Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information for:

Temperature-regulated protein adsorption on a PNIPAm layer

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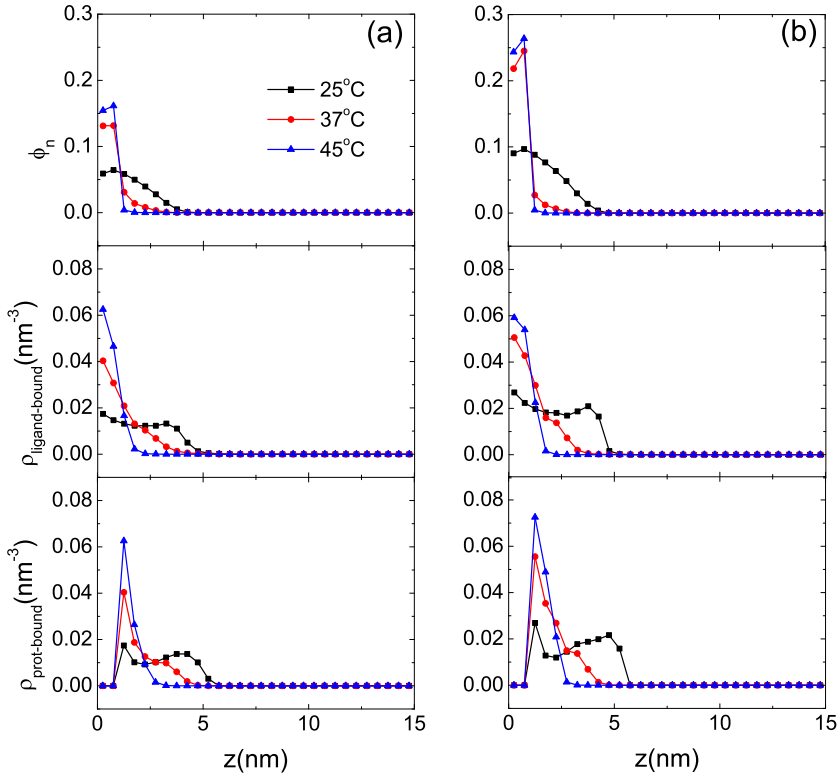

Fig. S1: Polymer volume fraction ϕ_n, number density of bound ligands $\rho_{ligand-bound}$, and number density of bound proteins $\rho_{prot-bound}$ (determined using the centers of the proteins) as a function of the distance from the surface when the temperature is 25^{o}C, 37^{o}C and 45^{o}C, respectively. (a) and (b) correspond to the two different cases of triple-responsive protein orientation shown in Fig. 2a, 2b. Parameters are the same as Fig. 2a, 2b, respectively.

1. Supplementary figures and discussions

Fig. S1 shows the volume fraction of PNIPAm, number density of the bound ligands and proteins of the two different cases (see Fig. 2) as a function of the distance from the surface, when temperature is 25 ^{o}C, 37 ^{o}C and 45 ^{o}C (note that the three temperatures are exactly in the three stages discussed in Fig. 2, respectively). At 25 ^{o}C, PNIPAm molecules in both cases are hydrophilic and randomly distribute between the surface to z=4 nm, which induces the random distribution of their end-bound ligands. Since the space for each bound protein under this situation is large due to long polymer length in z direction, the steric repulsion

between bound proteins is small, and has little effect on protein binding. Thus nearly all the ligands are bound with proteins, i.e., $\langle f_{bound} \rangle$ is close to 1.0 due to the strong specific interaction between ligands and proteins. Meanwhile, the orientation of bound proteins is approximately random, and the order parameter S is low. Notably, bound proteins in case of Fig. S1(b) is larger than that of Fig. S1(a) due to the higher polymer surface coverage so that the ligands are fully occupied by proteins, which induces a slight increase of protein order parameter as shown in Fig. 2. Further, the density distribution show the bound proteins form a bilayer structure, namely, a maximum in the bound density at 1.25 nm, corresponding to proteins in contact with the surface, and a second peak at 4.75 nm from the surface. This bilayer organization maximizes the number of ligand-protein bonding, even at the cost of some steric repulsion.

As temperature increases to 37 ^{o}C, PNIPAm molecules get hydrophobic quickly, and collapse onto substrate sharply. As a result, the mean length in z direction becomes shorter, the space for proteins to bind becomes smaller, and the steric repulsion between bound proteins get stronger. Besides that, local micro-phase separation starts, indicating that a few of polymers aggregate and crosslink into domains as explained in Fig. 3. These lead to a slight decrease of proteins bound to PNIPAm layer at high surface coverage in case of Fig. S1(b), but show no significant effect on the fraction of bound proteins in Fig. S1(a) at low polymer surface coverage, because more polymers in case of Fig. S1(b) participate in local micro-phase separation than that of Fig. S1(a), which result in a greater enhancement of steric repulsion between proteins bound to the same polymeric domain in Fig. S1(b). More importantly, in order to minimize the strong steric repulsion, the orientation of bound proteins in both cases tends to be uniform and the order parameter S rises. Meanwhile, the bilayer structure of bound proteins disappears. With a further increase to 45 ^{o}C in temperature, micro-phase separation takes place in most of the polymers, namely, many more polymeric domains formed by crosslinked PNIPAm appear. This enforces more proteins bound at high surface coverage layer fall off from the ligands in Fig. S1(b), so that the space for bound proteins get enlarged again, and the order parameter S is decreased, but cannot effect the fraction of proteins bound to polymer layer at low surface coverage in Fig. S1(a), only enforces more bound proteins get into the collapsed polymer layer to enlarge the distance between each other, and thus reduce the protein order parameter.

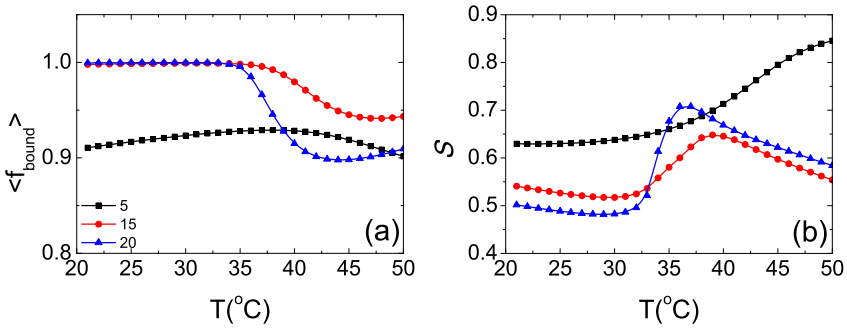

Fig. S2: The effect of PNIPAm length on the fraction of bound protein and protein order parameter as a function of temperature, when PNIPAm surface coverage is 0.08 nm^{-2}. Other parameters are same as Fig. 5c, 5d.

Fig. S2 shows the effect of PNIPAm length on thermo-responsive behavior of protein binding, when PNIPAm surface coverage is high, namely, 0.08 nm^{-2}. When PNIPAm is short, i.e., n=5, $\langle f_{bound} \rangle$ varies only a little around 0.9 as temperature rises, but the protein order gets enhanced visibly though the triple-responsive behavior disappears. As PNIPAm length increases to 15 and 20, the triple-responsive behavior appears, but the fraction of bound proteins is still thermo-responsive. More specifically, when temperature is below LCST, longer PNIPAm molecules benefit larger and lower ordered bound proteins due to the larger space caused by longer PNIPAm for proteins to bind. As temperature rises close to LCST, sharper increase of the protein order parameter takes place in longer PNIPAm system. With further increase of temperature, larger decline of bound protein and their order parameter appear caused by longer PNIPAm.

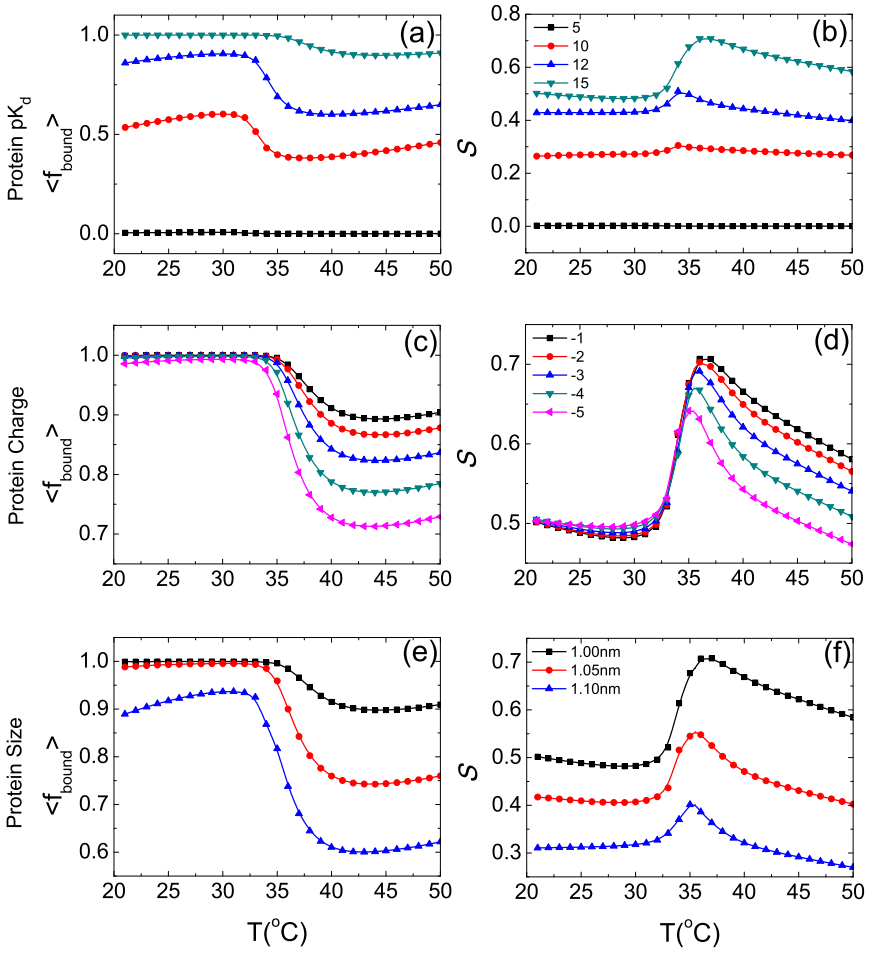

Fig. S3: The effect of (a), (b) protein dissociation constant, (c), (d) protein charge, and (e), (f) protein radius on the thermo-responsive fraction of bound protein (left column) and protein order parameter (right column), when PNIPAm surface coverage is 0.08 nm^{-2}. Other parameters are same as Fig. 6.

Fig. S3 shows the effect of protein property on the thermo-responsive binding, when PNIPAm surface coverage is 0.08 nm^{-2}. In general, the results are similar to that in Fig. 6, but we should notice that the fractions of bound protein under all these situations are always thermo-responsive, which is different from those at low surface coverage.

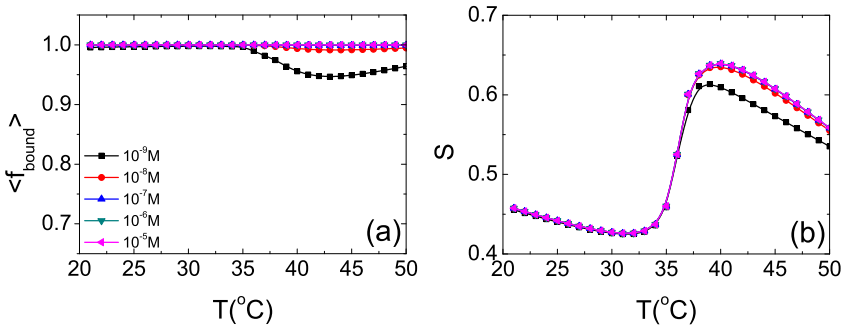

Fig. S4: The effect of protein concentration in bulk solution on the thermo-responsive behavior of protein binding. PNIPAm length n=20, PNIPAm surface coverage σ=0.05 nm^{-2}.

Fig. S4 shows the role of protein concentration in bulk solution on the thermo-responsive behavior. Interestingly, though changing protein concentration of bath can generate the transition between the two different cases (i.e., ligands are fully and invariably occupied or partially and responsively adsorbed by proteins, while the protein orientation in both cases is triple-thermo-responsive), the influence is not so significant as those shown in Fig. 6.

2. Simplified scheme for electroneutral proteins

In our modelling, each protein is modeled as a spherical particle of radius R_{prot} and charge Q_{prot} (in units of elemental charge). We consider spherical proteins with homogeneous charge distribution, namely, the distribution of charge of the proteins ($q_{prot}(z, z'')$) are independent of the protein orientation. When proteins are charged, salts are introduced to satisfy the constraint of global electroneutrality. However, when proteins are electroneutral (Q_{prot}=0), it is unnecessary to introduce salts into our system, namely, the Helmholtz free energy per unit area for the system in Fig. 1 gets into

$$\frac{\beta F}{A} = -\frac{S_p}{k_B A} + \frac{\beta F_{inter}}{A} - \frac{S_{mix}}{k_B A} - \frac{S_{prot}}{k_B A} + \frac{\beta F_{chem}}{A}, \tag{S1}$$

where the translational (mixing) entropy of small molecules (water molecule) is

$$-\frac{S_{mix}}{k_B A} = \int \rho_w(z)[\ln \rho_w(z) v_w - 1] dz, \tag{S2}$$

Therefore, the semigrand potential density is simplified as follows:

$$\frac{\beta W}{A} = \frac{\beta F}{A} - \beta\mu_{prot}\int[\int \rho_{prot}(z,z')dz' + (1-f_{unb}(z))\langle n_l(z)\rangle]dz + \int \beta\pi(z)[\rho_w(z)v_w + \langle\phi_n(z)\rangle + \langle\phi_{prot,free}(z)\rangle + \langle\phi_{prot,bound}(z)\rangle - 1]dz.$$

As a result, we do not need to consider the effect of electrostatic repulsion on the binding of electroneutral proteins.