Supporting Information

How the Conformations of an Internal Junction Contribute to Fold an RNA Domain

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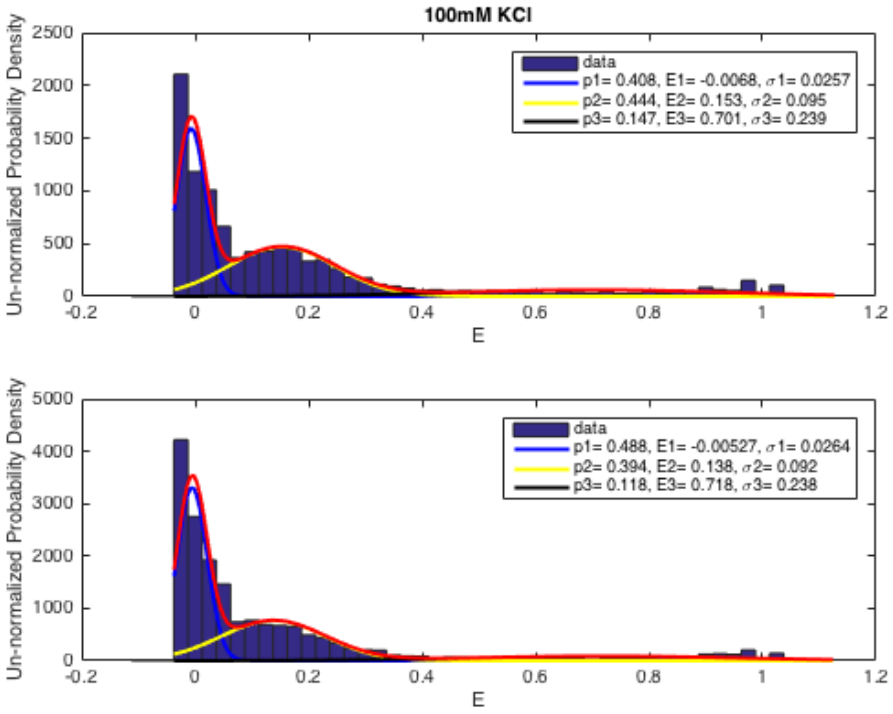
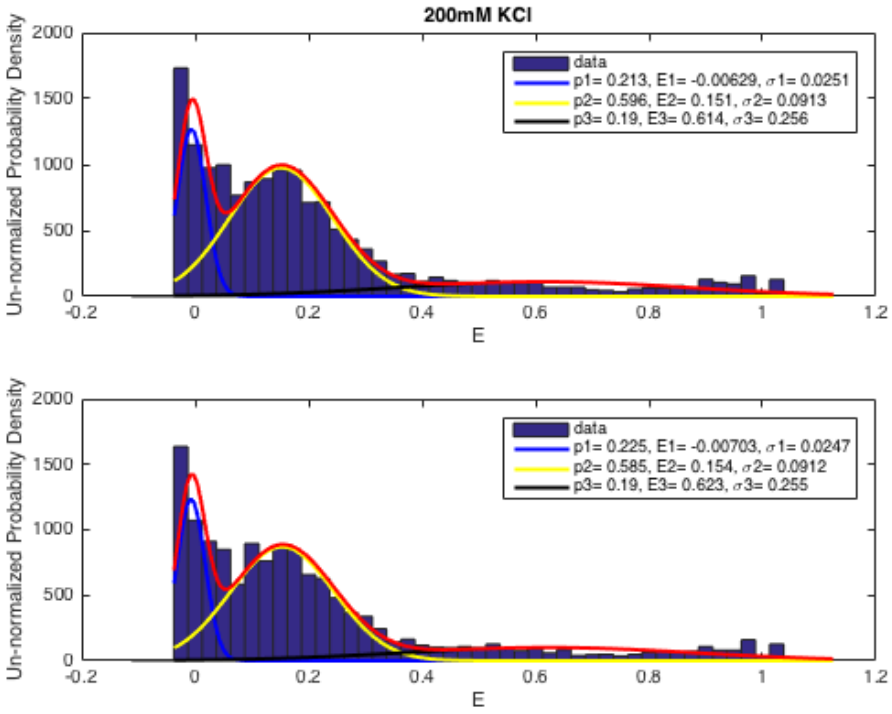
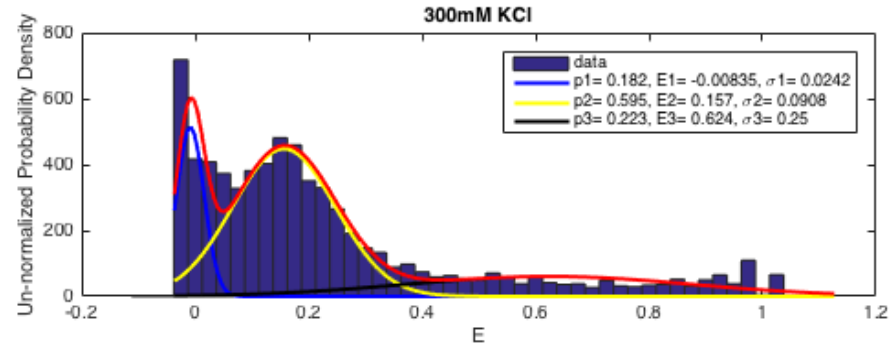
FRET Histograms and E_{FRET} Data

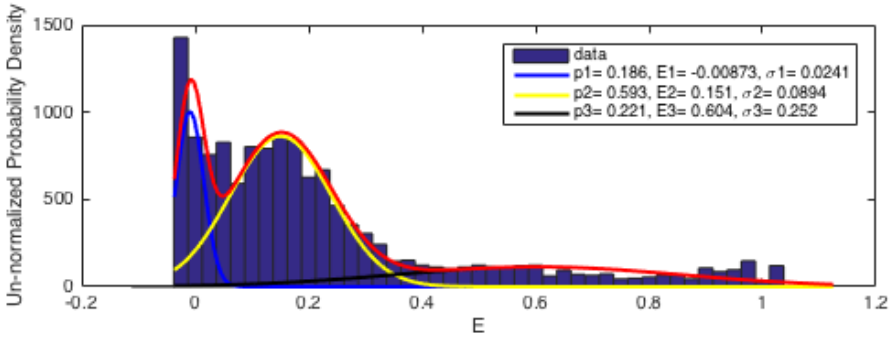
The following figures show the raw FRET data for conditions described in the text. Because the figures contain many panels, the caption is given at the start (top) of each series.

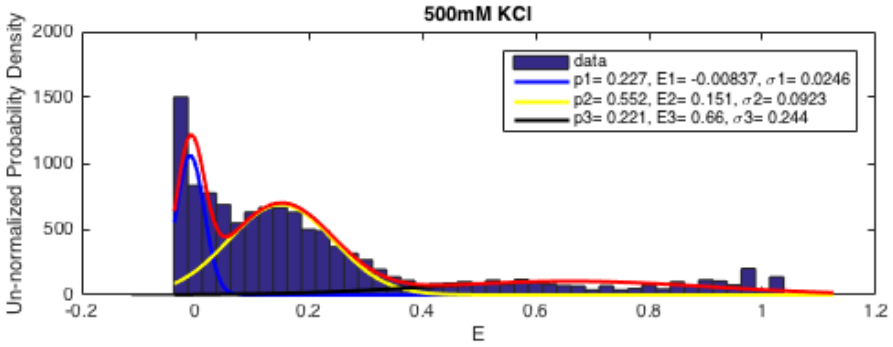
Figure S1 (below). FRET Histograms for H-JU_4U_5-H, D1-A (end labeled) in KCl solutions.

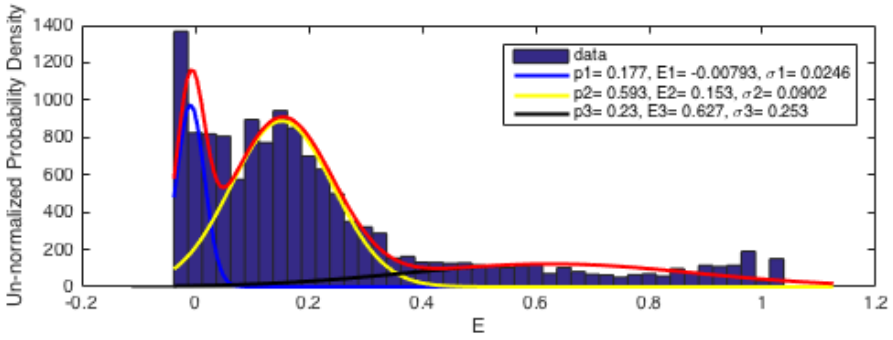



200mM KCl
Un-normalized Probability Density
E
data
p1= 0.213, E1= -0.00629, σ1= 0.0251
p2= 0.596, E2= 0.151, σ2= 0.0913
p3= 0.19, E3= 0.614, σ3= 0.256
Un-normalized Probability Density
E
data
p1= 0.225, E1= -0.00703, σ1= 0.0247
p2= 0.585, E2= 0.154, σ2= 0.0912
p3= 0.19, E3= 0.623, σ3= 0.255


300mM KCl
Un-normalized Probability Density
E
data
p1= 0.182, E1= -0.00835, σ1= 0.0242
p2= 0.595, E2= 0.157, σ2= 0.0908
p3= 0.223, E3= 0.624, σ3= 0.25


Un-normalized Probability Density
E
data
p1= 0.186, E1= -0.00873, σ1= 0.0241
p2= 0.593, E2= 0.151, σ2= 0.0894
p3= 0.221, E3= 0.604, σ3= 0.252


500mM KCl
Un-normalized Probability Density
E
data
p1= 0.227, E1= -0.00837, σ1= 0.0246
p2= 0.552, E2= 0.151, σ2= 0.0923
p3= 0.221, E3= 0.66, σ3= 0.244


Un-normalized Probability Density
E
data
p1= 0.177, E1= -0.00793, σ1= 0.0246
p2= 0.593, E2= 0.153, σ2= 0.0902
p3= 0.23, E3= 0.627, σ3= 0.253

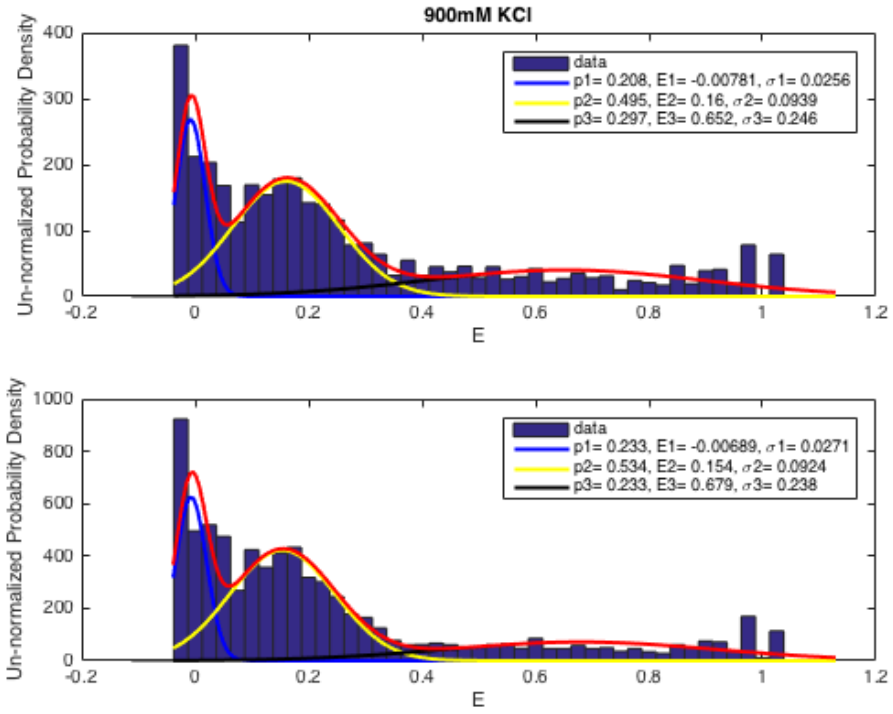
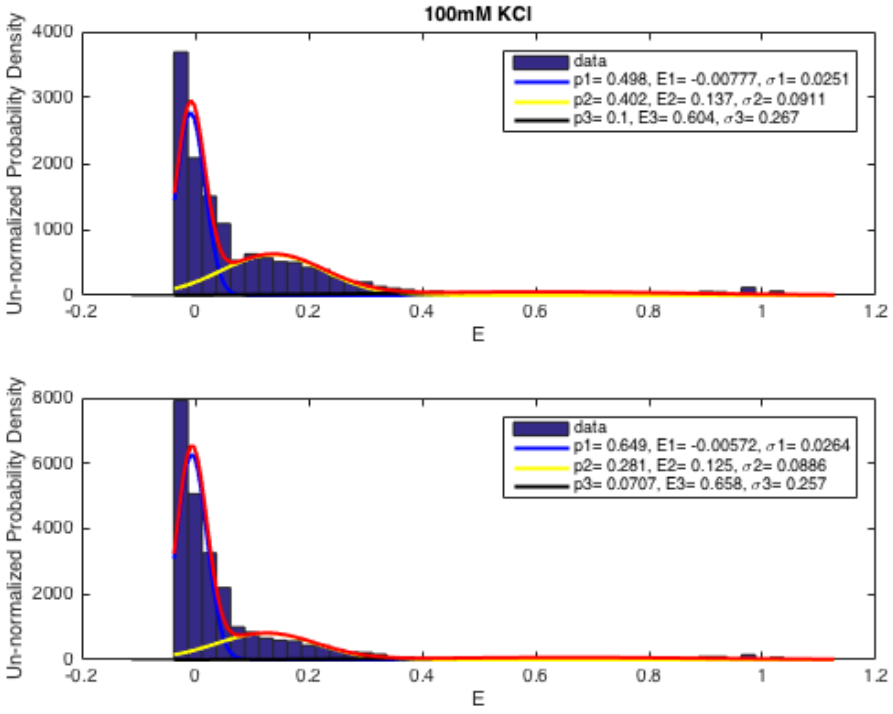
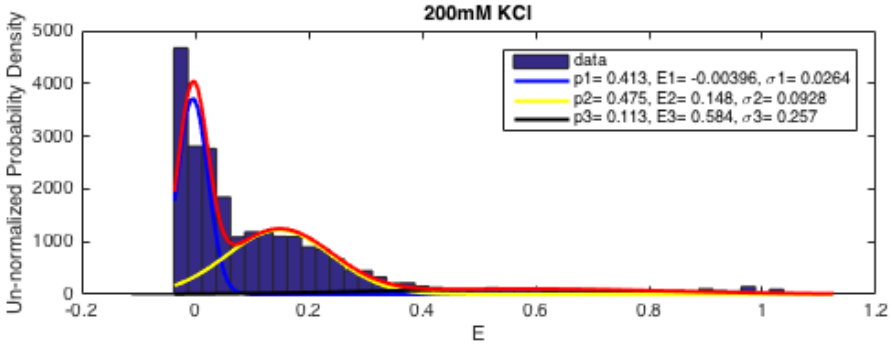
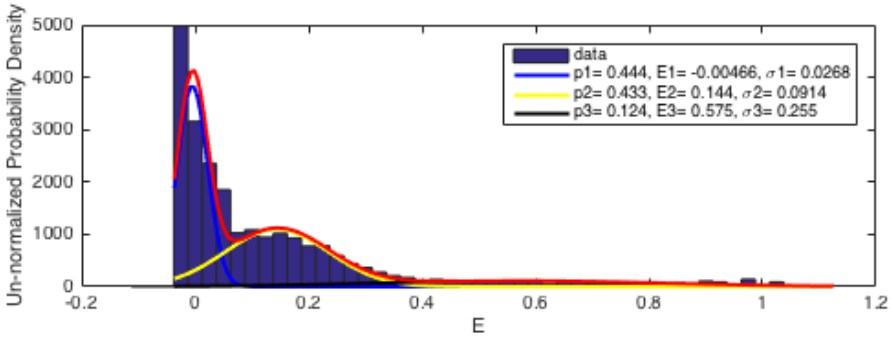
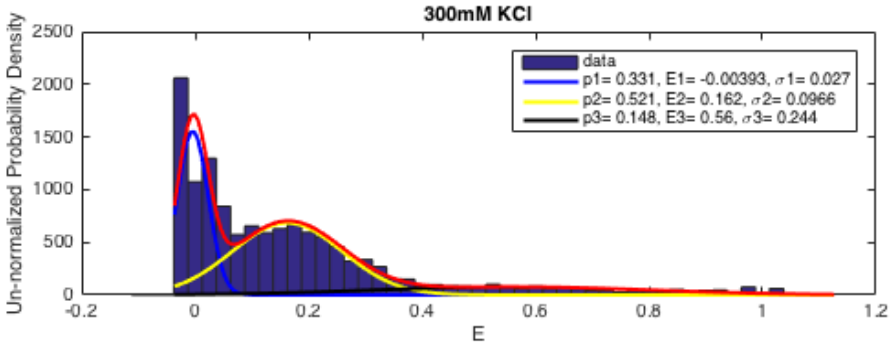


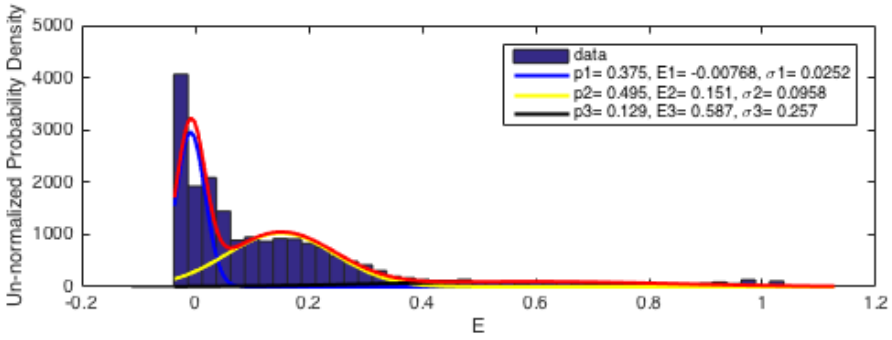
Figure S2 (below). FRET Histograms for H-J5/5a-H, D1-A (end labeled) in KCl solutions.

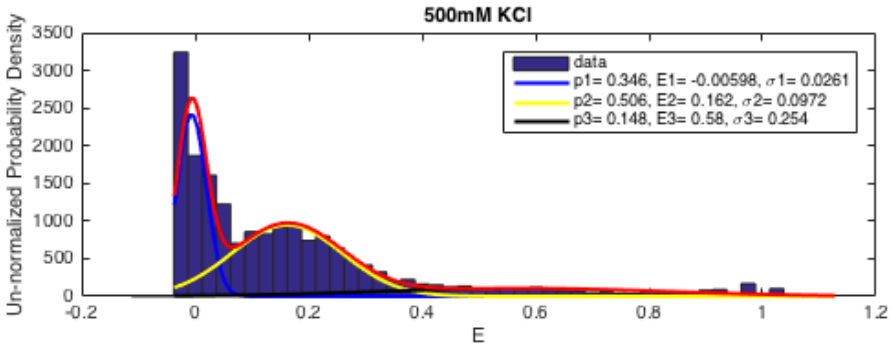


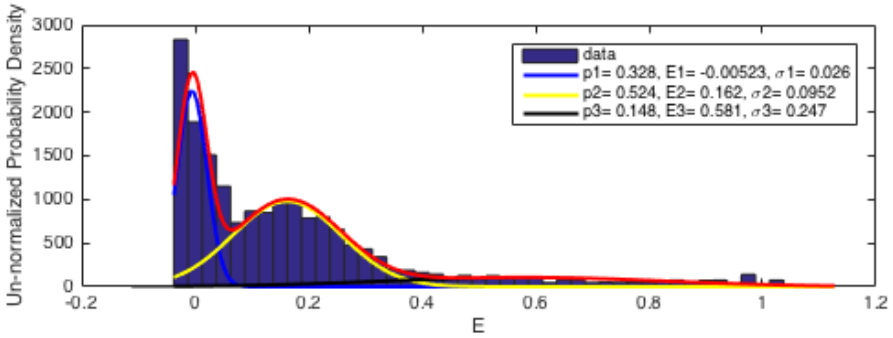

200mM KCl
Un-normalized Probability Density
E
data
p1= 0.413, E1= -0.00396, σ1= 0.0264
p2= 0.475, E2= 0.148, σ2= 0.0928
p3= 0.113, E3= 0.584, σ3= 0.257

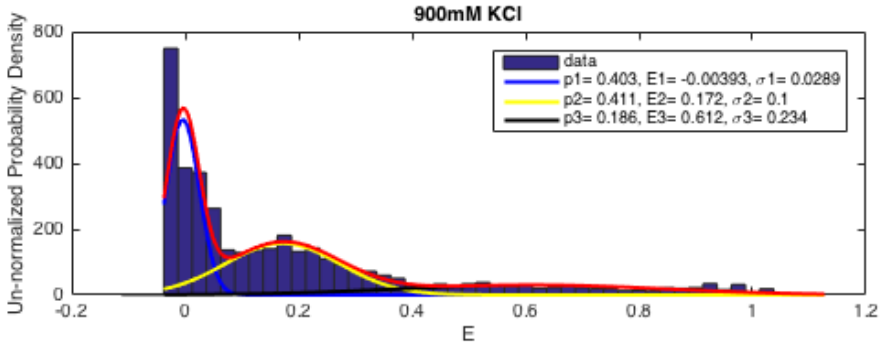

Un-normalized Probability Density
E
data
p1= 0.444, E1= -0.00466, σ1= 0.0268
p2= 0.433, E2= 0.144, σ2= 0.0914
p3= 0.124, E3= 0.575, σ3= 0.255


300mM KCl
Un-normalized Probability Density
E
data
p1= 0.331, E1= -0.00393, σ1= 0.027
p2= 0.521, E2= 0.162, σ2= 0.0966
p3= 0.148, E3= 0.56, σ3= 0.244


Un-normalized Probability Density
E
data
p1= 0.375, E1= -0.00768, σ1= 0.0252
p2= 0.495, E2= 0.151, σ2= 0.0958
p3= 0.129, E3= 0.587, σ3= 0.257


500mM KCl
Un-normalized Probability Density
E
data
p1= 0.346, E1= -0.00598, σ1= 0.0261
p2= 0.506, E2= 0.162, σ2= 0.0972
p3= 0.148, E3= 0.58, σ3= 0.254


Un-normalized Probability Density
E
data
p1= 0.328, E1= -0.00523, σ1= 0.026
p2= 0.524, E2= 0.162, σ2= 0.0952
p3= 0.148, E3= 0.581, σ3= 0.247


900mM KCl
Un-normalized Probability Density
E
data
p1= 0.403, E1= -0.00393, σ1= 0.0289
p2= 0.411, E2= 0.172, σ2= 0.1
p3= 0.186, E3= 0.612, σ3= 0.234

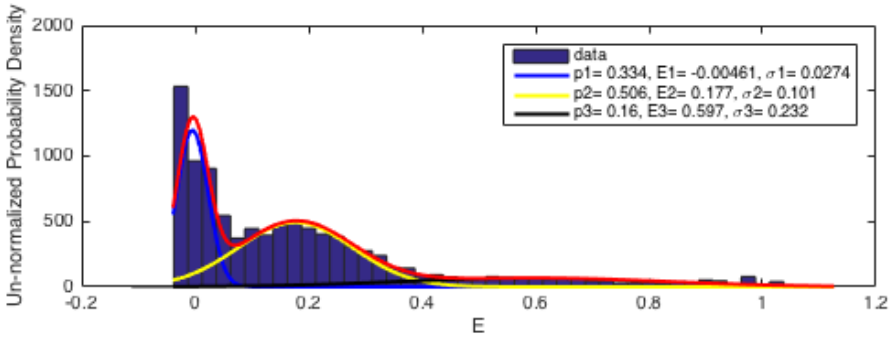
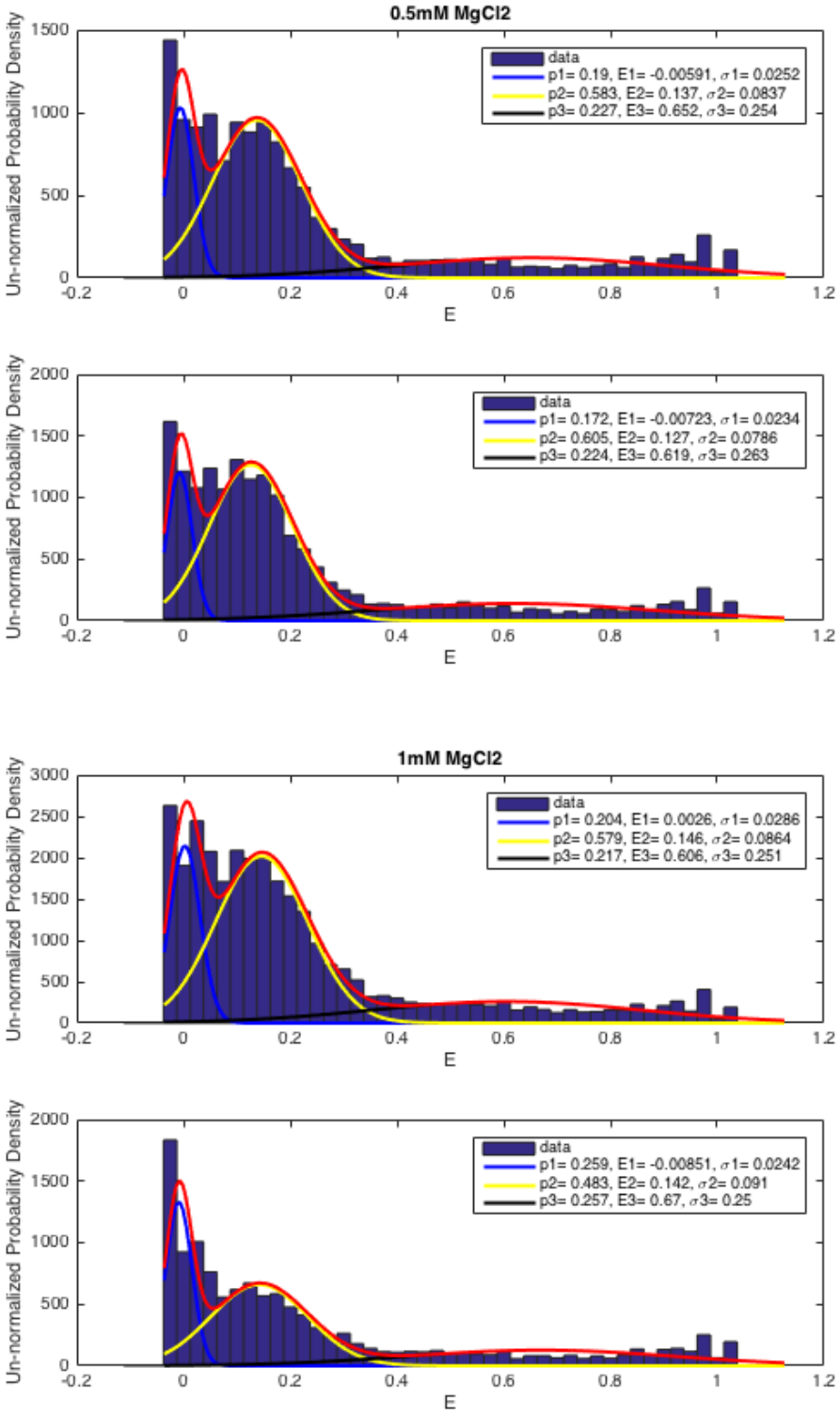
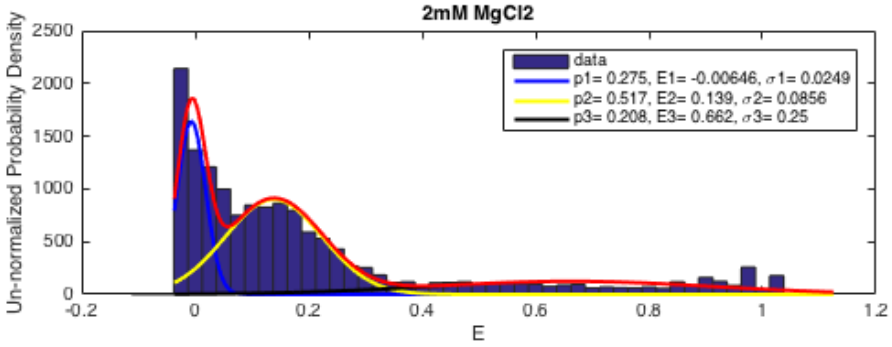
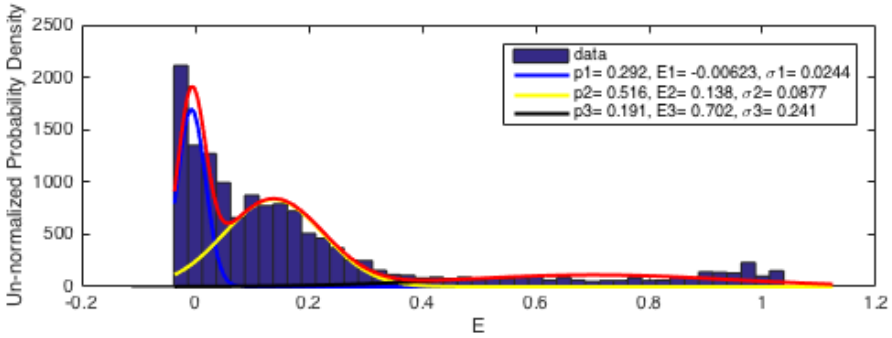
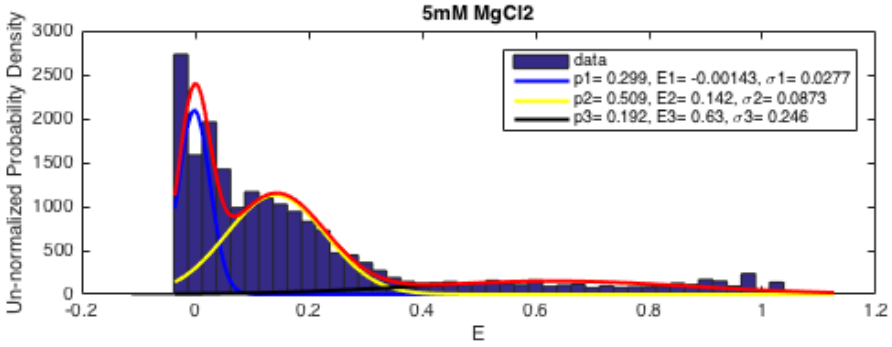

Un-normalized Probability Density
E
data
p1= 0.334, E1= -0.00461, σ1= 0.0274
p2= 0.506, E2= 0.177, σ2= 0.101
p3= 0.16, E3= 0.597, σ3= 0.232

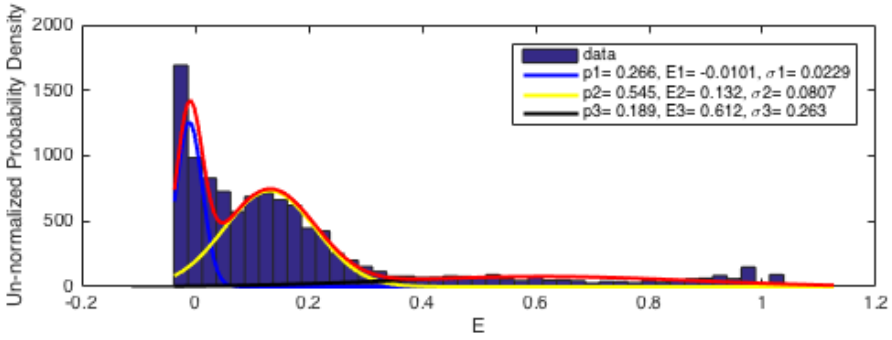
Figure S3 (below). FRET Histograms for H-JU_4U_5-H, D1-A (end labeled) in $MgCl_2$ solutions.

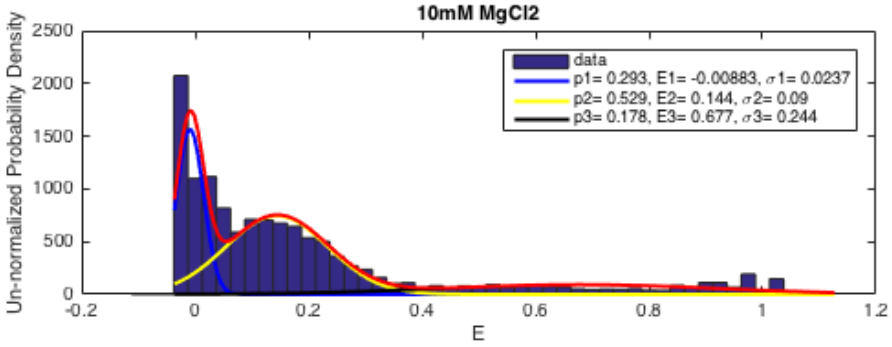


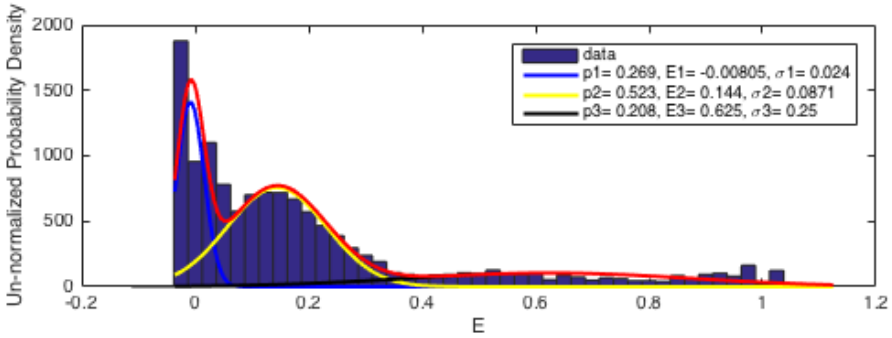

2mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.275, E1= -0.00646, σ1= 0.0249
p2= 0.517, E2= 0.139, σ2= 0.0856
p3= 0.208, E3= 0.662, σ3= 0.25

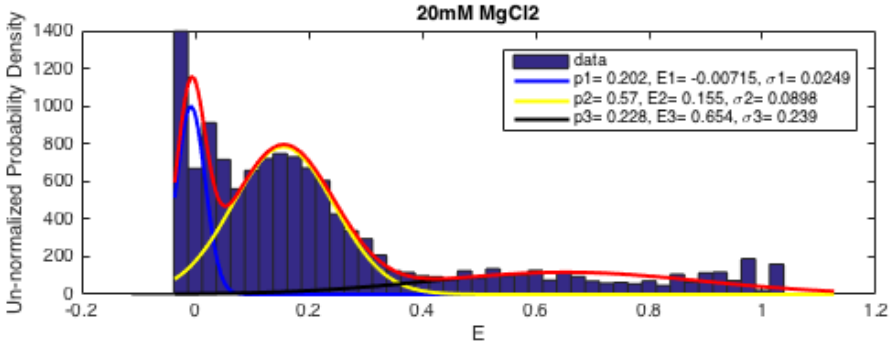

Un-normalized Probability Density
E
data
p1= 0.292, E1= -0.00623, σ1= 0.0244
p2= 0.516, E2= 0.138, σ2= 0.0877
p3= 0.191, E3= 0.702, σ3= 0.241

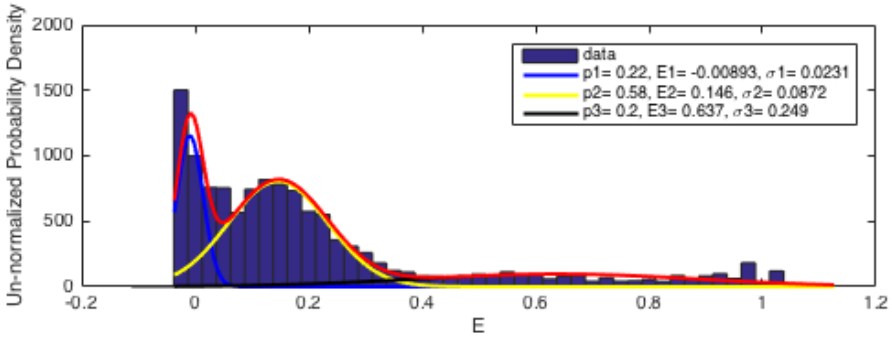

5mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.299, E1= -0.00143, σ1= 0.0277
p2= 0.509, E2= 0.142, σ2= 0.0873
p3= 0.192, E3= 0.63, σ3= 0.246

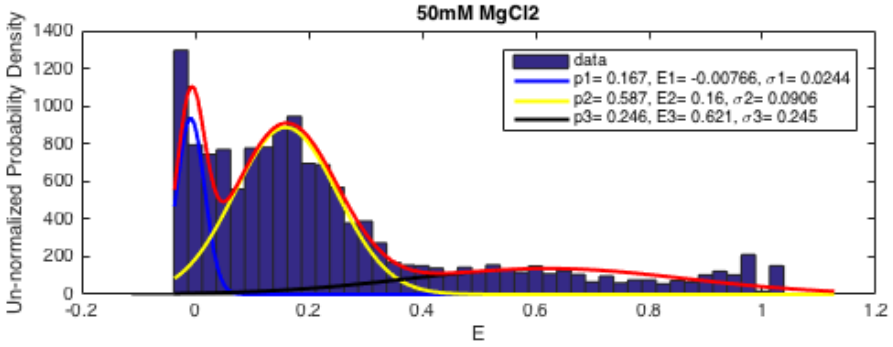

Un-normalized Probability Density
E
data
p1= 0.266, E1= -0.0101, σ1= 0.0229
p2= 0.545, E2= 0.132, σ2= 0.0807
p3= 0.189, E3= 0.612, σ3= 0.263

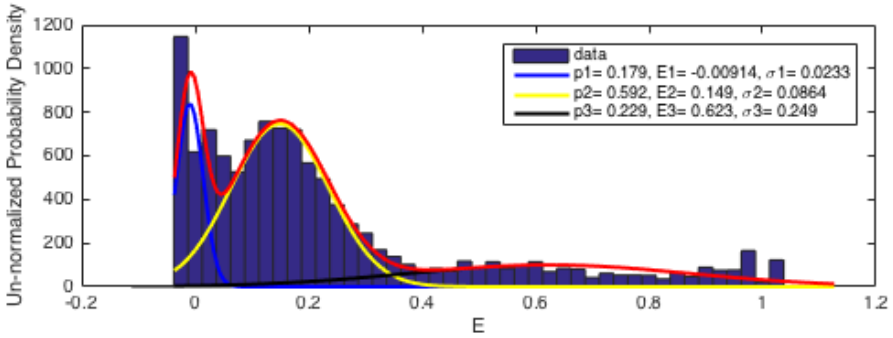

10mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.293, E1= -0.00883, σ1= 0.0237
p2= 0.529, E2= 0.144, σ2= 0.09
p3= 0.178, E3= 0.677, σ3= 0.244

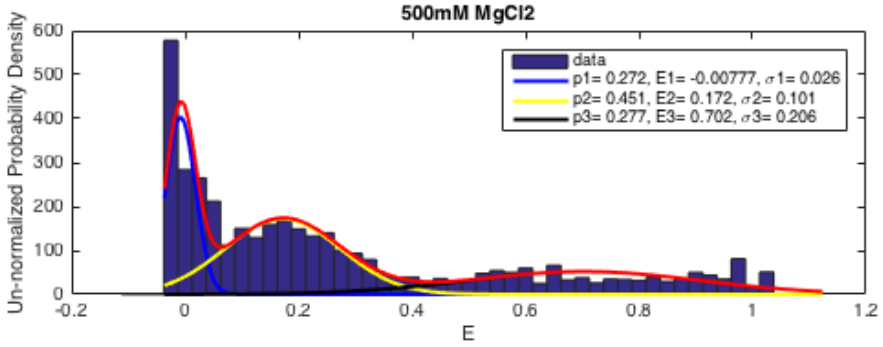

Un-normalized Probability Density
E
data
p1= 0.269, E1= -0.00805, σ1= 0.024
p2= 0.523, E2= 0.144, σ2= 0.0871
p3= 0.208, E3= 0.625, σ3= 0.25


20mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.202, E1= -0.00715, σ1= 0.0249
p2= 0.57, E2= 0.155, σ2= 0.0898
p3= 0.228, E3= 0.654, σ3= 0.239


Un-normalized Probability Density
E
data
p1= 0.22, E1= -0.00893, σ1= 0.0231
p2= 0.58, E2= 0.146, σ2= 0.0872
p3= 0.2, E3= 0.637, σ3= 0.249


50mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.167, E1= -0.00766, σ1= 0.0244
p2= 0.587, E2= 0.16, σ2= 0.0906
p3= 0.246, E3= 0.621, σ3= 0.245


Un-normalized Probability Density
E
data
p1= 0.179, E1= -0.00914, σ1= 0.0233
p2= 0.592, E2= 0.149, σ2= 0.0864
p3= 0.229, E3= 0.623, σ3= 0.249


500mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.272, E1= -0.00777, σ1= 0.026
p2= 0.451, E2= 0.172, σ2= 0.101
p3= 0.277, E3= 0.702, σ3= 0.206

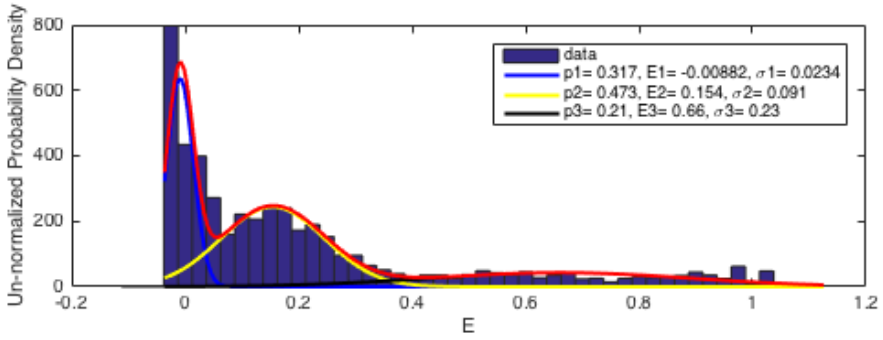
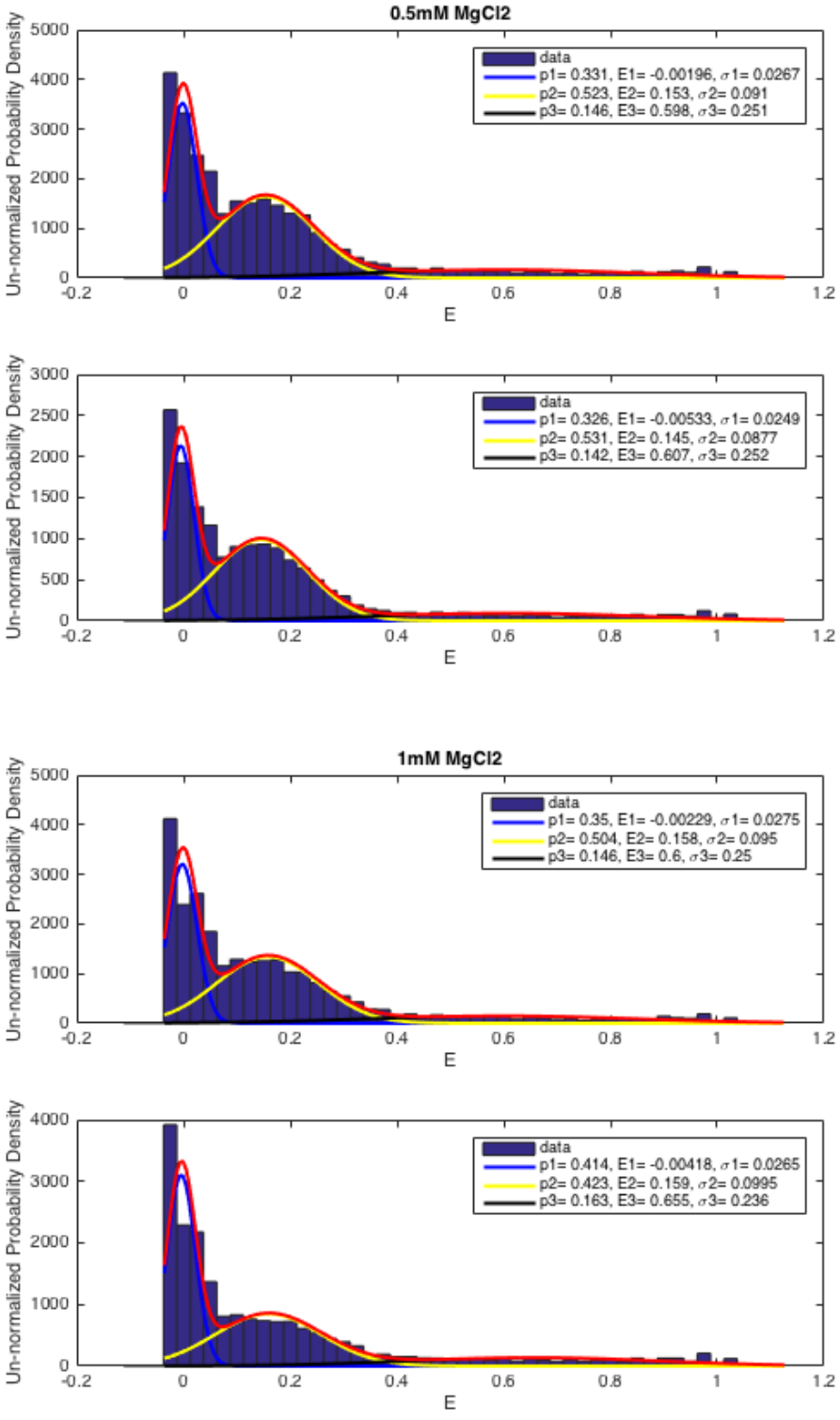
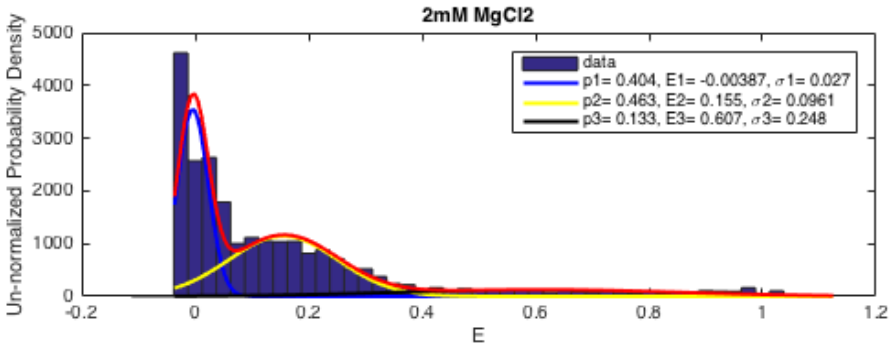
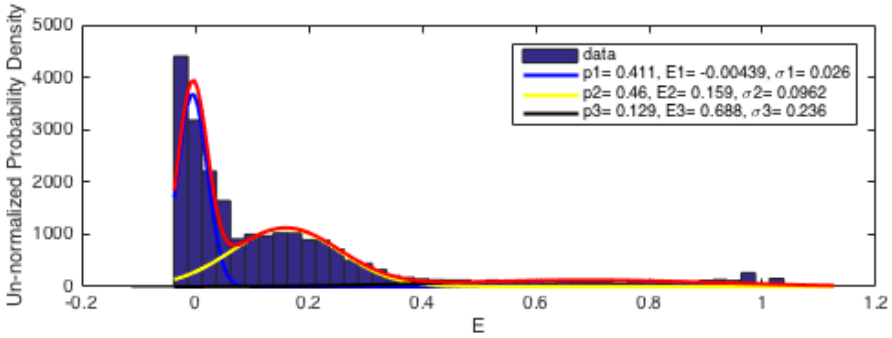
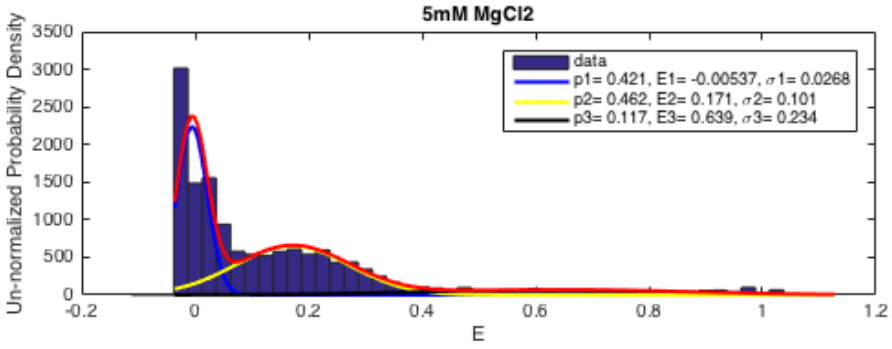

Un-normalized Probability Density
E
data
p1= 0.317, E1= -0.00882, σ1= 0.0234
p2= 0.473, E2= 0.154, σ2= 0.091
p3= 0.21, E3= 0.66, σ3= 0.23

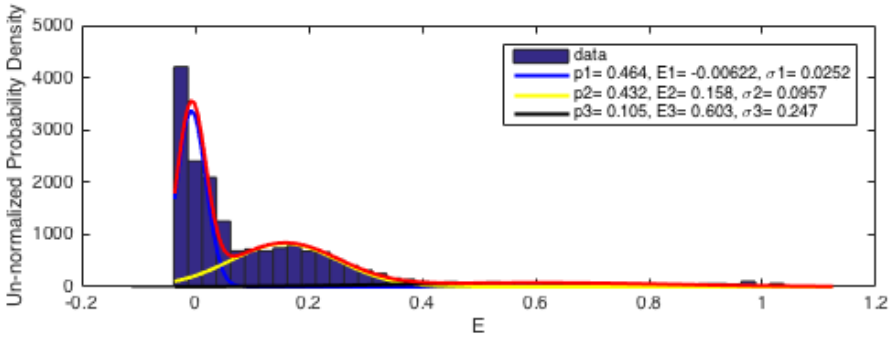
Figure S4 (below). FRET Histograms for H-J5/5a-H, D1-A (end labeled) in $MgCl_2$ solutions.

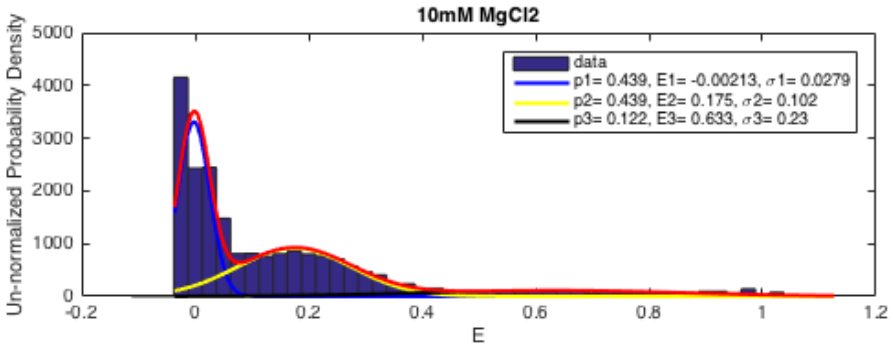


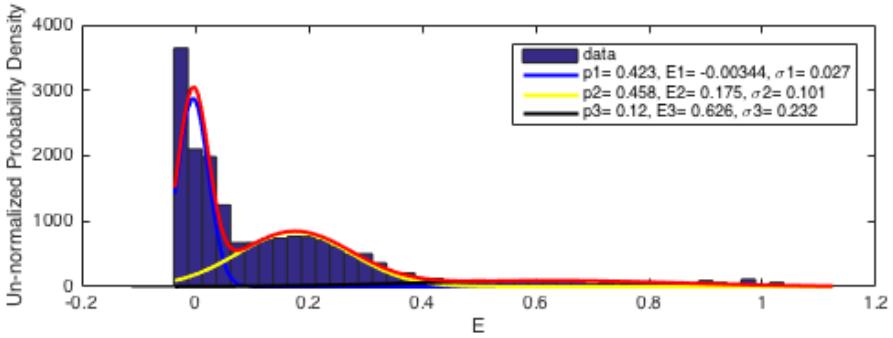

2mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.404, E1= -0.00387, σ1= 0.027
p2= 0.463, E2= 0.155, σ2= 0.0961
p3= 0.133, E3= 0.607, σ3= 0.248

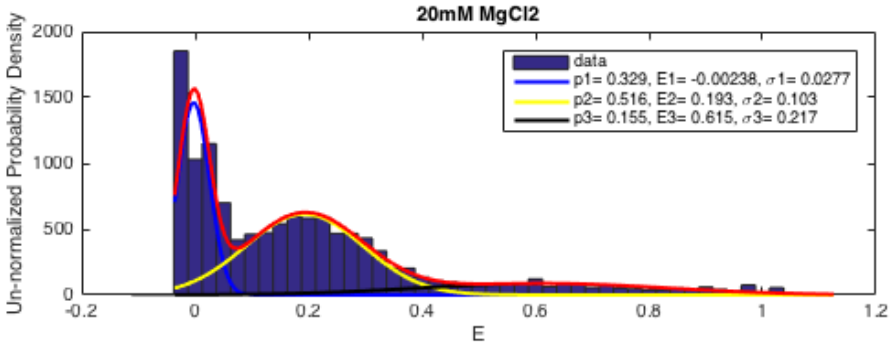

Un-normalized Probability Density
E
data
p1= 0.411, E1= -0.00439, σ1= 0.026
p2= 0.46, E2= 0.159, σ2= 0.0962
p3= 0.129, E3= 0.688, σ3= 0.236

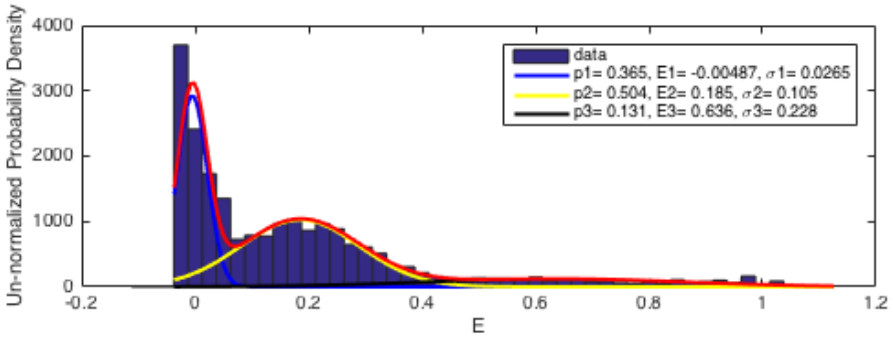

5mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.421, E1= -0.00537, σ1= 0.0268
p2= 0.462, E2= 0.171, σ2= 0.101
p3= 0.117, E3= 0.639, σ3= 0.234

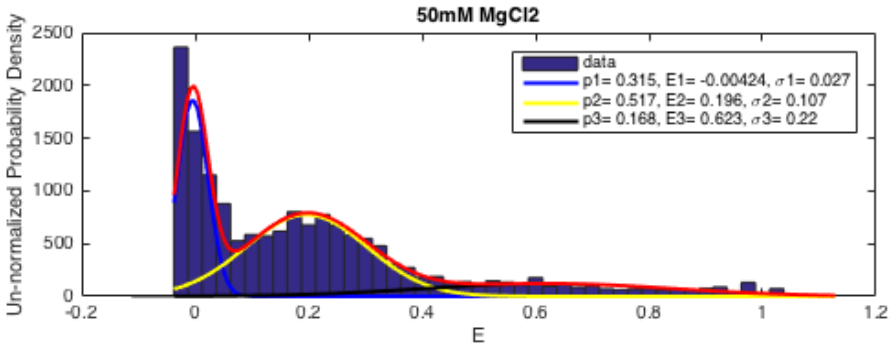

Un-normalized Probability Density
E
data
p1= 0.464, E1= -0.00622, σ1= 0.0252
p2= 0.432, E2= 0.158, σ2= 0.0957
p3= 0.105, E3= 0.603, σ3= 0.247

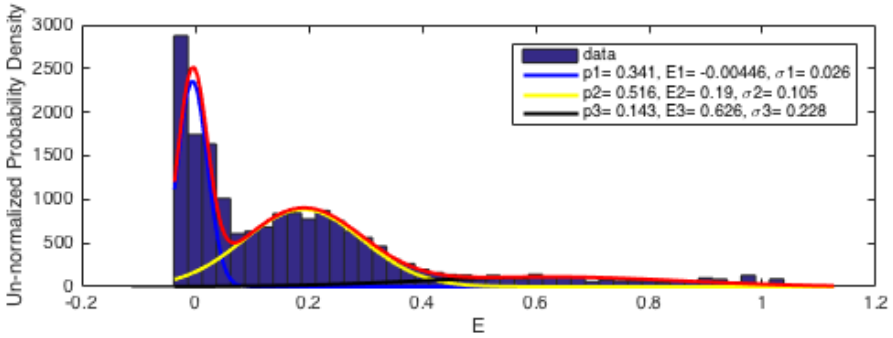

10mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.439, E1= -0.00213, σ1= 0.0279
p2= 0.439, E2= 0.175, σ2= 0.102
p3= 0.122, E3= 0.633, σ3= 0.23

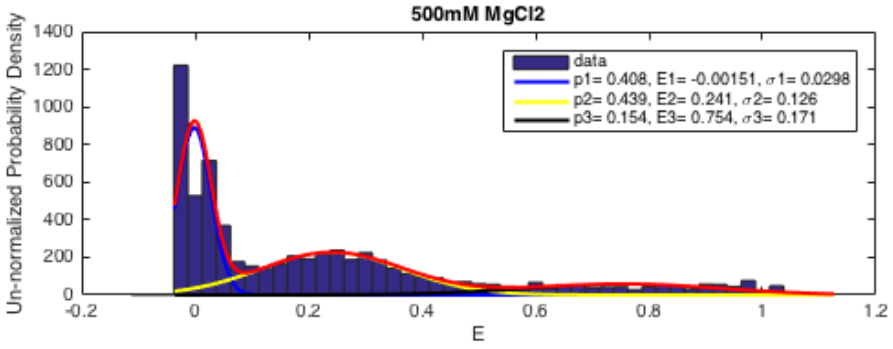

Un-normalized Probability Density
E
data
p1= 0.423, E1= -0.00344, σ1= 0.027
p2= 0.458, E2= 0.175, σ2= 0.101
p3= 0.12, E3= 0.626, σ3= 0.232


20mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.329, E1= -0.00238, σ1= 0.0277
p2= 0.516, E2= 0.193, σ2= 0.103
p3= 0.155, E3= 0.615, σ3= 0.217


Un-normalized Probability Density
E
data
p1= 0.365, E1= -0.00487, σ1= 0.0265
p2= 0.504, E2= 0.185, σ2= 0.105
p3= 0.131, E3= 0.636, σ3= 0.228


50mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.315, E1= -0.00424, σ1= 0.027
p2= 0.517, E2= 0.196, σ2= 0.107
p3= 0.168, E3= 0.623, σ3= 0.22


Un-normalized Probability Density
E
data
p1= 0.341, E1= -0.00446, σ1= 0.026
p2= 0.516, E2= 0.19, σ2= 0.105
p3= 0.143, E3= 0.626, σ3= 0.228


500mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.408, E1= -0.00151, σ1= 0.0298
p2= 0.439, E2= 0.241, σ2= 0.126
p3= 0.154, E3= 0.754, σ3= 0.171

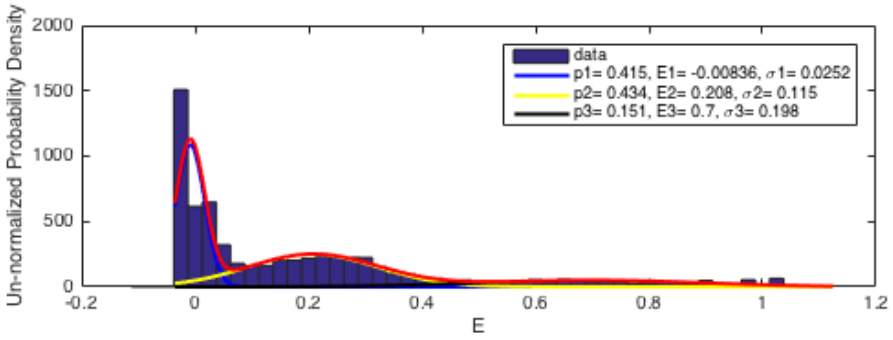
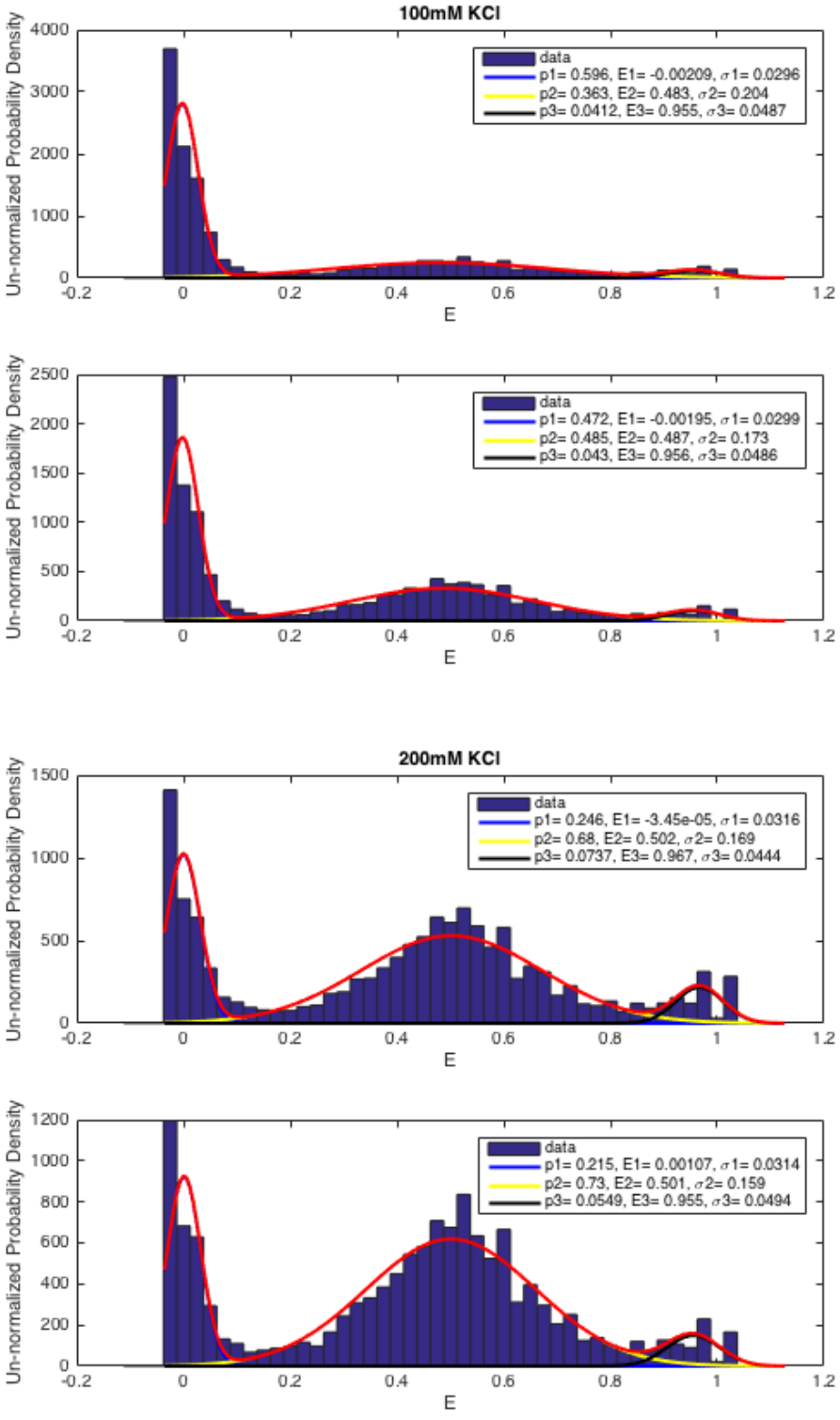
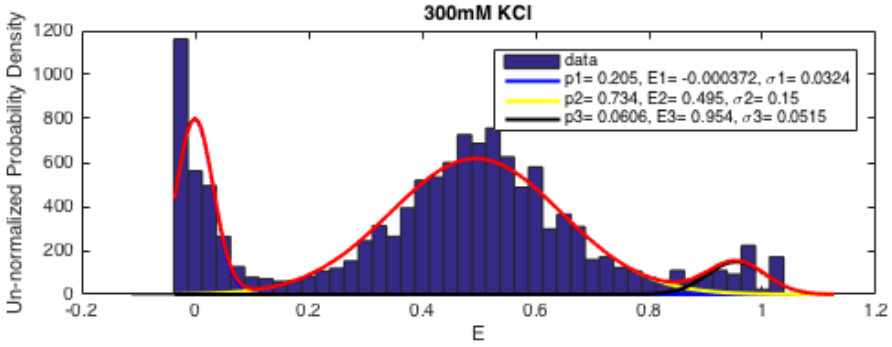
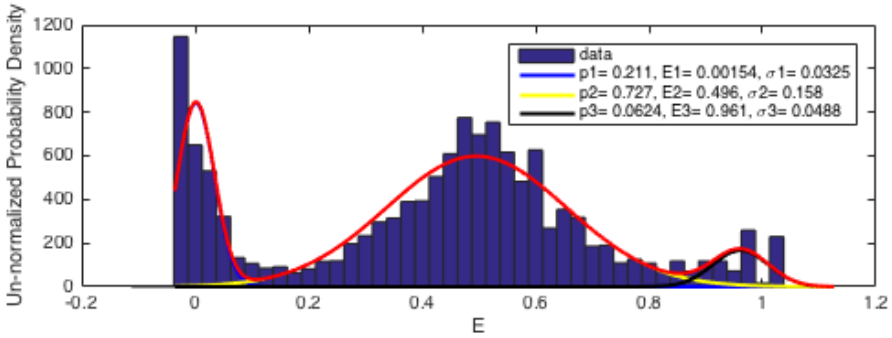
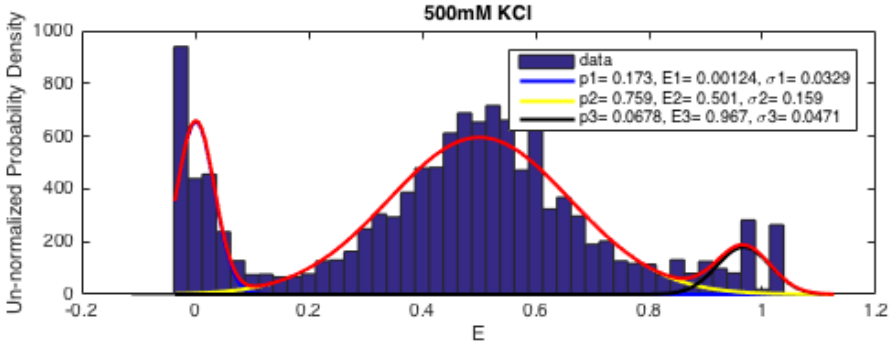

Un-normalized Probability Density
E
data
p1= 0.415, E1= -0.00836, σ1= 0.0252
p2= 0.434, E2= 0.208, σ2= 0.115
p3= 0.151, E3= 0.7, σ3= 0.198

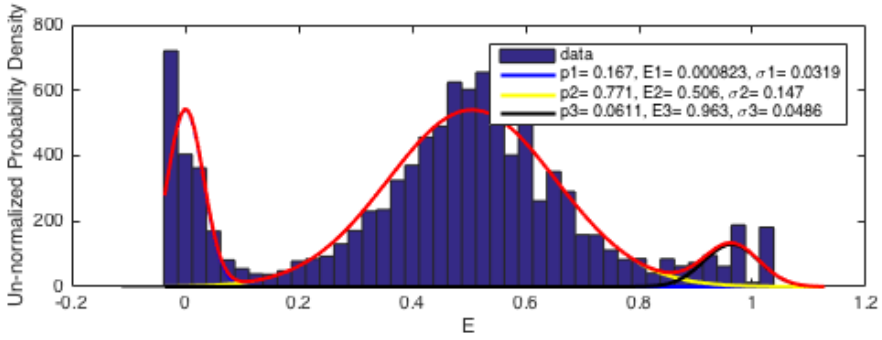
Figure S5 (below). FRET Histograms for H-JU_4U_5-H, D2-A (side labeled) in KCl solutions.




300mM KCl
Un-normalized Probability Density
E
data
p1= 0.205, E1= -0.000372, σ1= 0.0324
p2= 0.734, E2= 0.495, σ2= 0.15
p3= 0.0606, E3= 0.954, σ3= 0.0515


Un-normalized Probability Density
E
data
p1= 0.211, E1= 0.00154, σ1= 0.0325
p2= 0.727, E2= 0.496, σ2= 0.158
p3= 0.0624, E3= 0.961, σ3= 0.0488


500mM KCl
Un-normalized Probability Density
E
data
p1= 0.173, E1= 0.00124, σ1= 0.0329
p2= 0.759, E2= 0.501, σ2= 0.159
p3= 0.0678, E3= 0.967, σ3= 0.0471


Un-normalized Probability Density
E
data
p1= 0.167, E1= 0.000823, σ1= 0.0319
p2= 0.771, E2= 0.506, σ2= 0.147
p3= 0.0611, E3= 0.963, σ3= 0.0486

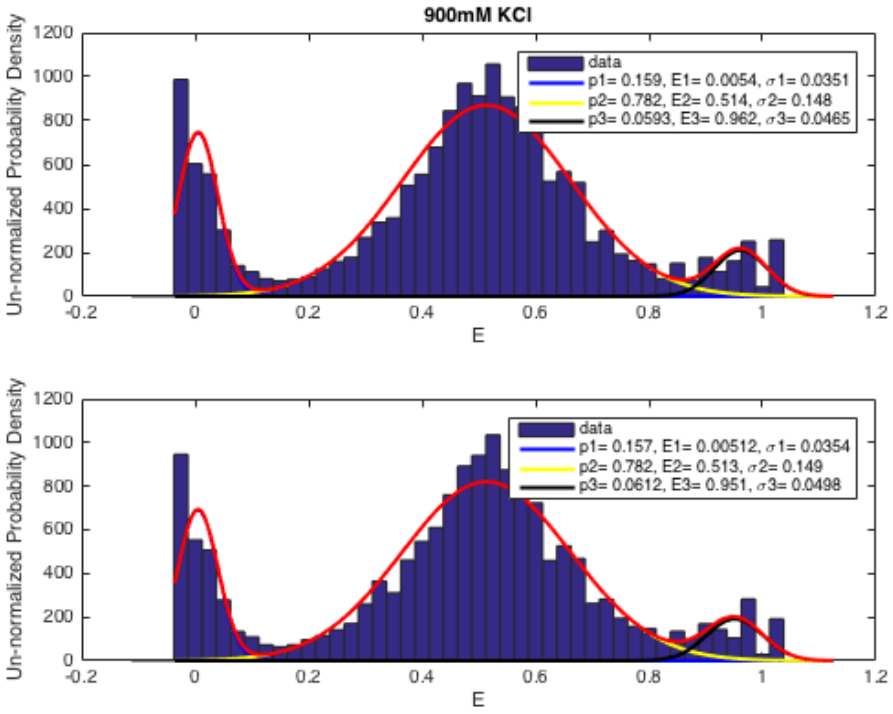
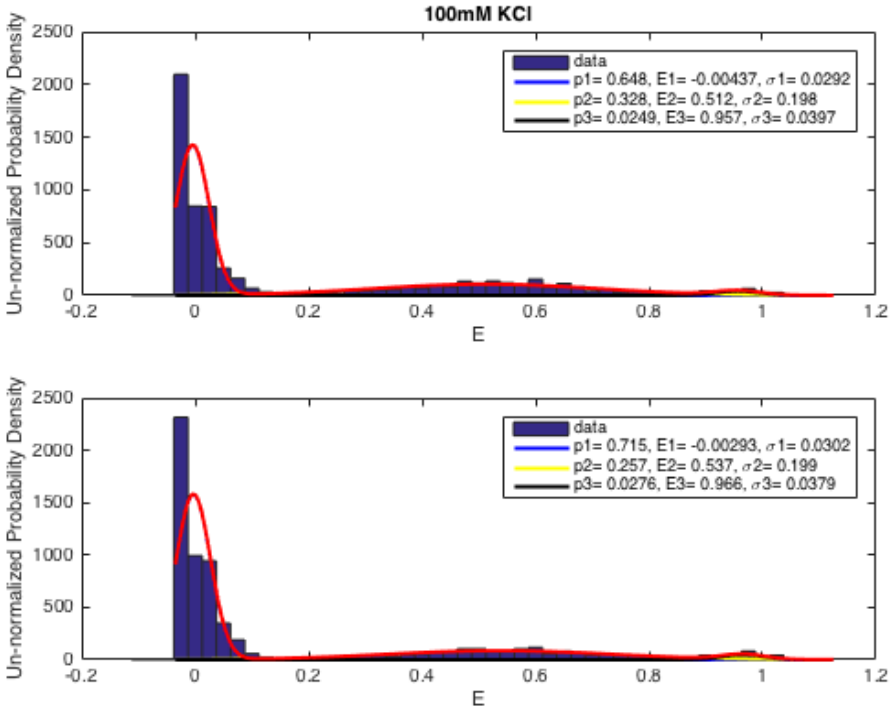
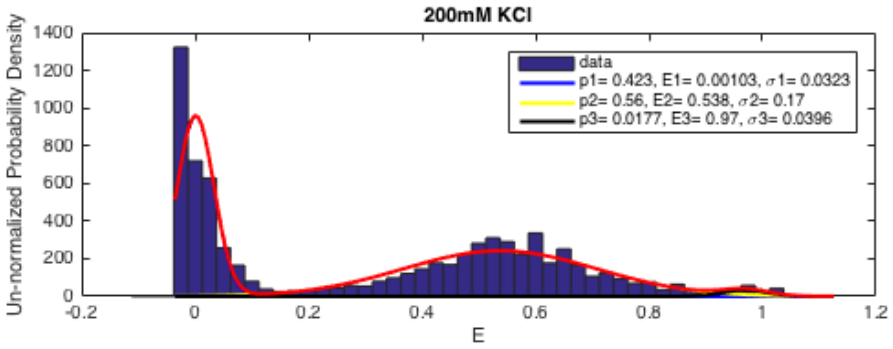
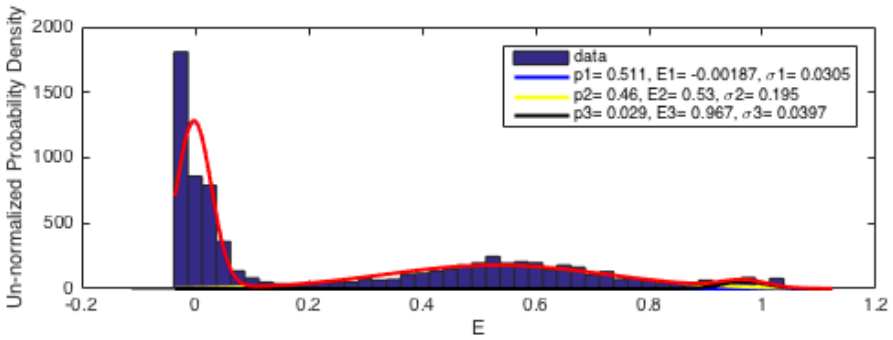
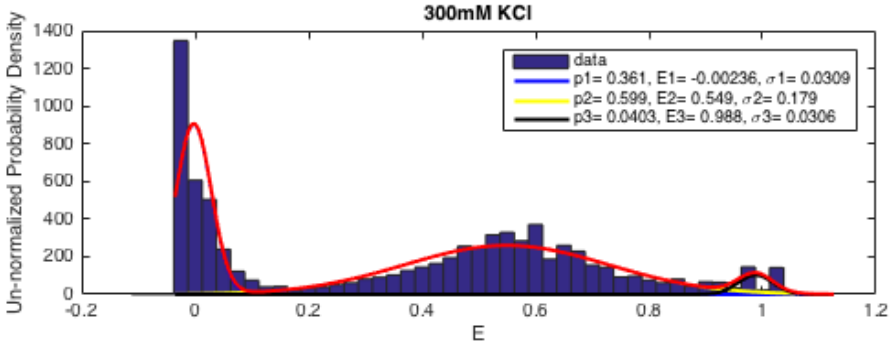


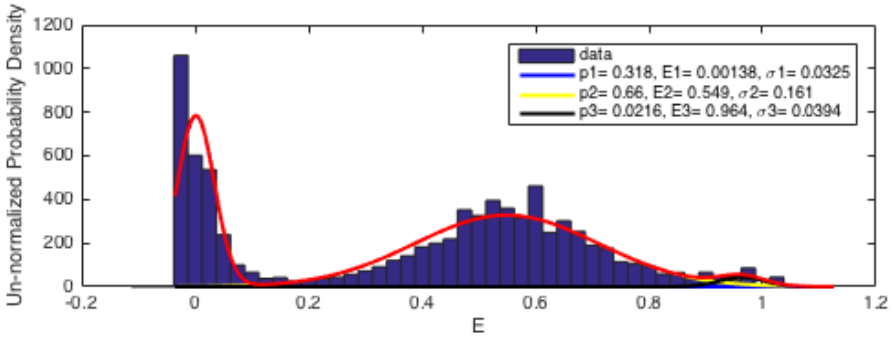
Figure S6 (below). FRET Histograms for H-J5/5a-H, D2-A (side labeled) in KCl solutions.

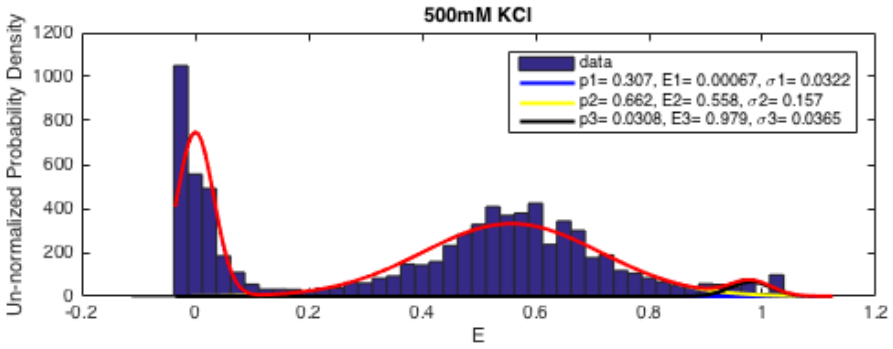


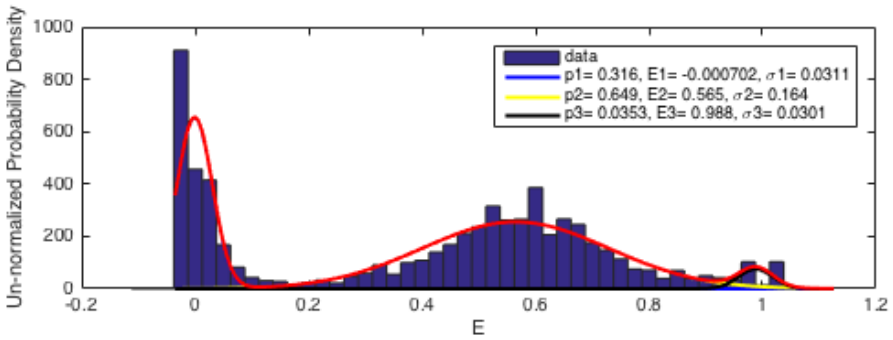

200mM KCl
Un-normalized Probability Density
E
data
p1= 0.423, E1= 0.00103, σ1= 0.0323
p2= 0.56, E2= 0.538, σ2= 0.17
p3= 0.0177, E3= 0.97, σ3= 0.0396

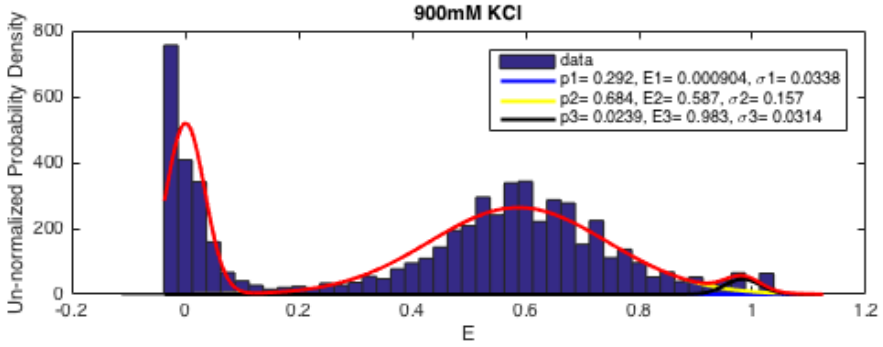

Un-normalized Probability Density
E
data
p1= 0.511, E1= -0.00187, σ1= 0.0305
p2= 0.46, E2= 0.53, σ2= 0.195
p3= 0.029, E3= 0.967, σ3= 0.0397


300mM KCl
Un-normalized Probability Density
E
data
p1= 0.361, E1= -0.00236, σ1= 0.0309
p2= 0.599, E2= 0.549, σ2= 0.179
p3= 0.0403, E3= 0.988, σ3= 0.0306


Un-normalized Probability Density
E
data
p1= 0.318, E1= 0.00138, σ1= 0.0325
p2= 0.66, E2= 0.549, σ2= 0.161
p3= 0.0216, E3= 0.964, σ3= 0.0394


500mM KCl
Un-normalized Probability Density
E
data
p1= 0.307, E1= 0.00067, σ1= 0.0322
p2= 0.662, E2= 0.558, σ2= 0.157
p3= 0.0308, E3= 0.979, σ3= 0.0365


Un-normalized Probability Density
E
data
p1= 0.316, E1= -0.000702, σ1= 0.0311
p2= 0.649, E2= 0.565, σ2= 0.164
p3= 0.0353, E3= 0.988, σ3= 0.0301


900mM KCl
Un-normalized Probability Density
E
data
p1= 0.292, E1= 0.000904, σ1= 0.0338
p2= 0.684, E2= 0.587, σ2= 0.157
p3= 0.0239, E3= 0.983, σ3= 0.0314

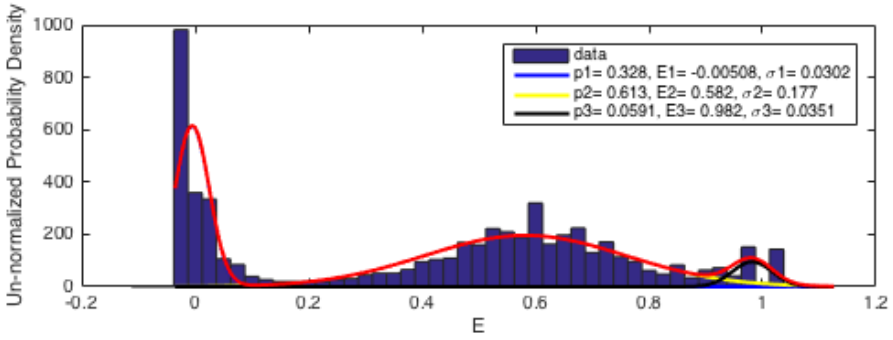
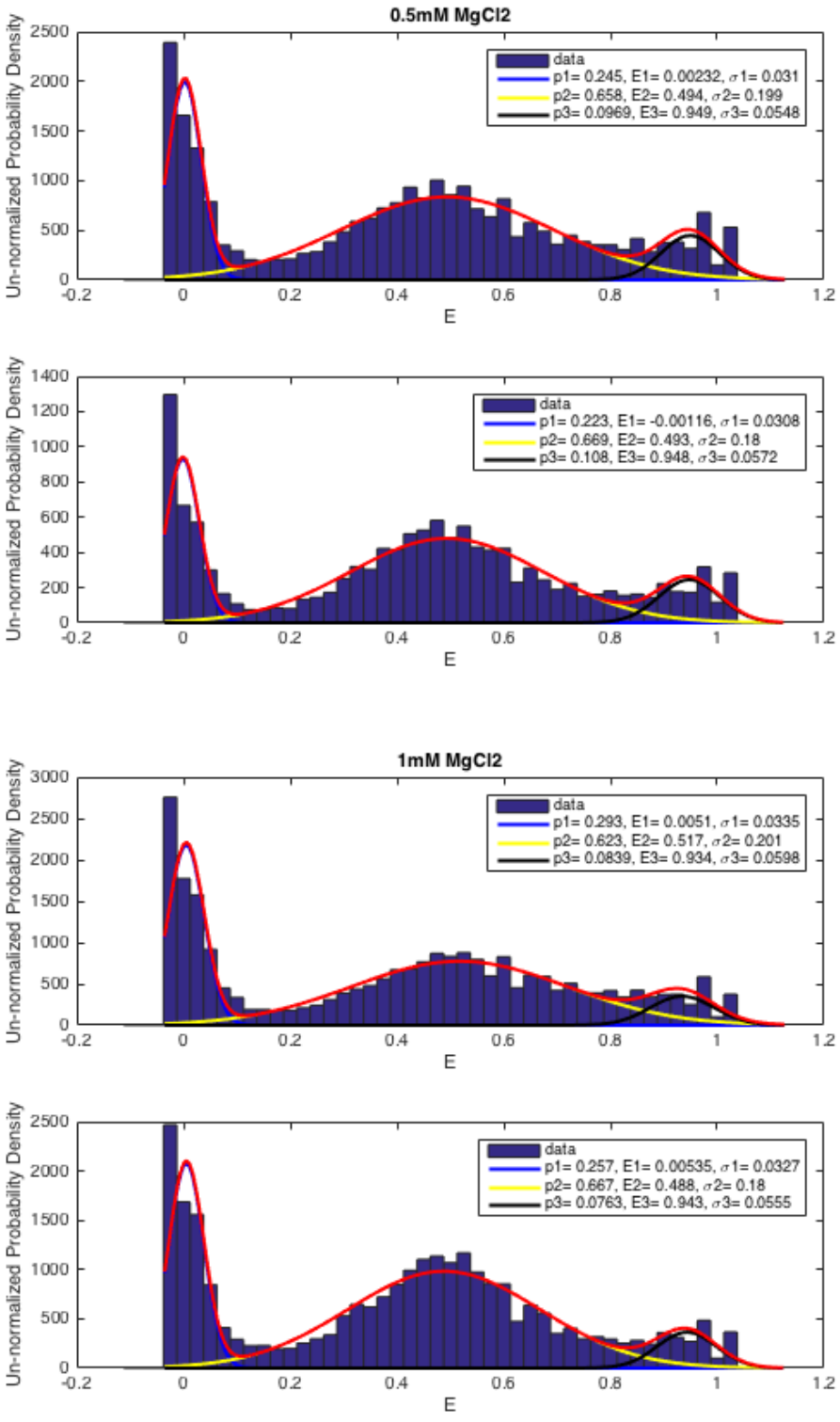
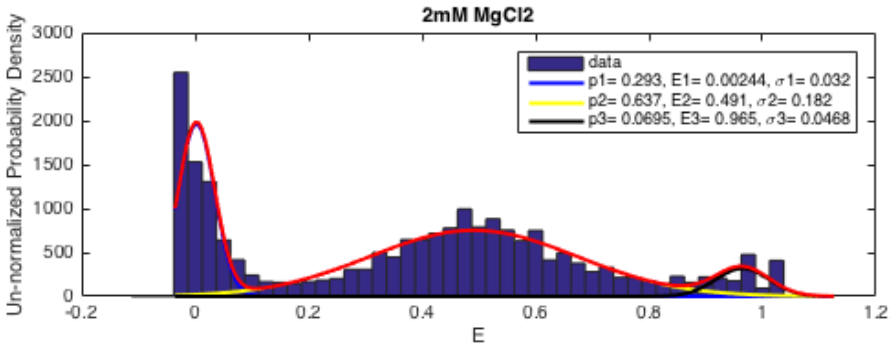
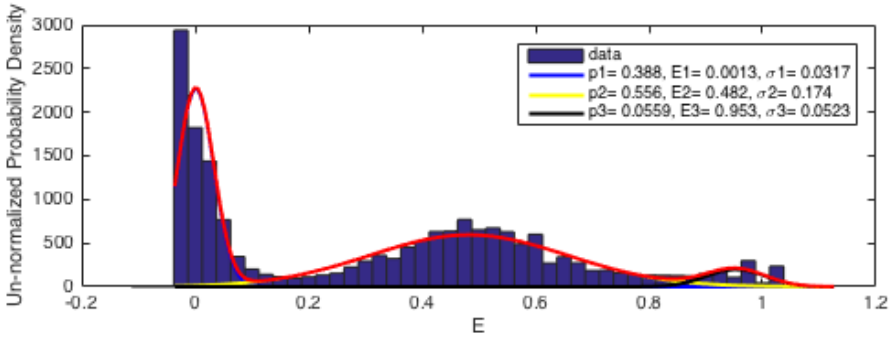
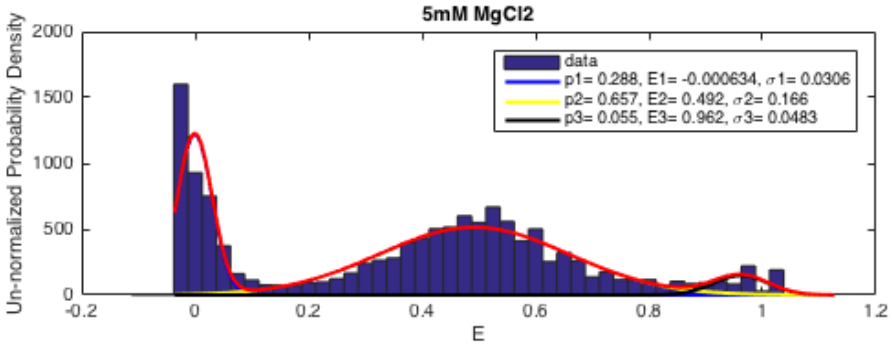

Un-normalized Probability Density
E
data
p1= 0.328, E1= -0.00508, σ1= 0.0302
p2= 0.613, E2= 0.582, σ2= 0.177
p3= 0.0591, E3= 0.982, σ3= 0.0351

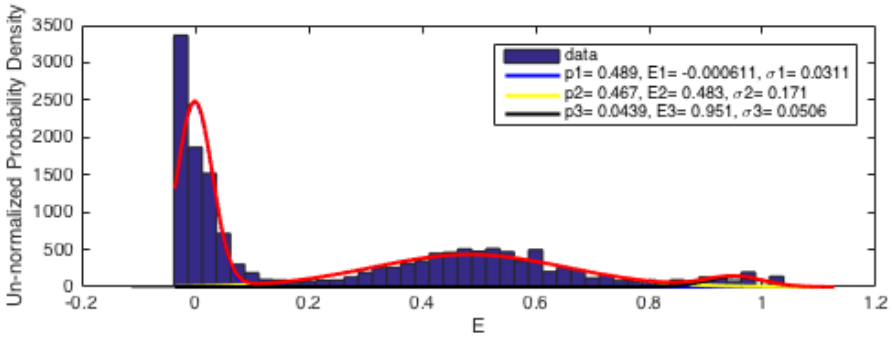
Figure S7 (below). FRET Histograms for H-JU_4U_5-H, D2-A (side labeled) in $MgCl_2$ solutions.

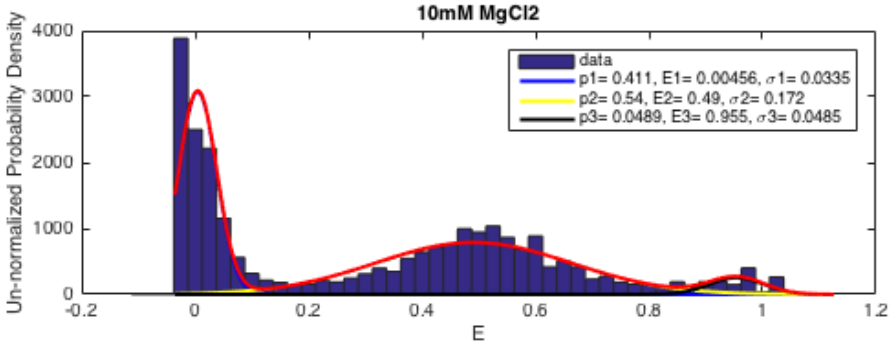


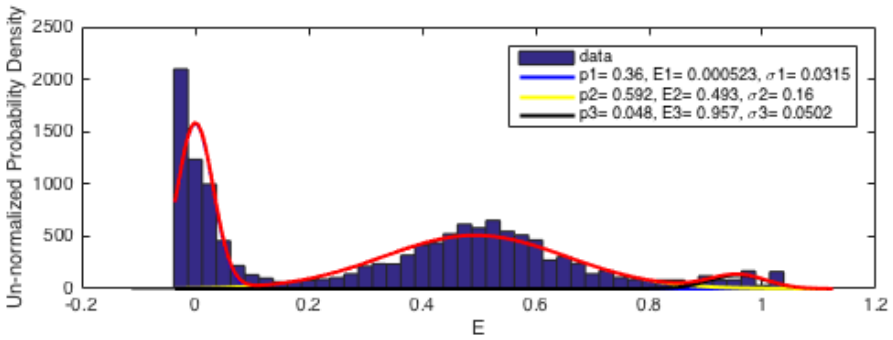

2mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.293, E1= 0.00244, σ1= 0.032
p2= 0.637, E2= 0.491, σ2= 0.182
p3= 0.0695, E3= 0.965, σ3= 0.0468

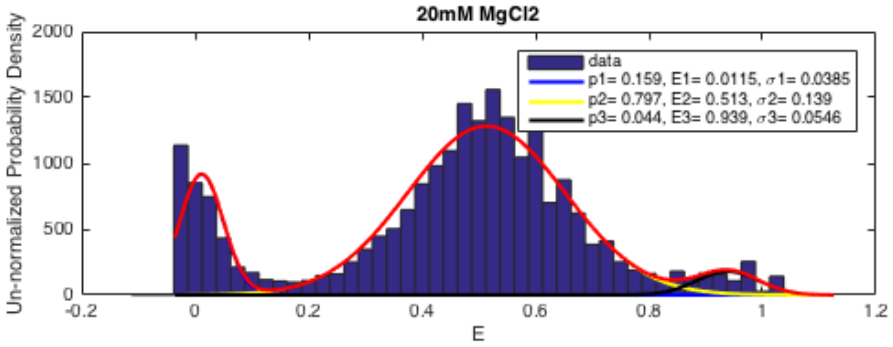

Un-normalized Probability Density
E
data
p1= 0.388, E1= 0.0013, σ1= 0.0317
p2= 0.556, E2= 0.482, σ2= 0.174
p3= 0.0559, E3= 0.953, σ3= 0.0523

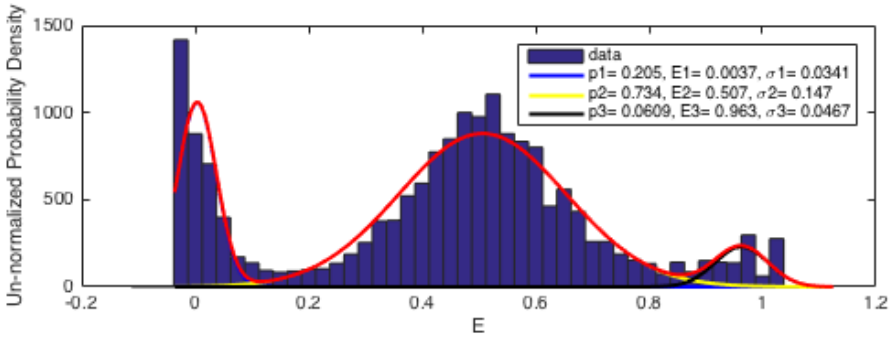

5mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.288, E1= -0.000634, σ1= 0.0306
p2= 0.657, E2= 0.492, σ2= 0.166
p3= 0.055, E3= 0.962, σ3= 0.0483

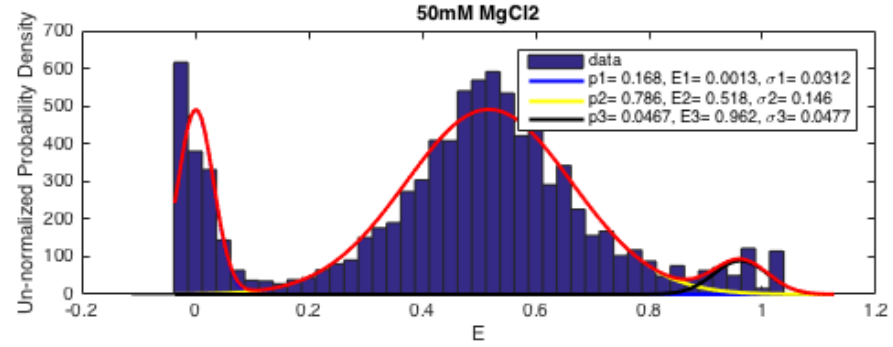

Un-normalized Probability Density
E
data
p1= 0.489, E1= -0.000611, σ1= 0.0311
p2= 0.467, E2= 0.483, σ2= 0.171
p3= 0.0439, E3= 0.951, σ3= 0.0506

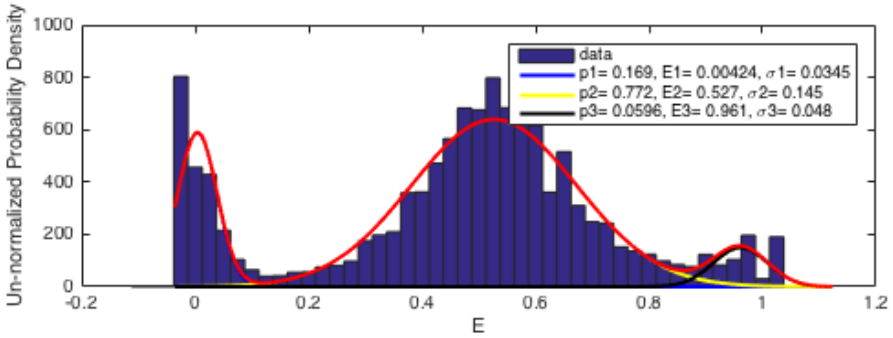

10mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.411, E1= 0.00456, σ1= 0.0335
p2= 0.54, E2= 0.49, σ2= 0.172
p3= 0.0489, E3= 0.955, σ3= 0.0485

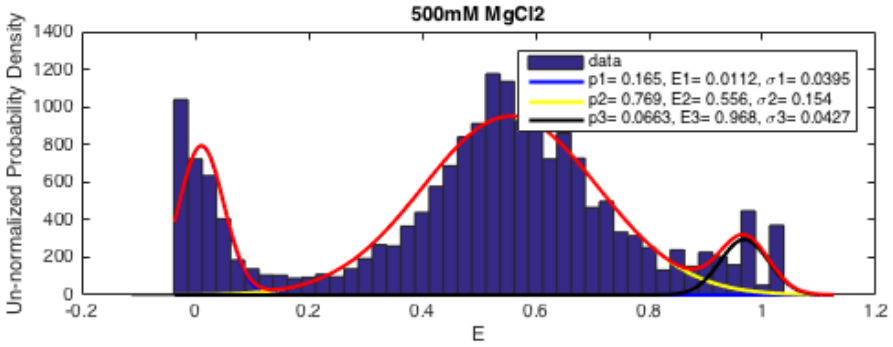

Un-normalized Probability Density
E
data
p1= 0.36, E1= 0.000523, σ1= 0.0315
p2= 0.592, E2= 0.493, σ2= 0.16
p3= 0.048, E3= 0.957, σ3= 0.0502


20mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.159, E1= 0.0115, σ1= 0.0385
p2= 0.797, E2= 0.513, σ2= 0.139
p3= 0.044, E3= 0.939, σ3= 0.0546


Un-normalized Probability Density
E
data
p1= 0.205, E1= 0.0037, σ1= 0.0341
p2= 0.734, E2= 0.507, σ2= 0.147
p3= 0.0609, E3= 0.963, σ3= 0.0467


50mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.168, E1= 0.0013, σ1= 0.0312
p2= 0.786, E2= 0.518, σ2= 0.146
p3= 0.0467, E3= 0.962, σ3= 0.0477


Un-normalized Probability Density
E
data
p1= 0.169, E1= 0.00424, σ1= 0.0345
p2= 0.772, E2= 0.527, σ2= 0.145
p3= 0.0596, E3= 0.961, σ3= 0.048


500mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.165, E1= 0.0112, σ1= 0.0395
p2= 0.769, E2= 0.556, σ2= 0.154
p3= 0.0663, E3= 0.968, σ3= 0.0427

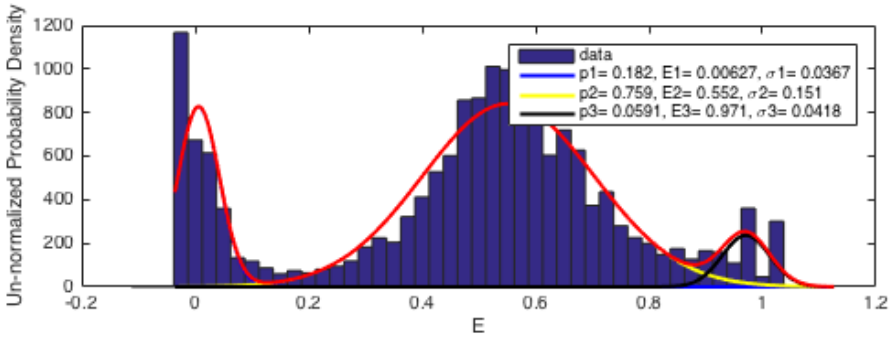
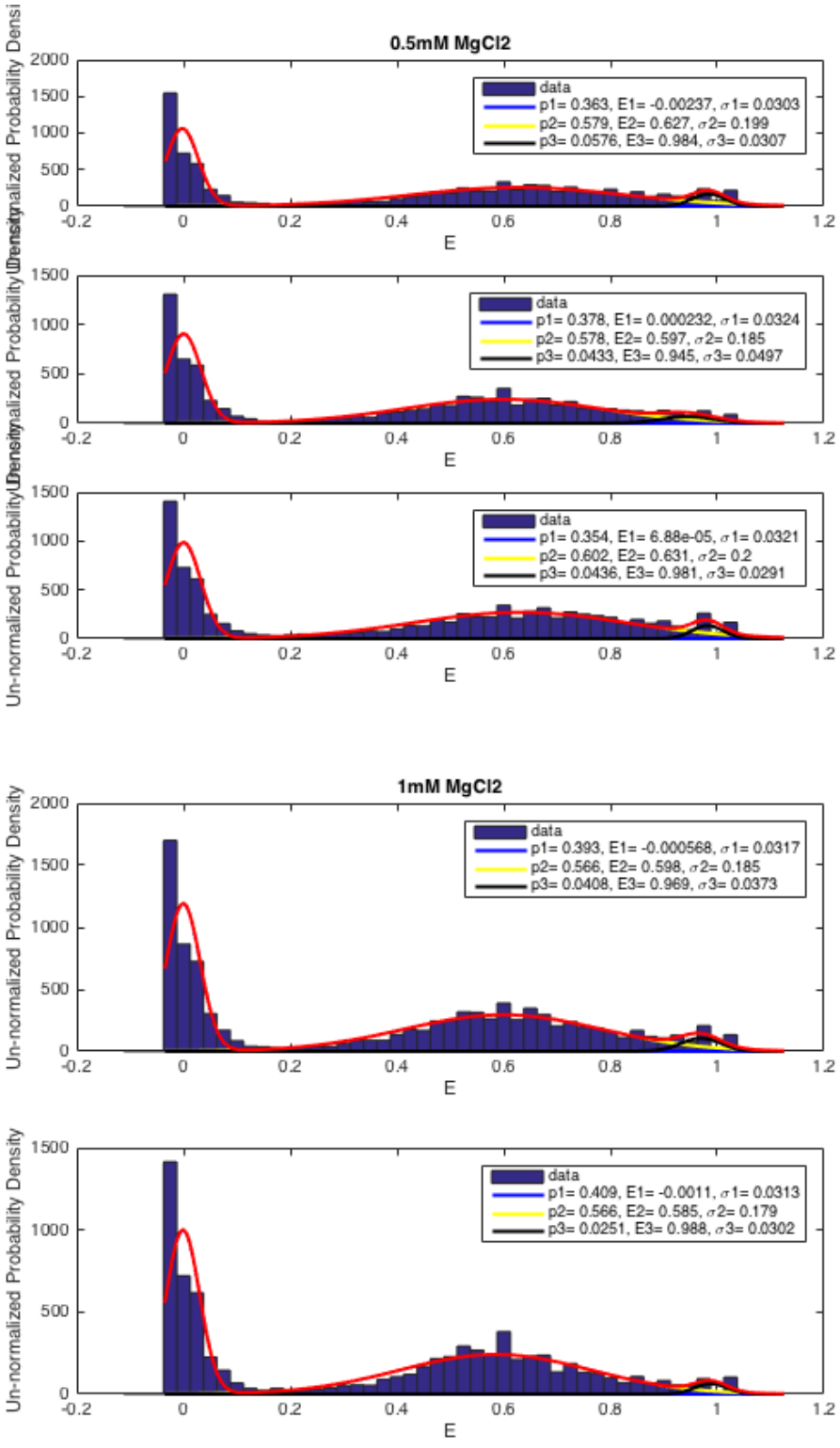
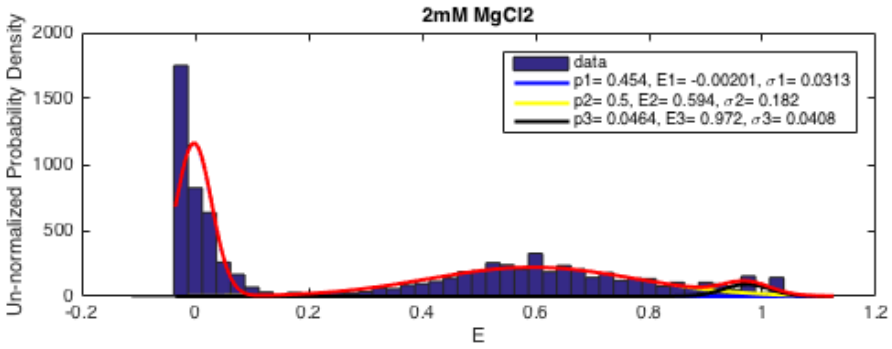
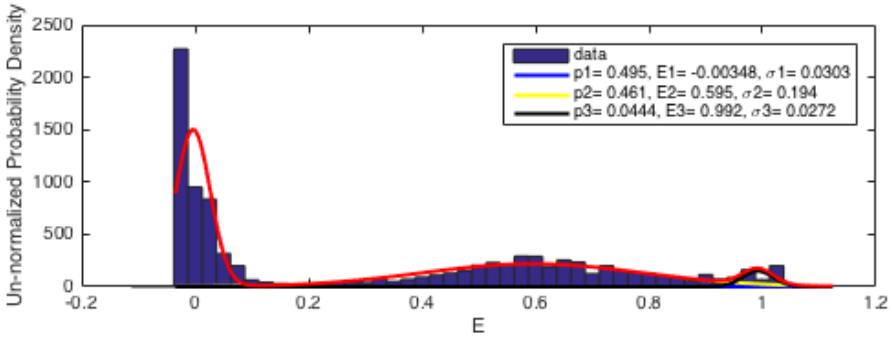
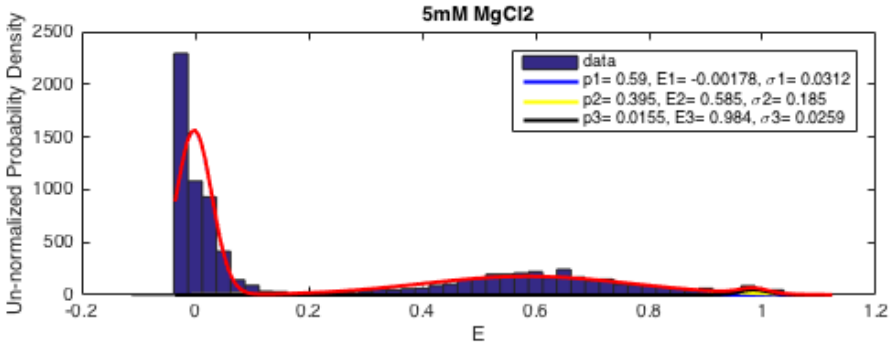

Un-normalized Probability Density
E
data
p1= 0.182, E1= 0.00627, σ1= 0.0367
p2= 0.759, E2= 0.552, σ2= 0.151
p3= 0.0591, E3= 0.971, σ3= 0.0418

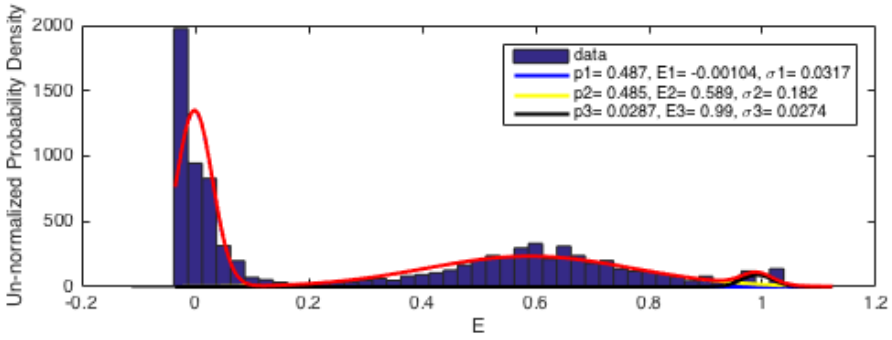
Figure S8 (below). FRET Histograms for H-J5/5a-H, D2-A (side labeled) in $MgCl_2$ solutions.

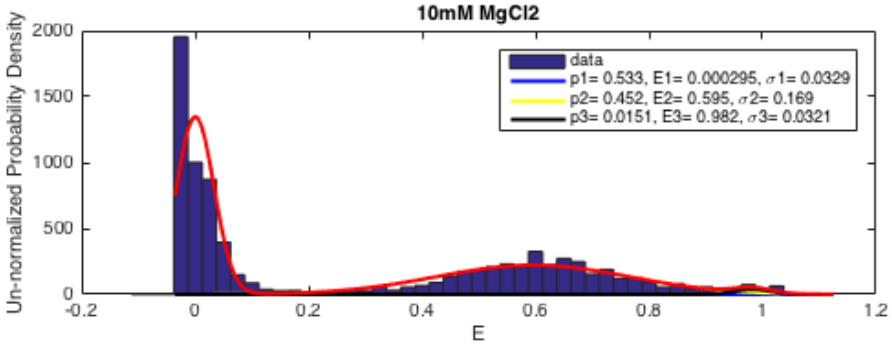


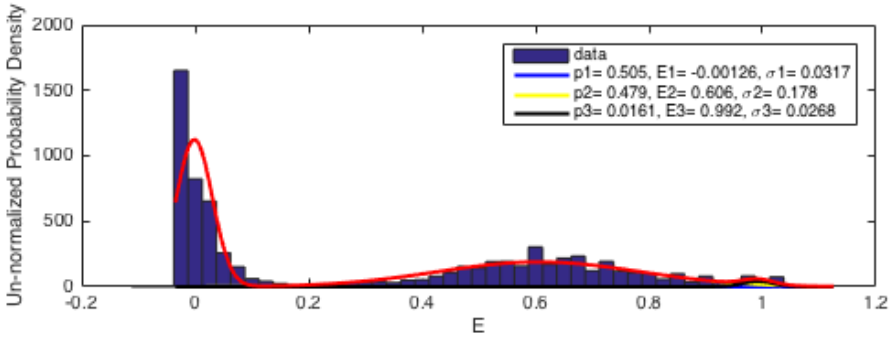

2mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.454, E1= -0.00201, σ1= 0.0313
p2= 0.5, E2= 0.594, σ2= 0.182
p3= 0.0464, E3= 0.972, σ3= 0.0408

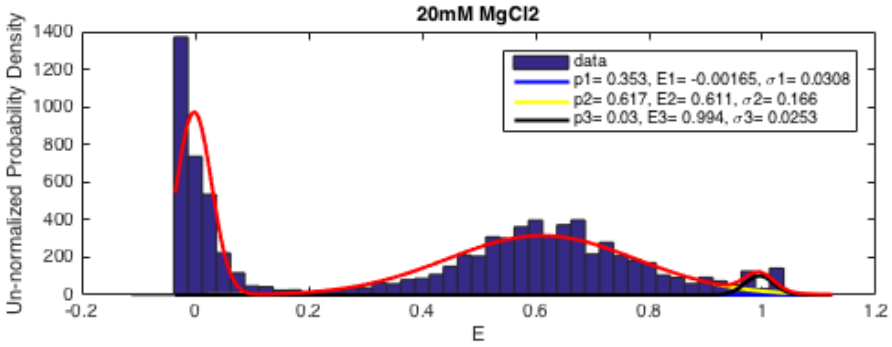

Un-normalized Probability Density
E
data
p1= 0.495, E1= -0.00348, σ1= 0.0303
p2= 0.461, E2= 0.595, σ2= 0.194
p3= 0.0444, E3= 0.992, σ3= 0.0272

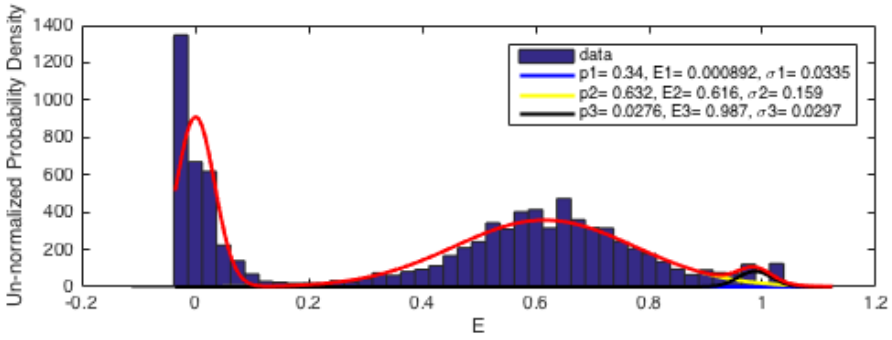

5mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.59, E1= -0.00178, σ1= 0.0312
p2= 0.395, E2= 0.585, σ2= 0.185
p3= 0.0155, E3= 0.984, σ3= 0.0259

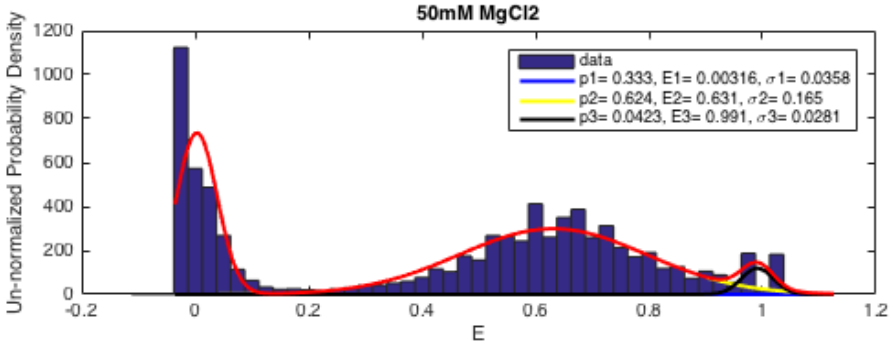

Un-normalized Probability Density
E
data
p1= 0.487, E1= -0.00104, σ1= 0.0317
p2= 0.485, E2= 0.589, σ2= 0.182
p3= 0.0287, E3= 0.99, σ3= 0.0274

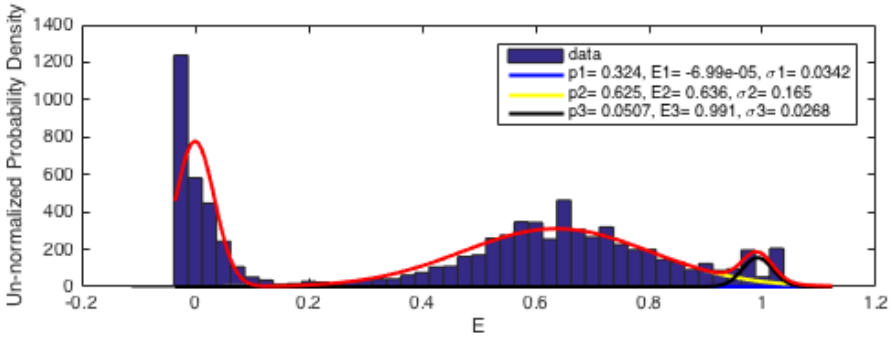

10mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.533, E1= 0.000295, σ1= 0.0329
p2= 0.452, E2= 0.595, σ2= 0.169
p3= 0.0151, E3= 0.982, σ3= 0.0321

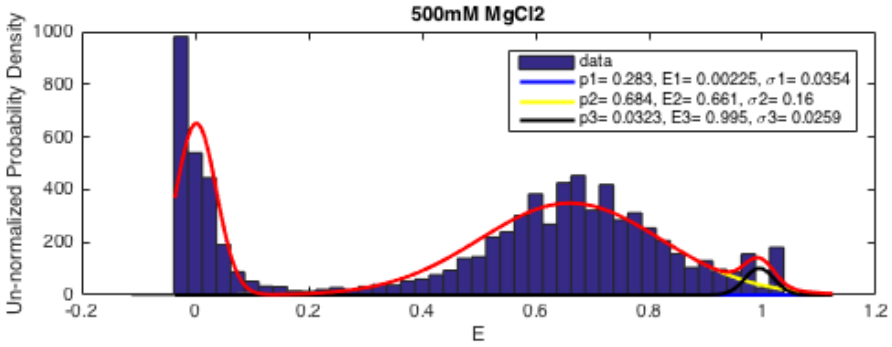

Un-normalized Probability Density
E
data
p1= 0.505, E1= -0.00126, σ1= 0.0317
p2= 0.479, E2= 0.606, σ2= 0.178
p3= 0.0161, E3= 0.992, σ3= 0.0268


20mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.353, E1= -0.00165, σ1= 0.0308
p2= 0.617, E2= 0.611, σ2= 0.166
p3= 0.03, E3= 0.994, σ3= 0.0253


Un-normalized Probability Density
E
data
p1= 0.34, E1= 0.000892, σ1= 0.0335
p2= 0.632, E2= 0.616, σ2= 0.159
p3= 0.0276, E3= 0.987, σ3= 0.0297


50mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.333, E1= 0.00316, σ1= 0.0358
p2= 0.624, E2= 0.631, σ2= 0.165
p3= 0.0423, E3= 0.991, σ3= 0.0281


Un-normalized Probability Density
E
data
p1= 0.324, E1= -6.99e-05, σ1= 0.0342
p2= 0.625, E2= 0.636, σ2= 0.165
p3= 0.0507, E3= 0.991, σ3= 0.0268


500mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.283, E1= 0.00225, σ1= 0.0354
p2= 0.684, E2= 0.661, σ2= 0.16
p3= 0.0323, E3= 0.995, σ3= 0.0259

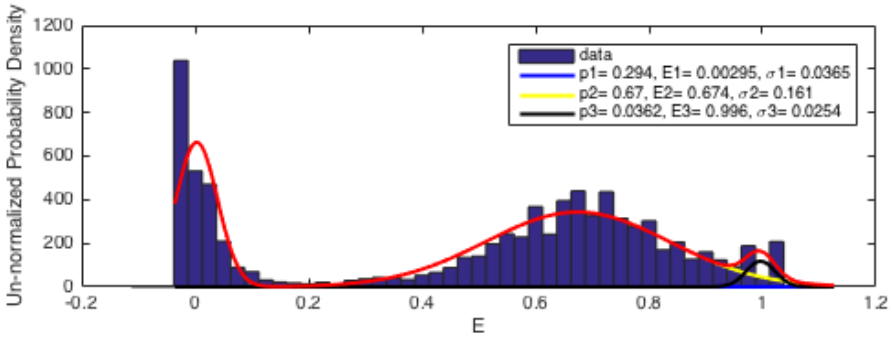
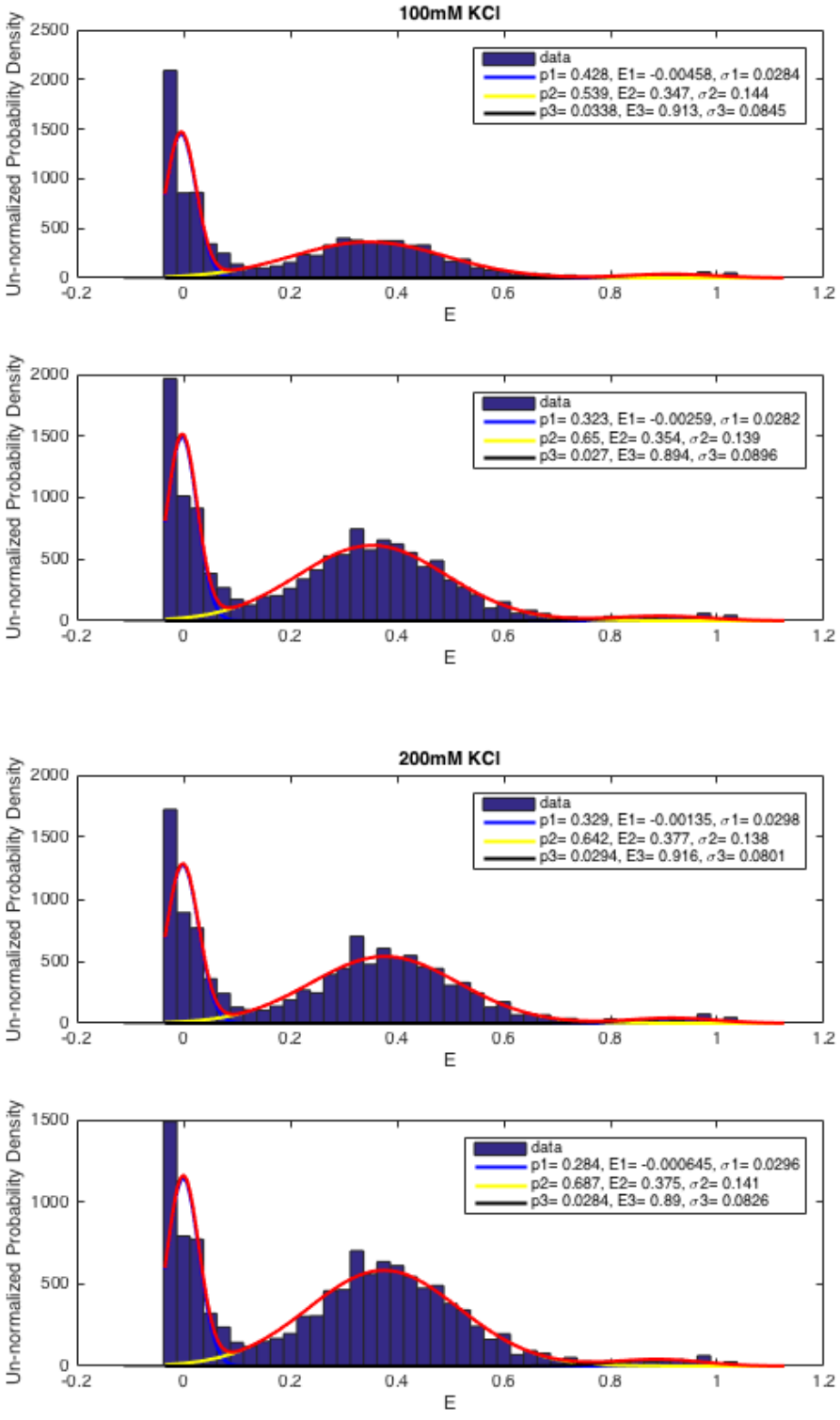
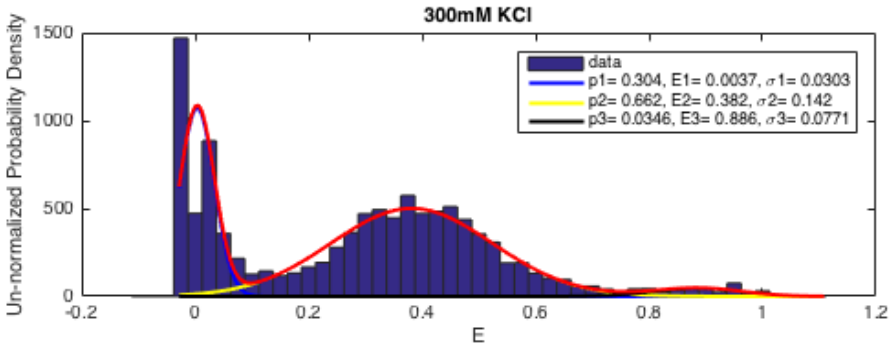
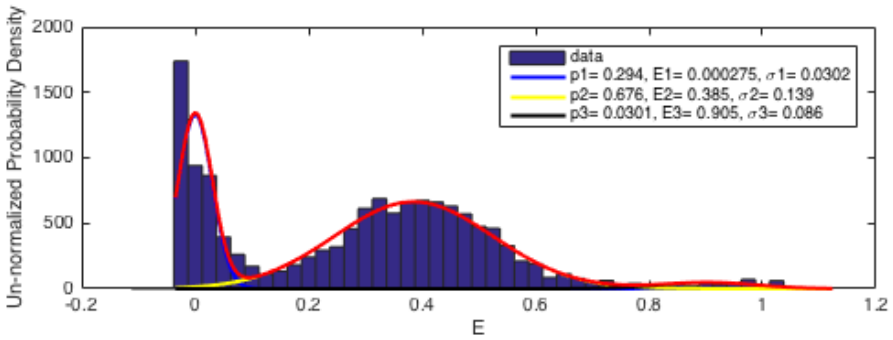
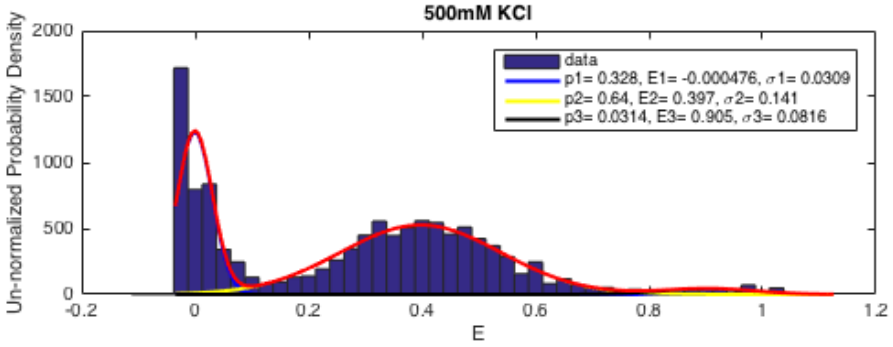

Un-normalized Probability Density
E
data
p1= 0.294, E1= 0.00295, σ1= 0.0365
p2= 0.67, E2= 0.674, σ2= 0.161
p3= 0.0362, E3= 0.996, σ3= 0.0254

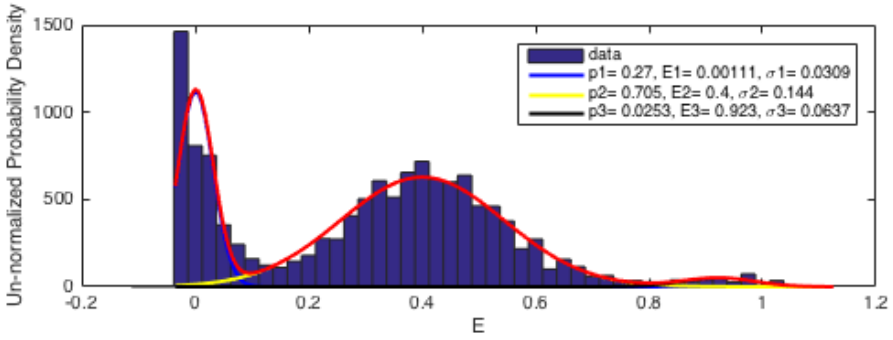
Figure S9 (below). FRET Histograms for H-JBP-H, D2-A (side labeled) in KCl solutions.




300mM KCl
Un-normalized Probability Density
E
data
p1= 0.304, E1= 0.0037, σ1= 0.0303
p2= 0.662, E2= 0.382, σ2= 0.142
p3= 0.0346, E3= 0.886, σ3= 0.0771


Un-normalized Probability Density
E
data
p1= 0.294, E1= 0.000275, σ1= 0.0302
p2= 0.676, E2= 0.385, σ2= 0.139
p3= 0.0301, E3= 0.905, σ3= 0.086


500mM KCl
Un-normalized Probability Density
E
data
p1= 0.328, E1= -0.000476, σ1= 0.0309
p2= 0.64, E2= 0.397, σ2= 0.141
p3= 0.0314, E3= 0.905, σ3= 0.0816


Un-normalized Probability Density
E
data
p1= 0.27, E1= 0.00111, σ1= 0.0309
p2= 0.705, E2= 0.4, σ2= 0.144
p3= 0.0253, E3= 0.923, σ3= 0.0637

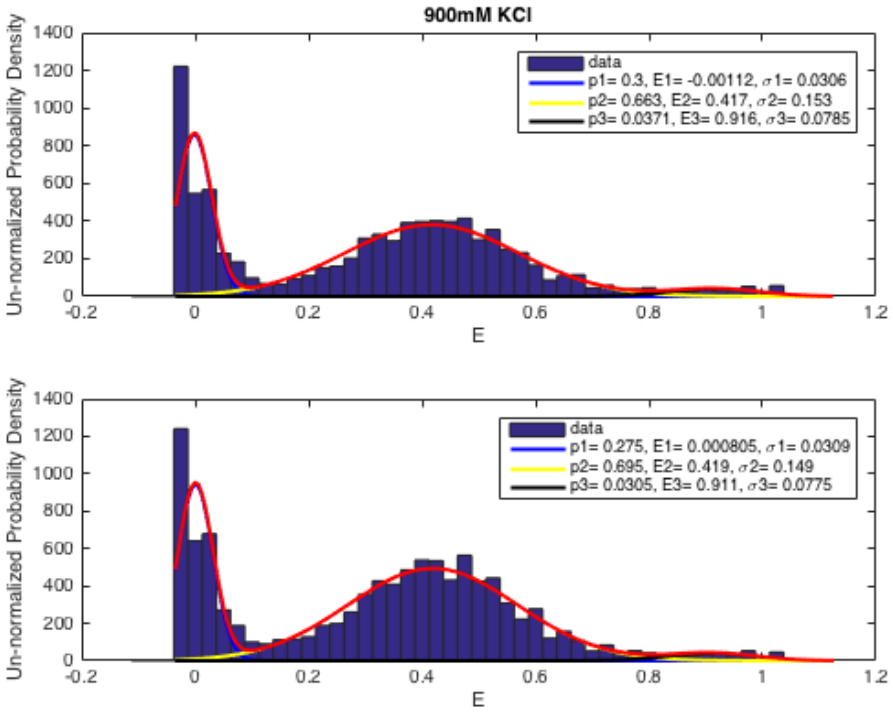
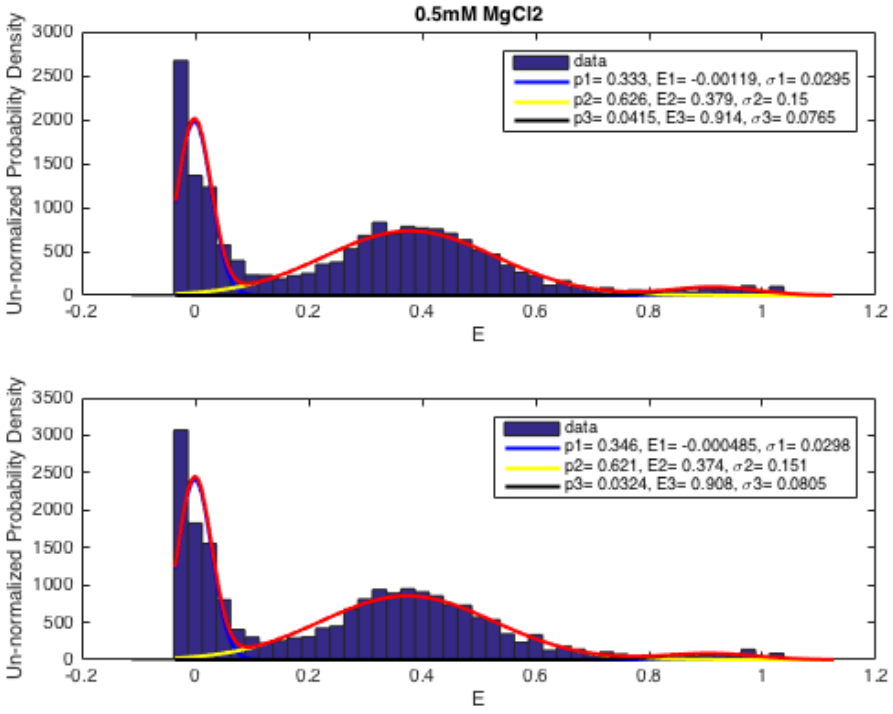
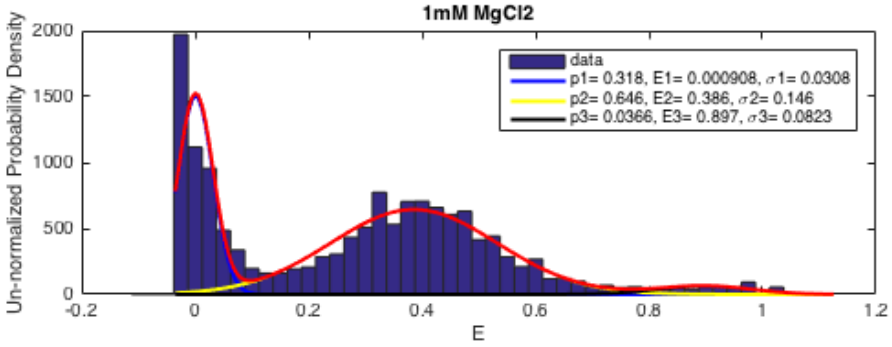
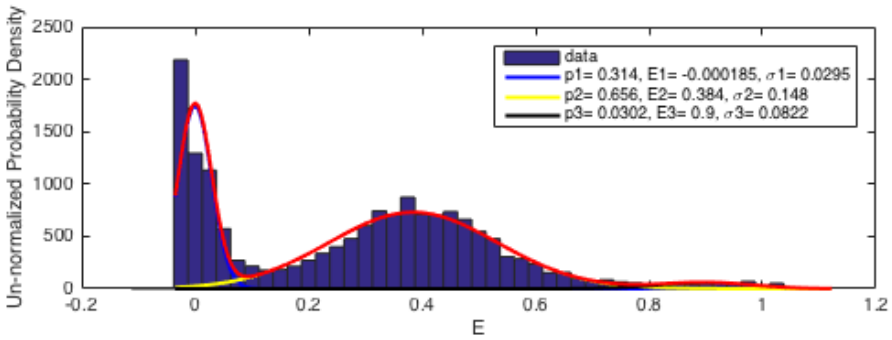
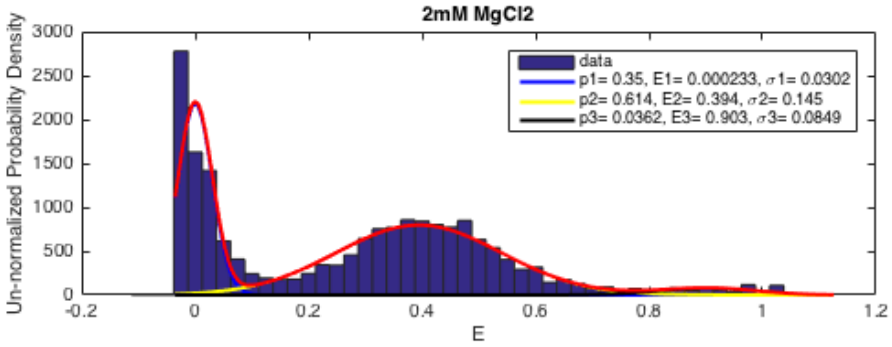


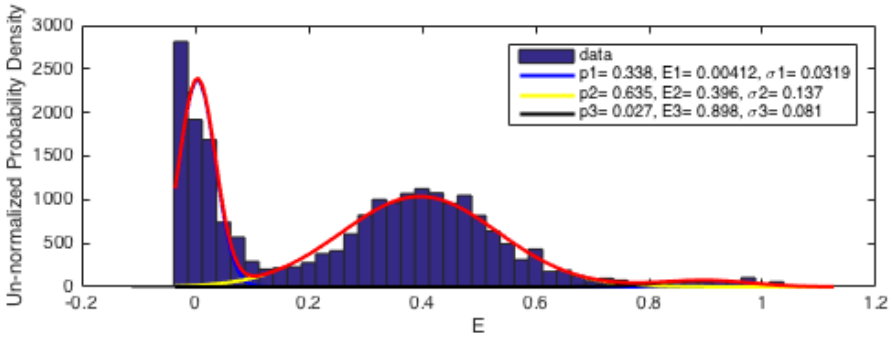
Figure S10 (below). FRET Histograms for H-JBP-H, D2-A (side labeled) in $MgCl_2$ solutions.

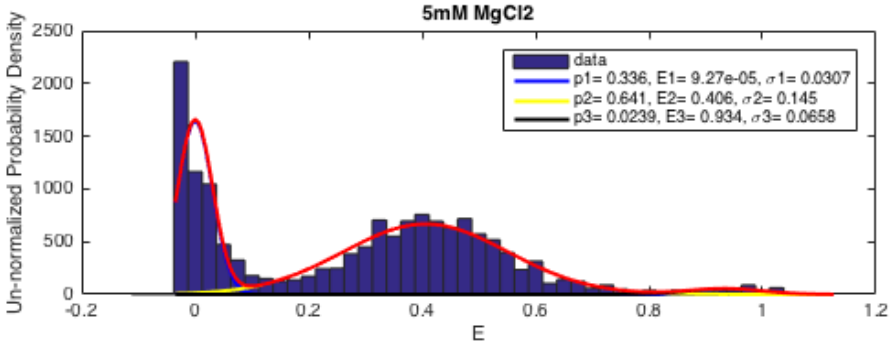


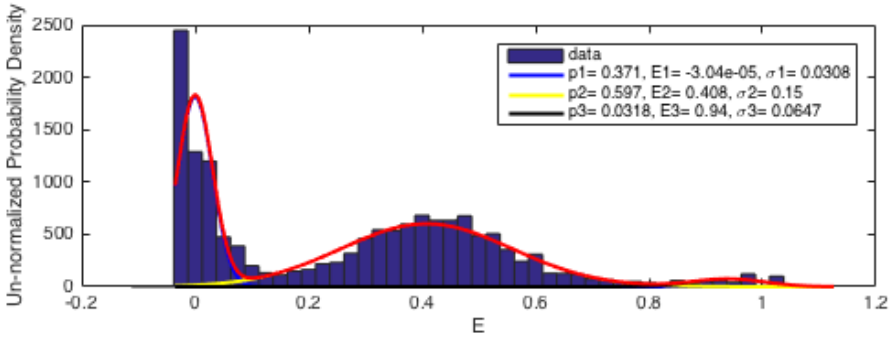

1mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.318, E1= 0.000908, σ1= 0.0308
p2= 0.646, E2= 0.386, σ2= 0.146
p3= 0.0366, E3= 0.897, σ3= 0.0823

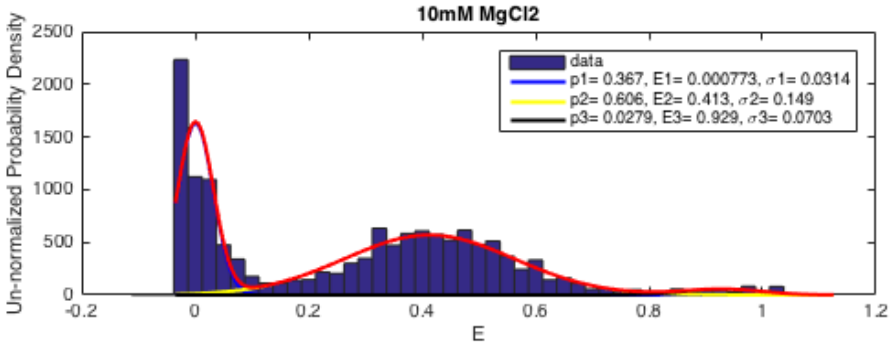

Un-normalized Probability Density
E
data
p1= 0.314, E1= -0.000185, σ1= 0.0295
p2= 0.656, E2= 0.384, σ2= 0.148
p3= 0.0302, E3= 0.9, σ3= 0.0822

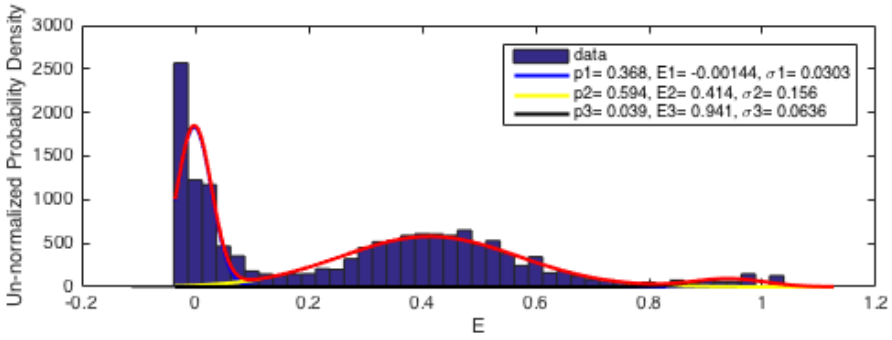

2mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.35, E1= 0.000233, σ1= 0.0302
p2= 0.614, E2= 0.394, σ2= 0.145
p3= 0.0362, E3= 0.903, σ3= 0.0849

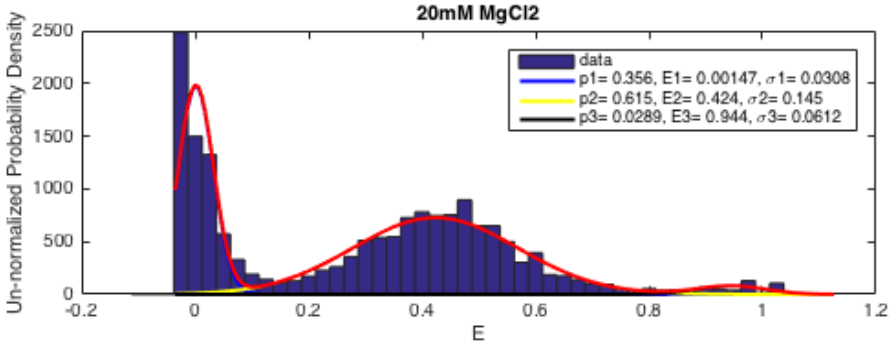

Un-normalized Probability Density
E
data
p1= 0.338, E1= 0.00412, σ1= 0.0319
p2= 0.635, E2= 0.396, σ2= 0.137
p3= 0.027, E3= 0.898, σ3= 0.081

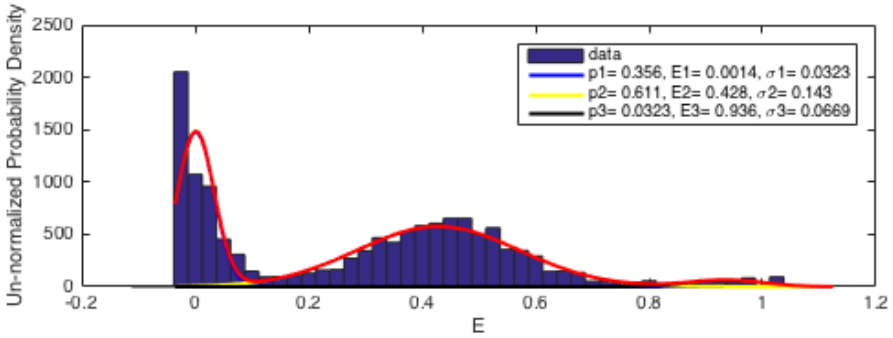

5mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.336, E1= 9.27e-05, σ1= 0.0307
p2= 0.641, E2= 0.406, σ2= 0.145
p3= 0.0239, E3= 0.934, σ3= 0.0658

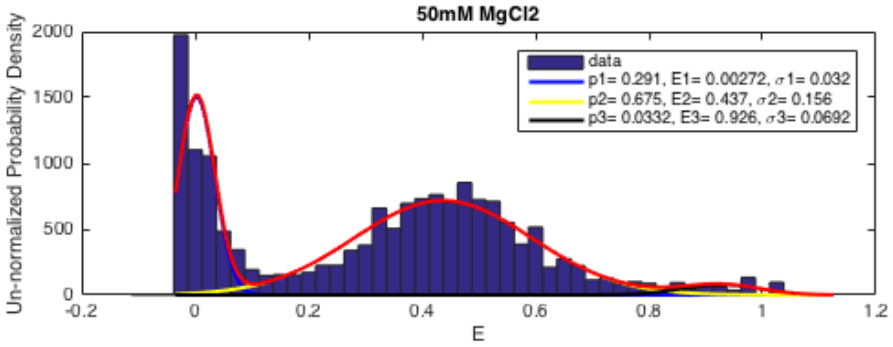

Un-normalized Probability Density
E
data
p1= 0.371, E1= -3.04e-05, σ1= 0.0308
p2= 0.597, E2= 0.408, σ2= 0.15
p3= 0.0318, E3= 0.94, σ3= 0.0647

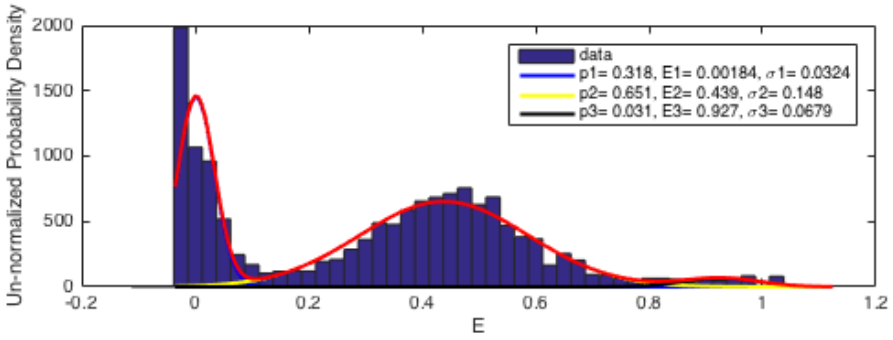

10mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.367, E1= 0.000773, σ1= 0.0314
p2= 0.606, E2= 0.413, σ2= 0.149
p3= 0.0279, E3= 0.929, σ3= 0.0703

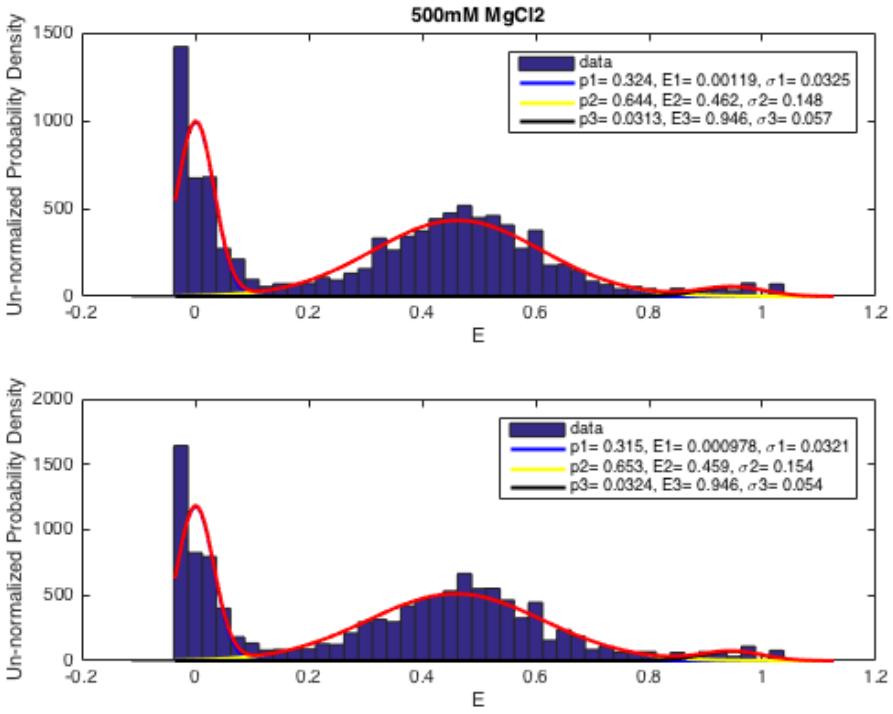

Un-normalized Probability Density
E
data
p1= 0.368, E1= -0.00144, σ1= 0.0303
p2= 0.594, E2= 0.414, σ2= 0.156
p3= 0.039, E3= 0.941, σ3= 0.0636


20mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.356, E1= 0.00147, σ1= 0.0308
p2= 0.615, E2= 0.424, σ2= 0.145
p3= 0.0289, E3= 0.944, σ3= 0.0612


Un-normalized Probability Density
E
data
p1= 0.356, E1= 0.0014, σ1= 0.0323
p2= 0.611, E2= 0.428, σ2= 0.143
p3= 0.0323, E3= 0.936, σ3= 0.0669


50mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.291, E1= 0.00272, σ1= 0.032
p2= 0.675, E2= 0.437, σ2= 0.156
p3= 0.0332, E3= 0.926, σ3= 0.0692


Un-normalized Probability Density
E
data
p1= 0.318, E1= 0.00184, σ1= 0.0324
p2= 0.651, E2= 0.439, σ2= 0.148
p3= 0.031, E3= 0.927, σ3= 0.0679


500mM MgCl2
Un-normalized Probability Density
E
data
p1= 0.324, E1= 0.00119, σ1= 0.0325
p2= 0.644, E2= 0.462, σ2= 0.148
p3= 0.0313, E3= 0.946, σ3= 0.057
Un-normalized Probability Density
E
data
p1= 0.315, E1= 0.000978, σ1= 0.0321
p2= 0.653, E2= 0.459, σ2= 0.154
p3= 0.0324, E3= 0.946, σ3= 0.054

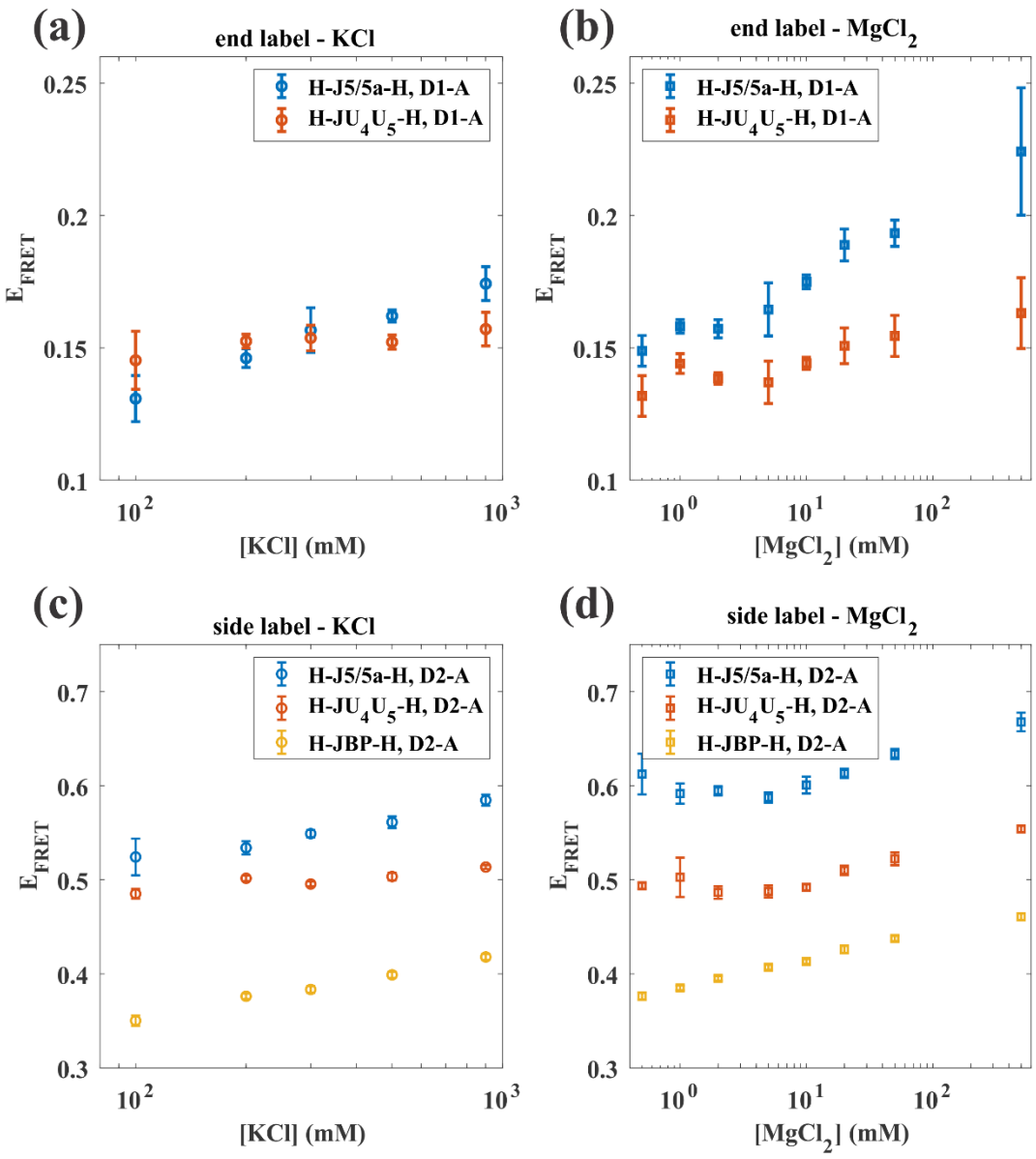


Figure S11. Measured effects on E_{FRET} of end-label donor dye position (D1-A dye pair) as a function of (a) KCl and (b) $MgCl_2$ for H-J5/5a-H and H-JU_4U_5-H isolated junction constructs and side-label donor dye position (D2-A dye pair) in (c) KCl and (d) $MgCl_2$ concentrations for H-J5/5a-H, H-JU_4U_5-H and H-JBP-H junction constructs.

Accessible Volume Simulations

All the sterically possible positions of fluorophores for the H-JBP-H RNA A-form duplex are calculated as a 3D probabilistic distribution by Accessible volume (AV) simulations[1]. The software inputs are dye and linker parameters[2], atomic coordinates of the macromolecule to be labeled, and the attachment point of the dye linker on the molecule. The software computes the space which the dye can explore without steric clashes with the macromolecular surface. We used the Nucleic Acid Builder web server[3] to build an A-form RNA helix with the same sequence as the H-JBP-H. Using the AV simulations, we calculated 3D probabilistic distributions for the donor and acceptor, P_D and P_A respectively, both of which are normalized.

$$\int P(\vec{r})\, d\vec{r} = 1 \tag{1}$$

The average distance between the donor and acceptor is then

$$\langle R_{FRET} \rangle = \iint \|\overrightarrow{r_D} - \overrightarrow{r_A}\|\, P_D(\overrightarrow{r_D}) P_A(\overrightarrow{r_A})\, \mathrm{d}\overrightarrow{r_D} \mathrm{d}\overrightarrow{r_A} \tag{2}$$

where $\overrightarrow{r_D}$ and $\overrightarrow{r_A}$ are donor and acceptor positions with respect to certain origin. The expected E_{FRET} can therefore be calculated.

$$\langle E_{FRET} \rangle = \frac{1}{1 + \left(\frac{\langle R_{FRET} \rangle}{R_0}\right)^6} \tag{3}$$

Note that we assume fast dye reorientation and relatively slow conformational dynamics.

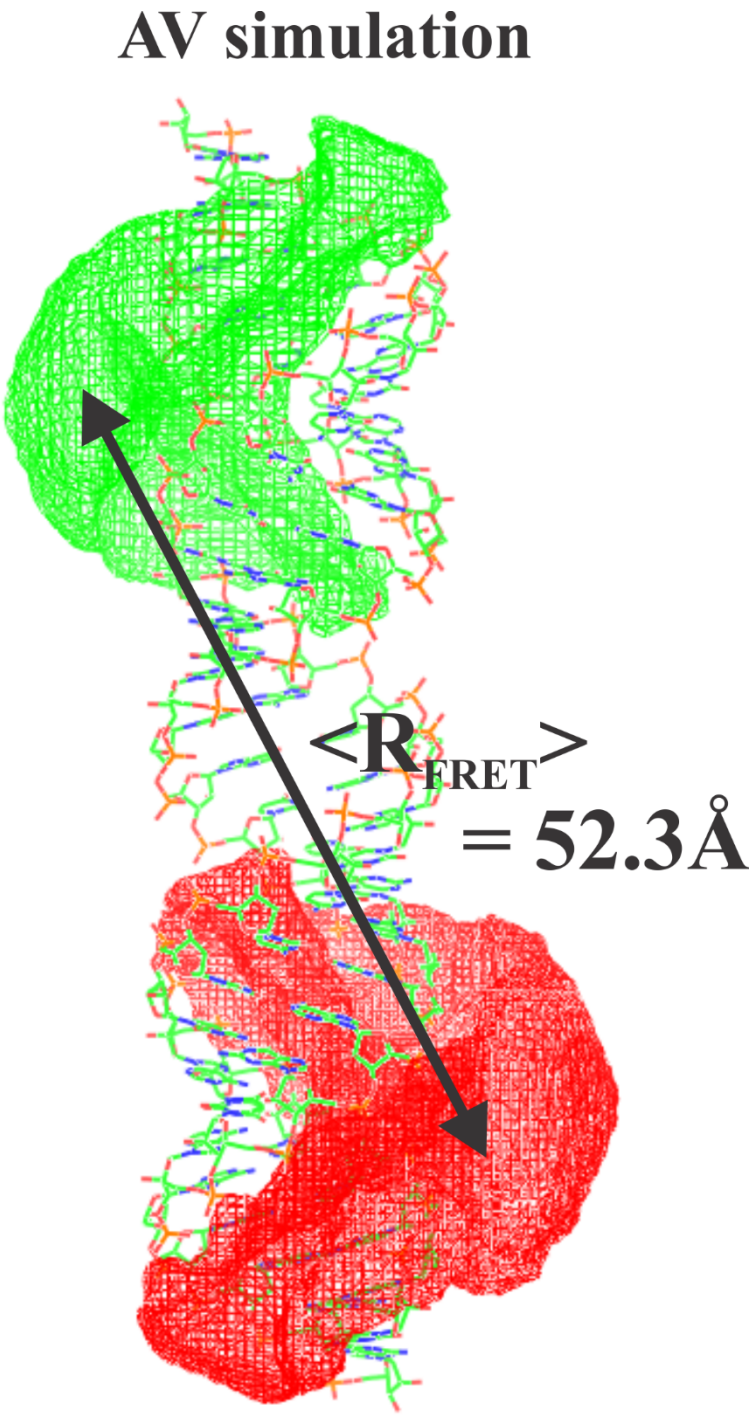


Figure S12. Representative accessible volumes of donor (green) and acceptor (red) dyes around the base-paired control RNA. The expected distance, $\langle R_{FRET} \rangle$ is 52.3 Å.

Comparison of smFRET Data Between DNA and RNA Duplex

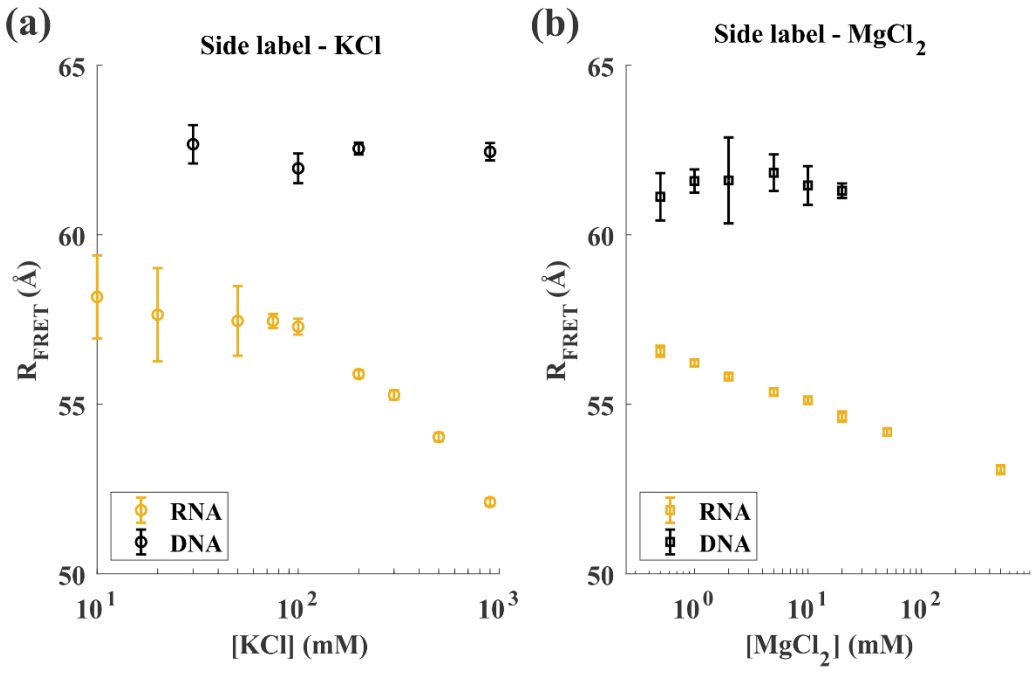


Figure S13. Comparison between RNA and DNA duplexes of the same length using the D2-A dye pair position in (a) KCl and (b) $MgCl_2$ solutions. The DNA B-form duplex remains stable throughout salt species and concentrations while RNA A-form duplex is twisted as ion strength increases.

REFERENCES

1. Muschielok A, Michaelis J. FRETnps Tools Documentation Starting FRETnpsTools. *Simulation*. 2008.
2. Sindbert S, Kalinin S, Nguyen H, et al. Accurate distance determination of nucleic acids via förster resonance energy transfer: Implications of dye Linker length and rigidity. *J Am Chem Soc*. 2011;133(8):2463-2480. doi:10.1021/ja105725e.
3. Macke TJ, Case DA. Modeling Unusual Nucleic Acid Structures BT - Molecular Modeling of Nucleic Acids. *Mol Model Nucleic Acids*. 2009;682:379-393. doi:10.1021/bk-1998-0682.ch024.