Supporting Information

The human biomolecular corona of liposomal doxorubicin: The overlooked factor in anticancer drug delivery

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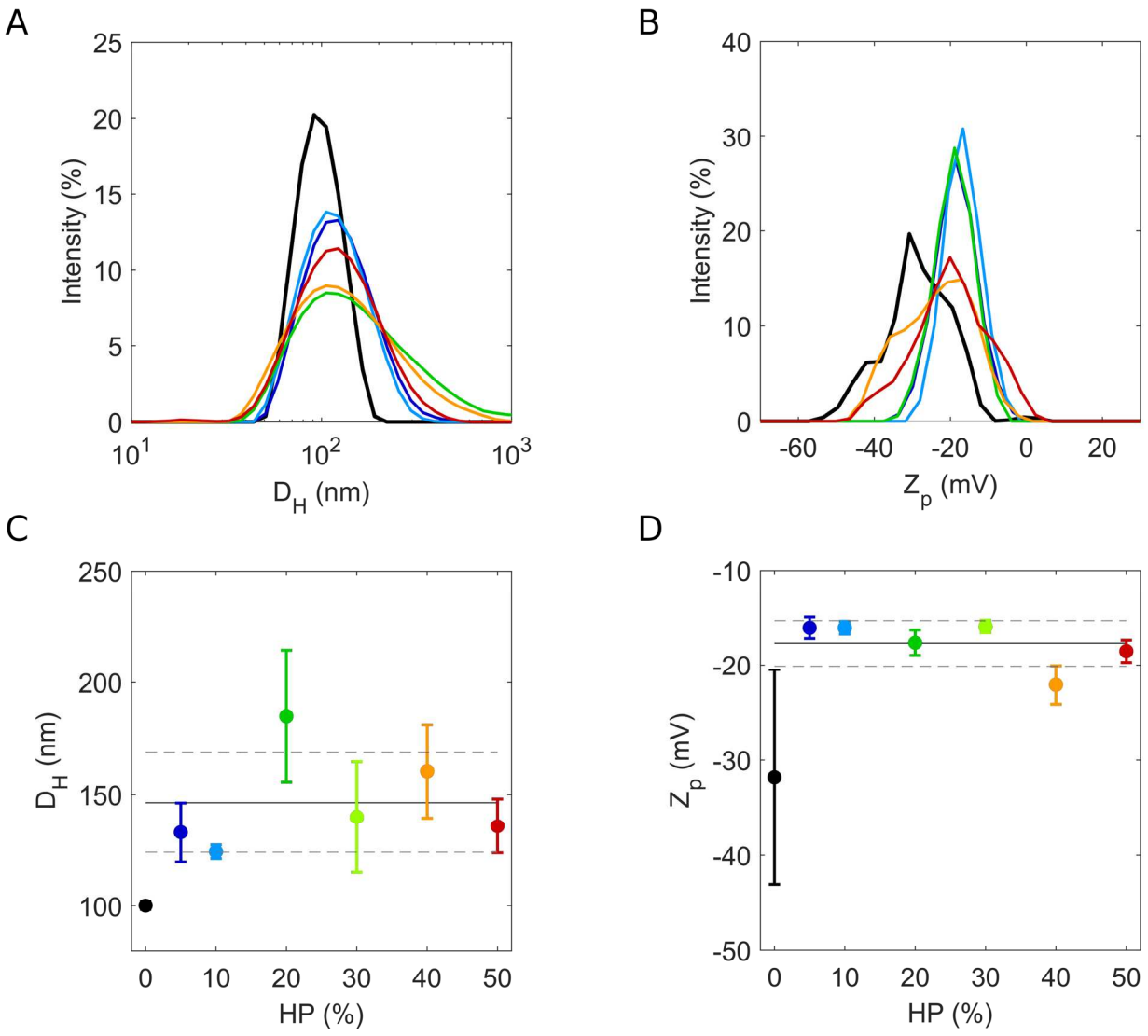

Figure S1. (A) Intensity dynamic light scattering (DLS) distributions of empty (i.e. not loaded with doxorubicin) Doxoves® (black curve) and empty Doxoves®-HP complexes: HP= 5% (dark blue curve); HP= 10% (light curve); HP= 20% (dark green curve); HP= 30% (light green curve); HP= 40% (orange curve); HP= 50% (red curve). (B) Zeta-potential distributions of empty Doxoves® (black curve) and Doxoves® liposome-HP complexes. (C) Hydrodynamic diameter of empty Doxoves® (black circle) and empty Doxoves®-HP complexes. (D) Zeta-potential of empty Doxoves® (black circle) and empty Doxoves®-HP complexes. In panels B-D, the same color code than panel A was used.

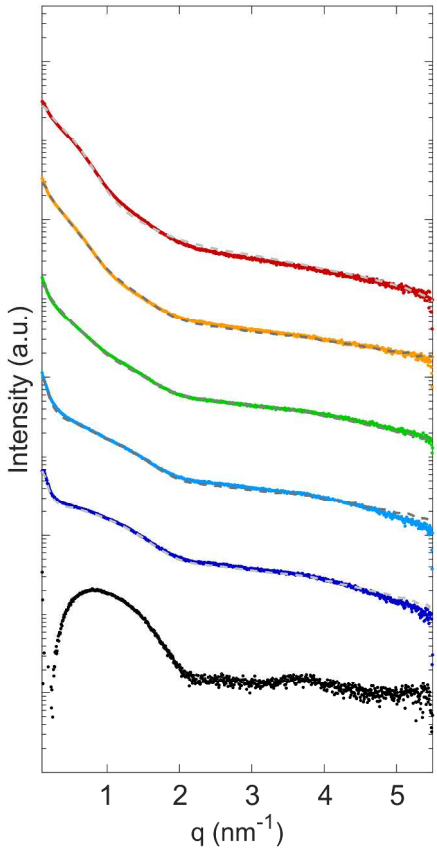

Figure S2. Synchrotron small-angle X-ray scattering (SAXS) patterns of empty Doxoves® (black points) and empty Doxoves®-HP complexes: HP= 5% (dark blue points); HP= 10% (light blue points); HP= 20% (dark green points); HP= 40% (orange points); HP= 50% (red points). Dashed lines are the best fits to the data using weighted linear combinations of the SAXS patterns of empty Doxoves® and HP.

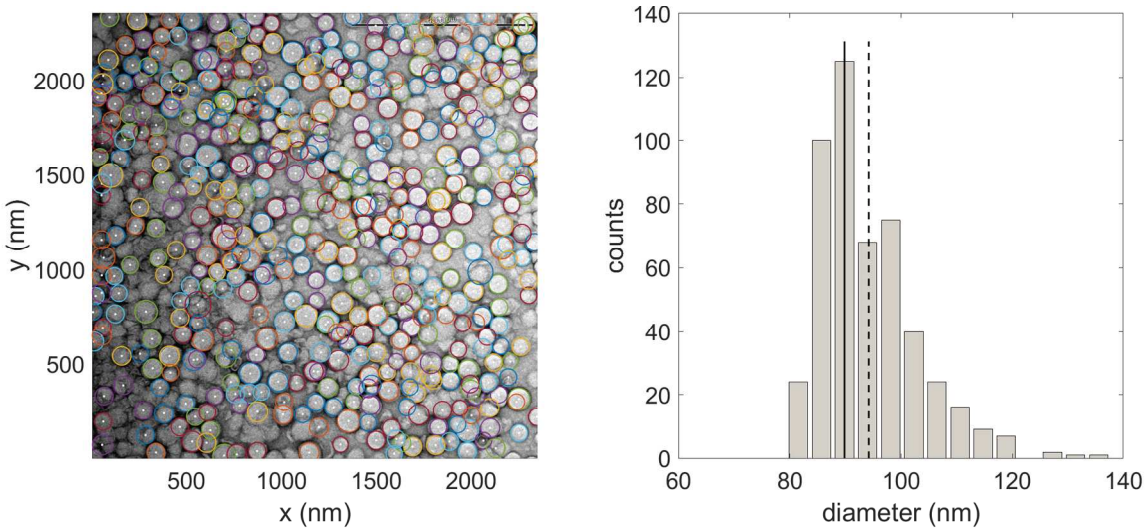

Figure S3. (A) Transmission electron microscopy (TEM) image of empty (i.e. not loaded with doxorubicin) Doxoves®. Scale bar: 1 micrometer. (B) Size distribution of empty Doxoves®. Solid and dashed lines indicate median and average of size distribution respectively. When empty Doxoves® was incubated with HP, no evidence of rupture vesicles was found (data not reported).

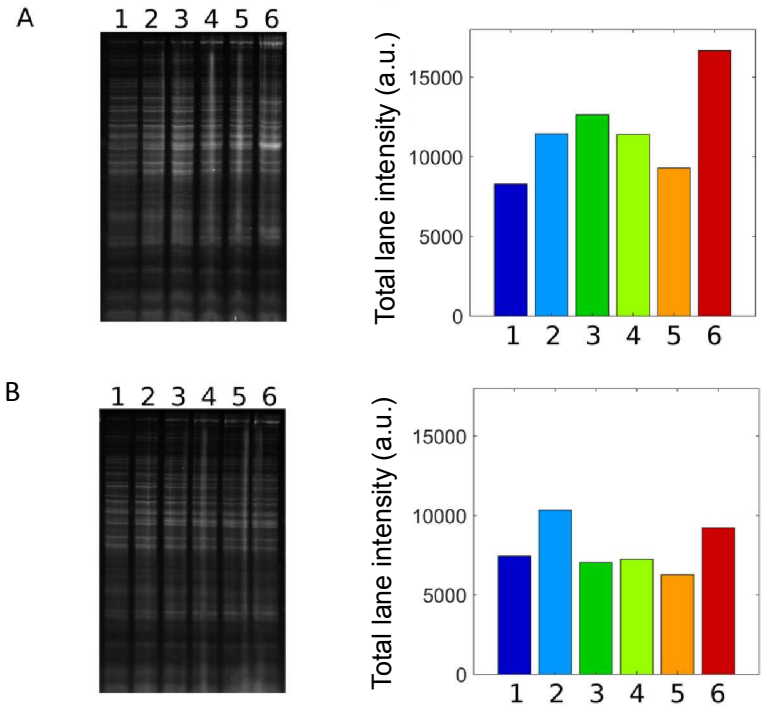

Figure S4. (A) Representative sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel image of plasma proteins obtained from Doxoves® following 1-hour exposure to human plasma (HP) at 37 °C (left). Histograms represent the total intensity of proteins recovered from lanes 1-6. (right) (B) Representative SDS-PAGE gel image of plasma proteins obtained from empty (i.e. not loaded with doxorubicin) Doxoves® following 1-hour exposure to HP at 37 °C. (B) Histograms represent the total intensity of proteins recovered from lanes 1-6. (Lanes 1-6: HP= 5%, 10%, 20%, 30%, 40% and 50%). As SDS-PAGE experiments were only aimed at investigating the evolution of the BC as a function of HP, ladder was not used. Precise identification of plasma proteins was made by nano-liquid chromatography tandem mass spectrometry.

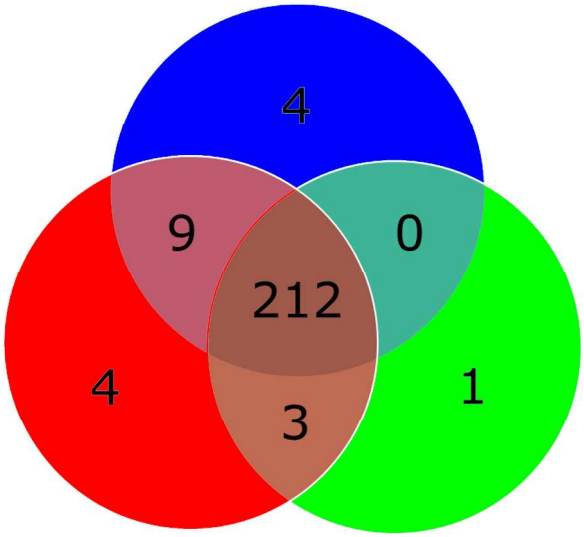

Figure S5. Venn diagram reporting the number of plasma proteins adsorbed to the surface of empty Doxoves® after 1 h incubation with human plasma (HP) at 37°C: HP=5% (blue), HP=20% (green), and HP =50% (red).

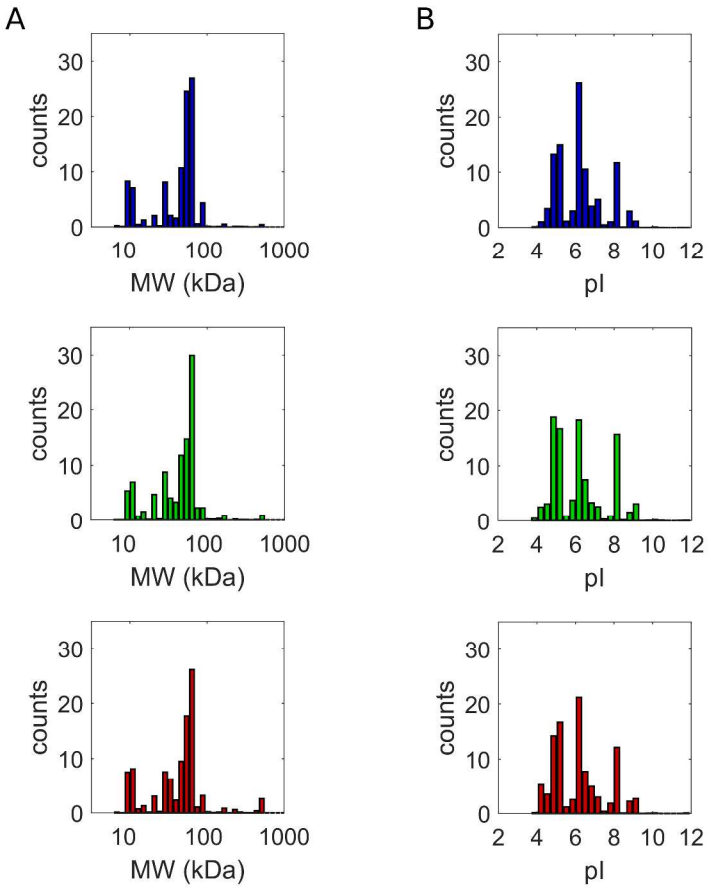

Figure S6. (A) Relative protein abundance (RPA) of corona proteins classified according to their calculated molecular weight (MW) for empty Doxoves®-HP complexes. (B) RPA of identified plasma proteins classified according to their calculated isoelectric point (pI) for empty Doxoves®-HP complexes. Color code: HP=5% (blue histograms), HP=20% (green histograms), and HP =50% (red histograms).

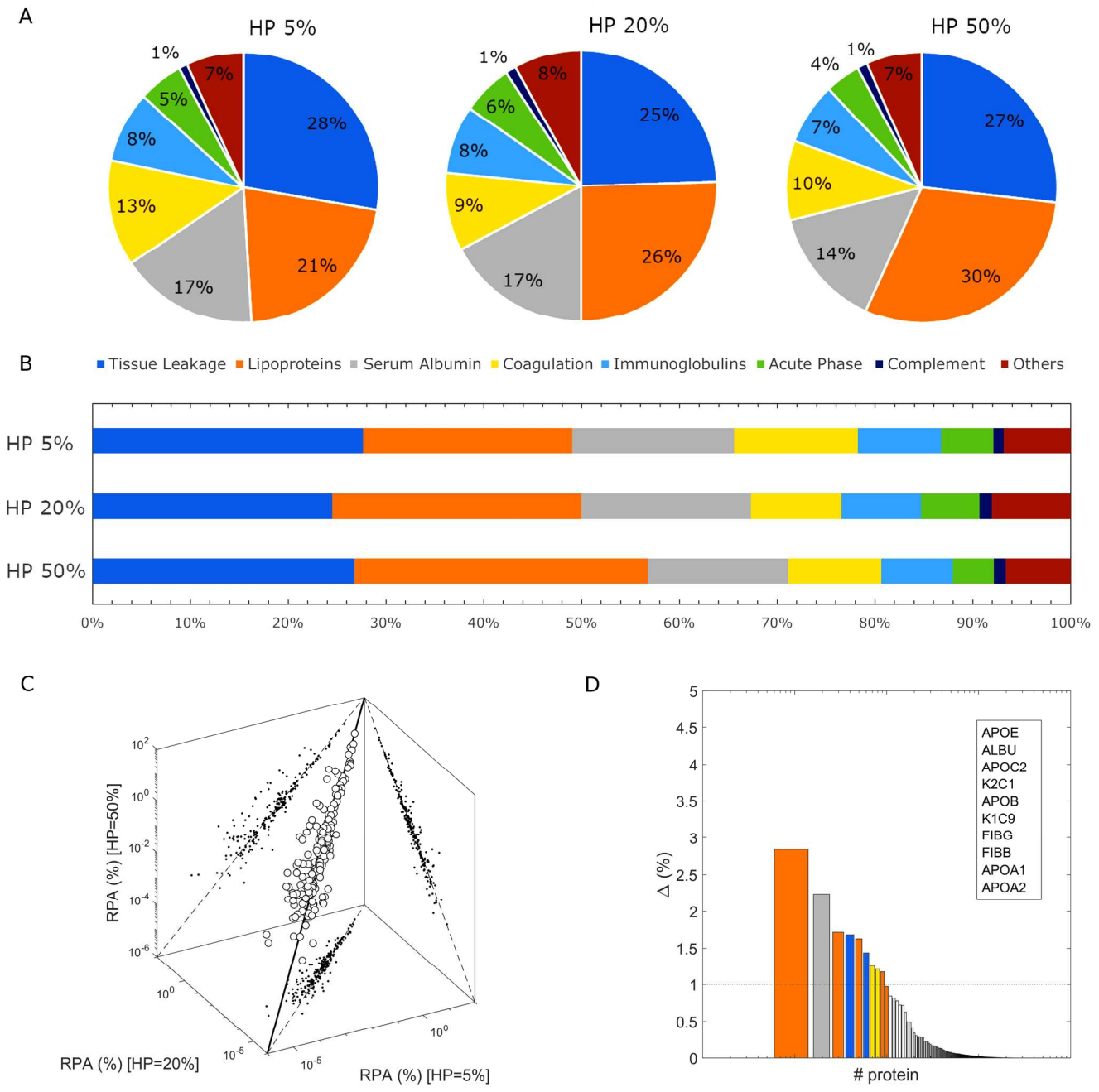

Figure S7. Bioinformatic classification of corona proteins found in the biomolecular corona of empty Doxoves® after 1 h incubation with human plasma (HP) at 37°C as determined by nanoLC/MS-MS. Proteins were classified according to their physiological functions at different HP concentration: (A) pie charts, (B) histograms. (C) Scatter plot depicting the measured relative protein abundances under different conditions of plasma concentrations (HP=5%, HP=20%, HP=50%). The diagonal represents an ideal reference distribution of data points with equal protein abundances under the investigated conditions. (D) Distance\Delta between each of the experimental data points and the reference diagonal. Results are sorted in descending order to rank the identified corona proteins according to their sensitivity to the plasma concentration.