Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information for

Toward Hemocompatible Self-assembling Antimicrobial Nanofibers: Understanding the Synergistic Effect of Supramolecular Structure and PEGylation on Hemocompatibility

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Materials and supplies. MBHA rink amide resin, Fmoc-protected amino acids, 2-(6-Chloro-1-H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) were purchased from Novabiochem. Piperidine, 5(6)-Carboxyfluorescein (FAM), N-acetylcysteine (NAC) and N-2-hydroxyethylpiperazine-N-2-ethane-sulfonic acid (HEPES) was purchased from Sigma-Aldrich. PEG750-COOH was purchased from Rapp Polymere. All other reagents and solvents for peptide synthesis and purification were purchased from Fisher Scientific and used as received. Annexin-V-FLUOS was provided by Roche (Germany). Fluo-3/AM was supplied by Invitrogen (USA). 2',7'-dichlorofluorescin diacetate (DCFH-DA) was purchased from Beyotime (China). Fetal bovine serum (FBS) was purchased from Gibco (AU). Penicillin-Streptomycin and L-Glutamine were provided by Gibco (USA).

CD spectroscopy. MDPs were dissolved and diluted in Tris buffer (pH 7.4, 20 mM) to reach a final concentration of 100 μM for CD measurements (Jasco-J715 spectrometer). Samples were loaded in a 1 mm cuvette and the data were collected from 250 nm to 190 nm at room temperature with a scan rate of 100 nm/min, a response time of 2 sec and a bandwidth of 1 nm. Final spectra were an average of three scans. Ellipticity measured in mDeg was converted to molar residual ellipticity via the formula $\theta=(mDeg*1000)/(c*n*l)$, where c is the concentration of the peptide solution expressed in mM, n is the number of amino acids in the peptide sequence and l is the path length of the cell used in mm. Temperature dependent CD experiment was performed by monitoring the ellipticity at 205 nm from 5 °C to 95 °C with a heating rate of 0.5 °C/min. The transition temperature was determined by applying first derivative function on the melting curve.

Negatively stained TEM. MDPs were dissolved and diluted in Tris buffer (pH=7.4, 20 mM) to reach a final concentration of 100 μM. 10 μl peptide solution was dropped onto a holey carbon grid (TED PELLA 01824). After 1 min, the excess solution was carefully removed with filter paper and the sample was stained by adding 10 μl of 2 wt% uranyl acetate solution for 1 min. The excess staining solution was removed with filter paper and the TEM sample was allowed to dry for overnight before imaging on a JEOL 2010 high-resolution transmission electron microscope.

SAXS Characterization. The SAXS experiments were performed using the automated BM29 bioSAXS beamline at the ESRF, Grenoble, France. Technical details were referred to the established literature protocol.[1] The data were obtained using an energy of 12.5 keV and a detector distance 2.87 m covering a Q-range ($Q=4\pi \sin(\theta/2)/\lambda$, λ is the wavelength, θ is the scattering angle) of about 0.0047 $Å^{-1} < Q < 0.5$ $Å^{-1}$. The data were calibrated to absolute intensity scale using water as a primary standard. The data were analysed using the Indirect Fourier transform (IFT)[2] routine implemented in the data program GNOM in the ATSAS package.[3] This model-independent approach allows the pair distribution function, p(r), describing the correlation between pairs of scattering points to be determined.

Erythrocyte preparation. All human subjects involved in the study were approved through written informed consent by the Medical Ethnics Committee of the Second Affiliated Hospital affiliated with the Third Medical University. Leukocyte-free erythrocytes from healthy donors were used shortly after donation (stored no more than 24 hrs) and were provided by the Chongqing Blood Center. Hematocrit was adjusted to 0.4% with Ringer solution (125 mM NaCl, 32 mM HEPES, 5 mM glucose, 5 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, pH7.4).

Measurement of intracellular ROS. HRBCs were incubated with MDPs at varying concentrations for 24 hours at 37°C and 5% CO_2 with 95% humidity. RBCs were washed with Ringer solution for three times followed by incubation with 10 μM DCFH-DA in Ringer solution in dark for 30 min at 37°C. Fluorescence intensity were measured by FACS Calibur (BD, USA) in fluorescence channel FL-1 (488 nm excitation and 530 nm emission). The data were analyzed using Flowjo software (Treestar, USA).

Measurement of cytosolic Ca^{2+}. HRBCs were incubated with MDPs at varying concentrations for 24 hours at 37°C and 5% CO_2 with 95% humidity. RBCs were resuspended in Ringer solution containing 5 mM of $CaCl_2$. 5 μM of Fluo-3 AM was added to measure cytosolic Ca^{2+}. After 30 min incubation at 37°C, RBC was washed three times using Ringer solution containing 5 mM $CaCl_2$. Fluorescence intensity of Fluo-3 was measured by FACS Calibur (BD, USA) in fluorescence channel FL-1 (488 nm

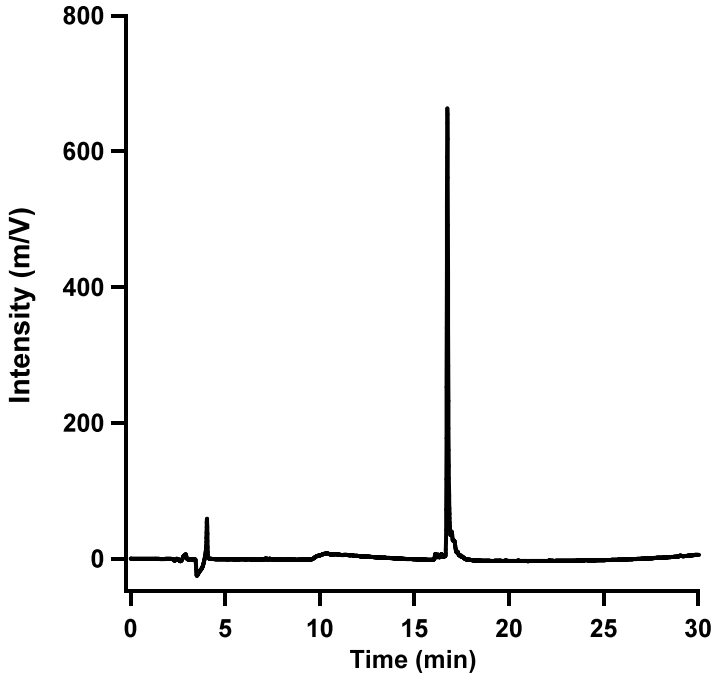
excitation and 530 nm emission). The data were analyzed using Flowjo software (Treestar, USA).

Bacterial culture. In this work, *E.coli* (25922) was purchased from ATCC *and P. aeruginosa* (15442) *S. aureus (6538)* were ordered from Presque Isle Cultures. All bacteria were cultured in MHB (Fisher) under constant shaking at 100 rpm at 37ºC. Bacteria were used upon reaching the mid-exponential growth phase.

Minimum inhibitory concentration (MIC) measurements. MICs of P-W362 and P-3W62 against *E.coli, P. aeruginosa* and *S. aureus* was measured by following the broth micro-dilution method. First, bacterial suspension was diluted to approximately 10^5 CFU/mL in MHB medium. Then, 10 μL of peptide solution at varying concentrations (800, 400, 200, 100, 50 and 25 μM) was added to 90 μL bacterial solution in a 96-well plate. Bacterial suspensions UV absorbance at 600 nm was measured after 18 hrs of incubation for *E. coli* and *P. aeruginosa,* and 36 hrs of incubation for *S. aureus*. The MIC was determined as the minimum concentration of the peptides required to completely inhibit the growth of bacteria by using pure broth as a negative control. Each MIC test was repeated 3 times using 3 replicates.

Table 1. MIC results of W362, 3W62, P-W362, and P-3W62 against *E. coli, P. aeruginosa* and *S. aureus*.

Peptide sequence	MIC (μM)		
	E.C.	**P.A.**	**S.A.**
W362	20	20	10
3W62	80	80	20
P-W362	20	20	10
P-3W62	80	80	10



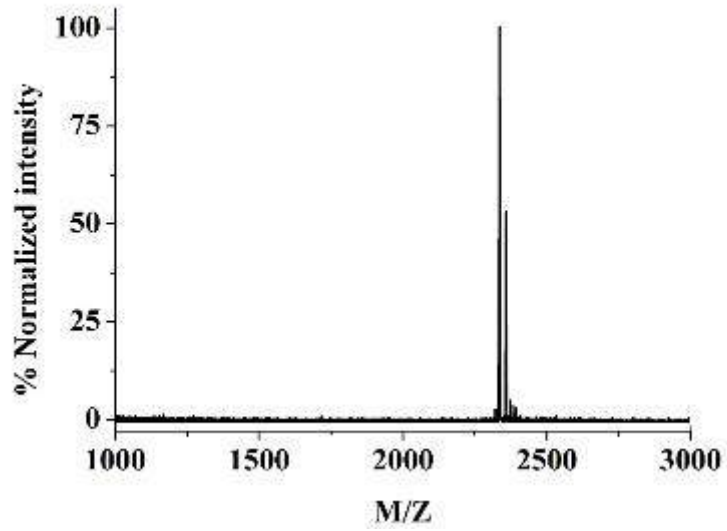


Figure S1. (a) HPLC and MALDI spectra of purified W362

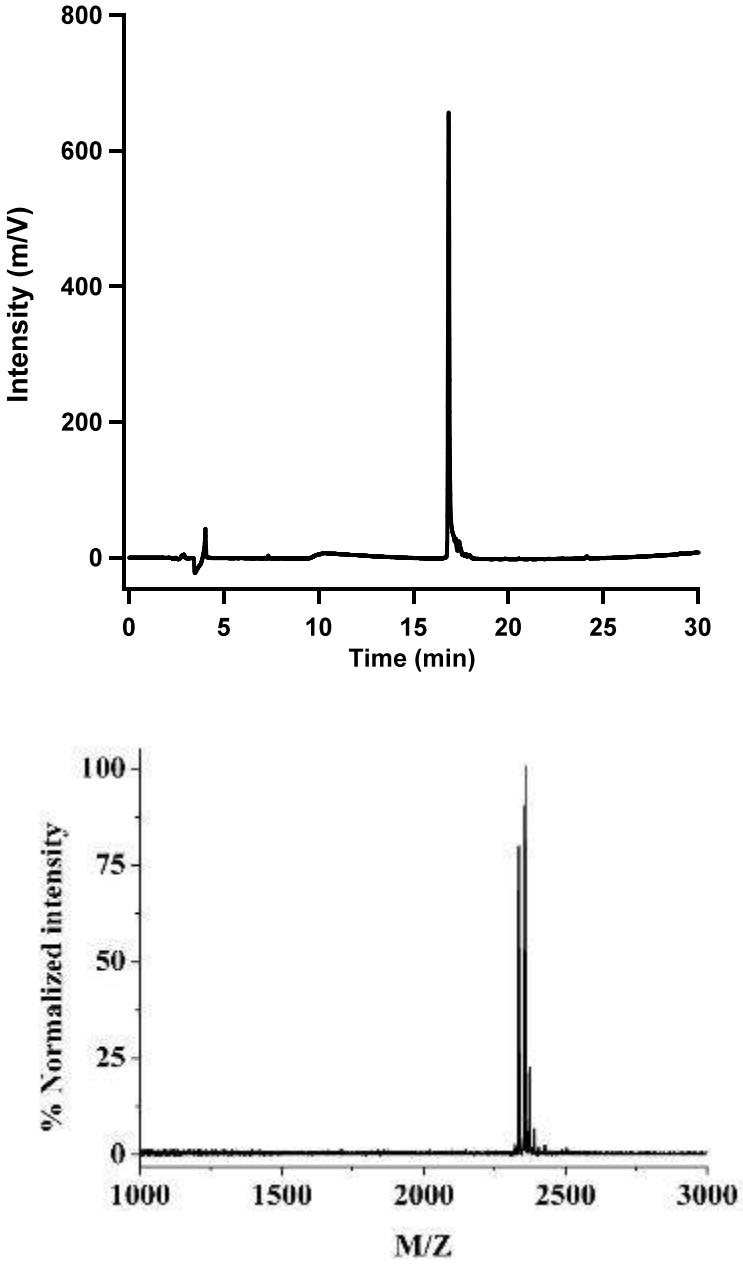


Figure S1. (b) HPLC and MALDI spectra of purified 3W62

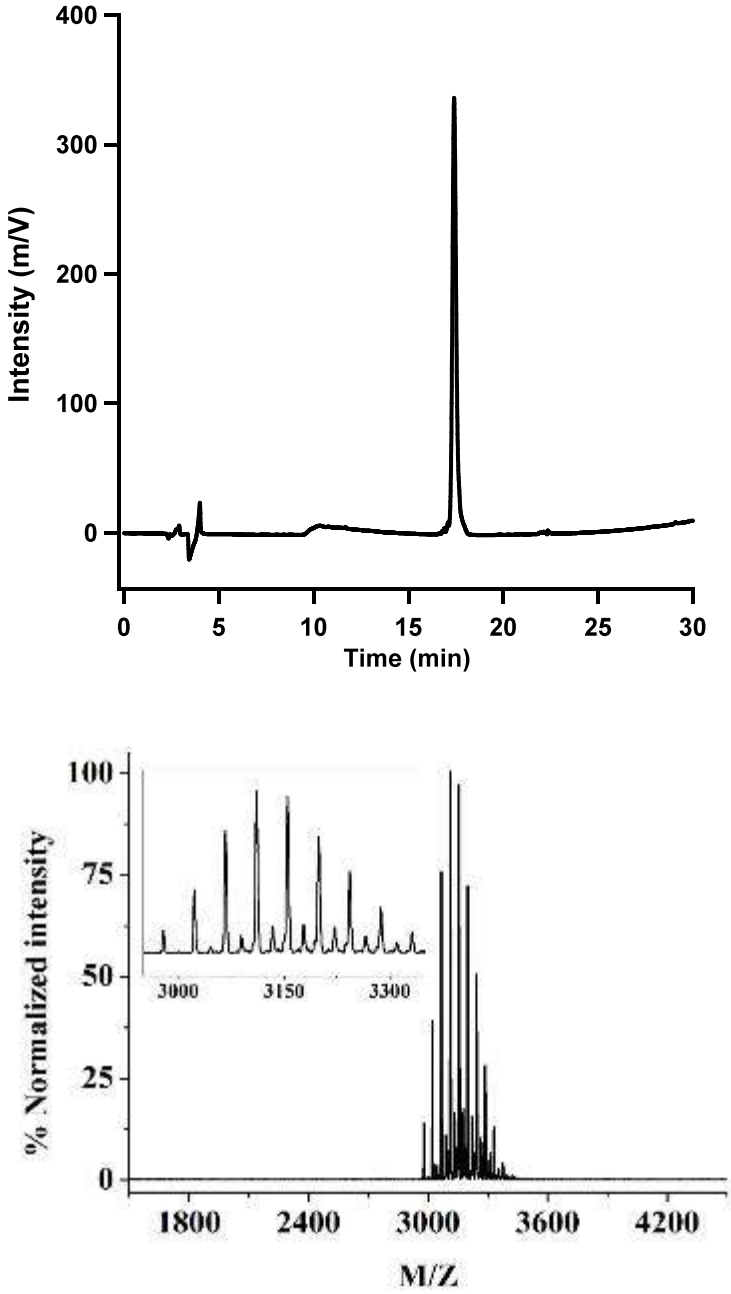


Figure S1. (c) HPLC and MALDI spectra of purified P-W362

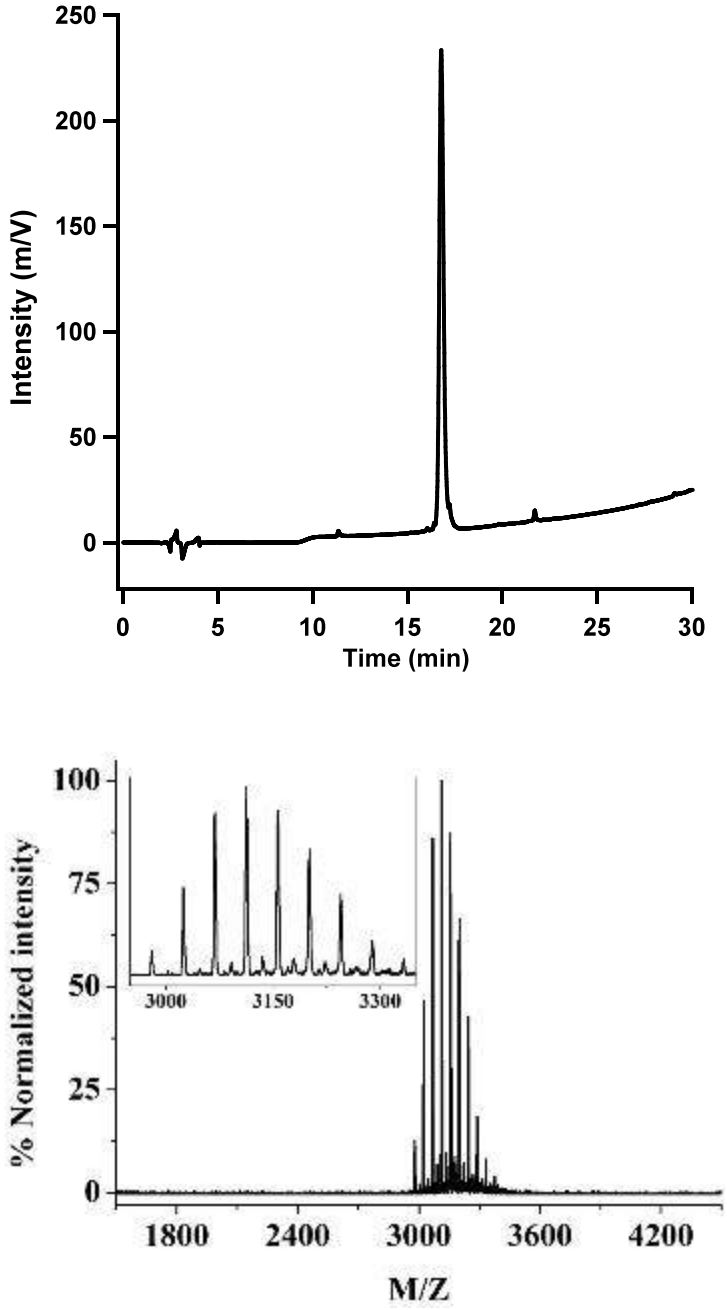


Figure S1. (d) HPLC and MALDI spectra of purified P-3W62.

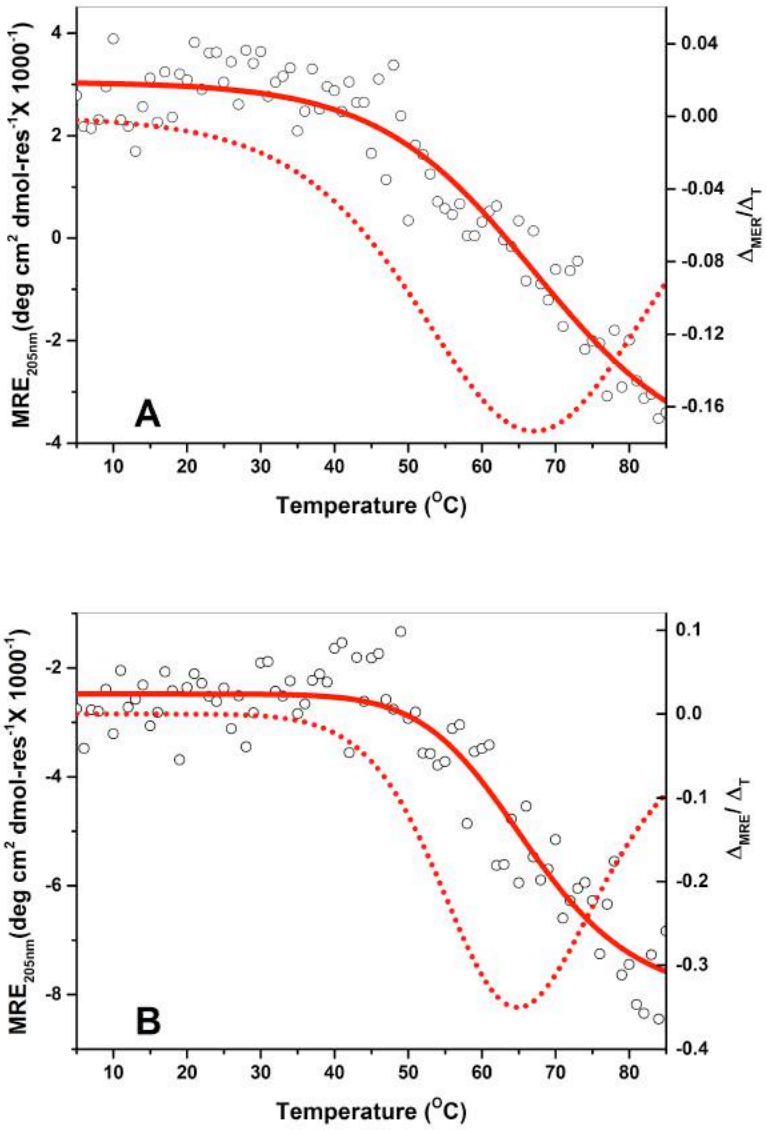


Figure S2. Melting curves of 3W62 (top) and P-3W62 (bottom) to qualitatively compare the supramolecular stability of β-sheet nanofibers. Black circle: experimental data by monitoring the ellipticity at 205 nm as a function of temperature. Solid red curve: fitted data. Dashed red curve: first derivative function to determine Tm. Tm (3W62) = 67 °C. Tm (P-3W62) = 65 °C.

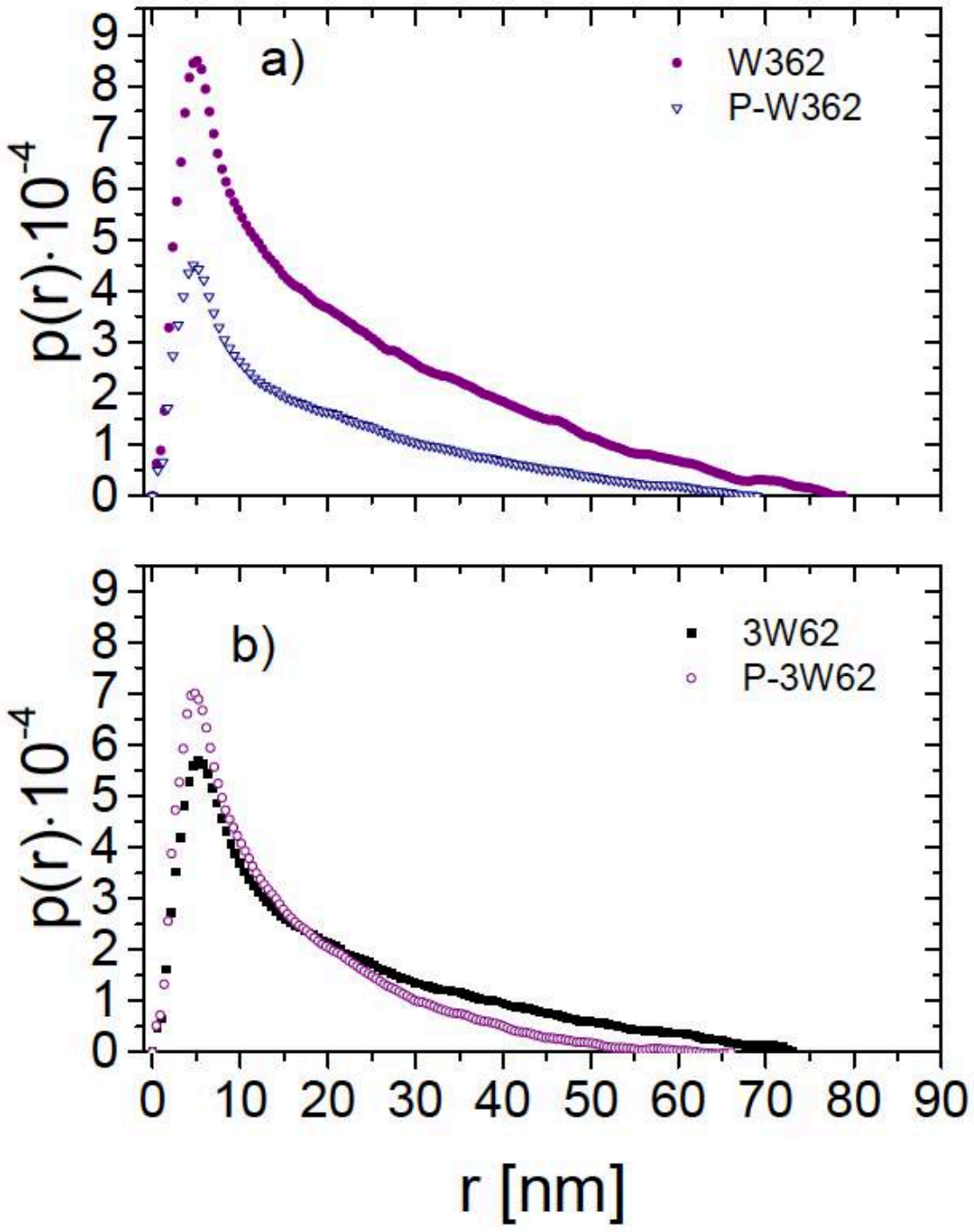


Figure S3: The extracted pair distribution function obtained from IFT showing the presence of elongated nanostructure for all MDP assembly.

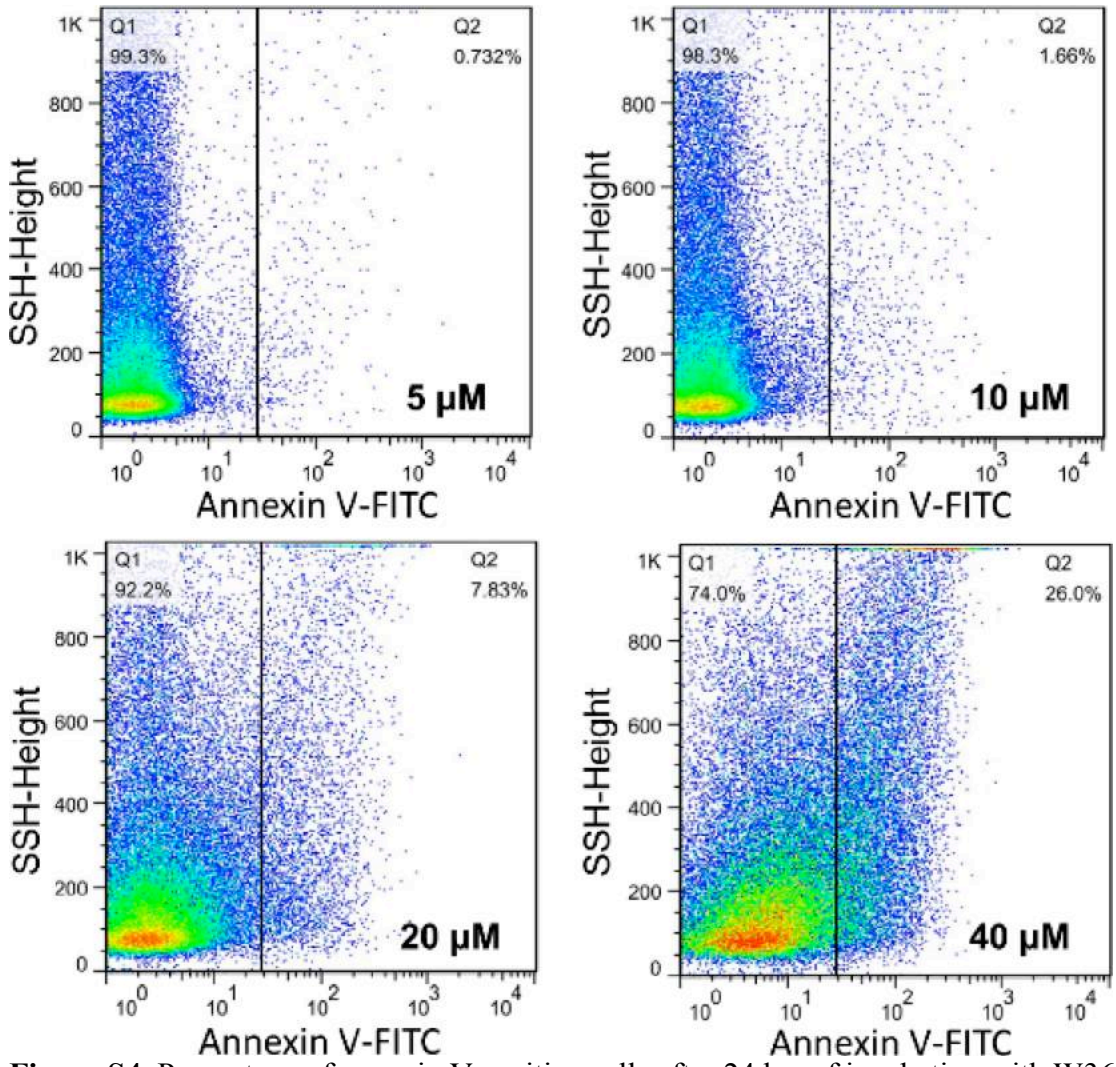


Figure S4. Percentage of annexin V positive cells after 24 hrs of incubation with W362 at 5 µM, 10 µM, 20 µM, 40 µM determined by flow cytometry.

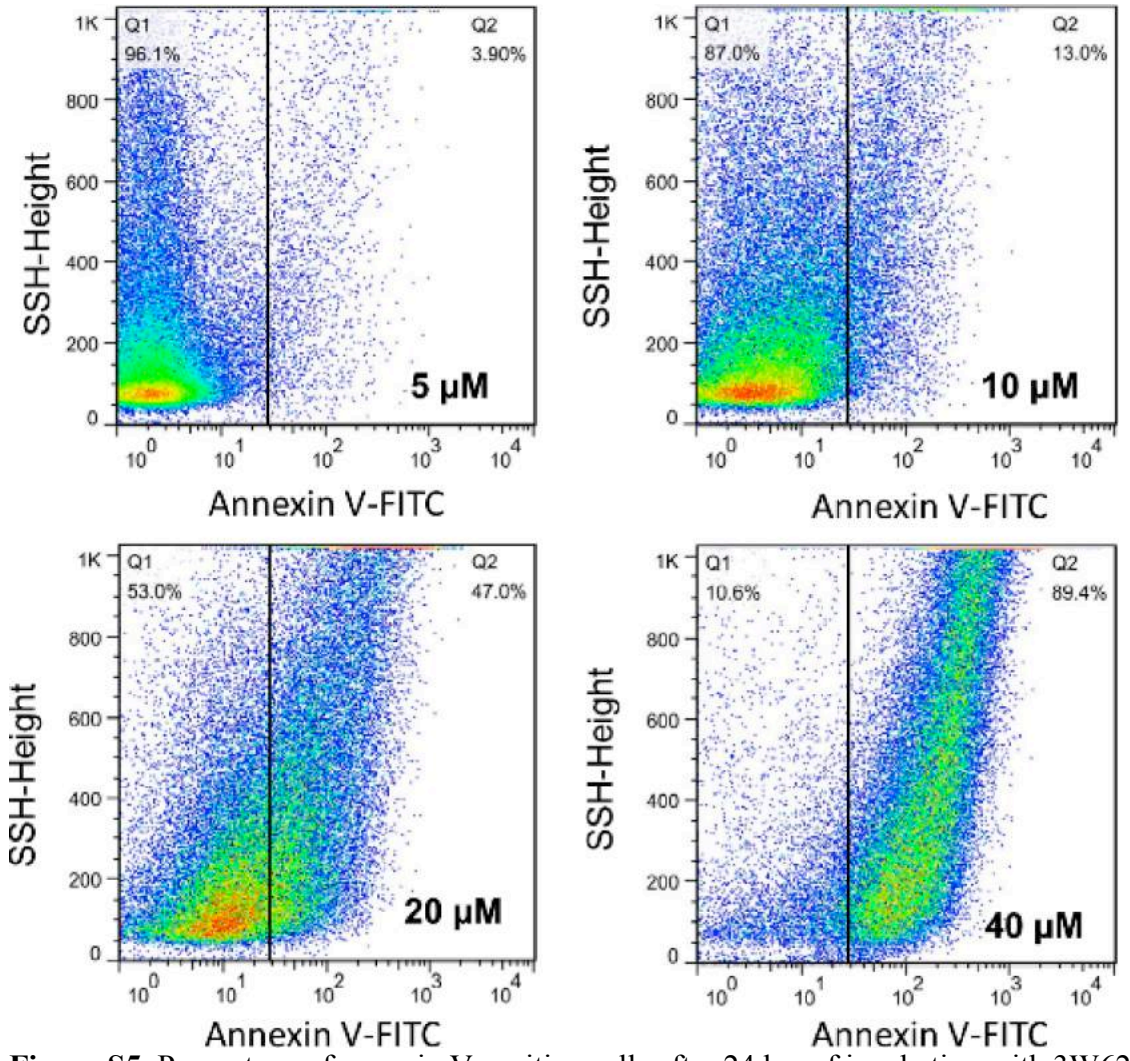


Figure S5. Percentage of annexin V positive cells after 24 hrs of incubation with 3W62 at 5 µM, 10 µM, 20 µM, 40 µM determined by flow cytometry.

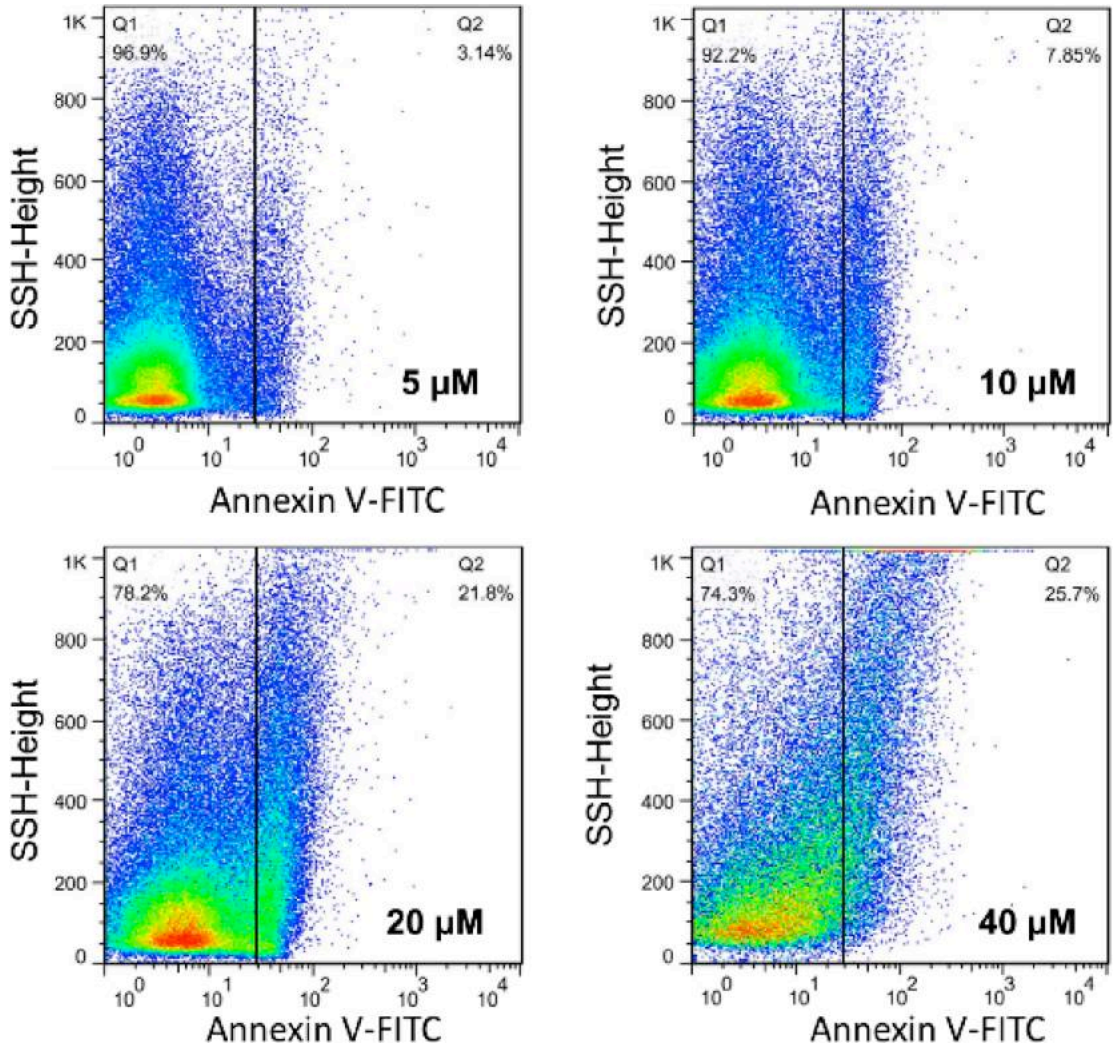


Figure S6. Percentage of annexin V positive cells after 24 hrs of incubation with P-W362 at 5 µM, 10 µM, 20 µM, 40 µM determined by flow cytometry.

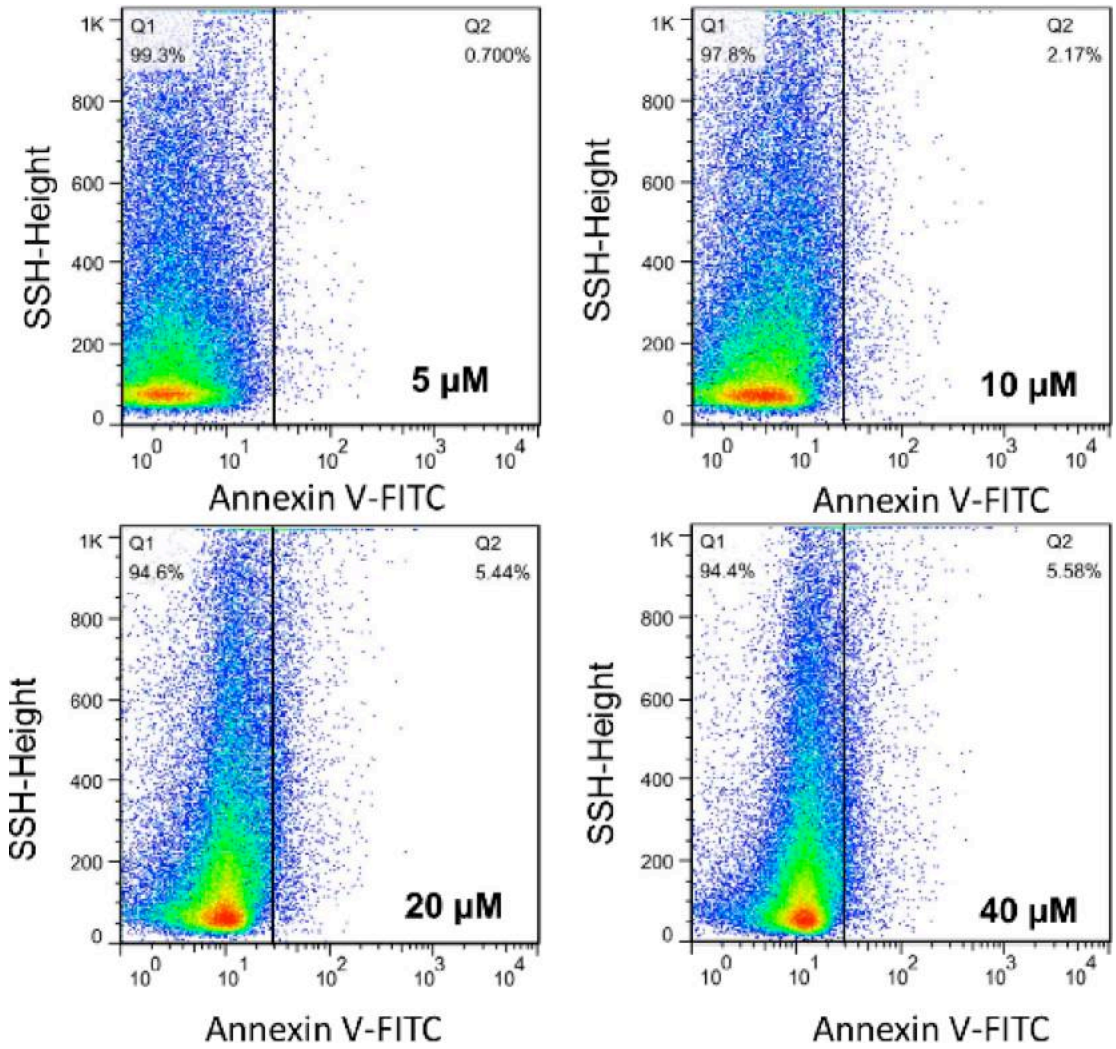


Figure S7. Percentage of annexin V positive cells after 24 hrs of incubation with P-3W62 at 5 µM, 10 µM, 20 µM, 40 µM determined by flow cytometry.

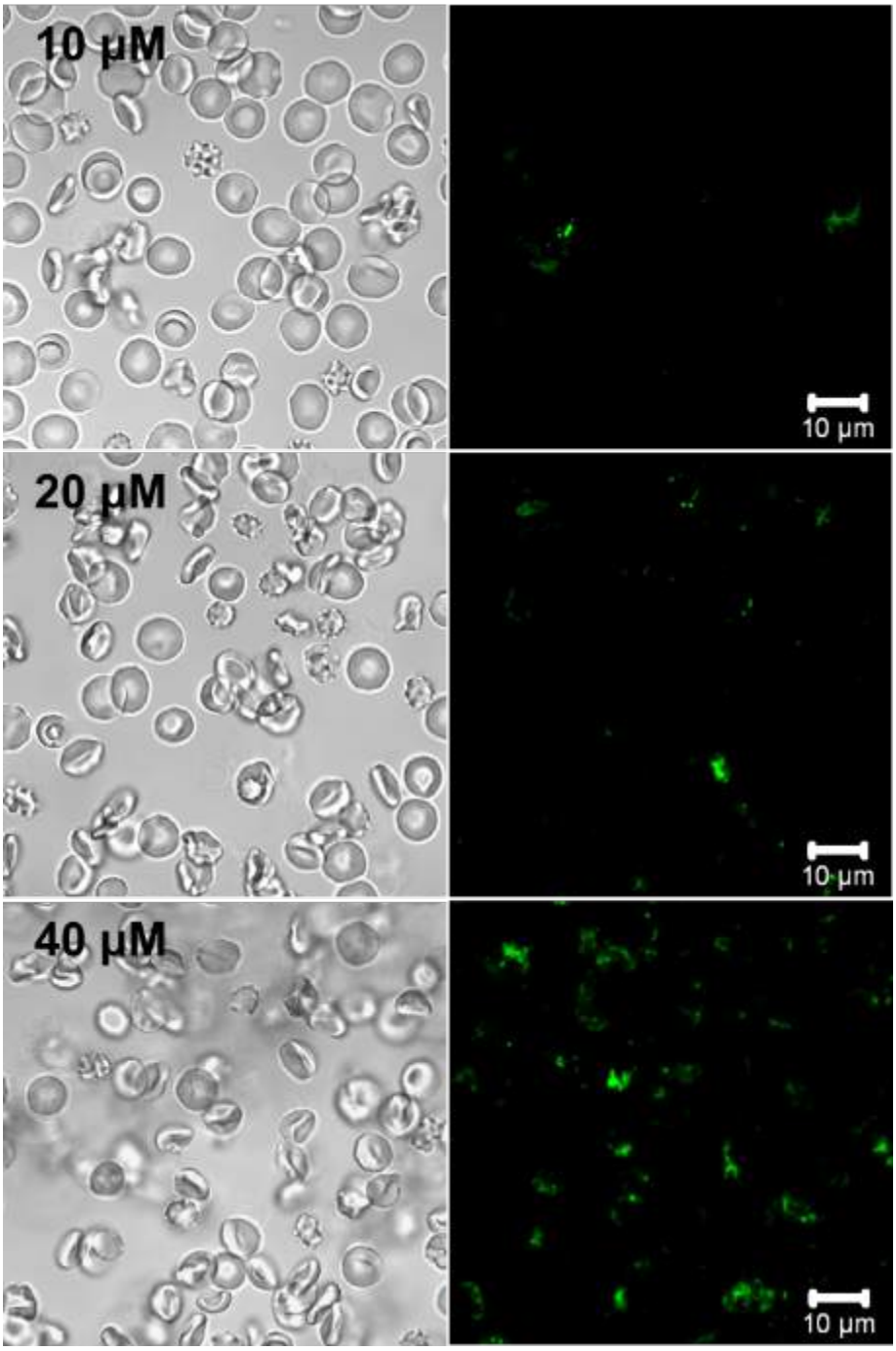


Figure S8. Confocal bright field and fluorescence images of fluorescein labeled annexin V positive RBCs after 24 hrs of incubation with W362 at 10 µM, 20 µM, 40 µM.

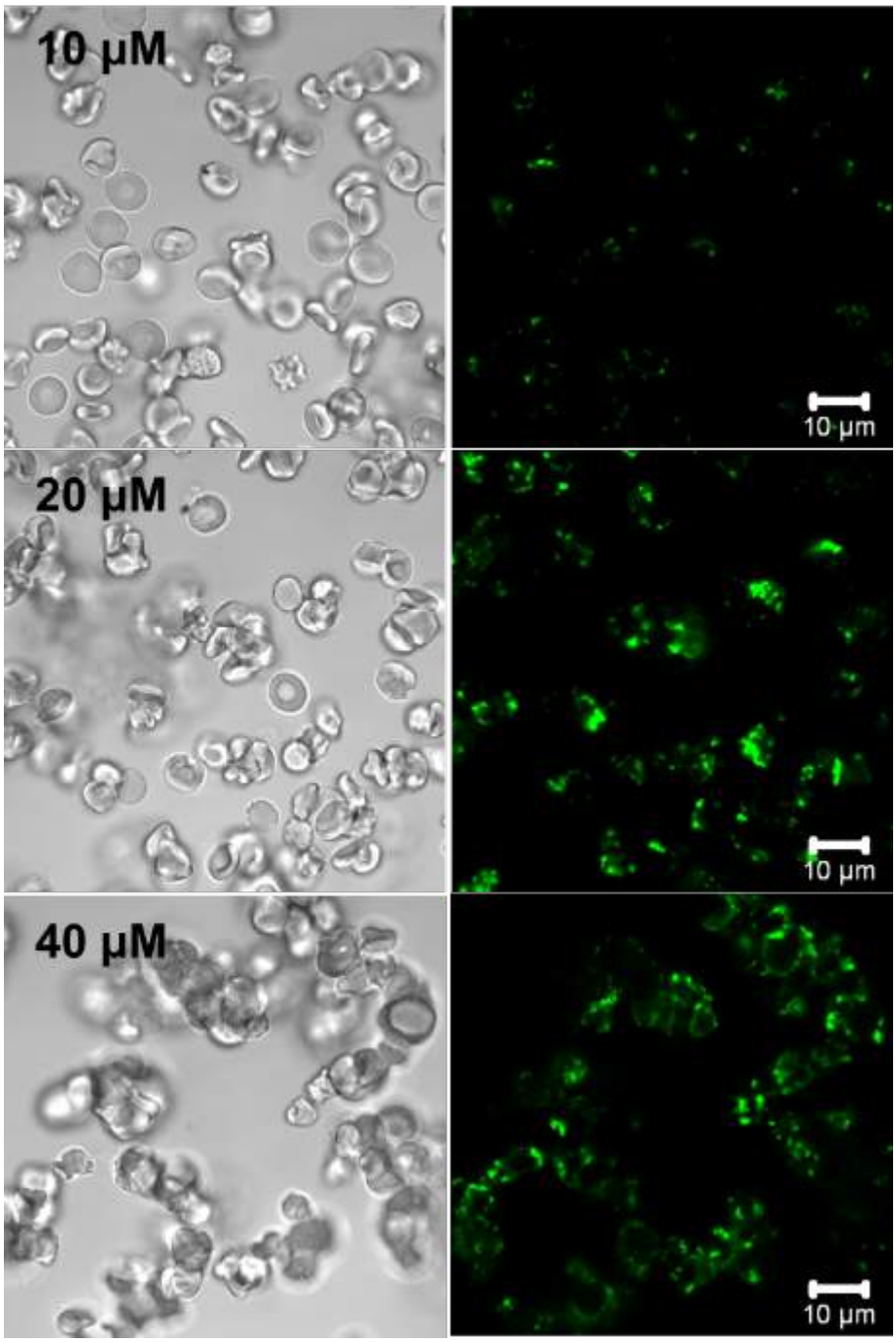


Figure S9. Confocal bright field and fluorescence images of fluorescein labeled annexin V positive RBCs after 24 hrs of incubation with 3W62 at 10 µM, 20 µM, 40 µM.

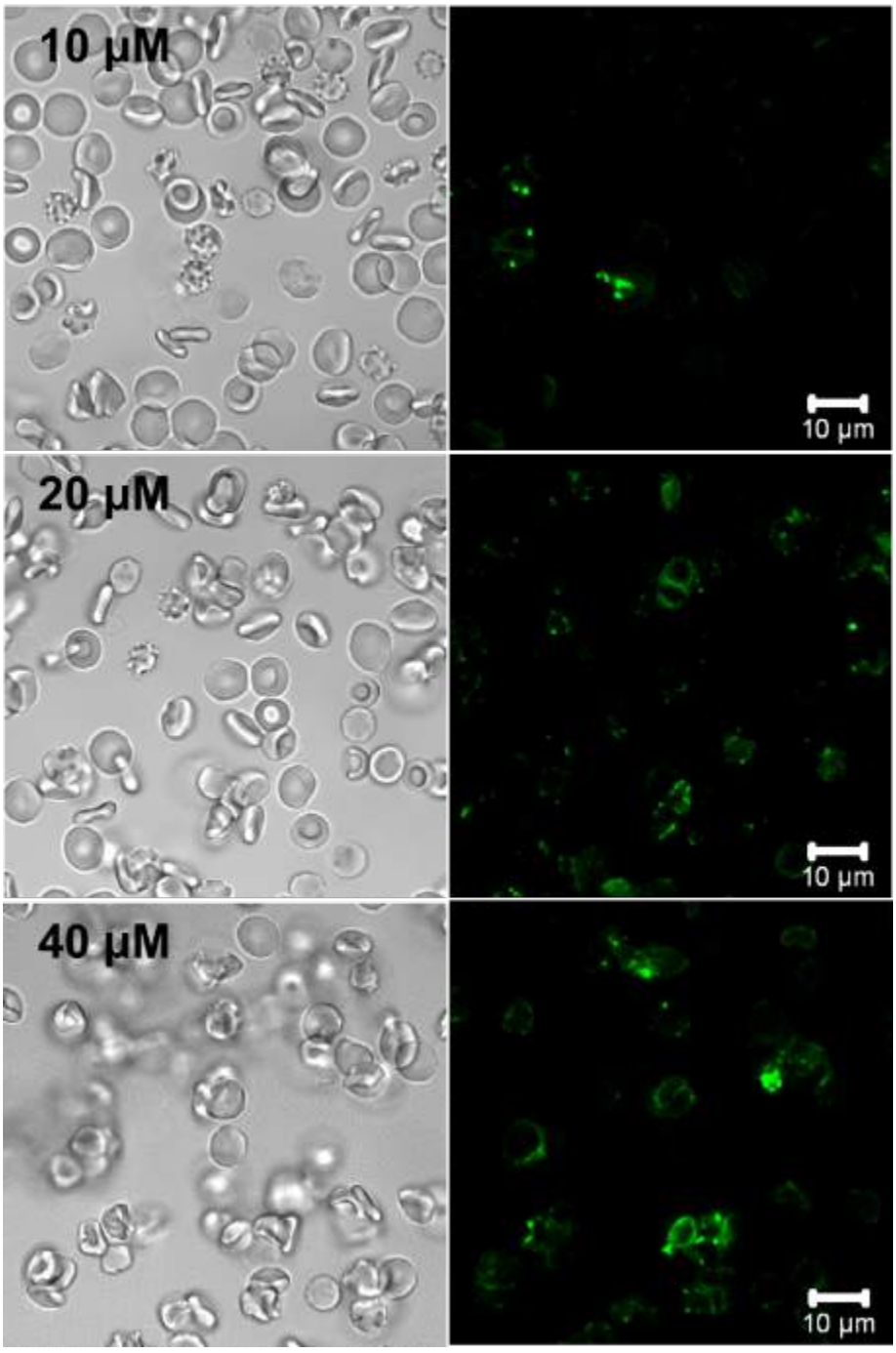


Figure S10. Confocal bright field and fluorescence images of fluorescein labeled annexin V positive RBCs after 24 hrs of incubation with P-W362 at 10 µM, 20 µM, 40 µM.

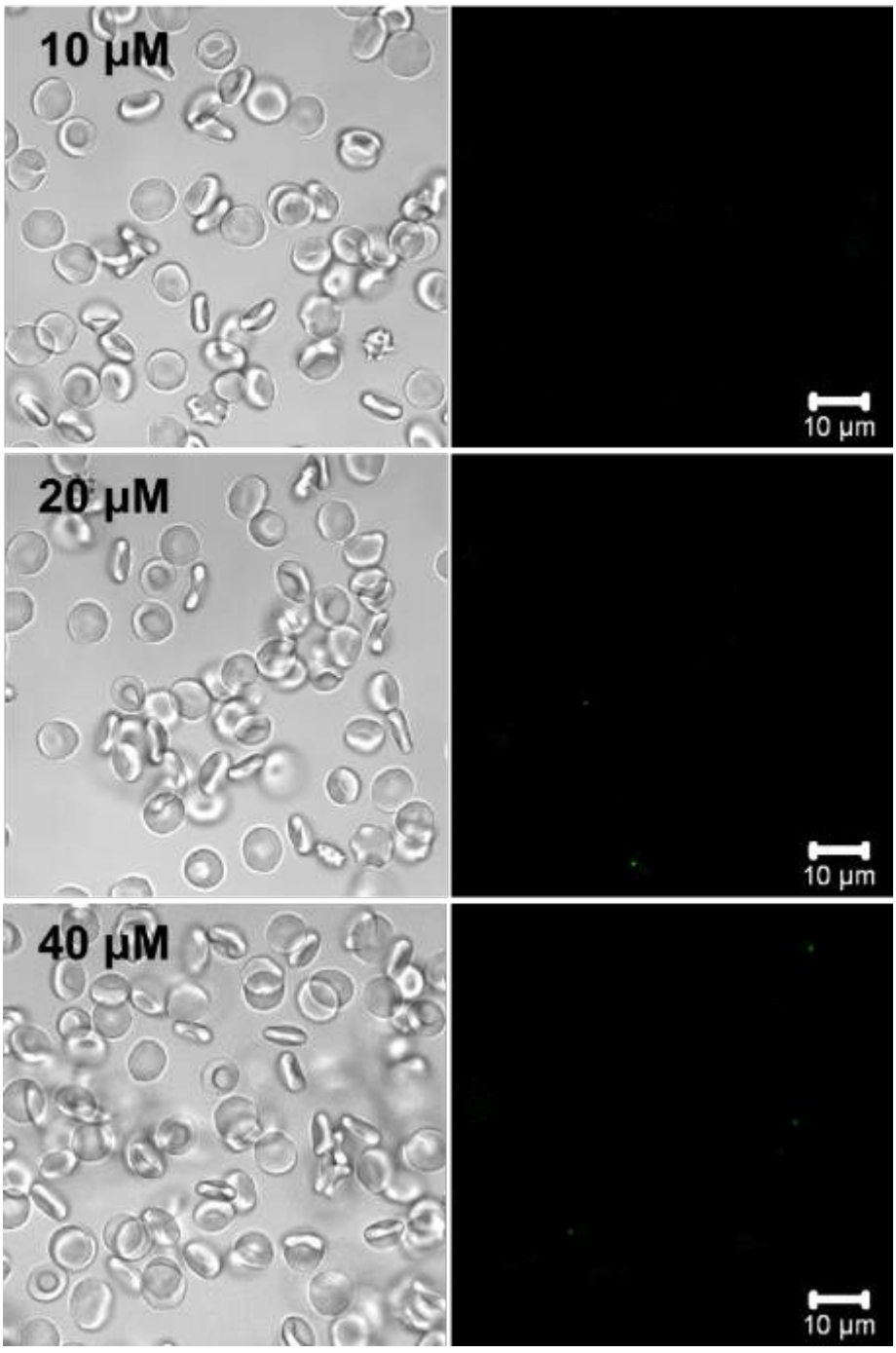


Figure S11. Confocal bright field and fluorescence images of fluorescein labeled annexin V positive RBCs after 24 hrs of incubation with P-3W62 at 10 µM, 20 µM, 40 µM.

References

1. P. Pernot, A. Round, R. Barrett, A. D. Antolinos, A. Gobbo, E. Gordon, J. Huet, J. Kieffer, M. Lentini, M. Mattenet, C. Morawe, C. Mueller-Dieckmann, S. Ohlsson, W. Schmid, J. Surr, P. Theveneau, L. Zerrad and S. McSweeney, *J. Synchrot. Radiat.*, 2013, **20**, 660-664.
2. O. Glatter, *J. Appl. Crystallogr.*, 1977, **10**, 415-421.
3. M. V. Petoukhov, D. Franke, A. V. Shkumatov, G. Tria, A. G. Kikhney, M. Gajda, C. Gorba, H. D. T. Mertens, P. V. Konarev and D. I. Svergun, *J. Appl. Crystallogr.*, 2012, **45**, 342-350.