Supplementary Information

Proto-Isomerization of Indigo and Isoindigo Dyes Confirmed by Gas-Phase Infrared Ion Spectroscopy

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Supplementary Figures

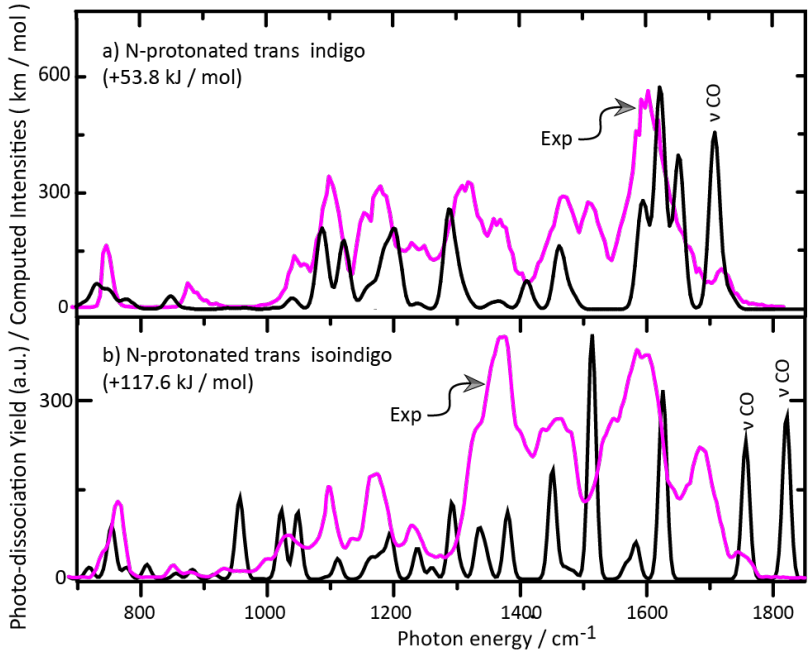

Figure S1: Gas-phase IRMPD spectra of protonated indigo (a) and protonated isoindigo (b) compared with computed spectra for N-protonated species in their *trans* configurations.

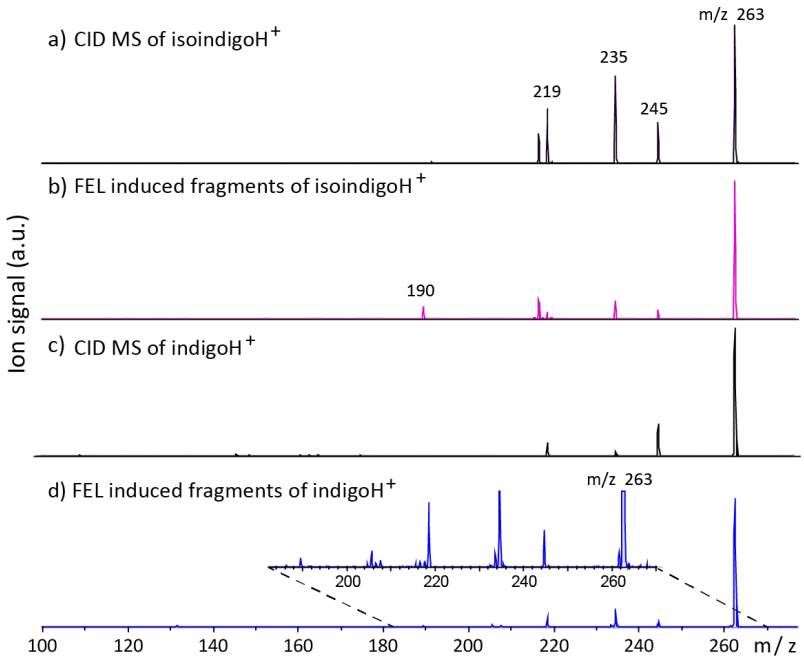

Figure S2 Mass spectra: CID MS (a) and on-resonance FEL induced photo-fragments at fixed IR laser frequency (b) of the protonated isoindigo (*m/z* 263). Similarly, CID MS (c) and FEL induced photo-fragments at fixed IR laser frequency (d) of the protonated indigo (*m/z* 263) which are very similar to the CID mass-fragments reported in the past[1,2].

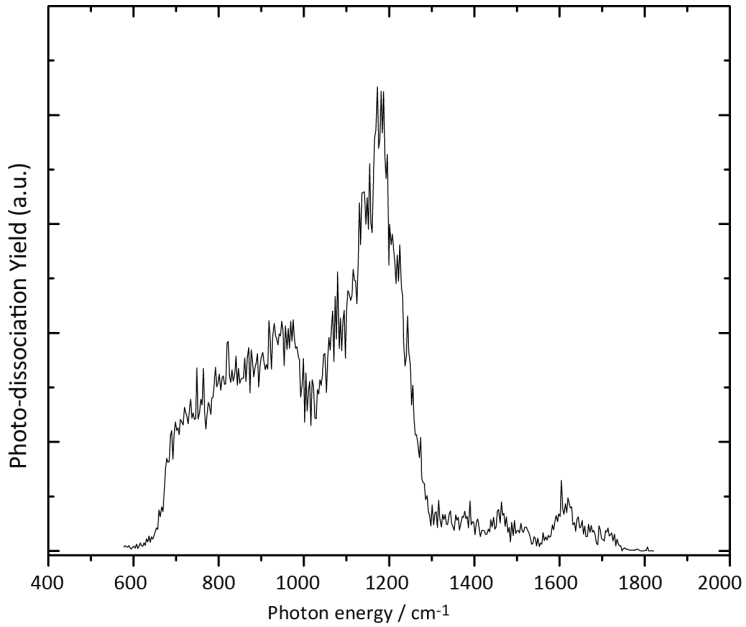

Figure S3 IRMPD spectrum of the mass isolated *m/z* 263 background ion. See main text for detailed discussion.

Cartesian coordinates of optimized structures at B3LYP/6-31++G(d,p)

Neutral indigo (trans)

Atom	X	Y	Z
6	-2.921431000	-0.674574000	0.000000000
6	-2.825724000	0.737738000	-0.000030000
6	-3.970762000	1.536533000	0.000016000
6	-5.208731000	0.888273000	0.000096000
6	-5.316760000	-0.514993000	0.000117000
6	-4.168018000	-1.305440000	0.000065000
6	-1.554706000	-1.209474000	-0.000048000
6	-0.681307000	0.000551000	-0.000105000
1	-3.905724000	2.620130000	-0.000012000
1	-6.113836000	1.488963000	0.000137000
1	-6.298139000	-0.977952000	0.000175000
1	-4.225711000	-2.389674000	0.000083000
6	0.681307000	-0.000554000	-0.000084000
6	1.554705000	1.209472000	-0.000033000
6	2.825725000	-0.737738000	-0.000010000
6	2.921430000	0.674574000	0.000008000
6	3.970765000	-1.536531000	0.000022000
6	4.168016000	1.305443000	0.000059000
6	5.208732000	-0.888269000	0.000070000

1	3.905728000	-2.620128000	0.000013000
6	5.316759000	0.514998000	0.000089000
1	4.225707000	2.389677000	0.000071000
1	6.113839000	-1.488957000	0.000097000
1	6.298137000	0.977958000	0.000128000
7	-1.489421000	1.119883000	-0.000157000
1	-1.111326000	2.058805000	-0.000012000
7	1.489423000	-1.119885000	-0.000050000
1	1.111331000	-2.058808000	-0.000043000
8	1.144247000	2.374852000	-0.000013000
8	-1.144249000	-2.374859000	-0.000058000

protonated indigo - cis

Atom	X	Y	Z
6	2.946190000	0.486684000	-0.031591000
6	2.855565000	-0.917026000	0.023589000
6	3.982290000	-1.722871000	0.063569000
6	5.225681000	-1.071280000	0.043581000
6	5.335881000	0.328001000	-0.014948000
6	4.192833000	1.125089000	-0.052302000
6	1.598734000	1.015819000	-0.041890000
6	0.685490000	-0.195536000	-0.002955000
1	3.919854000	-2.805312000	0.106313000
1	6.129113000	-1.672278000	0.073312000
1	6.318146000	0.787007000	-0.029989000
1	4.254679000	2.207523000	-0.094335000
6	-0.692478000	-0.189509000	-0.020324000
6	-1.590424000	0.966800000	0.022390000
6	-2.838730000	-0.944864000	-0.019236000
6	-2.919506000	0.470329000	0.033345000
6	-3.985275000	-1.734726000	-0.060566000
6	-4.170276000	1.124168000	0.066863000
6	-5.212020000	-1.069225000	-0.035564000
1	-3.936144000	-2.817673000	-0.103152000
6	-5.310535000	0.340551000	0.031428000
1	-4.222460000	2.206544000	0.114189000
1	-6.124287000	-1.657076000	-0.064676000
1	-6.290626000	0.803793000	0.053653000
7	1.494859000	-1.292988000	0.024086000
1	1.186990000	-2.240386000	0.190107000
7	-1.500309000	-1.326654000	-0.006658000
1	-1.198109000	-2.233408000	-0.332295000
8	1.222930000	2.196510000	-0.066110000
8	-1.269072000	2.220088000	0.056952000
1	-0.250382000	2.351558000	0.005797000

protonated indigo - trans

Atom	X	Y	Z
6	-2.938186000	-0.691001000	0.000001000
6	-2.869878000	0.713234000	-0.000005000
6	-4.003038000	1.507213000	-0.000007000
6	-5.241741000	0.840732000	-0.000005000
6	-5.330407000	-0.557282000	0.000001000
6	-4.172135000	-1.342273000	0.000005000
6	-1.566108000	-1.202587000	0.000008000
6	-0.686442000	0.055287000	-0.000003000
1	-3.951624000	2.591102000	-0.000010000
1	-6.152295000	1.431373000	-0.000007000
1	-6.305241000	-1.032406000	0.000003000
1	-4.221748000	-2.426296000	0.000009000
6	0.696138000	0.044676000	-0.000004000
6	1.599582000	1.149585000	0.000000000
6	2.787713000	-0.781748000	-0.000015000
6	2.912634000	0.649761000	0.000006000
6	3.922700000	-1.606965000	-0.000019000
6	4.198127000	1.253671000	0.000019000
6	5.160351000	-0.984735000	-0.000005000
1	3.833909000	-2.688016000	-0.000029000
6	5.303242000	0.432739000	0.000012000
1	4.314085000	2.333632000	0.000028000
1	6.056240000	-1.597626000	-0.000008000
1	6.298936000	0.862169000	0.000019000
7	-1.507641000	1.117412000	-0.000004000
1	-1.189897000	2.079508000	-0.000014000
7	1.464460000	-1.117708000	-0.000026000
1	1.055242000	-2.044895000	0.000026000
8	-1.120009000	-2.338119000	0.000029000
8	1.130666000	2.406751000	0.000003000
1	1.844099000	3.062642000	0.000008000

Neutral isoindigo (trans)

Atom	X	Y	Z
6	-2.865742000	-0.952290000	-0.000404000
6	-2.005941000	0.178634000	0.000013000
6	-2.618629000	1.444272000	0.000897000
6	-4.014282000	1.547017000	0.001317000
6	-4.824965000	0.409611000	0.000864000
6	-4.250332000	-0.866566000	-0.000002000
6	-0.752653000	-1.843949000	-0.001396000
6	-0.611235000	-0.320946000	-0.000479000
1	-2.010750000	2.333707000	0.001210000
1	-4.467654000	2.533261000	0.001988000
1	-5.906373000	0.509989000	0.001185000
1	-4.864757000	-1.761848000	-0.000345000
6	0.611230000	0.321024000	-0.000292000

6	2.005915000	-0.178616000	0.000316000
6	0.752706000	1.844006000	-0.000801000
6	2.865750000	0.952277000	0.000318000
6	2.618548000	-1.444276000	0.000998000
6	4.250338000	0.866511000	0.000930000
6	4.014200000	-1.547068000	0.001629000
1	2.010636000	-2.333689000	0.000994000
6	4.824922000	-0.409692000	0.001584000
1	4.864809000	1.761761000	0.000907000
1	4.467541000	-2.533327000	0.002138000
1	5.906326000	-0.510109000	0.002061000
8	0.097077000	-2.724745000	-0.002215000
8	-0.096983000	2.724798000	-0.001535000
7	-2.100852000	-2.111909000	-0.001206000
1	-2.449705000	-3.058904000	-0.001761000
7	2.100902000	2.111929000	-0.000328000
1	2.449849000	3.058889000	-0.000588000

protonated isoindigo - cis

Atom	X	Y	Z
6	2.949690000	0.088929000	-0.169332000
6	1.669484000	-0.466927000	0.076843000
6	1.595856000	-1.819368000	0.452319000
6	2.764165000	-2.580157000	0.502341000
6	4.005001000	-2.010659000	0.189335000
6	4.116939000	-0.651322000	-0.138867000
6	1.530174000	1.864495000	-0.214714000
6	0.695487000	0.612776000	-0.008272000
1	0.656024000	-2.269941000	0.739857000
1	2.710473000	-3.623311000	0.794097000
1	4.900698000	-2.622407000	0.222214000
1	5.083040000	-0.201214000	-0.341544000
6	-0.685435000	0.615204000	0.000940000
6	-1.666692000	-0.471684000	-0.078976000
6	-1.516820000	1.842764000	0.206473000
6	-2.942886000	0.083659000	0.165126000
6	-1.599312000	-1.822681000	-0.450049000
6	-4.116842000	-0.645432000	0.136804000
6	-2.773934000	-2.578730000	-0.495421000
1	-0.662922000	-2.280967000	-0.736587000
6	-4.012611000	-2.006835000	-0.185514000
1	-5.079846000	-0.187066000	0.336230000
1	-2.722630000	-3.623395000	-0.782646000
1	-4.910514000	-2.615045000	-0.216563000
8	1.162552000	3.057019000	-0.264275000
8	-1.173961000	3.069501000	0.278932000
1	-0.119687000	3.184357000	0.040366000
7	2.809689000	1.473333000	-0.375151000

1	3.563666000	2.134111000	-0.516399000
7	-2.790232000	1.473536000	0.359857000
1	-3.536799000	2.140443000	0.516577000

protonated isoindigo - trans

Atom	X	Y	Z
6	-2.857287000	-0.948610000	-0.133662000
6	-1.976280000	0.152448000	0.104410000
6	-2.538424000	1.375636000	0.541945000
6	-3.914084000	1.487696000	0.675304000
6	-4.753926000	0.395426000	0.384543000
6	-4.235745000	-0.840050000	-0.013225000
6	-0.762297000	-1.827514000	-0.364751000
6	-0.630284000	-0.322046000	-0.055734000
1	-1.907091000	2.217265000	0.787196000
1	-4.348905000	2.422061000	1.012565000
1	-5.828763000	0.507875000	0.487757000
1	-4.887750000	-1.684959000	-0.207410000
6	0.607926000	0.318574000	-0.057870000
6	1.982764000	-0.200031000	0.099086000
6	0.793105000	1.729056000	-0.336441000
6	2.866270000	0.884451000	-0.082167000
6	2.541775000	-1.436092000	0.456471000
6	4.243205000	0.807844000	0.043065000
6	3.930412000	-1.539753000	0.589725000
1	1.918585000	-2.304930000	0.604280000
6	4.773819000	-0.443312000	0.379529000
1	4.882402000	1.672699000	-0.103568000
1	4.360263000	-2.499536000	0.856026000
1	5.847642000	-0.556873000	0.483465000
8	0.116320000	-2.641186000	-0.558744000
8	-0.170072000	2.589661000	-0.622265000
1	0.154569000	3.442626000	-0.952796000
7	-2.116493000	-2.076435000	-0.447813000
1	-2.482119000	-3.003438000	-0.618425000
7	2.090489000	2.032550000	-0.366898000
1	2.477538000	2.944262000	-0.579425000

SI References

1. Zou, P. and H.L. Koh, *Determination of indican, isatin, indirubin and indigotin in Isatis indigotica by liquid chromatography/electrospray ionization tandem mass spectrometry.* Rapid communications in mass spectrometry, 2007. **21**(7): p. 1239-1246.

2. Puchalska, M., et al., *Identification of indigoid dyes in natural organic pigments used in historical art objects by high-performance liquid chromatography coupled to electrospray ionization mass spectrometry.* Journal of mass spectrometry, 2004. **39**(12): p. 1441-1449.