Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Functional Protein Shells Fabricated from the Self-Assembling Protein Sheets of Prokaryotic Organelles

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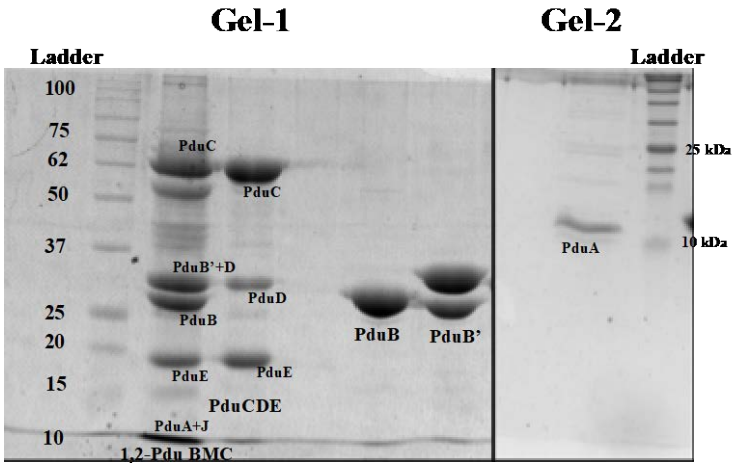


Fig. S1: SDS page of 1,2-Pdu BMC showing band for the major shell protein PduA/B/B' and enzyme PduCDE which is a dimer-trimer complex. The shell proteins PduA, B and B' have the molecular weight of 11, 25 and 28 kDa whereas PduCDE has a molecular weight of 110 kDa. Major shell proteins (PduA/B/BB) and enzyme PduCDE are expressed individually and purified by Ni-NTA chromatography. For PduB, PduB' and PduCDE 12 % gel is carried out (Gel-1). Because of the low molecular weight of PduA (11 kDa), a separate 18 % SDS PAGE is shown for the protein PduA (Gel-2).

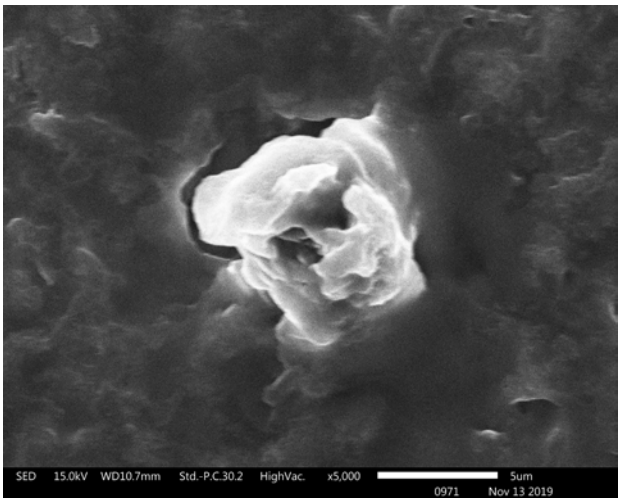


Fig. S2: SEM image for the PS fabricated from the K26A protein, mutated form of PduA

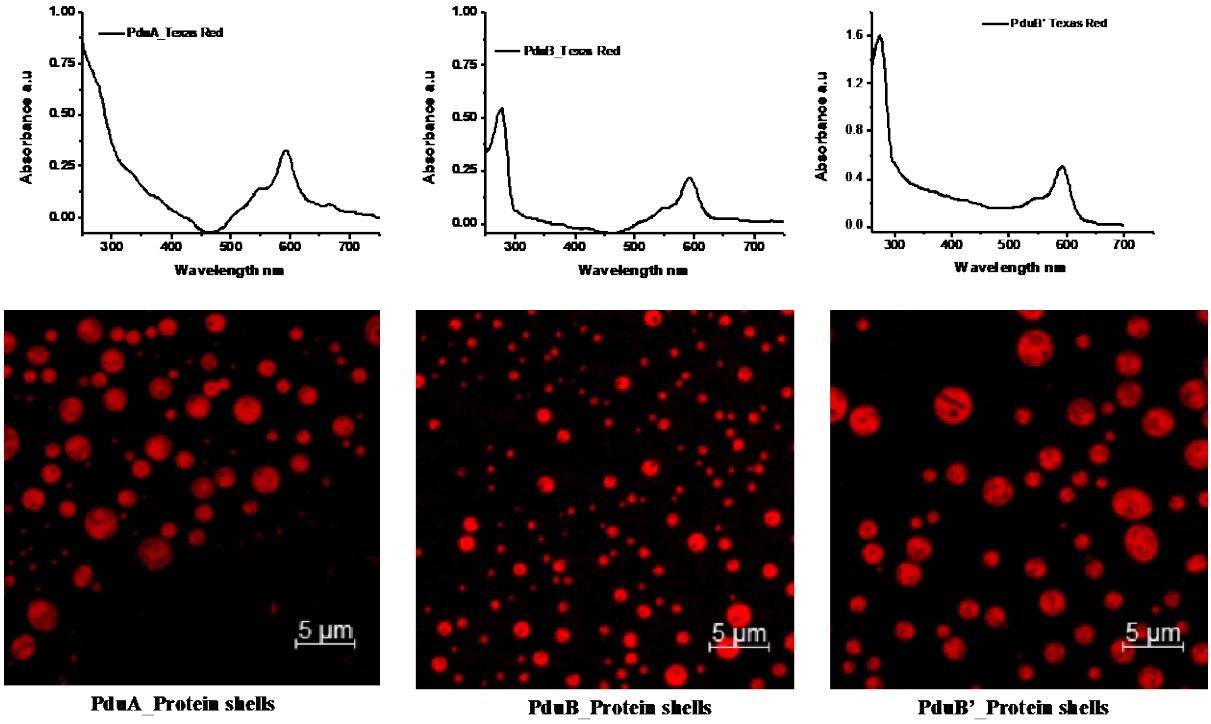


Fig. S3: Major SPs (PduA/B/B') are tagged with Texas red and the tagging is confirmed by the UV spectra of the conjugated protein (upper panel). The peak around 590 nm is the dye tagged PSs. The dye tagged SPs are used for the fabrication of PSs and is observed under confocal microscopy. The PSs are observed as spherical closed shell bodies. As they are formed by the assembly and folding of sheet having various dimension we observed poly-dispersed PSs when scanned under confocal microscope. The confocal fluorescent image for the protein shell shown here is representative image with a scale of 5 μm.

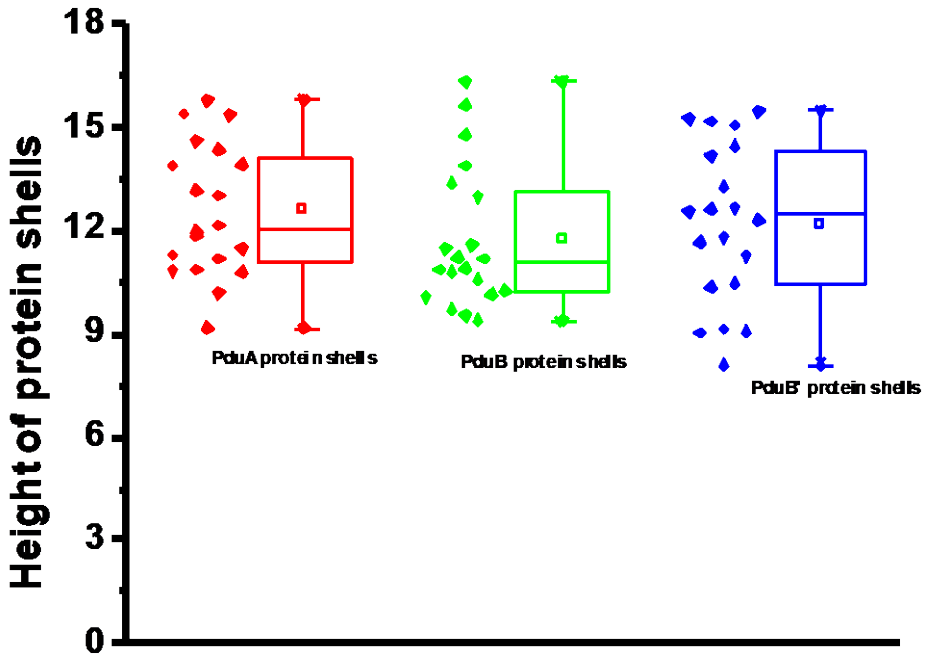


Fig. S4: Average height of the protein shells measured by AFM. The average height observed for various PSs fabricated from Pdu SPs is 12 ± 0.5 nm (Mean ± Standard error of mean).

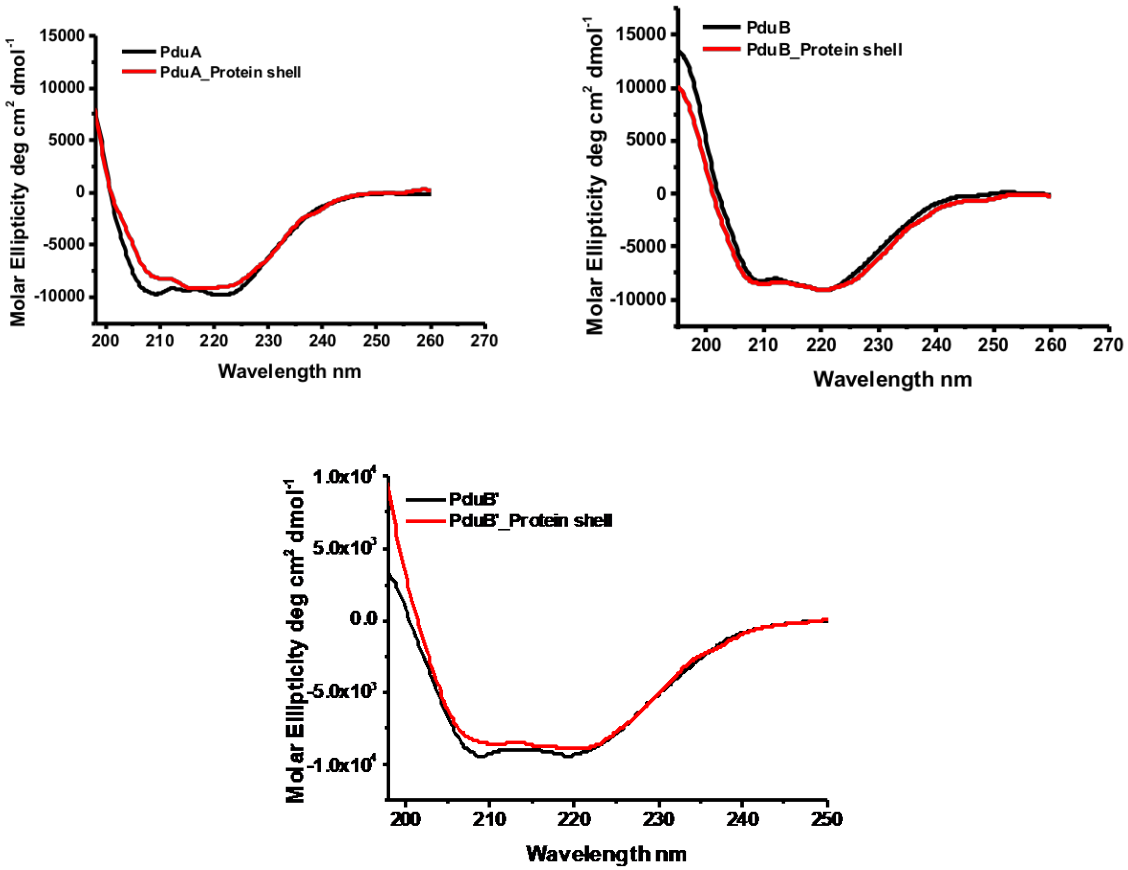


Fig. S5: Circular dichroism for the PSs fabricated from the SP PduA/B/B' do not show any change in their secondary structure as the two spectra completely overlay. No change in the characteristic alpha peak at 222 or 208 nm is observed after protein shell formation. When estimated by K2D3 for the secondary structure content, the SPs and the PSs have same alpha helical content. For all the PSs no change in the conformation is observed with the fabrication as shown in Table S2.

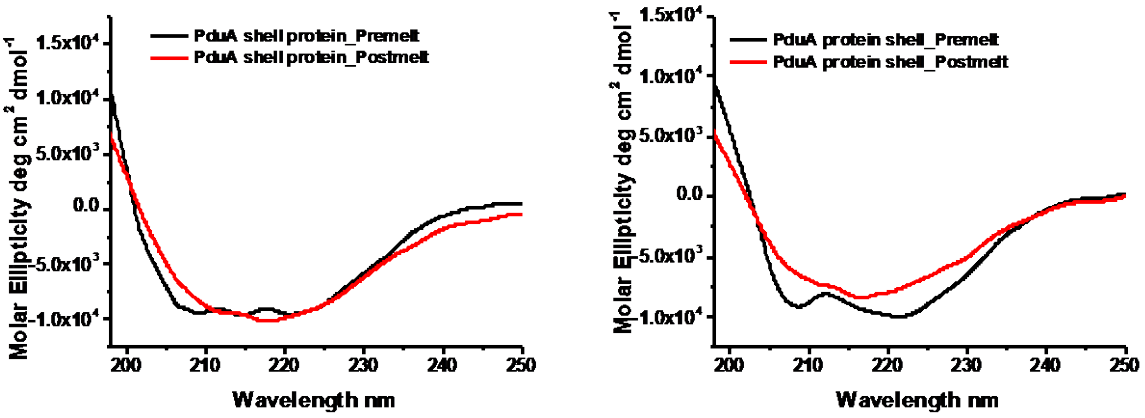


Fig. S6: The estimation of the secondary structure content determined by K2D3 shows no change in the conformation as shown in Table S4 and also shown in above overlaid spectra (the post melt CD spectra overlays the pre-melt for PSs).

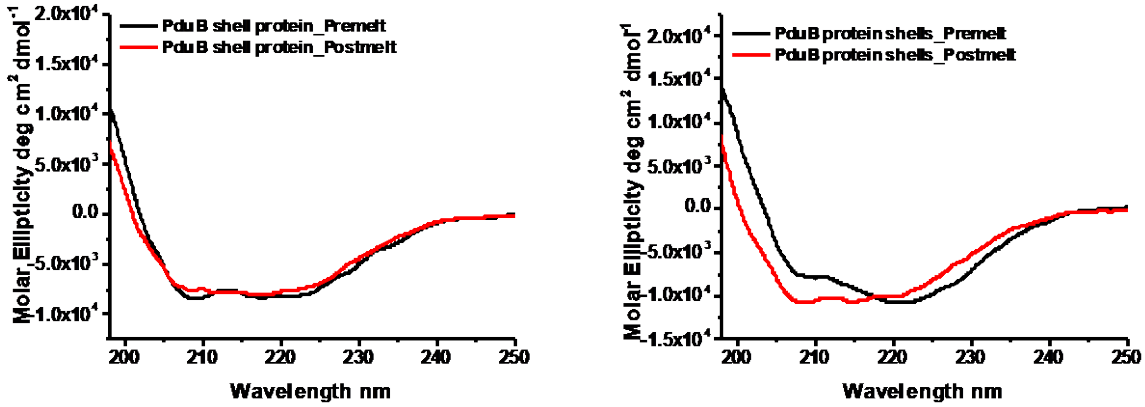


Fig. S7: The estimation of the secondary structure content done by K2D3 shows no change in the conformation as shown in Table S4 and also shown in above overlaid spectra (the post melt CD spectra overlays the pre-melt for PSs).

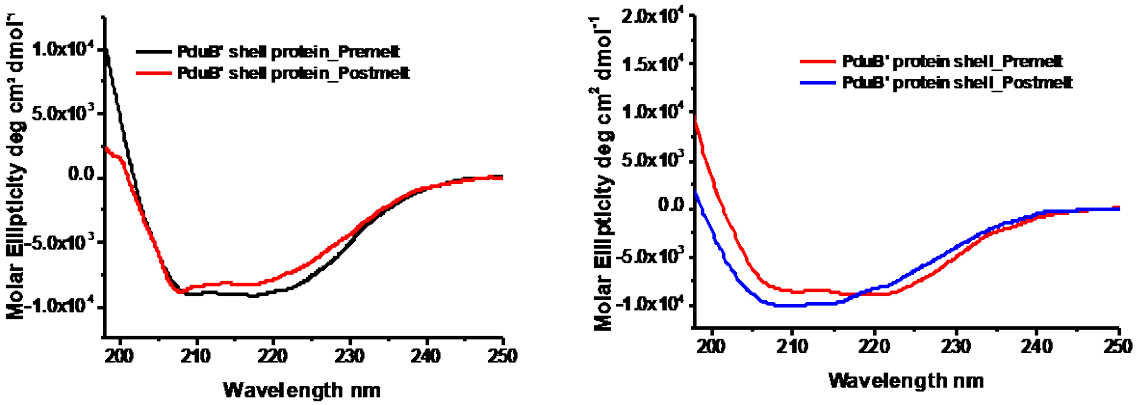


Fig. S8: The PSs are also thermally stable similar to SPs in terms of loss of secondary structure conformation, as the post melt spectra for the PSs overlays the pre-melt spectra. The estimation of the secondary structure content done by K2D3 shows no change in the conformation as shown in Table S4.

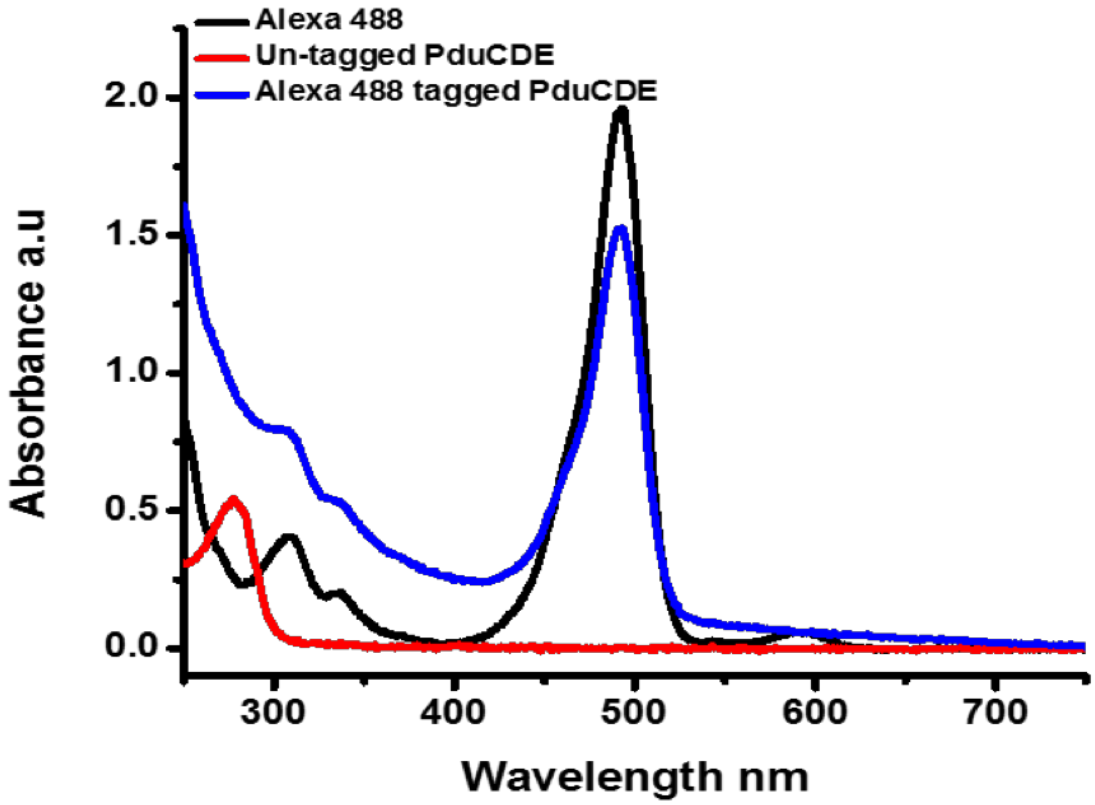


Fig. S9: UV-visible spectra of the dye tagged (Alexa 488) PduCDE shows that the protein has been tagged. The tagging is carried out by mixing the protein and the dye in a ratio of 1:5 and incubating it overnight at 4 °C. The untagged dye is removed from the mixture with the help of PD-10 column and the tagging of protein is checked with UV. The blue curve shows the tagged PduCDE having peak for Alexa 488 (black curve) and also the protein (red curve). The absorption spectra for Alexa 488 shows the characteristic peak at around 490 nm (black curve).

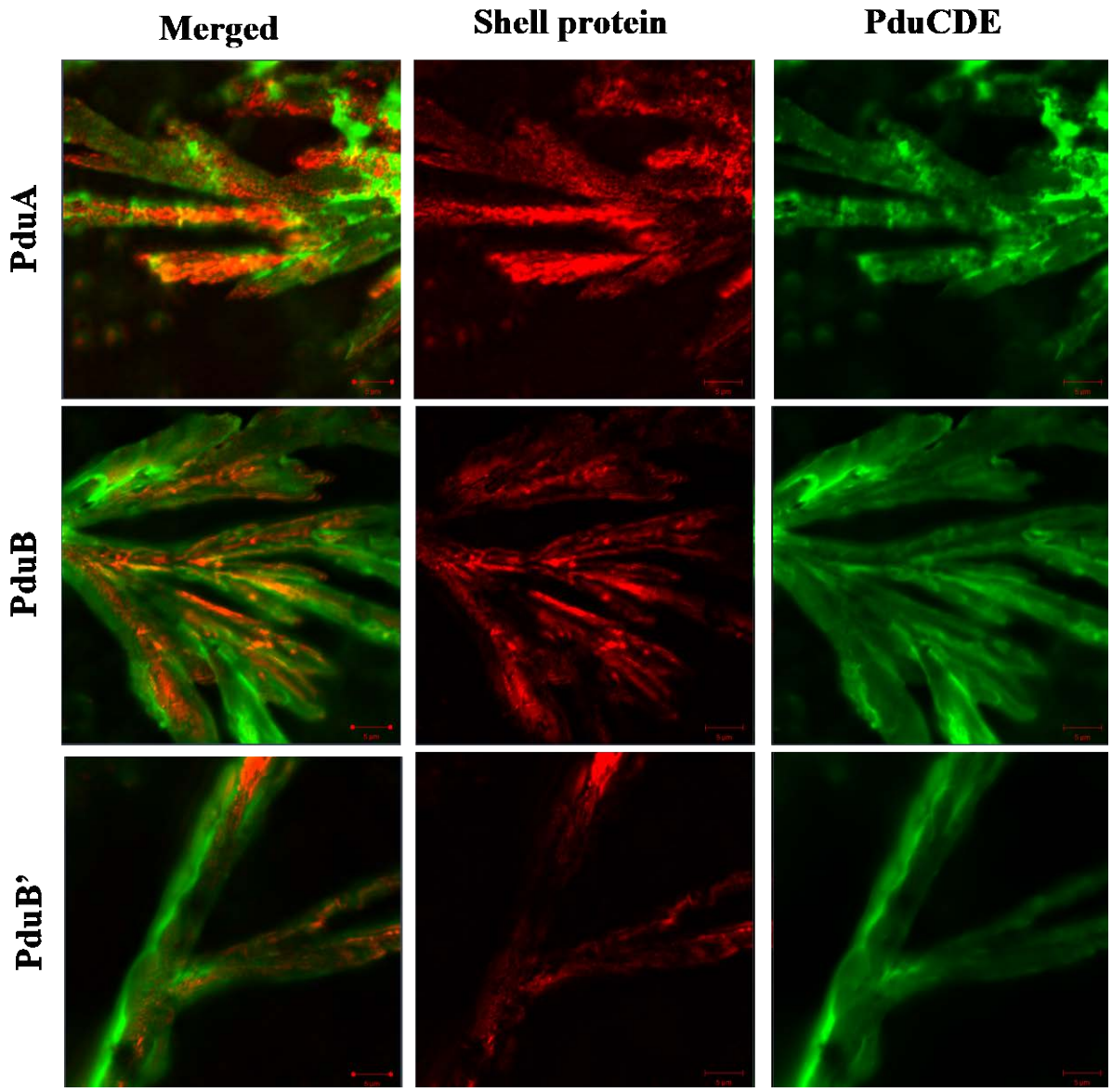


Fig. S10: Physical mixture of dye labelled PduCDE (Alexa 488) and SPs(PduA/B/B') (Texas Red) shown as fluorescent images proves the interaction of the enzyme with the shell proteins. As these shell proteins are having tendency to self-assemble they can be seen as extended sheet in the microscopic images. Scale bar in all the images is 5 μm.

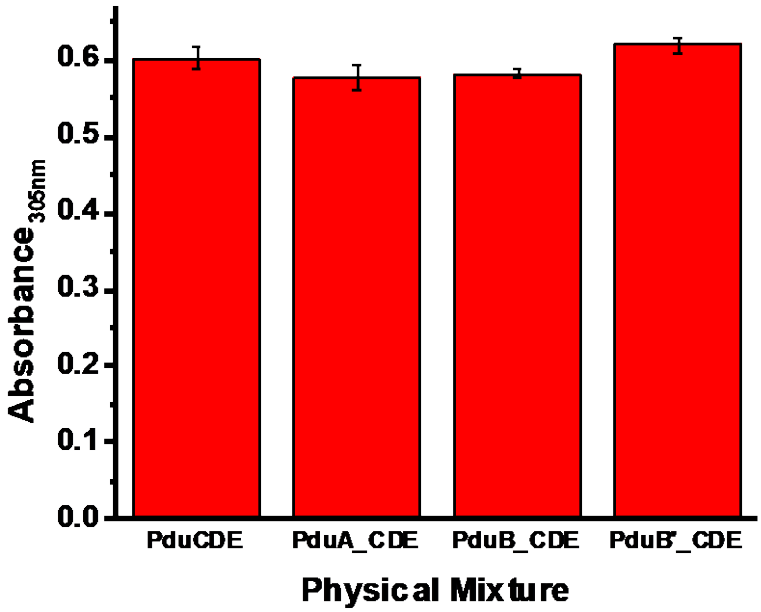


Fig. S11: Diol-dehydratase assay of the enzyme PduCDE present in the physical mixture of major SP PduA/B/B' and enzyme. As shown in Fig. S11 that in the physical mixture the two co-localizes but the similar absorbance at 305 nm for the free enzyme and the enzyme in physical mixture suggests that SPs doesn't destabilizes the enzyme.

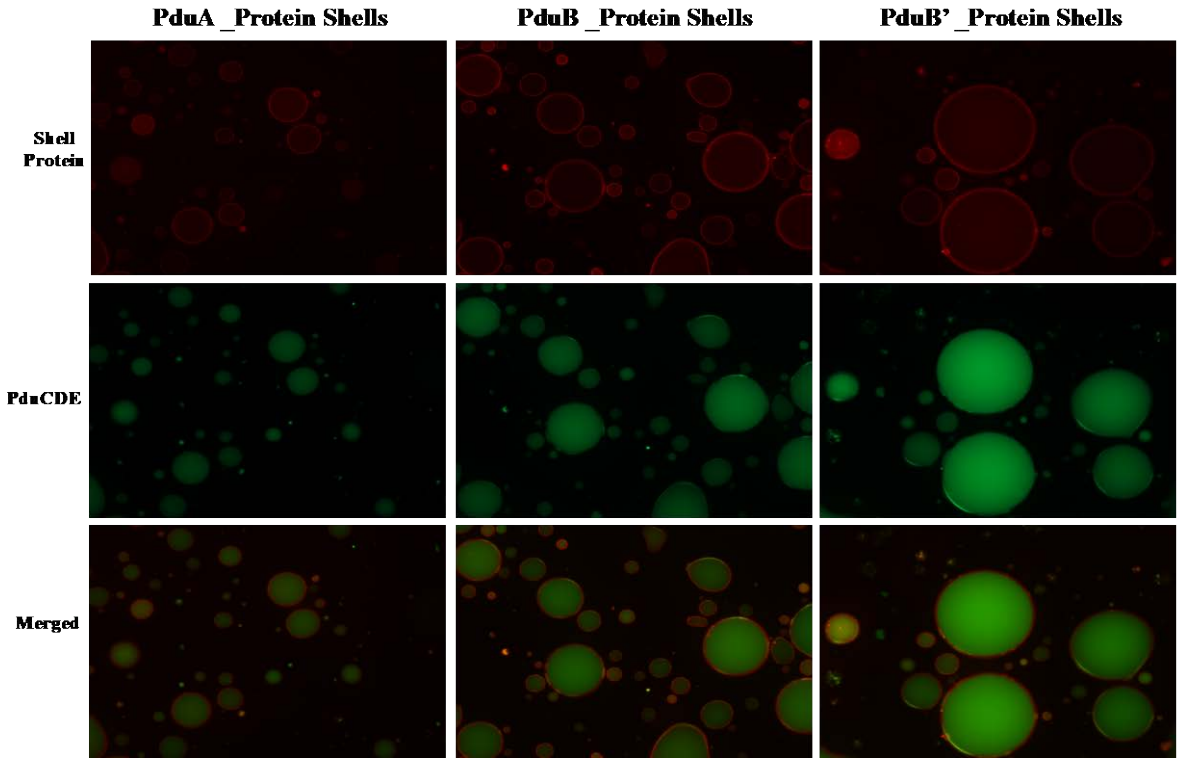


Fig. S12: Co-localization and encapsulation of PduCDE (Alexa 488 tagged) in the PSs fabricated from the shell proteins of 1,2-PduBMC is studied by fluorescence microscope. The scale bar in the above figure is 5 μm.

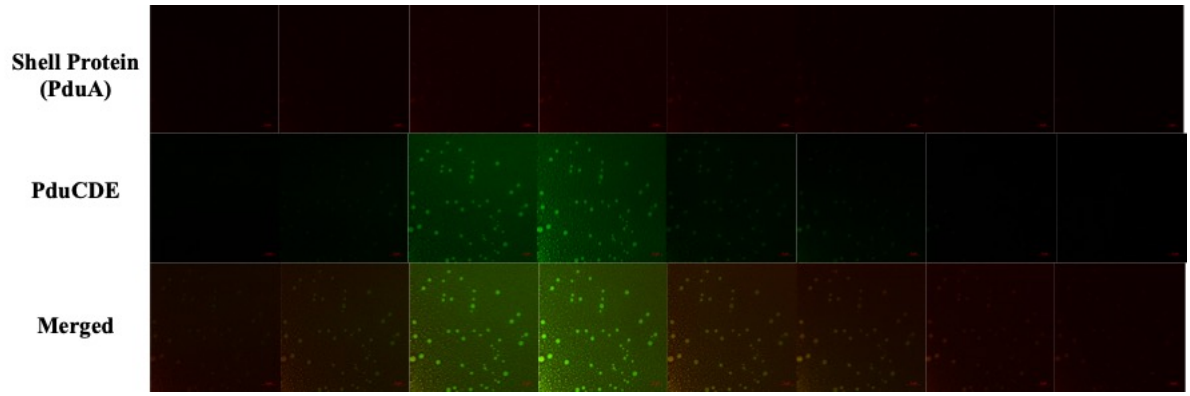


Fig. S13: Confocal microscopic images for the enzyme encapsulated PSs. PduA (tagged with Texas Red) is mixed with the enzyme PduCDE (tagged with Alexa 488) and PSs are fabricated and observed under confocal fluorescence microscope. The z-stacked confocal images show the encapsulation of enzyme inside the PSs. The scale bar in all of them is 5 μm.

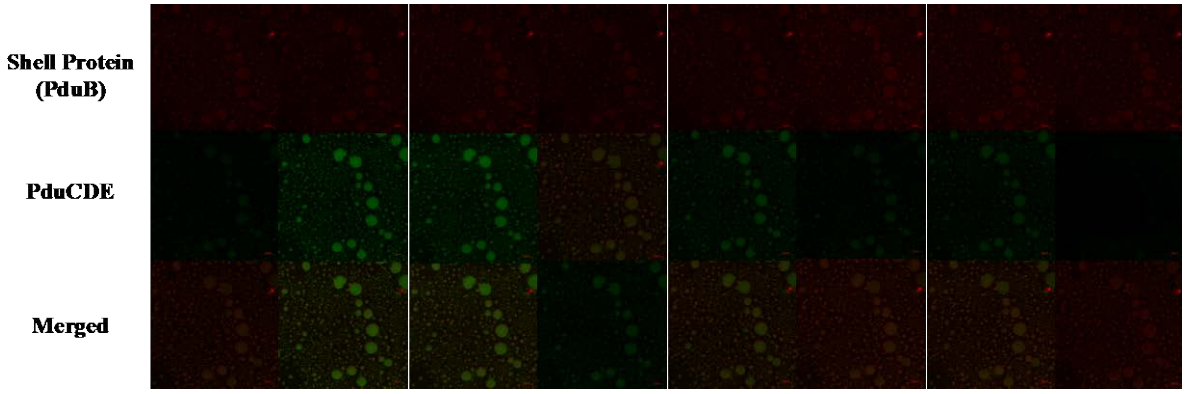


Fig. S14: Confocal microscopic images for the enzyme encapsulated PSs. PduB (tagged with Texas Red) is mixed with the enzyme PduCDE (tagged with Alexa 488) and PSs are fabricated and observed under confocal fluorescence microscope. The z-stacked confocal images show the encapsulation of enzyme inside the PSs. The scale bar in all of them is 5 μm.

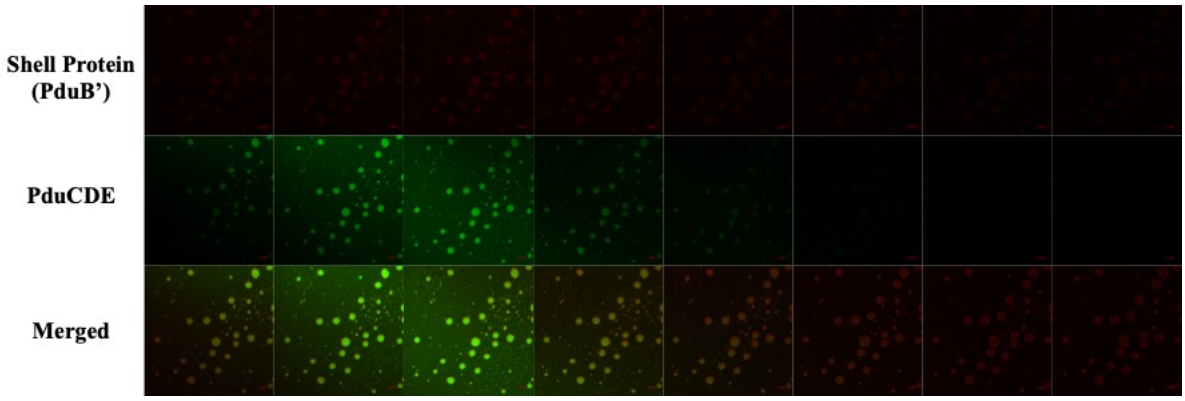


Fig. S15: Confocal microscopic images for the enzyme encapsulated PSs. PduB' (tagged with Texas Red) is mixed with the enzyme PduCDE (tagged with Alexa 488) and PSs are fabricated and observed under confocal fluorescence microscope. The z-stacked confocal images show the encapsulation of enzyme inside the PSs. The scale bar in all of them is 5 μm.

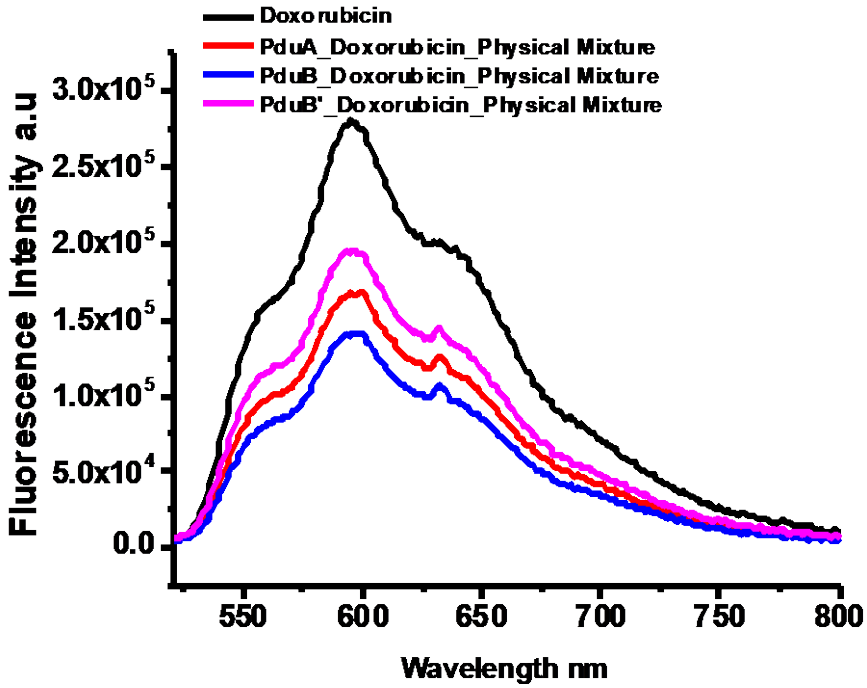


Fig. S16: Quenching of doxorubicin fluorescence in presence of SPs shows that they interact with the doxorubicin.

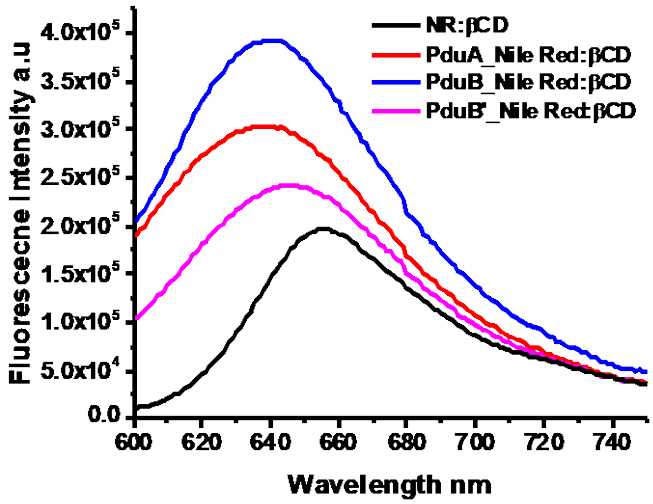


Fig. S17: Interaction of shell proteins with NR encapsulated in cyclodextrin as shown for the physical mixture of NR and shell proteins. This physical mixture is employed for fabrication of protein shells. The NR encapsulated protein shells are employed for studying the release through dialysis method.

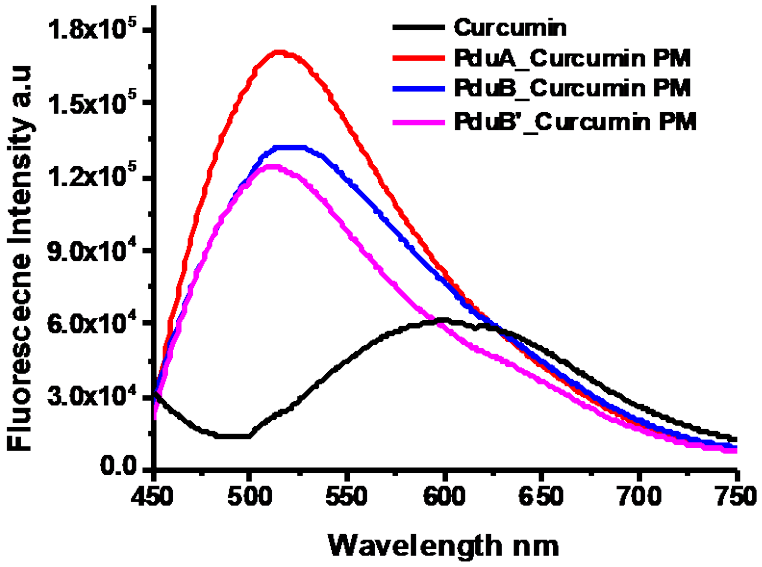


Fig S18: Interaction of shell proteins with curcumin as shown for the physical mixture of curcumin and shell proteins. This physical mixture is employed for fabrication of protein shells. The NR encapsulated protein shells are employed for studying the release through dialysis method.

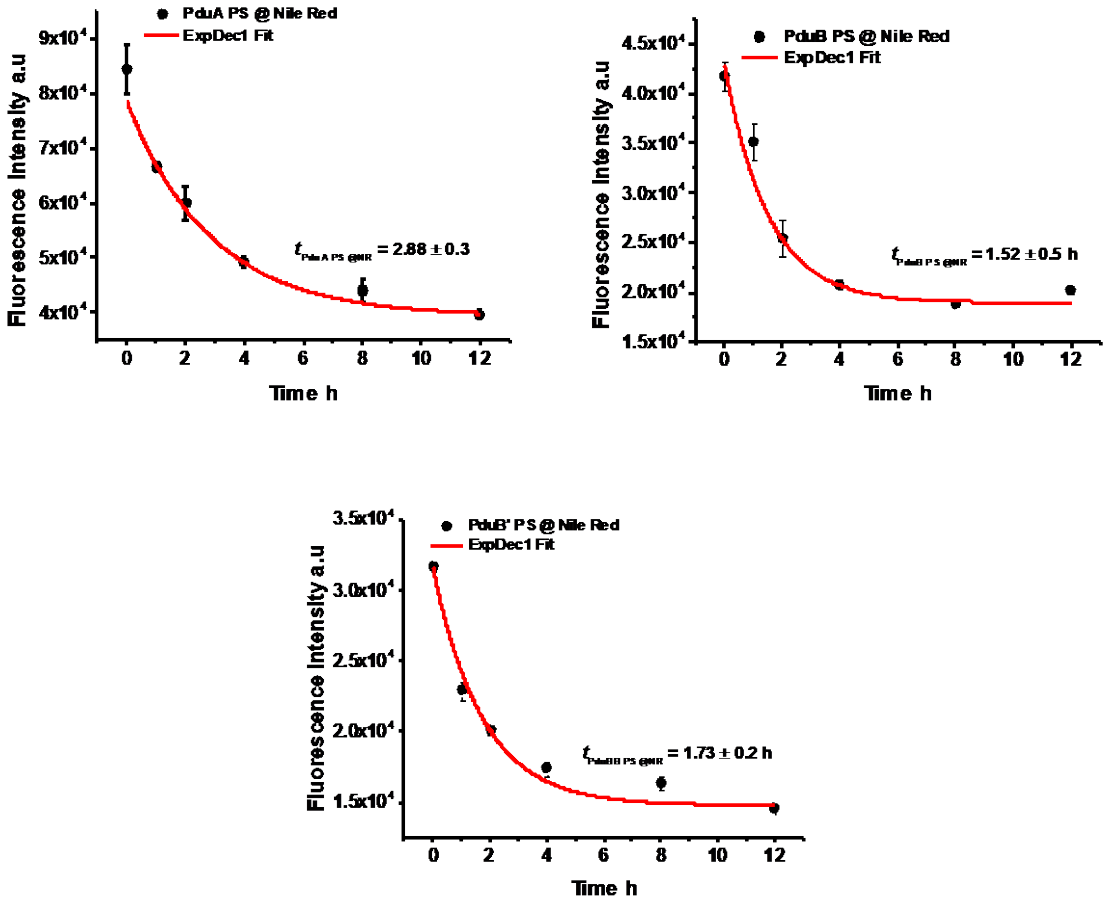


Fig. S19: Release of encapsulated NR from PSs of the shell proteins (NR being highly hydrophobic in nature is first encapsulated in beta-cyclodextrin then is encapsulated inside the PSs).

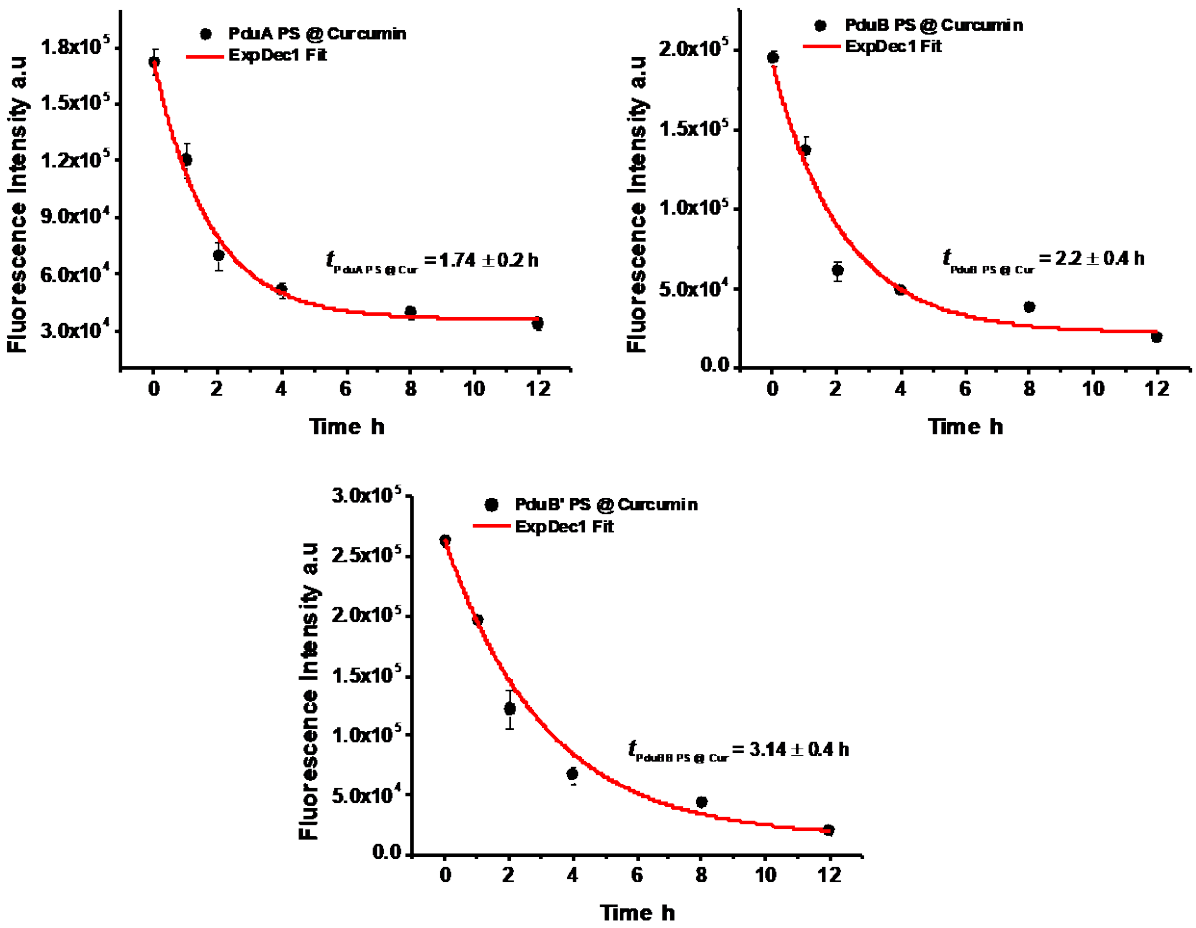


Fig. S20: Release of encapsulated curcumin from PSs of the shell proteins suggest different functional properties of the shell proteins.

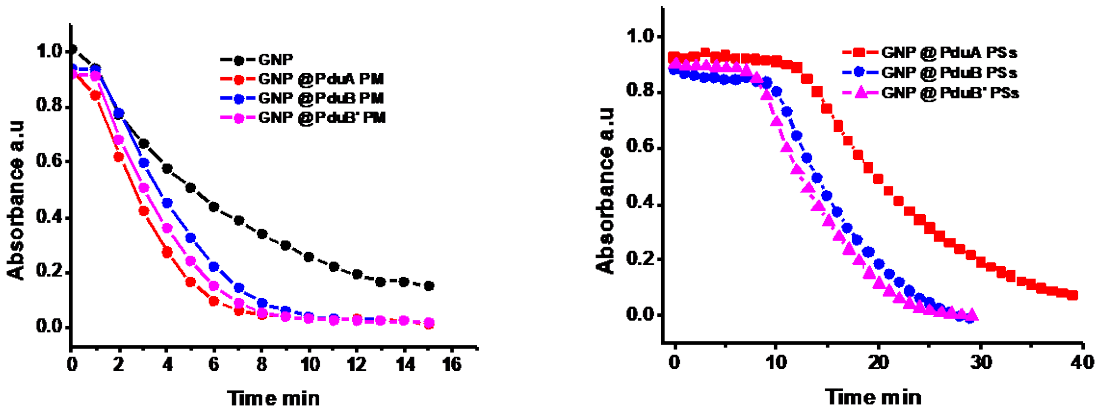


Fig. R21: Conversion of p-nitro phenol to p-amino phenol by the GNP in the physical mixture and also in the PSs encapsulated system

Supplementary Tables

Supplementary Table S1: % Distribution for the major shell protein in 1,2-Pdu BMC

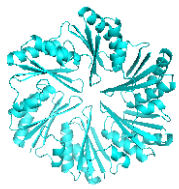
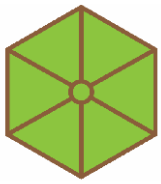
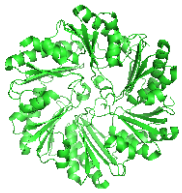
Shell Protein	Amino Acid Sequence	Structure
PduA	MQQEALGMVE TKGLTAAIEA ADAMVKSANV MLVGYEKIGS GLVTVIVRGD VGAVKAATDA GAAAARNVGE VKAVHVIPRP HTDVEKILPK GIS	Hexamer[1]  PduA(PDB ID: 3NGK) *Salmonella typhimurium*  Hexamer Exist as hexamer, extended sheet and also open nanostructures when excessively expressed Mol. Wt. = 10 kDa
PduB	MAEKSCSLTE FVGTAIGDTL GLVIANVDTA LLDAMKLEKR YRSIGILGAR TGAGPHIMAA DEAVKATNTE VVSIELPRDT KGGAGHGSLI ILGGNDVSDV KRGIEVALKE LDRTFGDVYG NEAGHIELQY TARASYALEK AFGAPIGRAC GIIVGAPASV GVLMADTALK SANVEVVAYS SPAHGTSFSN EAILVISGDS GAVRQAVTSA REIGKTVLAT LGSEPKNDRP SYI	Trimer[2] (Pseudohexamer)  PduB(PDB ID: 4FAY) *Lactobacillus reuteri*  Trimer (Pseudohexamer) Exist as trimer and as extended sheet, open nanostructures when expressed excessively. Mol. Wt. = 25 kDa
PduB'	MSSNELVEQI MAQVIARVAT PEQQAIPGQP QPIRETAMAE KSCSLTEFVG TAIGDTLGLV IANVDTALLD AMKLEKRYRS IGILGARTGA GPHIMAADEA VKATNTEVVS IELPRDTKGG AGHGSLIILG GNDVSDVKRG IEVALKELDR TFGDVYGNEA GHIELQYTAR ASYALEKAFG APIGRACGII VGAPASVGVL MADTALKSAN VEVVAYSSPA HGTSFSNEAI LVISGDSGAV RQAVTSAREI GKTVLATLGS EPKNDRPSYI	Trimer[3] (Pseudohexamer)  Trimer (Pseudohexamer) Exist as trimer and as extended sheet, open nanostructures when expressed excessively. PduB' crystal structure has not been solved yet the amino acid sequence has 80 % similarity with that of PduB except that of 37 extra amino acid at the N terminal which has a less conserved disordered region, but the oligomeric state is similar to that of PduB. Mol. Wt. = 28 kDa

Table S1: % Distribution for the major shell protein of 1,2-Pdu BMC content as reported in literature shows that PduA, B and B' makes around 68 % of the total shell protein content. PduJ (not shown in table) is a structural homologue of PduA with 98 % similarity and makes around 18 % of the total shell protein content.

Supplementary Table S2: % distribution of secondary structure						
	PduA		**PduB**		**PduB'**	
	Protein	**Protein shells**	**Protein**	**Protein shells**	**Protein**	**Protein shells**
α- Helix	93.2	93.1	86.14	86.12	83.94	83.94
β- sheet	0.11	0.11	0.74	0.71	1	1.04

Table S2: % Distribution for the secondary structure component (alpha helix and beta sheet) of major shell proteins of Pdu BMC and their derived protein shells as determined by K2D3 (http://cbdm-01.zdv.uni-mainz.de/~andrade/k2d3/).[4] These protein shells have mainly alpha helix in their secondary structure with negligible beta sheet. Formation of protein shells do not affect the conformation of the shell proteins because neither chemical modification is carried out nor any harsh chemical or physical treatment is applied for the fabrication.

Supplementary Table S3: Size distribution of PSs (SEM)	
Protein	**Size of protein shells (Mean ± SE)**
PduA	**1086 ±184**
PduB	**1127 ± 195**
PduB'	**950 ± 130**

Table S3: Average size distribution of PSs fabricated from the major shell proteins of 1,2-Pdu BMC as observed by SEM. The average distribution is mean of more than 100 PSs observed from the 5 different frames of three different set of samples.

Supplementary Table S4: % distribution of secondary structure in PSs as function of temperature						
	PduA		**PduB**		**PduB'**	
	Protein	**Protein shells**	**Protein**	**Protein shells**	**Protein**	**Protein shells**
α- Helix	93.2	93.1	86.12	86.03	83.94	83.17
β- sheet	0.11	0.11	0.71	0.65	1.04	0.85

Table S4: % Distribution for the secondary structure component (alpha helix and beta sheet) of pre-melt PSs and post melt PSs determined by K2D3. Though mainly having alpha helical structure these SPs are quite thermally stable, which eventually is reflected in the thermal stability of the PSs as no change in the conformation is observed. The overlapping pre-melt and post-melt CD spectra as shown in Fig S7-S9 warranty the thermal stability of the PSs. http://cbdm-01.zdv.uni-mainz.de/~andrade/k2d3/[4]

Supplementary Table S5: Thermal stability of PSs			
Proteins	**SP T_m**	**PS T_m**	**ΔT**
PduA	58±1°C	66±2°C	8°C
PduB	76±2°C	93±3°C	17°C
PduB'	70±1°C	ND	ND

Table S5: Thermal stability of PSs is higher as compared to individual SPs as we observed higher T_m for the protein shells (determined by CD spectroscopy). For PduB' as observed in Figure 2.f the change in CD spectra for the PSs as a function of temperature is not observed and thus T_m is not determined for the same.

Supplementary Table S6: Rate of formation of purpurogallin formation	
Composition	**Rate of purpurogallin formation (M/min)**
CytC	$10.9 \pm 0.22 * 10^{-6}$
PduA_PSs@ CytC	$12.7 \pm 0.1 * 10^{-6}$
PduB_ PSs@ CytC	$4.28 \pm 0.78 * 10^{-6}$
PduB'_ PSs@ CytC	$2.14 \pm 0.13 * 10^{-6}$

Table S6: Rate of formation of purpurogallin by the CytC encapsulated inside the protein shells

Supplementary Table S7: Rate of Vit.B_{12} release		
	t_1 (SPs) h	**t_1 (PSs) h**
PduA	13.12 ± 6	2.41 ± 0.18
PduB	25.11 ± 14	2.16 ± 0.04
PduB'	11.89 ± 4	2.94 ± 0.6

Table S7: Rate of Vit.B_{12} release from the protein shells of PduA/B/B'

References

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