Supplementary material:

From macro to micro - a comparative study

Systematic comparison between different types of peptide arrays (macro vs. micro-arrays) was not described so far in the literature, probably because such experiments are very expensive and time-consuming to conduct only for a comparative purpose. Here we demonstrate such an experiment. Two sets of peptides arrays were ordered (supplementary Figure 1): (i) A macro-array produced by the JPT Company [1]. The peptides were synthesized using the standard SPOT synthesis technique on cellulose sheets (PepSpot™). (ii) A micro-array produced by the INTAVIS Company [2]. The peptides were synthesized using the SC2 technique on glass slides (CelluSpots). The peptide sequences were identical in both arrays, which contained 174 peptides derived from the sequence of the CFTR protein (cystic fibrosis transmembrane conductance regulator) for a full list of all peptides see supplementary Table 2.

The CFTR protein is known to interact with the Hsp90 protein (heat shock protein 90) [3] and the peptide array experiments were carried out to identify the precise Hsp90 - binding sites in CFTR. All the binding assays were carried out by incubating recombinant Hsp90 protein with both peptide arrays. Both binding assays were similar in terms of the buffers, proteins and image analysis that were used. Hsp90 is a molecular chaperone and it is thus expected to bind a wide range of peptides with different binding affinities. Indeed, our studies showed that in both arrays Hsp90 bound different peptides and gave diverse signal intensities. Surprisingly, the two arrays showed somewhat different results, and only 12 observed peptides were identical in both arrays (supplementary Table 1).

The difference between the two arrays can be attributed to the nature of the array and the way the experiments are carried out: (i) the concentrations of peptide per spot were different. Synthesis of a macro-array is expected to yield peptide amounts at the pmol range, whereas for a micro-array such yield is expected to be at the nmol range [4]. High peptide density can be advantageous in case of relatively low binding affinities. Though, in some cases of chaperones (e.g. the chaperone DnaK), lower peptides concentrations were preferable since the protein did not enter the spots of the high density arrays [5]. (ii) Since each array was synthesized by a different technique, the quality and purity of the synthesized peptides in each spot might be different. Hence the results could be influenced by unsuccessful synthesis or by-products. (iii) Differences in binding can also arise from technical differences in the binding assay.

For example, the binding assay using the macro-arrays includes a key step of electrotransfer to PVDF membranes (performed in order to enable reusing of the array and fixation of the protein on the PVDF membrane). In micro-arrays the binding detection is performed directly on the glass slide (slides are disposable). Washing steps that are performed directly on the slide can affect the binding and eliminate relatively weak interactions. (iv) The different solid phase supports may influence the accessibility of the protein, and unspecific interaction of the protein with the membrane may also play a role.

To compare the properties of the Hsp90-binding peptides from both arrays, we analysed their various properties, such as charge and hydrophobicity. We measured their net charges at pH = 7.0 and their hydrophilicity. All calculations were done by Innovagen's Peptide Property Calculator [6]. As a control, we examined both parameters also for the nonbinding peptides from both arrays. Furthermore, we compared the distribution of charge and hydrophilicity within the groups of peptides bearing similar binding intensities in order to see if there is any correlation between these parameters and the binding intensity. The results (Supplementary Table 1, Supplementary Figure 1) show that in both arrays the Hsp90-binding peptides have a positive charge at pH = 7.0, while the non-binding peptides have a negative charge. In the micro-array the binding intensity correlated with the positive charge. In the macro-array there were no significant differences between the different intensity groups in this regard. As for the hydrophilicity, in the micro-array the average hydrophilicity of all binding peptides is lower then the average of all non binding peptides, indicating an overall hydrophobic character for the binding peptides. The macro-array results showed the opposite trend: the binding peptides were on average more hydrophilic than the non-binding peptides, and there was no correlation between the hydrophilicity and the binding intensity.

The described results are only array screening results and were not validated in solution. We conclude that the properties of the patterns may look different, depending on conditions and type of array. The conclusions of our experiments do not favor one commercial company over the other. Rather, these results emphasize the differences between micro-arrays and macro-arrays, regardless of which manufacturer makes them.

References

1. GmbH, J.P.T., http://www.jpt.com/index.htm.
2. INTAVIS, B.I., http://www.intavis.com/en/.
3. Loo, M.A., et al., *Perturbation of Hsp90 interaction with nascent CFTR prevents its maturation and accelerates its degradation by the proteasome.* Embo J, 1998. **17**(23): p. 6879-87.
4. Blackwell, H.E., *Hitting the SPOT: small-molecule macroarrays advance combinatorial synthesis.* Curr Opin Chem Biol, 2006. **10**(3): p. 203-12.
5. Rudiger, S., et al., *Substrate specificity of the DnaK chaperone determined by screening cellulose-bound peptide libraries.* Embo J, 1997. **16**(7): p. 1501-7.
6. Calculator, I.s.P.P., http://innovagen.com/custom-peptide-synthesis/peptide-property-calculator.

Supplementary Table 1: Binding between the HSP90 protein and peptides derived from the CFTR protein sequence: comparison of macro-array vs. micro array

Binding intensity	# binding pep. in Macro-array	# binding pep. in Micro-array	# Identical binding pep in both Macro/Micro- arrays
Very strong	5	7	3
Strong	10	7	2
Medium	16	10	4
Weak	14	12	3
Total	45	36	12

Supplementary Table 2: The peptides in the array derived from the CFTR protein.

#General	Sequence	Residues
1	MQRSPLEKASVVSKL	1-15
2	ASVVSKLFFSWTRPI	9-23
3	FSWTRPILRKGYRQR	17-31
4	RKGYRQRLELSDIYQ	25-39
5	SDIYQIPSVDSADNL	35-49
6	QIPSVDSADNLSEKL	39-53
7	DNLSEKLEREWDREL	47-61
8	REWDRELASKKNPKL	55-69
9	SKKNPKLINALRRCF	63-77
10	NALRRCFFWR	71-80
11	RIIASYDPDNKEERS	104-118
12	NKEERSIAIYL	113-123
13	HPAIFGLHHIGMQMR	139-153
14	HIGMQMRIAMFSLIY	147-161
15	AMFSLIYKKTLKLSS	155-169
16	KTLKLSSRVLDKISI	163-177
17	VLDKISIGQLVSLLS	171-185
18	LVSLLSNNLNKFDEG	180-194
19	LIWELLQAS	214-222
20	RMMMKYRDQRAGKIS	242-256
21	MKYRDQRAGKISERL	245-259
22	RAGKISERLVITS	251-263
23	GKISERLVITSEMIE	253-267
24	SEMIENIQSVKAYCW	263-277
25	VKAYCWEEAMEKMIE	272-286
26	AMEKMIENLRQTELK	280-294
27	LRQTELKLTRKAAYV	288-302
28	LKLTRKAAYVRYFNS	293-307
29	AAYVRYFNSSAFFFS	299-313
30	TRQFPWAVQTWYDSL	351-365

31	QTWYDSLGAINKIQD	359-373
32	AINKIQDFLQKQEYK	367-381
33	LQKQEYKTLEYNLTT	375-389
34	LEYNLTTTEVVMENV	383-397
35	TTTEVVMENVTAFWE	388-402
36	NVTAFWEEGFGELFE	396-410
37	FWEEGFGELFEKAKQ	400-414
38	LFEKAKQNNNNRKTS	408-422
39	NNNRKTSNGDDSLFF	416-430
40	FFSNFSLLGTPVLKD	429-443
41	GTPVLKDINFKIERG	437-451
42	GSTGAGKTSLLMMIM	458-472
43	ELEPSEGKIKHSGRI	474-488
44	ISFCSQFSWIMPGT	488-501
45	FSWIMPGTIKENIIF	494-508
46	TIKENIIFGVSYDE	501-514
47	TIKENIIGVSYDE	501-514
48	FGVSYDEYRYRSVIK	508-522
49	YDEYRYRSVIKACQL	512-526
50	VIKACQLEEDISKFA	520-534
51	QLEEDISKFAEKDNI	525-539
52	AEKDNIVLGEGGITL	534-548
53	LGEGGITLSGGQRAR	541-555
54	SGGQRARISLARAVY	549-563
55	SLARAVYKDADLYLL	557-571
56	DADLYLLDSPFGYLD	565-579
57	SPFGYLDVLTEKEIF	573-587
58	LDVLTEKEIFESCVC	578-592
59	IFESCVCKLMANKTR	586-600
60	LMANKTRILVTSKME	594-608
61	KTRILVTSKMEHLKK	598-612
62	KMEHLKKADKILILH	606-620
63	DKILILHEGSSYFYG	614-628
64	GSSYFYGTFSELQNL	622-636
65	TFSELQNLQPDFSSK	629-643
66	PDFSSKLMGCDSFDQ	638-652
67	GCDSFDQFSAERRNS	646-660
68	SAERRNSILTETLHR	654-668
69	LTETLHRFSLEGDAP	662-676
70	SLEGDAPVSWTETKK	670-684
71	SWTETKKQSFKQTGE	678-692
72	SFKQTGEFGEKRKNS	686-700
73	GEKRKNSILNPINSI	694-708
74	ILNPINSIRKFSIVQK	702-716
75	KFSIVQKTPLQMNGI	710-724
76	PLQMNGIEEDSDEPL	718-732
77	EDSDEPLERRLSLVP	726-740
78	RRLSLVPDSEQGEAI	734-748
79	SEQGEAILPRISVIS	742-756
80	PRISVISTGPTLQAR	750-764

81	GPTLQARRRQSVLNL	758-772
82	RQSVLNLMTHSVNQG	766-780
83	THSVNQGQNIHRKTT	774-788
84	QNIHRKTTASTRKVSL	782-796
85	STRKVSLAPQANLTE	790-804
86	PQANLTELDIYSRRL	798-812
87	DIYSRRLSQETGLEI	806-820
88	QETGLEISEEINEED	814-828
89	EEINEEDLKECFFDD	822-836
90	KECFFDDMESIPAVT	830-844
91	ESIPAVTTWNTYLRY	838-852
92	WNTYLRYITVHKSLI	846-860
93	TVHKSLIFVLIWCLV	854-868
94	LWLLGNTPLQDKGNS	881-895
95	LQDKGNSTHSRNNSY	889-903
96	HSRNNSYAVIITSTS	897-911
97	RGLPLVHTLITVSKI	933-947
98	LITVSKILHHKMLHS	941-955
99	HHKMLHSVLQAPMST	949-963
100	LQAPMSTLNTLKAGG	957-971
101	NTLKAGGILNRFSKD	965-979
102	TLNTLKAGGILNRFS	973-987
103	FSKDIAILDDLLPLT	976-990
104	QTSQQLKQLESEGRS	1035-1049
105	LESEGRSPIFTHLVT	1043-1057
106	IFTHLVTSLKGLWTL	1051-1065
107	LKGLWTLRAFGRQPY	1059-1073
108	AFGRQPYFETLFHKA	1067-1081
109	ETLFHKALNLHTANW	1075-1089
110	NLHTANWFLYLSTLR	1083-1097
111	LYLSTLRWFQMR	1091-1102
112	TGEGEGRVG	1122-1130
113	SIDVDSLMRSVSRVF	1150-1164
114	RSVSRVFKFIDMPTE	1158-1172
115	FIDMPTEGKPTKSTK	1166-1180
116	KPTKSTKPYKNGQLS	1174-1188
117	YKNGQLSKVMIIENS	1182-1196
118	VMIIENSHVKKDDIW	1190-1204
119	VKKDDIWPSGGQMTV	1198-1212
120	SGGQMTVKDLTAKYT	1206-1220
121	DLTAKYTEGGNAILE	1214-1228
122	GGNAILENISFSISP	1222-1236
123	ISFSISPGQRVGLLG	1230-1244
124	QRVGLLGRTGSGKST	1238-1252
125	TGSGKSTLLSAFLRL	1246-1260
126	LSAFLRLLNTEGEIQ	1254-1268
127	NTEGEIQIDGVSWDS	1262-1276
128	DGVSWDSITLQQWRK	1270-1284
129	TLQQWRKAFGVIPQK	1278-1292
130	FGVIPQKVFIFSGTF	1286-1300

131	FIFSGTFRKNLDPYE	1294-1308
132	KNLDPYEQWSDQEIW	1302-1316
133	WSDQEIWKVADEVGL	1310-1324
134	VADEVGLRSVIEQFP	1318-1332
135	SVIEQFPGKLDFVLV	1326-1340
136	CVLSHGHKQLMCLAR	1334-1348
137	GGCVLSHGHKQLMCL	1342-1356
138	HKQLMCLARSVLSKA	1350-1364
139	RSVLSKAKILLLDEP	1358-1372
140	ILLLDEPSAHLDPVT	1366-1380
141	AHLDPVTYQIIRRTL	1374-1388
142	QIIRRTLKQAFADCT	1382-1396
143	QAFADCTVILCEHRI	1390-1404
144	ILCEHRIEAMLECQQ	1398-1412
145	AMLECQQFLVIEENK	1406-1420
146	LVIEENKVRQYDSIQ	1414-1428
147	RQYDSIQKLLNERSL	1422-1436
148	LLNERSLFRQAISPS	1430-1444
149	RQAISPSDRVKLFPH	1438-1452
150	RVKLFPHRNSSKCKS	1446-1460
151	NSSKCKSKPQIAALK	1454-1468
152	PQIAALKEETEEEVQ	1462-1476
153	ALKEETEEEVQDTRL	1466-1480
154	TTEVVMENVTAFWEEGFGELFEKAK	389-413
155	EEGFGELFEKAKQNNNNRKTSNGDDSLF	402-429
156	KTSNGDDSLFFSNFSLL	420-436
157	VLKDINFKIERGQLLAVAGS	440-459
158	GQLLAVAGSTGAGKTSLLMMIMG	451-473
159	GKTSLLMMIMGELEPSEGKIKH	463-484
160	GKIKHSGRISFCSQFSWIMPG	480-500
161	IMPGTIKENIIFGVSYDEYRYRSVIK	497-522
162	EYRYRSVIKACQLEEDISKFAEKDN	514-538
163	VLGEGGITLSGGQRARISLARAVYK	540-564
164	SGGQRARISLARAVYKDADLYLLDS	549-573
165	DLYLLDSPFGYLDVLTEKEIFESCV	567-591
166	VLTEKEIFESCVCKLMANKTRILVT	580-604
167	NKTRILVTSKMEHLKKADKILILHE	597-621
168	DKILILHEGSSYFYGTFSELQNLQP	614-638
169	TFSELQNLQPDFSSKLMG	629-646
170	QIPSVDSADNLSEKLEREWDRELASKKNPK	39-68
171	MQMRIAMFSLIYKKTLKLSSRVLDK	150-174
172	IGQLVSLLSNNLNKFDEG	177-194
173	EMIENIQSVKAYCWEEAMEKMIE	264-286
174	ISFCSQFSWIMPGTIKENIIFGVSYDE	488-514

*An array of peptides derived from the CFTR protein was screened for binding Hsp90 by immunoblot experiments with Hsp90 antibody.

Supplementary Figure 1:

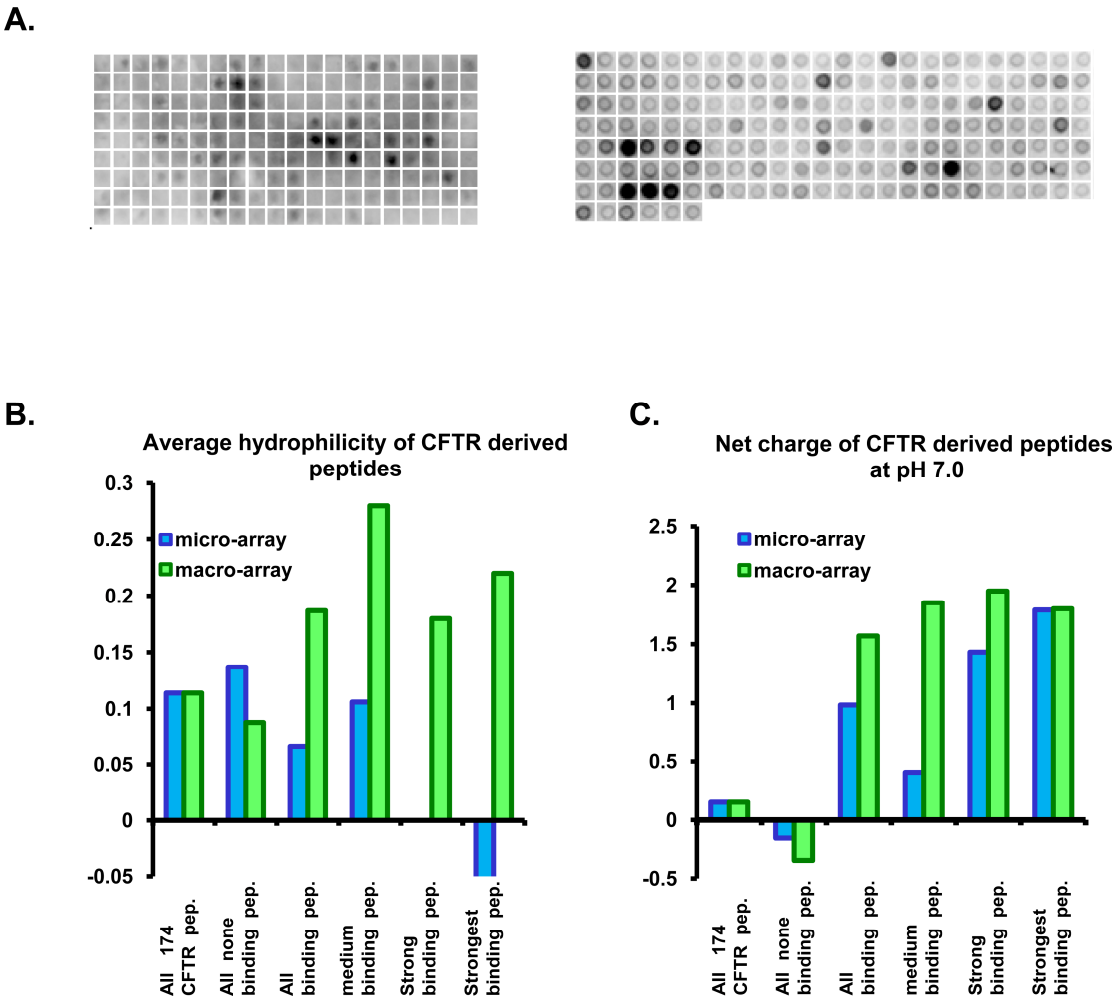

Figure 1. Comparative analysis between macro- and micro- array screening results, demonstrated for HSP90 protein binding to CFTR derived peptides; ***(A)*** Comparison of a cellulose-bound macro-array (*top*) and microscope slide micro-array (*bottom*), both containing 174 peptides derived from CFTR (original size). Recombinant Hsp90 protein with an N-terminal His-tag was expressed in E. coli and purified on Poros 20MC metal chelate media followed by Poros 20HQ anion exchange media. Binding of 231 nM Hsp90 to both peptide arrays was screened as described [9]. The detection of the binding was done by fluoroluminescence using primary anti-Hsp90 antibody and Alkaline Phosphatase conjugated secondary antibody. ***(B-C)*** Comparison between the observed binding peptides from both arrays, for matching the known profile of chaperone-binding peptides: high hydrophobicity and positive charge. Electrostatic charges (B) and hydrophilicity (C) were compared between all the binding peptides and all the non-binding peptides, as well as within peptides from the same groups categorized according to intensities of the binding, observed on the arrays. All calculations were performed using Innovagen's Peptide Property Calculator [6].