Defining the Quality of "Good Cholesterol"

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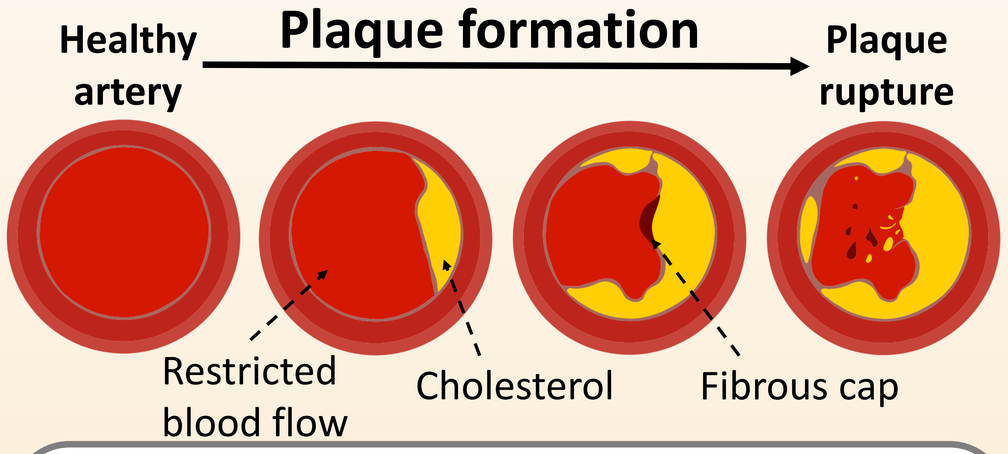
1. Challenges of treating Cardiovascular Diseases (CVD)

CVD is a leading cause of death worldwide. We accumulate arterial cholesterol throughout our lives. Treatment is usually provided at a late stage of plaque formation, CVD symptoms, for example:

- Statins can lower "bad cholesterol" production and accumulation, and lifestyle changes are a preventative measure.

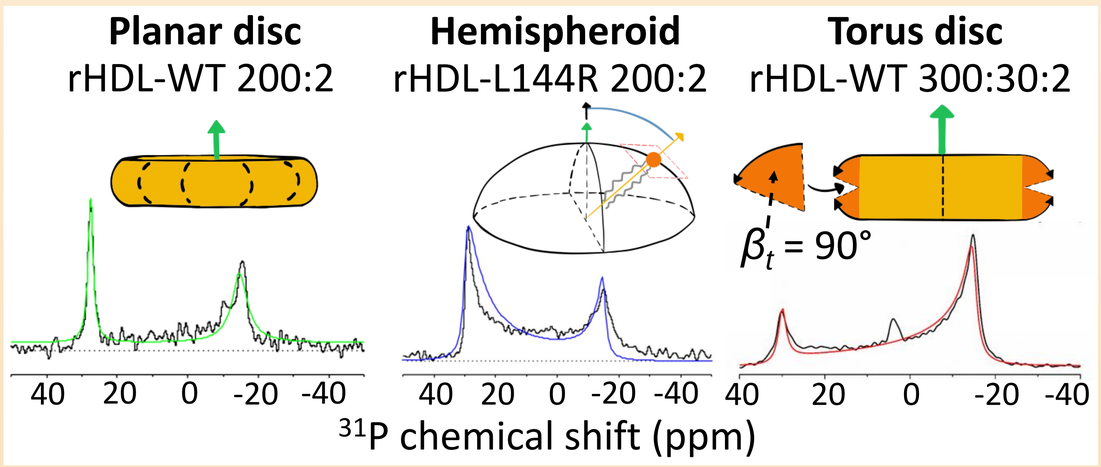
Our approach:

- Molecular level structural and functional characterisation of high-density lipoprotein (HDL) nanoparticles, "good cholesterol".
- HDL transports cholesterol from arterial plaques for excretion and recycling.
- HDL has three key molecular component targets – phospholipids: cholesterol: apolipoprotein A-I (apoA-I).



3. Detecting Particle Morphology[2,3]

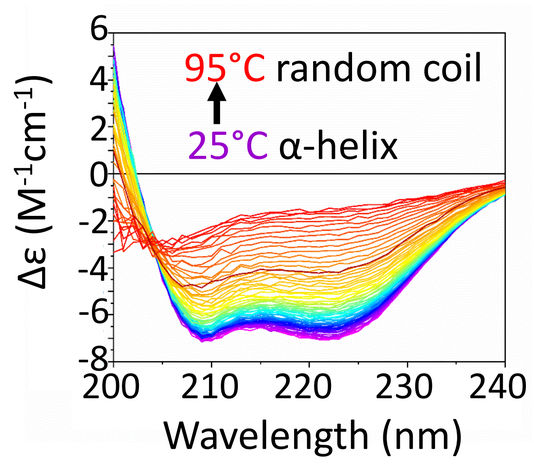
- Novel method to detect three distinct morphologies using ^{31}P solid-state NMR.
- NMR lineshapes sensitive to surface lipid curvature.
- Overcomes limitations of conventional techniques, i.e., TEM, DLS.



2. Investigating apoA-I structure

- HDL-bound apoA-I interacts with cell receptors and enzymes in reverse cholesterol transport.
- ApoA-I is the major protein in nascent HDL (70 %).
- Amyloidogenic and atheroprotective mutants could affect HDL function.

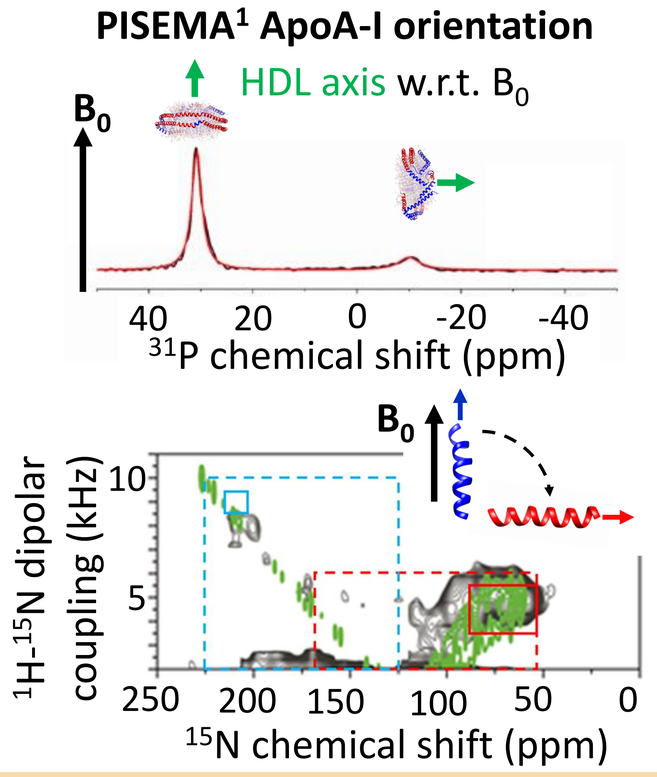
Circular dichroism



Protein thermal stability

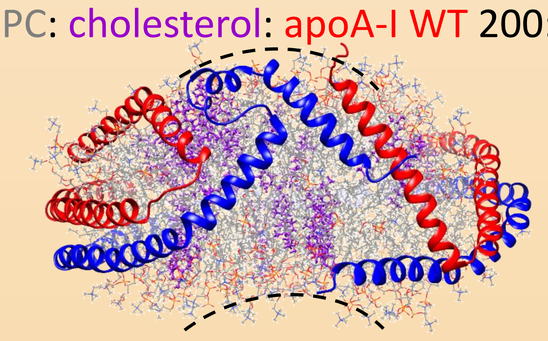
- T_m1 – lipid-bound apoA-I changes
- T_m2 – apoA-I dissociation

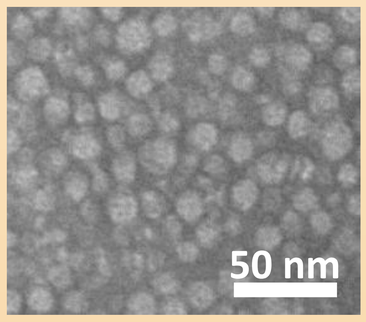
PISEMA[1] ApoA-I orientation



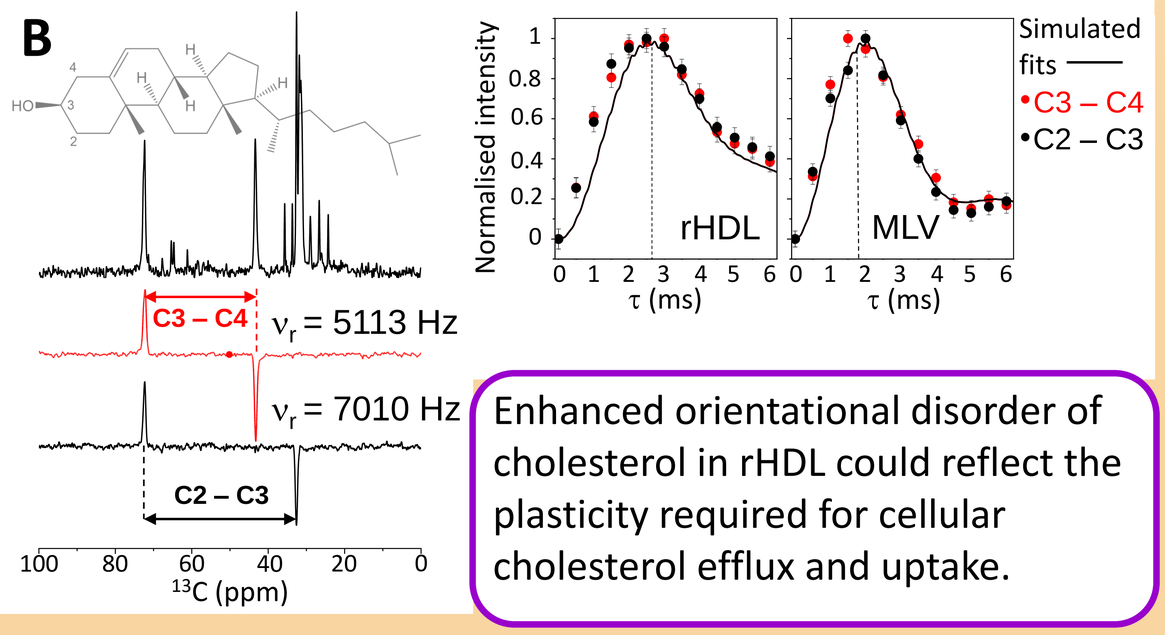
Nascent HDL AAMD Model[4]

POPC: cholesterol: apoA-I WT 200:20:2





Build-up and decay of **C2–C3** and **C3–C4** ^{13}C difference intensities as a function of the ZQ excitation period τ of the NMR pulse sequence



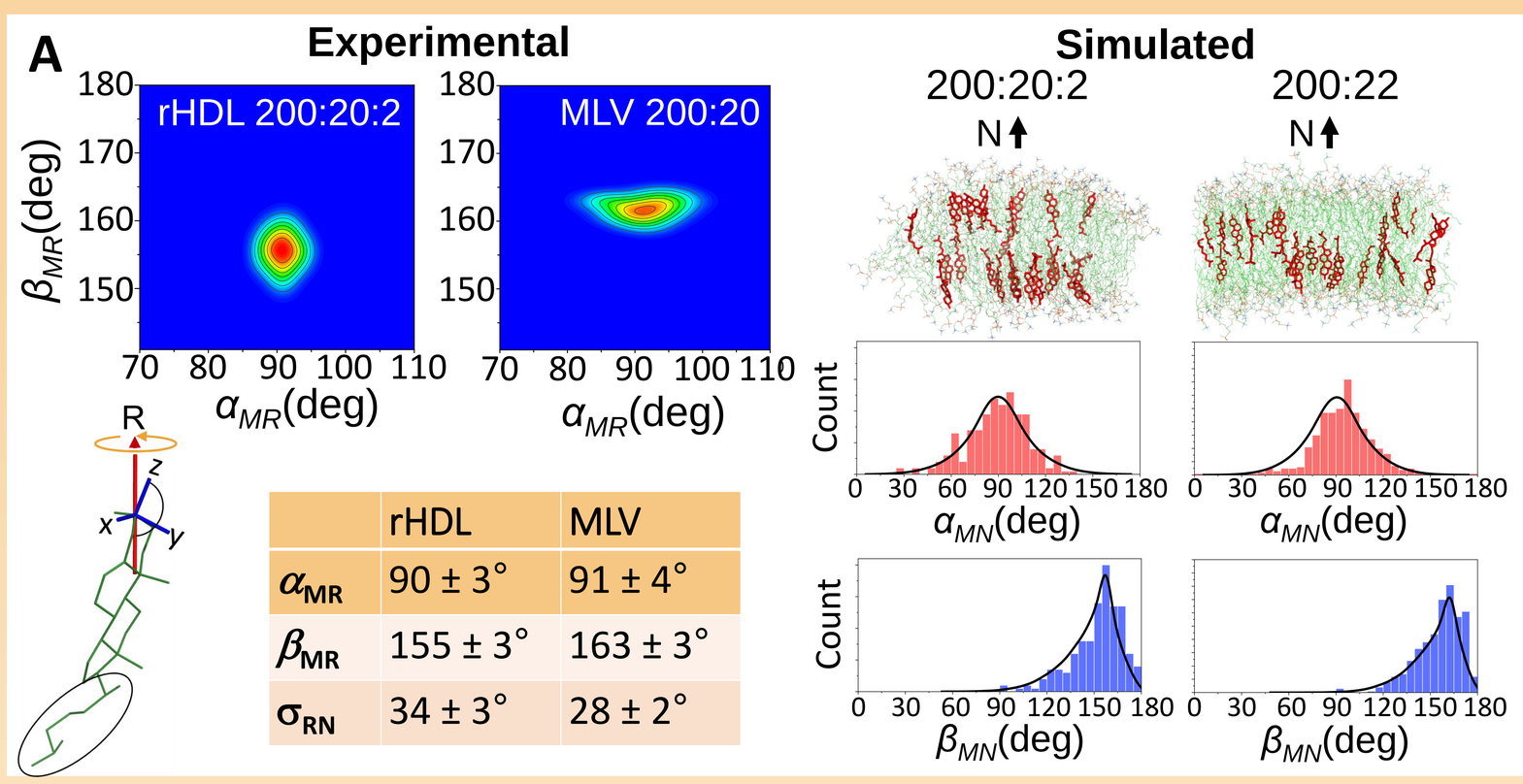
Enhanced orientational disorder of cholesterol in rHDL could reflect the plasticity required for cellular cholesterol efflux and uptake.

4. Detection of cholesterol orientation and dynamics using CP-MAS ssNMR[5]

A direct comparison of rHDL and multilamellar vesicles (MLVs) + [2,3,4-$^{13}C_3$]-cholesterol using ssNMR measurements of dynamically averaged ^{13}C-^{13}C and ^{13}C-^{1}H dipolar couplings.

A. Subtle differences between the average cholesterol orientation in the two cholesterol environments. Excellent agreement between experimental and simulated computational model data.

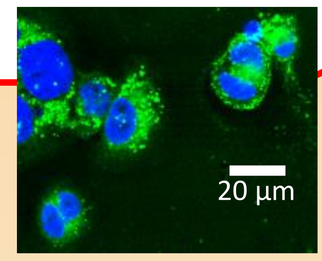
B. Different scaling extents of the ^{13}C- ^{13}C dipolar couplings → different cholesterol dynamic behaviour in rHDL-C vs. MLVs. Cholesterol samples a much greater range of orientations in rHDL.



	rHDL	MLV
α_{MR}	90 ± 3°	91 ± 4°
β_{MR}	155 ± 3°	163 ± 3°
σ_{RN}	34 ± 3°	28 ± 2°

5. Future work

- Apply structural characterisation to rHDL with a variety of compositions, e.g., cholesterol content, amyloidogenic/ atheroprotective apoA-I.
- *In vitro* cholesterol efflux and uptake cellular assays.
- Characterise clinical plasma HDL to develop a method to predict CVD risk.



BODIPY-cholesteryl ester loaded THP-1 macrophages

References

1. F. M. Marassi and S. J. Opella, *J Magn Reson*, **2000**, 144, 150-155.
2. S. Lau and D. A. Middleton, *Angew Chem Int Ed.*, **2020**, 59, 18126-18130.
3. F. Picard *et al.*, *Biophys J*, **1999**, 77, 888-902.
4. M. Pourmousa *et al*, *PNAS USA*, **2018**, 115, 5163-5168.
5. S. Lau and D. A. Middleton, *PCCP*, **2022**, 24, 23651-22366.