

Lock and key colloids

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New functional materials can in principle be created using colloids that self-assemble into a desired structure by means of a programmable recognition and binding scheme. This idea has been explored by attaching ‘programmed’ DNA strands to nanometre^{1–3} and micrometre^{4,5} sized particles and then using DNA hybridization to direct the placement of the particles in the final assembly. Here we demonstrate an alternative recognition mechanism for directing the assembly of composite structures, based on particles with complementary shapes. Our system, which uses Fischer’s lock-and-key principle⁶, employs colloidal spheres as keys and monodisperse colloidal particles with a spherical cavity as locks that bind spontaneously and reversibly via the depletion interaction. The lock-and-key binding is specific because it is controlled by how closely the size of a spherical colloidal key particle matches the radius of the spherical cavity of the lock particle. The strength of the binding can be further tuned by adjusting the solution composition or temperature. The composite assemblies have the unique feature of having flexible bonds, allowing us to produce flexible dimeric, trimeric and tetrameric colloidal molecules as well as more complex colloidal polymers. We expect that this lock-and-key recognition mechanism will find wider use as a means of programming and directing colloidal self-assembly.

The three essential ingredients of our scheme are key particles, lock particles and the depletion interaction. The key particles are simple spheres made of silica, poly(methyl methacrylate) or polystyrene, with sizes depending on the targeted geometry of the final assembly and on the physicochemical properties that we want to build in (see Methods for synthesis details). The lock particles, a new kind of colloid with a single spherical cavity (Fig. 1), are produced through two consecutive polymerizations of a monodisperse silicon oil emulsion of 3-methacryloxypropyl trimethoxysilane (TPM)⁷. The synthesis protocol (sketched schematically in Fig. 1a) starts with the solubilization of TPM in water by slow hydrolysis, followed by rapid polymerization via a polycondensation reaction to give low-molecular-weight TPM oligomers. The oligomers phase separate to give charge-stabilized oil-in-water emulsion droplets that are grown to the desired size by seeded growth. A second polymerization of the oil phase, now acting on the acrylate moieties of the TPM oligomers, creates the cavities: the radical polymerization grows a rigid cross-linked shell around the droplets and simultaneously causes the contraction of the polymerizing liquid core, with the contraction driving a controlled shell buckling that results in spherical cavities forming on the particles (see Fig. 1b and c). All oil droplets buckle to form lock particles, and a typical synthesis yields approximately 0.1 g of particles per millilitre of reaction mixture. The method, which is described in more detail in the Methods, routinely produces bulk quantities of 20–500 ml or approximately 10^{10} – 10^{13} particles.

Short-range attraction between the surfaces of the lock and key particles turns the complementary geometry of our simple building blocks—a spherical key fitting into the cavity of a lock—into a site-specific lock-and-key interaction. We induce such a short-range attraction by adding a non-adsorbing water-soluble polymer—the

depletant—to the system, causing depletion interactions^{8,9} which have their origin in the entropy associated with the centre of mass of the polymers. That is, each colloidal particle is surrounded by an exclusion layer whose thickness is given by the radius r_p of a polymer molecule; the polymer is excluded from this region because its centre cannot approach the colloid surface any closer than r_p (Fig. 2a). If the surfaces of two colloidal particles come closer than $2r_p$, their exclusion volumes overlap and the total volume from which polymers are excluded decreases by the amount of their overlapping exclusion volumes ΔV . This increases the total volume available to the polymers by ΔV and hence increases the entropy of the polymers, which reduces the free energy of the system by:

$$\Delta F_d \approx k_B T n_p \Delta V \quad (1)$$

where n_p is the number density of polymers, T is the temperature, and k_B is Boltzmann’s constant. The free energy reduction ΔF_d associated with two particles coming together represents the binding energy due to the depletion interaction and is determined, according to equation (1), by the overlap volume ΔV and the number density of polymers n_p ,

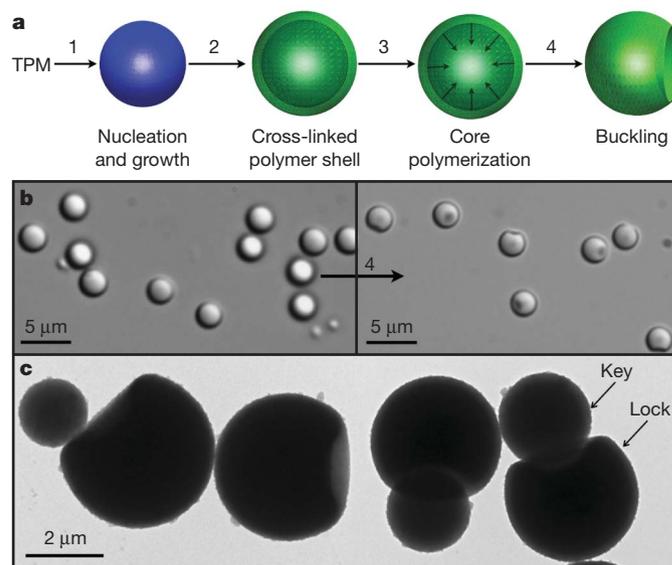


Figure 1 | Fabrication of lock particles. **a**, Diagram showing the synthetic steps involved in the preparation of particles with well-defined spherical cavities. Monodisperse silicon oil droplets are (1) nucleated from a homogeneous solution of hydrolysed 3-methacryloxypropyl trimethoxysilane monomer, and (2) encapsulated into cross-linked polymer shells. The liquid core (3) contracts when polymerized and (4) drives a controlled shell buckling that forms spherical cavities. **b**, This last step is easily followed by optical microscopy. After polymerization, cavities are visible as darker spots on the particles surfaces. **c**, The complementary fit between the locks and the spherical keys (here silica), is clearly visible in this transmission electron microscope image.

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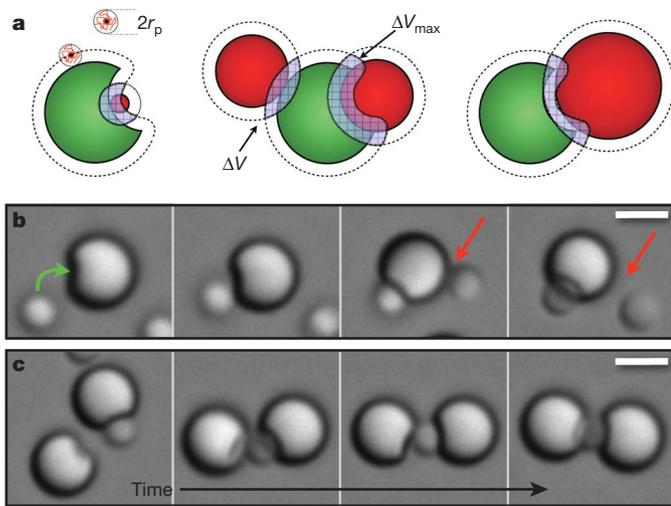


Figure 2 | Lock–key interactions. **a**, The depletion attraction potential between lock and key is proportional to the overlapping excluded volume ΔV , which attains a maximum ΔV_{\max} for the configuration in which the key particle, by virtue of its size and position, precisely fits into the spherical cavity of a lock particle. For all other configurations, $\Delta V < \Delta V_{\max}$. Because the depletion interaction is also proportional to the density n_p of the polymer depletant in solution, the interaction can be tuned by adjusting n_p so that it is sufficiently strong to bind two particles only for the lock-and-key configuration. **b, c**, Snapshots from a movie showing an example of depletion-driven self-assembly of lock and key particles. The site-specificity of the interactions is captured in sequence **b** in which arrows indicate examples of successful (green) and unsuccessful (red) lock–key binding. Scale bars, 2 μm .

while the range of the interaction is given by the polymer diameter $2r_p$. Figure 2a illustrates how spherical key particles of varying sizes interact with a given lock particle, with particles experiencing a depletion attraction when their surfaces come within $2r_p$ of each other. Clearly, the overlap of the exclusion volumes, and hence the depletion attraction, is maximized when the cavity of the lock particle is occupied by a spherical key particle whose radius matches that of the cavity.

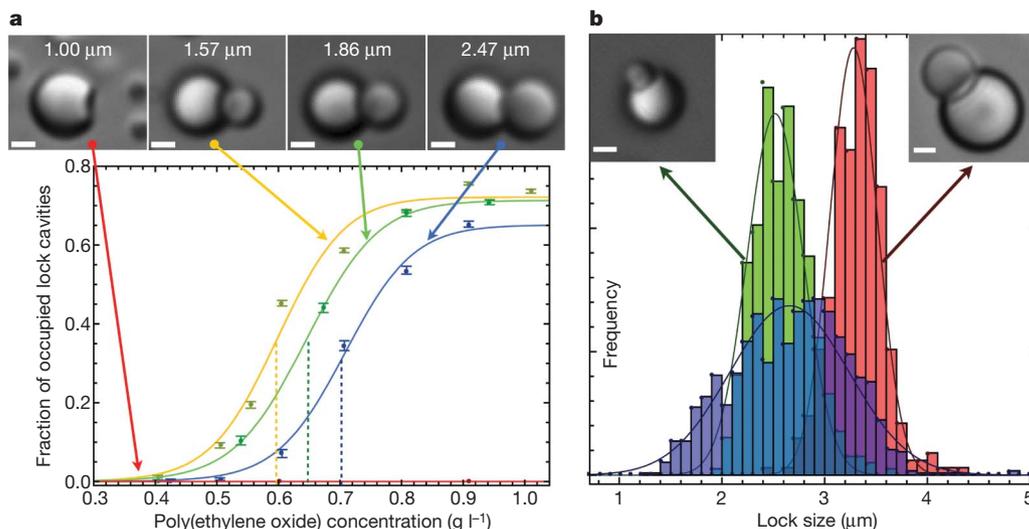


Figure 3 | Selectivity of the lock–key reversible binding. **a**, Experimentally measured lock–key association curves for different key particles along with fits to the model described in the text (see Supplementary Materials for more detail). Each point represents the ratio n_{LK}/n_L of the number of bound lock–key complexes to the number of lock particles in the sample. The values of n_{LK} and n_L were obtained by counting the number of locks and lock–key complexes in different areas of the samples, with $n_L \approx 10^3$ for a typical data point. The error bars reflect the statistical error $(n_{LK})^{0.5}/n_L$ associated with the finite data sample. The assembly is driven by depletion attractions and the lock–key selectivity is provided by the degree of their excluded volume overlap. 1.57- μm spherical keys maximize the overlap and assemble into lock

We demonstrate site-specific binding by mixing aqueous suspensions of lock and key particles, and using poly(ethylene oxide) with a molecular weight of 600,000 as the depletant polymer, unless stated otherwise. Optical microscopy observations reveal that key particles dock and bind only to the cavity of the lock particle. This behaviour is captured in the sequence of movie frames presented in Fig. 2b: two key particles approach a single lock particle by random Brownian motion; the particle encountering the concave cavity of the lock particle docks and binds while the particle that encounters the convex exterior fails to bind. (Supplementary Movie 1 shows the capture of the key by the lock particle and the subsequent motion of the bound lock-and-key pair in full.) The exposed surface of the key particle can accommodate the docking of a second lock particle, as illustrated in Fig. 2c. Docking of more than two lock particles is possible as well, a point we shall return to shortly.

This site-specific binding is reversible and can be controlled by varying the depletant concentration n_p and thereby the binding energy; see equation (1). The measured equilibrium fraction of bound lock-and-key pairs as a function of depletant concentration for four different sizes of key particles in Fig. 3a shows that there is no binding at low depletant concentration, where the depletion interaction is not sufficiently strong to overcome the electrostatic repulsion and the loss of configurational entropy that occurs when two particles bind. As the concentration is increased past a critical value n_p^c , binding occurs and the fraction of bound particles increases with increasing depletant concentration.

An essential requirement for programmable self assembly is selectivity, namely the ability of basic building blocks to recognize the right pieces with which to bind in a ‘sea’ of foreign objects. Selectivity in our system is driven by geometry, in that surfaces that fit together maximize the overlap of excluded volume and their binding is thus favoured. To investigate selectivity we therefore quantify the influence of size mismatch on lock-and-key binding, by measuring the number of bound lock-and-key complexes as a function of depletant concentration for a given lock size and three different key sizes. As expected, the concentration of depletant required to observe binding is lowest when the radii of the sphere and cavity are well matched (Fig. 3a,

cavities at the lowest depletant concentration (dashed yellow line). For a poor match the assembly occurs at higher depletant concentration; however, while the overlapping volume of large (2.47 μm) keys in contact with cavity rims is still sufficient to give specific lock–key binding, smaller keys (1 μm) do not bind at all. Insets show the different sizes of keys. **b**, Two different sets of monodisperse keys ($2R = 1.54 \mu\text{m}$ and $2.47 \mu\text{m}$; see insets), each mixed with a polydisperse population of locks (blue), selectively bind to their best-matching complementary particles. As a result, the lock particle size distribution in the assembled lock–key complexes (green and red) peaks at two different values. Scale bars, 1 μm .

$2R = 1.57 \mu\text{m}$). As mismatch increases, the concentration of depletant required for binding also increases. However, the shift on either side of ideal matching is not symmetric: small keys (Fig. 3a, $2R = 1.00 \mu\text{m}$) fail to bind for relatively small deviations from ideal size matching, whereas larger keys (Fig. 3a, $2R = 1.86 \mu\text{m}$, $2.47 \mu\text{m}$) exhibit lock-and-key binding well above ideal size matching. This asymmetry can be understood simply in terms of the overlap of excluded volume, which is significantly larger for a large sphere in contact with the lock cavity rim than it is for a small sphere inside the lock cavity (see Fig. 2a).

We also prepared two suspensions containing the same polydisperse distribution of lock particles. Because the cavity radii scale with lock particle size, these suspensions have a polydisperse distribution of cavity radii. We added monodisperse spherical keys with a diameter of $1.54 \mu\text{m}$ to one suspension and monodisperse keys with a diameter of $2.47 \mu\text{m}$ to the other, then progressively increased the concentration of depletant to n_p^c and measured the size distribution of locks that were bound to keys. As shown in Fig. 3b, keys with diameters of $1.54 \mu\text{m}$ and $2.47 \mu\text{m}$ bind to two distinct lock populations centred at lock diameters of $2.59 \mu\text{m}$ and $3.32 \mu\text{m}$, respectively, thus further illustrating the selectivity of the lock–key binding.

The binding and unbinding of locks L and keys K can be modelled by considering the change in free energy associated with the reaction $L + K \leftrightarrow LK$, where LK represents a bound lock–key complex. Locks and keys have a purely entropic free energy associated with their configurations within the sample volume $V^{10,11}$. The lock–key complexes are bound with energy E_b and the configurational entropy of their centres of mass is similar to that of an unbound lock or key, but in addition they have a configurational entropy associated with fluctuations in relative position within a small binding volume V_b .

Treating the unbound and bound species as dilute gases and equating the corresponding chemical potentials (see Supplementary Materials), we obtain the law of mass action for our reaction:

$$\frac{n_{LK}n_0}{n_L n_K} = e^{-[E_b - k_B T \ln(V_b/n_0)]} \quad (2)$$

where n_0 is the total number density of locks and n_L , n_K and n_{LK} are the equilibrium densities of unbound locks, unbound keys and bound lock–key complexes. The binding energy between locks and keys, E_b , minimizes the sum of the attractive depletion interaction and the repulsive screened Coulomb interaction between the negatively charged locks and keys. For binding to occur, E_b must be

attractive and large enough to overcome the loss in configurational free energy that accompanies the binding of two particles.

The measured fraction of bound locks and keys n_{LK} is consistent with the binding energy E_b calculated with a simple model, which approximates the depletion and Coulomb interactions as occurring between flat plates with an effective area that depends on how well the lock and key surfaces match. With a Coulomb potential given by the measured zeta potential of the particles (70 mV) and the salt concentration, and a depletant radius of 57 nm that corresponds to the radius of gyration of the polymer used, the remaining energetics are determined only by geometry. Although precise values are not known a priori, fitting the model results to the experimental data gives geometrical factors that are consistent with the size and goodness of the match between the locks and keys (see Supplementary Materials for details).

The lock and key particles used thus far are charge-stabilized, which produces a soft repulsive screened Coulomb potential competing with the depletion attraction. Preparing locks and keys with a grafted layer of hydrophilic polymer on their surfaces leads to short-range, nearly hard-sphere steric repulsion. Figure 4a compares binding curves for identical lock and key particles, but stabilized either by charge or by a layer of Pluronic F108 copolymer grafted onto the particle surfaces. The data show that the transition in the sterically stabilized system is sharper and occurs at a significantly lower concentration of depletant (0.28 g l^{-1} versus 0.61 g l^{-1}), demonstrating that the lock-and-key interaction can be tuned to a considerable extent.

Controlling the binding and unbinding of particles is essential for manipulating their self-assembly, and temperature is a more convenient control parameter than depletant concentration. We therefore demonstrate that microgel particles that change size when temperature is changed can also be used as the depletant in our system and thus provide direct control over the range and depth of the depletion potential (by changing ΔV in equation (1)). We use poly(*N*-isopropylacrylamide) (pNIPAM) particles¹² which, in their swollen hydrated state at room temperature, have a size close to that of the previously used poly(ethylene oxide) polymer so that lock-and-key binding occurs at a similar depletant concentration. When heated above their lower critical solution temperature (about 40°C), the microgel particles shrink (Fig. 4b, main panel) sufficiently that the

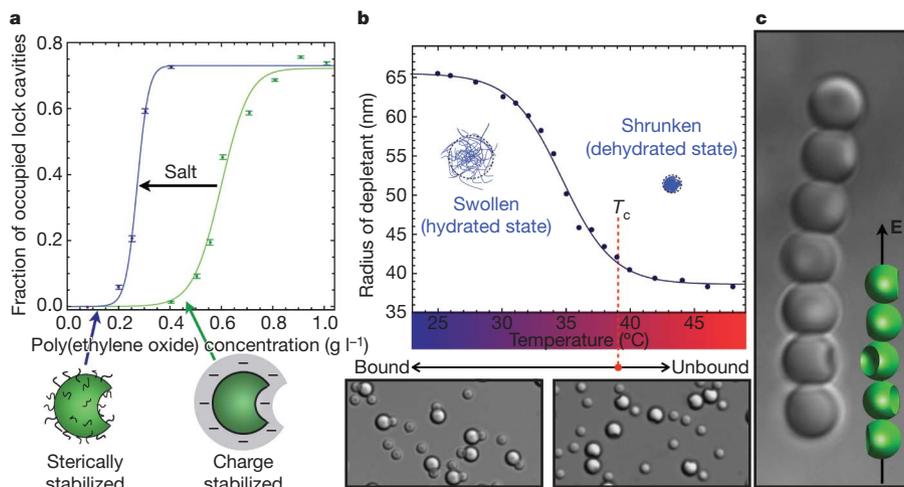


Figure 4 | Temperature-controlled lock-key self-assembly and electric field manipulation. **a**, Observed dependence of lock–key binding on depletant concentration, with error bars reflecting the statistical error $(n_{LK})^{0.5}/n_L$ associated with the finite data sample. When the concentration of salt is increased to the point that it is necessary to add a steric layer (Pluronic F108, average relative molecular weight 14,600) to the particles to prevent aggregation, a significantly lower depletant concentration is needed to drive the assembly, resulting in a shifted and sharper lock–key binding transition.

b, Thermosensitive microgel particles can be used as a depletant to implement a simple temperature switch for the lock–key binding reaction (bound; see left inset). At the transition temperature T_c the depletant shrinks to a size too small to give an effective lock–key attraction and the key particles are released (unbound; see right inset). **c**, The anisotropic shape of the lock particles allows the use of an external electric field E to control the orientation of the lock cavities and to assemble lock particles in ‘daisy-chain’-like structures.

range and depth of the attractive depletion potential falls below that required for binding. As a result, all locks release their bound keys (Fig. 4b, images at bottom; see also Supplementary Movie 2). Particle assembly can also be controlled by an externally applied electric field: as illustrated in Fig. 4c, the shape-anisotropic lock particles organize into chains with all cavities arranged perpendicularly to the field.

A striking and unique feature of our lock-and-key assemblies is that the bonds between particles are flexible. Spherical keys fitting inside matching lock cavities form ball-and-socket joints held together by the depletion force. The absence of chemical bonds at the junction allows keys to rotate within the cavity of their locks. When two or more locks are bound to a single key, this rotation becomes visible under an optical microscope (see Fig. 5, and Supplementary Movies 3 to 5). The assembly of monomers, dimers, trimers and tetramers occurs simply by progressively increasing the size of the central key particle to make room for extra locks to dock (Fig. 5 and Supplementary Fig. 1). This flexibility provides an additional degree of freedom that, together with the reversible nature of the binding, facilitates annealing during the assembly of larger structured aggregates. In the absence of spherical keys, locks with sufficiently large cavities assemble in a head-to-tail fashion into polymeric worm-like chains that diffuse and grow in solution (see Fig. 5d and Supplementary movie 5), provided the depletant concentration is sufficiently high to drive binding. Many other configurations are possible as well, including snowman-like particle assemblies that form when monodisperse keys are mixed with two different sets of locks (Fig. 5c).

Taken together, our observations demonstrate that the self-organization of colloidal particles by means of directional, selective and reversible interactions can be essentially reduced to a simple geometrical problem. This allows the lock-and-key interactions to direct the assembly of basic building blocks regardless of their composition and surface chemistry; this should offer unprecedented opportunities for engineering 'smart' composite particles, new functional materials and microscopic machinery with mobile parts.

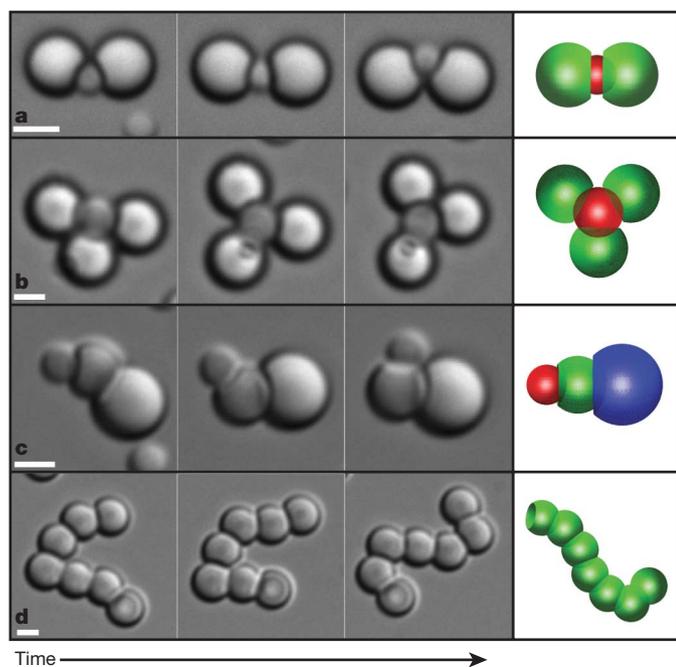


Figure 5 | Flexibility of the lock-key junctions in self-assembled colloidal molecules and polymers. Time-lapse optical microscopy images (left three columns), and schematics (rightmost column), show the flexibility of lock-key bonds in various assemblies (a–d), which are confined to two dimensions by being placed on a glass microscope slide. The absence of irreversible chemical bonds between the building blocks allows these ball-in-socket joints to move freely. Scale bars, 2 μm .

METHODS SUMMARY

The precursor emulsion for the synthesis of lock particles is prepared by a modification of a hydrolysis and polymerization method originally described in ref. 7. Typically, 750 μl of TPM are first hydrolysed in 15 ml of deionized water and then mixed to 30 ml of aqueous NH_3 at 0.4M. The ammonia catalyses a polycondensation reaction yielding low-molecular-weight TPM oligomers that in water rapidly phase-separate, forming a charge-stabilized emulsion. The resulting oil-in-water emulsion droplets are grown to the desired final size by feeding the emulsion with a solution of hydrolysed TPM at 0.2M. To form the cavities, the emulsion is further polymerized by adding a radical initiator (potassium persulphate, $[\text{KPS}] = 0.45 \text{ mM}$) and heating at 75 $^\circ\text{C}$ for 6 h. Specially modified locks with a polyacrylamide coating are prepared to prevent aggregation when mixed with pNIPAM particles. The coating is formed by mixing 20 ml of the lock suspension with 80 mg of acrylamide and 20 mg of KPS and heating at 75 $^\circ\text{C}$ for 3 h. Key particles of silica are prepared by the Stöber method¹³. Poly(methyl methacrylate) and styrene particles are prepared by a standard surfactant-free emulsion polymerization¹⁴. pNIPAM particles were synthesized according to the method described in ref. 15. After preparation, all the colloidal systems are washed and redispersed in deionized water. The binding curves in Fig. 3 are obtained by measuring the fraction of lock-key complexes recorded in a series of microscopy images after an equilibration time of 30 min. Thermo-reversible lock-key binding experiments are performed by gluing samples on heated microscope slides coated with indium tin oxide. Electric-field-induced lock assembly is performed by bringing lock suspensions in contact with two parallel indium electrodes 200 μm apart with a typical alternating-current electric potential of 20 V at 50 kHz.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions S.S. designed the lock synthesis, synthesized all the colloidal systems, designed and performed the experiments and analysed data. W.T.M.I. designed the experiments, performed field manipulation experiments, analysed data and theoretically modelled the system. D.J.P. and P.M.C. conceived of the depletion-induced colloidal lock-and-key interaction, initiated and supervised the research. The manuscript was written by S.S., W.T.M.I. and D.J.P.

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