S1) Impact of rebinding in the *in vivo* occupancy profiles of one-step binding drugs under different dosing paradigms.

When dosing complies with the constant $[L_{max}]/K_D$ ratio paradigm, increasing the "firmness" of rebinding by increasing k_1 is able to bring about longer- lasting occupancy and eventually also to "depress" the occupancy profile (i.e. to delay the attainment of peak occupancy and to decrease its magnitude). As shown in Figure S1, this effect is clearly outspoken for the constant $[L_{max}]/K_D$ ratio dosing paradigm. This is because an adjustment of the dosage compensates for the k_1- mediated decrease of the drug's K_D but not for the genuine effect rebinding (since it does not impact K_D). In contrast, the decreased K_D is no longer compensated for when the dosing proceeds according to the constant $[L_{max}]$ paradigm. The $[L_{max}]/K_D$ ratio thus increases and this effect now adds-up to the genuine effect of rebinding (Figure). Finally, the effect of rebinding is appreciably less pronounced when the dosing proceeds according to the constant $[L_{max}]$ - constant K_D dosing paradigm since it is largely offset by a higher k_2 to keep the K_D constant.

S2) Impact of target turnover on the *in vivo* occupancy profiles of one-step binding drugs in the absence or presence of rebinding.

In vivo protein turnover (degradation and synthesis) usually takes place with half-live between a couple of hours and a day [1]. For Figure S2, such target turnover is now also included to monitor its impact on the *in vivo* occupancy profiles one-step binding drugs. To this end, RL is now able to convert to R (i.e. the initial unbound target) with a first order rate constant k_t (Panel A). While the highest of the presently- explored turnover half-lives are in the usual range, the lower ones were included to better illustrate the impact of this mechanism on the occupancy profiles. Indeed, this impact increases when the turnover is faster.

Dosing complies with the constant $[L_{max}]/K_D$ ratio paradigm for the presented simulations and graphs from left to right (Panel B) correspond to increasing half-lives for drug dissociation. An eye-catching observation is that the impact of target turnover is most pronounced when the drug dissociates slowly. Both in the absence and presence of rebinding, this impact essentially brings about lower peak occupancy and faster subsequent decrease

thereof. This observation meets the viewpoint that the occupancy will not necessarily increase upon multiday dosing for drugs that dissociate very slowly [2]. The reason for why the impact of target turnover is most pronounced for slow- dissociating drugs has to do with the fact [L_{max}] remains constant in each individual graph regardless of k_t, (since this parameter does not affect the actual K_D of the binding process) whereas drug dissociation and target turnover act in concert to convert RL into R (Panel A). This increases the drug's apparent k_{off} (i.e. now $k_t + k_2$) and, consequently, also decreases the apparent [L_{max}]/K_D ratio (i.e. now k_1.[L_{max}]/($k_t + k_{2)}$). Each of those changes is able to modify the drug's occupancy profile in its own right and their magnitude is positively correlated with the k_t/k_2 ratio.

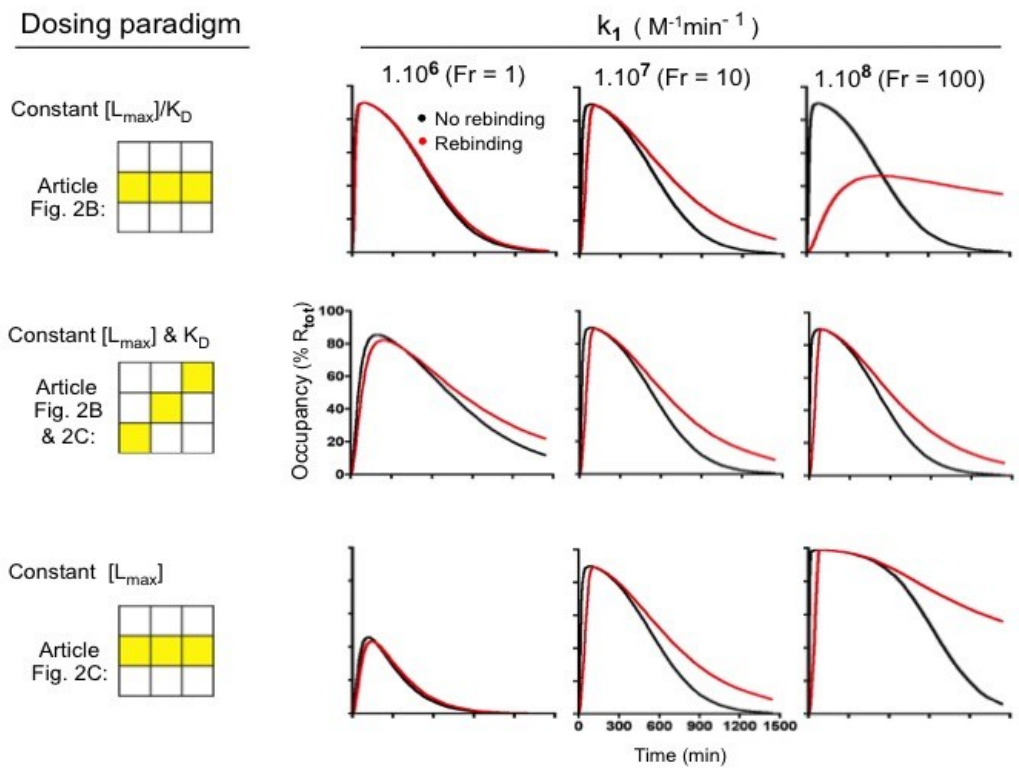
Finally, it is of note that, even in the presence of target turnover, rebinding can still affect the occupancy curves in a quite similar way as increasing the dissociation half-life.

References:

1) E. Eden et al. *Science*, 2011, **331**, 764.

2) R. H. A. Folmer, *Drug Discov. Today,* 2018, **23**, 12.

Figure S1.

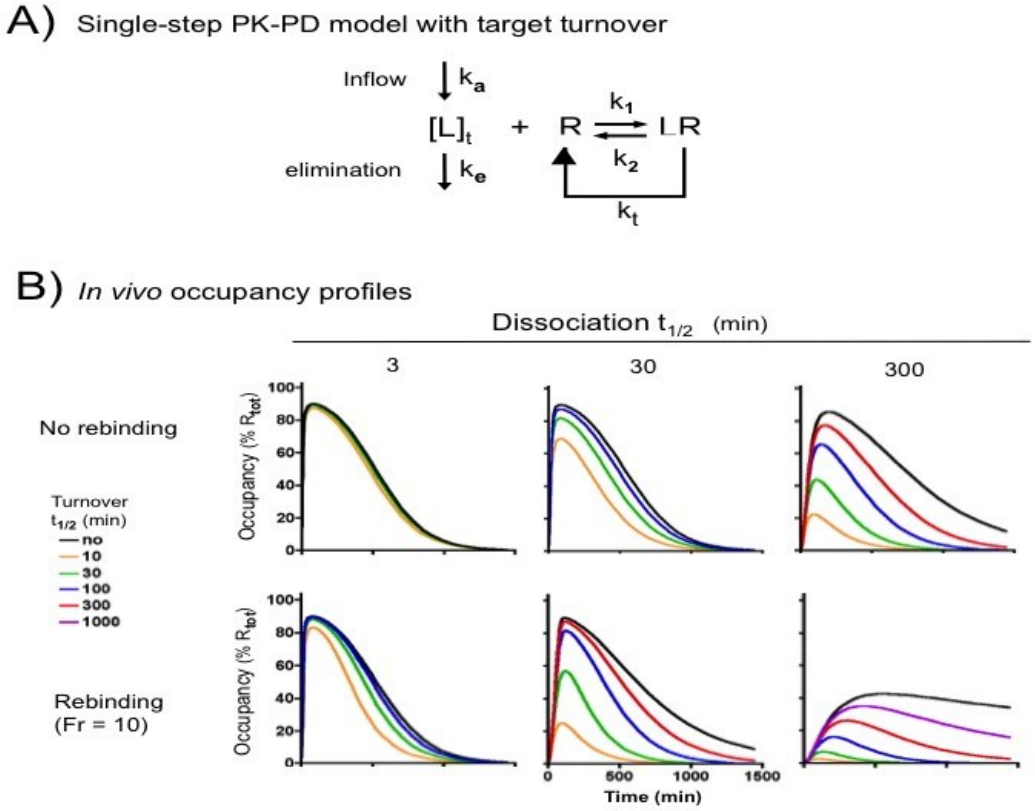


Comparison of the impact of the different dosing paradigms on the effect of increasing k_1 in the presence of rebinding for one-step binding drugs.

The grids at the left side refer to schematic representations of the k_1-k_2 combinations that were examined in Panels B and/or C of Figure 2 of the article. The highlighted cases therein refer to the combinations that were presently used for simulating the occupancy profiles shown at the right side. Curves in black refer to the absence of rebinding and those in red to the presence of rebinding (with F_r being proportional to k_1).

Parameters for all: $k_a = 0.023$ min^{-1} ($t_{1/2}$ = 30 min) and $k_e = 0.00575$ min^{-1} ($t_{1/2}$ = 2 h); for the constant $[L_{max}]/K_D$ ratio dosing paradigm: $[L_{max}] = 9 \times K_D$ and $k_2 = 2.3.10^{-2}$ min^{-1}; for the constant $[L_{max}]$ – constant K_D paradigm: $[L_{max}] = 9 \times K_D$ and k_2 ranges from $2.3.10^{-3}$ to $2.3.10^{-1}$ min^{-1} (from left to right.); for the constant $[L_{max}]$ paradigm: $[L_{max}]$ = 20.7 nM and $k_2 = 2.3.10^{-2}$ min^{-1}.

Figure S2.



Impact of target turnover on the *in vivo* occupancy profiles of one-step binding drugs in the absence or presence of rebinding.

Panel A) One-step binding model in where [L] evolves during 24 h according to the Bateman function in where k_a and k_e are the first-order rate constants for drug's inflow into and elimination from the target- containing body compartment. R and L stand for the target and the drug, k_1 for the second-order association rate constant, k_2 for the first-order dissociation rate constant and k_t for the first-order rate constant for target turnover (which converts RL into L in the present model). Of note is that turnover of R itself will not affect its concentration.

Panel B) Impact of target turnover on the *in vivo* occupancy profiles without (top) and with rebinding (bottom, with constant rebinding factor", F_r = 10). Dosing complies with the constant $[L_{max}]/K_D$ ratio paradigm (here $[L_{max}]$ = 9 x K_D to yield a maximal target occupancy '$[RL_{max}]$' of 90 % for instant equilibrium binding). Only the most pertinent curves are shown in each graph.

Parameters for all: k_a = 0.023 min^{-1} ($t_{1/2}$ = 30 min), k_e = 0.00575 min^{-1} ($t_{1/2}$ = 2 h) and k_1 = 1.10^6 $M^{-1}.min^{-1}$. k_2 And k_f (in min^{-1}) correspond to 0.69/(the indicated half- lives).