Supplementary Information for:

Impact of soft protein interactions on the excretion, extent of receptor occupancy and tumor accumulation of ultrasmall metal nanoparticles: a compartmental model simulation

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Ordinary differential equations for Model 1.

The ODEs below pertain to Model 1 as described in Fig. 1a. The ODEs can be easily modified to reflect the different NP-protein stoichiometries used in the actual simulations (see Eqs. 1 and 2). The ODEs are solved numerically given the rate constants and initial boundary conditions as specified under *Section 2.1–Parameter values*. Numerical integration was performed with the program Polymath (http://www.polymath-software.com).

$$d[NP]/dt = -2k_{on,P}[NP][P] + k_{off,P}[NP \cdot P_1] - k_{on,R}[NP][R] + k_{off,R}[NP \cdot R] - k_{clear0}[NP]$$

$$d[P]/dt = -2k_{on,P}[NP][P] + k_{off,P}[NP \cdot P_1] - k_{on,P}[NP \cdot P_1][P] + 2k_{off,P}[NP \cdot P_2]$$

$$d[NP \cdot P_1]/dt = 2k_{on,P}[NP][P] - k_{off,P}[NP \cdot P_1] - k_{on,P}[NP \cdot P_1][P] + 2k_{off,P}[NP \cdot P_2] - k_{on,R}[NP \cdot P_1][R] + k_{off,R}[NP \cdot P_1 \cdot R] - k_{clear1}[NP \cdot P_1]$$

$$d[NP \cdot P_2]/dt = k_{on,P}[NP \cdot P_1][P] - 2k_{off,P}[NP \cdot P_2] - k_{clear2}[NP \cdot P_2]$$

$$d[R]/dt = -k_{on,R}[NP][R] + k_{off,R}[NP \cdot R] - k_{on,R}[NP \cdot P_1][R] + k_{off,R}[NP \cdot P_1 \cdot R]$$

$$d[NP \cdot R]/dt = k_{on,R}[NP][R] - k_{off,R}[NP \cdot R]$$

$$d[NP \cdot P_1 \cdot R]/dt = k_{on,R}[NP \cdot P_1][R] - k_{off,R}[NP \cdot P_1 \cdot R]$$

$$d[NP_{out}]/dt = k_{clear0}[NP] + k_{clear1}[NP \cdot P_1] + k_{clear2}[NP \cdot P_2]$$

<u>Ordinary differential equations for Model 2.</u>

       The ODEs below pertain to Model 2 as described in Fig. 1b. The ODEs can be easily modified to reflect the different NP-protein stoichiometries used in the actual simulations. The ODEs are solved numerically given the rate constants and initial boundary conditions as specified under *Section 2.2–Parameter values*. Numerical integration was performed with the program Polymath.

$$d[NP]/dt = -2k_{on,P}[NP][P] + k_{off,P}[NP \cdot P_1] - k_{PT0}[NP] + (V_T/V_P) \times k_{TP0}[NP^T] - k_{clear0}[NP]$$

$$d[P]/dt = -2k_{on,P}[NP][P] + k_{off,P}[NP \cdot P_1] - k_{on,P}[NP \cdot P_1][P] + 2k_{off,P}[NP \cdot P_2]$$

$$d[NP \cdot P_1]/dt = 2k_{on,P}[NP][P] - k_{off,P}[NP \cdot P_1] - k_{on,P}[NP \cdot P_1][P] + 2k_{off,P}[NP \cdot P_2] - k_{PT1}[NP \cdot P_1] + (V_T/V_P) \times k_{TP1}[NP^T \cdot P_1^T] - k_{clear1}[NP \cdot P_1]$$

$$d[NP \cdot P_2]/dt = k_{on,P}[NP \cdot P_1][P] - 2k_{off,P}[NP \cdot P_2] - k_{PT2}[NP \cdot P_2] + (V_T/V_P) \times k_{TP2}[NP^T \cdot P_2^T] - k_{clear2}[NP \cdot P_2]$$

$$d[NP^T]/dt = -2k_{on,P}[NP^T][P^T] + k_{off,P}[NP^T \cdot P_1^T] + (V_P/V_T) \times k_{PT0}[NP] - k_{TP0}[NP^T]$$

$$d[P^T]/dt = -2k_{on,P}[NP^T][P^T] + k_{off,P}[NP^T \cdot P_1^T] - k_{on,P}[NP^T \cdot P_1^T][P^T] + 2k_{off,P}[NP^T \cdot P_2^T]$$

$$d[NP^T \cdot P_1^T]/dt = 2k_{on,P}[NP^T][P^T] - k_{off,P}[NP^T \cdot P_1^T] - k_{on,P}[NP^T \cdot P_1^T][P^T] + 2k_{off,P}[NP^T \cdot P_2^T] + (V_P/V_T) \times k_{PT1}[NP \cdot P_1] - k_{TP1}[NP^T \cdot P_1^T]$$

$$d[NP^T \cdot P_2^T]/dt = k_{on,P}[NP^T \cdot P_1^T][P^T] - 2k_{off,P}[NP^T \cdot P_2^T] + (V_P/V_T) \times k_{PT2}[NP \cdot P_2] - k_{TP2}[NP^T \cdot P_2^T]$$

$$d[NP_{out}]/dt = k_{clear0}[NP] + k_{clear1}[NP \cdot P_1] + k_{clear2}[NP \cdot P_2]$$

where V_P and V_T denote the volumes of the plasma and tumor compartments, respectively. As the ratio V_P/V_T represents only a scaling factor, an arbitrary value of 1 is assumed for simplicity.

Table S1. Experimental plasma clearance data for ultrasmall metal NPs.

Entry	NP core	NP coating	HD (nm)	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (h)	%ID in urine at 24 h p.i.[4]	Ref.
1	Ag[1]	GSH	3.1	1.6	22.2	51	[1]
2	Ag-Au[1]	GSH	3.1	2.4	21.4	53	[1]
3	Ag-Au[1]	GSH	3.1	3.5	20.3	49	[1]
4	Au[1]	GSH	3.1	5.1	16.5	46	[1]
5	Au	PEG	5.5	56.1	9.2	∼ 55	[2]
6	Au	GSH	3.3	5.4	8.5	∼ 55	[2]
7	Au[2]	GSH	∼ 1	7.1	11.2	19	[3]
8	Au[2]	GSH	∼ 1	11.5	9.5	23	[3]
9	Au[2]	GSH	∼ 1	4.9	10.3	27	[3]
10	Au[2]	GSH	∼ 1	4.5	12.3	52	[3]
11	Au	Gly-Cys	3.1	2.5	4.3	42	[4]
12	Au	Cys	2.7	3.2	4.9	21	[4]
13	Au	GSH	3.0	5.0	12.7	∼ 45	[5]
14	Au[3]	GSH	3.3	3.5	7.0	39	[6]
15	Au[3]	GSH	3.3	2.6	3.3	43	[6]
16	Au[3]	GSH	3.3	1.5	0.79	71	[6]
17	Au	GSH	3.0	0.73	8.1	52	[7]

1. NPs of different core compositions but same surface coating. The Ag:Au ratios are (from top to bottom): 1:0, 8.4:1, 0.64:1 and 0:1.
2. Atomically precise nanoclusters with formulas $Au_{10-11}GSH_{10-11}$, $Au_{15}GSH_{13}$, $Au_{18}GSH_{14}$ and $Au_{25}GSH_{18}$ (from top to bottom). Here the $t_{1/2\alpha}$ and $t_{1/2\beta}$ were calculated by fitting of Eq. 3 to the data provided in the Supplementary Table 3 in ref. [3].
3. Pharmacokinetic data was recorded using the same NPs under different injection doses. Here the $t_{1/2\alpha}$ and $t_{1/2\beta}$ were calculated from the data provided in the Supplementary Fig. S7 in ref. [6].
4. Values of %ID in the urine are included for reference. Entries 1-4: %ID at 48 h p.i.

Table S2. Experimental plasma clearance data for some protein drugs. Selected proteins cover a range of molecular weights from 7 to 80 KDa. Clearance data for smaller molecular tracers (first two rows) and larger proteins (last three rows) are also shown for comparison.

Molecule	MW (KDa)	HD (nm)[1]	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (h)	Ref.
^{177}Lu-DOTA	0.58	1.5	0.17	0.34	[8]
IR Dye	1.1	1.9	6.3	0.98	[9]
Affibody	7	3.5	14.7	23	[10]
Affibody dimer	15.6	4.5	13.4	5.8	[10]
scFv	27	5.5	12.9	6.3	[10]
scFv	28	5.5	5	4	[11]
scDb	54.5	6.9	10.2	5.6	[12]
scFv dimer	55	6.9	35.4	13.8	[10]
taFv	56	7.0	9	26	[11]
Minibody	80	7.8	62.7	9.9	[10]
scDb-PEG40[2]	95	8.3	31	13	[12]
taFv-HSA[3]	121	9.0	37	40	[11]
IgG	145	9.5	43.8	39	[12]

1. The hydrodynamic diameter (HD) of all molecules was estimated as HD = $1.82 \times MW^{0.333}$ for consistency (ref. [10])
2. PEGylated recombinant diabody
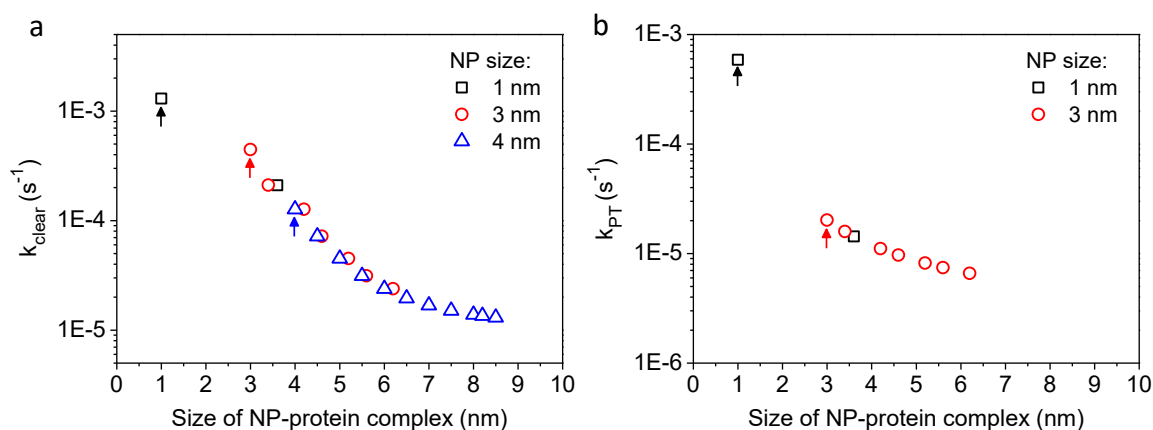3. Recombinant antibody-albumin fusion protein

Figure S1. Systemic clearance (a) and vascular extravasation (b) rates for NPs and NP-protein complexes as a function of compound size. Sizes were calculated according to Eqs. 1 and 2, whereas k_{clear} and k_{PT} were estimated with Eqs. 5 and 9, respectively. NPs of 1, 3 and 4 nm in size have maximum binding capacities of 1, 6 and 10 plasma proteins, respectively. Naked NPs are indicated with arrows.
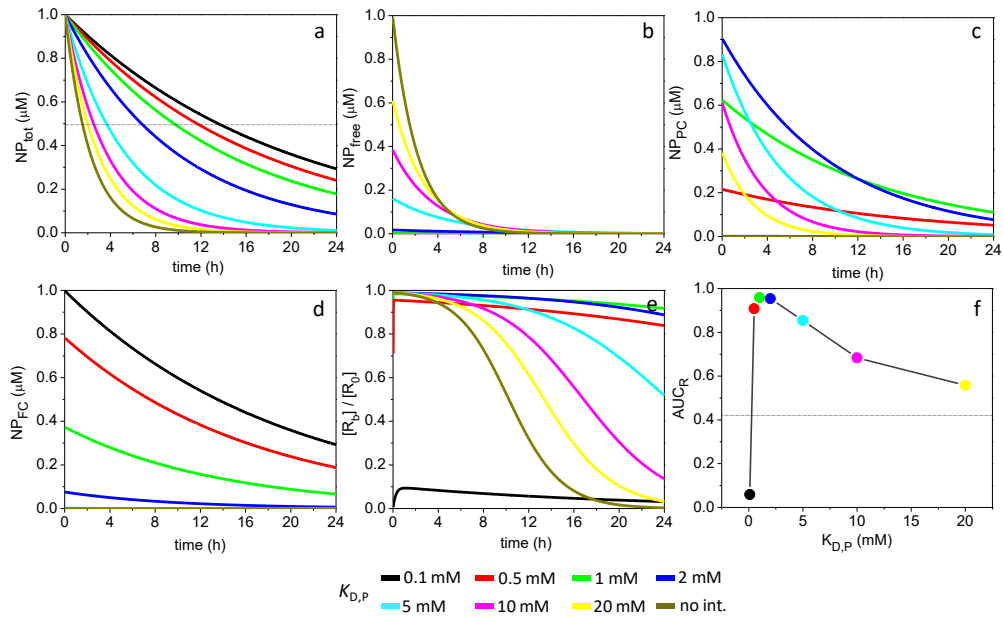
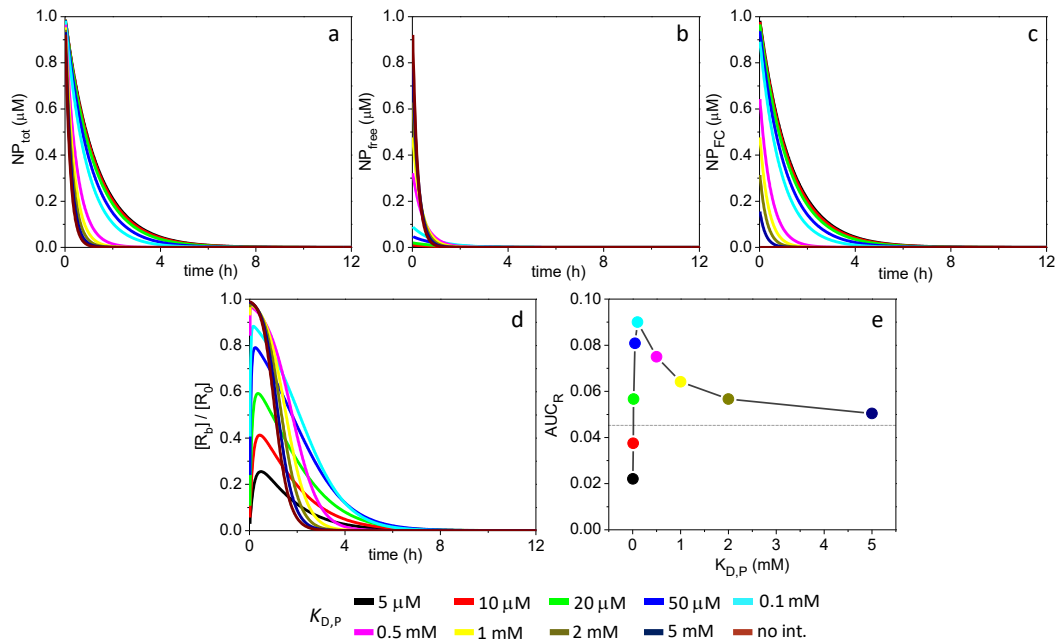**Figure S2. Time course of NP species in the compartment and extent of receptor occupancy for *Sim #2*. (a)** Time course of NP_{tot}. The half-life for systemic clearance of NP_{tot} for each value of $K_{D,P}$ is given by the intercept of the dashed line with each corresponding decay curve. **(b)** Time course of NP_{free}. **(c)** Time course of NP_{PC}. **(d)** Time course of NP_{FC}. **(e)** Receptor occupancy as a function of time. **(f)** Area under the curve for receptor occupancy as a function of $K_{D,P}$; calculated from e) with Eq. 7. Dashed line marks the calculated value of AUC_R (0.43) in the absence of soft interactions.
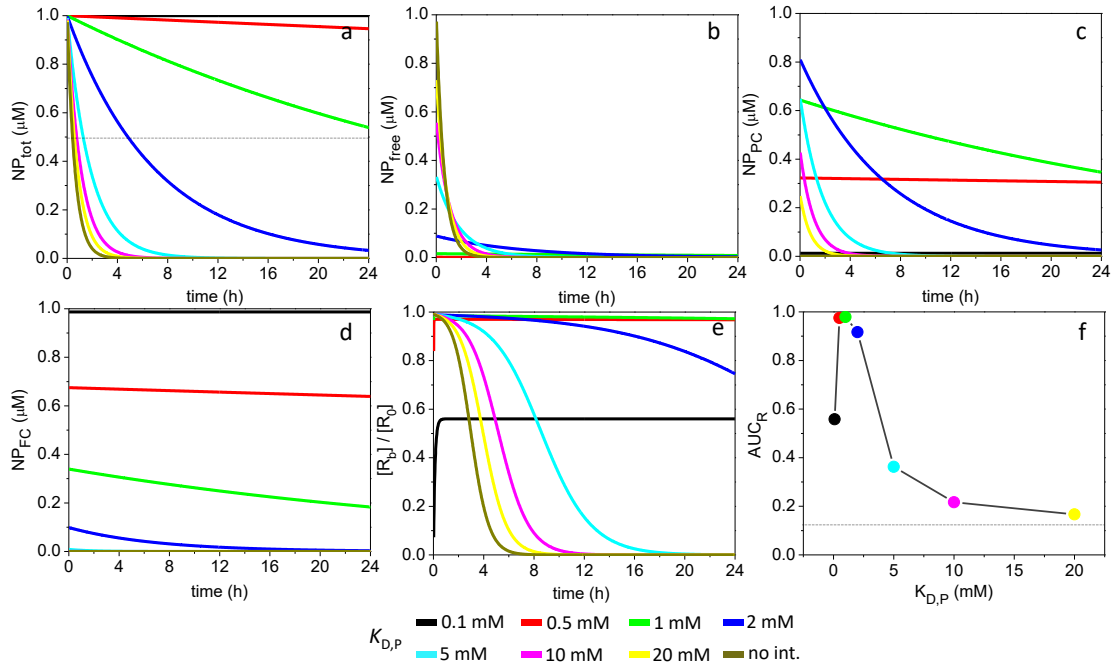

Figure S3. Time course of NP species in the compartment and extent of receptor occupancy for *Sim #3*. (a) Time course of NP_{tot}. **(b)** Time course of NP_{free}. **(c)** Time course of NP_{FC}. **(d)** Receptor occupancy as a function of time. **(e)** Area under the curve for receptor occupancy as a function of $K_{D,P}$; calculated from d) with Eq. 7. Dashed line marks the calculated value of AUC_R (0.046) in the absence of soft interactions.
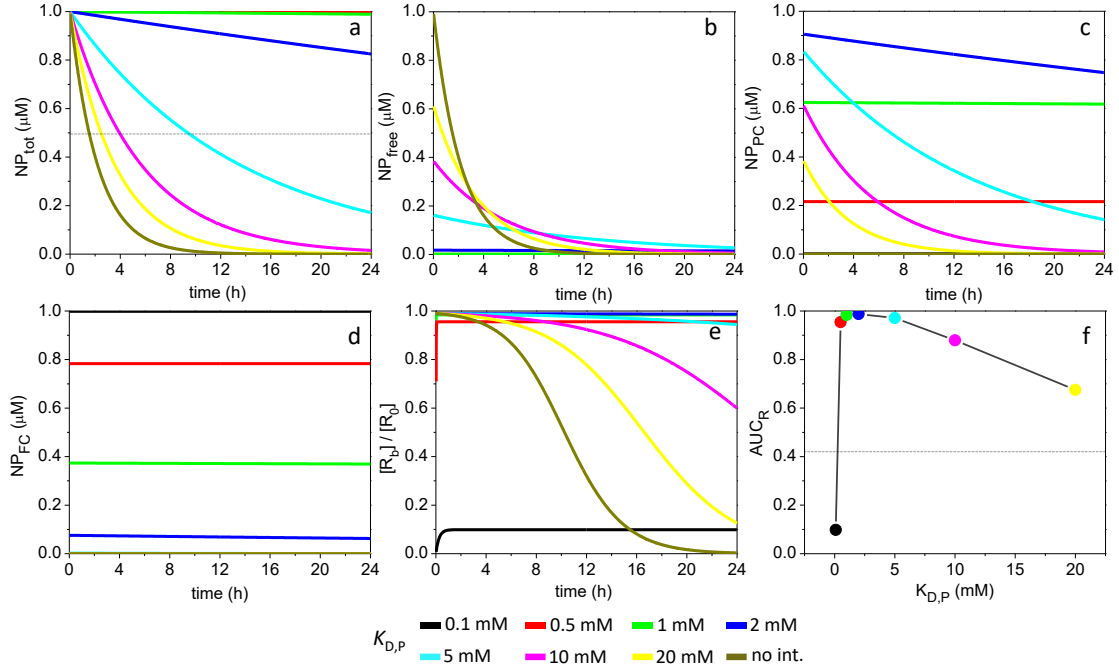
Figure S4. Time course of NP species in the compartment and extent of receptor occupancy for *Sim #4*. (a) Time course of NP_{tot}. The half-life for systemic clearance of NP_{tot} for each value of $K_{D,P}$ is given by the intercept of the dashed line with each corresponding decay curve. **(b)** Time course of NP_{free}. **(c)** Time course of NP_{PC}. **(d)** Time course of NP_{FC}. **(e)** Receptor occupancy as a function of time. **(f)** Area under the curve for receptor occupancy as a function of $K_{D,P}$; calculated from e) with Eq. 7. Dashed line marks the calculated value of AUC_R (0.12) in the absence of soft interactions.

Figure S5. Time course of NP species in the compartment and extent of receptor occupancy for *Sim #5*. (a) Time course of NP_{tot}. The half-life for systemic clearance of NP_{tot} for each value of $K_{D,P}$ is given by the intercept of the dashed line with each corresponding decay curve. **(b)** Time course of NP_{free}. **(c)** Time course of NP_{PC}. **(d)** Time course of NP_{FC}. **(e)** Receptor occupancy as a function of time. **(f)** Area under the curve for receptor occupancy as a function of $K_{D,P}$; calculated from e) with Eq. 7. Dashed line marks the calculated value of AUC_R (0.43) in the absence of soft interactions.
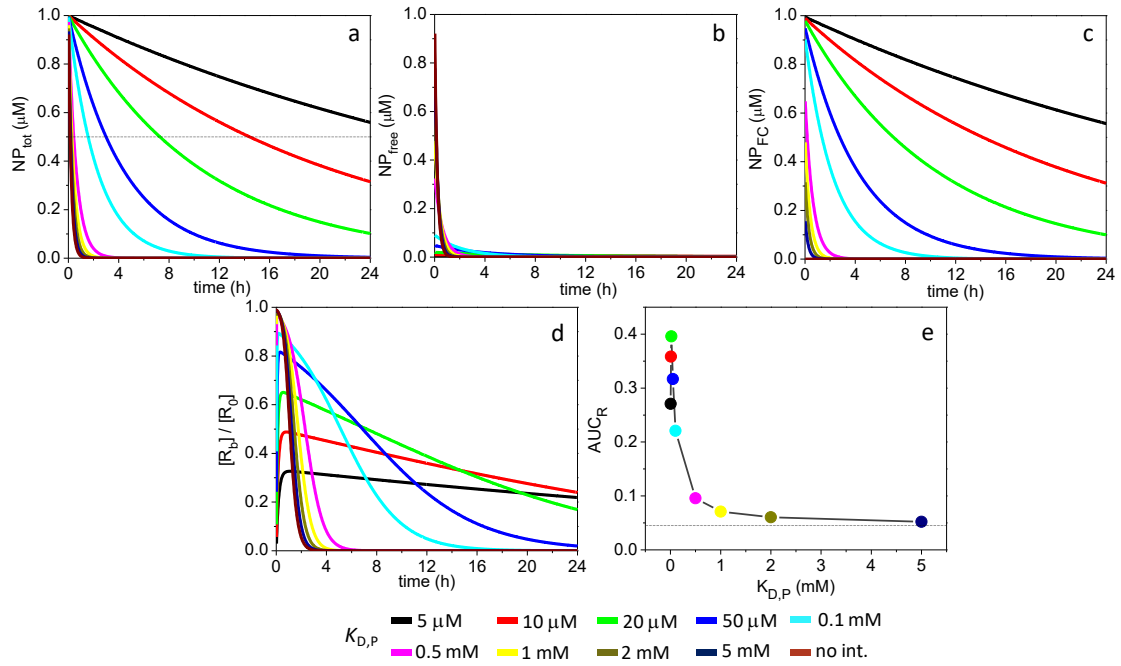
Figure S6. Time course of NP species in the compartment and extent of receptor occupancy for *Sim #6*. (a) Time course of NP_{tot}. The half-life for systemic clearance of NP_{tot} for each value of $K_{D,P}$ is given by the intercept of the dashed line with each corresponding decay curve. **(b)** Time course of NP_{free}. **(c)** Time course of NP_{FC}. **(d)** Receptor occupancy as a function of time. **(e)** Area under the curve for receptor occupancy as a function of $K_{D,P}$; calculated from d) with Eq. 7. Dashed line marks the calculated value of AUC_R (0.046) in the absence of soft interactions.
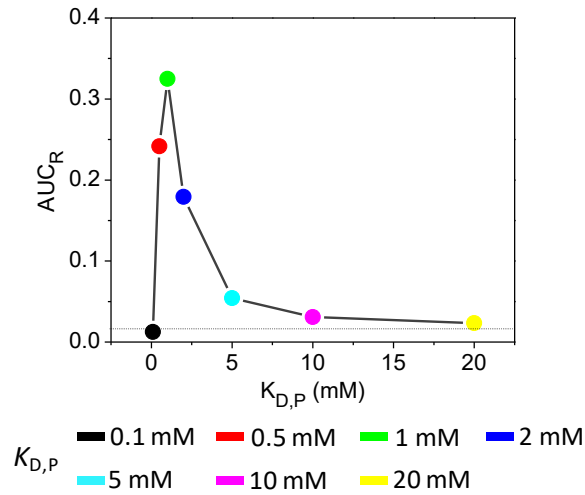

Figure S7. Extent of receptor occupancy assuming the model parameters in *Sim #7*. Area under the curve for receptor occupancy as a function of $K_{D,P}$. Dashed line marks the calculated value of AUC_R (0.018) in the absence of soft interactions.
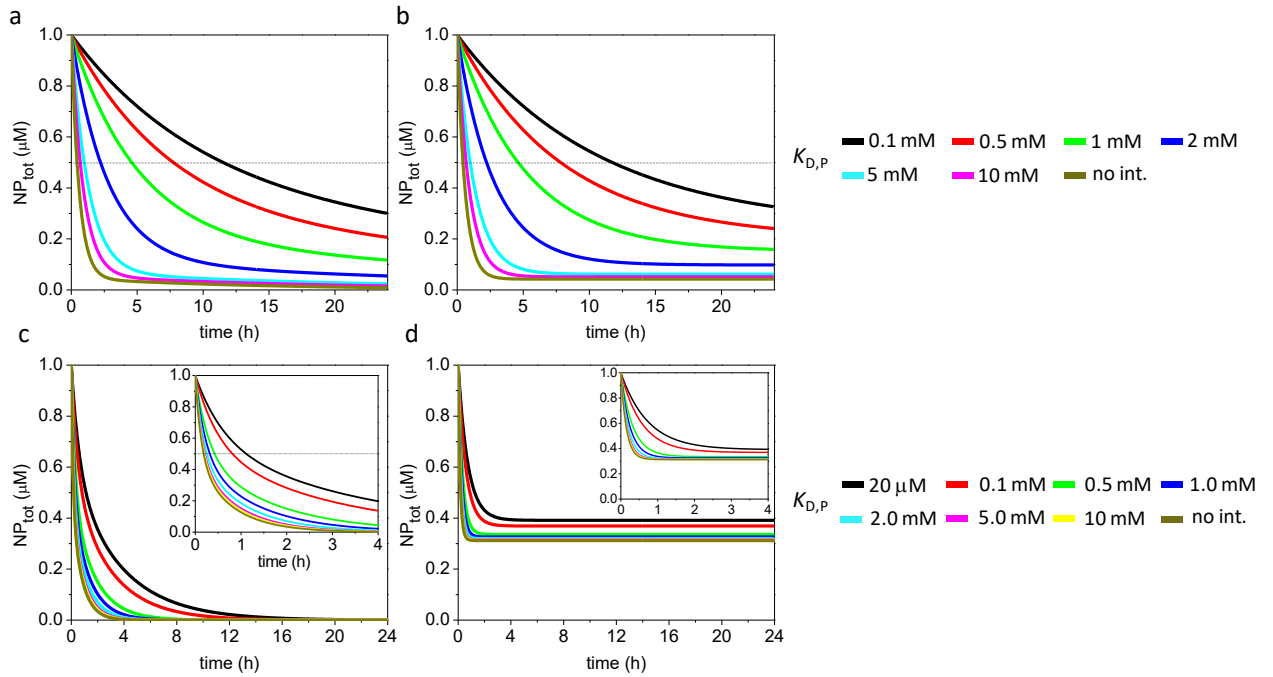
Figure S8. Time course of NP_{tot} assuming the model parameters in *Sim #9* (a), *#10* (b), *#11* (c) and *#12* (d). Half-life for systemic clearance of NP_{tot} for each value of $K_{D,P}$ is given by the intercept of the dashed line with each corresponding decay curve. In d), the NP_{tot} concentration does not fall down to 'zero' because a significant amount of NPs that have extravasated remain trapped in the peripheral compartment.
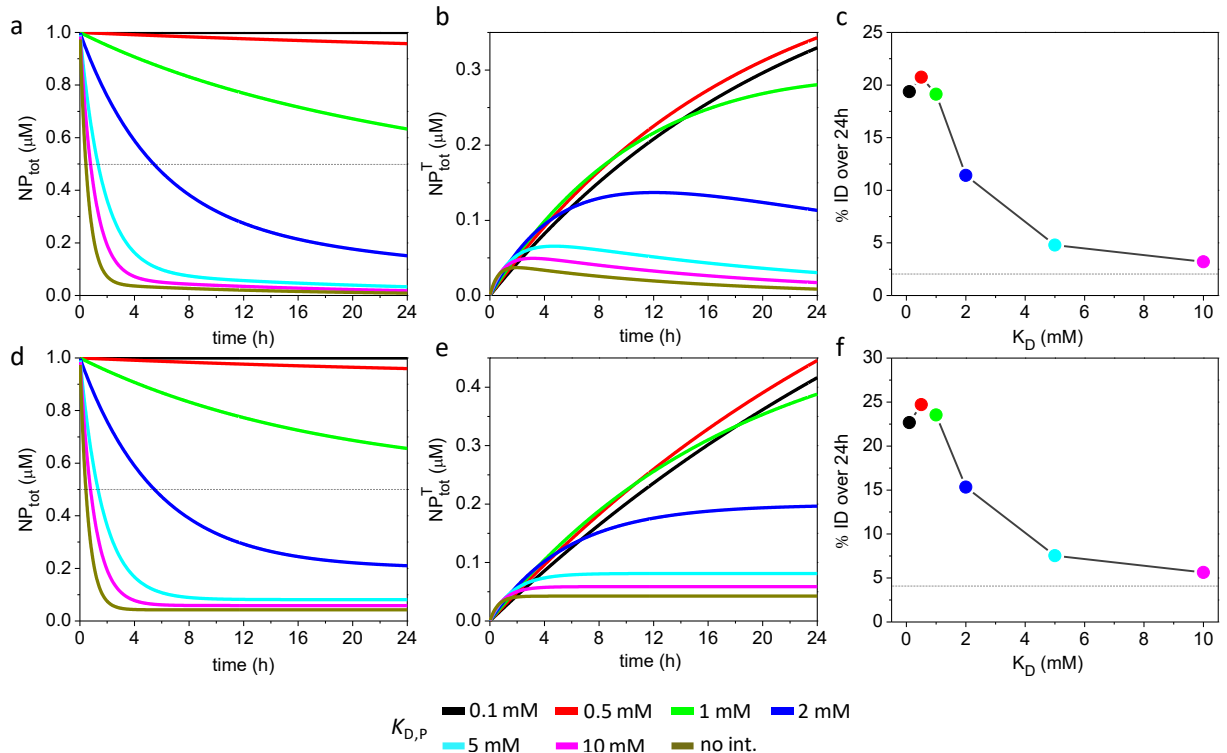

Figure S9. Time course of NP species and %ID assuming the model parameters in *Sim #13* and *#14*. (a) Time course of NP_{tot}, **(b)** time course of NP^T_{tot}, and **(c)** %ID as a function of $K_{D,P}$ for *Sim #13*. **(d,e,f)** Same for *Sim #14*. Values of %ID were calculated from b) and e) with Eq. 10. Half-life for systemic clearance of NP_{tot} for each value of $K_{D,P}$ is given by the intercept of the dashed line with each corresponding decay curve in a) and d). Dashed lines in c) and f) mark the calculated values of %ID (2 and 4.2%) in the absence of soft interactions.

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