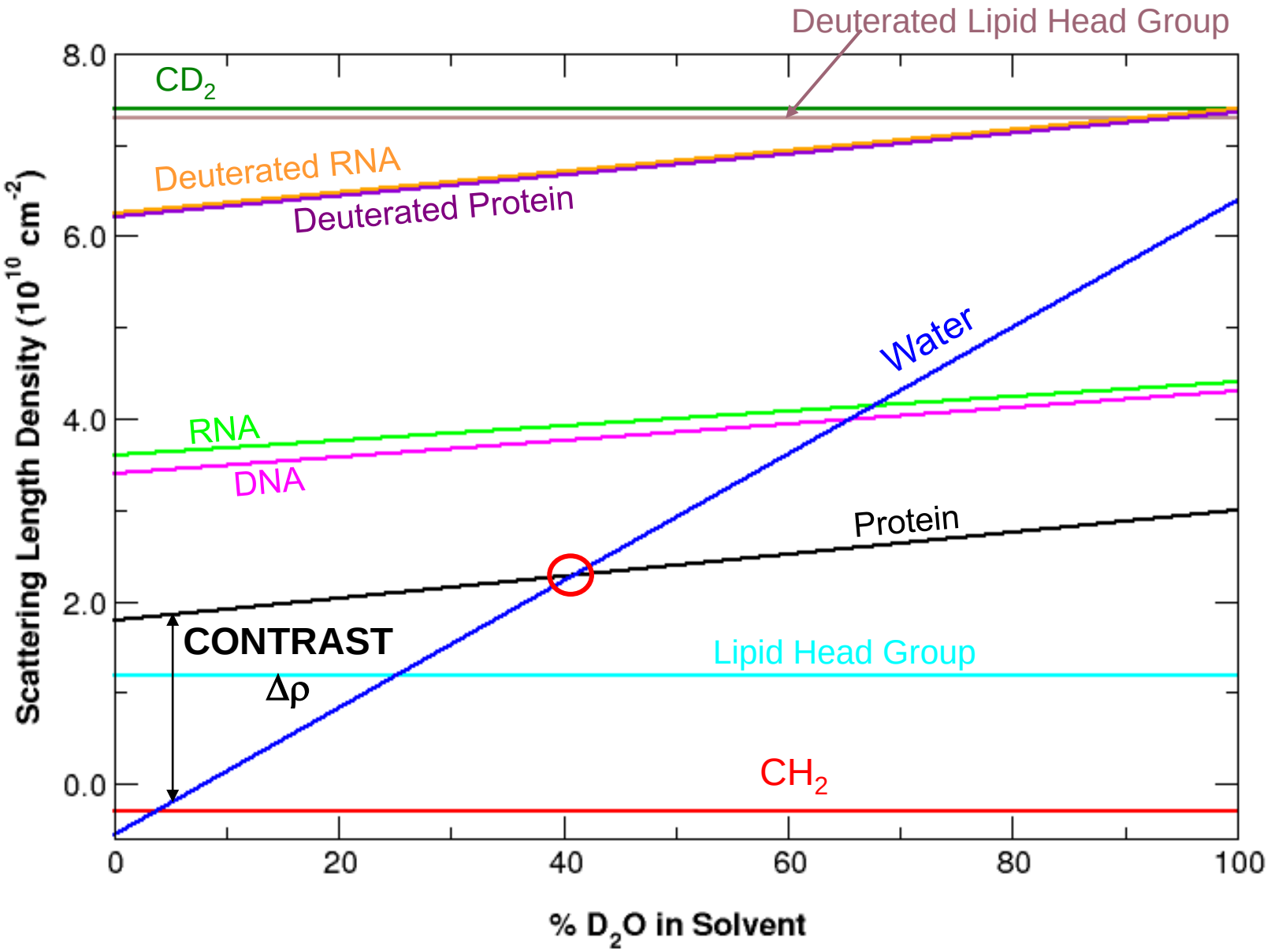


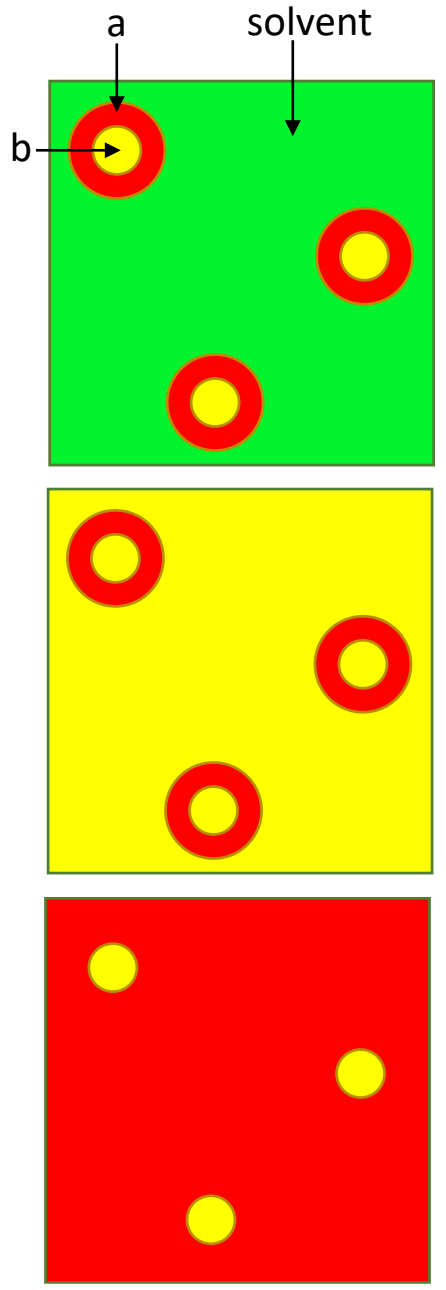
Contrast Variation





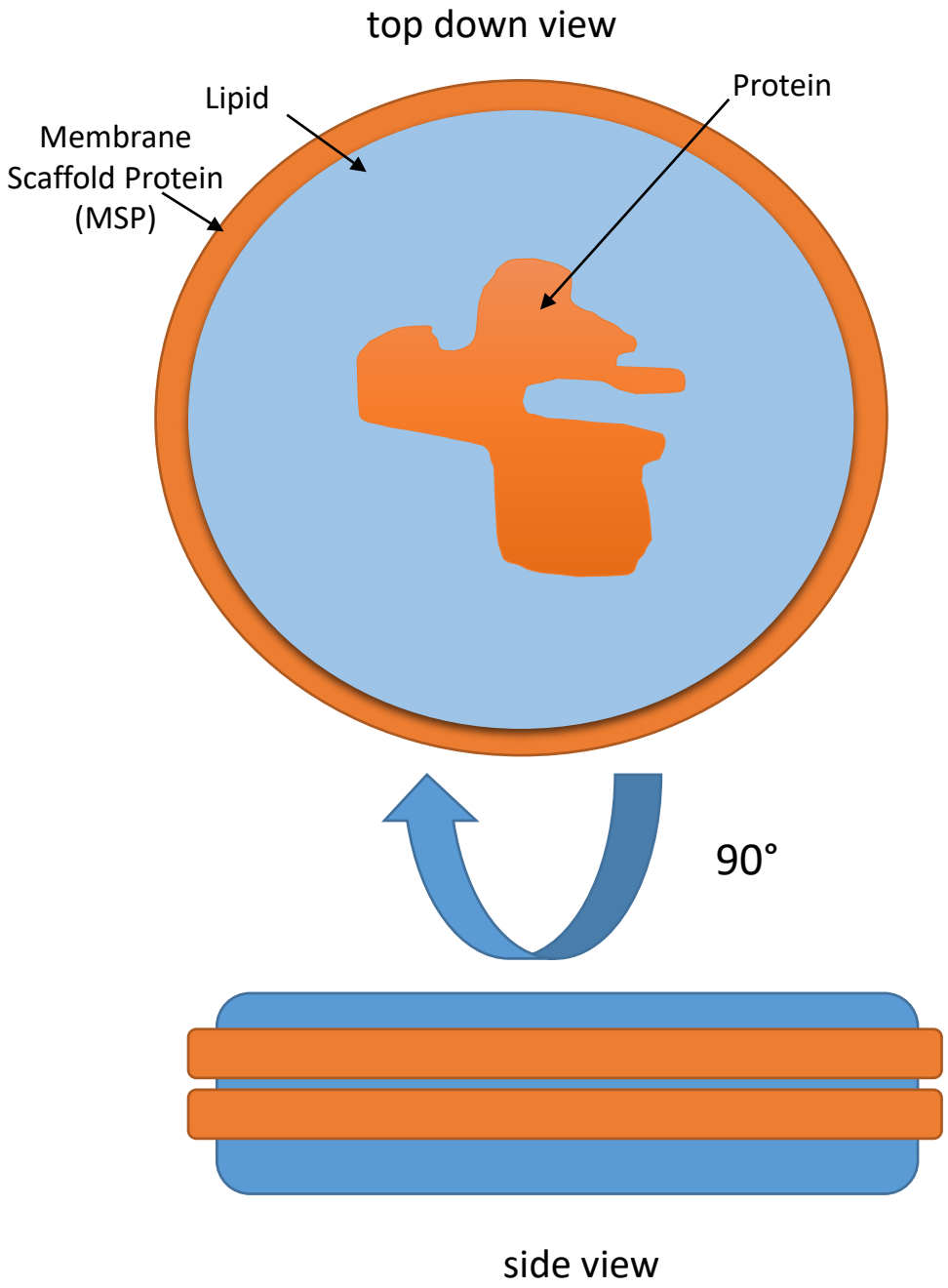
Contrast Variation



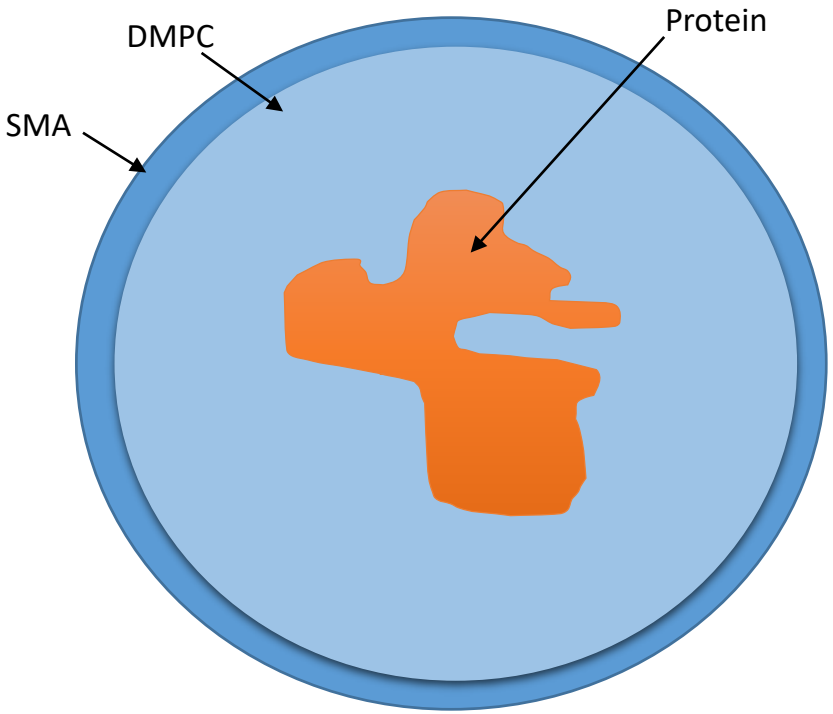


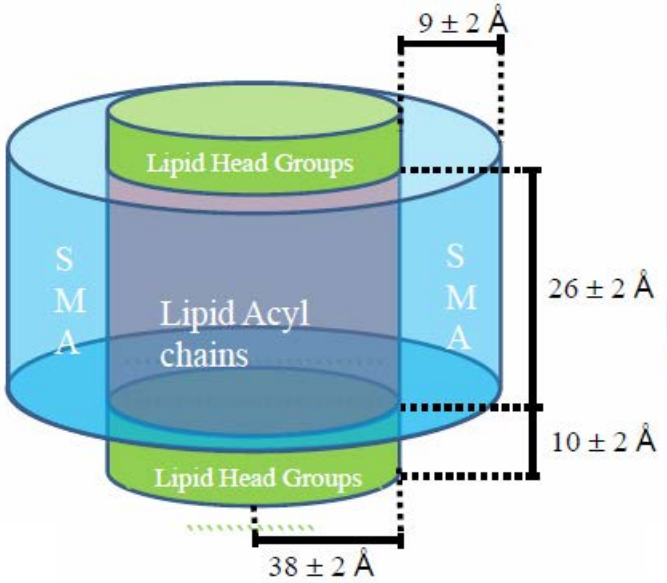
Contrast Variation and MSP

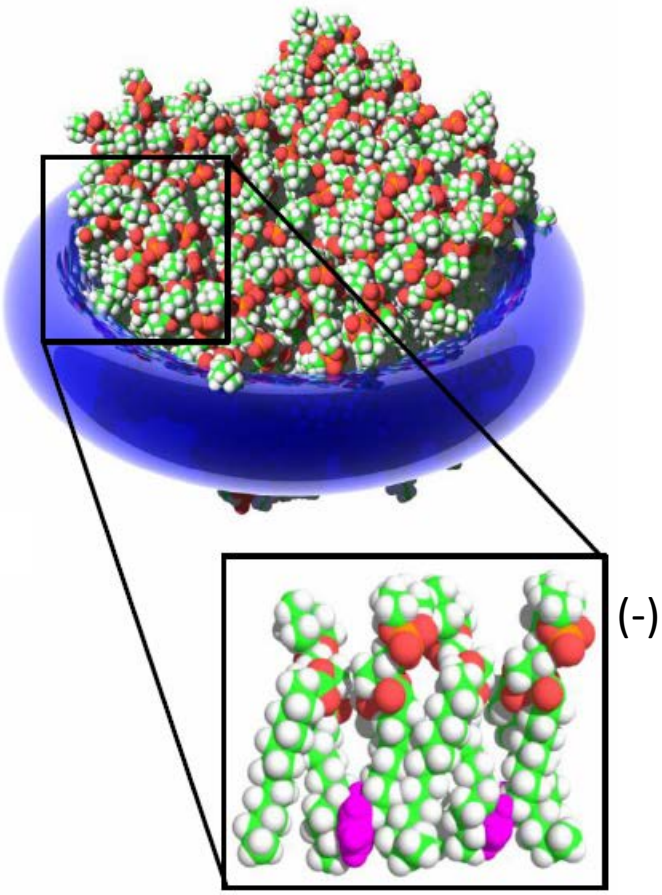
- Small-angle neutron scattering (SANS) interacts directly with the nucleus of the atoms being studied
- Hydrogen and deuterium coherent scattering lengths
- Contrast variation substitutes deuterium for hydrogen to change the scattering length density and differentiate two or more structures



Styrene/maleic acid polymer (SMA) scattering length densities match DMPC lipid, leaving a clearly defined protein







DMPC Lipid

Figure 1

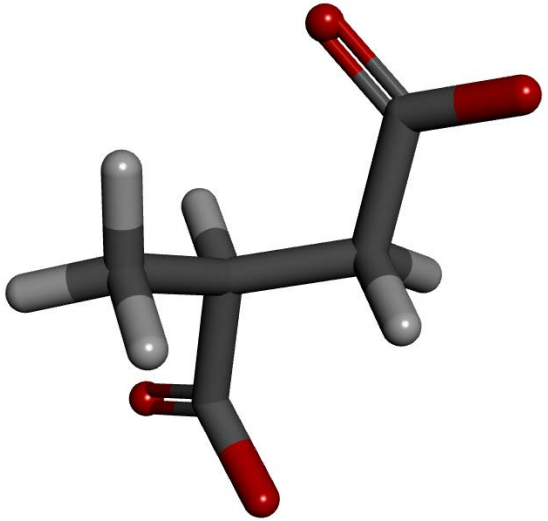
(Figure 1) Jamshad, M.; Grimard, V.; Idini, I.; Knowles, T.; Dowle, M.; Schofield, N.; Sridhar, P.; Lin, Y.; Finka, R.; Wheatley, M.; Thomas, O.; Palmer, R.; Overduin, M.; Govaerts, C.; Ruysschaert, J.; Edler, K.; Dafforn, T. *Nano Res.* 2014, *8*, 774-789.

Overview

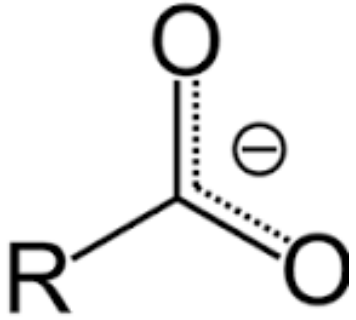
- We created a model of a nanodisc wrapped by SMA polymer
  - Force field parameters for SMA did not exist, so we created them
  - Validated our parameters by studying lone polymers
  - Constructed models of lipid systems to study interaction with SMA polymer
  - Calculated SANS profiles for SMA nanodiscs

Force Field Parameterization

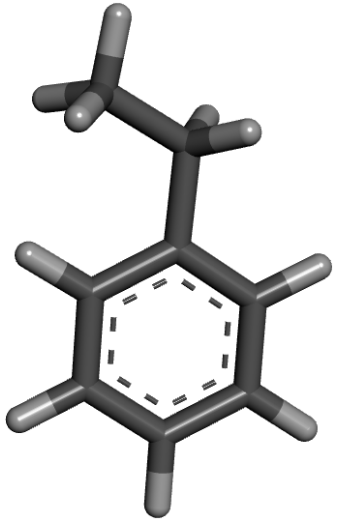
- CGENFF
  - Developed through collaboration and funded by the NSF
  - Generated a parameter file from a given starting structure through analogy with known structures
  - Provided a strong starting point for quantum mechanical validation of the parameterization (ongoing)



Maleic acid .mol2 structure



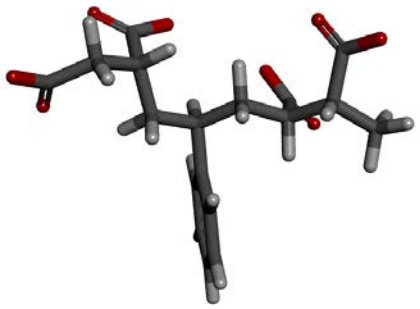
Carboxylate structure



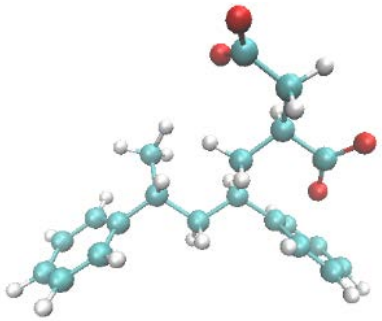
Styrene .mol2 structure

Constructing SMA Polymer

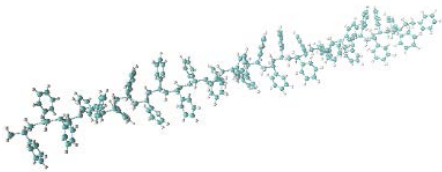
- Internal coordinates written based on minimized structures to define the individual styrene/maleic acid residues
- Python script defined the sequence and modified dihedrals between residues to construct a pre-wrapped starting structure
- Five trimers: SSS (35%), MSS/SSM (54%), MSM (11%), and SMS (Never MM)



.mol2 trimer constructed in maestro

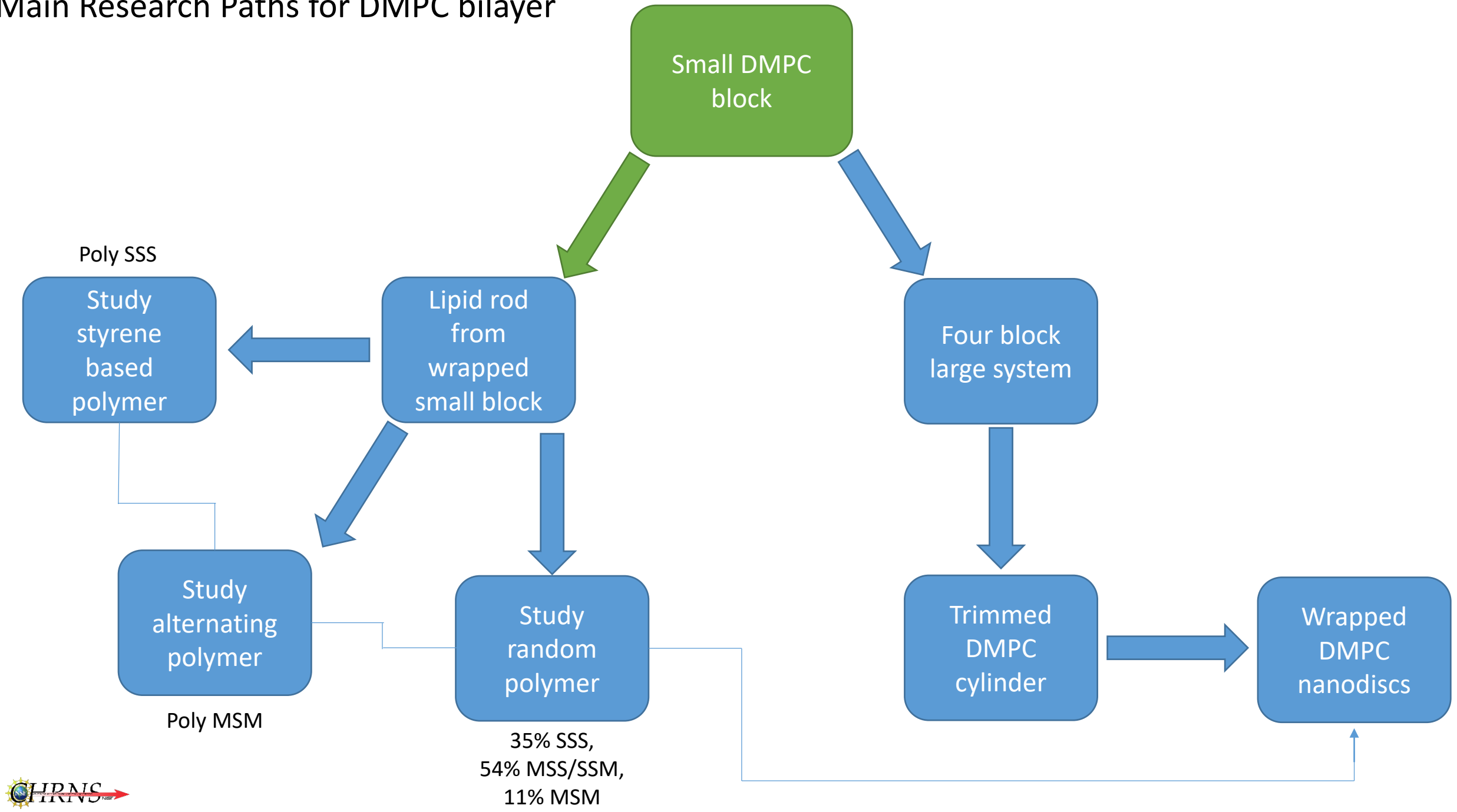


minimized trimer

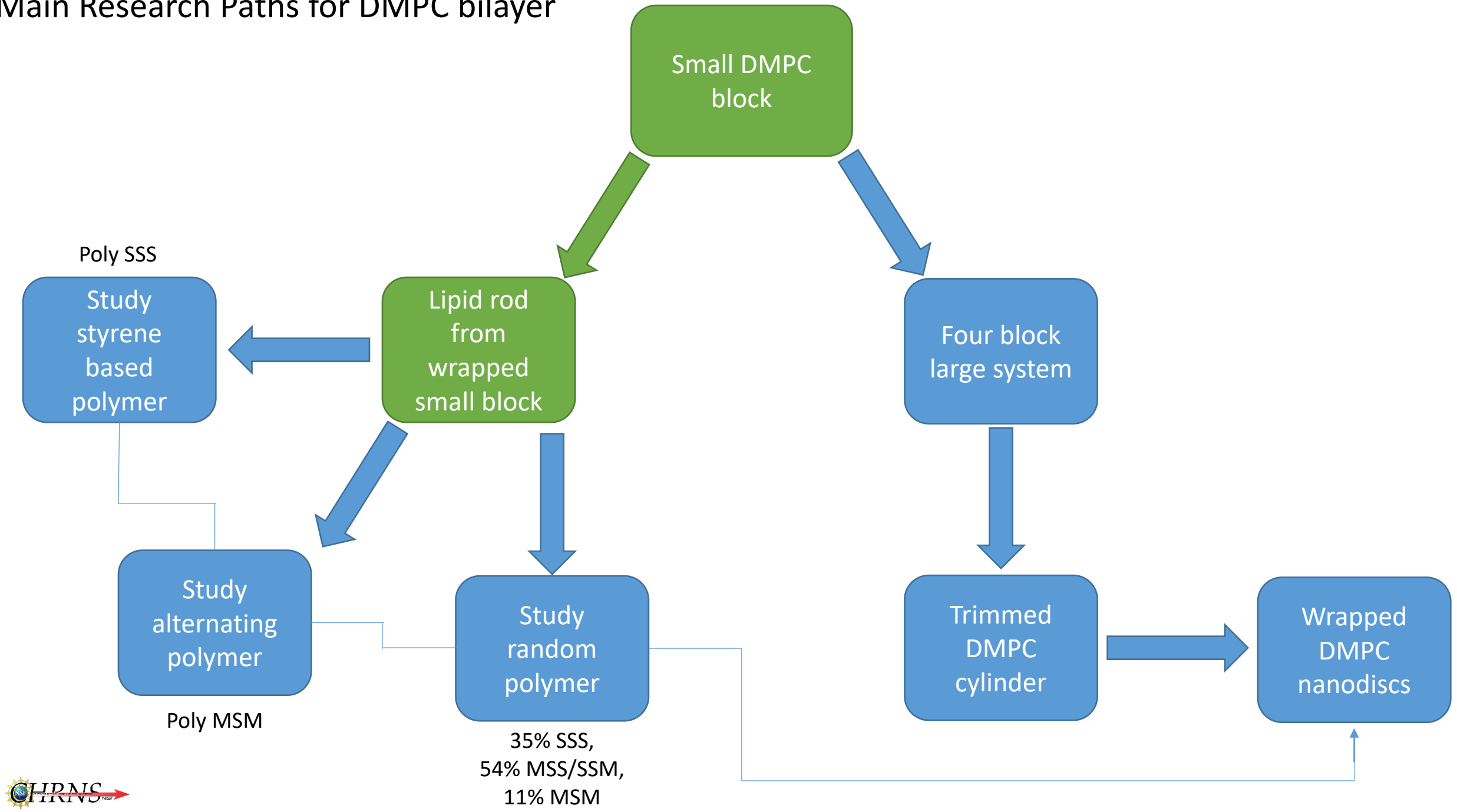


30 residue styrene based polymer

Main Research Paths for DMPC bilayer

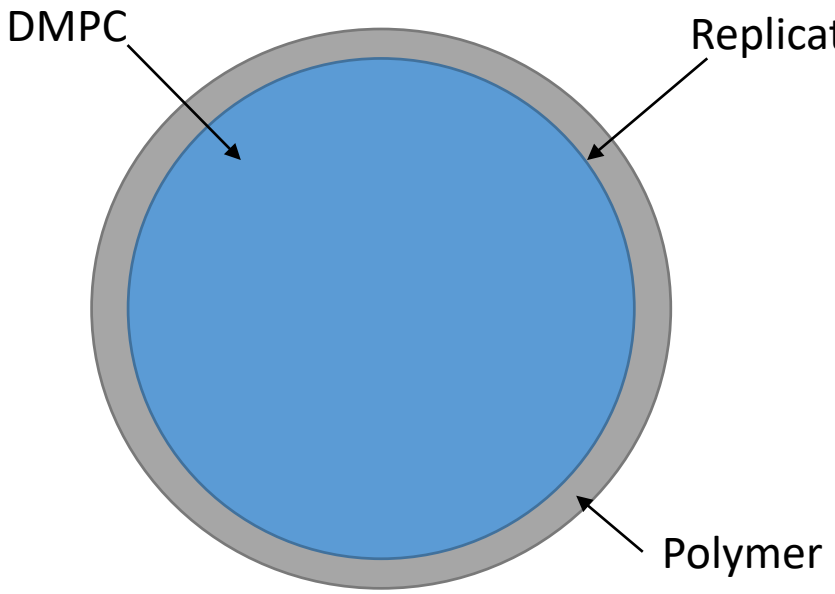


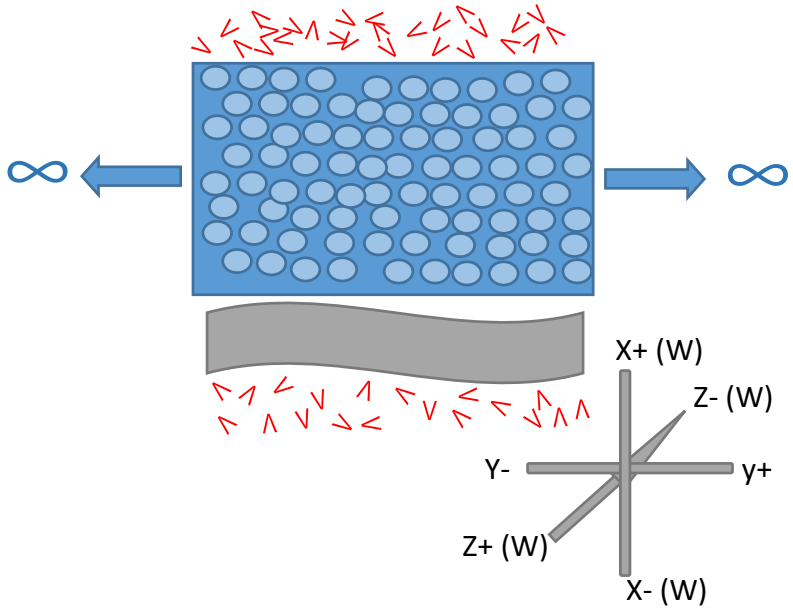
Main Research Paths for DMPC bilayer

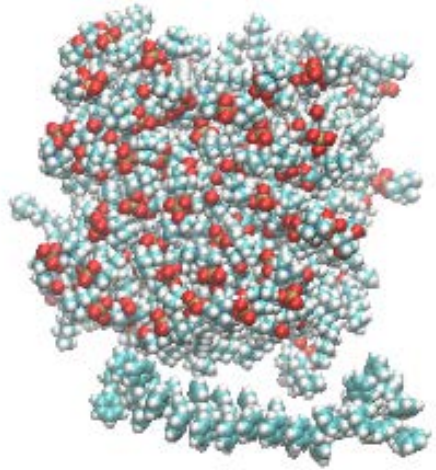


Lipid Rod

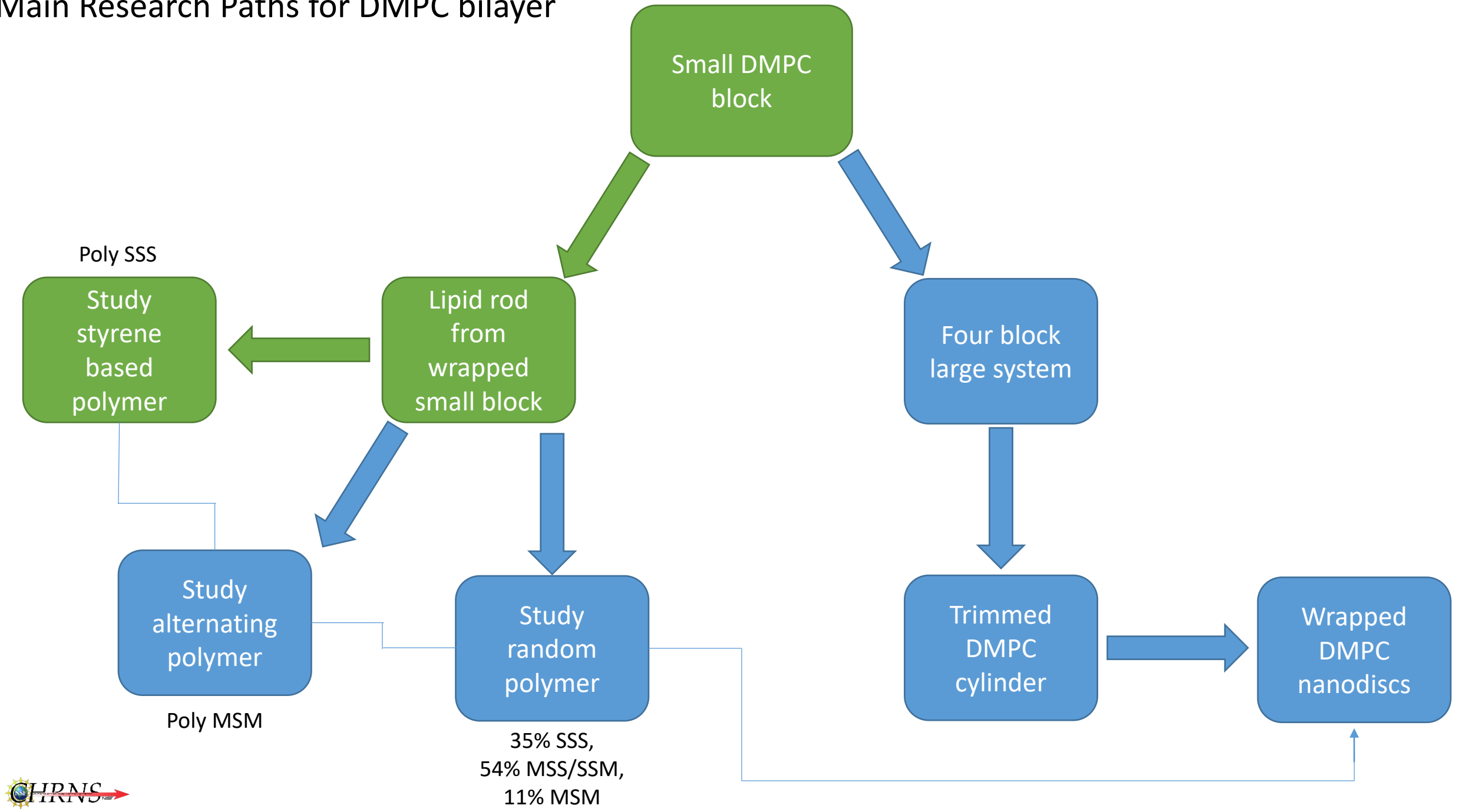
- We created a lipid rod from the small DMPC block
- This enabled us to analyze the polymer interaction with the bilayer
- Simulation cell wrapped to create the properties of a rod while retaining short computation time





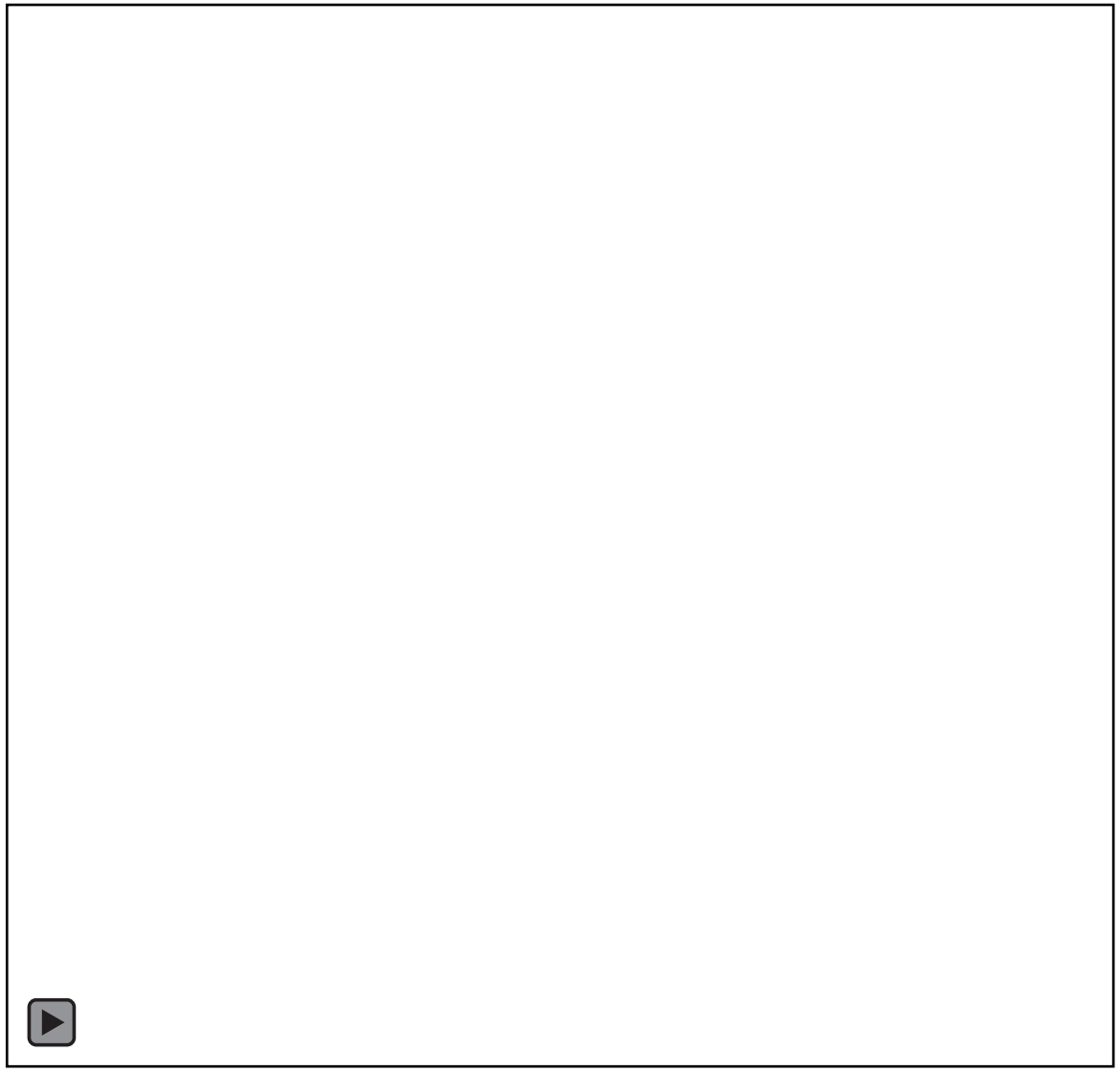


Main Research Paths for DMPC bilayer



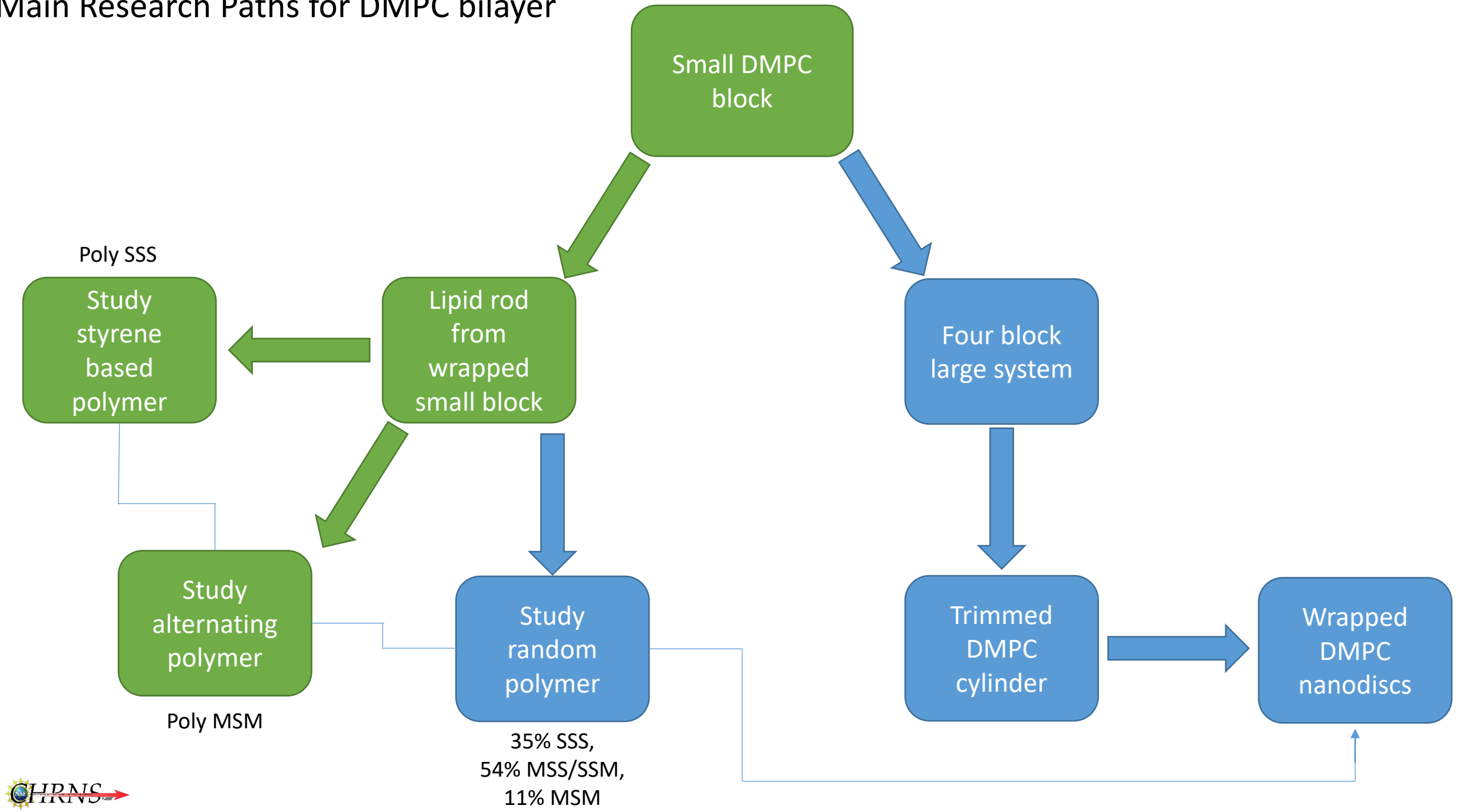
Bilayer Interaction with Pure Styrene Polymer

- Initial simulations of the small block used pure styrene polymers to confirm intercalation with the lipid tails
- Small box model was limited by harmonic constraints on the lipids
- Pure styrene residue chain strongly interacts since it is not amphipathic yet

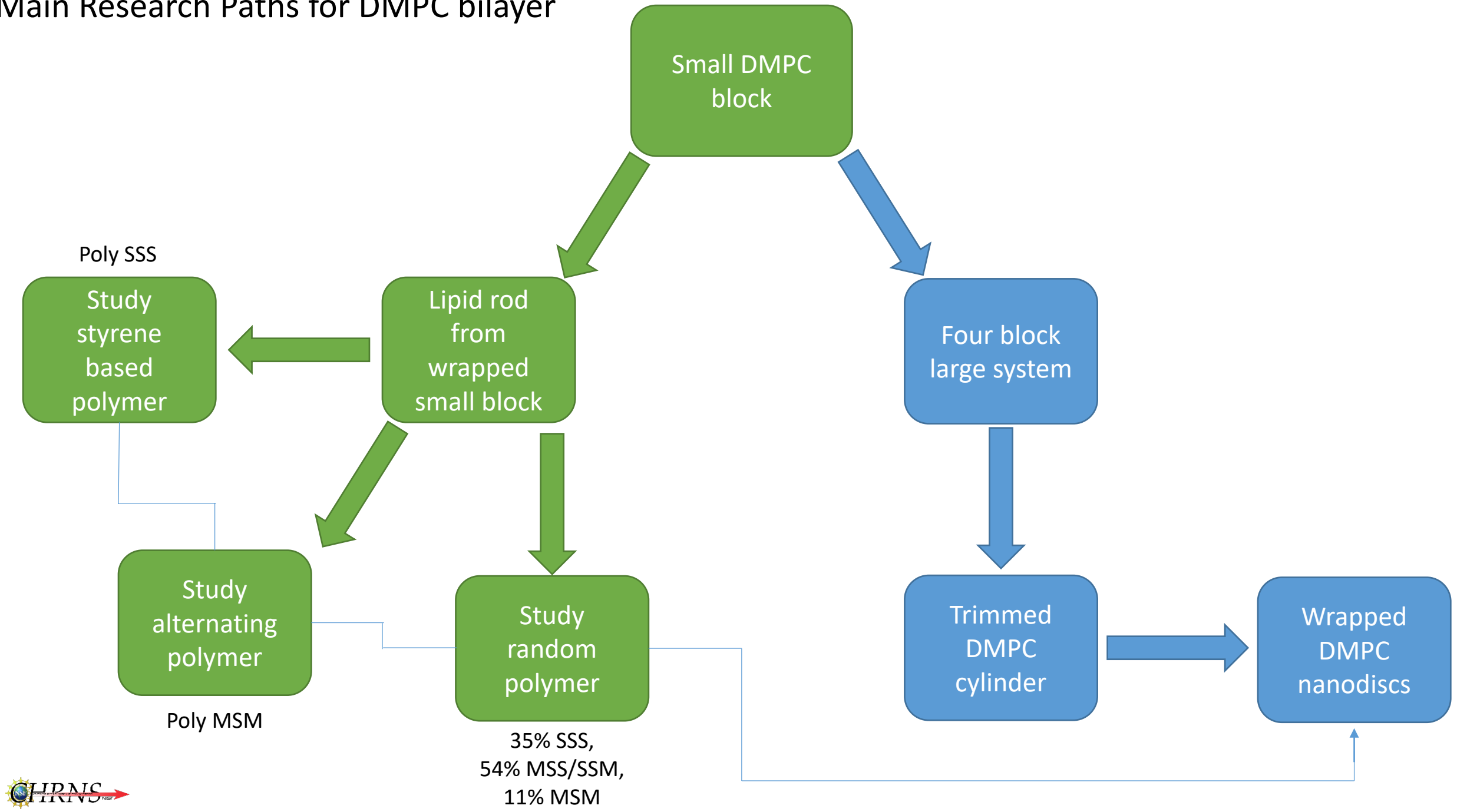


0.2 ns NPT run

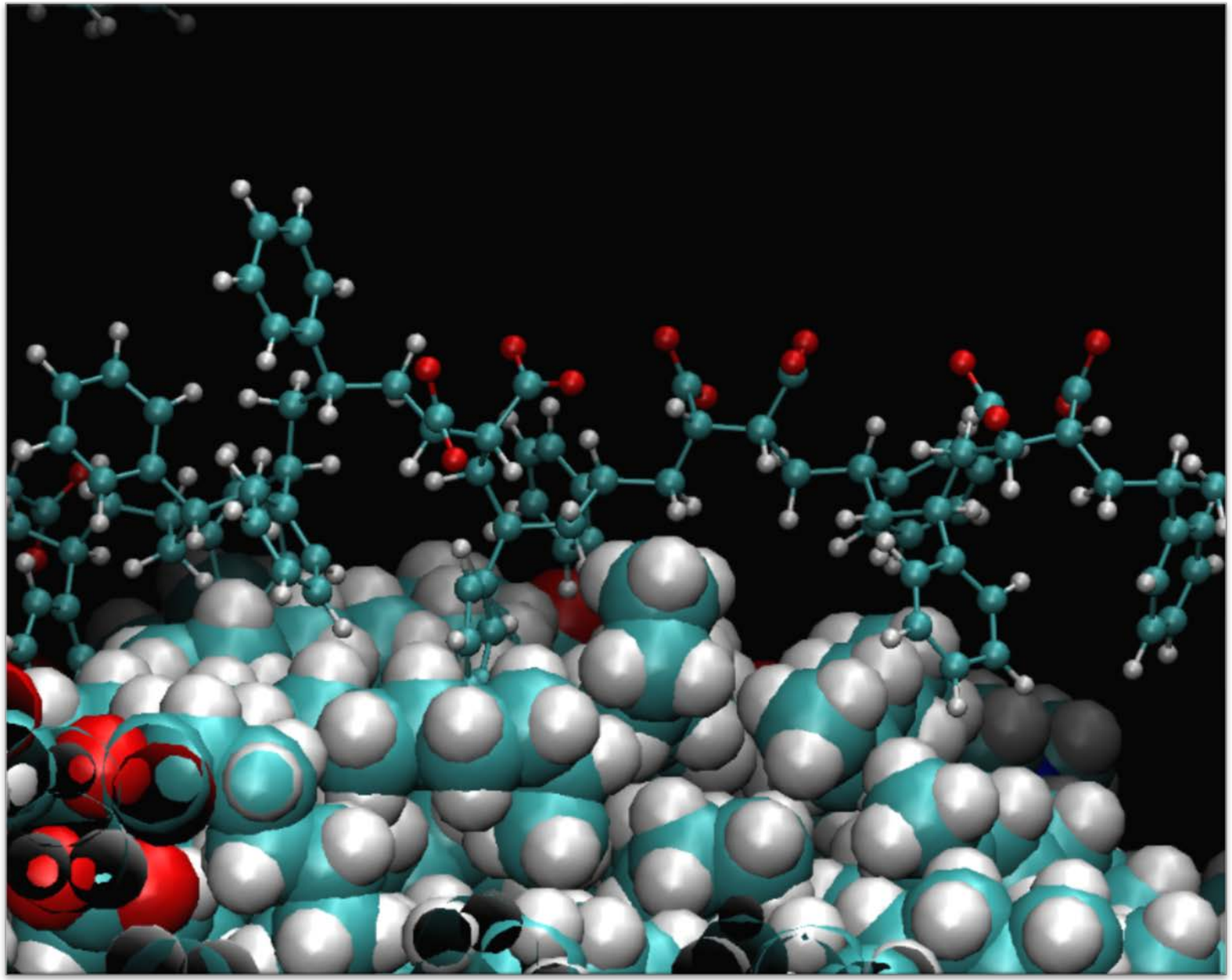
Main Research Paths for DMPC bilayer



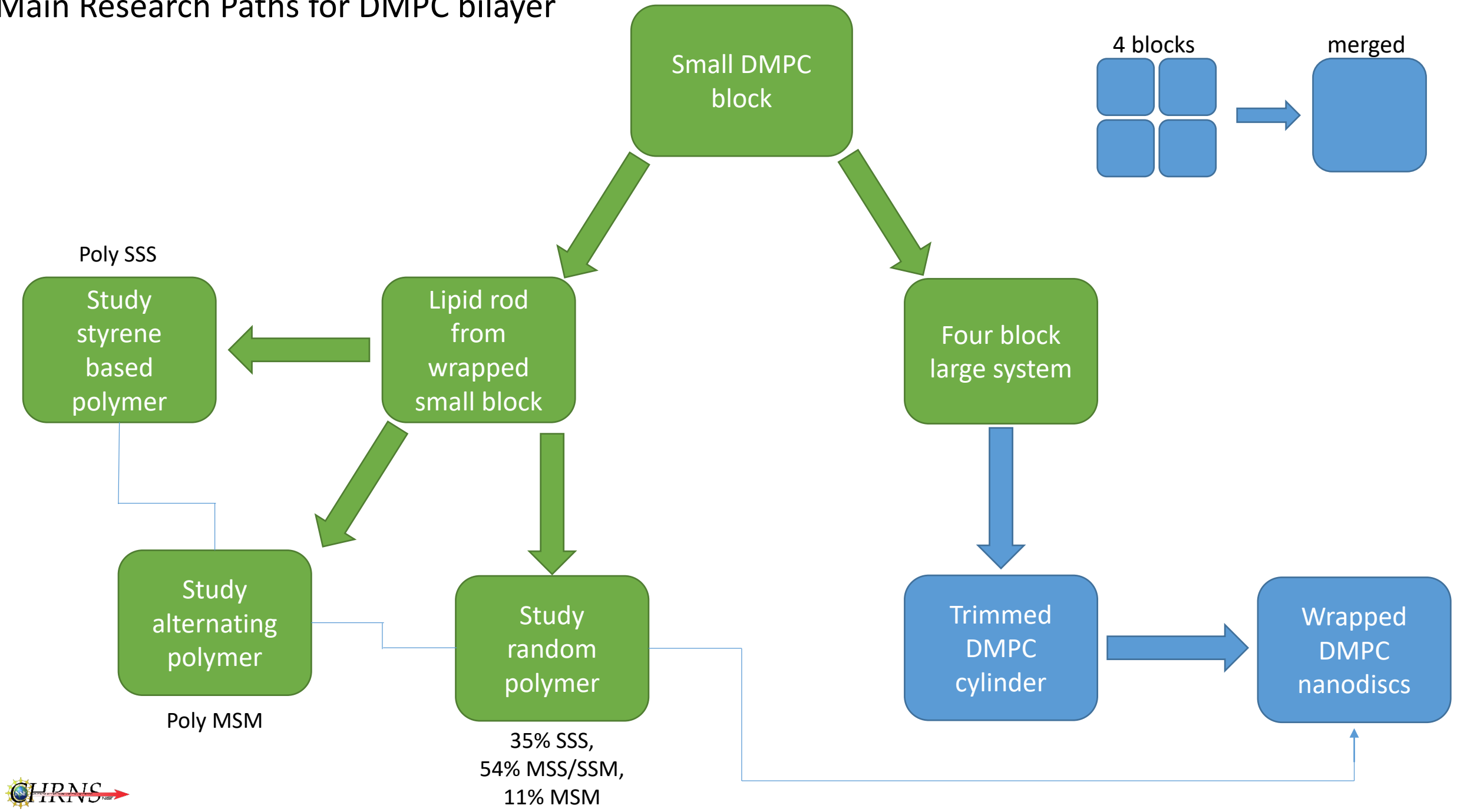
Main Research Paths for DMPC bilayer



0.2 ns random composition orientation



Main Research Paths for DMPC bilayer



Constructing the Lipid Bilayer

- Following design process from the small block we made the full nanodisc bilayer
- The solvent (water) no longer interfered, so harmonic constraints were removed
- Packing density and dimensions match experimental results

1, NVT
Fixed lipid heads, harmonic water constraints

→

2, NPT
Harmonic lipid heads and water constraints, keep_water_out.tcl force bias

→

3, NPT
Harmonic lipid head constraint, keep_water_out.tcl force bias

↓

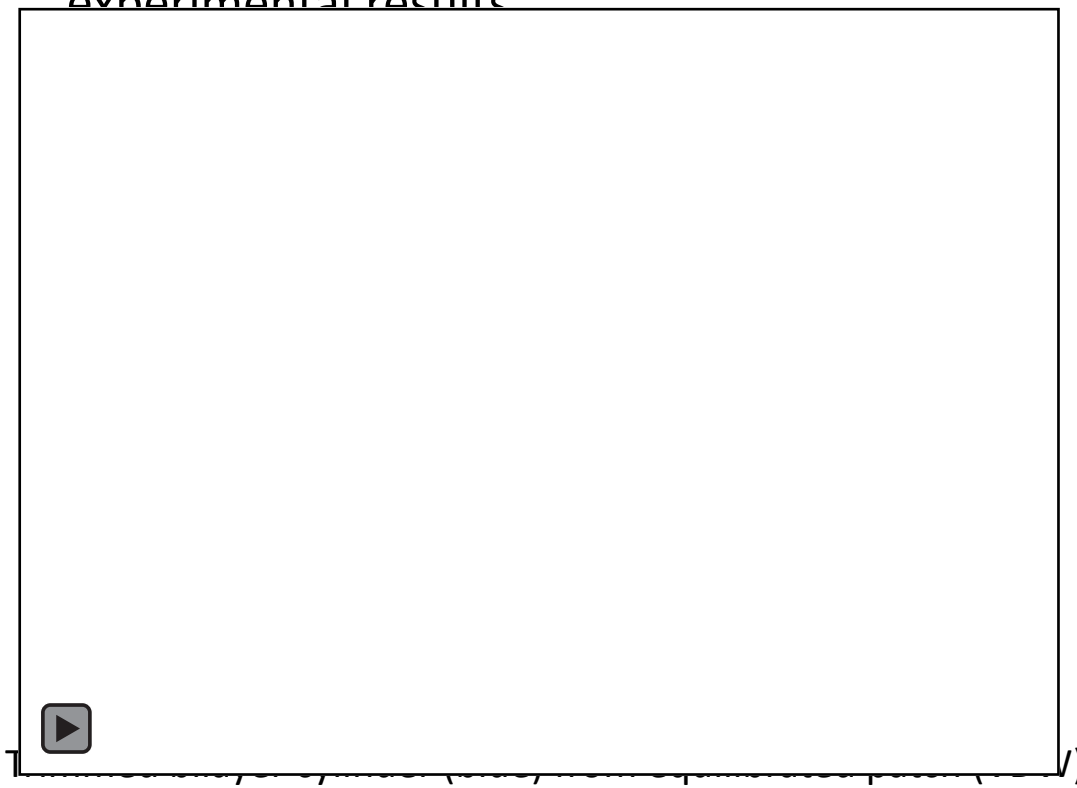
4, NPT
All constraints removed (crashed timestep 11,400)

↓

5, NPT
(restart) Increase Langevin Damping

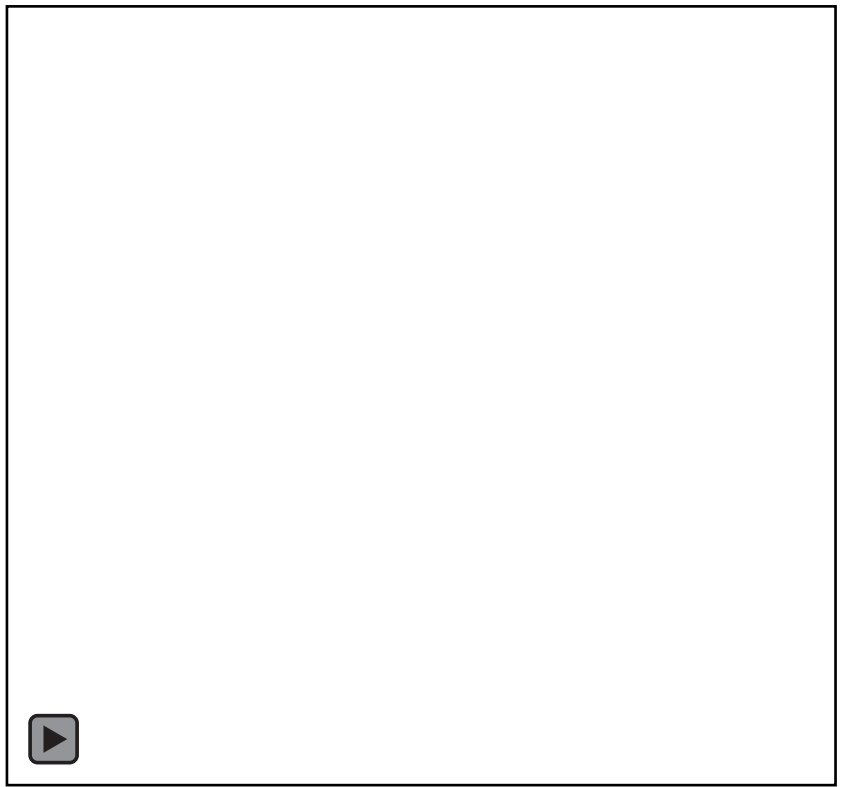
↓

6, NPT
Decrease Langevin damping, do production run

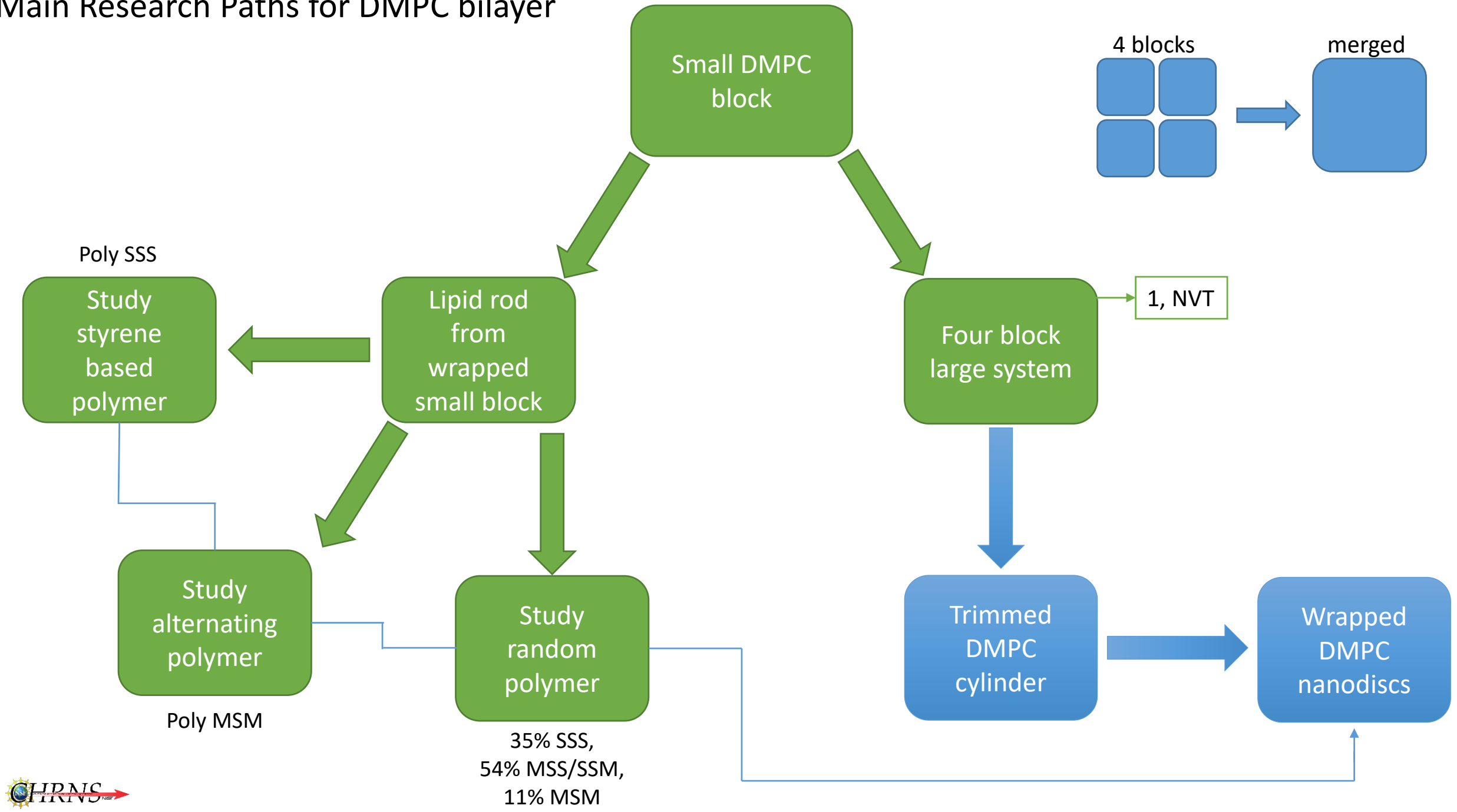


Trimmed bilayer cylinder (side) from equilibrated patch (TOP)

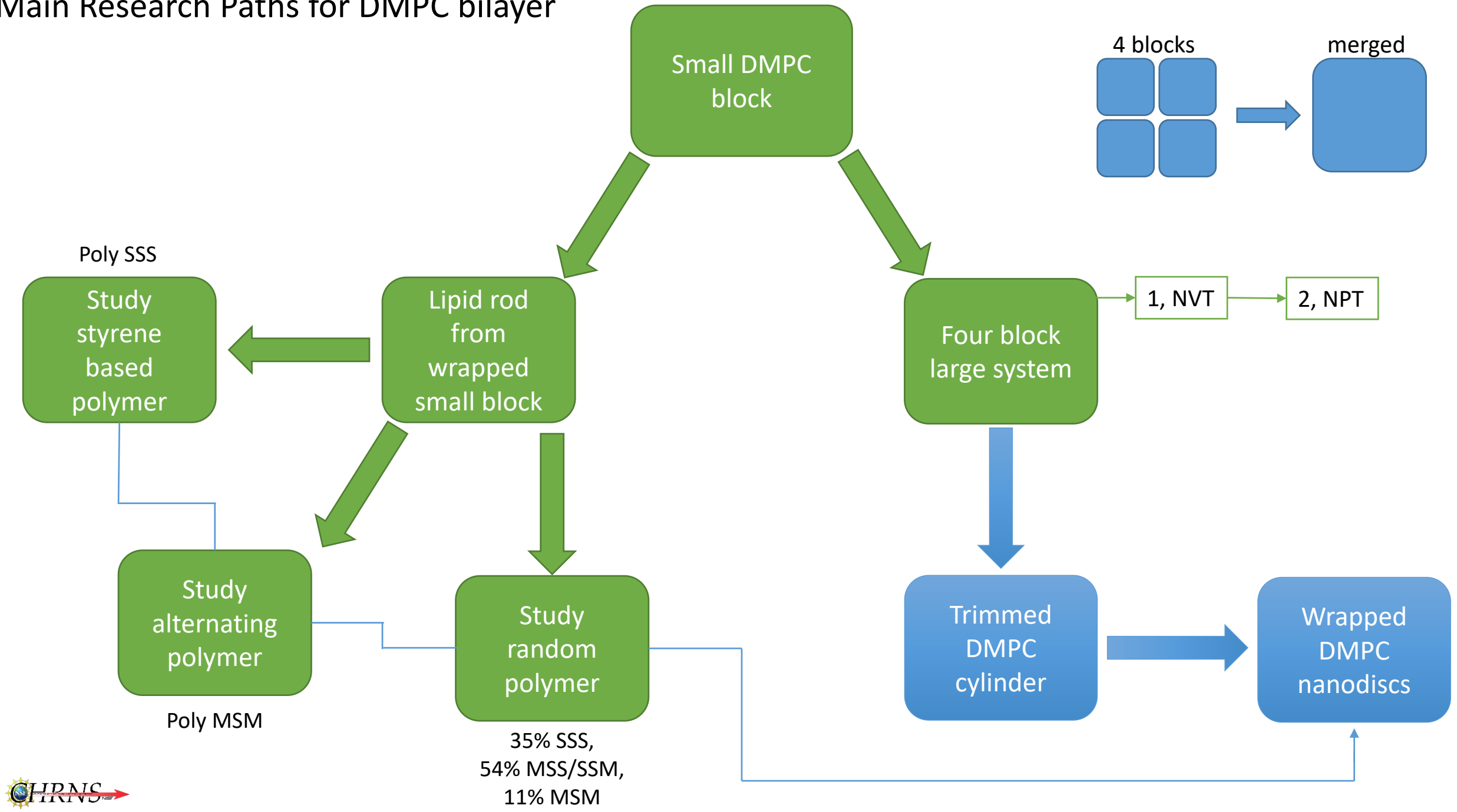
general large bilayer equilibration



Main Research Paths for DMPC bilayer



Main Research Paths for DMPC bilayer



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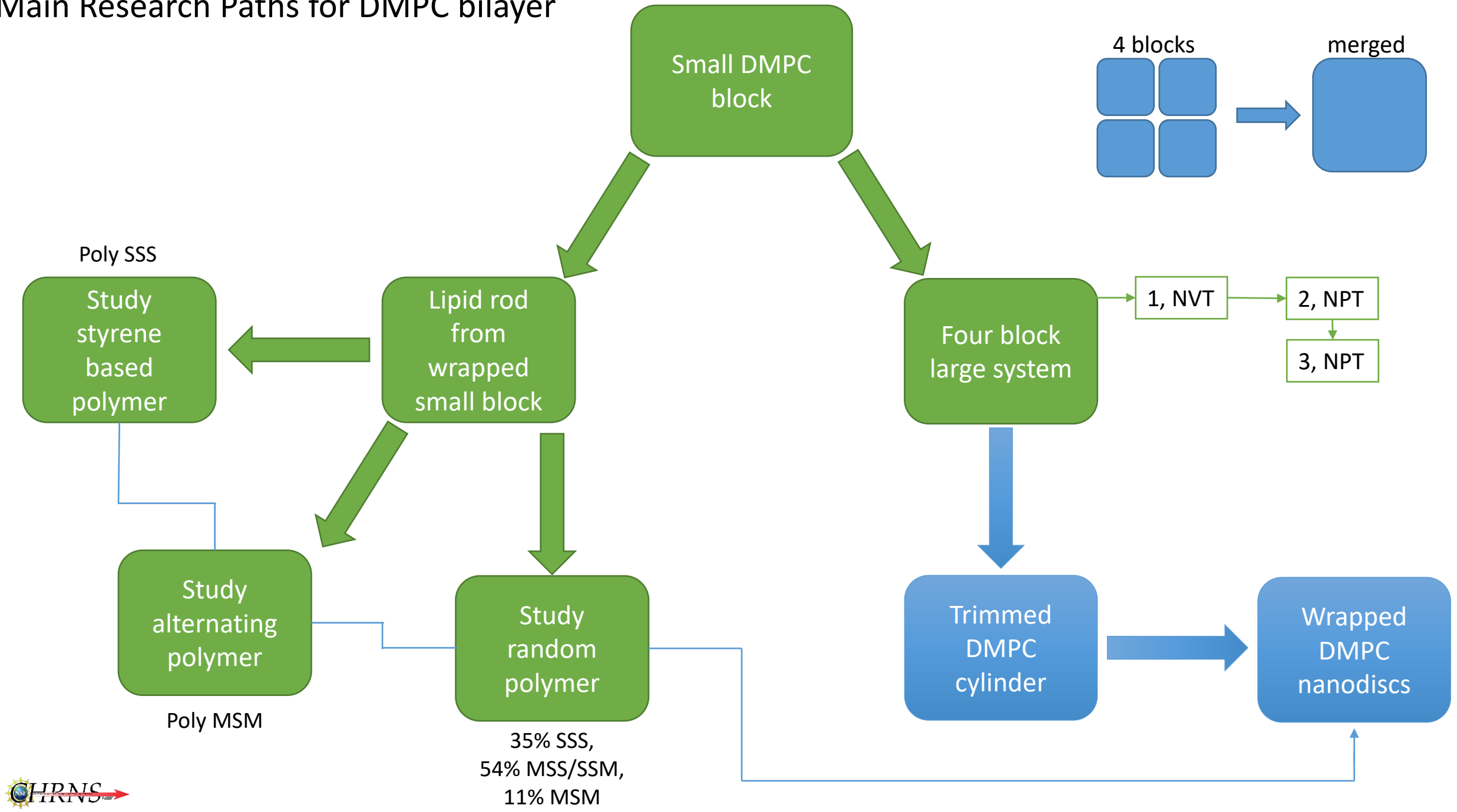

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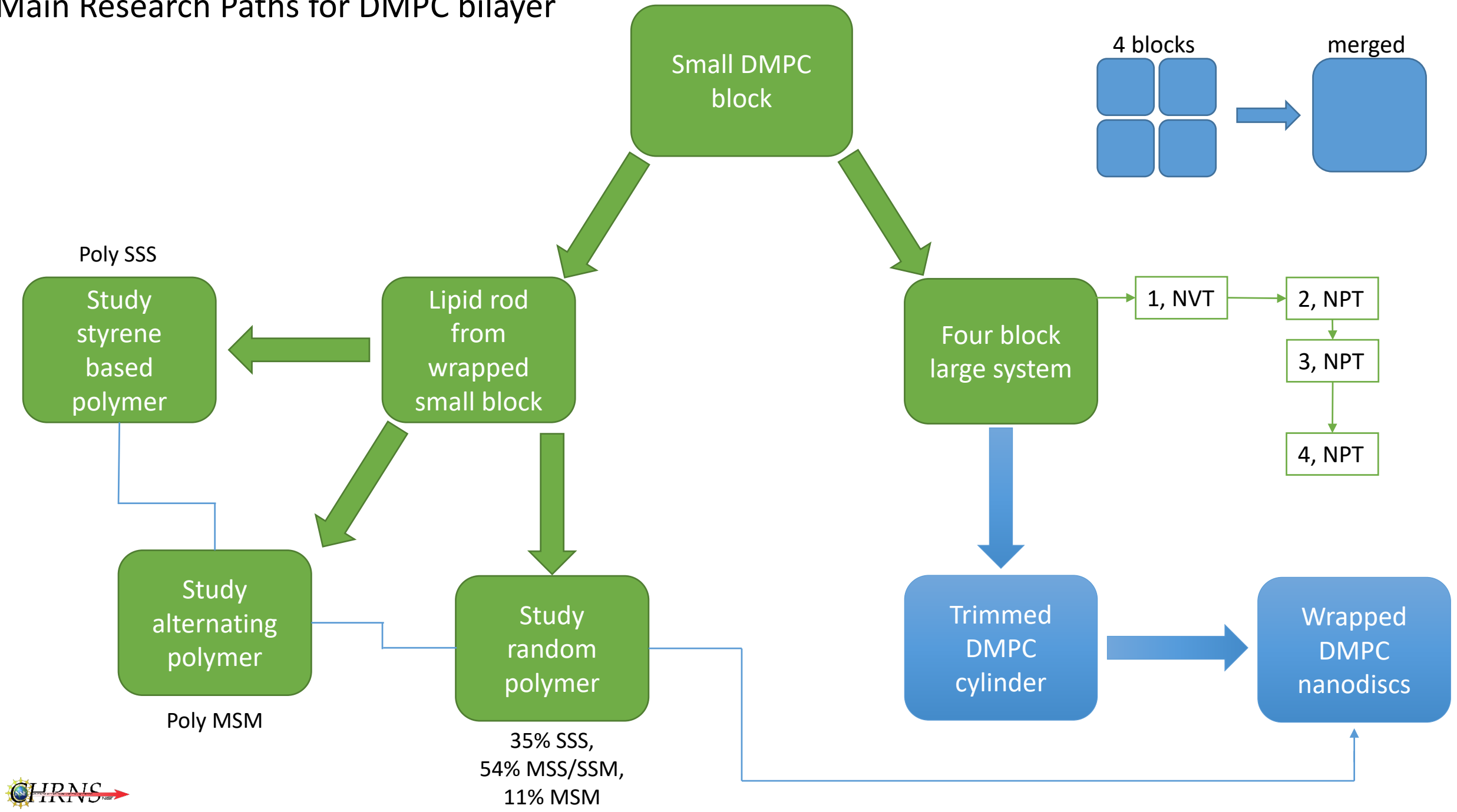
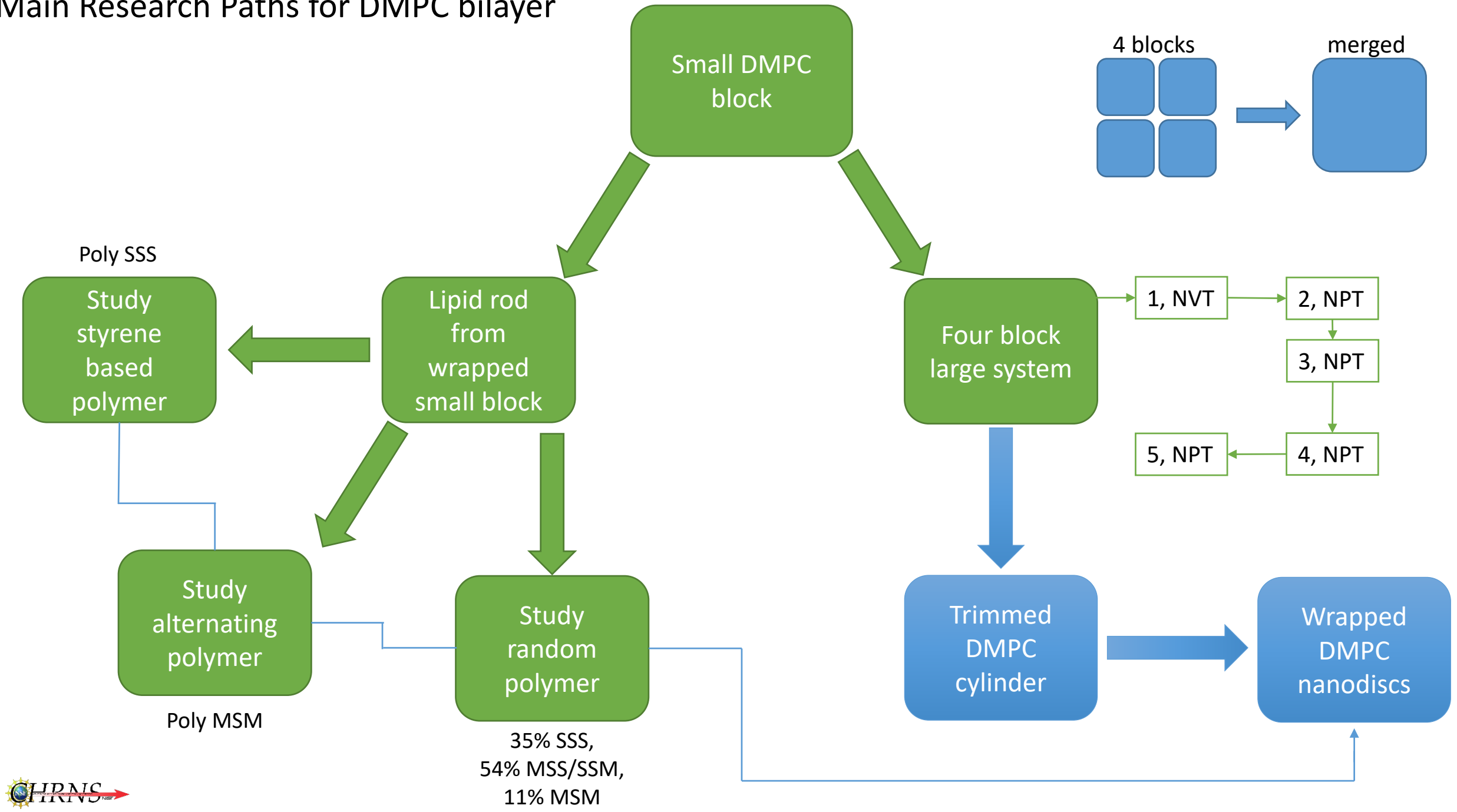


Main Research Paths for DMPC bilayer



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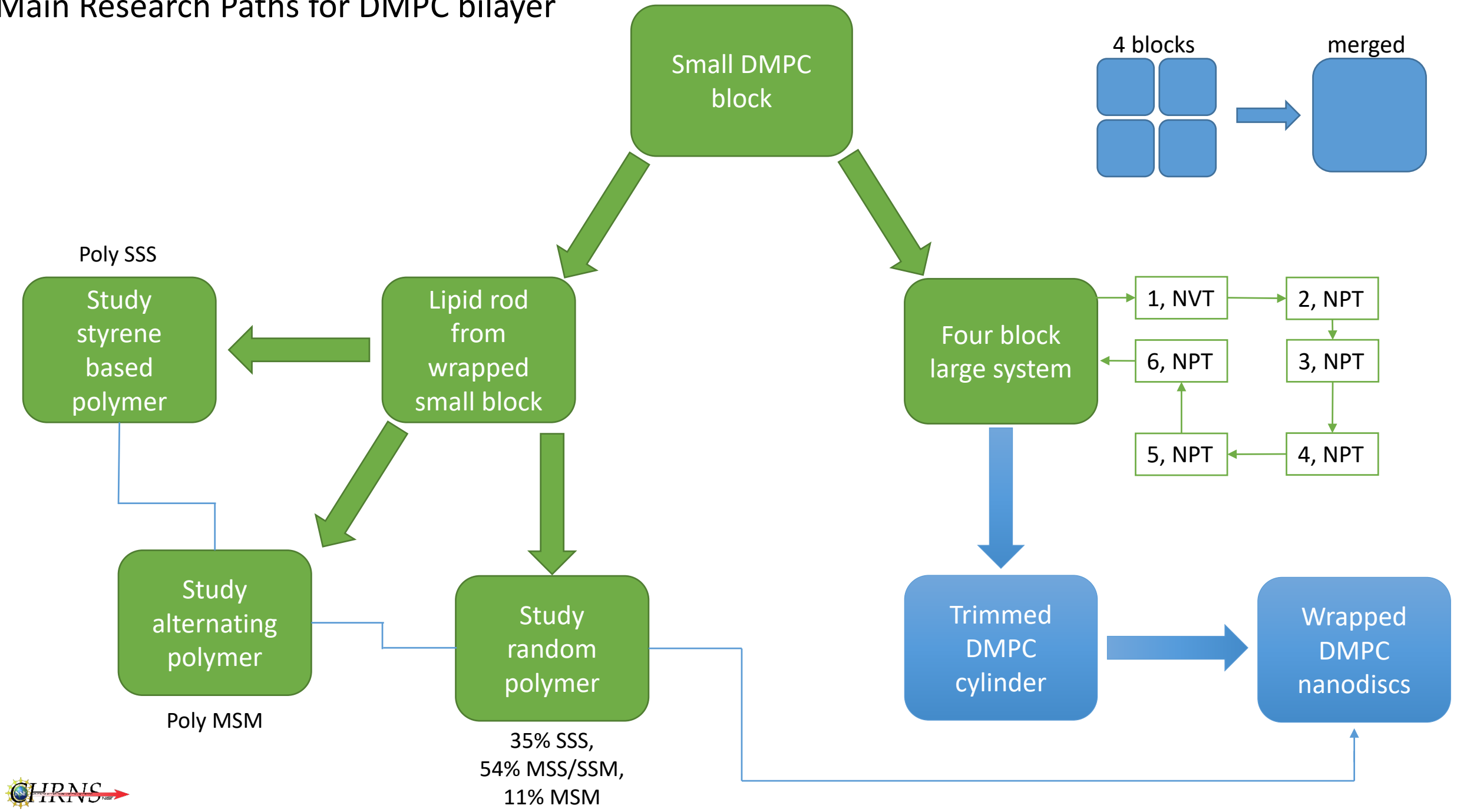

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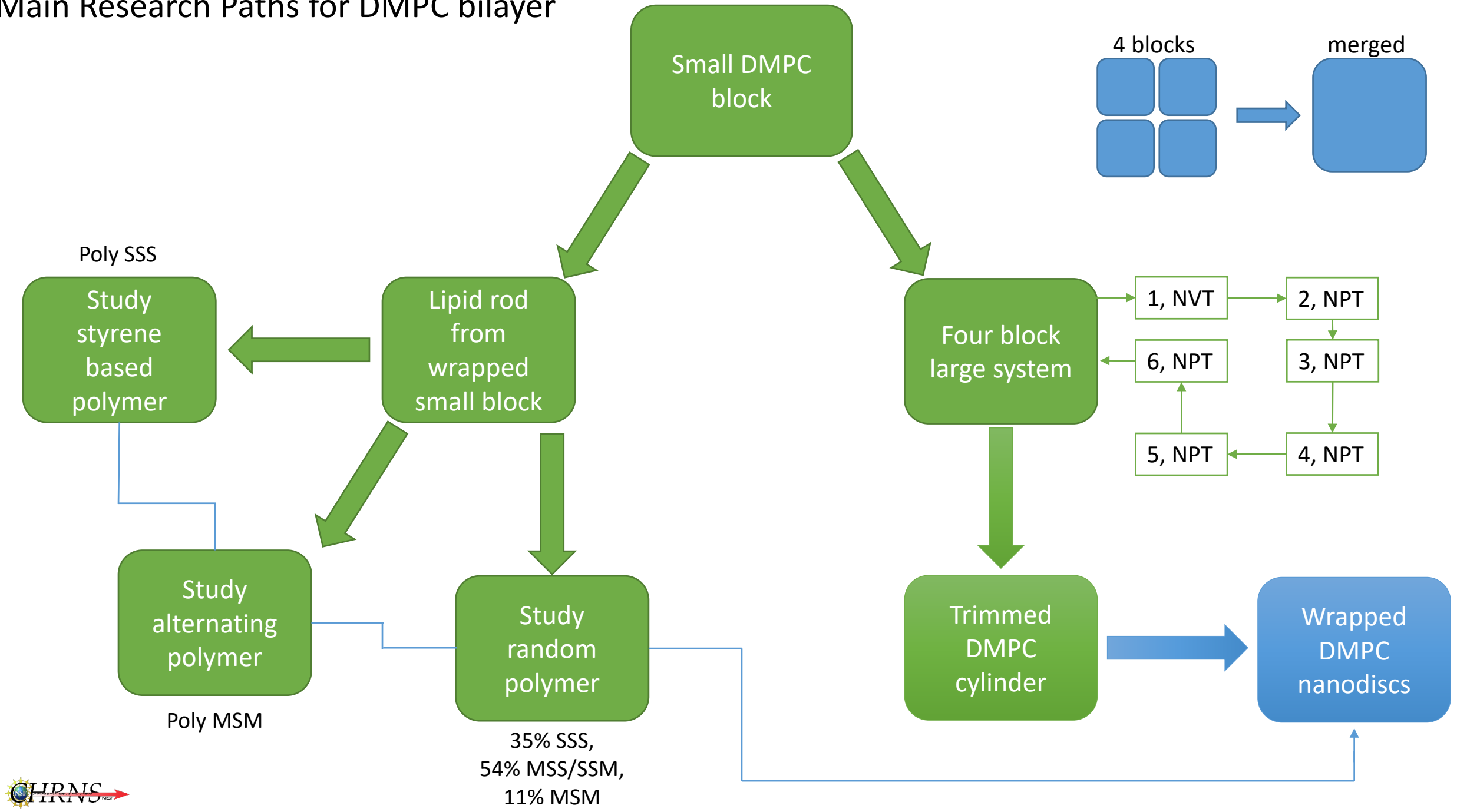
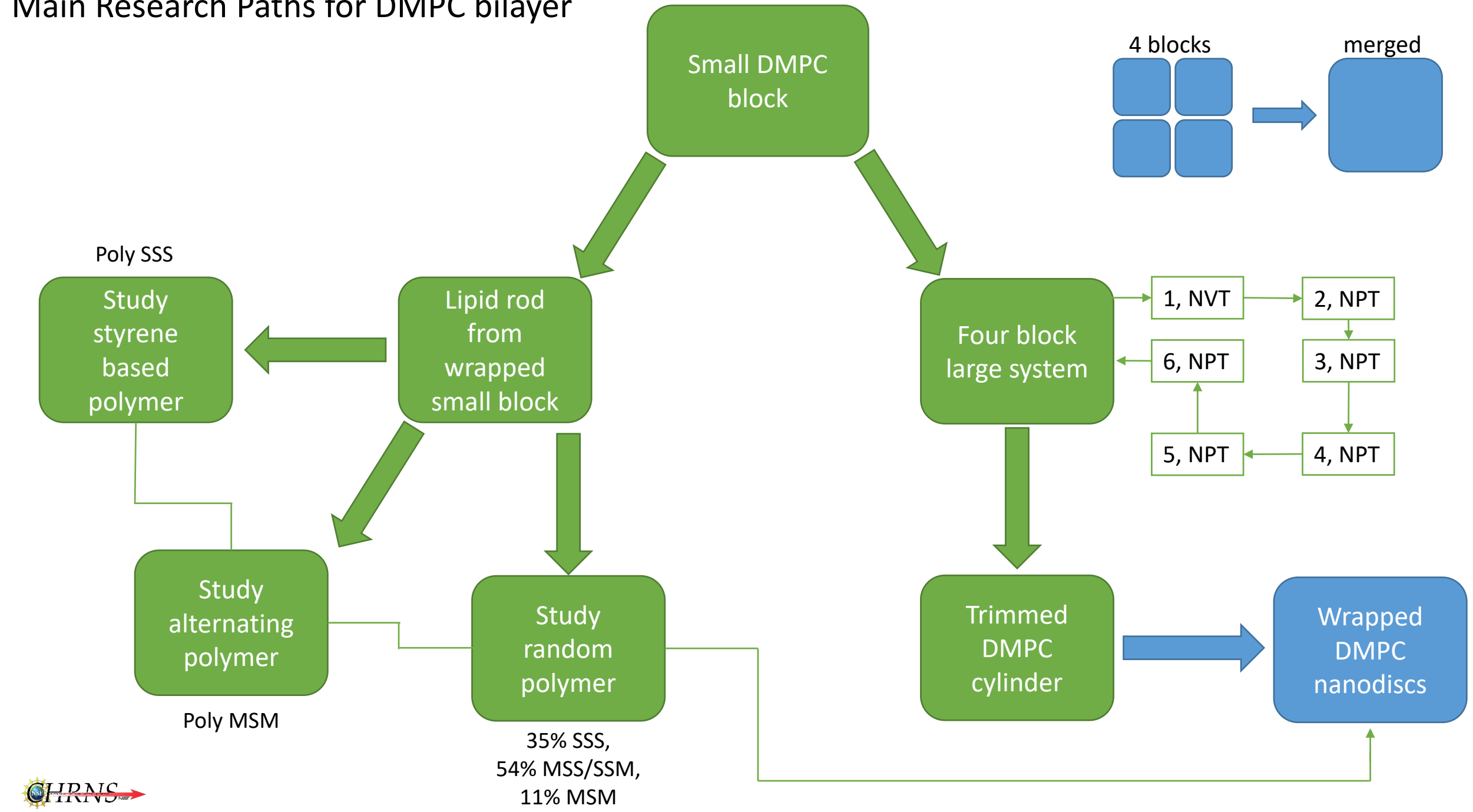
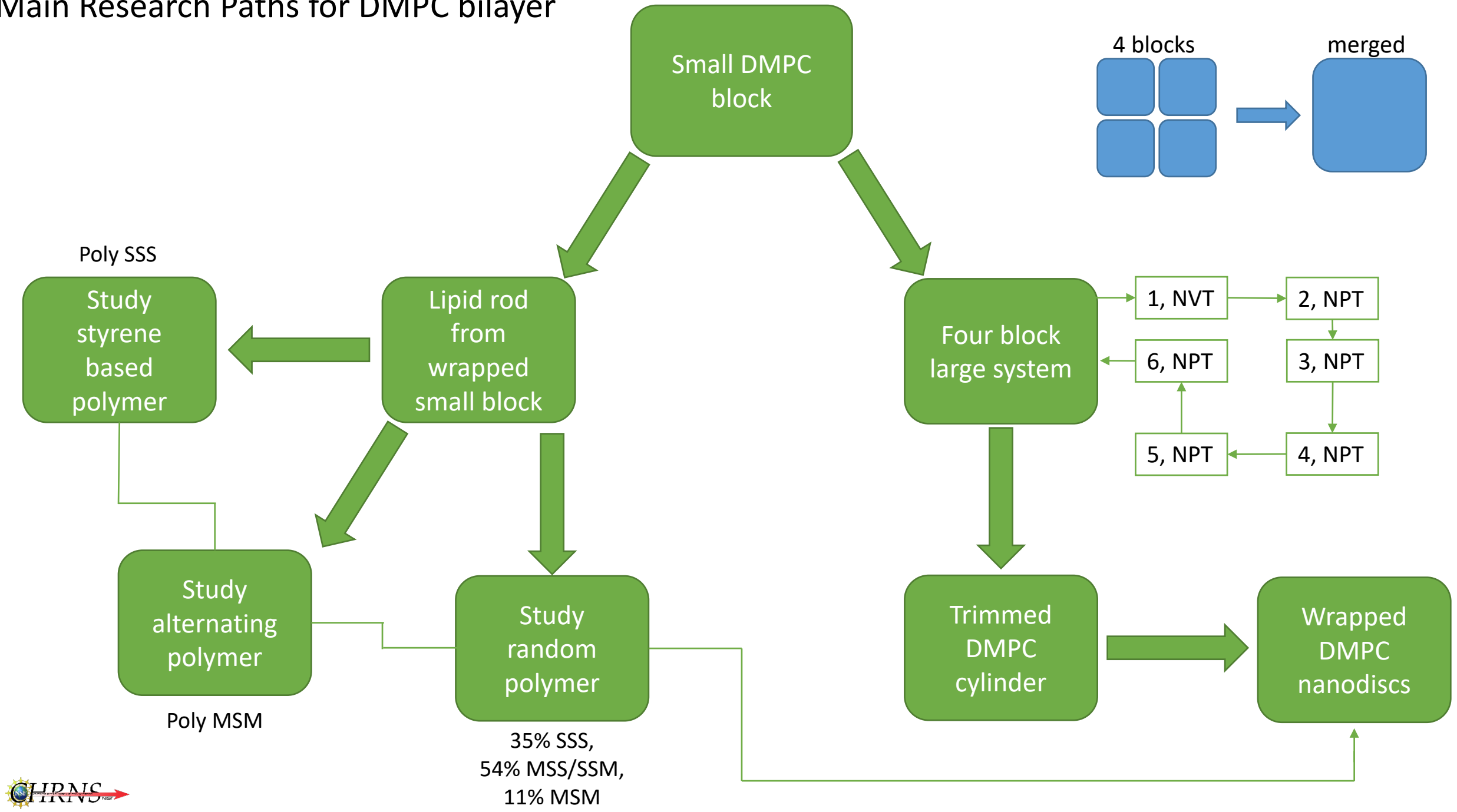


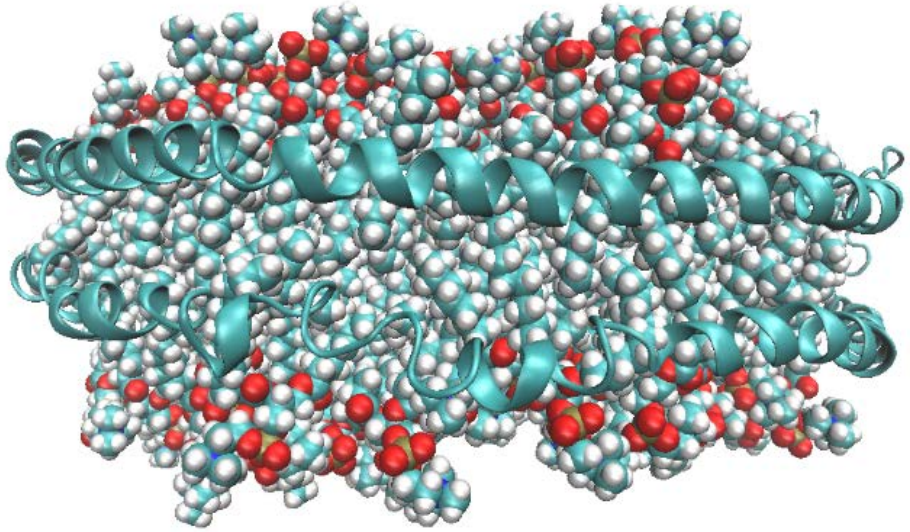
Main Research Paths for DMPC bilayer



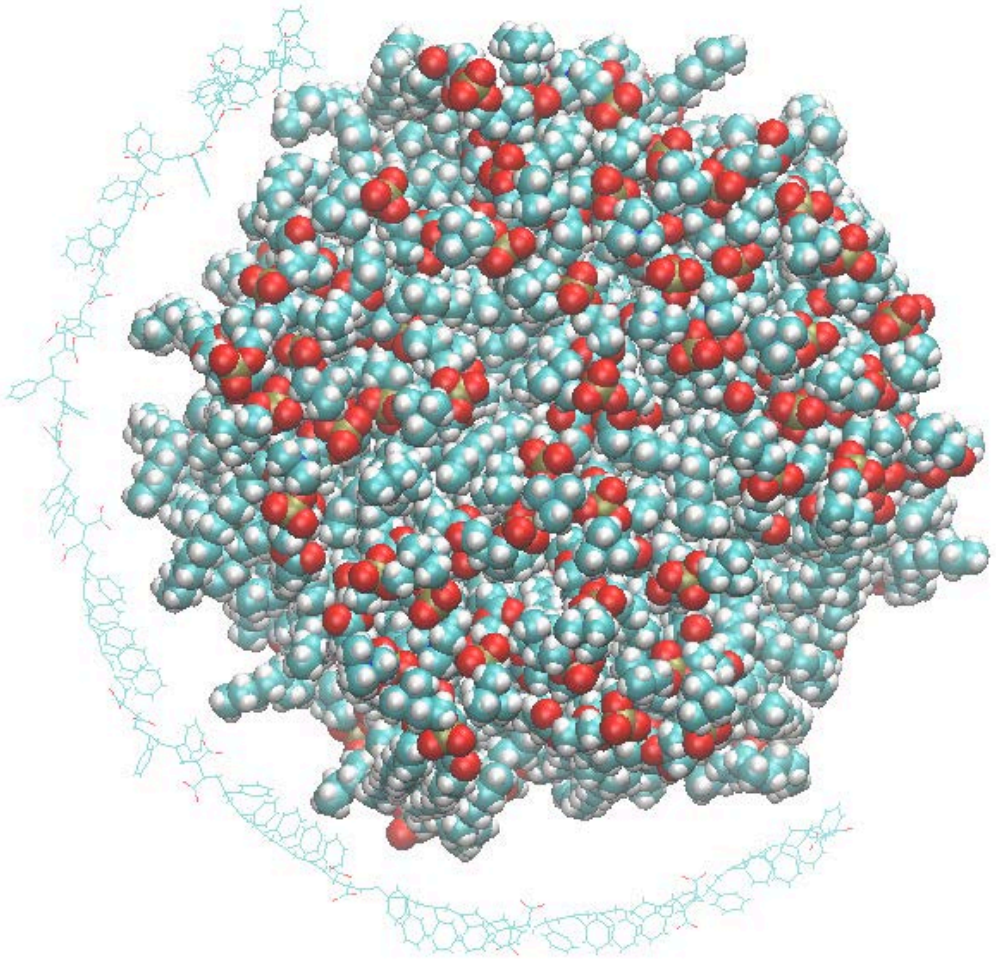
Main Research Paths for DMPC bilayer



Wrapped Nanodiscs



MSP wrapped nanodisc



SMA wrapped nanodisc (in progress)

Ongoing Work

- Various known proteins need to be simulated with our nanodisc
- Theoretical scattering profiles and contrast variation from the sample proteins should match known
- Quantum mechanical refinement of parameterization
- Validate the structure of the lipid by comparing the thickness of the bilayer and the surface per lipid head group
- Continue with the lipid rod/polymer interaction computational experiments

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