Supporting Information

Optimal His-tag design for efficient $[^{99m}Tc(CO)_3]^+$ and $[^{188}Re(CO)_3]^+$ labeling of proteins for molecular imaging and radionuclide therapy by analysis of peptide arrays.

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Background

Table S1. Summary of literature methods and radiochemical yields for labeling proteins with $[^{99m}Tc][Tc(CO)_3]^+$. RY = radiochemical yield; RP = radiochemical purity (post-purification where purification was required).

Ref.	Labelling Conditions	Labelling time	RY (%)	Purification required	RP (%)	Specific Activity
Mees et al. 2012 (1)	37°C, blown dry with N2	1-1.5 h	40-50%	Yes	>95%	8.9-10.4 MBq/μg
Shah et al. 2012 (2)	37°C, PBS at pH 7.4	2 h	98%			
Cortez-Retamozo et al. 2008 (3)	52°C, CO_3^{2-} buffer pH 8 and PBS pH 7.4	1 h		Often	98%	
Teran et al. 2011 (4)	50°C, H_2O for	50 min	95%			20 mCi/μg
Orlova et al. 2006 (5)	50°C, PBS buffer	40 min	60%			
Deyev et al. 2003 (6)			95%			2GBq/mg
Tait et al. 2002 (7)	37°C, 0.5/0.1M HEPES pH 7.4	1 h	80%	Yes	98%	
Chen et al. 2008 (8)	37°C	3 h	90%	Yes	90%	
Tavare et al. 2009 (9)	37°C, PBS, pH 7.4	30 min	95%	Yes	100%	
Zahnd et al. 2010 (10)	37°C, 0.6M phosphate buffer, pH 7	1 h				
Tolmachev et al. 2010 (11)	50°C, PBS	1 h	80%	Yes	95%	2 MBq/μg
Bidlingmaier et al. 2009 (12)	37°C, PBS buffer	1 h		Yes		
Berndorff et al. 2006 (13)	37°C, 0.1M HEPES and PBS	1 h	54%	Yes	91%	21 MBq/μmol

Results

Table S2 (continues over page). Peptide sequences in the Celluspots™ Serine/Threonine Kinase Substrate I (STKS1) and their positions on the glass slide. Peptide sequences containing his residues are highlighted in red. The grid layout to which grid positions refer is shown in Table S3.

no	*Grid position*	*kinase*	*sequence*	*to array*
1	A 1	control	**N-W-S-H-P-Q-F-E-K-X-X-X-X**	▶
2	A 2	neg. control	.space	▶
3	A 3	70-kDakinase	Q-N-R-S-G-A-M-S-P-M-S-W-N-S-D	▶
4	A 4	AFK	E-R-G-Y-S-F-T-T-T-A-E-R-E-I-V	▶
5	A 5	AMPK_group	T-L-V-N-R-K-V-S-Q-R-R-V-D-F-C	▶
6	A 6	AMPK_group	**S-Q-R-Q-R-S-T-S-T-P-N-V-H-M-V**	▶
7	A 7	AMPK_group	S-P-R-V-R-T-L-S-G-S-R-P-P-L-L	▶
8	A 8	AMPK_group	**Y-V-A-S-N-R-R-S-I-F-F-R-T-S-H**	▶
9	A 9	AMPK_group	S-D-G-E-F-L-R-T-S-C-G-S-P-N-Y	▶
10	A10	AMPK_group	P-V-R-M-R-R-N-S-F-T-P-L-S-S-S	▶
11	A11	AMPK_group	**S-S-G-S-P-A-N-S-F-H-F-K-E-A-W**	▶
12	A12	AMPK_group	G-K-I-K-R-L-R-S-Q-V-Q-V-S-L-E	▶
13	A13	AMPK_group	S-P-R-R-R-R-R-ST-F-S-S-L-S-N-S	▶
14	A14	ATM	P-Y-P-G-I-D-L-S-Q-V-Y-E-L-L-E	▶
15	A15	ATM	S-L-A-F-E-E-G-S-Q-S-T-T-I-S-S	▶
16	A16	ATM	**Q-K-G-E-L-S-R-S-P-S-P-F-T-H-T**	▶
17	A17	ATM	E-N-V-K-Y-S-S-S-Q-P-E-P-R-T-G	▶
18	A18	ATM	K-D-L-K-L-G-V-S-Q-Q-T-I-F-S-V	▶
19	A19	ATM	E-Q-Q-L-F-Y-I-S-Q-P-G-S-S-V-V	▶
20	A20	ATM	A-L-R-L-L-D-S-S-Q-I-V-I-I-S-A	▶
21	A21	ATM	S-Q-E-S-E-D-Y-S-Q-P-S-T-S-S-S	▶
22	A22	ATM	E-T-W-S-L-P-L-S-Q-N-S-A-S-E-L	▶
23	A23	ATM	**L-S-D-T-D-S-H-S-Q-D-L-G-S-P-E**	▶
24	A24	ATM	**S-K-L-L-M-I-I-S-Q-K-D-T-F-H-S**	▶
25	B 1	ATM	S-S-L-E-L-S-S-ST-Q-P-E-S-S-S-S	▶
26	B 2	ATR	**L-V-Q-G-I-S-F-S-Q-P-T-C-P-D-H**	▶
27	B 3	AuroraAkinase	P-P-D-Q-R-R-L-S-E-T-S-V-N-T-E	▶
28	B 4	AuroraAkinase	K-A-E-F-C-N-K-S-K-Q-P-G-L-A-R	▶
29	B 5	AuroraAkinase	**R-E-E-V-P-R-R-S-G-L-S-A-G-H-R**	▶
30	B 6	AuroraAkinase	**D-R-N-T-F-R-H-S-V-V-V-P-Y-E-P**	▶
31	B 7	AuroraAkinase	K-R-S-S-R-R-D-ST-K-L-P-A-L-K-R	▶
32	B 8	AuroraBkinase	A-P-S-L-R-R-K-T-M-C-G-T-L-D-Y	▶
33	B 9	AuroraBkinase	K-N-K-I-A-K-E-T-N-N-K-K-K-E-F	▶
34	B10	AuroraBkinase	I-T-S-A-A-R-R-S-Y-V-S-S-G-E-M	▶
35	B11	AuroraBkinase	A-T-K-A-A-R-K-S-A-P-A-T-G-G-V	▶
36	B12	AuroraBkinase	**K-P-R-Y-H-K-R-T-S-S-A-V-W-N-S**	▶
37	B13	AuroraBkinase	**S-Q-N-H-K-R-K-T-I-S-K-I-P-A-P**	▶

38	B14	AuroraBkinase	K-R-P-Q-S-A-T-S-N-V-F-A-M-F-D	▶
39	B15	AuroraBkinase	I-T-S-A-A-R-R-ST-S-A-G-E-G-P-P	▶
40	B16	BCKDK	**T-Y-R-I-G-H-H-S-T-S-D-D-S-S-A**	▶
41	B17	CaM-KI_group	R-F-I-I-G-S-V-S-E-D-N-S-E-D-E	▶
42	B18	CaM-KI_group	**G-G-V-K-K-R-K-S-S-S-S-V-H-L-M**	▶
43	B19	CaM-KII_group	N-V-A-S-R-M-E-S-T-G-V-M-G-N-I	▶
44	B20	CaM-KII_group	E-Q-L-S-R-E-L-S-T-L-R-N-L-F-K	▶
45	B21	CaM-KII_group	D-M-K-V-R-K-S-S-T-Q-E-E-I-K-K	▶
46	B22	CaM-KII_group	I-T-G-K-N-R-P-S-S-G-S-L-I-Q-V	▶
47	B23	CaM-KII_group	I-T-L-E-R-G-N-S-G-L-G-F-S-I-A	▶
48	B24	CaM-KII_group	**V-T-G-P-R-L-V-S-N-H-S-L-H-E-T**	▶
49	C 1	CaM-KII_group	**P-K-Y-S-R-Q-F-S-L-E-H-V-H-G-S**	▶
50	C 2	CaM-KII_group	A-R-I-R-A-A-K-S-G-S-A-N-A-Y-M	▶
51	C 3	CaM-KII_group	K-L-K-E-R-W-G-S-N-E-L-P-A-E-E	▶
52	C 4	CaM-KII_group	R-R-K-R-R-V-V-T-K-A-Y-K-E-P-L	▶
53	C 5	CaM-KII_group	A-G-L-R-R-Q-V-ST-L-E-E-P-A-Q-A	▶
54	C 6	CaM-KIV	P-Q-L-A-S-K-Q-S-M-V-N-S-L-P-T	▶
55	C 7	CaM-KIV	R-P-L-G-R-T-Q-S-A-P-L-P-Q-N-A	▶
56	C 8	CaM-KIV	K-L-S-S-P-A-L-S-A-S-A-S-D-G-T	▶
57	C 9	CaM-KIV	R-P-L-S-R-A-Q-ST-A-P-A-S-A-G-T	▶
58	C10	CCDPK	S-A-I-R-R-A-S-T-I-E-M-P-Q-Q-A	▶
59	C11	CCDPK	Q-Q-L-A-R-E-T-S-V-D-P-D-M-R-K	▶
60	C12	CCDPK	T-D-G-N-F-L-K-T-S-C-G-S-P-N-Y	▶
61	C13	CCDPK	D-P-G-S-V-L-S-T-A-C-G-T-P-G-Y	▶
62	C14	CCDPK	D-P-G-S-R-L-S-ST-A-C-G-T-P-G-Y	▶
63	C15	CDK	**E-E-P-S-P-L-P-S-P-T-A-S-P-N-H**	▶
64	C16	CDK	E-P-G-V-E-R-S-S-P-S-K-C-P-S-L	▶
65	C17	CDK	D-S-S-R-A-P-S-S-P-R-P-P-G-S-T	▶
66	C18	CDK	R-P-N-P-C-A-Y-T-P-P-S-L-K-A-V	▶
67	C19	CDK	T-P-A-P-R-Q-S-S-P-S-K-S-S-A-S	▶
68	C20	CDK	F-P-P-L-N-S-V-S-P-S-P-L-M-L-L	▶
69	C21	CDK	**S-G-H-F-T-M-R-S-P-F-K-C-D-A-C**	▶
70	C22	CDK	D-V-S-P-Y-S-L-S-P-V-S-N-K-S-Q	▶
71	C23	CDK	S-G-D-S-D-A-S-S-P-R-S-N-C-S-D	▶
72	C24	CDK	A-E-N-S-R-L-Q-T-P-G-G-G-S-K-T	▶
73	D 1	CDK	L-P-E-N-N-V-L-S-P-L-P-S-Q-A-M	▶
74	D 2	CDK	**G-T-N-R-C-F-G-S-F-R-H-S-P-Y-E**	▶
75	D 3	CDK	N-P-G-G-R-P-I-T-P-P-R-N-S-A-K	▶
76	D 4	CDK	A-A-L-R-Q-L-R-S-P-R-R-A-Q-A-P	▶
77	D 5	CDK	A-V-I-P-I-N-G-S-P-R-T-P-R-R-G	▶
78	D 6	CDK	V-R-Y-I-K-E-N-S-P-C-V-T-P-V-S	▶
79	D 7	CDK	N-S-S-D-T-V-T-S-P-Q-R-A-G-P-L	▶
80	D 8	CDK	S-G-Y-S-S-P-G-S-P-G-T-P-G-S-R	▶
81	D 9	CDK	S-S-P-S-T-P-S-ST-P-S-K-S-S-A-P	▶
82	D10	CDK1	F-L-E-G-C-A-C-T-P-E-R-M-A-E-A	▶
83	D11	CDK1	K-K-P-F-K-C-F-T-P-K-G-S-S-L-K	▶
84	D12	CDK1	**T-E-Y-S-Q-G-A-S-P-Q-P-Q-H-Q-L**	▶
85	D13	CDK1	R-P-V-S-S-A-A-S-V-Y-A-G-A-G-G	▶

86	D14	CDK1	T-D-S-A-T-I-V-S-P-P-P-S-S-P-P	▶
87	D15	CDK1	F-S-D-P-W-G-G-S-P-A-K-P-S-T-N	▶
88	D16	CDK1	**M-D-C-L-T-F-G-S-P-V-L-M-R-H-L**	▶
89	D17	CDK1	R-E-Y-Q-Q-R-N-S-P-G-V-P-T-G-A	▶
90	D18	CDK1	S-L-P-Q-A-T-V-T-P-P-R-K-E-E-R	▶
91	D19	CDK1	T-K-N-G-L-P-G-S-R-P-G-S-P-E-R	▶
92	D20	CDK1	K-E-P-S-E-V-P-T-P-K-R-P-R-G-R	▶
93	D21	CDK1	Q-E-P-T-G-E-P-S-P-K-R-P-R-G-R	▶
94	D22	CDK1	P-N-K-E-L-P-P-S-P-E-K-K-T-K-P	▶
95	D23	CDK1	**T-Q-G-H-P-D-G-T-P-P-K-L-D-T-A**	▶
96	D24	CDK1	S-F-K-K-Q-E-K-T-P-K-T-P-K-G-P	▶
97	E 1	CDK1	T-S-C-A-S-L-D-S-P-G-R-I-K-R-K	▶
98	E 2	CDK1	G-R-Y-L-T-Q-E-T-N-K-V-E-T-Y-K	▶
99	E 3	CDK1	G-G-L-I-E-P-D-T-P-G-R-V-P-L-D	▶
100	E 4	CDK1	S-L-I-V-P-G-K-S-P-T-R-K-K-S-G	▶
101	E 5	CDK1	K-E-L-Q-R-Q-A-S-P-S-I-V-I-A-L	▶
102	E 6	CDK1	G-A-G-G-Y-T-Q-S-P-G-G-F-G-S-P	▶
103	E 7	CDK1	D-P-Q-Q-L-Q-L-S-P-L-K-G-L-S-L	▶
104	E 8	CDK1	R-G-A-L-V-R-G-T-P-V-R-G-A-I-T	▶
105	E 9	CDK1	L-F-Q-L-G-P-P-S-P-V-K-M-P-S-P	▶
106	E10	CDK1	L-D-E-P-N-P-N-S-P-A-N-S-Q-A-A	▶
107	E11	CDK1	T-E-P-S-L-P-G-ST-P-V-R-P-S-S-A	▶
108	E12	CDK11	P-A-A-A-P-A-S-S-S-D-P-A-A-A-A	▶
109	E13	CDK2	A-D-L-G-E-V-R-T-P-E-P-P-E-S-L	▶
110	E14	CDK2	N-S-L-T-P-K-S-T-P-V-K-T-L-P-F	▶
111	E15	CDK2	I-N-K-K-Q-A-T-S-P-A-S-K-K-P-A	▶
112	E16	CDK2	R-V-K-A-L-P-L-S-P-R-K-R-L-G-D	▶
113	E17	CDK2	N-E-E-A-K-R-K-S-P-K-K-K-E-K-C	▶
114	E18	CDK2	R-N-K-L-K-P-K-ST-P-S-K-K-L-K-A	▶
115	E19	CDK4	K-R-S-F-A-P-S-T-P-L-T-G-R-R-Y	▶
116	E20	CDK4	E-R-G-K-L-P-E-S-P-K-R-A-E-E-I	▶
117	E21	CDK4	S-K-A-L-R-I-S-T-P-L-T-G-V-R-Y	▶
118	E22	CDK4	S-M-D-A-R-P-S-ST-P-L-A-G-V-R-Y	▶
119	E23	CDK5	S-I-K-S-E-P-I-S-P-P-R-D-R-M-T	▶
120	E24	CDK5	T-R-K-S-A-P-S-S-P-T-L-D-C-E-K	▶
121	F 1	CDK5	G-F-K-S-S-P-A-ST-P-K-A-D-G-A-A	▶
122	F 2	CDK7	**G-L-A-K-S-F-G-S-P-N-R-A-Y-T-H**	▶
123	F 3	CDK7	**P-E-E-F-I-S-L-S-P-P-H-E-A-L-D**	▶
124	F 4	CDK7	R-R-R-K-K-R-T-S-I-E-T-N-I-R-V	▶
125	F 5	CDK7	E-I-V-P-S-P-P-S-P-P-P-L-P-R-I	▶
126	F 6	CDK7	**G-I-P-I-R-V-Y-T-H-E-V-V-T-L-W**	▶
127	F 7	CDK7	Y-S-Y-Q-M-A-L-T-P-V-V-V-T-L-W	▶
128	F 8	CDK7	G-S-P-K-R-A-L-ST-P-E-V-V-T-L-W	▶
129	F 9	CDPK	S-E-T-T-K-S-A-S-F-L-K-G-R-A-A	▶
130	F10	CHK1	**E-V-V-G-G-T-D-S-S-M-D-V-F-H-L**	▶
131	F11	CHK1	**P-A-L-K-R-S-H-S-D-S-L-D-H-D-I**	▶
132	F12	CHK1	D-E-L-G-R-L-C-ST-G-A-F-V-E-S-L	▶
133	F13	CHK2	P-L-L-S-R-M-G-S-L-R-A-P-V-D-E	▶

134	F14	CHK2	P-P-L-F-P-I-K-S-F-V-K-T-K-C-K
135	F15	CHK2	S-G-L-Y-R-S-P-S-M-P-E-N-L-N-R
136	F16	CHK2	L-F-T-Q-R-Q-N-S-A-P-A-R-M-L-S
137	F17	CHK2	G-L-T-K-R-Q-K-ST-M-S-A-T-M-P-E
138	F18	CK	P-P-A-P-G-N-A-S-E-S-E-E-D-R-S
139	F19	CK	**A-V-H-Y-L-D-E-T-E-Q-W-E-K-F-G**
140	F20	CK	E-D-P-D-I-P-E-S-Q-M-E-E-P-A-A
141	F21	CK	**A-D-D-Y-H-P-E-ST-E-M-E-E-H-A-A**
142	F22	CK1delta	**N-R-M-G-Q-A-G-S-T-I-S-N-S-H-A**
143	F23	CK1delta	**A-G-S-T-I-S-N-S-H-A-Q-P-F-D-F**
144	F24	CK1epsilon	A-L-P-G-K-A-E-S-V-A-S-L-T-S-Q
145	G 1	CK1_group	S-R-C-S-S-L-S-S-L-S-S-A-E-D-E
146	G 2	CK1_group	G-A-T-T-T-A-P-S-L-S-G-K-G-N-P
147	G 3	CK1_group	R-M-G-Q-L-R-G-S-A-T-R-A-L-P-P
148	G 4	CK1_group	G-I-P-V-R-C-Y-S-A-E-V-V-T-L-W
149	G 5	CK1_group	G-D-D-D-D-A-Y-S-D-T-E-T-T-E-A
150	G 6	CK1_group	N-E-A-A-A-R-F-T-L-G-S-P-L-T-S
151	G 7	CK1_group	E-P-P-L-S-Q-E-T-F-S-D-L-W-K-L
152	G 8	CK1_group	S-T-L-S-S-S-S-ST-L-E-S-S-P-S-G
153	G 9	CK2	**H-K-A-E-L-Q-G-S-D-E-D-E-H-V-R**
154	G10	CK2	F-D-G-I-W-K-A-S-F-T-T-F-T-V-T
155	G11	CK2	**D-Y-F-L-L-S-H-S-L-L-P-A-L-C-D**
156	G12	CK2	K-E-R-D-K-E-V-S-D-D-E-A-E-E-K
157	G13	CK2	K-E-R-E-K-E-I-S-D-D-E-A-E-E-E
158	G14	CK2	T-S-G-E-D-T-L-S-D-S-D-D-E-D-D
159	G15	CK2	G-G-R-E-R-L-A-S-T-N-D-K-G-S-M
160	G16	CK2	**G-P-R-V-W-Y-V-S-N-I-D-G-T-H-I**
161	G17	CK2	**G-Y-L-R-K-P-K-S-M-H-K-R-F-F-V**
162	G18	CK2	**S-K-E-S-E-H-D-S-D-E-S-S-D-D-D**
163	G19	CK2	L-V-R-S-R-E-V-S-V-D-E-G-R-A-C
164	G20	CK2	K-R-G-W-I-P-A-S-F-L-E-P-L-D-S
165	G21	CK2	V-D-G-S-G-D-T-S-S-N-E-E-I-G-S
166	G22	CK2	V-D-G-T-G-D-T-S-S-E-E-D-E-D-E
167	G23	CK2	Y-G-G-F-T-E-E-S-G-D-D-E-Y-Q-G
168	G24	CK2	**R-I-C-M-R-N-F-S-R-S-D-H-L-T-T**
169	H 1	CK2	I-Y-P-W-M-R-S-S-G-T-D-R-K-R-G
170	H 2	CK2	E-F-D-T-N-Y-A-T-D-D-D-I-V-F-E
171	H 3	CK2	A-S-S-S-T-S-V-T-P-D-V-S-D-N-E
172	H 4	CK2	K-A-A-R-V-L-G-S-E-G-E-E-E-D-E
173	H 5	CK2	**G-K-K-T-K-F-A-S-D-D-E-H-D-E-H**
174	H 6	CK2	R-A-A-M-F-P-E-T-L-D-E-G-M-Q-I
175	H 7	CK2	A-E-K-Y-A-K-E-S-L-K-E-E-D-E-S
176	H 8	CK2	E-E-E-E-E-E-S-ST-D-D-E-E-E-E-E
177	H 9	CK2alpha	D-K-E-N-G-S-V-S-T-S-E-T-P-P-P
178	H10	CSFR1	I-E-S-Y-E-G-N-S-Y-T-F-I-D-P-T
179	H11	DAPK_group	R-R-E-E-R-S-L-S-A-P-G-N-L-L-T
180	H12	DAPK_group	A-R-K-K-W-K-Q-S-V-R-L-I-S-L-C
181	H13	DAPK_group	I-I-M-D-S-S-I-S-K-Q-A-L-S-E-I

182	H14	DAPK3	K-Q-T-A-R-K-S-T-G-G-K-A-P-R-K
183	H15	DAPK3	K-K-R-P-Q-R-A-T-S-N-V-F-A-M-F
184	H16	DAPK3	R-L-G-K-R-V-L-S-K-L-Q-S-P-S-R
185	H17	DAPK3	S-S-R-R-R-A-I-S-E-T-E-E-N-S-D
186	H18	DAPK3	**Q-G-R-K-R-R-Q-T-S-M-T-D-F-Y-H**
187	H19	DAPK3	A-G-R-K-R-R-S-ST-S-G-V-E-F-S-D
188	H20	DNA-PK	**E-K-E-E-D-H-I-S-I-S-S-L-A-E-G**
189	H21	DNA-PK	L-S-P-I-D-M-E-S-Q-E-R-I-K-A-E
190	H22	DNA-PK	L-T-P-M-F-V-E-T-Q-A-S-Q-G-T-L
191	H23	DNA-PK	**E-K-K-T-K-I-R-S-L-H-N-K-L-L-N**
192	H24	DNA-PK	Q-Q-A-T-T-G-V-S-Q-E-T-S-E-N-P
193	I 1	DNA-PK	L-T-V-L-N-A-F-S-Q-A-P-S-T-M-Q
194	I 2	DNA-PK	L-S-E-T-D-I-S-ST-Q-E-E-S-S-A-G
195	I 3	Eg3kinase	V-Q-N-K-R-R-R-S-V-T-P-P-E-E-Q
196	I 4	ERA	L-G-Q-K-I-S-I-T-S-R-K-A-Q-T-T
197	I 5	ERTPK	R-E-L-V-E-P-L-T-P-S-G-E-A-P-N
198	I 6	ERTPK	P-G-E-T-P-P-L-S-P-I-D-M-E-S-Q
199	I 7	ERTPK	L-L-P-T-P-P-L-S-P-S-R-R-S-G-L
200	I 8	ERTPK	L-L-P-T-P-P-L-ST-P-S-R-R-S-G-L
201	I 9	GRK_group	K-A-Y-G-N-G-Y-S-S-N-G-N-T-G-E
202	I10	GRK_group	Q-E-A-P-E-R-A-S-S-V-Y-T-R-S-T
203	I11	GRK_group	R-S-Q-E-L-R-K-T-F-K-E-I-I-C-C
204	I12	GRK_group	V-A-N-Q-D-P-V-S-P-S-L-V-Q-G-R
205	I13	GRK_group	E-S-G-E-D-E-S-ST-S-S-D-S-S-G-E
206	I14	GRK-1	P-L-G-D-D-E-A-S-A-T-V-S-K-T-E
207	I15	GRK-1	A-V-S-K-A-E-T-ST-Q-T-A-P-A-A-E
208	I16	GRK-2	K-D-E-K-K-E-E-S-E-E-S-D-D-D-M
209	I17	GRK-2	**L-C-E-D-L-P-G-T-E-D-F-V-G-H-Q**
210	I18	GRK-2	A-T-A-R-E-R-V-T-A-C-T-P-S-D-G
211	I19	GRK-2	E-R-A-L-T-E-D-S-T-Q-T-S-D-T-A
212	I20	GRK-2	P-G-M-E-G-L-G-T-D-I-T-V-I-C-P
213	I21	GRK-2	Y-E-D-D-E-E-E-S-E-A-Q-G-P-K
214	I22	GRK-2	G-M-D-E-M-E-F-T-E-A-E-S-N-M-N
215	I23	GRK-2	R-E-D-D-T-E-D-ST-E-D-T-S-G-S-G
216	I24	GRK-3	V-K-A-L-D-F-R-T-P-R-N-A-K-I-V
217	J 1	GRK-4	E-S-M-R-R-S-V-S-E-A-A-L-A-Q-P
218	J 2	GRK-5	V-L-D-I-E-Q-F-S-T-V-K-G-V-N-L
219	J 3	GRK-5	**D-F-V-G-H-Q-G-T-V-P-S-D-N-I-D**
220	J 4	GRK-6	V-A-K-L-L-E-G-T-G-S-E-A-S-S-T
221	J 5	GRK-6	P-A-L-V-R-S-A-S-S-D-T-S-E-E-L
222	J 6	GSK-3_group	**H-L-Q-P-G-H-P-T-P-P-P-T-P-V-P**
223	J 7	GSK-3_group	E-S-E-Q-S-M-D-S-E-E-P-D-S-R-G
224	J 8	GSK-3_group	R-V-K-E-E-P-P-S-P-P-Q-S-P-R-V
225	J 9	GSK-3_group	**Q-Q-Q-S-Y-L-D-S-G-I-H-S-G-A-T**
226	J10	GSK-3_group	N-A-S-G-S-T-S-T-P-A-P-S-R-T-A
227	J11	GSK-3_group	Q-K-R-R-E-I-L-S-R-R-P-S-Y-R-K
228	J12	GSK-3_group	E-E-V-D-L-A-C-T-P-T-D-V-R-D-V
229	J13	GSK-3_group	E-E-E-E-E-A-P-ST-P-P-P-S-P-P-G

230	J14	GSK-3beta	P-P-S-S-T-D-R-S-P-Y-E-K-V-S-A	▶
231	J15	GSK-3beta	Y-R-Y-P-R-P-A-S-V-P-P-S-P-S-L	▶
232	J16	GSK-3beta	**V-E-V-D-A-A-V-T-P-E-E-R-H-L-S**	▶
233	J17	GSK-3beta	P-D-L-K-N-V-K-S-K-I-G-S-T-E-N	▶
234	J18	GSK-3beta	**D-E-G-H-S-N-S-S-P-R-H-S-E-A-A**	▶
235	J19	GSK-3beta	**Q-A-R-A-H-G-L-S-L-I-P-S-T-G-L**	▶
236	J20	GSK-3beta	**M-K-I-D-E-P-S-T-P-Y-H-S-M-M-G**	▶
237	J21	GSK-3beta	L-L-D-E-Y-N-V-T-P-S-P-P-G-T-V	▶
238	J22	GSK-3beta	**H-V-Q-R-V-M-R-T-P-G-C-Q-S-P-G**	▶
239	J23	GSK-3beta	**V-P-P-S-V-P-L-ST-P-E-P-S-P-H-S**	▶
240	J24	HRI	I-E-G-M-I-L-L-S-E-L-S-R-R-R-I	▶
241	K 1	IKK_group	A-K-E-L-D-Q-G-S-L-C-T-S-F-V-G	▶
242	K 2	IKK_group	S-D-E-F-R-P-R-S-K-S-Q-S-S-S-N	▶
243	K 3	IKK_group	T-E-S-I-T-A-T-S-P-A-S-M-V-G-G	▶
244	K 4	IKK_group	L-F-E-F-R-P-R-S-K-S-Q-S-S-G-S	▶
245	K 5	IKK_group	A-R-V-G-G-A-S-S-L-E-N-T-V-D-L	▶
246	K 6	IKK_group	**L-P-A-P-A-H-H-S-F-H-L-A-L-S-N**	▶
247	K 7	IKK_group	D-E-L-R-D-S-D-S-V-C-D-S-G-V-E	▶
248	K 8	IKK_group	R-P-R-S-R-S-G-ST-P-S-S-S-S-S-S	▶
249	K 9	IKK-beta	S-G-D-E-D-F-S-S-I-A-D-M-D-F-S	▶
250	K10	IKK-beta	Q-D-V-L-G-E-E-S-P-L-G-K-P-A-M	▶
251	K11	IKK-beta	P-G-D-E-D-F-S-ST-I-A-D-A-D-F-S	▶
252	K12	ILK	**Q-K-R-H-A-R-V-T-V-K-Y-D-R-R-E**	▶
253	K13	ILK	V-R-R-Q-G-K-V-T-V-K-Y-D-R-K-E	▶
254	K14	ILK	S-M-A-D-V-K-F-S-F-Q-C-P-G-R-M	▶
255	K15	ILK	**R-H-Q-Q-G-K-V-T-V-K-Y-D-R-K-E**	▶
256	K16	ILK	**R-P-H-F-P-Q-F-S-Y-S-A-S-S-T-A**	▶
257	K17	ILK	**R-K-Q-H-A-R-V-ST-V-K-Y-D-R-R-E**	▶
258	K18	IPL1	S-N-N-Q-R-R-K-T-I-F-V-E-D-F-P	▶
259	K19	IPL1	**V-N-L-Q-K-I-D-S-N-L-S-F-C-F-H**	▶
260	K20	IPL1	L-R-L-K-K-N-I-S-M-D-D-D-D-A-L	▶
261	K21	IPL1	**K-D-I-R-L-K-E-S-L-A-P-F-D-N-H**	▶
262	K22	IPL1	R-N-S-V-S-R-L-S-I-N-Q-L-G-S-L	▶
263	K23	IPL1	S-A-T-E-Y-R-L-S-I-G-S-A-P-T-S	▶
264	K24	IPL1	**N-S-I-N-R-R-K-ST-I-L-S-F-A-T-H**	▶
265	L 1	JNK_group	F-S-F-D-T-D-R-S-P-A-P-M-S-C-D	▶
266	L 2	JNK_group	D-T-E-G-R-P-P-S-P-P-P-T-S-T-P	▶
267	L 3	JNK_group	**I-H-F-W-S-S-L-S-P-V-A-P-L-S-P**	▶
268	L 4	JNK_group	G-L-G-G-G-A-A-S-P-P-A-A-S-P-F	▶
269	L 5	JNK_group	D-L-F-A-S-S-S-ST-P-P-A-A-S-P-P	▶
270	L 6	KIS	V-R-V-S-N-G-S-P-S-L-E-R-M-D-A	▶
271	L 7	KIS	**G-S-H-G-Q-T-P-S-P-G-A-L-P-L-G**	▶
272	L 8	KIS	G-R-D-S-R-S-G-S-P-M-A-R-R	▶
273	L 9	MAP2K_group	S-R-G-G-A-R-A-S-P-A-T-Q-P-P-P	▶
274	L10	MAP2K_group	P-S-D-L-L-P-M-S-P-S-V-Y-A-V-L	▶
275	L11	MAP2K_group	A-N-L-D-S-A-Q-S-P-G-P-S-W-P-A	▶
276	L12	MAP2K_group	**H-D-H-T-G-F-L-T-E-Y-V-A-T-R-W**	▶
277	L13	MAP2K_group	A-G-T-S-F-M-M-T-P-Y-V-V-T-R-Y	▶

278	L14	MAP2K_group	S-S-M-S-T-E-Q-T-L-A-S-D-T-D-S	▶
279	L15	MAP2K_group	D-G-S-A-V-N-G-T-S-S-A-E-T-N-L	▶
280	L16	MAP2K_group	**E-P-I-C-S-V-N-T-P-R-E-V-T-L-H**	▶
281	L17	MAP2K_group	E-Y-P-E-P-Y-A-S-P-P-Q-P-G-L-P	▶
282	L18	MAP2K_group	V-S-G-Q-L-I-D-S-M-A-N-S-F-V-G	▶
283	L19	MAP2K_group	S-S-G-Q-L-P-A-ST-P-A-V-S-T-L-A	▶
284	L20	MAP2K1	P-E-V-L-R-P-E-T-P-R-P-V-D-I-G	▶
285	L21	MAP2K1	P-F-R-D-S-P-L-S-S-R-L-L-D-D-G	▶
286	L22	MAP2K1	I-L-P-F-T-P-P-V-V-K-R-L-L-G-W	▶
287	L23	MAP2K1	D-F-P-K-K-P-L-T-P-Y-F-R-F-F-M	▶
288	L24	MAP2K1	D-G-R-M-V-Q-L-S-P-P-A-L-A-A-P	▶
289	M 1	MAP2K1	V-A-P-P-V-P-A-T-P-Y-E-A-F-D-P	▶
290	M 2	MAP2K1	**K-K-K-S-E-P-S-S-P-D-H-G-S-S-T**	▶
291	M 3	MAP2K1	D-T-P-A-A-P-K-ST-P-P-P-L-D-G-V	▶
292	M 4	MAP2K2	**G-S-R-T-A-P-Y-T-P-N-L-P-H-H-Q**	▶
293	M 5	MAP2K2	S-A-L-S-Y-L-Q-S-P-I-T-T-S-P-S	▶
294	M 6	MAP2K2	T-E-L-E-P-L-C-T-P-V-V-T-C-T-P	▶
295	M 7	MAP2K3	R-Q-A-D-S-E-M-T-G-Y-V-V-T-R-W	▶
296	M 8	MAP2K4	I-S-G-Q-L-V-D-S-I-A-K-T-R-D-A	▶
297	M 9	MAP2K4	L-V-D-S-I-A-K-T-R-D-A-G-C-R-P	▶
298	M10	MAP2K6	K-R-K-S-L-V-G-T-P-Y-W-M-A-P-E	▶
299	M11	MAP3K1	N-C-E-L-P-L-L-T-P-C-S-K-A-V-M	▶
300	M12	MAP3K7	G-S-P-S-I-R-C-S-S-V-S	▶
301	M13	MAP3K7	**C-D-I-Q-T-H-M-T-N-N-K-G-S-A-A**	▶
302	M14	MAP3K7	**S-D-I-I-I-H-G-ST-A-A-K-G-A-A-A**	▶
303	M15	MAP3K8	**T-G-D-Y-I-P-G-T-E-T-H-M-A-P-E**	▶
304	M16	MAP4K4	S-T-E-V-K-E-D-S-A-Y-G-S-Q-S-V	▶
305	M17	MAPK_group	S-R-D-P-V-A-R-T-S-P-L-Q-T-P-A	▶
306	M18	MAPK_group	K-M-K-N-K-P-R-S-P-V-V-E-L-S-K	▶
307	M19	MAPK_group	S-S-S-S-P-P-G-T-P-S-P-A-D-A-K	▶
308	M20	MAPK_group	**R-R-L-K-G-P-G-T-P-A-F-P-H-Y-L**	▶
309	M21	MAPK_group	C-A-D-V-P-L-L-T-P-S-S-K-E-M-M	▶
310	M22	MAPK_group	K-I-K-Q-E-V-E-S-P-T-D-K-S-G-N	▶
311	M23	MAPK_group	S-A-Y-G-G-L-T-S-P-G-L-S-Y-S-L	▶
312	M24	MAPK_group	**L-H-P-P-P-Q-L-S-P-F-L-Q-P-H-G**	▶
313	N 1	MAPK_group	I-D-E-N-C-L-L-S-P-L-A-G-E-D-D	▶
314	N 2	MAPK_group	W-N-L-V-S-P-D-S-P-R-S-I-D-S-N	▶
315	N 3	MAPK_group	**A-P-P-P-Q-P-P-T-P-A-L-P-H-P-P**	▶
316	N 4	MAPK_group	**Y-S-H-K-G-H-L-S-E-G-L-V-T-K-W**	▶
317	N 5	MAPK_group	L-S-L-P-S-T-Q-S-L-N-I-K-S-E-P	▶
318	N 6	MAPK_group	N-F-D-F-V-T-E-T-P-L-E-G-D-F-A	▶
319	N 7	MAPK_group	D-D-I-E-Q-W-F-T-E-D-P-G-P-D-E	▶
320	N 8	MAPK_group	E-S-P-L-C-P-L-S-P-L-E-A-G-D-L	▶
321	N 9	MAPK_group	**H-S-M-S-V-P-T-T-P-T-L-G-F-S-T**	▶
322	N10	MAPK_group	D-T-E-F-T-S-R-T-P-K-D-S-P-G-I	▶
323	N11	MAPK_group	E-L-I-L-K-P-P-S-P-I-S-E-A-P-R	▶
324	N12	MAPK_group	N-F-S-S-S-P-S-T-P-V-G-S-P-Q-G	▶
325	N13	MAPK_group	S-S-P-P-P-P-L-ST-P-L-L-P-P-S-A	▶

326	N14	MAPK1	G-P-L-A-P-P-A-S-P-G-P-F-A-T-R	▶
327	N15	MAPK1	D-G-P-Q-L-P-A-S-P-N-P-T-T-T-A	▶
328	N16	MAPK1	G-G-L-P-E-V-A-T-P-E-S-E-E-A-F	▶
329	N17	MAPK1	Y-P-S-M-P-A-F-S-P-G-P-G-I-K-E	▶
330	N18	MAPK1	A-I-K-V-E-P-A-S-P-P-Y-Y-S-E-K	▶
331	N19	MAPK1	G-A-P-T-E-P-A-ST-P-P-P-Y-K-G-S	▶
332	N20	MAPK10	**G-D-R-C-P-H-G-S-P-Q-G-P-L-A-P**	▶
333	N21	MAPK10	P-G-P-F-A-T-R-S-P-L-F-I-F-V-R	▶
334	N22	MAPK10	D-K-S-T-Q-T-P-S-P-P-C-Q-A-F-N	▶
335	N23	MAPK10	D-S-A-I-D-T-W-S-P-S-E-W-Q-M-A	▶
336	N24	MAPK10	G-D-L-A-P-T-A-ST-P-G-F-F-A-A-R	▶
337	O 1	MAPK11	G-F-S-K-N-C-G-S-P-G-S-S-Q-L-S	▶
338	O 2	MAPK12	G-W-D-S-P-P-A-S-P-L-Q-R-Q-P-S	▶
339	O 3	MAPK12	P-L-Q-R-Q-P-S-S-P-G-P-T-P-R-N	▶
340	O 4	MAPK12	G-L-D-R-P-P-S-ST-P-G-P-R-P-P-N	▶
341	O 5	MAPK13	G-T-E-E-K-C-G-S-P-R-V-R-T-L-S	▶
342	O 6	MAPK14	**I-H-F-W-S-T-L-S-P-I-A-P-R-S-P**	▶
343	O 7	MAPK14	Q-A-T-Q-P-L-A-T-P-V-V-S-V-T-T	▶
344	O 8	MAPK14	M-P-W-P-E-P-Q-S-P-R-V-L-P-N-G	▶
345	O 9	MAPK14	E-E-K-E-R-T-F-S-F-C-G-T-I-E-Y	▶
346	O10	MAPK14	K-E-D-L-P-V-I-T-I-D-P-A-S-P-Q	▶
347	O11	MAPK14	**Q-M-V-N-G-A-H-S-A-S-T-L-D-E-A**	▶
348	O12	MAPK14	T-P-T-E-P-P-A-ST-P-V-L-P-P-Q-G	▶
349	O13	MAPK4	L-V-T-K-W-Y-R-S-P-R-L-L-L-S-P	▶
350	O14	MAPK6	M-T-I-L-Q-A-P-T-P-A-P-S-T-I-P	▶
351	O15	MAPK7	S-A-L-Q-G-F-N-S-P-G-M-L-S-L-G	▶
352	O16	MAPK7	A-N-P-S-P-P-P-S-P-S-Q-Q-I-N-L	▶
353	O17	MAPK8	Q-T-E-P-Q-D-R-S-P-A-P-M-S-C-D	▶
354	O18	MAPKAPK2	S-R-S-L-Y-A-S-S-P-G-G-V-Y-A-T	▶
355	O19	MAPKAPK2	K-G-F-R-R-A-V-S-E-L-D-A-K-Q-A	▶
356	O20	MAPKAPK2	F-S-L-L-R-G-P-S-W-D-P-F-R-D-W	▶
357	O21	MAPKAPK2	T-A-L-Y-K-S-L-S-V-P-A-A-S-T-A	▶
358	O22	MAPKAPK2	K-L-I-D-R-T-E-S-L-N-R-S-I-E-K	▶
359	O23	MAPKAPK2	R-L-T-G-R-S-T-S-L-V-E-G-R-S-C	▶
360	O24	MAPKAPK2	C-S-L-E-R-Q-L-S-L-E-Q-E-V-Q-Q	▶
361	P 1	MAPKAPK2	T-T-S-T-R-T-Y-S-L-G-S-A-L-R-P	▶
362	P 2	MAPKAPK2	G-Q-G-A-P-G-P-S-L-T-G-S-P-W-P	▶
363	P 3	MAPKAPK2	P-R-L-L-R-S-L-ST-L-G-G-S-S-A-P	▶
364	P 4	MARK_group	**N-V-R-S-K-V-G-S-T-E-N-I-K-H-Q**	▶
365	P 5	MARK_group	**K-A-Q-A-K-V-G-S-L-D-N-V-G-H-L**	▶
366	P 6	MFPK	S-T-S-T-P-A-P-S-R-T-A-S-F-S-E	▶
367	P 7	MHCK	E-Q-G-R-G-R-S-S-V-Y-S-C-P-Q-D	▶
368	P 8	MHCK	G-A-G-A-K-K-M-S-T-Y-N-V-P-Q-N	▶
369	P 9	MHCK	K-A-G-D-E-K-A-ST-K-Y-K-E-P-Q-D	▶
370	P10	MLCK	Y-I-V-E-R-Q-K-T-Q-T-K-L-V-N	▶
371	P11	MLCK	R-R-A-A-E-G-S-S-N-V-F-S-M-F-D	▶
372	P12	MLCK	K-R-P-Q-R-A-T-ST-N-V-F-A-M-F-D	▶
373	P13	MST1	D-T-M-A-K-R-N-T-V-I-G-T-P-F-W	▶

374	P14	NEK	E-E-G-T-F-R-S-S-I-R-R-L-S-T-R	▶
375	P15	NEK2	L-G-Y-P-F-A-L-S-K-S-S-M-Y-T-V	▶
376	P16	NEK2	E-K-K-K-P-N-A-T-R-P-V-T-P-P-R	▶
377	P17	NEK6	**P-P-F-N-P-N-V-S-G-P-N-E-L-R-H**	▶
378	P18	NEK6	**E-G-G-Q-L-N-E-S-M-D-H-G-G-V-E**	▶
379	P19	NEK6	P-D-D-T-L-N-D-ST-A-D-A-E-S-L-E	▶
380	P20	NEK9	**S-K-T-T-A-A-H-S-L-V-G-T-P-Y-Y**	▶
381	P21	NLK	**S-H-A-V-H-P-L-T-P-L-I-T-Y-S-D**	▶
382	P22	NLK	**T-Y-S-D-E-H-F-S-P-G-S-H-P-S-H**	▶
383	P23	control	**A-W-R-H-P-Q-F-G-G-X-X-X-X**	▶
384	P24	neg. control	Bio-X-X-X-X-X-X-X-X-X-X-X-X	▶

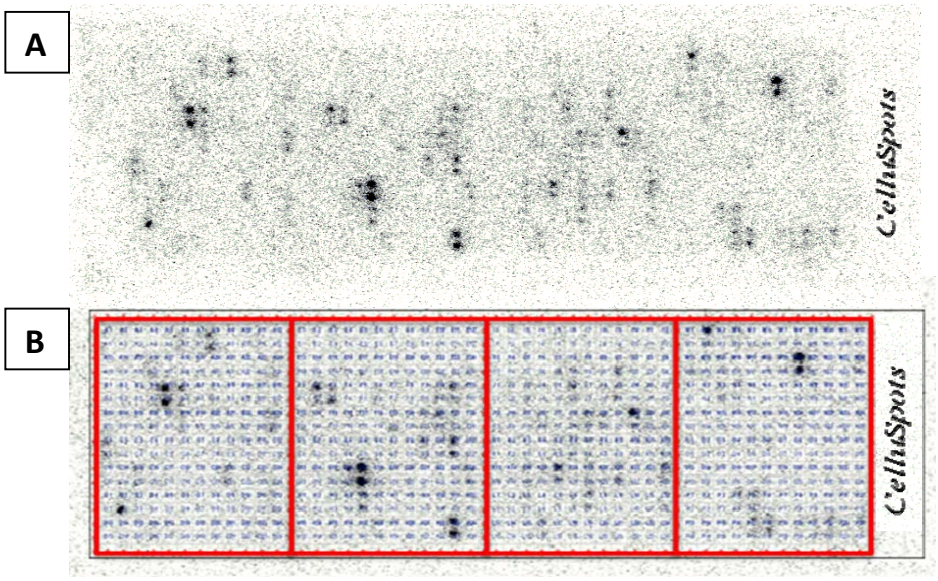


Figure S1. (A) Phosphor image of the $[^{99m}Tc][Tc(CO)_3]^+$ radiolabeled STKS-1 Celluspots™ peptide array post 48 hour wash in 70% acetonitrile in H_2O. The black dots represent the peptides that have been radiolabelled. **(B)** An overlay of the phosphor image on the Celluspots™ template.

Rationale for design of bespoke His-tagged peptide array

Sequences were included to evaluate classes of peptides to address pre-existing questions and questions arising from analysis of the STKS-1 array, loosely classified as follows:

- His/Cys Peptides: 10 peptide sequences for comparison with homogeneous solution phase labeling. These were based on the CKLAAALEHHHHHH labeling sequence engineered at the C-terminal of the C2A protein for $[^{99m}Tc][Tc(CO)_3]^+$ labelling (9). These 10 sequences contained different His-tags and are present in duplicate with and without an adjacent CK dipeptide: CKLAAALEHHHHHH, LAAALEHHHHHH, CKLAAALEHHHH, LAAALEHHHH, CKLAAALEHAHAHA, LAAALEHAHAHA, CKLAAALEHAHA, LAAALEHAHA, CKLAAALEHAAH, LAAALEHAAH. Each of these was synthesized separately as described elsewhere in this Supplementary Information for homogeneous labeling in solution.

- Distance from Cys (N-terminal His-tag): His/Cys sequences in which the position of the Cys has been varied with respect to the His residues. Cys is positioned between 7 and 1 amino acids from the nearest His. The His-tag is at the N-terminus (Nt) of the sequence.

- C-terminal His-tag: His/Cys peptide sequences with a C-terminal His-Tag.

- His/Cys tag with no Lys: His/Cys sequences without the Lys residues.

- His-tag in the middle: His-tag is embedded within the sequence (i.e. non-terminal) and surrounded by amino acids on either side. The surrounding amino acids are based on the composition of the His/Cys peptide and include Leu, Ala, Glu, Cys or Lys.

- Double cysteine: Two Cys residues within a His containing peptide sequence. One is on the N-terminal and one is on the C-terminal side of the His.

- HHHCHHH: Sequences with a Cys separating two sets of 3 sequential His residues

- Proline: Proline has been included in the His containing sequences at different positions e.g. HHHPHHH, HHHHHHP, HHHPHHHXXXP and HHHHHHXXXP, where X is another amino acid.

- Methionine: Methionine residues have been included in the His containing sequences to replace the Cys (based on the assumption that its interaction with Tc(I) or Re(I) would be similar to Cys but it cannot be oxidized to form disulfide bonds and hence may be more generally useful in proteins.

- Uncharged: His-containing sequences with no charged amino acids. Sequences include hydrophobic spacers such as Leu, Ala or Gly.

- Negatively charged amino acids: Sequences with 2 or more Glu and/or Asp residues surrounding the His.

- Positively charged amino acids: Sequences with 2 or more Arg and/or Lys residues surrounding the His.

- Serine: Sequences with multiple Ser residues surrounding the His.

- Catalytic sequences: Sequences with $(His)_6$-Tags and amino acid combinations that form catalytic triads that may affect the reactivity of His e.g. Ser, Asp and His.

- Controls: 4 spots on the CelluspotTM peptide array surface: 2 blank spots on the cellulose membrane and two randomly generated peptide sequences that contain no His. All other amino acids are included at least once in one of the sequences.

- HEHEHE Tag: Two His-containing labeling sequences previously published by Tolmachev et al.(11) for the radiolabeling of proteins with $[^{99m}Tc(CO)_3]^+$.

Table S3. Peptide array layout showing locations of grid numbers. Amino acid sequences corresponding to grid codes are listed in Tables S2 and S4.

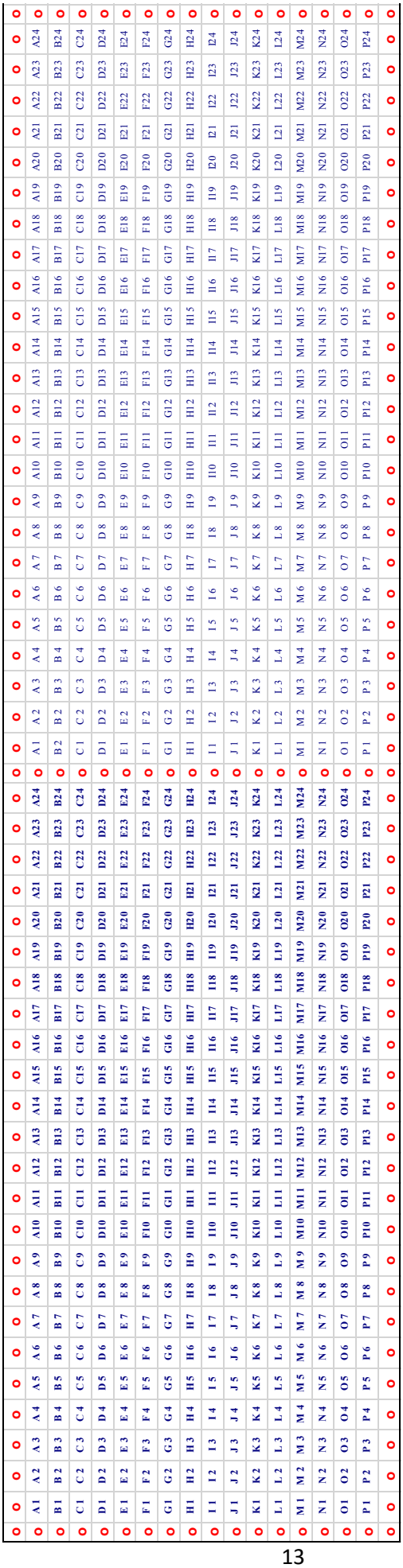


Table S4 (continued on following pages). Amino acid sequences in His-tagged peptide array. Position of each peptide in the array is given by the grid code which refers to Table S3. Doa = 3,6-dioxa-octanoic acid. Grid codes correspond to the layout shown in Table S3.

no	*Grid code*	*sequence*	*Reason for inclusion*	*N-terminus*
1	A 1	HHHHHHELAAAL Doa	10 SOLUTION PEPTIDES	acetylated
2	A 2	HHHHHHELAAALKC Doa		acetylated
3	A 3	AHAHAHELAAAL Doa		acetylated
4	A 4	AHAHAHELAAALKC Doa		acetylated
5	A 5	HHHHELAAAL Doa		acetylated
6	A 6	HHHHELAAALKC Doa		acetylated
7	A 7	AHAHELAAAL Doa		acetylated
8	A 8	AHAHELAAALKC Doa		acetylated
9	A 9	HAAHELAAAL Doa		acetylated
10	A10	HAAHELAAALKC Doa		acetylated
11	A11	HHHHHHELAAALCK Doa	N-TERMINAL CYSTEINE	acetylated
12	A12	HHHHHHELAAACLK Doa		acetylated
13	A13	HHHHHHELAACALK Doa		acetylated
14	A14	HHHHHHELACAALK Doa		acetylated
15	A15	HHHHHHELCAAALK Doa		acetylated
16	A16	HHHHHHECLAAALK Doa		acetylated
17	A17	HHHHHHCELAAALK Doa		acetylated
18	A18	AHAHAHELAAALCK Doa		acetylated
19	A19	AHAHAHELAAACLK Doa		acetylated
20	A20	AHAHAHELAACALK Doa		acetylated
21	A21	AHAHAHELACAALK Doa		acetylated
22	A22	AHAHAHELCAAALK Doa		acetylated
23	A23	AHAHAHECLAAALK Doa		acetylated
24	A24	AHAHAHCELAAALK Doa		acetylated
25	B 1	AHAHELAAALCK Doa		acetylated
26	B 2	AHAHELAAACLK Doa		acetylated
27	B 3	AHAHELAACALK Doa		acetylated
28	B 4	AHAHELACAALK Doa		acetylated
29	B 5	AHAHELCAAALK Doa		acetylated
30	B 6	AHAHECLAAALK Doa		acetylated
31	B 7	HAAHCELAAALK Doa		acetylated
32	B 8	HAAHELAAALCK Doa		acetylated
33	B 9	HAAHELAAACLK Doa		acetylated
34	B10	HAAHELAACALK Doa		acetylated
35	B11	HAAHELACAALK Doa		acetylated
36	B12	HAAHELCAAALK Doa		acetylated
37	B13	HAAHECLAAALK Doa		acetylated
38	B14	HAAHCELAAALK Doa		acetylated
39	B15	CKLAAALEHHHHHH Doa	C-TERMINAL CYSTEINE	acetylated
40	B16	CKLAAALEHAHAHA Doa		acetylated

41	B17	CKLAAALEHHHH Doa		acetylated
42	B18	CKLAAALEHAHA Doa		acetylated
43	B19	CKLAAALEHAAH Doa		acetylated
44	B20	KCLAAALEHHHHHH Doa	DISTANCE FROM CYSTEINE (C-terminal cysteine)	acetylated
45	B21	KLACAALEHHHHHH Doa		acetylated
46	B22	KLAAACLEHHHHHH Doa		acetylated
47	B23	KLAAALECHHHHHH Doa		acetylated
48	B24	HHHHHHELAAALC Doa	NO LYSINE	acetylated
49	C 1	AHAHAHELAAALC Doa		acetylated
50	C 2	HHHHELAAALC Doa		acetylated
51	C 3	AHAHELAAALC Doa		acetylated
52	C 4	HAAHELAAALC Doa		acetylated
53	C 5	HHHHHHELAACAL Doa		acetylated
54	C 6	HHHHHHELCAAAL Doa		acetylated
55	C 7	HHHHHHCELAAAL Doa		acetylated
56	C 8	AHAHAHELAACAL Doa		acetylated
57	C 9	AHAHAHELCAAAL Doa		acetylated
58	C10	AHAHAHCELAAAL Doa		acetylated
59	C11	HHHHELAACAL Doa		acetylated
60	C12	HHHHELCAAAL Doa		acetylated
61	C13	HHHHCELAAAL Doa		acetylated
62	C14	AHAHELAACAL Doa		acetylated
63	C15	AHAHELCAAAL Doa		acetylated
64	C16	AHAHCELAAAL Doa		acetylated
65	C17	HAAHELAACAL Doa		acetylated
66	C18	HAAHELCAAAL Doa		acetylated
67	C19	HAAHCELAAAL Doa		acetylated
68	C20	LAAALEHHHHHH Doa	No Cys/Lys (C/N-term His-tag)	acetylated
69	C21	LAAALEHAHAHA Doa		acetylated
70	C22	LAAALEHAHA Doa		acetylated
71	C23	LAAALEHAAH Doa		acetylated
72	C24	ALEHHHHHHELAKC Doa	NON-TERMINAL HIS-TAG, INCLUDING C, K	acetylated
73	D 1	ALEHHHHHHELAC Doa		acetylated
74	D 2	ALEHHHHHHELCAK Doa		acetylated
75	D 3	ALEHHHHHHELCA Doa		acetylated
76	D 4	ALEHHHHHHCELAK Doa		acetylated
77	D 5	ALEHHHHHHCELA Doa		acetylated
78	D 6	ALEAHAHAHELAKC Doa		acetylated
79	D 7	ALEAHAHAHELAC Doa		acetylated
80	D 8	ALEAHAHAHELCAK Doa		acetylated
81	D 9	ALEAHAHAHELCA Doa		acetylated
82	D10	ALEAHAHAHCELAK Doa		acetylated
83	D11	ALEAHAHAHCELA Doa		acetylated

84	D12	ALEAHAHELAKC	Doa		acetylated
85	D13	ALEAHAHELCAK	Doa		acetylated
86	D14	ALEAHAHCELAK	Doa		acetylated
87	D15	ALEAHAHELAC	Doa		acetylated
88	D16	ALEAHAHELCA	Doa		acetylated
89	D17	ALEAHAHCELA	Doa		acetylated
90	D18	ALEHAAHELAKC	Doa		acetylated
91	D19	ALEHAAHELCAK	Doa		acetylated
92	D20	ALEHAAHCELAK	Doa		acetylated
93	D21	ALEHAAHELAC	Doa		acetylated
94	D22	ALEHAAHELCA	Doa		acetylated
95	D23	ALEHAAHCELA	Doa		acetylated
96	D24	ALEHHHHHHELAK	Doa		acetylated
97	E 1	ALEHHHHHHELA	Doa		acetylated
98	E 2	ALEAHAHAHELAK	Doa		acetylated
99	E 3	ALEAHAHAHELA	Doa	NON-TERMINAL HIS-TAG (NO C, K)	acetylated
100	E 4	ALEAHAHELAK	Doa		acetylated
101	E 5	ALEAHAHELA	Doa		acetylated
102	E 6	ALEHAAHELAK	Doa		acetylated
103	E 7	ALEHAAHELA	Doa		acetylated
104	E 8	CLEHHHHHHELC	Doa		acetylated
105	E 9	KLCEHHHHHHECLK	Doa		acetylated
106	E10	KLECHHHHHHCELK	Doa	DOUBLE CYSTEINE	acetylated
107	E11	KLEHHHHHHELK	Doa		acetylated
108	E12	CKLEHHHHHHELKC	Doa		acetylated
109	E13	HHHCHHHELAAAL	Doa		acetylated
110	E14	HHHCHHHGLAAAL	Doa		acetylated
111	E15	HHHCHHHRLAAAL	Doa	CYSTEINE WITHIN HIS-TAG (compare E, D, K, R, G, S, K, M)	acetylated
112	E16	HHHMHHHGLAAAL	Doa		acetylated
113	E17	HHHMHHHELAAAL	Doa		acetylated
114	E18	HHHCHHHDLAAAL	Doa		acetylated
115	E19	HHHCHHHSLAAAL	Doa		acetylated
116	E20	HHHCHHHKLAAAL	Doa		acetylated
117	E21	HHHHHHELAPALKC	Doa		acetylated
118	E22	HHHHHHELAPALC	Doa		acetylated
119	E23	HHHHHHELAPAL	Doa		acetylated
120	E24	HHHPHHHELAAALKC	Doa		acetylated
121	F 1	HHHPHHHELAAALC	Doa	PROLINE	acetylated
122	F 2	HHHPHHHELAAAL	Doa		acetylated
123	F 3	HHHPHHHELAPALKC	Doa		acetylated
124	F 4	HHHPHHHELAPALC	Doa		acetylated
125	F 5	HHHPHHHELAPAL	Doa		acetylated
126	F 6	HHHHHHGLAPALKC	Doa		acetylated

127	F 7	HHHHHHGLAPALC	Doa		acetylated
128	F 8	HHHPHHHGLAPALKC	Doa		acetylated
129	F 9	HHHPHHHGLAPALC	Doa		acetylated
130	F10	HHHPHHHGLAPAL	Doa		acetylated
131	F11	HHHPHHHGLAAALKC	Doa		acetylated
132	F12	HHHPHHHGLAAALC	Doa		acetylated
133	F13	HHHPHHHGGPGKC	Doa		acetylated
134	F14	HHHPHHHGGPGC	Doa		acetylated
135	F15	HHHHHHPGGGC	Doa		acetylated
136	F16	HHHPHHHPGGGC	Doa		acetylated
137	F17	HHHHHHRLAPALKC	Doa		acetylated
138	F18	HHHHHHRLAPALC	Doa		acetylated
139	F19	HHHPHHHRLAPALKC	Doa		acetylated
140	F20	HHHPHHHRLAPALC	Doa		acetylated
141	F21	HHHPHHHRLAPAL	Doa		acetylated
142	F22	HHHPHHHRLAAALKC	Doa		acetylated
143	F23	HHHPHHHRLAAALC	Doa		acetylated
144	F24	HHHHHHELAPAM	Doa		acetylated
145	G 1	HHHHHHELAPAKM	Doa		acetylated
146	G 2	HHHPHHHGGPGKM	Doa		acetylated
147	G 3	HHHPHHHGGPGM	Doa		acetylated
148	G 4	HHHPHHHPGGGM	Doa		acetylated
149	G 5	HHHHHHPGGGM	Doa		acetylated
150	G 6	HHHHHHPELAAALKC	Doa		acetylated
151	G 7	HHHHHHPELAAALC	Doa		acetylated
152	G 8	HHHHHHPELAAAL	Doa		acetylated
153	G 9	HHHHHHPELAAALM	Doa		acetylated
154	G10	HHHPHHHELAAALM	Doa		acetylated
155	G11	HHHPHHHPELAAAL	Doa		acetylated
156	G12	HHHPHHHPELAAALKC	Doa		acetylated
157	G13	HHHPHHHPELAAALC	Doa		acetylated
158	G14	HHHHHHELAAALKM	Doa		acetylated
159	G15	HHHHHHELAAALM	Doa		acetylated
160	G16	HHHHHHELAAMALK	Doa		acetylated
161	G17	HHHHHHELMAAALK	Doa		acetylated
162	G18	HHHHHHMELAAALK	Doa		acetylated
163	G19	HHHHHHELAAMAL	Doa		acetylated
164	G20	HHHHHHELMAAAL	Doa	METHIONINE	acetylated
165	G21	HHHHHHMELAAAL	Doa		acetylated
166	G22	MKLEHHHHHHELKM	Doa		acetylated
167	G23	KLEMHHHHHHMELK	Doa		acetylated
168	G24	MLEHHHHHHELM	Doa		acetylated
169	H 1	LEMHHHHHHMEL	Doa		acetylated
170	H 2	MKLEAHAHELKM	Doa		acetylated

171	H 3	MLEAHAHELM	Doa		acetylated
172	H 4	KLEMAHAHMELK	Doa		acetylated
173	H 5	LEMAHAHMEL	Doa		acetylated
174	H 6	KLMEHHHHHHEMLK	Doa		acetylated
175	H 7	LMEHHHHHHEML	Doa		acetylated
176	H 8	MKLEAHAHAHELKM	Doa		acetylated
177	H 9	KLMEAHAHAHEMLK	Doa		acetylated
178	H10	KLEMAHAHAHMELK	Doa		acetylated
179	H11	MLEAHAHAHELM	Doa		acetylated
180	H12	MLEAHAHAHELM	Doa		acetylated
181	H13	LEMAHAHAHMEL	Doa		acetylated
182	H14	HHHHHHAMAAAM	Doa		acetylated
183	H15	HMHHMHLAAAL	Doa		acetylated
184	H16	HHHHHHGMGGGM	Doa		acetylated
185	H17	HHHHHHGGGGM	Doa		acetylated
186	H18	AHAHAMAAAM	Doa		acetylated
187	H19	HAAHAMAAAM	Doa		acetylated
188	H20	HHHHHHGLAAALM	Doa		acetylated
189	H21	HHHHHHELAAALM	Doa		acetylated
190	H22	HHHHHHRLAAALM	Doa		acetylated
191	H23	HHHHHHDLAAALM	Doa		acetylated
192	H24	HHHHHHSLAAALM	Doa		acetylated
193	I 1	HHHHHHRLAAALKM	Doa		acetylated
194	I 2	HHHHHHDLAAALKM	Doa		acetylated
195	I 3	HHHHHHGLAAALKM	Doa		acetylated
196	I 4	HHHHHHSLAAALKM	Doa		acetylated
197	I 5	KMLEHHHHHHELMK	Doa		acetylated
198	I 6	HHHHHHLAAAL	Doa		acetylated
199	I 7	HHHHHHLAAALKC	Doa		acetylated
200	I 8	HHHHHHLAAALC	Doa		acetylated
201	I 9	HHHHLAAAL	Doa		acetylated
202	I10	HHHHLAAALKC	Doa		acetylated
203	I11	HHHHLAAALC	Doa		acetylated
204	I12	AHAHAHLAAAL	Doa		acetylated
205	I13	AHAHAHLAAALKC	Doa	UNCHARGED RESIDUES	acetylated
206	I14	AHAHAHLAAALC	Doa		acetylated
207	I15	AHAHLAAAL	Doa		acetylated
208	I16	AHAHLAAALKC	Doa		acetylated
209	I17	AHAHLAAALC	Doa		acetylated
210	I18	HAAHLAAAL	Doa		acetylated
211	I19	HAAHLAAALKC	Doa		acetylated
212	I20	HAAHLAAALC	Doa		acetylated
213	I21	HHHHHHALEEELKC	Doa	NEGATIVE RESIDUES (E)	acetylated
214	I22	HHHHHHALEEEL	Doa		acetylated

215	I23	HHHHHHALEEELC	Doa		acetylated
216	I24	EHEHEHELAAAL	Doa		acetylated
217	J 1	EHEHEHELAAALC	Doa		acetylated
218	J 2	EHEHEHELAAALKC	Doa		acetylated
219	J 3	EHEHEHGLAAAL	Doa		acetylated
220	J 4	EHEHEHGLAAALC	Doa		acetylated
221	J 5	EHEHEHGLAAALKC	Doa		acetylated
222	J 6	EHEHELAAAL	Doa		acetylated
223	J 7	EHEHELAAALC	Doa		acetylated
224	J 8	EHEHGLAAAL	Doa		acetylated
225	J 9	EHEHGLAAALC	Doa		acetylated
226	J10	HEEHELAAAL	Doa		acetylated
227	J11	HEEHELAAALC	Doa		acetylated
228	J12	HHHHHHEEAAEE	Doa		acetylated
229	J13	HHHHHHEEAAEEC	Doa		acetylated
230	J14	HHHHHHGEGGE	Doa		acetylated
231	J15	HHHHHHGEGGEC	Doa		acetylated
232	J16	EHEHEHGEGGE	Doa		acetylated
233	J17	EHEHEHGEGGEC	Doa		acetylated
234	J18	HHHHHHEGGGE	Doa		acetylated
235	J19	HHHHHHEGGGEC	Doa		acetylated
236	J20	EHEHGGAAE	Doa		acetylated
237	J21	EHEHGGAAEC	Doa		acetylated
238	J22	EHEHEHGGAAE	Doa		acetylated
239	J23	EHEHEHGGAAEC	Doa		acetylated
240	J24	GEEHHHHHHEEG	Doa		acetylated
241	K 1	GEEHHHHHHEEGC	Doa		acetylated
242	K 2	GGEHHHEHHHEGG	Doa		acetylated
243	K 3	GGEHHHEHHHEGGC	Doa		acetylated
244	K 4	HHHHHHDLAAAL	Doa		acetylated
245	K 5	HHHHHHDLAAALC	Doa		acetylated
246	K 6	HHHHHHDLAAALKC	Doa		acetylated
247	K 7	HHHHHHALDDDLKC	Doa		acetylated
248	K 8	HHHHHHALDDDL	Doa		acetylated
249	K 9	HHHHHHALDDDLC	Doa		acetylated
250	K10	DHDHDHDLAAAL	Doa		acetylated
251	K11	DHDHDHDLAAALC	Doa	NEGATIVE RESIDUE (D)	acetylated
252	K12	DHDHDHDLAAALKC	Doa		acetylated
253	K13	DHDHDLAAAL	Doa		acetylated
254	K14	DHDHDLAAALC	Doa		acetylated
255	K15	HDDHDLAAAL	Doa		acetylated
256	K16	HDDHDLAAALC	Doa		acetylated
257	K17	HHHHHHDDAADD	Doa		acetylated
258	K18	HHHHHHDDAADDC	Doa		acetylated

259	K19	HHHHHHGDGGD Doa		acetylated
260	K20	HHHHHHGDGGDC Doa		acetylated
261	K21	DHDHDHGDGGD Doa		acetylated
262	K22	DHDHDHGDGGDC Doa		acetylated
263	K23	HHHHHHDGGGD Doa		acetylated
264	K24	HHHHHHDGGGDC Doa		acetylated
265	L 1	DHDHGGAAD Doa		acetylated
266	L 2	DHDHGGAADC Doa		acetylated
267	L 3	DHDHDHGGAAD Doa		acetylated
268	L 4	DHDHDHGGAADC Doa		acetylated
269	L 5	GDDHHHHHHDDG Doa		acetylated
270	L 6	GDDHHHHHHDDGC Doa		acetylated
271	L 7	GGDHHHDHHHDGG Doa		acetylated
272	L 8	GGDHHHDHHHDGGC Doa		acetylated
273	L 9	HHHHHHDAEAD Doa	NEGATIVE RESIDUES (E & D)	acetylated
274	L10	HHHHHHDAEADKC Doa		acetylated
275	L11	HHHHHHDAEADC Doa		acetylated
276	L12	EHEHEHGGAADC Doa		acetylated
277	L13	EHEHEHGGAAD Doa		acetylated
278	L14	DHDHDHGGAAE Doa		acetylated
279	L15	DHDHDHGGAAEC Doa		acetylated
280	L16	HHHHHHDDAEE Doa		acetylated
281	L17	HHHHHHDDAEEC Doa		acetylated
282	L18	DHDHGGAAE Doa		acetylated
283	L19	DHDHGGAAEC Doa		acetylated
284	L20	EHEHGGAAD Doa		acetylated
285	L21	EHEHGGAADC Doa		acetylated
286	L22	HHHHHHEAAAD Doa		acetylated
287	L23	HHHHHHEAAADC Doa		acetylated
288	L24	GEEHHHHHHDDGC Doa		acetylated
289	M 1	GEEHHHHHHDDG Doa		acetylated
290	M 2	GGEHHHDHHHEGG Doa		acetylated
291	M 3	GGEHHHDHHHEGGC Doa		acetylated
292	M 4	HHHHHHRLAAAL Doa	POSITIVE RESIDUE (R)	acetylated
293	M 5	HHHHHHRLAAALC Doa		acetylated
294	M 6	HHHHHHRLAAALKC Doa		acetylated
295	M 7	HHHHHHALRRRL Doa		acetylated
296	M 8	HHHHHHALRRRLKC Doa		acetylated
297	M 9	HHHHHHALRRRLC Doa		acetylated
298	M10	RHRHRHRLAAAL Doa		acetylated
299	M11	RHRHRHRLAAALC Doa		acetylated
300	M12	RHRHRHRLAAALKC Doa		acetylated
301	M13	RHRHRLAAAL Doa		acetylated
302	M14	RHRHRLAAALC Doa		acetylated

303	M15	RHRHRLAAALKC	Doa	acetylated
304	M16	HRRHRLAAAL	Doa	acetylated
305	M17	HRRHRLAAALC	Doa	acetylated
306	M18	HHHHHHRRAARR	Doa	acetylated
307	M19	HHHHHHRRAARRC	Doa	acetylated
308	M20	HHHHHHGRGGR	Doa	acetylated
309	M21	HHHHHHGRGGRC	Doa	acetylated
310	M22	HHHHHHRGGGR	Doa	acetylated
311	M23	HHHHHHRGGGRC	Doa	acetylated
312	M24	HHHHHHRARAR	Doa	acetylated
313	N 1	HHHHHHRARARC	Doa	acetylated
314	N 2	RHRHRHGRGGR	Doa	acetylated
315	N 3	RHRHRHGRGGRC	Doa	acetylated
316	N 4	RHRHGGAAR	Doa	acetylated
317	N 5	RHRHGGAARC	Doa	acetylated
318	N 6	RHRHRHGGAAR	Doa	acetylated
319	N 7	RHRHRHGGAARC	Doa	acetylated
320	N 8	GRRHHHHHHRRG	Doa	acetylated
321	N 9	CRRHHHHHHRRC	Doa	acetylated
322	N10	GGRHHHRHHHRGG	Doa	acetylated
323	N11	GGRHHHRHHHRGGC	Doa	acetylated
324	N12	HHHHHHSLAAAL	Doa	acetylated
325	N13	HHHHHHSLAAALKC	Doa	acetylated
326	N14	HHHHHHSLAAALC	Doa	acetylated
327	N15	HHHHHHALSSSL	Doa	acetylated
328	N16	HHHHHHALSSSLC	Doa	acetylated
329	N17	SHSHSHSLAAAL	Doa	acetylated
330	N18	SHSHSHSLAAALC	Doa	acetylated
331	N19	SHSHSHSLAAALKC	Doa	acetylated
332	N20	SHSHSLAAAL	Doa	acetylated
333	N21	SHSHSLAAALC	Doa	acetylated
334	N22	HHHHHHSSAASS	Doa	acetylated
335	N23	HHHHHHSSAASSC	Doa	acetylated
336	N24	SHSHSHGSGGS	Doa	acetylated
337	O 1	SHSHSHGSGGSC	Doa	acetylated
338	O 2	HHHHHHSGGGS	Doa	acetylated
339	O 3	HHHHHHSGGGSC	Doa	acetylated
340	O 4	HHHHHHSASAS	Doa	acetylated
341	O 5	HHHHHHSASASC	Doa	acetylated
342	O 6	GSSHHHHHHSSG	Doa	acetylated
343	O 7	GSSHHHHHHSSGC	Doa	acetylated
344	O 8	GGSHHHSHHHSGG	Doa	acetylated
345	O 9	GGSHHHSHHHSGGC	Doa	acetylated
346	O10	HHHHHHKLAAAL	Doa	acetylated

SERINE, S

LYSINE, K

347	O11	HHHHHHALKKKL Doa		acetylated
348	O12	HHHHHHALKKKLC Doa		acetylated
349	O13	KHKHKHKLAAAL Doa		acetylated
350	O14	KHKHKHKLAAALC Doa		acetylated
351	O15	HHHHHHKKAAKK Doa		acetylated
352	O16	HHHHHHKKAAKKC Doa		acetylated
353	O17	KHKHKHGKGGK Doa		acetylated
354	O18	KHKHKHGKGGKC Doa		acetylated
355	O19	HHHHHHKGGGK Doa		acetylated
356	O20	HHHHHHKGGGKC Doa		acetylated
357	O21	HHHHHHKAKAK Doa		acetylated
358	O22	HHHHHHKAKAKC Doa		acetylated
359	O23	GKKHHHHHHKKG Doa		acetylated
360	O24	GKKHHHHHHKKGC Doa		acetylated
361	P 1	GGKHHHKHHHKGG Doa		acetylated
362	P 2	GGKHHHKHHHKGGC Doa		acetylated
363	P 3	AHAAHELAAAL Doa	RANDOM	acetylated
364	P 4	AHAAHELAAALKC Doa		acetylated
365	P 5	REEVPRRSGLSAGHR Doa	INTER-ARRAY COMPARISON	acetylated
366	P 6	GYLRKPKSMHKRFFV Doa		acetylated
367	P 7	EKKTKIRSLHNKLLN Doa		acetylated
368	P 8	RRLKGPGTPAFPHYL Doa		acetylated
369	P 9	.space	UNMODIFIED CONTROL	empty
370	P10	HAc	BLANK ACETYLATED CONTROL	acetylated
371	P11	DGGHHHDHHHGGD Doa	CATALYTIC	acetylated
372	P12	DGGHHHHHHGGD Doa		acetylated
373	P13	HHHHHHGRCRG Doa		acetylated
374	P14	RCRGHHHHHHGRCR Doa		acetylated
375	P15	HHHHHHGDCDG Doa		acetylated
376	P16	DCDGHHHHHHGDCD Doa		acetylated
377	P17	HHHHHHGQCSG Doa		acetylated
378	P18	SCQGHHHHHHGQCS Doa		acetylated
379	P19	SGGHHHHHHGGD Doa		acetylated
380	P20	GGSHHHHHHDGG Doa		acetylated
381	P21	GQLVNEREGASPPWY Doa	RANDOM	acetylated
382	P22	RKNDDSTYAGMIMFE Doa		acetylated
383	P23	GSSHHHHHHLQVDNK Doa	HEHEHE COMPARISON	acetylated
384	P24	MHEHEHEAENKFNKE Doa		acetylated

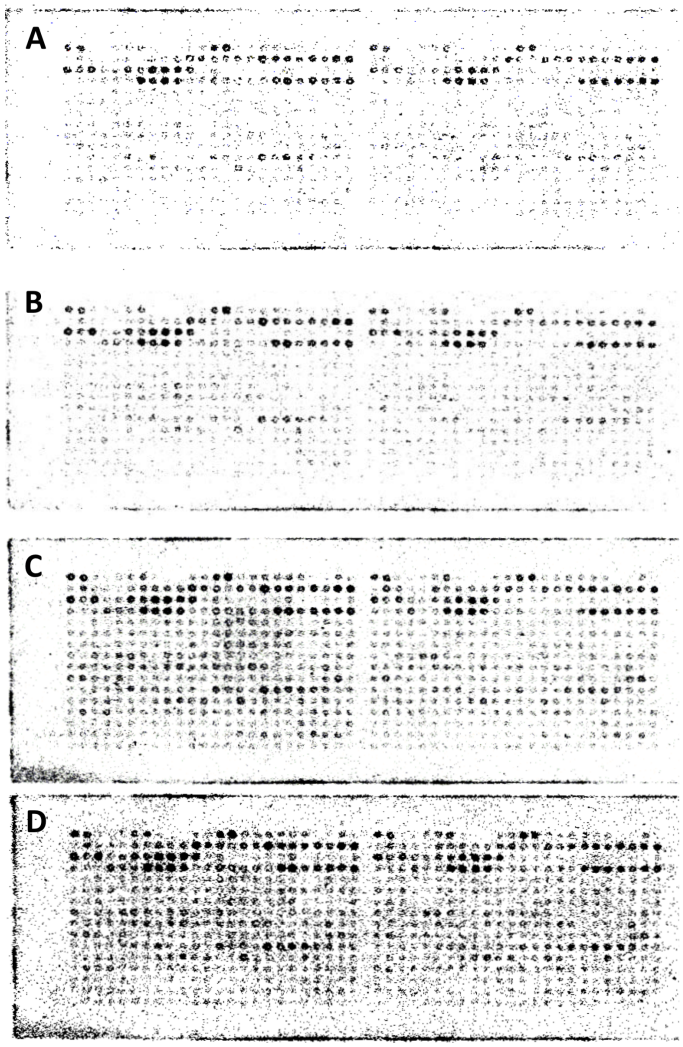


Figure S2. Phosphor image of the $[^{99m}Tc(CO)_3]^+$ radiolabeled His-Tagged CelluspotTM peptide array. Each black spot represents a peptide sequence. **A)** Image after 15 minutes total incubation time, **B)** Image after 30 minute total incubation time, **C)** Image after 60 minute total incubation time and **D)** Image after 120 minute incubation time.

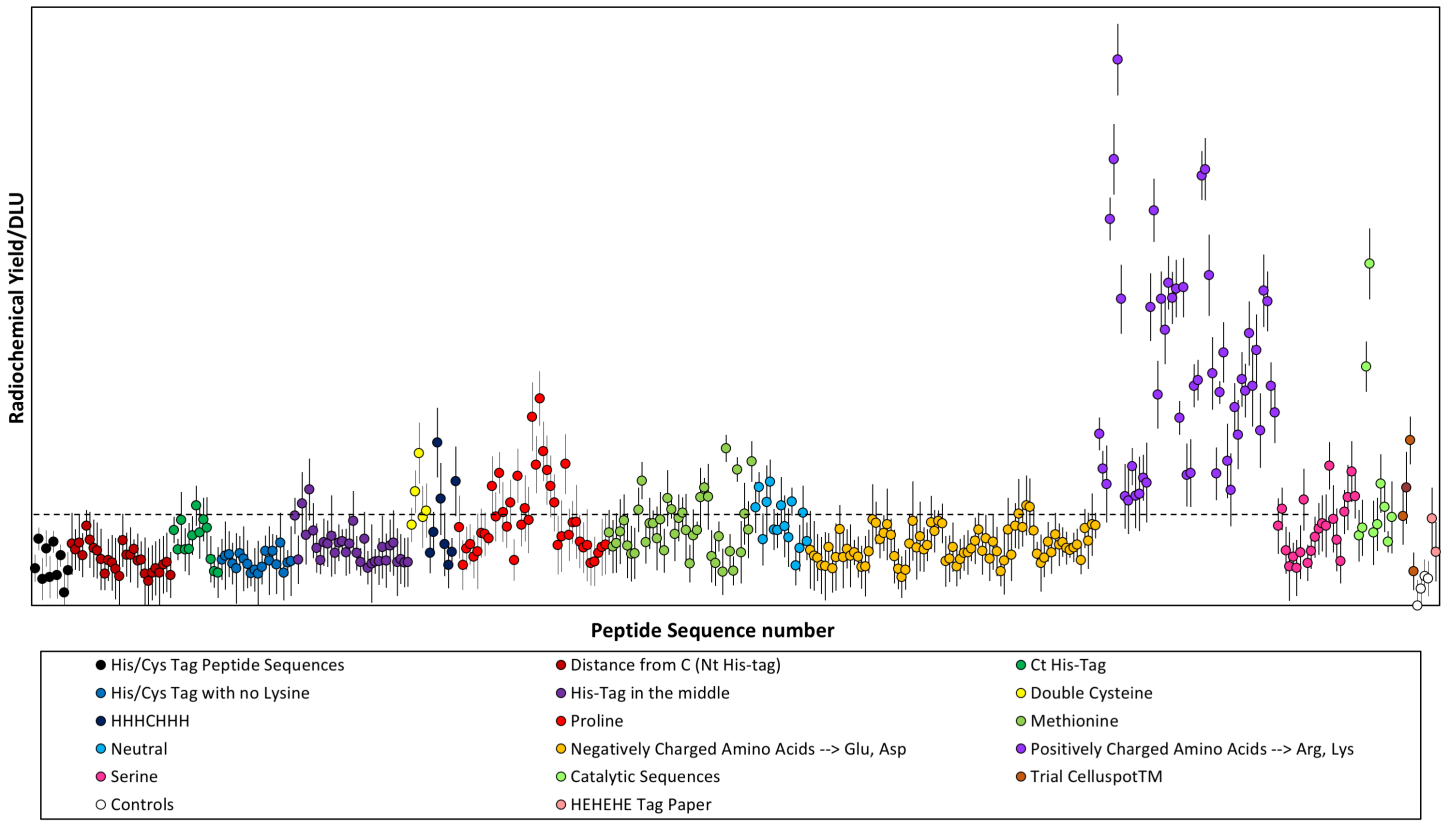


Figure S3. Radiochemical yield of all 384 peptides on the Celluspots^{TM} His-tagged peptide array post radiolabeling with $[^{99m}Tc][Tc(CO)_3]^+$ (15 minutes incubation time) in PBS at pH 7.4. Results and standard deviations are calculated based on 6 normalized sets of data. The peptides are categorized and color-coded according to their characteristic features of the amino acids that surround the His residues and the arrangement of these amino acids with regards to the His residues. The black horizontal broken line on the graph emphasises the clear boundary between the ten His/Cys tag peptides labeled in solution (black category) and the peptides containing 2 or more Arg/Lys residues (purple category). The extent of the positive charge influence on labeling is highlighted in the two categories "Proline" (red) and "Catalytic Sequences" (lime green, extreme right). Within these categories it is possible to see some individual sequences with a superior radiochemical yield in comparison to all other sequences in that category. These "superior" sequences all contain at least 2 Arg residues. For example in the "Catalytic Sequences" category, of the 8 peptides only two contain multiple Arg residues, GRCRGHHHHHH and RCRGHHHHHHGRCR, and these are the two that show a preferential coordination to $[^{99m}Tc(CO)_3]^+$.

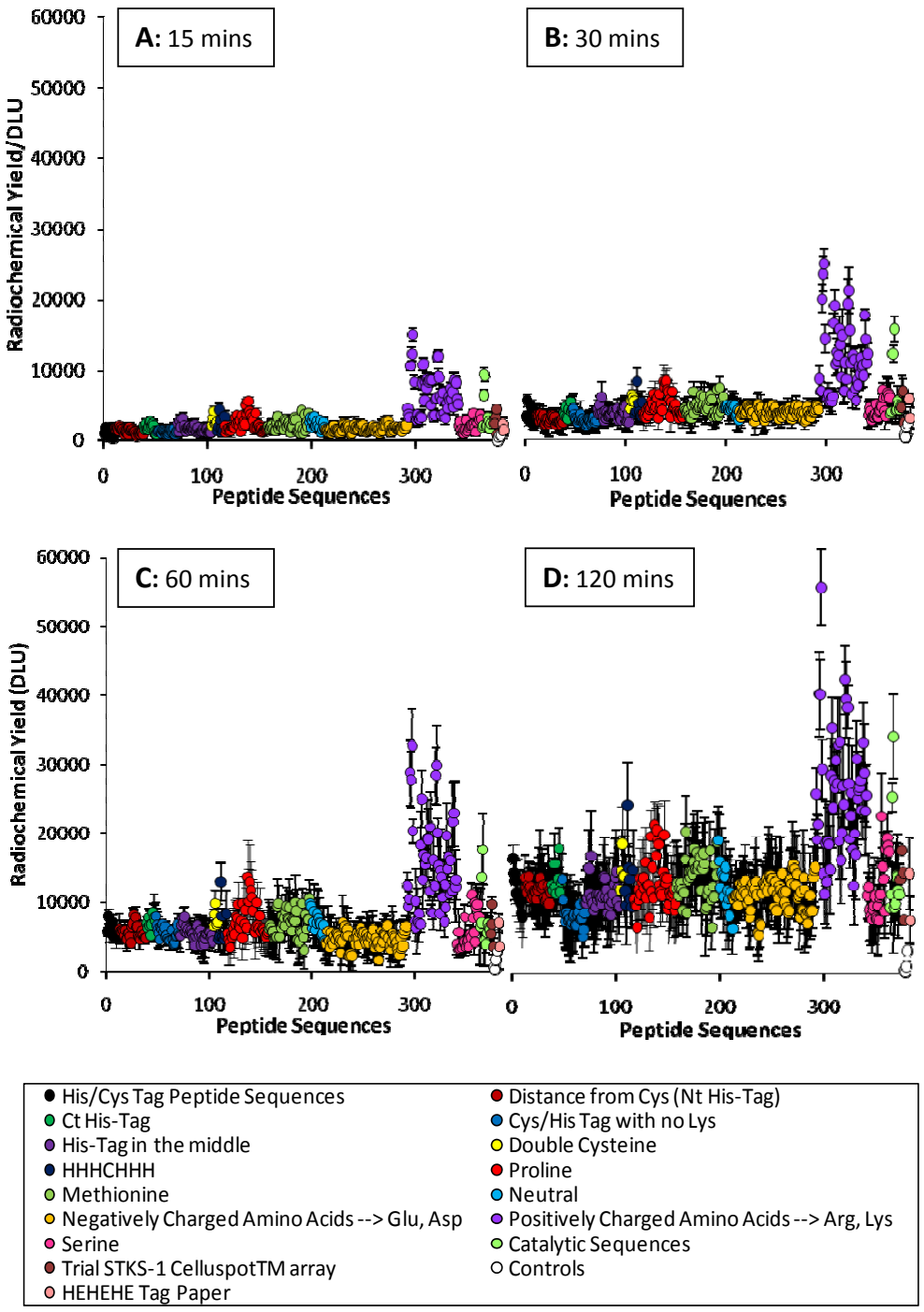


Figure S4. Time dependence of radiochemical yield of all 384 peptides on the His-Tagged Celluspot™ peptide array post radiolabeling with $[^{99m}Tc(CO)_3]^+$ in PBS at pH 7.4, showing increased labeling with time. Trends evident at 120 min are already emerging by 15 min. Results and standard deviations are calculated based on 6 sets of data. **A)** 15 min, **B)** 30 min, **C)** 60 min, **D)** 120 min.

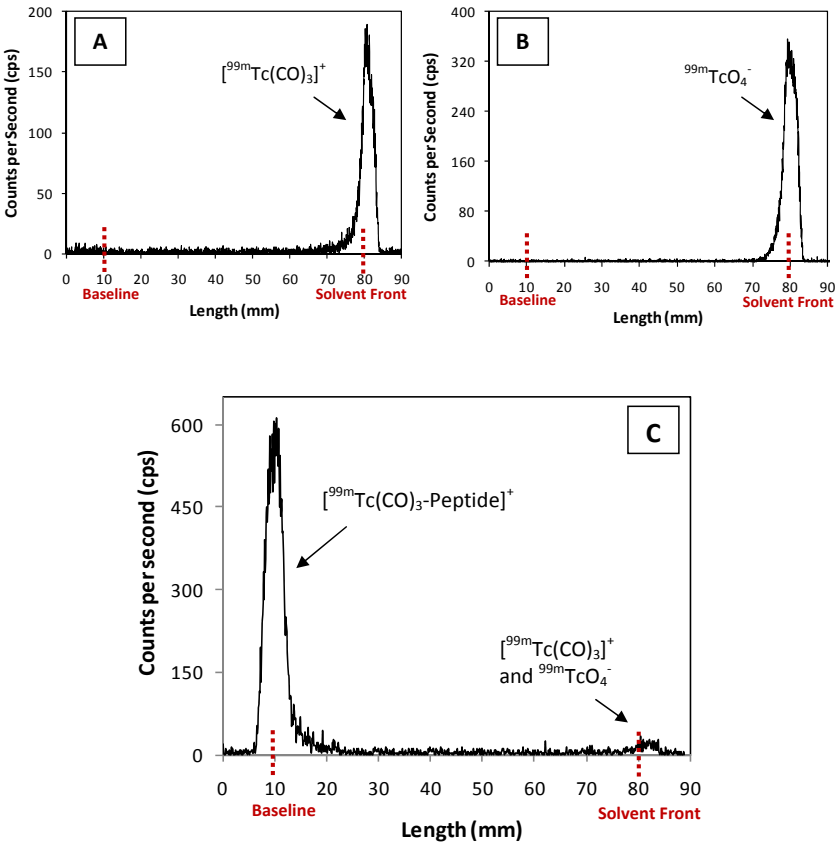


Fig. S5. Exemplar iTLC quality control of labeling of synthesized soluble peptides. iTLC-SA strips (7.5 x 90 mm) had an origin at 10 mm and a solvent front at 80 mm. A mobile phase of citrate buffer at pH 5.5 was used. **A)** Unbound $[^{99m}Tc(CO)_3]^+$, Rf = 1; **B)** Unreduced $^{99m}TcO_4^-$, Rf = 1; **C)** Cys/His-Tag labelling reaction with $[^{99m}Tc(CO)_3]^+$. The $[^{99m}Tc(CO)_3$-peptide] conjugate had Rf = 0 in all cases.

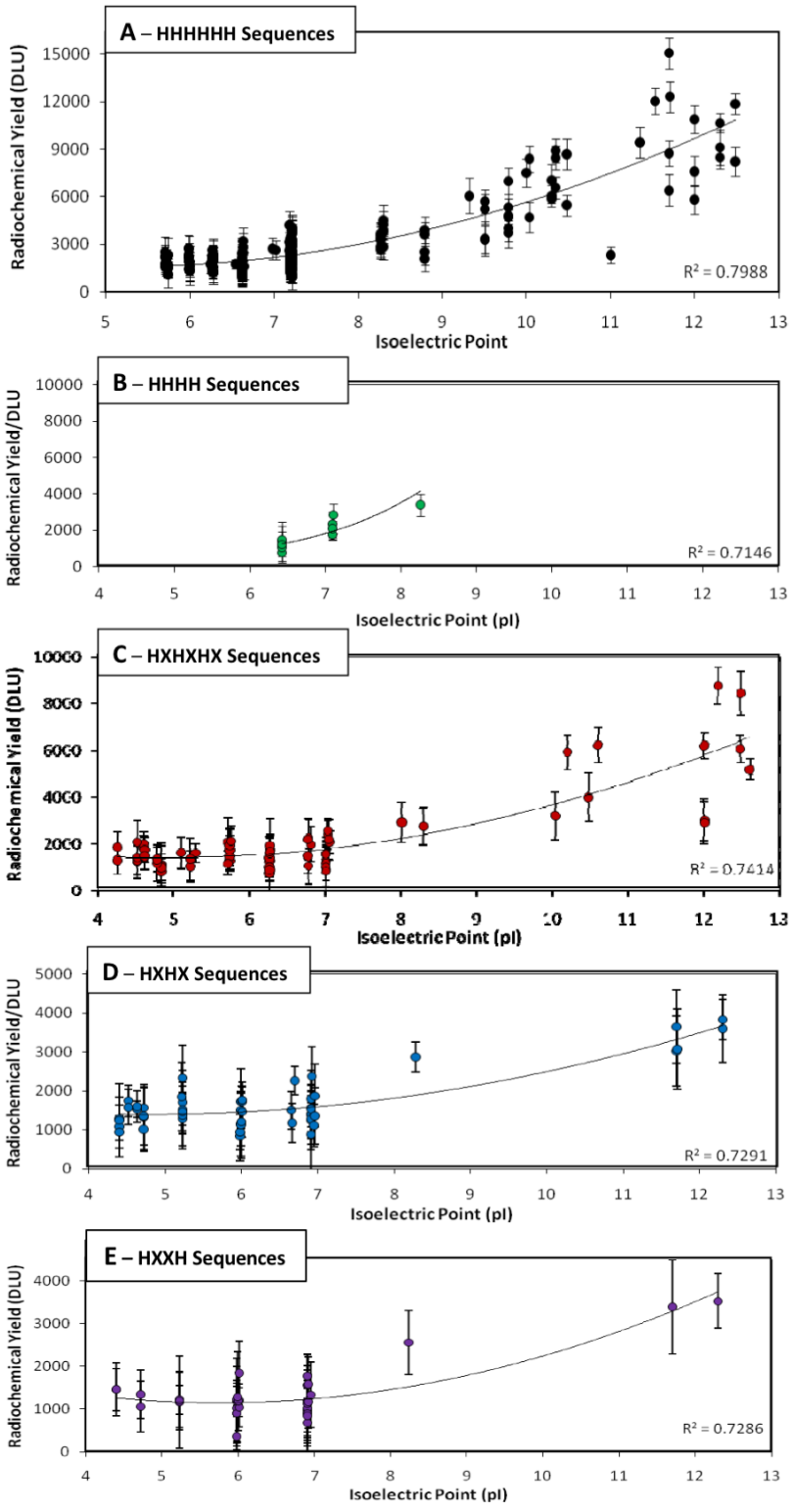


Figure S6. A comparison between the radiochemical yield and pI of the peptide sequences, classified according to types of His tag (number and position/interruption of His residues in the sequences). **A)** 6 His residues, HHHHHH; **B)** 4 His residues, HHHH; **C)** 3 His residues in a HXHXHX motif; **D)** 2 His residues in a HXHX motif; and, **E)** 2 His residues in a HXXH motif.

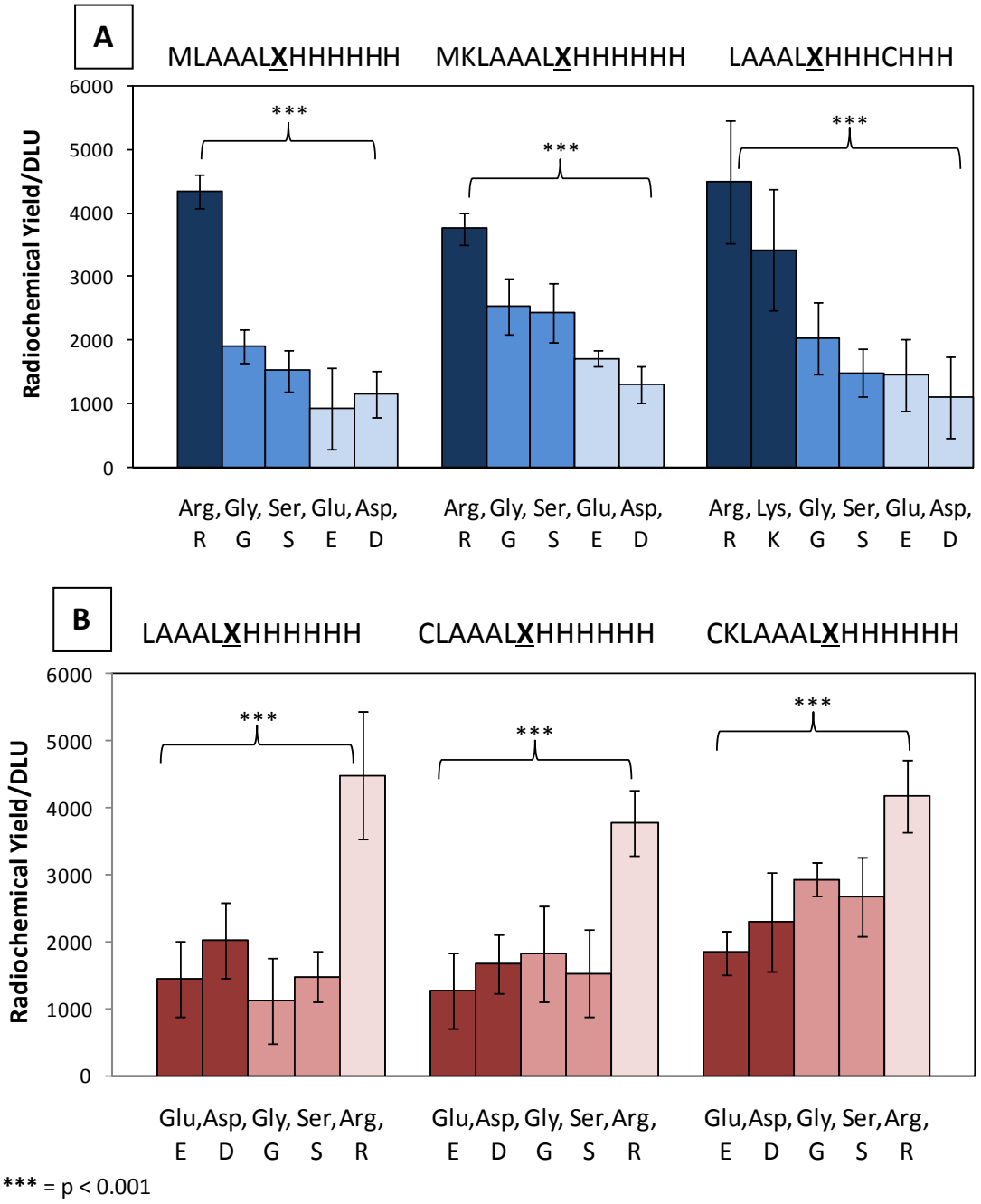


Figure S7. Comparison between the radiochemical yield of labelling sequences containing an Arg, Lys, Gly, Ser, Glu and Asp amino acid adjacent to the His tag. A single amino acid has been "point-mutated" in the X position within each sequence **A:** MLAAALXHHHHHH, MKLAAALXHHHHHH and LAAALXHHHCHHH sequences, **B:** LAAALXHHHHHH, CLAAALXHHHHHH, CKLAAALXHHHHHH sequences. There is a significant enhancement ($p < 0.001$) when X = arginine.

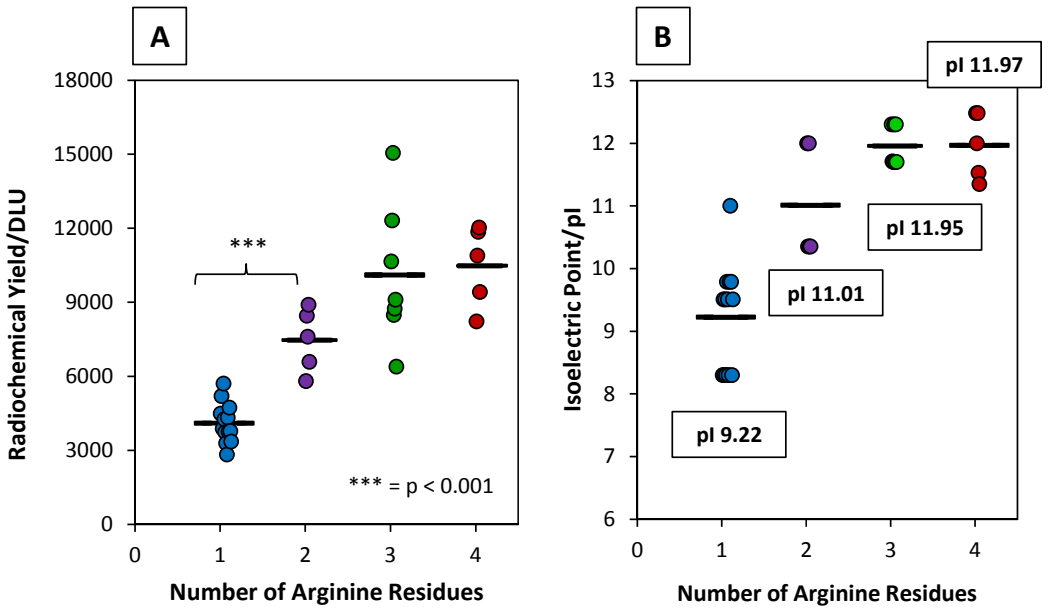


Figure S8. Effect of multiple arginine residues: number of Arg residues within a peptide sequence plotted against the radiochemical yield of the sequence (**A**) and pI (**B**, mean pI shown for each group). All sequences include a His-Tag, HHHHHH and the Arg residues are positioned on either side of the His_6-Tag, both sides of the His_6-Tag or in between the His residues. Increasing the number of Arg residues increases both labeling yield and pI compared to a single Arg residue.

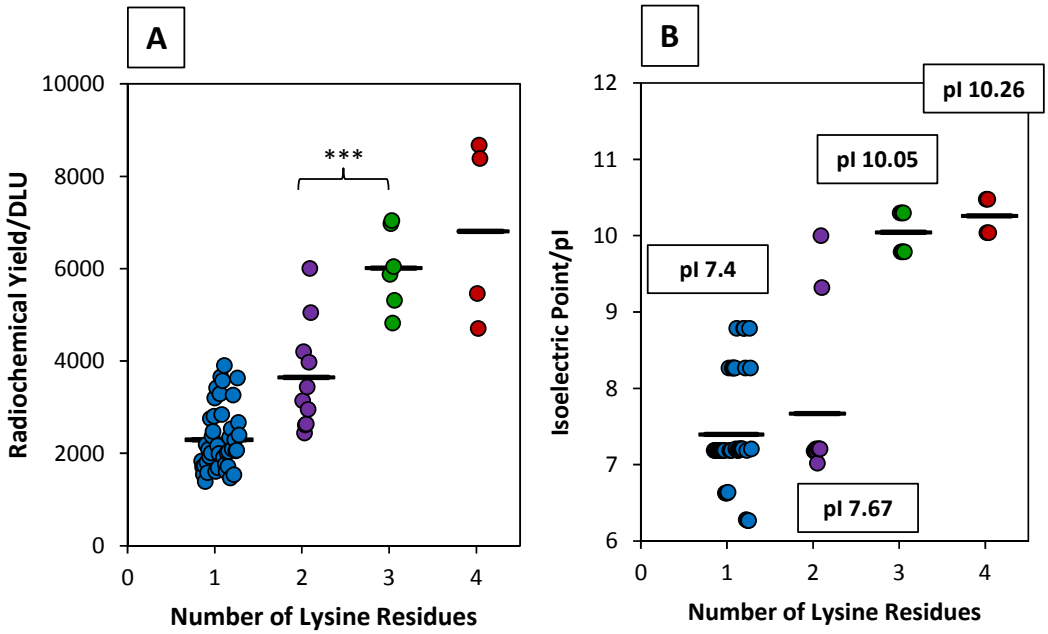


Figure S9. Effect of lysine residues on labeling efficiency: number of Lys residues within a peptide sequence plotted against the radiochemical yield of the sequence (**A**) and pI (**B**). All sequences include a His-Tag, HHHHHH and the Lys are positioned: on either side of the His_6-Tag, both sides of the His_6-Tag or in between the His residues. Increasing the number of lysine residues enhances both labeling efficiency and pI.

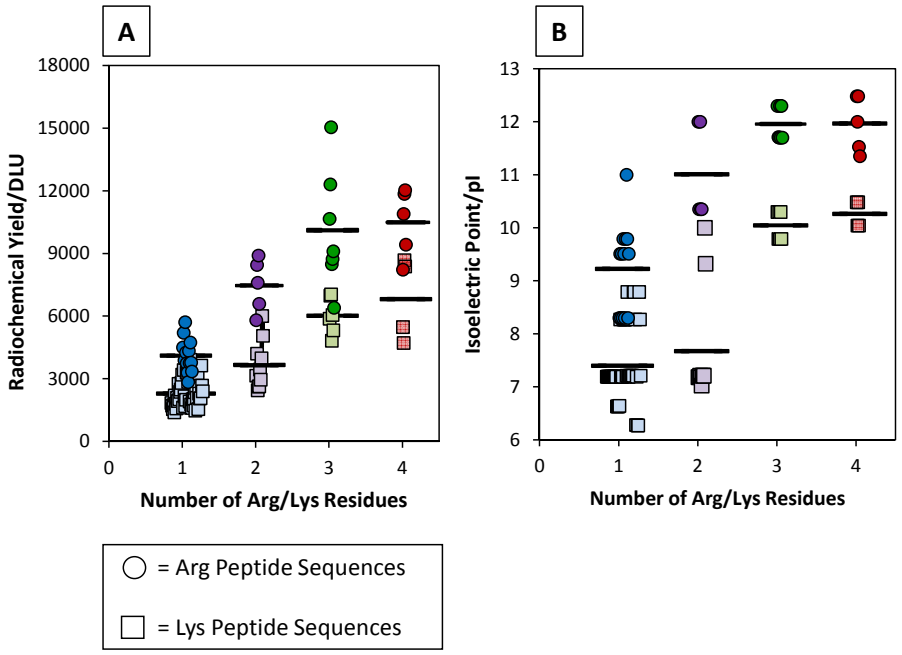


Figure S10. Combined data for the number of Lys and Arg residues within a peptide sequence plotted against the radiochemical yield of the sequence (**A**) and pI (**B**). Arg containing sequences are better than Lys containing sequences at improving radiochemical yield. All sequences include a His_6-Tag, HHHHHH.

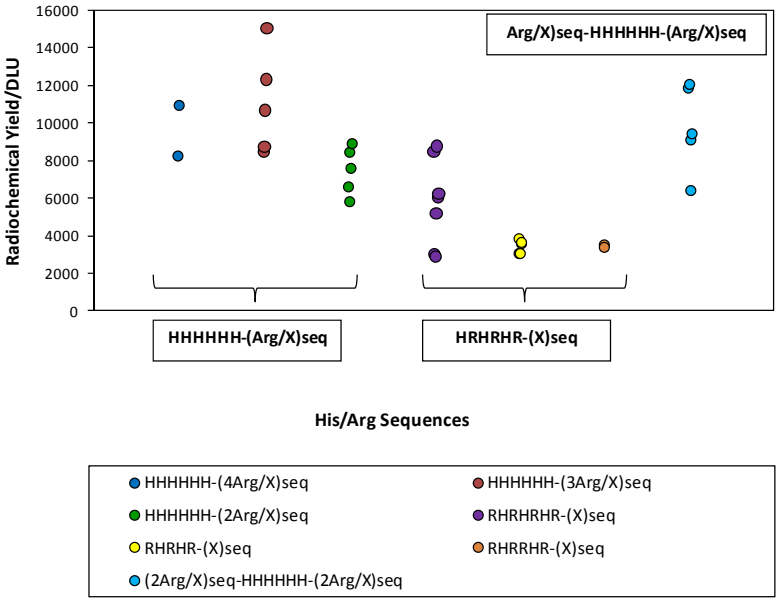


Figure S11. Comparisons between positions of Arg residues with respect to the His amino acids. The sequences have been categorized according to the positioning of the His residues: **HHHHHH-(Arg/X)**, **HRHRHR-(X)** and **(Arg/X)-HHHHHH-(Arg/X)**. X = any amino acid other than Arg or His. Placing arginine residues either side of the histidine sequence increases labeling efficiency more effectively than interrupting the histidine sequence.

Table S5. Occurrence of Cys residues in the ten most efficiently labeled sequences of the His tag array. Cysteine-containing residues are shaded.

Sequence	Labeling efficiency rank	Labeling efficiency, DLU
HHHHHHALRRRLC	1	15049
HHHHHHALRRRLKC	2	12308
CRRHHHHHHRRC	3	12029
GRRHHHHHHRRG	4	11858
HHHHHHRRAARRC	5	10894
HHHHHHALRRRL	6	10656
RCRGHHHHHHGRCR	7	9419
GGRHHHRHHHRGG	8	9107
HHHHHHRGGGRC	9	8897
RHRHRHGRGGRC	10	8767

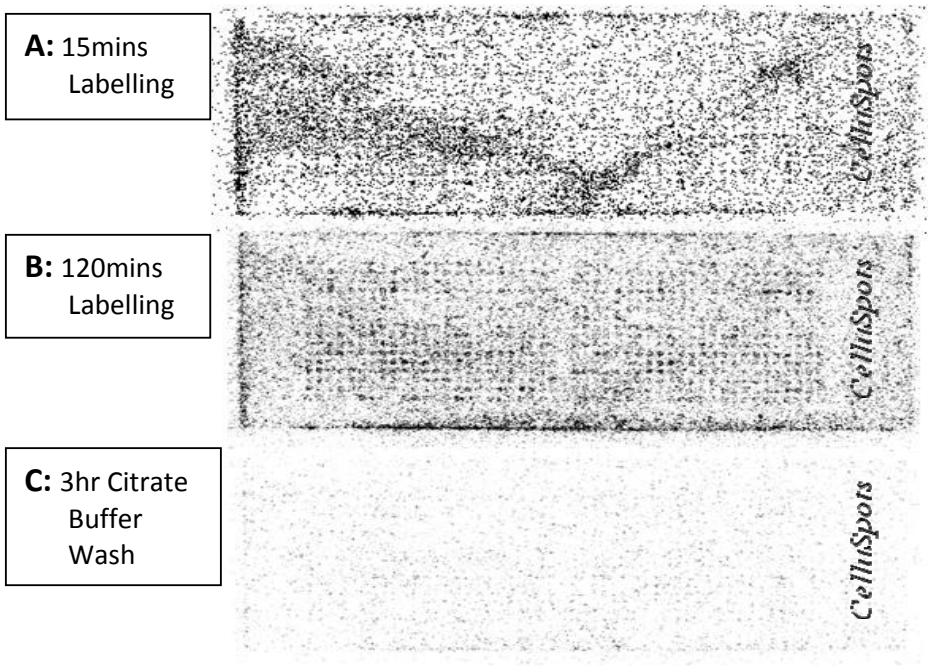


Figure S12. Labeling in citrate buffer: phosphor images of the $[^{99m}Tc(CO)_3]^+$ radiolabeled His-Tagged Celluspots™ array after radiolabeling in citrate buffer at pH 5.1, rather than PBS. **A)** Image after radiolabeling for 15 min; **B)** Image after radiolabeling for 120 min; **C)** Image after incubation for 120 min followed by exposure to a His containing solution for 3h. Labeling at pH 5.1 leads to peptide labeling that is much less efficient than in PBS, much less selective for positively charged residues and much less stable towards loss of label.

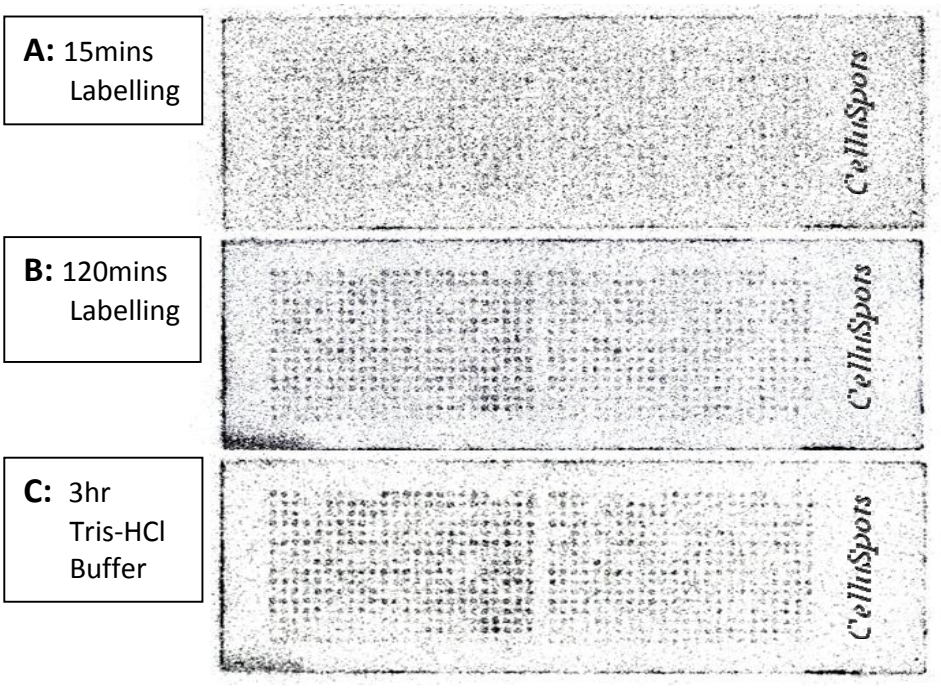


Figure S13. Labeling in Tris-HCl buffer (pH 8.8): phosphor images of the $[^{99m}Tc(CO)_3]^+$ radiolabeled His-Tagged Celluspots™ peptide array after radiolabeling in Tris-HCl buffer at pH 8.8, rather than PBS. **A)** Image after radiolabeling for 15 min; **B)** Image after radiolabeling for 120 min; **C)** Image after incubation for 120 min followed by exposure to Tris-HCl buffer for 3h. Labeling at pH 5.1 leads to peptide labeling that is much less efficient than in PBS and much less selective for positively charged residues.

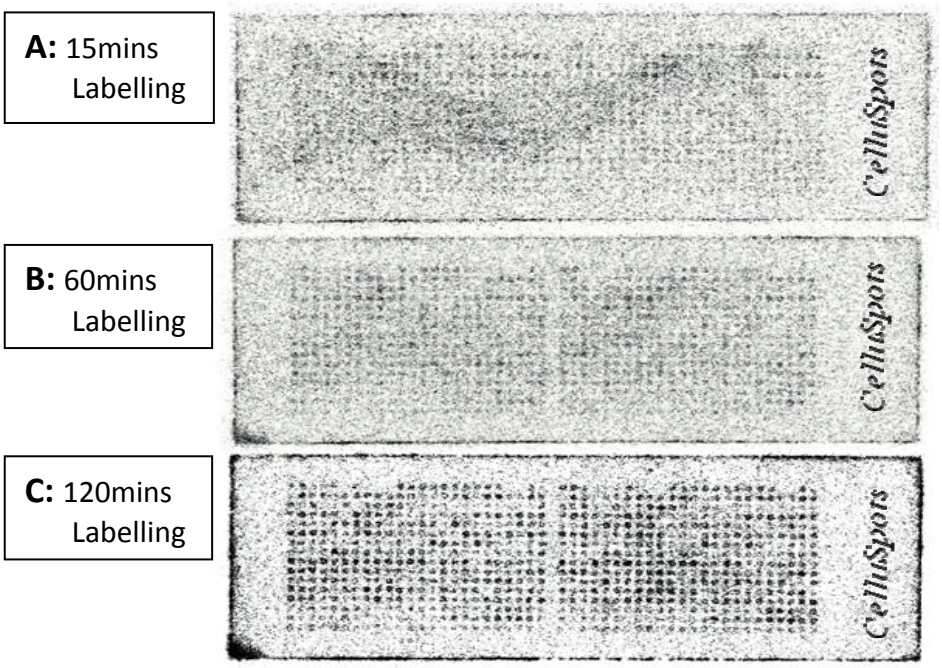


Figure S14. Labeling in Tris-HCl buffer (pH 7.4): phosphor images of the $[^{99m}Tc][Tc(CO)_3]^+$ radiolabeled His-Tagged Celluspot™ peptide array after radiolabeling in Tris-HCl buffer at pH 8.8, rather than PBS. **A)** Image after radiolabeling for 15 min; **B)** Image after radiolabeling for 120 min; **C)** Image after radiolabeling for 120 min. Labeling is more efficient than in Tris-HCl buffer at pH 8.8 but is not comparable to labeling in PBS.

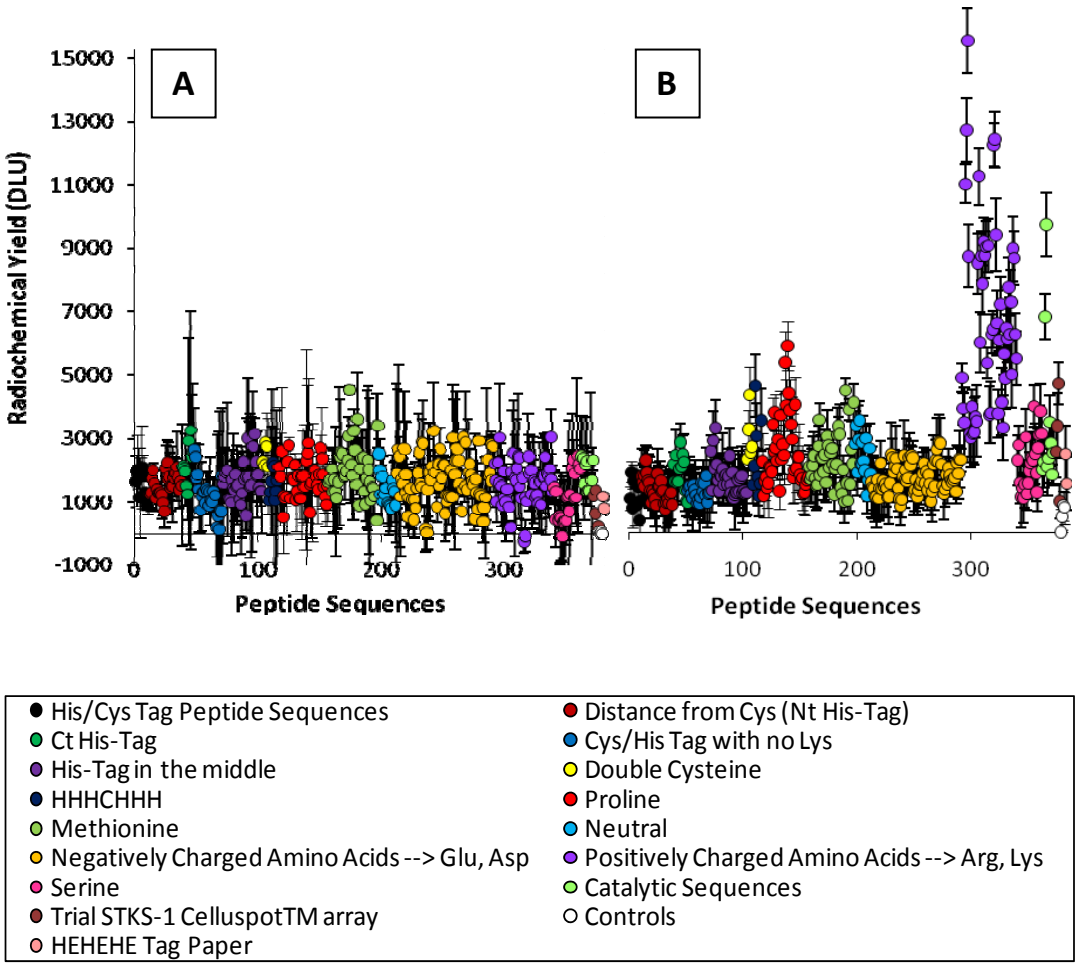


Figure S15: Comparison of $[^{99m}Tc][Tc(CO)_3]^+$ labeling of all sequences in tris-HCl pH 5.1 and in PBS. **A)** 120 min reaction time in citrate buffered solution at pH 5.1 and, **B)** 15 min reaction time in PBS solution. The graphs have been plotted to the same scale. Labeling is faster in PBS and dramatically more selective for positively charged sequences.

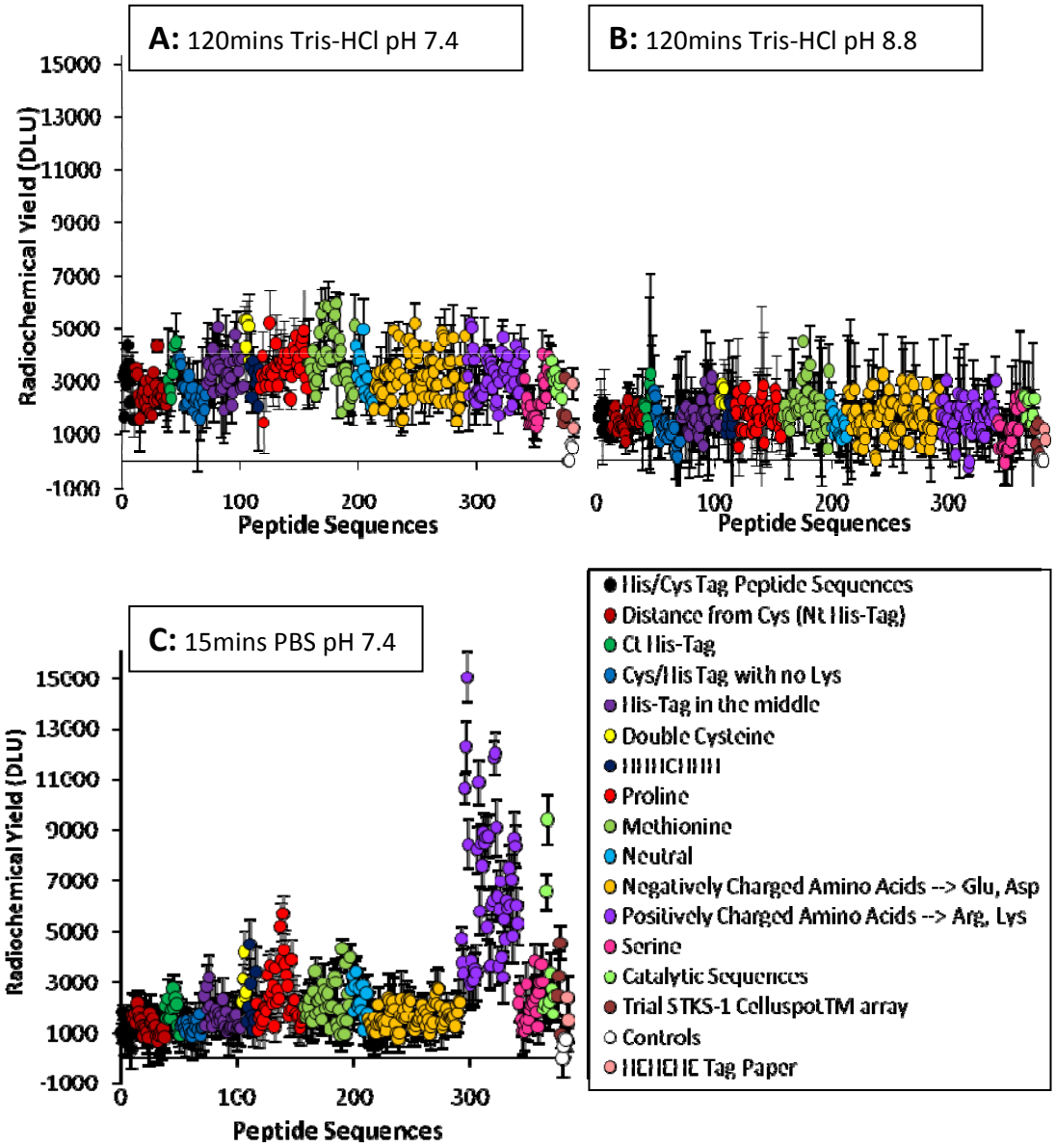


Figure S16: Comparisons of $[^{99m}Tc][Tc(CO)_3]^+$ labeling yields in tris-HCl buffer and PBS. **A)** After 120 min reaction time in Tris-HCl buffer, pH 7.4; **B)** After 120 min reaction time in Tris-HCl buffer, pH 8.8; **C)** After 15 min reaction time PBS. The graphs have been plotted to the same scale and the data normalized to the background values. Labeling in Tris-HCl is less efficient and less selective than in PBS.

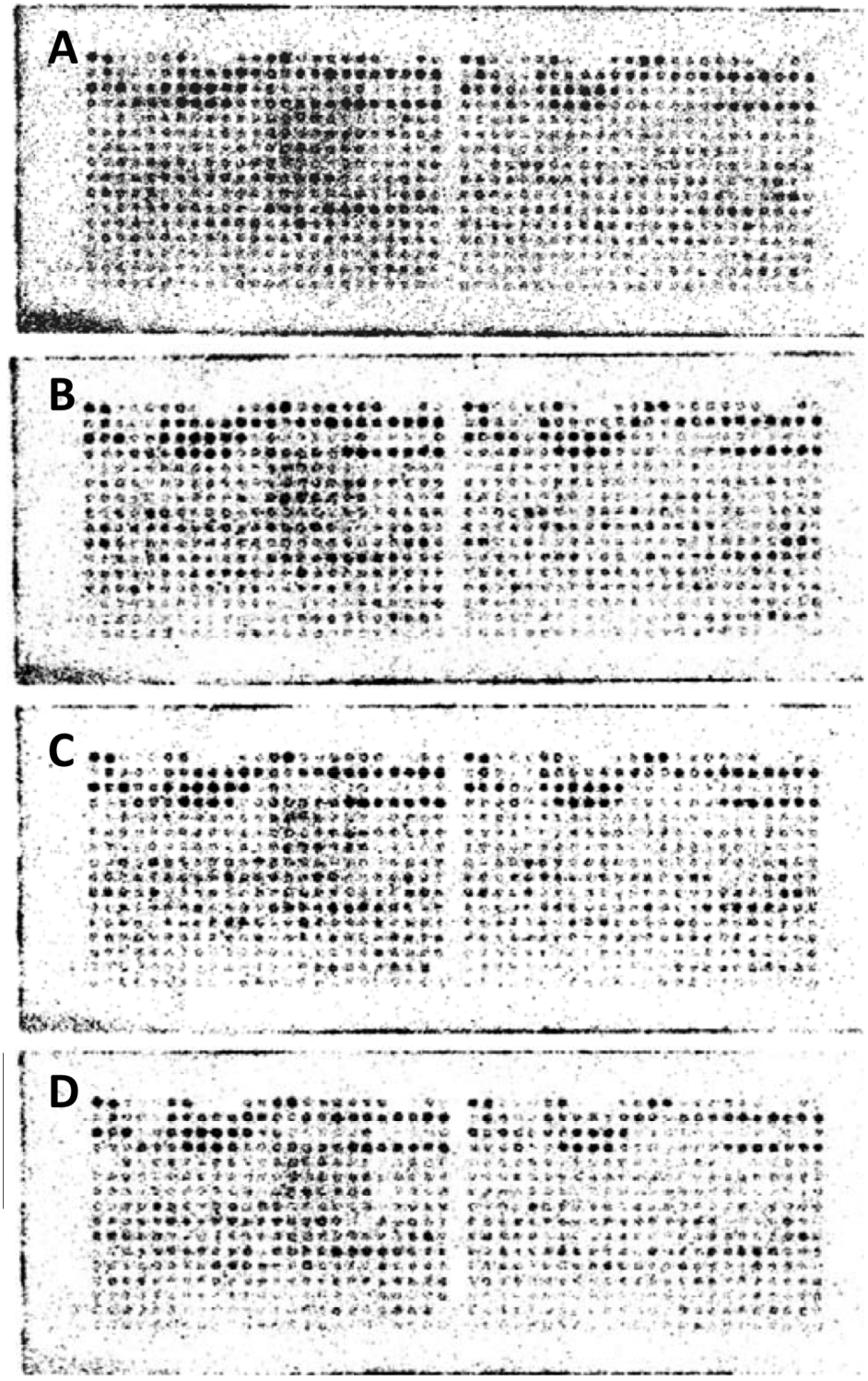


Figure S17. Phosphor image of the $[^{99m}Tc][Tc(CO)_3]^+$ radiolabeled His-Tagged Celluspots™ peptide array after 120 min labeling followed by incubation in PBS for up to 24 h. **A)** Image after radiolabeling for 120 min; **B)** Image after incubation of labeled array (A) in PBS buffer for 1 h; **C)** Image after incubation in PBS buffer for 2 h; **D)** Image after incubation in PBS buffer for 24 h. ^{99m}Tc complexes of the peptides labeled under these conditions are stable in PBS.

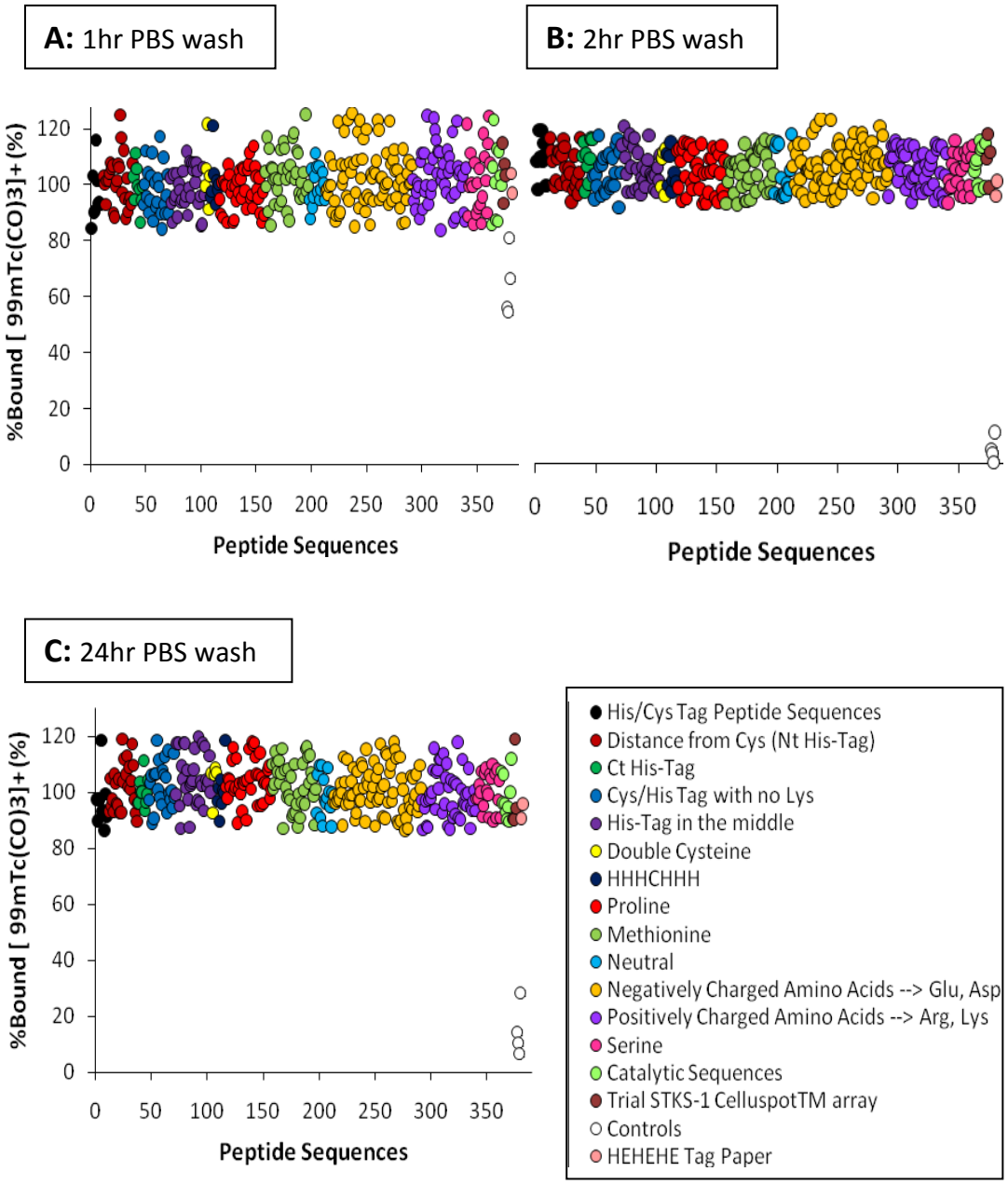


Figure S18. Radiolabel stability in PBS. Calculated % $[^{99m}Tc][Tc(CO)_3]^+$ remaining on all peptide sequences (labeled in PBS) after exposure to fresh non-radioactive PBS buffer for 1 h, 2 h and 24 h. The data are presented according to the categories previously established based on the characteristics of the peptide sequences. **A)** Exposure to PBS buffer for 1 h; **B)** Exposure to PBS buffer for 2 h; **C)** Exposure to PBS buffer for 24 h. The peptides in the "Controls" category (white circles), which show major loss of radiolabel, correspond to 2 spots on the array with no peptide sequences and 2 that do not contain His residues.

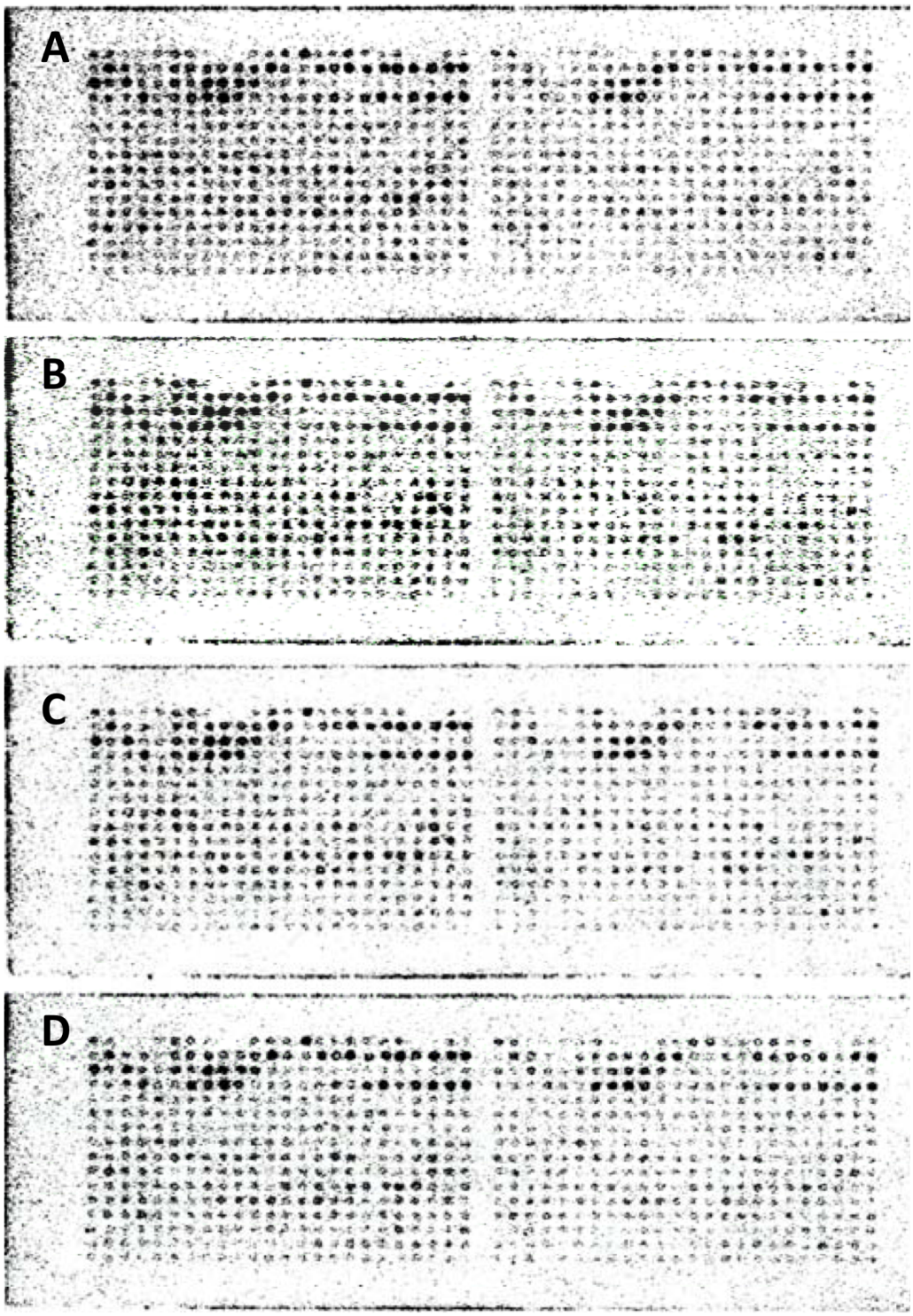


Figure S19. Radiolabel stability in serum. Phosphor image of the $[^{99m}Tc][Tc(CO)_3]^+$ radiolabeled His-Tagged Celluspot™ peptide array (labeled in PBS for 120 min) and after incubation in human serum for up to 24 h. **A)** Image after radiolabeling for 120 min; **B)** Image after incubation of labeled array (A) in PBS for 1 h; **C)** Image after incubation in PBS for 2 h; **D)** Image after incubation in PBS for 24h. Labeled peptides are highly stable and resistant to transchelation in serum.

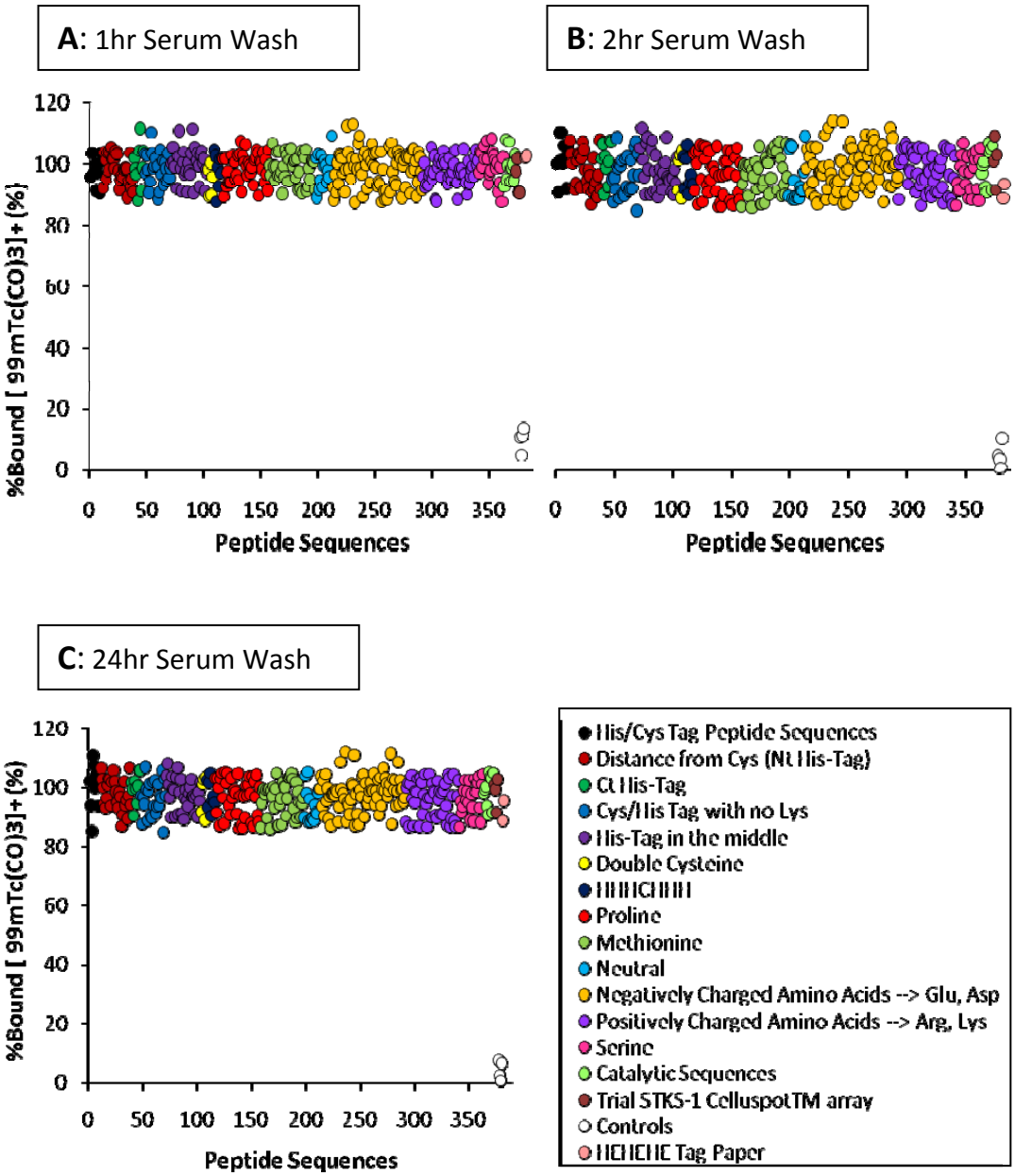


Figure S20. Serum stability. Calculated % [^{99m}Tc][Tc(CO)$_3$]$^+$ remaining on all peptide sequences (labeled in PBS for 120 min) after exposure to human serum for 1 h, 2 h and 24 h. The data are presented according to the categories previously established based on the characteristics of the peptide sequences. **A)** Exposure to human serum for 1 h; **B)** Exposure to human serum for 2 h; **C)** Exposure to human serum for 24 h. The peptides in the "Controls" category (white circles), which show major loss of radiolabel, correspond to 2 spots on the array with no peptide sequences and 2 that do not contain His residues. The labeled peptides are resistant to transchelation in serum over 24 h.

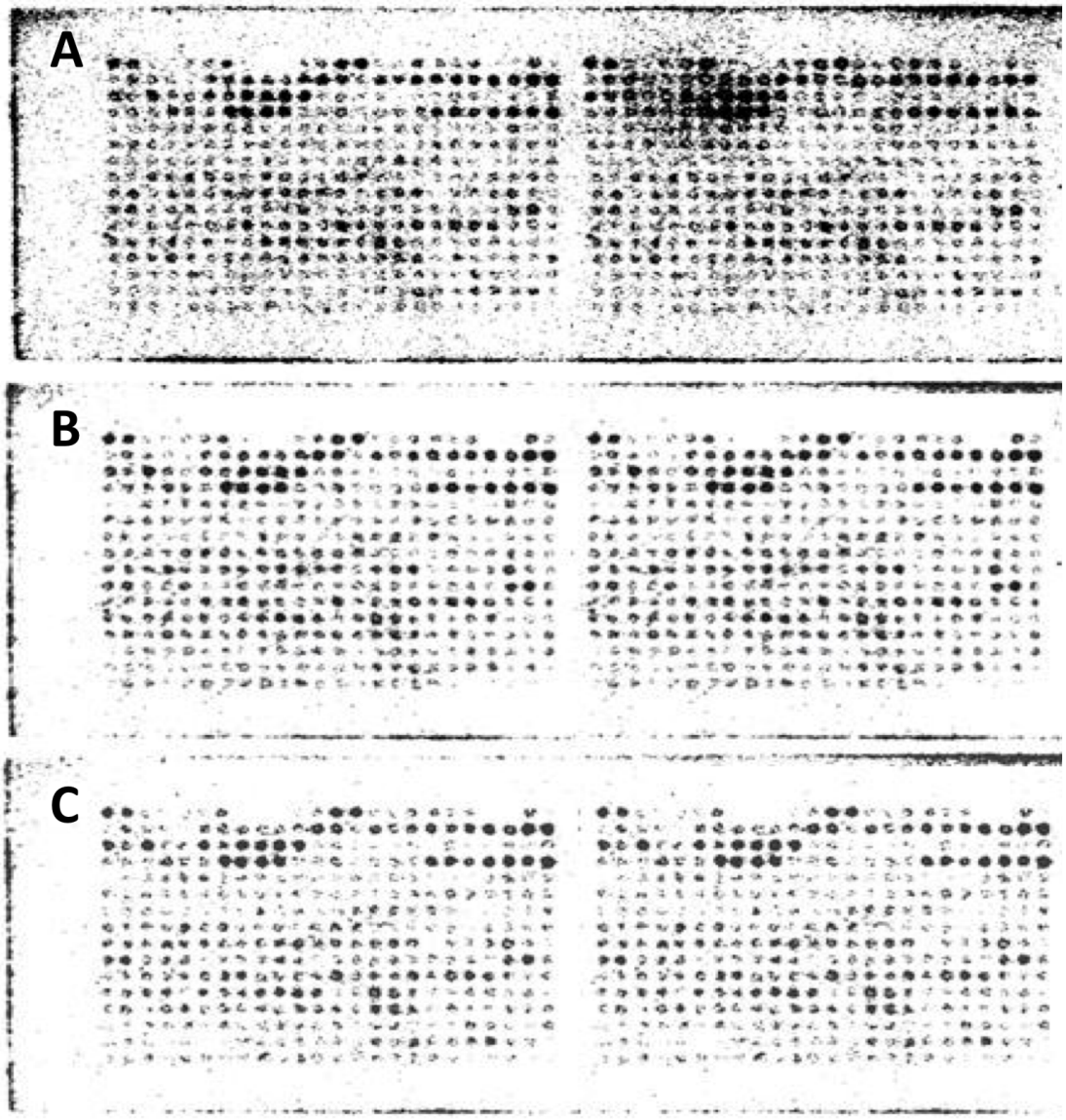


Figure S21. Resistance to transchelation with cysteine and histidine. Phosphor image of the $[^{99m}Tc][Tc(CO)_3]^+$ radiolabelled His-Tagged Celluspot™ peptide array (labeled for 120 min) followed by incubation in large excess of Cys and His containing solutions for 3 h consecutively. **A)** Image after radiolabeling for 120 min; **B)** Image after incubation of labeled array (A) in an excess Cys solution; and **C)** Image after incubation of Cys-treated array (B) in an excess His solution.

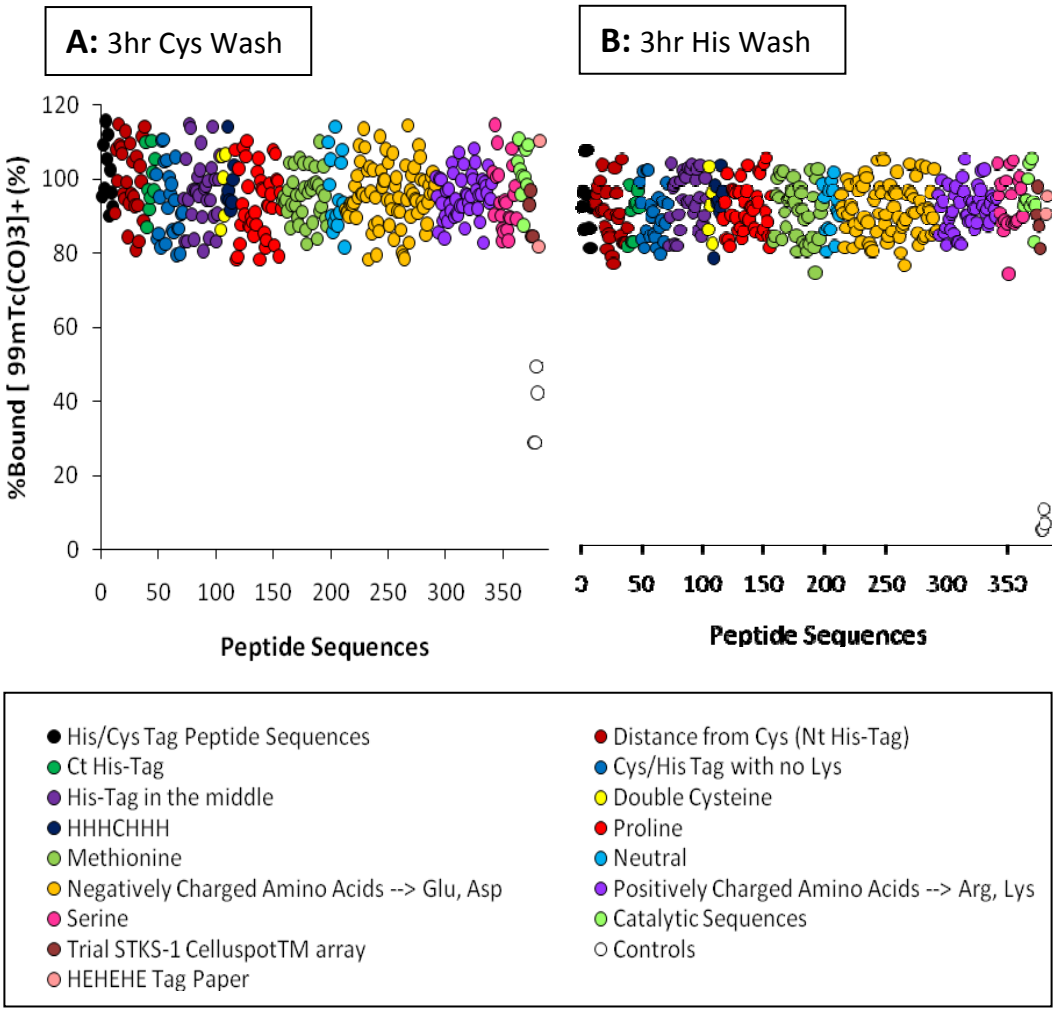


Figure S22. Resistance to transchelation with cysteine and histidine. Calculated % $[^{99m}Tc][Tc(CO)_3]^+$ remaining bound to all labeled (in PBS for 120 min) peptide sequences after exposure to excess Cys and His solutions for 3 h each consecutively. **A)** Exposure to a Cys rich solution for 3 h. **B)** Exposure to His rich solution for 3 h. Labeled peptides are highly resistant to transchelation with cysteine and histidine.

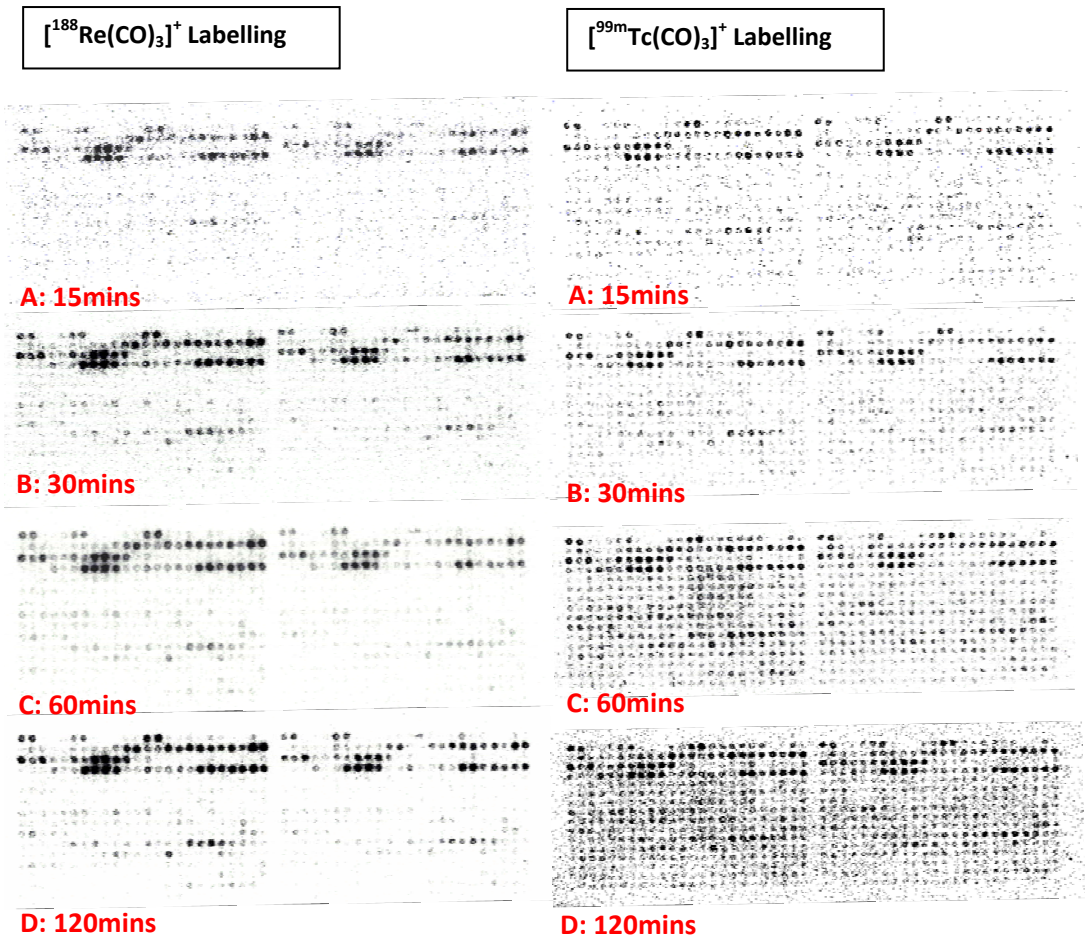


Figure S23: Comparison of phosphor images of the $[^{188}Re(CO)_3]^+$ (left) and $[^{99m}Tc][Tc(CO)_3]^+$ (right) labeling of the His-tagged Celluspots™ peptide arrays. A) Image after 15 min total incubation time, B) Image after 30 min total incubation time, C) Image after 60 min total incubation time and D) Image after 120 min total incubation time. Despite the poorer resolution of the ^{188}Re images, the analogy between the two radionuclides in selectivity among the spots is evident.

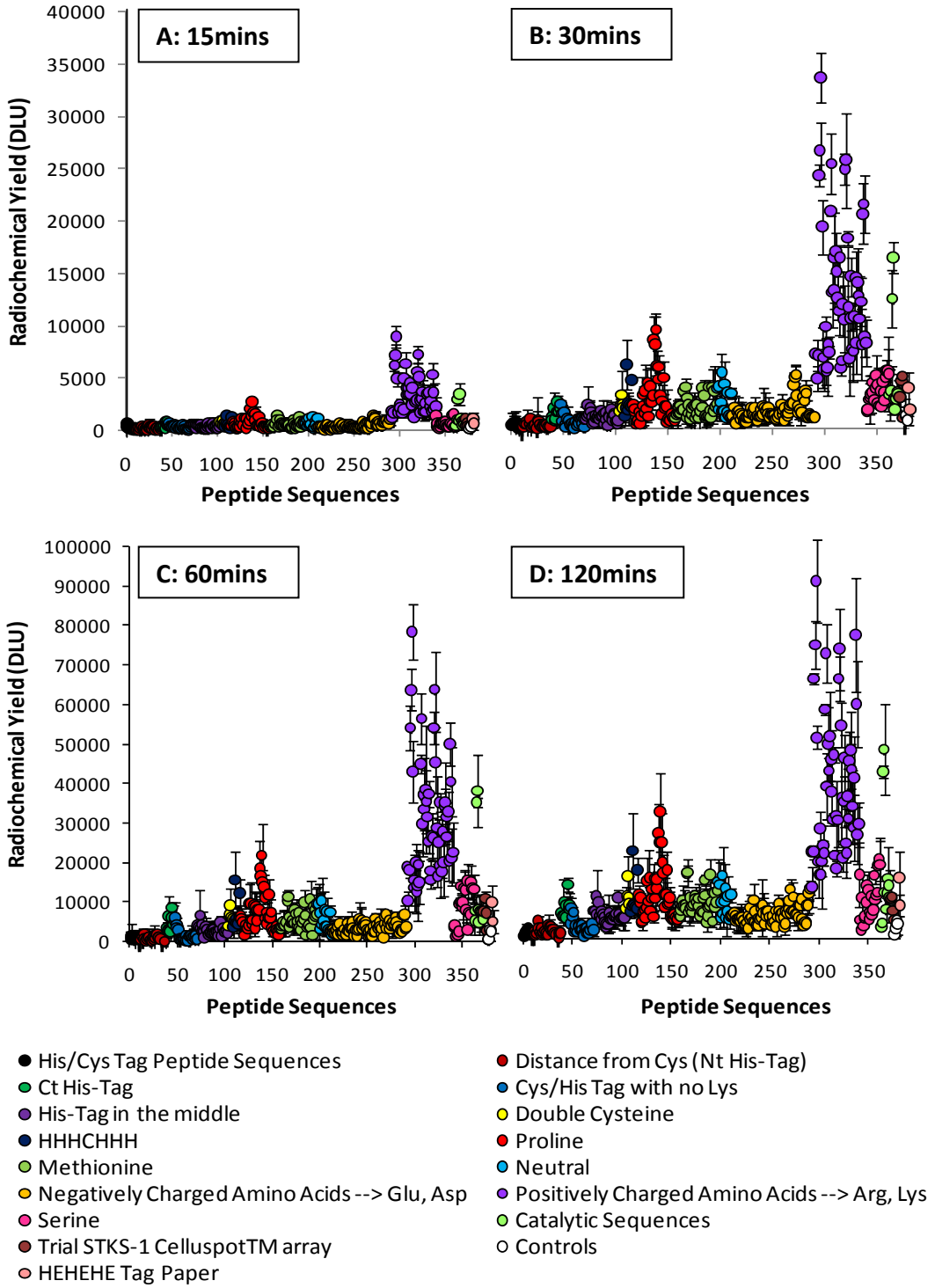


Figure S24: Rhenium-188 labelling. Radiochemical yield of all 384 peptides on the His-Tagged Celluspots™ peptide array post radiolabeling with $[^{188}Re(CO)_3]^+$ in PBS at pH 7.4. Results and standard deviations are calculated based on 2 sets of data. The sequence categories are listed in the legend and a description is provided above. **A)** after 15 min incubation, **B)** after 30 min incubation, **C)** after 60 min incubation and **D)** after 120 min incubation. The similarity of the profiles in this figure with those of the ^{99m}Tc analogs shown in Fig. S5 is striking.

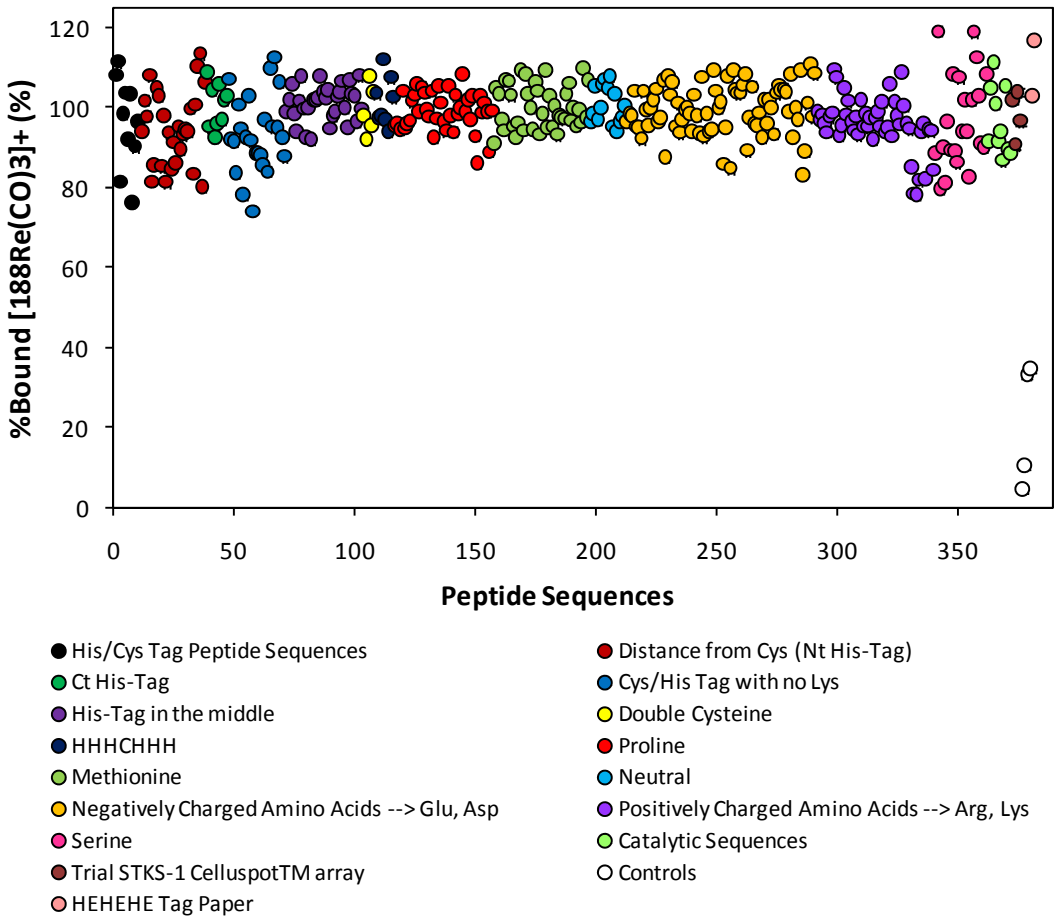


Figure S25. Serum stability of ^{188}Re-labeled peptides: the % of ^{188}Re bound to each spot in the array after labeling with $[^{188}Re(CO)_3]^+$ in PBS that remained after exposure to human serum for 48 h is shown. The attachment of ^{188}Re to the peptides is highly resistant to transchelation in serum, as is the case for the ^{99m}Tc analogs.

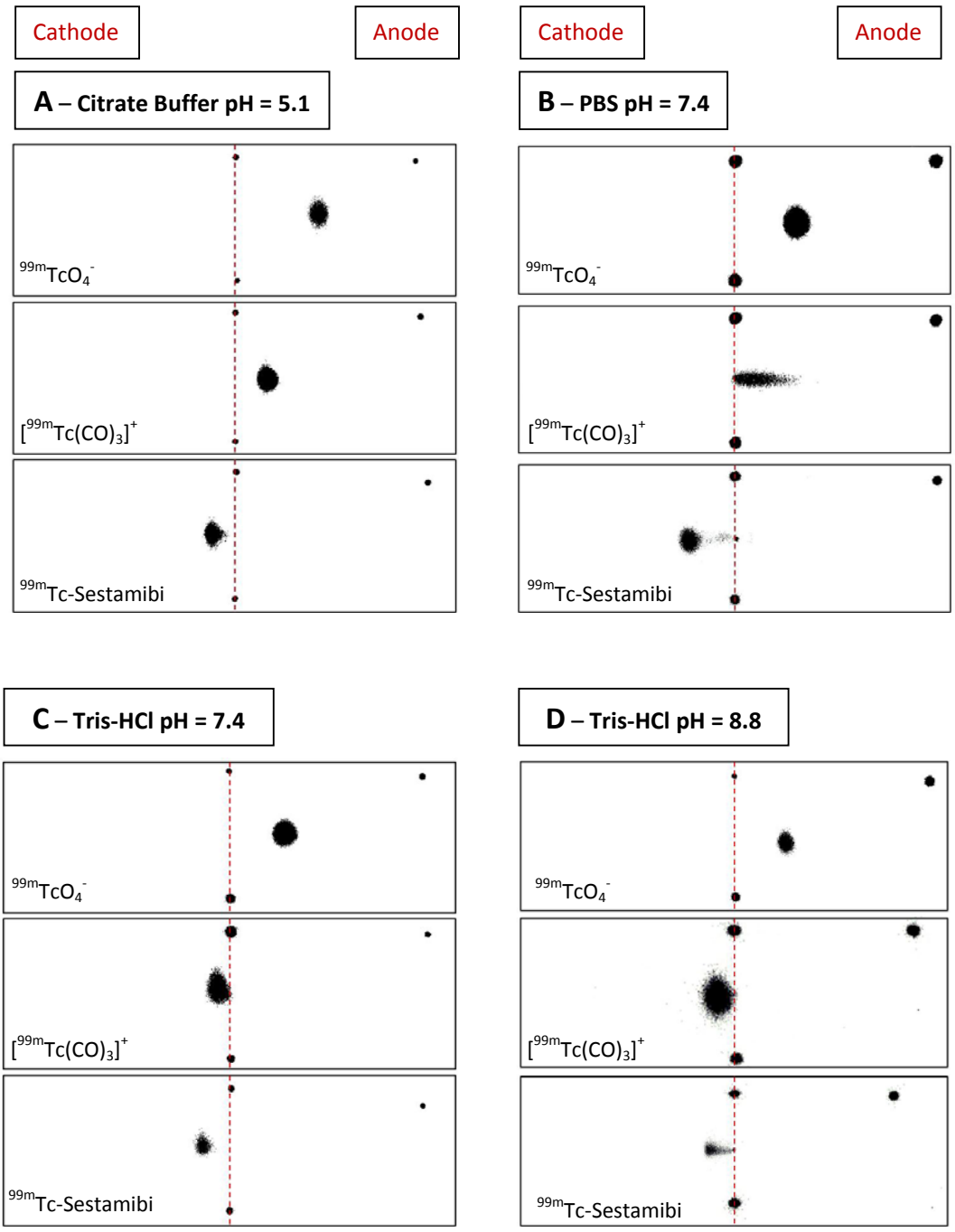


Figure S26. Images obtained from the electrophoresis of $[^{99m}Tc][TcO_4]^-$, ^{99m}Tc-Sestamibi and $[^{99m}Tc][Tc(CO)^3]^+$ in various buffers after 1 h (4 mA current). The two black spots joined by a vertical red dashed line represent the starting point of the radioactive complexes before application of the electrical field and the black spot in the top right hand corner indicates the position of the anode during the experiment. Electrophoresis was performed in different buffers: **A)** Citrate buffer at pH 5.1, **B)** PBS at pH 7.4, **C)** Tris-HCl buffer at pH 7.4 and **D)** Tris-HCl buffer at pH 8.8. While $[^{99m}Tc][TcO_4]^-$ and ^{99m}Tc-sestamibi in all media behaved as expected as an anion and a cation, respectively, the "$[^{99m}Tc][Tc(CO)_3]^+$" unexpectedly behaved as an anion in PBS and citrate buffer.

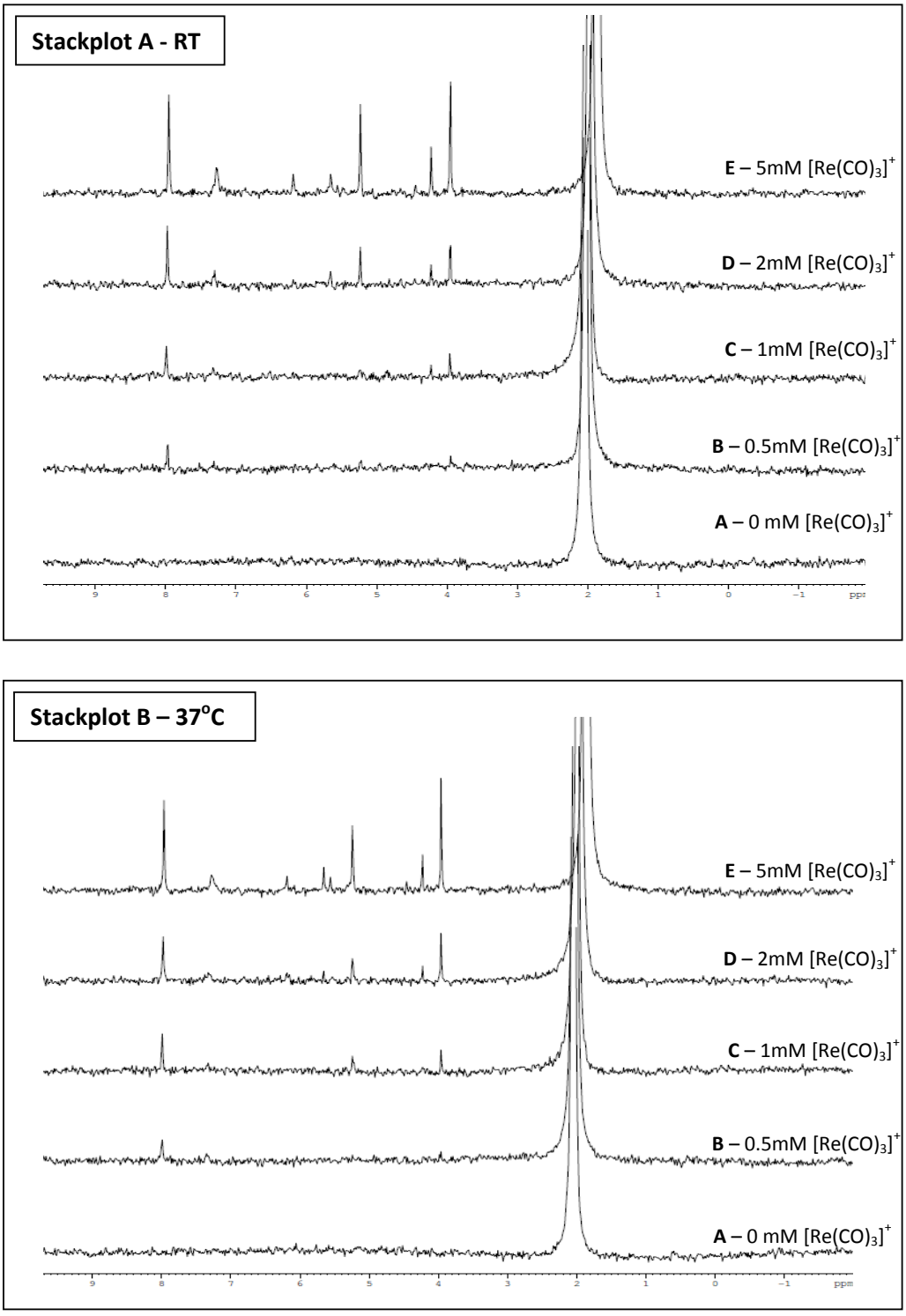


Figure S27. ^{31}P NMR spectra of increasing concentrations of $[Re(CO)_3]^+$ in a 100 mM phosphate buffered solution at pH 7.4. **Stackplot A)** Spectra obtained after incubation for 30 min at RT. **Stackplot B)** Spectra obtained after 30 min incubation at 37°C. New species, most likely due to coordination of phosphate to Re, are formed when $[Re(CO)_3]^+$ and phosphate are combined; there is little difference in their rate of formation at room temperature and 37°C.

Expression and purification of model proteins

All scFv fragments were successfully constructed, expressed and purified. The sequence of each was confirmed by DNA sequencing. The "labeling sequences" were defined as the 13 amino acids at the C-terminal of the protein which includes the His_6-tag and 7 of its surrounding amino acid residues. A His_6-tag in combination with multiple Arg residues was successfully incorporated as the labeling sequence at the C-terminus of J591scFvJWT.

The control proteins were chosen due to the minimal number of Arg residues at the C- terminal in close proximity to the His_6-tag. Apart from the labeling sequence, the J591scFv protein has an identical amino acid composition to the J591scFvJWT fragment. Its labeling sequence, KRAAALEHHHHHH, with a pI of 8.79, contains 1 Arg and 1 Lys residue 6 and 7 amino acids away, respectively, from the His_6-tag. To analyze the effect of this Arg on labeling, an additional version of the J591scFv was used for comparison, humanized (hu) J591scFv, with a labeling sequence not containing the Arg. The huJ591scFvscFv labeling sequence, IKLAAALEHHHHHH, has a pI of 7.21. The Lys 7 amino acids away from the His_6- tag is still present. 6C7.1scFv and 6C7.1-CscFv have identical amino acid composition other than an additional Cys residue at the C-terminus of the His-tag in the 6C7.1-CscFvys scFv. The labeling sequences for 6C7.1scFv and 6C7.1-CscFv are PTAAALEHHHHHH and TAAALEHHHHHHC respectively and the pI is 6.53 for both.

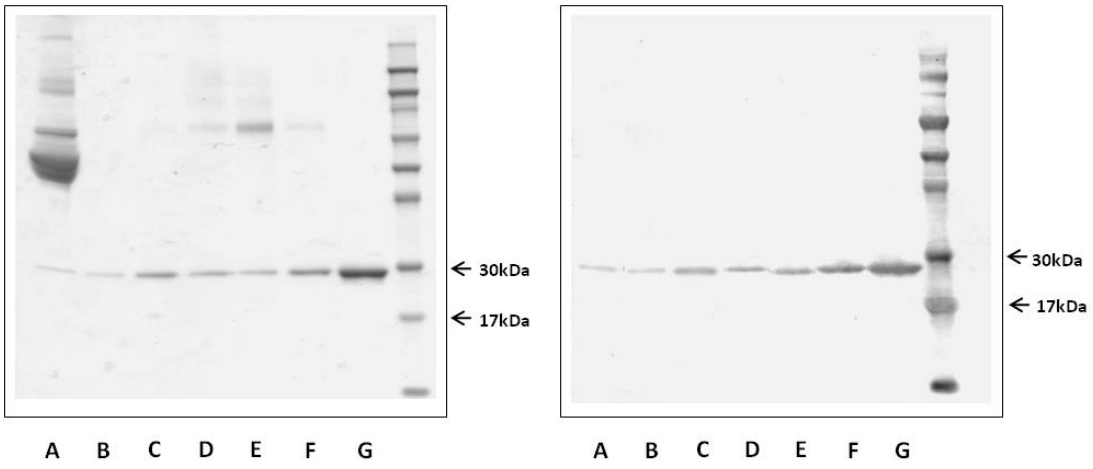
The J591scFvJWT non-reduced and reduced samples revealed a single band representing the J591scFvJWT monomer at 28 KDa (Fig. S28). This implies that the protein folding has not been compromised and a homogenous J591scFvJWT protein has been formed. As a result of the successful expression, J591scFvJWT was reproduced and purified in parallel with the J591scFv control protein for further comparative studies including radiolabeling efficiencies and binding to PSMA.

The large scale production of J591scFvJWT and J591scFv produced 2 mg/L and 7 mg/L respectively of the purified proteins. The results suggest that the JWT Tag influences the expression of the J591scFv protein resulting in smaller yields of the protein. Production and purification steps were monitored by SDS PAGE and Western blotting and are displayed in Figs. S28 and S29 for the J591scFvJWT and J591scFv respectively. The J591scFvJWT appears as a monomer of 28 KDa. Lanes A-C in the SDS-PAGE monitor the progress of extracting J591scFvJWT from the culture supernatatant. His_6 recombinant proteins such as J591scFvJWT have a high affinity and selectivity for the Ni- NTA and as a result, the majority of protein impurities present in the supernatant, lane A, are discarded in the flowthrough of the Ni-NTA purification system. Other impurities from the culture media non-specifically bound to the NiNTA are removed with addition of the Ni-NTA washing buffer containing 35 mM imidazole, lane B. J591scFvJWT was eluted from the column by competition with a 250 mM imidazole solution in the Ni-NTA elution buffer, lane C.

Three peaks appeared in the SEC purification step of the J591scFvJWT and samples from each were loaded onto the SDS PAGE and can be seen in lanes D-F. The first peak corresponded to serum proteins from culture media, row D, whilst the second peak corresponded to dimeric aggregates of J591scFvJWT protein, row E. The dimeric aggregates formed were non-covalent dimers and on the SDS-PAGE appear as single monomers at 28 KDa due to the SDS procedure disrupting the secondary and tertiary structures of the protein. J591scFvJWT was eluted as a

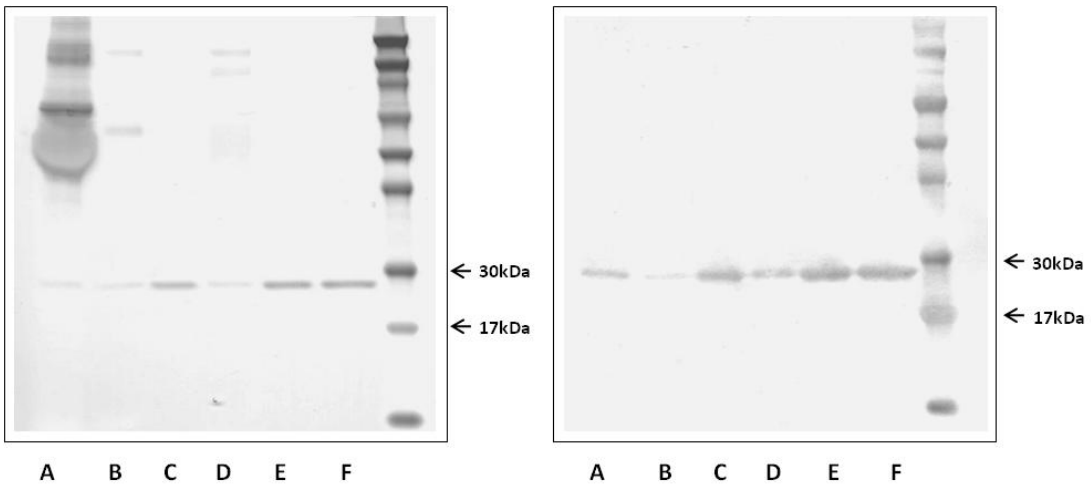
purified protein in the third peak, lane F, and concentrated to 1.3 mg/ml, lane G. The corresponding western blot confirms the appearance of the J591scFvJWT as a single band at 28 KDa on the SDS-PAGE. The primary antibody used in the western blot, antiPentaHis, specifically detects the $(His)_6$ tag.

SEC HPLC analysis confirms that for J591scFvJWT, the protein exists as a monomer, retention time 9:10-9:30 min, with only small amounts of scFv dimers present (Fig. S30). J591scFv and huJ591scFv show a higher degree of dimer formation.



Row	Sample
A	Culture Supernatant
B	35mM imidazole wash of the NiNTA column
C	J591scFvJWT elution from NiNTA column (250 mM imidazole)
D	SEC Purification: Fraction 1 – BSA Protein
E	SEC Purification: Fraction 2 – Non-covalent dimers of J591scFvJWT
F	SEC Purification: Fraction 3 – Purified monomeric J591scFvJWT
G	Concentrated J591scFvJWT – 1.3 mg/ml

Figure S28. SDS-PAGE and Western Blot of the extraction and purification process of the J591scFvJWT protein from the culture supernatant**. Left**: SDS-PAGE with lanes from A-G. **Right:** Western blot of the SDS-PAGE with lanes from A-G. J591scFvJWT sample runs as a single band corresponding to the size of the monomer, 28 KDa.



Row	Sample
A	Culture Supernatant
B	35 mM imidazole wash of the NiNTA column
C	J591scFv elution from NiNTA column (250 mM imidazole)
D	SEC Purification: Fraction 1 – BSA Protein
E	SEC Purification: Fraction 2 – Non-covalent dimers of J591scFv
F	SEC Purification: Fraction 3 – Purified monomeric J591scFv

Figure S29. SDS-PAGE and Western blot of the extraction and purification process of the J591scFv protein from the culture supernatant**. Left**: SDS-PAGE with lanes from A-F. **Right:** Western blot of the SDS-PAGE with lanes from A-F. J591scFv sample runs as a single band corresponding to the size of the monomer, 27 KDa.

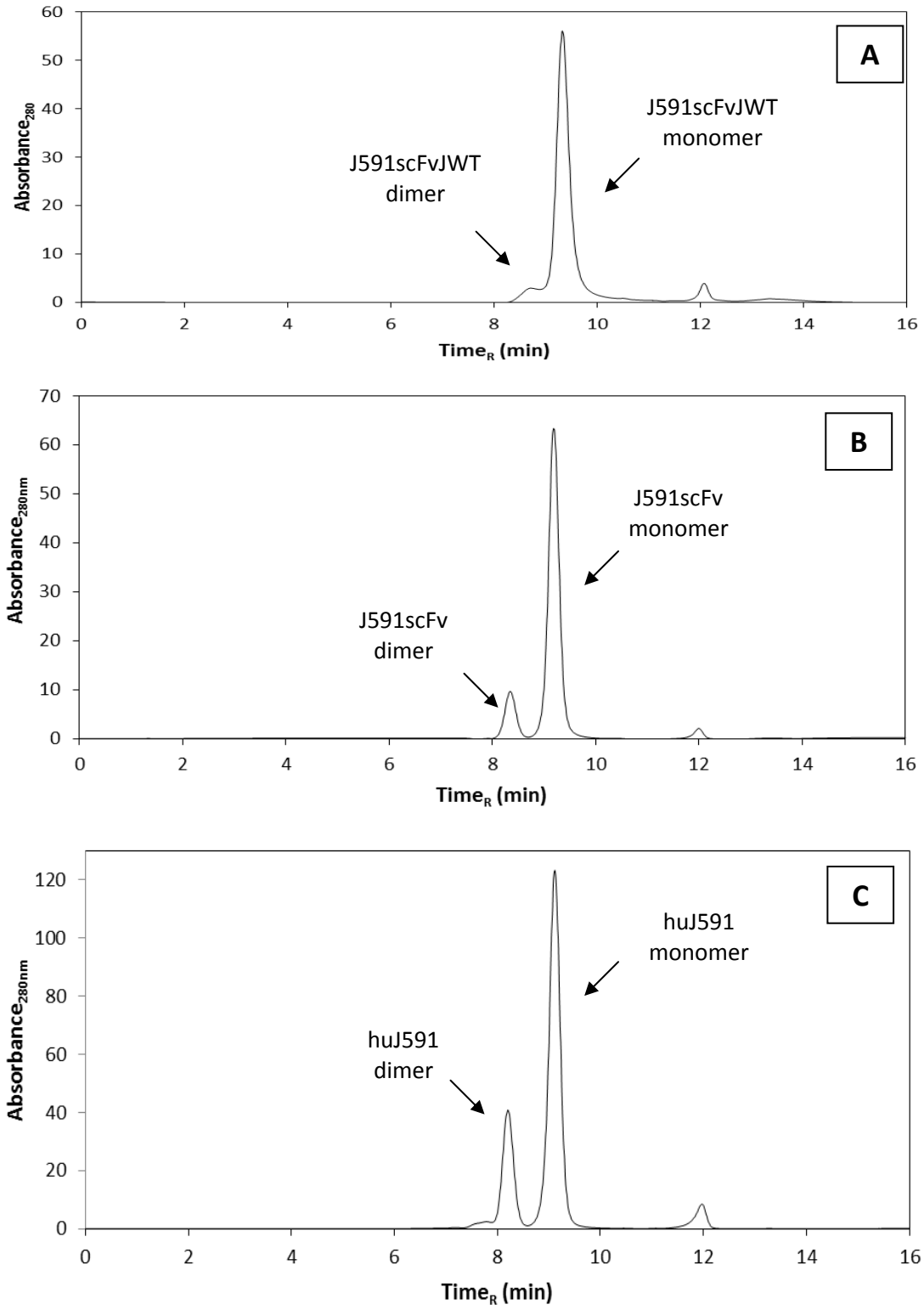


Figure S30. HPLC SEC analysis of the scFv proteins shows elution primarily as a single monomeric species in the region of 9:10 - 9:30 min. The peak in the region between 8:15-8:30 min represents the non-covalent dimers of the scFv protein. **A)** J591scFvJWT protein; **B)** J591scFv protein; **C)** huJ591scFv protein.

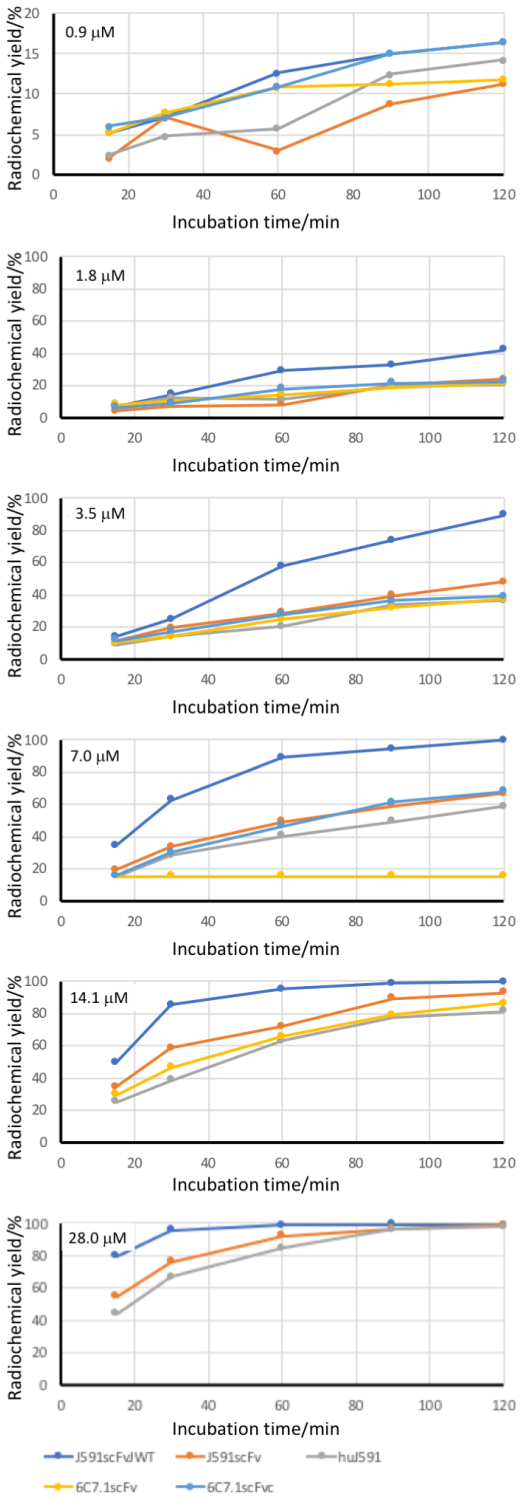


Figure S31. Labeling efficiency of five His-tagged proteins with $[^{99m}Tc][Tc(CO)_3]^+$ in PBS during 120 min at 37°C, at protein concentrations from 0.9 to 28 mM. Only J591scFvJWT (top) contains an optimized His-tag. There are no data for the highest and the highest two concentrations respectively of 6C7.1scFv and 6C7.1scFvCys because restricted quantities were available.

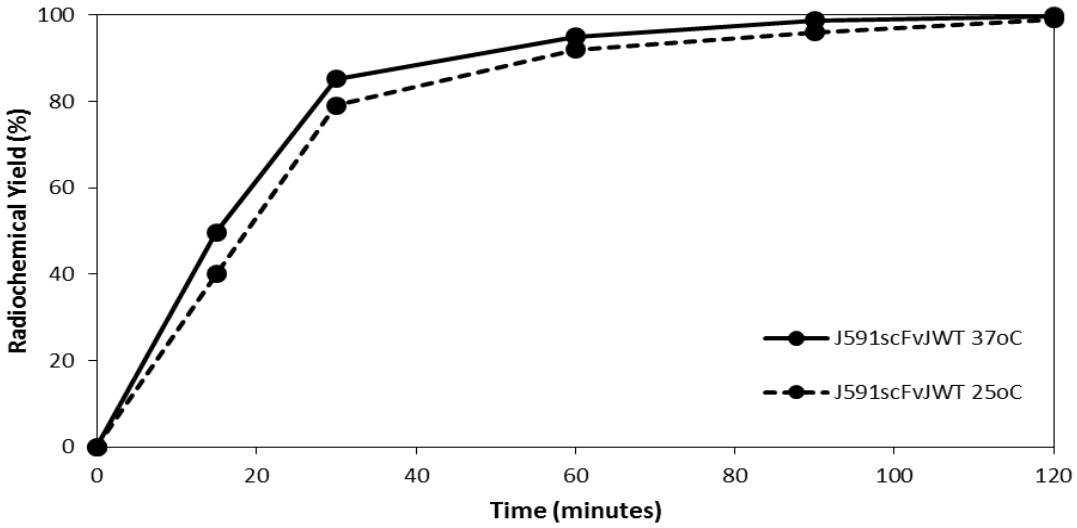
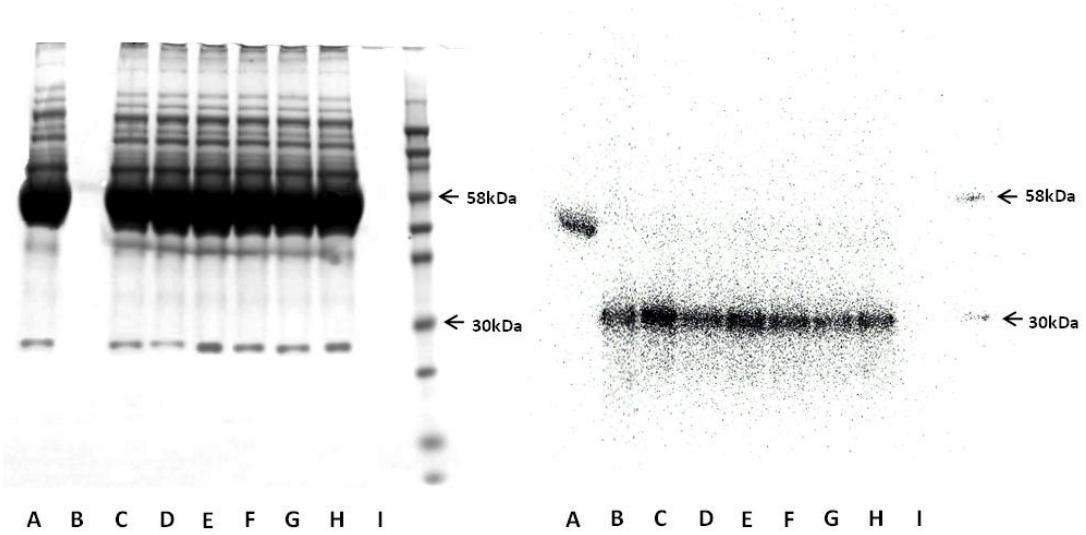


Figure S32. Comparison of [^{99m}Tc][Tc(CO)$_3$]$^+$ radiolabeling efficiency of J591scFvJWT at 37°C and 25°C in PBS. The protein concentration for both experiments was 14.1 µM.

Table S6. Protein concentrations and incubation times at which scFv proteins demonstrated a radiochemical yield greater than or equal to 95%. Only the proteins and radiochemical yield achieved that were greater than 95% have been included in the table.

	15min	30min	60min	90min	120min
28.2uM		**J591scFvJWT** = 96%	**J591scFvJWT** = 99%	**J591scFvJWT** = 99% **J591scFv** = 97% **huJ591scFv** = 96%	**J591scFvJWT** = 100% **J591scFv** = 99% **huJ591scFv** = 98%
14.1uM			**J591scFvJWT** = 95%	**J591scFvJWT** = 98%	**J591scFvJWT** = 99%
7uM				**J591scFvJWT** = 95%	**J591scFvJWT** = 99%
3.5uM					
1.76uM					
0.88uM					



A = Serum Proteins + $[^{99m}Tc(CO)_3]^+$

B = J591(scFv)LRRRLAHHHHHH-Tc^{99m}

C = J591(scFv)-Tc^{99m} & Serum at 0 min

D = J591(scFv)-Tc^{99m} & Serum at 15 min

E = J591(scFv)-Tc^{99m} & Serum at 30 min

F = J591(scFv)-Tc^{99m} & Serum at 60 min

G = J591(scFv)-Tc^{99m} & Serum at 120 min

H = J591(scFv)-Tc^{99m} & Serum at 240 min

I = $[^{99m}Tc(CO)_3]^+$

Figure S33. SDS-PAGE Coomassie staining (left) and autoradiograph (right) for the serum stability analysis of J591scFvJWT for 4 hours at 37°C.

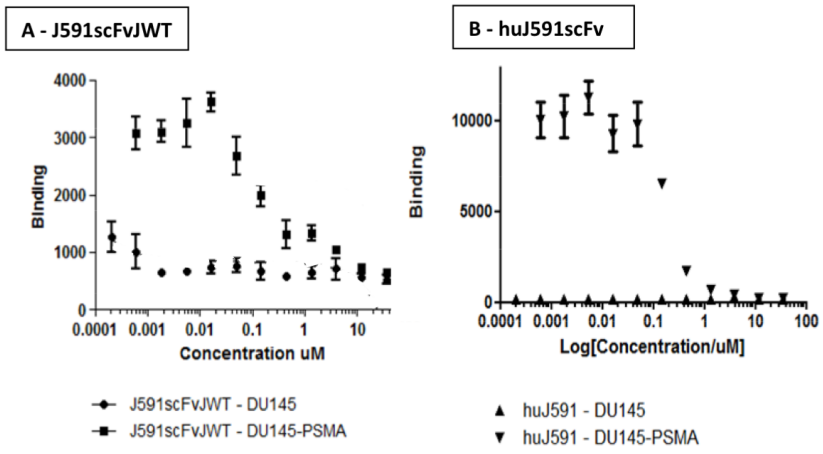


Figure S34. Cell binding competition assay using DU145 and DU145 PSMA positive cells. Competition is between 1 nM $[^{99m}Tc][Tc(CO)_3]^+$ labeled protein and the unlabeled protein as a cold competitor. **A)** J591scFvJWT protein, **B)** huJ591scFv protein.

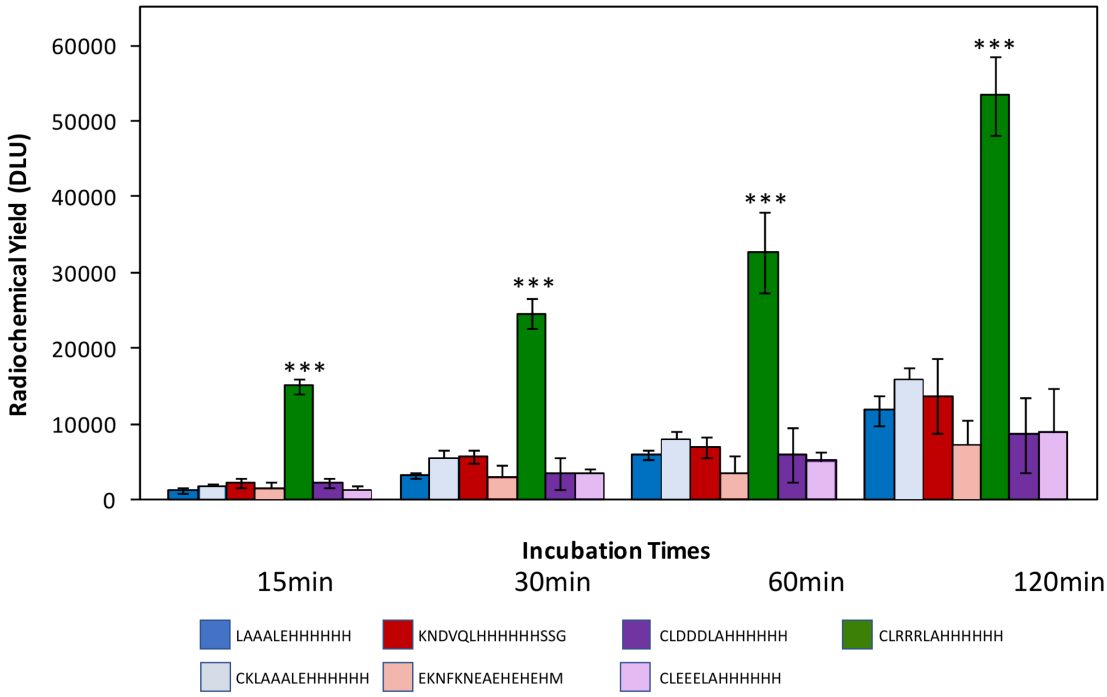


Figure S35 Comparison of labeling efficiency of optimal (highest labeling efficiency, CLRRRLAHHHHHH, green) sequence in the array with labeling efficiencies of comparable ^{99m}Tc-labeled sequences from the literature which are featured in the array. Dark blue and light blue are pairs representing the effect of adding CK;[9] Dark red and light red represent a comparison of HHHHHH with HEHEHE;[11] Dark purple and light purple represent the effect of substituting three negatively charged residues (aspartate and glutamate, respectively) for arginines in the optimized sequence. The optimized sequence has almost an order of magnitude higher labeling efficiency than the mean of the others.

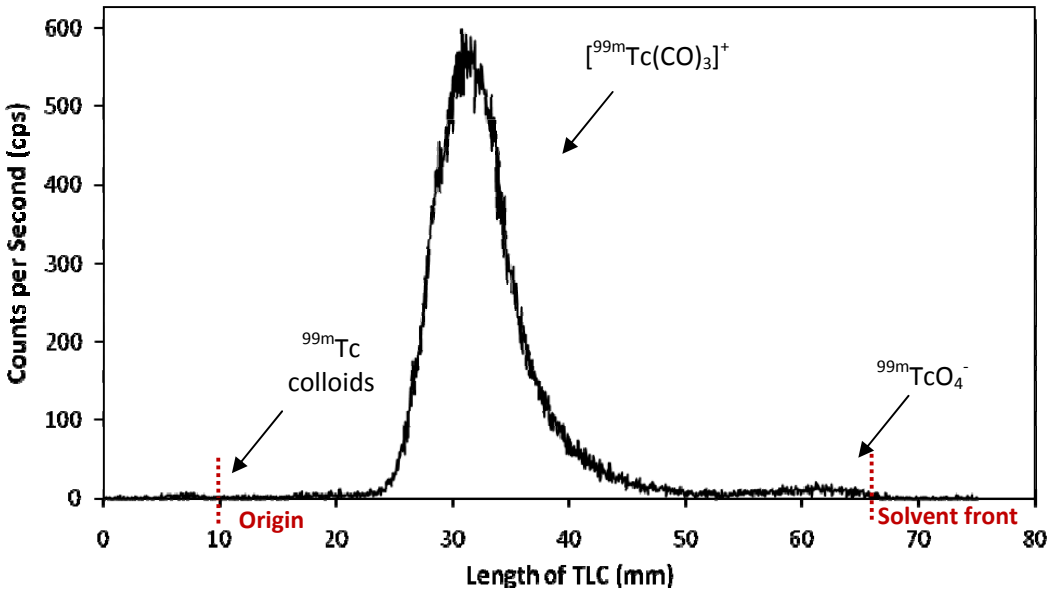


Figure S36. TLC quality control of $[^{99m}Tc][TcO_4]^-$ conversion to $[^{99m}Tc][Tc(CO)_3]^+$ for use in the radiolabeling of the scFv fragments: **Rf = 0.9** for $^{99m}TcO_4^-$, **Rf = 0** for ^{99m}Tc colloids and **Rf = 0.2-0.8** for $[^{99m}Tc(CO)_3(H_2O)_3]^+$. Silica gel $60F_{254}$ TLC plates (Merck Millipore, Darmstadt, Germany) with a mobile phase of 1% HCl in methanol were used.

Methods

Peptide synthesis

Peptides were synthesized in the Biomolecular Analysis Laboratory, University of Kent, using a PSSM-8 Multiple Peptide Synthesizer (Shimadzu). Analytical LC-ESMS was performed on a system comprising an Agilent 1200 Series Liquid Chromatography and an Agilent 6520 Accurate Mass Q-TOF mass spectrometer with a dual ESI ion source. Data analysis was carried out using the corresponding Agilent MassHunter Workstation Qualitative Analysis Software (Agilent Technologies, Cheshire, UK). Preparative HPLC was carried out using a LKB Bromma 2152 LC Controller Liquid Chromatography system (Rollabiotech, UK). The 10 Cys/His-Tag peptide analogs (Table S5) were synthesized on an automated peptide synthesizer using standard solid-state 9-fluorenylmethyloxycarbonyl (Fmoc) chemistry on a 20 µmol scale. NovaSyn TGT resin 0.2mmol/g substitution, (Novabiochem) in dimethylformamide (DMF) was used. The Fmoc amino acids (NovaBioChem) were added in 8 fold excess. A combination of HBTU, HOBt + H_2O and DIEA were used as the coupling reagents in a 1:1, 1:1, and 1:2 stoichiometric ratio to the amino acids (amino acid-reagent) respectively. Fmoc deprotection was achieved with a 30% piperidine solution in DMF. Peptide sequences were removed from the resin using 2 ml of a cleavage cocktail containing TFA:H_2O:EDT:TIS in a 94:2.5:2.5:1 ratio. Incubation in the cleavage cocktail was carried out for 2 hours and precipitation of the peptide was then induced by addition of 3 x 20 ml of ice cold diethyl ether. The peptide precipitate was dried using a lyophilizer. Peptide purification was carried out by preparative RP HPLC LC-ESMS and a Vydac 218TP C-18 column using a 15 ml/min flow rate and the following gradient: time(min):%B 0:10,

5:10, 10:25, 40:75, 45:100, 50:100, 51:10, 70:10, 71:10 where A was H_2O with 0.1% TFA and B was 30% H_2O/70% ACN and 0.09% TFA. The purified fraction was lyophilized to provide a white solid that was stored at -80 ^oC. Electrospray mass spectroscopy (ESI-MS) analysis confirmed the correct molecular weight of each peptide as is shown in Table S6. The peptides were purified via HPLC and approximately 10 mg of the pure products were obtained in each case.

Table S7. Methodology and summary of the 10 Cys/His-tag peptide sequences synthesized for comparison of homogenous radiolabeling with array labeling. Each peptide was one of a pair with and without a CysLys dipeptide. For every pair a different number and arrangement of His residues was present. Each sequence has an identical counterpart in the solid phase array.

Peptide Sequence	**Sequence "type"**
LAAALEHHHHHH	Generic $(His)_6$-Tag – HHHHHH
CKLAAALEHHHHHH	Generic $(His)_6$-Tag – HHHHHH
LAAALEHAHAHA	HXHXHX
CKLAAALEHAHAHA	HXHXHX
LAAALEHHHH	Abbreviated His-Tag
CKLAAALEHHHH	Abbreviated His-Tag
LAAALEHAHA	HXHX
CKLAAALEHAHA	HXHX
LAAALEHAAH	HXXH
CKLAAALEHAAH	HXXH

Table S8. Summary of the ESI-MS data for the 10 synthesized Cys/His-Tag peptide sequences.

Peptide	Peptide Sequence	Expected MW (g/mol)	Obtained MW (g/mol)
1	LAAALEHHHHHH	1408.69	1408.72
2	CKLAAALEHHHHHH	1639.79	1639.31
3	LAAALEHAHAHA	1210.62	1210.58
4	CKLAAALEHAHAHA	1441.72	1441.72
5	LAAALEHHHH	1134.57	1134.59
6	CKLAAALEHHHH	1365.67	1365.42
7	LAAALEHAHA	1139.58	1139.38
8	CKLAAALEHAHA	1370.69	1370.66
9	LAAALEHAAH	1139.58	1139.60
10	CKLAAALEHAAH	1370.69	1370.71

Model protein construction, expression and purification

Protein purification was carried out using the AKTA-FPLC-900 System with a fraction collector-950 (GE Healthcare Life Sciences) using a Ni-NTA column (Superflow Cartridge, Qiagen, Manchester, UK) and a Superdex 75 HR 10/30 column (GE Healthcare, Little Chalfont, UK) for gel filtration. All buffers were prepared in-house with chemicals and solvents obtained from Sigma-Aldrich (Dorset, UK) unless otherwise specified. Sterile water was used to prepare the buffers. Vivaspin2 ultracentrifugation tubes with a 5,000 molecular weight cut-off were purchased from Sartorius (Epsom, UK) for protein concentration and protein concentration measured by UV spectrometry using a Nanodrop 2000c spectrophotometer (ThermoScientific, UK). Proteins were analyzed using SDS-PAGE analysis with NuPAGE 12% gels (Invitrogen, Paisley, UK), MOPS buffer (Life Technologies, Paisley UK) and an Invitrogen Novex Mini Cell chamber (Life Technologies). For western blot development, antiPentaHis, goat anti mouse – horse radish peroxidise (Gam:HRP) and SigmaFastDAB were obtained from Qiagen, Millipore and Sigma-Aldrich respectively. The Novex Sharp prestained protein standard for SDS-PAGE and Western Blot was obtained from Life Technologies. High performance liquid chromatography (HPLC) analysis was carried out on an Agilent 1200 series system with an in-line UV (280 nm) detector and a BioSepSEC-s 2000 (Phenomenex, Cheshire, UK) size exclusion column (SEC). DNA sequencing was performed by Beckman Coulter Genomics, Takeley, UK. For radiolabelling experiments and quality controls, materials were obtained and equipment used as previously described for the radiolabeling of the Cys/His-Tag Peptides. DU145 cells and the DU145 cells expressing PSMA (DU145-PSMA) were described previously.(14)

A single chain fragment variable (scFv) of J591 in VH-VL orientation was PCR amplified from the SFG P28z vector and subcloned into a hybrid expression vector based on pSecTag2 (Life Technologies) and pIRES-eGFP (Clonetech) sequences.(15) J591JWT-CYS and J591JWT were generated by insertion of annealed overlapping oligonucleotides (Integrated DNA Technologies) with NotI/EcoRI overhangs replacing the original (His)6 sequence in the expression vector.

Oligo sequences:

JWT-CYS_forward: 5’ ggccgcATGCCTGAGAAGAAGGCTGGCCCACCACCACCACCACTGAG 3’

JWT-CYS reverse: 5’ AATTCTCAGTGGTGGTGGTGGTGGGCCAGCCTTCTTCTCAGGCATgc 3’

JWT-forward: 5’ ggccgcACTGAGAAGAAGGCTGGCCCACCACCACCACCACTGAG 3’

JWT- reverse: 5’ gcACTGAGAAGAAGGCTGGCCCACCACCACCACCACCACTGAGAATT 3’

A published, humanized sequence of the J591 scFv was synthesized (Geneart) and subcloned into the target expression vector. The sequence coding for the 6C7.1scFv was kindly provided by Prof S. Duebel, University of Braunscheig, Germany. The 6C7.1scFv sequence was PCR amplified from a source vector and subcloned into the target expression vector. 6C7.1scFv scFv with a C-terminal cysteine (6C7.1-CscFv) was used as an additional control. All sequences were verified by DNA sequencing.

For protein production, HEK393T cells were transfected with the respective expression vector and transfected cells were selected with 100 µg/ml of Zeocin. Cells were then expanded to triple layer flasks and culture supernatants containing the recombinant protein were collected.

Purification was achieved by a combination of immobilized metal ion chromatography and size exclusion chromatography. The scFvs were extracted from HEK293T culture supernatant on the AKTA-FPLC using Ni-NTA chromatography with a 5 ml Ni-NTA column using a binding buffer of 50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH8, and an elution buffer of 50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, pH8.

A size exclusion gel filtration step (SEC, Superdex 75 HR 10/30, PBS pH 7.4) using the AKTA-FPLC further purified the antibody fragments. The purified monomeric proteins in PBS at pH 7.4 were concentrated using VivaSpin membrane spin filter columns with a 5,000 molecular weight cut-off to > 0.9 mg/ml. Protein concentration was measured by UV spectrometry with a UV absorption at 280 nm using a Nanodrop spectrophotometer. A molar extinction coefficient and molecular weight of the respective protein was determined from the primary amino acid sequence using the ProtParam online tool, assuming all cysteines are present as cystines. These data are displayed in Table S7. All aliquots were stored in PBS at pH 7.4 at -80^{o}C.

Purity of the scFv protein fragments was assessed by SDS-PAGE/Coomassie brilliant blue staining, by analytical size exclusion HPLC and a Western blot. 12% precast polyacrylamide gels (Nu-PAGE) were used to separate and visualize the proteins. A Novex Sharp prestained protein standard was used as a reference. Gels were either visualized with Coomassie Brilliant blue G250 staining or proteins were transferred to nitrocellulose membranes for subsequent Western blot detection. For Western blotting, proteins were transferred to nitrocellulose membranes and detected with anti-Penta His and goat anti mouse:HRP, with SigmaFast DAB as the HRP substrate.

Table S9. Extinction coefficient and molecular weight data for the primary amino acid sequence for all 7 antibody fragments expressed. The labelling sequence of each protein is also listed and its pI. Data obtained using ExPASy ProtParam online tool assuming all cysteines are present as (reduced) cysteines.

Protein	Extinction Coefficient (E_{280nm}) (M^{-1} cm^{-1})	$MW_{monomer}$ (kDa)	Labelling Sequence	pI of Labelling Sequence	Comments
J591scFvJWT	51130	28.23	ALRRRLAHHHHHH	12.40	scFv against J591mAb with JWT-tag
J591scFv	51130	27.07	KRAAALEHHHHHH	6.62	scFv against J591mAb with His_6-tag
huJ591scFv	51130	27.28	IKAAALEHHHHHH	6.62	Humanised scFv against J591mAb with His_6-tag
C67.1 scFv	48610	28.78	PTAAALEHHHHHH	6.62	scFv against VCAM-1 with His_6 tag
C67.1-C scFv	48610	28.89	TAAALEHHHHHHC	6.62	scFv against VCAM-1 with Cys/His_6-tag

References

1. Mees, G., Dierckx, R., Mertens, K., Vermeire, S., Van Steenkiste, M., Reutelingsperger, C., D'Asseler, Y., Peremans, K., Van Damme, N., Van de Wiele, C. (2012) ^{99m}Tc-labeled tricarbonyl his-CNA35 as an imaging agent for the detection of tumor vasculature. *J. Nucl. Med.*, *53*, 464-471.
2. Shah, S. Q., Khan, A. U., Khan, M. R. (2012) $^{99m}Tc(CO)_3$-Ibritumomab tiuxetan, a novel radioimmunoimaging (RII) agent of B cell non-Hodgkin's lymphoma (NHL). *Biol. Chem.*, *393*, 71-75.
3. Virna Cortez-Retamozo, T. L., Caveliers, V., Olive, L., Gainkam, T., Hernot, S., Packeu, A., De Vos, F., Vanhove, C., Muyldermans, S., De Baetselier, P., et al. (2008) ^{99m}Tc-labeled nanobodies: a new type of targeted probes for imaging antigen expression, *Current Radiopharmaceuticals*, *1*, 37-41.

4. Teran, M. A., Martinez, E., Reyes, A. L., Paolino, A., Vital, M., Esperon, P., Pacheco, J. P., Savio, E. (2011) Biological studies in animal models using $[^{99m}Tc(CO)_3]^+$ recombinant annexin V as diagnostic agent of apoptotic processes. *Nucl. Med. Biol.*, *38*, 279-285.
5. Orlova, A., Nilsson, F. Y., Wikman, M., Widstrom, C., Stahl, S., Carlsson, J., Tolmachev, V. (2006) Comparative in vivo evaluation of technetium and iodine labels on an anti-HER2 affibody for single-photon imaging of HER2 expression in tumors. *J. Nucl. Med.*, *47*, 512-519.
6. Deyev, S. M., Waibel, R., Lebedenko, E. N., Schubiger, A. P., Pluckthun, A. (2003) Design of multivalent complexes using the barnase-barstar module. *Nat. Biotechnol.*, *21*, 1486-1492.
7. Tait, J. F., Smith, C., Gibson, D. F. (2002) Development of annexin V mutants suitable for labeling with Tc(I)-carbonyl complex. *Bioconjugate Chem.*, *13*, 1119-1123.
8. Chen, W. J., Yen, C. L., Lo, S. T., Chen, K. T., Lo, J. M. (2008) Direct ^{99m}Tc labeling of Herceptin (trastuzumab) by $^{99m}Tc(I)$ tricarbonyl ion. *Appl. Radiat. Isot.*, *66*, 340-345.
9. Tavare, R., Torres Martin De Rosales, R., Blower, P. J., Mullen, G. E. (2009) Efficient site-specific radiolabeling of a modified C2A domain of synaptotagmin I with $[^{99m}Tc(CO)_3]^+$: a new radiopharmaceutical for imaging cell death. *Bioconjugate Chem.*, *20*, 2071-2081.
10. Zahnd, C., Kawe, M., Stumpp, M. T., de Pasquale, C., Tamaskovic, R., Nagy- Davidescu, G., Dreier, B., Schibli, R., Binz, H. K., Waibel, R., et al. (2010) Efficient tumor targeting with high-affinity designed ankyrin repeat proteins: effects of affinity and molecular size. *Cancer Res.*, *70*, 1595-1605.
11. Tolmachev, V., Hofstrom, C., Malmberg, J., Ahlgren, S., Hosseinimehr, S. J., Sandstrom, M., Abrahmsen, L., Orlova, A., Graslund, T. (2010) HEHEHE-tagged affibody molecule may be purified by IMAC, is conveniently labeled with $[^{99m}Tc(CO)_3]^+$, and shows improved biodistribution with reduced hepatic radioactivity accumulation, *Bioconjugate Chem.*, *21*, 2013-2022.
12. Bidlingmaier, S., He, J., Wang, Y., An, F., Feng, J., Barbone, D., Gao, D., Franc, B., Broaddus, V. C., Liu, B. (2009) Identification of MCAM/CD146 as the target antigen of a human monoclonal antibody that recognizes both epithelioid and sarcomatoid types of mesothelioma. *Cancer Res.*, *69*, 1570-1577.
13. Berndorff, D., Borkowski, S., Moosmayer, D., Viti, F., Muller-Tiemann, B., Sieger, S., Friebe, M., Hilger, C. S., Zardi, L., Neri, D., et al. (2006) Imaging of tumor angiogenesis using ^{99m}Tc-labeled human recombinant anti-ED-B fibronectin antibody fragments. *J. Nucl. Med.*, *47*, 1707-1716.
14. Kampmeier, F., Williams, J. D., Maher, J., Mullen, G. E., Blower, P.J. (2014) Design and preclinical evaluation of a Tc-99m-labelled diabody fragment of mAb J591 for SPECT imaging of prostate specific membrane antigen (PSMA). *EJNMMI Res.*, 4, 13.
15. Maher, J., Brentjens, R. J., Gunset, G., Riviere, I., Sadelain, M. (2002) Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRzeta /CD28 receptor, *Nat. Biotechnol.*, *20*, 70-75.