Electronic Supplementary Material (ESI) for Analyst.

Supplementary material to

Biorecognition Antifouling Coatings in Complex Biological Fluids: A Review of Functionalization Aspects

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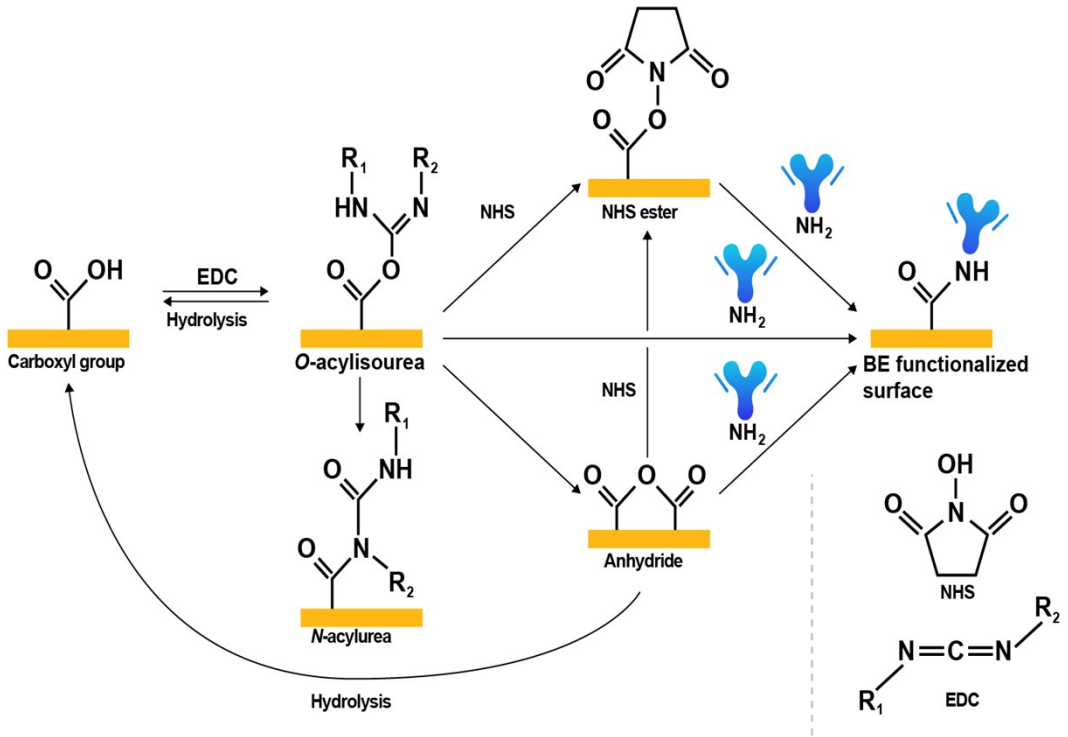

Figure S1: Scheme of functionalization reactions using EDC/NHS.

Table S1: The effect of functionalization on fouling from bodily fluids for a selection of antifouling (ultra-low and low fouling) platforms relevant for the review

Platform	Medium (bodily fluid)	Exposition time [min]	Fouling before functionalization [ng/cm²]	Method of functionalization	Method of deactivation of residual intermediates (if relevant)	Biorecognition element	Fouling after functionalization [ng/cm²]	Probing method	Ref.
Poly(carboxybetaine) platforms									
pCBAA	HBP [a] (100%)	10	2.73	EDC/NHS	hydrolysis (PBS, pH 7.4, 50min)	anti-Ecoli / anti-Salmonella	18.54	SPR	1
	HBP [a] (100%)	7	< 0.2 (below LOD)	EDC/NHS	hydrolysis (buffer pH 8-9, 21 min)	anti-Salmonella	2.6 ± 1.1	SPR	2
	HBP (50%)						1.2 ± 0.4		
	HBP [a] (100%)	10	~ 6	EDC/NHS	hydrolysis (10 mM Na_2CO_3+ 300 mM NaCl, pH 10, 10 min)	anti-TSH	~ 9	SPRi	3
						anti-ALCAM			
						anti-HCG			
	HBS [b] (100%)	10	~ 0 (below LOD)	EDC/NHS	hydrolysis during immobilization	anti-Salmonella	~ 0 (below LOD)	SPR	4
Two-layer hierarchical architecture of highly dense layer of pCBAA and second loose layer of pCBAA on top	HBS [b] (100%)	10	1.6	EDC/NHS	hydrolysis (10 mM Na_2CO_3 + 300 mM NaCl, pH 10, 10 min)	anti-TSH antibody	0.5	SPR	5
	HBP [a] (100%)	10	< 5	EDC/NHS	hydrolysis (10 mM Na_2CO_{3v}+ 300 mM NaCl, pH 10, 10 min, followed by 10 mM sodium acetate, pH 5)	anti-TSH antibody	< 5	SPR	6
pCBAA hydrogel thin films with carboxybetaine diacrylamide crosslinker	HBS [b] (100%)	10	4.7 ± 1.4 (extracted from plot)	EDC/NHS	hydrolysis followed by possible covalent interaction with GLY	anti-TSH antibody	5.9 ± 1.7 (extracted from plot)	SPR	7

[a] Human Blood Plasma; [b] Human Blood Serum

(Continues)

Table S1 *(Continuation)*

Platform	Medium (bodily fluid)	Exposition time [min]	Fouling before functionalization [ng/cm²]	Method of functionalization	Method of deactivation of residual intermediates (if relevant)	Biorecognition element	Fouling after functionalization [ng/cm²]	Probing method	Ref.
Poly(carboxybetaine) platforms									
p(CBMAA)	HBP [a] (100%)	10	11.1 ± 2.8	EDC/NHS	hydrolysis (10 mM sodium borate + 10 mM imidazole + 10 mM NaCl, pH 8, 30min)	anti-Salmonella	25.4 ± 4.1	SPR	[8]
p(CBMAA 7.5 mol%-co-HPMAA)			0				2.6 ± 1.2		
p(CBMAA 15 mol%-co-HPMAA)	HBP [a] (100%)	10	2.9 ± 0.8	EDC/NHS	hydrolysis (10mM sodium borate buffer +150 mM NaCl+10mM imidazole)	anti-Salmonella	11.6 ± 2.8	SPR	[9]
	HBP [a] (100%)		4.9 ± 2.0	EDC/NHS	1M glycine	anti-Salmonella	6.6 ± 3.6	SPR	[8]
						NH2-ON-probes	4.9 ± 2.3		
p(CBMAA 30 mol%-co-HPMAA)	HBS [b] (10%)	10	0	EDC/NHS	hydrolysis (PBS, 90 min)	Hepatitis B surface antigen	0	SPR	[10]
	SLV10% [c]	10	below LOD	EDC/NHS	hydrolysis (PBS, 90 min)	Hepatitis B surface antigen	below LOD	SPR	[11]
	SLV100% [d]		bellow LOD			not measured	not measured		
	HBP [a] (100%)		10.0 ± 3.5		hydrolysis (10 mM sodium borate + 10mM imidazole + 10mM NaCl, pH 8, 30min)	anti-Salmonella antibody	23.4 ± 3.9	SPR	[8]

[a] Human Blood Plasma; [b] Human Blood Serum; [c] Saliva (10%, supernatant after centrifugation); [d] Saliva (10%, supernatant after centrifugation)

(Continues)

Table S1 *(Continuation)*

Platform	Medium (bodily fluid)	Exposition time [min]	Fouling before functionalization [ng/cm²]	Method of functionalization	Method of deactivation of residual intermediates (if relevant)	Biorecognition element	Fouling after functionalization [ng/cm²]	Probing method	Ref.
p(SBMAA 3mol%-co-CBMAA 15 mol%-co-HPMAA 82 mol%)	HBP [a] (100%)	10	5.3 ± 1.1	EDC/NHS	1M AEAA	anti-bacterial antibody	10.9 ± 3.2	SPR	[12]
PEG(3)-Dendrimer carboxybetaine	HBP [a] (10%)	30	2.40	EDC/NHS	blocking with tris(hydroxymethyl)amino methane hydrochloride (0.5 M)	anti-ARG1 N-terminus antibody	~175 (extracted from plot)	SPRi	[13]
$Catechol_2$ -$pCBMA_2$	HBP [a] (100%)	10	8.9 ± 3.4	EDC/NHS	hydrolysis (buffer, pH 8-9, 21 min)	anti-ALCAM	9.5 ± 4.1	SPR	[14]
	HBS [b] (100%)		11.0 ± 5.0				NOT SHOWN		
$DOPA_2$–$pCBMA_2$	HBS [b]) (100%)	10	11.7 ± 3.0	EDC/NHS	hydrolysis (10 mM sodium phosphate + 300 mM NaCl, pH 8.2, 10 min)	anti-ALCAM	55 (ALCAM naturally occurs in plasma)	SPR	[15]
	HBP [a] (100%)		6.3 ± 0.9			not tested	not tested		
$DOPA_2$–$pCBMA_2$	FBS [e] (centrifuge-filtered)	5	~2 (extracted from plot)	EDC/NHS	hydrolysis (10 mM borate buffer + 0.75 M NaCl, pH 8.2, 10 min)	mouse monoclonal anti-ALCAM antibody	~31 (extracted from plot)	Suspended Micro-channel Resonator	[16]
DOPA-pCBMA	HBP [a] (100%)	15	~ 11 pm (extracted from plot)	EDC/NHS	hydrolysis (10 mM HEPES, 300 mM NaCl, pH 8.2)	Immunoglobulin control antibodies	~54 pm (extracted from plot)	Silicon microring resonator	[17]
Platforms with end hydroxyl group									
pHEMA	HBP [a] (100%)	10	16.2	DSC/DMAP in DMF	ethanolamine	anti-Ecoli O157 or anti-Salmonella	61.7	SPR	[1]

[a] Human Blood Plasma; [b] Human Blood Serum; [e] Fetal Bovine Serum

(Continues)

Table S1 *(Continuation)*

Platform	Medium (bodily fluid)	Exposition time [min]	Fouling before functionalization [ng/cm²]	Method of functionalization	Method of deactivation of residual intermediates (if relevant)	Biorecognition element	Fouling after functionalization [ng/cm²]	Probing method	Ref.
Platforms with end hydroxyl group									
p(HPMAA)	HBP [a] (100%)	15	< 0.03 (SPR detection limit)	DSC/DMAP	hydrolysis (PBS, 24 h)	Ab against peptide-glycan-polysaccha-ride antigen unique to group Gstreptococcus	< 0.03 (SPR detection limit)	SPR	[18]
PEG platforms									
Mixed SAMs of C11−EG6−COOH and C11−EG4−OH	HBP [a] (100%)	10	147	EDC/NHS	ethanolamine	anti-Ecoli O157 or anti-Salmonella	135	SPR	[1]
			147			anti-Ecoli O157 or anti-Salmonella, BSA blocked surface	23		
DNA probe and $(CH_2)_{15}$-COOH with covalently bound BSA	HBP [a] (10%)	10	5	DNA hybridiza-tion	none	anti-ALCAM conjugated with DNA	2.9 ± 0.6	SPRi	[19]
						anti-hCG conjugated with DNA probe	3 ± 1		

[a] Human Blood Plasma

(Continues)

Table S1 *(Continuation)*

Platform	Medium (bodily fluid)	Exposition time [min]	Fouling before functionalization [ng/cm²]	Method of functionalization	Method of deactivation of residual intermediates (if relevant)	Biorecognition element	Fouling after functionalization [ng/cm²]	Probing method	Ref.
PEG platforms									
poly(MeOEGMA-b-GMA) + NaN3	HBP [a] (100%)	15	32 (extracted from plot)	catalyst-free SPAAC reaction	none	biotin conjugated to dibenzocyclooctyne	88 (extracted from plot)	SPR	[20]
Structured polymer brush p(MeOEGMA-b-GMA) + NaN3 as antifouling and DBCO-biotin as functionalizable layer			88 (extracted from plot)			streptavidin and biotinylated mouse anti-IgG	25 (extracted from plot)		
methyl ether methacrylate (MeOEGMA) end-group nucleophilic substitution with azide			17 (extracted from plot)			biotin conjugated to dibenzocyclooctyne	18 (extracted from plot)		
poly(MeOEGMA)-DBCO-biotin brush			18 (extracted from plot)			streptavidin and biotinylated mouse anti-IgG	23 (extracted from plot)		

[a] Human Blood Plasma

(Continues)

Table S1 *(Continuation)*

Platform	Medium (bodily fluid)	Exposition time [min]	Fouling before functionalization [ng/cm²]	Method of functionalization	Method of deactivation of residual intermediates (if relevant)	Biorecognition element	Fouling after functionalization [ng/cm²]	Probing method	Ref.
DNA tetrahedron probes	cell lysate (100%)	30	N/A (platform itself s functional)	none	none	DNA-probe	0	SPR	21
	WB [f] (5%)						0		
	red cells (9.85e8 CFU/mL)						5.6 ± 1.4		
	HBP [a] (100%)						8.0 ± 2.1		
	HBS [b] (100%)						7.7 ± 1.2		
ssDNA surface passivated by mercaptohexanol	HBP [a] (100%)	30	N/A (platform itself s functional)	none	none	DNA-probe	41.5 ± 4.0		
	HBS [b] (100%)						68.2 ± 7.5		
Ionic liquid SAM (1-(carboxymethyl)-3-(mercaptododecyl)-imidazoliumbromide)	HBS [b] (100%)	not defined	99	NHS/EDC	EA	anti-human IgG antibody	45	SPR	22
Biotynylated poly(acrylic acid)-(ethylene oxide)n monolayer adsorbed on top of multilayer poly(ethylene imine)-[(poly(styrene sulfonate)/poly(allylamine hydrochloride)]$_3$	FBS [e] (10%)	10	< 1	none	none	streptavidin - biotinylated antiovalbumin	<1	QCM	23
1-(carboxyethyl)-3-(12-mercaptododecyl)-1H-imidazolium bromide $[(HS)^{12}C_{12}(COOH)^{5}C_5im]^+ Br^-$ ionic liquid	Breast cancer cell lysates - MCF-7 and SK-BR-3 (50%)	20	Irreversible fouling 191 ± 45, real time nonspecific fouling 6 ± 4	NHS	cell lysate	polyclonal rabbit anti-HER2	Irreversible fouling not shown, real time nonspecific fouling 61 ± 8	SPR	24

[a] Human Blood Plasma; [b] Human Blood Serum; [e] Fetal Bovine Serum; [f] Whole Blood

(Continues)

Table S1 *(Continuation)*

Platform	Medium (bodily fluid)	Exposition time [min]	Fouling before functionalization [ng/cm²]	Method of functionalization	Method of deactivation of residual intermediates (if relevant)	Biorecognition element	Fouling after functionalization [ng/cm²]	Probing method	Ref.
IgG-molecular imprinted hydrogel based on acrylamide, methylene N,N´ bis (acrylamide) and N-isopropylacrylamide crosslinked monomers	FBS [e] (50%)	30	Data not shown for FBS [e] (50%)	Imprinted hydrogel	none	Cavity for IgG detection	Charge-transfer resistance change of 2.063%	EIS	[25]
peptide CPPPPNQNQNQNQDHWRGWVA mixed with CPPP-PNQNQNQNQ	FBS [e] (100%)	30	N/A (platform itself s functional)	none	none	last part of peptide HWRGWVA selectively binds Fc region of human IgG	Signal suppression ~1%	DPV	[26]
peptide CPPPPNQNQNQNQDHWRGWVA mixed with CPPP-PNQNQNQNQ	FBS [e] (100%)	180	N/A (platform itself s functional)	non	none	last part of peptide HWRGWVA selectively binds Fc region of human IgG	Signal suppression of ~11%	DPV	[26]
Mixed SAM from peptide EKEKEKE-PPPPC, ATP binding aptamer 5'-SH-(CH2)6-ACCTGGGGGAGTATTGCGGAGGAAGGT-3' and 6-mercaptohexanol	HBP [a] (10%)	not defined	N/A (platform itself s functional)	none	none	SH-aptamer self-assembled with antifouling peptide	Charge-transfer resistance change of ~10% (extracted from plot)	EIS	[27]
Mixed SAM from PEG, ATP binding aptamer 5'-SH-(CH2)6-ACCTGGGGGAGTATTGCGGAGGAAGGT-3' and 6-mercaptohexanol	HBP [a] (10%)	not defined	N/A (platform itself s functional)	none	none	SH-aptamer simultaneously self-assembled with thiolated PEG	Charge-transfer resistance change of ~64 (extracted from plot)	EIS	[28]

[a)] Human Blood Plasma; [e)] Fetal Bovine Serum

Table S2: The effect of functionalization on fouling from foodstuff for a selection of antifouling (ultra-low and low fouling) platforms relevant for the review.

Platform	Medium (foodstuff)	Exposition time [min]	Fouling before functionalization [ng/cm²]	Method of functionalization	Method of deactivation of residual intermediates (if relevant)	Biorecognition element	Fouling after functionalization [ng/cm²]	Probing method	Ref.
Poly(carboxybetaine) platforms									
pCBAA	milk (100%)	10min	0.83	EDC/NHS	hydrolysis (PBS. pH 7.4. 50min)	anti-Salmonella	1.81	SPR	1
	milk (100%)	10	0.2	EDC/NHS	hydrolysis (10 mM sodium borate buffer +150 mM NaCl+10 mM imidazole)	anti-Salmonella	4.1	SPR	9
	spinach (10%)		2.8				16.8		
	cucumber (10%)		0.25				8.1		
	hamburger (10%)		0				2.2		
	lettuce (10%)		0.14				4.3		
	cucumber (10%)	15	<0.2 (below LOD)	EDC/NHS	hydrolysis (PBS, pH 10)	anti-Ecoli	6.2 ± 1.7	SPR	29
	hamburger (10%)						3.4 ± 1.3		
	orange extract (100%)	10	1.45	EDC/NHS	hydrolysis (PBS pH 7.4, 50 min)	anti-Ecoli / anti-Salmonella	1.4	SPR	1
	tomato extract (100%)		1.05				0.06		
	cucumber extract (100%)		1.8				3.09		
p(CBMAA)	milk (100%)	10	0.6	EDC/NHS	hydrolysis (10 mM sodium borate buffer +150 mM NaCl + 10 mM imidazole)	anti-Salmonella	20.4	SPR	9
	spinach (10%)		2.74				12.2		
	cucumber (10%)		2.1				3.4		
	hamburger (10%)		2.2				4.5		
	lettuce (10%)		2.4				4.9		

(Continues)

Table S2 *(Continuation)*

Platform	Medium (foodstuff)	Exposition time [min]	Fouling before functionalization [ng/cm²]	Method of functionalization	Method of deactivation of residual intermediates (if relevant)	Biorecognition element	Fouling after functionalization [ng/cm²]	Probing method	Ref.
Poly(carboxybetaine) platforms									
p(CBMAA 7.5 mol%-co-HPMAA)	milk (100%)	10	0	EDC/NHS	hydrolysis (10 mM sodium borate buffer +150 mM NaCl + 10 mM imidazole)	anti-Salmonella	0.6	SPR	9
	spinach (10%)		0				1.1		
	cucumber (10%)		0				0		
	hamburger (10%)		0				0		
	lettuce (10%)		0				0		
p(CBMAA 15 mol%-co-HPMAA)	milk (100%)		0				1.7		
	spinach (10%)		0.5				3.4		
	cucumber (10%)		0.3				0.05		
	hamburger (10%)		0				0		
	lettuce (10%)		0.2				0		
p(CBMAA 30 mol%-co-HPMAA)	milk (100%)		0				6.8		
	spinach (10%)		1.2				4.2		
	cucumber (10%)		1.4				2.8		
	hamburger (10%)		0				2.5		
	lettuce (10%)		1.4				3.5		

(Continues)

Table S2 *(Continuation)*

Platform	Medium (foodstuff)	Exposition time [min]	Fouling before functionalization [ng/cm²]	Method of functionalization	Method of deactivation of residual intermediates (if relevant)	Biorecognition element	Fouling after functionalization [ng/cm²]	Probing method	Ref.
Platforms with end hydroxyl group									
pHEMA	orange (100%)	10	1.6	DSC/DMAP in DMF	EA	anti-Ecoli O157 or anti-Salmonella	10.5	SPR	1
	tomato (100%)		2.6				14.5		
	cucumber (100%)		5.3				26.7		
	milk (100%)		5.7				23.6		
	fresh-whole fat milk (100%)	30	~0	DSC/DMAP in DMF	hydrolysis (PBS, pH 7.4, 24h)	anti-Cronobacter (ex situ immobilization)	~0	SPR	30
	whole-fat milk from powder (Laktino) (10%)		~0				~0		
	infant formulation from powder (Sunar™) (10%)		~0				~0		

(Continues)

Table S2 *(Continuation)*

Platform	Medium (foodstuff)	Exposition time [min]	Fouling before functiona-lization [ng/cm^2]	Method of functiona-lization	Method of deactivation of residual intermediates (if relevant)	Biorecognition element	Fouling after functiona-lization [ng/cm^2]	Probing method	Ref.
PEG platforms									
Mix of HS−C11−EG6−COOH and HS−C11−EG4−OH	orange (100%)	10	21.12	EDC/NHS	ethanolamine	anti-Ecoli O157 or anti-Salmonella	2.6	SPR	1
	tomato (100%)		9.13				7.51		
	cucumber (100%)		51.9				18.52		
	milk (100%)		154.25				6.88		
	orange (100%)		21.12			anti-Ecoli O157 or anti-Salmonella. BSA blocked surface	2.28		
	tomato (100%)		9.13				2.64		
	cucumber (100%)		51.9				4.4		
	milk (100%)		154.25				6.24		
Other platforms									
Hyaluronic acid-grafted Au	soybean milk (10mg/mL. centrifuge-filtered 0.22um)	10	0.6	EDC/NHS	1M ethanolamine	anti-BSA	0.67	SPR	31
	cow milk (100%. centrifuge-filtered 0.22um)		9.8				17		

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