

Figure S1

Resin	Flowthrough	Chase	100 mM NaCl	150 mM NaCl	200 mM NaCl	250 mM NaCl	300 mM NaCl	350 mM NaCl	400 mM NaCl	450 mM NaCl	500 mM NaCl	Strip
Q Ceramic HyperD 20	2.753	3.662	1.171	1.258	1.733	1.511	1.013	1.114	0.767	1.541	3.125	4.496
Q Ceramic HyperD F	2.436	3.710	0.658	0.789	1.192	0.997	0.817	0.788	0.836	2.417	3.085	4.052
Q Hypercel	2.175	3.656	0.658	0.924	1.069	2.783	4.533	2.367	0.983	0.664	0.378	0.787
MacroPrep DEAE	2.251	3.512	1.979	1.632	1.776	2.159	3.396	3.008	2.222	1.557	2.168	3.667
MacroPrep High Q	2.308	3.384	2.335	1.935	1.774	1.498	1.696	1.414	2.694	3.795	2.750	3.949
Nuvia Q	2.502	3.546	1.609	2.123	2.251	1.776	2.041	1.801	1.473	2.036	2.888	4.427
UnoSphere Q	1.855	3.437	1.189	1.552	1.498	1.243	1.370	2.245	2.629	1.659	0.738	1.356
ANX Sepharose 4FF High Sub	2.065	3.811	1.992	1.735	1.569	1.457	1.747	2.262	3.434	3.369	2.288	4.019
Capto DEAE	2.078	3.543	0.956	1.110	1.106	1.041	1.035	1.442	2.684	2.672	1.803	2.358
Capto Q	2.155	3.552	1.030	1.224	1.208	1.132	1.160	1.141	1.790	2.557	2.213	3.697
DEAE Sepharose FF	2.241	3.533	2.147	1.928	2.011	2.328	3.756	3.482	1.652	0.988	0.756	0.475
Q Sepharose XL	1.954	3.530	0.846	0.951	0.979	0.776	0.830	0.820	0.859	1.523	2.276	4.140
Source 30Q	2.475	3.786	2.259	1.873	1.928	2.367	3.425	3.265	2.098	1.027	1.893	2.914
Eshmuno Q	2.246	3.461	1.191	1.441	1.238	1.020	1.186	1.376	2.548	2.487	1.356	2.557
Fractogel EMD DEAE (M)	2.349	3.563	1.984	1.918	1.965	1.950	2.255	2.529	2.394	1.874	1.938	3.829
Fractogel EMD TMAE (M)	2.280	3.720	3.456	2.388	2.195	2.293	3.108	3.246	2.240	1.349	1.154	3.824
Fractogel EMD TMAE Hicap (M)	2.671	3.453	2.329	2.687	2.219	2.177	2.144	1.570	1.777	2.893	1.268	3.982
Hypercel Star AX	2.302	3.198	0.465	0.396	0.439	0.438	0.427	0.372	0.378	0.383	1.544	4.390
POROS 50D	2.637	3.429	2.241	1.843	1.973	1.901	2.764	3.736	2.668	2.126	2.047	4.071
POROS 50 HQ	2.796	3.445	2.704	2.103	1.835	1.565	1.496	1.867	2.458	1.536	3.284	
POROS XQ	2.406	3.200	1.015	1.520	1.662	1.453	1.502	1.219	1.666	2.608	1.485	4.115
POROS 50 PI	2.479	3.646	1.057	0.773	0.607	0.512	0.495	0.470	0.445	0.508	2.206	4.246
TOYOPEARL NH2-750F	3.174	3.506	0.887	0.621	0.512	0.443	0.413	0.375	0.360	0.295	0.347	1.977
TOYOPEARL DEAE-650M	2.174	3.864	3.853	3.916	4.409	3.814	4.081	1.230	0.347	0.086	1.355	0.073
TOYOPEARL DEAE-650C	2.117	3.835	3.736	4.290	4.139	3.457	1.873	0.442	0.107	0.058	0.046	0.064
TOYOPEARL GigaCap Q-650M	2.648	3.578	1.950	2.089	1.874	1.745	1.952	1.526	1.265	1.736	2.720	3.896
TOYOPEARL GigaCap Q-650S	3.037	3.493	2.456	2.380	2.112	2.117	2.450	1.921	1.609	1.464	2.532	3.786
TOYOPEARL SuperQ-650C	2.186	3.418	1.599	1.688	1.759	1.614	2.884	4.105	2.391	0.958	0.506	1.300
TOYOPEARL SuperQ-650M	2.328	3.612	2.197	2.164	2.100	1.833	1.993	4.242	3.200	1.298	0.646	2.018
TOYOPEARL QAE 550C	2.400	3.310	2.352	2.129	1.857	1.606	1.665	1.591	1.668	2.006	3.743	3.706
TOYOPEARL Q-600C AR	2.105	3.535	0.884	0.821	0.712	0.541	0.482	0.418	0.327	0.266	0.391	4.412
TOYOPEARL GigaCap DEAE-650M	2.139	3.629	1.968	1.945	1.838	1.927	1.827	1.986	3.288	3.533	2.599	4.184

Resin	Flowthrough	Chase	100 mM NaCl	150 mM NaCl	200 mM NaCl	250 mM NaCl	300 mM NaCl	350 mM NaCl	400 mM NaCl	450 mM NaCl	500 mM NaCl	Strip
Q Ceramic HyperD 20	1.489	1.231	0.702	0.921	1.170	0.717	0.333	0.181	-0.042	0.140	NR	NR
Q Ceramic HyperD F	1.574	1.248	0.506	0.787	1.124	0.616	0.293	0.145	0.112	0.085	0.067	0.126
Q Hypercel	1.564	1.264	0.460	0.376	0.300	0.199	0.196	0.149	0.141	0.102	0.066	0.082
MacroPrep DEAE	1.632	1.508	1.333	0.888	0.505	0.273	0.237	0.191	0.201	0.186	0.156	0.157
MacroPrep High Q	1.652	1.411	1.493	1.254	0.883	0.423	0.284	0.193	0.162	0.108	0.073	0.217
Nuvia Q	1.607	1.267	1.223	1.409	1.433	1.101	0.797	0.471	0.291	0.155	0.120	0.159
UnoSphere Q	1.580	1.244	0.872	1.132	1.034	0.598	0.364	0.155	0.078	0.047	0.039	0.090
ANX Sepharose 4FF High Sub	1.604	1.368	1.150	1.198	1.278	1.037	0.575	0.235	0.178	0.123	0.090	0.172
Capto DEAE	1.450	1.142	0.481	0.547	0.489	0.401	0.257	0.153	0.114	0.065	NR	NR
Capto Q	1.552	1.238	0.646	0.662	0.524	0.317	0.219	0.131	0.105	0.061	0.047	0.056
DEAE Sepharose FF	1.516	1.297	1.061	1.116	0.906	0.389	0.174	0.093	0.061	0.061	0.029	0.021
Q Sepharose XL	1.570	1.261	0.504	0.622	0.719	0.536	0.332	0.189	0.100	0.063	0.056	0.036
Source 30Q	1.584	1.449	1.369	1.119	0.568	0.218	0.141	0.086	0.075	0.084	0.041	0.049
Eshmuno Q	1.566	1.246	0.804	1.034	1.081	0.726	0.395	0.165	0.084	0.053	0.032	0.037
Fractogel EMD DEAE (M)	1.553	1.367	1.148	0.941	0.737	0.464	0.263	0.179	0.149	0.128	0.091	0.053
Fractogel EMD TMAE (M)	1.592	1.470	1.251	1.228	0.761	0.404	0.196	0.115	0.094	0.088	0.070	0.128
Fractogel EMD TMAE Hicap (M)	1.604	1.271	1.310	1.452	1.279	0.814	0.429	0.222	0.166	0.090	0.339	0.201
Hypercel Star AX	1.596	1.207	0.373	0.381	0.396	0.342	0.330	0.276	NR	NR	NR	NR
POROS 50D	1.620	1.395	1.542	1.298	0.795	0.331	NR	NR	NR	NR	0.196	0.043
POROS 50 HQ	1.613	1.446	1.570	1.441	1.258	0.873	NR	NR	NR	NR	NR	NR
POROS XQ	1.572	1.186	0.963	1.241	1.121	0.922	0.846	0.568	0.285	0.166	0.116	0.236
POROS 50 PI	1.619	1.378	1.108	0.947	0.706	0.451	0.307	0.198	0.164	0.137	0.123	0.197
TOYOPEARL NH2-750F	1.667	1.417	0.405	0.255	0.215	0.166	0.164	0.130	0.130	0.123	0.114	0.313
TOYOPEARL DEAE-650M	1.634	1.445	1.096	0.491	0.295	0.240	0.263	0.164	0.041	0.013	0.113	0.019
TOYOPEARL DEAE-650C	1.590	1.421	0.961	0.439	0.252	0.231	0.178	0.048	0.020	0.011	0.435	0.009
TOYOPEARL GigaCap Q-650M	1.563	1.265	1.321	1.344	1.219	0.904	0.703	0.505	NR	NR	NR	NR
TOYOPEARL GigaCap Q-650S	1.613	1.323	1.425	1.357	1.159	0.916	NR	NR	NR	NR	0.376	0.000
TOYOPEARL SuperQ-650C	1.586	1.255	1.208	1.162	0.798	0.437	NR	NR	NR	NR	NR	NR
TOYOPEARL SuperQ-650M	1.559	1.269	1.423	1.210	0.617	0.271	0.177	0.111	0.102	0.110	0.067	0.068
TOYOPEARL QAE 550C	1.557	1.237	1.167	1.247	1.316	1.113	0.821	0.396	0.249	0.166	0.073	0.221
TOYOPEARL Q-600C AR	1.539	1.247	0.506	0.642	0.815	0.545	0.295	0.156	0.107	0.071	0.053	0.080
TOYOPEARL GigaCap DEAE-650M	1.575	1.247	1.298	1.281	1.077	0.836	0.603	0.503	0.429	0.264	0.158	0.264

LEGEND

NR

LOW

HIGH

Resin	Flowthrough	Chase	100 mM NaCl	150 mM NaCl	200 mM NaCl	250 mM NaCl	300 mM NaCl	350 mM NaCl	400 mM NaCl	450 mM NaCl	500 mM NaCl	Strip
Q Ceramic HyperD 20	1.506	1.291	0.899	1.083	1.254	1.026	0.517	0.294	0.291	0.215	0.185	0.176
Q Ceramic HyperD F	1.521	1.342	0.813	0.926	1.182	0.959	0.609	0.325	0.234	0.155	0.150	0.166
Q Hypercel	1.534	1.258	0.823	0.396	0.315	0.213	0.205	0.173	0.174	0.148	0.127	0.126
MacroPrep DEAE	1.444	1.396	1.194	0.562	0.272	0.197	0.250	0.216	0.254	0.227	0.181	0.131
MacroPrep High Q	1.458	1.389	1.430	1.246	0.950	0.536	0.375	0.265	0.238	0.187	0.132	0.237
Nuvia Q	1.550	1.245	1.244	1.467	1.452	1.181	0.845	0.479	0.315	0.189	0.239	0.249
UnoSphere Q	1.517	1.258	0.908	1.187	1.093	0.752	0.455	0.246	0.130	0.080	0.065	0.106
ANX Sepharose 4FF High Sub	1.445	1.295	1.264	1.331	1.403	1.235	0.741	0.399	0.318	0.234	0.170	0.266
Capto DEAE	1.378	1.220	0.428	0.482	0.467	0.378	0.248	0.172	0.170	0.109	0.073	0.058
Capto Q	1.491	1.250	0.648	0.661	0.508	0.336	0.281	0.202	0.181	0.131	0.217	0.106
DEAE Sepharose FF	1.487	1.310	1.193	1.148	0.789	0.312	0.178	0.136	0.110	0.067	0.027	0.018
Q Sepharose XL	1.495	1.226	0.377	0.519	0.588	0.447	0.327	0.203	0.149	0.111	0.389	0.066
Source 30Q	1.501	1.384	1.315	1.117	0.596	0.244	0.162	0.115	0.107	0.119	0.068	0.062
Eshmuno Q	1.514	1.255	0.784	1.117	1.129	0.772	0.427	0.198	0.123	0.081	0.211	0.067
Fractogel EMD DEAE (M)	1.473	1.373	0.964	0.782	0.630	0.351	0.261	0.191	0.176	0.138	0.075	0.049
Fractogel EMD TMAE (M)	1.540	1.432	1.227	1.248	0.907	0.543	0.294	0.167	0.160	0.148	0.126	0.138
Fractogel EMD TMAE Hicap (M)	1.527	1.261	1.392	1.473	1.285	0.914	0.555	0.470	0.276	0.170	0.074	0.255
Hypercel Star AX	1.434	1.246	0.486	0.416	0.466	0.296	0.283	0.196	0.231	0.160	0.157	0.157
POROS 50D	1.528	1.377	1.341	0.998	0.510	0.271	0.231	0.184	0.170	0.130	0.370	0.094
POROS 50 HQ	1.537	1.442	1.510	1.405	1.264	0.891	0.588	0.366	0.304	0.201	0.150	0.442
POROS XQ	1.511	1.255	1.078	1.374	1.314	1.081	0.855	0.515	0.313	0.228	0.156	0.197
POROS 50 PI	1.550	1.414	0.925	0.785	0.771	0.599	0.470	0.283	0.261	0.214	0.121	0.288
TOYOPEARL NH2-750F	1.559	1.373	0.444	0.286	0.253	0.054	0.400	0.275	0.227	0.201	0.158	0.361
TOYOPEARL DEAE-650M	1.555	1.385	1.005	0.442	0.337	0.304	0.328	0.171	0.064	0.030	0.132	0.025
TOYOPEARL DEAE-650C	1.481	1.357										

Figure S2

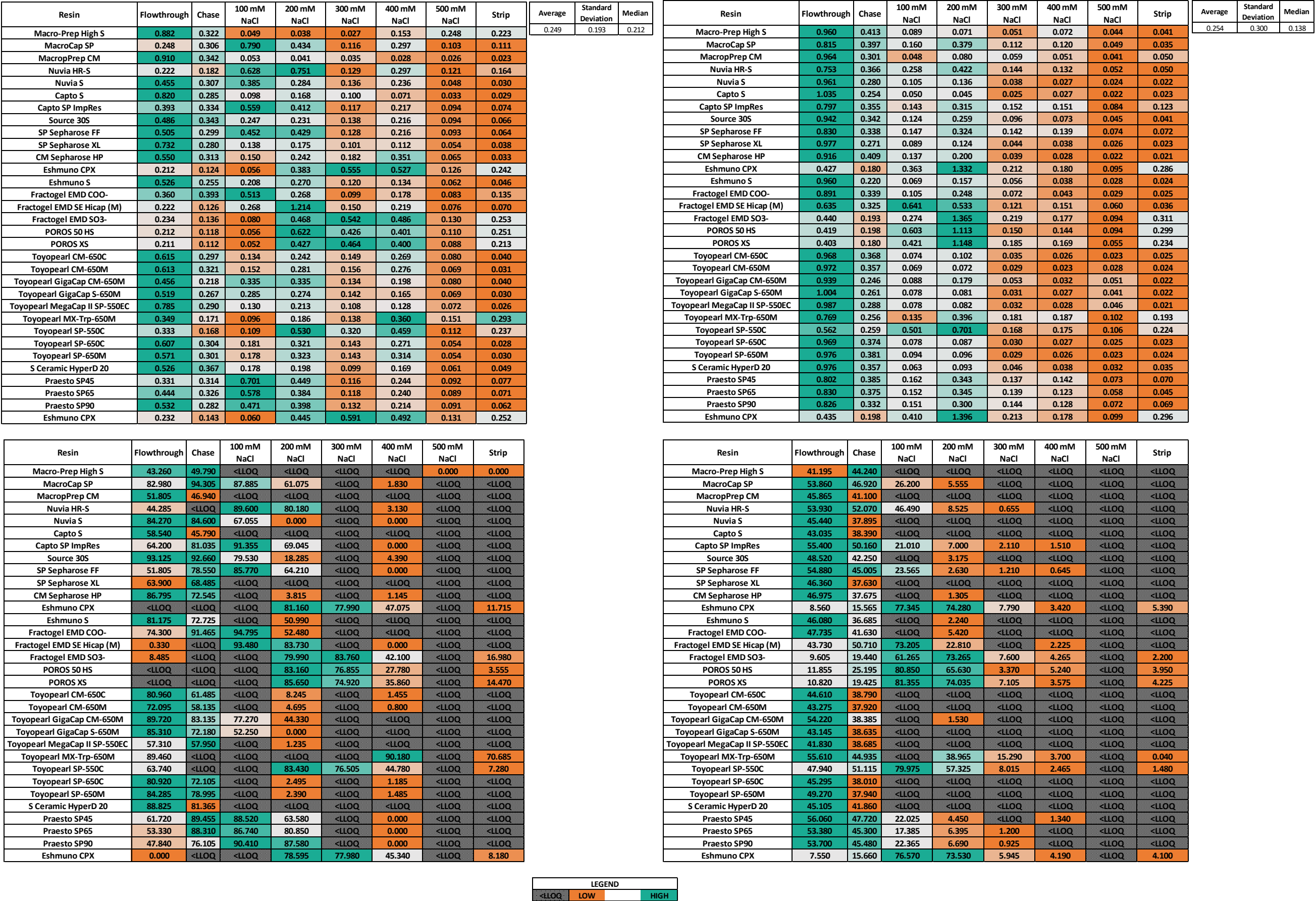


Figure S3

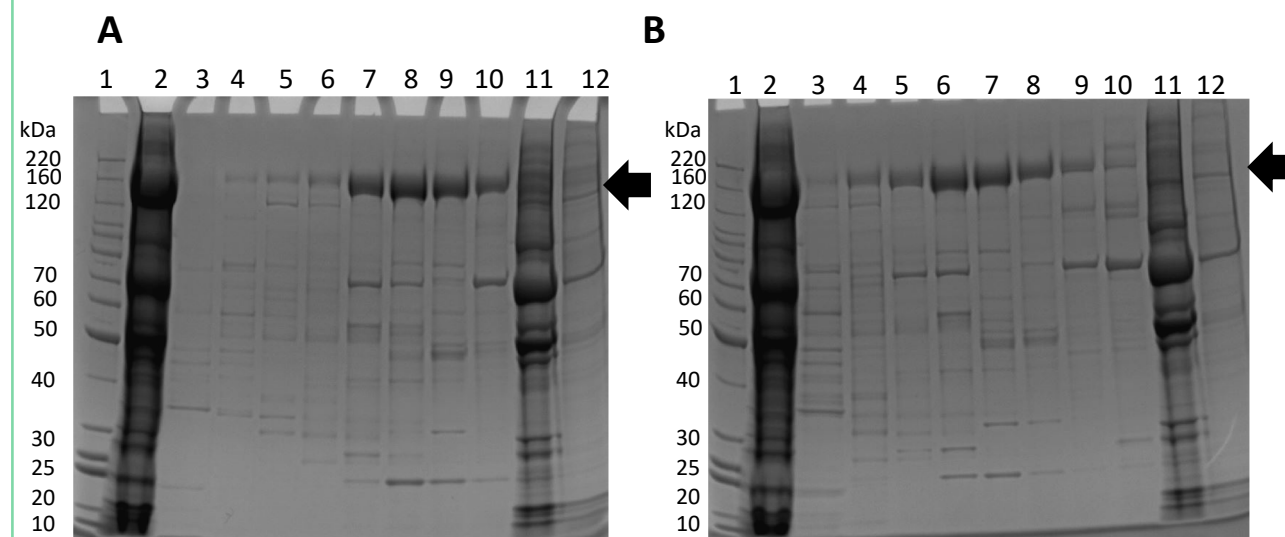


Figure S3. AEX Capture Step Buffer Selection SDS-PAGE. 25 mM MES pH 6.5 running buffer (A) and 25 mM Sodium Phosphate, pH 6.5 buffer (B), where well 1 is the benchmark protein ladder, well 2 is the TFF retentate (POROS 50 D load), well 3 is the flowthrough fraction, wells 4 through 11 are wash fractions in running buffer with 50 mM NaCl (4), 75 mM NaCl (5), 100 mM NaCl (6), 125 mM NaCl (7), 150 mM NaCl (8), 175 mM NaCl (9), 200 mM NaCl (10), and 500 mM NaCl (11), and the 1M NaCl strip in running buffer is well 12.

Figure S4

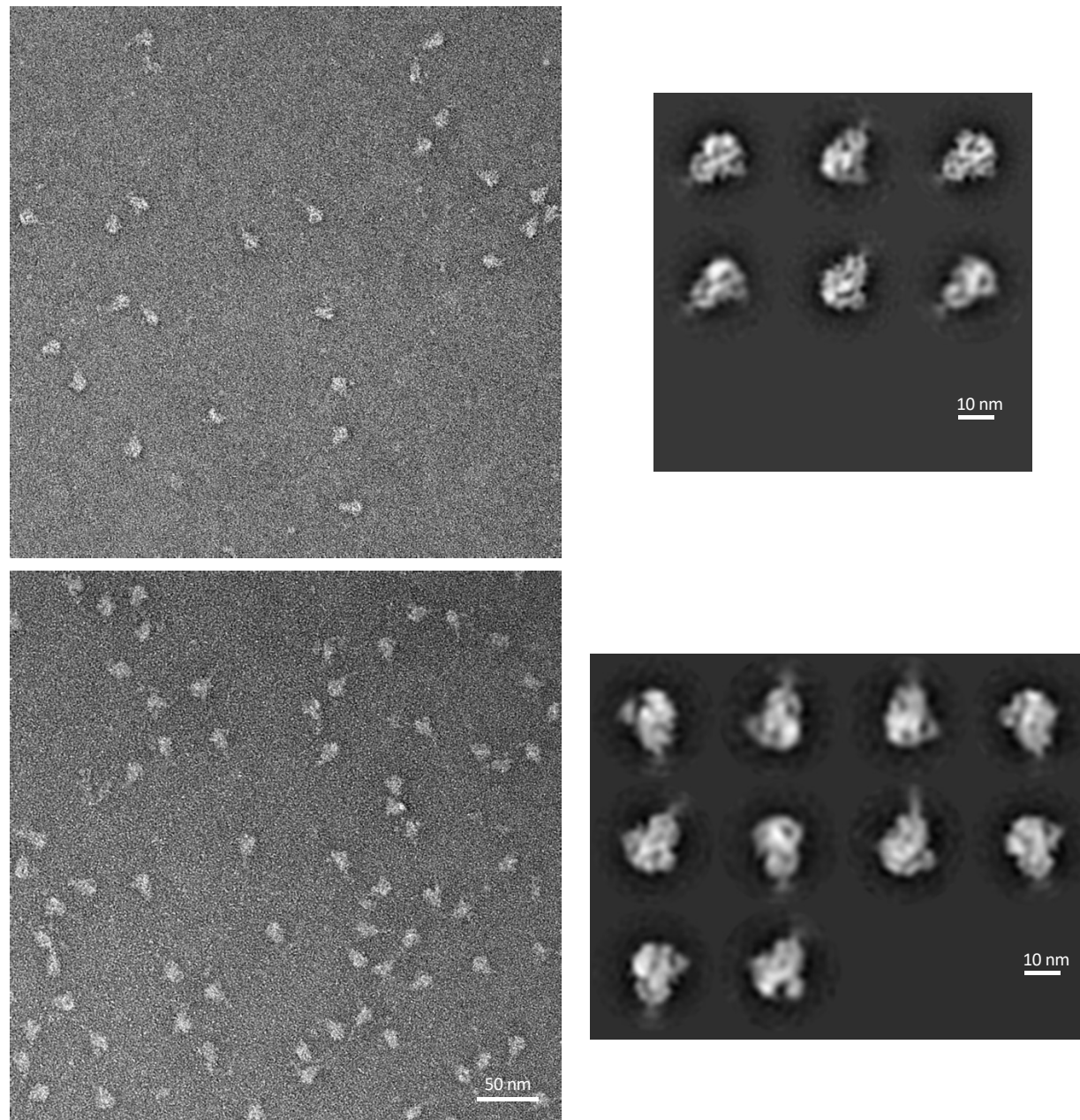


Figure S4. Cation Exchange proof of concept NS-EM. Top row: SP650M flow through. Bottom row: Nuvia HR-S elution. Left column: representative images at 100,000x. Right column: 2D Classes.

Figure S5

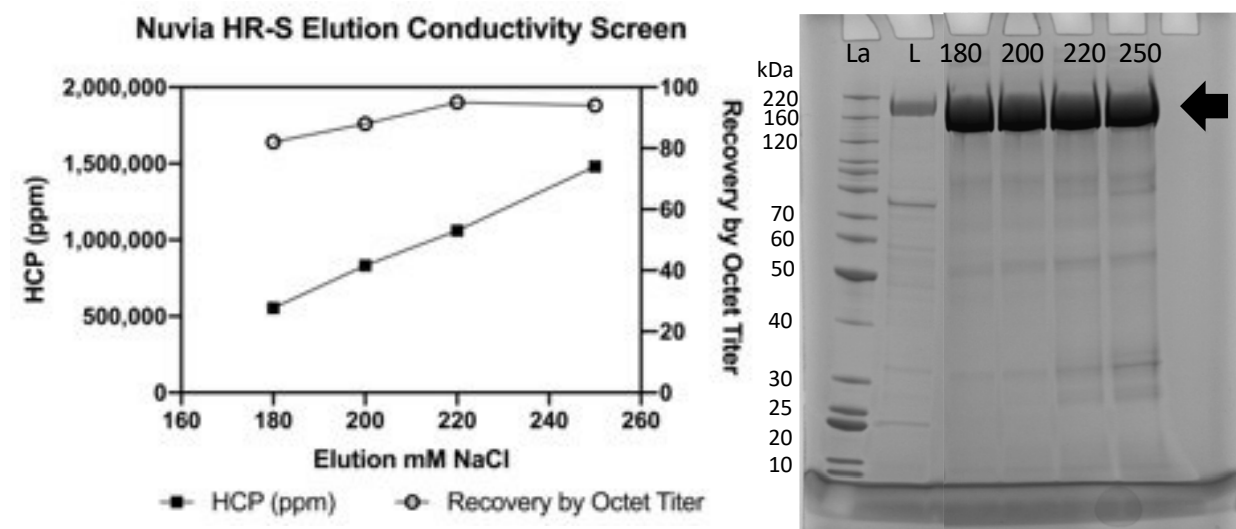


Figure S5. Nuvia HR-S Elution Buffer Selection. Left: HCP (ppm) (closed square, left axis) and Recovery by Octet Titer (open circle, right axis) are plotted against Elution mM NaCl ranging from 180 mM NaCl to 250 mM NaCl. Right: Samples are assessed by SDS-PAGE. La: ladder, L: load; numbers represent mM NaCl elution.

Table S1

Unit Operation	Operation Step	Purification Process
Clarification	Feed	6.5 L Cell culture harvest
	Filter Train	0.11 m ² Clarisolve 20MS, 0.11 m ² Millistak+ F0HC, 0.26 m ² 0.8/0.2 µm sterile filter
	Flux	60 LMH (based on F0HC area)
TFF1	Load Material	Clarified cell culture harvest
	Filter area	0.5 m ²
	MWCO	300 kDa
	Feed flux	330 LMH
	TMP	10 psi
	Equilibration buffer	25 mM MES, 25 mM NaCl pH 6.5
	Ultrafiltration and Diafiltration	5X/5X
	Diafiltration buffer	25 mM MES, 25 mM NaCl pH 6.5
AEX 1	Resin	POROS 50 D
	Load Material	TFF1 Retentate & chase pool, 0.8/0.2 µm filtered
	Loading capacity	20-25 mg/mL-r (total protein by A280)
	Equilibration buffer	25 mM MES, 25 mM NaCl pH 6.5
	Wash buffer	25 mM MES, 100 mM NaCl pH 6.5
	Elution buffer	25 mM MES, 190 mM NaCl pH 6.5
Low pH treatment	Conditioning solution	5 N HCl
	Hold time	60 minutes
CEX 1	Resin	Toyopearl SP-650M
	Load Material	Conditioned, low pH treated capture step eluate
	Conditioning solution	50 mM Sodium Citrate pH 4.0
	Loading capacity	< 15 mg/mL-r (total protein by A280)
	Equilibration & chase buffer	50 mM Sodium Citrate, 50 mM NaCl pH 4.0
CEX 2	Resin	Nuvia HR-S
	Load Material	SP-650M flow through and chase
	Loading capacity	30 mg/mL-r (total protein by A280)
	Equilibration buffer	50 mM Sodium Citrate, 50 mM NaCl pH 4.0
	Wash buffer	50 mM Sodium Citrate, 50 mM NaCl pH 4.0
	Elution buffer	50 mM Sodium Citrate, 180 mM NaCl pH 4.0
20 nm Filtration	Load Material	Nuvia HR-S eluate
	Pre-Filter	Viresolve Shield
	20 nm Filter	Viresolve Pro
	Loading capacity	< 10 g/m ²
TFF2	Load Material	20 nm filtrate
	Loading capacity	15 g/m ²
	Feed flux	300 LMH
	MWCO	100 kDa
	TMP	7.3 psi
	Equilibration buffer	50 mM Sodium Citrate, 180 mM NaCl pH 4.0
	Diafiltration buffer	10 mM Histidine, 150 mM NaCl, 5% (w/v) Sucrose pH 5.5
	Ultrafiltration and Diafiltration	2X./20X

Table S1. Process Parameters. Specific process parameters for each unit operation of the HexaPro proof of concept are listed.