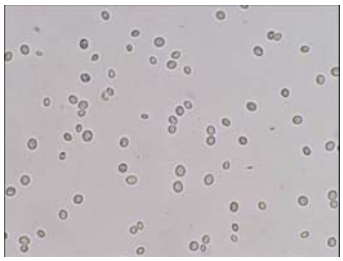
Supporting Online Material

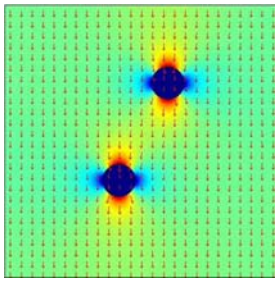
On-Chip Dielectrophoretic Co-Assembly of Live Cells and Particles into Responsive Biomaterials

Shalini Gupta[†], Rossitza G. Alargova[‡], Peter K. Kilpatrick[§] and Orlin D. Velev*

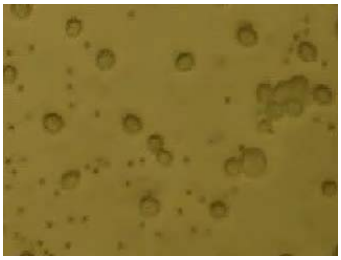
Description of Movies



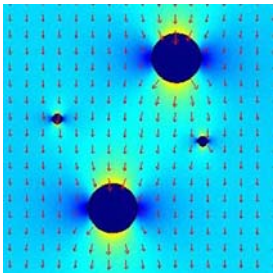
Movie 1: Electric field directed assembly of cells into chains. AC electric field of 13 V mm^{-1} and 100 Hz between two parallel coplanar electrodes applied across a suspension of 0.01 % w/v *S. cerevisiae* yeast cells in PBS. The cells align into chains parallel to the electric field. This movie has been speeded up 40×.



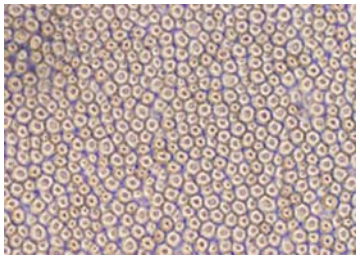
Movie 2: Numerical simulation of cell chain assembly in electric field. 2D electrostatic computation of effective yeast cell polarizability in PBS performed using FEMLAB. The electric field distribution around the cells illustrates how cells arrange into chains collinear to the applied field due to positive dielectrophoresis.



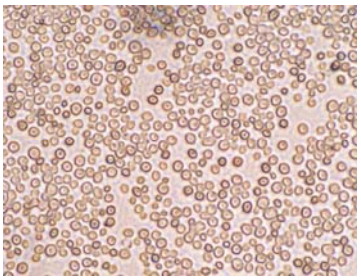
Movie 3: Experimentally observed co-assembly of yeast cells and latex particles. AC electric field of 10 V mm^{-1} and 30 Hz between two parallel coplanar electrodes applied across a mixture of 0.1 % w/v yeast cells and 0.1 % w/v 1 μm sulfate stabilized latex microspheres in DI water. The cells and particles align alternatively along the field direction. This movie has been speeded up 8×.



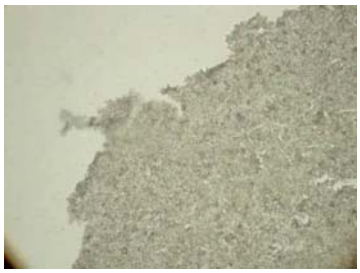
Movie 4: Numerical simulation of a two cell – two particle co-assembly process. 2D electrostatic computation of dynamic assembly of yeast cells and 1 μm latex spheres in DI water performed using FEMLAB. The electric field distribution and effective polarizabilities show how one of the latex microspheres gets entrapped between the cells due to positive dielectrophoresis.



Movie 5: 2D array formation of cells using 4-point electrodes. Yeast cells (0.08 % w/v) aligned alternatively in two orthogonal directions using an AC electric field of 75 V and 30 Hz in the four-electrode chip. The cells assemble into 2D arrays. The movie was taken over 30 min at 1 frame min^{-1}.



Movie 6: Sedimentation of cells in the absence of electric fields. No 2D array structure was formed when yeast cells (0.08 % w/v) were allowed to freely sediment under gravity in the absence of electric field. The movie was taken over 30 min at 1 frame min^{-1}.



Movie 7: 3D manipulation of permanent cell membrane. Manipulation of a freely suspended, single-cell thick, permanent yeast cell membrane in an external magnetic field in real-time.

Movie 8: Folding of permanent cell membrane. Folding of a permanent yeast cell membrane controlled with a small magnet (not seen) in real-time.

(B) Coupling Protocols for Protein Conjugation to Colloidal Particles

1. Conjugation of FITC-labeled Concanavalin A Lectin to 1.0 μm Fluorescent Aldehyde/Sulfate Latex Microspheres (Ref.: protocol adapted from "*Coupling of Proteins to IDC UltraClean Aldehyde/Sulfate Latex*", Interfacial Dynamics Corp., OR)

The following reagents were purchased from Interfacial Dynamics Corp. (OR): 0.025 M phosphate buffered saline at pH 6.2 or PBS-I, 0.1 M phosphate buffered saline at pH 7.2 or PBS-II, storage buffer containing 0.1 M phosphate buffered saline at pH 7.2.

Preparation of latex suspension. 0.5 mL of 2 % w/v aldehyde/sulfate latex microspheres in DI water were homogeneously mixed with 1 mL of PBS-I. The solution was centrifuged at 3000g for 20 min and the pellet was resuspended in 1 mL of PBS-I. Recentrifugation of the solution at 2700g for 20 min and resuspension in 0.5 mL PBS-I resulted in a final particle concentration of 2 % w/v.

Functionalization of latex microspheres with Concanavalin A lectin. A 0.1 % w/v lectin solution in 0.5 mL of 1:1 v/v PBS-I and PBS-II mixture was added to the above 2 % w/v latex suspension in 0.5 mL PBS-I. The lectin concentration was kept in excess to ensure complete coverage of the particle surface. The latex-lectin mixture was incubated overnight with constant gentle mixing at room temperature. The mixture was centrifuged at 3000g for 20 min to remove any unbound protein. The pellet was resuspended in 10 mL of PBS-II and the process was repeated twice. The lectin-functionalized latex microspheres were finally resuspended in 1 mL of storage buffer to obtain ≈ 1 % w/v particle concentration. The suspension was stored at 4°C until further use.

2. Chemical Conjugation of FITC-labeled Concanavalin A Lectin to 1.8 μm Amine-Terminated Magnetic Iron Oxide Particles (Ref.: protocol adapted from data Sheet #546 provided by Bangs Laboratories, Inc., IN)

All reagents used were purchased from Bangs Laboratories Inc. (IN) and are listed in Table S1.

Activation of magnetic particles. 5 mL of amine-terminated 1.8 μm magnetic particles were vigorously mixed with 20 mL of Coupling Buffer. The mixture was washed three times by centrifugation at 8000g for 10 min and resuspension of the pellet in 20 mL of Coupling Buffer. 10 mL of 5 % w/v glutaraldehyde was added to the suspension and the mixture was then rotated at room temperature for 3 h. The magnetic particles were pulled to one side perpendicular to the gravity using an external magnet and the unreacted glutaraldehyde was replaced by the Coupling Buffer. The mixture was then washed three times at 8000g for 10 min and the particles were finally suspended in Coupling Buffer to obtain a concentration of ≈ 1 % w/v.

Functionalization of magnetic microspheres with Concanavalin A lectin.

A 0.4 % w/v lectin solution was prepared fresh in 2 mL of Coupling Buffer. 75 μL of this solution was diluted with 1 mL of Coupling Buffer and set aside for coupling efficiency determination after labeling as 'Pre-Coupling Solution'. The remaining was added to the above 20 mL of 1 % w/v glutaraldehyde-activated magnetic particles in Coupling Buffer. The mixture was shaken vigorously and rotated for 16 - 24 h at room temperature. The magnetic particles were pulled to one side perpendicular to the direction of gravity using an external magnet and the supernatant was separated. The supernatant was labeled 'Post-Coupling Solution' and set aside for coupling efficiency determination. 10 mL of Glycine Quenching Solution was added to the suspension. The mixture was shaken vigorously and then rotated for 30 min at room temperature.

Washing and dilution of the conjugated magnetic particles. The particles were magnetically separated and the supernatant was aspirated. 100 mL of Wash Buffer was added and the suspension was shaken vigorously. The particles were again magnetically separated perpendicular to gravity and the supernatant was removed. This supernatant was added to the 'Post-Coupling Solution' for coupling efficiency determination. The above steps were repeated 3 times and the lectin-conjugated particles were finally stored in Wash Buffer at 4°C at a concentration of ≈ 1 % w/v.

Coupling efficiency. Absorbances of the Pre- and Post-Coupling Solutions with respect to a blank Coupling Buffer solution were measured using a Jasco V550 spectrophotometer at 280 nm wavelength. The coupling efficiency for lectin uptake was calculated to be > 60% using the formula shown below where D is the Dilution Factor.

$$\frac{OD_{280}\text{Pre-coupling solution x } (D_1) - OD_{280}\text{ Post-coupling supernatant x } (D_2) \text{ x } 100}{OD_{280}\text{Pre-coupling solution x } (D_1)}$$

Table S1. A list of reagents used for conjugating Concanavalin A lectin to amine-terminated magnetic iron oxide particles.

Solution	Composition	Materials	Preparation Instructions
Coupling Buffer	0.01 M pyridine	0.8 mL pyridine	Add 0.8 mL pyridine to 900 mL distilled water. Adjust to pH 6.0 with 6 N HCl. Fill to 1 L with water.
Glutaraldehyde	5 % glutaraldehyde	5 mL 25% glutaraldehyde	In a hood, add 5 mL of glutaraldehyde to 20 mL Coupling Buffer.
Glycine Quenching Solution	1 M glycine	7.5 g glycine	Dissolve 7.5 g glycine in 90 mL distilled water and adjust to pH 8.0 with 10 N NaoH. Fill to 100 mL with water.
Wash Buffer	0.01 M Tris 0.1 % NaN_3 0.1 % w/v BSA 0.15 M NaCl 0.001 M EDTA	1.21 g Tris 1.0 g NaN_3 1 g BSA 8.7 g NaCl 0.37 g EDTA	Dissolve solids in 900 mL distilled water. Adjust to pH 7.4 with 10 N NaOH or 6 N HCl as required. Fill to 1 L with water.

3. Physical Adsorption of Fibronectin to 1.0 μm Sulfate-Stabilized Latex Microspheres (Ref.: "*Working with FluoSpheres Fluorescent Microspheres*" from Molecular Probes, OR)

0.5 mL of 1.0 μm sulfate-stabilized latex suspension (1 % v/w in DI water) was washed twice in DI water by centrifuging at 3000g for 20 min. 10 μL of 0.1 % w/v fibronectin solution was homogeneously added to the latex suspension. The mixture was vortexed for 1.5 h and then refrigerated overnight. The unbound fibronectin was removed by centrifuging the suspension at 3000g for 20 min and replacing the supernatant with 0.1 mM PBS solution. 5 μL of 10 % w/v bovine serum albumin (BSA) was added to the final suspension to prevent non-specific aggregation of the fibronectin-conjugated latex particles.

(C) Polarizability Calculations Using Multi-Shell Models

Table S2. Numerical parameters for calculating effective polarizability of yeast (*S. cerevisiae*) cells. The values of these parameters were taken from Ref. [35, 56].

Cytoplasm dielectric constant	ε_2	5.31×10^{-10}	$C^2\ J^{-1}\ m^{-1}$
Cytoplasm conductivity	σ_2	0.5	$S\ m^{-1}$
Membrane capacitance	c_m	0.01	$F\ m^{-2}$
Membrane transconductance	g_m	0	$S\ m^{-2}$
Cell wall dielectric constant	ε_1	5.75×10^{-10}	$C^2\ J^{-1}\ m^{-1}$
Cell wall conductivity	σ_1	0.1	$S\ m^{-1}$
Inner radius	R	2.0×10^{-6}	m
Outer radius	R_o	2.5×10^{-6}	m
Double layer thickness	Δ	3.0×10^{-9}	m
Debye length	κ^{-1}	9.60×10^{-9}	m
Zeta potential	ζ	-30	mV
Counter-ionic layer dielectric constant	ε_{EDL}	1.66×10^{-12}	$C^2\ J^{-1}\ m^{-1}$
Counter-ionic layer conductivity	σ_s	8.61×10^{-5}	$S\ m^{-1}$
Medium dielectric constant	ε_m	6.9×10^{-10}	$C^2\ J^{-1}\ m^{-1}$
Medium conductivity	σ_m	0.017	$S\ m^{-1}$
Angular frequency	ω	628	$Rad\ s^{-1}$
Effective cell dielectric constant	ε_1''	1.48×10^{-8}	$C^2\ J^{-1}\ m^{-1}$
Effective cell conductivity	σ_1''	0.039	$S\ m^{-1}$

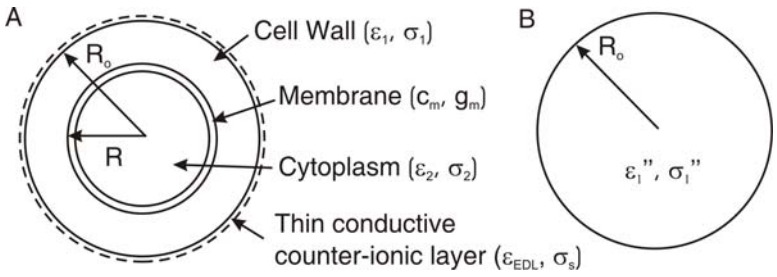


Figure S1. (A) Multi-shell model for the polarizability of yeast cell. **(B)** Simplified model of yeast cell with effective polarizability equivalent to the multi-shell type structure in (A).

We developed a numerical procedure for evaluating the effective polarizability of yeast cells as a function of frequency. In this model, the multi-shelled structure of the yeast cell was substituted by a homogeneous sphere of complex permittivity $\underline{\varepsilon_1}'' = \varepsilon_1'' - \frac{j}{\omega}\sigma_1''$ (Figure S1B), the effective polarizability of which was equivalent to that of the original yeast cell (Figure S1A). The procedure includes the following steps -

1. The complex permittivities of the various shells comprising the yeast cell were expressed

Cytoplasm: $$\underline{\varepsilon_2} = \varepsilon_2 - \frac{j}{\omega}\sigma_2 \qquad (1)$$

Membrane: $$\underline{c_m} = c_m - \frac{j}{\omega} g_m \qquad (2)$$

Cell wall: $$\underline{\varepsilon_1} = \varepsilon_1 - \frac{j}{\omega}\sigma_1 \qquad (3)$$

Counter-ionic layer: $$\underline{\varepsilon_{EDL}} = \varepsilon_{EDL} - \frac{j}{\omega}\sigma_s \qquad (4)$$

2. The complex permittivities of the cytoplasm and the membrane were replaced with a homogeneous sphere of equivalent complex permittivity.
3. The complex permittivities of the equivalent homogeneous sphere in step 2 and the cell wall were replaced with a homogeneous sphere of equivalent complex permittivity.
4. The complex permittivities of the equivalent homogeneous sphere in step 3 and the thin counter-ionic layer were replaced with a homogeneous sphere of equivalent complex permittivity.

The final expression for the complex permittivity of the yeast cell in terms of the shell parameters is given by equation (5). The values of each parameter are given in Table S2.

$$\underline{\varepsilon_1}'' = \underline{\varepsilon_1}\left[\frac{\left(\frac{R_o}{R}\right)^3 + 2\left(\frac{\underline{\varepsilon_2}' - \underline{\varepsilon_1}}{\underline{\varepsilon_2}' + 2\underline{\varepsilon_1}}\right)}{\left(\frac{R_o}{R}\right)^3 - \left(\frac{\underline{\varepsilon_2}' - \underline{\varepsilon_1}}{\underline{\varepsilon_2}' + 2\underline{\varepsilon_1}}\right)}\right] + \underline{\varepsilon_{EDL}} \qquad (5)$$

where, $\underline{\varepsilon_2}' = \dfrac{\underline{c_m} R \underline{\varepsilon_2}}{\underline{c_m} R + \underline{\varepsilon_2}}$, $\varepsilon_{EDL} = \dfrac{2\Delta\varepsilon_m}{R}$ and $\sigma_s = 2\sigma_m R_o^{-1} \kappa^{-1} \exp\left(\dfrac{ze\xi}{2kT} - 1\right)$

Table S2. Numerical parameters for calculating the effective polarizability of 1 μm latex particles. The values of these parameters were taken from Ref. [56] and from the information data sheet provided by the vendor, Interfacial Dynamics Corp. (OR).

Particle dielectric constant	ε_p	2.26×10^{-11}	$C^2\ J^{-1}\ m^{-1}$
Particle conductivity	σ_p	1.0×10^{-15}	$S\ m^{-1}$
Particle radius	R_p	5.0×10^{-7}	m
Medium dielectric constant	ε_m	6.95×10^{-10}	$C^2\ J^{-1}\ m^{-1}$
Medium conductivity	σ_m	0.017	$S\ m^{-1}$
Surface charge density	γ	9.5×10^{-2}	$C\ m^{-2}$
Sodium ion mobility	μ_{Na}	5.24×10^{-8}	$m^2\ S\ C^{-1}$
Particle surface conductivity	σ_s	0.02	$S\ m^{-1}$
Effective particle conductivity	σ_p'	0.02	$S\ m^{-1}$

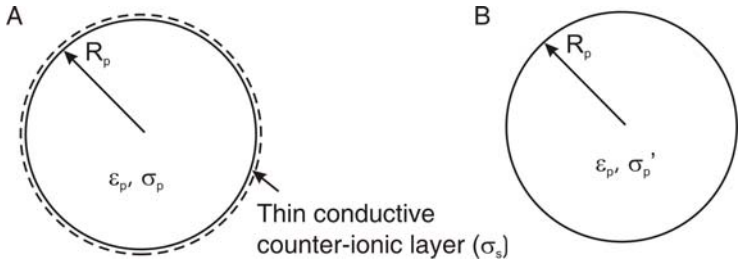


Figure S2. Models of latex microspheres in aqueous media: **(A)** Thin conductive counter-ionic layer surrounding the latex microsphere. **(B)** Simplified structure with effective polarizability equivalent to the shell type structure in (A).

A model similar to the one described above for yeast cells was used to calculate the effective polarizability of latex particles in Figure S2A. The equivalent conductivity of the simplified structure in Figure S2B was given by equation (6).

$$\sigma_p' = \sigma_p + \sigma_s \qquad (6)$$

where $\sigma_s = \frac{\gamma \mu_{Na}}{R}$.