Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

An ultrastable conjugate of silver nanoparticle and protein formed through weak interactions

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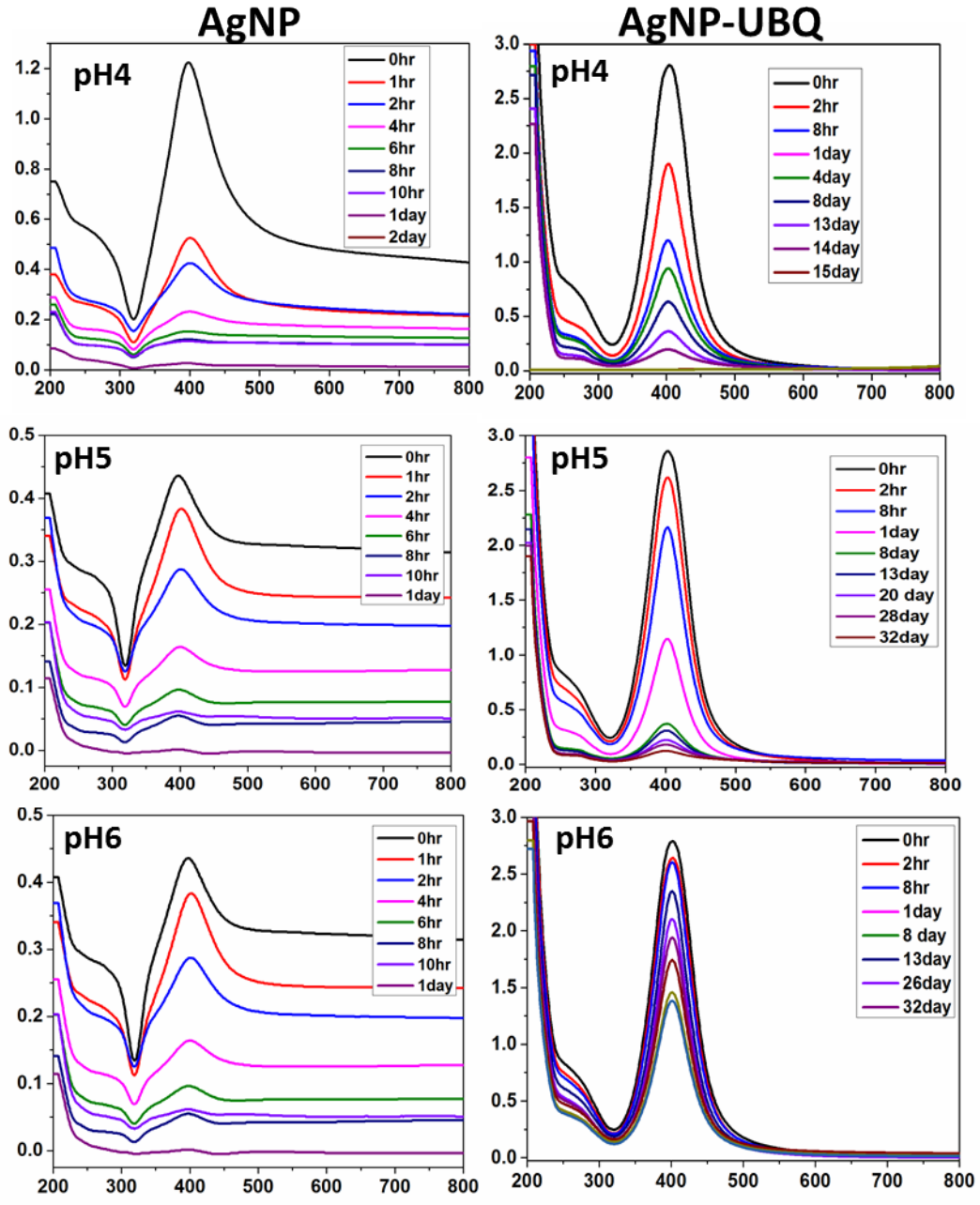

Figure S1 a: Stability of SNP and SNP-UBQ conjugate at different pH (4,5,6)

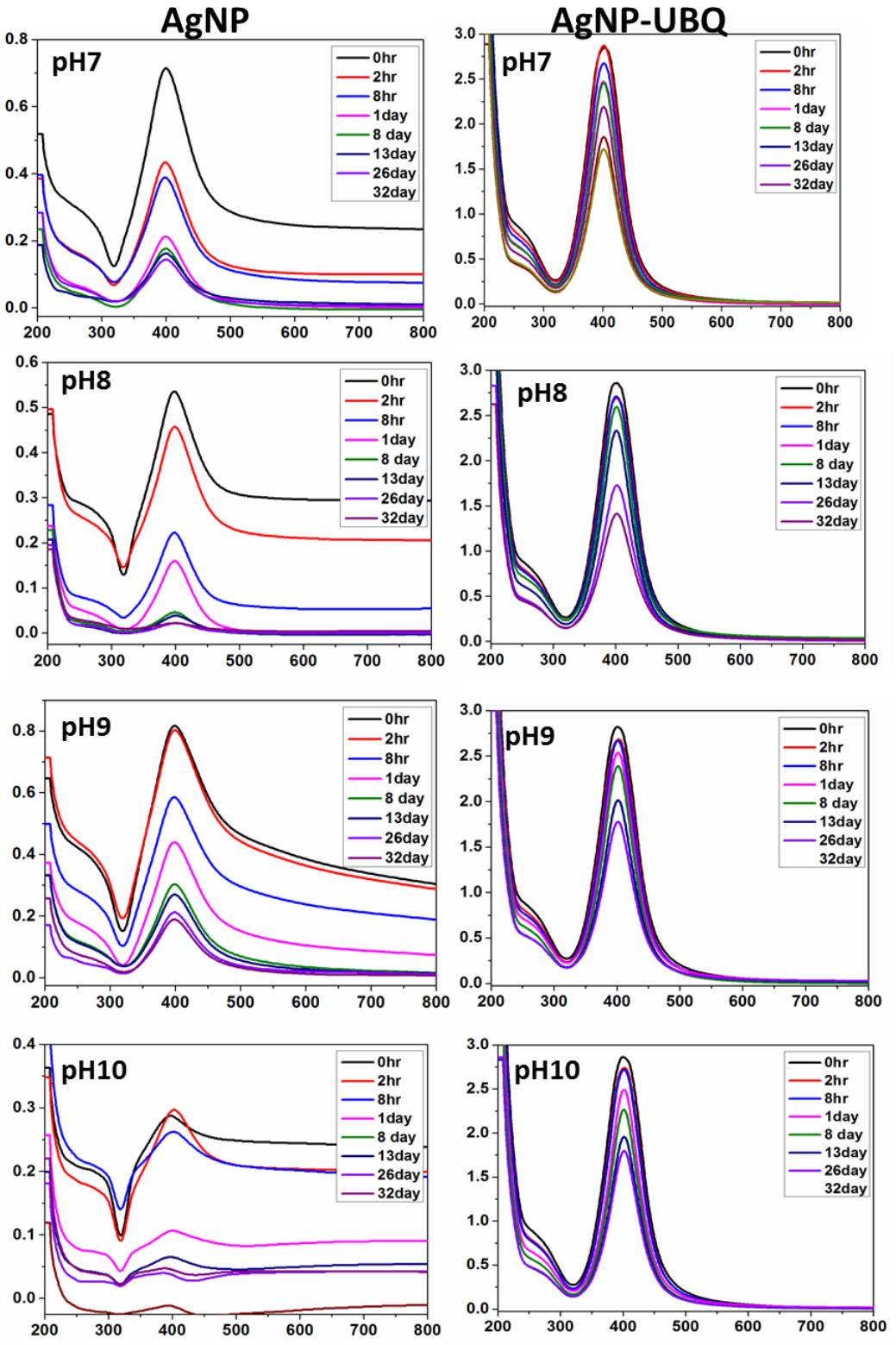

Figure S1 b: Stability of SNP and SNP-UBQ conjugate at different pH (7,8,9,10)

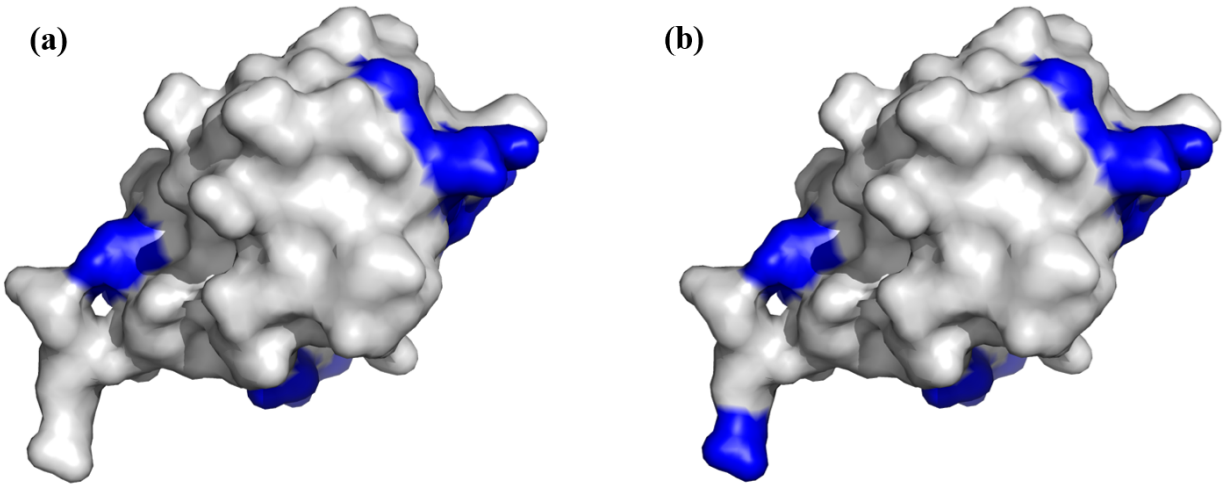

Figure S2: (a) Ubiquitin residue which shows decrease in λ_{noe} values upon interaction with AgNP mapped on to the protein surface. (b) Residues which show decrease in amide proton T_2 upon interacting with AgNP as compared to the free protein.

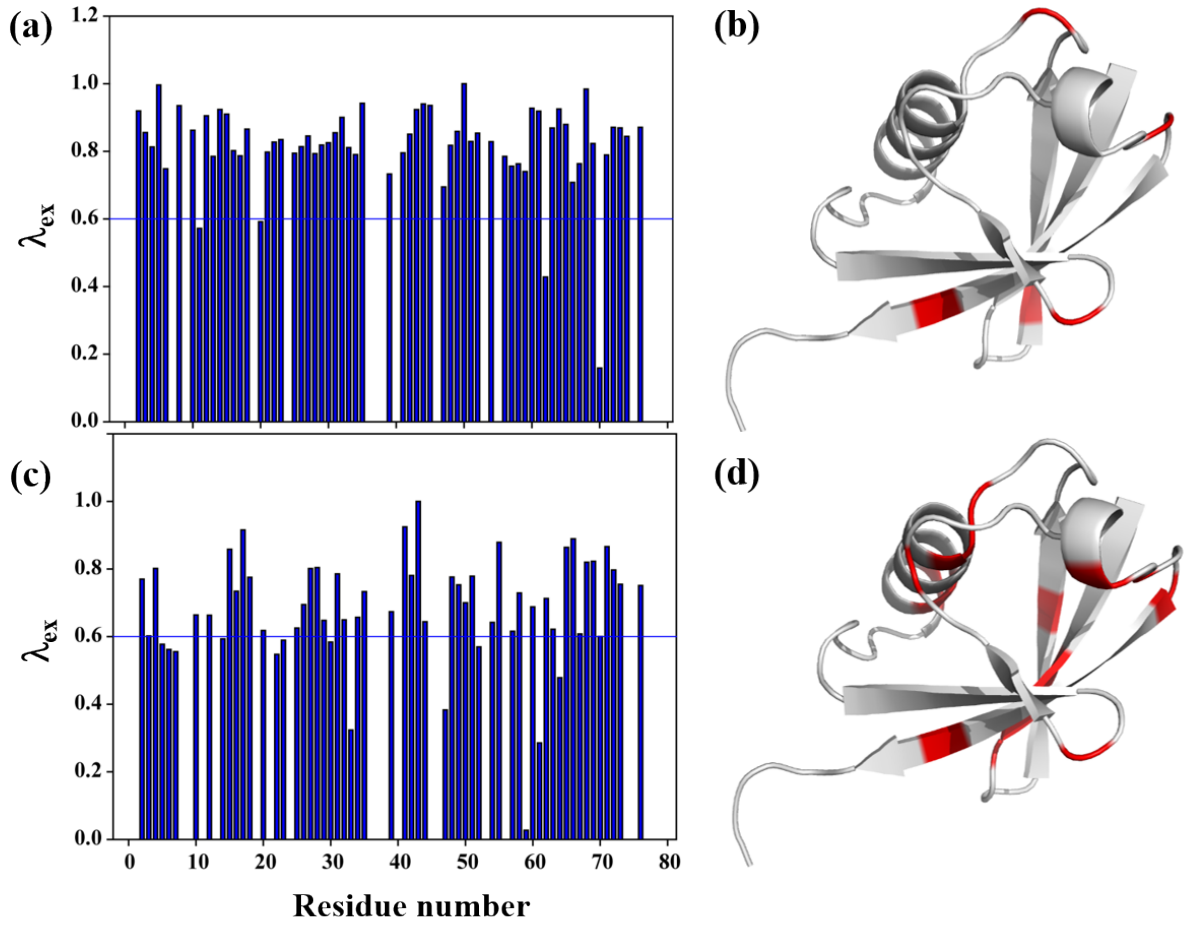

Figure S3: A plot of λ_{ex} obtained for (a) free ubiquitin and (c) AgNP-Ubq. We have considered a threshold value (indicated by the solid line) for discriminating highly solvent accessible residues from the rest of the protein. Note that a decrease in λ_{ex} implies higher solvent exposure. (b) and (d) Residues that show higher λ_{ex} values than the threshold in free protein (c) and in AgNP bound form (d) mapped on to the 3D structure of protein.

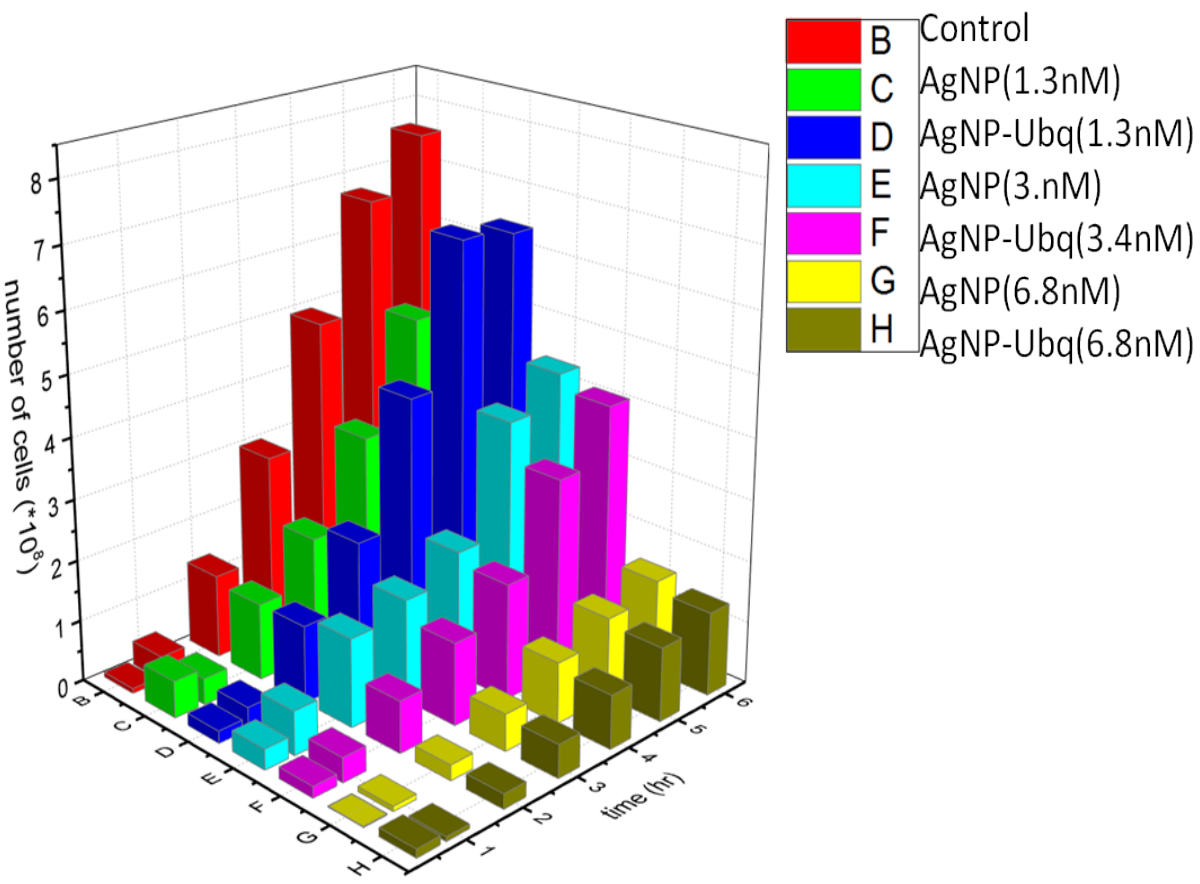

Figure S4 Growth kinetics of *E. coli*: Bacterial suspensions treated with AgNPs and AgNP-UBQ for 6 h at different concentrations of AgNP and AgNP-Ubq, 1.3 nM, 3.4 nM and 6.8 nM respectively. To examine the bacterial growth rate and to determine the growth kinetics in the presence of silver nanoparticles, *E. coli* cells were grown in 25 ml of LB medium supplemented with 1.3, 3.4, and 6.8 nM of nanoparticles (AgNP , AgNP-Ubq) as the final concentration. The growth kinetic curves were established by bacterial cell counting using Petroff Hausser counter in conjunction with Leitz phase contrast microscope (Laborlux K Wild MPS12).

Text S1: Energy of AgNP-Ubiquitin interaction

A calculation of the energy of the system, which consists of attractive, electrostatic repulsive and steric interactions illuminates the extent to which each of these factors contribute to the overall stability. The overall energy of AgNP-Ubq interaction can be written as sum of the three individual interactions as:

$$\begin{aligned} E_{total} = \; & 64\pi RkTn^{\infty}\kappa^{-2}tanh^{2}\left(\frac{ze\psi_0}{4kT}\right)exp(-\kappa d) \\ & -\frac{A}{6}\left[\frac{2}{s^2-4}+\frac{2}{s^2}+\ln\left(\frac{s^2-4}{s^2}\right)\right] \\ & +\frac{100R\delta^2}{d\pi\sigma^2}\,kT\exp(\frac{-\pi d}{\delta}) \end{aligned} \qquad (1)$$

where the first and the third term are the electrostatic (using Derjaguin approximation) and steric repulsive energy, respectively and the second term represents the van der Waals attractive force; κ is the Debye-Hückel length, R represents the radius of the nanoparticle, n^{∞} represents the ionic concentration; ψ_0 is the zeta potential; d is the distance between the surface of two nanoparticles at which the energy is being evaluated, A is the Hamaker constant ($3.0*10^{-19}$ J) , $s=(2R+d)/2R$, δ is the length of the ubiquitin molecule which is adsorbed on the surface of the nanoparticle (taken equal to the diameter of ubiquitin: 2.5 nm) and σ represents the diameter of the area occupied by one ubiquitin molecule on the surface of AgNP (which is obtained by dividing the surface area of AgNP by the number of ubiquitin molecules adsorbed assuming the surface is uniformly occupied by the protein molecules). The value of the Hamaker constant was obtained from that reported by Tai et al. (J.-T. Tai, C.-S. Lai, H.-C. Ho, Y.-S. Yeh, H.-F. Wang, R.-M. Ho and D.-H. Tsai, *Langmuir*, 2014, **30**, 12755-12764). The last term representing the steric repulsion is a modification of the de Gennes model (P. G. de Gennes, *Advances in Colloid and Interface Science*, 1987, **27**, 189-209) as proposed by Korgel et al.(B. A. Korgel, S. Fullam, S. Connolly and D. Fitzmaurice, *The Journal of Physical Chemistry B*, 1998, **102**, 8379-8388). Figure S5 shows a plot of interaction energy (E_{total}) as a function of distance between the surface of the nanoparticles ('*d*' in Equation 3 above) for two cases: (i) Electrostatic repulsion and van der Waals attraction (first two terms of the above equation). The electrostatic repulsion has been considered assuming a zeta-potential value of 30 mV as seen for unconjugated AgNPs (Figure 2A**)** and (ii) all the three terms of Equation 3 above are considered assuming a zeta potential of 10 mV, which is close to the observed values in the case with conjugated AgNP-Ubq (See Figure 2A of Main text).

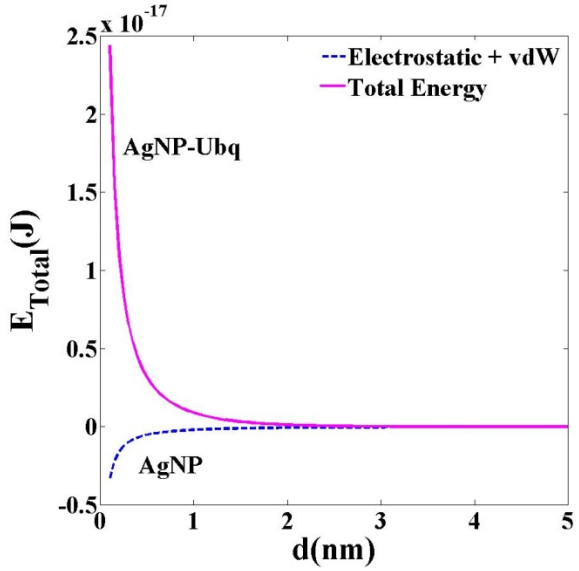

Figure S5: A plot of the total energy given by Equation 3 as a function of the interparticle distance. The following values were used for the calculation: R=15 nm; A=3.0*10^{-19} J; ψ_0 (zeta potential)= -30 mV for AgNP and ζ = -10 mV for AgNP-Ubq; δ=2.5 nm; κ^{-1} = 1.5 nm (calculated based on 50 mM NaCl).

It is evident that in the absence of the protein, the repulsive forces are not enough to overcome the van der Waals forces of attraction, which results in the aggregation of the nanoparticles. On the other hand, in the presence of the steric repulsive interactions which happens in AgNP-Ubq, the particles do not aggregate. While steric interactions can explain the stability of AgNP-Ubq conjugate observed from pH 6 to pH 10, the relatively weak stability at pH 4 and pH 5 (Figure 3 of Main text) is attributed to the weak interaction of ubiquitin with AgNP at these pH.