SUPPLEMENTAL INFORMATION

Multimodal tracking of (controlled) *Staphylococcus aureus* infections in mice

Mick M. Welling [a#], Clarize M. de Korne [a,c], Silvia. J. Spa [a], Danny M. van Willigen [a], Albertus W. Hensbergen [a], Anton Bunschoten [a,b], Nikolas Duszenko [a,c], Wiep Klaas Smits [d], Meta Roestenberg [c], Fijs W. B. van Leeuwen [a, b]

[a] Interventional Molecular Imaging Laboratory, Department of Radiology, Leiden University Medical Center, Leiden, the Netherlands

[b] Laboratory of BioNanoTechnology, Department of Agrotechnology and Food Sciences, Wageningen University & Research, Wageningen, the Netherlands

[c] Department of Parasitology and Department of Infectious Diseases, Leiden University Medical Center, Leiden, the Netherlands

[d] Department of Medical Microbiology, Section Experimental Bacteriology, Leiden University Medical Center, Leiden, the Netherlands

Corresponding author; Mick M. Welling, E-mail: m.m.welling@lumc.nl

Table S1. The biodistribution of I.M. administered ^{99m}Tc-UBI$_{29-41}$-Cy5-N3 labelled *S. aureus*. Data (expressed as the mean ± SD of the percentage of the injected dose per gram tissue (%ID/g) of 3 observations) were calculated based on the radioactive counts measured in various tissues at 4 h, 20 h, and 28 h post-injection of the radioactive tracer.

Tissue	**%ID/g**		
	4 h p.i.	**20 h p.i.**	**28 h p.i.**
Blood	1.98 ± 0.53	0.15 ± 0.11	0.13 ± 0.03
Thyroid gland	1.37 ± 0.32	0.79 ± 1.07	1.14 ± 0.66
Salivary gland	6.12 ± 1.23	3.36 ± 5.09	2.51 ± 1.90
Heart	0.83 ± 0.16	0.46 ± 0.70	0.37 ± 0.25
Lungs	1.40 ± 0.37	0.59 ± 0.91	0.40 ± 0.30
Bladder	82.06 ± 33.50	10.85 ± 15.04	3.64 ± 2.70
Liver	8.75 ± 1.09	4.77 ± 6.33	4.15 ± 3.74
Spleen	0.89 ± 0.33	0.86 ± 1.37	1.19 ± 0.47
Kidneys	33.67 ± 6.30	11.26 ± 16.72	8.67 ± 7.61
Stomach	3.90 ± 4.66	2.93 ± 4.07	2.87 ± 2.25
Intestines	17.90 ± 14.05	1.43 ± 1.69	1.96 ± 0.72
Infected muscle	51.91 ± 19.53	42.12 ± 21.52	44.44 ± 25.70
Control muscle	0.66 ± 0.21	0.63 ± 0.42	1.38 ± 1.33
Brains	0.13 ± 0.04	0.06 ± 0.08	0.08 ± 0.03
Excretion (%ID; cumulative)	42.90 ± 22.47	78.21 ± 8.33	83.16 ± 8.32
Infection-to-blood ratio	27.99 ± 13.17	301.20 ± 120.83	359.20 ± 227.78
Control-to-blood ratio	0.35 ± 0.15	4.23 ± 2.51	9.76 ± 7.84

Figure S1. Chemical synthesis pathway of UBI_{29-41}-Cy5-N_3. **a)** NaN_3, DMF, 50°C; **b)** 6-bromohexanoic acid, CH_3CN, 60°C; **c)** CH_3CN, 55°C; **d)** DiPEA, Ac_2O, CH_2Cl_2, 0 °C to R.T.; **e)** NaOAc, EtOH, R.T.; **f)** HSPyU, DiPEA, DMSO, R.T.; **g)** UBI_{29-41}, 0.1M phosphate buffer pH 8.5/DMSO 3:1, R.T.

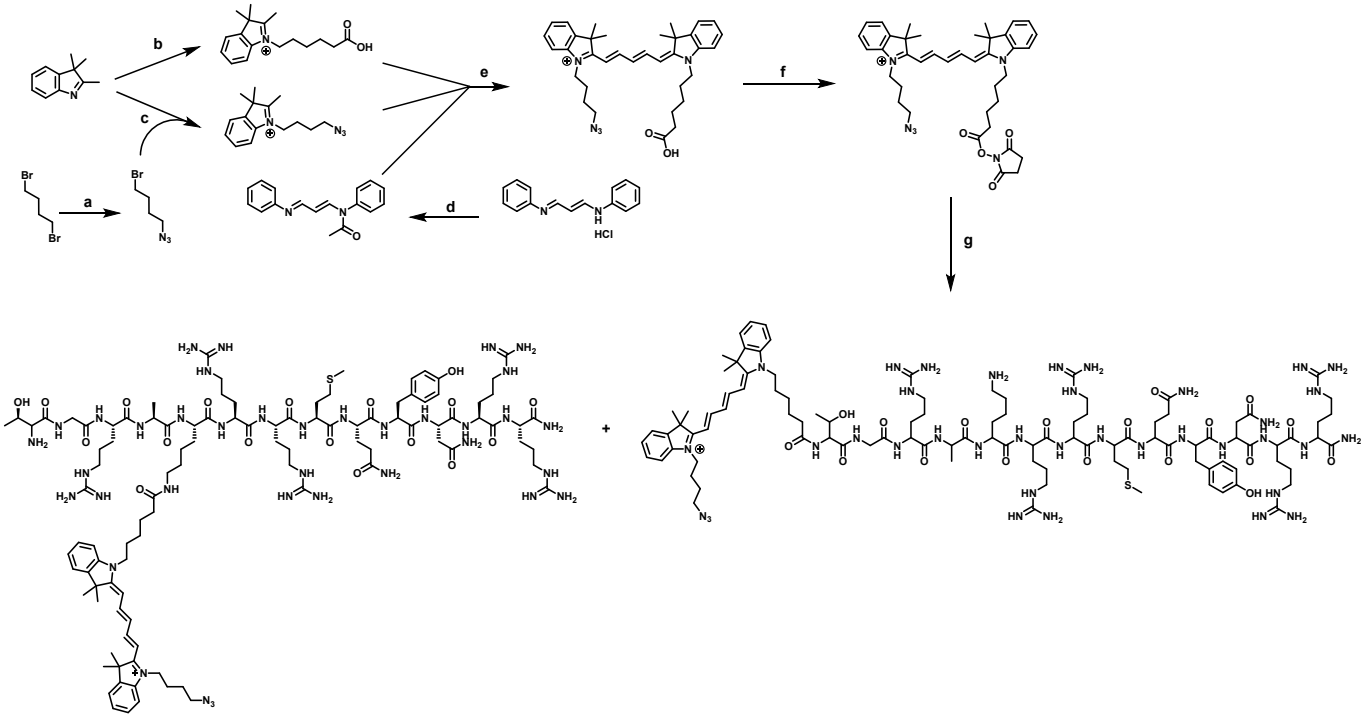

Figure S2. MALDI-TOF spectrum of 1-(4-azidobutyl)-2-((1E,3E)-5-((E)-1-(5-carboxypentyl)-3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-3,3-dimethyl-3H-indol-1-ium.

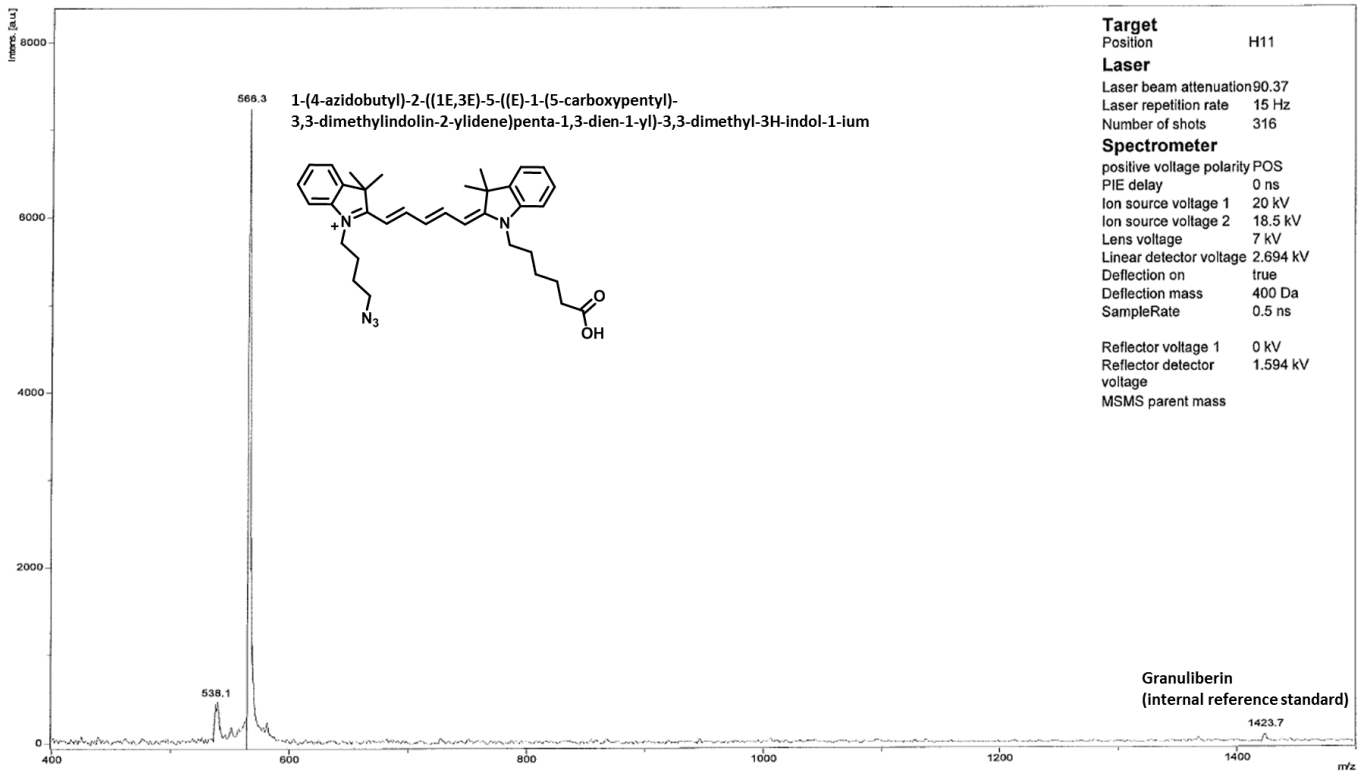

Figure S3. MALDI-TOF spectrum of 1-(4-azidobutyl)-2-((1E,3E)-5-((E)-1-(6-((2,5-dioxopyrrolidin-1-yl)oxy)-6-oxohexyl)-3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-3,3-dimethyl-3H-indol-1-ium.

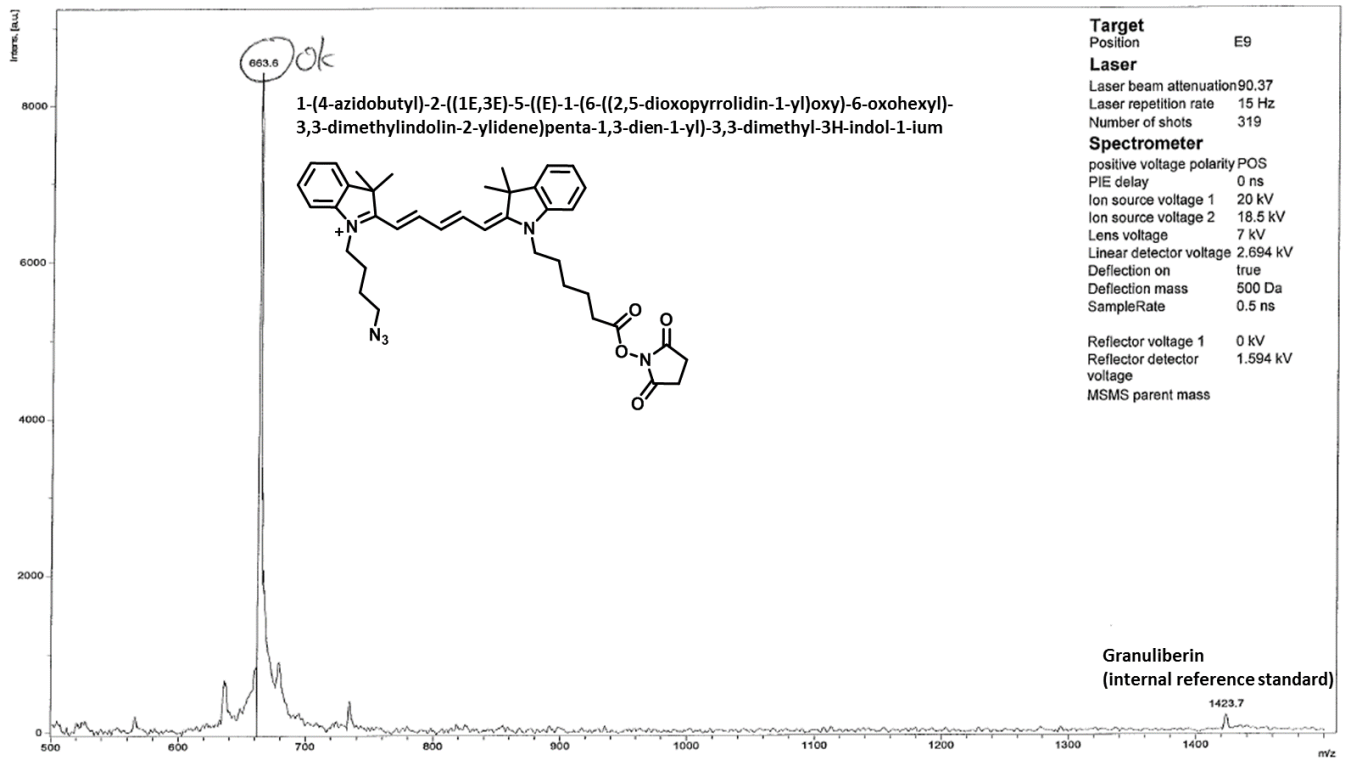

Figure S4a. ^{1}H-NMR spectrum of 1-(4-azidobutyl)-2-((1E,3E)-5-((E)-1-(6-((2,5-dioxopyrrolidin-1-yl)oxy)-6-oxohexyl)-3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-3,3-dimethyl-3H-indol-1-ium.

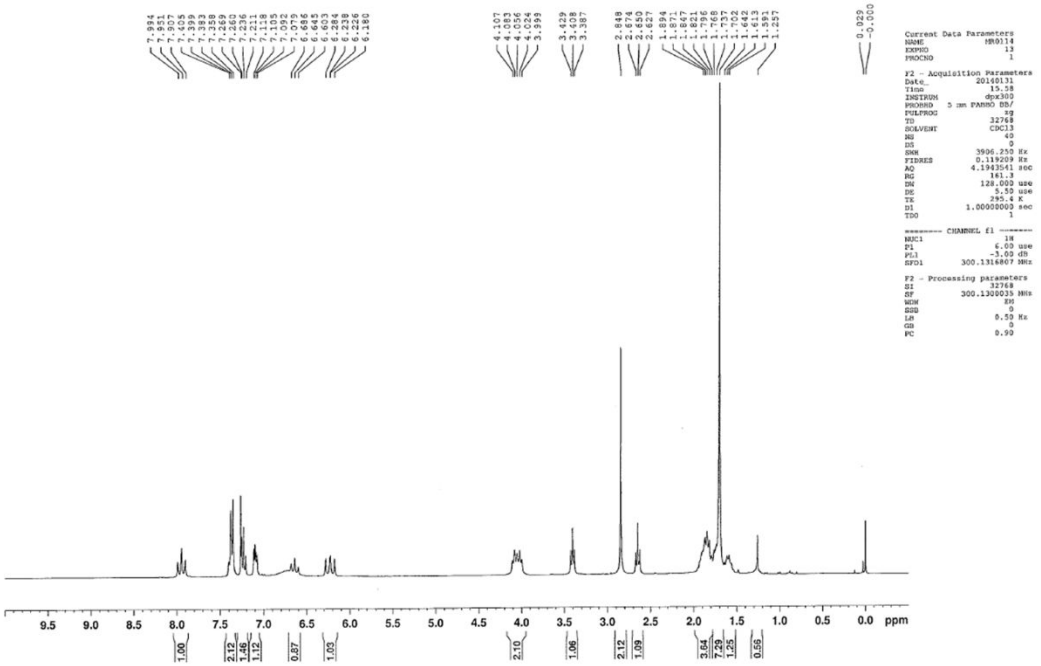

Figure S4b. Zoomed in on the aromatic peak area of the ^{1}H-NMR spectrum of 1-(4-azidobutyl)-2-((1E,3E)-5-((E)-1-(6-((2,5-dioxopyrrolidin-1-yl)oxy)-6-oxohexyl)-3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-3,3-dimethyl-3H-indol-1-ium.

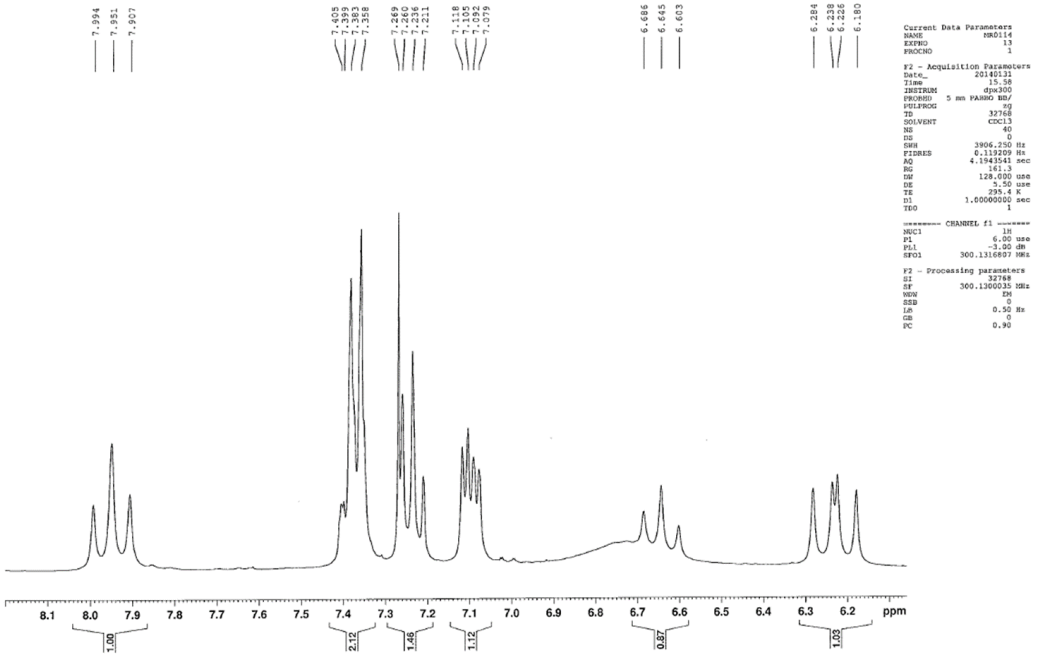

Figure S5. HPLC reverse-phase chromatography of the purification and characterization of UBI_{29-41}-Cy5-N_3. Chromatogram with the collected product peaks depicted by red arrow/lines.

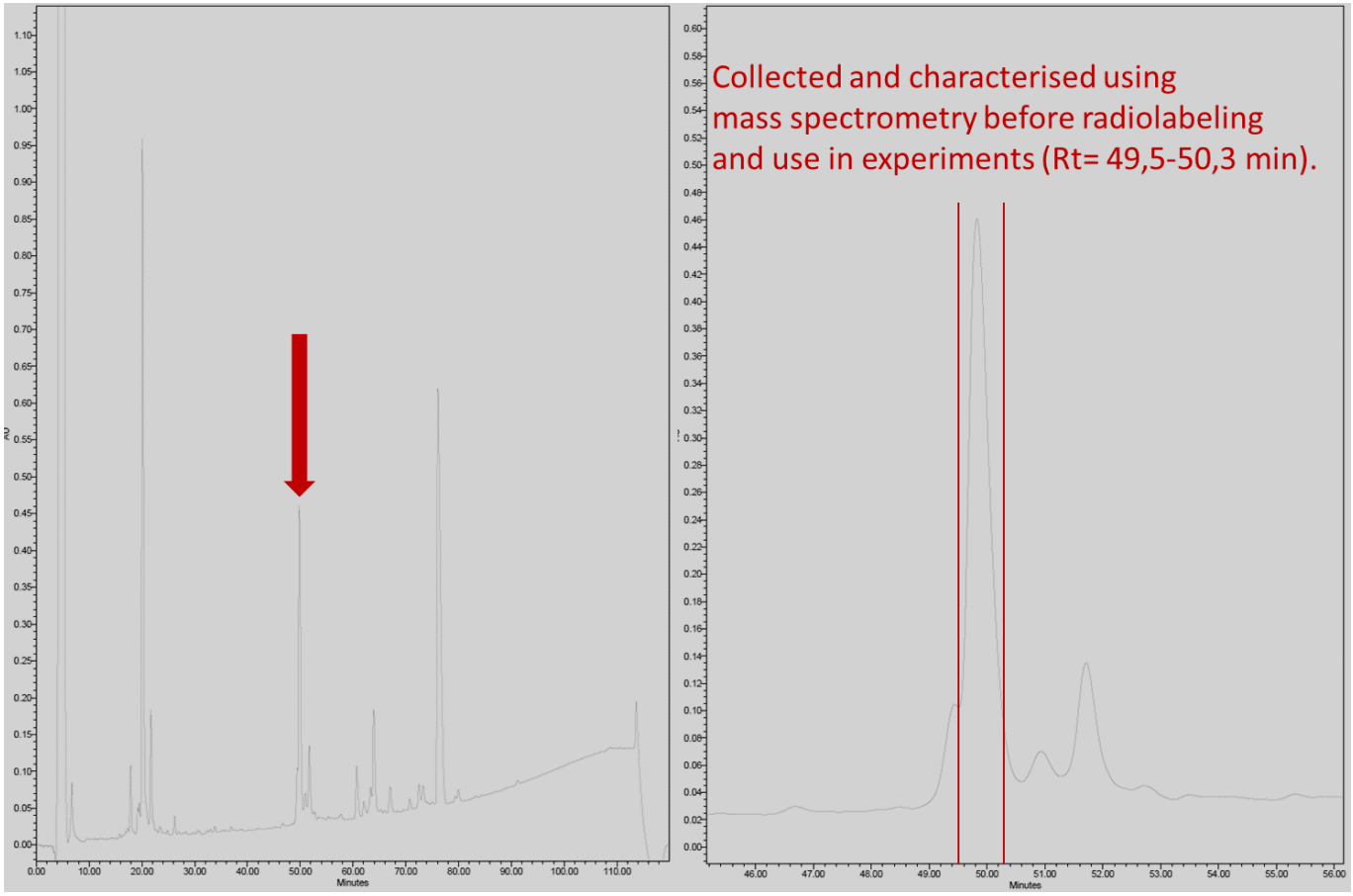

Figure S6. MALDI-TOF spectrum of HPLC purified UBI_{29-41}-Cy5-N_3. The red asterisk represents an alternative attachment point of the dye. This, however, will not change the recorded mass.

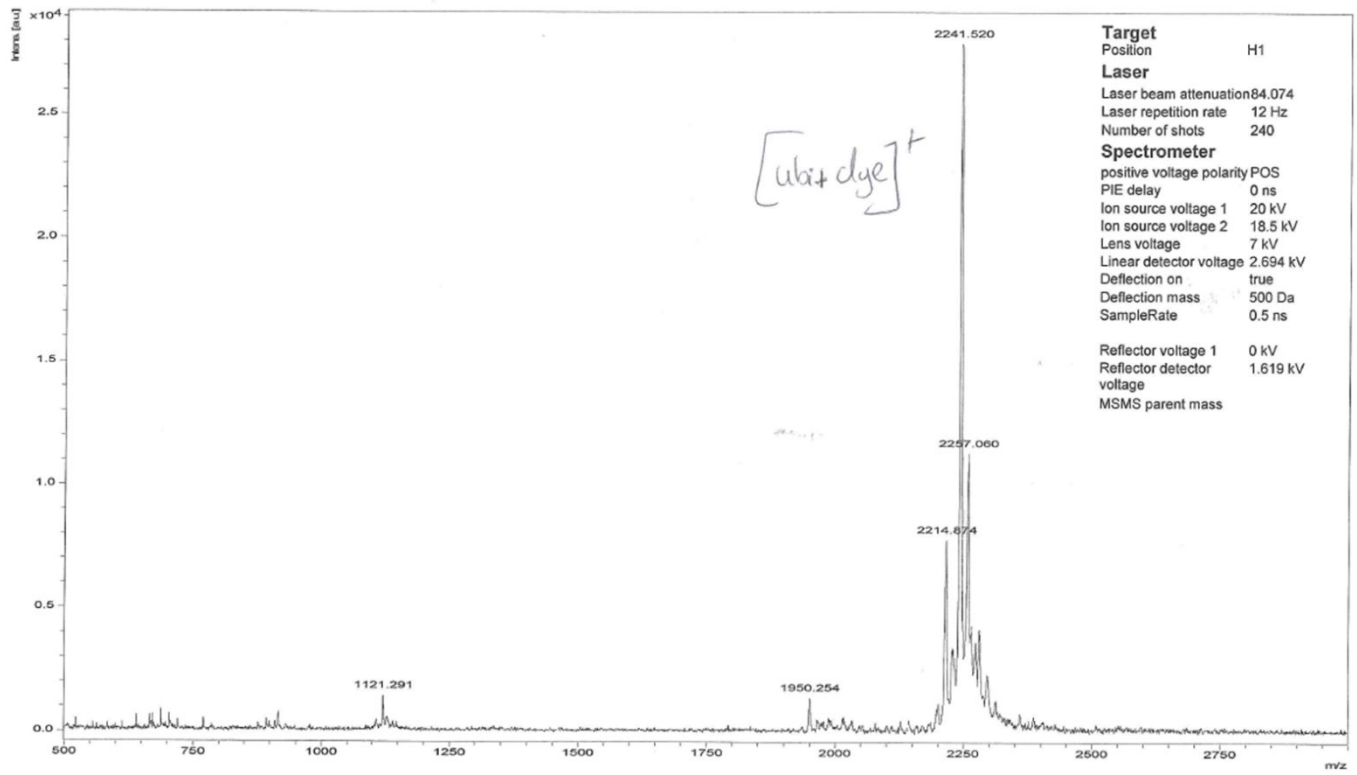

Figure S7. Schematic depiction of ^{99m}Tc labeled UBI$_{29-41}$-Cy5-N$_3$ according to Meléndez-Alafort *et al*. Nucl Med Biol. 2003;30(6):605. However, it is likely that other binding modes are also present in solution.

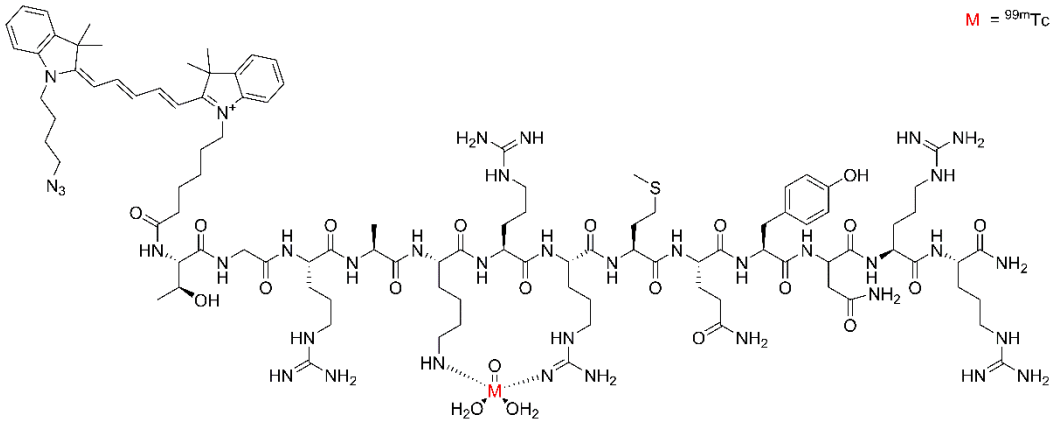

Figure S8. A) BHI agar plates containing serial dilutions of *S. aureus* from urine samples. **B)** assessment of viable bacteria in urine at various intervals after I.M. injection of $1x10^7$ CFU bacteria. Control = urine collected from non-infected mice. BDL = CFU's below detection limit. **C)** Radioactivity (^{99m}Tc expressed as %ID) and fluorescence (Cy5, expressed as Total Flux (p/s)/pixel) measurements in urine samples. Data are expressed as the mean of 3 observations. Error bars represent the standard deviation.

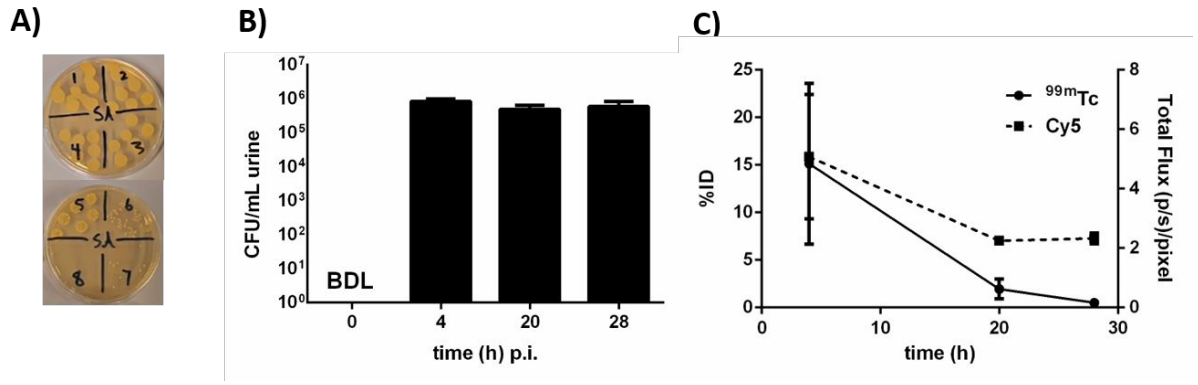

Figure S9. Confocal microscopic imaging of phagocytosis of UBI_{29-41}-Cy5-N_3 labeled *S. aureus* by human macrophages. Images were taken at t = 0 (A), 6 (B), and 30 (C) min. A few examples are highlighted with arrows indicating free (white arrow) or phagocytosed (yellow arrow) *S. aureus*. Grey = bright field, blue = Hoechst, red = Cy5. D) = UBI_{29-41}-Cy5-N_3 labeled dead *S. aureus*.