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2D HEALTH

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Using Cationic Pyrene Derivatives to Produce Aqueous Graphene Dispersions with Exceptional Stability and Biocompatibility

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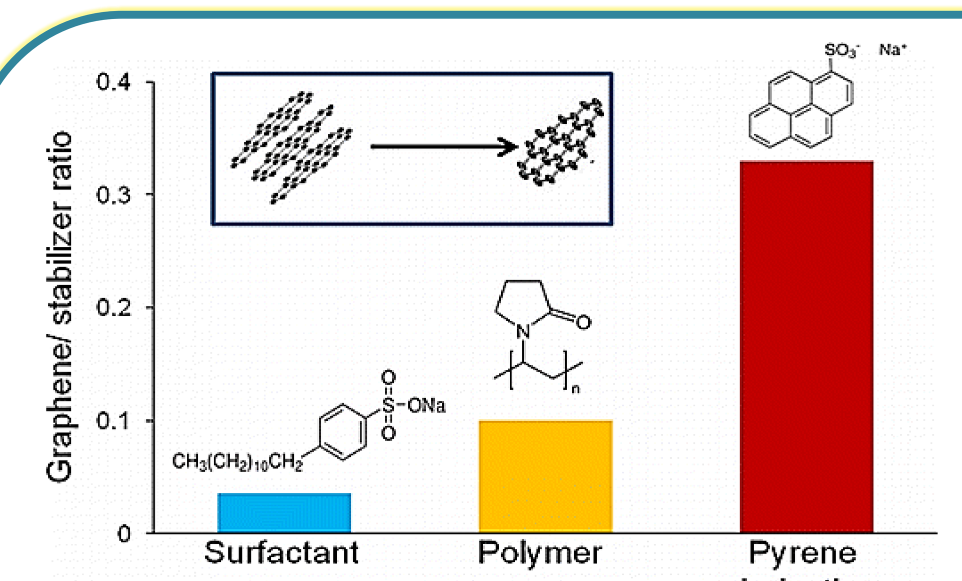
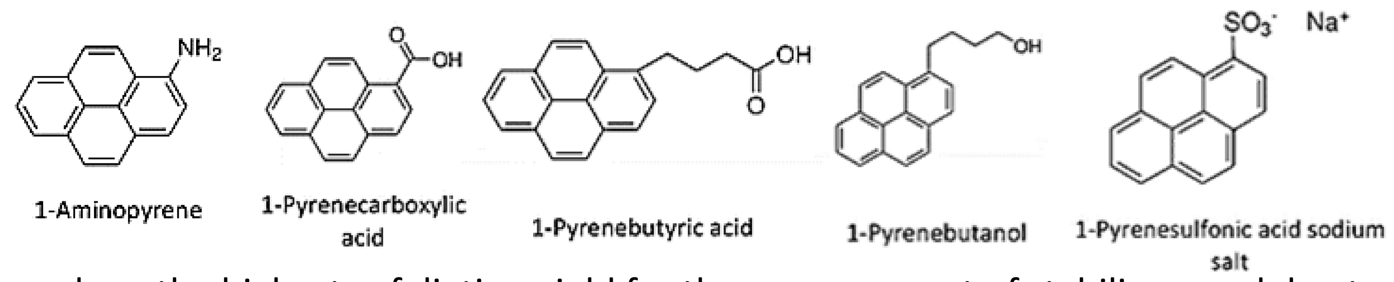
INTRODUCTION



Parviz, D., et al., Acs Nano, 6, 8857 (2012)

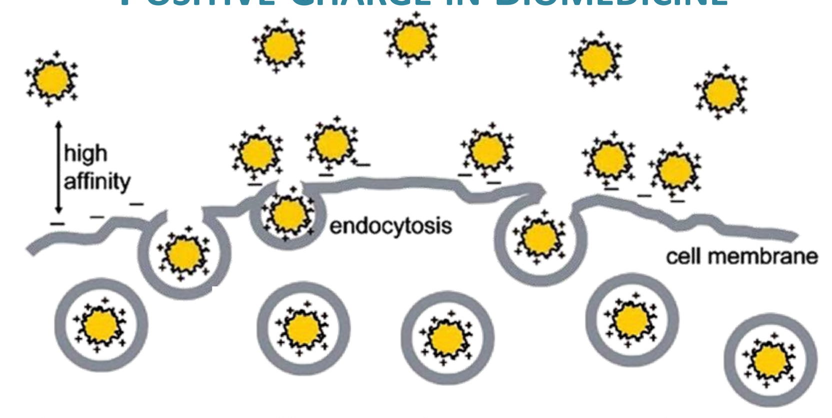
Liquid Phase Exfoliation (LPE) of Graphene in Water

Dispersant-assisted LPE of graphene is achieved by using an amphiphilic molecule, *i.e.* a molecule with hydrophobic part for adsorption on the basal plane of 2D crystals and hydrophilic/ionic functional groups for preventing restacking of exfoliated 2D crystals through electrostatic repulsion and/or steric effect.



- Amongst different types of stabilizers, **pyrene derivatives** show the highest exfoliation yield for the same amount of stabilizer used due to the efficient adsorption by strong π - π interaction between pyrene base to graphene surface. *Parviz, D., et al., Acs Nano, 6, 8857 (2012); Lee, D., Chem. Comm., 47, 8259 (2011)*
- High yield and high quality 2D materials have been obtained using sulfonic pyrene (negatively charged) as exfoliating agent. *Yang, H.,et al., Carbon, 53, 357-365, (2013); Yang, H., et al., 2D Materials 1.1, 011012 (2014).*

Positive Charge in Biomedicine

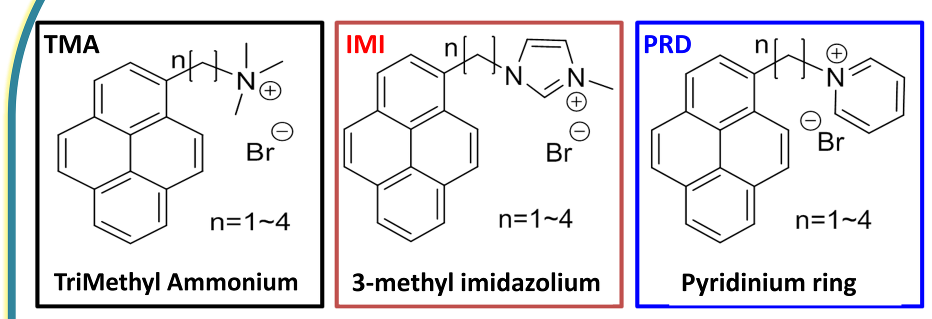


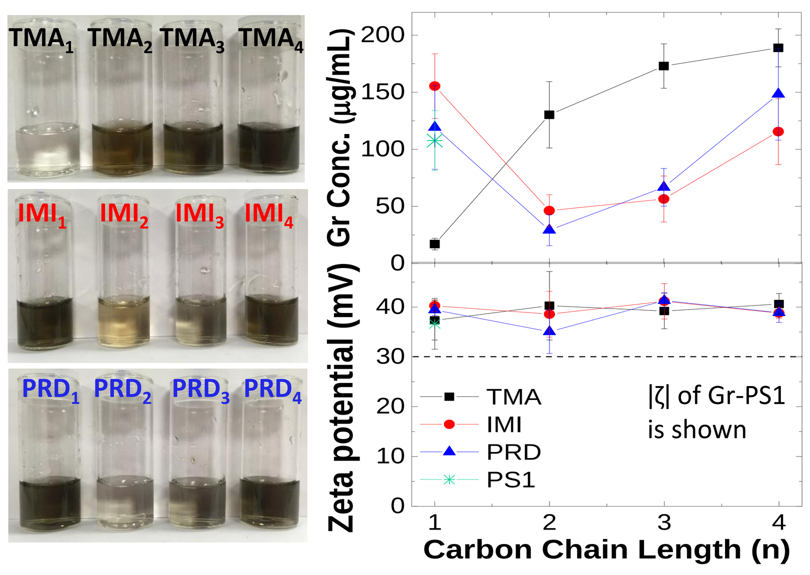
Positive charge can offer advantages such as better cellular internalisation or complex formation with negatively charged biomolecules through favourable electrostatic interactions .

Aim of this work: Preparation of positively charged graphene dispersion in water using pyrene derivatives by a simple one-step liquid-phase exfoliation for biomedical application utilising the positive surface charge.

GRAPHENE EXFOLIATION STUDY

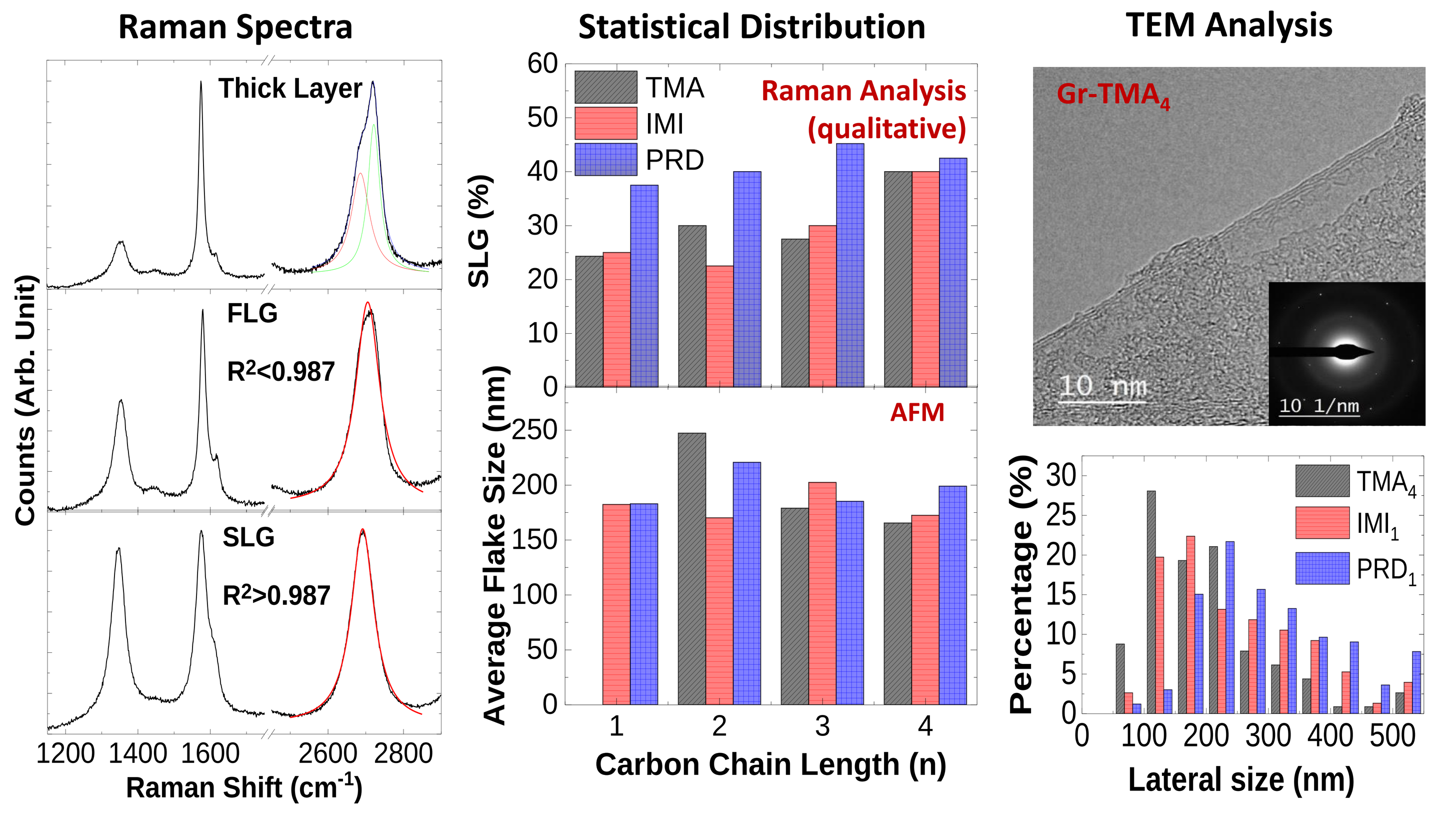
Graphene Exfoliation





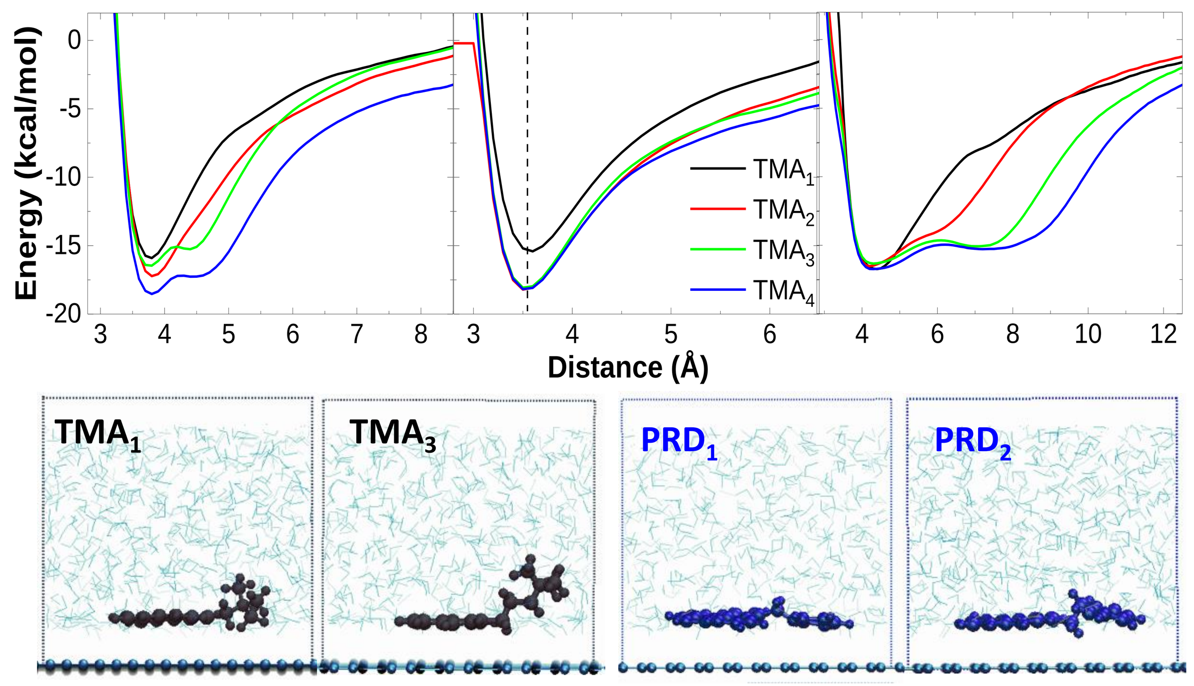
- 12 different Py⁺ were used for graphene exfoliation in water.
- The concentration was determined with **UV-Vis spectroscopy** and Beer- Lambert law (α = 24.6 mL mg^{-1} cm^{-1} @ 660nm).
- **Zeta potential (ζ)** was measured for estimation of the dispersion stability. Typically, aqueous colloidal dispersion with ζ > |30| mV are marked as stable.

Characterization of Graphene Nanosheets



- The **Raman spectroscopy** is used with a qualitative protocol for thickness analysis [*Shin, Y., et al., Carbon 102, 357-366, (2016)*]. By fitting a single Lorentzian lineshape on the 2D peak, single-layer graphene (SLG), few-layer sheets (FLG) and thick layer (>10 layers) was distinguished by evaluating the fitting coefficient (R^2).
- **Atomic force microscopy (AFM)** was used to assess the morphology, thickness and lateral size distribution of the dispersed graphene nanosheets.
- **Transmission electron microscopy (TEM)** was used on selected samples to assess the morphology and confirm Raman and AFM analysis.
- The exfoliated graphene shows thickness and size distribution of **typical LPE graphene (~30% SLG and ~200 nm lateral size)**.

Molecular Dynamics Simulation

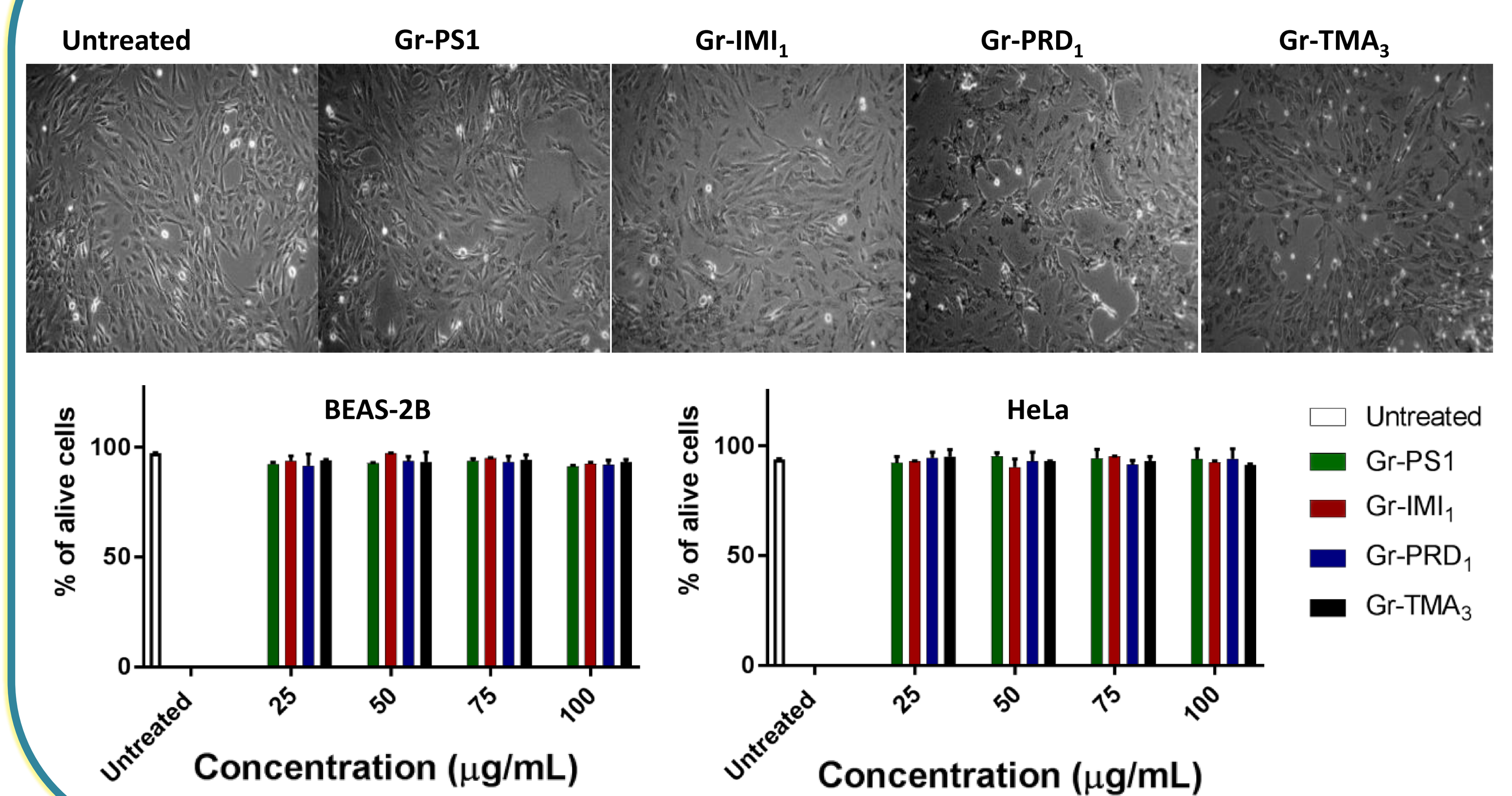


- Potential mean force (PMF) curves were obtained from MD simulation using adaptive biasing force technique, NAMD code & CHARMMs force field for TMA and PRD pyrene derivatives.
- MD simulation results shows that for localized charge (TMA series), the graphene exfoliation yield increases with increasing distance between the pyrene base and the functional group by better separation of the pyrene adsorption on graphene surface and the charge solvation with water molecules.
- For delocalized charge (PRD series), the graphene exfoliation yield is better when the functional group is also adsorbed on the graphene surface, which could be hindered at certain carbon chain length (e.g. n = 2 or 3)

Shin, Y, et al. *Nanoscale* 12.23 (2020): 12383

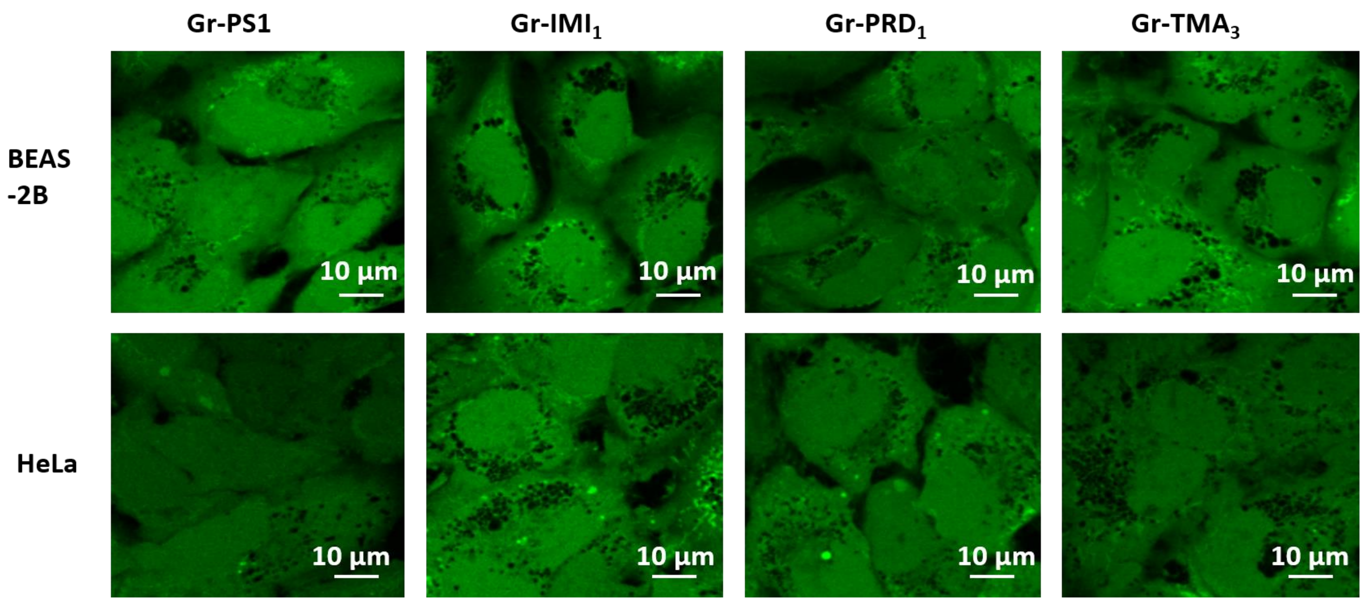
BIOMEDICAL STUDY

Optical Microscopy and Flow Cytometry



- For biomedical application, three **Gr⁺**s with highest exfoliation efficiency were chosen (Gr-IMI$_1$, Gr-PRD$_1$, and Gr-TMA$_3$), and compared with **Gr⁻** prepared with PS1 to see the effect of surface charge.
- Cytotoxicity of the 4 graphene dispersons were tested at various concentration up to 100 μg/ml for 24 hours using optical microscopy and flow cytometry with PI/annexin V. No cytotoxicity was observed for all 4 types of graphene dispersions.

Live Cell Imaging



- Cellular uptake of the 4 graphene flakes were observed using live cell imaging: all three **Gr⁺** showed better cellular uptake than **Gr⁻** showing the positive surface charge enhances cellular uptake capacity.

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CONCLUSION

- Using **various pyrene derivatives** showed that localised charge at longer distance lead to effective separation between the adsorption of pyrene and the stabilisation of the functional group in water for better exfoliation.
- Positively charged graphene dispersions in water with **high concentration** and **good stability** were obtained.
- Graphene dispersions prepared with pyrene derivatives showed **excellent colloidal stability** and **biocompatibility** and **positively charged graphene** showed better cellular uptake, offering **high potential in many biomedical applications**, especially as a delivery vector.

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