Supporting Information

A Sulfonated Diblock Copolymer Exhibits Reversible Antiplatelet Activity and Enables Aqueous Dipyridamole Formulation

Aleksandra Frackiewicz*[a], Karol Depczynski[a], Magdalena Obłoza[b], Shin-ichi Yusa[c], Amarjargal Altangerel[d], Amir Fahmi[d], Dariusz Pawlak[a], Krzysztof Szczubiałka[b], Andrzej Mogielnicki[a] and Bartlomiej Kalaska**[a]

[a] Department of Pharmacodynamics, Medical University of Bialystok, Mickiewicza 2C St., 15-089 Bialystok, Poland. E-mail: *aleksandra.frackiewicz@umb.edu.pl, **bartlomiej.kalaska@umb.edu.pl

[b] Faculty of Chemistry, Jagiellonian University, Gronostajowa 2 St., 30-387 Krakow, Poland

[c] Department of Applied Chemistry, Graduate School of Engineering, University of Hyogo, 167 Shosha, Himeji 671-2280, Japan

[d] Faculty of Technology and Bionics, Rhine-Waal University of Applied Sciences, Marie-Curie-Str. 1, 47533 Kleve, Germany

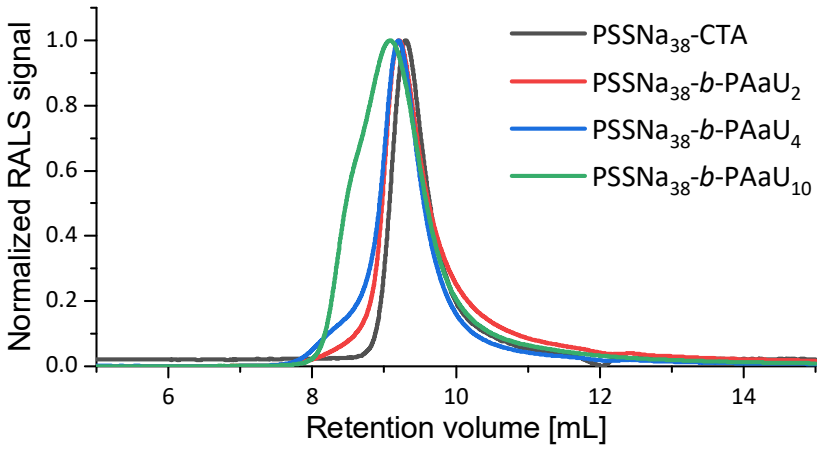

Figure S1. GPC chromatograms of $PSSNa_{38}$-*b*-$PAaU_n$ copolymers measured at a 0.8 ml/min flow rate. A 0.1 M $NaNO_3$ aqueous solution containing 20% v/v acetonitrile was used as eluent.

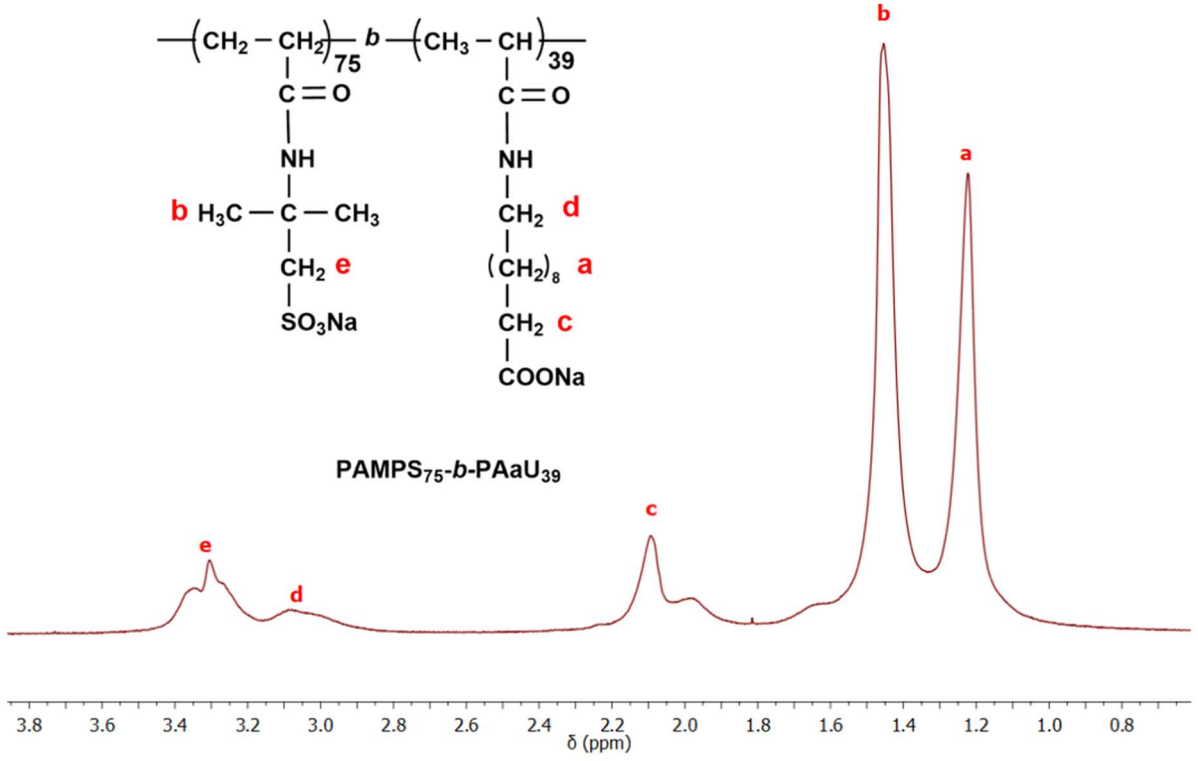

Figure S2. ^{1}H NMR spectrum for $PAMPS_{75}$-*b*-$PAaU_{39}$ at C_p = 10 g/L in D_2O at pH 10. DP_n of the PAaU block was estimated by comparing the intensities of signals *c* and *b*.

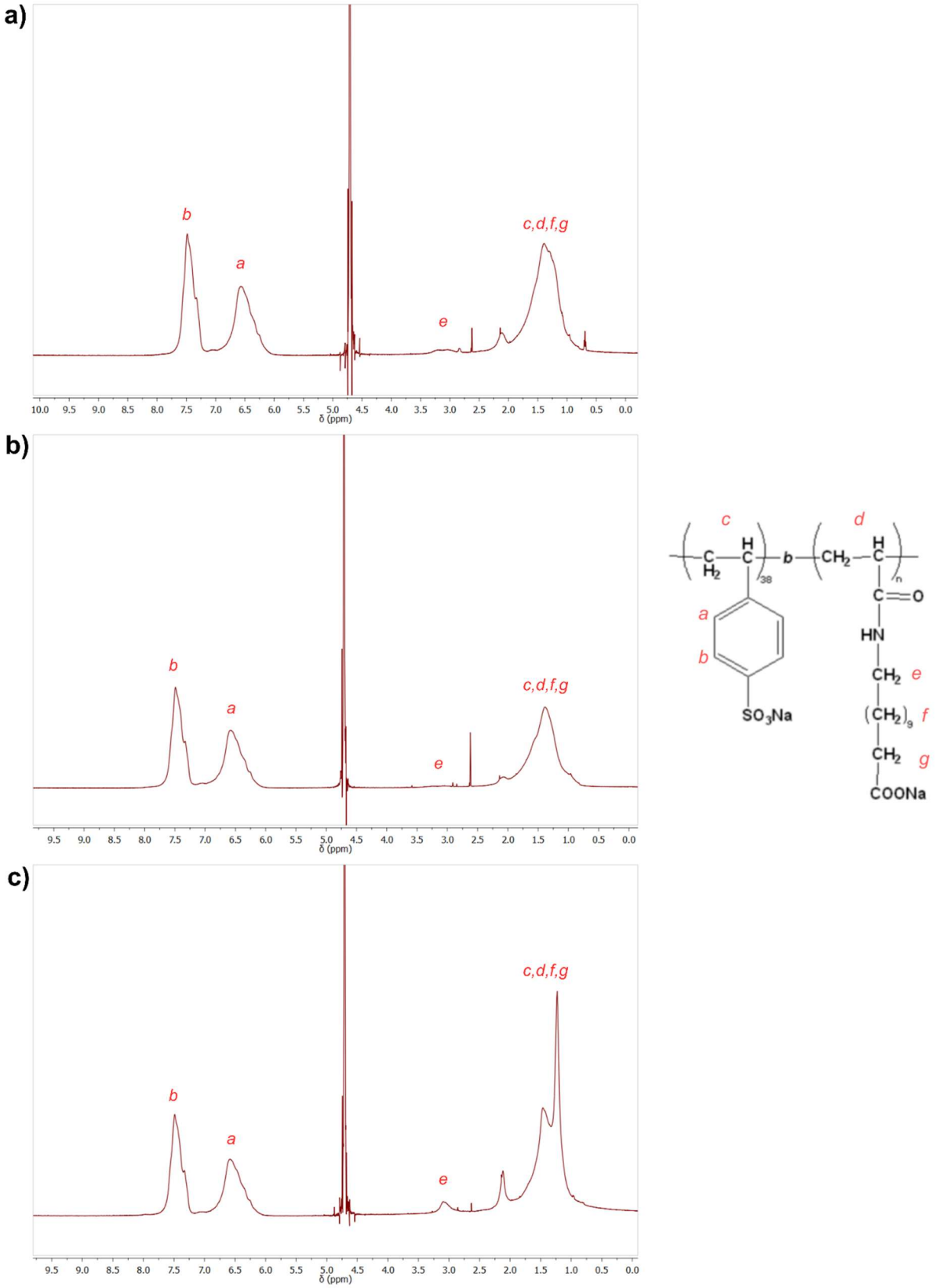

Figure S3. ^{1}H NMR spectra of PSSNa$_{38}$-*b*-PAaU$_2$ (a), PSSNa$_{38}$-*b*-PAaU$_4$ (b), and PSSNa$_{38}$-*b*-PAaU$_{10}$ (c) in D_2O.

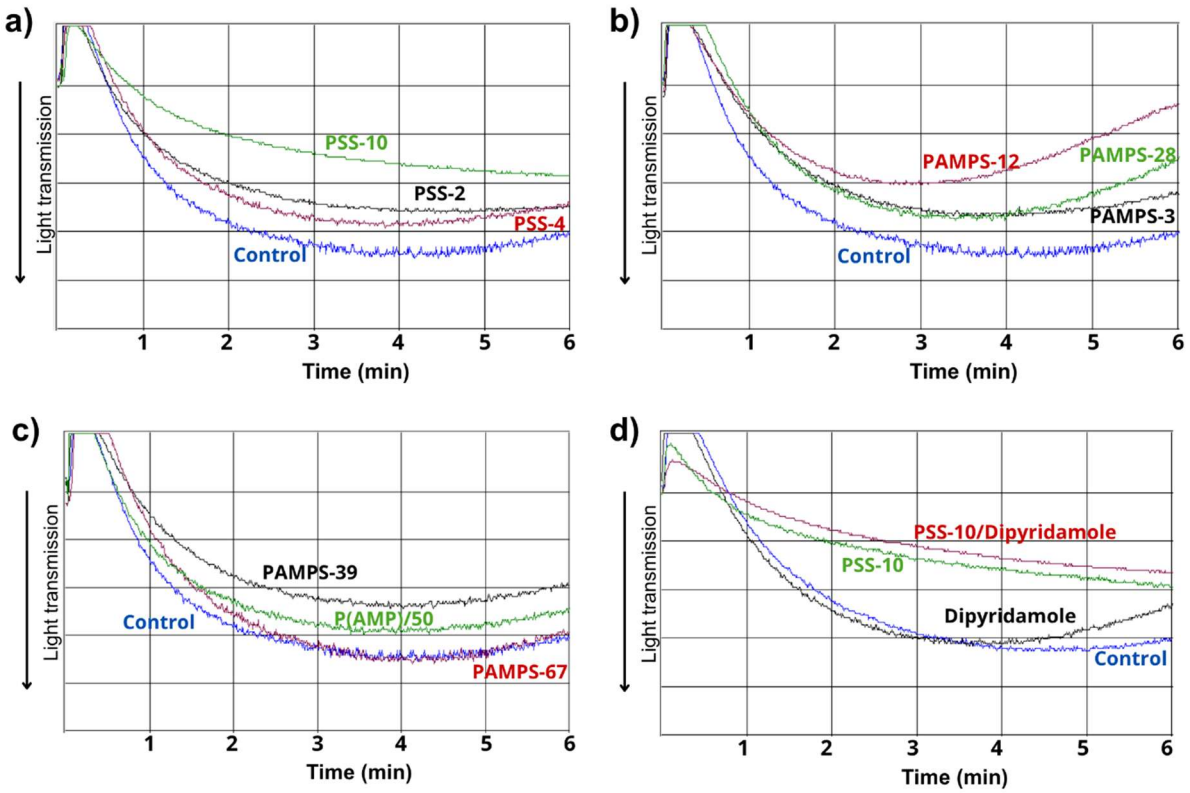

Figure S4. Representative raw aggregation tracings of **a)** $PSSNa_{38}$-*b*-$PAaU_2$ (PSS-2; 100 µg/mL), $PSSNa_{38}$-*b*-$PAaU_4$ (PSS-4; 100 µg/mL), $PSSNa_{38}$-*b*-$PAaU_{10}$ (PSS-10; 100 µg/mL), **b)** $PAMPS_{75}$-*b*-$PAaU_3$ (PAMPS-3; 100 µg/mL), $PAMPS_{75}$-*b*-$PAaU_{12}$ (PAMPS-12; 100 µg/mL), $PAMPS_{75}$-*b*-$PAaU_{39}$ (PAMPS-39; 100 µg/mL), **c)** $PAMPS_{75}$-*b*-$PAaU_{67}$ (PAMPS-67; 100 µg/mL), P($AMPS_{50}$-*ran*-AaU_{50}) (P(AMP)/50; 100 µg/mL), and **d)** dipyridamole (10 µg/mL), $PSSNa_{38}$-*b*-$PAaU_{10}$ (PSS-10; 190 µg/mL), and $PSSNa_{38}$-*b*-$PAaU_{10}$/dipyridamole system (PSS-10/D; 200 µg/mL) recorded by the Chronolog aggregometer in PRP.

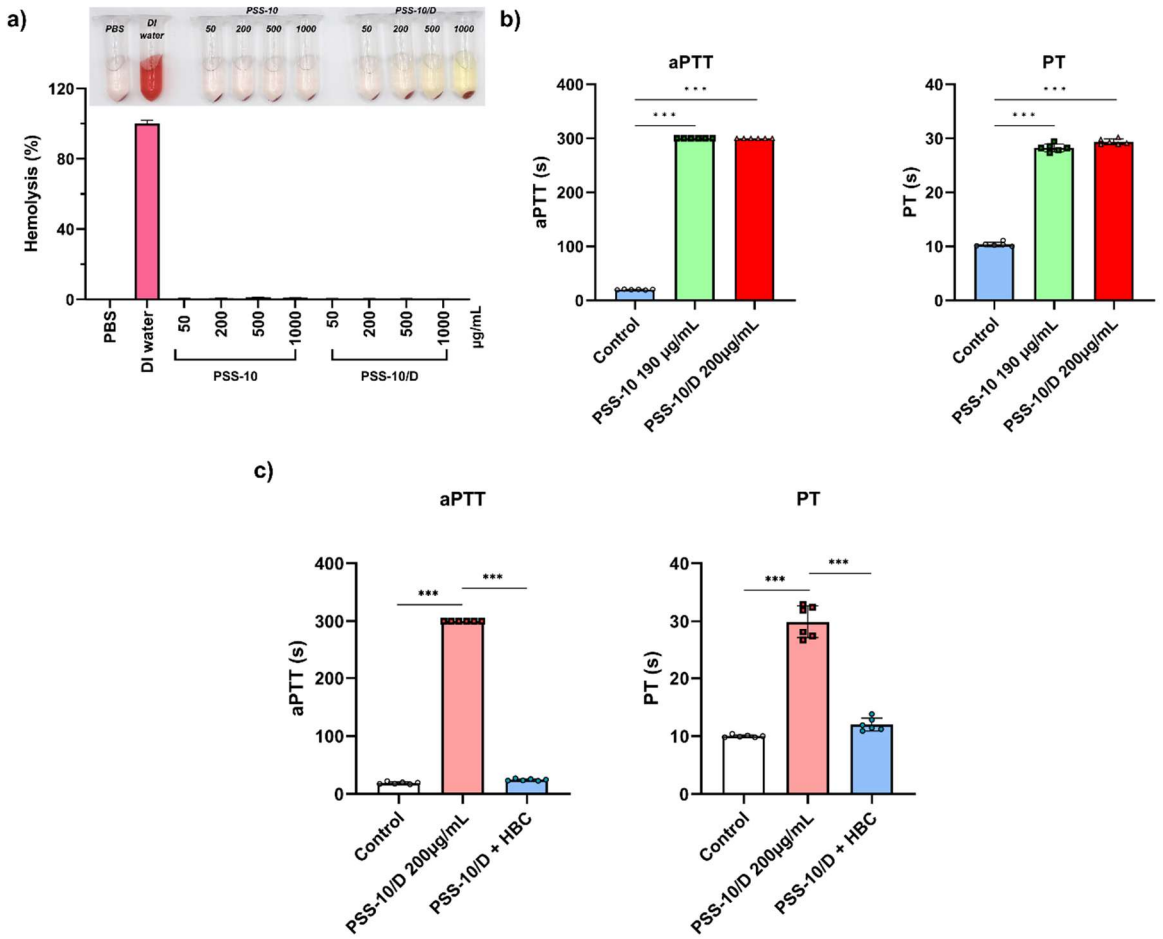

Figure S5. Hemocompatibility, activated partial thromboplastin time (aPTT) and prothrombin time (PT)-based assessment of anticoagulant activity, and heparin-binding copolymer (HBC)-mediated reversal of the anticoagulant effect of the PSSNa$_{38}$-*b*-PAaU$_{10}$/dipyridamole system (PSS-10/D) *in vitro*. a) Effect of PSSNa$_{38}$-*b*-PAaU$_{10}$ (PSS-10) and PSS-10/D on the mean percentage of total hemolysis *in vitro* in rat blood, compared to the negative control (PBS) and the positive control (deionized (DI) water). The image shows no hemolytic effect induced by the polymer or the polymer-dipyridamole system at the tested concentrations. **b)** Effects of the PSS-10 (190 µg/mL) and PSS-10/D (200 µg/mL) on plasma coagulation parameters, including aPTT and PT (n = 6). **c)** Reversal of the anticoagulant effect of PSS-10/D by complexation with HBC, assessed by aPTT and PT in plasma (n = 6). Data are presented as mean ± SD; individual points represent independent measurements. ***$p < 0.001$.

Table S1. Reaction mixture composition for the synthesis of $PSSNa_{38}$-*b*-$PAaU_n$ polymers.

Samples	Concentration		
	[AaU] (M)	**[PSSNa-CTA] (mM)**	**[ACVA] (mM)**
$PSSNa_{38}$-*b*-$PAaU_2$	0.5	92.0	5.0
$PSSNa_{38}$-*b*-$PAaU_4$	0.5	46.0	1.5
$PSSNa_{38}$-*b*-$PAaU_{10}$	0.5	23.0	1.0

Table S2. Number- and weight-average molecular weight (M_n and M_w), dispersion index (M_w/M_n) of $PSSNa_{38}$ - CTA (macro-CTA) and $PSSNa_{38}$-*b*-$PAaU_n$ copolymers, and the number-average degree of polymerization (DP_n) of the PAaU blocks.

Polymer	**M_n ($\cdot 10^4$) (GPC)**	**M_w ($\cdot 10^4$) (GPC)**	**M_w/M_n**	**DP_n (PAaU, NMR)**	**DP_n (PAaU, EA)**
$PSSNa_{38}$-CTA	0.81	0.95	1.17	-	-
$PSSNa_{38}$-*b*-$PAaU_2$	0.92	1.16	1.27	2	2
$PSSNa_{38}$-*b*-$PAaU_4$	1.53	1.91	1.25	4	4
$PSSNa_{38}$-*b*-$PAaU_{10}$	1.95	2.62	1.34	10	10

Table S3. Molecular weight and compositions of the $PAMPS_{75}$-*b*-$PAaU_m$ and P($AMPS_{50}$-*ran*-AaU_{50}) polymers.

Polymer	M_n [a] ($\cdot 10^4$) (NMR)	M_w [b] ($\cdot 10^4$) (GPC)	M_w/M_n	DP_n (PAMPS, GPC)[c]	DP_n (PAaU, NMR)[d]
$PAMPS_{75}$-*b*-$PAaU_{12}$	1.98	4.53	1.42	75	12
$PAMPS_{75}$-*b*-$PAaU_{39}$	2.73	9.36	1.23	75	39
$PAMPS_{75}$-*b*-$PAaU_{67}$	3.51	2.93	1.40	75	67
P($AMPS_{50}$-*ran*-AaU_{50})	4.38[b]	9.90[e]	2.26[e]	50[f]	50[f]

[a] Determined by ^{1}H NMR.

[b] Determined by GPC in H_2O/CH_3CN (80/20, v/v) 0.1 M $NaNO_3$ solution calibrated with poly (sodium 4-styrenesulfonate) standards.

[c] Number-average degree of polymerization of the PAMPS blocks determined by GPC in H_2O/CH_3CN (80/20, v/v) 0.1 M $NaNO_3$ solution calibrated with poly (sodium styrene sulfonate) standards.

[d] Number-average degree of polymerization of the PAaU blocks determined by ^{1}H NMR in D_2O.

[e] Determined by GPC using a mixed solvent of water and DMF (50/50, v/v) containing 50 mM LiBr as the eluent.

[f] % mol, determined by ^{1}H NMR in D_2O at 95ºC.

Table S4. The values of hydrodynamic diameter (d), and zeta potentials for $PSSNa_{38}$-*b*-$PAaU_n$, $PAMPS_{75}$-*b*-$PAaU_m$, and P($AMPS_{50}$-*ran*-AaU_{50}) copolymers (c = 1 mg/ml in PBS, pH 7.4, T = 25°C).

	Copolymer	d [nm]	PDI	Zeta potential
1	$PSSNa_{38}$-*b*-$PAaU_2$	5.88±0.17	0.41±0.13	-29.4±1.8
2	$PSSNa_{38}$-*b*-$PAaU_4$	5.96±0.20	0.41±0.45	-31.6±3.4
3	$PSSNa_{38}$-*b*-$PAaU_{10}$	13.02±1.13	0.28±0.01	-27.5±2.9
4	$PAMPS_{75}$-*b*-$PAaU_{12}$	7.38±0.82	0.37±0.12	-24.3±2.2
5	$PAMPS_{75}$-*b*-$PAaU_{39}$	23.2±0.5	0.32±0.06	-25.7±0.8
6	$PAMPS_{75}$-*b*-$PAaU_{67}$	19.9±1.1	0.23±0.01	-33.3±2.1
7	P($AMPS_{50}$-*ran*-AaU_{50})	16.0±2.6	0.38±0.07	-24.2±1.2