Supporting Information (SI)

Evidence of Oral Translocation of Anionic G6.5 Dendrimers in Mice

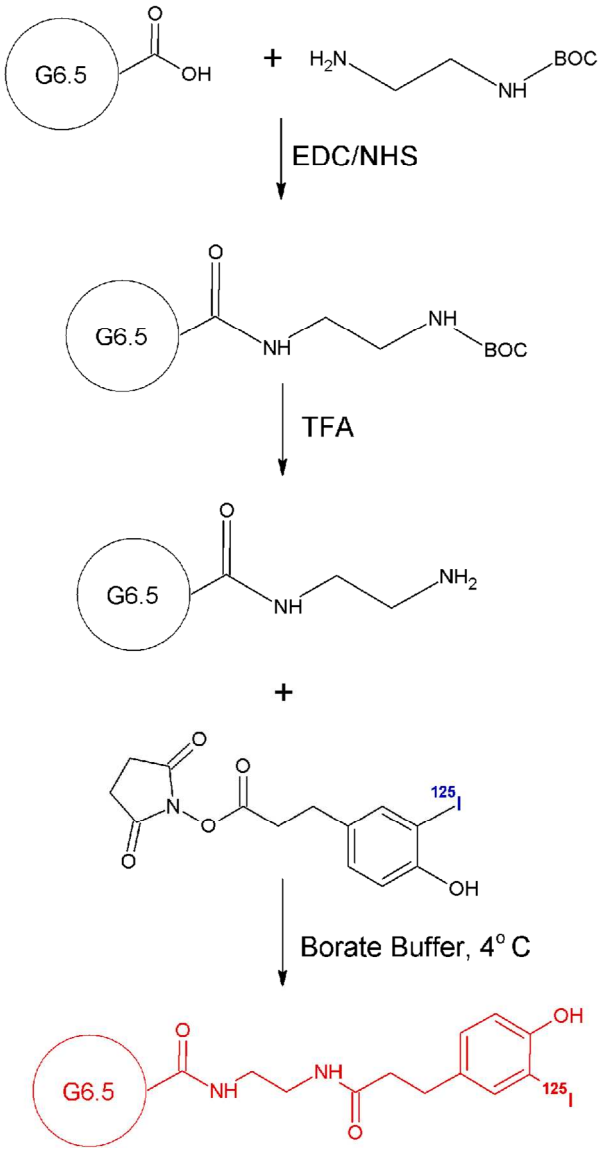
Giridhar Thiagarajan[‡, †], *Shraddha Sadekar*[§, †], *Khaled Greish*[§, †, 1] *Abhijit Ray*[§, †], *and Hamidreza Ghandehari*[§, ‡, †, *]

[‡] Department of Bioengineering, [§] Department of Pharmaceutics and Pharmaceutical Chemistry, and [†] Utah Center for Nanomedicine, Nano Institute of Utah, University of Utah, Salt Lake City, Utah 84112, United States

[1]Present address: Department of Pharmacology & Toxicology, Otago School of Medical Sciences, University of Otago, Dunedin, New Zealand

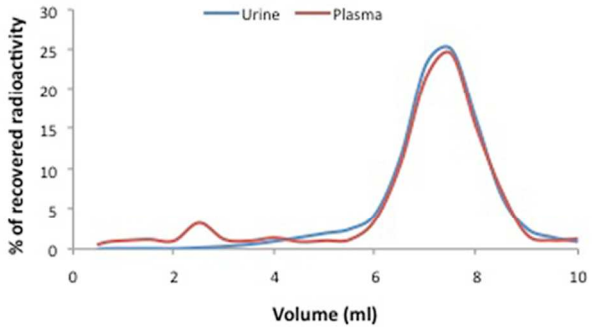
Corresponding author: Hamidreza Ghandehari, Utah Center for Nanomedicine, Nano Institute of Utah, University of Utah, Salt Lake City, UT 84112, USA. Tel: +(001) 801 587 1566. Fax: +(001) 801 585 0575. E-mail: hamid.ghandehari@pharm.utah.edu

Supplementary Figure 1



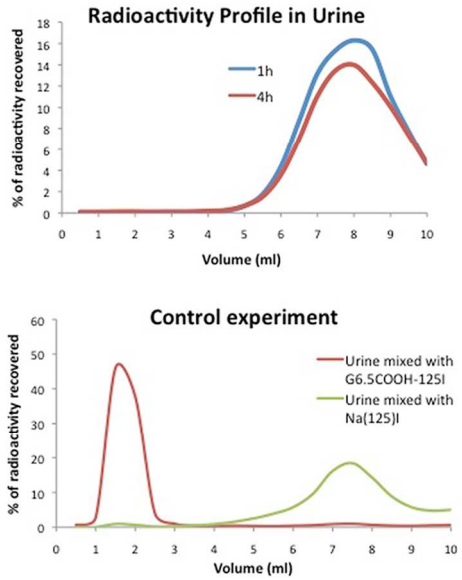
SI – 1. Scheme depicting the radiolabeling of anionic G6.5 PAMAM dendrimers. Anionic dendrimers were initially modified with ethylene diamine and then reacted with [^{125}I] Bolton-Hunter reagent.

Supplementary Figure 2



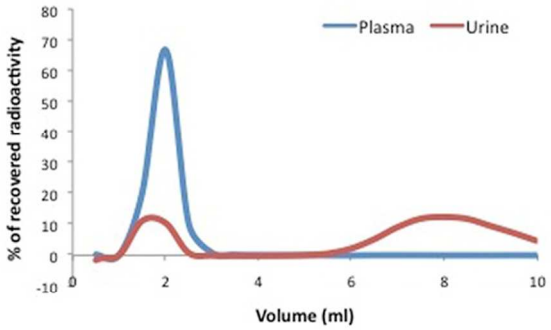
SI – 2. Control size exclusion experiments to validate PD-10 methods. Radioactivity profile from a PD-10 column loaded with ^{125}I-labeled Bolton-Hunter reagent in plasma and urine. Proteins and salts in these physiological fluids do not seem to affect the elution profile of small molecular weight Bolton-Hunter reagent.

Supplementary Figure 3



SI – 3. Size exclusion radioactivity profile of urine from mice orally administered with radiolabeled G6.5-COOH. Results show predominant radioactivity corresponding to small molecules was excreted in the urine. Bottom panel indicates control experiment with ^{125}I and radiolabeled G6.5-COOH externally mixed with urine from mice to validate the size exclusion methods.

Supplementary Figure 4



Sample	Plasma Profile		Urine Profile	
Peak volume	1.0-3.6	6.5-7.6	0.8-3.0	4.7-10
% Area under curve (AUC)	100	0.0	23.7	76.3

SI – 4. Radioactivity profile in plasma of CD-1 mice intravenously injected with radiolabeled G6.5-COOH indicates no release of small molecular weight compounds in blood (blue) under in vivo conditions after 4h. The urine profile (red) shows 76.3% of the area under the curve in the small molecular weight region.

SI- Table 1: Clinical parameters evaluated for toxicity in CD-1 mice

Organ system	Observation sign
Respiratory	Cyanosis: bluish appearance of tail, mouth, foot pads
	Nostril discharges: red/colorless
	Dyspnea: labored breathing
Motor activities	Tremors: limbs /entire body
	Unusual locomotion/low body posture
	Somnolence: drowsiness
Ocular signs	Lacrimation
	Ptosis: dropping of upper eyelids
	Exophthalmos: eye protrusion
Cardiovascular signs	Vasodilatation: redness of skin
	Vasoconstriction: whitening of skin
	Cold body
Salivation	Hair around mouth is wet
Gastrointestinal	Solid, dried, scant
Droppings	Loss of fluid, water stool
Fur/skin	Ruffling
Bleeding	Evidence of bleeding from any orifice
Body weight	Measured daily for 10 days