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Supporting Information

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Triggered Sorting and Co-Assembly of Genetically Engineered Protein Microdomains in the Cytoplasm

*Pu Shi, Yi-An Lin, Martha Pastuszka, Honggang Cui, and J. Andrew MacKay**

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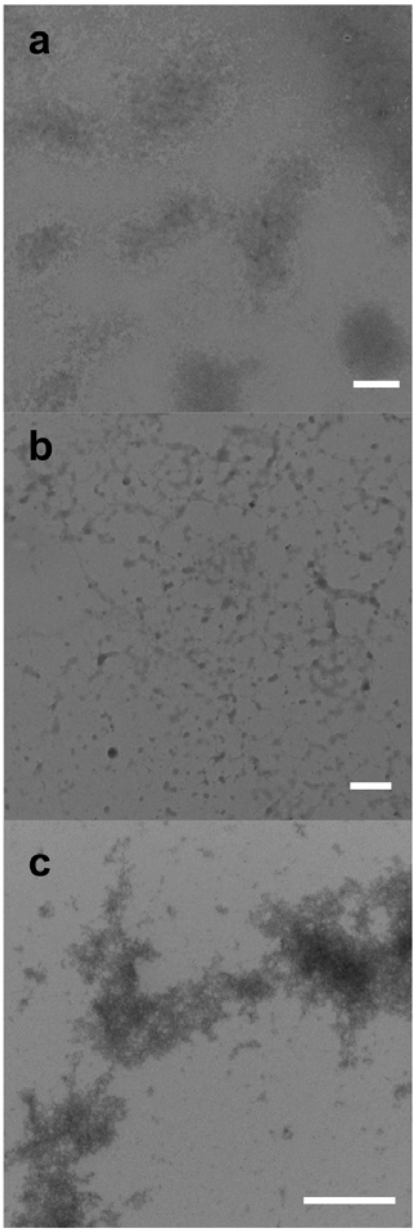
Supporting Information

S1. Negative staining TEM imaging confirms that ELP monoblocks assemble large Genetically Engineered Protein Microdomains (GEPMs).

Unlike the diblock ELP S48I48, which forms small uniform particles (Figure 1e), ELP monoblocks I24, V96 and V192 form large polydisperse objects when dried above their transition temperature, which can be imaged using negative staining TEM. The size and shape of these structures vary widely, which is consistent with their observation using confocal imaging (Figure 2).



Supplementary Figure S1. Micron-scale GEPMs assembled by monoblock ELPs can be observed using negative staining TEM imaging. Using 2% uranyl acetate negative staining, large GEPMs formed by ELP monoblocks were imaged by regular TEM imaging. **a, b** and **c** are I24, V96 and V192 (100μM, in DI H_2O) samples respectively. Bar length = 500nm.

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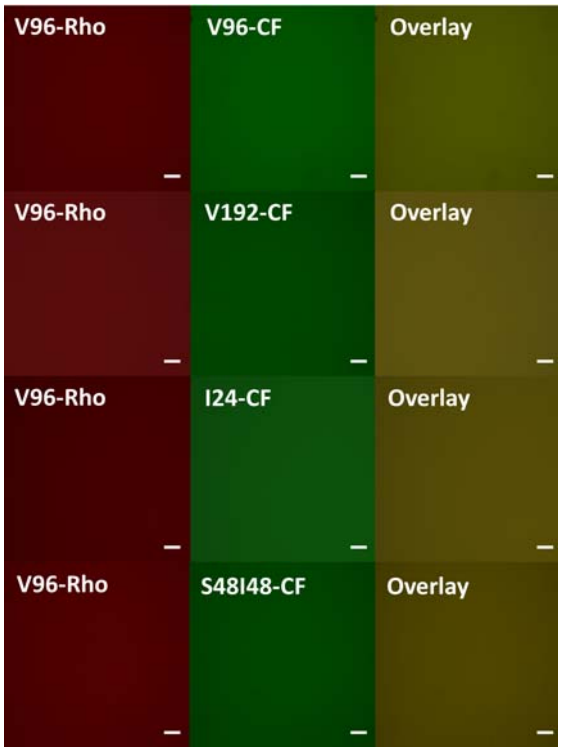
S2. Rhodamine (Rho) and carboxyfluorescein (CF) labeled ELPs are uniformly dispersed below their transition temperature (T_t) *in vitro*

When imaged *in vitro* below their common T_t (28 ^{o}C), ELP samples in all four groups were uniformly mixed and highly colocalized (Supplementary Figure S2); however, they did not form genetically engineered protein microdomains (GEPMs) until raised above this assembly temperature (Figure 2). The un-transitioned Rho and CF labeled ELP samples showed diffuse red and green fluorescence respectively.



Supplementary Figure S2. Confocal imaging of mixtures of Rho and CF labeled ELPs below the matched transition temperature. Mixed in a glass bottom dish, the Rho and CF labeled ELP samples emitted diffuse red and green fluorescence respectively lacking any formation of GEPM structures. The yellow overlay fluorescence confirms the two samples were uniformly mixed prior to heating (Figure 2). Scale bar = 50 µm.

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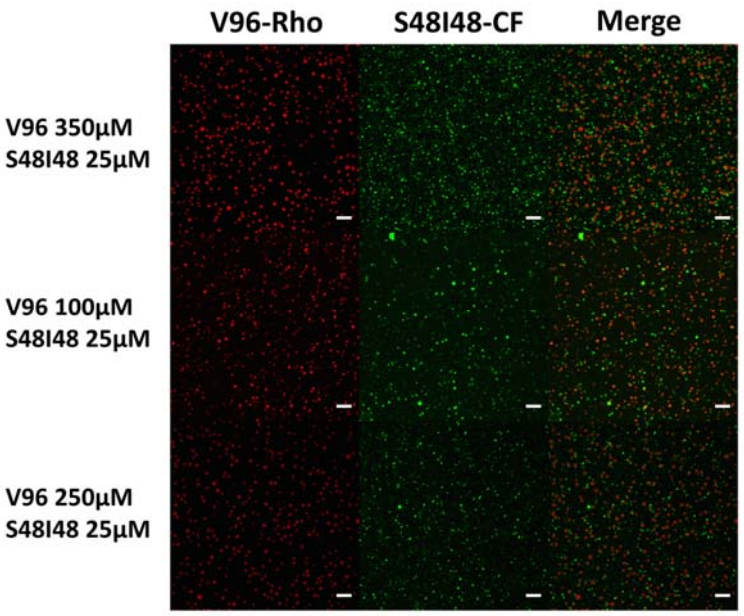
S3. Spatial sorting between GEPMs formed by monoblock and diblock ELPs is conserved over a range of mixing concentrations.

Besides the ratio tested in Figure 2 (V96: S48I48 = 250 μM: 25 μM), two additional mixing ratios of monoblock and diblock ELPs (V96: S48I48 = 350 μM : 25 μM and 100 μM : 25 μM) were examined to discover whether *in vitro* self-sorting was concentration dependent. As shown in Supplementary Figure S3, the other two mixing ratios (top two rows) show similar spatial self-sorting of GEPMs. Different from the original mixing ratios that were selected to match the T_t (28 ^{o}C), in these two new mixing ratios the ELPs transition at slightly different temperatures. 250 μM V96 and 25 μM S48I48 both phase separate at 28 ^{o}C. Using the regression line for the correlation of concentration and T_t, the T_t of V96 at 350 μM and 100 μM are 27.7 and 29.7 ^{o}C respectively.



Supplementary Figure S3. Self-sorting between monoblock and diblock GEPMs is concentration independent. Confocal imaging was used to observe mixtures of ELPs *in vitro*. While holding the concentration of the diblock S48I48-CF constant (25 μM), three concentrations of V96-Rho (100, 250, and 350 μM) were evaluated. In all three mixing ratios, monoblock and diblock ELPs assembled into distinct GEPMs, which suggests that the spatial sorting properties of these ELPs are relatively concentration independent. Scale bar = 10 μm.