Supporting Information

Probing the binding mechanism of photoresponsive azobenzene polyamine derivatives with human serum albumin

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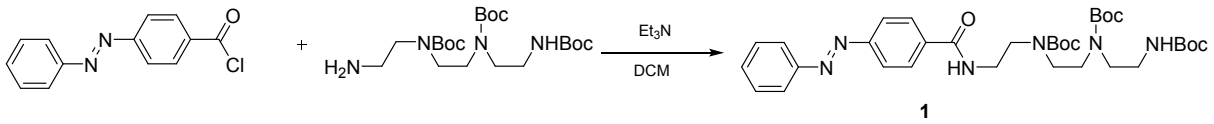
Synthesis and characterization

Polyamines were synthesized according to Alnylam Pharmaceuticals patent procedure.[1]

Synthesis of **1**: (tert-butyl (E)-(2-((tert-butoxycarbonyl)(2-((tert-butoxycarbonyl)amino)ethyl)amino)ethyl)(2-(4-(phenyldiazenyl)benzamido)ethyl)carbamate):



To a solution of tert-butyl (2-aminoethyl)(2-((tert-butoxycarbonyl)(2-((tert-butoxycarbonyl)amino)ethyl)amino)ethyl)carbamate (0.3 g, 0.67 mmol, 1 eq.) in DCM (10 mL), Et_3N (0.21 mL, 1.47 mmol, 2.2 eq.) and (E)-4-(phenyldiazenyl)benzoyl chloride) (0.18 g, 0.74 mmol, 1.1 eq.) were added. The reaction mixture was stirred overnight and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography on SiO_2 in gradient from DCM do 10%MeOH/DCM. **1** was obtained as orange-red semi solid (70 mg, 23%).

^{1}H NMR (601 MHz, $CDCl_3$) δ:

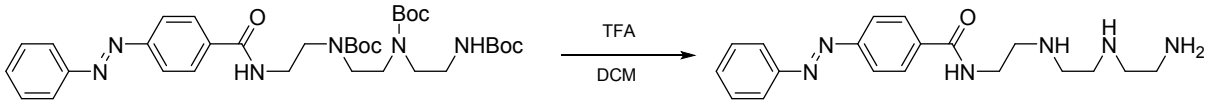
8.12 – 7.86 (m, 6H), 7.57 – 7.38 (m, 3H), 3.68 – 3.45 (m, 4H), 3.42 – 3.17 (m, 8H), 1.52 – 1.35 (m, 27H).

^{13}C NMR (151 MHz, $CDCl_3$) δ:

166.8, 157.5, 156.2, 156.0, 154.2, 152.6, 131.6, 129.2, 128.8, 128.1, 123.1, 122.8, 81.1, 80.8, 80.4, 47.5, 47.3, 46.8, 46.4, 45.8, 45.5, 40.7, 39.4, 28.5, 28.5.

HRMS m/z (ESI): Calculated $[M+H]^+$655.3819, Found:655.3811

Synthesis of **2**: (E)-N-(2-((2-((2-aminoethyl)amino)ethyl)amino)ethyl)-4-(phenyldiazenyl)benzamide:



1 **2**

1 (70 mg, 106,9 µmol, 1 eq.) was dissolved in DCM (2 mL) and TFA was added (0.2 mL) and reaction mixture was stirred overnight. Solvent was removed under reduced pressure and **2** was obtained in TFA salt form as a red semi solid (70 mg, 94%).

^{1}H NMR (601 MHz, D_2O) δ:

7.92 (d, *J* = 8.0 Hz, 2H), 7.80 – 7.77 (m, 4H), 7.64 – 7.57 (m, 3H),

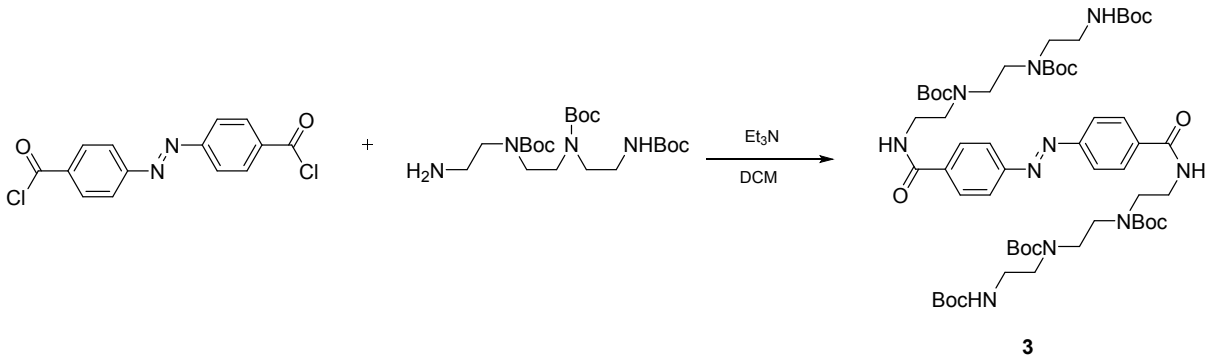
3.83 (t, *J* = 5.7 Hz, 2H), 3.71 (dt, *J* = 10.9, 5.7 Hz, 4H), 3.67 (t, *J* = 7.1 Hz, 2H), 3.57 (t, *J* = 7.1 Hz, 2H), 3.52 (t, *J* = 5.7 Hz, 2H).

^{13}C NMR (151 MHz, D_2O) δ:

172.9, 156.8, 154.7, 137.4, 135.1, 132.3, 131.3, 125.6, 125.4, 51.0, 47.6, 46.5, 46.1, 39.3, 38.2.

HRMS m/z (ESI): Calculated $[M+H]^+$ = 697.2032, Found: 697.2026

Synthesis of **3**: di-tert-butyl (((4,4'-(diazene-1,2-diyl)bis(benzoyl))bis(azanediyl))bis(ethane-2,1-diyl))(E)-bis((2-((tert-butoxycarbonyl)(2-((tert-butoxycarbonyl)amino)ethyl)amino)ethyl)carbamate):



To a solution of Tert-butyl (2-aminoethyl)(2-((tert-butoxycarbonyl)(2-((tert-butoxycarbonyl)amino)ethyl)amino)ethyl)carbamate (0.58 g, 1.3 mmol, 2.2 eq.) in DCM (10 mL), Et_3N (0.18 mL, 1.3 mmol, 2.2 eq.) and azobenzene-4,4′-dicarbonyl dichloride (0.18 g, 0.59 mmol, 1 eq.) were added. Reaction mixture was stirred overnight and solvent was removed under reduced pressure. The crude product was purified by flash chromatography on SiO_2 using EtOAC as eluent. **3** was obtained as a red semi solid (0.3 g, 44%).

^{1}H NMR (601 MHz, $CDCl_3$) δ:

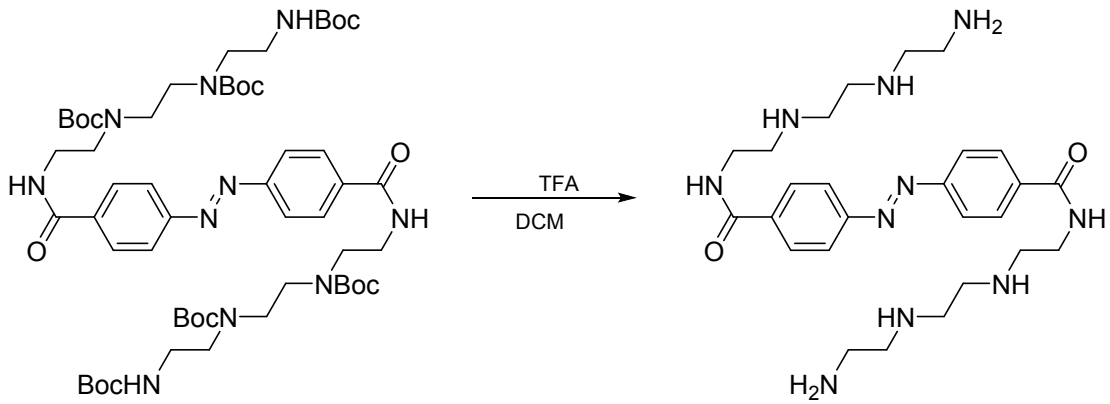
8.10 – 7.90 (m, 8H), 7.80 (s, 1H), 7.66 (s, 1H), 4.98 (s, 1H), 4.77 (s, 1H), 3.66 – 3.48 (m, 8H), 3.42 – 3.19 (m, 16H), 1.48 – 1.37 (m, 54H).

^{13}C NMR (151 MHz, $CDCl_3$) δ:

166.7, 157.6, 154.1, 136.4, 128.2, 123.1, 81.1, 80.9, 80.5, 47.3, 46.8, 46.5, 45.9, 45.5, 41.1, 40.9, 39.4, 28.5, 28.5**.**

HRMS m/z (ESI): Calculated $[M+Na]^+$ = 1149.6536, Found: 1149.6506

Synthesis of **4**: (E)-4,4'-(diazene-1,2-diyl)bis(N-(2-((2-((2-aminoethyl)amino)ethyl)amino)ethyl)benzamide):



3 **4**

3 (0.3 g, 0.27 mmol, 1 eq.) was dissolved in DCM (5 mL) and TFA was added (1 mL) and reaction mixture was stirred overnight. The solvent was removed under reduced pressure and **4** was obtained in TFA salt form as a red semi solid (0.287 g , 90%).

^{1}H NMR (601 MHz, D_2O) δ:

7.95 (d, *J* = 8.4 Hz, 4H), 7.87 (d, *J* = 8.4 Hz, 4H), 3.83 (t, *J* = 5.6 Hz, 4H), 3.68 – 3.63 (m, 8H), 3.60 (t, *J* = 7.0 Hz, 4H), 3.53 – 3.47 (m, 8H).

^{13}C NMR (151 MHz, D_2O) δ:

173.2, 156.9, 138.2, 131.5, 125.9, 51.2, 47.7, 46.6, 46.2, 39.4, 38.3.

HRMS m/z (ESI): Calculated $[M+Na]^+$ = 1210.3083, Found: 1210.3094

Computation

MarvinSketch package was used to predict the pK_a values of polyamine side chains. The *ab initio* calculations were performed using the Gaussian 09 program package (Revision D.01).[2] DFT method with B3LYP[3] functional was used with 6-31G(p) as a basis. To include solvent the SCRF-PCM model was used. All optimized geometries were confirmed as a minimum by frequency analysis. Electrostatic potential surface (ESP) were calculated at 0.02 isovalue and 0.000400 density, the scales are given in the corresponding Figures S1 and S2. Based on the difference energies of the *trans* and *cis* forms the thermodynamic stability of the isomers were estimated:

Table S1. Thermodynamic stability of the *trans* forms of **Azo-4N** and **Bis-Azo-4N**.

Compound	Stability of *trans* form compared to *cis* [kcal/mol]
Azo-4N	-12.8
bis-Azo-4N	-13.0

Cartesian coordinates for **Azo-4N** (*trans*)

C	2.25474300	0.54463200	1.51175000
C	3.59008900	0.90937900	1.35390600
C	4.42530700	0.17219600	0.49850900
C	3.91426100	-0.94035800	-0.19573700
C	2.57819000	-1.29740300	-0.04047600
C	1.73150200	-0.55339700	0.80764600
H	1.60218900	1.10232300	2.17248500
H	4.00720000	1.75963900	1.88097000
H	4.57192500	-1.51011200	-0.83947600
H	2.20900900	-2.17601800	-0.55870700
N	5.77221400	0.63030100	0.40796700
N	6.53723100	-0.04893100	-0.36051100
C	7.88316200	0.39829000	-0.46501000
C	8.40443800	1.52415900	0.20304200
C	8.70729800	-0.36967700	-1.30545800

C	9.74302200	1.86920800	0.02383100
H	7.75533400	2.10496800	0.84644100
C	10.04839400	-0.01860900	-1.48001500
H	8.27712600	-1.22991400	-1.80615900
C	10.56771200	1.10072300	-0.81617200
H	10.15031200	2.73514900	0.53508900
H	10.68416700	-0.61182200	-2.12810200
C	0.29305200	-0.89501600	1.01814900
O	-0.33339300	-0.52104900	2.04867100
N	-0.32989100	-1.62192900	0.04214600
C	-1.71098800	-2.07158700	0.18736100
H	-1.86246500	-2.40280700	1.21897700
H	-1.86371200	-2.92462800	-0.47912100
H	0.14309200	-1.81887000	-0.82722200
C	-2.68638600	-0.92954500	-0.14677600
H	-2.53106200	-0.56052000	-1.16168900
H	-2.57319400	-0.11160300	0.56538100
N	-4.13108100	-1.40309400	-0.06530200
H	-4.27704700	-2.16044600	-0.74374300
C	-5.14802800	-0.29771700	-0.32996500
H	-4.86868500	0.16128800	-1.28081400
H	-5.04383900	0.43654700	0.46898900
C	-6.57252500	-0.85926100	-0.37518100
H	-6.63559500	-1.67572800	-1.11743200
H	-6.83103000	-1.28921400	0.59898300
N	-7.49967400	0.24477400	-0.64335000
H	-7.38645100	0.61223900	-1.58741700

C	-8.91227000	-0.03149700	-0.33654800
H	-8.96707900	-0.38600100	0.69979600
H	-9.33754500	-0.82920100	-0.97252300
C	-9.74951500	1.24913700	-0.48702000
H	-9.33946600	2.01819700	0.17783200
H	-9.66601700	1.62160400	-1.51700000
C	-11.21561400	0.97742000	-0.15140500
H	-11.65858600	0.23844300	-0.82154200
H	-11.34060700	0.64163000	0.87974100
N	-12.05551700	2.24403400	-0.29210300
H	-12.02144100	2.60853700	-1.24992000
H	-13.03760800	2.05897100	-0.06341000
H	-11.72035600	2.98039500	0.33782400
H	-4.30804500	-1.80322300	0.86412100
H	11.60857900	1.37601400	-0.95023700

Cartesian coordinates for **Azo-4N** (*cis*)

N	-6.45334544	1.80918622	-0.61372670
N	-5.30613806	2.30565961	-0.40202758
C	-4.11326765	1.55488979	-0.09557550
C	-3.67888435	0.47555131	-0.88611559
C	-3.27965108	2.06812985	0.91207144
C	-2.42769060	-0.09373975	-0.65096359
H	-4.30825929	0.08960123	-1.67907877
C	-2.04770628	1.47078799	1.16341970
H	-3.60981191	2.92496834	1.48818013
C	-1.59975153	0.39274231	0.37812448

H	-2.12473033	-0.93883133	-1.25956435
H	-1.40804215	1.84143979	1.95520140
C	-0.25970097	-0.18231983	0.69453809
O	0.29326682	0.00945694	1.81414931
C	-6.82098779	0.43591527	-0.36747994
C	-7.75722030	-0.10702399	-1.26389123
C	-6.42836362	-0.29000490	0.77280025
C	-8.24871949	-1.39831667	-1.06036106
H	-8.08031317	0.48967356	-2.10978993
C	-6.95335190	-1.56706359	0.98680313
H	-5.74452750	0.14312575	1.49277211
C	-7.84962841	-2.13021294	0.06675144
H	-8.95357608	-1.82460206	-1.76584488
H	-6.66392867	-2.12264521	1.87238077
H	-8.24625093	-3.12541085	0.23660505
N	0.36523820	-0.91686011	-0.27535650
C	1.65406936	-1.55723296	-0.02872813
H	1.77285001	-2.37701531	-0.74153645
H	1.64837794	-1.97107618	0.98394787
H	-0.02371535	-0.97891969	-1.20416769
C	2.79034635	-0.53045773	-0.17276747
H	2.83988144	-0.13409396	-1.18803514
H	2.65200970	0.28559499	0.53709573
N	4.14659601	-1.15607989	0.12651126
H	4.14045599	-1.54472349	1.07726458
C	5.29991923	-0.16600026	-0.00175245
H	5.28166708	0.22280752	-1.02014246

H	5.09076321	0.64598495	0.69790669
C	6.64795562	-0.83094234	0.30086398
H	6.86779204	-1.58481563	-0.46233094
H	6.60934711	-1.35103535	1.27334213
N	7.68467809	0.20392974	0.23687604
H	7.62513940	0.85700628	1.01515592
H	4.30656061	-1.94418401	-0.51287014
C	9.06015962	-0.23718352	-0.01334602
H	9.03241359	-1.05350605	-0.74285788
H	9.56800198	-0.62129455	0.88813895
C	9.83698071	0.94836935	-0.59535308
H	9.81899662	1.80855210	0.07630507
H	9.43275496	1.24059141	-1.56438948
N	11.30522289	0.59399191	-0.80417640
H	11.41178553	-0.18669702	-1.46061864
H	11.82173301	1.39318885	-1.18577910
H	11.75368722	0.32654358	0.07858346

Cartesian coordinates for **bis-Azo-4N** (*trans*)

C	3.88176400	-1.47479800	-1.44825300
C	2.49015000	-1.45464300	-1.50737300
C	1.73321300	-1.87247100	-0.40037300
C	2.37973100	-2.32200200	0.76650900
C	3.77001000	-2.33515800	0.82298700
C	4.53558700	-1.90082000	-0.27939900
H	4.47740000	-1.15859300	-2.29583600
H	1.96937900	-1.11807700	-2.39621000

H	1.78510100	-2.65767500	1.60627400
H	4.25269400	-2.71358700	1.71762200
N	0.32036800	-1.80400100	-0.56286700
N	-0.37491100	-2.18699600	0.44006100
C	-1.78759700	-2.11052700	0.28342700
C	-2.43754400	-1.62910700	-0.86871000
C	-2.54146300	-2.54606700	1.38563800
C	-3.82802700	-1.59707700	-0.91362400
H	-1.84555800	-1.28109200	-1.70534400
C	-3.93289100	-2.50795500	1.33770800
H	-2.01822300	-2.90733400	2.26323900
C	-4.59085600	-2.04490500	0.18531600
H	-4.30998300	-1.19285600	-1.79732000
H	-4.52645900	-2.83481900	2.18257600
C	6.02970900	-1.89059400	-0.27738400
O	6.69178100	-1.89846200	-1.35232600
N	6.65958500	-1.85526400	0.93528100
C	8.11546500	-1.90176200	1.03800900
H	8.49326200	-2.62113600	0.30516100
H	8.37717500	-2.25266200	2.03933600
H	6.12809300	-1.73893100	1.78532400
C	8.71223700	-0.51003500	0.76703700
H	8.37146500	0.21908500	1.50361500
H	8.44689900	-0.17339700	-0.23570100
N	10.23288600	-0.53663700	0.84081300
H	10.52429900	-0.85371600	1.77344300
C	10.86852500	0.81981700	0.55402500

H	10.44497400	1.51927500	1.27804300
H	10.56329900	1.11334000	-0.45087100
C	12.39555100	0.75904900	0.66448700
H	12.69312500	0.31291600	1.63068900
H	12.79944900	0.11562800	-0.12504700
N	12.92019300	2.11294800	0.45984100
H	12.68217600	2.73056600	1.23472600
C	14.34842900	2.19913600	0.11688400
H	14.52546300	1.55138900	-0.75008200
H	15.00482600	1.83772900	0.92919300
C	14.71528400	3.64950300	-0.23794300
H	14.07225900	3.98056700	-1.06170900
H	14.50928200	4.29768600	0.62462000
C	16.18802600	3.76257200	-0.62989900
H	16.85312800	3.46342400	0.18234100
H	16.42020100	3.16878800	-1.51605500
N	16.55314300	5.20446000	-0.97343800
H	16.40781700	5.82484200	-0.17013200
H	17.53795800	5.27532200	-1.24990600
H	15.98274900	5.55606300	-1.74986200
H	10.59160400	-1.22513000	0.16865000
C	-6.08518800	-2.02807600	0.20383500
O	-6.73123000	-2.07257500	1.28765200
N	-6.73593800	-1.97531500	-0.99684200
C	-8.19470800	-1.97411700	-1.06738400
H	-8.58345900	-2.67749100	-0.32406100
H	-8.48871600	-2.31818900	-2.06166200

H	-6.22074400	-2.01859600	-1.86323400
C	-8.73696800	-0.56168700	-0.79031400
H	-8.38897400	0.15100400	-1.53926000
H	-8.43743800	-0.22758900	0.20380800
N	-10.25910400	-0.53855400	-0.82717400
H	-10.58220600	-0.84027900	-1.75457500
C	-10.84603800	0.83521700	-0.51741600
H	-10.40601000	1.52963000	-1.23645800
H	-10.52267700	1.10548100	0.48819000
C	-12.37511300	0.82559200	-0.61493300
H	-12.69363500	0.41512900	-1.59021600
H	-12.79221600	0.17402000	0.16080300
N	-12.85596700	2.18868400	-0.36856700
H	-12.59998400	2.82212200	-1.12479100
C	-14.28015300	2.30905000	-0.01902500
H	-14.47815400	1.63232400	0.82074900
H	-14.94898400	2.00350900	-0.84407900
C	-14.59888400	3.75446200	0.39733200
H	-13.95535000	4.02489600	1.24260600
H	-14.35965100	4.43349700	-0.43246000
C	-16.07222300	3.90277800	0.77499500
H	-16.73401500	3.67764600	-0.06336700
H	-16.34334600	3.26815500	1.62068800
N	-16.38642500	5.33613300	1.19553400
H	-16.16995800	5.99699200	0.44177400
H	-17.37975400	5.43915600	1.42756300
H	-15.84145400	5.60894000	2.02022500

H	-10.62450700	-1.22011300	-0.15106600

Cartesian coordinates for **bis-Azo-4N** (*cis*)

N	-0.62393600	4.92143800	0.22441700
N	0.62410000	4.90026500	0.47753200
C	1.45738500	3.70770400	0.37836800
C	1.43494300	2.85330300	-0.73478700
C	2.43503400	3.54803500	1.37238400
C	2.38382300	1.83794100	-0.84408300
H	0.69285500	2.98665200	-1.51079600
C	3.35160500	2.50829800	1.27555200
H	2.45588300	4.23801400	2.20672900
C	3.34903600	1.65852400	0.15862200
H	2.34155200	1.17654400	-1.70121800
H	4.09491200	2.34286800	2.04475700
C	4.39168900	0.58226800	0.13967400
O	5.01452600	0.25319600	1.18127500
C	-1.43980700	3.72759000	0.03423100
C	-2.39992200	3.78508200	-0.98766000
C	-1.42158000	2.64481900	0.92695300
C	-3.30306700	2.74069800	-1.14298300
H	-2.41873600	4.64477100	-1.64589100
C	-2.35661000	1.62036900	0.78734300
H	-0.69387500	2.60916100	1.72685000
C	-3.30397000	1.66028200	-0.24710600
H	-4.03385400	2.74462000	-1.94139800
H	-2.31682500	0.78443500	1.47559800

N	4.65517300	-0.01614100	-1.06321200
C	5.60204000	-1.13849200	-1.13382900
H	5.50054600	-1.61269600	-2.11160300
H	5.35332900	-1.86041100	-0.34982300
H	4.27727700	0.34866200	-1.93047000
C	7.03652700	-0.61177500	-0.90827700
H	7.33181900	0.09572100	-1.68267300
H	7.09347500	-0.16244100	0.08221200
N	8.05083300	-1.76706600	-0.94453500
H	7.77108900	-2.48367100	-0.25418000
C	-4.33351900	0.59791300	-0.48793000
O	-4.96208000	0.52713100	-1.57476600
N	-4.57874000	-0.28404700	0.53018000
C	-5.51571700	-1.39830100	0.32589400
H	-5.39345000	-2.10509800	1.14835600
H	-5.27631400	-1.89280400	-0.62025200
H	-4.19227900	-0.14628400	1.45722900
C	-6.95592200	-0.84446800	0.26767100
H	-7.22959500	-0.34592100	1.19722400
H	-7.03769100	-0.16838600	-0.58259000
N	-7.97321500	-1.97712700	0.05107800
H	-7.72942000	-2.49672500	-0.80902200
H	-7.91386900	-2.64618100	0.83698700
C	-9.42267300	-1.46335100	-0.06553200
H	-9.64919800	-0.92985200	0.85663400
H	-9.43543300	-0.77486100	-0.91203000
C	-10.41041300	-2.62762100	-0.26774300

H	-10.40436400	-3.27102300	0.61694900
H	-10.12096500	-3.23695800	-1.13718700
C	9.49144800	-1.31294800	-0.63878100
H	9.73403700	-0.54468400	-1.37461500
H	9.48742900	-0.88661000	0.36352200
C	10.47131900	-2.49886000	-0.71590400
H	10.39759100	-2.99811200	-1.69438900
H	10.22315700	-3.22974500	0.05944400
N	11.81101100	-1.94414500	-0.41800800
H	12.19043000	-1.44018400	-1.22517100
N	-11.75106700	-2.01031700	-0.38344200
H	-11.90122100	-1.62275400	-1.31950800
H	8.02686100	-2.21622500	-1.87532200
C	-12.90139000	-2.83746600	0.04475500
H	-13.28851200	-3.50827500	-0.73717800
H	-12.58674000	-3.45027300	0.89450900
C	-13.98830700	-1.84721200	0.49653800
H	-13.64650400	-1.26542500	1.35077900
H	-14.29146900	-1.18512200	-0.31537500
C	12.80683600	-2.88856800	0.13658000
H	13.35948300	-3.45765600	-0.62634900
H	12.28426400	-3.60131700	0.78095100
C	13.77101300	-2.04126700	0.98382700
H	13.24654400	-1.58176400	1.81974700
H	14.27049400	-1.27861100	0.38540000
N	14.89023600	-2.92405600	1.58064100
H	15.54453300	-2.35519700	2.14180800

H	14.49692700	-3.65503900	2.19606200
N	-15.26573700	-2.59561800	0.94062700
H	-15.07310800	-3.21466800	1.74504400
H	-15.99851800	-1.92515500	1.22422700
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H	-15.64357600	-3.17304900	0.17142000

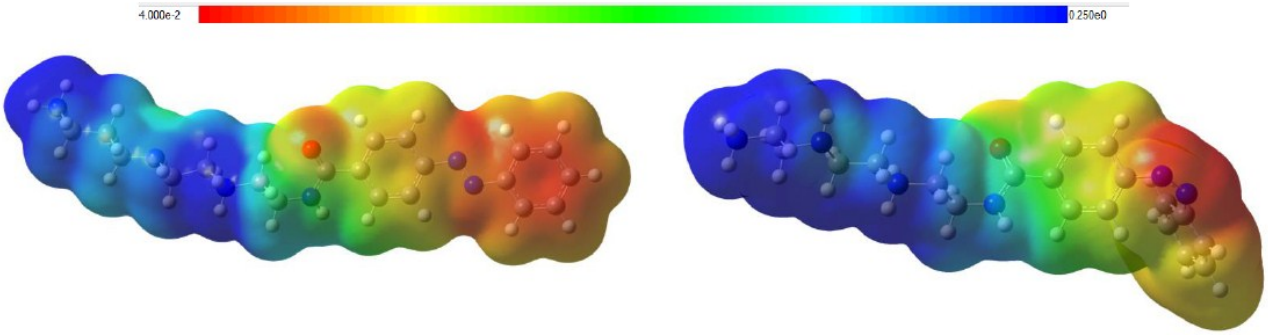

Figure S1. ESP of **Azo-4N** in *trans* (left) and *cis* (right) configurations.

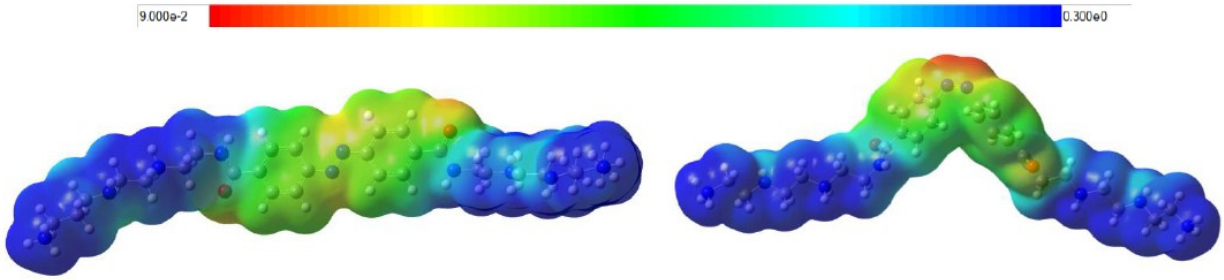

Figure S2. ESP of **bis-Azo-4N** in *trans* (left) and *cis* (right) configurations.

Calculation of the positive charges along the polyamine substituents

As previously mentioned, the amine pK_a values for both **Azo-4N** and **bis-Azo-4N** were determined by using the MarvinSketch software.

The number of charges along the azobenzene substituents can be estimated by using the following equations:

Azo-4N: pK_{a1}: 4.37; pK_{a2}: 8.62; pK_{a3}: 9.55

$$n = \frac{\frac{K_{a1}K_{a2}}{10^{-2pH}} + 2\frac{K_{a1}}{10^{-pH}} + 3}{1 + \frac{K_{a1}}{10^{-pH}} + \frac{K_{a1}K_{a2}}{10^{-2pH}} + \frac{K_{a1}K_{a2}K_{a3}}{10^{-3pH}}} \quad (1)$$

bis-Azo-4N: pK_{a1}: 4.07; pK_{a2}: 4.67; pK_{a3}: 8.32; pK_{a4}: 9.08; pK_{a5}: 9.34; pK_{a6}: 9.68

$$n = \frac{\frac{K_{a1}K_{a2}K_{a3}K_{a4}K_{a5}}{10^{-5pH}} + 2\frac{K_{a1}K_{a2}K_{a3}K_{a4}}{10^{-4pH}} + 3\frac{K_{a1}K_{a2}K_{a3}}{10^{-3pH}} + 4\frac{K_{a1}K_{a2}}{10^{-2pH}} + 5-}{1 + \frac{K_{a1}}{10^{-pH}} + \frac{K_{a1}K_{a2}}{10^{-2pH}} + \frac{K_{a1}K_{a2}K_{a3}}{10^{-3pH}} + \frac{K_{a1}K_{a2}K_{a3}K_{a4}}{10^{-4pH}} + \frac{K_{a1}K_{a2}K_{a3}K_{a4}K_{a5}}{10^{-5pH}} + \frac{K_{a}}{}}$$

The calculated values are:

$n_{\textbf{Azo-4N}} = 2$

$n_{\textbf{bis-Azo-4N}} = 3.8$

Assessing the absence of aggregation

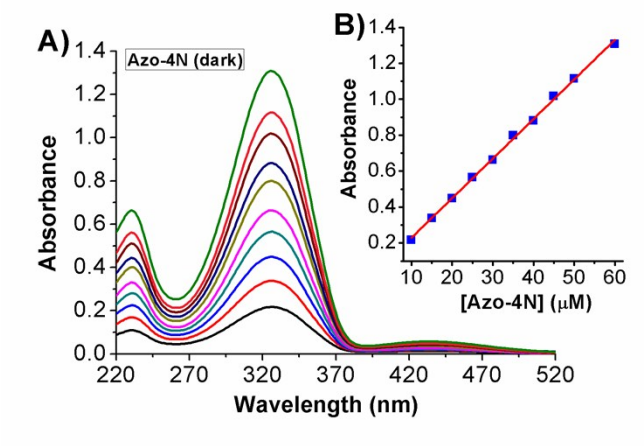

Figure S3. A) plot of absorbance *vs.* **Azo-4N** concentration. B) regression line for the plot of Abs *vs.* [**Azo-4N** (dark)]. [**Azo-4N** (dark)] =1 to 6 × 10^{-5} M.

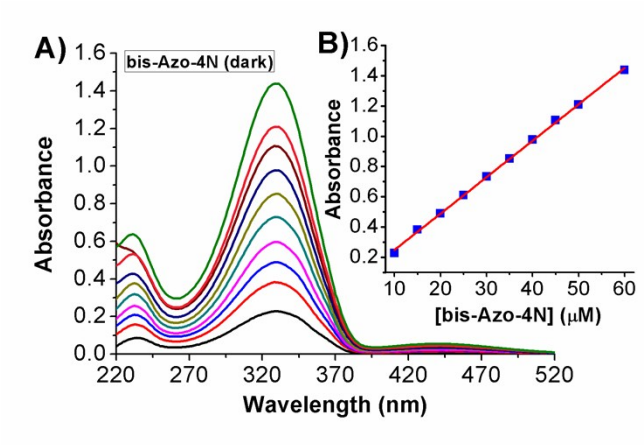

Figure S4. A) plot of absorbance *vs.* **bis-Azo-4N** concentration. B) regression line for the plot Abs *vs.* [**bis-Azo-4N** (dark)]. [**bis-Azo-4N** (dark)] =1 to 6 × 10^{-5} M.

Influence of the UV irradiation on the HSA template

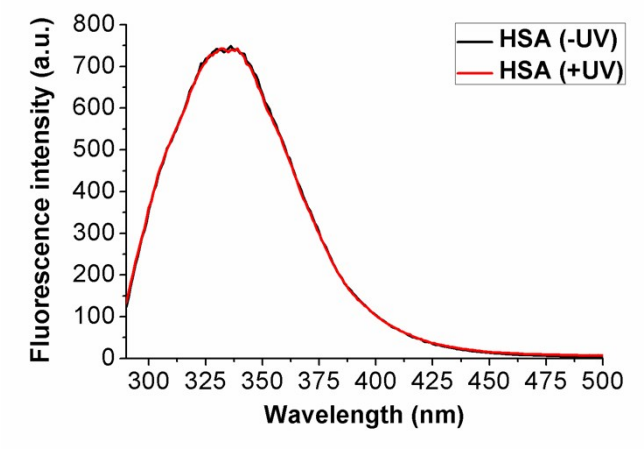

Figure S5. Emission spectra of the native HSA solution in the absence (black line) and presence (red line) of 90 minutes UV irradiation.

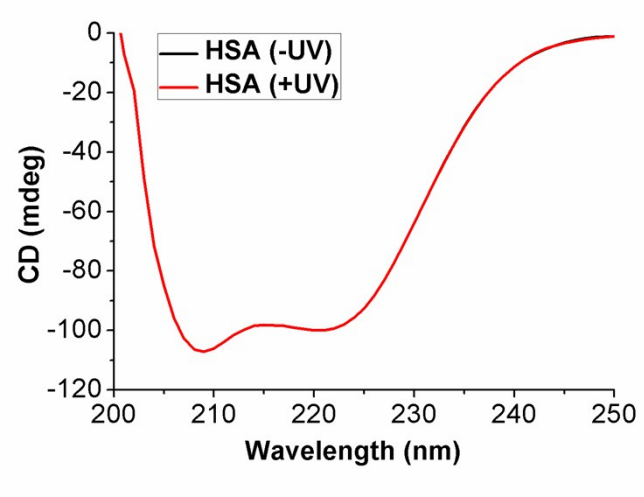

Figure S6. CD spectra of the native HSA solution in the absence (black line) and presence (red line) of 90 minutes UV irradiation. The two curves are almost superimposable.

Quenching of the HSA emission by bis-Azo-4N

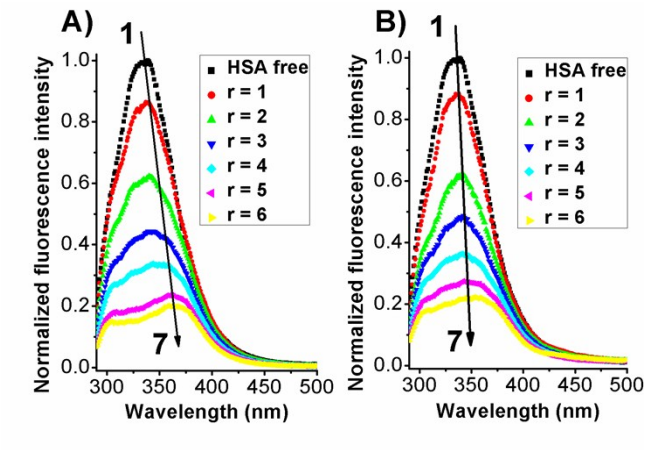

Figure S7. Fluorescence emission spectra of HSA in the absence (black line) and presence of **bis-Azo-4N** in **A)** *trans* form (-UV) and **B)** *cis* form (+UV). [HSA] = 1×10^{-5} M and [**bis-Azo-4N** (dark)] = $0 - 6 \times 10^{-5}$ M. r is the molar ratio defined as [**bis-Azo-4N** (dark)] / [HSA].

Scatchard plots

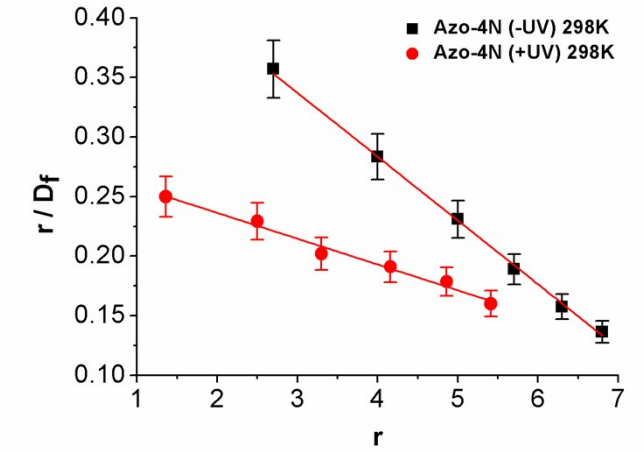

Figure S8. Scatchard plots for the HSA-**Azo-4N** (+/-UV) systems at 298K. [HSA] = 1 × 10^{-5} M, [**Azo-4N** (+/- UV)] = 0 to 4.8 × 10^{-5} M.

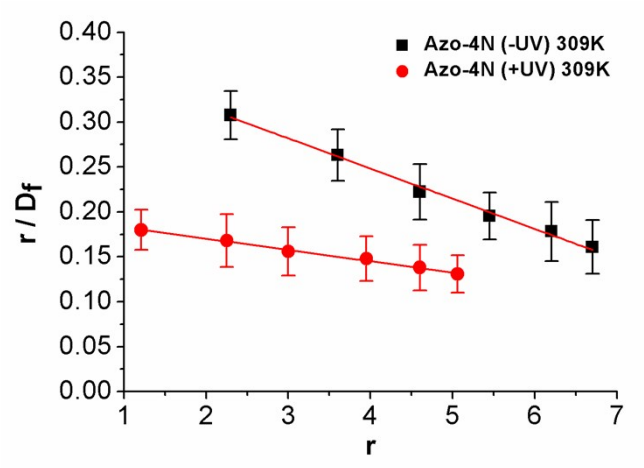

Figure S9. Scatchard plots for the HSA-**Azo-4N** (+/-UV) systems at 309K. [HSA] = 1 × 10^{-5} M, [**Azo-4N** (+/- UV)] = 0 to 4.8 × 10^{-5} M.

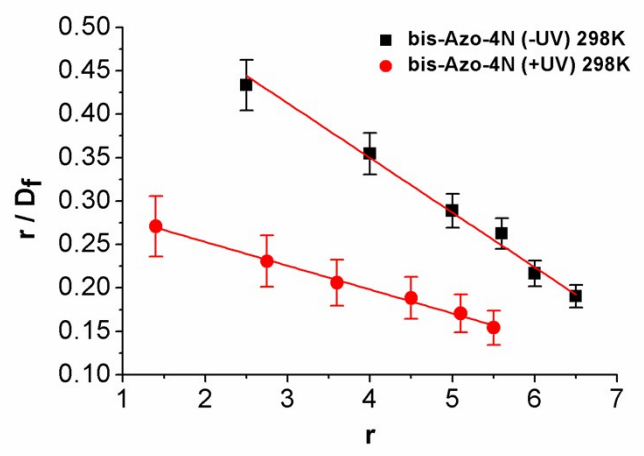

Figure S10. Scatchard plots for the HSA-**bis-Azo-4N** (+/-UV) systems. [HSA] = 1 × 10^{-5} M, [**bis-Azo-4N** (+/- UV)] = 0 to 4.2 × 10^{-5} M.

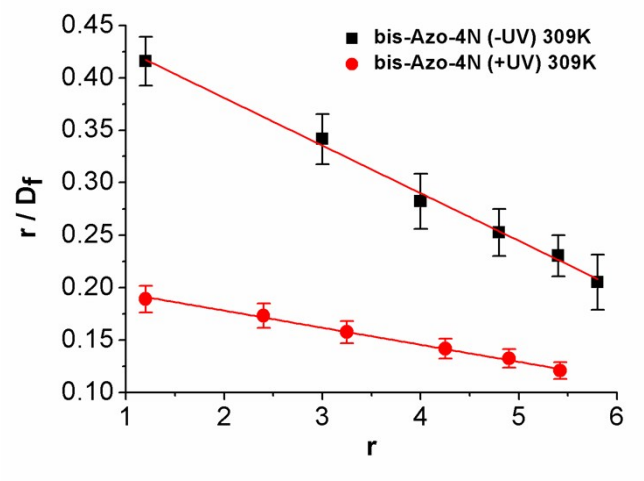

Figure S11. Scatchard plots for the HSA-**bis-Azo-4N** (+/-UV) systems. [HSA] = 1 × 10^{-5} M, [**bis-Azo-4N** (+/- UV)] = 0 to 4.2 × 10^{-5} M.

FRET

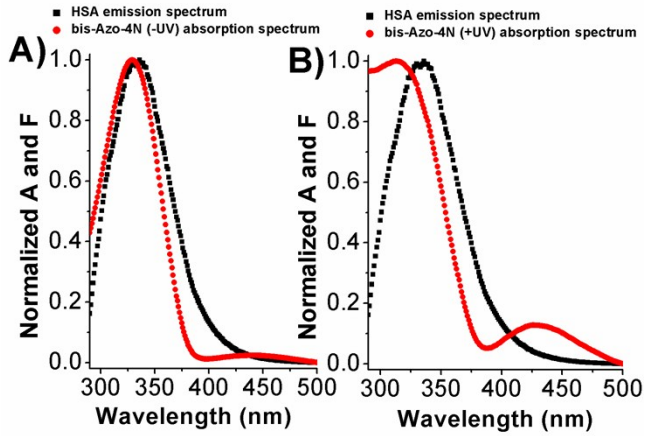

Figure S12. Overlap between the HSA emission spectrum and **A) bis-Azo-4N** (-UV) and **B) bis-Azo-4N** (+UV) absorption spectrum. [HSA] = [**bis-Azo-4N** (+/- UV)] = 1 × 10^{-5} M.

Site-marker experiments

In order to confirm the results achieved by using the FRET theory, we performed displacement binding assay by using the well-known HSA site IIA binder Warfarin. As shown in Figure S13 the azobenzene derivatives were able to displace the anticoagulant agent which resulted in the

fluorescence intensity of the HSA-Warfarin complex being efficiently quenched. This results along with the evidences discussed in the main text unambiguously confirm that the photosensitive polyamines used in this work preferentially bind within the protein subdomain IIA.

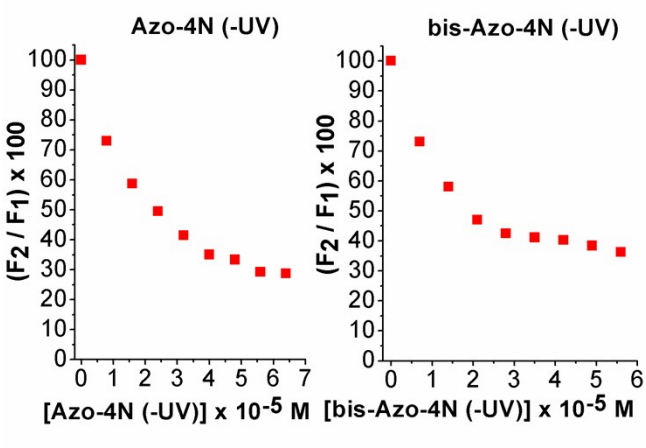

Figure S13. Site-marker competition experiment between Warfarin and **Azo-4N** (-UV) (left panel) and **bis-Azo-4N** (-UV) (right panel). [HSA] = [Warfarin] = 1×10^{-5} M; [**Azo-4N** (-UV)] = 0 to 5.6×10^{-5} M; [**bis-Azo-4N** (-UV)] = 0 to 6.4×10^{-5} M. F_2 and F_1 are the fluorescence intensities of the HSA-Warfarin complex in the presence and absence of the photoswitches, respectively.

CD spectra of HSA-bis-Azo-4N (+/- UV) systems

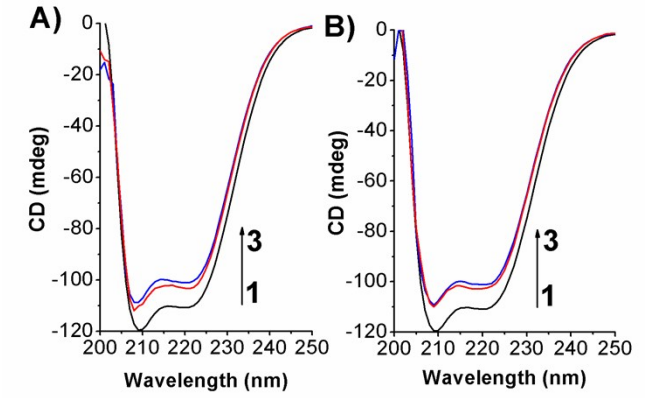

Figure S14. CD spectra of HSA in its free state and complexed form by **A) bis-Azo-4N** (-UV) and **B) bis-Azo-4N** (+UV) at 298K. [HSA] = 1×10^{-6} M and [**bis-Azo-4N** (dark)] = 3 and 5×10^{5} M.

Thermodynamic parameters (Table S2)

Table S2. Thermodynamic parameters (ΔH, ΔS and ΔG) of the interaction between the azobenzene derivatives and HSA.

	Azo-4N (-UV)			**Azo-4N** (+UV)		
T (K)	ΔH^0 (kJ mol^{-1})	ΔS^0 (J mol^{-1} K^{-1})	ΔG^0 (kJ mol^{-1})	ΔH^0 (kJ mol^{-1})	ΔS^0 (J mol^{-1} K^{-1})	ΔG^0 (kJ mol^{-1})
298	-34.6 ± 1.5	-24.9 ± 2	-27.1 ± 0.1	-30.5 ± 2	-19.9 ± 2.3	-24.7 ± 0.3
309	/	/	-26.8 ± 0.6	/	/	-24.4 ± 0.1

	bis-Azo-4N (-UV)			**bis-Azo-4N** (+UV)		
T (K)	ΔH^0 (kJ mol^{-1})	ΔS^0 (J mol^{-1} K^{-1})	ΔG^0 (kJ mol^{-1})	ΔH^0 (kJ mol^{-1})	ΔS^0 (J mol^{-1} K^{-1})	ΔG^0 (kJ mol^{-1})
298	-30.2 ± 1.8	-9.3 ± 1.2	-27.5 ± 0.2	-31.4 ± 3.1	-20.4 ± 2.2	-25.3 ± 0.1
309	/	/	-27.3 ± 0.1	/	/	-25.1 ± 0.1

FRET parameters (Table S3)

Table S3. Overlap integral (J), critical distance (R_0), efficiency of energy transfer (E), average distance D-A (r) and rate constant of energy transfer (k_{ET}) calculated according to the FRET theory.

	J ($\times 10^{-14}$ cm^3 M^{-1})	R_0 (nm)	E	r (nm)	k_{ET} ($\times 10^8$ s^{-1})
Azo-4N (-UV)	2.04	2.76	0.46	2.94	1.94
Azo-4N (+UV)	0.69	2.30	0.28	2.95	0.64
bis-Azo-4N (-UV)	2.62	2.88	0.39	3.21	1.48
bis-Azo-4N (+UV)	1.12	2.50	0.37	2.81	1.40

Secondary structure components (Table S4)

Table S4. Secondary structure variations of the native HSA upon complexation with the photochromes at different molar ratios (r). r = [Photochrome (+/- UV)] / [HSA free].

	α-helix (%)		β-sheet (%)	
HSA free	52		9.2	
molar ratios (r)	r = 30	r = 50	r = 30	r = 50
Azo-4N (-UV)	44.24	43.11	11.52	12.11
Azo-4N (+UV)	44.66	44.21	11.74	13.07
bis-Azo-4N (-UV)	47.51	45.81	10.96	11.26
bis-Azo-4N (+UV)	47.22	46.01	11.23	11.54

References

(1) Alnylam Pharmaceuticals, Inc. Patent: WO2008/42973 A2, **2008**.

(2) Gaussian 09, Revision E.01, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2009.

(3) A. D. Becke, *J. Chem. Phys.* 1993, **98**, 1372-1377.