Tech Session 4: Nanomedicine and Nanoscale Delivery

Bruno Silva

CONTROLLED RELEASE SOCIETY
CRS 2023 ANNUAL MEETING & EXPOSITION
JULY 24-28, 2023 Paris Hotel » Las Vegas, NV, USA
THE FUTURE OF DELIVERY SCIENCE

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Welcome
Bienvenue

INTERNATIONAL IBERIAN NANOTECHNOLOGY LABORATORY

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Materials Science and Technology

A Criterion for Predicting Stable Assembly of Lipid–Polymer–Nucleic Acid Nanoparticles

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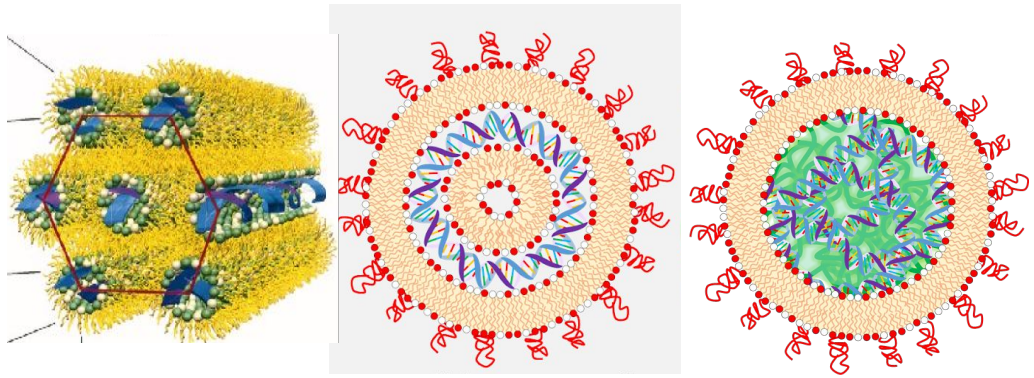
Bio-Nano Assembly group @ Empa

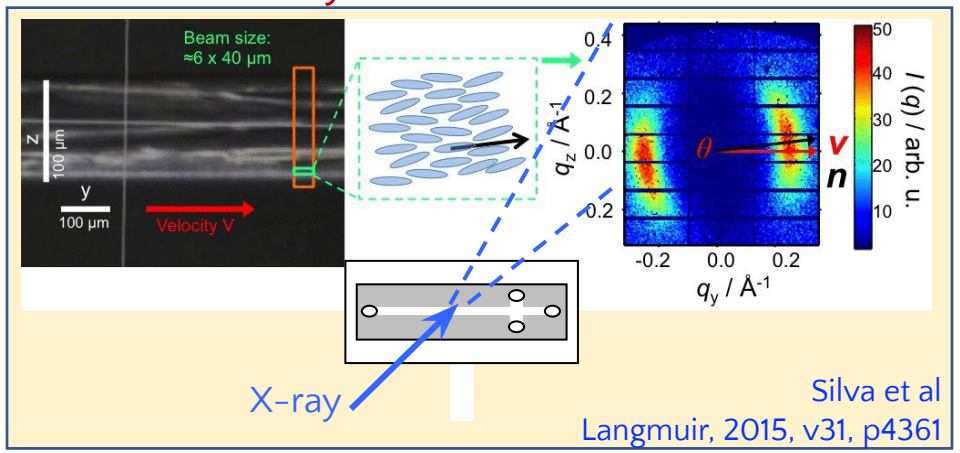
We develop new bio-nano-inspired nanosystems for health,
and small-angle scattering (and beyond) methods & concepts to understand them

Emphasis: on nonviral systems for gene therapeutics

- DNA-Lipid complexes, LCs & NPs



- Microfluidics for directed assembly
- in-situ X-ray



Silva et al
Langmuir, 2015, v31, p4361

Complex designs to overcome complex tasks

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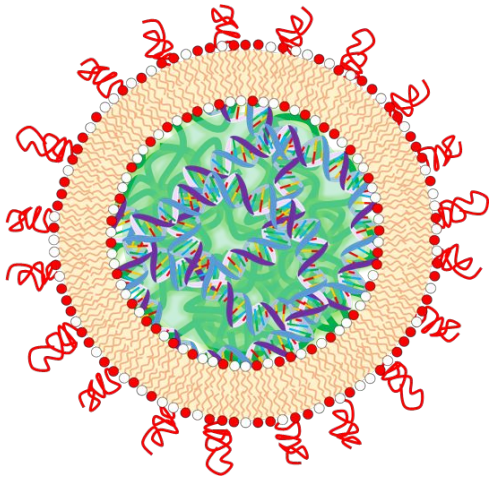
A successful gene-delivery nanosystems must overcome several biological barriers

Low circulation time (inactivation by protein coating and removal) → **Cell targeting and internalization** → **Endosomal release (PEG dilemma)** → **Trafficking to nucleus** → **Gene Release**

Lipid Envelope

Targeting peptides (RGD, KALA, etc)

Diffusible PEG coating



Polymer Core

NLS tags (SV40)

Destabilization for release (e.g. disulfide bonds)

Lipid–polymer core shell NPs (LPNPs)

Harashima et al, JCR, 2014, v190, p593
Torchilin et al, Int. J. Pharm. 2012, v427, p3

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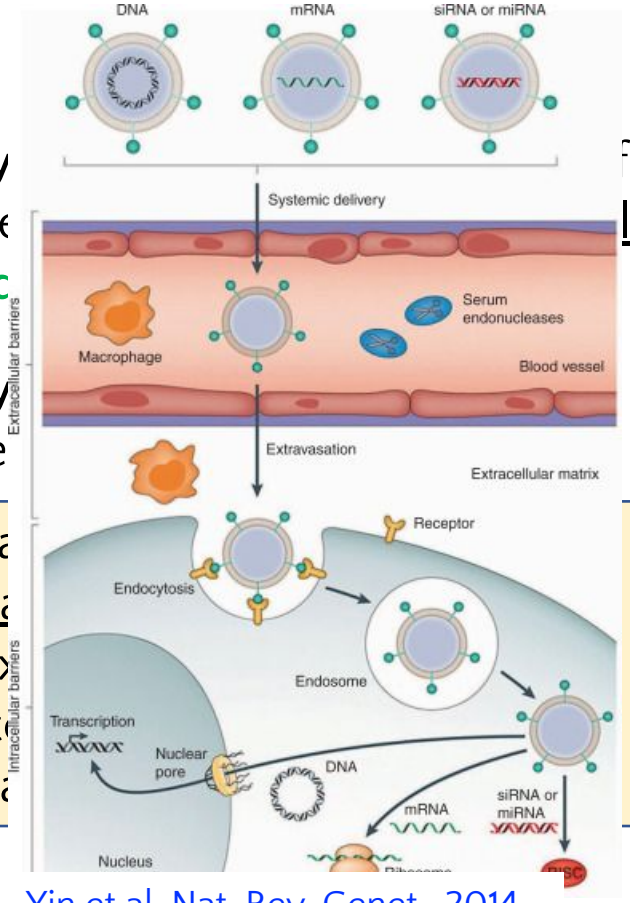
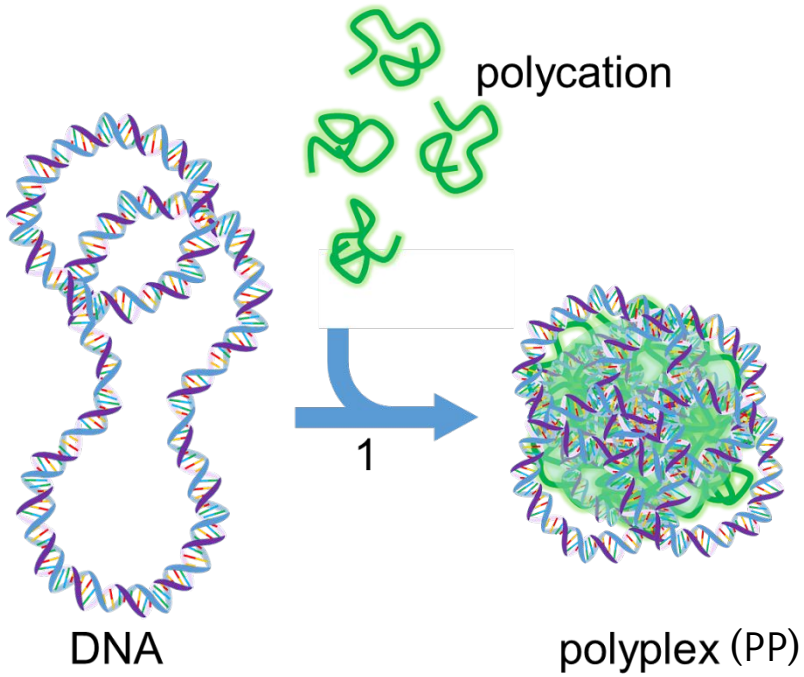
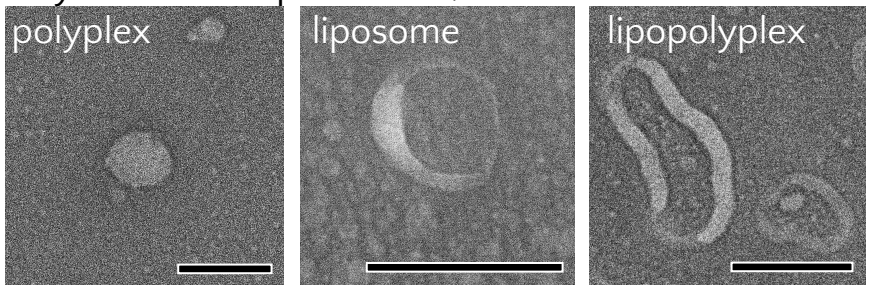


Yin et al, Nat. Rev. Genet., 2014

LPNPs: liposome–polycation–DNA nanoparticles



1) Charge inversion drives assembly – **Electrostatics** (identical to LbL)
2) For final particle positive charge, core should be negative (excess DNA)
- Poly-l-lysine used as polycation (ubiquitous)
- PEGylated Cat. Liposomes (80% DOTAP, 10% DOPC, 10% PEG)



3) Other pairwise interactions need to be considered
4) Liposomes may displace DNA from polycation
(c.f. Cat. Liposome - nucleosomes, Berezhnoy, Korolev, Nordenskiöld, Adv. Colloid Int. Sci. 2014)

5) Difficult to characterize with conventional methods

TEM methods reveal some details, but:
- How many particles have all three components?
- What is the optimal ratio between Polymer, DNA & Lipid?

FCCS - Fluorescence Cross-Correlation Spectroscopy

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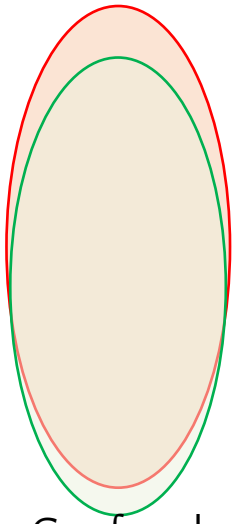
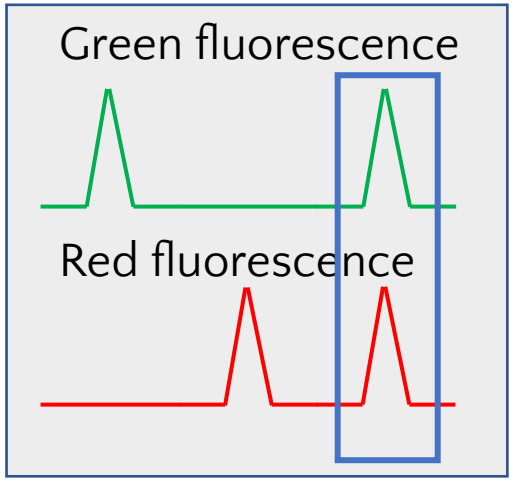
Diffusing particles



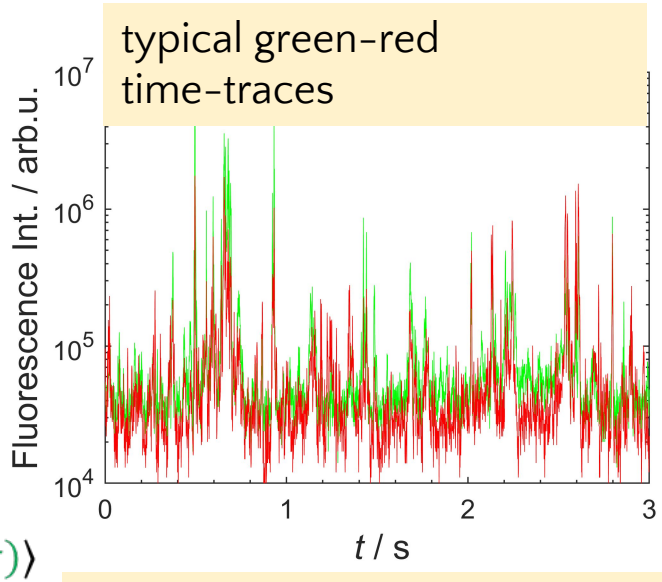
Confocal green/red volume
w ~ 400-500 nm
h ~ 1.7-1.9 µm



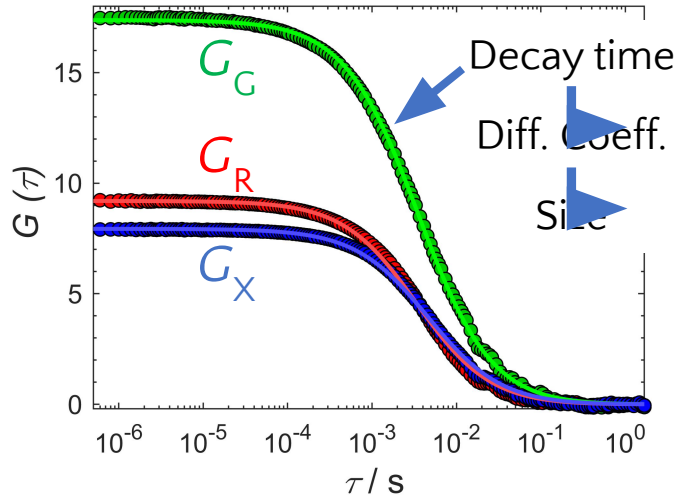
Correlation between 2 signals: co-localization

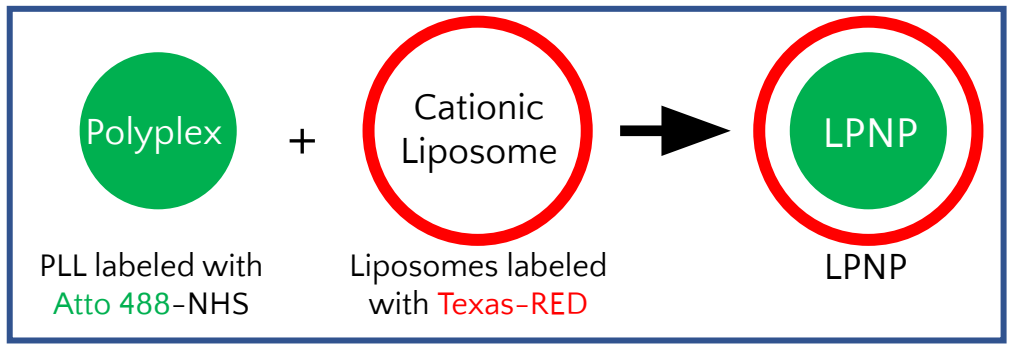
$$G_X(\tau) = \frac{\langle \delta F_R(t) \delta F_G(t+\tau) \rangle}{\langle F_R(t) \rangle \langle F_G(t) \rangle}$$

typical green-red time-traces



typical auto/cross-correlation





Bacia & Schwille, Nature Protocols, 2007

FCCS – Fluorescence Cross-Correlation Spectroscopy

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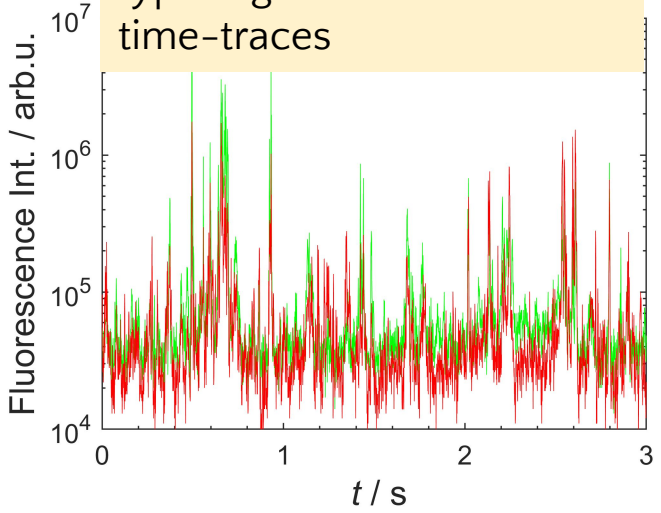
Amplitudes of auto- and cross-correlation depend on particle concentration

$$A_G = \sum_i \eta_{i,G}^2 N_{i.G} \Big/ \left(\sum_i \eta_{i,G} N_{i.G} \right)^2$$

$$A_R = \sum_i \eta_{i,R}^2 N_{i.R} \Big/ \left(\sum_i \eta_{i,R} N_{i.R} \right)^2$$

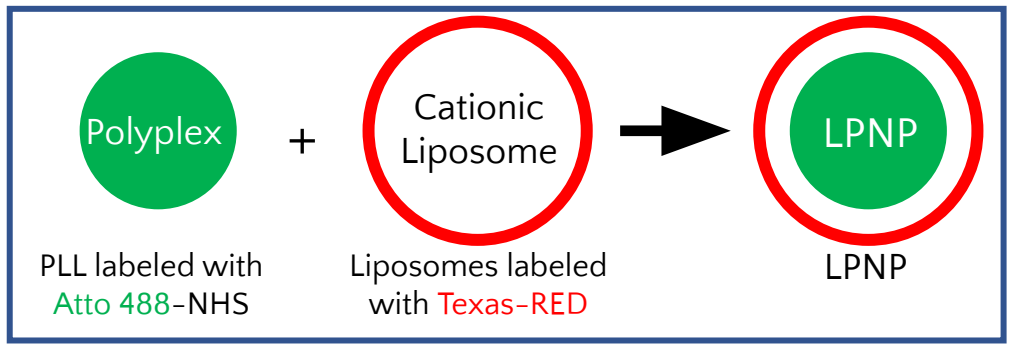
$$A_{RG} = \sum_i \eta_{i,G} \eta_{i,R} N_{i.RG} \Big/ \left[\left(\sum_i \eta_{i,G} N_{i.G} \right) \left(\sum_i \eta_{i,R} N_{i.R} \right) \right]$$

typical green-red time-traces



Auto-correlation A_G & A_R $\propto 1/C_i$ – inversely proportional to concentration

Cross-correlation A_{RG} $\propto C_{i.RG}$ – <u>proportional</u> to conc. of <u>Co-localized</u> species



Bacia & Schwille, Nature Protocols, 2007

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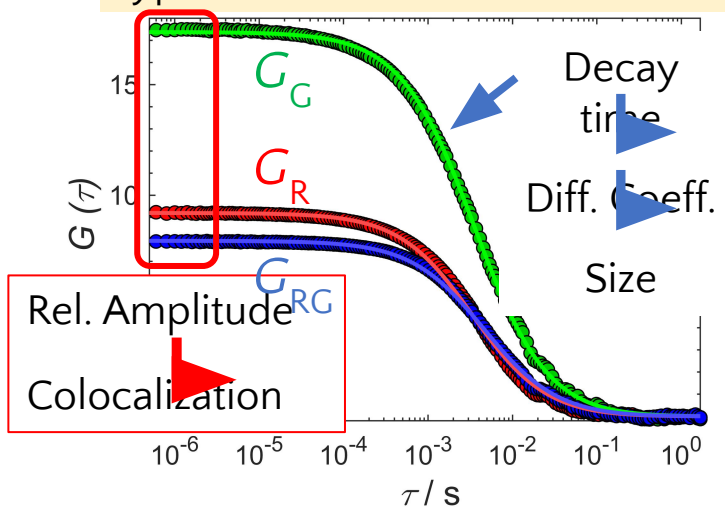

FCCS – Fluorescence Cross-Correlation Spectroscopy

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For **1:1 binding** between **red** & **green** species and **no cross-talk**

$$A_G = 1/(N_{PP.free} + N_{LPNP})$$

$$A_R = 1/(N_{Lip.free} + N_{LPNP})$$

$$A_X = N_{LPNP}/\left((N_{PP.free} + N_{LPNP})(N_{Lip.free} + N_{LPNP})\right)$$

Fraction of coated PPs

$$\frac{A_X}{A_R} = \frac{N_{LPNP}}{N_{LPNP} + N_{PP.free}}$$

Fraction of used lips.

$$\frac{A_X}{A_G} = \frac{N_{LPNP}}{N_{LPNP} + N_{Lip.free}}$$

Relative values of the amplitudes G(0) of Green, Red, and Cross-correlation

Extent of association between PPs and Lipos to form LPPs

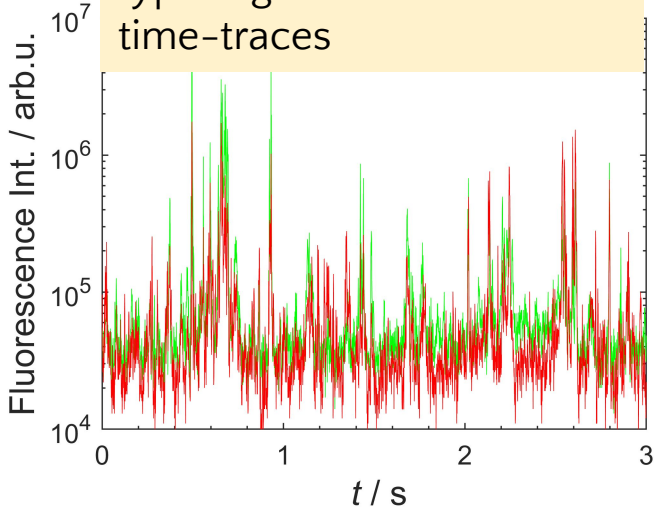
PLL labeled with Atto 488-NHS

Liposomes labeled with Texas-RED

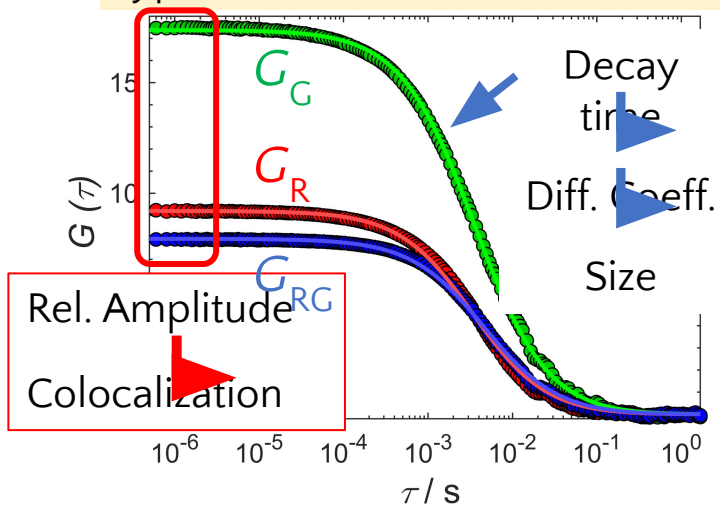
LPNP

Bacia & Schwille, Nature Protocols, 2007

typical green-red time-traces



typical auto/cross-correlation



FCCS quantifies colocalization between Lipos. & cores

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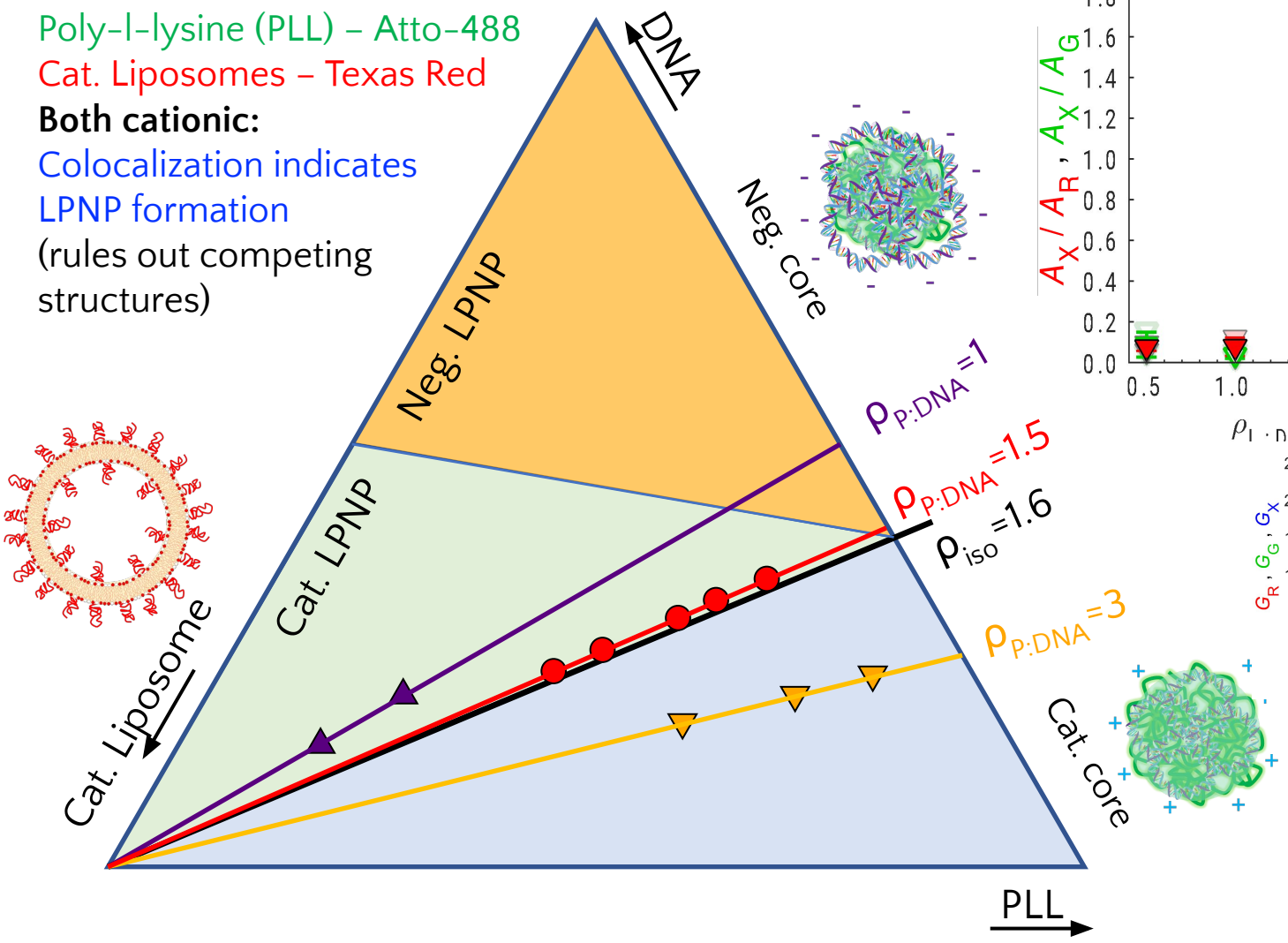
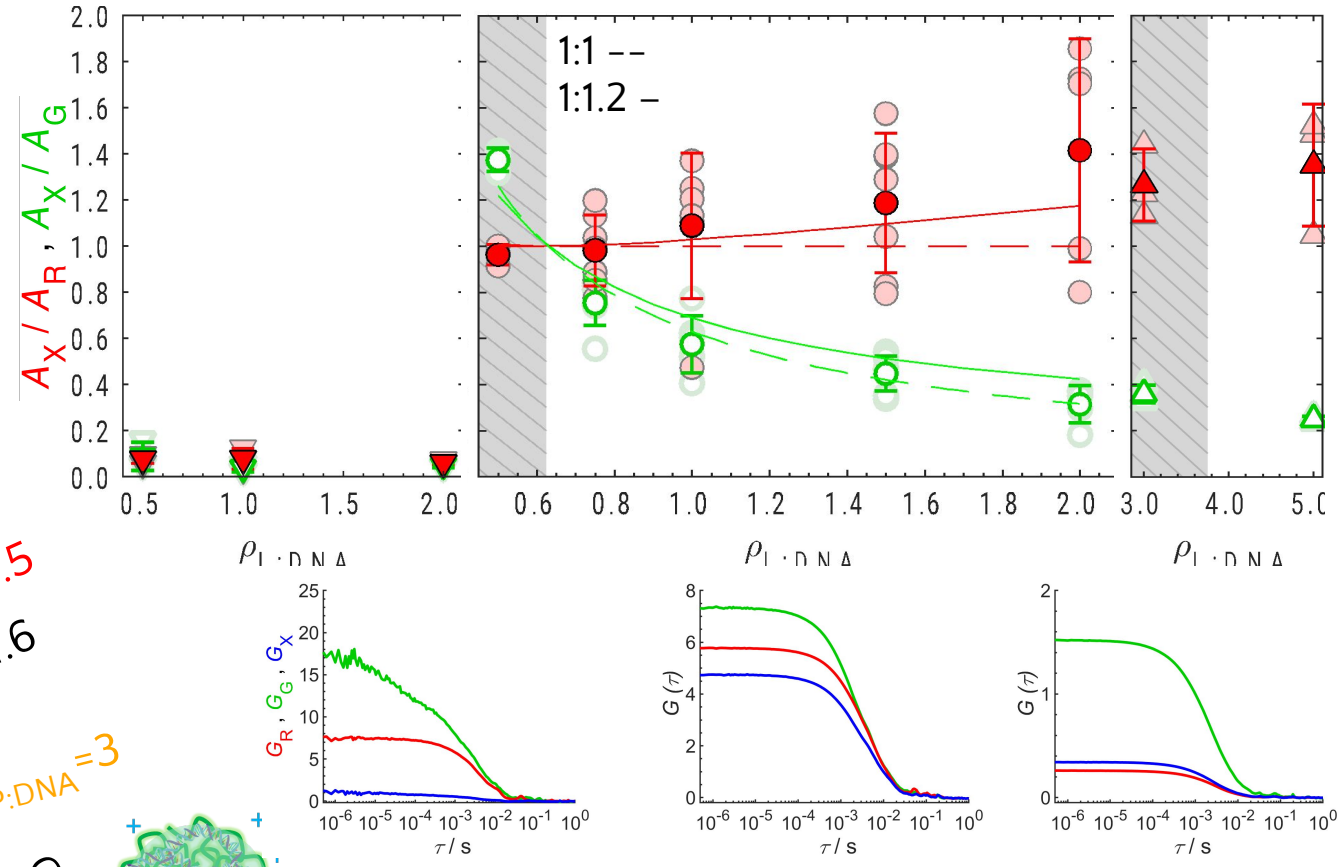
Labels:
Poly-l-lysine (PLL) – Atto-488
Cat. Liposomes – Texas Red
Both cationic:
Colocalization indicates LPNP formation
(rules out competing structures)





Fraction of coated PPs

$$\frac{A_X}{A_R} \approx \frac{N_{LPNP}}{N_{LPNP} + N_{PP.free}}$$

Fraction of used liposomes

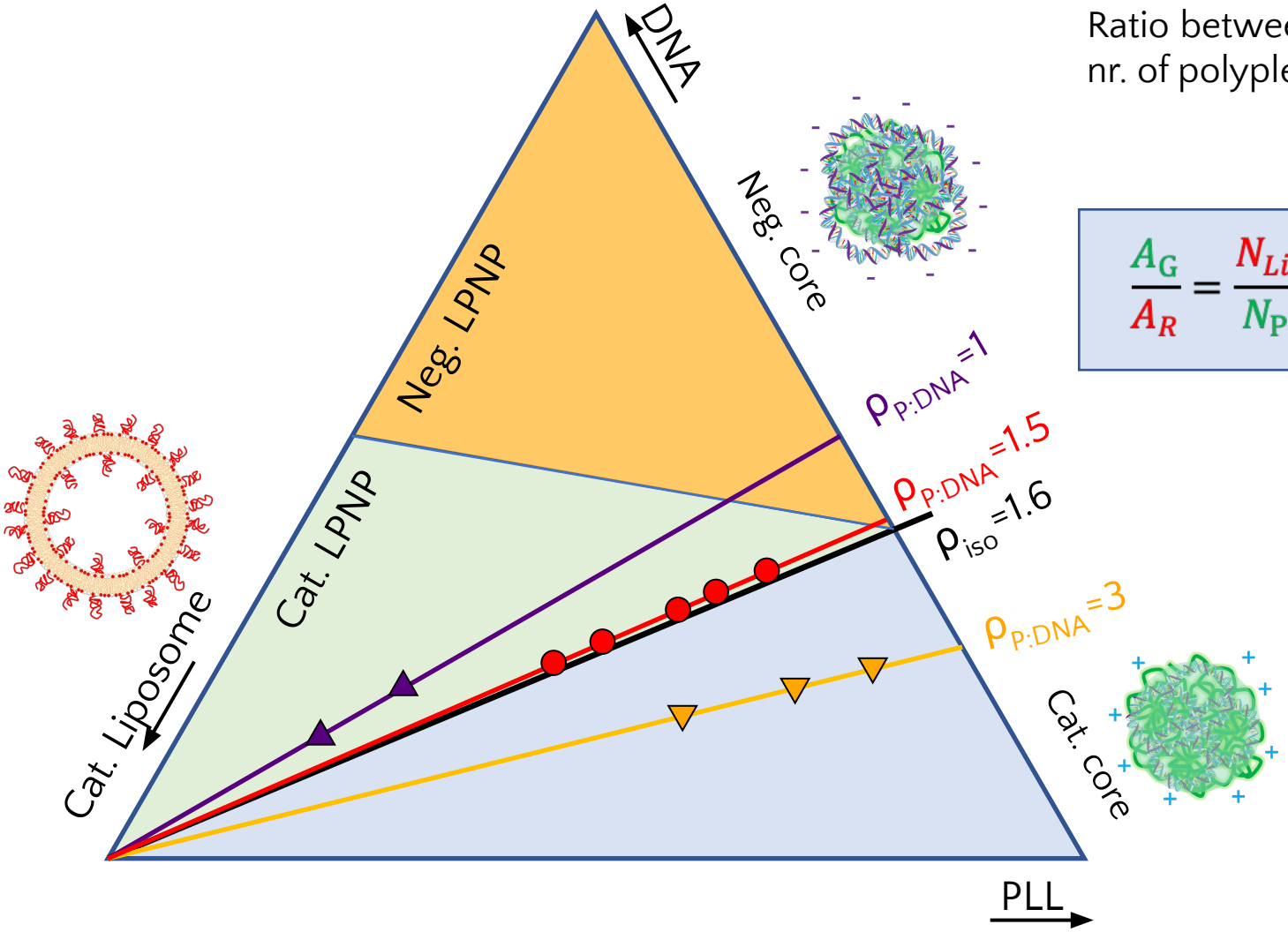
$$\frac{A_X}{A_G} \approx \frac{N_{LPNP}}{N_{LPNP} + N_{Lip.free}}$$

Paris, Gaspar, Coelho, De Beule, Silva, bioRxiv, doi: 10.1101/2022.02.06.479316

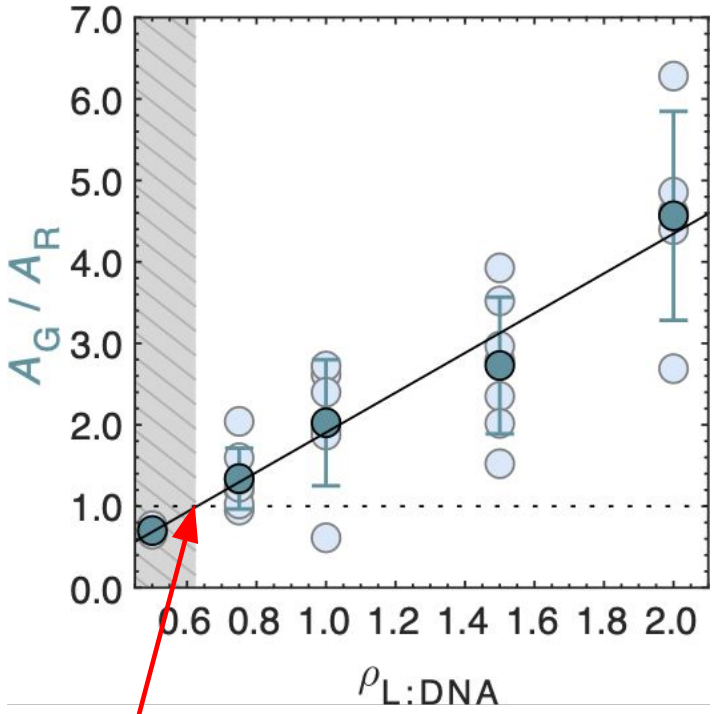
FCCS identifies critical ratio between nr of cores & lipos.

Since only slight deviation from 1:1 stoichiometry (n<1.5):

Ratio between total nr. of liposomes (Liposomes + LPNPs) and total nr. of polyplexes (PPs + LPNPs) can be obtained from the A_G/A_R ratio



$$\frac{A_G}{A_R} = \frac{N_{Lip.Total}}{N_{PP.Total}}$$



Colloidal stability occurs when: Liposomes to PPs nr ratio is at least 1 ($\rho_N \geq 1$)

Paris, Gaspar, Coelho, De Beule, Silva, bioRxiv, doi: 10.1101/2022.02.06.479316

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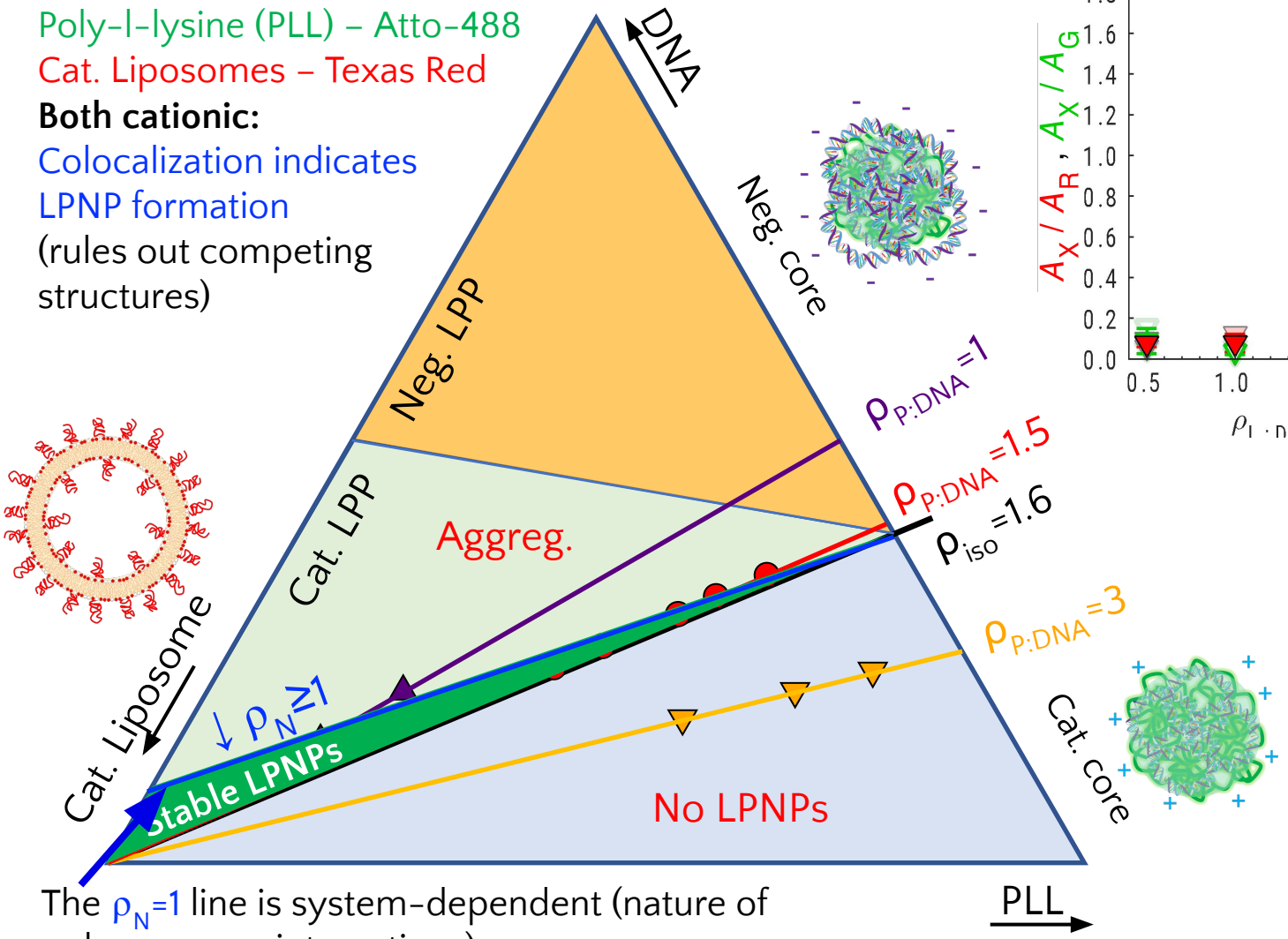
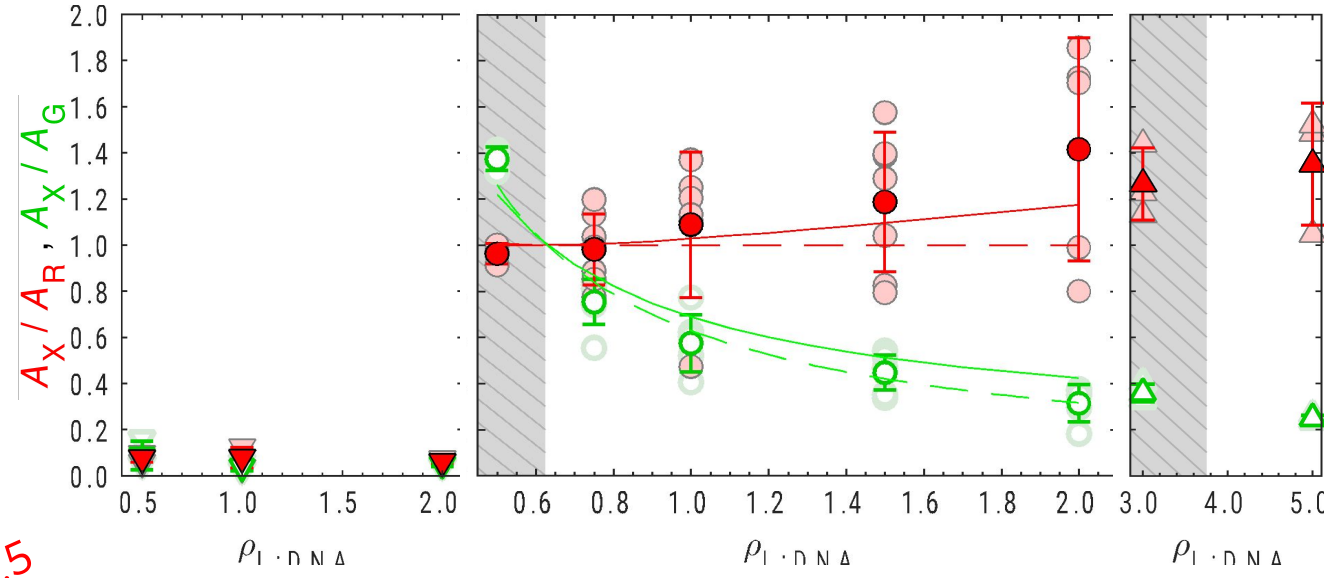
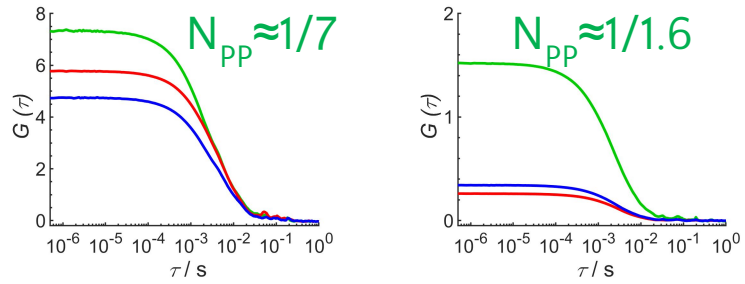


The ρ_N=1 line is system-dependent (nature of polymer-core interactions)



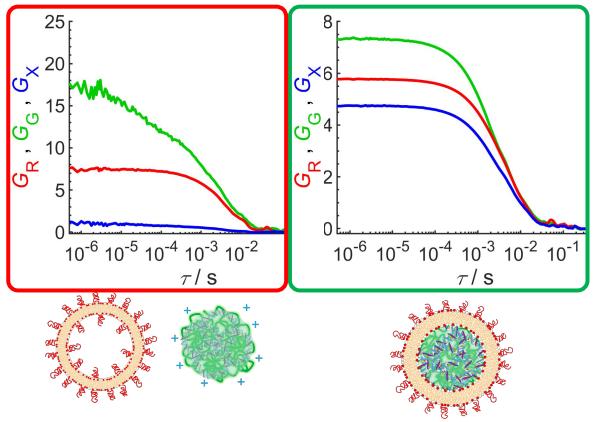


The nr. of PPs is ca. 3.5 times greater for $\rho_{P:DNA}$=1, requiring also more liposomes to fulfill ρ_N≥1 criteria

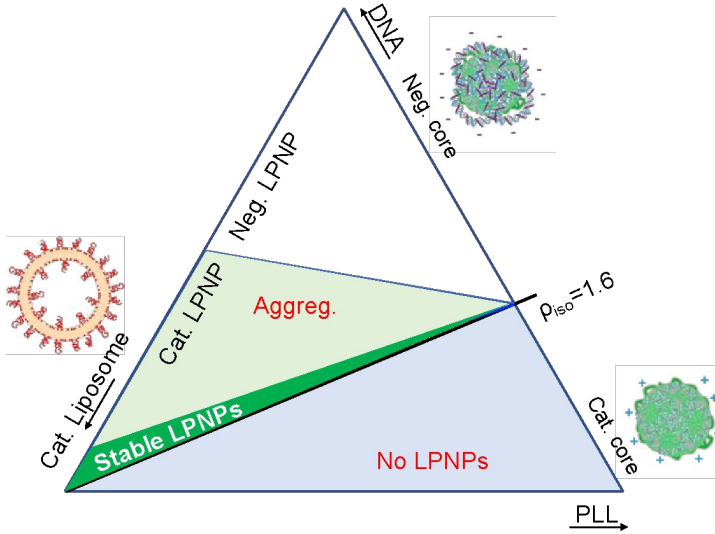
Paris, Gaspar, Coelho, De Beule, Silva, bioRxiv, doi: 10.1101/2022.02.06.479316

Conclusions

- **FCCS** is a **powerful** tool for hybrid LPNPs characterization
  - **quantitative information** about the **core-shell association** (difficult to obtain by traditional techniques)
  - **insight** on forces/factors influencing association – LPNP synthesis optimization



- For Polylysine LPNPs
  - Association always between oppositely-charged particles (in simple buffer)
  - The liposome to polyplex number ratio **must** be at least 1 ($\rho_N \geq 1$) to ensure every core is coated and avoid aggregation
  - Polyplex (**core**) with **slight overcharge** – to avoid coexistence of species



Paris, Gaspar, Coelho, De Beule, Silva, bioRxiv, doi: 10.1101/2022.02.06.479316

Acknowledgments

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Soft Matter for Therapeutics Lab at INL

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Thank you for your Attention

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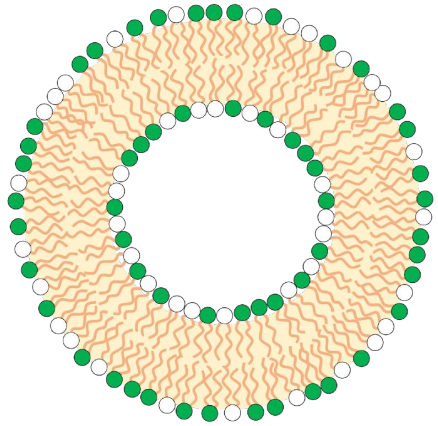
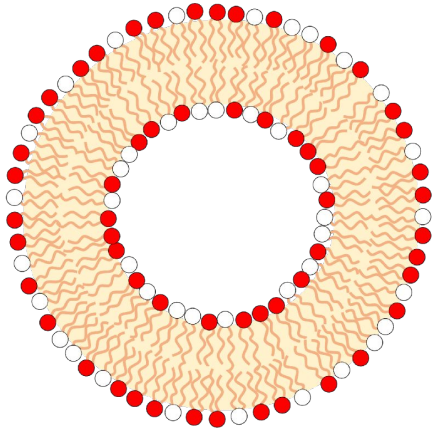
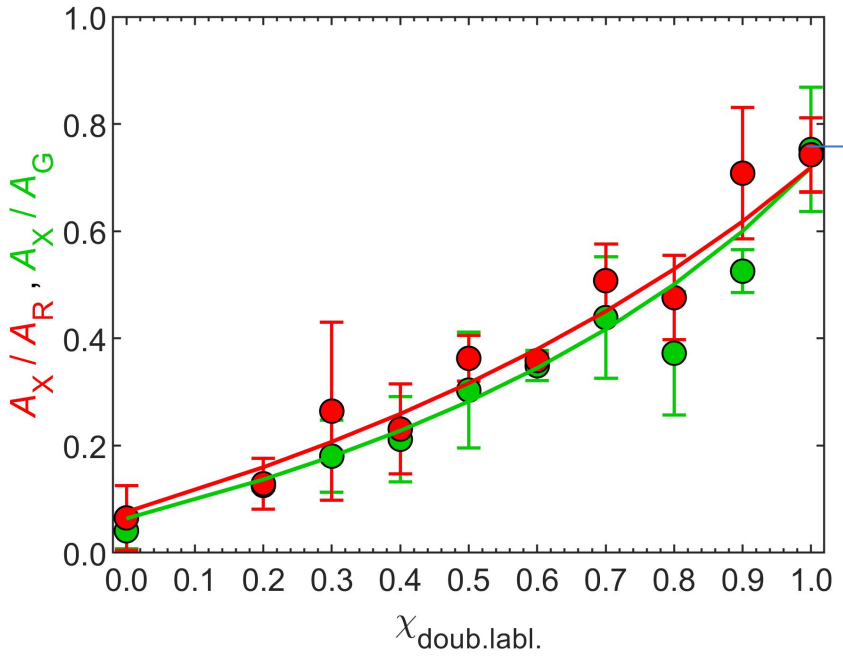
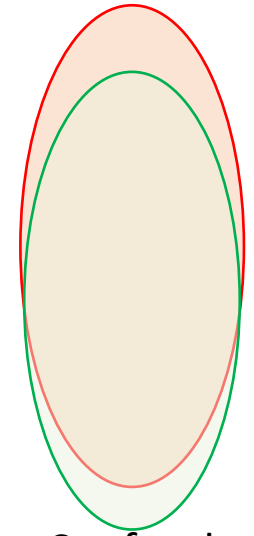
FCCS: control measurements and volume overlap



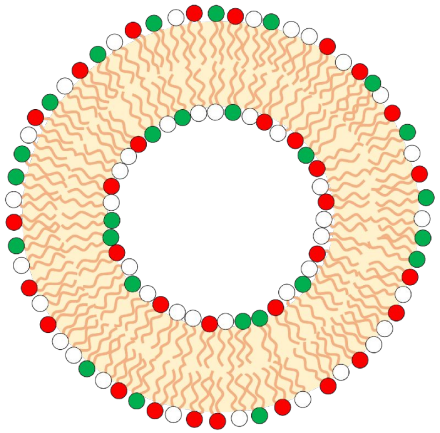




Overlap volume correction: **72%**



Confocal green/red vol.



Similar to: Werner, Ebenhan, Haupt, Bacia, ChemPhysChem, 2018, 19, 3436