Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

Supplementary Information

Development of Immunoliposomes Containing Cytotoxic Gold Payloads Against HER2-Positive Breast Cancers

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S1: Structures of gold(I) Complexes

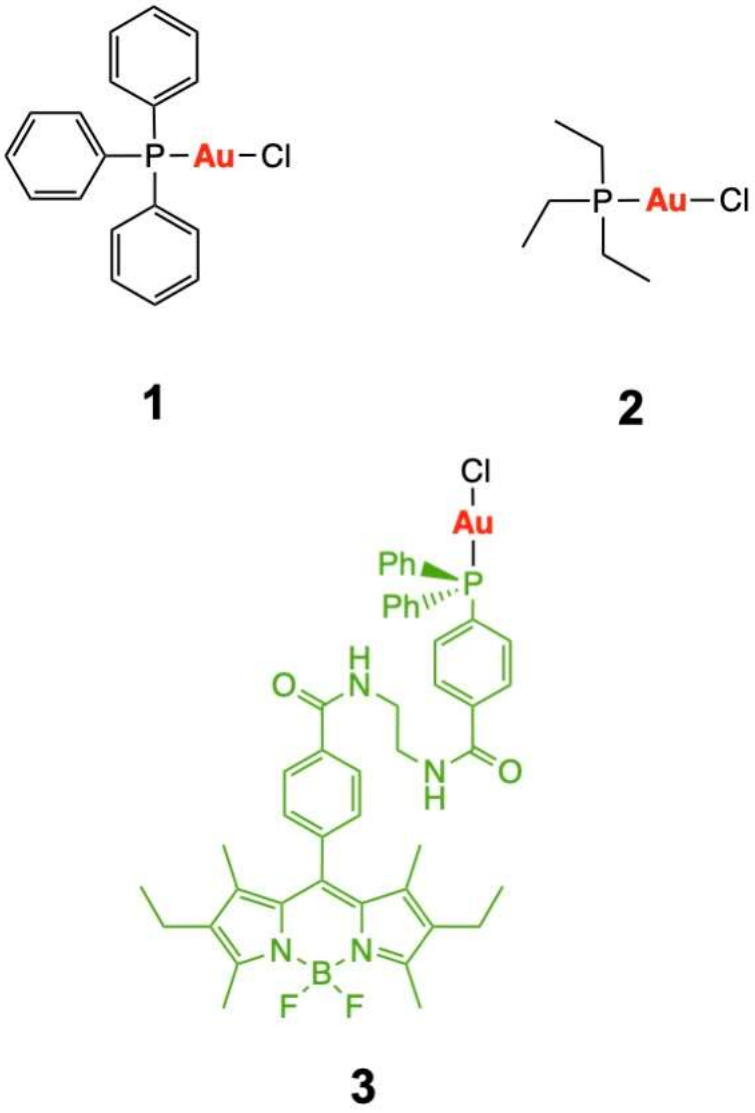


Figure S1: Structure of gold(I) phosphane-containing payloads [AuCl(PPh_3)] (**1**), [AuCl(PEt_3)] (**2**), [AuCl(PPh_2-BODIPY)] (**3**) encapsulated into immunolipsomes.

S2: Dynamic light scatter

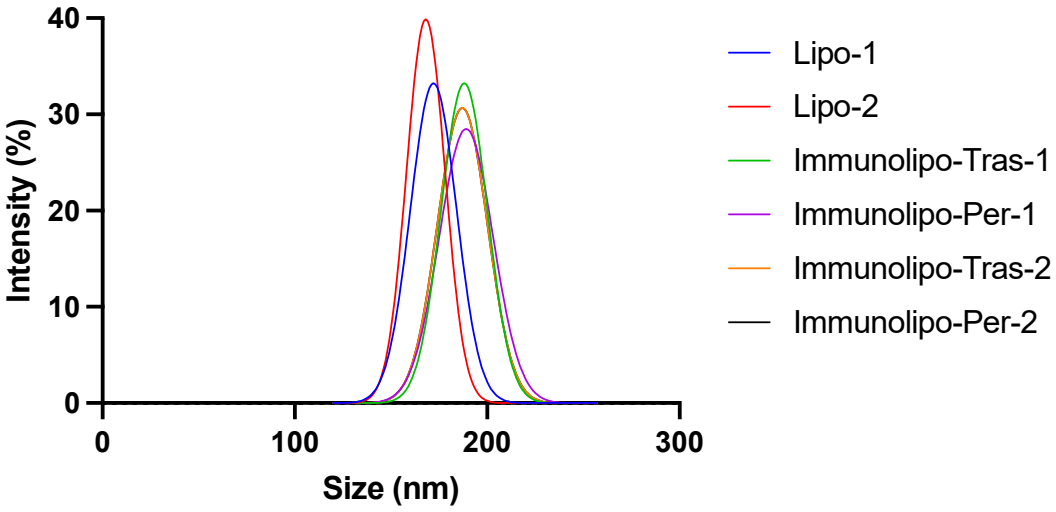


Figure S2: Measurements of liposome and immunoliposome size by DLS.

S3. Engraftment

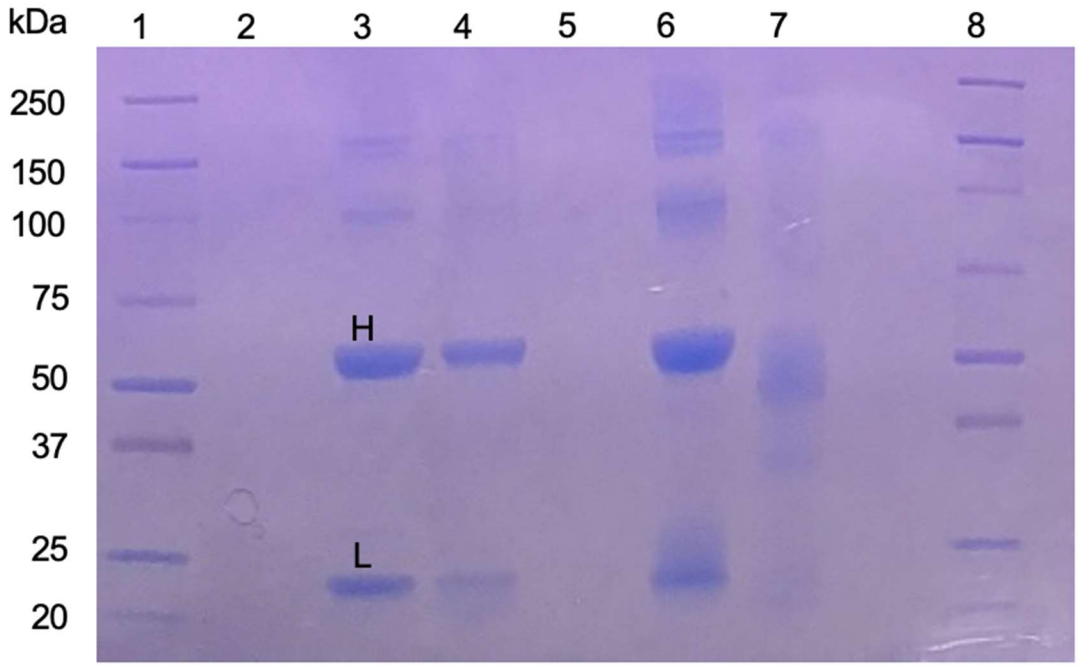


Figure S3. Representative SDS-PAGE gel of antibody engraftment on liposome surface. Protein markers (L1 and L8), Lipo-1 (L2), reduced Trastuzumab (thiolation by Traut's reagent) indicating heavy (H) and light (L) chains (L3), Immunolipo-Tras-1 in identical bands indicating presence of antibody (L4), antibody-free liposomes (L5), reduced Pertuzumab again indicating heavy and light chains (L6), Immunolipo-Per-1 (L7).

S4. Stability assays

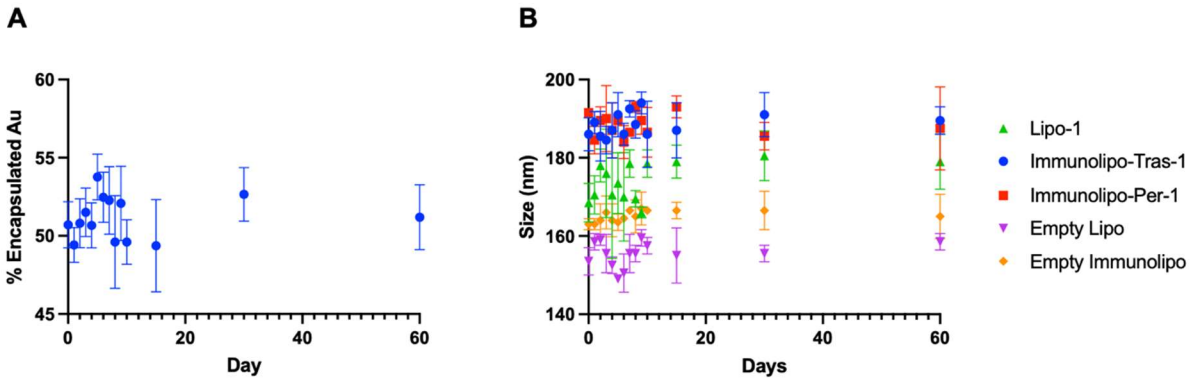


Figure S4. (A) Encapsulation efficiency of liposomal [AuCl(PPh_3)] (**1**) over 60 days with storage in PBS in 4°C. (B) Stability of size of liposomes and immunoliposomes over 60 days.

S5. Optimized immunoliposomes

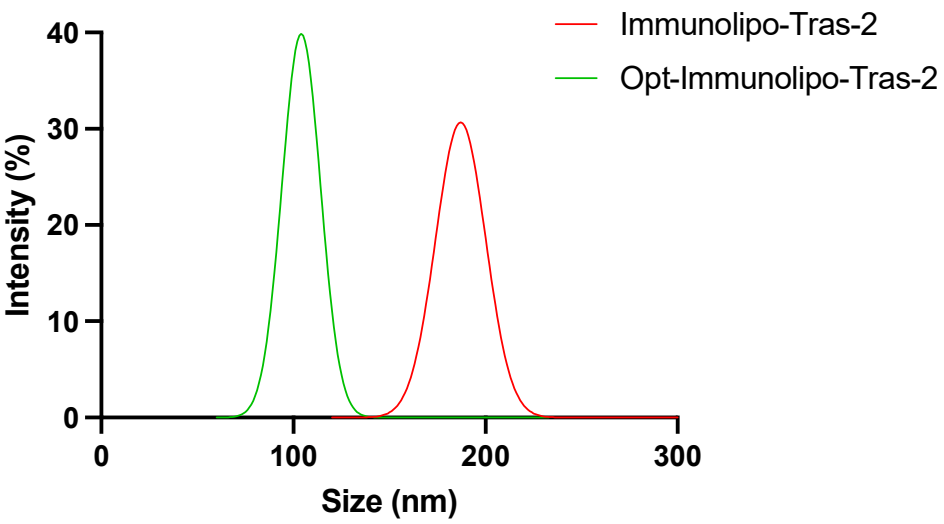


Figure S5: Measurements of larger and optimized immunoliposome size by DSL.

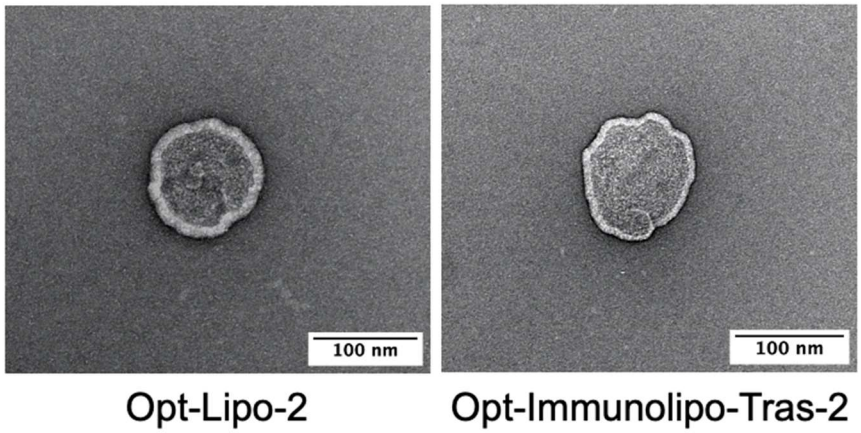


Figure S6. TEM images of reduced size liposome (left) or immunoliposome (right) encapsulating [AuCl(PEt_3) (**2**) following uranyl acetate staining. Scale bar indicates size of liposomes to be ~100 nm in diameter. 150,000-200,000x magnification.

S6. Encapsulation of [AuCl(PPh$_2$-BODIPY)]

Table S1. Properties of liposomes and immunoliposomes encapsulating [AuCl(PPh$_2$-BODIPY)] (**3**). Stability measurements of size, PdI, Zeta potential, encapsulation and engraftment remain unchanged in storage in PBS at 4°C for up to 60 days.

	Lipo-3	**Immunolipo-Tras-3**	**Opt-Immunolipo-Tras-3**
Size	172 ± 9	188 ± 7	105 ± 9
Polydispersity Index	0.1	0.1	0.05
Zeta Potential	neutral	neutral	neutral
Concentration	9.4 x 10^{10}	1.2 x 10^{9}	6.4 x 10^{7}
Encapsulation Efficiency	30%	35%	32%
Engraftment Efficiency	-	30%	31%

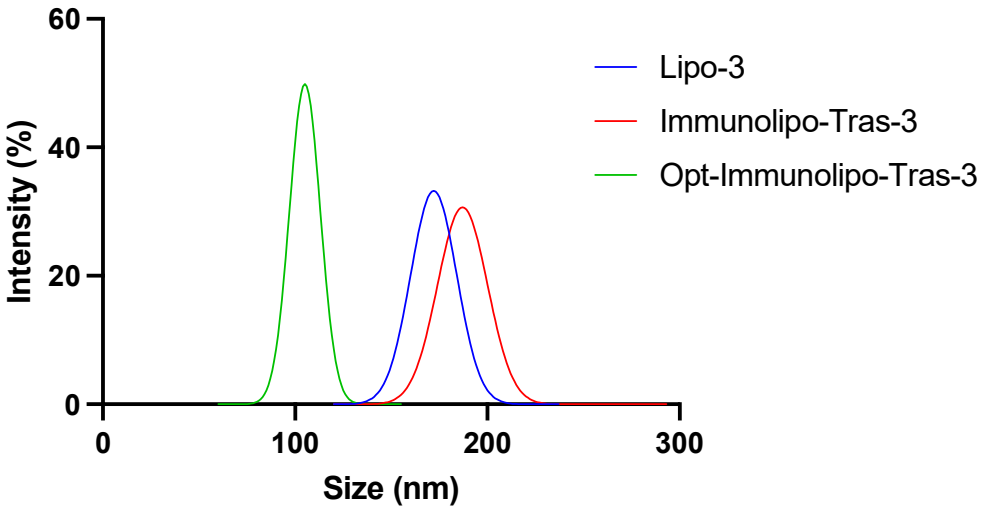


Figure S7. Size measurements of liposomes and immunoliposomes encapsulating **3** by DLS.

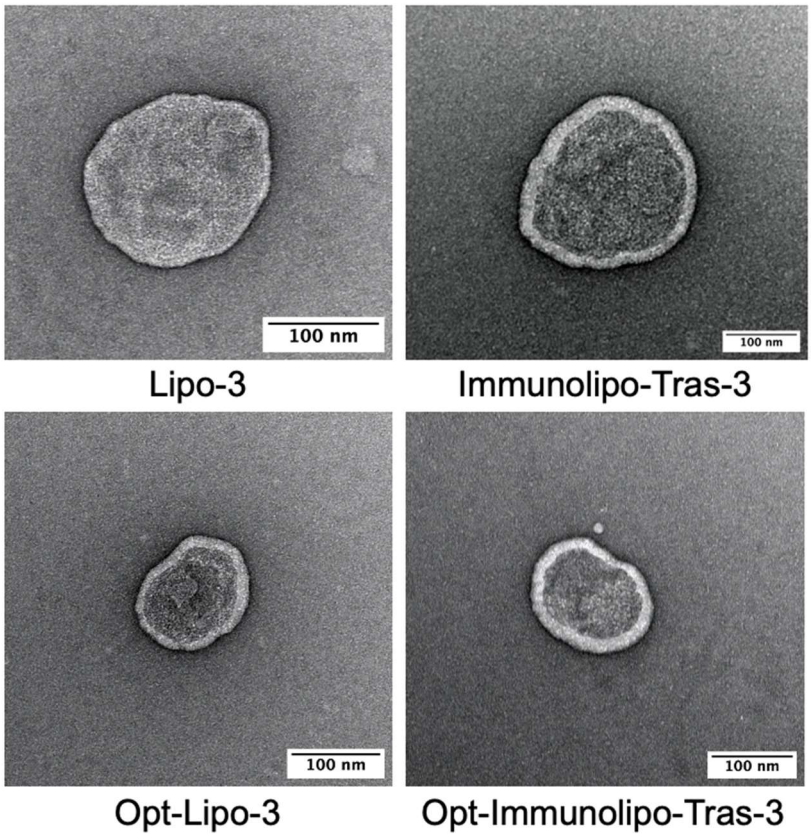
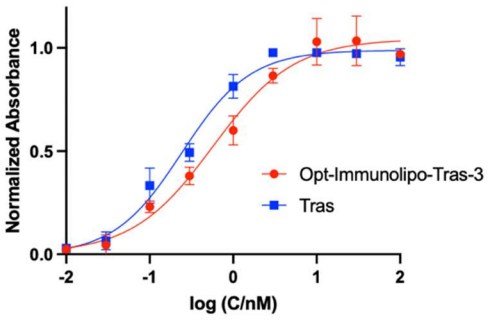


Figure S8. TEM images of liposomes or immunoliposomes encapsulating [AuCl(PPh_2-BODIPY) (**3**) following uranyl acetate staining. Top left: 200 nm liposomes encapsulating **3**, top right: 200 nm immunoliposomes encapsulating **3**, bottom left: 100 nm liposomes encapsulating **3**, bottom right: 100 nm immunoliposomes encapsulating **3.** 150,000-200,000x magnification.

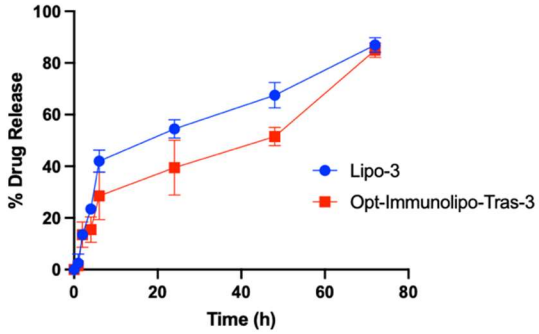
A



B

Opt-IL-Tras-3	Tras
0.574	0.243

C



D

Drug	**IC_{50} (μM)**
3	>60
Lipo-3	54.7 ± 7.3
Opt-Immunolipo-Tras-3	52.6 ± 5.4

Figure S9. (A) ELISA binding affinity assay between free antibodies and immunoliposomes to HER2. 2.5h incubation on pre-coated HER2 plate followed by colorimetric quantification of TMB substrate. Abs measured at 450 nm. (B) EC_{50} quantification from ELISA. IL: immunoliposome. (C) Drug release of [AuCl(PPh_2-BODIPY)] (**3**). from liposomes vs. Trastuzumab-engrafted immunoliposomes in human serum at 37°C for up to 72 hours. Comparable drug release for both with 87% released for **Lipo-3** and 85% for **Opt-Immunolipo-Tras-3**. (D) IC_{50} values in cell line BT-474 for **3**, **Lipo-3** and **Opt-Immunolipo-Tras-3.**

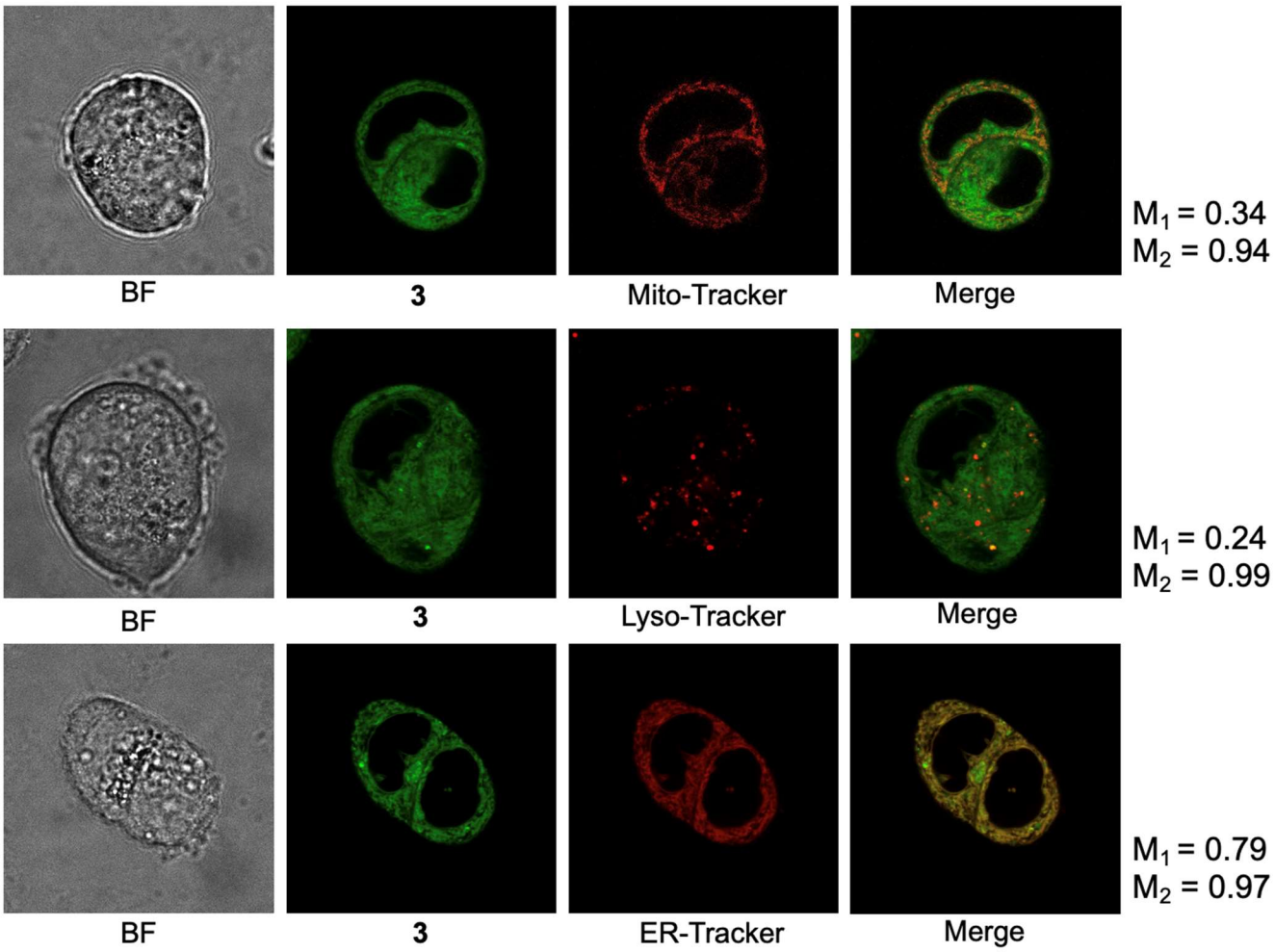


Figure S10. Confocal microscopy images of live BT-474 cells treated with **3** (IC_{10}) and MitoTracker Red FM (top), LysoTracker Red DND-99 (middle), and ER-Tracker Red (bottom). The image overlay (Merge, right) of both fluorescent channels shows the colocalization of **3** and organelle trackers. Manders' colocalization coefficient M_1 reflects the fraction of total signal emitted by **3** that overlaps with the organelle tracker, and M_2 reflects the fraction of the total signal emitted by the organelle tracker that overlaps with that of