General

Background for the movies can be found in Schuck P, Sedimentation Patterns of rapidly reversible protein interactions. Biophysical Journal (2010) 98 (in press), to which they serve as Supplementary Material. More movies can be generated for different conditions using the Effective Particle Explorer of the software SEDPHAT (to be found in the Options Menu under Interaction Calculator).

The movies are meant to illustrate the principle of co-migration between interacting molecules A and B, migrating at velocities v_a and v_b, respectively (with $v_a < v_b$), and forming complexes AB that migrate at a velocity v_{ab}. The presentation is faithful with regard to the relative concentrations of species (following mass action law), the relative lifetimes of states, and the relative velocities of all species and the reaction boundary. Similar movies can be generated in the public domain software SEDPHAT for user-determined parameters.

To show the principle, we plot only a subset of the interacting molecules of A (green triangles), B (blue squares), and AB (overlapping squares with triangles). Arbitrarily, to facilitate observation of the movement of a single particle, a single copy of A was painted orange, and a single copy of B was painted red. The molecules are linked by a sequence of interactions and exchange. Without loss of generality of the principle, we choose the initial particles such that all A is initially in the complex state. The total number of particles displayed is roughly proportional to their total concentration.

Even though in EPT the chemical reaction is assumed to be very fast, i.e. the lifetime of the complex is short relative to the characteristic transport time, to illustrate the concept of the coupled sedimentation it is necessary to assign finite lifetimes to the complexes. The lifetime of the free A and free B states then follows from the ratio of complex to free concentrations in the reaction boundary.

In the plot, the horizontal axis reflects distance in space (sedimentation to the right). The species' sedimentation velocities are visualized by their rate of horizontal movement. In contrast, the vertical direction in the cartoon is solely used to distinguish the different particles, without spatial meaning. Accordingly, the vertical velocity is only a visualization of the exchange of binding partners between particles. The vertical dotted line migrating to the right indicates the velocity of the reaction boundary.

Movie S1

In movie S1, the total concentrations of A and B are 1 K_D and 2.366 K_D, respectively, and mass action law was used to determine the total concentration of complex and free states of each species. The relative velocities of the species were fixed to va/vab = 0.2, and vb/vab = 0.6 (all in arbitrary units). Under these conditions, the system is at the phase transition line and there is no undisturbed boundary.

It can be discerned that the complex migrates faster than the average velocity of the reaction boundary, but when it dissociates both products free A and free B start falling behind. Since free B is slower than the complex but still faster than free A, it loses ground more slowly than free A. For this reason, it will stay free for longer time than free A. Free A has to recombine more quickly in order to catch up again with the reaction boundary. The quicker recombination of A enables a smaller copy number of A (as compared to B) to serve time in the complex and to co-migrate with the reaction boundary. This can be discerned from the fact that only 3 triangles are shared by 7 squares. The relative velocity of the reaction boundary $v_{a\cdots b}/v_{ab}$ is 0.707.

Movie S2

The second movie follows the same principles as described in the legend of movie S1, with the same species' velocities as in movie S1. However, the total concentrations of A and B are 4 K_D and 6 K_D, respectively. As in S1, this system is precisely at the phase transition line, and there is no undisturbed boundary. As a consequence of the higher concentration, the saturation of the complex is higher, i.e., the fractional time spent in the free state decreases for both A and B. Therefore, the velocity of the boundary is higher. Because A is not free as long as under the conditions of movie 1, it cannot as efficiently 'multi-task' to serve as binding partner for as many copies of B, and the stoichiometry is higher at 2/3. The velocity is higher than before, and $v_{a \cdots b}/v_{ab}$ is 0.800.

Movie S3

The third movie also follows the same principles as described in the legend of movie S1, with the same species' velocities as in movies S1 and S2. However, the total concentrations of A and B are now lower, with 0.5 K_D and 1.701 K_D, respectively. Again, this system is precisely at the phase transition line, and there is no undisturbed boundary. As a consequence of the lower concentration, most of the molecules stay free, and the complex occupies only a small fractional time. Therefore, a single copy of A can recombine with more copies of, with a stoichiometry of 0.29 (approximately 2/7). The velocity $v_{a \cdots b}/v_{ab}$ is 0.669.